Synthesis and Structural Features of -Fluorocarbonyl Systems

CRAIG AARON FISHER

Electronic Supplementary Material (ESI) for Green Chemistry.

Sustainable synthesis of enantiopure fluorolactam derivatives by a selective direct fluorination - amidase strategy

Nicky J. Willis,[a] Craig A. Fisher, [b] Catherine M. Alder, [a] Antal Harsanyi, [b] Lena Shukla*[a] Joseph P. Adams,[a] and Graham Sandford*[b]

SUPPORTING INFORMATION 1

Experimental details

SI-1.1 General information

Unless otherwise stated, commercially available reagents were used as supplied. All reactions requiring anhydrous conditions were conducted in vacuum-dried apparatus under an inert atmosphere of nitrogen. Analytical thin-layer chromatography (TLC) was performed on silica gel plates (0.25 mm) pre-coated with a fluorescent indicator. All column chromatography was carried out using either Silicagel LC60A (40–63 micron) purchased from Fluorochem or a Biotage SP4 automated purification system.

NMR Spectroscopy: Proton, carbon and fluorine nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR and ^{19}F NMR) were recorded on a Bruker 400 Ultrashield (^{1}H NMR at 400 MHz; ^{13}C NMR at 101 MHz; ^{19}F NMR at 376 MHz) spectrometer. ^{1}H, ^{13}C and ^{19}F spectroscopic data are reported as follows: chemical shift (ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant in Hz and assignment.

Accurate Mass: Accurate mass analysis was performed on either a Bruker MaXis Impact QtoF or a Xevo QtoF mass spectrometer (Waters Ltd, UK) equipped with an accurate solids analysis probe (ASAP).

Crystallography Data: Crystallographic data was recorded with a Rigaku R-Axis SPIDER IP diffractometer equipped with a Cryostream (Oxford Cryosystems) low temperature device at 120 K.
UPLC-MS: The UPLC analysis was conducted on an Acquity UPLC CSH C18 column (50mm x 2.1mm x i.d. 1.7µm packing diameter) at 40 ^{o}C.

Chiral HPLC Chromatography: Chiral HPLC analysis was carried out using an Agilent 1100 series instrument (see SI-3 for method and column parameters).

Infra-Red (IR) Spectroscopy: Infrared spectra were recorded in the range 4000-600 cm^{-1}, using either a Perkin Elmer Spectrum One FTIR spectrometer or a Perkin Elmer 1600 Series FTIR fitted with an ATR probe. Only major absorbances are reported.

Specific Rotation: Specific rotation was measured using a Perkin Elmer Model 341 polarimeter.

Melting Point Determination: Melting points were measured with either a Gallenkamp or Stuart automatic SMP40 melting point machine apparatus at atmospheric pressure and are uncorrected.

Hydrolase Screening Collection: Hydrolases screened during this investigation were obtained from the following sources:

EST X01 - EST X50; Johnson Matthey Xzyme Esterase system (50 enzymes), purchased from Johnson Matthey.

JM EST001 and JM EST002; Johnson Matthey Enzyme system, commercially available preparations from Johnson Matthey.

Candida antarctica lipase B (CAL-B) (NZL-102-IMB), *Thermomyces lanuginosus* lipase, mutant (NZL-105-IMB), 'Bulky substrates' lipase (NZL-107-IMB), 'Broad range' lipase (NZL-108-IMB), Julich esterase 007

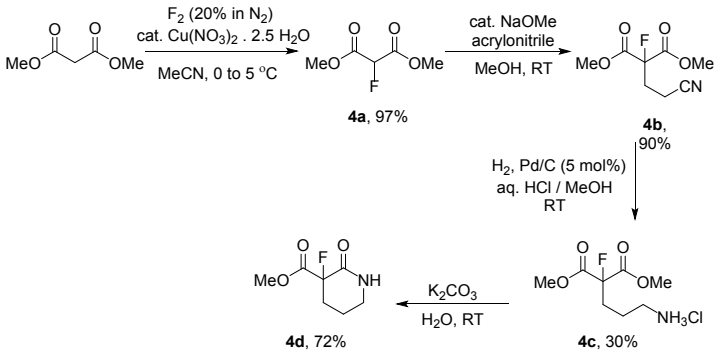
(EL09), *Candida rugosa* lipase AY30, Subtilisin A, Papain, Lipase AYS 'amano' and *Pseudomonas stutzeri;* Novacta amidation system, formerly commercially available from Novacta.

Fermase CAL-B refers to Fermase CAL-B 10,000, a commercially available preparation from Fermase.

Pig liver esterase (PLE); commercially available preparation from Sigma Aldrich.

Codexis EST 002; commercially available preparation from Codexis.

