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### Synthesis of Competitive Inhibitors of Phospholipase A 2 (PLA 2 )

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## SYNTHESIS OF COMPETITIVE INHIBITORS OF PHOSPHOLIPASE A<sub>2</sub> (PLA<sub>2</sub>)

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*The synthetic routes for preparation of several phospholipid analogues such as lithium alkyl-3-alkoxy allylvinyl-sn-glycerol **5** and **6** and lithium trans-2-amidomethyl phosphate **7** and lithium cholesterol ester phosphate **8** containing ether, amide, and phosphate ester moieties are described.*

**Keywords:** Inhibitor; phospholipase A<sub>2</sub> (PLA<sub>2</sub>); phospholipid

### INTRODUCTION

This article focuses on the synthesis of inhibitor for the class of 14-KDa secreted phospholipase A<sub>2</sub> (PLA<sub>2</sub>)<sup>1–4</sup> which catalyzes the hydrolysis of the sn-2-acyl chain of naturally occurring phospholipids **1** at the 2-position of the glycerol backbone into a 1-acyllysophospholipids **2** and fatty acids; it is one of the best examples of heterogeneous interfacial catalysts in biological systems (see Figure 1).<sup>5–10</sup>

The venom of poisonous snakes contains this class of enzymes known as phospholipases, enzymes that cause the breakdown of phospholipids. Venom of the Indian cobra (Nag naja), which kills several thousand people each year, and the eastern diamondback rattlesnake (*Crotalus adamanteus*) both among other things contain phospholipase A<sub>2</sub>. Lysolecithin, the phospholipid breakdown product of this reaction, acts as a detergent and dissolves the membranes of red blood cells.<sup>11</sup>

Various forms of PLA<sub>2</sub> are distributed widely, and they are abundant in pancreatic digestive fluid, venoms, and inflammatory exudates. Recently, several phospholipid analogues<sup>12</sup> (e.g., **3** and **4**)<sup>13</sup> containing

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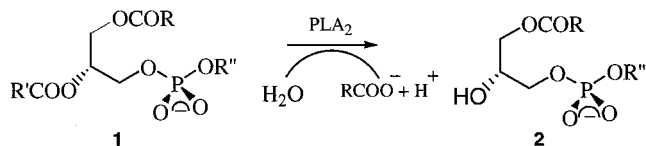


FIGURE 1

a phosphonate or amide function in place of the ester at the 2-position of the glycerol backbone were synthesized (Figure 2).<sup>14,15</sup>

These compounds were shown as inhibitors of the phospholipase A<sub>2</sub> enzyme. As an extension to the study of **3** and **4**, compounds **5–8** containing phosphate and amide moieties were synthesized (Figure 3).

## RESULTS AND DISCUSSION

The basic procedure for the synthesis of the lithium glycerol-sn-2-phosphoesters **5**, **6** is outlined in Scheme 1, and for lithium phosphodimethyl ester amide **7** and lithium cholesterol phosphate ester **8** are outlined in (Schemes 2 and 3) respectively.<sup>16–18</sup>

In all cases the isolated yields were over 50%, however no attempt was made to optimize the synthetic procedures. The structure of the intermediates and the final products were consistent with their Bruker 250 MHz <sup>1</sup>H NMR (CDCl<sub>3</sub>). The mass spectra were run on Nicolet FT-2000. All reagents and chromatographic silica gel (60 Å, 230–400 mesh) were from Aldrich. TLC plates were from Analtech.

## EXPERIMENTAL SECTION

### Preparation of 1-Allyloxy-3-octyloxy-propan-2-ol (12a) and 1-Allyloxy-3-octadecyloxy-propan-2-ol (12b)

To a solution of 20 mmol vinyl allyl ether epoxide **9** and under stream of nitrogen was added 22 mmol alcohol **10** or **11** in dry distilled methylene chloride (30 ml) in round bottom flask were added 5 drops of BF<sub>3</sub> in diethyl ether (Fluka, distilled). The solvent was removed and the residue

