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A new method for the synthesis of a phosphonic acid analogue of phosphoserine via a novel 1,1-difluorophosphonate intermediate

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Abstract

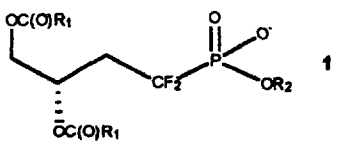
The synthesis of [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid, an analogue of phosphoserine, is reported by two different routes; one route gave a racemic mixture, and the other, by using the strategy of a coupling reaction with a chiral compound, gave a single enantiomer.

Keywords: Phosphoserine analogue; 1,1-Difluorophosphane intermediate; Synthesis

1. Introduction

Phosphonic acids have been widely studied as analogues of naturally occurring phosphates, in an effort to design biologically useful molecules [1]. The introduction of two fluorine atoms onto the methylene carbon often leads to a remarkable enhancement of biological activity [2,3]. To evaluate this hypothesis, a number of studies have been conducted to examine the efficacy of 1,1-difluoroalkyl phosphonates as analogues of natural phosphates and, in many cases, these difluorophosphonates offered significant advantages over their nonfluorinated counterparts, as enzyme inhibitors or alternate substrates [4,5].

Martin et al. [4] prepared a series of 1,1-difluoroalkyl phosphonates related to compound (**1**) as potential inhibitors of phosphatidyl choline and phosphatidyl inositol specific phosphalipase C's



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R_1 = alkyl or unsaturated alkyl

R_2 = choline or inositol

Blackburn and co-workers [5] also demonstrated that the electronic properties of pyrophosphate were more effectively mimicked by its difluoromethylene *bis*-phosphonic acid analog than by methylene *bis*-phosphonic acid.

Aminophosphonic acids are becoming increasingly important as analogues of natural amino acids. Serine phosphate is unique among these amino acids, for it occurs both in the membrane proteins and the membrane phospholipid and phosphatidylserine [6]. Thus, the synthesis of phosphoserine peptides and their analogues has received considerable attention in the chemical literature.

PP1, PP2A, PP2B and PP2C, are members of the protein phosphoserine phosphatase family of enzymes and, in signal transduction events [7], are important as mediators. They also have the ability to dephosphorylate peptides [7] and, therefore, those peptides which contain a hydrolytically stable, and effective phosphoserine mimic are, potentially, inhibitors of this family of enzymes.

Although Berkowitz et al. [8] reported on the synthesis of (α, α-difluoroalkyl) phosphonate protected phosphoserine analogue in a form appropriate for solid phase peptide synthesis, there are generally few methods available which lead to phosphoserine analogues.

In order to explore the potential biological activity of the amino acids phosphonates, the synthesis of [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**9**) an analogue of phosphoserine, via a novel 1,1-difluoro intermediate (**6**), is described in this paper. This analogue is expected to have an important biological activity, and it is of interest for biological testing as a potential inhibitor of the phosphoserine phosphatase class of enzymes.

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2. Results and discussion

The strategy employed in the preparation of phosphono amino-acid (**9**) is outlined in Scheme 1.

Diethyl(bromodifluoromethyl)phosphonate (**3**) was prepared from triethyl phosphite (**2**) and dibromodifluoromethane [9]. Reaction of (**3**) with zinc dust gave the stable [(diethoxyphosphinyl)difluoromethyl] zinc bromide (**4**) [10] which was reacted with 2-bromoacrylic acid to afford the novel [4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (**5**). Esterification of (**5**) using methanol in the presence of $HCl_{(gas)}$ provided (**6**). Azide displacement of the bromine group of (**6**), and subsequent hydrogenation of the azido group of (**7**), resulted in the salt (**8**). Reaction of the salt (**8**) with iodotrimethylsilane and subsequent dissolution in water gave the target a-amino phosphonic acid (**9**), a phosphoserine analogue, as a white solid in 82% yield.

Compound (**9**) is, however, racemic. In order to isolate each enantiomer, the synthesis of (**9**) followed an alternative strategy, outlined in Scheme 2.

Compound (**5**) was coupled with [(1S)-endo]-(-)-borneol. Dicyclohexyl carbodiimide (DCC) was used to activate the carboxyl group of the compound (**5**). Reaction of DCC with the carboxyl group of the compound (**5**) formed the *O*-acylisourea intermediate (**10**), a highly activated acylating agent, which was expected to react with borneol, yielding a mixture of diastereisomers (**11**).

