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# Design and Synthesis of Conformationally Restricted Phospholipids as Phospholipase A2 Inhibitors

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The use of conformationally restricted phospholipids **1** and **2** has been employed to understand the conformational preference of phospholipase A2 (PLA2) for substrate phospholipids. Inhibition of porcine pancreatic PLA2 with **1** and **2** indicated a two- to fivefold preference for the distal isomer **2** over the proximal isomer **1.** Based upon these studies, both side-chains of the substrate phospholipid appear to occupy the lipid binding domains near the active site with the side-chains further apart most preferred by PLA2.

**Key words: PLA2 substrate conformation, modeling, active-site inhibitors, phospholipid side chain orientation, pancreatic PLA2**

Phospholipase A2 (PLA2) is a hydrolase responsible for the release of fatty acids from the two position of membrane phospholipids [1]. In particular, the liberation of arachidonic acid, a mediator of inflammation, is regulated by PLA2 [1–3]. The ability to specifically inhibit PLA2 has been the focus of several labs [4–6] for the potential discovery of anti-inflammatories.

To further understand the mode of substrate-PLA2 binding, efforts have been directed at using phospholipids as probes. In earlier work by deHaas and coworkers [7], the minimum substrate requirement for PLA2 was shown to be the acylester glycolphosphorylcholine. This discovery led to the preparation of a variety of alkylphosphorylcholines [8–12], which were shown to protect the active site $His^{48}$ from alkylation by *para*-bromophenacyl bromide, a histidine alkylating reagent. Extending this observation to the triglyceride phosphorylcholines [13–18] demonstrated the relative importance of the upper side chain for active site phospholipid binding [17,18]. Typically, greater PLA2 inhibition was observed when the upper side-chain length was extended. These results suggested the upper side-chain orients the triglyceride to afford optimum substrate binding to PLA2. This is consistent with the lower $K_M$ associated with triglyceride phospholipid over the glycol phospholipid [8–12]. Recent studies [19,20] aimed at the preparation of potential transition-state analogs have resulted in the preparation of glycol and triglyceride phosphorylcholines with difluoroketone substitution of cleavage site.

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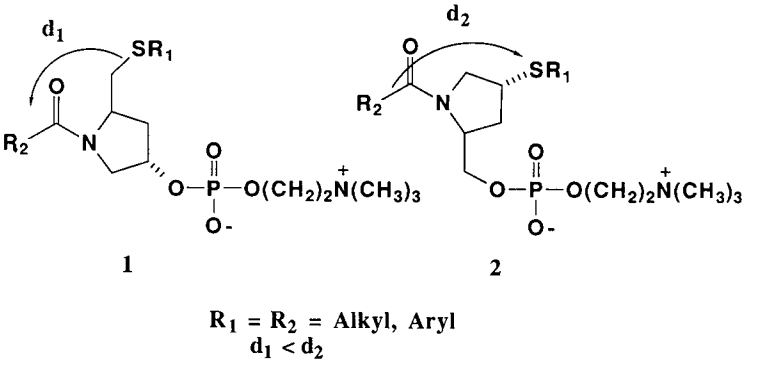



Fig. 1. Conformationally restricted phospholipids **1** (proximal) and **2** (distal).

Central to these approaches has been the retention of the phosphorylcholine moiety with several mono- and diacylglyceride modifications. Only recently [21–25] has a study been performed on a conformationally restricted triglyceride phospholipid substrate. This approach restricts the inherent flexibility of the substrate phospholipid which permits PLA2 to select the preferred side-chain orientation. In this fashion, the preferred orientation of the side-chains by PLA2 could be evaluated. To test this approach further, we have targeted the conformationally restricted phospholipids **1** and **2** (Fig. 1). These targets have the phosphorylcholine anchor along with the two alkylsubstituents to permit the enzymatic evaluation of the different side-chain substitution patterns. The principal difference between **1** and **2** is the distance $\mathbf{d_1}$ and $\mathbf{d_2}$ between both side-chains. Since $\mathbf{d_1}$ is much smaller than $\mathbf{d_2}$, the conformationally restricted phospholipid **1** will be called the proximal isomer while the phospholipid **2** will be called the distal isomer.

## MATERIALS AND METHODS

### Synthesis of Conformationally Restricted Phospholipids

To prepare the targeted phospholipids **1** and **2,** we employed the commercially available (Sigma) amino acid, N-CBZ-4(S)-hydoxy-L-proline (**3**). While this will control the C4 substitution stereochemistry, the epimerizable center at C2 has the potential to introduce disastereomers. Through a series of synthetic manipulations a variety of conformationally restricted phospholipids were prepared (Figs. 2–5). While attempts were made to selectively prepare the diastereomerically pure material, all synthetic efforts were unsuccessful. All biological evaluations were performed on the same mixture of diastereosmers. A synthetic route was identified that specifically provides the pure trans isomer **1d** (Fig. 4). The versatility of this synthetic approach has permitted the introduction of the amides in place of the carbamates. Synthetic details for these cyclic phospholipids are shown below. Unless otherwise stated all reactions were performed under a nitrogen atmosphere. All NMRs were conducted on a GE 300MHz with all chemical shifts relative to TMS. All phospholipid samples were lyophilized (3× benzene) before collecting any analytical data. Elemental analysis of phospholipids were conducted at Galbraith Laboratories (Knoxville, TN).

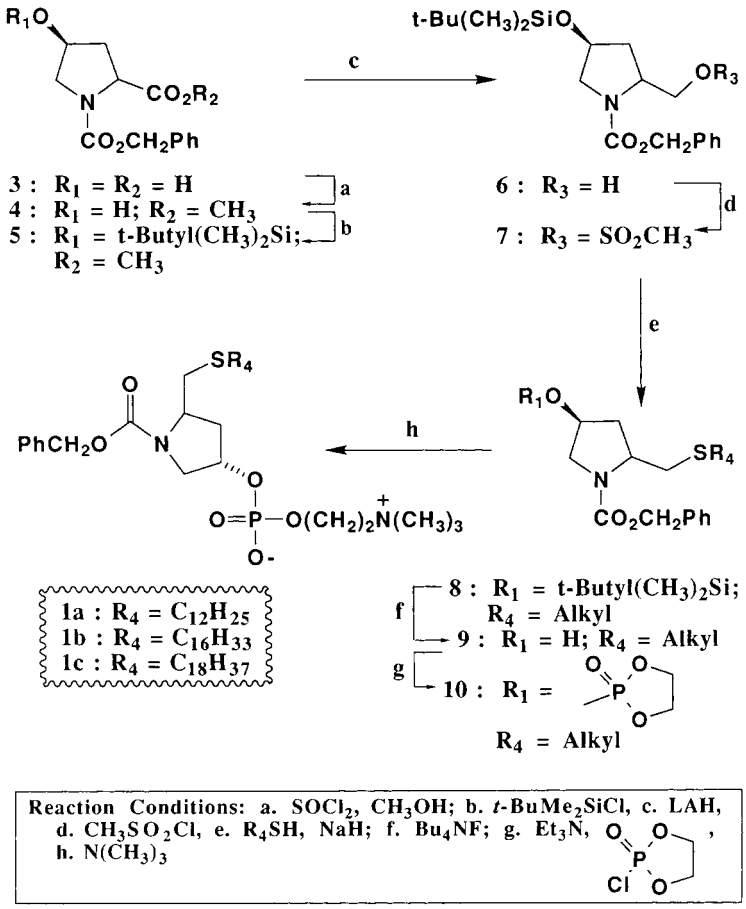



Fig. 2. Synthetic scheme for proximal, conformationally restricted phospholipid **1**.

