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α-Amino-β-fluorocyclopropanecarboxylic acids as a new tool for drug development: Synthesis of glutamic acid analogs and agonist activity towards metabotropic glutamate receptor 4

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ABSTRACT

Herein we describe the diastereoselective synthesis of glutamic acid analogs and the evaluation of their agonist activity towards metabotropic glutamate receptor subtype 4 (mGluR4). These analogs are based on a monofluorinated cyclopropane core substituted with an α-aminoacid function. The potential of this new building block as a tool for the development of a novel class of drugs is demonstrated with racemic analog **11a** that displayed the best agonist activity with an EC50 of 340 nM.

1. Introduction

Glutamic acid (Glu **1**, Fig. 1) is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). It is involved in numerous disorders and synaptic receptors of glutamate have been extensively studied. It appeared that glutamate activates ionotropic receptors (iGluR) as well as metabotropic receptors (mGluR). iGlu receptors are responsible for the migration of Ca^{2+}, Na^{+}, K^{+} and Mg^{2+} ions through cellular membranes, thus triggering excitatory post-synaptic current, whereas mGlu receptors are G-protein coupled receptors (GPCR) that regulate the activity of either ion channels or enzymes via the modulation of second messenger concentration.[1] Eight subtypes of mGluR have been identified and classified into three groups, according to sequence similarity, mechanism of transduction and agonist pharmacology.[2]

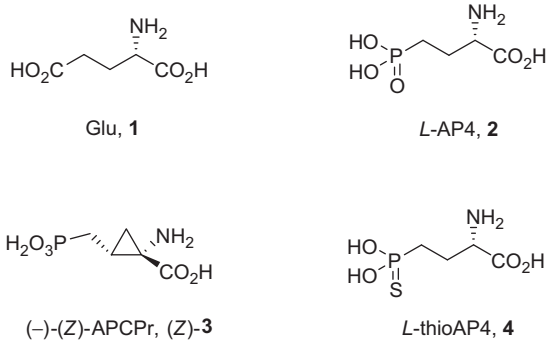

Figure 1. Glutamic acid and agonist analogs.

Even though the preparation of iGlu receptor agonists has been studied,[3] the glutamate metabotropic receptors have become the most valuable therapeutic targets.[4–9] In particular, group III mGlu receptors (mGluR4, mGluR6-8) are mostly presynaptic receptors that inhibit the production of cyclic adenosine monophosphate (cAMP) by adenylyl cyclase, thus reducing glutamate release in the synapse and, consequently, post-synaptic iGluR activation.[10] Excessive glutamate release is involved in numerous pathology and group III mGlu receptors play an important role in disorders such as anxiety,[11] pain,[12–15] tumor cell growth[16] or neurodegenerative disorders such as Parkinson's disease (PD).[5,17–20] Interestingly, mGluR4 has been shown to play a specific role in the regulation of neurotransmission in the basal ganglia in rat models of PD, suggesting that selective agonists of this subtype could have significant utility for the symptomatic treatment of this neuropathology.[21–23]

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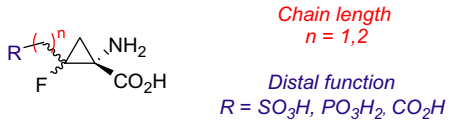


Figure 2. General scheme of targeted molecules.

Numerous efficient agonists of group III mGlu receptors, cyclic or linear analogs of glutamate, have been discovered.[24–29] It was found that the nature of the distal acidic function is essential to increase both selectivity and affinity for group III mGlu receptor. Hence, *L*-2-amino-4-phosphonobutyric acid (*L*-AP4 **2**) shows a 20-fold increase in affinity for mGluR4 when compared with glutamic acid. (−)-(*Z*)-1-amino-2-phosphonomethylcyclopropane carboxylic acid ((−)-(*Z*)-APCPr, (*Z*)-**3**),[30] the cyclopropanic analog of *L*-AP4, has a comparable affinity at mGluR4, showing that the 1-aminocyclopropylcarboxylic acid building block is compatible with these receptors.[31] Interestingly, exchanging the phosphonic acid function of *L*-AP4 for a thiophosphonic acid resulted in a two times increase of activity at mGluR4.[29] Acher et al. established a direct link between the stronger second acidity of thiophosphonic acid function of *L*-2-amino-4-thiophosphonobutyric acid (*L*-thioAP4 **4**) and the better activity at mGlu4 receptor.

As part of our program directed toward the preparation of novel fluorinated building blocks and their incorporation into bioactive compounds, we developed a method of synthesis of highly functionalized fluorinated cyclopropanes and designed potentially active glutamic acid analogs (Fig. 2).

The structural diversity of existing agonists of mGlu4 receptor permits a concrete evaluation of the potential of monofluorinated cyclopropane building-block for the development of new drug candidates. In that way we prepared functional and structural cyclopropanic analogs of glutamic acid (**1**) based on agonists *L*-AP4 **2** and APCPr **3**. It is well known that physico-chemical properties (acidity and basicity of substituents, global lipophilicity, bond length, electronical shape) of a molecule and, in particular, of a cyclopropane ring can be significantly modified by introducing a fluorine atom.[32,33] According to the results obtained with *L*-thioAP4 (**4**), we envisioned that the inductive effect of the fluorine atom could confer to the distal function a stronger acidity, thus increasing the activity at the mGluR4.

2. Chemistry

Recently, our team developed rapid and atom economical syntheses of fluorinated scaffolds using ethyl dibromofluoroacetate **4**, diethylzinc and a carbonyl compound.[34–37] We envisioned applying this strategy to the synthesis of monofluorinated cyclopropane via Michael induced ring closure (MIRC) reaction using appropriate Michael acceptors. Although Et_2Zn is known to promote the polymerization of acrylates,[38] the protected aminoacrylate **5** was found to give the corresponding fluorinated cyclopropane **6** with 63% yield and 2:1 dr (Scheme 1). Relative configurations of the substituents of diastereoisomers were determined by 19F-1H HOESY 2D NMR of the mixture (Fig. 3). A correlation between hydrogen atoms of the methyl ester group and the fluorine atom is observed for the minor isomer, thus indicating a *E* configuration for this isomer.

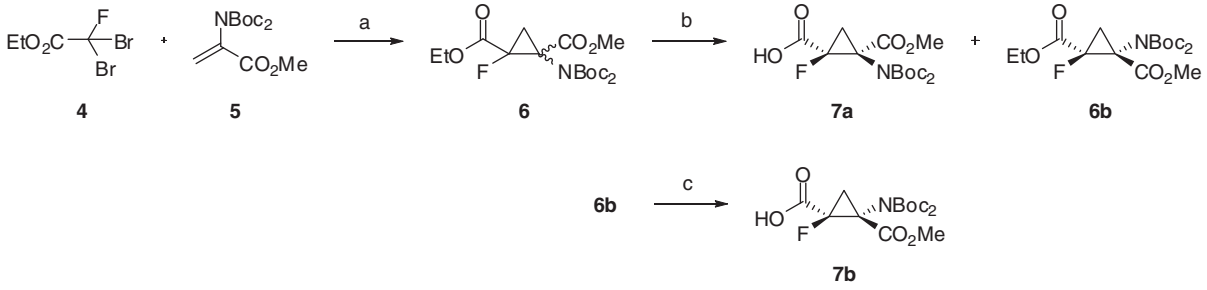
The cyclopropane diester **6** can be diastereoselectively and regioselectively saponified using lithium hydroxyde at 0 °C,[39] thus giving after acid-basic extraction the corresponding *Z* isomer of carboxylic acid **7a** and the *E* isomer of diester **6b** as pure diastereoisomers. Then, diester **6b** can be saponified regioselectively using lithium hydroxide at room temperature Scheme 2.

To evaluate the influence of the α-amino-β-fluorocyclopropanecarboxylic acid scaffold on the binding property of a molecule, we decided to synthesize racemic mixtures of constrained analogs of glutamic acid. At first, we prepared 1-amino-2-fluoro-2-phosphonomethylcyclopropane carboxylic acid (FAP4), a fluorinated cyclopropane analog of APCPr **3** in a 4 steps sequence. The *Z* isomer **7a** was reduced to alcohol **8a** which was activated by mesylation and iodination to undergo an Arbuzov condensation with triethylphosphite, thus leading to the corresponding phosphonate **10a**. Deprotection upon treatment with concentrated HCl in AcOH gave analog (±)-(*Z*)-FAP4 **11a** in 21% overall yield from the carboxylic acid **7a**. Unfortunately, applying the sequence to the *E* isomer **7b** did not succeed.

The synthesis of homologated analogs, 1-amino-2-(2-carboxyethyl)-2-fluorocyclopropanecarboxylic acids (FAC5), is depicted in Scheme 3. Aldehydes **12a** and **12b** were obtained from alcohols **8a** and **8b** by oxidation with IBX in nearly quantitative yield. These aldehydes were homologated to give compounds **13a**, **15a** and **15b** via Horner-Wadsworth-Emmons condensation followed by hydrogenation of resulting alkenes. The analog bearing a carboxylic acid **14a** (±)-(*Z*)-FAC5 was obtained after saponification of ester functions and removal of protecting groups of the amine function in acidic conditions (23% from **7a**). Both *Z* and *E* isomers of 1-amino-2-fluoro-2-phosphonoethylcyclopropanecarboxylic acid (FAP5), (±)-(*Z*)-FAP5 **16a** and (±)-(*E*)-FAP5 **16b,** were obtained after deprotection of all protecting groups using concentrated HCl in AcOH (21% and 5% from **7a** and **6b**, respectively).

1-Amino-2-carboxymethyl-2-fluorocyclopropanecarboxylic acid (FAC4) analogs (±)-(*Z*)-FAC4 **18a** and (±)-(*E*)-FAC4 **18b** were synthesized from corresponding alcohols **8a** and **8b** through a Mitsunobu reaction with 2-hydroxy-2-methylpropanenitrile followed by hydrolysis of the nitrile function and aminoacid protections with HCl 6 N (4% and 12% from **7a** and **6b**, respectively). It appeared that the amine function of **8b** needs to be previously monodeprotected with LiBr for the Mitsunobu reaction to occur (Scheme 4). Only the *Z* diastereoisomer was tested as agonist of mGluR4 receptor.

