Aspartyl Phosphonates and Phosphoramidates: The First Synthetic Inhibitors of Bacterial Aspartate-Semialdehyde Dehydrogenase

Russell J. Cox,* Jennifer S. Gibson, and María Belén Mayo Martín[a]

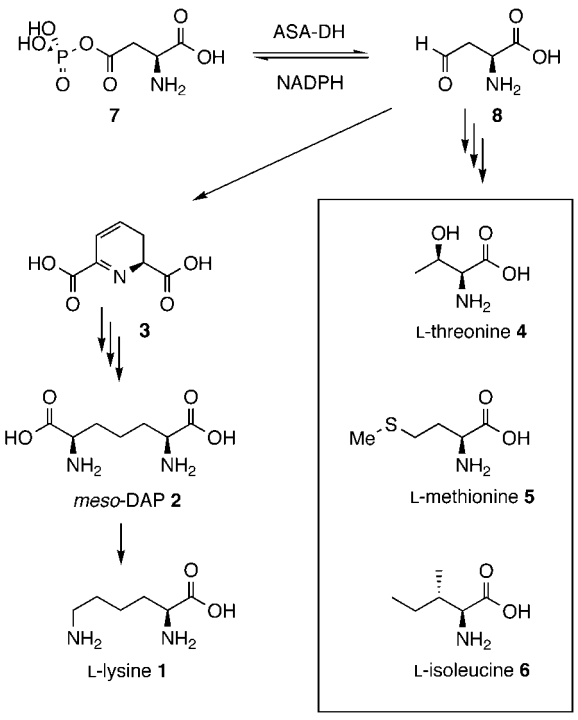
The synthesis of methylene phosphonate, difluoromethylene phosphonate and phosphoramidate analogues of aspartyl phosphate, together with reduced analogues, is described. These compounds were shown to be effective inhibitors of aspartate-semialdehyde dehydrogenase (ASA-DH) from Escherichia coli. *However, despite the structural similarity of the compounds, different patterns of inhibition were observed, indicative of two phases of recognition and binding. Correlation between measured inhibition constants with pK_a values supports the theory that binding at the phosphate binding site is optimised for singly ionised phosphate analogues.*

KEYWORDS:

antibiotics · dehydrogenases · inhibitors · phosphonates · phosphoramidates

Introduction

The enzyme aspartate-semialdehyde dehydrogenase (ASA-DH, E.C. 1.2.1.11)[1] lies at the start of the bacterial pathways leading to L-lysine (**1**), via diaminopimelic acid (DAP, **2**),[2] and to other amino acids such as L-threonine (**4**), L-methionine (**5**) and L-isoleucine (**6**) (Scheme 1).[3] Because of the requirement for amino acids by bacterial protein biosynthesis and the absence of ASA-DH from mammalian metabolism, ASA-DH could be a useful target for the development of new classes of antibacterial compounds. This potential is heightened when it is recognised that DAP (**2**) also plays a crucial role in bacterial replication because of its role as a cross-linking element in the peptidoglycan layer of the cell wall of both Gram-positive and Gram-negative organisms.[4] ASA-DH is also operative in fungi where it is known that inhibitors of aspartate metabolism show antifungal activity.[5]



Scheme 1. *Role of ASA-DH in the microbial biosynthesis of cell wall and protein components.*

The mechanism of ASA-DH has been investigated with classical kinetic methods[6] and the recent publication of an X-ray crystal structure has supported many of the mechanistic conclusions.[7] For the forward (biosynthetic) reaction aspartyl-γ-phosphate (**7**) acts as the substrate (Scheme 2). Upon binding to ASA-DH, the γ-carbonyl group of **7** is intercepted by an active site nucleophilic cysteine sulphur atom, forming a (presumed) tetrahedral intermediate **9** which then expels inorganic phosphate to form the enzyme-bound thiolester **10**. The 4-pro*R* hydride from nicotinamide adenine dinucleotide phosphate, reduced form, (NADPH) is then transferred to the thiolester carbonyl group, forming a second (presumed) tetrahedral intermediate **11** which collapses to release aspartate-β-semialdehyde (ASA, **8**) and ASA-DH.

On the basis of this mechanistic rationale, we designed a series of potential inhibitors of ASA-DH by using the substrate structure **7** as a starting point. The enzyme utilises phosphate as an excellent nucleofuge and we reasoned that attenuation of the leaving group ability could provide inhibitory compounds.

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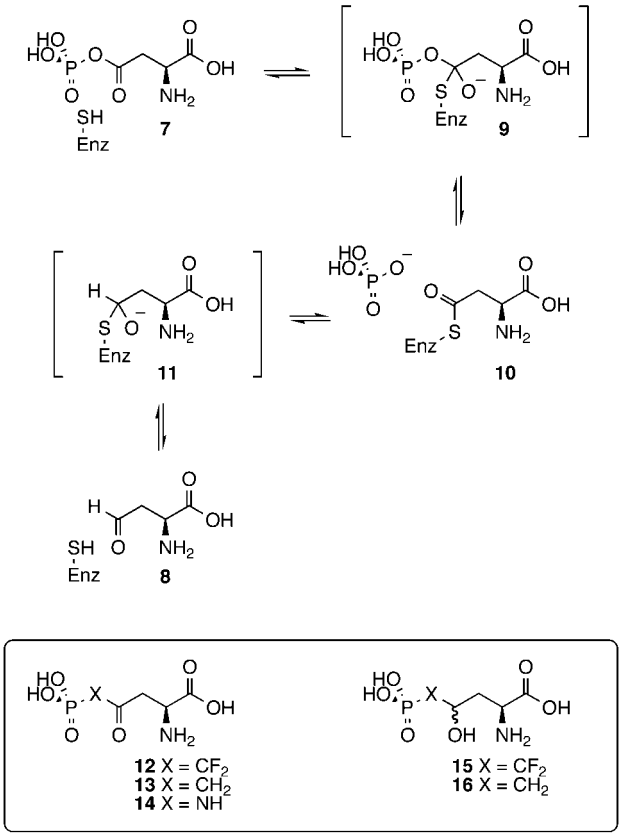


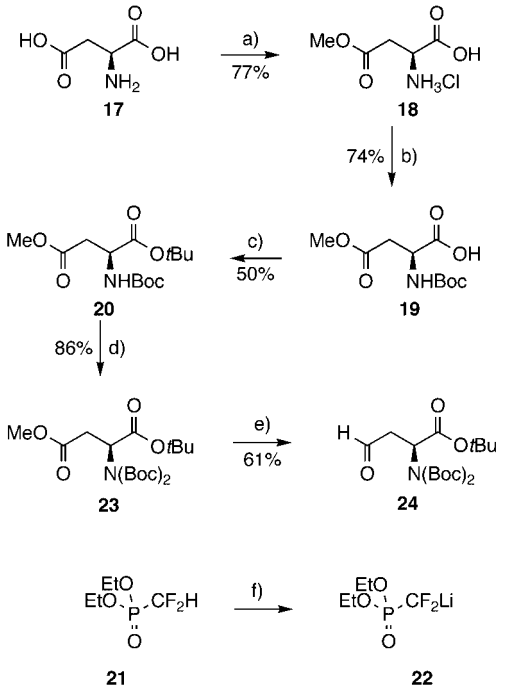
Scheme 2. *Likely chemical mechanism of ASA-DH and structures of substrate and intermediate analogues* ***12*** *–* ***16****.*

Thus, difluorophosphonate **12**[8] and phosphonate **13** were considered as potential substrate mimics. In the case of the difluorophosphonate **12** it could reasonably be expected that enhanced electrophilicity of the γ-carbonyl group could lead to significant covalent attachment to the active site nucleophile. The effect of adjacent fluorine atoms on the pK_a value of phosphonic acids has also been investigated and in some cases it has been claimed that difluoromethylene phosphonates can effectively mimic phosphates in terms of both steric factors and pK_a value.[9] The phosphoramidate **14** was also considered as a candidate inhibitor. Although the γ-carbonyl group of **14** is unlikely to possess significant electrophilic character, the pK_a value of the phosphoramidate closely matches that of the phosphate. We also considered the use of the reduced phosphonate analogues **15** and **16** which, although unable to form covalent linkages to ASA-DH, might mimic the presumed tetrahedral intermediates.

Results

Difluoromethylene Phosphonates

Differential protection of the carboxylate groups of aspartic acid has been well investigated. In order for the later selective attachment of the phosphonate at the γ-carbonyl group we decided to use methyl ester protection at this position. Thus, L-aspartic acid (**17**) was selectively monomethylated at the γ-carbonyl group by treatment with one equivalent of thionyl chloride in methanol (Scheme 3) following the method of Schwarz et al.[10–12] The protected amino acid was precipitated as the hydrochloride salt **18** which was then *N*-*tert*-butoxycarbonyl (Boc) protected under standard conditions to give the carboxylic acid **19**. Finally *tert*-butyl ester formation was achieved by an *N*-ethyl-*N*′-(3-dimethyl-aminopropyl)carbodiimide (EDCI) mediated coupling reaction with *t*BuOH to afford the desired protected aspartate **20**.



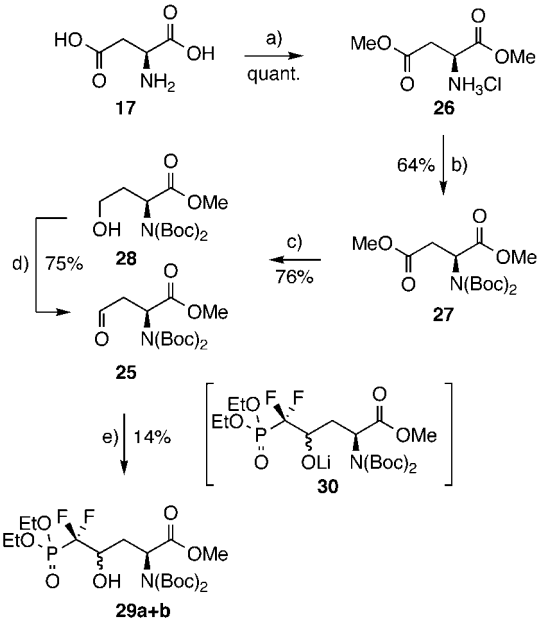
Scheme 3. *Synthesis of differentially protected* L*-aspartate and aspartate semialdehyde. a)* $SOCl_2$*, MeOH,* $-10\,^{\circ}C$*; b)* Boc_2O*, MeOH,* $NaHCO_3$*, RT; c) t*BuOH*,* CH_2Cl_2*, EDCI, DMAP, RT; d)* Boc_2O*, NaH, THF,* Δ*; e) DIBALH,* Et_2O*,* $-78\,^{\circ}C$*; f) LDA, THF,* $-78\,^{\circ}C$*. Boc* = tert-*butyloxycarbonyl, DIBALH* = *diisobutylaluminium hydride, DMAP* = *4-dimethylaminopyridine, EDCI* = N-*ethyl*-N′-*(3-dimethyl-aminopropyl)carbodiimide, LDA* = *lithium diisopropylamide, THF* = *tetrahydrofuran.*

Berkowitz et al. have shown that methyl esters can be treated with the lithium difluorophosphonate **22** to afford protected ketodifluoromethylene phosphonates in a single step.[13] However, treatment of the protected aspartate **20** with the preformed lithium salt **22** did not result in a productive reaction. Subsequent double Boc protection to give the fully protected aspartate **23** was then achieved in order to remove the acidic carbamate proton (Scheme 3). Compound **23** was also unreactive towards **22**. Percy and co-workers have recently reported the successful use of $CeCl_3$ to improve the yields of these reactions.[14] However, even under these conditions, no significant product formation was observed.

It was clear from these results that the γ-methyl ester was insufficiently electrophilic to react with **22**. We then considered increasing the reactivity of the electrophile by forming the corresponding aldehyde. The mixed ester **23** was treated with diisobutylaluminium hydride (DIBALH) to afford the correspond-

ing aldehyde **24** (Scheme 3), but this too was unreactive towards the lithium anion **22**.

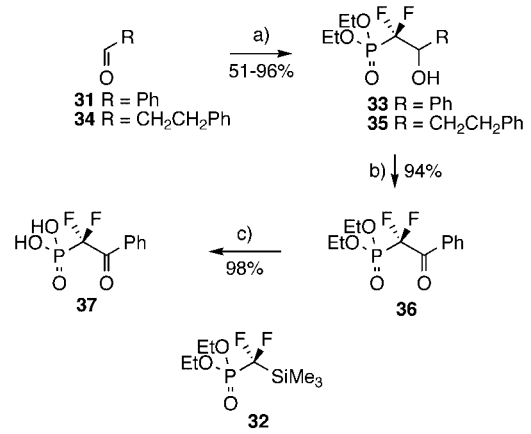
In order to simplify the synthesis, and to reduce possible steric crowding of the aldehyde, we chose to synthesise the methyl ester aldehyde **25** according to the method of Vederas and Sutherland. Thus, L-aspartic acid **17** was treated with refluxing HCl in anhydrous MeOH to form the bis-ester **26** (Scheme 4). Double Boc protection then afforded the fully protected aspartate **27**.[10, 15] The diester **27** was treated with DIBALH to form a mixture of the desired aldehyde **25** (71%), the corresponding alcohol **28** (5%) and unreacted starting material **27** (10%).[16, 17] There was no indication of reduction of the α-ester. The alcohol **28** could be smoothly oxidised to the aldehyde **25** with the Dess–Martin periodinane[18] in 75% yield.



Scheme 4. *Synthesis of fully protected L-aspartate semialdehyde and attempted conversion into difluoromethylene phosphonate. a) MeOH, HCl, Δ; b) Boc_2O, THF, NaH Δ; c) DIBALH, Et_2O, $-78\,^{\circ}C$, 71% for **25**, 5% for **28**, 10% for **27**; d) Dess–Martin periodinane, CH_2Cl_2, RT; e) **22** (7.5 equiv), THF, $-78\,^{\circ}C$.*

Treatment of aldehyde **25** with one equivalent of **22**, however, did not result in formation of the desired secondary alcohol. A high excess (7.5 equivalents) of **22** gave a low yield (approximately 14%) of the diastereomers **29a** and **29b**, but the yield could not be increased. We reasoned that under highly basic reaction conditions, a retro-aldol reaction of the intermediate oxyanion **30** would be favoured. We therefore attempted to perform a similar reaction under more neutral conditions.

