Exploring the Molecular Basis of Multiple Herbicide Resistance in Black Grass (Alopecurus myosuroides)

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Exploring the Molecular Basis of Multiple Herbicide Resistance in Black Grass (*Alopecurus myosuroides*)

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Abstract

Modern agriculture couples the management of invasive weed species with enhancing crop yields through the intensive use of herbicides. As a result of herbicides being the primary method of weed control in agronomic crops, herbicide resistance has evolved and there has been a vast increase in the occurrence and distribution of herbicide resistant weeds. Many of the most problematic weeds have now evolved multiple herbicide resistance (MHR), which is associated with an enhanced ability to detoxify xenobiotics, enabling the weed to survive herbicide application irrespective of the mode of action. As herbicide resistance is a major limiting factor to food security in global agriculture, effective methods for weed management are sought.

This work focuses on MHR in black grass (*Alopecurus myosuroides*), one of the most damaging weeds of winter cereals. Previous work by Edwards et al. described that black grass populations showing MHR, exhibit an upregulation in the expression of a specific phi-class glutathione transferase (*Am*GSTF1), which is thought to have a direct regulatory control on metabolism. The importance of *Am*GSTF1 in MHR has been confirmed both genetically by transgene experiments and chemically through inhibition experiments. This offers the opportunity to develop potential herbicide synergists, which are active towards *Am*GSTF1 and restore herbicide control in multiple herbicide resistant black grass. Considering the rapid spread of MHR in grass weeds and the limited development of new herbicides, synergists offer an important alternative strategy in counteracting resistance in the field.

Recently a new class of *Am*GSTF1 inhibitors, derived from flavonoids, could be identified. Initial efforts focused on the synthesis of a series of flavone analogues, mainly with structural changes of the C-ring and different substituents in the 5-position. A particular emphasis was put on increasing the aqueous solubility and other physicochemical properties of the compounds, in order to increase the bioavailability in the plant.

Although the potency of the initial lead molecule could not be increased *in vitro*, 5,7-dimethoxy-2-(1’,3’-thiazol-2’-yl)-4H-chromen-4-one, with a significantly increased aqueous solubility, showed good results in *in vivo* studies in black grass and acted as a synergist to the herbicide pinoxaden. The black grass plants died, when pinoxaden (10µl of a 15 µM solution) was applied to plants, which were pre-treated with the thiazole (10 µl of a 2 mM solution), but survived the herbicide treatment, if no synergist was applied prior.

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Abbreviations

4-HPPD	4-Hydroxyphenyl-pyruvatedioxygenase
µw	Microwave
AA	Ascorbic acid
ABC	ATP-binding cassette
ACCase	Acetyl Coenzyme A carboxylase
ADP	Adenosine diphosphate
ALS	Acetolactate synthase
ASAP	Atmospheric Solids Analysis Probe
ATP	Adenosine triphosphate
ATR	Attenuated total reflection
Boc	*tert*-Butyloxycarbonyl
Cbz	Carboxybenzyl
CDNB	4-Chloro-1,3-dinitrobenzene
CNBF	4-Chloro-7-nitro-benzoxadiazole
conc.	Concentrated
COSY	Correlation spectroscopy
CuTC	Copper(I) thiophene-3-carboxylate
CyJohnPhos	(2-Biphenyl)dicyclohexylphosphine
CYP450	Cytochrome P450 monooxygenase
DCC	*N,N′*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DEPT	Distorsionless Enhancement by Polarisation Transfer
DHA	Dehydroascorbate

DHAR	Dehydroascorbat reductase
DHP	7,8-Dihydro-pteroate synthase
DIPA	Diisopropylamine
DIPEA	Diisopropylethylamine
DMAC	Dimethylacetamide
DMAD	Dimethylacetylenedicarboxylate
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DOXP	1-Deoxy-D-xylulose 5-phosphate
DTB	Desthiobiotin
DTT	Dithiothreitol
E. coli	Escherichia coli
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic acid
EPSPS	5-Enolypyruvyl-shikimate-3-phosphate synthase
eq	Equivalents
ES	Electrospray
Fd	Ferrodoxin
FNR	Ferrodoxin-NADP$^+$-reductase
FPLC	Fast protein liquid chromatography
GC-MS	Gas chromatography–mass spectrometry
GPOX	Glutathione peroxidase
GSH	Glutathione
GST	Glutathione *S*-transferase

GT	Glycosyltransferase
HABA	2-(4-Hydroxyphenylazo)benzoic acid
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HPLC	High performance liquid chromatography
HRAC	Herbicide-Resistance Action Committee
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence spectroscopy
IPTG	Isopropyl β-D-1-thiogalactopyranoside
IR	Infrared
Is	Internal standard
JohnPhos	(2-Biphenyl)di-*tert*-butylphosphine
LB	Luria-Bertani broth
LC-MS	Liquid chromatography–mass spectrometry
LDA	Lithium diisopropylamid
LED	Light-emitting diode
LiHMDS.	Lithium bis(trimethylsilyl)amide
m-CPBA	*Meta*-chlorperoxybenzoic acid
MALDI	Matrix assisted desorption/ionization
MAPEGs	Membrane associated proteins involved in eicosanoid and glutathione metabolism
MDR	Multidrug resistance
MeCN	Acetonitrile
MHR	Multiple herbicide resistance
MTBE	Methyl *tert*-butyl ether

$NADP^+$	Nicotinamide adenine dinucleotide phosphate
n.d.	Not determined
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
NTSR	Nontarget-site resistance
OEC	Oxygen evolving complex
p	Product
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PCy	Plastocyanin
PCy_3	Tricyclohexylphosphine
$Pd_2(dba)_3$	Tris(dibenzylideneacetone)dipalladium
PDS	Phytoene desaturase
PEG	Polyethylene glycol
Protox	Protoporphyrinogen oxidase
PS I	Photosystem I
PS II	Photosystem II
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
QTOF	Quadrupole-time-of-flight
rt	Room temperature
SAR	Structure activity relationships
sat.	Saturated
SDS	Sodium dodecyl sulfate
sm	Starting material

SPhos	2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl
TCHQD	Tetrachlorohydroquinone dehalogenase-like
TFA	Trifluoroacetic acid
TFP	Tris(2-furyl)phosphine
THF	Tetrahydfofuran
TLC	Thin layer chromatography
TQD	Tandem quadrupole detector
TSR	Target-site resistance
UDPG	Uridine diphosphate-glucose
UGT	UDP-dependent glycosyltransferase
UPLC	Ultra performance liquid chromatography
UV	Ultraviolet
WSSA	Weed Science Society of America
WT	Wilde type
XPhos	2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbipheny

Declaration

The work described in this thesis was carried out in the Department of Chemistry, Durham University and at Syngenta International Research Centre, Jealott's Hill, Berkshire between October 2013 and May 2017. All work is the author's own, unless otherwise stated. This work has not been previously submitted for a degree at this or any other institution.

Statement of Copyright

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1 Introduction

1.1 Overview

The work in this thesis is aimed towards the development of a herbicide synergist, which inhibits the effects of multiple herbicide resistance in black grass (*Alopecurus myosuroides*).

This thesis comprises 5 chapters. Chapter 2 will give an overview of previous work within the group and outline the aims of this project. Chapter 3 will discuss the work undertaken and a conclusion will be drawn in chapter 4. The chemical and biological experimental procedures will be detailed in chapter 5. The remainder of this chapter outlines the background to the project.

1.2 Herbicide resistance

1.2.1 Introduction to herbicide resistance

In the last century herbicides have revolutionised the control of weeds and made a big contribution towards the increase of crop yields (Figure 1).[1]

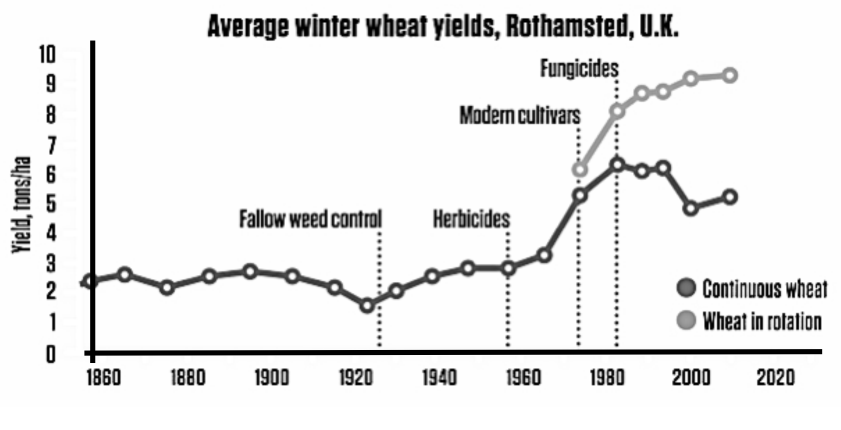


Figure 1: Increase in wheat yield from controlling weeds and diseases.[2]

However, the high selective pressure caused by the application of herbicides has resulted in the evolutionary adaption of weeds which over the last 40 years resulted in a dramatic increase in the frequency (Figure 2) and diversity of herbicide resistant weed biotypes. This poses a substantial threat to the sustainability of agriculture both locally and globally. With the growth in global population leading to increasing demands for food production, the fast rise of herbicide resistant weeds threatens food security.

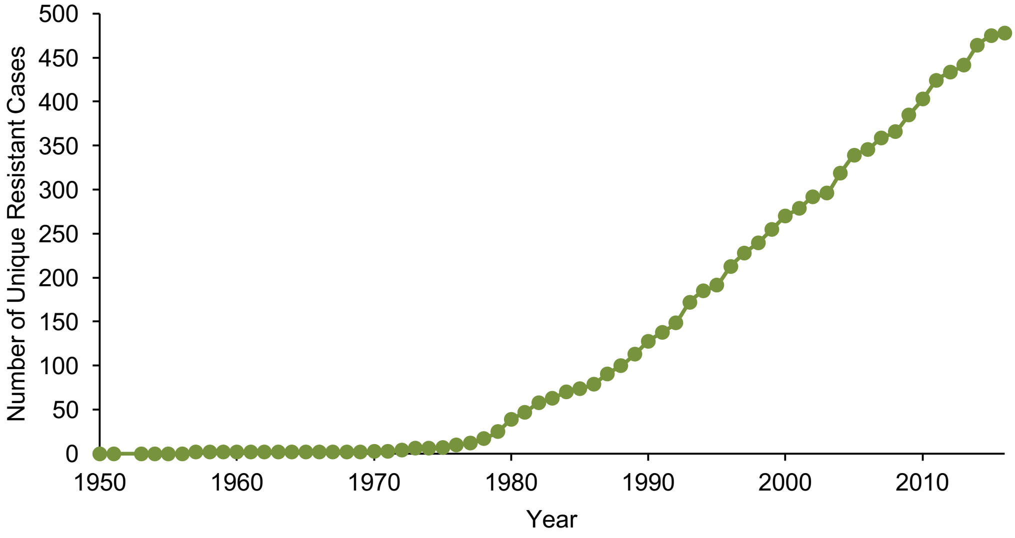
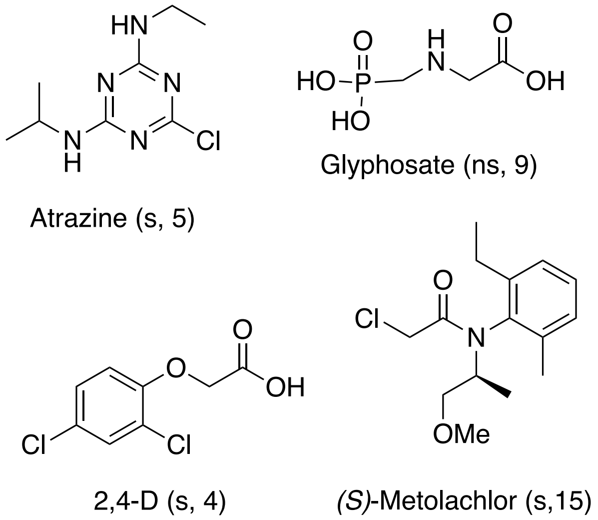


Figure 2: Chronological increase in unique herbicide resistant weed cases.[1]

The International Survey of Herbicide Resistant Weeds[3] reports the occurrence of herbicide resistant weeds worldwide and shows that there are currently 252 herbicide resistant weed species globally (147 dicots and 105 monocots). Weeds have evolved resistance to 23 of the 26 known herbicide sites of action and to 161 different herbicides, and there have been reports of herbicide resistant weeds in 91 crops in 67 countries.[3] As not all countries or regions are equally represented in this survey, the actual occurrence of herbicide resistant weeds may well be much higher.

1.2.2 Herbicides

Herbicides are chemicals that kill plants or inhibit their growth. They can either be selective or non-selective (Figure 3).



Atrazine (s, 5)

Glyphosate (ns, 9)

2,4-D (s, 4)

(S)-Metolachlor (s,15)

Figure 3: Some of the most widely used selective (s) and non-selective (ns) herbicides; Numbers in brackets indicate herbicide class.

Selective herbicides are toxic to some plant species but less toxic, or not toxic at all to others. They target specific weeds or weed categories without causing damage to the crop and therefore allow weed control. Herbicide selectivity can be enhanced through the use of herbicide safeners, which selectively protect crop plants from herbicide damage, without reducing the activity in the target weed.[4] Non-selective herbicides, such as glyphosate,[5] eliminate all plants and are applied to control weeds before crop planting, or weeds in plantation crops like rubber or oil palm. They can also be used together with crops, which have been genetically engineered to be resistant to non-selective herbicides.

Herbicides can be further classified based on their mode of action.[6-7] They target only a couple of processes and proteins within a cell (Figure 4).

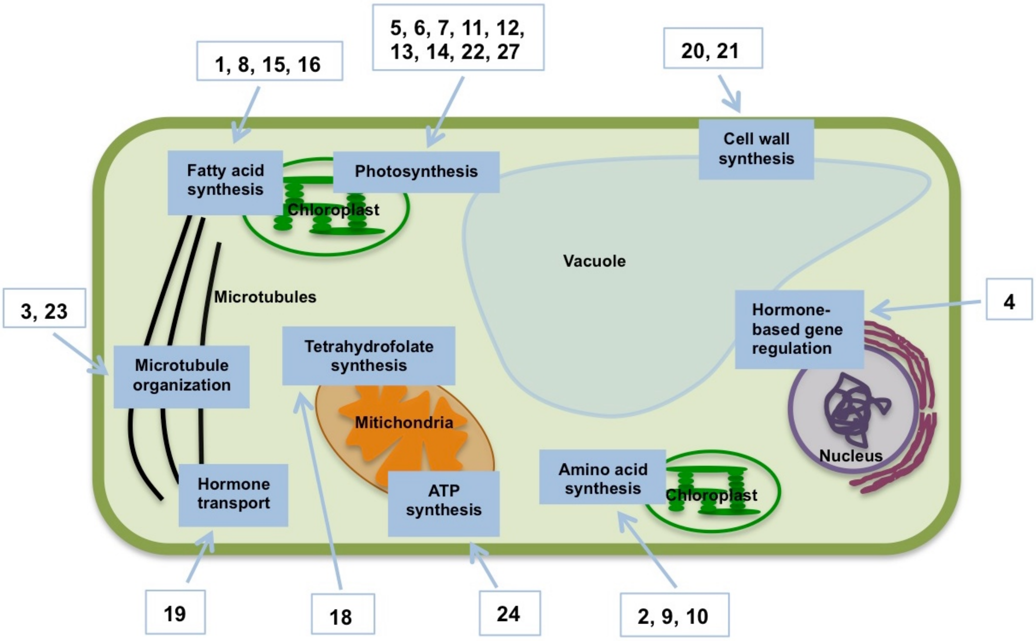


Figure 4: Herbicide sites of action (numbers indicate herbicide class (WSSA)).

The Weed Science Society of America (WSSA) herbicide classification system assigns each herbicide group a number,[6] whereas the Herbicide-Resistance Action Committee (HRAC) classification system allocates a letter to each group (Table 1).[7]

Group	Site of action	Examples
1 (A)	Inhibitors of acetyl CoA carboxylase (ACCase)	Clodinafop, Fenoxaprop, Pinoxaden
2 (B)	Inhibitors of acetolactate synthase (ALS)	Nicosulfuron, Chlorsulfuron, Imazapyr
3 (K1)	Inhibitors of microtubule assembly	Trifluralin, Thiazopyr
4 (O)	Synthetic auxins	Dichlorprop, 2,4-D
5 (C1)	Inhibitors of photosynthesis at photosystem II (PS II) site A	Atrazine, Simazine
6 (C3)	Inhibitors of photosynthesis at PS II site B	Bentazon, Ioxynil
7 (C2)	Inhibitors of photosynthesis at PS II site A; different binding behaviour from group 5	Chlortoluron, Diuron
8 (N)	Inhibitors of lipid synthesis; not ACCase inhibition	Cycloate, Pebulate
9 (G)	Inhibitor of 5-enolypyruvyl-shikimate-3-phosphate synthase (EPSPS)	Glyphosate
10 (H)	Inhibitor of glutamine synthase	Glufosinate
11 (F3)	Inhibitors of carotenoid biosynthesis (unknown target)	Amitrole, Aclonifen

12 (F1)	Inhibitors of the phytoene desaturase (PDS)	Norflurazon, Fluridone
13 (F4)	Inhibitor of 1-deoxy-D-xyulose 5-phosphate (DOXP) synthase	Clomazone
14 (E)	Inhibitors of protoporphyrinogen oxidase (Protox)	Lactofen, Sulfentrazone
15 (K3)	Inhibitors of synthesis of very long–chain fatty acids	Alachlor, Flufenacet, Metolachlor
16 (N)	Unknown	Ethofumesate
17 (Z)	Unknown	DSMA, MSMA
18 (I)	Inhibitor of 7,8-dihydropteroate synthase (DHP)	Asulam
19 (P)	Inhibitors of indoleacetic acid transport	Naptalam, Diflufenzppyr
20 (L)	Inhibitor of cell wall synthesis site A	Dichlobenil
21 (L)	Inhibitor of cell wall synthesis site B	Isoxaben
22 (D)	Photosystem I (PS I) electron diverters	Diquat, Paraquat
23 (K2)	Inhibitor of mitosis	Carbetamide
24 (M)	Membrane disruptors (uncouplers)	Dinoterb
25 (Z)	Unknown	Flamprop
26 (Z)	Unknown	Difenzoquat, Pelargonic acid
27 (F2)	Inhibitors of 4-hydroxyhenyl-pyruvatedioxygenase (4-HPPD)	Isoxaflutole, Mesotrione

Table 1: Herbicide classification according to WSSA (numbers) and HRAC (letters in brackets). [6-7]

1.2.2.1 Herbicides prone to resistance

The herbicide groups, which are most affected by herbicide resistance are ALS inhibitors, PS II site A inhibitors, ACCase inhibitors and EPSPS inhibitors (Table 2).

Group	Site of action	Herbicide resistant monocots	Herbicide resistant dicots	Total
2	ALS inhibitors	62	97	159
5	PS II site A inhibitors	23	51	74
1	ACCase inhibitors	48	0	48
9	EPSPS inhibitor	17	19	36

Table 2: Occurrence of herbicide-resistant weed species to herbicide sites of action.[1]

Evolution of resistance is based on several factors. Most importantly, it depends on the characteristics of the weed species treated, such as fecundity, breeding system, generation time, fitness in the absence of herbicide, seed longevity, gene flow by pollen and seed, and seed dormancy, and the number of individuals treated over time. The number, frequency, dominance and fitness of genes that confer resistance to each herbicide site of action play an important role as well.[8]

1.2.2.1.1 Acetolactate Synthase (ALS) inhibitors (2)

ALS-inhibiting herbicides, which can also be called acetohydroxy acid synthase inhibitors, include imidazolinone, pyrimidinylthio-benzoate, sulfonylamino-carbonyltriazolinone, sulfonylurea and triazolopyrimidine herbicides.[6] The inhibition of ALS, a common enzyme which is essential for the biosynthesis of the branched-chain amino acids isoleucine, valine and leucine leads to starvation of the plants (Figure 5).[9-10]

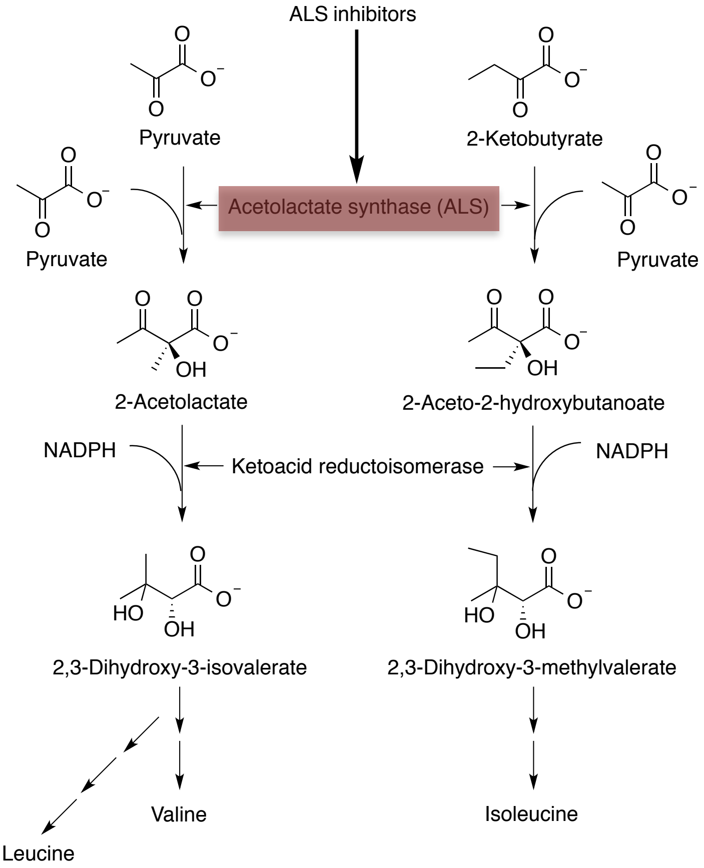


Figure 5: The branched-chain amino acid biosynthetic pathway.

The fact that ALS inhibitors are among the most widely used herbicides in the world, that they target many species and that ALS is very prone to gene point mutations that confer resistance, has led to a large number of resistant weeds.[8-9] There are currently amino acid substitutions at eight sites conferring resistance to ALS herbicides. Substitutions at Pro 197 are most common, followed by substitutions at Trp 574 and Ser 653 (Figure 6). Amino acid numbers are standardized to *Arabadopsis thaliana L.* ALS.

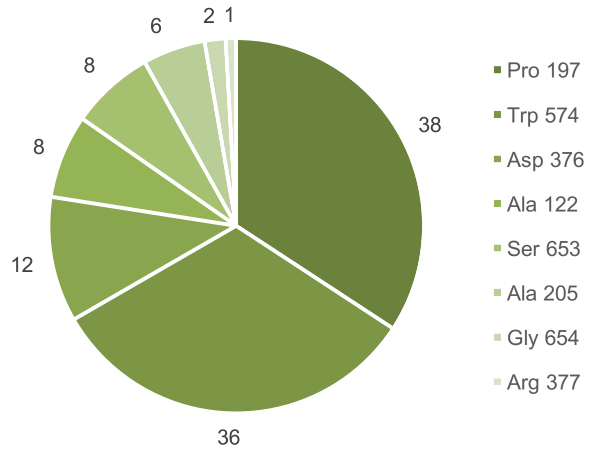


Figure 6: Number of species with ALS mutations by amino acid residue.[3]

Some of those mutations result in cross resistance (*cf.* Chapter 1.2.3.2.1).[11] Even though target-site resistance is the most common form of resistance among ALS inhibitors, there have also been reports of multiple herbicide resistance[12-13] and other non-target-site resistance mechanisms (*cf.* Chapter 1.2.3.2.2).[14-15]

1.2.2.1.2 Photosystem II (PS II) inhibitors (5)

Phenyl-carbamates, pyridazinones, triazines, triazinones, triazolinones, and uracils are examples for herbicides that inhibit photosynthesis.[6] They bind to the Q_B-site of the PS II complex, herbicides from groups 5, 6 and 7 thereby only differ in the position of the pocket, where they bind to the Q_B-site on the D1 protein, and compete with the exchangeable plastoquinone within the Q_B binding domain which interrupts the electron flow (Figure 7).[16]

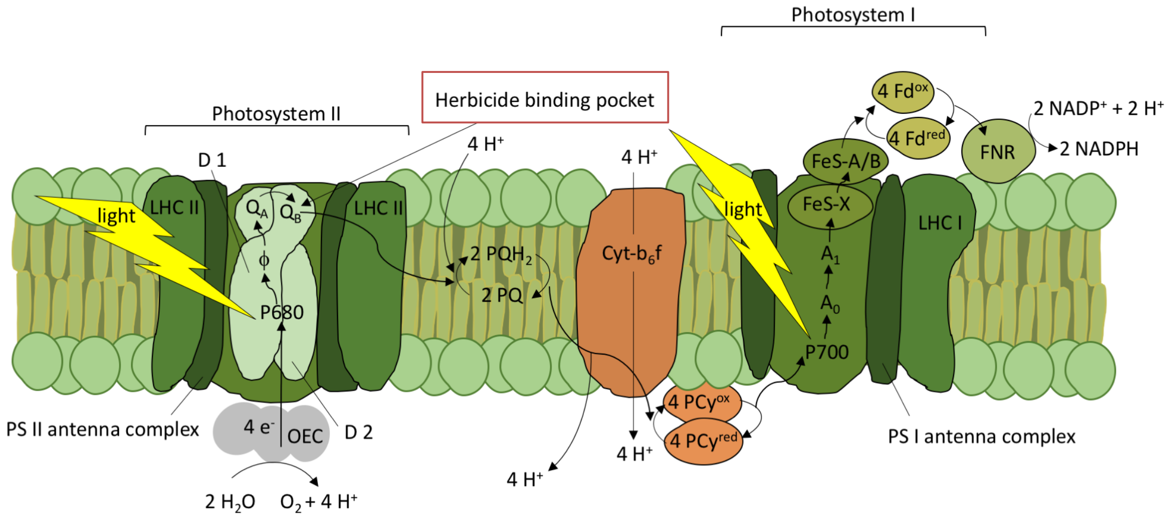


Figure 7: Scheme of the light-induced electron chain from water oxidation to the reduction of high-energy molecules. Photosystem II (PS II) uses light energy (through excitation of a special chlorophyll pair called P680) to oxidise two molecules of H_2O into one molecule of molecular oxygen (catalysed by the oxygen-evolving complex (OEC)). Electrons are transferred to a pheophytin (Φ) from P680*, which in turn extracts electrons from H_2O. Then electrons are further transferred step-wise to a plastoquinone at the Q_A site (D 2 protein), to a plastoquinone at the Q_B site (D 1 protein) and via a group of cytochrome proteins, called the b_6-f complex (Cyt-b_6f), and the electron-carrier molecule plastocyanin (PCy), through the chain to Photosystem I (PS I). At PSI, absorbed photons result in the excitation of the dimeric chlorophyll P700, which starts the electron transport and is then reduced by PCy. Electrons are rapidly transferred step-wise through a series of acceptors to the iron-sulfur proteins FeS-X, FeS-A and FeS-B, and finally to the ferredoxin (Fd), which in turn reduces $NADP^+$ to NADPH in a reaction catalysed by ferrodoxin-$NADP^+$-reductase (FNR).

Plants die because of severe oxidative stress. The interruption of electron flow causes the generation of triplet chlorophyll, which can interact with oxygen to form singlet oxygen. Carotenoids, which would normally quench triplet chlorophyll or singlet oxygen, cannot cope with the abundance of those reactive species when the electron flow is blocked. [16-17]

PS II inhibitors have been used extensively, which made them prone to resistance. Atrazine (Figure 3) especially, has been used almost solely in the 1960s to 1990s to control weeds in maize in Europe and the USA.[8] Most cases of resistance are due to one mutation (Ser264 to Gly) in the D1 protein, conferring resistance to triazines (e.g. atrazine). Different cross resistance patterns to other PS II inhibitors can be observed in different weed species.[18] There are also reports of some multiple herbicide resistance cases.[19]

1.2.2.1.3 Acetyl Coenzyme A Carboxylase (ACCase) inhibitors (1)

ACCase-inhibiting herbicides comprise two chemical families: aryloxyphenoxy propionates and cyclohexanediones.[6] Over a long period wheat production was dependent on ACCase inhibitors for grass control over large areas.[8] Herbicides in this class inhibit acetyl-coenzyme A carboxylase in grasses, an enzyme that catalyses the first step in the synthesis of fatty acids through the ATP-dependent carboxylation of acetyl coenzyme A to malonyl coenzyme A (Figure 8).[20]

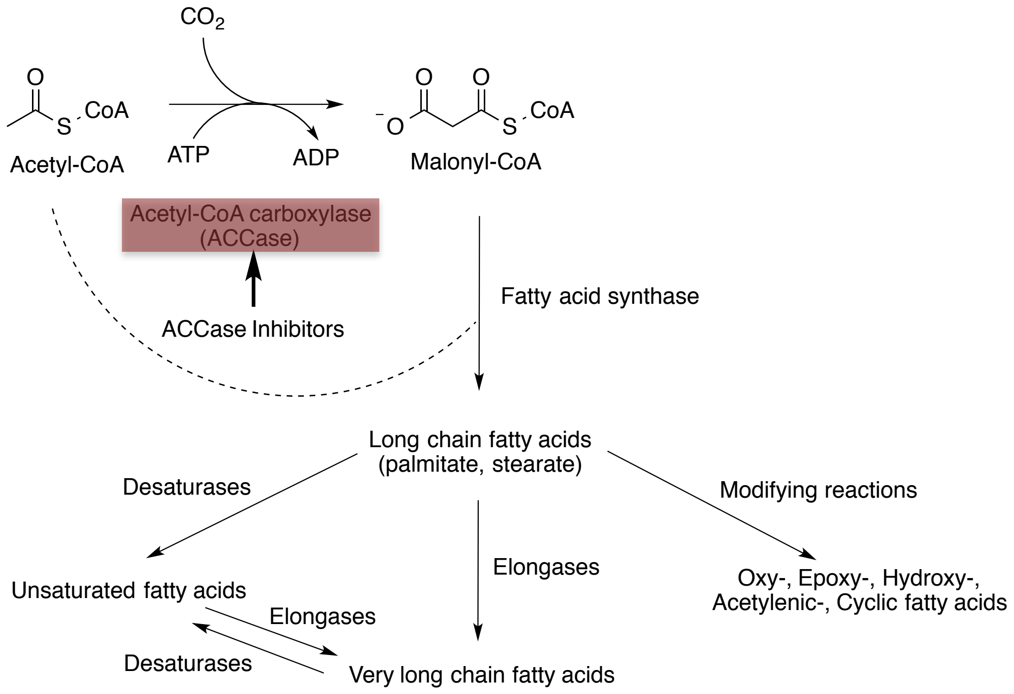


Figure 8: Fatty acid biosynthesis.

The blocked fatty acid biosynthesis alters the cell membrane integrity and the plant dies. Cytotoxic hydroperoxides, caused by the disrupted fatty acid biosynthesis, have also been proposed to be responsible for plant death.[20]

Resistance to ACCase inhibitors occurs in many cases due to target-site amino acid substitutions in the ACCase gene, but multiple herbicide resistance based on enhanced metabolism is widespread (*cf*. Chapter 1.2.3.2.2.1).[20]

1.2.2.1.4 5-Enolypyruvyl-shikimate-3-phosphate synthase inhibitors (9)

This group only consists of one herbicide, glyphosate.[6] Glyphosate, however, is one of the most important herbicides. It inhibits EPSP synthase of all higher plants in the biosynthetic pathway of aromatic amino acids (Figure 9). [10, 21]

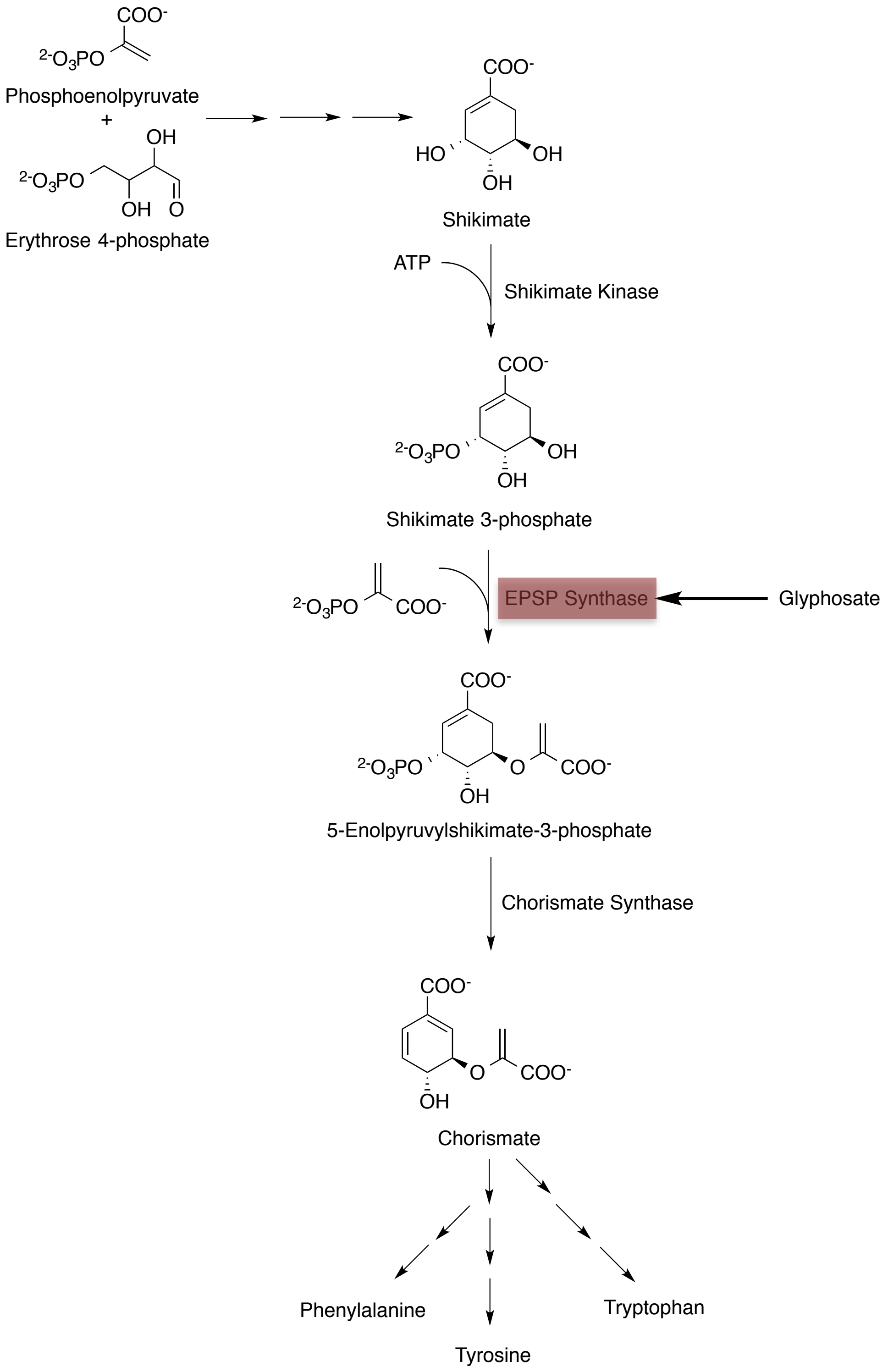


Figure 9: The aromatic amino acid biosynthetic pathway.

Plant death has been associated with the depletion of the aromatic amino acids phenylalanine, tyrosine and tryptophan or with shortage of carbon for other essential pathways due to the increased carbon flow to the shikimate pathway in the treated plant.[5]

Since glyphosate was first introduced in 1974 it has become the most important herbicide worldwide. 90% of all transgenic plants worldwide are glyphosate resistant.[5] Due to the reliance solely on glyphosate for weed control on massive areas in glyphosate resistant crops and orchards, there is a very high selection pressure for resistance and resistant weeds are evolving rapidly (Figure 10).[22]

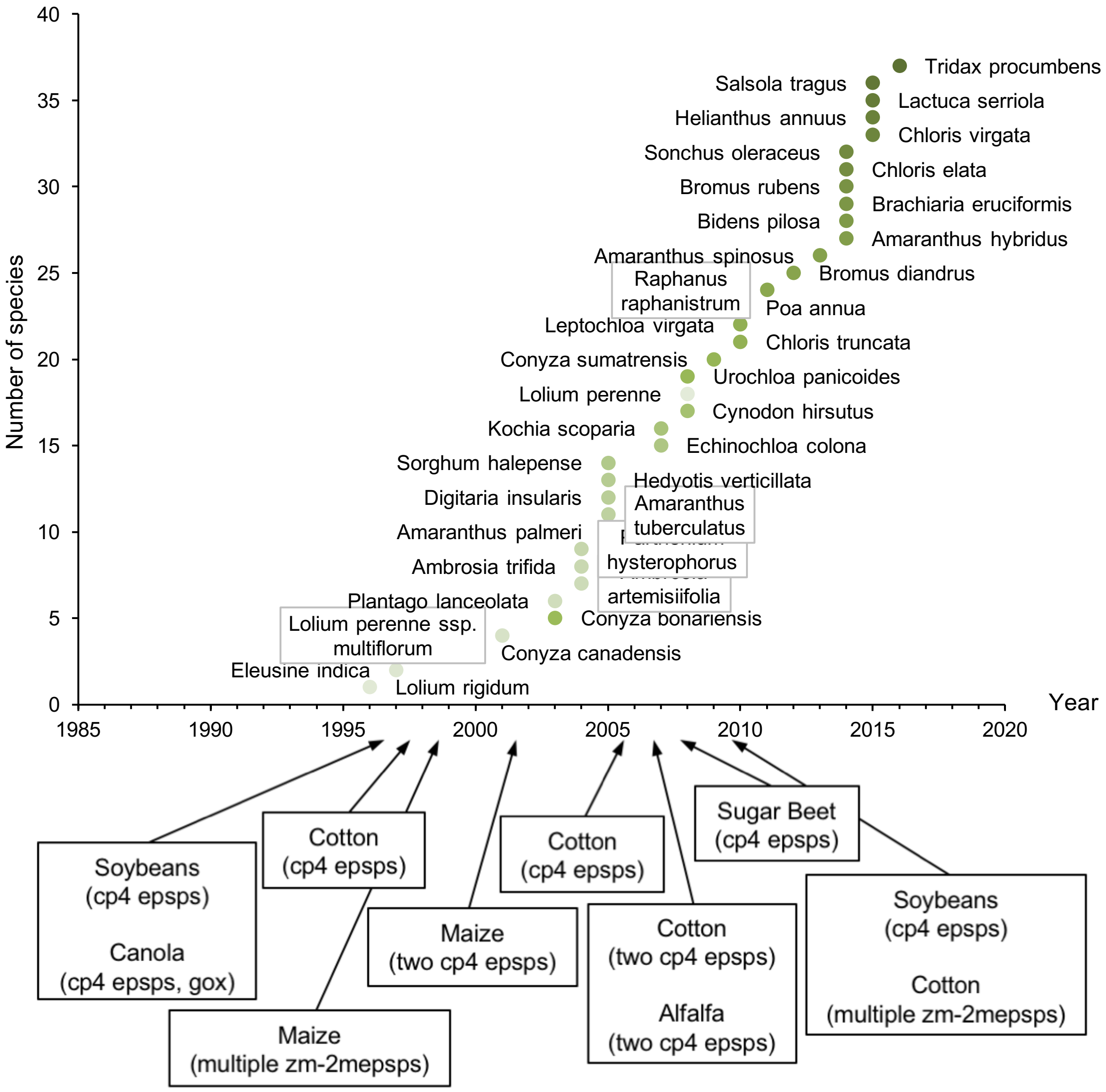


Figure 10: Chronological increase in glyphosate-resistant weeds worldwide and commercial introduction of transgenic crops resistant to glyphosate. Resistance gene(s) are indicated in brackets; *cp4 epsps*: microbial EPSP encoding gene; *zm-2mepsps*: mutated maize EPSP encoding gene; *gox*: glyphosate oxidoreductase encoding gene.[3]

Weeds have evolved nine different mechanisms of resistance to glyphosate, most prominently target-site resistance and resistance due to reduced translocation in the plant (*cf*. Chapter 1.2.3.2.2).[22] Worryingly a lot of glyphosate resistant weeds are reported to be also resistant to other herbicides.[3, 22]

1.2.3 Resistance Pathways

1.2.3.1 Target-site resistance versus nontarget-site resistance

There are two types of mechanism observed for herbicide resistance, target-site resistance (TSR) and non-target-site resistance (NTSR).[23-24] TSR develops from mutations in the gene encoding the protein targeted by the herbicide, which causes the herbicide to be less efficient. NTSR describes mechanisms complementary to changes at the target-site, enabling a plant to survive herbicide application.[25-27] Herbicide action can be divided into three steps: 1. penetration of the herbicide into the plant; 2. translocation and accumulation of the herbicide at the target site; 3. binding of the herbicide to the biological target, causing a phenotypic response such as the disruption of vital metabolic pathways or structural function leading to plant death (Figure 11).[28]

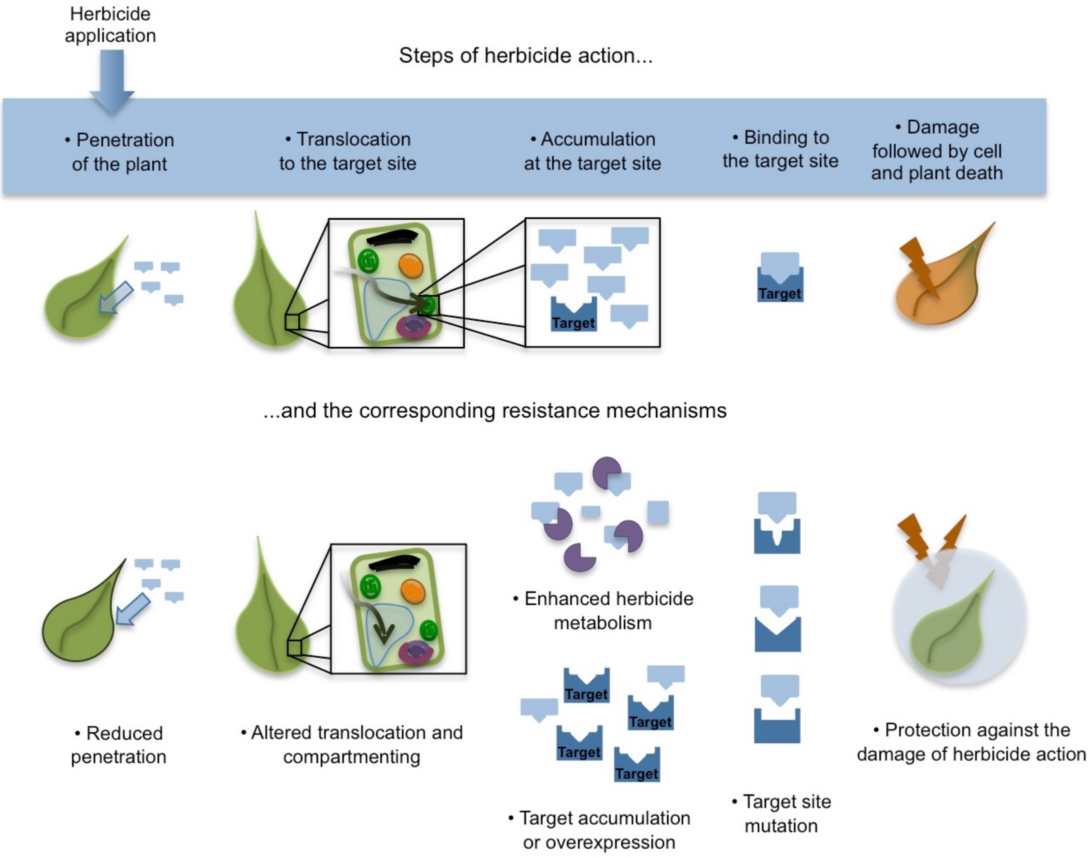


Figure 11: Steps of herbicide action and the corresponding resistance mechanisms.

To combat this, nature has developed resistance mechanisms for every single step. Mechanisms changing the efficacy of herbicide binding to the target enzyme belong to TSR. Any mechanisms interfering with at least one of the other steps belong to NTSR.

1.2.3.2 Cross-resistance

Cross-resistance occurs when plants have a single mechanism that provides resistance to two or more herbicides from different chemical families, this cross-resistance can be triggered by a single gene modification which contributes to a single mechanism, but also by more than one gene, all contributing to the same mechanism.[11, 18, 24] The mechanism can be target-site-based (*cf.* Chapter 1.2.3.2.1) as well as non-target-site based (*cf.* Chapter 1.2.3.2.2).

1.2.3.2.1 Cross resistance based on TSR

Target-site-based cross resistance is normally restricted to herbicides with the same site of action. However, it does not always result in resistance to all herbicide classes with a similar mode of action or all herbicides within a certain class.[18]

For example, resistance to ALS-inhibitors can be caused by numerous single-point mutations of the ALS-enzyme, with different cross resistance patterns depending on the mutation. The Asp376Glu (amino acid number standardized to *Arabadopsis thaliana L.* ALS) mutation can cause resistance to ALS-inhibitors from imidazolinone, pyrimidinylthiobenzoate, sulfonylaminocarbonyltriazolinone, sulfonylurea and triazolopyrimidine herbicide families (Figure 12).[11, 29] Ser653 substitutions on the other hand confer resistance to imidazolinone herbicides and to a lesser extent to pyrimidinylthiobenzoate herbicides.[11]

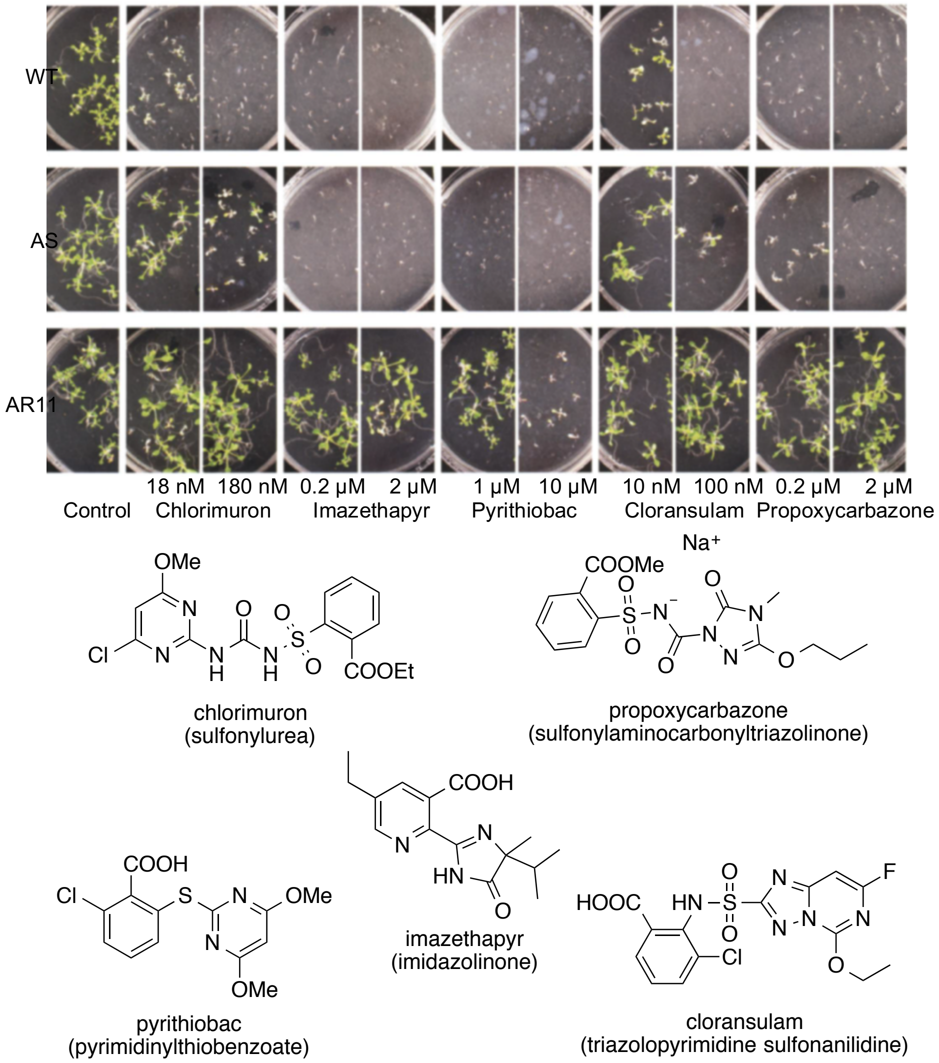


Figure 12: *Arabidopsis thaliana* seedling growth on media containing no selection agent, chlorimuron, imazethapyr, cloransulam, and propoxycarbazone. Growth of wilde type (WT) and transgenic *Arabidopsis* expressing the ALS gene from an ALS inhibitor-susceptible (AS) and –resistant (AR11) smooth pigweed biotype are shown.[29]

1.2.3.2.2 Cross resistance based on NTSR

NTSR weeds often show a central defence mechanism, which is responsible for cross resistance across herbicide sites of actions. One non-target-site based mechanism for cross resistance is enhanced metabolism, which is also referred to as multiple herbicide resistance (MHR).[30] MHR is distinct from multiple resistance (*cf.* Chapter 1.2.3.3) which is a term used to describe weed populations that exhibit more than one resistance mechanism. Other non-target-site based mechanisms for cross resistance include reduced herbicide penetration and translocation, enhanced levels of gibberellins, gene amplification or overexpression and protection against the damage of herbicide action.

Reduced herbicide penetration is caused by different physical and chemical properties of the cuticle in resistant plants.[25] For ALS-inhibitors from both imidazolinone, and sulfonylurea herbicide families, a reduction in penetration has been described in a common sunflower population.[14]

Enhanced levels of gibberellins, plant hormones that regulate growth, confer resistance to triallate (thiocarbamate herbicide) and difenzoquat (pyrazolium herbicide), causing rapid shoot growth that prevents sufficient herbicide from reaching the site of action.[31]

Reduced translocation is the major basis of resistance for glyphosate and the bipyridylium herbicides, paraquat and diquat, but there is also evidence for cross resistance. Reduced translocation has been suggested to be the resistance mechanism for the glutamine synthase inhibitor glufosinate and glyphosate in Lolium perenne.[32]

Gene amplification or overexpression increases the production of the target enzyme. Therefore, higher concentrations of herbicide are needed to reach the target site to inhibit the enzyme and cause death. For example, herbicide resistance against sulfonylureas and a imidazolinone herbicide due to amplification of a mutant ALS gene has been shown in tobacco.[15]

Protection against the damage of herbicide action is another NTSR mechanism. Two GSTs have been shown to confer protection against oxidative stress caused by ACCase inhibitors in black grass.[33-34] These inhibitors not only disrupt fatty acid synthesis, but also cause the release of reactive oxygen species that damage cell components. The GSTs protect the cell against oxidative damage, thereby giving the resistant plant time to degrade the herbicide.

1.2.3.2.2.1 Multiple Herbicide Resistance (MHR)

In the same way that multidrug resistance (MDR) is a major drawback to improved healthcare, MHR is a serious threat to weed management. In contrast to MDR, which is most frequently due to active transporters that pump a wide range of drugs, including antibiotics, antimalarials and cancer chemotherapeutics, out of human cells[35], participation in MHR has been well established for four gene families: P450 monooxygenases (CYP450s), glutathione *S*-transferases (GSTs), to some extend UDP-dependent glycosyltransferases (UGTs) and ATP-binding cassette (ABC) transporters.[26]

MHR is not only the most important aspect of NTSR it is also the most studied.[26, 36] The detoxification of plants is a multistep process involving several types of enzymes. Most herbicides can be detoxified by plants to some extent, but the ability to metabolize the herbicide might be insufficient to stop the weed species from being killed. Selective herbicides often rely on the fact that the herbicide is more rapidly metabolised in the crop than in the weed, especially in grasses.[37] MHR is most problematic in black grass and rye grass, which compete with cereal crops.[23] There is still little known about the exact mechanisms that cause enhanced metabolism and many enzymes might be involved.[26, 36] Nevertheless, there are indications that MHR can be monogenic, but the coordinated regulation of detoxifying genes and interaction among the enzymes seem to be the more important mechanism.[26]

1.2.3.3 Multiple resistance

Multiple resistance refers to plants with more than one resistance mechanism. Multiple resistance can for example arise from pyramiding of multiple TSR-traits.[11] It is usually caused through accumulation of resistance genes via pollen flow or is the result of selection of different resistance mechanisms by herbicides that differ in their sites of action.

Over the last 30 years the number of weeds resistant to 2 or more herbicide sites of action, caused by either cross resistance or multiple resistance, has increased dramatically and there are now reports of weeds resistant to herbicides with up to eleven different sites of action (Figure 13).[3]

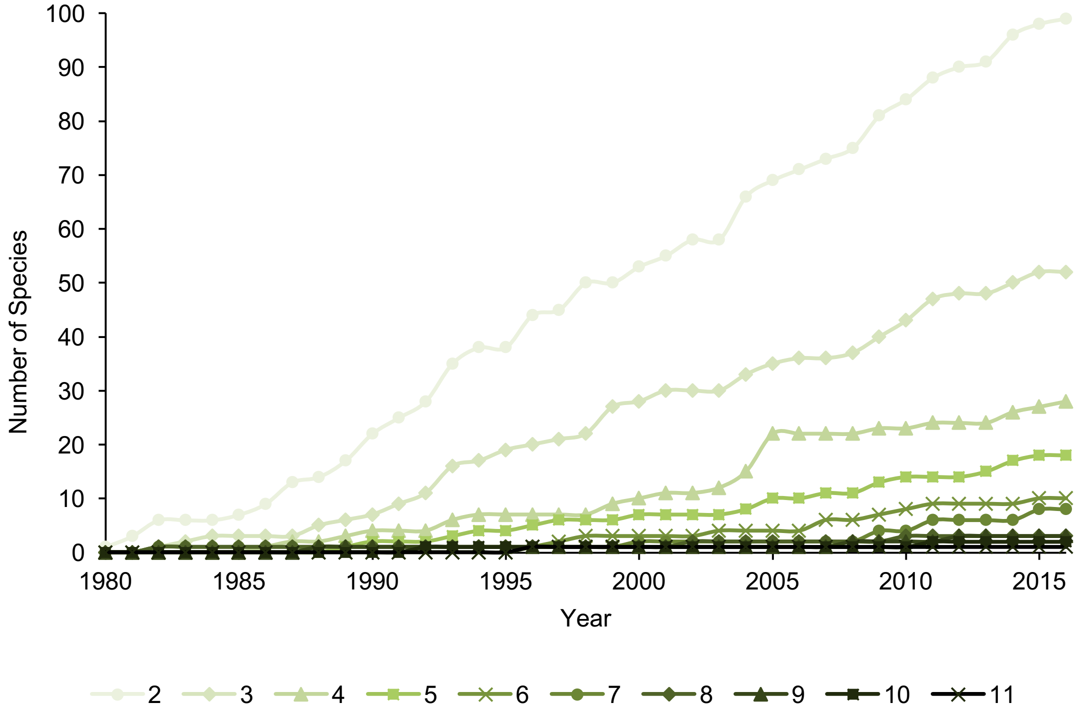


Figure 13: Increase in the number of weeds resistant to two or more herbicide sites of action.[3]

1.2.4 Herbicide resistance in black grass

Black grass (*Alopecurus myosuroides*) is an annual or winter annual tufted grass weed, which propagates by seeds (Figure 14).

Figure 14: Black Grass.

Most seeds germinate in late summer or early autumn. It is perfectly adapted to winter cereal production, which has led to a rapid increase of black grass. The large populations and widespread distribution in cropping areas, rapid seed bank turnover, high reproductive capacity, allogamous reproduction and genetic and phenotypic plasticity all favour the accumulation of herbicide resistance mechanisms.[18] Black grass has been reported as a problematic weed worldwide, but it is most serious in Europe, particularly England, France and Germany.[38] It belongs to the weed species resistant to the most number of sites of action (Figure 15).[3]

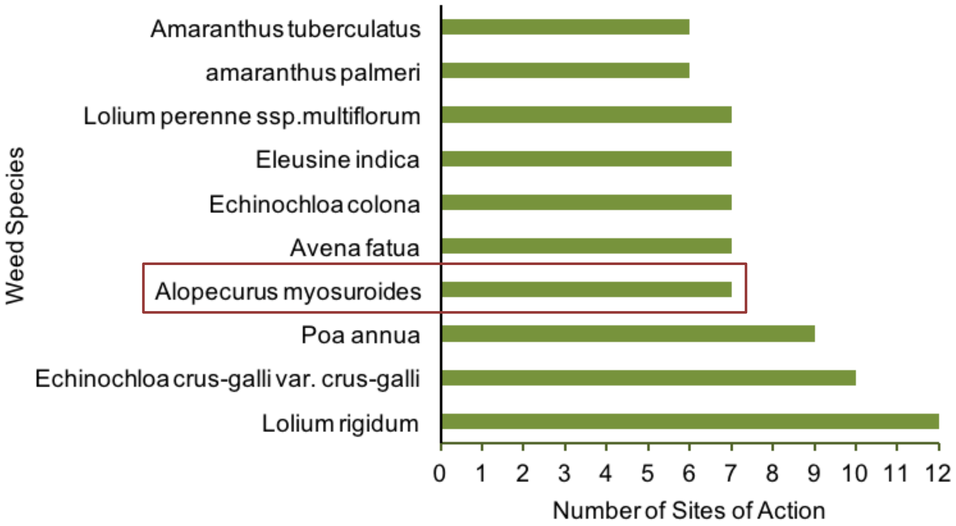


Figure 15: Resistant species by the number of sites of action.[3]

Numerous herbicide resistant biotypes have been documented and both TSR and NTSR occur frequently in field populations of black grass.[39-43] A random survey in England in 2002 indicated that 80% of the black grass samples demonstrated resistance to at least one herbicide such as fenoxaprop-P, sethoxydim (both ACCase inhibitor), chlorotoluron and isoproturon (both PSII inhibitor).[44]

TSR mostly concerns ACCase inhibitors[40, 45-48] and ALS inhibitors[48-49], which is alarming because the control is mainly based on those two modes of action.[50] Analysis of the geographical structure of resistance to ACCase inhibitors across six countries demonstrated the wide spread occurrence of seven mutant resistant ACCase alleles in black grass populations (Figure 16).[40]

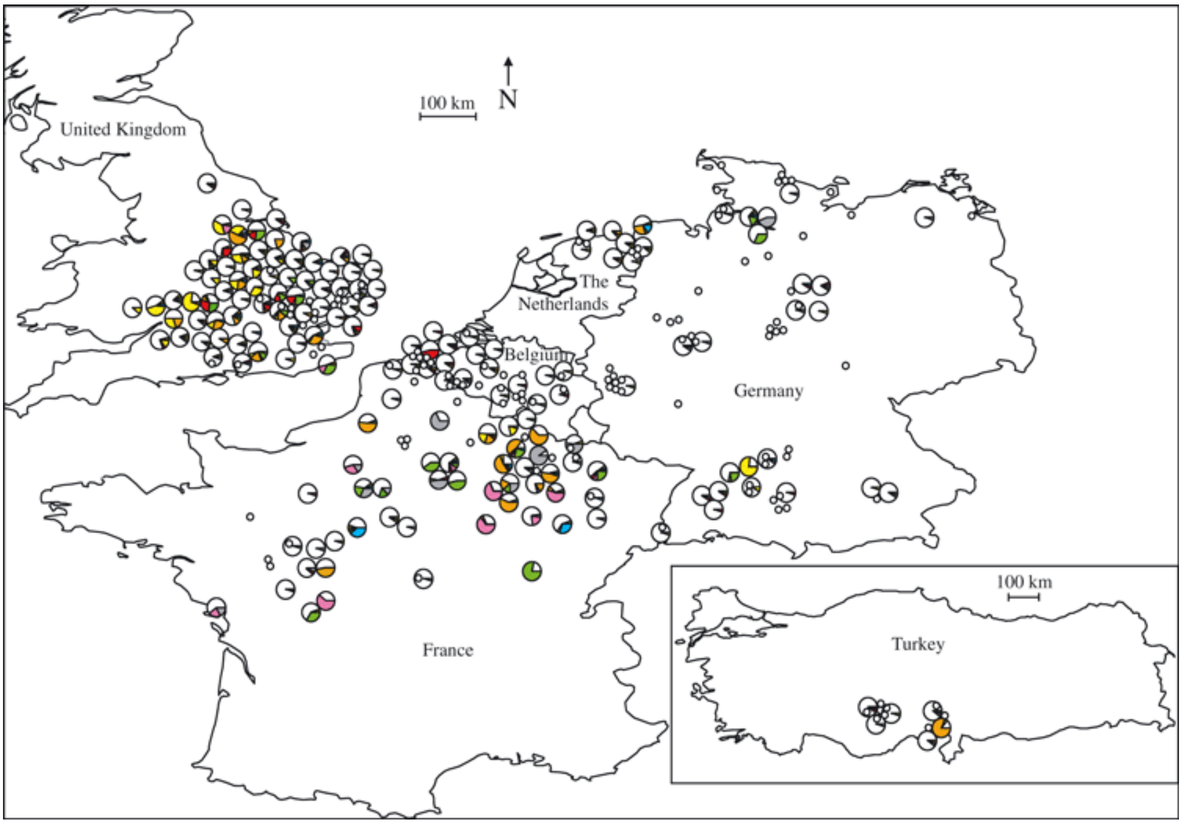


Figure 16: Geographical distribution of mutant herbicide-resistant variants of the target protein ACCase among 297 populations of black grass in Europe. Pie charts show the respective frequencies of the mutant acetyl-CoA carboxylase variants detected in populations. White, wild-type allele; yellow, C-L1781; orange, T-L1781; red, C-C2027; purple, T-C2027; green, N2041; blue, G2078; grey, A2096. Populations where no mutant variant was detected are shown as small, white circles.[40]

The study also reported that only a fraction of genes involved in resistance to ACCase inhibitors are mutant ACCase alleles and NTSR mechanisms are responsible for most of this resistance.

NTSR in black grass is in many cases attributed to MHR, but protection against the damage of herbicide action has also been reported.[33-34] MHR in black grass was first detected in a population from Peldon in Essex, England[51-52] and has since been reported in populations across Europe. Herbicide resistance in the Peldon population could not be explained by reduced target site sensitivities and studies demonstrated that the more extensive metabolism in the resistant biotypes could be reduced by CYP450 inhibitors.[51, 53-54] Since then, the involvement of CYP450s in MHR was suggested for other black grass populations across Europe.[55-58]

In addition, higher GST activities were shown to be responsible for MHR in some black grass populations.[30, 34, 58-60] MHR was associated with an increased expression of *Am*GSTF1, a member of the plant-specific phi (F) class of GST. *Am*GSTF1 showed a higher activity as a glutathione peroxidase (GPOX), catalysing the reduction of peroxides, rather than detoxification of herbicides[34] and seems to have a direct regulatory control on metabolism.[30]

Compared to herbicide sensitive black grass, the plants from the Peldon population also contained higher levels of *O*-glucosyltransferases activity.[61]

1.2.5 Dealing with herbicide resistance

With an increase in herbicide resistance, new developments and other methods are needed to control weeds. Up to a certain point agronomic practices can be very important tools for weed control. Strategies include rotating crops, optimization of planting dates and seeding rates, mechanical methods such as tillage[24] and hand-weeding as a last resort. Methods to prevent, or at least delay herbicide resistance, include the rotation of herbicide mode of action and the use of herbicide mixtures.[8]

TSR may be overcome using herbicides with a different mode of action, but this is increasingly difficult, as industry has not provided a herbicide with a new mode of action in more than 30 years. Although controversial, a solution to this could be new genetically modified herbicide resistant crops, as old herbicides could be used in new ways.[8]

MHR however is the most difficult to deal with. One possibility could be the application of synergists, which inhibit the effects of MHR. A parallel could be drawn to MDR in cancer, where the inhibition of drug detoxifying proteins has been the focus of medicinal chemistry programs.[62-63] There are a variety of compounds, which have been shown to inhibit herbicide detoxification caused by CYP450s. Malathion synergized imazethapyr activity in a resistant population of Digitaria sanguinalis[64] and chlorosulfuron activity in herbicide resistant Lolium rigidum[12-13]. 1-Aminobenzotriazole has been shown to decrease the metabolism of simazine[13, 65], chlorotoluron[13] and diclofop[13] in herbicide resistant Lolium rigidum and piperonyl butoxid reduced the metabolism of simazine[13] and chlorotoluron[13], also in Lolium rigidum. In the same way piperonyl butoxide and 1-aminobenzotriazole acted in synergism with the herbicides isoproturon, chlorproturon, haloxyfop and clodinafop propargyl and malathion with the herbicide flupysulfuron in MHR black grass.[58] The GST inhibitor 4-chloro-7-nitro-benzoxadiazole increased the toxicity of chlorotoluron, fenoxaprop-p-ethyl and clodinafop-propargyl, when applied as a synergist[30] and tridiphane reduced the metabolism of fenoxaprop-p-ethyl[58] in herbicide resistant black grass.

1.3 Herbicide Metabolism in Plants

Herbicide metabolism is a multi-enzyme process that can be arranged into four phases (Figure 17). [26, 36, 66]

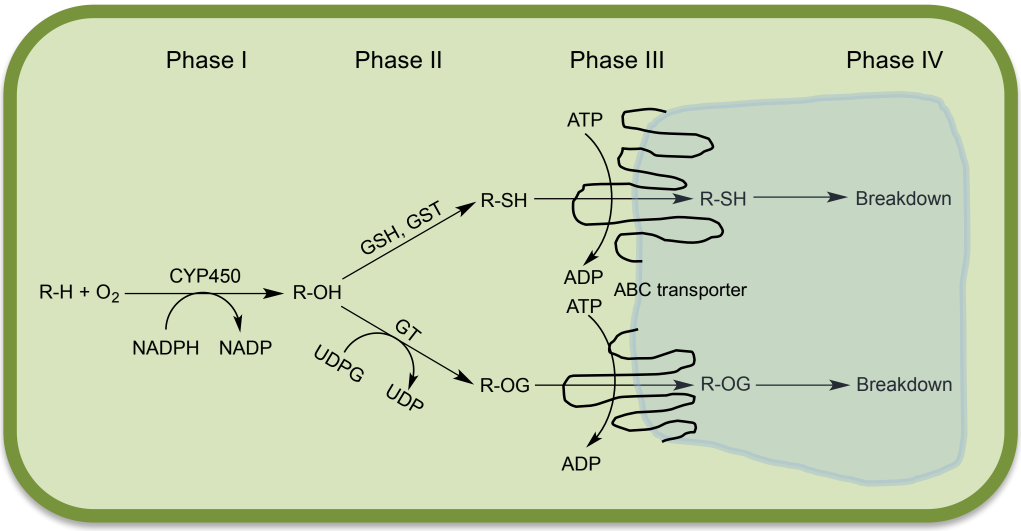


Figure 17: Schema of CYP450, GST, UGT and ABC transporter gene-encoded detoxification activities.

In phase I molecules are activated for further conjugation by other enzymes in phase II, which most commonly involves oxidations by cytochrome P450 monooxygenases (CYP450s) or mixed function oxidases and hydrolyses by carboxylesterases. With a few exceptions CYP450s are localized in the endoplasmic reticulum membrane with the remainder of the protein in the cytosol. CYP450s are heme protein-dependent oxidase systems, which need NADPH and/or NADH to cleave atmospheric oxygen and functionalize a molecule.[67] CYP450s can catalyse different reactions, but common for herbicides are hydroxylations of aromatic rings and alkyl groups and dealkylations (Figure 18).[36] Through the incorporation of the oxygen, the activity towards phase II enzymes is enhanced.

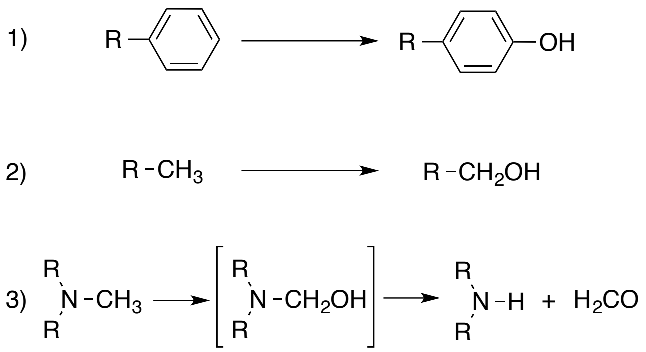


Figure 18: Common CYP450 catalyzed reactions. 1) Aryl hydroxylation. 2) Alkyl hydrokylation. 3) Heteroatom release (N-dealkylation).

In phase II xenobiotics are normally conjugated to bulky hydrophilic molecules such as thiols or sugars. One example for phase II enzymes are glycosyltransferases (GTs), which catalyse the conjugation of glucose to a variety of lipophilic small molecule acceptors, such as plant metabolites, phytotoxins and xenobiotics, altering their properties.[68]. Depending on the functional group, which is conjugated to the sugar (-OH, -COOH, -NH_2), GTs can be divided into *O*-glycosyltransferases or *N*-glycosyltransferases. Usually uridindiphosphate-glucose (UDPG) is required as a sugar-donor.[36, 68] Another example is glutathione *S*-transferases (GSTs). Because MHR in black grass has been associated with an increased expression of *Am*GSTF1,[30] GSTs are discussed in more detail below (*cf.* Chapter 1.3.1.1).

In phase III conjugated molecules are actively transported to the vacuole or extracellular spaces by enzymes such as the ATP-binding cassette (ABC) drug transporter proteins. ABC transporters acquire their energy from hydrolysis of ATP, which takes place in the ATP binding cassette domain and use it to transport molecules through membranes. They can detoxify herbicides through compartmenting herbicides and their metabolites. ABC transporters are also responsible for the uptake of nutrients and a broad variety of other transport functions, such as sequestration of secondary metabolites, translocation of fatty acids and phospholipids or regulation of ion channels.[69]

Phase IV detoxification involves reactions such as further degradation, secondary conjugation and incorporation into cell wall constituents.

Degradation of the ACCase inhibitor fenoxaprop illustrates how herbicide metabolism is a multistep process involving the coordinated action of the enzymes highlighted above (Figure 19).

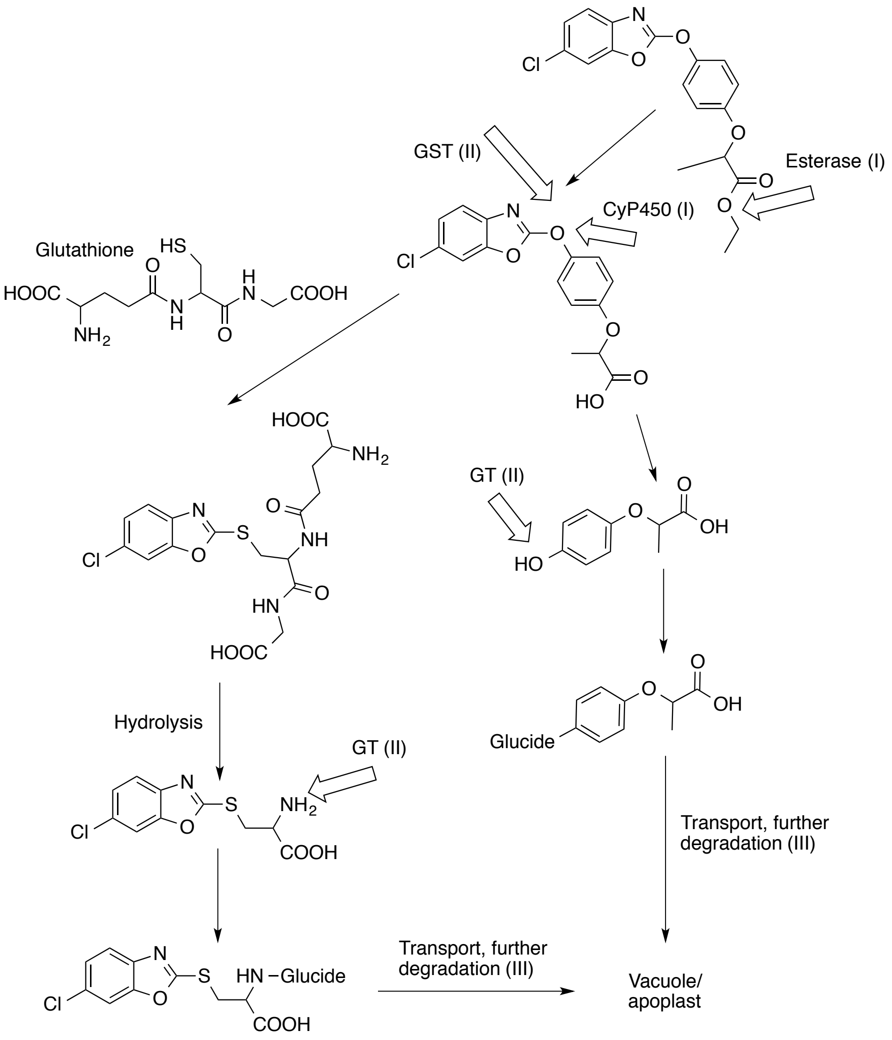


Figure 19: Metabolism of the ACCase inhibitor fenoxaprop in grasses.[25] I, II and III refer to phase I, II and III in plant metabolism.

1.3.1.1 Glutathione *S*-transferases

Plant GSTs are an ancient enzyme superfamily and have multiple and diverse functions. They catalyse the reaction of reduced glutathione (γ-L-Glu-L-Cys-Gly; GSH) with an electrophilic substrate R-X by displacement of the leaving group to form a polar *S*-glutothionylated product R-SG (Figure 20). [70-72]

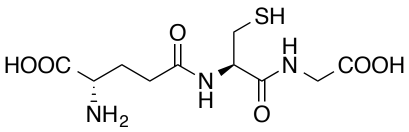


Figure 20: Glutathione.

Soluble plant GSTs can be classified according to their sequence identity, genome organizations, reactivities and kinetic properties. This gives rise to seven evolutionary distinct classes, tau (U), phi (F), theta (T), zeta (Z), lambda (L), dehydroascorbat reductase (DHAR) and tetrachlorohydroquinone dehalogenase-like (TCHQD), the latter being only mentioned for completeness (Figure 21).[72-73] Tau, phi, lambda and DHAR enzymes are plant specific whereas theta and zeta enzymes have animal counterparts. Additionally, GST activity is shown by membrane associated proteins involved in eicosanoid and glutathione metabolism (MAPEGs)[74], but those are not discussed in more detail.

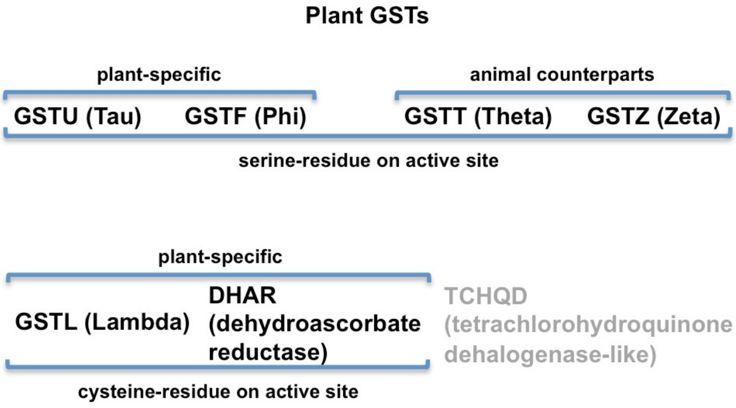


Figure 21: Classes of plant GSTs.

1.3.1.1.1 Structure of GSTs

In general, soluble GSTs are hetero- or homodimeric proteins of about 50 kDa.[70] Lambda and DHAR enzymes, however, appear monomeric by gel filtration. [75] Sequence identity within a class is >40%, between classes it is lower at less than 20%.[72] Despite their diversity in the sequence, the overall structures are quite similar. Each subunit has a distinct folding pattern, the 'GST fold', which consists of an N-terminal domain, with β-strands and α-helices and a C-terminal domain with right-handed α-helices (Figure 22).[72]

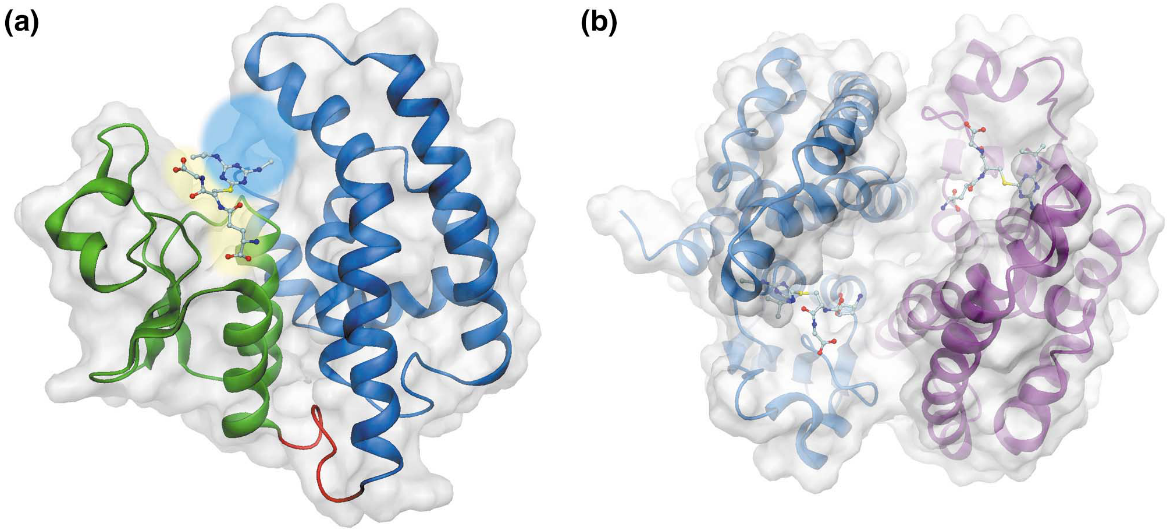


Figure 22: GST structure and substrate binding. (a) GST subunit (*Zm*GSTF1), with the N-terminal domain in green and the C-terminal domain in blue. A glutathione conjugate of the herbicide atrazine is shown binding at the active site; the G site is highlighted in yellow and the H site is highlighted in blue. (b) Atrazine-glutathione conjugates bound to the active site of each subunit of the *Zm*GSTF1 homodimer.[70]

Each catalytic subunit contains a GSH binding site (the G-site) in the N-terminal domain and a binding site for the hydrophobic substrate (the H-site) in the C-terminal domain connected by a linker region of 5-10 residues. The G-site is a highly-conserved region. Tau, phi, theta and zeta enzymes have a serine-residue at the target site, which is involved in the formation and stabilisation of the reactive thiolate anion of GSH. This thiolate anion acts as the nucleophile in the addition or substitution reaction with the hydrophobic co-substrate.[70, 72] Lambda and DHAR enzymes have a cysteine residue at the target site, which alters the function of the protein, as they cannot directly catalyse the *S*-glutathionylation of a substrate at the H-site any longer. [75] The H-site is not so strongly conserved and has a larger variability, which determines the substrate specificity. [70, 72] Quite high mobility of the G and H site upon determination of the crystal structure, suggests that GST subunits might undergo significant changes when binding the substrate.[70]

GSTs can also act as ligand-binding proteins, which means they can bind compounds such as plant hormones and flavonoids into a specific site, the L-site, in a non-substrate manner. There is still little known about L-sites in GSTs and their location can differ.[72, 76]

1.3.1.1.2 Function of GSTs

GSTs have a whole variety of functions, catalytic and non-catalytic, and the functions of many GSTs are still poorly understood (Figure 23).

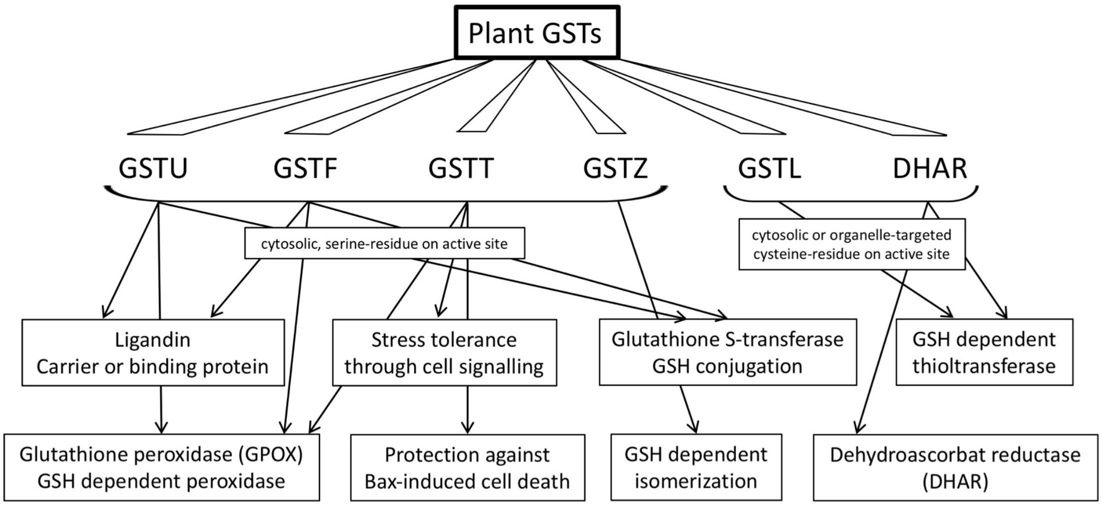


Figure 23: Functions of GSTs in plants.

A key role is their ability to detoxify compounds such as herbicides by conjugation with GSH as part of phase II metabolism. Typical conjugation reactions include nucleophilic aromatic substitutions and Michael-type additions. The *S*-glutathionylated products are then transported into the vacuole by phase III proteins such as ABC transporters prior to proteolytic processing (Figure 24, A).[70]

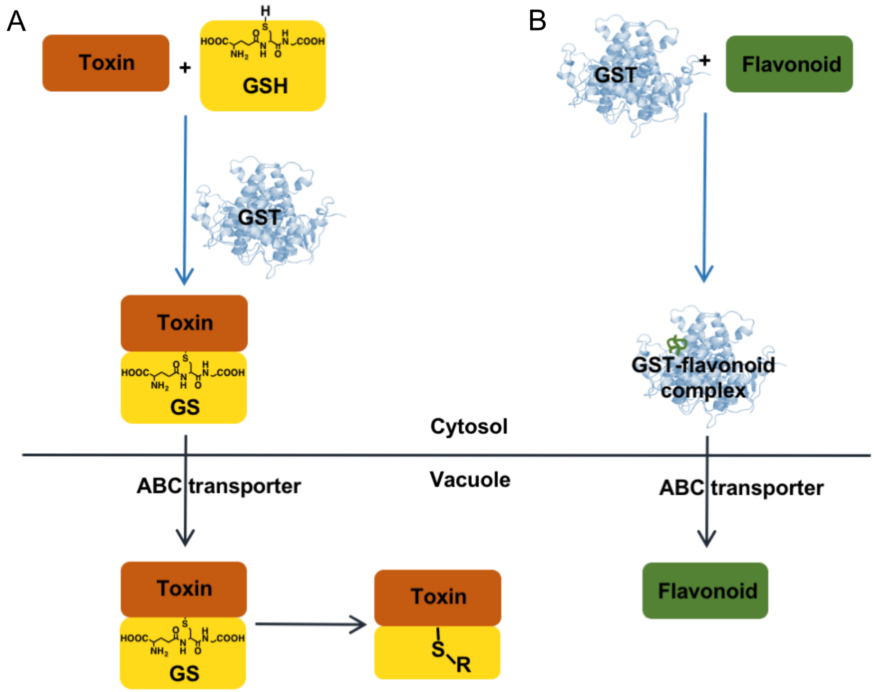


Figure 24: GST functions in phase II metabolism. A: Detoxification of toxins by conjugation with GSH; B: Transport of flavonoid pigments to the vacuole.

There is little evidence that natural products are *S*-glutathionylated in a similar way. Such activities are hard to show because of the instability and the fast turnover of potentially conjugated products, however, there is strong evidence that at least some function as GSH-conjugating enzymes.[73] As mentioned before, GSTs also work as non-enzymatic carriers or ligandins, and are involved in the intercellular transport of flavonoids (Figure 24, B).[77-78] AN9, a petunia GST, was shown to be involved in anthocyanin biosynthesis. It was suggested that AN9 is required for anthocyanin transport from the cytoplasm, where those flavonoid pigments are synthesized, to the vacuole for storage, as AN9 was shown to bind flavonoids, but no GSH conjugation could be detected.[77] This hypothesis was further supported by studies on the cytoplasmic and tonoplast-localised Arabidopsis TT19 (*At*GSTF12), showing that the protein can directly bind cyanidin and cyanidin-3-O-glucoside.[79] Recently, evidence of non-catalytic ligand-binding sites in a GST from

Arabidopsis thaliana, *At*GSTF2, was provided by X-ray crystallography. Two distinct non-catalytic binding sites were identified for small heterocyclic ligands such camalexin and quercetin.[76]

Especially theta, phi and tau GSTs also show GPOX activity (Figure 25, B). They use GSH to reduce organic hydroperoxides of fatty acids and nucleic acids to monohydroxyalcohols and therefore prevent the degradation of the hydroperoxides to cytotoxic aldehyde derivatives.[70, 72] Another role of GSTs in stress metabolism has been demonstrated by expressing a tau GST from tomato in yeast and showing that it can suppress apoptosis induced by the Bax protein, preventing oxidative damage (Figure 25, A).[80]

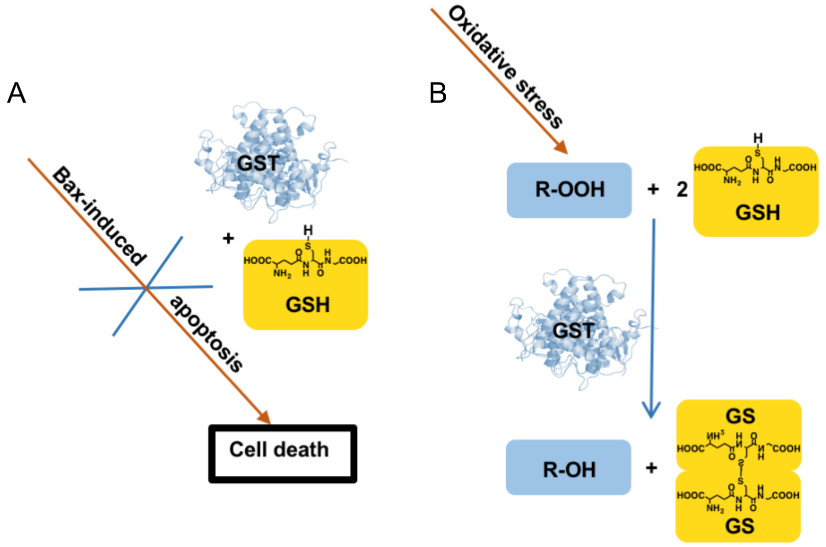


Figure 25: GST functions in stress metabolism. A: Protection against Bax-induced cell death; B: GPOX activity.

The fact, that a specific tau GST and GSH are required for the induction of the genes encoding enzymes of flavonoid biosynthesis by UV light in parsley, suggests GSTs could also be involved in stress tolerance through cell signalling (Figure 26, A).[81] GSTs also show the ability to catalyse GSH dependent isomerizations. One example is the isomerization of maleylacetoacetate to fumarylacetoacetate, a part of the tyrosine degradation pathway (Figure 26, B).[82]

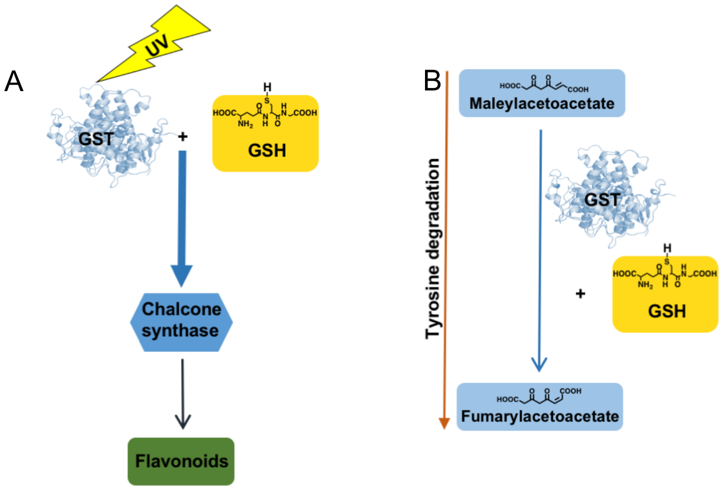


Figure 26: GST functions in signalling and phase I metabolism. A: Stress tolerance through cell signalling; B: GSH dependent isomerization of maleylacetoacetate to fumarylacetoacetate.

As mentioned before DHARs and GSTLs have cysteine residues in their active site. These cysteines can reversibly form mixed disulfides with thiols. DHARs, as their name suggests, are known to reduce dehydroascorbate (DHA) to ascorbic acid (AA), as part of the ascorbate-glutathione cycle, by oxidizing glutathione to glutathione disulfide (Figure 27, B). GSTLs are believed to follow a similar catalytic mechanism, but with oxidized anthocyanidins and tocopherols as substrates.[83] Furthermore DHARs and GSTLs show thioltransferase activity using 2-hydroxylethyl disulfide as a substrate, which means they could have a regulatory and protective role through reversible thiolation and dethiolation of proteins, but substrates are unknown (Figure 27, A).[72]

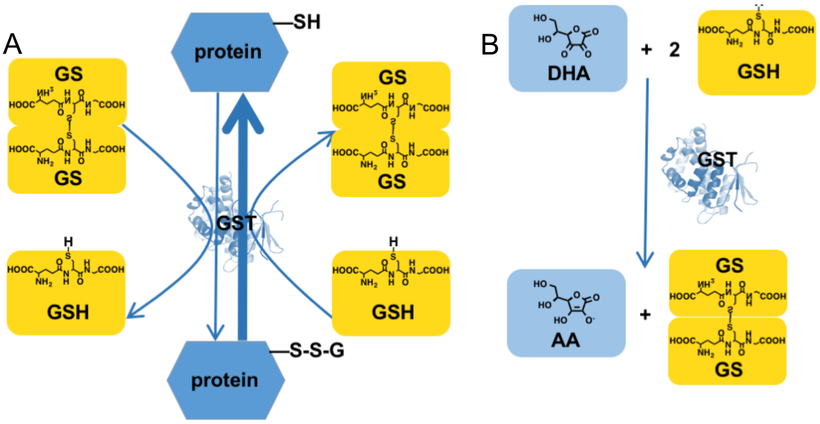


Figure 27: Functions of DHARs and GSTLs. A: Thioltransferase activity; B: Reduction of DHA.

2 Previous work and aims

2.1 Previous work

The evolution of NTSR in black grass has led to a loss of chemical control in cereal crops. MHR in black grass was first reported in 1982 in Peldon, Essex and has since become a major problem in the UK and across Europe.[38] MHR in black grass is associated with an enhanced level of detoxifying enzymes, particularly GSTs which seem to play an important part in this process (*cf.* Chapter 1.2.4).[30, 33-34] The group of Robert Edwards first reported the enhanced level of GSTs in MHR black grass ("Peldon").[34] They showed that the level of *Am*GSTF1, as well as *Am*GSTU1, was increased in herbicide resistant black grass compared to the wild-type. *Am*GSTF1 was 20 times more present in crude plant extracts of MHR plants, which corresponds to 0.2% of the total protein of the MHR plants.[30] *Am*GSTF1, in contrast to *Am*GSTU1, showed only limited activity in detoxifying herbicides, but high GPOX activity, suggesting, the ability of GSTs to reduce organic hydroperoxides plays a role in addition to their ability to catalyse conjugation with glutathione. Another study showed that the use of safeners in wild-type black grass induces similar antioxidant and detoxifying pathways to those in MHR black grass.[33]

This upregulation of *Am*GSTF1 in MHR black grass is similar to the enhanced expression of a pi class GST (GSTP1) in multiple drug resistant tumors in humans. GSTP1 detoxifies drugs directly and reduces organic hydroperoxides but also has important signalling functions promoting drug resistance.[84] Further investigations were carried out to demonstrate this regulatory role in MHR weeds.[30] *Am*GSTF1 was expressed in *Arabidopsis thaliana* and the *Am*GSTF1 transformants were tested for herbicide tolerance using a combination of spraying whole plants, as well as germination phytotoxicity studies (Figure 28). *Am*GSTF1 transformants (line 8 and 12) were more resistant to the herbicides chlorotoluron, alachlor and atrazine and showed changes in metabolism similar to those in NTSR weeds. These changes were not due to changes in gene expression, but regulatory control of plant metabolism triggered by *Am*GSTF1, which led to an accumulation of protective compounds (glutathione, anthocyanins and flavonoids).[30]

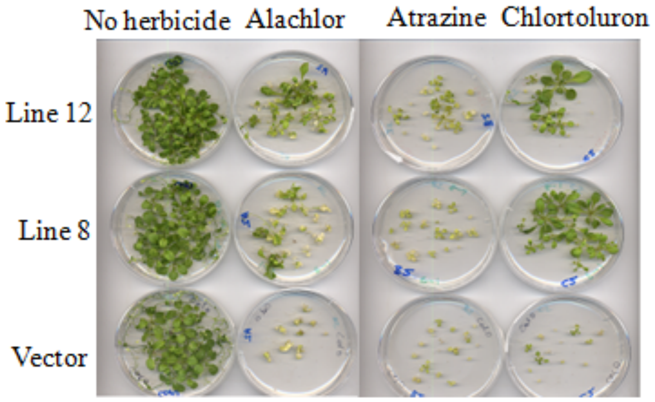
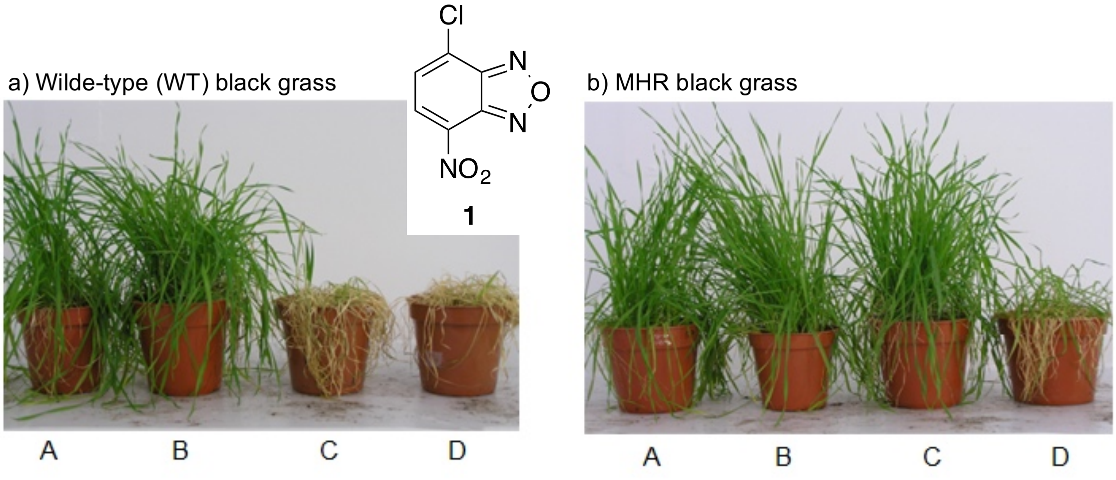


Figure 28: Herbicide resistance of transgenic Arabidopsis expressing *Am*GSTF1.

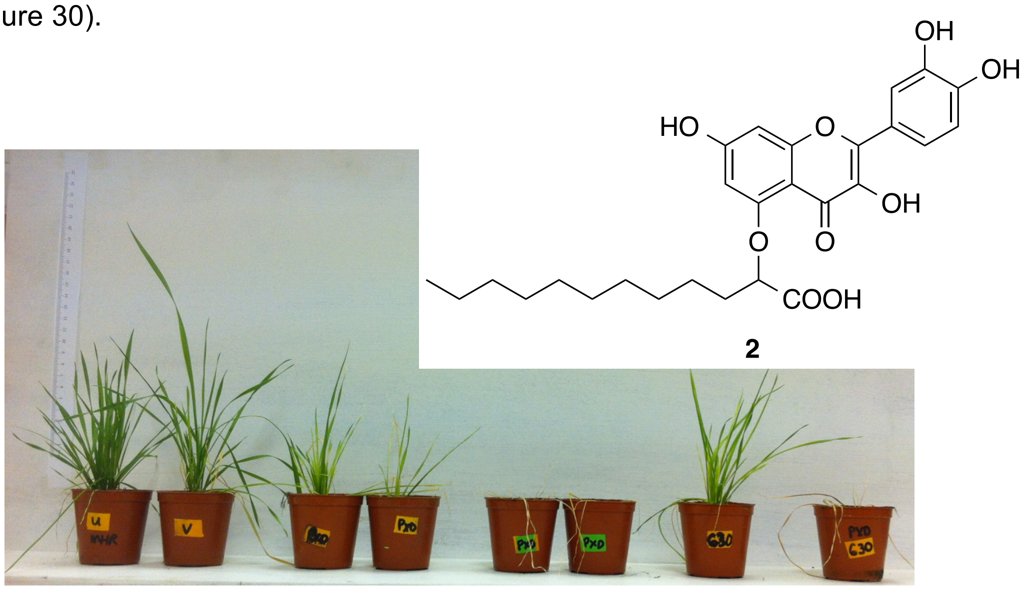
The importance of *Am*GSTF1 was further confirmed by the identification of 4-chloro-7-nitro-benzoxadiazole (CNBF) **1** acting as a synergist. CNBF **1** is a known inhibitor for GSTP1 and multiple drug resistance.[84] It was shown to inhibit *Am*GSTF1 in a 4-Chloro-1,3-dinitrobenzene (CDNB) assay and enhance the herbicidal activity of chlorotoluron (Figure 29), fenoxaprop-p-ethyl and clodinafop-propargyl towards MHR black grass, when applied to MHR plants (“Peldon”).


a) Wilde-type (WT) black grass

b) MHR black grass

Figure 29: Effect of CNBF 1, a known GST inhibitor, on MHR in black-grass. (a) Sensitive and (b) MHR Peldon black grass plants were treated at 12 days with either formulation or CNBF, before an application of either formulation only (control) or the herbicide chlortoluron. A) Formulation control B) CNBF only C) Chlorotoluron only D) Chlorotoluron and CNBF.

Compounds that act as synergists and restore the activity of herbicides towards MHR weeds could offer a very important strategy to overcome MHR. Therefore, further work was carried out in our group to develop potential herbicide synergists.[85] A range of CNBF **1** analogues, compounds with the benzoxadiazole scaffold, substituted purines and different thiazopyrimidines were synthesized and tested for their activity in a CDNB assay and on plants in spray trials. However, no improvements to the initial hit could be made, the lead compound was difficult to optimize and the inherent toxicity of a lot of analogues was difficult to overcome. Ligand fishing was used as a high throughput method to find new potential synergists. Ligand fishing is an expedient method in which potential ligands can be screened for their ability to bind to a receptor. A compound cocktail is injected onto a strep-tactin column where an immobilized protein is present. Following elution of the solvent, the protein is removed from the column, precipitated and the remaining supernatant analyzed by HPLC.[86] *Am*GSTF1 was found to be capable of binding a number of different flavonoids and inhibition could be confirmed by CDNB-assay and spray trials. The initial screening of a library of flavonoids afforded the quercetin analogue **2** as the lead compound, showing the highest inhibition and synergistic effects with pinoxaden on black grass (Figure 30).



2

Figure 30: Effect of 2, a known GST inhibitor, on MHR in black-grass. MHR black grass plants were treated at 14 days with either formulation or 2, before an application of either formulation only (control) or the herbicide pinoxaden. A) Untreated, B) Formulation control, C) Pinoxaden only, D) 2 only, E) Pinoxaden and 2.

To optimize the potential synergist, further analogues derived from chrysin were synthesized and tested with a focus on alkylation at the 7-hydroxy position, substitution of the 7-hydroxy group with a range of amines and some variation of the 5-oxyacetic acid derivatives. Early structure-activity relationships and the synthesis performed is summarized in Figure 31.

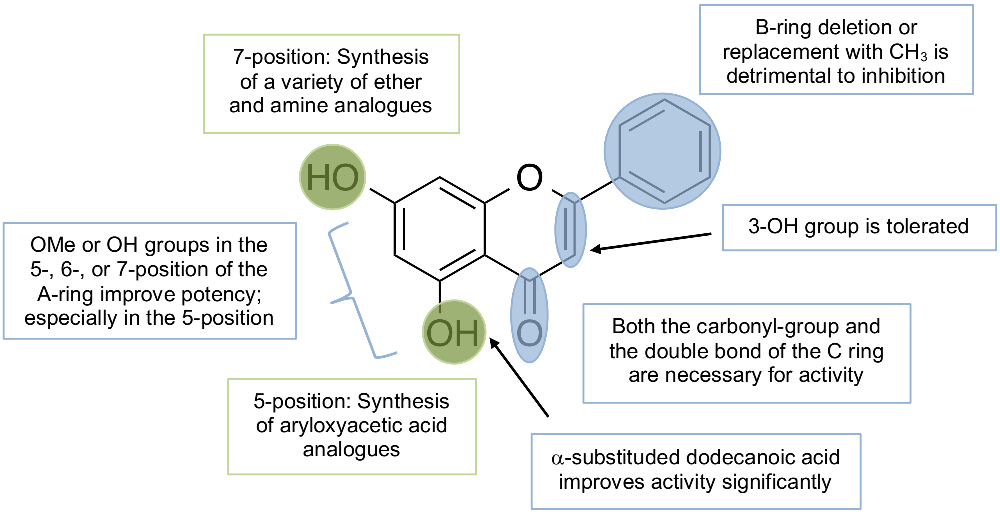
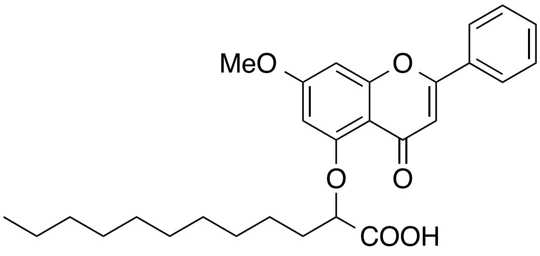


Figure 31: Summary of early structure-activity relationships (blue) and synthesis performed (green).

These studies afforded compound **3** (Figure 32) with a similar structure to **1** and the same level of inhibition towards *Am*GSTF1 (71% ± 5% inhibition in the CDNB assay at a 1 µM level). This work provides the basis of this project.



3

Figure 32: Lead compound.

2.2 Project aims

The primary objective of this project was to develop potential herbicide synergists through structure activity relationship studies. Having identified flavonoid **3** as the lead compound, initial work would focus on the synthesis of further analogues, especially with variations in the C ring and of the substituent in the 5-position (Figure 33) and on testing them for inhibition activity towards *Am*GSTF1. This data would inform the synthesis of further synergists.

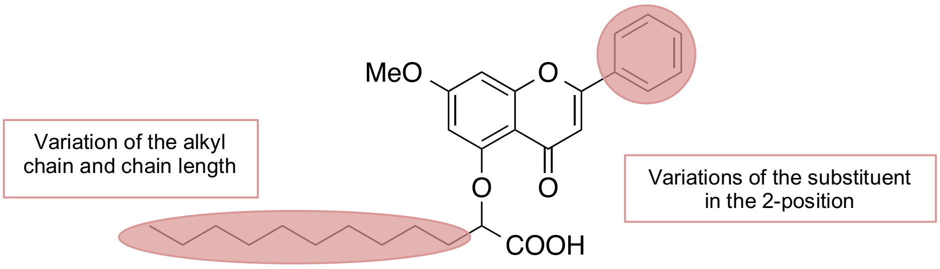


Figure 33: Investigation of flavonoid analogues.

Furthermore, this project aimed to gain a deeper insight into the protein-ligand interactions between the flavonoid inhibitors and *Am*GSTF1 through photoaffinity labelling.

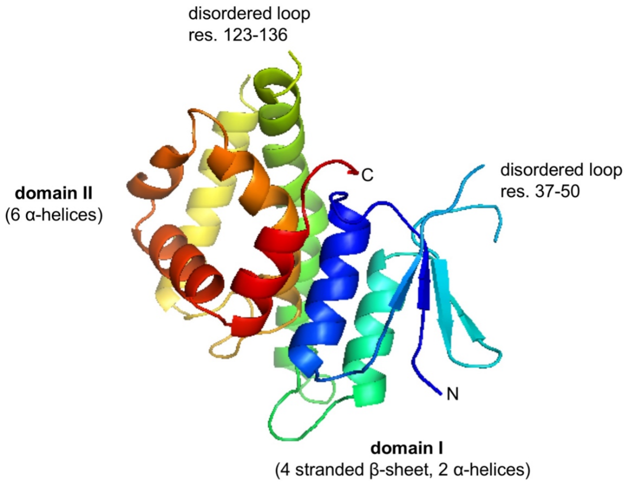


Figure 34: Crystal structure of *Am*GSTF1.

Identification of the binding pocket would allow to use the crystal structure of *Am*GSTF1 (Figure 34) for a structure based design and a more targeted optimization of ligands.

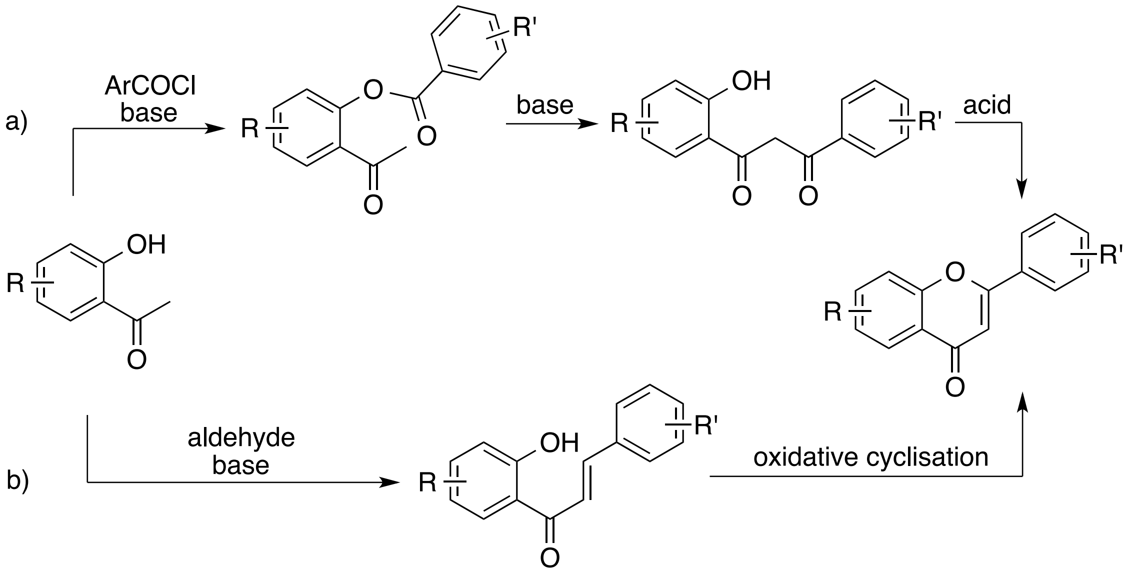
3 Results and Discussion

As flavonoids showed good inhibition of *Am*GSTF1 in the CDNB assay and synergising effects of herbicides in the spray trials, it was of interest to synthesise analogues in order to develop structure activity relationships (SAR). In particular it was deemed important to explore any variations of the substituent at the 2-position and 5-position, and investigate their ability to bind to *Am*GSTF1. Furthermore, efforts were put into gaining a deeper insight into the interactions between *Am*GSTF1 and its ligands.

3.1 Chemical Synthesis

3.1.1 Variations of the substituent in the 2-position

One of the most common methods to synthesise flavones is the Baker-Venkataraman transformation (Scheme 1, a).[87-89] In this procedure, a hydroxyacetophenone is reacted with a benzoyl chloride to form the ester. This benzoyl ester is then treated with base to form the 1,3-diketone. Under basic conditions the diketone cyclises to form the flavone.



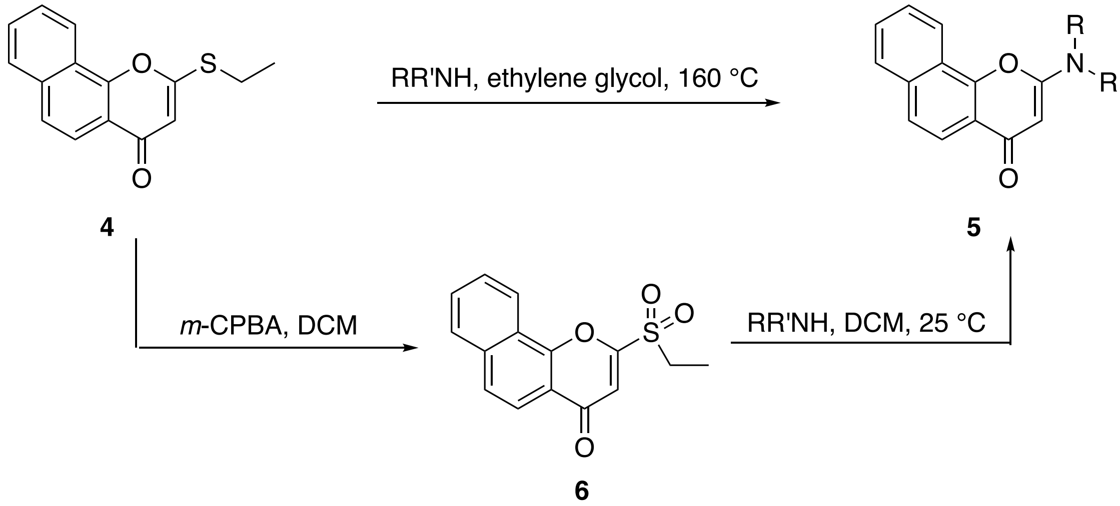
Scheme 1: Flavone synthesis.

Alternatively, 2-substituted 4H-chromen-4-ones can be obtained from the 2-hydroxyacetophenone and a benzaldehyde via the corresponding hydroxychalcone (Scheme 1, b).[90-93] In both cases the substituent in the 2-position is introduced in the first step of the linear synthesis.

In order to obtain analogues with different substituents in the 2-position more efficiently, ways were sought to synthesise 5,7-dimethoxy-4H-chromen-4-ones with a leaving group in the 2-position This leaving group could then be displaced, which would allow diversification at a later step in the synthesis, compared to the more conventional synthetic routes. In order to accelerate the synthesis process, analogues were synthesised with a simple methoxy group in the 5-position.

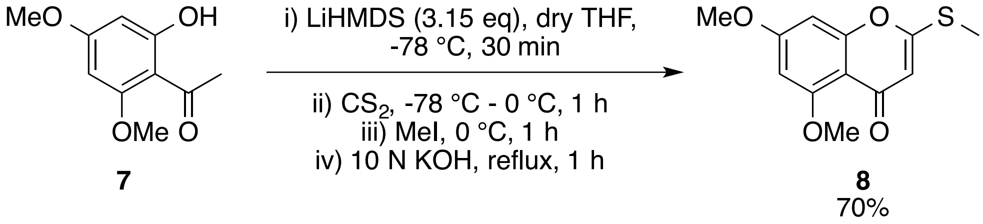
3.1.1.1 Synthesis of 2-amino-5,7-dimethoxy-4H-chromen-4-ones

A review of the literature for the synthesis of 2-amino-4H-chromen-4-ones revealed a procedure by Griffin et al. suggesting the facile displacement of thioethers or sulfones with amines was possible, Scheme 2.[94] Consequently the initial goal was the efficient preparation of a 2-thiochromenone.



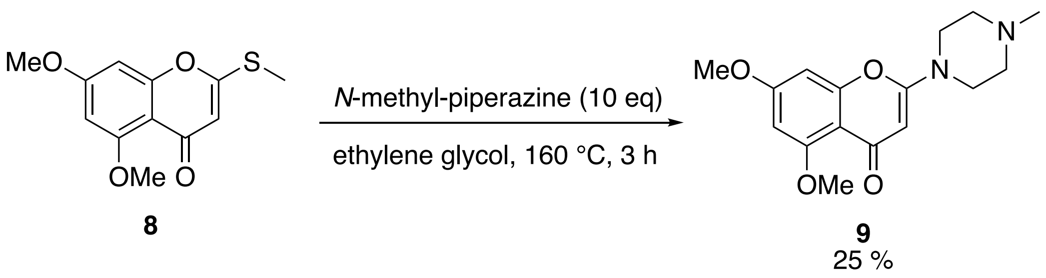
Scheme 2: Synthesis of 2-amino-benzo[h]chromen-4-ones by Griffin.[94]

The preparation of similar thioethers to **4** had previously been reported by Lee and Pak[95] and following this precedent, 2-hydroxy-4,6-dimethoxyacetophenone **7** was treated with lithium bis(trimethylsilyl)amide (LiHMDS). Subsequent, sequential addition of CS_2, MeI and 10 N KOH, followed by aqueous workup afforded, after recrystallization from THF, the pure product 5,7-dimethoxy-2-methylthio-4H-4-chromenone **8** in 70% yield (Scheme 3). The formation of the desired chromenone **8** was confirmed by presence of a peak in the liquid chromatography–mass spectrometry (LC-MS) spectrum (*m/z* (ES+) = 253 MH^+) in combination with appearance of a 3H singlet at 2.53 ppm in the ^1H-NMR spectrum for the SMe-group.



Scheme 3: Synthesis of 5,7-dimethoxy-2-methylthio-4H-4-chromenone 8.

Following the Griffin procedure, thiochromenone **8** was heated with *N*-methyl-piperazine in ethylene glycol at 160°C (Scheme 4). After 3 h, analysis by thin layer chromatography (TLC) showed that all starting material was consumed. Aqueous workup and column chromatography yielded 25% of the desired 2-amino-4H-chromen-4-one **9**, which was confirmed by the corresponding peak in LC-MS (*m/z* (ES+) = 305 MH^+) and a disappearance of the SMe-signal in the ^1H-NMR spectrum. The low yield was attributed to decomposition arising from the high reaction temperature.

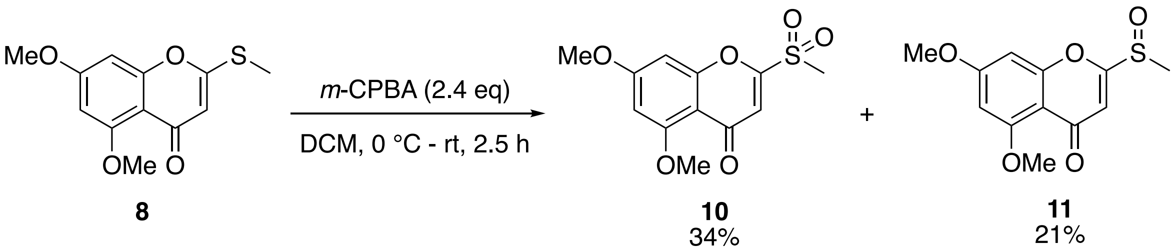


Scheme 4: Synthesis of 5,7-dimethoxy-2-(4'-methylpiperazin-1'-yl)-4H-chromen-4-one 9.

Due to the rather low yield, an alternative synthesis, which was also shown by Griffin et al.[94], was used. They reported a method in which they had converted the thioether **4** to the corresponding sulfone **6** first, before reacting it with the amine (Scheme 2).

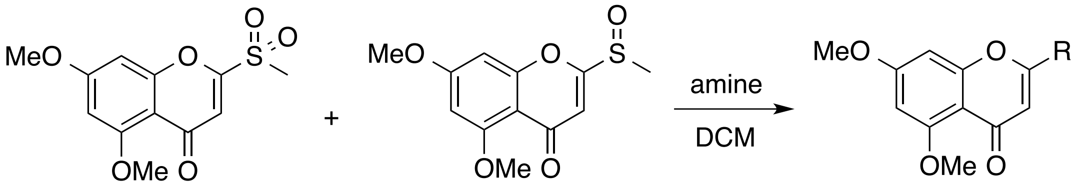
However, initial attempts to reproduce the oxidation proved not to be straight-forward, as treatment with 2.4 equivalents of *meta*-chloroperoxybenzoic acid (*m*-CPBA) afforded a mixture of the desired sulfone **10** together with the intermediate sulfoxide **11** (Scheme 5) confirmed by two peaks in the LC-MS chromatogram (*m/z* (ES+) = 285 MH$^+$ and *m/z* (ES+) = 269 MH$^+$). The two compounds could be isolated in 34% (**10**) and 21% yield (**11**) respectively. The low combined yield can be explained by high losses during the purification and separation of the two compounds.

Attempts to enhance the conversion of **10** by prolonged reaction times or higher stoichiometries of *m*-CPBA were not successful.



Scheme 5: Synthesis of 5,7-dimethoxy-2-(methylsulfonyl)-4H-chromen-4-one 10.

Consequently, it was decided to test whether the sulfoxide **11** would also act as a leaving group. A small-scale experiment in which **11** was reacted with morpholine afforded the desired 5,7-dimethoxy-2-morpholino-4H-chromen-4-one **12**, as confirmed by a peak in LC-MS (*m/z* (ES+) = 292 MH^+). As a result, the crude oxidation product was directly converted to the amine.



crude mixture of **10** & **11** (10:1)

12 - **15**

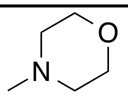
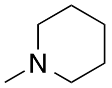
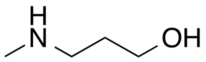
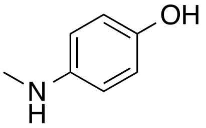
Entry	Starting material	Product	R	Reaction conditions	Yield over 2 steps
1	morpholine	12		46 h, rt - reflux	32%
2	piperidine	13		20 h, rt	50%
3	3-amino-propan-1-ol	14		20 h, rt	33%
4	4-aminophenol	15		20 h, rt	-

Table 3: Synthesis of 2-amino-5,7-dimethoxy-4H-chromen-4-ones (12-15).

A range of amines were reacted with the crude mixture of **10** and **11** until no starting material could be detected by TLC and LC-MS analyses or no further conversion was observed (Table 3). Simple aliphatic or alicyclic amines (entries 1 – 3) worked well, but less nucleophilic species such as 4-aminophenol (entry 4) were not viable.

The potential synergists were tested for inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1). As the non-aromatic amines in the 2-position seem to lower inhibition, attention was turned to analogues with aryl substituents in the 2-position.

3.1.1.2 Synthesis of 2-aryl-5,7-dimethoxy-4H-chromen-4-ones

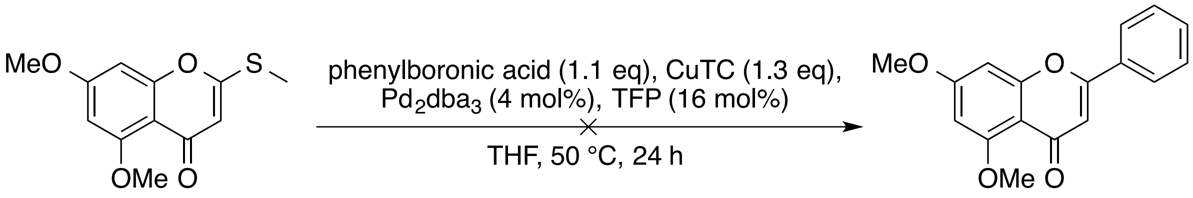
3.1.1.2.1 Liebeskind-Srogl cross-coupling

Analysis of the target suggested that cross-coupling of a suitable aryl nucleophile with a chromenone featuring a leaving group would offer a convenient approach for the synthesis of a variety of analogues. With thioether **8** already successfully synthesised, a cross-coupling reaction described by Liebeskind and Srogl[96], in which a new carbon-carbon bond is formed from a organosulfur compound, such as a thioester or thioether, and an aryl boronic acid using a metal catalyst (Scheme 6), was explored.

$$\text{Het}-\text{SR'} \quad + \quad \text{RB(OH)}_2 \xrightarrow[\substack{\text{CuTC (1.2 - 1.3 eq)} \\ \text{THF, 50 °C, 18 h}}]{\text{cat Pd}} \text{Het}-\text{R}$$

Scheme 6: Liebeskind-Srogl cross coupling.

Cross coupling of thioether **8** with phenylboronic acid was carried out under standard conditions reported by Liebeskind and Srogl[96] (Scheme 7), using the tris(dibenzylideneacetone)dipalladium ($Pd_2(dba)_3$)/tris(2-furyl)phosphine (TFP) catalytic system and copper(I) thiophene-3-carboxylate (CuTC). Repeated checks by TLC and LC-MS showed mainly starting material and that no product was formed, even after 24 h.

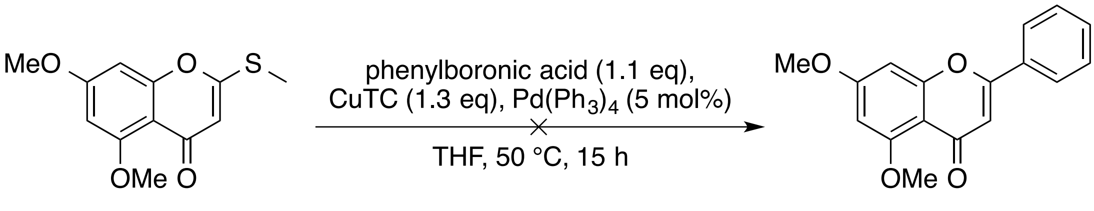


8

16

Scheme 7: Attempted synthesis of 5,7-dimethoxy-2-phenyl-4H-chromen-4-one 16 using a $Pd_2(dba)_3$/TFP catalytic system.

A review of the literature revealed that the use of tetrakis(triphenylphosphine)palladium ($Pd(Ph_3)_4$) instead of $Pd_2(dba)_3$/TFP may be beneficial.[97] However, attempts using this catalyst led to the same result (Scheme 8), suggesting that the starting material is not a suitable substrate for this cross coupling reaction, which works most efficiently in π-deficient heteroaromatic thioethers such as 2-(methylthio)pyrazines or 2-(methylthio)benzothiazoles[96]. Consequently, the approach for the synthesis of 2-substituted 4H-chromen-4-ones was modified. Since the thioether was not a good enough leaving group in the cross coupling reaction, a better leaving group in the 2-position was needed.

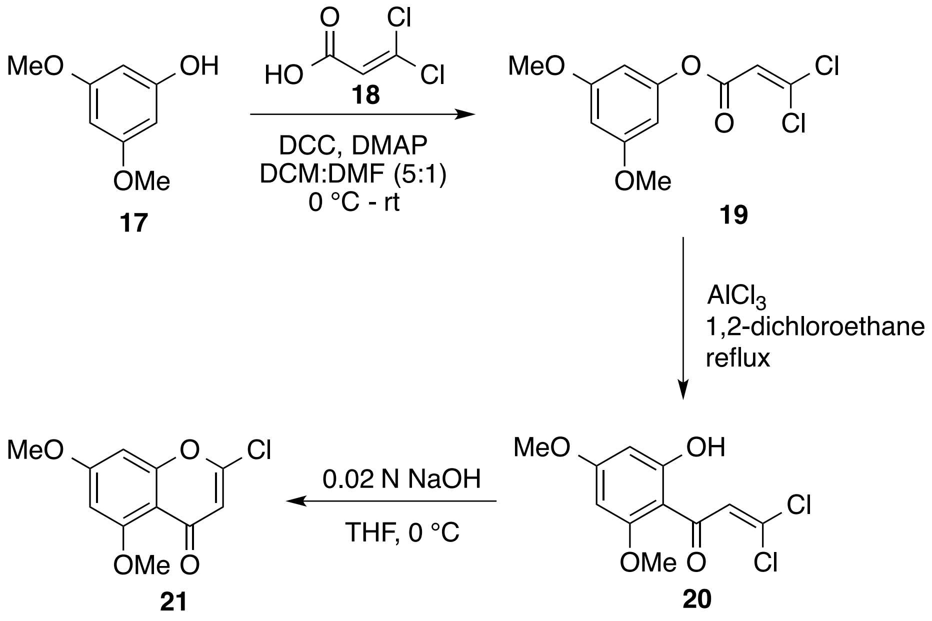


8 **16**

Scheme 8: Attempted synthesis of 5,7-dimethoxy-2-phenyl-4H-chromen-4-one 16 using $Pd(Ph_3)_4$ as a catalyst.

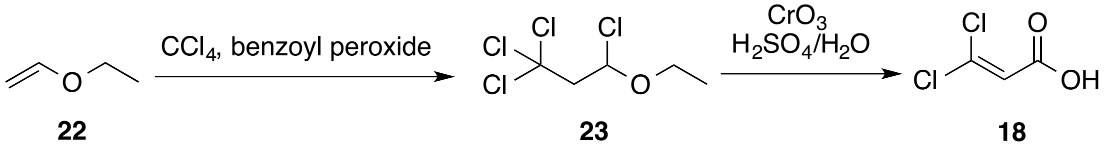
3.1.1.2.2 Suzuki Miyaura cross-coupling

In the Suzuki Miyaura reaction halides are coupled with boronic acids, a large number of which are readily available. Since the Suzuki cross coupling also allowed a convergent synthesis of flavones, this was the approach of choice for the creation of a small library of compounds. A literature research revealed a procedure by Kraus et al.[98], which gives access to 2-chloro-chromen-one **21** (Scheme 9) via esterification of phenol **17** with acid **18** and subsequent Fries rearrangement and cyclisation.



Scheme 9: Synthesis of 2-chloro-5,7-dimethoxy-4H-chromen-4-one 21 by Kraus et al.[98]

Starting material **18** had to be synthesised, as it is not commercially available. Following a procedure by Schroth et al.[99] (Scheme 10) ethyl vinyl ether **22** was reacted with tetrachloromethane (CCl_4) through radical addition using benzoylperoxide to form ethyl-(1,3,3,3-tetrachloropropyl)ether **23.** Removal of excess CCl_4 under reduced pressure afforded the crude product with only minor impurities. A doublet of doublets at 5.96 ppm in the ^{1}H NMR spectrum, assigned to the CH-group and another two doublet of doublets at 3.52 and 3.42 ppm assigned to the two protons of the CH_2-group next to it, confirmed the formation of the product.

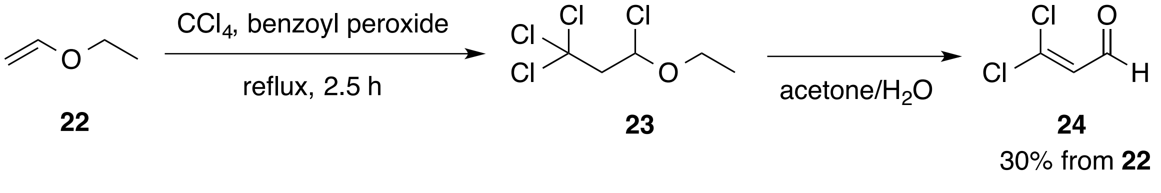


Scheme 10: Synthesis of 3,3-dichloroacrylic acid 18 by Schroth et al.[99]

On the basis of NMR-analysis it was suspected that **23** was undergoing fast decomposition, consequently the crude product was not further purified by distillation and used directly in subsequent reactions in order to minimise further losses.

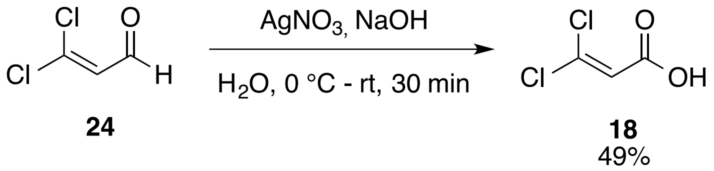
Initial attempts to generate 3,3-dichloroacrylic acid **18** followed the one-pot procedure described by Schroth et al.[99], combining the hydrolysis and the oxidation, using sulfuric acid and CrO_3 (Scheme 10). However, despite multiple attempts and and paying particular attention to the temperature profile, no evidence for the desired product could be obtained. Analysis of the crude product by ^{1}H-NMR spectroscopic analysis suggested that mostly 3,3,3-trichloropropanoic acid was afforded, as the spectrum showed a single singlet at 3.85 ppm.

Consequently, a reaction with ethyl-(1,3,3,3-tetrachloropropyl)ether **23** and sulfuric acid was conducted. ^{1}H-NMR spectroscopic analysis showed that 3,3,3-trichloropropanal was formed, with a doublet at 3.70 ppm and a triplet at 9.86 ppm in the ^{1}H-NMR spectrum. This suggested that the elimination-step to give the double bond was not working. Therefore ethyl-(1,3,3,3-tetrachloropropyl)ether **34** was converted to 3,3-dichloroacrolein **24** in an independent step following a method described by Hanack et al.[100]. In this, crude **23** was mixed with acetone and H_2O. The acetone was removed by distillation through a packed column. Aqueous workup followed by a vacuum distillation afforded the desired product **24** in 30% yield over the two steps (Scheme 11), showing two doublets at 9.87 ppm and 6.44 ppm in the ^{1}H-NMR spectrum. The rather low yield can be explained due to losses in the distillation-process because of condensation reactions of the product.



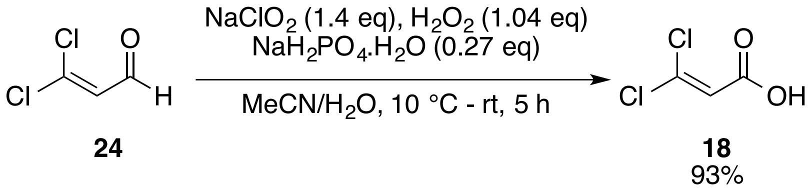
Scheme 11: Synthesis of 3,3-dichloroacrolein 24.

Attempts to oxidise the aldehyde **24** to the acid **18** using CrO_3 failed, but a protocol by Stack et al.[101] using Ag_2O, prepared in situ from $AgNO_3$, afforded the product in 49% yield after purification (Scheme 12). ^{1}H-NMR spectroscopic analysis showed the characteristic singlet at 6.42 ppm. As this is an expensive method and the yield not high (even dropping to 33% when the reaction was conducted on a larger scale), other methods were sought from the literature.



Scheme 12: Oxidation of aldehyde 24 to acid 18 using Ag_2O.

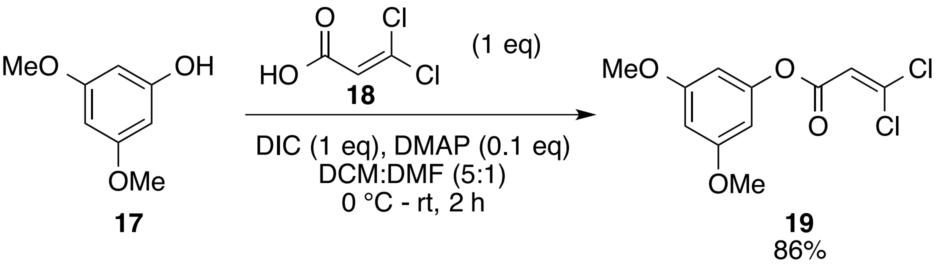
Dalcanale et al.[102] describe a general, very mild method for the oxidation of aldehydes with sodium chlorite and hydrogen peroxide as a scavenger for the formed hypochlorous acid. These conditions were applied to 3,3-dichloroacrolein **24** (Scheme 13). Aqueous $NaClO_2$ was added to a solution of **24** and H_2O_2 in aqueous acetonitrile buffered with NaH_2PO_4. Purification yielded 93% of 3,3-dichloroacrylic acid **18**.



Scheme 13: Oxidation of aldehyde 24 to acid 18 using sodium chlorite.

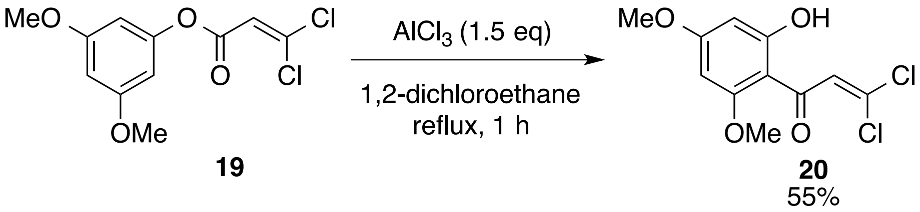
With 3,3-dichloroacrylic acid **18** in hand, the synthesis of 2-chloro-5,7-dimethoxy-4H-chromen-4-one **21** could now be pursued. For the first step, the Steglich esterification (Scheme 14), 3,5-dimethoxyphenol **17**, was coupled with 3,3-dichloroacrylic acid **18** using 4-dimethylaminopyridine (DMAP) and diisopropylcarbodiimide (DIC), as it is easier to handle than DCC. Purification yielded 86% of 3,5-dimethoxyphenyl 3,3-dichloroacrylate

19, confirmed by the presence of the corresponding peak in the LC-MS chromatogram (*m/z* (ES+) = 277 MH^+).



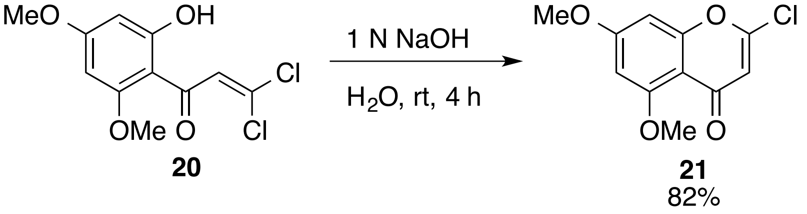
Scheme 14: Synthesis of 3,5-dimethoxyphenyl 3,3-dichloroacrylate 19.

For the second step, initial attempts to achieve a Fries rearrangement using 1.1 eq of $AlCl_3$ in 1,2-dichloroethane resulted in low conversion and it was found that higher conversion required more equivalents of $AlCl_3$ than reported by Kraus et al.[98] With 1.5 eq of $AlCl_3$ 3,3-dichloro-1-(2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one **20** could be produced in 55% yield (Scheme 15), characterized by a signal in the ^{1}H-NMR at 13.46 ppm for the OH-group, confirming the successful rearrangement. The two doublets at 6.08 and 5.92 ppm for the two aromatic protons confirmed the 1,2-rearrangement rather than the 1,4-rearrangement.



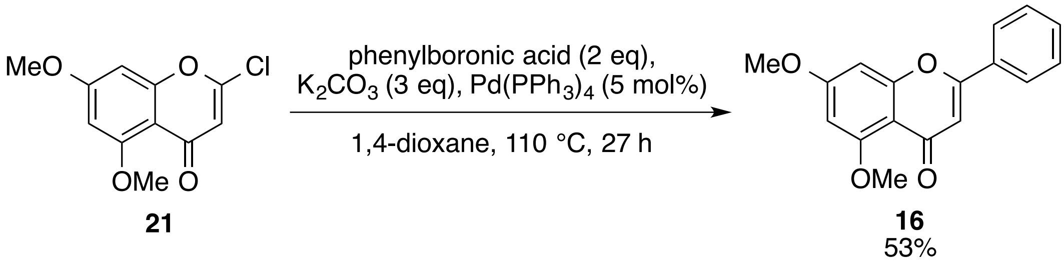
Scheme 15: Synthesis of 3,3-dichloro-1-(2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one 20.

First attempts to cyclise compound **20** involved dissolution in THF and addition of 0.02 N NaOH. This reaction however resulted in no conversion at all and only starting material was recovered. Several different attempts, which included a longer reaction time, elevated temperature and stronger base led to the same result. Significantly, if the base was too strong, decomposition was observed. A search of the literature revealed a similar procedure by Levas et al.[103], which suggested dispersion of the starting material **20** in H_2O and then slow addition of aqueous NaOH. This approach (Scheme 16) turned out to work well and following purification the product 2-chloro-chromenone **21** was obtained in 82% yield, as confirmed by the appearance of the corresponding peak in the LC-MS chromatogram (*m/z* (ES+) = 263 MH^+).



Scheme 16: Synthesis of 2-chloro-5,7-dimethoxy-4H-chromen-4-one 17.

With **21** successfully synthesised, it was then necessary to couple it with a range of aryl boronic acids. In a preliminary Suzuki-Miyaura cross coupling reaction, **21** was coupled with phenylboronic acid in the presence of $Pd(PPh_3)_4$ and K_2CO_3 following the Kraus[98] procedure. Product **16** was confirmed by the presence of the corresponding peak in the LC-MS chromatogram (*m/z* (ES+) = 283 MH^+) and disappearance of the characteristic chlorine isotope pattern, albeit in only 53% yield (Scheme 17). In order to identify optimum cross coupling conditions, a condition screening was then undertaken.



Scheme 17: Suzuki-coupling of 21 with phenylboronic acid.

To determine the yield of the product in the optimization experiments, without the necessity to isolate the product in every case, a calibration using LC-MS was established.

For a quantitative analysis, for both the product (p) **16** and the starting material (sm) **21**, an internal standard (is), chromone, was added to the samples. A good separation with retention times of about 1.9 (is), 2.6 (sm **21**) and 2.9 min (p **16**) could be achieved (Figure 35).

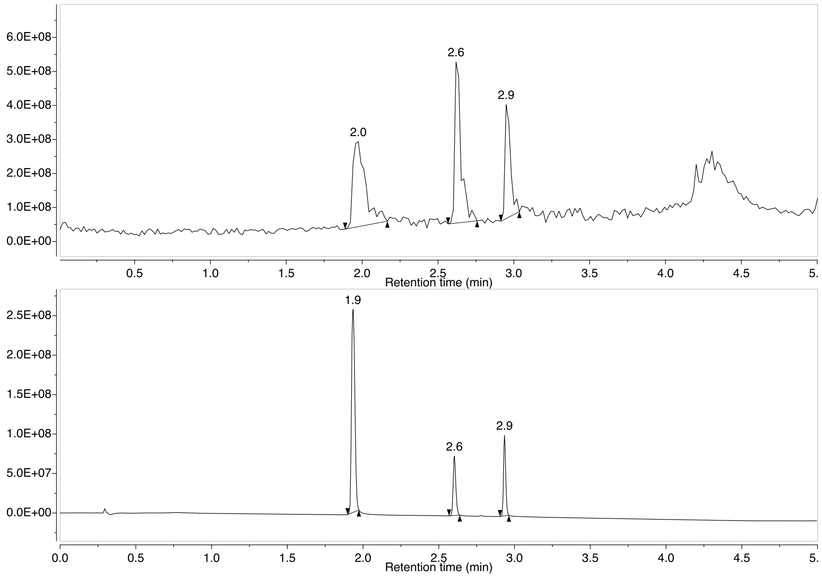


Figure 35: Total ion currant (TIC) chromatogram (ES^+) and total absorbance chromatogram of a mixture of is, sm and p; Acquity UPLC (Waters Ltd, UK) with a 1.7 μm Acquity UPLC BEH C18 column; mobile phase of water containing formic acid (0.1%) (A):acetonitrile (B) (gradient: 0.2 min 5% B, then up to 95% B after 4 min, constant 95% B till 4.5 min, down to 5% B after 5 min); constant flow rate of 0.6 ml/min; absorbance data was acquired from 210 to 400 nm using an Acquity photodiode array detector.

Figure 36 and Figure 37 show the calibration curves for the starting material and the product, using chromone as an internal standard. In both cases it is linear for the ratio of c_p/c_{is} and c_{sm}/c_{is} respectively, in a range from 0.025 – 1 with a very good correlation coefficient.

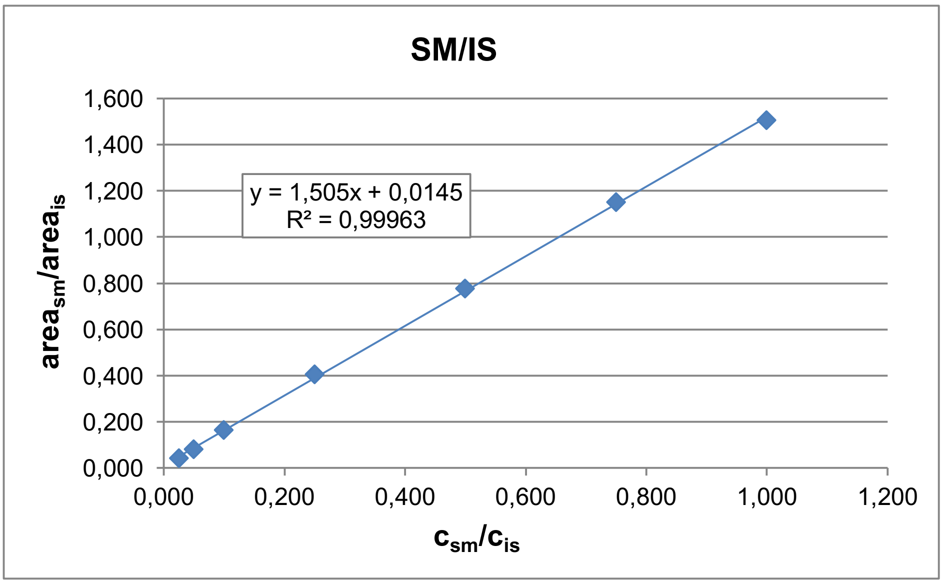


Figure 36: Calibration curve for the starting material 21.

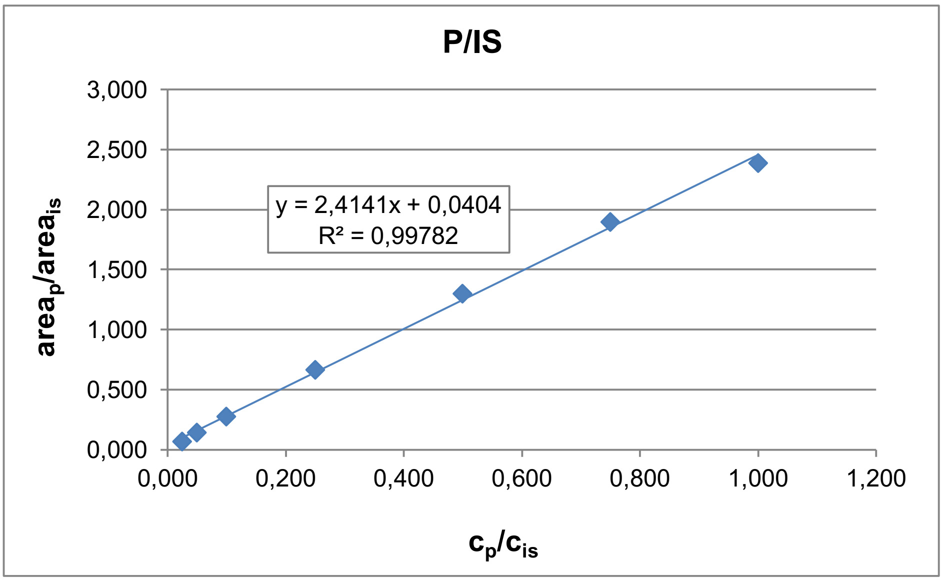
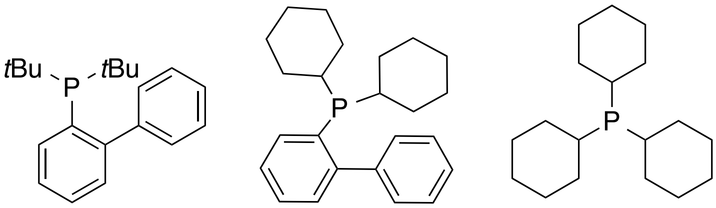


Figure 37: Calibration curve for the product 16.

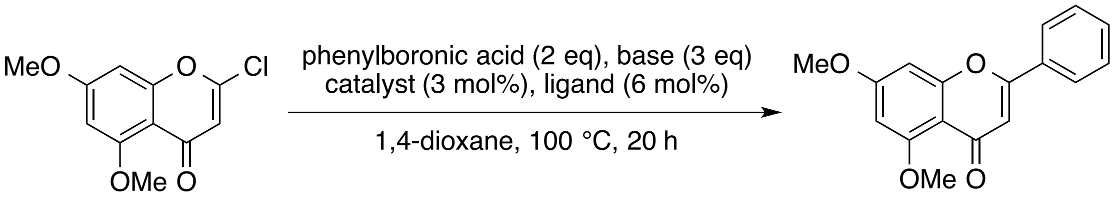
A literature search showed that the phosphine ligands (2-biphenyl)di-*tert*-butylphosphine (JohnPhos **22**), (2-biphenyl)dicyclohexylphosphine (CyJohnPhos **23**) and tricyclohexylphosphine (PCy_3 **24**) (Figure 38) work very well for Suzuki-couplings with chlorides.[104-105] Those ligands, together with the catalysts $Pd_2(dba)_3$ and palladium(II)acetate ($Pd(OAc)_2$) and the bases KF, Cs_2CO_3 and K_3PO_4 were used in the screening.



22 **23** **24**

Figure 38: Phosphine ligands 22, 23 and 24.

In Table 4 the results of the screening are summarized. The yield of the product and the starting material were determined by LC-MS as described before using an internal standard.



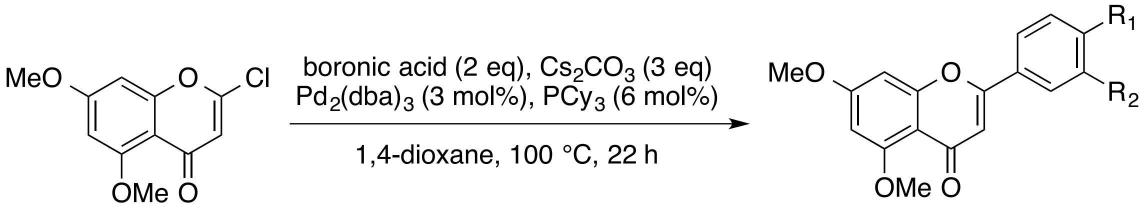
21 **16**

Entry	Catalyst	Ligand	Base	Yield[a]
1	$Pd(OAc)_2$	JohnPhos	KF	84%
2	$Pd(OAc)_2$	CyJohnPhos	KF	100%
3	$Pd(OAc)_2$	PCy_3	KF	100%
4	$Pd(OAc)_2$	JohnPhos	Cs_2CO_3	44%
5	$Pd(OAc)_2$	CyJohnPhos	Cs_2CO_3	100%
6	$Pd(OAc)_2$	PCy_3	Cs_2CO_3	100%
7	$Pd_2(dba)_3$	JohnPhos	KF	99%
8	$Pd_2(dba)_3$	CyJohnPhos	KF	100%
9	$Pd_2(dba)_3$	PCy_3	KF	100%
10	$Pd_2(dba)_3$	JohnPhos	Cs_2CO_3	49%
11	$Pd_2(dba)_3$	CyJohnPhos	Cs_2CO_3	92%
12	$Pd_2(dba)_3$	PCy_3	Cs_2CO_3	100%
13[b]	$Pd(OAc)_2$	JohnPhos	K_3PO_4	98%
14[b]	$Pd(OAc)_2$	CyJohnPhos	K_3PO_4	87%
15[b]	$Pd(OAc)_2$	PCy_3	K_3PO_4	96%

Table 4: Condition screening for Suzuki-coupling reaction. [a] Determined by LC-MS using an internal standard. [b] Toluene was used instead of 1,4-dioxane as solvent.

Most conditions proved to work in excellent yield. Only some, where JohnPhos **22** was used as a ligand (Table 4, entries 1, 4 and 10) showed lower yields.

Conditions from entry 12, using PCy_3 **24**, $Pd_2(dba)_3$ and Cs_2CO_3 were chosen for further reactions and a library of compounds was synthesised. Satisfyingly, a variety of different 2-aryl-5,7-dimethoxy-4H-chromen-4-ones (**16**, **25 - 32**) could be obtained in good isolated yields (Table 5, entries 1 - 9).



21

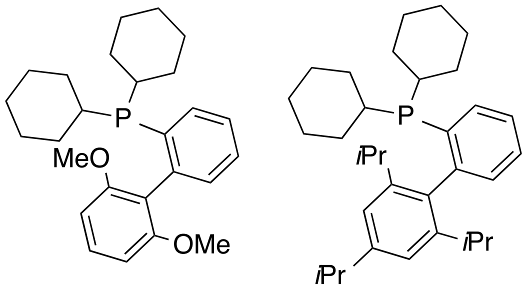
16, **25 - 35**

Entry	Starting material	Product	R_1	R_2	Yield
1	phenylboronic acid	**16**	H	H	83%
2	4-fluorophenylboronic acid	**25**	F	H	50%
3	4-methoxyphenylboronic acid	**26**	OMe	H	92%
4	3-biphenylboronic acid	**27**	H	phenyl	63%
5	4-ethylphenylboronic acid	**28**	ethyl	H	74%
6	3-methoxyphenylboronic acid	**29**	H	OMe	75%
7	3,4-dimethoxyphenylboronic acid	**30**	OMe	OMe	71%
8	3,4-(methylenedioxy)phenylboronic acid	**31**	methylenedioxy		30%
9	4-methoxycarbonylphenylboronic acid	**32**	COOMe	H	68%
10	4-bromophenylboronic acid	**33**	Br	H	-
11	3-hydroxyphenylboronic acid	**34**	H	OH	-
12	4-(methylhydroxy)phenylboronic acid	**35**	CH_2OH	H	-

Table 5: Synthesis of different 2-aryl-5,7-dimethoxy-4H-chromen-4-ones (16, 25-35).

The attempt to couple 4-bromophenylboronic acid led to an inseparable mixture of mono and decoupled product, as detected by LC-MS (Table 5, entry 10). Cross coupling of 3-hydroxyphenylboronic acid and 4-(methylhydroxy)phenylboronic acid worked but because of the limited solubility of the products, no pure compound could be obtained (Table 5, entries 11 and 12).

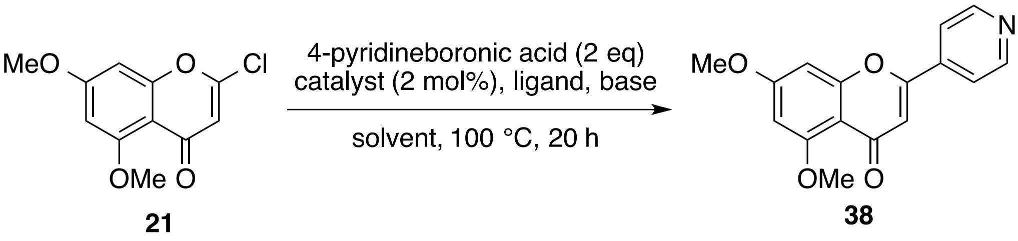
For the coupling reactions with 3-pyridineboronic acid, 4-pyridineboronic acid and 2-furanylboronic acid, no formation of product could be detected under these conditions. Therefore, a further set of different conditions for the Suzuki coupling of heteroaromatic boronic acids was screened (Table 6). The screening was conducted on small scale and the conversion of the starting material was determined by ^{1}H-NMR. Entries 1 and 2 are conditions, which are known to work well for heteroaromatic boronic acids[106], using



36 **37**

Figure 39: Phosphine ligands 36 and 37.

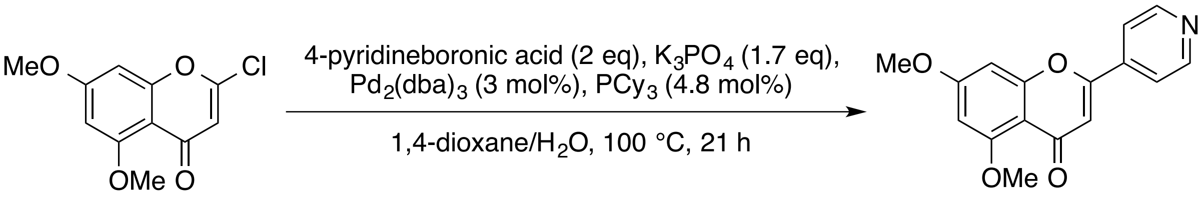
2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos **36**) and 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos **37**) as ligands (Figure 39). Full conversion of starting material **21** and formation of the desired 2-pyridylchromenone **38**, by a peak in the LC-MS, could be observed for entry 1 and 2, but purification of a reaction on larger scale using conditions from entry 2 showed that mainly 2-butoxy-5,7-dimethoxy-4H-chromen-4-one was formed. Changing the solvent to 1,4-dioxane led to poor conversion. Entries 3 - 5 are similar to conditions previously used (Table 4), which showed good results in the coupling of phenylboronic acid. With 4-pyridineboronic acid, however, those conditions led to poor conversion.



Entry	Catalyst	Ligand	Base	Solvent	Conversion
1	$Pd_2(dba)_3$	XPhos (8 mol%)	K_3PO_4 (2 eq)	*n*-butanol	100%
2	$Pd_2(dba)_3$	SPhos (8 mol%)	K_3PO_4 (2 eq)	*n*-butanol	100%
3	$Pd_2(dba)_3$	JohnPhos (8 mol%)	KF (3 eq)	1,4-dioxane	7%
4	$Pd_2(dba)_3$	CyJohnPhos (8 mol%)	KF (3 eq)	1,4-dioxane	22%
5[a]	$Pd(OAc)_2$	PCy_3 (6 mol%)	K_3PO_4 (3 eq)	toluene	12%
6	$Pd_2(dba)_3$	PCy_3 (4.8 mol%)	K_3PO_4 (1.7 eq)	1,4-dioxane /H_2O	100%

Table 6: Condition screening for Suzuki-coupling reaction of heteroaromatic boronic acids. [a] 3 mol% $Pd(OAc)_2$.

Another literature search revealed a procedure by Kudo et al.[107] for Suzuki cross-coupling reactions of nitrogen heterocycles (Table 6 ,entry 6) using $Pd_2(dba)_3$ as a catalyst, PCy_3 as a ligand, K_3PO_4 as a base and a mixture of 1,4-dioxane and H_2O as solvent. Pleasingly, these conditions resulted in 100% conversion of starting material. However, purification was difficult and eventually only successful following basic extraction and column chromatography leading to a low isolated yield of **38** (Scheme 18).



21

38
15%

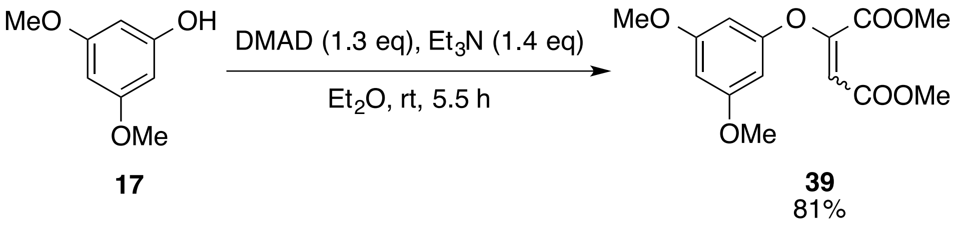
Scheme 18: Synthesis of 5,7-dimethoxy-2-(pyrid-4'-yl)-4H-chromen-4-one 38.

Similarly attempts to cross couple **21** and 3-pyridineboronic acid showed formation of product but no pure compound could be obtained. However, when 2-furanylboronic acid was used, no product could be observed.

The synthesised analogues were tested for inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1). The data indicates that a phenyl ring in the 2-position is beneficial for inhibition, but further modifications on the phenyl-ring did not significantly affect the activity of these compounds. Compound **27** with biphenyl in the 2-position and compound **28** with a 4-ethyl-substituted phenyl-ring, also show similar inhibition, which suggests that steric bulk does not have a major effect in this position.

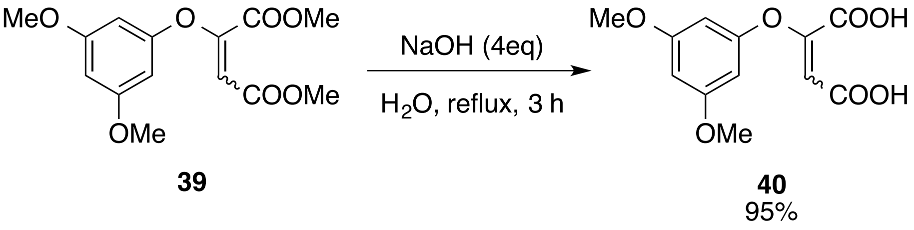
3.1.1.3 Synthesis of 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamides

As a further addition to the structure activity relationship investigations, carboxamides, which can be derived from 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxylic acid **41**, were synthesised. Following a procedure by Stoermer et al.[108] for the synthesis of chromone-2-carboxylic acids, addition of triethylamine and dimethylacetylenedicarboxylate (DMAD) to a solution of phenol **16** afforded a 1:0.9 mixture of E:Z isomer of the diester **39** in 81% yield (Scheme 19). Formation was confirmed by LC-MS (*m/z* (ES+) = 297 MH$^+$).



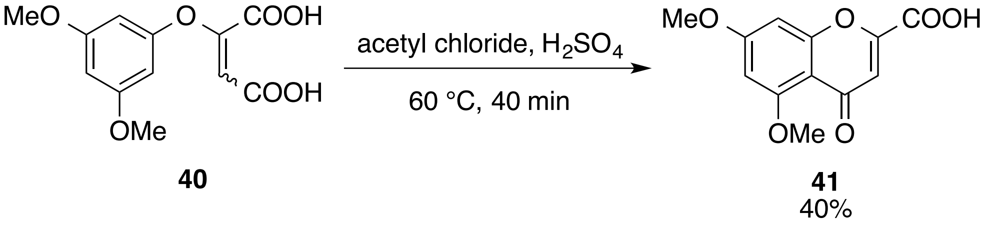
Scheme 19: Synthesis of dimethyl (E,Z)-2-(3',5'-dimethoxyphenoxy)ethene-1-2-dicarboxylate 39.

In the next step the diester **39** was hydrolysed into the diacid **40** to afford a 1:0.8 mixture of E:Z isomers (Scheme 20), as indicated by a loss of the OCH$_3$ signals in the ^{1}H-NMR spectrum and the presence of the two corresponding peaks in the LC-MS chromatogram (*m/z* (ES+) = 269 MH$^+$).



Scheme 20: Synthesis of (E,Z)-2-(3',5'-dimethoxyphenoxy)ethene-1-2-dicarboxylic acid 40.

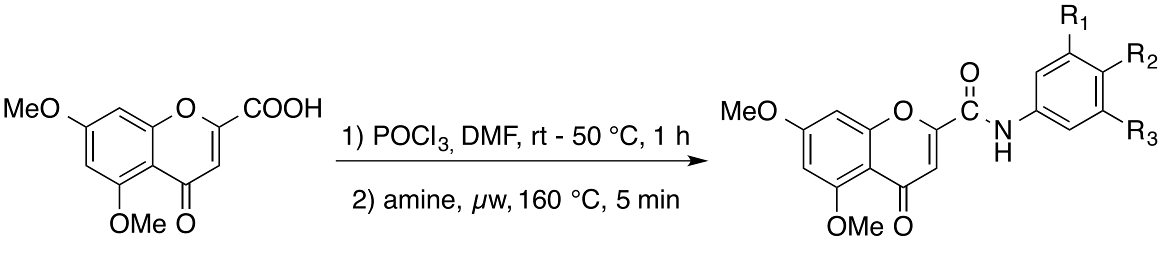
The final cyclisation step was achieved by addition of sulfuric acid to a solution of diacid **40** in acetyl chloride (Scheme 21). Purification afforded the product **41** in 40% yield, confirmed by the presence of the respective peak in the LC-MS chromatogram (*m/z* (ES+) = 297 MH$^+$).



Scheme 21: Synthesis of 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxylic acid 41.

For the formation of aromatic amides from chromone-2-carboxylic acid **41**, a literature search revealed a microwave assisted synthesis by Cagide et al.[109], whereby the acyl chloride was synthesised in situ from acid **41** and $POCl_3$ and then further converted to the carboxamide with the amine. This procedure proved to be viable and the reaction was carried out for different amines, affording the desired carboxamides **42** – **44** in moderate yields (Table 7).

As this set of carboxamides did not show significantly higher inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1) and the solubility proved to be an issue, no further carboxamide analogues were synthesised and the method was not further optimised. Attention was shifted to analogues with different substituents in the 5-position.



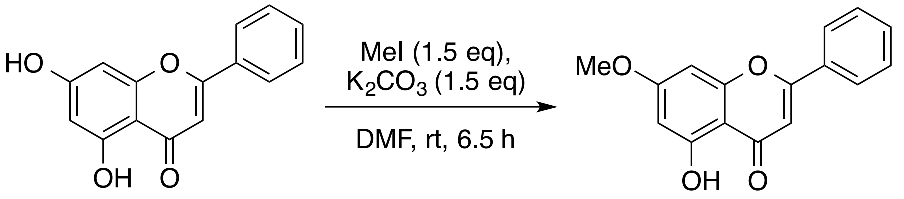
41 **42 - 44**

Entry	Starting material	Product	R_1	R_2	R_3	Yield
1	aniline	**42**	H	H	H	48%
2	3,5-dimethoxyaniline	**43**	OMe	H	OMe	32%
3	*p*-methylaniline	**44**	H	Me	H	44%

Table 7: Synthesis of carboxamides 42 – 44.

3.1.2 Variations of the substituent in the 5-position

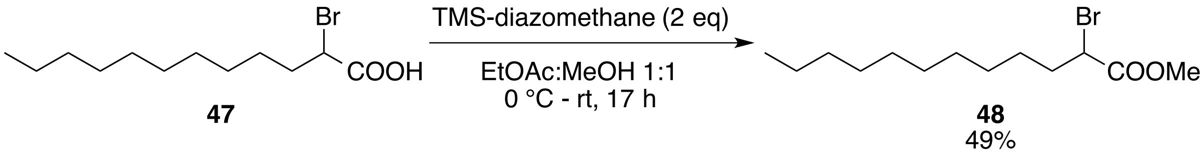
As discussed in Chapter 2.1, replacing the methyl group of the methoxy substituent in the 5-position with α-substituted dodecanoic acid, improves the inhibition activity towards *Am*GSTF1 significantly. To further extend the structure activity relationship investigations, methods were sought to synthesise different acids with a leaving group in the α-position, which could be used in an alkylation reaction with 5-hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one **46**. **46** was easily accessible from chrysin **45** by the selective alkylation of the hydroxyl-group in the 7-position with MeI (Scheme 22), which was described in the literature before.[110] LC-MS showed the formation of product (*m/z* (ES+) = 269 MH^+) with full conversion of starting material and only insignificant amounts of the dialkylated product.



45 **46**
86%

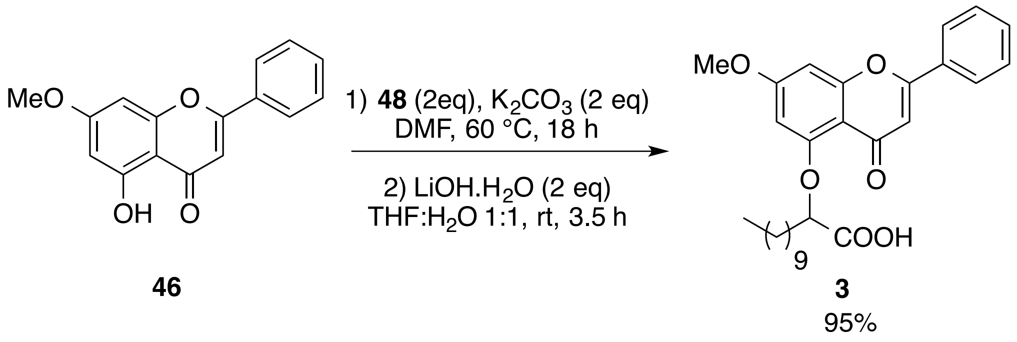
Scheme 22: Synthesis of 5-hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one 46.

3 was synthesised as previously reported by Straker, [85] to use it as a reference in biological testing. Following this precedent, methyl 2-bromododecanoate **48** could be obtained from the commercially available 2-bromododecanoic acid **47** by methylation with TMS-diazomethane in 49% yield (Scheme 23), confirmed by the additional peak in the ^{1}H-NMR spectrum at 3.78 ppm.



Scheme 23: Methylation of acid 47 to form ester 48.

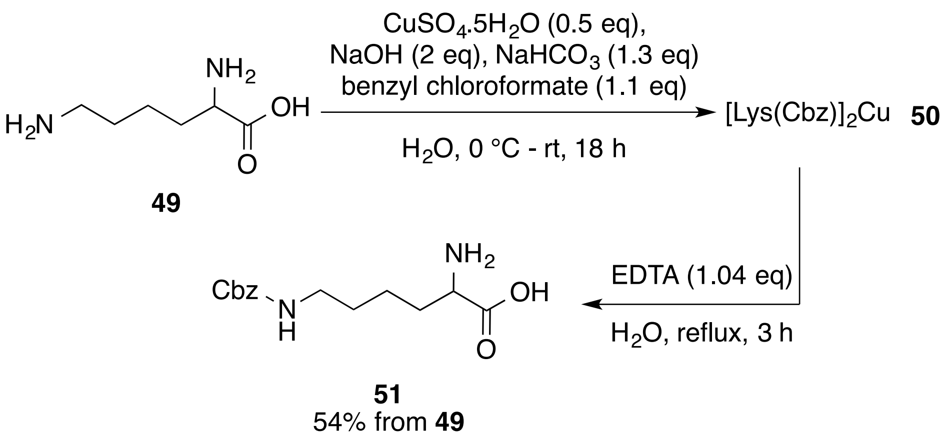
46 was alkylated at 60 °C with methyl 2-bromododecanoate **48** using K_2CO_3 as a base, full conversion was confirmed by the corresponding peak in LC-MS (*m/z* (ES+) = 467 MH^+). Subsequently the crude ester was hydrolysed using $LiOH.H_2O$ in a 1:1 mixture of THF and H_2O (Scheme 24), affording **3** in 95% yield over 2 steps. Successful hydrolysis was verified by disappearance of the methyl signal at ∂ = 3.75 ppm in ^{1}H-NMR.



Scheme 24: Synthesis of 2-(7-methoxy-4-oxo-2-phenyl-4H-chromen-5-oxy)dodecanoic acid 3.

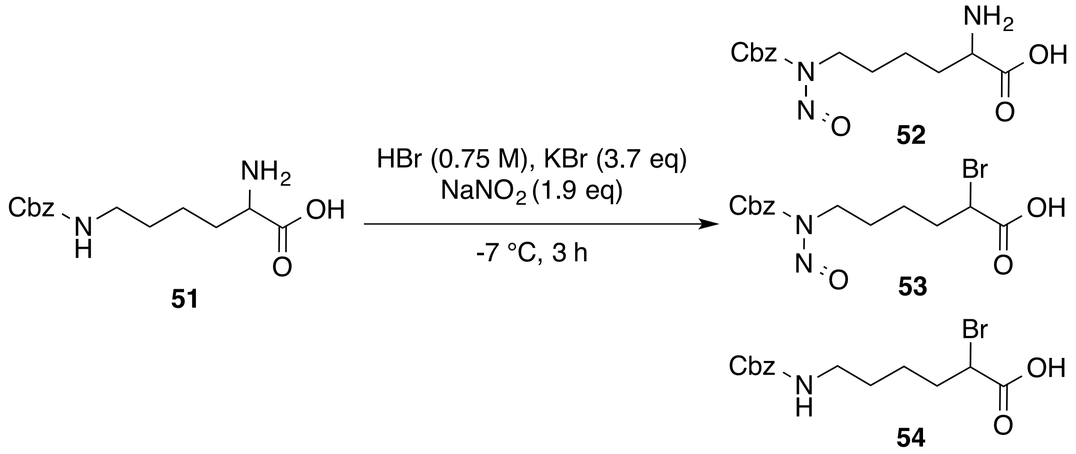
3.1.2.1 Synthesis of α-bromo acids from amino acids

Not many α-bromo acids are commercially available. A well-known method to obtain α-bromo acids is from the corresponding amino acid by diazotization of the amine in the presence of a bromide source.[111-112] Lysine **49** was the amino acid of choice, as it could serve as a building block, offering the ability to elongate the chain by amide coupling with the side chain amino group. Starting from DL-lysine **49**, H-Lys(Cbz)-OH **51** was prepared by a method similar to that described by Balajthy et al.[113] reacting the primary amino and carboxyl group with $CuSO_4$ to form the stable chelate complex, which facilitates the selective protection of the side chain amino-group (Scheme 25). Ethylenediaminetetraacetic acid (EDTA) was used in order to liberate the Cbz-protected amino acid from the copper complex **50**.



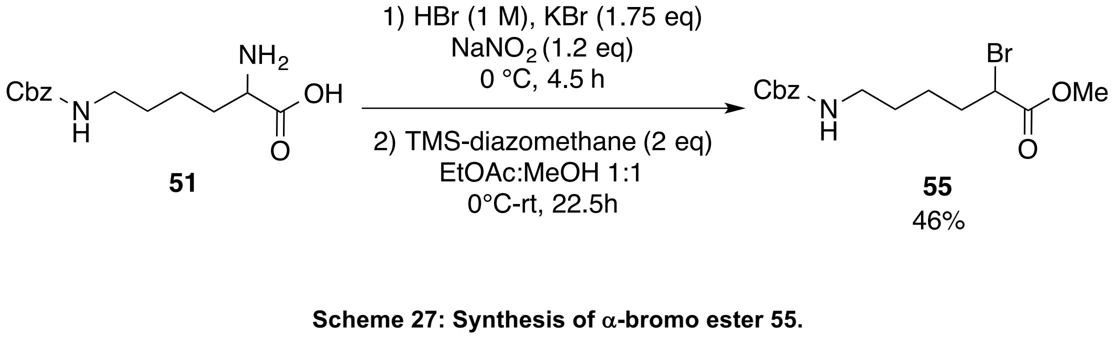
Scheme 25 Cbz-protection of lysine.

Following a procedure by Ellman et al.[112] for amino acids with diverse, protected side chain functionality, KBr, followed by $NaNO_2$, was added to a 0.75 M HBr solution at -7 °C, and after slow addition of **51**, the mixture was stirred for 3 h at -7 °C (Scheme 26). LC-MS analysis showed almost full conversion of the starting material, but in addition to small amounts of the product **54** (m/z (ES^-) = 342 ($[M-H]^-$, Br^{79}), 344 ($[M-H]^-$, Br^{81})), another two peaks with m/z (ES^-) = 371 ($[M-H]^-$, Br^{79}), 373 ($[M-H]^-$, Br^{81}), being the major one, and m/z (ES^-) = 309 $[M-H]^-$ could be observed. The increment of 29 Da compared to the expected mass for the starting material **51** and the desired product **54** respectively, suggested the formation of the nitroso equivalents **52** and **53**. The position of the nitroso-group in compound **52** is somewhat ambiguous, but following the reaction by LC-MS over 5 h, showed that in the beginning mainly nitroso compound **52** was formed which was consequently transformed into **53**. This data confirms the suggested structure for **52**. Compound **53** was isolated and ^{1}H-NMR spectroscopic analysis showed the characteristic downfield shift of the 6-H_2 signal to 3.75 ppm, due to the nitrosamine.



Scheme 26: α-Bromo acid synthesis.

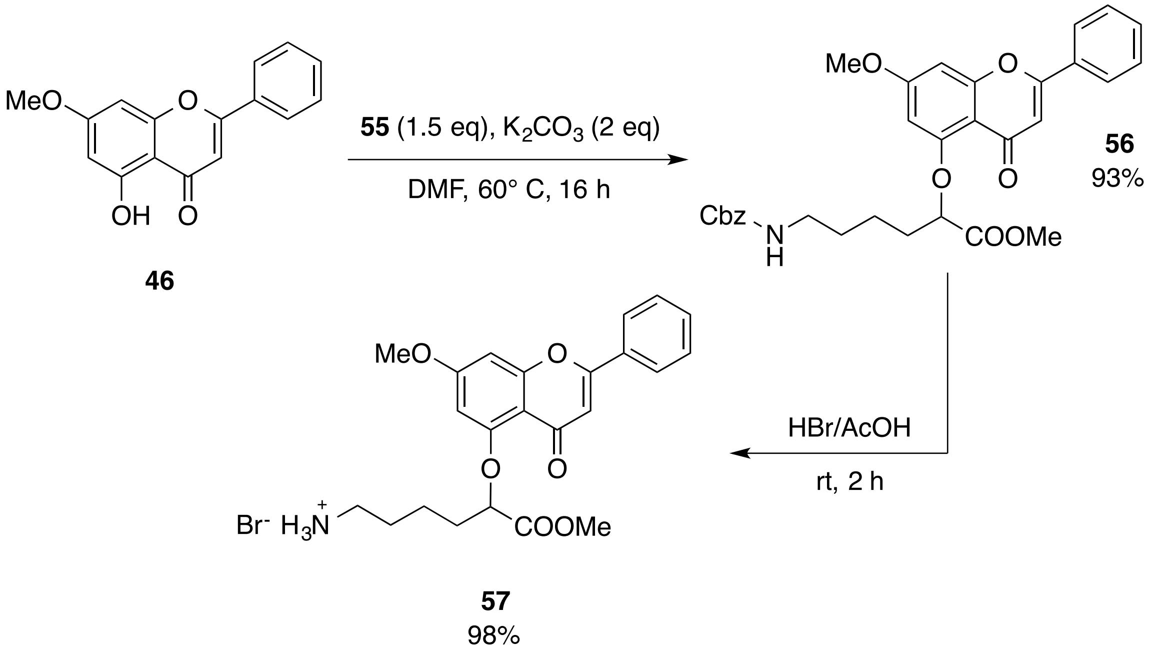
Using the Boc-protected version of lysine, which was previously used by Ellman et al.[112] showed similar results.



Scheme 27: Synthesis of α-bromo ester 55.

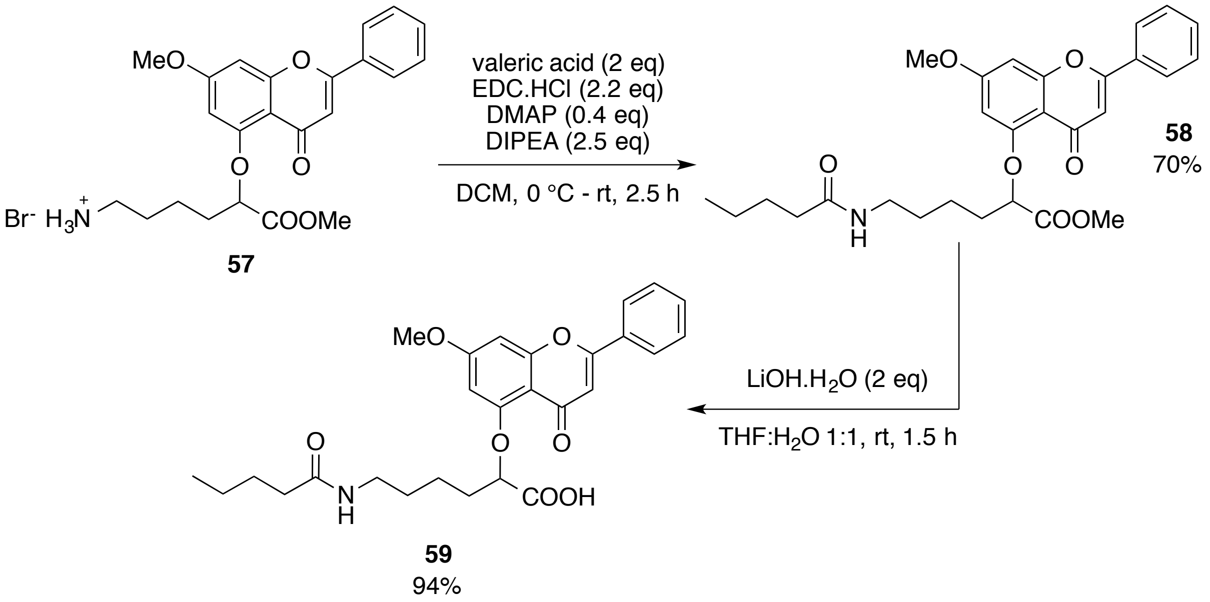
Better yields could be obtained, when the order of addition was changed and conditions previously reported by Angelovski et al.[114] were applied. KBr and H-Lys(Cbz)-OH **51** were dissolved in HBr (1 M). Then $NaNO_2$ was added portionwise at 0 °C over 1 h and the mixture was stirred for 3.5 h at 0 °C. Although **53** could still be observed by LC-MS, α-bromo ester **55** could be obtained in 46% yield over two steps after methylation with TMS-diazomethane, confirmed by the corresponding peak in LC-MS (*m/z* (ES+) = 358 (MH^+, Br^{79}), 360 (MH^+, Br^{81}).

With **55** successfully synthesised, it was used to alkylate 5-hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one **46** with K_2CO_3 as a base in 93% yield (Scheme 28), full conversion was confirmed by LC-MS (*m/z* (ES+) = 546 MH^+). Subsequent deprotection afforded the ammonium bromide salt **57** in 98% yield (Scheme 28).



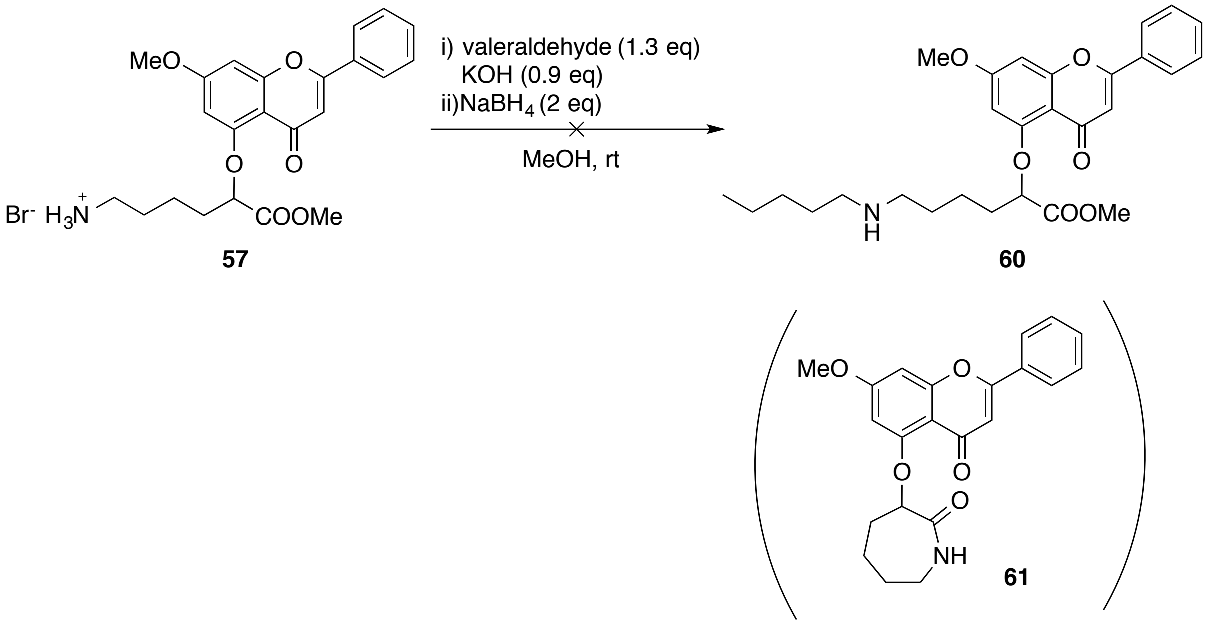
Scheme 28: Synthesis of 6-methoxy-2-(7-methoxy-4-oxo-2-phenyl-4H-chromen-5-oxy)-6-oxo-hexylammonium bromide 57.

In order to maintain a chain length of 10, which would allow to compare the inhibition activity towards *Am*GSTF1 with **3**, **57** was coupled to valeric acid, using standard amide coupling conditions (Scheme 29). LC-MS (*m/z* (ES+) = 496 MH^+) showed full conversion of starting material and product **58** was obtained in 70% yield. Hydrolysis with $LiOH.H_2O$ afforded the final product **59** in 94% yield. Successful hydrolysis was verified by disappearance of the methyl signal in ^1H-NMR.



Scheme 29: Synthesis of 2-(7-methoxy-4-oxo-2-phenyl-4H-chromen-5-oxy)-6-((1-oxo-pentylamino)hexanoic acid 59.

As the amide functionality led to a drop in inhibition activity (*cf.* Chapter 3.2.1.1), attempts were undertaken to elongate the chain by reductive amination. Therefore, the amine salt was stirred in MeOH with KOH and valeraldehyde before adding $NaBH_4$ (Scheme 30).



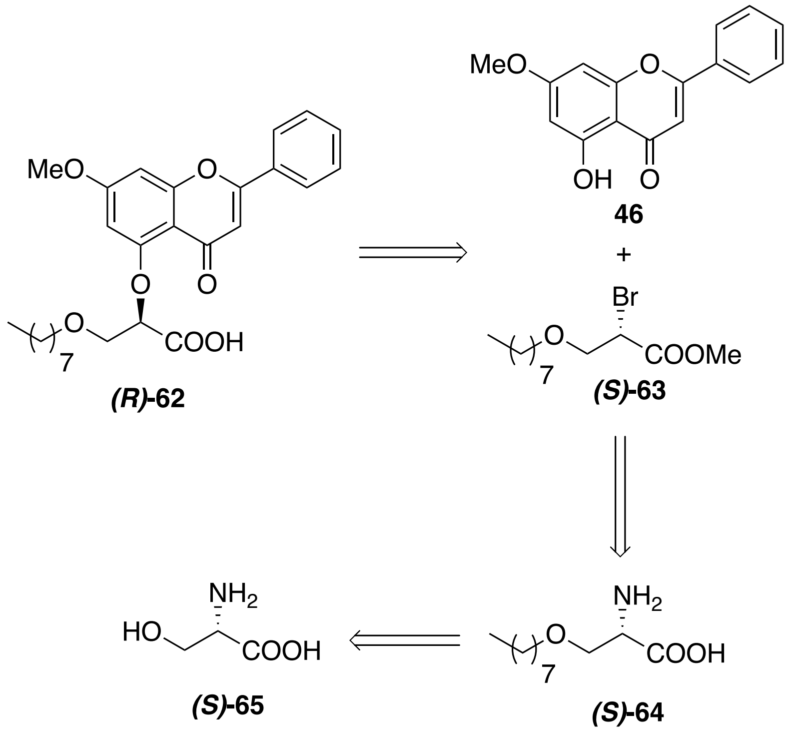
Scheme 30: Reductive amination of 57.

LC-MS analysis showed two main signals, one for starting material **57** (*m/z* (ES+) = 412-MH$^+$) and another peak (*m/z* (ES+) = 380 MH$^+$), whose mass corresponds to the ε-lactam **61**.

Following a one pot procedure by Abdel-Magid et al.,[115] where sodium triacetoxyborohydride was used as a reducing agent , no product was observed either and mainly starting material **57** was detected by LC-MS.

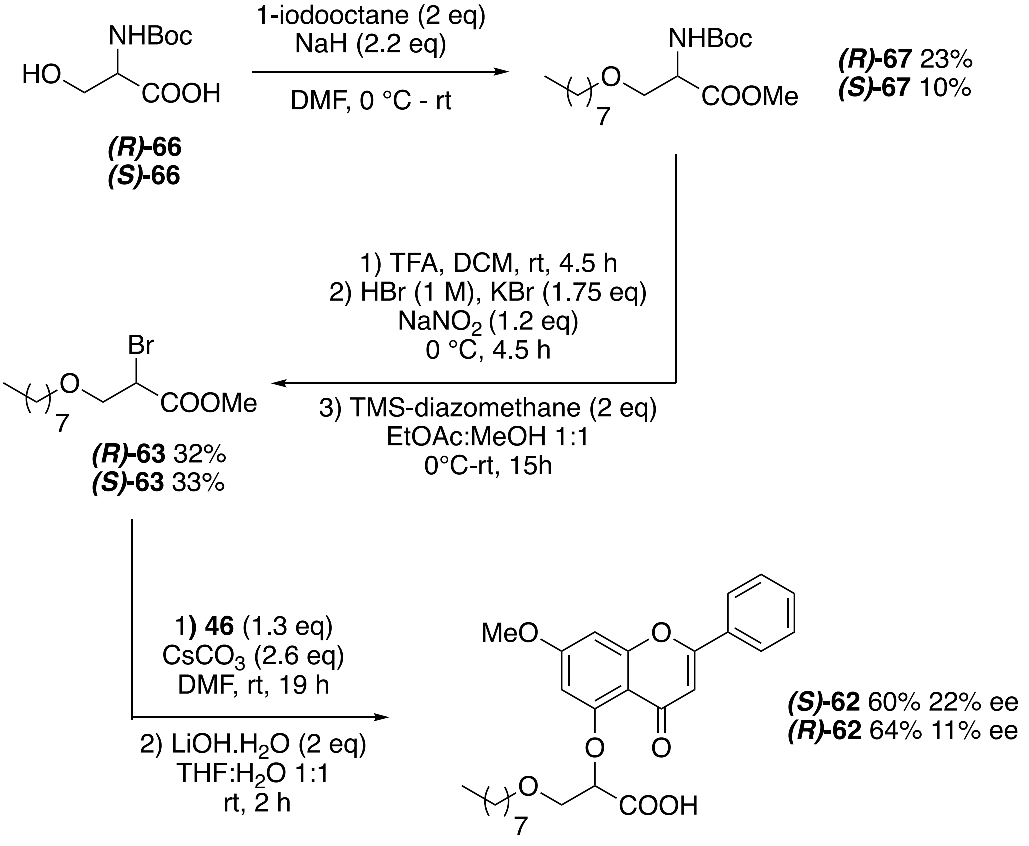
3.1.2.2 Chiral synthesis

2-(7-Methoxy-4-oxo-2-phenyl-4H-chromen-5-oxy)dodecanoic acid **3** has a stereogenic centre in the 2-position and it was of interest to test whether the configuration of the molecule has an effect on the activity. The two enantiomers can either be obtained by chiral synthesis of an analogue or by chiral HPLC-separation of the racemic compound.



Scheme 31: Retrosynthetic analysis of *(R)*-62.

As previously discussed in Chapter 3.1.2.1 α-bromo acids can be obtained from amino acids and if chiral amino acids are used, they can be converted into enantiomerically enriched α-bromo acids with retention of configuration.[111-112] So the analogue ***(R)*-62** can be obtained from L-Serine (Scheme 31).



Scheme 32: Attempt of enantioselective synthesis.

In order to access both enantiomers Boc-D-Ser-OH ***(R)*-66** and Boc-L-Ser-OH ***(S)*-66** were alkylated with 1-iodooctane using NaH as a base, resulting in low conversion and 23% of ***(R)*-67** and 10% of ***(S)*-67** respectively. Boc-Deprotection, bromination and methylation by TMS-diazomethane afforded ***(R)*-63** and ***(S)*-63** in 32% and 33% over three steps. The same conditions as discussed before were applied for the bromination. The final alkylation step with **46** needed some optimization, as surprisingly neither the use of K_2CO_3 nor NaH in dimethylformamide (DMF) gave any product. The reasons for this difference in reactivity compared to the non-oxygenated analogue were not obvious. Ultimately using $CsCO_3$ resulted in product formation confirmed by the corresponding peak in LC-MS (*m/z* (ES+) = 483 MH^+). Hydrolysis with $LiOH.H_2O$ proceeded in full conversion (LC-MS (*m/z* (ES+) = 469 MH^+)) and afforded ***(S)*-62** in 60% and ***(R)*-62** in 64% yield (Scheme 32). Disappointingly, at this step chiral HPLC analysis showed low ee for both ***(S)*-62** and ***(R)*-62** (Figure 40). It is assumed that enantiomeric erosion occurred during the bromination step, but the reasons are not obvious.

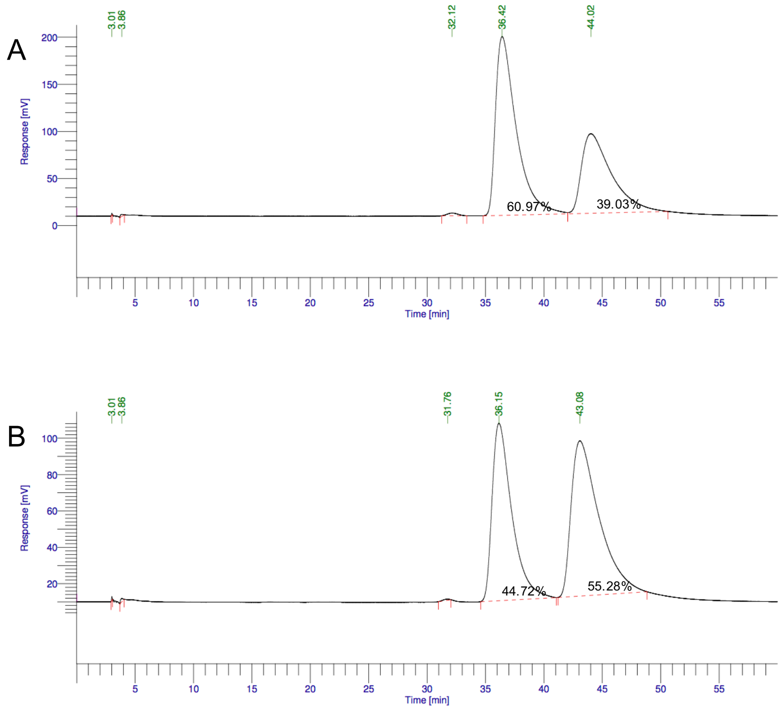
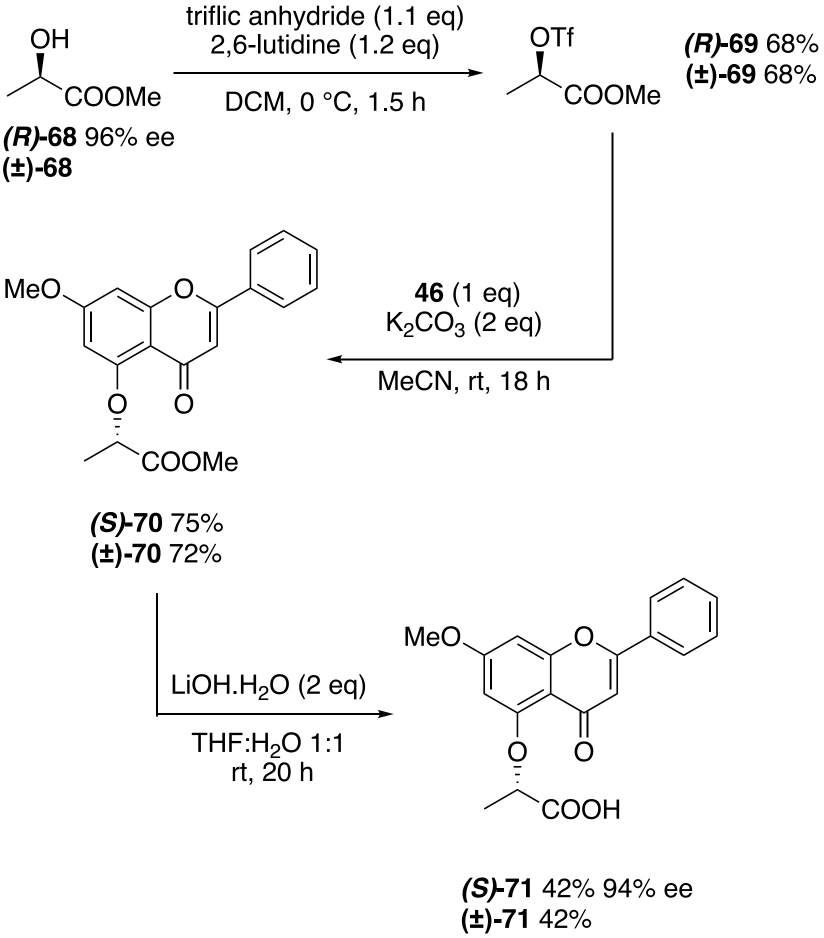


Figure 40: Chiral HPLC analysis of *(S)*-62 (A) and *(R)*-62 (B) using a Daical Chiralpak IA column.

An alternative method to access a chiral analogue was the use of (+)-methyl D-lactate ***(R)*-68** as a chiral starting material (Scheme 33). It could be easily converted into the triflate ***(R)*-69**, using triflic anhydride and 2,6-lutidine, confirmed by the characteristic quartet at 118.6 for CF_3 in the ^{13}C-NMR spectrum. S_N2-type alkylation of **46** with K_2CO_3 or $CsCO_3$ in DMF at 60 °C led to no product formation, but using K_2CO_3 in MeCN at rt and subsequent hydrolysis afforded the product ***(S)*-71** (LC-MS (*m/z* (ES+) = 341 MH^+)) in 42% yield and 94% ee, which was again determined by chiral HPLC (Figure 41).



Scheme 33: Chiral synthesis of R-2-(7'-methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-oxy)propionic acid *(S)*-71.

Following the same steps, 2-(7'-methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-oxy)propionic acid **(±)-71** was prepared from methyl DL-lactate **(±)-68** (Figure 33), in order to have the racemic compound as a comparison in biological testing.

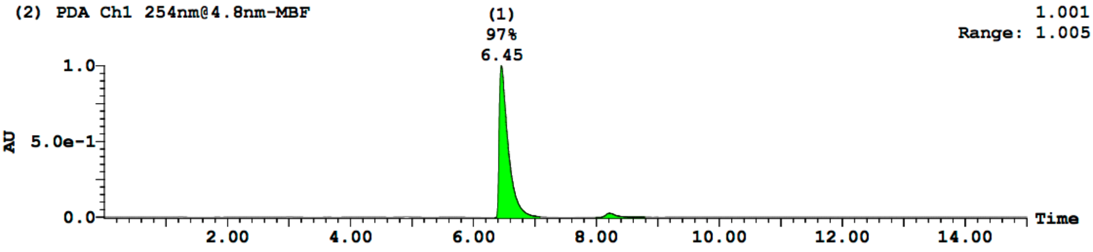
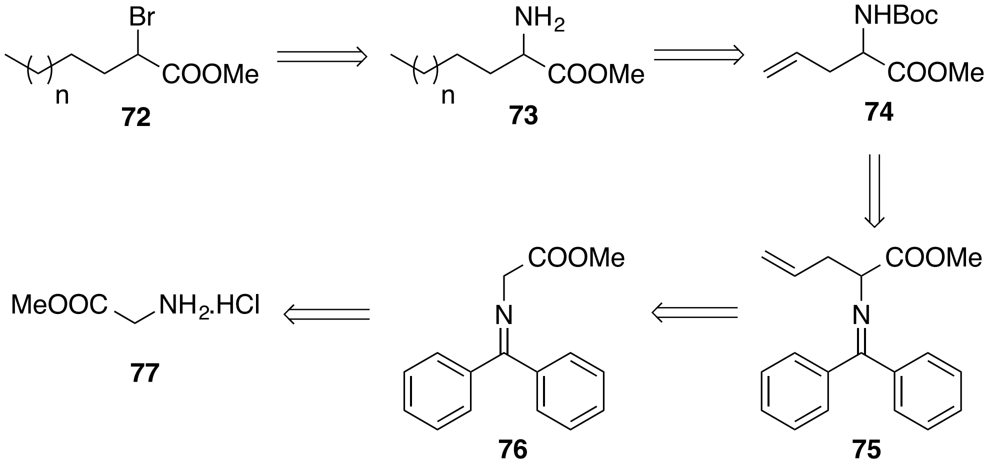


Figure 41: Chiral HPLC analysis of *(S)*-71 using a Daical Chiralpak ID-3 column.

Compounds ***(S)*-71** and **(±)-71** were tested for inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1) and showed an inhibition of 5% ± 4% and 14% ± 3% at a 10 µM level respectively. Although the relative difference in activity is quite big, a conclusion was difficult to draw because of the low absolute inhibition. In order to validate this result, **3** was resolved by chiral HPLC.[116] These two enantiomers were tested as well and showed similar inhibition values (further discussed in Chapter 3.2.1.1). As the configuration of the molecule has, if any, only a very small effect on the activity, the chiral synthesis of analogues was not further pursued.

