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Jason A. Davy[a]; Zhongyi Wang[a]; Robert H. Notter[b]; Adrian L. Schwan[a]
[a] Department of Chemistry, University of Guelph, Guelph, ON, Canada [b] Department of Pediatrics, University of Rochester, Rochester, NY, USA

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Synthesis of sulfur-containing glycerophospholipids

JASON A. DAVY†, ZHONGYI WANG†, ROBERT H. NOTTER‡ and ADRIAN L. SCHWAN*†

†Department of Chemistry, University of Guelph, Guelph, ON, Canada, N1G 2W1
‡Department of Pediatrics, University of Rochester, Rochester, NY 14642, USA

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The inclusion of one or more sulfur atoms at the backbone of glycerophospholipids or phosphonolipids has created a number of new non-natural substrates for investigation in medicinal chemistry and enzymology. This review, containing 138 references, summarizes research contributions to date, with an emphasis on the synthetic organic chemistry that provides the lipids.

Keywords: Synthesis; Phospholipids; Phosphonolipids; Sulfur; Lipid analogs; Biological activity; Lipases

The biological importance of both natural and non-natural phospholipids cannot be overstated; thus, it is hardly surprising that this class of compounds continues to fascinate researchers [1–8]. A growing interest in the synthesis of new phospholipids has been bolstered by applications in membrane engineering [9, 10], the pursuit of vehicles suitable for introducing photodegradable molecules into membranes [11], and by a general ambition to surpass the bioactivity of natural compounds [12–16]. Sulfur-containing glycerophospholipids and phosphonolipids are important among the collection of synthetic glycerolipids. For instance, thioether derivatives of glycerophospholipids or phosphonolipids have been targeted as potential therapeutic pharmacologic agents against cancer, infection, or respiratory disease as detailed later, while thiolester and thiophosphate functionalities are valuable for studying phospholipase kinetics and related applications.

Issues of stereochemistry, undesirable rearrangements, and the compatibility of protecting groups often complicate the synthesis of sulfur-containing targets. Although a number of the recent reviews [17–23] have sections addressing the synthetic protocols for sulfur-containing lipids, there appears to be no review focused solely on glycerophospholipids possessing sulfur in place of oxygen in the glycerol backbone.

In this paper, we will present the applications of and the synthetic progress towards selected sulfur-containing glycerophospholipid or phosphonolipid compounds. Particular stress is placed on useful synthetic strategies for the inclusion of the sulfur atom or sulfur-containing

*Corresponding author. Email: schwan@uoguelph.ca

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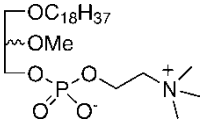
functionality. While the effects of sulfur substitution on the properties of the resulting lipids are discussed, the reader is also urged to consult the primary literature for full details pertaining to the allied applications in medicinal chemistry or enzymology. The layout of the following sections is based on the structure of the lipids and not necessarily on their biological role(s). Sulfur-containing lipids with a single thioether or thiolester functionality at the *sn*-1 position or *sn*-2 position are discussed in sections 1 and 2, followed by di-thio derivatives in section 3, and *sn*-3 sulfur-containing lipids in section 4.

1. Sulfur-containing *sn*-1 phospholipids

The first class of sulfur-containing phospholipids and phosphonolipids discussed comprises lipids with a thioether or thiolester functionality at the *sn*-1 position [24]. Sulfur-containing *sn*-1 phospholipids encompass a wide spectrum of biological roles ranging from synthetic lung surfactants [15] to antitumor [25, 26] and anti-HIV [27–30] agents, to analogs [26, 31] of the experimental anticancer drug (±)-1-*O*-octadecyl-2-O-methyl-glycero-3-phosphocholine (ET-18-OCH_3, **1**) [32] and platelet activating factor (PAF) [16].



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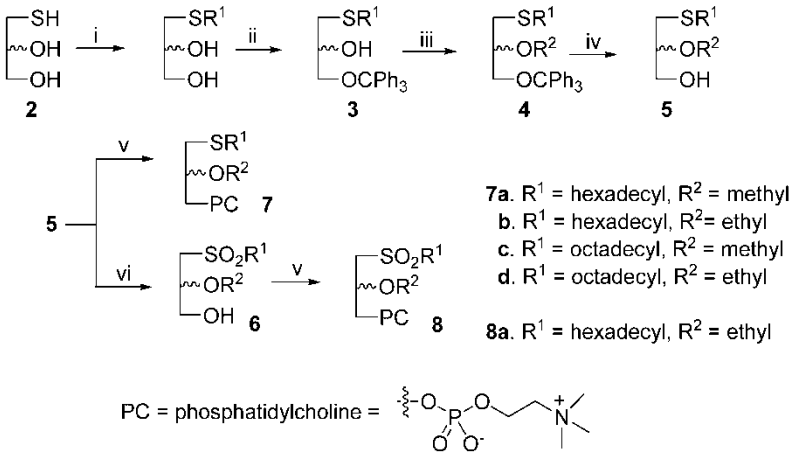
Tactics for the syntheses of thioethers and thiolesters tend to differ substantially. Thioethers can be prepared by having fattyalkyl thiols attack an electrophilic site or through the alkylation of thiols at the *sn*-1 position. Synthesis of thiolesters, on the other hand, invariably requires the acylation of a thiol. In either case, the enhanced nucleophilicity of sulfur compared to oxygen is the key to the preparation. Thioether *sn*-1 lipids are described in section 1.1 below, followed by discussion of thiolester *sn*-1 lipids in section 1.2.

1.1 *Thioether lipids*

The fundamental starting point for a large number of *sn*-1 sulfur containing lipids is commercially available 1-thioglycerol (**2**, 1-mecaptoglycerol, 1-mercapto-2,3-propanediol) [33]. The less nucleophilic hydroxyl groups do not encumber sulfur alkylation of this substrate. An example of the value of **2** has been demonstrated in the synthesis of a series of sulfur analogs of alkyl lysophospholipids.

To access such compounds, Piantadosi and coworkers [25] synthesized (±)-1-*S*(or SO_2)-fattyalkyl-2-*O*-alkyl-thioglycero-3-phosphocholines (**7**, **8**, scheme 1). Thioglycerol (**2**) was alkylated with hexadecyl or octadecyl bromide in alcoholic KOH [34]. The primary alcohol was subsequently protected with trityl chloride allowing alkylation at the *sn*-2 position with methyl or ethyl iodide in the presence of sodium hydride, affording **4**. Deprotection using BF_3·MeOH [35] provided a sulfide (**5**) ready for headgroup installation or for oxidation to the sulfone (**6**) with potassium hydrogen persulfate [36]. Phosphocholines were prepared from both **5** and **6** using phosphorus oxychloride and choline tosylate.

Alkyl lysophospholipids (ALP) are generally pursued for their antineoplastic behavior. These particular sulfur lipids were targeted out of the likelihood that they possess enhanced lipophilicity [25], permitting facile inclusion of the lipid into the cell membrane. Furthermore, *sn*-1 sulfur analogs had been shown previously to have antineoplastic activity *in vitro* [26].



Reagents and conditions: i) R^1Br, C_2H_5OH-KOH; ii) TrCl, pyridine; iii). NaH, R^2I; iv). BF_3MeOH, CH_2Cl_2; v) 1. $POCl_3$, Et_3N; 2. choline OTs, pyridine, $CHCl_3$; vi) $KHSO_5$.

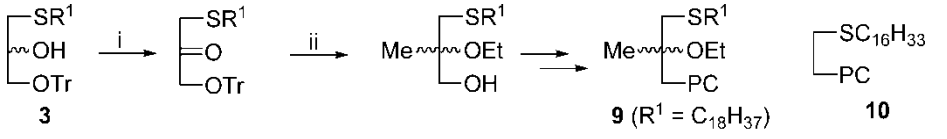
SCHEME 1

ALP analogs (**7**) are at least as active as the recognized standard ET-18-OCH_3 against HL-60 cells; in fact, lipid **7b** is twice as potent as ET-18-OCH_3 in clonogenic assays [25].

Mavromoustakos *et al.* [37] prepared an optically pure form of 1-thiohexadecyl-2-*O*-methyl-3-phosphocholine (**7a**) (*vide infra*) [38] and studied its conformational properties in organic solvents and in micelles. Using NMR spectroscopy to examine the molecular dynamics of the lipid, the authors observed a compact conformation in which the hexadecyl chain is in close proximity to the glycerol backbone and the polar headgroup, ostensibly accounting for its interactions within the cellular membrane [37].

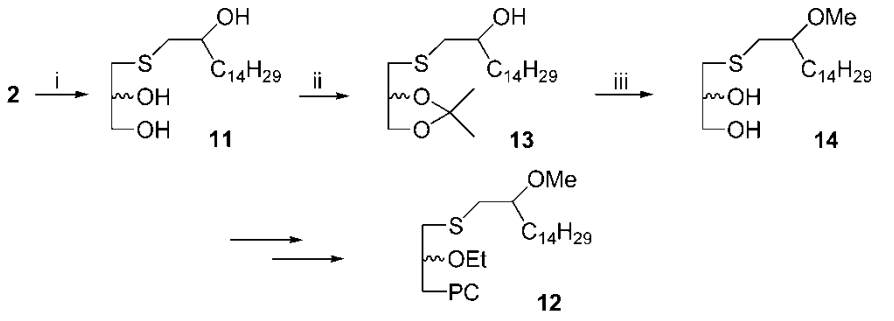
Further study in this area by Piantadosi and coworkers [30, 39, 40] focused on the synthesis and properties of a larger library of thio, oxy and amidoalkyl lipids with either phosphocholine or quaternary ammonium head groups (scheme 2). One such analog was accessed through oxidation of trityl protected sulfide **3** using dicyclohexylcarbodiimide (DCC)/dimethyl sulfoxide (DMSO) [41] to give a ketone at the *sn*-2 carbon [40]. This ketone was converted to a tertiary alcohol using methylmagnesium iodide. Alkylation, detritylation, and phosphorylation afforded lipid **9** [40]. Another derivative (**10**) was prepared by alkylation of 2-mercaptoethanol and phosphocholine placement on the hydroxyl group [30].

Lipid analog **12** (scheme 3) represents another sulfur-containing analog constructed from 1-thioglycerol [40]. In this instance, sulfur alkylation was performed with 1,2-epoxyhexadecane offering triol **11**. Acetalization of the vicinal diol component of **11**, affording **13**, permitted selective methylation of the side chain alcohol. After deacetalization [40], the resulting diol (**14**) could be developed into lipid **12** in accordance with scheme 1.



Reagents and conditions: i) DMSO, DCC; ii) 1. MeMgI; 2. EtI, NaH; 3. pTSA.

SCHEME 2



Reagents and conditions: i) 1,2-epoxyhexadecane, C_2H_5OH-KOH; ii) acetone, cat. H_2SO_4 iii) 1. NaH, MeI; 2. aq. HCl, MeOH.

