Synthesis of acid-labile diplasmenyl lipids for drug and gene delivery applications. Chem Phys Lipids

ARTICLE *in* CHEMISTRY AND PHYSICS OF LIPIDS · JULY 1999

Impact Factor: 2.42 · DOI: 10.1016/S0009-3084(99)00033-X · Source: PubMed

ELSEVIER

Chemistry and Physics of Lipids
99 (1999) 145–153

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

Synthesis of acid-labile diplasmenyl lipids for drug and gene delivery applications

Jeremy A. Boomer, David H. Thompson *

Department of Chemistry, Purdue University, West Lafayette, IN 47907-1393, USA

Received 19 January 1999; accepted 22 February 1999

Abstract

The low pH environments characteristic of endosomal compartments and ischemic tissues provide an intrinsic pathway for triggering site-specific contents release from appropriately designed delivery vehicles. Accordingly, research in this group has focused on the design, synthesis and application of novel acid-sensitive lipids that will undergo facile lamellar (L_α) to hexagonal (H_{II}) phase transitions within these acidic sites. Previously, it has been demonstrated that plasmenylcholine-type lipids have excellent acid hydrolysis and contents release kinetics (Gerasimov et al., Biochim. Biophys. Acta. 1324 (1997) 200–214; Rui et al., J. Am. Chem. Soc. 120 (1998) 11213–11218). This paper describes the synthesis of three new acid sensitive lipids, based on a chiral 1,2-di-*O*-(1Z′,9Z′-octadecadienyl)-*sn*-glycerol (**6**) platform, displaying phosphocholine (**7**), poly(ethyleneoxide) (**8**), and *O*-carbamoyl-*N*-diethylenetriamine (**10**) headgroups. Intermediate **6** was obtained in 28% overall yield via a six step synthesis from (*S*)-(+)-2,2-dimethyl-1,2-dioxolane-4-methanol. Subsequent conversion to the final products was acheived in moderate (**7** and **10**) to excellent yields (**8**). © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Acid-sensitive; Cationic lipid; Vinyl ether; Controlled release

1. Introduction

The use of liposomes as in vivo drug and gene delivery vehicles has received much attention in recent years (Gerasimov et al., 1996; Lasic, 1997; Chonn and Cullis, 1998; Gerasimov et al., in press). The efficacy of these formulations relies, in large part, on their ability to extend drug circulation times and deliver their cargo site-specifically. Liposomal circulation lifetimes have been substantially improved via modification with a sterically-stabilizing poly (ethyleneoxide) (or polyethyleneglycol, PEG) corona (Lasic and Needham, 1995; Lasic, 1997). Incorporation of active targeting agents such as folate-, antibody-, or galactose-conjugates may further enhance site-

* Corresponding author. Fax: +1-765-496-2592.
E-mail address: davethom@chem.purdue.edu (D.H. Thompson)

PII: S0009-3084(99)00033-X

specific cellular uptake (Gerasimov et al., in press), however, formulations lacking specific contents release mechanisms are predisposed towards eventual lysosomal degradation. This obstacle has prompted the development of liposomal triggering mechanisms for efficient cytoplasmic contents release that are both reliable and biocompatible. Recent advances in enzymatic, thermal, photochemical, and acid triggered release have been reviewed (Gerasimov et al., 1996; Gerasimov et al., in press).

The intrinsic low pH environment of endosomes and ischemic tissues has motivated the design of several different mechanisms for acid triggered release. One promising strategy utilizes the lamellar (L_α) to hexagonal (H_{II}) phase transition of phosphatidylethanolamine vesicles, stabilized by incorporation of pH-sensitive cosurfactants (Litzinger and Huang, 1992). Others have employed pH-sensitive proteins (Parente et al., 1990; Vogel et al., 1996) and polymers (Thomas et al., 1996; Kono et al., 1997), whose morphological changes upon acidification are meant to promote membrane destabilization, contents leakage, and fusion. Research in our group is currently focused on the design, synthesis and application of novel acid-sensitive lipids that are also capable of promoting these membrane transitions within acidic and/or oxidative environments. This work has centered on vinyl ether-containing plasmenylcholine type lipids (Fig. 1) which possess excellent acid hydrolysis and contents release kinetics (Gerasimov et al., 1997; Rui et al., 1998). In particular, the utility of acid-induced membrane destabilization by this class of materials was recently demonstrated using folate-targeted diplasmenylcholine (Fig. 1B) liposomes; $>80\%$ cytoplasmic delivery of liposomal contents and nearly 6000-fold increase in 1-β-arabinofuranosylcytosine (Ara-C) efficacy relative to free drug was observed (Rui et al., 1998). In addition to their inherent acid lability, vinyl ether bonds also exhibit facile photooxidative cleavage in the presence of $^1O_2$. Plasmenylcholine based liposome formulations have been shown to rapidly and efficiently release their contents upon exposure to $^1O_2$ generated in situ (Thompson et al., 1996; Wymer et al., 1998), thus expanding their potential therapeutic application to non-acidic environments.

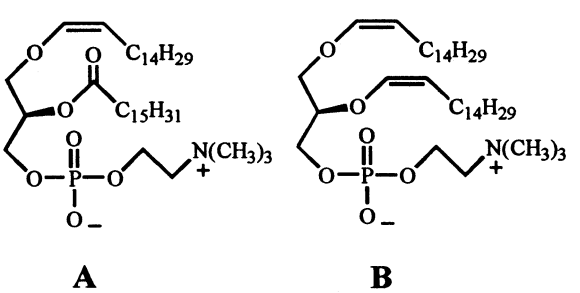

Fig. 1. (A) Plasmenylcholine; (B) diplasmenylcholine.