3.1.2.3 Cross metathesis

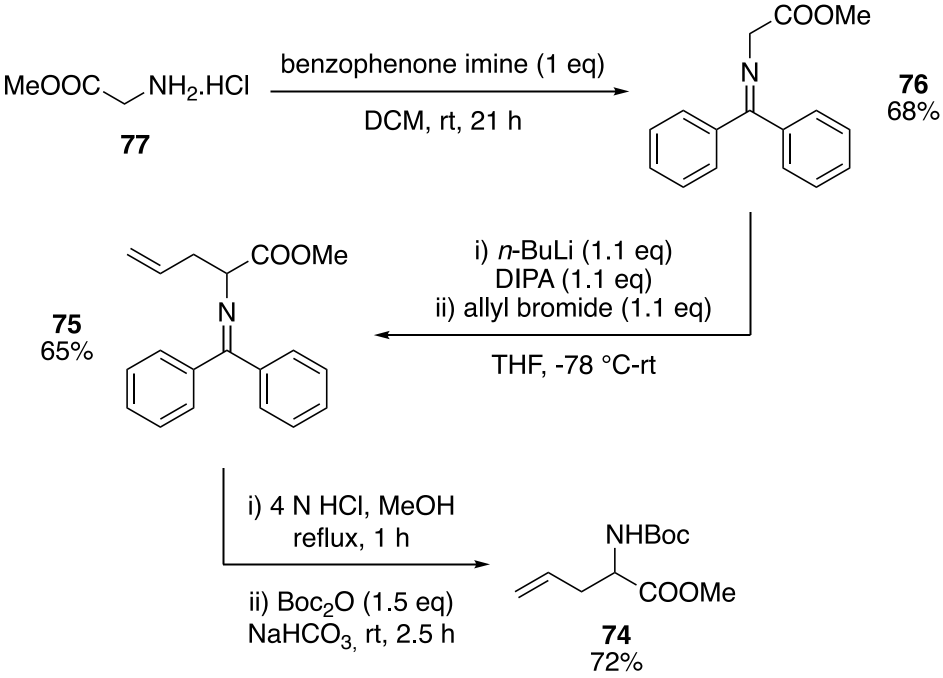
Due to the decrease in activity, which was observed for 2-(7-methoxy-4-oxo-2-phenyl-4H-chromen-5-oxy)-6-((1-oxo-pentylamino)hexanoic acid **59** compared to 2-(7-methoxy-4-oxo-2-phenyl-4H-chromen-5-oxy)dodecanoic acid **3** (*cf.* Chapter 3.1.2.1), methods were sought to synthesise alkanoic acids of varying chain length with a leaving group in the α-position.



Scheme 34: Retrosynthetic analysis of 72.

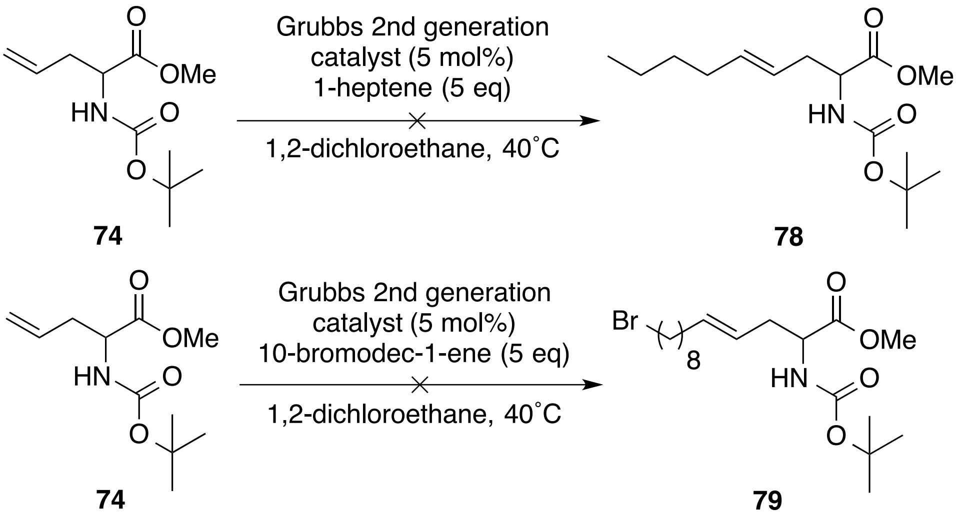
Retrosynthetic analaysis (Scheme 34) suggested that chain elongation could be achieved by cross metathesis of Boc-protected methyl 2-amino-pent-4-enoate **74** with different alkenes, followed by reduction, Boc-deprotection and bromination (described in Chapter 3.1.2.1). **74** can be obtained by alkylation of the benzophenone imine of glycine **76** and subsequent imine hydrolysis and Boc-protection. Asymmetric alkylation methods for **76** are established by Corey, Lygo and others, [117-120] so this method could be potentially used for a chiral synthesis as well.

Following a literature procedure, glycine methyl ester hydrochloride **77** was stirred in DCM with benzophenone imine to afford **76** in good yield. The Schiff base was alkylated with allylbromide using LDA as a base and Boc-protected methyl 2-amino-pent-4-enoate **74** was obtained by imine hydrolysis with HCl and Boc-protection using Boc_2O under basic conditions (Scheme 35).[121]



Scheme 35: Synthesis of Boc-protected methyl 2-amino-pent-4-enoate 74.

With **74** in hand, standard olefin cross metathesis conditions[122] were applied to elongate the carbon chain with 1-heptene and 10-bromodec-1-ene respectively, the bromo-group of which could be used for further functionalizations (Scheme 36). However, no product formation could be observed in either reaction. This might be due to chelation by the carbonyl group to the ruthenium, sequestering the catalyst in an unproductive form. Fürstner et al. reported such chelation for γ,δ-unsaturated carbonyl compounds. [123-124]

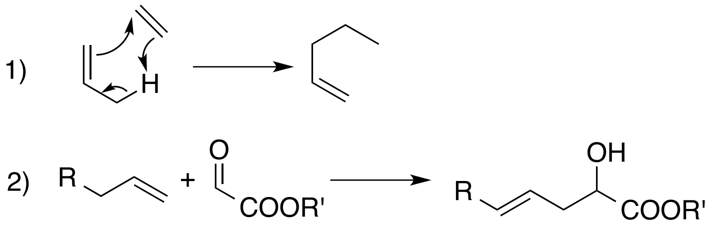


Scheme 36: Cross metathesis.

Attempts to alkylate Schiff base **76** with non-allylic alkyl bromides were not successful. As this synthesis route did not seem to be viable, alternative ways were pursued.

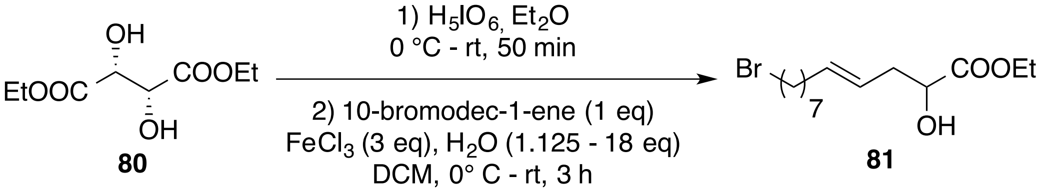
3.1.2.4 Ene-reaction

The ene-reation is a useful synthetic method for carbon-carbon bond formation. It refers to the reaction of an alkene with an allylic hydrogen (ene) with a compound containing a double or triple bond (enophile) to form a new bond with migration of the ene double bond by 1,5-hydrogen shift (Scheme 37,1)). Lewis acids can accelerate the reaction for enophiles containing basic groups through complexation.[125] If glyoxylate is used as an enophile, α-hydroxy acids can be obtained (Scheme 37, 2)), the hydroxyl-group of which could be transformed into a leaving group by triflation.



Scheme 37: Ene-reaction.

Using similar conditions to those reported by Agouridas et al.[126], ethyl glyoxalate was reacted with 10-bromodec-1-ene and an excess of ferric chloride (3 eq) in DCM. Ethyl glyoxalate was obtained from diethyl L-tartrate **80** by treatment with periodic acid[127] and used in the next step without further purification. Formation of ethyl 12-bromo-2-hydroxy-dodec-4-enoate **81** was confirmed by GC-MS (*m/z* = 320 (M^+, Br^{79}), 322 (M^+, Br^{81})), but the reaction gave unreliable results, which depended on the batch of ferric chloride used.



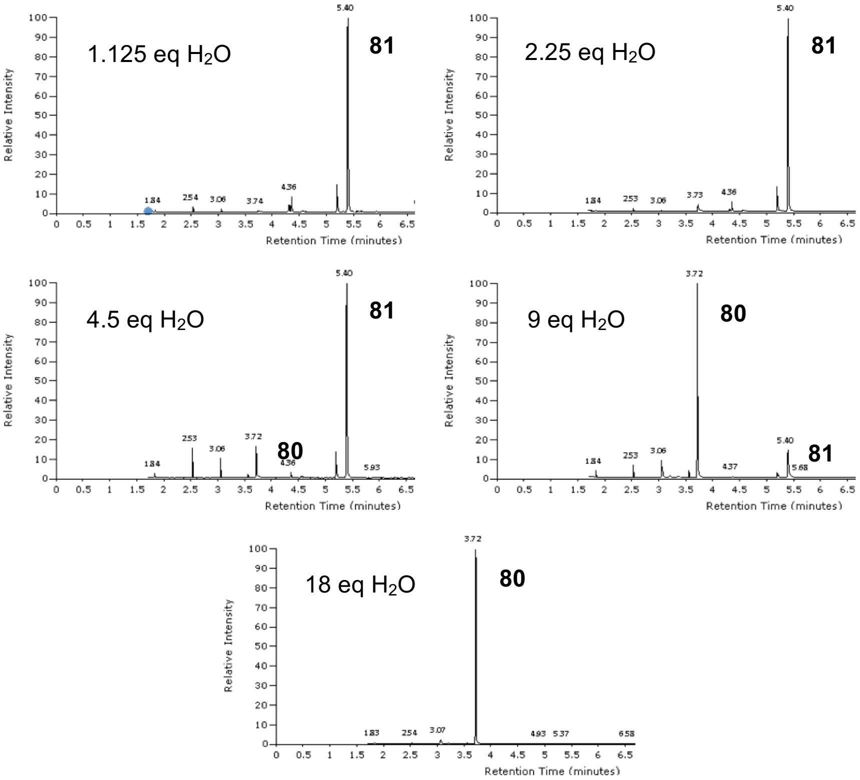
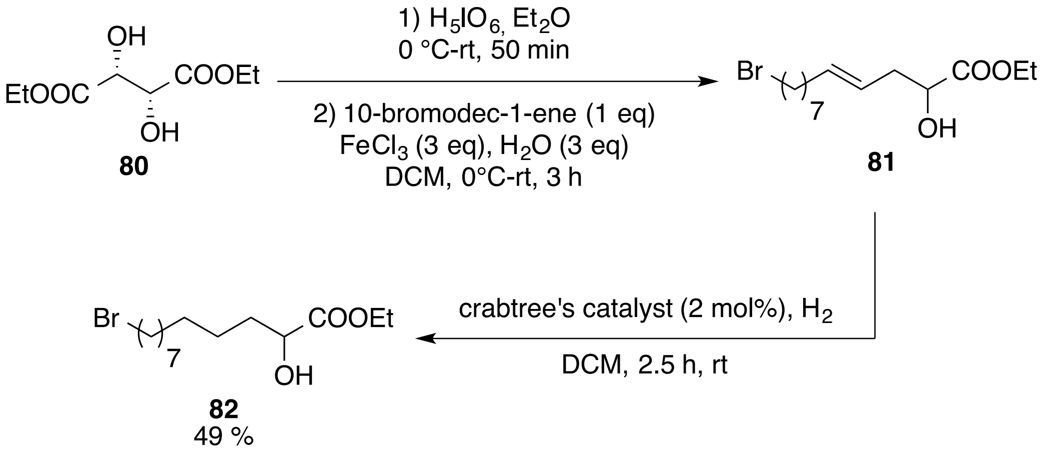


Figure 42: GC spectra of ene reaction using different equivalents of H_2O.

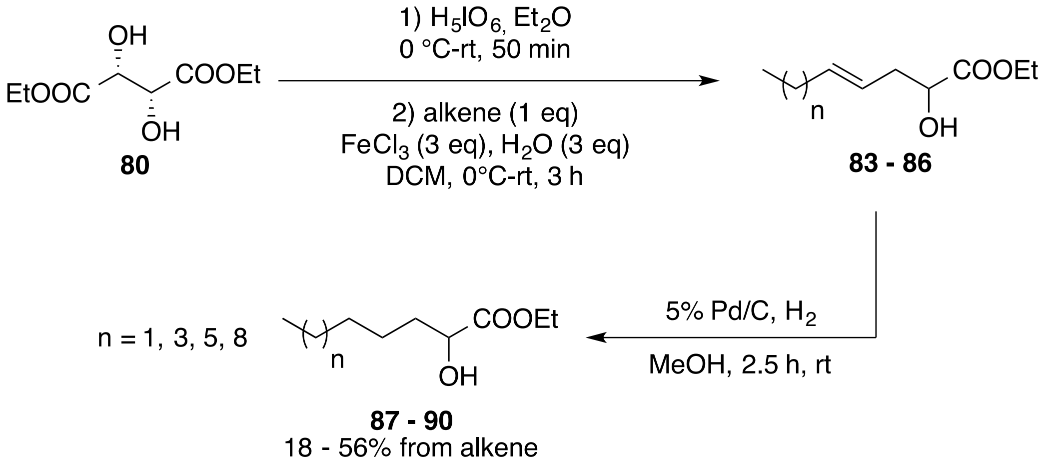
As ferric chloride is very hygroscopic, the influence of H_2O on the reaction was investigated. The reaction was conducted under an argon atmosphere using newly purchased anhydrous ferric chloride. No reaction could be observed for anhydrous ferric chloride alone. When 1.125 or 2.25 eq of H_2O were added to the reaction, GC-MS analysis showed full conversion of starting material to product, but for 4.5 and 9 eq only partial conversion and for 18 eq H_2O no reaction was observed (Figure 42), indicating that the fully hydrated $FeCl_3.6H_2O$ is not accelerating the reaction. The use of 3 eq of H_2O proved to give good results and was applied in all subsequent ene reactions.

With **81** successfully synthesised, methods to reduce the double bond were sought. As expected, 10% Pd/C under hydrogen atmosphere in methanol, not only reduced the alkene, but also led to some debromination. Using the more selective, homogeneous hydrogenation catalyst, Wilkinson's catalyst ($RhCl(PPh_3)_3$)[128] under hydrogen atmosphere in toluene gave almost full conversion of **81** to **82** (GC-MS (*m/z* = 322 (M^+, Br^{79}), 324 (M^+, Br^{81})), but a very high catalyst loading of 30 mol% was necessary. Changing to the more active cationic Crabtree's catalyst ($[Ir(cod)(PCy_3)(py)]PF_6$)[129] under hydrogen atmosphere in DCM resulted in full conversion from **81** to **82** with a catalyst loading of only 2 mol% and **82** could be afforded in 49% yield over three steps (Scheme 38). **82** was subsequently used for the synthesis of photoaffinity label **160** (*cf.* Chapter 3.1.4).



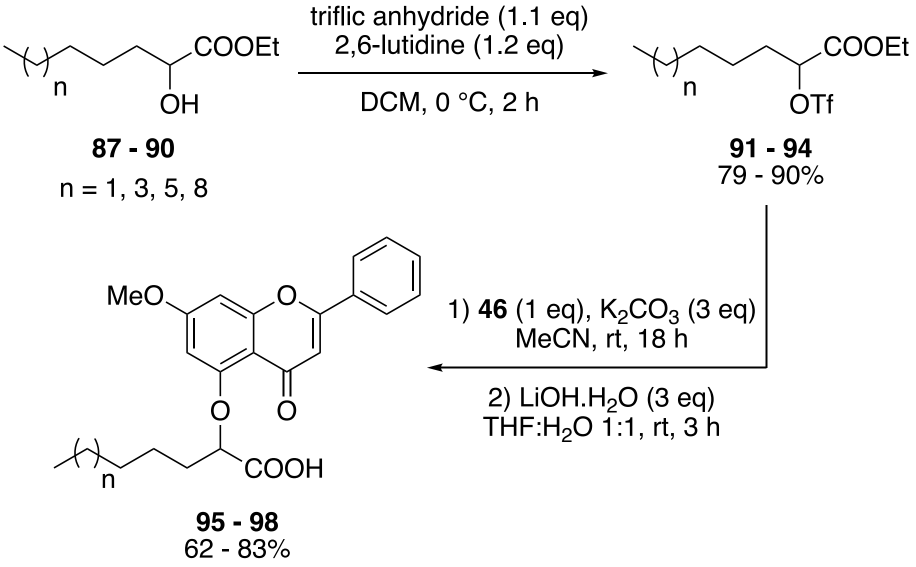
Scheme 38: Synthesis of ethyl 12-bromo-2-hydroxy-dodecanoate 82.

In order to investigate the influence of the alkyl chain length on the inhibition activity towards *Am*GSTF1, α-hydroxy acids with 5, 7, 9 and 12 carbons were synthesised, following the same steps, but using Pd/C for the hydrogenation (Scheme 39).



Scheme 39: Synthesis of 87 – 90.

Ethyl 2-hydroxy-tetradecanoate **90** could be afforded in a good yield of 56% over 3 steps, but the yield dropped for ethyl 2-hydroxy-undecanoate **89**, ethyl 2-hydroxy-nonanoate **88** and ethyl 2-hydroxy-heptanoate **87** with 31, 20 and 18% respectively, which probably can be attributed to the volatility of pentene, heptane and nonene.



Scheme 40: Synthesis of 95 – 99.

The α-hydroxy acids **87** – **90** were then used to synthesise the flavonoid analogues **95** – **98** in good yields (Scheme 40), applying the same reaction conditions as described before (*cf.* Chapter 3.1.2.2). **87** – **90** were converted into the respective triflate analogue, using triflic anhydride and 2,6-lutidine, confirmed in each case by the characteristic quartet around 118.6 for CF_3 in the ^{13}C-NMR spectrum. Subsequent alkylation and hydrolysis afforded the products **95 – 98** in good to excellent yield.

The analogues showed an increase in inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1) with an increase of the alkyl chain length.

3.1.3 Improving Aqueous Solubility

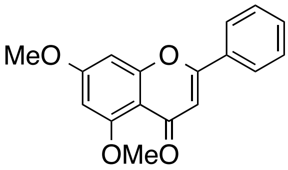
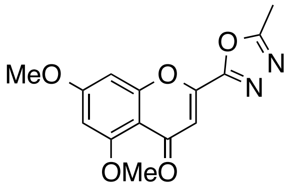
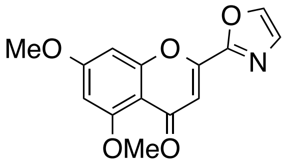
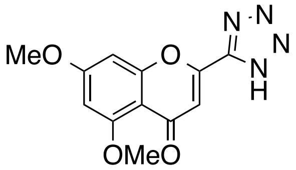
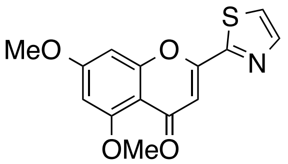
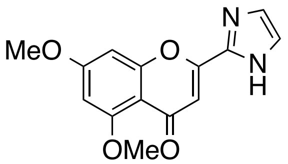
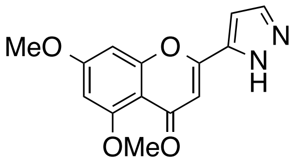
Improving the aqueous solubility of the flavonoid compounds became an important issue of this project and different approaches are discussed below.

Similar ranges of simple physicochemical parameters (molecular mass, logP, hydrogen-bond donors and acceptors, rotatable bonds), which have been correlated with oral bioavailability of drugs by Lipnski's rule of 5[130], apply to the bioavailability of agrochemicals in plants. The most significant difference is that agrochemicals have a lower number of hydrogen bond donors.[131-132] Lipinski's rule of 5 indicates that logP, the logarithm of the octanol/water partition coefficient, should be ≤ 5, **3**, however, has an estimated logP of 7.1 and it's aqueous solubility is too low to detect.[133]

Four different approaches to potentially improve the aqueous solubility were chosen. The phenyl group was replaced by a variety of different heterocycles and an SO_2Me-substituent, which can lower the lipophilicity of a compound, was incorporated. Furthermore, substituents were introduced to possibly change the overall planar structure of **16** and disrupt π-π stacking interactions and attempts were undertaken to replace the long alkyl chain with a polyethylene glycol (PEG)-like chain.

3.1.3.1 Heterocyclic analogues

In order to improve the logP value, analogues of **16** with a variety of different heterocycles in the 2-position were synthesised, focusing on those for which initial predictions, using the ChemAxon program Instant JChem, had shown a lowering of logP values (Table 8). Even though these predicted logP values are only an approximation, the decrease in comparison to the predicted logP value of **16** can serve as a guideline.

Entry	Compound	Compound Number	Predicted logP
1		16	2[a]
2		99	0
3		100	0
4		101	0
5		102	1
6		103	0
7		104	1

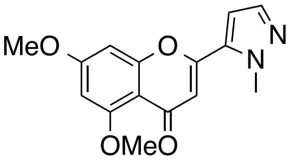
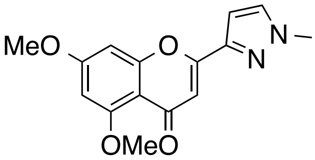
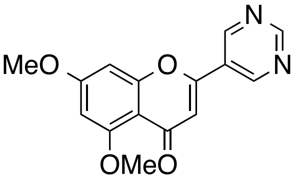
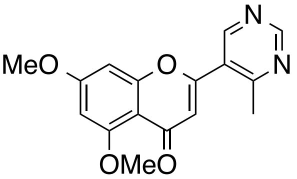
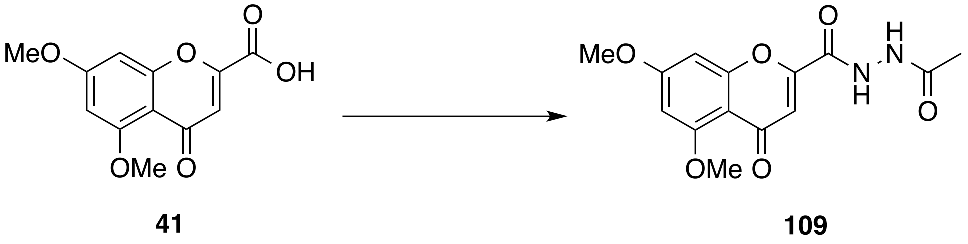
8		105	0
9		106	1
10		107	0
11		108	0

Table 8: Predicted logP values using the ChemAxon program Instant JChem;
[a] Measured logP of 3.19[133].

Retrosynthetic analysis showed, that analogues **99**, **100**, **101** and **102** could all be traced back to 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxylic acid **41**. The 1,3,4-oxadiazole analogue **99** could be obtained by cyclisation of the carboxylic acid, 2-acetylhydrazide **109**. Initially synthesis of **109** was attempted via formation of the acid chloride intermediate and subsequent addition of acethydrazide (Table 9, Entries 1 - 5). Heating acid **41** in thionyl chloride with a few drops of DMF at reflux before concentrating the reaction mixture and adding acethydrazide, led to decomposition of the starting material and no product could be detected by LC-MS (Table 9, Entry 1). Using the much milder oxalyl chloride in DCM instead, gave a very low conversion (Table 9, Entry 2), probably due to the low solubility of **41** in DCM. The same conditions used in Chapter 3.1.1.3 for the synthesis of carboxamides (Table 9, Entry 3), afforded a complex mixture of the desired product **109**, cyclised product **99** and side products, the most abundant being *N,N*-dimethyl 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide, which forms due to decomposition of the solvent DMF. Attempts to drive the reaction towards cyclised product **99** using different temperatures and reaction times were unsuccessful, as *N,N*-dimethyl 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide was the main product.

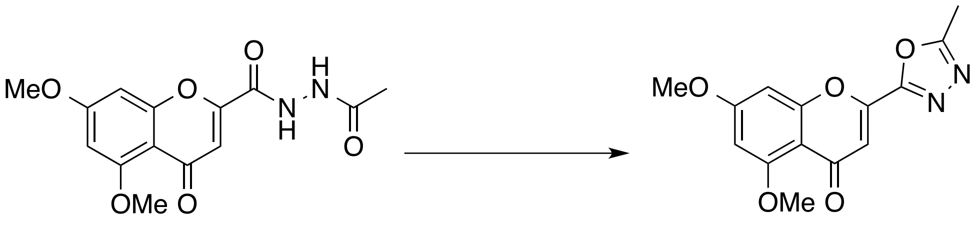


Entry	Conditions acid chloride formation	Conditions hydrazide formation	Yield
1[a]	$SOCl_2$ (20 eq), DMF (cat) reflux, 3 h	acethydrazide (1.2 eq) *N*-methylmorpholine (1 eq) DCM, rt, 18 h	decomposition[b]
2[a]	oxalyl chloride (5 eq) DMF (cat) DCM, 0 °C - rt, 4 h	acethydrazide (1.2 eq) *N*-methylmorpholine (1 eq) DCM, rt, 18 h	low conversion[b]
3	$POCl_3$ (1 eq), DMF rt – 50 °C, 1 h	acethydrazide (1 eq) µw, 160°C, 5 min	Complex mixture of **109**, cyclised product **99** and side products[b]
4	$POCl_3$ (1 eq), DMF rt – 50 °C, 1 h	acethydrazide (1 eq) rt, 18 h	71%[c]
5	$POCl_3$ (1 eq), DMF (cat) DCM, rt – 35 °C, 3 h	acethydrazide (1 eq) rt, 18 h	low conversion[b]
6	-	PyBOP (1.1 eq), DIPEA (2.2 eq) acethydrazide (1.1 eq) DMF, rt, 18 h	71%

Table 9: Hydrazide synthesis. a reaction mixture concentrated after 1. step; b Analysis by LC-MS; c 0.25 mmol scale, more side products on larger scale.

At small scale, **109** could be obtained in good yield, as confirmed by the presence of the corresponding peak in the LC-MS chromatogram (*m/z* (ES+) = 307 MH^+), when acethydrazide was added to the acid chloride at rt and stirred overnight (Table 9, Entry 4). If the reaction was conducted at large scale, more side products formed, which made purification difficult. Using DCM as a solvent and only catalytic amounts of DMF for the acid chloride formation with $POCl_3$ resulted in a very low conversion (Table 9, Entry 5), again probably due to the low solubility of **41** in DCM. Alternatively classic amide coupling conditions[134] were applied, with (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) as the coupling reagent (Table 9, Entry 6). **109** was obtained in 71% yield (LC-MS (*m/z* (ES+) = 307 MH$^+$)).

With **109** in hand, different cyclisation conditions were adopted. There are several methods for the cyclodehydration of diacylhydrazines[135], one of the most popular is the use of $POCl_3$ as a dehydrating agent.[136] However, stirring **109** with $POCl_3$ at 80 °C overnight, led to decomposition of the starting material (Table 10, Entry 1).



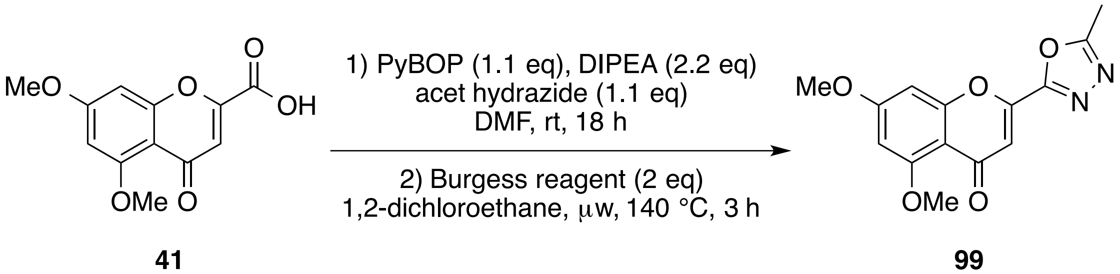
109 **99**

Entry	Conditions	Yield
1	$POCl_3$ (15 eq) 80 °C, 18 h	decomposition[a]
2	tosyl chloride (5.25 eq) pyridine, 80 °C, 18 h	9%
3	triflic anhydride (1.5 eq) PH_3PO (3 eq), DCM rt – 50 °C, 20 h	Low conversion[a]
4	Burgess reagent (1.5 eq) THF, μw, 110 °C, 3.5 h	21%

Table 10: 1,3,4-Oxadiazole synthesis; a Analysis by LC-MS.

Using tosyl chloride/pyridine[137] instead, showed full conversion of **109** to **99** in LC-MS (*m/z* (ES+) = 289 MH$^+$), but **99** could be obtained in only 9% yield after purification (Table 10, Entry 2). Cyclising **109** with triflic anhydride and Ph_3PO,[138] resulted in some product formation, observed by LC-MS, but even after 20 h, only low conversion (Table 10, Entry 3). Using the dehydrating agent Burgess reagent (Methyl *N*-(triethylammoniosulfonyl)-

carbamate) under microwave conditions for the cyclisation,[139] gave **99** in 21% yield (Table 10, Entry 4). Changing the solvent to 1,2-dichloroethane and using a higher temperature afforded **99** in 36% yield over two steps (Scheme 41).

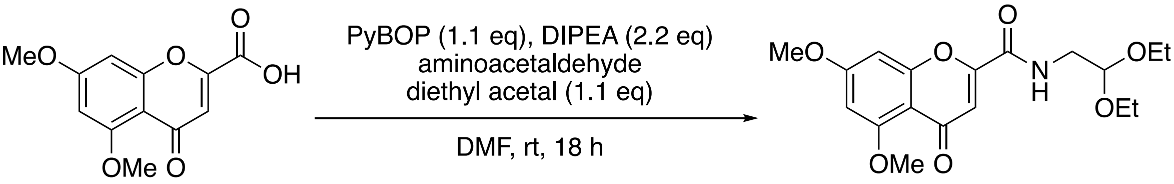


41

99
36%

Scheme 41: Synthesis of 5,7-dimethoxy-2-(5'-methyl-1',3',4'-oxadiazol-2'-yl)-4H-chromen-4-one 99.

A literature review revealed that oxazoles can be directly obtained from the corresponding *N*-(2,2-dimethoxyethyl)-carboxamide using Eaton's reagent (phosphorus pentoxide, 7.7 wt% in methanesulfonic acid).[140]

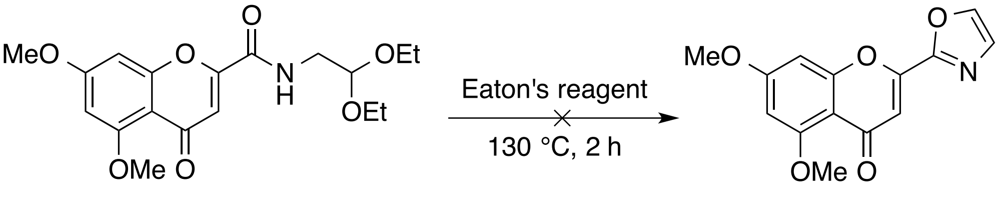


41

110
71%

Scheme 42: Synthesis of *N*-(2',2'-diethoxyethyl)-5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide 110.

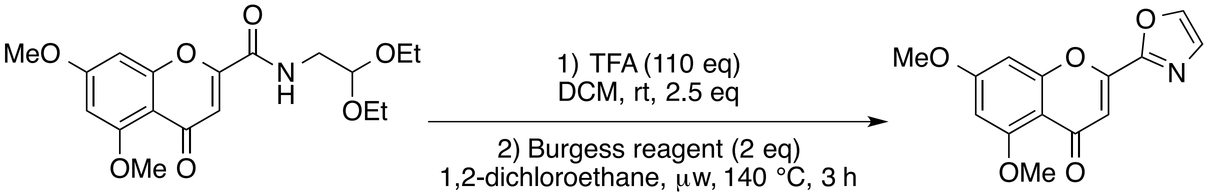
Therefore, *N*-(2',2'-diethoxyethyl)-5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide **110** was synthesised using the same coupling conditions as for **109** (Scheme 42) (LC-MS (*m/z* (ES+) = 366 MH^+)). **110** was then heated with Eaton's reagent to 140 °C (Scheme 43). LC-MS analysis showed full conversion of the starting material, but in addition to small amounts of the product (*m/z* (ES+) = 274 MH^+), another two peaks with (*m/z* (ES+) = 251 MH^+) and (*m/z* (ES+) = 250 MH^+), as the major one, could be observed, probably corresponding to the acid **41** and the primary amide, respectively.



110 **100**

Scheme 43: Attempt of oxazole synthesis using Eaton's reagent.

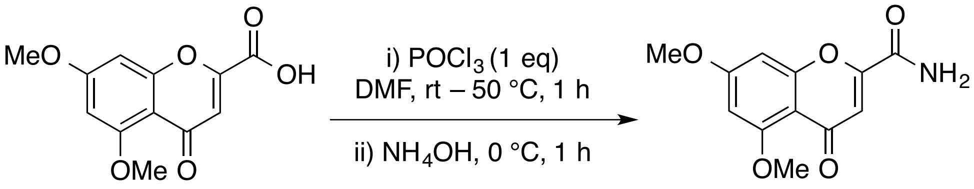
As Burgess reagent was successfully used in the synthesis of oxadiazole **99**, acetal **110** was deprotected using trifluoroacetic acid (TFA) and directly cyclised applying the same conditions (Scheme 44). Oxazole **100** was obtained in 28 % yield, confirmed by the corresponding peak in the LC-MS (*m/z* (ES+) = 274 MH^+) spectrum.



110 **100** 28%

Scheme 44: Synthesis of 5,7-dimethoxy-2-(1',3'-oxazol-2'-yl)-4H-chromen-4-one 100.

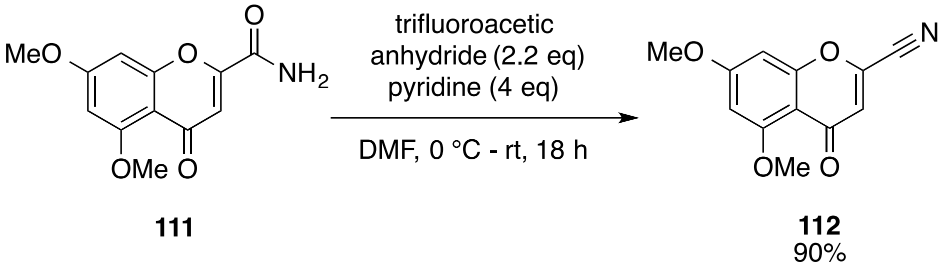
Nitriles can act as a starting material for both tetrazoles[141] and thiazoles[142] and can themselves be synthesised from the corresponding carboxamide. Carboxamide **111** was obtained, by slowly dropping the in situ synthesised acid chloride into 35% NH_4OH, in 73% yield (LC-MS (*m/z* (ES+) = 250 MH^+)) (Scheme 45).



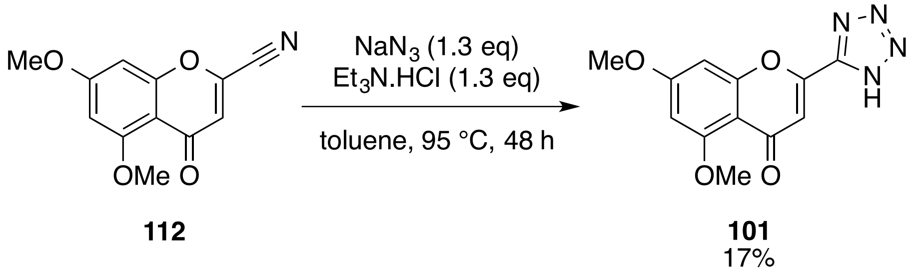
41 **111** 73%

Scheme 45: Synthesis of 5,7-dimethoxy-4H-chromen-4-one-2-carboxamide 111.

111 could be converted into nitrile **112** using trifluoroacetic anhydride and pyridine.[143] The product was obtained in excellent yield and formation was confirmed by the appearance of a peak in the LC-MS chromatogram (*m/z* (ES+) = 232 MH$^+$).



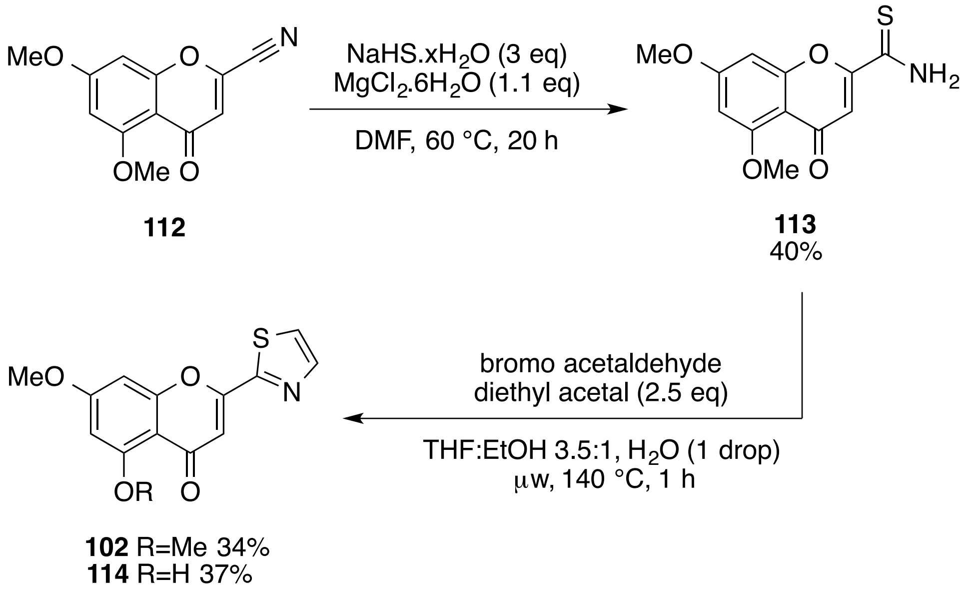
Scheme 46: Synthesis of 5,7-dimethoxy-4H-chromen-4-one-2-carbonitrile 112.



Scheme 47: Synthesis of 5,7-dimethoxy-2-(1H-1’,2’,3’,4’-tetrazol-5’-yl)-4H-chromen-4-one 101.

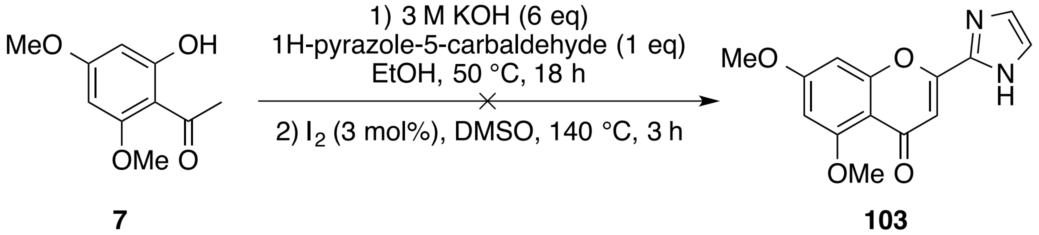
Tetrazole **102** (LC-MS (*m/z* (ES+) = 275 MH$^+$)) was afforded by the reaction of sodium azide with nitrile **101**, in the presence of an ammonium salt (Scheme 47).[141]

The thiazole ring of compound **102** was assembled via Hantzsch thiazole synthesis. The thioamide precursor **113** was prepared from nitrile **112**. Following a literature procedure[142], addition of sodium hydrogen sulfide and magnesium chloride to a solution of nitrile **112** in DMF readily afforded thioamide **113**, albeit heating was required. As suggested by Käsnänen et al.,[144] reacting thioamide **113** with bromoacetaldehyde diethyl acetal under microwave conditions afforded thiazole **102** (LC-MS (*m/z* (ES+) = 290 MH$^+$)) (Scheme 48).[142] Addition of small amounts of H_2O improved the conversion significantly. A mixture of THF and EtOH was used as a solvent, in order to reach a higher reaction temperature in the microwave, which also improved the conversion of the starting material. In addition to the desired product **102**, the demethylated thiazole **114** (LC-MS (*m/z* (ES+) = 276 MH^+)), could be isolated in 37% yield. Stereochemistry was confirmed in 2D-NMR. The HMBC experiment showed a correlation between the C-4a, the C-5 and the C-6 carbon signal and the signal for the OH-group.



Scheme 48: Synthesis of 5,7-dimethoxy-2-(1',3'-thiazol-2'-yl)-4H-chromen-4-one 102.

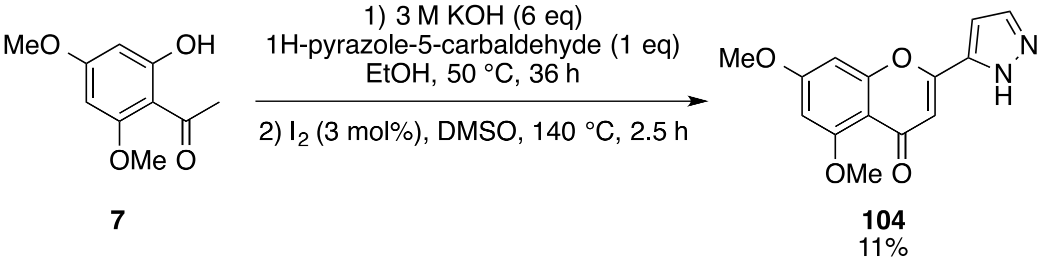
Another method to obtain 4H-chromen-4-ones with various substituents in the 2-position, is to prepare them from the corresponding hydroxychalcones.[90, 145]



Scheme 49: Attempted synthesis of pyrazole 103.

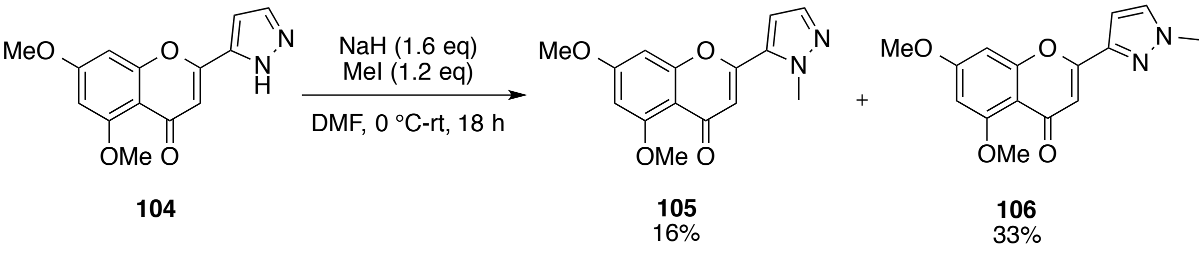
In order to afford the hydroxychalcone, acetophenone **7**, together with 1H-pyrazole-5-carbaldehyde, was stirred in 3 M KOH in ethanol. No reaction occurred at rt, but at 50 °C product was formed in an aldol condensation (Scheme 49), confirmed by both LC-MS (*m/z* (ES+) = 275 MH^+) and ^{1}H-NMR analysis with the two characteristic doublets at 8.03 and 7.55 ppm corresponding to the two alkene protons. No product could be obtained from the oxidative cyclisation reaction with I_2 in dimethyl sulfoxide (DMSO). The main product observed by LC-MS (*m/z* (ES+) = 275 MH^+), which was also confirmed by ^{1}H-NMR, was the flavanone.

Using the same conditions with 1H-pyrazole-5-carbaldehyde, product **104** (LC-MS (*m/z* (ES+) = 273 MH^+)) was obtained in 11% yield over the two steps. The rather low yield is due to a low conversion in the first step.



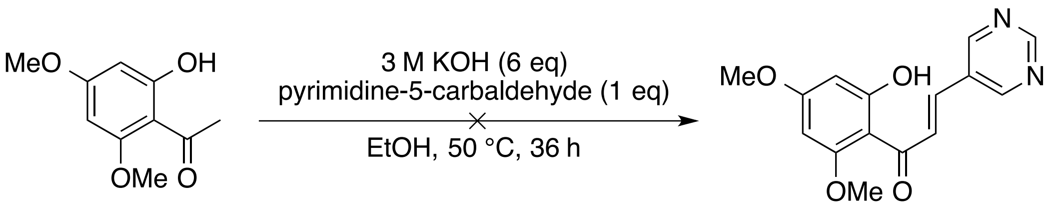
Scheme 50: Synthesis of pyrazole 104.

To see how an alkylation might affect the activity, **104** was methylated with MeI, using NaH as a base to afford the two isomers **105** and **106** in 16 and 33% yield respectively (Scheme 51). The constitutional isomers were characterized by 2D-NMR. A correlation between the signal for the proton in the 3-position and the signal for the NCH_3-group in the NOESY experiment for **105** confirmed the structure of the isomer.



Scheme 51: Methylation of 104.

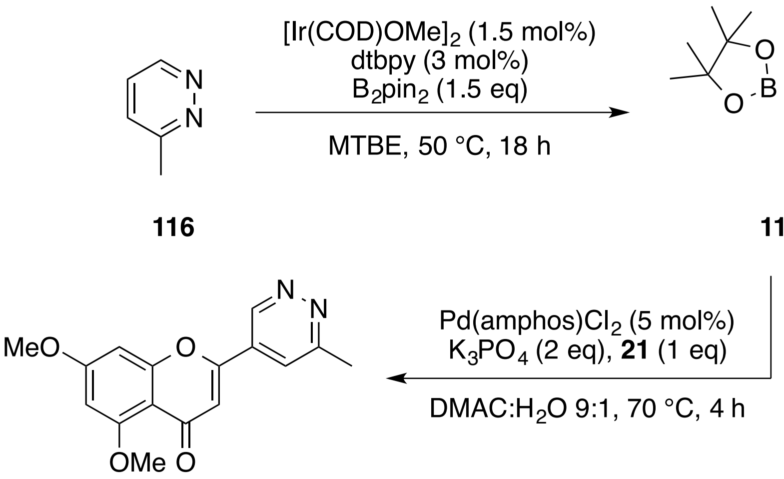
As no product formation could be observed in the aldol condensation between acetophenone **7** and pyrimidine-5-carbaldehyde (Scheme 52), which might be due to decomposition of the aldehyde, a different synthetic pathway was pursued to obtain a pyridazine analogue.



7 **115**

Scheme 52: Attempted synthesis of 107.

The pyridazine analogue **111** was obtained by a tandem one-pot C-H-borylation, Suzuki-Miyaura cross coupling reaction (Scheme 53).[146-147] 3-Methylpyridazine **116** was selectively borylated in the 5-position due to the steric hindrance of the methyl group. The reaction mixture was then concentrated and to the crude boronate ester was added Pd(amphos)Cl_2, K_3PO_4, chloride **21** and a mixture of dimethylacetamide (DMAC) and H_2O. **108** was afforded in 31% yield (LC-MS (*m/z* (ES+) = 299 MH^+)).

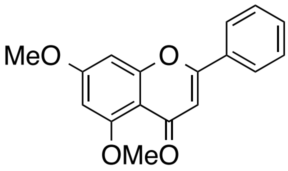
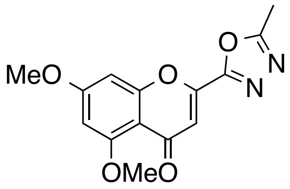
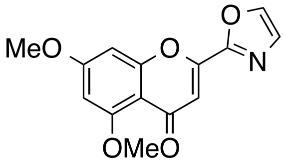
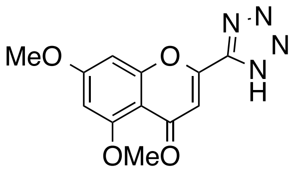
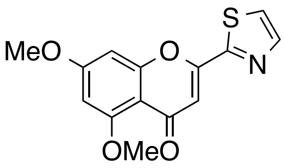
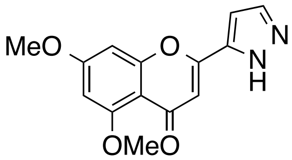


116 **117**

108
31% from **116**

Scheme 53: Synthesis of 5,7-dimethoxy-2-(6'-methylpyridazin-4'-yl)-4H-chromen-4-one 108.

Calculated logP values and aqueous solubility for all heterocyclic analogues are summarised in Table 11. As predicted, the logP decreased for all the heterocyclic analogues. The aqueous solubility slightly decreased the different pyrazole analogues **104**, **105** and **106**, but improved for all the other compounds, most significantly for the thiazole analogue **102**, for which the aqueous solubility increased almost twenty-fold. For tetrazole **101** the aqueous solubility was measured around pH 2 to obtain the solubility of the non-ionized form, but solubility is higher at higher pH values.

Entry	Compound	Compound Number	logP	Aqueous solubility (ppm)	Aqueous solubility pH
1		16	3.19	25.0	7.22
2		99	0.99	70.1	7.21
3		100	1.59	88.3	7.19
4		101	1.23	15.9	2.15
5		102	2.1	467.7	7.20
6		104	1.82	16.7	7.23

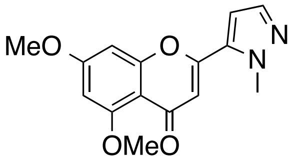
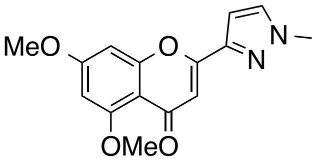
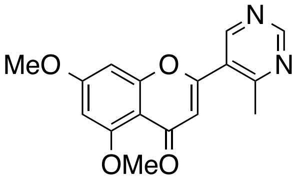
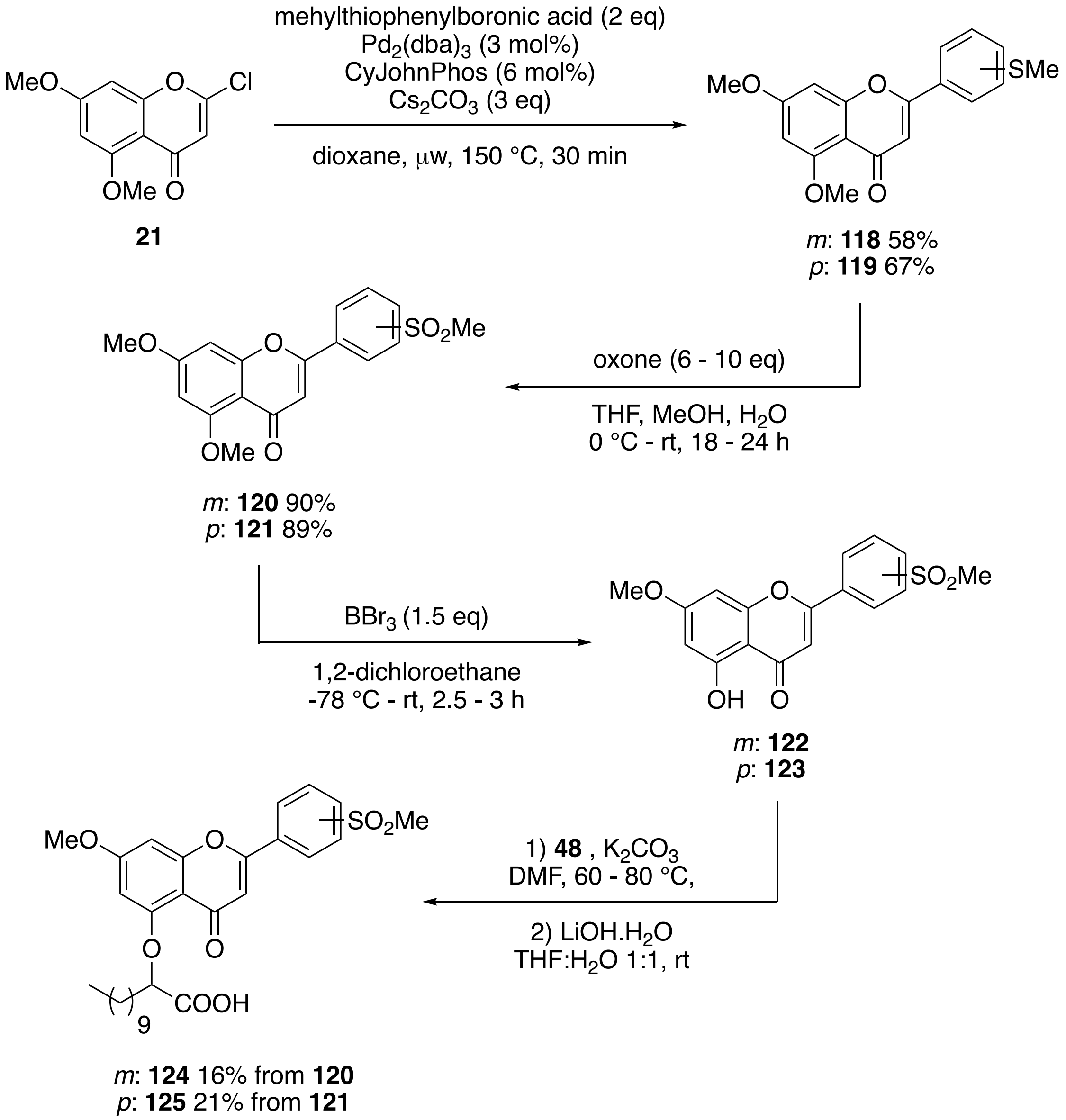
7		105	1.84	16.9	7.22
8		106	1.83	21.4	7.22
9		108	1.32	148.6	7.22

Table 11: LogP values and aqueous solubility for heterocyclic analogues compared to 16; all measurements were conducted by Syngenta at Jealotts Hill.

The heterocyclic analogues were also tested for inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1). They all showed a comparable, but slightly lower inhibition in comparison to 5,7-dimethoxy-2-phenyl-4H-chromen-4-one **16**, with the exception of thiazole **102**, which showed a slightly higher inhibition of *Am*GSTF1.

3.1.3.2 Analogues with an SO_2Me-substituent

As the SO_2Me-substituent can lower the lipophilicity of a compound, two analogues of **16** with said group in the *meta* or *para* position of the phenyl ring, **120** and **121**, were synthesised (Scheme 54) to improve the aqueous solubility. In order to compare the activity with **3**, α-substituted dodecanoic acid was attached.



Scheme 54: Synthesis of 124 and 125.

Compound **21** was coupled with 4- and 3-methylthiophenylboronic acid respectively, conditions were selected from the previous screening of Suzuki coupling conditions (Table 4). Microwave heating instead of conventional heating was chosen to shorten reaction times. Products **118** and **119** were obtained in 58 and 67% yield, formation of both was shown by LC-MS (*m/z* (ES+) = 329 MH$^+$). Oxidation of the thioether with an excess of Oxone® afforded **120** and **121** in good yield. A shift of the ^{13}C-NMR methyl-signal from around 15 ppm to about 45 ppm in both cases confirmed the oxidation. Selective demethylation with BBr$_3$ in the 5-position, which is known in literature[148] and subsequent alkylation with **48** and hydrolysis of the ester afforded **124** and **125** in 16 and 21% over 3 steps (LC-MS (*m/z* (ES+) = 545 MH^+)).

The logP values for the two analogues are summarised in Table 12.

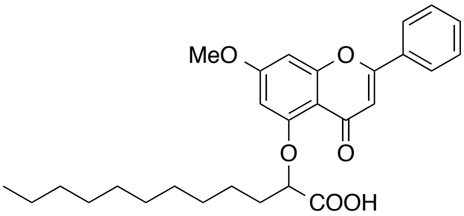
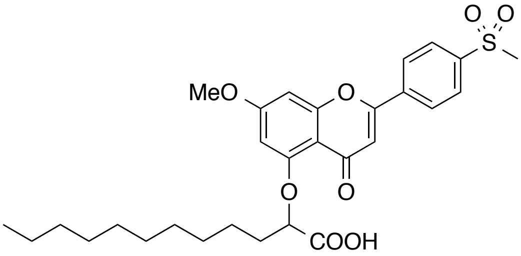
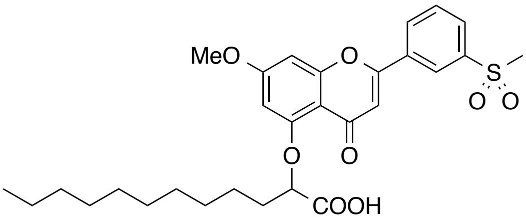
Entry	Compound	Compound Number	logP
1		3	7.1
2		125	6.92
3		124	6.91

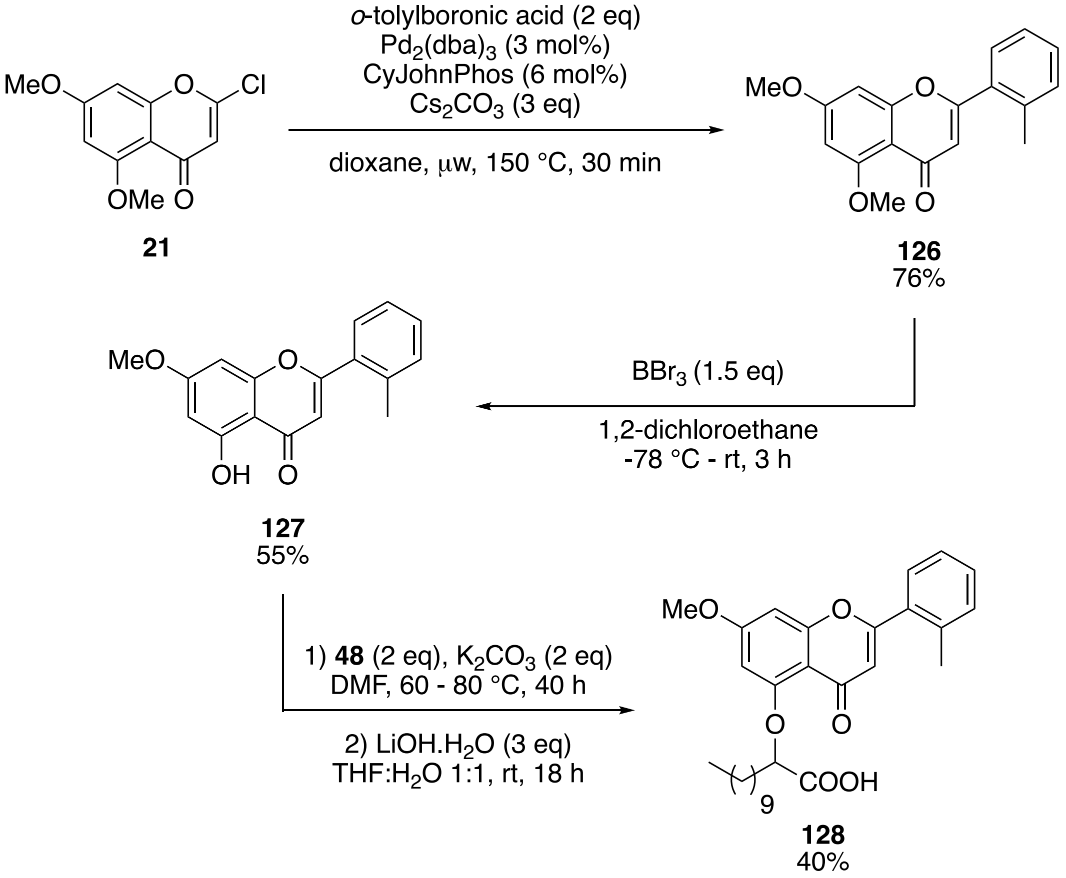
Table 12: LogP values for the SO_2Me –analogues 124 and 124 compared to 3; all measurements were conducted by Syngenta at Jealotts Hill; values are estimates, due to difficulties measuring them accurately as they are very high.

Although the logP values improved slightly, the aqueous solubility for **124** and **125** was still too low to detect. Both potential synergists **124** and **125** were tested for inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1). In both cases the inhibition dropped compared to **3** although less for **125**.

3.1.3.3 Change of the planar structure

The crystal structure of **16** shows that the phenyl ring is only slightly twisted in respect to the essentially planar 4H-chromen-4-one.[149] Therefore the introduction of substituents in the *ortho*-position of the phenyl ring or 3-position of the 4H-chromen-4-one could improve the aqueous solubility by twisting the phenyl ring further and disrupting π-π stacking interactions.

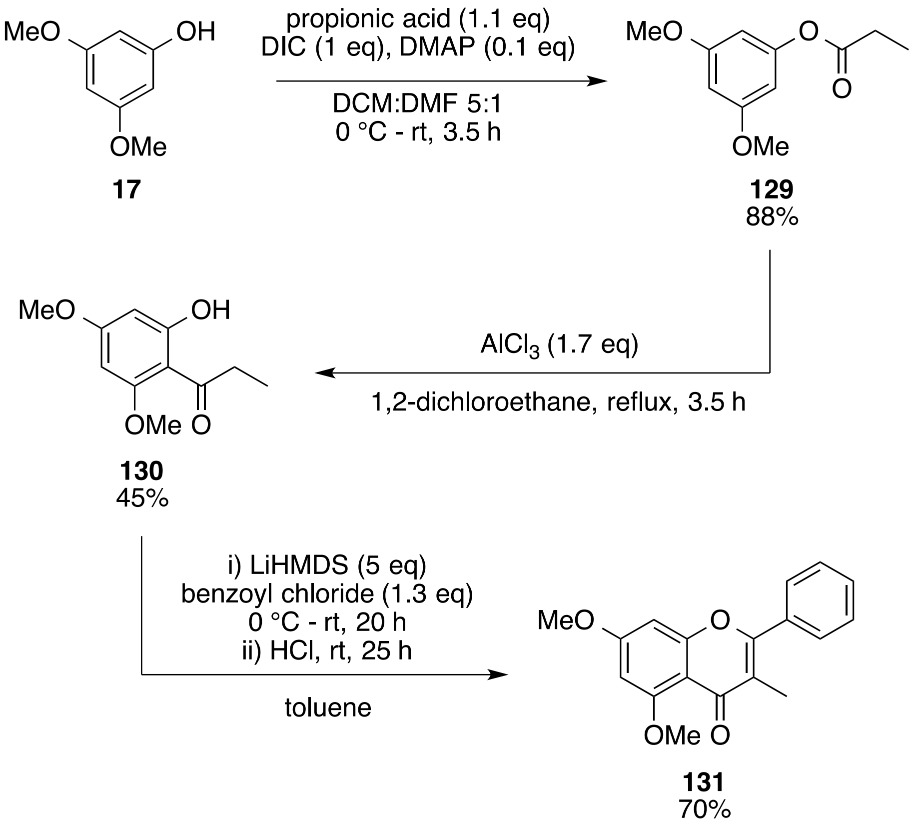
Compound **128**, with a methyl group in the *ortho*-position of the phenyl ring, was synthesised in a similar manner as for **124** and **125** (Scheme 55). Suzuki coupling of **21** with *o*-tolylboronic acid, followed by selective demethylation, alkylation and hydrolysis afforded **128** (LC-MS (*m/z* (ES+) = 481 MH^+)) with moderate to good yields in the individual steps.



Scheme 55: Synthesis of 128.

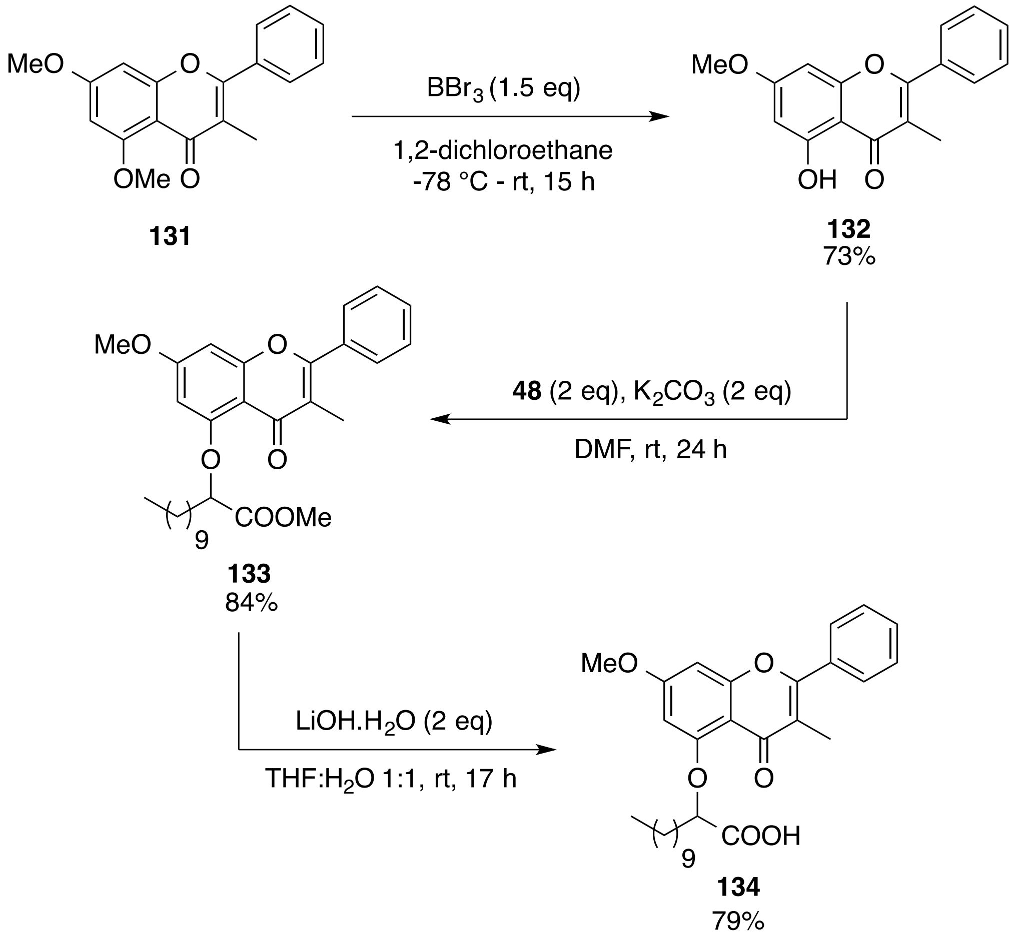
In order to introduce a substituent in the 3-position of the 4H-chromen-4-one, the heterocyclic skeleton was build up starting from propiophenone **130**. Following a procedure by Rocha et al.[150], propiophenone **130** was reacted with benzoyl chloride in the presence of an excess amount of LiHMDS, which was made in situ from *n*-Buli and 1,1,1,3,3,3-hexamethyldisilazane, and then treated with HCl (Scheme 56). Under these conditions **131** was obtained in 70% yield with the ^{1}H-NMR spectrum in accordance to literature.[150]

Propiophenone **130** itself was obtained from phenol **17** using similar Steglich esterification and Fries rearrangement conditions (Scheme 56) to the ones used in Chapter 3.1.1.2.2.



Scheme 56: Synthesis of 5,7-dimethoxy-3-methyl-2-phenyl-4H-chromen-4-one 131.

Again selective demethylation, alkylation and hydrolysis afforded **134** (LC-MS (*m/z* (ES+) = 481 MH$^+$)) as an analogue of **3** in good yield (Scheme 57).



Scheme 57: Synthesis of 134.

The logP values for **128** and **134** are summarised in Table 13. The logP value decreased for **128** by almost 1.5, but inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1) was also significantly lower compared to **3,** both might be attributed to a twisted structure.

For **134** neither the logP value nor the inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1) changed significantly.

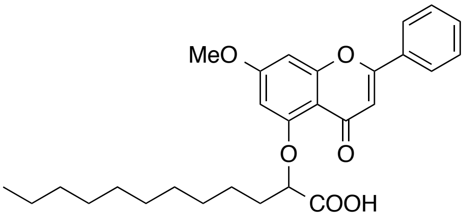
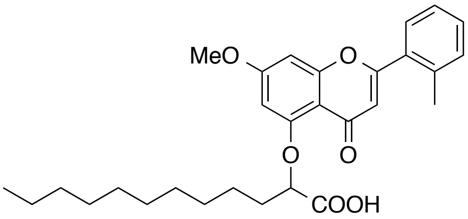
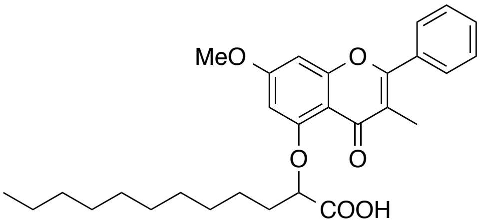
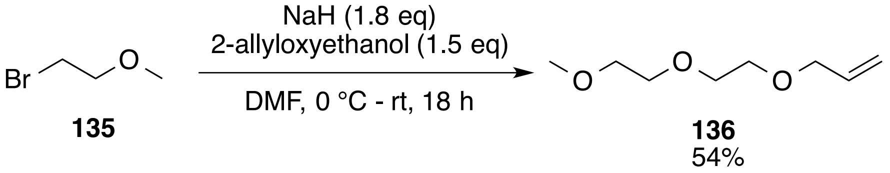
Entry	Compound	Compound Number	logP
1		3	7.1
2		128	5.75
3		134	7.4

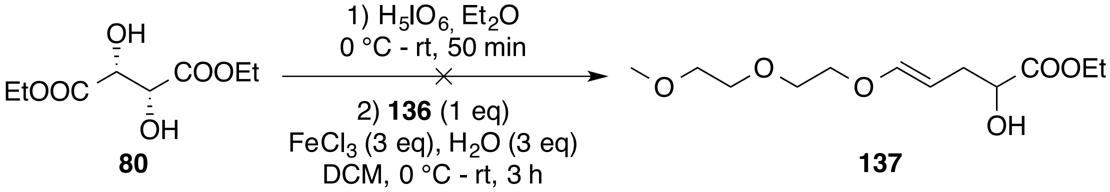
Table 13: LogP values for analogues 128 and 134 compared to 3; all measurements were conducted by Syngenta at Jealotts Hill; values are estimates, due to difficulties measuring them accurately as they are very high.

3.1.3.4 Incorporation of a PEG-like chain

Another approach to improve the aqueous solubility of **3** was to replace the long alkyl chain with a PEG-like chain. Therefore, alkene **136** was synthesised (Scheme 58) and used in an ene-reaction (Scheme 59), however no formation of product **137** could be observed in GC-MS or crude ^{1}H-NMR.

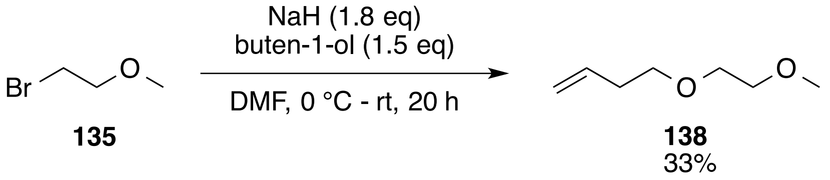


Scheme 58: Synthesis of 3-(2''-methoxy-(2'-ethoxyethoxy))-1-propene 136.



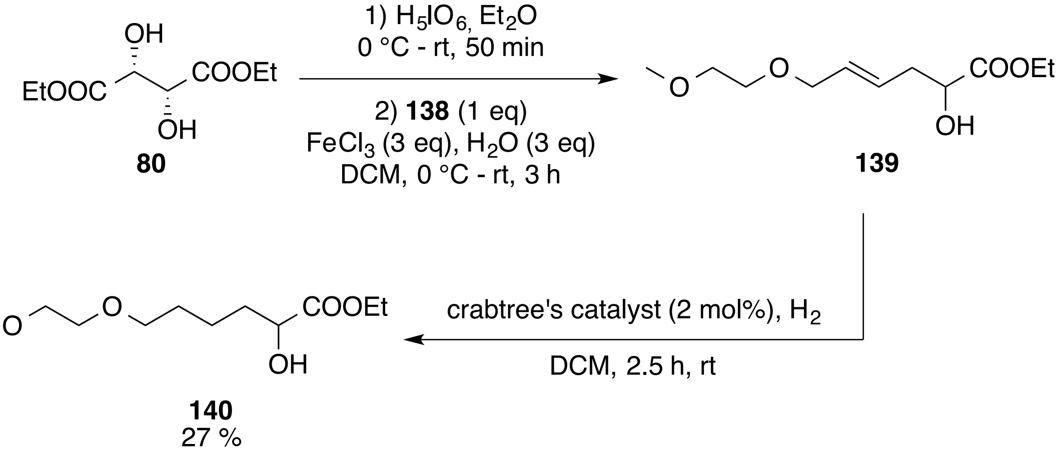
Scheme 59: Ene-reaction with 136.

As the allyl ether might be responsible for that, alkene **138** was synthesised, featuring an additional CH_2-group (Scheme 60).



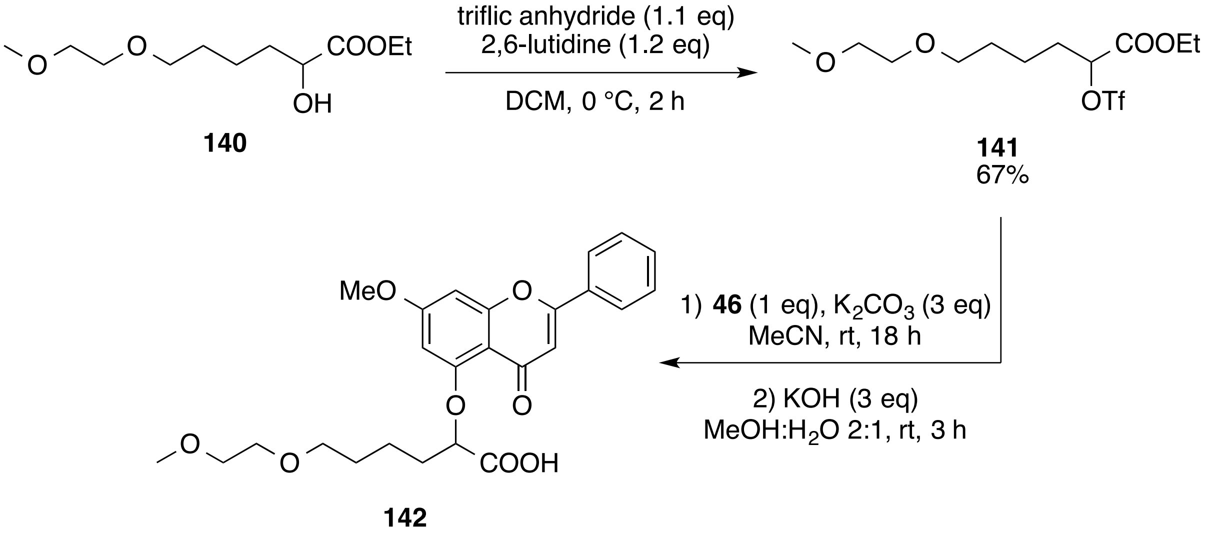
Scheme 60: Synthesis of 4-(2'-methoxyethoxy)-1-butene 138.

Using **138** instead of **136** in the ene-reaction proved to be successful and **139** could be obtained (GC-MS (*m/z* = 232 M^+)), which was directly reduced to **140** (GC-MS (*m/z* = 234 M^+)) (Scheme 61). Crabtree's catalyst was used for the hydrogenation instead of Pd/C as the later conditions resulted in no product formation. The rather low yield can be explained by the difficult purification.



Scheme 61: Synthesis of ethyl 2-hydroxy-6-(2'-methoxyethoxy)hexanoate 140.

140 was converted into the triflate **141**, using triflic anhydride and 2,6-lutidine (Scheme 62), confirmed by the characteristic quartet at 118.6 for CF_3 in the ^{13}C-NMR spectrum. **46** was alkylated at rt with **141** using K_2CO_3 as a base, product formation was confirmed by LC-MS (*m/z* (ES+) = 485 MH^+).



Scheme 62: Attampted synthesis of 142.

Attempts to hydrolyse the crude ester using $LiOH.H_2O$ in a 1:1 mixture of THF and H_2O failed and no conversion of the starting material could be observed by LC-MS. When KOH, which is less prone to complexation, in a 2:1 mixture of MeOH and H_2O was used instead, **142** could be detected in LC-MS (*m/z* (ES+) = 457 MH^+) and the formation was confirmed in the crude ^{1}H-NMR spectrum, however no pure product could be obtained by flash column chromatography.

3.1.4 Synthesis of photoaffinity labels

Photoaffinity labelling is an important method to study protein-ligand interactions and to identify binding sites in proteins.[151-153] Photolabile ligands can be used as chemical probes, which covalently bind their target upon activation by light. MALDI-MS and LC-MS analysis of the ligand-labelled protein can be used to identify mass additions due to ligand attachment. Through subsequent tryptic digestion and MS-based sequencing the amino acid sequence of the ligand-binding region can be obtained which offers valuable information about the binding site (Figure 43).[154]

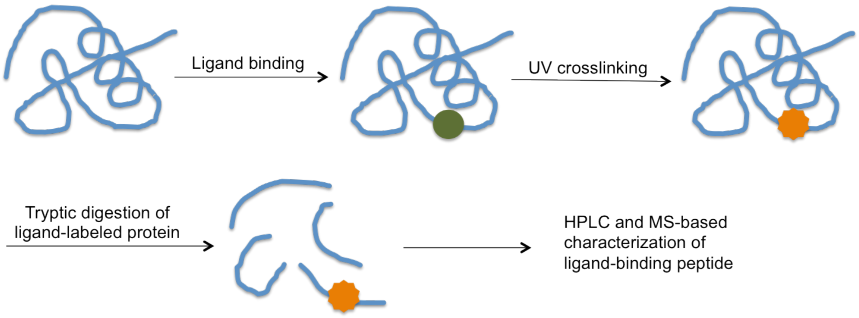


Figure 43: Photoaffinity labelling

The main groups used for photoaffinity labelling are azides, diazirines, and benzophenones, which form nitrenes, carbenes and radicals as reactive intermediates, respectively, when irradiated with light (Figure 44).[155]

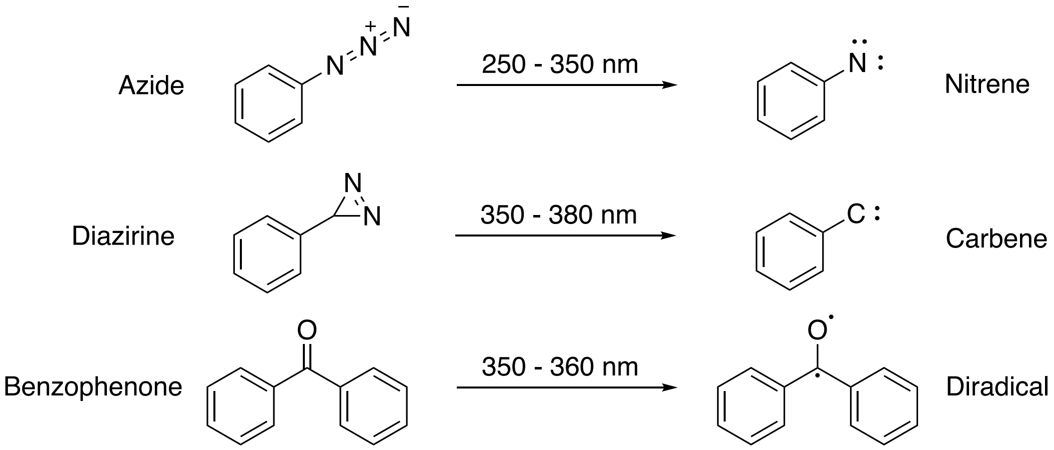
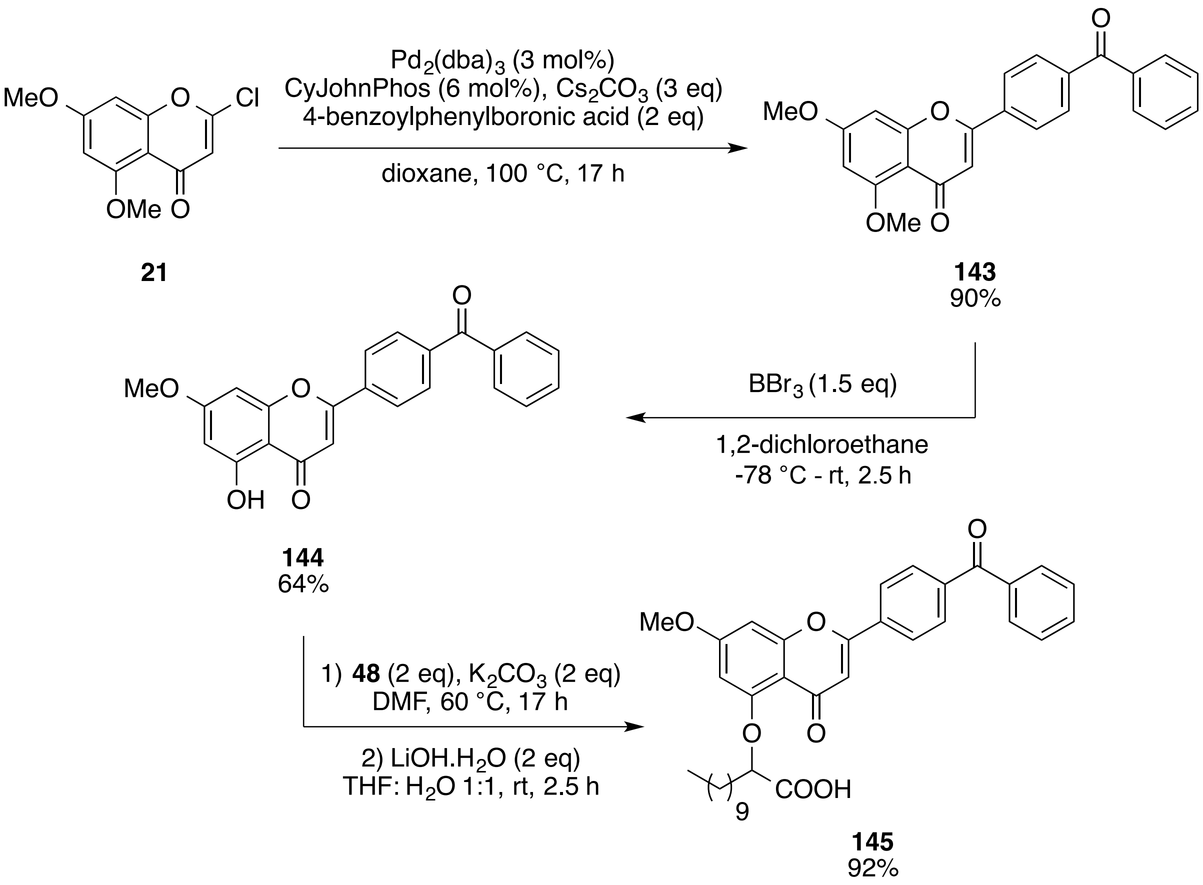


Figure 44: Photoaffiniy functional groups and their reactive intermediates.

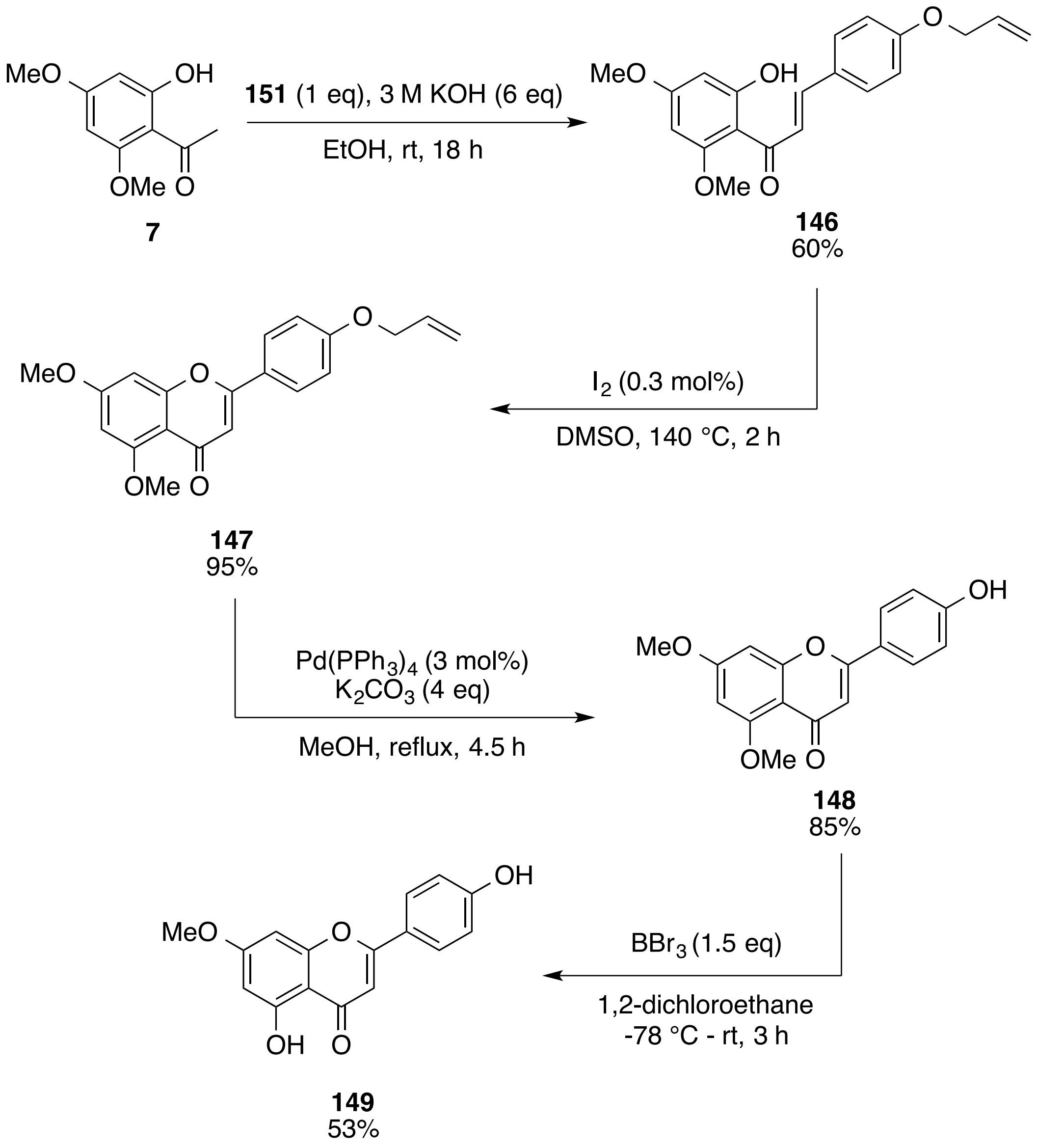
Benzophenone analogues were the probes of choice, as they are chemically more stable than azides or diazarines and can be manipulated in ambient light. Benzophenones can be activated at rather high wavelengths of 350 – 360 nm, which reduces the potential damage to the protein and they preferentially react with C-H bonds, even in the presence of solvent water and bulk nucleophiles. They can, however, require longer irradiation times and their bulkiness can affect the interaction between the ligand and the target protein.[156-158]

In order to see the how the benzophenone group affects the activity towards *Am*GSTF1, four analogues with the group attached at different parts of the molecule were synthesised.



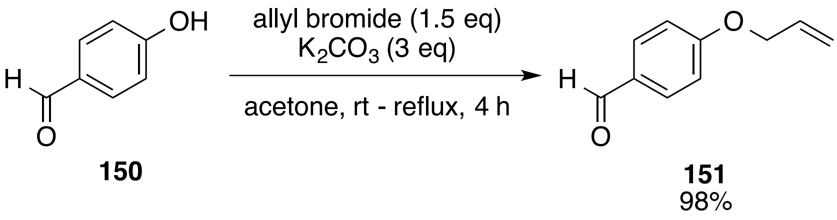
Scheme 63: Synthesis of photoaffinity label 145.

Suzuki coupling of chloride **21** with 4-benzoylphenylboronic acid gave access to an analogue with the benzophenone moiety directly attached to the 4H-chromen-4-one core. **143** (LC-MS (*m/z* (ES+) = 387 MH$^+$)) was obtained in excellent yield and selective demethylation, alkylation and hydrolysis afforded **145** (LC-MS (*m/z* (ES+) = 571 MH$^+$)) in good yield (Scheme 63). Photoaffinity label **153**, where the benzophenone group is attached further away from the core structure, was synthesised using chromenone **149** as a starting material (Scheme 66).



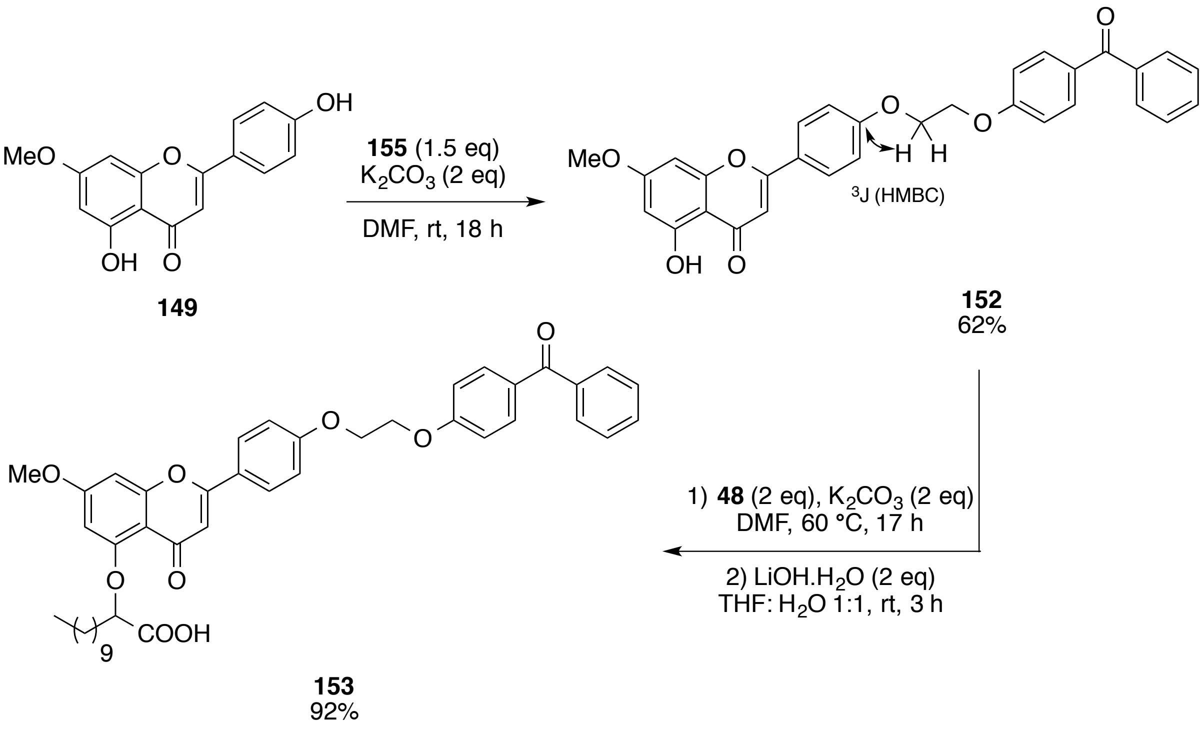
Scheme 64: Synthesis of 5-hydroxy-7-methoxy-2-(4'-hydroxyphenyl)-4H-chromen-4-one 149.

149 was obtained following a literature procedure.[145] Base-catalyzed aldol condensation of 2-hydroxyacetophenone **7** with 4-allyloxybenzaldehyde **151** afforded chalcone **146** in 60% yield (Scheme 64). 4-Allyloxybenzaldehyde **151**, required as starting material was synthesised by alkylation of 4-hydroxybenzaldehyde **150** with allyl bromide using K_2CO_3 in acetone (Scheme 65).



Scheme 65: Synthesis of 4-allyloxybenzaldehyde 151.

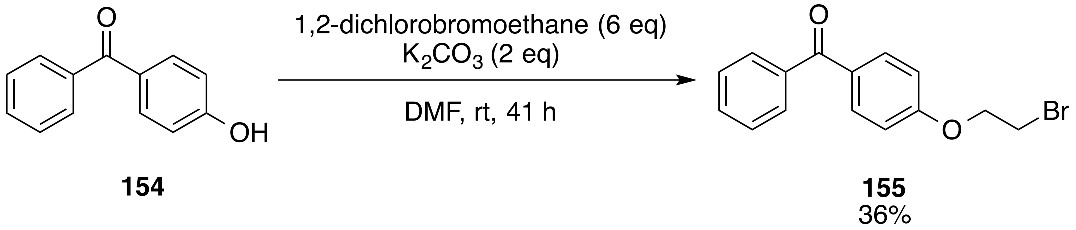
146 was then cyclized with catalytic amounts of iodine in DMSO to give the desired product **147** in excellent yield (Scheme 64). Appearance of a singlet at 6.72 instead of the two doublets at 7.81 and 7.66 in the ^{1}H-NMR spectrum confirmed the cyclisation. The allyl protecting group was cleaved with catalytic amounts of $Pd(PPh_3)_4$ using K_2CO_3 in methanol at reflux to obtain **148** in good yield. Selective demethylation with BBr_3 in the 5-position gave 5-hydroxy-7-methoxy-2-(4'-hydroxyphenyl)-4H-chromen-4-one **149** (LC-MS (*m/z* (ES+) = 285 MH$^+$)) in 53% yield (Scheme 64).



Scheme 66: Synthesis of photoaffinity label 153.

4-Hydroxybenzophenone attached to a linker was used in an alkylation reaction with **149** to give **152** in good yield (Scheme 66). Formation was confirmed by the corresponding peak in LC-MS (*m/z* (ES+) = 509 MH^+) and stereoselectivity was verified by 2D-NMR. The HMBC experiment showed a correlation between the C-4' carbon signal and the signal for the CH_2-group. Alkylation with **48** in the 5-position and hydrolysis of the methyl ester afforded photoaffinity label **153** in 92% yield over the two steps (LC-MS (*m/z* (ES+) = 707 MH^+)).

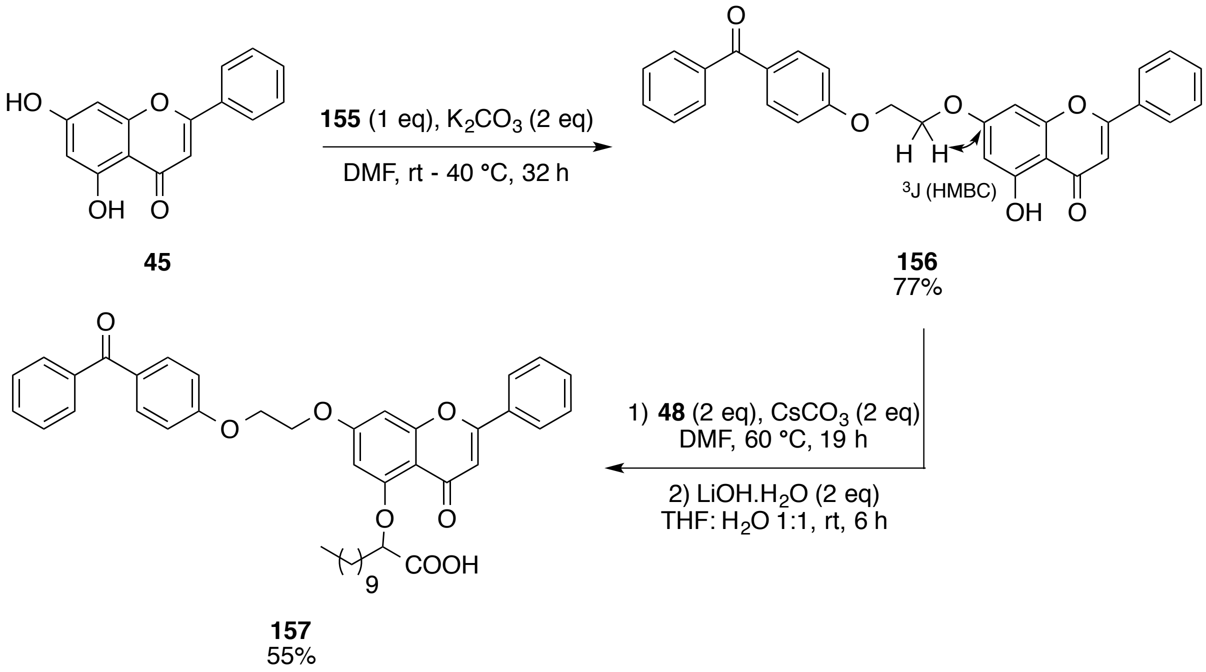
The alkyl linker was connected to 4-hydroxybenzophenone by reaction with an excess of 1,2-dibromoethane and K_2CO_3 in DMF (Scheme 67).



Scheme 67: Synthesis of 4-(2''-bromoethoxy)benzophenone 155.

Compound **155** was also used to attach the benzophenone moiety in the 7-position of the 4H-chromen-4-one (Scheme 68). Reaction of **45** with **155** and K_2CO_3 in DMF resulted in the stereoselective alkylation, though elevated temperatures were required to improve the conversion. Formation of **156** was confirmed by the presence of the corresponding peak in LC-MS (*m/z* (ES+) = 479 MH^+) and 2D-NMR. The HMBC experiment showed a correlation between the C-5 carbon signal and the signal for the CH_2-group.

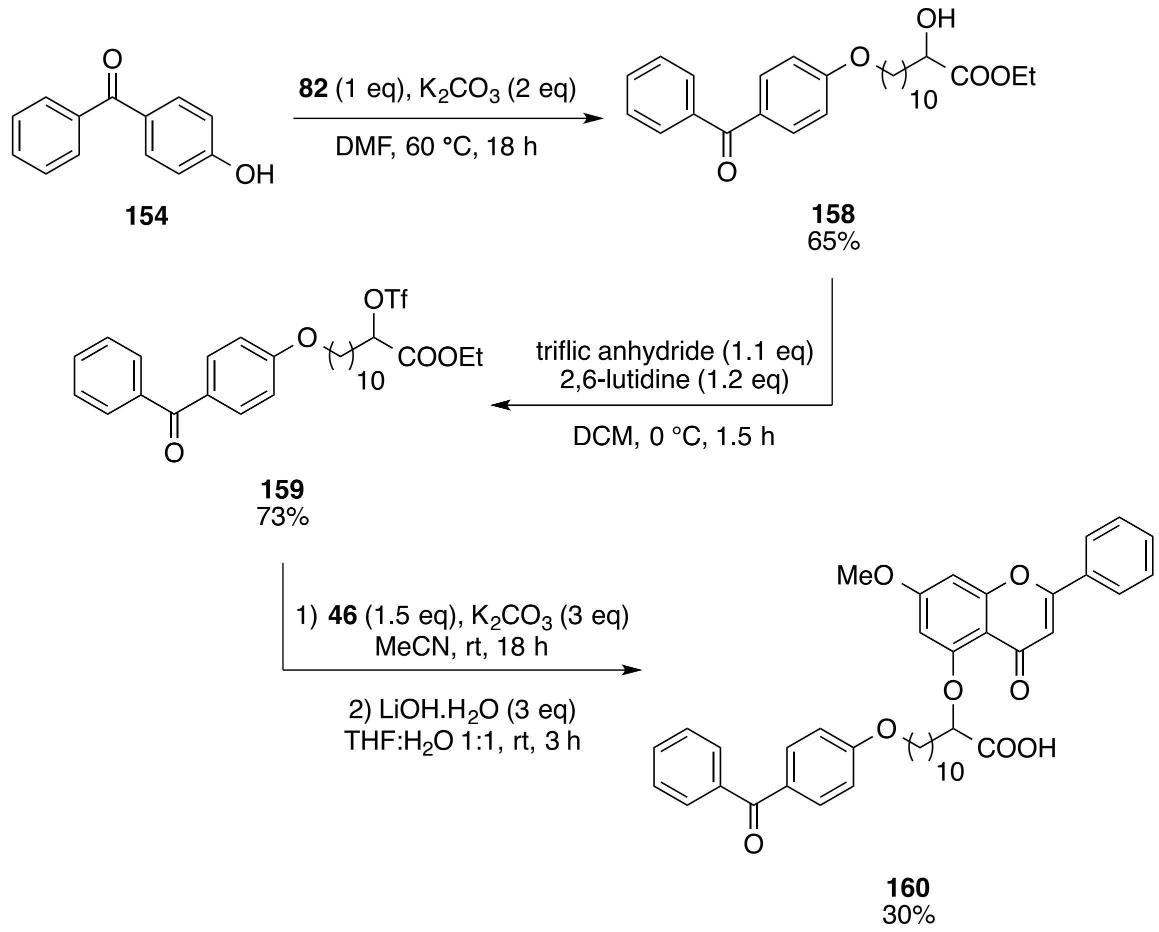
Alkylation with **48** in the 5-position and hydrolysis of the methyl ester afforded **157** in 55% yield over the two steps (LC-MS (*m/z* (ES+) = 677 MH^+)). The alkylation conditions had to be changed, however, Cs_2CO_3 was used instead of K_2CO_3 as no product formation was observed using the latter.



Scheme 68: Synthesis of photoaffinity label 157.

For the last photoaffinity probe, the benzophenone group was attached at the long alkyl chain (Scheme 69). α-Hydroxy acid **82**, whose synthesis was described in Chapter 3.1.2.4, was stirred with **154** and K_2CO_3 in DMF in an alkylation reaction to afford **158** in 65% yield (LC-MS (*m/z* (ES+) = 441 MH$^+$)). Using the same conditions as before, to form the triflate, **158** was reacted with triflic anhydride and 2,6-lutidine. Product formation was confirmed by the characteristic quartet at 118.6 for the CF_3 group in the ^{13}C-NMR spectrum. Subsequent alkylation and hydrolysis afforded the product **160** (LC-MS (*m/z* (ES+) = 663 MH$^+$)) in moderate yield.

All the photoaffinity labels were tested for inhibition of *Am*GSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1). **153** and **157** showed a lower inhibition than **3**, but for **145** and **160** the inhibition was unchanged. Therefore, those two labels were considered for further labelling experiments. (*cf.* Chapter 3.2.2.1)



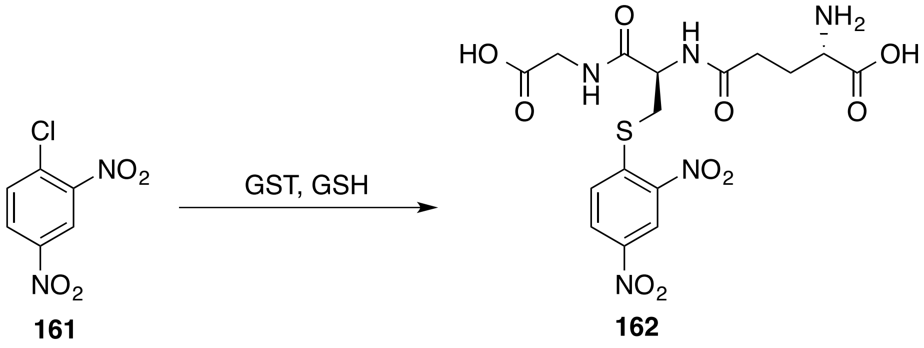
Scheme 69: Synthesis of photoaffinity label 154.

3.2 Biological Testing

3.2.1 Synergist evaluation

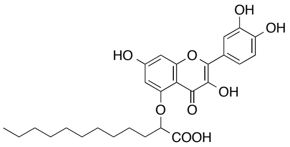
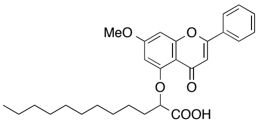
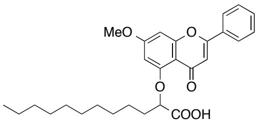
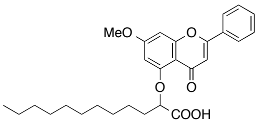
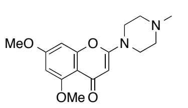
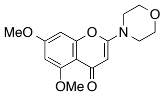
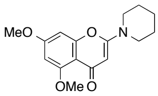
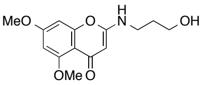
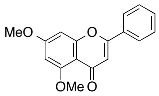
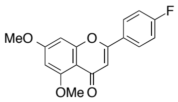
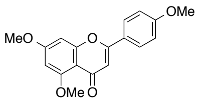
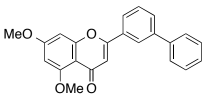
3.2.1.1 CDNB assay

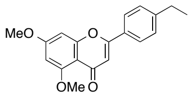
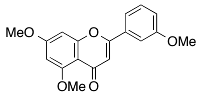
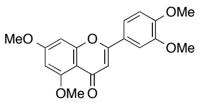
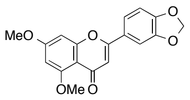
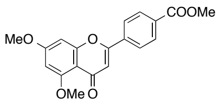
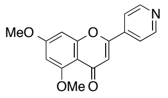
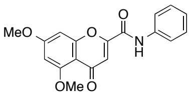
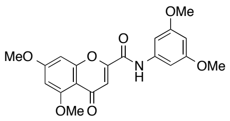
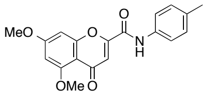
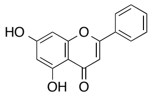
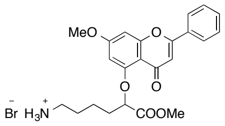
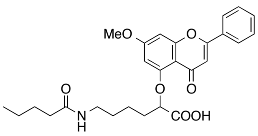
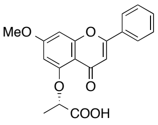
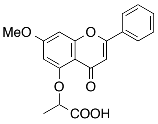
In order to determine whether a compound acts as a GST inhibitor or not, a CDNB assay was performed. A CDNB assay is a colorimetric assay, which is based on the GST-catalysed conjugation of GSH to 1-chloro-2,4-dinitrobenzene (CDNB **161**) (Scheme 70). This reaction forms the dinitrophenyl thioether **162** that can be spectrophotometrically detected at 340 nm.[159] As almost all GSTs have an affinity for CDNB, the CDNB assay is commonly used to determine GSH conjugating activity.

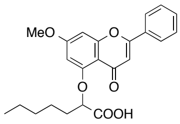
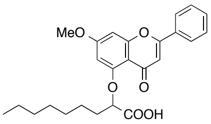
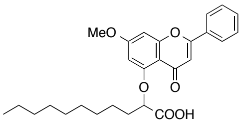
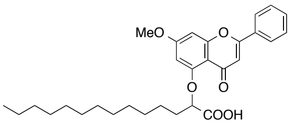
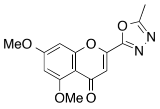
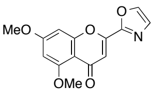
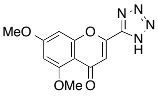
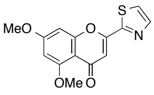
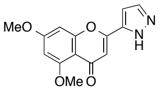
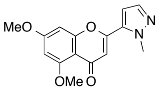
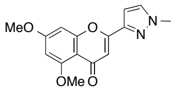
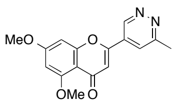
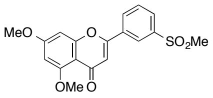
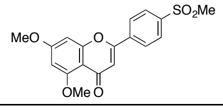


Scheme 70: Reaction in the CDNB assay.

*Am*GSTF1 was obtained by overexpression in Escherichia coli (E. coli) cells. Therefore, competent E. coli cells were transformed using strep-tagged-AmGSTF1, cloned into a pET Strep vector. Selection and propagation of the transformed cells was followed by Isopropyl β-D-1-thiogalactopyranoside (IPTG) induction of protein expression. AmGSTF1 was purified by fast protein liquid chromatography (FPLC) using a streptacin column. All inhibitors were tested at a final concentration of 1, 10 or 100 μM in the CDNB assay. Values were reported as % inhibition of the specific enzyme activity and are summarized in Table 14. The results are discussed in more detail below.

Entry	Compound	Number	Inhibition 100 µM	Inhibition 10 µM	Inhibition 1 µM
1		**2**	100% ± 13%	96% ± 2%	70% ± 1%
2		**3**	100% ± 4%	99% ± 6%	71% ± 5%
3	 **E1**	**3 E1**	n.d.	n.d.	46% ± 8%
4	 **E2**	**3 E2**	n.d.	n.d.	68% ± 3%
5		**9**	16% ± 3%	n.d.	n.d.
6		**12**	37% ± 4%	0% ± 3%	n.d.
7		**13**	67% ± 5%	12% ± 4%	n.d.
8		**14**	12% ± 1%	n.d.	n.d.
9		**16**	70% ± 12%	20% ± 3%	n.d.
10		**25**	78% ± 13%	23% ± 11%	n.d.
11		**26**	100% ± 3%	15% ± 4%	n.d.
12		**27**	87% ± 7%	12% ± 2%	n.d.

13		**28**	80% ± 7%	11% ± 7%	n.d.
14		**29**	79% ± 3%	15% ± 8%	n.d.
15		**30**	100% ± 3%	11% ± 6%	n.d.
16		**31**	33% ± 4%	12% ± 5%	n.d.
17		**32**	58% ± 14%	20 % ±5%	n.d.
18		**38**	50% ± 6%	10 % ±4%	n.d.
19		**42**	49% ± 3%	26% ± 14%	n.d.
20		**43**	56% ± 5%	19% ± 6%	n.d.
21		**44**	64% ± 6%	33% ± 13%	n.d.
22		**45**	100% ± 10%	47% ± 5%	n.d.
23		**57**	n.d.	25% ± 10%	n.d.
24		**59**	n.d.	39% ± 2%	6% ± 1%
25		**(*S*)-71**	n.d.	5% ± 4%	n.d.
26		**(±)-71**	n.d.	14% ± 3%	n.d.

27		**95**	n.d.	69% ± 6%	4% ± 7%
28		**96**	n.d.	86% ± 1%	40% ± 3%
29		**97**	n.d.	97% ± 7%	58% ± 4%
30		**98**	n.d.	90% ± 3%	75% ± 3%
31		**99**	53% ± 3%	8% ± 2%	n.d.
32		**100**	64% ± 2%	11% ± 1%	n.d.
33		**101**	53% ± 5%	13% ± 7%	n.d.
34		**102**	83% ± 3%	23% ± 3%	n.d.
35		**104**	59% ± 2%	6% ± 3%	n.d.
36		**105**	58% ± 7%	9% ± 7%	n.d.
37		**106**	64% ± 3%	19% ± 8%	n.d.
38		**108**	64% ± 5%	8% ± 2%	n.d.
39		**120**	60% ± 7%	11% ± 3%	n.d.
40		**121**	35% ± 10%	14% ± 2%	n.d.

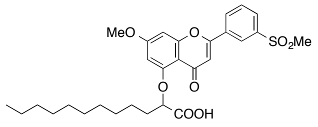
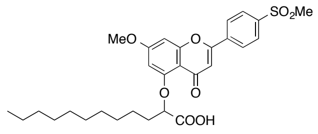
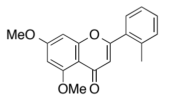
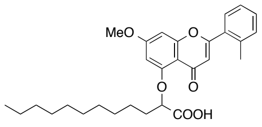
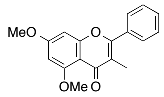
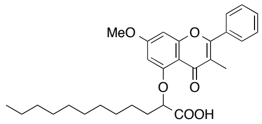
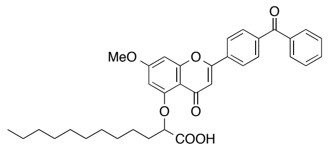
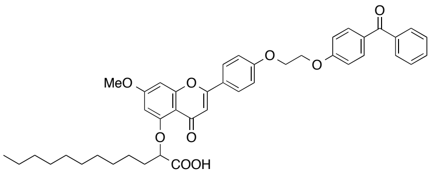
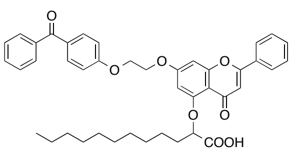
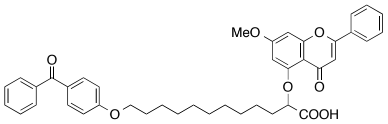
41		**124**	n.d.	83% ± 1%	24% ± 3%
42		**125**	n.d.	94% ± 2%	40% ± 2%
43		**126**	36% ± 1%	9% ± 5%	n.d.
44		**128**	n.d.	82% ± 1%	27% ± 2%
45		**131**	n.d.	17% ± 1%	n.d.
46		**134**	n.d.	100% ± 2%	70% ± 1%
47		**145**	n.d.	81% ± 4%	71% ± 3%
48		**153**	n.d.	30% ± 6%	n.d.
49		**157**	n.d.	47% ± 5%	n.d.
50		**160**	n.d.	78% ± 4%	57% ± 4%

Table 14: Inhibition of *Am*GSTF1 in the CDNB assay. Values are reported as % inhibition of the specific enzyme activity. Each value is the result of three replicate measurements.

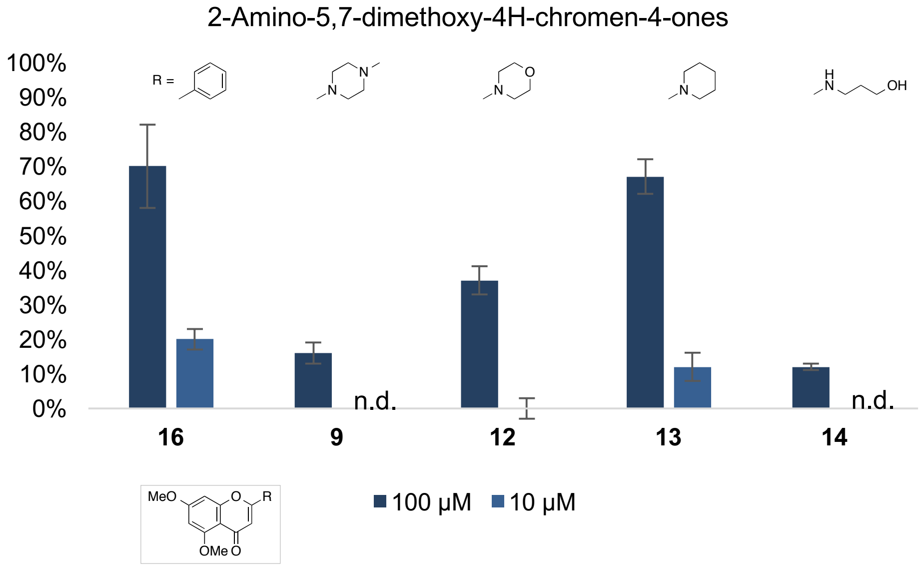


Figure 45: Inhibition of *Am*GSTF1 caused by 2-amino-5,7-dimethoxy-4H-chromen-4-ones in the CDNB assay compared to phenyl analogue 16. Each value is the result of three replicate measurements.

With the exception of **13**, the non-aromatic amines in the 2-position (Table 14, entries 5-8) seem to lower the inhibition of *Am*GSTF1 significantly compared to 5,7-dimethoxy-2-phenyl-4H-chromen-4-one **16** (Figure 45), suggesting that either the electron donating effect of the amine is unfavourable or the aromatic ring in this position is important.

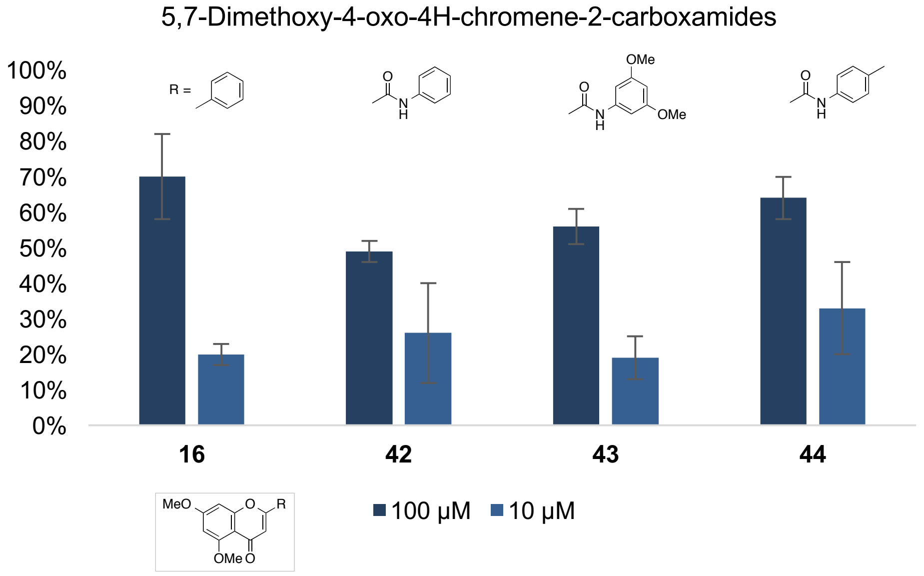


Figure 46: Inhibition of *Am*GSTF1 caused by 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamides in the CDNB assay compared to phenyl analogue 16. Each value is the result of three replicate measurements.

Carboxamides **42** – **44** (Table 14, entries 19-21) had a similar potency to compound **16** at a 10 µM level, but a slightly lower inhibition at a 100 µM level (Figure 46). As the amide group is planar, it is a linker between the C ring and the aryl group, but does not change the overall planar structure of the molecule.

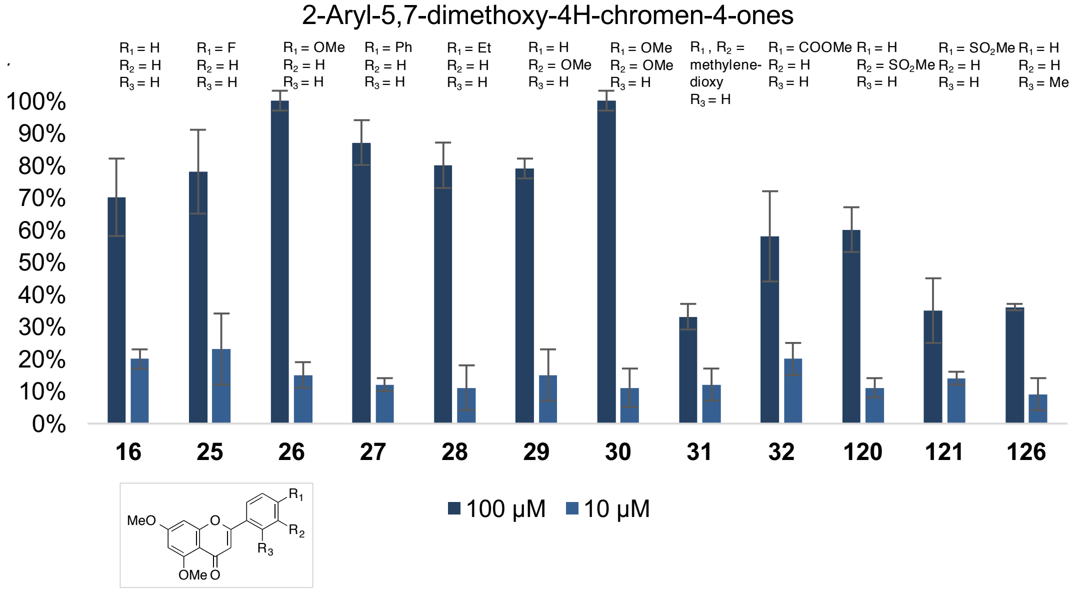


Figure 47: Inhibition of *Am*GSTF1 caused by 2-aryl-5,7-dimethoxy-4H-chromen-4-ones in the CDNB assay. Each value is the result of three replicate measurements.

Most substituents on the phenyl-ring of the 2-aryl-5,7-dimethoxy-4H-chromen-4-ones did not significantly affect the activity of these compounds (Figure 47). Compound **27** with a biphenyl in the 2-position and compound **28** with a 4-ethyl-substituted phenyl ring, retained their activity and showed a similar inhibition, which indicates that steric bulk in those positions is tolerated. The *p*-OMe substituent (**26**, **30**) slightly improved the inhibition of *Am*GSTF1 at 100 µM, which might suggest that a hydrogen bond acceptor in this position is beneficial. The 1,3-benzodioxole analogue **31** an alternative to the dimethoxy derivative **30**, however, had a significantly lower potency at 100 µM. A *p*-CO_2Me substituent (**32**), as well as a SO_2Me group in the *para* (**121**) or *meta* (**120**) position led to a decrease in activity at 100 µM. As the *o*-methyl substituent of analogue **126** possibly twists the phenyl ring in respect to the planar 4H-chromen-4-one, the low inhibition of *Am*GSTF1 might be explained by a different molecular geometry.

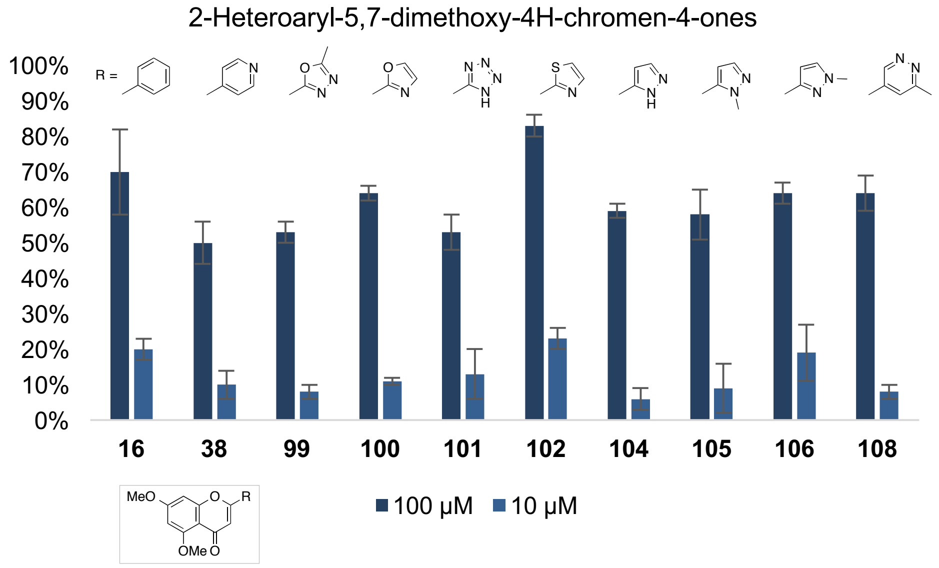


Figure 48: Inhibition of *Am*GSTF1 caused by 2-heteroaryl-5,7-dimethoxy-4H-chromen-4-ones in the CDNB assay compared to phenyl analogue 16. Each value is the result of three replicate measurements.

All heteroaryl analogues, except thiazole **102**, had a marginally lower potency compared to compound **16** at a 10 and 100 µM level (Figure 48). Thiazole **102** showcased a slightly higher activity than **12** at both concentrations.

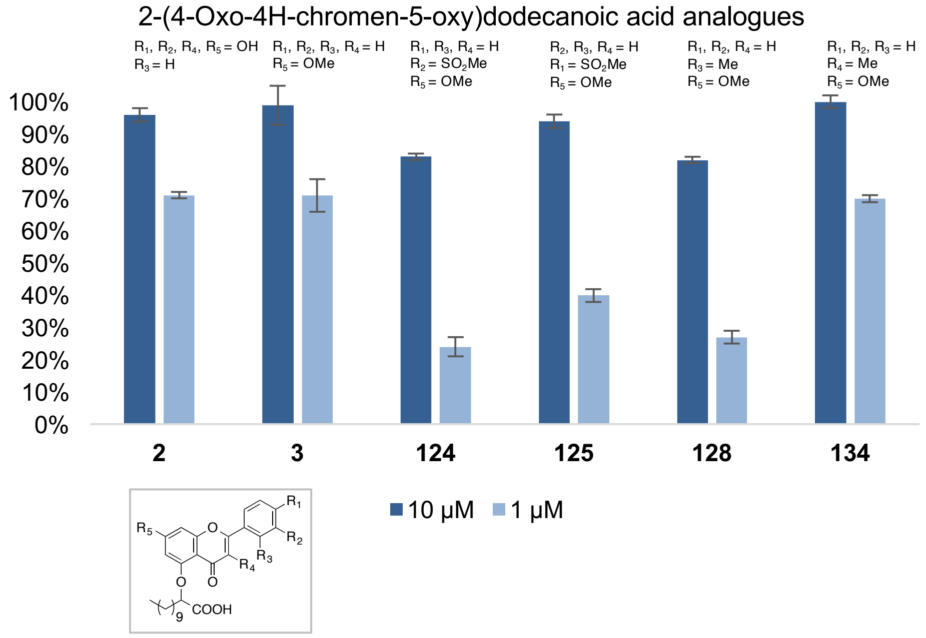


Figure 49: Inhibition of *Am*GSTF1 caused by 2-(4-oxo-4H-chromen-5-oxy)dodecanoic acid analogues in the CDNB assay. Each value is the result of three replicate measurements.

As discussed in Chapter 2.1, compound **2** and **3** have a very similar activity towards *Am*GSTF1 in the CDNB assay at 10 and 1 µM (Figure 49). The same is true for compound **134**, the methyl substituent in the 3-position of the 4H-chromen-4-one had no effect on potency. The decreased activity of the *o*-methyl substituted analogue **128** and the *p*-SO_2Me and *m*-SO_2Me substituted analogues **125** and **124** compared to **3**, especially at a 1 µM level, was consistent with the loss in activity for compounds **121**, **120** and **126** compared to **16**.

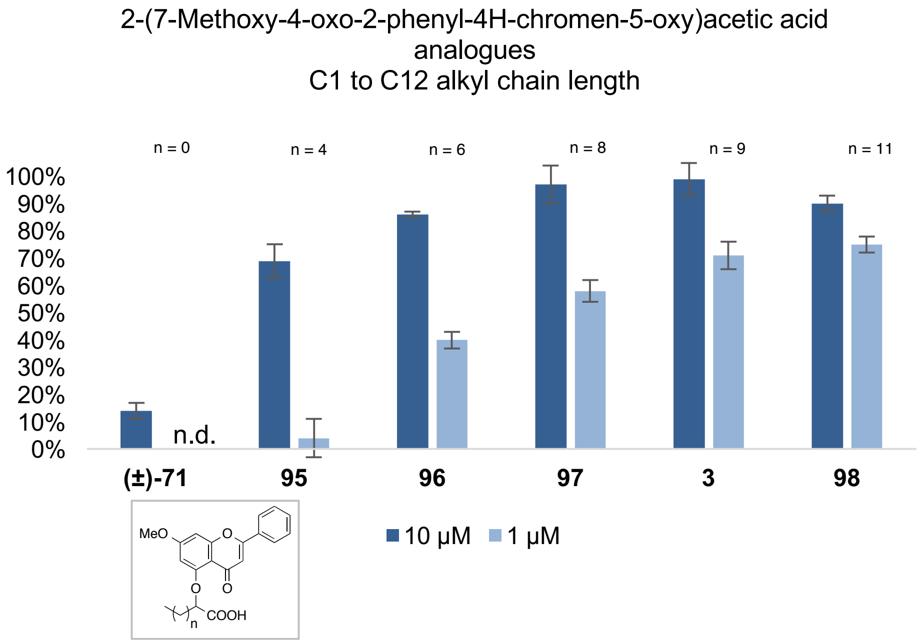


Figure 50: Influence of the alkyl chain length on the inhibition of *Am*GSTF1 in the CDNB assay. Each value is the result of three replicate measurements.

The inhibition activity of analogues of **3** with varying alkyl chain length at 10 and 1 µM increased with the number of carbon atoms (Figure 50). The heptanoic acid analogue **95** had a significantly higher activity than the propanoic acid compound **(±)-71**. Extending the chain by a further two (**96**) and four (**97**) carbons led to another huge increase in potency, which then slightly levelled for the dodecanoic (**3**) and tetradecanoic (**98**) acid analogue.

In order to determine a possible effect of the configuration on the inhibition activity, the two enantiomers of compound **3** were tested in the CDNB assay (Figure 51). One of the enantiomers had the same potency as the racemic compound, whereas the other enantiomer showed a slightly decreased activity at 1 µM, suggesting, that, if any, the stereochemistry has a small influence on activity.

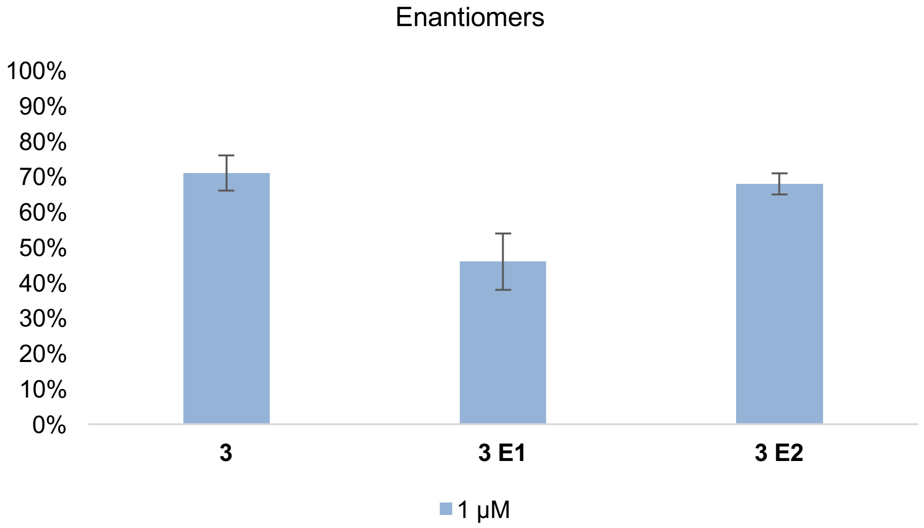


Figure 51: Influence of the configuration on the inhibition of *Am*GSTF1 in the CDNB assay. Each value is the result of three replicate measurements.

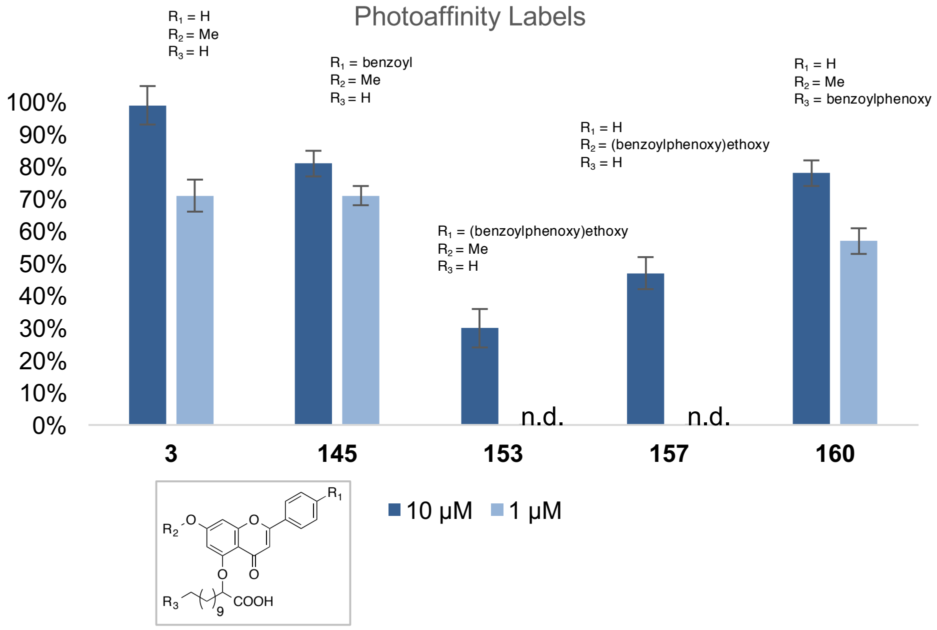


Figure 52: Inhibition of *Am*GSTF1 caused by the photoaffinity labels in the CDNB assay. Each value is the result of three replicate measurements.

The inhibition activity of the photoaffinity labels is shown in Figure 52. Both label **145** and **160** retained most of their potency, suggesting the bulkiness of the benzophenone moiety is not affecting the interaction between the ligand and the protein. For the other two labels the activity decreased significantly.

3.2.1.2 Thermal shift assay

A different method to analyse potential synergists is the thermal shift assay, which measures the change in protein melting temperature upon binding of a ligand to the protein.[160] In the thermofluor assay, a dye, SYPRO orange[161], with a low fluorescence in a polar environment, but a high fluorescence in a non-polar environment, is added to a protein solution and the fluorescence of the solution is monitored while heating. As the protein is unfolding, the hydrophobic core is exposed and the signal increases until the protein is completely denatured. The melting temperature T_m can be determined by the temperature where half the protein population is unfolded. The assay can be performed in 96-well plate using a standard quantitative PCR instrument.[162]

A preliminary test was performed to see how much DMSO could be added without destabilizing the enzyme (Figure 53) and the results indicated that less than about 1.5 % should be used, as higher concentrations decreased T_m.

a)

	1	2	3	4	5	6	7	8	9	10	11	12
A	59.1	32.6	45.3	50.6	55.3	58.0	59.3	59.1	59.4	59.2	59.3	59.0
B	59.2	32.9	45.4	50.4	54.3	56.7	57.9	59.0	58.9	58.9	59.4	59.2

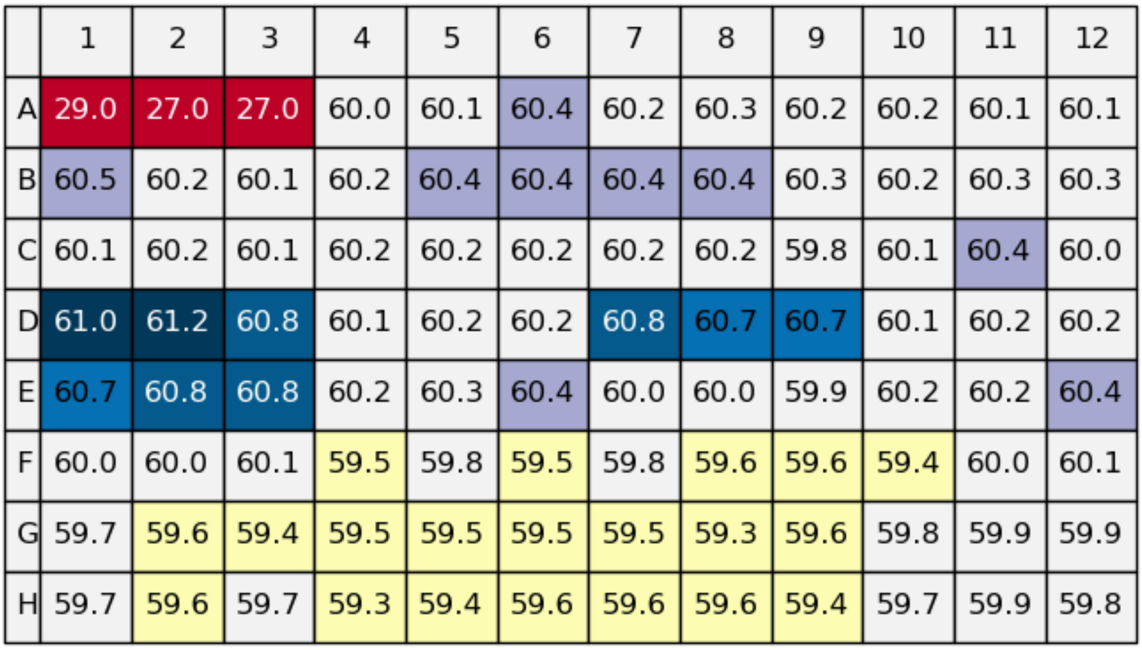
b)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.2	50.2	25.2	12.7	6.45	3.325	1.763	0.981	0.591	0.395	0.298	0.249
B	0.2	50.2	25.2	12.7	6.45	3.325	1.763	0.981	0.591	0.395	0.298	0.249

Figure 53: a) Results of the thermal shift assay showing T_m for each well. Decreases in melting temperature are indicated by a colour change from yellow to red (largest decrease). b) Final concentration of DMSO [%]. Experiments were done in duplicate.

The results of the compound screen (Figure 54) indicated three areas that increase the thermal stability, positions D1-D3, D7-D9 and E1-E3, corresponding to compounds **3**, **134** and **2** at a 10 µM concentration. Those three compounds are the only aryloxydodecanoic acid analogues in this screen, which again emphasises the importance of this substituent in the 5-position and confirms those compounds as the most potent binding partners of *Am*GSTF1. All other compounds showed, if any only a very low increase or decrease in melting temperature.

a)



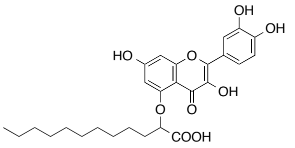
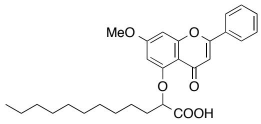
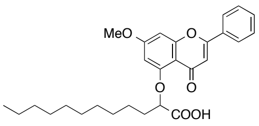
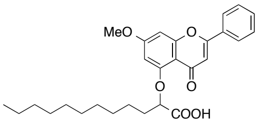
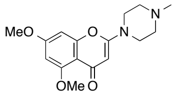
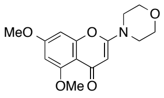
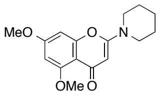
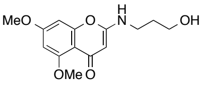
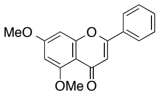
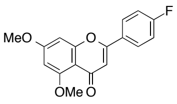
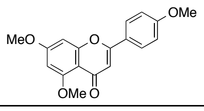
	1	2	3	4	5	6	7	8	9	10	11	12
A	29.0	27.0	27.0	60.0	60.1	60.4	60.2	60.3	60.2	60.2	60.1	60.1
B	60.5	60.2	60.1	60.2	60.4	60.4	60.4	60.4	60.3	60.2	60.3	60.3
C	60.1	60.2	60.1	60.2	60.2	60.2	60.2	60.2	59.8	60.1	60.4	60.0
D	61.0	61.2	60.8	60.1	60.2	60.2	60.8	60.7	60.7	60.1	60.2	60.2
E	60.7	60.8	60.8	60.2	60.3	60.4	60.0	60.0	59.9	60.2	60.2	60.4
F	60.0	60.0	60.1	59.5	59.8	59.5	59.8	59.6	59.6	59.4	60.0	60.1
G	59.7	59.6	59.4	59.5	59.5	59.5	59.5	59.3	59.6	59.8	59.9	59.9
H	59.7	59.6	59.7	59.3	59.4	59.6	59.6	59.6	59.4	59.7	59.9	59.8

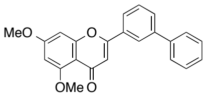
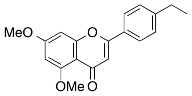
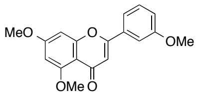
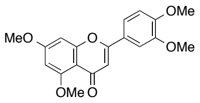
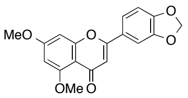
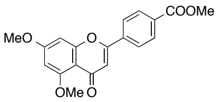
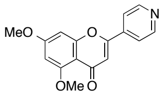
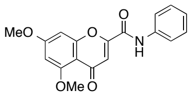
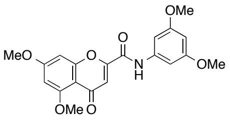
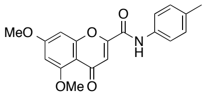
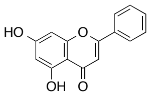
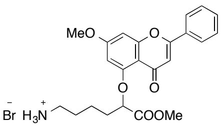
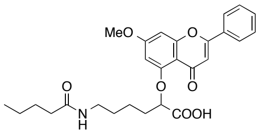
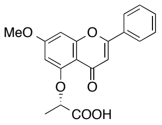
b)

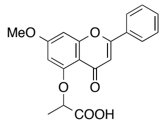
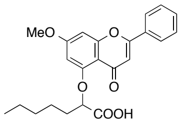
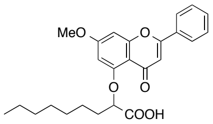
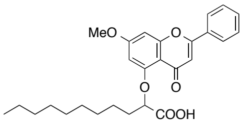
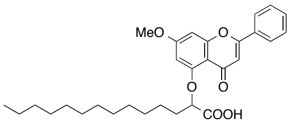
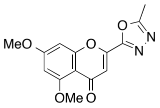
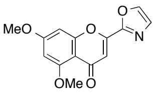
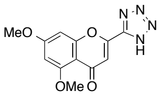
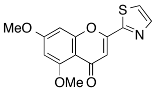
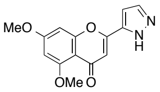
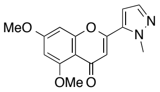
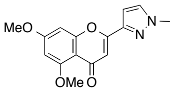
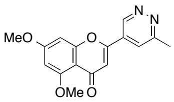
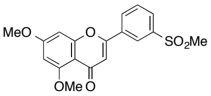
	1	2	3	4	5	6	7	8	9	10	11	12
A	No dye/ compound	No dye/ compound	No dye/ compound	No compound	No compound	No compound	**45** (10μM)	**45** (10μM)	**45** (10μM)	**45** (1μM)	**45** (1μM)	**45** (1μM)
B	**46** (10μM)	**46** (10μM)	**46** (10μM)	**46** (1μM)	**46** (1μM)	**46** (1μM)	**16** (10μM)	**16** (10μM)	**16** (10μM)	**16** (1μM)	**16** (1μM)	**16** (1μM)
C	**132** (10μM)	**132** (10μM)	**132** (10μM)	**132** (1μM)	**132** (1μM)	**132** (1μM)	**131** (10μM)	**131** (1μM)	**131** (1μM)	**131** (1μM)	**131** (1μM)	**131** (1μM)
D	**3** (10μM)	**3** (10μM)	**3** (10μM)	**3** (1μM)	**3** (1μM)	**3** (1μM)	**134** (10μM)	**134** (10μM	**134** (10μM	**134** (1μM)	**134** (1μM)	**134** (1μM)
E	**2** (10μM)	**2** (10μM)	**2** (10μM)	**2** (1μM)	**2** (1μM)	**2** (1μM)	**59** (10μM)	**59** (10μM)	**59** (10μM)	**59** (1μM)	**59** (1μM)	**59** (1μM)
F	**27** (10μM)	**27** (10μM)	**27** (10μM)	**27** (1μM)	**27** (1μM)	**27** (1μM)	**28** (10μM)	**28** (10μM)	**28** (10μM)	**28** (1μM)	**28** (1μM)	**28** (1μM)
G	**26** (10μM)	**26** (10μM)	**26** (10μM)	**26** (1μM)	**26** (1μM)	**26** (1μM)	**29** (10μM)	**29** (10μM)	**29** (10μM)	**29** (1μM)	**29** (1μM)	**29** (1μM)
H	**30** (10μM)	**30** (10μM)	**30** (10μM)	**30** (1μM)	**30** (1μM)	**30** (1μM)	**25** (10μM)	**25** (10μM)	**25** (10μM)	**25** (1μM)	**25** (1μM)	**25** (1μM)

Figure 54: a) Results of the thermal shift assay showing T_m for each well. Increases in melting temperature are indicated by a colour change from light-blue to dark–blue (largest increase), decreases from yellow to red (largest decrease). b) Compound tested; final concentration of either 1 or 10 μM; Experiments were done in triplicate.

These initial results suggested the use of the thermal shift assay as an alternative orthogonal screening method for ligand identification and a full screening of all synthesised analogues was conducted by Becky Eno, the results of which are summarised in Table 15.

Entry	Compound	Number	ΔT_m 100 µM	ΔT_m 10 µM	ΔT_m 1 µM
1		**2**	1.10	2.17	0.20
2		**3**	1.17	0.23	0.30
3	 **E1**	**3 E1**	0.47	0.23	0.20
4	 **E2**	**3 E2**	1.20	0.47	0.20
5		**9**	0.20	-0.03	0
6		**12**	0.20	0.23	0.06
7		**13**	0.30	0.13	0.23
8		**14**	0.07	0.17	0.23
9		**16**	0.3	0.33	0.23
10		**25**	0.27	0.33	0.17
11		**26**	0.70	0.17	0.23

12		**27**	-0.1	0.13	0.13
13		**28**	0	0.10	30
14		**29**	-0.17	0.2	0.33
15		**30**	0.17	-0.03	0.30
16		**31**	0.33	0.17	0.27
17		**32**	0.27	0.30	0.27
18		**38**	0.23	0.27	0.23
19		**42**	0.03	0.03	0.17
20		**43**	0.13	0.50	0.20
21		**44**	0.33	0.10	0.20
22		**45**	0.13	0.03	0.20
23		**57**	0.57	0.43	0.20
24		**59**	1.10	0.30	0.13
25		***(S)*-71**	0.47	0.23	0.30

26		**(±)-71**	1.10	0.20	0.20
27		**95**	2.33	0.43	0.17
28		**96**	2.93	0.70	0.27
29		**97**	0.90	0.70	0.40
30		**98**	1.43	0.73	0.20
31		**99**	-0.27	-0.17	-0.10
32		**100**	-0.23	-0.17	-0.10
33		**101**	-0.43	-0.27	-0.23
34		**102**	-0.50	-0.47	-0.40
35		**104**	-0.03	-0.07	-0.30
36		**105**	-0.10	-0.03	0
37		**106**	-0.17	-0.03	0
38		**108**	-0.33	-0.13	-0.07
39		**120**	0.30	0.10	0.20

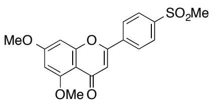
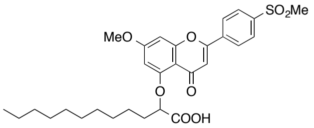
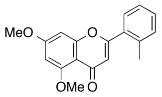
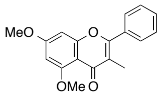
40		**121**	0.30	0.20	0.13
41		**125**	0.87	0.17	0.17
42		**126**	-0.03	-0.03	-0.13
43		**131**	0.20	0.23	0.07

Table 15: Results of the thermal shift assay showing ΔT$_m$ for each compound at a final concentration of 1, 10 or 100 µM. Increases in melting temperature are indicated in blue.

As observed in the preliminary results, ΔT$_m$, the difference between protein melting temperature and the melting temperature upon binding of a ligand, increases most significantly for the aryloxyacetic acid analogues.

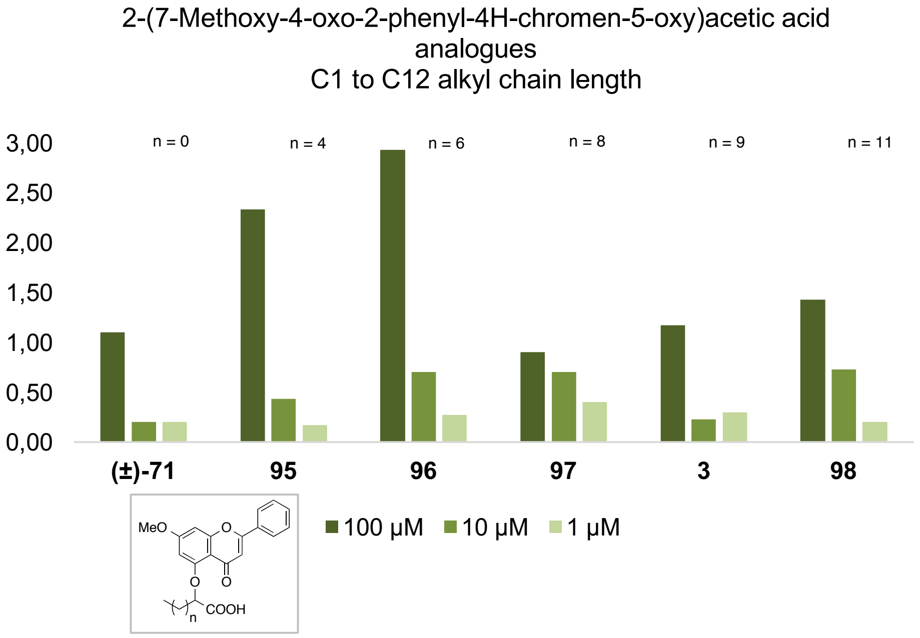


Figure 55: Influence of the alkyl chain length on ΔT$_m$ at a final concentration of 1, 10 or 100 µM.

ΔT_m of nonanoic acid analogue **96** was significantly higher than ΔT_m of propanoic acid compound **(±)-71** and heptanoic acid analogue **95**, which was comparable with the activity of those analogues in the CDNB assay. The decrease of ΔT_m for analogue **97** with an even longer alkyl chain might be due to the limited aqueous solubility, which decreases with the chain length.

Other than in the CDNB assay, the configuration of the flavonoid seemed to have as slightly bigger effect on ΔT_m (Table 15, Entries 3 and 4).

3.2.2 Target site identification

It is important to gain a deeper insight into the interactions between *Am*GSTF1 and its inhibitors. Although the crystal structure of *Am*GSTF1 has been solved, the ligand binding site is unknown. Identification of the binding pocket would allow a structure based design and a more targeted optimization of ligands.

3.2.2.1 Photoaffinity labelling experiments

As mentioned in Chapter 3.1.4, photoaffinity labelling is an effective method in identifying binding sites in proteins. The evaluation of the four synthesised photoaffinity labels in the CDNB assay for their ability to inhibit *Am*GSTF1 (*cf.* Chapter 3.2.1.1) showed that compounds **145** and **160** have very similar activity to that of the parent structure **3**, indicating that the benzophenone moiety does not affect the binding. Therefore those two labels were used for crosslinking experiments.

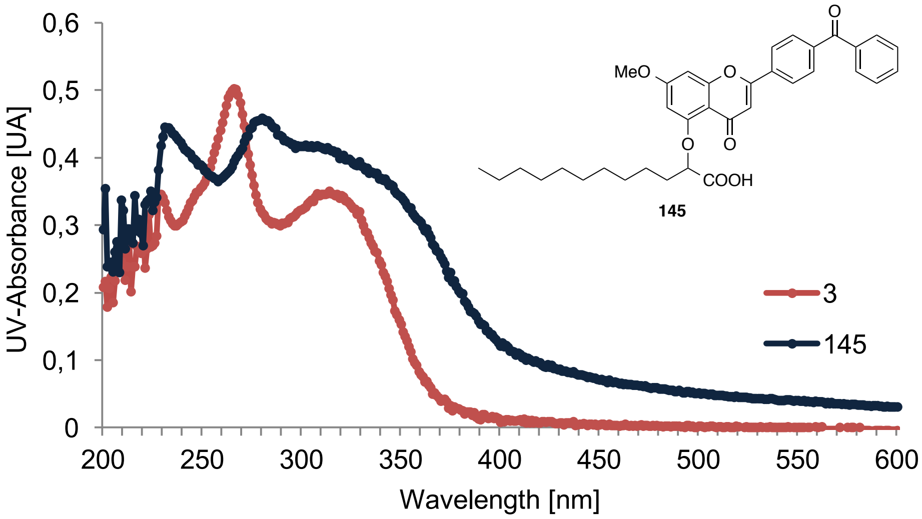


Figure 56: UV-Absorbance spectra of 145 and 3 (solution in H_2O + 2% DMSO); Spectra were measured at room temperature.

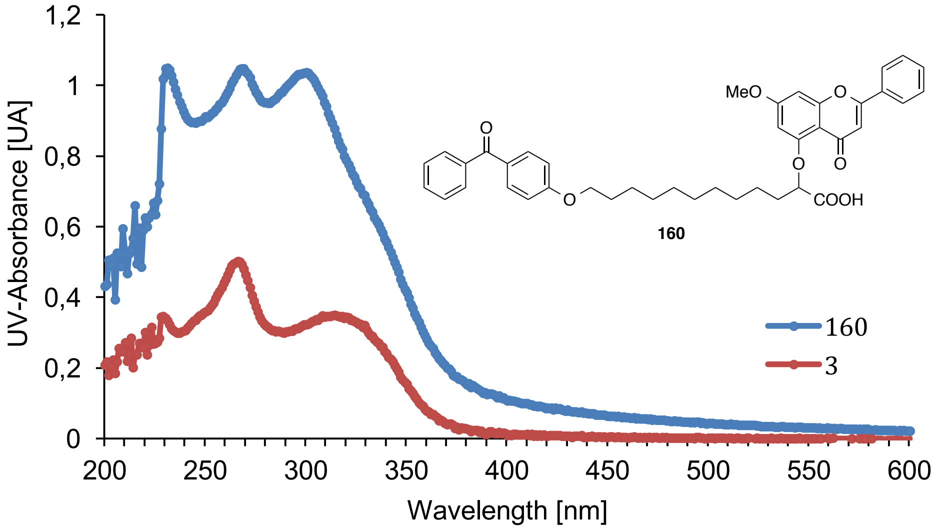


Figure 57: UV-Absorbance spectra of 160 and 3 (solution in H_2O + 2% DMSO); Spectra were measured at room temperature.

Benzophenones are activated at wavelengths between 350 and 360 nm, a range that avoids damage of the target protein.[156] UV-Absorbance spectra of the two compounds (Figure 56 and Figure 57) illustrate absorbance at higher wavelengths compared to compound **3** though maybe not as pronounced as hoped.

To form a protein ligand-complex *Am*GSTF1 was incubated with an approximately 10-fold molar excess of compound **145** or **160** respectively in PBS-buffer and 1% DMSO for 10 min on ice. The protein ligand complex was irradiated for 10 or 30 min with either a laser (355 nm) or an UV-LED lamp (365 nm) at rt. Control experiments with no label were performed to rule out protein damage from the irradiation. Samples were desalted and analysed by electrospray ionization on a QTOF instrument. Unfortunately, no proof of labelling was observed. The photoaffinity labels only seemed to facilitate decomposition of the protein compared to the control experiments.

3.2.2.2 Tests for dimer dissociation

As mentioned in Chapter 1.3.1.1.2, GSTs can act as carrier proteins or ligandins of compounds including plant hormones, plant metabolites and flavonoids.[71-72] Those molecules bind to GSTs in a non-substrate manner at a specific site, the L-site and the exact localisation and nature of this site can vary hugely. Recently Ahmad et al. reported structural evidence for non-catalytic binding sites in the *arapidopsis thaliana* GST *At*GSTF2.[76] Two L-sites were identified, one at the periphery of the dimer (L1) and one situated at the dimer interface (L2). In the complex of the flavonoid quercetin and *At*GSTF2, quercetin was found only at the L2 site (Figure 58). This poses the question, of whether the flavonoid inhibitors of AmGSTF1 bind in the same manner. As the inhibition activity towards AmGSTF1 in the CDNB assay (*cf.* Chapter 3.2.1.1) increases significantly with the alkyl chain length of aryloxyacetic acid analogues (**(±)-71**, **95**, **96**, **97**, **3** and **97**), it was speculated that the alkyl chain might be sticking out of a binding pocket and disrupting the protein-protein interactions at the dimer interface. As most GSTs are only active as dimers, any disruption of the interface could lead to dissociation and this may be the mechanism of the observed inhibition. Consequently, experiments were conducted to test this theory.

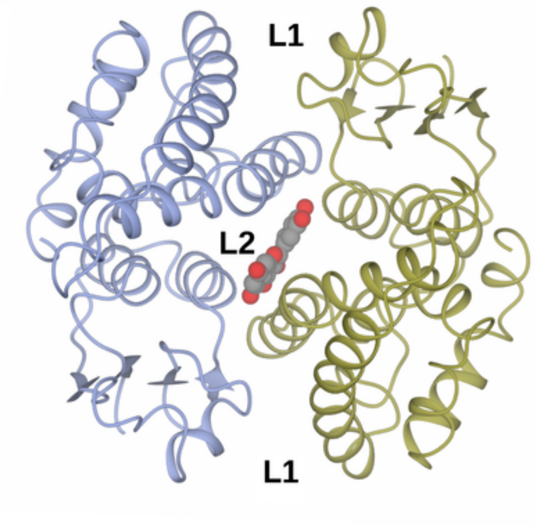


Figure 58: Quercetin-complex with AtGSTF2-dimer, showing location of the two binding sites L1 and L2.[76]

3.2.2.2.1 Native Protein Gel

The simplest and most direct way to test whether the inhibitors are disrupting protein-protein interactions and causing GST-dimer dissociation was running a native protein gel. Proteins are usually separated by polyacrylamide gel electrophoresis (PAGE) under denaturing and often reducing conditions in the presence of a detergent like sodium dodecyl sulfate (SDS). Consequently, in SDS PAGE, *Am*GSTF1 is present in its monomeric form. SDS shields the respective charge of the proteins and provides all proteins with a negative charge, which results in a separation according to the protein weight. In native protein gels the mobility of proteins depends on a number of factors in addition to molecular weight, including protein shape and charge. Because no detergent is present and no denaturing or reducing conditions are applied, the tertiary and quaternary structure of proteins is retained.[163]

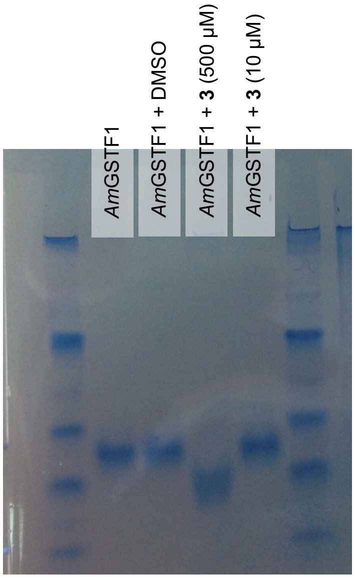


Figure 59: Native-PAGE of *Am*GSTF1 in the presence of different concentrations of inhibitor 44.

*Am*GSTF1 was incubated with two different concentrations of inhibitor **3** (10 and 500 µM) and subsequently a native PAGE was performed (Figure 59). At the higher inhibitor concentration, the protein band travels faster within the gel, which could be due to dimer dissociation. However, as only very high concentrations of inhibitor showed a positive result, further experiments were conducted, looking into a possible dimer dissociation of *Am*GSTF1.

3.2.2.2.2 Fluorescence anisotropy

To confirm the dimeric nature of AmGSTF1 in the presence of inhibitor **3**, a steady state fluorescence anisotropy experiment was performed (Figure 60). Fluorescence anisotropy correlates to the rotational diffusion of a macromolecule and therefore to its molecular weight. *Am*GSTF1 was labelled with the fluorophore HiLyte Fluor 488, which was used by Fabrini et al.[164] to examine the monomer-dimer equilibrium of GSTs in a fluorescence anisotropy experiment.

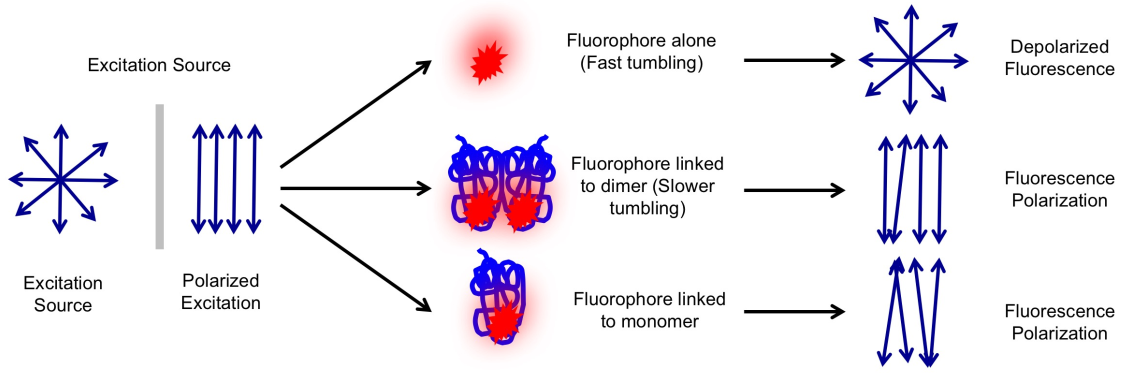


Figure 60: Theory behind the fluorescence anisotropy experiment.

Upon excitation with polarized light the emission of HiLyte Fluor 488-labeled *Am*GSTF1 is also polarized. Anisotropy describes the extent of polarization and its origin is the existence of transition moments for absorption and emission, that lie along specific directions within the fluorophore. The anisotropy depends on the viscosity of the solvent and the size and shape of the molecule, as it depends on the angular displacement between absorption and emission of the photon, which is caused by rotational diffusion. Hence the anisotropy of the dimer is expected to be higher than for the monomer. Small molecules, such as the unbound fluorophore, are fast tumbling and the rate of rotational diffusion is faster than the rate of emission, which is therefore depolarized and the anisotropy is close to zero. The maximum anisotropy value that can theoretically be observed is 0.4, when there are no depolarization processes and absorption and emission dipoles are colinear. [165] The fluorescence anisotropy was measured for two different protein concentrations (20 and 100 nm) and four different concentrations of inhibitor **3** (1, 10, 100 and 200 µM).

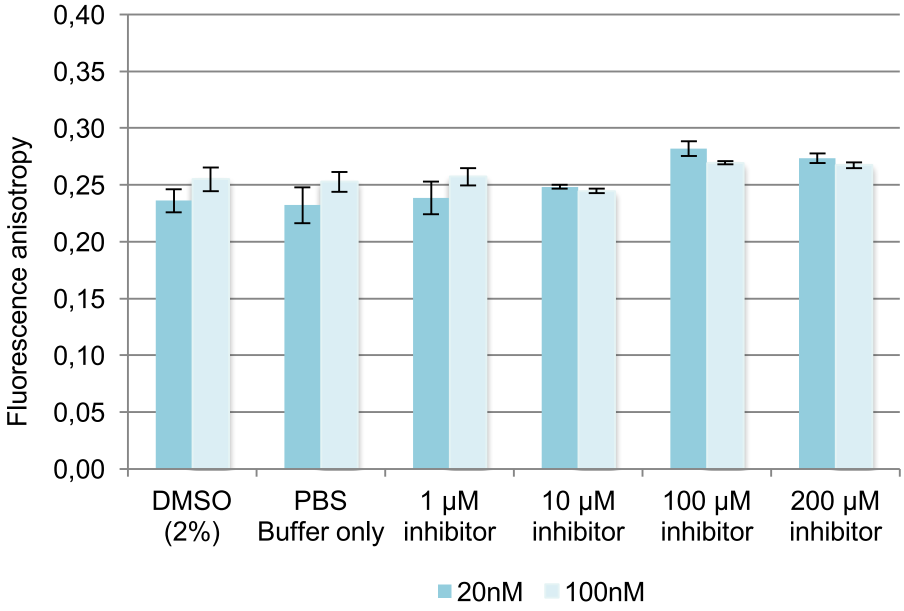


Figure 61: Steady-state anisotropy of the HiLyte Fluor 488 emission of labelled *Am*GSTF1 in the presence of different concentrations of inhibitor 3. Appropriate controls with buffer only and DMSO only were performed and each value is the result of eight replicate measurements.

No appreciable variations in the steady-state anisotropy were found (Figure 61). The measured fluorescence anisotropy was not dependent on the inhibitor concentration and the expected decrease in anisotropy for the dimer dissociation could not be observed, suggesting that no such dissociation is taking place.

3.2.2.3 Tests on plants

The CDNB assay is a relatively quick and easy method to screen a number of compounds, but as *Am*GSTF1 shows only limited GSH conjugation activity and most importantly is believed to have important signalling functions promoting MHR, it can only give a first indication. The assay cannot reflect the complicated mechanisms in MHR black grass.

3.2.2.3.1 Maize

It is well established that GSTs in maize play an important role in protecting the crop from phytotoxic effects caused by herbicides.[60, 166-167] Herbicide selectivity for weeds versus maize can be caused by either a higher constitutive GST expression or an accumulation of GSTs following a treatment with herbicide safeners.[168-169] One of the two major GST enzymes in maize identified to be responsible for herbicide detoxification is the constitutively expressed GSTI[169], which is now described as *Zm*GSTF1 due to a new classification system.[60]

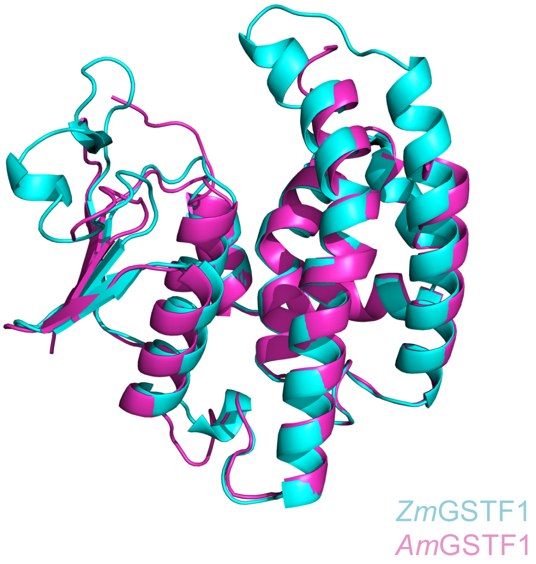


Figure 62: Comparison of the *Zm*GSTF1 and *Am*GSTF1 crystal structure.

*Zm*GSTF1 has a 63.4% sequence identity to *Am*GSTF1 and its crystal structure[170] is very similar to that of *Am*GSTF1 (Figure 62).

Because of those properties in maize, it was considered whether synergists might have a similar effect on maize and if they could be tested on maize instead of black grass. This would simplify the testing, as maize is much easier to germinate and grows faster.

Previous tests on black grass had been conducted using a Potter Precision spray tower, a chemical spraying apparatus, to achieve some reproducibility. The spray tower was increased in height by 10 cm to accommodate a plant instead of a petri dish, which it was built for (Figure 63). Measuring the collected volume following spraying of a known start volume allowed to calculate an approximate field rate in l/ha (Figure 63). It is, however, difficult to translate this into an actual field rate and to know how much of the solution is hitting the plant. It was deemed more appropriate to describe the dosage in terms of standard measurements such as concentration of active ingredient and volume of formulation.

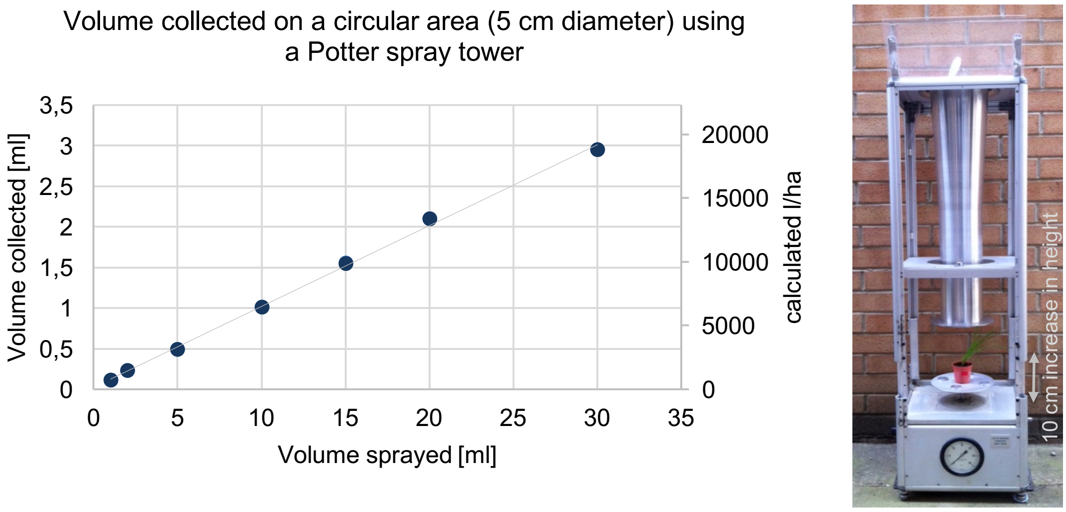
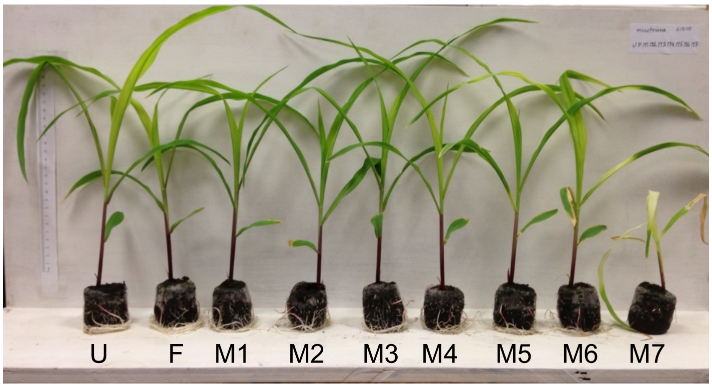


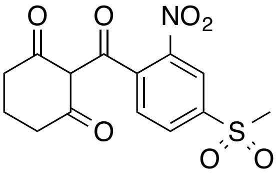
Figure 63: Calculated approximate field rate using the spray tower at 15 psi and modified Potter spray tower.

Before any synergists could be tested, experiments focused on finding an appropriate herbicide concentration, which would not kill the plant. The herbicides were formulated as an emulsion using 2 % DMSO, and 0.5 % Agridex, a non-ionic surfactant, in H_2O. Multiple concentrations of the herbicides mesotrione **163** (7.8125 µM to 500 µM), nicosulfuron **164** (15.625 µM to 1 mM), chlortoluron **165** (31.25 µM to 1 mM), pinoxaden **166** (31.25 µM to

1 mM) and clodinafop-propargyl **167** (31.25 µM to 1 mM) were sprayed on maize (Figure 64 to Figure 68). Mesotrione **163** and nicosulforon **164** are used to control weeds in maize fields and chlortoluron **165**, pinoxaden **166** and clodinafop-propargyl **167** are herbicides MHR black grass is resistant to and have previously been used for synergist tests on black grass. The plants were sprayed 7 days after planting and 20 ml of every solution was used. Appropriate controls including untreated plants and formulation only were included. Pictures were taken 7 days after the treatment.

Mesotrione

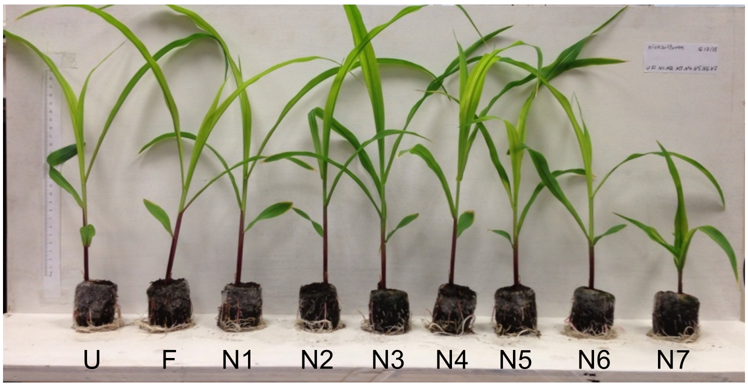


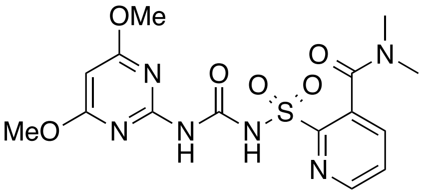


163

Figure 64: U: Untreated; F: Formulation only; M: Mesotrione; 1: 7.8125 µM; 2: 15.625 µM; 3: 31.25 µM; 4: 62.5 µM; 5: 125 µM; 6: 250 µM; 7: 500 µM;

Nicosulfuron

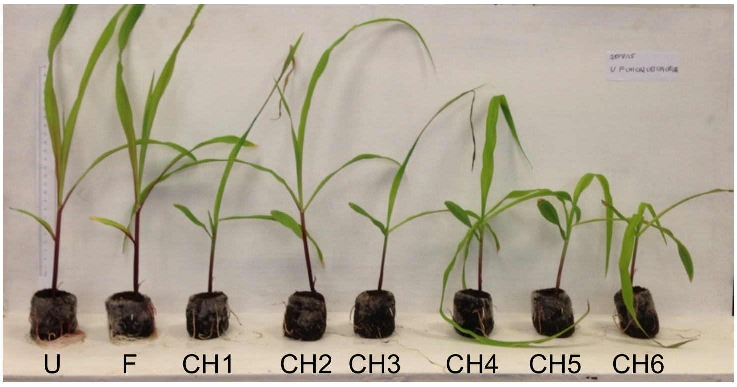


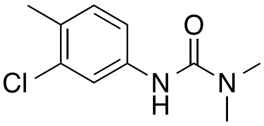


164

Figure 65: U: Untreated; F: Formulation only; N: Nicosulfuron; 1: 15.625 µM; 2: 31.25 µM; 3: 62.5 µM; 4: 125 µM; 5: 250 µM; 6: 500 µM; 7: 1 mM;

Chlortoluron

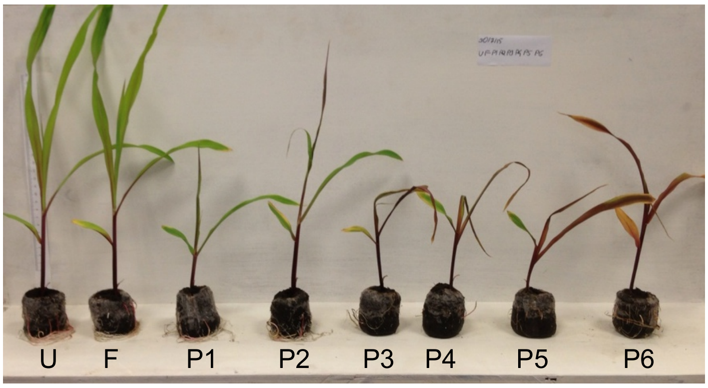


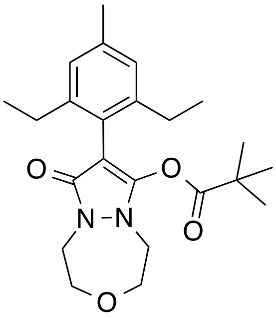


165

Figure 66: U: Untreated; F: Formulation only; Ch: Chlortoluron; 1: 31.25 µM; 2: 62.5 µM; 3: 125 µM; 4: 250 µM; 5: 500 µM; 6: 1 mM;

Pinoxaden

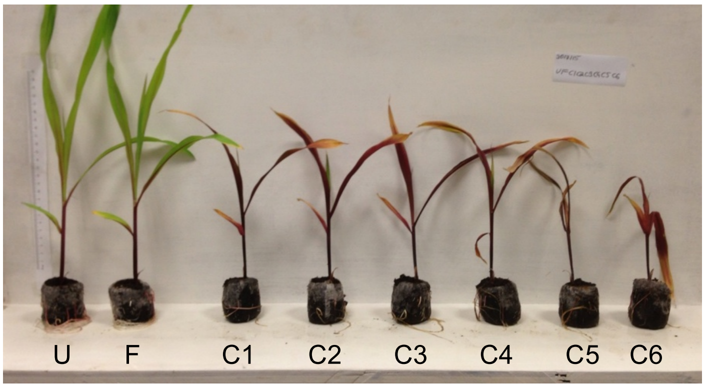


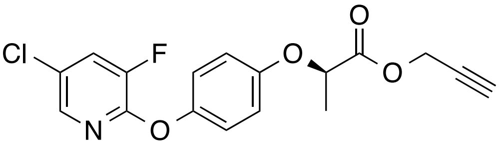


166

Figure 67: U: Untreated; F: Formulation only; P: Pinoxaden; 1: 31.25 µM; 2: 62.5 µM; 3: 125 µM; 4: 250 µM; 5: 500 µM; 6: 1 mM;

Clodinafop-propargyl





167

Figure 68: U: Untreated; F: Formulation only; C: Clodinafop-propargyl; 1: 31.25 µM; 2: 62.5 µM; 3: 125 µM; 4: 250 µM; 5: 500 µM; 6: 1 mM;

In order to see if DMSO alone could have an impact on the plant, different concentrations of DMSO were sprayed on maize (1 to 20%, Figure 69), but no effect could be observed.

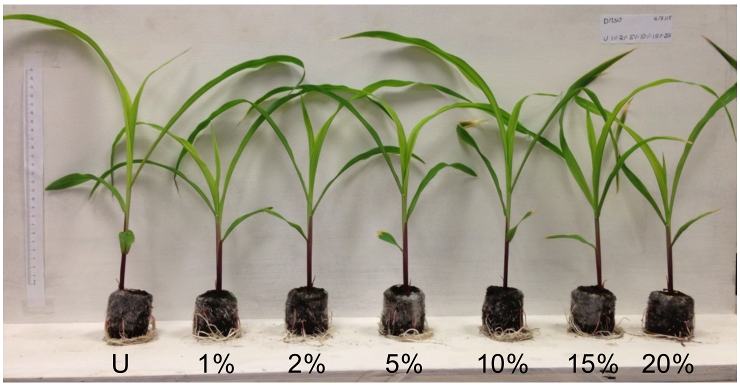


Figure 69: U: Untreated; % DMSO in H_2O.

As for mesotrione **163** (Figure 64) and nicosulfuron **164** (Figure 65) only high herbicide concentrations led to stunted growth and discolouration in the case of mesotrione **163**, concentrations of 30 µM and 250 µM were picked for further experiments. Concentrations of 30 µM and 60 µM were chosen for chlortoluron **165** (Figure 66) and because pinoxaden **166** (Figure 67) and clodinafop-propargyl **167** (Figure 68) showed effects on the plant at much lower concentrations, concentrations of 7.5 µM and 15 µM were selected for the spray trials.

Having optimised the herbicide concentrations, synergism spray trials were carried out with flavonoid **3** and CNBF **1**. The synergists were formulated identically to the herbicides at a 2 mM concentration. 20 ml of the synergist or formulation only emulsion were sprayed on the plant 7 days after planting. 24 h later either a herbicide solution or a formulation only control was applied. The plants were assessed 7 days after the treatment and again appropriate controls were carried out.

Mesotrione

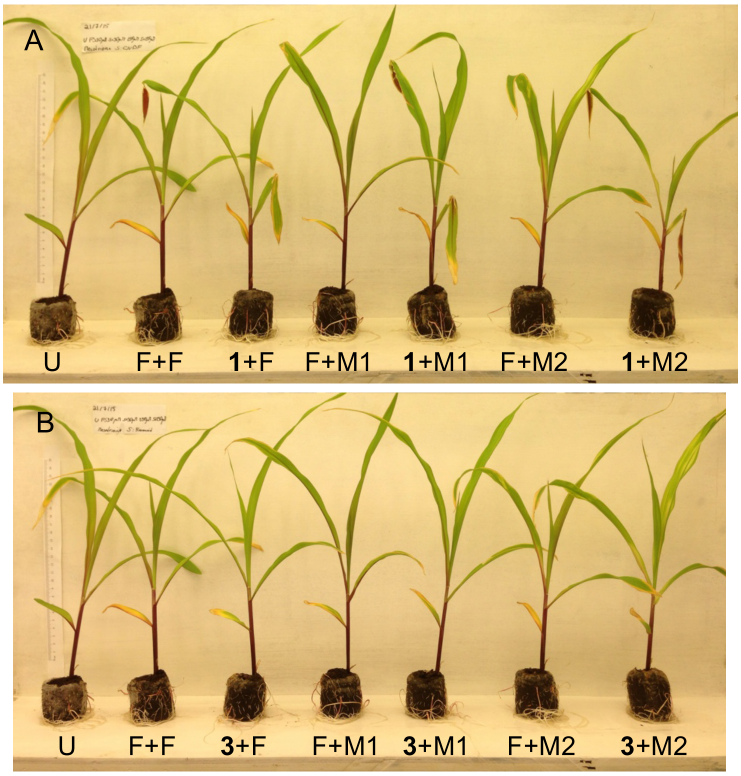


Figure 70: U: Untreated; F: Formulation only; M1: 30 µM Mesotrione; M2: 250 µM Mesotrione; 3: 2 mM synergist; 1: 2 mM synergist;

Nicosulfuron

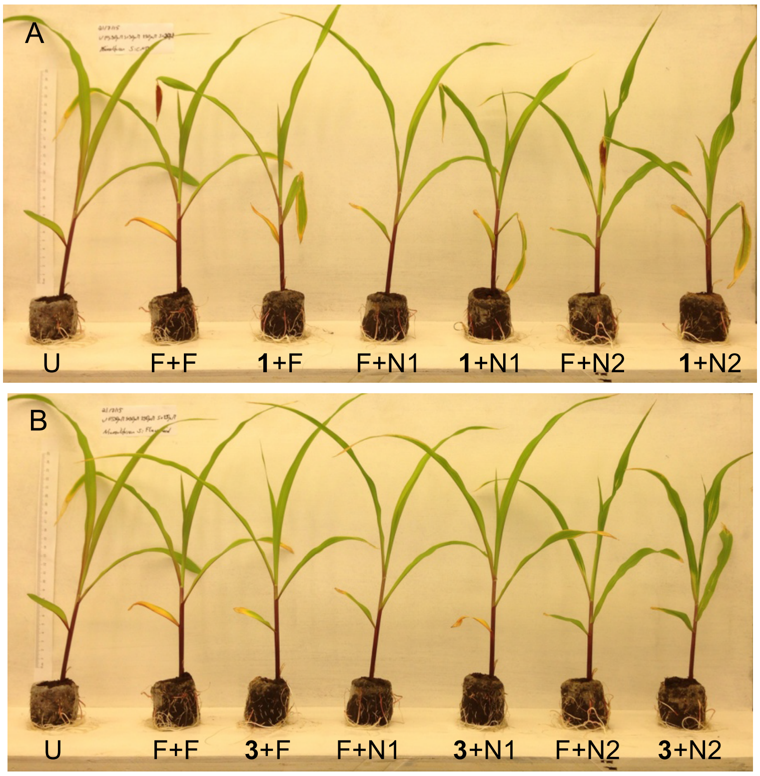


Figure 71: U: Untreated; F: Formulation only; M1: 30 µM Nicosulfuron; M2: 250 µM Nicosulfuron; 3: 2 mM synergist; 1: 2 mM synergist.

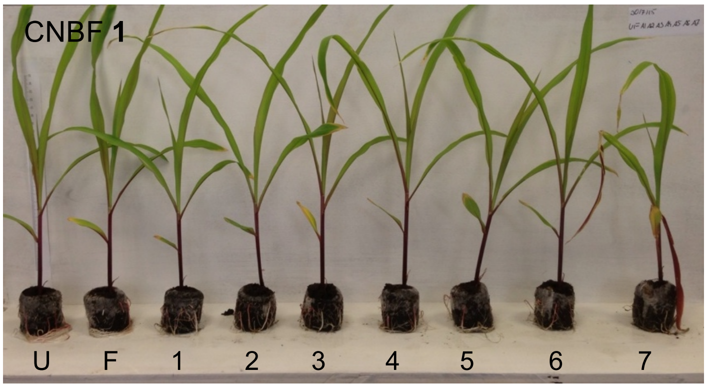


Figure 72: U: Untreated; F: Formulation only; 1: 31.25 µM; 2: 62.5 µM; 3: 125 µM; 4: 250 µM; 5: 500 µM; 6: 1 mM; 7: 2 mM;

Because the 2 mM solution of CNBF **1** caused the maize leaves to bend down and their tips to eventually die in the spray trials with mesotrione **163** (Figure 70, A) and nicosulfuron **164** (Figure 71, A), different concentrations of CNBF **1** were assessed for their effects on maize (Figure 72). The concentration was reduced to 1 mM for the next experiments.

Chlortoluron

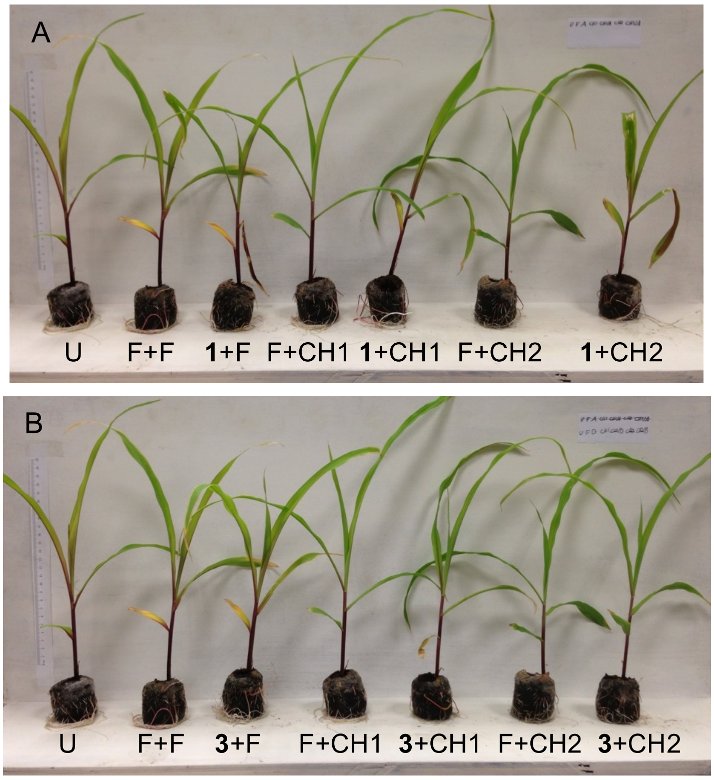


Figure 73: U: Untreated; F: Formulation only; CH1: 30 µM Chlortoluron; CH2: 60 µM Chlortoluron; 3: 2 mM synergist; 1: 1 mM synergist;

Pinoxaden

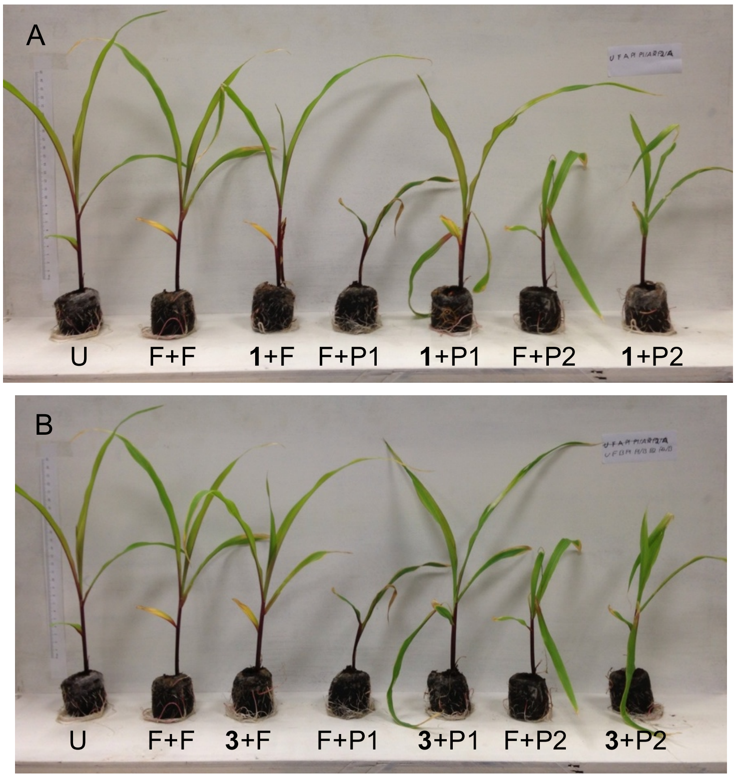


Figure 74: U: Untreated; F: Formulation only; P1: 7.5 μM Pinoxaden; P2: 15 μM Pinoxaden; 3: 2 mM synergist; 1: 1 mM synergist;

Clodinafop-propargyl

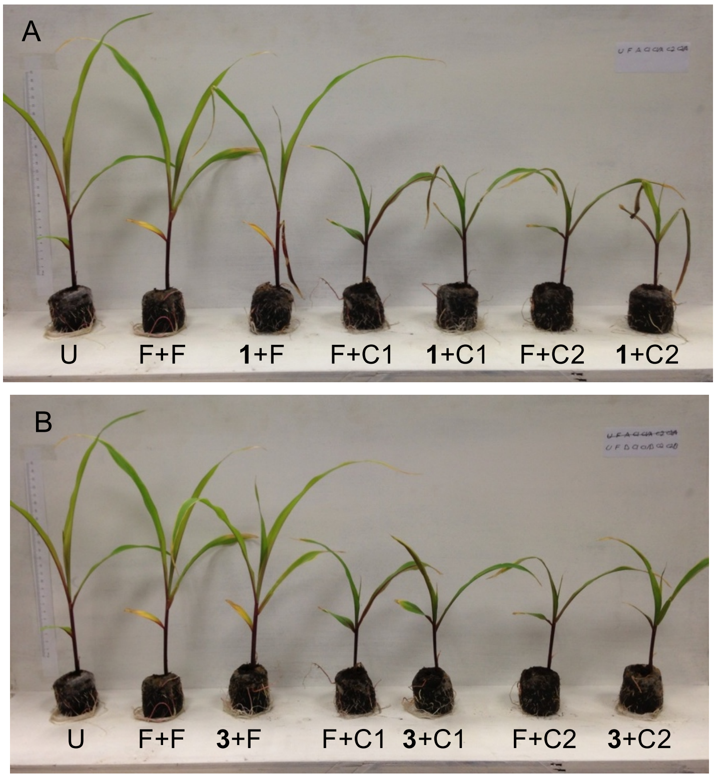


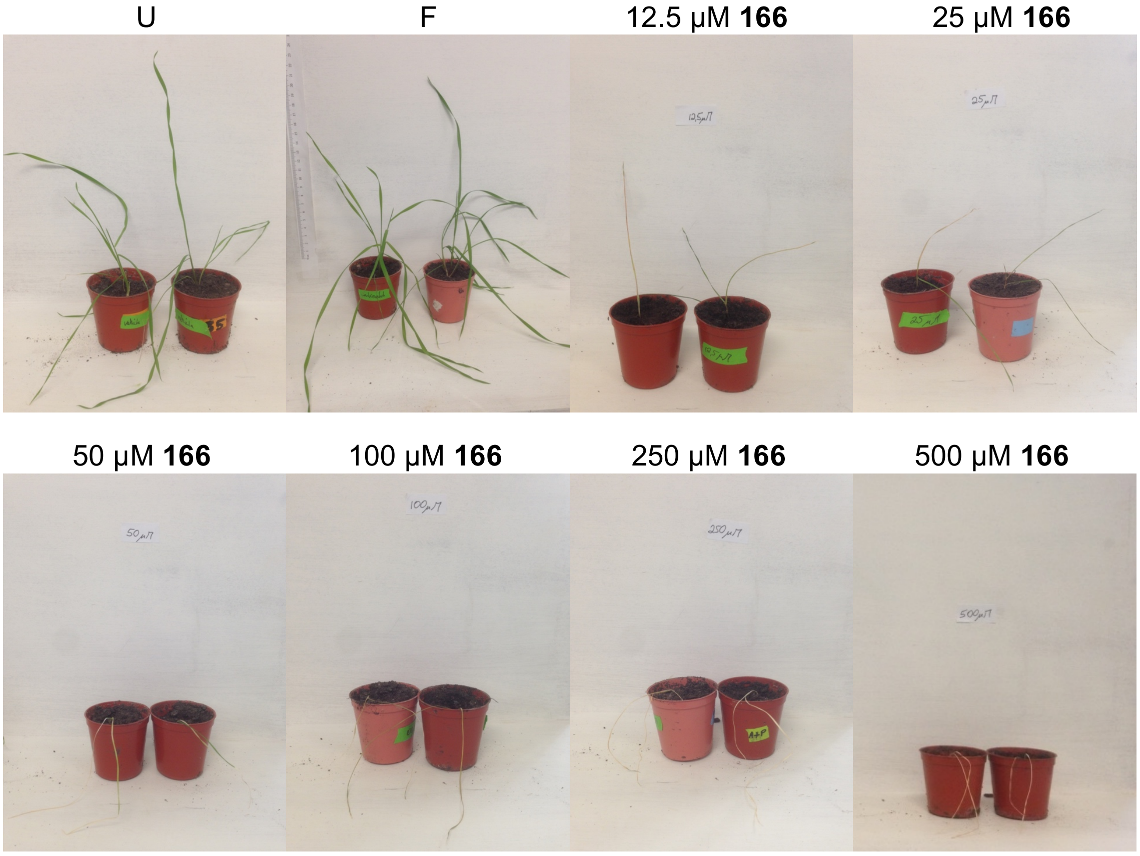
Figure 75: U: Untreated; F: Formulation only; C1: 7.5 μM Clodinafop-propargyl; C2: 15 μM Clodinafop-propargyl; 3: 2 mM synergist; 1: 1 mM synergist;

However, no synergistic effects could be observed for either flavonoid **3** or CNBF **1** with any of the herbicides used (Figure 70, Figure 71 and Figure 73 to Figure 75). Consequently, potential synergists were tested on black grass.

3.2.2.3.2 Black Grass

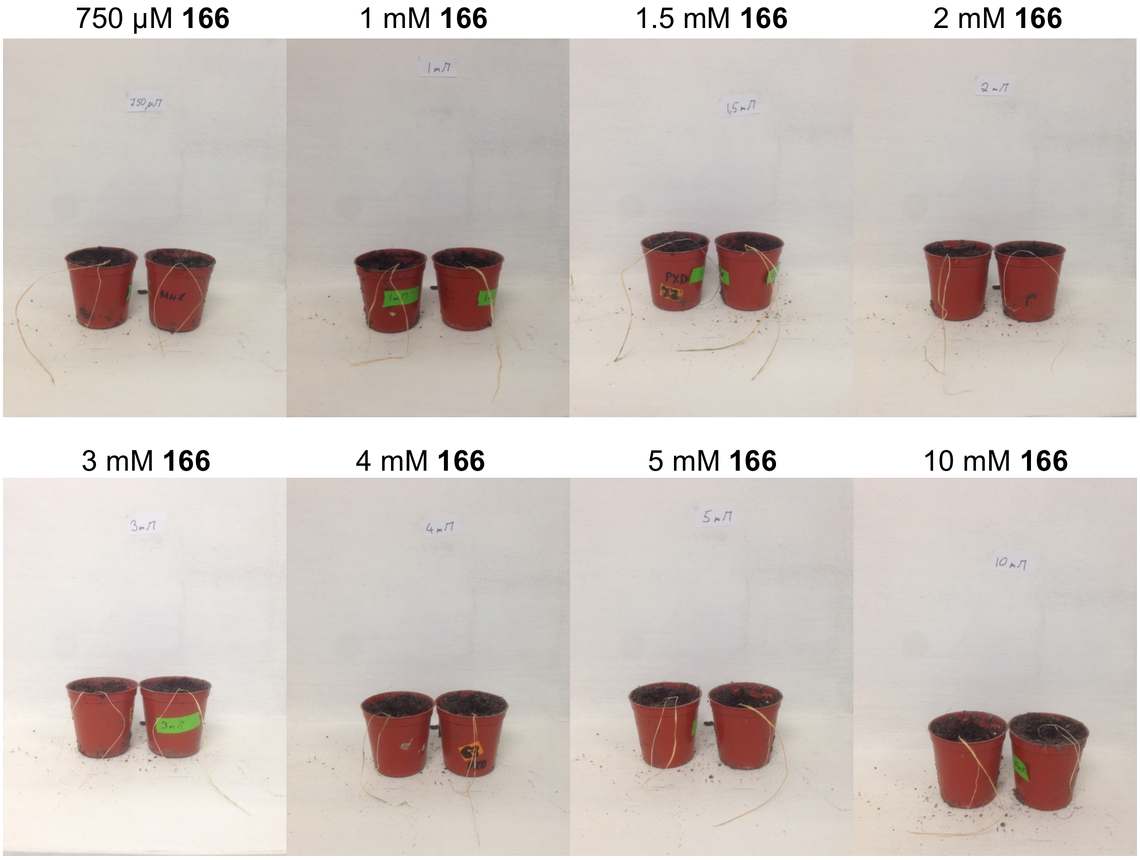
In order to evaluate their ability to act in synergism with the herbicide pinoxaden **166**, a number of compounds were tested directly on black grass.

Pinoxaden **166** is a selective post emergence herbicide, that inhibits the enzyme Acetyl-CoA-Carboxylase. It was developed to control annual grass weeds, including black grass, in cereal crops.[171] In order to determine the lethal pinoxaden **166** dose in WT black grass, different concentrations of pinoxaden **166**, ranging from 12.5 µM to 10 mM, were applied to the plant (Figure 76).



U F 12.5 µM **166** 25 µM **166**

50 µM **166** 100 µM **166** 250 µM **166** 500 µM **166**



750 µM **166** 1 mM **166** 1.5 mM **166** 2 mM **166**

3 mM **166** 4 mM **166** 5 mM **166** 10 mM **166**

Figure 76: Determination of a lethal pinoxaden 166 dose in WT black grass; U: Untreated, F: Formulation;

The herbicide was formulated as an emulsion in water, using 5 % acetone and 0.5 % Adigor, which is a methylated rapeseed oil based adjuvant. Five 2 µl drops of the emulsion were applied with a micropipette 14 days after germination at the two leaf stage. This form of application, rather than spraying the plants using the spray tower, was chosen because of the easy reproducibility and the much lower amount of synergist that is needed.

For comparison one plant was left untreated and one was treated with formulation only. All experiments were done in duplicate and plants were assessed 21 days after the treatment.

As even the lowest concentration of 12.5 µM proved to be lethal, another set of experiments was designed, this time also taking the formulation parameters into account (Figure 77).