## Synthesis of Proximal Conformationally Restricted Phospholipid 1

The reaction of N-CBZ-4(S)-hydroxy-L-proline (**3**) (6.1 g, 25.3 mmol) in anhydrous methanol (350 ml) with thionyl chloride (1.85 ml, 25.3 mmol) was initially conducted at 0°C allowed to reach room temperature then heated to reflux for 1 h. After cooling the reaction mixture was neutralized with aqueous sodium bicarbonate, concentrated in vacuo to afford the methyl ester **4** (6.13 g) in greater than 95% yield. It was evident from the NMR data that an inseparable mixture of diastereomers (65:35) were obtained in this reaction [26,27]. Attempts to use other methods for esterification were equally unsuccessful and the mixture was carried through the following synthetic sequences—NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.47 (m, 1H, NCH), 3.80 and 3.50 (65:35) (s, 3H, $OCH_3$), 3.70–3.60 (m, 3H, CHOH,$NCH_2$), 2.60 (bs, 1H, OH), 2.15 (m, 2H, $CH_2$).

Protection of the 4-hydoxyl group was effected by combining **4** (16 g, 69 mmol) with *tert*-butylchlorodimethylsilane (Aldrich, 13.6 g, 89.6 mmol) and imidazole (7.1 g, 103 mmol) in dry DMF (70 ml) at 0°C initially, then permitting the reaction mixture to reach room temperature over 2 h. The reaction mixture was quenched with water,

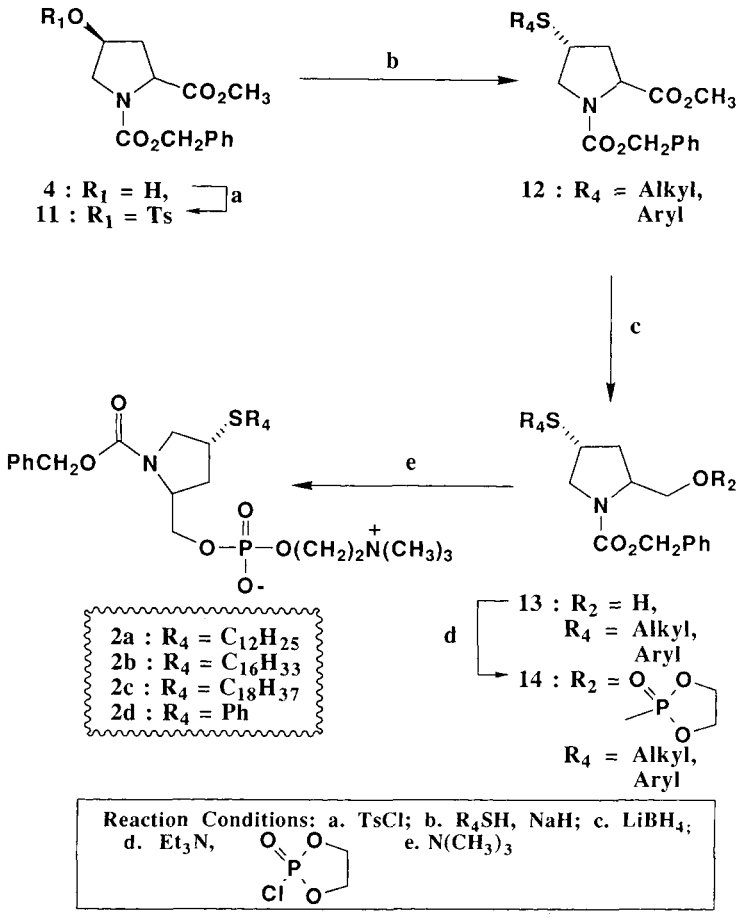



Fig. 3. Synthetic scheme for distal, conformationally restricted phospholipid **2.**

extracted with dichloromethane (4× 100 ml), and the combined organics were dried over magnesium sulfate and concentrated in vacuo. The crude residue was subjected to silica gel chromatography ($R_f$ (**5**) = 0.45 [diethyl ether in hexane, 1:1]) to give **5** in excellent yield (24 g, 99%) (Fig. 2). NMR ($CDCl_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.47 (m, 1H, NCH), 3.80 and 3.50 (65:35) (s, 3H, $OCH_3$), 3.70–3.60 (m, 3H, CHOSi,$NCH_2$), 2.15 (m, 2H, $CH_2$), 0.85 (s, 9H, $C(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

Chemoselective reduction of the ester **5** (47.3 g, 135 mmol) with lithium borohydride (8.92 g, 406 mmol) occurred promptly (1 h) in dry THF (540 ml) at 0°C. The reaction mixture was diluted with dichloromethane (1 liter) and ice followed by neutralization with 1N oxalic acid. The aqueous layer was extracted further with dichloromethane (3× 200 ml) and the combined organics were washed with water and saturated sodium chloride and dried over magnesium sulfate. After filtration and concentration, the residue was promptly subjected to silica gel chromatography ($R_f$(**6**) = 0.20 [diethyl ether in hexane, 1:1]) provided the pure alcohol **6** (31 g, 71% yield). NMR ($CDCl_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.15 (m,