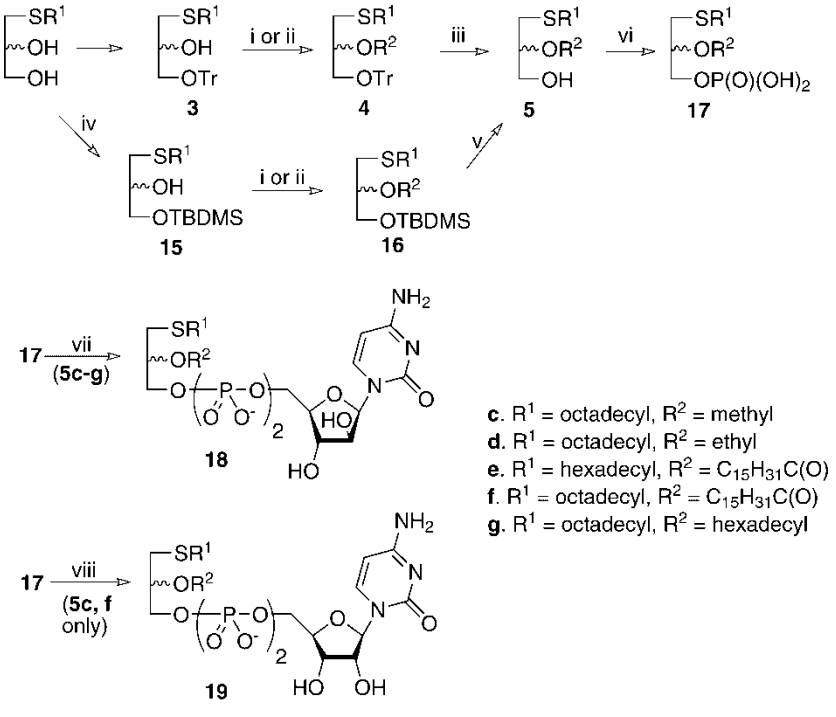
SCHEME 3

Although interesting as new synthetic compounds, sulfur-containing lipids prepared as in schemes 2 and 3 have been found to rank after those with *sn*-1 amido and oxygen linkages in terms of their anti-HIV properties [30]. Some sulfur-containing glycerolipid analogs with quaternary nitrogen-based headgroups exhibit antineoplastic activity comparable to ET-18-OMe [40]. However, in studies based on the HL-60 leukemic cell system, ET-18-OMe exhibited higher activity than sulfur-containing lipids **7b**, **10** and **12** [40]. Whereas the 2-mercaptoethanol-based lipid **10** proved more active and less toxic than **7b** in the anti-HIV assay [30], the reverse was true for *in vitro* antineoplastic activity [40].

Also deploying 1-thioglycerol (**2**) as a convenient starting point, Hong and coworkers [42, 43] synthesized some 1-β-D-arabinofuranosylcytosine (*ara*-C) conjugates (scheme 4). To this end, sulfur alkylation was followed by either tritylation or *sn*-3 silylation with tert-butyldimethylsilyl chloride in the presence of imidazole and DMF [44]. Trityl compounds **4** were then deprotected to produce 2-*O*-alkyl alcohols **5c**, **d** and **g** in good yield [43]. However, both of the 2-acyl congeners **5e** and **f** were produced in only 15% yield due to significant acyl migration to the 3-position. Improved access was achieved with the TBDMS-protected derivatives **15** and **16**. Fluoride-induced desilylation of **16e**, **f** yielded alcohols **5e**, **f** in ca. 60% yield, although acyl migration remained an issue [43]. Substituted glycerols **5c-g** were phosphorylated with $POCl_3$ and Et_3N [45] to form acids **17** [43].

Acids **17** represent immediate precursors to the series of ara-C conjugates. Thus, condensation of **17c-g** with *ara*-CMP morpholidate [46] in pyridine gave **18c-g** with yields ranging from 15–38% (scheme 4) [43]. Likewise, condensation of **17c** and **f** with cytidine 5′-phosphoromorpholidate [47] gave **19c** and **f**, the C2-epimers of **18c** and **f**, in 29–31% yield [43]. Biological evaluation of conjugates **18e, f** revealed improved activity compared to ET-18-OMe, *ara*-C, and their equimolar mixtures [43]. Using a synthetic protocol comparable to that of scheme 4 and employing TBDMS protection, a collection of ten compounds of structure **18** with different *sn*-1-thioalkyl (C_{10-18}) and *sn*-2-fattyacyl (C_{12-18}) substituents have also been prepared [48].

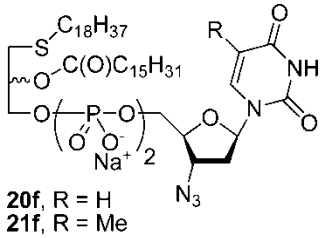
Several studies [43, 45, 48, 49] have reached the general conclusion that a thioether at the *sn*-1 position and an *sn*-2 fatty acyl chain can improve the antitumor activity of lipids, suggesting that compounds such as those in scheme 4 hold potential as pro-drugs of *ara*-C. The chemistry of pyrophosphate-linked sulfur-containing lipids also encompasses some anti-HIV nucleoside conjugates [27]. Linkage of (±)-1-S-octadecyl-2-O-palmitoyl-1-thioglycerol 3-phosphate (**17f**) to nucleoside 5′-monophosphoromorpholidates by established protocols [43, 45] afforded conjugates **20–22f** [27]. Acting as pro-drugs, compounds **20f** and **21f** delivered AZT (3′-azido-3′-deoxythymidine) and AzddU (3′-azido-2′,3′-dideoxyuridine),



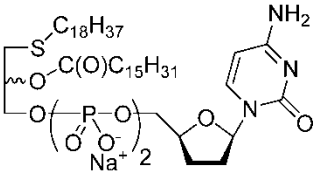
Reagents and conditions: i) NaH, R^2I; ii) $C_{15}H_{31}C(O)Cl$, py, C_6H_6 iii) BF_3MeOH, CH_2Cl_2; iv) TBDMSCl, Im, DMF; v) TBAF/THF, HOAc; vi) $POCl_3$, Et_3N; vii) pyridine, *ara*-CMP-morpholidate; viii) pyridine, cytidine 5' phosphoromorpholidate.

SCHEME 4

respectively, and exhibited longer half-lives than when administered by a direct introduction method [27]. Furthermore, when administered in a micellar formulation at concentrations as low as 0.58 μM, conjugate **20f** showed high *in vitro* anti-HIV activity, protecting 80% of HIV-infected SEM cells [27]. The extra lipophilicity of the long chain alkylthio (*vs* alkoxy) derivatives is believed to be an important contributor to facile and efficacious micelle formation [48].



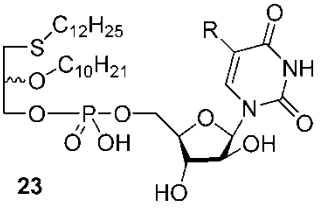
20f, R = H
21f, R = Me



22f

The use of ether/thioether-linked lipids rather than ester-linked lipids as pro-drug conjugates has also proved successful [50]. Compounds **23** and **24** represent conjugates of the chemotherapy drugs *ara*-C and gemcitabine (dFdC), respectively, and were prepared using methods already introduced [51, 52]. DFdC conjugate **24** proved superior to **23** against a number of

tumor cell lines, likely because it formed lipid soluble aggregates able to pass through the cell membrane more readily [50].



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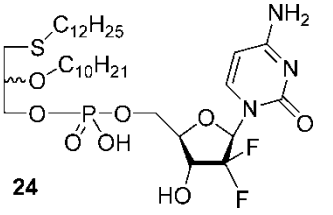
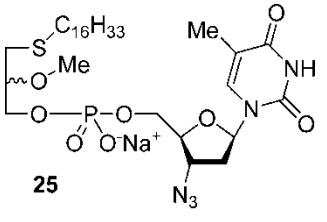


24

Other conjugates, including compounds **25**, **26** and **27** have also been prepared. Although the thioether of **25** provided no advantages over analogs possessing ether or amide linkages, this compound did show increased cytotoxicity for uninfected CEM-SS cells compared to the amide-linked congener [51]. Conjugate **26** was prepared by adaptations to the *sn*-2 ether side chain [28]. This latter compound exhibited activity and selectivity equal or superior to AZT in a number of assays, including a test for antiviral activity against wild-type HIV-1. Moreover, it was not cytotoxic to five different cell types [28]. Tritiated analog **27** (3′-azido-3′-deoxy-5′-(1-[9,10-^{3}H]-*S*-octadecylthio-2-*O*-methoxypropyl)-phosphothymidine-[methyl-^{3}H]) was synthesized in order to establish which bond breaking processes were important in the cell [53].



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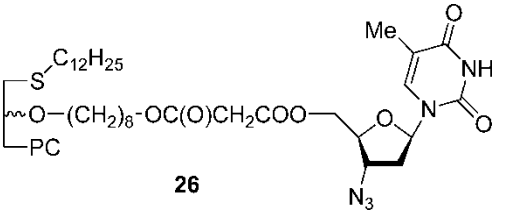

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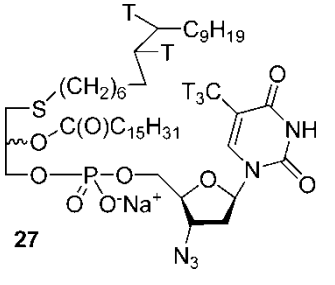
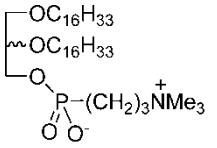


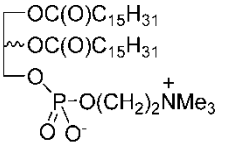
27

Another application for ether/thioether-linked lipids involves their use in synthetic lung surfactants [54, 55]. Endogenous pulmonary surfactant is essential for normal breathing, and its deficiency or dysfunction gives rise to potentially lethal respiratory failure in premature infants with the respiratory distress syndrome (RDS) and in patients of any age with clinical acute lung injury (ALI) or the acute respiratory distress syndrome (ARDS) [56]. Native lung surfactant is a mixture of glycerophospholipids and specific apoproteins synthesized in type II pneumocytes in the alveolar epithelium [56]. The predominant surface tension lowering lipid component in native lung surfactant is **29**, dipalmitoyl phosphatidylcholine (DPPC). Diether phosphonolipid analog **28** is a very promising substitute for DPPC in synthetic exogenous lung surfactant preparations [54, 55, 56–61]. Not only can **28** match the ability of DPPC to reach extremely low surface tensions in compressed surface films, but it also exhibits superior adsorption and film spreading behaviors while incorporating a resistance to degradation by

lytic phospholipase enzymes that can be present in the pulmonary interstitium and alveoli during inflammatory injury [54, 55–61].



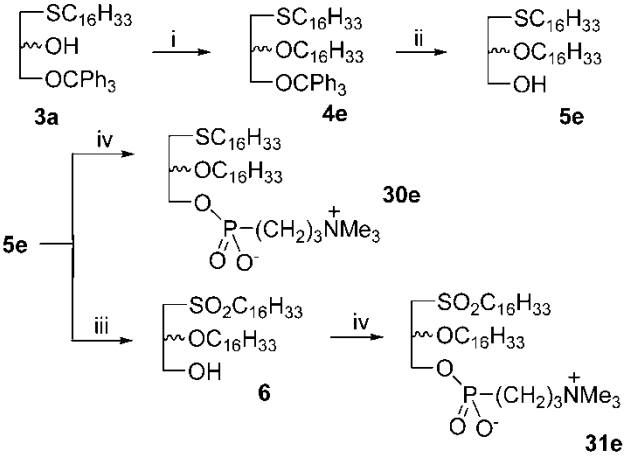
28



29, DPPC

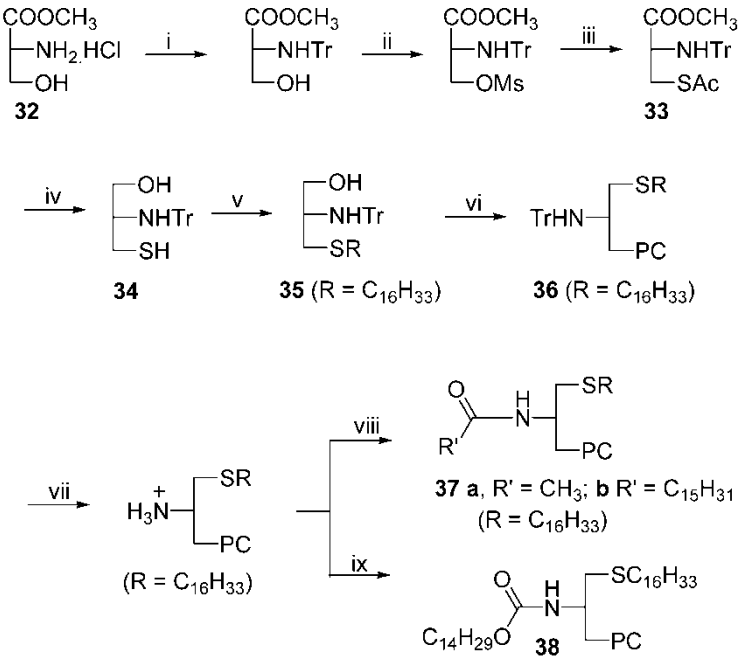
Recently, sulfur-containing analogs of **28** have been prepared using a protocol closely related to that of scheme 1 [54, 55]. Small differences in preparative methodology included modified conditions for alkylation at the *sn*-2 position [62], the employment of p-TsOH in aqueous MeOH for detritylation, and the use of *m*CPBA for the sulfide to sulfone oxidation. Attachment of the phosphocholine group is detailed in scheme 5 [54]. The thio-containing lipid **30e** was found to be less surface-active than lipid **28** as a synthetic lung surfactant component based on assessments using the Wilhelmy balance and pulsating bubble surfactometer [54, 55]. However, the sulfonyl-containing lipid **31e** was similar to **28** in reaching extremely low surface tensions <1 mN/m in studies on the Wilhelmy balance and pulsating bubble [54, 55]. As with **28**, lipid **31e** is both chemically and biophysically resistant to inhibition by phospholipase A_2 [55].