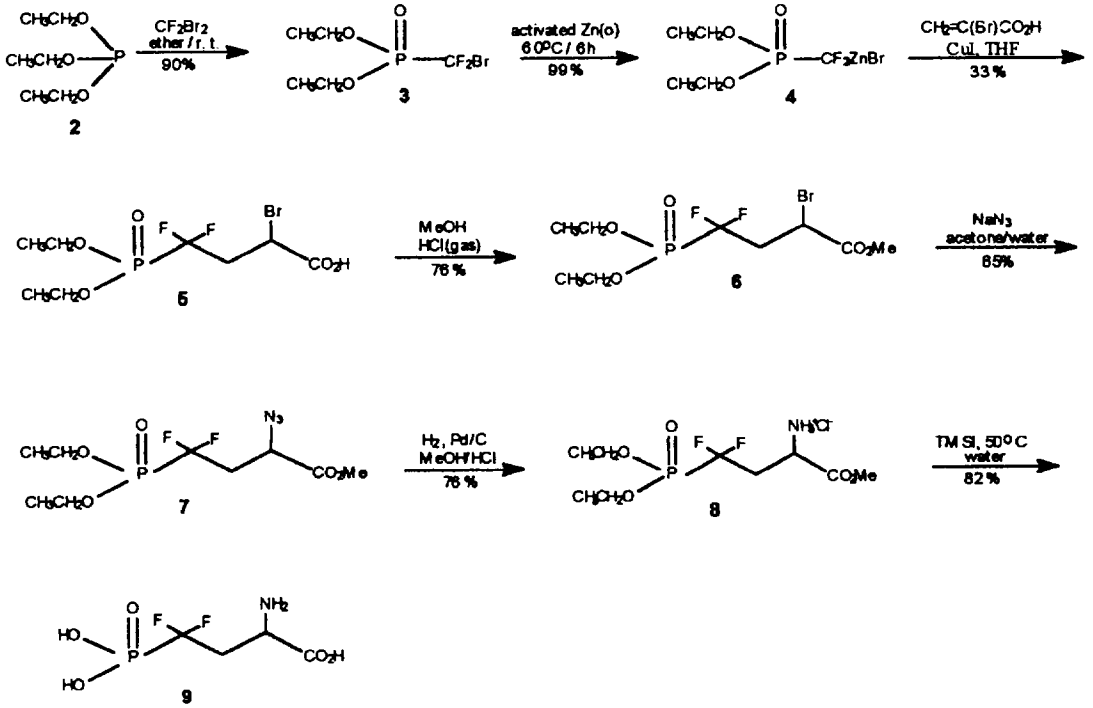
The intramolecular reaction of the intermediate (**10**) was a competing and unwanted side-reaction leading to the formation of the N-acyl urea, thus causing problems in the synthesis of compound (**11**), due to difficulty of purification, and also by reducing the yield of the reaction. This problem was avoided when the compound (**10**) was reacted with borneol using 4-dimethylaminopyridine (DMAP) as catalyst. Then, compound (**11**) was formed in 95% yield with no formation of *N*-acylurea as a by-product.

The nucleophilic displacement of bromine by the azido group in (**11**) yielded compound (**12**). Reduction of (**12**) by catalytic hydrogenation over 5% Pd/C using methanol as a solvent in the presence of $HCl_{(conc.)}$, afforded a mixture of amine salts, (**13S**) and (**13R**), which were separated by column chromatography.

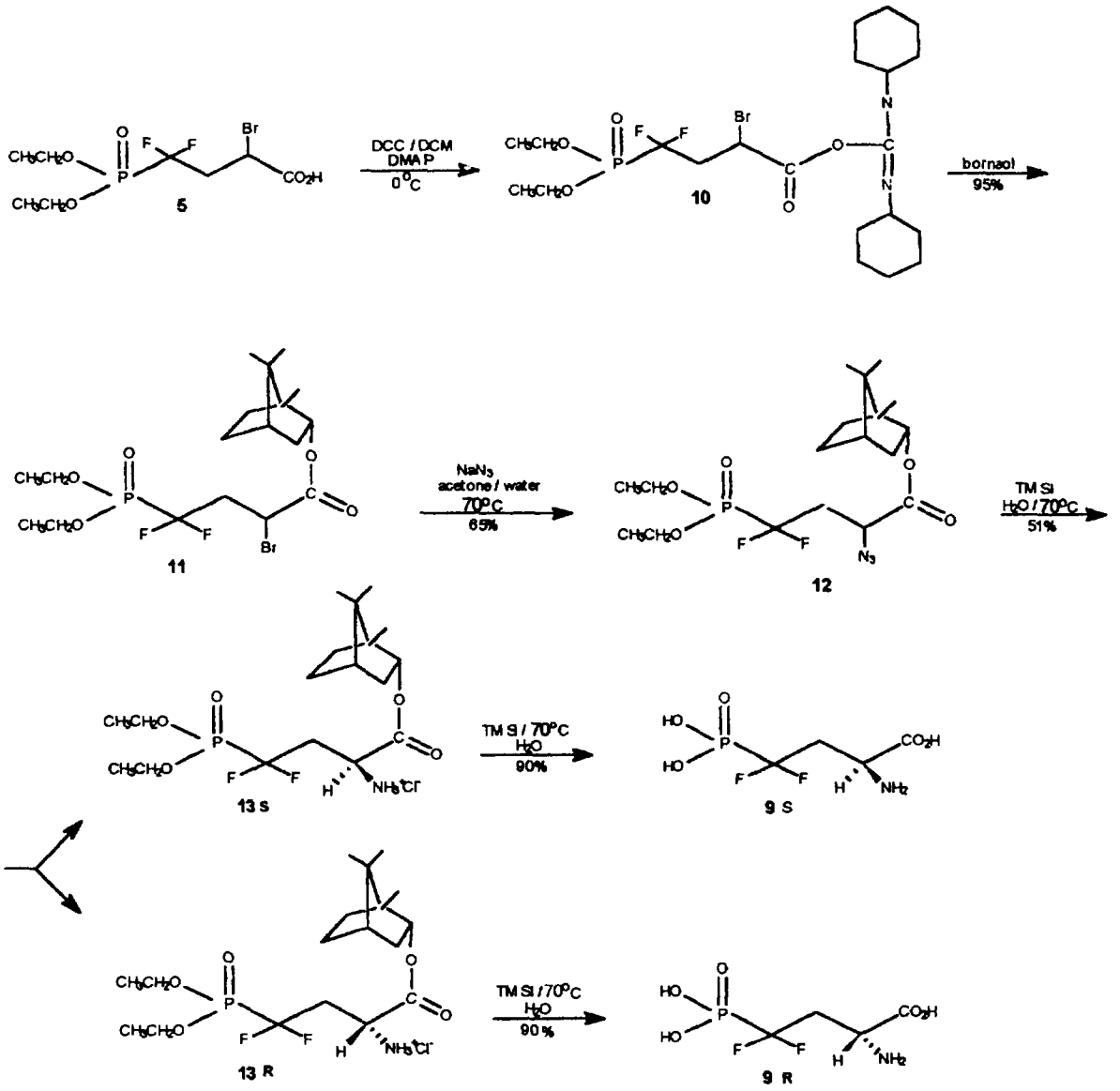
Deprotection of the diethoxyphosphinyl group and the bornyl ester of the amino salts (**13S**) and (**13R**) with TMSI followed by hydrolysis in water, yielded the title compound. The product (**9S**), as expected, was optically pure $[\alpha]_D = 30.4°$ (c 4.60, H_2O). However, racemization of (**9S**) ocurred upon standing in aqueous solution for one week. Since the resulting compound, [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**9S**), is a strong acid, ionisation could lead to autoracemization. The expected (**R**) stereochemistry for (**9R**) was not observed. Autoracemization of the compounds (**13R**) probably ocurred faster than (**9S**), just after the reactin with TMSI.