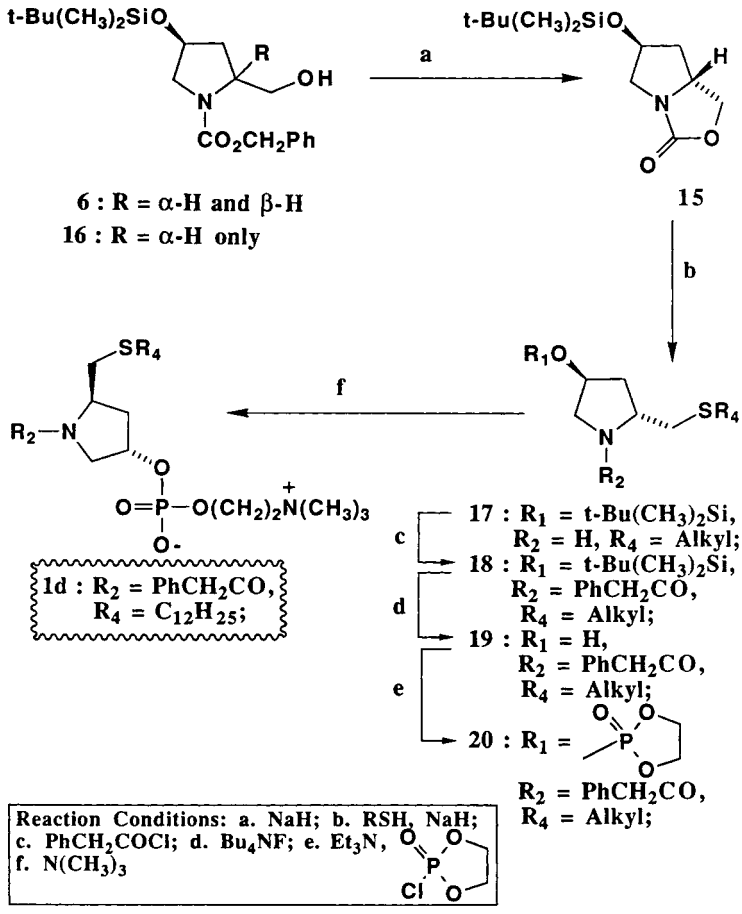



Fig. 4. Synthetic scheme for proximal, conformationally restricted phospholipid **1d** with exclusively 2-trans stereochemistry.

1H, NCH), 3.70–3.60 (m, 3H, CHOSi,$NCH_2$), 3.40–3.30 (m, 2H, $CH_2O$), 2.50 (m, 1H, OH), 2.15 (m, 2H, $CH_2$), 0.85 (s, 9H, $C(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

To activate this alcohol **6** (2.0 g, 6.2 mmol) was dissolved in dichloromethane (20 ml) at 0°C and treated by the dropwise addition of methanesulfonyl chloride (0.74 ml, 9.3 mmol) followed by triethylamine (1.3 ml, 9.3 mmol). The reaction was complete as judged by TLC after 1 h and was diluted with dichloromethane (100 ml) and quenched with cold (0°C) 1N oxalic acid (100 ml). The organic layer was then washed with water (100 ml), saturated sodium bicarbonate (2× 100 ml), saturated sodium chloride (100 mL), and dried over magnesium chloride. After filtration and concentration the crude mesylate **7** was obtained (2.3 g, 93%), which was used directly in the next reaction. NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.15 (m, 1H, NCH), 3.70–3.60 (m, 3H, CHOSi,$NCH_2$), 3.60–3.45 (m, 2H, $CH_2O$), 2.10 (singlets, 3H, $SO_2CH_3$), 2.15 (m, 2H, $CH_2$), 0.85 (s, 9H, $C(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

Displacement of the mesylate **7** (3.68 g, 9.3 mmol) in dry THF (28 ml) was achieved by the addition of dodecyl mercaptan (2.70 ml, 11.1 mmol) dissolved in 3 ml of

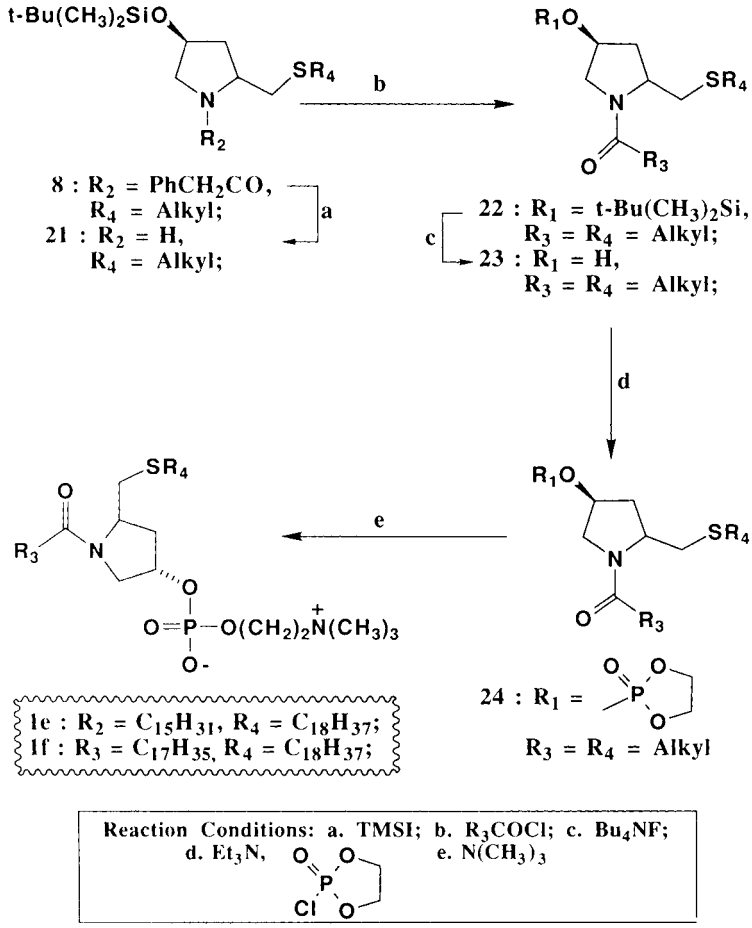



Fig. 5. Synthetic scheme for proximal, conformationally restricted phospholipids **1e** and **1f** with amide side-chains.

THF followed by the portionwise addition of sodium hydride (50% oil dispersion, 0.67 g, 13.9 mmol). After stirring for 1 h at 0°C, the reaction mixture gradually warmed up to reflux to effect complete conversion as determined by TLC. The reaction was cooled, diluted with diethyl ether (100 ml), and quenched by slow addition of ice water. Careful neutralization with 1N oxalic acid is followed by extraction of the aqueous with additional portions of diethyl ether (2× 75 ml). The collective organic fractions were washed one time each with water (100 ml) and saturated sodium chloride (100 ml) and dried over magnesium sulfate. After filtration and concentration, the residue was chromatographed on silica gel ($R_f$ (**8a**) = 0.89 [diethyl ether in hexane, 1:1]) to provide desired thioether **8a** (3.50 g, 74% yield). NMR ($CDCl_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.15 (m, 1H, NCH), 3.70–3.50 (m, 3H, CHOSi,$NCH_2$), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.60–1.10 (bs, 20H, alkyl), 0.85 (s, 9H, $C(CH_3)_3$), 0.80 (ill-defined triplet, 3H, $CH_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