SI-1.2 Initial unoptimised synthesis of racemic 4a-d (Scheme 4)



Synthesis of fluoromalonate **4a** by selective direct fluorination using fluorine gas has been described previously: a) R.D. Chambers, J. Hutchinson, *J. Fluorine Chem.* **1998**, *92*, 45-52; b) A. Harsanyi, G. Sandford, *Green Chem.* **2015**, *17*, 3000-3009.

Synthesis of dimethyl 2-(2-cyanoethyl)-2-fluoromalonate 4b

Dimethyl fluoromalonate **4a** (1.50 g, 10 mmol) was added to a solution of sodium methoxide (0.05 g sodium, 2 mmol) in methanol (40 mL) before acrylonitrile (1.06 g, 20 mmol) was added and the solution stirred at RT. After 1 h the solvent was evaporated, water added (100 mL), extracted with ethyl acetate (3 x 50 mL), washed with brine (50 mL), dried ($MgSO_4$) and concentrated to yield *dimethyl 2-(2-cyanoethyl)-2-fluoromalonate* **4b** (1.82 g, 90%) as a clear oil; ([MH]$^+$, 204.0652. $C_8H_{11}FNO_4$ requires: [MH]$^+$, 204.0672); IR (neat, cm^{-1}) 2962, 2253, 1748, 1438; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (6H, s, OCH_3), 2.60 – 2.49 (4H, m, CH_2); ^{19}F NMR (376 MHz, $CDCl_3$) δ - 167.85 – - 168.04 (m); ^{13}C NMR (101 MHz, chloroform-*d*) δ 165.42 (d, $^2J_{CF}$ 25.3, C=O), 117.88 (s, CN), 92.73 (d, $^1J_{CF}$ 201.0, C-F), 53.86 (s, CH_3O), 30.16 (d, $^2J_{CF}$ 21.5, CH_2), 11.48 (d, $^3J_{CF}$ 5.5, CH_2); *m/z* (ASAP) 204.1 (100 %, [MH]$^+$), 162.1 (25).

Synthesis of dimethyl 2-(3-aminopropyl)-2-fluoromalonate, hydrochloride salt 4c

Dimethyl 2-(2-cyanoethyl)-2-fluoromalonate **4b** (10.16 g, 50 mmol), 10% Pd/C (2.66 g, 5 mol %) and c. HCl (5 mL) in methanol (25 mL) were reacted in a Parr hydrogenator (H_2, 45 psi). After 2 h the solution was filtered through celite, concentrated and the solid was washed with ethyl acetate (20 mL) to yield *dimethyl 2-(3-aminopropyl)-2-fluoromalonate, hydrochloride salt* **4c** (3.60 g, 30 %) as a white solid; mp 147-148 ^{o}C; ([M - Cl]$^+$, 208.0978. $C_8H_{15}FNO_4$ requires [M - Cl]$^+$, 208.0985); IR (neat, cm^{-1}) 3016, 2942, 1748, 1580, 1437, 1249, 1033; ^{1}H NMR (400 MHz, methanol-d_4) δ 3.87 (6H, s, OCH_3), 3.08 – 2.98 (2H, m, CH_2), 2.32 (2H, ddd, $^3J_{HF}$ 23.1, $^3J_{HH}$ 9.2, $^3J_{HH}$ 6.9, CH_2), 1.89 – 1.77 (2H, m, CH_2); ^{19}F NMR (376 MHz, methanol-d_4) δ -167.20 (t, $^3J_{HF}$ 23.1); ^{13}C NMR (101 MHz, methanol-d_4) δ 167.60 (d, $^2J_{CF}$ 25.8, C=O), 95.57 (d, $^1J_{CF}$ 197.4, C-F), 54.10 (s, CH_3O), 40.21 (s, CH_2NH_2), 32.18 (d, $^2J_{CF}$ 21.6, *C*H_2-CF), 22.38 (d, $^3J_{CF}$ 3.2, CH_2); *m/z* (ASAP) 208.1 (100%, [M - Cl]$^+$), 191 (14), 176 (8).