Isomers of 1-amino-2-fluoro-2-sulfomethylcyclopropane carboxylic acid (FAS4), (±)-(*Z*)-FAS4 **20a** and (±)-(*E*)-FAS4 **20b,** were



Scheme 1. Reagents and conditions: (a) Et_2Zn, THF, rt; (b) LiOH, THF:H_2O, 0 °C; (c) LiOH, THF:H_2O, rt.

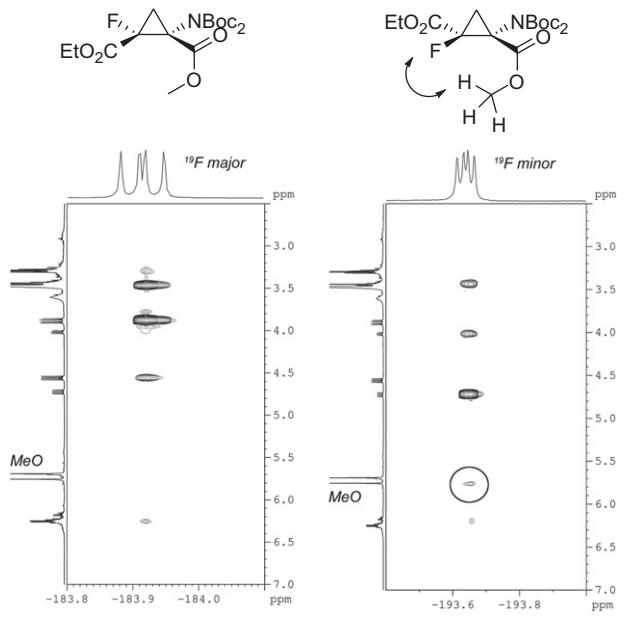


Figure 3. 19F-1H HOESY 2D NMR of the 2:1 diastereoisomeric mixture of **6**.

prepared in a similar way to carboxylic acid analogs using thioacetic acid as the nucleophile in the Mitsunobu reaction (Scheme 5). One-pot oxidation and hydrolysis with HCl 6 N afforded the desired compounds (52% and 10% from **7a** and **6b**, respectively).

Finally, to examine the influence of an extra binding group next to the phosphonic acid function, 1-amino-2-fluoro-2-(hydroxy-(phosphono)methyl)cyclopropanecarboxylic acid isomers, (±)-(*Z*)-HFAP4 **22a** and (±)-(*E*)-HFAP4 **22b,** were prepared. Starting from aldehydes **12a** and **12b**, diethylphosphite was added and subsequent deprotection with concentrated HCl in AcOH (66% and 19% from **21a** and **21b**, respectively) gave the desired products Scheme 6.

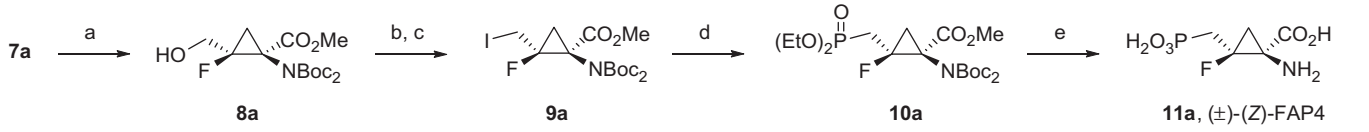
3. Pharmacological assays on recombinant mGluRs

The function of nine derivatives of α-amino-β-fluorocyclopropanecarboxylic acid was tested functionally on mGlu4 transiently transfected in HEK293 cells with a chimeric G protein allowing coupling of the receptor to the phospholipase-C (PLC) signaling cascade. Activation of this transduction pathway was determined measuring intracellular Ca^{2+} release using Fluo4 as a fluorescent probe.

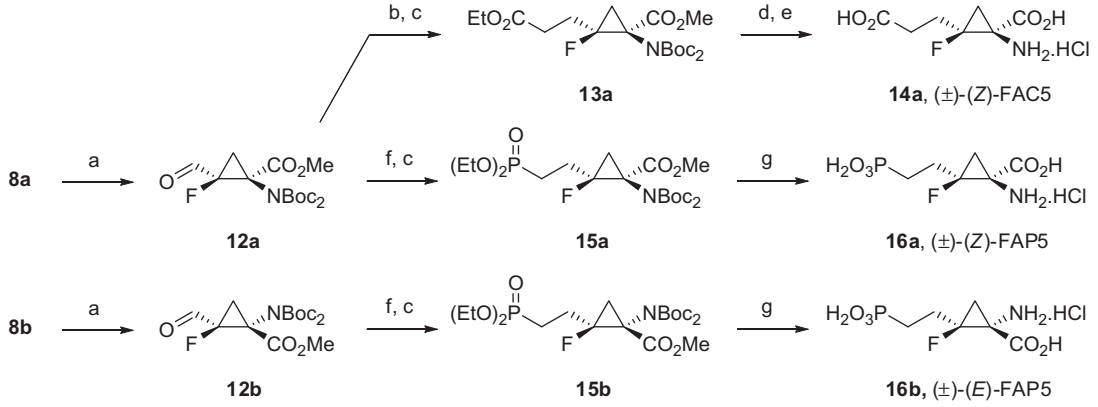
In a first series of experiments, a single dose of each derivative was added to the mGlu4 receptor expressing cells (Fig. 4A). At 100 μM, compound (*Z*)-FAP4 (**11a**) induced an activation of mGlu4 that was comparable to that of the natural agonist glutamate, which was used as a positive control in these experiments, while all other compounds induced a lower level of activation.

In a second series of experiments, dose-response experiments were performed with the six most active compounds in order to determine their potency, EC50 values are shown in Table 1. Dose-response curves of the 3 most active compounds, (*Z*)-FAP4 (**11a**), (*Z*)-FAS4 (**20a**) and (*Z*)-HFAP4 (**22a**), are depicted on Fig. 4B. The most potent compound is (*Z*)-FAP4 (**11a**). With an EC50 value of 0.34 ± 0.08 μM (n = 3), compound **11a** was almost 10-times more potent than glutamate (EC50 value of 2.88 ± 0.69 μM, n = 3) while its maximal efficacy was comparable to that of glutamate (Fig. 4B). In comparison, (*Z*)-HFAP4 (**22a**) and (*Z*)-FAS4 (**20a**) were less potent, with EC50 values of 27.4 ± 10.6 μM (n = 3) and 85.6 ± 15.6 μM (n = 3), respectively.

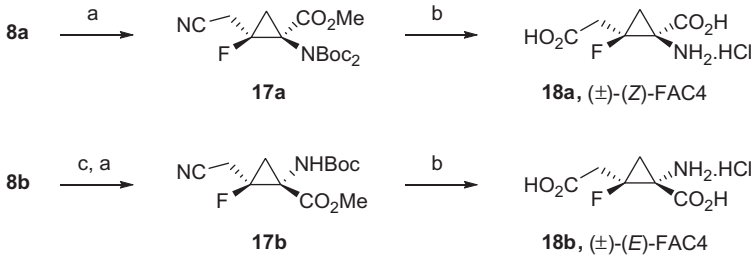
(*E*)-APCPr (*E*)-**3** has been reported to be 18-times less potent than *L*-AP4 at mGlu4 receptor.[41] In comparison, fluorinated analog **11a** is 2.6-times less potent than *L*-AP4. Although pharmacological experiment was different, this indicates that the racemic analog **11a** is about 7-times more potent than the corresponding non-fluorinated racemic analog (*E*)-APCPr (*E*)-**3** (Table 1). Acher's group observed that the second acidity of the distal function plays an important role in the recognition of agonists by mGlu4 receptor.[29] Consequently, to rationalize the increase in activity resulting from the introduction of a fluorine atom, dissociation constants of (*Z*)-



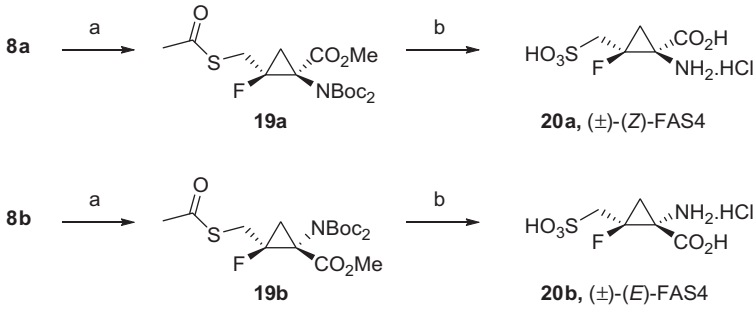
Scheme 2. Reagents and conditions: (a) BH_3 1 M in THF, 0 °C to rt; (b) MsCl, Et_3N, Et_2O, 0 °C to rt; (c) NaI, TBAI, acetone, reflux; (d) $P(OEt)_3$, sealed tube, 120 °C; (e) HCl 12 N, AcOH, 80 °C then Dowex.



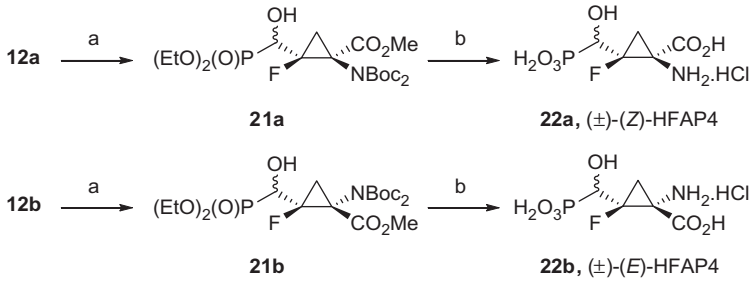
Scheme 3. Reagents and conditions: (a) IBX, EtOAc, reflux; (b) $(EtO)_2P(O)CH_2CO_2Et$, LiBr, Et_3N, THF, rt; (c) H_2, Pd/C, THF, rt; (d) LiOH, THF:H_2O, rt; (e) HCl 12 N, AcOH, rt; (f) $(EtO)_2P(O)CH_2P(O)(OEt)_2$, LiBr, Et_3N, THF, rt; (g) HCl 12 N, AcOH, reflux.



Scheme 4. Reagents and conditions: (a) 2-hydroxy-2-methylpropanenitrile, DBAD, PPh_3, rt; (b) HCl 6 N, 80 °C; (c) LiBr, CH_3CN, 60 °C.



Scheme 5. Reagents and conditions: (a) $CH_3C(O)SH$, DBAD, PPh_3, rt; (b) HCOOH, H_2O_2, rt then HCl 6 N, 80 °C.



