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SYNTHESIS OF PHOSPHONATE ANALOGUES OF *MYO*-INOSITOL PHOSPHOLIPIDS

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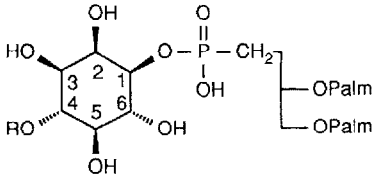
ABSTRACT. The phosphonate analogues **I** and **II** of PtdIns and PtdIns[4]P, respectively, were prepared by alkylation of the α-lithio anion of a properly protected **D**-*myo*-inositol methylphosphonate diester with 1,2-isopropylidene-*sn*-glycerol 3-trifluoromethanesulfonate, followed by protective group manipulation and deprotection

It is now well established that cleavage of phosphatidylinositol 4,5-*bis*phosphate (PtdIns[4,5]P_2) by receptor mediated phospholipase C upon stimulation by neurotransmitters, hormones and growth factors[1], results in the formation of two second messengers *e g* **D**-*myo*-inositol 1,4,5-*tris*phosphate[2] and 1,2-diacylglycerol[3] The supply of PtdIns[4,5]P_2 is constantly replenished by sequential phosphorylation of phosphatidylinositol (PtdIns)[4] Initially, PtdIns is phosphorylated by a specific PtdIns 4-kinase to give phosphatidylinositol 4-phosphate (PtdIns[4]P), which in turn is phosphorylated by a PtdIns[4]P 5-kinase to generate the key lipid PtdIns[4,5]P_2.

Since the pioneering work of Shvets and others[5], the synthesis of inositol phospholipids and analogues thereof has received renewed attention[6] For instance, phosphorothioate analogues of PtdIns have been prepared[7] and applied to determine the stereochemical course of the cleavage reaction by PtdIns-specific phospholipase C

Apart from this, it has been shown[8] that phosphonate analogues of PtdIns are effective phospholipase C inhibitors For example, the phosphonate analogue **I** (see Figure) proved to be a potent anti-inflammatory and analgesic agent. The racemic isosteric analogue **I** of PtdIns was prepared[8] by trichloroacetonitrile-mediated condensation of 2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol[9] with 3,4-dipalmitoyloxybutyl-1-phosphonic acid[10], followed by removal of all benzyl protecting groups As part of a programme directed towards the preparation of *myo*-inositol phospholipids and phosphate analogues thereof, we now describe a versatile approach towards the synthesis of the optically active phosphonate analogues **I** and **II** of PtdIns and PtdIns[4]P, respectively

Figure

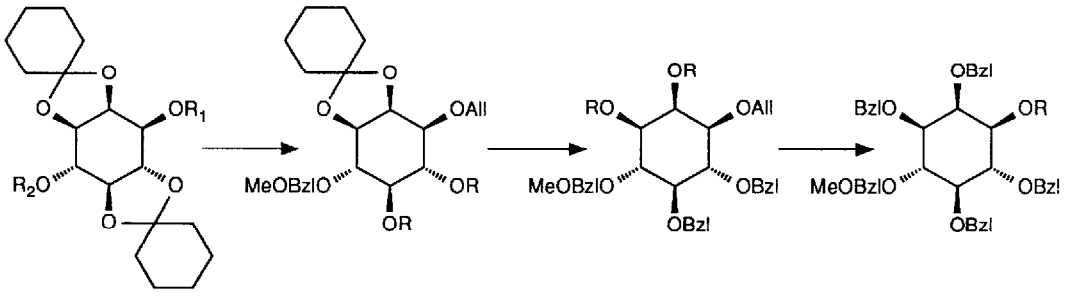


I R = H
II R = P(O)(OH)$_2$

The synthesis of the phosphonate analogues **I** and **II** comprises three main stages, (a) the preparation of a suitably protected **D**-*myo*-inositol derivative, (b) the introduction of the optically active phosphotidic acid and finally the removal of all protecting groups

In the first stage, 1,2.4,5-di-*O*-cyclohexylidene inositol (**1**)[11] was converted into the properly protected **D**-*myo*-inositol derivative **8** as depicted in Scheme 1 Thus, compound **1** was regioselectively allylated with allyl bromide in the presence of BaO/Ba(OH)$_2$ in DMF[12] for 72 h at 20°C to afford, after work-up and purification, the crystalline mono-allyl derivative **2**[13] (m p. 116 5-117 5°C) in a yield of 68% Subsequent *p*-methoxybenzylation (*p*MeOBzlCl/NaH) of **2**, followed by selective removal of the 4,5-cyclohexylidene group (0 1M $HOCH_2CH_2OH$ in CH_2Cl_2) from **3** gave, after