Synthesis of methyl 3-fluoro-2-oxo-3-piperidinecarboxylate 4d

Dimethyl 2-(3-aminopropyl)-2-fluoromalonate, hydrochloride salt **4c** (0.73 g, 3.0 mmol) and K_2CO_3 (0.50 g, 3.6 mmol) in water (5 mL) was stirred for 5 min before the solvent was evaporated. The resulting solid was washed with ethyl acetate (3 x 15 mL) and the filtrate concentrated to give *methyl 3-fluoro-2-oxo-3-piperidinecarboxylate* **4d** (0.38 g, 72 %) as a white solid; mp 115-116 ^oC; ($[MH]^+$, 176.0718. $C_7H_{11}FNO_3$ requires $[MH]^+$, 176.0723); IR (neat, cm^{-1}) 3200, 3074, 2968, 2888, 1760, 1668, 1435; ^1H NMR (400 MHz, chloroform-*d*) δ 7.49 (1H, s, NH), 3.85 (3H, s, OCH_3), 3.45 – 3.37 (2H, m, CH_2), 2.42 – 2.20 (2H, m, CH_2), 2.04 – 1.89 (2H, m, CH_2); ^{19}F NMR (376 MHz, chloroform-*d*) δ - 156.07 (dd, $^3J_{HF}$ 28.2, $^3J_{HF}$ 20.0); ^{13}C NMR (101 MHz, chloroform-*d*) δ 168.68 (d, $^2J_{CF}$ 26.2, NH-C=O), 165.13 (d, $^2J_{CF}$ 22.4, CO_2), 90.87 (d, $^1J_{CF}$ 190.8, C-F), 53.29 (s, CH_3O), 42.22 (s, CH_2-NH), 31.25 (d, $^2J_{CF}$ 22.4, CH_2-CF), 18.26 (d, $^3J_{CF}$ 2.6, CH_2); *m/z* (ASAP) 176 (100 %, $[MH]^+$), 162 (9).

SI-1.3 Representative procedure for hydrolase desymmetrisation of dimethyl 2-(3-aminopropyl)-2-fluoromalonate, hydrochloride salt

Materials

Standard Solutions were made up according to the following methods.

Standard Solution 01

Dimethyl 2-(3-aminopropyl)-2-fluoromalonate, hydrochloride salt (628 mg, 2576 μmol) and 2-methyl-2-butanol (100 mL) were mixed in a 100 mL volumetric flask to give a pale green solution.

Standard Solution 02

Methyl 1,4-dimethoxybenzene (174 mg, 1257 μmol) and anhydrous MeOH (100 mL) were mixed in a 100 mL volumetric flask to give a clear solution.

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Method

To individual 1.5 mL Eppendorf vials was added 4 mg of each of the appropriate 62 enzymes described above in SI-1.

Standard solution 01 (680 μL) was added to each of the vials *via* Gilson pipette and the vials were placed in an Eppendorf shaker at 20 ^{o}C at 1400 rpm for 16 h.

Negative Control

An Eppendorf vial containing **Standard solution 01** (680 μL) was placed in "position 01" on every Eppendorf shaker used, such that each instrument had a 'negative control'.

Sampling

After 16 h aliquots (136 μL) were removed from the reaction mixtures and the samples were diluted in 250 μL HPLC vials with **Standard Solution 02** (164 μL). The diluted samples were filtered using Sartorius Vivaspin ultra-centrifuge 5 kDa filter (20 ^{o}C, 13,500 rpm, 30 min). The samples were analyzed by chiral HPLC (**SI-3**) and UPLC and percentage conversions were calculated according to calibration curve (**SI-3**). The reaction mixtures were heated to 40 ^{o}C for a further 5 days and sampled using the same method.

Results

Hydrolases given in SI-1

Table S3 Anhydrous screening results

Hydrolase	Day 1 Yield (%)	Day 6 Yield (%)	Hydrolase	Day 1 Yield (%)	Day 6 Yield (%)
X1	33	22	X32	13	18
X2	22	19	X33	18	23
X3	2	19	X34	13	26
X4	37	19	X35	17	9
X5	28	11	X36	13	10
X6	41	17	X37	14	38
X7	15	23	X38	14	-
X8	16	-	X39	9	21
X9	11	26	X40	18	50
X10	10	18	X41	11	17
X11	16	13	X42	15	42
X12	10	17	X43	10	19
X13	25	10	X44	7	28
X14	16	13	X45	9	-
X15	13	18	X46	11	41
X16	10	17	X47	13	32
X17	14	20	X48	14	31
X18	30	20	X49	15	-
X19	9	8	X50	13	21
X20	19	15	Est01	10	25
X21	12	22	Est02	16	2
X22	20	19	N1	16	17
X23	11	16	N2	12	17
X24	8	25	N3	10	9
X25	10	22	N4	12	29
X26	15	33	N5	10	15
X27	11	-	N6	12	15
X28	13	44	N7	23	17
X29	12	25	N8	11	15
X30	11	18	N9	8	17
X31	12	27	N10	12	23

In all these experiments there was significantly more product formation than in the negative controls [0% 1 day; ~1% 3 days] indicating that the enzyme preparation has a significant effect on reaction rate. Chiral HPLC indicated that all the products were racemic.