3. Conclusion

In conclusion we present a convenient and practical synthesis of [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**9**), an analogue of phosphoserine, by two different routes, that also lead to a novel 1,1 difluoroalkyl phosphonate (**5**) with the added advantage of using an easily commercially available starting material.



Scheme 1.



Scheme 2.

4. Experimental section

Melting points were determined on commercially available apparatus (Electrothermal melting point apparatus), or Büchi 510, and are uncorrected. Elemental microanalyses were carried out using a Carlo Erba 1106 Elemental Analyser. Infrared spectra were recorded in the range of 4000–600 cm^{-1}, using a Perkin Elmer 1600 FT-IR spectrophotometer and peaks are reported (ν_{max}) in wave numbers (cm^{-1}). Spectra of liquid samples were taken as Nujol mulls, or in chloroform solution, as indicated.

Proton NMR (^1H nmr) spectra were recorded on a Jeol GX FT-270 (270 MHz) spectrometer although, where indicated, a Jeol GX FT-400 (400 MHz) spectrometer was used. Carbon 13 magnetic resonance (^{13}C nmr) spectra were recorded on a Jeol GX FT-270 spectrometer operating at 67.8 MHz and using 90 and 135 DEPT pulse sequences to aid multiplicity determination. Chemical shifts (δ) are expressed in parts per million downfield from internal tetramethylsilane (TMS). Mass spectra were recorded using a VG Analytical 7070 E instrument with a VG 2000 data system. Electron ionisation (E.I.) was produced using an ionising potential of 70 eV. Chemical ionisation (C.I.) was employed using isobutane as the reagent gas although, where indicated, ammonia was also used.

All general reagents and solvents were purified and dried when required, using the methods described in D.D. Perrin, W.L.F. Armarego, and D.R. Perrin, "Purification of Laboratory Chemicals", Pergamon Press, Oxford, 1980.

Diethyl bromodifluoromethane phosphonate (**3**), [δ ($CDCl_3$) 1.4 (3H, t) and 4.3–4.4 (2H, m)] were prepared by the reaction of triethyl phosphite with dibromodifluoromethane at room temperature and using diethyl ether as solvent [9].

[(Diethoxyphosphinyl)difluoromethyl] zinc bromide (**4**), [δ ($CDCl_3$) 1.4 (3H, t) and 4.2–4.3 (2H, m)] were

prepared by the reaction of (**3**) with acid-washed zinc powder at room temperature using dry THF as solvent [10].

4.1. [4,4-Difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoic acid (5)

To a solution of (**4**) (1.30 g, 3.90 mmol) in 3 ml of dry THF, was added a catalytic amount of cuprous iodide (0.2 g). Then, 2-bromoacrylic acid (0.71 g, 4.70 mmol), dissolved in 3 ml of dry THF was added dropwise at room temperature, and the mixture was stirred for 4 days. The mixture was filtered and poured into 10 ml of brine and extracted with ether (3 × 10 ml). The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The product was purified using column chromatography on silica gel with chloroform/methanol/acetic acid (95:4:1) as eluent to give the product in 33% yield. R_f = 0.46($CHCl_3$:MeOH:AcOH 90:8:2); ν_{max}(liquid film)/cm^{-1} 3459(COOH), 3057, 2981, 1739(C=O), 1596, 1489, 1446, 1373, 1243(P=O), 1174, 1046, 941, 904, 846, 758, 704; δ_H($CDCl_3$) 1.39 (t, $C\underline{H}_3CH_2OP$, J = 7.05 Hz), 2.59–2.83 (m, $CF_2C\underline{H}H$), 3.09–3.34 (m, $CF_2CH\underline{H}$), 4.25–4.36 (m, $CH_3C\underline{H}_2OP$), 4.55 (dd, $CF_2CH_2C\underline{H}Br$, $J_{2,3b}$ = 4.39 Hz, $J_{2,3a}$ = 9.28 Hz); δ_C($CDCl_3$) 16.2 (d, $\underline{C}H_3CH_2OP$, $J_{C,P}$ = 5.5 Hz), 3.49 (s, $CF_2CH_2\underline{C}H(Br)$), 39.3 (dd, $CF_2\underline{C}H_2CH(Br)$, $J_{C,F}$ = 36.35 Hz, $J_{C,P}$ = 19.85 Hz), 65.5 (d, $CH_3\underline{C}H_2OP$, $J_{C,P}$ = 8.9 Hz), 118.7 (td, $\underline{C}F_2$, $J_{C,P}$ = 219.2 HZ, $J_{C,F}$ = 262.2 Hz), 171.6 (s, C=O); δ_F ($CDCl_3$) −112.1 (dddd, $J_{F,F}$ = 301.7 Hz, $J_{F,P}$ = 105.2 Hz, $J_{3b,F}$ = 25.4 Hz, $J_{3a,F}$ = 12.7 Hz, 1F), −113.2(dddd, $J_{F,F}$ = 301.7 Hz, $J_{F,P}$ = 105.7 Hz, $J_{3b,F}$ = 25.5 Hz, $J_{3a,F}$ = 11.6 Hz, 1F); δp($CDCl_3$) 5.08 (t, ^{1}H decoupled, $J_{P,F}$ = 104 Hz; m, ^{1}H coupled, $J_{P,3a}$ = $J_{P,3b}$ = 4.03 Hz); m/z(C.I.) 339,341 (MH^+, 98%), 321,323(M^+-OH, 20), 293,295 (M^+-CO_2H, 7). Anal. Calcd. for $C_8H_{14}BrF_2O_5P$: C, 28.3; H, 4.2; Found: C, 28.6; H, 4.3%.