Scheme 1



1 $R_1 = R_2 = H$ **4** R = H **6** R = H **8** R = H

2 R_1 = All, R_2 = H **5** R = Bzl **7** R = Bzl **9** R = Cam

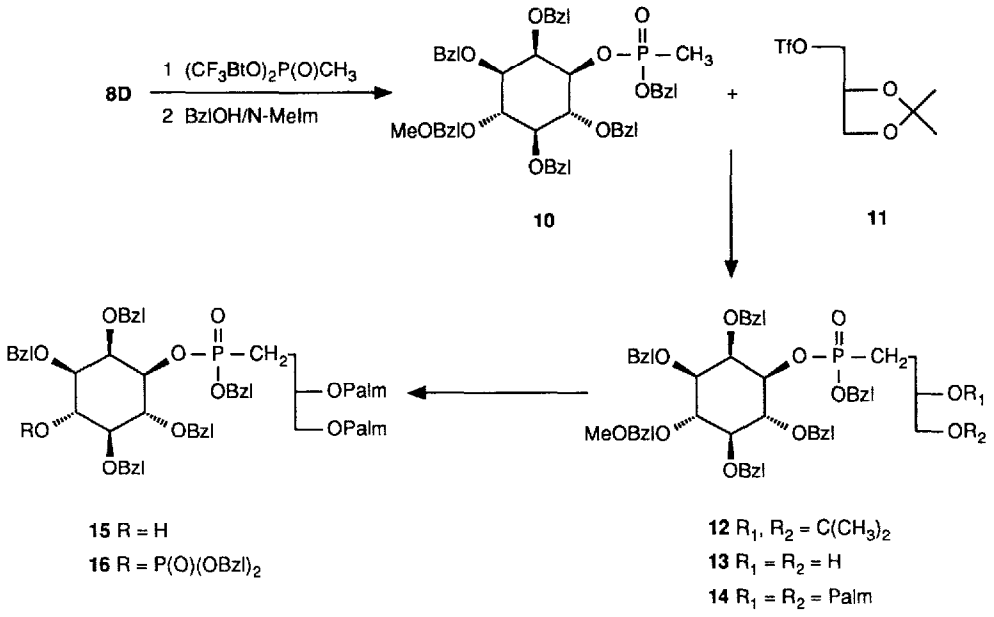
3 R_1 = All, R_2 = pMeOBzl

column chromatography, compound **4** (m.p. 142-143°C) in a yield of 71%. After benzylation (BzlBr/NaH) of **4**, the 1,2-*cis*-cyclohexylidene group from **5** was removed by treatment with 0.05N HCl in MeOH to give crystalline **6** (m.p. 112.5-113.5°C) in 89% yield. Benzylation (BzlBr/NaH) of compound **6** furnished the fully protected *myo*-inositol **7** in a yield of 98%. Isomerization[14] of the allyl group in **7** with 1,5-cyclooctadiene-*bis*[methyldiphenylphosphine]-iridium hexafluorophosphate[15] (activated with H_2 for 2 min) in 1,2-dichloroethane, followed by hydrolysis of the intermediate *trans*-prop-1-enyl group with 0.1N HCl in CH_2Cl_2/MeOH (1/1, v/v), resulted in the isolation of the racemic 1-OH-derivative **8** (m.p. 69.5-70.5°C) in 95% yield. Optical resolution of the alcohol **8** could be realized by converting it with (-)-camphanic acid chloride into the corresponding diastereomeric camphanate esters **9**[16]. Separation of these diastereomers by silica gel column chromatography gave **9L** (46% yield, m.p. 141-141.5°C, $[\alpha]_D^{20}$ +13.3°, c 1, $CHCl_3$) and **9D** (43% yield, m.p. 152.5-153.5°C, $[\alpha]_D^{20}$ -18.8°, c 1, $CHCl_3$) in greater than 98% diastereomeric excess, as gauged by ^{1}H-NMR spectroscopy. Hydrolysis with 0.2N NaOH in dioxane/MeOH/H_2O (14/5/1, v/v/v) of the camphanate-ester groups of the individual diastereomers afforded the enantiomers **8L**[17] (m.p. 73.5-74.5°C, $[\alpha]_D^{20}$ +12.2°, c 1, $CHCl_3$) and **8D** (m.p. 73.5-74.5°C, $[\alpha]_D^{20}$ -12.2°, c 1, $CHCl_3$) in 98% and 99% yield, respectively.