Scheme 6. Reagents and conditions: (a) $HP(O)(OEt)_2$, Et_3N, THF, rt; (b) HCl 12 N, AcOH, reflux.

FAP4 **11a** and (*E*)-APCPr (*E*)-**3** were calculated using I-Lab 2.0 (Fig. 5).[42] According to calculated pK_{a3}, at physiological pH, APCPr **3** exists as a mix of monoanionic phosphonic acid and dianionic phosphonic acid (pK_{a3} = 6.7). In comparison, at this pH, the phosphonic acid function of FAP4 **11** is exclusively in dianionic form (pK_{a3} = 6.0). These differences in ionization states can account for the better binding of FAP4 **11** with basic residues in the mGlu4 receptor.

4. Materials and methods

4.1. Cell culture and transfection of recombinant mGluRs

Pharmacological experiments were performed in HEK293 cells. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum, penicillin and streptomycin (100 U/mL final)(GIBCO-BRL-Life Technologies, Inc. Cergy Pontoise, France). Twenty four hours prior to functional experiments, mGlu4 was transiently transfected in HEK293 cells by electroporation as described elsewhere,[43] together with a chimeric Gq/Gi-protein which couples the activation of the receptor to the PLC pathway and EAAC1, a high affinity glutamate transporter in order to avoid any influence of glutamate released by the cells in the assay medium and plated in 96-well microplates for functional assays.

4.2. Functional assay: intracellular calcium measurements

In our experiments, thanks to the use of the chimeric Gq/Gi-protein, $mGlu_4$ receptor activation of PLC induces the production of inositol phosphate (IP) which in turn induces intracellular Ca^{2+} release. Receptor activity can thus be determined by measurement of Ca^{2+} release, as already described.[44] We and others have previously reported that assays measuring the PLC pathway are more easily handled and gave more accurate results than the classical measurement of the inhibition of the forskolin-activated adenylyl-cyclase activity and that the pharmacology of these receptors was not altered.[45]

For intracellular calcium measurements, cells expressing mGluRs were loaded with Ca^{2+}-sensitive fluorescent dye Fluo-4 AM (Invitrogen, Cergy Pontoise, France) dissolved in Hanks' balanced salt solution (HBSS, Invitrogen, Cergy Pontoise, France) containing 2.5 mM Probenicid (Sigma-Aldrich Chemie, Saint-Quentin Fallavier, France) for 1 h at 37 °C, then washed and incubated with HBSS containing probenecid. A drug plate was prepared with the various concentrations of agonist to be tested and drug solution was added in each well after 20 s of recording. Fluorescence signals (excitation 485 nm, emission 525 nm) were measured by using the fluorescence microplate reader Flexstation III (Molecular Devices, Saint Grégoire, France) at sampling intervals of 1.5 s for 60 s. All points are realized in triplicate. The dose-response

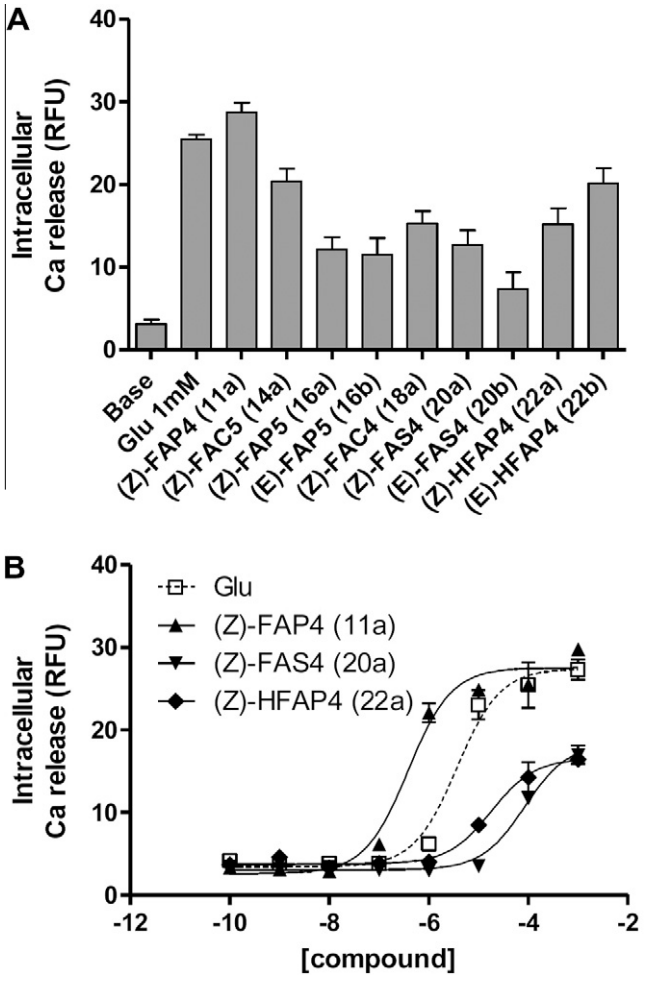


Figure 4. Activity of α-amino-β-fluorocyclopropanecarboxylic acid derivatives on mGlu4. Rat clone of mGlu4 was transiently transfected in HEK293 cells together with a chimeric G-protein and the high affinity glutamate transporter EAAC1. Receptor activity was determined by measurement of the release of intracellular calcium resulting from receptor activation upon ligand addition. (A) Effect of a single dose of each compound (100 μM) on mGlu4 activity, as compared to glutamate (1 mM). (B) Dose-dependent activation of mGlu4 by (*Z*)-FAP4 (**11a**), (*Z*)-FAS4 (**20a**), (*Z*)-HFAP4 (**22a**) and glutamate. Data presented are representative of $n = 3$ experiments. Each point corresponds to the mean ± SEM of triplicate.

Table 1
Agonist activities at mGlu4 receptor

Agonist		EC_{50} (μM)[a]
L-Glu	**1**	2.88 ± 0.696
L-AP4	**2**	0.13 ± 0.02[b]
(±)-(*E*)-APCPr	**(*E*)-3**	1.84 ± 0.186[c]
(±)-(*Z*)-FAP4	**11a**	0.34 ± 0.084
(±)-(*Z*)-FAC5	**14a**	609 ± 27
(±)-(*Z*)-FAC4	**18a**	465 ± 119
(±)-(*Z*)-FAS4	**20a**	85.6 ± 15.6
(±)-(*Z*)-HFAP4	**22a**	27.4 ± 10.6
(±)-(*E*)-HFAP4	**22b**	1400 ± 446

[a] Data are the mean ± SEM of 3 separate experiments.
[b] For this data, $n = 34$, see Ref. [40]
[c] Value calculated from Ref. [41] using *L*-AP4 as reference compound (EC_{50} (*E*)-**3** = 7.9 ± 0.8 μM with EC_{50} **2** = 0.43 ± 0.2 μM).

curves were fitted using the GraphPad Prism program and the following equation: y=[(ymax−ymin)/(1+(x/EC50)n)]+ymin where EC50 is the concentration of the compound necessary to obtain the half maximal effect and n is the Hill coefficient.

5. Conclusion

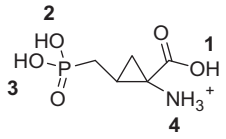
A range of racemic fluorinated cyclopropane analogs of glutamic acid have been synthesized and tested at mGluR4 receptors. Among these compounds, (*Z*)-FAP4 (**11a**) is almost 10-times more potent than glutamate. Moreover, using *L*-AP4 as reference compound, it is 7-times more potent than the racemic non-fluorinated analog (±)-(*E*)-APCPr,[41] thus highlighting the potential of monofluorinated cyclopropane bearing an aminoacid function as building-block for the development of new bioactive compounds. Further explorations concerning the synthesis of pure enantiomers of (*Z*)-FAP4 (**11a**) and racemic (*E*)-FAP4 are currently underway in our laboratory.

6. Experimental section

Reagents were commercial grade and were used as received unless otherwise noted. The structures of all tested compounds were consistent with their ^{1}H, ^{13}C, ^{19}F NMR, and mass spectra, and were judged to be >95% pure by combustion analysis. Experiments involving organometallic reagents were carried out under argon atmosphere. All moisture-sensitive reactants were handled under argon atmosphere. Low temperature experiments were carried out by cooling down the flasks with an acetone bath frozen by dry-ice. The flasks were equipped with septum caps. All commercial solvents were distilled before use: THF and Et_2O were distilled from sodium benzophenone ketyl under nitrogen atmosphere, DMF and DMSO over BaO and CH_2Cl_2 over P_2O_5. TLC was performed on Merck 60F-250 silica gel plates and column chromatography over silica gel SI 60 (230–240 mesh). Flash column chromatography purifications were carried out using silica gel (70–230 mesh). ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and ^{19}F NMR ($CFCl_3$ as external reference) were recorded at 300.13, 75.47, 121.49 and 282.40 MHz. Abbreviations used for peak multiplicity are s: singlet, d: doublet, t: triplet, q: quadriplet, m: multiplet, b: broad singlet, bm: broad multiplet. *J* was used to indicate coupling constant in Hertz. IR spectra were recorded on a Perkin-Elmer 1420. Absorption bands are reported in cm^{-1}. Elemental analyses were performed on CARLO ERBA 1106. Mass spectrums were performed on MCD HF 5970 quadripolar for electronic impact or on Thermofinnigan Navigator 2.1 for Electrospray.