In a series of control reactions, benzaldehyde (**31**) was treated with the trimethylsilyl (TMS) protected phosphonate **32** at $0\,^{\circ}C$ in the presence of catalytic fluoride (CsF, tetrabutylammonium fluoride (TBAF) or tetrabutylammonium tribromide (TBAT)) to give the expected benzylic alcohol **33**, as reported by Obayashi and Kondo, in excellent yield (Scheme 5).[19] The use of TBAF as the fluoride source gave significantly better yields (98%) than those reported in the literature[19] when using CsF (58%).



Scheme 5. *Synthesis of difluoromethylene phosphonates. a) **32**, THF, TBAF (10 mol%), $-78\,^{\circ}C$, 96% for **33**, 51% for **35**; b) Dess–Martin periodinane, CH_2Cl_2, RT; c) TMSI, then ion exchange. TBAF = tetrabutylammonium fluoride, TMSI = trimethylsilyl iodide.*

Hydrocinnamaldeyde (**34**) also reacted smoothly to give the corresponding secondary alcohol **35** under these conditions.

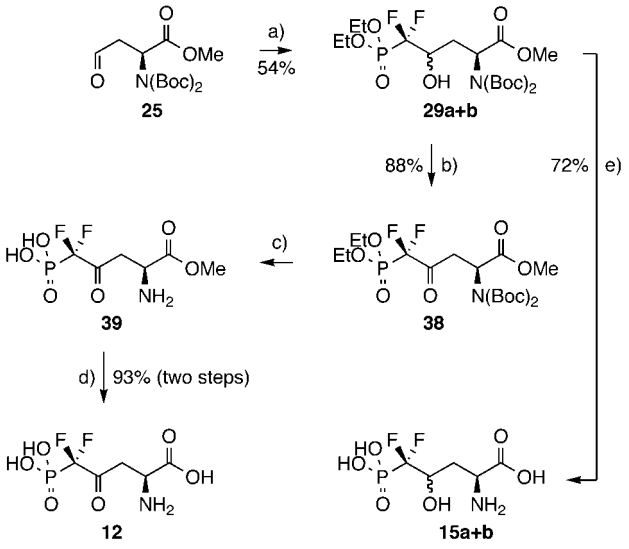
Under the catalytic fluoride conditions the intermediate alkoxides are presumably trapped as trimethylsilyl ethers which are not prone to retro-aldol reactions. The silyl ethers are then cleaved during workup and purification to give the relatively stable alcohol. The benzylic alcohol **33** was then treated with the Dess–Martin periodinane. This smoothly afforded the desired ketophosphonate **36**. Deprotection was achieved by treatment of the ketone **36** with trimethylsilyl iodide (TMSI) and the resulting ketophosphonate **37** was purified by anion exchange chromatography.

The synthetic aldehyde **25** was then used under identical conditions to give the expected mixture of diastereomers of the secondary alcohols **29a** and **29b** in 55% yield after optimisation (Scheme 6). Although the yield of the reaction was moderate, the balance of unreacted aldehyde **25** could be recovered from the reaction. Oxidation of the alcohols **29a** + **b** with the Dess–Martin periodinane then gave the desired fully protected ketophosphonate **38**.

Deprotection was achieved in two steps. Treatment with an excess of TMSI cleaved the ethyl esters and the two Boc groups, but, rather surprisingly, left the methyl ester intact; thus, the partially deprotected compound **39** was formed. However, further treatment with aqueous KOH rapidly hydrolysed **39** and gave the desired amino acid **12**, which was purified by ion exchange chromatography. The purified target compound was isolated as a 6:4 mixture of hydrate and keto forms. Alcohols **29a** + **b** were also deprotected and purified following the same procedure.

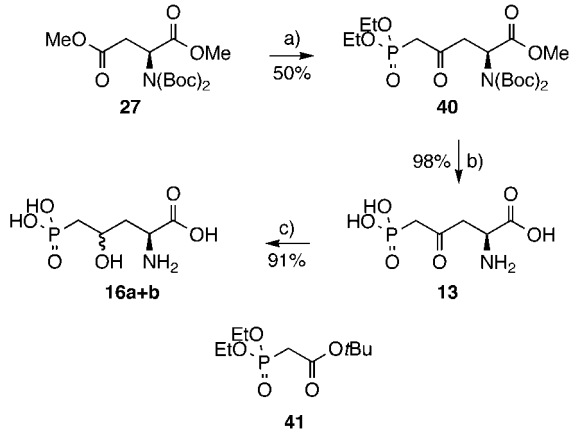
Methylene phosphonates

The nonfluorinated D-enantiomer of **13** has been previously reported as a potent *N*-methyl-D-aspartate (NMDA) antagonist.[20] Two synthetic approaches have been considered. That used by Baldwin et al. has featured ring opening of an appropriate β-



Scheme 6. *Synthesis of difluoromethylene phosphonates. a)* ***32****, THF, TBAF (10 mol%), −78 °C, 3:1 mixture of diastereomers; b) Dess–Martin periodinane, CH_2Cl_2, RT; c) TMSI; d) aq KOH, then ion exchange; e) TMSI, then KOH, then ion exchange.*

lactam,[21] while Whitten et al. have described the addition of $LiCH_2P(O)(OEt)_2$ to methyl esters.[20] We realised that modification of our procedure could also provide access to **13**. Thus, treatment of the bis-methyl ester **27** with lithium diethylmethylene phosphonate afforded the desired ketophosphonate **40** in approximately 50% yield (Scheme 7). The *tert*-butyl ester **41** was



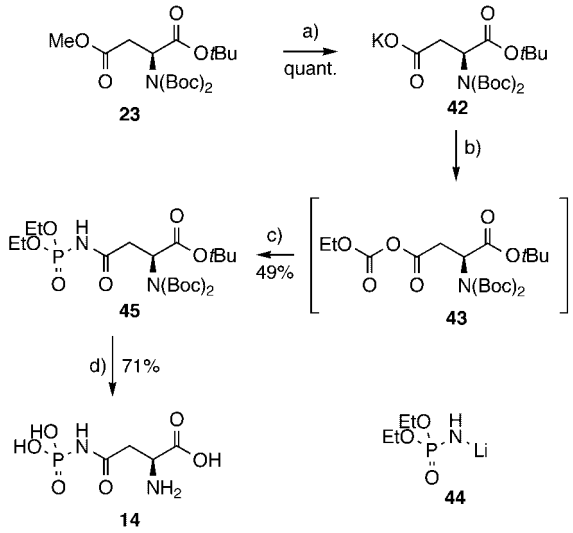
Scheme 7. *Synthesis of methylene phosphonates. a) $LiCH_2P(O)(OEt)_2$, THF, −78 °C; b) aq HCl, Δ, then ion exchange; c) aq $NaBH_4$, 0 °C, then ion exchange, 2:1 ratio of diastereomers.*

observed as a byproduct in low yield, presumably as a consequence of attack at the carbamate group. The nonfluorinated anion is clearly significantly more reactive than its difluorinated analogue **22** which did not react under similar conditions. The ketophosphonate **40** was deprotected by treatment with refluxing aqueous HCl followed by ion exchange chromatography to give the desired **13**. Treatment of **13** with $NaBH_4$ afforded a diastereomeric mixture of the secondary alcohols **16a** + **b**.

Phosphoramidate

N-Phosphoryl amides have been synthesised by the treatment of primary amides with $ClP(O)(OEt)_2$. We thus attempted to treat the methyl ester **23** with ammonia in order to produce a suitable amide. However, treatment of **23** with liquid ammonia at reflux temperature (−33 °C) for 12 h failed to give more than 1% conversion (LC–MS analysis). Similar results were obtained with refluxing NH_3/tetrahydrofuan (THF) and refluxing NH_3/H_2O. We therefore considered adding an intact P–N unit to a suitable aspartate derivative. It is clear, from the failed reactions with ammonia and the difficulties in adding lithium nucleophiles to methyl esters such as **23**, that the aspartate coupling partner would have to possess enhanced electrophilicity. In this case, the use of an aldehyde would be inappropriate because of the ease of retro-aldol reactions and acid chloride functionality would be incompatible with the Boc and *t*Bu protection.

We therefore treated the methyl ester **23** with 1 equivalent of KOH in H_2O/CH_3CN to afford the potassium salt **42** (Scheme 8). This was soluble in anhydrous CH_2Cl_2, and treatment with ethyl



Scheme 8. *Synthesis of γN-phosphoryl asparagine. a) KOH (1.0 equiv), CH_3CN/H_2O, 40 °C; b) CH_2Cl_2, EtOCOCl, RT; c)* ***44****, RT; d) TMSI (5.0 equiv), CH_2Cl_2/CH_3CN, 0 °C → RT, then ion exchange.*

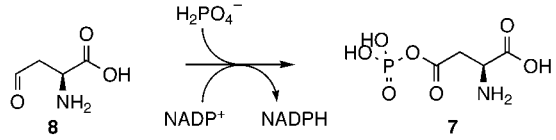
chloroformate then provided the mixed carbonate anhydride **43**. This active ester was then treated with an excess of the lithium salt **44** of commercially available diethyl phosphoramidate in situ. This afforded the desired fully protected *N*-phosphoryl amide **45** in good yield. Deprotection was achieved under mild conditions by using 5.0 equivalents of TMSI at 0 °C to afford **14** after extraction into H_2O and lyophilisation. Use of excess TMSI, elevated temperatures or extended exposure to aqueous acid conditions resulted in the formation of aspartic acid and asparagine as observed by TLC and LC–MS.

Enzyme assays

ASA-DH is most conveniently assayed in the "reverse" biosynthetic direction due to the instability of the substrate aspartyl

phosphate (**7**). ASA (**8**) itself was synthesised from allylglycine in a simple procedure involving ozonolysis in 1 M aqueous HCl followed by treatment with dimethyl sulfide.[22] The resulting aqueous solution containing ASA (**8**) and dimethyl sulfoxide (DMSO) was stable when stored at −20 °C for prolonged periods, showing no diminished activity in ASA-DH assays over time.

The assay procedure was performed by using L-ASA (**8**; 0.35 mM), inorganic phosphate (15 mM) and $NADP^+$ (150 μM, Scheme 9). These conditions were based on the published



Scheme 9. *Assay procedure for ASA-DH. ASA (**8**) was prepared by reductive ozonolysis of allylglycine.[22] The production of NADPH was monitored spectrophotometrically at 340 nm.*

Michaelis constant (K_M) values of the substrates.[6] Under these conditions satisfactory rates of NADPH formation were observed at 340 nm of around 3 μM min^{-1} (that is, 2% $NADP^+$ conversion min^{-1}, Figure 1). In initial inhibition assays we added the difluoromethylene phosphonate **12** (1 – 10 mM) to standard ASA-DH assay mixtures, but we were disappointed to observe no apparent inhibition of the reaction.

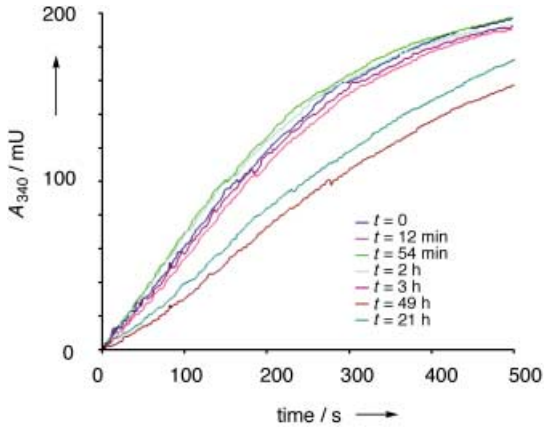


Figure 1. *Typical plot of NADPH production versus time for a standard ASA-DH assay containing ASA-DH (2.8 μg), 350 μM ASA (**8**), 150 μM $NADP^+$ and 15 mM phosphate in 0.2 M tris(hydroxymethyl)aminomethane (pH 8.6). The enzyme was tested for denaturation at 37 °C over the indicated time.*

We reasoned that under the high phosphate concentrations required to force the enzyme to run in the reverse direction, the active site may be significantly occupied by phosphate, effectively blocking the binding by **12**. In order to test this theory we preincubated ASA-DH with **12** in the absence of all substrates at 37 °C for varying periods of time. Aliquots of the enzyme/**12** solution were then tested for residual activity. In the absence of phosphate, **12** does inhibit ASA-DH in a time- and concentration-dependent manner (Figure 2). The inhibition is not irreversible

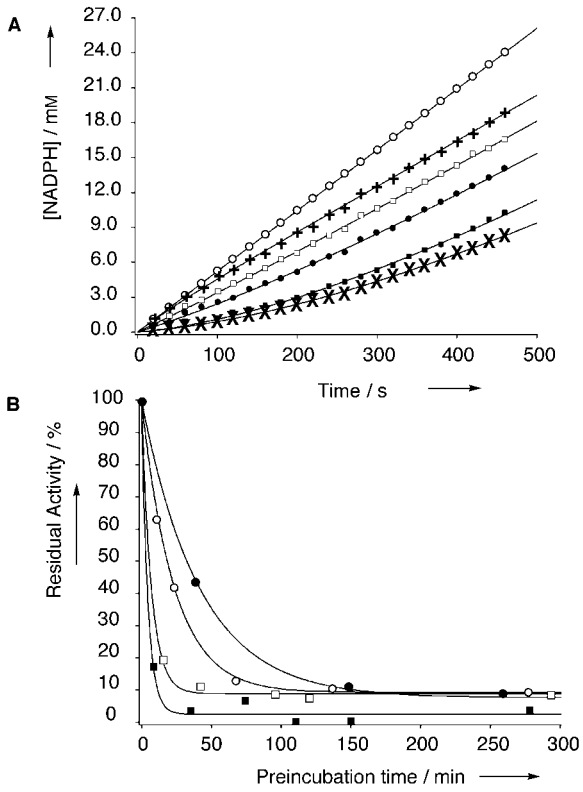


Figure 2. *Inhibition of ASA-DH by **12**. A) Inhibition of ASA-DH by preincubation with **12** at 0.66 mM. ○ uninhibited reaction; + preincubation with **12** for 0 min; □ preincubation with **12** for 12 min; ● preincubation with **12** for 25 min; ■ preincubation with **12** for 68 min; × preincubation with **12** for 138 min. B) Rate of inhibition of ASA-DH at varying concentrations of **12** (○ 0.66 mM; □ 2.5 mM; ■ 5.0 mM) without phosphate and at 2.5 mM **12** (●) in the presence of 15 mM phosphate.*

however, as indicated by the fact that 100% inhibition was not observed. In addition, when enzyme that had been treated with **12** for some time was diluted into the assay reaction, the activity of the inhibited enzyme slowly recovers, resulting in curved plots. The effect is most clearly observed in the enzyme samples which are initially most inhibited (for example, crosses in Figure 2 A).