Enantiopure *sn*-1 thioether-containing lipids have been targeted by many research groups and several synthetic strategies have been adopted. Again, pursuing sulfur analogs of PAF [25], Bhatia and Hajdu [63, 64] identified D-serine methyl ester (**32**) as a starting point for the stereospecific synthesis of 1-thioalkyl-2-acylaminodeoxy-*sn*-glycero-3-phosphocholines (scheme 6). To begin, the amine from the serine derivative (**32**) was protected with a trityl group. The hydroxy group was then activated by mesylation enabling its displacement by thiolacetate to afford thiolester **33** [64]. Subsequent exposure to $LiAlH_4$ effected the reduction of both ester and thiolester groups to mercapto alcohol **34**. Alkylation of **34** with hexadecyl iodide yielded thioether **35**. Treatment with the phosphorylating reagent, 2-chloro-2-oxo-1,3,2-dioxaphospholane in benzene, led to the cyclic triester intermediate which, upon



Reagents and conditions: i) $C_{16}H_{33}Br$, KOH, DMSO; ii) *p*TsOH, 95% MeOH; iii) *m*CPBA, CH_2Cl_2; iv) 1. $Cl_2P(O)(CH_2)_3Br$, Et_3N, $CHCl_3$; 2. aq. Me_3N, $CHCl_3/CH_3CN/iPrOH$, 2 days, 60 °C.

SCHEME 5

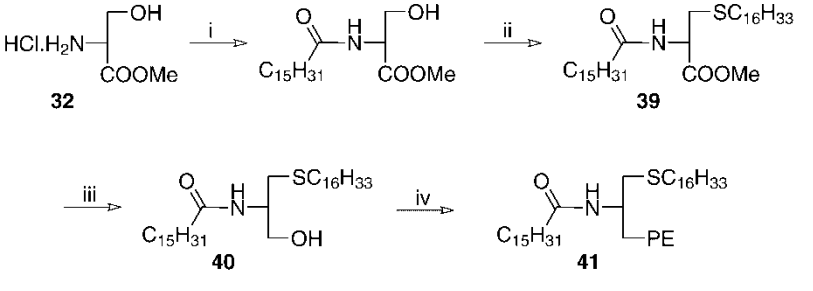


Reagents and conditions: i) TrCl, Et_3N, $CHCl_3$; ii) $MeSO_2Cl$, DMAP, $CHCl_3$; iii) $MeC(O)S^-K^+$, MeCN; iv) $LiAlH_4$, THF; v) $CH_3(CH_2)_{15}I$, MeONa, MeOH; vi) 1. 2-chloro-2-oxo-1,3,2- dioxaphospholane, Et_3N, benzene, 2. MeCN, $(CH_3)_3N$, 65 °C; vii) CF_3COOH, $CHCl_3$;viii) RCOCl, DMAP, $CHCl_3$; ix) $CH_3(CH_2)_{13}OCOC_6H_4$-NO_2-p, DMAP, $CHCl_3$.

SCHEME 6

exposure to anhydrous trimethylamine in acetonitrile, gave the N-protected phosphocholine **36** [63, 64]. TFA was used for the deprotection of **36**, freeing a nitrogen which could be functionalized to amides **37** or carbamate **38**, the former of which inhibits phospholipase A_2 found in bee venom [63].

A complementary and apparently shorter preparation of 1-thio-2-amido phospholipids was presented by Yu and coworkers [65] in 1990. The blueprint (scheme 7) commenced by securing



Reagents and conditions: i) $C_{15}H_{31}COCl$, DMAP, Et_3N, $CHCl_3$; ii) 1. TsCl, Et_3N, $CHCl_3$, 2. $C_{16}H_{33}SH$, MeONa/MeOH; iii) $LiBH_4$, MeOH; iv) 1. $PhthCH_2CH_2OPOCl_2/Et_3N$, py, 2. NH_2NH_2, abs. EtOH.

SCHEME 7

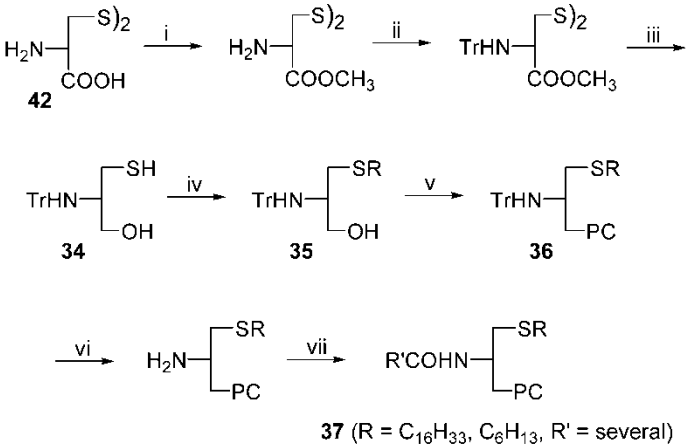
an acyl group in on D-serine methyl ester (**32**). The alcohol was converted to a tosylate, which allowed thiolate substitution forming sulfide **39**. Reduction of the ester to a carbinol created a glycerol analog (**40**) ready for head group chemistry [65]. In this instance, the phosphorylethanolamine (PE) group was put in place using 2-phthalimidoethylphosphoryl dichloride [66] followed by hydrazinolysis. Lipid **41** (1-hexadecylthio-2-(palmitoylamido)-1,2-dideoxy-*sn*-glycerol-3-phosphorylethanolamine) was obtained in 30% yield from **39** [65].

Lipid **41** along with several other lipids including **37b**, **38**, and DPPC, were evaluated for their inhibition of cobra venom phospholipase A_2 (*Naja naja naja*) [65]. In an assay that involved the lysis of S-C bonds of dithiolester-based lipids (vide infra), **41** exhibited an IC_{50} of 0.45 μM, a value superior to seven other lipids. The 2-amido group is generally responsible for tighter binding in the active site, and the thioether lipid **37b** was significantly more active than its ether analog [65].

An alternative to the use of a serine derivative for the preparation of enantiopure thiolipids is the direct deployment of a sulfur-containing amino acid. Thus, Yu and Dennis [67] documented D-cystine (**42**) as a suitable starting material. Using a plan that had parallels to scheme 6, 1-thio-2-(tritylamino)-1,2-dideoxy-*sn*-glycerol **34** was prepared by esterification, tritylation, and then reduction of D-cystine. Sulfur functionalization was achieved with iodohexane or iodohexdecane in the presence of sodium methoxide to form **35** [67]. Phosphorylation of **35** with 2-bromoethyl phosphorodichloridate, and reaction with trimethylamine offered phosphocholine **36**. After detritylation and acylation by acids or acyl chlorides of different chain lengths, a library of 1-(alkylthio)-2-(acylamino)-1,2-dideoxy-*sn*-glycerol-3-phosphocholines (**37**) was created (scheme 7) [67]. Although the text of the paper does not explicitly indicate which analogs of **37** were prepared, analysis of the tables of inhibition data suggest there were 12 versions of **37** where $R = C_6H_{13}$, $R' = H$ through $C_{11}H_{23}$ and 15 analogs where $R = C_{16}H_{33}$, $R' = H$ through $C_{19}H_{39}$ [65]. The ability of thioether amide lipids to inhibit phospholipase A_2 activity [67, 68] was the primary motivation for the synthesis of this collection of lipids **37**. The short chain series of lipids (**37**, $R = C_6H_{13}$) was used to establish the key interactions of the monomeric lipid. It was confirmed that the *sn*-2 amide side chain was crucial for efficient binding. The fatty alkyl analogs (**37**, $R = C_{16}H_{33}$), on the other hand, were evaluated in micellar form. Enzyme inhibition was found to be far less dependent on *sn*-2 chain lengths of these latter compounds [67].

Still within the realm of 1-thio-2-acylamino-3-phosphocholines, Garrigues [69] prepared (*S*)-2′-aza-3′-thio-PAF analog (**43**) in enantiopure form (scheme 9). First, a suitable protocol was established on the racemate [69]. To secure the desired enantiomer, commercially available (*S*)-*N*-acetylcysteine (**44**) was S-alkylated with 1-bromooctadecane. The carboxyl group was converted to a mixed anhydride, which then was responsive to sodium borohydride reduction (scheme 9). The phosphocholine group was then affixed to 2-acetylamino-3-octadecylthiopropanol (**45**) using 2-chloro-2-oxo-1,3,2-dioxaphospholane, followed by trimethylamine [69]. The method does not require the carboxylic acid to be in the ester form [69].

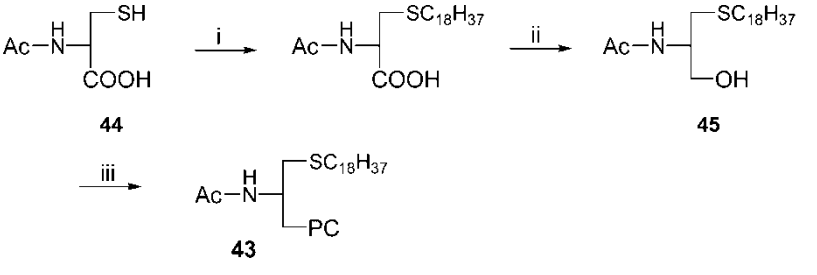
Bhatia and Hajdu [38] studied the stereospecific synthesis of thioether phospholipids by using an L-glyceric acid derivative as a chiral precursor (scheme 5). Accordingly, commercially available 2,3-isopropylidine-L-methyl glycerate **46** was converted in three steps to carbinol **47**, the first of which created L-methyl glycerate [70] (scheme 10). The alcohol group of **47** was activated by nosylation and reacted with potassium thiolacetate in acetonitrile providing a thiolester [38]. Next, reductive cleavage by $LiAlH_4$ produced thiol **48** (scheme 10). After *S*-octadecylation, (+)−**4c** was deprotected to alcohol (−)−**5c**. Applying 2-chloro-2-oxo-1,3,2-dioxaphospholane in the presence of Et_3N formed the cyclic triester; a ring which, when opened with Me_3N, yielded the thioether phosphocholine (−)−**7c**. The overall sequence is an extension of a preparation of enantiopure *sn*-1 fattyalkyl ethers introduced in the same



37 (R = $C_{16}H_{33}$, C_6H_{13}, R' = several)

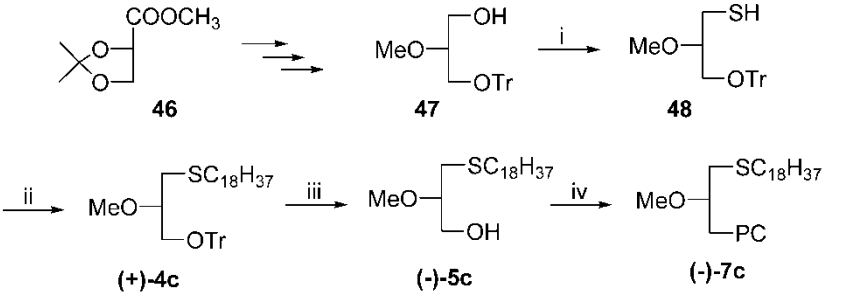
Reagents and condition: i) $SOCl_2$, MeOH, reflux; ii) TrCl, Et_3N, $CHCl_3$; iii) $LiAlH_4$, THF; iv) RI, MeONa/MeOH; v) 1. $BrCH_2CH_2OPOCl_2$, py, $CHCl_3$, 2. Me_3N, $CHCl_3$/2-propanol/DMF (1:5:5); vi) CF_3COOH; vii) R'COCl, Et_3N, $CHCl_3$ or R'COOH, 1, 1'-carbonyldiimidazole.