In a similar fashion, **8b** and **8c** were prepared with the exception that in the preparation of **8c** the reaction temperature did not exceed 50°C and the reaction was kept at this temperature for 24 h. Higher temperatures lead to much lower yields. **8b:** (83% yield); ($R_f$ (**8b**) = 0.90 [diethyl ether in hexane, 1:1]); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.15 (m, 1H, NCH), 3.70–3.50 (m, 3H, CHOSi,$NCH_2$), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.60–1.10 (bs, 28H, alkyl), 0.85 (s, 9H, $C(CH_3)_3$), 0.80 (ill-defined triplet, 3H, $CH_3$), 0.05 (s, 6H, $Si(CH_3)_3$). **8c:** (77% yield); ($R_f$ (**8a**) = 0.95 [diethyl ether in hexane, 1:1]); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.15 (m, 1H, NCH), 3.70–3.50 (m, 3H, CHOSi,$NCH_2$), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.60–1.10 (bs, 32H, alkyl), 0.85 (s, 9H, $C(CH_3)_3$), 0.80 (ill-defined triplet, 3H, $CH_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

The silylether **8a** (6.20 g, 12.3 mmol) was dissolved in THF (64 mL) and treated with tetrabutylammonium fluoride (Aldrich, 18.4 ml, 18.4 mmol) at room temperature for 18 h. When complete by TLC, the reaction was combined with water (100 ml), and the THF was removed in vacuo to leave an aqueous solution which was extracted with dichloromethane (3× 100 ml). The combined organics were washed with saturated sodium chloride (100 ml), dried over magnesium sulfate, and concentrated. Silica gel purification ($R_f$ (**9a**) = 0.15 [diethyl ether in hexane, 1:1]) afforded **9a** (4.0 g, 83% yield). NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.15 (m, 1H, NCH), 3.70–3.50 (m, 3H, CHO,$NCH_2$), 2.50 (bs, 1H, OH), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.60–1.10 (bs, 20H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$).

In the same fashion, **9b** and **9c** were prepared. **9b:** (87% yield); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.15 (m, 1H, NCH), 3.70–3.50 (m, 3H, CHO,$NCH_2$), 2.50 (bs, 1H, OH), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.60–1.10 (bs, 28H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). **9c:** (79% yield); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.15 (m, 1H, NCH), 3.70–3.50 (m, 3H, CHO,$NCH_2$), 2.50 (bs, 1H, OH), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.60–1.10 (bs, 32H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$).

Conversion of these alcohols **9** to the phosphorylcholine was performed using the method of Thuong and Chabrier [28] to prepare the desired phospholipids. The general method for the preparation of **1a, 1b,** and **1c** is shown below. The azeotropically dried (benzene) **9a** (356 mg, 0.91 mmol) is dissolved in dry THF (4.6 ml) and stirred at 0°C. To this cold solution (0°C) was added dry triethylamine (0.19 ml, 1.36 mmol) followed by the dropwise addition of 2-chloro-2-oxo-1,2,3-dioxaphospholane (195 mg, 1.36 mmol) and the reaction was followed by TLC ($R_f$ = 0.35 [1% methanol in diethyl ether]). After completion (3 h), the reaction mixture was diluted with cold THF (30 ml) and magnesium sulfate was added, stirred for 10 min, then filtered and concentrated. The residue was azeotroped with benzene (two times) and was then dissolved in dry acetonitrile (4 ml; in the case of **9b** and **9c** dry chloroform was used to assist compound transfer) and placed in the reaction tube. Gaseous anhydrous trimethylamine (0.17 g, 27 mmol) was bubbled into this reaction vessel, sealed, then heated at 70°C for 30 h. At this time, the sealed tube was opened and the contents were concentrated and subjected to HPLC purification ($R_f$ (**1a**) = 0.25 [chloroform:methanol:water; 65:25:4]) to give pure **1a** (243 mg, 48% overall yield from **9a**). NMR (10% $CD_3OD$ in $CDCL_3$): 7.40–7.20 (m,

5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.75 (m, 1H, POCH), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.60–1.10 (bs, 20H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). Elemental analysis calculated for $C_{30}H_{53}N_2O_6PS$: 59.97% C, 8.89% H, 4.66% N, 5.16% P, 5.34% S; found: 59.70% C, 8.67% H, 4.57% N, 4.98% P, 5.13% S. **1b:** (43% yield); NMR (10% $CD_3OD$ in $CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.75 (m, 1H, POCH), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.60–1.10 (bs, 28H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). Elemental analysis calculated for $C_{34}H_{61}N_2O_6PS$: 62.17% C, 9.36% H, 4.26% N, 4.72% P, 4.88% S; found: 62.00% C, 9.11% H, 4.35% N, 4.58% P, 4.67% S. **1c:** (40% yield); NMR (10% $CD_3OD$ in $CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.75 (m, 1H, POCH), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.60–1.10 (bs, 32H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). Elemental analysis calculated for $C_{36}H_{65}N_2O_6PS$: 63.13% C, 9.57% H, 4.09% N, 4.52% P, 4.68% S; found: 62.81% C, 9.18% H, 3.91% N, 4.33% P, 4.54% S.

## Synthesis of Distal Conformationally Restricted Phospholipid 2

The N-CBZ-4(S)-hydroxy-proline methyl ester (**4**) (54.83 g, 183 mmol) was dissolved in dichloromethane (724 ml) and exposed to p-toluenesulfonyl chloride (59.23 g, 312 mmol) and dimethylaminopyridine (4.47 g, 37 mmol) initially at 0°C and eventually stirred at room temperature for 18 h (Fig. 3). The reaction was quenched with the addition of 1N oxalic acid and the organic fraction was washed with water (200 ml) and saturated sodium bicarbonate (3× 200 ml) and dried over magnesium sulfate. Filtration and concentration afforded the residue, which was chromatographed ($R_f$ (**11**) = 0.70 [5% methanol in chloroform]), to give pure **11** (79.31 g, 95% yield). NMR ($CDCL_3$): 7.75 (m, 2H, CH=), 7.40–7.20 (m, 7H, Ph, CH=), 5.20–5.00 (m, 2H, $CH_2O$), 4.47 (m, 1H, NCH), 3.80 and 3.50 (65:35) (s, 3H, $OCH_3$), 3.70–3.60 (m, 3H, CHOTs,$NCH_2$), 2.45 (two singlets, 3H, $CH_3$), 2.15 (m, 2H, $CH_2$).

Exposure of **11** with an alkyl or aryl mercaptan in the presence of sodium hydride as outline above for the preparation **8** generated the thioethers **12a, 12b, 12c,** and **12d. 12a:** (53% yield); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.47 (m, 1H, NCH), 3.80 and 3.50 (65:35) (s, 3H, $OCH_3$), 3.70–3.60 (m, 2H, $NCH_2$), 2.15 (m, 2H, $CH_2$), 2.00 (m, 3H, $CHSCH_2$), 1.60–1.10 (bs, 20H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). **12b:** (58% yield); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.47 (m, 1H, NCH), 3.80 and 3.50 (65:35) (s, 3H, $OCH_3$), 3.70–3.60 (m, 2H, $NCH_2$), 2.15 (m, 2H, $CH_2$), 2.00 (m, 3H, $CHSCH_2$), 1.60–1.10 (bs, 28H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). **12c:** (54% yield); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.47 (m, 1H, NCH), 3.80 and 3.50 (65:35) (s, 3H, $OCH_3$), 3.70–3.60 (m, 2H, $NCH_2$), 2.15 (m, 2H, $CH_2$), 2.00 (m, 3H, $CHSCH_2$), 1.60–1.10 (bs, 32H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). **12d:** (62% yield); NMR ($CDCL_3$): 7.50–7.20 (m, 10H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.47 (m, 1H, NCH), 3.80 and 3.50 (65:35) (s, 3H, $OCH_3$), 3.70–3.60 (m, 2H, $NCH_2$), 2.15 (m, 2H, $CH_2$), 2.00 (m, 1H, CHSPh).