Evidence for binding at the active site was obtained by performing the inhibition reactions in the presence of inorganic phosphate—significant protection from inhibition was observed when inorganic phosphate was present. Based on this assay procedure, an inhibition constant (K_i) value of 95 μM was calculated for **12**.

We next examined inhibition by a mixture of the diastereomeric alcohols **15a** + **b**. In both preincubation and direct assays this mixture of compounds did not appear to show significant inhibition. The same lack of activity was displayed by the mixture of nonfluorinated diastereomeric alcohols **16a** + **b**.

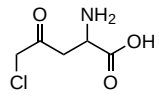
The nonfluorinated ketophosphonate **13** was then tested in preincubation assays. In contrast to the activity observed for **12**, this compound showed both relatively weak and slower inhibition under these conditions. However, in direct assays this compound caused much more evident inhibition in the presence of phosphate. Standard Michaelis – Menten analysis

of this inhibition (varying concentrations of ASA (**8**), phosphate and **13**) revealed that **13** inhibits ASA-DH competitively versus ASA with $K_i = 750\ \mu M$ and noncompetitively versus phosphate with $K_i = 2.13$ mM (Figure 3 A).

The phosphoramidate **14** was also tested as an inhibitor of ASA-DH. No apparent time-dependent inhibition was observed when **14** was incubated with ASA-DH alone. However, **14** showed clear competitive inhibition versus ASA ($K_i = 214 \pm 120\ \mu M$) and competitive inhibition ($K_i = 92 \pm 40\ \mu M$) when assayed versus inorganic phosphate in the standard activity assay (Figure 3 B).

Discussion

Because of its position at the start of branching pathways to diverse amino acids in bacteria, ASA-DH is potentially a good target for the de novo design of antibacterial compounds. Despite this fact surprisingly little information has been gathered regarding inhibition of ASA-DH. The compounds described here are the first systematically designed and synthesised compounds shown to inhibit the enzyme. Other compounds are known to inhibit ASA-DH, however. The chloroketone **46** is an irreversible inhibitor of ASA-DH, presumably because of covalent bond formation with the nucleophilic active site cysteine.



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Kish and Viola have recently reported results of a study on the substrate and inhibition properties of various metal oxyanions as mimics of phosphate.[23] A range of species were studied; arsenate and vanadate were found to be effective substrates of ASA-DH, while perrhenate, tungstate, phosphonate, tellurate and, most effectively, periodate were found to be inhibitors. It was suggested that charge on oxygen might be the key to binding, although the best inhibitor, periodate, has an anomalous oxyanion charge. Surprisingly pK_a values of the species were not considered. When we correlate the pK_a values of the inhibitory/substrate species with their respective binding constants (Figure 4), a clear preference for pK_a values in the region of 7.5 – 8 for good inhibitors/substrates is evident.

Our results also support the importance of pK_a values of the binding species at the phosphate site. The substrate for the reaction, aspartyl phosphate (**7**), would be expected to have a second phosphate pK_a value in the region of 4.8 – 5.4.[24] The difluoromethylene phosphonate **12** would likely have a relatively low second phosphate pK_a value (values in the range of 4.2 – 5.0 have been reported).[25] This compound shows very poor ability to compete with phosphate, as would be expected. On the other hand, the methylene phosphonate **13** competes much better in the presence of phosphate. pK_a values in the region of 6.1 have been measured for similar compounds.[24] The phosphoramidate **14** has a slightly higher pK_a value (values in the range of 6.2 – 6.4 have been reported)[26] and our inhibition experiments show a much better inhibitory profile compared to the methylene phosphonate **13**.

These observations are rationalised by observation of the active site of ASA-DH from recently reported crystallographic

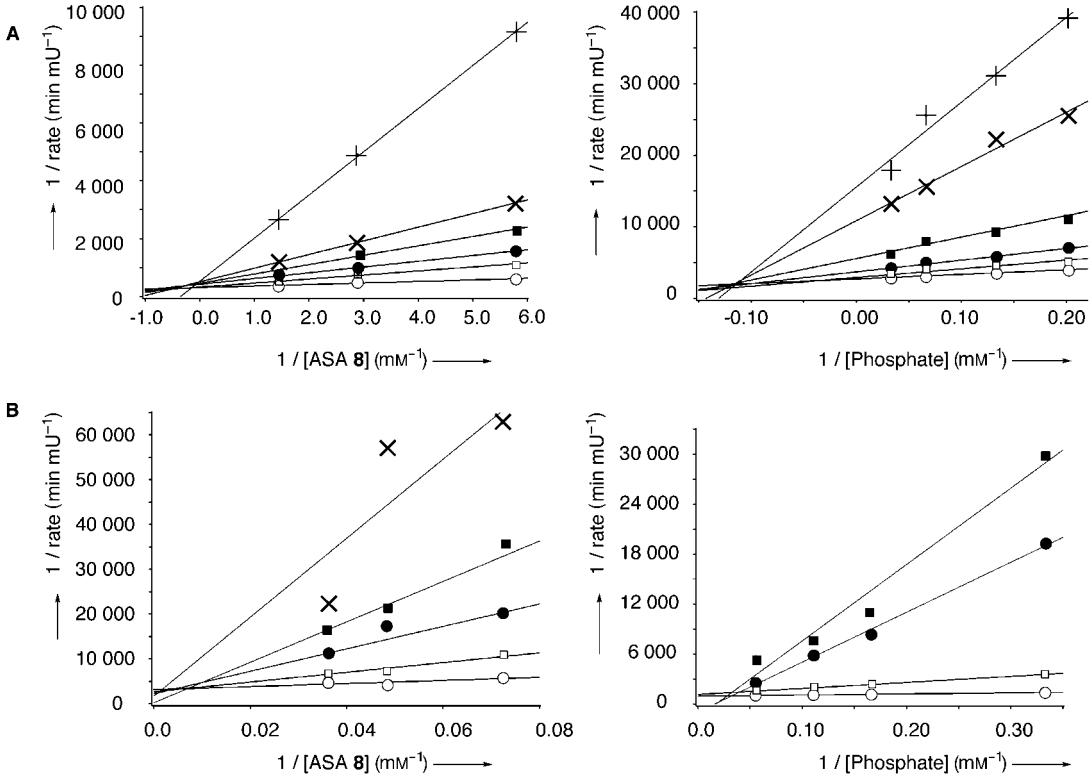

Figure 3. *Inhibition of ASA-DH by **13** and **14**. Plots of 1/rate versus 1/[substrate] for: A) **13**; ○ uninhibited reaction; □ 1.0 mM; ● 2.5 mM; ■ 5.0 mM; × 10 mM; + 20 mM; B) **14**; ○ uninhibited reaction; □ 1.0 mM; ● 1.5 mM; ■ 2.0 mM; × 2.5 mM. Left-hand panels show inhibition against ASA (**8**), right-hand panels show inhibition against inorganic phosphate.*

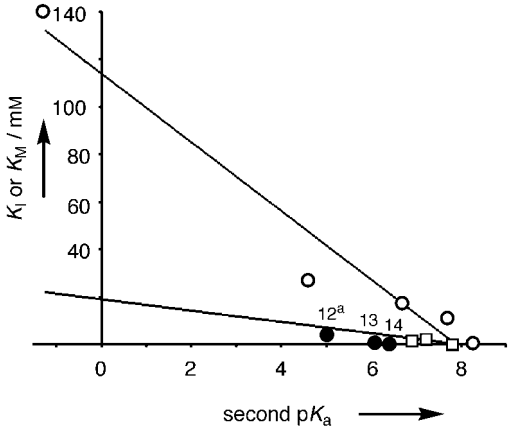

Figure 4. *Relationship between second pK_a values of inhibitory and substrate species and measured binding constants. ○: Inhibitory activity of (from left) perrhenate, tungstate, phosphonate, tellurate and periodate. □: Substrate activity of (from left) arsenate, phosphate and vanadate. ●: Compounds from this study as indicated (a = estimated value). K_I and K_M data were taken from the work of Kish and Viola.[23]*

investigations.[7, 27, 28] Generation of a model structure of **12** covalently bound to Cys135 reveals potential substrate binding residues (Figure 5). In the model structure a single positively charged species (Arg267) interacts with bound phosphate, while

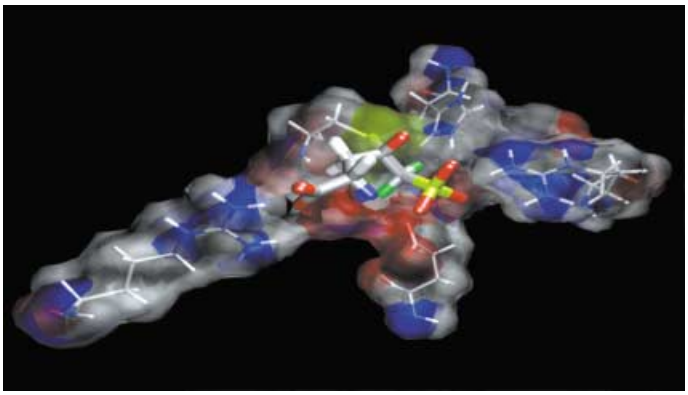

Figure 5. *Model of **12** (centre) covalently bound to Cys135 (rear) in the active site of ASA-DH and showing key potential binding residues Arg267 (right), Arg102 (left), Glu241 (below) and catalytic His274 (rear). The model was generated by using the PDB coordinates for ASA-DH (file: 1gl3.pdb) and the flexible ligand docking package Gold (Cambridge Crystallographic Data Centre). The figure was constructed with the VMD Molecular Graphics program[37] and rendered with PovRay.*

the ASA carboxylate is bound by Arg102. Arg267 has already been implicated as a substrate binding residue by site-directed mutagenesis experiments.[29] This implies that the species bound at the phosphate binding site should have a single negative charge, that is, it should be a singly ionised anion. Species with a high second pK_a value will better fulfil this criterion, while more acidic species, such as tungstate and the difluoromethylene phosphonate **12**, will be doubly ionised and less likely to be recognised at the active site.

The effect of fluorine on **12** is, however, more complex than merely lowering the phosphate pK_a value. Its inductive activation of the adjacent γ-carbonyl group, evinced by the observation of stable hydrate forms of **12** and **39**, makes covalent attachment to the active site thiol of ASA-DH energetically more favourable. Because of this, **12** shows good inhibition of ASA-DH (in the absence of phosphate). Lack of fluorine in compound **13** results in very poor time-dependent (that is, likely covalent) inhibition, while **14**, with its deactivated amide γ-carbonyl group, shows no detectable time-dependent inhibition.

Future inhibitor design will have to heed both phosphate mimic pK_a values and polarisation of the carbonyl group to ensure maximal inhibition. Steric considerations may also be important as the tetrahedral alcohols **15** and **16** all showed negligible inhibition of ASA-DH.

Experimental Section

All reagents were used without further purification unless otherwise stated. The Dess–Martin periodinane[18] was prepared according to the improved method of Ireland and Liu.[30] TLC was carried out on Merck glass plates coated with 0.2 mm silica gel, eluted with the indicated solvent and visualised with ultraviolet light (254 nm) or developed with permanganate, phosphomolybdic acid, ninhydrin (with previous HCl treatment if needed) or *o*-anysaldehyde solutions and heated with a hot-air gun. Merck Kieselgel 60 was used for flash chromatography according to the method of Still et al.[31]

IR absorption spectra were measured on a Perkin Elmer FT-IR Paragon 1000 machine with oil or solid samples mounted directly over the diamond cell. Melting points were obtained on an electrothermal melting point apparatus and are uncorrected. Optical rotations were measured with Perkin-Elmer 141 and 241C polarimeters in 1 dm cells. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

NMR spectra were recorded on JEOL Δ270, JEOL Λ300, JEOL Δ400 and JEOL Λ500 spectrometers at the indicated frequency. Chemical shifts of samples dissolved in $CDCl_3$ are reported in ppm downfield from tetramethylsilane, while shifts of samples in D_2O are reported downfield from sodium 3-(trimethylsilyl) propionate. ^{31}P NMR was referenced to phosphoric acid and ^{19}F NMR was referenced to trifluoroacetic acid. ^{13}C NMR spectra were obtained under broad-band proton-decoupled conditions {^{1}H}. ^{31}P NMR spectra were obtained under the indicated conditions.

Mass spectra were determined with a Micromass Autospec mass spectrometer by EI at a potential of 70 eV or by CI. LC–MS analyses were run with a Waters/Micromass system comprising a Waters 600 LC system equipped with both Waters 996 photodiode array and platform MS detectors running in ES$^+$ mode. Chromatographic separations were achieved with a Phenomenex C_8 reverse-phase column (4.6 × 250 mm) run at 1 mL min^{-1}. Solvent A: 0.1% TFA in water. Solvent B: 0.05% TFA in CH_3CN. Samples (20 μL) of approximately 1 mg mL^{-1} were injected. The gradient was as follows: 0 min, 0%B; 13 min, 99% B; 17 min, 99% B; 18 min, 0% B; 20 min, 0% B. The void volume of the system was 3.0 mL. Data analysis was performed with MassLynx v3.3 software. Elemental analyses were carried out in the microanalytical laboratories of the University of Bristol.

Enzyme assays: Stock solutions were made with Milli-Q water and ACS grade reagents. ASA-DH was expressed and purified from

recombinant *Escherichia coli* (provided by Dr. A. Hadfield, Univeristy of Bristol). The decrease or increase in β-NADPH concentration was observed at 340 nm over 600 seconds in a Pharmacia-LKB Ultrospec III spectrophotometer equipped with a water-heated (37 °C) cuvette holder. The buffer solutions were prewarmed to 37 °C before use by immersion in a water bath. The other stock solutions (see below) were stored on ice.

The assay was performed in the following way: Buffer solution (910 μL, 0.2 M tris-(hydroxymethyl)aminomethane (Tris), 1.0 mM ethylenediamintetraacetate (EDTA), pH 8.6), phosphate solution (10 μL, 1.5 M) ASA-DH solution (20 μL, 14 μg mL^{-1}) and ASA (**8**)[22] (30 μL, 11.5 mM) were introduced, in that order, into a 1000-μL quartz cuvette which was placed in the spectrophotometer. Then, $NADP^+$ (30 μL, 5 mM) was added, and the subsequent reaction was monitored at 340 nm over 600 s. Data points were collected every 2 s and the plots were analysed with Microsoft Excel.