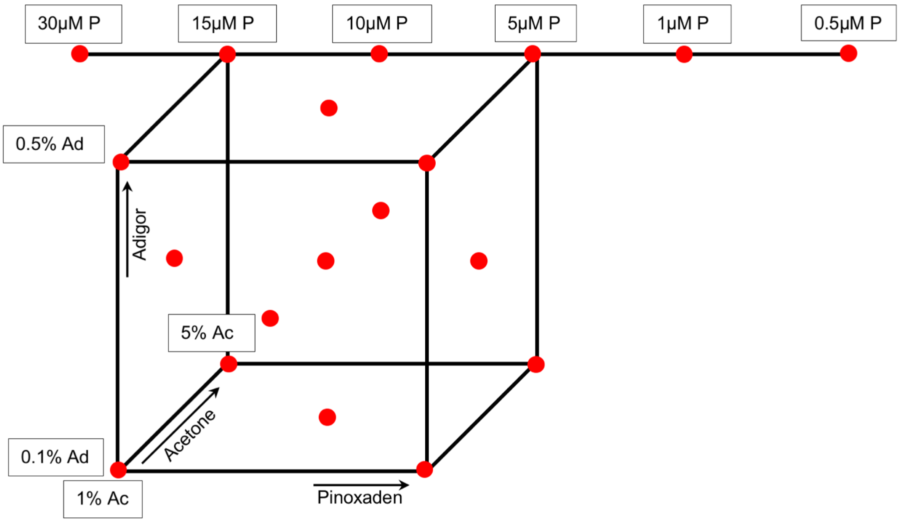
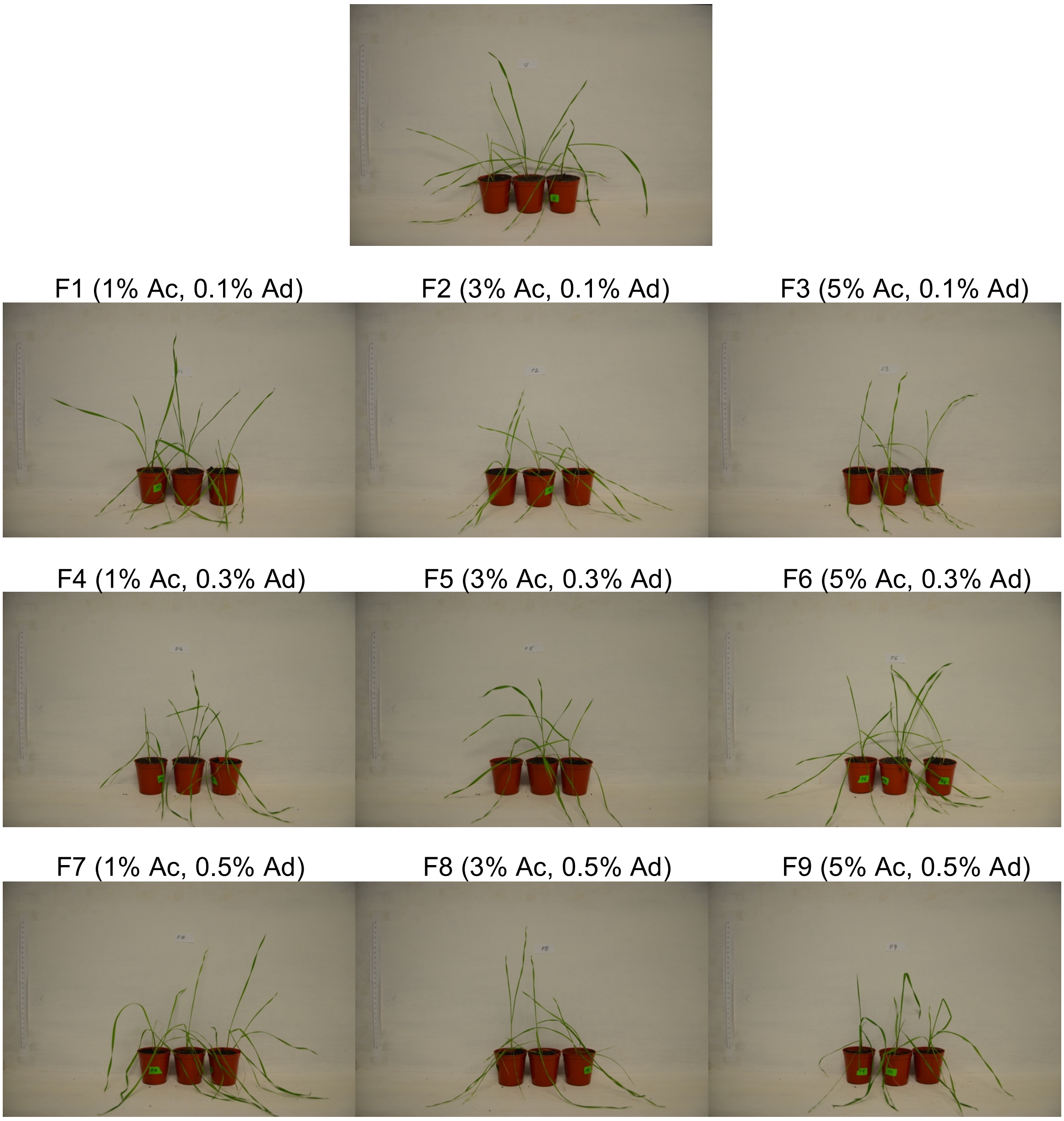


Figure 77: Set of experiments to determine a lethal pinoxaden dose in WT black grass; each dot represents an experiment; Ad: Adigor, Ac: Acetone, P: Pinoxaden 166;

The concentration of pinoxaden ranged from 0.5 µM to 30 µM, using the same formulation parameters as before. Additionally, the percentage of Adigor and acetone were varied for 5 µM, 10 µM and 15 µM pinoxaden.

No big differences were observed, comparing the WT black grass plants treated with different formulations to untreated WT black grass (Figure 78) after a period of 21 days.

10 µM (Figure 80) and 15 µM (Figure 81) pinoxaden **166** proved to be lethal for WT black grass regardless of the formulation used. For 5 µM pinoxaden (Figure 79), however, some plants seemed to survive the treatment for formulations F1, F3, F5 and F7. Stunted growth was observed but not all of the plants died. With formulation F9 5 µM pinoxaden was a lethal dose for all three plants. Further reduction of the pinoxaden concentration to 1 µM and 0.5 µM proved to be not lethal (Figure 82).



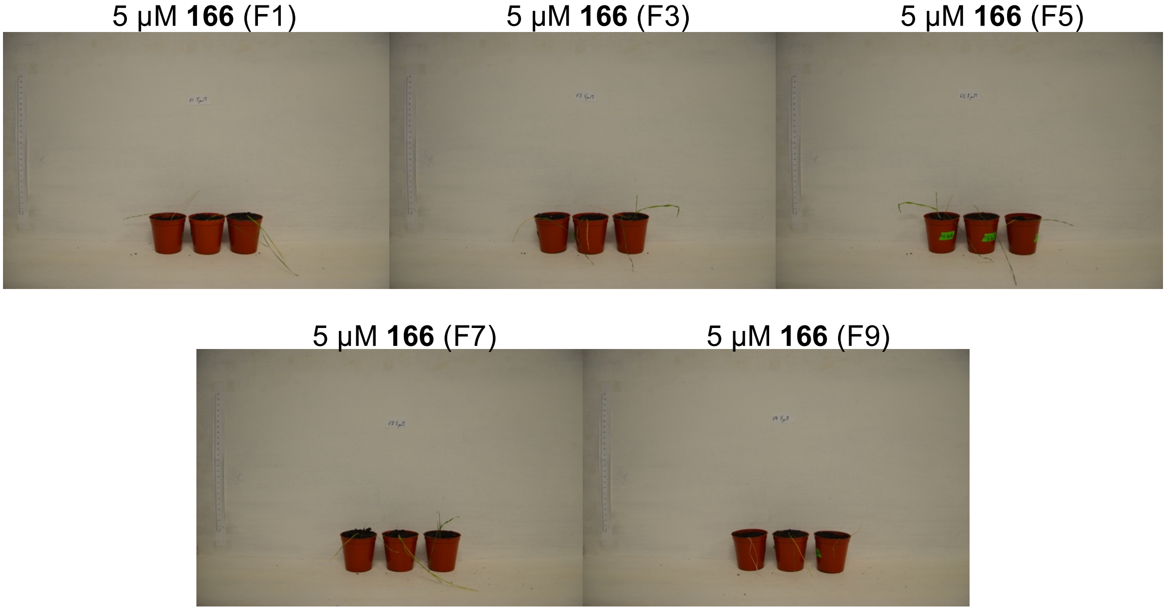
U

F1 (1% Ac, 0.1% Ad)
F2 (3% Ac, 0.1% Ad)
F3 (5% Ac, 0.1% Ad)

F4 (1% Ac, 0.3% Ad)
F5 (3% Ac, 0.3% Ad)
F6 (5% Ac, 0.3% Ad)

F7 (1% Ac, 0.5% Ad)
F8 (3% Ac, 0.5% Ad)
F9 (5% Ac, 0.5% Ad)

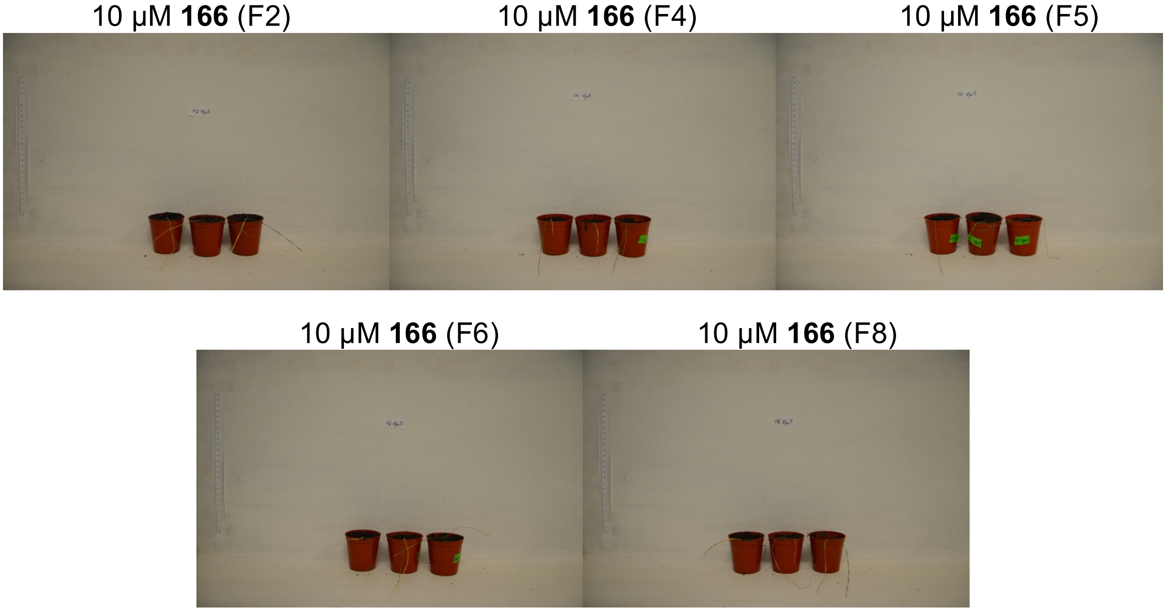
Figure 78: Comparison of WT black grass treated with different formulations (F1 – F9) to untreated WT black grass (U); Ad: Adigor, Ac: Acetone;



5 µM **166** (F1) 5 µM **166** (F3) 5 µM **166** (F5)

5 µM **166** (F7) 5 µM **166** (F9)

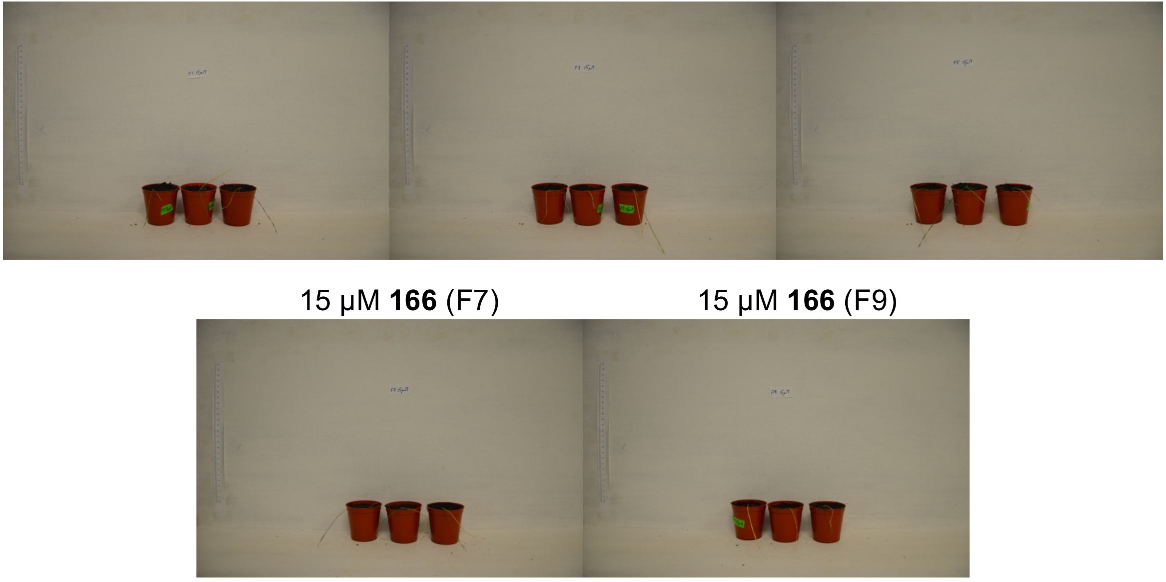
Figure 79: Comparison of different formulations for the treatment of WT black grass (F1, F3, F5, F7, F9) with a 5 µM pinoxaden 166 concentration;



10 µM **166** (F2) 10 µM **166** (F4) 10 µM **166** (F5)

10 µM **166** (F6) 10 µM **166** (F8)

Figure 80: Comparison of different formulations for the treatment of WT black grass (F2, F4, F5, F6, F8) with a 10 µM pinoxaden 166 concentration;



15 µM **166** (F1)

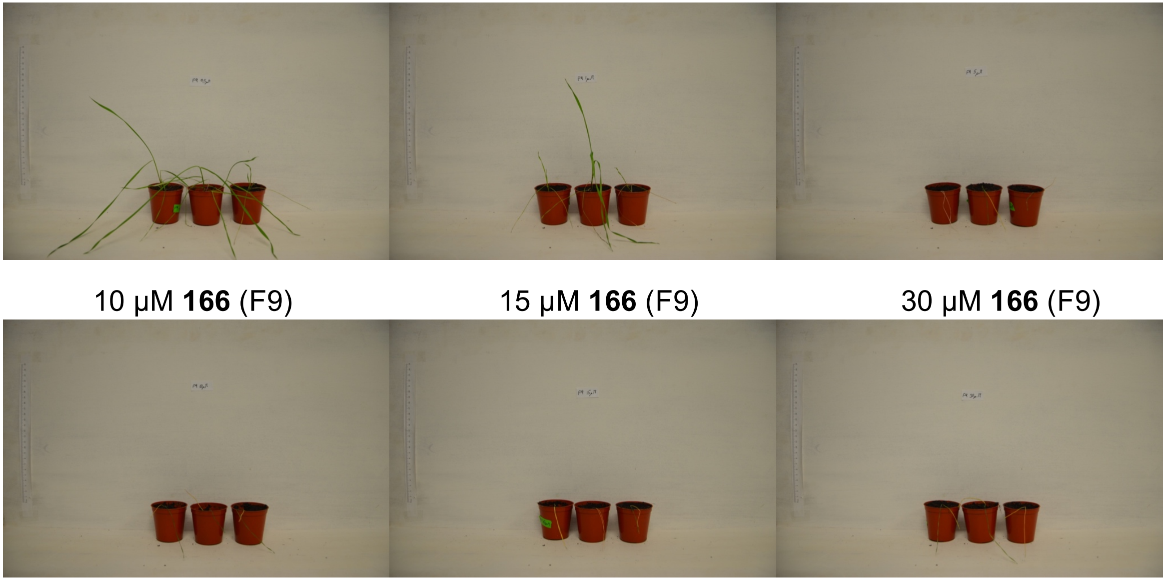
15 µM **166** (F3)

15 µM **166** (F5)

15 µM **166** (F7)

15 µM **166** (F9)

Figure 81: Comparison of different formulations for the treatment of WT black grass (F1, F3, F5, F7, F9) with a 15 µM pinoxaden 166 concentration;



0.5 µM **166** (F9)

1 µM **166** (F9)

5 µM **166** (F9)

10 µM **166** (F9)

15 µM **166** (F9)

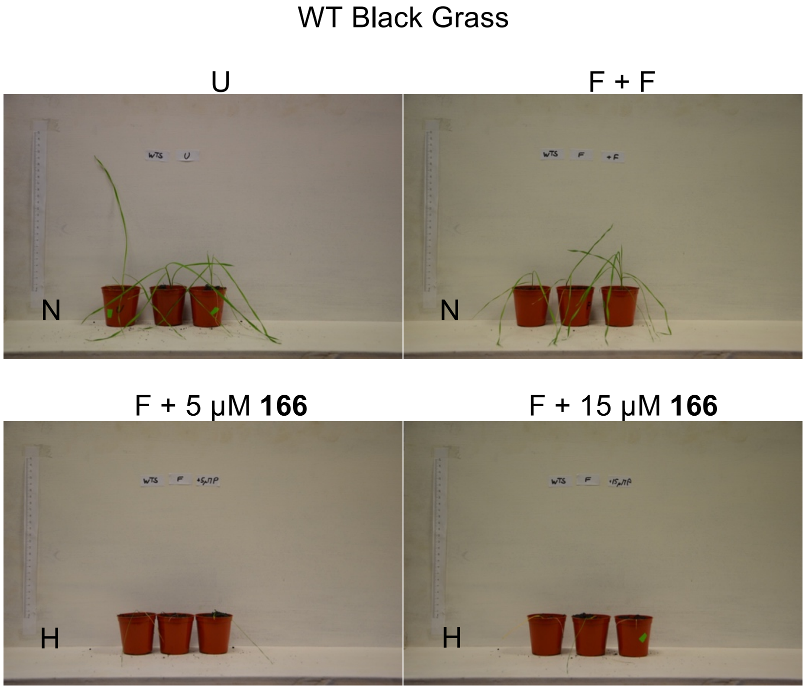
30 µM **166** (F9)

Figure 82: Determination of a lethal pinoxaden 166 dose in WT black grass;
F9 (5% Ac, 0.5% Ad);

To test the ability of compounds to act in synergism with pinoxaden **166** on MHR black grass, seven inhibitors were selected for trials. Compound **16** was chosen in order to have a comparison to the other compounds and see the effects of structural changes. To find out if the alkyl chain length of the substituent in the 5-position has an impact on the synergistic effect, analogues **(±)-71**, **95** and **3** were picked. Compound **125** was selected to see if the SO_2Me-group might have an effect on plant uptake and show different results compared to compound **3**. Both tetrazole **101** and thiazole **102** were selected for their better physicochemical properties compared to **16**.

As 5 µM pinoxaden **166** was the lowest lethal dose for WT black grass and it showed consistent results with formulation F9, this herbicide formulation was chosen for the tests on MHR black grass. Additionally, experiments were carried out using 15 µM pinoxaden **166** with formulation F9. The synergists were formulated identically at a 2 mM concentration. Compound **102** was additionally tested at a 6 mM, 0.6 mM and 0.2 mM concentration. Appropriate controls were carried out, including untreated plants, formulation only and herbicide only for WT black grass (Figure 83) and MHR black grass (Figure 84). In all cases 10 µl of the emulsion/suspension were applied with a micropipette. The black grass plants were treated 14 days post emergence with either a synergist or a formulation only control. 24 h later either a herbicide solution or a formulation only control was applied. The synergistic effect and consequently herbicide damage on the plant was rated from no damage (N) to low damage (L), medium damage (M) and high damage (H).

As expected, a dose of 5 or 15 µM pinoxaden **166** was lethal for WT black grass (Figure 83), but not MHR black grass (Figure 84).



WT Black Grass

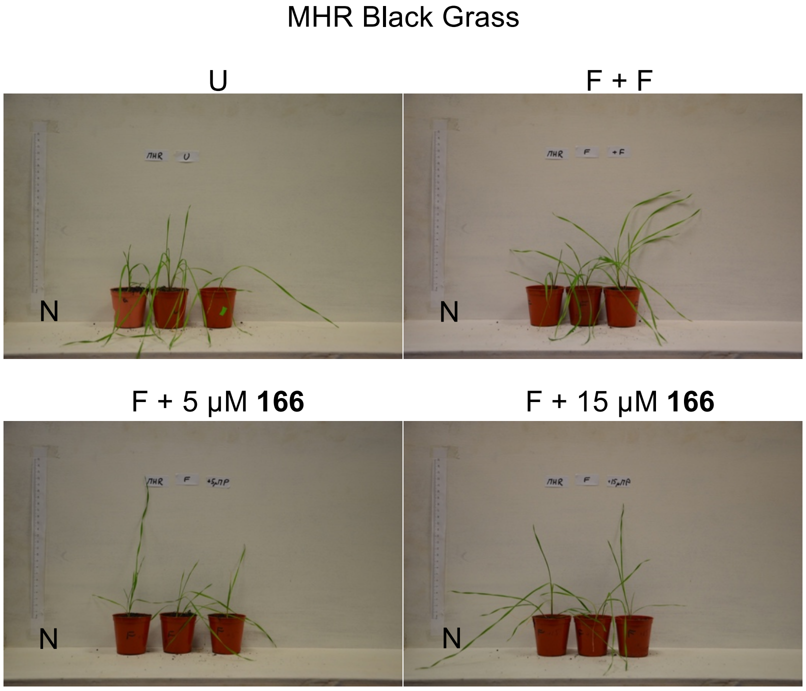
U

F + F

F + 5 µM **166**

F + 15 µM **166**

Figure 83: Effects of pinoxaden 166 on WT black grass; U: Untreated, F: Formulation;



MHR Black Grass

U

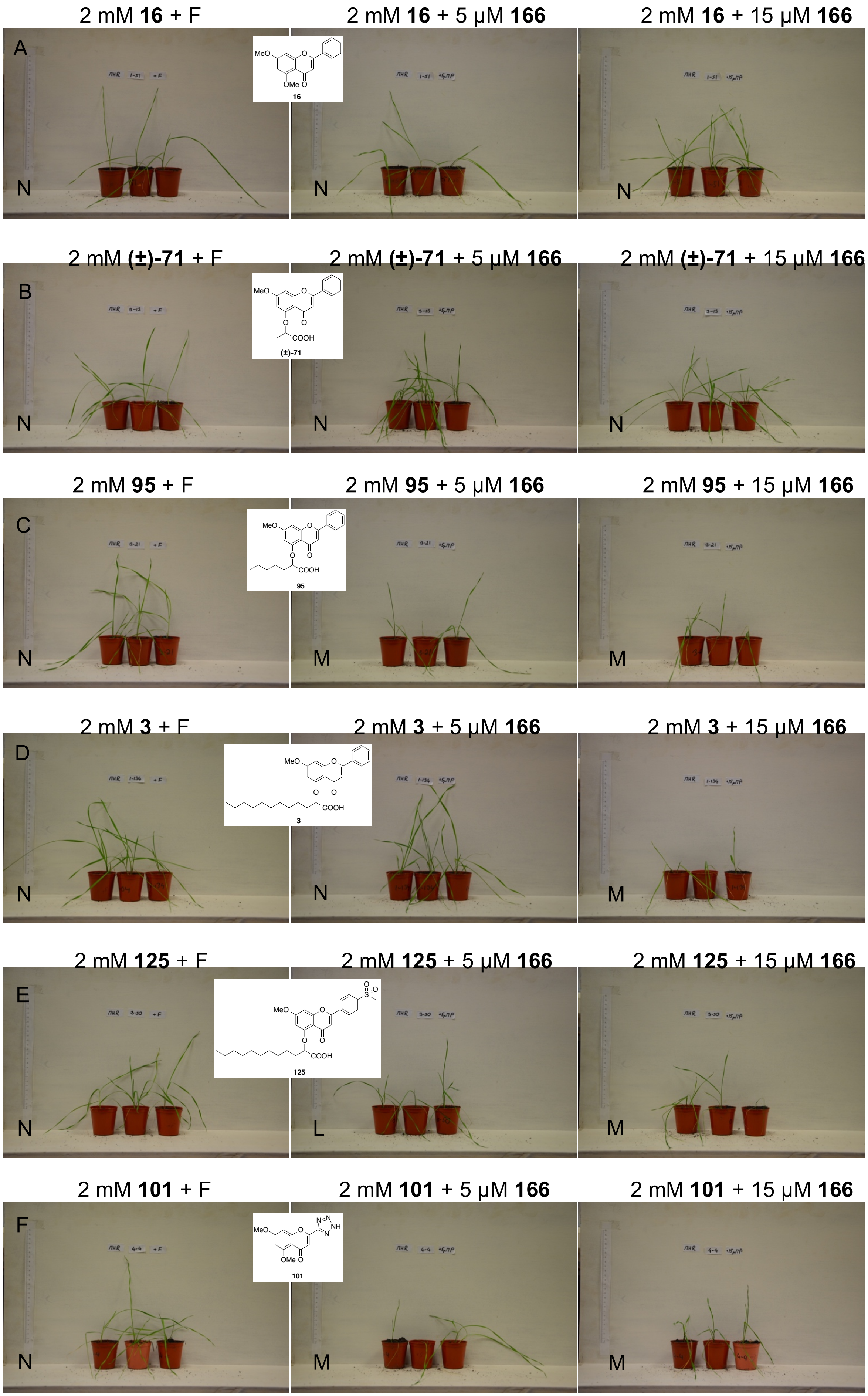
F + F

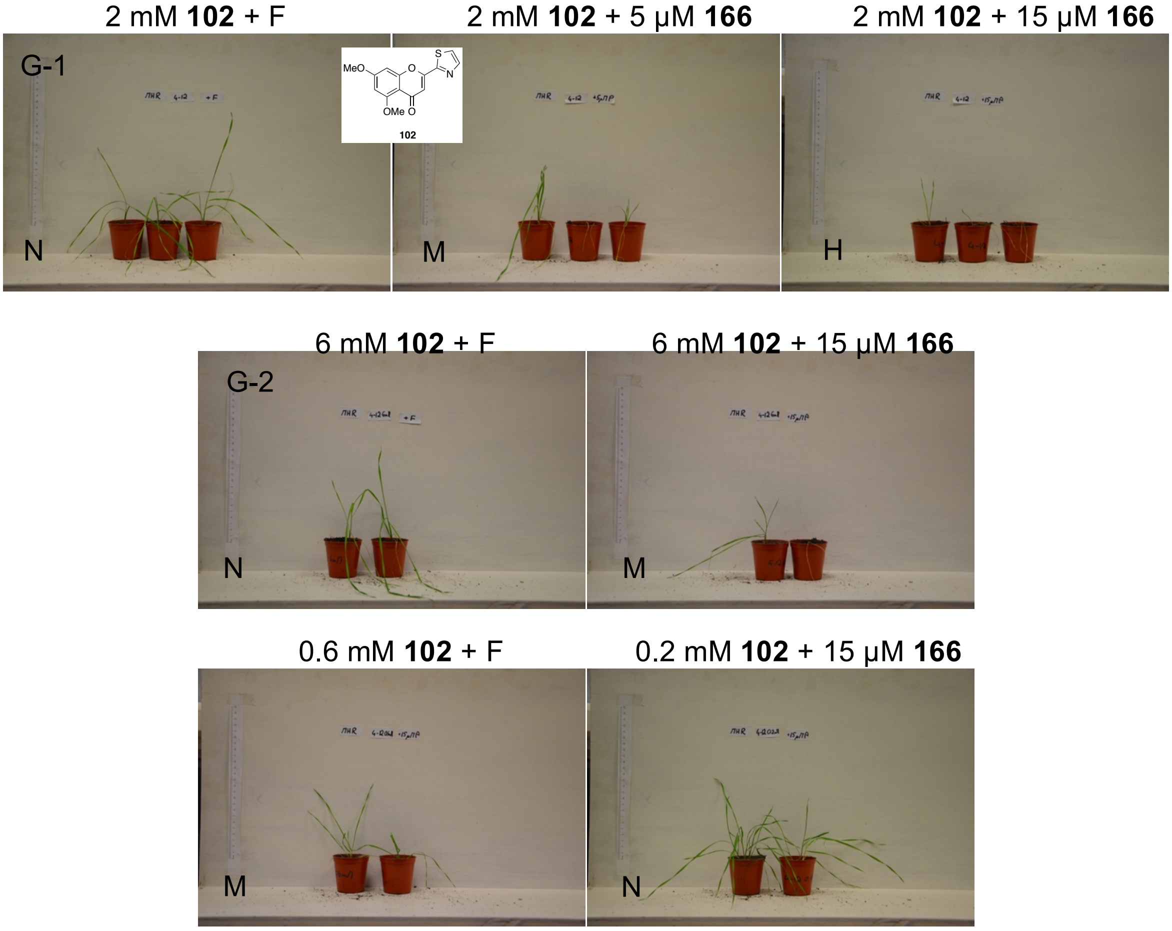
F + 5 µM **166**

F + 15 µM **166**

Figure 84: Effects of pinoxaden 166 on MHR black grass; U: Untreated, F: Formulation;

Dimethoxychromenone **16** was not effective at synergising the effects of pinoxaden **166** in MHR black grass (Figure 85, A), which is consistent with the lower inhibition activity in the CDNB assay. A clear relationship between synergising activity and alkyl chain length of the substituent in the 5-position could be observed. Compound **(±)-71** was inactive (Figure 85, B), but compounds **95** (Figure 85, C) and **3** (Figure 85 ,D) showed some synergising effect in combination with a 15 µM pinoxaden **166** solution, though not big enough to kill the plant. The same is true for compound **125** (Figure 85, E). Compound **95** (Figure 85, C) also enhanced the effects of pinoxaden **166** at a 5 µM concentration, which might be explained with a slightly better solubility and uptake compared to **3** due to the shorter chain. Tetrazole **101** showed some synergising effect, especially in combination with a 15 µM pinoxaden **166** solution (Figure 85, F). The plants were not killed, but stunted growth was observed. Thiazole **102** enhanced the effects of pinoxaden **166** significantly (Figure 85, G-1). All the plants died, when 2 mM **102** was used in combination with a 15 µM pinoxaden **166** solution and the plants showed at least severely stunted growth when 2 mM **102** was used in combination with a 5 µM pinoxaden **166** solution. At a 0.6 mM concentration **102** still showed some synergising effect in combination with a 15 µM pinoxaden **166** solution, but for the lower concentration no effect was apparent (Figure 85, G-2). The higher activity on plants compared to **16** for both **101**, which shows a slightly lower inhibition activity in the CDNB assay than **16**, and **102**, which shows a slightly higher inhibition activity, can probably be explained by better solubility and uptake. Pleasingly none of the synergists caused damage to the plant, if applied alone, which allows to rule out inherent toxicity.


2 mM **16** + F
2 mM **16** + 5 µM **166**
2 mM **16** + 15 µM **166**
A
MeO
OMe O
16
N
N
N
2 mM **(±)-71** + F
2 mM **(±)-71** + 5 µM **166**
2 mM **(±)-71** + 15 µM **166**
B
MeO
O
COOH
(±)-71
N
N
N
2 mM **95** + F
2 mM **95** + 5 µM **166**
2 mM **95** + 15 µM **166**
C
MeO
O
COOH
95
N
M
M
2 mM **3** + F
2 mM **3** + 5 µM **166**
2 mM **3** + 15 µM **166**
D
MeO
O
COOH
3
N
N
M
2 mM **125** + F
2 mM **125** + 5 µM **166**
2 mM **125** + 15 µM **166**
E
MeO
O
S
COOH
125
N
L
M
2 mM **101** + F
2 mM **101** + 5 µM **166**
2 mM **101** + 15 µM **166**
F
MeO
N=N
NH
OMe O
101
N
M
M



2 mM **102** + F

2 mM **102** + 5 µM **166**

2 mM **102** + 15 µM **166**

6 mM **102** + F

6 mM **102** + 15 µM **166**

0.6 mM **102** + F

0.2 mM **102** + 15 µM **166**

Figure 85: Synergising effects of compounds 16, (±)-71, 95, 3, 125, 101 and 102 on MHR black grass treated with pinoxaden 166; F: Formulation; Rating from no damage (N) to low damage (L), medium damage (M) and high damage (H).

4 Conclusion and Future Work

4.1 Binding site identification

Although photoaffinity labels, which maintained inhibition activity towards *Am*GSTF1 in the CDNB assay, were successfully synthesized, no proof of labeling could be observed in the irradiation experiments.

Future work should focus on the incorporation of alternative photoreactive groups. Especially flavonoid derivatives featuring a aryldiazarine, the most commonly used photoaffinity group, may offer a promising alternative, as diazarines are much easier activated than benzophenones.

Alternatively, obtaining a crystal structure of the protein-ligand complex through cocrystallization or soaking techniques could be a possible way to gain a better understanding of the protein ligand interactions between the flavonoid inhibitors and *Am*GSTF1, informing a more targeted optimization of the inhibitors.

A preliminary steady state fluorescence anisotropy experiment did not confirm the initial theory, that the inhibitors are disrupting protein-protein interactions and causing GST-dimer dissociation.

4.2 Synergist development

A library of 44 flavonoid analogues was synthesised, focusing on different substituents in the 2-position and modifications of the long alkyl chain. Each analogue was screened for its ability to inhibit *Am*GSTF1 in a CDNB assay, which led to a number of interesting SAR discoveries summarised below (Figure 86).

A variety of alternative 2-substituents including amides, cyclic amines and a variety of arenes were introduced from the corresponding acid **41**, thioether **8** and chloride **21**. Whilst a broad range of substituents could be tolerated at C-2, higher activity appeared to reside in aromatic substituents. The high tolerance to structural changes suggests that the flavonoids might not bind to *Am*GSTF1 in a tight binding pocket

A series of derivatives bearing a long alkyl chain with an α-carboxylate group in the 5-position were successfully prepared using the iron promoted ene reaction between glyoxalate and the appropriate terminal alkene as a key step. Activity increased with increasing chain length, from an inhibition of only 14% ±3% at a 10 μM level for the propanoic acid analogue **(±)-71** to 75% ±3% inhibition at a 1 μM level for the tetradecanoic acid analogue **98**, plateauing at lengths beyond 10 carbons. Reduction of flexibility through incorporation of an amide in the chain was not advantageous.

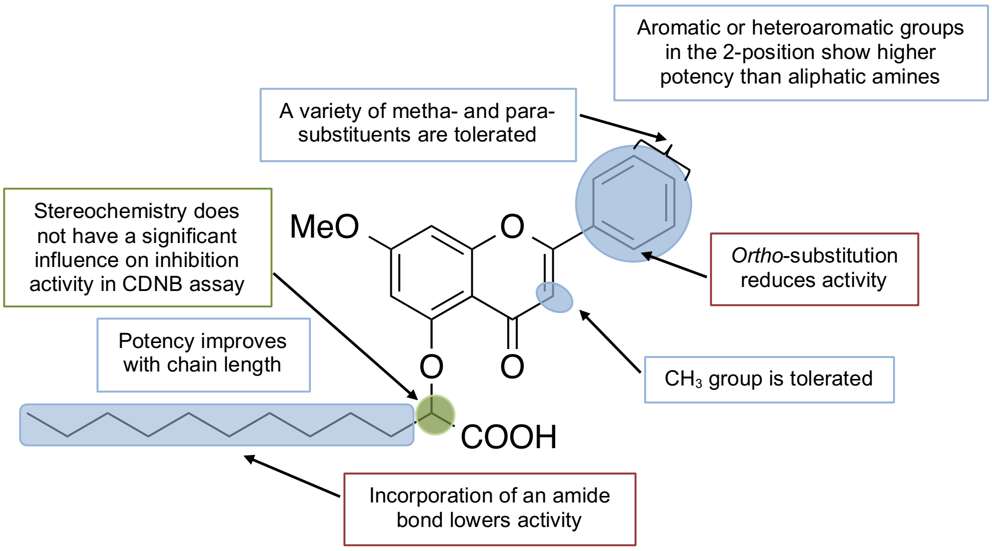
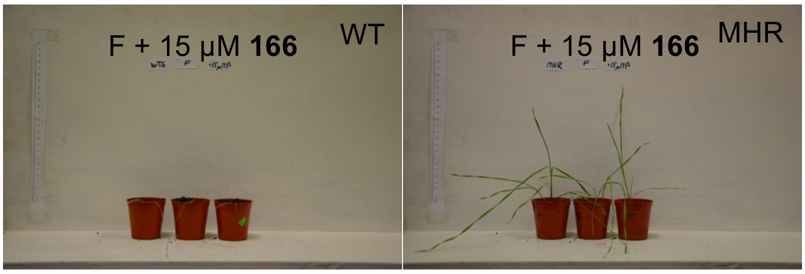


Figure 86: Summary of SARs.

A particular emphasis was put on increasing the aqueous solubility, as the synergist has to reach the target site in the plant in order to be effective, intrinsic potency is not sufficient. Four different strategies were explored: replacing the long alkyl chain with comparably sized and equally flexible but more soluble PEG unit, introducing substituents to change the overall planar structure of the molecule, incorporating an SO_2Me-substituent, which can lower the lipophilicity of a compound and exploring more polar heteroaromatic nuclei at the 2-position. Whilst the former three strategies were not successful, replacing the phenyl group of 5,7-dimethoxy-2-phenyl-chromen-4-one **16** with heteroarenes improved the aqueous solubility. Oxadiazole **99**, oxazole **100**, terazole **101** and thiazole **102** were generated following conventional heterocycle synthesis strategies starting from acid **41**. Pyridazine **108** could be obtained by cross-coupling with the boronate ester generated in one pot by Ir-catalysed C-H borylation and pyrazole **104** could be accessed via the corresponding hydroxychalcone. All heteroarenes showed a comparable inhibition of AmGSTF1 in the CDNB assay (53-83% at 100µM) in comparison to the phenyl analogue **16** (70% ±12% at 100µM). Initial testing of these compounds for aqueous solubility revealed that most of the heterocycles showed enhancements relative to the parent phenyl compound, but thiazole **102** was dramatically more soluble.



2 mM **102** + F   2 mM **102** + 15 µM **166**

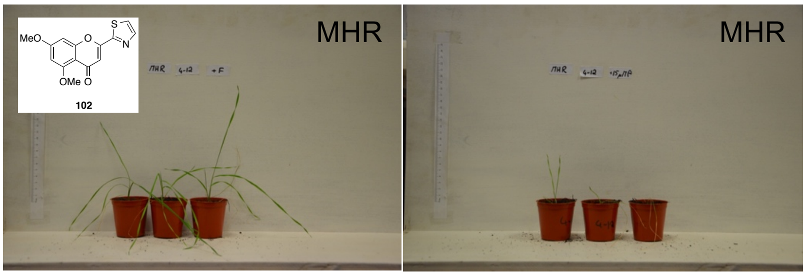


Figure 87: Synergising effects of thiazole 102 on MHR black grass treated with pinoxaden 166; F: Formulation;

Even though the potency of the initial lead molecule in the CDNB assay could not be increased, analogues with improved physicochemical properties were obtained, which is crucial for the application on plants. In plant experiments with black grass, **102** acted as a synergist to the herbicide pinoxaden **166** (Figure 87) and exhibited better results than other analogues, with higher activity in the CDNB assay, which might be associated with the better physicochemical properties.

4.2.1 Testing of synergists

As the tests directly on black grass showed, biochemical activity does not necessarily translate to efficacy in the plant. With the role of *Am*GSTF1 in black grass not yet completely understood, judging the relevance of the CDNB assay is difficult and as is designing a new assay with more relevance to assess synergists. Although the thermal shift assay offers an orthogonal method to the CDNB assay, the same problems persist. This makes evaluation of new synergists challenging, as black grass experiments cannot be used for high throughput screening.

4.2.2 Synergist selectivity

For future applications of the synergists it was important to test whether the synergists selectively inhibit *Am*GSTF1 over other GSTs and whether the activity profile differs. Therefore, a preliminary experiment was conducted and a couple of compounds were tested for their inhibition of *At*GSTF8 in the CDNB assay. *At*GSTF8 is a phi-class GST in Arabidopsis thaliana.[172] It was selected because of its relatively high CDNB conjugating activity, its accessibility and because it originates from the same GST class as *Am*GSTF1. The *A*tGSTF8 construct was provided by Robert Edwards (Newcastle University) and overexpression and purification of the protein was conducted by Becky Eno (Durham University).

Four different inhibitors were selected for tests. Dimethoxychromenone **16** was chosen as a simple flavonoid whose inhibition towards *Am*GSTF1 is not very high. The aryloxytetradecanoic acid analogue **98** was selected as it showed the highest inhibition towards *Am*GSTF1 out of all synergists and compound **(±)-71** was picked to see if the alkyl chain length of the substituent in the 5-position had a similar effect on the activity towards *At*GSTF8. Thiazole **102** was included because of its remarkable synergistic effects in black grass.

The inhibition of dimethoxychromenone **16** towards *At*GSTF8 is similar to the one towards *Am*GSTF1 in the CDNB assay at a 10 µM level (Figure 88). Compound **(±)-71** with α-substituted propanoic acid showed a higher inhibition towards *Am*GSTF1 and the aryloxytetradecanoic acid analogue **98** is significantly more active towards *Am*GSTF1. Thiazole **102**, however, showed a higher inhibition activity towards *At*GSTF8 compared to *Am*GSTF1. This high inhibition activity towards other GSTs could potentially be problematic in future applications, because it could lead to undesirable effects in other plants.

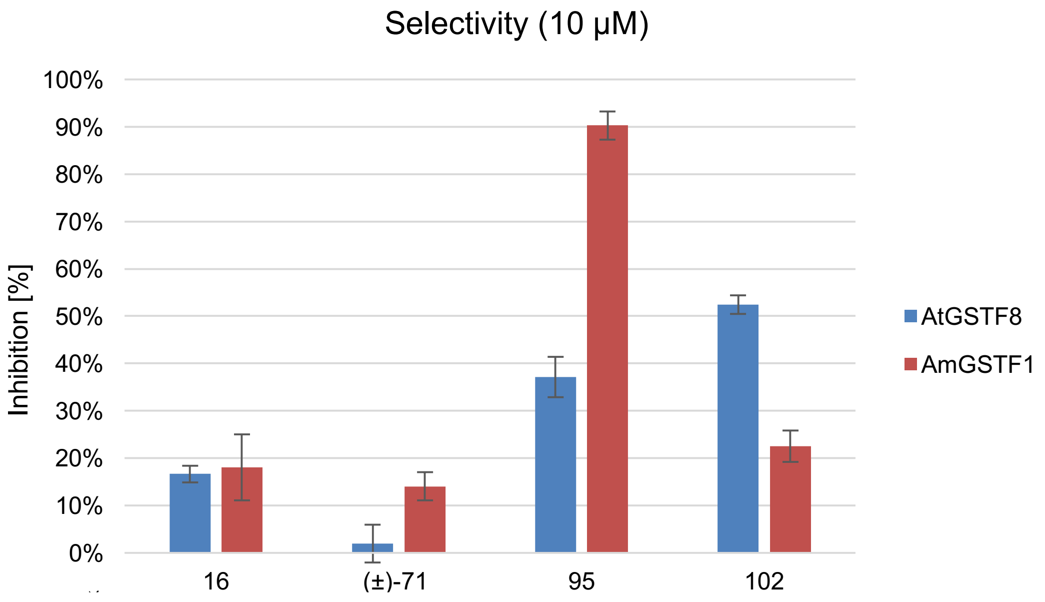
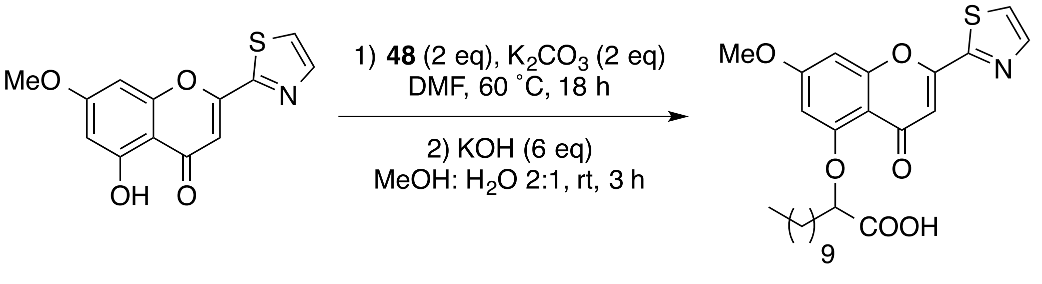


Figure 88: A Inhibition of *At*GSTF8 compared to *Am*GSTF1 at 10 µM in a CDNB assay; Experiments are conducted in triplicate.

As the substitution with α-substituted acids in the 5-position could establish a selectivity for *Am*GSTF1 over *At*GSTF8 for the phenyl analogue, 5-substituted thiazole **168** was synthesised (Scheme 71) and tested.



114

168
40%

Scheme 71: Synthesis of analogue 168.

Pleasingly **168** showed a higher inhibition activity towards *Am*GSTF1 compared to *At*GSTF8 (Figure 89, A). The inhibition is also comparable to the corresponding phenyl analogue **3** (Figure 89, B).

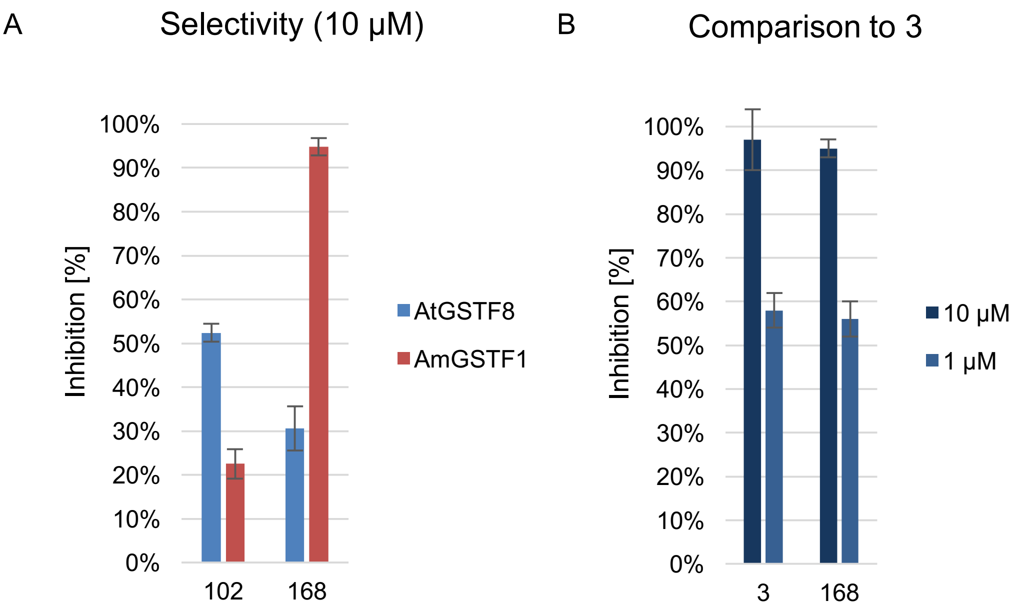


Figure 89: A Inhibition of *At*GSTF8 compared to *Am*GSTF1 by compounds 102 and 168 at 10 µM in a CDNB assay; B Inhibition of *Am*GSTF1 by compounds 3 and 168 at 10 and 1 µM in a CDNB assay; Experiments are conducted in triplicate.

In order to investigate the synergistic effects of **168**, the compound needs to be tested directly on black grass in the future. Modifications to further improve the physicochemical properties might be necessary.

5 Experimental Part

5.1 Chemical Synthesis

5.1.1 General Notes

Chemicals: All chemicals were purchased from commercial suppliers and were used without further purification unless otherwise stated.

Dry Solvents: 1,2-Dichloroethane and methyl-*tert*-butyl-ether (MTBE) were purchased anhydrous from Sigma Aldrich or Acros respectively. All other dry reaction solvents were dried using an Innovative Technology Solvent Purification System and stored under argon.

Column chromatography: Flash column chromatography was performed on a CombiFlash® System from Teledyne Isco equipped with an UV-light detector using prepacked silica RediSep Rf cartridges with the stated solvent gradient. Crude mixtures to be purified were dry loaded onto silica prior to loading on the column.

LC-MS: Purity of all final compounds was checked by LC-MS. LC-MS analyses were conducted on a TQD mass spectrometer (Waters Ltd, UK), which was equipped with an Acquity UPLC, using an Acquity UPLC BEH C18 (2.1 mm × 50 mm, 1.7 μm) column, and an electrospray ion source. Absorbance data were acquired from 210 to 400 nm using an Acquity photodiode array detector.

GC-MS: GC-MS analyses were performed on a Shimadzu QP2010-Ultra using electron ionization (EI). The mass spectrometer was equipped with either an Rxi-5Sil MS column (0.15 μm × 10 m × o.15 mm) for non-polar compounds or an Rxi-17Sil MS column (0.15 μm × 10 m × o.15 mm) for polar compounds.

ASAP: ASAP measurements were performed on a LCT Premier XE mass spectrometer and ASAP ion source.

Chiral HPLC-analysis: Chiral analyses were conducted on a PerkinElmer Series 200 HPLC equipped with a diode array detector analysing at 254 nm, using a Daicel Chiralpak IA column (250 × 4.6 mm, 5 µm) or a Chiralpak ID-3 column (250 × 4.6 mm, 3 µm).

Chiral separation: Chiral separation was performed on a Waters 2525 preparative HPLC, equipped with a 2767 sample manager and a Waters 2996 UV detector PhotoArray fraction manager, using a Daicel Chiralpak IC column (250 × 21 mm, 5 µm).

HRMS: HRMS measurements were carried out on a QToF Premier mass spectrometer (Waters Ltd, UK) with an electrospray ion source or a LCT Premier XE mass spectrometer with an ASAP ion source.

IR spectroscopy: Infrared (IR) spectra were recorded on a Perkin-Elmer RX I FT-IR spectrometer via use of a Pike ATR accessory in the range of 3500 – 600 cm^{-1}. Assigned peaks are reported in wavenumbers (cm^{-1}).

Melting points: Melting points were measured in open capillary tubes using a Thermo Scientific™ Melting Point Apparatus and are uncorrected.

Microwave: Microwave reactions were performed in septum-containing, crimp capped, sealed vials in a Emrys^{TM} Optimizer microwave unit from Personal Chemistry. The reported times are hold times.

NMR-spectroscopy: NMR-spectra were recorded in $CDCl_3$, d^6-DMSO, CD_3OD or D_2O solutions on a Bruker Advance-400 (^{1}H, ^{13}C, ^{19}F, DEPT, COSY), Varian Inova-600 (^{1}H, ^{13}C, ^{19}F, HSQC, HMBC, COSY, NOESY) or Varian VNMRS-700 (^{1}H, ^{13}C, ^{19}F, HSQC, HMBC, COSY, NOESY) spectrometer, using the solvent peak ($CDCl_3$: δ = 7.26 ppm (^{1}H), δ = 77.16 ppm (^{13}C), d^6-DMSO: δ = 2.50 ppm (^{1}H), δ = 39.52 ppm (^{13}C) CD_3OD: δ = 3.31 ppm (^{1}H), δ = 49.00 ppm (^{13}C), D_2O: δ = 4.79 ppm (^{1}H)) as reference. For some spectra tetramethylsilane (TMS) was used as an internal standard. ^{13}C spectra were run in proton-decoupled mode. Chemical shift values (δ) are reported in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz). The multiplicity is indicated by singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or a combination thereof. Assignment of spectra was carried out using 2D HSQC, HMBC, COSY and NOESY techniques.

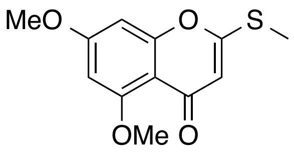
Optical Rotation: Measurements were performed on an AA-10 polarimeter from Optical Activity Ltd. and are stated alongside temperature, solvent and concentration in mg ml^{-1}.

TLC: TLC analyses were performed on pre-coated aluminium-backed plates (Silica gel 60 F254, Merck). Signals were visualized with UV-light (254 nm and 365 nm) or by staining with potassium permanganate in water where necessary.

5.1.2 Variations of the substituent in the 2-position

5.1.2.1 Synthesis of 2-amino-5,7-dimethoxy-4H-chromen-4-ones

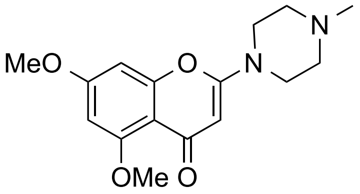
5,7-Dimethoxy-2-methylthio-4H-chromen-4-one **8**



n-BuLi (15.1 ml of a 2.5 M solution in hexane, 37.8 mmol, 3.15 eq) was added dropwise over 30 min to a solution of 1,1,1,3,3,3-hexamethyldisilazane (8.25 ml, 39.6 mmol, 3.3 eq) in dry THF (30 ml) under an argon atmosphere at -78 °C. After 10 min the mixture was allowed to warm to 0 °C and stirred for another 10 min. The reaction mixture was recooled to -78°C and a solution of 2-hydroxy-4,6-dimethoxyacetophenone (2.36 g, 12 mmol, 1 eq) in dry THF (9 ml) added dropwise. After stirring for 30 min, CS_2 (1.08 ml, 18 mmol, 1.5 eq) was added in one portion and the reaction mixture allowed to warm to 0°C. After 1 h MeI (1.65 ml, 26.4 mmol, 2.2 eq) was added dropwise over 10 min and stirring continued for another 1 h at rt. 10 N KOH (6 ml) was added and the reaction mixture heated under reflux for 1 h. Then H_2O (20 ml) was added and THF removed under reduced pressure. DCM (20 ml) was added to dissolve the precipitated product and the reaction mixture extracted with DCM (3 × 10 ml). The combined organic layers were dried over Mg_2SO_4 and concentrated. Recrystallization from THF then afforded the title chromenone (2.11 g, 70%) as a pale orange solid.

m.p.: 189 - 191 °C; ν_{max} (ATR): 1621 (C=O), 1585, 1427, 1319, 1304, 1154, 1126, 1106, 1064, 917, 831, 821 cm^{-1}; δ_H (d^6-DMSO, 400 MHz): 6.65 (d, *J* = 2.3 Hz, 1H, 8-H), 6.48 (d, *J* = 2.3 Hz, 1H, 6-H), 6.00 (s, 1H, 3-H), 3.85 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 2.53 (s, 3H, SCH_3); δ_C (d^6-DMSO, 100 MHz): 173.1 (C-4), 166.3 (C-2), 163.5 (C-7), 160.3 (C-5), 159.5 (C-8a), 108.1 (C-3), 107.6 (C-4*a*), 96.3 (C-6), 92.9 (C-8), 56.1 (OCH_3), 56.0 (OCH_3), 13.3 (SCH_3); HRMS (ES^+) found $[M+H]^+$ 253.0537, $C_{12}H_{12}O_4S$ requires *M* 253.0535.

5,7-Dimethoxy-2-(4'-methylpiperazin-1'-yl)-4H-chromen-4-one **9**



A round bottom flask was charged with 5,7-dimethoxy-2-methylthio-4H-4-chromenone **8** (130 mg, 0.5 mmol, 1 eq), *N*-methyl-piperazine (0.55 ml, 5 mmol, 10 eq) and ethylene glycol (5 ml). The reaction mixture was heated to 160 °C and stirred for 3 h. After cooling to rt, H_2O (5 ml) was added and the reaction mixture extracted with DCM (3 × 2 ml). The combined organic layers were washed with H_2O (1.5 ml) and dried over Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography (12 g silica gel, DCM:MeOH 50:1 - 9:1) yielded the title amine (39 mg, 25%) as a colourless solid.

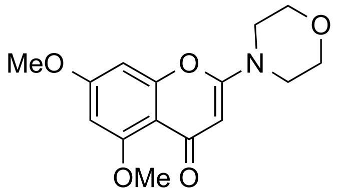
m.p.: 71 – 73 °C; ν_{max} (ATR): 1634 (C=O), 1589, 1558, 1412, 1247, 1162, 1110, 1000, 808 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 6.33 (d, *J* = 2.3 Hz, 1H, 8-H), 6.32 (d, *J* = 2.3 Hz, 1H, 6-H), 5.33 (s, 1H, 3-H), 3.91 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.45 (t, *J* = 5.2 Hz, 4H, CH_2), 2.50 (t, *J* = 5.2 Hz, 4H, CH_2), 2.34 (s, 3H, CH_3); δ_C ($CDCl_3$, 100 MHz): 177.7 (C-4), 163.0 (C-2), 161.3 (C-7), 160.7 (C-5), 157.5 (C-8a), 107.6 (C-4a), 95.9 (C-6), 92.5 (C-8), 88.6 (C-3), 56.5 (OCH_3), 55.7 (OCH_3), 54.2 (CH_2), 46.2 (NCH_3), 44.7 (CH_2); HRMS (ES^+) found $[M+H]^+$ 305.1516, $C_{16}H_{20}N_2O_4$ requires *M* 305.1501.

General Procedure A

5,7-Dimethoxy-2-methylthio-4H-chromen-4-one **8** (1.009 g, 4 mmol, 1 eq) was suspended in DCM (28 ml). *Meta*-chloroperoxybenzoic acid (75%, 2.945 g, 12.8 mmol, 3.2 eq) was slowly added at 0 °C and the reaction mixture heated to 35 °C and stirred for 5.5 h. H_2O

(16 ml) was added and the reaction mixture extracted with DCM (4 × 15 ml). The combined organic layers were washed consecutively with saturated (sat.) aqueous $Na_2S_2O_5$ (15 ml), sat. aqueous $NaHCO_3$ (2 × 15 ml) and sat. aqueous NH_4Cl (15 ml), dried over Na_2SO_4 and concentrated. A fraction of the crude 5,7-dimethoxy-2-(methylsulfonyl)-4H-chromen-4-one (1/8, 96 mg) was mixed with the amine (3.4 mmol) in DCM and stirred at rt or under reflux respectively.

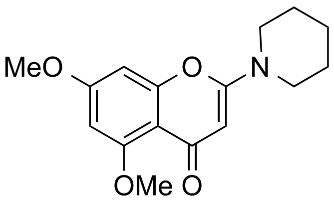
5,7-Dimethoxy-2-morpholino-4H-chromen-4-one **12**



5,7-Dimethoxy-2-morpholino-4H-chromen-4-one **12** was synthesised according to General procedure A, using morpholine (296 mg) and stirring for 20 h at rt followed by 26 h heating under reflux. The crude reaction mixture was concentrated and purified by flash column chromatography (12 g silica gel, DCM:MeOH 97:3 - 19:1), which afforded the title product (47 mg, 32%) as a colourless solid.

m.p.: 165 - 168 °C; ν_{max} (ATR): 1634 (C=O), 1593, 1558, 1242, 1242, 1108, 810 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 6.36 - 6.30 (m, 2H, 6-H, 8-H), 5.33 (s, 1H, 3-H), 3.92 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.80 (t, *J* = 5.0 Hz, 4H, 3'-H_2, 5'-H_2), 3.40 (t, *J* = 5.0 Hz, 4H, 2'-H_2, 6'-H_2); δ_C ($CDCl_3$, 100 MHz): 177.6 (C-4), 163.1 (C-7), 161.4 (C-2), 160.8 (C-5), 157.5 (C-8a), 107.7 (C-4a), 96.0 (C-6), 92.5 (C-8), 88.8 (C-3), 66.1 (C-3', C-5'), 56.6 (OCH_3), 55.7 (OCH_3), 44.9 (C-2', C-6'); HRMS (ES$^+$) found $[M+H]^+$ 292.1186, $C_{15}H_{17}NO_5$ requires *M* 292.1185.

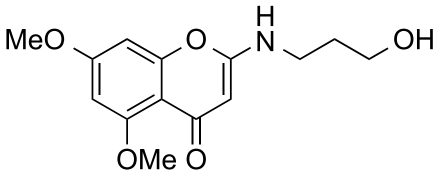
5,7-Dimethoxy-2-(piperidin-1-yl)-4H-chromen-4-one **13**



5,7-Dimethoxy-2-(piperidin-1-yl)-4H-chromen-4-one **13** was synthesised according to General procedure A, using piperidine (290 mg) and stirring for 20 h at rt. The crude reaction mixture was concentrated and EtOAc (20 ml) added. The organic layer was washed with sat. aqueous $NaHCO_3$ (2 × 15 ml) and the reaction mixture was concentrated again. Flash column chromatography (12 g silica gel, DCM:MeOH 97:3 - 19:1) yielded the title amine (72 mg, 50%) as a colourless solid.

m.p.: 79 - 81 °C; ν_{max} (ATR): 1627 (C=O), 1595, 1556, 1397, 1245, 1202, 1159, 1140, 1117, 807, 744, 742 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 6.34 (d, *J* = 2.3 Hz, 1H, 8-H), 6.32 (d, *J* = 2.3 Hz, 1H, 6-H), 5.37 (s, 1H, 3-H), 3.91 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.45 - 3.38 (m, 4H, 2'-H_2, 6'-H_2), 1.70 - 1.61 (m, 6H, 3'-H_2, 4'-H_2, 5'-H_2); δ_C ($CDCl_3$, 100 MHz): 177.6 (C-4), 162.9 (C-7), 161.2 (C-2), 160.7 (C-5), 157.5 (C-8a), 107.5 (C-4a), 95.8 (C-6), 92.5 (C8), 88.1 (C-3), 56.6 (OCH_3), 55.7 (OCH_3), 46.0 (C-2', C-6'), 25.3 (C-3', C-5'), 24.3 (C-4'); HRMS (ES^+) found $[M+H]^+$ 290.1377, $C_{16}H_{19}NO_4$ requires *M* 290.1392.

2-(3’-Hydroxypropylamino)-5,7-dimethoxy-4H-chromen-4-one **14**

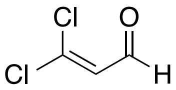


2-(3’-Hydroxypropylamino)-5,7-dimethoxy-4H-chromen-4-one **14** was synthesised according to General procedure A, using 3-aminopropan-1-ol (290 mg) and stirring for 20 h at rt. The crude reaction mixture was concentrated, H_2O (20 ml) added, and the precipitate collected by filtration. The aqueous layer was extracted with DCM (4 × 15 ml), the combined organic layers were dried over Na_2SO_4 and the extracts were combined with the precipitated product. The reaction mixture was concentrated and purified by flash column chromatography (12 g silica gel, DCM:MeOH 9:1), which yielded the title product (47 mg, 33%) as a colourless solid.

m.p.: 143 - 146 °C; ν_{max} (ATR): 3380, 3196, 1640 (C=O), 1593, 1549, 1330, 1202, 1077, 804 cm^{-1}; δ_H (CD_3OD, 400 MHz): 6.52 (d, *J* = 2.3 Hz, 1H, 8-H), 6.46 (d, *J* = 2.3 Hz, 1H, 6-H), 5.21 (s, 1H, 3-H), 3.88 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.67 (t, *J* = 6.1 Hz, 2H, 3’-H), 3.34 (t, 7.1 Hz, 2H, 1’-H), 1.89 – 1.81 (m, 2H, 2’-H_2); δ_C (CD_3OD, 100 MHz): 179.6 (C-4), 165.0 (C-2), 164.5 (C-7), 161.7 (C-5), 158.6 (C-8a), 107.3 (C-4a), 96.7 (C-6), 93.8 (C-8), 85.62 (C-3), 60.2 (C-3’), 56.4 (OCH_3), 56.3 (OCH_3), 39.5 (C-1’), 32.7 (C-2’); HRMS (ES^+) found $[M+H]^+$ 280.1183, $C_{14}H_{17}NO_5$ requires *M* 280.1185.

5.1.2.2 Synthesis of 2-aryl-5,7-dimethoxy-4H-chromen-4-ones

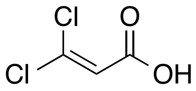
3,3-Dichloroacrolein[100] **24**



Benzoyl peroxide (70%, 714 mg, 2 mmol, 0.008 eq) in CCl_4 (300 ml) was heated under reflux. Ethyl vinyl ether (24.0 ml, 250 mmol, 1 eq) was added dropwise over 10 min and the reaction mixture stirred for 2.5 h. The remaining CCl_4 was removed under reduced pressure. The synthesised ethyl-(1,3,3,3-tetrachloropropyl)ether **23** was diluted with acetone (90 ml) and added dropwise to a mixture of acetone (90 ml) and H_2O (136 ml). Acetone was removed by distillation through a packed column at 95 °C. The residue was extracted with Et_2O (4 × 30 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The title aldehyde (9.5 g, 30%) could be obtained by vacuum distillation as a light yellow liquid.

b.p.: 35 °C (10 mbar) (lit.[100] b.p. 124 °C/1 bar); δ_H ($CDCl_3$, 400 MHz): 9.87 (d, J = 6.8 Hz, 1H, 1-H), 6.44 (d, J = 6.8 Hz, 1H, 2-H); δ_C ($CDCl_3$, 100 MHz): 188.3 (C-1), 144.9 (C-3), 124 (C-2); m/z GC-MS 126 (M^+, Cl^{35}), 128 (M^+, Cl^{35}/Cl^{37}), 143 (M^+, Cl^{37}).

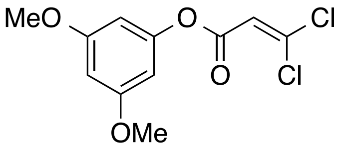
3,3-Dichloroacrylic acid **18**



A round bottom flask was charged with 3,3-dichloroacrolein **24** (3.125 g, 25 mmol, 1 eq), MeCN (25 ml), a solution of $NaH_2PO_4.H_2O$ (931 mg, 6.75 mmol, 0.27 eq) in H_2O (10 ml) and H_2O_2 (2.24 ml of a 35 wt. % solution in H_2O, 26 mmol, 1.04 eq). To the resulting mixture was added a solution of $NaClO_2$ (80 %, 3.957 g, 3.5 mmol, 1.4 eq) in H_2O (35 ml) over 2 h whilst maintaining the temperature at 10 °C. The reaction mixture was stirred for another 2 h at 10 °C and then allowed to warm to rt and stirred for 1 h, until evolution of O_2 ceased. $Na_2S_2O_3$ was added and the reaction mixture acidified with 1 M HCl and extracted with EtOAc (3 × 30 ml). The combined organic layers were dried over Na_2SO_4 and concentrated, which afforded the title acid (3.262 g, 93%) as a colourless solid.

m.p.: 76 - 77 °C (lit.[99] m.p. 77°C); ν_{max} (ATR): 3064 (COO-H), 2862 (COO-H), 2693 (COO-H), 2589 (COO-H), 1678 (C=O), 1581, 1213, 894, 851, 666 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 6.42 (s, 1H, 2-H); δ_C ($CDCl_3$, 100 MHz): 167.4 (C-1), 140.9 (C-3), 119.32 (C-2); m/z LC-MS (ES^-) 139 ($[M-H]^-$, Cl^{35}), 141 ($[M-H]^-$, Cl^{35}/Cl^{37}), 143 ($[M-H]^-$, Cl^{37}).

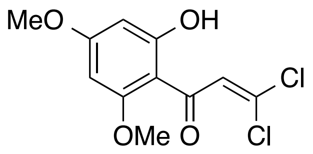
3’,5’-Dimethoxyphenyl 3,3-dichloroacrylate[98] **19**



A round bottom flask was charged with 3,5-dimethoxyphenol (3.31 g, 21.5 mmol, 1 eq), 3,3-dichloroacrylic acid **18** (3.33 g, 23.6 mmol, 1.1 eq) and 4-(dimethylamino)-pyridine (DMAP) (260 mg, 2.15 mmol, 0.1 eq) and evacuated and flushed with argon three times. DCM (18 ml) and DMF (3.6 ml) were added and the solution was cooled to 0 °C. Diisopropylcarbodiimide (3.33 ml, 21.5 mmol, 1 eq) was added dropwise and after 10 min, the reaction mixture was allowed to warm to rt and stirred for another 2 h. The mixture was filtered through Celite® and washed with 1 M HCl (2 × 10 ml), sat. aqueous $NaHCO_3$ (2 × 10 ml) and with brine (10 ml). The organic layer was dried over Na_2SO_4 and concentrated. The title product (5.09 g, 86%) was afforded by flash column chromatography (40 g silica gel, hexane:EtOAc 93:7 - 4:1) as a colourless solid.

m.p.: 50 - 52 °C; ν_{max} (ATR): 1745 (C=O), 1591, 1456, 1135, 1046, 963, 825 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 6.58 (s, 1H, 2-H), 6.36 (t, *J* = 2.2 Hz, 1H, 4’-H), 6.30 (d, *J* = 2.2 Hz, 2H, 2’-H, 6’-H), 3.77 (s, 6H, 2 × OCH_3); δ_C ($CDCl_3$, 100 MHz): 161.3 (C-3’, C-5’), 160.6 (C-1), 151.7 (C-1’), 140.1 (C-3), 119.3 (C-2), 100.1 (C-2’, C-6’), 98.7 (C-4’), 55.7 (2 × OCH_3); m/z LC-MS (ES^+) 155 $[M-Cl_2CCO+H]^+$, 277 ($[M+H]^+$, Cl^{35}), 279 ($[M+H]^+$, Cl^{35}/Cl^{37}), 281 ($[M+H]^+$, Cl^{37}).

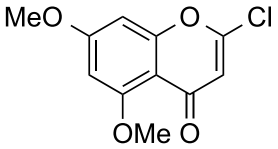
3,3-Dichloro-1-(2'-hydroxy-4',6'-dimethoxyphenyl)prop-2-en-1-one[98] **20**



3',5'-Dimethoxyphenyl 3,3-dichloroacrylate **19** (5.01 g, 18.1 mmol, 1 eq) was dissolved in dry 1,2-dichloroethane (300 ml) and added to a slurry of $AlCl_3$ (3.61, 27.1 mmol, 1.5 eq) in dry 1,2-dichloroethane (100 ml) under an argon atmosphere at 0 °C. Then the reaction mixture was heated under reflux and stirred for 1 h. After cooling down, the mixture was poured over a 1:1 mixture of ice and 1 M HCl (approx. 500 ml) and stirred for 30 min. The organic layer was separated and the aqueous layer was extracted with DCM (3 × 250 ml). The combined organic layers were washed with H_2O (200 ml) and brine (200 ml), dried over Na_2SO_4 and concentrated. The title product (2.76 g, 55%) was obtained by flash column chromatography (40 g silica gel, hexane:EtOAc 20:1 - 10:1) as a yellow solid.

m.p.: 85 - 87 °C; ν_{max} (ATR): 3090 (OH), 3010 (OH), 2951 (OH), 1621 (C=O), 1588, 1557, 1216, 1164, 1110, 948, 820, 736, 753 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 13.46 (s, 1H, OH), 7.36 (s, 1H, 2-H), 6.08 (d, *J* = 2.3 Hz, 1H, Ar-H), 5.92 (d, *J* = 2.3 Hz, 1H, Ar-H), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 189.3 (C-1), 168.3 (C-4'), 167.2 (C-6'), 162.3 (C-2'), 131.5 (C-3), 129.2 (C-2), 106.0 (C-1'), 94.0 (C-5'), 91.4 (C-3'), 56.2 (OCH_3), 55.9 (OCH_3); m/z LC-MS (ES^+) 181 $[M-Cl_2CCH]^+$, 277 ($[M+H]^+$, Cl^{35}), 279 ($[M+H]^+$, Cl^{35}/Cl^{37}), 281 ($[M+H]^+$, Cl^{37}).

2-Chloro-5,7-dimethoxy-4H-chromen-4-one [98] **21**



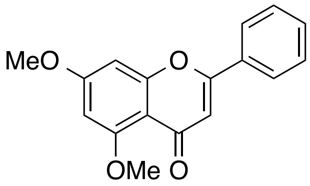
3,3-Dichloro-1-(2’-hydroxy-4’,6’-dimethoxyphenyl)prop-2-en-1-one **20** (2.64 g, 9.5 mmol, 1 eq) was dispersed in H_2O (95 ml). The reaction mixture was stirred vigorously for 4 h, adding 1 N NaOH (9.5 ml) over the first hour. 1 N HCl was added to neutralize the reaction mixture and the addition of EtOAc (100 ml) dissolved the precipitated product. The aqueous layer was extracted with EtOAc (3 × 50 ml) and the combined organic layers were washed with H_2O (50 ml) and brine (50 ml), dried over Na_2SO_4 and concentrated. Flash column chromatography (40 g silica gel, DCM:MeOH 97:3) yielded the title product (1.88 g, 82%) as a light yellow solid.

m.p.: 158 - 160 °C; ν_{max} (ATR): 1643 (C=O), 1602, 1319, 1160, 1114, 1088, 914, 847, 828 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 6.46 (d, *J* = 2.3 Hz, 1H, 8-H), 6.38 (d, *J* = 2.3 Hz, 1H, 6-H), 6.25 (s, 1H, 3-H), 3.93 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 176.2 (C-4), 164.4 (C-7), 161.2 (C-5), 160.1 (C-2), 153.5 (C-8a), 112.5 (C-3), 108.4 (C-4a), 97.0 (C-6), 92.9 (C-8), 56.6 (OCH_3), 56.0 (OCH_3); m/z LC-MS (ES^+) 241 ($[M+H]^+$, Cl^{35}), 243 ($[M+H]^+$, Cl^{37}), 263 ($[M+Na]^+$, 265 ($[M+Na]^+$, Cl^{37}).

General procedure B

In a reaction tube of a carousel reactor 2-chloro-5,7-dimethoxy-4H-chromen-4-one **21** (60 mg, 0.25 mmol, 1 eq), boronic acid (0.50 mmol, 2 eq), $Pd_2(dba)_3$ (6.9 mg, 3 mol%), PCy_3 (4.2 mg, 6 mol%) and Cs_2CO_3 (244 mg, 0.75 mmol, 3 eq) were suspended in dry dioxane (1.25 ml) under a nitrogen atmosphere. The reaction mixture was heated to 100 °C and stirred for 22 h.

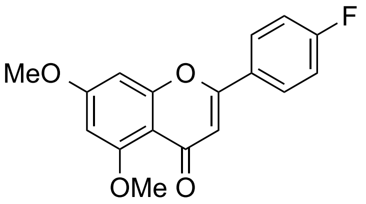
5,7-Dimethoxy-2-phenyl-4H-chromen-4-one[98] **16**



5,7-Dimethoxy-2-phenyl-4H-chromen-4-one **16** was synthesised according to General procedure B, using phenylboronic acid (61 mg). DCM (2 ml) and H_2O (2 ml) were added to the reaction mixture and the layers separated using a phase separator. The organic layer was concentrated and the residue was diluted with DCM (1 ml). The title product (59 mg, 83%) was obtained as a colourless solid by filtration through silica gel, using DCM (10 ml) to wash and then a 1:1 mixture of DCM and MeOH (10 ml) to elute the product.

m.p.: 135 - 138 °C; ν_{max} (ATR): 1644 (C=O), 1606, 1345, 1156, 1116, 820, 764, 694 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.95 - 7.85 (m, 2H, 2’-H, 6’-H), 7.57 - 7.47 (m, 3H, 3’-H, 4’-H, 5’-H), 6.71 (s, 1H, 3-H), 6.60 (d, *J* = 2.3 Hz, 1H, 8-H), 6.41 (d, *J* = 2.3 Hz, 1H, 6-H), 3.99 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.6 (C-4), 164.1 (C-7), 160.9 (C-5), 160.7 (C-2), 159.9 (C-8a), 131.6 (C-1’), 131.2 (C-4’), 129.0 (C-3’, C-5’), 126.0 (C-2’, C-6’), 109.4 (C-4a), 109.1 (C-3), 96.2 (C-6), 92.8 (C-8), 56.5 (OCH_3), 55.8 (OCH_3); m/z LC-MS (ES^+) 283 $[M+H]^+$.

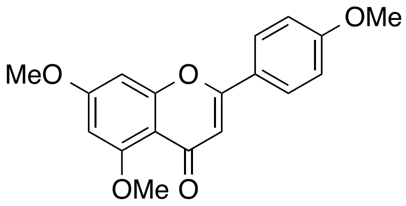
2-(4’-Fluorophenyl)-5,7-dimethoxy-4H-chromen-4-one[173] **25**



2-(4’-Fluorophenyl)-5,7-dimethoxy-4H-chromen-4-one **25** was synthesised according to General procedure B, using 4-fluorophenylboronic acid (70 mg). DCM (2 ml) and H_2O (2 ml) were added to the reaction mixture and the layers separated using a phase separator. The organic layer was concentrated and the residue was diluted in DCM (1 ml). The mixture was filtrated through silica gel, using DCM (10 ml) followed by 1:1 DCM:MeOH (10 ml) to elute the product. Flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 24:1) yielded the title product (38 mg, 50%) as a colourless solid.

m.p.: 235 - 237 °C (lit.[173] m.p. 236.5 – 238.5 °C); ν_{max} (ATR): 1650 (C=O), 1602, 1343, 1216, 1198, 1163, 1117, 1100, 832, 812 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.91 - 7.85 (m, 2H, 2’-H, 6’-H), 7.24 - 7.15 (m, 2H, 3’-H, 5’-H), 6.63 (s, 1H, 3-H), 6.57 (d, J = 2.3 Hz, 1H, 8-H), 6.39 (d, J = 2.3 Hz, 1H, 6-H), 3.97 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.4 (C-4), 164.5 (d, $^1J_{CF}$ = 252.5 Hz, C-4’), 164.1 (C-7), 160.9 (C-5), 159.8 (C-2), 159.7 (C-8a), 128.1 (d, $^3J_{CF}$ = 8.7 Hz, C-2’, C-6’), 127.8 (d, $^4J_{CF}$ = 3.3 Hz, C-1’), 116.2 (d, $^2J_{CF}$ = 22.0 Hz, C-3’, C-5’), 109.2 (C-4a), 108.9 (d, J = 1.0 Hz, C-3), 96.2 (C-6), 92.8 (C-8), 56.5 (OCH_3), 55.8 (OCH_3); δ_F ($CDCl_3$, 400 MHz): 107.94; m/z LC-MS (ES^+) 301 $[M+H]^+$.

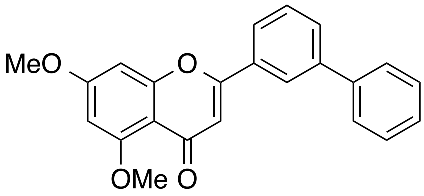
5,7-Dimethoxy-2-(4'-methoxyphenyl)-4H-chromen-4-one[98] **26**



5,7-Dimethoxy-2-(4'-methoxyphenyl)-4H-chromen-4-one **26** was synthesised according to General procedure B, using 4-methoxyphenylboronic acid (76 mg). DCM (2 ml) and H_2O (2 ml) were added to the reaction mixture and the layers separated using a phase separator. The organic layer was concentrated and the residue was diluted in DCM (1 ml). The mixture was filtrated through silica gel, using DCM (10 ml) to wash and then a 1:1 mixture of DCM and MeOH (10 ml) to elute the product. Flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 24:1) afforded the title product (72 mg, 92%) as a colourless solid.

m.p.: 155 - 157 °C; ν_{max} (ATR): 1640 (C=O), 1602, 1594, 1347, 1254, 1214, 1194, 1162, 1120, 1100, 1030, 830 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.85 - 7.79 (m, 2H, 3'-H, 5'-H), 7.03 - 6.97 (m, 2H, 2'-H, 6'-H), 6.59 (s, 1H, 3-H), 6.56 (d, *J* = 2.3 Hz, 1H, 8-H), 6.37 (d, *J* = 2.3 Hz, 1H, 6-H), 3.96 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.6 (C-4), 163.9 (C-7), 162.0 (C-4'), 160.9 (C-5), 160.6 (C-2), 159.8 (C-8a), 127.6 (C-3',C-5'), 123.8 (C-1'), 114.3 (C-2', C-6'), 109.2 (C-4a), 107.7 (C-3), 96.1 (C-6), 92.8 (C-8), 56.4 (OCH_3), 55.7 (OCH_3), 55.5 (OCH_3); m/z LC-MS (ES^+) 313 $[M+H]^+$.

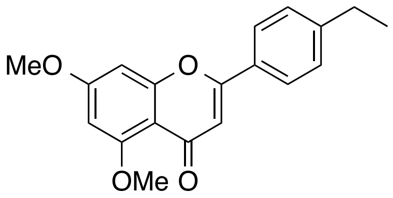
2-(3’-Biphenyl)-5,7-dimethoxy-4H-chromen-4-one **27**



2-(3’-Biphenyl)-5,7-dimethoxy-4H-chromen-4-one **27** was synthesised according to General procedure B, using 3-biphenylboronic acid (99 mg). DCM (2 ml) and H_2O (2 ml) were added to the reaction mixture and the layers separated using a phase separator. The organic layer was concentrated and the residue was diluted in DCM (1 ml). The mixture was filtrated through silica gel, using DCM (10 ml) to wash and then a 1:1 mixture of DCM and MeOH (10 ml) to elute the product. Flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 24:1) yielded the title product (57 mg, 50%) as a colourless solid.

m.p.: 190 - 193 °C; ν_{max} (ATR): 1649 (C=O), 1603, 1336, 1213, 1154, 1119, 1103, 818, 762, 700 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.05 (td, *J* = 1.9, 0.5 Hz, 1H, 2’-H), 7.82 (ddd, *J* = 7.8, 1.9, 1.1 Hz, 1H, 6’-H), 7.70 (ddd, *J* = 7.8, 1.9, 1.1 Hz, 1H, 4’-H), 7.67 - 7.59 (m, 2H, 2’’-H, 6’’-H), 7.57 - 7.37 (m, 4H, 5’-H, 3’’-H, 4’’-H, 5’’-H), 6.73 (s, 1H, 3-H), 6.57 (d, *J* = 2.3 Hz, 1H, 8-H), 6.36 (d, *J* = 2.3 Hz, 1H, 6-H), 3.94 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.6 (C-4), 164.1 (C-7), 160.9 (C-5), 160.5 (C-2), 159.9 (C-8a), 142.1 (C-3’), 140.3 (C-1’’), 132.1 (C-1’), 129.9 (C-Ar), 129.4 (C-Ar), 129.0 (C-Ar), 127.9 (C-Ar), 127.2 (C-Ar), 124.8 (C-Ar), 124.7 (C-Ar), 109.4 (C-4a), 109.3 (C-3), 96.3 (C-6), 92.9 (C-8), 56.5 (OCH_3), 55.8 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 359.1295, $C_{23}H_{18}O_4$ requires *M* 359.1283.

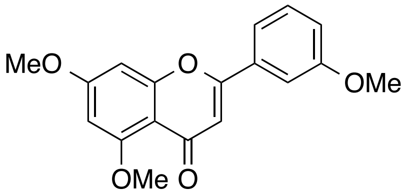
2-(4’-Ethylphenyl)-5,7-dimethoxy-4H-chromen-4-one **28**



2-(4’-Ethylphenyl)-5,7-dimethoxy-4H-chromen-4-one **28** was synthesised according to General procedure B, using 4-ethylphenylboronic acid (0.0750 g). DCM (2 ml) and H_2O (2 ml) were added to the reaction mixture and the layers separated using a phase separator. The organic layer was concentrated and the residue was diluted in DCM (1 ml). The mixture was filtrated through silica gel, using DCM (10 ml) to wash and then a 1:1 mixture of DCM and MeOH (10 ml) to elute the product. Flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 24:1) afforded the title product (57 mg, 74%) as a colourless solid.

m.p.: 126 - 129 °C; ν_{max} (ATR): 1644 (C=O), 1606, 1343, 1214, 1158, 1114, 838, 820 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.82 - 7.77 (m, 2H, 2’-H, 6’-H), 7.36 - 7.31 (m, 2H, 3’-H, 5’-H), 6.66 (s, 1H, 3-H), 6.58 (d, *J* = 2.3 Hz, 1H, 8-H), 6.38 (d, *J* = 2.3 Hz, 1H, 6-H), 3.97 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 2.73 (q, *J* = 7.6 Hz, 2H, CH_2), 1.29 (t, *J* = 7.6 Hz, 3H, CH_3); δ_C ($CDCl_3$, 100 MHz): 177.7 (C-4), 164.0 (C-7), 160.9 (C-5), 160.8 (C-2), 159.9 (C-8a), 148.0 (C-4’), 128.9 (C-1’), 128.5 (C-3’, C5’), 126.0 (C-2’, C-6’), 109.3 (C-4a), 108.5 (C-3), 96.1 (C-6), 92.8 (C-8), 56.4 (OCH_3), 55.8 (OCH_3), 28.8 (CH_2), 15.3 (CH_3); HRMS (ES^+) found $[M+H]^+$ 311.1280, $C_{19}H_{18}O_4$ requires *M* 311.1283.

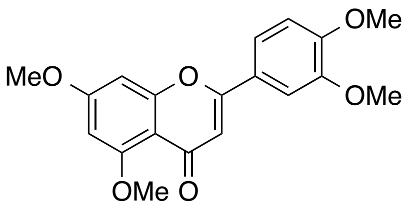
5,7-Dimethoxy-2-(3'-methoxyphenyl)-4H-chromen-4-one[174] **29**



5,7-Dimethoxy-2-(3'-methoxyphenyl)-4H-chromen-4-one **29** was synthesised according to General procedure B, using 3-methoxyphenylboronic acid (76 mg). The reaction mixture was filtered through Celite® and concentrated. DCM (2 ml) and H_2O (2 ml) were added and the layers separated using a phase separator. The organic layer was concentrated and the title product (58 mg, 75%) was afforded by flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 19:1) as a light yellow solid.

m.p.: 143 - 146 °C (lit.[174] m.p. 147 – 148 °C); ν_{max} (ATR): 1634 (C=O), 1592, 1456, 1350, 1279, 1120, 1107, 1058, 818 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.45 (dt, J = 8.0, 1.2 Hz, 1H, 6'-H), 7.43 - 7.35 (m, 2H, 2'-H, 5'-H), 7.03 (ddd, J = 7.8, 2.5, 1.2 Hz, 1H, 4'-H), 6.66 (s, 1H, 3-H), 6.56 (d, J = 2.3 Hz, 1H, 8-H), 6.37 (d, J = 2.3 Hz, 1H, 6-H), 3.95 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.7 (C-4), 164.2 (C-7), 161.0 (C-5), 160.5 (C-2), 160.1 (C-8a), 160.0 (C-3'), 133.0 (C-1'), 130.1 (C-5'), 118.5 (C-6'), 117.0 (C-4'), 111.4 (C-2'), 109.5 (C-4a), 109.4 (C-3), 96.3 (C-6), 93.0 (C-8), 56.6 (OCH_3), 55.9 (OCH_3), 55.6 (OCH_3); m/z LC-MS (ES$^+$) 313 [M+H]$^+$.

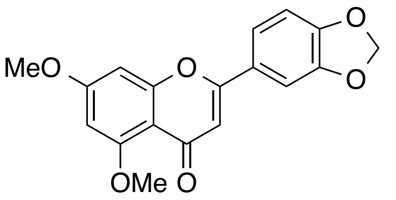
5,7-Dimethoxy-2-(3’,4’-dimethoxyphenyl)-4H-chromen-4-one[98] **30**



5,7-Dimethoxy-2-(3’,4’-dimethoxyphenyl)-4H-chromen-4-one **30** was synthesised according to General procedure B, using 3,4-dimethoxyphenylboronic acid (91 mg). The reaction mixture was filtered through Celite® and concentrated. DCM (2 ml) and H_2O (2 ml) were added and the layers separated using a phase separator. The organic layer was concentrated and the title product (61 mg, 71%) was obtained by flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 19:1) as a colourless solid.

m.p.: 191 - 192 °C; ν_{max} (ATR): 1644 (C=O), 1600, 1355, 1254, 1220, 1138, 1118, 1018, 831, 805 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.51 (dd, J = 8.5, 2.1 Hz, 1H, 6’-H), 7.32 (d, J = 2.1 Hz, 1H, 2’-H), 6.96 (d, J = 8.5 Hz, 1H, 5’-H), 6.61 (s, 1H, 3-H), 6.56 (d, J = 2.3 Hz, 1H, 8-H), 6.38 (d, J = 2.3 Hz, 1H, 6-H), 3.99 - 3.94 (m, 9H, 3 × OCH_3), 3.92 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.8 (C-4), 164.1 (C-7), 161.0 (C-5), 160.8 (C-2), 160.0 (C-8a), 151.8 (C-4’), 149.4 (C-3’), 124.2 (C-1’), 119.6 (C-6’), 111.2 (C-5’), 109.4 (C-4a), 108.7 (C-2’), 108.1 (C-3), 96.2 (C-6), 93.0 (C-8), 56.6 (OCH_3), 56.2 (OCH_3), 56.2 (OCH_3), 55.9 (OCH_3); m/z LC-MS (ES$^+$) 343 $[M+H]^+$.

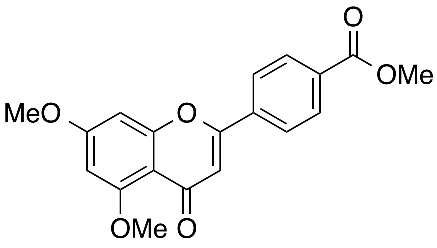
5,7-Dimethoxy-2-(3’,4’-methylenedioxyphenyl)-4H-chromen-4-one **31**



5,7-Dimethoxy-2-(3’,4’-methylenedioxyphenyl)-4H-chromen-4-one **31** was synthesised according to General procedure B, using 3,4-methylenedioxyphenylboronic acid (83 mg). The reaction mixture was filtered through Celite® and concentrated. DCM (2 ml) and H_2O (2 ml) were added and the layers separated using a phase separator. The organic layer was concentrated and the pure title product (25 mg, 30%) was afforded by flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 19:1) as a colourless solid.

m.p.: 233 - 236 °C; ν_{max} (ATR): 1652 (C=O), 1612, 1451, 1328, 1105, 921, 807 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.43 (dd, *J* = 8.2, 1.8 Hz, 1H, 6’-H), 7.31 (d, *J* = 1.8 Hz, 1H, 2’-H), 6.91 (d, *J* = 8.2 Hz, 1H, 5’-H), 6.58 - 6.53 (m, 2H, 3-H, 8-H), 6.37 (d, J = 2.3 Hz, 1H, 6-H), 6.06 (s, 2H, OCH_2O), 3.95 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.7 (C-4), 164.1 (C-7), 161.1 (C-5), 160.5 (C-2), 159.9 (C-8a), 150.4 (C-4’), 148.5 (C-3’), 125.7 (C-1’), 121.1 (C-6’), 109.4 (C-4a), 108.8 (C-5’), 108.3 (C-3), 106.2 (C-2’), 102.0 (CH_2), 96.3 (C-6), 92.9 (C-8), 56.6 (OCH_3), 55.9 (OCH_3); m/z LC-MS (ES$^+$) 327 [M+H]$^+$.

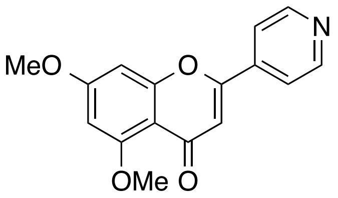
5,7-Dimethoxy-2-(4'-methoxycarbonylphenyl)-4H-chromen-4-one **32**



5,7-Dimethoxy-2-(4'-methoxycarbonylphenyl)-4H-chromen-4-one **32** was synthesised according to General procedure B, using 4-methoxycarbonylphenylboronic acid (90 mg). The reaction mixture was filtered through Celite® and concentrated. DCM (2 ml) and H_2O (2 ml) were added and the layers separated using a phase separator. The organic layer was concentrated and flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 19:1) yielded the title product (58 mg, 68%) as a colourless solid.

m.p.: 202 - 204 °C; ν_{max} (ATR): 1715 (C=O), 1640 (C=O), 1594, 1567, 1277, 1218, 1109, 1058, 826, 772, 699 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.18 - 8.13 (m, 2H, 4'-H, 5'-H), 7.97 - 7.92 (m, 2H, 2'-H, 6'-H), 6.74 (s, 1H, 3-H), 6.59 (d, J = 2.3 Hz, 1H, 8-H), 6.40 (d, J = 2.3 Hz, 1H, 6-H), 3.99 - 3.95 (m, 6H, $COOC\mathbf{H}_3$, OCH_3), 3.93 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.5 (C-4), 166.4 ($\mathbf{C}OOCH_3$), 164.4 (C-7), 161.1 (C-5), 160.0 (C-2), 159.5 (C-8a), 135.8 (C-1'), 132.4 (C-4'), 130.2 (C-3', C-5'), 126.0 (C-2', C-6'), 110.5 (C-3), 109.5 (C-4a), 96.5 (C-6), 93.0 (C-8), 56.6 (OCH_3), 56.0 (OCH_3), 52.6 ($COO\mathbf{C}H_3$); HRMS (ES^+) found $[M+H]^+$ 341.1035, $C_{19}H_{16}O_6$ requires *M* 341.1025.

5,7-Dimethoxy-2-(pyrid-4’-yl)-4H-chromen-4-one[107] **38**

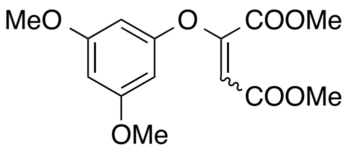


2-Chloro-5,7-dimethoxy-4H-chromen-4-one **21** (60 mg, 0.25 mmol, 1 eq), 4-pyridineboronic acid (62 mg, 0.50 mmol, 2 eq), $Pd_2(dba)_3$ (4.6 mg, 2 mol%), Cy_3P (3.4 mg, 4.8 mol%), K_3PO_4 (0.33 ml of a 1.27 M solution in H_2O, 0.425 mmol, 1.7 eq) and dioxane (0.67 ml) were mixed in a reaction tube of a carousel reactor under a nitrogen atmosphere. The reaction mixture was heated to 100°C and stirred for 22 h. After allowing to cool to rt, the crude mixture was filtered through Celite® and concentrated The residue was dissolved in 1 N HCl (5 ml) and washed with DCM (3 ml). Then 1 N NaOH was added to the aqueous layer until it was basic and the product was extracted DCM (3 × 5 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. Flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 19:1) yielded the title product (11 mg, 15%) as a colourless solid.

m.p.: 229 - 230 °C; ν_{max} (ATR): 1652 (C=O), 1594, 1413, 1349, 1322, 1200, 1166, 1119, 822, 646 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.84 - 8.75 (m, 2H, 3’-H, 5’-H), 7.77 - 7.71 (m, 2H, 2’-H, 6’-H), 6.79 (s, 1H, 3-H), 6.60 (d, *J* = 2.3 Hz, 1H, 8-H), 6.42 (d, *J* = 2.3 Hz, 1H, 6-H), 3.98 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.1 (C-4), 164.6 (C-7), 161.1 (C-5), 159.9 (C-2), 157.9 (C-8a), 150.9 (C-3’, C-5’), 139.1 (C-1’), 119.6 (C-2’, C-6’), 111.1 (C-3), 109.6 (C-4a), 96.6 (C-6), 93.0 (C-8), 56.6 (OCH_3), 56.0 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 284.0903, $C_{16}H_{13}NO_4$ requires *M* 284.0923.

5.1.2.3 Synthesis of 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamides

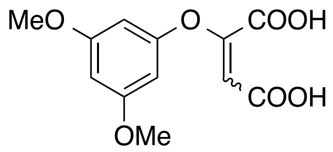
Dimethyl (E,Z)-2-(3',5'-dimethoxyphenoxy)ethene-1-2-dicarboxylate **39**



3,5-Dimethoxyphenol **17** (920 mg, 6 mmol, 1 eq) was dissolved in dry diethylether (9 ml) under an argon atmosphere. First Et_3N (1.17 ml, 8.4 mmol, 1.4 eq) and then dimethylacetylenedicarboxylate (0.96 ml, 7.8 mmol, 1.3 eq) were added and the solution was stirred at rt for 5.5 h. The reaction mixture was washed with 1 N HCl (3 ml), H_2O (3 ml) and brine (3 ml), dried over Na_2SO_4 and concentrated. Flash column chromatography (40 g silica gel, hexane:EtOAc 9:1 - 4:1) afforded the title ester (1:0.9 mixture of E:Z isomers, 1.439 g, 81 %) as a colourless solid.

m.p.: 56 - 58 °C; δ_H ($CDCl_3$, 400 MHz): 6.57 (s, 1H, 1-H (E)), 6.33 (t, J = 2.2 Hz, 1H, 4'-H (Z)), 6.26 (d, J = 2.2 Hz, 2H, 2'-H, 6'-H (Z)), 6.17 (t, J = 2.2 Hz, 1H, 4'-H (E)), 6.12 (d, J = 2.2 Hz, 2H, 2'-H, 6'-H (E)), 5.24 (s, 1H, 1-H (Z)), 3.92 (s, 3H, COOC**H**$_3$ (Z)), 3.77 - 3.74 (m, 15H, 5 × OC**H**$_3$), 3.71 (s, 3H, COOC**H**$_3$ (E)), 3.67 (s, 3H, COOC**H**$_3$ (Z)); δ_C ($CDCl_3$, 100 MHz): 166.0 (C-2 (E/Z)), 163.9 (C-2 (E/Z)), 163.4 (**C**OOCH$_3$ (Z)), 162.7 (**C**OOCH$_3$ (E)), 161.8 (C-3', C-5' (Z)), 161.6 (C-3', C-5' (E)), 160.5 (**C**OOCH$_3$ (Z)), 158.3 (C-1' (E)), 154.6 (C-1' (Z)), 149.6 (**C**OOCH$_3$ (E)), 115.5 (C-1 (E)), 99.3 (C-1 (Z)), 99.2 (C-2', C-6' (Z)), 98.7 (C-4' (Z)), 95.7(C-4' (E)), 95.0 (C-2', C-6' (E)), 55.7 (2 × O**C**H$_3$ (E/Z)), 55.5 (2 × O**C**H$_3$ (E/Z)), 53.2 (COO**C**H$_3$ (E)), 53.2 (COO**C**H$_3$ (Z)), 52.1 (COO**C**H$_3$ (E)), 51.89(COO**C**H$_3$ (Z)); HRMS (ES^+) found $[M+H]^+$ 297.0995, $C_{14}H_{16}O_7$ requires *M* 297.0974.

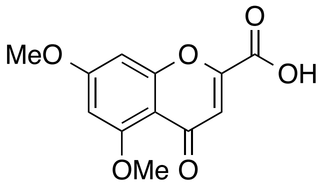
(E,Z)-2-(3’,5’-Dimethoxyphenoxy)ethene-1-2-dicarboxylic acid **40**



A 1:0.9 mixture of the E and Z isomer of dimethyl 2-(3’,5’-dimethoxyphenoxy)ethene-1-2-dicarboxylate **39** (1.25 g, 4.2 mmol, 1 eq) was heated under reflux with NaOH (680 mg, 16.9 mmol, 4 eq) in H_2O (6.3 ml) for 3 h. The reaction was allowed to cool to rt, washed with Et_2O (3 ml) and acidified to pH 1 with concentrated HCl. Et_2O (7 ml) was added to dissolve the precipitated product and the aqueous layer was extracted with Et_2O (3 × 5 ml). The combined organic layers were dried over Na_2SO_4 and concentrated, which afforded the title acid (1:0.8 mixture of E:Z isomers, 1.08 g, 95 %) as a yellow solid.

m.p.: 147 - 148 °C; δ_H (d^6-DMSO, 400 MHz): 6.51 (s, 1H, 1-H (E)), 6.44 (t, J = 2.2 Hz, 1H, 4’-H (Z)), 6.28 (d, J = 2.2 Hz, 2H, 2’-H, 6’-H (Z)), 6.122 (t, J = 2.2 Hz, 1H, 4’-H (E)), 6.05 (d, J = 2.2 Hz, 2H, 2’-H, 6’-H (E)), 5.23 (s, 1H, 1-H (Z)), 3.75 (s, 6H, O**C**H_3 (Z)), 3.71 (s, 6H, O**C**H_3 (E)); δ_C (d^6-DMSO, 100 MHz): 165.9 (C-2 (E/Z)), 164.5 (C-2 (E/Z)), 163.3 (**C**OOCH_3 (Z)), 163.0 (**C**OOCH_3 (E)), 161.4 (C-3’, C-5’ (Z)), 161.1 (C-3’, C-5’ (E)), 159.0 (**C**OOCH_3 (Z)), 158.2 (C-1’ (E)), 155.0 (C-1’ (Z)), 148.3 (**C**OOCH_3 (E)), 116.8 (C-1 (E)), 101.9 (C-1 (Z)), 98.4 (C-2’, C-6’ (Z)), 97.7 (C-4’ (Z)), 94.7 (C-4’ (E)), 94.5 (C-2’, C-6’ (E)), 55.7 (2 × O**C**H_3 (Z)), 55.4 (2 × O**C**H_3 (E)),; HRMS (ES$^+$) found $[M+H]^+$ 269.0691, $C_{12}H_{12}O_7$ requires *M* 269.0661.

5,7-Dimethoxy-4-oxo-4H-chromene-2-carboxylic acid **41**



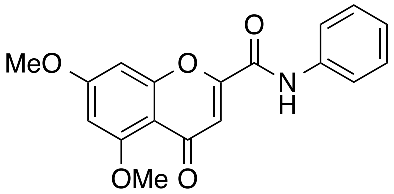
A 1:0.8 mixture of dimethyl 2-(3',5'-dimethoxyphenoxy)fumaric acid and dimethyl 2-(3',5'-dimethoxyphenoxy)maleic acid (1.04 g, 3.9 mmol, 1 eq) was dissolved in acetyl chloride (22 ml) and concentrated H_2SO_4 (0.88 ml) slowly added. The reaction mixture was heated to 60 °C and stirred for 10 min. The reaction was allowed to cool to rt and the acetyl chloride removed under reduced pressure (the removed acetyl chloride was carefully destroyed with H_2O). The reaction mixture was cooled with an ice bath and H_2O (approx. 65 ml) slowly added. The precipitate was collected by filtration and dried over Na_2SO_4 to afford the title acid (390 mg, 40 %) as a colourless solid.

m.p.: 248 - 250 °C; ν_{max} (ATR): 2934 (COO-H), 1748 (C=O), 1640 (C=O), 1594, 1219, 1139, 811, 672 cm^{-1}; δ_H (d^6-DMSO, 400 MHz): 6.72 (d, J = 2.3 Hz, 1H, 8-H), 6.64 (s, 1H, 3-H), 6.54 (d, J = 2.3 Hz, 1H, 6-H), 3.89 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); δ_C (d^6-DMSO, 100 MHz): 175.5 (C-4), 164.4 (C-7), 161.5 (COOH), 160.4 (C-5), 159.1 (C-8a), 150.6 (C-2), 115.4 (C-3), 109.1 (C-4a), 96.7 (C-6), 93.4 (C-8), 56.2 (OCH_3), 56.1 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 251.0587, $C_{12}H_{10}O_6$ requires *M* 251.0556.

General Procedure C

To a solution of 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxylic acid **41** (62 mg, 0.25 mmol, 1 eq) in dry DMF (0.34 ml) in a microwave vial, $POCl_3$ (38 g, 0.25 mmol, 1 eq) was added. The reaction mixture was stirred at rt for 10 min and successively heated to 50 °C and stirred for another 50 min. The amine (0.25 mmol, 1 eq) was added and the mixture heated for 5 min to 160 °C in a microwave. H_2O (4.5 ml) was added, after the reaction was allowed to cool to rt and the precipitated product isolated by filtration. Flash column chromatography (4 g silica gel, hexane:acetone 3:1 - 1:1 (3% Et_3N)) afforded the desired product.

N-Phenyl 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide **42**

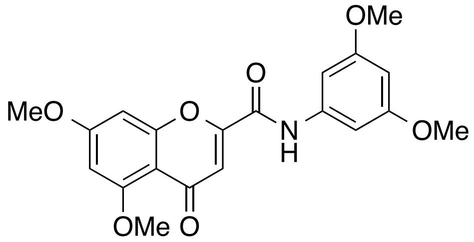


N-Phenyl 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide **42** was synthesised according to General procedure C, using aniline (23 mg). The title product (39 mg, 48%) was obtained as a colourless solid.

m.p.: 203 - 205 °C; ν_{max} (ATR): 1641 (C=O), 1595 (N-C=O), 1418, 1328, 1222, 1161, 1103, 857, 749, 692 cm^{-1}; δ_H (d^6-DMSO, 400 MHz): 10.57 (s, 1H, NH), 7.81 - 7.74 (m, 2H, 2'-H, 6'-H), 7.46 - 7.37 (m, 2H, 3'-H, 5'-H), 7.23 - 7.16 (m, 1H, 4'-H), 6.87 (d, *J* = 2.3 Hz, 1H, 8-H), 6.72 (s, 1H, 3-H), 6.57 (d, *J* = 2.3 Hz, 1H, 6-H), 3.92 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3); δ_C (d^6-DMSO, 100 MHz): 175.3 (C-4), 164.2 (C-7), 160.4 (C-5), 158.7 (C-8a), 157.7 (CONH), 153.0 (C-2), 137.5 (C-1'), 128.8 (C-3', C-5'), 124.9 (C-4'), 121.1 (C-2', C-

6’), 112.8 (C-3), 108.9 (C-4a), 96.7 (C-6), 93.6 (C-8), 56.2 (OCH_3), 56.0 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 326.1044, $C_{18}H_{15}NO_5$ requires *M* 326.1028.

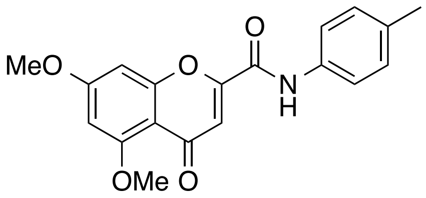
N-(3’,5’-Dimethoxyhenyl) 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide **43**



N-(3’,5’-Dimethoxyhenyl) 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide **43** was synthesised according to General procedure C, using 3,5-dimethoxyaniline (38 mg). The title product (31 mg, 32%) was obtained as a colourless solid.

m.p.: 220 - 222 °C; ν_{max} (ATR): 1651 (C=O), 1601 (N-C=O), 1543, 1455, 1418, 1338, 1262, 1202, 1151, 1054, 822 cm^{-1}; δ_H (d^6-DMSO, 400 MHz): 10.47 (s, 1H, NH), 7.07 (d, J = 2.2 Hz, 2H, 2’-H, 6’-H), 6.87 (d, J = 2.3 Hz, 1H, 8-H), 6.71 (s, 1H, 3-H), 6.57 (d, J = 2.3 Hz, 1H, 6-H), 6.35 (t, J = 2.2 Hz, 1H, 4’-H), 3.92 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.76 (s, 6H, 2x OCH_3); δ_C (d^6-DMSO, 100 MHz): 175.3 (C-4), 164.2 (C-7), 160.4 (C-5), 160.4 (C-3’), 158.7 (C-8a), 157.7 (CONH), 152.8 (C-2), 139.2 (C-1’), 112.8 (C-3), 108.9 (C-4a), 99.2 (C-2’, C-6’), 96.8 (C-4’), 96.7 (C-6), 93.6 (C-8), 56.2 (OCH_3), 56.1 (OCH_3), 55.2 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 386.1249, $C_{20}H_{19}NO_7$ requires *M* 386.1240.

N-(4’-Methylphenyl) 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide **44**

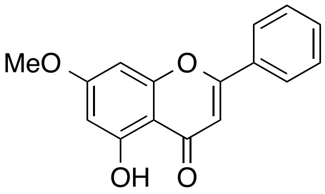


N-(4’-Methylphenyl) 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide **44** was synthesised according to General procedure C, using *p*-methylaniline (27 mg). The title product (38 mg, 44%) was obtained as a colourless solid.

m.p.: 198 - 200 °C; ν_{max} (ATR): 1645 (C=O), 1597 (N-C=O), 1531, 1316, 1163, 1122, 1104, 1071, 806 cm^{-1}; δ_H (d^6-DMSO, 400 MHz): 10.51 (s, 1H, NH), 7.67 - 7.63 (m, 2H, 2’-H, 6’-H), 7.23 - 7.19 (m, 2H, 3’-H, 5’-H), 6.87 (d, J = 2.3 Hz, 1H, 8-H), 6.70 (s, 1H, 3-H), 6.57 (d, J = 2.3 Hz, 1H, 6-H), 3.92 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 2.30 (s, 3H); δ_C (d^6-DMSO, 100 MHz): 175.3 (C-4), 164.2 (C-7), 160.4 (C-75, 158.7 (C-8a), 157.5 (CONH), 153.1 (C-2), 135.0 (C-1’), 134.0 (C-4’), 129.2 (C-3’, C-5’), 121.1 (C-2’, C-6’), 112.7 (C-3), 108.9 (C-4a), 96.7 (C-6), 93.6 (C-8), 56.2 (OCH_3), 56.0 (OCH_3), 20.5 (CH_3); HRMS (ES^+) found $[M+H]^+$ 340.1202, $C_{19}H_{17}NO_5$ requires *M* 340.1185.

5.1.3 Variations of the substituent in the 5-position

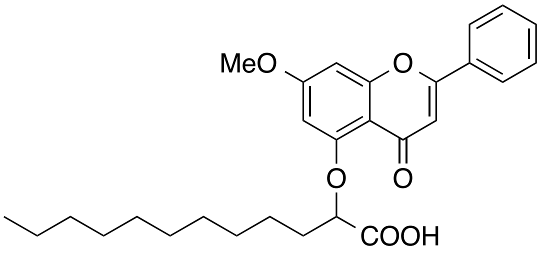
5-Hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one[175] **46**



To a solution of chrysin **45** (1.53 g, 6 mmol, 1 eq) in dry DMF (30 ml), K_2CO_3 (1.24 g, 9 mmol, 1.5 eq) and iodomethane (0.56 ml, 9 mmol, 1.5 eq) were added and the reaction mixture was stirred for 6.5 h at rt. 0.1M HCl (60 ml) was added to the mixture and the product was extracted with DCM (3 × 60 ml). The combined organic layers were washed with H_2O (3 × 40 ml), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The title product (1.39 g, 86%) was obtained by flash column chromatography (40 g silica gel, hexane:EtOAc 9:1 - 0:10) as a yellow solid.

m.p.: 166 - 167 °C (lit.[175] m.p. 252 – 254 °C); ν_{max} (ATR): 3072 (OH), 2952 (OH), 1663 (C=O), 1587, 1351, 1201, 1159, 1040, 848, 806, 768, 693 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 12.73 (s, 1H, OH), 7.93 - 7.85 (m, 2H, 2'-H, 6'H), 7.59 - 7.48 (m, 3H, 3'-H, 4'-H, 5'-H), 6.67 (s, 1H, 3-H), 6.51 (d, *J* = 2.3 Hz, 1H, 8-H), 6.38 (d, *J* = 2.3 Hz, 1H, 6-H), 3.89 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 182.7 (C-4), 165.8 (C-7), 164.1 (C-2), 162.4 (C-5), 158.0 (C-8a), 132.0 (C-4'), 131.5 (C-1'), 129.2 (C-3', C-5'), 126.5(C-2', C-6'), 106.1 (C-3), 105.9 (C-4a), 98.4 (C-6), 92.9 (C-8), 56.0 (OCH_3); m/z LC-MS (ES^+) 269 $[M+H]^+$.

2-(7'-methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-oxy)dodecanoic acid[85] **3**

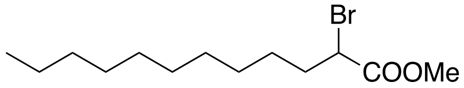


A solution of methyl 2-bromododecanoate **48** (294 mg, 1 mmol, 2 eq) in dry DMF (1 ml) was added to a mixture of 5-hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one (134 mg, 0.5 mmol, 1 eq) and K_2CO_3 (138 mg, 1 mmol, 2 eq) in dry DMF (5 ml) under an argon atmosphere. The reaction mixture was stirred at 60 °C for 18 h. H_2O (6 ml) was added and the product was extracted with EtOAc (3 × 15 ml). The combined organic layers were washed with H_2O (10 ml), dried over Na_2SO_4 and concentrated. The crude product was dissolved in a 1:1 mixture of THF (6.5 ml) and H_2O (6.5 ml) and $LiOH.H_2O$ (42 mg, 1 mmol, 2 eq) was added. The reaction mixture was stirred for 3.5 h at rt. The mixture was acidified with 1M HCl to pH 3 - 4 and extracted with EtOAc (3 × 15 ml). The combined organic layers were washed with brine (20 ml), dried over Na_2SO_4 and concentrated. Flash column chromatography (12 g silica gel, DCM:MeOH 1:0 - 4:1) afforded the title product (222 mg, 95%) as a colourless solid.

m.p.: 98 - 99 °C (lit.[85] m.p. 97 – 101 °C); ν_{max} (ATR): 2917 (COOH), 2848, 1748 (C=O), 1640 (C=O), 1598, 1353, 1194, 1159, 1107 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.95 - 7.88 (m, 2H, 2''-H, 6''-H), 7.62 - 7.51 (m, 3H, 3''-H, 4''-H, 5''-H), 6.79 (s, 1H 3'-H), 6.75 (d, *J* = 2.2 Hz, 1H, 8'-H), 6.50 (d, *J* = 2.2 Hz, 1H, 6'-H), 4.86 (t, *J* = 5.3 Hz, 1H, 2-H), 3.96 (s, 3H, OCH_3), 2.23 - 2.11 (m, 2H, 3-H_2), 1.67 – 1.51 (m, 2H, 4-H_2), 1.43 - 1.23 (m, 14H, 7 × CH_2), 0.89 (t, J=6.9 Hz, 3H, 12-H_3); δ_C ($CDCl_3$, 100 MHz): 179.0 (C-4'), 172.5 (C-1), 164.9 (C-7'), 162.9 (C-2'), 159.6 (C-8a'), 159.0 (C-5'), 132.1 (C-4''), 131.1 (C-1''), 129.3 (C-3'', C-5''), 126.4 (C-2'', C6''), 109.6 (C-4a'), 108.4 (C-3'), 101.4 (C-6'), 95.3 (C-8'), 82.0 (C-2), 56.2 (OCH_3), 33.1 (C-3), 32.0 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.8 (C-4), 22.8 (CH_2), 14.3 (C-12)*;* m/z LC-MS (ES^+) 467 $[M+H]^+$.

Synthesis of α-bromo acids from amino acids

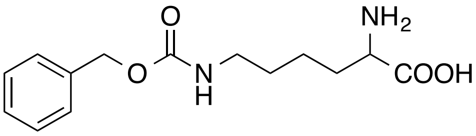
Methyl 2-bromododecanoate[176] **48**



α-Bromododecanoic acid **47** (2.23 g, 8 mmol, 1 eq) was dissolved in a 1:1 mixture of EtOAc (40 ml) and MeOH (40 ml) and TMS-diazomethane (8 ml, 16 mmol, 2 eq) was added at 0 °C. The reaction mixture was stirred for 17 h while allowing to warm to rt. The solvent was removed under reduced pressure, sat. aqueous $NaHCO_3$ (100 ml) was added and the product was extracted with DCM (4 × 80 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The title product (1.14 g, 49%) was obtained by flash column chromatography (24 g silica gel, hexane:EtOAc 1:0 - 4:1) as a colourless oil.

ν_{max} (ATR): 2924, 2854, 1743 (C=O), 1150 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 4.22 (dd, *J* = 7.8, 7.0 Hz, 1H, 2-H_2), 3.78 (s, 3H, $COOCH_3$), 2.11 - 1.87 (m, 2H, 3-H_2), 1.48 - 1.18 (m, 16H, 4-H_2, 5-H_2, 6-H_2, 7-H_2, 8-H_2, 9-H_2, 10-H_2, 11-H_2), 0.88 (t, *J* = 6.8 Hz, 3H, 12-H_3); δ_C ($CDCl_3$, 100 MHz): 170.6 (C-1), 53.0 (COO**C**H_3), 45.9 (C-2), 35.1 (CH_2), 32.0 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (2 × CH_2), 29.0 (CH_2), 27.4 (CH_2), 22.8 (CH_2), 14.3 (C-12); m/z ASAP (ES) 293 ($[M+H]^+$,Br^{79}), 295 ($[M+H]^+$,Br^{81}).

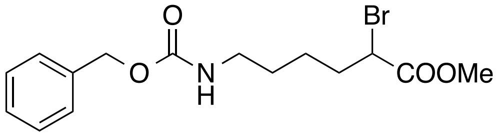
6-((Benzyloxycarbonyl)amino)-2-aminohexanoic acid[177] **51**



A solution of D,L-lysine.HCl **49** (3.65 g, 20 mmol, 1 eq) in aqueous NaOH (20 ml of a 2M solution, 40 mmol, 2 eq) was added to a solution of $CuSO_4.5H_2O$ (2.50 g, 10 mmol, 0.5 eq) in H_2O (8 ml) and cooled to 0°C. $NaHCO_3$ (2.18 g, 26 mmol, 1.3 eq) and benzyl chloroformate (3.75 g, 3.14 ml, 22 mmol, 1.1 eq) were added and the reaction mixture was stirred for 1 h at 0 °C and a further 17 h at rt. The blue precipitate Cu[lys(Cbz)]$_2$ **50** was collected, washed with H_2O and added to a boiling suspension of EDTA (6.08 g, 20.8 mmol, 1.04 eq) in H_2O (40 ml). The mixture was stirred for 3 h under reflux. After cooling to rt, 3N NaOH was added in order to neutralize the mixture and precipitate the crude product, which was collected by filtration. Recrystallization from H_2O afforded the title product (3.05 g, 54%) as a white solid.

m.p.: 250 - 252°C (decomposition) (lit.[177] m.p. 252 – 254 °C); ν_{max} (ATR): 3311, 2925, 1685 (C=O), 1576, 1539, 1282 cm^{-1}; δ_H (D_2O, 400 MHz): 7.49 - 7.34 (m, 5H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H), 5.10 (s, 2H, $ArCH_2$), 3.72 (t, *J* = 6.2 Hz, 1H, 2-H), 3.13 (t, *J* = 6.7 Hz, 2H, 6-H_2), 1.92 – 1.79 (m, 2H, 3-H_2), 1.57 – 1.48 (m, 2H, 5-H_2), 1.44 - 1.31 (m, 2H, 4-H_2); δ_C (d^6-DMSO, 100 MHz): 171.0 (C-1), 156.2 (NH-COOR), 137.3 (C-1'), 128.4 (C-3', C-5'), 127.8 (C-4'), 127.8 (C-2', C-6'), 65.2 ($ArCH_2$), 51.9 (C-2), 40.0 (C-6), 29.6 (C-3), 28.9 (C-5), 21.7 (C-4)*;* m/z LC-MS (ES$^+$) 281 [M+H]$^+$.

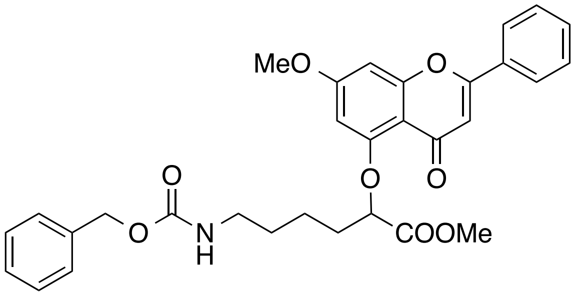
Methyl 6-((benzyloxycarbonyl)amino)-2-bromohexanoate[178] **55**



6-((Benzyloxycarbonyl)amino)-2-aminohexanoic acid **51** (1.85 g, 6.6 mmol, 1 eq) and KBr (1.38 g, 11.6 mmol, 1.75 eq) were dissolved in HBr (14.87 ml of a 1M solution in H_2O, 14.9 mmol, 2.25 eq). $NaNO_2$ (0.55 g, 14.9 mmol, 1.2 eq) was added at 0 °C over 1 h and the mixture was stirred for 3.5 h at 0 °C. After the addition of concentrated (conc.) H_2SO_4 (6.6 ml), the reaction mixture was stirred for another 10 min at 0 °C and then allowed to warm to rt. H_2O (10 ml) was added and the product was extracted with EtOAc (3 × 15 ml). The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated. The crude intermediate was dissolved in EtOAc (25 ml) and MeOH (25 ml) and TMS-diazomethane (6.6 ml, 13.2 mmol, 2 eq) was added at 0 °C. The reaction mixture was stirred for 22.5 h while allowing to warm to rt. The solvent was removed under reduced pressure and flash column chromatography (40 g silica gel, hexane:EtOAc 9:1 - 7:3) yielded the title product (1.08 g, 46%) as a colourless oil.

v_{max} (ATR):3340 (NH), 2952 (NH), 1695 (C=O), 1521, 1243, 1151, 696 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 7.38 - 7.29 (m, 5H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H), 5.09 (s, 2H, $ArCH_2$), 4.82 (br s, 1H, NH), 4.21 (t, *J* = 7.3 Hz, 1H, 2-H), 3.77 (s, 3H, $COOCH_3$), 3.20 (q, *J* = 6.4 Hz, 2H, 6-H_2), 2.11 - 1.96 (m, 2H, 3-H_2), 1.58 - 1.46 (m, 3H, 4-H_2, 5-H_2), 1.42 - 1.34 (m, 1H, 4-H_2); δ_C ($CDCl_3$, 175 MHz): 170.3 (C-1), 156.5 (NH-COOR), 136.7 (C-1'), 128.6 (C-3', C-4', C-5'), 128.2 (C-2', C-6'), 66.8 ($ArCH_2$), 53.1 ($COOCH_3$), 45.5 (C-2), 40.8 (C-6), 34.5 (C-3), 29.3 (C-5), 24.5 (C-4); m/z LC-MS (ES^-) 242 ($[M-H]^-$,Br^{79}), 244 ($[M-H]^-$,Br^{81}).

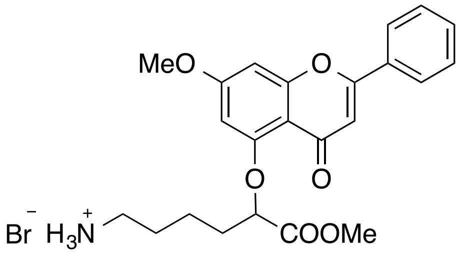
Methyl 6-((benzyloxycarbonyl)amino)-2-(7''-methoxy-4''-oxo-2''-phenyl-4''H-chromen-5''-oxy)hexanoate **56**



A solution of methyl 6-((benzyloxycarbonyl)amino)-2-bromohexanoate **55** (637 mg, 1.8 mmol, 1.5 eq) in dry DMF (3 ml) was added to a mixture of 7-methoxychrysin **46** (318 mg, 1.2 mmol, 1 eq) and K_2CO_3 (327 mg, 2.4 mmol, 2 eq) in dry DMF (7 ml) under an argon atmosphere. The reaction mixture was stirred at 60°C for 16 h. H_2O (10 ml) was added and the product was extracted with EtOAc (3 × 30 ml). The combined organic layers were washed with H_2O (10 ml) and brine (10 ml), dried over Na_2SO_4 and the solvent was evaporated. Flash column chromatography (40 g silica gel, hexane:EtOAc 9:1 - 0:1) afforded the title product (600 mg, 93%) as a colourless solid.

m.p.: 109 - 111 °C; ν_{max} (ATR): 3406 (NH), 2951 (NH), 1721 (C=O), 1640 (C=O), 1598, 1163, 1120, 769 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 7.83 – 7.80 (m, 2H, 2'''-H, 6'''-H), 7.54 - 7.47 (m, 3H, 3'''-H, 4'''-H, 5'''-H), 7.38 - 7.23 (m, 5H, 2'-H, 3'-H, 4'-H, 5'-H, 6'-H), 6.61 (d, *J* = 2.3 Hz, 1H, 8''-H), 6.57 (s, 1H, 3''-H), 6.22 (d, *J* = 2.3 Hz, 1H, 6''-H), 5.60 - 5.56 (br m, 1H, NH), 5.11 - 5.05 (m, 2H, $ArCH_2$), 4.67 (dd, *J* = 7.6, 4.3 Hz, 1H, 2-H), 3.88 (s, 3H, OCH_3), 3.75 (s, 3H, $COOCH_3$), 3.33 – 3.23 (m, 2H, 6-H_2), 2.17 – 2.03 (m, 2H, 3-H_2), 1.80 - 1.57 (m, 4H, 4-H_2, 5-H_2); δ_C ($CDCl_3$, 175 MHz): 177.2 (C-4''), 171.6 (C-1), 163.9 (C-7''), 160.9 (C-2''), 160.0 (C-8a''), 159.0 (C-5''), 156.8 (NHC=O), 137.1 (C-1'), 131.7 (C-1'''), 131.3 (C-4'''), 129.1 (C-3''', C-5'''), 128.5 (C-3', C-5'), 128.0 (C-2', C-6'), 128.0 (C-4'), 126.1 (C-2''', C-6'''), 110.1 (C-4a''), 109.3 (C-3''), 99.2 (C-6''), 94.3 (C-8''), 78.2 (C-2), 66.5 ($ArCH_2$), 55.9 (OCH_3), 52.5 (COO**C**H_3), 40.4 (C-6), 32.0 (C-3), 29.0 (C-5), 21.8 (C-4); HRMS (ES^+) found $[M+H]^+$ 546.2136, $C_{31}H_{32}NO_8$ requires *M* 546.2128.

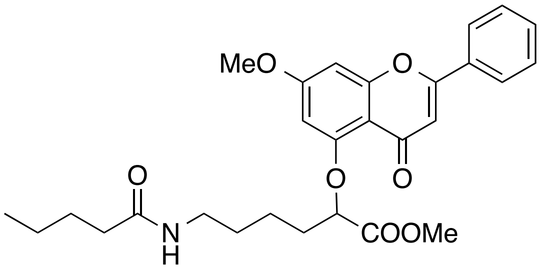
6-Methoxy-5-(7’-methoxy-4’-oxo-2’-phenyl-4’H-chromen-5’-oxy)-6-oxo-hexylammonium bromide **57**



HBr (0.5 ml, 33 wt. % solution in AcOH) was added to methyl 6-((benzyloxycarbonyl)amino)-2-(7’’-methoxy-4’’-oxo-2’’-phenyl-4’’H-chromen-5’’-oxy) hexanoate **56** (259 mg, 0.5 mmol, 1 eq) under an argon atmosphere and the mixture was stirred for 2 h. After adding dry Et_2O (25 ml) and stirring for another 10 min, the solid was collected by filtration, washed with Et_2O and dried, which yielded the title product (230 mg, 98%) as a yellow solid.

m.p.: 144 - 145 °C (decomposition); ν_{max} (ATR): 2851 (NH), 1739 (C=O), 1631 (C=O), 1593, 1366, 1208 cm^{-1}; δ_H (d^6-DMSO, 700 MHz): 8.08 - 8.02 (m, 2H, 2’’-H, 6’’-H), 7.68 (br s, 3H, NH_3^+), 7.62 - 7.54 (m, 3H, 3’’-H, 4’’-H, 5’’-H), 6.95 (d, *J* = 2.3 Hz, 1H, 8’-H), 6.75 (s, 1H, 3’-H), 6.30 (d, *J* = 2.3 Hz, 1H, 6’-H), 4.98 (t, *J* = 5.9 Hz, 1H, 5-H), 3.88 (s, 3H, OCH_3), 3.70 (s, 3H, $COOCH_3$), 2.85 – 2.80 (m, 2H, 1-H_2), 1.98 – 1.93 (m, 2H, 4-H_2), 1.68 - 1.51 (m, 4H, 2-H_2, 3-H_2); δ_C (d^6-DMSO, 175 MHz): 175.5 (C-4’), 170.9 (C-6), 163.4 (C-7’), 159.8 (C-2’), 159.2 (C-8a’), 158.1 (C-5’), 131.5 (C-4’’), 130.8 (C-1’’), 129.1 (C-3’’, C-5’’), 126.0 (C-2’’, C-6’’), 108.8 (C-4a’), 108.2 (C-3’), 98.4 (C-6’), 94.3 (C-8’), 76.4 (C-5), 56.1 (OCH_3), 52.2 (COO**C**H_3), 38.7 (C-1), 31.4 (C-4), 26.5 (C-2), 21.3 (C-3); HRMS (ES^+) found $[M+H]^+$ 412.1765, $C_{23}H_{26}NO_6$ requires *M* 412.1760.

Methyl 2-(7’-methoxy-4’-oxo-2’-phenyl-4’H-chromen-5’-oxy)-6-((1’’’-oxo-pentylamino)hexanoate **58**

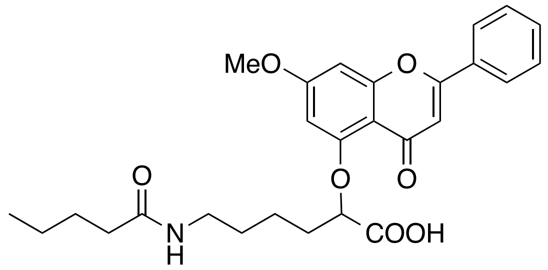


Valeric acid (0.11 ml, 1.02 mmol, 2 eq) was added to a solution of *N*-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (214 mg, 1.12 mmol, 2.2 eq) in dry DCM (7.5 ml) at 0 °C and the mixture was stirred for 30 min. After the addition of DMAP (25 mg, 0.20 mmol, 0.4 eq), *N,N*-diisopropylethylamine (164 mg, 1.27 mmol, 2.5 eq) and 6-methoxy-5-(7’-methoxy-4’-oxo-2’-phenyl-4’H-chromen-5’-oxy)-6-oxo-hexylammonium bromide **57** (250 mg, 0.51 mmol, 1 eq) the reaction mixture was stirred for 2.5 h at rt. Sat. aqueous NH_4Cl solution (20 ml) was added and the mixture was diluted with EtOAC (60 ml). The organic layer was separated and washed with sat. aqueous NH_4Cl solution (2 × 30 ml), sat. aqueous $NaHCO_3$ solution (30 ml) and brine (30 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and flash column chromatography (24 g silica gel, EtOAc) yielded the title product (175 mg, 70%) as a colourless solid.

m.p.: 108 - 110 °C; v_{max} (ATR): 3280 (NH), 2954, 2930, 2862, 1756 (C=O), 1646 (C=O), 1609, 1163, 1124 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.92 - 7.82 (m, 2H, 2’’-H, 6’’-H), 7.58 - 7.45 (m, 3H, 3’’-H, 4’’-H, 5’’-H), 6.90 – 6.83 (br m, 1H, NH), 6.61 (d, *J* = 2.3 Hz, 1H, 8’-H), 6.59 (s, 1H, 3’-H), 6.18 (d, *J* = 2.3 Hz, 1H, 6’-H), 4.67 (dd, *J* = 7.6, 4.0 Hz, 1H, 2-H), 3.87 (s, 3H, OCH_3), 3.76 (s, 3H, $COOCH_3$), 3.44 - 3.23 (m, 2H, 6-H_2), 2.27 - 2.19 (m, 2H, 2’’’-H_2), 2.19 - 1.98 (m, 2H, 3-H_2), 1.87 - 1.53 (m, 6H, 4-H_2, 5-H_2, 3’’’-H_2), 1.37 – 1.27 (m, 2H, 4’’’-H_2), 0.87 (t, *J* = 7.3 Hz, 3H, 5’’’-H_3); δ_C ($CDCl_3$, 100 MHz): 177.4 (C-4’), 174.2 (C-1’’’), 171.5 (C-1), 164.2 (C-7’), 161.5 (C-2’), 160.2 (C-8a’), 159.1 (C-5’), 131.6 (C-4’’), 131.5 (C-1’’), 129.2

(C-3'', C-5''), 126.2 (C-2'', C-6''), 109.6 (C-4a'), 108.8 (C-3'), 98.7 (C-6'), 94.1 (C-8'), 77.8 (C-2), 56.0 (OCH_3), 52.5 ($COOCH_3$), 38.4 (C-6), 36.4 (C-2'''), 31.6 (C-3), 28.3 (C-5/C-3'''), 28.1 (C-5/C-3'''), 22.7 (C-4'''), 21.6 (C-4), 14.0 (C-5'''); HRMS (ES^+) found $[M+H]^+$ 496.2344, $C_{28}H_{34}NO_7$ requires *M* 496.2335.

2-(7'-Methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-oxy)-6-((1'''-oxo-pentylamino)-hexanoic acid **59**

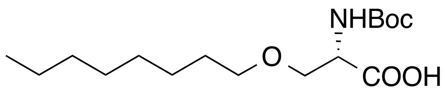


Methyl 2-(7'-methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-oxy)-6-((1'''-oxo-pentylamino)-hexanoate **58** (110 mg, 0.22 mmol, 1 eq) was dissolved in a 1:1 mixture of THF (5 ml) and H_2O (5 ml) and $LiOH.H_2O$ (19 mg, 0.44 mmol, 2 eq) was added. The reaction mixture was stirred for 1.5 h at rt and then neutralized with 1M HCl. The solvent was removed under reduced pressure. Flash column chromatography (24 g silica gel, DCM:MeOH 19:1 - 7:3) afforded the title product (100 mg, 94%) as a colourless solid.

m.p.: 186 - 188 °C (decomposition); ν_{max} (ATR): 3265 (NH), 2934, 2870, 1635 (C=O), 1628 (C=O), 1591, 1162, 1109 cm^{-1}; δ_H (d^6-DMSO, 700 MHz): 8.09 - 7.99 (m, 2H, 2''-H, 6''-H), 7.74 (t, *J* = 5.6 Hz, 1H, NH), 7.63 - 7.53 (m, 3H, 3''-H, 4''-H, 5''-H), 6.83 (br s, 1H, 8'-H), 6.78 (s, 1H, 3'-H), 6.43 (br s, 1H, 6'-H), 4.52 (br t, *J* = 5.7 Hz, 1H, 2-H), 3.85 (s, 3H, OCH_3), 3.01 – 2.91 (m, 2H, 6-H_2), 1.99 (t, *J* = 7.5 Hz, 2H, 2'''-H_2), 1.88 – 1.78 (m, 2H, 3-H_2), 1.46 – 1.31 (m, 6H, 4-H_2, 5-H_2, 3'''-H_2), 1.22 – 1.16 (m, 2H, 4'''-H_2), 0.80 (t, *J* = 7.4 Hz, 3H, 5'''-H_2); δ_C (d^6-DMSO 175 MHz): 176.8 (C-4'), 172.3 (C-1), 171.8 (C-1'''), 163.9 (C-7'), 160.2 (C-2'), 159.2 (C-8a'), 158.7 (C-5'), 131.6 (C-4''), 130.7 (C-1''), 129.1 (C-3'', C-5''), 126.0 (C-2'', C-6''), 108.4 (C-4a'), 107.9 (C-3'), 97.9 (C-6'), 93.0 (C-8'), 79.5 (C-2), 55.9 (OCH_3), 38.4 (C-6), 35.1 (C-2'''), 30.9 (C-3), 29.1 (C-5), 27.4 (C-3'''), 22.0 (C-4), 21.8 (C-4'''), 13.7 (C-5'''); HRMS (ES^+) found $[M+H]^+$ 482.2184, $C_{27}H_{32}NO_7$ requires *M* 482.2179.

5.1.3.1 Chiral synthesis

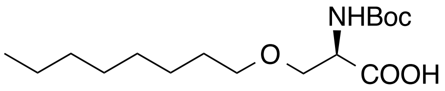
(S)-2-(*t*-Butyloxycarbonylamino)-3-(octyloxy)propanoic acid ***(S)*-67**



Boc-L-Ser-OH ***(S)*-66** (718 mg, 3.5 mmol, 1 eq) was dissolved in dry DMF (17.5 ml) under an argon atmosphere and cooled to 0 °C. NaH (308 mg, 7.7 mmol, 2.2 eq) was added and the reaction mixture was stirred for 45 min before the addition of 1-iodooctane (0.63 ml, 3.5 mmol, 1 eq). The mixture was allowed to warm to rt and stirred at rt for 24 h. H_2O (60 ml) was added and the reaction mixture was washed with Et_2O (50 ml) prior to acidifying with 3M HCl to pH 2-3. The product was extracted with EtOAc (5 × 50 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. Flash column chromatography (24 g silica gel, DCM:MeOH 1:0 - 9:1 + 1% AcOH) yielded the title product (255 mg, 23%) as a colourless oil.

$[\alpha]^{20}_{D}$ (c = 1.00 g/100 ml, $CHCl_3$): +19; ν_{max} (ATR): 2928, 2857, 1717 (C=O), 1161, 1118 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 5.36 (br d, *J* = 8.3 Hz, 1H, NH), 4.48 - 4.36 (br m, 1H, 2-H), 3.88 (dd, *J* = 9.4, 3.4 Hz, 1H, 3-H), 3.64 (dd, *J* = 9.4, 4.2 Hz, 1H, 3-H), 3.46 (t, *J* = 6.7 Hz, 2H, 1'-H_2), 1.58 – 1.52 (m, 2H, 2'-H_2), 1.45 (s, 9H, 3 × CH_3), 1.37 - 1.18 (m, 10H, 5 × H_2), 0.88 (t, *J* = 6.8 Hz, 3H, 8'-H_3); δ_C ($CDCl_3$, 150 MHz): 174.5 (C-1), 155.9 (NHCOOR), 80.6 (**C**(CH_3)$_3$), 72.1 (C-1'), 70.1 (C-3), 53.7 (C-2), 32.0 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 28.5 (3 × CH_3), 26.1 (CH_2), 22.8 (CH_2), 14.2 (C-8'); HRMS (ASAP) found $[M+H]^+$ 318.2286, $C_{16}H_{32}NO_5$ requires *M* 318.2280.

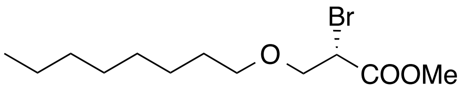
(R)-2-(*t*-Butyloxycarbonylamino)-3-(octyloxy)propanoic acid ***(R)*-67**



Boc-D-Ser-OH ***(R)*-66** (3.08 g, 15 mmol, 1 eq) was dissolved in dry DMF (75 ml) under an argon atmosphere and cooled to 0 °C. NaH (1.32 g, 33 mmol, 2.2 eq) was added and the reaction mixture was stirred for 40 min before the addition of 1-iodooctane (5.4 ml, 30 mmol, 2 eq). The mixture was allowed to warm to rt and stirred at rt for 20 h. H_2O (200 ml) was added and the reaction mixture was washed with Et_2O (200 ml) prior to acidifying with 3M HCl to pH 2-3. The product was extracted with EtOAc (5 × 80 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. Flash column chromatography (24 g silica gel, DCM:MeOH 1:0 - 9:1 + 1%AcOH) afforded the title product (460 mg, 10%) as a colourless oil.

$[\alpha]^{20}_{D}$ (c = 1.00 g/100 ml, $CHCl_3$): -18; ν_{max} (ATR): 2928, 2857, 1717 (C=O), 1161, 1118 cm^{-1}; δ_H ($CDCl_3$, 600 MHz): 5.36 (br d, *J* = 7.7 Hz, 1H, NH), 4.47 - 4.38 (br m, 1H, 2-H), 3.88 (dd, *J* = 9.0, 2.9 Hz, 1H, 3-H), 3.64 (dd, *J* = 9.0, 4.4 Hz, 1H, 3-H), 3.46 (t, *J* = 6.7 Hz, 2H, 1'-H_2), 1.59 – 1.52 (m, 2H, 2'-H_2), 1.46 (s, 9H, 3 × CH_3), 1.33 - 1.17 (m, 10H, 5 × H_2), 0.88 (t, *J* = 7.0 Hz, 3H, 8'-H_3); δ_C ($CDCl_3$, 150 MHz): 174.8 (C-1), 155.9 (NHCOOR), 80.5 (**C**(CH_3)$_3$), 72.0 (C-1'), 70.1 (C-3), 53.7 (C-2), 32.0 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 28.4 (3 × CH_3), 26.1 (CH_2), 22.8 (CH_2), 14.2 (C-8'); HRMS (ASAP) found $[M-H]^-$ 316.2112, $C_{16}H_{30}NO_5$ requires *M* 316.2124.

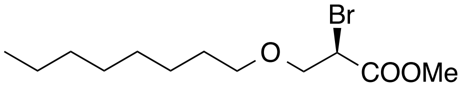
Methyl *(S)*-2-(bromo)-3-(octyloxy)propanoate ***(S)*-63**



For the Boc-deprotection *(S)*-2-(*t*-butyloxycarbonylamino)-3-(octyloxy)propanoic acid ***(S)*-67** (470 mg, 1.48 mmol, 1 eq) was dissolved in DCM (22 ml), triflouroacetic acid (1.48 ml) was added and the reaction mixture was stirred for 4.5 h at rt. The solvent was removed under reduced pressure and the intermediate together with KBr (529 mg, 4.44 mmol, 3 eq) was dissolved in HBr (3.33 ml of a 1M solution in H_2O, 3.33 mmol, 2.25 eq). $NaNO_2$ (123 mg, 1.78 mmol, 1.2 eq) was added at 0 °C over 1 h and the mixture was stirred for 3.5 h at 0 °C. After the addition of conc. H_2SO_4 (0.15 ml), the reaction mixture was stirred for another 10 min at 0 °C and then allowed to warm to rt. H_2O (10 ml) was added and the product was extracted with EtOAc (3 × 15 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude intermediate was dissolved in EtOAc (4.5 ml) and MeOH (4.5 ml) and TMS-diazomethane (2.22 ml, 4.44 mmol, 3 eq) was added at 0 °C. The reaction mixture was stirred for 15 h while allowing to warm to rt. The solvent was removed under reduced pressure and flash column chromatography (24 g silica gel, hexane:EtOAc 1:0 - 4:1) yielded the title product (138 mg, 32%) as a colourless oil.

$[\alpha]^{20}_D$ (c = 1.00 g/100 ml, $CHCl_3$): -21; ν_{max} (ATR): 2926, 2856, 1747 (C=O), 1114 cm^{-1}; δ_H ($CDCl_3$, 600 MHz): 4.31 (dd, *J* = 8.5, 5.8 Hz, 1H, 2-H), 3.92 (dd, *J* = 10.3, 8.5 Hz, 1H, 3-H), 3.79 (s, 3H, $COOCH_3$), 3.74 (dd, *J* = 10.3, 5.8 Hz, 1H, 3-H), 3.52 – 3.44 (m, 2H, 1'-H_2), 1.58 - 1.49 (m, 2H, 2'-H_2), 1.36 - 1.19 (m, 10H, 5 × H_2), 0.87 (t, *J* = 7.0 Hz, 3H, 8'-H); δ_C ($CDCl_3$, 150 MHz): 169.3 (C-1), 72.0 (C-1'), 71.6 (C-3), 53.2 (COO**C**H_3), 41.8 (C-2), 32.0 (CH_2), 29.6 (C-2'), 29.5 (CH_2), 29.4 (CH_2), 26.1 (CH_2), 22.8 (CH_2), 14.2 (C-8'); HRMS (ASAP) found $[M+H]^+$ 295.0901, $C_{12}H_{24}O_3{}^{79}Br$ requires *M* 295.0909.

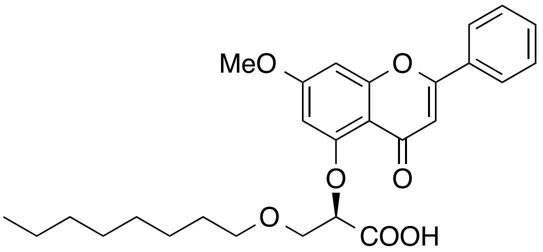
Methyl *(R)*-2-(bromo)-3-(octyloxy)propanoate ***(R)*-63**



For the Boc-deprotection *(R)*-2-(*t*-butyloxycarbonylamino)-3-(octyloxy)propanoic acid ***(R)*-67** (410 mg, 1.29 mmol, 1 eq) was dissolved in DCM (20 ml), triflouroacetic acid (1.29 ml) was added and the reaction mixture was stirred for 4.5 h at rt. The solvent was removed under reduced pressure and the intermediate together with KBr (461 mg, 3.87 mmol, 3 eq) was dissolved in HBr (2.91 ml of a 1M solution in H_2O, 2.91 mmol, 2.25 eq). $NaNO_2$ (107 mg, 1.55 mmol, 1.2 eq) was added at 0 °C over 1 h and the mixture was stirred for 3.5 h at 0 °C. After the addition of conc. H_2SO_4 (0.13 ml), the reaction mixture was stirred for another 10 min at 0 °C and then allowed to warm to rt. H_2O (10 ml) was added and the product was extracted with EtOAc (3 × 15 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude intermediate was dissolved in EtOAc (4 ml) and MeOH (4 ml) and TMS-diazomethane (1.94 ml, 3.88 mmol, 3 eq) was added at 0 °C. The reaction mixture was stirred for 15 h while allowing to warm to rt. The solvent was removed under reduced pressure and flash column chromatography (24 g silica gel, hexane:EtOAc 1:0 - 4:1) yielded the title product (125 mg, 33%) as a colourless oil.

$[\alpha]^{20}_D$ (c = 1.00 g/100 ml, $CHCl_3$): +40; ν_{max} (ATR): 2926, 2856, 1747 (C=O), 1114 cm^{-1}; δ_H ($CDCl_3$, 600 MHz): 4.31 (dd, *J* = 8.5, 5.8 Hz, 1H, 2-H), 3.92 (dd, *J* = 10.3, 8.5 Hz, 1H, 3-H), 3.80 (s, 3H, $COOCH_3$), 3.74 (dd, *J* = 10.3, 5.8 Hz, 1H, 3-H), 3.53 – 3.44 (m, 2H, 1'-H_2), 1.58 - 1.50 (m, 2H, 2'-H_2), 1.34 - 1.20 (m, 10H, 5 × H_2), 0.88 (t, *J* = 7.0 Hz, 3H, 8'-H); δ_C ($CDCl_3$, 150 MHz): 169.3 (C-1), 72.0 (C-1'), 71.6 (C-3), 53.2 (COO**C**H_3), 41.8 (C-2), 32.0 (CH_2), 29.6 (C-2'), 29.5 (CH_2), 29.4 (CH_2), 26.1 (CH_2), 22.8 (CH_2), 14.2 (C-8'); HRMS (ASAP) found $[M+H]^+$ 295.0917, $C_{12}H_{24}O_3{}^{79}Br$ requires *M* 295.0909.

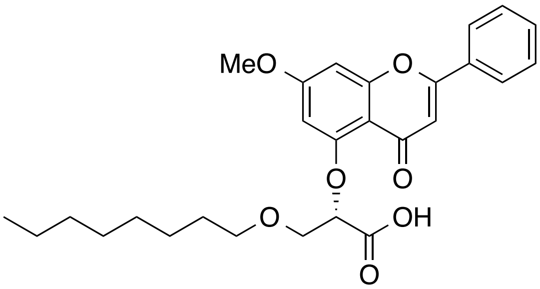
(R)-2-(7'-methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-oxy)-3'-(octyloxy)propanoic acid ***(R)*-62**



A solution of methyl *(S)*-2-(bromo)-3-(octyloxy)propanoate ***(S)*-63** (55 mg, 0.19 mmol, 1 eq) in dry DMF (1 ml) was added to a mixture of 7-methoxy-chrysin **46** (65 mg, 0.24 mmol, 1.3 eq) and Cs_2CO_3 (158 mg, 0.48 mmol, 2.6 eq) under an argon atmosphere. The reaction mixture was stirred at rt for 19 h. H_2O (15 ml) was added and the product was extracted with DCM (3 × 20 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude intermediate was dissolved in a 1:1 mixture of THF (9.2 ml) and H_2O (9.2 ml) and $LiOH.H_2O$ (16 mg, 0.37 mmol, 2 eq) was added. The reaction mixture was stirred for 2 h at rt. The mixture was acidified with 1M HCl to pH 6 and extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4 and concentrated. Flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 9:1) afforded the title product (52 mg, 60%) as a colourless solid.

$[\alpha]^{20}_D$ (c = 1.00 g/100 ml, $CHCl_3$): -17; 11% ee (determined by chiral HPLC analysis); m.p.: 126 - 127°C; ν_{max} (ATR): 2916, 2854, 1730 (C=O), 1631, 1616, 1596, 1363, 1166, 1123, 843 cm^{-1}; δ_H ($CDCl_3$, 600 MHz): 7.93 - 7.85 (m, 2H, 2''-H, 6''-H), 7.59 - 7.48 (m, 3H, 3''-H, 4''-H, 5''-H), 7.06 (d, *J* = 2.3 Hz, 1H, 6'-H), 6.77 (s, 1H, 3'-H), 6.75 (d, *J* = 2.3 Hz, 1H, 8'-H), 4.94 (dd, *J* = 8.8, 2.2 Hz, 1H, 2-H), 4.23 (dd, *J* = 10.9, 2.2 Hz, 1H, 3-H), 3.92 (s, 3H, OCH_3), 3.87 (dd, *J* = 10.9, 8.8 Hz, 1H, 3-H), 3.62 – 3.51 (m, 2H, 1'''-H_2), 1.66 - 1.59 (m, 2H, 2'''-H_2), 1.40 - 1.19 (m, 10H, 5 × H_2), 0.87 (t, *J* = 7.0 Hz, 3H, 8'''-H_3); δ_C ($CDCl_3$, 150 MHz): 179.0 (C-4'), 170.2 (C-1), 165.1 (C-7'), 163.0 (C-2'), 159.7 (C-5'), 159.2 (C-8a'), 132.1 (C-4’’), 131.1 (C-1’’), 129.3 (C-3’’, C-5’’), 126.4 (C-2’’, C-6’’), 109.4 (C-4a’), 108.1 (C-3’), 103.1 (C-6’), 96.3 (C-8’), 83.3 (C-2), 72.2 (C-1’’’), 72.0 (C-3), 56.2 (OCH_3), 32.0 (CH_2), 29.8 (C-2’’’), 29.6 (CH_2), 29.4 (CH_2), 26.3 (CH_2), 22.8 (CH_2), 14.2 (C-8’’’); HRMS (ES^+) found $[M+H]^+$ 469.2240, $C_{27}H_{33}O_7$ requires *M* 469.2226.

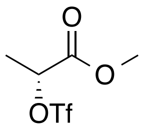
(S)-2-(7’-Methoxy-4’-oxo-2’-phenyl-4’H-chromen-5’-oxy)-3-(octyloxy)propanoic acid ***(S)*-62**



A solution of methyl *(R)*-2-(bromo)-3-(octyloxy)propanoate ***(R)*-63** (55 mg, 0.19 mmol, 1 eq) in dry DMF (1 ml) was added to a mixture of 7-methoxy-chrysin **46** (65 mg, 0.24 mmol, 1.3 eq) and Cs_2CO_3 (158 mg, 0.48 mmol, 2.6 eq) under an argon atmosphere. The reaction mixture was stirred at rt for 19 h. H_2O (15 ml) was added and the product was extracted with DCM (3 × 20 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude intermediate was dissolved in a 1:1 mixture of THF (9.2 ml) and H_2O (9.2 ml) and $LiOH.H_2O$ (16 mg, 0.37 mmol, 2 eq) was added. The reaction mixture was stirred for 2 h at rt. The mixture was acidified with 1M HCl to pH 6 and extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4 and concentrated. Flash column chromatography (4 g silica gel, DCM:MeOH 1:0 - 9:1) afforded the title product (56 mg, 64%) as a colourless solid.

$[\alpha]^{20}_D$ (c = 1.00 g/100 ml, $CHCl_3$): +23; 22% ee (determined by chiral HPLC analysis); m.p.: 126 - 127 °C; ν_{max} (ATR): 2916, 2854, 1730 (C=O), 1631, 1616, 1596, 1363, 1166, 1123, 843 cm^{-1}; δ_H ($CDCl_3$, 600 MHz): 7.932 - 7.87 (m, 2H, 2’’-H, 6’’-H), 7.59 - 7.50 (m, 3H, 3’’-H, 4’’-H, 5’’-H), 7.06 (d, *J* = 2.2 Hz, 1H, 6’-H), 6.76 (s, 1H, 3’-H), 6.75 (d, *J* = 2.2 Hz, 1H, 8’-H), 4.93 (dd, *J* = 8.8, 1.9 Hz, 1H, 2-H), 4.22 (dd, *J* = 10.9, 2.2 Hz, 1H, 3-H), 3.92 (s, 3H, OCH_3), 3.87 (dd, *J* = 10.9, 8.8 Hz, 1H, 3-H), 3.62 – 3.50 (m, 2H, 1'''-H_2), 1.66 - 1.59 (m, 2H, 2'''-H_2), 1.40 - 1.20 (m, 5 × H_2), 0.87 (t, *J* = 7.0 Hz, 3H, 8'''-H_3); δ_C ($CDCl_3$, 150 MHz): 179.0 (C-4'), 170.2 (C-1), 165.1 (C-7'), 163.0 (C-2'), 159.5 (C-5'), 159.1 (C-8a'), 132.1 (C-4''), 131.0 (C-1''), 129.3 (C-3'', C-5''), 126.4 (C-2'', C-6''), 109.4 (C-4a'), 108.1 (C-3'), 103.0 (C-6'), 96.3 (C-8'), 83.2 (C-2), 72.1 (C-1'''), 72.0 (C-3), 56.1 (OCH_3), 31.9 (CH_2), 29.8 (C-2'''), 29.5 (CH_2), 29.4 (CH_2), 26.3 (CH_2), 22.8 (CH_2), 14.2 (C-8'''); HRMS (ES^+) found $[M+H]^+$ 469.2236, $C_{27}H_{33}O_7$ requires *M* 469.2226.

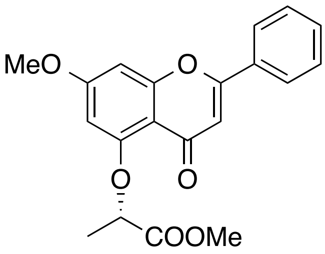
(R)-Methyl 2-(((trifluoromethyl)sulfonyl)oxy)propanoate[179] ***(R)*-69**



(+)Methyl D-lactate ***(R)*-68** (0.19 ml, 2 mmol, 1 eq) was dissolved in dry DCM (2 ml) at 0 °C under a nitrogen atmosphere. 2,6-Lutidine (0.28 ml, 2.4 mmpl, 1.2 eq) and subsequently triflic anhydride (0.37 ml, 2.2 mmol, 1.1 eq) were added and the reaction was stirred at 0°C for 1.5h. H_2O (2 ml) was slowly added and the two layers were separated. The organic layer was dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) afforded the title product (321 mg, 68%) as a colourless oil.

v_{max} (ATR): 1755 (C=O), 1201, 1143, 931 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 5.24 (q, J = 7.0 Hz, 1H, 2-H), 3.86 (s, 3H, COOCH3), 1.72 (d, J = 7.0 Hz, 3H, 3-H); δ_C ($CDCl_3$, 101 MHz): 168.0 (C-1), 118.6 (q, $^1J_{CF}$ = 319.4 Hz, CF_3), 80.0 (C-2), 53.5 (COO**C**H_3), 18.2 (C-3); δ_F ($CDCl_3$, 376 MHz): -75.2;

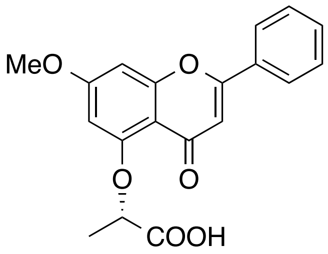
(S)-Methyl 2-(7'-methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-oxy)propionate ***(S)*-70**



A mixture of 5-hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one **46** (161 mg, 0.6 mmol, 1 eq), *(R)*-Methyl 2-(((trifluoromethyl)sulfonyl)oxy)propanoate ***(R)*-69** (213 mg, 0.9 mnmol, 1.5 eq) and K_2CO_3 (166 mg, 1.2 mmol, 2 eq) in dry MeCN (1 ml) under a nitrogen atmosphere was stirred at rt overnight. H_2O (2 ml) was added to the reaction mixture. The aqueous phase was extracted with EtOAc (3 × 2 ml), the organic extracts collected, washed with brine, dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, iso-hexane:EtOAc 7:3 - 0:1) yielded the title product (160 mg, 75%) as a colourless solid.

$[\alpha]^{20}_{D}$ (c = 1.00 g/100 ml, $CHCl_3$): -42 ; m.p.: 149 – 150 °C; ν_{max} (ATR): 3060, 3000, 2947, 1753 (C=O), 1633 (C=O), 1606, 1202, 1135, 1109 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.93 - 7.81 (m, 2H, 2''-H, 6''-H), 7.5 - 7.43 (m, 3H, 4''-H, 3''-H, 5''-H), 6.66 (d, *J* = 2.3 Hz, 1H, 8'-H), 6.64 (s, 1H, 3'-H), 6.39 (d, *J* = 2.3 Hz, 1H, 6'-H), 4.81 (q, *J* = 6.8 Hz, 1H, 2-H), 3.88 (s, 3H, OCH_3), 3.77 (s, 3H,$COOCH_3$), 1.76 (d, *J* = 6.8 Hz, 3H, 3-H); δ_C ($CDCl_3$, 101 MHz): 177.1 (C-4'), 172.4 (C-1), 163.7 (C-7'), 160.9 (C-2'), 159.9 (C-8a'), 158.7 (C-5'), 131.7 (C1''), 131.4 (C-4''), 129.1 (C-3'', C-5''), 126.1 (C-2'', C-6''), 110.6 (C-4a'), 109.1 (C-3'), 101.6 (C-6'), 95.3 (C-8'), 75.7 (C-2), 55.9 (OCH_3), 52.4 ($COOCH_3$), 18.6 (C-3); HRMS (ES^+) found $[M+H]^+$ 355.1168, $C_{20}H_{19}O_6$ requires *M* 355.1182.

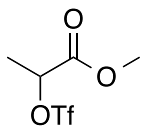
(S)-2-(7'-Methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-oxy)propionic acid ***(S)*-71**



(S)-Methyl 2-(7'-methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-oxy)propionate ***(S)*-70** (89 mg, 0.25 mmol, 1 eq) and LiOH.H_2O (21 mg, 0.5 mmol, 2 eq) were stirred in a 1:1 mixture of H_2O (2 ml) and THF (2 ml) at rt for 20 h. Sat. aqueous $NaHCO_3$ (5 ml) was added and the mixture was washed with EtOAc (2 × 5 ml). The aqueous layer was acidified to pH 2 with 2N HCl and the product was extracted with DCM (3 × 5 ml). The organic extracts were collected, dried over $MgSO_4$ and concentrated. The title product (36 mg, 42%) was obtained as a colourless solid without further purification.

$[\alpha]^{20}_{D}$ (c = 1.00 g/100 ml, $CHCl_3$): +103; 94% ee (determined by chiral HPLC analysis); m.p.: 161 – 163 °C; ν_{max} (ATR): 2992 (COOH), 1739 (C=O), 1625 (C=O), 1598, 1363, 1161, 1113cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 13.64 (br. s, 1H, COOH), 7.95 - 7.84 (m, 2H, 2''-H, 6''-H), 7.62 - 7.48 (m, 3H,4''-H, 3''-H, 5''-H), 6.76 (s, 1H, 3'-H), 6.73 (d, *J* = 2.3 Hz, 1H, 8'-H), 6.47 (d, *J* = 2.3 Hz, 1H, 6'-H), 4.82 (q, *J* = 6.9 Hz, 1H, 2-H), 3.94 (s, 3H, OCH_3), 1.81 (d, *J* = 6.9 Hz, 3H, 3-H_3); δ_C ($CDCl_3$, 101 MHz): 178.8 (C-4'), 172.8 (C-1), 164.9 (C-7'), 162.9 (C-2'), 159.6 (C-8a'), 158.6 (C-5'), 132.1 (C-4''), 131.1 (C-1''), 129.3 (C-3'', C-5''), 126.4 (C-2'', C-6''), 109.5 (C-4a'), 108.4 (C-3'), 101.3 (C-6'), 95.3 (C-8'), 78.0 (C-2), 56.3 (OCH_3), 19.1 (C-3); HRMS (ES$^+$) found $[M+H]^+$ 341.1015, $C_{19}H_{17}O_6$ requires *M* 341.1025.

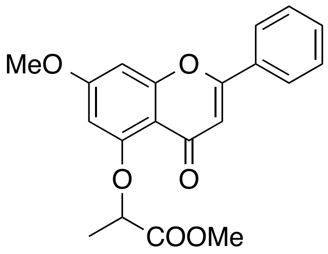
Methyl 2-(((trifluoromethyl)sulfonyl)oxy)propanoate[179] **(±)-69**



Methyl lactate **(±)-68** (0.19 ml, 2 mmol, 1 eq) was dissolved in dry DCM (2 ml) at 0 °C under a nitrogen atmosphere. 2,6-Lutidine (0.28 ml, 2.4 mmpl, 1.2 eq) and subsequently triflic anhydride (0.37 ml, 2.2 mmol, 1.1 eq) were added and the reaction was stirred at 0 °C for 1.5 h. H_2O (2 ml) was slowly added and the two layers were separated. The organic layer was dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) afforded the title product (322 mg, 68%) as a colourless oil.

ν_{max} (ATR): 1733 (C=O), 1206, 1144, 936 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 5.24 (q, J = 7.0 Hz, 1H, 2-H), 3.85 (s, 3H, $COOCH_3$), 1.71 (d, J = 6.9 Hz, 3H, 3-H); δ_C ($CDCl_3$, 101 MHz): 168.0 (C-1), 118.6 (q, $^1J_{CF}$ = 319.4 Hz, CF_3), 80.0 (C-2), 53.5 ($COO\mathbf{C}H_3$), 18.2 (C-3); δ_F ($CDCl_3$, 376 MHz): -75.3;

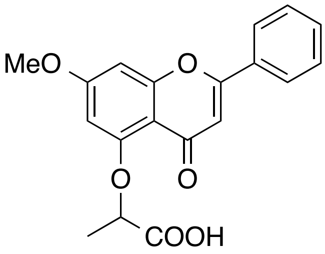
2-(7’-Methoxy-4’-oxo-2’-phenyl-4’H-chromen-5’-oxy)propionate **(±)-70**



A mixture of 5-hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one **46** (161 mg, 0.6 mmol, 1 eq), methyl 2-(((trifluoromethyl)sulfonyl)oxy)propanoate **(±)-69** (213 mg, 0.9 mnmol, 1.5 eq) and K_2CO_3 (166 mg, 1.2 mmol, 2 eq)in dry MeCN (1 ml) under a nitrogen atmosphere was stirred at rt overnight. H_2O (2 ml) was added to the reaction mixture. The aqueous phase was extracted with EtOAc (3 × 2 ml), the organic extracts collected, washed with brine, dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, iso-hexane:EtOAc 7:3 - 0:1) yielded the title product (154 mg, 72%) as a colourless solid.

m.p.: 149 – 150 °C; v_{max} (ATR): 3066, 3001, 2945, 1754 (C=O), 1641 (C=O), 1608, 1215, 1134, 1106 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.94 - 7.80 (m, 2H, 2’’-H, 6’’-H), 7.60 - 7.42 (m, 3H, 4’’-H, 3’’-H, 5’’-H), 6.66 (d, *J* = 2.3 Hz, 1H, 8’-H), 6.64 (s, 1H, 3’-H), 6.39 (d, *J* = 2.3 Hz, 1H, 6’-H), 4.81 (q, *J* = 6.8 Hz, 1H, 2-H), 3.88 (s, 3H, OCH_3), 3.77 (s, 3H,$COOCH_3$), 1.76 (d, *J* = 6.8 Hz, 3H, 3-H); δ_C ($CDCl_3$, 101 MHz): 177.1 (C-4’), 172.4 (C-1), 163.7 (C-7’), 160.9 (C-2’), 159.9 (C-8a’), 158.7 (C-5’), 131.7 (C1’’), 131.4 (C-4’’), 129.1 (C-3’’, C-5’’), 126.1 (C-2’’, C-6’’), 110.6 (C-4a’), 109.1 (C-3’), 101.6 (C-6’), 95.3 (C-8’), 75.7 (C-2), 55.9 (OCH_3), 52.4 ($COOCH_3$), 18.6 (C-3); HRMS (ES$^+$) found $[M+H]^+$ 355.1177, $C_{20}H_{19}O_6$ requires *M* 355.1182.

2-(7’-Methoxy-4’-oxo-2’-phenyl-4’H-chromen-5’-oxy)propionic acid **(±)-71**

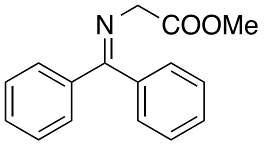


2-(7’-Methoxy-4’-oxo-2’-phenyl-4’H-chromen-5’-oxy)propionate **(±)-70** (89 mg, 0.25 mmol, 1 eq) and LiOH.H_2O (21 mg, 0.5 mmol, 2 eq) were stirred in a 1:1 mixture of H_2O (2 ml) and THF (2 ml) at rt for 20 h. Sat. aqueous $NaHCO_3$ (5 ml) was added and the mixture was washed with EtOAc (2 × 5 ml). The aqueous layer was acidified to pH 2 with 2N HCl and the product was extracted with DCM (3 × 5 ml). The organic extracts were collected, dried over $MgSO_4$ and concentrated. The title product (36 mg, 42%) was obtained as a colourless solid without further purification.

m.p.: 161 – 163 °C; ν_{max} (ATR): 2946 (COOH), 1741 (C=O), 1613 (C=O), 1589, 1361, 1165, 1125 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 13.64 (br. s, 1H, COOH), 7.95 - 7.84 (m, 2H, 2’’-H, 6’’-H), 7.60 - 7.47 (m, 3H,4’’-H, 3’’-H, 5’’-H), 6.75 (s, 1H, 3’-H), 6.73 (d, *J* = 2.3 Hz, 1H, 8’-H), 6.47 (d, *J* = 2.3 Hz, 1H, 6’-H), 4.81 (q, *J* = 6.9 Hz, 1H, 2-H), 3.94 (s, 3H, OCH_3), 1.81 (d, *J* = 6.9 Hz, 3H, 3-H_3); δ_C ($CDCl_3$, 101 MHz): 178.8 (C-4’), 172.8 (C-1), 164.9 (C-7’), 162.9 (C-2’), 159.6 (C-8a’), 158.6 (C-5’), 132.1 (C-4’’), 131.1 (C-1’’), 129.3 (C-3’’, C-5’’), 126.4 (C-2’’, C-6’’), 109.5 (C-4a’), 108.4 (C-3’), 101.3 (C-6’), 95.3 (C-8’), 78.0 (C-2), 56.3 (OCH_3), 19.1 (C-3); HRMS (ES^+) found $[M+H]^+$ 341.1026, $C_{19}H_{17}O_6$ requires *M* 341.1025.

5.1.3.2 Cross metathesis

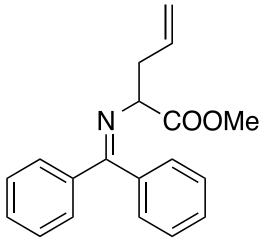
Methyl 2-[(diphenylmethylene)amino]acetate[121] **76**



Benzophenone imine (2 ml, 12 mmol, 1 eq) was added to a suspension of glycine methyl ester hydrochloride **77** (1.51 g, 12 mmol, 1 eq) in dry DCM (40 ml) and the reaction mixture was stirred for 21 h at rt. NH_4Cl was removed by filtration and the solution was concentrated. The crude product was dissolved in Et_2O (50 ml) and washed with H_2O (50 ml). The organic layer was dried over dried over Na_2SO_4 and concentrated. Flash column chromatography (80 g silica gel, hexane:EtOAc 9:1 - 3:2) yielded the title product (2.07 g, 68%) as a colourless waxy solid.

ν_{max} (ATR): 1754 (C=O), 1625, 1385, 1197, 691 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.71 - 7.61 (m, 2H, Ar-H), 7.51 - 7.30 (m, 6H, Ar-H), 7.23 - 7.11 (m, 2H, Ar-H), 4.22 (s, 2H, 2-H), 3.75 (s, 3H, $COOCH_3$); δ_C ($CDCl_3$, 101 MHz): 172.0 (C-1), 171.2 (Ph_2**C**=N), 139.4 (C-1’), 136.1 (C-1’’), 130.6 (C-4’), 129.0 (C-4’’), 128.9 (C-Ar), 128.8 (C-Ar), 128.2 (C-Ar), 127.8 (C-Ar), 55.8 (C-2), 52.1 (COO**C**H_3); m/z LC-MS (ES^+) 254 $[M+H]^+$.

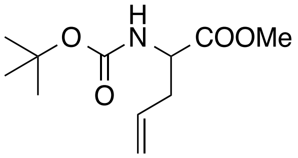
Methyl 2-[(diphenylmethylene)amino]pent-4-enoate[121] **75**



n-BuLi (1.6 M solution in hexanes, 1.38 ml, 2.2 mmol, 1.1 eq) was slowly added to a solution of DIPA (0.31 ml, 2.2 mmol, 1.1 eq) in dry THF (18 ml) at 0°C under an argon atmosphere and stirred for 40 min. The solution was cooled to -78°C and methyl 2-[(diphenylmethylene)amino]acetate **76** (507 mg, 2 mmol, 1 eq) dissolved in dry THF (2ml) was added dropwise. After stirring for 40 min, allyl bromide (0.19 ml, 2.2 mmol, 1.1 eq) was added dropwise and the reaction was stirred another 1.5 h at -78°C. The mixture was further stirred overnight, while allowing to warm to rt and the poured onto a 1:1 mixture of H_2O and water (50 ml). The product was extracted with EtOAc (3 × 50 ml) and the combined organic layers were dried over Na_2SO_4 and concentrated. Flash column chromatography (40 g silica gel, toluene:EtOAc 1:0 - 9:1) afforded the title product (381 mg, 65%) as a colourless oil.

ν_{max} (ATR): 2955, 2384, 1702 (C=O), 1491, 1167, 841 cm^{-1}; δ_H ($CDCl_3$, 600 MHz): 7.69 - 7.60 (m, 2H, Ar-H), 7.49 - 7.29 (m, 6H, Ar-H), 7.21 - 7.13 (m, 2H, Ar-H), 5.67 (ddt, *J* = 17.2, 10.2, 7.1 Hz, 1H, 4-H), 5.10 - 5.00 (m, 2H, 5-H), 4.17 (dd, *J* = 7.9, 5.2 Hz, 1H, 2-H), 3.72 (s, 3H, $COOCH_3$), 2.73 - 2.59 (m, 2H, 3-H); δ_C ($CDCl_3$, 151 MHz): 172.5 (C-1), 170.8 (Ph_2**C**=N), 139.7 (C-1’), 136.5 (C-1’’), 134.4 (C-4), 130.5 (C-4’), 129.0 (C-Ar), 128.8 (C-4’’), 128.6 (C-Ar), 128.2 (C-Ar), 128.1 (C-Ar), 117.8 (C-5), 65.4 (C-2), 52.2 (COO**C**H_3), 38.3 (C-3); m/z LC-MS (ES^+) 294 $[M+H]^+$.

Methyl 2-[(*tert*-butoxycarbonyl)amino]pent-4-enoate[121] **74**

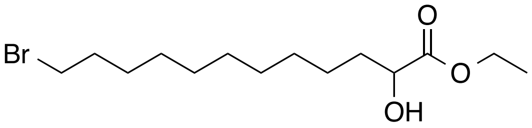


A mixture of methyl 2-[(diphenylmethylene)amino]pent-4-enoate **75** (359 mg, 1.22 mmol, 1 eq) and 4N HCl (1.51 ml) in MeOH (5 ml) was heated under reflux for 1 h and subsequently allowed to cool to rt. The reaction mixture was extracted with Et_2O (2 × 4 ml). The aqueous layer was basified with $NaHCO_3$ to pH 8, followed by addition of Boc_2O (534 mg, 2.45 mmol, 1.5 eq) and the reaction was stirred at rt for 2.5 h. The reaction volume was reduced under vacuum, H_2O (10 ml) was added and the product was extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4 and concentrated. The title product (203 mg, 72%) was obtained by flash column chromatography (24 g silica gel, hexane:EtOAc 1:0 - 4:1) as a colourless oil.

ν_{max} (ATR): 3376 (NH), 29770, 2879, 1714 (C=O), 1490, 1172, 838 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 5.72 - 5.65 (m, 1H, 4-H), 5.15 - 5.10 (m, 2H, 5-H_2), 5.03 (br. d, J = 8.2 Hz, 1H, NH), 4.42 - 4.33 (m, 1H, 2-H), 3.73 (s, 3H, $COOCH_3$), 2.58 – 2.43 (m , 2H, 3-H), 1.43 (s, 9H, $C(CH_3)_3$); δ_C ($CDCl_3$, 175 MHz): 172.7 (C-1), 155.3 (C=O), 132.4 (C-4), 119.2 (C-5), 80.1 (**C**$(CH_3)_3$), 53.1 (C-2), 52.4 ($COOCH_3$), 36.9 (C-3), 28.5 (C(**C**$H_3)_3$); m/z LC-MS (ES$^+$) 230 $[M+H]^+$.

5.1.3.3 Ene-reaction

Ethyl 12-bromo-2-hydroxydodecanoate **82**



To a solution of (+)-diethyl L-tartrate **80** (0.31 ml, 1.8 mmol, 0.6 eq) in dry Et_2O (1.5 ml) at 0°C, periodic acid (410 mg, 1.8 mmol, 0.6 eq) was slowly added over 30 min. The reaction mixture was stirred another 20 min at rt, filtered through Celite® and concentrated. The crude product was dissolved in dry DCM (10 ml) and added to a slurry of ferric chloride (1.460 g, 9 mmol, 3 eq) in dry DCM (14 ml) and H_2O (0.16 ml, 9 mmol, 3 eq). The mixture was cooled to 0 °C and 10-bromo-1-decene (0.60 ml, 3 mmol, 1 eq) was added before the mixture was stirred for 3 h at rt. The reaction mixture was washed with 0.1 N HCl (25 ml), dried over $MgSO_4$, filtered through silica gel and concentrated.

The hydrogenator flask was charged with Crabtree catalyst (48 mg, 2 mol%) and the crude product from the previous step, dissolved in dry DCM (12 ml). The flask was purged with nitrogen and then flushed with hydrogen (1.5 bar) and stirred for 2.5 h at rt. The reaction mixture was concentrated and the title product (478 mg, 49%) was afforded as a colourless oil by flash column chromatography (40 g silica gel, iso-hexane:EtOAc 1:0 - 7:3).

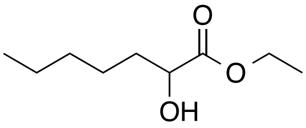
ν_{max} (ATR): 3508 (OH), 2925, 2854, 1730 (C=O), 1208, 1094 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 4.28 - 4.20 (m, 2H, O-C**H₂**-CH₃), 4.16 (ddd, *J* = 7.4, 5.6, 4.1 Hz, 1H, 2-H), 3.40 (t, J = 6.9 Hz, 2H, 12-H_2), 2.74 (d, J = 5.6 Hz, 1H, OH), 1.92 - 1.70 (m, 3H, 11-H_2, 3-H), 1.68 - 1.56 (m, 1H, 3-H), 1.55 - 1.13 (m, 17H, 7 × CH_2, O-CH_2-C**H₃**); δ_C ($CDCl_3$, 101 MHz): 175.6 (C-1), 70.6 (C-2), 61.7 (O-**C**H_2-CH_3), 34.6 (C-3), 34.2 (C-12), 33.0 (C-11), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 28.9 (CH_2), 28.3 (CH_2), 24.8 (CH_2), 14.4 (O-CH_2-**C**H_3); HRMS (ASAP) found $[M+H]^+$ 323.1217, $C_{14}H_{28}O_3{}^{79}Br$ requires *M* 323.1222.

General Procedure D

To a solution of (+)-diethyl L-tartrate **80** (0.17 ml, 1.5 mmol, 0.6 eq) in dry Et_2O (1.5 ml) at 0 °C, periodic acid (342 mg, 1.5 mmol, 0.6 eq) was slowly added over 30 min. The reaction mixture was stirred another 20 min at rt, filtered through Celite® and concentrated. The crude product was dissolved in dry DCM and added to a slurry of ferric chloride (1.217 g, 7.5 mmol, 3 eq) in dry DCM and H_2O (0.14 ml, 7.5 mmol, 3 eq). The mixture was cooled to 0 °C and alkene (2.5 mmol, 1 eq) was added before the mixture was stirred for 3 h at rt. The reaction mixture was washed with 0.1 N HCl, dried over $MgSO_4$, filtered through silica gel and concentrated.

The hydrogenator flask was charged with 5% Pd/C (62% H_2O, 80 mg) and the crude product from the previous step, dissolved in dry MeOH. The flask was purged with nitrogen and then flushed with hydrogen (1.5 bar) and stirred for 2.5 h at rt. The reaction mixture was concentrated and the title product was afforded by flash column chromatography.

Ethyl 2-hydroxyheptanoate[180] **87**

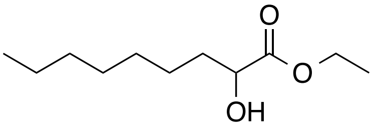


The reaction was carried out according to General procedure D using 1-pentene (0.27 ml, 2.5 mmol, 1 eq). Flash column chromatography (24 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) yielded the title product (137 mg, 31%)) as a colourless oil.

ν_{max} (ATR): 3472 (OH), 2931, 2862, 1730 (C=O), 1199, 1132 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 4.28 - 4.20 (m, 2H, O-C$\mathbf{H_2}$-CH_3), 4.16 (ddd, *J* = 7.5, 5.7, 4.2 Hz, 1H, 2-H), 2.79 - 2.65 (m, 1H, OH), 1.84 - 1.70 (m, 1H, 3-H), 1.67 - 1.58 (m, 1H, 3-H), 1.52 - 1.23 (m, 9H, 3 × CH_2, O-CH_2-C$\mathbf{H_3}$), 0.94 - 0.83 (m, 3H, 7-H_3); δ_C ($CDCl_3$, 101 MHz): 175.6 (C-1), 70.6 (C-2), 61.7

(O-**C**H_2-CH_3), 34.5 (CH_2), 31.6 (CH_2), 24.5 (CH_2), 22.6 (CH_2), 14.4 (O-CH_2-**C**H_3), 14.1 (C-7); HRMS (ASAP) found $[M+H]^+$ 175.1326, $C_9H_{19}O_3$ requires *M* 175.1334.

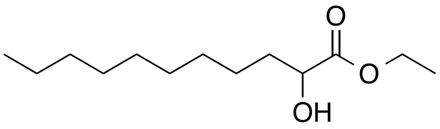
Ethyl 2-hydroxynonanoate **88**



The reaction was carried out according to General procedure D using 1-heptene (0.35 ml, 2.5 mmol, 1 eq). Flash column chromatography (24 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) yielded the title product (100 mg, 20%)) as a colourless oil.

ν_{max} (ATR): 3480 (OH), 2925, 2857, 1731 (C=O), 1204, 1132, 1092 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 4.30 - 4.20 (m, 2H, O-C**H_2**-CH_3), 4.16 (ddd, *J* = 7.3, 5.3, 4.2 Hz, 1H, 2-H), 2.73 (d, *J* = 5.3 Hz, 1H, OH), 1.84 - 1.71 (m, 1H, 3-H), 1.69 - 1.56 (m, 1H, 3-H), 1.55 - 1.10 (m, 13H, 5 × CH_2, O-CH_2-C**H_3**), 0.94 - 0.79 (m, 3H, 9-H_3); δ_C ($CDCl_3$, 101 MHz): 175.6 (C-1), 70.6 (C-2), 61.7 (O-**C**H_2-CH_3), 34.6 (CH_2), 31.9 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 24.9 (CH_2), 22.8 (CH_2), 14.4 (O-CH_2-**C**H_3), 14.2 (C-9); HRMS (ASAP) found $[M+H]^+$ 203.1632, $C_{11}H_{23}O_3$ requires *M* 203.1647.

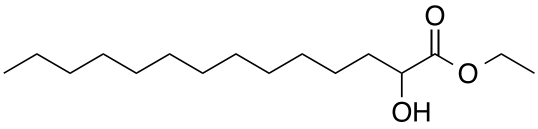
Ethyl 2-hydroxyundecanoate[181] **89**



The reaction was carried out according to General procedure D using 1-nonene (0.43 ml, 2.5 mmol, 1 eq). Flash column chromatography (24 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) yielded the title product (103 mg, 18%)) as a colourless oil.

ν_{max} (ATR): 3438 (OH), 2916, 2849, 1734 (C=O), 1195, 1087 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 4.28 - 4.21 (m, 2H, O-C**H$_2$**-CH$_3$), 4.16 (ddd, *J* = 7.4, 5.8, 4.2 Hz, 1H, 2-H), 2.72 (dd, *J* = 5.8, 2.2 Hz, 1H, OH), 1.84 - 1.71 (m, 1H, 3-H), 1.68 - 1.56 (m, 1H, 3-H), 1.53 - 1.10 (m, 17H, 7 × CH$_2$, O-CH$_2$-C**H$_3$**), 0.87 (t, *J* = 6.8 Hz, 3H, 11-H$_3$); δ_C ($CDCl_3$, 101 MHz): 175.6 (C-1), 70.6 (C-2), 61.8 (O-**C**H$_2$-CH$_3$), 34.6 (CH$_2$), 32.0 (CH$_2$), 29.7 (CH$_2$), 29.6 (CH$_2$), 29.5 (CH$_2$), 29.4 (CH$_2$), 24.9 (CH$_2$), 22.8 (CH$_2$), 14.4 (O-CH$_2$-**C**H$_3$), 14.3 (C-11); HRMS (ASAP) found $[M+H]^+$ 231.1959, $C_{13}H_{27}O_3$ requires *M* 231.1960.

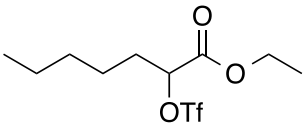
Ethyl 2-hydroxytetradecanoate **90**



The reaction was carried out according to General procedure D using 1-dodecene (0.56 ml, 2.5 mmol, 1 eq). Flash column chromatography (24 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) yielded the title product (379 mg, 56%)) as a colourless solid.

m.p.: 48 – 50 °C; ν_{max} (ATR): 3460 (OH), 2917, 2849, 1732 (C=O), 1197, 1093 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 4.28 - 4.21 (m, 2H, O-C**H₂**-CH₃), 4.19 - 4.11 (m, 1H, 2-H), 2.71 (d, *J* = 5.7 Hz, 1H, OH), 1.83 - 1.72 (m, 1H, 3-H), 1.68 - 1.56 (m, 1H, 3-H), 1.54 - 1.12 (m, 23H, 10 × CH_2, O-CH_2-C**H₃**), 0.88 (t, *J* = 6.7 Hz, 3H, 14-H_3); δ_C ($CDCl_3$, 101 MHz): 175.6 (C-1), 70.6 (C-2), 61.8 (O-**C**H_2-CH_3), 34.6 (CH_2), 32.1 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 24.9 (CH_2), 22.8 (CH_2), 14.4 (O-CH_2-**C**H_3), 14.3 (C-14); HRMS (ASAP) found $[M+H]^+$ 273.2427, $C_{16}H_{33}O_3$ requires *M* 273.2430.

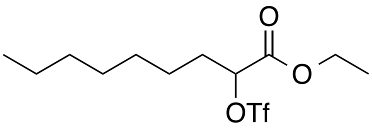
Ethyl 2-(((trifluoromethyl)sulfonyl)oxy)heptanoate **91**



Ethyl 2-hydroxyheptanoate **87** (87 mg, 0.5 mmol, 1 eq) was dissolved in dry DCM (1 ml) at 0 °C under a nitrogen atmosphere. 2,6-Lutidine (0.07 ml, 0.6 mmol, 1.2 eq) and subsequently triflic anhydride (0.09 ml, 0.55 mmol, 1.1 eq) were added and the reaction was stirred at 0 °C for 2 h. H_2O was slowly added and the two layers were separated. The organic layer was dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) afforded the title product (121 mg, 79%) as a colourless oil.

ν_{max} (ATR): 2936, 2876, 1736 (C=O), 1417, 1199, 1144, 954 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 5.10 (t, *J* = 6.2 Hz, 1H, 2-H), 4.39 - 4.22 (m, 2H, O-C**H$_2$**-CH$_3$), 2.06 - 1.90 (m, 2H, 3-H), 1.53 - 1.19 (m, 9H, 3 × CH$_2$, O-CH$_2$-C**H$_3$**), 1.00 - 0.82 (m, 3H, 7-H$_3$); δ_C ($CDCl_3$, 101 MHz): 167.3 (C-1), 118.5 (q, $^1J_{CF}$ = 319.5 Hz, CF$_3$), 83.9 (C-2), 62.9 (O-**C**H$_2$-CH$_3$), 32.0 (CH$_2$), 30.9 (CH$_2$), 24.1 (CH$_2$), 22.3 (CH$_2$), 14.0 (O-CH$_2$-**C**H$_3$), 13.9 (C-7); δ_F ($CDCl_3$, 376 MHz): -74.95.

Ethyl 2-(((trifluoromethyl)sulfonyl)oxy)nonanoate **92**

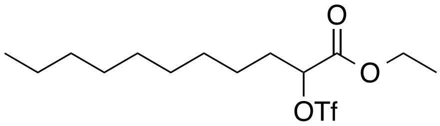


Ethyl 2-hydroxynonanoate **88** (81 mg, 0.4 mmol, 1 eq) was dissolved in dry DCM (0.8 ml) at 0 °C under a nitrogen atmosphere. 2,6-Lutidine (0.06 ml, 0.48 mmol, 1.2 eq) and subsequently triflic anhydride (0.07 ml, 0.44 mmol, 1.1 eq) were added and the reaction was stirred at 0 °C for 2 h. H_2O was slowly added and the two layers were separated. The organic layer was dried over $MgSO_4$ and concentrated. Flash column chromatography

(12 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) afforded the title product (110 mg, 82%) as a colourless oil.

ν_{max} (ATR): 2932, 2861, 1763 (C=O), 1417, 1200, 1144, 922 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 5.10 (t, *J* = 6.1 Hz, 1H, 2-H), 4.37 - 4.22 (m, 2H, O-C**H₂**-CH₃), 2.10 - 1.88 (m, 2H, 3-H), 1.53 - 1.17 (m, 13H, 5 × CH_2, O-CH_2-C$\mathbf{H_3}$), 0.95 - 0.82 (m, 3H, 9-H_3); δ_C ($CDCl_3$, 101 MHz): 167.4 (C-1), 118.6 (q, $^1J_{CF}$ = 319.5 Hz, CF_3), 84.0 (C-2), 62.8 (O-**C**H_2-CH_3), 32.1 (CH_2), 31.7 (CH_2), 29.0 (CH_2), 28.9 (CH_2), 24.6 (CH_2), 22.7 (CH_2), 14.2 (O-CH_2-**C**H_3), 14.1 (C-9); δ_F ($CDCl_3$, 376 MHz): -74.94.

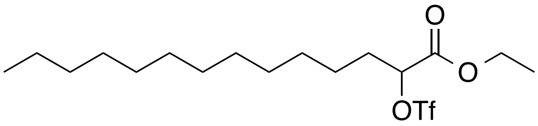
Ethyl 2-(((trifluoromethyl)sulfonyl)oxy)undecanoate **93**



Ethyl 2-hydroxyundecanoate **89** (81 mg, 0.35 mmol, 1 eq) was dissolved in dry DCM (0.8 ml) at 0 °C under a nitrogen atmosphere. 2,6-Lutidine (0.05 ml, 0.48 mmol, 1.2 eq) and subsequently triflic anhydride (0.06 ml, 0.44 mmol, 1.1 eq) were added and the reaction was stirred at 0 °C for 2 h. H_2O was slowly added and the two layers were separated. The organic layer was dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) afforded the title product (116 mg, 91%) as a colourless oil.

ν_{max} (ATR): 2929, 2858, 1764 (C=O), 1418, 1201, 1145, 921 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 5.10 (t, *J* = 6.1 Hz, 1H, 2-H), 4.40 - 4.20 (m, 2H, O-C$\mathbf{H_2}$-CH_3), 2.13 - 1.82 (m, 2H, 3-H), 1.50 - 1.20 (m, 17H, 7 × CH_2, O-CH_2-C$\mathbf{H_3}$), 0.88 (t, $^1J_{CF}$ = 6.7 Hz, 3H, 11-H_3); δ_C ($CDCl_3$, 101 MHz): 167.4 (C-1), 118.6 (q, *J* = 319.7 Hz, CF_3), 84.0 (C-2), 62.8 (O-**C**H_2-CH_3), 32.1 (CH_2), 32.0 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 24.6 (CH_2), 22.8 (CH_2), 14.2 (O-CH_2-**C**H_3), 14.1 (C-11); δ_F ($CDCl_3$, 376 MHz): -74.92.

Ethyl 2-(((trifluoromethyl)sulfonyl)oxy)tetradecanoate **94**



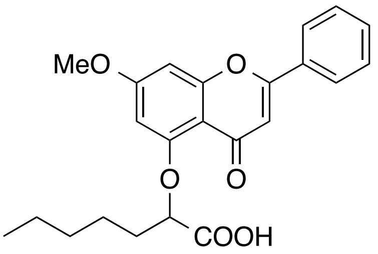
Ethyl 2-hydroxy-tetradecanoate **90** (163 mg, 0.6 mmol, 1 eq) was dissolved in dry DCM (1.5 ml) at 0 °C under a nitrogen atmosphere. 2,6-Lutidine (0.08 ml, 0.72 mmol, 1.2 eq) and subsequently triflic anhydride (0.11 ml, 0.66 mmol, 1.1 eq) were added and the reaction was stirred at 0 °C for 2 h. H_2O was slowly added and the two layers were separated. The organic layer was dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, iso-hexane:EtOAc 1:0 - 4:1) afforded the title product (219 mg, 90%) as a colourless oil.

ν_{max} (ATR): 2926, 2856, 1764 (C=O), 1418, 1201, 1144, 921 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 5.10 (t, *J* = 6.1 Hz, 1H, 2-H), 4.38 - 4.22 (m, 2H, O-C**H_2**-CH_3), 2.05 - 1.92 (m, 2H, 3-H), 1.54 - 1.06 (m, 23H, 10 × CH_2, O-CH_2-C**H_3**), 0.88 (t, *J* = 6.8 Hz, 3H, 14-H_3); δ_C ($CDCl_3$, 101 MHz): 167.4 (C-1), 118.6 (q, $^1J_{CF}$ = 319.5 Hz, CF_3), 84.0 (C-2), 62.8 (O-**C**H_2-CH_3), 32.1 (CH_2), 32.1 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 24.6 (CH_2), 22.8 (CH_2), 14.3 (O-CH_2-**C**H_3), 14.1 (C-14); δ_F ($CDCl_3$, 376 MHz): -74.94.

General Procedure E

A mixture of 5-hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one **46** (101 mg, 0.375 mmol, 1.5 eq), triflate (0.25 mnmol, 1 eq) and K_2CO_3 (104 mg, 0.75 mmol, 3 eq) in dry MeCN (1 ml) under a nitrogen atmosphere was stirred at rt overnight. H_2O (2 ml) was added to the reaction mixture. The aqueous phase was extracted with EtOAc (3 × 2 ml), the organic extracts collected, washed with brine, dried over $MgSO_4$ and concentrated. The crude product together with LiOH.H_2O (31 mg, 0.75 mmol, 3 eq) was stirred in a 1:1 mixture of H_2O (3 ml) and THF (3 ml) at rt for 3 h. The mixture was acidified to pH 2-3 with 1N HCl and the aqueous phase was extracted with DCM (3 × 5 ml). The combined organic layers were dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, DCM:MeOH 1:0 - 19:1) yielded in the product.

2-[(7’-Methoxy-4’-oxo-2’-phenyl-4’H-chromen-5’-yl)oxy]heptanoic acid **95**

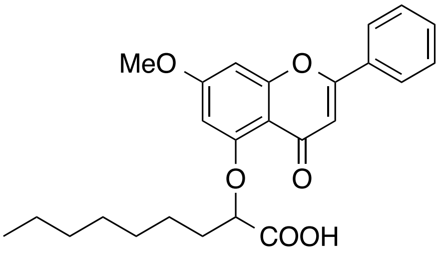


Following General procedure E using ethyl 2-(((trifluoromethyl)sulfonyl)oxy)heptanoate **91** (77 mg, 0.25 mnmol, 1 eq) gave the title product (61 mg, 62%) as a colourless solid.

m.p.: 104 – 105 °C; ν_{max} (ATR): 2929, 2860, 1743 (C=O), 1630 (C=O), 1592, 1354, 1161, 1110, 768 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 13.67 (br. s, 1H, COOH), 7.95 - 7.84 (m, 2H, 2’’-H, 6’’-H), 7.60 - 7.47 (m, 3H, 4’’-H, 3’’-H, 5’’-H), 6.76 (s, 1H, 3’-H), 6.73 (d, *J* = 2.2 Hz, 1H, 8’-H), 6.47 (d, *J* = 2.2 Hz, 1H, 6’-H), 4.83 (t, *J* = 5.3 Hz, 1H, 2-H), 3.94 (s, 3H, OCH_3), 2.23 - 2.07 (m, 2H, 3-H_2), 1.66 - 1.45 (m, 2H, 4-H_2), 1.42 - 1.22 (m, 4H, 2 × CH_2), 0.89 (t, *J* = 7.1

Hz, 3H, 7-H$_3$); δ$_C$ (CDCl$_3$, 101 MHz): 179.0 (C-4'), 172.5 (C-1), 164.9 (C-7'), 162.9 (C-2'), 159.6 (C-8a'), 159.0 (C-5'), 132.1 (C-4''), 131.1 (C-1''), 129.3 (C-3'', C-5''), 126.4 (C-2'', C-6''), 109.6 (C-4a'), 108.4 (C-3'), 101.4 (C-6'), 95.2 (C-8'), 82.0 (C-2), 56.2 (OCH$_3$), 33.0 (C-3), 31.7 (CH$_2$), 24.5 (C-4), 22.6 (CH$_2$), 14.1 (C-7); HRMS (ES$^+$) found [M+H]$^+$ 397.1663, $C_{23}H_{25}O_6$ requires *M* 397.1651.

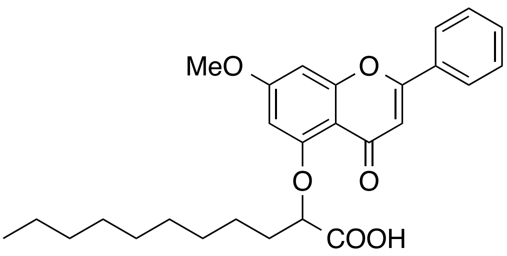
2-[(7'-Methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-yl)oxy]nonanoic acid **96**



Following General procedure E using ethyl 2-(((trifluoromethyl)sulfonyl)oxy)nonanoate **92** (84 mg, 0.25 mnmol, 1 eq) gave the title product (65 mg, 61%) as a colourless solid.

m.p.: 143 – 145 °C; v_{max} (ATR): 2926, 2852, 1739 (C=O), 1610 (C=O), 1591, 1357, 1168, 766 cm^{-1}; δ$_H$ (CDCl$_3$, 400 MHz): 13.59 (br. s, 1H, COOH), 7.96 - 7.83 (m, 2H, 2''-H, 6''-H), 7.61 - 7.47 (m, 3H, 4''-H, 3''-H, 5''-H), 6.76 (s, 1H, 3'-H), 6.73 (d, *J* = 2.2 Hz, 1H, 8'-H), 6.47 (d, *J* = 2.2 Hz, 1H, 6'-H), 4.83 (t, *J* = 5.3 Hz, 1H, 2-H), 3.94 (s, 3H, OCH$_3$), 2.25 - 2.06 (m, 2H, 3-H$_2$), 1.67 - 1.46 (m, 2H, 4-H$_2$), 1.42 - 1.21 (m, 8H, 4 × CH$_2$), 0.87 (t, *J* = 6.7 Hz, 3H, 9-H$_3$); δ$_C$ (CDCl$_3$, 101 MHz): 179.0 (C-4'), 172.6 (C-1), 164.9 (C-7'), 162.9 (C-2'), 159.6 (C-8a'), 159.0 (C-5'), 132.1 (C-4''), 131.1 (C-1''), 129.3 (C-3'', C-5''), 126.4 (C-2'', C-6''), 109.6 (C-4a'), 108.4 (C-3'), 101.4 (C-6'), 95.2 (C-8'), 82.0 (C-2), 56.2 (OCH$_3$), 33.0 (C-3), 31.9 (CH$_2$), 29.5 (CH$_2$), 29.2 (CH$_2$), 24.8 (C-4), 22.8 (CH$_2$), 14.2 (C-9); HRMS (ES$^+$) found [M+H]$^+$ 425.1967, $C_{25}H_{29}O_6$ requires *M* 425.1964.