The next stage in the synthesis of the phosphonate analogues **I** and **II** of PtdIns and PtdIns[4]P consisted (see Scheme 2) of the introduction of the phosphotidic acid function[18]. In the first step, the alcohol **8D** was reacted with a slight excess of the new bifunctional phosphonylating agent *bis*(1-[6-trifluoromethyl]benzotriazolyl)methylphosphonate[19] (1.1 eq) in dioxane for 15 min at 20°C to give the putative (1-[6-trifluoromethyl]benzotriazolyl)methylphosphonate intermediate. *In situ* treatment of the latter with benzyl alcohol (2 eq) in the presence of *N*-methylimidazole (5 eq) gave, after 1 h at 20°C, the methylphosphonate **10** (diastereomeric mixture, ratio 1/3; δ_p 31.07 and 32.88 ppm) in an overall yield of 90%. Treatment of the α-lithio methylphosphonate, prepared from one diastereomer of the methylphosphonate **10**[20] (1 mmol) and butyllithium (1 mmol) in THF (10 ml) at -78°C, with 1,2-isopropylidene-*sn*-glycerol 3-trifluoromethanesulfonate **11**[21] (1.05 mmol) at -40°C for 1 h, yielded after work-up and purification, derivative **12**. Hydrolysis of the isopropylidene function of **12** with 0.05N HCl in MeOH gave, after work-up and purification, diol **13** (δ_p 35.36 ppm) in 45% overall yield. Acylation of **13** with palmitoyl chloride (2 eq) and pyridine (2 eq) in CH_2Cl_2 for 2 h at 35°C furnished the fully protected *myo*-inositol phospholipid analogue **14** (δ_p 33.00 ppm) in 84% yield. Cleavage of the *p*-methoxybenzyl group with 2.5% CF_3COOH in CH_2Cl_2 gave the 4-OH-derivative **15** (δ_p 33.12 ppm) in a yield of 86%. Finally, hydrogenolysis (H_2/Pd(OH)$_2$/(EtOAc/EtOH/H_2O=70/25/5, v/v/v)) of compound **15** under pressure for 16 h gave **I**[22], which was isolated as the homogeneous ammonium-salt.

On the other hand, phosphitylation of compound **15** with *N,N*-diisopropyl dibenzyl phosphoramidite[23], in the presence of 1*H*-tetrazole, followed by oxidation of the intermediate phosphite-triester with *t*-BuOOH[24], afforded the fully protected *myo*-inositol phospholipid analogue **16** (δ_p -0.91 and 33.15 ppm) in a yield of 85%. Deprotection of compound **16**, as described above, furnished the ammonium-salt of **II**[22].

Scheme 2



The results presented in this paper nicely illustrate the usefulness of the protected methylphosphonate derivative **10** Thus, the corresponding α-lithio anion of **10** could be readily alkylated to give an easy access to the phosphonate analogues I and II, which are valuable tools to study in detail the inhibition of phospholipase C Furthermore, we believe that the approach described herein promises to be of great value for the future synthesis of other isosteric phosphonate analogues of naturally occurring phosphate esters

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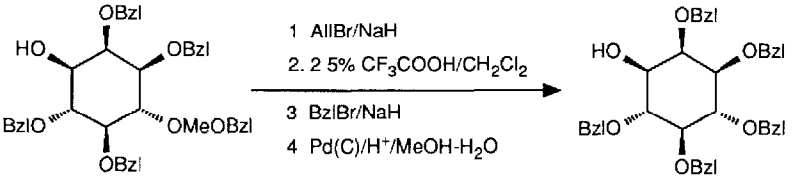
17 The absolute configuration of **8L** was confirmed by its transformation into 2,3,4,5,6-penta-*O*-benzyl-L-*myo*-inositol and comparison of the observed $[\alpha]_D^{20}$-value (+8 8°, c 1, $CHCl_3$) with the reported[9] one (+9 2°, c 3 25, $CHCl_3$)