The synthetic compounds **12** – **16** were then tested for inhibition by using standard procedures.

(2*S*,4*RS*)-(5,5-Difluoro-4-oxo-2-amino-5-phosphoryl)butyric acid (12): Under anhydrous nitrogen, a solution of (2*S*)-methyl-2-[bis(*tert*-butyloxycarbonyl)]amino-4-oxo-5,5-difluoro-5-(diethoxy)-phosphoryl pentanoate (**38**, 200 mg, 386 μmol) in a mixture of anhydrous CH_2Cl_2 (6 mL) and anhydrous CH_3CN (6 mL) was cooled to 0 °C. Freshly distilled trimethylsilyl iodide (400 μL, 2.66 mmol) was added and the solution was stirred for 30 min. The reaction mixture was allowed to reach RT and then stirred for a further 1.5 h. Deionised water (12 mL) was added to the mixture, which was then stirred for 30 min. The aqueous layer was separated and washed with EtOAc (5 × 10 mL). The aqueous solution was lyophilised to give a dark yellow solid (180 mg) and pertinent analysis showed that, apart from the methyl ester group, all other protecting groups were removed. A 45:55 mixture of the ketone and hydrate forms of **39** was obtained. ^{1}H NMR (300 MHz, D_2O, hydrate and keto forms) 4.40 – 4.20 (m, 1H, αCH), 3.70 – 3.17 (m, 2H, βCH, hydrate), 3.63 (s, 3H, OCH_3), 2.44 – 2.29 (m, 1H, βCH), 2.25 (dd, 1H, ^2J(H,H) = 15.4 Hz, ^3J(H,H) = 9.0 Hz, βCH) ppm; ^{13}C NMR (75.5 MHz, D_2O, hydrate and keto forms): δ = 200.3 (m, $\underline{C}OCF_2$), 170.5 (CO_2Me, hydrate), 169.4 (CO_2Me), 124.4 – 110.3 (m, $\underline{C}F_2$), 95.2 – 94.2 (m, $C(OH)_2$, hydrate), 54.2 (OCH_3), 54.0 (OCH_3, hydrate), 49.2 (αCH, hydrate), 47.7 (αCH), 38.1 – 37.5 (m, $\beta\underline{C}H_2$, hydrate), 34.2 – 33.5 (m, $\beta\underline{C}H_2$) ppm; LC – MS (ES^+, CH_3CN/H_2O): R_T = 3.2 min, *m/z* (%): 523 (11, [2*M*]H$^+$), 303 (14, [*M*+CH_3CN]H$^+$), 284 (4, [*M*+Na]$^+$), 280 (75, [*M* hydrate]H$^+$), 262 (100, [*M* ketone]H$^+$).

A solution of KOH (2 mL, 5 M) was added dropwise to an aqueous solution (6 mL) of the hydrate and ketone. The mixture was stirred at RT for 5 h and the solvent was evaporated to give a yellow solid that was purified on a cation-exchange column (Dowex AG50-Wx8). The ninhydrin-positive and LC – MS-positive ([*M*]H$^+$ = 248) fractions were collected. The aqueous residue was lyophilised and a light grey solid was obtained as a 2:3 mixture of the ketone and hydrate forms of (2*S*)-**12** (7.3 mg, 353 μmol, 93 %). $[\alpha]_D^{23}$ − 2.2 (*c* = 0.5 in H_2O); IR (solid): 3169 (N–H), 2922 (C–H), 1736 (C=O), 1617 (NH_2, N–H), 1173 (P(O)OH, P=O), 1056 (C–F) cm^{-1}; ^{1}H NMR (400 MHz, D_2O): δ = 4.05 (dd, 1H, ^3J(H,H) = 4.1, 8.05 Hz, αCH, hydrate and keto), 3.39 (dd, 1H, ^2J(H,H) = 19.8 Hz, ^3J(H,H) = 4.1 Hz, βCH, keto), 3.32 (dd, 1H, ^2J(H,H) = 20.1 Hz, ^3J(H,H) = 7.8 Hz, βCH, keto), 2.35 (dd, 1H, ^2J(H,H) = 15.4 Hz, ^3J(H,H) = 4.4 Hz, βCH, hydrate), 2.17 (dd, 1H, ^2J(H,H) = 15.4 Hz, ^3J(H,H) = 8.2 Hz, βCH, hydrate) ppm; ^{19}F NMR (283 MHz, D_2O): δ = − 120.2 (br d, 2F, ^2J(F,P) = 84.1 Hz, keto), − 122.6 (dd, 1F, ^2J(F,F) = 301.8 Hz, ^2J(F,P) = 90.6 Hz, hydrate), − 123.5 (dd, 1F, ^2J(F,F) = 300.0 Hz, ^2J(F,P) = 0.3 Hz, hydrate) ppm; ^{31}P NMR (122 MHz, D_2O, {^{1}H}): δ = 0.7 (t, ^2J(F,P) = 85 Hz, keto), 3.3 (dd, ^2J(F,P) = 87 Hz, ^2J(F,P) = 89 Hz, hydrate) ppm; ^{13}C NMR (75.5 MHz, D_2O): δ = 70.1 (m, CF_2), 65.2 (CHOH), 55.5 (αCH), 54.0 (OCH_3), 52.9 (OCH_2CH_3), 30.3 $\beta\underline{C}H_2$), 28.6 (C–$\underline{C}H_3$), 16.9 ($\underline{C}H_3$–CH_2) ppm; MS (EI$^+$): *m/z* (%): 355 (18), 341 (16), 281 (72), 267 (18, [*M* hydrate+H]H$^+$), 248 (25, [*M* ketone]H$^+$), 229 (42), 207 (78), 190 (32), 185 (6, [*M* hydrate − PO_3H_2]$^+$), 167 (6, [*M* ketone − PO_3H_2]$^+$), 137 (98), 135 (100), 128 (36), 125 (48), 119 (60); LC – MS (ES^+, CH_3CN/H_2O): R_T = 3.5 min, *m/z* (%): 495 (20, [2*M*]H$^+$), 290 (45, [*M*+CH_3CN]H$^+$), 270 (50, [*M*+Na]$^+$), 266 (75, [*M*+H_2O]H$^+$), 248 (100, [*M*]H$^+$).

(2*S*)-2-Amino-4-oxo-5-phosphoryl pentanoic acid (13)[21, 32]: (2*S*)-Methyl-2-[bis(*tert*-butyloxycarbonyl)]amino-4-oxo-5-(diethoxy)phosphoryl pentanoate (**40**, 670 mg, 1.50 mmol) was dissolved in 5 M HCl solution (5 mL). The solution was heated under reflux for 3 h. After cooling to RT, the mixture was washed with ethyl acetate (4 × 40 mL). The aqueous solution was evaporated to afford a solid product which was dissolved in H_2O. The aqueous solution was passed through a cation-exchange column (Dowex AG50-Wx8). The ninhydrin-positive and LC – MS-positive ([*M*]H$^+$ = 212) fractions were combined and freeze-dried, to give the product **13** as a colourless solid (315 mg, 1.49 mmol, 98 %). $[\alpha]_D^{25}$ − 5.2 (*c* = 0.44 in H_2O), (ref. [21]: $[\alpha]_D^{25}$: − 5.4 (*c* = 0.25 in H_2O)); IR (solid): $\tilde{\nu}_{max}$ = 2910 (C–H), 1706 (C=O), 1595 (NH_2, N–H), 1198 (P(O)OH, P=O) cm^{-1}; ^{1}H NMR (270 MHz, D_2O): δ = 4.23 (dd, 1H, ^3J(H,H) = 5.8, 4.5 Hz, αCH), 3.46 – 3.12 (m, 2H, βCH), 3.05 (dd, 2H, ^2J(H,H) 21.8, ^2J(H,P) = 1.32 Hz, $\delta\underline{C}H_2P$) ppm; after D_2O exchange ^{1}H NMR (300 MHz, D_2O): δ = 4.22 (dd, 1H, ^3J(H,H) = 5.8, 4.5 Hz, αCH), 3.43 – 3.26 (m, 2H, βCH) ppm; ^{31}P NMR (122 MHz, D_2O, {^{1}H}): δ = 15.0 (s) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 203.6 (d, ^2J(C,P) = 7 Hz, γC=O), 171.1 (CO_2H), 49.0 (d, ^1J(C,P) = 153 Hz, $\delta\underline{C}H_2P$), 48.4 (αCH), 42.8 ($\beta\underline{C}H_2$) ppm; LC – MS (ES^+, CH_3CN/H_2O): R_T = 4.0 min, *m/z* (%): 423 (32, [2*M*]H$^+$), 271 (8, [*M*+CH_3CN+H_2O]H$^+$), 253 (12, [*M*+CH_3CN]H$^+$), 212 (100, [*M*]H$^+$).

***γN*-Phosphoryl-L-asparagine (14)**: (2*S*)-γ*N*-diethoxyphosphoryl-α*N*,α*N*-bis(*tert*-butyloxycarbonyl)-L-asparagine *tert*-butyl ester (**45**; 160 mg, 305 μmol) was dissolved in anhydrous CH_2Cl_2 (6 mL) and anhydrous CH_3CN (6 mL) and the solution was cooled to 0 °C under dry N_2. Freshly distilled trimethylsilyl iodide (234 μL, 1.53 mmol) was added to the mixture and the reaction was stirred at 0 °C for 1 h. The solution was then allowed to warm to RT and stirred for another 2 h. Finally distilled water was added (3 mL) and the mixture was stirred for 1 h at RT. The crude reaction mixture was washed successively with CH_2Cl_2 (3 × 7 mL) and EtOAc (3 × 7 mL). The aqueous layer was collected, the solvent was evaporated and the orange mixture was purified on a cation-exchange column (Dowex AG50-Wx8). Ninhydrin-positive and LC – MS-positive ([*M*]H$^+$ = 213) fractions were collected and the mixture was lyophilised. **14** was obtained as a colourless solid (47 mg, 221 μmol, 72 %). $[\alpha]_D^{24}$ + 2.7 (*c* = 0.5 in CH_2Cl_2); mp: 168 – 169 °C; IR (oil); $\tilde{\nu}_{max}$ = 3427 (O–H), 3170 (CONH, N–H), 2843 (C–H), 1668 (N–H), 1429 (OH), 1180 (P(O)OH, P=O) cm^{-1}; ^{1}H NMR (300 MHz, D_2O): δ = 4.31 (dd, 1H, ^3J(H,H) = 5.9, 4.95 Hz, αCH), 3.05 (dd, 1H, ^2J(H,H) = 18.3 Hz, ^3J(H,H) = 5.9 Hz, $\beta\underline{C}H$H), 2.97 (dd, 1H, ^2J(H,H) = 18.3 Hz, ^3J(H,H) = 4.9 Hz, $\beta\underline{C}H$H) ppm; ^{31}P NMR (121 MHz, D_2O, {^{1}H}): δ = 0.7 (s) ppm; ^{31}P NMR (121 MHz, D_2O): δ = 0.7 (m) ppm; ^{13}C NMR (75 MHz, D_2O): δ = 193.3 (d, ^2J(C,P) = 38 Hz, γCO), 177.2 (CO_2H), 45.1 (αCH), 28.8 (d, ^3J(C,P) = 2.9 Hz, $\beta\underline{C}H_2$) ppm; LC – MS (ES^+, CH_3CN/H_2O): R_T = 2.7 min, *m/z* (%): 254 (5, [*M*+CH_3CN]H$^+$), 213 (8, [*M*]H$^+$), 143 (7), 142 (100), 119 (5, [*M* − $NHPO_3$]H$^+$), 101 (86); MS (EI$^+$): *m/z* (%): 254 (60 [*M*+CH_3CN]H$^+$), 225 (35), 213 (5, [*M*]H$^+$), 153 (24), 127 (100).

(2*S*,4*RS*)-5,5-Difluoro-4-hydroxy-2-amino-5-phosphoryl pentanoic acid (15): (2*S*-4*RS*)-Methyl-2-[bis(*tert*-butyloxycarbonyl)]amino-4-hydroxy-5,5-difluoro-5-(diethoxyphosphoryl) pentanoate (**29**) (66.7 mg, 130 μmol) was dissolved in a 1:1 mixture of anhydrous CH_2Cl_2 (1.5 mL) and CH_3CN (1.5 mL) and the resulting mixture was cooled to 0 °C. Freshly distilled trimethylsilyl iodide (99 μL, 663 μmol) was added to the solution which was stirred for 20 min. The reaction

mixture was allowed to reach RT and then stirred for 1.5 h. After adding water (2 mL) the mixture was stirred for further 10 min. The aqueous layer was collected, washed with EtOAc (5 × 3 mL) and the solvent was evaporated. The solid was redissolved in water and lyophilised to give a dark yellow solid (54 mg, 109.2 μmol, 84%). Pertinent analysis showed that all protecting groups except the methyl ester had been removed. Two diastereomers (**A**, minor, and **B**, major) were observed. ^{1}H NMR (300 MHz, D_2O): $\delta = 4.25-4.12$ (m, 1H, αCH, **A**), 4.30–4.12 (m, 1H, γCHOH, **A**), 4.30–4.12 (m, 1H, γCH, **B**), 4.2 (t, 1H, ^3J(H,H) = 6.1 Hz, αCH, **B**), 3.74 (s, 3H, OCH_3, **A**), 3.72 (s, 3H, OCH_3, **B**), 2.46 (ddd, 1H, ^2J(H,H) = 15.4 Hz, ^3J(H,H) = 6.2, 2.1 Hz, βCH, **B**), 2.41–2.30 (m, 1H, βCH, **A**), 2.26–2.14 (m, 1H, βCH, **A**), 2.06 (ddd, 1H, ^2J(H,H) = 15.8 Hz, ^3J(H,H) = 9.2, 4.8 Hz, βCH, **B**) ppm; ^{19}F NMR (283 MHz, D_2O): $\delta = -119.25$ (ddd, 1F, ^2J(F,F) = 294.2 Hz, ^2J(F,P) = 90.45 Hz, ^3J(H,F) = 8.2 Hz, **B**), −119.4 (ddd, 1F, ^2J(F,F) = 295.1 Hz, ^2J(F,P) = 91.3 Hz, ^3J(H,F) = 10.7 Hz, **A**) −126.6(ddd, 1F, ^2J(F,F) = 294.0 Hz, ^2J(F,P) = 90.95 Hz, ^3J(H,F) = 17.2 Hz, **A**); −126.75 (ddd, 1F, ^2J(F,F) = 294.0 Hz, ^2J(F,P) = 90.2 Hz, ^3J(H,F) = 17.2 Hz, **B**) ppm; ^{31}P NMR (122 MHz, D_2O, {^{1}H}): $\delta = 3.5$ (t, ^2J(F,P) = 91 Hz, **B**), 3.4 (t, ^2J(F,P) = 91 Hz, **A**) ppm; LC–MS (ES$^+$, CH_3CN/H_2O): R_T = 3.65 min (**B**), 3.75 min (**A**), *m/z* (%): 526 (10, $[2M]H^+$), 305 (8, $[M+CH_3CN]H^+$), 264 (100, $[M]H^+$), 204 (5, $[M-CO_2Me]^+$), 186 (6, $[M-CO_2Me-H_2O]H^+$).