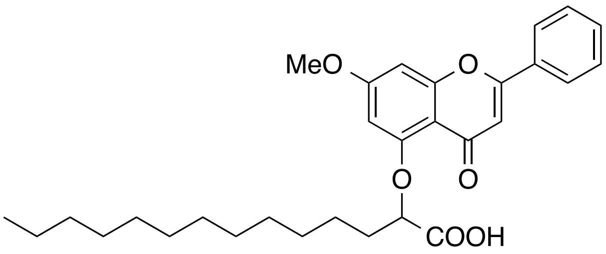
2-[(7’-Methoxy-4’-oxo-2’-phenyl-4’H-chromen-5’-yl)oxy]undecanoic acid **97**



Following General procedure E using ethyl 2-(((trifluoromethyl)sulfonyl)oxy)undecanoate **93** (91 mg, 0.25 mnmol, 1 eq) gave the title product (90 mg, 80%) as a colourless solid.

m.p.: 96 – 98 °C; ν_{max} (ATR): 2914, 2851, 1728 (C=O), 1617 (C=O), 1597, 1363, 1165, 1111, 855 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 13.68 (br. s, 1H, COOH), 7.95 - 7.82 (m, 2H, 2’’-H, 6’’-H), 7.64 - 7.46 (m, 3H, 4’’-H, 3’’-H, 5’’-H), 6.76 (s, 1H, 3’-H), 6.73 (d, *J* = 2.2 Hz, 1H, 8’-H), 6.47 (d, *J* = 2.2 Hz, 1H, 6’-H), 4.83 (t, *J* = 5.3 Hz, 1H, 2-H), 3.94 (s, 3H, OCH_3), 2.30 - 1.99 (m, 2H, 3-H_2), 1.70 - 1.44 (m, 2H, 4-H_2), 1.43 - 1.16 (m, 12H, 6 × CH_2), 0.87 (t, *J* = 6.6 Hz, 3H, 11-H_3); δ_C ($CDCl_3$, 101 MHz): 179.0 (C-4’), 172.5 (C-1), 164.9 (C-7’), 162.9 (C-2’), 159.6 (C-8a’), 159.0 (C-5’), 132.1 (C-4’’), 131.1 (C-1’’), 129.3 (C-3’’, C-5’’), 126.4 (C-2’’, C-6’’), 109.6 (C-4a’), 108.4 (C-3’), 101.4 (C-6’), 95.2 (C-8’), 82.0 (C-2), 56.2 (OCH_3), 33.0 (C-3), 32.0 (CH_2), 29.6 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.8 (C-4), 22.8 (CH_2), 14.3 (C-11) ; HRMS (ES^+) found $[M+H]^+$ 453.2285, $C_{27}H_{33}O_6$ requires *M* 453.2277.

2-[(7'-Methoxy-4'-oxo-2'-phenyl-4'H-chromen-5'-yl)oxy]tetradecanoic acid **98**

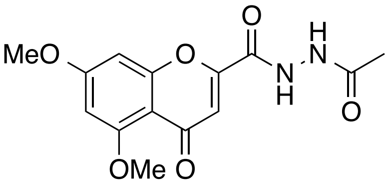


Following General procedure E using ethyl 2-(((trifluoromethyl)sulfonyl)oxy)-tetradecanoate **94** (101 mg, 0.25 mmol, 1 eq) gave the title product (0.103 g, 83%) as a colourless solid.

m.p.: 99 – 101 °C; ν_{max} (ATR): 2916, 2848, 1745 (C=O), 1639 (C=O), 1597, 1355, 1194, 1159 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 13.68 (br. s, 1H, COOH), 7.93 - 7.86 (m, 2H, 2''-H, 6''-H), 7.60 - 7.49 (m, 3H, 4''-H, 3''-H, 5''-H), 6.76 (s, 1H, 3'-H), 6.73 (d, *J* = 2.2 Hz, 1H, 8'-H), 6.47 (d, *J* = 2.2 Hz, 1H, 6'-H), 4.83 (t, *J* = 5.3 Hz, 1H, 2-H), 3.94 (s, 3H, OCH_3), 2.22 - 2.07 (m, 2H, 3-H_2), 1.66 - 1.46 (m, 2H, 4-H_2), 1.45 - 1.10 (m, 12H, 6 × CH_2), 0.87 (t, *J* = 6.8 Hz, 3H, 14-H_3); δ_C ($CDCl_3$, 101 MHz): 179.0 (C-4'), 172.6 (C-1), 164.9 (C-7'), 162.9 (C-2'), 159.6 (C-8a'), 159.0 (C-5'), 132.1 (C-4''), 131.0 (C-1''), 129.3 (C-3'', C-5''), 126.4 (C-2'', C-6''), 109.5 (C-4a'), 108.3 (C-3'), 101.4 (C-6'), 95.2 (C-8'), 82.0 (C-2), 56.2 (OCH_3), 33.0 (C-3), 32.1 (CH_2), 29.8 (2 × CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 24.8 (C-4), 22.8 (CH_2), 14.3 (C-14); HRMS (ES$^+$) found $[M+H]^+$ 495.2762, $C_{30}H_{39}O_6$ requires *M* 495.2747.

5.1.4 Improving Solubility

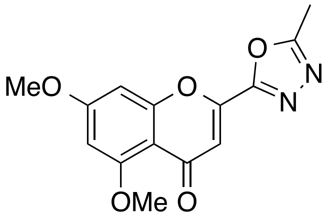
5,7-Dimethoxy-4-oxo-4H-chromene-2-carboxylic acid, 2'-acetylhydrazide **109**



5,7-Dimethoxy-4-oxo-4H-chromene-2-carboxylic acid **41** (125 mg, 0.5 mmol, 1 eq), acethydrazide (41 mg, 0.55 mmol, 1.1 eq) and PyBOP (286 mg, 0.55 mmol, 1.1 eq) were dissolved in dry DMF (1.5 ml) and DIPEA (0.19 ml, 1.1 mmol, 2.2 eq) was added. The reaction mixture was stirred at rt overnight and then concentrated. The title product (108 mg, 71%) was obtained by flash column chromatography (12 g silica gel, DCM:MeOH 1:0-9:1) and subsequent trituration with DCM as a colourless solid.

m.p.: 282 – 285 °C (decomposition); ν_{max} (ATR): 3241 (NH), 1657 (C=O), 1606 (C=O), 1352, 1164, 1110, 495 cm^{-1}; δ_H (d^6-DMSO, 700 MHz): 10.82 (br. s, 1H, NH), 10.08 (s, 1H, NH), 6.76 (d, *J* = 2.4 Hz, 1H, 8-H), 6.58 (s, 1H, 3-H), 6.56 (d, *J* = 2.4 Hz, 1H, 6-H), 3.89 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 1.94 (s, 3H, CH_3); δ_C (d^6-DMSO, 176 MHz): 175.0 (C-4), 168.3 ($CH_3\mathbf{C}$=O), 164.2 (C-7), 160.4 (C-5), 158.6 (C-8a), 158.2 (C=O), 152.0 (C-2), 112.8 (C-3), 108.9 (C-4a), 96.6 (C-6), 93.4 (C-8), 56.2 (OCH_3), 55.9 (OCH_3), 20.5 (CH_3); HRMS (ES^+) found $[M+H]^+$ 307.0941, $C_{14}H_{15}N_2O_6$ requires *M* 307.0930.

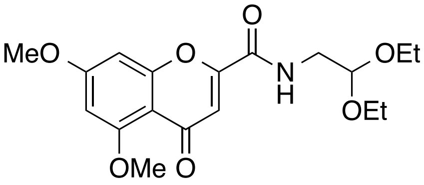
5,7-Dimethoxy-2-(5’-methyl-1’,3’,4’-oxadiazol-2’-yl)-4H-chromen-4-one **99**



5,7-Dimethoxy-4-oxo-4H-chromene-2-carboxylic acid **41** (125 mg, 0.5 mmol, 1 eq), acethydrazide (41 mg, 0.55 mmol, 1.1 eq) and PyBOP (286 mg, 0.55 mmol, 1.1 eq) were dissolved in dry DMF (1.5 ml) and DIPEA (0.19 ml, 1.1 mmol, 2.2 eq) was added. The reaction mixture was stirred at rt overnight and then concentrated. The crude product and Burgess reagent (238 mg, 1 mmol, 2 eq) were dissolved in dry 1,2-dichloroethane under an argon atmosphere and heated to 140 °C for 3 h in a microwave. The title product (51 mg, 36%) was obtained by flash column chromatography (24 g silica gel, DCM:MeOH 1:0-19:1) and subsequent trituration with DCM as a colourless solid.

m.p.: 231 – 234 °C (decomposition); ν_{max} (ATR): 1637 (C=O), 1608, 1569, 1315, 1136, 1073, 819 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 6.90 (s, 1H, 3-H), 6.64 (d, *J* = 2.3 Hz, 1H, 8-H), 6.40 (d, *J* = 2.3 Hz, 1H, 6-H), 3.95 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 2.67 (s, 3H, CH_3); δ_C ($CDCl_3$, 176 MHz): 176.0 (C-4), 165.4 (C-4’), 165.0 (C-7), 161.2 (C-5), 159.7 (C-8a), 159.1 (C-1’), 146.7 (C-2), 114.0 (C-3), 110.0 (C-4a), 97.1 (C-6), 93.2 (C-8), 56.6 (OCH_3), 56.1 (OCH_3), 11.3 (CH_3); HRMS (ES^+) found $[M+H]^+$ 289.0828, $C_{14}H_{13}N_2O_5$ requires *M* 289.0824.

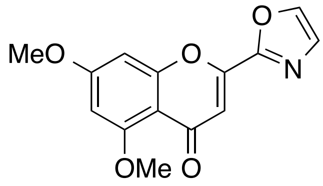
N-(2',2'-Diethoxyethyl)-5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide **110**



5,7-Dimethoxy-4-oxo-4H-chromene-2-carboxylic acid **41** (250 mg, 1 mmol, 1 eq), aminoacetaldehyde diethyl acetal (0.15 ml, 1.1 mmol, 1.1 eq) and PyBOP (572 mg, 1.1 mmol, 1.1 eq) were dissolved in dry DMF (3 ml) and DIPEA (0.38 ml, 2.2 mmol, 2.2 eq) was added. The reaction mixture was stirred at rt overnight and then concentrated. The title product (330 mg, 90%) was obtained by flash column chromatography (40 g silica gel, DCM:MeOH 1:0-19:1) as a colourless solid.

m.p.: 106 – 108 °C (decomposition); ν_{max} (ATR): 3497 (NH), 2976, 1658 (C=O), 1608 (C=O), 1345, 1057, 826 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 7.04 (t, *J* = 6.0 Hz, 1H, NH), 6.96 (s, 1H, 3-H), 6.47 (d, *J* = 2.3 Hz, 1H, 8-H), 6.38 (d, *J* = 2.3 Hz, 1H, 6-H), 4.62 (t, *J* = 5.1 Hz, 1H, 2'-H), 3.93 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.81 - 3.73 (m, 2H, 1'-H), 3.67 - 3.51 (m, 4H, 2 × $OC\mathbf{H_2}CH_3$), 1.25 (t, *J* = 7.0 Hz, 6H, 2 × $OCH_2C\mathbf{H_3}$); δ_C ($CDCl_3$, 176 MHz): 177.1 (C-4), 164.7 (C-7), 161.3 (C-5), 159.6 (C=O), 159.1 (C-8a), 152.4 (C-2), 114.1 (C-3), 109.9 (C-4a), 100.6 (C-2'), 96.7 (C-6), 93.0 (C-8), 63.5 (C-1'), 56.6 (OCH_3), 56.0 (OCH_3), 42.4 (2 × $O\mathbf{C}H_2CH_3$), 15.5 (2 × $OCH_2\mathbf{C}H_3$); HRMS (ES^+) found $[M+H]^+$ 366.1552, $C_{18}H_{24}NO_7$ requires *M* 366.1553.

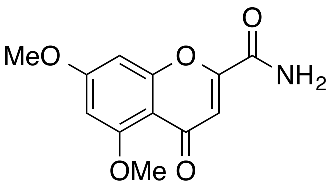
5,7-Dimethoxy-2-(1’,3’-oxazol-2’-yl)-4H-chromen-4-one **100**



TFA (1.68 ml, 22 mmol, 110 eq) was added to a solution of *N*-(2’,2’-diethoxyethyl)-5,7-dimethoxy-4-oxo-4H-chromene-2-carboxamide **110** (73 mg, 0.2 mmol, 1 eq) in dry DCM (2.5 ml) under an argon atmosphere. The reaction mixture was stirred at rt for 2.5 h and then concentrated. The crude product and Burgess reagent (238 mg, 1 mmol, 2 eq) were dissolved in dry 1,2-dichloroethane under an argon atmosphere and heated to 140 °C for 3 h in a microwave. The reaction mixture was concentrated and the title oxazole (16 mg, 28%) obtained by flash column chromatography (12 g silica gel, Et_2O:MeOH 1:0-9:1) as a colourless solid.

m.p.: 205 – 208 °C (decomposition); ν_{max} (ATR): 1657 (C=O), 1610, 1332, 1116, 828 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 7.84 (d, J = 0.8 Hz, 1H, 4’-H), 7.39 (d, J = 0.8 Hz, 1H, 3’), 6.94 (s, 1H, 3-H), 6.66 (d, J = 2.3 Hz, 1H, 8-H), 6.40 (d, J = 2.3 Hz, 1H, 6-H), 3.95 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3); δ_C ($CDCl_3$, 176 MHz): 176.6 (C-4), 164.7 (C-7), 161.1 (C-5), 159.8 (C-8a), 155.1 (C-1’), 149.1 (C-2), 140.9 (C-4’), 129.8 (C-3’), 112.5 (C-3), 110.0 (C-4a), 96.9 (C-6), 93.2 (C-8), 56.6 (OCH_3), 56.1 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 274.0686, $C_{14}H_{12}NO_5$ requires *M* 274.0715.

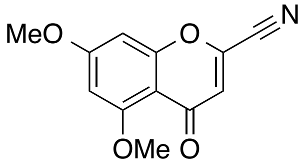
5,7-Dimethoxy-4H-chromen-4-one-2-carboxamide[182] **111**



Phosphorus oxychloride (0.15 ml, 1.6 mmol, 1 eq) was added to a solution of 5,7-dimethoxy-4-oxo-4H-chromene-2-carboxylic acid **41** (400 mg, 1.6 mmol, 1 eq) in dry DMF (3.2 ml). The reaction mixture was stirred 10 min at rt and another 50 min at 50 °C and then added to ammonium hydroxide solution (35% NH_3 in H_2O, 3.2 ml) at 0 °C and stirred for 1 h. The resulting precipitate was collected by filtration and dried. The title carboxamide (291 mg, 73%) was obtained by flash column chromatography (40 g silica gel, DCM:MeOH 1:0-9:1) as a colourless solid.

m.p.: 268 – 269 °C (decomposition) (lit.[182] m.p.: 268 – 270 °C); ν_{max} (ATR): 3519 (NH), 3385 (NH), 3144 (NH), 1652 (C=O), 1608 (C=O), 1330, 1069, 639 cm^{-1}; δ_H (d^6-DMSO , 700 MHz): 8.40 (s, 1H, NH), 8.07 (s, 1H, NH), 6.77 (d, *J* = 2.4 Hz, 1H, 8-H), 6.56 (s, 1H, 3-H), 6.54 (d, *J* = 2.4 Hz, 1H, 6-H), 3.88 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); δ_C (d^6-DMSO, 176 MHz): 175.5 (C-4), 164.1 (C-7), 160.7 ($CONH_2$), 160.4 (C-5), 158.7 (C-8a), 153.1 (C-2), 112.2 (C-3), 108.8 (C-4a), 96.5 (C-6), 93.4 (C-8), 56.2 (OCH_3), 55.9 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 250.0686, $C_{12}H_{12}NO_5$ requires *M* 250.0715.

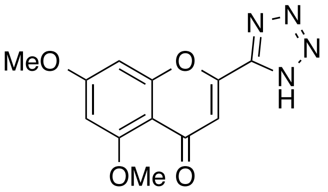
5,7-Dimethoxy-4H-chromen-4-one-2-carbonitrile[182] **112**



Trifluoroacetic anhydride (0.21 ml, 1.54 mmol, 2.2 eq) and pyridine (0.23 ml, 2.81 mmol, 4 eq) were added to a solution of 5,7-dimethoxy-4H-chromen-4-one-2-carboxamide **111** (175 mg, 0.70 mmol, 1 eq) in dry DMF (3.5 ml) at 0 °C under an argon atmosphere. The reaction mixture was stirred at rt overnight and concentrated. Flash column chromatography (24 g silica gel DCM:MeOH 1:0-19:1) afforded the title nitrile (145 mg, 90%) as a colourless solid.

m.p.: 188 – 190 °C (lit.[182] m.p.: 189 – 191 °C); ν_{max} (ATR): 2253 (CN), 1652 (C=O), 1608, 1341, 1157, 1078, 852, 799 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 6.66 (s, 1H, 3-H), 6.46 (d, *J* = 2.3 Hz, 1H, 8-H), 6.40 (d, *J* = 2.3 Hz, 1H, 6-H), 3.94 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3); δ_C ($CDCl_3$, 176 MHz): 174.2 (C-4), 165.3 (C-7), 161.3 (C-5), 159.9 (C-8a), 135.9 (C-2), 122.1 (C-3), 111.9 (CN), 110.3 (C-4a), 97.4 (C-6), 93.0 (C-8), 56.7 (OCH_3), 56.2 (OCH_3); HRMS (ES$^+$) found $[M+H]^+$ 232.0614, $C_{12}H_{10}NO_4$ requires *M* 232.0610.

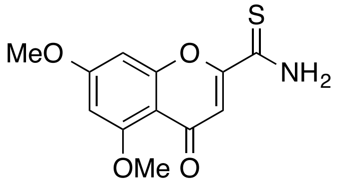
5,7-Dimethoxy-2-(1H-1’,2’,3’,4’-tetrazol-5’-yl)-4H-chromen-4-one[182] **101**



Dry toluene (0.75 ml) was added to 5,7-dimethoxy-4H-chromen-4-one-2-carbonitrile **112** (75 mg, 0.33 mmol, 1 eq), NaN_3 (28 mg, 0.43 mmol, 1.3 eq) and $Et_3N.HCl$ (59 mg, 0.43 mmol, 1.3 eq) under an argon atmosphere. The reaction mixture was stirred at 95 °C for 48 h. H_2O (10 ml) was added, the aqueous phase was separated and the organic phase was further washed with H_2O. The combined aqueous layers were cooled to 0 °C, aqueous sodium nitrite solution (20 wt%, 0.1 ml) and sulfuric acid (20 wt%, 0.1 ml) were added and the solution was stirred for 30 min. The product was extracted with EtOAc (5 × 20 ml) and the combined organic layers were dried over Na_2SO_4 and concentrated. The title tetrazole (15 mg, 17%) was obtained by recrystallization from MeOH as a colourless solid.

m.p.: 242 – 243 °C (lit.[182] m.p.: 244 – 245 °C); ν_{max} (ATR): 1651 (C=O), 1602, 1312, 1161, 1092, 834 cm^{-1}; δ_H (d^6-DMSO, 700 MHz): 6.80 (s, 1H, 3-H), 6.73 (d, *J* = 2.3 Hz, 1H, 8-H), 6.57 (d, *J* = 2.3 Hz, 1H, 6-H), 3.92 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3); δ_C (d^6-DMSO, 176 MHz): 174.6 (C-4), 164.2 (C-7), 160.5 (C-5), 159.0 (C-8a), 152.8 (C-1’), 149.7 (C-2), 111.8 (C-3), 108.8 (C-4a), 96.7 (C-6), 93.3 (C-8), 56.2 (OCH_3), 56.1 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 275.0787, $C_{12}H_{11}N_4O_4$ requires *M* 275.0780.

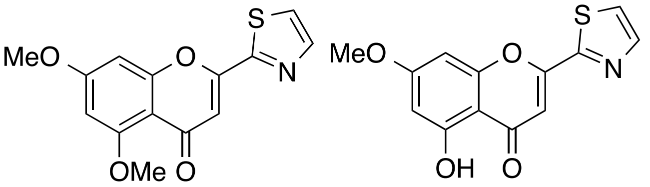
5,7-Dimethoxy-4H-chromen-4-one-2-carbothioamide **113**



5,7-Dimethoxy-4H-chromen-4-one-2-carbonitrile (1.340 g, 5.8 mmol, 1 eq) was added to a slurry of NaHS.xH_2O (70%, 1.392 g, 17.4 mmol, 3 eq) and $MgCl_2$.6H_2O (1.296 g, 6.4 mmol, 1.1 eq) in DMF (10 ml) and stirred at 60 °C for 20 h. H_2O (50 ml) was added and the resulting precipitate was collected, washed with H_2O, resuspended in 1N HCl and stirred for 30 min. The precipitate was collected again, washed with $CHCl_3$ to remove unreacted starting material, and dried. Flash column chromatography (40 g silica gel DCM:MeOH 19:1-17:3) afforded the title carbothioamide (629 mg, 41%) as a yellow solid.

m.p.: 247 - 249 °C (decomposition); ν_{max} (ATR): 3382, 3301, 3198, 1623 (C=O), 1595, 1416, 1224, 1125, 826 cm^{-1}; δ_H (d^6-DMSO, 700 MHz): 10.51 (s, 1H, NH), 10.06 (s, 1H, NH), 6.87 (s, 1H, 3-H), 6.87 (d, *J* = 2.3 Hz, 1H, 8-H), 6.54 (d, *J* = 2.3 Hz, 1H, 6-H), 3.88 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); δ_C (d^6-DMSO, 176 MHz): 187.9 ($CSNH_2$) 175.3 (C-4), 164.2 (C-7), 160.3 (C-5), 158.2 (C-8a), 155.0 (C-2), 114.0 (C-3), 108.5 (C-4a), 96.6 (C-6), 93.5 (C-8), 56.2 (OCH_3), 55.9 (OCH_3); HRMS (ES$^+$) found $[M+H]^+$ 266.0500, $C_{12}H_{12}NO_4S$ requires *M* 266.0487.

5,7-Dimethoxy-2-(1’,3’-thiazol-2’-yl)-4H-chromen-4-one **102** & 5-Hydroxy-7-methoxy-2-(1’,3’-thiazol-2’-yl)-4H-chromen-4-one **114**

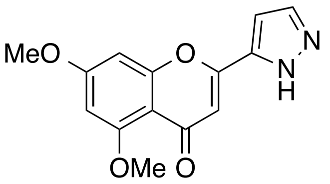


5,7-Dimethoxy-4H-chromen-4-one-2-carbothioamide (121 mg, 0.46 mmol, 1 eq) and bromoacetaldehyde diethyl acetal (226 mg, 1.14 mmol, 2.5 eq) were suspended in THF (2 ml) and EtOH (0.57 ml) and a drop of H_2O was added. The reaction mixture was heated to 140 °C for 1 h in a microwave and subsequently concentrated. The title thiazole **102** (45 mg, 34%) and the demethylated product **114** (47 mg, 37%) were obtained by flash column chromatography (24 g silica gel, hexane:(EtOAc:EtOH 3:1) 9:1-2:3), both as a light yellow solid.

5,7-Dimethoxy-2-(1’,3’-thiazol-2’-yl)-4H-chromen-4-one **102**: m.p.: 169 – 171 °C; ν_{max} (ATR): 3107, 3067, 2945, 1644 (C=O), 1604, 1331, 1158, 1096, 829 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 8.01 (d, *J* = 3.1 Hz, 1H, 3’-H), 7.60 (d, *J* = 3.1 Hz, 1H, 4’-H), 7.02 (s, 1H, 3-H), 6.58 (d, *J* = 2.3 Hz, 1H, 8-H), 6.39 (d, *J* = 2.3 Hz, 1H, 6-H), 3.95 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3); δ_C ($CDCl_3$, 176 MHz): 176.9 (C-4), 164.5 (C-7), 161.2 (C-5), 159.7 (C-1’), 159.6 (C-8a), 154.6 (C-2), 145.1 (C-3’), 122.5 (C-4’), 109.9 (C-3), 109.9 (C-4a), 96.7 (C-6), 93.0 (C-8), 56.6 (OCH_3), 56.0 (OCH_3); HRMS (ES$^+$) found $[M+H]^+$ 290.0489, $C_{14}H_{12}NO_4S$ requires *M* 290.0487.

5-Hydroxy-7-methoxy-2-(1’,3’-thiazol-2’-yl)-4H-chromen-4-one **114**: m.p.: 204 - 206 °C; ν_{max} (ATR): 3112, 3087, 3059, 1658 (C=O), 1613, 1579, 1321, 1193, 1155, 968 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 12.60 (s, 1H, OH), 8.05 (d, *J* = 3.1 Hz, 1H, 3’-H), 7.66 (d, *J* = 3.1 Hz, 1H, 4’-H), 7.09 (s, 1H, 3-H), 6.52 (d, *J* = 2.3 Hz, 1H, 8-H), 6.40 (d, *J* = 2.3 Hz, 1H, 6-H), 3.90 (s, 3H, OCH_3); δ_C ($CDCl_3$, 176 MHz): 182.2 (C-4), 166.0 (C-7), 162.5 (C-5), 159.2 (C-1'), 157.5 (C-8a), 157.4 (C-2), 145.4 (C-3'), 123.3 (C-4'), 106.6 (C-3), 106.4 (C-4a), 98.8 (C-6), 93.0 (C-8), 56.1 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 276.0338, $C_{13}H_{10}NO_4S$ requires *M* 276.0331.

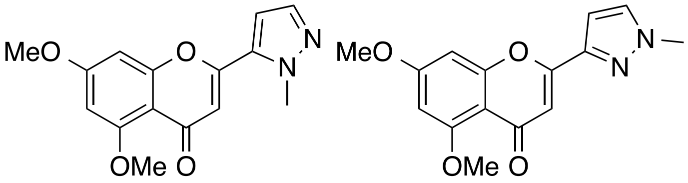
5,7-Dimethoxy-2-(1’H-pyrazol-3’-yl)-4H-chromen-4-one **104**



KOH (10 ml of a 3M solution in EtOH, 12 mmol, 6 eq) was added to a mixture of 2-hydroxy-4,6-dimethoxyacetophenone **7** (392 mg, 2 mmol, 1 eq) and 1H-pyrrazole-5-carbaldehyde (0.19 ml, 2 mmol, 1 eq). The reaction mixture was stirred for 36 h at 50 °C and subsequently acidified to pH 2 with 3M HCl and extracted with DCM (3 × 10 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. Iodine (15 mg, 0.3 mol%) was added to a stirred solution of the crude product in DMSO (3 ml) at 50 °C. Then the reaction mixture was heated to 140 °C and stirred for 2.5 h. After cooling to rt, H_2O (20 ml) and DCM (20 ml) were added. The layers were separated and the aqueous layer was extracted two more times with DCM (2 × 20 ml). The combined organic layers were washed with aqueous $Na_2S_2O_3$ solution (0.5%, 50 ml), dried over $MgSO_4$ and concentrated. The title product (62 mg, 11%) was obtained by flash column chromatography (24 g silica gel, Et_2O:MeOH 1:0-8:2) as a colourless solid.

m.p.: 255 – 257 °C (decomposition); ν_{max} (ATR): 3250 (NH), 1651 (C=O), 1606, 1324, 1132, 818, 766 cm^{-1}; δ_H (d^6-DMSO, 700 MHz 13.49 (s, 1H, NH), 7.99 - 7.91 (m, 1H, 4’-H), 6.95 - 6.88 (m, 1H, 5’-H), 6.75 (d, *J* = 2.4 Hz, 1H, 8-H), 6.56 (s, 1H, 3-H), 6.51 (d, *J* = 2.4 Hz, 1H, 6-H), 3.89 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); δ_C (d^6-DMSO, 176 MHz): 175.3 (C-4), 163.6 (C-7), 160.3 (C-5), 159.1 (C-8a), 156.3 (C-2), 143.4 (C-1’), 130.6 (C-4’), 108.7 (C-4a), 107.2 (C-3), 104.3 (C-5’), 96.3 (C-6), 93.3 (C-8), 56.1 (OCH_3), 56.0 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 273.0903, $C_{14}H_{13}N_2O_4$ requires *M* 273.0875.

5,7-Dimethoxy-2-(1’-methyl-1’H-pyrazol-5’-yl)-4H-chromen-4-one **105** & 5,7-Dimethoxy-2-(1’-methyl-1’H-pyrazol-3’-yl)-4H-chromen-4-one **106**

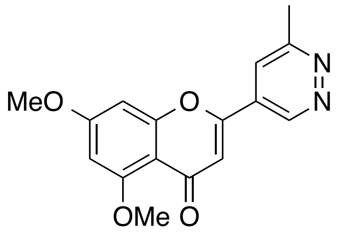


5,7-Dimethoxy-2-(1’H-pyrazol-3’-yl)-4H-chromen-4-one **104** (95 mg, 0.35 mmol, 1 eq) was added to a slurry of NaH (60% dispersed in mineral oil, 22 mg, 0.56 mmol, 1.6 eq) in dry DMF (1 ml) at 0 °C under an argon atmosphere and the resulting mixture was stirred for 15 min at 0 °C and another 15 min at rt. MeI (0.03 ml, 0.42 mmol, 1.2 eq) was added at 0 °C and the mixture was stirred another at rt overnight. H_2O (1 ml) was added and the product was extracted with DCM (3 × 2 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. Flash column chromatography (24 g silica gel, hexane:(EtOAc:EtOH 3:1) 4:1-1:4) afforded the title products **105** (16 mg, 16%) and **106** (33 mg, 33%) as colourless solids.

5,7-Dimethoxy-2-(1’-methyl-1’H-pyrazol-5’-yl)-4H-chromen-4-one **105**: m.p.: 180 – 181 °C; ν_{max} (ATR): 1651 (C=O), 1607, 1354, 1112, 840, 783 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.52 (d, J = 2.1 Hz, 1H, 4’-H), 6.69 (d, J = 2.1 Hz, 1H, 5’-H), 6.53 - 6.42 (m, 2H, 8-H, 3-H), 6.39 (d, J = 2.3 Hz, 1H, 6-H), 4.16 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3); δ_C ($CDCl_3$, 151 MHz): 176.8 (C-4), 164.5 (C-7), 161.2 (C-5), 159.7 (C-8a), 153.0 (C-2), 138.8 (C-4’), 134.8 (C-1’), 112.0 (C-3), 109.3 (C-4a), 108.8 (C-5’), 96.6 (C-6), 92.8 (C-8), 56.6 (OCH_3), 56.0 (OCH_3), 39.8 (CH_3); HRMS (ES^+) found $[M+H]^+$ 287.1034, $C_{15}H_{15}N_2O_4$ requires *M* 287.1032.

5,7-Dimethoxy-2-(1’-methyl-1’H-pyrazol-3’-yl)-4H-chromen-4-one **106**: m.p.: 169 – 171 °C; ν_{max} (ATR): 1650 (C=O), 1604, 1328, 1202, 1079, 772 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.44 (d, *J* = 2.3 Hz, 1H, 4’-H), 6.74 - 6.65 (m, 2H, 5’-H, 3-H), 6.62 (d, *J* = 2.3 Hz, 1H, 8-H), 6.36 (d, *J* = 2.3 Hz, 1H, 6-H), 4.01 (s, 3H, CH_3), 3.94 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3); δ_C ($CDCl_3$, 151 MHz): 177.6 (C-4), 164.0 (C-7), 161.0 (C-5), 159.9 (C-8a), 156.2 (C-2), 144.6 (C-1’), 131.9 (C-4’), 109.8 (C-4a), 109.0 (C-3), 105.6 (C-5’), 96.4 (C-6), 93.2 (C-8), 56.6 (OCH_3), 55.9 (OCH_3), 39.8 (CH_3); HRMS (ES^+) found $[M+H]^+$ 287.1035, $C_{15}H_{15}N_2O_4$ requires *M* 287.1032.

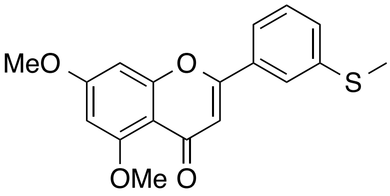
5,7-Dimethoxy-2-(6’-methylpyridazin-4’-yl)-4H-chromen-4-one **108**



To a solution of $[Ir(COD)OMe]_2$ (7.5 mg, 3 mol%), dtbpy (6 mg, 6 mol%) and B_2pin_2 (286 mg, 1.125 mmol, 3 eq) in degassed, dry MTBE (1.9 ml) under an argon atmosphere, 3-methylpyridazine (0.07 ml, 0.75 mmol, 2 eq) was added. The reaction was stirred at 50 °C overnight and subsequently concentrated. To the crude intermediate under an argon atmosphere were added $Pd(amphos)Cl_2$ (13.3 mg, 5 mol%), K_3PO_4 (159 mg, 0.75 mmol, 2 eq), 5,7-dimethoxy-2-chloro-4H-chromen-4-one **21** (90 mg, 0.375 mmol, 1 eq) and a 9:1 mixture of DMAC (0.9 ml) and H_2O (0.1 ml) as solvent. The mixture was stirred at 70 °C for 4 h and then filtered through Celite® and concentrated. The reaction mixture was diluted with H_2O and extracted with DCM (3 × 5 ml). The organic extracts were collected, dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, hexane:(EtOAc:EtOH 3:1) 4:1 - 0:1) gave the title product (35 mg, 31 %) as a colourless solid.

m.p.: 279 – 282 °C (decomposition); ν_{max} (ATR): 1652 (C=O), 1613, 1341, 1162, 1124, 831 cm^{-1}; δ_H ($CDCl_3$, 600 MHz): 9.45 (d, *J* = 2.1 Hz, 1H, 2’-H), 7.71 (d, *J* = 2.1 Hz, 1H, 6’-H), 6.82 (s, 1H, 3-H), 6.59 (d, *J* = 2.3 Hz, 1H, 8-H), 6.42 (d, *J* = 2.3 Hz, 1H, 6-H), 3.97 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 2.85 (s, 3H, CH_3); δ_C ($CDCl_3$, 151 MHz): 176.5 (C-4), 164.8 (C-7), 161.3 (C-5), 160.7 (C-5’), 159.9 (C-8a), 155.8 (C-2), 145.0 (C-2’), 130.0 (C-1’), 122.1 (C-6’), 112.2 (C-3), 109.7 (C-4a), 96.9 (C-6), 93.0 (C-8), 56.7 (OCH_3), 56.1 (OCH_3), 22.7 (CH_3); HRMS (ES^+) found $[M+H]^+$ 299.1014, $C_{16}H_{15}N_2O_4$ requires *M* 299.1032.

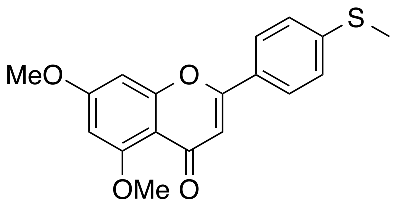
5,7-Dimethoxy-2-(3’-methylthiophenyl)-4H-chromen-4-one **118**



In a microwave vial 2-chloro-5,7-dimethoxy-4H-chromen-4-one **21** (120 mg, 0.5 mmol, 1 eq) 3-(methylthio)phenylboronic acid (168 mg, 1 mmol, 2 eq), $Pd_2(dba)_3$ (14 mg, 3 mol%), CyJohnPhos (11 mg, 6 mol%) and Cs_2CO_3 (489 mg, 1.5 mmol, 3 eq) were suspended in dioxane (2.5 ml) under a nitrogen atmosphere. The reaction mixture was heated in the microwave to 150 °C for 30 min. After allowing to cool to rt, the crude mixture was filtered through Celite® and concentrated The residue was dissolved in H_2O (15 ml) and EtOAc (15 ml). The layers were separated and the aqueous layer was extracted two more times with EtOAc (2 × 25 ml). The combined organic layers were washed with H_2O (25 ml), dried over Mg_2SO_4 and concentrated. The title product (96 mg, 58%) was afforded by reversed phase column chromatography (H_2O:MeCN 0:1 - 1:0, 0.1% TFA) as a light yellow solid.

m.p.: 169 – 171 °C; ν_{max} (ATR): 1639 (C=O), 1607, 1345, 1212, 1119, 816 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.76 - 7.68 (m, 1H, 2’-H), 7.65 - 7.56 (m, 1H, 6’-H), 7.45 - 7.30 (m, 2H, 5’-H, 4’-H), 6.67 (s, 1H, 3-H), 6.57 (d, *J* = 2.3 Hz, 1H, 8-H), 6.38 (d, *J* = 2.3 Hz, 1H, 6-H), 3.96 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 2.55 (s, 3H, SCH_3); δ_C ($CDCl_3$, 101 MHz): 177.7 (C-4), 164.3 (C-7), 161.0 (C-5), 160.3 (C-2), 160.0 (C-8a), 140.0 (C-3’), 132.3 (C-1’), 129.4 (C-5’), 129.1 (C-4’), 123.8 (C-2’), 122.7 (C-6’), 109.5 (C-3), 109.4 (C-4a), 96.4 (C-6), 93.0 (C-8), 56.6 (OCH_3), 56.0 (OCH_3), 15.9 (SCH_3); HRMS (ES^+) found $[M+H]^+$ 329.0842, $C_{18}H_{17}O_4S$ requires *M* 329.0842.

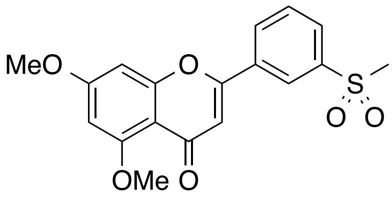
5,7-Dimethoxy-2-(4'-methylthiophenyl)-4H-chromen-4-one **119**



In a microwave vial 2-chloro-5,7-dimethoxy-4H-chromen-4-one **21** (60 mg, 0.25 mmol, 1 eq) 4-(methylthio)phenylboronic acid (84 mg, 0.5 mmol, 2 eq), $Pd_2(dba)_3$ (7 mg, 3 mol%), CyJohnPhos (5 mg, 6 mol%) and Cs_2CO_3 (244 mg, 0.75 mmol, 3 eq) were suspended in dioxane (1.25 ml) under a nitrogen atmosphere. The reaction mixture was heated in the microwave to 150 °C for 30 min. After allowing to cool to rt, the crude mixture was filtered through Celite® and concentrated The residue was dissolved in H_2O (10 ml) and EtOAc (10 ml). The layers were separated and the aqueous layer was extracted two more times with EtOAc (2 × 25 ml). The combined organic layers were washed with H_2O (25 ml), dried over Mg_2SO_4 and concentrated. The title product (55 mg, 67%) was afforded by reversed phase column chromatography (H_2O:MeCN 0:1 - 1:0, 0.1% TFA) as a light yellow solid.

m.p.: 163 – 165 °C; ν_{max} (ATR): 1643 (C=O), 1599, 1347, 1119, 817 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.86 - 7.71 (m, 2H, 2'-H, 6'-H), 7.40 - 7.28 (m, 2H, 3'-H, 5'-H), 6.64 (s, 1H, 3-H), 6.56 (d, *J* = 2.3 Hz, 1H, 8-H), 6.38 (d, *J* = 2.3 Hz, 1H, 6-H), 3.96 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 2.54 (s, 3H, SCH_3).; δ_C ($CDCl_3$, 101 MHz): 177.8 (C-4), 164.2 (C-7), 161.0 (C-5), 160.6 (C-2), 160.0 (C-8a), 143.5 (C-4'), 127.8 (C-1'), 126.3 (C-3', C-6'), 125.9 (C-3', C-5'), 109.4 (C-4a), 108.4 (C-3), 96.3 (C-6), 93.0 (C-8), 56.6 (OCH_3), 55.9 (OCH_3), 15.2 (SCH_3); HRMS (ES^+) found $[M+H]^+$ 329.0843, $C_{18}H_{17}O_4S$ requires *M* 329.0848.

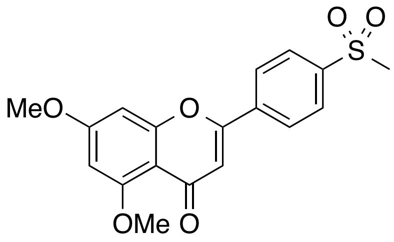
2-(3’-Methanesulfonylphenyl)-5,7-dimethoxy-4H-chromen-4-one **120**



To a solution of the 5,7-dimethoxy-2-(3’-methylthiophenyl)-4H-chromen-4-one **118** (82 mg, 0.25 mmol, 1 eq) in MeOH (1.25 mL) and THF (1.25 mL) was added a solution of Oxone® (768 mg, 2.5 mmol, 10 eq) in H_2O (2 mL) dropwise at 0 °C. The resulting mixture was stirred at rt overnight. The solution was extracted with DCM (3 × 5 ml), the combined organic layers washed with brine and dried over anhydrous Mg_2SO_4. The resulting solution was filtered and concentrated under reduced pressure. The title product, a colourless solid, (81 mg, 90%) was used in the following step without further purification.

m.p.: 239 – 241 °C (decomposition; ν_{max} (ATR): 1650 (C=O), 1611, 1349, 1299, 1144, 1121 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.55 - 8.44 (m, 1H, 2’-H), 8.18 - 8.02 (m, 2H, 4’-H, 6’-H), 7.80 - 7.67 (m, 1H, 5’-H), 6.75 (s, 1H, 3-H), 6.63 (d, *J* = 2.3 Hz, 1H, 8-H), 6.41 (d, *J* = 2.3 Hz, 1H, 6-H), 3.95 (d, *J* = 10.5 Hz, 6H, 2 × OCH_3), 3.13 (s, 3H, SO_2CH_3); δ_C ($CDCl_3$, 101 MHz): 177.2 (C-4), 164.6 (C-7), 161.1 (C-5), 160.0 (C-8a), 158.3 (C-2), 141.9 (C-3’), 133.4 (C-1’), 130.9 (ArC), 130.4 (C-5’), 129.7 (ArC), 125.0 (C-2’), 110.4 (C-3), 109.4 (C-4a), 96.8 (C-6), 93.0 (C-8), 56.6 (OCH_3), 56.1 (OCH_3), 44.7 (SO_2CH_3); HRMS (ES^+) found $[M+H]^+$ 361.0750, $C_{18}H_{17}O_6S$ requires *M* 361.0746.

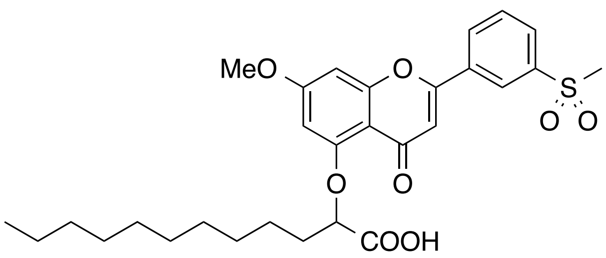
2-(4’-Methanesulfonylphenyl)-5,7-dimethoxy-4H-chromen-4-one **121**



To a solution of 5,7-dimethoxy-2-(4’-methylthiophenyl)-4H-chromen-4-one **119** (33 mg, 0.1 mmol, 1 eq) in MeOH (0.5 mL) and THF (0.5 mL) was added a solution of Oxone® (184 mg, 0.6 mmol, 6 eq) in H_2O (1 mL) dropwise at 0 °C. The resulting mixture was stirred at rt. After 24 h LC-MS analysis still showed the presence of starting material. Another 4 eq of Oxone® (123 mg, 4 mmol, 4 eq) in H_2O (0.5 ml) were added and the mixture was stirred for another 24 h. The solution was then extracted with DCM (2 × 5 ml), the combined organic layers washed with brine and dried over anhydrous Mg_2SO_4. The resulting solution was filtered and concentrated under reduced pressure. Flash column chromatography (12 g silica gel, DCM:MeOH 1:0 - 19:1) afforded the title product (32 mg, 89%) as a colourless solid.

m.p.: 299 – 300 °C (decomposition); v_{max} (ATR): 1640 (C=O), 1343, 1148, 1120, 839, 775 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.15 - 8.00 (m, 4H, 2’-H, 6’-H, 3’-H, 5’-H), 6.75 (s, 1H, 3-H), 6.59 (d, *J* = 2.3 Hz, 1H, 8-H), 6.41 (d, *J* = 2.3 Hz, 1H, 6-H), 3.97 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.11 (s, 3H, CH_3); δ_C ($CDCl_3$, 101 MHz): 177.2 (C-4), 164.6 (C-7), 161.2 (C-5), 160.0 (C-8a), 158.4 (C-2), 142.6 (C-4’), 136.9 (C-1’), 128.2 (2 × ArC), 126.9 (2 × ArC), 111.1 (C-3), 109.5 (C-4a), 96.7 (C-6), 93.0 (C-8), 56.7 (OCH_3), 56.0 (OCH_3), 44.6 (CH_3); HRMS (ES^+) found $[M+H]^+$ 361.0749, $C_{18}H_{17}O_6S$ requires *M* 361.0746.

2-{[2’-(3’’-Methanesulfonylphenyl)-7’-methoxy-4’-oxo-4’H-chromen-5’-yl]oxy}dodecanoic acid **124**

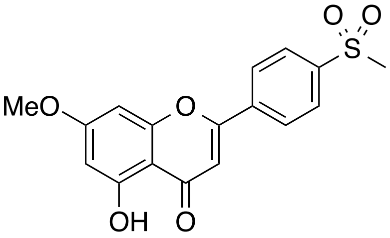


BBr_3 (1 M solution in DCM, 0.3 ml, 0.3 mmol, 1.5 eq) was added to a solution of 2-(3’-methanesulfonylphenyl)-5,7-dimethoxy-4H-chromen-4-one **120** (72 mg, 0.2 mmol, 1 eq) in dry 1,2-dichloroethane (2 ml) under a nitrogen atmosphere, while cooling in a dry ice/acetone-bath. The reaction mixture was allowed to warm to rt and stirred for 3 h, before pouring over a 1:1 mixture of ice and H_2O (approx. 20 ml). The product was extracted with DCM (3 × 20 ml) and the combined organic layers were washed with brine (30 ml), dried over Mg_2SO_4 and concentrated. A solution of methyl 2-bromododecanoate **48** (59 mg, 0.2 mnmol, 1 eq) in dry DMF (0.5 ml) was added to a mixture of crude product and K_2CO_3 (28 mg, 0.2 mmol, 1 eq) in dry DMF (0.5 ml) under an argon atmosphere. The reaction mixture was stirred at 80 °C overnight and after allowing the mixture to cool to rt, it was filtered through Celite® and concentrated. The crude product together with $LiOH.H_2O$ (13 mg, 0.3 mmol, 1.5 eq) was stirred in a 1:1 mixture of H_2O (1 ml) and THF (1 ml) at rt for 24 h. The mixture was acidified to pH 2-3 with 1N HCl and the aqueous phase was extracted with DCM (3 × 5 ml). The organic extracts were collected, dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, Et_2O then DCM:MeOH 19:1) afforded the title product (17 mg, 10 %) as a colourless semi solid.

ν_{max} (ATR): 2925, 2854, 1746 (C=O), 1638 (C=O), 1607, 1356, 1150 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.68 – 8.35 (m, 1H, 2’’-H), 8.33 - 7.92 (m, 2H, 4’’-H, 6’’-H), 7.91 – 7.62 (m, 1H, 5’’-H), 6.83 (s, 1H, 3’-H), 6.79 (d, *J* = 2.2 Hz, 1H, 8’-H), 6.49 (d, *J* = 2.2 Hz, 1H, 6’-H), 4.84 (t,

J = 5.2 Hz, 1H, 2-H), 3.96 (s, 3H, OCH_3), 3.15 (s, 3H, SO_2CH_3), 2.25 - 2.05 (m, 2H, 3-H_2), 1.64 - 1.45 (m, 2H, 4-H_2), 1.41 - 1.19 (m, 14H, 7 × CH_2), 0.93 - 0.82 (m, 3H, 12-H_3); δ_C ($CDCl_3$, 101 MHz): 178.5 (C-4'), 172.3 (C-1), 165.3 (C-7'), 160.3 (C-8a'), 159.5 (C-5'), 158.9 (C-2'), 142.1 (C-3''), 132.7 (C-1''), 131.2 (ArC), 130.6 (C-5''), 130.4 (ArC), 125.2 (C-2''), 109.6 (C-3'), 109.5 (C-4a'), 101.7 (C-6'), 95.2 (C-8'), 81.9 (C-2), 56.5 (OCH_3), 44.7 (SO_2CH_3), 33.0 (C-3), 32.0 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.8 (C-4), 22.8 (CH_2), 14.3 (C-12); HRMS (ES^+) found $[M+H]^+$ 545.2211, $C_{29}H_{37}O_8S$ requires M 545.2209.

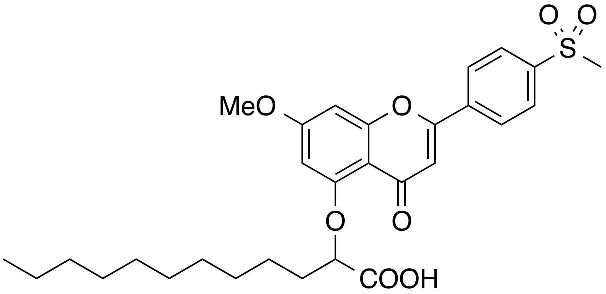
5-Hydroxy-2-(4'-methanesulfonylphenyl)-7-methoxy-4H-chromen-4-one **123**



BBr_3 (1 M solution in DCM, 0.3 ml, 0.3 mmol, 1.5 eq) was added to a solution of 2-(4'-methanesulfonylphenyl)-5,7-dimethoxy-4H-chromen-4-one **121** (72 mg, 0.2 mmol, 1 eq) in dry 1,2-dichloroethane (2 ml) under a nitrogen atmosphere, while cooling in a dry ice/acetone-bath. The reaction mixture was allowed to warm to rt and stirred for 2.5 h, before pouring over a 1:1 mixture of ice and H_2O (approx. 20 ml). The product was extracted with DCM (3 × 20 ml) and the combined organic layers were washed with brine (30 ml), dried over Mg_2SO_4 and concentrated. The title product (46 mg, 66%) was afforded as a yellow solid by flash column chromatography (12 g silica gel, DCM:MeOH 0:1 - 9:1).

m.p.: 251 – 253 °C (decomposition); ν_{max} (ATR): 3070 (OH), 1661 (C=O), 1623, 1272, 1143, 822, 778 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 12.53 (s, 1H, OH), 8.22 - 7.99 (m, 4H, 4 × ArH), 6.74 (s, 1H, 3-H), 6.53 (d, J = 2.2 Hz, 1H, 8-H), 6.41 (d, J = 2.2 Hz, 1H, 6-H), 3.90 (s, 3H, OCH$_3$), 3.12 (s, 3H, SO$_2$CH$_3$); δ_C (CDCl$_3$, 101 MHz): 182.2 (C-4), 166.2 (C-7), 162.5 (C-5), 161.6 (C-2), 157.8 (C-8a), 143.3 (C-4'), 136.6 (C-1'), 128.4 (2 × ArC), 127.3 (2 × ArC), 108.0 (C-3), 106.00 (C-4a), 98.7 (C-6), 93.1 (C-8), 56.1 (OCH$_3$), 44.6 (SO$_2$CH$_3$).; HRMS (ES$^+$) found [M+H]$^+$ 347.0585, $C_{17}H_{15}O_6S$ requires *M* 347.0589.

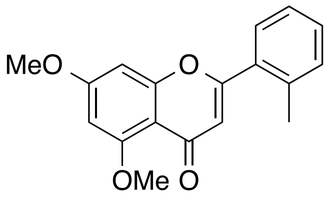
2-{[2'-(4''-Methanesulfonylphenyl)-7'-methoxy-4'-oxo-4'H-chromen-5'-yl]oxy}dodecanoic acid **125**



A solution of methyl 2-bromododecanoate **48** (59 mg, 0.2 mnmol, 2 eq) in dry DMF (0.5 ml) was added to a mixture of 5-hydroxy-2-(4'-methanesulfonylphenyl)-7-methoxy-4H-chromen-4-one **123** (35 mg, 0.1 mmol, 1 eq) and K_2CO_3 (28 mg, 0.2 mmol, 2 eq) in dry DMF (0.5 ml) under an argon atmosphere. The reaction mixture was stirred at 60 °C for 20 h and at 80 °C for another 20 h. After allowing the reaction mixture to cool to rt, it was filtered through Celite® and concentrated. The crude product together with $LiOH.H_2O$ (13 mg, 0.3 mmol, 3 eq) was stirred in a 1:1 mixture of H_2O (1 ml) and THF (1 ml) at rt overnight. The mixture was acidified to pH 2-3 with 1N HCl and the aqueous phase was extracted with DCM (3 × 5 ml). The organic extracts were collected, dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, Et_2O then DCM:MeOH 19:1) afforded the title product (20 mg, 37 %) as a colourless semi solid.

ν_{max} (ATR): 2924, 2854, 1745 (C=O), 1636 (C=O), 1601, 1356, 1152, 1111 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.22 - 7.97 (m, 4H), 6.84 (s, 1H), 6.75 (d, *J* = 2.2 Hz, 1H), 6.49 (d, *J* = 2.2 Hz, 1H), 4.85 (t, *J* = 5.2 Hz, 1H), 3.95 (s, 3H), 3.12 (s, 3H), 2.22 - 2.08 (m, 2H), 1.64 - 1.45 (m, 2H), 1.42 - 1.14 (m, 18H), 0.86 (t, *J* = 6.8 Hz, 3H); δ_C ($CDCl_3$, 101 MHz): 178.5 (C-4'), 172.3 (C-1), 165.3 (C-7'), 160.4 (C-8a'), 159.5 (C-5'), 159.0 (C-2'), 143.3 (C-4''), 136.1 (C-1''), 128.4 (2 × ArC), 127.3 (2 × ArC), 110.2 (C-3'), 109.5 (C-4a'), 101.5 (C-6'), 95.2 (C-8'), 81.8 (C-2), 56.4 (OCH_3), 44.6 (SO_2CH_3), 32.9 (C-3), 32.0 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.7 (C-4), 22.8 (CH_2), 14.3 (C-12); HRMS (ES^+) found $[M+H]^+$ 545.2204, $C_{29}H_{37}O_8S$ requires *M* 545.2209.

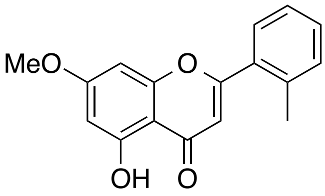
5,7-Dimethoxy-2-(2'-methylphenyl)-4H-chromen-4-one **126**



In a microwave vial 2-chloro-5,7-dimethoxy-4H-chromen-4-one **21** (120 mg, 0.5 mmol, 1 eq) *o*-tolylboronic acid (136 mg, 1 mmol, 2 eq), $Pd_2(dba)_3$ (14 mg, 3 mol%), CyJohnPhos (11 mg, 6 mol%) and Cs_2CO_3 (489 mg, 1.5 mmol, 3 eq) were suspended in dioxane (2.5 ml) under a nitrogen atmosphere. The reaction mixture was heated in the microwave to 150 °C for 30 min. After allowing to cool to rt, the crude mixture was filtered through Celite® and concentrated The residue was dissolved in H_2O (15 ml) and EtOAc (15 ml). The layers were separated and the aqueous layer was extracted two more times with EtOAc (2 × 25 ml). The combined organic layers were washed with H_2O (25 ml), dried over Mg_2SO_4 and concentrated. The title product (112 mg, 76%) was afforded by reversed phase column chromatography (H_2O:MeCN 0:1 - 1:0, 0.1% TFA)

m.p.: 107 – 109 °C; v_{max} (ATR): 1642 (C=O), 1605, 1333, 1158, 1102, 723 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.55 - 7.46 (m, 1H, 6'-H), 7.44 - 7.35 (m, 1H, 4'-H), 7.35 - 7.27 (m, 2H, 3'-H, 5'-H), 6.48 (d, *J* = 2.3 Hz, 1H, 8-H), 6.39 (d, *J* = 2.3 Hz, 1H, 6-H), 6.36 (s, 1H, 3-H), 3.96 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 2.47 (s, 3H, CH_3); δ_C ($CDCl_3$, 101 MHz): 177.7 (C-4), 164.3 (C-7), 163.4 (C-2), 161.1 (C-5), 160.3 (C-8a), 136.8 (C-2'), 132.4 (C-1'), 131.3 (ArC), 130.6 (C-4'), 129.2 (C-6'), 126.3 (ArC), 113.5 (C-3), 109.2 (C-4a), 96.4 (C-6), 92.8 (C-8), 56.6 (OCH_3), 55.9 (OCH_3), 20.7 (CH_3); HRMS (ES^+) found $[M+H]^+$ 297.1132, $C_{18}H_{17}O_4$ requires *M* 297.1127.

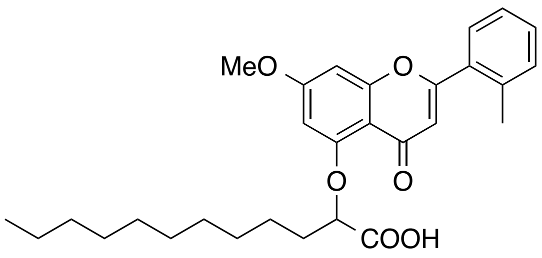
5-Hydroxy-7-methoxy-2-(2’-methylphenyl)-4H-chromen-4-one **127**



BBr_3 (1 M solution in DCM, 0.45 ml, 0.45 mmol, 1.5 eq) was added to a solution of 5,7-dimethoxy-2-(2’-methylphenyl)-4H-chromen-4-one **126** (89 mg, 0.3 mmol, 1 eq) in dry 1,2-dichloroethane (2.5 ml) under a nitrogen atmosphere, while cooling in a dry ice/acetone-bath. The reaction mixture was allowed to warm to rt and stirred for 3 h, before pouring over a 1:1 mixture of ice and H_2O (approx. 20 ml). The product was extracted with DCM (3 × 20 ml) and the combined organic layers were washed with brine (30 ml), dried over Mg_2SO_4 and concentrated. The title product (47 mg, 55%) was afforded as a yellow solid by flash column chromatography (12 g silica gel, DCM:MeOH 19:1).

m.p.: 148 – 150 °C; v_{max} (ATR): 3077 (OH), 3004, 2925, 1658 (C=O), 1618, 1377, 1157, 812 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 12.72 (s, 1H, OH), 7.53 - 7.47 (m, 1H, 6’-H), 7.46 - 7.39 (m, 1H, 4’-H), 7.36 - 7.29 (m, 2H, 3’-H, 5’-H), 6.42 (d, *J* = 2.3 Hz, 1H, 8-H), 6.39 (d, *J* = 2.3 Hz, 1H, 6-H), 6.34 (s, 1H, 3-H), 3.87 (s, 3H, OCH_3), 2.48 (s, 3H, CH_3); δ_C ($CDCl_3$, 101 MHz): 182.5 (C-4), 166.7 (C-2), 165.8 (C-7), 162.4 (C-5), 158.2 (C-8a), 137.0 (C-2’), 132.3 (C-1’), 131.5 (ArC), 131.1 (C-4’), 129.3 (C-6’), 126.4 (ArC), 110.4 (C-3), 105.7 (C-4a), 98.3 (C-6), 92.7 (C-8), 56.0 (OCH_3), 20.7 (CH_3); HRMS (ES^+) found $[M+H]^+$ 283.0961, $C_{17}H_{15}O_4$ requires *M* 283.0970.

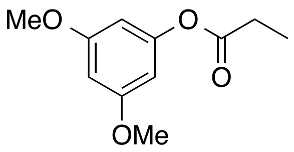
2-{[7’-Methoxy-2’-(2’’-methylylphenyl)-4’-oxo-4’H-chromen-5’-yl]oxy}dodecanoic acid **128**



A solution of methyl 2-bromododecanoate **48** (59 mg, 0.2 mmol, 2 eq) in dry DMF (0.5 ml) was added to a mixture of 5-hydroxy-7-methoxy-2-(2’-methylylphenyl)-4H-chromen-4-one **127** (28 mg, 0.1 mmol, 1 eq) and K_2CO_3 (28 mg, 0.2 mmol, 2 eq) in dry DMF (0.5 ml) under an argon atmosphere. The reaction mixture was stirred at 60 °C for 20 h and at 80 °C for another 20 h. After allowing the reaction mixture to cool to rt, it was filtered through Celite® and concentrated. The crude product together with $LiOH.H_2O$ (13 mg, 0.3 mmol, 3 eq) was stirred in a 1:1 mixture of H_2O (1 ml) and THF (1 ml) at rt overnight. The mixture was acidified to pH 2-3 with 1N HCl and the aqueous phase was extracted with DCM (3 × 5 ml). The organic extracts were collected, dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, Et_2O then DCM:MeOH 19:1) afforded the title product (17 mg, 35 %) as a colourless semi solid.

ν_{max} (ATR): 2924, 2854, 1747 (C=O), 1635 (C=O), 1607, 1354, 1162, 1105 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 13.65 (s, 1H), 7.53 - 7.38 (m, 2H), 7.37 - 7.27 (m, 2H), 6.63 (d, *J* = 2.2 Hz, 1H), 6.48 (d, *J* = 2.2 Hz, 1H), 6.44 (s, 1H), 4.84 (t, *J* = 5.3 Hz, 1H), 3.91 (s, 3H), 2.48 (s, 3H), 2.22 - 2.09 (m, 2H), 1.65 - 1.46 (m, 2H), 1.41 - 1.20 (m, 14H), 0.90 - 0.83 (m, 3H); δ_C ($CDCl_3$, 101 MHz): 178.9 (C-4’), 172.50 (C-1), 165.4 (C-7’), 164.9 (C-2’), 159.8 (C-8a’), 159.0 (C-5’), 136.9 (C-2’’), 131.9 (C-1’’), 131.5 (ArC), 131.2 (C-4’’), 129.3 (C-6’’), 126.5 (ArC), 112.8 (C-3’), 109.4 (C-4a’), 101.4 (C-6’), 95.1 (C-8’), 81.9 (C-2), 56.2 (OCH_3), 33.0 (C-3), 32.0 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.8 (C-4), 22.8 (CH_2), 20.7 (CH_3), 14.3 (C-12); HRMS (ES$^+$) found [M+H]$^+$ 481.2599, $C_{29}H_{37}O_6$ requires *M* 481.2590.

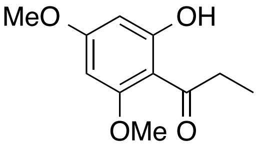
3’,5’-Dimethoxyphenylpropanoate **129**



3,5 Dimethoxyphenol **17** (4.62 g, 30 mmol, 1 eq), DMAP (370 mg, 3 mmol, 0.1 eq) and propionic acid (2.46 ml, 33 mmol, 1.1 eq) were dissolved in a 5:1 mixture of DCM (25 ml) and DMF (5 ml) under an argon atmosphere and cooled to 0 °C. N,N’-Diisopropylcarbodiimide (4.65 ml, 30 mmol, 1 eq) was added dropwise and after 5 min, the reaction mixture was allowed to warm to rt and stirred for another 3.5 h. The mixture was filtered through Celite® and washed with 1 M HCl (2 × 50 ml), sat. aqueous $NaHCO_3$ (2 × 50 ml) and with brine (50 ml). The organic layer was dried over Na_2SO_4 and concentrated. The title product (5.53 g, 88%) was afforded by flash column chromatography (80 g silica gel, hexane:EtOAc 20:1-4:1) as a colourless oil.

ν_{max} (ATR): 1758, 1594 (C=O), 1126, 1050 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 6.34 (t, *J* = 2.3 Hz, 1H, 4’-H), 6.26 (d, *J* = 2.3 Hz, 2H, 2’-H, 6’-H), 3.77 (s, 6H, 2 × OCH_3), 2.57 (q, *J* = 7.6 Hz, 2H, 2-H_2), 1.26 (t, *J* = 7.6 Hz, 3H, 1-H_3).; δ_C ($CDCl_3$, 175 MHz): 172.9 (C-3), 161.3 (C-3’, C-5’), 152.5 (C-1’), 100.3 (C-2’, C-6’), 98.4 (C-4’), 55.6 (2 × OCH_3), 27.9 (C-2), 9.2 (C-1); HRMS (ES^+) found $[M+H]^+$ 211.0965, $C_{11}H_{15}O_4$ requires *M* 211.0970.

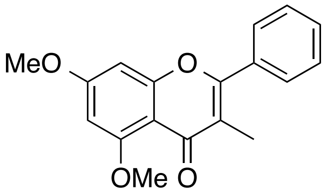
3-(2'-Hydroxy-4',6'-dimethoxyphenyl)propan-3-one[183] **130**



3',5'-Dimethoxyphenylpropanoate **129** (5.28 g, 25.1 mmol, 1 eq) was dissolved in dry 1,2-dichloroethane (300 ml) and added to a slurry of $AlCl_3$ (5.69 g, 42.7 mmol, 1.7 eq) in dry 1,2-dichloroethane (100 ml) under an argon atmosphere at 0 °C. Then the reaction mixture was heated under reflux and stirred for 3.5 h. After cooling to rt, the mixture was poured over a 1:1 mixture of ice and 1 M HCl (approx. 500 ml) and stirred for 30 min. The organic layer was separated and the aqueous layer was extracted with DCM (3 × 200 ml). The combined organic layers were washed with H_2O (200 ml) and brine (200 ml), dried over Na_2SO_4 and concentrated. The title product (2.38 g, 45%) was obtained by flash column chromatography (80 g silica gel, toluene) as a colourless solid.

m.p.: 108 – 109 °C (lit.[183] m.p. 106 – 107 °C); ν_{max} (ATR): 2974, 2927, 1592 (C=O), 1422, 1217, 1155, 1116, 824 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 14.10 (s, 1H, OH), 6.07 (d, *J* = 2.4 Hz, 1H, 3'-H), 5.92 (d, *J* = 2.4 Hz, 1H, 5'-H), 3.85 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.02 (q, *J* = 7.2 Hz, 2H, 2-H_2), 1.15 (t, *J* = 7.2 Hz, 3H, 1-H_3); δ_C ($CDCl_3$, 100 MHz): 206.5 (C-3), 167.7 (C-2'), 165.9 (C-4'), 163.0 (C-6'), 105.9 (C-1'), 93.8 (C-3'), 90.9 (C-5'), 55.7 (2 × OCH_3), 37.6 (C-2), 8.7 (C-1); m/z LC-MS (ES^+) 211 $[M+H]^+$.

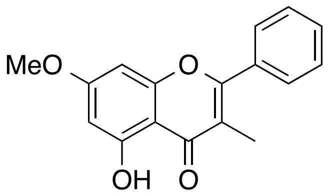
5,7-Dimethoxy-3-methyl-2-phenyl-4H-chromen-4-one[184] **131**



1,1,1,3,3,3-Hexamethyldisilazane (7.21 g, 44.7 mmol, 5.25 eq) was dissolved in dry THF (30 ml) under an argon atmosphere. The solution was cooled to -78 °C and n-BuLi (17.02 ml of a 2.5 M solution in hexane, 42.5 mmol, 5 eq) was added dropwise over 20 min. The reaction mixture was stirred for 10 min, then it was allowed to warm to 0 °C and stirred a further 20 min. The solution of in situ synthesised LiHMDS was added to a solution of 3-(2'-hydroxy-4',6'-dimethoxyphenyl)propan-3-one **130** (1.79 g, 8.5 mmol, 1 eq) in dry toluene (45 ml) at 0 °C. The mixture was stirred for 30 min at 0 °C and after the addition of benzoyl chloride (1.28 ml, 11.1 mmol, 1.3 eq) the reaction mixture was allowed to warm to rt and stirred for 20 h. Conc. HCl (30.6 ml) was carefully added and the reaction mixture was stirred for another 25 h at rt, before pouring over a 1:1 mixture of ice and H_2O (approx. 300 ml). The product was extracted with EtOAc (3 × 200 ml) and the combined organic layers were dried over Na_2SO_4 and concentrated. Two flash column chromatography purifications (80 g silica gel, DCM:MeOH 10:0-9:1, and 80 g silica gel, hexane:EtOAc 5:1-0:1) yielded the title product (1.78 g, 70%) as a colourless solid.

m.p.: 181 - 183°C (lit.[184] m.p. 178 °C); ν_{max} (ATR): 1609 (C=O), 1349, 1201, 1107, 695 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.65 - 7.59 (m, 2H, 2'-H, 6'-H), 7.54 - 7.46 (m, 3H, 3'-H, 4'-H, 5'-H), 6.44 (d, *J* = 2.3 Hz, 1H, 8-H), 6.35 (d, *J* = 2.3 Hz, 1H, 6-H), 3.95 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 2.09 (s, 3H, CH_3); δ_C ($CDCl_3$, 100 MHz): 177.9 (C-4), 163.9 (C-5), 161.0 (C-7), 159.9 (C-8a), 158.4 (C-2), 133.6 (C-1'), 130.0 (C-4'), 129.0 (C-2', C-6'), 128.5 (C3', C5'), 118.6 (C3), 108.3 (C-4a), 96.0 (C-6), 92.4 (C-8), 56.5 (OCH_3), 55.8 (OCH_3), 11.8 (CH_3)*;* m/z LC-MS (ES^+) 297 $[M+H]^+$.

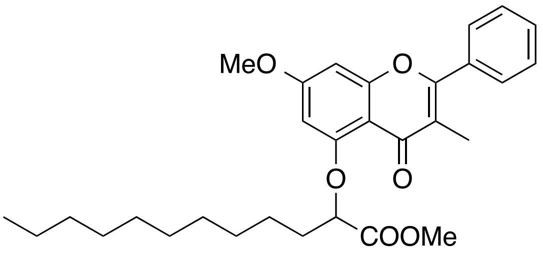
5-Hydroxy-7-methoxy-3-methyl-2-phenyl-4H-chromen-4-one[185] **132**



BBr_3 (2.25 ml of a 1 M solution in DCM, 2.25 mmol, 1.5 eq) was added to a solution of 5,7-dimethoxy-3-methyl-2-phenyl-4H-chromen-4-one **131** (450 mg, 1.5 mmol, 1 eq) in dry 1,2-dichloroethane (12.5 ml) under an argon atmosphere, while cooling in a dry ice/acetone-bath. The reaction mixture was allowed to warm to rt and stirred for 15 h, before pouring over a 1:1 mixture of ice and H_2O (approx. 80 ml). The product was extracted with EtOAc (3 × 100 ml) and the combined organic layers were washed with brine (60 ml), dried over Na_2SO_4 and concentrated. The title product (310 mg, 73%) was afforded by flash column chromatography (24 g silica gel, DCM:MeOH 10:0-9:1) as a colourless solid.

m.p.: 163 - 164 °C (lit.[185] m.p. 157 °C); ν_{max} (ATR): 3012 (OH), 2845 (OH), 1658 (C=O), 1602, 1348, 1203, 1152, 773, 696 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 12.94 (s, 1H, OH), 7.65 - 7.59 (m, 2H, 2'-H, 6'-H), 7.55 - 7.50 (m, 3H, 3'-H, 4'-H, 5'-H), 6.39 (d, *J* = 2.3 Hz, 1H, 8-H), 6.37 (d, *J* = 2.3 Hz, 1H, 6-H), 3.86 (s, 3H, OCH_3), 2.12 (s, 3H, CH_3); δ_C ($CDCl_3$, 100 MHz): 182.9 (C-4), 165.6 (C-7), 162.2 (C-2), 161.6 (C-5), 157.9 (C-8a), 133.1 (C-1'), 130.6 (C-4'), 129.1 (C-2', C-6'), 128.7 (C3', C5'), 116.0 (C3), 104.9 (C-4a), 98.1 (C-6), 92.1 (C-8), 55.9 (OCH_3), 11.0 (CH_3)*;* m/z LC-MS (ES^+) 283 $[M+H]^+$.

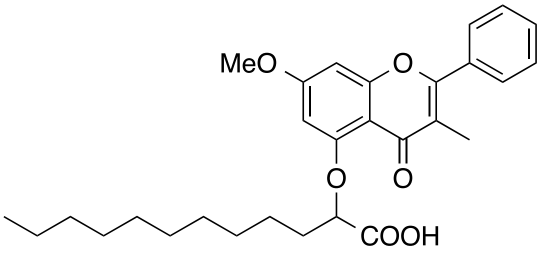
Methyl 2-(7'-methoxy-4'-oxo-3'-methyl-2'-phenyl-4'H-chromen-5'-oxy)dodecanoate **133**



A solution of methyl 2-bromododecanoate **48** (312 mg, 1.06 mmol, 2 eq) in dry DMF (1 ml) was added to a mixture of 5-hydroxy-7-methoxy-3-methyl-2-phenyl-4H-chromen-4-one **132** (0.150 g, 0.53 mmol, 1 eq) and K_2CO_3 (147 mg, 1.06 mmol, 2 eq) in dry DMF (4 ml) under an argon atmosphere. The reaction mixture was stirred at rt for 24 h. K_2CO_3 was collected by filtration through Celite® and the solvent was removed under reduced pressure. Flash column chromatography (12 g silica gel, hexane:EtOAc 20:1-4:1) yielded the title product (222 mg, 84%) as a colourless solid.

m.p.: 66 - 67°C; ν_{max} (ATR): 2920, 2849, 1744 (C=O), 1645 (C=O), 1609, 1336, 1165, 1142, 1116, 821 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.64 - 7.56 (m, 2H, 2''-H, 6''-H), 7.54 - 7.45 (m, 3H, 3''-H, 4''-H, 5''-H), 6.47 (d, *J* = 2.3 Hz, 1H, 8'-H), 6.22 (d, *J* = 2.3 Hz, 1H, 6'-H), 4.69 (dd, *J* = 7.3, 5.4 Hz, 1H, 2-H), 3.82 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 2.22 - 2.00 (m, 5H, CH_3, 3-H_2), 1.67 - 1.56 (m, 2H, 4-H_2), 1.43 - 1.20 (m, 14H, 7 × CH_2), 0.87 (t, J=6.8 Hz, 3H, 12-H_3); δ_C ($CDCl_3$, 100 MHz): 177.3 (C-4'), 172.0 (C-1), 163.5 (C-7'), 159.9 (C-8a'), 159.1 (C-5'), 158.3 (C-2'), 133.6 (C-1''), 130.0 (C-4''), 129.0 (C-2'', C-6''), 128.5 (C-3'', C-5''), 118.5 (C-3'), 108.8 (C-4a'), 98.6 (C-6'), 93.6 (C-8'), 78.3 (C-2), 55.8 (OCH_3), 52.3 (COO**C**H_3), 32.9 (C-3), 32.0 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 25.1 (C-4), 22.8 (CH_2), 14.3 (C-12), 11.8 (CH_3); HRMS (ES$^+$) found $[M+H]^+$ 495.2738, $C_{30}H_{39}O_6$ requires *M* 495.2747.

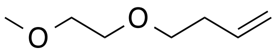
2-(7’-Methoxy-4’-oxo-3’-methyl-2’-phenyl-4’H-chromen-5’-oxy)dodecanoic acid **134**



Methyl 2-(7-methoxy-4-oxo-3-methyl-2-phenyl-4H-chromen-5-oxy)dodecanoate **133** (145 mg, 0.29 mmol, 1 eq) was dissolved in a 1:1 mixture of THF (7.5 ml) and H_2O (7.5 ml) and $LiOH.H_2O$ (25 mg, 0.58 mmol, 2 eq) was added. The reaction mixture was stirred for 17 h at rt. The mixture was acidified with 1M HCl to pH 6 and extracted with EtOAc (3 × 15 ml). The combined organic layers were washed with brine (20 ml), dried over Na_2SO_4 and the solvent was removed under reduced pressure. Flash column chromatography (24 g silica gel, DCM:MeOH 1:0-9:1) afforded the title product (111 mg, 79%) as a colourless solid.

m.p.: 103 - 105 °C; ν_{max} (ATR): 2920, 2854, 1732 (C=O), 1604 (C=O), 1359, 1202, 1161, 1107, 690 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 13.81 (s, 1H, COOH), 7.64 - 7.58 (m, 2H, 2’’-H, 6’’-H), 7.56 - 7.49 (m, 3H, 3’’-H, 4’’-H, 5’’-H), 6.59 (d, *J* = 2.2 Hz, 1H, 8’-H), 6.44 (d, *J* = 2.3 Hz, 1H, 6’-H), 4.81 (t, *J* = 5.4 Hz, 1H, 2-H), 3.89 (s, 3H, OCH_3), 2.18 - 2.10 (m, 5H, CH_3, 3-H_2), 1.63 - 1.48 (m, 2H, 4-H_2), 1.40 - 1.20 (m, 14H, 5-H_2, 6-H_2, 7-H_2, 8-H_2, 9-H_2, 10-H_2, 11-H_2), 0.87 (t, *J* = 7.1 Hz, 3H, 12-H_3); δ_C ($CDCl_3$, 175 MHz): 179.2 (C-4’), 172.6 (C-1), 164.5 (C-7’), 160.6 (C-2’), 159.3 (C-8a’), 158.9 (C-5’), 132.8 (C-1’’), 130.6 (C-4’’), 129.0 (C-2’’, C-6’’), 128.7 (C-3’’, C-5’’), 118.3 (C-3’), 108.3 (C-4a’), 101.3 (C-6’), 94.7 (C-8’), 82.0 (C-2), 56.1 (OCH_3), 33.1 (C-3), 32.0 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.9 (C-4), 22.8 (CH_2), 14.2 (C-12), 11.8 (CH_3)*;* HRMS (ES^+) found $[M+H]^+$ 481.2599, $C_{29}H_{37}O_6$ requires *M* 481.2590.

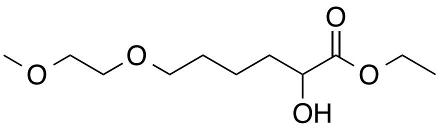
4-(2'-Methoxyethoxy)-1-butene **138**



3-Buten-1-ol (5.16 ml, 60 mmol, 1.5 eq) was added to a slurry of NaH (60% dispersed in mineral oil, 2.88 g, 72 mmol, 1.8 eq) in dry DMF (50 ml) at 0 °C under an argon atmosphere and the resulting mixture was stirred for 45 min at 0 °C. 2-Bromoethyl methyl ether **135** (3.76 ml, 40 mmol, 1 eq) was added and the mixture was stirred another 20 h at rt. The mixture was neutralized with 1 N HCl and extracted with Et_2O (3 × 100 ml). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. Flash column chromatography (80 g silica gel, hexane:EtOAc 1:0-7:3) afforded the title product (2.55 g, 33%) as a colourless oil.

ν_{max} (ATR): 2873, 1099, 751 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 5.82 (ddt, *J* = 17.2, 10.2, 6.7 Hz, 1H, 2-H), 5.09 (dq, *J* = 17.2, 1.7 Hz, 1H, 1-H), 5.03 (ddt, *J* = 10.2, 2.1, 1.2 Hz, 1H, 1-H), 3.62 - 3.57 (m, 2H, 1'-H_2), 3.56 - 3.50 (m, 4H, 4-H_2, 2'-H_2), 3.39 (s, 3H, OCH_3), 2.36 (qt, *J* = 6.9, 1.4 Hz, 2H, 3-H_2); δ_C ($CDCl_3$, 175 MHz): ^{13}C NMR (176 MHz, $CDCl_3$) δ 135.3 (C-2), 116.5 (C-1), 72.1 (C-2'), 70.9 (C-4), 70.2 (C-1'), 59.2 (OCH_3), 34.2 (C-3).

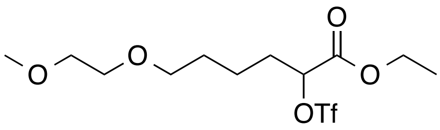
Ethyl 2-hydroxy-6-(2'-methoxyethoxy)hexanoate **140**



To a solution of (+)-diethyl L-tartrate **80** (0.38 ml, 2.2 mmol, 0.6 eq) in dry Et_2O (2 ml) at 0 °C, periodic acid (501 mg, 2.2 mmol, 0.6 eq) was slowly added over 30 min. The reaction mixture was stirred another 20 min at rt, filtered through Celite® and concentrated. The crude product was dissolved in dry DCM (10 ml) and added to a slurry of ferric chloride (1.946 g, 12 mmol, 3 eq) in dry DCM (12 ml) and H_2O (0.19 ml, 12 mmol, 3 eq). The mixture was cooled to 0 °C and 4-(2'-methoxyethoxy)-1-butene (521 mg, 4 mmol, 1 eq) dissolved in dry DCM (10 ml) was added before the mixture was stirred for 3 h at rt. The reaction mixture was washed with 0.1 N HCl (25 ml), dried over $MgSO_4$, filtered through silica gel and concentrated. A flask was charged with Crabtree catalyst (64 mg, 2 mol%) and the crude product from the previous step, dissolved in dry DCM (16 ml). The flask was purged with nitrogen and then flushed with hydrogen and stirred with a hydrogen balloon attached for 2.5 h at rt. The reaction mixture was concentrated and the title product (255 mg, 27%) was afforded as a colourless oil by flash column chromatography (80 g silica gel, chloroform:EtOAc 9:1 – 7:3).

ν_{max} (ATR): 3446 (OH), 2867, 1733 (C=O), 1111 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 4.26 - 4.20 (m, 2H, $OC\mathbf{H_2}CH_3$), 4.15 (dt, *J* = 7.6, 4.7 Hz, 1H, 2-H), 3.60 - 3.49 (m, 4H, 1'-H_2, 2'-H_2), 3.46 (t, *J* = 6.7 Hz, 2H, 6-H_2), 3.37 (s, 3H, OCH_3), 2.81 (d, *J* = 5.5 Hz, 1H, OH), 1.83 - 1.77 (m, 1H, 3-H), 1.69 - 1.56 (m, 3H, 3-H, 5-H_2), 1.54 - 1.41 (m, 2H, 4-H_2), 1.29 (t, *J* = 7.1 Hz, 3H, $OCH_2C\mathbf{H_3}$); δ_C ($CDCl_3$, 101 MHz): 175.4 (C-1), 72.1 (C-2'), 71.3 (C-6), 70.5 (C-2), 70.2 (C-1'), 61.8 ($O\mathbf{C}H_2CH_3$), 59.2 (OCH_3), 34.3 (C-3), 29.4 (C-5), 21.6 (C-4), 14.3 ($OCH_2\mathbf{C}H_3$); HRMS (ASAP) found $[M+H]^+$ 235.1544, $C_{11}H_{23}O_5$ requires *M* 235.1545.

Ethyl 6-(2'-methoxyethoxy)-2-(((trifluoromethyl)sulfonyl)oxy)hexanoate **141**

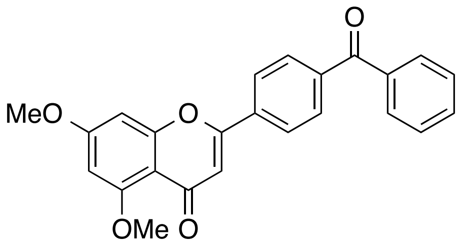


Ethyl 2-hydroxy-6-(2'-methoxyethoxy)hexanoate **140** (100 mg, 0.43 mmol, 1 eq) was dissolved in dry DCM (0.9 ml) at 0 °C under a nitrogen atmosphere. 2,6-Lutidine (0.06 ml, 0.51 mmol, 1.2 eq) and subsequently triflic anhydride (0.08 ml, 0.47 mmol, 1.1 eq) were added and the reaction was stirred at 0 °C for 2.5 h. H_2O was slowly added and the two layers were separated. The organic layer was dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, hexane:EtOAc 1:0 - 4:1) afforded the title product (105 mg, 67%) as a colourless oil.

ν_{max} (ATR): 2934, 1760 (C=O), 1417, 1204, 1144, 928 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 5.10 (t, J = 6.1 Hz, 1H, 2-H), 4.34 - 4.25 (m, 2H, $OC\mathbf{H_2}CH_3$), 3.61 - 3.51 (m, 4H, 1'-H_2, 2'-H_2), 3.47 (t, J = 6.3 Hz, 2H, 6-H_2), 3.38 (s, 3H, OCH_3), 2.06 - 2.00 (m, 2H, 3-H_2), 1.70 - 1.60 (m, 2H, 5-H_2), 1.58 - 1.50 (m, 2H, 4-H_2), 1.32 (t, J = 7.1 Hz, 3H, $OCH_2C\mathbf{H_3}$); δ_C ($CDCl_3$, 101 MHz): 167.2 (C-1), 118.6 (q, $^1J_{CF}$ = 321.3 Hz, CF_3), 83.8 (C-2), 72.1 (C-1'), 70.8 (C-6), 70.3 (C-2'), 62.8 ($O\mathbf{C}H_2CH_3$), 59.2 (OCH_3), 31.9 (C-3), 28.9 (C-5), 21.5 (C-4), 14.1 ($OCH_2\mathbf{C}H_3$); δ_F ($CDCl_3$, 376 MHz): -74.88;

5.1.5 Synthesis of photoaffinity labels

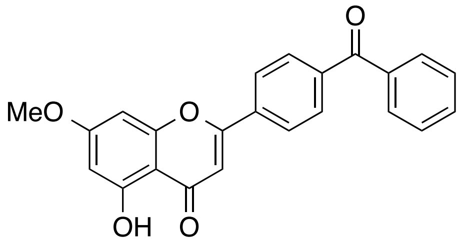
2-(4’-Benzoylphenyl)-5,7-dimethoxy-4H-chromen-4-one **143**



2-Chloro-5,7-dimethoxy-4H-chromen-4-one **21** (120 mg, 0.5 mmol, 1 eq), 4-benzoylphenylboronic acid (226 mg, 1 mmol, 2 eq), $Pd_2(dba)_3$ (13.8 mg, 3 mol%), CyJohnPhos (10.6 mg, 6 mol%) and Cs_2CO_3 (489 mg, 1.5 mmol, 3 eq) were suspended in dioxane (2.5 ml) under an argon atmosphere. The reaction mixture was heated to 100 °C and stirred for 17 h. After allowing to cool to rt, the crude mixture was filtered through Celite® and concentrated The residue was dissolved in H_2O (20 ml) and EtOAc (20 ml). The layers were separated and the aqueous layer was extracted two more times with EtOAc (2 × 50 ml). The combined organic layers were washed with H_2O (50 ml), dried over Na_2SO_4 and concentrated. The title product (173 mg, 90%) was obtained by flash column chromatography (4 g silica gel, DCM:EtO_2:MeOH 1:1:0-2:2:1) as a colourless solid.

m.p.: 187 - 188 °C; ν_{max} (ATR): 1641 (C=O), 1605 (C=O), 1279, 1160, 1117, 831, 699 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 8.02 - 7.94 (m, 2H, 2’-H, 6’-H), 7.95 - 7.87 (m, 2H, 3’-H, 5’-H), 7.86 - 7.76 (m, 2H, 2’’-H, 6’’-H), 7.67 - 7.57 (m, 1H, 4’’-H), 7.55 - 7.46 (m, 2H, 3’’-H, 5’’-H), 6.76 (s, 1H, 3-H), 6.60 (d, *J* = 2.3 Hz, 1H, 8-H), 6.40 (d, *J* = 2.3 Hz, 1H, 6-H), 3.97 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 195.9 (C=O), 177.4 (C-4), 164.4 (C-7), 161.1 (C-5), 160.0 (C-8a), 159.5 (C-2), 139.8 (C-4’), 137.2 (C-1’’), 135.2 (C-1’), 133.0 (C-4’’), 130.6 (C-3’, C-5’), 130.2 (C-2’’, C6’’), 128.6 (C-3’’, C-5’’), 126.0 (C-2’, C-6’), 110.5 (C-3), 109.5 (C-4a), 96.5 (C-6), 93.0 (C-8), 56.6 (OCH_3), 56.0 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 387.1241, $C_{24}H_{19}O_5$ requires *M* 387.1232.

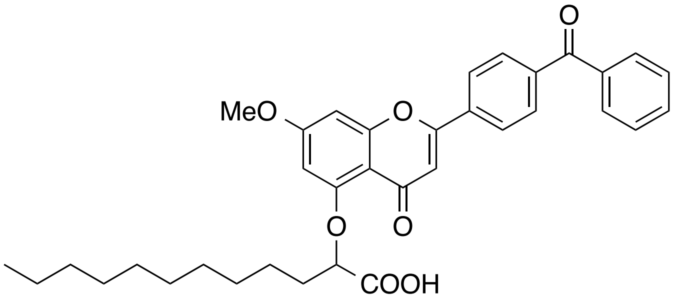
2-(4'-Benzoylphenyl)-5-hydroxy-7-methoxy-2-4H-chromen-4-one **144**



BBr_3 (0.34 ml of a 1 M solution in heptanes, 0.34 mmol, 1.5 eq) was added to a solution of 2-(4-benzoylphenyl)-5,7-dimethoxy-4H-chromen-4-one **143** (87 mg, 0.23 mmol, 1 eq) in dry 1,2-dichloroethane (2 ml) under an argon atmosphere, while cooling in a dry ice/acetone-bath. The reaction mixture was allowed to warm to rt and stirred for 2.5 h, before pouring over a 1:1 mixture of ice and H_2O (approx. 25 ml). The product was extracted with DCM (3 × 30 ml) and the combined organic layers were washed with brine (40 ml), dried over Na_2SO_4 and concentrated. The title product (75 mg, 90%) was afforded by flash column chromatography (12 g silica gel, $CHCl_3$:MeOH 1:0-9:1) as a colourless solid.

m.p.: 189 - 191°C; ν_{max} (ATR): 1648 (C=O), 1617 (C=O), 1277, 1158, 699 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 12.63 (s, 1H, OH), 8.03 - 7.97 (m, 2H,2'-H, 6'-H), 7.96 - 7.90 (m, 2H, 3'-H, 5'-H), 7.86 - 7.80 (m, 2H, 2''-H, 6''-H), 7.67 - 7.60 (m, 1H, 4''-H), 7.55 - 7.49 (m, 2H, 3''-H, 5''-H), 6.74 (s, 1H, 3-H), 6.53 (d, *J* = 2.3 Hz, 1H, 8-H), 6.39 (d, *J* = 2.2 Hz, 1H, 6-H), 3.89 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 195.7 (C=O), 182.4 (C-4), 166.0 (C-7), 162.7 (C-2), 162.4 (C-5), 157.9 (C-8a), 140.3 (C-4'), 137.1 (C-1''), 134.8 (C-1'), 133.1 (C-4''), 130.7 (C-3', C-5'), 130.2 (C-2'', C-6''), 128.7 (C-3'', C-5''), 126.3 (C-2', C-6'), 107.2 (C-3), 106.0 (C-4a), 98.5 (C-6), 92.9 (C-8), 56.0 (OCH_3).; HRMS (ES^+) found $[M+H]^+$ 373.1081, $C_{23}H_{17}O_5$ requires *M* 373.1076.

2-(2’-(4’’-Benzoylphenyl)-7’-methoxy-4’-oxo-2-4H-chromen-5’-oxy)dodecanoic acid **145**

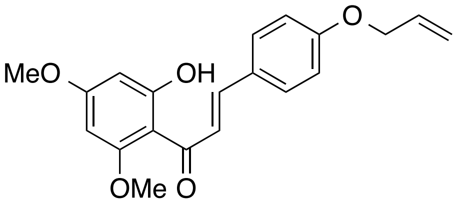


A solution of methyl 2-bromododecanoate **48** (95 mg, 0.32 mmol, 2 eq) in dry DMF (1 ml) was added to a mixture of 2-(4-benzoylphenyl)-5-hydroxy-7-methoxy-2-4H-chromen-4-one **144** (60 mg, 0.16 mmol, 1 eq) and K_2CO_3 (45 mg, 0.32 mmol, 2 eq) under an argon atmosphere. The reaction mixture was stirred at 60 °C for 17 h. H_2O (10 ml) was added and the product was extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with H_2O (20 ml), dried over Na_2SO_4 and concentrated. The crude product was dissolved in a 1:1 mixture of THF (4 ml) and H_2O (4 ml) and LiOH.H_2O (14 mg, 0.32 mmol, 2 eq) was added. The reaction mixture was stirred for 2.5 h at rt. The mixture was acidified with 1M HCl to pH 5 - 6 and extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4 and concentrated. Flash column chromatography (12 g silica gel, Et_2O, then DCM:MeOH 9:1) afforded the title product (59 mg, 65%) as a colourless semi-solid.

ν_{max} (ATR): 2923, 2852, 1742 (C=O), 1634 (C=O), 1599, 1277, 1107, 698 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 13.48 (s, 1H, COOH), 8.07 - 7.98 (m, 2H, 2’’-H, 6’’-H), 7.98 - 7.90 (m, 2H, 3’’-H, 5’’-H), 7.86 - 7.78 (m, 2H, 2’’’-H, 6’’’-H), 7.69 - 7.58 (m, 1H, 4’’’-H), 7.55 – 7.50 (m, 2H, 3’’’-H, 5’’’-H), 6.84 (s, 1H, 3’-H), 6.75 (d, *J* = 2.2 Hz, 1H, 8’-H), 6.49 (d, *J* = 2.2 Hz, 1H, 6’-H), 4.85 (t, *J* = 5.3 Hz, 1H, 2-H), 3.95 (s, 3H, OCH_3), 2.24 - 2.08 (m, 2H, 3-H_2), 1.66 - 1.45 (m, 2H, 4-H_2), 1.43 - 1.16 (m, 14H, 5-H_2, 6-H_2, 7-H_2, 8-H_2, 9-H_2, 10-H_2, 11-H_2), 0.87 (t, *J* = 6.8 Hz, 3H, 12-H_3); δ_C ($CDCl_3$, 100 MHz): 195.7 (C=O), 178.7 (C-4’), 172.4 (C-1), 165.1 (C-7’), 161.6 (C-8a’), 159.6 (C-2’), 159.0 (C-5’), 140.4 (C-4’’), 137.0 (C-1’’’), 134.4 (C-1’’), 133.2 (C-4’’’), 130.7 (C-3’’, C-5’’), 130.2 (C-2’’’, C-6’’’), 128.7 (C-3’’’, C-5’’’), 126.3

(C-2'', C-6''), 109.6 (C-4a'), 109.6 (C-3'), 101.5 (C-6'), 95.2 (C-8'), 81.9 (C-2), 56.3 (OCH_3), 33.0 (C-3), 32.0 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.8(C-4), 22.8 (CH_2), 14.3 (C-12); HRMS (ES^+) found $[M+H]^+$ 571.2683, $C_{35}H_{39}O_7$ requires *M* 571.2696.

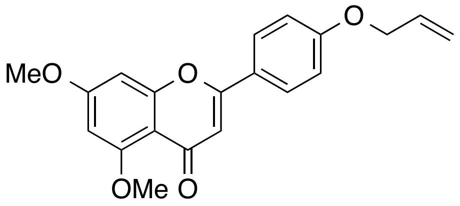
trans-3-(4’-Allyloxyphenyl)-1-(2’’-hydroxy-4’’,6’’-dimethoxyphenyl)-propenone[145] **146**



KOH (20 ml of a 3M solution in EtOH, 60 mmol, 6 eq) was added to a mixture of 2-hydroxy-4,6-dimethoxyacetophenone **7** (1.96 g, 10 mmol, 1 eq) and 4-allyloxybenzaldehyde **151** (1.62 g, 10 mmol, 1 eq). The reaction mixture was stirred for 18 h at rt and subsequently acidified to pH 5 with 1M HCl and extracted with DCM (3x 50 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The title product (2.05 g, 60%) was obtained by flash column chromatography (50 g silica gel, $CHCl_3$) as a yellow solid.

m.p.:106 - 108 °C(lit.[145] m.p. 99 – 100 °C); ν_{max} (ATR):3114 (OH), 2974, 2355, 1603 (C=O), 1542, 1203, 1151, 825 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 14.41 (s, 1H, OH), 7.81 (d, *J* = 15.5 Hz, 2-H), 7.66 (d, *J* = 15.5 Hz, 3-H), 7.59 - 7.52 (m, 2H, 2’-H, 6’-H), 6.98 - 6.91 (m, 2H, 3’-H, 5’-H), 6.11 (d, *J* = 2.4 Hz, 1H, 3’’-H), 6.10 - 6.01 (m, 1H, C**H**=CH_2), 5.97 (d, *J* = 2.4 Hz, 1H, 5’’-H), 5.43 (ap dq, *J* = 17.2, 1.5 Hz, 1H, O-CH_2-CH=C**Htrans**H), 5.32 (ap dq, *J* = 10.5, 1.5 Hz, 1H, O-CH_2-CH=CH**Hcis**), 4.59 (ap dt, *J* = 5.3, 1.5 Hz, 2H, O-C**H$_2$**-CH=CH_2), 3.92 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 192.7 (C-1), 168.5 (C-2’’), 166.2 (C-4’’), 162.6 (C-6’’), 160.5 (C-4’), 142.6 (C-3), 133.0 (O-CH_2-**C**H=CH_2), 130.2 (C-2’, C-6’), 128.6 (C-1’), 125.3 (C-2), 118.2 (O-CH_2-CH=**C**H_2), 115.3 (C-3’, C-5’), 106.5 (C-1’’), 94.0 (C-3’’), 91.4 (C-5’’), 69.0 (O-**C**H_2-CH=CH_2), 56.0 (OCH_3), 55.7 (OCH_3); m/z LC-MS (ES^+) 341 $[M+H]^+$.

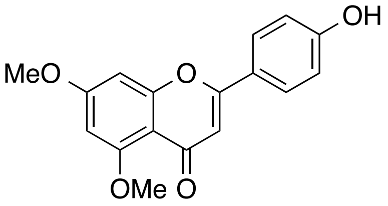
5,7-Dimethoxy-2-(4'-allyloxyphenyl)-4H-chromen-4-one[145] **147**



Iodine (45 mg, 0.3 mol%) was added to a stirred solution of trans-3-(4''-allyloxyphenyl)-1-(2'-hydroxy-4',6'-dimethoxyphenyl)-propenone **146** (1.85 g, 5.4 mmol, 1 eq) in DMSO at 50 °C. Then the reaction mixture was heated to 140 °C and stirred for 2 h. After cooling to rt, H_2O (40 ml) and DCM (40 ml) were added. The layers were separated and the aqueous layer was extracted two more times with DCM (2 × 40 ml). The combined organic layers were washed with an aqueous $Na_2S_2O_3$ solution (0.5%, 50 ml), dried over Na_2SO_4 and concentrated. The title product (1.75 g, 95%) was obtained by flash column chromatography (12 g silica gel, EtO_2:MeOH 1:0-4:1) as a colourless solid.

m.p.: 169 - 171 °C (lit.[145] m.p. 171 – 172 °C); ν_{max} (ATR): 1640 (C=O), 1603, 1163, 1104, 825 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.86 - 7.80 (m, 2H, 2'-H, 6'-H), 7.05 - 6.98 (m, 2H, 3'-H, 5'-H), 6.72 (s, 1H, 3-H), 6.57 (d, *J* = 2.3 Hz, 1H, 8-H), 6.38 (d, *J* = 2.3 Hz, 1H, 6-H), 6.07 (ddt, *J* = 17.3, 10.5, 5.3 Hz, 1H, C**H**=CH_2), 5.44 (ap dq, *J* = 17.3, 1.5 Hz, 1H, O-CH_2-CH=C**Htrans**H), 5.33 (ap dq, *J* = 10.5, 1.5 Hz, 1H, O-CH_2-CH=CH**Hcis**), 4.62 (ap dt, *J* = 5.3, 1.5 Hz, 2H, O-C**H_2**-CH=CH_2), 3.96 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3); δ_C ($CDCl_3$, 100 MHz): 177.8 (C-4), 164.3 (C-7), 161.4 (C-4''), 161.2 (C-5), 161.1 (C-2), 160.0 (C-8a), 132.7 (O-CH_2-**C**H=CH_2), 127.9 (C-2', C-6'), 123.9 (C-1'), 118.4 (O-CH_2-CH=**C**H_2), 115.3 (C-3', C-5'), 109.0 (C-4a), 107.4 (C-3), 96.4 (C-6), 93.0 (C-8), 69.1 (O-**C**H_2-CH=CH_2), 56.6 (OCH_3), 55.9 (OCH_3); m/z LC-MS (ES^+) 339 $[M+H]^+$.

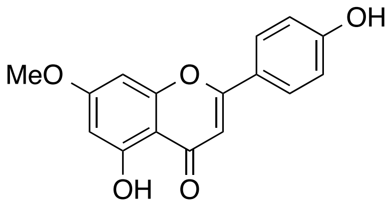
5,7-Dimethoxy-2-(4’-hydroxyphenyl)-4H-chromen-4-one[186] **148**



A suspension of 5,7-dimethoxy-2-(4’-allyloxyphenyl)-4H-chromen-4-one **147** (1.55 g, 5.2 mmol, 1 eq), $Pd(PPh_3)_4$ (180 mg, 3 mol%) and K_2CO_3 (2.87 g, 20.8 mmol, 4 eq) in dry MeOH (90 ml) was stirred at reflux for 4,5 h under an argon atmosphere. After cooling the reaction mixture to rt, excess K_2CO_3 was removed by filtration through Celite®. The filtrate was added to H_2O (100 ml) and the solution was acidified to pH 3 with 1M HCl. An off-white precipitate was formed, which was collected by filtration and dried under reduced pressure. The title product, a yellow solid, (1.32 g, 85%) was used for upcoming steps without further purification.

m.p.: 290 – 292 °C (lit.[186] m.p. 289 – 290 °C); ν_{max} (ATR): 3172 (OH), 1631 (C=O), 1596, 1349, 1107, 829 cm^{-1}; δ_H (d^6-DMSO, 400 MHz): 10.22 (s, 1H, OH), 7.91 - 7.85 (m, 2H, 2’-H, 6’-H), 6.93 - 6.87 (m, 2H, 3’-H, 5’-H), 6.83 (d, *J* = 2.3 Hz, 1H, 8-H), 6.59 (s, 1H, 3-H), 6.49 (d, *J* = 2.3 Hz, 1H, 6-H), 3.89 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); δ_C (d^6-DMSO, 100 MHz): 175.6 (C-4), 163.5 (C-7), 160.5 (C-4’’), 160.2 (C-2) , 160.0 (C-5), 159.1 (C-8a), 127.8 (C-2’, C-6’), 121.3 (C-1’), 115.8 (C-3’, C-5’), 108.2 (C-4a), 106.1 (C-3), 96.1 (C-6), 93.3 (C-8), 56.1 (OCH_3), 55.9 (OCH_3); m/z LC-MS (ES^+) 299 $[M+H]^+$.

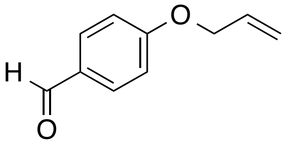
5-Hydroxy-2-(4’-hydroxyphenyl)-7-methoxy-4H-chromen-4-one[187] **149**



BBr_3 (3.02 ml of a 1 M solution in DCM, 3 mmol, 1.5 eq) was added to a solution of 5,7-dimethoxy-2-(4’-hydroxyphenyl)-4H-chromen-4-one **148** (600 mg, 2 mmol, 1 eq) in dry 1,2-dichloroethane (18 ml) under an argon atmosphere, while cooling in a dry ice/acetone-bath. The reaction mixture was allowed to warm to rt and stirred for 3 h, before adding H_2O (approx. 50 ml). The product was collected by filtration and dried under reduced pressure. The filtrate was further extracted with DCM (2 × 30 ml) and the combined organic layers were dried over Na_2SO_4 and concentrated. The resulting solid was combined with the collected precipitate and recrystallized from methanol to yield the title product (293 mg, 51%).

m.p.: 289 – 291 °C (lit.[188] m.p.: 290 – 292 °C); ν_{max} (ATR): 3251 (OH), 1663 (C=O), 1589, 1499, 1161, 818 cm^{-1}; δ_H (CD_3OD, 400 MHz): 7.92 - 7.85 (m, 2H, 2’-H, 6’-H), 6.97 - 6.90 (m, 2H, 3’-H, 5’-H), 6.67 (d, *J* = 2.3 Hz, 1H, 8-H), 6.65 (s, 1H, 3-H), 6.36 (d, *J* = 2.3 Hz, 1H, 6-H), 3.90 (s, 3H, OCH_3); δ_C (d^6-DMSO, 176 MHz): 181.9 (C-4), 165.1 (C-7), 164.0 (C-2), 161.3 (C-4’), 161.2 (C-5), 157.2 (C-8a), 128.5 (C-2’, C-6’), 121.0 (C-1’), 115.9 (C-3’, C-5’), 104.6 (C-4a), 103.0 (C-3), 97.9 (C-6), 92.7 (C-8), 56.0 (OCH_3); m/z LC-MS (ES^+) 285 $[M+H]^+$.

4-Allyloxybenzaldehyde [189] **151**

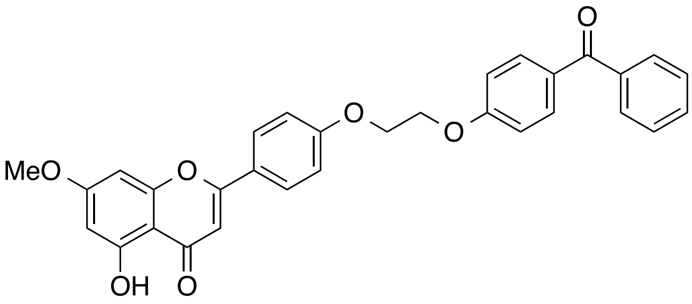


K_2CO_3 (6.79 g, 49.1 mmol, 3 eq) and allyl bromide (2.13 ml, 24.6 mmol, 1.5 eq) were added to a solution of 4-hydroxybenzaldehyde **150** (2.00 g, 16.4 mmol, 1 eq) in acetone (20 ml). The reaction mixture was stirred at rt for 2 h and subsequently heated to reflux for another 2 h. The solvent was removed under reduced pressure and flash column chromatography (24 g silica gel, hexane:EtOAc 1:0-7:3) afforded the title product (2.38 g, 89%) as a colourless oil.

ν_{max} (ATR): 1694 (C=O), 1683, 1601, 1576, 1508, 1313, 1258, 1163, 996, 833 cm^{-1} δ_H ($CDCl_3$, 400 MHz): 9.88 (s, 1H, CHO), 7.89 - 7.78 (m, 2H, 2-H, 6-H), 7.08 - 6.97 (m, 2H, 3-H, 5-H), 6.05 (ddt, *J* = 17.2, 10.5, 5.3 Hz, 1H, C**H**=CH_2), 5.43 (ap dq, *J* = 17.2, 1.5 Hz, 1H, CH=C**Htrans**H), 5.33 (ap dq, *J* = 10.5, 1.5 Hz, 1H, CH=CH**Hcis**), 4.62 (ap dt, *J* = 5.3, 1.5 Hz, 2H, C**H$_2$**-CH=CH_2); δ_C ($CDCl_3$, 100 MHz): 190.9 (CHO), 163.7 (C-4), 132.4 (**C**H=CH_2), 132.1 (C-2, C-6), 130.2 (C-1), 118.5 (CH=**C**H_2), 115.1 (C-3, C-5), 69.1 (**C**H_2-CH=CH_2)*;* m/z LC-MS (ES$^+$) 163 [M+H]$^+$.

2-{4’-[2’’-(4’’’-Benzoylphenoxy)ethoxy]phenyl}-5-hydroxy-7-methoxy-4H-chromen-4-one

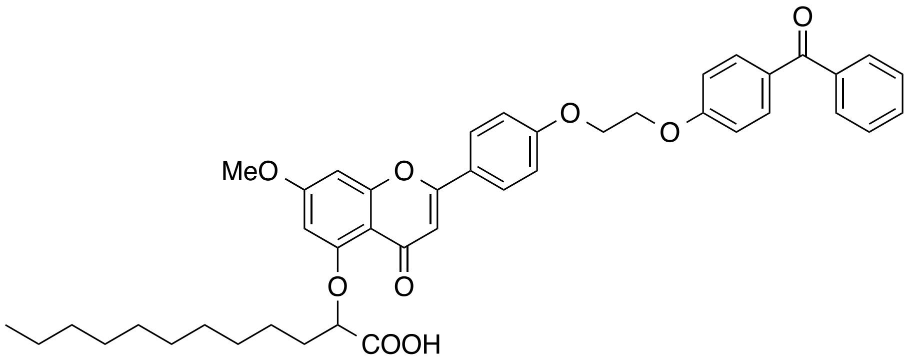
152



4-(2’’-Bromoethoxy)benzophenone **155** (343 mg, 1.125 mmol, 1.5 eq) was added to a suspension of 5-hydroxy-7-methoxy-2-(4’-hydroxyphenyl)-4H-chromen-4-one **149** (213 mg, 0.75 mmol, 1 eq) and K_2CO_3 (207 mg, 1.5 mmol, 2 eq) in dry DMF (2 ml) under an argon atmosphere and the reaction mixture was stirred at rt overnight. H_2O (10 ml) was added and the product was extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with brine (20 ml), dried over Na_2SO_4 and concentrated. The title product (236 mg, 62%) was afforded by flash column chromatography (24 g silica gel, DCM:Et_2O 9:1-1:1) as a light yellow solid.

m.p.: 181 – 184 °C; v_{max} (ATR): 1644 (C=O), 1599, 1250, 1158, 833 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 12.79 (s, 1H), 7.90 - 7.80 (m, 4H, 3’-H, 5’-H, 2’’’-H, 6’’’-H), 7.78 - 7.73 (m, 2H, 2’’’’-H, 6’’’’-H), 7.60 - 7.55 (m, 1H, 4’’’-H), 7.51 - 7.45 (m, 2H, 3’’’’-H, 5’’’’-H), 7.12 - 7.06 (m, 2H, 2’-H, 6’-H), 7.05 - 7.00 (m, 2H, 3’’’-H, 5’’’-H), 6.59 (s, 1H, 3-H), 6.49 (d, *J* = 2.3 Hz, 1H, 8-H), 6.37 (d, *J* = 2.3 Hz, 1H, 6-H), 4.49 - 4.40 (m, 4H, 1’’-H_2, 2’’-H_2), 3.88 (s, 3H, OCH_3); δ_C ($CDCl_3$, 176 MHz): 195.6 (C=O), 182.6 (C-4), 165.6 (C-7), 163.9 (C-2), 162.4 (C-5), 162.2 (C-1’’’), 161.6 (C-4’), 157.9 (C-8a), 138.3 (C-1’’’’), 132.7 (C-2’’’, C-6’’’), 132.2 (C-4’’’’), 130.9 (C-4’’’), 129.9 (C-2’’’’, C-6’’’’), 128.4 (C-3’’’’, C-5’’’’), 128.3 (C-3’, C-5’), 124.3 (C-1’), 115.3 (C-2’, C-6’), 114.3 (C-3’’’, C-5’’’), 105.7 (C-4a), 104.7 (C-3), 98.2 (C-6), 92.8 (C-8), 66.7 (CH_2), 66.6 (CH_2), 56.0 (OCH_3); HRMS (ES^+) found $[M+H]^+$ 509.1615, $C_{31}H_{25}O_7$ requires *M* 509.1600.

2-[(2’-{4’’-[2’’’-(4’’’’-Benzoylphenoxy)ethoxy]phenyl}-7’-methoxy-4’-oxo-4’H-chromen-5’-yl)oxy]dodecanoic acid **153**

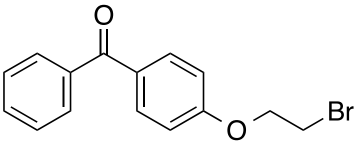


A solution of methyl 2-bromododecanoate **48** (136 mg, 0.46 mmol, 2 eq) in dry DMF (0.5 ml) was added to a mixture of 2-{4’-[2’’-(4’’’-benzoylphenoxy)ethoxy]phenyl}-5-hydroxy-7-methoxy-4H-chromen-4-one **152** (118 mg, 0.23 mmol, 1 eq) and K_2CO_3 (64 mg, 0.46 mmol, 2 eq) in dry DMF (1 ml) under an argon atmosphere. The reaction mixture was stirred at 60 °C for 17 h. H_2O (10 ml) was added and the product was extracted with EtOAc (3 × 10 ml). The combined organic layers were washed with brine (20 ml), dried over Na_2SO_4 and concentrated. The crude intermediate was dissolved in a 1:1 mixture of THF (5 ml) and H_2O (5 ml) and $LiOH.H_2O$ (19 mg, 0.46 mmol, 2 eq) was added. The reaction mixture was stirred for 3 h at rt. The mixture was acidified with 1M HCl to pH 4-5 and extracted with EtOAc (3 × 10 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. Flash column chromatography (4 g silica gel, DCM:MeOH 1:0-9:1) afforded the title product (150 mg, 92%) as a colourless semi-solid.

ν_{max} (ATR): 3025 (COO-H), 2919, 2851, 1739 (C=O), 1628 (C=O), 1598, 1244, 1163, 1111, 831 cm^{-1}; δ_H ($CDCl_3$, 600 MHz): 7.91 - 7.82 (m, 4H, 2’’’’-H, 6’’’’-H, 2’’-H, 6’’-H), 7.80 - 7.74 (m, 2H, 2’’’’’-H, 6’’’’’-H), 7.61 - 7.55 (m, 1H, 4’’’’’-H), 7.52 – 7.45 (m, 2H, 3’’’’’-H, 5’’’’’-H), 7.13 - 7.07 (m, 2H, 3’’-H, 5’’-H), 7.06 - 7.00 (m, 2H, 3’’’’-H, 5’’’’-H), 6.71 (d, *J* = 2.3 Hz, 1H, 8’-H), 6.69 (s, 1H, 3’-H), 6.47 (d, *J* = 2.3 Hz, 1H, 6’-H), 4.83 (t, *J* = 5.3 Hz, 1H, 2-H), 4.46 (s, 4H, 1’’’-H_2, 2’’’-H_2), 3.93 (s, 3H, OCH_3), 2.19 - 2.07 (m, 2H, 3-H), 1.66 - 1.46 (m, 1H, 4-H), 1.42 - 1.18 (m, 14H, 7 × CH_2), 0.87 (t, J = 7.0 Hz, 3H, 12-H_3); δ_C ($CDCl_3$, 151

MHz): 195.6 (C=O), 178.9 (C-4’), 172.6 (C-1), 164.7 (C-7’), 162.8 (C-2’), 162.2 (C-1’’’’), 161.7 (C-4’’), 159.5 (C-8a’), 159.0 (C-5’), 138.3 (C-1’’’’’), 132.7 (C-3’’’’, C-5’’’’), 132.2 (C-4’’’’’), 130.9 (C-4’’’’), 129.9 (C-2’’’’’, C-6’’’’’), 128.4 (C-3’’’’’, C-5’’’’’), 128.2 (C-2’’, C-6’’), 123.8 (C-1’’), 115.4 (C-3’’, C-5’’), 114.3 (C-2’’’’, C-6’’’’), 109.5 (C-4a’), 107.0 (C-3’), 101.4 (C-6’), 95.3 (C-8’), 82.1 (C-2), 66.8 (C-1’’’), 66.6 (C-2’’’), 56.2 (OCH_3), 33.1 (CH_2), 32.0 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.9 (C-4), 22.8 (CH_2), 14.3 (C-12); HRMS (ES^+) found $[M+H]^+$ 707.3232, $C_{43}H_{47}O_9$ requires *M* 707.3220.

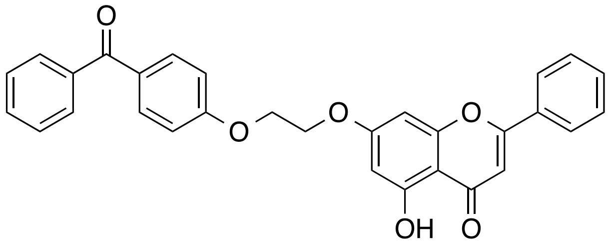
4-(2''-Bromoethoxy)benzophenone[190] **155**



1,2-Dibromoethane (5.18 ml, 60 mmol, 6 eq) was added to a suspension of 4-hydroxybenzophenone **154** (1.98 g, 10 mmol, 1 eq) and K_2CO_3 (2.76 g, 20 mmol, 2 eq) in dry DMF (30 ml) under an argon atmosphere and the reaction mixture was stirred at rt for 41 h. H_2O (50 ml) was added and the product was extracted with DCM (3 × 50 ml). The combined organic layers were washed with H_2O (100 ml) and brine (100 ml), dried over Na_2SO_4 and concentrated. The title product (1.10 g, 36%) was afforded by flash column chromatography (24 g silica gel, hexane:EtOAc 1:0-4:1) as a colourless solid.

m.p.: 72 – 74 °C (lit.[190] m.p.: 74 – 75 °C); ν_{max} (ATR): 1652 (C=O), 1600, 1253, 697 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 7.88 - 7.80 (m, 2H, 2-H, 6-H), 7.79 - 7.71 (m, 2H, 2'-H, 6'-H), 7.60 - 7.54 (m, 1H, 4'-H), 7.51 - 7.44 (m, 2H, 3'-H, 5'-H), 7.03 - 6.94 (m, 2H, 3-H, 5-H), 4.38 (t, J = 6.2 Hz, 2H, 1''-H_2), 3.68 (t, J = 6.2 Hz, 2H, 2''-H_2); δ_C ($CDCl_3$, 176 MHz): 195.6 (C=O), 161.8 (C-4), 138.3 (C-1'), 132.7 (C-2, C-6), 132.1 (C-4'), 131.0 (C-1), 129.9 (C-2', C-6'), 128.4 (C-3', C-5'), 114.3 (C-3, C-5), 68.0 (C-1''), 28.8 (C-2''); HRMS (ES^+) found $[M+H]^+$ 305.0202, $C_{15}H_{14}O_2{}^{79}Br$ requires M 305.0177.

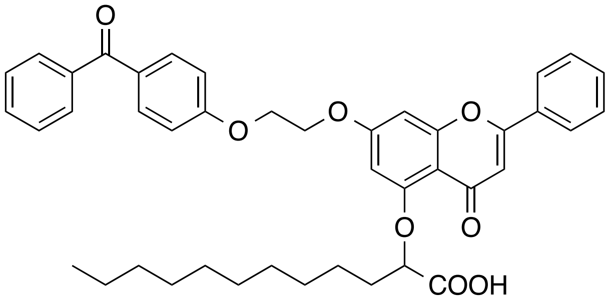
7-[2’-(4’’-Benzoylphenoxy)ethoxy]-5-hydroxy-2-phenyl-4H-chromen-4-one **156**



A flask was charged with 4-(2’’-bromoethoxy)benzophenone **155** (153 mg, 0.5 mmol, 1 eq), chrysin **45** (0.127 g, 0.5 mmol, 1 eq) and K_2CO_3 (0.138 g, 1 mmol, 2 eq) in dry DMF (2 ml) under an argon atmosphere and the reaction mixture was stirred at rt overnight and another 24 h at 40 °C. H_2O (10 ml) was added and the product was extracted with DCM (3 × 10 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The title product (185 mg, 77%) was afforded by flash column chromatography (12 g silica gel, DCM:MeOH 0:1-9:1) as a light yellow solid.

m.p.: 186 – 187 °C; v_{max} (ATR): 1657 (C=O), 1604, 1350, 1259, 1166 cm^{-1}; δ_H ($CDCl_3$, 600 MHz): 12.76 (s, 1H, OH), 8.00 - 7.79 (m, 4H, 2’’’’-H, 6’’’’-H, 3’’-H, 5’’-H), 7.80 - 7.71 (m, 2H, 2’’’-H, 6’’’-H), 7.68 - 7.40 (m, 6H, 4’’’-H, 4’’’’-H, 3’’’-H, 5’’’-H, 3’’’’-H, 5’’’’-H), 7.07 - 6.97 (m, 2H, 2’’-H, 6’’-H), 6.69 (s, 1H, 3-H), 6.57 (d, *J* = 2.3 Hz, 1H, 8-H), 6.44 (d, *J* = 2.3 Hz, 1H, 6-H), 4.45 (s, 4H, 1’-H_2, 2’-H_2); δ_C ($CDCl_3$, 151 MHz): 195.6 (C=O), 182.7 (C-4), 164.5 (C-7), 164.3 (C-2), 162.5 (C-5), 162.1 (C-1’’), 157.9 (C-8a), 138.3 (C-1’’’’), 132.7 (C-3’’, C-5’’), 132.1 (C-4’’’’), 132.1 (C-4’’’), 131.4 (C-1’’’’), 130.9 (C-4’’), 129.9 (C-2’’, C-6’’), 129.3 (C-3’’’, C-5’’’), 128.4 (C-3’’’’, C-5’’’’), 126.5 (C-2’’’’, C-6’’’’), 114.3 (C-2’’, C-6’’), 106.2 (C-4a), 106.1 (C-3), 98.7 (C-6), 93.6 (C-8), 67.0 (C-1’), 66.4 (C-2’); HRMS (ES$^+$) found [M+H]$^+$ 479.1499, $C_{30}H_{23}O_6$ requires *M* 479.1495.

2-({7’-[2’’-(4’’’-Benzoylphenoxy)ethoxy]-4’-oxo-2’-phenyl-4’H-chromen-5’-yl}oxy)dodecanoic acid **157**

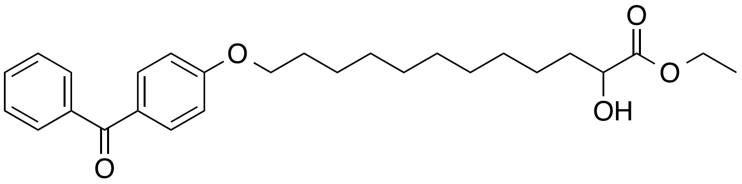


A solution of methyl 2-bromododecanoate **48** (123 mg, 0.42 mmol, 2 eq) in dry DMF (0.5 ml) was added to a mixture of 7-[2’-(4’’-benzoylphenoxy)ethoxy]-5-hydroxy-2-phenyl-4H-chromen-4-one (0.100 g, 0.21 mmol, 1 eq) and Cs_2CO_3 (0.136 g, 0.42 mmol, 2 eq) in dry DMF (2 ml) under an argon atmosphere. The reaction mixture was stirred at 60 °C for 19 h. H_2O (10 ml) was added and the product was extracted with DCM (3 × 10 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude intermediate was dissolved in a mixture of THF and H_2O (1:1, 5 ml) and $LiOH.H_2O$ (18 mg, 0.42 mmol, 2 eq) was added. The reaction mixture was stirred for 6 h at rt. The mixture was acidified with 1M HCl to pH 4-5 and extracted with DCM (3 × 10 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. Flash column chromatography (12 g silica gel, Et_2O:MeOH 1:0-9:1) afforded the title product (78 mg, 55%) as a colourless semi-solid.

$ν_{max}$ (ATR): 2922, 2851 1742 (C=O), 1630 (C=O), 1590, 1252, 1256, 1168 cm^{-1}; $δ_H$ ($CDCl_3$, 600 MHz): 13.60 (s, 1H, COOH), 8.06 - 7.80 (m, 4H, 2’’’’’-H, 6’’’’’-H, 3’’’-H, 5’’’-H), 7.80 - 7.71 (m, 2H, 2’’’’-H, 6’’’’-H), 7.64 - 7.40 (m, 6H, 4’’’’-H, 4’’’’’-H, 3’’’’-H, 5’’’’-H, 3’’’’’-H, 5’’’’’-H), 7.07 - 6.98 (m, 2H, 2’’’-H, 6’’’-H), 6.79 (d, *J* = 2.2 Hz, 1H, 8’-H), 6.77 (s, 1H, 3’-H), 6.54 (d, *J* = 2.2 Hz, 1H, 6’-H), 4.84 (t, *J* = 5.3 Hz, 1H, 2-H), 4.49 (s, 4H, 1’’-H_2, 2’’-H_2), 2.22 - 2.08 (m, 2H, 3-H_2), 1.65 - 1.48 (m, 2H, 4-H_2), 1.42 - 1.16 (m, 14H, 7 × CH_2), 0.86 (t, *J* =

7.0 Hz, 3H, 12-H_3); δ_C ($CDCl_3$, 151 MHz): 195.6 (C=O), 178.9 (C-4’), 172.4 (C-1), 163.7 (C-7’), 163.0 (C-2’), 161.9 (C-1’’’), 159.5 (C-8a’), 159.1 (C-5’), 138.2 (C-1’’’’’), 132.8 (C-3’’’, C-5’’’), 132.2 (C-4’’’’’), 132.2 (C-4’’’’), 131.1 (C-4’’’), 131.0 (C-1’’’’), 129.9 (C-2’’’’, C-6’’’’), 129.3 (C-3’’’’, C-5’’’’), 128.4 (C-3’’’’’, C-5’’’’’), 126.4 (C-2’’’’’, C-6’’’’’), 114.3 (C-2’’’, C-6’’’), 109.9 (C-4a’), 108.4 (C-3’), 101.7 (C-6’), 95.9 (C-8’), 82.0 (C-2’), 67.3 (C-1’’), 66.3 (C-2’’), 33.0 (C-3), 32.0 (CH_2), 29.7 (CH_2), 29.7(CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.8 (C-4), 22.8 (CH_2), 14.3 (C-12); HRMS (ES^+) found $[M+H]^+$ 677.3135, $C_{42}H_{45}O_8$ requires *M* 677.3114.

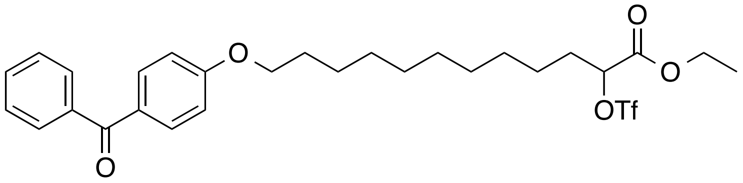
Ethyl 12-(4'-benzoylphenoxy)-2-hydroxydodecanoate **158**



A mixture of 4-hydroxybenzophenone **154** (178 mg, 0.9 mmol, 1.5 eq), ethyl 12-bromo-2-hydroxydodecanoate **82** (194 mg, 0.6 mmol, 1 eq) and K_2CO_3 (166 mg, 1.2 mmol, 2 eq) in dry DMF (5 ml) under a nitrogen atmosphere was stirred at 60 °C overnight. H_2O (5 ml) was added and the aqueous phase extracted with EtOAc (3 × 5 ml). The organic extracts were collected, washed with brine, dried over $MgSO_4$ and concentrated. The title product (171 mg, 65%) was afforded by reversed phase column chromatography (H_2O:MeCN 0:1 - 1:0, 0.1% TFA) as a colourless solid.

m.p.: 50 – 52 °C; ν_{max} (ATR): 3488 (OH), 2919, 2850, 1724 (C=O), 1639 (C=O), 1603, 1254, 692 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.87 - 7.78 (m, 2H, 3'-H, 5'-H), 7.79 - 7.71 (m, 2H, 2''-H, 6''-H), 7.60 - 7.52 (m, 1H, 4''-H), 7.52 - 7.40 (m, 2H, 3''-H, 5''-H), 7.02 - 6.87 (m, 2H, 2'-H, 6'-H), 4.24 (qd, *J* = 7.2, 1.1 Hz, 2H, O-C**H_2**-CH_3), 4.16 (dt, *J* = 7.2, 5.0 Hz, 1H, 2-H), 4.03 (t, *J* = 6.5 Hz, 2H, 12-H_2), 2.74 (d, *J* = 5.0 Hz, 1H, OH), 1.86 - 1.71 (m, 3H, 3-H, 11-H_2), 1.69 - 1.57 (m, 1H, 3-H), 1.55 - 1.14 (m, 17H, 7 × CH_2, O-CH_2-C**H_3**); δ_C ($CDCl_3$, 101 MHz): 195.7 (C=O), 175.6 (C-1), 163.0 (C-1'), 138.5 (C-1''), 132.7 (C-3', C-5'), 132.0 (C-4''), 130.0 (C-4'), 129.9 (C-2'', C-6''), 128.3 (C-3'', C-5''), 114.1 (C-2', C-6'), 70.6 (C-2), 68.4 (C-12), 61.8 (O-**C**H_2-CH_3), 34.6 (C-3), 29.6 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 26.1 (CH_2), 24.9 (CH_2), 14.4 (O-CH_2-**C**H_3); HRMS (ES^+) found $[M+H]^+$ 441.2653, $C_{27}H_{37}O_5$ requires *M* 441.2641.

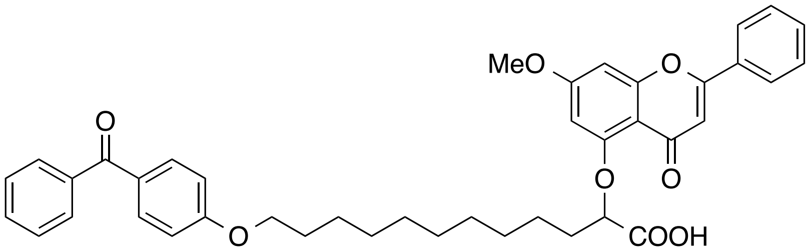
Ethyl 12-(4’-benzoylphenoxy)-2-(((trifluoromethyl)sulfonyl)oxy)dodecanoate **159**



Ethyl 12-(4’-benzoylphenoxy)-2-hydroxy-dodecanoate **158** (132 mg, 0.3 mmol, 1 eq) was dissolved in dry DCM (0.5 ml) at 0 °C under a nitrogen atmosphere. 2,6-Lutidine (0.04 ml, 0.36 mmol, 1.2 eq) and subsequently triflic anhydride (0.06 ml, 0.33 mmol, 1.1 eq) were added and the reaction was stirred at 0 °C for 1.5 h. H_2O was slowly added and the two layers separated. The organic layer was dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, iso-hexane:EtOAc 1:0 – 4:1) afforded the title product (125 mg, 73%) as a colourless oil.

ν_{max} (ATR): 2930, 2856, 1760 (C=O), 1653 (C=O), 1599, 1252, 1202, 1144, 921 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 7.88 - 7.79 (m, 2H, 3’-H, 5’-H), 7.78 - 7.70 (m, 2H, 2’’-H, 6’’-H), 7.60 - 7.53 (m, 1H, 4’’-H), 7.51 - 7.43 (m, 2H, 3’’-H, 5’’-H), 6.99 - 6.90 (m, 2H, 2’-H, 6’-H), 5.10 (t, *J* = 6.1 Hz, 1H, 2-H), 4.37 - 4.23 (m, 2H, O-C**H₂**-CH₃), 4.04 (t, *J* = 6.5 Hz, 2H, 12-H_2), 2.06 - 1.95 (m, 2H, 3-H_2), 1.87 - 1.76 (m, 2H, 11-H_2), 1.56 - 1.21 (m, 17H, 7 × CH_2, O-CH_2-C**H₃**); δ_C ($CDCl_3$, 101 MHz): 195.8 (C=O), 167.3 (C-1), 163.0 (C-1’), 138.5 (C-1’’), 132.8 (C-3’, C-5’), 132.0 (C-4’’), 130.0 (C-4’), 129.9 (C-2’’, C-6’’), 128.3 (C-3’’, C-5’’), 118.6 (q, *J* = 321.7 Hz, CF_3), 114.2 (C-2’, C-6’), 83.9 (C-2), 68.4 (C-12), 62.8 (O-**C**H_2-CH_3), 32.1 (C-3), 29.6 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 26.1 (CH_2), 24.6 (CH_2), 14.1 (O-CH_2-**C**H_3); δ_F ($CDCl_3$, 376 MHz): -74.89;

12-(4’-Benzoylphenoxy)-2-[(7’’’-methoxy-4’’’-oxo-2’’’-phenyl-4’’’H-chromen-5’’’-yl)oxy]dodecanoic acid **160**

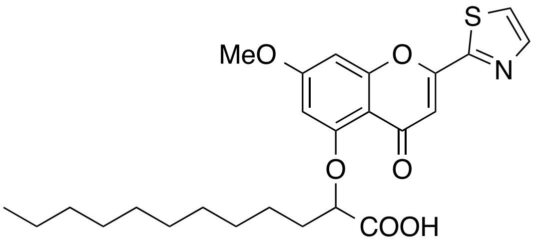


A mixture of 5-hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one **46** (101 mg, 0.375 mmol, 1.5 eq), ethyl 12-(4-benzoylphenoxy)-2-[hydroxy-dioxo-(trifluoromethyl)-sulfanyl]dodecanoate **160** (143 mg, 0.25 mnmol, 1 eq) and K_2CO_3 (104 mg, 0.75 mmol, 3 eq) in dry MeCN (1 ml) under a nitrogen atmosphere was stirred at rt overnight. H_2O (2 ml) was added to the reaction mixture. The aqueous phase was extracted with EtOAc (3 × 2 ml), the organic extracts collected, washed with brine, dried over $MgSO_4$ and concentrated. The crude product together with LiOH.H_2O (0.031 g, 0.75 mmol, 3 eq) was stirred in a 1:1 mixture of H_2O (3 ml) and THF (3 ml) at rt for 3h. The mixture was acidified to pH 2-3 with 1N HCl and the aqueous phase was extracted with DCM (3 × 5 ml). The organic extracts were collected, dried over $MgSO_4$ and concentrated. Flash column chromatography (12 g silica gel, EtO_2 then DCM:MeOH 19:1) gave the title product (50 mg, 30 %) as a colourless semi solid.

v_{max} (ATR): 2925, 2854, 1743 (C=O), 1633 (C=O), 1595, 1253, 1162, 909, 728 cm^{-1}; δ_H ($CDCl_3$, 400 MHz): 13.70 (br. s, 1H, COOH), 7.99 - 7.85 (m, 2H, 2’’’’-H, 6’’’’-H), 7.85 - 7.72 (m, 4H, 3’-H, 5’-H, 2’’-H, 6’’-H), 7.60 - 7.41 (m, 6H, 4’’-H, 4’’’’-H, 3’’-H, 5’’-H, 3’’’’-H, 5’’’’-H), 7.00 - 6.88 (m, 2H, 2’-H, 6’-H), 6.76 (s, 1H, 3’’’-H), 6.73 (d, *J* = 2.2 Hz, 1H, 8’’’-H), 6.47 (d, *J* = 2.2 Hz, 1H, 6’’’-H), 4.84 (t, *J* = 5.3 Hz, 1H, 2-H), 4.03 (t, *J* = 6.6 Hz, 2H, 12-H_2), 3.94 (s, 3H, OCH_3), 2.24 - 2.08 (m, 2H, 3-H_2), 1.87 - 1.75 (m, 2H, 11-H_2), 1.65 - 1.25 (m, 14H, 7 × CH_2); δ_C ($CDCl_3$, 101 MHz): 195.7 (C=O), 179.0 (C-4’’’), 172.5 (C-1), 164.9 (C-7’’’), 163.0

(C-1’), 163.0 (C-2’’’), 159.6 (C-8a’’’), 159.0 (C-5’’’), 138.5 (C-1’’), 132.7 (C-3’, C-5’), 132.1 (C-4’’’’), 132.0 (C-4’’), 131.1 (C-1’’’’), 130.0 (C-4’), 129.9 (C-2’’, C-6’’), 129.3 (C-3’’’’, C-5’’’’), 128.3 (C-3’’, C-5’’), 126.4 (C-2’’’’, C-6’’’’), 114.2 (C-2’, C-6’), 109.6 (C-4a’’’), 108.4 (C-3’’’), 101.5 (C-6’’’), 95.2 (C-8’’’), 82.0 (C-2), 68.4 (C-12), 56.2 (OCH_3), 33.0 (C-3), 29.6 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 26.1 (CH_2), 24.8 (CH_2); HRMS (ES^+) found $[M+H]^+$ 663.2961, $C_{41}H_{43}O_8$ requires *M* 663.2958.

2-{[7’-Methoxy-2’-(2’’-methylylphenyl)-4’-oxo-4’H-chromen-5’-yl]oxy}dodecanoic acid **168**



A solution of methyl 2-bromododecanoate **48** (107 mg, 0.36 mmol, 2 eq) in dry DMF (0.6 ml) was added to a mixture of 5-hydroxy-7-methoxy-2-(1’,3’-thiazol-2’-yl)-4H-chromen-4-one **114** (50 mg, 0.18 mmol, 1 eq) and K_2CO_3 (50 mg, 0.36 mmol, 2 eq) under an argon atmosphere. The reaction mixture was stirred at 60 °C for 18 h. H_2O (5 ml) was added and the product was extracted with DCM (3 × 5 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was dissolved in a 2:1 mixture of MeOH (2 ml) and H_2O (1 ml) and KOH (61 mg, 1.08 mmol, 2 eq) added. The reaction mixture was stirred for 3.5 h at rt. The mixture was acidified with 1M HCl to pH 3 - 4 and extracted with DCM (3 × 5 ml). The combined organic layers were dried over Na_2SO_4 and concentrated. Flash column chromatography (12 g silica gel, hexane:(EtOAc:EtOH 3:1) 4:1-0:1) afforded the title product (34 mg, 40 %) as a colourless semi-solid.

ν_{max} (ATR): 2924, 2854, 1749 (C=O), 1635 (C=O), 1600, 1409, 1347, 1165, 1104 cm^{-1}; δ_H ($CDCl_3$, 700 MHz): 8.06 (d, *J* = 3.1 Hz, 1H, 4’’-H), 7.67 (d, *J* = 3.1 Hz, 1H, 3’’-H), 7.14 (s, 1H 3’-H), 6.73 (d, *J* = 2.2 Hz, 1H, 8’-H), 6.48 (d, *J* = 2.2 Hz, 1H, 6’-H), 4.84 (t, *J* = 5.2 Hz, 1H, 2-H), 3.94 (s, 3H, OCH_3), 2.21 - 2.07 (m, 2H, 3-H_2), 1.62 – 1.47 (m, 2H, 4-H_2), 1.40 - 1.19 (m, 14H, 7 × CH_2), 0.87 (t, J=7.1 Hz, 3H, 12-H_3); δ_C ($CDCl_3$, 100 MHz): 178.4 (C-4'), 172.3 (C-1), 165.2 (C-7'), 159.1 (C-8a'), 158.9 (C-5'), 158.8 (C-1''), 156.3 (C-2'), 145.5 (C-4''), 123.4 (C-3''), 110.0 (C-4a'), 109.0 (C-3'), 101.5 (C-6'), 95.2 (C-8'), 81.8 (C-2), 56.3 (OCH_3), 32.9 (C-3), 32.0 (CH_2), 29.7 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 24.7 (CH_2), 22.8 (CH_2), 14.3 (C-12)*;* HRMS (ES^+) found $[M+H]^+$ 474.1937, $C_{25}H_{32}NO_6S$ requires *M* 474.1950.

5.2 Biological Testing

5.2.1 General Notes

Chemicals: All chemicals and molecular biology reagents were purchased from commercial suppliers and were used without further purification unless otherwise stated.

Buffers were made using ultrapure water (ddH_2O), purified by a Milli-Q® Integral Water Purification System for Ultrapure Water.

5.2.2 Preparation of buffers and other solutions

Luria-Bertani broth (LB)

Tryptone (5.0 g), yeast extract (2.5 g) and sodium chloride (5.0 g) were added to distilled water (500 ml) and the pH was adjusted to 7 with NaOH solution. The resulting solution was heated in an autoclave for 20 min.

HEPES buffer I (20 mM HEPES, 150 mM NaCl, 1 mM EDTA-NaOH, pH 7.6)

HEPES free acid (4.77 g) and sodium chloride (8.77 g) were dissolved in 800 ml ddH_2O and 4 ml of EDTA (0.25 M solution, pH 8) were added. The pH was adjusted to 7.6 with 10 N NaOH and the volume was brought up to 1 l with ddH_2O.

HEPES buffer II (20 mM HEPES-NaOH, 150 mM NaCl, pH 7.6)

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (4.77 g) and sodium chloride (8.77 g) were dissolved in 800 ml ddH_2O and the pH was adjusted to pH 7.6 with 10 N NaOH. Subsequently the volume was brought up to 1 l with ddH_2O.

Potassium phosphate buffer (0.1 M, pH 6.5)

K_2HPO_4 (33 ml of a 1 M solution in H_2O) and KH_2PO_4 (67 ml of 1 M solution in H_2O) were mixed and the pH was adjusted to 6.5. The combined stock solutions were diluted to 1 l with ddH_2O and the pH was checked again.

Phosphate-buffered saline (PBS buffer) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2HPO_4, pH 7.4)

Sodium chloride (8.01 g), potassium chloride (0.20 g), Na_2HPO_4 (1.41 g) and KH_2PO_4 (0.24 g) were dissolved in 800 ml ddH_2O and the pH was adjusted to pH 7.4. Subsequently the volume was brought up to 1 l with ddH_2O.

5.2.3 Protein expression and purification

Transforming E. coli cells

Competent cells (Rosetta™ 2(DE3)) were defrosted on ice, plasmid DNA, *Am*GSTF1 containing a Strep-tag, cloned into a pET Strep vector, (2 μl) was added and the cells were left on ice for about 30 min. The cells were heat shocked by putting them in a 42 °C water bath for 1 min, then they were transferred back on ice and cooled for 2 min. 1 ml of LB (prewarmed to 37 °C) was added and the cells were incubated for 1 h at 37 °C. Inoculated (50 µg/ml kanamycin, 35 µg/ml chloramphenicol) agar plates were streaked using a sterile inoculation loop over the flame of a Bunsen burner. The plates were incubated at 37 °C overnight.

Expression of *Am*GSTF1

Kanamycin (final concentration 50 μg/ml) and chloramphenicol (final concentration 35 μg/ml) were added to 10 ml of LB. A colony from a LB-agar plate was selected and added to the inoculated LB using a sterile pipette tip. The mixture was incubated at 37 °C overnight, shaking at 200 rpm. The culture was then added to inoculated (50 μg/ml kanamycin, 35 μg/ml chloramphenicol) LB (500 ml) and incubated again at 37 °C till OD_{600} reached 0.5 to 0.8.

In order to induce the expression of *Am*GSTF1, IPTG (50 µl of a 1 M solution in H_2O) was added to the culture and it was further incubated at 37 °C overnight at 150 rpm. The whole procedure was carried out over the flame of a Bunsen burner. The cells were obtained by centrifugation (5000 rpm, 15 min) and the media was decanted off. The cell pellets were stored at -20 °C.

Purification of *Am*GSTF1

The frozen cells were thawed and suspended in HEPES buffer I. A 5 ml aliquot of the re-suspended E. coli cells in a 15 ml falcon tube was taken and sonicated at 0 °C (3 × 30 s). Dithiothreitol (DTT) (10 μl of a 1 M solution in H_2O) and avidin (10 μl of a 1 mg/ml solution in 10% glycerol) were added to the lysate and the mixture was centrifuged (4000 rpm, 15 min). Protein purification was carried out on an ÄKTA-FPLC system (GE Healthcare Life Sciences). The supernatant was loaded onto a 1 mL Strep-tactin Superflow High Capacity column which was pre-equilibrated with HEPES buffer I at a flow rate of 1 ml/min. All unbound protein was removed from the column. Addition of desthiobiotin (DTB) (5 mL of a 2.5 mM solution in HEPES buffer I) eluted *Am*GSTF1 and single fractions (1 ml) were collected which were stored at 4 °C. The column was regenerated with 2-(4-hydroxyphenylazo)benzoic acid (HABA) (5 ml of a 1 mM solution in HEPES buffer I). The concentration of the protein fractions was determined by absorbance measurements at 280 nm (NanoDrop 2000 spectrophotometer, Thermo Scientific). Purity was checked by SDS-PAGE. In some cases, *Am*GSTF1 was further purified using a GSTrap™ 4B column (GE Healthcare). *Am*GSTF1 was buffer exchanged into either HEPES buffer II or PBS buffer for storage at -80 °C. The dialysis procedure was performed with an approx. 100-fold dilution in a GeBaFlex-tube (molecular weight cut-off: 6000 - 8000) at 4 °C overnight.

SDS-PAGE: SDS-PAGE was performed according to the method described by Laemmli.[191] The gels were composed of 12% resolving gel and 4% stacking gel (Mini-PROTEAN® TGX™ Precast Gel) and ran at constant voltage of 200 V for 30 min in a Mini-PROTEAN Tetra cell. After electrophoretic separation, the gel was stained with InstantBlue™. A prestained SDS-PAGE Standard (Bio-Rad, low range) was used for molecular weight estimation.

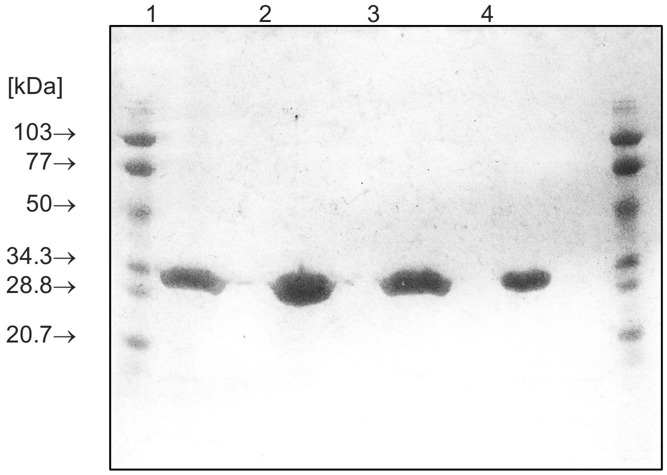


Figure 90: SDS PAGE gel (separating gels: 12% T) of *Am*GSTF1, obtained from the final purification step. Four different fractions were analysed (1-4) and the gel was stained with Coomassie Blue.

5.2.4 CDNB assay

Potassium phosphate buffer (890 μl, 0.1 M, pH 6.5) was warmed to 30 °C in a 1 ml cuvette and *Am*GSTF1 (25 μl of a 1 mg/ml solution in HEPES buffer II (pH 7.6)) and CDNB (25 μl of a 40 nM solution in EtOH) added. Inhibitor (10 μl of a 10 mM, 1 mM or 100 μM solution in DMSO, leading to a final concentration of 100 µM, 10 µM or 1 µM respectively) was added and the cuvette transferred to a Beckman DU 530 UV-Vis spectrophotometer. Following the addition of GSH (50 µl of a 100 mM solution in H_2O), the increase in UV absorbance was measured at 340 nm over 30 s and converted to a reaction rate. Using DMSO instead of the inhibitor solution and HEPES buffer II instead of the enzyme solution determined the chemical reaction rate. The specific enzyme activity was measured in the absence of inhibitor. All measurements were carried out in triplicate and the chemical reaction rate was deducted from the other measurements in order to remove the background chemical reaction. Values were reported as % inhibition of the specific enzyme activity.

5.2.5 Thermal Shift Assay

4 µl 5000X SYPRO Orange in DMSO were added to 1 ml of a 2 mg/ml solution of *Am*GSTF1 in HEPES buffer I. 10 µl of the resulting solution were pipetted into each well of a standard 96 well PCR plate (Starlab, semi-skirted PCR plates (FAST)). 10 µl of inhibitor solution (2 or 20 µM, ddH_2O + 2% DMSO) were added into the 96 well plates for a final inhibitor concentration of 1 or 10 µM. The plate was sealed and spun at 1000 rpm for 2 min. Fluorescence data was collected on an Applied Biosystems 7500 FAST Real-Time PCR System with excitation at 485 nm and the emission at 510 nm was used for data analysis. Temperature was held for 1 minute per degree from 24 to 95 degrees. The data was analysed using the data analysis program NAMI.[162]

5.2.6 Photoaffinity labelling

UV-absorbance measurements

UV-absorbance of the photoaffinity labels (solution in H_2O + 2% DMSO) was measured with an ATI UNICAM UV2 UV/Vis spectrometer at room temperature.

Labelling experiments

*Am*GSTF1 (100 µl, 1 mg/ml in PBS buffer) was incubated with a solution of compound **145** or **160** (100 µl, 200 µM in PBS buffer + 2% DMSO) for 10 min on ice. Irradiation was performed with a UV-LED (365 nm, 100 mW) for 30 min or a laser (355 nm, Q-switched Nd: YAG laser (Spectra Physics GER-150-10), 10 ns pulse, 10 Hz, 50 mJ per pulse) for 10 or 30 min at rt. Control experiments with no label were performed to rule out protein damage from the irradiation.

ESI-MS Analysis: Samples were desalted prior to mass spectrometry with a Waters MassPREP on-line desalting cartridge (2.1 x 10 mm) used with an Acquity UPLC (Waters Ltd, UK) (mobile phase gradient of water containing formic acid (0.1%) (A):acetonitrile (B): 2 min 5% B, then up to 80% B after 4 min, constant 80% B till 5 min, further up to 95% B at 5.1 min, back down to 5% B after 6 min and constant 5% B till 9 min; constant flow rate of 0.4 ml/min). ESI-MS measurements were then carried out on a QToF Premier mass spectrometer (Waters Ltd, UK) with an electrospray ion source. Protein data were processed using Masslynx 4.1 and deconvoluted using MaxEnt 1 to show the nominal neutral mass of the protein.

5.2.7 Native protein gel

*Am*GSTF1 (0.5 mg/ml) was incubated on ice for 30 min with two different concentrations of inhibitor **3** (10 and 500 µM final concentration). Native PAGE was carried out in a non-denaturing 4 – 20% polyacrylamide gel (Mini-PROTEAN® TGX™ Precast Gel). The gel was run at 4 °C in a Tris-glycine buffer (100 ml of Bio-Rad 10 × Tris/glycine diluted to 1 l, 25 mM Tris, 192 mM glycine) at 200 V in a Mini-PROTEAN Tetra cell. Samples were diluted with an equal volume of native sample buffer (Bio-Rad premixed protein sample buffer for native PAGE, containing 62.5 mM Tris-HCl, pH 6.8, 40% glycerol, 0.01%

bromophenol blue), before loaded onto the gel. After electrophoretic separation, the gel was stained with Coomassie Blue. Invitrogen™ Novex™ NativeMARK™ unstained protein standard was used to check if a good separation was achieved.

5.2.8 Fluorescence anisotropy

Labeling of *Am*GSTF1 with HiLyte Fluor™ 488

*Am*GSTF1 (1 mg/ml) was reduced with a 10-fold molar excess of tris(2-carboxyethyl) phosphine (TCEP) and then incubated at rt for 2 h with 400 µM HiLyte Fluor™ 488 C2 maleimide (AnaSpec) in the presence of PBS buffer. The solution was passed through two gel filtration chromatography columns (disposable PD 10 desalting column, GE Healthcare). The fractions containing the labelled protein were analysed for their protein content by absorbance at 280 nm (NanoDrop 2000 spectrophotometer, Thermo Scientific). The contribution of HiLyte Fluor™ 488 to the absorption was estimated by the absorption value at 507 nm multiplied by 0.2 and subtracted from the total absorbance before calculation of protein concentration using an extinction coefficient of 42525 M^{-1} cm^{-1} for the GST monomer.

Fluorescence anisotropy measurements

100 µl of the HiLyte Fluor™ 488 labelled *Am*GSTF1 solution (40 nm or 200 nm) in PBS-buffer were pipetted into each well of a standard 96 well plate. 100 µl solution of inhibitor **3** (2, 20, 200 or 400 µM, PBS-buffer + 4% DMSO) were added into the 96 well plates for a final inhibitor concentration of 1, 10, 100 and 200 µM. Fluorescence anisotropy experiments were performed on a BioTek Synergy™ H4 Hybrid Microplate Reader. The HiLyte Fluor™ 488 fluorescence anisotropy was measured with λ_{ex} at 485 nm ± 20 nm and λ_{em} at 528 nm ± 20 nm. Each anisotropy value is the average of eight replicates.

5.2.9 Synergist tests on plants

Maize

For all experiments maize of the Garland variety was used, provided by Syngenta. The seeds were soaked in water overnight and then individually sown at the surface of peat pellets with the tip cap showing up. For faster germination the pellets were placed on a tray and covered with cling film for the first two days. The maize was grown in a growth room at 25 °C during day (16 h) and 22 °C during night (8 h).

For the spray trials on maize a modified Potter Precision spray tower, a chemical spraying apparatus, was used. In order to accommodate a plant, the spray tower was increased in height by 10 cm (*cf.* Chapter 3.2.2.3.1). Spray trials were conducted using optimised concentrations of active ingredients, as follows:

Entry	Compound	Compound Number	Role	Concentrations
1	Mesotrione	157	Herbicide	30 μM, 250 μM
2	Nicosulfuron	158	Herbicide	30 μM, 250 μM
3	Chlortoluron	159	Herbicide	30 μM, 60 μM
4	Pinoxaden	155	Herbicide	7.5 μM, 15 μM
5	Clodinafop-propargyl	160	Herbicide	7.5 μM, 15 μM
6	Flavonoid	3	Synergist	2 mM
7	CNBF	1	Synergist	1 mM, 2 mM

Table 16: All compounds formulated using DMSO (2%) and Agridex (0.5%).

20 ml of the synergist or formulation only emulsions were sprayed onto the plant 7 days after sowing. 24 h later the same amount of herbicide or formulation only solution was applied. The modified Potter spray tower was used at 15 psi. The plants were assessed 7 days after the treatment.

Black grass

Seeds for the MHR bitotype of black grass (Peldon) and the respective herbicide-susceptible WT black grass were provided by Syngenta. Black grass seeds were sown densely in a tray on top of compost (John Innes No. 2), which was pressed down prior to sowing and approximately 0.5 – 1 cm of vermiculite were sprinkled on top of the seeds. Plants were grown in a growth chamber (Environmental test chamber MLR-350H, Sanyo Ltd.) with a 16 h light/8 h dark cycle at 20 °C during days and 16 °C during nights. After 5 days (WT black grass) or 7 days (MHR black grass) respectively the plants germinated and 5 days later seedlings were transplanted into individual pots (6 cm diameter).

The optimised concentrations of active ingredients used for synergism experiments are summarized in Table 17.

Entry	Compound	Role	Concentrations
1	Pinoxaden	Herbicide	5 μM, 15 μM
2	Flavonoids	Synergist	2 mM[a]

Table 17: All compounds formulated using acetone (5%) and Adigor (0.5%).
a Additionally a synergist concentration of 0.2, 0.6 and 6 mM was tested for thiazole 102.

Five 2 μl drops of the synergist or formulation only emulsions were applied with a micropipette 14 days after germination at the two leave stage. As some of the synergists are not completely soluble under these conditions, they were applied as suspensions and sonicated prior to use. The drops were distributed evenly on each plant, two drops on each leave and one at the node. 24 h later 10 μl of herbicide or formulation only solution was applied in the same way. The plants were assessed 21 days after the treatment.

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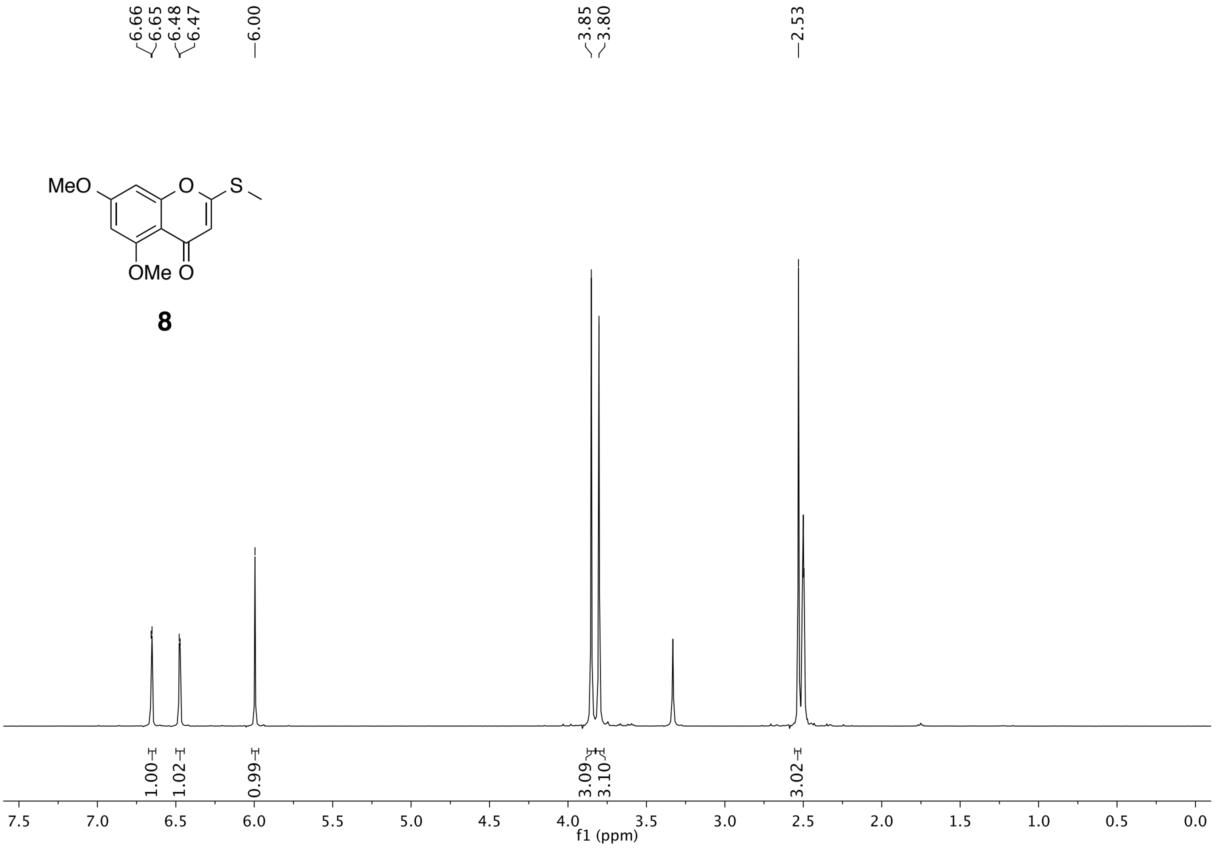
Appendix

NMR spectra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6.48
6.47
6.00
3.85
3.80
2.53
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O
S
OMe
O
8
1.00
1.02
0.99
3.09
3.10
3.02
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
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1.5
1.0
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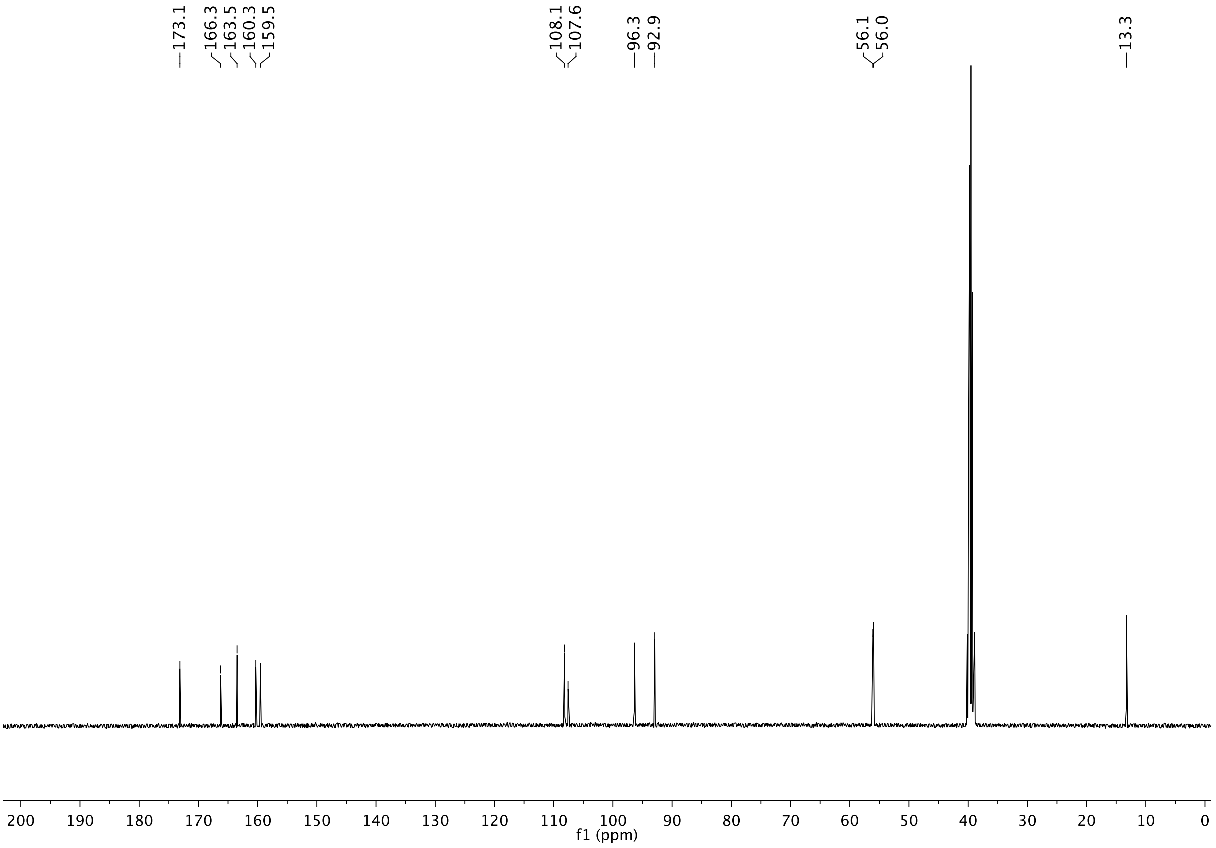
0.0
f1 (ppm)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163.5
160.3
159.5
108.1
107.6
96.3
92.9
56.1
56.0
13.3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


6.34
6.33
6.32
6.31
5.33
3.91
3.84
3.46
3.45
3.44
2.51
2.50
2.48
2.34
MeO
O
N
N
OMe
O
9
0.96
1.02
1.00
3.03
3.13
4.05
4.09
3.09
7.5
7.0
6.5
6.0
5.5
5.0
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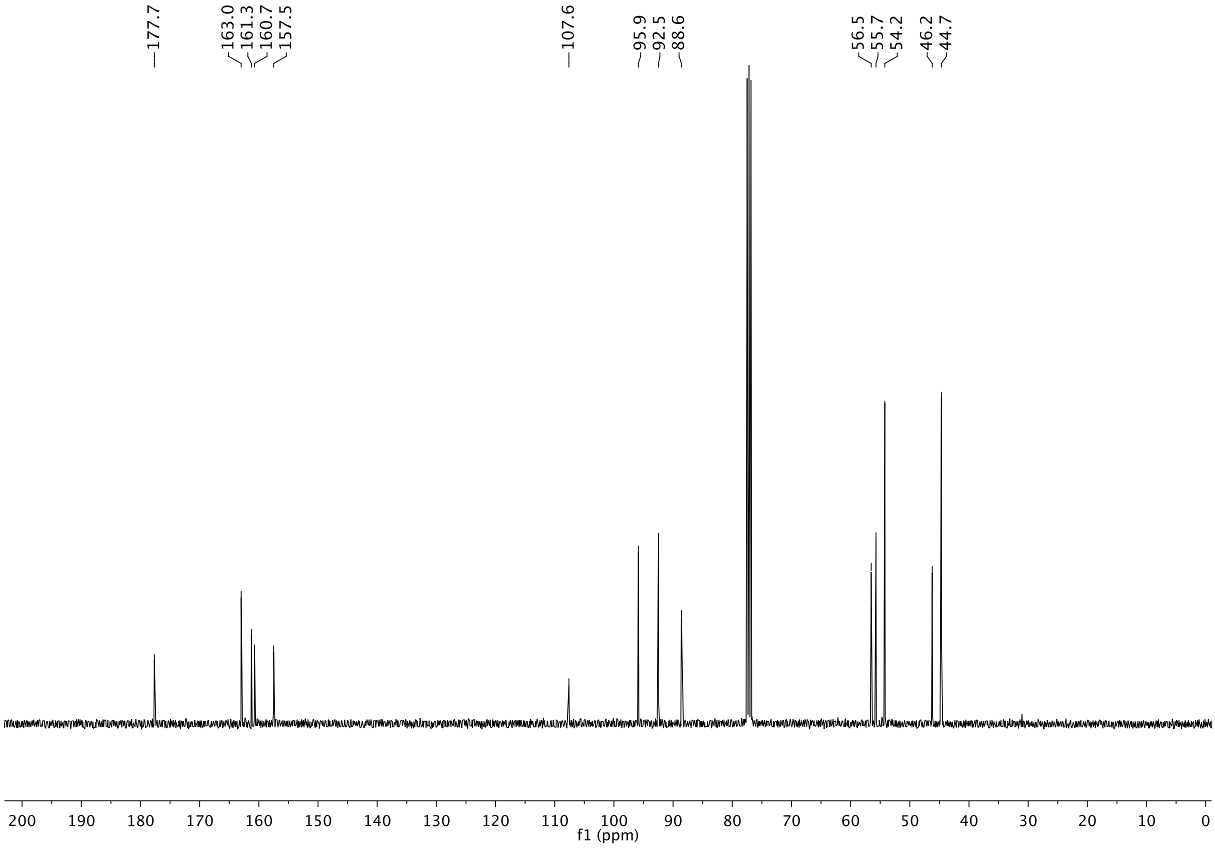
f1 (ppm)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107.6
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92.5
88.6
56.5
55.7
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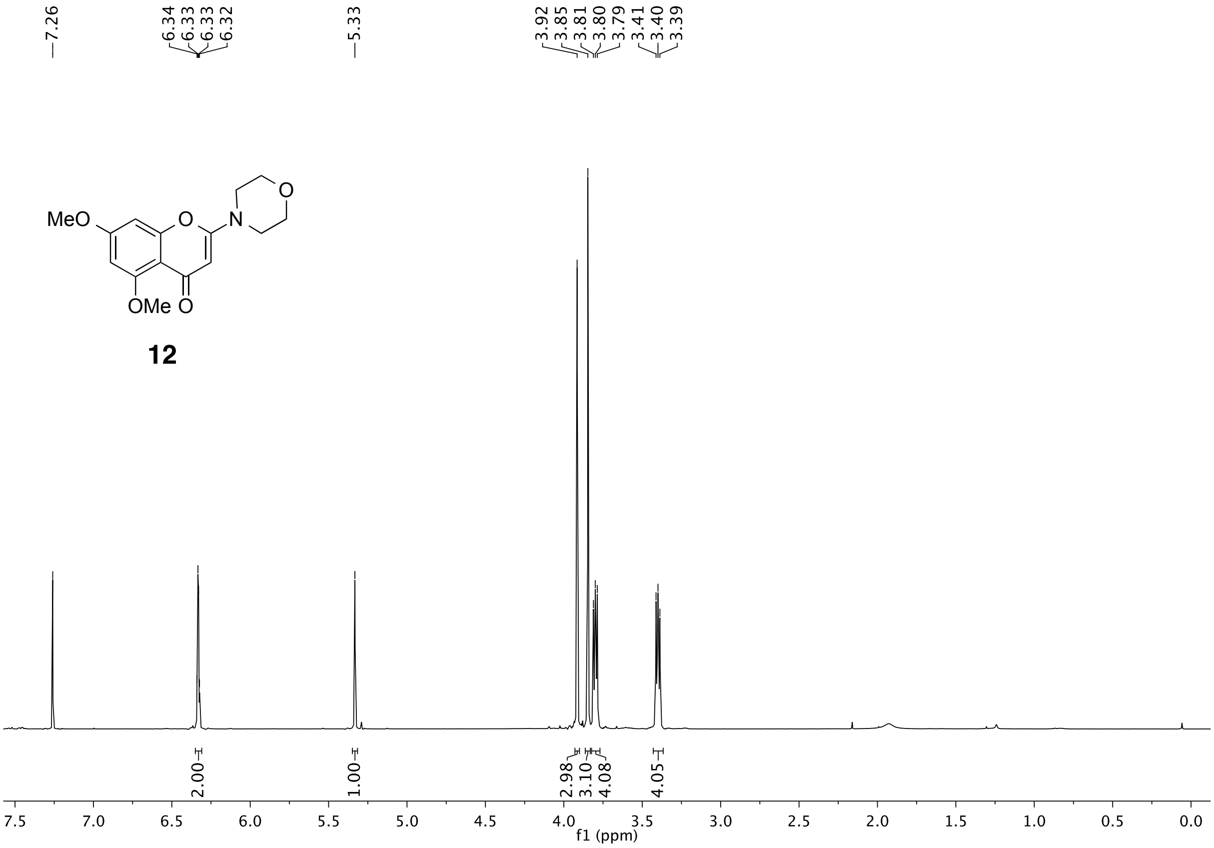
44.7
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



12

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6.33
6.33
6.32
5.33
3.92
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3.81
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3.41
3.40
3.39
MeO
O
N
O
OMe
O
2.00
1.00
2.98
3.10
4.08
4.05
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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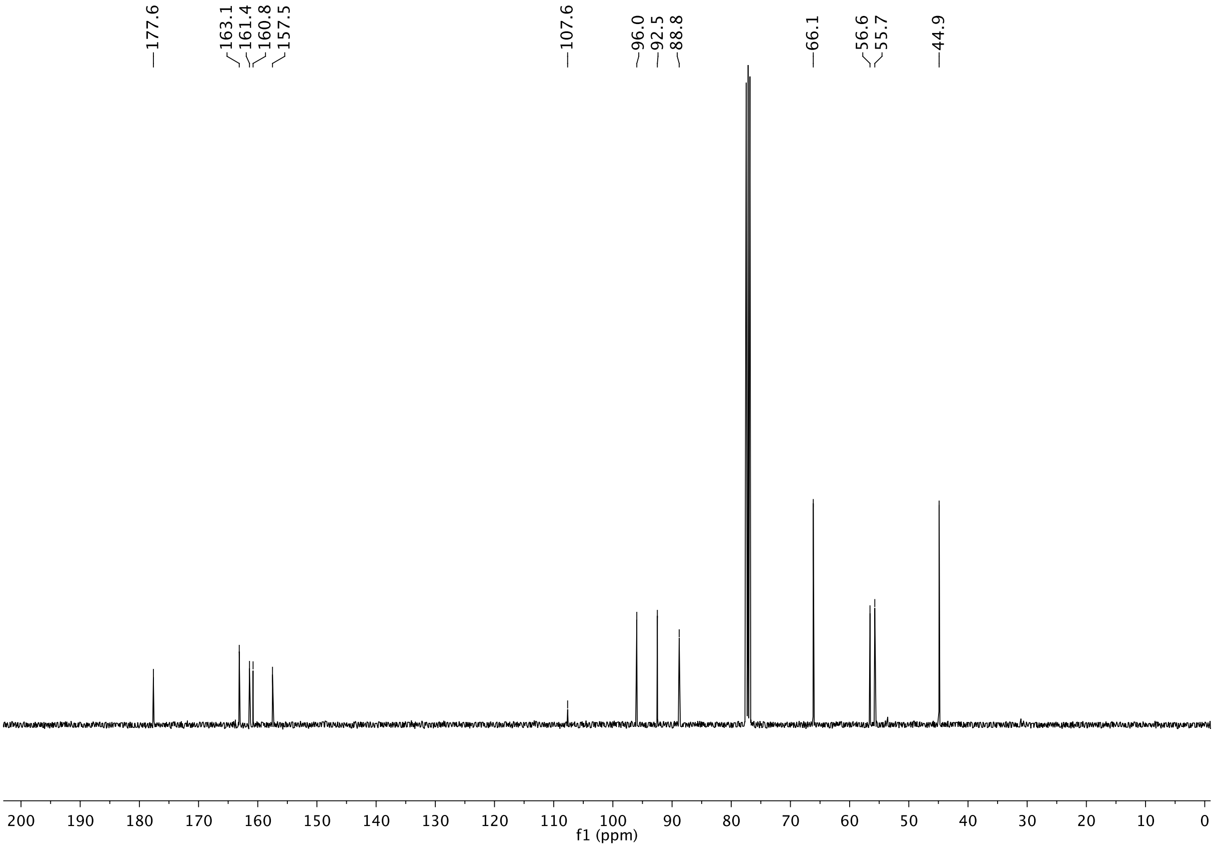
f1 (ppm)



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107.6
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66.1
56.6
55.7
44.9
200
190
180
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140
130
120
110
100
90
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70
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50
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10
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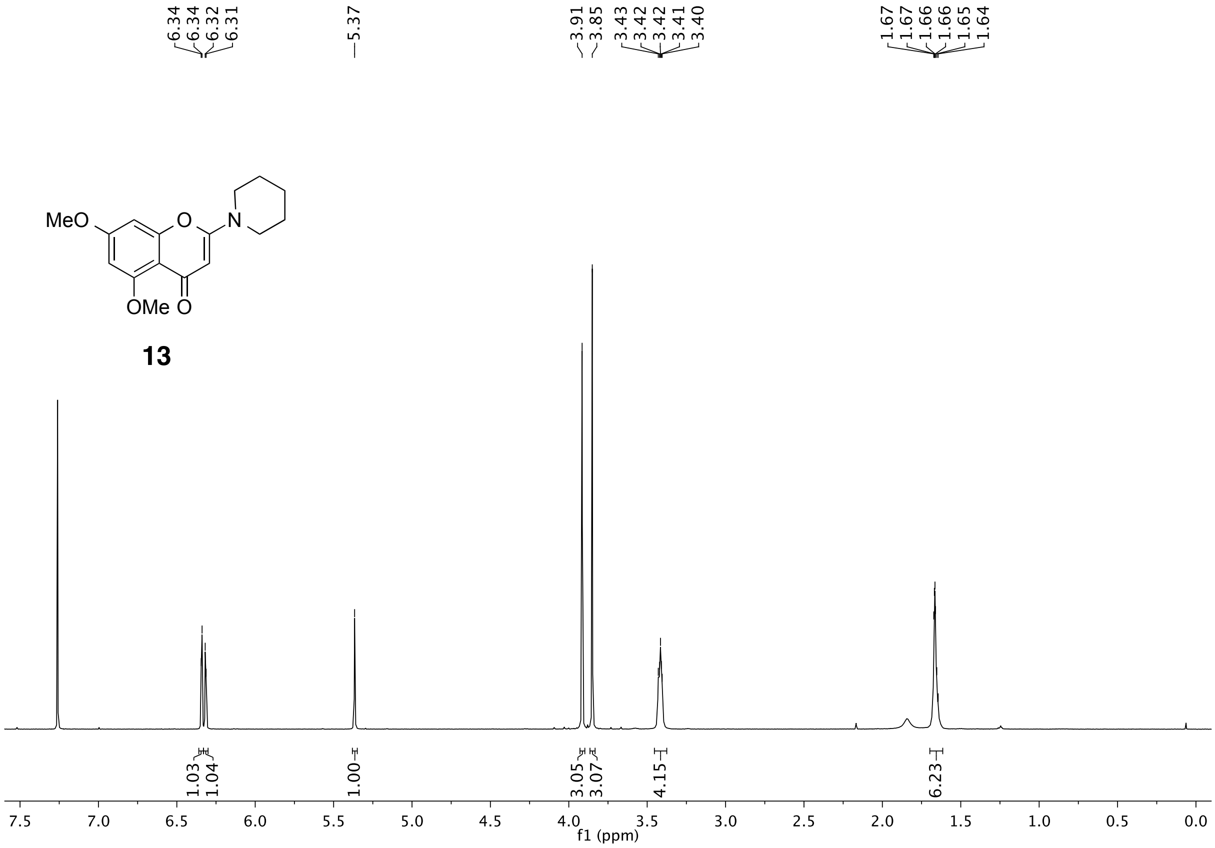
f1 (ppm)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1.67
1.67
1.66
1.66
1.65
1.64
MeO
O
N
OMe
O
13
1.03
1.04
1.00
3.05
3.07
4.15
6.23
7.5
7.0
6.5
6.0
5.5
5.0
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3.5
3.0
2.5
2.0
1.5
1.0
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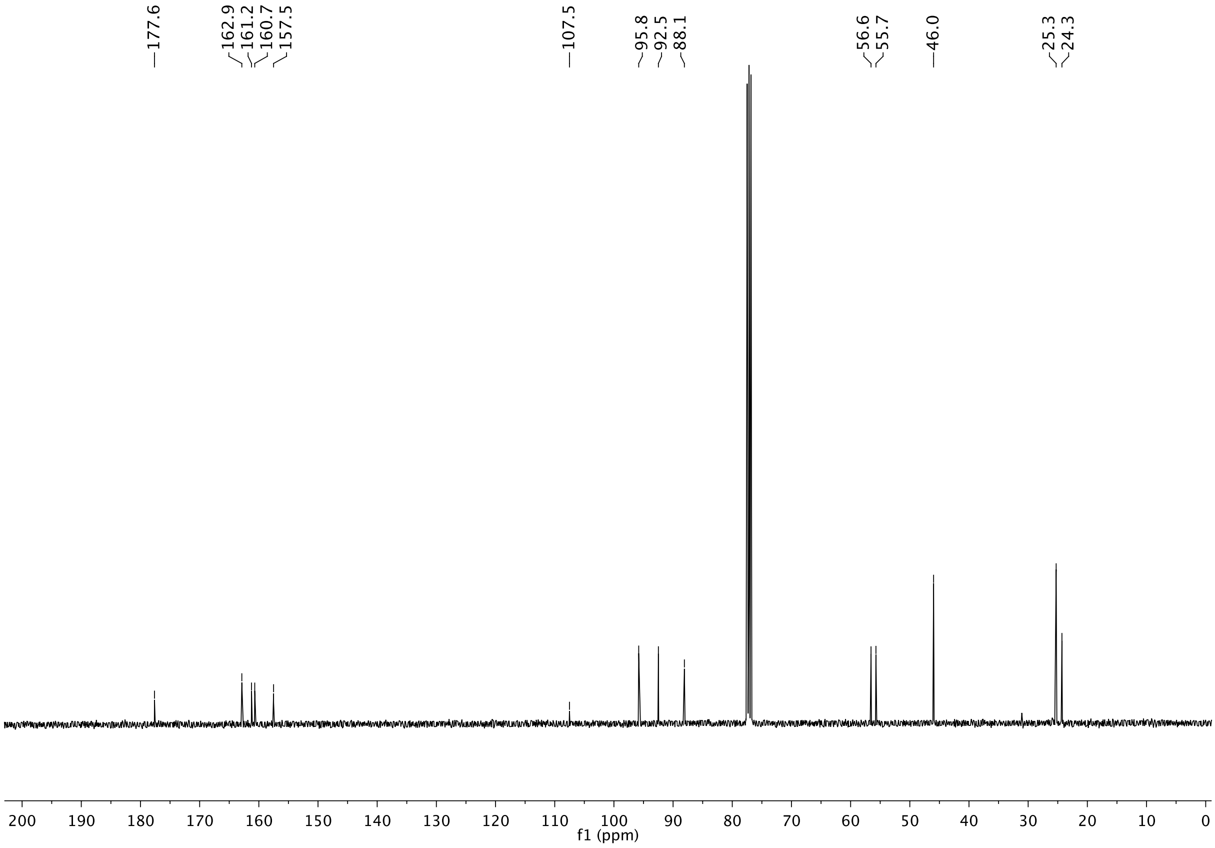
0.0
f1 (ppm)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107.5
95.8
92.5
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24.3
200
190
180
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90
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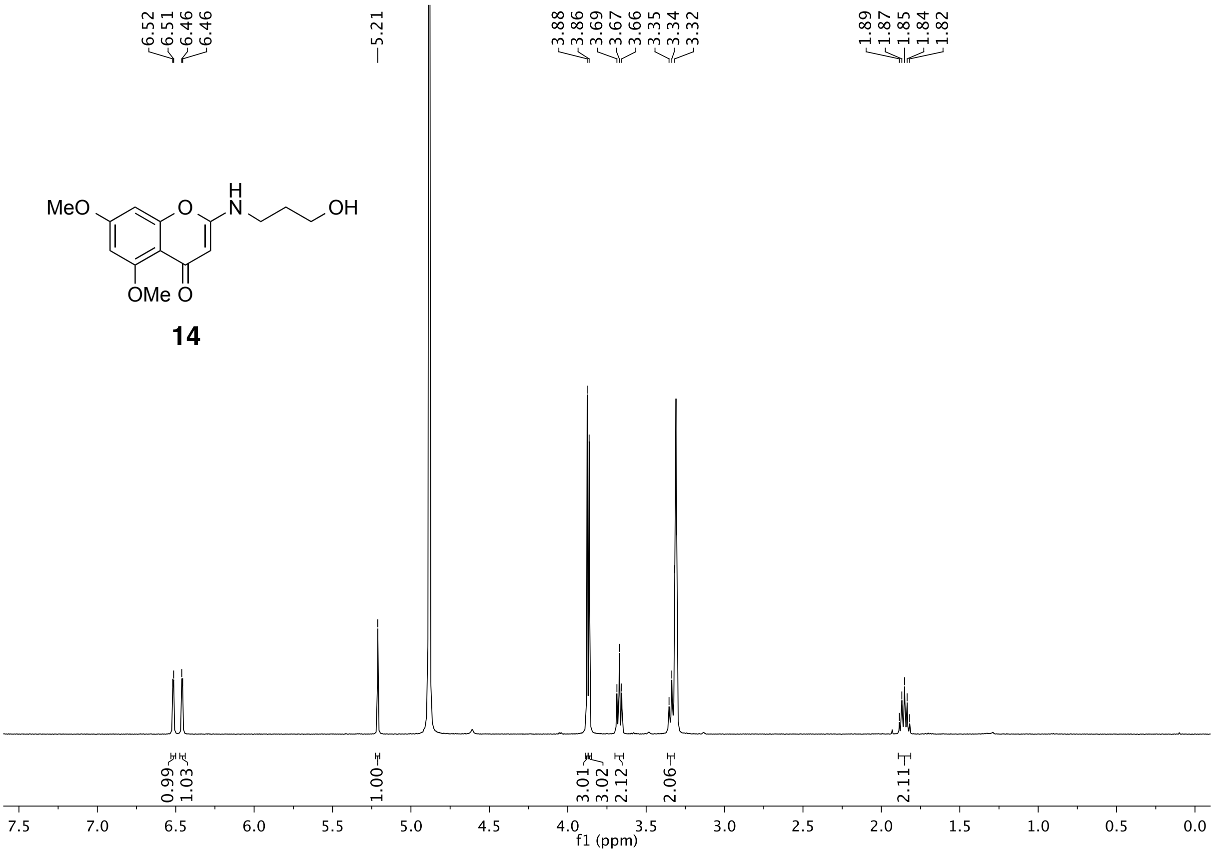
50
40
30
20
10
0
f1 (ppm)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OH
OMe
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1.03
1.00
3.01
3.02
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2.06
2.11
7.5
7.0
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5.5
5.0
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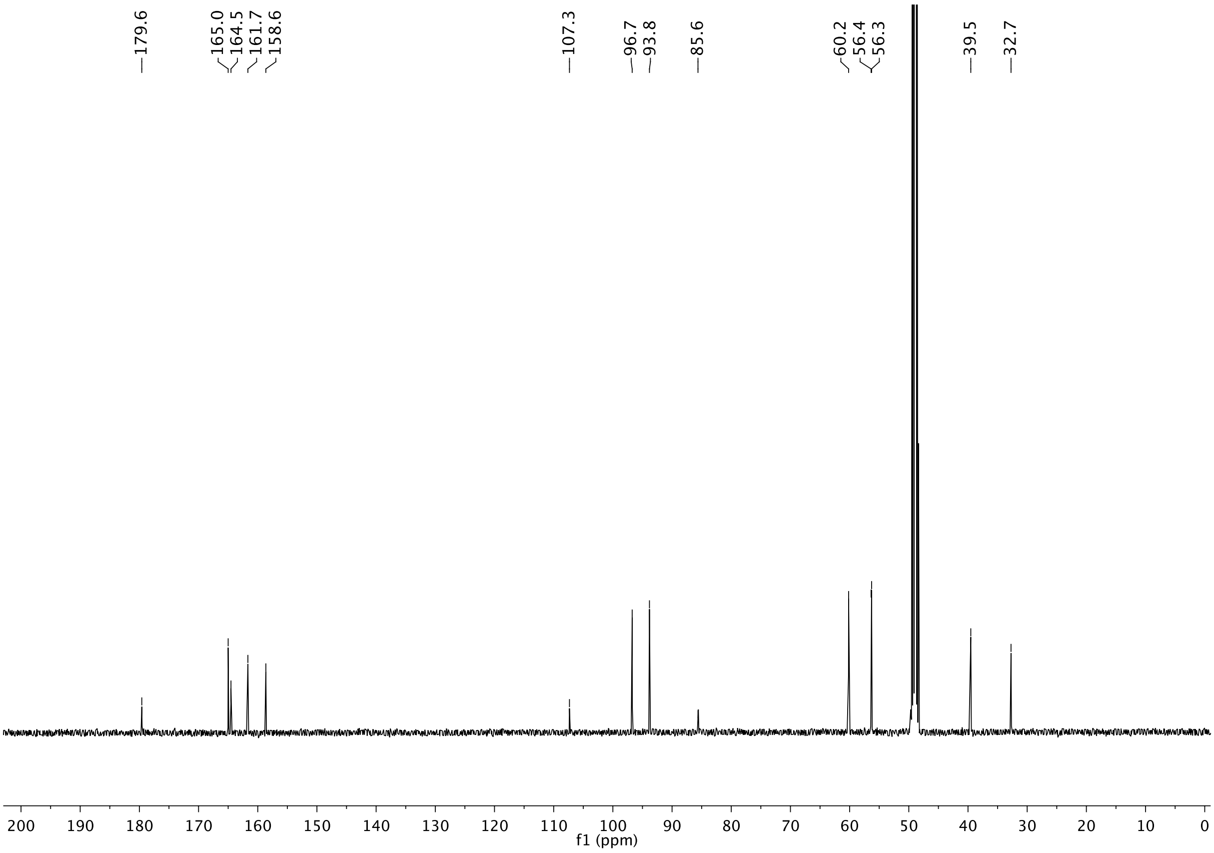
f1 (ppm)

14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56.4
56.3
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32.7
200
190
180
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160
150
140
130
120
110
100
90
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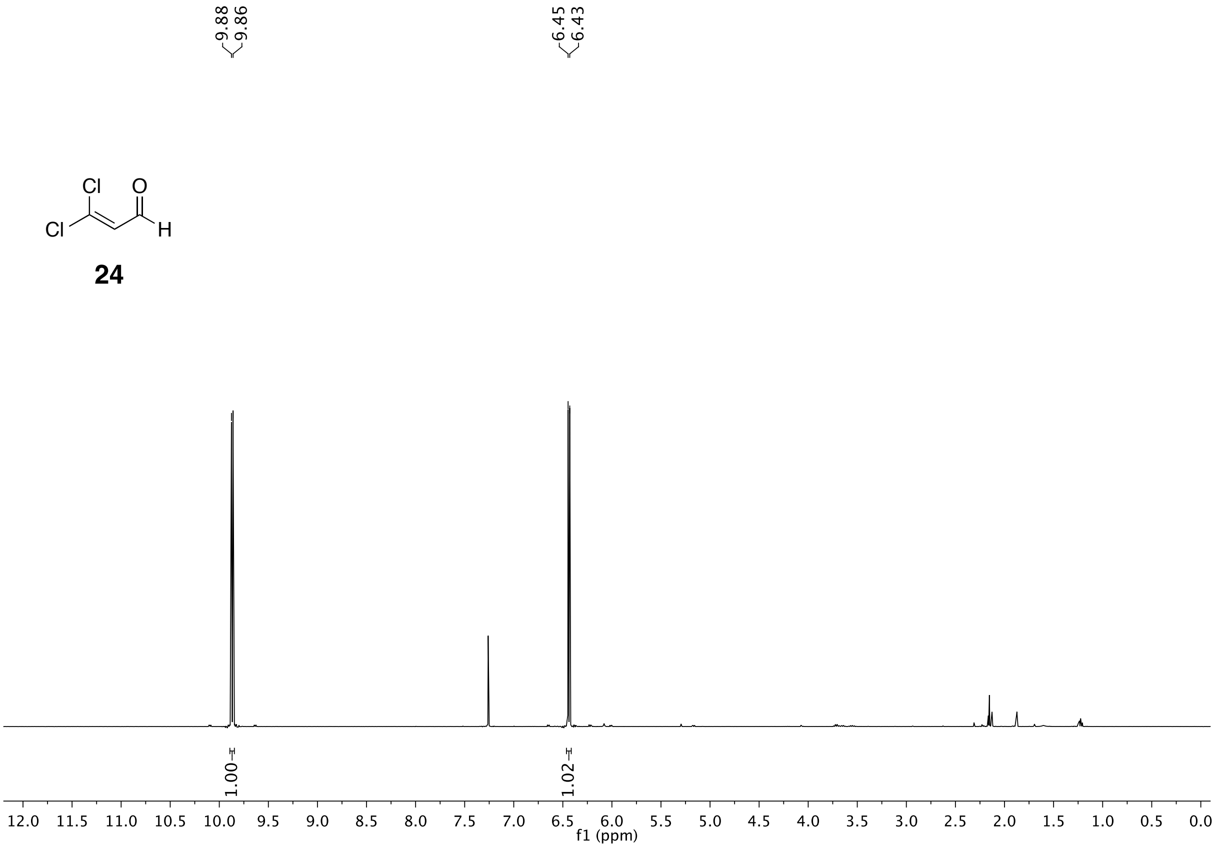
70
60
50
40
30
20
10
0
f1 (ppm)



24

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6.43
Cl
Cl
O
H
1.00
1.02
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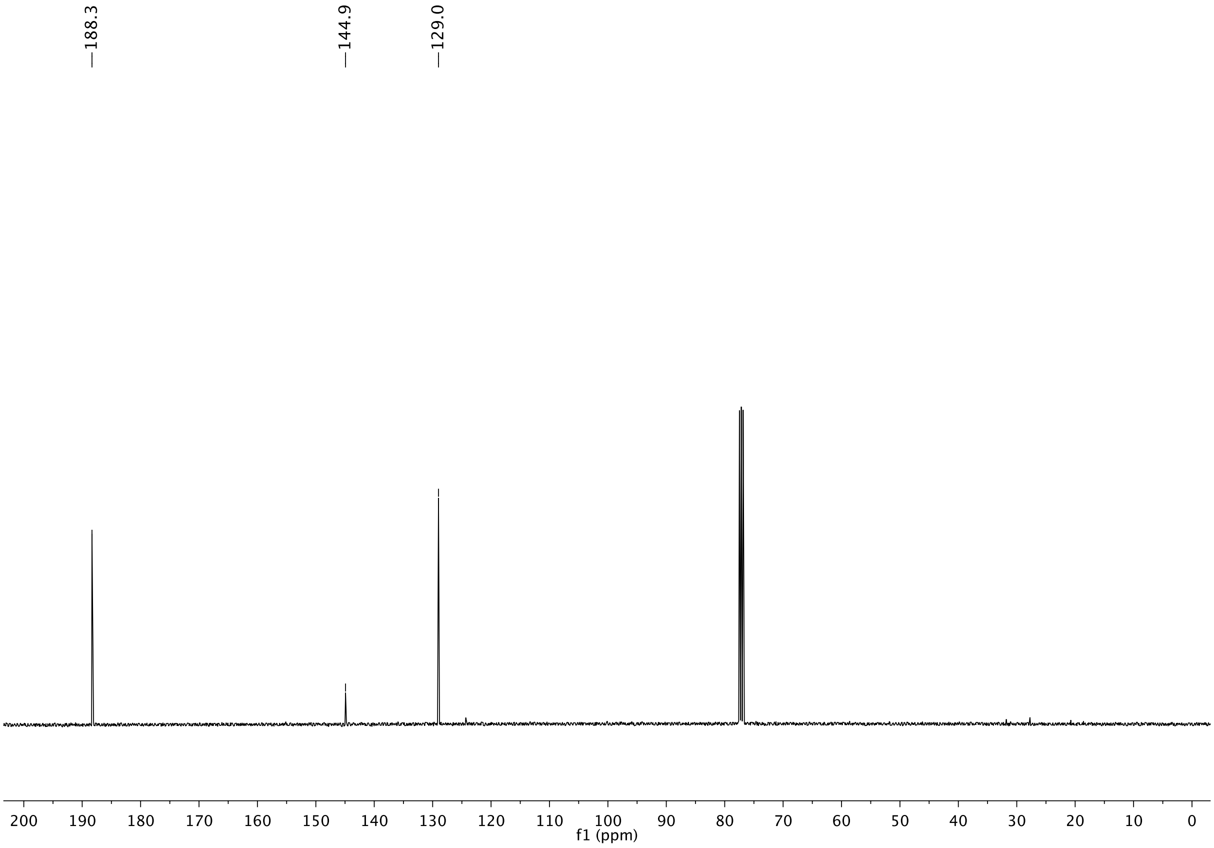
f1 (ppm)



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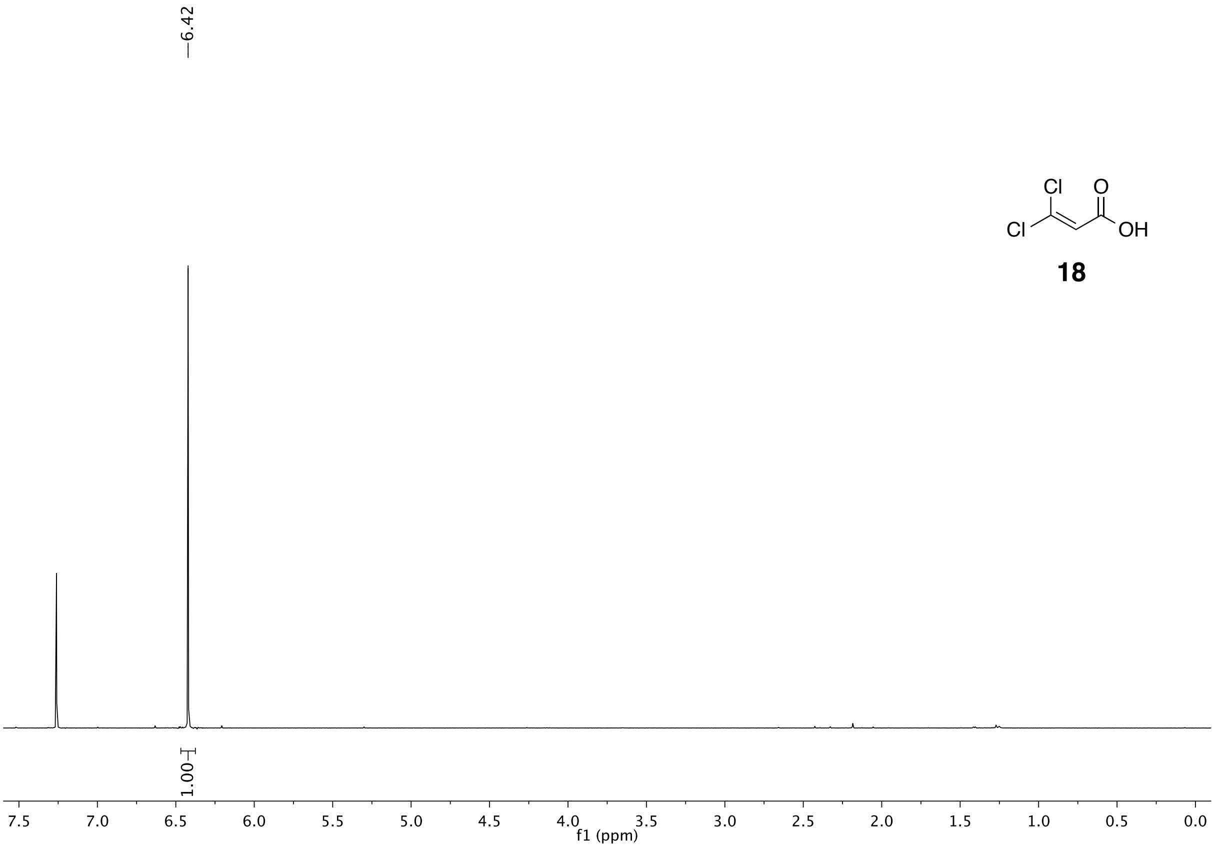
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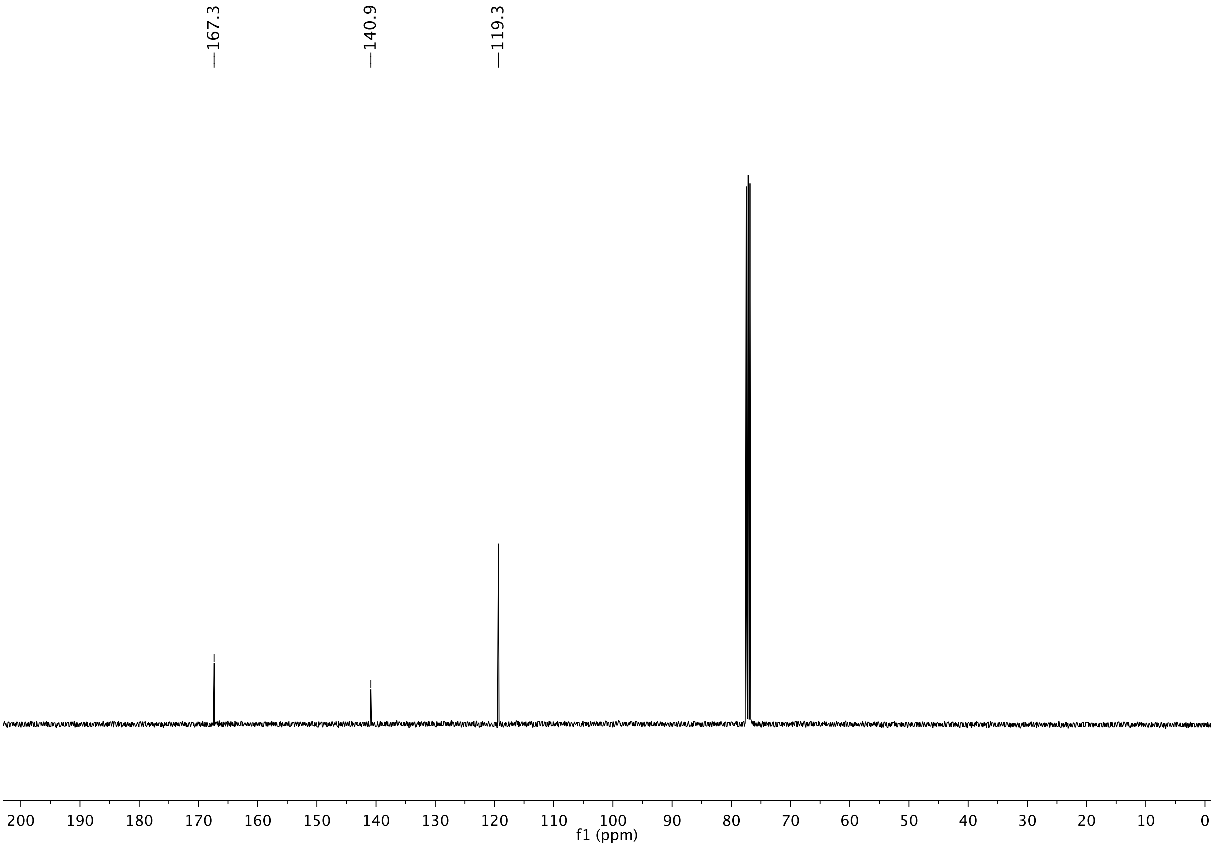
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