This paper describes the synthesis of three new lipids constructed from a chiral 1,2-di-*O*-(1Z′,9Z′-octadecadienyl)-*sn*-glycerol (**6**, Fig. 2) core similar to that found in Fig. 1B. Modifications of the existing synthetic route (Rui and Thompson, 1994, 1996) allow for the incorporation of oleoyl side chains on a chiral glycerol backbone. Further elaboration of this intermediate with phosphocholine (**7**), poly(*n*)ethylene oxide (**8**; $n \cong 115$, PEG5000), or diethylenetriamine (**10**) headgroups produces triggerable materials that may be used for drug delivery and cellular transfection applications (Fig. 3). The intrinsic lability of the vinyl ether functionality under both acidic and oxidative conditions, formation of the naturally occurring (and more reactive) Z-configuration, and introduction of the polar headgroups without destruction of the vinyl ether linkages represent the primary challenges to the synthesis of these materials. A detailed description of their preparation is outlined below.

2. Results and discussion

A reaction scheme for the synthesis of 1,2-di-*O*-(1Z′,9Z′-octadecadienyl)-*sn*-glycerol (**6**), the common intermediate in the syntheses of compounds **7**, **8** and **10** is shown in Fig. 2. A previous synthesis of 1,2-di-*O*-(1Z′-hexadecenyl)-*rac*-glycerol utilized commercially available 1,2-dihexadecoyl-*rac*-glycerol as starting material. Given the limited commercial availability of 1,2-dioleoyl-*sn*-glycerol and the potential need for a diverse class

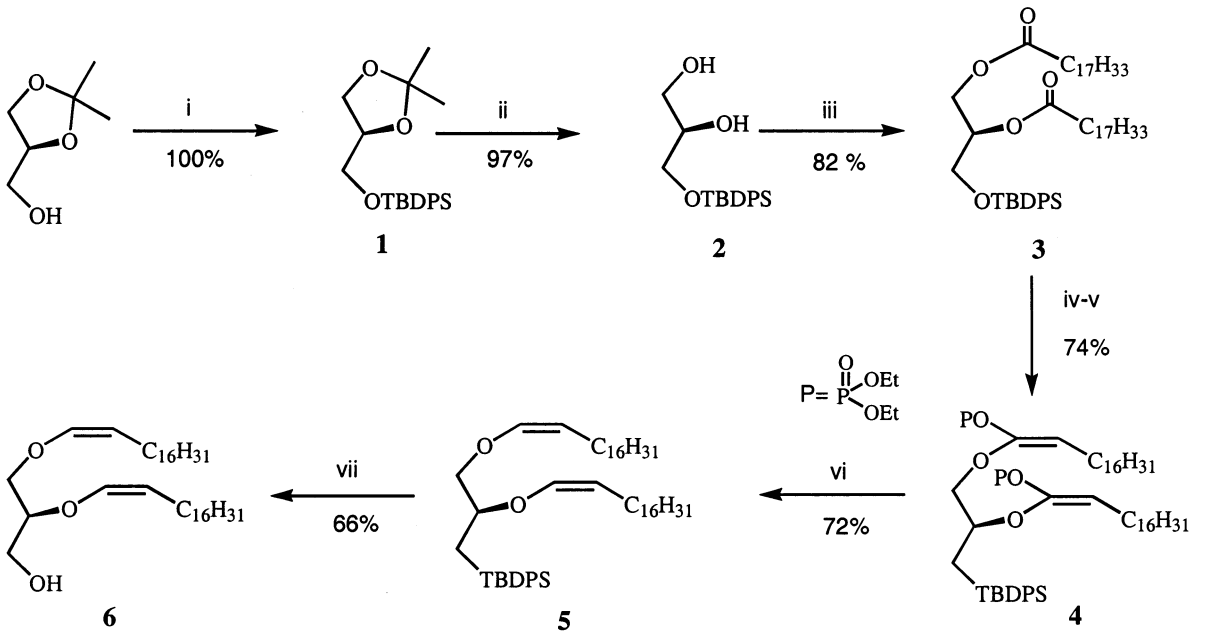

Fig. 2. Synthetic route to platform **6**. (i) *t*-Butyldiphenylsilylchloride (TBDPSCl), imidazole; (ii) HCl, MeOH; (iii) oleoyl-Cl, pyridine; (iv) lithium diisopropylamide (LDA); (v) diethylchlorophosphate, hexamethylphosphoramide (HMPA); (vi) $Pd(PPh_3)_4$, Et_3Al; (vii) tetrabutylammonium fluoride (TBAF), tetrabutylammonium hydroxide (TBAH).

of unnatural 1,2-di-*O*-(1Z′-alkenyl)-*sn*-glycerols, a general route to 1,2-diacyl-*sn*-glyceryl-3-*t*-butyldiphenyl silyl ether **3** was developed starting from the readily available precursor (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol. Protection with *t*-butyldiphenylsilylchloride (TBDPSCl) in the presence of imidazole (Hanessian and Lavallee, 1975) gives **1,** which is converted to diol **2** using ethanolic HCl. Several methods for selective removal of the acetonide were explored. The use of 2 N HCl (Ohgi et al., 1977), trifluoracetic acid (Leblanc et al., 1986), or acetic acid typically resulted in complete deprotection, generating glycerol as the primary product. Improved selectivity was observed with pyridinium tosylate (Van Rijsbergen et al., 1983), although it was often slow and inefficient (20–60% yield). Konosu's 1,3-propanedithiol method (Konosu and Oida, 1991) was quite effective, however, the ethanolic HCl methodology was finally chosen since the reaction times were shorter and the conditions milder. The use of TBDPS protection was based on its observed robustness during the vinyl ether transformation sequence (Fig. 2, iv–vi) and subsequent ease of removal under conditions compatible with vinyl ether linkages (Rui and Thompson, 1994, 1996). Finally, double acylation (Rui, 1996) of diol **2** generates 3-*O*-(*t*-butyldiphenylsilyl)-1,2-di-*O*-(9Z′-octadecenyl)-*sn*-glycerol (**3**) in 82% isolated yield.

