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Note

Synthesis of acylphosphonyl analogues of phosphatidyl-*myo*-inositol as inhibitors of phosphatidylinositol-specific phospholipase C

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It is now well-known that *myo*-inositol 1,4,5-trisphosphate and diacylglycerol are released upon phosphatidylinositol-specific phospholipase C (PI-PLC) catalysed cleavage of phosphatidylinositol 4,5-bisphosphate after agonist stimulation of several cell surface receptors [1]. The PI-PLC is an ubiquitous enzyme and it is involved in various cellular functions such as signal transduction [2] and cleavage of hydrophobic anchors from membrane proteins [3].

A specific and potent inhibitor of PLC could be a useful pharmacological tool, but to date, the inhibitors found among natural substances are not useful in therapy [4]. Syntheses of analogues of natural *myo*-inositol phosphates and phosphatidyl-*myo*-inositol have been reported, but none of them is a potent and specific inhibitor of PI-PLC [5].

Phosphonates have been used previously in the study of lipases [6] and, recently, the preparation [7] and the effects on the activity [8] of several bacterial PI-specific phospholipase C (PI-PLC) of phosphonate derivatives of *myo*-inositol were reported.

We now describe the synthesis and the inhibitory activity on PI-PLC from human platelets of two acylphosphonates of *myo*-inositol.

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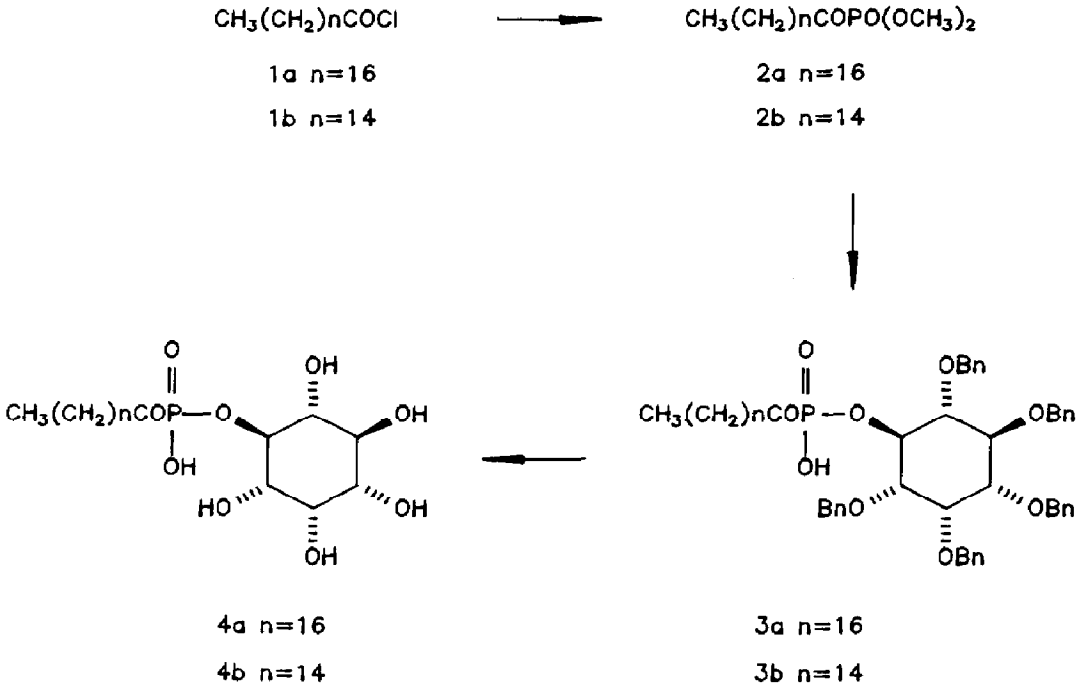

Scheme 1.

The synthetic route to the *myo*-inositol esters of acylphosphonates is shown in Scheme 1. The first step in the synthesis is the formation of a carbon–phosphorus bond. A convenient method to establish such a bond is given by the Arbuzov reaction [9], where trialkyl phosphites are made to react with acyl halides, leading to acylphosphonate diesters.

Dimethyl acylphosphonates were formed by adding dropwise, at room temperature, an equimolar amount of trimethyl phosphite to the corresponding acyl chlorides **1a** and **1b** with yields of 90–95%, respectively. The resulting acylphosphonates **2a** and **2b** were transesterified with trimethylsilyl bromide in dry dichloromethane and, after evaporation of the solvent at reduced pressure, the products were coupled with (±)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (**PBI**) [10] in dry pyridine and triethylamine in the presence of 2,4,6-triisopropylbenzensulfonyl chloride (TPSCl). The products **3a** and **3b** were isolated by gradient elution on a column of silica gel in yields of 21–22%, respectively, and debenzylated using BF_3-etherate in ethanethiol [11] (yields 70–43%, respectively) to give **4a** and **4b**.

