Synthesis and Properties of New Bisphosphatidylcholine Lipids

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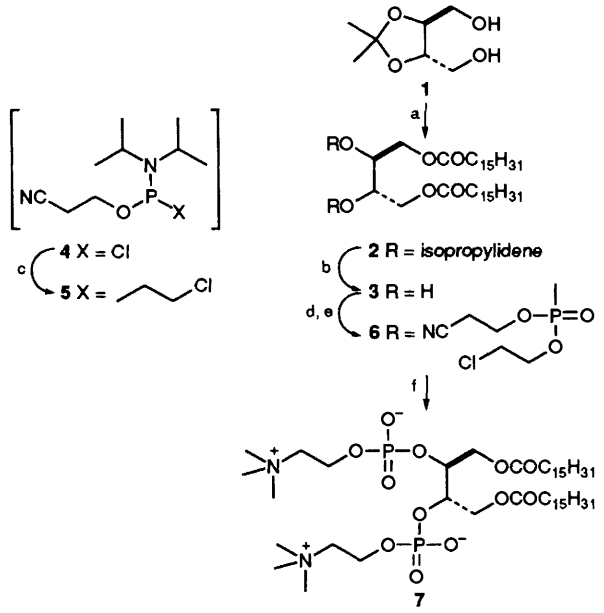
The synthesis of novel bisphosphatidylcholine lipids using a phosphoramidite coupling scheme is reported.

Phospholipases A_2 (PLA_2) are enzymes that selectively hydrolyse the fatty acid ester at the *sn*-2 position of membrane diacylphospholipids. They are believed to be an important factor in the release of arachidonic acid and subsequent formation of eicosanoid pro-inflammatory mediators. Inhibition of these enzymes has recently been studied in detail in the context of PLA_2 being a pharmacological target.[1]

Recent X-ray crystal structures of a secretory PLA_2 (s-PLA_2) have been helpful in explaining the catalytic mechanism of this enzyme-PLA_2, particularly the active site chemistry.[2] Some mechanistic details of s-PLA_2 remain unclear, especially those related to the s-PLA_2 'lipid interface-activation'[3,4] phenomenon. This activation makes PLA_2 much more active at the lipid–water interface than in solution. Given that the lipid–water interface is important for the expression of full catalytic activity, our phospholipid kinetic inhibition studies, using lipid vesicles made up with varying ratios of the non-hydrolysable (+)-phosphatidylcholine (*sn*-1-PC) and its naturally occurring (−)-enantiomer (*sn*-3-PC) suggest that the simultaneous binding of *two* neighbouring phospholipids is a requisite for interfacial activation.[5] This conclusion is further supported by the fact that natural bisphosphatidylglycerols (*i.e.* cardiolipins) are substrates for the cobra s-PLA_2.[6] In order to study this 'dual phospholipid binding requirement,' we have synthesized and measured some physical properties of bisphosphatidylcholine lipids **7** and **8** (Schemes 1 and 2). We also believe that kinetic studies of s-PLA_2 hydrolysis of these new bisphosphatidylcholine lipids may provide information regarding the interfacial activation mechanism.

Because bisphosphatidylcholine lipids **7** and **8** are not glycerol esters, hemisynthetic strategies are not viable. We have prepared compounds **7** and **8** by a total synthesis method involving mild phosphorylation conditions based on a phosphoramidite coupling.[7] This technology allows for the total control of the hetero-bis-esterification of the phosphate group and avoids formation of a cyclic compound[8] and acyl migration. The synthetic strategy permits a large choice of functionalized fatty acid esters including those with double bonds. Control of the stereocentres is facile and depends on the configuration of the starting product, (*i.e.* L-tartaric acid or L-threitol for **7** and D-mannitol for **8**).

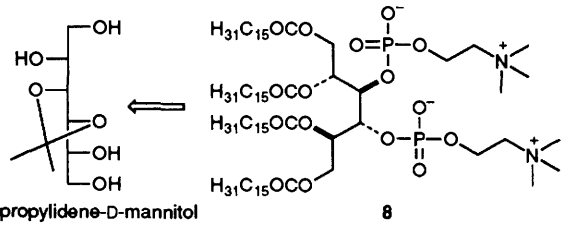


Scheme 1 *Reagents and conditions*: a, palmitic acid, DCC, DMAP, CH_2Cl_2, 20 °C, 16 h; 96%; b, 80% TFA, 20 °C, 1 h; 95%; c, 2-chloroethanol, Et_3N, THF, 20 °C, 1 h; d, **5**, tetrazole, THF, 20 °C, 16 h; e, 30% H_2O_2; 73%; Me_3N, MeCN–$CHCl_3$, NaI, 65 °C, 24 h; 55%