Treatment of **3** with 2.8 equivalents lithium diisopropylamide (LDA) generated in situ at −78°C, followed by *O*-trapping of the bis-enolate with four equivalents of diethylchlorophosphate in hexamethylphosphoramide (HMPA), generates the bisvinyl phosphate intermediate **4** (Charbonnier et al., 1987; Jackson et al., 1989). Reduction with triethylaluminum in the presence of palladium (0) catalyst (immediately following chromatographic isolation of **4**) yields 1,2-di-*O*-(1Z′,9Z′-octadecadienyl)-3-*O*-(*t*-butyldiphenylsilyl)-*sn*-glycerol (**5**), in 53% overall yield from **3**. The success of this reaction was highly dependent on the color and physical appearance of the palladium catalyst (yellow–orange crystalline samples generally performing better than yellow–green

powders). The principle contaminants in this transformation were ethyl-coupled product (material bearing 1′-ethyl-1Z′,9Z′-octadecadienyl sidechains (Takai et al., 1980; Sato et al., 1981)) and the starting diester **3** (Jackson et al., 1989), arising from ethyl transfer from the ethyl(vinylether)Pd complex and vinyl phosphate rearrangement, respectively.

Desilylation of **5** with tetrabutylammonium fluoride (TBAF) (Hanessian and Lavallee, 1975) affords 1,2-di-*O*-(1Z′,9Z′-octadecadienyl)-*sn*-glycerol (**6**) in 66% yield (35% overall yield from **3**). The addition of small amounts of tetrabutylammonium hydroxide (TBAH, 30% in water) significantly increases the reaction rate and ensures a basic solution, thereby preventing hydrolytic decomposition of the vinyl ether species (occasionally observed in its absence).

Alcohol **6** was then subjected to three different types of polar headgroup modifications (Fig. 3). A phosphocholine headgroup (compound **7**), previously introduced via condensation with 2-chloro-1,3,2-dioxaphospholane-2-oxide with subsequent ring opening amination with trimethylamine (Rui and Thompson, 1996), was installed in this case using a phosphorus oxychloride/choline tosylate sequence, significantly reducing reaction time (Brockerhoff and Ayengar, 1979). Addition of **6** to a chloroform solution of phosphorus oxychloride, followed by sequential addi-