SI-1.4 Methyl 3-fluoro-2-oxopiperidine-3-carboxylate 4d stability test

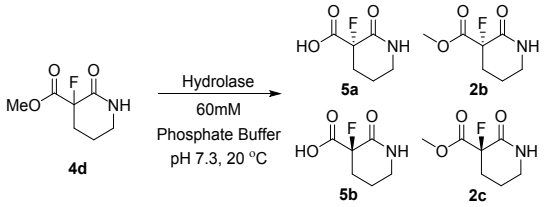
Methyl 3-fluoro-2-oxopiperidine-3-carboxylate **4d** (5 mg) was added to a 1.5 mL Eppendorf tube in a pre-cooled shaking block (10 ºC). Buffer [0.06M Na_2HPO_4: 0.06M KH_2PO_4 (3:1, pH 7.3, 0.5 mL)] was added and the tube was shaken at a pre-set temperature at 750 rpm for 16 h. An aliquot (0.1 mL) of reaction mixture was diluted with D_2O and analysed by ^{19}F NMR spectroscopy (**Table S4**).

Table S4 Stability data

Entry	Temperature (ºC)	Hydrolysis (%)
1	20	0
2	25	0
3	30	6.7
4	35	13.8

Therefore, **4d** is stable in 0.06M Na_2HPO_4: 0.06M KH_2PO_4 Buffer (3:1, pH 7.3) at 20-25 ºC for 16 h and enzymatic bio-transformations in this media can be investigated in the 20-25 ºC temperature range.

SI-1.5 General procedure for analytical scale achiral hydrolase resolution of 4d



To a 1.5 mL Eppendorf vial containing hydrolase (4 mg) was added an aqueous solution of methyl 3-fluoro-2-oxopiperidine-3-carboxylate **4d** (25.8mM in 60mM Na_2HPO_4: 60mM KH_2PO_4 Buffer; 3:1; pH 7.3, 20 °C, 400 µL, 10.3 µmol). The vessel was shaken at 20 °C, 750 rpm for 7 h before methyl 1,4-dimethoxybenzene (9.37 mMol in MeOH, 1100 µL, 10.3 µmol) was added as an internal standard. The reaction mixture was shaken at 20 °C, 1100 rpm for a further 5 min.

After sufficient mixing, an aliquot (400 µL) was taken and filtered using Sartorius Stedmin 5 kDa centrifuge filters (15 °C, 13,000 rpm, 12 min). The filtrates were analyzed by UPLC-MS (**SI-3**) and reaction conversion was calculated based on relative UV response to the internal standard (**SI-1.5; SI-3.3**).

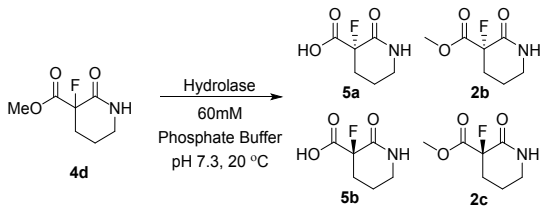
Samples with promising conversion values (10 – 60 %, **Table S6**) were concentrated at 20 °C (0.5 mbar) and organic materials were resuspended in methanol (50 µL), filtered and analysed by chiral HPLC (**SI-1.5, Table S6; SI-3.3**).

Table S5 Chiral resolution screening

Entry	Hydrolase	Yield (%)	Entry	Hydrolase	Yield (%)	Entry	Hydrolase	Yield (%)
1	JM X1	13	20	JM X20	17	39	JM X39	47
2	JM X2	3	21	JM X21	90	40	JM X40	5
3	JM X3	23	22	JM X22	5	41	JM X41	19
4	JM X4	2	23	JM X23	16	42	JM X42	3
5	JM X5	57	24	JM X24	59	43	JM X43	66
6	JM X6	2	25	JM X25	4	44	JM X44	39
7	JM X7	17	26	JM X26	3	45	JM X45	53
8	JM X8	2	27	JM X27	2	46	JM X46	2
9	JM X9	2	28	JM X28	2	47	JM X47	12
10	JM X10	3	29	JM X29	4	48	JM X48	50
11	JM X11	2	30	JM X30	23	49	JM X49	3
12	JM X12	42	31	JM X31	24	50	JM X50	28
13	JM X13	4	32	JM X32	13	51	JM EST 101	8
14	JM X14	30	33	JM X33	3	52	JM EST 102	100
15	JM X15	20	34	JM X34	3	53	PLE	100
16	JM X16	9	35	JM X35	19	54	CAL-B 10,000	51
17	JM X17	2	36	JM X36	0	55	PSL	0
18	JM X18	2	37	JM X37	14	56	Codexis EST002	99
19	JM X19	24	38	JM X38	16			