18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50
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30
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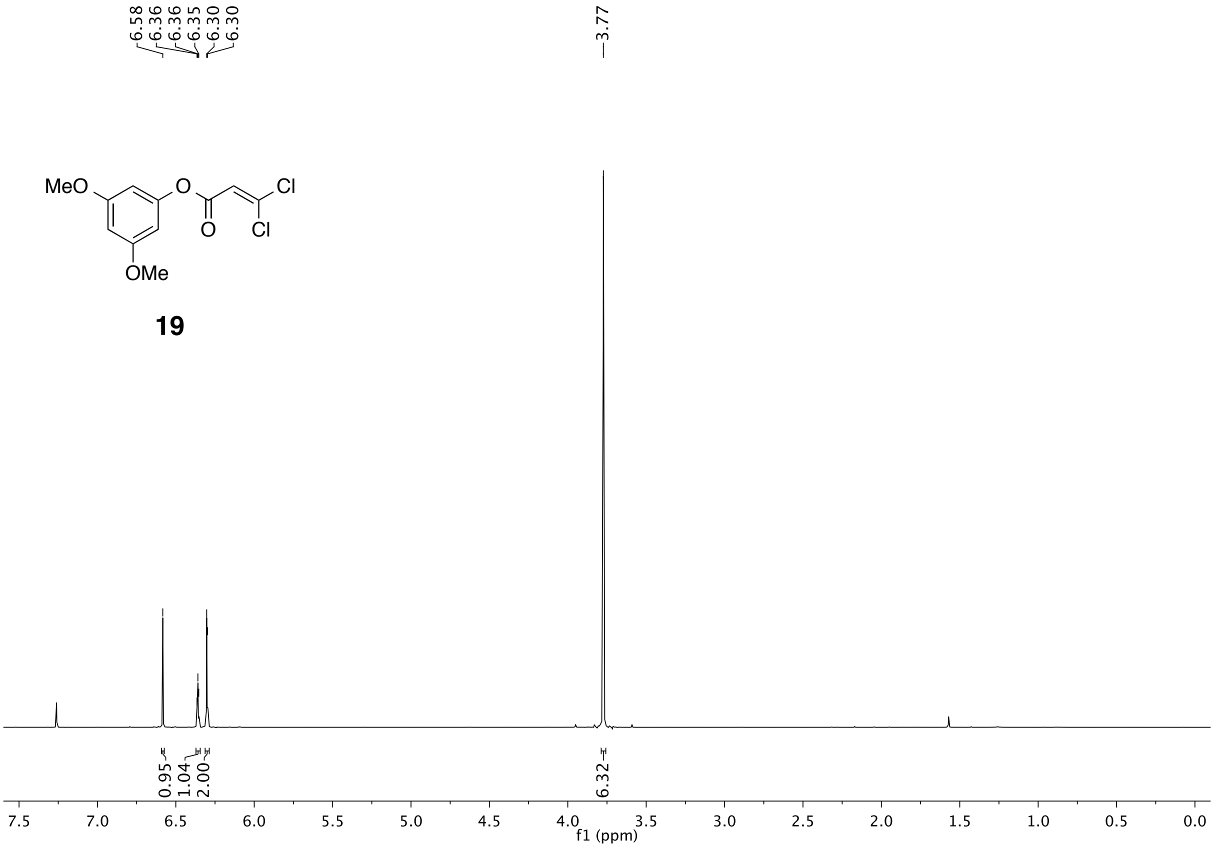
10
0
f1 (ppm)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Cl
OMe

19

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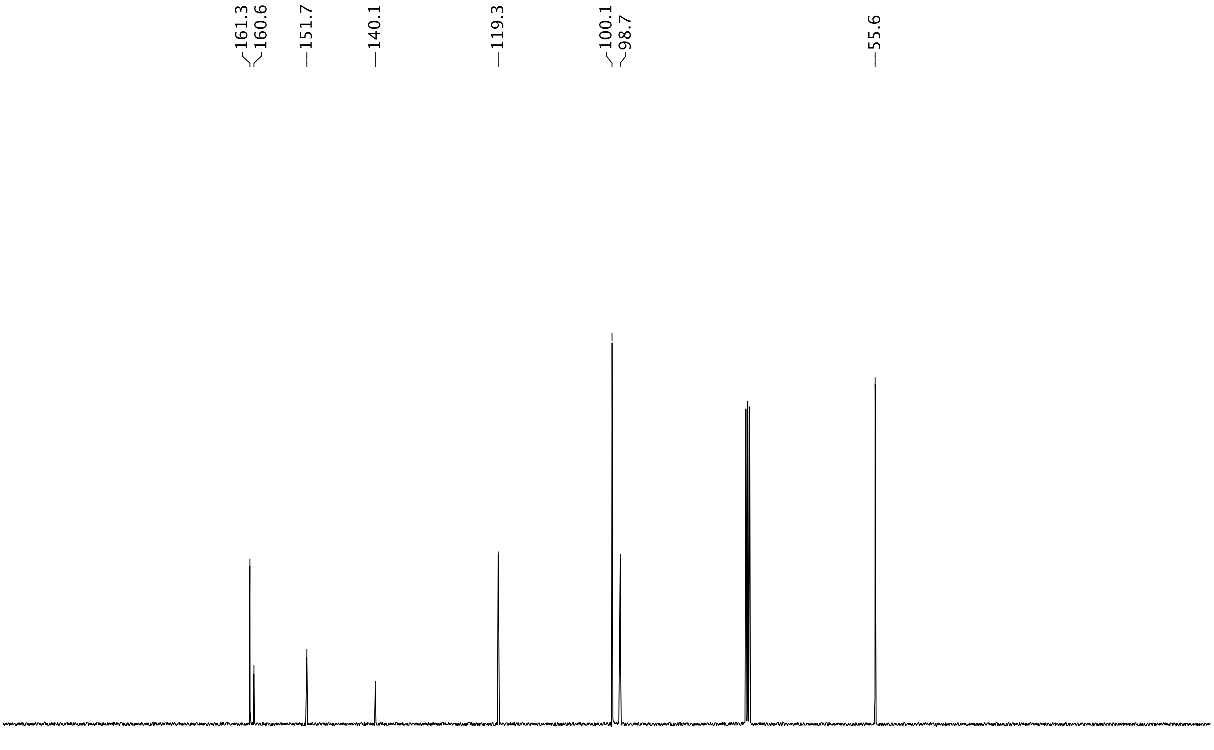
0.0
f1 (ppm)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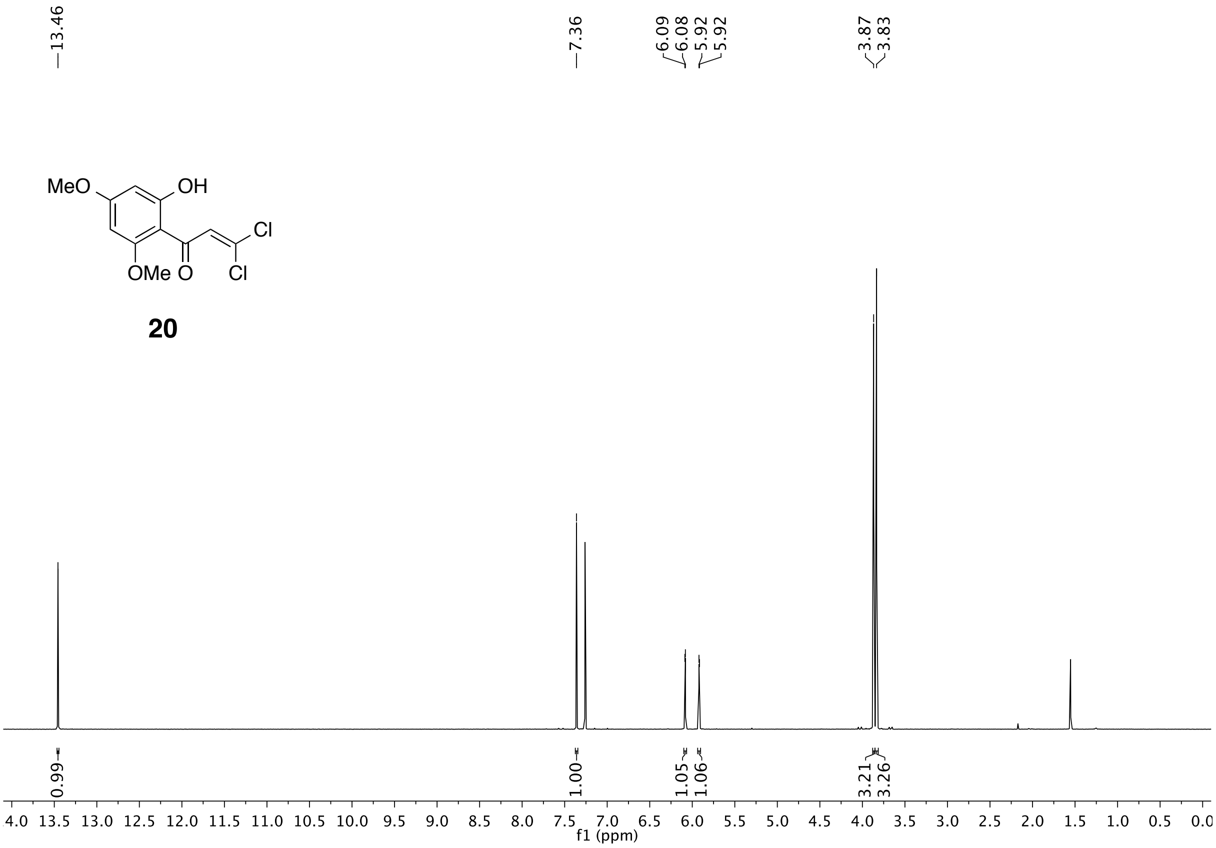
f1 (ppm)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1.00
1.05
1.06
3.21
3.26
4.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
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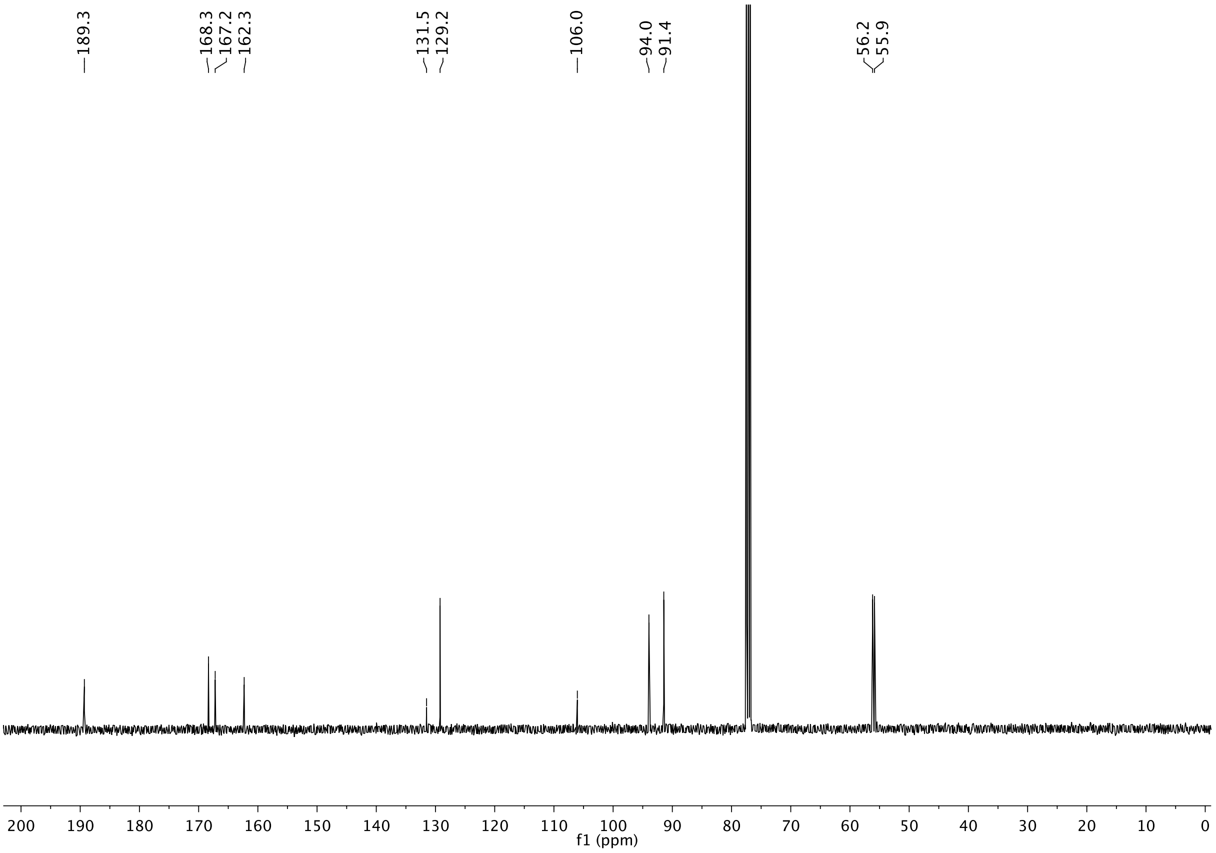
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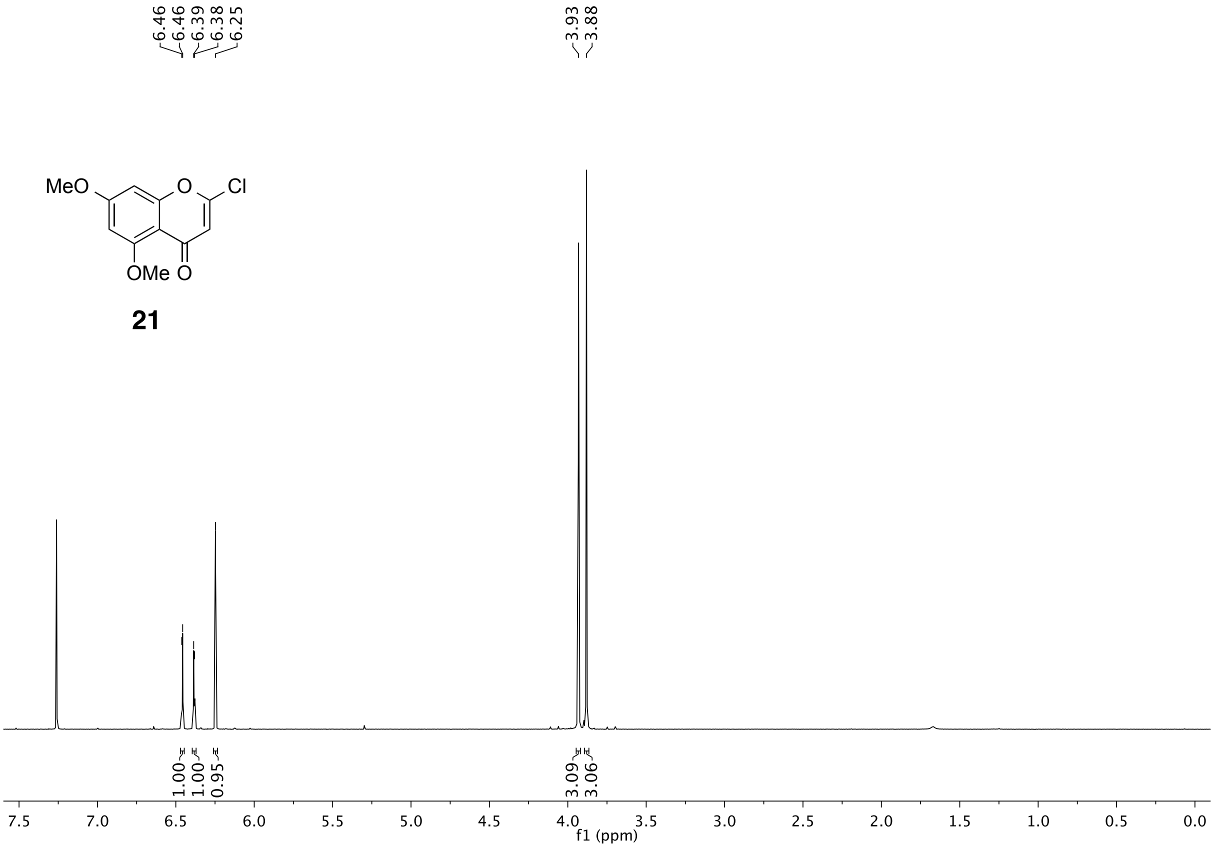

21

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Cl
OMe
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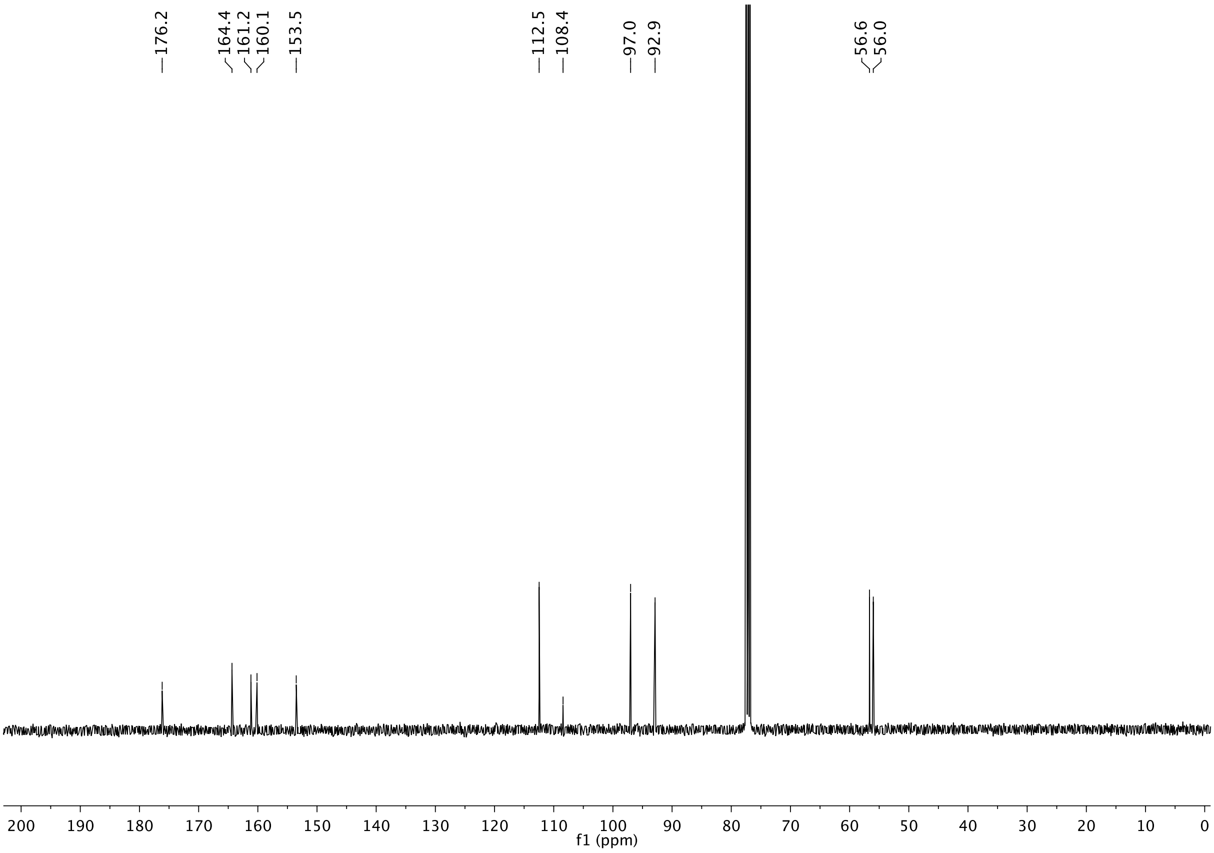

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56.6
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200
190
180
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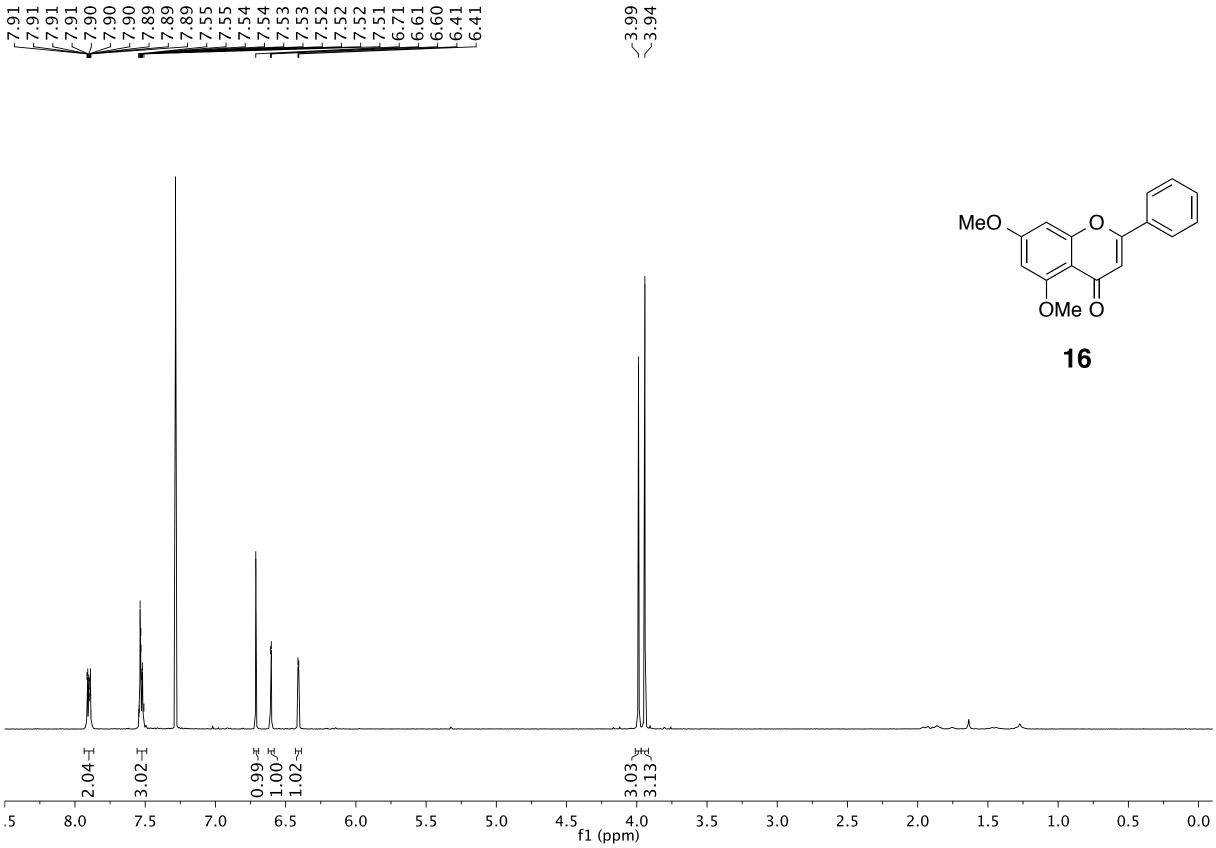
f1 (ppm)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6.41
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3.94
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OMe
O
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3.02
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1.00
1.02
3.03
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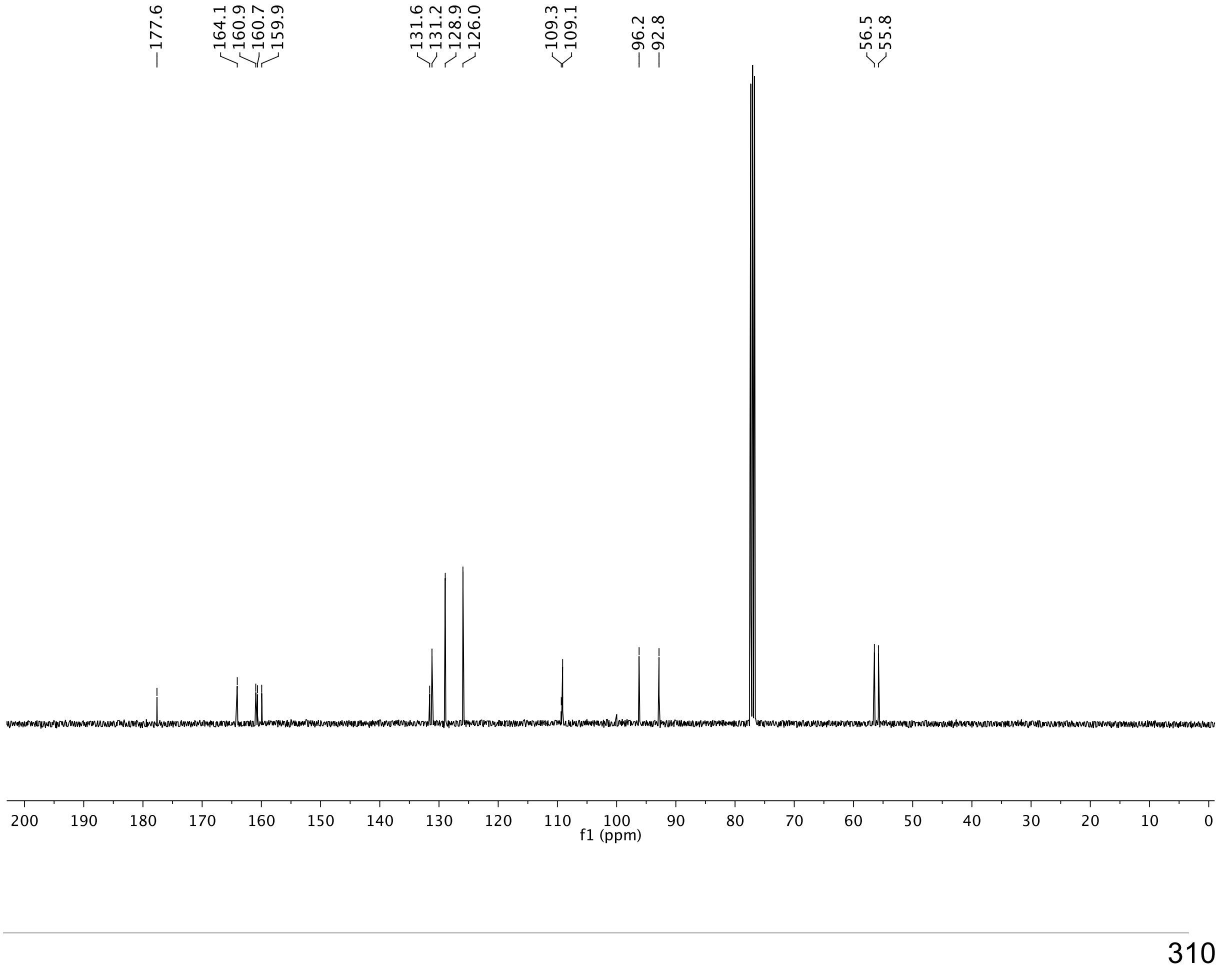
1.5
1.0
0.5
0.0
f1 (ppm)

16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128.9
126.0
109.3
109.1
96.2
92.8
56.5
55.8
200
190
180
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90
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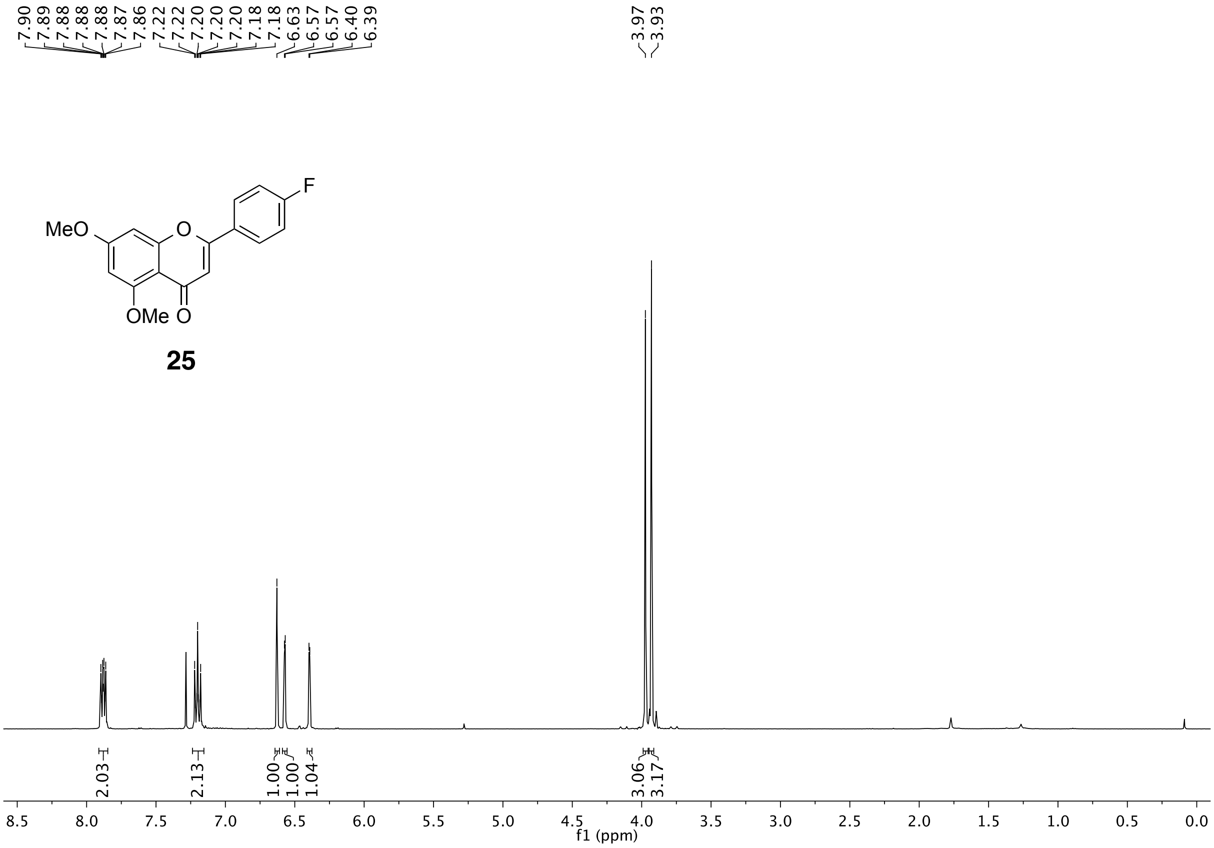
f1 (ppm)



7.90
7.89
7.88
7.88
7.88
7.87
7.86
7.22
7.22
7.20
7.20
7.20
7.18
6.63
6.57
6.57
6.40
6.39
3.97
3.93
MeO
O
F
OMe
O
2.03
2.13
1.00
1.00
1.04
3.06
3.17
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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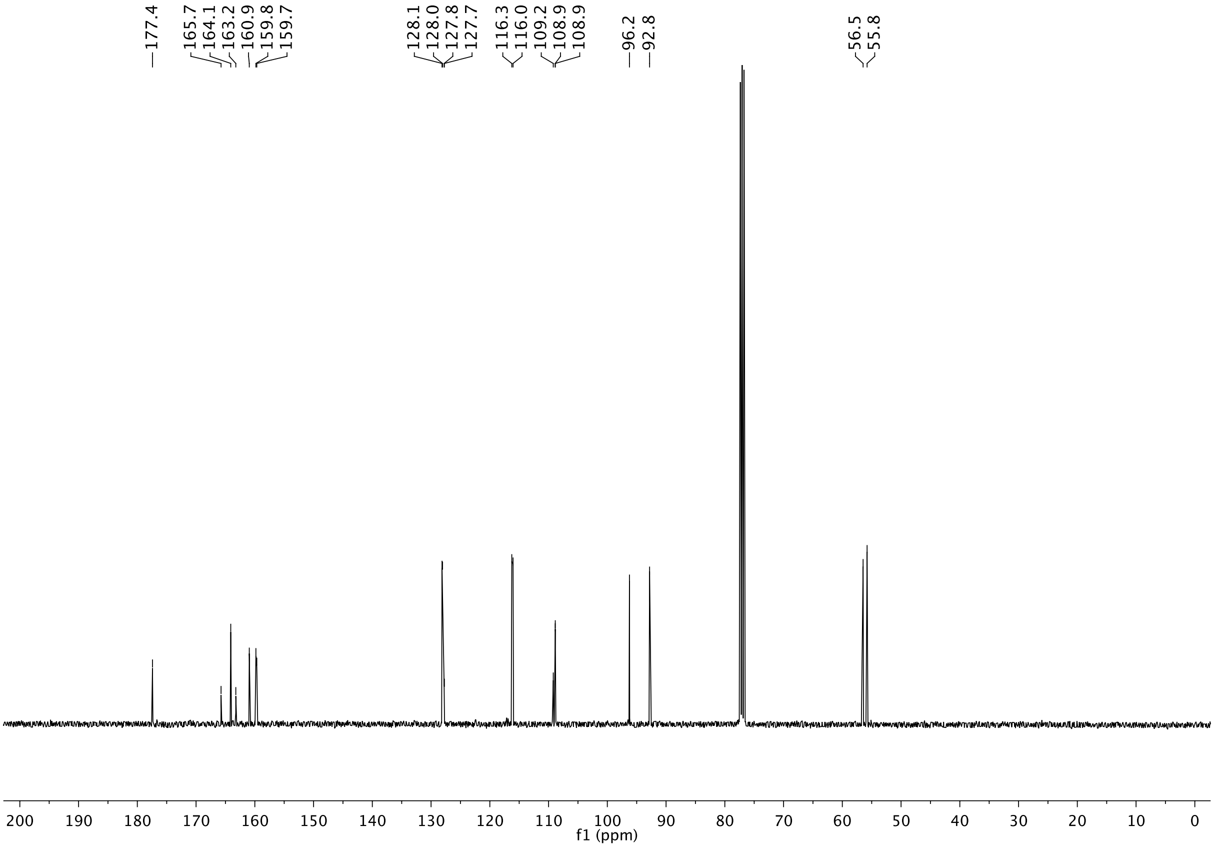
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

25



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159.8
159.7
128.1
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127.8
127.7
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116.0
109.2
108.9
108.9
96.2
92.8
56.5
55.8
200
190
180
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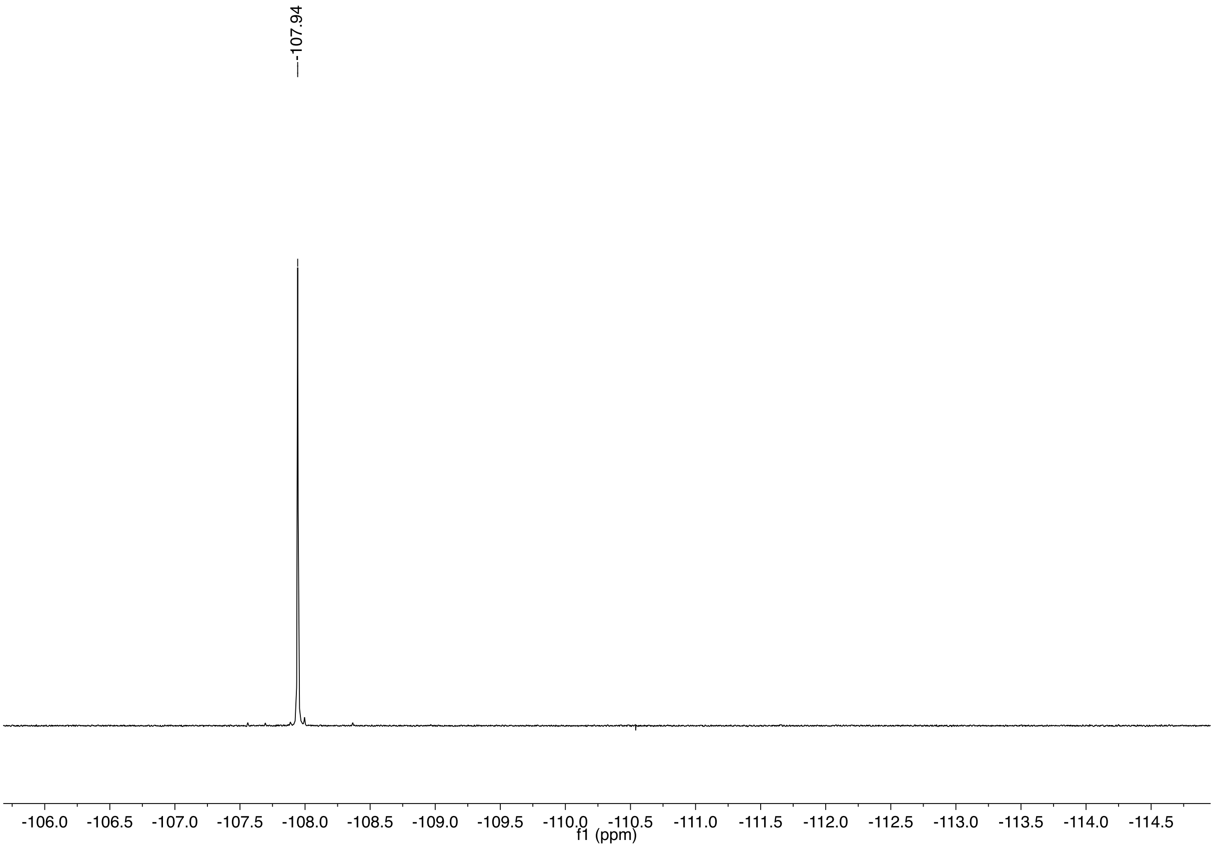
f1 (ppm)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-108.5
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-109.5
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-113.0
-113.5
-114.0
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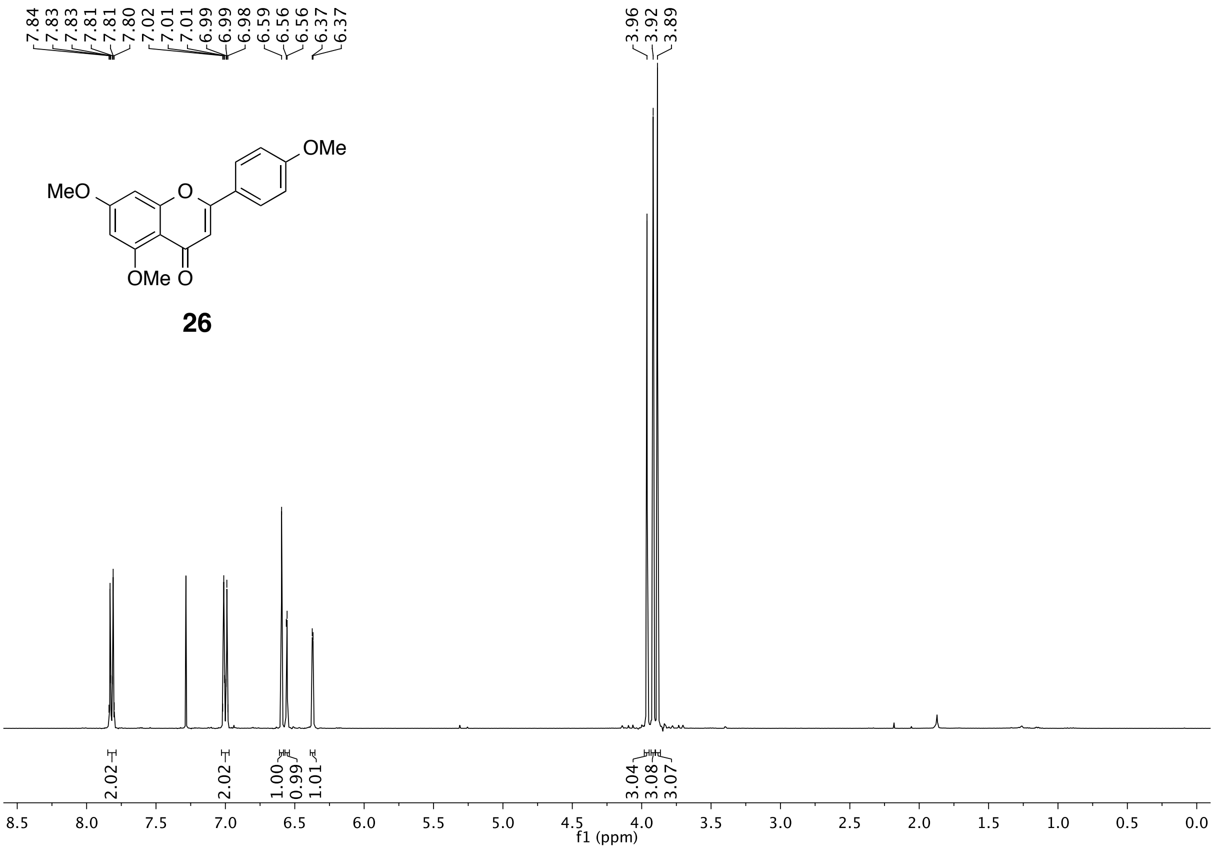
f1 (ppm)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7.01
7.01
6.99
6.99
6.98
6.59
6.56
6.56
6.37
6.37
3.96
3.92
3.89
MeO
OMe
O
OMe O
26
2.02
2.02
1.00
0.99
1.01
3.04
3.08
3.07
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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4.5
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3.0
2.5
2.0
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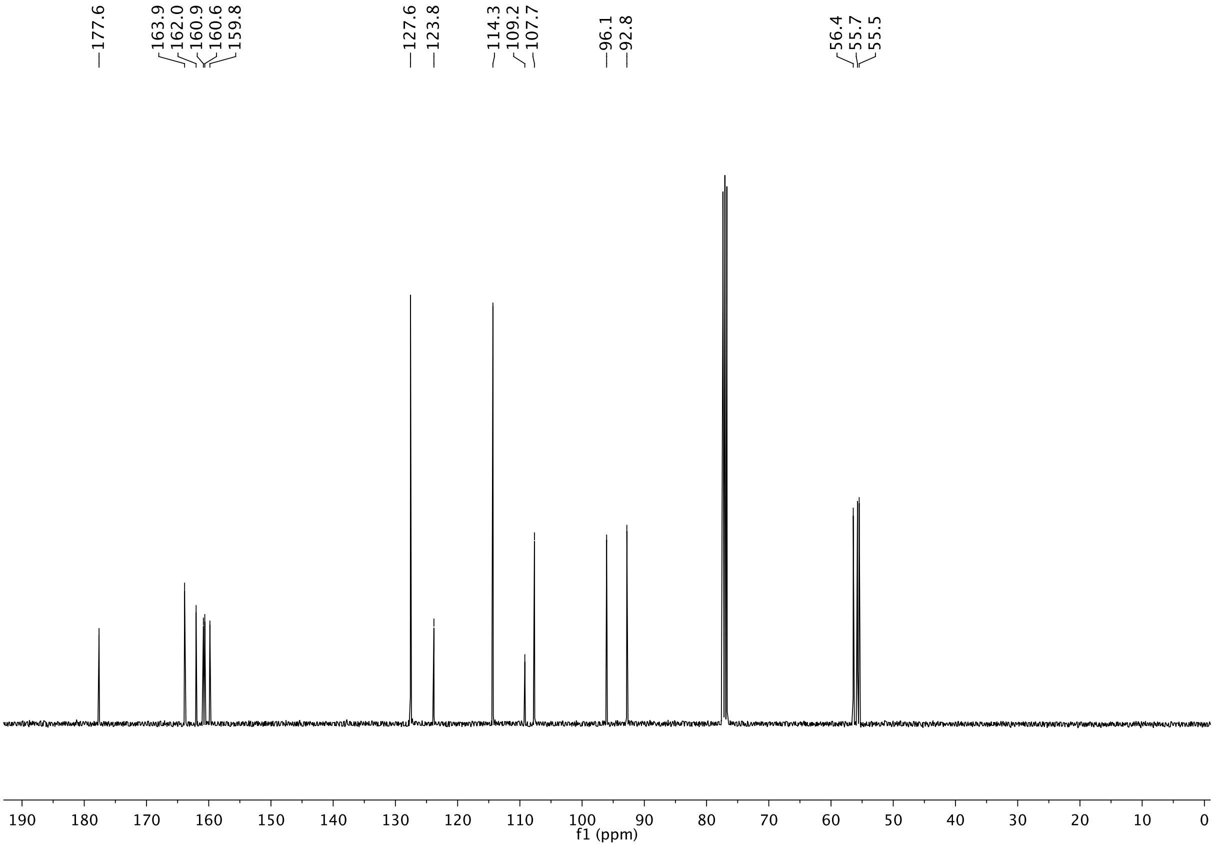
0.0
f1 (ppm)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159.8
127.6
123.8
114.3
109.2
107.7
96.1
92.8
56.4
55.7
55.5
190
180
170
160
150
140
130
120
110
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90
80
70
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50
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30
20
10
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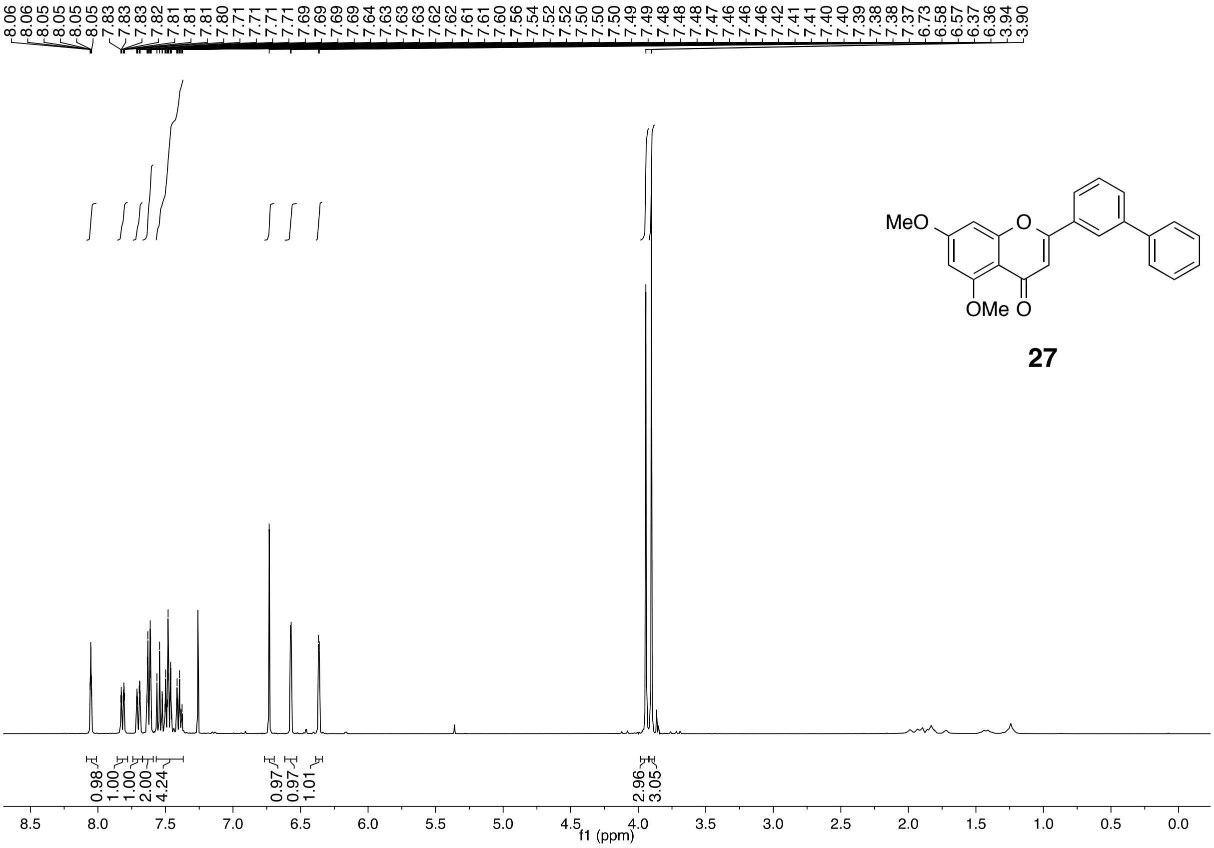
f1 (ppm)


8.06
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7.82
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7.80
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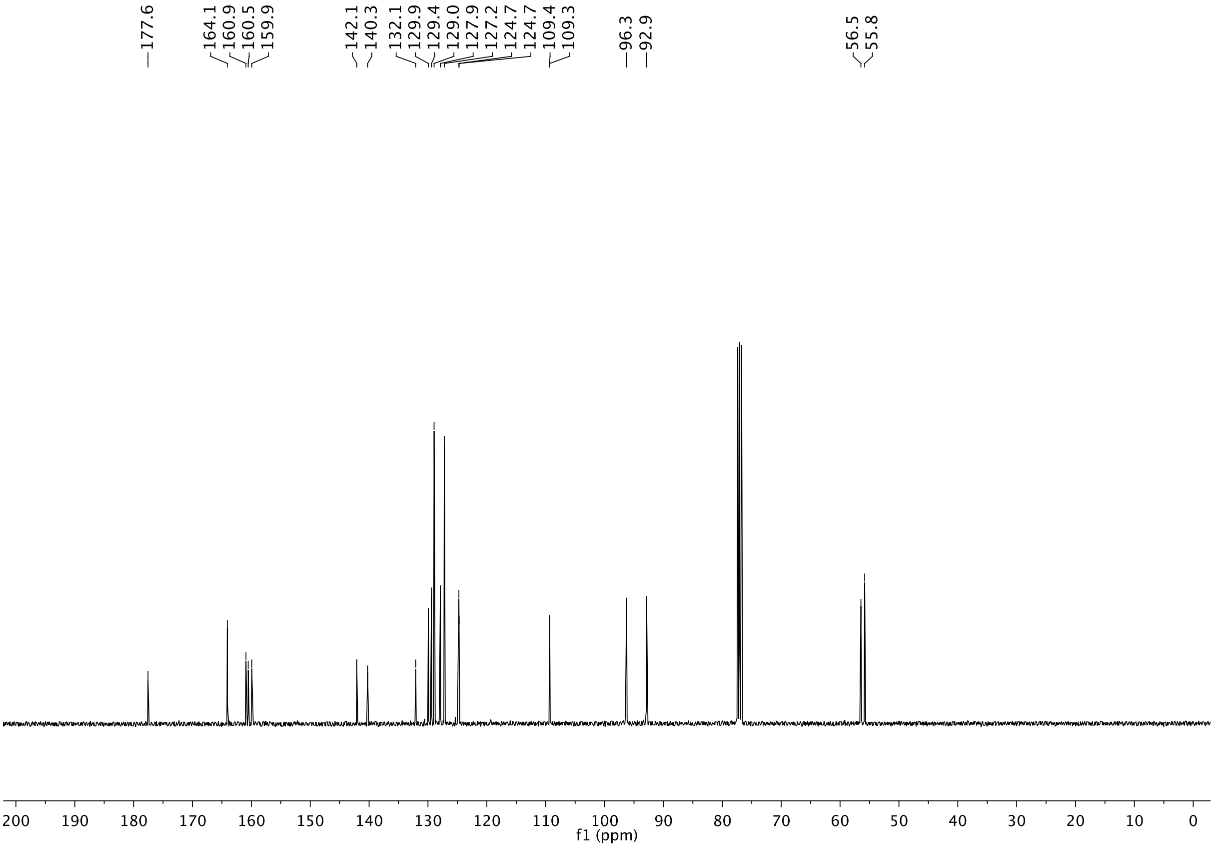
7.71
7.71
7.69
7.69
7.69
7.64
7.63
7.63
7.62
7.61
7.60
7.56
7.54
7.52
7.52
7.50
7.50
7.49
7.49
7.48
7.48
7.47
7.46
7.46
7.42
7.41
7.40
7.40
7.39
7.38
7.37
6.73
6.58
6.57
6.37
6.36
3.94
3.90
MeO
O
OMe
O
0.98
1.00
1.00
2.00
4.24
0.97
0.97
1.01
2.96
3.05
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

27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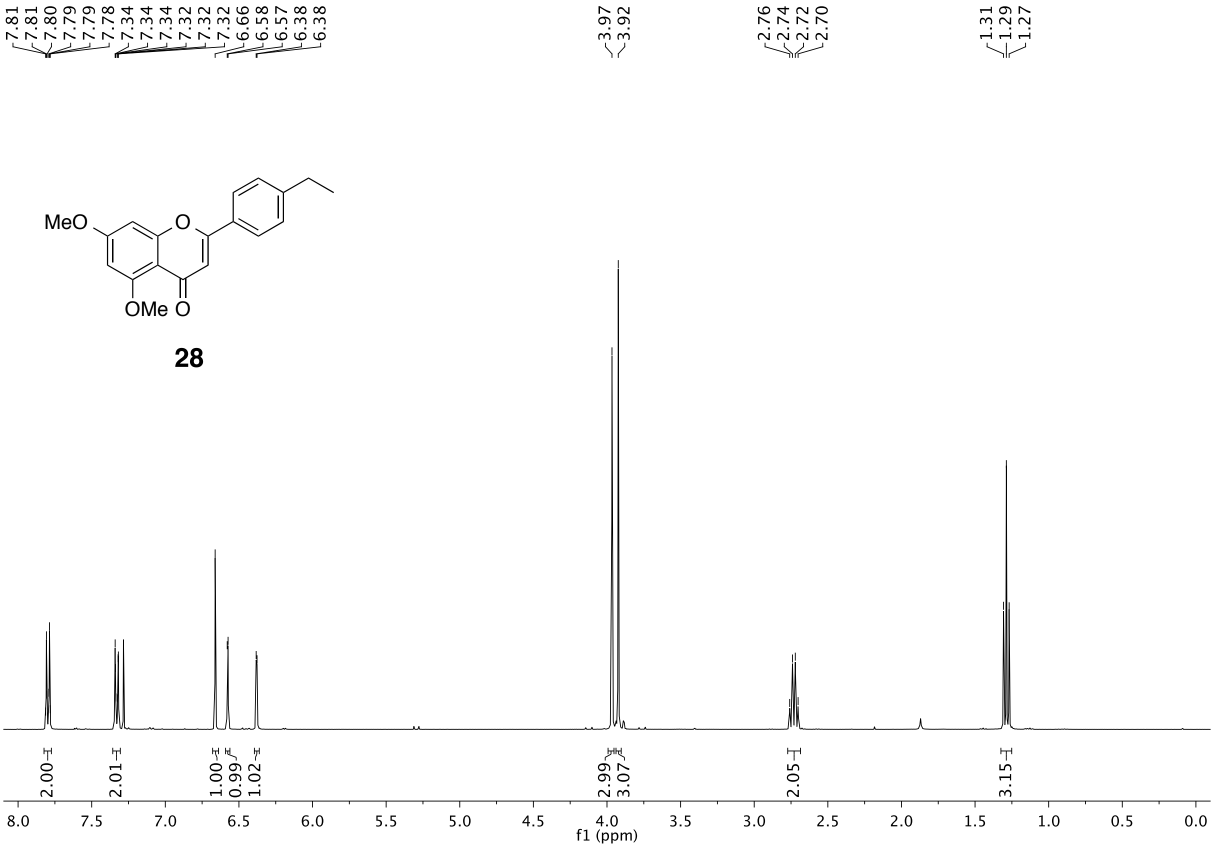
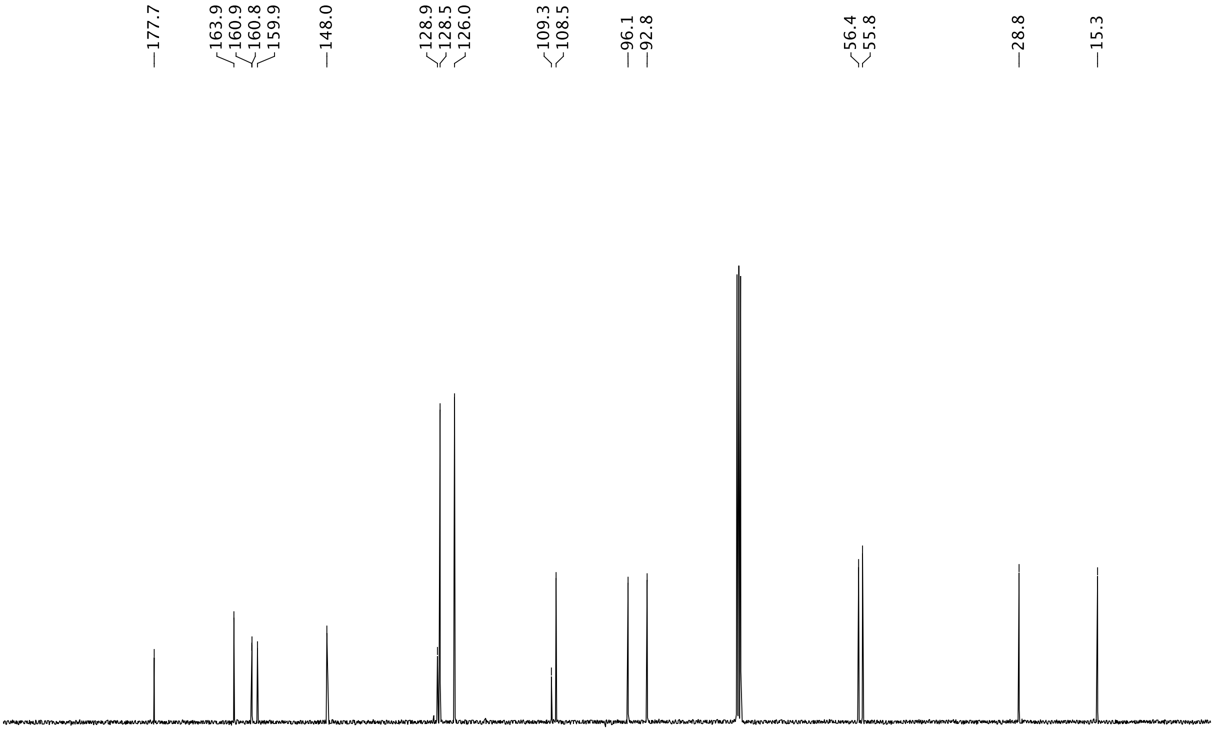
160.9
160.5
159.9
142.1
140.3
132.1
129.9
129.4
129.0
127.9
127.2
124.7
124.7
109.4
109.3
96.3
92.9
56.5
55.8
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



28

7.81
7.81
7.80
7.79
7.79
7.78
7.34
7.34
7.34
7.32
7.32
7.32
6.66
6.58
6.57
6.38
6.38
3.97
3.92
2.76
2.74
2.72
2.70
1.31
1.29
1.27
MeO
O
OMe
O
2.00
2.01
1.00
0.99
1.02
2.99
3.07
2.05
3.15
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



177.7
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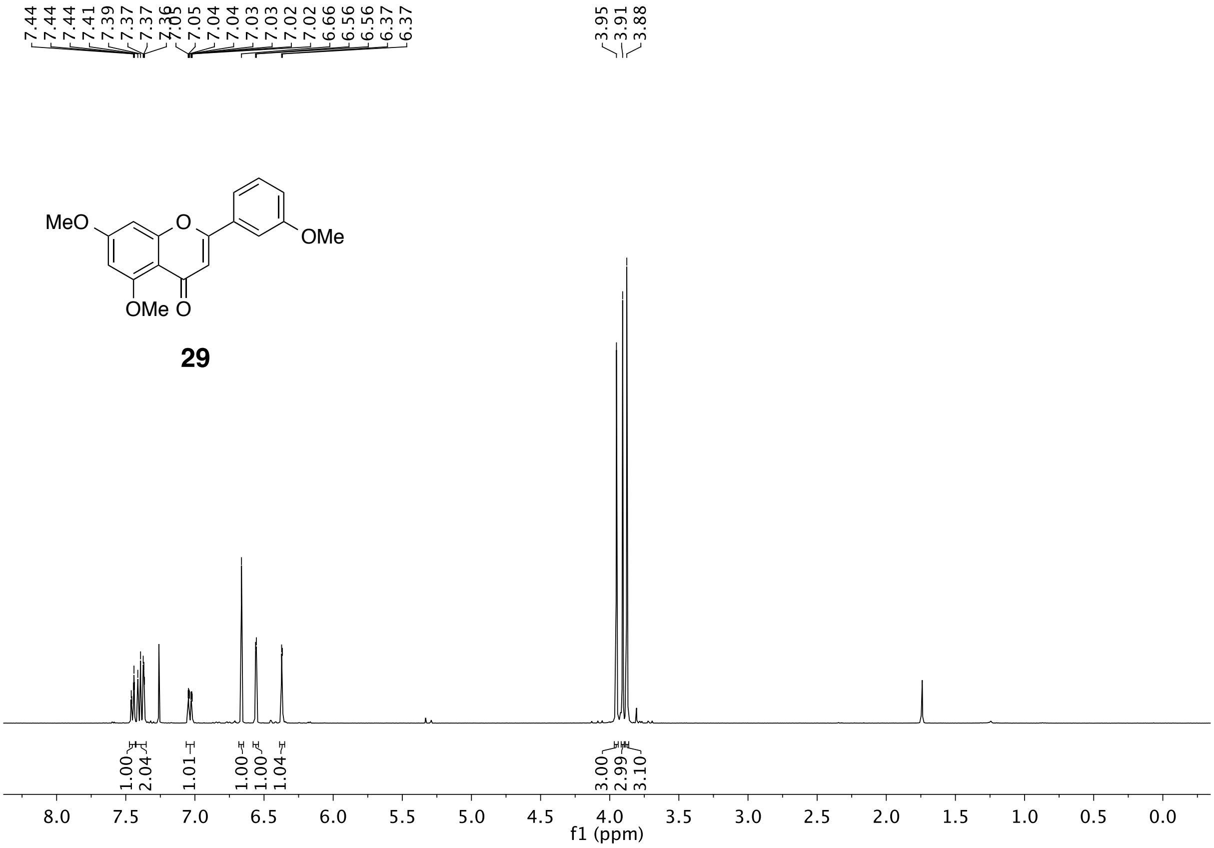
160.9
160.8
159.9
148.0
128.9
128.5
126.0
109.3
108.5
96.1
92.8
56.4
55.8
28.8
15.3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.37
7.37
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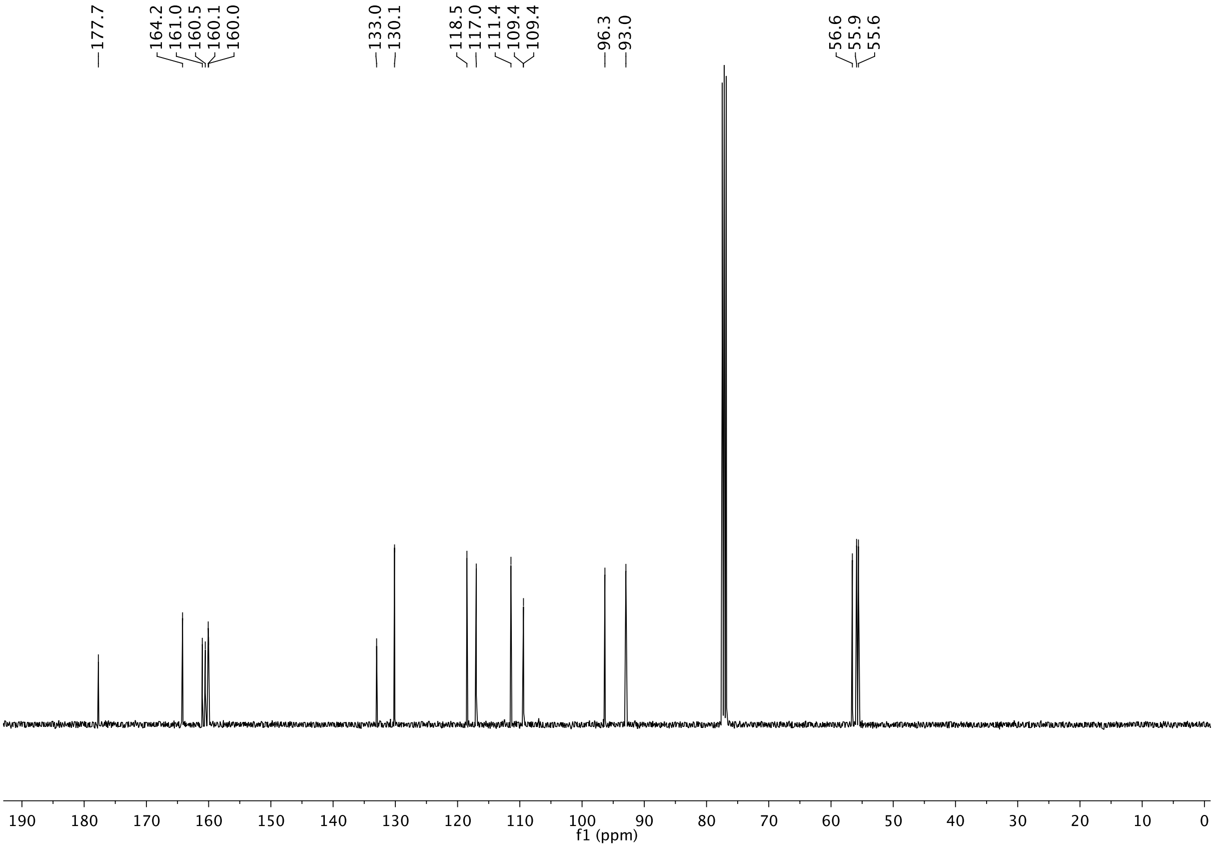
7.05
7.05
7.04
7.04
7.03
7.03
7.02
7.02
6.66
6.56
6.56
6.37
6.37
3.95
3.91
3.88
MeO
O
OMe
OMe
O
29
1.00
2.04
1.01
1.00
1.00
1.04
3.00
2.99
3.10
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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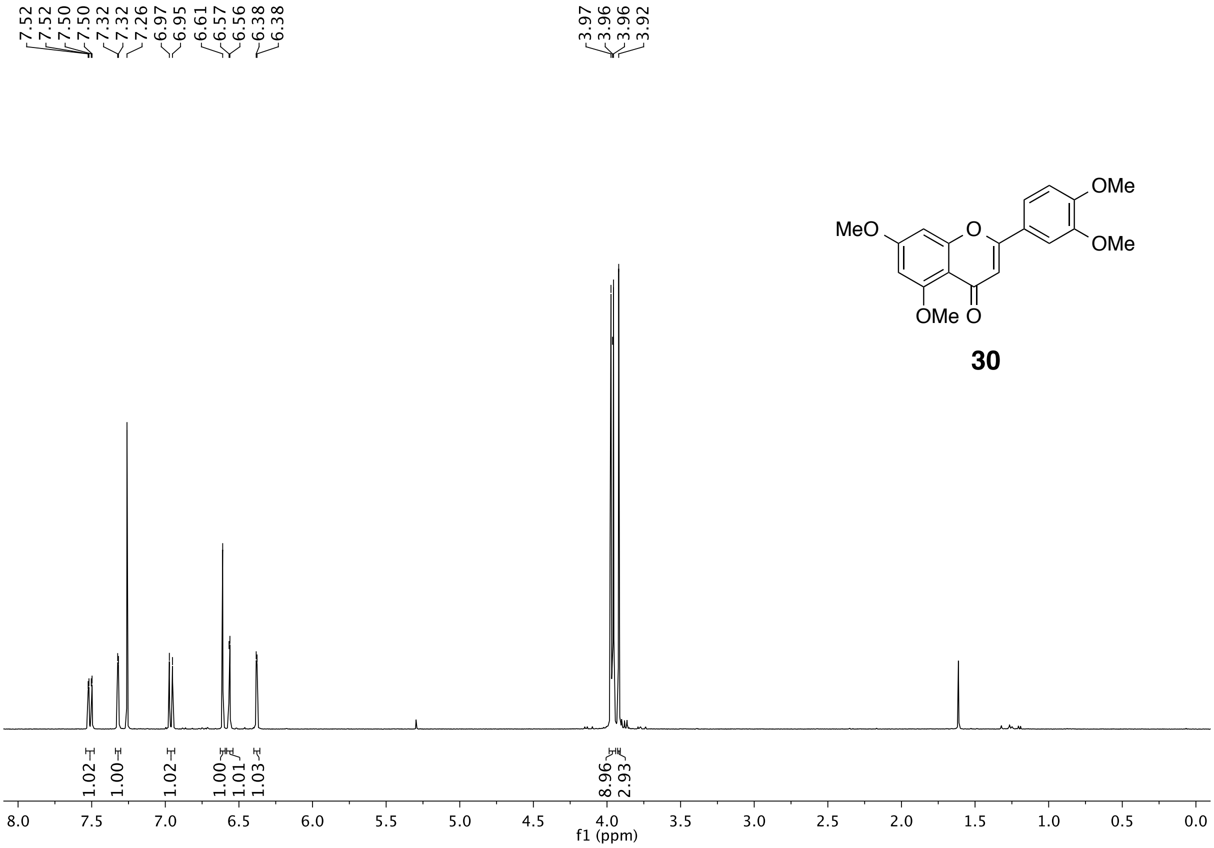
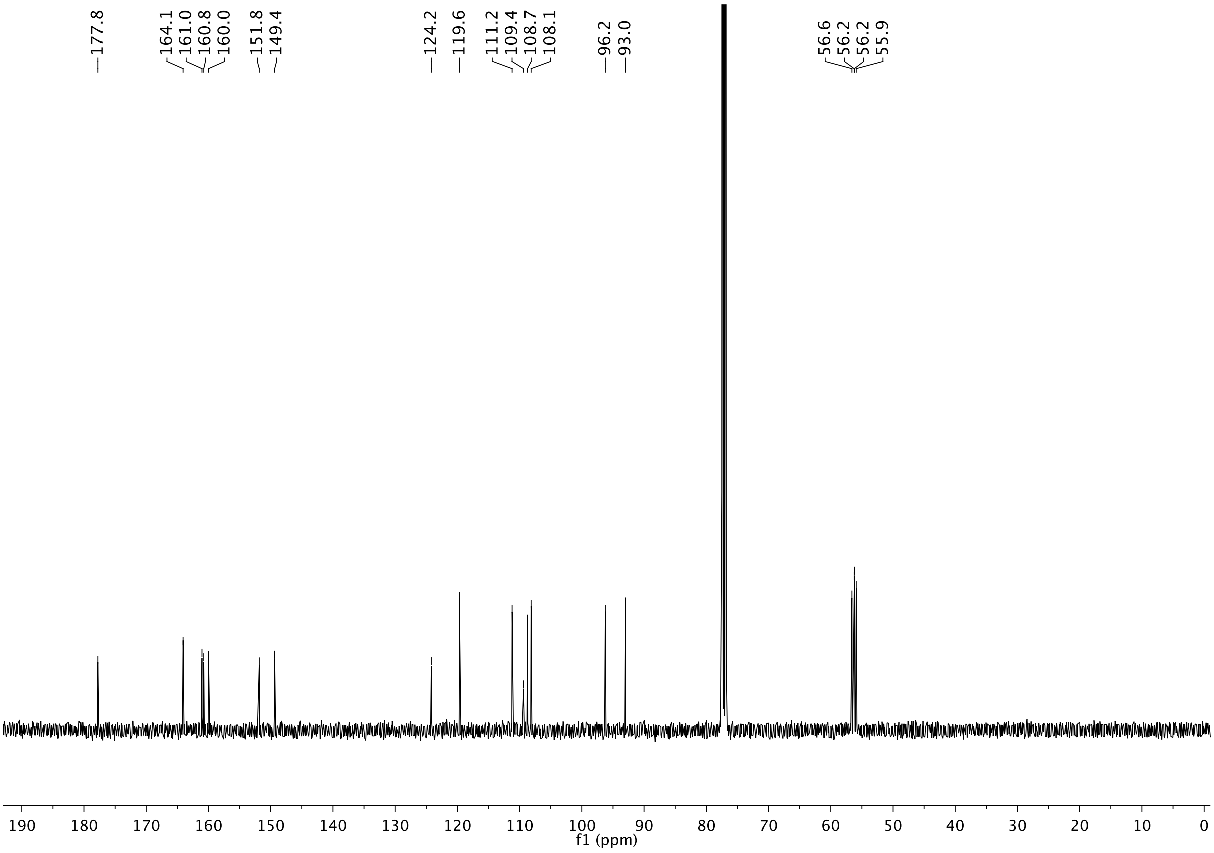
161.0
160.5
160.1
160.0
133.0
130.1
118.5
117.0
111.4
109.4
109.4
96.3
93.0
56.6
55.9
55.6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



30

7.52
7.52
7.50
7.50
7.32
7.32
7.26
6.97
6.95
6.61
6.57
6.56
6.38
6.38
3.97
3.96
3.96
3.92
MeO
O
OMe
OMe
OMe
O
1.02
1.00
1.02
1.00
1.01
1.03
8.96
2.93
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



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160.0
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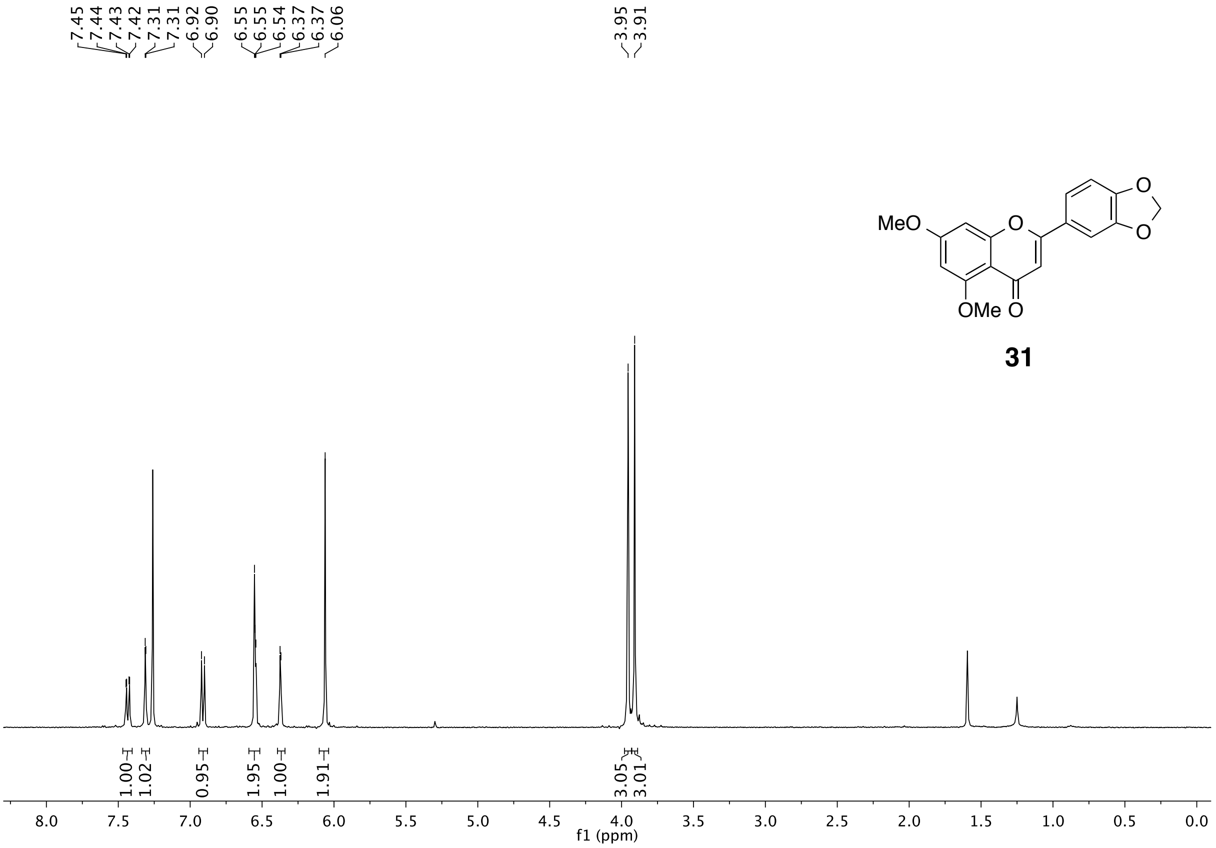
149.4
124.2
119.6
111.2
109.4
108.7
108.1
96.2
93.0
56.6
56.2
56.2
55.9
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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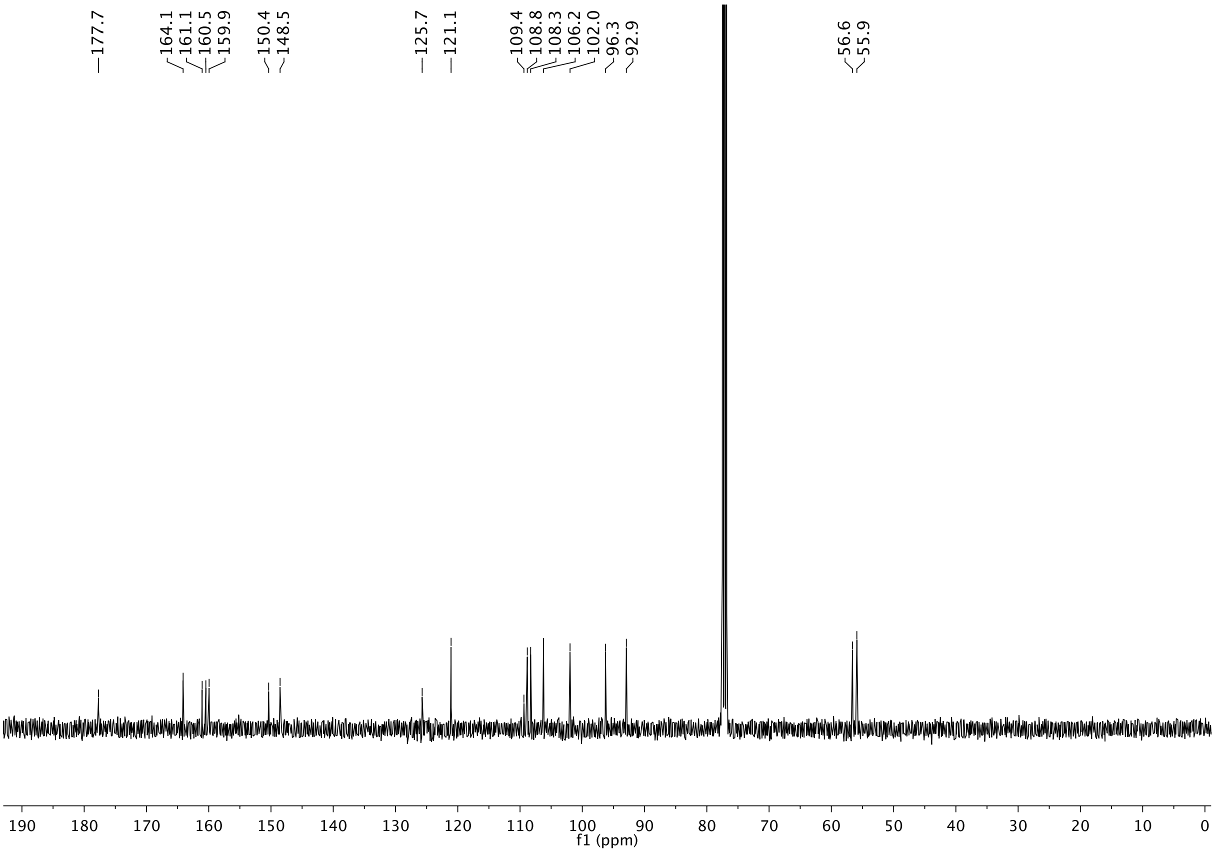
7.42
7.31
7.31
6.92
6.90
6.55
6.55
6.54
6.37
6.37
6.06
3.95
3.91
MeO
O
O
O
OMe
O
1.00
1.02
0.95
1.95
1.00
1.91
3.05
3.01
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

31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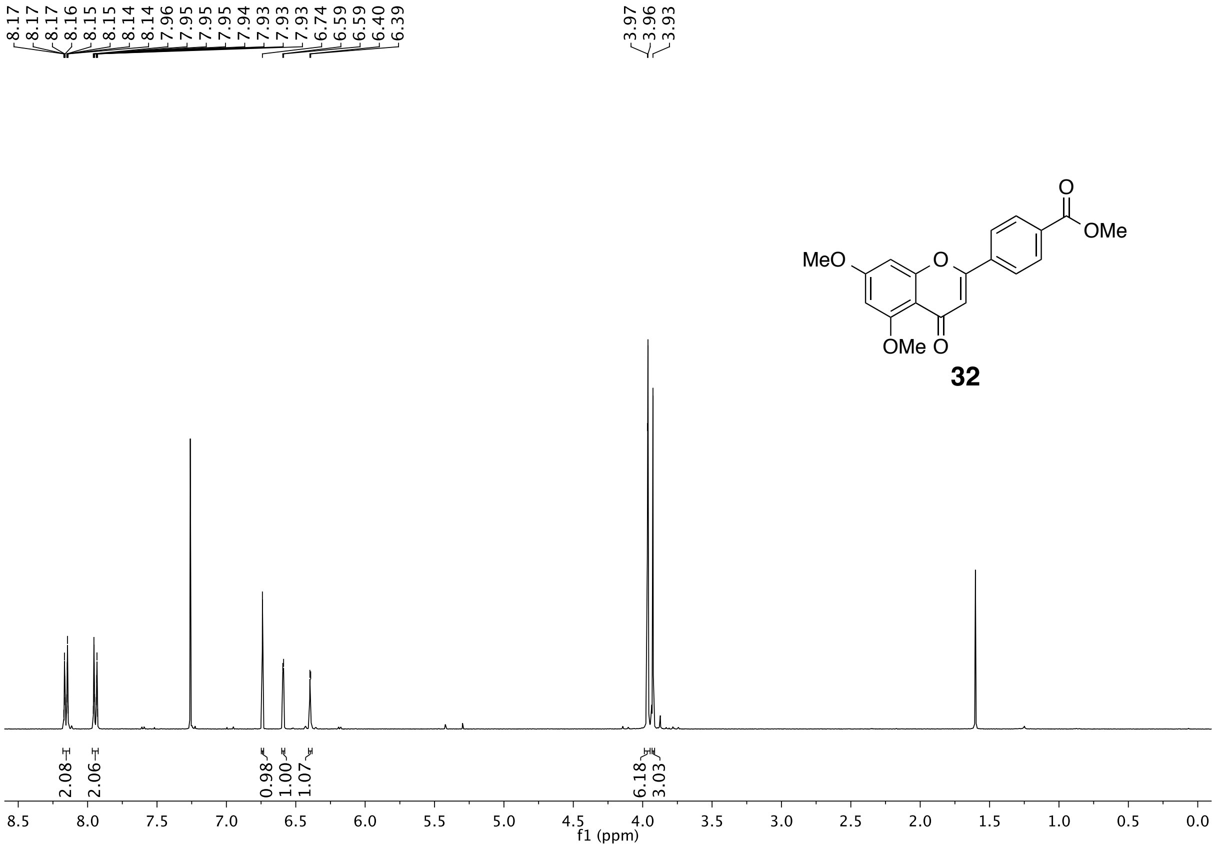
161.1
160.5
159.9
150.4
148.5
125.7
121.1
109.4
108.8
108.3
106.2
102.0
96.3
92.9
56.6
55.9
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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8.14
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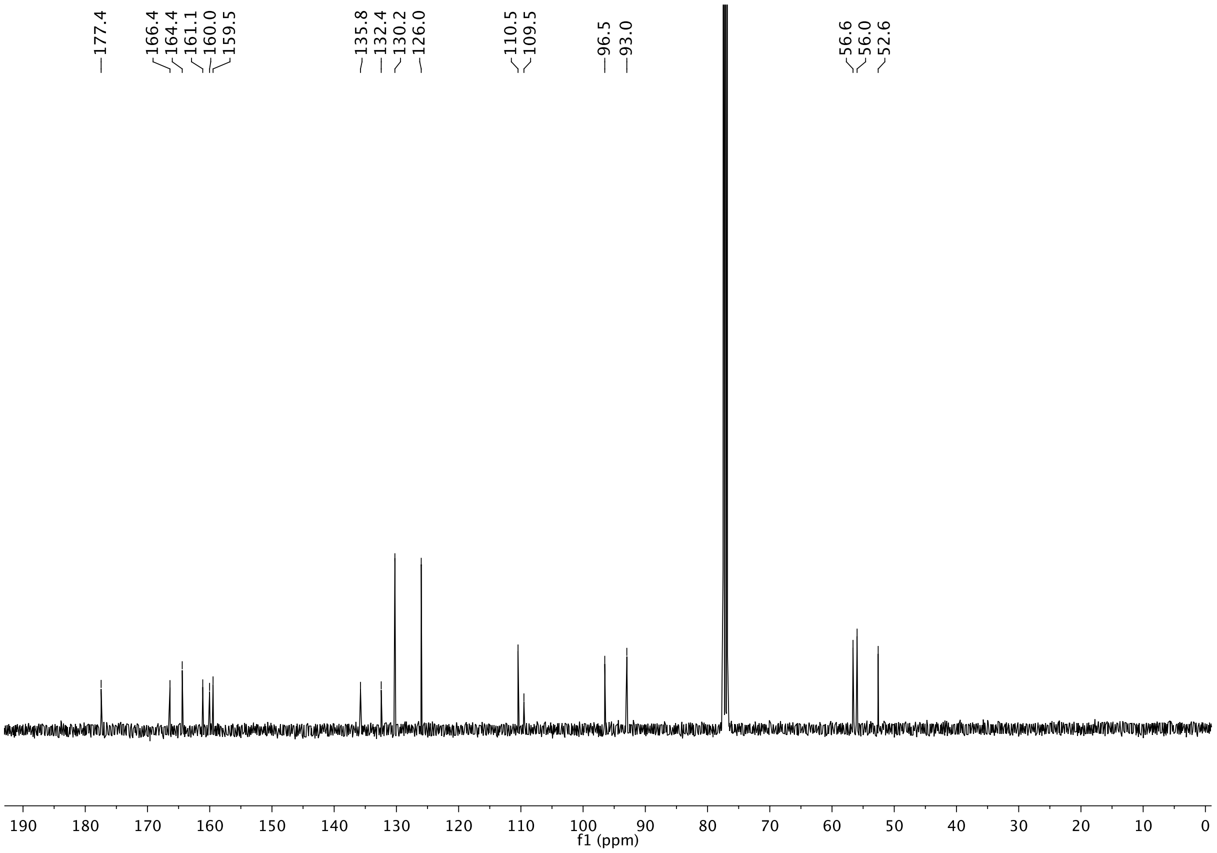
7.95
7.95
7.94
7.93
7.93
7.93
6.74
6.59
6.59
6.40
6.39
3.97
3.96
3.93
MeO
O
O
OMe
OMe
O
2.08
2.06
0.98
1.00
1.07
6.18
3.03
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

32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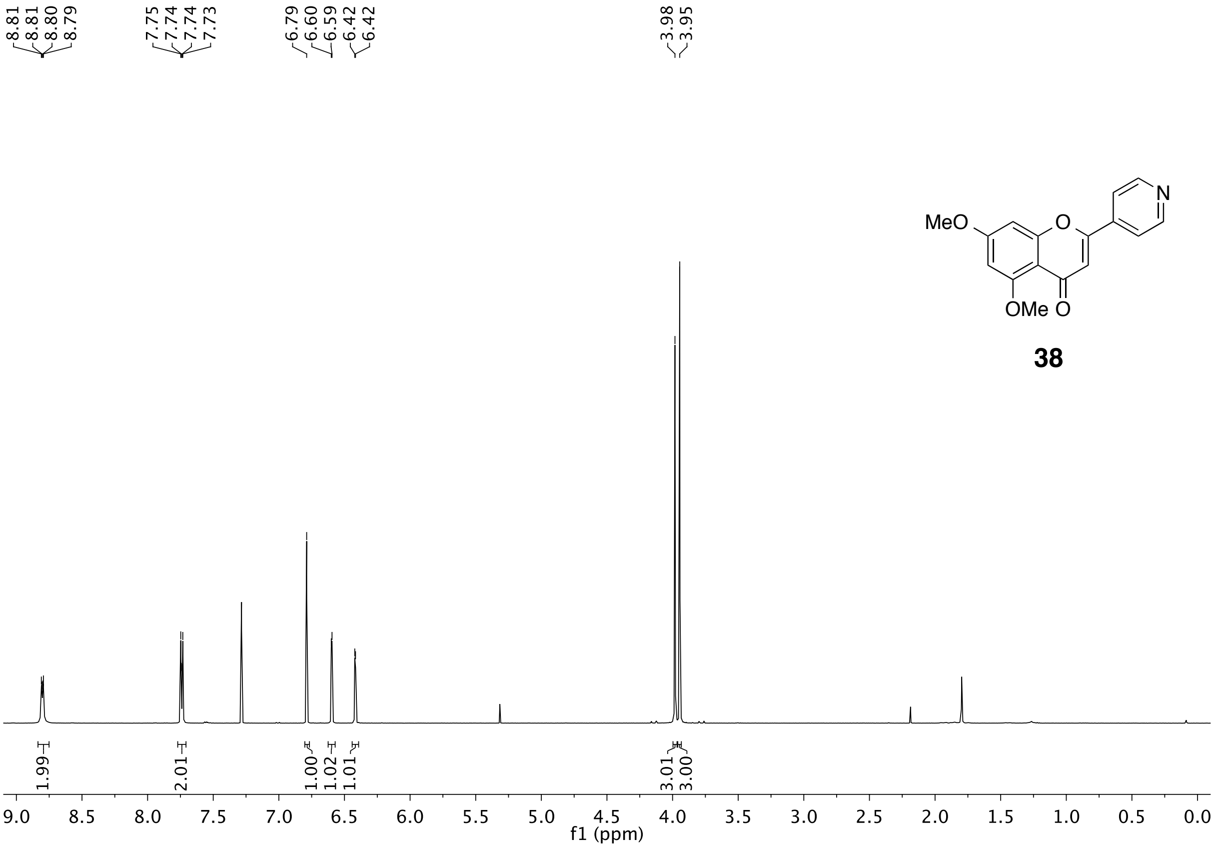
164.4
161.1
160.0
159.5
135.8
132.4
130.2
126.0
110.5
109.5
96.5
93.0
56.6
56.0
52.6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.74
7.74
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6.60
6.59
6.42
6.42
3.98
3.95
MeO
O
N
OMe
O
1.99
2.01
1.00
1.02
1.01
3.01
3.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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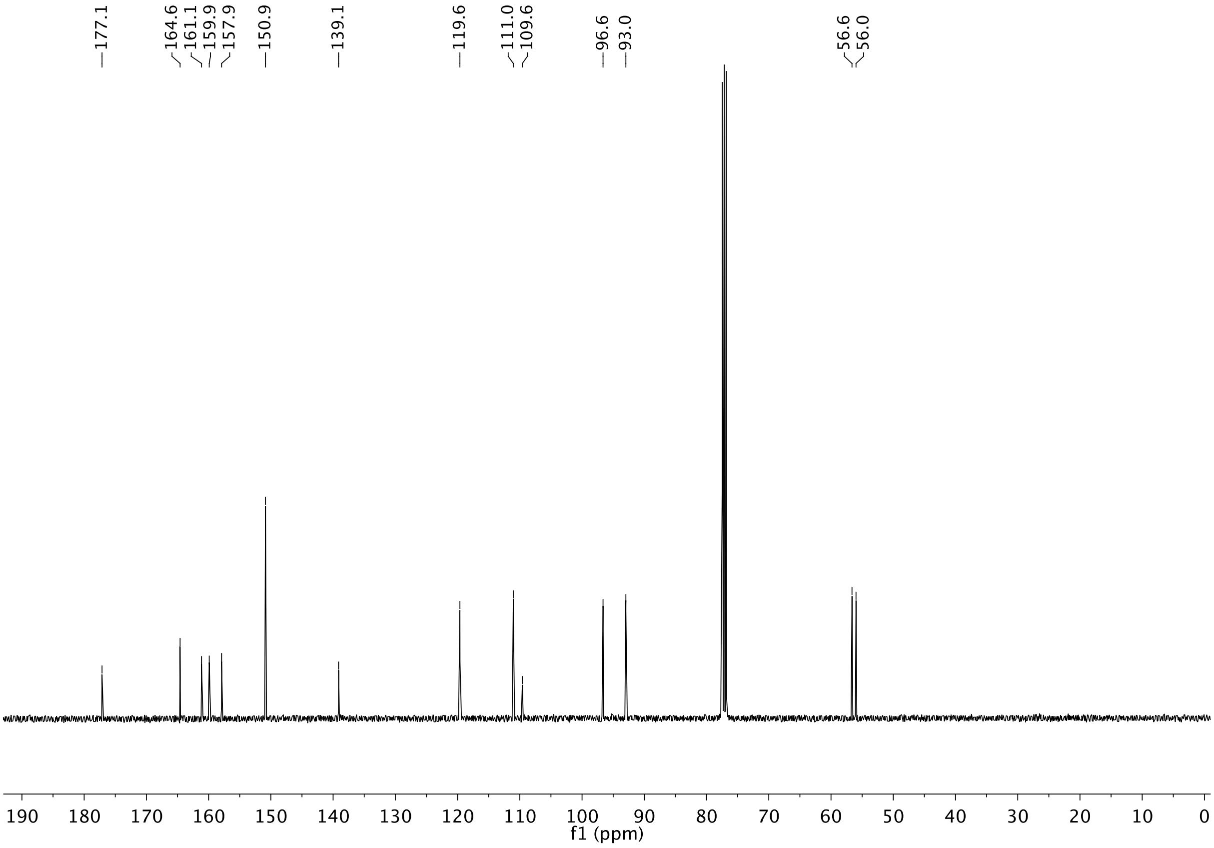
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

38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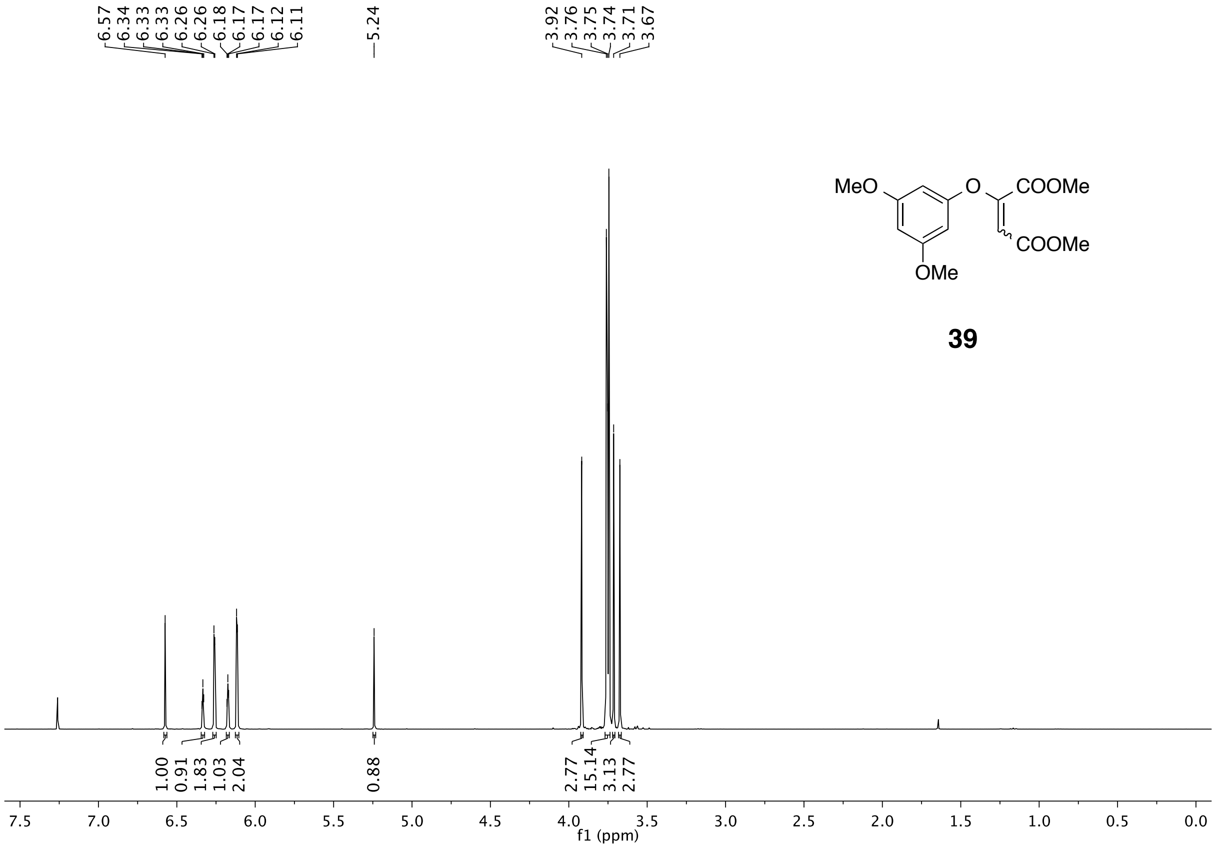
157.9
150.9
139.1
119.6
111.0
109.6
96.6
93.0
56.6
56.0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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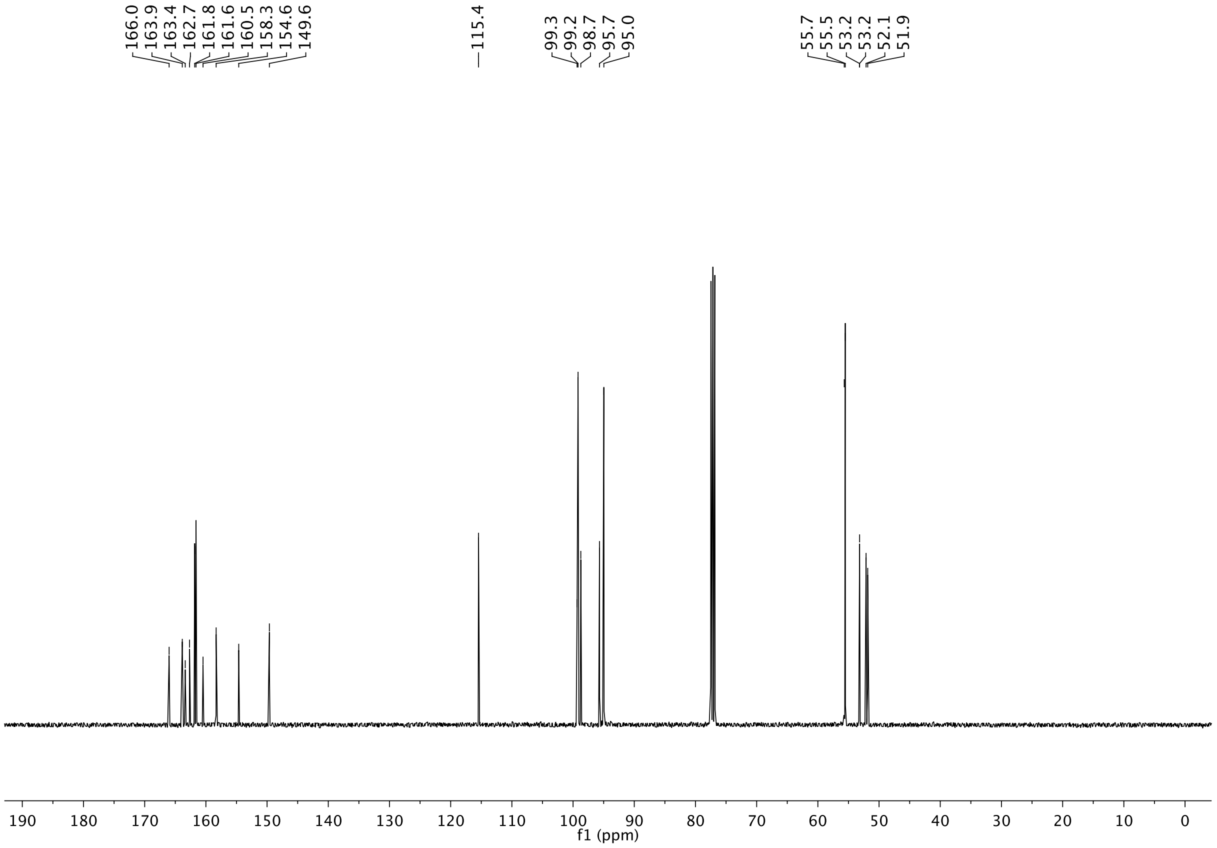
6.26
6.26
6.18
6.17
6.17
6.12
6.11
5.24
3.92
3.76
3.75
3.74
3.71
3.67
MeO
O
COOMe
COOMe
OMe
1.00
0.91
1.83
1.03
2.04
0.88
2.77
15.14
3.13
2.77
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

39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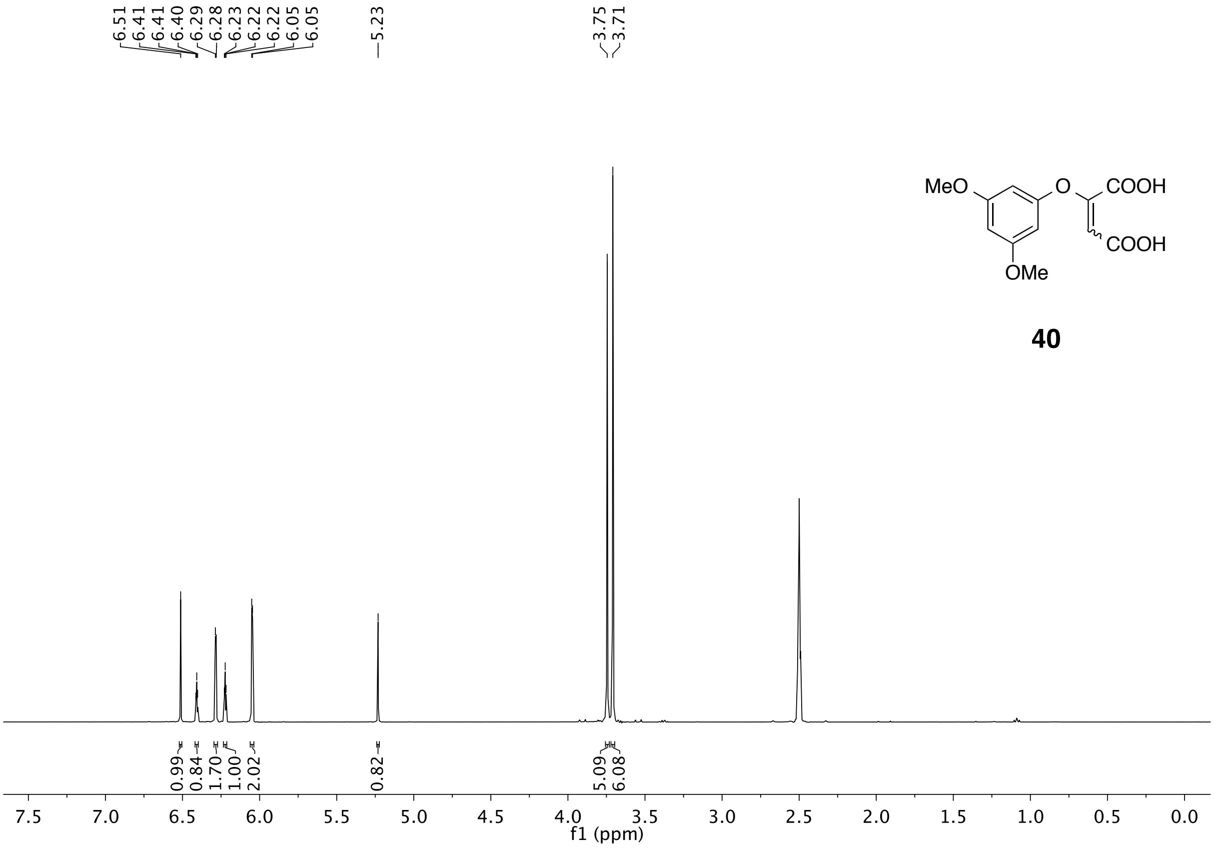
162.7
161.8
161.6
160.5
158.3
154.6
149.6
115.4
99.3
99.2
98.7
95.7
95.0
55.7
55.5
53.2
53.2
52.1
51.9
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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6.41
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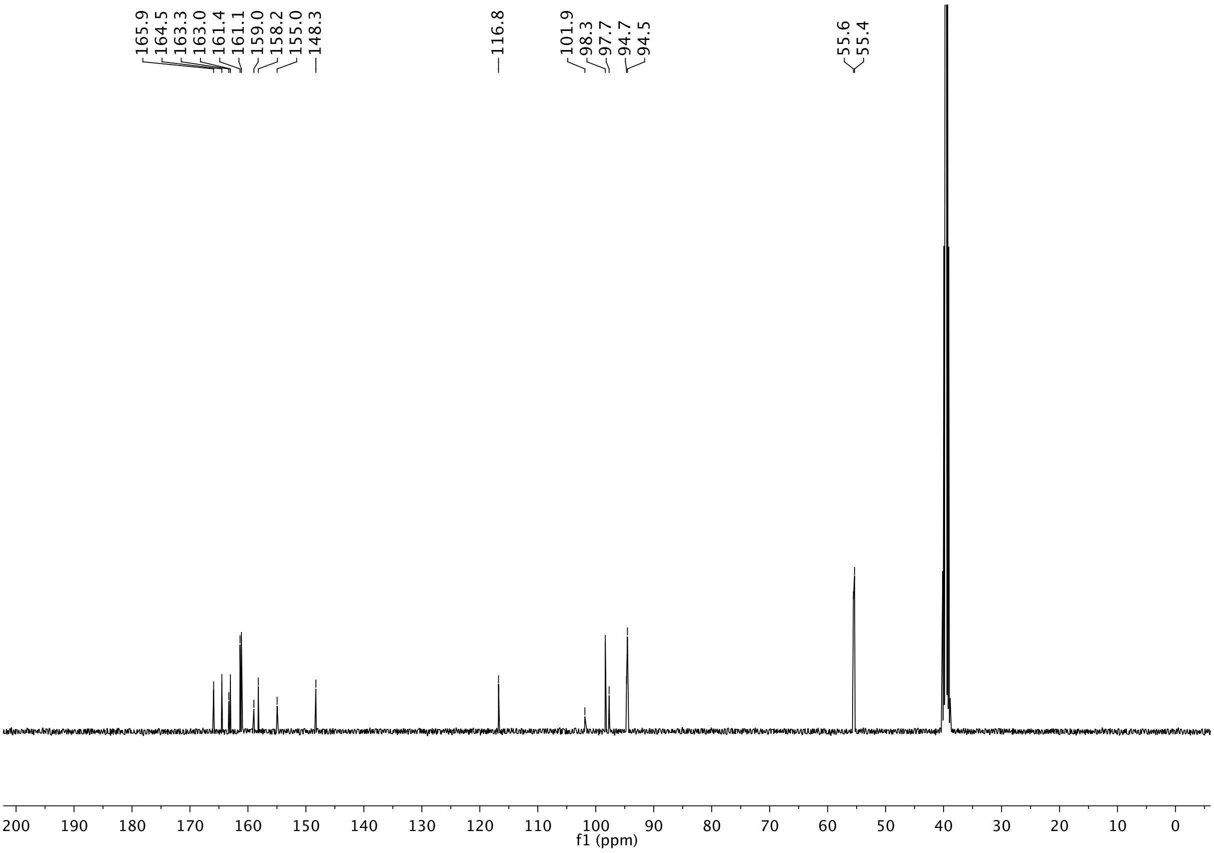
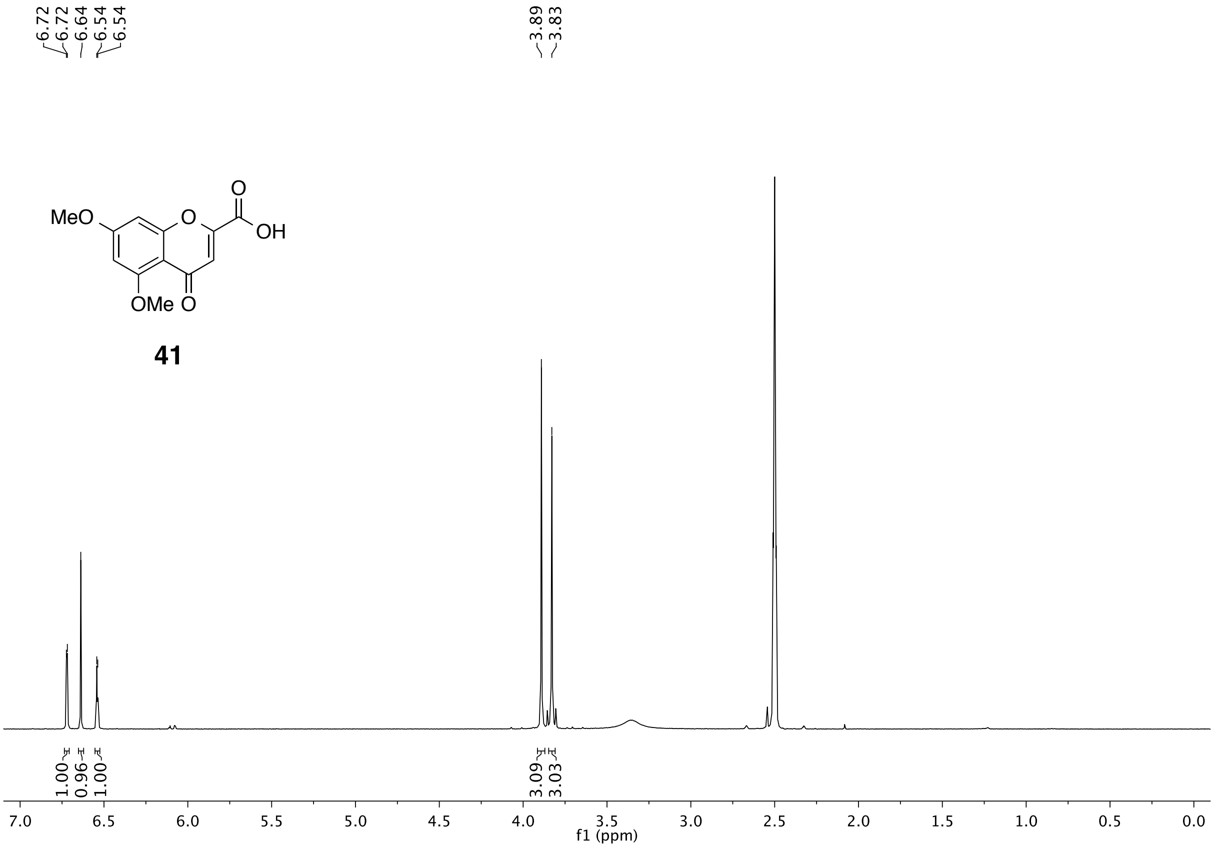
6.29
6.28
6.23
6.22
6.22
6.05
6.05
5.23
3.75
3.71
MeO
O
COOH
COOH
OMe
0.99
0.84
1.70
1.00
2.02
0.82
5.09
6.08
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

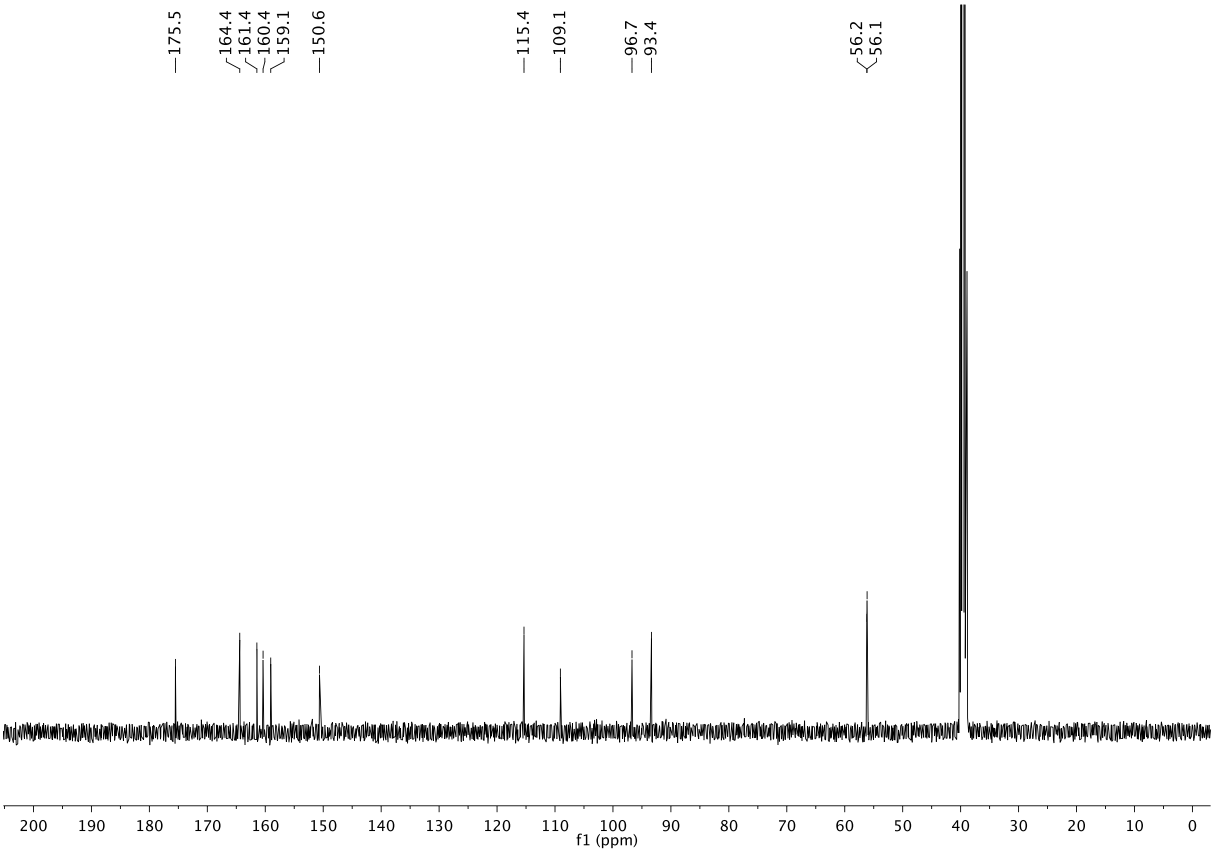
40


165.9
164.5
163.3
163.0
161.4
161.1
159.0
158.2
155.0
148.3
116.8
101.9
98.3
97.7
94.7
94.5
55.6
55.4
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

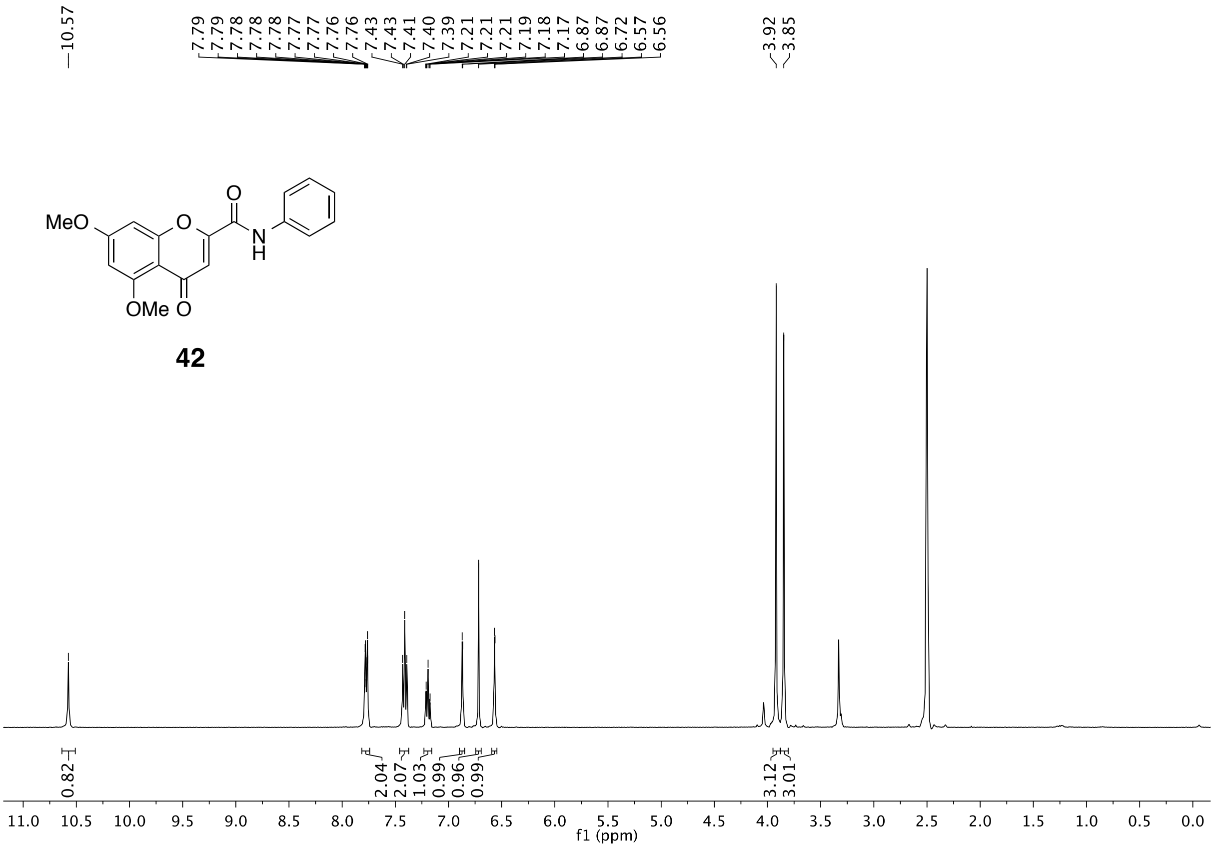


41

6.72
6.72
6.64
6.54
6.54
3.89
3.83
MeO
O
O
OH
OMe
O
1.00
0.96
1.00
3.09
3.03
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



175.5
164.4
161.4
160.4
159.1
150.6
115.4
109.1
96.7
93.4
56.2
56.1
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.79
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7.77
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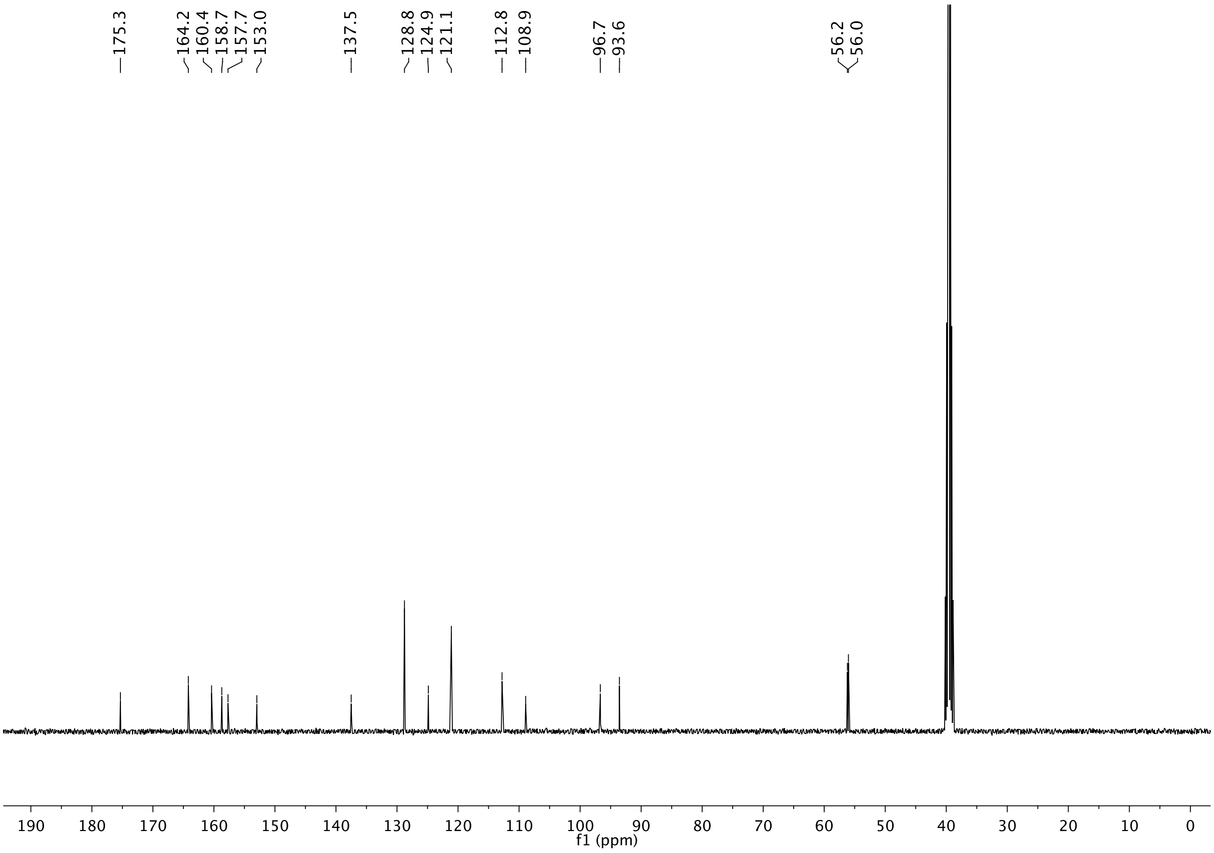
7.41
7.40
7.39
7.21
7.21
7.21
7.19
7.18
7.17
6.87
6.87
6.72
6.57
6.56
3.92
3.85
MeO
O
O
N
H
OMe
O
42
0.82
2.04
2.07
1.03
0.99
0.96
0.99
3.12
3.01
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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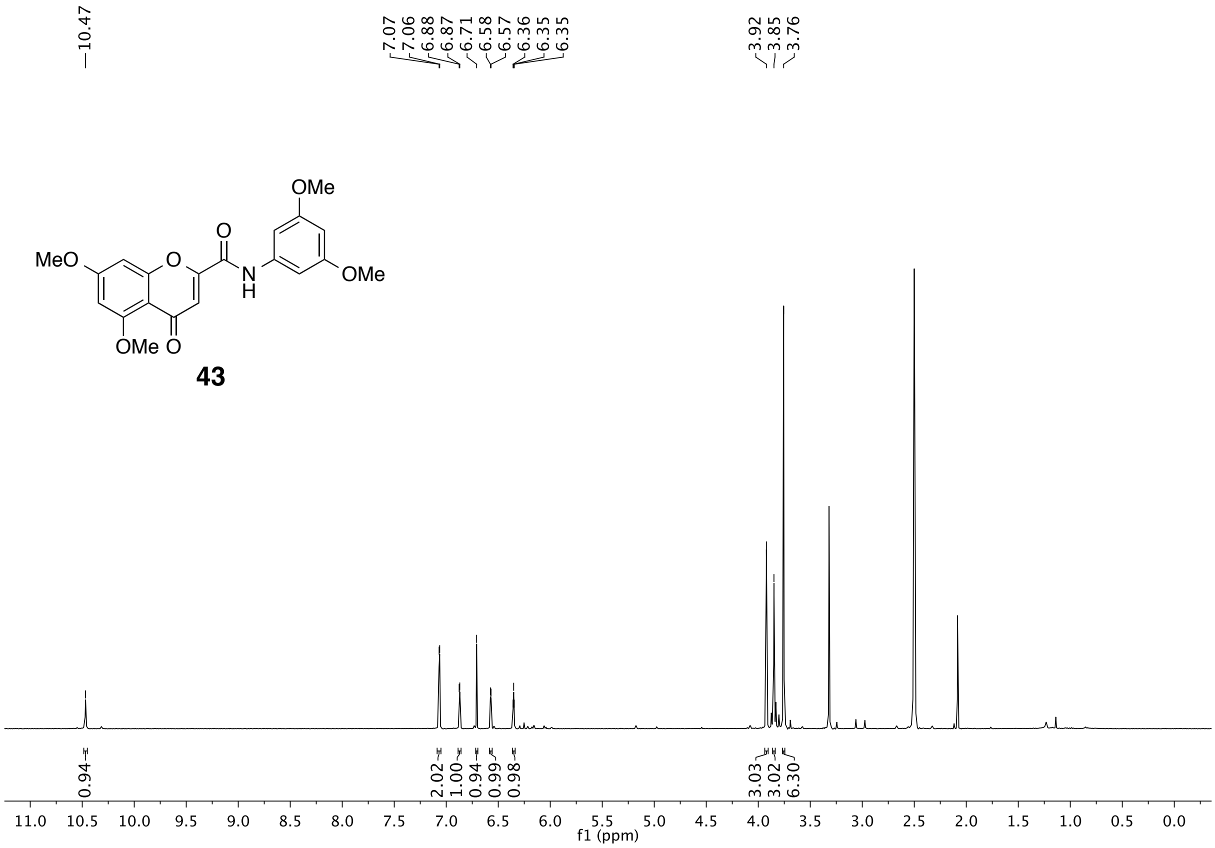
164.2
160.4
158.7
157.7
153.0
137.5
128.8
124.9
121.1
112.8
108.9
96.7
93.6
56.2
56.0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

OMe
O
MeO
O
N
H
OMe
OMe
O

43



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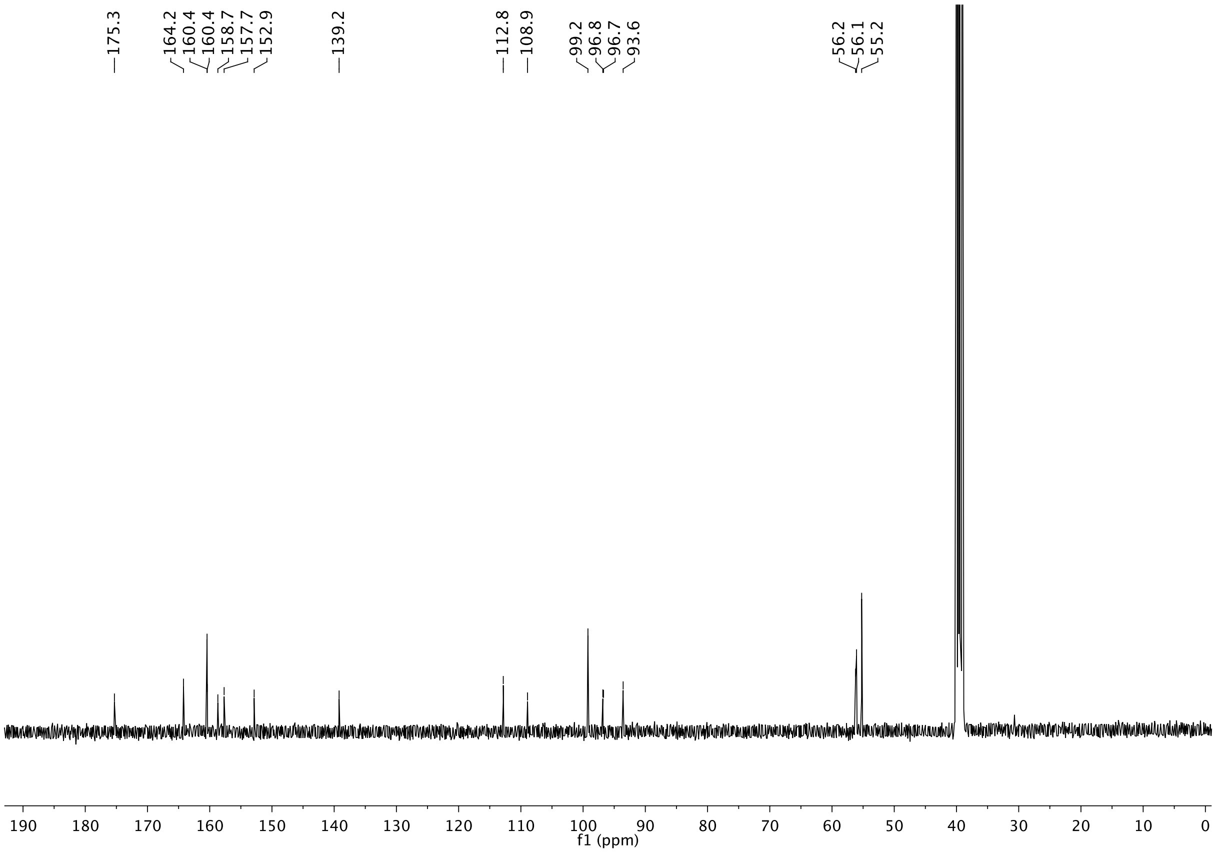
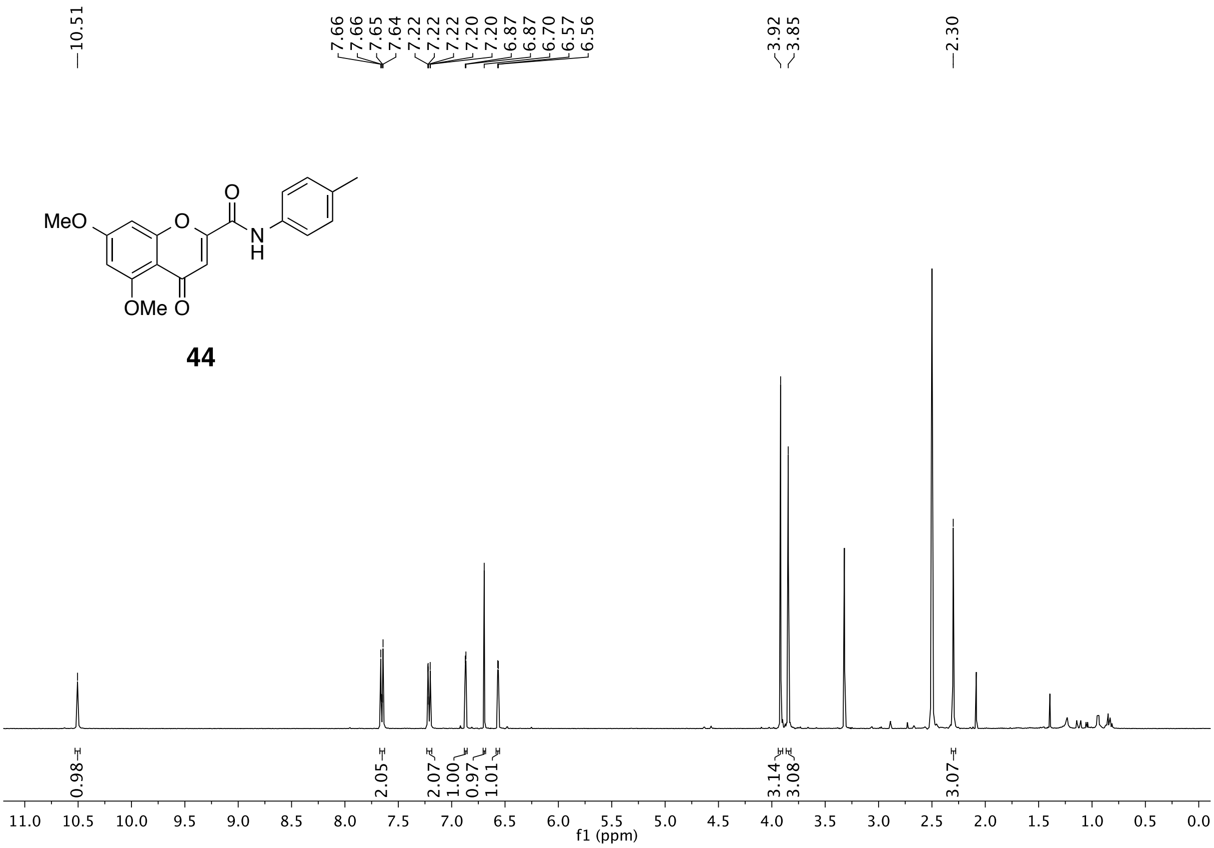
7.07
7.06
6.88
6.87
6.71
6.58
6.57
6.36
6.35
6.35
3.92
3.85
3.76
0.94
2.02
1.00
0.94
0.99
0.98
3.03
3.02
6.30
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



175.3
164.2
160.4
160.4
158.7
157.7
152.9
139.2
112.8
108.9
99.2
96.8
96.7
93.6
56.2
56.1
55.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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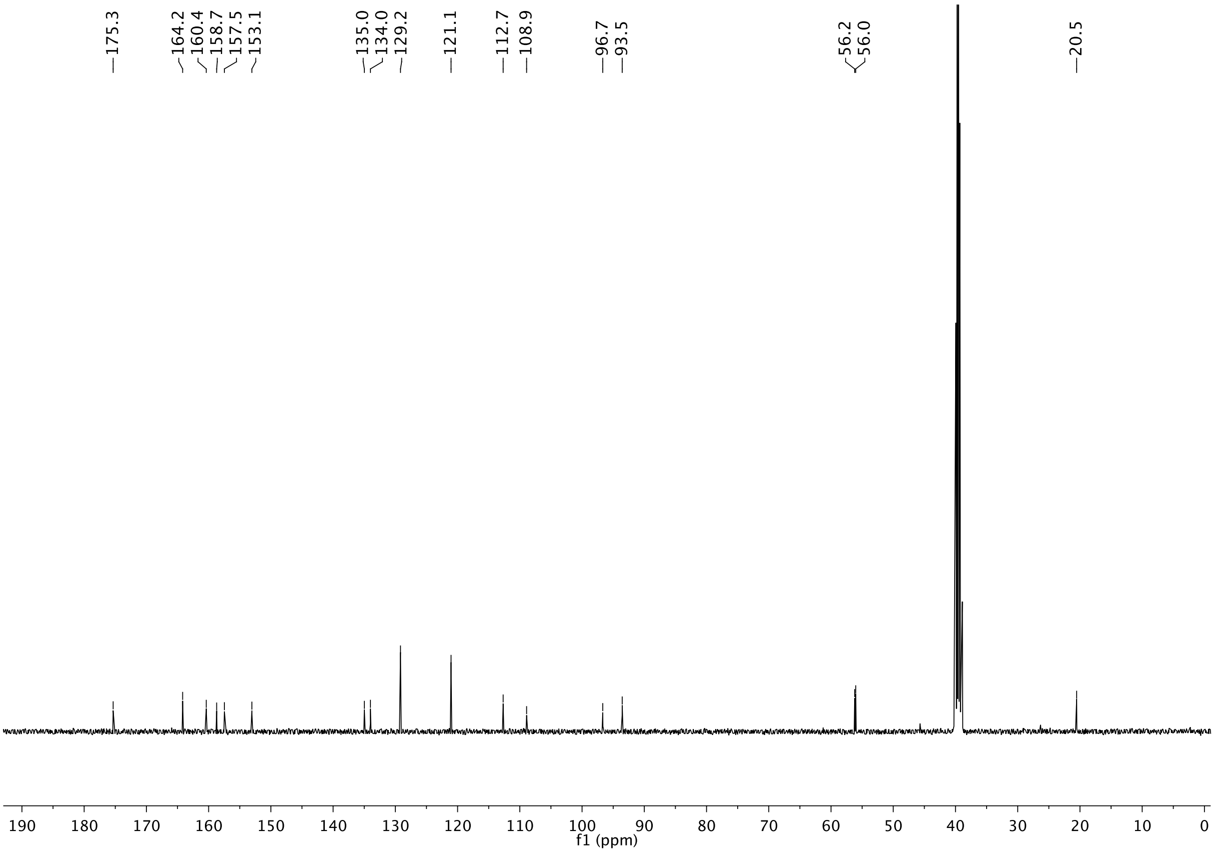
7.22
7.20
7.20
6.87
6.87
6.70
6.57
6.56
3.92
3.85
2.30
MeO
O
O
N
H
OMe
O
44
0.98
2.05
2.07
1.00
0.97
1.01
3.14
3.08
3.07
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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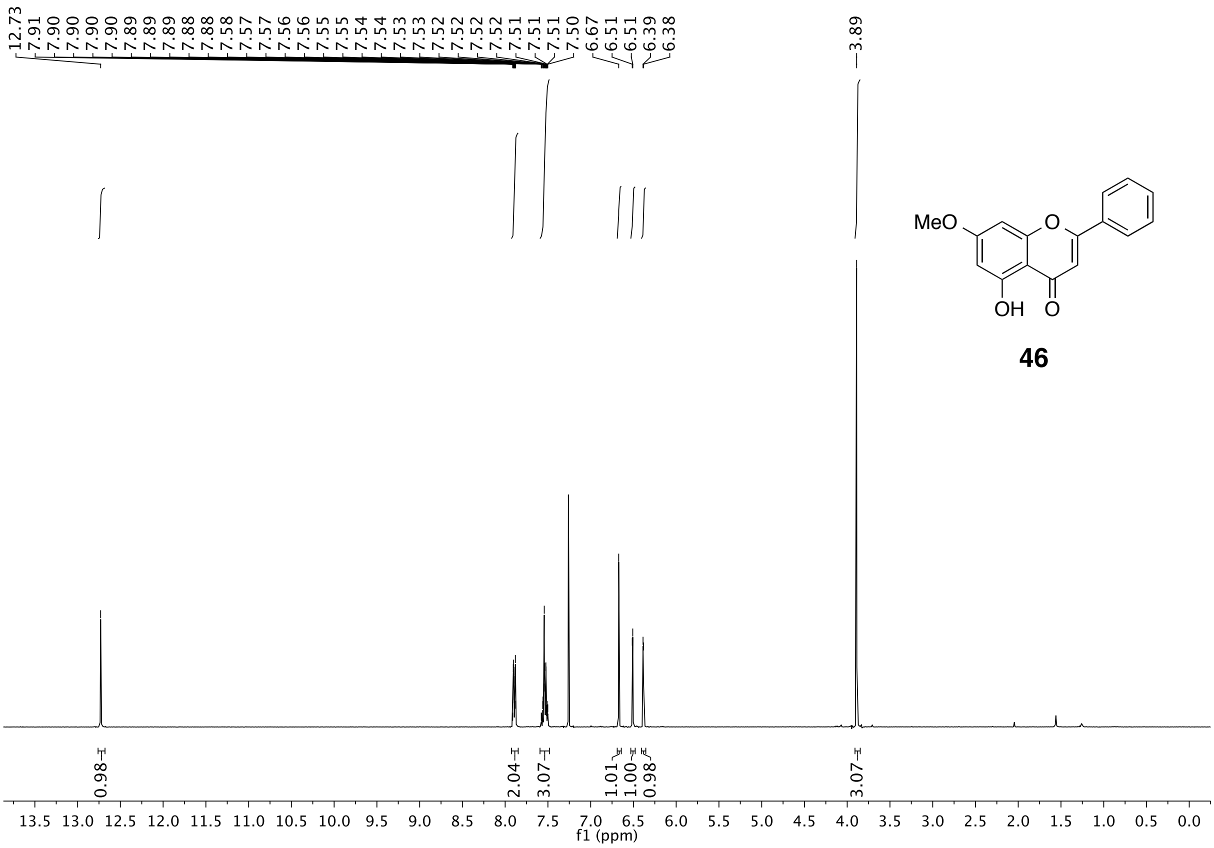
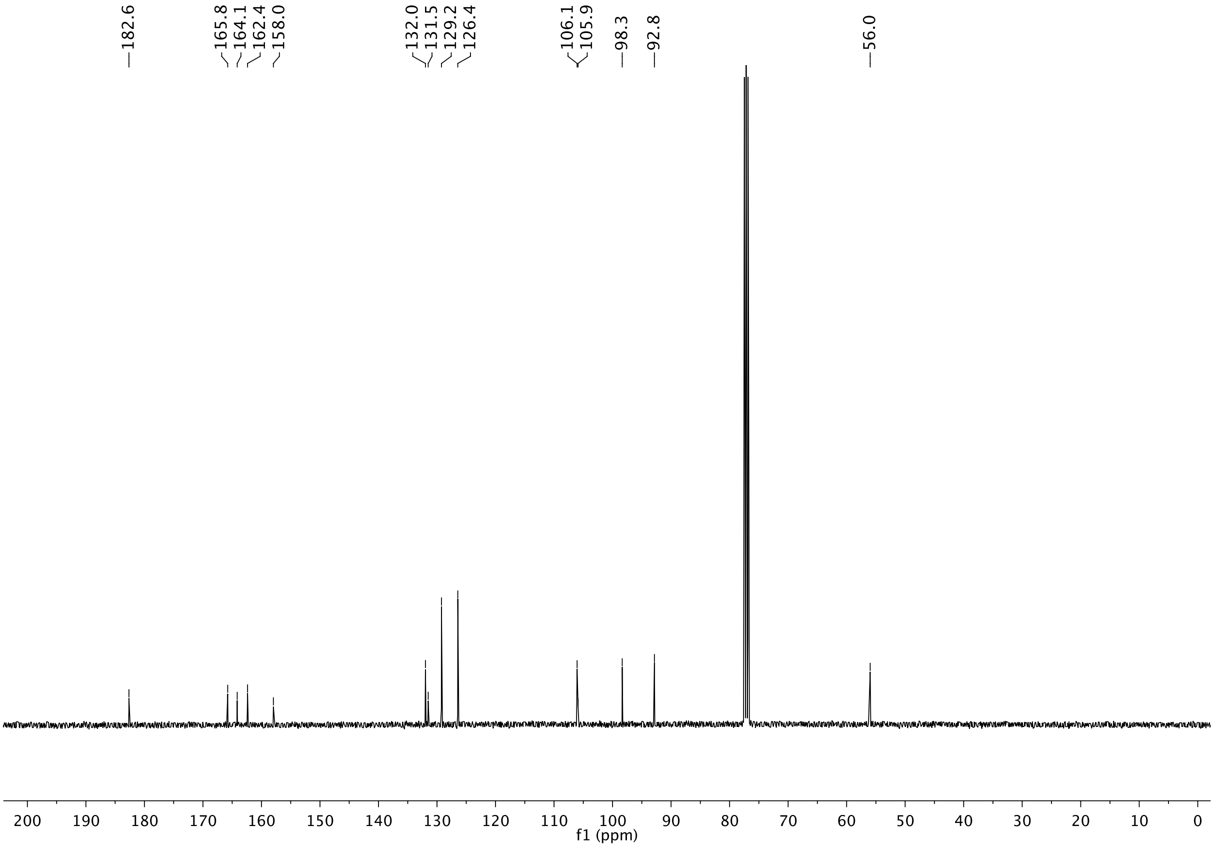
158.7
157.5
153.1
135.0
134.0
129.2
121.1
112.7
108.9
96.7
93.5
56.2
56.0
20.5
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


12.73
7.91
7.90
7.90
7.90
7.90
7.89
7.89
7.89
7.88
7.88
7.58
7.57
7.57
7.56
7.56
7.55
7.55
7.54
7.54
7.53
7.53
7.52
7.52
7.52
7.52
7.51
7.51
7.51
7.50
6.67
6.51
6.51
6.39
6.38
3.89
MeO
O
OH
O
0.98
2.04
3.07
1.01
1.00
0.98
3.07
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

46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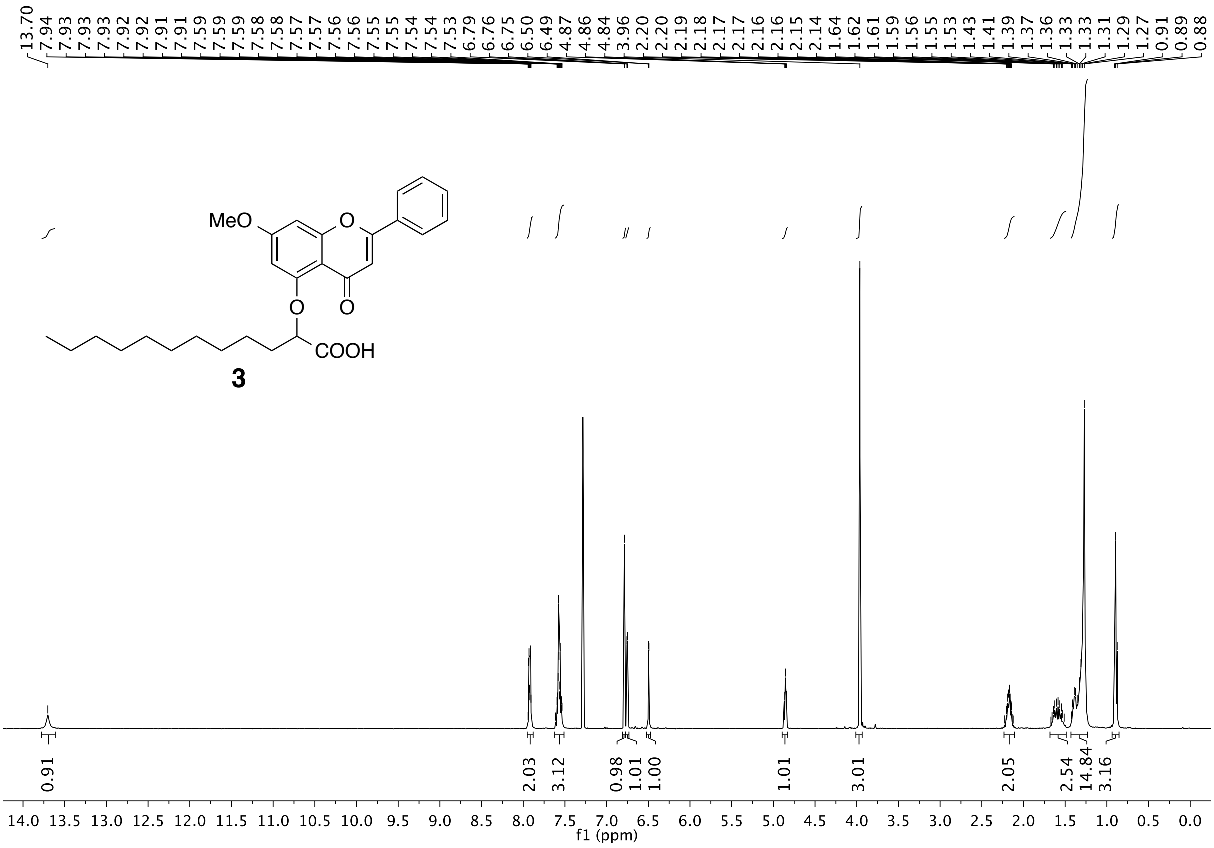
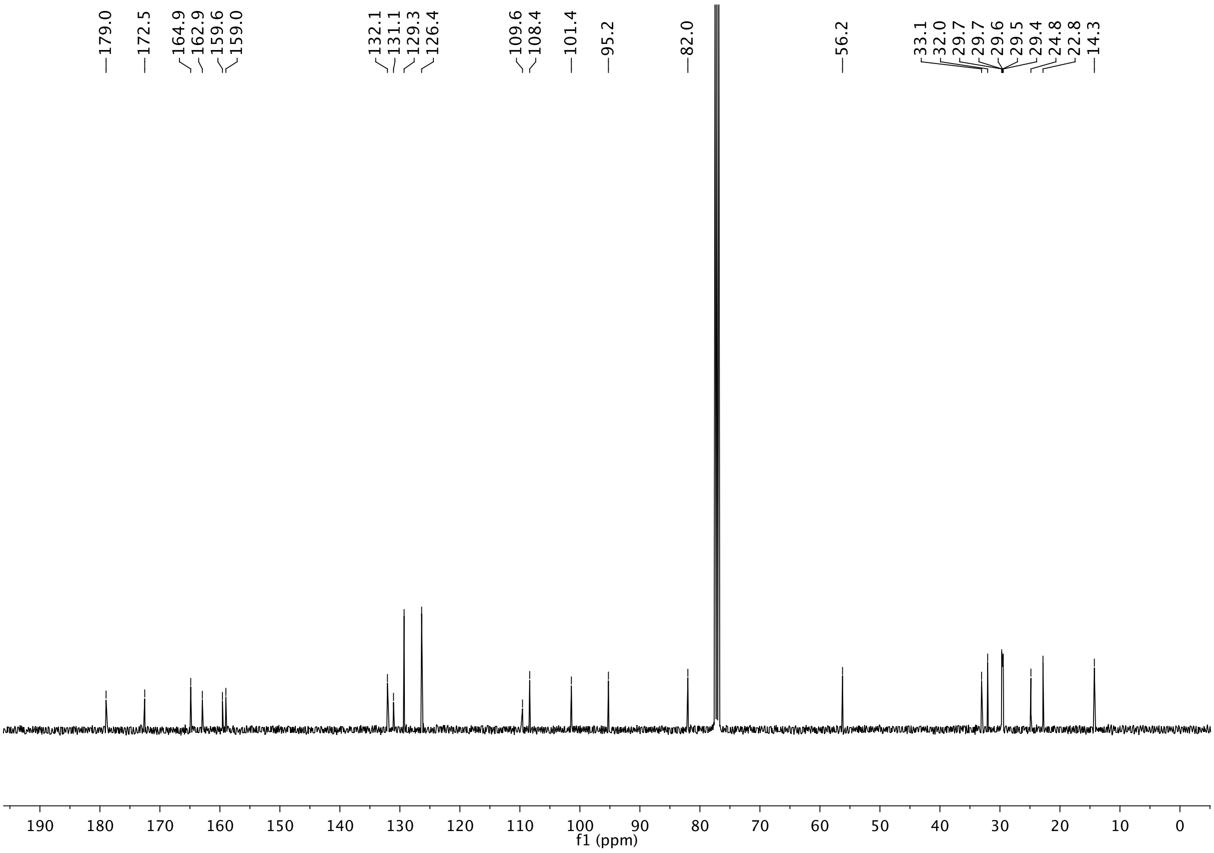
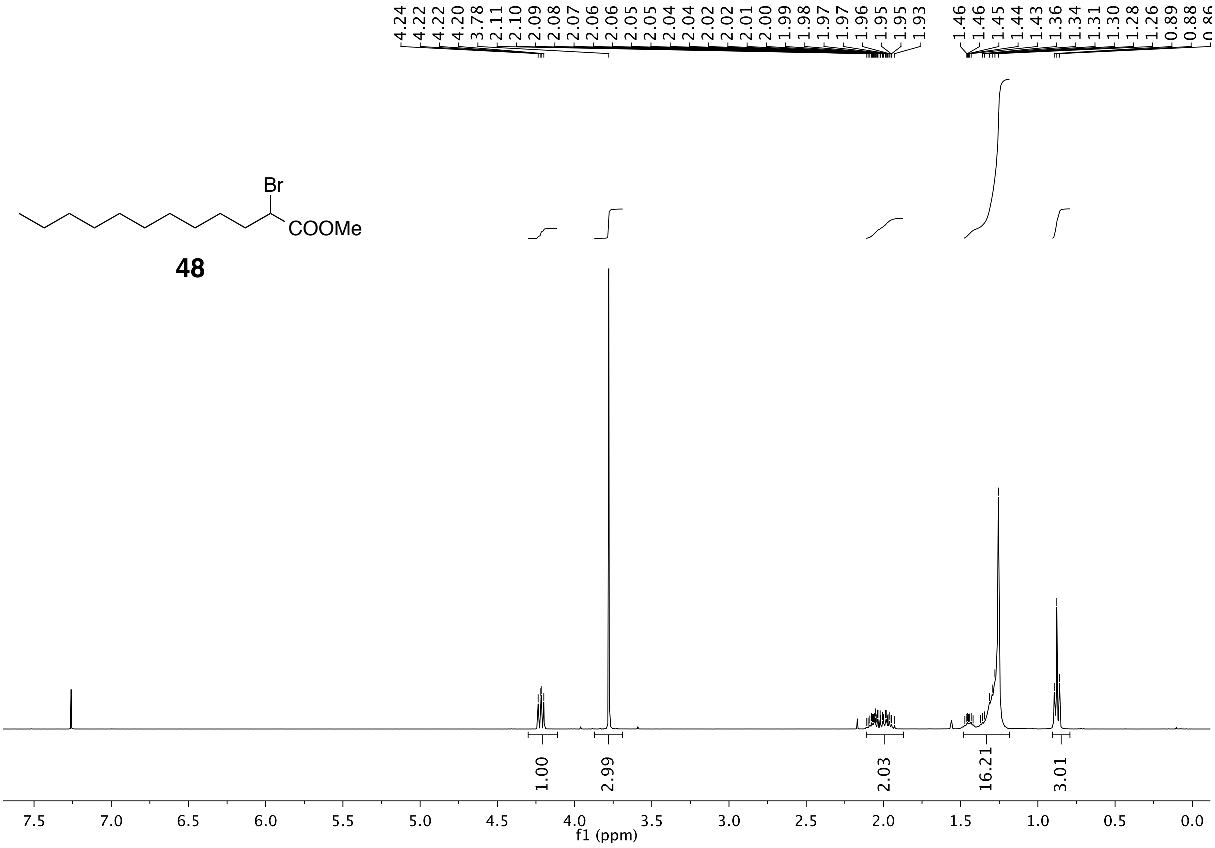
165.8
164.1
162.4
158.0
132.0
131.5
129.2
126.4
106.1
105.9
98.3
92.8
56.0
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


13.70
7.94
7.93
7.93
7.92
7.92
7.91
7.91
7.59
7.59
7.59
7.58
7.58
7.57
7.57
7.56
7.56
7.55
7.55
7.54
7.54
7.53
6.79
6.76
6.75
6.50
6.49
4.87
4.86
4.84
3.96
2.20
2.20
2.19
2.18
2.17
2.17
2.16
2.16
2.15
2.14
1.64
1.62
1.61
1.59
1.56
1.55
1.53
1.43
1.41
1.39
1.37
1.36
1.33
1.33
1.31
1.29
1.27
0.91
0.89
0.88
MeO
O
O
O
COOH
3
0.91
2.03
3.12
0.98
1.01
1.00
1.01
3.01
2.05
2.54
14.84
3.16
14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


179.0
172.5
164.9
162.9
159.6
159.0
132.1
131.1
129.3
126.4
109.6
108.4
101.4
95.2
82.0
56.2
33.1
32.0
29.7
29.7
29.6
29.5
29.4
24.8
22.8
14.3
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


4.24
4.22
4.22
4.20
3.78
2.11
2.10
2.09
2.08
2.07
2.06
2.06
2.05
2.05
2.04
2.04
2.02
2.02
2.01
2.00
1.99
1.98
1.97
1.97
1.96
1.95
1.95
1.93
1.46
1.46
1.45
1.44
1.43
1.36
1.34
1.31
1.30
1.28
1.26
0.89
0.88
0.86
Br
COOMe
1.00
2.99
2.03
16.21
3.01
7.5
7.0
6.5
6.0
5.5
5.0
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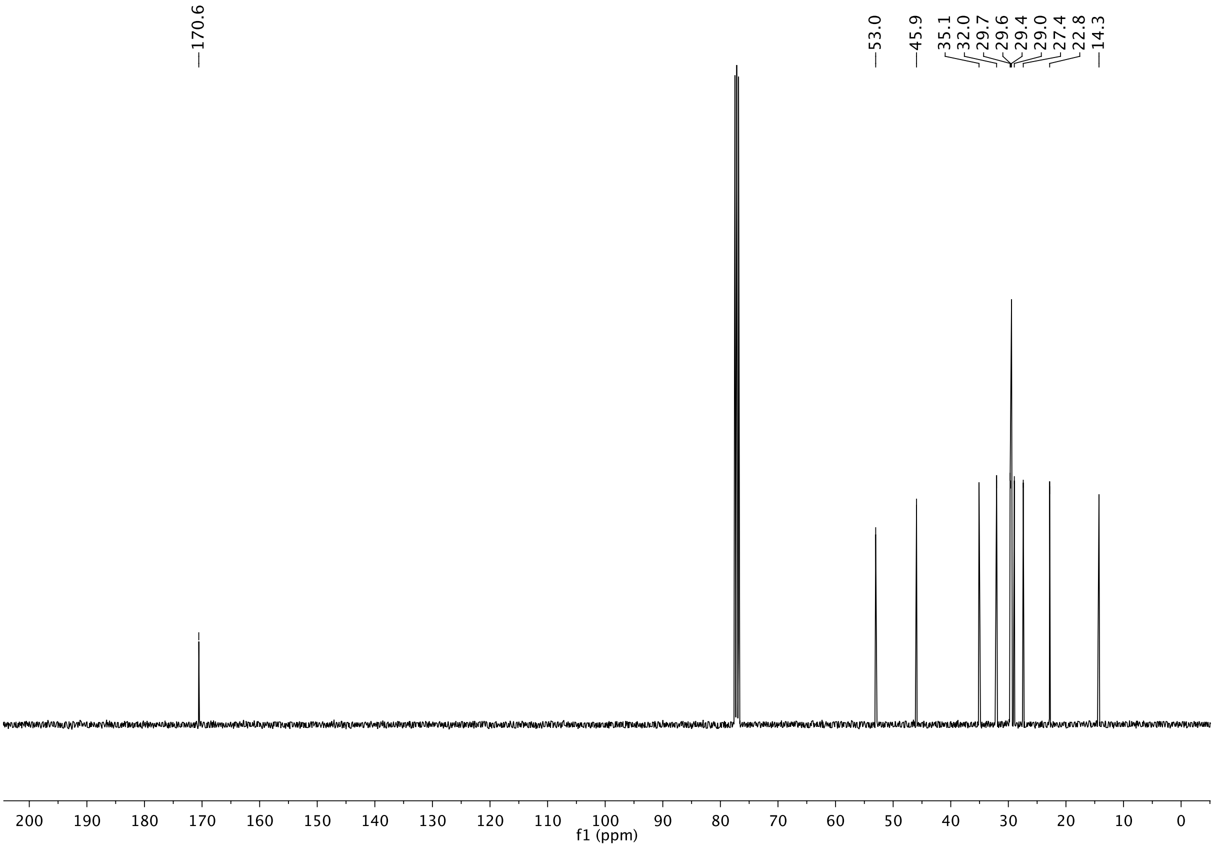
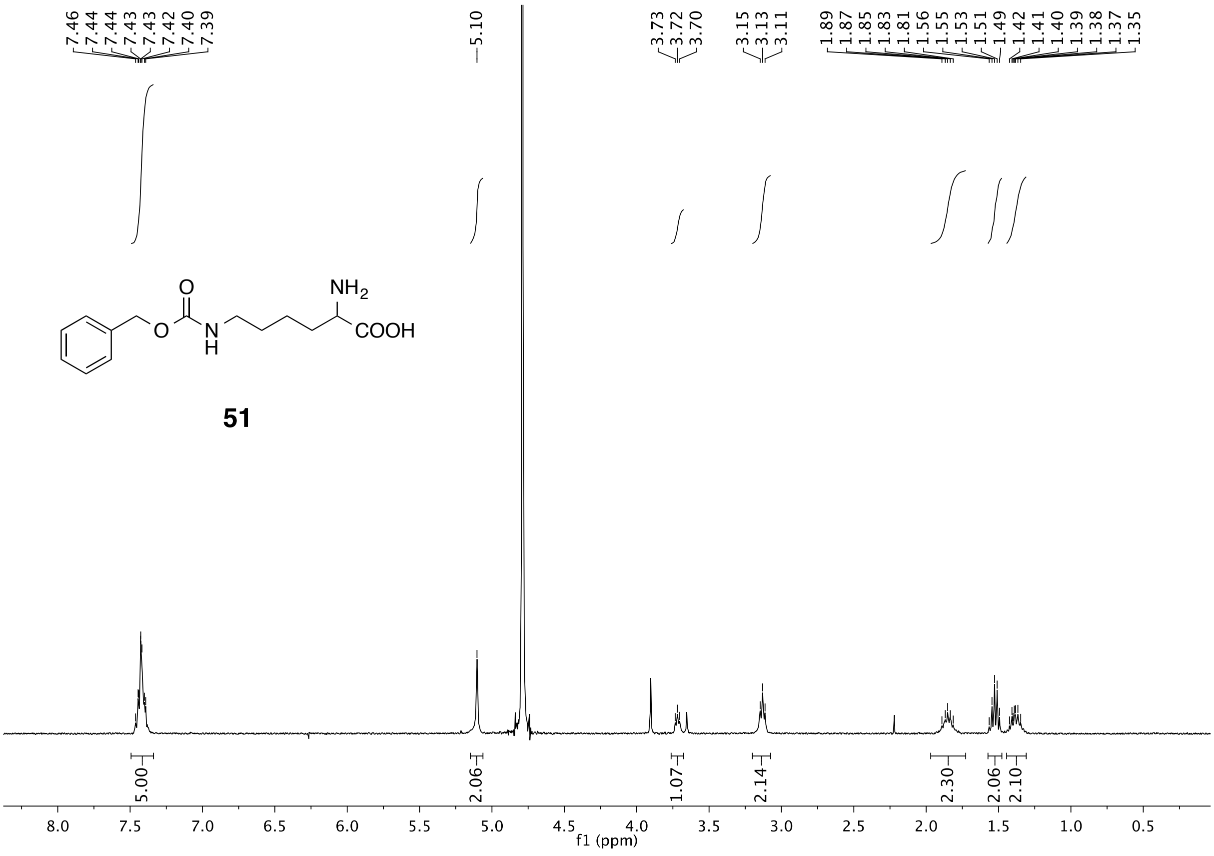
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

48


170.6
53.0
45.9
35.1
32.0
29.7
29.6
29.4
29.0
27.4
22.8
14.3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.44
7.44
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7.39
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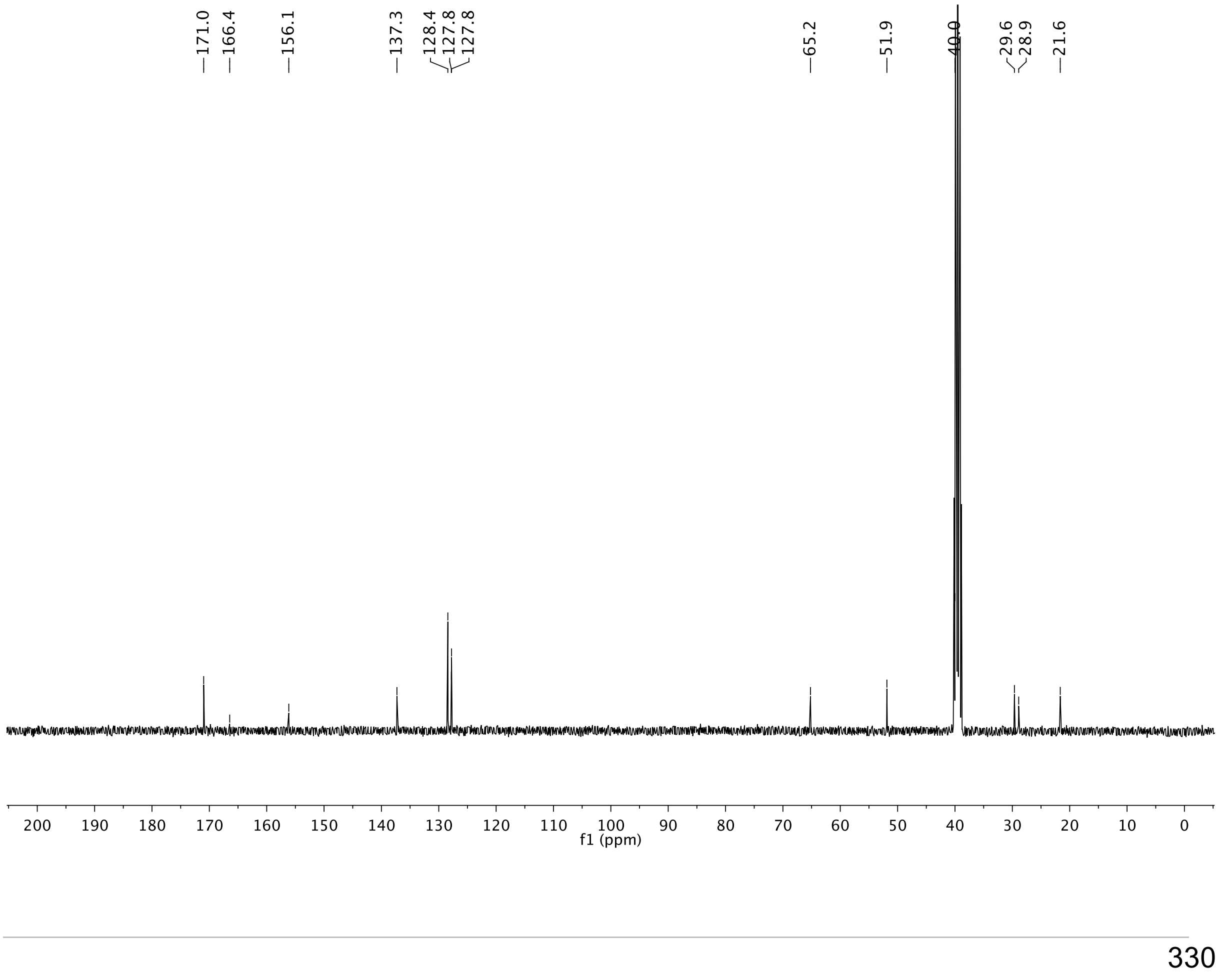
3.72
3.70
3.15
3.13
3.11
1.89
1.87
1.85
1.83
1.81
1.56
1.55
1.53
1.51
1.49
1.42
1.41
1.40
1.39
1.38
1.37
1.35
O
NH2
O
N
H
COOH
5.00
2.06
1.07
2.14
2.30
2.06
2.10
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

51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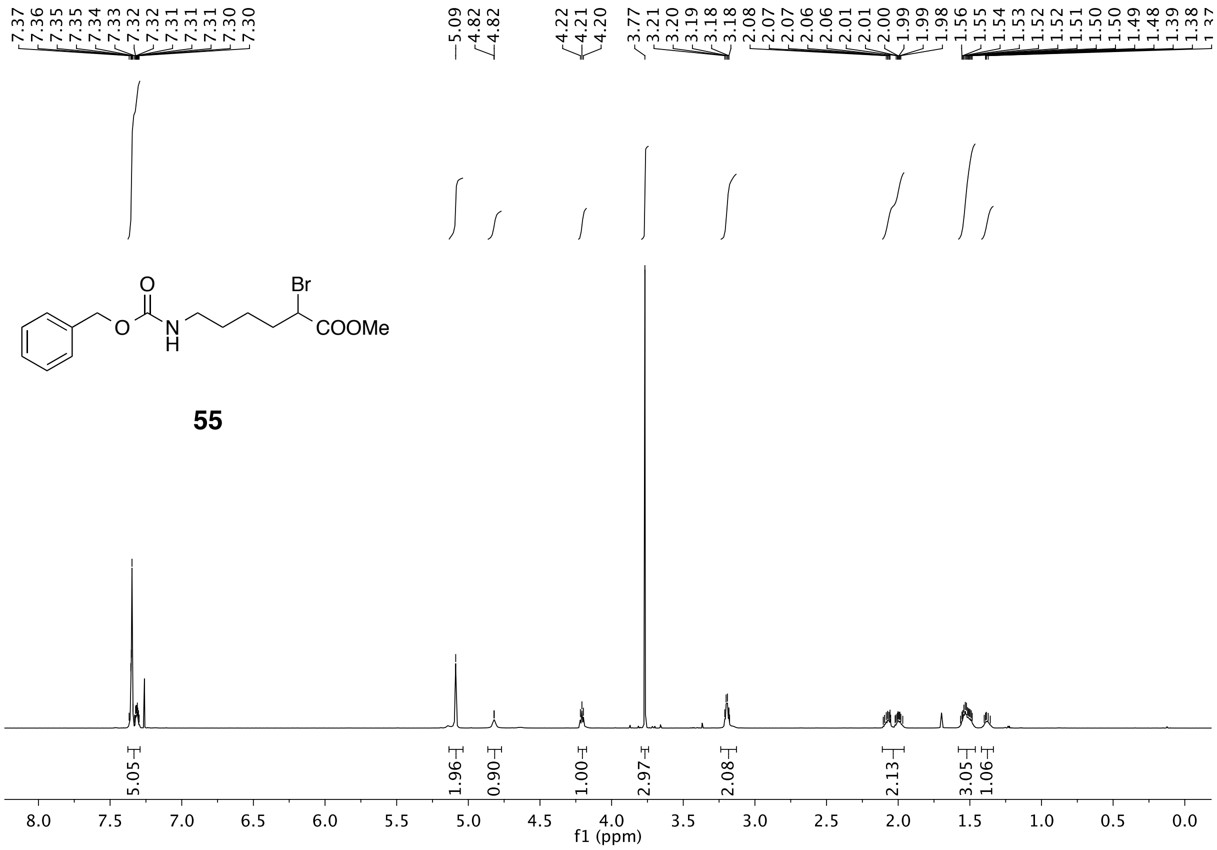
166.4
156.1
137.3
128.4
127.8
127.8
65.2
51.9
40.0
29.6
28.9
21.6
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.32
7.32
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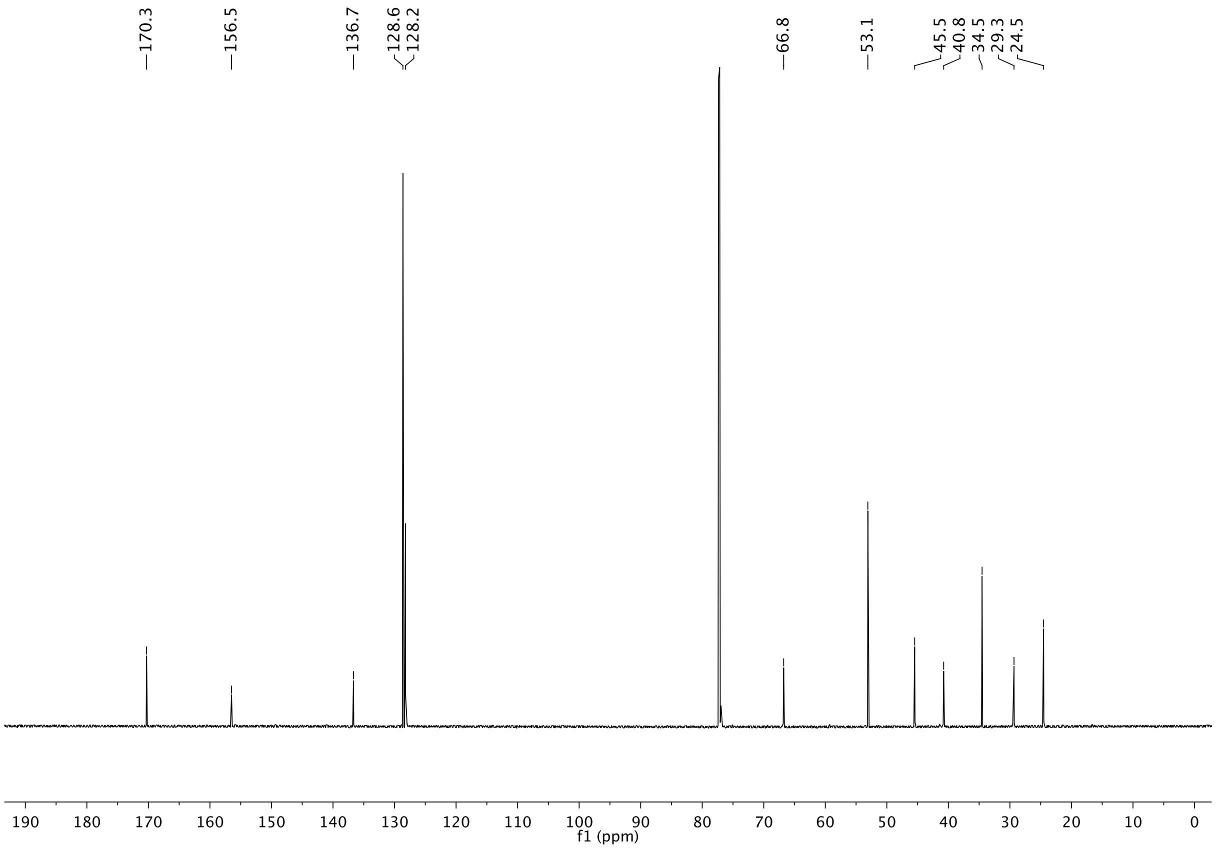
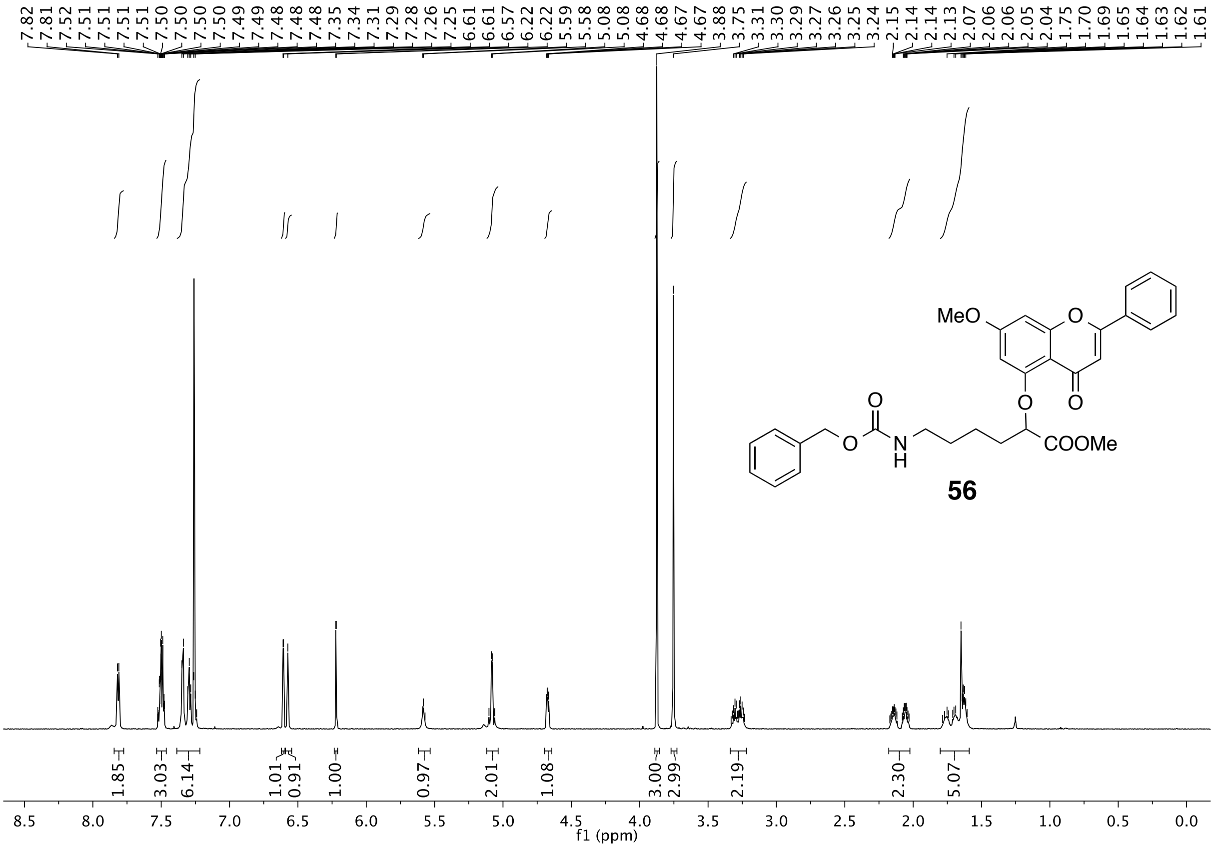
7.30
5.09
4.82
4.82
4.22
4.21
4.20
3.77
3.21
3.20
3.19
3.18
3.18
2.08
2.07
2.07
2.06
2.06
2.01
2.01
2.00
1.99
1.99
1.98
1.56
1.55
1.54
1.53
1.52
1.52
1.51
1.50
1.50
1.49
1.48
1.39
1.38
O
Br
O
N
H
COOMe
5.05
1.96
0.90
1.00
2.97
2.08
2.13
3.05
1.06
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

55


170.3
156.5
136.7
128.6
128.2
66.8
53.1
45.5
40.8
34.5
29.3
24.5
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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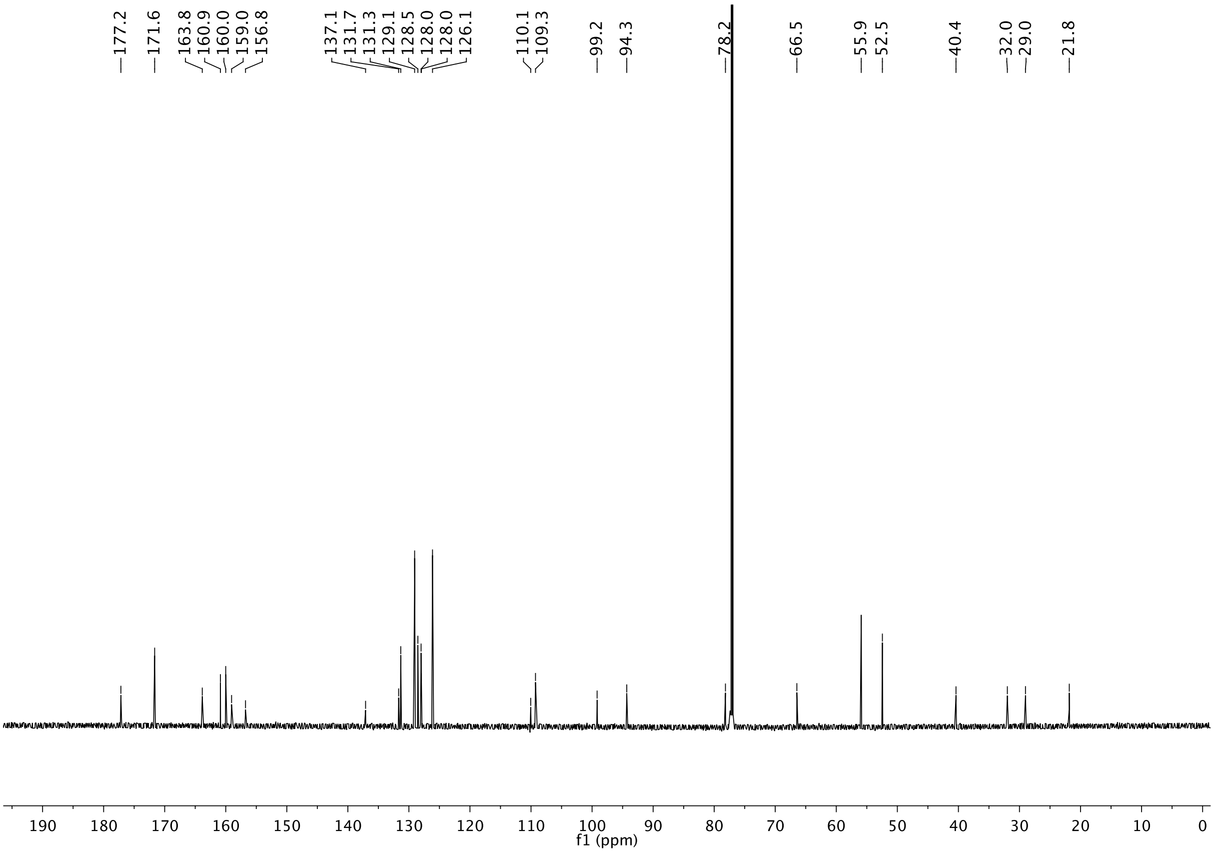
7.48
7.35
7.34
7.31
7.29
7.28
7.26
7.25
6.61
6.61
6.57
6.22
6.22
5.59
5.58
5.08
5.08
4.68
4.68
4.67
3.88
3.75
3.31
3.30
3.29
3.27
3.26
3.25
3.24
2.15
2.14
2.14
2.13
2.07
2.06
2.06
2.05
2.04
1.75
1.70
1.69
1.65
1.64
1.63
1.62
1.61
MeO
O
O
O
O
N
H
COOMe
56
1.85
3.03
6.14
1.01
0.91
1.00
0.97
2.01
1.08
3.00
2.99
2.19
2.30
5.07
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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131.3
129.1
128.5
128.0
128.0
126.1
110.1
109.3
99.2
94.3
78.2
66.5
55.9
52.5
40.4
32.0
29.0
21.8
190
180
170
160
150
140
130
120
110
100
90
80
70
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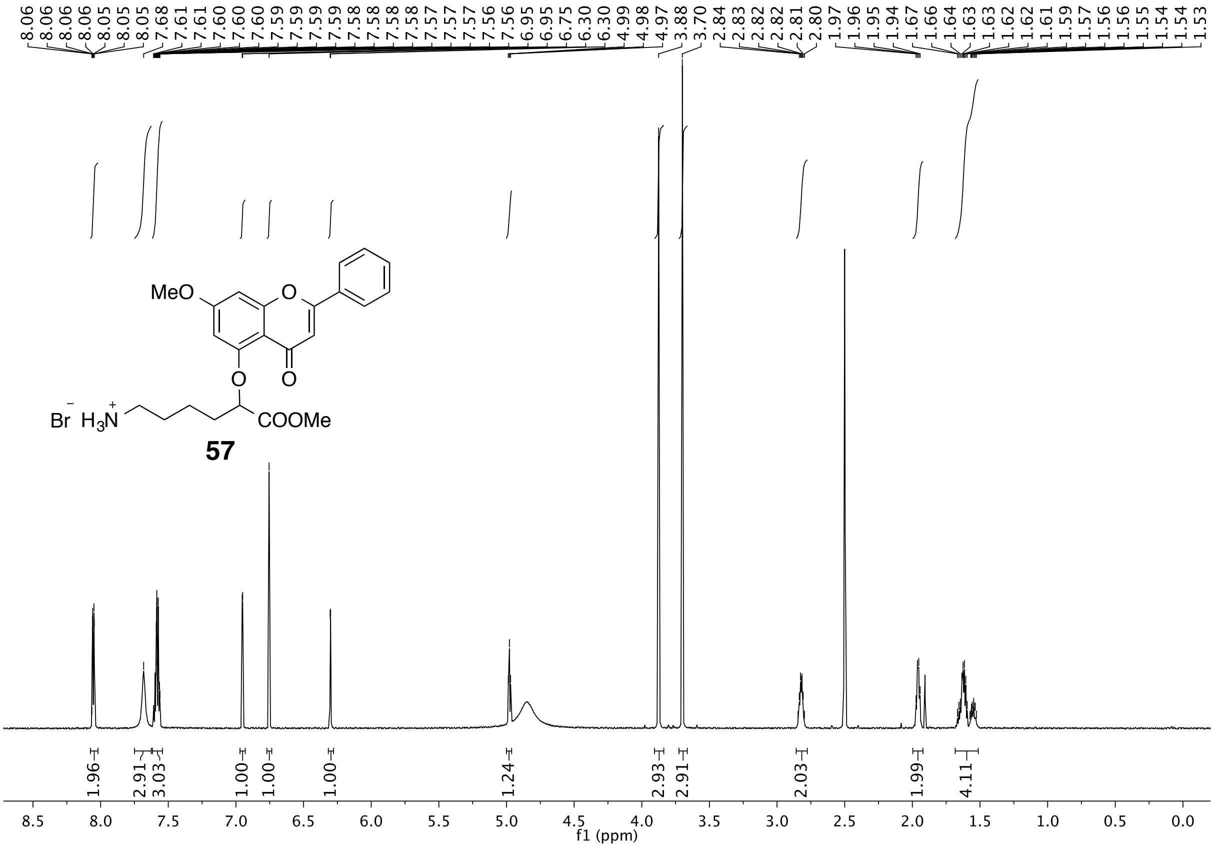
50
40
30
20
10
0
f1 (ppm)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7.57
7.56
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6.75
6.30
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4.97
3.88
3.70
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2.82
2.82
2.81
2.80
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1.96
1.95
1.94
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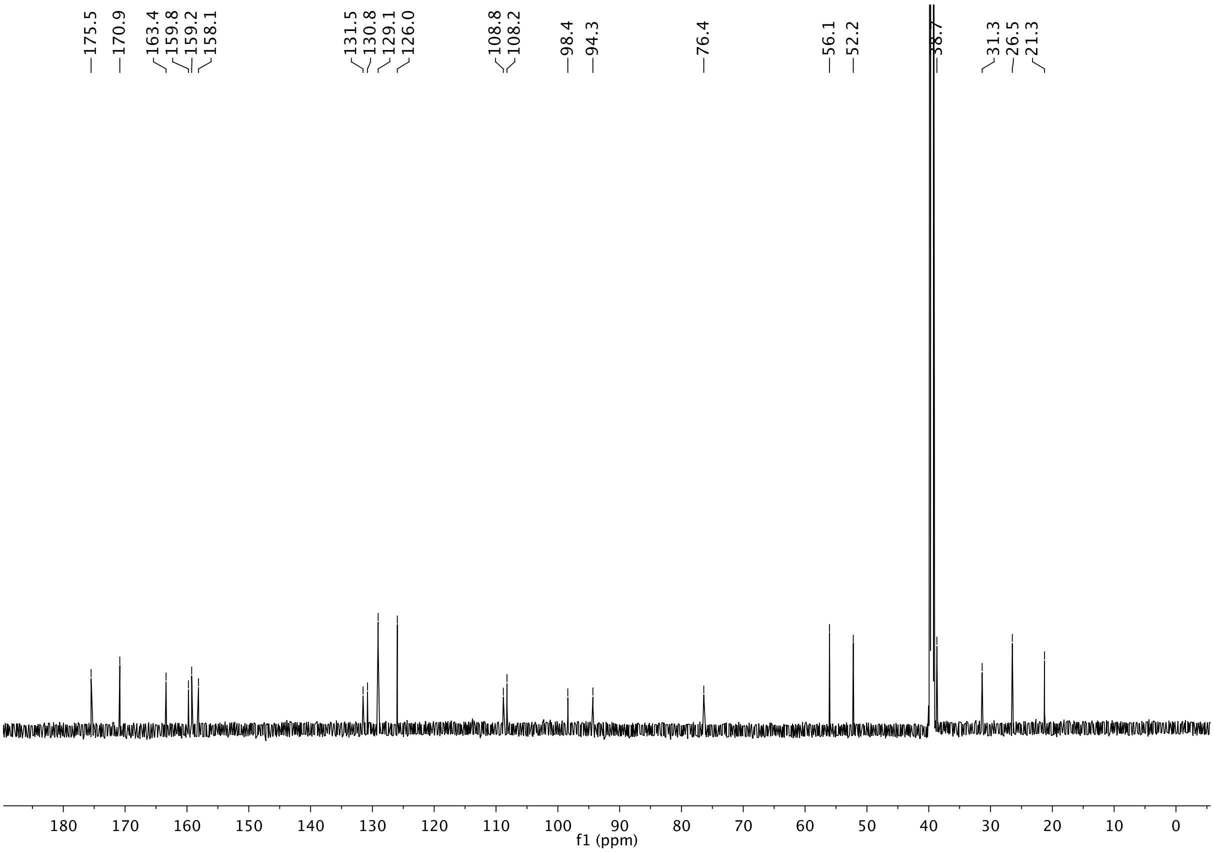
1.63
1.63
1.62
1.62
1.61
1.59
1.57
1.56
1.56
1.55
1.54
1.54
1.53
MeO
O
O
O
Br⁻ H3N⁺
COOMe
1.96
2.91
3.03
1.00
1.00
1.00
1.24
2.93
2.91
2.03
1.99
4.11
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

57


175.5
170.9
163.4
159.8
159.2
158.1
131.5
130.8
129.1
126.0
108.8
108.2
98.4
94.3
76.4
56.1
52.2
38.7
31.3
26.5
21.3
180
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160
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140
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120
110
100
90
80
70
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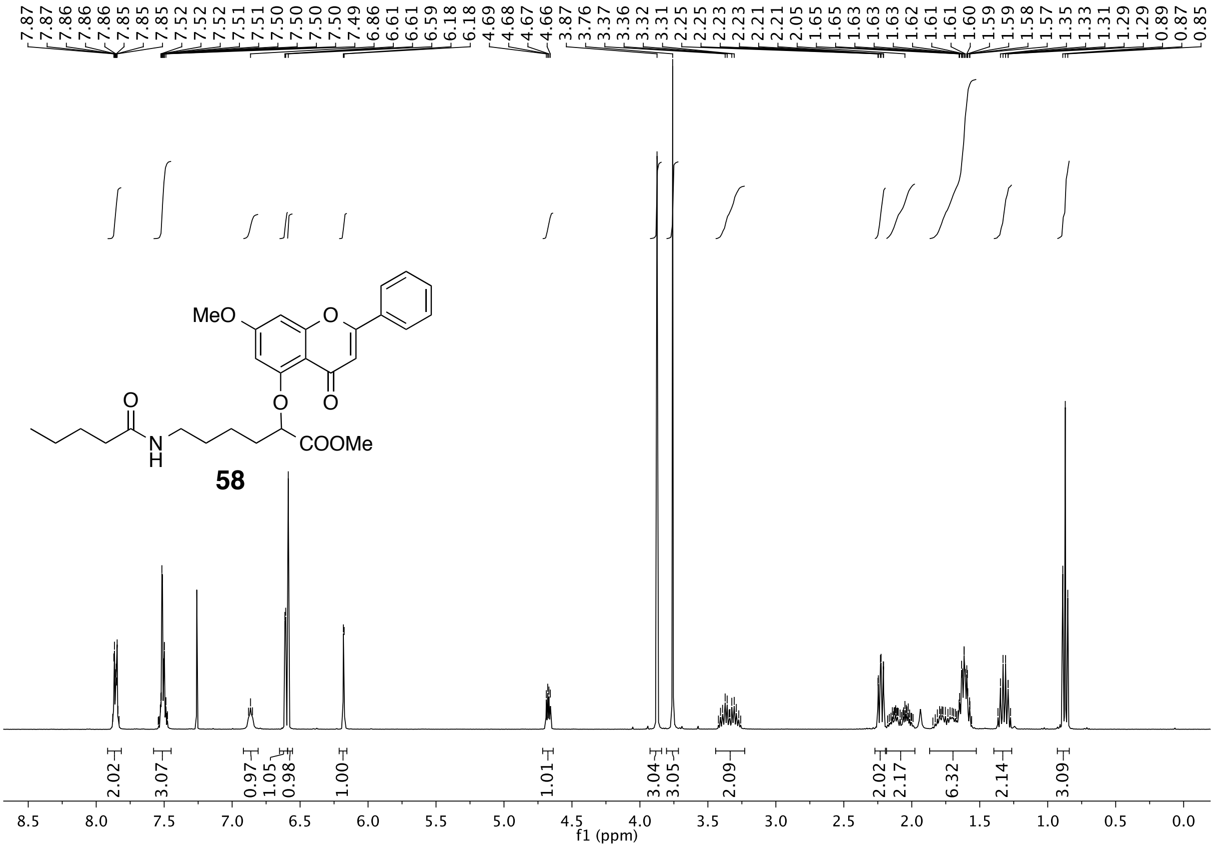
50
40
30
20
10
0
f1 (ppm)



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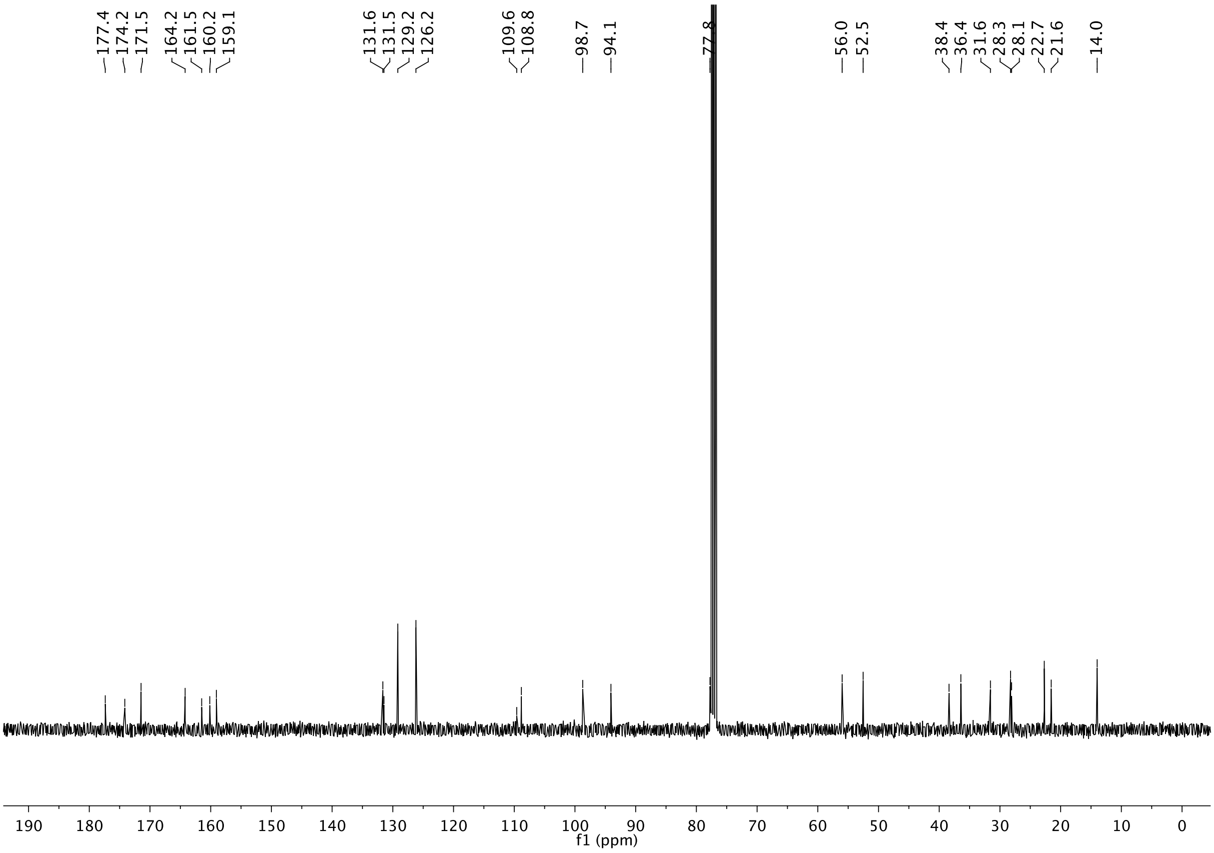
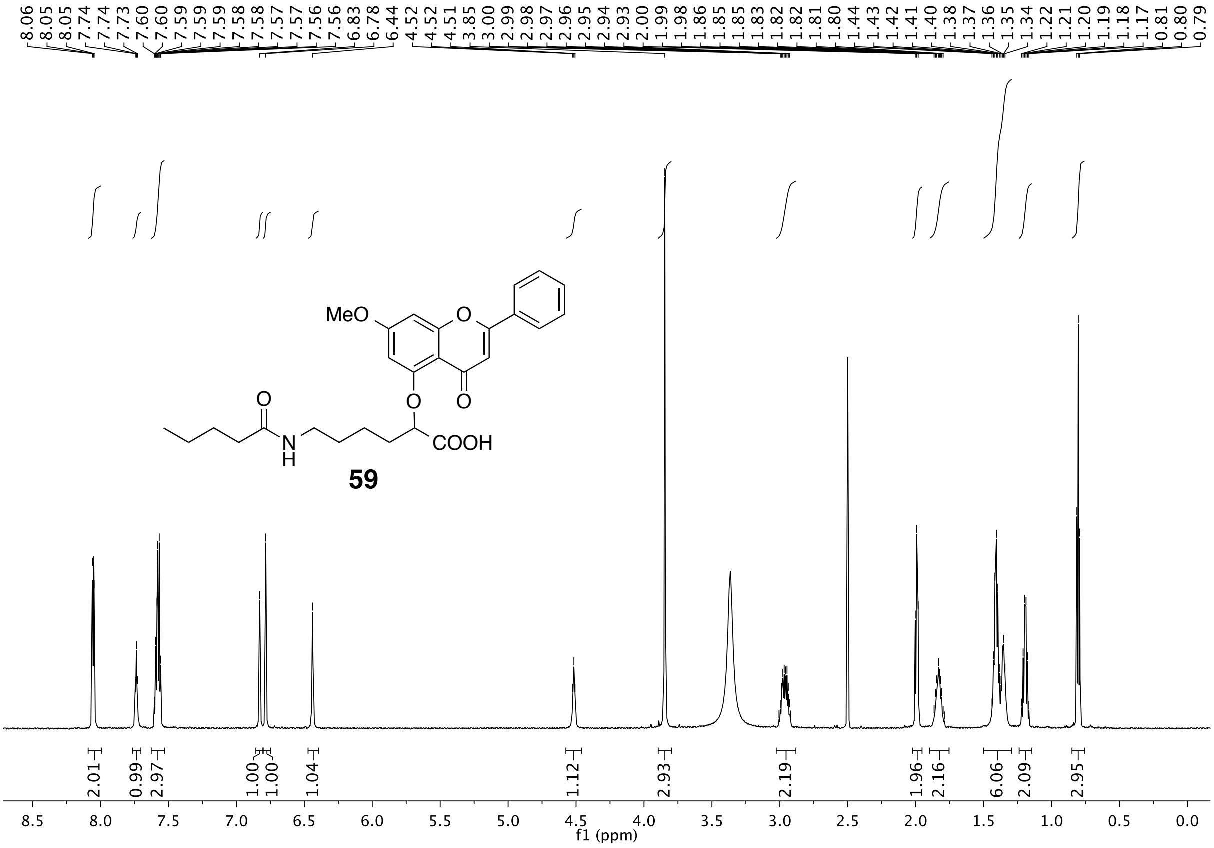
7.87 7.87 7.86 7.86 7.86 7.85 7.85 7.85 7.52 7.52 7.52 7.51 7.51 7.50 7.50 7.50 7.50 7.49 6.86 6.61 6.61 6.59 6.18 6.18 4.69 4.68 4.67 4.66 3.87 3.76 3.37 3.36 3.32 3.31 2.25 2.25 2.23 2.23 2.21 2.21 2.05 1.65 1.65 1.63 1.63 1.63 1.62 1.61 1.61 1.60 1.59 1.59 1.58 1.57 1.35 1.33 1.31 1.29 0.89 0.87 0.85
MeO
O
O
O
O
N
H
COOMe
2.02 3.07 0.97 1.05 0.98 1.00 1.01 3.04 3.05 2.09 2.02 2.17 6.32 2.14 3.09
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)