SCHEME 8



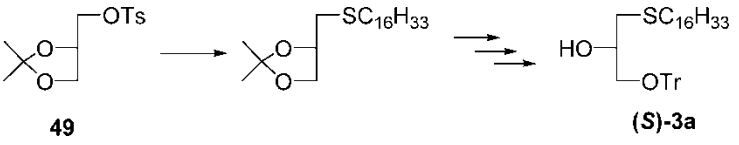
Reagents and conditions: i) $C_{18}H_{37}Br$, KOH, EtOH; ii) 1. *i*-C_4H_9OCOCl, Et_3N, THF 2. $NaBH_4$, H_2O; iii) 1. 2-chloro-2-oxo-1,3,2-dioxaphospholane, Et_3N, THF 2. Me_3N, CH_3CN.

SCHEME 9



Reagents and conditions: i) 1. *p*-$O_2NC_6H_4SO_2Cl$, DMAP, $CHCl_3$, 2. $CH_3COS^-K^+$, MeCN, 3. $LiAlH_4$, ether; ii) $C_{18}H_{37}I$, MeONa/MeOH; iii) HCl, $CHCl_3$-MeOH; iv) 1. 2-chloro-2-oxo-1,3,2-dioxaphospholane, Et_3N, C_6H_6, 2. Me_3N, MeCN, 65°C.

SCHEME 10



49 **(*S*)-3a**

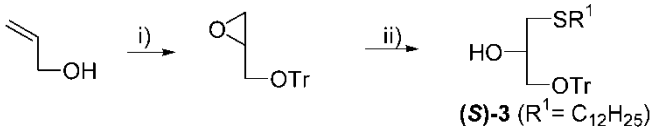
SCHEME 11

publication as non-hydrolyzable PAF analogs [38]. Though a lone thiolipid was prepared by Bhatia and Hajdu [38], the synthetic method is sufficiently flexible to permit various alkyl groups at both the *sn*-1 and *sn*-2 positions.

Another viable source of the optically enriched glycerol unit is chiral isopropylideneglycerol tosylate or solketal tosylate (**49**) [71] (scheme 11). The alcohol precursor is commercially available or can be made through the cleavage of mannose [72]. This gambit has been used to generate a series of phospholipids bearing a tetrahedral phosphonate functionality at the *sn*-2 site [71], and also a series of 1-*S*-fattyalkylthioglycerol derivatives of Foscarnet, a drug used for the treatment of AIDS-related vision problems [73].

Alternatively, gycidol represents one of the most direct implementations of the glycerol framework. Enantiopure glycidol is now commercially available in both forms, but the Sharpless epoxidation protocol also serves as a point of access to these compounds [74, 75] (scheme 12). Hendrickson found the one-pot sequence of Sharpless epoxidation and tritylation of allyl alcohol afforded tritylated glycidol in about 50% yield and >98% ee [75]. The utility of this method is compounded by the fact that ring-opening with dodecyl mercaptan, a representative thiol, occurred exclusively at the *sn*-1 position. The overall protocol has been incorporated into the syntheses of thioether and thiolester [75] lipids, not to mention *sn*-2 phosphonate [76] analogs of phospholipids.

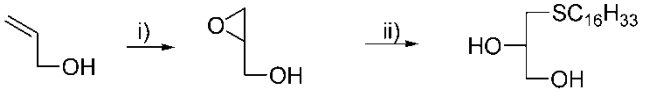
Obviating the tritylation step of scheme 12, Byun and Bittman [74] showed that under the proper conditions, thiols could open glycidol directly (scheme 13). After Sharpless epoxidation, the regioselective addition of hexadecanethiol was achieved in the presence of a 10-fold excess of $NaBH_4$. Proceeding with either of the glycidol enantiomers permitted the chiral synthesis of both ET-18-OMe thio analogs [74]. Building on of this work, Bittman *et al.* [77] used 3-hexadecyl glycerol to construct a butanephosphonate lipid analog ET-18-OMe.



(*S*)-3 (R^1= $C_{12}H_{25}$)

Reagents and conditions: i) 1. $Ti(O\text{-}iPr)_4$, cumene hydroperoxide, L-(+)-diisopropyl tatrate; 2. TrCl, Et_3N; ii) cat. BuLi, $C_{12}H_{25}SH$, THF.

SCHEME 12



Reagents and conditions: i) 1. $Ti(O\text{-}iPr)_4$, cumene hydroperoxide, L-(+)-diisopropyl tatrate; 2. $P(OMe)_3$; ii) 1. $NaBH_4$, $C_{16}H_{33}SH$, iPrOH; 2. NaOH.

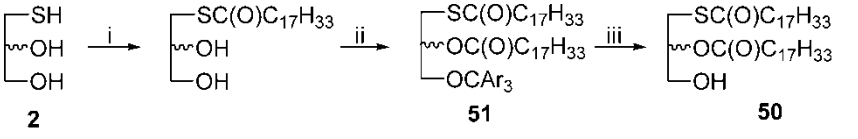
SCHEME 13

1.2 *Thiolester lipids*

Despite the similarities between alkylation and acylation, the methods outlined for sulfur alkylation are not necessarily amenable to sulfur acylation. Indeed the selectivity of sulfur over oxygen when reacting with alkyl halides is seldom observed in acylation reactions. Hence, the monoacylation of thioglycerol (**2**) may give a mixture of products [78] and, depending on reaction conditions or subsequent treatment, the immediate product may succumb to acyl migration [79].

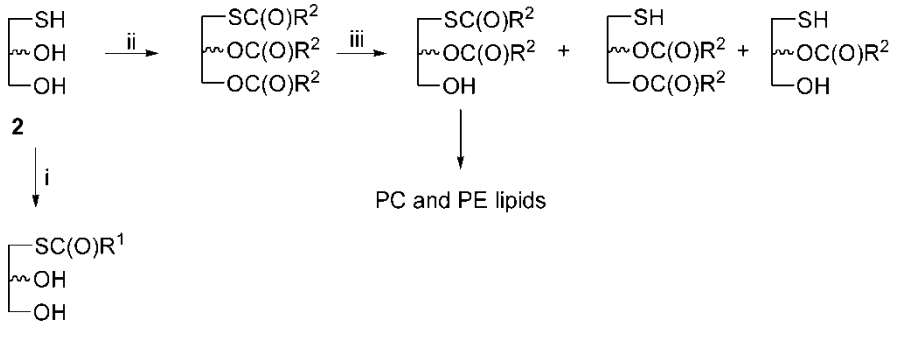
Kucera *et al.* [80] developed conditions for the selective and efficient preparation of 1-*S*-oleyl-2-*O*-oleylglycerol (**50**) from **2** through consecutive *S*-acylation, *sn*-3-protection, *sn*-2-acylation and then deprotection (scheme 14). No attempt was made towards purification until *sn*-2-acylation, after which the product, 3-*O*-dimethoxytrityl-1-*S*-oleyl-2-*O*-oleylglycerol (**51**), could be isolated in respectable 73% from **2** [80]. Presumably, the researchers chose to implement the purification step only after full occupation of all the nucleophilic atoms; thus, precluding any unwanted isomerization. After deprotection, the PC phospholipid preparation was finalized [80].

Alternatively, thioglycerol can be fully acylated at all nucleophilic sites and then, using *Rhizopus delemar* lipase, deacylated at the *sn*-3 position [81]. The reaction is low yielding, unselective and does not proceed completely, yet it afforded adequate alcohol (26%) for conversion to PC and PE derivatives (scheme 15) [82]. The direct and selective sulfur acylation of thioglycerol, used in excess, was also reported in this study [82]. The acylation was achieved in 30% yield, though no mention was made about other reaction products.



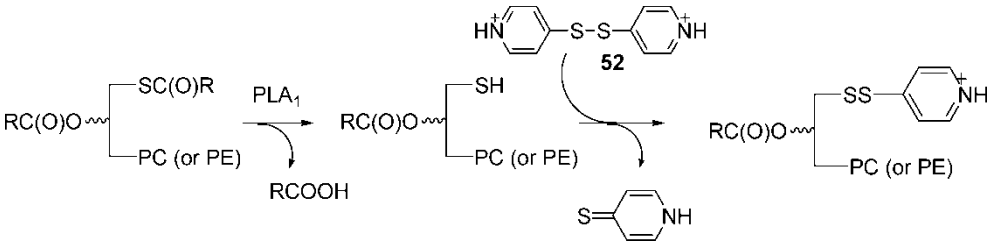
Reagents and conditions: i) 0.5 equiv. oleoyl chloride, Et_3N, C_6H_6/DMF, 30 min. 0 °C ii) 1. $(MeOC_6H_4)_2PhCCl$, py, 72 h, 25 °C, 2. oleoyl chloride, py/DMF, 24 h, 25 °C, 73% overall; iii) $B(OH)_3$, $B(OMe)_3$, 42%.

SCHEME 14



Reagents and conditions: i) 0.25 equiv. R^1 RC(O)Cl, Et_2O, py, 30% (R^1 = C_9H_{19} or $C_{15}H_{31}$); ii) 5 equiv. $R^2C(O)Cl$, $CHCl_3$, py, 95%; iii) *R. delemar*, 0.2 M NaOAc (aq), $CaCl_2$ (R^2 = C_9H_{19}).

SCHEME 15



SCHEME 16

Other investigations in the glycerol series have indicated that thioacetic and thiobenzoic acids regioselectively ring-open glycidol and glycidol esters [79]. Having prepared scalemic trityl-protected glycidol (scheme 12), Hendrickson and Hendrickson [75] and showed that thioldecanoic acid added to the oxirane in high yield (94%), placing a thiolester at the *sn*-1 position of the glycerol backbone. Efficient s*n*-2 acylation (99%) and deprotection afforded cleanly the phosphocholine precursor although no yields were offered for the deprotection or for the PC installation [75].

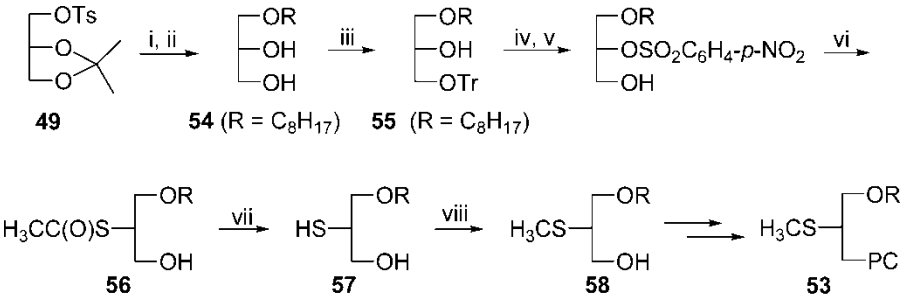
Thiolester *sn*-1 phospholipid analogs are also utilized as substrates for assessing phospholipase A_1 (PLA_1) activity. PLA_1 selectively hydrolyzes the thiolester group at the *sn*-1 position, and an assay focused on the thiol functionality has been developed to assess this enzymatic activity [80, 82]. The nucleophilicity of the thiol and its position on the glycerol backbone allowed hydrolyzed thiolesters to be distinguished from typical esters. As shown in scheme 16, the presence of commercially available 4,4′-dithiopyridine (DTP, Aldrithiol 4®, **52**) during enzymatic hydrolysis permits the reaction of thiol and diaryl disulfide. The leaving group takes the form of 4-thiopyridone, which has an absorption maximum at 324 nm [82]. Given these properties, the growth of the 324 nm absorption can be monitored by continuous spectrophotometry and related to kinetic parameters of PLA_1 activity [80]. The assay is also attractive for assessing the amount of synthetic lipid with sulfur at the *sn*-1 position. 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) has also been used for this purpose [78].