4.2. Methyl[4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butanoate (6)

To the solution of (**5**) (0.0035 mmol, 1.09 g) in 25 ml of dry methanol $HCl_{(gas)}$ was added to the solution until no more was absorbed. The $HCl_{(aq.)}$ resulting from the reaction and the excess of methanol were removed under reduced pressure. The product was purified using column chromatography on silica gel with ethyl acetate/petrol (b.p. 60–80 °C) (3:7) as the eluent, giving a pale oil in 76.4% yield; R_f = 0.48(petrol (b.p. 60–80 °C)-ethyl acetate 1:1); ν_{max}(liquid film)/cm^{-1} 2987, 1749(C=0), 1439, 1371, 1276(P=O), 1209, 1116, 1020, 981, 796, 761, 573; δ_H($CDCl_3$) 1.39 (t, $C\underline{H}_3CH_2OP$, J = 7.05 Hz), 2.62–2.82 (m, $CF_2C\underline{H}H$), 3.13–3.27 (m, $CF_2CH\underline{H}$), 3.81 (s, $OC\underline{H}_3$), 4.23–4.34 (m, $CH_3C\underline{H}_2OP$), 4.57 (dd, $CF_2CH_2C\underline{H}(Br)$, $J_{2,3b}$ = 9.61 Hz, $J_{2,3a}$ = 4.12 Hz); δ_C($CDCl_3$) 15.72 (d, $\underline{C}H_3CH_2OP$, $J_{C,P}$ = 5.5 Hz), 34.18 (d, $CF_2CH_2\underline{C}H(Br)$, $J_{C,F}$ = 6.6 Hz), 38.9 (q, $CF_2\underline{C}H_2CH(Br)$, $J_{C,F}$ = 36.35 Hz, $J_{C,P}$ = 20.95 Hz), 52.6 (s, $O\underline{C}H_3$), 64.3 (d, $CH_3\underline{C}H_2OP$, $J_{C,P}$ = 6.6 Hz), 118.2 (td, $\underline{C}F_2$ $J_{C,P}$ = 216.0Hz, $J_{C,F}$ = 259.0Hz), 168.8 (s, C=O); δ_F ($CDCl_3$) −112.3 (dddd, $J_{F,F}$ = 301.7Hz, $J_{F,P}$ = 103.5Hz, $J_{3b,F}$ = 22.5Hz, $J_{3a,F}$ = 15.1Hz, 1F), −113.4(dddd, $J_{F,F}$ = 301.7 Hz, $J_{F,P}$ = 103.1 Hz, $J_{3b,F}$ = 22.5 Hz, $J_{3a,F}$ = 13.9 Hz, 1F); δ_P($CDCl_3$) 5.15 (t, ^{1}H decoupled, $J_{P,F}$ = 104 Hz); m/z(C.I.) 353,355 (MH^+, 100%), 321,323(M^+-CO_2H, 8); m/z(E.I.) 352; 354 (M^+, 353.98663 $C_9H_{16}O_5F_2BrP$ requires 353.98272, 6).

4.3. Methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-azido] butanoate (7)

A solution of (**6**) (5.74 mmol, 2.02 g) in 15 ml of acetone and 15 ml of water was treated with sodium azide (9.23 mmol, 0.6 g), and stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure and poured into EtOAc. The mixture was extracted with ethyl acetate (3 × 20 ml). The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed (petrol (b.p. 60–80 °C)-ethyl acetate 1:1) to yield the title compound as a pale oil in 65.0% yield; R_f = 0.65(petrol (b.p. 60–80 °C)-ethyl acetate 1:1); ν_{max}(liquid film)/cm^{-1} 2986, 2123(N_3), 1749(C=O), 1272(P=O), 1019; δ_H($CDCl_3$) 1.40 (t, $C\underline{H}_3CH_2OP$, J = 6.87 Hz), 2.34–2.51 (m, $CF_2C\underline{H}H$), 2.67–2.83 (m, $CF_2CH\underline{H}$), 3.83 (s, $OC\underline{H}_3$), 4.26–4.35 (m, $CH_3C\underline{H}_2OP$ and $CF_2CH_2C\underline{H}(N_3)$); δ_C($CDCl_3$) 16.2 (d, $\underline{C}H_3CH_2OP$, $J_{C,P}$ = 3.6 Hz), 35.3 (td, $CF_2\underline{C}H_2CH(N_3)$, $J_{C,F}$ = 20.7 Hz, J_{CP} = 15.9 Hz), 55.84 (q, $CF_2CH_2\underline{C}H(N_3)$, $J_{C,F}$ = 9.2 Hz, $J_{C,P}$ = 5.5 Hz), 53.04 (s, $O\underline{C}H_3$), 64.9 (d, $CH_3\underline{C}H_2OP$, $J_{C,P}$ = 5.5 Hz), 118.79(td, $\underline{C}F_2$ $J_{C,P}$ = 216.9 Hz, $J_{C,F}$ = 216.9 Hz), 169.41 (s, C=O); δ_F ($CDCl_3$) −111.5 (dddd, $J_{F,F}$ = 300.7 Hz, $J_{F,P}$ = 103.5 Hz, $J_{3b,F}$ = 25.5 Hz, $J_{3a,F}$ = 11.6 Hz, 1F), −112.9(dddd, $J_{F,F}$ = 300.7 Hz, $J_{F,P}$ = 104.7 Hz, $J_{3b,F}$ = 24.8 Hz, $J_{3a,F}$ = 12.7 Hz, 1F); δ_P($CDCl_3$) 5.50 (t, ^{1}H decoupled, $J_{P,F}$ = 104 Hz); m/z(C.I.) 316 (MH^+, 316.0874 $C_9H_{16}O_5F_2N_3P$ requires 316.0874, 100%), 288(12), 268(40), 228(15); m/z(E.I.) 316 (M^+, 2%), 284(M^+-OMe, 2), 228(30), 200(34), 172(100).