177.4 174.2 171.5 164.2 161.5 160.2 159.1 131.6 131.5 129.2 126.2 109.6 108.8 98.7 94.1 77.8 56.0 52.5 38.4 36.4 31.6 28.3 28.1 22.7 21.6 14.0
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.56
6.83
6.78
6.44
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4.52
4.51
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2.97
2.96
2.95
2.94
2.93
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1.98
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1.81
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1.35
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1.22
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0.80
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MeO
O
O
O
O
N
H
COOH
59
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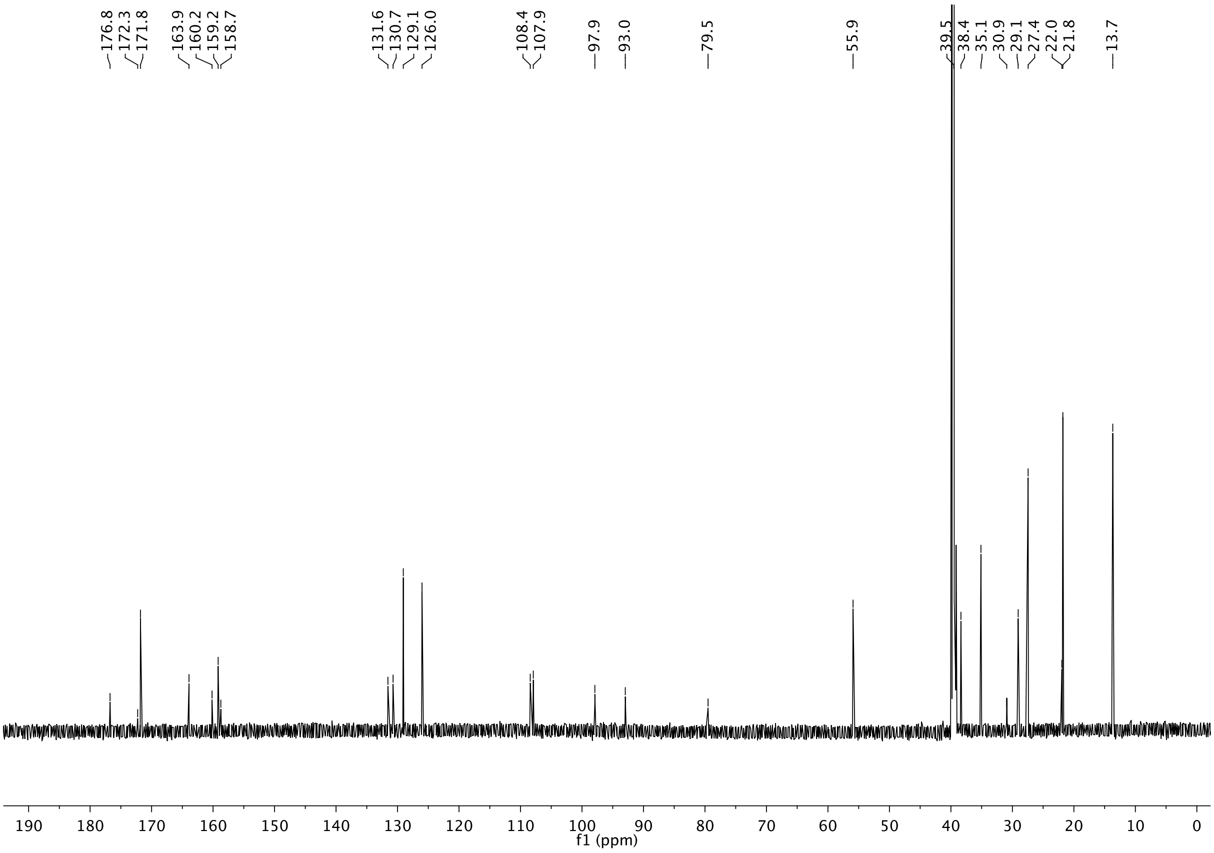
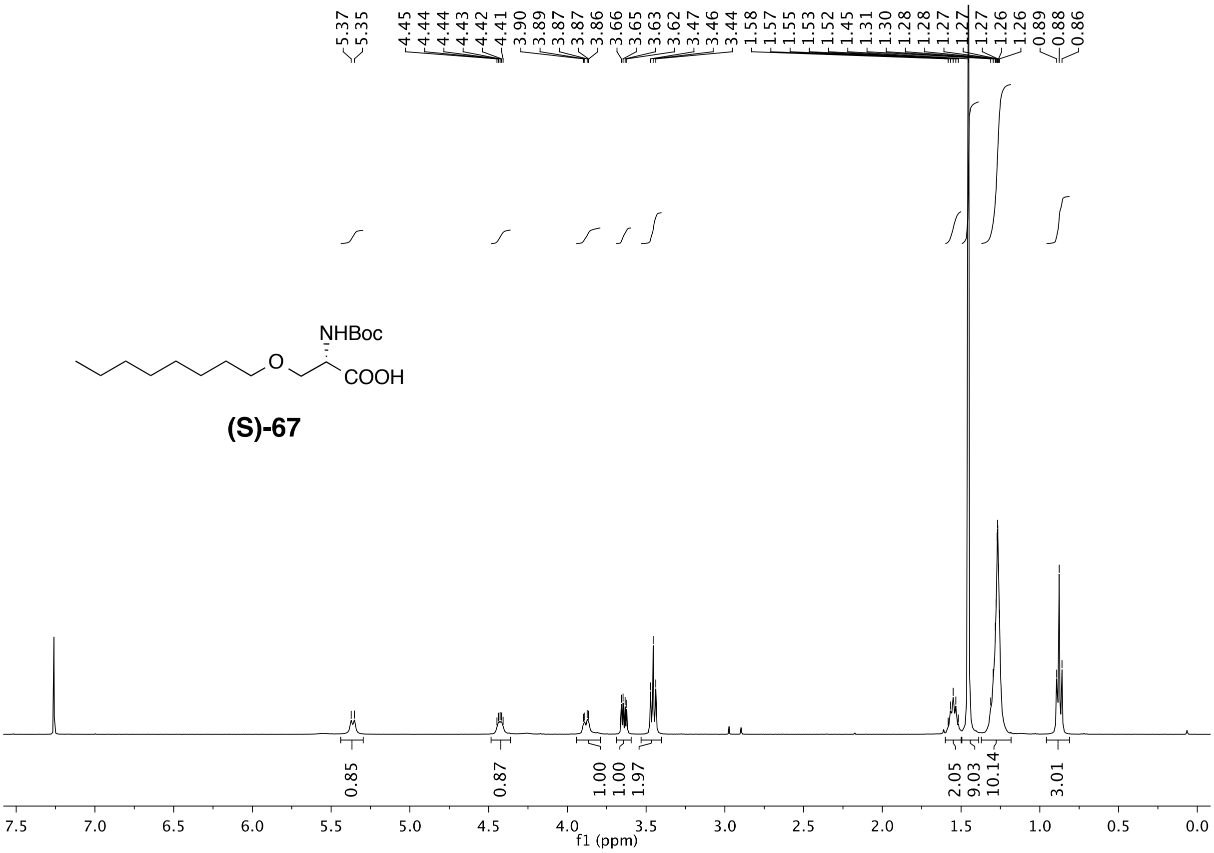
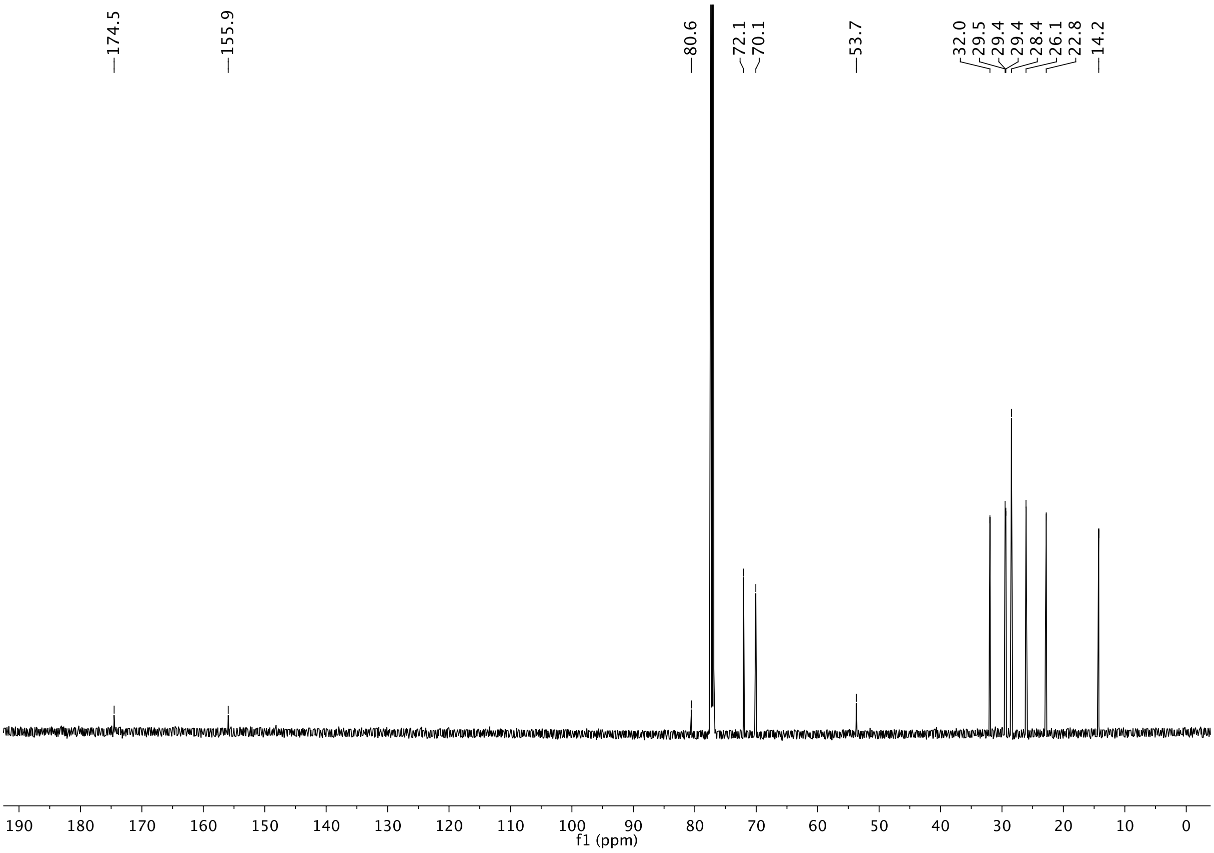
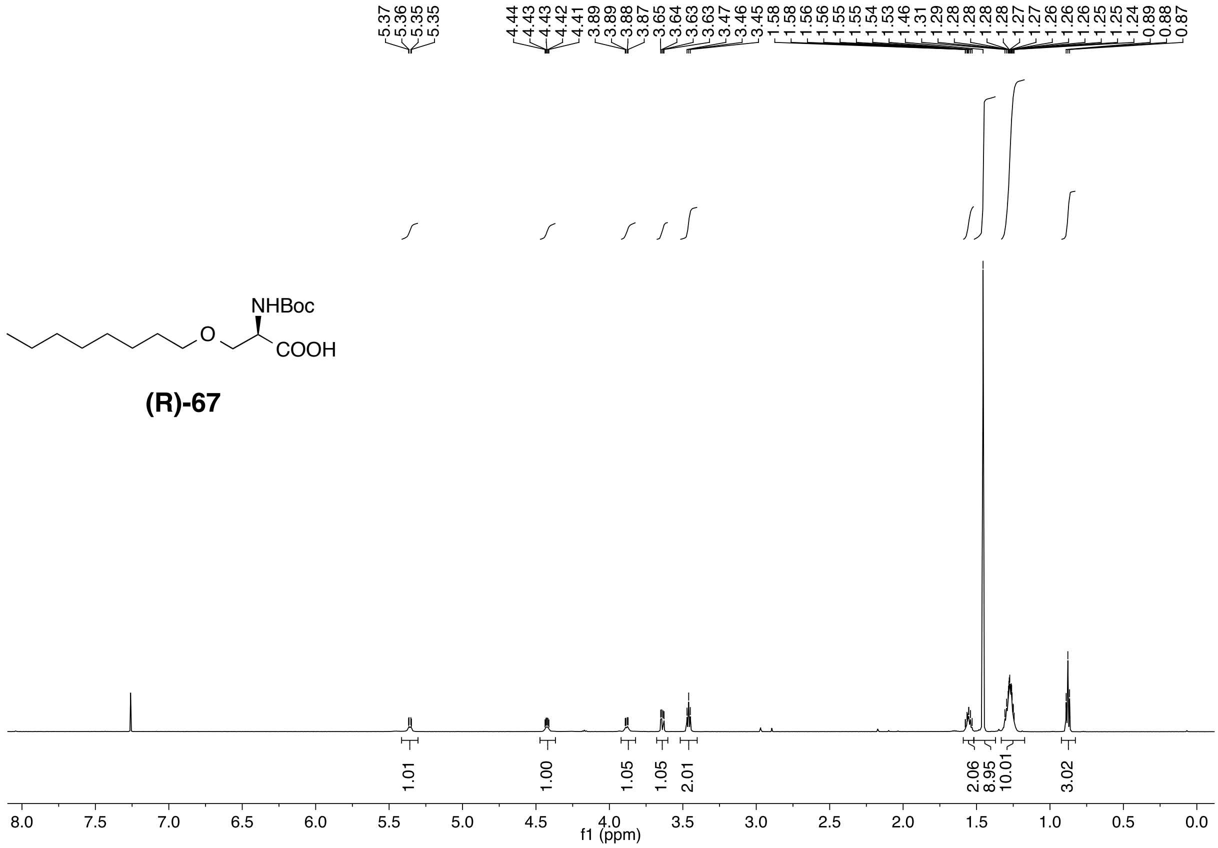
1.04
1.12
2.93
2.19
1.96
2.16
6.06
2.09
2.95
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


176.8
172.3
171.8
163.9
160.2
159.2
158.7
131.6
130.7
129.1
126.0
108.4
107.9
97.9
93.0
79.5
55.9
39.5
38.4
35.1
30.9
29.1
27.4
22.0
21.8
13.7
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

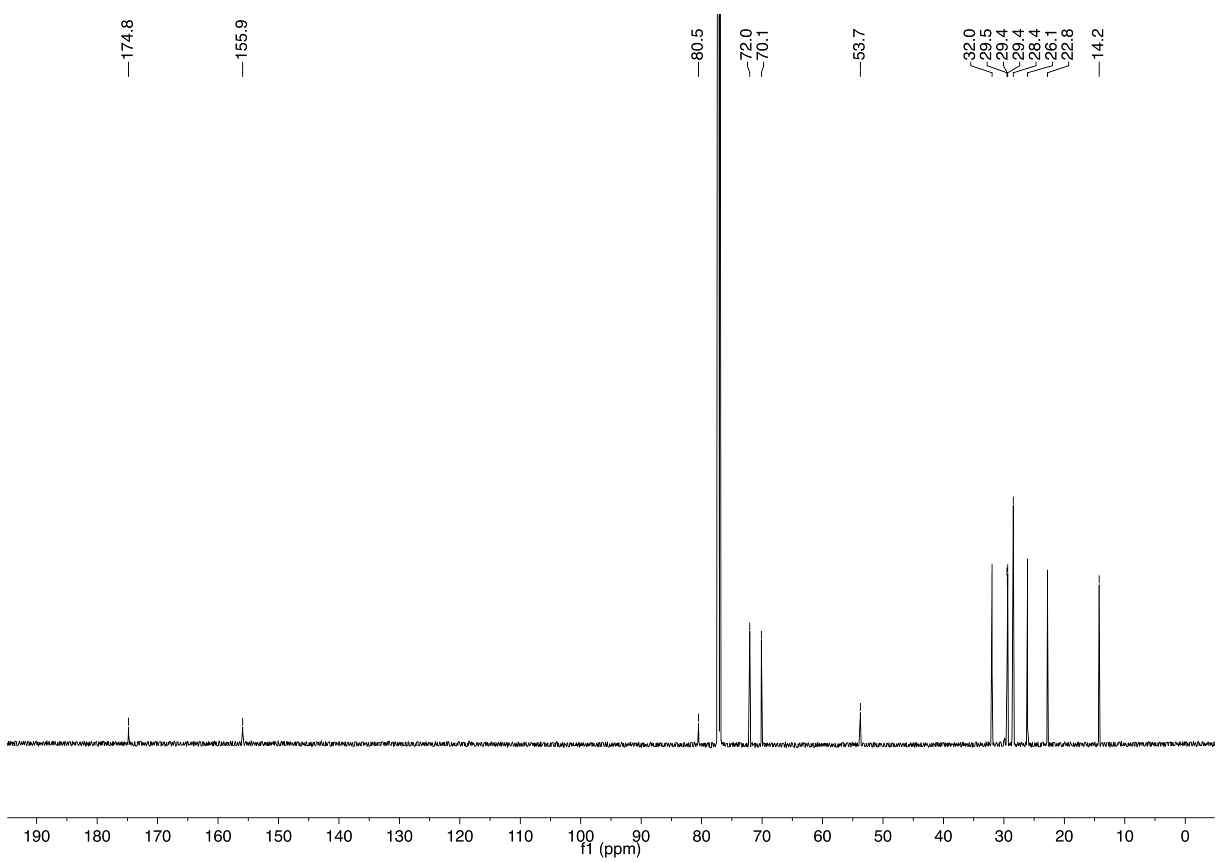

5.37
5.35
4.45
4.44
4.44
4.43
4.42
4.41
3.90
3.89
3.87
3.87
3.86
3.66
3.65
3.63
3.62
3.47
3.46
3.44
1.58
1.57
1.55
1.53
1.52
1.45
1.31
1.30
1.28
1.28
1.27
1.27
1.27
1.26
1.26
0.89
0.88
0.86
NHBoc
O
COOH
0.85
0.87
1.00
1.00
1.97
2.05
9.03
10.14
3.01
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

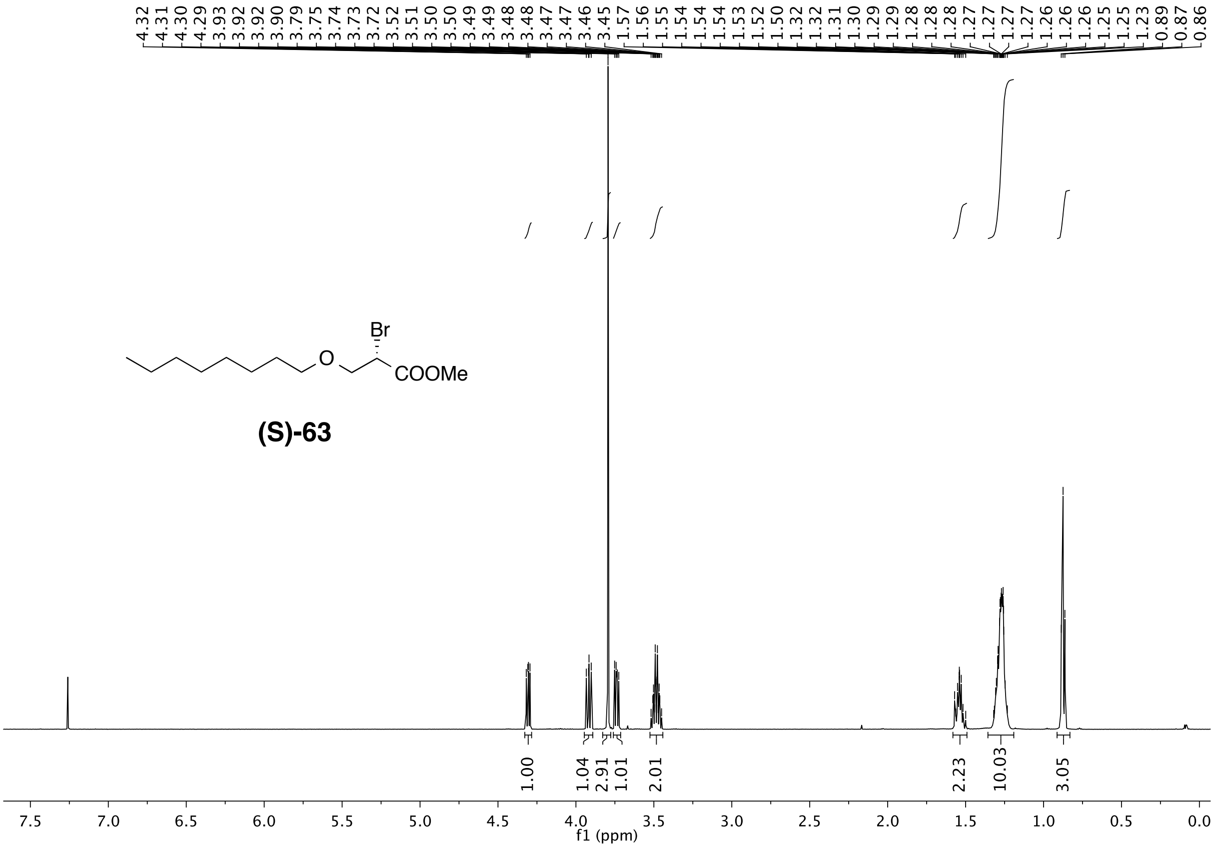
(S)-67


174.5
155.9
80.6
72.1
70.1
53.7
32.0
29.5
29.4
29.4
28.4
26.1
22.8
14.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


5.37
5.36
5.35
5.35
4.44
4.43
4.43
4.42
4.41
3.89
3.89
3.88
3.87
3.65
3.64
3.63
3.63
3.47
3.46
3.45
1.58
1.56
1.56
1.55
1.55
1.54
1.53
1.46
1.31
1.29
1.28
1.28
1.28
1.28
1.27
1.27
1.26
1.26
1.25
1.25
1.24
0.89
0.88
0.87
NHBoc
O
COOH
1.01
1.00
1.05
1.05
2.01
2.06
8.95
10.01
3.02
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

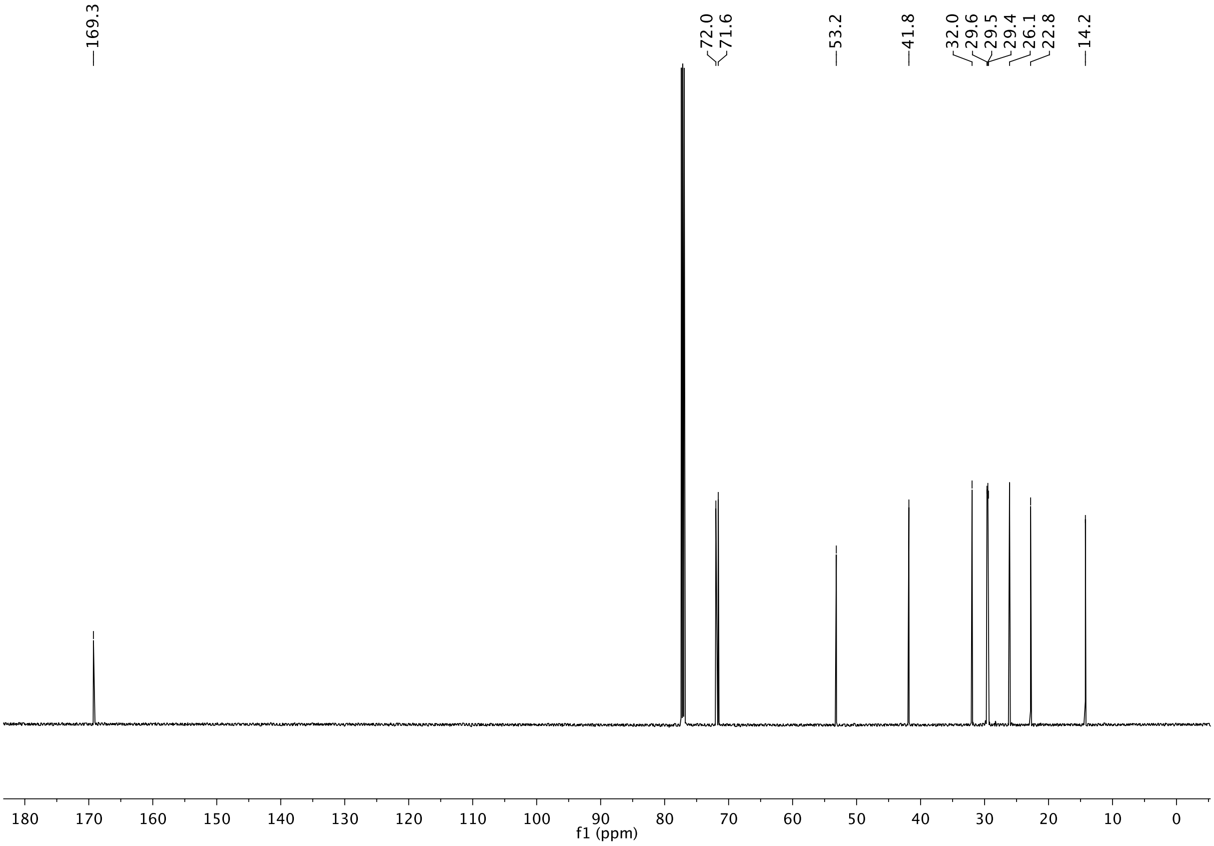
(R)-67


174.8
155.9
80.5
72.0
70.1
53.7
32.0
29.5
29.4
29.4
28.4
26.1
22.8
14.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

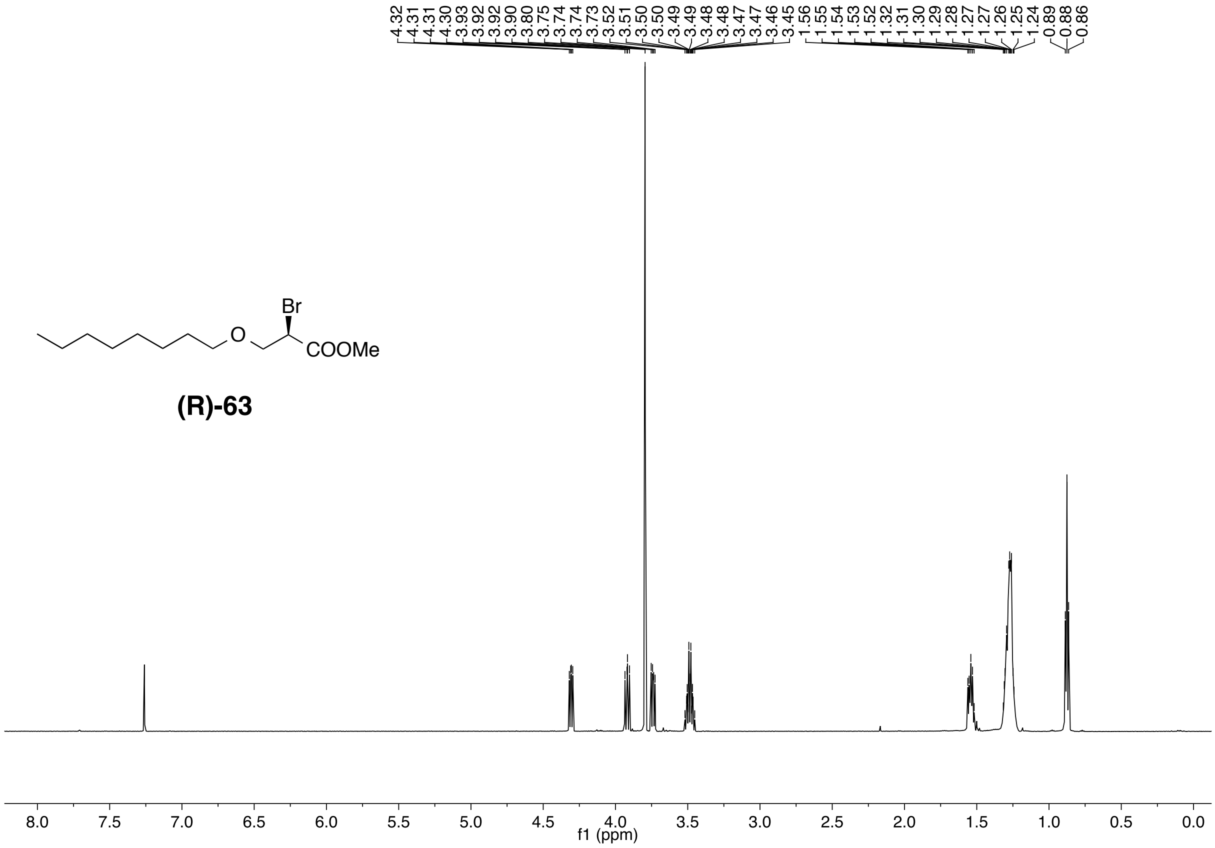


(S)-63

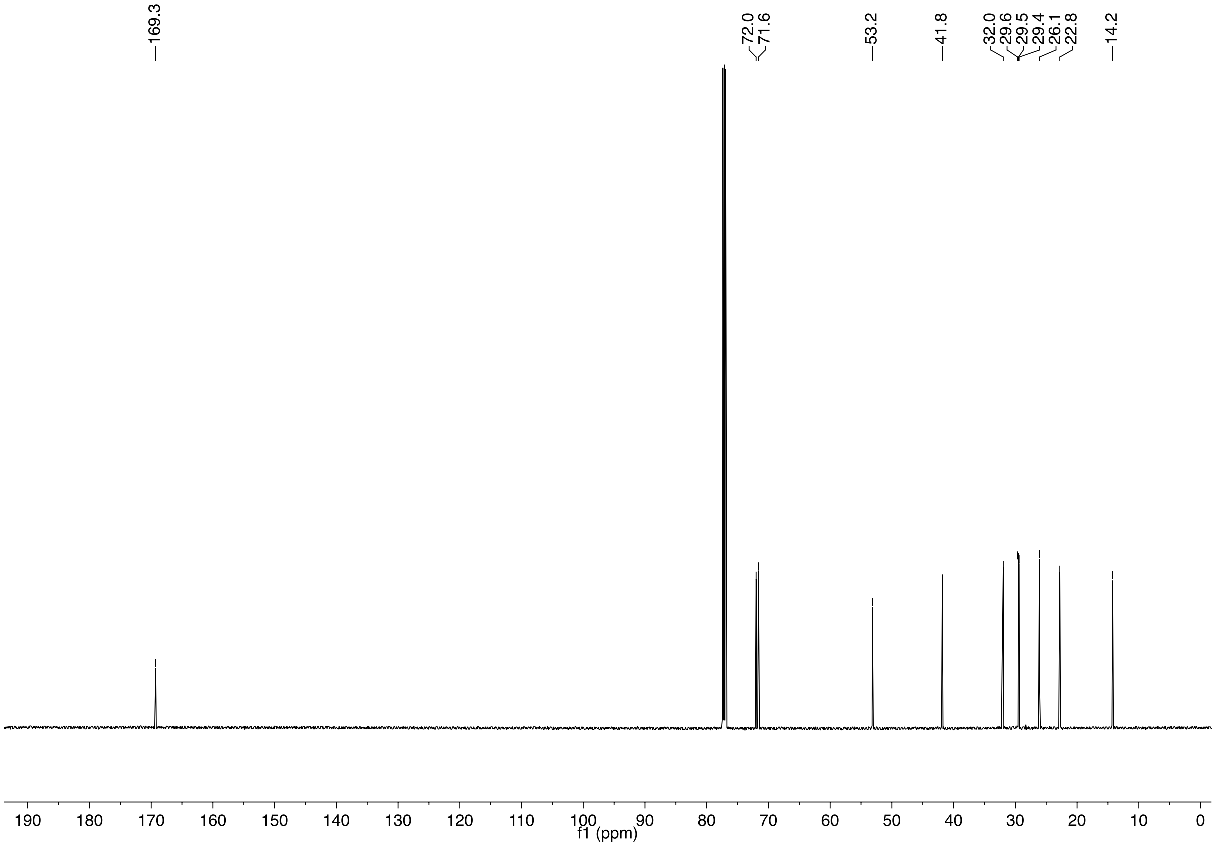
4.32
4.31
4.30
4.29
3.93
3.92
3.92
3.90
3.79
3.75
3.74
3.73
3.72
3.52
3.51
3.50
3.50
3.49
3.49
3.48
3.48
3.47
3.47
3.46
3.45
1.57
1.56
1.55
1.54
1.54
1.54
1.53
1.52
1.50
1.32
1.32
1.31
1.30
1.29
1.29
1.28
1.28
1.28
1.27
1.27
1.27
1.27
1.26
1.26
1.25
1.25
1.23
0.89
0.87
0.86
Br
O
COOMe
1.00
1.04
2.91
1.01
2.01
2.23
10.03
3.05
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

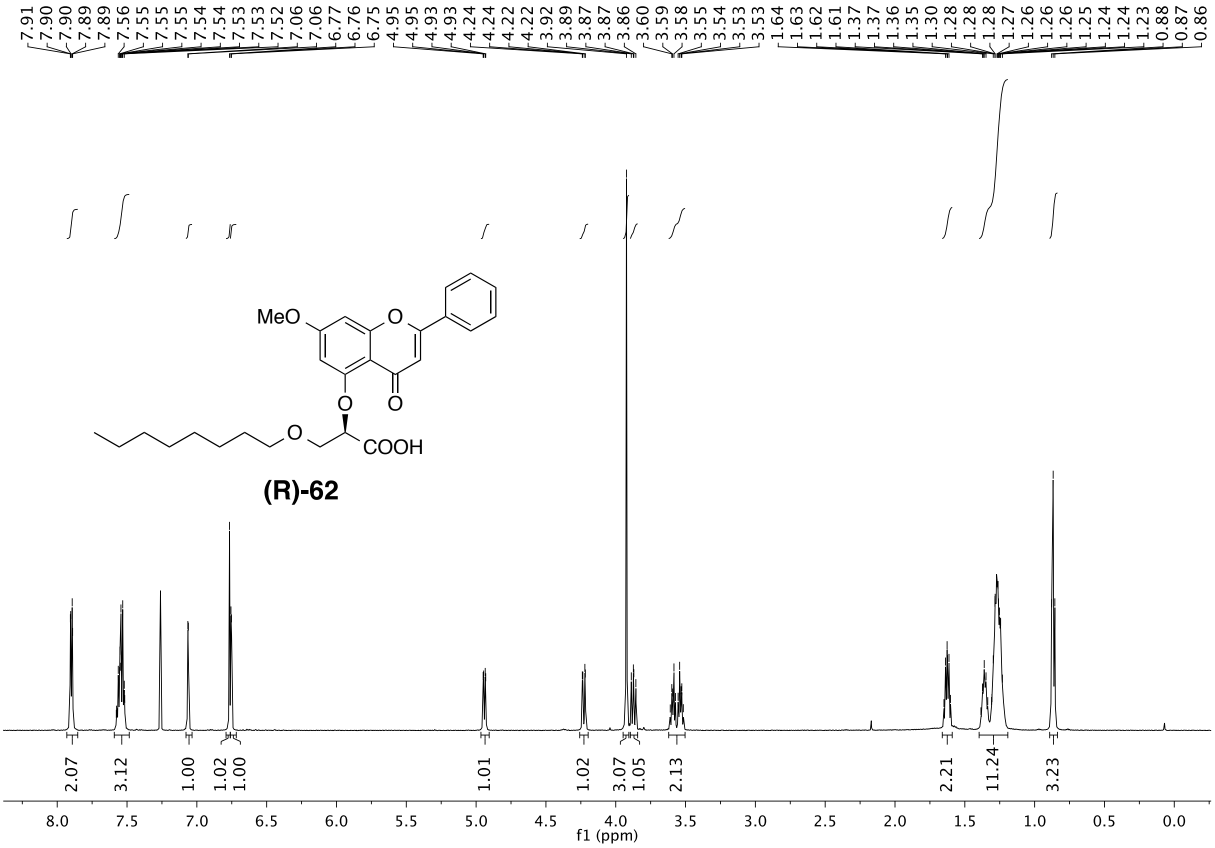


169.3
72.0
71.6
53.2
41.8
32.0
29.6
29.5
29.4
26.1
22.8
14.2
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


(R)-63

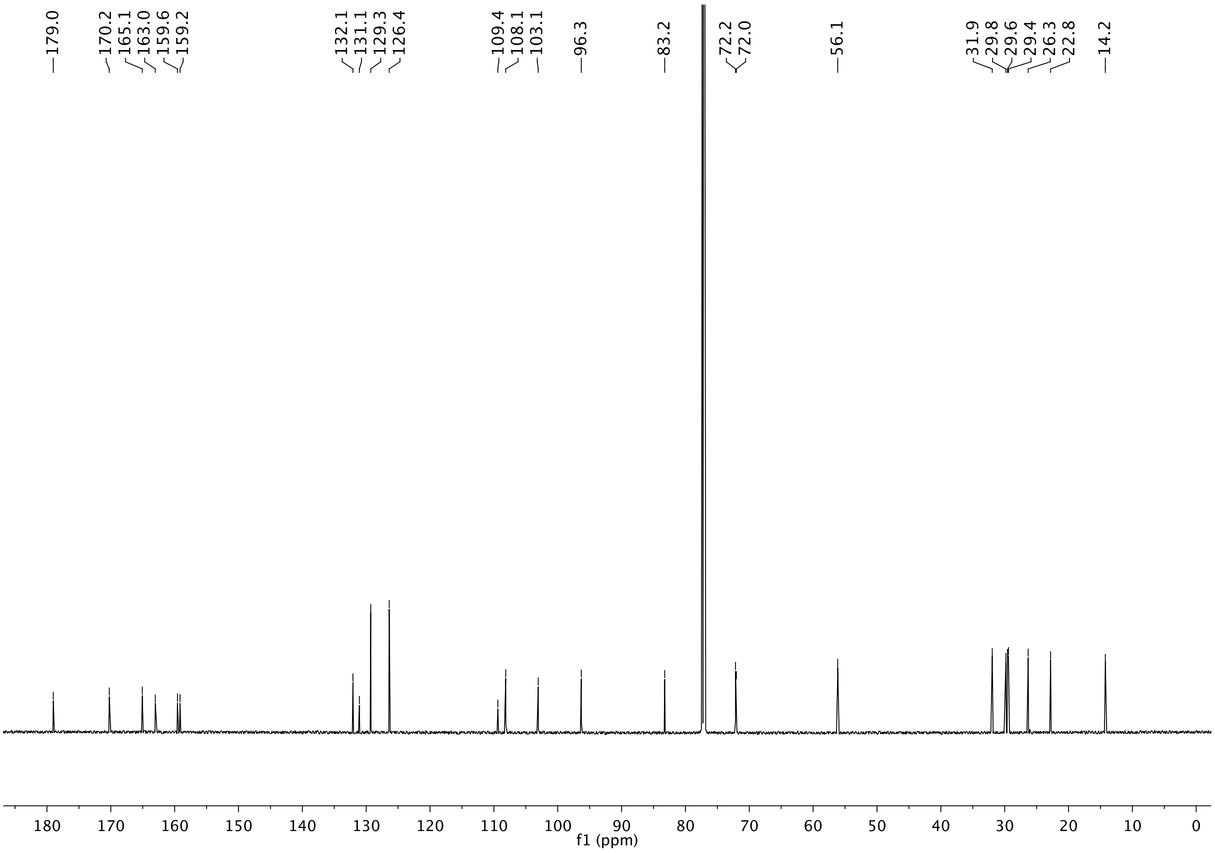
4.32
4.31
4.31
4.30
3.93
3.92
3.92
3.90
3.80
3.75
3.74
3.74
3.73
3.52
3.51
3.50
3.50
3.49
3.49
3.48
3.48
3.47
3.47
3.46
3.45
1.56
1.55
1.54
1.53
1.52
1.32
1.31
1.30
1.29
1.28
1.27
1.27
1.26
1.25
1.24
0.89
0.88
0.86
Br
O
COOMe
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


169.3
72.0
71.6
53.2
41.8
32.0
29.6
29.5
29.4
26.1
22.8
14.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

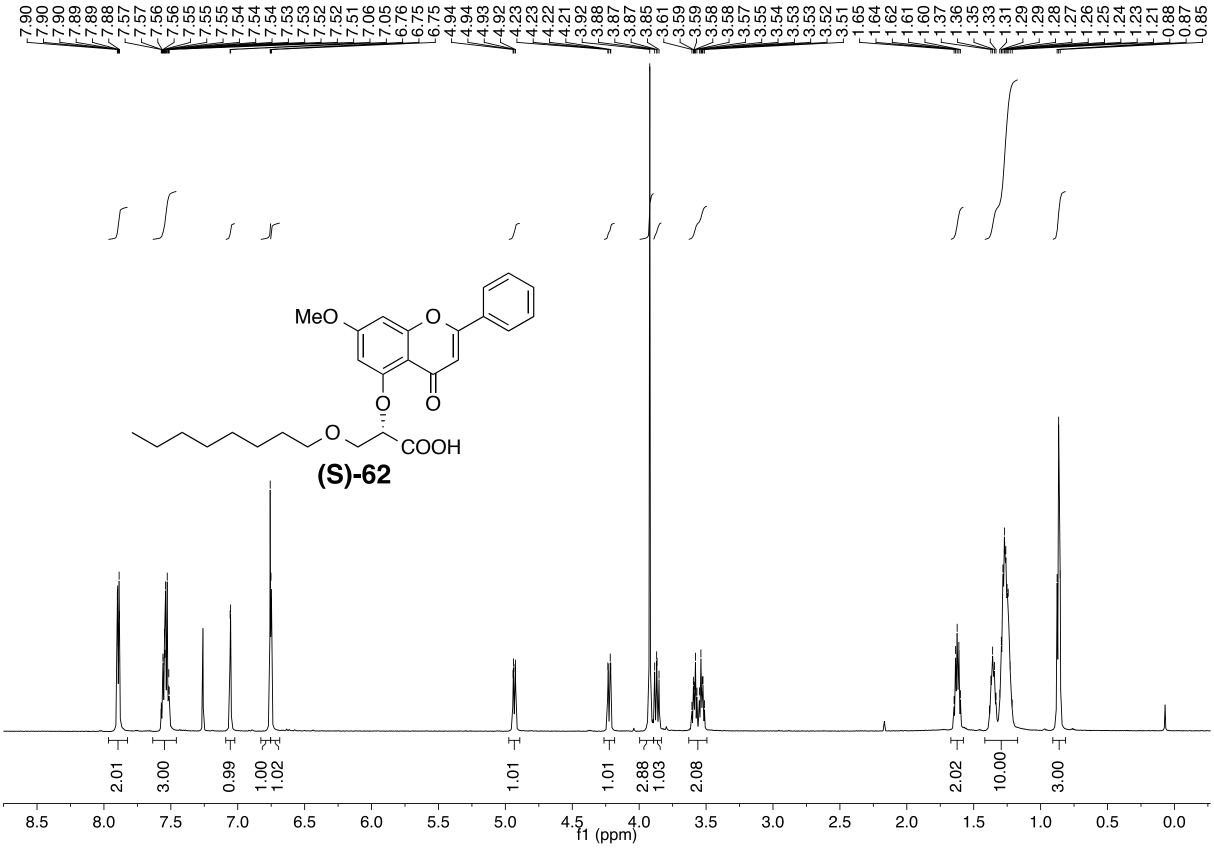


(R)-62

7.91
7.90
7.90
7.89
7.89
7.56
7.55
7.55
7.54
7.54
7.53
7.53
7.52
7.06
6.77
6.76
6.75
4.95
4.95
4.93
4.93
4.24
4.24
4.22
4.22
3.92
3.89
3.87
3.87
3.86
3.60
3.59
3.58
3.55
3.54
3.53
3.53
1.64
1.63
1.62
1.61
1.37
1.36
1.35
1.30
1.28
1.28
1.28
1.27
1.26
1.26
1.26
1.25
1.24
1.24
1.23
0.88
0.87
0.86
MeO
O
O
O
O
COOH
2.07
3.12
1.00
1.02
1.00
1.01
1.02
3.07
1.05
2.13
2.21
11.24
3.23
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

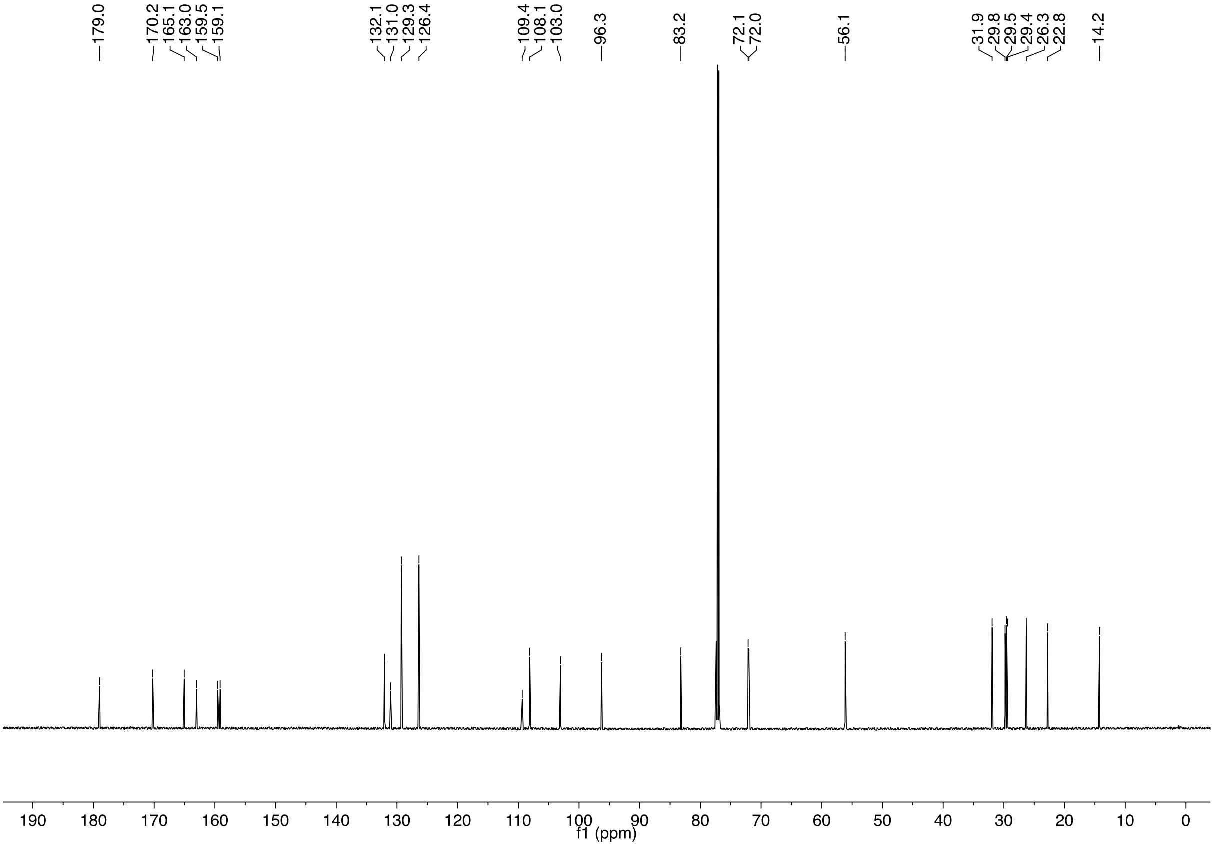


179.0
170.2
165.1
163.0
159.6
159.2
132.1
131.1
129.3
126.4
109.4
108.1
103.1
96.3
83.2
72.2
72.0
56.1
31.9
29.8
29.6
29.4
26.3
22.8
14.2
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

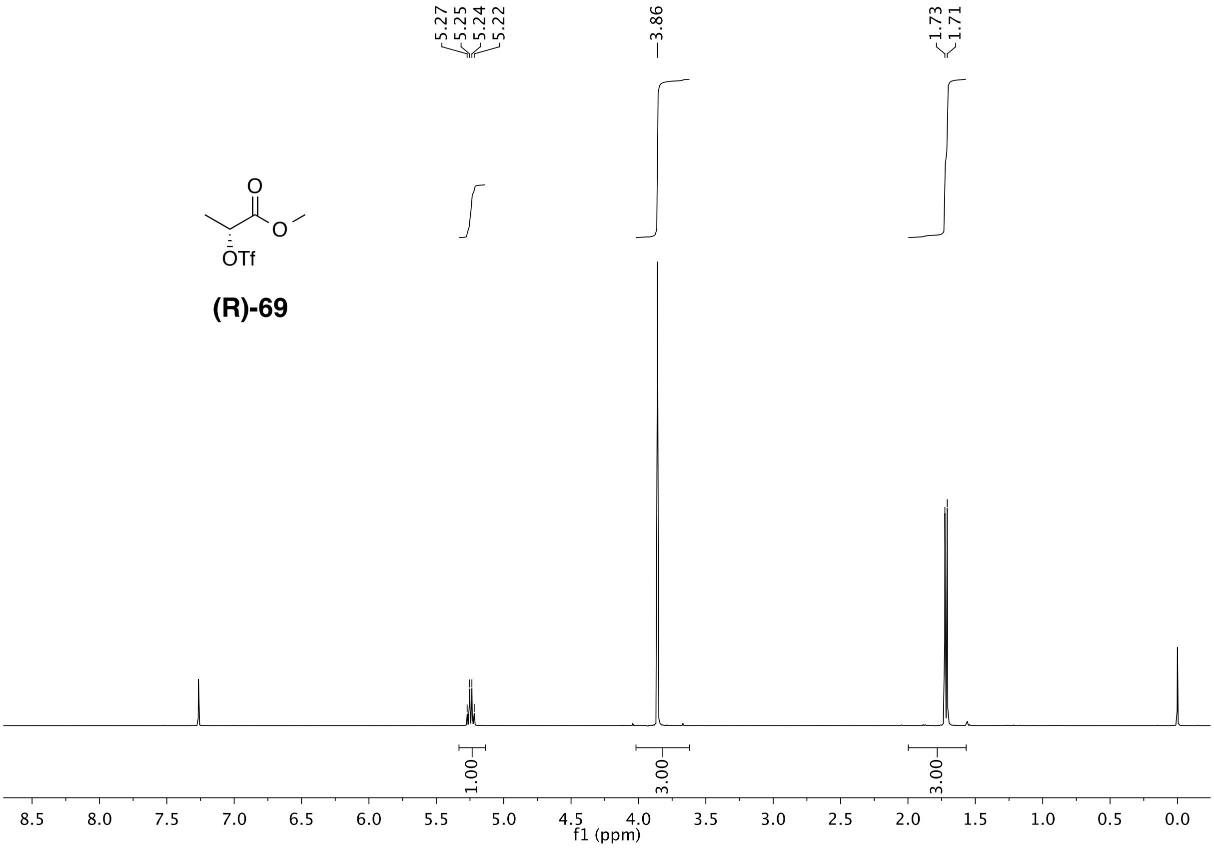


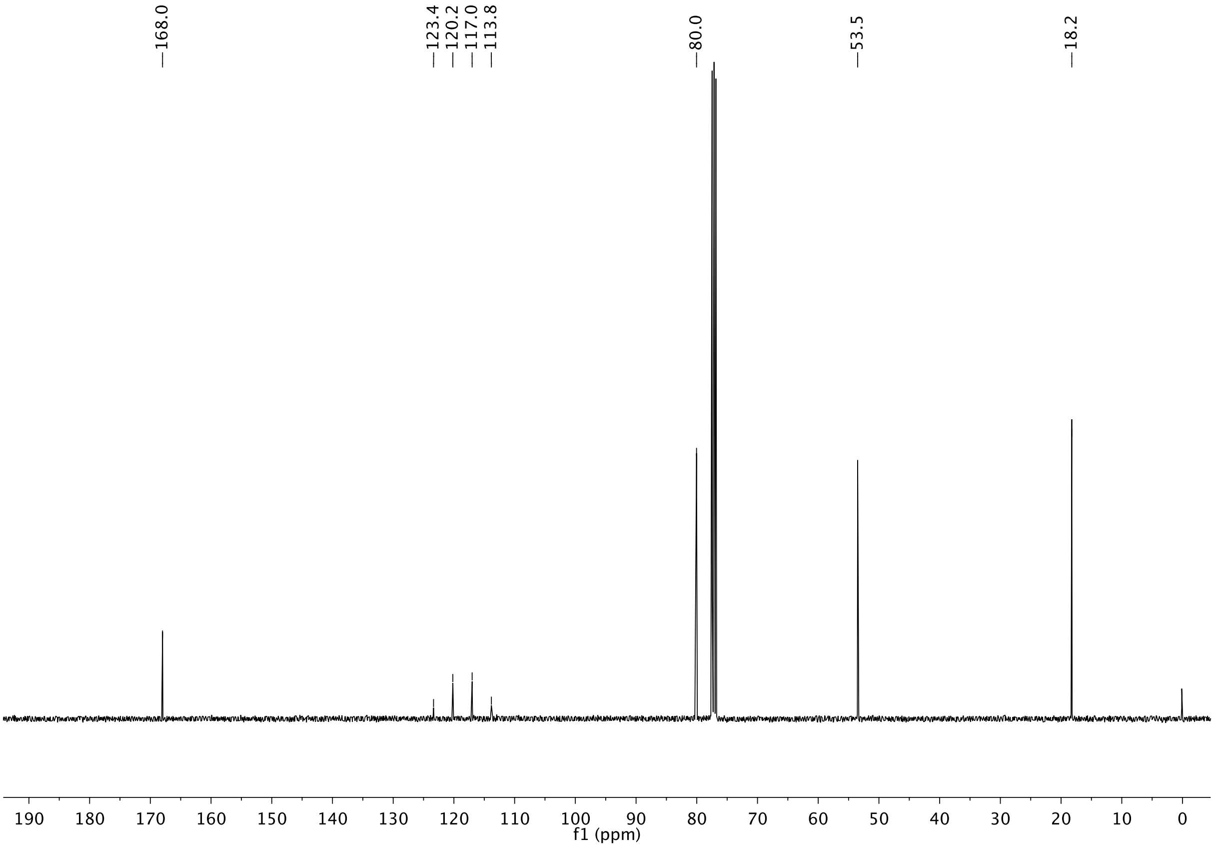
(S)-62

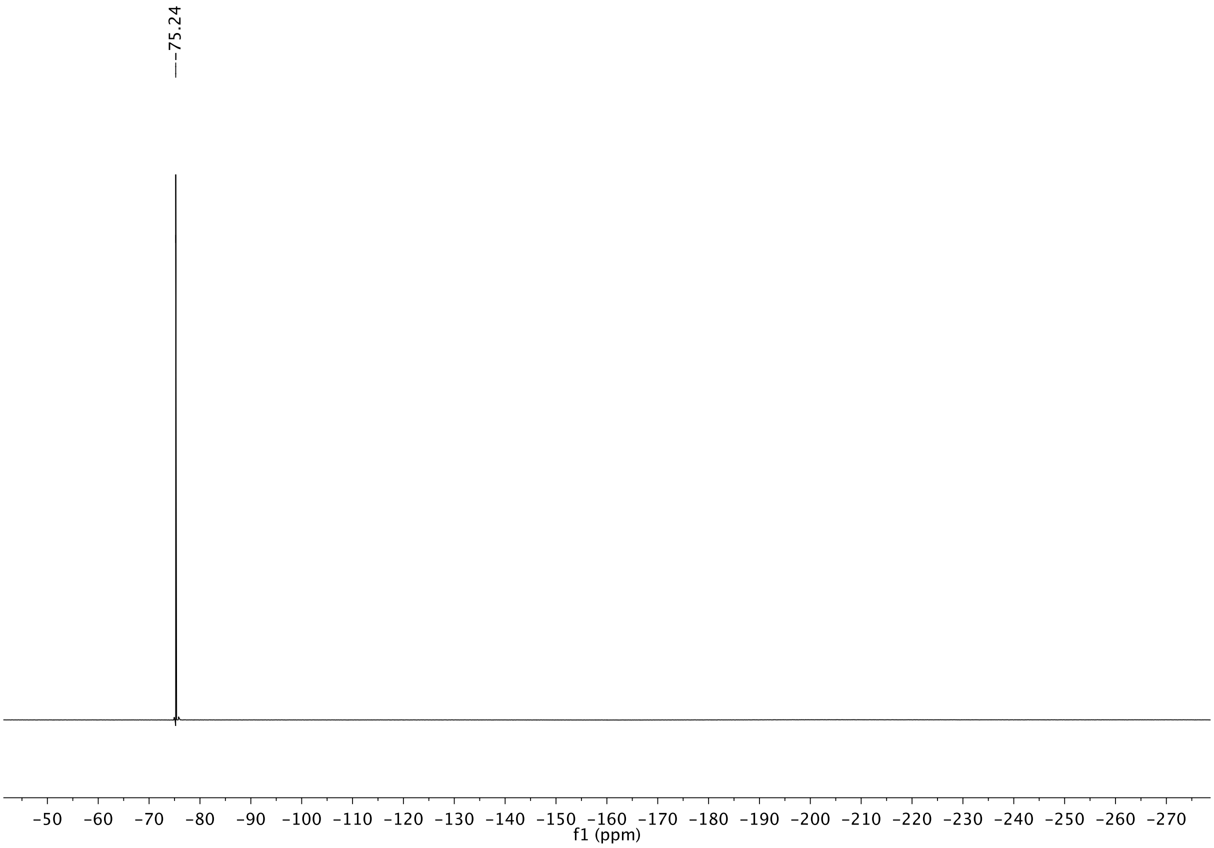
7.90
7.90
7.90
7.89
7.89
7.88
7.57
7.57
7.56
7.56
7.55
7.55
7.55
7.54
7.54
7.54
7.53
7.53
7.52
7.52
7.51
7.06
7.05
6.76
6.75
6.75
4.94
4.94
4.93
4.92
4.23
4.23
4.22
4.21
3.92
3.88
3.87
3.87
3.85
3.61
3.59
3.59
3.58
3.58
3.57
3.55
3.54
3.53
3.53
3.52
3.51
1.65
1.64
1.62
1.61
1.60
1.37
1.36
1.35
1.33
1.31
1.29
1.29
1.28
1.27
1.26
1.25
1.24
1.23
1.21
0.88
0.87
0.85
MeO
O
O
O
O
COOH
2.01
3.00
0.99
1.00
1.02
1.01
1.01
2.88
1.03
2.08
2.02
10.00
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

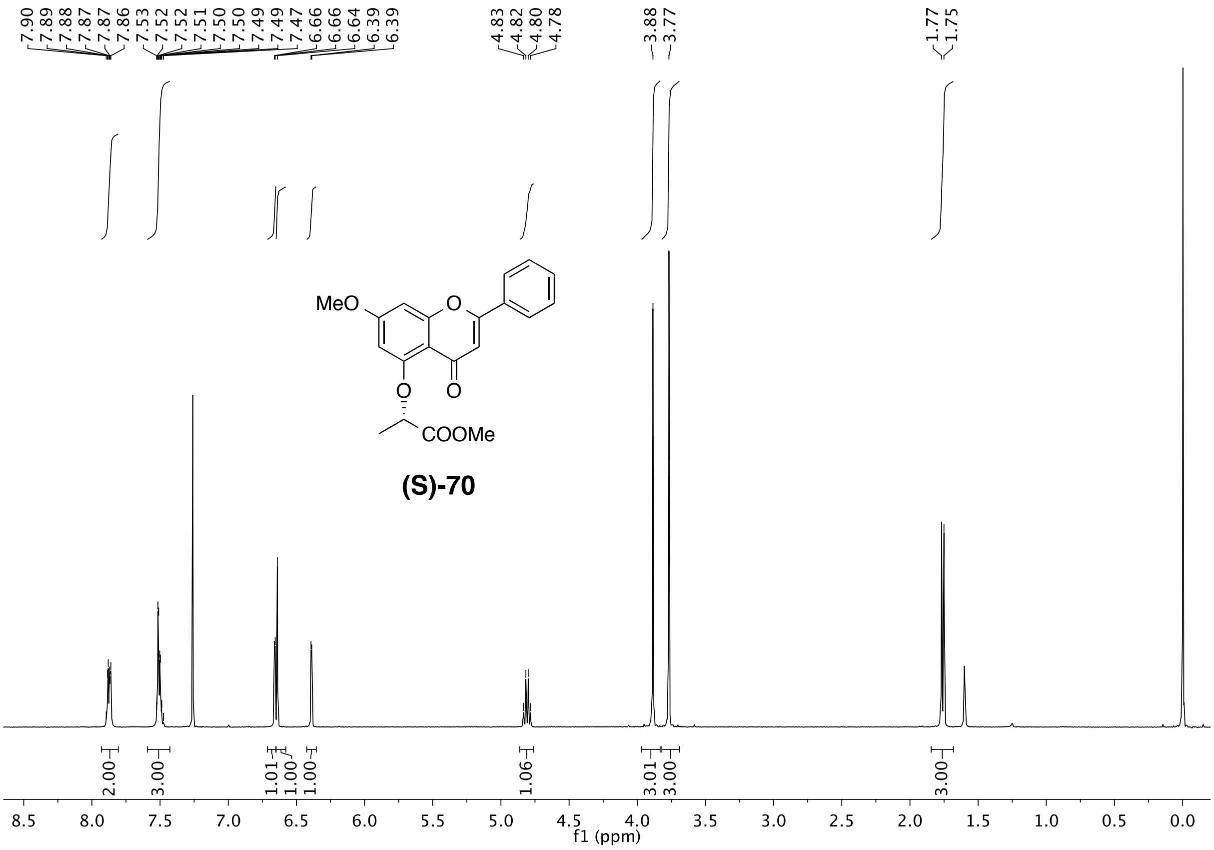


179.0
170.2
165.1
163.0
159.5
159.1
132.1
131.0
129.3
126.4
109.4
108.1
103.0
96.3
83.2
72.1
72.0
56.1
31.9
29.8
29.5
29.4
26.3
22.8
14.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

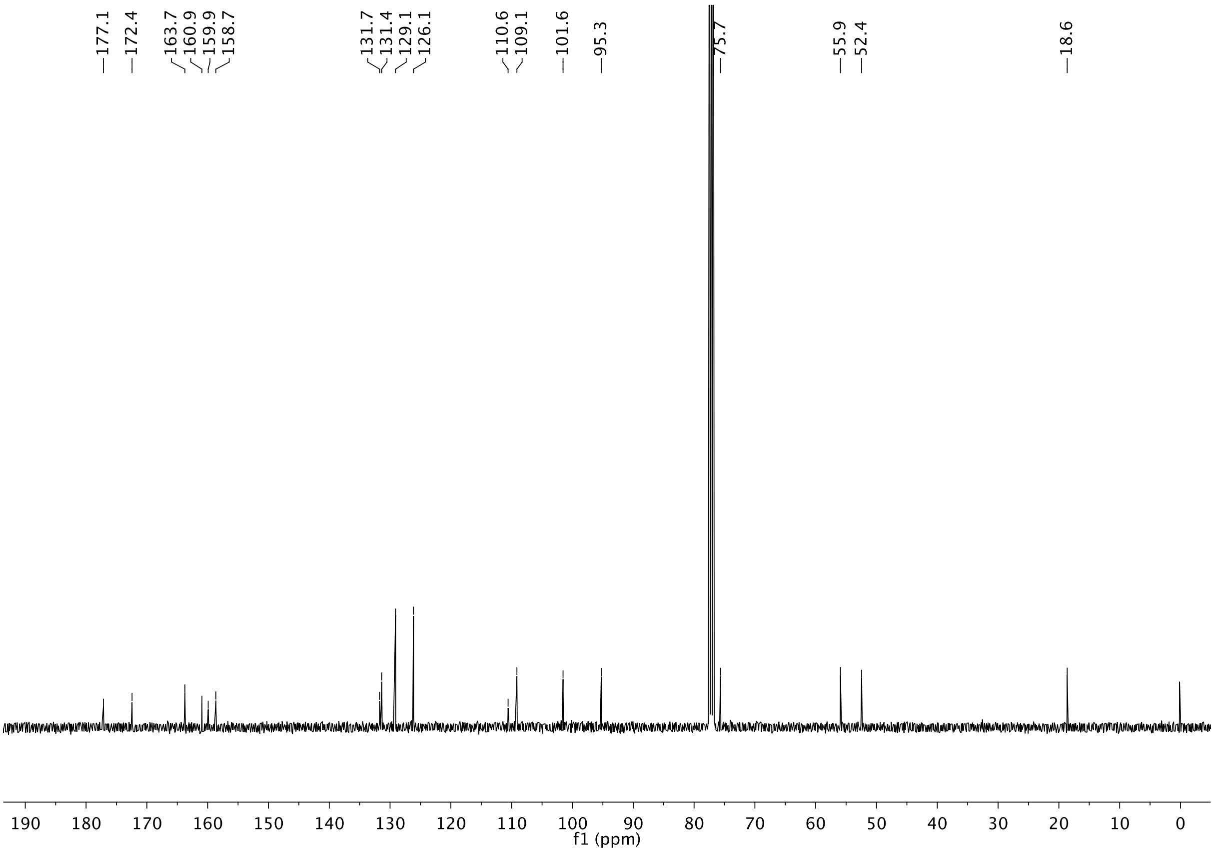

(R)-69
5.27
5.25
5.24
5.22
3.86
1.73
1.71
O
O
OTf
1.00
3.00
3.00
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

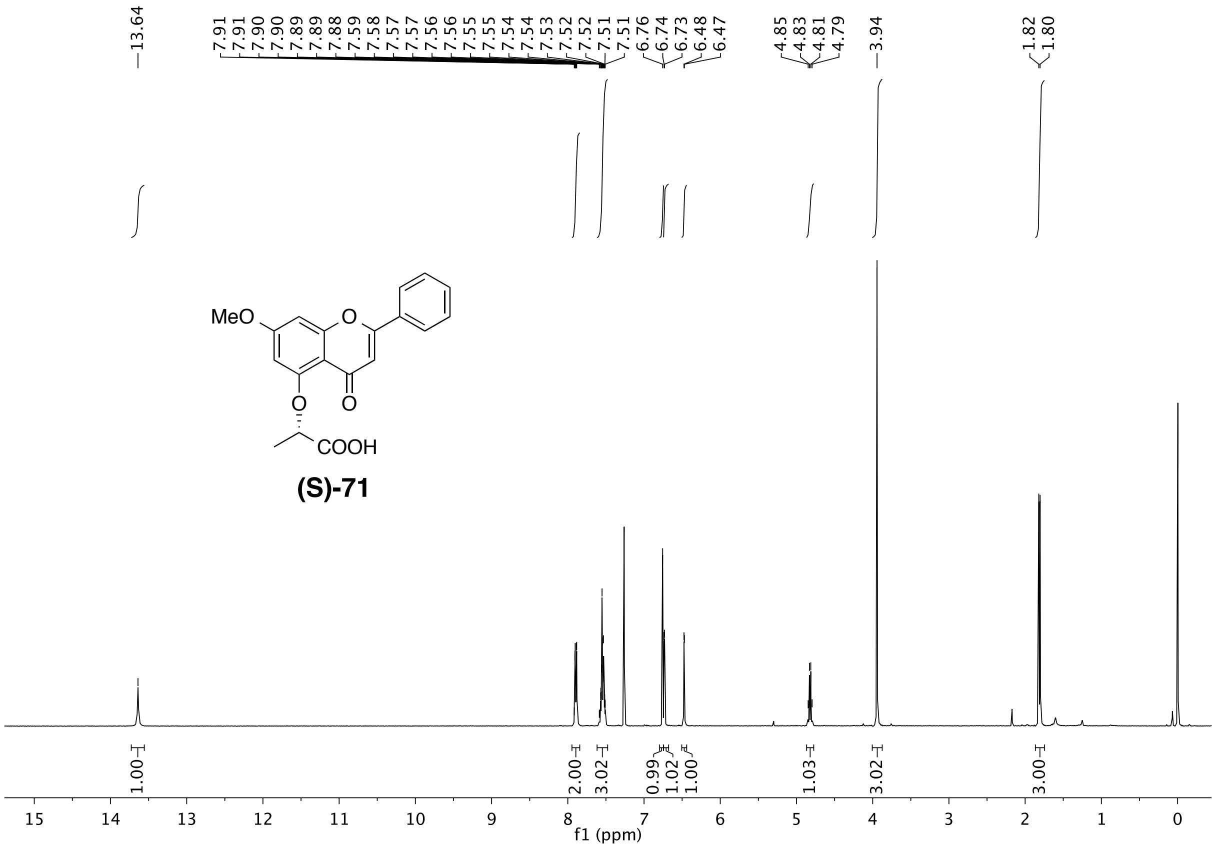

168.0
123.4
120.2
117.0
113.8
80.0
53.5
18.2
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


−75.24
−50
−60
−70
−80
−90
−100
−110
−120
−130
−140
−150
−160
−170
−180
−190
−200
−210
−220
−230
−240
−250
−260
−270
f1 (ppm)

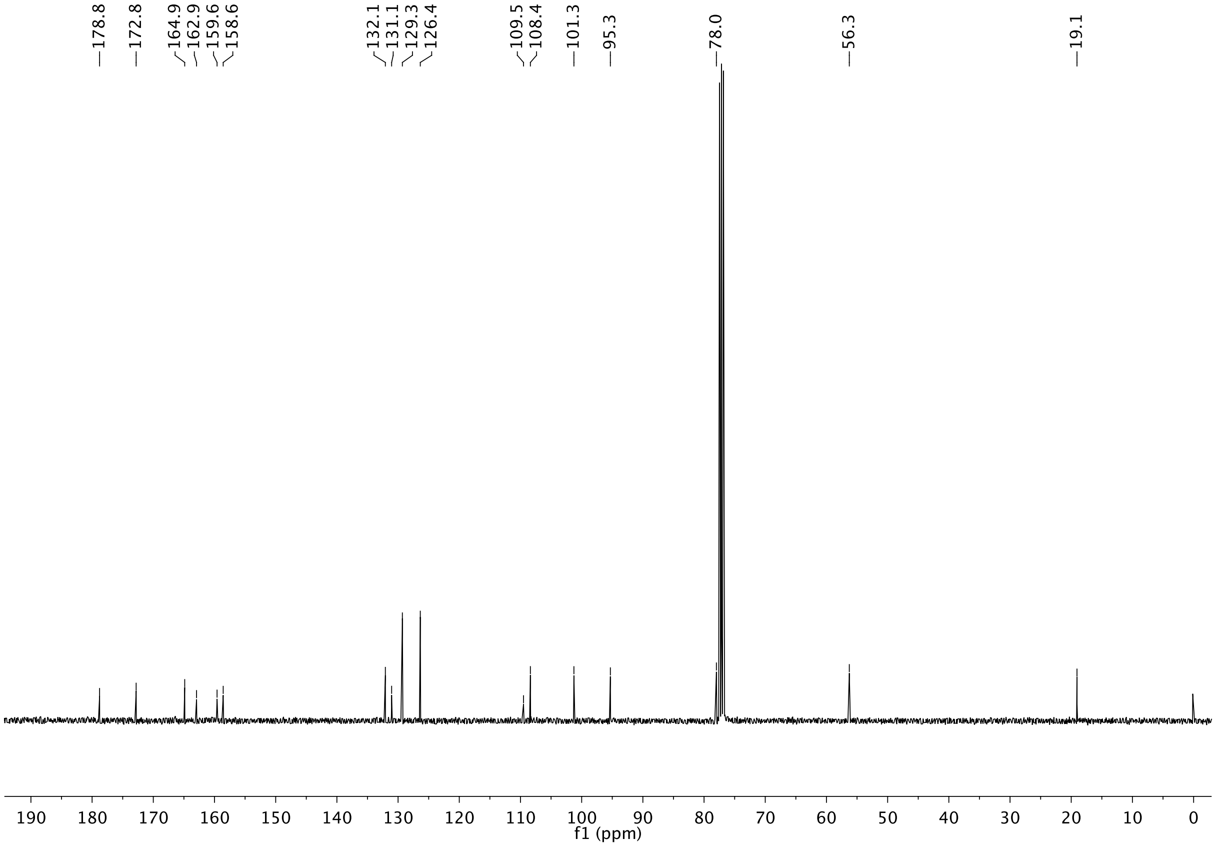

7.90
7.89
7.88
7.87
7.87
7.86
7.53
7.52
7.52
7.51
7.50
7.50
7.49
7.49
7.47
6.66
6.66
6.64
6.39
6.39
4.83
4.82
4.80
4.78
3.88
3.77
1.77
1.75
MeO
O
O
O
COOMe
2.00
3.00
1.01
1.00
1.00
1.06
3.01
3.00
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

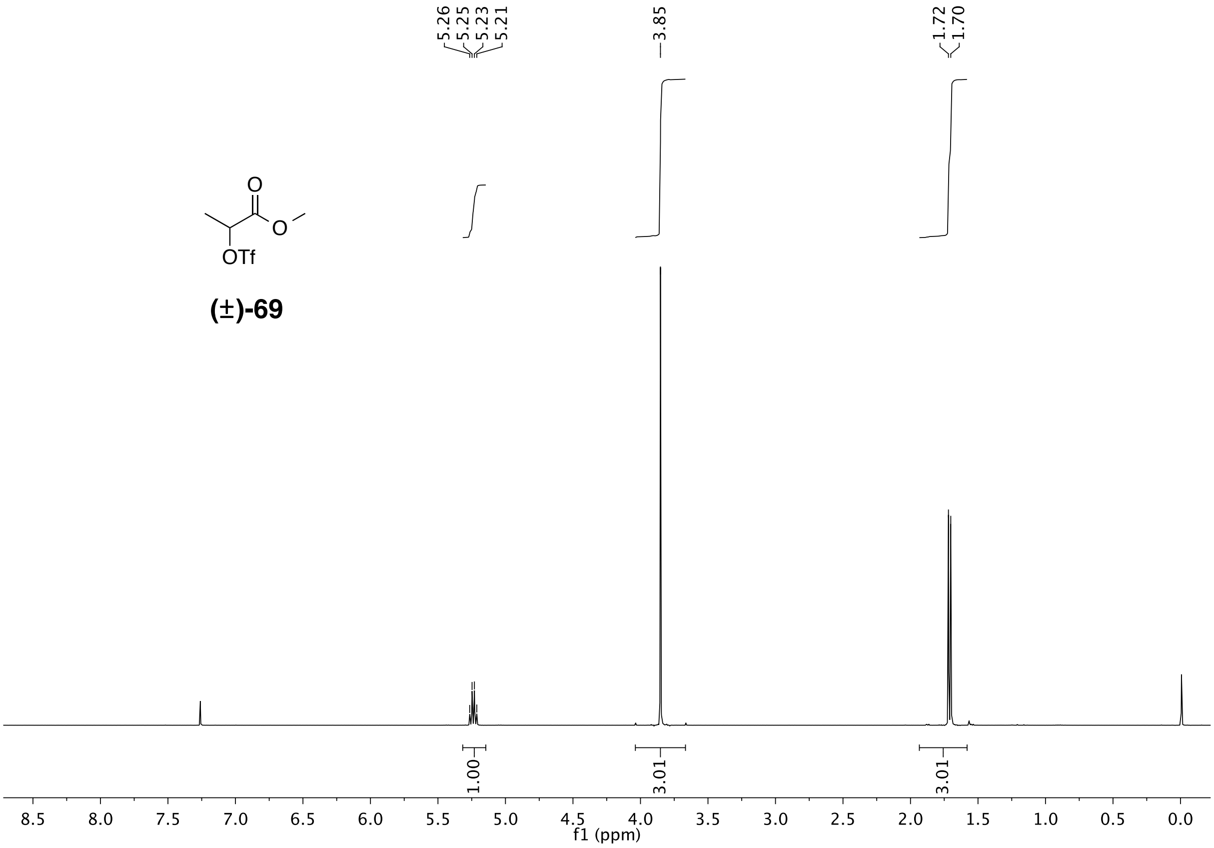
(S)-70


177.1
172.4
163.7
160.9
159.9
158.7
131.7
131.4
129.1
126.1
110.6
109.1
101.6
95.3
75.7
55.9
52.4
18.6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

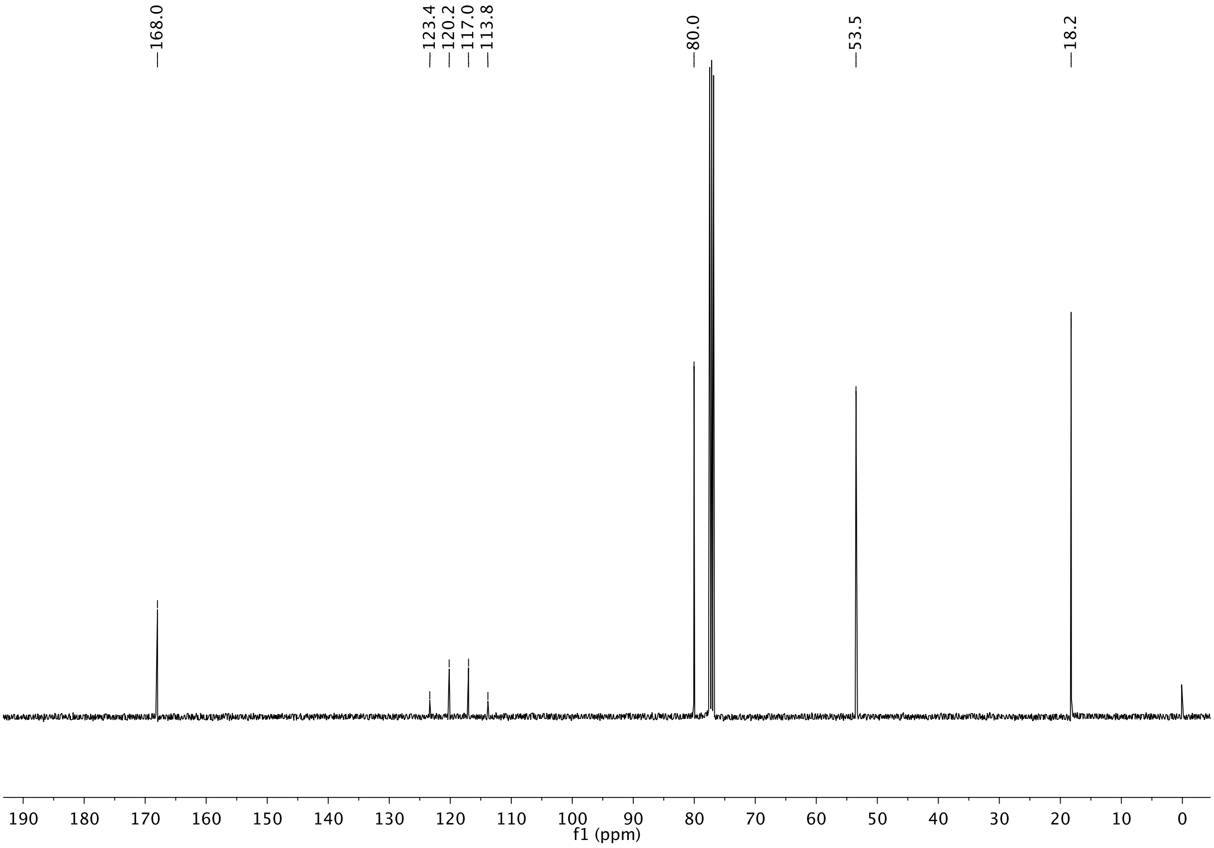

13.64
7.91
7.91
7.90
7.90
7.89
7.89
7.88
7.59
7.58
7.57
7.57
7.56
7.56
7.55
7.55
7.54
7.54
7.53
7.52
7.52
7.51
7.51
6.76
6.74
6.73
6.48
6.47
4.85
4.83
4.81
4.79
3.94
1.82
1.80
MeO
O
O
O
COOH
1.00
2.00
3.02
0.99
1.02
1.00
1.03
3.02
3.00
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

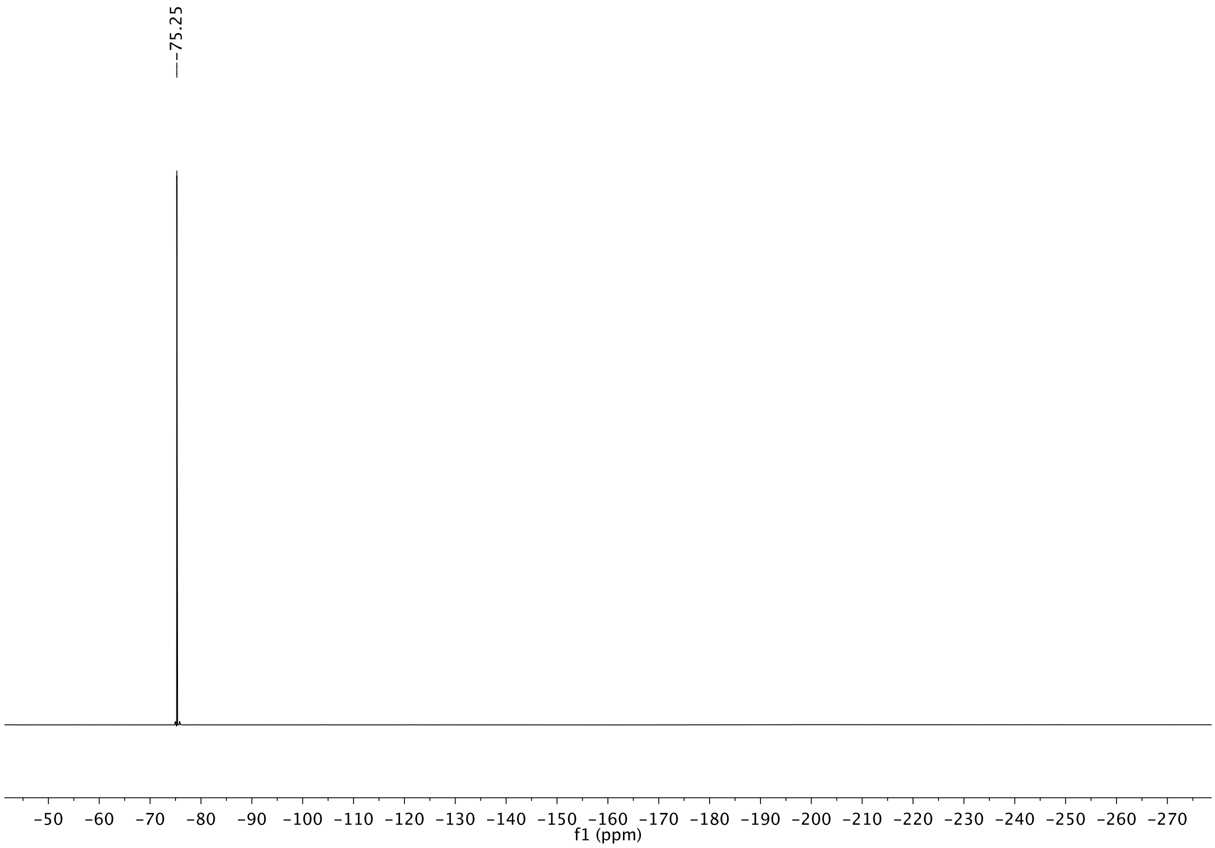
(S)-71

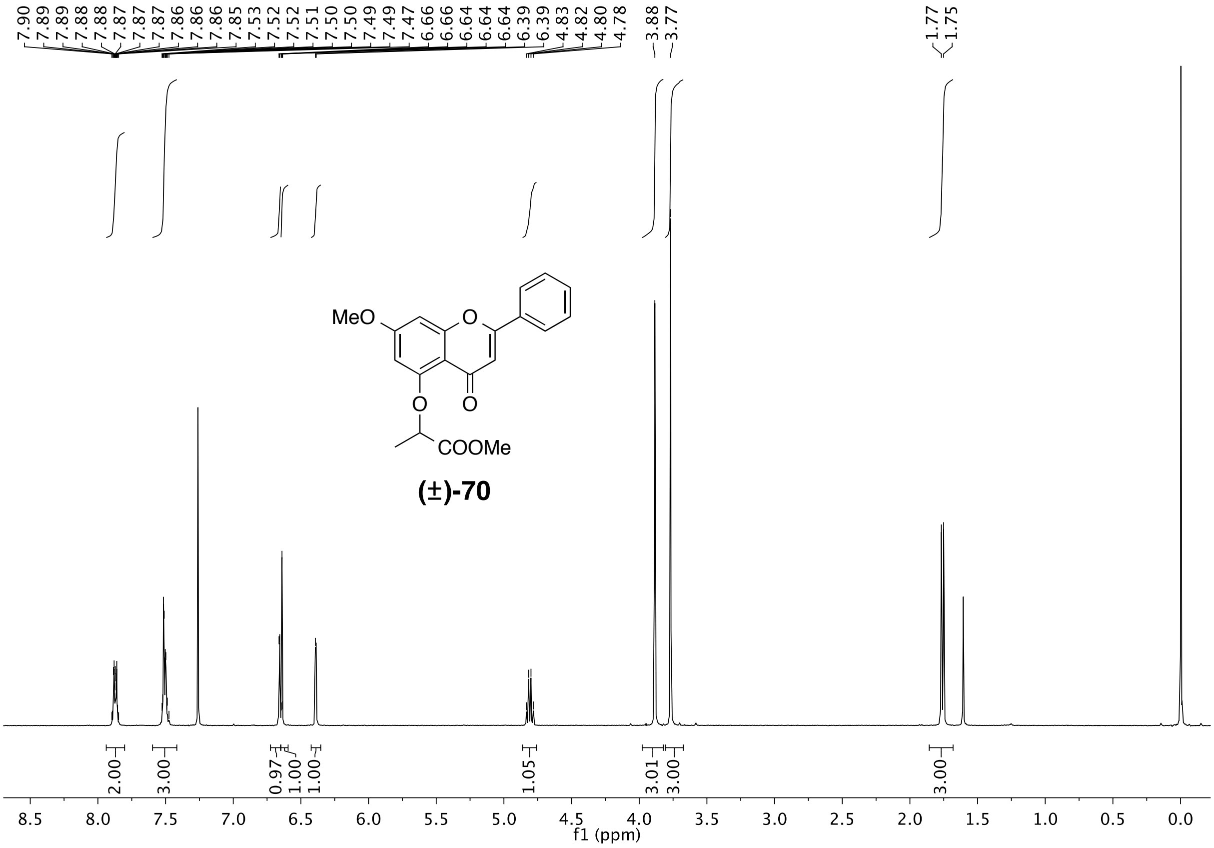

178.8
172.8
164.9
162.9
159.6
158.6
132.1
131.1
129.3
126.4
109.5
108.4
101.3
95.3
78.0
56.3
19.1
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

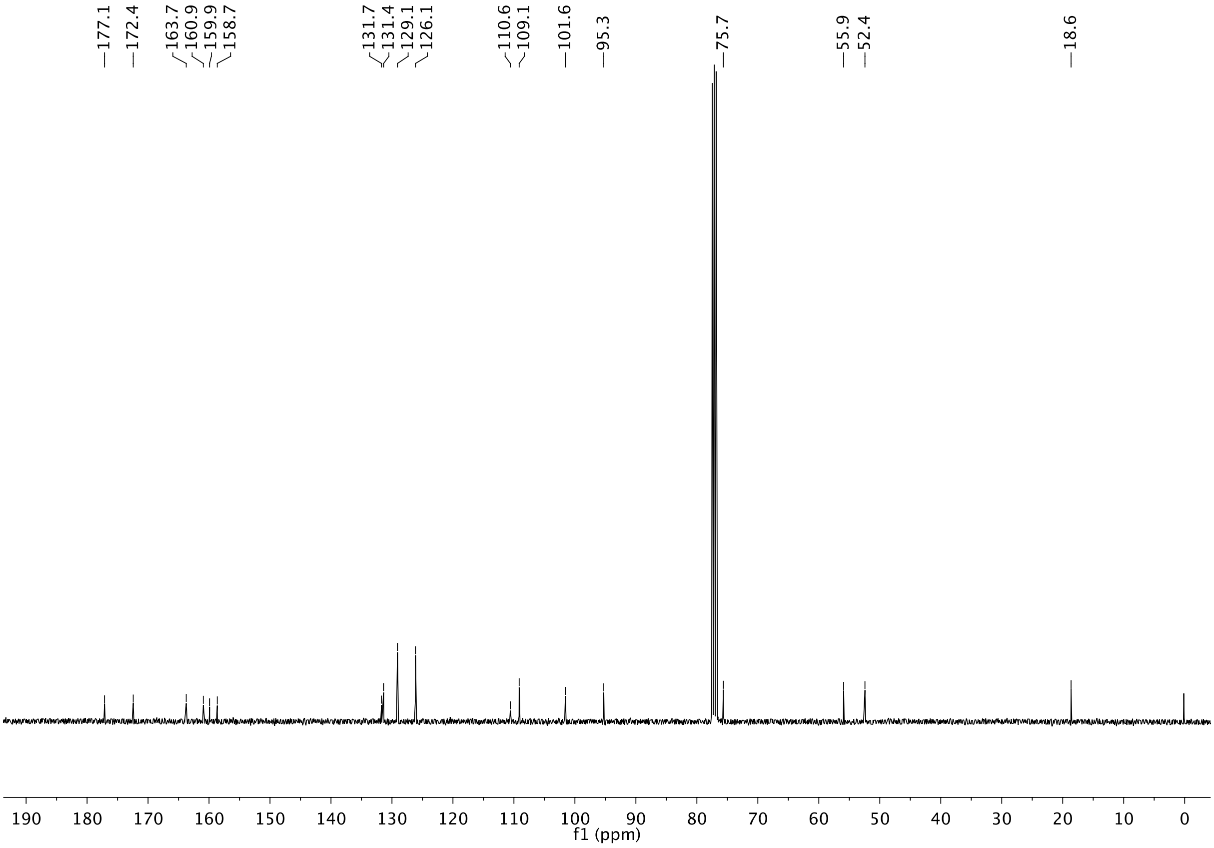

5.26
5.25
5.23
5.21
3.85
1.72
1.70
O
O
OTf
1.00
3.01
3.01
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

(±)-69


168.0
123.4
120.2
117.0
113.8
80.0
53.5
18.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


−75.25
−50
−60
−70
−80
−90
−100
−110
−120
−130
−140
−150
−160
−170
−180
−190
−200
−210
−220
−230
−240
−250
−260
−270
f1 (ppm)


7.90 7.89 7.89 7.88 7.88 7.87 7.87 7.87 7.86 7.86 7.86 7.85 7.53 7.52 7.52 7.51 7.50 7.50 7.49 7.49 7.47 6.66 6.66 6.64 6.64 6.64 6.39 6.39 4.83 4.82 4.80 4.78 3.88 3.77 1.77 1.75
MeO
O
O
O
COOMe
(±)-70
2.00
3.00
0.97
1.00
1.00
1.05
3.01
3.00
3.00
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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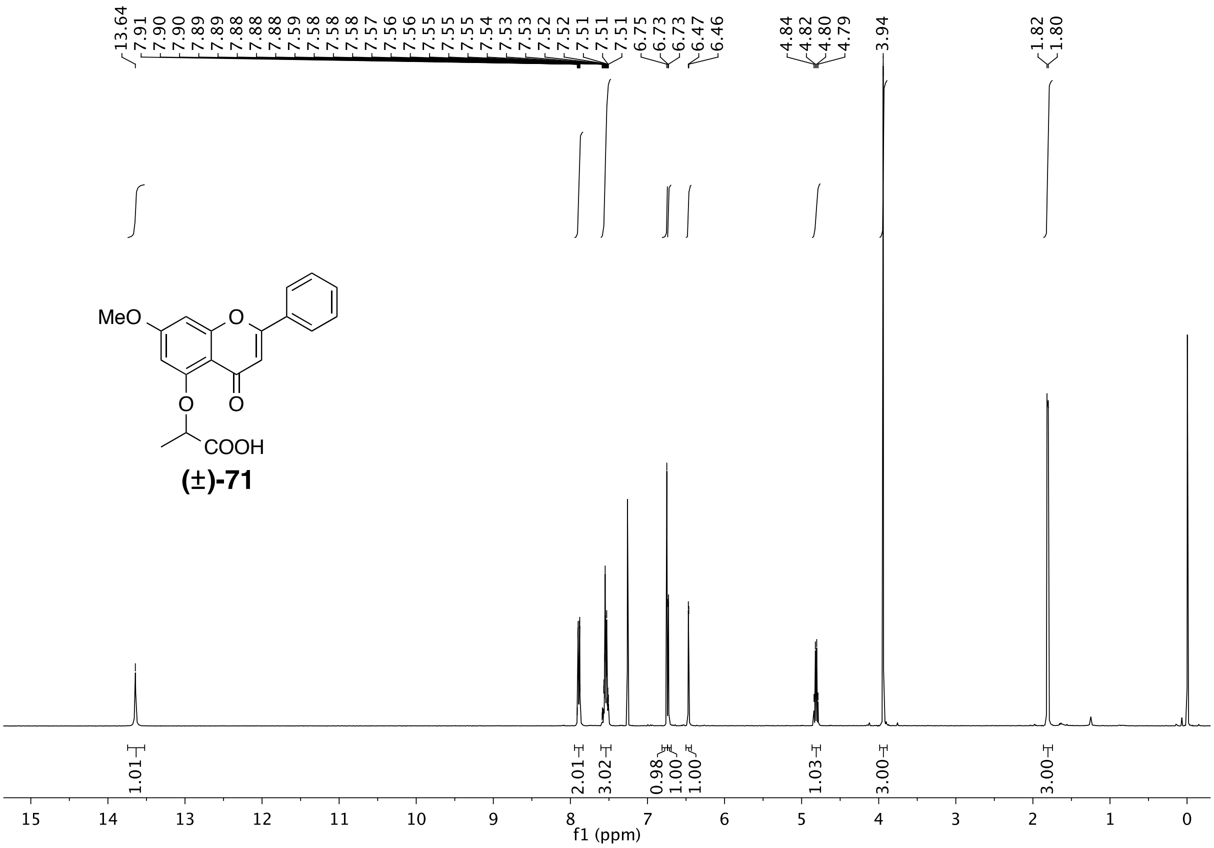
f1 (ppm)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7.55
7.54
7.53
7.53
7.52
7.52
7.51
7.51
7.51
6.75
6.73
6.73
6.47
6.46
4.84
4.82
4.80
4.79
3.94
1.82
1.80
MeO
O
O
O
COOH
(±)-71
1.01
2.01
3.02
0.98
1.00
1.00
1.03
3.00
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13
12
11
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9
8
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5
4
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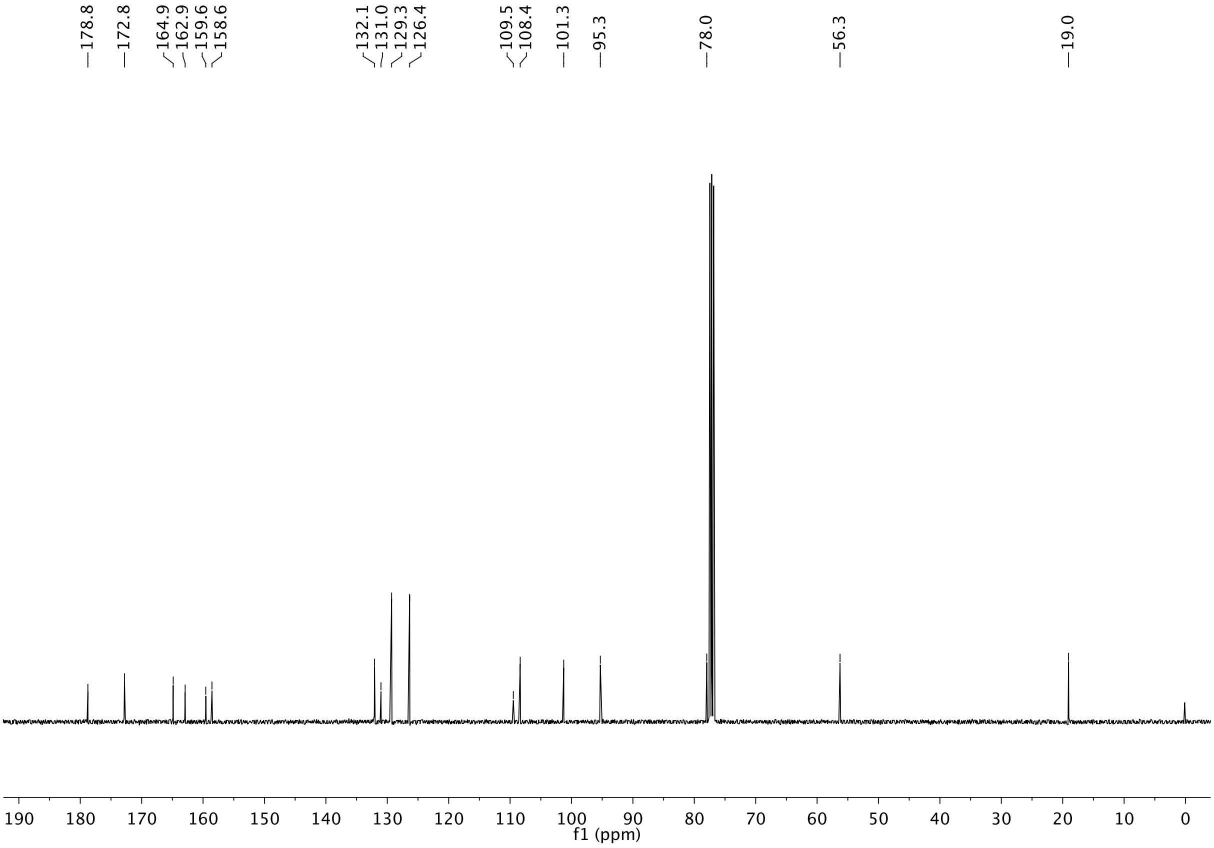
f1 (ppm)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158.6
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131.0
129.3
126.4
109.5
108.4
101.3
95.3
78.0
56.3
19.0
190
180
170
160
150
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130
120
110
100
90
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50
40
30
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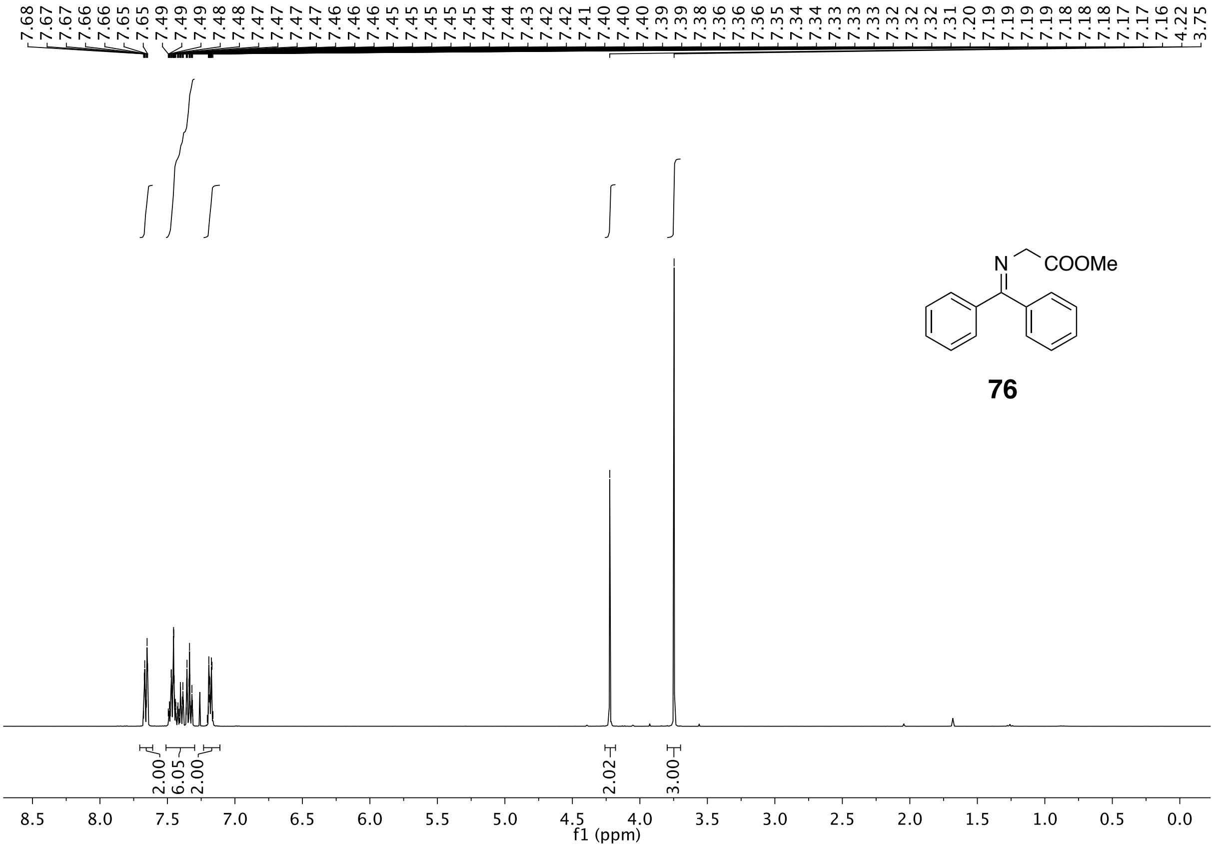
f1 (ppm)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7.33
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7.32
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7.31
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7.19
7.19
7.19
7.19
7.18
7.18
7.18
7.17
7.16
4.22
3.75
N
COOMe
76
2.00
6.05
2.00
2.02
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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4.0
3.5
3.0
2.5
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1.5
1.0
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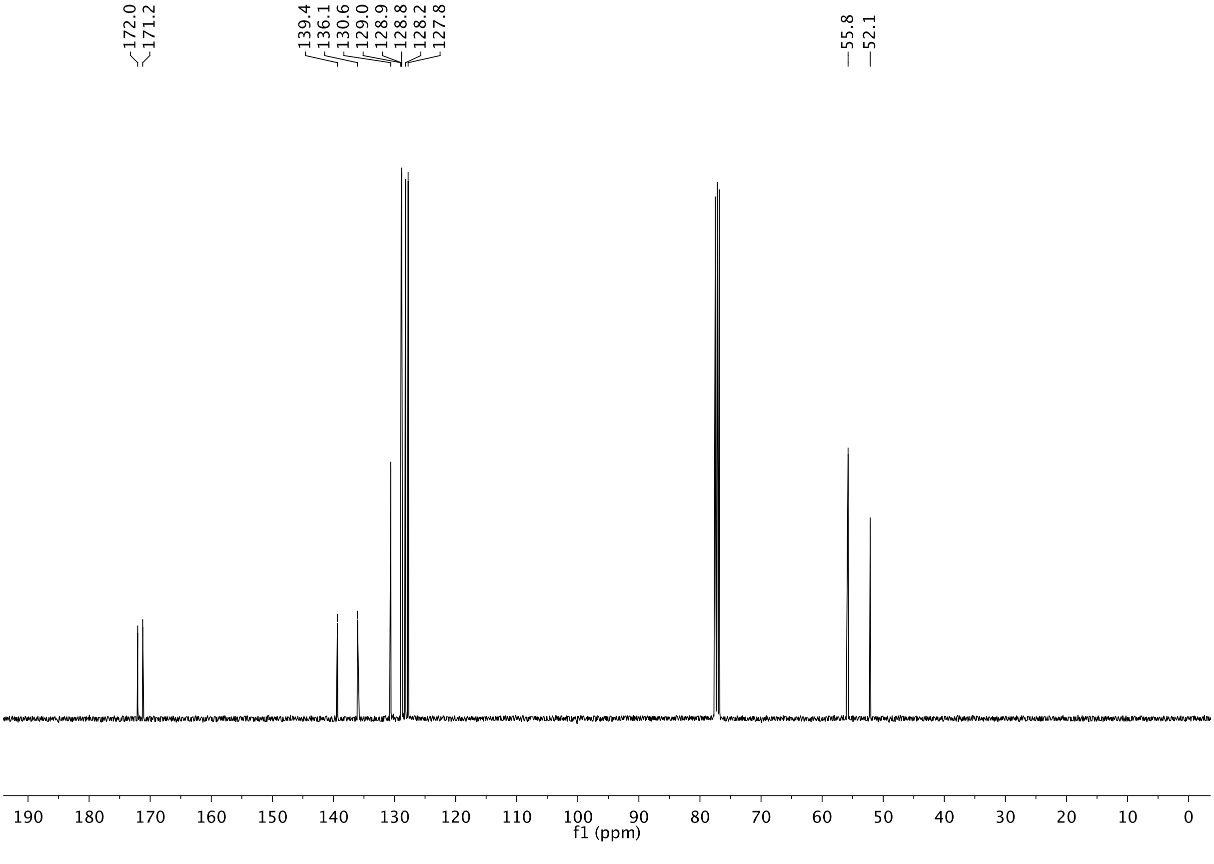
f1 (ppm)


172.0
171.2
139.4
136.1
130.6
129.0
128.9
128.8
128.2
127.8
55.8
52.1
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
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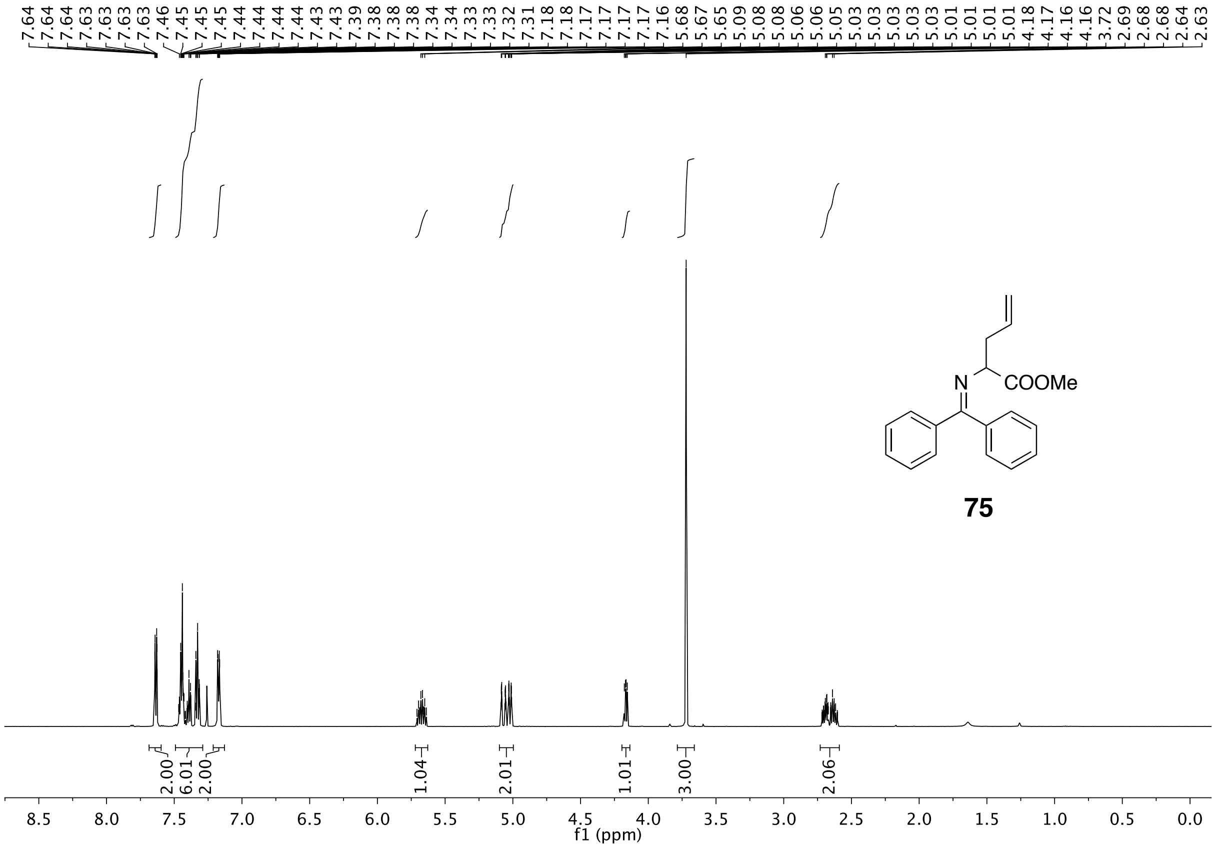
30
20
10
0
f1 (ppm)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7.44
7.43
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7.18
7.17
7.17
7.17
7.17
7.16
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5.67
5.65
5.09
5.08
5.08
5.06
5.05
5.03
5.03
5.03
5.03
5.01
5.01
5.01
4.18
4.17
4.16
3.72
2.69
2.68
2.68
2.64
2.63
N
COOMe
2.00
6.01
2.00
1.04
2.01
1.01
3.00
2.06
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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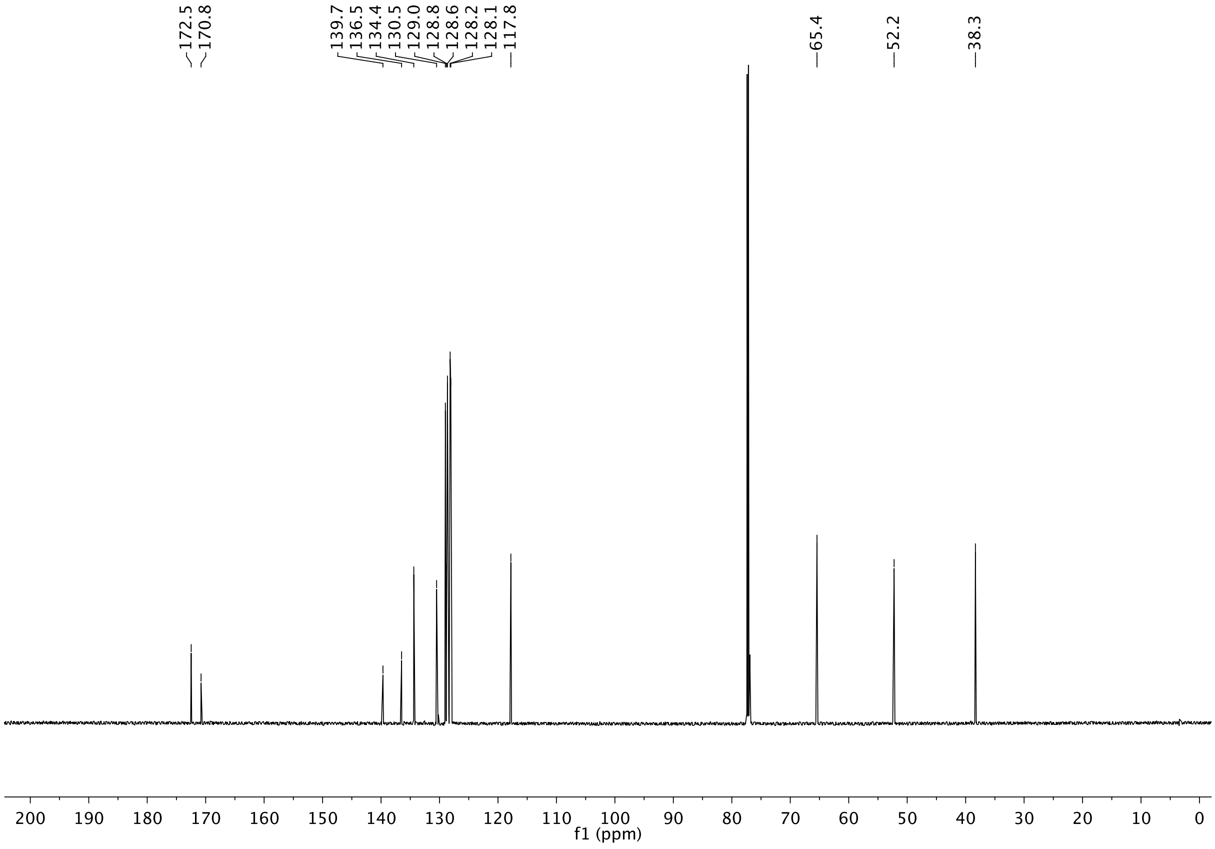
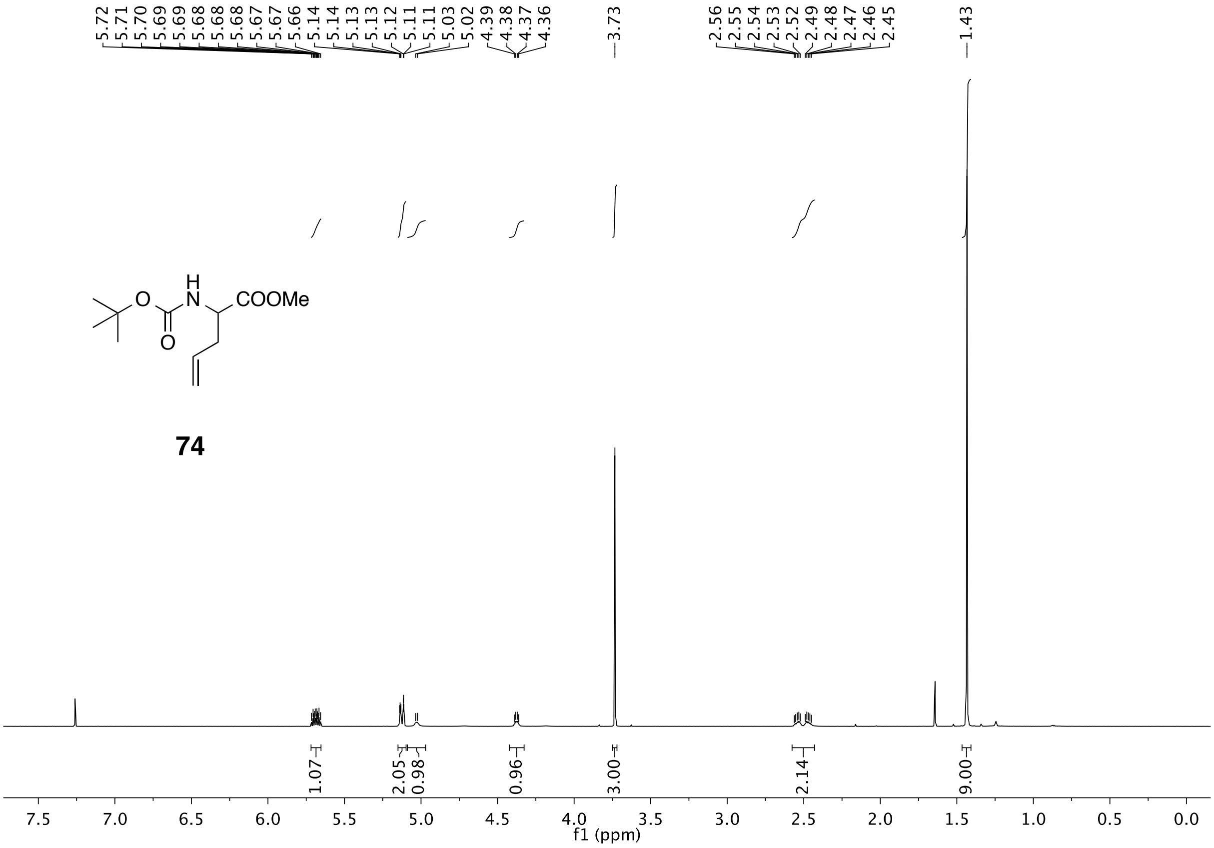
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

75


172.5
170.8
139.7
136.5
134.4
130.5
129.0
128.8
128.6
128.2
128.1
117.8
65.4
52.2
38.3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


74
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5.71
5.70
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5.68
5.67
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5.66
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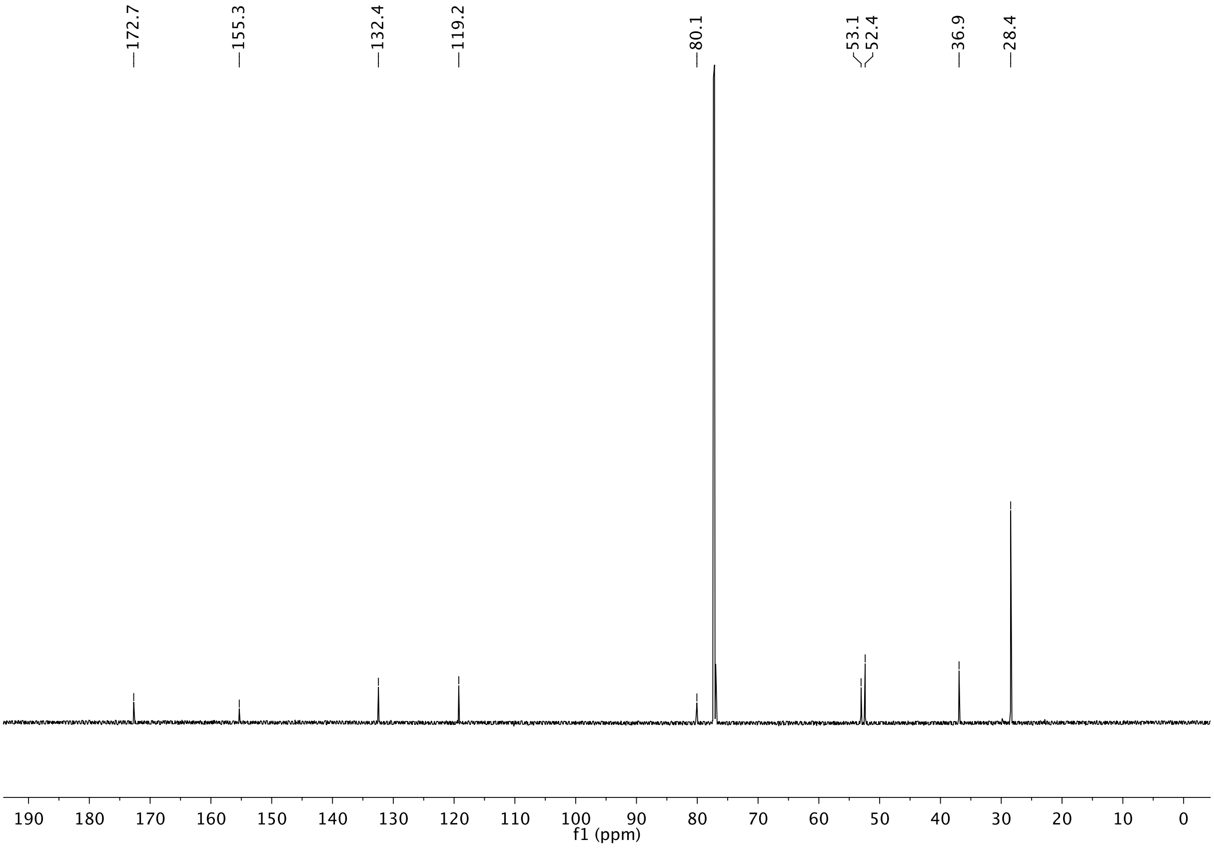
5.14
5.13
5.13
5.12
5.11
5.11
5.03
5.02
4.39
4.38
4.37
4.36
3.73
2.56
2.55
2.54
2.53
2.52
2.49
2.48
2.47
2.46
2.45
1.43
H
O
N
COOMe
O
1.07
2.05
0.98
0.96
3.00
2.14
9.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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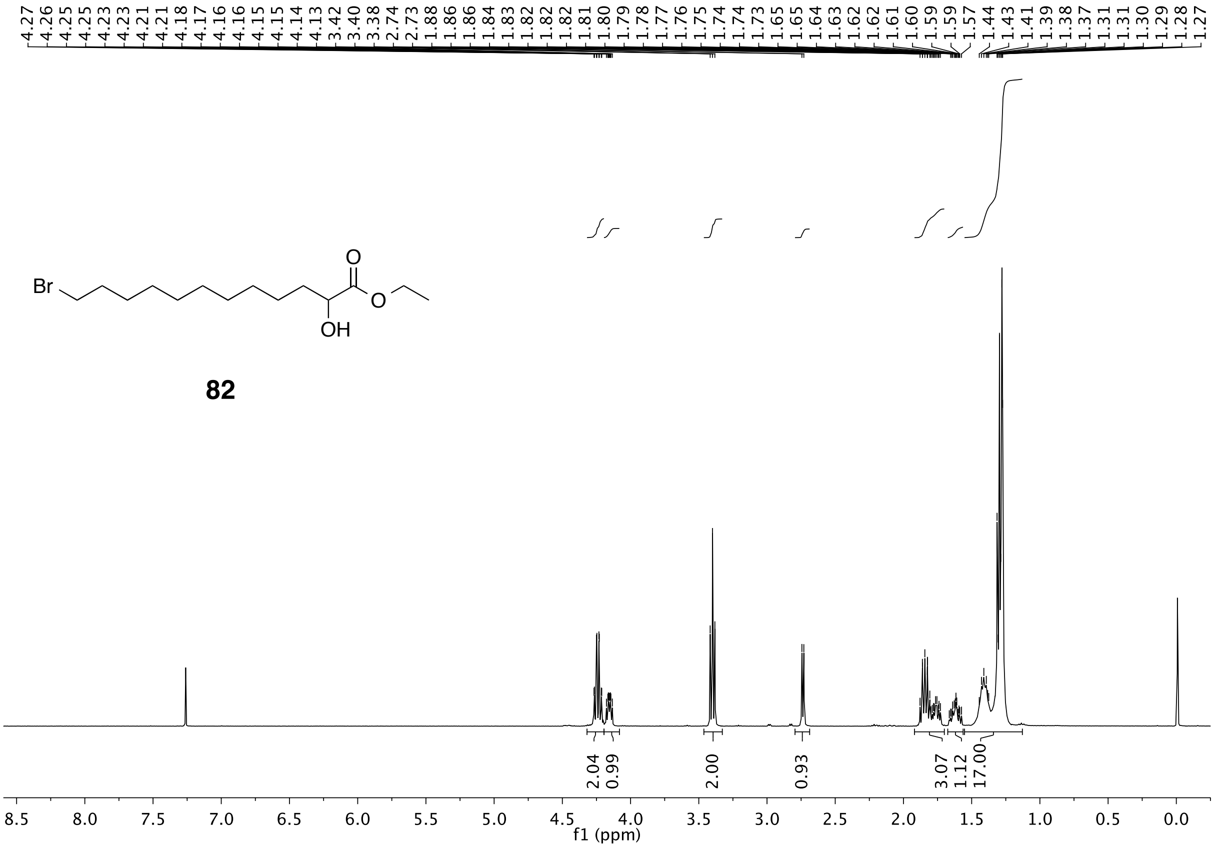
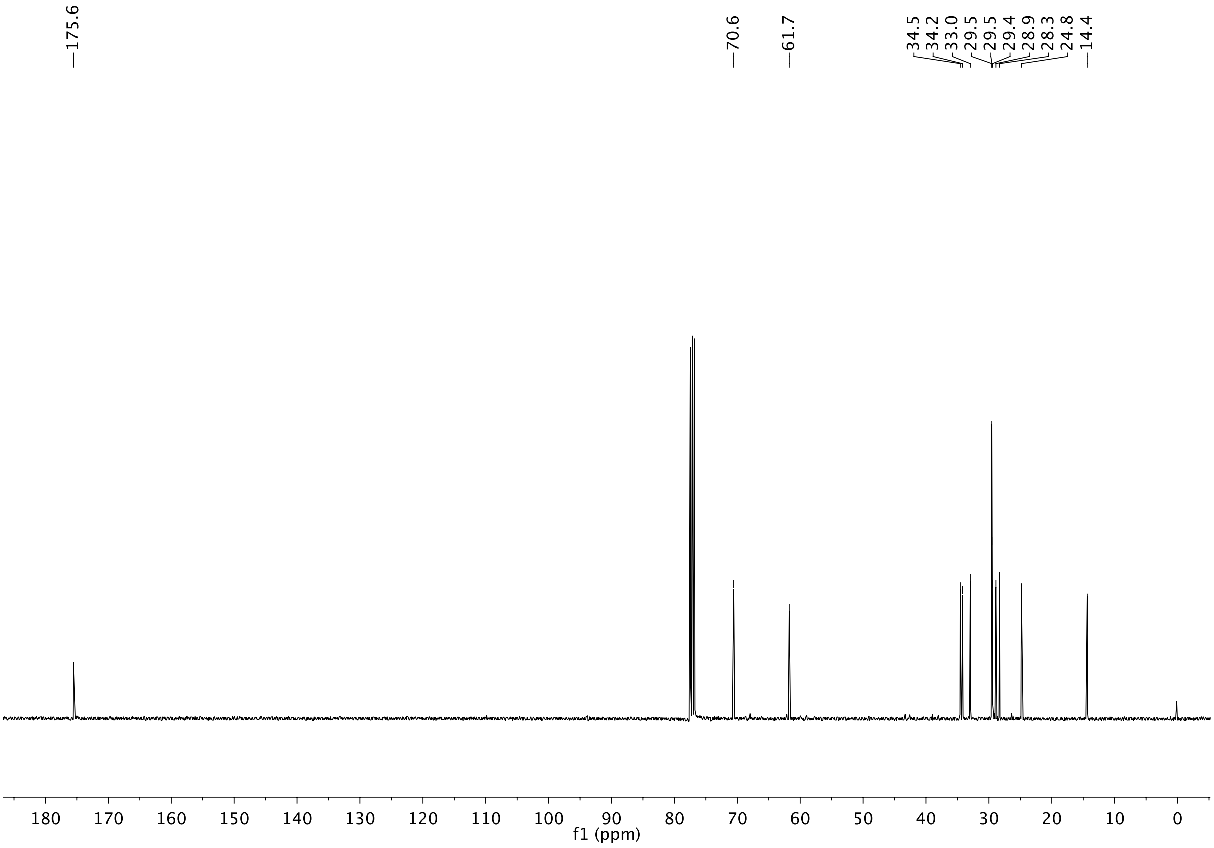
155.3
132.4
119.2
80.1
53.1
52.4
36.9
28.4
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


4.27 4.26 4.25 4.25 4.23 4.23 4.21 4.21 4.18 4.17 4.16 4.16 4.15 4.15 4.14 4.13 3.42 3.40 3.38 2.74 2.73 1.88 1.86 1.86 1.84 1.83 1.82 1.82 1.82 1.81 1.80 1.79 1.78 1.77 1.76 1.75 1.74 1.74 1.73 1.65 1.64 1.63 1.62 1.61 1.60 1.59 1.59 1.57 1.44 1.43 1.41 1.39 1.38 1.37 1.31 1.31 1.30 1.29 1.28 1.27
Br
O
O
OH
2.04
0.99
2.00
0.93
3.07
1.12
17.00
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

82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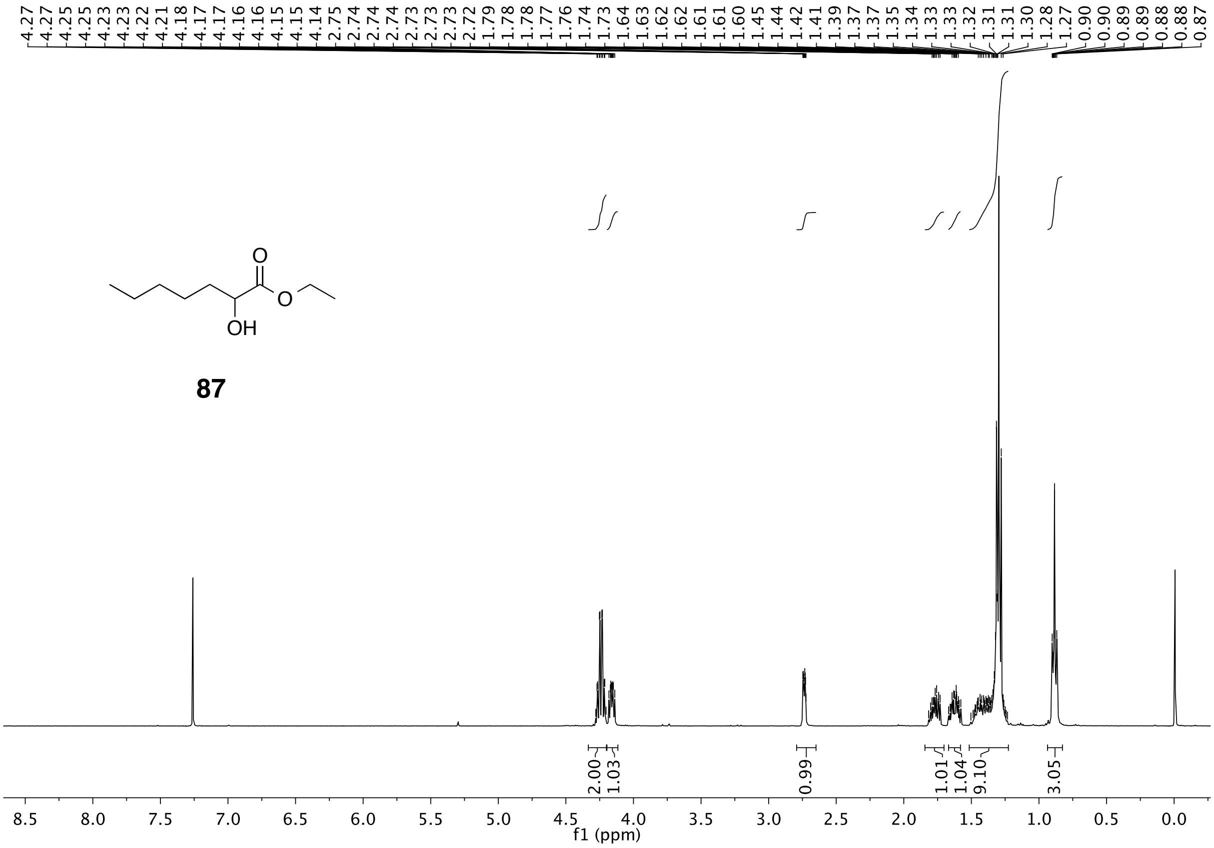
70.6
61.7
34.5 34.2 33.0 29.5 29.5 29.4 28.9 28.3 24.8
14.4
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



87

4.27
4.27
4.25
4.25
4.23
4.23
4.22
4.21
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4.17
4.17
4.16
4.16
4.15
4.15
4.14
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2.74
2.74
2.73
2.73
2.73
2.72
1.79
1.78
1.78
1.77
1.76
1.74
1.73
1.64
1.63
1.62
1.61
1.61
1.60
1.45
1.44
1.42
1.41
1.39
1.37
1.37
1.35
1.34
1.33
1.33
1.32
1.31
1.31
1.30
1.28
1.27
0.90
0.90
0.89
0.88
0.88
0.87
O
O
OH
2.00
1.03
0.99
1.01
1.04
9.10
3.05
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
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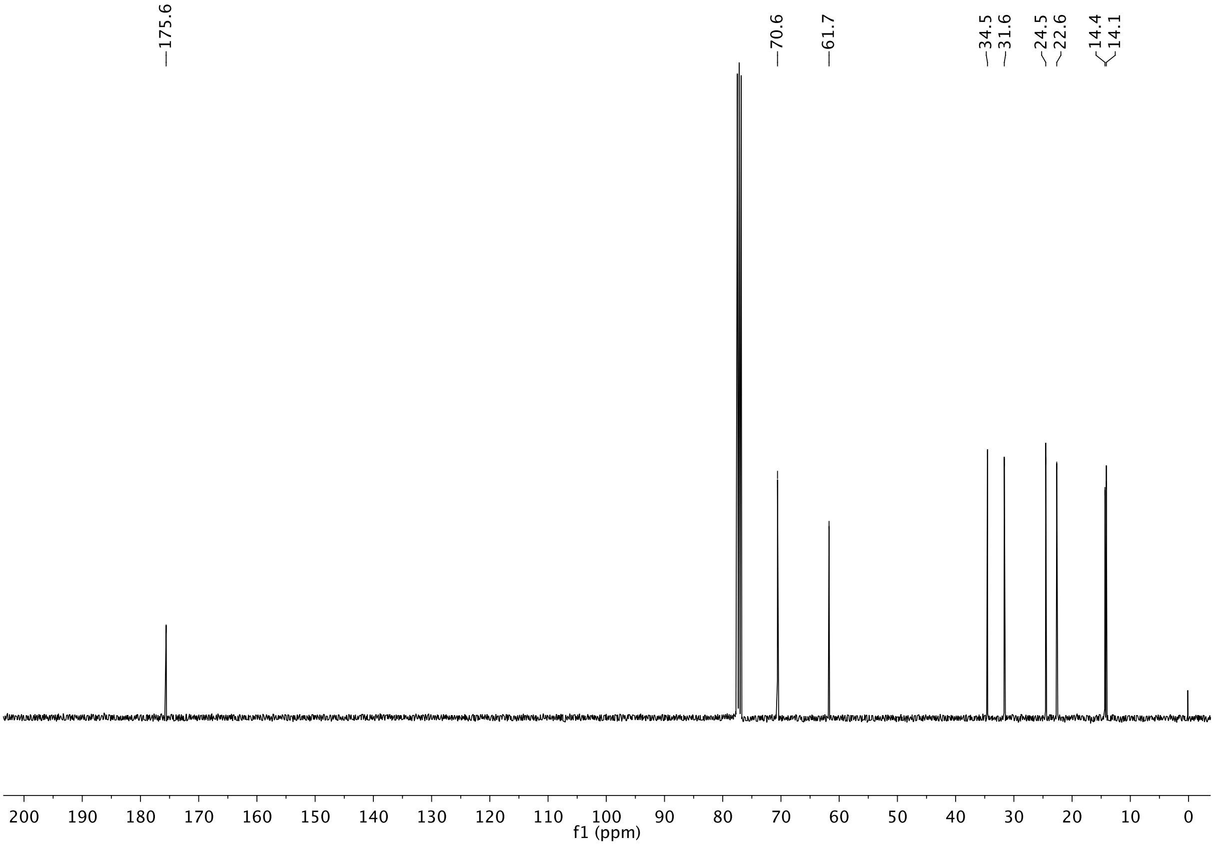
0.5
0.0
f1 (ppm)



175.6
70.6
61.7
34.5
31.6
24.5
22.6
14.4
14.1
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
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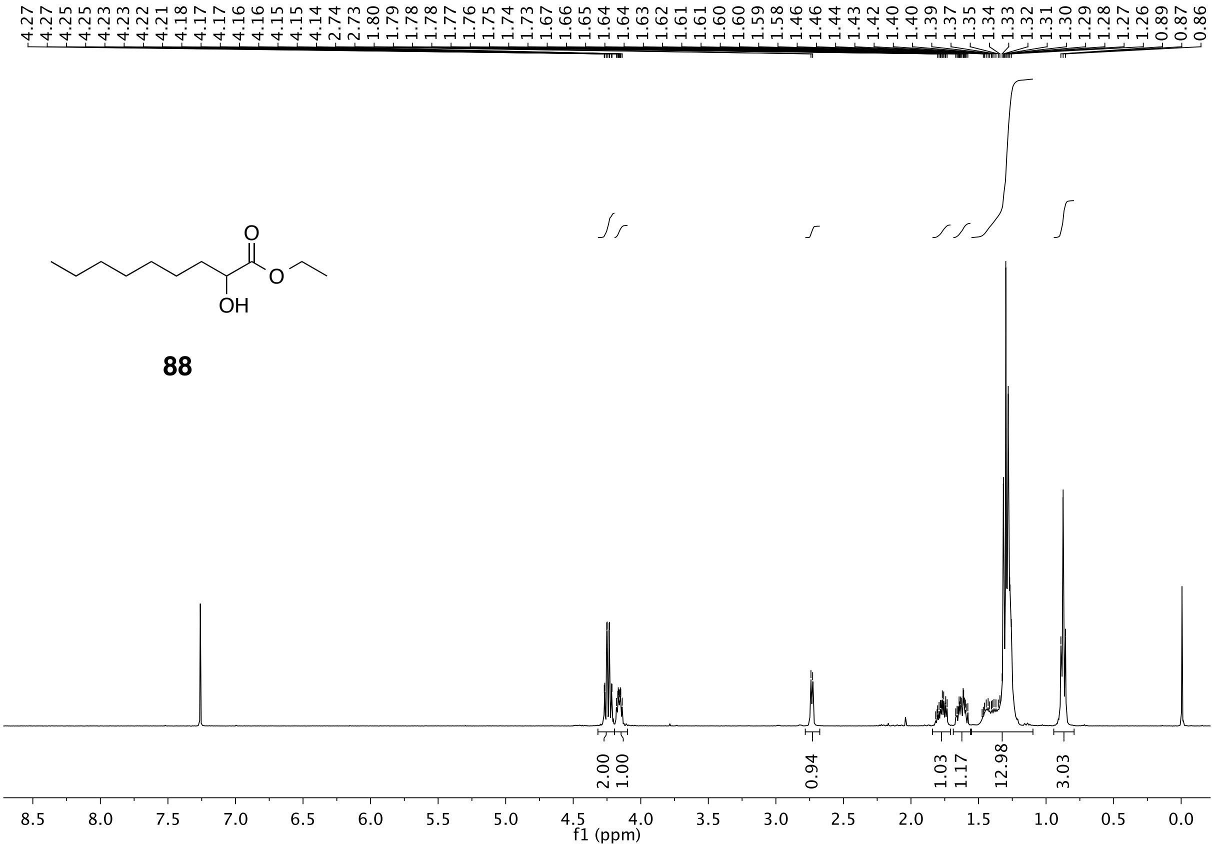
20
10
0
f1 (ppm)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4.16
4.15
4.15
4.14
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2.73
1.80
1.79
1.78
1.78
1.77
1.76
1.75
1.74
1.73
1.67
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1.39
1.37
1.35
1.34
1.33
1.32
1.31
1.30
1.29
1.28
1.27
1.26
0.89
0.87
0.86
O
O
OH
2.00
1.00
0.94
1.03
1.17
12.98
3.03
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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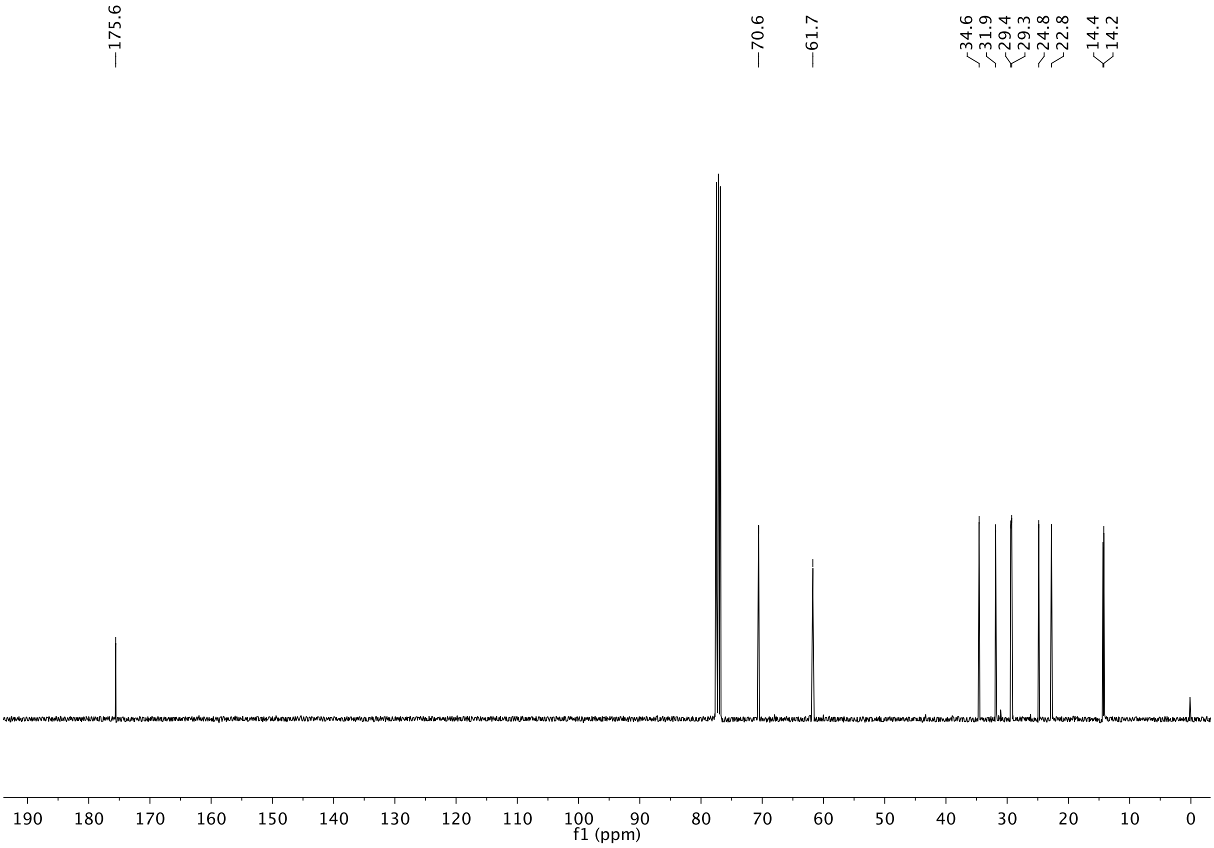
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

88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34.6
31.9
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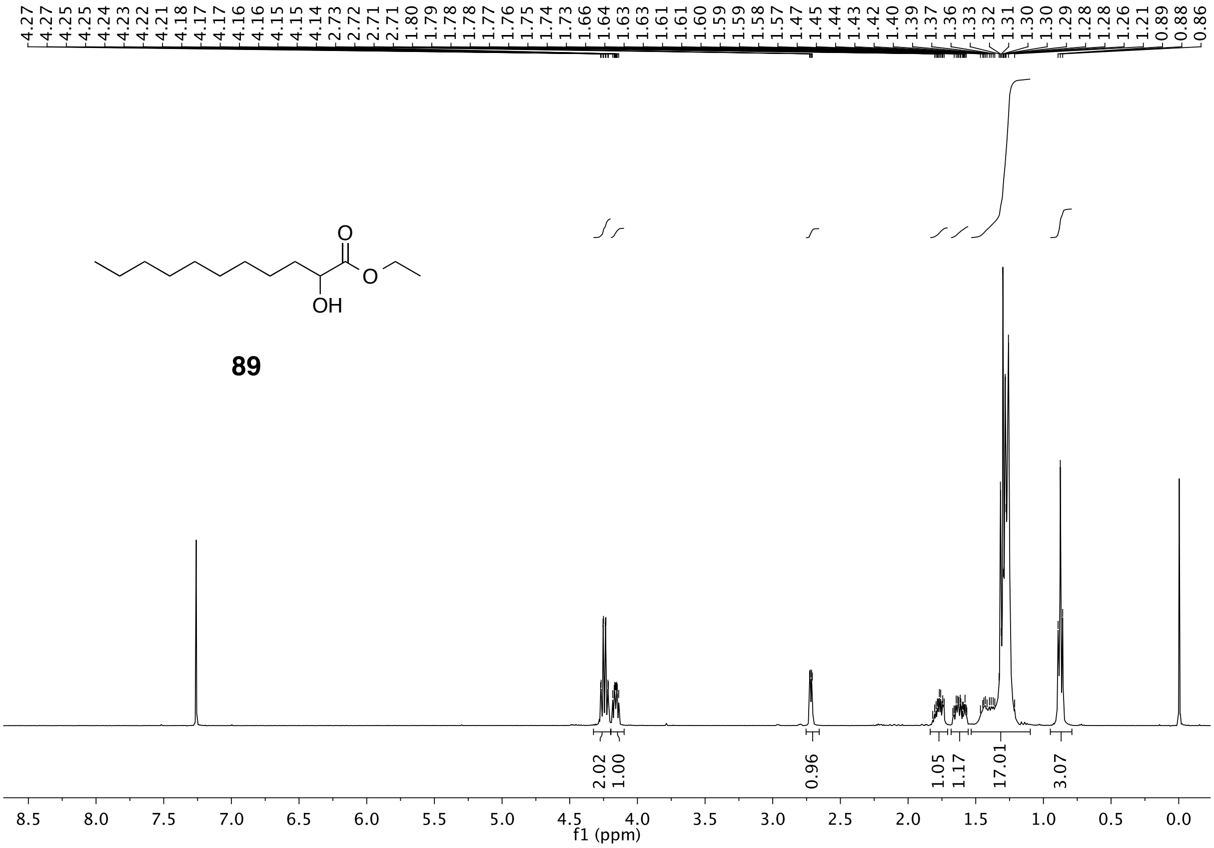
29.3
24.8
22.8
14.4
14.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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4.16
4.15
4.15
4.14
2.73
2.72
2.71
2.71
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1.79
1.78
1.78
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1.74
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1.58
1.57
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1.45
1.44
1.43
1.42
1.40
1.39
1.37
1.36
1.33
1.32
1.31
1.30
1.29
1.28
1.28
1.26
1.21
0.89
0.88
0.86
O
O
OH
2.02
1.00
0.96
1.05
1.17
17.01
3.07
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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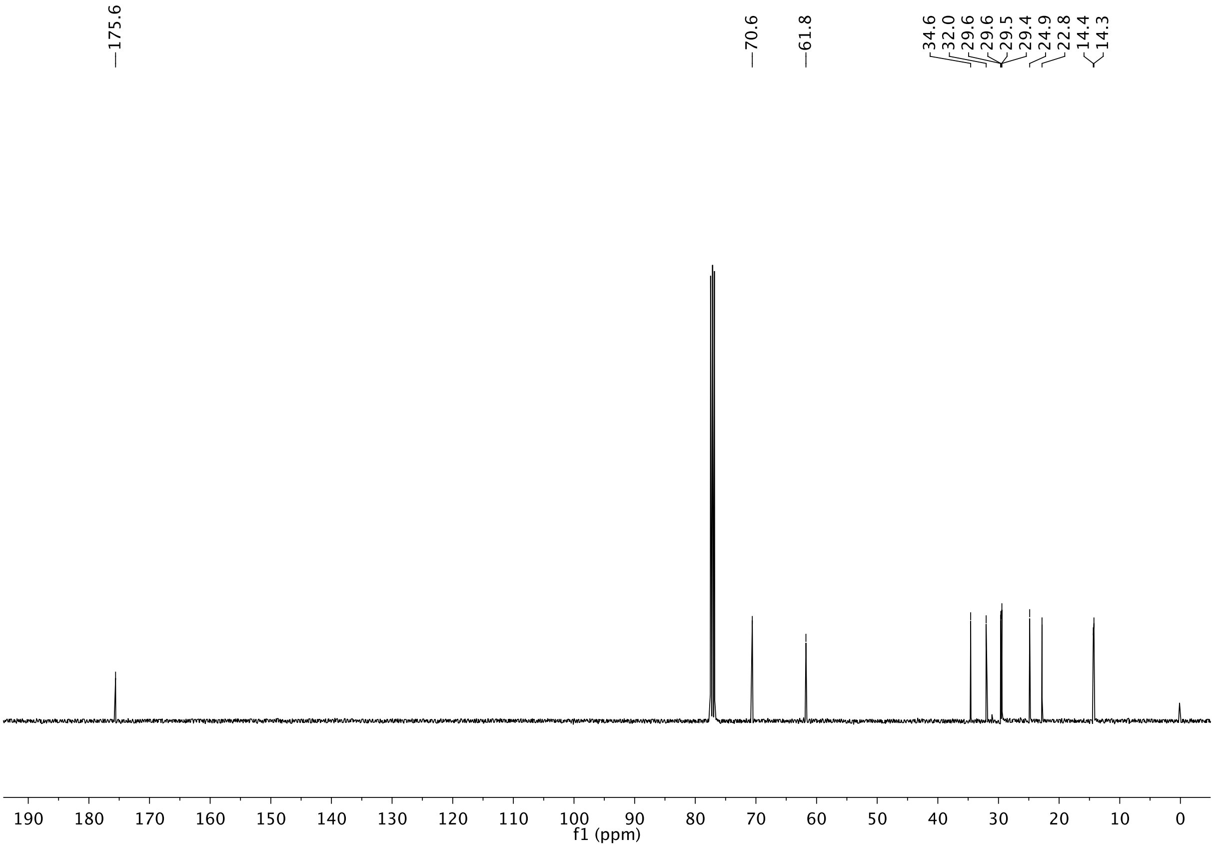
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

89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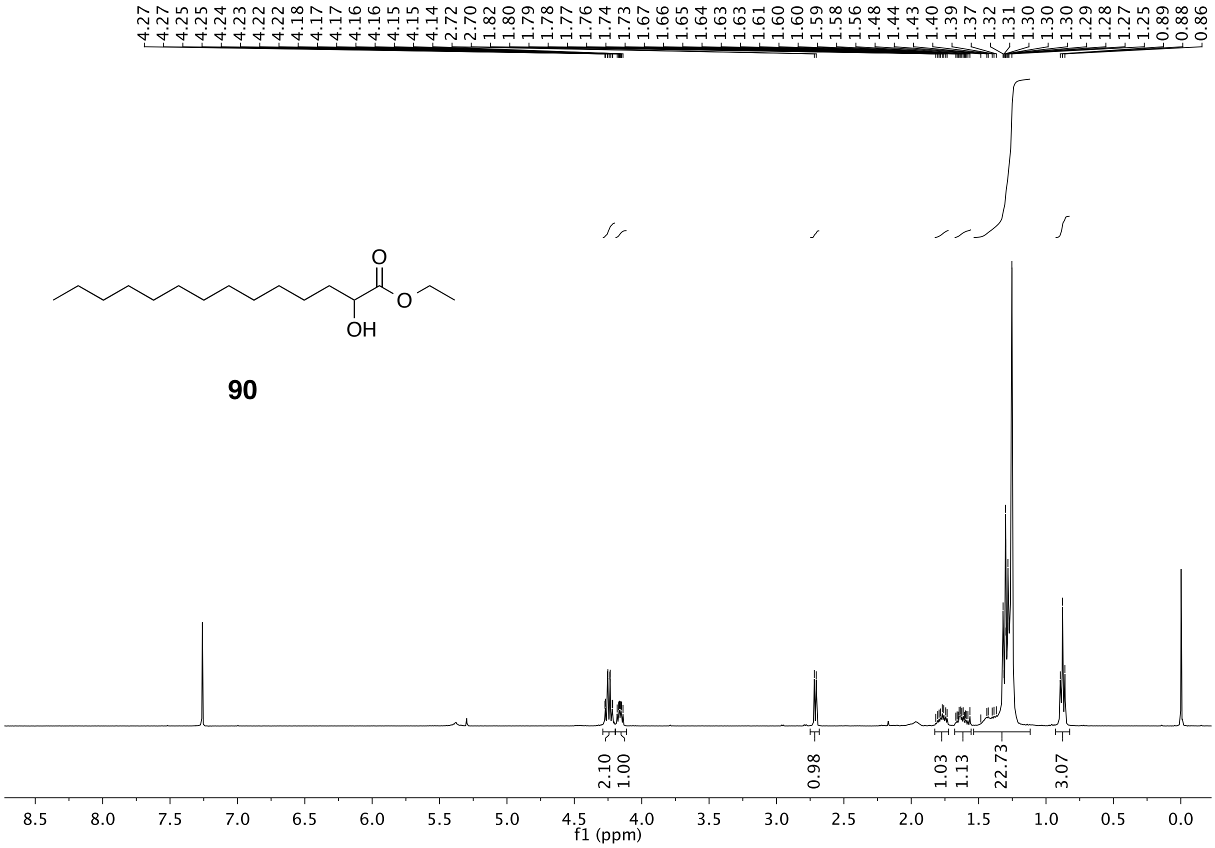
32.0
29.6
29.6
29.5
29.4
24.9
22.8
14.4
14.3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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4.16
4.16
4.15
4.15
4.14
2.72
2.70
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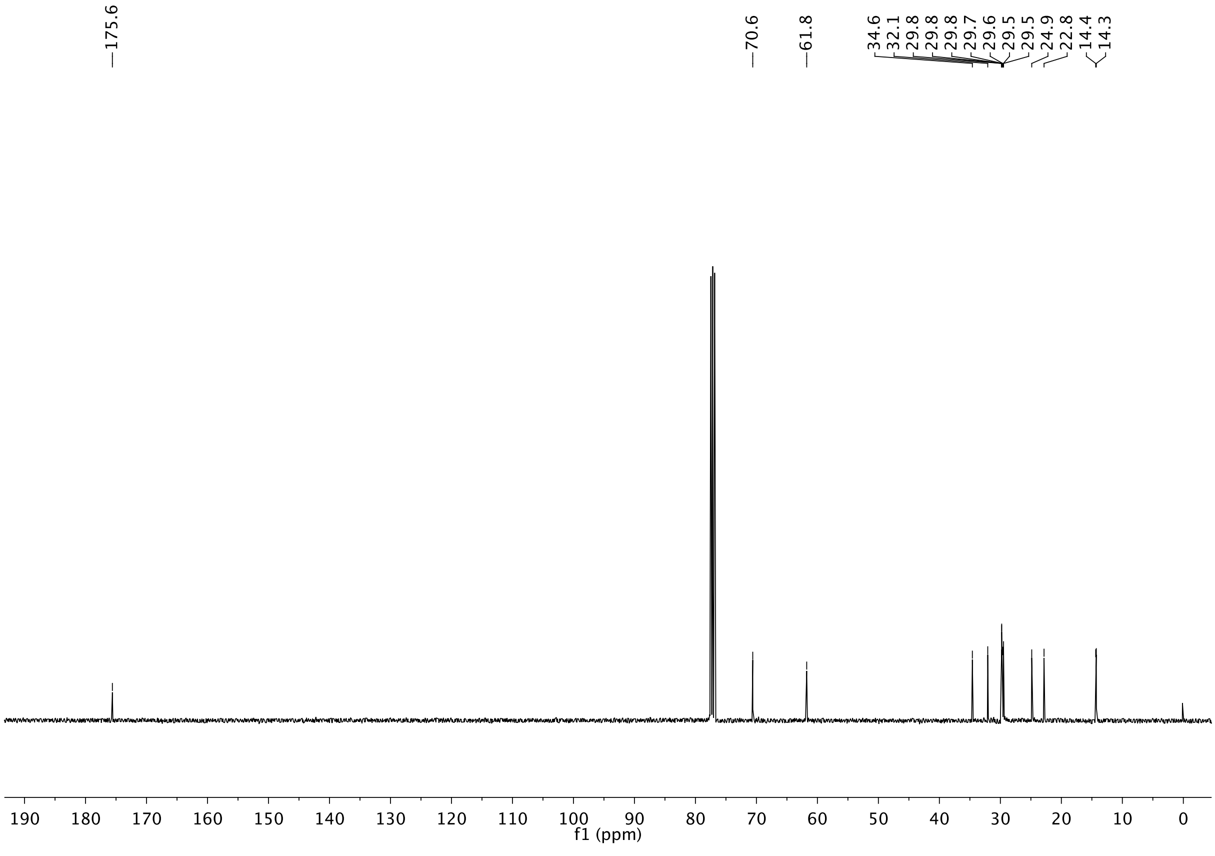
1.80
1.79
1.78
1.77
1.76
1.74
1.73
1.67
1.66
1.65
1.64
1.63
1.63
1.61
1.60
1.60
1.59
1.58
1.56
1.48
1.44
1.43
1.40
1.39
1.37
1.32
1.31
1.30
1.30
1.30
1.29
1.28
1.27
1.25
0.89
0.88
0.86
O
O
OH
2.10
1.00
0.98
1.03
1.13
22.73
3.07
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

90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61.8
34.6
32.1
29.8
29.8
29.8
29.7
29.6
29.5
29.5
24.9
22.8
14.4
14.3
190
180
170
160
150
140
130
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110
100
90
80
70
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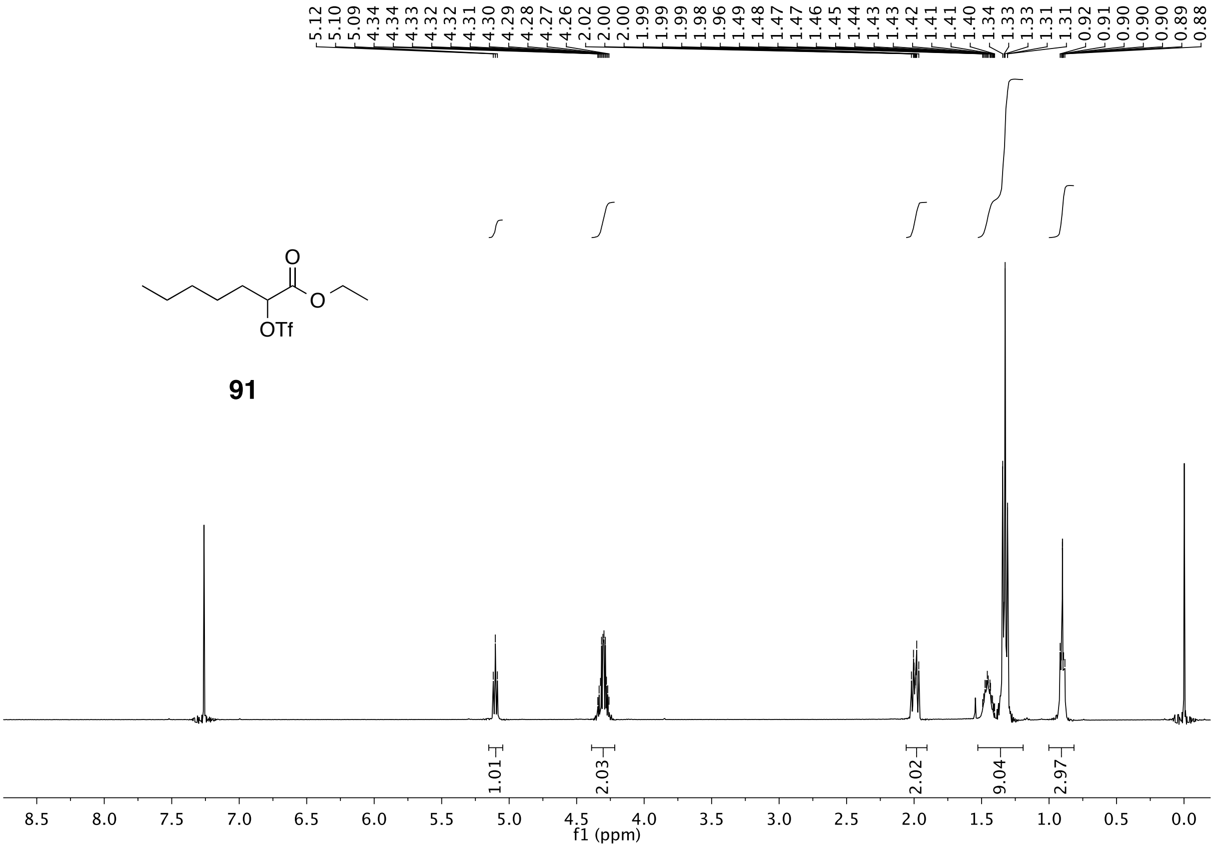
50
40
30
20
10
0
f1 (ppm)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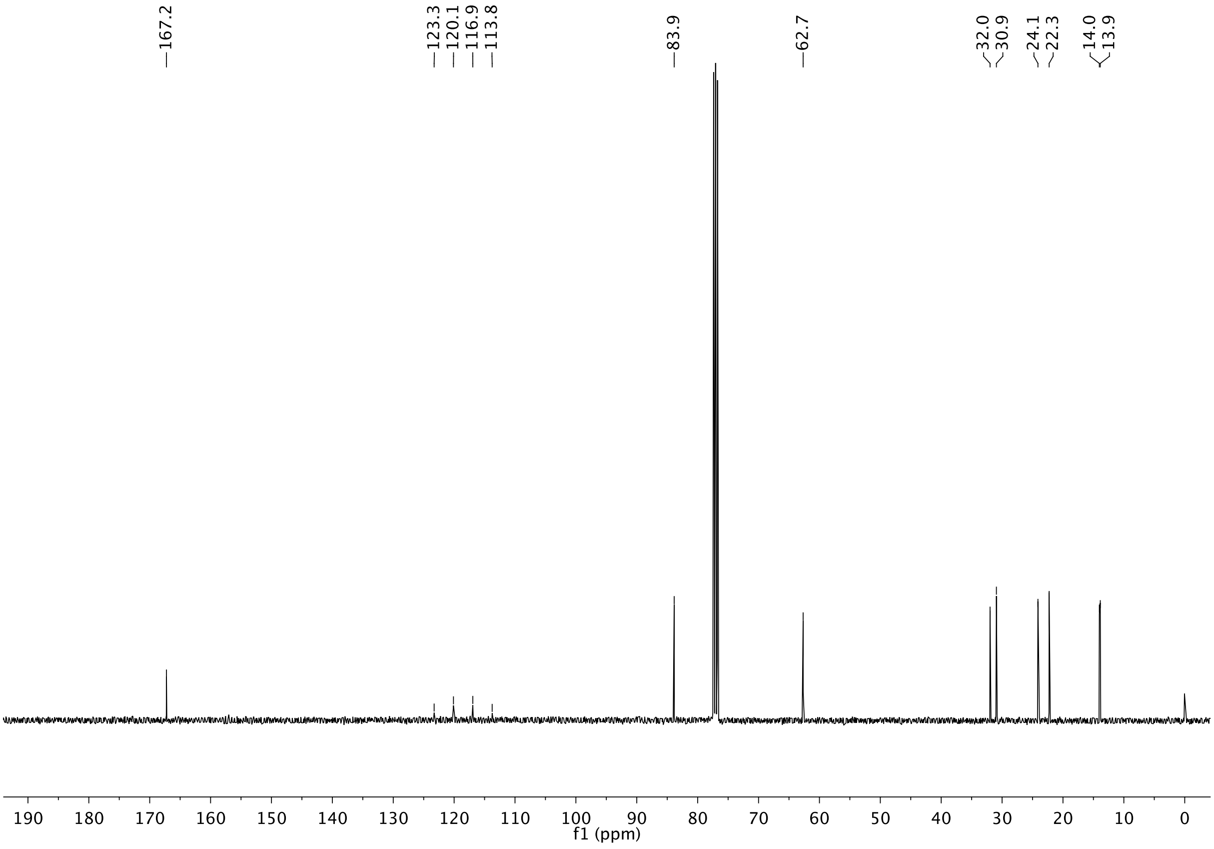
4.31
4.30
4.29
4.28
4.27
4.26
2.02
2.00
2.00
1.99
1.99
1.98
1.98
1.96
1.49
1.48
1.47
1.47
1.46
1.45
1.44
1.43
1.43
1.42
1.41
1.41
1.40
1.34
1.33
1.33
1.31
1.31
0.92
0.91
0.90
0.90
0.90
0.89
0.88
O
O
OTf
1.01
2.03
2.02
9.04
2.97
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

91


167.2
123.3
120.1
116.9
113.8
83.9
62.7
32.0
30.9
24.1
22.3
14.0
13.9
190
180
170
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120
110
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90
80
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40
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20
10
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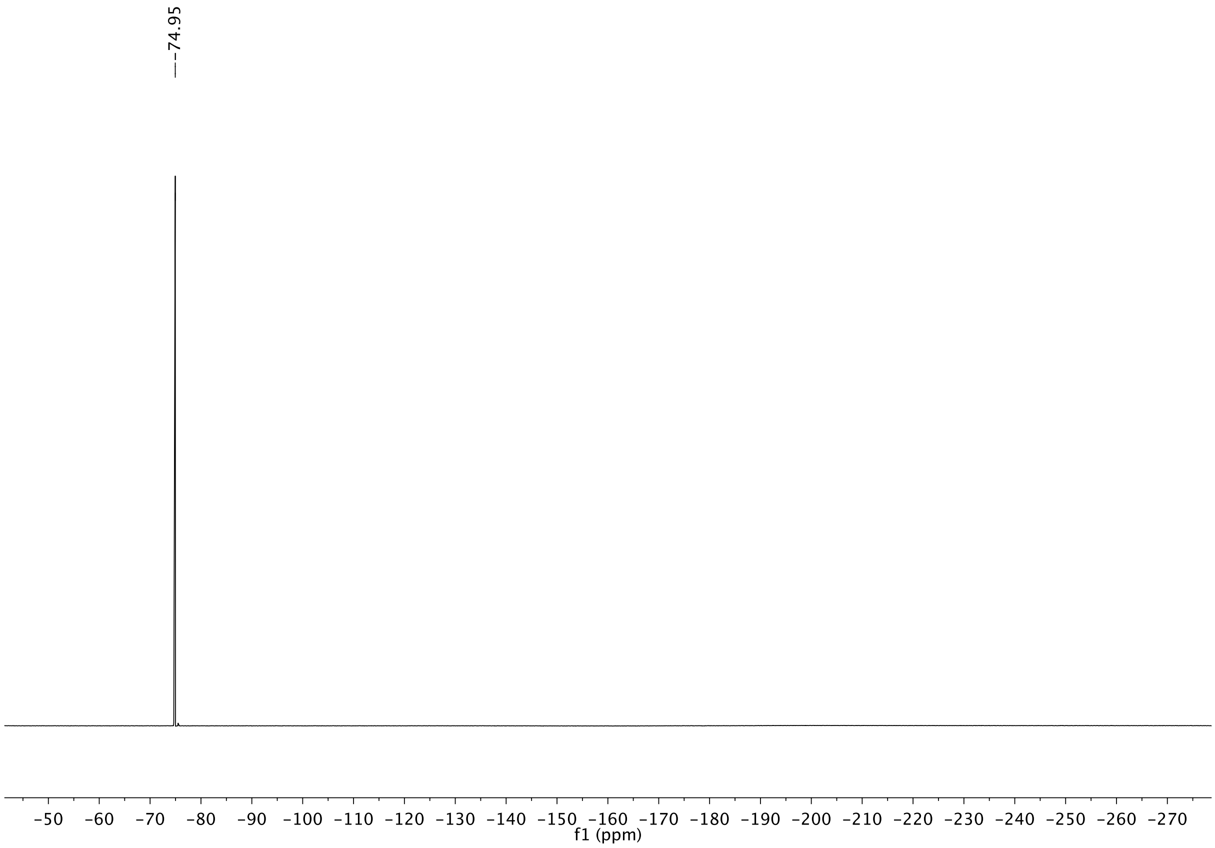
f1 (ppm)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−60
−70
−80
−90
−100
−110
−120
−130
−140
−150
−160
−170
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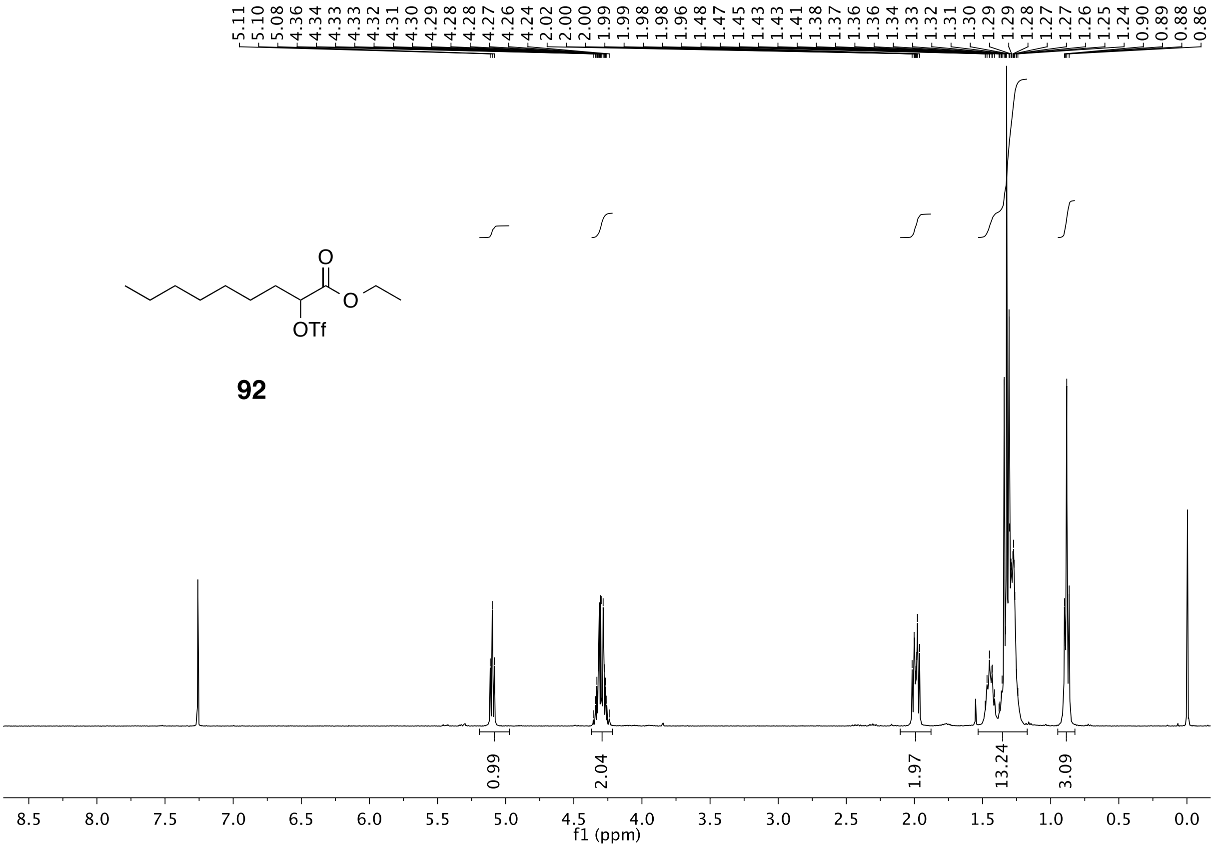
−250
−260
−270
f1 (ppm)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4.34
4.33
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4.32
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4.30
4.29
4.28
4.28
4.27
4.26
4.24
2.02
2.00
2.00
1.99
1.99
1.98
1.98
1.96
1.48
1.47
1.45
1.43
1.43
1.41
1.38
1.37
1.36
1.36
1.34
1.33
1.33
1.32
1.31
1.30
1.29
1.29
1.28
1.27
1.27
1.26
1.25
1.24
0.90
0.89
0.88
0.86
O
O
OTf
0.99
2.04
1.97
13.24
3.09
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
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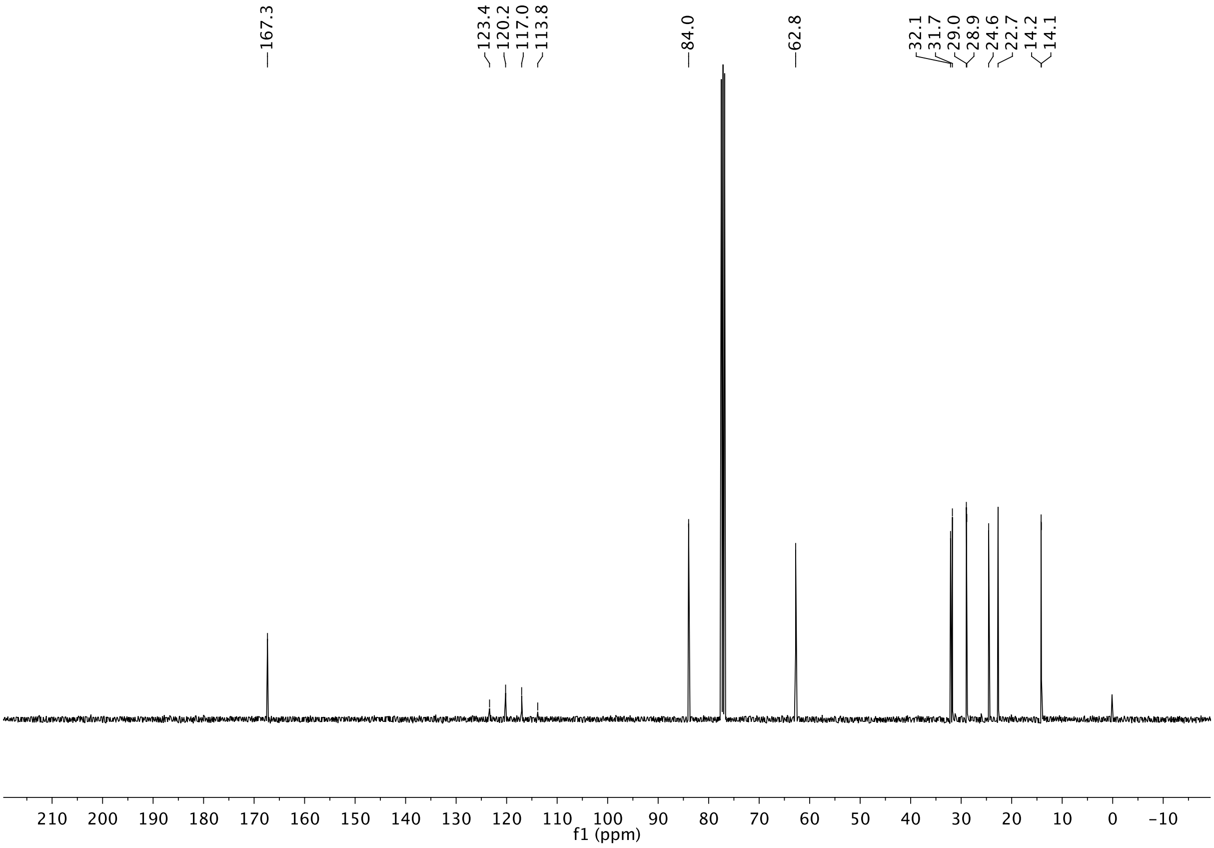
0.0
f1 (ppm)

92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113.8
84.0
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29.0
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24.6
22.7
14.2
14.1
210
200
190
180
170
160
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140
130
120
110
100
90
80
70
60
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40
30
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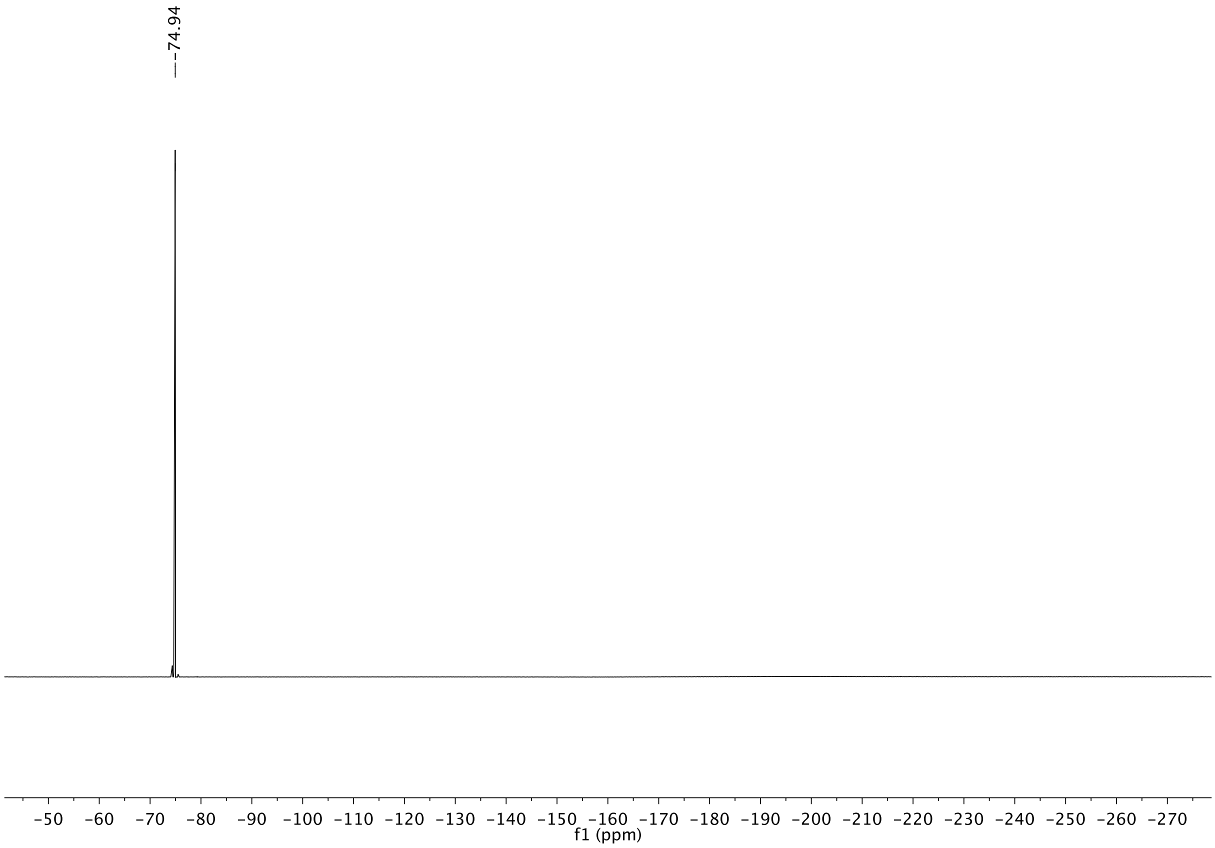
−10
f1 (ppm)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-180
-190
-200
-210
-220
-230
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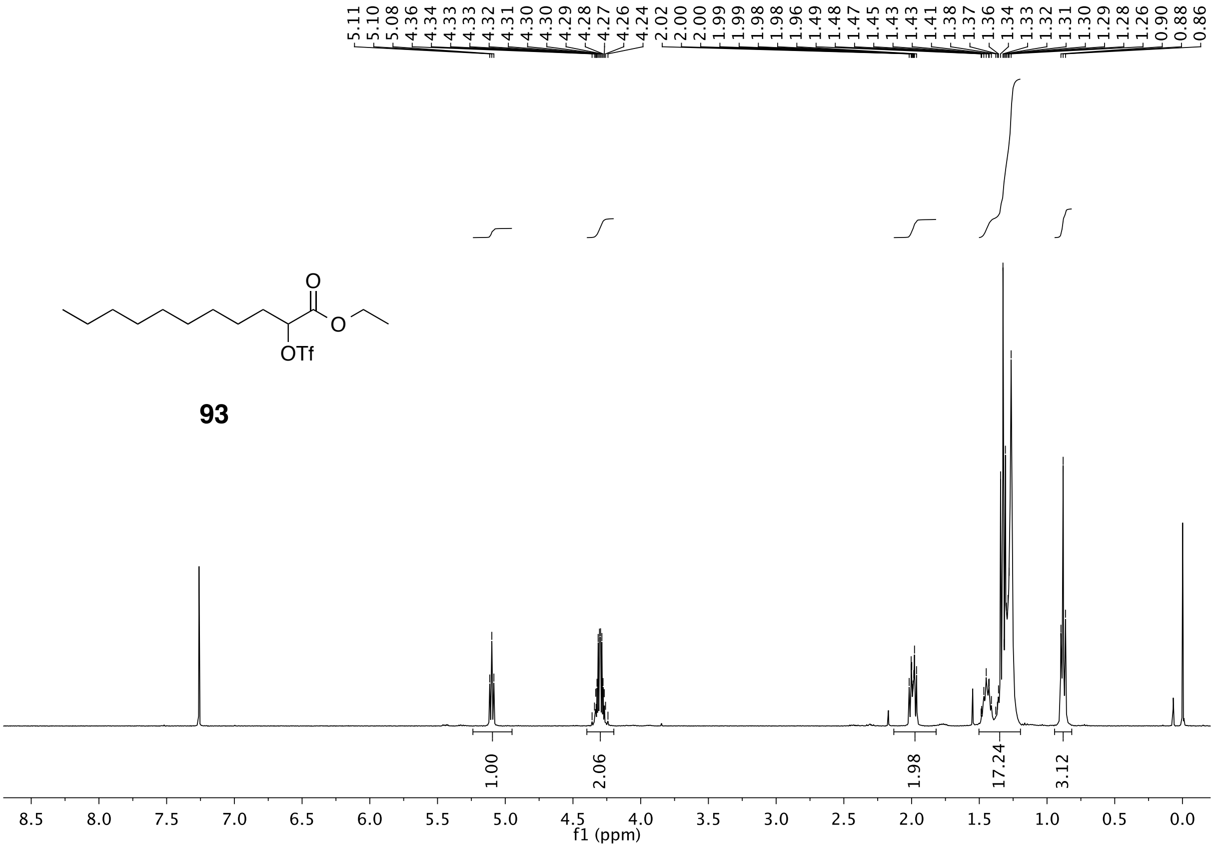
-260
-270
f1 (ppm)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4.28
4.27
4.26
4.24
2.02
2.00
1.99
1.99
1.98
1.98
1.96
1.49
1.48
1.47
1.45
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1.43
1.41
1.38
1.37
1.36
1.34
1.33
1.32
1.31
1.30
1.29
1.28
1.26
0.90
0.88
0.86
O
O
OTf
1.00
2.06
1.98
17.24
3.12
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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2.0
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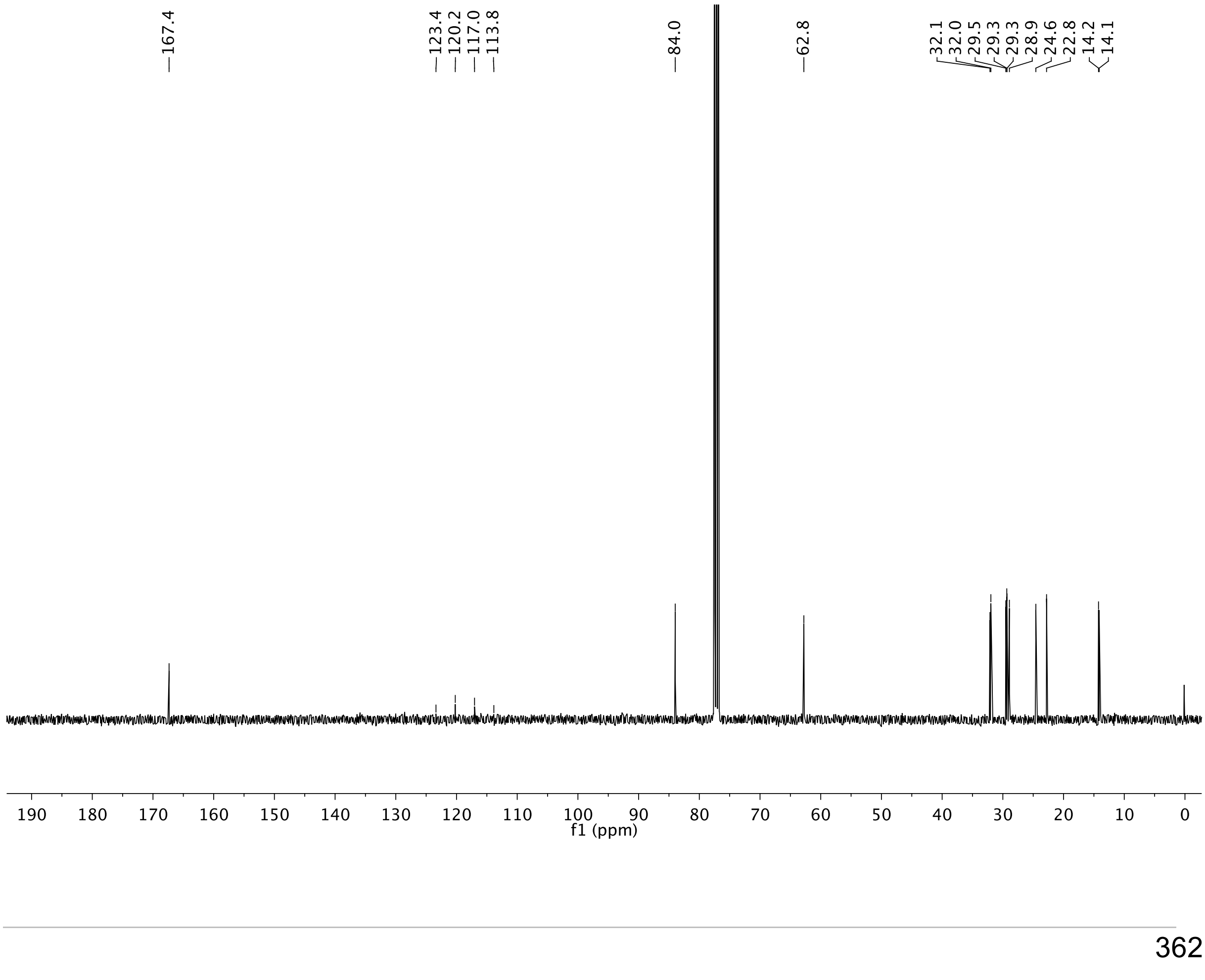
0.0
f1 (ppm)

93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24.6
22.8
14.2
14.1
190
180
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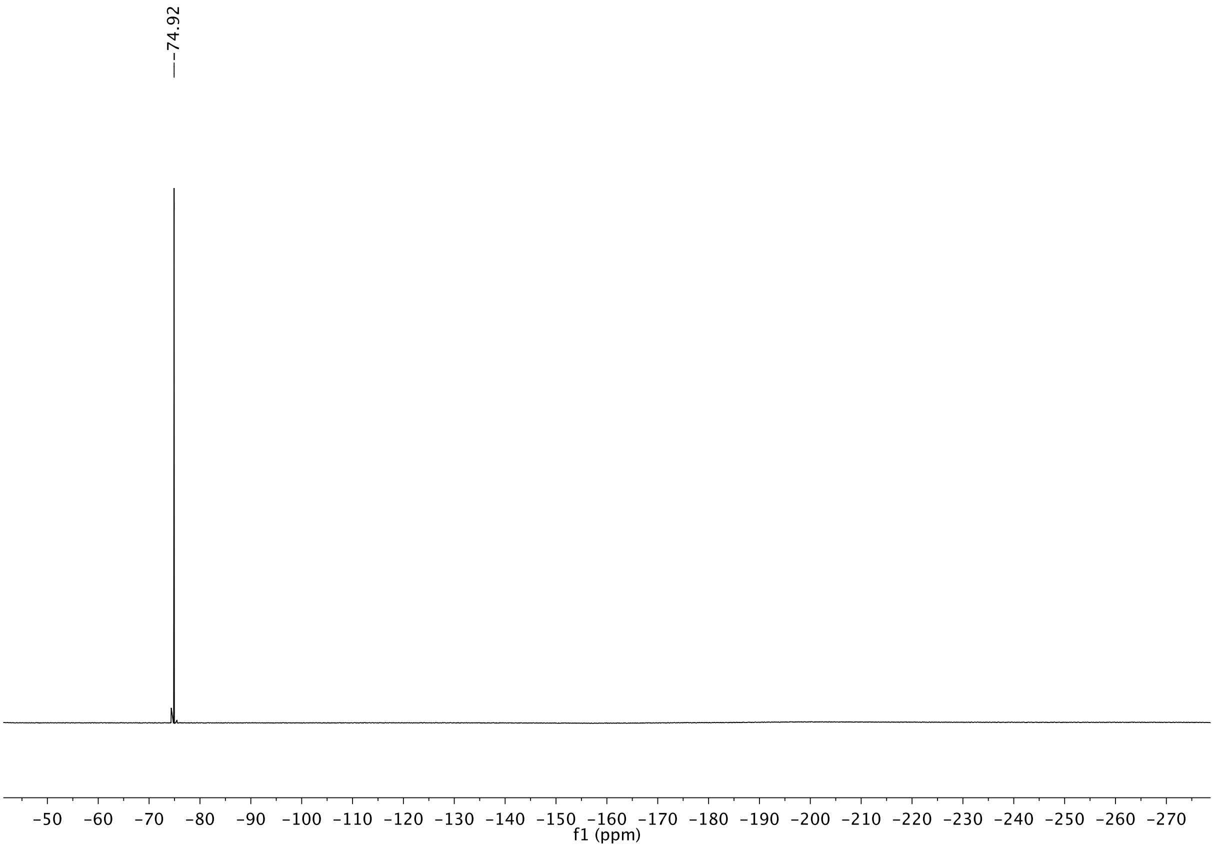
f1 (ppm)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-120
-130
-140
-150
-160
-170
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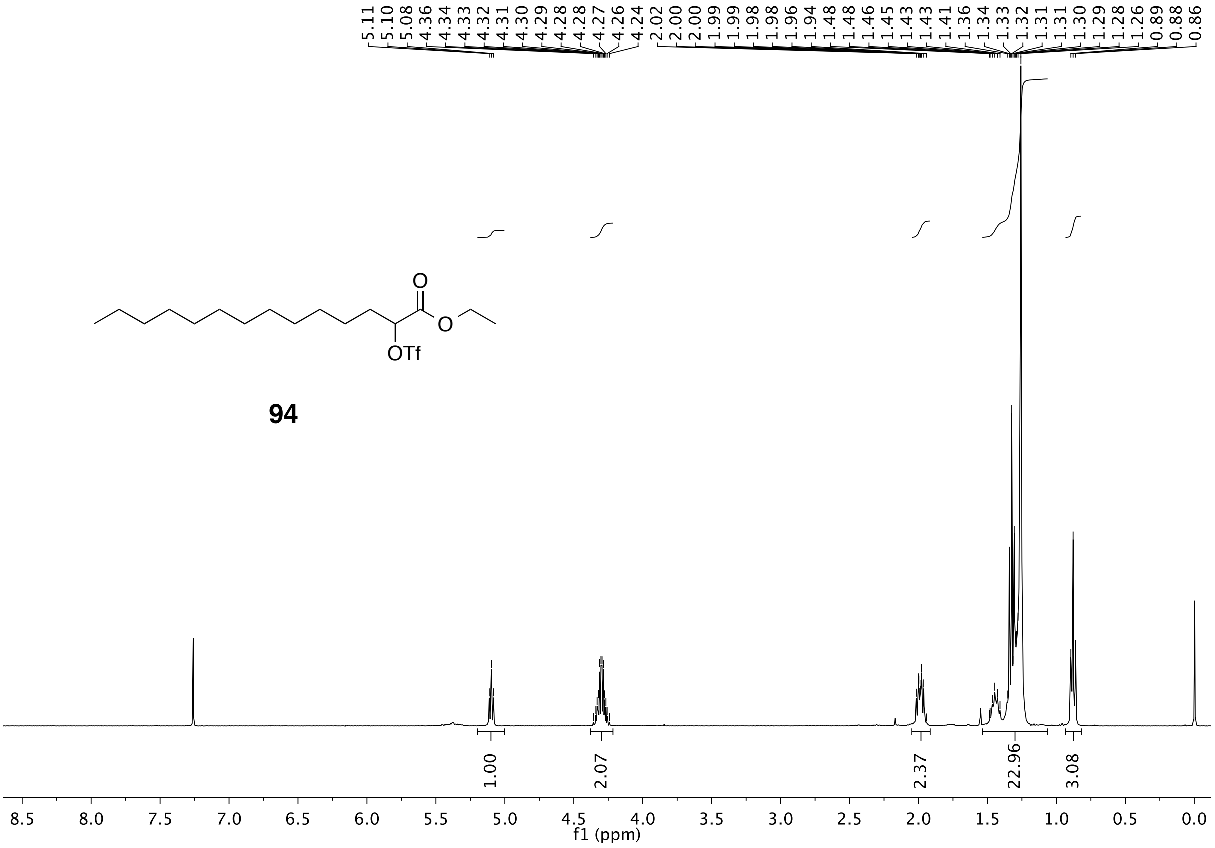
-190
-200
-210
-220
-230
-240
-250
-260
-270
f1 (ppm)