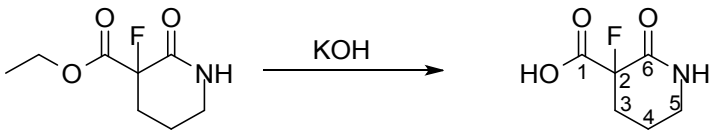
Table S5 Yield Key	0-10	10-25	25-50	50-60	60-100

Table S6 Chiral resolution analysis



Entry	Hydrolase	Yield (%)	Acid 5		Ester 2	
			ee	Stereo.	*ee*	Stereo.
1	**JM X1**	13	58	(*R*)	23	(*S*)
2	**JM X3**	23	46	(*R*)	70	(*S*)
3	**JM X5**	57	0	(±)	0	(±)
4	**JM X7**	17	0	(±)	0	(±)
5	**JM X12**	42	>95	(*S*)	16	(*R*)
6	**JM X14**	30	>95	(*S*)	62	(*R*)
7	**JM X15**	20	6	(*R*)	5	(*S*)
8	**JM X19**	24	20	(*R*)	3	(*S*)
9	**JM X20**	17	>95	(*S*)	24	(*R*)
10	**JM X23**	16	>95	(*S*)	21	(*R*)
11	**JM X24**	59	0	(±)	0	(±)
12	**JM X30**	23	86	(*S*)	47	(*R*)
13	**JM X31**	24	7	(*R*)	2	(*S*)
14	**JM X32**	13	4	(*R*)	5	(*S*)
15	**JM X35**	19	>95	(*S*)	19	(*R*)
16	**JM X37**	14	>95	(*S*)	11	(*R*)
17	**JM X38**	16	7	(*S*)	3	(*R*)
18	**JM X39**	47	52	(*R*)	45	(*S*)
19	**JM X41**	19	>95	(*S*)	11	(*R*)
20	**JM X44**	39	67	(*S*)	>99	(*S*)
21	**JM X45**	53	60	(*R*)	84	(*S*)
22	**JM X47**	12	52	(*R*)	63	(*S*)
23	**JM X48**	50	49	(*R*)	71	(±)
24	**JM X50**	28	>95	(*S*)	37	(*R*)
25	**CAL-B 10,000**	51	>95	(*R*)	>95	(*S*)

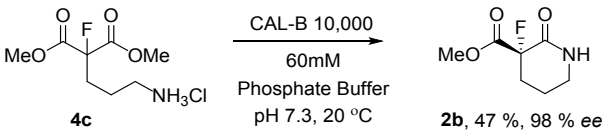
SI-1.6 Synthesis of racemic 3-fluoro-2-oxopiperidine-3-carboxylic acid 5 as a standard



A mixture consisting of KOH (0.27 g, 4.80 mmol), 3-fluoro-2-oxopiperidine-3-carboxylate **4d** (0.89 g, 4.70 mmol) and ethanol (5 mL) was stirred at RT for 16 h. The mixture was concentrated to give a white solid (0.83 g) which was washed with TBME (2 x 5 mL) and EtOH (2 x 5mL) to give a glassy white solid (0.80 g). The solid was placed on a watch-glass and dissolved in H_2O (0.8 mL). The resultant aqueous solution was cooled in an ice bath (0 ºC, external) before acidification to pH 4 by dropwise addition of 25% aq. HCl (0.1 mL). After 15 min a white crystalline suspension had formed and, NaCl was added to the ice bath (-3 ºC external) to assist the crystallisation process and, after a further 5 min, the acidic aqueous solution was carefully removed *via* syringe.

The remaining white crystalline solid was triturated with water (0.5 mL) and TBME (3 x 5 mL) at -3 ºC (external) and dried in a vacuum oven at RT to give racemic *3-fluoro-2-oxopiperidine-3-carboxylic acid* **5** (0.38 g, 50 %) as white needles; mp 101-103 ºC; ([MH]$^+$, 162.0561, $C_6H_9FNO_3$ requires [MH]$^+$, 162.0561); IR (neat, cm^{-1}) 3498, 3216, 3084w, 2960, 2884, 1663, 1620, 1493; ^{1}H NMR (400 MHz, MeOH-d_4) δ 3.39 – 3.33 (2H, m, CH_2NH), 2.43 – 2.27 (1H, m, CH), 2.22 – 2.08 (1H, m, CH), 2.06 – 1.93 (1H, m, CH), 1.91 – 1.77 (1H, m, CH); ^{19}F NMR (376 MHz, MeOH-d_4) δ -148.39 (dd, $^3J_{HF}$ 25.3, $^3J_{HF}$ 19.2); ^{13}C NMR (101 MHz, MeOH-d_4) δ 174.8 (d, $^2J_{CF}$ 22.9, OCNH), 169.9 (d, $^2J_{CF}$ 23.9, CO_2H), 93.5 (d, $^1J_{CF}$ 185.6, CF), 43.0 (s, CH_2NH), 32.9 (d, $^2J_{CF}$ 23.3, CH_2CF), 20.11 (d, $^3J_{CF}$ 3.1, C-4).