2. Sulfur-containing *sn*-2 phospholipids

Previous sections have demonstrated the need for judicious use of protecting groups for the preparation of *sn*-1 sulfur-containing lipids, particularly when a distinct group is to occupy the *sn*-2 position. Similar considerations apply to the case when the sulfur-containing moiety is at the *sn*-2 position and a different group occupies the *sn*-1 position. Another prevalent theme in many preparations of *sn*-2 sulfur-containing lipids is the use of a sulfonate ester at the *sn*-2 position. This group conveniently facilitates S_N2 reactions with both alkanethiols and selected thioacids as discussed below.

2.1 *Thioether lipids*

Exemplifying this technique, the stereospecific synthesis of 1-hexadecyl-2-*S*-methyl-2-deoxyglycerophosphocholine (**53**) brings into play a nitrobenzenesulfonate (nosylate) at the *sn*-2 position (scheme 17) [83]. Alkylation and deprotection of solketal tosylate (**49**) provide diol **54**, which is tritylated at the *sn*-3 alcohol. Reacting **55** ($R = C_{16}H_{33}$) with nitrobenzenesulfonyl chloride equips the *sn*-2 position for sulfur substitution [83]. However, it has been



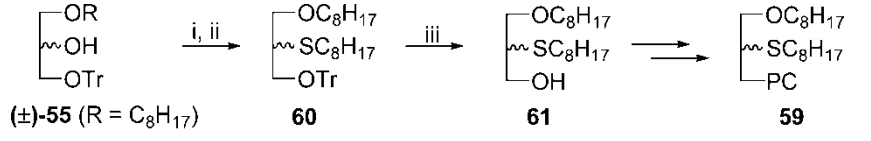
Reagents and conditions: i) $CH_3(CH_2)_{15}OH$, NaH, THF; ii) HCl, MeOH; (65%, 2 steps) iii) Ph_3CCl, Et_3N, toluene, 73%; iv) p-$O_2NC_6H_4SO_2Cl$, DMAP, $CHCl_3$; v) HCl, MeOH-$CHCl_3$; (76%, 2 steps) vi) $CH_3COS^-K^+$, CH_3CN, 85%; vii) $LiBH_4$, Et_2O, rt, 70%; viii) CH_3I, $NaOCH_3$, CH_3OH, 57%.

SCHEME 17

reported to be essential to remove the trityl group before thioacetate could effectively displace the nosylate, affording thiolacetate **56** [84]. Caution is also required to prevent the sulfur-to-oxygen migration of the acetyl group, and Ellman's reagent offers a useful check for free thiol (scheme 17) [83]. Reduction to the thiol (**57**) using, not $LiAlH_4$ but rather $LiBH_4$, and then methylation gave lipid precursor **58**. PC installation using 2-chloro-2-oxo-1,3,2-dioxophospholane completed the phospholipid synthesis [83]. Thiolacetate **56** and thiol **57** were also converted to *sn*-2 thioacyl phospholipids (*vide infra*), confirming the overall usefulness of this preparative route [83]. The phosphocholine formed in scheme 17, along with a number of synthetic phospholipids, demonstrated a high therapeutic index when tested for their selective toxicity toward neoplastic cells [85].

To study the structure-activity of phospholipid analog towards inhibition of phospholipase A_2 activity in ionophore A23187 stimulated macrophages, Letourneux *et al.* [86] prepared racemic 1-octyl-2-octylthio-2-deoxyglycerophosphocholine (**59**). They found it had a higher inhibitory effect than mepacrine, dexamethasone or bromophenacyl bromide at corresponding concentrations; although, it was less effective than *sn*-2 ether or amide analogs. The lipids were chosen for this study based on their resistance to lipase hydrolysis.

Secondary alcohol **55** ($R = C_8H_{17}$) (scheme 18) was prepared from solketal in two steps by way of the mesylate [87]. The hydroxy group was activated with tosyl chloride, permitting nucleophilic displacement [86]. The resulting octylthio derivative **60** was deprotected with $ZnBr_2$-MeOH to give **61**. PC installation using 2-chloro-2-oxo-1,3,2-dioxophospholane ensued (scheme 18) [86]. No mention of reaction yields or information on the difficulty of the



Reagents and conditions: i) TsCl, DMAP, CH_2Cl_2; ii) $C_8H_{17}SH$, NaH, THF; iii) $ZnBr_2$-MeOH, CH_2Cl_2.

SCHEME 18

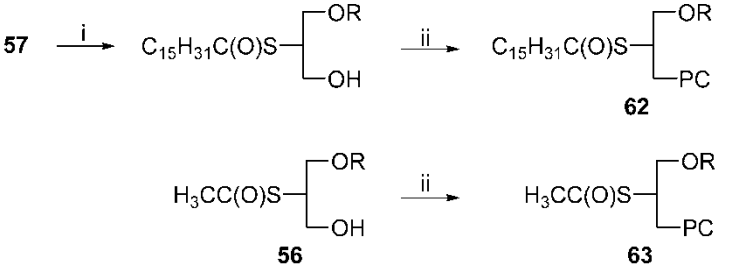
thiolate substitution on the tosylate was given in this paper, and experimental descriptions provided minimal detail. Presumably, the thiolate, a stronger nucleophile compared to thioacetate, can effect the substitution more readily but this was not specifically documented [86].

2.2 *Thiolester lipids*

As previously indicated, thiols resulting from lipase action on thiolester linkages permit the quantitation of kinetic parameters for the lipase. Phospholipase A_2 is specific for the *sn*-2 ester linkage, and it follows that *sn*-2 acylthio linkages can be similarly engaged to evaluate the kinetics and specificity of different forms of this enzyme. Again, the creation of the thiol group during hydrolysis permits the use of spectrophotometric analytical techniques [88, 89]. Accordingly, a number of *sn*-2 acylthio-containing phospholipid analogs have been prepared to evaluate the activity of phospholipase A_2 enzymes.

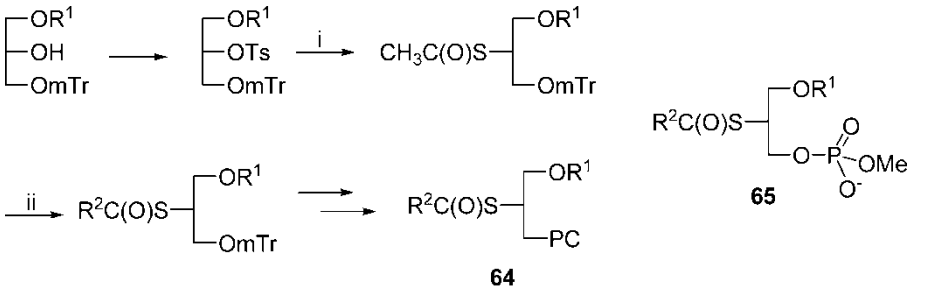
As in scheme 17, thiolacetate **56** can act as a source of *sn*-2 thioether lipids [83], but it can also itself be converted to a phosphocholine. Indeed, **56** was used as a thiolester containing substrate by Bhatia and Hajdu [83, 84] to evaluate the two common phosphorylation methods. In the comparison, phosphorylation by 2-chloro-2-oxo-1,3,2-dioxophospholane and subsequent amination proceeded in 65% yield, whereas the use of 2-bromoethyl phosphorodichloridate [84] followed by amination yielded 47% of phospholipid [83]. Thiol **57** was readily acylated, selectively placing a longer chain at the *sn*-2 sulfur. Next, 2-chloro-2-oxo-1,3,2-dioxophospholane was adopted to effect PC incorporation and eventual phospholipid synthesis. The stereochemical purities of lipids **62** and **63,** assessed using bee-venom phospholipase A_2, were very high. In addition, PAF analog **63** has proved to be a potent hypertensive agent [83, 84].

Aarsman *et al.* [88] synthesized a series of short-chain phospholipids analogs with acylthiolester bonds at the *sn*-2-position (scheme 20). Reminiscent of the sulfur chemistry in scheme 17, the synthetic procedure called for an initial alkylation 1,2-isopropylidene-*sn*-glycerol [90]. However, deviations from aforementioned chemistry include use of tosylate rather than nosylate, methoxytrityl as the *sn*-3 protecting group, and the successful reaction of potassium thiolacetate in a crowded steric environment about the *sn*-2 position. Lipids **64**, with R^1 = octyl or hexadecyl and R^2 = methyl, pentyl or hexyl, can be used with spectrophotometric assays of PLA_2 from *porcine pancreas* which has also confirmed the expected stereochemical configuration [88]. The same protocol was employed by Wheeler *et al.* [91] to construct the backbone of a large number of a phospholipid analogs (**65**), which included significant aryl, allyl and alkyl variations at R^1. Lipids **65** were part of a larger collection of phospholipids



Reagents and conditions: i) $CH_3(CH_2)_{14}C(O)Cl$, DMAP/$CHCl_3$, rt. 20 h, 90%;
ii) 1. 2-chloro-2-oxo-1,3,2-dioxophospholane, Et_3N/C_6H_6, rt, 24 h,
2. Me_3N/CH_3CN, 65 °C, 24 h (**62**: 35%; **63**: 65%).

SCHEME 19



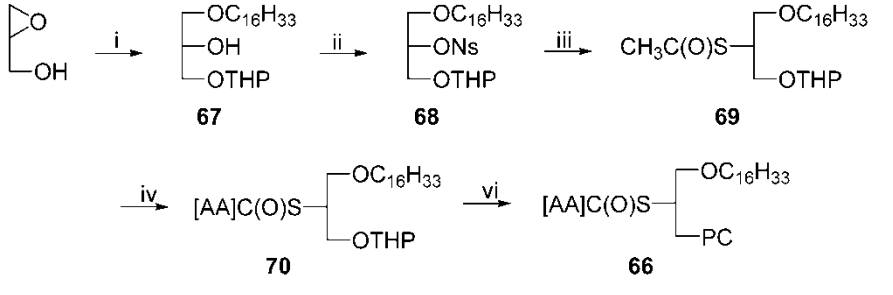
Reagents and conditions: i) $CH_3COS^-K^+$, DMF; ii) 1. $NaBH_4$, 2. R^2COCl.

SCHEME 20

synthesized to probe the active site of human synovial fluid phospholipase A_2, an enzyme known to contribute to tissue inflammation [91].

Dennis and coworkers [89] reported a non-radioactive, spectrophotometric, microtiterplate assay for human cystolic phospholipase A_2 (cPLA$_2$) utilizing 1-hexadecyl-2-arachidonylthio-2-deoxy-*sn*-glycero-3-phosphorylcholine (**66**), a phosphatidylcholine derivative with an arachidonoylthiolester at the *sn*-2 position and an alkyl ether at the *sn*-1 position (scheme 21). This lipid is specific for cPLA$_2$ and is much better suited for high throughput screening of cPLA$_2$ inhibitors than are conventional radioactive assays. The assay provides a highly sensitive assessment of enzymatic activity through the use of 5,5′-dithiobis(2-nitrobenzoic acid) (Ellman's reagent, DTNB) as a thiol-sensitive reagent [89]. The chemistry parallels that of scheme 16, producing 5-mercapto-2-nitrobenzoic acid, which has an absorption maximum of 410 nm. Thus, cPLA$_2$ inhibitory activity can be determined spectrophotometrically in 96-well microtiter plates [89]. Studies of phospholipase A_2-induced release of arachidonate have important implications related to prostanoid production and membrane signal transduction [92, 93].