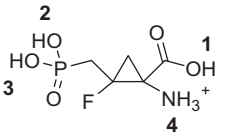
6.1. 2-Ethyl 1-methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-fluorocyclopropane-1,2-dicarboxylate 6

A solution under nitrogen of **5** (20.5 g, 68.2 mmol, 1 equiv) and ethyl dibromofluoroacetate (19.6 mL, 136.4 mmol, 2 equiv) in dry THF (60 mL) was heated at 50 °C. Diethyl zinc 1 N in hexanes (137 mL, 137 mmol, 2 equiv) was added dropwise over 2 h via a syringe pump and the resulting mixture was stirred 3 h at this temperature. After cooling at room temp, the mixture was poured into a stirred mixture of water (150 mL) and diethylether (150 mL). The heterogeneous mixture was filtered through a pad of celite, organic layer was separated and aqueous layer was extracted with diethylether. Organic layers were assembled, washed with 100 mL of brine and dried over $MgSO_4$. Removal of solvent afford 33 g of crude product which was purified by column chromatography (5% ethyl acetate, 1% triethylamine in cyclohexane) to afford 17.4 g (63%) of pure **6** as an unseparable mixture of *Z* (**6a**) and *E* (**6b**) diastereoisomers (67:33); R_f (30% EtOAc in cyclohexane) = 0.51; *Z* isomer (**6a**): ^{1}H NMR ($CDCl_3$), δ 4.29 (q, $J = 7.2$ Hz, 2H), 3.72 (s, 3H), 2.61 (dd, $J = 9.0$, 15.9, 1H), 1.90 (dd, $J = 9.0$, 20.7 Hz, 1H), 1.50 (s, 18H), 1.32 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 168.0 (d, $J = 2$ Hz), 164.5 (d, $J = 26$ Hz), 151.4, 83.8, 81.4 (d, $J = 242$ Hz), 62.6, 53.1, 46.8 (d, $J = 10$ Hz), 28.1, 22.7 (d, $J = 8$ Hz), 14.3; ^{19}F NMR ($CDCl_3$) δ −185.5 (dd, $J = 16$, 21 Hz); *E* isomer (**6b**):



APCPr

pK_{a1} 1.4, pK_{a2} 2.5, pK_{a3} 6.7, pK_{a4} 9.1



FAP4

pK_{a1} 0.8, pK_{a2} 2.1, pK_{a3} 6.0, pK_{a4} 7.5

Figure 5. Calculated pK_a values of (*E*)-APCPr (*E*)-**3** and (*Z*)-FAP4 **11a** using I-Lab 2.0.[42]

^{1}H NMR ($CDCl_3$) δ 4.22 (q, *J* = 7.2 Hz, 2H), 3.78 (s, 3H), 2.79 (dd, *J* = 8.4, 17.8 Hz, 1H), 2.06 (dd, *J* = 8.4, 10.8 Hz, 1H), 1.49 (s, 9H), 1.47 (s, 9H), 1.31 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.6, 165.7 (d, *J* = 35 Hz), 151.4, 83.7, 81.9 (d, *J* = 249 Hz), 62.8, 53.5, 48.5 (d, *J* = 14 Hz), 28.3, 28.0, 27.2 (d, *J* = 9 Hz), 14.2; ^{19}F NMR ($CDCl_3$) δ −195.5 (dd, *J* = 11, 18 Hz); MS (ESI$^+$) *m/z* 428.2 [M+Na]$^+$. Anal. Calcd for $C_{18}H_{28}FNO_8$: C, 53.33; H, 6.96; N, 3.45. Found: C, 52.95; H, 6.64; N, 3.71.

6.2. (*Z*)-2-(Bis(*tert*-butoxycarbonyl)amino)-1-fluoro-2-(methoxycarbonyl)cyclopropanecarboxylic acid 7a

Compound 6 (19.83 g, 48.9 mmol, 1 equiv) was solubilised in a solution of THF:water 10:1 (300 mL) and was cooled at 0 °C. A molar solution of lithium hydroxide (73 mL, 73 mmol, 1.5 equiv) was added dropwise and the mixture was stirred at this temperature for 2 h. The solution was then acidified to pH 2 by addition of a solution of HCl 1 N and extracted with EtOAc (3 × 200 mL). Solvents were removed and the oily mixture was taken up in Et_2O. Desired acid **7a** in its sodium salt form was extracted with Na_2CO_3 0.5 M (3 × 100 mL), the organic layer was washed with brine, dried over $MgSO_4$ and concentrated under vacuo to afford 7.05 g (36%) of **6b**. The aqueous layer was acidified at 0 °C by addition of HCl 4 N to pH 2, extracted with diethylether (4 × 100), dried over $MgSO_4$ and concentrated to furnish 11.43 g (62%) of **7a**; R_f (80% EtOAc in cyclohexane) = 0.21; ^{1}H NMR ($CDCl_3$) δ 8.54 (s, 1H), 3.72 (s, 3H), 2.63 (dd, *J* = 9.2, 16.9 Hz, 1H), 1.95 (dd, *J* = 9.2, 20.8 Hz, 1H), 1.48 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 168.3, 166.6 (d, *J* = 26 Hz), 151.8, 84.5, 81.3 (d, *J* = 241 Hz), 53.5, 46.8 (d, *J* = 10 Hz), 28.1, 26.7 (d, *J* = 9 Hz); ^{19}F NMR ($CDCl_3$) δ −183.3 (dd, *J* = 17, 21 Hz); MS (ESI$^-$) *m/z* 376.7 [M−H]$^-$. Anal. Calcd for $C_{16}H_{24}FNO_8$: C, 50.92; H, 6.41; N, 3.71. Found: C, 51.13; H, 6.65; N, 3.76.

6.3. (*E*)-2-(Bis(*tert*-butoxycarbonyl)amino)-1-fluoro-2-(methoxycarbonyl)cyclopropanecarboxylic acid 7b

The diester **6b** (1.64 g, 4.04 mmol, 1 equiv) was solubilised in a THF:water mix (5:2) (70 mL) and was cooled at 0 °C. A molar solution of lithium hydroxide (4.45 mL, 4.45 mmol, 1.1 equiv) was added dropwise and the mixture was allowed to warm to room temperature and stirred for 20 h. The solution was then acidified by addition of a solution of HCl 1 N to pH 3 and extracted with diethylether (200 mL). Organic layer was dried over $MgSO_4$ and concentrated to furnish 1.31 g (86%) of **7b**; ^{1}H NMR ($CDCl_3$) δ 7.50 (s, 1H), 3.75 (s, 3H), 2.77 (dd, *J* = 8.3, 17.5 Hz, 1H), 2.04 (dd, *J* = 8.3, 10.6 Hz, 1H), 1.43 (s, 9H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 169.1 (d, *J* = 26 Hz), 166.5, 151.2, 151.1, 84.4, 84.1, 81.5 (d, *J* = 249 Hz), 53.6, 46.6 (d, *J* = 14 Hz), 28.0 (d, *J* = 11 Hz), 27.8, 27.8; ^{19}F NMR ($CDCl_3$) δ −195.3 (dd, *J* 11, 18 Hz); MS (ESI$^-$) *m/z* 376.4 [M−H]$^-$.

6.4. (*Z*)-Methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-fluoro-2-(hydroxymethyl)cyclopropanecarboxylate 8a

The carboxylic acid **7a** (16.0 g, 42.5 mmol, 1 equiv) was dissolved at 0 °C in anhydrous THF (250 mL) under argon. A 1 M solution of BH_3.THF (130 mL, 130 mmol, 3 equiv) was added dropwise over a period of 30 min. The reaction was allowed to warm to room temperature and stirred overnight. Upon completion (TLC and ^{19}F NMR monitoring), it was then quenched by dropwise addition of water (150 mL), extracted with Et_2O (100 mL), ethyl acetate (2 × 100 mL), washed with brine (100 mL) and dried over magnesium sulfate. Removal of solvents and purification by column chromatography (5% ethyl acetate, 1% triethylamine in cyclohexane) afforded 15.07 g (97%) of pure desired alcohol **8a**.; R_f (50% EtOAc in cyclohexane) = 0.48; ^{1}H NMR ($CDCl_3$) δ 4.28 (dd, *J* = 13.1, 15.9 Hz, 1H), 4.08 (dd, *J* = 13.1, 30.5 Hz, 1H), 3.75 (s, 3H), 3.06 (s, 1H), 2.21 (dd, *J* = 8.6, 17.4 Hz, 1H), 1.80 (dd, *J* = 8.6, 22.0 Hz, 1H), 1.51 (s, 9H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.2, 153.2, 152.0, 86.4 (d, *J* = 233 Hz), 84.1, 83.6, 61.3 (d, *J* = 33 Hz), 53.4, 44.8 (d, *J* = 9 H), 28.3, 28.2, 27.2 (d, *J* = 9 Hz); ^{19}F NMR ($CDCl_3$) δ −177.8 (dddd, *J* = 16, 17, 22, 30 Hz); MS (ESI$^+$) *m/z* 385.9 [M+Na]$^+$.

6.5. (*E*)-Methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-fluoro-2-(hydroxymethyl)cyclopropanecarboxylate 8b

Carboxylic acid **7b** (3.40 g, 9.0 mmol, 1 equiv) was solubilised in anhydrous THF (30 mL) under argon. A 1 M solution of BH_3.THF (63 mL, 63 mmol, 7 equiv) was added dropwise over a period of 20 min. The reaction was allowed to warm to room temperature and stirred overnight. Upon completion (TLC and ^{19}F NMR monitoring), it was then quenched with 2 M HCl (20 mL). Water was added (50 mL), and the mixture was extracted with Et_2O. Organic phases were combined, washed with NH_4Cl, water, brine, dried over magnesium sulfate and concentrated. The resulting oily crude oil was purified by column chromatography (9% ethyl acetate, 1% triethylamine in cyclohexane) to afford 3.16 g (97%) of pure desired alcohol **8b**; R_f (50% EtOAc in cyclohexane) = 0.48; ^{1}H NMR ($CDCl_3$) δ 4.12 (dd, *J* = 11.1, 23.7 Hz, 1H), 3.79 (s, 3H), 3.67 (ddd, *J* = 2.1, 13.8, 23.4 Hz, 1H), 2.55 (dd, *J* = 8.7, 20.5 Hz, 1H), 1.57 (s, 9H), 1.49 (s, 9H), 1.15 (dd, *J* = 8.6, 11.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 167.9, 154.7, 152.2, 86.3 (d, *J* = 240 Hz), 84.9, 84.2, 62.6 (d, *J* = 20 Hz), 53.4, 47.5 (d, *J* = 16 Hz), 28.3, 28.2, 23.5 (d, *J* = 10 Hz); ^{19}F NMR ($CDCl_3$) δ −191.0; MS (ESI$^+$): *m/z* 363.8 [M+H]$^+$.