SI-1.7 Telescoped Formation of 2b with catalyst recycling



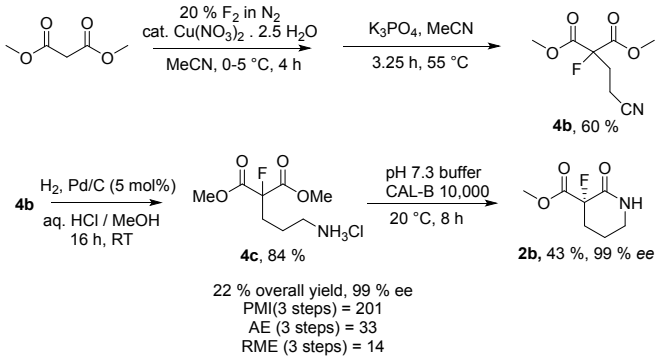
4c **2b**, 47 %, 98 % *ee*

No loss of CAL-B activity after 3 uses

To a 15 mL flask was added 3:1 0.06M Na_2HPO_4: 0.06M KH_2PO_4 buffer (4 mL, pH 7.3, 20 °C) followed by **4c** (500 mg, 2.05 mmol) in small portions using 0.5M NaOH to buffer the solution to pH 7.3. The solution was filled to 8 mL total volume by addition of 0.06M Na_2HPO_4: 0.06M KH_2PO_4 buffer (3:1, pH 7.3) to give a 257 mM solution.

Fermase immobilised CAL-B 10,000 (500 mg) was added to the reaction vessel which was shaken at 20 °C for 8 h. The reaction mixture was poured onto a Waters Oasis HLB 12cc 500mg LP Extraction cartridge containing a fluted filter paper under reduced pressure. Further buffer (3.5 mL) was added to the cartridge until the acid by-product was fully eluted (*ca*.1 mL per min) and the washed enzyme was removed for re-use. The ester was eluted from the cartridge with 10% formic acid (3.5 mL) and the solution was concentrated under reduced pressure at 0 °C to give (*S*)-*methyl 3-fluoro-2-oxopiperidine-3-carboxylate* **2b** (170 mg, 47 %, 98 % *ee*) as a white solid; $[\alpha]_D^{20}$ +14.393° (*c* 1.00, MeCN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.26 (1H, br s), 3.89 (3H, s), 3.53 - 3.37 (2H, m), 2.51 - 2.22 (2H, m), 2.14 - 1.89 (2H, m); ^{19}F NMR (376 MHz, $CDCl_3$) δ -153.06 (dd, $^3J_{HF}$ 20.0, $^3J_{HF}$ 28.0); absolute stereochemistry was determined by X-ray crystallography (**SI-4**) and purity determined by HPLC (**SI-3**).

SI-1.8 Telescoped synthesis of 4b (Scheme 6)



General procedure: base screening experiment

Dimethyl fluoromalonate **4a** was added to a solution of base and other additive as appropriate in methanol or acetonitrile before acrylonitrile was added and the solution stirred for the stated time (Table) at RT. Analysis of the crude reaction mixture by ^{19}F NMR spectroscopy gave reaction conversion.

Table S9 Michael addition reactions using different bases

Base	Dimethyl fluoromalonate	Acrylonitrile	Other	Solvent /mL	Time /h	Conv* /%
K_2CO_3, 2.76 g, 1.3 eq.	1.5 g, 10 mmol	0.58 g, 1.1 eq.	Aliquat 336 0.2 g, 0.05 eq.	MeOH 40	0.5	(≥ 99)
NaOMe, 0.05 g Na, 0.2 eq.	1.5 g, 10 mmol	1.06 g, 2 eq.	-	MeOH 40	1	90
NaOMe, 0.46 g Na, 0.2 eq.	15 g, 100 mmol	5.8 g, 1.1 eq.	-	MeOH 150	0.5	94
DBU 0.30 g, 0.2 eq.	1.5 g, 10 mmol	0.64 g, 1.2 eq.	-	MeCN 10	2	(≥ 99)
DBU 0.76 g, 0.25 eq.	2.64 g, 20 mmol	1.33 g, 1.25 eq.	$Cu(NO_3)_2{\cdot}2.5H_2O$ 0.47 g, 0.1 eq.	MeCN 20	17	(20)
DBU 1.52 g, 0.5 eq.	2.64 g, 20 mmol	1.33 g, 1.25 eq.	$Cu(NO_3)_2{\cdot}2.5H_2O$ 0.47 g, 0.1 eq.	MeCN 20	2	(23)