In terms of specific synthesis methods, Dennis and coworkers [89] prepared lipid **66** starting from (*R*)-glycidol. After THP protection and alkoxide attack placing a hexadecyloxy group at the *sn*-1 position of alcohol **67**, the preparation closely followed scheme 20 [89]. Achiwa and coworkers [16] also completed the synthesis of lipid **66** from **67**, but utilized methods requiring a greater number of synthetic steps to obtain this alcohol. Nonetheless, high yields



[AA] = $CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2$-

Reagents and conditions: i) 1. 2,4-dihydro-2H-pyran, pyridinium p-toluenesulfonate, 50%; 2. $CH_3(CH_2)_{15}OH$, NaOH, 32%; ii) through vi), see Table 1.

SCHEME 21

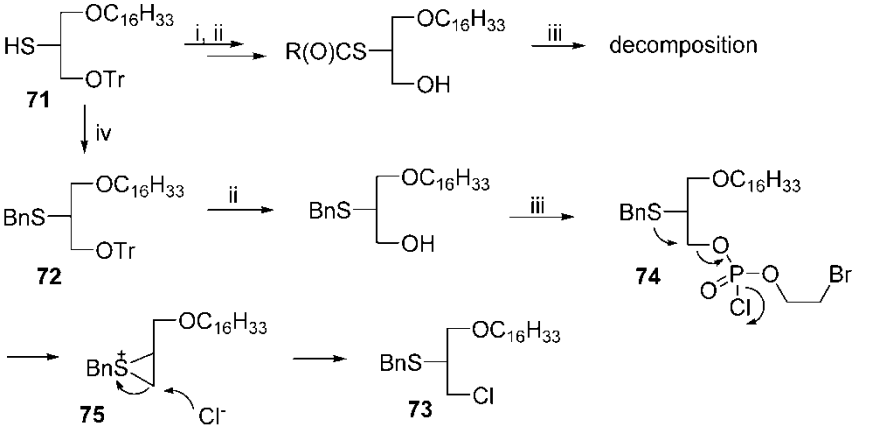
Table 1. Comparative yields of steps converting alcohol **67** to phosphocholines in the studies of Dennis *et al.* [89] and Achiwa *et al.* [16].

	Dennis *et al.* [89]		Achiwa *et al.* [16]	
Reaction step (product)	reagent/conditions	yield	reagent/conditions	yield
nitrobenzenesulfonation (**68**)	p-$NO_2C_6H_4SO_2Cl$/1 eq. DMAP, CH_2Cl_2, rt	78%	p-$NO_2C_6H_4SO_2Cl$/cat. DMAP/py, CH_2Cl_2, rt	80%
sn-2 thioacetate substitution (**69**)	$CH_3C(O)S^-K^+$, CH_3CN, 50 °C, 8 h.	60%	$CH_3C(O)S^-K^+$, CH_3CN, reflux 3 h.	95%
thiolacetate reduction and sulfur acylation (**70**)	1. $LiBH_4$, THF, reflux 2. Arachidonic acid, CDI, $CHCl_3$, rt, 3 h.[a]	75% 91%	1. $LiAlH_4$, THF, rt 2. arachidonic acid, DEPC, DMF, Et_3N, rt, 2 h.[b]	87% (2 steps)
Removal of THP and PC introduction (**66**)	1. PPTS, EtOH, 55 °C 2. $POCl_3$ and 3. choline tosylate	n/a 40% over 2 steps	1. PPTS, EtOH, 55 °C 2. $BrCH_2CH_2P(O)Cl_2$, Et_3N and 3. Me_3N	55% over 3 steps

a) CDI = 1, 1′-carbonyldimidazole; b) DEPC = diethyl phosphorocyanidate

helped to justify the practicability of their approach [16]. Table 1 compares the common steps of Dennis *et al.* [89] and Achiwa *et al.* [16] on the basis of reagent choice and yield. The paper of Achiwa *et al.* [16] is also noteworthy in claiming that the chemistry of scheme 19 [84] is prone to racemization by oxirane formation during the thiolacetylation. Thus the utility this alternative synthesis pathway is further ensconced [16].

Fuji *et al.* [94] endeavored to prepare lipid **66** on a multigram scale using the Dennis method, but the overall yield from (R)-glycidol was only 2%. As a different approach, thiol **71** (scheme 22) was prepared using (*S*)-solketal following established methods including placement of a mesylate at the *sn*-2 position, subsequent thioacetate substitution, and methanolysis [94]. Although the sulfur could be acylated with a number of groups and detritylated, subsequent phosphorylation reactions failed. Benzylation at sulfur to give thioether **72** was a possible alternative for protection, but neither 2-chloro-2-oxo-1,3,2-dioxaphospholane nor (2-bromoethyl)phosphodichloridate could bring about phosphorylation. The reaction using benzyl protection was monitored and chloride **73** was the isolated product. Scheme 22 demonstrates a logical mechanism leading to chloride **73**, one that calls for sulfur anchimeric



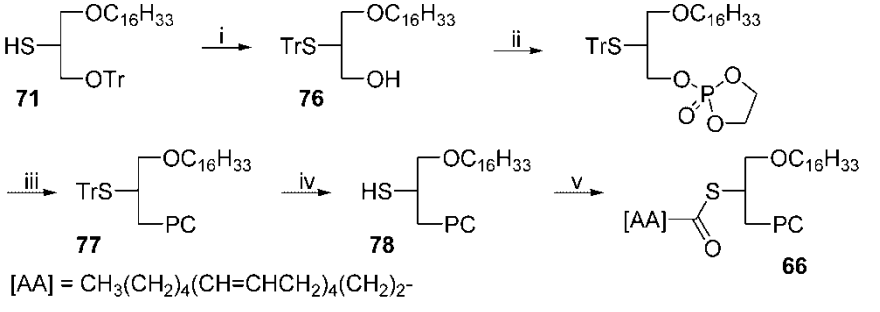
Reagents and conditions: i) acylation; ii) detritylation; iii) phosphorylation attempt; iv) BnBr, DMF, aq NaOH, 15 min., rt, 95%.

SCHEME 22

assistance to decompose intermediate phosphorus compound **74**. Counterattack of chloride on thiiranium ion **75** affords **73** in 85% yield by the (2-bromoethyl)phosphodichloridate method [94].

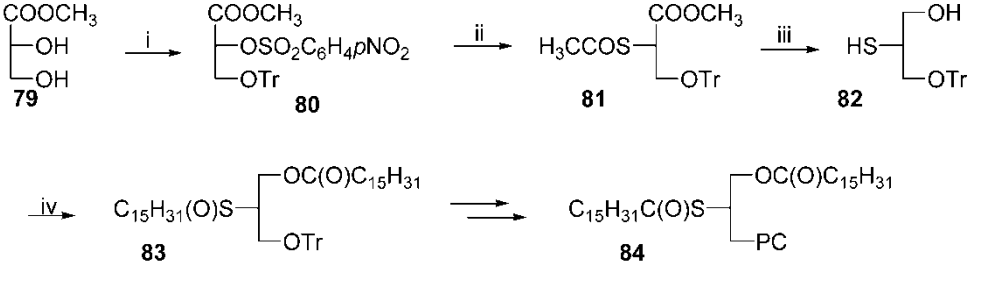
To solve this problem, the authors deliberately induced the participation of the sulfur as a means to its own protection [94]. Hence thiol **71** was treated with BF_3-Et_2O in CH_2Cl_2 to bring about an oxygen-to-sulfur migration of the trityl group. The reaction proceeded in 85% yield to afford alcohol **76**, which could be phosphorylated to phosphocholine **77** (scheme 23). As hoped, the tritylated sulfur atom refrained from anchimeric participation, presumably due to steric implications. However, detritylation of **77** was not trivial. A sequence of silver nitrate and pyridine followed by hydrogen sulfide and pyridine offered conditions sufficiently effective and mild to free thiol **78**. The immediate and specific *sn*-2 placement of arachidonic acid (aa) was achieved in high yield and the overall synthetic protocol supplied >15 g of lipid **66** [94].

The literature provides only a single technique for the preparation of *sn*-1-acyl *sn*-2-thioacyl glycerophospholipids [95] (scheme 24). This method begins with 2,3-isopropylidine-L-methyl glycerate (*ent*-**46**), which can be readily converted to D-glycerate ester **79** followed by tritylation and nosylation to afford **80** [95]. This latter *sn*-1-protected nosylate can then be converted to the *sn*-2 thiolester **81**, with LAH treatment reducing both the carboxylic and thiolesters. The resulting mercaptoalcohol (**82**) can be doubly acylated with 2 equiv. of palmitoyl chloride



Reagents and conditions: i) BF_3-Et_2O, CH_2Cl_2 (85%); ii) 2-chloro-2-oxo-1,3,2-dioxaphospholane, Et_3N; iii) Me_3N (2 steps, 70%);
iv) 1. $AgNO_3$, py, 2. H_2S, py; v) arachidonic acid, DCC, DMAP, CH_2Cl_2 (76% from **77**).

SCHEME 23



Reagents and conditions: i) 1. N-trityl pyridinium BF_4^-, CH_3CN; 2. $pNO_2C_6H_4SO_2Cl$, DMAP, py, $CHCl_3$, 89%; ii) $CH_3COS^-K^+$, CH_3CN, 81%; iii) $LiAlH_4$, ether, 60%;
iv) 2 eq. $CH_3(CH_2)_{14}C(O)Cl$, DMAP, $CHCl_3$, 92%.

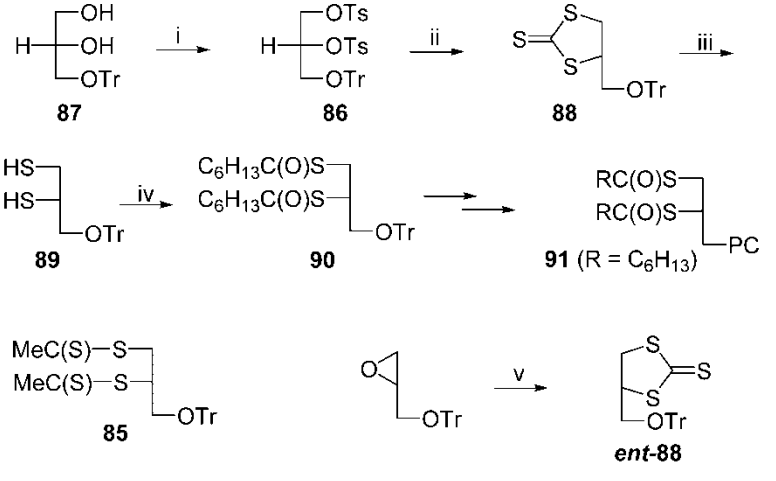
SCHEME 24

giving **83**. Detritylation, without acyl migration, followed by phosphorylation and trimethylamination completed the synthesis of thiophosphatidylcholine **84** [95]. Lipid **84** has been evaluated for its reactivity toward bee venom phospholipase A_2 [96]. Hydrolysis proceeded at the C-S bond with absolute specificity, but at a rate approximately 10X slower than the oxygen analog DPPC. Nevertheless, it was assessed to be suitable for the evaluation of PLA_2 enzymes [96].

