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Isomerization of all-(*E*)-Retinoic Acid Mediated by Carbodiimide Activation – Synthesis of ATRA Ether Lipid Conjugates

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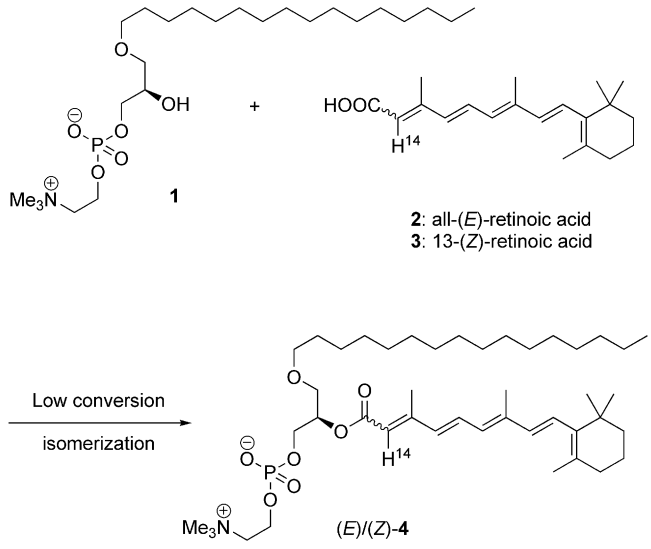
Treatment of the lysolipid 1-*O*-hexadecyl-*sn*-phosphatidylcholine with all-(*E*)-retinoic acid, DCC and DMAP resulted in poor acylation and caused (*Z*)/(*E*) isomerization of the α–β double bond. In the presence of a proton source, the carbodiimide-activated all-(*E*)-retinoic acid undergoes fast isomerization to give a final mixture of (13*E*)/(13*Z*) isomers in a 3:1 ratio. Similar treatment of (13*Z*)-retinoic acid leads to the same isomer ratio. The isomerization was circumvented successfully by using a Mitsunobu reaction, which provided an efficient synthesis of all-(*E*)-retinoic acid *sn*-2-conjugated to phosphatidylcholine and phosphatidylglycerol etherlipids.

Introduction

Liposomes are efficient drug-delivery systems for oncology, and a number of such systems are on the market or in clinical development.[1] We have recently disclosed novel types of drug-delivery systems, where the active drug is covalently linked to the lipid constituting the carrier system and liberated in situ by secretory phospholipase A_2 ($sPLA_2$).[2,3] In extension of these findings, we were interested in synthesizing *sn*-1 ether lipids with all-(*E*)-retinoic acid (ATRA) in the *sn*-2 position. ATRA is known to have cytotoxic properties towards a range of cancer cells and as such constitutes an interesting compound for cancer treatment.[4] Furthermore, ATRA is a highly lipophilic compound sensitive to acid and light that could potentially benefit from a liposomal formulation and the protection offered by localization to the liposome membrane.

Based on our experience with prodrugs with the anticancer agent chlorambucil in the *sn*-2 position, we have investigated the synthesis of (*E*)-**4** from lysolipid **1**[3] and ATRA (**2**) under standard acylation conditions.[5] However, when **1** was treated with excess **2**, dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in dichloromethane the conversion was poor and most retinoic acid formed either the anhydride or the *N*-acylurea byproduct. Furthermore, the isolated ester **4** was a mixture of two inseparable isomers. In the ^{1}H NMR spectrum, H^{14} gave rise to two signals with the proton of the major isomer resonating at δ = 5.8 ppm and the minor at δ = 5.6 ppm, data that is consistent with the isomers being esters of all-(*E*)-retinoic acid (**2**) and (13*Z*)-retinoic acid (**3**), respectively (Scheme 1).[6]



Scheme 1. DCC/DMAP-mediated ester coupling with concurrent isomerization.