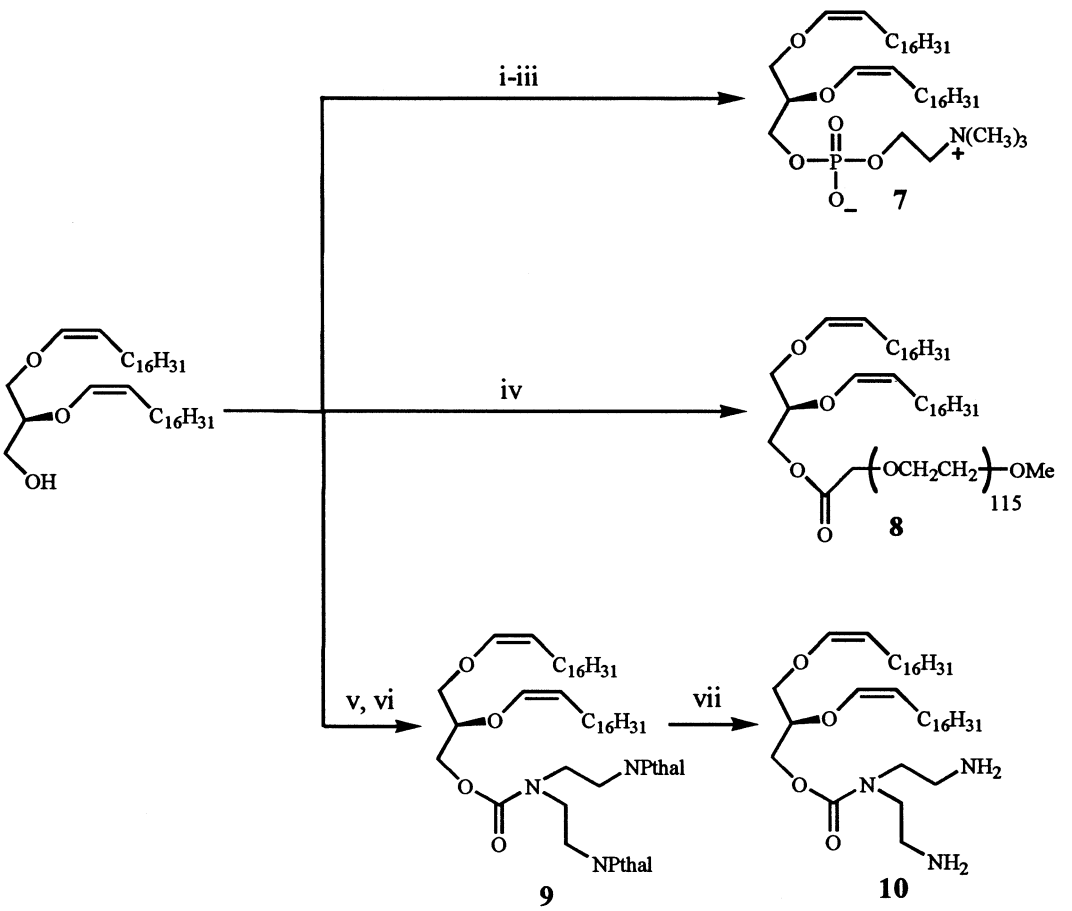

Fig. 3. Headgroup modifications (Pthal = pthalamidyl). (i) $POCl_3$, Et_3N; (ii) choline tosylate; (iii) H_2O; (iv) dicyclohexylcarbodiimide (DCC), *N*,*N*-dimethyl-4-aminopyridine (DMAP), methoxy-poly(ethyleneoxide)-carboxymethyl (MPEGA); (v) dipyridyl carbonate (DPC), Et_3N; (vi) *N*,*N*′-dipthalamidyldiethylenetriamine (PDETA); (vii) $H_2NNH_2{\cdot}H_2O$.

tion of choline tosylate and water, was conducted in the presence of excess, freshly distilled triethylamine to buffer the acid generated. Subsequent chromatography followed by lyophilization generated a white precipitate in 35% yield (10% overall yield from (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol). Shorter reaction times, simplified purification, and comparable isolated yields underscore the utility of this route.

Dicyclohexylcarbodiimide (DCC) mediated coupling of **6** with methoxy-poly(ethyleneoxide)-carboxymethyl (MPEGA, avgerage MW = 5000) in the presence of *N*,*N*-dimethyl-4-aminopyridine (DMAP) catalyst followed by ether precipitation afforded ester **8** in 93% yield (26% overall yield from (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol). Similar condensations utilizing 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and DMAP gave products containing impurities that proved difficult to remove using the precipitation/ether trituration technique.

The diethylenetriamine cationic headgroup in compound **10** was installed via standard carbamate coupling methodology utilizing dipyridyl carbonate (DPC) as the linking agent. Briefly, alcohol **6** was added to DPC generating a mixed carbonate intermediate. Following extraction with 5% $NaHCO_3$ and saturated NaCl, the remaining 2-hydroxypyridine was displaced with *N*,*N*′-dipthalamidyldiethylenetriamine (PDETA) giving carbamate **9** in 69% isolated yield (Garrigues and Vidaud, 1988). After column chromatography, carbamate **9** was deprotected with hydrazine hydrate (Garrigues and Vidaud, 1988), affording diamine **10** as its free base in 56% isolated yield from **6** (16% overall yield from (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol).

3. Conclusions

A facile synthesis of 1,2-di-*O*-(1Z′,9Z′-octadecadienyl)-*sn*-glycerol (**6**) from a chiral glycerol precursor via a revised synthetic sequence, and its subsequent headgroup modifications are described. This pathway is readily amenable to a wide range of sidechain and headgroup functionalities. These compounds are currently under investigation for their activity in dePEGylative liposomal contents release (**8**) and DNA tranfection (**10**) applications.

4. Experimental

2,2′-Dipyridyl carbonate was purchased from TCI-America. MPEG5000 was purchased from Shearwater Polymers. All other compounds were purchased from Aldrich and used without further purification unless otherwise stated. Triethylamine and diisopropylamine were distilled from CaH_2. All solvents were spectrophotometric grade and were dried over an appropriate desiccant, then distilled before use. Reactions were performed under an argon atmosphere.

Column chromatography was typically performed on 230–400 mesh silica gel using high grade solvents to elute the compounds. Thin layer chromatography was performed using Baker-flex IB-F plates (J.T. Baker) and visualized using UV, I_2 adsorption, $KMnO_4$/heat, and/or molybdic acid/heat.

^{1}H-NMR, and ^{13}C-NMR spectra were recorded on a 4.7 T spectrometer. Chemical shifts are reported in PPM relative to the residual solvent peaks as the internal standard.

*4.1. (R)-2,2-Dimethyl-1,3-dioxolane-4-t-butyldiphenylsilylmethanol (**1**)*

(*S*)-(+)-2,2-Dimethyl-1,3-dioxolane-4-methanol (26.5 g, 200.5 mmol) and imidazole (21.1 g, 309.9 mmol) were dissolved in 250 ml THF. *t*-Butyldiphenylsilyl chloride (61.0 g, 222.6 mmol) in 100 ml THF was added dropwise via addition funnel over 15 min. The reaction was stirred for 2 h and then divided into two 175 ml portions. Each portion was added to 700 ml ether and extracted with 3×600 ml H_2O. The combined ether layers were dried over $MgSO_4$, filtered, evaporated, and dried in vacuo. A faint yellow oil (77.1 g, 100% yield) was recovered. The product was used without further purification. TLC (1:1 hexane: ether, UV): $R_f = 0.6$. ^{1}H-NMR ($CDCl_3$): 1.1 (s, 9H), 1.38 (s, 3H), 1.42 (s, 3H), 3.75 (m,

2H), 3.86 (m, 1H), 4.1 (m, 1H), 4.22 (m, 1H), 7.43 (m, 6H), 7.70 (m 4H).