6.6. (*Z*)-Methyl 1-(bis(tert-butoxycarbonyl)amino)-2-fluoro-2-(iodomethyl)cyclopropanecarboxylate 9a

To a stirred solution of alcohol **8a** (644 mg, 1.77 mmol, 1 equiv) and triethylamine (0.21 mL, 3.63 mmol, 2.05 equiv) at 0 °C in Et_2O (10 mL) was added dropwise methansulfonyl chloride (0.50 mL, 2.66 mmol, 1.5 equiv). The resulting suspension was allowed to warm to room temperature under stirring until completion (TLC monitoring). The reaction was then quenched by addition of water (5 mL) and brine was added (5 mL). Organic layer was extracted with Et_2O (3 × 10 mL), dried over $MgSO_4$ and concentrated. The resulting crude product was purified by column chromatography (5–10% ethyl acetate, 1% triethylamine in cyclohexane) to afford 752 mg (96%) of pure mesylated alcohol; R_f (50% EtOAc in cyclohexane) = 0.53; ^{1}H NMR ($CDCl_3$) δ 4.94 (dd, *J* = 12.7, 28.0 Hz, 1H), 4.78 (ddd, *J* = 1.5, 12.7, 18.7 Hz, 1H), 3.76 (s, 3H), 3.08 (s, 3H), 2.23 (dd,

J = 8.8, 17.0 Hz, 1H), 1.86 (ddd, J = 1.5, 8.8, 20.7 Hz, 1H), 1.49 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 169.5 (d, J = 2 Hz), 151.9, 83.8, 82.8 (d, J = 236 Hz), 67.2 (d, J = 21 Hz), 53.6, 45.3 (d, J = 9 Hz), 38.2, 28.3, 28.2, 27.6 (d, J = 9 Hz); ^{19}F NMR ($CDCl_3$) δ −180.1. To a solution of the mesylated alcohol (4.30 g, 9.74 mmol, 1 equiv) in dry acetone (200 mL) was added sodium iodide (11.6 g, 77.92 mmol, 8 equiv) and tetra *n*-butylammonium iodide (1.80 g, 4.87 mmol, 0.5 equiv). The resulting mixture was stirred and heated at reflux until completion (^{19}F NMR and TLC monitoring). Solvent was removed, the resulting oily crude product was taken up in ethyl acetate, washed with $Na_2S_2O_3$ 10% aqueous solution, water, brine and dried over $MgSO_4$. Removal of solvent afford 5.17 g of crude product which was purified by column chromatography (5% ethyl acetate, 1% triethylamine in cyclohexane) to afford 3.18 g (68%) of pure desired product; R_f (20% EtOAc in cyclohexane) = 0.52; ^{1}H NMR ($CDCl_3$) δ 3.99 (dd, J = 11.5, 38.1 Hz, 1H), 3.75 (s, 3H), 3.65 (ddd, J = 1.8, 11.5, 9.9 Hz, 1H), 1.99 (dd, J = 8.7, 17.6 Hz, 1H), 1.76 (ddd, J = 1.8, 8.7, 21.1 Hz, 1H), 1.49 (s, 9H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 169.7 (d, J = 2 Hz), 151.6, 151.4, 85.4 (d, J = 232 Hz), 83.4, 83.3, 53.2, 50.2 (d, J = 9 Hz), 30.5 (d, J = 9 Hz), 28.1, 28.1, 2.0 (d, J = 23 Hz); ^{19}F NMR ($CDCl_3$) δ −168.8 (dddd, J_{FH} = 10, 18, 21, 38 Hz); MS (ESI$^+$) m/z 496.1 [M+Na]$^+$. Anal. Calcd for $C_{16}H_{25}FINO_6$: C, 40.60; H, 5.32; N, 2.96. Found: C, 40.62; H, 5.34; N, 2.88.

6.7. (*Z*)-Methyl-1-(bis(*tert*-butoxycarbonyl)amino)-2-((diethoxyphosphoryl)methyl)-2-fluorocyclopropanecarboxylate 10a

A mixture under argon of **9a** (238 mg, 0.50 mmol, 1 equiv) in distilled triethylphosphite (3 mL) was heated at 120 °C in a sealed tube until complete conversion (^{19}NMR and TLC monitoring). The excess of phosphite was then removed under vacuo without heating and the resulting oily crude product was purified by column chromatography (30% ethyl acetate, 1% triethylamine in cyclohexane) to afford 91 mg (37%) of **10a**; R_f (20% EtOAc in cyclohexane) = 0.35; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J = 7.0 Hz, 6H), 1.41 (s, 9H), 1.43 (s, 9H), 1.77 (dd, J = 8.8, 21.7 Hz, 1H), 2.12 (dd, J = 8.8, 14.3 Hz, 1H), 2.48 (m, 1H), 2.70 (ddd, J = 15.8, 21.1, 37.1 Hz, 1H), 3.67 (s, 3H), 4.14–4.01 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 16.3, 16.4, 26.6 (dd, J = 13, 142 Hz), 28.3–27.8, 27.9, 28.0, 44.5 (dd, J = 10, 16 Hz), 52.9, 61.9 (d, J = 7 Hz), 62.2 (d, J = 7 Hz), 81.7 (dd, J = 8, 232 Hz), 82.8, 83.1, 151.5, 169.7; ^{19}F NMR ($CDCl_3$) δ −168.4; ^{31}P NMR ($CDCl_3$) δ 25.0; MS (ESI$^+$) m/z 484.1 [M+H]$^+$. Anal. Calcd for $C_{20}H_{35}FNO_9P$: C, 40.69; H, 7.30; N, 2.90. Found: C, 49.85; H, 7.32; N, 2.63.

6.8. (*Z*)-1-Amino-2-fluoro-2-(phosphonomethyl)cyclopropanecarboxylic acid

6.8.1. (*Z*)-FAP4 11a

The precursor **10a** (90 mg, 0.19 mmol) was heated at 80 °C in a 1:1 mixture of acetic acid and hydrochloric acid until complete conversion (^{19}NMR and TLC monitoring). Solvents were removed, the crude solid product was taken up in HCl 1 N solution (10 mL), washed with diethylether (5 mL), dichloromethane (5 mL), lyophilised and purified on Dowex column to furnish 41 mg (88%) of pure **11a**; ^{1}H NMR (D_2O) δ 2.72–2.41 (m, 2H), 2.05 (dd, J = 9.2, 12.6 Hz, 1H), 1.98 (bs, 1H); ^{13}C NMR (D_2O) δ 170.6, 82.0 (dd, J = 8, 228 Hz), 49.1, 30.4 (dd, J = 21, 139 Hz), 24.7; ^{19}F NMR (D_2O) δ −173.1; ^{31}P NMR (D_2O) δ 21.7; MS (ESI$^-$) m/z 212.1 [M−H]$^-$. Anal. Calcd for $C_5H_9FNO_5P$: C, 28.18; H, 4.26; N, 6.57. Found: C, 28.34; H, 4.34; N, 6.32.

6.9. General procedure for oxidation with IBX

To a solution of **8a** or **8b** (1 equiv) in EtOAc (0.5 M), IBX (3 equiv) was added. The resulting suspension was heated under stirring at reflux. End of reaction was monitored by TLC. Solvent was removed, the resulting white heterogeneous oil was taken up in diethyl ether and filtered. The filtrate was concentrated under reduced pressure to afford quantitatively the corresponding aldehyde which can either be used without further purification or purified by column chromatography (5% ethyl acetate in cyclohexane).

6.10. (*Z*)-Methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-fluoro-2-formylcyclopropanecarboxylate 12a

99% yield; R_f (30% EtOAc in cyclohexane) = 0.45; ^{1}H NMR ($CDCl_3$) δ 9.66 (d, J = 14.2 Hz, 1H), 3.79 (s, 3H), 2.18 (dd, J = 9.0, 15.6 Hz, 1H), 2.18 (dd, J = 9.0, 19.6 Hz, 1H), 1.47 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 191.2, 169.4 (d, J = 2 Hz), 85.0 (d, J = 244 Hz), 84.2, 53.7, 48.7 (d, J = 9.2 Hz), 28.5 (d, J = 7.2 Hz), 28.2; ^{19}F NMR ($CDCl_3$) δ −193.2 (ddd, J = 14, 16, 20 Hz); MS (ESI$^+$) m/z 745.3 [2 M+Na]$^+$. Anal. Calcd for $C_{16}H_{24}FNO_7$: C, 53.18; H, 6.69; N, 3.88. Found: C, 53.34; H, 6.78; N, 3.77.

6.11. (*E*)-Methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-fluoro-2-formylcyclopropanecarboxylate 12b

99% yield; R_f (50% EtOAc in cyclohexane) = 0.66; ^{1}H NMR ($CDCl_3$) δ 9.65 (d, J = 4.5 Hz, 1H), 3.80 (s, 3H), 2.81 (dd, J = 8.4, 17.5 Hz, 1H), 2.01 (dd, J = 8.4, 11.1 Hz, 1H), 1.49 (s, 9H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 191.3 (d, J = 36 Hz), 166.2, 151.8, 151.5, 86.7 (d, J = 246 Hz), 84.5, 84.2, 53.7, 49.4 (d, J = 13 Hz), 28.3, 28.1, 25.8 (d, J = 8.3 Hz); ^{19}F NMR ($CDCl_3$) δ −203.6 (ddd, J = 5, 11, 18 Hz); Anal. Calcd for $C_{16}H_{24}FNO_7$: C, 53.18; H, 6.69; N, 3.88. Found: C, 53.47; H, 6.76; N, 4.06.