18 In a preliminary experiment it was found that alkylation of the α-lithio anion of dibenzyl methylphosphonate with triflate **11** afforded, after hydrolysis of the isopropylidene function, subsequent palmitoylation and hydrogenolysis, the corresponding phosphonate analogue of phosphatidic acid in an overall yield of 35%
19 *Bis*(1-[6-trifluoromethyl]benzotriazolyl)methylphosphonate was prepared by dropwise addition of commercially available methylphosphonic dichloride (5 mmol) in dioxane (5 ml) to a cooled solution (0°C) of 1-hydroxy-6-trifluoromethylbenzotriazole (10 mmol) and pyridine (10 mmol) in dioxane (20 ml) After stirring for 1 h at 20°C, the salts were removed by filtration The thus obtained 0 2M stock solution of $(CF_3BtO)_2P(O)CH_3$ could be stored for several weeks at -20°C
20 Separation of the individual diastereomeric methylphosphonate diesters **10** could be accomplished by silica gel column chromatography (elution, *n*-hexane/ethyl acetate, 1/0 to 1/1, v/v. Only the lower-running diastereomer was used to facilitate the interpretation of the ^{1}H- and ^{13}C-NMR spectra.
21 The triflate **11** was prepared starting from commercially available 1,2-isopropylidene-*sn*-glycerol (R W Binkley, M.G Ambrose and D G Hehemann, J Org Chem **45**, 4387 (1980))
22 Compound I ^{31}P-NMR ($CH_2Cl_2/CH_3OH/H_2O$, 70/30/3, v/v/v) δ 27 56 ppm. ^{1}H-NMR ($CDCl_3/CD_3OD/D_2O$, 70/30/3, v/v/v) δ 0 88 (t, 6 H, 2 x CH_3, palmitoyl, J = 7.0 Hz), 1.20-1.46 (m, 48 H, 24 x CH_2, palmitoyl), 1 54-1 78 and 1 82-1 95 (m, 8 H, 2 x H-1 (butyl), 2 x H-2 (butyl) and 2 x C(=O)CH_2CH_2, palmitoyl), 2 30 (t, 2 H, C(=O)CH_2, palmitoyl, J = 7 5 Hz), 2 32 (t, 2 H, C(=O)CH_2, palmitoyl, J = 7 5 Hz), 3 25 (dd, 1 H, H-5 (inositol), $J_{5,6}$ = 9 0 Hz), 3 46 (dd, 1 H, H-3 (inositol), $J_{3,4}$ = 10 0 Hz), 3 63 (dd, 1 H, H-4 (inositol), $J_{4,5}$ = 9 0 Hz), 3 74 (dd, 1 H, H-6 (inositol), $J_{6,1}$ = 10 0 Hz), 4 03 (m, 1 H, H-1 (inositol), $J_{1,2}$ = 2 5 Hz), 4 04 (dd, 1 H, H-4a (butyl), $J_{4a,4b}$ = 12 0 Hz), 4 14 (dd, 1 H, H-2 (inositol), $J_{2,3}$ = 2 5 Hz), 4 29 (dd, 1 H, H-4b (butyl)), 5.13 (m, 1 H, H-3 (butyl), $J_{3,4a}$ = 7 5 Hz, $J_{3,4b}$ = 3 0 Hz).
Compound II· ^{31}P-NMR ($CH_2Cl_2/CH_3OH/H_2O$, 70/30/3, v/v/v) δ 2 51 and 26 99 ppm ^{1}H-NMR ($CDCl_3/CD_3OD/D_2O$, 70/30/3, v/v/v) δ 0 89 (t, 6 H, 2 x CH_3, palmitoyl, J = 7 0 Hz), 1 21-1 31 (m, 48 H, 24 x CH_2, palmitoyl), 1 52-1 80 and 1 83-1.97 (m, 8 H, 2 x H-1 (butyl), 2 x H-2 (butyl) and 2 x C(=O)CH_2CH_2, palmitoyl), 2 30 (t, 2 H, C(=O)CH_2, palmitoyl, J = 8 0 Hz), 2 33 (t, 2 H, C(=O)CH_2, palmitoyl, J = 8 0 Hz), 3 48 (br, 1 H, H-5 (inositol)), 3 67 (br, 1 H, H-3 (inositol)), 3 81 (br, 1 H, H-6 (inositol)), 4 00-4 25 (m, 2 H, H-1 (inositol) and H-2 (inositol)), 4 05 (dd, 1 H, H-4a (butyl), $J_{4a,4b}$ = 12.0 Hz), 4 32 (dd, 1 H, H-4b (butyl)), 4 19 (br, 1 H, H-4 (inositol)), 5 13 (m, 1 H, H-3 (butyl), $J_{3,4a}$ = 7 5 Hz, $J_{3,4b}$ = 3 0 Hz)
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