Chemoselective reduction of the methyl ester **12** was performed in the same fashion as **6** with lithium borohydride in THF to afford the rigid triglycerides **13. 13a:** (87% yield); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.10 (m, 1H, NCH), 3.70–3.60 (m, 2H, $NCH_2$), 3.40–3.30 (m, 2H, $CH_2O$), 2.25 (bs, 1H, OH), 2.15 (m, 2H, CH2), 2.00 (m, 3H, $CHSCH_2$), 1.60–1.10 (bs, 20H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). **13b:** (89% yield); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.10 (m, 1H, NCH), 3.70–3.60 (m, 2H, $NCH_2$), 3.40–3.30 (m, 2H, $CH_2O$), 2.25 (bs, 1H, OH), 2.15 (m, 2H, $CH_2$), 2.00 (m, 3H, $CHSCH_2$), 1.60–1.10 (bs, 28H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). **13c:** (84% yield); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.10 (m, 1H, NCH), 3.70–3.60 (m, 2H, $NCH_2$), 3.40–3.30 (m, 2H, $CH_2O$), 2.25 (bs, 1H, OH), 2.15 (m, 2H, CH2), 2.00 (m, 3H, $CHSCH_2$), 1.60–1.10 (bs, 32H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). **13d:** (92% yield); NMR ($CDCL_3$): 7.40–7.20 (m, 10H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.10 (m, 1H, NCH), 3.70–3.60 (m, 2H, $NCH_2$), 3.40–3.30 (m, 2H, $CH_2O$), 2.25 (bs, 1H, OH), 2.15 (m, 2H, $CH_2$), 2.00 (m, 1H, CHSPh).

Phosphorylcholine introduction of **13** was effected by the modified Thuong [28] procedure shown above for the preparation of **2a, 2b, 2c,** and **2c. 2a:** (42% yield); NMR (10% $CD_3OD$ in $CDCL_3$) 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.80 (m, 2H, $POCH_2$), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.25 (m, 2H, $CH_2$), 2.00 (m, 3H, $CH_2SCH_2$), 1.60–1.10 (bs, 20H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). Elemental analysis calculated for $C_{30}H_{53}N_2O_6PS$: 59.97% C, 8.89% H, 4.66% N, 5.16% P, 5.34% S; found: 59.81% C, 8.74% H, 4.53% N, 4.90% P, 5.19% S. **2b:** (47% yield); NMR (10% $CD_3OD$ in $CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.80 (m, 2H, $POCH_2$), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.25 (m, 2H, $CH_2$), 2.00 (m, 3H, $CH_2SCH_2$), 1.60–1.10 (bs, 28H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). Elemental analysis calculated for $C_{34}H_{61}N_2O_6PS$: 62.17% C, 9.36% H, 4.26% N, 4.72% P, 4.88% S; found: 61.90% C, 9.08% H, 3.99% N, 4.51% P, 4.71% S. **2c:** (44% yield); NMR (10% $CD_3OD$ in $CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.80 (m, 2H, $POCH_2$), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.25 (m, 2H, $CH_2$), 2.00 (m, 3H, $CH_2SCH_2$), 1.60–1.10 (bs, 32H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). Elemental analysis calculated for $C_{36}H_{65}N_2O_6PS$: 63.13% C, 9.57% H, 4.09% N, 4.52% P, 4.68% S; found: 62.93% C, 9.29% H, 3.98% N, 4.36% P, 4.42% S. **2d:** (57% yield); NMR (10% $CD_3OD$ in $CDCL_3$): 7.40–7.20 (m, 10H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.80 (m, 2H, $POCH_2$), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.25 (m, 2H, $CH_2$), 2.00 (m, 3H, $CH_2SPh$). Elemental analysis calculated for $C_{24}H_{33}N_2O_6PS$: 56.79% C, 6.36% H, 5.52% N, 6.10% P, 6.32% S; found: 56.57% C, 6.25% H, 5.27% N, 5.84% P, 6.15% S.

## Synthesis of Proximal Conformationally Restricted Phospholipid 1d With Exclusively 2-Trans Stereochemistry

As an alternative to the preparation of the thioether **8,** the alcohol **6** (24.0 g, 63.4 mmol) dissolved in THF (50 ml) was added dropwise to a cold (0°C) solution of sodium hydride (50% oil dispersion, 4.6 g, 95 mmol). This intramolecular cyclization reaction was kept at 0°C for 2 h then stirred at room temperature. The reaction mixture was

poured into diethyl ether (100 ml) and ice, and the organic fraction was further extracted with water (3× 200 ml), and saturated sodium chloride (200 mL). After filtration and concentration, the residue was subjected to silica gel chromatography, which provided (Fig. 4) the product **15** (10.26 g, 60% yield, $R_f = 0.20$ [diethyl ether in hexane, 1:1]) and unreacted starting material **16** (7.60 g, 32% yield, $R_f = 0.10$ [diethyl ether in hexane, 1:1]). The bicyclic carbamate **15** was recrystalized from hexane to give rodlike crystals {m.p. = 93–94°C}. With the chiral integrity of the C4 position retained, the absolute stereochemistry of the bicyclic system **15** was established by X-ray analysis [J.C. Calabrese, unpublished data] showing that the 2-hydrogen is *syn* to the 4β-*tert*-butyldimethylsilyloxy group. NMR ($CDCL_3$): 4.50 (m, 2H, $OCH_2$, CONCH), 4.30 (m, 1H, SiOCH), 4.20 (m, 1H, $OCH_2$), 3.80 (dd, J = 15Hz, 8Hz, 1H, $CONCH_2$), 3.00 (dd, J = 15Hz, 3Hz, $CONCH_2$), 2.00 (dd, J = 13Hz,7Hz, 1H, $CH_2$), 1.50 (m, 1H, $CH_2$).