The solid was dissolved in distilled water (1 mL), a solution of KOH (0.5 mL, 2.0 M, 58 mmol) was added dropwise and the resulting solution was then stirred overnight at RT. The solvent was removed by freeze-drying and the obtained yellow-brown solid was purified by cation-exchange chromatography (Dowex AG50-Wx8). Ninhydrin-positive and LC–MS-positive ($[M]H^+ = 250$) fractions were collected together and lyophilised to give a mixture of diastereomers (**A**, minor, and **B**, major) of **15a** + **b** as a light grey solid (23 mg, 93.6 μmol, 72% overall). $[\alpha]_D^{25}$: +11.5 (c = 2.3 in H_2O); IR (solid): $\tilde{\nu}_{max}$ = 3139 (N–H), 3139 (O–H), 2892 (C–H), 1616 (NH_2, N–H), 1440 (O–H), 1160 (P(O)OH, P=O), 1033 (C–F) cm^{-1}; ^{1}H NMR (300 MHz, D_2O): δ = 4.30–4.05 (m, 1H, αCH, **A** + **B**), 4.30–4.05 (m, 1H, γCH, **A** + **B**), 2.48–2.40 (m, 1H, βCH, **A**), 2.41–2.30 (m, 1H, βCH, **B**), 2.32–2.21 (m, 1H, βCH, **B**), 2.06–1.96 (m, 1H, βCH, **A**) ppm; ^{19}F NMR (283 MHz, D_2O): $\delta = -19.7$ (ddd, 1F, ^2J(F,F) = 284.1 Hz, ^2J(F,P) = 82.2 Hz, ^3J(H,F) = 12.7 Hz, **A**), −120.6 (ddd, 1F, ^2J(F,F) = 282.65 Hz, ^2J(F,P) = 82.3 Hz, ^3J(H,F) = 11.9 Hz, **B**), −123.8 (ddd, 1F, ^2J(F,F) = 284.9 Hz, ^2J(F,P) = 81.1 Hz, ^3J(H,F) = 13.6 Hz, **B**), −24.55 (ddd, 1F, ^2J(F,F) = 284.9 Hz, ^2J(F,P) = 82.2 Hz, ^3J(H,F) = 14.7 Hz, **A**) ppm; ^{31}P NMR (122 MHz, D_2O, {^{1}H}): $\delta = 1.7$ (t, ^2J(F,P) = 86 Hz, **B**), 1.65 (t, ^2J(F,P) = 86.5 Hz, **A**) ppm; ^{13}C NMR (75.45 MHz, D_2O): $\delta = 171.5$ (CO_2H, **A** + **B**), 124.5–115.0 (m, CF_2, **A**), 119.7 (ddd, ^2J(C,F) = 264 Hz, ^2J(C,F) = 261 Hz, ^2J(C,P) = 189 Hz, CF_2, **B**), 69.1–68.2 (m, γCHOH, **B**), 68.4–67.6 (m, γCHOH, **A**), 50.7 (αCH, **B**), 50.5 (αCH, **A**), 30.2 (βCH_2, **B**), 29.8–29.7 (βCH_2, **A**) ppm; LC–MS (ES$^+$, CH_3CN/H_2O): R_T = 3.5 min (**B**), 3.6 min (**A**), *m/z* (%): 386 (35), 250 (100, $[M]H^+$), 208 (17), 170 (75).

(2*S*,4*RS*)-2-Amino-4-hydroxy-5-phosphono pentanoic acid (16):[32] (2*S*)-2-Amino-4-oxo-5-phosphono pentanoic acid (**13**, 93.2 mg, 440 μmol) was dissolved in distilled water (1.5 mL) and cooled to 0 °C. $NaBH_4$ (40 mg, 98%, 1.036 mmol) was added to the solution which was then stirred for 2 h at 0 °C. The reaction was monitored by TLC (NH_3/isopropanol (50:50), R_f ketone = 0.32, R_f alcohol = 0.28) and LC–MS. The solution was acidified to pH 3 (HCl, 1 M) and allowed to reach RT. The solvent was removed by freeze-drying. The sample was purified by cation exchange (Dowex AG50-Wx8). Ninhydrin-positive and LC–MS-positive ($[M]H^+ = 214$) fractions were collected and the sample was lyophilised to give a light-yellow solid (85.6 mg, 402 μmol, 91%). A mixture of two diastereomers (**A**, minor, and **B**, major) of **16** was obtained in a 2:1 ratio. $[\alpha]_D^{25}$: +6.6 (c = 0.93 in H_2O); IR (solid); $\tilde{\nu}_{max}$ = 3170 (O–H), 3018 (N–H), 2852 (C–H), 1588 (NH_2, N–H), 1238 (P(O)OH, P=O) cm^{-1}; ^{1}H NMR (300 MHz, D_2O): $\delta = 4.00-3.85$ (m, 1H, γCH, **B**), 3.91–3.75 (m, 1H, γCH, **A**), 3.68 (dd, 1H, ^3J(H,H) = 8.25, 3.5 Hz, αCH), 3.58 (dd, 1H, ^3J(H,H) = 8.6, 3.7 Hz, αCH, **A**), 1.94 (ddd, 1H, ^2J(H,H) = 15.2 Hz, ^3J(H,H) = 8.6, 2.15 Hz, βCH, **A**), 2.08–2.01 (m, 1H, βCH, **B**), 1.79 (ddd, 1H, ^2J(H,H) = 15.2 Hz, ^3J(H,H) = 10.3, 3.7 Hz, βCH, **A**), 1.72–1.56 (m, 2H, δCH_2P, **A**), 1.70–1.56 (m, 1H, βCH, **B**), 1.70–1.56 (m, 2H, δCH_2P, **B**) ppm; ^{13}C NMR (75 MHz, D_2O) 174.9 (CO_2H, **B**), 174.8 (CO_2H), 67.4 (br s, αCH, **B**), 65.5 (d, ^4J(C,P) = 1.87 Hz, αCH), 54.3 (d, ^2J(C,P) = 4 Hz, γCH, **B**), 52.8 (d, ^2J(C,P) = 4 Hz, γCH), 38.2 (d, ^3J(C,P) = 11 Hz, βCH, **B**), 38.1 (d, ^3J(C,P) = 11 Hz, βCH_2), 36.7 (d, ^1J(C,P) = 129 Hz, δCH_2P, **B**), 36.5 (d, ^1J(C,P) = 129 Hz, δCH_2P) ppm; ^{31}P NMR (121 MHz, D_2O, {^{1}H}): $\delta = 24.9$ (s, **A**), 20.6 (s, **B**) ppm; LC–MS (ES$^+$, CH_3CN/H_2O): R_T = 3.4 min (**A** + **B**), *m/z* (%): 488 (100%), 427 (13, $[2M]H^+$), 356 (38), 255 (10, $[M+CH_3CN]H^+$), 214 (66, $[M]H^+$).

***α*-*tert*-Butyl-*γ*-methyl-*N*,*N*-bis(*tert*-butyloxycarbonyl)-L-aspartate (23)**: A mixture of **20**[33] (0.65 g, 2.14 mmol), sodium hydride (60% in oil, 0.13 g, 3.25 mmol) and *tert*-butyloxycarbonyl anhydride (0.7 g, 97%, 3.60 mmol) was stirred in anhydrous THF (35 mL). The solution was heated under reflux for 4 h. The reaction was quenched with water and extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were collected, dried over anhydrous Na_2SO_4 and filtered, and the solvent was removed under reduced pressure to give a yellow oil (1.2 g). Purification by flash column chromatography (from EtOAc/hexane (15:85), R_f = 0.27, to EtOAc/hexane (20:80), R_f = 0.32) yielded the product **23** as a colourless solid (0.7 g, 1.85 mmol, 86.2%). $[\alpha]_D^{25}$: −13.0 (c = 2.01 in CH_2Cl_2); mp: 64–66 °C (from EtOAc/petroleum ether (40:60)); IR (solid): $\tilde{\nu}_{max}$ = 2982 (C–H), 2937 (C–H), 1731 (C=O), 1720 (C=O), 1368 (C–$(CH_3)_3$), 1175 (C–O), 1108 (C–O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.27$ (dd, 1H, ^3J(H,H) = 7.3, 6.5 Hz, αCH), 3.63 (s, 3H, OMe), 3.16 (dd, 1H, ^2J(H,H) = 16.4 Hz, ^3J(H,H) = 7.3 Hz, βCH), 2.61 (dd, 1H, ^2J(H,H) = 16.4 Hz, ^3J(H,H) = 6.5 Hz, βCH), 1.44 (s, 18H, N–$(CO_2C(CH_3)_3)_2$), 1.37 (s, 9H, C–$(CO_2C(CH_3)_3)$ ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta = 171.3$ (CO_2Me), 168.6 (CO_2tBu), 152.0 (NCO), 83.1 ($C(CH_3)_3$), 81.9 ($C(CH_3)_3$), 55.7 (αCH), 51.8 (OCH_3), 35.4 (βCH_2), 28.0 (N–$(CO_2C(CH_3)_3)_2$), 27.8 ($CCO_2C(CH_3)_3$) ppm; MS (CI$^+$): *m/z* (%): 347 (4, $[M-C_4H_9]H^+$), 304 (29, $[M-$Boc+H$]H^+$), 292 (26), 248 (9), 204 (6, $[M-2$Boc+2H$]H^+$), 192 (61), 148 (84, $[M-2$Boc$-C_4H_9+3H]H^+$), 102 (79, $[CO_2C_4H_9]H^+$), 57 (100, $[C_4H_9]^+$); elemental analysis: calcd (%) for $C_{19}H_{33}NO_8$: C 56.56, H 8.24, N 3.47; found: C 56.56, H 8.27, N 3.54.

***tert*-Butyl-*N*,*N*-bis(*tert*-butyloxycarbonyl)-L-aspartate-*γ*-semialdehyde (24)**: A solution of **23** (202 mg, 0.5 mmol) in anhydrous diethyl ether (2.5 mL) was cooled to −78 °C. DIBALH (600 μL, 1.0 M, 0.60 mmol) was added by syringe. The reaction was quenched with water (0.5 mL) after 10 min and the solution was allowed to warm to RT over 30 min. The mixture was dried with anhydrous Na_2SO_4 and filtered through a Celite layer. The pad was washed with diethyl ether. The solvent was evaporated to yield a colourless oil (208 mg). The oil was purified by column chromatography (EtOAc/hexane (20:80), R_f = 0.31) to give **24** (123.0 mg, 329 μmol, 61%) as a colourless solid. $[\alpha]_D^{24}$: −6.0 (c = 1.13 in CH_2Cl_2); IR (solid): $\tilde{\nu}_{max}$ = 2963 (C–H), 2927 (C–H), 1736 (C=O), 1700 (C=O), 1367 (C–$(CH_3)_3$), 1010 (C–O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 9.79$ (dd, 1H, ^3J(H,H) = 1.5, 1.3 Hz, γCHO), 5.42 (dd, 1H, ^3J(H,H) = 7.0, 6.0 Hz, αCH), 3.37 (ddd, 1H, ^2J(H,H) = 17.6 Hz, ^3J(H,H) = 7.0, 1.5 Hz, βCH), 2.77 (ddd, 1H, ^2J(H,H) = 17.6 Hz, ^3J(H,H) = 6.0, 1.3 Hz, βCH), 1.52 (s, 18H, N–$(CO_2C(CH_3)_3)_2$), 1.44 (s, 9H, $CCO_2C(CH_3)_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$); $\delta = 198.8$ (CHO), 168.6 (CCO_2), 152.1 (NCO), 83.3 (N–$(CO_2C(CH_3)_3)_2$), 82.2 ($CCO_2C(CH_3)_3$), 53.7 (αCH), 44.6 (βCH_2), 28.0 (N–$(CO_2C(CH_3)_3)_2$), 27.8 ($CCO_2C(CH_3)_3$) ppm; MS (EI$^+$): *m/z* (%): 373 (12, $[M]^+$), 57 (100); LC–MS (ES$^+$, CH_3CN/H_2O): R_T = 17.3 min, *m/z* (%): 374 (5, $[M]^+$), 318 (6, $[M-C_4H_9+H]$), 274 (4, $[M-$Boc+H$]H^+$), 262 (19,

[$M-2C_4H_9+2H$]H^+), 218 (19, [$M-Boc-C_4H_9+2H$]H^+), 206 (42, [$M-3C_4H_9+3H$]H^+), 162 (100); elemental analysis: calcd for $C_{18}H_{31}NO_7$: C 57.89, H 8.37, N 3.75; found: C 57.56, H 8.27, N 3.84.