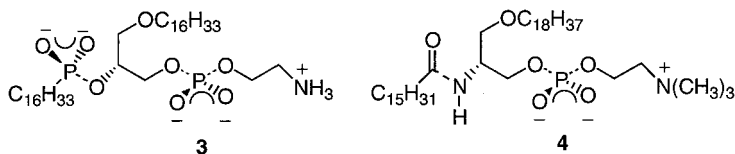


FIGURE 2

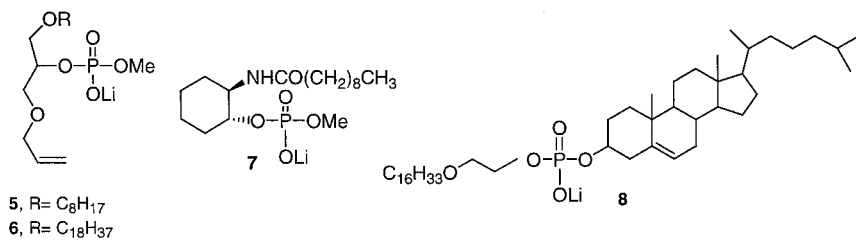
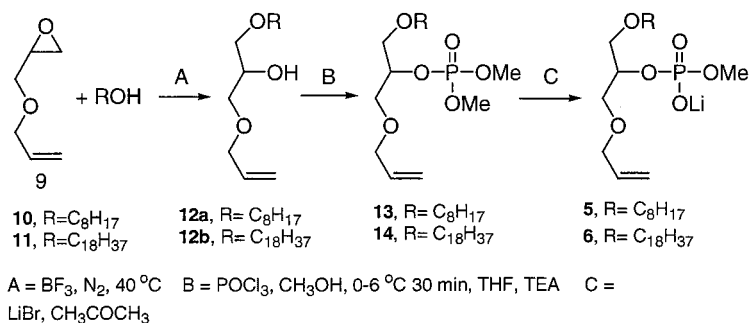
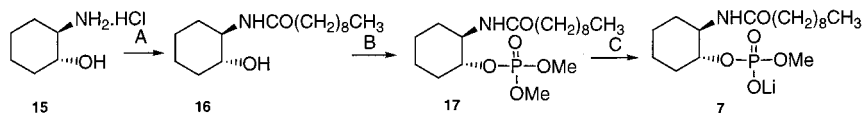


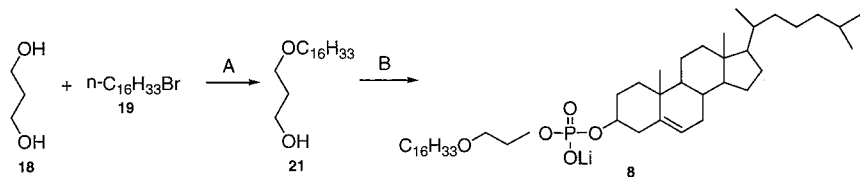
FIGURE 3



SCHEME 1



SCHEME 2



A = (CH<sub>3</sub>)<sub>3</sub>COK, tert-amylalcohol    20    B = 1) POCl<sub>3</sub>, THF, 2) 5,6-cholesten-3-ol 22 3) LiBr, acetone

SCHEME 3

was pumped. The crude material was taken on column of silicagel, compounds **12a** and **12b** were eluted with mixture of EtOAc: ligroin (7:93 v/v), and latter with EtOAc: ligroin (20:80 v/v), and finally with CHCl<sub>3</sub>: ether (90:10 v/v) 55 fractions were recovered, fractions 37–52 were pure product. MS for **12a** =  $m/z$  244 (M<sup>+</sup>), for **12b** =  $m/z$  384 (M<sup>+</sup>).

### Preparation of Phosphoric Acid 1-Allyloxymethyl-2-octadecyloxy-ethyl Ester Dimethyl Ester (**14**) (the Same Procedure Could be Applied for Preparation of (**13**))

To a three-neck round bottom flask equipped with an addition funnel and drying tube, at 0–6°C (ice bath) was added 0.34 ml (3.6 mmol, 1.2 eq) of POCl<sub>3</sub> (Aldrich freshly distilled). To this from addition funnel was added dropwise solution of 1.16 g (3 mmol) secondary alcohol **12** (**12a** = 1-Allyloxy-3-octyloxy-propan-2-ol or **12b** = 1-Allyloxy-3-octadecyloxy-propan-2-ol and 1 ml (2.4 eq, 7.2 mmol,  $d = 0.726$ ) three ethyl amine (TEA) in 15 ml THF dropwise added, the temperature was kept at 0°C, and allowed to react for 30 min. After completion of addition, the reaction mixture was allowed to warm up to r.t. for 48 h, then heated to 60°C for 30 min. The TLC was run, and to this was added 10 ml brine and the resulting solution extracted with 2 × 15 ml hexane. The hexane layer was combined and dried over MgSO<sub>4</sub>. After removal of solvent, the weight of residue was 1.07 g; TLC showed only one spot. MS for **13** =  $m/z$  352 (M<sup>+</sup>), for **14**  $m/z$  = 492 (M<sup>+</sup>).

### Preparation of Lithium Salt of (**5**) and (**6**)

To a 25 ml round-bottom flask was added 1.07 g (2.17 mmol) phosphodimethyl ester and 0.246 g (2.82 mmol, 1.3 eq) LiBr and 10 ml reagent grade acetone. The reaction was heated to reflux overnight. A white solid precipitated on cooling to –20°C, was filtered and washed with cold acetone; if no solid precipitated, the mixture was concentrated and the residue was chromatographed on silicagel. 0.9 g of crude compound was taken on 25 g silicagel column. The solvent used was MeOH:CHCl<sub>3</sub>:NH<sub>4</sub>OH (10:88:2), fractions 27–32 (each collection 10 ml) gave a white crystal weighing 0.6 g.