4.4. Methyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butanoate hydrochloride salt (8)

Compound (7) (2.07 mmol, 0.65 g) in methanol (15 ml) and HCl (1.0 ml) was hydrogenolyzed over 5%-Pd/C in the usual manner. The mixture was stirred overnight and then filtered through celite. The solvents were evaporated under reduced pressure, then the reaction mixture was dissolved in water (10 ml) and was lyophilised to yield the title compound as a cream solid in 76.4% yield; R_f = 0.20($CHCl_3$-MeOH 1:1); ν_{max}(liquid film)/cm^{-1} 3435(OH), 2984, 1755(C=O), 1601(NH_3^+), 1245(P=O), 1020; δ_H(D_2O) 1.45 (t, $C\underline{H}_3CH_2O$, J = 7.05 Hz), 2.89–3.09 (m, $CF_2C\underline{H}_2$), 3.93 (s, $O\underline{CH}_3$), 4.38–4.49 (m, $CH_3C\underline{H}_2O$); 4.68 (dd, $CH_2C(\underline{H})NH_3 + Cl^-$, $J_{2,3b}$ = 7.51 Hz, $J_{2,3a}$ = 4.03 Hz); δ_C(MeOD) 17.0 (d, $\underline{C}H_3CH_2O$, $J_{C,P}$ = 5.5 Hz), 35.4 (q,

$CF_2\underline{C}H_2C(H)NH_3^+Cl^-$, $J_{C,F}=38.4$ Hz, $J_{CP}=19.1$ Hz), 48.7 (s, $CF_2CH_2\underline{C}(H)NH_3^+Cl^-$), 54.7 (s, $O\underline{C}H_3$), 67.3 (d, $CH_3\underline{C}H_2O$, $J_{C,P}=7.7$ Hz), 121.2(td, $\underline{C}F_2$ $J_{C,P}=219.5$ Hz, $J_{C,F}=261.4$ Hz), 169.6 (s, C=O); *m/z*(+ve FAB) 290(MH^+, 290.0980 $C_9H_{18}O_5F_2NP$ requires 290.0969, 100%), 262(5), 210(10).

4.5. *[4,4-Difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (9).*

Compound (**8**) (0.86 g, 2.63 mmol) was stirred with TMSI in excess (2.5 ml) without solvent at 60 °C for 7 days. The excess of silylating reagent and ethyl iodide were removed under reduced pressure to give the disilylated ester, which was dissolved in ether (30 ml), and then treated with water (30 ml) to provide the corresponding free amine and phosphonic acid, [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**9**). The compound (**9**) was lyophilised and purified by washing with methanol. The impurities stayed in the methanol, and the compound (**9**) precipitated as a white solid (0.472 g; 82% yield); m.p. = 220 °C; $\nu_{max}(D_2O)/cm^{-1}$ 3423(OH), 2527(NH_3^+), 1746(C=O), 1209(P=O); $\delta_H(D_2O)$ 2.64–2.80 (m, $CF_2C\underline{H}_2$-$CH(NH_3^+)$), 4.32 (d, $CF_2CH_2C\underline{H}(NH_3^+)$, $J_{H,H}=6.77$ Hz); $\delta_C(D_2O)$ 33.4 (q, $CF_2\underline{C}H_2CH(NH_3^+)$, $J_{C,F}=36.6$ Hz, $J_{CP}=18.9$ Hz), 47.5 (s, $CF_2CH_2\underline{C}H(NH_3^+)$), 120.5(td, $\underline{C}F_2J_{C,P}=195.5$ Hz, $J_{C,F}=259.2$ Hz), 170.7 (s, C=O); *m/z*(-ve FAB) 218(MH^-, 218.0039 $C_4H_8F_2NO_5P$ requires 218.0030, 75%), 184(30), 153(100); *m/z*(+ve FAB) 220 (MH^+, 35%), 191(35), 152(100), 138(95), 122(40); Anal. Calcd. for $C_4H_8F_2NO_5P+1.5\ H_2O$: C, 19.50; H, 4.47; N, 5.70; Found: C, 19.70; H, 4.31; N, 5.70%.