4.2. 3-O-(t-Butyldiphenylsilyl)-sn-glycerol (2)

(*R*)-2,2-Dimethyl-1,3-dioxolane-4-*t*-butyldiphenylsilylmethanol (**1**, 13.0 g, 35.1 mmol) was dissolved in 100 ml ethanol prior to the addition of a mixture of 7 ml concentrated HCl and 10 ml H_2O. The reaction was run for 12 min then quenched with 100 ml 4 N NaOH. The product was extracted with 3×100 ml ether. The combined ether layers were dried over $MgSO_4$, filtered, evaporated, and dried in vacuo. The crude reaction mixture was purified via silica gel chromatography using 1:1 hexane:ether to elute starting material, then ethyl acetate to elute the product. A white solid (7.1 g, 97% yield, based on consumed starting material) was isolated. TLC (1:1 hexane:ether, UV, $KMnO_4$): $R_f = 0.16$. ^{1}H-NMR ($CDCl_3$): 1.1 (s, 9H), 2.05 (bs, 2H), 3.6–3.9 (m, 5H), 7.43 (m, 6H), 7.70 (m 4H).

4.3. 3-O-(t-Butyldiphenylsilyl)-1,2-di-O-(9Z′-octadecenoyl)-sn-glycerol (3)

3-*O*-(*t*-Butyldiphenylsilyl)-*sn*-glycerol (**2**, 12.75g, 38.6 mmol) and pyridine (15.7 ml, 193 mmol) were dissolved in 130 ml THF and cooled to 0°C. Oleoyl chloride (45.0 ml, 115.7 mmol) was then added dropwise over 10 min. The solution was stirred at 0°C for 30 min, then room temperature overnight. Diethyl ether (200 ml) was added and the organic phase washed 2×150 ml H_2O. The ether layer was dried over $MgSO_4$, filtered, evaporated, and dried in vacuo. The crude mixture was purified in two batches via silica gel chromatography (8:1 hexane:ether). This purified oil, consisting of product and the acid anhydride sideproduct, was further purified via silica gel step gradient chromatography (hexane to elute the anhydride, followed by ether to elute the product). A faint yellow oil (27 g, 82% yield) was recovered. TLC (6:1 hexane:ether, UV, I_2): $R_f = 0.65$. ^{1}H-NMR ($CDCl_3$): 0.85 (t, $J = 7$ hz, 6H), 1.05 (s, 9H), 1.3 (m, 40H), 1.6 (m, 4H), 2.05 (m, 8H), 2.28 (t, $J = 10$ hz, 4 H), 3.78 (d, $J = 8$hz, 2H), 4.22 (dd, $J^1 = 8$hz $J^2 = 6$hz, 1H), 4.4 (dd, $J^1 = 8$hz $J^2 = 4$hz, 1H), 5.18 (m, 1H), 5.35 (t, $J = 6$hz, 4H), 7.42 (m, 6H), 7.65 (m, 4H).

4.4. 3-O-(t-Butyldiphenylsilyl)-1,2-di-O-(1′-diethylphosphonyl-1Z′,9Z′-octadecadienyl)-sn-glycerol (4)

*n*BuLi (2.5 M in hexane, 18 ml, 45 mmol) was added dropwise to diisopropylamine (10.0 ml, 71.3 mmol) at −78°C and stirred for 30 min. 3-*O*-(*t*-Butyldiphenylsilyl)-1,2-di-*O*-(9Z′-octadecenoyl)-*sn*-glycerol (**3**, 13.6 g, 15.8 mmol) in 50 ml THF was then added dropwise via syringe over 15 min. The reaction was stirred for 1.5 h prior to the rapid addition of five 20-ml aliquots of chlorodiethyl phosphate (9.2 ml, 63.5 mmol) in HMPA (90 ml), while maintaining the temperature at −78°C. When the addition was complete, the reaction turned dark orange/brown and solidified. It was then warmed just enough to resume stirring, then recooled for 30 min, warmed to room temperature, and stirred for an additional 1.5 h. The reaction mixture was then poured into 200 ml ether and washed 2×200 ml 5% $NaHCO_3$. The H_2O layers were extracted with 2×200 ml ether and the combined ether layers dried over $MgSO_4$, filtered, evaporated, and dried in vacuo. The crude product was purified via silica gel flash chromatography (1:1 hexane:ether). The product was isolated as a yellow oil (8.36 g, 74% yield, based on consumed starting material). TLC (Ether, UV, I_2): $R_f = 0.38$. ^{1}H-NMR ($CDCl_3$): 0.85 (t, $J = 7$hz, 6H), 1.05 (s, 9H), 1.3 (m, 46H), 2.05 (m,12H), 3.78 (m, 2H), 4.15 (m, 10H), 4.3–4.6 (m, 4H), 5.35 (t, $J = 6$hz, 4H), 7.42 (m, 6H), 7.65 (m, 4H).

4.5. 3-O-(t-butyldiphenylsilyl)-1,2-di-O-(1Z′,9Z′-octadecadienyl)-sn-glycerol (5)

3-*O*-(*t*-Butyldiphenylsilyl)-1,2-di-*O*-(1′-diethylphosphonyl-1Z′,9Z′-octadecadienyl)-*sn*-glycerol (**4,** **7**, 1.9 g,1.68 mmol) and tetrakis (triphenylphosphine)palladium (60 mg, 52 μmol) were dissolved in 20 ml hexane and cooled to 0°C. Triethylaluminum (1.0 M in hexanes, 5.9 ml, 5.9 mmol) was then added dropwise via syringe over 10 min. The reaction was stirred at 0°C for 1 h,

then at room temperature for another 2 h before filtering through a small silica plug with ether. The filtrate was evaporated and purified via silica gel chromatography (9:1 hexane:ether), giving 1.0 g of a faint yellow oil (72% yield). TLC (2:1 hexane: ether, UV, I_2): $R_f = 0.64$. ^{1}H-NMR ($CDCl_3$): 0.85 (t, $J = 7$hz, 6H), 1.05 (s, 9H), 1.3 (m, 40H), 2.05 (m, 12H), 3.70–4.0 (m, 5H), 4.35 (m, 2H), 5.35 (t, $J = 6$hz, 4H), 5.95 (m, 2H), 7.42 (m, 6H), 7.65 (m, 4H).