<sup>1</sup>H NMR compound **5**: 0.8 ppm (t, 3H,  $J = 7$  Hz), 1.3 ppm (s, 12H), 1.48 ppm (b, 1H), 3.6 ppm (m, 7H), 4.1 ppm (d, 3H,  $J = 7$  Hz), 4.4 ppm (b, 1H), 5.1–5.3 ppm (dd, 2H,  $J = 25$ ), 5.9 ppm (m, 1H), MS:  $m/z$  344 (M<sup>+</sup>), Anal. Calcd for C<sub>15</sub>H<sub>30</sub>O<sub>6</sub>PLi: C, 52.33, H, 8.78 Found, C, 52.51, H, 8.61.

<sup>1</sup>H NMR of compound **6**: 0.9 ppm (t, 5H,  $J = 7$  Hz), 1.21 ppm (s, 13H), 1.55 ppm (m, 2H), 3.57 ppm (m, 20H), 4 ppm (d, 9H,  $J = 13$  Hz), 5.16–5.36 ppm (dd, 2H,  $J = 25$  Hz), 5.8–6 ppm (m, 1H), MS:  $m/z$

484 (M<sup>+</sup>). Anal. Calcd. for C<sub>25</sub>H<sub>50</sub>O<sub>6</sub>PLi: C, 61.97, H, 10.40, Found, C, 61.89; H, 10.51.

### Preparation of Decanoic Acid (Trans-2-hydroxy-cyclohexyl)-amide (16)

To a 100 ml round-bottom flask and under stream of nitrogen was added 1g (6.59 mmol) of mainly trans-2-aminocyclohexanol hydrochloride. To this was added 10 ml water and 3.7 ml (4 eq, 26.36 mmol) of three ethyl amine. The solution was stirred, to this was added a solution of and 1.3 eq (0.9 eq, 5.9 mmol) of decanoyl chloride (98%, d = 0.919) in 10 ml THF. The solution was stirred at r.t. overnight.

The reaction was monitored by TLC, after completion the crude product was recrystallized from water and MeOH. The weight of pure product after two days of vacuum drying was 1.8 g.

<sup>1</sup>H NMR (250 MHz): 0.94 ppm (t, 3H, J = 7 Hz), 1.17 ppm (s, 16H), 1.58–1.8 ppm (m, 5H), 1.8–2.2 ppm (m, 2H), 2.18–2.4 (m, 1H), 3.2–3.4 (m, 2H), 3.58–3.8 (b, 1H, NH), 5.4 (b, 1H, OH). MS: m/z 269 (M<sup>+</sup>).

### Preparation of Trans-Phosphoric Acid-2-decanoylamino-cyclohexyl Ester Dimethyl Ester (17)

To a three-neck round-bottom flask equipped with a dropping funnel and CaCl<sub>2</sub> tube at 0°C was added 0.7 ml (7.4 mmol, 1.2 eq) of POCl<sub>3</sub> (Aldrich, freshly distilled). To this was added solution of 1.65 g (6.12 mmol) trans-amidocyclohexanol in 15 ml THF (dry and freshly distilled). The solution was cooled to 0°C and 2.5 ml (17.76 mmol ~ 2.8 eq) three ethylamine added dropwise over 1 h, and the solution allowed to stay at 0°C for ~4 h. The progress of reaction was monitored by TLC. After completion of reaction excess of MeOH and 1.75 ml (12.43 mmol) three ethylamine was added. The reaction mixture was allowed to warm to r.t. and then heated to 60°C for ~30 min. The reaction was then cooled to r.t. to this was added 10 ml brine and the mixture extracted with hexane. The hexane extract was dried and taken on column of silica gel. The weight of pure **17** was 0.56 g. TLC showed one spot. <sup>1</sup>H NMR (250 MHz): 0.95 ppm (t, 3H J = 7 Hz), 1.18 ppm (s, 16H), 1.59–1.8 ppm (m, 5H), 1.8–2.3 ppm (m, 2H), 2.18–2.4 (m, 1H), 3.2–3.4 ppm (m, 2H), 3.68 ppm (d, 6H), 5.4 ppm (b, 1H, OH), MS: m/z 377 (M<sup>+</sup>).