6.12. (*Z*)-Methyl 1-(bis(tert-butoxycarbonyl)amino)-2-(3-ethoxy-3-oxopropyl)-2-fluorocyclopropanecarboxylate 13a

A solution of aldehyde **12a** (380 mg, 1.05 mmol, 1 equiv), lithium bromide (184 mg, 2.1 mmol, 2 equiv) and triethyl phosphonoacetate (0.425 mL, 2.1 mmol, 2 equiv) in THF (5 mL) was stirred at room temp until lithium bromide was totally solubilised. Triethylamine (0.293 mL, 2.1 mmol, 2 equiv) was added dropwise and the mixture was stirred overnight. It was then filtered through a pad of celite, concentrated and purified by column chromatography (10% ethyl acetate, 1% triethylamine in cyclohexane) to afford 414 mg (91%) of the desired homologated compound; R_f (50% EtOAc in cyclohexane) = 0.74; ^{1}H NMR ($CDCl_3$) δ 7.18 (dd, J = 15.7, 25.2 Hz, 1H), 6.24 (d, J = 15.7 Hz, 1H), 4.19 (q, J = 7.0 Hz, 2H), 3.74 (s, 3H), 2.36 (dd, J = 8.6, 18.9 Hz, 1H), 1.95 (dd, J = 8.6, 21.4 Hz, 1H), 1.42 (s, 9H), 1.29 (s, 9H), 1.27 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 169.0 (d, J = 2 Hz), 165.7, 151.7, 151.4, 139.5 (d, J = 16 Hz), 122.7, 83.7, 83.4 (d, J = 235 Hz), 83.3, 60.9, 53.3, 48.1 (d, J = 9 Hz), 29.8 (d, J = 9 Hz), 28.3, 28.2, 14.5; ^{19}F NMR ($CDCl_3$) δ −179.8 (ddd, J = 17, 21, 26 Hz). A suspension of the homologated unsaturated ester (1.9 g, 4.4 mmol) and a catalytic amount of palladium on carbon in THF (20 mL) was saturated in hydrogen. This suspension was stirred at room temp until starting material was consumed and was filtered off. Solvent was removed and the crude product was purified by column chromatography (5% ethyl acetate, 1% triethylamine in cyclohexane) to afford 1.62 g (86%) of pure desired product; R_f (30% EtOAc in cyclohexane) = 0.54; ^{1}H NMR ($CDCl_3$) δ 4.12 (q, J = 7.0 Hz, 2H), 3.72 (s, 3H), 2.62–2.33 (m, 4H), 1.98 (dd, J = 8.3, 18.0 Hz, 1H), 1.65 (dd, J = 8.3, 20.0 Hz, 1H), 1.49 (s, 9H), 1.46 (s, 9H), 1.24 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.9, 170.0, 152.3, 152.1, 85.5 (d, J = 232 Hz), 83.1, 60.8, 53.0, 45.4 (d, J = 9 Hz), 29.8, 28.3, 28.2, 28.0, 25.5 (d, J = 20 Hz), 14.5; ^{19}F NMR ($CDCl_3$) δ −174.8; MS (ESI$^+$) m/z 456.00 [M+Na]$^+$. Anal. Calcd for

$C_{20}H_{32}FNO_8$: C, 55.42; H, 7.44; N, 3.23. Found: C, 55.69; H, 7.72; N, 3.14.

6.13. (*Z*)-1-Amino-2-(2-carboxyethyl)-2-fluorocyclopropane carboxylic acid hydrochloride

6.13.1. (*Z*)-FAC5 14a

A solution of **13a** (1.48 g, 3.4 mmol, 1 equiv) and lithium hydroxide (1.63 g, 68 mmol, 20 equiv) in THF:H_2O 2:1 (40 mL) was heated at reflux overnight. Organic solvent was removed under vacuo. The resulting aqueous solution was washed with diethyl ether and acidified to pH 1. Aqueous layer was then continuously extracted 5 h with diethyl ether. Solvent was removed and the resulting yellow oily solid was recrystallized from Et_2O/Pentane to furnish the saponified product; ^{1}H NMR ($CDCl_3$) δ 2.65–2.26 (m, 4H), 1.83 (dd, J = 7.7, 16.0 Hz, 1H), 1.52 (dd, J = 7.9, 15.1 Hz, 1H), 1.47 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 178.8, 175.6, 160.9, 87.3 (d, J = 230 Hz), 83.0, 43.9 (d, J = 9 Hz), 31.2, 31.0, 29.3 (d, J = 21 Hz), 28.6 (d, J = 9 Hz); ^{19}F NMR ($CDCl_3$) δ −180.5; MS (ESI^-) m/z 390.9 $[M-H]^-$. Anal. Calcd for $C_{17}H_{26}FNO_8$: C, 52.17; H, 6.70; N, 3.58. Found: C, 51.82; H, 6.75; N, 3.36. A solution of the diacid (135 mg, 0.345 mmol) in 1:1 solution of concentrated HCl and acetic acid was stirred 24 h at room temp. Solvents were removed, the resulting brown solid was taken up in water washed with ethyl acetate and lyophilised to furnish 56 mg (71%) of pure **14a** as a white solid; ^{1}H NMR (D_2O) δ 2.03–1.85 (m, 1H), 1.92 (d, J = 19.8 Hz, 1H), 1.25 (d, J = 6.4 Hz, 1H); ^{13}C NMR (D_2O) δ 176.9, 169.0, 82.3 (d, J = 229 Hz), 39.5 (d, J = 11 Hz), 29.3 (d, J = 1 Hz), 24.4 (d, J = 20 Hz), 22.0 (d, J = 10 Hz); ^{19}F NMR (D_2O) δ −178.5; MS (ESI^+) m/z 456.0 $[M+Na]^+$.

6.14. General procedure for the preparation of 15a and 15b

A solution of aldehyde **12a** or **12b** (1 equiv), lithium bromide (2 equiv) and tetraethylmethylenediphosphonate (2 equiv) in THF (0.5 M) was stirred at room temp until lithium bromide was totally solubilised. Triethylamine (2 equiv) was added dropwise and the mixture was stirred overnight. It was then filtered through a pad of celite, concentrated and purified by column chromatography (10% ethyl acetate, 1% triethylamine in cyclohexane) to afford the desired homologated compound (79% for **12a**, 59% for **12b**). A suspension of compound and a catalytic amount of palladium on carbon in THF (20 mL) was saturated in hydrogen. This suspension was stirred at room temp until starting material was consumed and palladium on carbon was filtered off. Solvent was removed and the crude product was purified by column chromatography (5% ethyl acetate, 1% triethylamine in cyclohexane) to afford **15a** (51%) and **15b** (83%).

6.15. (*Z*)-Methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-(2-(diethoxyphosphoryl)ethyl)-2-fluorocyclopropanecarboxylate 15a

R_f (50% EtOAc in cyclohexane) = 0.29; ^{1}H NMR ($CDCl_3$) δ 4.05–3.90 (m, 4H), 3.59 (s, 3H), 2.30–2.15 (m, 2H), 2.02–1.75 (m, 3H), 1.53 (dd, J = 8.3, 21.5 Hz, 1H), 1.34 (s, 18H), 1.27 (dt, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 169.4, 152.0, 151.7, 85.3 (dd, J = 21, 233 Hz), 82.7, 83.1, 61.6, 61.5, 52.6, 45.1 (d, J = 9 Hz), 27.8, 27.7, 27.6, 23.3 (dd, J = 2, 21 Hz), 21.2 (d, J = 143 Hz), 16.3, 16.2; ^{19}F NMR ($CDCl_3$) δ −175.4; ^{31}P NMR ($CDCl_3$) δ 31.8 m MS (ESI^+) m/z 498.0 $[M+H]^+$.

6.16. (*E*)-Methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-(2-(diethoxyphosphoryl)ethyl)-2-fluorocyclopropanecarboxylate 15b

^{1}H NMR ($CDCl_3$) δ 4.09–3.96 (m, 4H), 3.68 (s, 3H), 2.60–2.45 (m, 1H), 2.39 (ddd, J = 1.5, 8.5, 19.3 Hz, 1H), 2.07–2.00 (m, 1H), 1.90–1.69 (m, 1H), 1.68–1.51 (m, 1H), 1.44 (s, 9H), 1.40 (s, 9H), 1.24 (m, 6H), 1.11 (dd, J = 8.5, 12.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 168.2, 152.0, 151.8, 87.0 (dd, J = 22, 238 Hz), 83.6, 83.5, 62.0 (d, J = 4 Hz), 61.9 (d, J = 4 Hz), 52.9, 47.9 (dd, J = 4, 23 Hz), 28.1, 28.0, 26.2 (dd, J = 4, 23 Hz), 26.2, 21.8 (d, J = 144 Hz), 16.6, 16.5; ^{19}F NMR ($CDCl_3$) δ −185.8; MS (ESI^+) m/z 520.13 $[M+Na]^+$.

6.17. General procedure for the removal of protecting groups

Precursors **15a** or **15b** were heated at 80 °C in a 1:1 mixture of acetic acid and hydrochloric acid until complete conversion (^{19}NMR and TLC monitoring). Solvents were removed, the crude solid product was taken up in HCl 1 N solution (10 mL), washed with diethylether (5 mL), dichloromethane (5 mL), lyophilised and purified on Dowex column to furnish desired products **16a** (53%) and **16b** (15%).

6.18. (*Z*)-1-Amino-2-fluoro-2-(2-phosphonoethyl)cyclopropane carboxylic acid hydrochloride

6.18.1. (*Z*)-FAP5 16a

^{1}H NMR (D_2O) δ 2.40–1.93 (m, 5H), 1.70 (m, 1H); ^{13}C NMR (D_2O) δ 170.4, 81.2 (dd, J = 21, 227 Hz), 40.6 (dd, J = 2, 15 Hz), 24.3 (dd, J = 3, 22 Hz), 23.5 (d, J = 135 Hz), 21.8 (d, J = 10 Hz); ^{19}F NMR (D_2O) δ −181.7; ^{31}P NMR (D_2O) δ 24.3; MS (ESI^-) m/z 226.2 $[M-H]^-$. Anal. Calcd for $C_6H_{12}ClFNO_5P$: C, 27.34; H, 4.59; N, 5.31. Found: C, 27.18; H, 4.95; N, 5.16.

6.19. (*E*)-1-Amino-2-fluoro-2-(2-phosphonoethyl)cyclopropane carboxylic acid hydrochloride

6.19.1. (*E*)-FAP5 16b

^{1}H NMR (D_2O) δ 2.40–1.93 (m, 5H), 1.68 (m, 1H); ^{13}C NMR (D_2O) δ 168.5, 81.2 (dd, J = 18, 235 Hz), 41.8 (dd, J = 3, 14 Hz), 24.8 (dd, J = 3, 22 Hz), 22.4 (d, J = 134 Hz), 21.8 (d, J = 12 Hz); ^{19}F NMR (D_2O) δ −184.9; ^{31}P NMR (D_2O) δ 25.3; MS (ESI^-) m/z 226.3 $[M-H]^-$. Anal. Calcd for $C_6H_{12}ClFNO_5P$: C, 27.34; H, 4.59; N, 5.31. Found: C, 27.16; H, 4.95; N, 5.01.