The inhibitory activity of **4a** and **4b** on PI-PLC from human platelets was measured under conditions previously described [12]. The two compounds, at a concentration of 10^{-4} M, gave 50% inhibition.

Assuming that the acylphosphonic bond cannot be hydrolysed by PI-PLC, compounds **4a** and **4b** can be assumed to be true inhibitors thus differing from octadecylphosphodithionyl-1-*myo*-inositol [12] and a deoxy analogue of phosphatidylinositol [13]. The former, prepared by us within this program, was found to have similar activity, while the latter was found to be a twenty times less potent inhibitor of PI-PLC than **4a** and **4b** [13].

1. Experimental

General methods.—Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. TLC and column chromatography were performed on silica gel (E. Merck).

The ^{1}H NMR spectra were recorded at 25°C using a Bruker (200 MHz) spectrometer with the appropriate internal standard. IR spectra were recorded for solutions in $CHCl_3$ using a Mattson 3000 FTIR spectometer.

Dimethyl octadecanoylphosphonate (**2a**).—Trimethyl phosphite (0.7 mL, 6 mmol) was added dropwise to octadecanoyl chloride **1a** (2.0 mL, 6 mmol) under N_2. After 3 h at room temperature, TLC ($CHCl_3$) showed the absence of **1a**. The mixture was concentrated under vacuum to give a waxy product **2a** (2.26 g, 95%) which was used without further purification; ν_{max} 2930 (m), 2857 (m), 1697 (m), 1465 (w), 1260 (m), 1183 (w), 1038 (s), 838 (w) cm^{-1}. ^{1}H NMR data (200 MHz, $CDCl_3$): δ 3.85 (d, 6 H, J 10.7 Hz, OCH_3), 2.81 (t, 2 H, J 7.1 Hz, CH_2CO), 1.61 (t, 2 H, J 7.1 Hz, CH_2CH_2CO), 1.24 (s, 28 H, $CH_3(CH_2)_{14}$), 0.87 (t, 3 H, J 6.5 Hz, CH_3). Anal. Calcd for $C_{20}H_{41}O_4P$: C, 63.80; H, 10.98; P, 8.23. Found: C, 63.92; H, 11.00; P, 8.38.

Dimethyl hexadecanoylphosphonate (**2b**).—Using the same conditions as described for **2a**, **1b** (1.82 mL, 6 mmol) was treated with trimethyl phosphite (0.7 mL, 6 mmol) to give the waxy product **2b** (2.05 g, 98%) which was used without further purification; ν_{max} 2925 (m), 2854 (m), 1698 (m), 1464 (w), 1258 (m), 1183 (w), 1038 (s), 840 (w) cm^{-1}. ^{1}H NMR data (200 MHz, $CDCl_3$): δ 3.81 (d, 6 H, J 10.7 Hz, OCH_3), 2.77 (t, 2 H, J 7.2 Hz, CH_2CO), 1.59 (t, 2 H, J 7.0 Hz, CH_2CH_2CO), 1.22 (s, 24 H, $CH_3(CH_2)_{12}$), 0.85 (t, 3 H, J 6.5 Hz, CH_3). Anal. Calcd for $C_{18}H_{37}O_4P$: C, 62.04; H, 10.70; P, 8.89. Found: C, 62.02; H, 10.66; P, 8.90.

1-O-*octadecanoylphosphonyl-2,3,4,5,6-penta*-O-*benzyl*-myo-*inositol* (**3a**).—Bromotrimethylsilane (1.95 mL, 15 mmol) was added dropwise to a solution of **2a** (2.26 g, 5.7 mmol) in dry CH_2Cl_2 (10 mL) under N_2. After 1 h at room temperature, TLC (65:35:5 $CHCl_3$–CH_3OH–NH_4OH) showed the complete disappearance of **2a** (R_f 0.9). The solution was concentrated and the oily residue treated with dry pyridine (25 mL) and dry triethylamine (4.8 mL). Then (±)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (3.29 g, 5.22 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (5.7 g, 18.8 mmol) were added, and the mixture was stirred at room temperature under N_2. After 24 h, water (2 mL) was added to the mixture and, after concentration under reduced pressure, dry Et_2O was added. The resulting precipitate was filtered and the filtrate was concentrated to give an oily dark red residue which was chromatographed on SiO_2 eluting with $CHCl_3$ and 98:2 and 95:5 $CHCl_3$–CH_3OH to give **3a** as a syrup (1.88 g, 21%). ^{1}H NMR data (200 MHz, $CDCl_3$): δ 7.26 (m, 25 H, 5 Ph), 4.62 (m, 12 H, 5 CH_2Ph + H-1 + H-2), 4.35 (t, 1 H, J 10 Hz, H-6), 4.20 (t, 1 H, J 10 Hz, H-4), 3.53 (m, 2 H, H-5 + H-3), 2.85 (t, 2 H, J 10 Hz, CH_2CO), 1.26 (s, 30 H, $CH_3(CH_2)_{15}$), 0.86 (t, 3 H, J 10 Hz CH_3). Anal. Calcd for $C_{66}H_{84}O_9P$: C, 75.33; H, 8.05; P, 2.94. Found: C, 75.31; H, 8.06; P, 2.90.