4.6. *Bornyl [(4,4-difluoro-4-(diethoxyphosphinyl)-2-bromo] butyrate (11)*

To a stirred solution of (**6**) (3.42 g, 10.11 mmol) in anhydrous CH_2Cl_2 (30 ml) was added [(1S)-endo]-(-)-borneol (1.64 g, 10.62 mmol) and DMAP (10 mol%). DCC (2.19 g; 10.62 mmol) was added to the reaction mixture at 0 °C. The mixture was then stirred for 5 h, gradually reaching room temperature. Precipitated urea was then filtered off and the filtrate concentrated under reduced pressure. The residue was taken up in dichloromethane and, if necessary, filtered free of any further precipitated urea. Column chromatography (petrol (b.p. 60–80 °C)-ethyl acetate 9:1) yielded the desired ester as a colourless oil (3.76 g; 78%); $R_f=0.59$ (petrol–ethyl acetate 7:3); ν_{max}(liquid film)/cm^{-1} 2955, 2876, 1743(C=O), 1666, 1274(P=O), 1159, 1020; $\delta_H(CDCl_3)$ 0.79, 0.80, 0.81, 0.84 (s, $C\underline{H}_3$ 8', 9', 10'), 0.97(dd, $C\underline{H}_2$ 2', $J_{H(2'),H(2')}=13.9$ Hz, $J_{H(2'),H(1')}=3.3$ Hz), 1.14–1.25(m, $C\underline{H}_2$ 5'), 1.32 (t, $C\underline{H}_3CH_2OP$, $J=7.15$ Hz), 1.63 (t, $C\underline{H}$ 3', $J_{H(3'),(4')}=J_{H(3'),H(2')}=4.4$ Hz), 1.67–1.79 (m, $C\underline{H}_2$ 4'), 1.86–1.95 (m, $C\underline{H}_2$ 4'), 2.23–2.39 (m, $C\underline{H}_2$ 2'), 2.57–2.79 (m, $CF_2C\underline{H}_2CH(Br)$), 3.13–3.29 (m, $CF_2C\underline{H}_2CH(Br)$), 4.18–4.37 (m, $CH_3C\underline{H}_2OP$), 4.47–4.69 (m, $C\underline{H}$ 1'), 4.85–5.31 (m, $CF_2CH_2C\underline{H}(Br)$); $\delta_C(CDCl_3)$ 12.3 (C-10'), 15.4 (d, $\underline{C}H_3CH_2OP$, $J_{C,P}=5.5$ Hz), 17.8 (C-8'), 18.6 (C-9'), 26.0 (C-4'), 26.9 (C-5'), 34.9 (s, $CF_2CH_2\underline{C}H(Br)$), 35.0 (C-2'), 38.4 (q, $CF_2\underline{C}H_2CH(Br)$, $J_{C,F}=35.2$ Hz, $J_{C,P}=19.8$ Hz), 43.8 (C-3'), 47.1 (C-7'), 48.1 (C-6'), 63.9 (d, $CH_3\underline{C}H_2OP$, $J_{C,P}=6.6$ Hz), 81.0 (C-1'), 117.9 (td, $\underline{C}F_2$, $J_{C,P}=216.0$ Hz, $J_{C,F}=261.0$ Hz), 168.0 (s, C=O); *m/z*(C.I.) 475,477 (MH^+, 18%), 339,341(100), 321,323 (12). Anal. Calcd. for $C_{18}H_{30}BrF_2O_5P$: C, 45.5; H, 6.4; Found: C, 45.9; H, 6.5%.

4.7. *Bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-azido] butyrate (12)*

A solution of (**11**) (3.76 g, 7.91 mmol) in 20 ml of acetone and 10 ml of water was treated with sodium azide (0.57 g, 8.70 mmol) and stirred at 40 °C for 10 h. Then, the reaction mixture was concentrated under reduced pressure and extracted with ethyl acetate (3×15 ml). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. Column chromatography (petrol (b.p. 60–80 °C)-ethyl acetate 4:1) yielded the title compound as a pale oil (2.26 g; 65%); $R_f=0.28$ (petrol (b.p. 60–80 °C)-ethyl acetate 7:3); ν_{max}(liquid film)/cm^{-1} 2956, 2110(N_3), 1743(C=O), 1454, 1273(P=O), 1194, 1020; $\delta_H(CDCl_3)$ 0.86, 0.87, 0.89, 091 (s, $C\underline{H}_3$ 8', 9', 10'), 1.04(dt, $C\underline{H}_2$ 2', $J_{H(2'),H(2')}=13.9$ Hz, $J_{H(2'),H(1')}=J_{H(2'),H(3')}=3.7$ Hz), 1.23–1.31(m, $C\underline{H}_2$ 5'), 1.40 (t, $C\underline{H}_3CH_2OP$, $J=7.14$ Hz), 1.72 (t, $C\underline{H}$ 3', $J_{H(3'),(4')}=J_{H(3'),H(2')}=4.4$ Hz), 1.76–1.82 (m, $C\underline{H}_2$ 4'), 1.89–1.99 (m, $C\underline{H}_2$ 4'), 2.30–2.55 (m, $C\underline{H}_2$ 2' and $CF_2C\underline{H}_2CH(N_3)$), 2.65–2.85 (m, $CF_2C\underline{H}_2CH(N_3)$), 4.25–4.36 (m, $CF_2CH_2C\underline{H}(N_3)$ and $CH_3C\underline{H}_2OP$), 4.97–5.01 (m, $C\underline{H}$ 1'); $\delta_C(CDCl_3)$ 13.4 (C-10'), 16.3 (d, $\underline{C}H_3CH_2OP$, $J_{C,P}=5.5$ Hz), 18.8 (C-8'), 19.6 (C-9'), 27.1 (C-4'), 27.9 (C-5'), 35.3 (q, $CF_2\underline{C}H_2CH(Br)$, $J_{C,F}=20.1$ Hz, $J_{C,P}=16.5$ Hz), 36.6 (C-2'), 44.7 (C-3'), 47.9 (C-7'), 48.9 (C-6'), 56.1 (s, $CF_2CH_2\underline{C}H(Br)$), 64.8 (d, $CH_3\underline{C}H_2OP$, $J_{C,P}=7.3$ Hz), 82.7 (C-1'), 120.2 (td, $\underline{C}F_2$, $J_{C,P}=216.9$ Hz, $J_{C,F}=261.0$ Hz), 169.2 (s, C=O); δ_F ($CDCl_3$) −111.4 (ddddd, $J_{F,F}=300.5$ Hz, $J_{F,P}=104.2$ Hz, $J_{3b,F}=25.0$ Hz, $J_{3a,F}=19.7$ Hz, $J_{2,F}=12.7$ Hz, 1F), −112.5(ddddd, $J_{F,F}=300.5$ Hz, $J_{F,P}=104.9$ Hz, $J_{3b,F}=34.4$ Hz, $J_{3a,F}=23.9$ Hz, $J_{2,F}=13.9$ Hz 1F); $\delta_P(CDCl_3)$ 5.6 (t, ^1H decoupled, $J_{P,F}=105$ Hz; m, ^1H coupled, $J_{P,3a}\approx J_{P,3b}=6.9$ Hz); *m/z*(C.I.) 438 (MH^+, 18%), 302(100),228(8), 210(10),137(40). Anal. Calcd. for $C_{18}H_{30}F_2N_3O_5P$:C, 49.3; H, 6.9; N, 9.6; Found: C, 49.6; H, 7.1; N, 9.4%.