3. *sn*-1,2-Dithio lipids

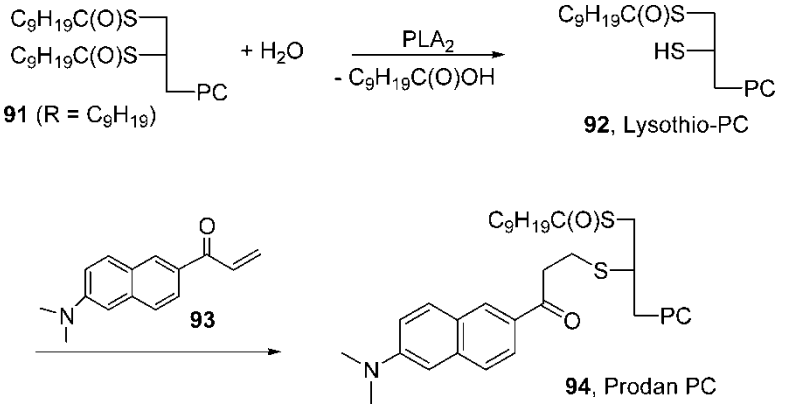
As with the monothioacylated lipid analogs outlined in the sections above, 1,2 bis(thioacyl) glycerophospholipids have been used to evaluate the specificity, mechanisms, and reactive character of phospholipase A_2 [65, 97–102] and other hydrolytic enzymes [80, 103]. Although the logical choice of commercially-available dithioglycerol as a starting material for dithio derivatives has been attempted, yields are low [104] or not reported [105] and alternative methods have been examined.

The generally accepted protocol for preparing *sn*-1,2-dithio lipids is by incorporating the two sulfur atoms in the form of trithiocarbonate, as was first reported by Hendrickson [106] (scheme 25). The discovery of the trithiocarbonates was somewhat fortuitous as the authors intended to prepare 1,2-bis(methylxanthate) **85** by the reaction of potassium methylxanthate and ditosylate **86**. The ditosylate (**86**) was secured through the reaction of 1-trityl glycerol **87** that, in turn, was prepared from D-mannitol. Reaction of **86** and *in situ* derived potassium methylxanthate did not provide a 1,2-bis(methylxanthate), but trithiocarbonate **88** was obtained instead. It was suggested that **85** could indeed have formed, but was rapidly converted to **88** under conditions where a small amount of water was present [106]. Trityl glycidol can also be stereospecifically converted to **88** using the *in situ* generated potassium methylxanthate reagent system [75, 106]. LAH reduction of **88** gave the dithiol **89**, which was immediately acylated at both sulfurs affording **90**. Again, without purification of **90**, the PC headgroup was attached using $P(O)Cl_3$ and choline tosylate to give the di-thio PC target **91** [106].



Reagents and conditions: i) TsCl, pyridine; ii) KOH, CS_2, acetone, MeOH, 40 °C, 24 h, 55%; iii) $LiAlH_4$, dry THF; iv) $C_6H_{13}C(O)Cl$, py, hexane, 66%; v) as per ii) at 50 °C, 6 h, 51%.

SCHEME 25



SCHEME 26

Although the trithiocarbonate method is the usual entrée to dithio lipids, improvements over the original experimental method have been offered [65, 91]. Rigorous purification of dithiol **89** eliminated the need for selected purification steps at later stages of the preparation. Moreover, the resulting expeditious chemistry prevents undesirable acyl migration [65, 91, 107]. In addition, Wheeler *et al.* [91] noted that methoxytrityl, rather than trityl protection, facilitated recrystallization of the trithiocarbonate intermediate.

Other lipids of the type **91** have been prepared, including a number of short chain derivatives [91, 101], the bis(decanoylthio) analog [65, 97–99, 108], and the bis(oleoylthio) analog [80]. Lipids **91** are convenient substrates for phospholipase D (cabbage) in transphosphatidylation reactions [98, 109], so that direct conversion of the bis(acylthioglycerol) to phosphatidylmethanol is also possible using this approach [101, 109].

Most *sn*-1,2-dithio lipids are prepared to assess the activity of different forms of phospholipase A_2 using spectrometric analysis techniques. Hendrickson *et al.* [102] have also used 1,2-bis(decanoylthio)-1,2-dideoxy-*sn*-glycero-3-phosphorylcholine (**91**, $R = C_9H_{19}$) as a fluorescent probe for assessing pancreatic and snake venom phospholipase A_2.

Acrylodan (**92**) is a polarity sensitive fluorescent reagent [110] that reacts with free thiols by a Michael addition (scheme 26). Incubating **91** ($R = C_9H_{19}$) with PLA_2 generates lysothio-PC (**93**) *in situ*, which then reacts with **92** making Prodan-PC (**94**). Adduct **94** shows a maximum absorption in ethanol at 370 nm. The emission maximum is sensitive to the polarity of the environment, ranging from 530 nm in water to 498 nm in ethanol [102].

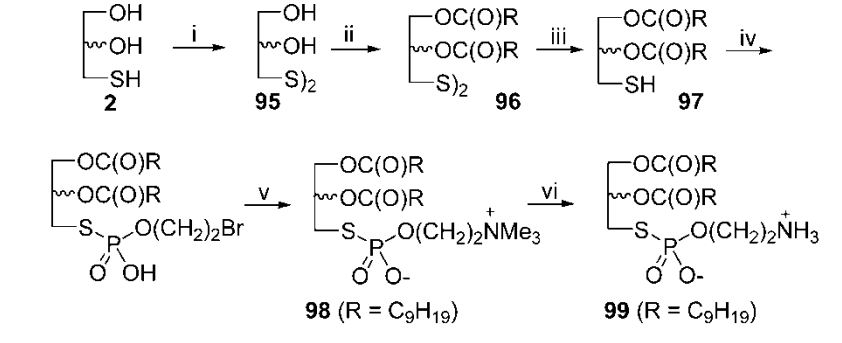
4. Sulfur containing *sn*-3 phospholipids

Incorporating a sulfur atom at the *sn*-3 position of a glycerophospholipid means that adaptations are made to the phosphorus-containing group, as opposed to the carboxylic ester linkage of the other positions. The thiophosphate group is prone to phospholipase C hydrolysis [111, 112] and, in analogy with thiols released from other positions, can be used to evaluate the kinetics of this enzyme by functionalizing the sulfur. This technique has been applied mostly to phosphatidylinositols [113–115].

Both nucleophilic and electrophilic-based protocols have been developed for the construction of the thiophosphate headgroup in *sn*-3 sulfur-containing lipids. For example, 1-thioglycerol (**2**), either in free or acetal form, is a common starting material because of the nucleophilic sulfur. However, the sulfur must initially be protected to permit oxygen acylation

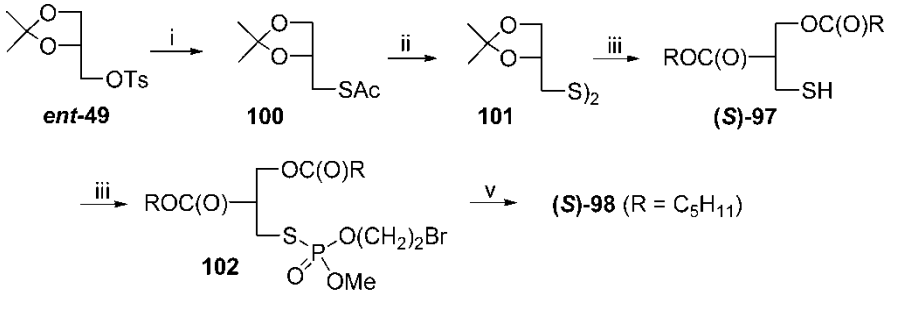
or alkylation. One mode of sulfur protection is through disulfide formation. Cox *et al.* [112] called on $FeCl_3$/air to effect disulfide formation from 1-thioglycerol (**2**). Disulfide **95** could then be peracylated and reduction of **96** with dithiothreitol (DTT) in the presence of alkaline ethanol (pH = 9.6) regenerated the thiol (**97**) (scheme 27). PC installation and eventual formation of **98** followed an established protocol [78] involving the sulfur of **97** selectively reacting at the phosphorus of 2-bromoethylphosphoryldichloride. Thiophosphatidyl ethanolamine analog **99** was accessed through transphosphatidylation of **98** by phospholipase D from green cabbage in the presence of ethanolamine (scheme 27) [112]. Various R groups are amenable to this chemistry, and the initial sulfur oxidation can also be effected with H_2O_2 [111]. Disulfide **95** can be monoacylated at both *sn*-1 positions in low yield (20%) [116], permitting the introduction of different acyl groups at the *sn*-2 positions.

A related preparative method has the diol component of **95** protected as an acetal in a protocol that tolerates the use of chiral glycerol systems such as solketal tosylate (***ent*-49**) [117]. The tosylate is converted to thiolacetate **100** and then to disulfide **101** (scheme 28). The subsequent protocol is similar to that of scheme 27, eventually providing thiol **(*S*)-97** (R = C_5H_{11}). The thioPC headgroup is constructed by thiol and bromoethanol substitution on $MeOPCl_2$, and oxidation provides bromide **102**. This bromide is converted to lipid **(*S*)-98** by deprotection and



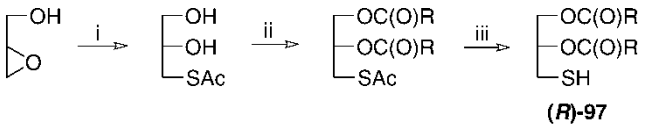
Reagents and conditions: i) $FeCl_3$, air, 70-80%; ii) 5 equiv. RC(O)Cl, pyridine, 75%; iii) DTT, EtOH, NH_4OH, 100%; iv) 1. $Br(CH_2)_2OP(O)Cl_2$, Et_3N, $CHCl_3$, 2. aq. KCl; v) Me_3N, MeOH, 35% from **97**; vi) $HO(CH_2)_2NH_2$, cabbage PLD, 36% as NaCl salt.

SCHEME 27



Reagents and conditions: i) $MeC(O)S^-K^+$, MeCN, 18-c-6, 97%; ii) NaOH, MeOH, air, 82%; iii) 1. *p*-TsOH, MeOH, 2. 4.3 equiv. RC(O)Cl, py, DMAP, 3. DTT, EtOH, NH_4OH, 48% from **101**; iv) 1. $MeOPCl_2$, $HO(CH_2)_2Br$, *i*$PrNEt_2$, 2. H_2O_2, 38%; v) 1. Me_3N, toluene, 2. MeCN, aq HF, 82%.

SCHEME 28



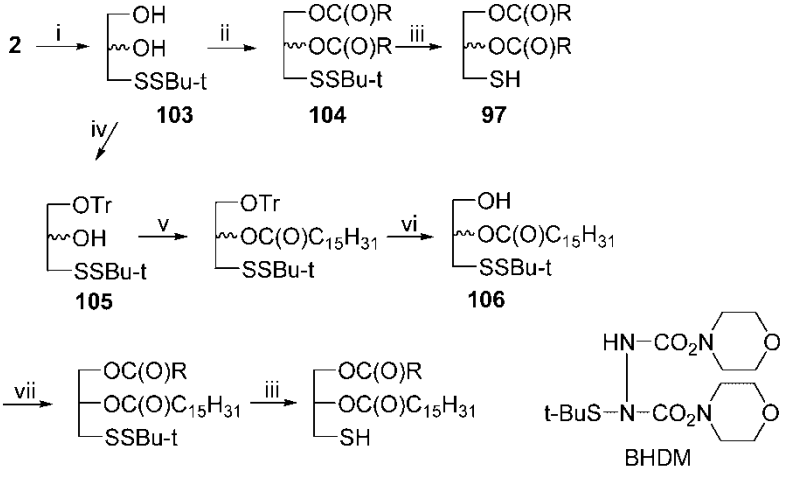
Reagents and conditions: i) MeC(O)SH, 4 °C, 7 d; ii) RC(O)Cl, py, 86%; iii) 1. $AgNO_3$, MeOH, 2. HCl/Et_2O, 80%.

SCHEME 29

amine substitution [117]. An alternative approach introduces a diethyl thiophosphate group prior to acetal removal, with deprotection and diacylation then followed by conversion of the diethyl thiophosphate to a thiophosphatitylinositol group [118].