6.20. (*Z*)-Methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-(cyanomethyl)-2-fluorocyclopropanecarboxylate 17a

Alcohol **8a** (554 mg, 1.52 mmol) was dissolved in anhydrous THF (5 mL) under an argon atmosphere. The mixture was cooled down to 0 °C and PPh_3 (786 mg, 3.0 mmol) was added as one portion. A solution of DBAD (702 mg, 3.0 mmol) in anhydrous THF (2 mL) was added dropwise and the mixture stirred for 30 min. α-hydroxyisobutyronitrile (275 μL, 3.0 mmol) in anhydrous THF (500 μL) was added dropwise. The mixture was stirred at this temperature for 30 min, and was then allowed to reach room temperature and stirred for a further 12 h. The reaction mixture was concentrated in vaccuo and recrystallised from Et_2O/petroleum ether (50:50) to eliminate phosphine oxide. The solid was discarded and the filtrate was concentrated and purified by column chromatography (EtOAc/cyclohexane 15:85). The compound could not be fully separated from the reduced DBAD residue and was transferred impure to the deprotection step as a yellow oily residue; ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 3H), 3.42 (dd, J = 17.5, 30.5 Hz, 1H), 3.20 (ddd, J = 1.1, 12.6, 17.5 Hz, 1H), 2.13 (dd, J = 8.8, 17.3 Hz, 1H), 1.92 (ddd, J = 1.1, 8.8, 21.1 Hz, 1H), 1.87 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 169.5, 151.8, 151.4, 115.5, 83.6, 80.7 (d, J = 236 Hz), 53.2, 44.8 (d, J = 9 Hz), 29.6, 27.4 (d, J = 48 Hz), 20.0 (d, J = 22 Hz); ^{19}F NMR ($CDCl_3$) δ −172.2 m MS (ESI^+) m/z 395.1 $[M+Na]^+$.

6.21. (*E*)-Methyl 1-(tert-butoxycarbonylamino)-2-(cyanomethyl)-2-fluorocyclopropanecarboxylate 17b

To a stirred solution of alcohol **8b** (349 mg, 0.96 mmol) in acetonitrile (10 mL) was added LiBr (250 mg, 2.9 mmol) as small portions. The reaction was heated to 60 °C and carefully monitored by ^{19}F NMR & TLC (cyclohexane/ EtOAC 50:50). After 7 h, the mixture was allowed to cool down to room temperature and stirred for a further 16 h. The solvent was then removed and the residue taken up into EtOAc (10 mL). The solution was washed with water (3 × 5 mL) and brine (2 × 5 mL), dried over $MgSO_4$ and evaporated. The yellow oily residue was purified by column chromatography (cyclohexane/ EtOAc 60:40) to afford a pure colourless oil (162 mg, 64% yield); ^{1}H NMR ($CDCl_3$) δ 5.42 (bs, 1H), 4.28 (m, 1H), 3.70 (s, 3H), 3.52 (m, 1H), 2.32 (dd, J = 7.9, 20.9 Hz, 1H), 1.39 (s, 9H), 1.29 (dd, J = 7.9, 11.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 168.8, 157.1, 84.9 (d, J = 236 Hz), 81.2, 62.7 (d, J = 21 Hz), 52.8, 42.2, 28.1, 23.4; ^{19}F NMR ($CDCl_3$) δ −193.3. Previous monodeprotected alcohol (44 mg, 0.16 mmol) was dissolved in anhydrous THF (0.7 mL) under an argon atmosphere. The mixture was cooled down to 0 °C and PPh_3 (84 mg, 0.32 mmol) was added as one portion. A solution of DBAD (74 mg, 0.32 mmol) in anhydrous THF (0.4 mL) was added dropwise and the mixture stirred for 30 min. α-hydroxyisobutyronitrile (30 μL, 0.32 mmol) in anhydrous THF (0.5 mL) was added dropwise. The mixture was stirred at this temperature for 30 min, and was then allowed to reach room temperature and stirred for a further 4 h. The reaction mixture was concentrated in vaccuo and recrystallised from Et_2O/petroleum ether (50:50) to eliminate the phosphine oxide. The solid was discarded and the filtrate was concentrated and purified by column chromatography (EtOAc/cyclohexane 15:85) to afford a colourless oil (22 mg, 51% yield); R_f (50% EtOAc in cyclohexane) = 0.23; ^{1}H NMR ($CDCl_3$) δ 5.26 (bs, 1H), 3.80 (s, 3H), 3.14 (dd, J = 17.7, 22.9 Hz, 1H), 3.08 (dd, J = 17.7, 24.1 Hz, 1H), 2.53 (dd, J = 8.4, 19.9 Hz, 1H), 1.58 (m, 1H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$), δ 167.7, 155.6, 115.4, 81.5, 80.8 (d, J = 240 Hz), 53.2, 42.5 (d, J = 13 Hz), 28.2, 24.3 (d, J = 10 Hz), 22.1 (d, J = 24 Hz); ^{19}F NMR ($CDCl_3$) δ −183.2 m MS (ESI^+) m/z 295.1 $[M+Na]^+$.

6.22. (*Z*)-1-Amino-2-(carboxymethyl)-2-fluorocyclopropane carboxylic acid hydrochloride

6.22.1. (*Z*)-FAC4 18a

Impure nitrile **17a** (92 mg, 0.25 mmol) was dissolved in 6 N HCl (4 mL). The reaction was heated up to 80 °C and stirred over 24 h. The solvent was then removed in vaccuo, and the chlorhydrate residue purified over a Dowex 1X4-400 anion exchange resin column (elution: gradient of AcOH 0.05–0.5 M). Fractions revealing with ninhydrin were combined and freeze-dried to give a white solid (14 mg); ^{1}H NMR (D_2O) δ 3.41 (t, J = 18.6 Hz, 1H), 3.15 (dd, J = 18.1, 33.5 Hz, 1H), 1.95 (dd, J = 9.6, 14.1 Hz, 1H), 1.88 (m, 1H); ^{13}C NMR (D_2O) δ 173.1, 169.8, 78.8 (d, J = 226 Hz), 39.5 (d, J = 10 Hz), 35.0 (d, J = 23 Hz), 21.2 (d, J = 9 Hz); ^{19}F NMR (D_2O) δ −178.9; MS (ESI^+) m/z 178.1 $[M+H]^+$. Anal. Calcd for $C_6H_9ClFNO_4$: C, 33.74; H, 4.25; N, 6.56. Found: C, 33.29; H, 4.31; N, 6.58.

6.23. (*E*)-1-Amino-2-(carboxymethyl)-2-fluorocyclopropane carboxylic acid hydrochloride

6.23.1. (*E*)-FAC4 18b

E-nitrile **17b** (20 mg, 0.073 mmol) was dissolved in 1 N HCl (2 mL). The reaction was heated up to 80 °C and stirred over 24 h. The solvent was then removed in vaccuo, the residue was triturated in Et_2O and filtered off. The white powder was taken up into water and freeze-dried to give a white solid (8 mg, 52% yield); ^{1}H NMR (D_2O) δ 3.71 (dd, J = 18.3, 59.5 Hz, 1H), 3.68 (dd, J = 16.9, 41.2 Hz, 1H), 2.62 (dd, J = 8.1, 19.6 Hz, 1H), 1.79 (t, J = 9.3 Hz, 1H); ^{13}C NMR (D_2O) δ 168.8, 168.0 (d, J = 10 Hz), 81.1 (d, J = 255 Hz), 50.4 (d, J = 7 Hz), 36.6 (d, J = 24 Hz), 28.0 (d, J = 11.0 Hz); ^{19}F NMR (D_2O) δ −194.1; MS (ESI^+) m/z 159.1 $[M+H-F]^+$. Anal. Calcd for $C_6H_9ClFNO_4$: C, 33.74; H, 4.25; N, 6.56. Found: C, 33.44; H, 3.95; N, 6.60.

6.24. (*Z*)-Methyl 2-(acetylthiomethyl)-1-(bis(*tert*-butoxy carbonyl)-amino)-2-fluorocyclopropanecarboxylate 19a

DIAD (324 μL, 1.64 mmol) was added dropwise to a stirred solution of PPh_3 (431 mg, 1.64 mmol) in anhydrous THF (5 mL) at 0 °C under argon. The mixture was stirred at this temperature for 30 min until formation of a white precipitate of Mitsunobu betaine, and a solution of thioacetic acid (120 μL, 1.64 mmol) and alcohol **8a** (299 mg, 0.82 mmol) in anhydrous THF (3 mL) was added slowly. The reaction was stirred at 0 °C for 1 hour, allowed to reach room temperature and stirred for a further hour. The solvent was removed in vaccuo and the residue taken up into a mixture of diethyl ether and cyclohexane (50:50 v/v) and triturated at 0 °C. The resulting white solid was filtered off and washed with Et_2O/cyclohexane. The filtrate was evaporated and the residue was purified by column chromatography (Ethyl acetate/cyclohexane, 20:80 v/v) to afford a colourless oil (130 mg, 38% yield); ^{1}H NMR ($CDCl_3$) δ 3.85 (dd, J = 14.8, 35.4 Hz, 1H), 3.77 (s, 3H), 3.55 (ddd, J = 1.5, 11.8, 13.8 Hz, 1H), 2.38 (s, 3H), 2.09 (dd, J = 8.5, 17.5 Hz, 1H), 1.71 (ddd, J = 1.5, 8.5, 20.9 Hz, 1H), 1.52 (s, 9H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 193.2, 168.5, 150.5, 83.4 (d, J = 232 Hz), 82.1, 51.9, 45.4 (d, J = 9 Hz), 29.4, 28.2 (d, J = 21 Hz), 27.0 (d, J = 17 Hz), 26.8; ^{19}F NMR ($CDCl_3$) δ −172.2; MS (ESI^+) m/z 444.2 $[M+Na]^+$. Anal. Calcd for $C_{18}H_{28}FNO_7S$: C, 51.29; H, 6.70; N, 3.32. Found: C, 51.26; H, 6.80; N, 2.09.