Bis-esterification of the 2,3-isopropylidene-L-threitol **1** with palmitic acid using dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) afforded the diester **2** in nearly 100% yield. The deprotection of the isopropylidene group of **2** is best performed in 80% aqueous trifluoroacetic acid (TFA) at 20 °C. Product **3** readily precipitates to give high yield without measurable acyl chain migration. The phosphorylation step requires the prior formation of the dialkyl phosphoramidite **5**. Thus, 2-cyanoethyl (*N,N*-diisopropylamino)chlorophosphinite **4** was condensed with choroethanol in tetrahydrofuran (THF) with triethylamine (Et_3N) to give **5** as a crude oil. A solution of **5** was immediately prepared in dry THF containing tetrazole, to which a solution of diol **3** in THF was added. After 16 h, an excess of hydrogen peroxide was added and the neutral phosphate triester **6** was readily isolated using silica gel column chromatography (73%). No isomerized product could be detected (TLC, 300 MHz ^1H NMR) and the formation of a cyclic phosphate ester from the vicinal diol groups in **3** is practically eliminated. Finally, complete deprotection was accomplished by treatment of **6** with trimethylamine at 65 °C in a pressure bottle containing chloroform and acetonitrile. Despite its high polarity, **7** was isolated and purified by silica gel column chromatography, using an eluent composed of methanol–choroform (1:2) and enough water to achieve saturation (solvent A). The diester **7**† was obtained as a white solid in 55% yield.

Using the same synthetic strategy, we were able to prepare 'bisglycerophospholipid' **8**‡ starting from 3,4-*O*-isopropylidene-D-mannitol (Scheme 2) in 20% overall yield. Other bisphosphatidylcholine lipids are presently being synthesized and their properties as PLA_2 substrates as well as full experimental details will be part of a forthcoming paper.

The monolayer forming properties of **8** were studied using the film balance technique (KSV, model 3000) using MilliQ water (25 °C) as the subphase. The isotherm for this sample exhibits features of stable phospholipid monolayers including a high collapse pressure and a mean molecular area of *ca.* 2 diacylphospholipids. This stability is particularly noteworthy because it suggests that **8** packs well into a lamellar form. Sonicated dispersions of **8** are able to entrap carboxyfluorescein dye,[9] suggesting that closed structures such as vesicles form under the same conditions used to form vesicles with diacylphospholipids. Compound **7** on the other hand is very



Scheme 2 Retrosynthetic pathway for the synthesis of the bisglycerophospholipid **8**

water soluble and probably aggregates in a micellar form; sonicated dispersions do not entrap carboxyfluorescein. An aqueous dispersion of **8** also exhibits phase transitions characteristic of conventional phospholipids. As measured by turbidity,[10] we find that **8** has a pretransition (49 °C) and a main transition (53 °C). These temperatures are about 10 °C greater than those of the related species, dipalmitoylphosphatidylcholine (DPPC). Evidently, linking the two glycerol backbones provides some added degree of stabilization to the solid-like gel phase. This class of bisphosphatidylcholine lipids therefore proves to be interesting in its relationship to diacylphospholipids.

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Footnotes

† TLC solvent A, R_f 0.16; $[\alpha]_D^{23}$ +7.5 (*c* 0.0302, $CDCl_3$); ^1H NMR (499.84 MHz, $CDCl_3$—MeOD, 2 : 1, MeOD reference) δ 0.84 (t, 6 H, *J* 6.9 Hz), 1.22 (s br, 48 H), 1.55 m, 4 H), 2.28 (t, 4 H, *J* 7.6 Hz), 3.18 (s 18 H, Me_3N^+), 3.58 (m, 4 H, CH_2N^+), 4.18 (m, 2 H, 2-, 3-H), 4.20–4.38 (m, 4 H, $CH_2OPO_3^-$), 4.44 (d br, 4 H, *J* 9.5 Hz, 1-, 4-H); ^{31}P NMR (81.0 MHz, $CDCl_3$–MeOD, 2 : 1) δ 0.53; MS (FAB$^+$, NBA) *m/z* (%) 951 (11) $[M + Na]^+$, 929 (100) $[MH]^+$, 871 (25), 870 (38) $[MH - (CH_3)_3N]^+$, 746 (23) $[M - C_5H_{13}NO_4P]^+$, 588 (15), 490 (10).

‡ TLC solvent A, R_f 0.26; $[\alpha]_D^{23}$ + 14.9 (*c* 0.0198, $CDCl_3$); ^1H NMR (200.0 MHz, $CDCl_3$–MeOD, 2 : 1, MeOD reference) δ 0.89 (t, 12 H, *J* 6.5 Hz), 1.27 (s br, 96 H), 1.55–1.68 (m, 8 H), 2.28 (t, 4 H, *J* 7.5 Hz, 2″-H), 2.40 (m, 4 H, 2′-H), 3.24 (s, 18 H, Me_3N^+), 3.62–3.75 (m, 4 H, CH_2N^+), 4.13 (dd, 2 H, J_1 12.1, J_2 8.9 Hz, 1/2 1-, 6-H), 4.26–4.47 (m, 6 H, $CH_2OPO_3^-$ and 1/2 1-, 6-H), 4.75 (dd, 2 H, J_1 12.3, J_2 2.1 Hz, 3, 4-H), 5.30 (m, 2 H, 2-, 5-H); ^{31}P NMR (81.0 MHz, $CDCl_3$–MeOD, 2 : 1) δ 0.97; MS (FAB$^+$, NBA) *m/z* (%) 1488 (9) $[M + Na]^+$, 1466 (20) $[MH]^+$, 1283 (31) $[M - C_5H_{13}NO_4P]^+$.

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