Having established the stereochemistry of this system we next endeavored to introduce the thioether side-chain by treating the cyclic carbamate **15** (10.0 g, 39 mmol) in dry DMF (125 ml) with dodecyl mercaptan (11.78 g, 58.3 mmol) followed by the portionwise addition of sodium hydride (50% oil dispersion, 2.80 g, 58.3 mmol) at 0°C. Letting the reaction slowly reaching room temperature followed by gentle heating (50°C) for 2 h gave exclusively the thioether **17.** Using nonpolar solvents (e.g., $CH_2Cl_2$) resulted in no product formation, but using polar solvents [29] (e.g., dimethylformamide) resulted in smooth conversion of the cyclic carbamate into the desired thioether **17** with concomitant decarboxylation. The reaction vessel was cooled to room temperature and the reaction was diluted with diethyl ether (250 ml), quenched with the slow addition of ice, and kept basic with saturated sodium bicarbonate solution (100 ml). The resultant aqueous phase was further extracted with diethyl ether (2× 100 ml) and the combined organics were extracted with saturated sodium chloride solution (100 ml). After drying over magnesium sulfate, the organic fraction was filtered and concentrated in vacuo to give a residue that was chromatographed on silica gel ($R_f$ (**17**) = 0.30 [diethyl ether in hexane, 1:1]) to give pure **17** (13.4 g) in 83% yield. NMR ($CDCL_3$): 3.70 (m, 1H, CHOSi), 3.50–3.20 (m, 3H, NCH, $NCH_2$), 2.15 (m, 2H, $CH_2$), 2.05 (d, J = 8Hz, 2H, $SCH_2$), 2.00 (t, J = 7.5, 2H, $SCH_2$), 1.80 (bs, 1H, NH), 1.60–1.10 (bs, 20H, alkyl), 0.85 (s, 9H, $C(CH_3)_3$), 0.80 (ill-defined triplet, 3H, $CH_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

Acylation of the amine **17** (13.4 g, 32.4 mmol) with benzyl chloroformate (6.60 g, 38.8 mmol) was conducted in dry dichloromethane (125 ml) in the presence of triethylamine (6.80 ml, 48.5 mmol) at 0°C. After 1 h, the reaction was diluted with dichloromethane (200 ml) and quenched with ice water (100 ml). Extraction of the aqueous layer with additional portions of dichloromethane (2× 100 ml) was followed by extraction of the combined organics with 1N oxalic acid (100 ml), water (100 ml), and saturated sodium chloride (100 ml). The resultant organic layer was dried over magnesium sulfate, filtered, and concentrated to give the desired carbamate **18** (13.7 g, 78% yield). The residue was used without any further purification in the next step ($R_f$ (**18**) = 0.55 [diethyl ether in hexane, 1:1]); NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.15 (m, 1H, NCH), 3.70–3.50 (m, 3H,CHOSi, $NCH_2$), 2.25 (m, 2H, $CH_2$), 2.05 (d, J = 8Hz, 2H, $SCH_2$), 2.00 (t, J = 7.5, 2H, $SCH_2$), 1.60–1.10 (bs, 20H, alkyl), 0.85 (s, 9H, $C(CH_3)_3$), 0.80 (ill-defined triplet, 3H, $CH_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

Deprotection of the 4β-*tert*-butyldimethylsilyloxy group of **18** (5.50 g, 10 mmol) was effected in THF (20 ml) in the presence of tetrabutylammonium fluoride (100 ml, 100 mmol, 1M in THF, Aldrich) at room temperature. After 18 h, water (50 ml) and diethyl ether (100 ml) was added to the reaction mixture and the organic fraction was washed with saturated sodium chloride (100 ml), dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography ($R_f$ (**19**) = 0.25 [diethyl ether in hexane, 1:1]) to give pure **19** (3.92 g) in 90% yield. NMR ($CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (m, 2H, $CH_2O$), 4.15 (m, 1H, NCH), 3.70–3.60 (m, 3H, CHO,$NCH_2$), 2.60 (m, 1H, OH), 2.25 (m, 2H, $CH_2$), 2.05 (d, J = 8Hz, 2H, $SCH_2$), 2.00 (t, J = 7.5, 2H, $SCH_2$), 1.60–01.10 (bs, 20H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$).

The phosphorylcholine moiety was introduced as previously described above to afford the pure trans conformationally restricted proximal phospholipid **1d** in 38% overall yield. NMR (10% $CD_3OD$ in $CDCL_3$): 7.40–7.20 (m, 5H, Ph), 5.20–5.00 (bs, 2H, $CH_2O$), 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.75 (m, 1H, POCH), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.25 (m, 2H, $CH_2$), 2.05 (d, J = 8Hz, 2H, $SCH_2$), 2.00 (t, J = 7.5, 2H, $SCH_2$), 1.60–1.10 (bs, 20H, alkyl), 0.80 (t, J = 7Hz, 3H, $CH_3$). Elemental analysis calculated for $C_{30}H_{53}N_2O_6PS$: 59.97% C, 8.89% H, 4.66% N, 5.16% P, 5.34% S; found: 59.59% C, 8.64% H, 4.41% N, 4.89% P, 5.03% S.

## Synthesis of Proximal Conformationally Restricted Phospholipids 1e and 1f With Amide Side-Chains

Given the improved PLA2 inhibition with the amides over the carbamates in our previous studies [17,18], we sought to prepare the amide series of potential inhibitors **1e** and **1f.** While attempts to remove the N-CBZ group of **8** with catalytic hydrogenolysis were unsuccessful, treatment of **8** (6.40 g, 10.1 mmol) in $CH_2Cl_2$ (50 ml) at −78°C with iodotrimethylsilane [30] (3.0 g, 15.2 mmol) smoothly afforded the desired amine **21** in 77% yield. The reaction mixture was stirred for 1 h at −78°C, then permitted to reach 0°C and kept stirring another 30 min. At this time, the reaction mixture was poured into ice-cold dichloromethane (100 ml) and the organic layer was extracted with saturated sodium bicarbonate (2× 100 ml) and saturated sodium bisulfite (100 ml), then dried over magnesium sulfate. After filtration and concentration, the residue was purified on silica gel ($R_f$ (**21**) = 0.30 [diethyl ether in hexane, 1:1]) to give **21** (3.88 g, 77% yield). NMR ($CDCL_3$): 3.70 (m, 1H, CHOSi), 3.50–3.20 (m, 3H, NCH, $NCH_2$), 2.15 (m, 2H, $CH_2$), 2.00 (ill-defined doublet and triplet, 4H, $SCH_2$), 1.80 (bs, 1H, NH), 1.60–1.10 (bs, 32H, alkyl), 0.85 (s, 9H, $C(CH_3)_3$), 0.80 (ill-defined triplet, 3H, $CH_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