Methyl-*N*,*N*-bis(*tert*-butyloxycarbonyl)-L-aspartate-γ-semi-aldehyde (25)[17, 34] and methyl-*N*,*N*-bis(*tert*-butyloxycarbonyl)-L-homoserinate (28):[17, 34]

Method A: Dimethyl-*N*,*N*-bis-(*tert*-butyloxycarbonyl)-L-aspartate[34] (**27**, 3.39 g, 9.39 mmol) was dissolved in anhydrous diethyl ether (60 mL) and cooled to $-78\,^{\circ}C$. DIBALH (13 mL, 1 M, 13.0 mmol) was added through a syringe and the reaction mixture was stirred for 15 min. The reaction was quenched with water (5 mL) and the mixture was stirred at RT for 30 min. The reaction mixture was treated with anhydrous Na_2SO_4 and filtered through a pad of Celite, then more diethyl ether was added to wash the Celite layer. Finally all the solvent was evaporated and a transparent oil was obtained (3.39 g). The oil was purified by flash column chromatography (EtOAc/petroleum ether (20:80), $R_f = 0.29$) to give **25** as a colourless solid (2.19 g, 6.62 mmol, 71 %). $[\alpha]_D^{24}$: -53.3 ($c = 2.25$ in $CHCl_3$), (ref. [34]: $[\alpha]_D^{24}$: -54.9 ($c = 2.0$ in $CHCl_3$)); IR (solid): $\tilde{\nu}_{max} = 2981$ (C–H), 1739 (C=O), 1699 (C=O), 1367 ($C(CH_3)_3$, C–H), 1114 (C–O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 9.80$ (t, 1 H, $^3J(H,H) = 0.9$ Hz, γCHO), 5.45 (dd, 1 H, $^3J(H,H) = 6.4$, 6.0 Hz, αCH), 3.70 (s, 3 H, OMe), 3.42 (ddd, 1 H, $^2J(H,H) = 17.6$ Hz, $^3J(H,H) = 6.4$, 0.9 Hz, βCH), 2.83 (ddd, 1 H, $^2J(H,H) = 17.6$ Hz, $^3J(H,H) = 6.0$, 0.9 Hz, βCH), 1.51 (s, 18 H, N–($CO_2C(CH_3)_3$)$_2$) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta = 198.4$ (CHO), 170.3 (CO_2Me), 151.7 (NCO), 83.7 ($C(CH_3)_3$), 54.7 (αCH), 52.6 (OCH_3), 45.0 (βCH_2), 28.0 ($C(CH_3)_3$) ppm; MS (CI^+): *m/z* (%): 331 (4, [M]$^+$), 281 (75), 102 (8, [$CO_2C_4H_9$]H^+), 57 (100, [C_4H_9]$^+$).

Following the elution of **25**, **28** was also isolated (EtOAc/petroleum ether 20:80, $R_f = 0.18$, changing eluent to EtOAc/petroleum ether 50:50, $R_f = 0.31$) as a colourless solid (150 mg, 450 μmol, 5 %). $[\alpha]_D^{25}$: -23.3 ($c = 1.23$ in MeOH), (Ref. [35] $[\alpha]_D^{25}$: -36.6 ($c = 1.58$ in MeOH)); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.00$ (dd, 1 H, $^3J(H,H) = 9.8$ Hz, $^3J(H,H) = 4.7$ Hz, αCH), 3.73 (s, 3 H, OCH_3), 3.79 – 3.67 (m, 1 H, γCH), 3.63 – 3.49 (m, 1 H, γCH), 2.64 – 2.47 (br s, 1 H, OH), 2.47 – 2.36 (m, 1 H, βCH), 2.07 – 1.97 (m, 1 H, βCH), 1.50 (s, 18 H, N–($CO_2C(CH_3)_3$)$_2$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) 171.4 (CO_2Me), 152.5 (NCO), 83.8 (N–($CO_2C(CH_3)_3$)$_2$), 59.0 (γCH_2OH), 55.5 (αCH), 52.4 (OCH_3), 33.0 (βCH_2), 28.0 (N–($CO_2C(CH_3)_3$)$_2$) ppm; MS (CI^+) *m/z* (%): 334 (3, [M]H^+), 278 (24), 234 (44, [M – Boc+H]$^+$), 222 (45), 202 (42, [M – Boc – CH_2OH]H^+), 160 (64), 146 (59), 134 (90, [M – 2Boc+2H]H^+), 102 (82, [CO_2tBu]H^+), 74 (54, [tBuOH]$^+$), 57 (100, [C_4H_9]$^+$); MS (EI^+): *m/z* (%): 333 (2, [M]$^+$), 303 (6, [M – CHOH]H^+), 277 (8, [$M-C_4H_9$]H^+), 274 (17, [$M-CO_2CH_3$]H^+), 259 (12, [$M-C_4H_9-H_2O$]H^+), 245 (6, [$M-O_2C_4H_9$]H^+), 233 (4, [M – Boc+H]H^+), 116 (5, [NCO_2tBu]H^+), 102 (6, [CO_2tBu]H^+), 74 (17, [tBuOH]$^+$), 57 (100, [C_4H_9]$^+$); LC – MS (ES^+, CH_3CN/H_2O): R_T = 15.9 min, *m/z* (%): 375 (7, [M+CH_3CN]$^+$), 334 (100, [M]H^+), 278 (30, [$M-C_4H_9$+H]H^+), 234 (15, [M – Boc+H]H^+), 178 (47, [M – Boc – C_4H_9+2H]H^+); HRMS (CI^+): calcd for [M]H^+, $C_{15}H_{28}NO_7$: 334.1866; found: 334.1847; calcd for [M – Boc+H]H^+, $C_{10}H_{20}NO_5$: 234.1341; found: 234.1342.

Method B: A solution of **28** (80 mg, 0.239 mmol) in anhydrous CH_2Cl_2 (2 mL) was added to a solution of Dess – Martin periodinane (305 mg, 0.719 mmol) in anhydrous CH_2Cl_2 (2 mL). After stirring for 30 min, the mixture was poured into a saturated aqueous solution of $NaHCO_3$ (10 mL) containing $Na_2S_2O_3$ (1.7 g). Diethyl ether was added to the mixture. Finally the organic layer was collected, dried (Na_2SO_4), and filtered, and the solvent was removed under reduced pressure to give a colourless oil (68 mg, 86 %), which was purified by flash column chromatography (EtOAc/hexane (25:75), $R_f = 0.32$) to afford **25** (59 mg, 0.18 mmol, 75 %).

(2*S*,4*RS*)-Methyl-2-[bis(*tert*-butyloxycarbonyl)]amino-4-hydroxy-5,5-difluoro-5-(diethoxyphosphoryl) pentanoate (29): Under an atmosphere of anhydrous N_2 a solution of **25** (2.86 g, 8.63 mmol) and **32** (2.28 mL, 9.06 mmol) in anhydrous THF (50 mL) was cooled to $-60\,^{\circ}C$. TBAF (1.0 M, 1.03 mL, 1.03 mmol) was added. A red coloration was observed at this point which turned to black-red with time. The solution was stirred overnight and allowed to reach RT. The solvent was evaporated and a red oil was obtained (4 g). The crude sample was purified by flash column chromatography (EtOAc/hexane (60:40), $R_f = 0.38$ and $R_f = 0.33$ for diastereomers A and B, respectively) to give **29** in a 1:3 mixture of the diastereomers A and B (2.37 g, 4.57 mmol, 53 %) as a colourless oil and also diastereomer B separately (95 mg, 183 μmol, 1 %) as a colourless oil.

Diastereomer A (minor): $[\alpha]_D^{27}$: -4.3 ($c = 3.0$ in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.08$ (dd, 1 H, $^2J(H,H) = 10.3$ Hz, $^3J(H,H) = 4.4$ Hz, αCH), 4.34 – 4.25 (m, 4 H, 2 × OCH_2CH_3), 4.04 – 3.95 (m, 1 H, γCHOH), 3.75 (s, 3 H, OCH_3), 3.66 (d, 1 H, $^3J(H,H) = 5.36$ Hz, OH), 3.30 (d, 1 H, $^3J(H,H) = 5.4$ Hz, γCHO*H*), 2.51 – 2.43 (m, 1 H, βCH), 2.33 – 2.27 (m, 1 H, βCH), 1.50 (s, 18 H, N–($CO_2C(CH_3)_3$)$_2$), 1.38 (t, 6 H, $^3J(H,H) = 7.12$ Hz, 2 × OCH_2CH_3) ppm; ^{19}F NMR (283 MHz, $CDCl_3$): $\delta = -116.7$ (ddd, 1 F, $^2J(F,F) = 305.0$ Hz, $^3J(F,P) = 102.5$ Hz, $^3J(H,F) = 6.2$ Hz), -126.1 (ddd, 1 F, $^2J(F,F) = 305.0$ Hz, $^3J(F,P) = 103.2$ Hz, $^3J(H,F) = 18.4$ Hz) ppm; ^{31}P NMR (162 MHz, $CDCl_3$, {^1H}): $\delta = 7.3$ (dd, $^3J(F,P) = 103.0$ Hz, $^3J(F,P) = 102.0$ Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 171.0$ (CO_2Me), 152.0 (NCO), 124.0 – 113.5 (m, CF_2), 83.7 (N–($CO_2C(CH_3)_3$)$_2$), 70.0 – 68.1 (m, γCHOH), 64.8 – 64.5 (m, OCH_2CH_3), 54.7 (αCH), 52.1 (OCH_3), 29.6 – 29.3 (m, βCH_2), 27.8 (C–CH_3), 16.2 (d, $^3J(C,P) = 1$ Hz, CH_3–CH_2) ppm; MS (CI^+): *m/z* (%) 548 (29, [M+CH_2=CH_2]H^+), 520 (12, [M]H^+), 464 (11, [$M-C_4H_9$+H]$^+$), 446 (2, [$M-(C_4H_9+H_2O)$]H^+), 420 (35, [M – Boc+H]H^+), 364 (21, [M – Boc – C_4H_9+2H]H^+), 346 (11, [M – Boc – $C_4H_9-H_2O$+2H]H^+), 320 (100, [M – 2Boc+2H]H^+), 57 (34, [C_4H_9]$^+$); HRMS (CI^+): calcd for [M]H^+, $C_{20}H_{37}NO_{10}F_2P$: 520.2127; found: 520.2123.

Diastereomer B (major): $[\alpha]_D^{27}$: -12.0 ($c = 0.423$ in CH_2Cl_2); mp: 58 – 60 °C (from CH_2Cl_2/Et_2O (25 : 75)); IR (solid): $\tilde{\nu}_{max} = 3309$ (OH), 2980 (C–H), 1748 (C=O), 1710 (C=O), 1367.5 ($C(CH_3)_3$, C–H), 1251 (P=O), 1144 (C–O), 1017 (C–F) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.19$ (dd, 1 H, $^3J(H,H) = 8.0$, 5.1 Hz, αCH), 4.43 – 4.30 (m, 1 H, γCHOH), 4.35 – 4.25 (m, 4 H, 2 × OCH_2CH_3), 3.73 (s, 3 H, OCH_3), 3.20 (d, 1 H, $^3J(H,H) = 5.4$ Hz, γCHO*H*), 2.74 (m, 1 H, βCH), 1.99 – 1.92 (m, 1 H, βCH), 1.50 (s, 18 H, N–($CO_2C(CH_3)_3$)$_2$), 1.39 (dt, 6 H, $^3J(H,H) = 7.1$ Hz, $^4J(H,P) = 1.4$ Hz, 2 × OCH_2CH_3) ppm; ^{19}F NMR (283 MHz, $CDCl_3$): $\delta = -117.3$ (ddd, 1 F, $^2J(F,F) = 304.1$ Hz, $^2J(F,P) = 100.0$ Hz, $^3J(H,F) = 6.2$ Hz), 126.47 (ddd, 1 F, $^2J(F,F) = 304.1$ Hz, $^2J(F,P) = 105.0$ Hz, $^3J(H,F) = 19.2$ Hz) ppm; ^{31}P NMR (121.4 MHz, $CDCl_3$, {^1H}): $\delta = 8.3$ (dd, $^2J(F,P) = 105$ Hz, $^2J(F,P) = 100$ Hz); ^{31}P NMR (121 MHz, $CDCl_3$): $\delta_P = 9.3 - 7.4$ (m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 171.1$ (CO_2Me), 151.8 (NCO), 124.0 – 113.5 (m, CF_2), 83.5 (N–($CO_2C(CH_3)_3$)$_2$), 65.0 – 64.9 (m, γCHOH), 54.9 (αCH), 53.4 (OCH_3), 52.3 (m, OCH_2CH_3), 30.9 – 30.7 (m, βCH_2), 28.0 (N–($CO_2C(CH_3)_3$)$_2$), 16.2 (d, $^3J(C,P) = 1$ Hz, CH_3–CH_2) ppm; MS (CI^+): *m/z* (%): 548 (29, [M+CH_2=CH_2]H^+), 520 (2, [M]H^+), 464 (11, [$M-C_4H_9$+H]H^+), 446 (2, [$M-C_4H_9-H_2O$+H]H^+), 420 (35, [M – Boc+H]H^+), 364 (21, [M – Boc – C_4H_9+2H]H^+), 346 (11, [M – Boc – $C_4H_9-H_2O$+2H]H^+), 320 (100, [M – 2Boc+2H]H^+), 57 (34, [C_4H_9]$^+$); HRMS (CI^+): calcd for [M]H^+, $C_{20}H_{37}NO_{10}F_2P$: 520.2127; found: 520.2123.