4.6. 1,2-di-O-(1Z′,9Z′-Octadecadienyl)-sn-glycerol (6)

TBAF (33.0 ml, 33.0 mmol) was added to a solution of 3-*O*-(*t*-butyldiphenylsilyl)-1,2-di-*O*-(1Z′,9Z′-octadecadienyl)-*sn*-glycerol (**5**, 9 g, 10.9 mmol) in 70 ml THF. Then TBAH (0.5 ml, 40% in H_2O) was added and the reaction stirred for 2 h. The solution was filtered through a small silica plug with ether, the eluent evaporated, and the crude oil purified via silica gel chromatography (9:1 hexane:ether). Since the silanol side-product had a similar R_f value under all conditions examined, further purification was not attempted. The recovered oil, containing product (4.2 g, 66% yield) and silanol, was used without further purification. Subsequent characterization data were collected using a sample isolated from exhaustive chromatographic purification. TLC (1:1 hexane:ether, I_2): $R_f = 0.52$. ^{1}H-NMR ($CDCl_3$): 0.85 (t, $J = 7$hz, 6H), 1.3 (m, 40H), 2.05 (m, 12H), 3.63–3.92 (m, 5H), 4.4 (m, 2H), 5.35 (t, $J = 6$hz, 4H), 5.9 (dt, $J^1 = 6$ hz, $J^2 = 3$hz, 1H), 6.05 (dt, $J^1 = 6$ hz, $J^2 = 3$hz, 1H).

4.7. 1,2-O-(1Z′,9Z′-Octadecadienyl)-sn-glycero-3-phosphocholine (7)

A solution of 1,2-di-*O*-(1Z′,9Z′-octadecadienyl)-*sn*-glycerol (**6**, 396 mg, 673 μmol) and triethylamine (188 μl, 1.35 mmol) in $CHCl_3$ (7 ml) was added dropwise to a solution of phosphorus oxychloride (75.3 μl, 808 μmol) in freshly distilled, ethanol free $CHCl_3$ (1 ml, distilled from P_2O_5) at 0°C. After 30 min, pyridine (1.8 ml, 22.2 mmol) was added dropwise via syringe over 5 min. After an additional hour of stirring, the mixture was warmed to room temperature, choline tosylate (278 mg, 1.0 mmol) added and the solution stirred for 1 h. The reaction was quenched with H_2O (60 μl, 2.83 mmol) and stirred for 30 min before evaporating and drying in vacuo. The crude product was purified via silica gel chromatography (gradient elution: 100% $CHCl_3$ to 50:50 $CHCl_3$:MeOH). Product fractions were combined, evaporated and chromatographed a second time under identical conditions. The isolated product was lyophilized, yielding a white precipitate (177 mg, 35% yield). TLC (65:35:6 $CHCl_3$:MeOH:NH_4OH, I_2): $R_f = 0.3$. ^{1}H-NMR ($CDCl_3$): 0.85 (t, $J = 7$hz, 6H), 1.3 (m, 40H), 2.05 (m, 12H), 3.35 (bs, 9H), 3.70–4.0 (m, 7H), 4.35 (m, 4H), 5.35 (t, $J = 6$hz, 4H), 5.9 (dt, $J^1 = 6$ hz, $J^2 = 3$hz, 1H), 6.05 (dt, $J^1 = 6$ hz, $J^2 = 3$hz, 1H). ^{13}C-NMR ($CDCl_3$) (Rui, 1996): 14.1, 22.7, 24.0, 24.1, 29.4–29.9, 31.9, 54.4, 60.0, 64.4, 71.5, 80.0, 107.4, 107.9, 129.9, 130.0, 144.2, 145.0. MS (EI/CI) (Rui, 1996): $m/z = 754$.

4.8. 1,2-O-(1Z′,9Z′-Octadecadienyl)-sn-glyceryl-3-(ω-methoxy-poly(ethyleneglycolate[115])) (8)

1,2-*O*-(1Z′,9Z′-Octadecadienyl)-*sn*-glycerol (**6**, 33 mg, 56.1 μmol), methoxy-poly(ethylene glycol)-carboxymethyl (MW 5000, 205 mg, 41 μmol), DCC (11 mg, 53 μmol), and *N*,*N*-dimethyl-4-aminopyridine (1 mg, 4.8 μmol) were stirred in 2 ml methylene chloride for 4 days. The resulting dicyclohexylurea crystals were removed via filtra tion and the solvent concentrated to 2 ml; this solution was dripped into cold diethyl ether and centrifuged at 3500 × *g* for 10 min. The ether was decanted and the pellet triturated with fresh ether and centrifuged. This cycle was repeated five times. The pellet was finally dissolved in methylene chloride, evaporated, dissolved in 2 ml Millipore purified water and lyophilized yielding 208 mg PEG containing material, as a white solid (93% yield). TLC (THF, I_2): $R_f = 0.24$. ^{1}H-NMR ($CDCl_3$): 0.85 (t, $J = 7$hz, 6H), 1.27 (bs, 40H), 2.05 (m, 12H), 3.2–4.41 (m, >460 H), 5.35 (t, $J = 6$ hz, 4H), 5.87 (dt, $J^1 = 6$ hz, $J^2 = 3$ hz, 1H), 5.94 (dt, $J^1 = 6$ hz, $J^2 = 3$ hz, 1H).