### Preparation of Lithium Salt of Trans-Phosphoric Acid 2-Decanoylamino-cyclohexyl Ester Methyl Ester (7)

To a 30 ml round-bottom flask was added 0.58 g (1.53 mmol) of phosphodimethyl ester amide and 0.16 g (1.2 eq, 1.85 mmol) LiBr and 10 ml

acetone (reagent grade). The flask was equipped with  $\text{CaCl}_2$  tube and heated to reflux for 6 h. After this time the solution changed to cloudy. The solution was cooled to  $-20^\circ\text{C}$  and was filtered. The filtrate was washed with acetone. The TLC showed only one spot. The weight of final compound was 0.28 g.  $^1\text{H}$  NMR (250 MHz): 0.94 ppm (t, 3H,  $J = 7$  Hz), 1.18 ppm (s, 16H), 1.6–1.8 ppm (m, 5H), 1.8–2.25 ppm (m, 2H), 2.18–2.4 (m, 1H), 3.2–3.4 ppm (m, 2H), 3.63 ppm (d, 3H), 5.2 (b, 1H, OH), MS:  $m/z$  369 ( $\text{M}^+$ ). Anal. Calcd. C, 55.25; H, 9.01, Found C, 55.51; H, 9.14.

### Preparation of 3-Hexadecyloxy-propan-1-ol Ether (21)

To a 250 ml three-neck round-bottom flask was added 9.2 g (120 mmol, 3 eq) of 1,3-propanediol and 50 ml tert-amylalcohol 99% and 7.01 g (60 mmol, 95%) potassium tert-butoxide. The flask was equipped with reflux condenser and addition funnel. The reaction run under  $\text{CaCl}_2$ , and heated to reflux. To this from addition funnel was added a solution of 12.2 g (12.21 ml,  $d = 0.999$ ) 1-bromohexadecane in 50 ml THF dropwise over period of 3 h. The reaction mixture was stirred for 50 h. The reaction mixture was cooled to r.t. and poured into a 50 ml  $\text{H}_2\text{O}$  and the water phase was acidified with 10% HCl. To the mixture was added 100 ml hexane and the organic phase was separated and to the aqueous phase was extracted with additional 100 ml hexane. The organic layers were combined and dried over  $\text{MgSO}_4$ . The solvent was removed. The weight of residue after vacuum drying was 10 g. The crude compound was recrystallized from pentane. The yield of pure product was 58%.  $^1\text{H}$  NMR: 0.88 ppm (t, 3H,  $J = 7$  Hz), 1.28 ppm (s, 28H), 1.8–2 (pentate, 2H,  $J = 7$  Hz), 2.23 ppm (b, 1H), 3.4 ppm (t, 2H,  $J = 7$  Hz), 3.6 (t, 2H,  $J = 7$  Hz), 3.8 (t, 2H,  $J = 7$  Hz).

$^1\text{H}$  NMR of proton decoupling of **21**: 0.85 ppm (t, 3H,  $J = 7$  Hz), 1.3 ppm (s, 28H), 3.4 ppm (t, 3H), 3.63 ppm (s, 2H), 3.93 ppm (s, 2H). MS:  $m/z$  300 ( $\text{M}^+$ ).

### Preparation of (8), Lithium Salt of Phosphoric Acid 17(1,5-Dimethyl-hexyl)-10, 13-Dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl] 2-Hexadecyloxy-ethyl ester

A three-neck round-bottom flask was equipped with addition funnel and  $\text{CaCl}_2$  drying tube. To the addition funnel was added 1.9 g (6.32 mmol) of hexadecanol ether **21** (purified by recrystallization), and 20 ml THF dry over  $\text{CaH}_2$ , NaOH) and 1.33 ml (1.5 eq, 9.4 mmol) triethyl

amine. This solution was added to the 0°C solution of 0.65 ml POCl<sub>3</sub> (redistilled, 7 mmol) dropwise over period of 45 min and allowed to stay at this condition for 210 min. The process of reaction was monitored by TLC. After completion of reaction to this was added solution of 2.56 g (1.2 eq, 6.95 mmol) of cholesterol (5,6-cholesten-3-ol) **22** in 25 ml dry THF over 30 min at the same temperature. The reaction allowed to stir while warming to r.t. overnight. Next day the reaction was stopped and to this was added 10 ml NaOH 10% and 10 ml H<sub>2</sub>O. The reaction mixture was heated to 60°C for 2 min, and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The CH<sub>2</sub>Cl<sub>2</sub> layer was separated. <sup>1</sup>H NMR of 8: 0.67 ppm (s, 3H), 0.87 ppm (m, 19H), 1–1.6 ppm (m, 46H), 1.6–2.18 ppm (b, 1H). (m, 6H), 2.2–2.4 ppm (m, 4H), 3.36–3.48 ppm (tt, 3H, J = 7 Hz), 3.93 ppm (bd, 2H). MS: m/z 754 (M<sup>+</sup>), Exact mass: Calcd. 754.6216; Foud, 754.6211.

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