1-O-*hexadecanoylphosphonyl-2,3,4,5,6-penta*-O-*benzyl*-myo-*inositol* (**3b**).—Using the conditions described for **3a**, **2b** (0.992 g, 2.85 mmol) was treated with bromotrimethylsilane (0.98 mL, 7.5 mmol) and then with (±)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (1.645 g, 2.61 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (2.85 g, 9.4 mmol) to give **3b**

as a syrup (504 mg, 0.54 mmol; 22%). ^{1}H NMR data (200 MHz, $CDCl_3$): δ 7.28 (m, 25 H, 5 Ph), 4.75 (m, 12 H, 5 CH_2Ph + H-1 + H-2), 4.17 (t, 1 H, *J* 10 Hz, H-6), 3.78 (m, 1 H, H-4), 3.42 (m, 2 H, H-5 + H-3), 2.70 (t, 2 H, *J* 12 Hz, CH_2CO), 1.23 (s, 26 H, $CH_3(CH_2)_{13}$), 0.85 (t, 3 H, *J* 12 Hz, CH_3). Anal. Calcd for $C_{64}H_{80}O_9P$: C, 75.05; H, 7.87; P, 3.02. Found: C, 75.02; H, 7.86; P, 3.00.

1-O-*octadecanoylphosphonyl*-myo-*inositol* (**4a**).—To a solution of **3a** (340 mg, 0.354 mmol) in dry CH_2Cl_2 (7 mL) ethanethiol (6.86 mL) and boron trifluoride etherate (1.4 mL) were added dropwise at room temperature under N_2. After 1 h, water was added and the aqueous phase was washed repeatly with Et_2O and lyophilized to give a solid residue which was purified by chromatography using 65:35:5 $CHCl_3$–CH_3OH–H_2O as eluent to give **4a** (126 mg, 0.247 mmol; 70%) as a white solid; mp 244–246°C (from CH_3OH–H_2O); ν_{max} 3360 (s, br), 2922 (s), 2850 (s), 1750 (w), 1189 (m), 1042 (m) cm^{-1}. ^{1}H NMR data (200 MHz, $CDCl_3$): δ 4.57–4.44 (m, 5 H, 5 OH), 3.92 (t, 1 H, J 3 Hz, H-2), 3.82 (m, 1 H, H-1), 3.45 (m, 2 H, H-6 + H-4), 2.74 (m, 2 H, CH_2CO), 1.59 (s, 30 H, $CH_3(CH_2)_{15}$), 0.87 (t, 3 H, J 10 Hz CH_3). Anal. Calcd for $C_{24}H_{47}O_9P$: C, 56.45; H, 9.28; P, 6.07. Found: C, 56.44; H, 9.31; P, 6.11.

1-O-*hexadecanoylphosphonyl*-myo-*inositol* (**4b**).—Using the conditions described for **4a**, **3b** (439 mg, 0.53 mmol) was treated with ethanethiol (10.2 mL) and boron trifluoride etherate (2.1 mL) to give **4b** as a white solid, further crystallised from CH_3OH–H_2O to give 110 mg (0.23 mmol, 43%) of **4b**; mp 236–238°C. ν_{max} 3360 (s, br), 2920 (s), 2852 (s), 1748 (w), 1184 (m), 1036 (m) cm^{-1}. ^{1}H NMR data (200 MHz, $CDCl_3$): δ 4.49 (m, 5 H, 5 OH), 3.93 (t, 1 H, *J* 2 Hz, H-2), 3.67–3.52 (m, 2 H, H-1 + H-6), 3.36 (m, 1 H, H-4), 3.10-2.88 (m, 4 H, H-5 + H-3 + CH_2CO), 1.24 (s, 26 H, $CH_3(CH_2)_{13}$), 0.85 (t, 3 H, *J* 8 Hz, CH_3). Anal. Calcd for $C_{22}H_{43}O_9P$: C, 54.76; H, 8.98; P, 6.42. Found: C, 54.80; H, 9.01; P, 6.40.

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