4.9. O-(1,2-O-1Z′,9Z′-octadecadiene-sn-glycerol)-N-(di-2-pthalamidyl ethyl amino)carbamate (9)

1,2-*O*-(1Z′,9Z′-Octadecadienyl)-*sn*-glycerol (**6**, 308 mg, 0.523 mmol), 2,2′-dipyridyl carbonate (274 mg, 1.26 mmol), and triethylamine (1.0 ml, 7.18 mmol) were stirred for 3 days in 10 ml CH_2Cl_2. The reaction mixture was extracted with 25 ml $NaHCO_3$ followed by 25 ml saturated NaCl. The CH_2Cl_2 layer was dried over Na_2SO_4, filtered, and evaporated. The resulting oil was dissolved in CH_2Cl_2 (10 ml). 1,5-Dipthalamidyldiethylenetriamine (315 mg, 0.868 mmol) was added and the reaction stirred for 3 days. The solution was evaporated and the crude mixture purified via flash chromatography (3:2 hexane:ether), yielding 350 mg of a clear oil (69% yield). TLC (1:2 hexane: ether, UV, acidic molybdate): $R_f = 0.27$. ^{1}H-NMR (C_6D_6): 0.91 (t, $J = 7$ hz, 6H), 1.2–1.55 (m, 40H), 2.08 (m, 8H), 2.28 (m, 4H), 3.39 (m, 4H), 3.58 (m, 4H), 3.68 (d, $J = 5$ hz, 2H), 3.79 (p, $J = 5$ hz, 1H), 3.98 (d, $J = 5$ hz, 2H), 4.45 (m, 2H), 5.50 (m, 4H), 6.02 (m, 2H), 6.89 (m, 4H), 7.47 (m, 4H). ^{13}C-NMR (C_6D_6): 14.3, 23.1, 24.5, 27.7, 29.7–30.3, 32.3, 36.0, 45.5, 46.5, 64.5, 71.7, 78.9, 107.3, 107.8, 123.0, 130.1, 130.3, 132.4, 132.7, 133.4, 133.6, 144.9, 145.8, 155.9, 167.7, 168.0. IR (neat): 715, 873, 1111, 1200, 1450, 1467, 1700, 1717, 1774, 2923.

4.10. O-(1,2-O-1Z′,9Z′-octadecadiene-sn-glycerol)-N-(bis-N,N-ethylamine)-carbamate (10)

O-(1,2-*O*-1Z′,9Z′-octadecadiene-*sn*-glycerol)-*N*-(di-2-pthalamidyl ethyl amino)carbamate (**9**, 306 mg, 0.313) was dissolved in 60 ml methanol and hydrazine hydrate (100 μl, 2.1 mmol) added. The reaction was stirred for 2 days. The solution was evaporated, yielding an off white precipitate. Chloroform (50 ml) was added and the precipitate side product removed via filtration. The filtrate was evaporated then dried in vacuo overnight yielding 179 mg of a faint yellow oil (80% yield). TLC (80:19.5:0.5 $CHCl_3$:MeOH: NH_4OH, molybdic acid/heat, I_2): $R_f = 0.85$. ^{1}H-NMR (C_6D_6): 0.91 (t, $J = 6$ hz, 6H), 1.15–1.55 (m, 40 H), 2.08 (m, 8H), 2.28 (m, 4H), 2.58 (bs, 4H), 2.9–3.2 (m, 4H), 3.58 (m, 2H), 3.79 (p, $J = 5$ hz, 1H), 4.19 (dd, $J^1 = 10$ hz, $J^2 = 6$ hz, 1H), 4.29 (dd, $J^1 = 10$ hz, $J^2 = 6$ hz, 1H), 4.43 (m, 2H), 5.50 (m, 4H), 5.83 (d, $J = 6$ hz, 1H), 5.92 (d, $J = 6$ hz, 1H). ^{13}C-NMR (C_6D_6): 14.4, 23.1, 24.5, 27.7, 29.7–30.2, 32.3, 36.0, 40.9, 41.1, 50.1, 50.3, 64.4, 71.5, 79.1, 107.1, 107.6, 107.8, 130.1, 144.8, 145.4, 156.2. IR (neat): 723, 769, 1110, 1196, 1257, 1378, 1423, 1466, 1665, 1701, 2854, 2925, 3360, 3371.

Acknowledgements

The authors wish to thank Drs Yuanjin Rui and Sean Sullivan for their assistance with the synthesis and Aniruddha Patwardhan for his invigorating discussions. This work was kindly supported by NIH grant 1 R01GM55266-01A2.

References

Brockerhoff, H., Ayengar, N.K.N., 1979. Improved synthesis of choline phospholipids. Lipids 14, 88–89.

Charbonnier, F., Moyano, A., Greene, A.E., 1987. Facile synthesis of chiral *O*-alkyl enol ethers. J. Org. Chem. 52, 2303–2306.

Chonn, A., Cullis, P.R., 1998. Recent advances in liposome technologies and their applications for systemic gene delivery. Adv. Drug Deliv. Rev. 30, 73–83.