When (R)-glycidol is used as the glycerol backbone and thioacetic acid is the source of sulfur, disulfide formation can be avoided (scheme 29) [113]. In this method, the thiolacetate unit serves as an effective sulfur-protecting group during the diacylation step. Although initially the conversion to thiol (*R*)-**97** proved difficult, it was finally effected in 80% yield by silver ion-induced methanolysis and then acidification. Phosphoramidite chemistry was invoked to complete the preparation of a thiophosphatidylinositol [113].

Using a mixed disulfide protocol, Moroder and co-workers [119, 120] prepared (±)-1,2-di-O-acyl-3-thioglycerols (**97**), which may also be used to synthesize thiophospholipids via scheme 30. The mixed *t*-butyl disulfide functionality was introduced to thioglycerol, using 1-(*t*-butylthiohydrazine)-1,2-dicarboxmorpholide (BHDM) [119, 120] as an electrophilic source of the *t*-butylthio group. After esterification of the two hydroxy groups of **103** with fatty acids, disulfide **104** was reduced using Bu_3P, releasing thiol **97**. Mixed carboxy esters can be introduced by protecting the *sn*-1 position of **103** with a trityl group, affording **105**. The remainder of the procedure is indicated in scheme 30. One noteworthy item in this otherwise standard procedure relates to the generation of free alcohol **106**. The deprotection of the trityl

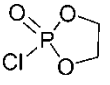


Reagents and Conditions: i) BHDM, 1N NaOH/dioxane, rt, 12h, 92%; ii) 3 equiv. RCO_2H, DMAP, DCC, rt,12h, 85-98%; iii) Bu_3P, CF_3CH_2OH, MeO*t*Bu, H_2O, rt, 12h, 95%; iv) TrCl, toluene, py, 20h, 95%; v) $R'CO_2H$, DMAP, DCC,rt, 12h, 89%; vi) $ZnBr_2$, CH_2Cl_2, MeOH, rt, 5min, 85%; viii) RCO_2H, DMAP, DCC, rt, 12h, 85-90%.

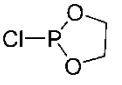
SCHEME 30

group with various reagents frequently resulted in acyl migration; however, this difficulty was minimized (1–2%) when silicic acid/boric acid column chromatography or $ZnBr_2$ were employed. The R(O) groups included common fatty acids and the retinoyl moiety [119]. The thiols were actually intended for selective attachment to proteins and peptides, but their structures are clearly feasible for elaboration into *sn*-3 thiophospholipids.

In addition to this technique for phosphorylating thiols, Mlotkowska and Markowsha [121, 122] have showed that dioxaphospholane chemistry is also amenable to thiophosphatidylcholine fabrication (scheme 31). Using (±)-2,3-di(stearyloxy)-1-propanethiol (**97**, R=$C_{17}H_{35}$) or its S-TMS derivative (**107**), thiophosphocholine **98** (R = $C_{17}H_{35}$) was formed, by way of 2-thio-2-oxo-1,3,2-dioxaphospholane intermediate **108** in yields of 52% and 70%, respectively [121]. Two mechanisms were offered for the formation of S-P bonds in **108** and the breaking the of S-Si bond in **107** [121]. Sulfur-substituted dioxaphospholane **108** is also accessible by reaction of **97** (R = $C_{17}H_{35}$) with 2-chloro-1,3,2-dioxaphospholane followed by nitrogen dioxide oxidation. If intermediate **109** is allowed to warm above 5 °C, a rearrangement generates thione **110** which can be modified to thiophosphonolipid **111** in 82% [122].

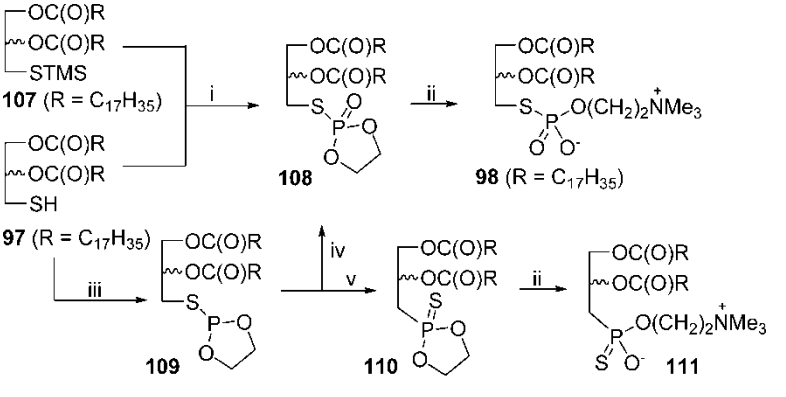


2-chloro-2-oxo-1,3,2-dioxaphospholane



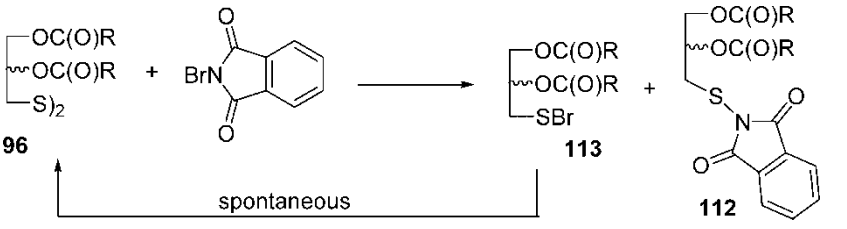
2-chloro-1,3,2-dioxaphospholane

The general mechanism for the electrophilic thiophosphate formation requires the sulfur to be attached to an appropriate leaving group. Phosphorus incorporation can then proceed through a Michaelis-Arbuzov reaction. Thiophthalimides, thiosuccinimides and sulfenyl chlorides are all suitable candidates for this chemistry. Sulfenyl chlorides are readily accessible by way of sulfuryl chloride oxidation of disulfides **96**. Michaelis-Arbuzov phosphorylation may be performed with dimethyl phosphate [123], 2,4-dichlorophenyl dimethyl phosphate [124], or 2-alkyl- or silyloxy substituted 1,3,2-dioxaphospholanes [125, 126]. All these options offer thiophosphate derivatives appropriate for conversion to thiophospholipids. However, the protocol that has been generally adopted, particularly for the formation of phosphatidylinositols, uses thiophthalimide derivatives.



Reagents and conditions: i) 2-chloro-2-oxo-1,3,2-dioxaphospholane, Et_3N, CH_2Cl_2; ii) Me_3N; iii) 2-chloro-1,3,2-dioxaphospholane, Et_3N, CH_2Cl_2; iv) N_2O_4, -40 °C; v) -40 °C.

SCHEME 31

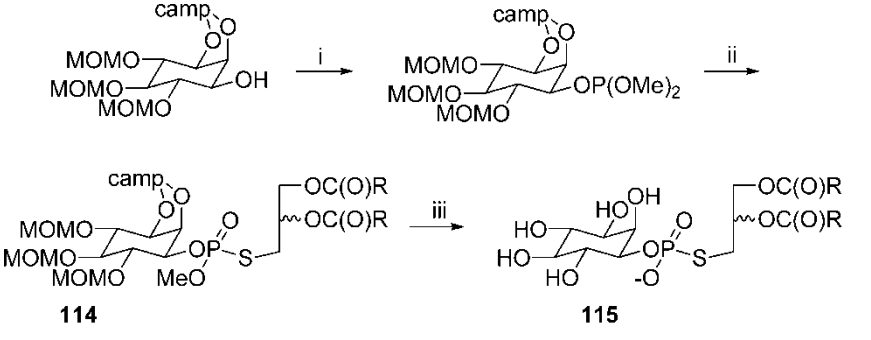


SCHEME 32

More specifically, the use of (±)-2,3-di(acyloxy)-1-propanesulfanyl phthalimides in combination with the Michaelis-Arbuzov reaction has proved synthetically useful and popular in preparing sn-3 sulfur-containing phosphatidylinositols (scheme 32). The requisite starting thiothalimides are formed from 2,3-di(acyloxy)-1-propanethiols using NBS, following an established radical chemistry protocol [127]. Although little experimental detail is offered in more recent publications, the reaction appears to generate thiophalimide **112** and a sulfenyl bromide (**113**), which is conveniently recovered as the disulfide.

Various phosphites offer dialkyl (or disilyl) thiophosphates via the Michaelis-Arbuzov protocol. Exceptionally rapid access to the desired headgroup is achieved when the phosphite is equipped with a protected phosphoinisitol (PI) group (scheme 33). Although the chemistry was initially established for hexadecylthio chains [128], it has since been tailored to glycerol-based substrates, most notably 1,2-dimyristoyloxypropane-3-thiophthalimide. A room temperature reaction of the thiophthalimide (**112**) and the phosphite makes the requisite S-P bond of **114**. Deprotection then affords the thioPI analog 1,2-dimyristoyloxypropane-3-thiophospho(1D-1-*myo*-inositol) (D-thio-DMPI, **115** (R,R = $C_{13}H_{27}$)) [115, 129].

The synthesis of **115** offered a thiophosphointositol analog appropriate for kinetic analysis of phosphoinositide specific phospholipase C (PI-PLC) enzymes. A continuous spectrophotometric assay involving DTP (**52**) capture of the released thiol was used (scheme 16) [115]. The specific PI-PLC under study was from *Bacillus cereus* [115]. The kinetics of PI-PLC kinetics *B. cereus* can be optimized by using L-thio-DMPI as a diluent [130]. Hendrickson [114] reported that a Δ(1-132) deletion mutant of mammalian PI-PLC δ_1 exhibited activity with D-thio-DMPI that was about 25% that of PI [114]. Hendrickson and Hendrickson [131] also prepared 1,2-dimyristoyloxypropane-3-thiophospho((±)-1-*myo*-inositol-4-phosphate) ((±)-thio-DMPI), and reported a high initial activity (30 μmol min^{-1} mg^{-1}) with Δ (1-132)-PI-PLC δ_1 that decreased due to rapid substrate depletion.



114 **115**

Reagents and conditions: (camp = chiral camphor-based protecting group)
i) $(MeO)_2PCl$, Et_3N, THF, -75 °C; ii) **109**, toluene, rt; iii) 1. LiBr, acetone, reflux, 4 h, 2. $HO(CH_2)_2SH$, BF_3Et_2O, CH_2Cl_2, 3h, rt.

SCHEME 33

A number of the methods yielding glycerol-3-thiophospholipid derivatives outlined earlier in this section have also been adapted for thioanalogs of PI [113, 116, 118]. A number of such derivatives were developed to target PI-PLC inhibition, with the principal variations being based on the *sn*-1 and *sn*-2 acyl groups. Some of these were evaluated against PI-PLC from *B. thuringiensis* [113].

5. Miscellaneous

Although not the subject of this review, it is important to mention that sulfur has been incorporated in a variety of additional places in lipid or lipid-related molecules. A small list includes the preparation of fattyalkylthiophospholipids [101, 129, 132], the placement of sulfur atom(s) to change the lipid's physical properties [133, 134], and the use of sulfur to facilitate attachment of photoactive functionality [133, 135]. The phosphorus atom in diether phospholipids can also be replaced with a sulfonium group [40], or simply with a sulfur attached to a glycosyl unit [136] representing the polar head group. The sulfur atom has also been called on to anchor phospholipids to surfaces [137, 138].

6. Conclusions

In order to access a variety of non-natural sulfur-containing glycerophospholipids and phosphonolipids, researchers have successfully applied the tools of organic synthesis to overcome reactivity and selectivity barriers. As described in this review, there are many available starting substrates, including an ample chiral pool, that possess functionality for the ready incorporation of sulfur, either as alkanethiolate or as thiocarboxylate. The placement of sulfur atoms at the *sn*-1 and/or *sn*-2 positions has created a number of useful and biologically-active lipid analogs with applications including pharmacologic agents (e.g., anti-tumor agents, anti-viral agents, synthetic lung surfactants) as well as compounds to assess the activity of different forms of phospholipase A_1, A_2, C, or D. Similarly, *sn*-3 thiophosphate-based lipids have found use for the inhibition of selected lipase enzymes.

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