6.25. (*E*)-Methyl 2-(acetylthiomethyl)-1-(bis(tert-butoxycarbonyl)-amino)-2-fluorocyclopropanecarboxylate 19b

A solution of DBAD (1.113 g, 4.83 mmol) in anhydrous THF (5 mL) was added dropwise to a stirred solution of PPh_3 (1.268 g, 4.83 mmol) in anhydrous THF (5 mL) at 0 °C under argon. The mixture was stirred at this temperature for 30 min until formation of a white precipitate of Mitsunobu betaine, and a solution of thioacetic acid (346 μL, 4.83 mmol) and alcohol **8b** (879 mg, 2.42 mmol) in anhydrous THF (3 mL) was added slowly. The reaction was stirred at 0 °C for 1 hour, allowed to reach room temperature and stirred for a further hour. The solvent was removed in vaccuo and the residue taken up into a mixture of diethyl ether and cyclohexane (50:50 v/v) and triturated at 0 °C. The resulting white solid was filtered off and washed with Et_2O/cyclohexane. The filtrate was evaporated and the residue was purified by column chromatography (Ethyl acetate/cyclohexane/Et_3N, 5:94.5:0.5 v/v) to afford a colorless oil (130 mg, 38% yield); ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 3H), 3.61 (ddd, J = 2.3, 10.0, 14.7 Hz, 1H), 3.25 (dd, J = 14.8, 36.3 Hz, 1H), 2.43 (ddd, J = 2.3, 10.9, 19.2 Hz, 1H), 2.35 (s, 3H), 1.52 (s, 9H), 1.48 (s, 9H), 1.29 (dd, J = 3.1, 11.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 194.4, 167.5, 151.6 (d, J = 19 Hz), 86.5 (d, J = 239 Hz), 83.5, 52.8, 48.7 (d, J = 14 Hz), 32.1 (d, J = 21 Hz), 30.3, 27.9, 25.9 (d, J = 10 Hz); ^{19}F NMR ($CDCl_3$) δ −181.9; MS (ESI^+) m/z 444.4 $[M+Na]^+$.

6.26. General procedure for the deprotection of Thioacetate 19a and 19b

Thioacetate **19a** (219 mg, 0.5 mmol) or **19b** (588 mg, 1.40 mmol) was dissolved in formic acid (0.2 N), and a 30% solution of aqueous hydrogen peroxide (2 mL/mmol) was added slowly. The mixture was stirred at room temperature until completion (14 h for **19a**, 72 h for **19b**). The solvent was removed

in vaccuo to afford the *N*-deprotected compound as the formate salt, as a white powder (prior recrystallization from EtOAc/MeOH (60:40) was necessary for **19b**). The formate salt was then dissolved in 6 N HCl (0.07 N). The reaction was heated up to 70–80 °C and stirred until completion (18 h for **19a**, 48 h for **19b**). The solvent was then removed in vaccuo.

6.27. (*Z*)-1-Amino-2-fluoro-2-(sulfomethyl) cyclopropane carboxylic acid hydrochloride

6.27.1. (*Z*)-FAS4 20a

The chlorhydrate residue was purified by water elution over a Dowex 50WX4-50 cation exchange resin column. Fractions revealing with ninhydrin were combined and freeze-dried to give a yellow solid. Trituration in Et_2O and filtration afforded the title compound (74 mg, yield 93%); R_f (50% EtOAc in cyclohexane) = 0.23; ^{1}H NMR ($CDCl_3$) δ 3.71 (m, 1H), 3.41 (dd, J = 15.6, 30.1 Hz, 1H), 2.00 (m, 1H), 1.93 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 168.4, 78.8 (d, J = 226 Hz), 49.3 (d, J = 21 Hz), 39.3 (d, J = 10 Hz), 21.8 (d, J = 9 Hz); ^{19}F NMR ($CDCl_3$) δ −180.7; MS (ESI$^-$) m/z 212.3 [M−H]$^-$. Anal. Calcd for $C_5H_9ClFNO_5S$: C, 24.06; H, 3.63; N, 5.61; S, 12.84. Found: C, 24.56; H, 3.45; N, 5.36; S, 14.03.

6.28. (*E*)-1-Amino-2-fluoro-2-(sulfomethyl)cyclopropane carboxylic acid hydrochloride

6.28.1. (*E*)-FAS4 20b

The chlorhydrate residue was recrystallised from hot water to afford a white powder (58 mg, yield 77%); ^{1}H NMR (D_2O) δ 3.63 (m, 2H), 2.46 (dd, J = 9.6, 20.7 Hz, 1H), 1.87 (t, J = 11.3 Hz, 1H); ^{13}C NMR (D_2O) δ 167.9, 77.3 (d, J = 235 Hz), 50.1 (d, J = 22 Hz), 41.5 (d, J = 15 Hz), 21.1 (d, J = 12 Hz); ^{19}F NMR (D_2O) δ −183.2; MS (ESI$^-$) m/z 212.3 [M−H]$^-$. Anal. Calcd for $C_5H_9ClFNO_5S$: C, 24.06; H, 3.63; N, 5.61; S, 12.84. Found: C, 24.47; H, 3.46; N, 5.65; S, 12.88.

6.29. General procedure for the preparation of 21a and 21b

To a solution under argon of aldehyde **12** (1 equiv) and triethylamine (0.25 equiv) in dry THF (0.6 M) was added diethyl phosphonate dropwise (1.1 equiv). The mixture was stirred at room temp until starting material was consumed (TLC monitoring). Solvent was removed, the resulting oily product was taken up in a 1:1 mixture of diethyl ether/ethyl acetate, washed with brine, dried over $MgSO_4$ and concentrated. Resulting crude product was purified by column chromatography (10% ethyl acetate, 1% triethylamine in cyclohexane) to afford pure desired product as a single diastereoisomer.

6.30. (*Z*)-Methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-((diethoxyphosphoryl)(hydroxy)methyl)-2-fluorocyclopropanecarboxylate 21a

R_f (50% EtOAc in cyclohexane) = 0.23; ^{1}H NMR ($CDCl_3$) δ 4.41 (ddd, J = 3.8, 13.2, 35.1 Hz, 1H), 4.18–3.97 (m, 5H), 3.65 (s, 3H), 2.27 (ddd, J = 1.7, 8.9, 17.4 Hz, 1H), 1.85 (dd, J = 8.9, 22.3 Hz, 1H), 1.42 (s, 9H), 1.39 (s, 9H), 1.26 (t, J = 7.0 Hz, 3H), 1.23 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 168.7, 153.5, 151.3, 84.8 (dd, J = 3, 235 Hz), 84.2, 83.3, 62.6 (dd, J = 22, 167 Hz), 63.2 (d, J = 7 Hz), 62.6 (d, J = 7 Hz), 53.1, 44.2 (dd, J = 10, 12 Hz), 27.8, 27.8, 27.7, 16.3, 16.3; ^{19}F NMR ($CDCl_3$) δ −182.8; ^{31}P NMR ($CDCl_3$) δ 18.5; MS (ESI$^+$) m/z 500.1 [M+H]$^+$. Anal. Calcd for $C_{20}H_{35}FNO_{10}P$: C, 48.09; H, 7.06; N, 2.80. Found: C, 47.86; H, 7.15; N, 2.77.

6.31. (*E*)-methyl 1-(bis(*tert*-butoxycarbonyl)amino)-2-((diethoxyphosphoryl)(hydroxy)methyl)-2-fluorocyclopropanecarboxylate 21b

R_f (50% EtOAc in cyclohexane) = 0.36; ^{1}H NMR ($CDCl_3$) δ 6.54 (bs, 1H), 5.14 (ddd, J = 3.8, 14.4, 31.7 Hz, 1H), 4.31–4.13 (m, 4H), 3.77 (s, 3H), 2.61–2.52 (m, 1H), 1.72–1.63 (m, 1H), 1.51 (s, 9H), 1.43 (s, 9H), 1.35 (t, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 167.7, 152.8, 152.7, 84.3, 79.0 (d, J = 251 Hz), 71.1 (dd, J = 21, 168 Hz), 64.4 (dd, J = 6 Hz), 63.6 (d, J = 7 Hz), 53.1, 41.3, 28.4, 27.8, 25.5, 16.5 (d, J = 10 Hz), 16.4 (d, J = 10 Hz); ^{19}F NMR ($CDCl_3$) δ −196.2; ^{31}P NMR ($CDCl_3$) δ 16.5; MS (ESI$^+$) m/z 499.8 [M+H]$^+$. Anal. Calcd for $C_{16}H_{24}FNO_8$: C, 48.09; H, 7.06; N, 2.80. Found: C, 48.12; H, 7.12; N, 2.81.

6.32. General procedure for the deprotection of 21a and 21b

A solution of **21** (1 equiv) in a 1:1 mixture of acetic acid and concentrated hydrochloric acid (0.05 M) was heated under reflux until completion (NMR and TLC monitoring). Solvents were removed, the resulting oily product was taken up in ethyl acetate, extracted with water, washed with EtOAc and dried to afford pure desired product as a single diastereoisomer.

6.33. (*Z*)-1-Amino-2-fluoro-2-(hydroxy(phosphono)methyl) cyclopropanecarboxylic acid hydrochloride

6.33.1. (*Z*)-HFAP4 22a

^{1}H NMR (D_2O) δ 3.76–3.58 (m, 1H), 1.70–1.50 (m, 1H), 1.22–1.14 (m, 1H); ^{13}C NMR (D_2O) δ 178.1, 85.5 (d, J = 226 Hz), 69.8 (dd, J = 24, 144 Hz), 43.5 (dd, J = 9, 9 Hz), 22.9 (d, J = 8 Hz); ^{19}F NMR (D_2O) δ −196.9; ^{31}P NMR (D_2O) δ 13.1; MS (ESI$^-$) m/z 227.9 [M−H]$^-$. Anal. Calcd for $C_5H_{10}ClFNO_6P$: C, 22.61; H, 3.80; N, 5.27. Found: C, 22.56; H, 4.02; N, 5.31.

6.34. (*E*)-1-Amino-2-fluoro-2-(hydroxy(phosphono)methyl) cyclopropanecarboxylic acid hydrochloride

6.34.1. (*E*)-HFAP4 22b

^{1}H NMR (D_2O) δ 4.27 (dd, J = 12.1, 20.5 Hz, 1H), 2.37 (ddd, J = 1.5, 9.7, 21.8 Hz, 1H), 1.96 (dd, J = 9.9, 12.4 Hz, 1H); ^{13}C NMR (D_2O) δ 167.9, 81.5 (dd, J = 5, 238 Hz), 68.0 (dd, J = 25, 156 Hz), 41.0 (dd, J = 3, 14 Hz), 21.3 (dd, J = 5, 12 Hz); ^{19}F NMR (D_2O) δ −186.2; ^{31}P NMR (D_2O) δ 15.0; MS (ESI$^+$) m/z 230.1 [M+H]$^+$. Anal. Calcd for $C_5H_{10}ClFNO_6P$: C, 22.61; H, 3.80; N, 5.27. Found: C, 22.58; H, 3.47; N, 4.94.

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