O
O
OTf

94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4.26
4.24
2.02
2.00
1.99
1.98
1.98
1.96
1.94
1.48
1.48
1.46
1.45
1.43
1.43
1.41
1.36
1.34
1.33
1.32
1.31
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1.30
1.29
1.28
1.26
0.89
0.88
0.86
1.00
2.07
2.37
22.96
3.08
8.5
8.0
7.5
7.0
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6.0
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3.0
2.5
2.0
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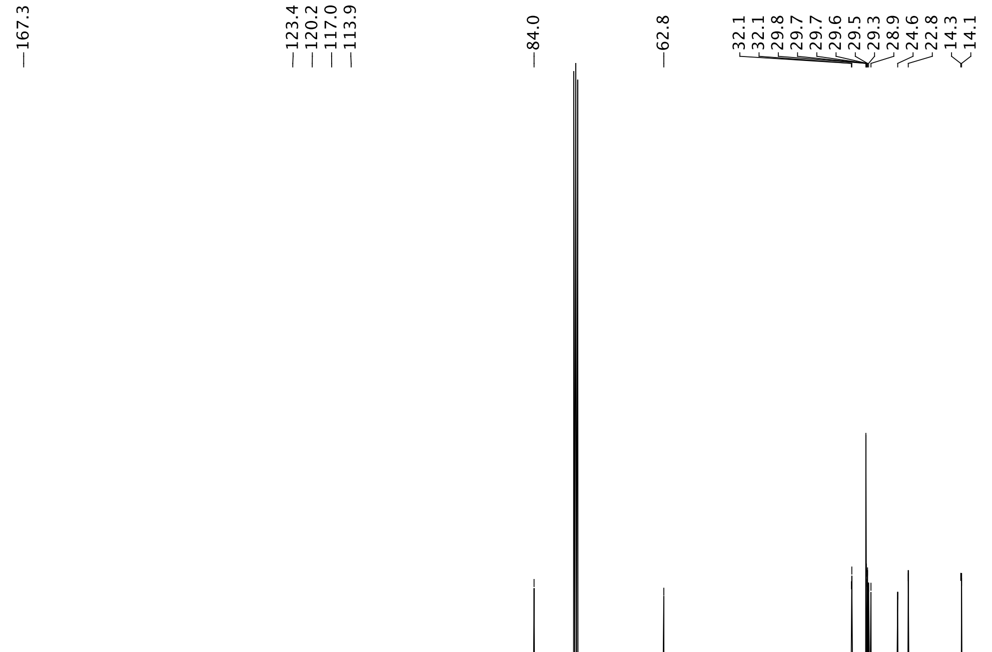
0.0
f1 (ppm)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29.7
29.6
29.5
29.3
28.9
24.6
22.8
14.3
14.1
190
180
170
160
150
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120
110
100
90
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50
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10
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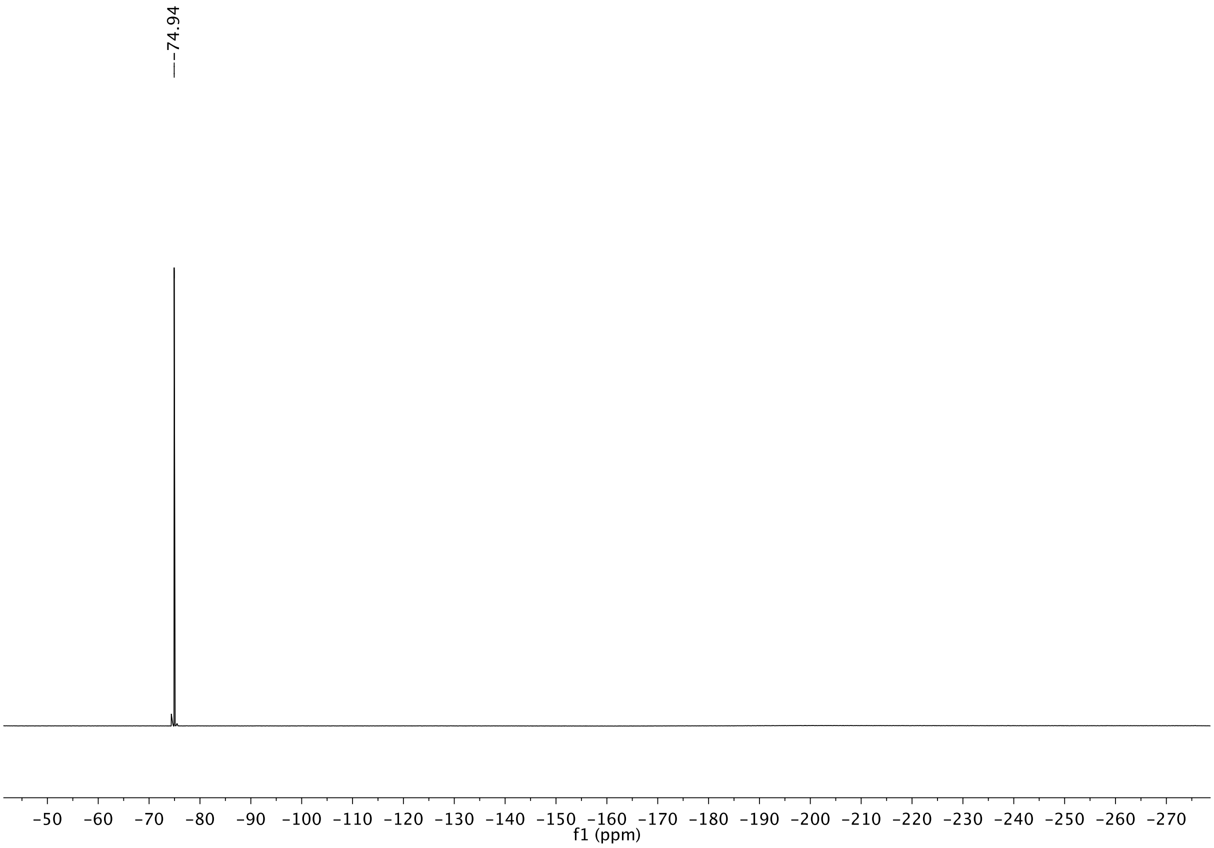
f1 (ppm)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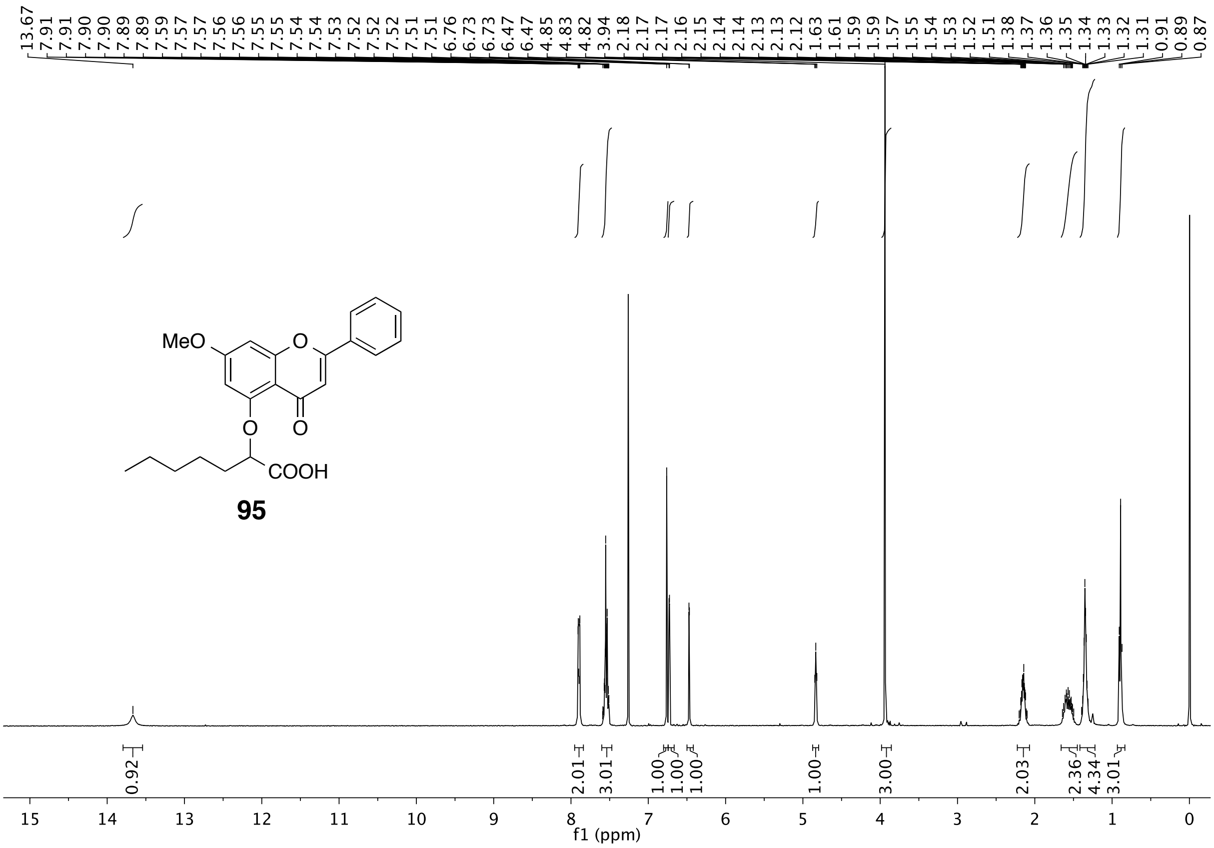
-190
-200
-210
-220
-230
-240
-250
-260
-270
f1 (ppm)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7.55
7.55
7.54
7.54
7.53
7.52
7.52
7.52
7.51
7.51
6.76
6.73
6.73
6.47
6.47
4.85
4.83
4.82
3.94
2.18
2.17
2.17
2.16
2.15
2.14
2.14
2.13
2.13
2.12
1.63
1.61
1.59
1.59
1.57
1.55
1.54
1.53
1.52
1.51
1.38
1.37
1.36
1.35
1.34
1.33
1.32
1.31
0.91
0.89
0.87
MeO
O
O
O
COOH
95
0.92
2.01
3.01
1.00
1.00
1.00
1.00
3.00
2.03
2.36
4.34
3.01
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13
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11
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9
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7
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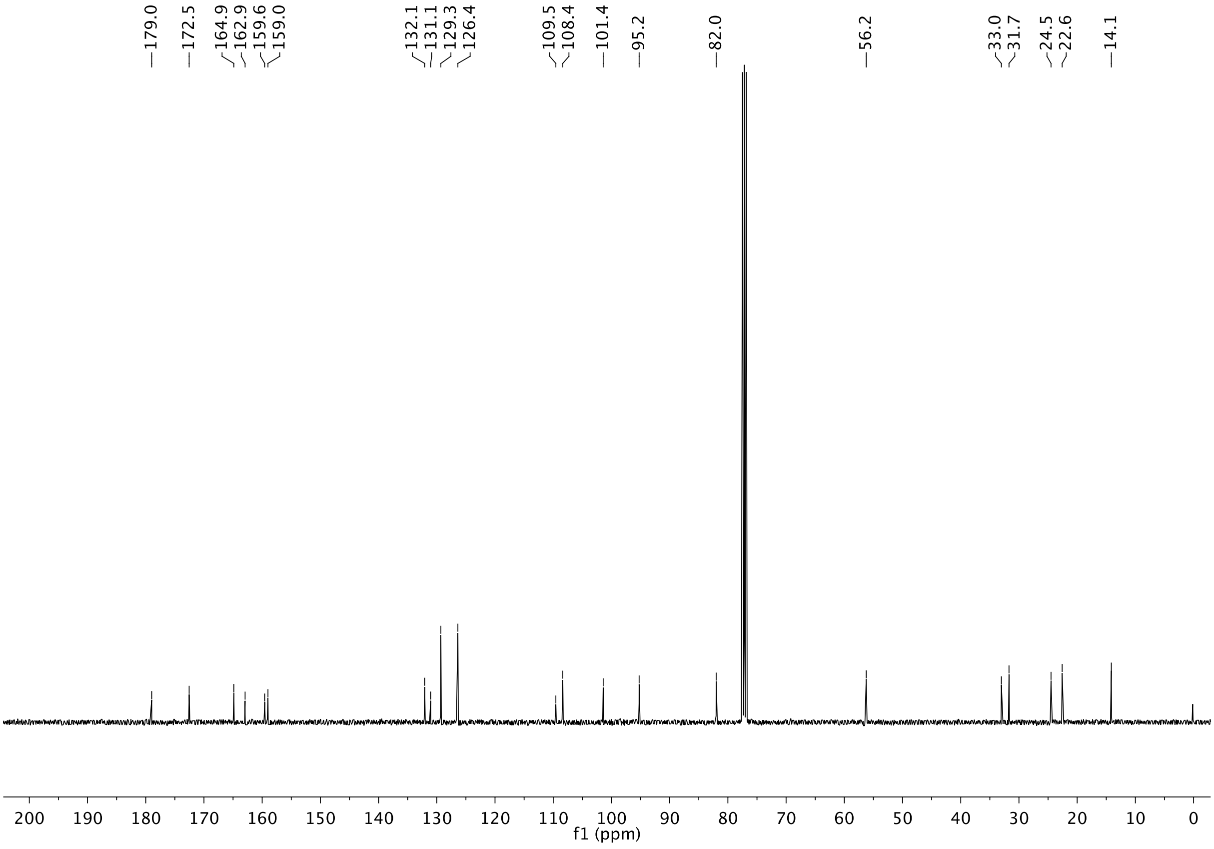
5
4
3
2
1
0
f1 (ppm)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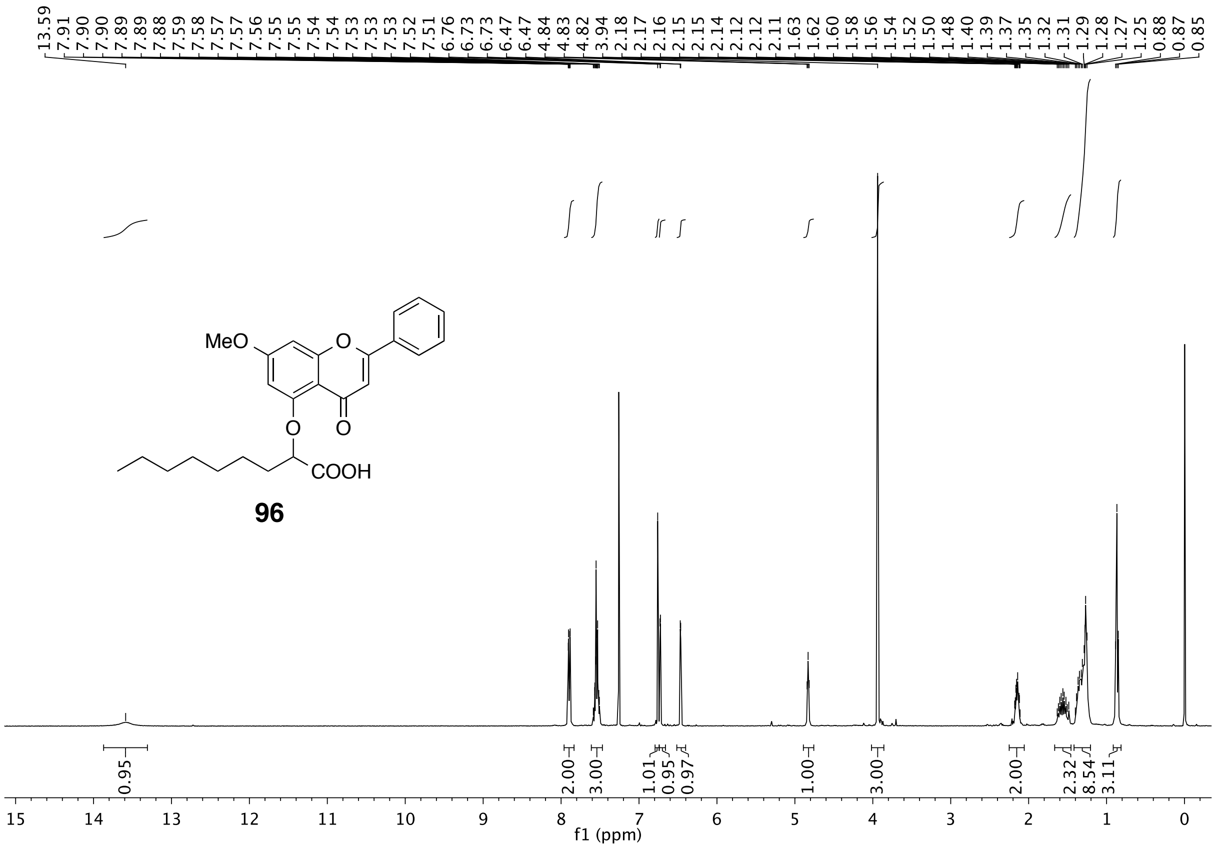
162.9
159.6
159.0
132.1
131.1
129.3
126.4
109.5
108.4
101.4
95.2
82.0
56.2
33.0
31.7
24.5
22.6
14.1
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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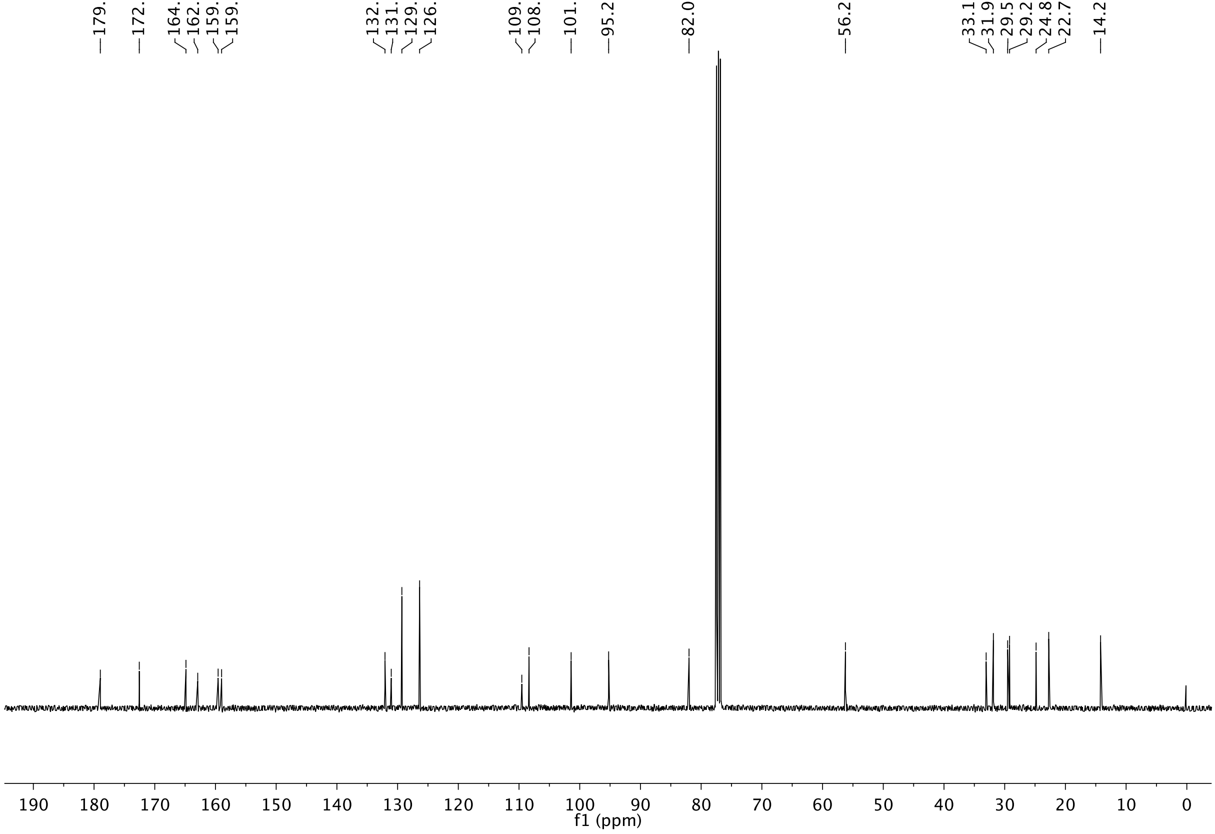
7.91 7.90 7.89 7.89 7.88 7.59 7.58 7.57 7.57 7.56 7.55 7.55 7.54 7.54 7.53 7.53 7.53 7.52 7.51 6.76 6.73 6.73 6.47 4.84 4.83 4.82 3.94 2.18 2.17 2.16 2.15 2.15 2.14 2.12 2.12 2.11 1.63 1.62 1.60 1.58 1.56 1.54 1.52 1.50 1.48 1.40 1.39 1.37 1.35 1.32 1.31 1.29 1.28 1.27 1.25 0.88 0.87 0.85
MeO
O
O
O
COOH
96
0.95
2.00
3.00
1.01
0.95
0.97
1.00
3.00
2.00
2.32
8.54
3.11
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
f1 (ppm)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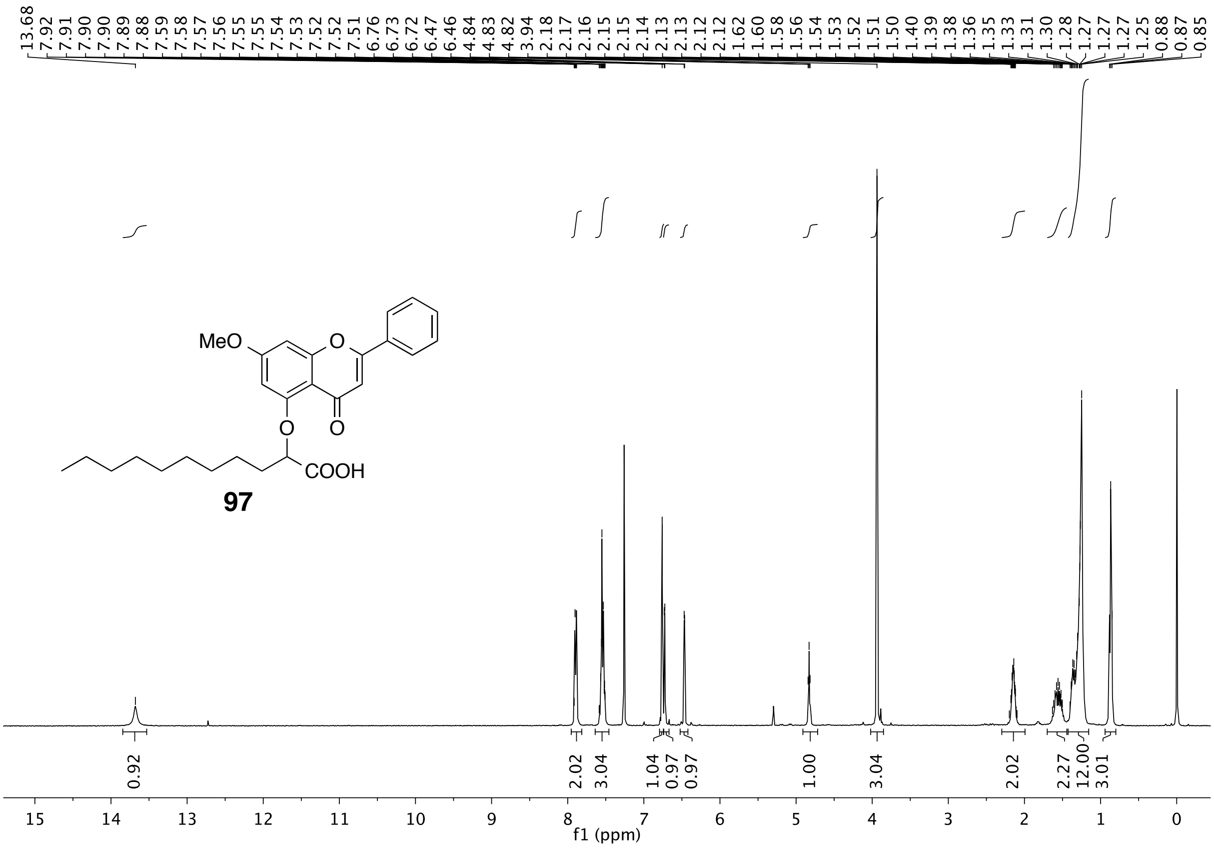
164.9 162.9 159.6 159.0
132.1 131.0 129.3 126.4
109.5 108.3
101.4
95.2
82.0
56.2
33.1 31.9 29.5 29.2 24.8 22.7
14.2
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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7.55
7.54
7.53
7.52
7.52
7.51
6.76
6.73
6.72
6.47
6.46
4.84
4.83
4.82
3.94
2.18
2.17
2.16
2.15
2.15
2.14
2.13
2.13
2.12
2.12
1.62
1.60
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1.56
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1.53
1.52
1.51
1.50
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1.39
1.38
1.36
1.35
1.33
1.31
1.30
1.28
1.27
1.27
1.27
1.25
0.88
0.87
0.85
MeO
O
O
O
COOH
97
0.92
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3.04
1.04
0.97
0.97
1.00
3.04
2.02
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12.00
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5
4
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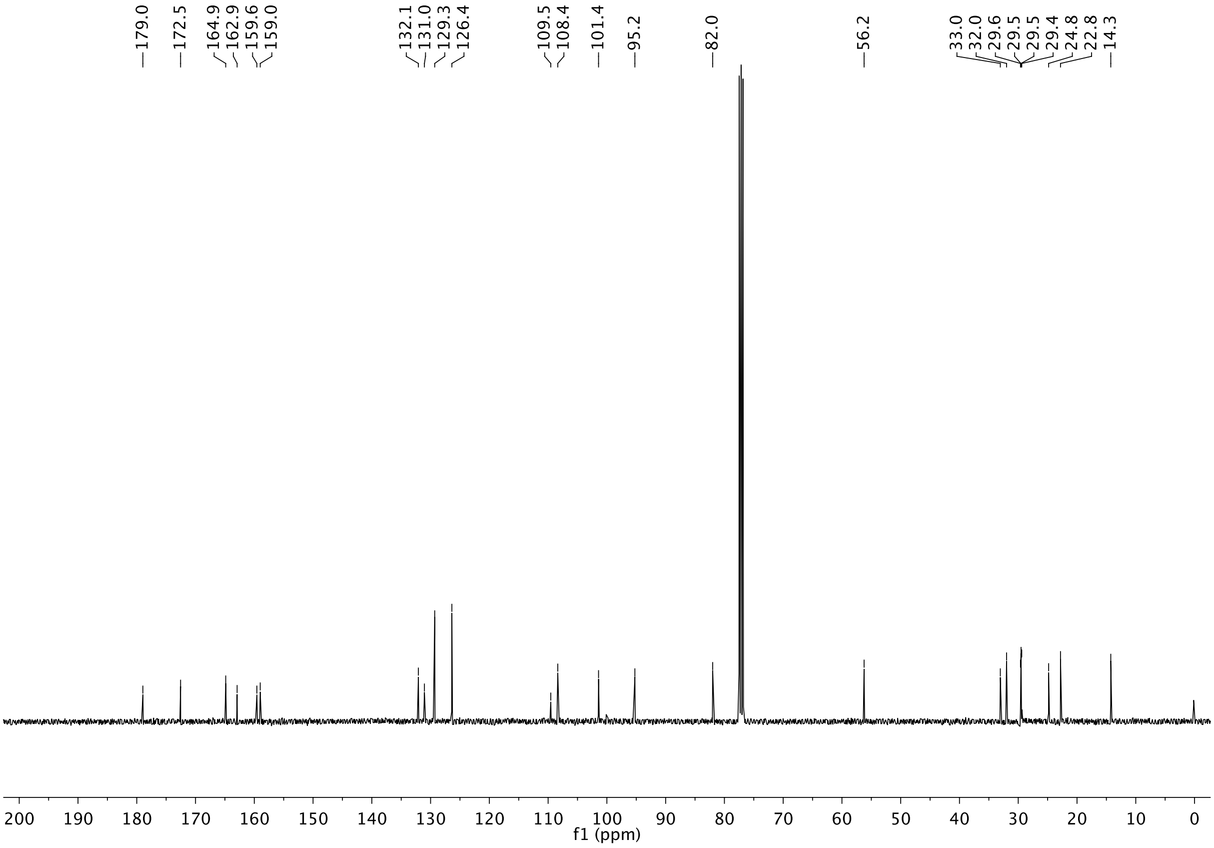
f1 (ppm)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129.3
126.4
109.5
108.4
101.4
95.2
82.0
56.2
33.0
32.0
29.6
29.5
29.5
29.4
24.8
22.8
14.3
200
190
180
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160
150
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130
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110
100
90
80
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50
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30
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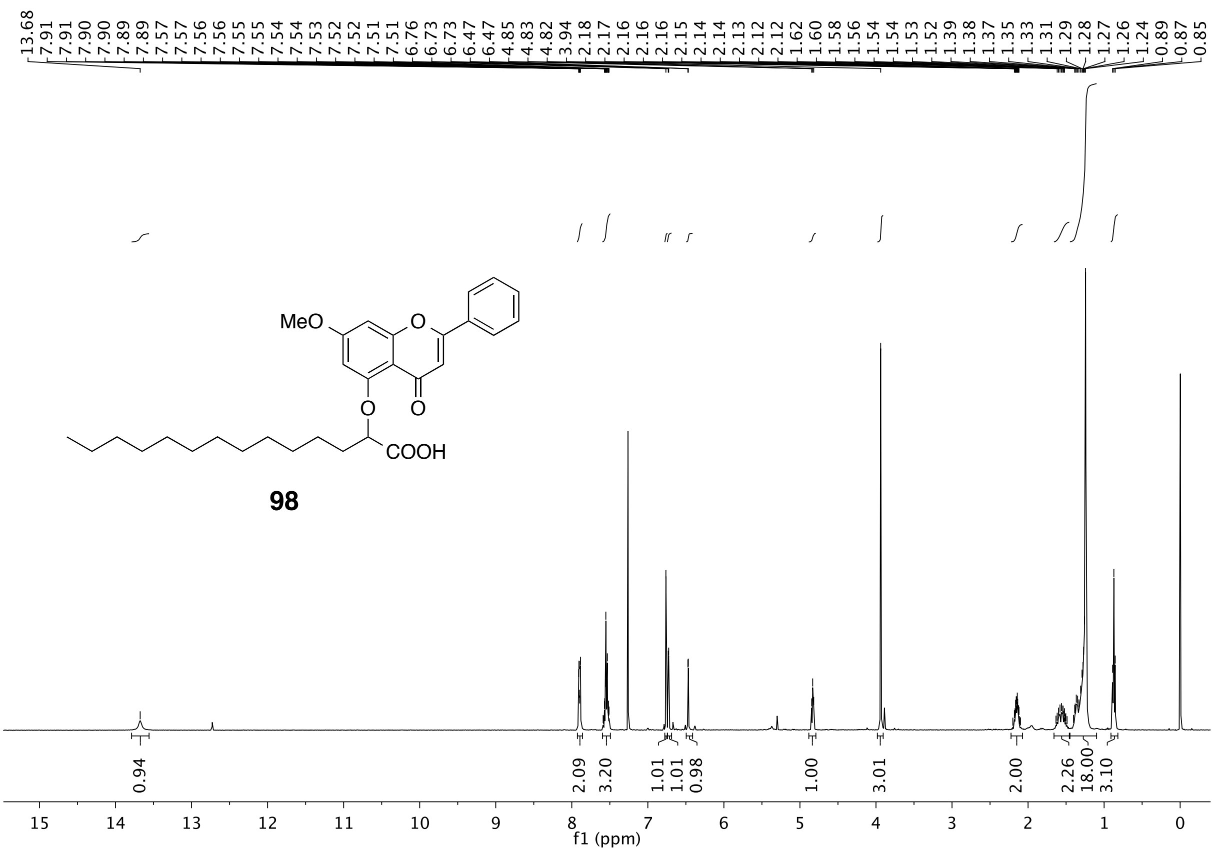
10
0
f1 (ppm)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7.55
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7.54
7.53
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7.51
6.76
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6.73
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4.83
4.82
3.94
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1.54
1.54
1.53
1.52
1.39
1.38
1.37
1.35
1.33
1.31
1.29
1.28
1.27
1.26
1.24
0.89
0.87
0.85
MeO
O
O
O
COOH
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1.01
0.98
1.00
3.01
2.00
2.26
18.00
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10
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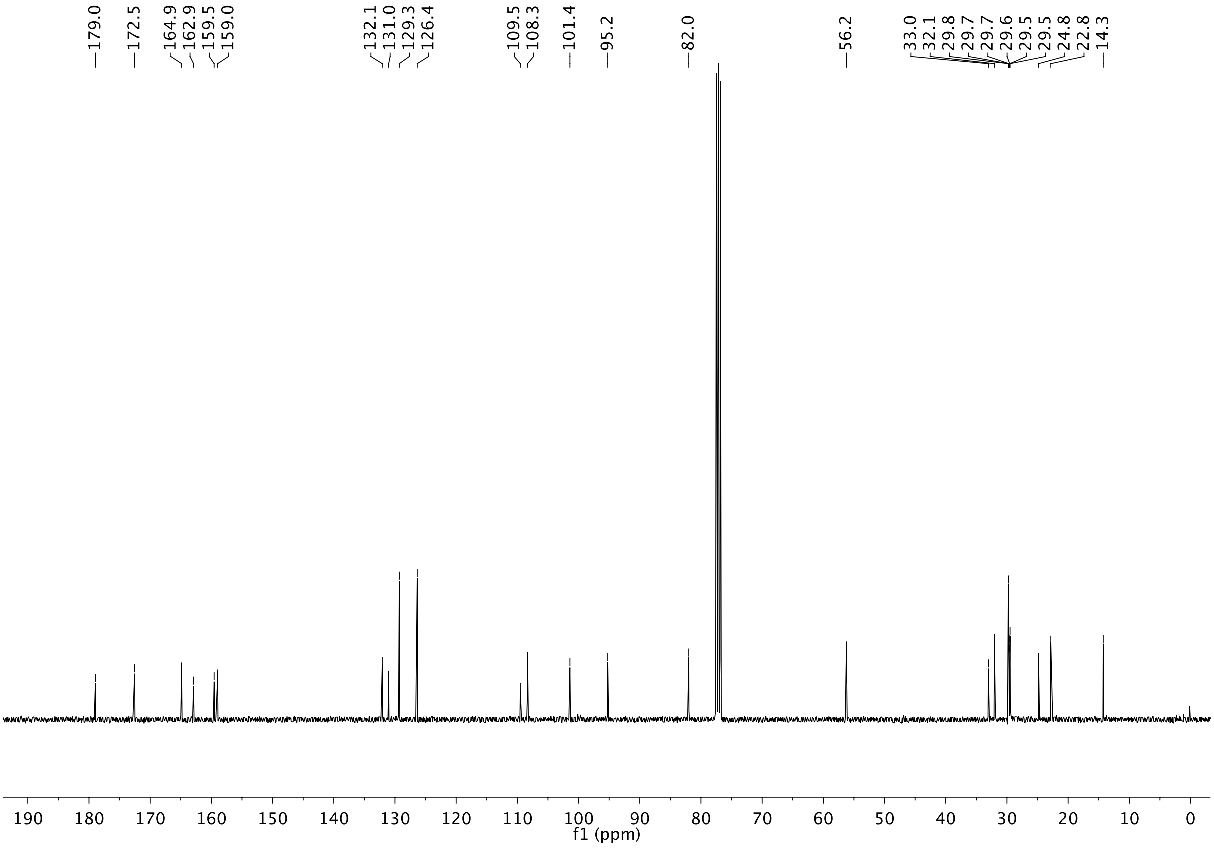
8
7
6
5
4
3
2
1
0
f1 (ppm)

98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131.0
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126.4
109.5
108.3
101.4
95.2
82.0
56.2
33.0
32.1
29.8
29.7
29.7
29.6
29.5
29.5
24.8
22.8
14.3
190
180
170
160
150
140
130
120
110
100
90
80
70
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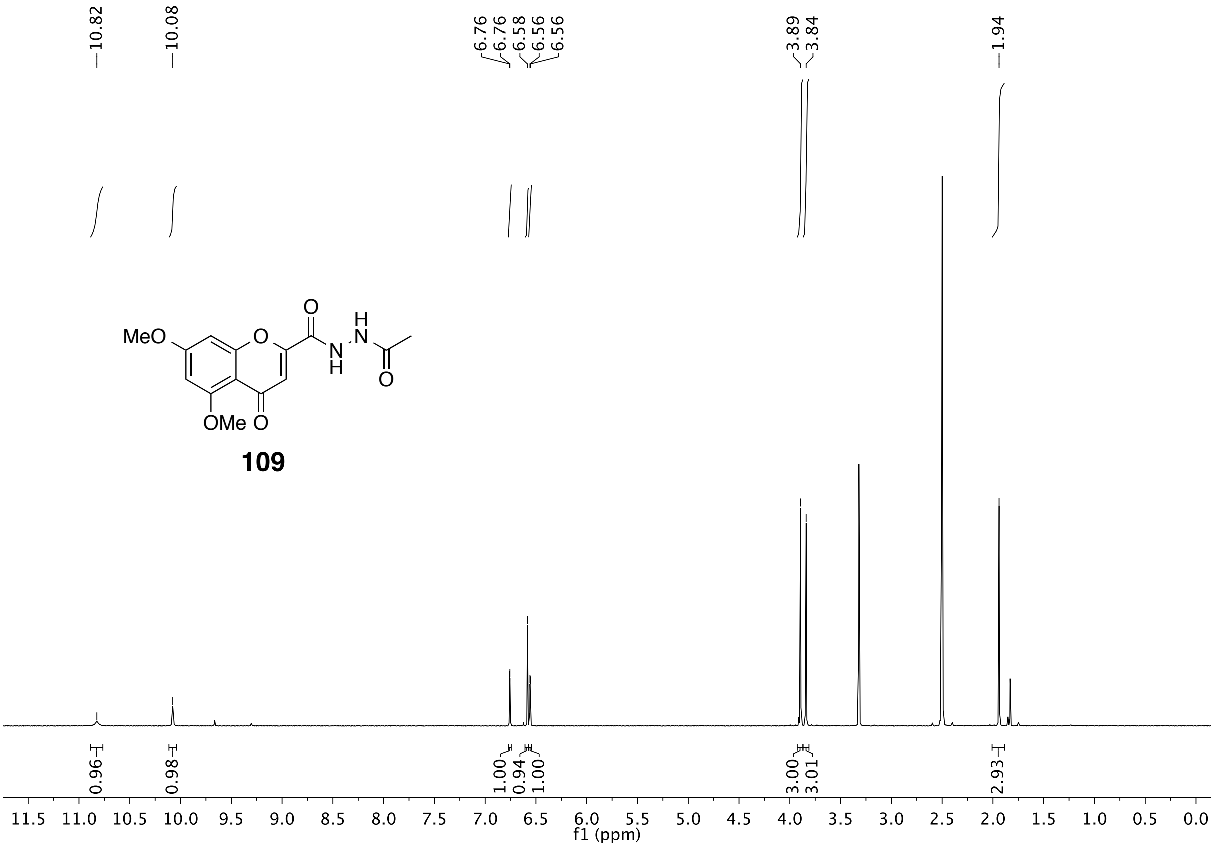
50
40
30
20
10
0
f1 (ppm)



109

10.82
10.08
6.76
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6.56
6.56
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3.84
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O
H
N
N
H
O
OMe
O
0.96
0.98
1.00
0.94
1.00
3.00
3.01
2.93
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
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6.5
6.0
5.5
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0.5
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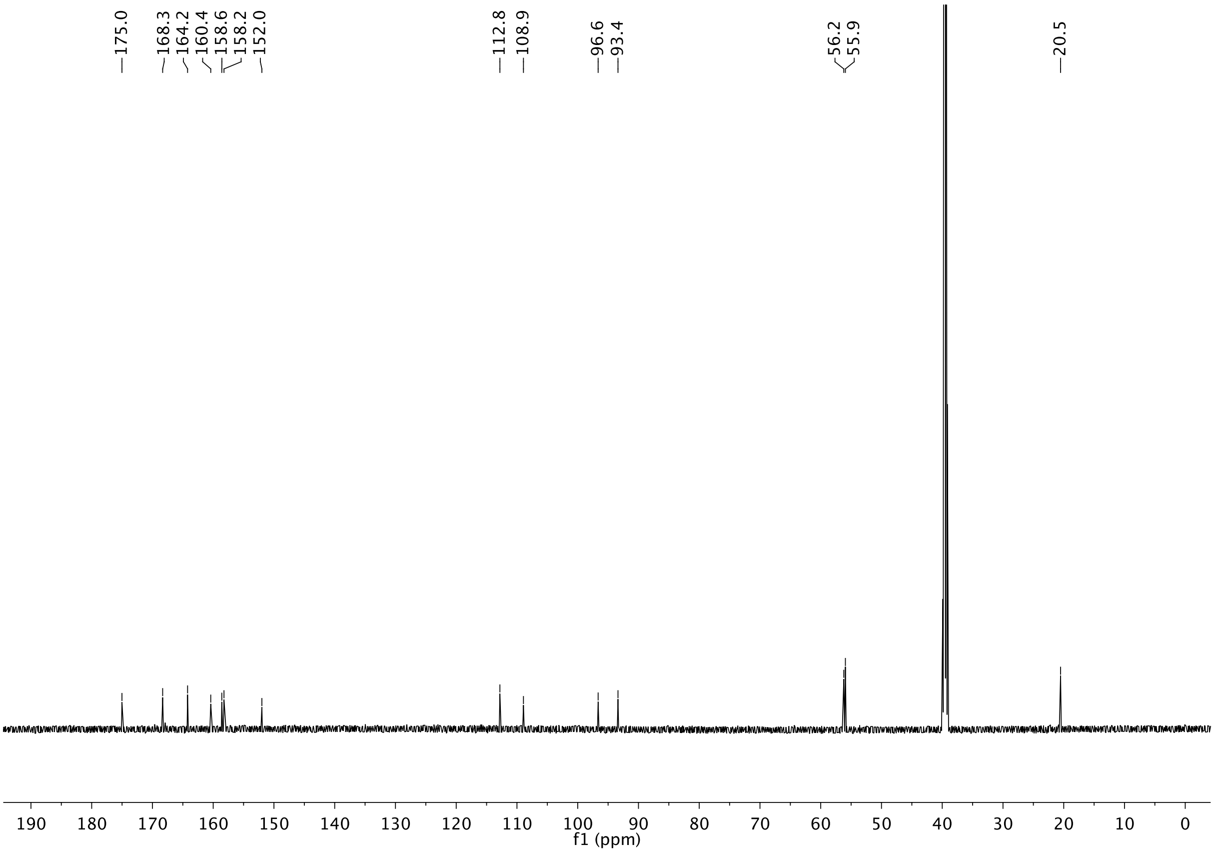
f1 (ppm)



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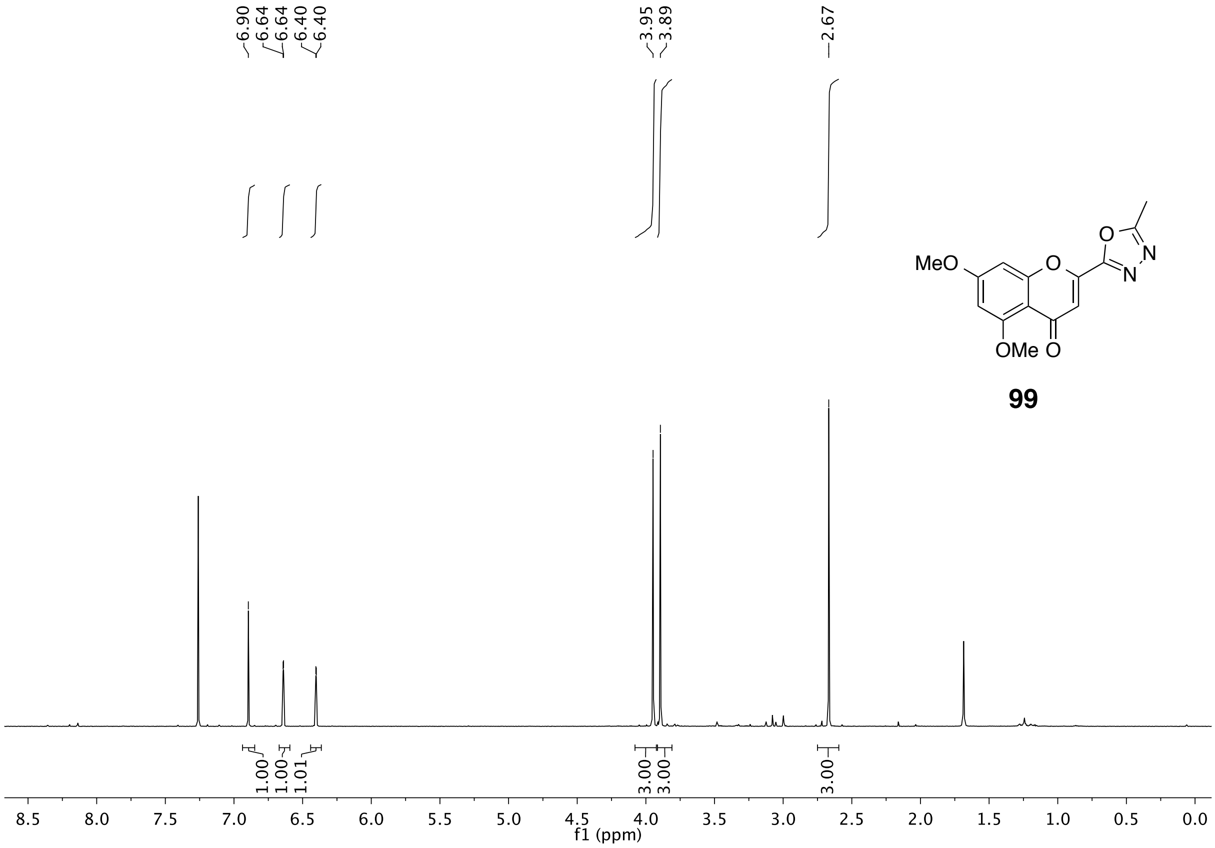
152.0
112.8
108.9
96.6
93.4
56.2
55.9
20.5
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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2.67
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O
N
N
OMe
O
1.00
1.00
1.01
3.00
3.00
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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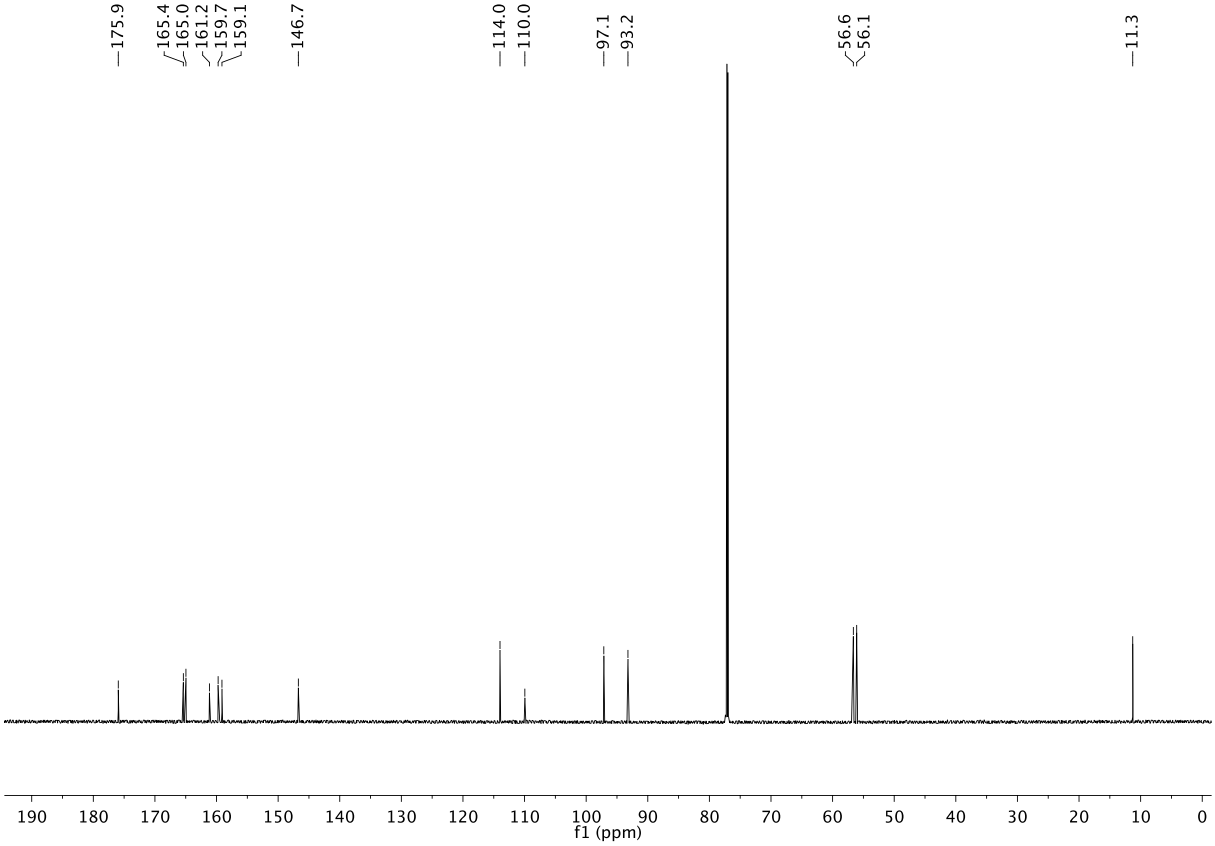
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

99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159.7
159.1
146.7
114.0
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93.2
56.6
56.1
11.3
190
180
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160
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100
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70
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50
40
30
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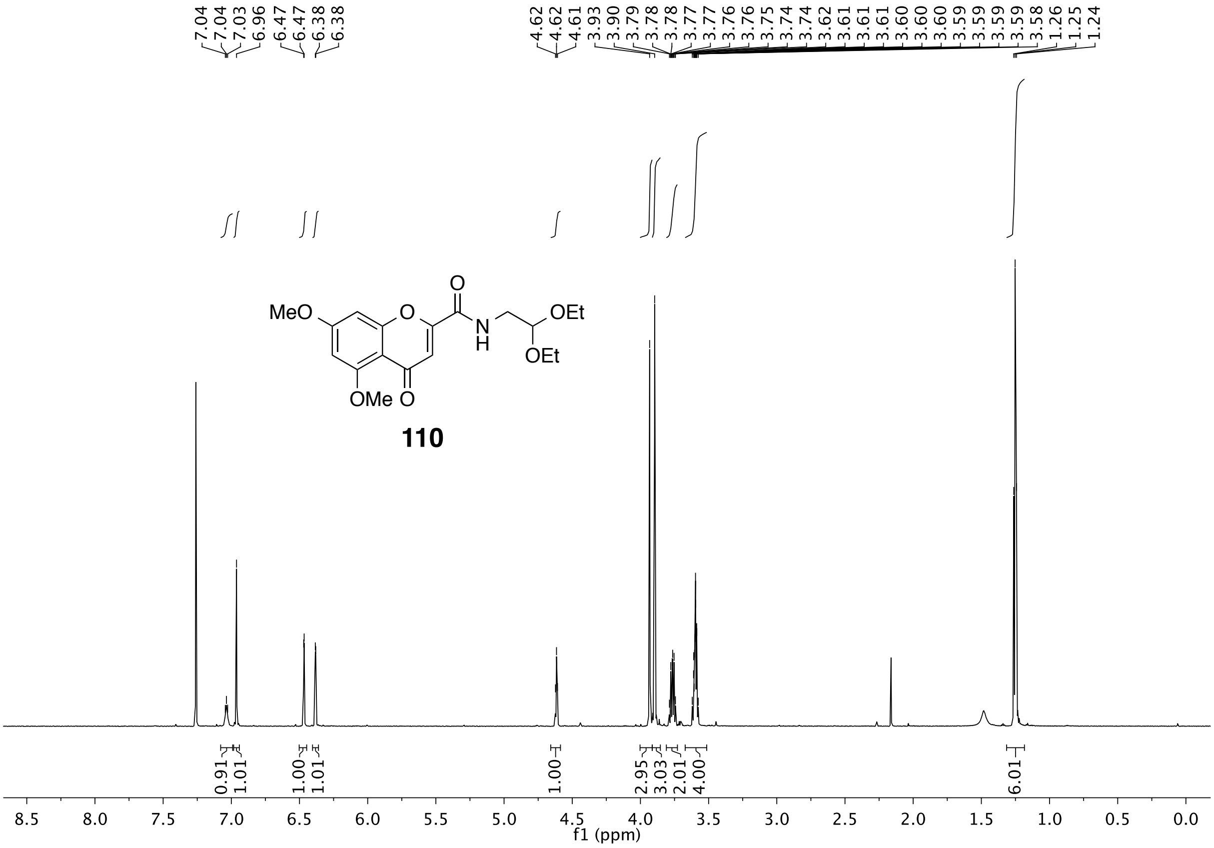
10
0
f1 (ppm)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3.78
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3.77
3.76
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3.75
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3.74
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3.59
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3.58
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1.25
1.24
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O
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H
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OEt
OMe
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1.00
1.01
1.00
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2.01
4.00
6.01
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1.5
1.0
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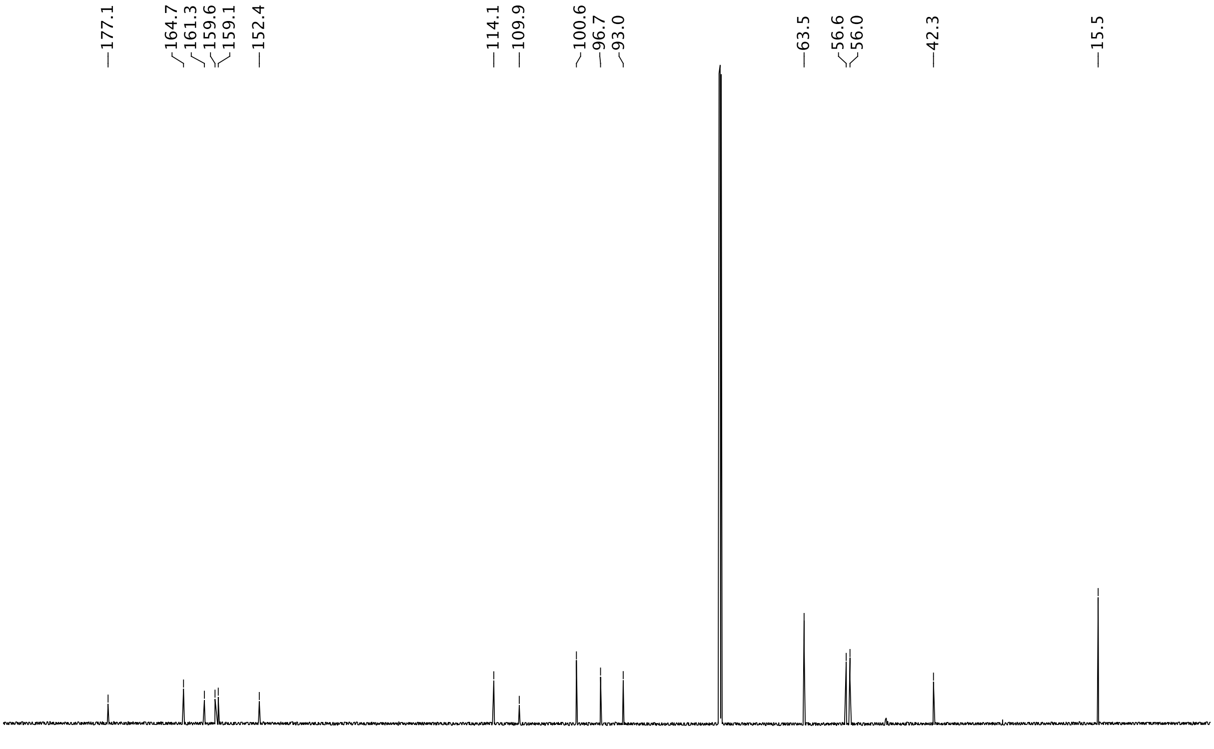
0.0
f1 (ppm)

110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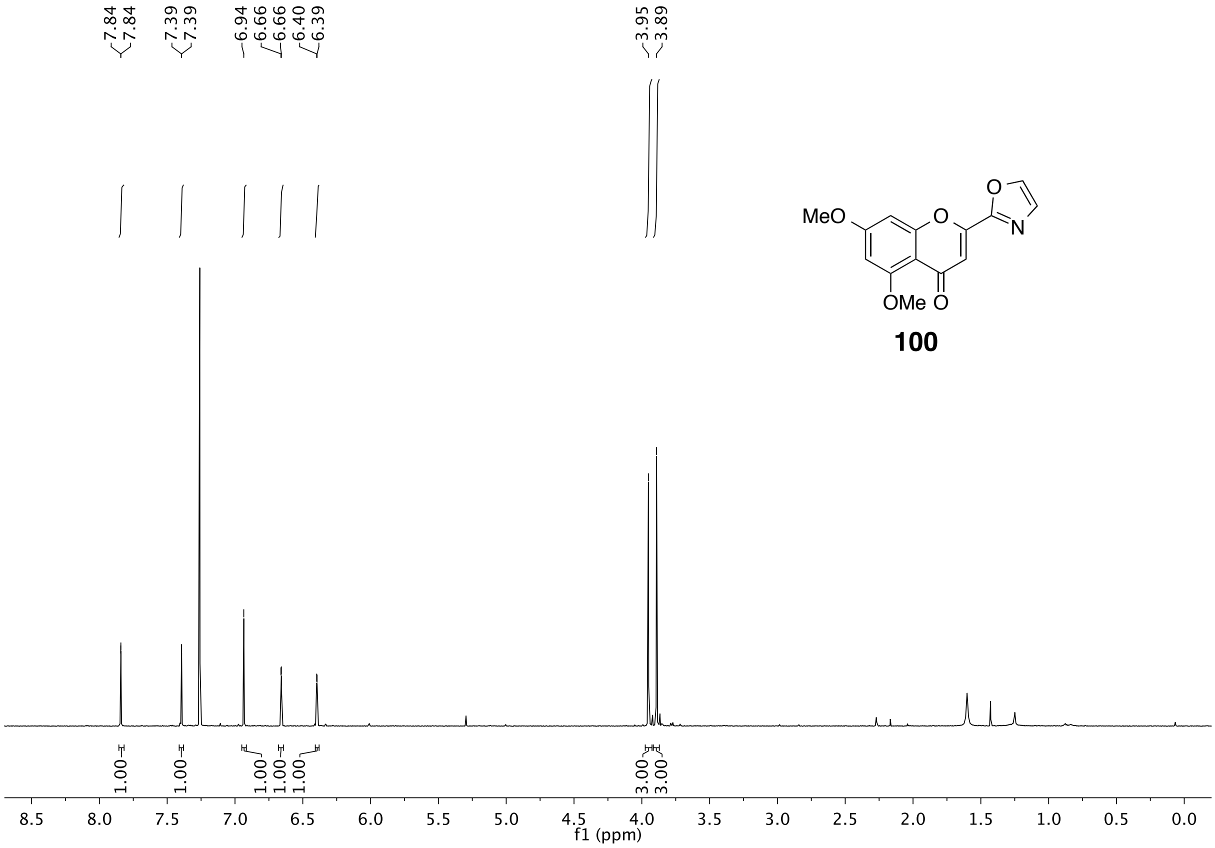
159.1
152.4
114.1
109.9
100.6
96.7
93.0
63.5
56.6
56.0
42.3
15.5
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.84
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7.39
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6.66
6.66
6.40
6.39
3.95
3.89
1.00
1.00
1.00
1.00
1.00
3.00
3.00
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

MeO
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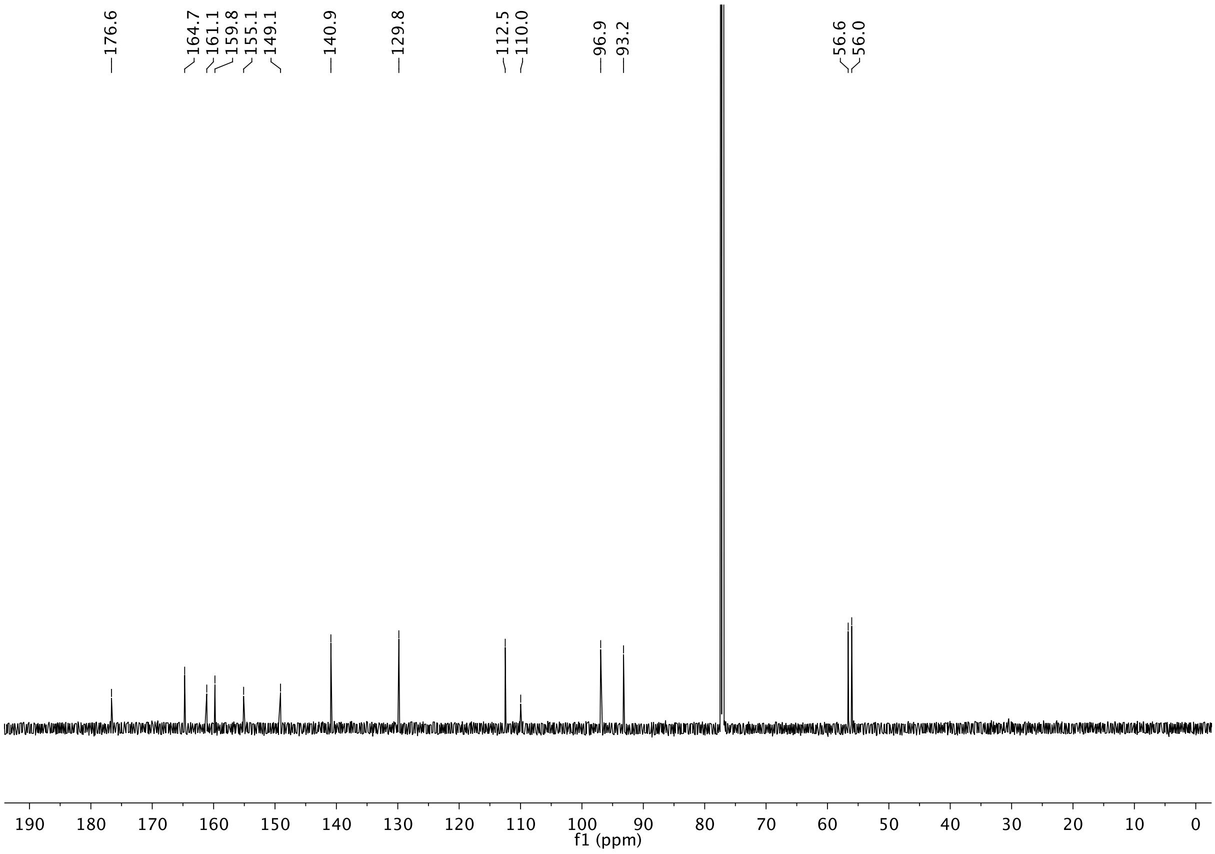
O
N
OMe
O

100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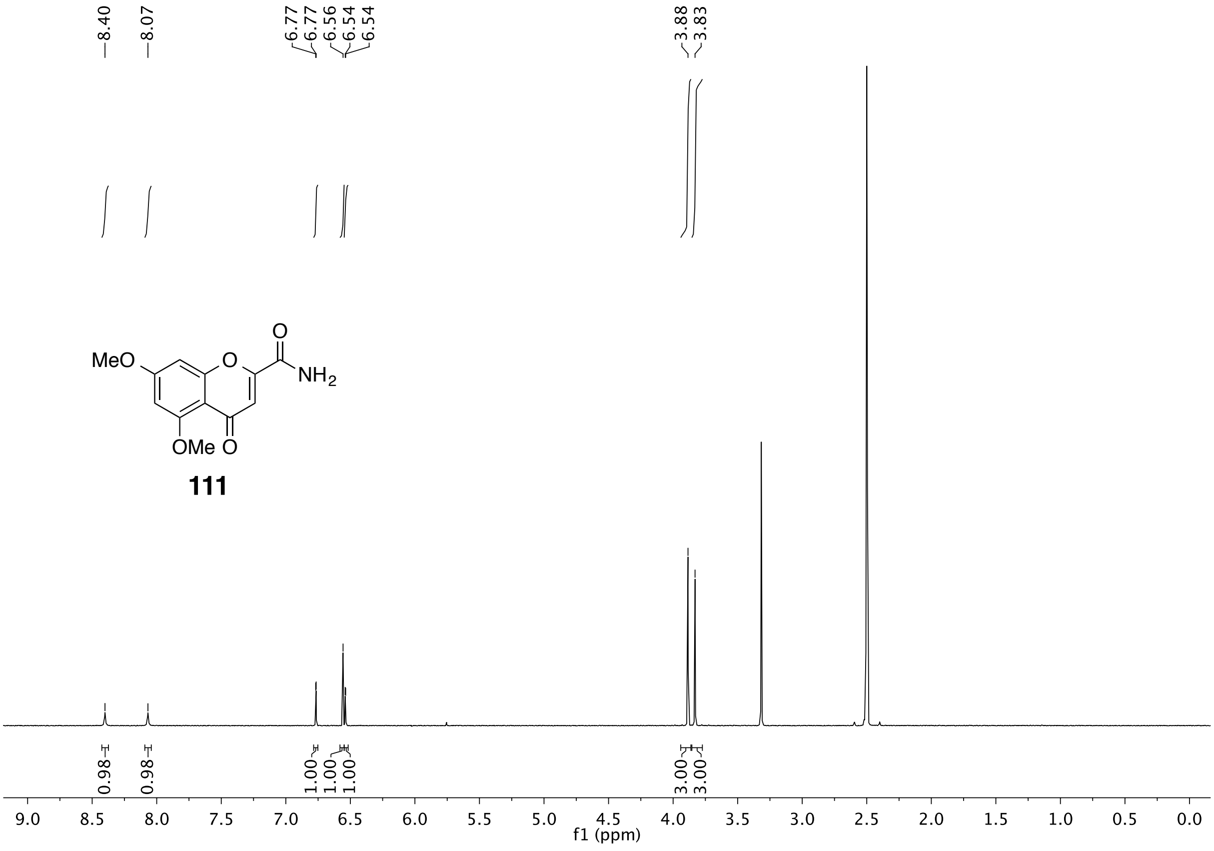
159.8
155.1
149.1
140.9
129.8
112.5
110.0
96.9
93.2
56.6
56.0
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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8.07
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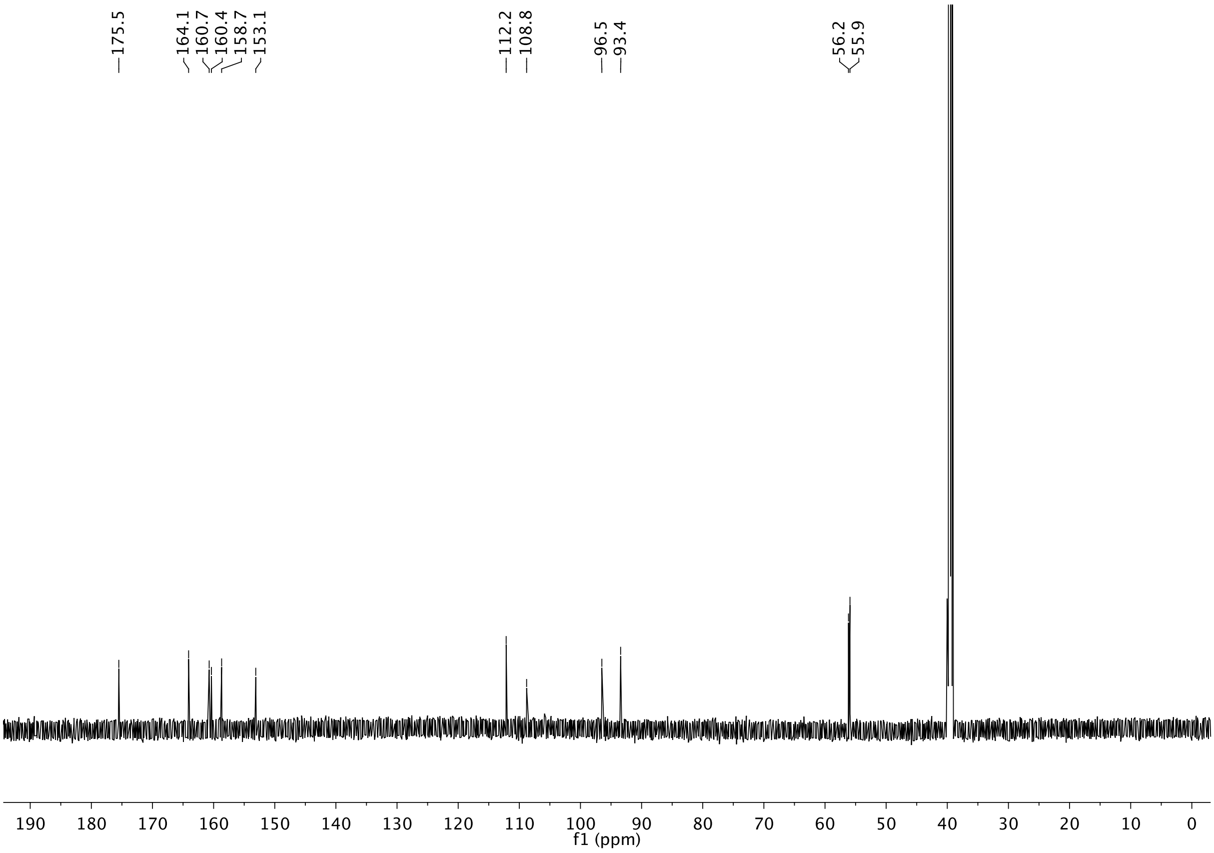
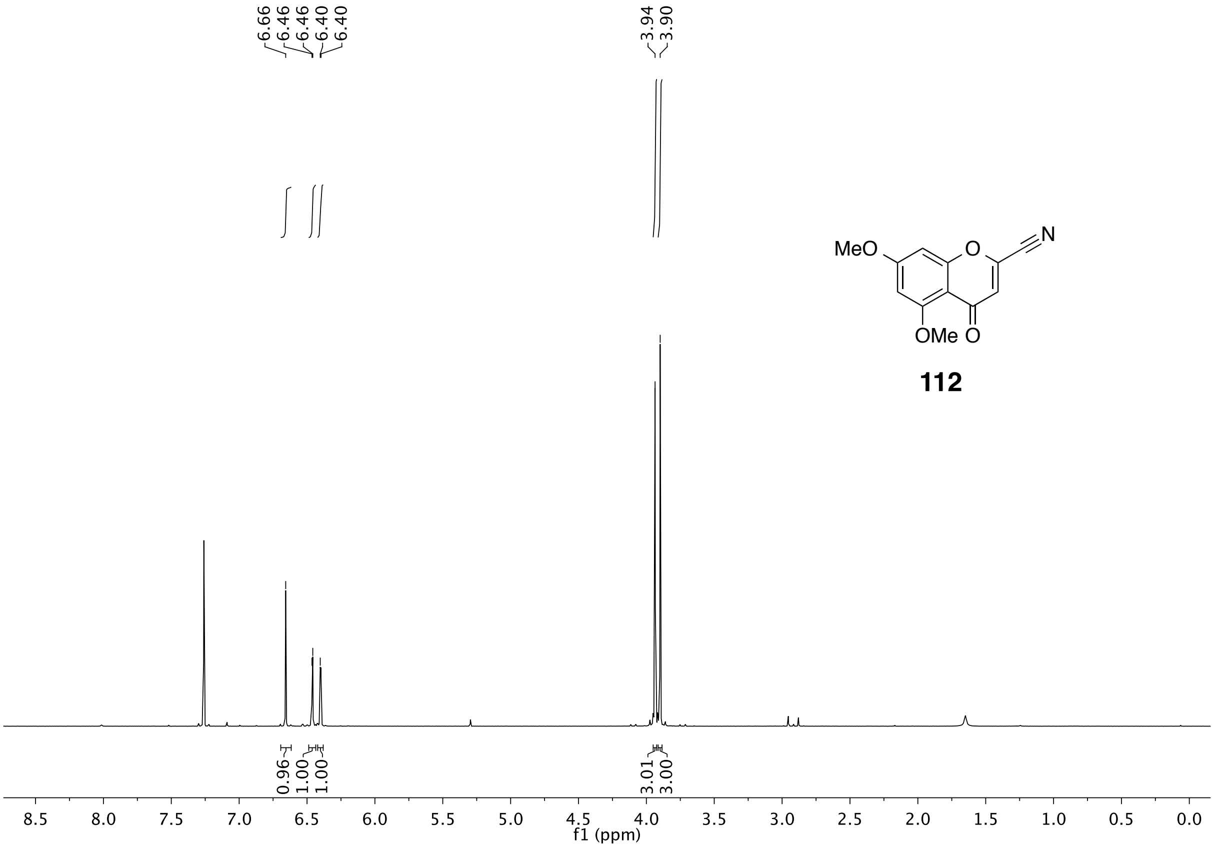
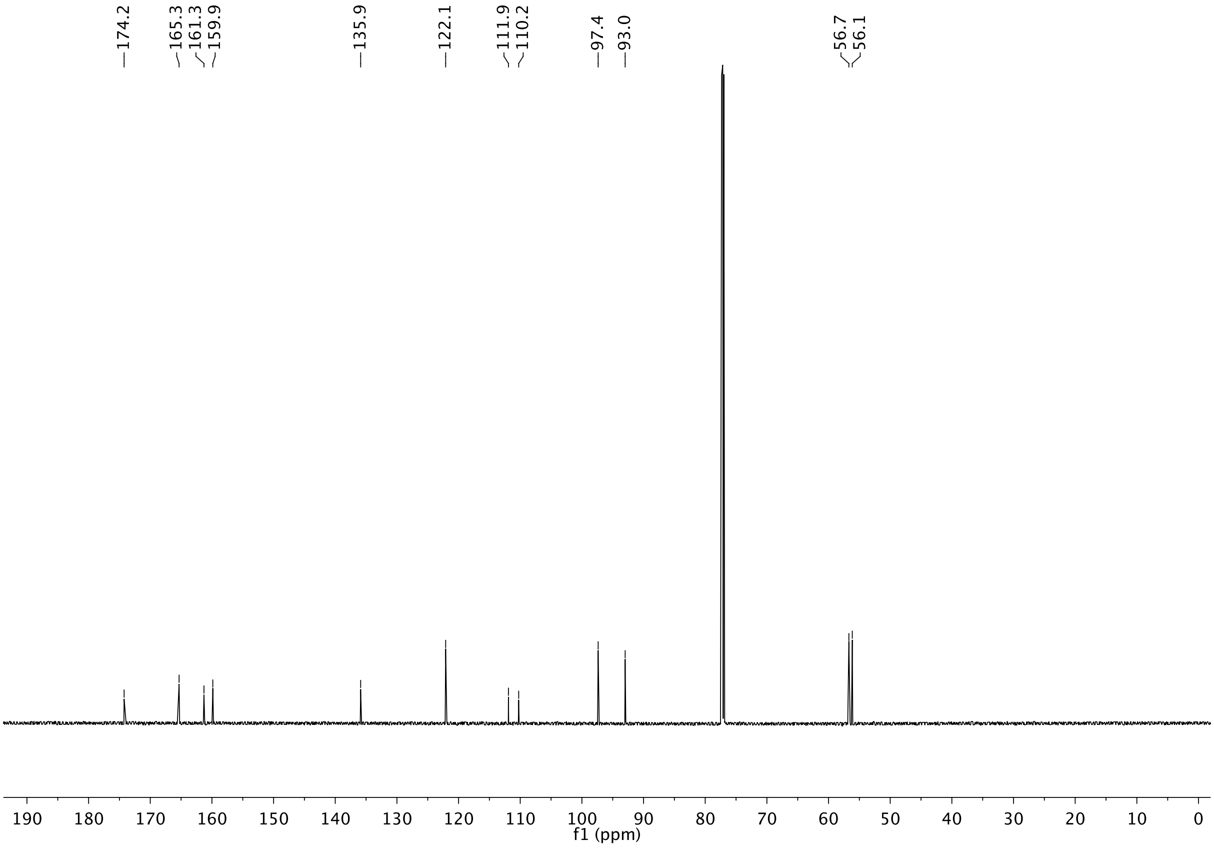
6.54
6.54
3.88
3.83
MeO
O
O
NH2
OMe
O
0.98
0.98
1.00
1.00
1.00
3.00
3.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

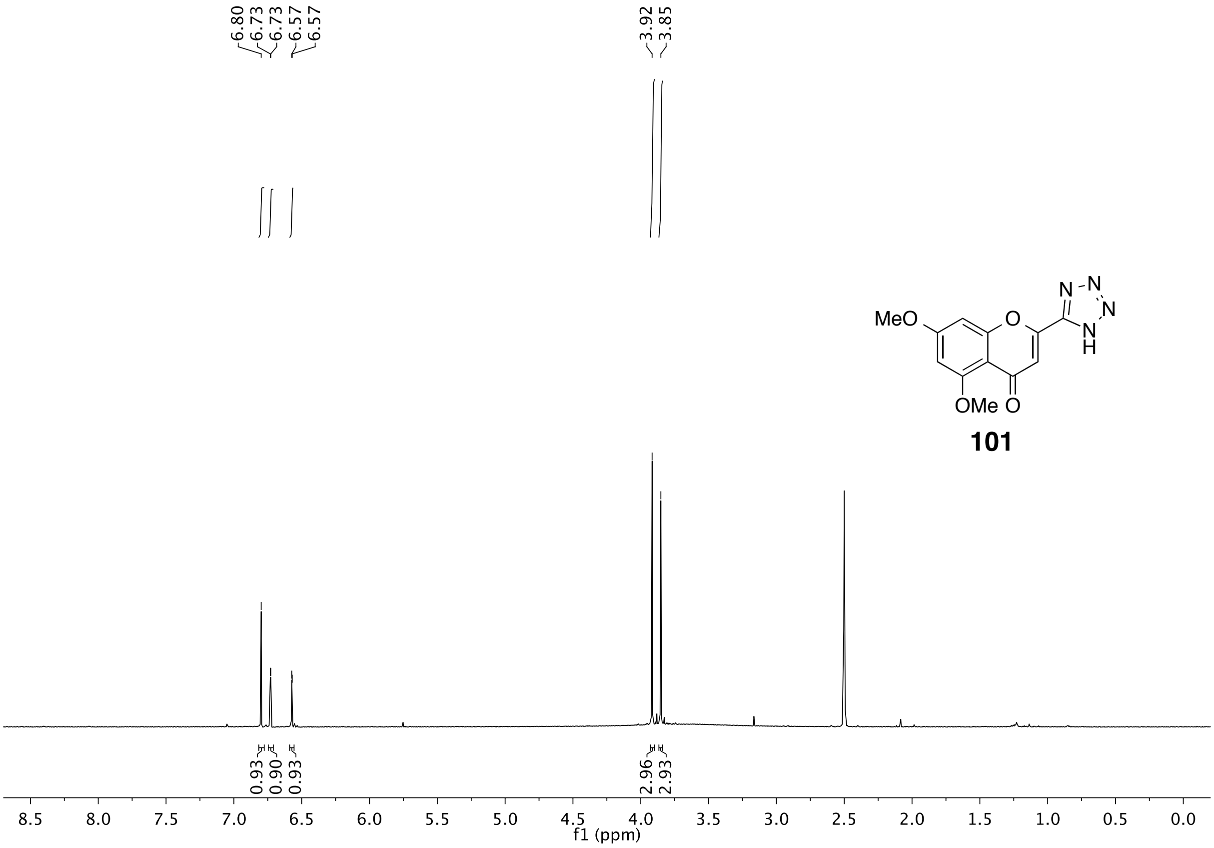
111

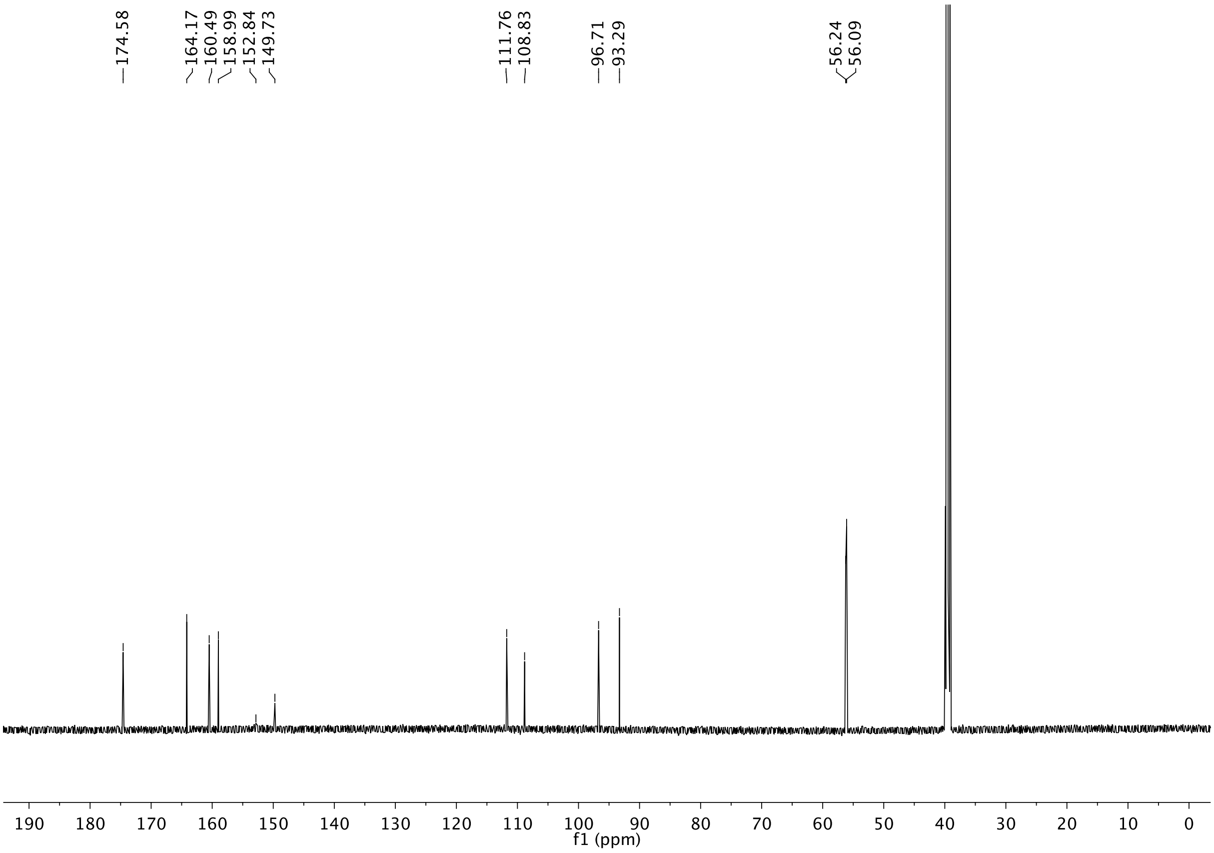

175.5
164.1
160.7
160.4
158.7
153.1
112.2
108.8
96.5
93.4
56.2
55.9
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

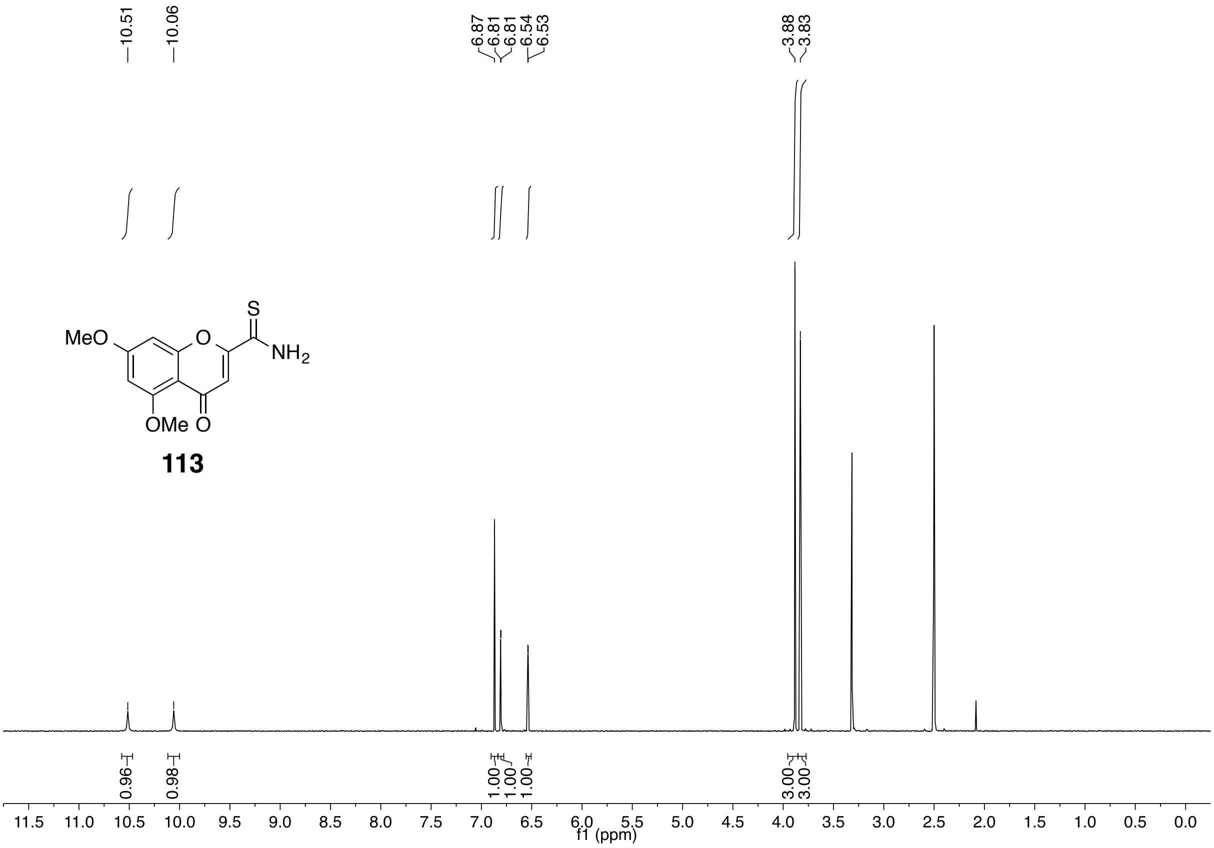

6.66
6.46
6.46
6.40
6.40
3.94
3.90
MeO
O
N
OMe O
0.96
1.00
1.00
3.01
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

112


174.2
165.3
161.3
159.9
135.9
122.1
111.9
110.2
97.4
93.0
56.7
56.1
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

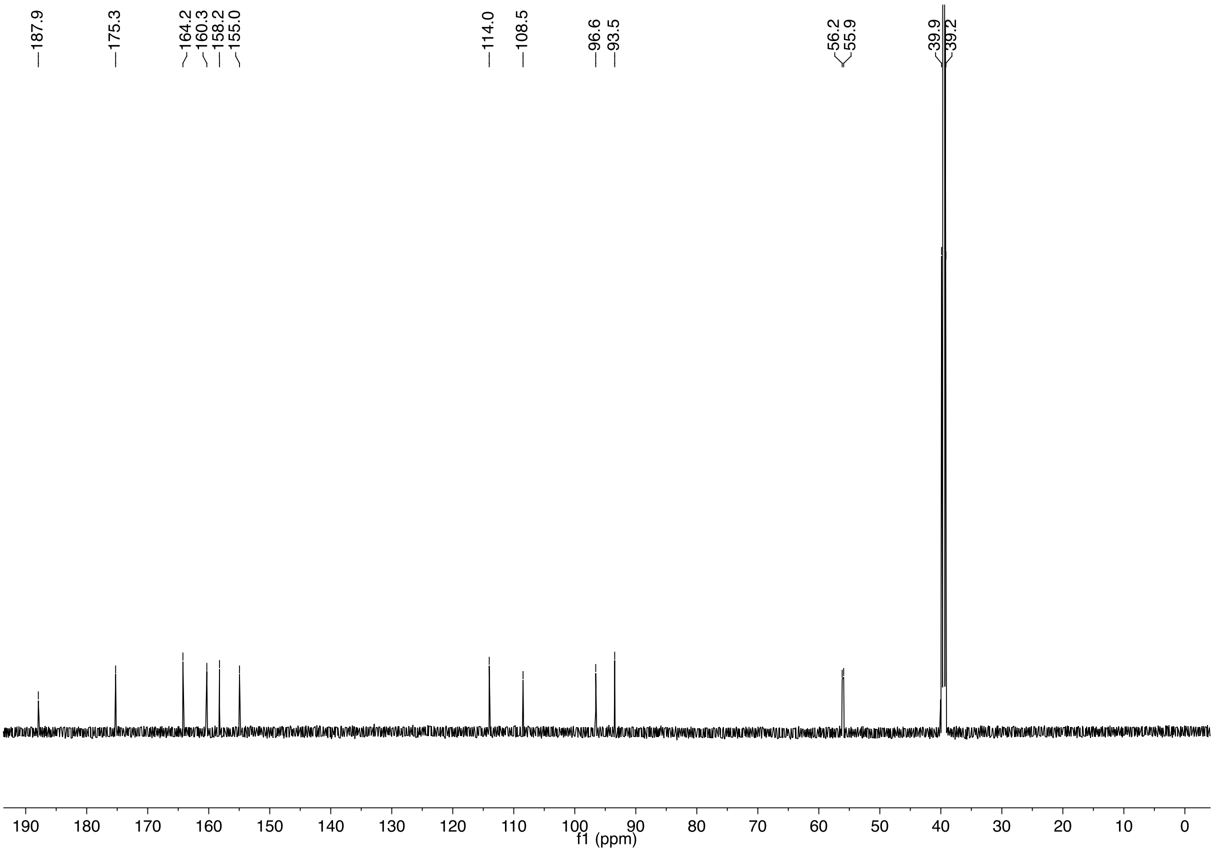

6.80
6.73
6.73
6.57
6.57
3.92
3.85
MeO
O
N
N
N
N
H
OMe
O
101
0.93
0.90
0.93
2.96
2.93
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


174.58
164.17
160.49
158.99
152.84
149.73
111.76
108.83
96.71
93.29
56.24
56.09
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

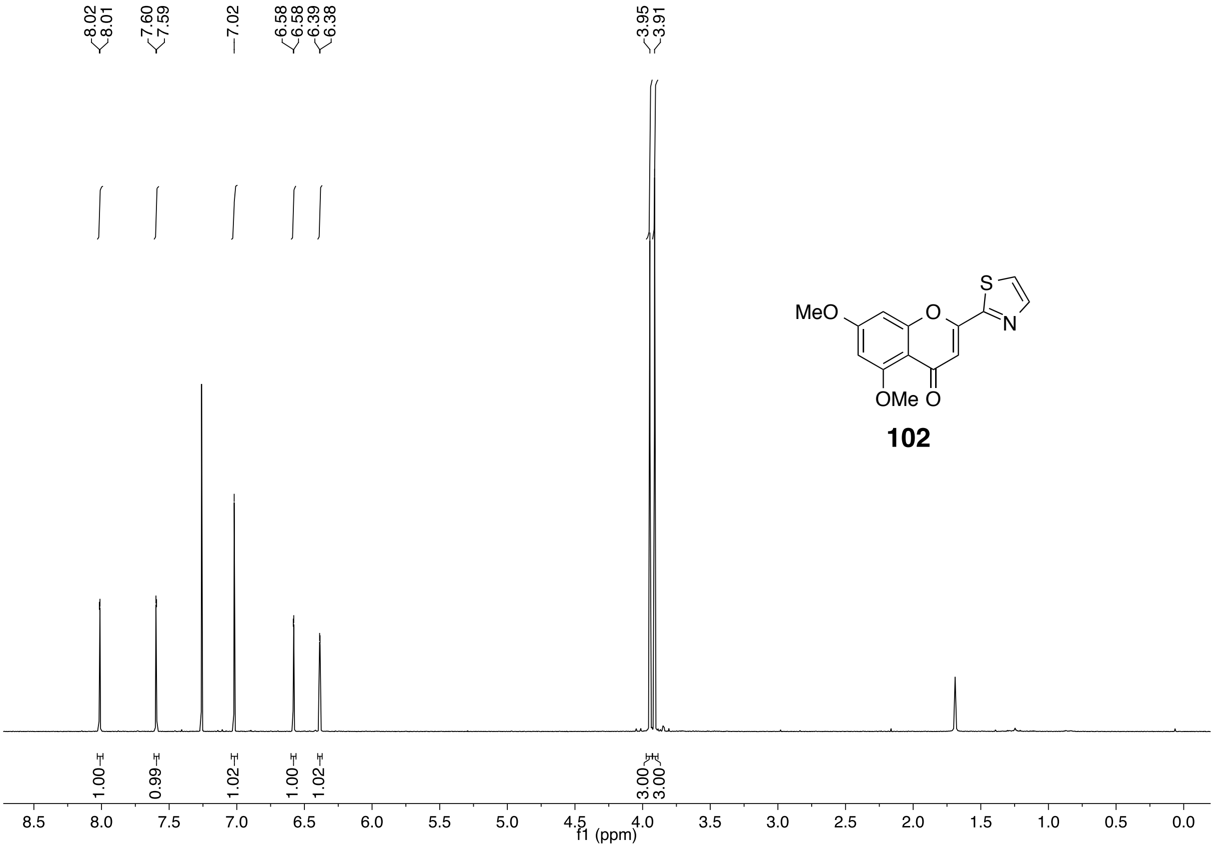


113

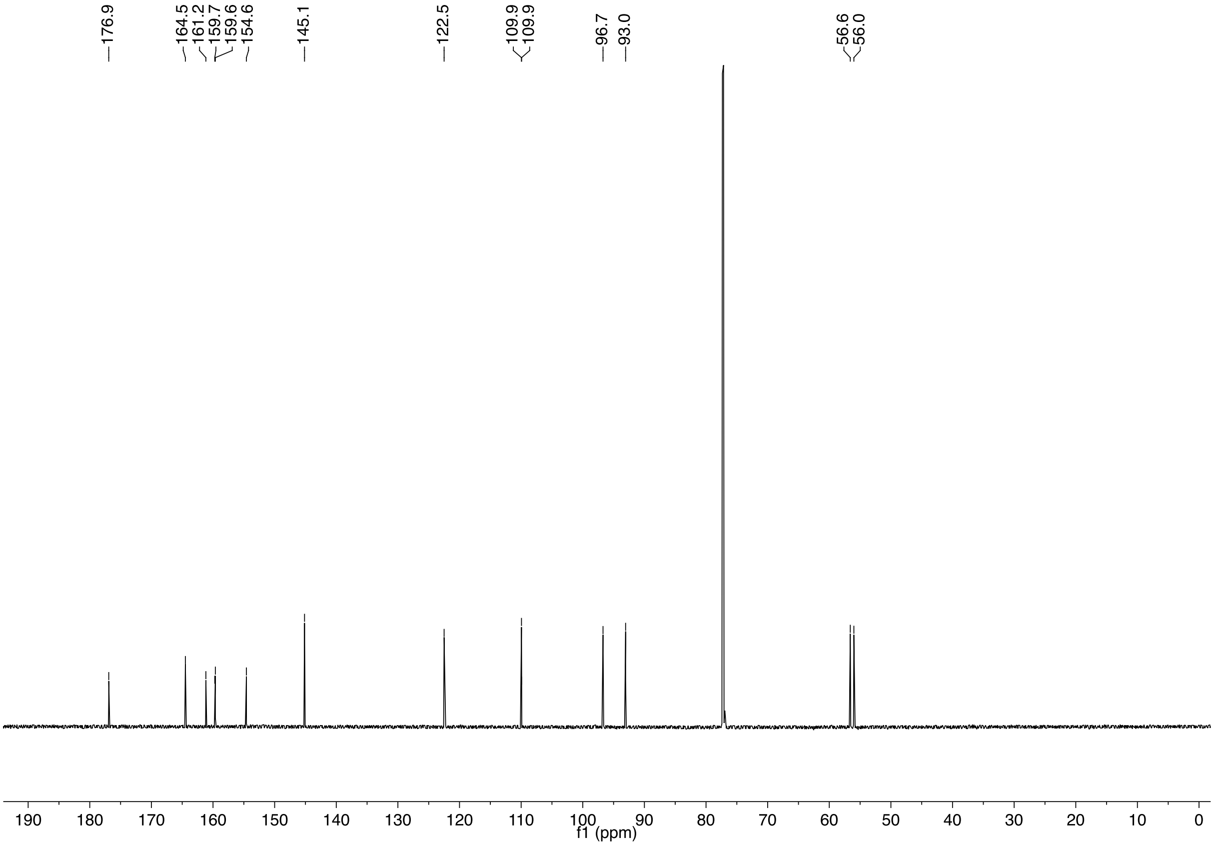
10.51
10.06
6.87
6.81
6.81
6.54
6.53
3.88
3.83
MeO
O
S
NH$_2$
OMe
O
0.96
0.98
1.00
1.00
1.00
3.00
3.00
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

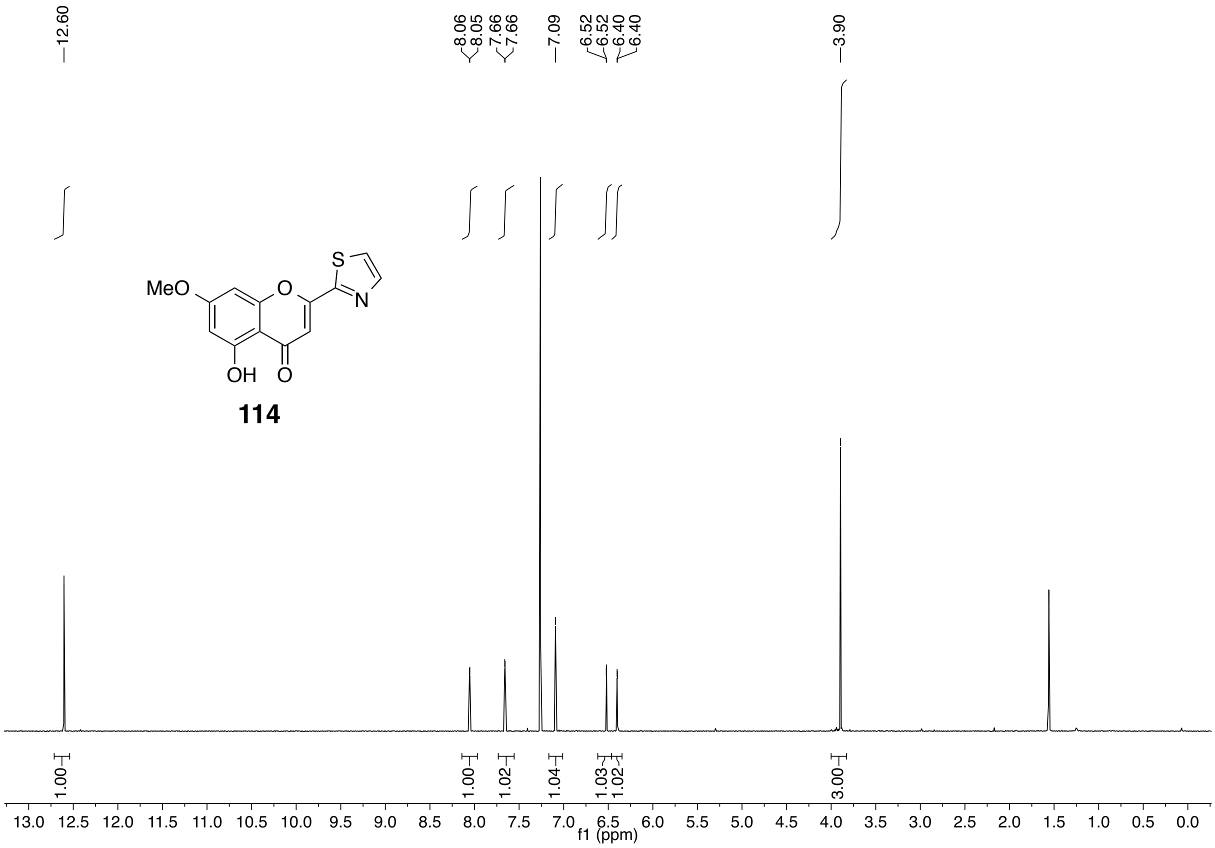


187.9
175.3
164.2
160.3
158.2
155.0
114.0
108.5
96.6
93.5
56.2
55.9
39.9
39.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

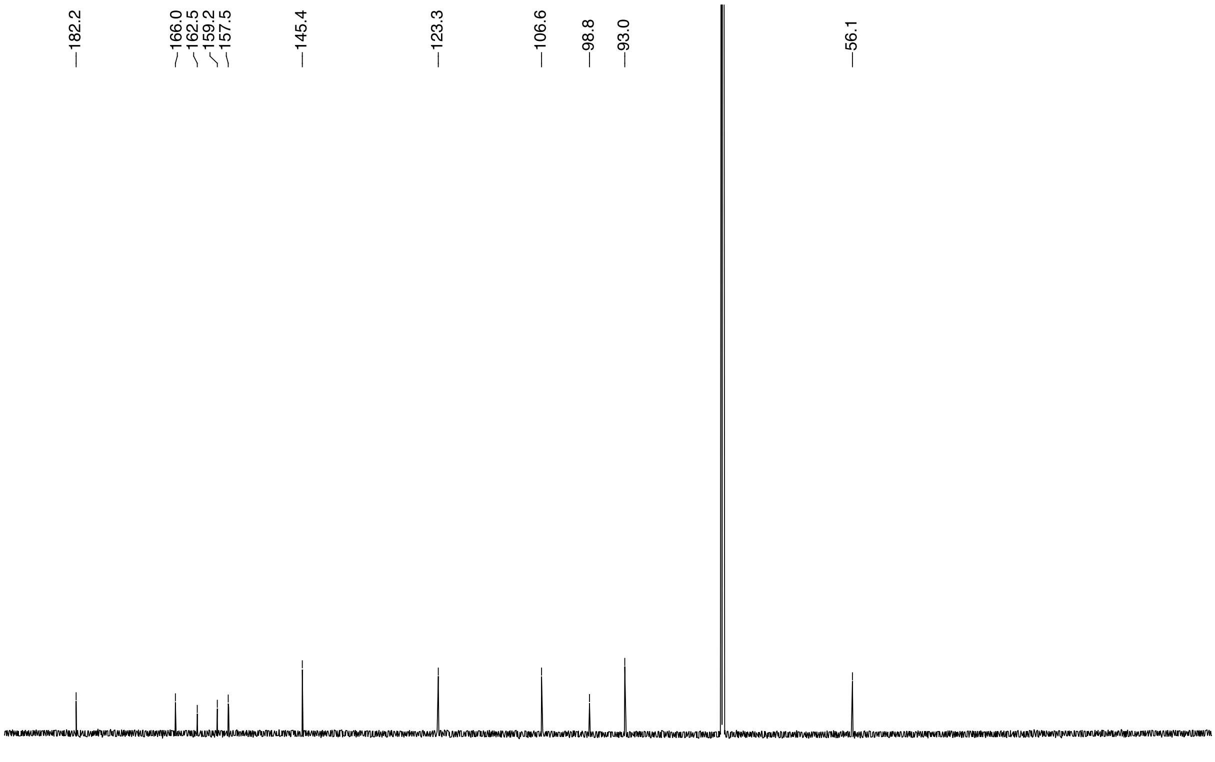

8.02
8.01
7.60
7.59
7.02
6.58
6.58
6.39
6.38
3.95
3.91
MeO
O
S
N
OMe
O
1.00
0.99
1.02
1.00
1.02
3.00
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

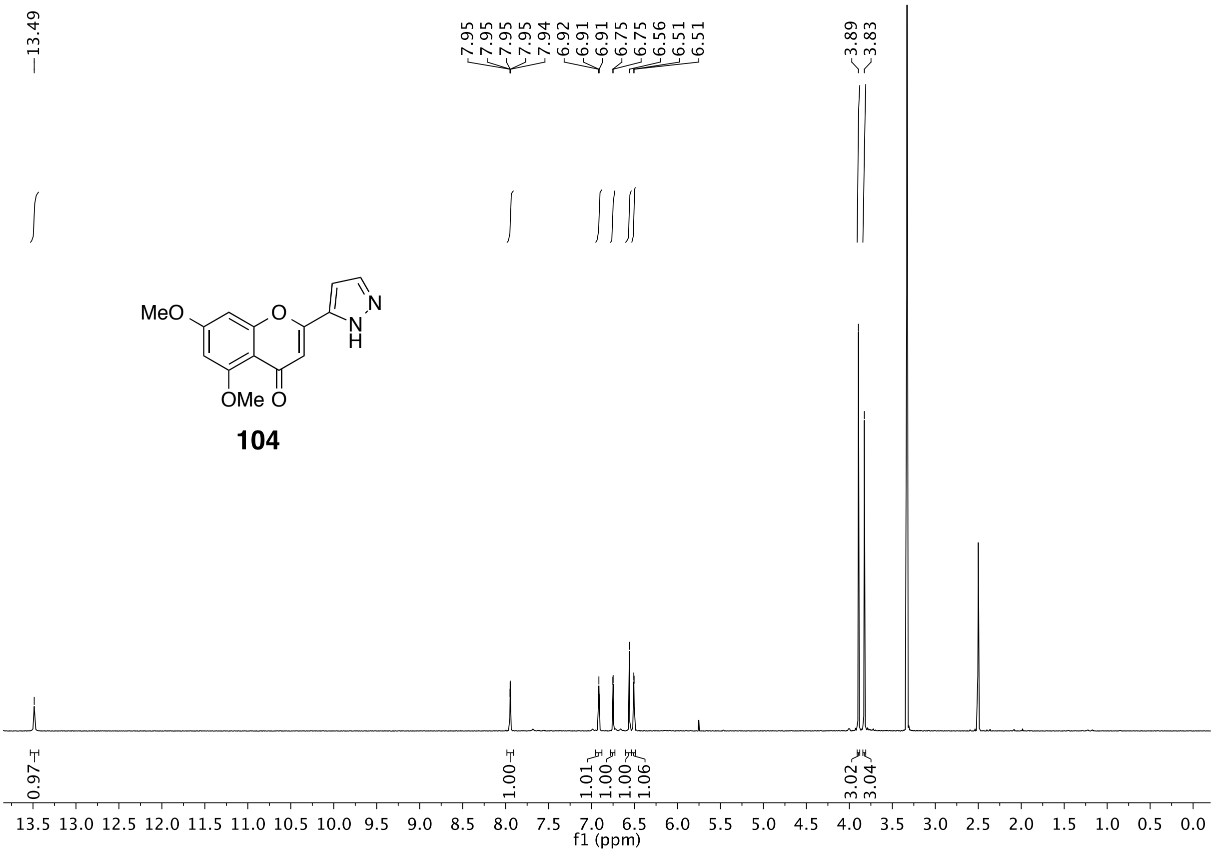
102


176.9
164.5
161.2
159.7
159.6
154.6
145.1
122.5
109.9
109.9
96.7
93.0
56.6
56.0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

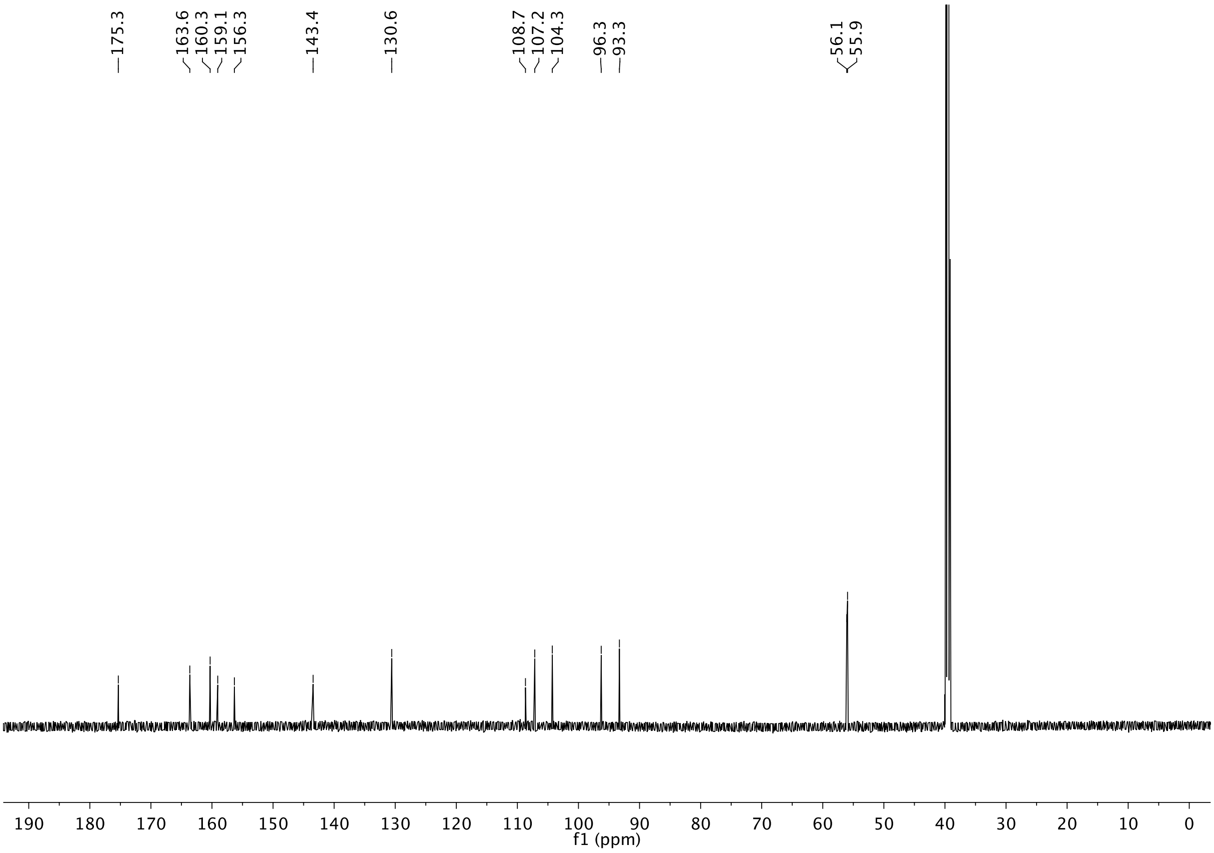

12.60
8.06
8.05
7.66
7.66
7.09
6.52
6.52
6.40
6.40
3.90
MeO
O
S
N
OH
O
1.00
1.00
1.02
1.04
1.03
1.02
3.00
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

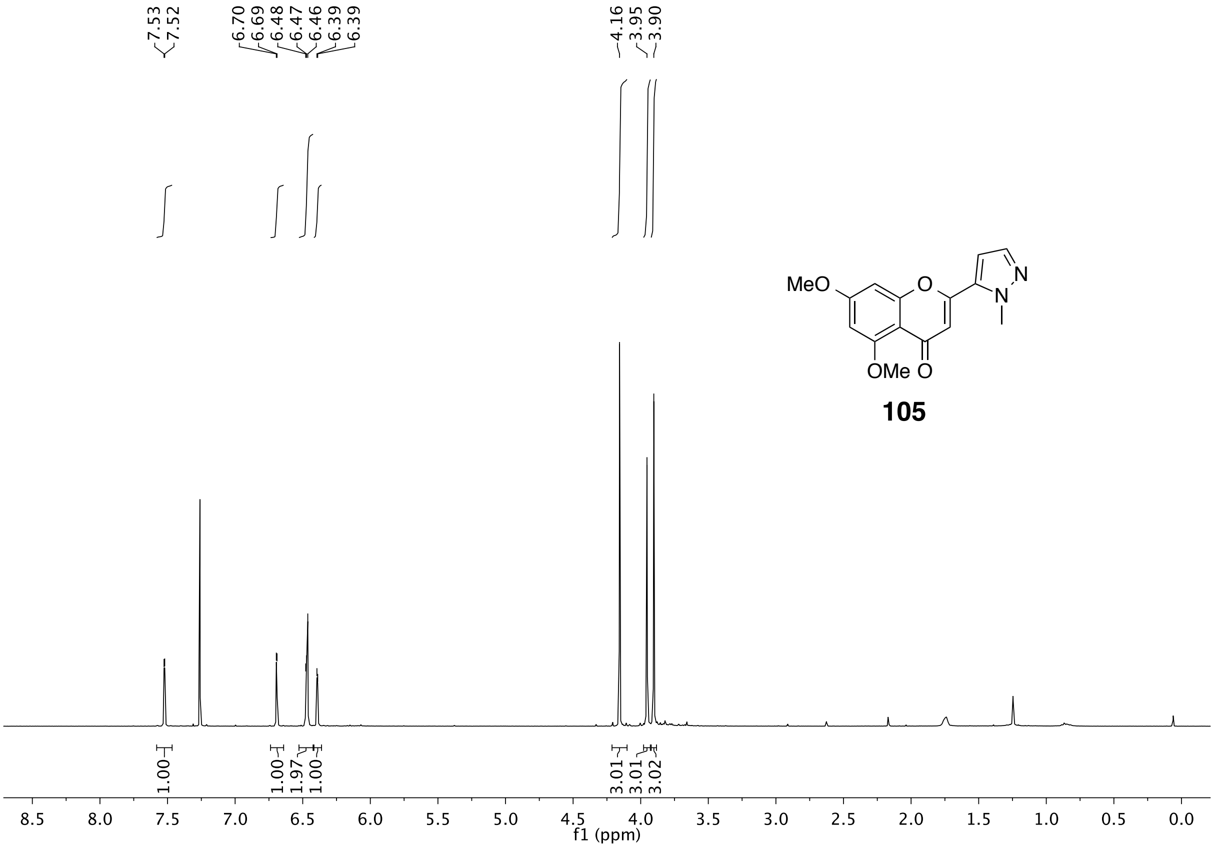
114


182.2
166.0
162.5
159.2
157.5
145.4
123.3
106.6
98.8
93.0
56.1
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


13.49
7.95
7.95
7.95
7.95
7.94
6.92
6.91
6.91
6.75
6.75
6.56
6.51
6.51
3.89
3.83
MeO
O
N
N
H
OMe
O
0.97
1.00
1.01
1.00
1.00
1.06
3.02
3.04
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

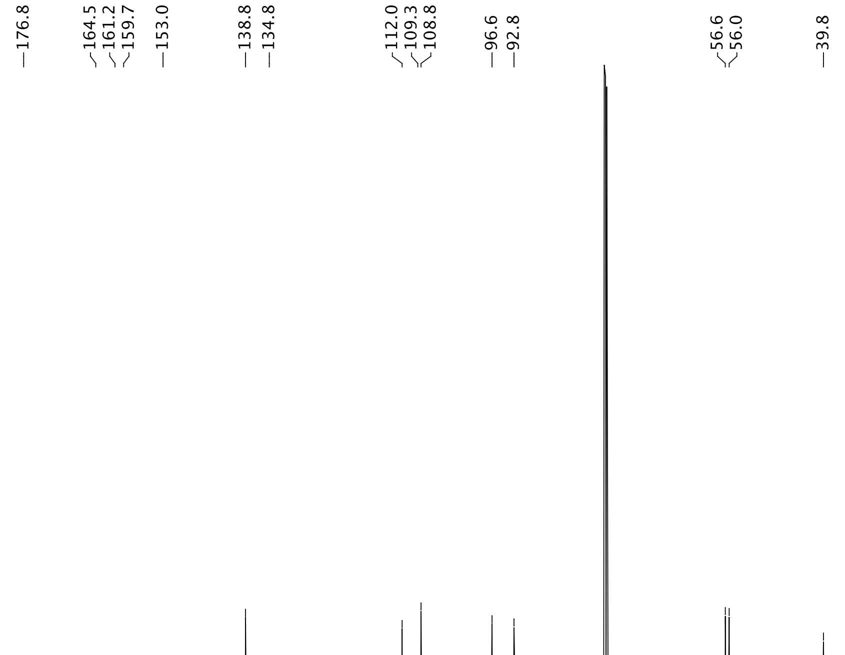
104


175.3
163.6
160.3
159.1
156.3
143.4
130.6
108.7
107.2
104.3
96.3
93.3
56.1
55.9
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

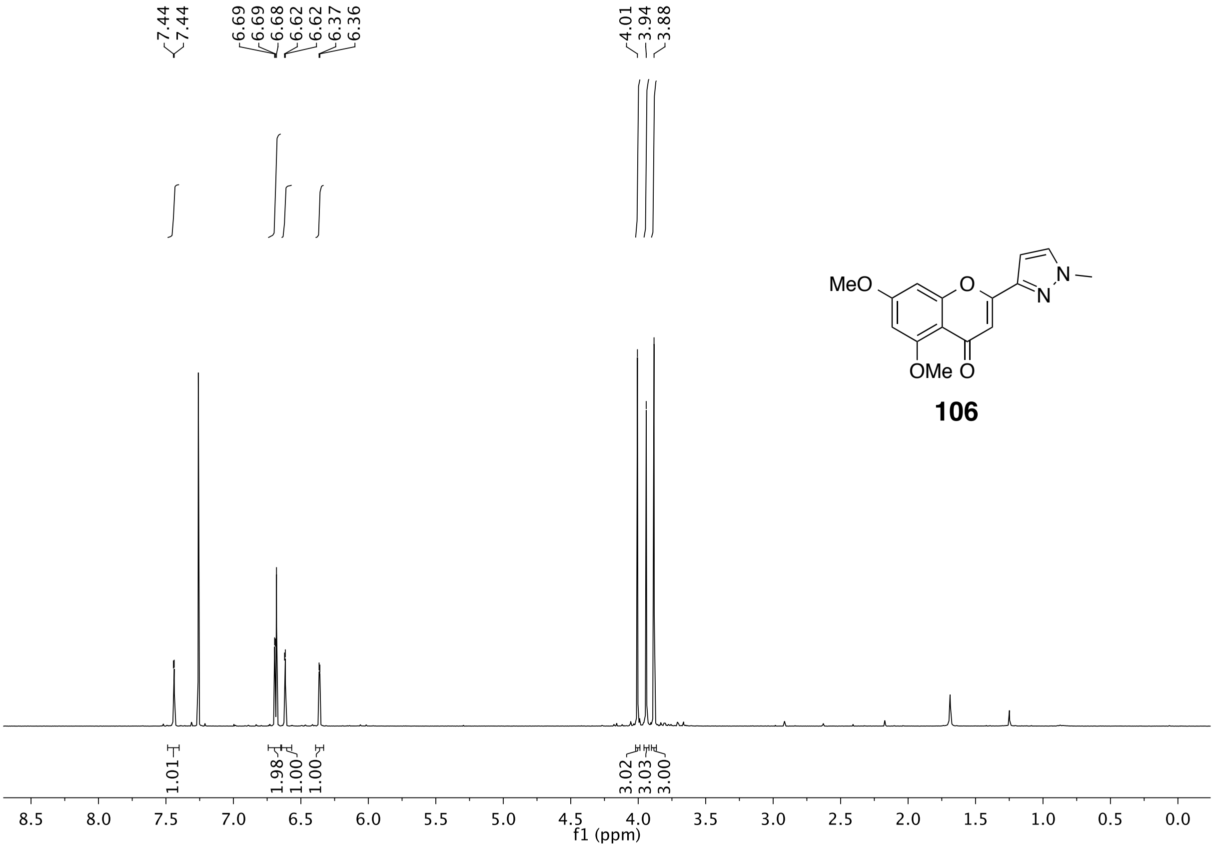


7.53
7.52
6.70
6.69
6.48
6.47
6.46
6.39
6.39
4.16
3.95
3.90
MeO
O
N
N
OMe O
1.00
1.00
1.97
1.00
3.01
3.01
3.02
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

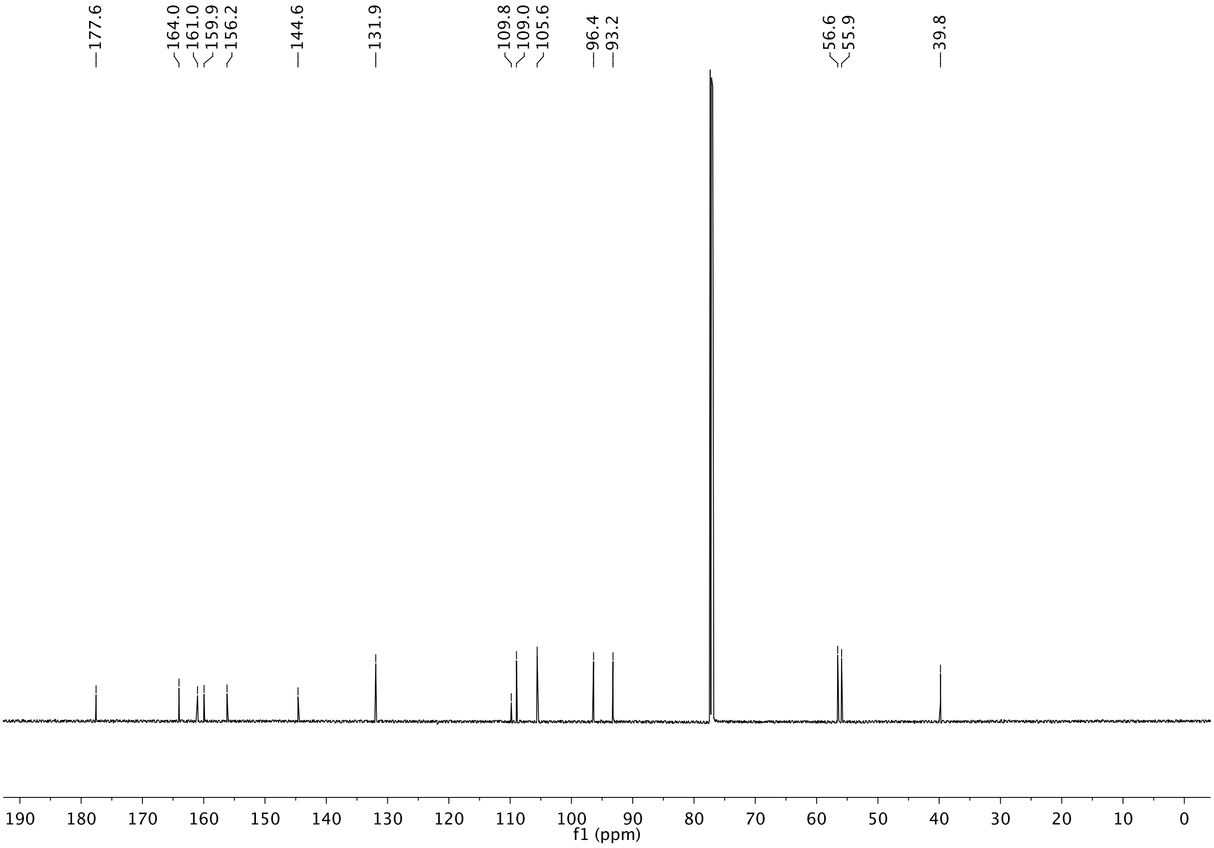
105

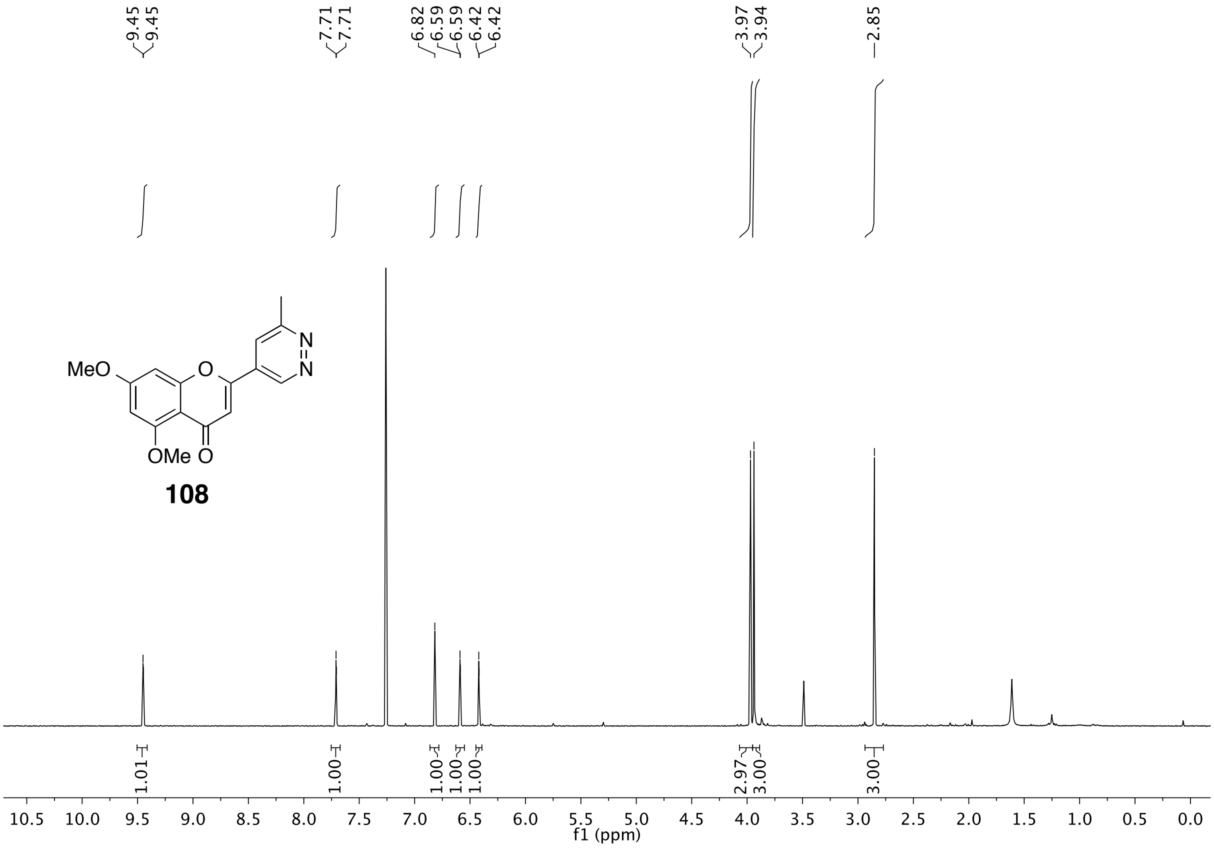


176.8
164.5
161.2
159.7
153.0
138.8
134.8
112.0
109.3
108.8
96.6
92.8
56.6
56.0
39.8
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

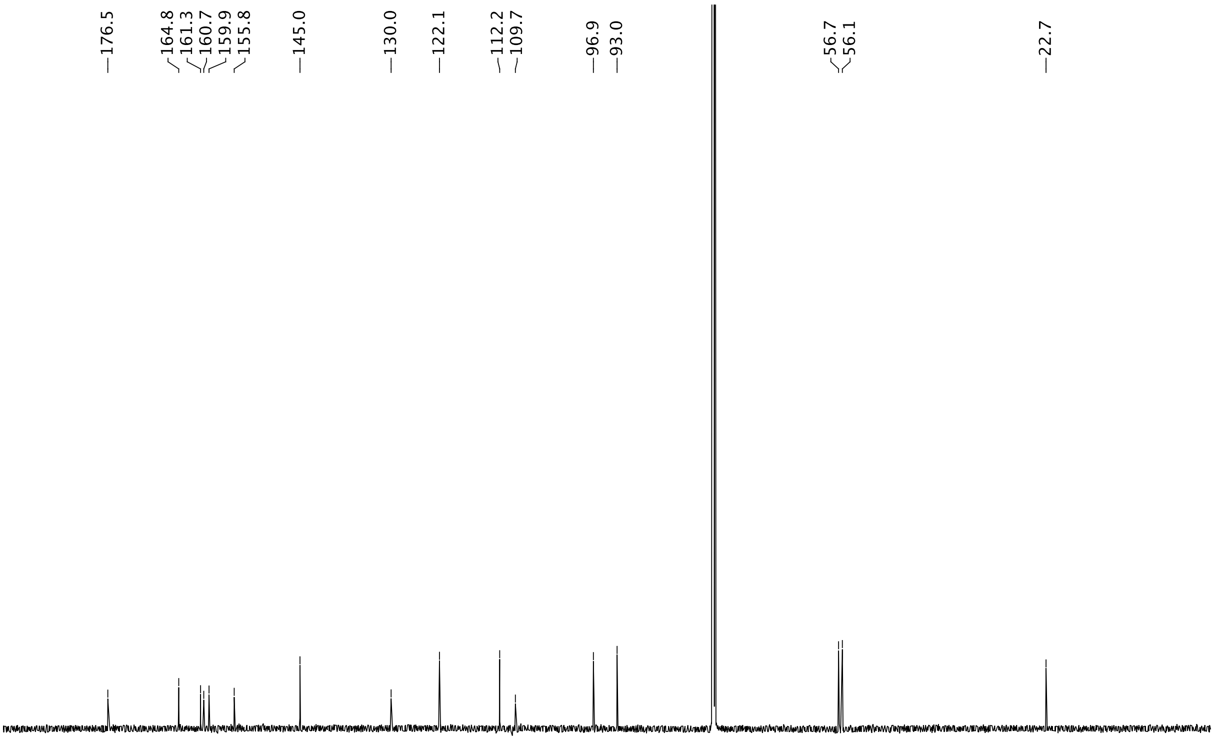

7.44
7.44
6.69
6.69
6.68
6.62
6.62
6.37
6.36
4.01
3.94
3.88
MeO
O
N
N
OMe
O
1.01
1.98
1.00
1.00
3.02
3.03
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

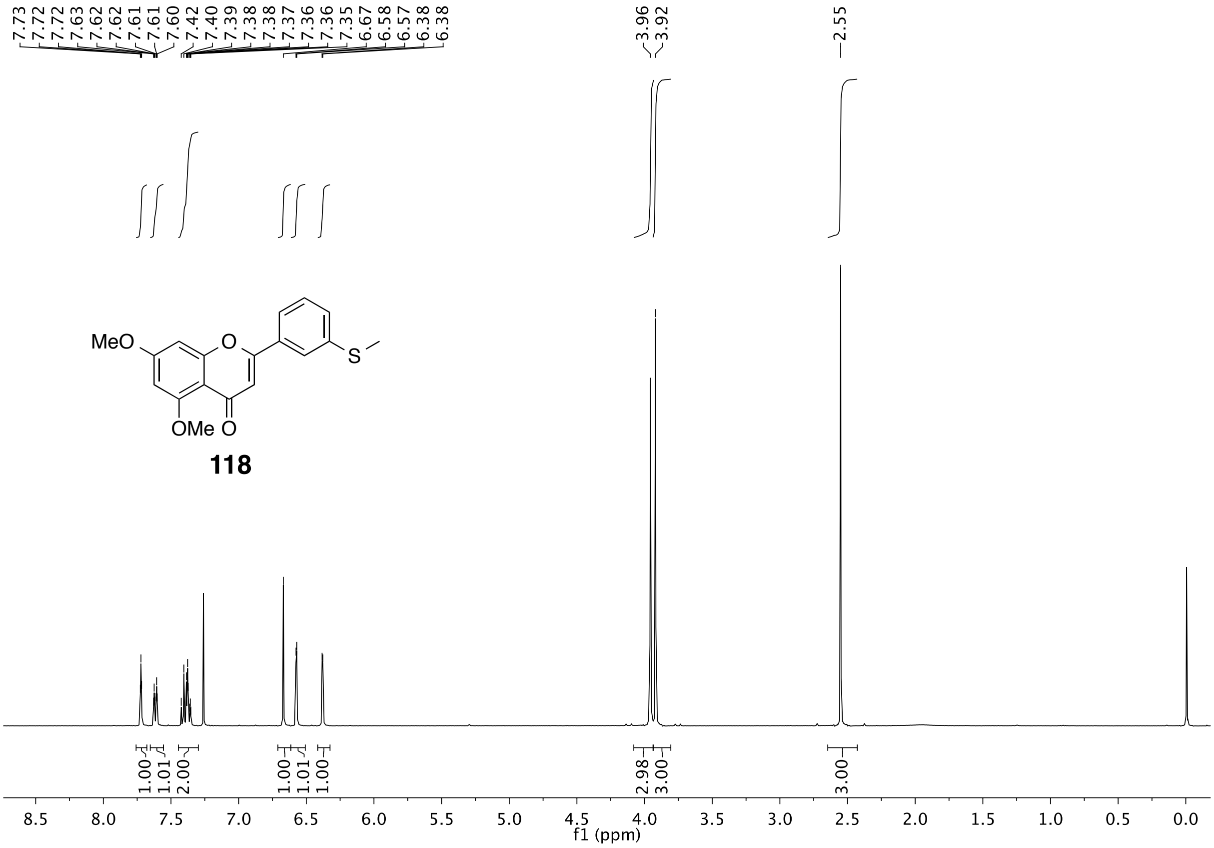
106


177.6
164.0
161.0
159.9
156.2
144.6
131.9
109.8
109.0
105.6
96.4
93.2
56.6
55.9
39.8
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

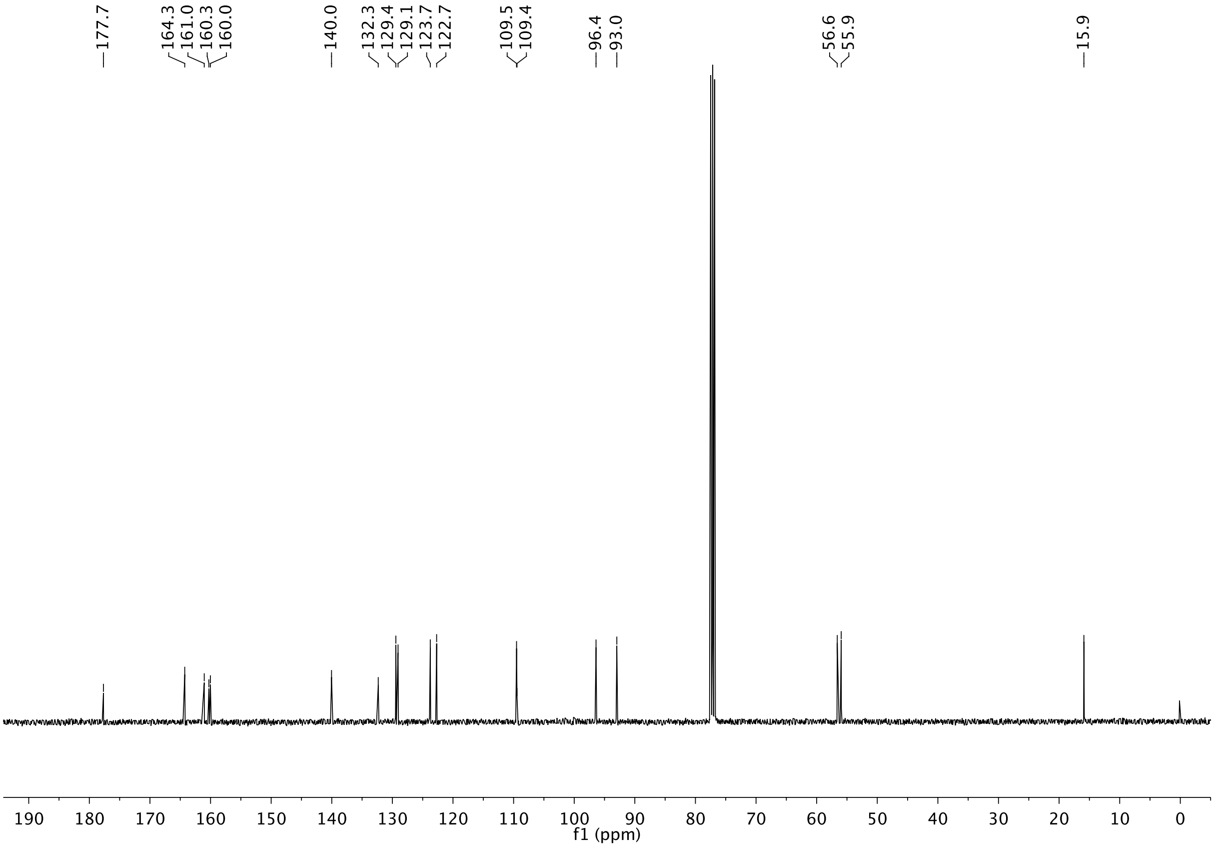

9.45
9.45
7.71
7.71
6.82
6.59
6.59
6.42
6.42
3.97
3.94
2.85
MeO
O
N
N
OMe
O
1.01
1.00
1.00
1.00
1.00
2.97
3.00
3.00
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

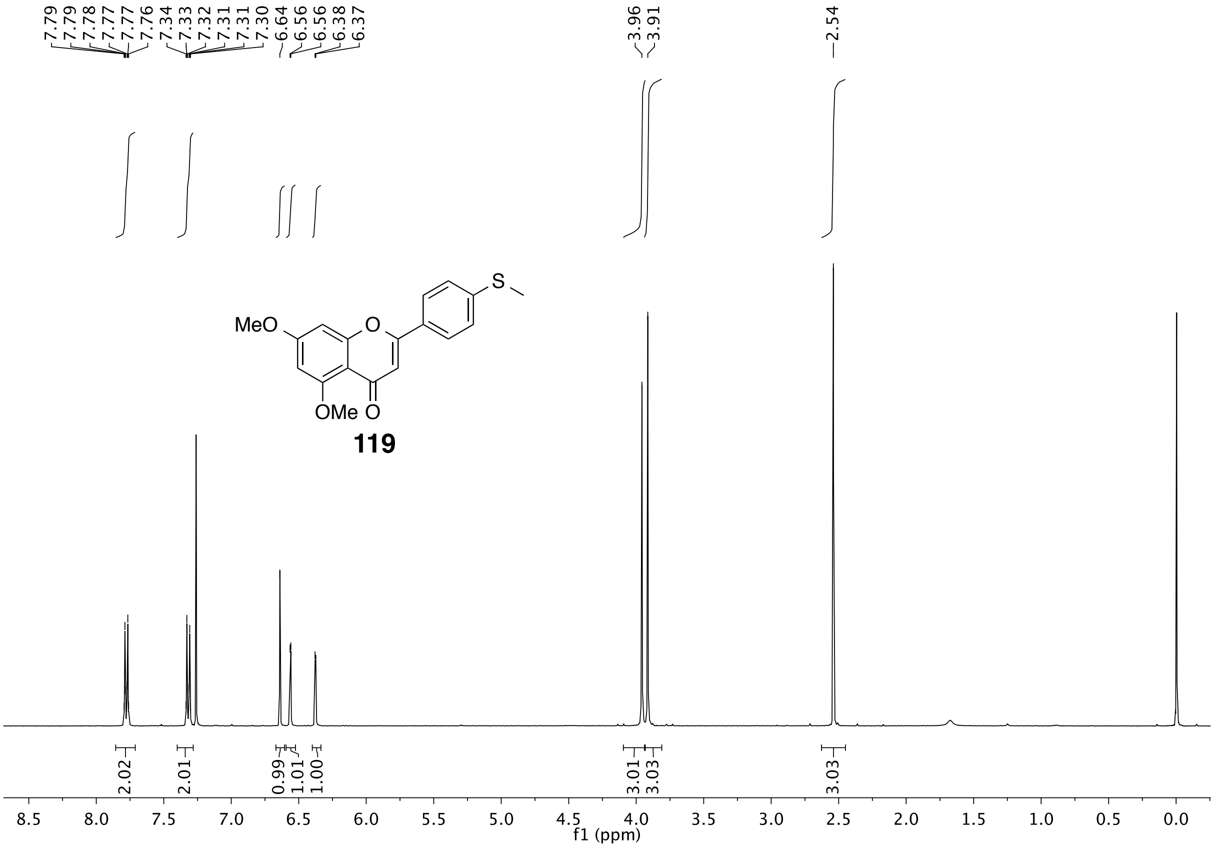
108


176.5
164.8
161.3
160.7
159.9
155.8
145.0
130.0
122.1
112.2
109.7
96.9
93.0
56.7
56.1
22.7
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

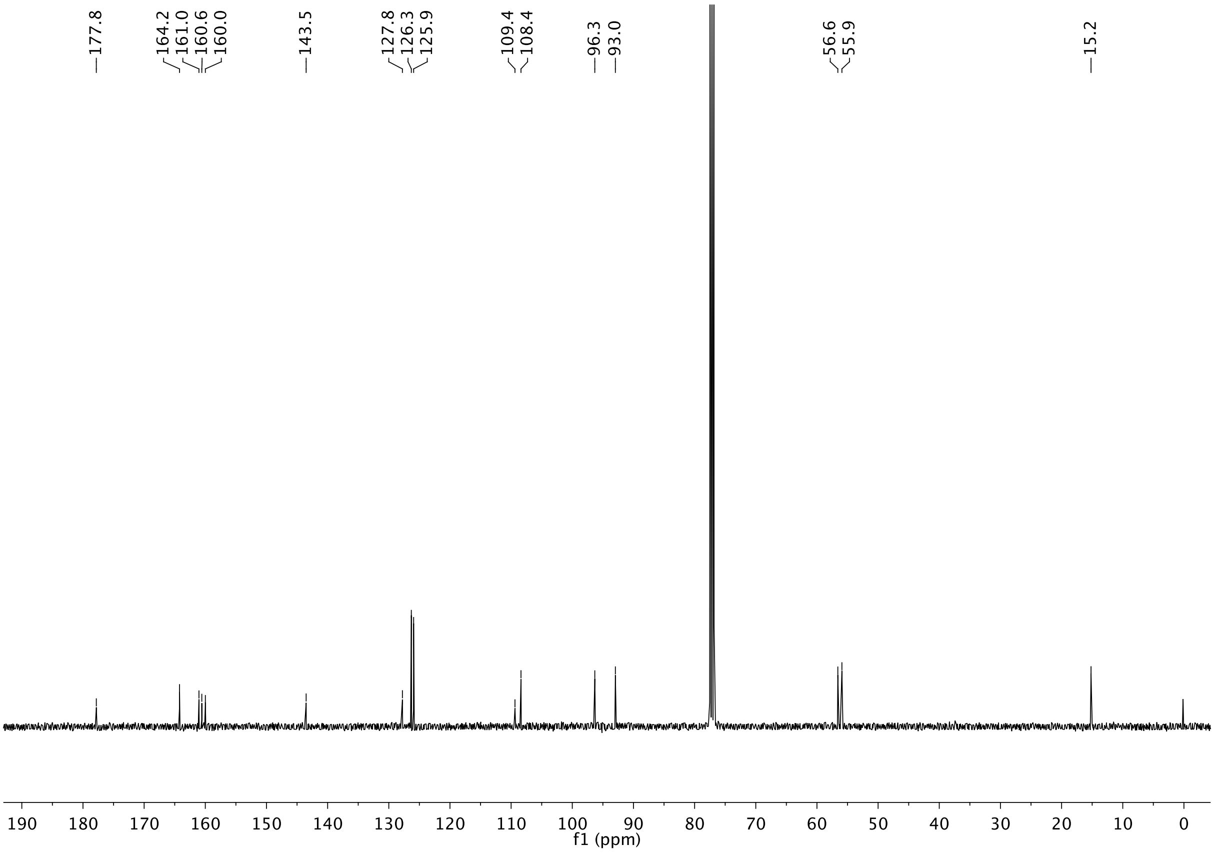

7.73
7.72
7.72
7.63
7.62
7.62
7.61
7.61
7.60
7.42
7.40
7.39
7.38
7.38
7.37
7.36
7.36
7.35
6.67
6.58
6.57
6.38
6.38
3.96
3.92
2.55
MeO
O
S
OMe O
1.00
1.01
2.00
1.00
1.01
1.00
2.98
3.00
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

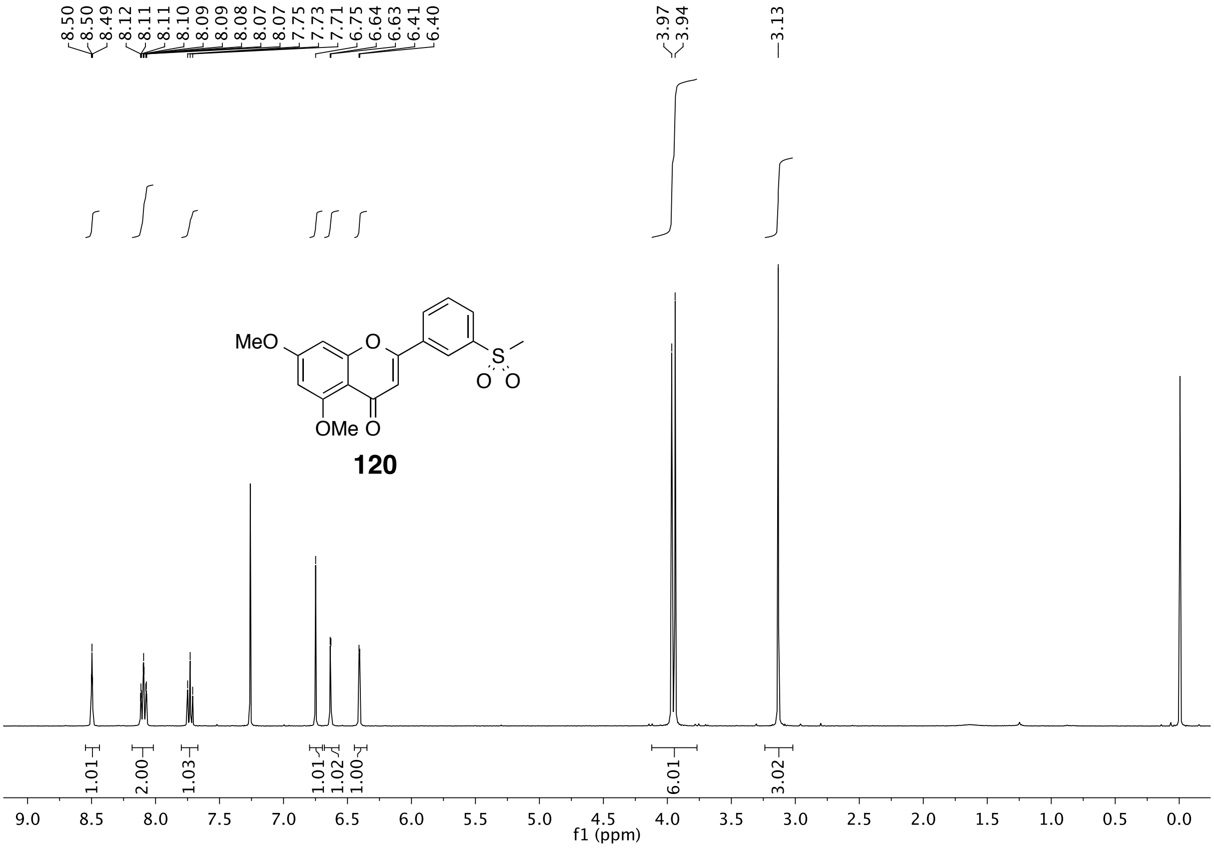
118

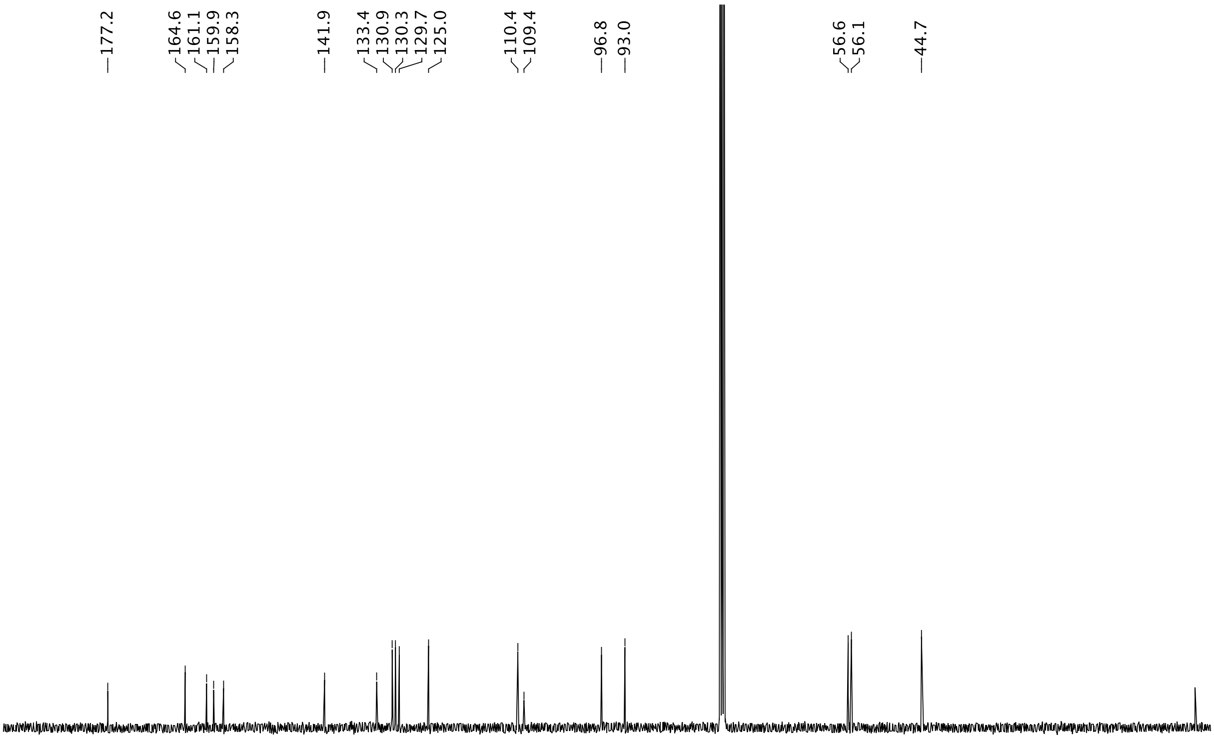

177.7
164.3
161.0
160.3
160.0
140.0
132.3
129.4
129.1
123.7
122.7
109.5
109.4
96.4
93.0
56.6
55.9
15.9
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

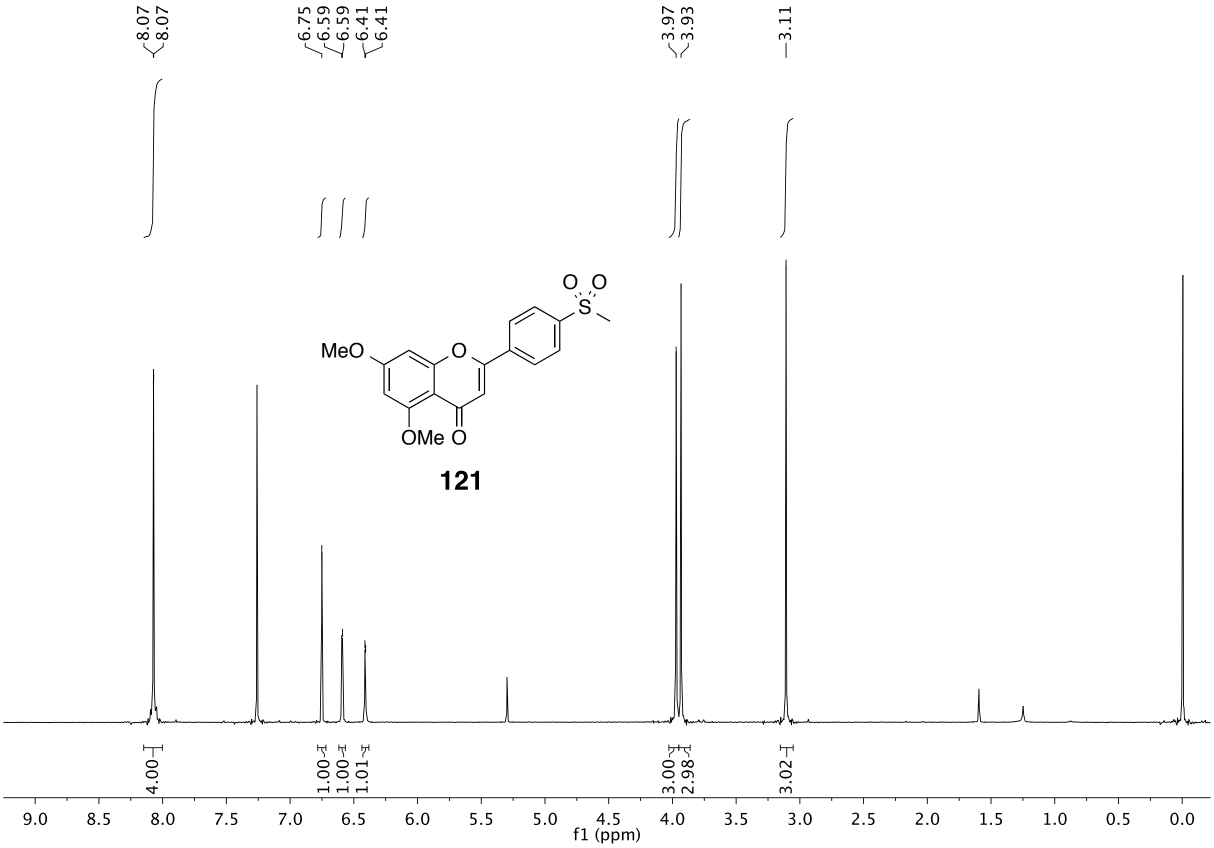

7.79
7.79
7.78
7.77
7.77
7.76
7.34
7.33
7.32
7.31
7.31
7.30
6.64
6.56
6.56
6.38
6.37
3.96
3.91
2.54
MeO
O
S
OMe
O
2.02
2.01
0.99
1.01
1.00
3.01
3.03
3.03
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

119

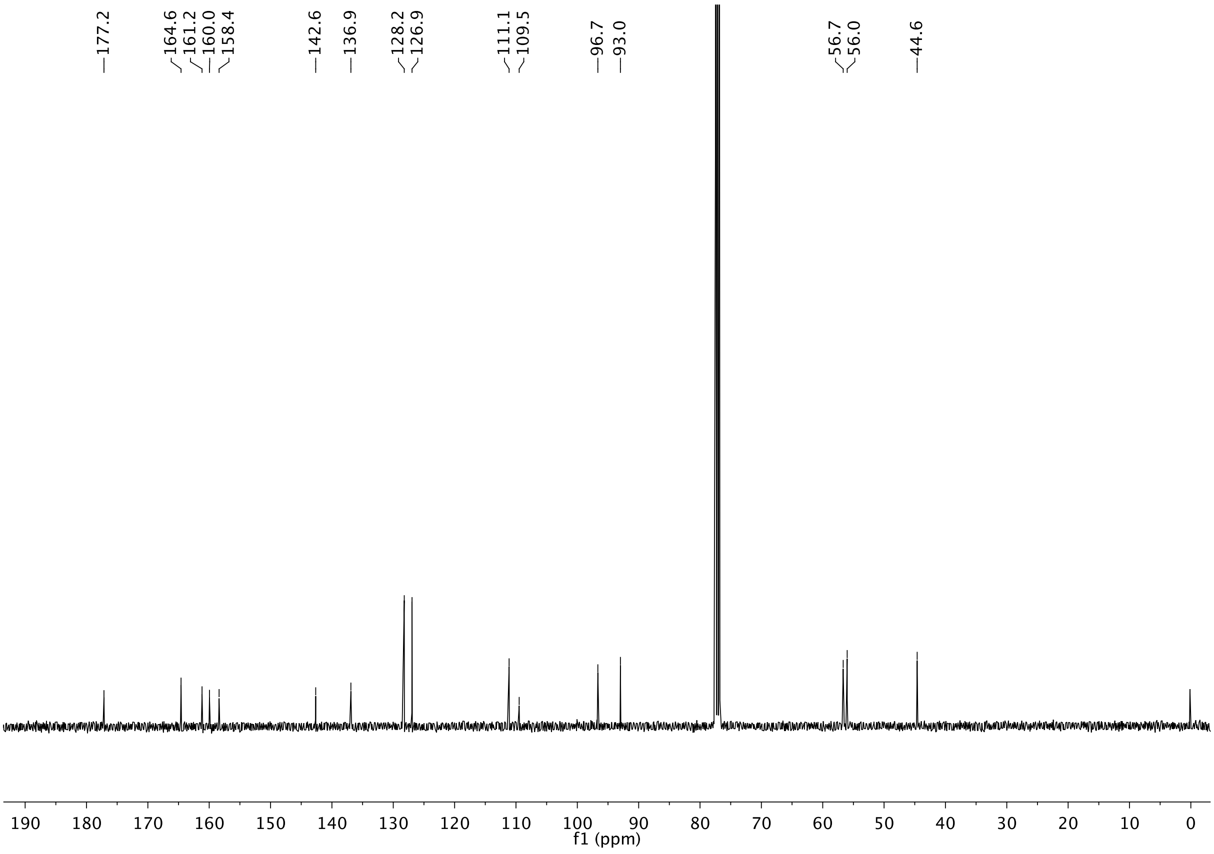

177.8
164.2
161.0
160.6
160.0
143.5
127.8
126.3
125.9
109.4
108.4
96.3
93.0
56.6
55.9
15.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

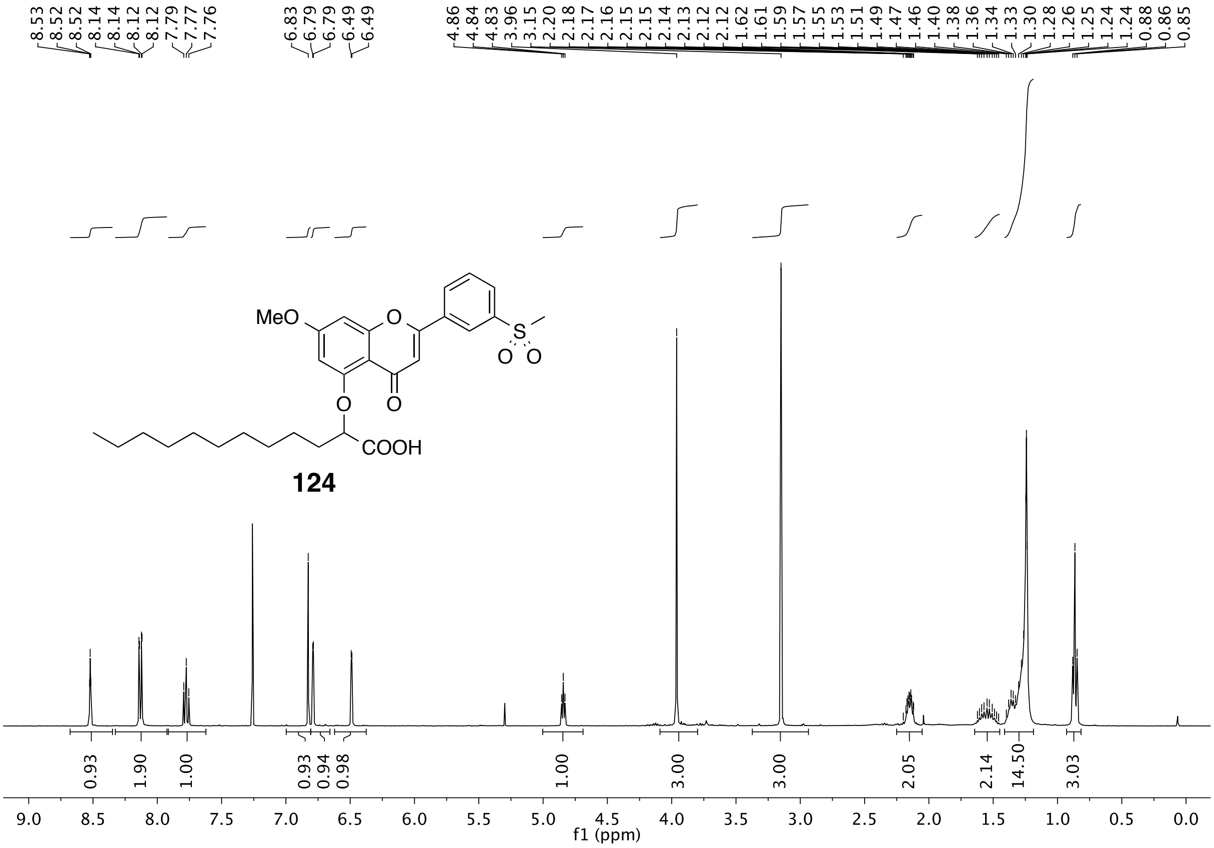

8.50
8.50
8.49
8.12
8.11
8.11
8.10
8.09
8.09
8.08
8.07
8.07
7.75
7.73
7.71
6.75
6.64
6.63
6.41
6.40
3.97
3.94
3.13
MeO
O
S
O
O
OMe
O
120
1.01
2.00
1.03
1.01
1.02
1.00
6.01
3.02
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


177.2
164.6
161.1
159.9
158.3
141.9
133.4
130.9
130.3
129.7
125.0
110.4
109.4
96.8
93.0
56.6
56.1
44.7
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

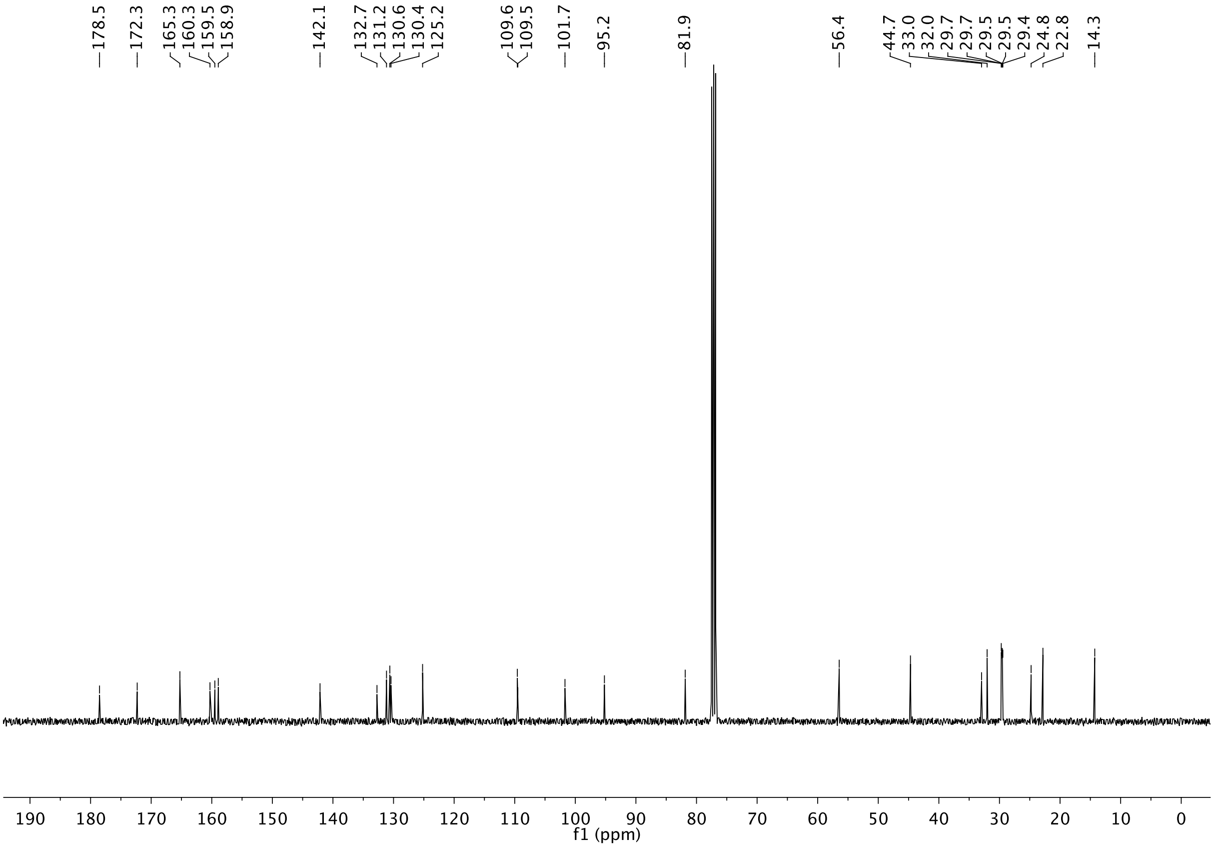

8.07
8.07
6.75
6.59
6.59
6.41
6.41
3.97
3.93
3.11
MeO
O
O
O
S
OMe
O
4.00
1.00
1.00
1.01
3.00
2.98
3.02
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

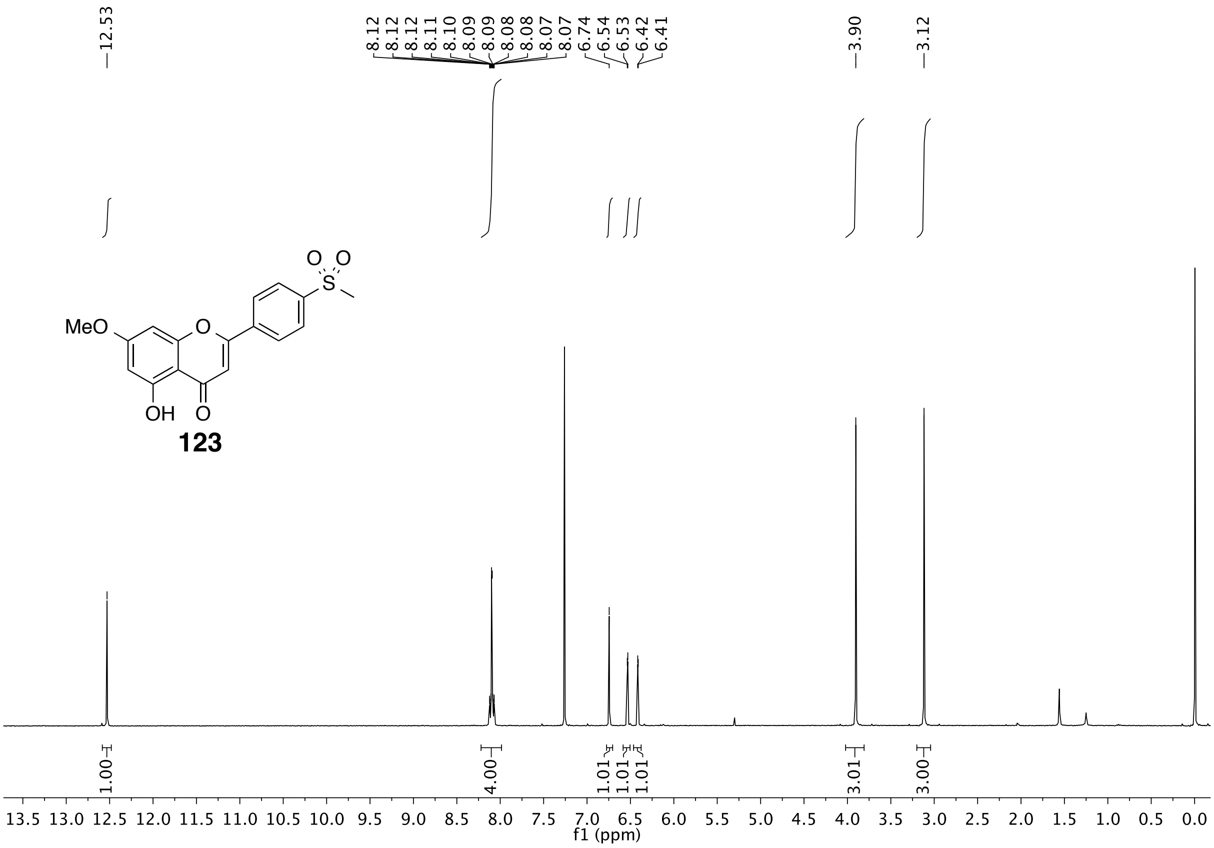
121


177.2
164.6
161.2
160.0
158.4
142.6
136.9
128.2
126.9
111.1
109.5
96.7
93.0
56.7
56.0
44.6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


8.53
8.52
8.52
8.14
8.14
8.12
8.12
7.79
7.77
7.76
6.83
6.79
6.79
6.49
6.49
4.86
4.84
4.83
3.96
3.15
2.20
2.18
2.17
2.16
2.15
2.14
2.13
2.12
2.12
1.62
1.61
1.59
1.57
1.55
1.53
1.51
1.49
1.47
1.46
1.40
1.38
1.36
1.34
1.33
1.30
1.28
1.26
1.25
1.24
1.24
0.88
0.86
0.85
MeO
O
S
O O
O
O
COOH
0.93
1.90
1.00
0.93
0.94
0.98
1.00
3.00
3.00
2.05
2.14
14.50
3.03
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

124


178.5
172.3
165.3
160.3
159.5
158.9
142.1
132.7
131.2
130.6
130.4
125.2
109.6
109.5
101.7
95.2
81.9
56.4
44.7
33.0
32.0
29.7
29.7
29.5
29.5
29.4
24.8
22.8
14.3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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8.12
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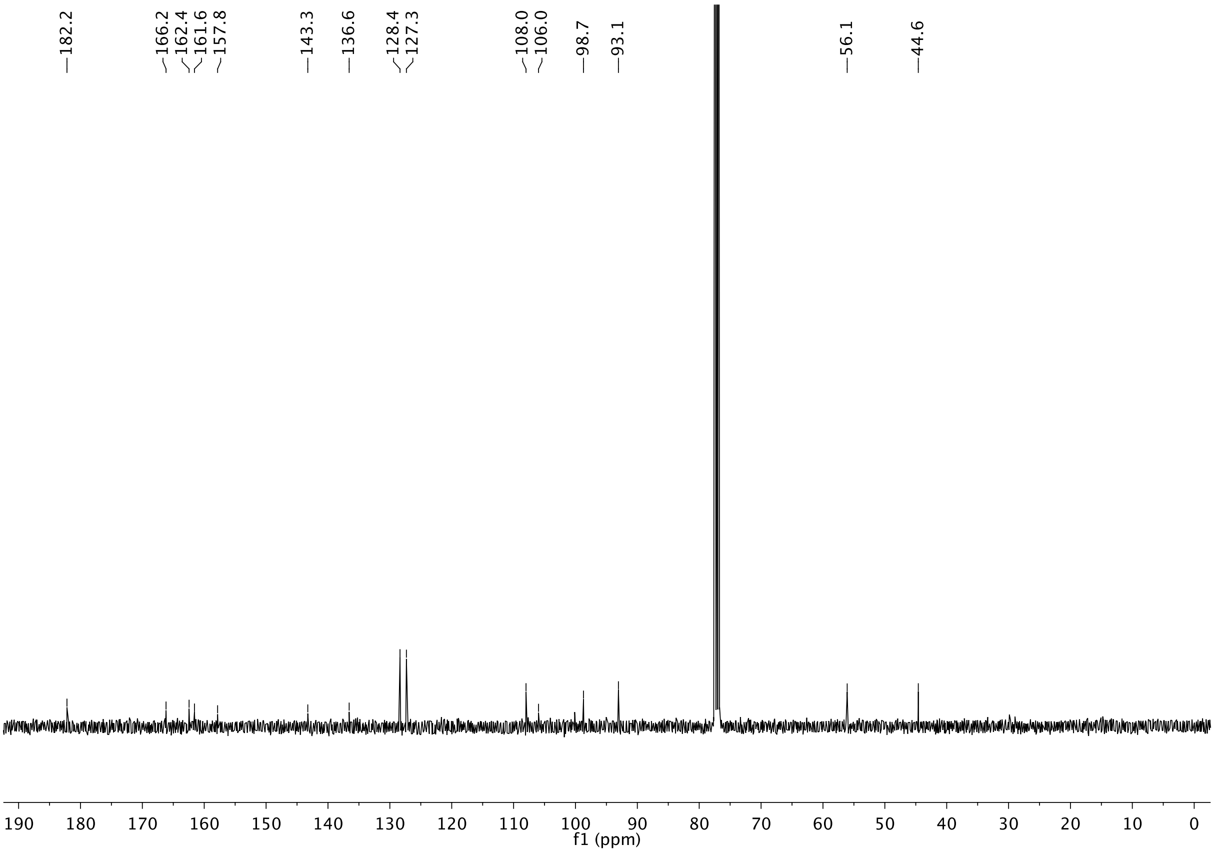
8.11
8.10
8.09
8.09
8.08
8.08
8.07
8.07
6.74
6.54
6.53
6.42
6.41
3.90
3.12
MeO
O
O
O
S
OH
O
123
1.00
4.00
1.01
1.01
1.01
3.01
3.00
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


182.2
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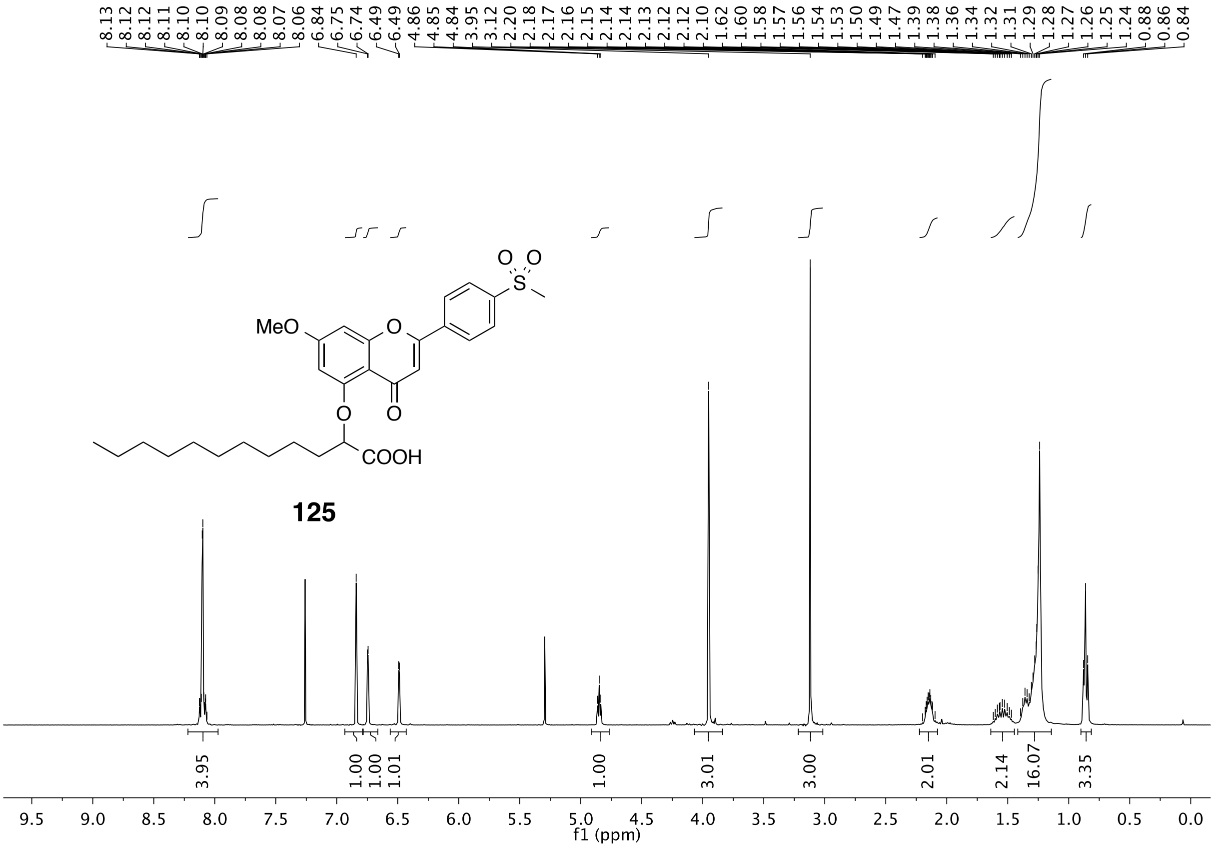
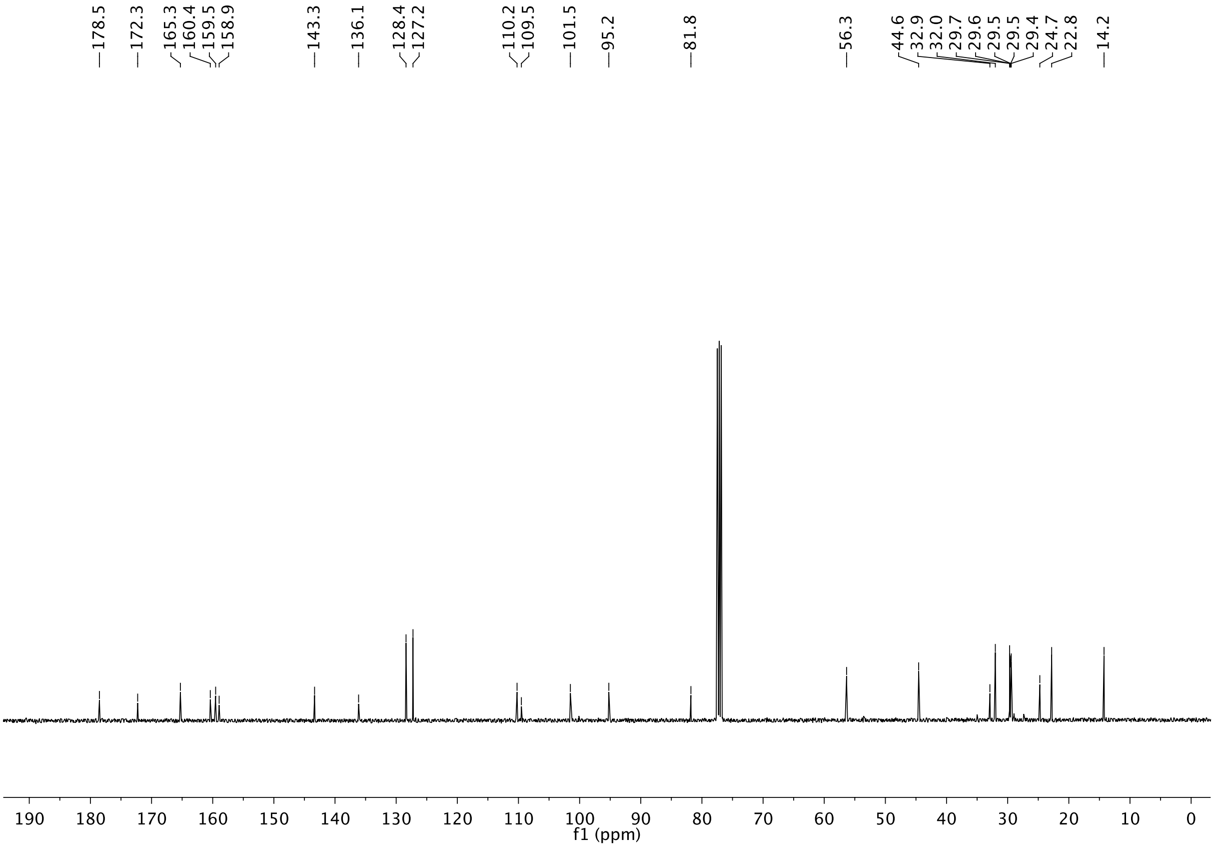
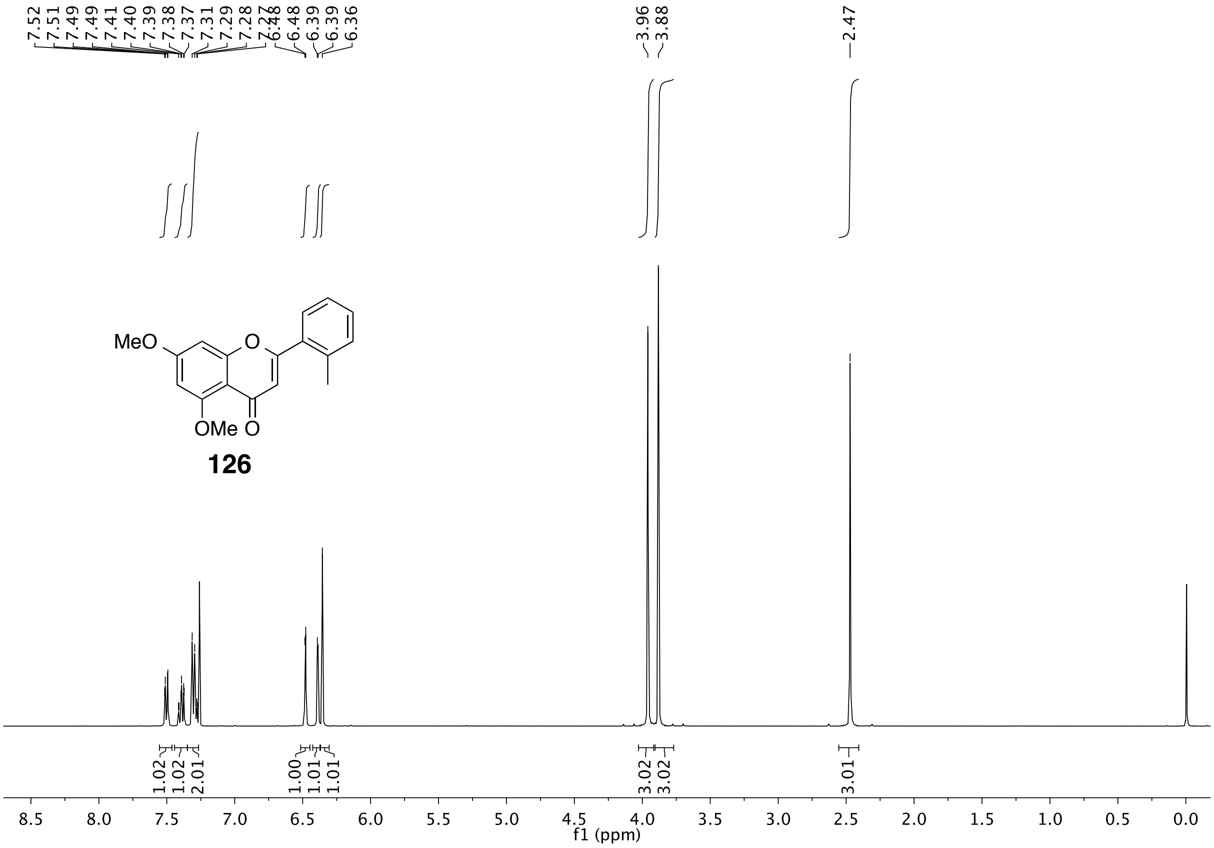
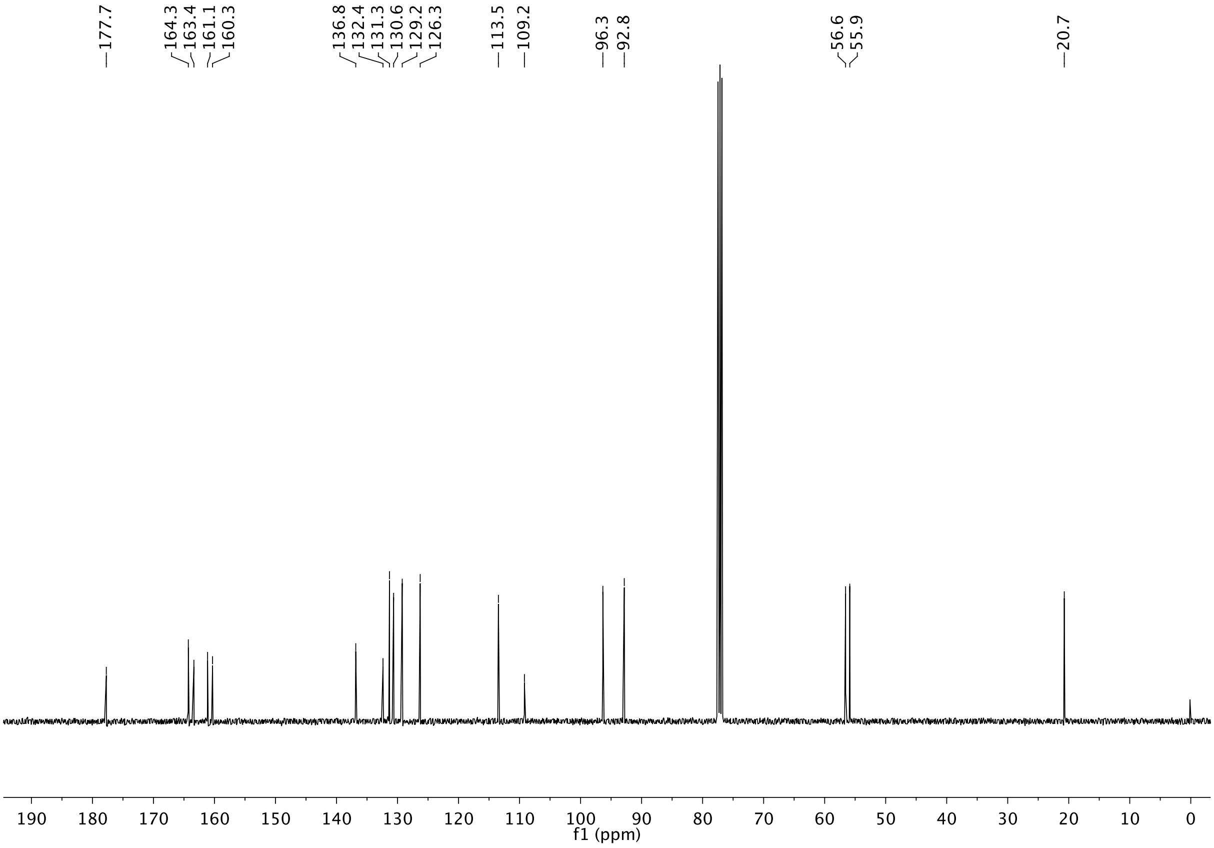
161.6
157.8
143.3
136.6
128.4
127.3
108.0
106.0
98.7
93.1
56.1
44.6
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

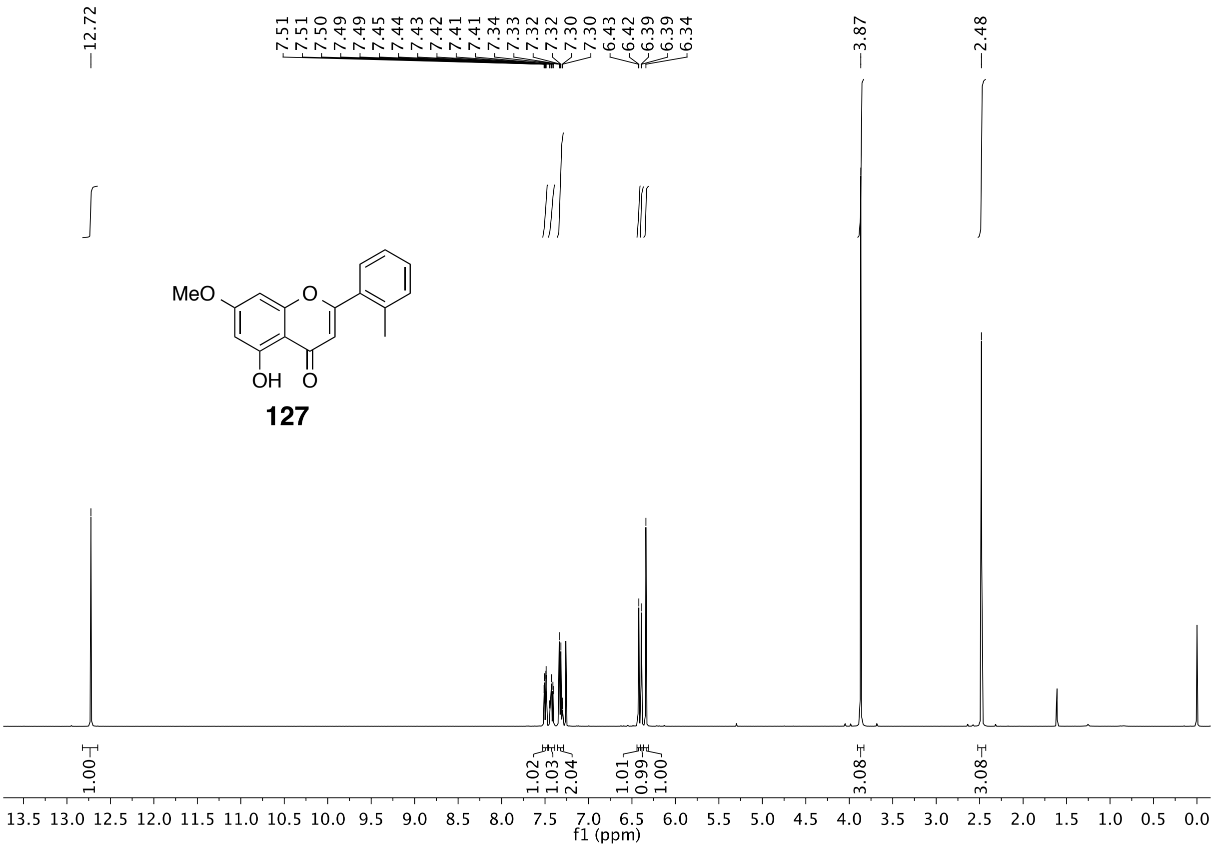

8.13
8.12
8.12
8.11
8.10
8.09
8.08
8.08
8.07
8.06
6.84
6.75
6.74
6.49
6.49
4.86
4.85
4.84
3.95
3.12
2.20
2.18
2.17
2.16
2.15
2.14
2.14
2.13
2.12
2.12
2.10
1.62
1.60
1.58
1.57
1.56
1.54
1.53
1.50
1.49
1.47
1.39
1.38
1.36
1.34
1.32
1.31
1.29
1.28
1.27
1.26
1.25
1.24
0.88
0.86
0.84
MeO
O
O
O
S
O
O
COOH
125
3.95
1.00
1.00
1.01
1.00
3.01
3.00
2.01
2.14
16.07
3.35
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


178.5
172.3
165.3
160.4
159.5
158.9
143.3
136.1
128.4
127.2
110.2
109.5
101.5
95.2
81.8
56.3
44.6
32.9
32.0
29.7
29.6
29.5
29.5
29.4
24.7
22.8
14.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.52
7.51
7.49
7.41
7.40
7.39
7.38
7.37
7.31
7.29
7.28
7.27
6.48
6.48
6.39
6.39
6.36
3.96
3.88
2.47
MeO
O
OMe
O
1.02
1.02
2.01
1.00
1.01
1.01
3.02
3.02
3.01
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

126


177.7
164.3
163.4
161.1
160.3
136.8
132.4
131.3
130.6
129.2
126.3
113.5
109.2
96.3
92.8
56.6
55.9
20.7
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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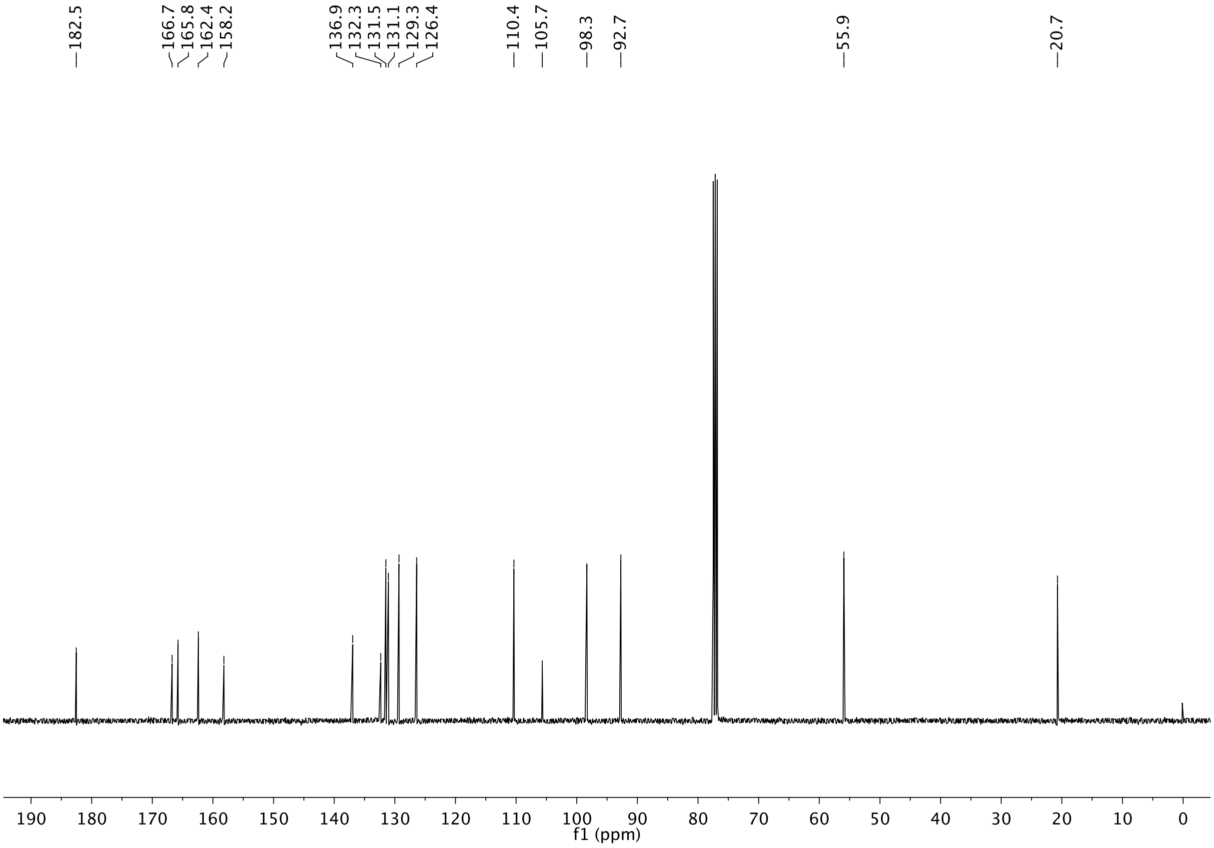
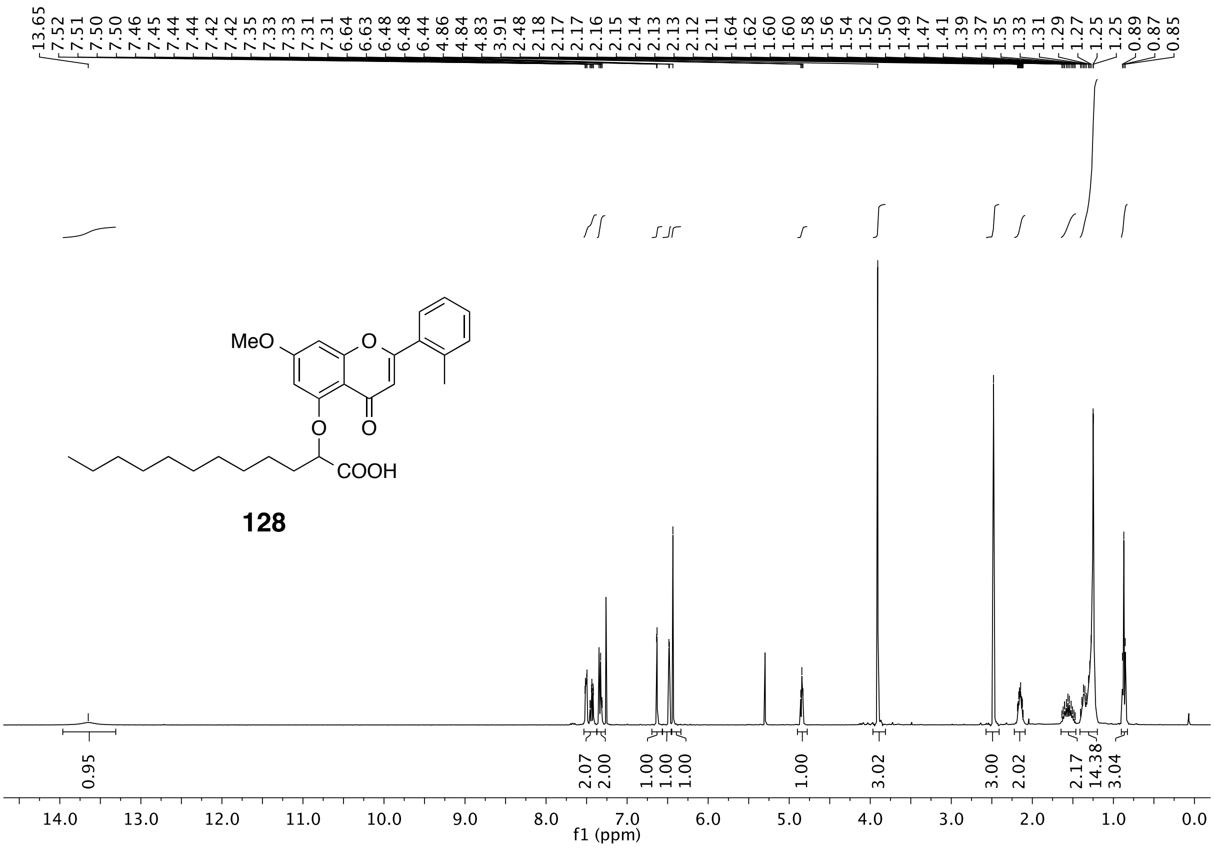
7.51
7.51
7.50
7.49
7.49
7.45
7.44
7.43
7.42
7.41
7.41
7.34
7.33
7.32
7.32
7.30
7.30
6.43
6.42
6.39
6.39
6.34
3.87
2.48
MeO
O
OH
O
1.00
1.02
1.03
2.04
1.01
0.99
1.00
3.08
3.08
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

127


182.5
166.7
165.8
162.4
158.2
136.9
132.3
131.5
131.1
129.3
126.4
110.4
105.7
98.3
92.7
55.9
20.7
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


13.65
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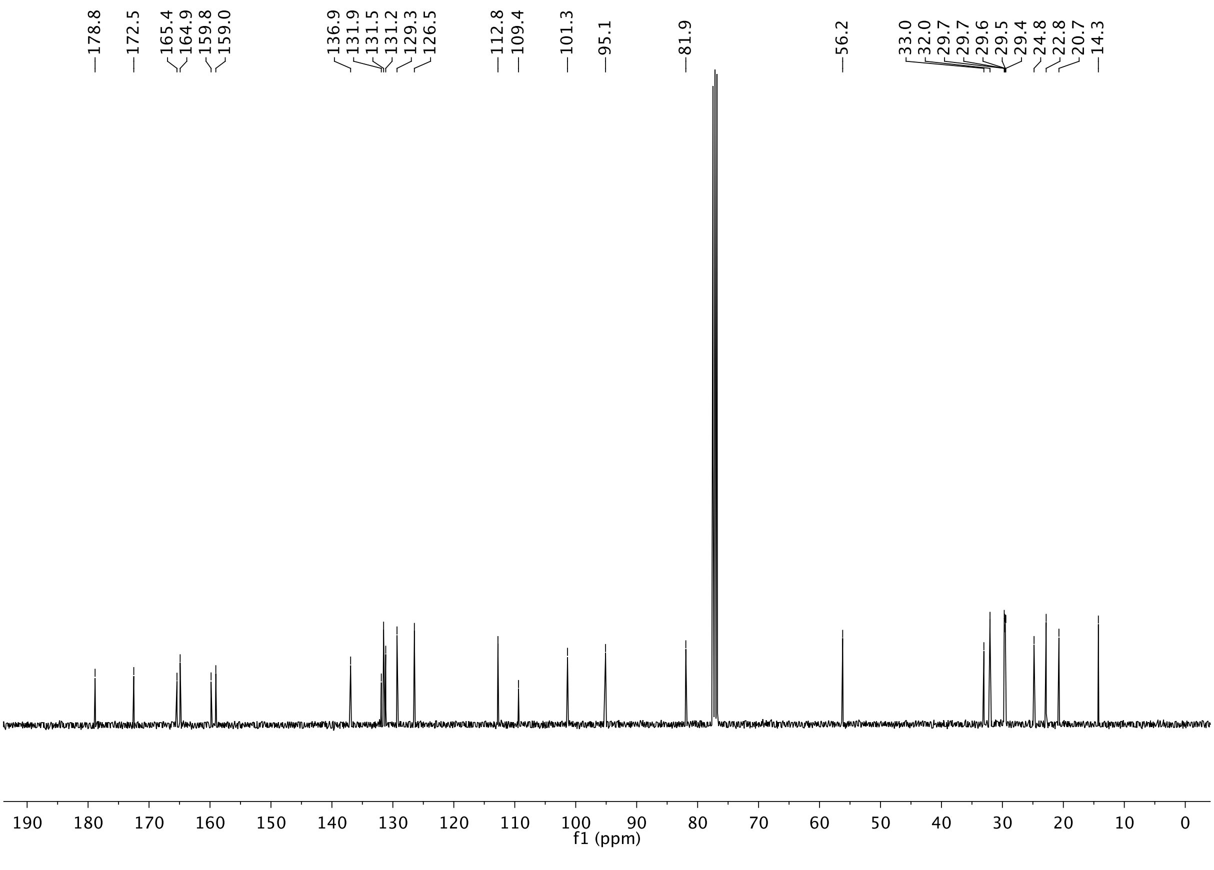
7.51
7.50
7.50
7.46
7.45
7.44
7.44
7.42
7.42
7.35
7.33
7.33
7.31
7.31
6.64
6.63
6.48
6.48
6.44
4.86
4.84
4.83
3.91
2.48
2.18
2.17
2.17
2.16
2.15
2.14
2.13
2.13
2.12
2.11
2.11
1.64
1.62
1.60
1.60
1.58
1.56
1.54
1.52
1.50
1.49
1.47
1.41
1.39
1.37
1.35
1.33
1.31
1.29
1.27
1.25
1.25
0.89
0.87
0.85
MeO
O
O
O
COOH
0.95
2.07
2.00
1.00
1.00
1.00
1.00
3.02
3.00
2.02
2.17
14.38
3.04
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