DBU 3.04 g, 1 eq.	2.64 g, 20 mmol	1.33 g, 1.25 eq.	$Cu(NO_3)_2{\cdot}2.5H_2O$ 0.47 g, 0.1 eq.	MeCN 20	1.5	(≥ 99)
K_3PO_4 0.13 g, 0.2 eq.	0.45 g, 3 mmol	0.19 g, 1.2 eq.	-	-	1.5	(≥ 99)
K_3PO_4 0.13 g, 0.2 eq.	0.45 g, 3 mmol	0.19 g, 1.2 eq	$Cu(NO_3)_2{\cdot}2.5H_2O$ 0.07 g, 0.1 eq.	MeCN 3	1.5	(0)
K_3PO_4 0.64 g, 1 eq.	0.45 g, 3 mmol	0.19 g, 1.2 eq	$Cu(NO_3)_2{\cdot}2.5H_2O$ 0.07 g, 0.1 eq.	MeCN 3	1.5	(≥ 99)
2-Methyl pyridine 0.06 g, 0.2 eq.	0.45 g, 3 mmol	0.19 g, 1.2 eq	-	-	1.5	(0)
2-Methyl pyridine 0.06 g, 0.2 eq.	0.45 g, 3 mmol	0.19 g, 1.2 eq	$Cu(NO_3)_2{\cdot}2.5H_2O$ 0.07 g, 0.1 eq.	MeCN 3	1.5	(0)

* Conversions determined by ^{19}F NMR analysis of crude reaction mixtures.

Optimised telescoped synthesis of 4b

Dimethyl malonate **4a** (26.40 g, 200 mmol) and copper (II) nitrate hemi(pentahydrate) (4.65 g, 20 mmol) were dissolved in acetonitrile (100 mL) and the mixture was cooled to 0-5 °C and stirred at 650 rpm using an overhead stirrer. After purging the system with N_2 for 5 minutes, fluorine gas (20 % *v/v* in N_2, 100 mL/min, 220 mmol) was introduced into the reaction mixture for 4 h 25 min. After purging with nitrogen for 5 min, potassium phosphate tribasic (anhydrous) (42.45 g, 200 mmol) was added to the reaction mixture and stirred. After 1 h the potassium phosphate was removed by filtration and washed with acetonitrile (2 x 20 mL) before a further portion of potassium phosphate (42.45 g, 200 mmol) was added to the solution which was heated to 55 ^oC. Acrylonitrile (12.73 g, 240 mmol) in acetonitrile (10 mL) was added over 30 min and the solution stirred. After a further 3.25 h the potassium phosphate was removed by filtration and washed with acetonitrile (3 x 20 mL) and the filtrate was concentrated *in vacuo*. Vacuum distillation (140 – 141 ^oC, 6 mbar) of the crude product yielded *dimethyl 2-(2-cyanoethyl)-2-fluoromalonate* **4b** (24.45 g, 60 %) as a clear oil; physical and spectroscopic data as above.

SI-1.9 Optimisation of the nitrile reduction for synthesis of 4c

Typical procedure

Dimethyl 2-(2-cyanoethyl)-2-fluoromalonate **4b** (360 mg, 1.90 mmol), 10% Pd/C (94 mg, 5 mol %) and conc. HCl (165 μL) in methanol (1.56 mL) were reacted in a glass hydrogenation vessel (H_2, 4 bar). After 8 h the solution was filtered through celite (100 mg) with methanol (400 μL) and evaporated to give *dimethyl 2-(3-aminopropyl)-2-fluoromalonate, hydrochloride salt* **4c** (434 mg, 99 %) as white crystals; physical and spectroscopic data as above.

Reactions were carried out as described above using varying quantities of Pd/C, HCl, H_2O and MeOH at appropriate temperatures as given in Table S8. The crude filtrates were diluted with MeOH-d_4 and analysed by ^{19}F NMR spectroscopy.

Table S10 Nitrile reduction optimisation

Ent.	Pd/C (mol %)	HCl (equiv.)	H_2O (wt %)	MeOH (wt %)	Conv. (%)*	Press. (bar)	Temp. (°C)
1	5.0	1.48	238	1028	24	3	25
2	5.0	1.48	45	1028	42	3	20
3	5.0	1.48	45	1028	47	3	30
4	5.0	1.48	45	1028	49	3	25
5	5.0	1.48	45	1028	31	1	25
6	5.0	1.48	45	1028	43	2	25
7	5.0	1.48	45	1028	57	4	25
8	2.5	1.48	45	1028	26	4	25
9	1.25	1.48	45	1028	28	4	25
10	0.63	1.48	45	1028	21	4	25
11	5.0	0.74	23	1028	94	4	25
12	5.0	0.37	11	1028	59	4	25
13	5.0	0.18	6	1028	23	4	25

14	5.0	0.37	11	514	24	4	25
15	5.0	0.74	23	514	100	4	25
16	2.5	0.37	11	514	24	4	25
17	2.5	0.74	23	514	53	4	25
18	5.0	1.11	35	514	100	4	25
19	5.0	1.11	35	343	100	4	25
20	5.0	1.11	35	257	74	4	25

* Conversion determined by ^{19}F NMR spectroscopy.