Garrigues, B., Vidaud, L., 1988. Synthesis of spermine and spermidine selectively substituted on the secondary amine functions. Bull. Soc. Chim. Belg. 97, 775–785.

Gerasimov, O.V., Rui, Y., Thompson, D.H., 1996. Triggered release from liposomes mediated by physically and chemically induced phase transitions. Surfactant Sci. Ser. 62, 679–746.

Gerasimov, O.V., Schwan, A., Thompson, D.H., 1997. Acid-catalyzed plasmenylcholine hydrolysis and its effect on bilayer permeability: a quantitative study. Biochim. Biophys. Acta 1324, 200–214.

Gerasimov, O.V., Boomer, J.A., Qualls, M.M., Thompson, D.H., in press. Cytosolic drug delivery using pH- and light-sensitive liposomes. Adv. Drug. Deliv. Rev.

Hanessian, S., Lavallee, P., 1975. Preparation and synthetic utility of tert-butyldiphenylsilyl ethers. Can. J. Chem. 53, 2975–2977.

Jackson, J.A., Hammond, G.B., Wiemer, D.F., 1989. Synthesis of α-phosphono lactones and esters through a vinyl phosphate-phosphonate rearrangement. J. Org. Chem. 54, 4750–4754.

Kono, K., Igawa, T., Takagishi, T., 1997. Cytoplasmic delivery of calcein mediated by liposomes modified with a pH-sensitive poly(ethylene glycol) derivative. Biochim. Biophys. Acta 1325, 143–154.

Konosu, T., Oida, S., 1991. Enantiocontrolled synthesis of the antifungal β-lactam (2R,5S)-2-(hydroxymethyl)clavam. Chem. Pharm. Bull. 39, 2212–2215.

Lasic, D.D., 1997. Recent developments in medical applications of liposomes: sterically stabilized liposomes in cancer therapy and gene delivery in vivo. J. Control. Rel. 48, 203–222.

Lasic, D.D., Needham, D., 1995. The 'Stealth' liposome: a prototypical biomaterial. Chem. Rev. (Washington, D.C.) 95, 2601–2628.

Leblanc, Y., Fitzsimmons, B.J., Adams, J., Perez, F., Rokach, J., 1986. The total synthesis of 12-HETE (12-hydroxyeicosatetraenoic acid) and 12,20-diHETE. J. Org. Chem. 51, 789–793.

Litzinger, D.C., Huang, L., 1992. Phosphatidylethanolamine liposomes: drug delivery, gene transfer and immunodiagnostic applications. Biochim. Biophys. Acta 1113, 201–227.

Ohgi, T., Kondo, T., and Goto, T., 1977. Total synthesis of nucleoside Q. Tetrahedron Lett. 4051–4054.

Parente, R.A., Nir, S., Szoka, F.C. Jr., 1990. Mechanism of leakage of phospholipid vesicle contents induced by the peptide GALA. Biochemistry 29, 8720–8728.

Rui, Y., 1996. Thesis, Department of Chemistry, Purdue University. Stereocontrolled total synthesis of plasmalogen and diplasmalogen lipids and their activities in an intracellular drug delivery system.

Rui, Y., Thompson, D.H., 1994. Stereocontrolled synthesis of plasmalogen-type lipids from glyceryl ester precursors. J. Org. Chem. 59, 5758–5762.

Rui, Y., Thompson, D.H., 1996. Efficient stereoselective synthesis of plasmenylcholines. Chem. Eur. J. 2, 1505–1508.

Rui, Y., Wang, S., Low, P.S., Thompson, D.H., 1998. Diplasmenylcholine-folate liposomes: an efficient vehicle for intracellular drug delivery. J. Am. Chem. Soc. 120, 11213–11218.

Sato, M., Takai, K., Oshima, K., Nozaki, H., 1981. Palladium(0) promoted alkylation of enol phosphates with organoaluminum compounds and its synthetic applications. Tetrahedron Lett. 22, 1609–1612.

Takai, K., Oshima, K., Nozaki, H., 1980. Carbon-carbon bond formation by cross-coupling of enol phosphates with organoaluminum compounds catalyzed by palladium(0) complex. Tetrahedron Lett. 21, 2531–2534.

Thomas, J.L., Devlin, B.P., Tirrell, D.A., 1996. Kinetics of membrane micellization by the hydrophobic polyelectrolyte poly(2-ethylacrylic acid). Biochim. Biophys. Acta 1278, 73–78.

Thompson, D.H., Gerasimov, O.V., Wheeler, J.J., Rui, Y., Anderson, V.C., 1996. Triggerable plasmalogen liposomes: improvement of system efficiency. Biochim. Biophys. Acta 1279, 25–34.

Van Rijsbergen, R., Anteunis, M.J.O., De Bruyn, A., 1983. Selective removal of the isopropylidene group in 4-*O*-protected 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose and the conformational impact of it. J. Carbohydr. Chem. 2, 395–404.

Vogel, K., Wang, S., Lee, R.J., Low, P.S., Chmielewski, J., 1996. Peptide-mediated release of folate-targeted liposome contents from endosomal compartments. J. Am. Chem. Soc. 118, 1581–1586.

Wymer, N.J., Gerasimov, O.V., Thompson, D.H., 1998. Cascade liposomal triggering: light-induced Ca^{2+} release from diplasmenylcholine liposomes triggers PLA_2-catalyzed hydrolysis and contents leakage from DPPC liposomes. Bioconjugate Chem. 9, 305–308.