128


178.8
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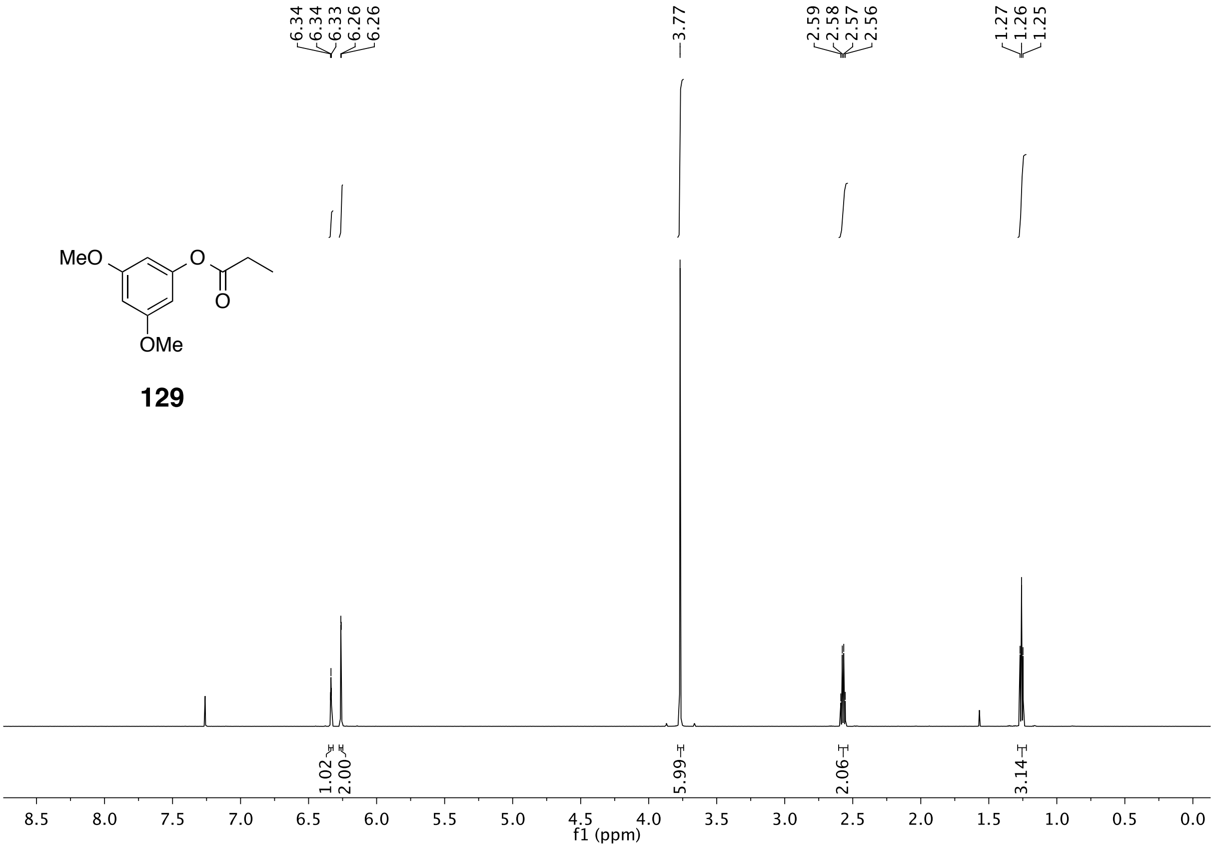
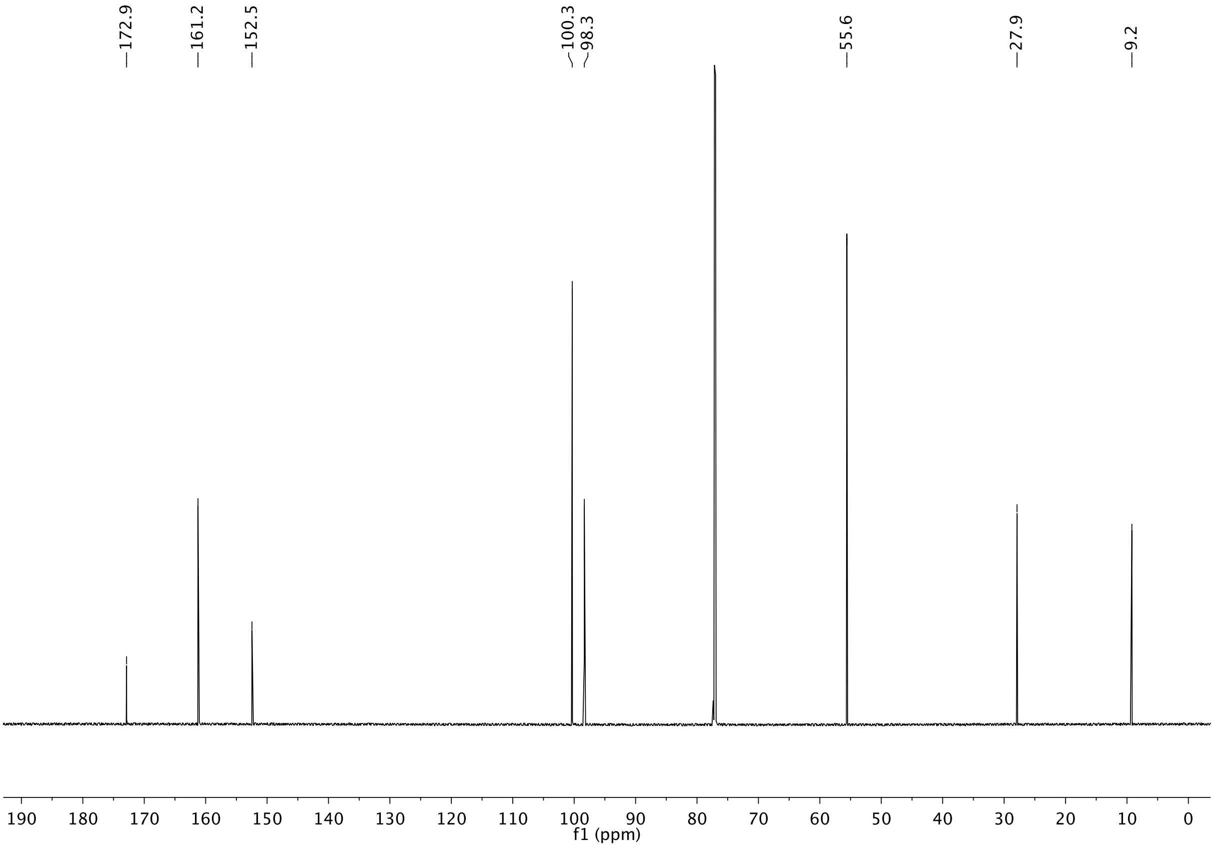
159.0
136.9
131.9
131.5
131.2
129.3
126.5
112.8
109.4
101.3
95.1
81.9
56.2
33.0
32.0
29.7
29.7
29.6
29.5
29.4
24.8
22.8
20.7
14.3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


6.34
6.34
6.33
6.26
6.26
3.77
2.59
2.58
2.57
2.56
1.27
1.26
1.25
MeO
O
O
OMe
1.02
2.00
5.99
2.06
3.14
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

129


172.9
161.2
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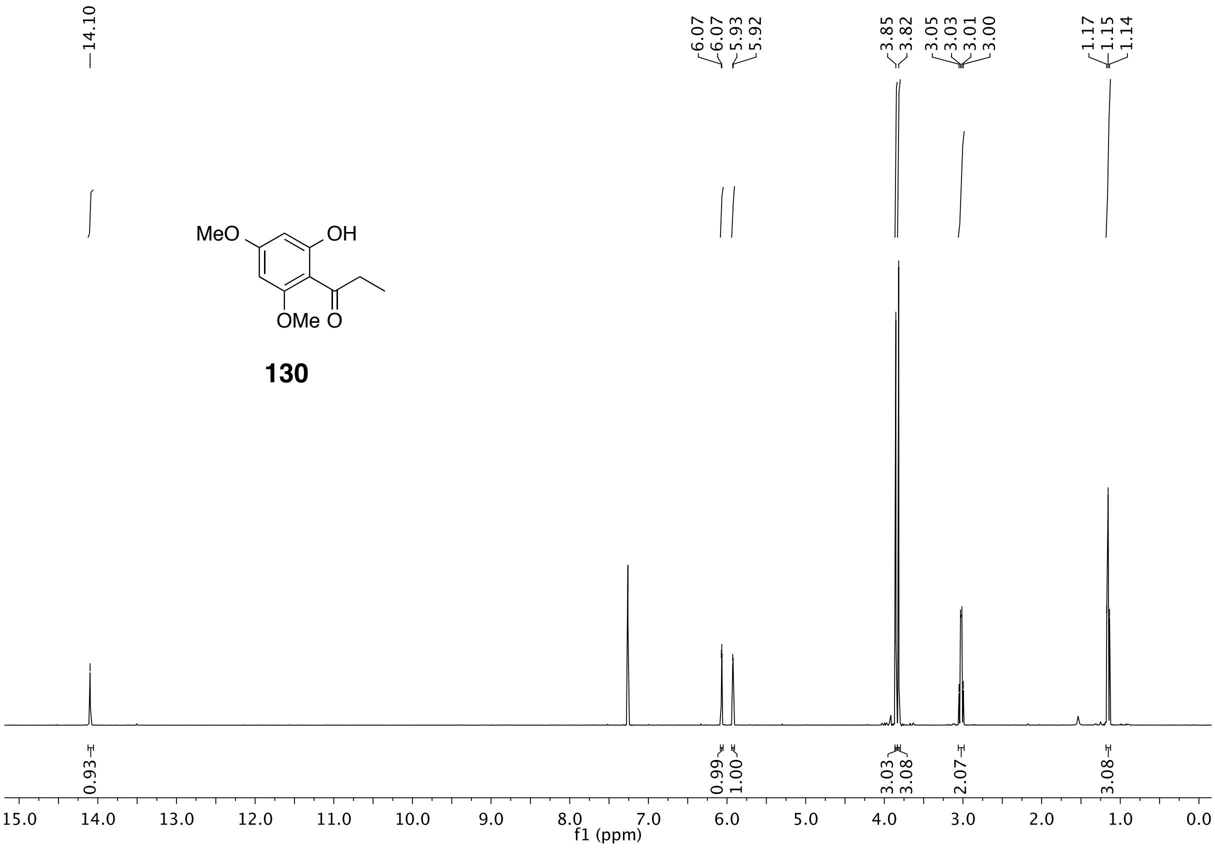
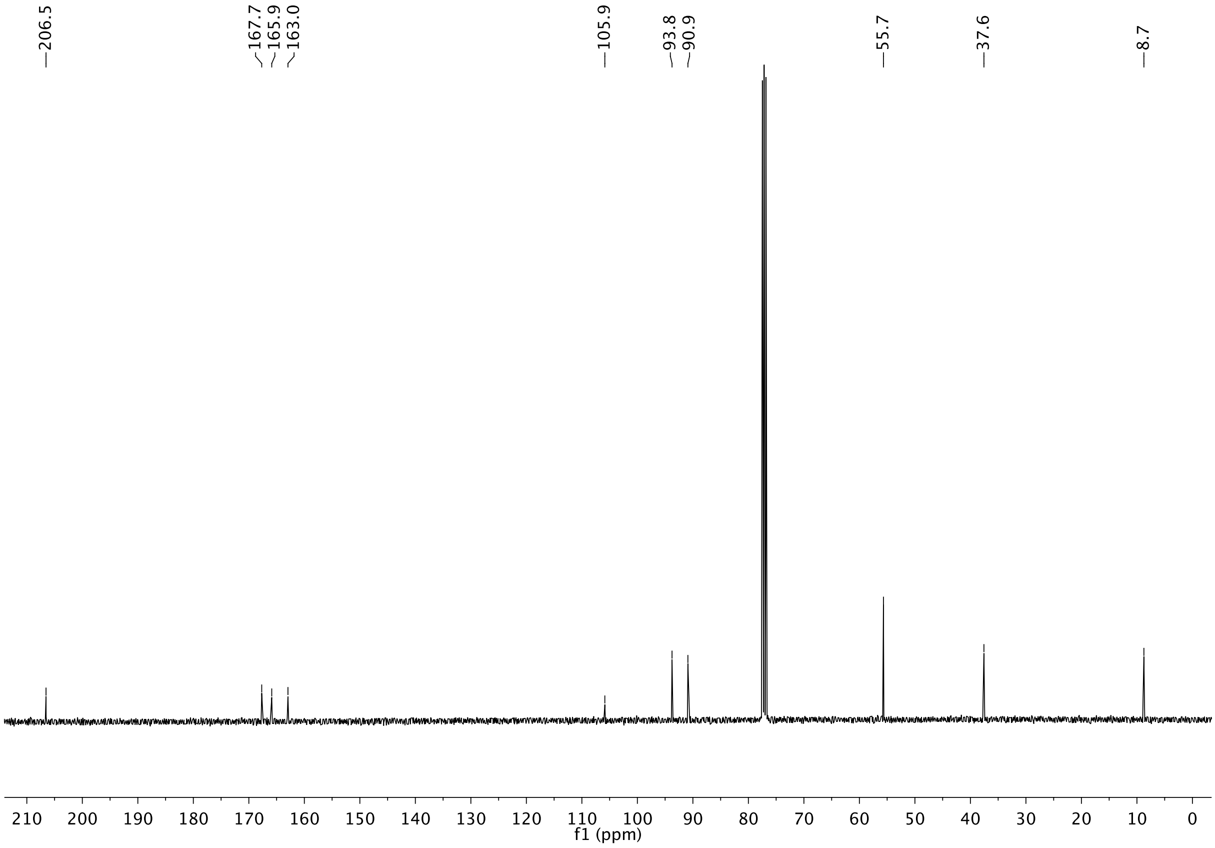
100.3
98.3
55.6
27.9
9.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


14.10
6.07
6.07
5.93
5.92
3.85
3.82
3.05
3.03
3.01
3.00
1.17
1.15
1.14
MeO
OH
OMe
O
0.93
0.99
1.00
3.03
3.08
2.07
3.08
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

130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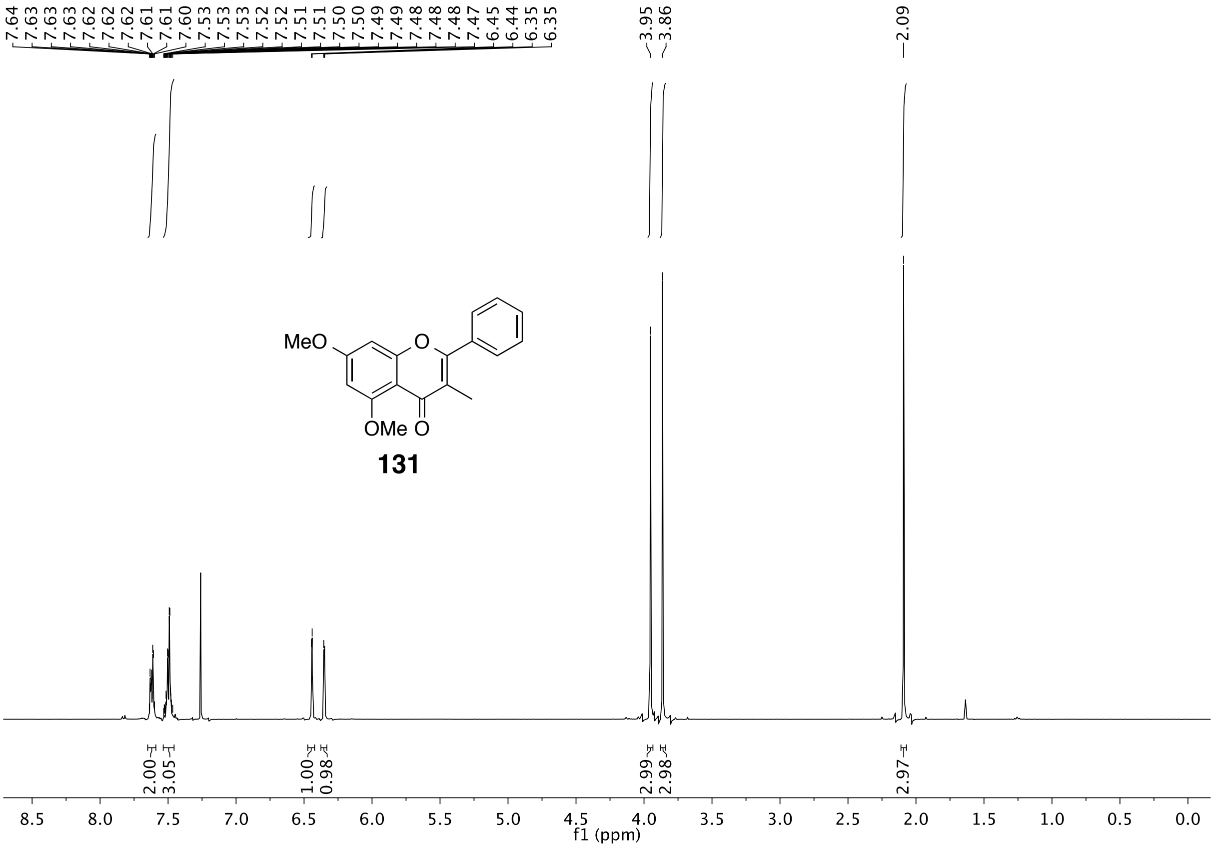
163.0
105.9
93.8
90.9
55.7
37.6
8.7
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.64
7.63
7.63
7.62
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7.61
7.61
7.60
7.53
7.53
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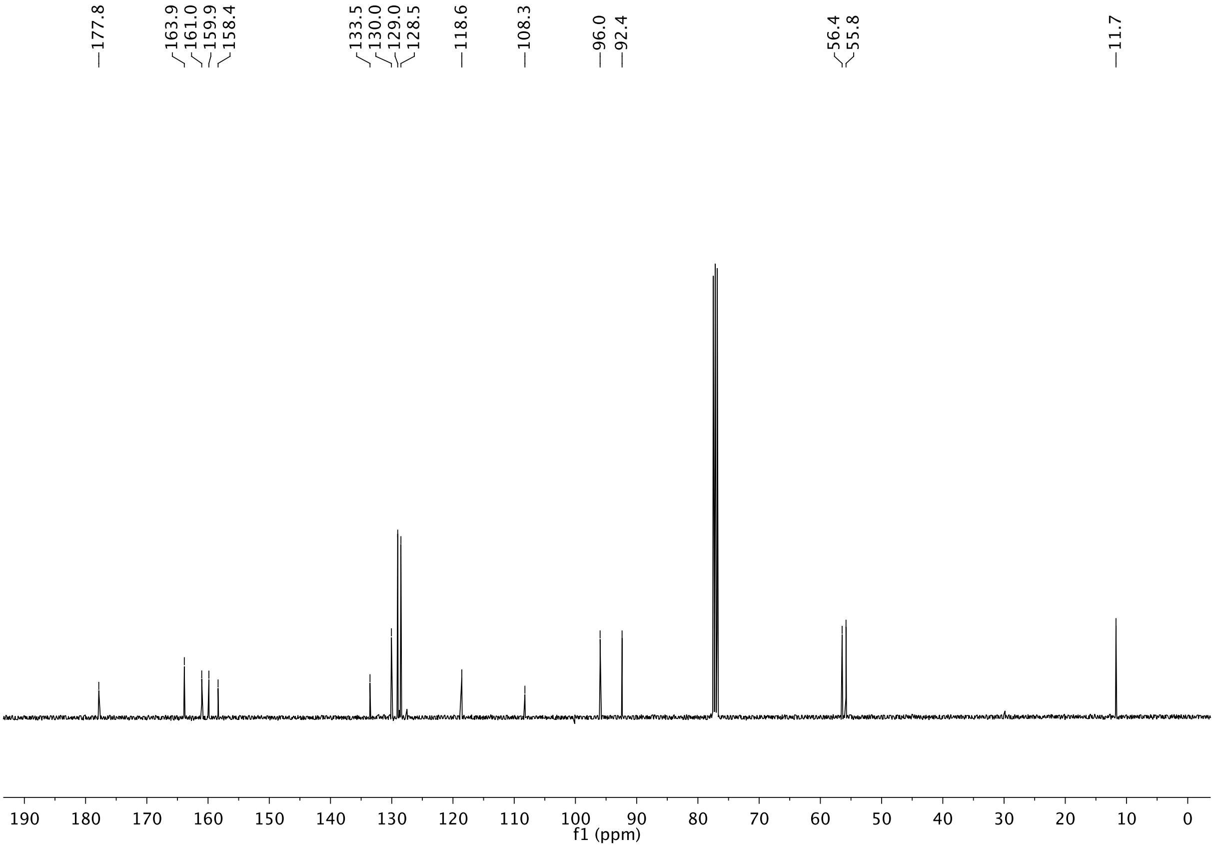
7.51
7.51
7.50
7.50
7.49
7.49
7.48
7.48
7.48
7.47
6.45
6.44
6.35
6.35
3.95
3.86
2.09
MeO
O
OMe
O
131
2.00
3.05
1.00
0.98
2.99
2.98
2.97
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


177.8
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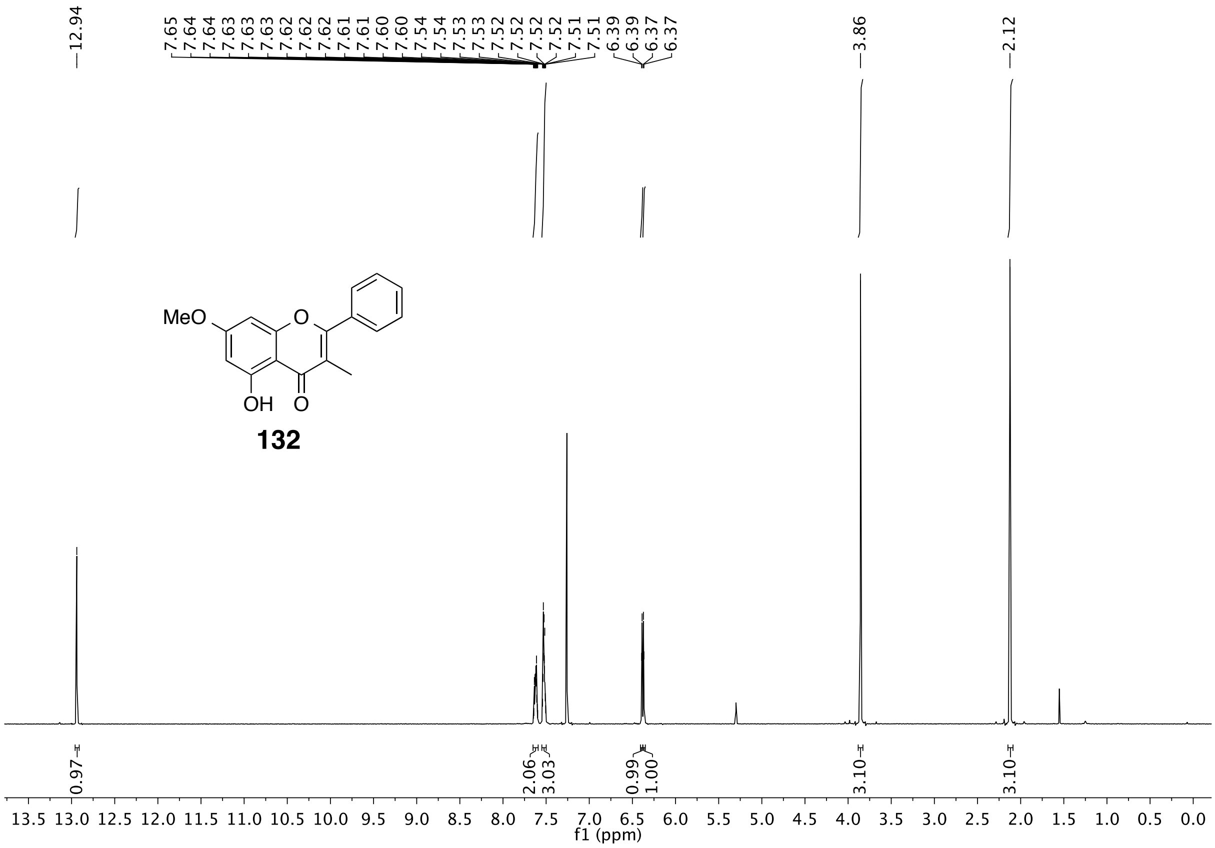
159.9
158.4
133.5
130.0
129.0
128.5
118.6
108.3
96.0
92.4
56.4
55.8
11.7
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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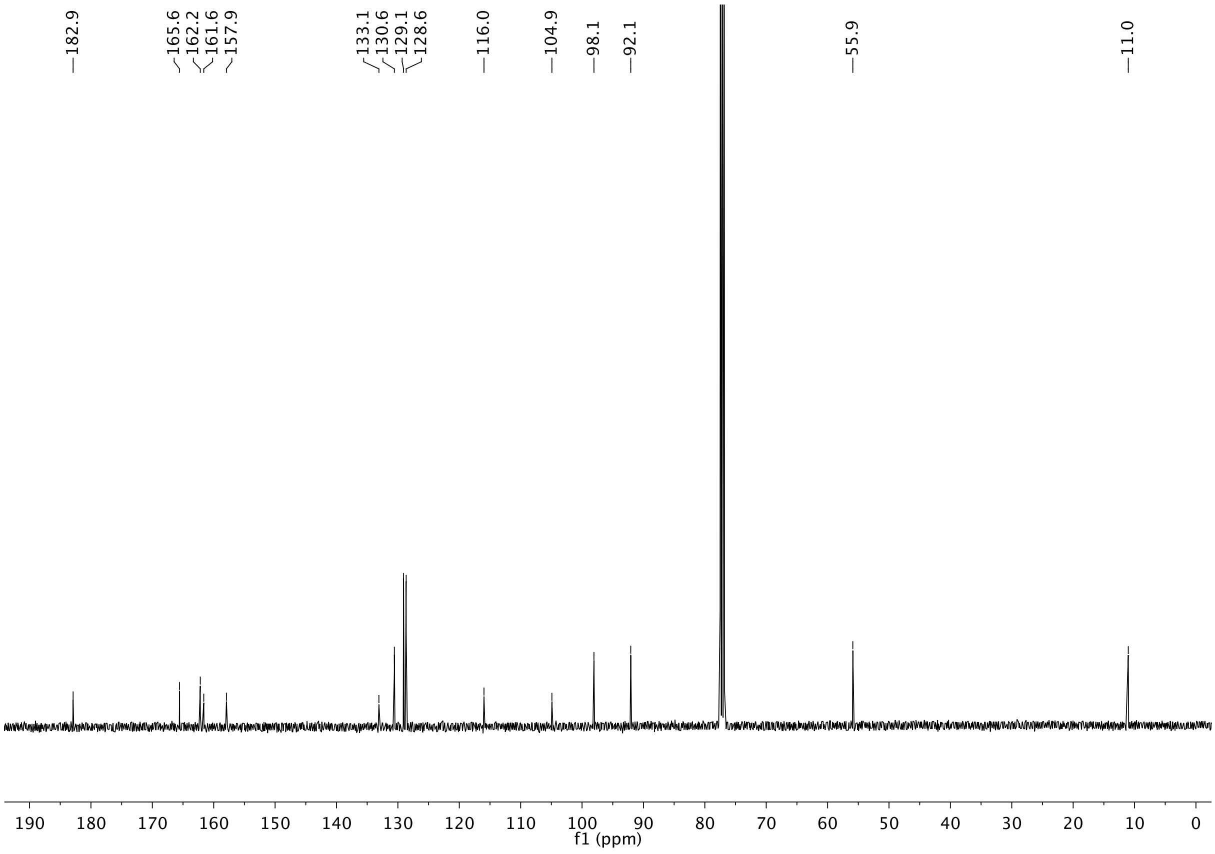
7.61
7.61
7.60
7.60
7.54
7.54
7.53
7.53
7.52
7.52
7.52
7.52
7.51
7.51
6.39
6.39
6.37
6.37
3.86
2.12
MeO
O
OH
O
0.97
2.06
3.03
0.99
1.00
3.10
3.10
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

132


182.9
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161.6
157.9
133.1
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129.1
128.6
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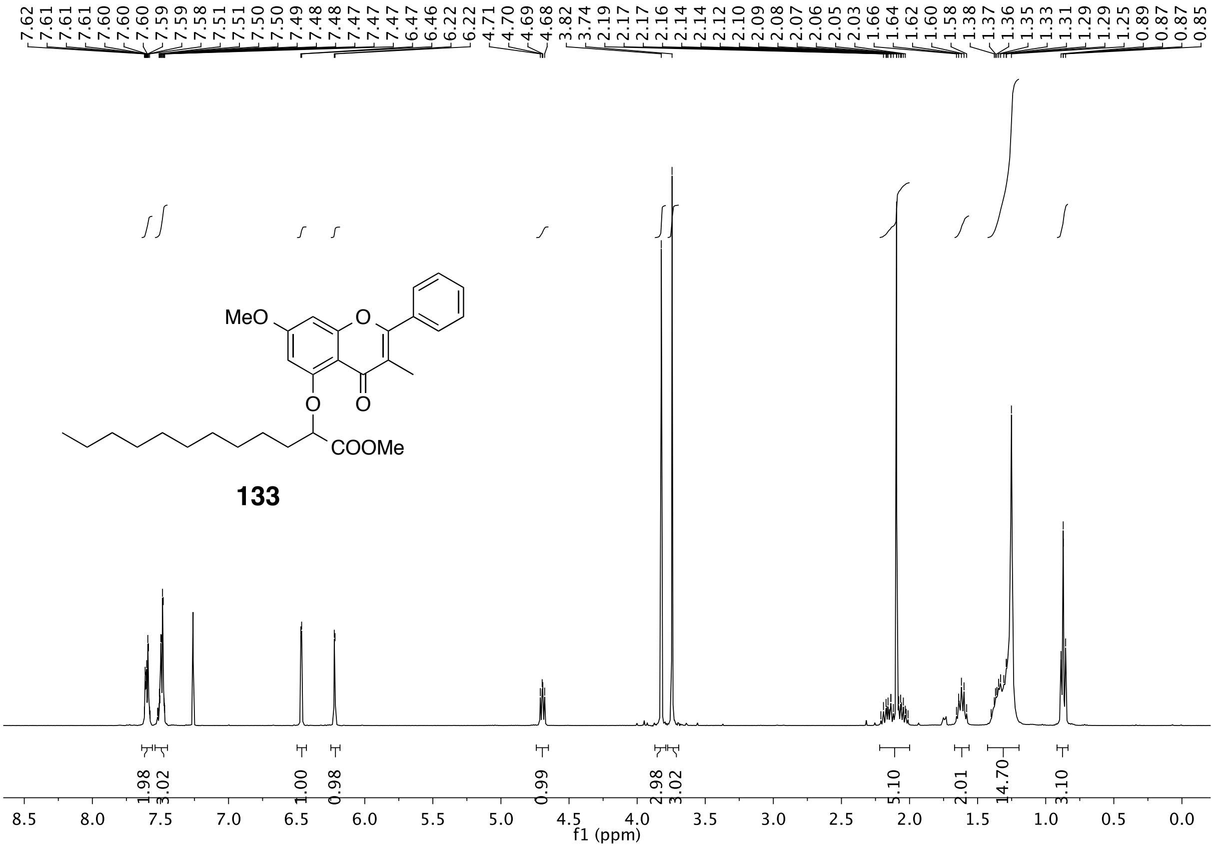
104.9
98.1
92.1
55.9
11.0
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


7.62
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7.59
7.58
7.51
7.51
7.50
7.50
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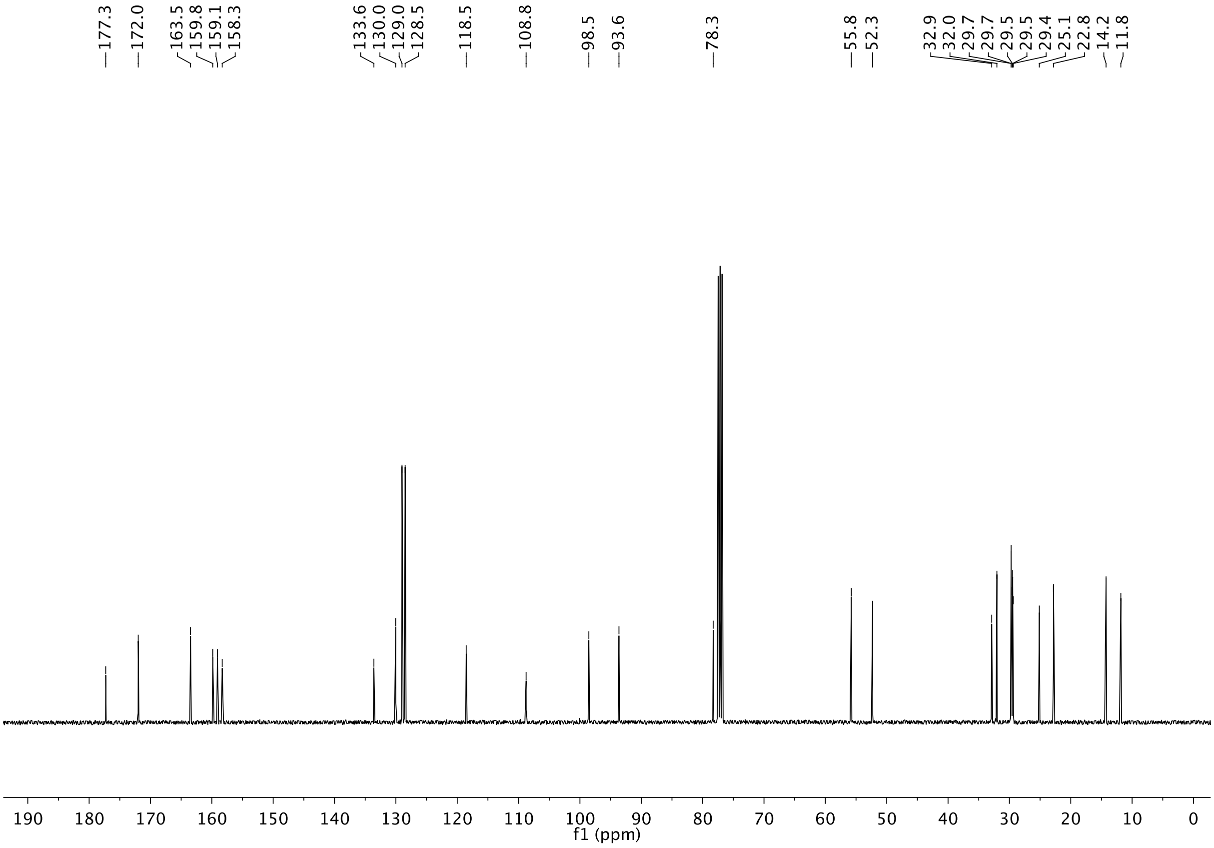
7.48
7.47
7.47
7.47
6.47
6.46
6.22
6.22
4.71
4.70
4.69
4.68
3.82
3.74
2.19
2.17
2.17
2.16
2.14
2.14
2.12
2.10
2.09
2.08
2.07
2.06
2.05
2.03
1.66
1.64
1.62
1.60
1.58
1.38
1.37
1.36
1.35
1.33
1.31
1.29
1.29
1.25
0.89
0.87
0.87
0.85
MeO
O
O
O
COOMe
1.98
3.02
1.00
0.98
0.99
2.98
3.02
5.10
2.01
14.70
3.10
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

133


177.3
172.0
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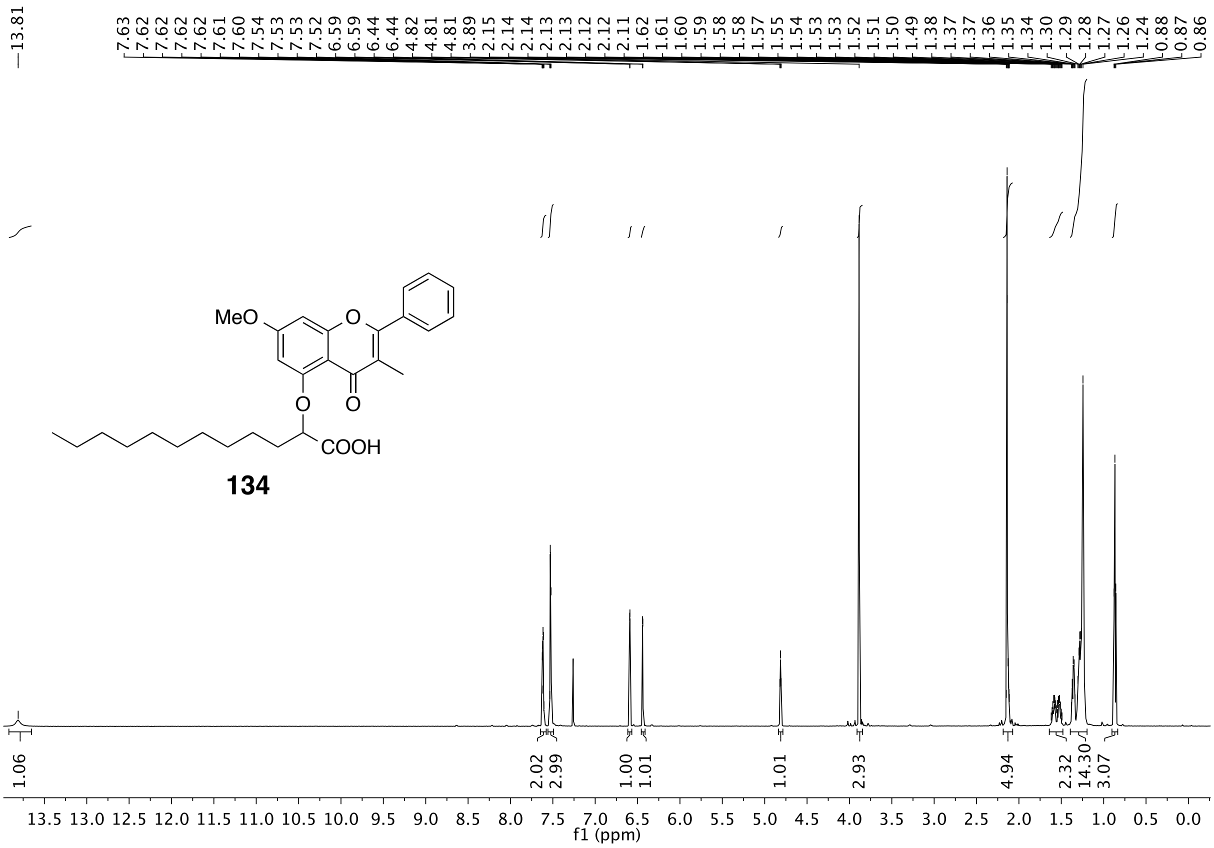
159.8
159.1
158.3
133.6
130.0
129.0
128.5
118.5
108.8
98.5
93.6
78.3
55.8
52.3
32.9
32.0
29.7
29.7
29.5
29.5
29.4
25.1
22.8
14.2
11.8
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


13.81
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7.62
7.62
7.62
7.62
7.61
7.60
7.54
7.53
7.53
7.52
6.59
6.59
6.44
6.44
4.82
4.81
3.89
2.15
2.14
2.14
2.13
2.13
2.12
2.12
2.11
1.62
1.61
1.60
1.59
1.58
1.58
1.57
1.55
1.54
1.53
1.53
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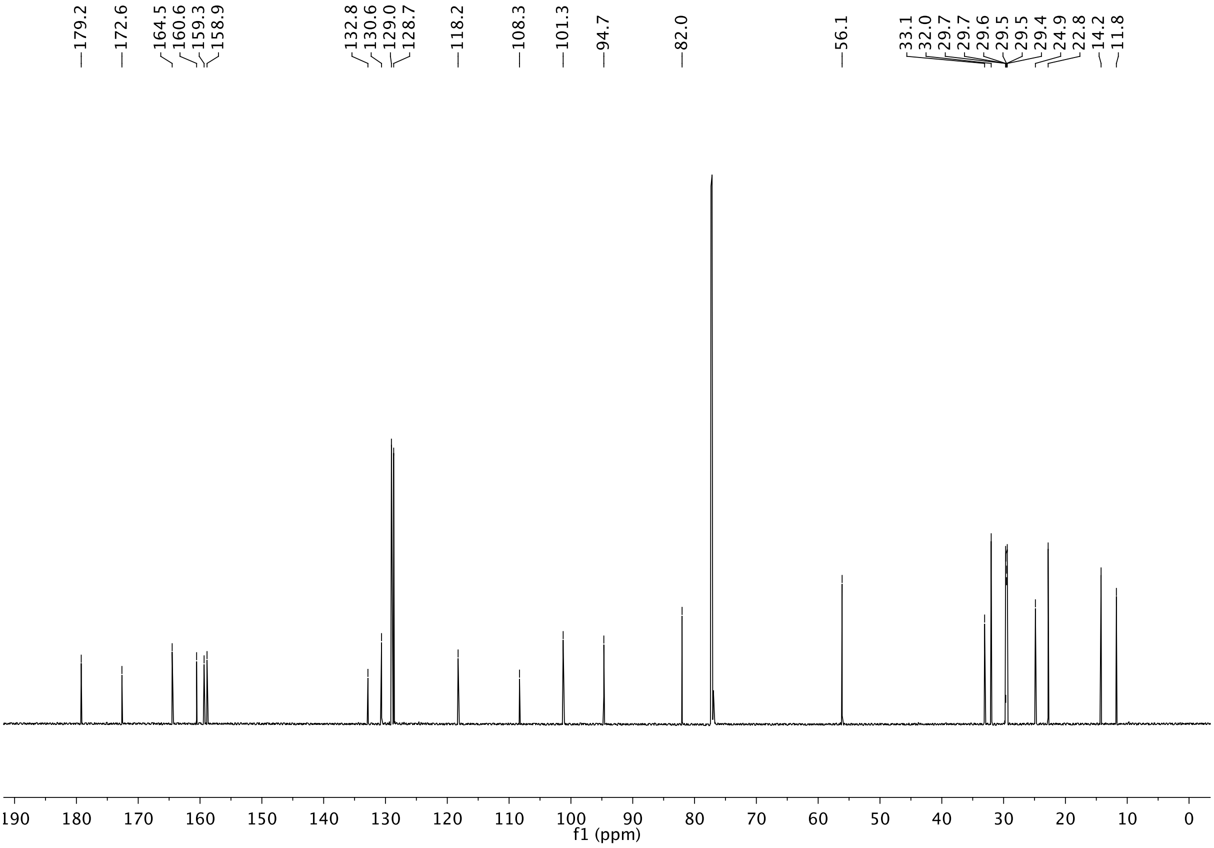
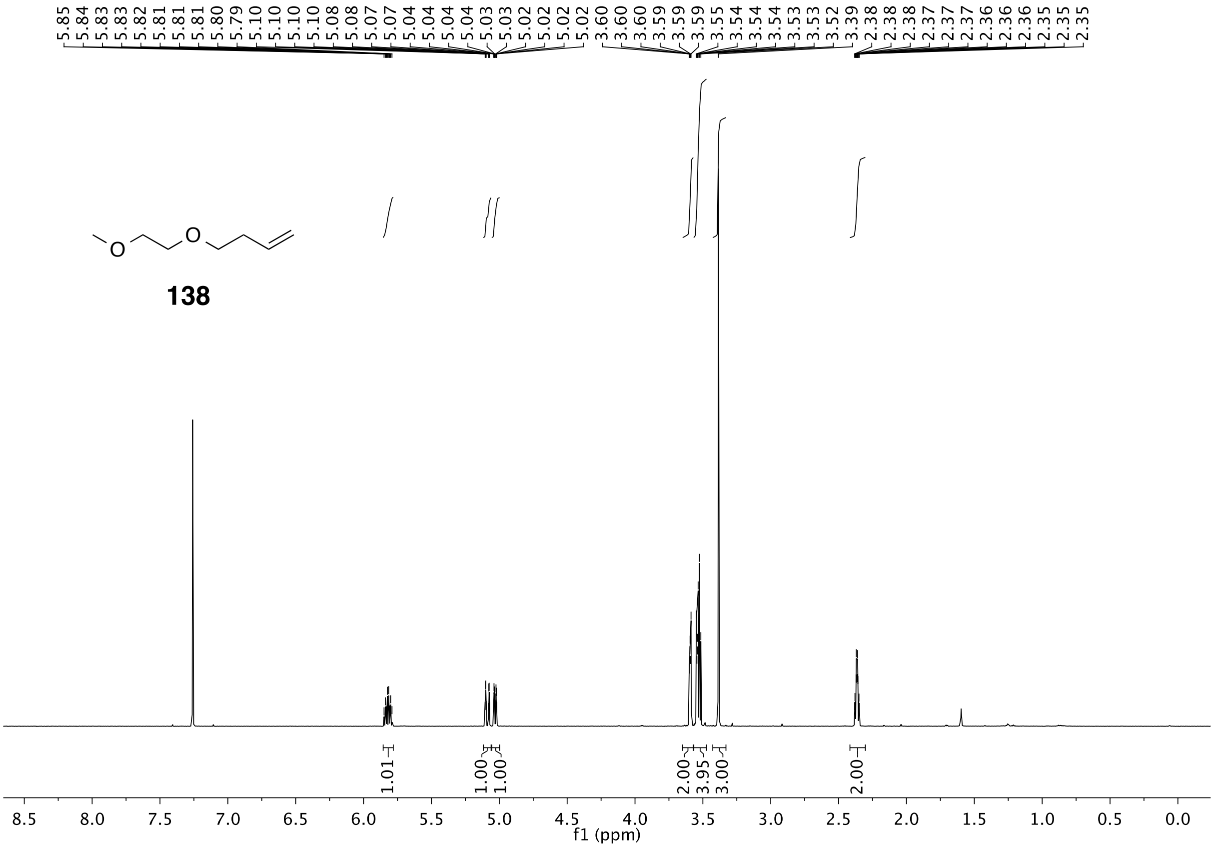
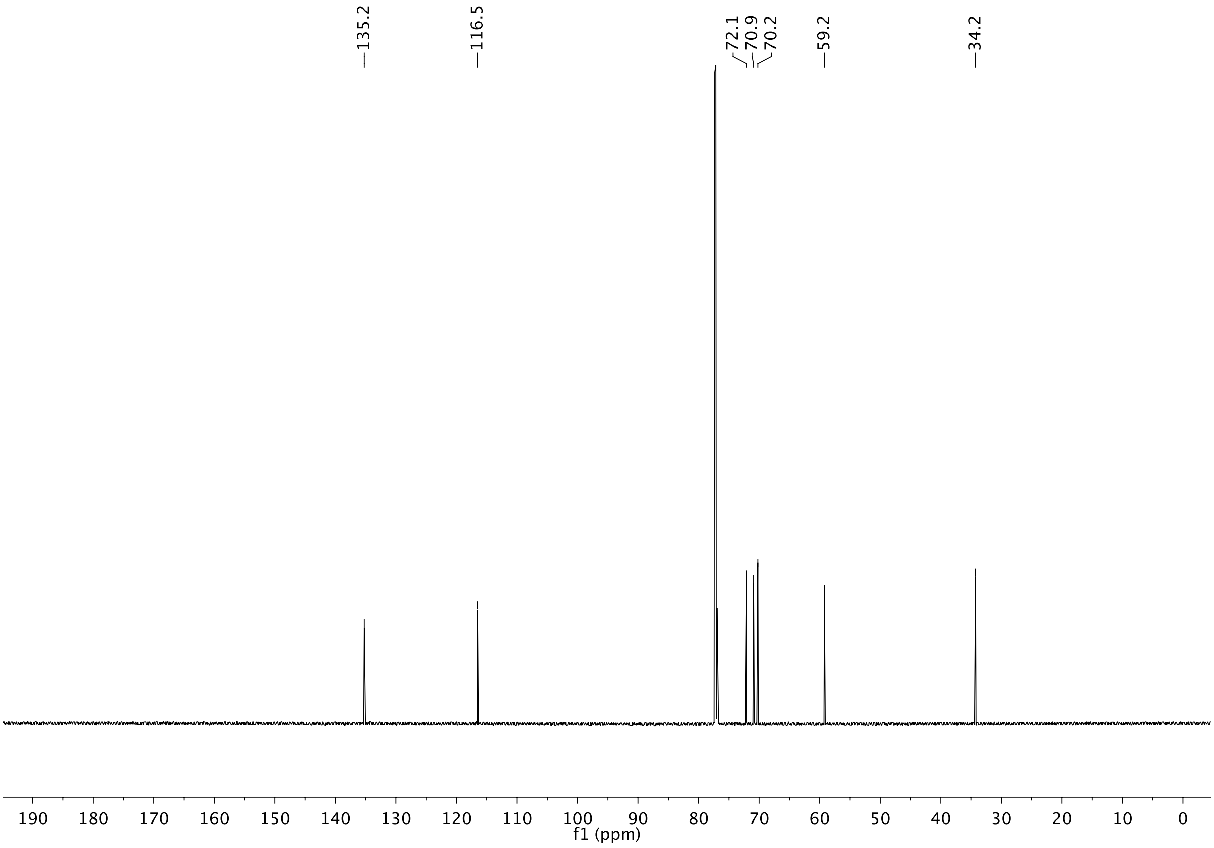
1.51
1.50
1.49
1.38
1.37
1.37
1.36
1.35
1.34
1.30
1.29
1.28
1.27
1.26
1.24
0.88
0.87
0.86
MeO
O
O
O
COOH
1.06
2.02
2.99
1.00
1.01
1.01
2.93
4.94
2.32
14.30
3.07
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

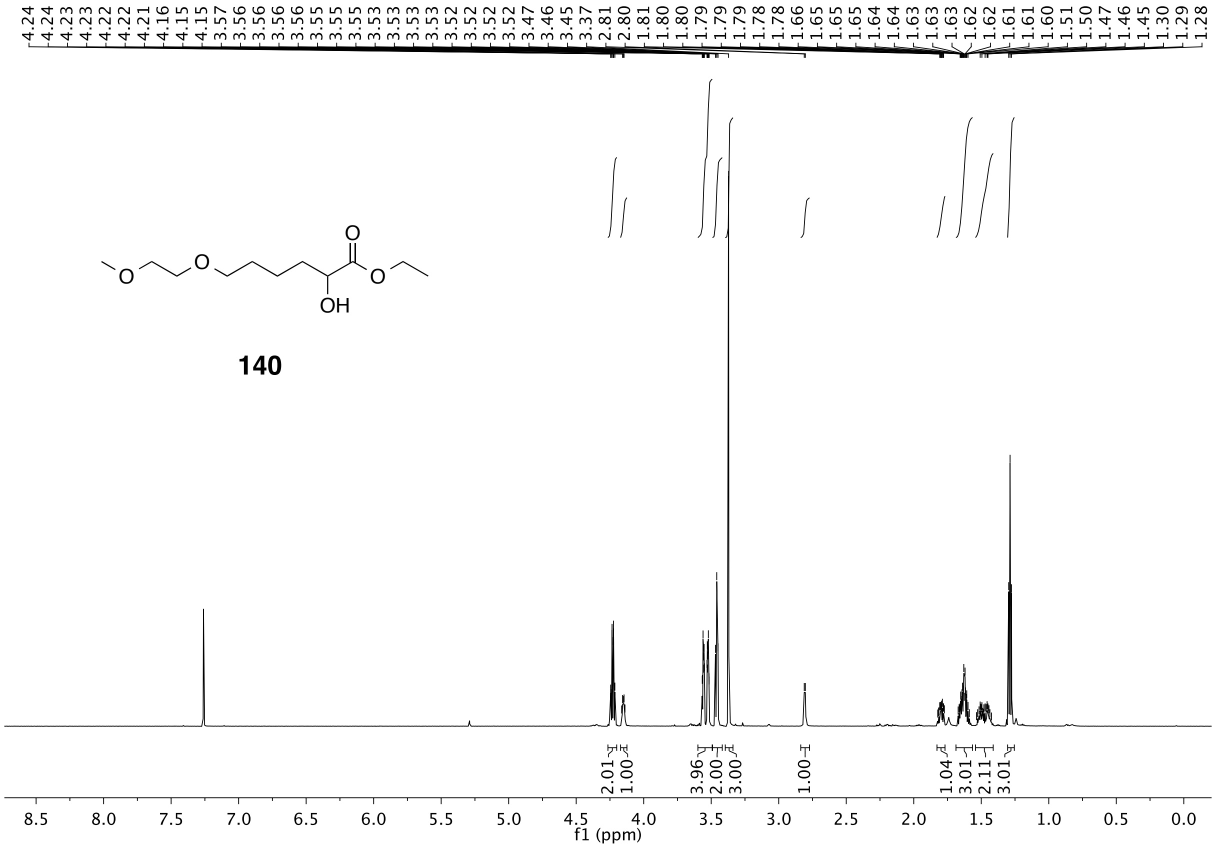
134


179.2
172.6
164.5
160.6
159.3
158.9
132.8
130.6
129.0
128.7
118.2
108.3
101.3
94.7
82.0
56.1
33.1
32.0
29.7
29.7
29.6
29.5
29.5
29.4
24.9
22.8
14.2
11.8
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

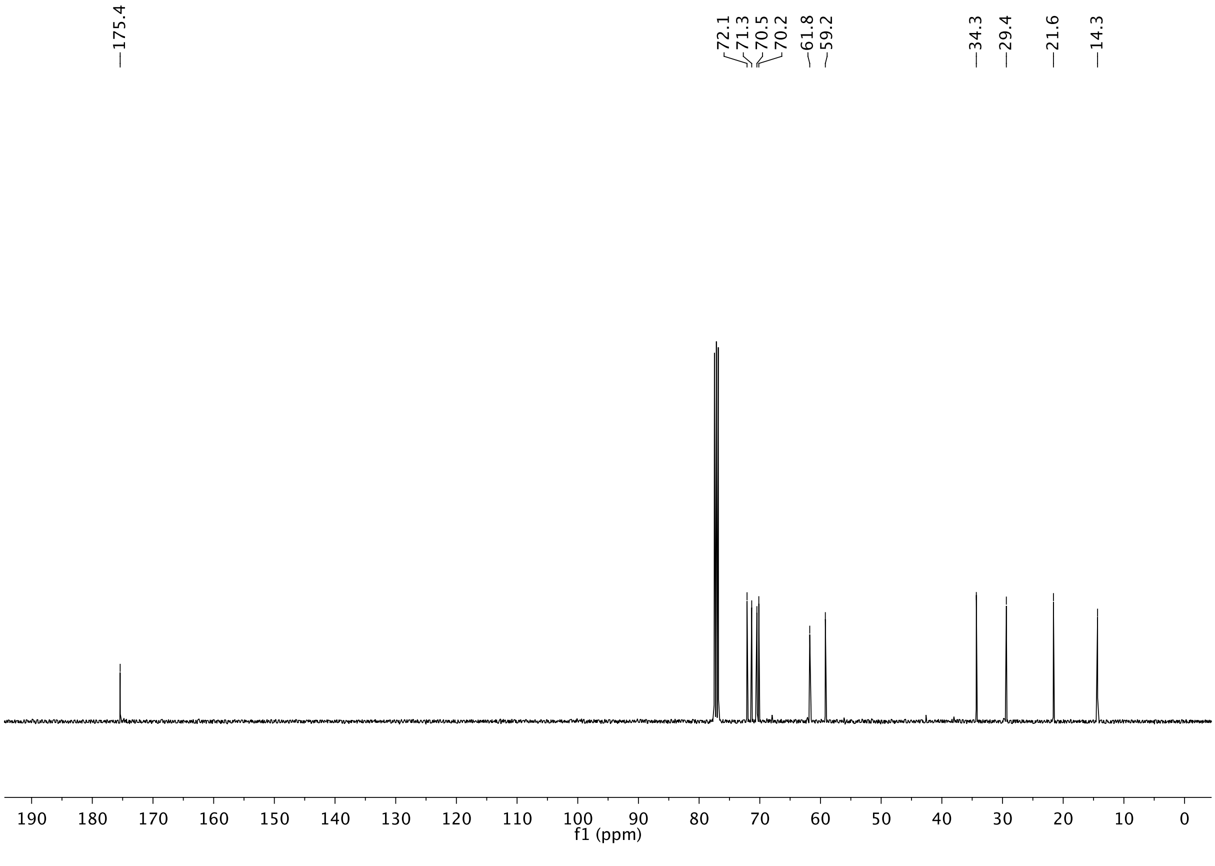

5.85
5.84
5.83
5.83
5.82
5.81
5.81
5.81
5.80
5.79
5.10
5.10
5.10
5.10
5.08
5.08
5.07
5.07
5.04
5.04
5.04
5.04
5.03
5.03
5.02
5.02
5.02
5.02
3.60
3.60
3.59
3.59
3.59
3.55
3.54
3.54
3.53
3.53
3.52
3.39
2.38
2.38
2.38
2.37
2.37
2.37
2.36
2.36
2.35
2.35
2.35
O
O
1.01
1.00
1.00
2.00
3.95
3.00
2.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

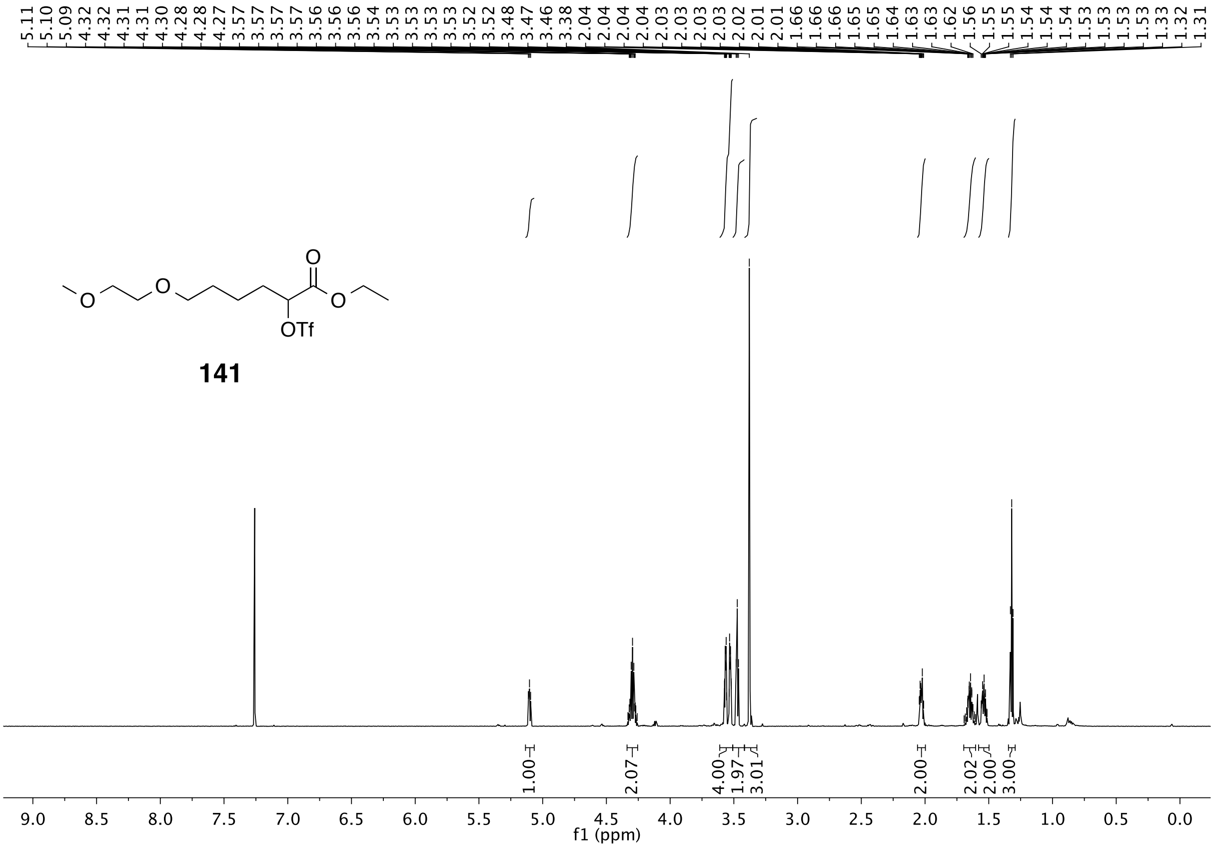
138

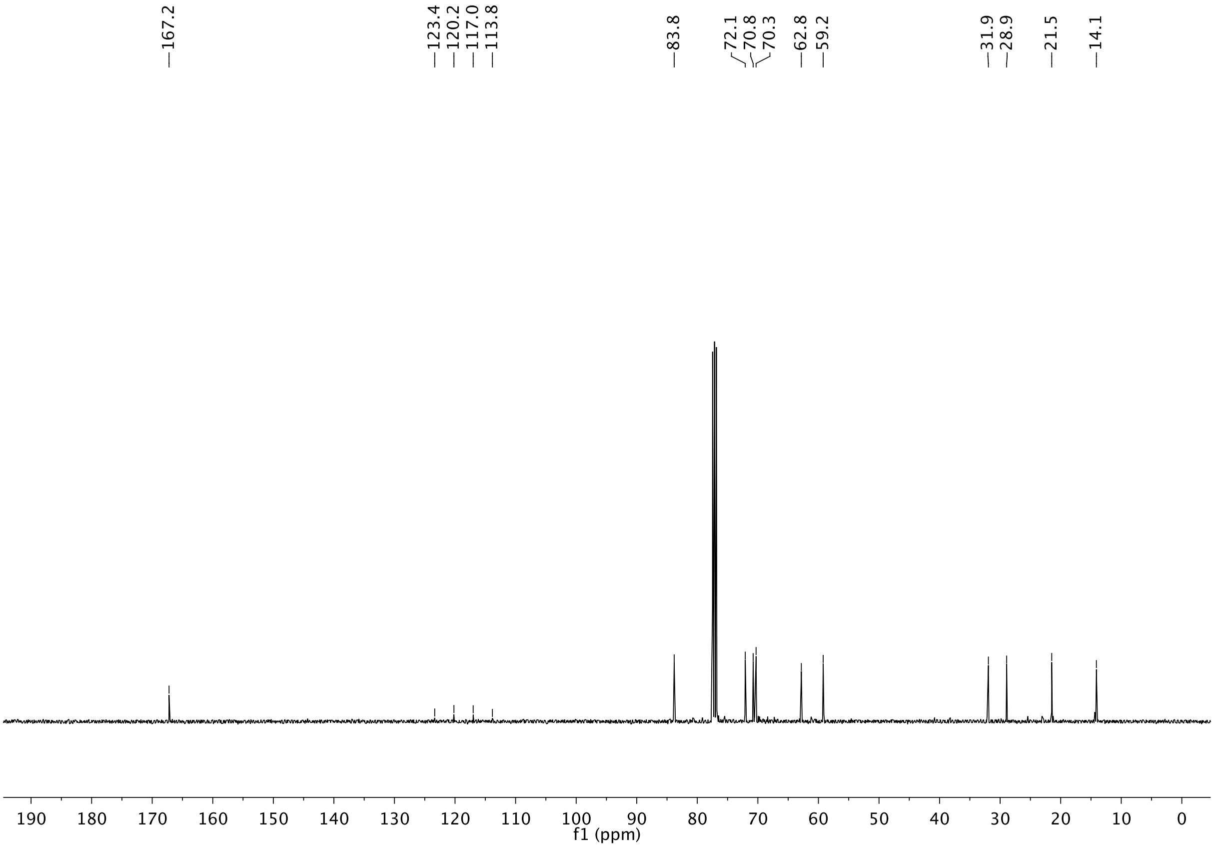

135.2
116.5
72.1
70.9
70.2
59.2
34.2
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

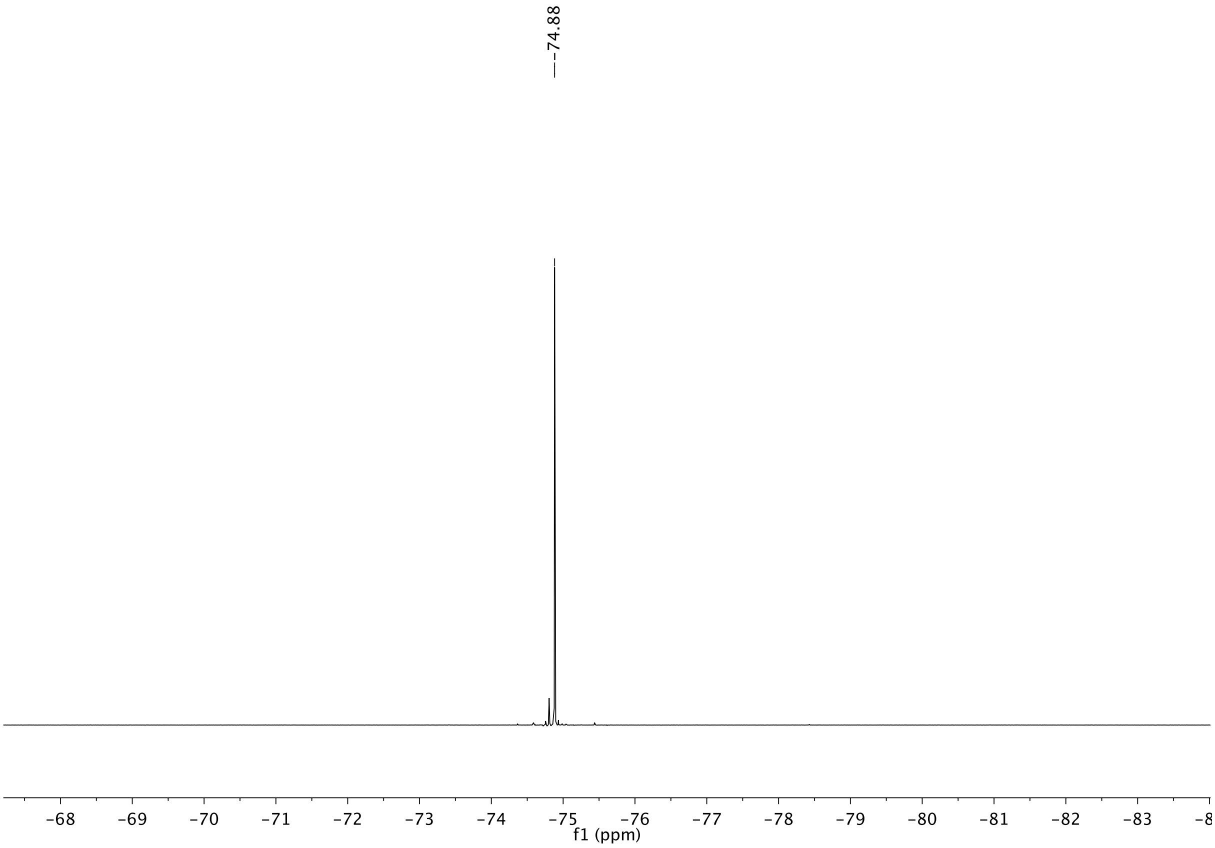

4.24
4.24
4.23
4.23
4.22
4.22
4.21
4.16
4.15
4.15
3.57
3.56
3.56
3.56
3.55
3.55
3.55
3.53
3.53
3.53
3.53
3.52
3.52
3.52
3.52
3.47
3.46
3.45
3.37
2.81
2.80
1.81
1.80
1.80
1.79
1.79
1.79
1.78
1.66
1.65
1.65
1.65
1.64
1.64
1.63
1.63
1.63
1.62
1.62
1.61
1.61
1.60
1.51
1.50
1.47
1.46
1.45
1.30
1.29
1.28
O
O
O
O
OH
2.01
1.00
3.96
2.00
3.00
1.00
1.04
3.01
2.11
3.01
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

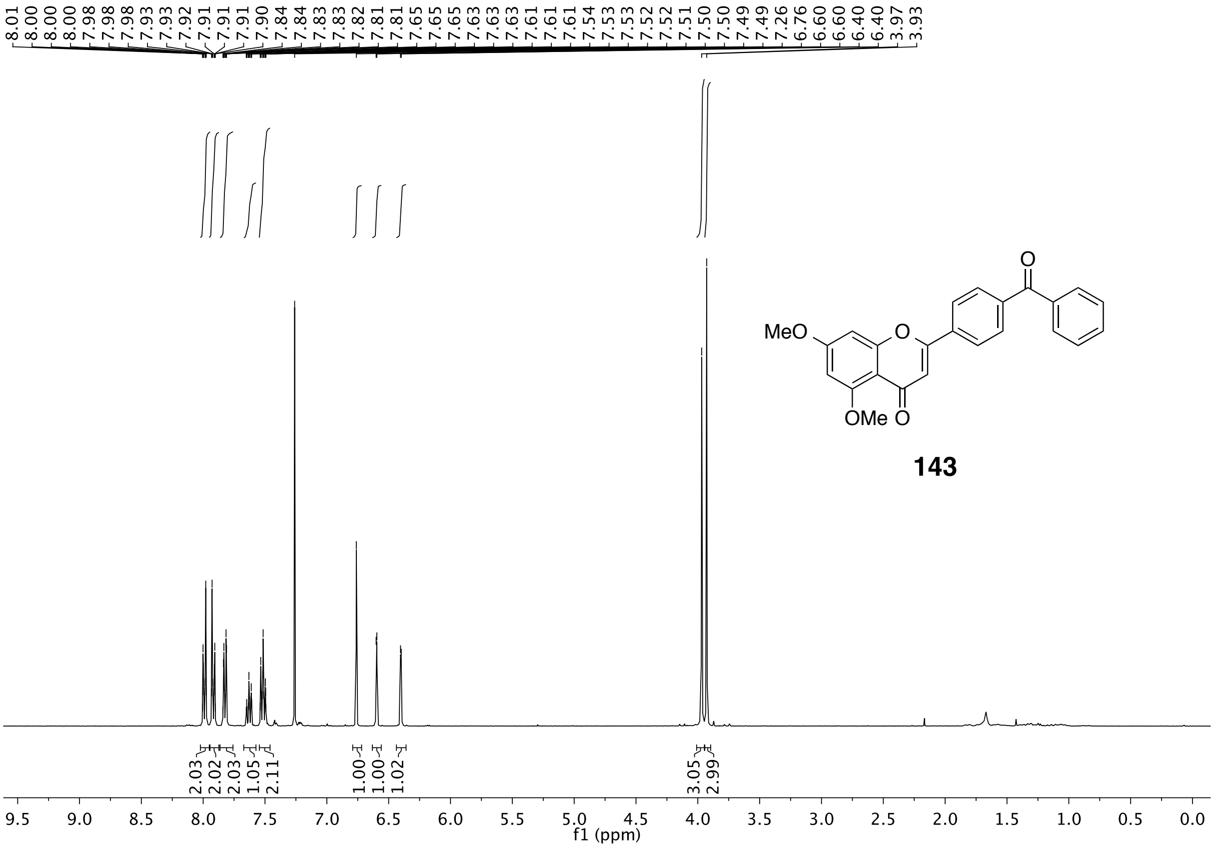
140


175.4
72.1
71.3
70.5
70.2
61.8
59.2
34.3
29.4
21.6
14.3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

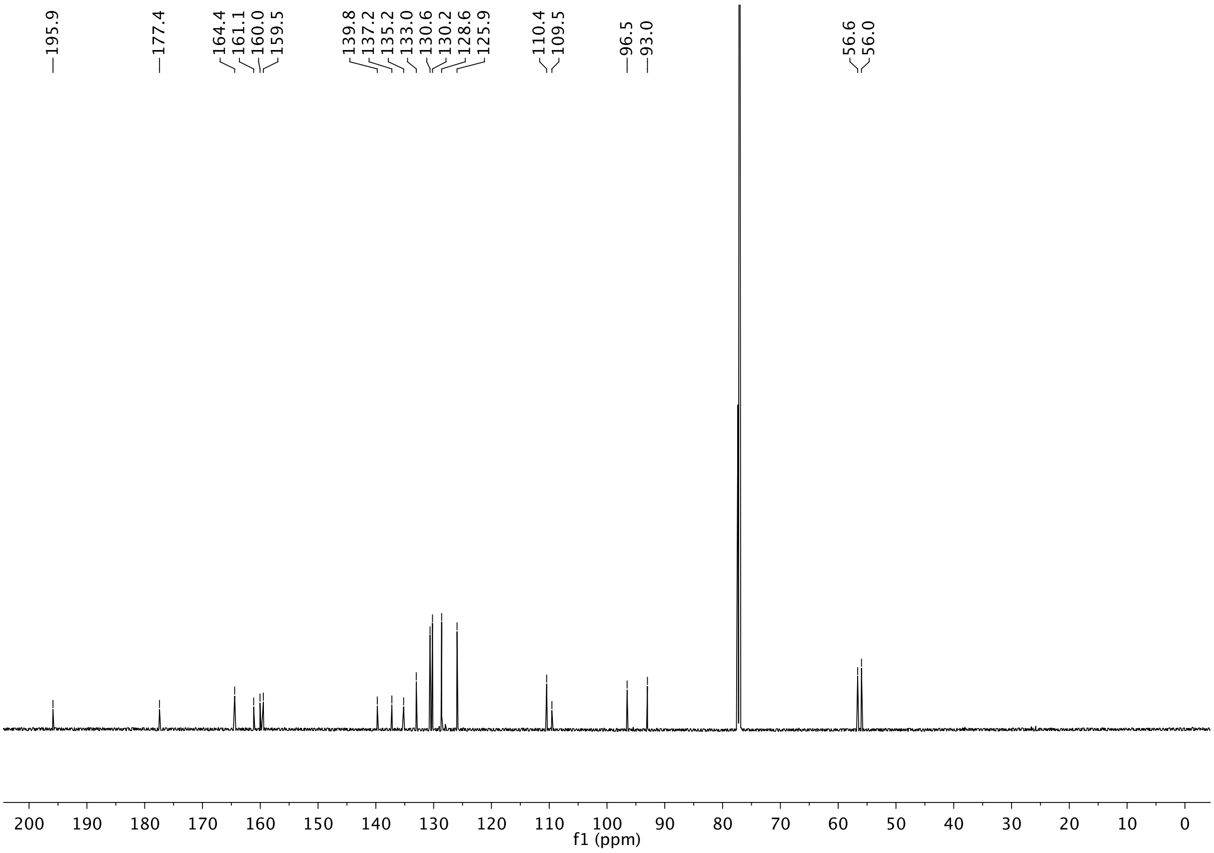

5.11
5.10
5.09
4.32
4.32
4.31
4.31
4.30
4.28
4.28
4.27
3.57
3.57
3.57
3.57
3.56
3.56
3.54
3.53
3.53
3.53
3.53
3.52
3.52
3.48
3.47
3.46
3.38
2.04
2.04
2.04
2.03
2.03
2.03
2.03
2.02
2.01
2.01
1.66
1.66
1.66
1.65
1.65
1.64
1.63
1.63
1.62
1.56
1.55
1.55
1.54
1.54
1.54
1.53
1.53
1.53
1.53
1.33
1.33
1.32
1.31
O
O
O
O
OTf
141
1.00
2.07
4.00
1.97
3.01
2.00
2.02
2.00
3.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

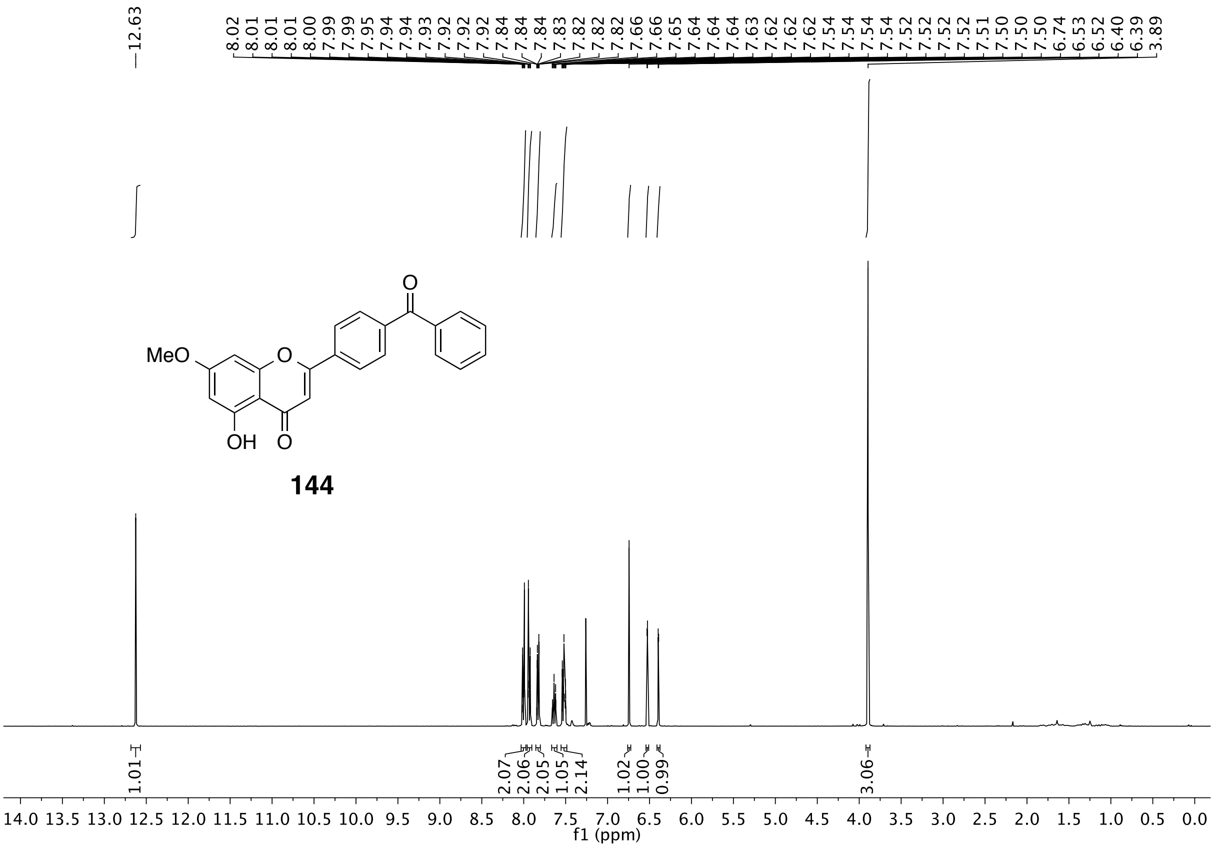

167.2
123.4
120.2
117.0
113.8
83.8
72.1
70.8
70.3
62.8
59.2
31.9
28.9
21.5
14.1
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


-74.88
-68
-69
-70
-71
-72
-73
-74
-75
-76
-77
-78
-79
-80
-81
-82
-83
f1 (ppm)

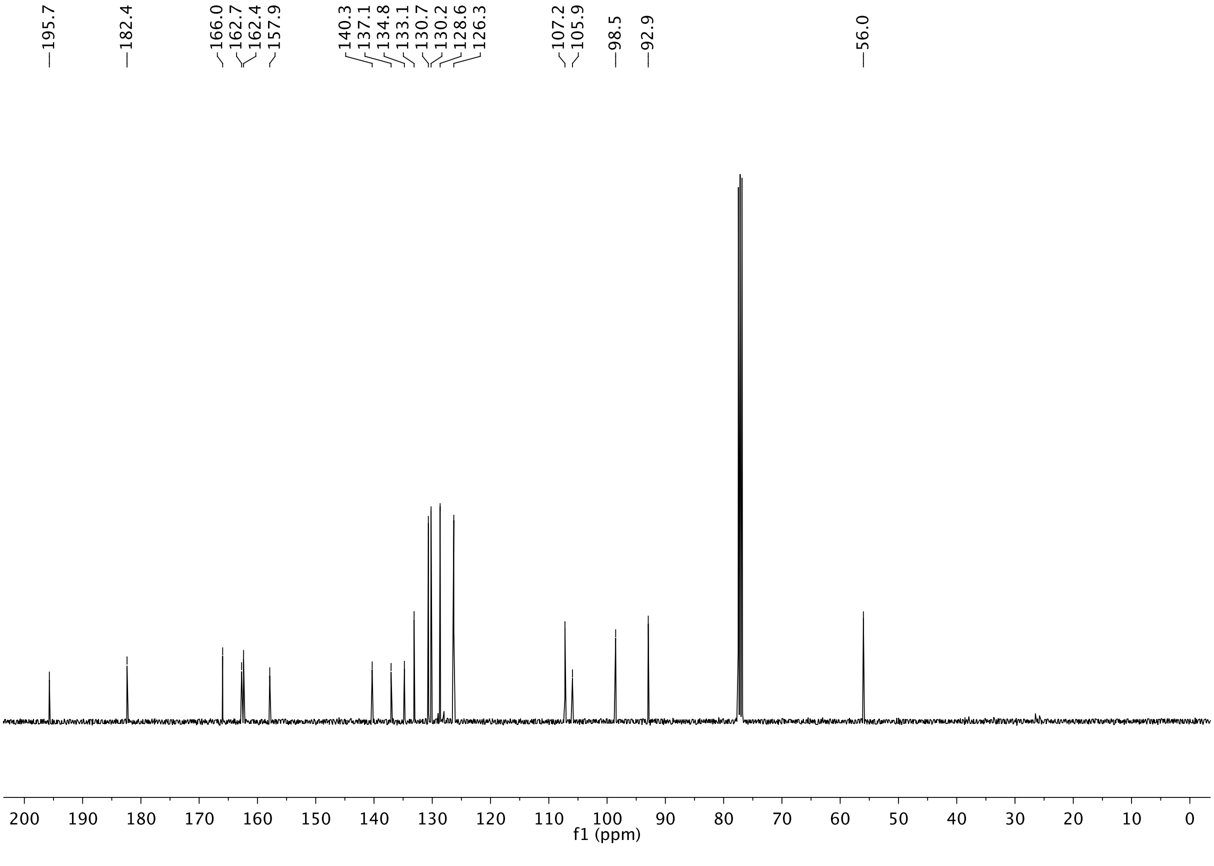

8.01
8.00
8.00
7.98
7.98
7.93
7.93
7.92
7.91
7.91
7.90
7.84
7.83
7.83
7.82
7.81
7.65
7.65
7.63
7.63
7.61
7.61
7.61
7.54
7.53
7.53
7.52
7.52
7.51
7.50
7.50
7.49
7.49
7.26
6.76
6.60
6.60
6.40
6.40
3.97
3.93
MeO
O
O
OMe
O
2.03
2.02
2.03
1.05
2.11
1.00
1.00
1.02
3.05
2.99
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

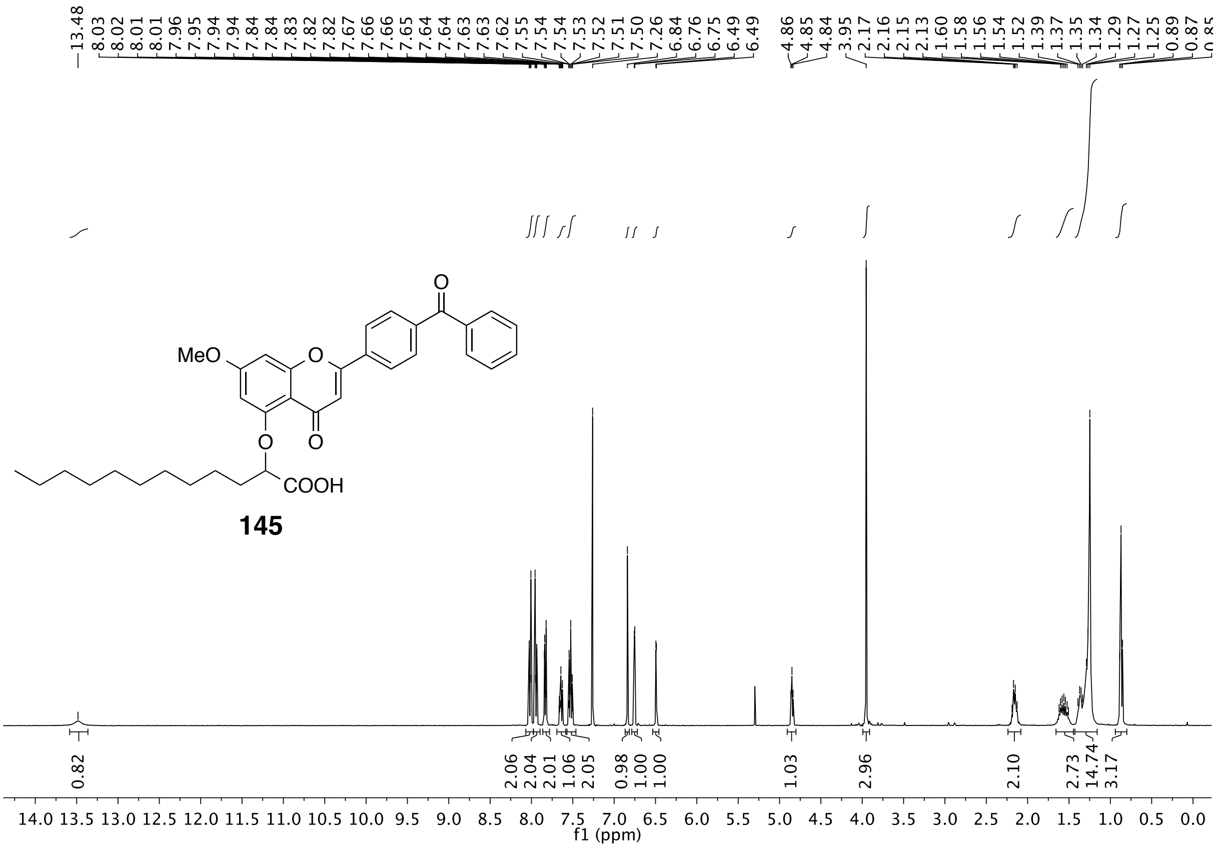
143


195.9
177.4
164.4
161.1
160.0
159.5
139.8
137.2
135.2
133.0
130.6
130.2
128.6
125.9
110.4
109.5
96.5
93.0
56.6
56.0
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

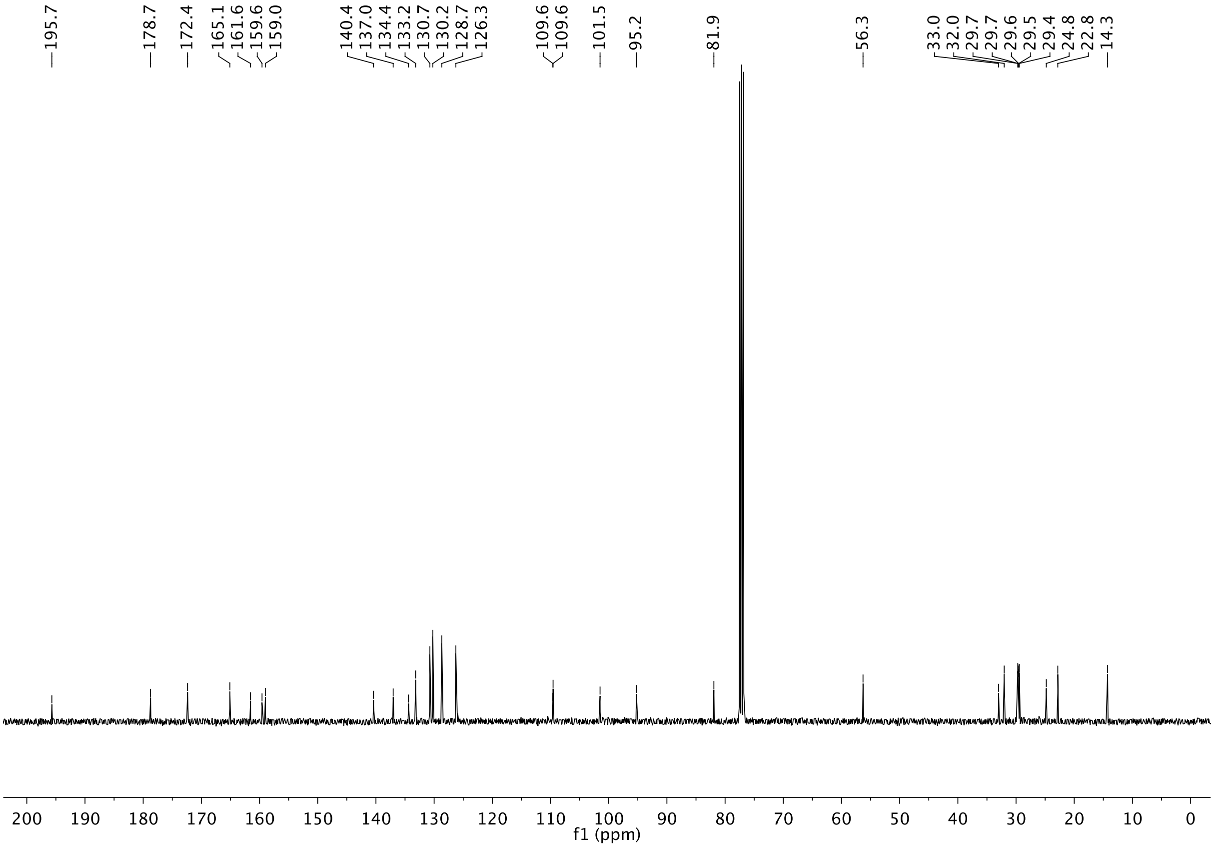

12.63
8.02 8.01 8.01 8.01 8.00 7.99 7.99 7.95 7.94 7.94 7.93 7.92 7.92 7.92 7.92 7.84 7.84 7.83 7.82 7.82 7.82 7.66 7.66 7.65 7.64 7.64 7.64 7.63 7.62 7.62 7.62 7.54 7.54 7.54 7.54 7.52 7.52 7.52 7.52 7.51 7.50 7.50 7.50 6.74 6.53 6.52 6.40 6.39 3.89
MeO
O
O
OH
O
1.01
2.07
2.06
2.05
1.05
2.14
1.02
1.00
0.99
3.06
14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

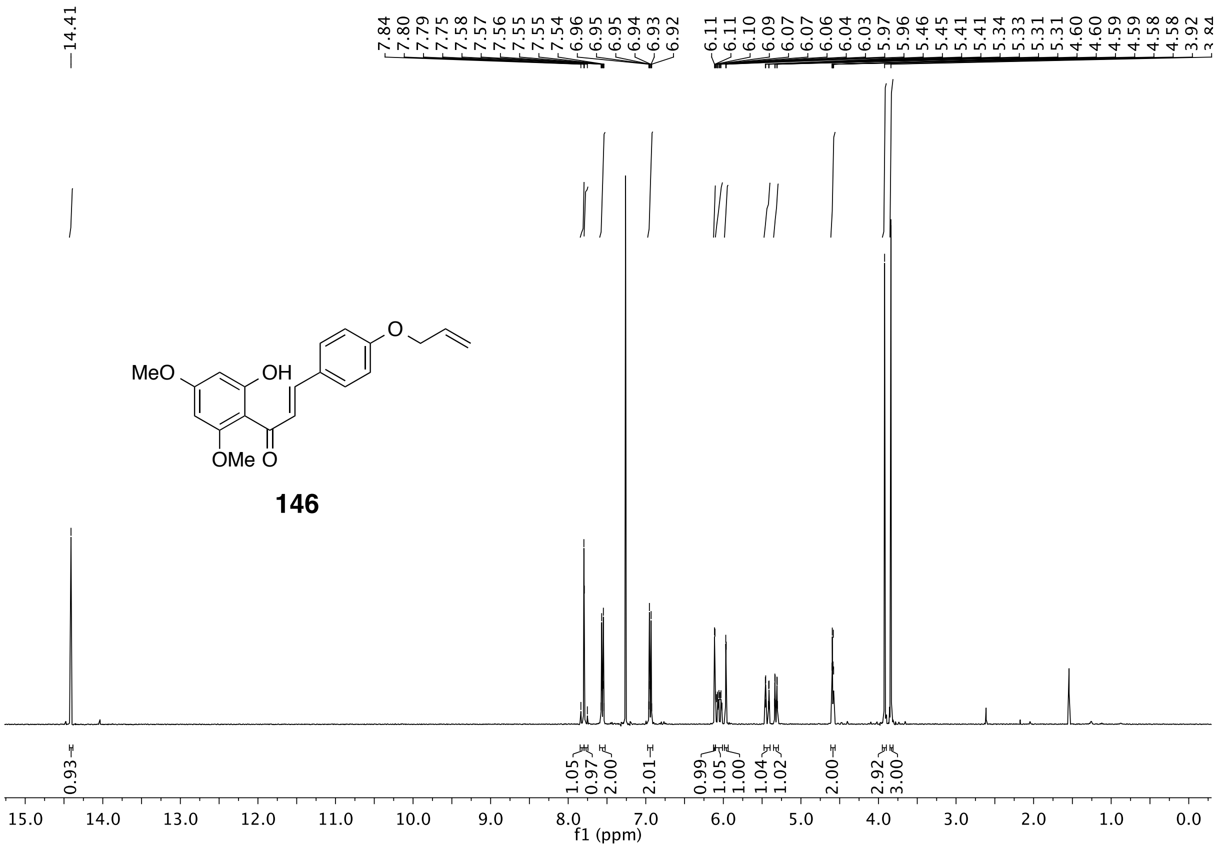
144

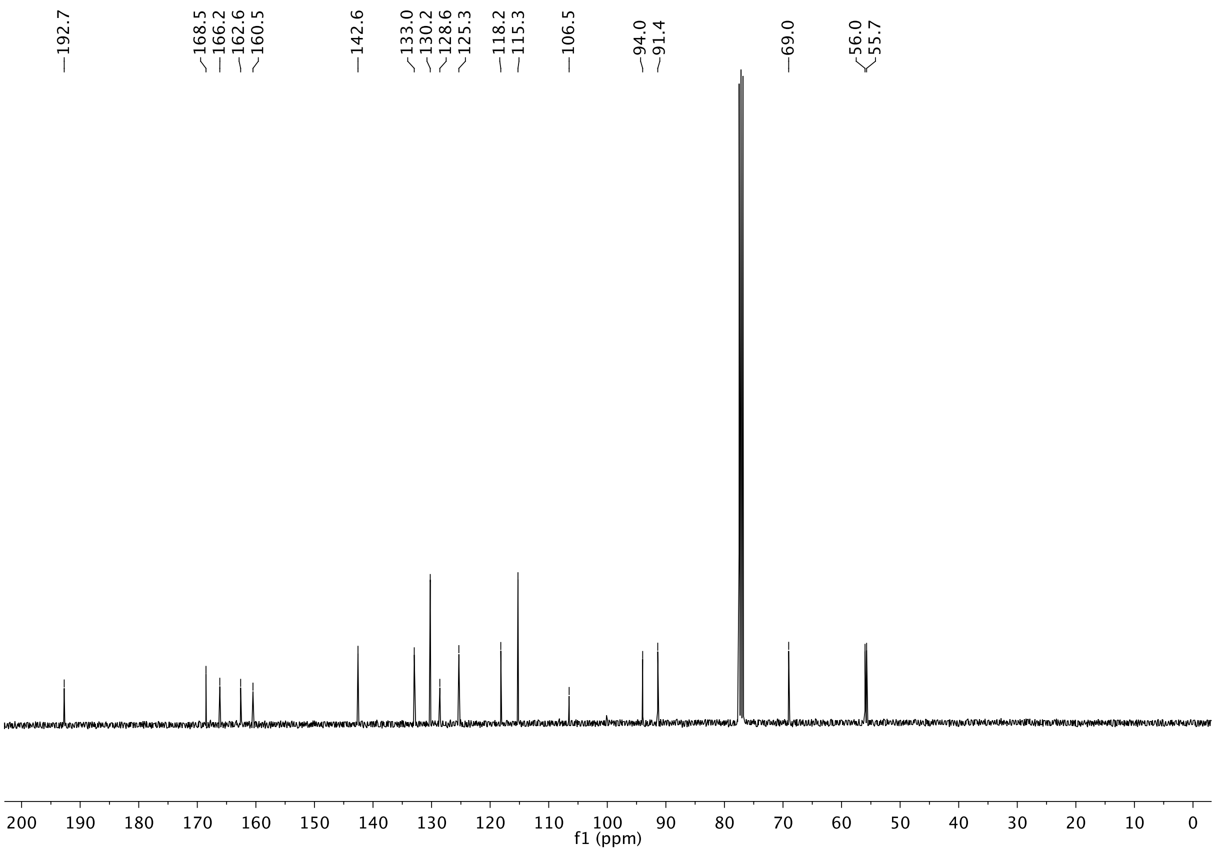

195.7
182.4
166.0 162.7 162.4 157.9
140.3 137.1 134.8 133.1 130.7 130.2 128.6 126.3
107.2 105.9
98.5
92.9
56.0
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

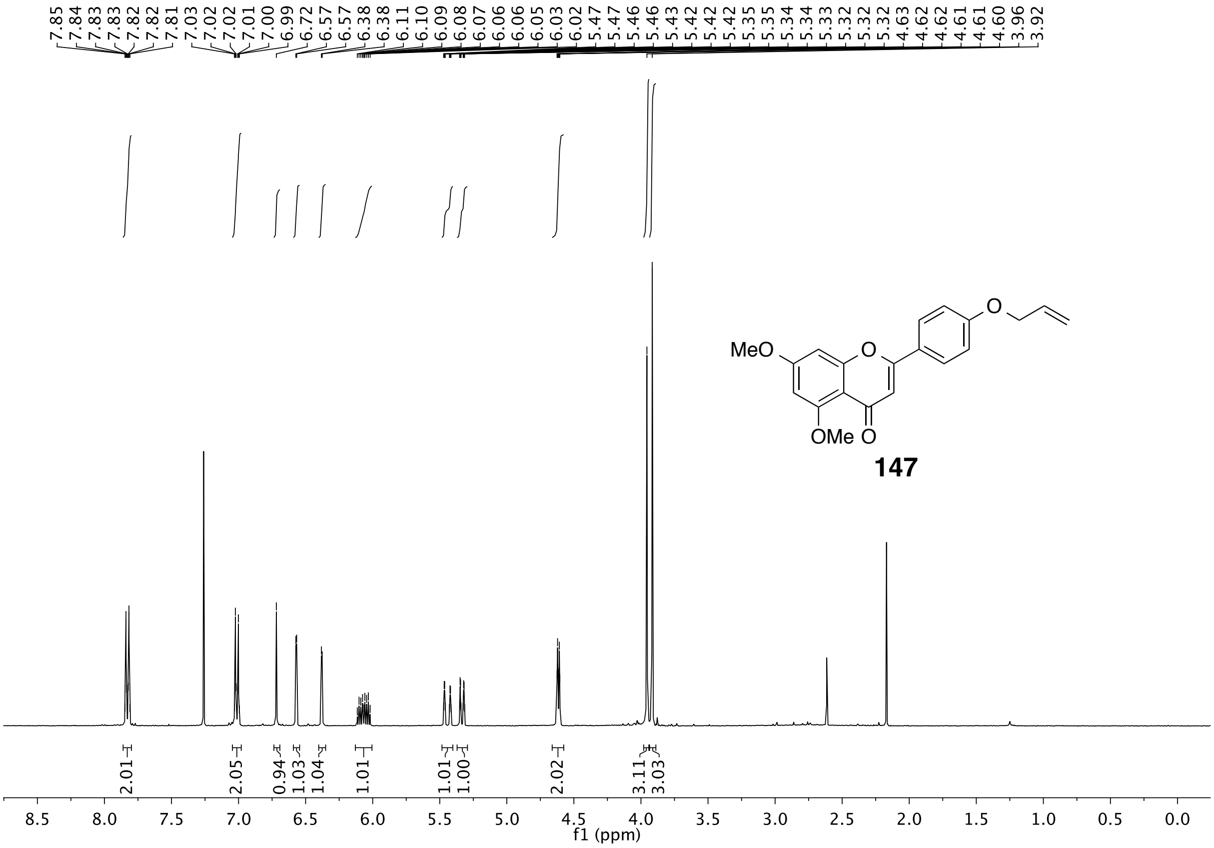

13.48
8.03
8.02
8.01
8.01
7.96
7.95
7.94
7.94
7.84
7.84
7.83
7.82
7.82
7.67
7.66
7.66
7.65
7.64
7.64
7.63
7.63
7.62
7.55
7.54
7.54
7.53
7.52
7.51
7.50
7.26
6.84
6.76
6.75
6.49
6.49
4.86
4.85
4.84
3.95
2.17
2.16
2.15
2.13
1.60
1.58
1.56
1.54
1.52
1.39
1.37
1.35
1.34
1.29
1.27
1.25
0.89
0.87
MeO
O
O
O
O
COOH
0.82
2.06
2.04
2.01
1.06
2.05
0.98
1.00
1.00
1.03
2.96
2.10
2.73
14.74
3.17
14.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

145

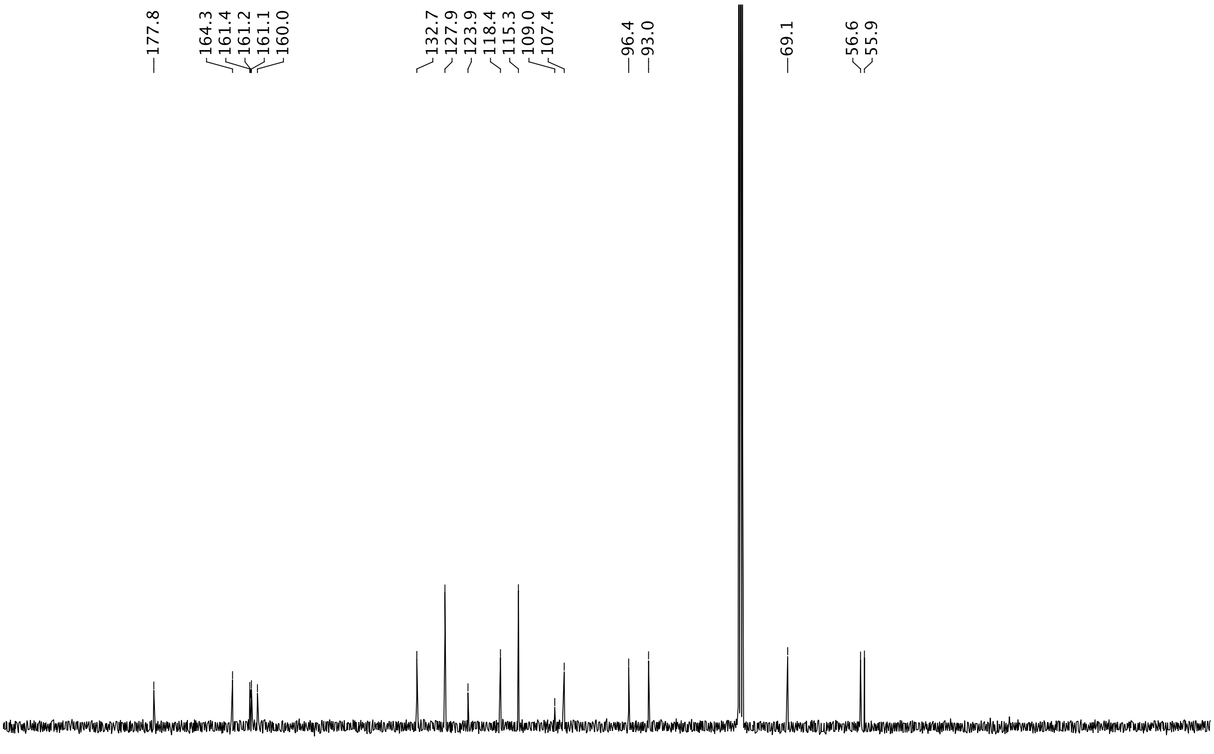

195.7
178.7
172.4
165.1
161.6
159.6
159.0
140.4
137.0
134.4
133.2
130.7
130.2
128.7
126.3
109.6
109.6
101.5
95.2
81.9
56.3
33.0
32.0
29.7
29.7
29.6
29.5
29.4
24.8
22.8
14.3
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

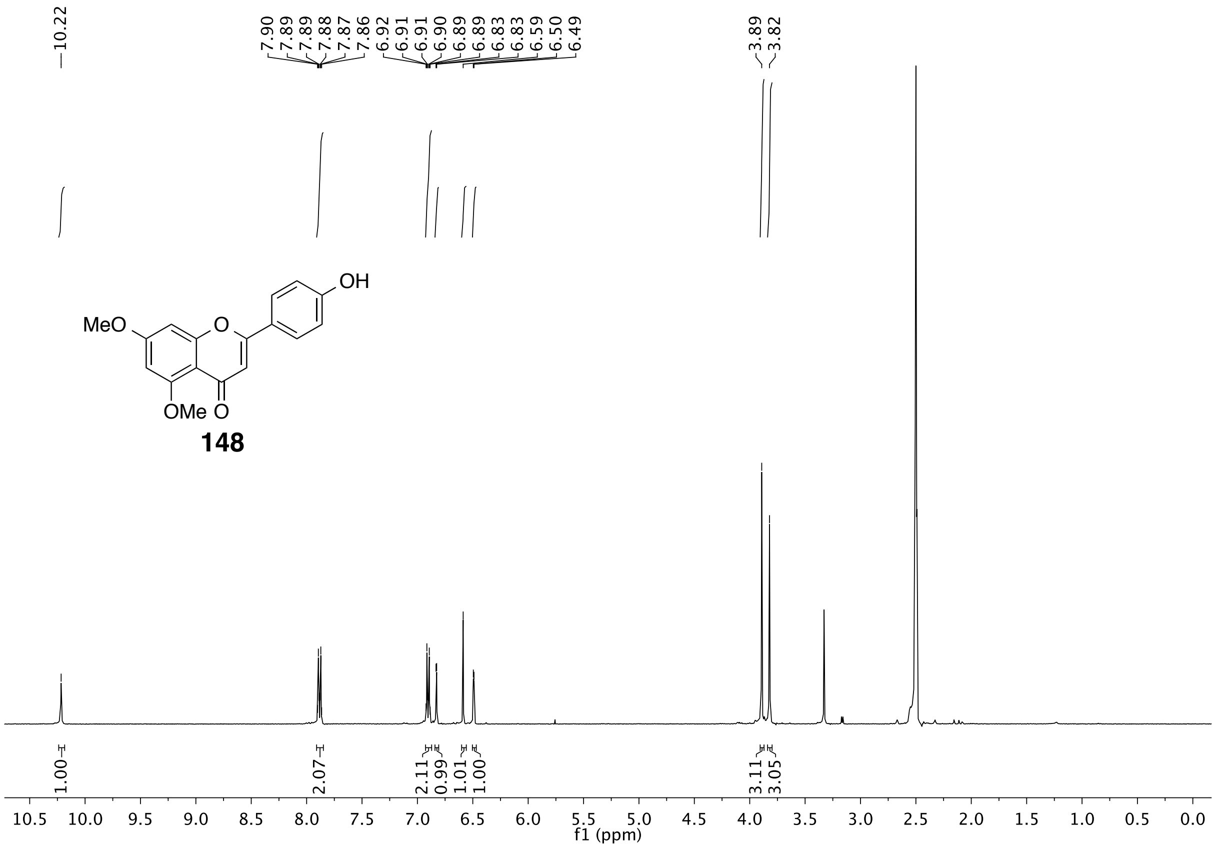

14.41
7.84
7.80
7.79
7.75
7.58
7.57
7.56
7.55
7.55
7.54
6.96
6.95
6.95
6.94
6.93
6.92
6.11
6.11
6.10
6.09
6.07
6.07
6.06
6.04
6.03
5.97
5.96
5.46
5.45
5.41
5.41
5.34
5.33
5.31
5.31
4.60
4.59
4.59
4.58
4.58
3.92
3.84
MeO
OH
O
OMe
O
146
0.93
1.05
0.97
2.00
2.01
0.99
1.05
1.00
1.04
1.02
2.00
2.92
3.00
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)


192.7
168.5
166.2
162.6
160.5
142.6
133.0
130.2
128.6
125.3
118.2
115.3
106.5
94.0
91.4
69.0
56.0
55.7
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

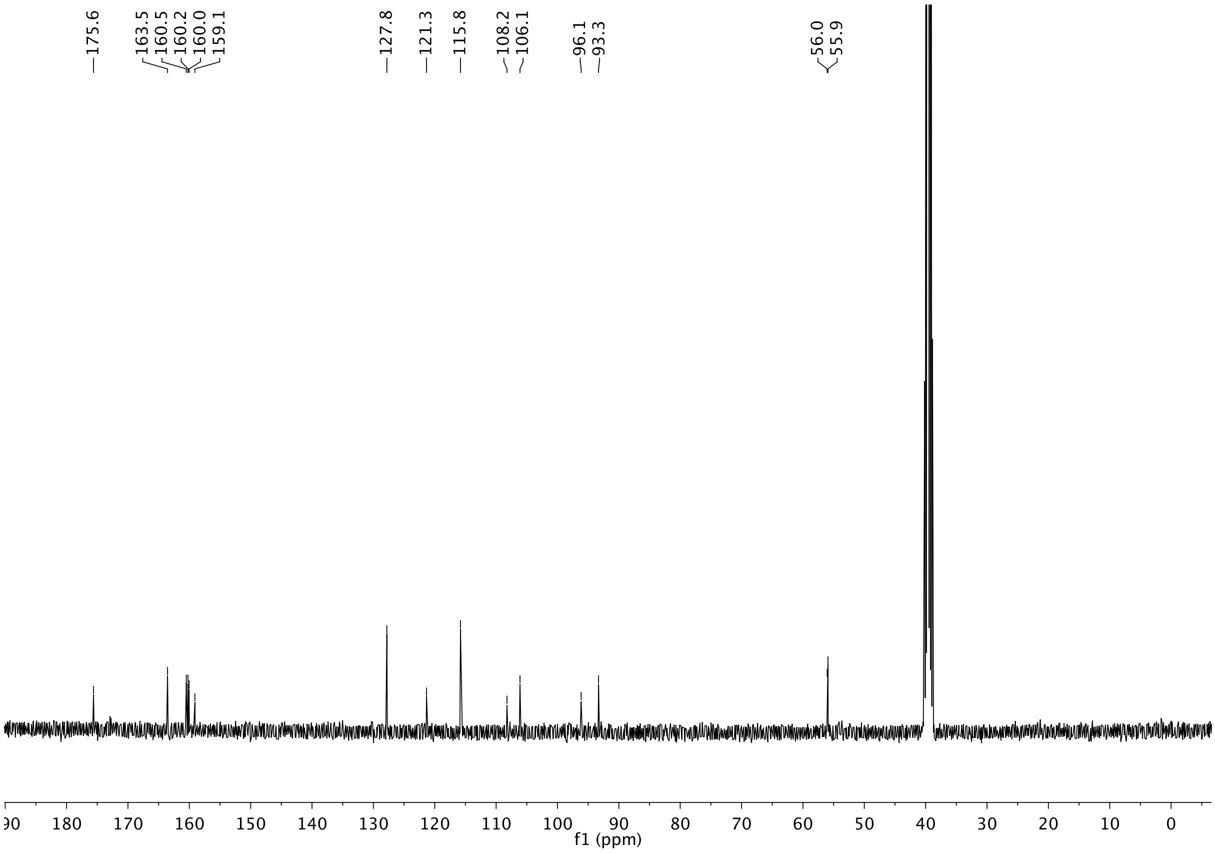

7.85
7.84
7.83
7.83
7.82
7.82
7.81
7.03
7.02
7.02
7.01
7.00
6.99
6.72
6.57
6.57
6.38
6.38
6.11
6.10
6.09
6.08
6.07
6.06
6.06
6.05
6.03
6.02
5.47
5.47
5.46
5.46
5.43
5.42
5.42
5.42
5.35
5.35
5.34
5.34
5.33
5.32
5.32
5.32
4.63
4.62
4.62
4.61
4.61
4.60
3.96
3.92
MeO
O
O
OMe O
2.01
2.05
0.94
1.03
1.04
1.01
1.01
1.00
2.02
3.11
3.03
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

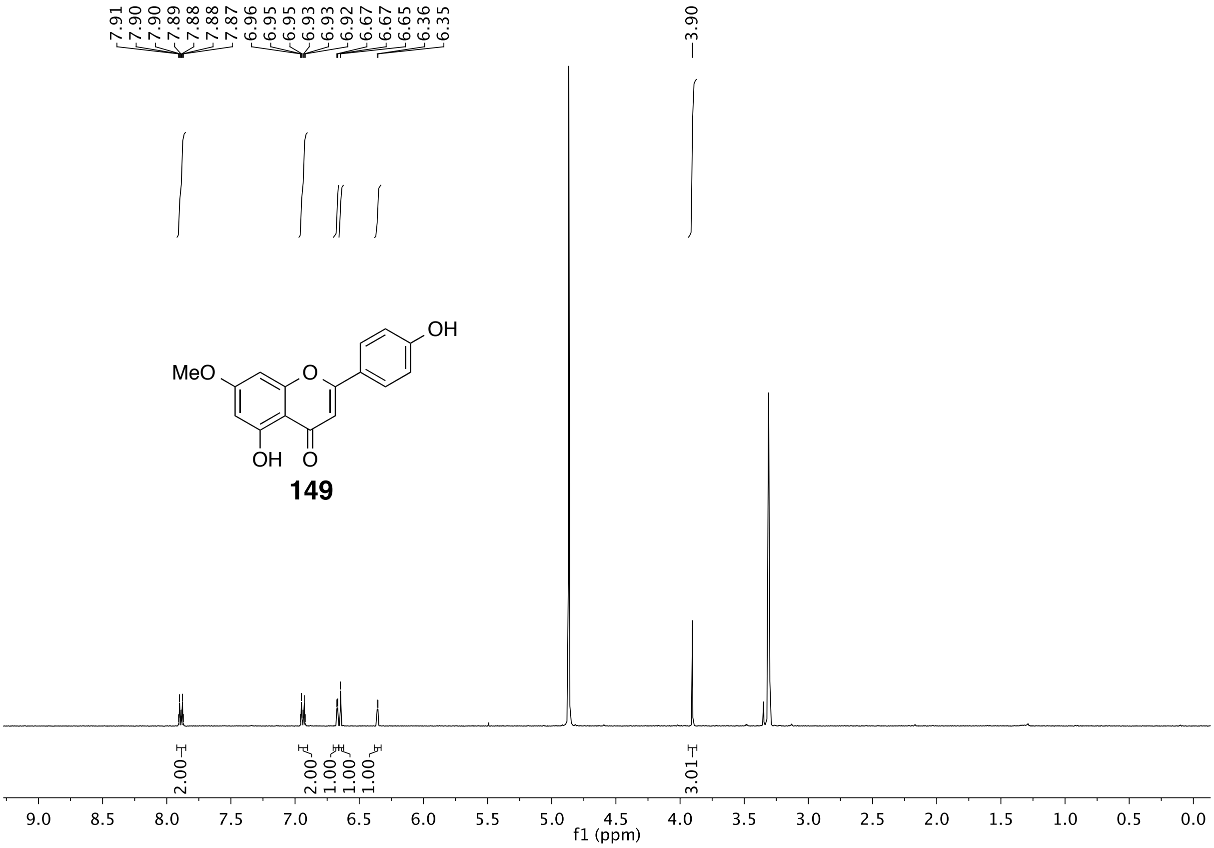
147


177.8
164.3
161.4
161.2
161.1
160.0
132.7
127.9
123.9
118.4
115.3
109.0
107.4
96.4
93.0
69.1
56.6
55.9
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

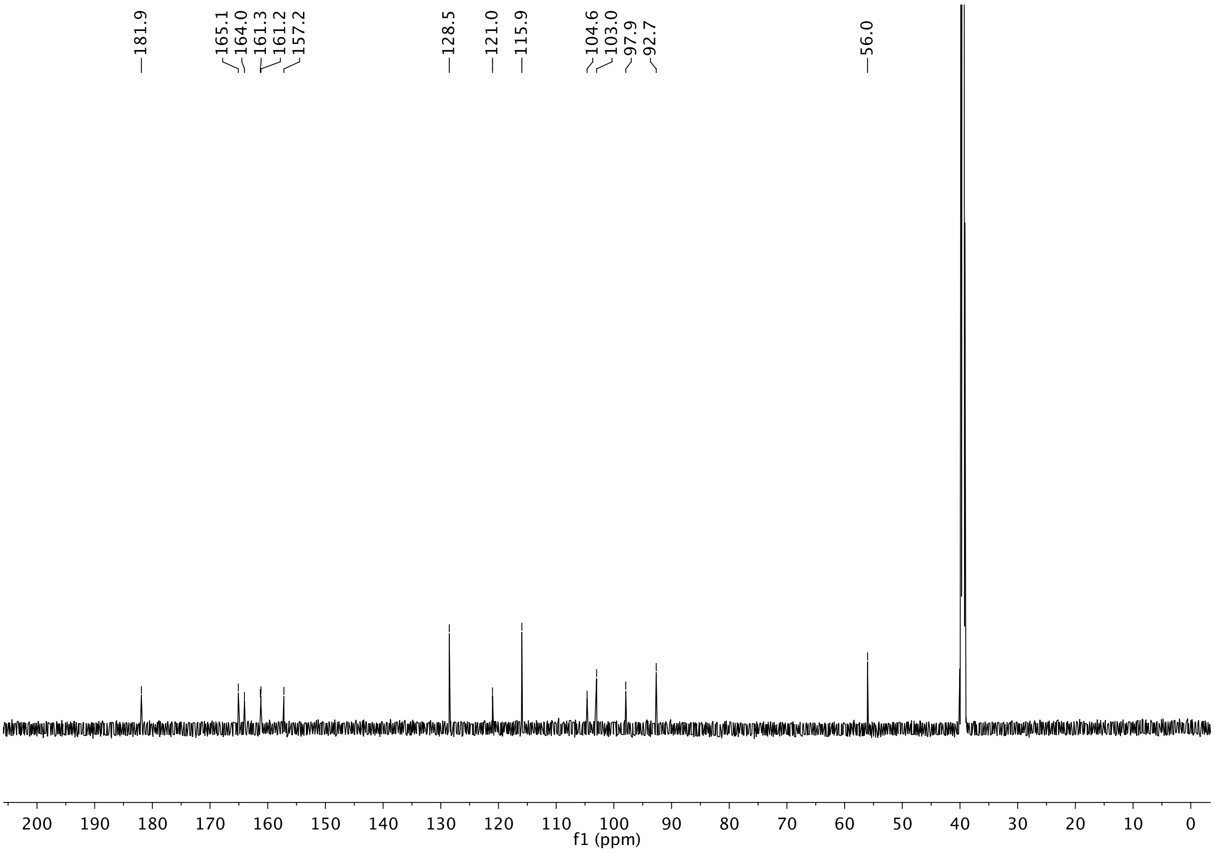

10.22
7.90
7.89
7.89
7.88
7.87
7.86
6.92
6.91
6.91
6.90
6.89
6.89
6.83
6.83
6.59
6.50
6.49
3.89
3.82
MeO
O
OH
OMe
O
1.00
2.07
2.11
0.99
1.01
1.00
3.11
3.05
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

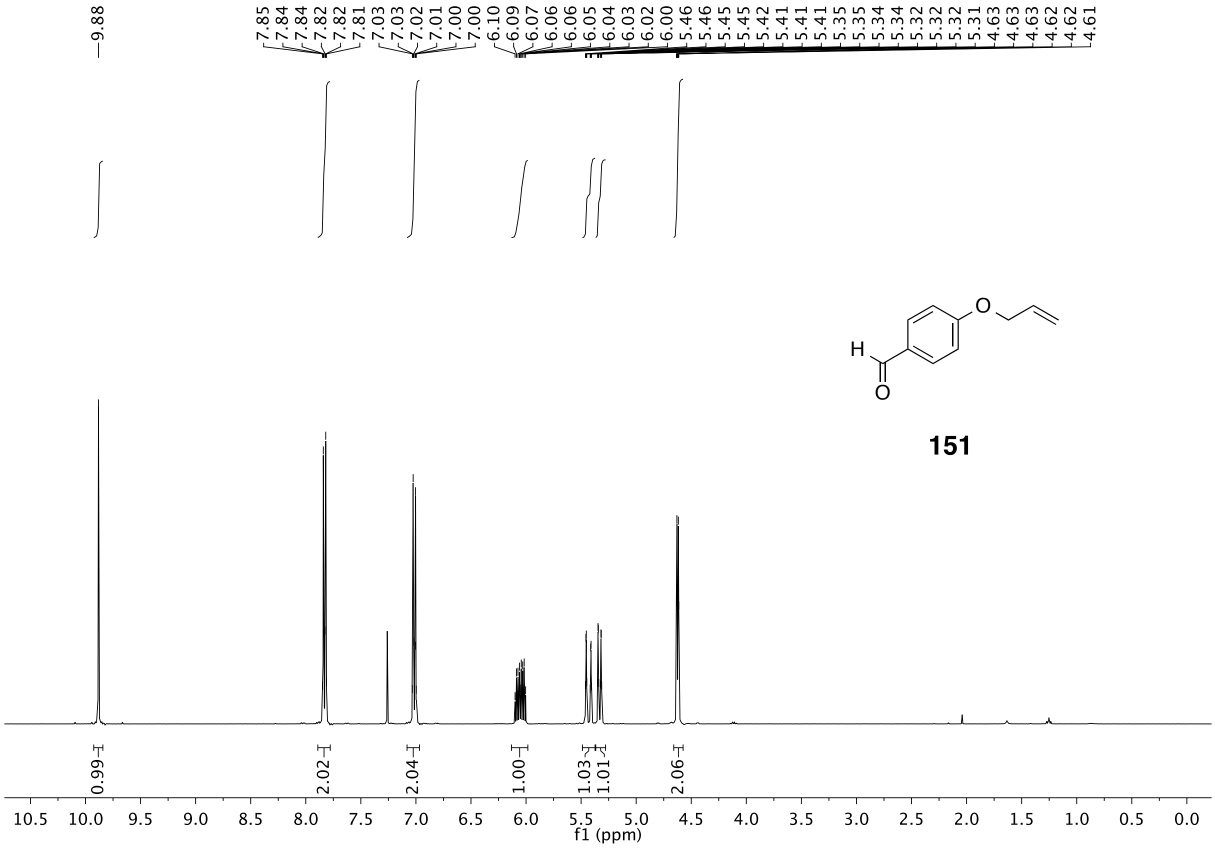
148


175.6
163.5
160.5
160.2
160.0
159.1
127.8
121.3
115.8
108.2
106.1
96.1
93.3
56.0
55.9
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

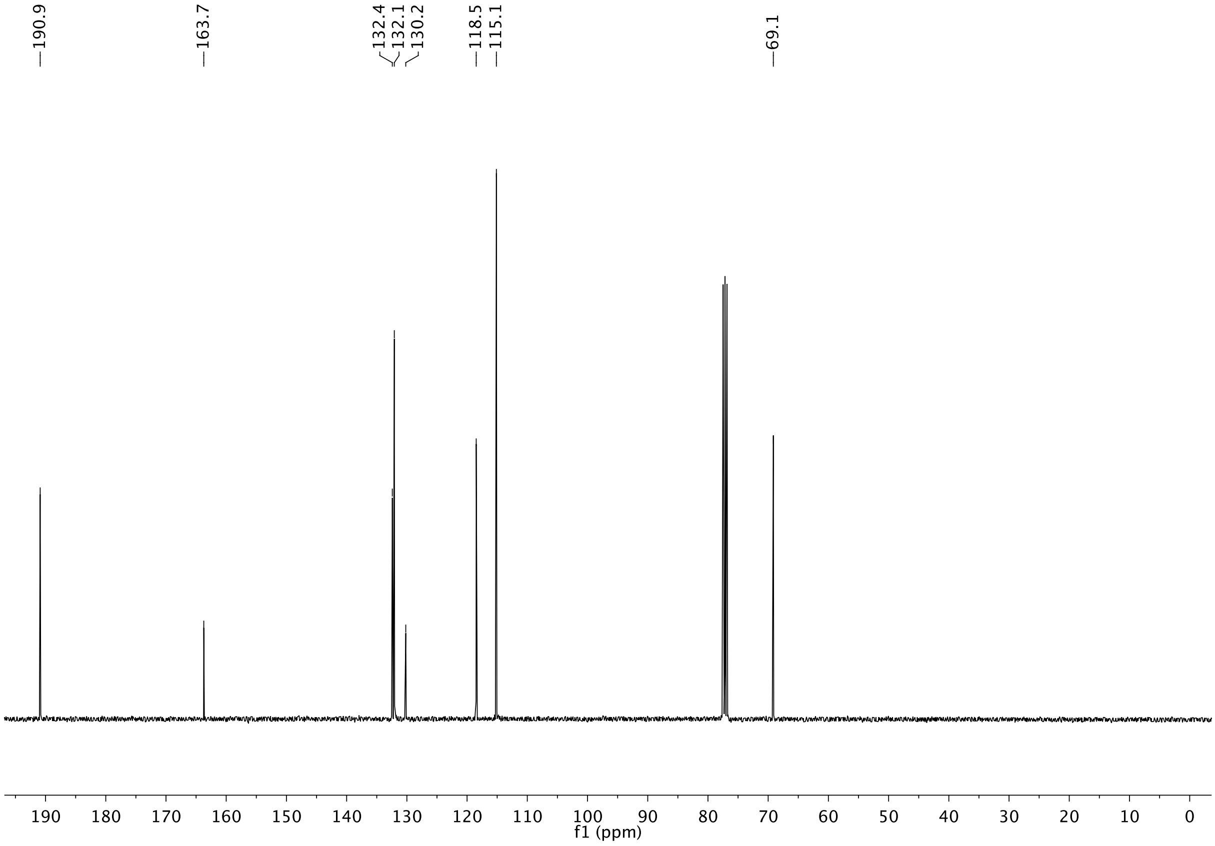

7.91
7.90
7.90
7.89
7.88
7.88
7.87
6.96
6.95
6.95
6.93
6.93
6.92
6.67
6.67
6.65
6.36
6.35
3.90
MeO
O
OH
OH
O
2.00
2.00
1.00
1.00
1.00
3.01
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

149


181.9
165.1
164.0
161.3
161.2
157.2
128.5
121.0
115.9
104.6
103.0
97.9
92.7
56.0
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


9.88
7.85
7.84
7.84
7.82
7.82
7.81
7.03
7.03
7.02
7.01
7.00
7.00
6.10
6.09
6.07
6.06
6.06
6.05
6.04
6.03
6.02
6.00
5.46
5.46
5.45
5.45
5.42
5.41
5.41
5.41
5.35
5.35
5.34
5.32
5.32
5.31
4.63
4.63
4.63
4.62
4.62
4.61
H
O
O
0.99
2.02
2.04
1.00
1.03
1.01
2.06
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

151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130.2
118.5
115.1
69.1
190
180
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140
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120
110
100
90
80
70
60
50
40
30
20
10
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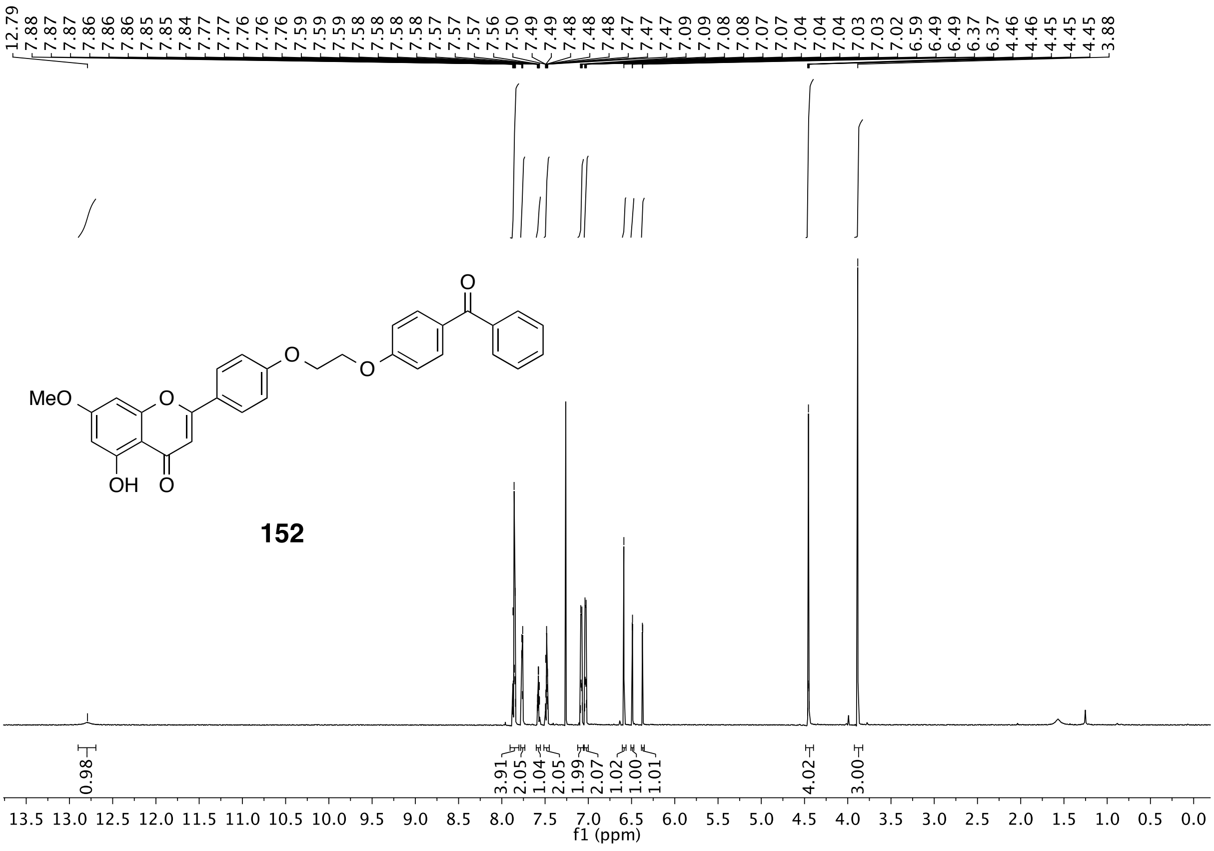
f1 (ppm)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7.77
7.76
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7.59
7.59
7.58
7.58
7.58
7.57
7.57
7.56
7.50
7.49
7.49
7.48
7.48
7.47
7.47
7.09
7.09
7.08
7.08
7.07
7.04
7.04
7.04
7.03
7.03
7.02
6.59
6.49
6.49
6.37
6.37
4.46
4.45
4.45
4.45
3.88
MeO
O
OH
O
O
O
O
0.98
3.91
2.05
1.04
2.05
1.99
2.07
1.02
1.00
1.01
4.02
3.00
13.5
13.0
12.5
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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3.5
3.0
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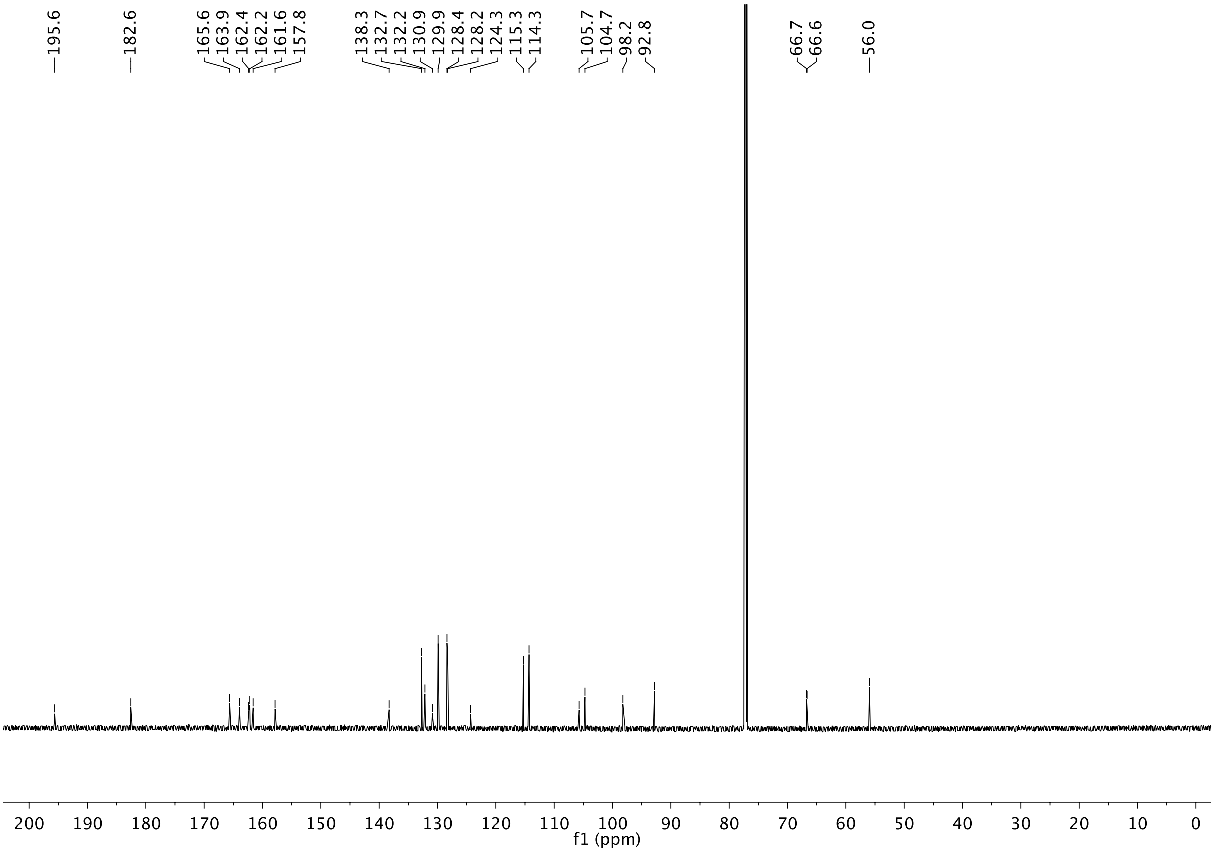
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

152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161.6
157.8
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132.7
132.2
130.9
129.9
128.4
128.2
124.3
115.3
114.3
105.7
104.7
98.2
92.8
66.7
66.6
56.0
200
190
180
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90
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30
20
10
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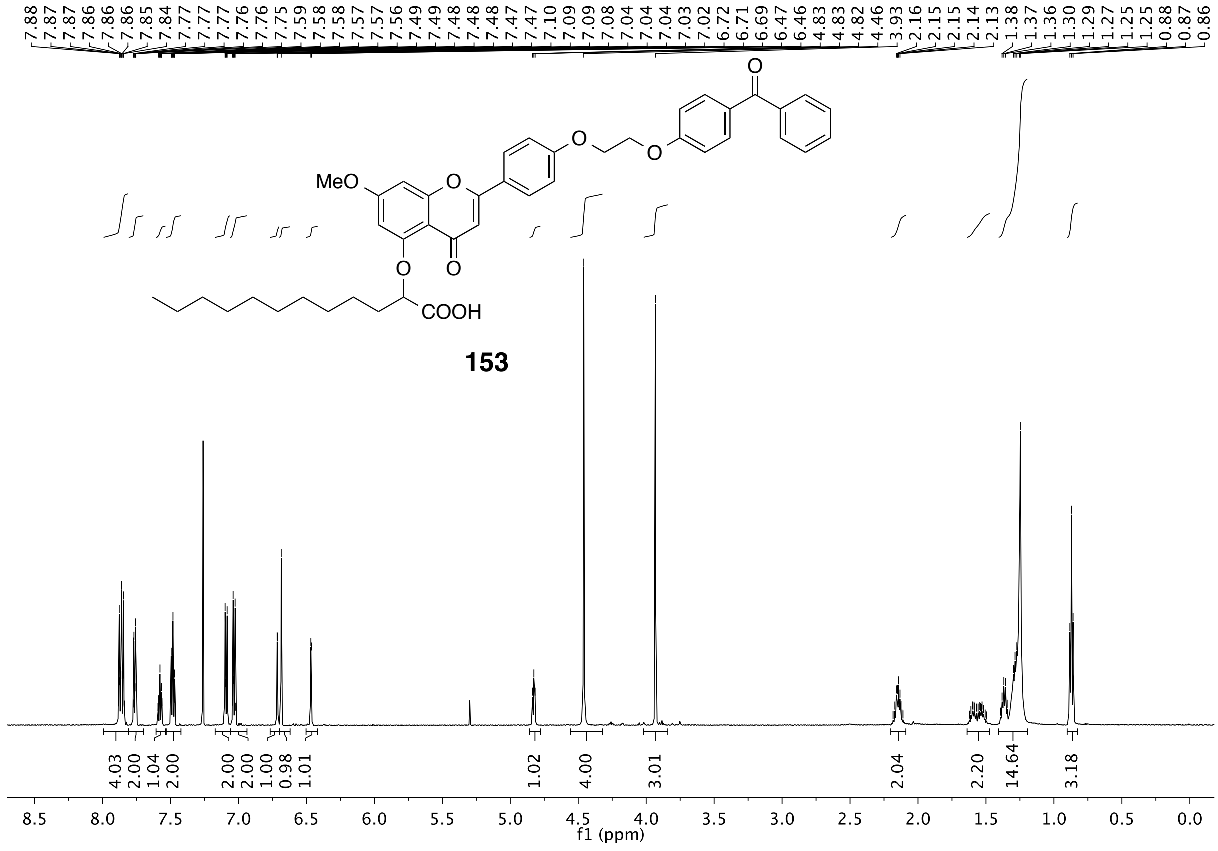
f1 (ppm)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7.58
7.57
7.57
7.56
7.49
7.49
7.48
7.48
7.47
7.47
7.10
7.09
7.08
7.04
7.04
7.03
7.02
6.72
6.71
6.69
6.47
6.46
4.83
4.83
4.82
4.46
3.93
2.16
2.15
2.15
2.14
2.13
1.38
1.37
1.36
1.30
1.29
1.27
1.25
0.88
0.87
0.86
O
O
O
MeO
O
O
O
COOH
4.03
2.00
1.04
2.00
2.00
2.00
1.00
0.98
1.01
1.02
4.00
3.01
2.04
2.20
14.64
3.18
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
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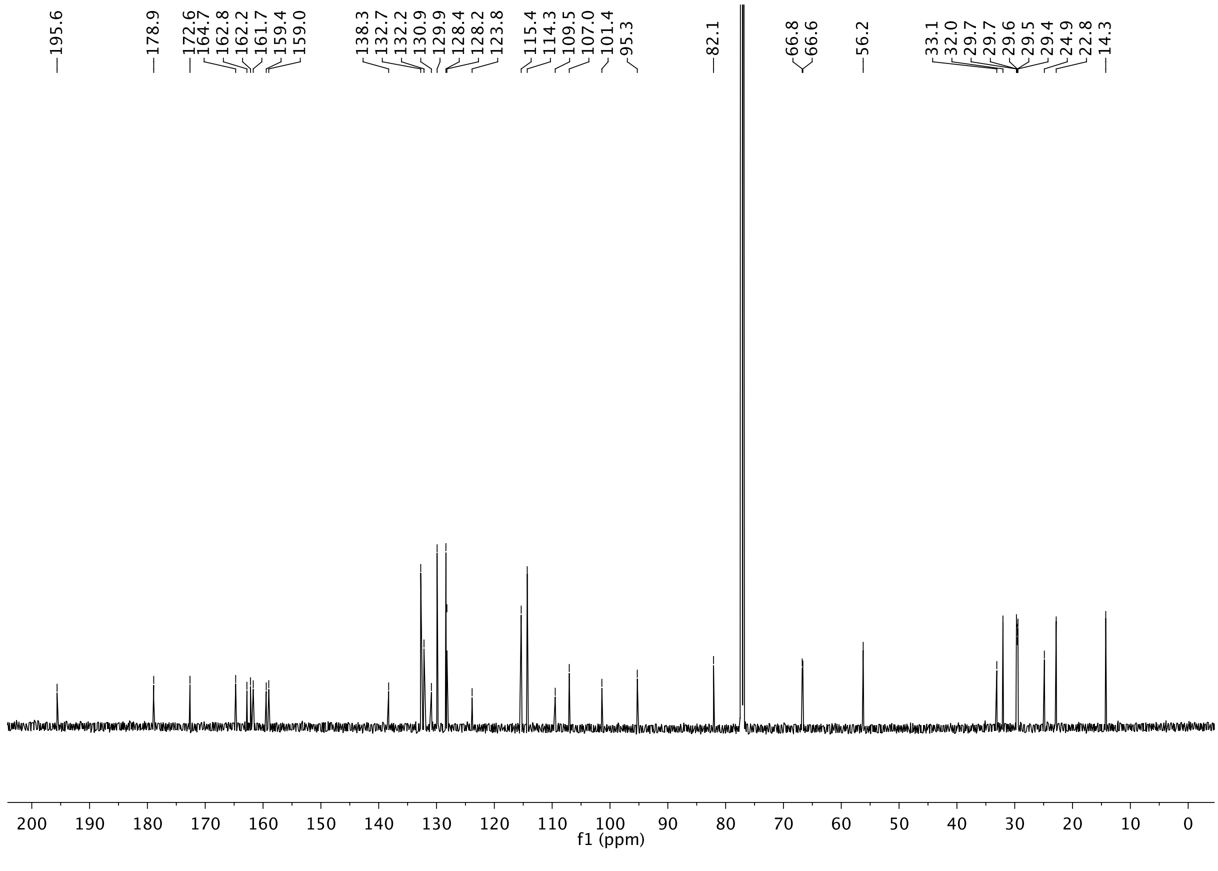
0.5
0.0
f1 (ppm)

153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172.6
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162.8
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161.7
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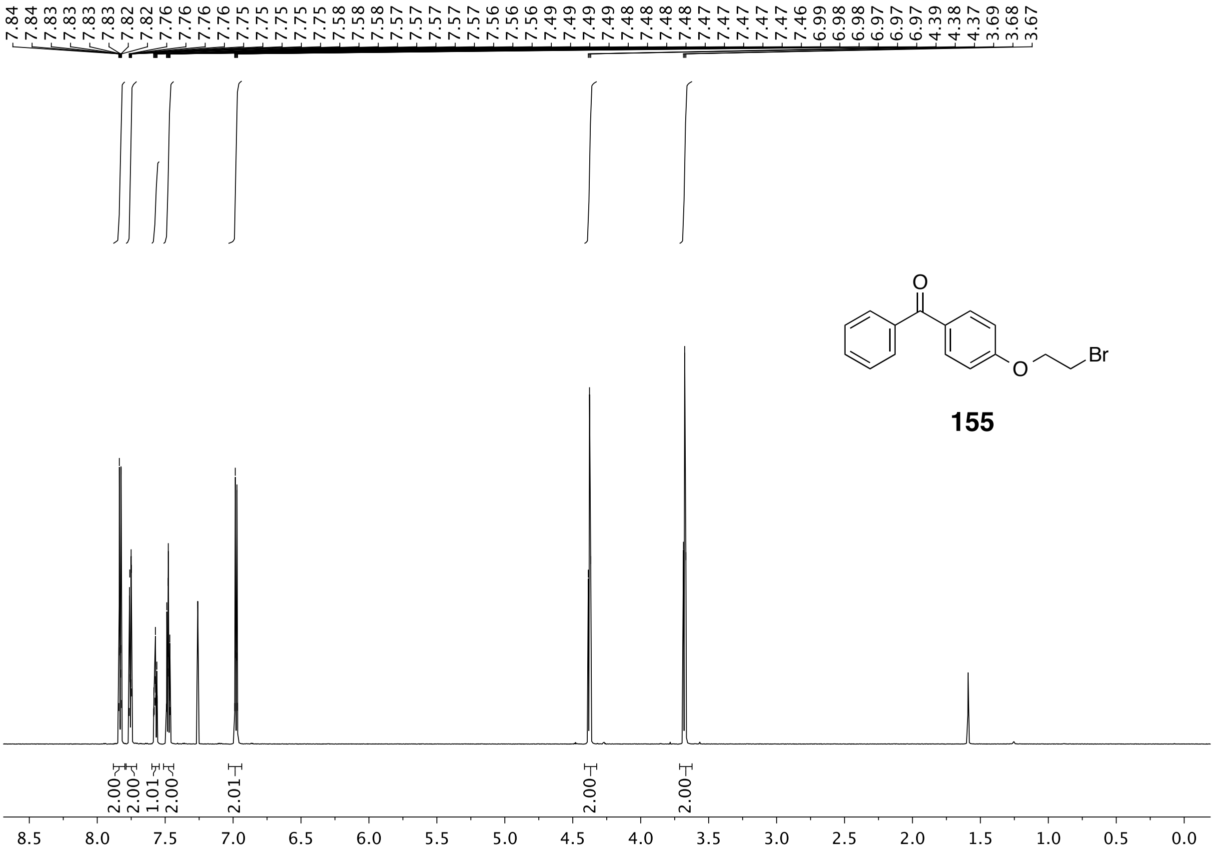
138.3
132.7
132.2
130.9
129.9
128.4
128.2
123.8
115.4
114.3
109.5
107.0
101.4
95.3
82.1
66.8
66.6
56.2
33.1
32.0
29.7
29.7
29.6
29.5
29.4
24.9
22.8
14.3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.83
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7.82
7.76
7.76
7.76
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7.75
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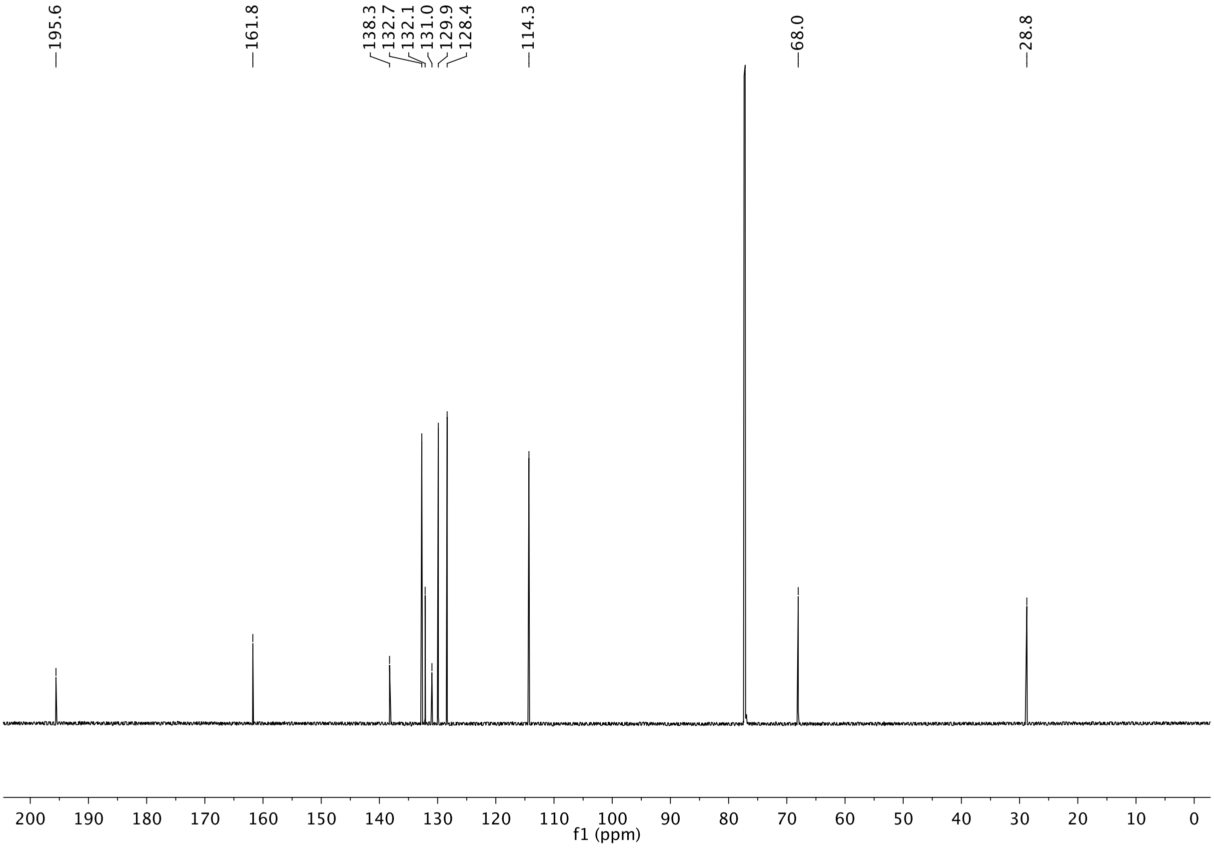
7.75
7.58
7.58
7.58
7.57
7.57
7.57
7.57
7.56
7.56
7.49
7.49
7.49
7.48
7.48
7.48
7.48
7.47
7.47
7.47
7.47
7.46
6.99
6.98
6.98
6.97
6.97
6.97
4.39
4.38
4.37
3.69
3.68
3.67
O
O
Br
155
2.00
2.00
1.01
2.00
2.01
2.00
2.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0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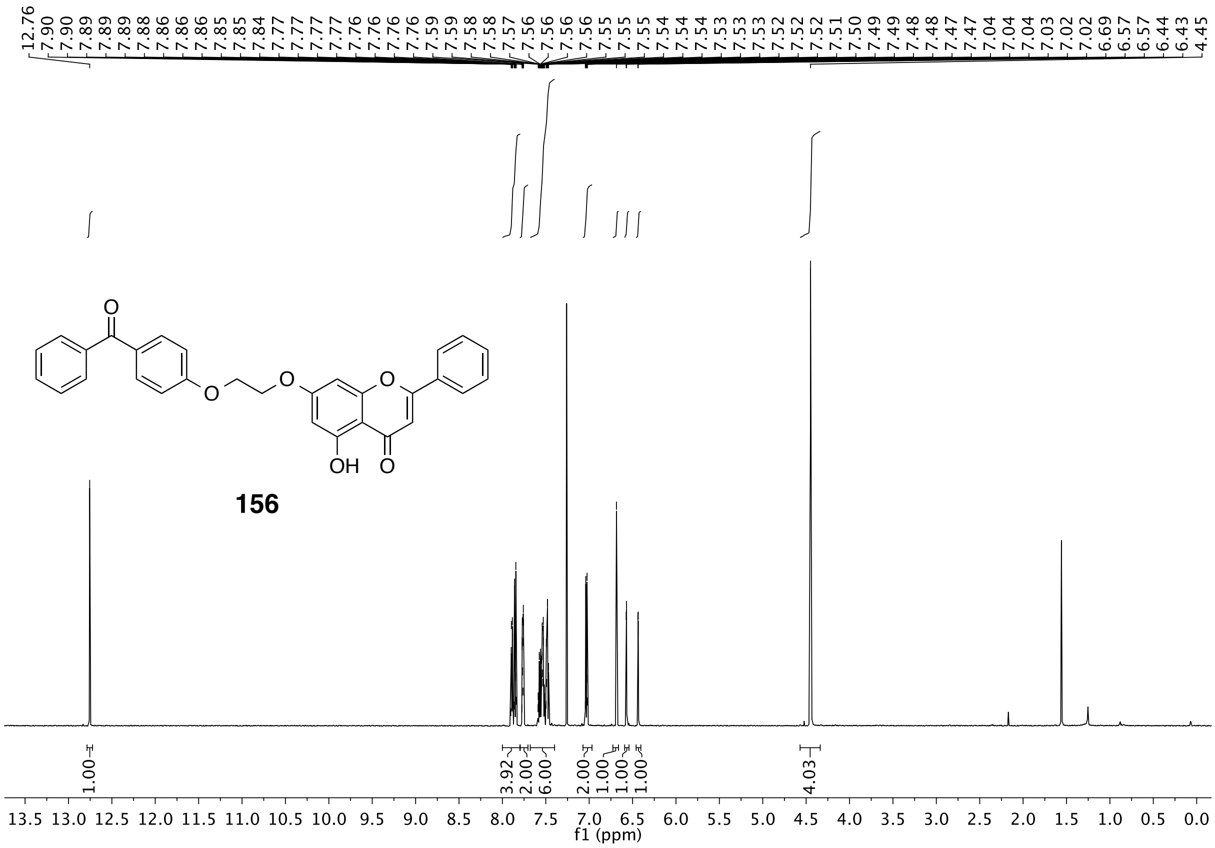
161.8
138.3
132.7
132.1
131.0
129.9
128.4
114.3
68.0
28.8
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.86
7.86
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7.85
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7.77
7.77
7.77
7.77
7.76
7.76
7.76
7.76
7.59
7.59
7.58
7.58
7.57
7.56
7.56
7.56
7.55
7.55
7.55
7.54
7.54
7.53
7.53
7.53
7.52
7.52
7.52
7.51
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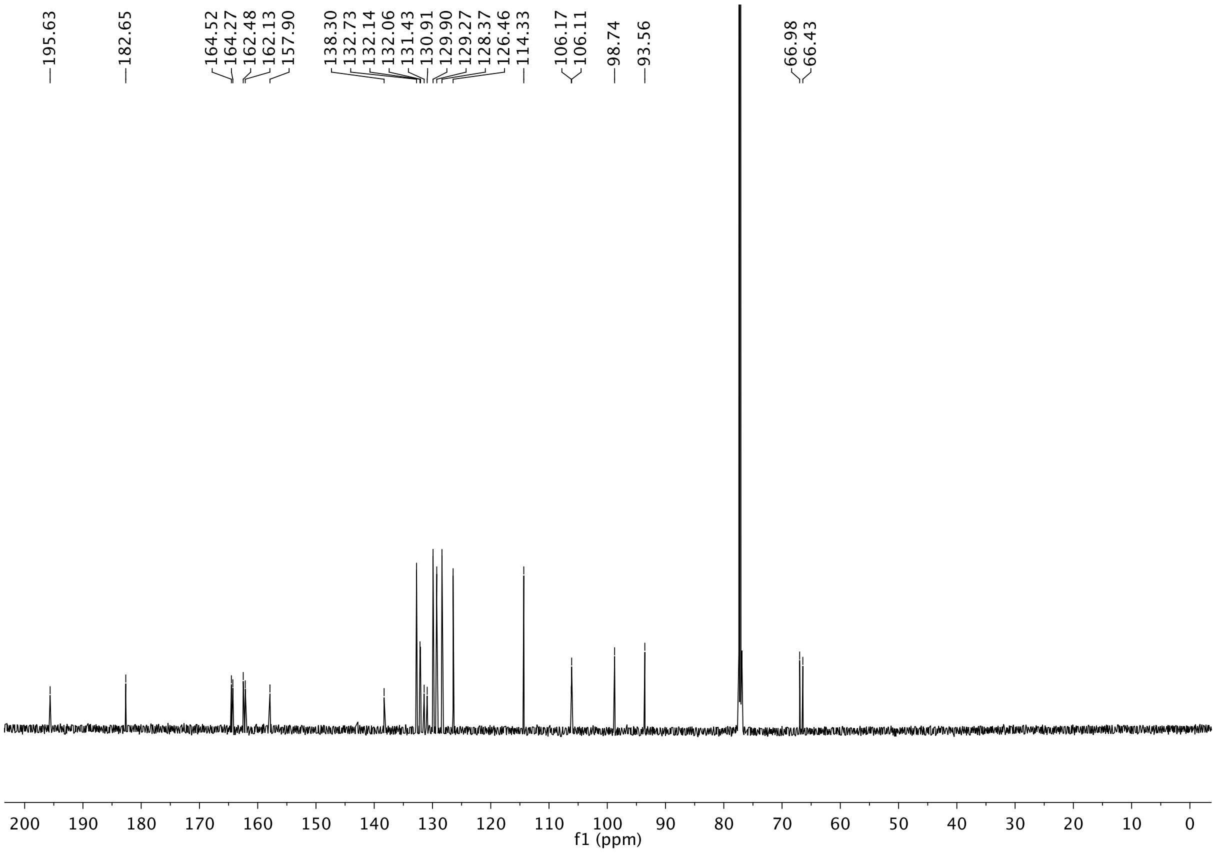
7.48
7.48
7.47
7.47
7.04
7.04
7.04
7.03
7.02
7.02
6.69
6.57
6.57
6.44
6.43
4.45
O
O
O
O
OH
O
1.00
3.92
2.00
6.00
2.00
1.00
1.00
1.00
4.03
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

156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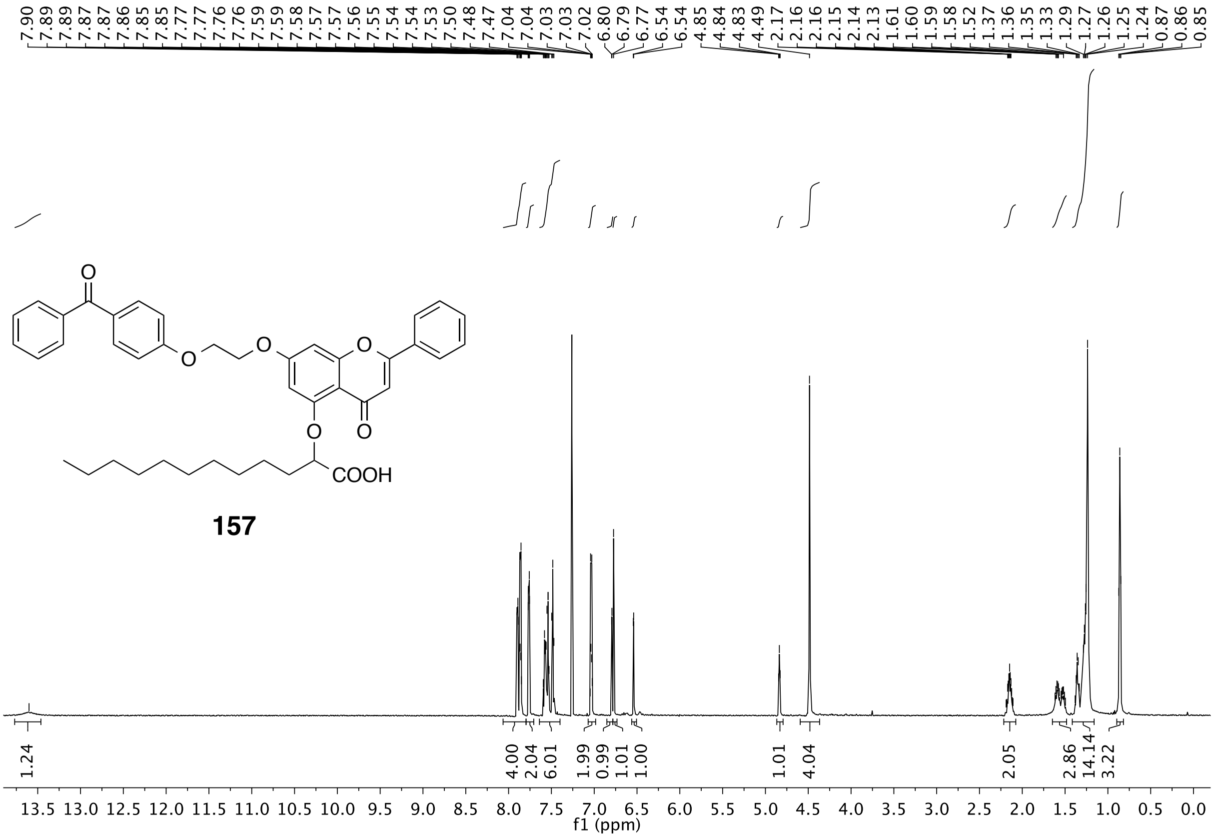
162.13
157.90
138.30
132.73
132.14
132.06
131.43
130.91
129.90
129.27
128.37
126.46
114.33
106.17
106.11
98.74
93.56
66.98
66.43
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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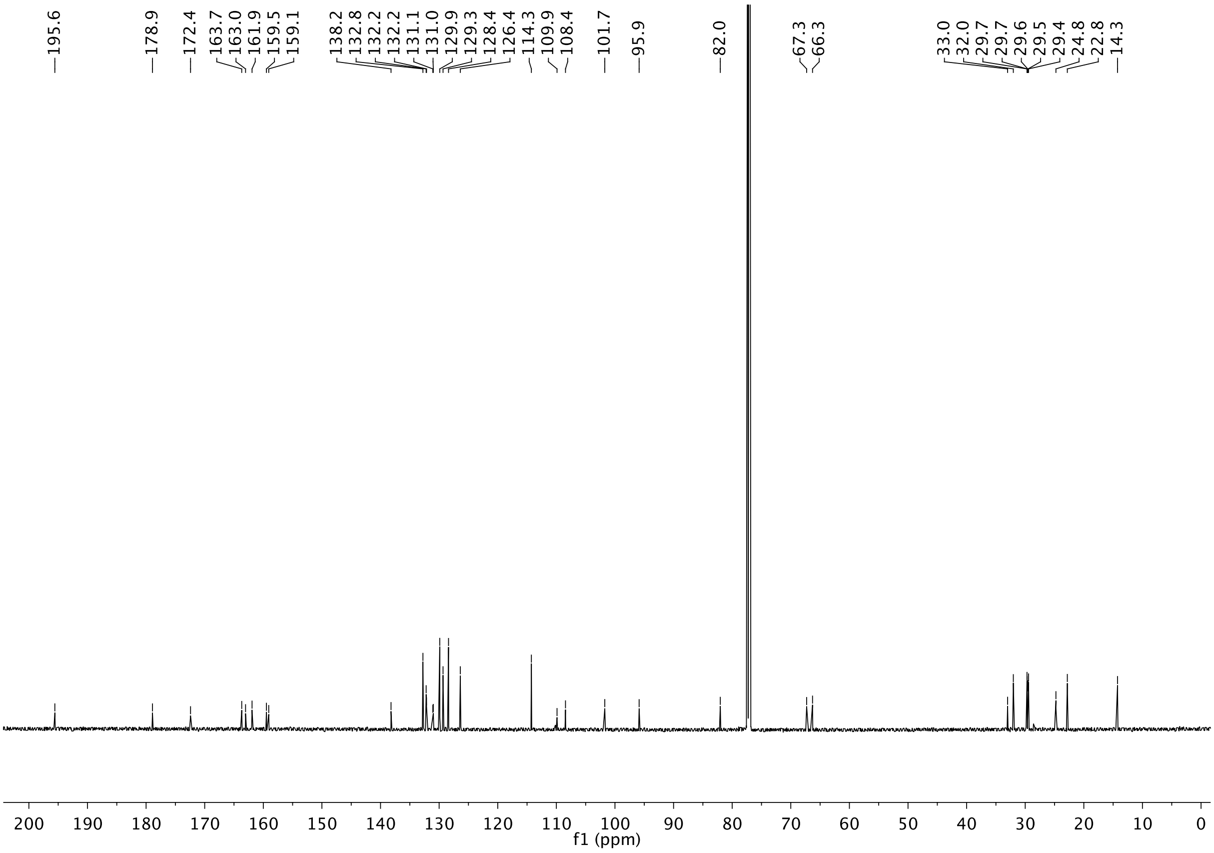
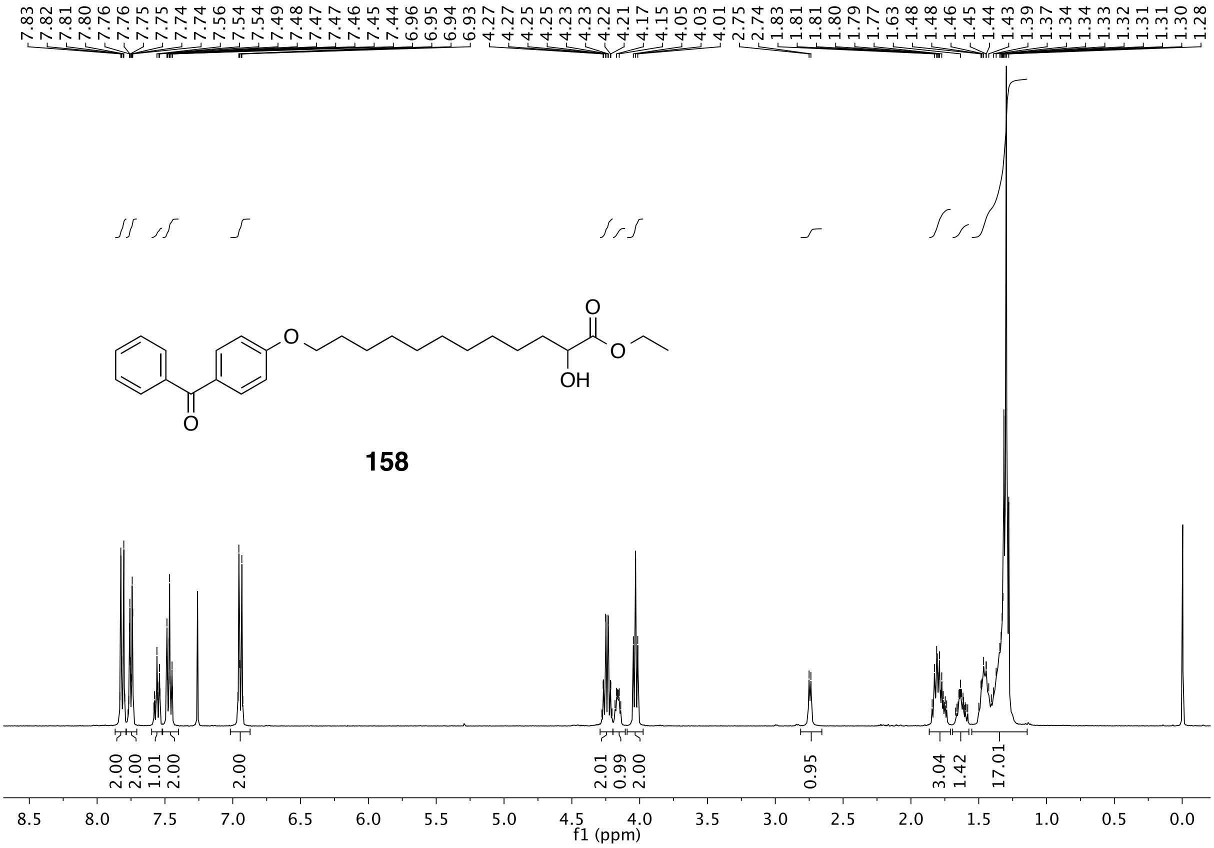
7.89
7.87
7.87
7.86
7.85
7.85
7.77
7.77
7.76
7.59
7.59
7.58
7.57
7.57
7.56
7.55
7.54
7.54
7.53
7.50
7.48
7.47
7.04
7.04
7.03
7.03
7.02
6.80
6.79
6.77
6.54
6.54
4.85
4.84
4.83
4.49
2.17
2.16
2.16
2.15
2.14
2.13
1.61
1.60
1.59
1.58
1.52
1.37
1.36
1.35
1.33
1.29
1.27
1.26
1.25
1.24
0.87
0.86
0.85
O
O
O
O
O
O
COOH
1.24
4.00
2.04
6.01
1.99
0.99
1.01
1.00
1.01
4.04
2.05
2.86
14.14
3.22
13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

157


195.6
178.9
172.4
163.7
163.0
161.9
159.5
159.1
138.2
132.8
132.2
132.2
131.1
131.0
129.9
129.3
128.4
126.4
114.3
109.9
108.4
101.7
95.9
82.0
67.3
66.3
33.0
32.0
29.7
29.6
29.5
29.4
24.8
22.8
14.3
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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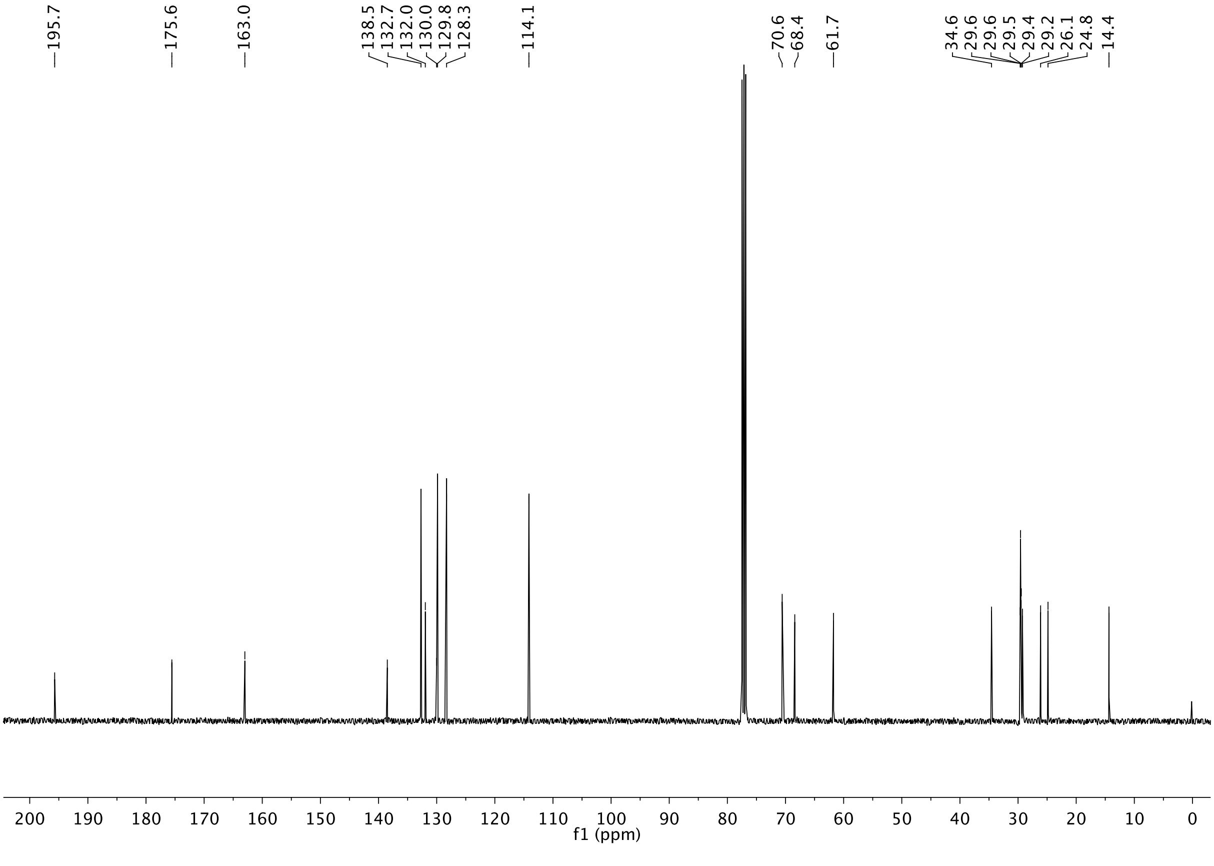
7.75
7.75
7.74
7.74
7.56
7.54
7.54
7.49
7.48
7.47
7.46
7.45
7.44
6.96
6.95
6.94
6.93
4.27
4.27
4.25
4.25
4.23
4.23
4.22
4.21
4.17
4.15
4.05
4.03
4.01
2.75
2.74
1.83
1.81
1.81
1.80
1.79
1.77
1.63
1.48
1.48
1.46
1.45
1.44
1.43
1.39
1.37
1.34
1.34
1.34
1.33
1.32
1.31
1.31
1.30
1.28
O
O
O
O
OH
2.00
2.00
1.01
2.00
2.00
2.01
0.99
2.00
0.95
3.04
1.42
17.01
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

158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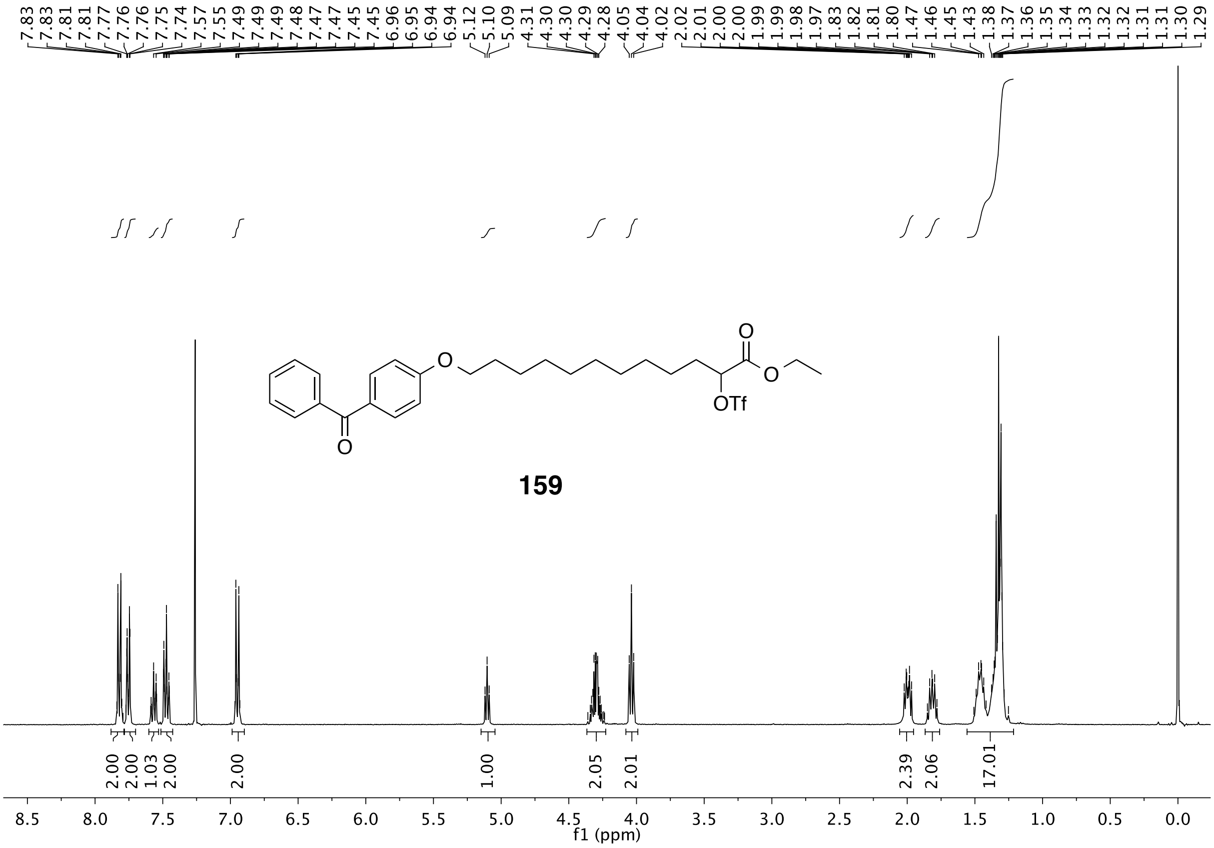
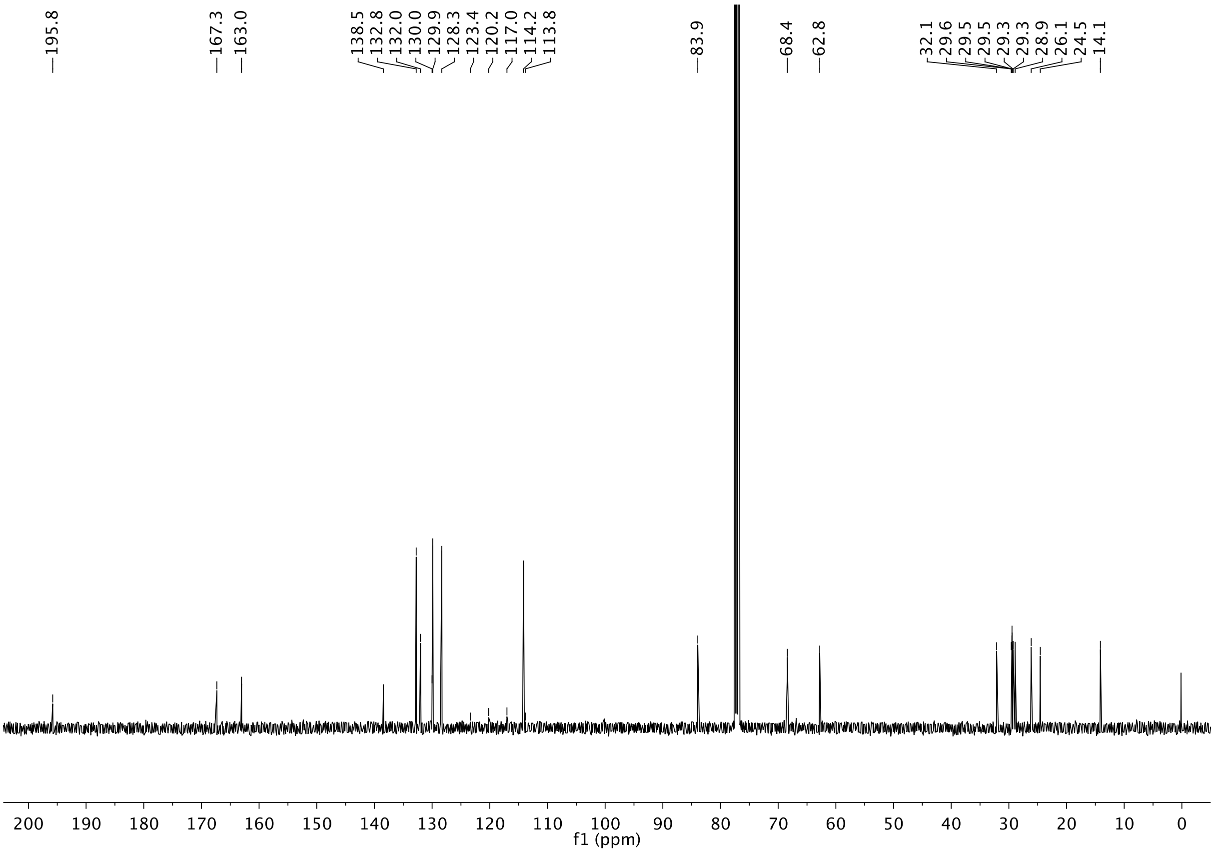
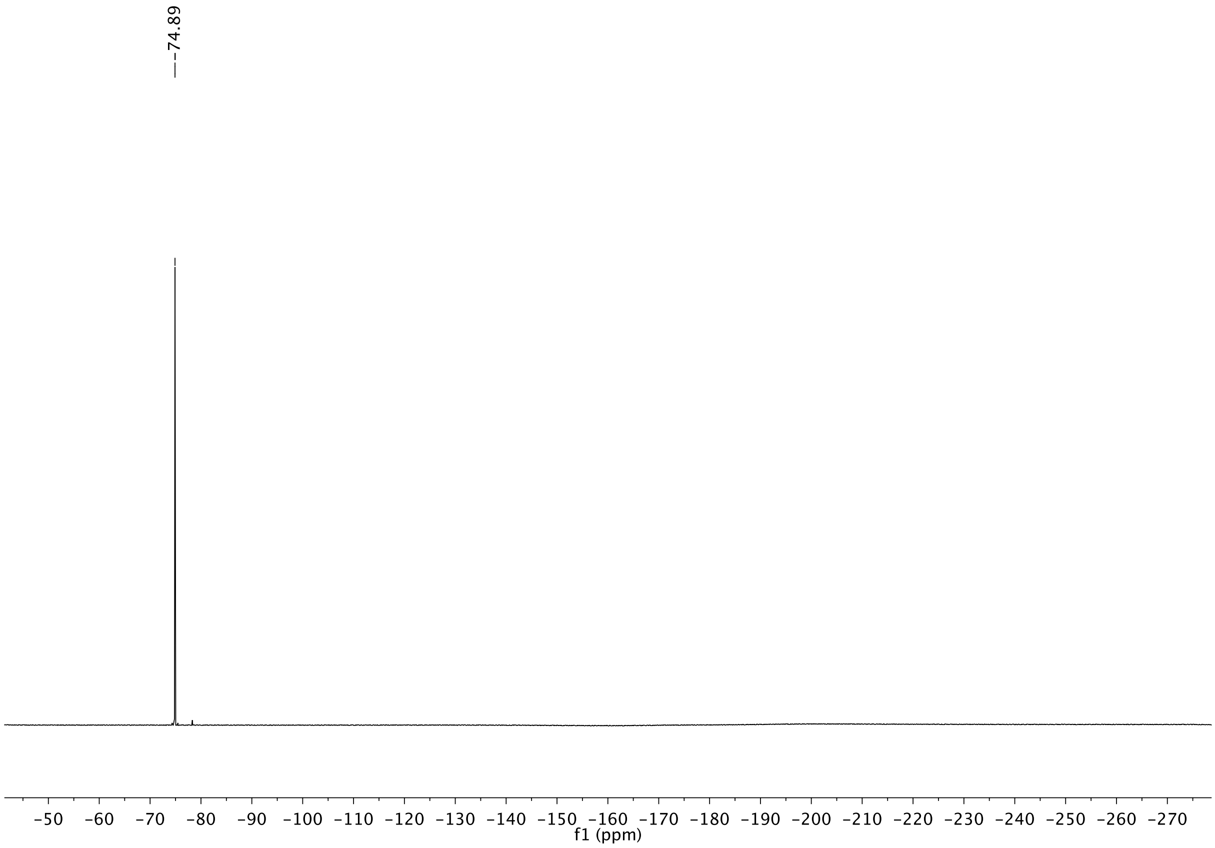
138.5
132.7
132.0
130.0
129.8
128.3
114.1
70.6
68.4
61.7
34.6
29.6
29.5
29.4
29.2
26.1
24.8
14.4
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


7.83 7.83 7.81 7.81 7.77 7.76 7.76 7.75 7.74 7.57 7.55 7.49 7.49 7.48 7.47 7.47 7.45 6.96 6.95 6.94 5.12 5.10 5.09 4.31 4.30 4.29 4.28 4.05 4.04 4.02 2.02 2.01 2.00 2.00 1.99 1.99 1.98 1.97 1.83 1.82 1.81 1.80 1.47 1.46 1.45 1.43 1.38 1.37 1.36 1.35 1.34 1.33 1.32 1.32 1.31 1.30 1.29
O
O
O
O
OTf
2.00 2.00 1.03 2.00 2.00 1.00 2.05 2.01 2.39 2.06 17.01
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

159


195.8
167.3 163.0
138.5 132.8 132.0 130.0 129.9 128.3 123.4 120.2 117.0 114.2 113.8
83.9
68.4 62.8
32.1 29.6 29.5 29.5 29.3 29.3 28.9 26.1 24.5 14.1
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


-74.89
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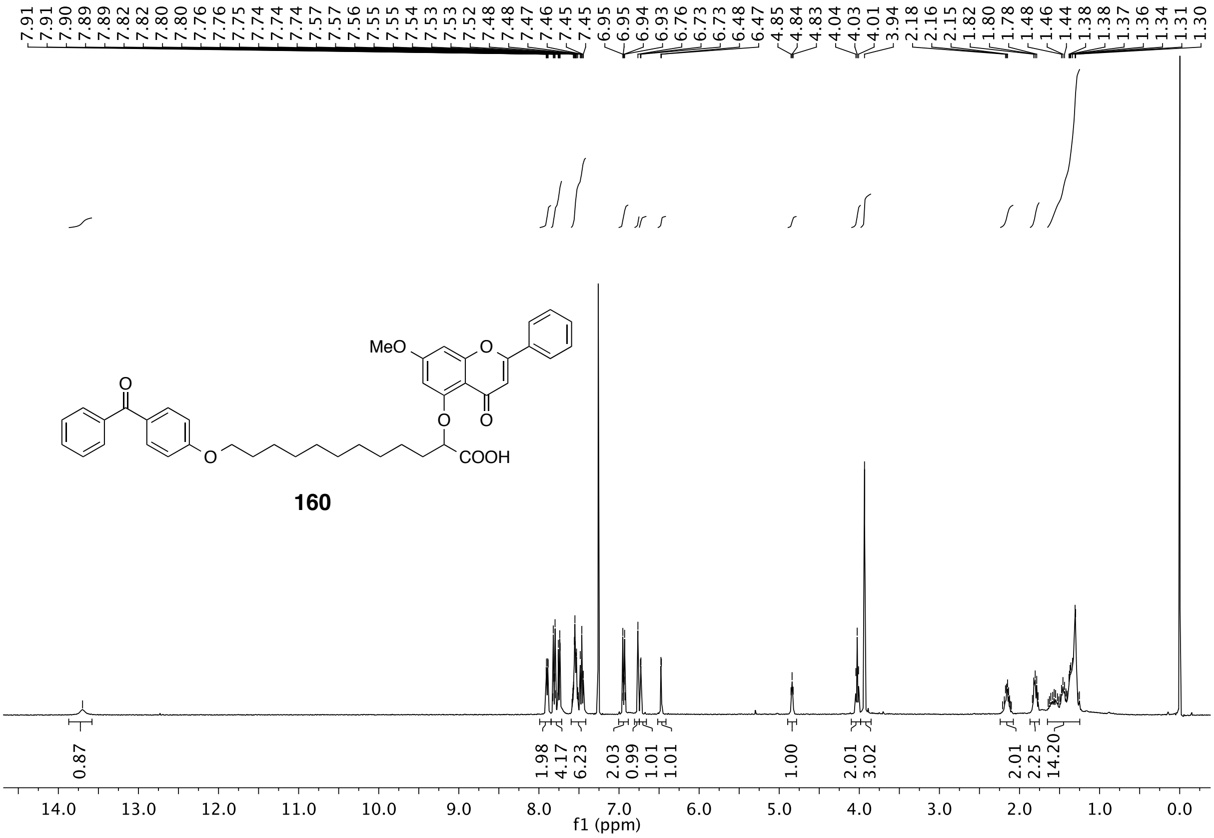
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
-250
-260
-270
f1 (ppm)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7.82
7.82
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7.80
7.76
7.76
7.75
7.74
7.74
7.74
7.57
7.57
7.56
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7.48
7.48
7.47
7.46
7.45
7.45
6.95
6.95
6.94
6.93
6.76
6.73
6.73
6.48
6.47
4.85
4.84
4.83
4.04
4.03
4.01
3.94
2.18
2.16
2.15
1.82
1.80
1.78
1.48
1.46
1.44
1.38
1.37
1.36
1.34
1.31
1.30
MeO
O
O
O
O
O
COOH
0.87
1.98
4.17
6.23
2.03
0.99
1.01
1.01
1.00
2.01
3.02
2.01
2.25
14.20
14.0
13.0
12.0
11.0
10.0
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6.0
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4.0
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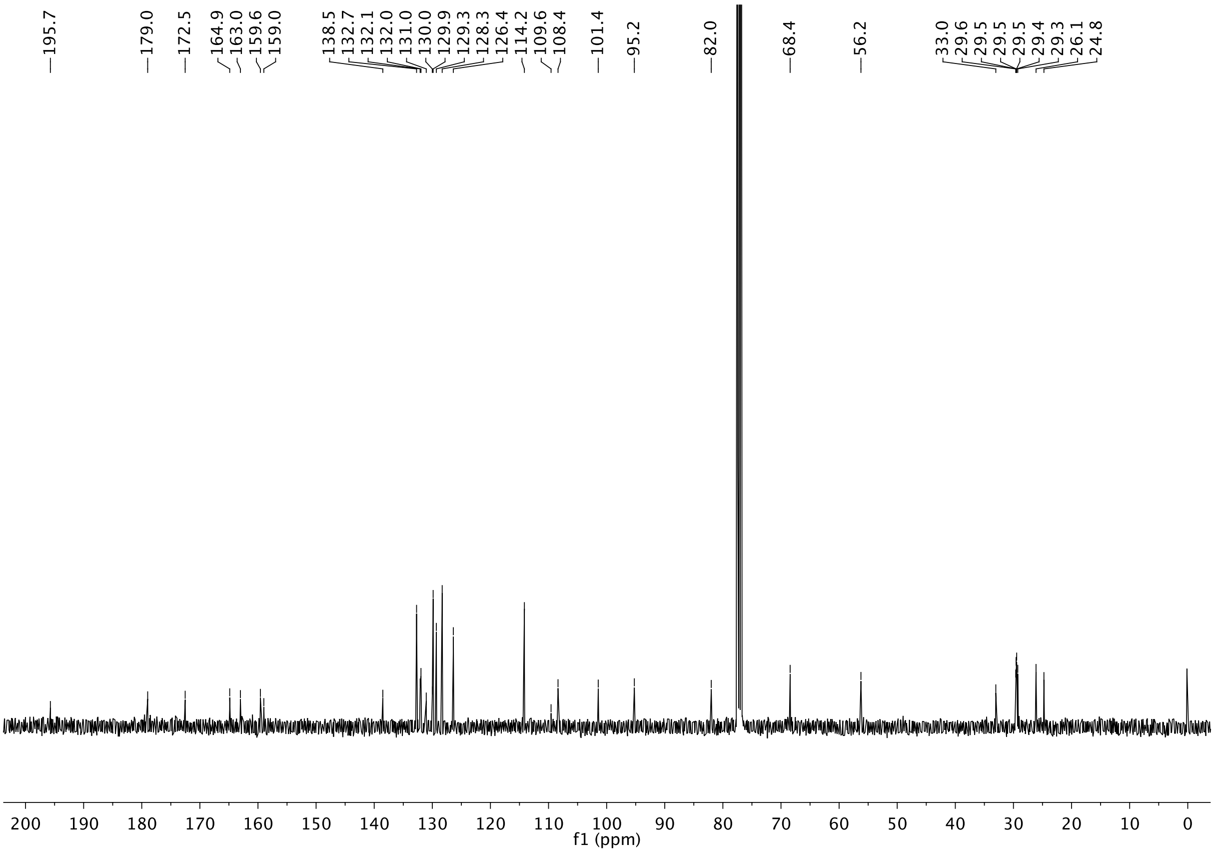
2.0
1.0
0.0
f1 (ppm)

160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164.9
163.0
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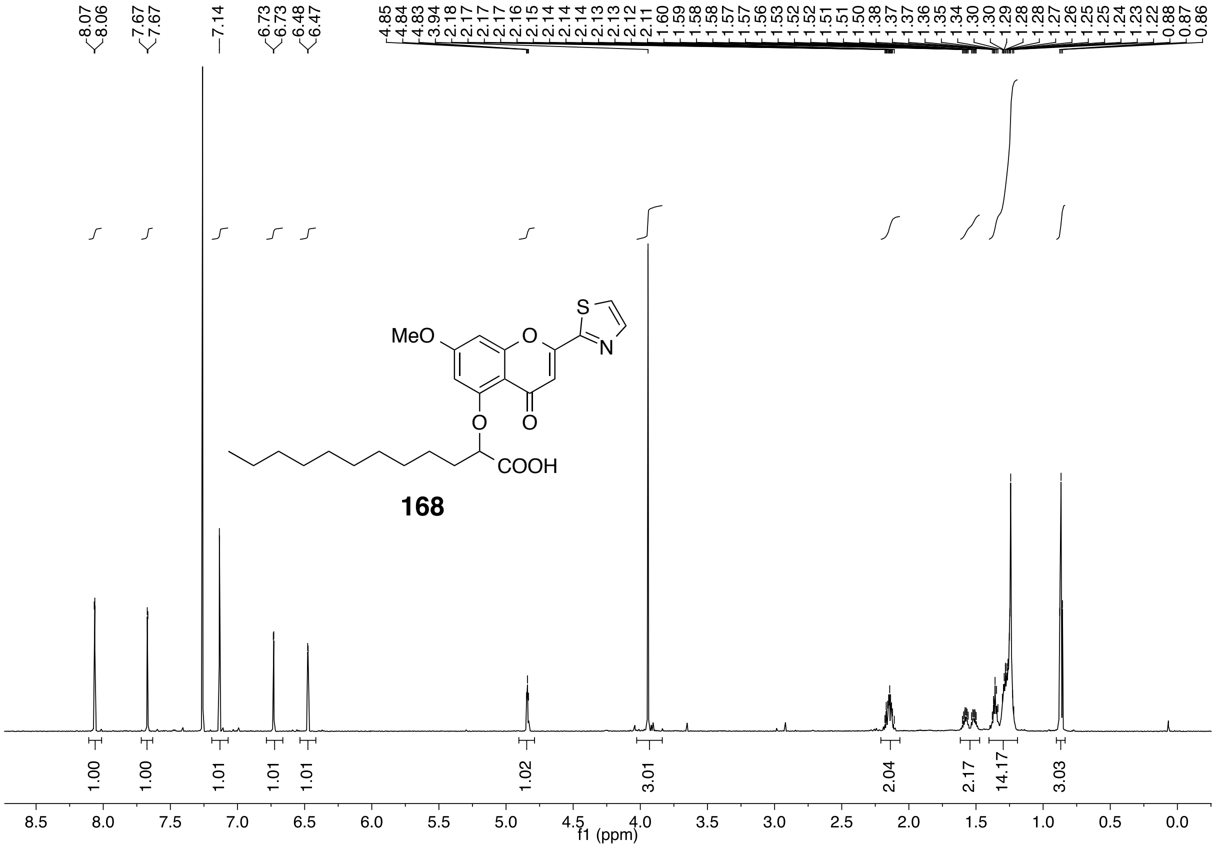
159.0
138.5
132.7
132.1
132.0
131.0
130.0
129.9
129.3
128.3
126.4
114.2
109.6
108.4
101.4
95.2
82.0
68.4
56.2
33.0
29.6
29.5
29.5
29.4
29.3
26.1
24.8
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


8.07
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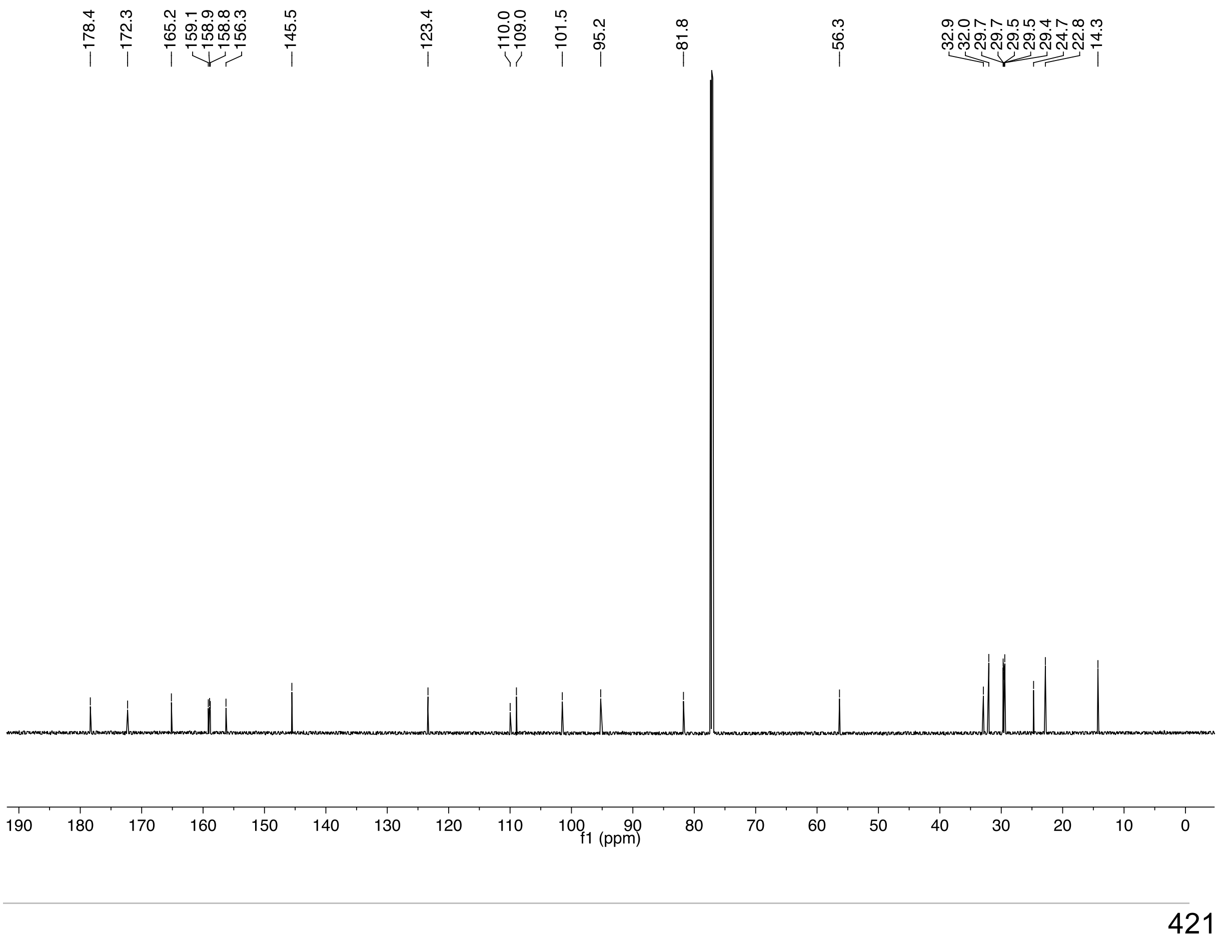
6.47
4.85
4.84
4.83
3.94
2.18
2.17
2.17
2.17
2.16
2.15
2.14
2.14
2.14
2.13
2.13
2.12
2.11
1.60
1.59
1.58
1.58
1.57
1.57
1.56
1.53
1.52
1.52
1.51
1.51
1.50
1.38
1.37
1.37
1.36
1.35
1.34
1.30
1.29
1.28
1.28
1.27
1.26
1.25
1.25
1.24
1.23
1.22
0.88
0.87
0.86
MeO
O
S
N
O
O
COOH
168
1.00
1.00
1.01
1.01
1.01
1.02
3.01
2.04
2.17
14.17
3.03
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


178.4
172.3
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159.1
158.9
158.8
156.3
145.5
123.4
110.0
109.0
101.5
95.2
81.8
56.3
32.9
32.0
29.7
29.7
29.5
29.5
29.4
24.7
22.8
14.3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)