Efficient acylation of the amine **21** (3.88 g, 7.77 mmol) was initiated in THF (30 ml) at 0°C with the dropwise addition of stearoyl chloride (1.90 g, 9.34 mmol) dissolved in THF (7 ml) followed by the addition of triethylamine (1.63 ml, 11.7 mmol). After stirring at 0°C for 1 h, the reaction mixture was stirred at room temperature for 6 h, at which time an excess of 1N lithium hydroxide (100 ml) was added and the reaction mixture was vigorously stirred for another 18 h. After separation of the organic fraction, the aqueous phase was extracted with diethyl ether (3× 100 ml) and all of the organic fractions were combined and extracted with saturated sodium bicarbonate solution (3× 100 ml), then dried over magnesium sulfate. Filtration and concentration gave a residue

consistent with the amide **22f** (3.73 g, 72% yield), which was used directly in the next step ($R_f$ (**22f**) = 0.80 [diethyl ether in hexane, 1:1]); NMR ($CDCL_3$): 4.30 (m, 1H, NCH), 3.70–3.60 (m, 3H, CHOSi,$NCH_2$), 2.30 (t, J = 8Hz, 2H, $COCH_2$), 2.15 (m, 2H, $CH_2$), 2.00 (ill-defined doublet and triplet, 4H, $SCH_2$), 1.80–1.00 (m, 62H, alkyl), 0.85 (s, 9H, $C(CH_3)_3$), 0.80–0.75 (two triplets, J = 7.5Hz, 6H, $CH_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

Using palmitoyl chloride with **21** as described above resulted in the formation of **22e** in 78% yield ($R_f$ (**22e**) = 0.75 [diethyl ether in hexane, 1:1]); NMR ($CDCL_3$): 4.30 (m, 1H, NCH), 3.70–3.60 (m, 3H, CHOSi,$NCH_2$), 2.30 (t, J = 8Hz, 2H, $COCH_2$), 2.15 (m, 2H, $CH_2$), 2.00 (ill-defined doublet and triplet, 4H, $SCH_2$), 1.80–1.00 (m, 58H, alkyl), 0.85 (s, 9H, $C(CH_3)_3$), 0.80–0.75 (two triplets, J = 7.5Hz, 6H, $CH_3$), 0.05 (s, 6H, $Si(CH_3)_3$).

Removal of the 4*β-tert*-butyldimethylsilyloxy group from **22e** and **22f** using tetrabutylammonium fluoride in THF as described for **19** generated the free purified alcohol **23e** and **23f. 23e:** (67% yield); ($R_f$ = 0.45 [diethyl ether in hexane, 1:1]); NMR ($CDCL_3$): 4.30 (m, 1H, NCH), 3.70–3.60 (m, 3H, CHO,$NCH_2$), 2.30 (t, J = 8Hz, 2H, $COCH_2$), 2.15 (m, 2H, $CH_2$), 2.00 (ill-defined doublet and triplet, 4H, $SCH_2$), 1.80–1.00 (m, 58H, alkyl), 0.80–0.75 (two triplets, J = 7.5Hz, 6H, $CH_3$). **23f:** (73% yield); ($R_f$ = 0.50 [diethyl ether in hexane, 1:1]); NMR ($CDCL_3$): 4.30 (m, 1H, NCH), 3.70–3.60 (m, 3H, CHO,$NCH_2$), 2.30 (t, J = 8Hz, 2H, $COCH_2$), 2.15 (m, 2H, $CH_2$), 2.00 (ill-defined doublet and triplet, 4H, $SCH_2$), 1.80–1.00 (m, 62H, alkyl), 0.80–0.75 (two triplets, J = 7.5 Hz, 6H, $CH_3$).

Phosphorylcholine incorporation of **23e** and **23f** proceeded as described above to provide **1e** and **1f. 1e:** (36% yield); ($R_f$ = 0.45 [chloroform:methanol:water; 65:25:4]); NMR (10% $CD_3OD$ in $CDCL_3$): 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.75 (m, 1H, POCH), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.30 (t, J = 8Hz, 2H, $COCH_2$), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.80–1.00 (bs, 58H, alkyl), 0.80–0.75 (two triplets, J = 7.5Hz, 6H, $CH_3$). Elemental analysis calculated for $C_{44}H_{92}N_2O_5PS$: 67.05% C, 11.25% H, 3.55% N, 3.93% P, 4.07% S; found: 66.83% C, 10.87% H, 3.23% N, 3.75% P, 3.88% S. **1f:** (33% yield); ($R_f$ = 0.55 [chloroform:methanol:water; 65:25:4]); NMR (10% $CD_3OD$ in $CDCL_3$): 4.25 (m, 2H, $POCH_2$), 4.05 (m, 1H, NCH), 3.75 (m, 1H, POCH), 3.60–3.50 (m, 2H, $CONCH_2$), 3.50 (m, 2H, $NCH_2$), 3.20 (s, 9H, $N(CH_3)_3$), 2.30 (t, J = 8Hz, 2H, $COCH_2$), 2.25 (m, 2H, $CH_2$), 2.00 (ill-defined triplet and doublet, 4H, $CH_2SCH_2$), 1.80–1.00 (bs, 62H, alkyl), 0.80–0.75 (two triplets, J = 7.5Hz, 6H, $CH_3$). Elemental analysis calculated for $C_{46}H_{88}N_2O_5PS$: 67.68% C, 11.36% H, 3.43% N, 3.79% P, 3.93% S; found: 67.31% C, 11.08% H, 3.09% N, 3.53% P, 3.60% S.

## Phospholipase A2 Assay

Phospholipase A2 activity was evaluated using porcine pancreatic PLA2 and a sonicated dispersion of 1-palmitoyl-2-[$C^{14}$]-arachidonoyl-phosphatidyl choline as the substrate [31]. Inhibitors **1** and **2** were mixed with buffers (25 mM Tris, 25 mM glycylglycine, 25 mM $CaCL_2$, 0.75 mM EDTA, pH = 8.5) and preincubated with porcine pancreatic PLA2 (Sigma, 25 ng) at 37°C for 2 min. The enzymatic reaction was initiated at 37°C with the addition of 0.04 $\mu$Ci 1-palmitoyl-2-[$C^{14}$]-arachidonoyl-phosphatidyl choline (54.5 $\mu$Ci/$\mu$mol, New England Nuclear) making the entire reaction volume 0.1 ml and 7 $\mu M$ in substrate. Under these conditions, the rate of substrate

hydrolysis was 5–7 pmol/min (15–20% of substrate hydrolyzed) in the absence of inhibitors. After 5 min, the reaction mixture was frozen with dry ice in ethanol, then triturated with 0.5 ml of solvent A (solvent A = ethyl acetate:acetic acid, 99:1 by volume). This mixture containing the liberated [$C^{14}$]-arachidonic acid and unreacted 1-palmitoyl-2-[$C^{14}$]-arachidonoyl-phosphatidyl choline was pipetted onto preconditioned (1 ml of solvent A) 3 ml silica gel columns (J.T. Baker, Philipsburg, NJ). Elution of [$C^{14}$]-arachidonic acid was completed with the 2 ml addition of solvent A. The remaining unreacted 1-palmitoyl-2-[$C^{14}$]-arachidonoyl-phosphatidyl choline was removed using two washes with 2 ml of solvent B (solvent B = ethyl acetate:methanol: water, 1:1:1). The radioactivity of column fractions derived from both solvents A and B was measured using a scintillation counter that converted CPMs to DPMs to correct for solvent quenching. In this fashion, at least 85% of the radioactivity was recovered. Each inhibitor was tested in duplicate at six different concentrations and was repeated at least three to five times to provide a concentration response profile. The average inhibition value for each concentration was plotted to identify for each compound an IC50, the concentration needed to inhibit 50% of substrate conversion by porcine pancreatic PLA2. Multiple determinations of the IC50 values routinely agree such that a threefold difference in IC50 value is much greater than two standard errors of the mean.