4.8. *Bornyl [4,4-difluoro-4-(diethoxyphosphinyl)-2-amino] butyrate hydrochloride salt (13S and 13R)*

Compound (**12**) (1.35 g, 31.0 mmol) in methanol (20 ml) and HCl (9.0 ml) was hydrogenolyzed over 5% Pd/C in the usual manner. The mixture was stirred overnight and then filtered through celite. The solvents were evaporated under reduced pressure, then the reaction was dissolved in

water (20 ml) and lyophilised. TLC indicated products (**13S** and **13R**) in 50.6% yield, which was separated by column cromatography (chloroform–methanol 99:1 to 95:5).

The first band eluted (R_f=0.75, chloroform–methanol 90:10), was collected and evaporated to afford *bornyl* [4-4-*difluoro*-4-(*diethoxyphosphinyl*)-2-*amino*] *butyrate hydrochloride salt* (***13R***); ν_{max}(liquid film)/cm^{-1} 3743, 2361, 1733(C=O), 1650, 1274(P=O), 1188, 1021; δ_H($CDCl_3$) 0.89, 0.90, 0.93, 0.96 (s, C$\underline{H}_3$ 8', 9', 10'), 10.6(dd, C$\underline{H}_2$ 2', $J_{H(2'),H(2')}$=13.83 Hz, $J_{H(2'),H(1')}$=3.2 Hz), 1.16 (t, C$\underline{H}$ 3', $J_{H(3'),(4')}=J_{H(3'),H(2')}$=7.15 Hz), 1.25–1.37(m, C$\underline{H}_2$ 5'), 1.43 (t, C$\underline{H}_3$CH$_2$OP, J=7.05 Hz), 1.69–1.99 (m, C$\underline{H}_2$ 4'), 2.00–2.36 (m, C$\underline{H}_2$ 4'), 2.41–2.76 (m, $CF_2C\underline{H}_2CH(NH_3^+Cl^-)$ and C$\underline{H}_2$ 2'), 3.73 (ddd, $CF_2CH_2C\underline{H}(NH_3^+Cl^-)$, $J_{2,3a}$=4.87 Hz, $J_{2,3b}$=7.63 Hz, $J_{2,F}$=3.35 Hz), 427–4.38 (m, $CH_3C\underline{H}_2OP$), 4.96–4.99 (m, C$\underline{H}$ 1'); δ_C($CDCl_3$) 13.3 (C-10'), 16.3 (s, $\underline{C}H_3CH_2OP$), 18.7 (C-8'), 19.6 (C-9'), 27.0 (C-4'), 27.9 (C-5'), 37.3 (q, $CF_2\underline{C}H_2CH(NH_3^+Cl^-)$, $J_{C,F}$=35.8 Hz, $J_{C,P}$=17.5 Hz), 41.9 (C-2'), 44.8 (C-3'), 47.7 (C-7'), 48.7 (C-6'), 55.2 (s, $CF_2CH_2\underline{C}H(NH_3^+Cl^-)$), 64.5 (d, $CH_3\underline{C}H_2OP$, $J_{C,P}$=5.5 Hz), 80.8 (C-1'), 119.6 (td, $\underline{C}F_2$, $J_{C,P}$=215.1 Hz, $J_{C,F}$=261.0 Hz), 174.1 (s, C=O); *m/z*(-ve FAB) 410.2 (MH^-, 100%), 398.1(12), 302.1(22), 274.1 (M^+-$C_{10}H_{17}$,10).