The reaction was repeated using 2M HCl determine whether the reaction would tolerate greater quantities of water (**Table 1, entry 1**) but several hydrolysis products and a lower conversion after 8 h was obtained (**entry 4**). At 20 ^{o}C the reaction proceeded slightly slower (**entry 2**) but warming the reaction to 30 ^{o}C gave rise to significant amounts of hydrolysis product. The reaction was also attempted at various H_2 pressures (**entries 5-7**) and at 4 bar the reaction proceeded faster (57 %) and with a very similar impurity profile as before. Reducing the catalyst loadings (**entries 8-10**) slowed the reaction considerably. Additionally, reducing the amount of aqueous 37 % HCl (**entries 11-13**) appeared to considerably suppress the formation of hydrolysis by-products, such that 0.74 equiv. gave excellent conversion to the amine HCl salt (**entry 11**). A 50 % reduction in the volume of methanol gave the desired product quantitatively (**entry 15**). Reducing the amount of HCl (**entry 13**) or Pd/C (**entries 16-17**) further at this concentration was found to retard reaction progress significantly. The reaction was repeated using 1.1 equiv. of HCl, such that the hydrochloride salt could be easily isolated after reduction and under these conditions (**entry 18**) the desired product was obtained quantitatively. Solvent volumes could be further reduced by one third without any decrease in conversion, giving the desired product in 99 % isolated yield (**entry 19**). However, reducing the solvent volumes even further led to lower yields (**entry 20**).

Scale-up of the nitrile reduction

To a Hastelloy autoclave 10 % Pd/C (2.62 g, 5 mol %) and conc. HCl (4.85 mL) were added. A solution of *dimethyl 2-(2-cyanoethyl)-2-fluoromalonate* **4b** (10 g, 49.2 mmol) in methanol (43.3 mL) was added and the vessel sealed. The vessel was pressurized with H_2 (4 bar) and the contents were stirred at 600 rpm. After 16 h the solution was filtered through celite (2 g) with methanol (20 mL) and evaporated to give crude **4c**. The solid was washed with methanol (2 x 20 mL) and acetone (2 x 15 mL) to give *dimethyl 2-(3-aminopropyl)-2-fluoromalonate, hydrochloride salt* **4c** (10.43 g, 84 %) as white crystals; physical and spectroscopic data as above.

SI-1.10 Scaled-up Telescoped Formation of 2b

To a 500 mL round bottomed flask was added 0.06M Na_2HPO_4: 0.06M KH_2PO_4 buffer (164 mL, 3:1, pH 7.3)followed by **4c** (10.00 g, 41.0 mmol) in small portions using 0.5M NaOH to buffer the solution to pH 7.3. The solution was filled to 328 mL total volume with further 0.06M Na_2HPO_4: 0.06M KH_2PO_4 buffer (3:1, pH 7.3) to give a 257mM solution.

Fermase immobilised CAL-B 10,000 (7.2 g) was added to the reaction mixture which was stirred at 100 rpm at 20 ^oC for 8 h. The reaction mixture was poured onto a Waters Oasis HLB 12 cc 5 g LP extraction cartridge under reduced pressure and water (30 mL) was added to the cartridge such that the acid was fully eluted (*ca.* 1 mL per min). The washed enzyme was removed and the ester was eluted with 20 % formic acid (30 mL). The solution was concentrated under reduced pressure at RT to give a solid, which was recrystallised from acetone (10 mL) to give (*S*)-*methyl 3-fluoro-2-oxopiperidine-3-carboxylate* **2b** (3.15 g, 44 %, >99 % *ee*) as white crystals; ^1H NMR (400 MHz, methanol-d_4) δ 3.81 (3H, s), 3.43 - 3.34 (2H, m), 2.47 - 2.14 (2H, m), 2.01 - 1.76 (2H, m); ^{19}F NMR (376 MHz, methanol-d_4) δ -153.06 (dd, $^3J_{HF}$ 20.0, $^3J_{HF}$ 28.0).

^{1}H and ^{19}F NMR spectra of (*S*)-methyl 3-fluoro-2-oxopiperidine-3-carboxylate **2b** prepared by scaled-up process.