(2*RS*)-Diethyl-[(1,1-difluoro-2-hydroxy-2-phenyl)ethyl] phosphonate (33):[19, 36] Diethyl-[difluoro(trimethylsilyl)methyl] phosphonate (125 μL, 96 %, 0.5 mmol) was dissolved in anhydrous THF (2 mL). Benzaldehyde (51 μL, 99 %, 0.5 mmol) was added and the solution was cooled to $-78\,^{\circ}C$. A solution of TBAF (1 M, 90 μL, 90 μmol) in anhydrous THF (2 mL) was prepared and cooled to 0 °C. The reaction mixture was stirred overnight and allowed to reach RT. Finally the

mixture was filtered, the solvent was evaporated and a red oil was obtained. The crude product was purified by silica chromatography (EtOAc/hexane (50:50), $R_f = 0.24$) to give **33** as a colourless oil which crystallised upon standing (141 mg, 0.48 mmol, 96%). Mp: 72–75 °C (EtOAc/petroleum ether (40:60)), (ref. [36]: 76–77 °C); IR (solid): $\tilde{\nu}_{max} = 3324$ (O–H), 3011 (C–H aromatic), 2988 (C–H), 2914 (C–H), 1250 (P=O), 1037 (P–O–alkyl), 1008 (C–F) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.54–7.32$ (m, 5H, Ar*H*), 5.20–5.05 (m, 1H, C*H*OH), 4.34–4.10 (m, 4H, $2 \times OCH_2$), 4.15–4.00 (br m, 1H, CHO*H*), 1.34 (dt, 3H, $^3J(H,H) = 7.0$ Hz, $^4J(H,P) = 0.6$ Hz, CH_3), 1.31 (dt, 3H, $^3J(H,H) = 7.2$ Hz, $^4J(H,P) = 0.7$ Hz, CH_3) ppm; ^{19}F NMR (121 MHz, $CDCl_3$): $\delta = -114.30$ (ddd, 1F, $^2J(F,F) = 304.1$ Hz, $^2J(F,P) = 99.5$ Hz, $^3J(H,F) = 6.2$ Hz), -125.12 (ddd, 1F, $^2J(F,F) = 304.1$ Hz, $^2J(F,P) = 105.1$ Hz, $^3J(H,F) = 20.1$ Hz) ppm; ^{31}P NMR (282 MHz, $CDCl_3$): $\delta = 6.9$ (dd, $^2J(F,P) = 105$ Hz, $^2J(F,P) = 99.5$ Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 134.6$ (m, ArC_1), 128.8 (Ar), 128.1 (Ar), 117.9 (ddd, $^1J(C,F) = 272$, 265 Hz, $^1J(C,P) = 205$ Hz, CF_2P), 73.5 (ddd, $^2J(C,F) = 26$, 22 Hz, $^2J(C,P) = 15$ Hz, CHOH), 65.0 (ddd, $^2J(C,P) = 14$ Hz, $^4J(C,F) = 7$, 2 Hz, $2 \times OCH_2$), 16.3 (d, $^3J(C,P) = 5$ Hz, CH_3), 16.2 (d, $^3J(C,P) = 5$ Hz, CH_3) ppm; MS (EI^+): *m/z* (%): 294 (35, $[M]^+$), 274 (67, $[M-F-H]^+$), 243 (58), 226 (76), 188 (74, $[CF_2P(O)(OEt)_2]^+$), 161 (51), 160 (44), 140 (100), 132 (83), 137 (10, $[P(O)(OEt)_2]^+$), 109 (47), 107 (48, $[C_6H_5COH]H^+$), 84 (75), 77 (52, $[C_6H_5]^+$); MS (CI^+): *m/z* (%): 294 (30, $[M]^+$), 221 (75), 188 (60), 161 (40), 140 (94), 132 (70), 84 (100), 77 (40), 74 (60); LC–MS (ES^+, CH_3CN/H_2O): $R_T = 14.6$ min, *m/z* (%) 589 (31, $[2M]H^+$) 354 (5), 336 (8, $[M+CH_3CN]H^+$), 317 (2, $[M+Na]^+$), 295 (100, $[M]H^+$), 277 (22, $[M-OH]^+$); HRMS (CI^+): calcd for $[M]H^+$, $C_{12}H_{18}O_4F_2P$: 295.0911; found: 295.0910.

(2*RS*)-Diethyl-[(1,1-difluoro-2-hydroxy-4-phenyl)butyl] phosphonate (35): Hydrocinnamaldehyde (86 μL, 97%, 627 μmol) was dissolved in anhydrous THF (5mL) and cooled to 0 °C. Diethyl-[difluoro(trimethylsilyl)methyl] phosphonate (77.64 μL, 96%, 307 μmol) and TBAF (1.0 M, 78 μL, 78 μmol) were added to the mixture. The reaction mixture was stirred for 7 h at RT. The solvent was evaporated under reduced pressure to give a red oil (360 mg) which was purified by silica chromatography (EtOAc/petroleum ether (30:70), $R_f = 0.20$) to give **35** as a colourless oil (103.6 mg, 0.322 mmol, 51%). IR (oil): $\tilde{\nu}_{max} = 3361$ (OH), 3028 (C–H), 2985 (C–H), 2934 (C–H),1252 (P=O), 1164 (P–O–alkyl), 1015 (C–F) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.31–7.16$ (m, 5H, Ar), 4.35–4.20 (m, 4H, OCH_2), 4.04–3.88 (m, 1H, C*H*OH), 3.31 (br s, 1H, OH), 2.98–2.90 (m, 1H, PhCH*H*), 2.75–2.65 (m, 1H, PhCH*H*), 2.10–1.89 (m, 2H, CH_2CHOH), 1.36 (br t, 6H, $^3J(H,H) = 7.3$, CH_3) ppm; ^{19}F NMR (282 MHz, $CDCl_3$): $\delta = -116.70$ (ddd, 1F, $^2J(F,F) = 303.5$ Hz, $^2J(F,P) = 101.5$ Hz, $^3J(H,F) = 7.35$ Hz), -125.45 (ddd, 1F, $^2J(F,F) = 303.5$ Hz, $^2J(F,P) = 105$ Hz, $^3J(H,F) = 18.4$ Hz) ppm; ^{31}P NMR (162 MHz, $CDCl_3$, $\{^1H\}$): $\delta = 7.65$ (dd, $^2J(F,P) = 105$, 101.5 Hz) ppm; ^{13}C NMR (100.5 MHz, $CDCl_3$); $\delta = 141.2$ (Ar), 128.5 (Ar), 128.4 (Ar), 126.0 (Ar), 118.05 (ddd, $^1J(C,F) = 274$, 263.5, $^1J(C,P) = 205$ Hz, CF_2P), 70.9 (ddd, $^2J(C,F) = 25$, 23 Hz, $^2J(C,P) = 14$ Hz, CHOH), 64.9–64.8 (m, O–CH_2), 31.25 (CH_2–Ar), 30.4–30.25 (m, CH_2–CHOH), 16.3 (d, $^3J(C,P) = 5$ Hz, CH_3) ppm; MS (CI^+): *m/z* (%): 323 (34, $[M]H^+$), 295 (18, $[M-C_2H_4]H^+$), 267 (14, $[M-C_4H_9+H]H^+$), 249 (18, $[M-C_4H_9-H_2O+H]H^+$), 155 (10, $[H_2C{-}COH{=}CPFO_3H_2]^+$), 117 (29, $[C_6H_5CH_2CH_2C]^+$), 105 (39, $[C_6H_5CH_2CH_2]^+$), 91 (100, $[C_6H_5CH]^+$); HRMS (CI^+): calcd for $[M]H^+$, $C_{14}H_{22}O_4F_2P$: 323.1224; found: 323.1218.

Diethyl [(1,1-difluoro-2-phenyl-2-oxo)ethyl] phosphonate (36):[14, 19, 36] A solution of **33** (196 mg, 666 μmol) in anhydrous CH_2Cl_2 (5 mL) was added to a solution of Dess–Martin periodinane (590 mg, 1.39 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction was quenched after 40 min with a saturated aqueous $NaHCO_3$ solution (20 mL) containing $Na_2S_2O_3$ (2.64 g). The organic layer was washed with a saturated solution of $NaHCO_3$ (20 mL), dried (Na_2SO_4), filtered and evaporated to give a colourless oil (245 mg). The crude product was purified by silica chromatography, eluting with EtOAc/petroleum ether (40:60, $R_f = 0.25$), to give **36** as a colourless oil (183 mg, 626 μmol, 94%). IR (oil): $\tilde{\nu}_{max} = 3021$ (C–H), 2988 (C–H), 2917 (C–H), 1694 (C=O), 1240 (P=O), 1095 (P–O–alkyl), 1017 (C–F) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.15$ (m, 2H, *o*-Ph), 7.65 (m, 1H, *p*-Ph), 7.51 (m, 2H, *m*-Ph), 4.42–4.25 (m, 4H, $2 \times OCH_2$), 1.38 (dt, 6H, $^3J(H,H) = 7.1$ Hz, $^4J(H,P) = 1.4$ Hz, $2 \times CH_3$) ppm; ^{19}F NMR (283 MHz, $CDCl_3$): $\delta = -110.0$ (d, $^2J(F,P) = 96.0$ Hz) ppm; ^{31}P NMR (162 MHz, $CDCl_3$, $\{^1H\}$): $\delta = 4.1$ (t, $^2J(F,P) = 96$ Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 188.8$ (td, $^2J(C,P) = 14$ Hz, $^2J(C,F) = 25$ Hz, CO), 134.85 (Ar), 132.1, (Ar), 130.5 (Ar), 128.75 (Ar), 120.0–110.1 (m, CF_2), 65.5 (d, $^2J(C,P) = 7$ Hz, CH_2), 16.4 (d, $^3J(C,P) = 6$ Hz, CH_3) ppm; MS (CI^+): *m/z* (%): 309 (18), 293 (6, $[M]H^+$), 292 (11, $[M]^+$), 247 (33, $[M-OEt]^+$), 219 (6), 156(8), 105 (100), 84 (11), 77 (61, $[C_6H_5]^+$), 65 (9) ppm; LC–MS (ES^+, CH_3CN/H_2O): $R_T = 11.4$ min, *m/z* (%): 495 (15, $[2M+Na]^+$), 473 (14, $[2M]H^+$), 319 (17), 300 (18, $[M+CH_3CN+Na]^+$), 278 (100, $[M+CH_3CN]H^+$), 259 (18, $[M+Na]^+$), 237 (85, $[M]H^+$); HRMS (CI^+): calcd for $[M]H^+$, $C_{12}H_{16}O_4F_2P$: 293.0754; found: 293.0761.

(1,1-Difluoro-2-oxo-2-phenyl-ethyl)phosphonic acid (37): Compound **36** (44 mg, 150.5 μmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) and CH_3CN (2 mL) under a dry nitrogen atmosphere and cooled to 0 °C. Freshly distilled TMSI (94 μL, 617 μmol) was added and a yellow solution was obtained. After 15 min stirring, the reaction mixture was warmed to RT. Water (2 mL) was added and the mixture was stirred for 30 min. The aqueous layer was collected and washed with EtOAc (5×5 mL). The aqueous solvent was evaporated under reduced pressure. The residue was lyophilised and **37** was obtained as a dark solid (36 mg, 148 μmol, 98%). ^1H NMR (400 MHz, D_2O): $\delta = 8.07$ (d, 2H, $^3J(H,H) = 7.8$ Hz, Ar *o*-H), 7.64 (t, 1H, $^3J(H,H) = 7.8$ Hz, Ar *p*-H), 7.48 (t, 2H, $^3J(H,H) = 7.8$ Hz, Ar *m*-H) ppm; ^{19}F NMR (282 MHz, D_2O): $\delta = -111.8$ (d, 2F, $^2J(F,P) = 86.0$ Hz) ppm; ^{31}P NMR (122 MHz, D_2O, $\{^1H\}$): $\delta = 0.6$ (t, $^2J(F,P) = 86$ Hz) ppm; ^{13}C NMR (75.5 MHz, D_2O): $\delta = 193.7–187.2$ (m, CO), 135.9 (Ar), 133.2 (Ar), 131.2 (Ar), 129.2 (Ar), 120.8–113.4 (m, CF_2) ppm; LC–MS (ES^+, CH_3CN/H_2O): $R_T = 11.4$ min, *m/z* (%): 495 (15, $[2M]Na^+$), 473 (14, $[2M]H^+$), 319 (17), 300 (18, $[M+CH_3CN]Na^+$), 278 (100, $[M+CH_3CN]H^+$), 259 (18, $[M]Na^+$), 237 (85, $[M]H^+$).

(2*S*)-Methyl-2-[bis(*tert*-butyloxycarbonyl)]amino-4-oxo-5,5-difluoro-5-(diethoxy)phosphoryl pentanoate (38): A solution of a mixture of diastereomers of **29** (260.0 mg, 500 μmol) in anhydrous CH_2Cl_2 (6 mL) was added to a solution of the Dess–Martin periodinane (530 mg, 1.25 mmol) in anhydrous CH_2Cl_2 (5 mL). The reaction was stirred for 45 min and quenched with a saturated aqueous solution of $NaHCO_3$ (42 mL) containing $Na_2S_2O_3$ (7.40 g). The mixture was stirred for 15 min and extracted with diethyl ether. The organic layer was washed with a saturated solution of $NaHCO_3$ (40×3 mL). Finally the organic layer was dried (Na_2SO_4) and filtered, and the solvent was evaporated. A colourless oil was obtained (240 mg). The crude product was purified by flash column chromatography (EtOAc/hexane (40:60), $R_f = 0.31$) to give the desired product as a colourless oil which crystallised from CH_2Cl_2 to afford colourless needles (227 mg, 439 μmol, 88%). $[\alpha]_D^{23}$: -11.85 ($c = 4.5$ in MeOH); mp: 115–117 °C (from CH_2Cl_2); IR (solid): $\tilde{\nu}_{max} = 2933$ (C–H), 2873 (OCH_3, C–H), 1744 (C=O), 1719 (C=O), 1368 ($C(CH_3)_3$), 1252 (P=O), 1016 (C–F) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 5.54$ (dd, 1H, $^3J(H,H) = 7.1$, 5.6 Hz, αCH), 4.3–4.4 (m, 4H, $2 \times OCH_2CH_3$), 3.80–3.89 (dd, 1H, $^2J(H,H) = 19.0$ Hz, $^3J(H,H) = 7.1$ Hz, βCH), 3.73 (s, 3H, OCH_3), 3.09–3.11 (dd, 1H, $^2J(H,H) = 19.0$ Hz, $^3J(H,H) = 5.6$ Hz, βCH), 1.51 (s, 18H, N–$(CO_2C(CH_3)_3)_2$), 1.39 (t, 6H, $^2J(H,H) = 7.1$ Hz, $2 \times OCH_2CH_3$) ppm; ^{19}F NMR (283 MHz, $CDCl_3$): $\delta = -118.6$ (dd, 1F, $^2J(F,F) = 315.7$ Hz, $^2J(F,P) = 97.0$ Hz), -117.1 (dd, 1F, $^2J(F,F) = 315.5$ Hz, $^2J(F,P) = 96.0$ Hz) ppm; ^{31}P NMR (162 MHz, $CDCl_3$, $\{^1H\}$): $\delta = 3.5$ (t, $^2J(F,P) = 97$ Hz) ppm; ^{31}P NMR (121 MHz, $CDCl_3$): $\delta_P = 3.5$ (tq, $^2J(F,P) = 97$ Hz, $^3J(H,P) = 8$ Hz) ppm; ^{13}C NMR

(75 MHz, $CDCl_3$): $\delta = 196.0$ (m, $COCF_2$), 169.8 (CO_2CH_3), 151.5 (NCO), 136.2 (m, CF_2P), 83.7 (N–($CO_2C(CH_3)_3)_2$), 65.5 (d, $^2J(C,P) = 7$ Hz, $2 \times OCH_2CH_3$), 53.3 (CH), 52.7 (OCH_3), 39.3 (βCH_2), 27.9 (N–($CO_2C(CH_3)_3)_2$), 16.3 (d, $^3J(C,P) = 5.6$ Hz, CH_3–CH_2) ppm; MS (Cl^+): *m/z* (%): 517 (1, $[M]^+$), 462 (18, $[M - C_4H_9+H]H^+$), 446 (32), 416 (20, $[M - Boc+H]H^+$), 390 (44), 318 (100, $[M - 2Boc+2H]H^+$) 301 (24), 188 (11, $[CF_2P(O)(OCH_2CH_3)_2]H^+$), 106 (14), 57 (85, $[C_4H_9]^+$) ppm; LC–MS (ES^+, CH_3CN/H_2O): $R_T = 17.3$ min, *m/z* (%): 577 (6, $[M+CH_3CN+H_2O]H^+$), 559 (6, $[M+CH_3CN]H^+$), 540 (18), 536 (22, $[M+H_2O]H^+$), 518 (47, $[M]H^+$), 462 (52, $[M - C_4H_9+H]H^+$), 418 (7, $[M - Boc+H]H^+$), 362 (18, $[M - Boc - C_4H_9+2H]H^+$), 318 (100, $[M - 2Boc+2H]H^+$), 301 (8, $[M - 2Boc - H_2O+2H]H^+$), 201 (28); elemental analysis: calcd for $C_{20}H_{34}F_2NO_{10}P$: C 46.42, H 6.62, N 2.71; found: C 46.49, H 6.17, N 2.42.