The second band eluted (R_f=0.65, chloroform–methanol 90:10), was collected and evaporated to afford *bornyl* [4-4-*difluoro*-4-(*diethoxyphosphinyl*)-2-*amino*] *butyrate hydrochloride salt* (***13S***); ν_{max}(liquid film)/cm^{-1} 3743, 2954, 1735(C=O), 1650, 1271(P=O), 1165, 1022; δ_H($CDCl_3$) 0.84, 0.85, 0.88, 0.91 (s, C$\underline{H}_3$ 8', 9', 10'), 0.99(dddd, C$\underline{H}_2$ 2', $J_{H(2'),H(2')}$=13.7 Hz, $J_{H(2'),H(1')}$=3.5 Hz, $J_{H(2'),H(3')}$=5.31 Hz), 1.19–1.32(m, C$\underline{H}_2$ 5'), 1.39 (t, C$\underline{H}_3$CH$_2$OP, J=7.05 Hz), 1.69 (t, C$\underline{H}$ 3', $J_{H(3'),(4')}=J_{H(3'),H(2')}$=4.4 Hz), 1.74–1.81 (m, C$\underline{H}_2$ 4'), 1.85–1.97 (m, C$\underline{H}_2$ 4'), 2.24–2.49 (m, $CF_2C\underline{H}_2CH(NH_3^+Cl^-)$ and C$\underline{H}_2$ 2'), 2.58–2.79 (m, $CF_2C\underline{H}_2CH(NH_3^+Cl^-)$), 3.79 (dd, $CF_2CH_2C\underline{H}(NH_3^+Cl^-)$, $J_{2,3a}$=4.58 Hz, $J_{2,3b}$=7.69 Hz), 424–4.35 (m, $CH_3C\underline{H}_2OP$), 4.89–4.94 (m, C$\underline{H}$ 1'); δ_C($CDCl_3$) 13.4 (C-10'), 16.3 (d, $\underline{C}H_3CH_2OP$, $J_{C,P}$=3.7 Hz), 18.7 (C-8'), 19.6 (C-9'), 27.0 (C-4'), 27.9 (C-5'), 36.5 (C-2'), 38.6 (q, $CF_2\underline{C}H_2CH(NH_3^+Cl^-)$, $J_{C,F}$=34.1 Hz, $J_{C,P}$=17.5 Hz), 44.8 (C-3'), 47.8 (s, $CF_2CH_2\underline{C}H(NH_3^+Cl^-)$), 48.8 (C-7'), 49.3 (C-6'), 64.6 (d, CH3CH2OP, JC,P=5.5 Hz), 81.2 (C-1'), 120.2 (td, CF2, JC,P=215.1 Hz, JC,F=260.6 Hz), 173.9 (s, C=O); *m/z*(+ve FAB) 412.3 (MH+, 100%), 276.1(60), 174.1(30).

5. (R,S)[4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (9)

(a) Compound (**13R**) (74.8 mg, 0.17 mmol) were stirred with TMSI in excess (0.05 ml) without solvent at 70 °C for 7 days. The excess of silylating reagent and ethyl iodide were removed under reduced pressure to give the disilylated ester, which was dissolved in ether (20 ml), and then treated with water (15 ml) to provide the corresponding free amine and phosphonic acid, (R)[4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**9R**) in 90% yield; . R_f=0.3(H_2O/NH_3/propanol 1:1:3); ν_{max}(D_2O)/cm^{-1} 3838, 3430, 1734(C=O), 1207(P=O); δ_H(D_2O) 2.73–2.87 (m, $CF_2C\underline{H}_2CH(NH_2)$), 4.29 (overlapped signal, $CF_2CH_2C\underline{H}(NH_2)$); δ_C(D_2O) 36.0 (d, $CF_2\underline{C}H_2CH(NH_2)$), $J_{C,F}$=14.7 Hz), 57.42 (s, $CF_2CH_2\underline{C}H(NH_2)$), $\underline{C}F_2$ (weak signal), 173.2 (s, C=O); *m/z*(+ve FAB) 220(MH^+, 12%), 192(10), 176(100).

(b) Compound (**13S**) (83.9 mg; 0.19 mmol) were stirred with TMSI in excess (0.2 ml) without solvent at 70 °C for 7 days. The excess of silylating agent and ethyl iodide were removed under reduced pressure to give the disilylated ester, which was dissolved in ether (20 ml), and then treated with water (15 ml) to provide the corresponding free amine and phosphonic acid (S), [4,4-difluoro-4-(dihydroxyphosphinyl)-2-amino] butanoic acid (**9S**) in 90% yield; R_f=0.2 (H_2O/NH_3/propanol 1:1:3); $[\alpha]_D$=30.4 (c=4.6 in water); ν_{max}(D_2O)/cm^{-1} 3853, 3743, 1650, 1206(P=O); δ_H(D_2O) 2.72–3.11 (m, $CF_2C\underline{H}_2CH(NH_2)$),4.5 (dd, $CF_2CH_2C\underline{H}(NH_2)$, $J_{2,3a}$=3.4 Hz, $H_{2,3b}$=8.9 Hz); δ_C(D_2O) 37.06 (td, $CF_2\underline{C}H_2CH(NH_2)$, $J_{C,F}$=11.5 Hz, J_{CP}=9.0 Hz), 51.47 (d, $CF_2CH_2\underline{C}H(NH_2)$, $J_{C,F}$=2.0 Hz), $\underline{C}F_2$ (weak signal), 174.9 (s, C=O); δ_F (D_2O) −112.2 (dddd, $J_{F,F}$=290.2 Hz, $J_{F,P}$=91.3 Hz, $J_{3b,F}$=23.1 Hz, $J_{3a,F}$=15.1 Hz, 1F), −113.2(dddd, $J_{F,F}$=290.2 Hz, $J_{F,P}$=91.3 Hz, $J_{3b,F}$=22.0 Hz, $J_{3a,F}$=16.2 Hz, 1F); δ_P(D_2O) 2.8 (t,^{1}H decoupled, $J_{P,F}$=91.2 Hz); *m/z*(+ve FAB) 220 (MH^+, 12%), 192(10), 176(100).

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