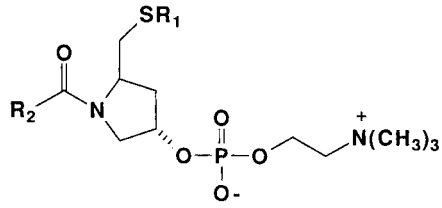
### Computer Molecular Modeling

By using an Evans and Sutherland PS300 with the computer graphics program, PSSHOW, several of these inhibitors were graphically evaluated. General computational methods applied were as previously described [6,8–12]. Porcine and bovine pancreatic PLA2 X-ray crystallographic coordinates were employed for this study [32]. In general, each structure was graphically inserted into the active site and run through a computer program to give an optimum steric and electronic fit affording an energy value. Two modeling requirements were established based upon previous biochemical evaluations [6–18]. The phosphorylcholine functionality was inserted specifically into the active site with the phosphate oxygen capable of coordination with the calcium in the active site of PLA2. The carbonyl oxygen of **1** and **2** were directed at this same coordination sphere with a water molecule within hydrogen bonding distance to histidine.[48] During the process, the inhibitor was fixed in a given conformation and allowed to dock into the active site. Several substrate and inhibitor conformations were considered by either manual or computer manipulation. Since these phospholipids **1** and **2** are conformationally restricted, the number of possible conformations were drastically reduced.

## RESULTS

Using the porcine pancreatic PLA2 assay described above, a summary of the PLA2 inhibition studies with conformationally restricted phospholipids **1** and **2** are shown respectively in Tables I and II. By evaluating isomeric materials in an PLA2 assay and with substrate concentrations very close to the inhibitor IC50, complications typically associated with a variety of reported PLA2 assays [33] (e.g., micelle formation, substrate-inhibitor interactions) are minimized. Comparisons of the IC50s between two isomers **1** vs. **2** (e.g., entry **1A** vs. **2a**) illustrates that the distal isomer **2** [IC50 = 20 $\mu$M (**1a**); 5.6 $\mu$M (**2a**)]. Additional potency was not observed with the amides **1e** and **1f** as anticipated.

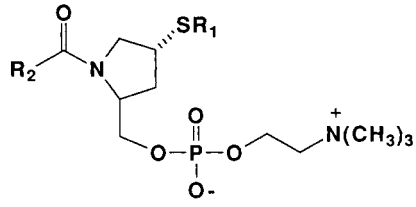
**TABLE I. Phospholipase A2 (PLA2) Activity of Proximal, Conformationally Restricted Phospholipid 1**



| Entry | $R_1$ | $R_2$ | IC50 ( μM) |
|---|---|---|---|
| 1a | $C_{12}H_{25}$ | $PhCH_2O$ | 20 |
| 1b | $C_{16}H_{33}$ | $PhCH_2O$ | 40 |
| 1c | $C_{18}H_{37}$ | $PhCH_2O$ | 25 |
| 1d | $C_{12}H_{25}$ | $PhCH_2O$ (trans) | 23 |
| 1e | $C_{18}H_{37}$ | $C_{15}H_{31}$ | 13 |
| 1f | $C_{18}H_{37}$ | $C_{17}H_{35}$ | 9 |

Based upon our earlier work [4,5], we proposed that the active site of porcine or bovine pancreatic PLA2 has two different lipid binding domains that can accommodate the substrate. By extending the upper side-chain length, greater potency was observed with the previous series of inhibitors. In the current case, by comparing the difference of the distal verses the proximal side-chain orientations an interesting result occurs. One striking feature is that the distal orientation **2** fits better into the active site than the corresponding proximal isomer **1** with both side-chains extending into the active site of porcine or bovine pancreatic PLA2. This would suggest that the 2 acyl chain of lecithins

**TABLE II. Phospholipase A2 (PLA2) Activity of Distal, Conformationally Restricted Phospholipid 2**



| Entry | $R_1$ | $R_2$ | IC50 ( μM) |
|---|---|---|---|
| 2a | $C_{12}H_{25}$ | $PhCH_2O$ | 5.6 |
| 2b | $C_{16}H_{33}$ | $PhCH_2O$ | 22.0 |
| 2c | $C_{18}H_{37}$ | $PhCH_2O$ | 4.7 |
| 2d | Ph | $PhCH_2O$ | 6.2 |

coil into the lipophilic pocket near phenyalanine 106 (bovine) while the upper 1-acyl chain tends to occupy some of the region near lipophilic residues of the N-terminus.

## CONCLUSIONS

We have identified and prepared conformationally restricted phospholipids **1** and **2.** This synthetic method has been shown to be very versatile for a variety of side-chain substitutions. These phospholipids **1** and **2** were shown to be inhibitors of porcine pancreatic PLA2 with an enzymatic two- to fivefold preference for distal isomer **2** over the proximal orientation **1.** This was supported by the direct comparisons of the enzymatic activity for the isomeric inhibitors which should avoid any physical artifacts. Given these biochemical results, computer molecular modeling using either the porcine or bovine X-ray crystal structure suggested that this preference was a result of an optimum occupation of both side-chains in the active site of PLA2. The N-phenyl-methoxy carbonyl group appeared to prefer extending into the active site near phenylalanine 106 (bovine PLA2) while the thioether tended to prefer inserting near the N-terminus loop region. While these modeling studies offer a view of how these inhibitors **1** and **2** may bind PLA2, there are still other alternative modes of binding that may explain the inhibitor selectivity displayed by PLA2.

While it is true these inhibitors did not inhibit PLA2 with greater potency than the corresponding acyclic versions [13–18], we feel they have given us greater insight in the conformational preference of PLA2. From these and our previous studies [16,17], a hypothetical mode of phospholipid binding to PLA2 has been presented. With these inhibitors in hand, biophysical studies (X-ray, NMR) are underway to determine the validity of this model. Finally, these PLA2 inhibitors **1** and **2** have allowed us to test the conformational preference of phospholipids for the active site of PLA2 (these inhibitors **1** and **2** are envisioned to have the potential biological properties associated with PLA2 inhibitors such as anti-inflammatory and antihypertensive activity). Our hope is that these studies will be very useful for future PLA2 inhibitor design.

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