(2*S*)-Methyl-2-[bis(*tert*-butyloxycarbonyl)]amino-4-oxo-5-(diethoxy) phosphoryl pentanoate (40) and *tert*-Butyl-2-(diethoxy) phosphoryl acetate (41): Diethyl methylphosphonate (2.41 mL, 16.0 mmol, 1.04 g mL^{-1}) was dissolved in anhydrous THF (10 mL) and cooled to $-78\,^\circ C$. Butyllithium (6.14 mL, 2.5 M, 15.3 mmol) was added dropwise and the reaction was stirred for 20 min. In a second flask **27**[34, 35] (1.12 g, 3.4 mmol) was dissolved in anhydrous THF (8 mL), cooled to $-78\,^\circ C$ and added dropwise through a cannula to the first mixture over a period of 30 min. The reaction mixture was stirred for 1 h at $-78\,^\circ C$. It was quenched with glacial acetic acid (875 μL, 15.3 mmol) and the mixture was allowed to reach RT gradually. The product was extracted with EtOAc (3×30 mL). The organic layers were collected, dried (Na_2SO_4), filtered and evaporated to yield a brown solid (2 g). The residue was purified by flash column chromatography, initially with EtOAc/petroleum ether eluent (60:40, $R_f = 0.20$) followed by EtOAc/petroleum ether (80:20, $R_f = 0.34$), to give the desired product **40** as an oil (810 mg, 1.6 mmol, 50%). $[\alpha]_D^{25}$: -5.23 ($c = 0.53$ in MeOH); IR (oil): $\tilde{\nu}_{max} = 2981$ (C–H), 1744 (C=O), 1718 (C=O), 1367 ($C(CH_3)_3$, C–H), 1252 (P=O), 1140 (C–O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.46$ (dd, 1H, $^3J(H,H) = 7.4$, 5.1 Hz, αCH), 4.20–4.06 (m, 4H, OCH_2), 3.67 (s, 3H, CO_2CH_3), 3.58 (dd, 1H, $^2J(H,H) = 17.85$ Hz, $^3J(H,H) = 7.4$ Hz, βCH), 3.18 (dt, 1H, $^2J(H,P) = 49.4$ Hz, $^2J(H,H) = 13.7$ Hz, δCH), 3.11 (dt, 1H, $^2J(H,P) = 48.9$ Hz, $^2J(H,H) = 13.7$ Hz, δCH), 2.90 (dd, 1H, $^2J(H,H) = 17.85$ Hz, $^3J(H,H) = 5.2$ Hz, βCH), 1.47 (s, 18H, N–($CO_2C(CH_3)_3)_2$), 1.30 (dt, 6H, $^3J(H,H) = 7.1$ Hz, $^3J(H,P) = 2.2$ Hz, $2 \times OCH_2CH_3$) ppm; ^{31}P NMR (121 MHz, $CDCl_3$, {^{1}H}): $\delta = 20.3$ (s) ppm; ^{31}P NMR (121 MHz, $CDCl_3$): $\delta_P = 20.30 - 20.06$ (m) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 198.4$ (d, $^2J(C,P) = 6$ Hz, γCO), 170.5 (CO_2Me), 151.7 (NCO), 83.5 (N–($CO_2C(CH_3)_3)_2$), 62.7 (d, $^2J(C,P) = 6$ Hz, OCH_2), 62.6 (d, $^2J(C,P) = 6$ Hz, OCH_2), 54.2 (αCH), 52.5 (OCH_3), 44.9 (βCH_2), 42.7 (d, $^1J(C,P) = 127$ Hz, δCH_2P), 28.0 (N–($CO_2C(CH_3)_3)_2$), 16.3 (CH_3–CH_2), 16.2 (CH_3–CH_2) ppm; –MS (ES^+, CH_3CN/H_2O): $R_T = 15.95$ min, *m/z* (%): 482 (100, $[M]H^+$); MS (Cl^+): *m/z* (%): 310 (18), 282 (100, $[M - 2Boc+2H]H^+$), 195 (28), 167 (12, $[COPO(OEt)_2]H^+$), 139 (11, $[HPO(OEt)_2]H^+$), 57 (12, $[C_4H_9]^+$); HRMS (Cl^+): calcd for $[M]H^+$, $C_{20}H_{37}NO_{10}P$: 482.2155; found: 482.2131.

tert-Butyl 2-(diethoxy)phosphoryl acetate (**41**) was also isolated from the column as a colourless oil ($R_f = 0.25$, ethyl acetate/petroleum ether (40:60); 43.0 mg, 0.17 mmol, 5%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.20 - 4.06$ (m, 4H, $2 \times OCH_2$), 2.85 (d, 2H, $^2J(H,P) = 21.7$ Hz, PCH_2), 1.44 (s, 9H, $C(CH_3)_3$), 1.34 (m, 6H, $2 \times OCH_2CH_3$) ppm; ^{31}P NMR (121 MHz, $CDCl_3$, {^{1}H}): $\delta = 21.4$ (s) ppm; ^{31}P = (121 MHz, $CDCl_3$) $\delta_P = 21.6 - 21.1$ (m) ppm; LC–MS (ES^+, CH_3CN/H_2O): $R_T = 14.5$ min, *m/z* (%): 275, (15, $[M]Na^+$), 253 (100, $[M]H^+$).

α-*tert*-Butyl-*N*,*N*-bis(*tert*-butyloxycarbonyl)-L-aspartate potassium salt (42): Compound (**23**) (2.58 g, 6.39 mmol) was dissolved in a 0.91 M KOH solution of CH_3CN/H_2O (1:1, 7 mL). The mixture was stirred for 30 h at RT. The crude mixture was washed with EtOAc (3×10 mL) to remove the unreacted starting material. The aqueous layer was separated, solvent was evaporated and **42** was obtained as a colourless oil that became a colourless solid upon standing (2.03 g, 4.76 mmol, 75.5%). $[\alpha]_D^{25}$: -5.67 ($c = 1.13$ in H_2O); mp: 112–115 °C; IR (oil): $\tilde{\nu}_{max} = 2979$ (C–H), 2935 (C–H), 1732 (C=O), 1728 (C=O), 1582 (N–CO), 1400 (CO_2^-), 1350 (C–$(CH_3)_3$, C–H), 1300 (C–$(CH_3)_3$, C–H), 1150 (C–O), 1150 (C–O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 5.23$ (dd, 1H, $^3J(H,H) = 7.3$, 6.2 Hz, αCH), 2.86 (dd, 1H, $^2J(H,H) = 15.75$ Hz, $^3J(H,H) = 7.3$ Hz, βCH), 2.44 (dd, 1H, $^2J(H,H) = 15.75$ Hz, $^3J(H,H) = 6.2$ Hz, βCH), 1.40 (s, 18H, N–($CO_2C(CH_3)_3)_2$), 1.35 (s, 9H, $CCO_2C(CH_3)_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 178.5$ (CO_2K), 171.3 (CCO_2tBu), 153.0 (NCO), 85.8 ($C(CH_3)$), 83.9 ($C(CH_3)_3$), 57.5 (αCH), 38.5 (βCH_2), 27.4 (N–($CO_2C(CH_3)_3)_2$), 27.3 ($CCO_2C(CH_3)_3)_2$) ppm; MS (Cl^+): *m/z* (%): 290 (14, $[M - K - Boc+H]H^+$), 234 (6, $[M - K - Boc - C_4H_9+2H]H^+$), 190 (10, $[M - K - 2Boc+2H]H^+$), 178 (42), 160 (10, $[H_9C_4O_2C(NH_2)CH_2CH_2]^+$), 134 (46, $[M - K - 2Boc - C_4H_9+3H]H^+$), 116 (30, $[H_9C_4O_2CCH_2]^+$), 102 (14, $[H_9C_4O_2CH]^+$), 88 (60, $[H_9C_4OC]^+$), 74 (40), 57 (100, $[H_9C_4]^+$); LC–MS (ES^+, CH_3CN/H_2O): $R_T = 16.9$ min, *m/z* (%): 446 (13, $[M+H_2O]H^+$), 390 (54, $[M - K+H]H^+$), 346 (48), 334 (52, $[M - K - C_4H_9+H]H^+$), 290 (49, $[M - Boc - K+2H]^+$), 178 (100).

(2*S*)-γ-*N*-Diethoxyphosphoryl-α*N*,α*N*-bis(*tert*-butyloxycarbonyl)-L-asparagine *tert*-butylester (45): The potassium salt **42** (515 mg, 1.20 mmol) was dissolved in anhydrous CH_2Cl_2 (11 mL) with some molecular sieves (4 Å, 1.6 mm pellets). Ethylchloroformate (855 μL, 1.135 g mL^{-1}, 970 μg, 8.674 mmol) was added dropwise and the mixture was stirred for 75 min. Some colourless solid was observed to precipitate at this point (KCl). In a second flask, diethylphosphoramidate (1.1 g, 97%, 6.9 mmol) was dissolved in anhydrous CH_2Cl_2 (5mL), cooled to $-78\,^\circ C$ and butyllithium (2.76 mL, 2.5 M, 6.9 mmol) was added dropwise. The reaction mixture was stirred for 30 min, allowing it to reach RT. The deprotonated phosphoramidate solution was added dropwise to the first reaction mixture and then stirred overnight at RT. Diluted HCl was added to the crude reaction mixture until pH 3 was achieved. The product was extracted with CH_2Cl_2 (4×50 mL), the organic layers were collected together, dried (Na_2SO_4), filtered and evaporated. A pale red oil was obtained (1.27 g). The crude mixture was purified by column chromatography, with EtOAc/petroleum ether (30:70, $R_f = 0.05$) as the initial eluent with the ratio of EtOAc being increased up to 100% ($R_f = 0.61$), to give **45** as a colourless oil (310 mg, 588 μmol, 49%). $[\alpha]_D^{25}$: -5.0 ($c = 0.88$ in CH_2Cl_2); IR (oil): $\tilde{\nu}_{max} = 3122$ (CONH, N–H), 2981 (C–), 2934 (C–H), 2256 (N–CO), 1734 (C=O), 1725 (C=O), 1366 ($C(CH_3)_3$, C–H), 1234 (P=O), 1141 (C–O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.32 - 8.08$ (br s, 1H, HN–P) 5.38 (dd, 1H, $^3J(H,H) = 4.5$, 7.6 Hz, αCH), 4.25–4.05 (m, 4H, $2 \times OCH_2$), 3.29 (dd, 1H, $^2J(H,H) = 16.0$ Hz, $^3J(H,H) = 7.6$ Hz, βCH), 2.6 (dd, 1H, $^2J(H,H) = 16.0$ Hz, $^3J(H,H) = 4.5$ Hz, βCH), 1.34 (m, 6H, $2 \times OCH_2CH_3$) ppm; ^{31}P NMR (121 MHz, $CDCl_3$, {^{1}H}): $\delta = -1.8$ (s) ppm; ^{31}P NMR (121 MHz, $CDCl_3$): $\delta_P = -1.8$ to -1.9 (m) ppm; ^{13}C NMR (75 MHz, D_2O): $\delta = 171.4$ (d, $^2J(C,P) = 38$ Hz, CO–NH–P), 168.9 (CO_2tBu), 151.9 ($tBuCO_2N$), 83.2 ($C(CH_3)_3$), 81.8 ($C(CH_3)_3$), 64.0 (d, $^2J(C,P) = 6$ Hz, OCH_2), 63.9 (d, $^2J(C,P) = 5$ Hz, OCH_2), 55.3 (αCH), 36.3 (d, $^3J(C,P) = 9$ Hz, βCH_2), 28.0 (N–($CO_2C(CH_3)_3)_2$), 27.8 ($CCO_2C(CH_3)_3$), 16.1 (d, $^3J(C,P) = 3$ Hz, CH_3–CH_2), 16.0 (d, $^3J(C,P) = 3$ Hz, CH_3–CH_2) ppm; LC–MS (ES^+, CH_3CN/H_2O): $R_T = 16.4$ min, *m/z* (%): 525 (100, $[M]H^+$), 425 (52, $[M - Boc+H]H^+$), 369 (24, $[M - Boc - C_4H_9+2H]H^+$), 325 (5, $[M - 2Boc+2H]H^+$), 269 (22, $[M - 2Boc - C_4H_9+3H]H^+$); MS ($Cl^+$): *m/z* (%): 525 (1, $[M]H^+$), 425 (12, $[M - Boc+H]H^+$), 369 (74, $[M - Boc - C_4H_9+2H]H^+$), 341 (20, $[M - Boc - C_4H_9 - C_2H_5+3H]H^+$), 325 (12, $[M - 2Boc+2H]H^+$), 313 (22, $[M - 2Boc - C_2H_5+3H]H^+$), 269 (20, $[M - 2Boc - C_4H_9+3H]H^+$), 251 (28, $[M - 2Boc - C_4H_9 - C_2H_5+4H]H^+$), 180 (18, $[CONHP(O)(OCH_2CH_3)_2]^+$). HRMS ($Cl^+$): calcd for $[M - Boc+H]H^+$, $C_{17}H_{34}N_2O_8P$: 425.4041; found: 425.2052.

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