Results and Discussion

To examine the acylation with ATRA and its concomitant isomerization we chose hexadecanol for acylation experiments to simplify spectral characterization and purification (Scheme 2 and Table 1). As displayed in Table 1 (Entry 1) our first choice of activation reagent was 3-[3-(di-

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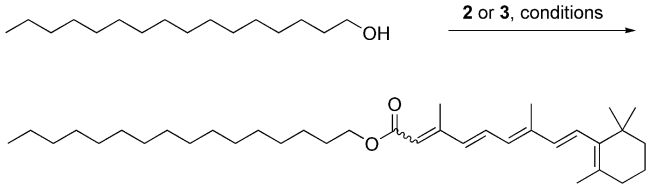
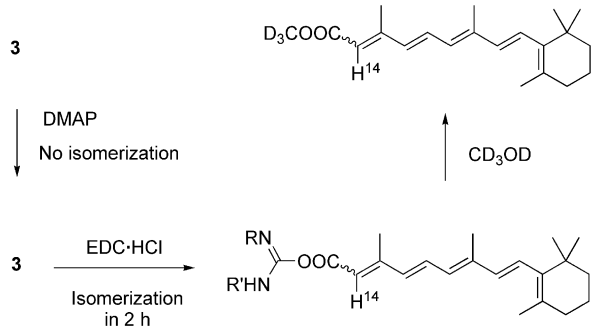
methylamino)propyl]-1-ethylcarbodiimide (EDC) hydrochloride hoping to minimize the formation of *N*-acylurea.[7,8] Indeed, the isolated yield was an excellent 98%, but isomerization was prominent with a (13*E*)/(13*Z*) isomer ratio of 3:1. Employing DCC instead (Entry 2) led to a lower yield of 80% but notably without the concomitant isomerization. Addition of pyridine hydrochloride (pyr·HCl) as a proton source (Entry 3) did not improve the yield but restored a (13*E*)/(13*Z*) isomer ratio of 3:1. At this point, it appeared that HCl was necessary for isomerization to occur, and another experiment employing tetrabutylammonium chloride (TBACl) as additive alongside DCC and DMAP (Entry 4) showed the chloride anion to have no significant effect on isomerization or yield. Simple treatment of retinoic acid with pyr·HCl in CD_2Cl_2 did not cause significant isomerization within 2 h (data not shown), which was well within the time frame of isomerization in the coupling experiments (vide infra). However, as indicated in Entry 5, employing EDC hydrochloride alone without DMAP did not cause any significant isomerization either. It is thus evident that both a proton source and DMAP are involved in the isomerization step.



Scheme 2. Reagents and conditions for acylation experiments.

In order to obtain a better understanding of the isomerization process, the reaction was monitored by ^{1}H NMR spectroscopy. All-(*E*)-retinoic acid (**2**) was dissolved in CD_2Cl_2 in an NMR tube, and reagents were added sequentially (Scheme 3, Figure 1 and Supporting Information).[9] DMAP addition did not cause any observable isomerization within 1 h, and EDC hydrochloride was added. A (13*E*)/(13*Z*) isomer ratio of ca. 3:1 for the activated intermediate was reached within 2 h, and this remained unchanged for 24 h (not shown). Thus, equilibrium had been reached, and quenching this mixture with [D_4]MeOH gave rise to two new signals conserving the 3:1 isomer ratio. Applying the same methodology with (13*Z*)-retinoic acid (**3**) resulted in the exact same (*E*)/(*Z*) = 3:1 ratio (see Supporting Information). Because this ratio is established within 2 h in the presence of the carbodiimide and HCl, it is evident that the isomerization process takes place only after formation of the activated intermediate, whereafter equilibrium is reached quickly.



Scheme 3. Sequential addition of coupling reagents in NMR experiment.

Naturally occurring retinoic acids exist in biological systems as mixtures of mainly all-(*E*)-retinoic acid, (13*Z*)-retinoic acid and (9*Z*)-retinoic acid and are all known to be efficacious in certain cancer treatments.[4] They have been shown to be interconverted enzymatically by glutathione *S*-methyltransferase,[10] and they may also be interconverted by treatment with thiol reagents.[11] Mechanistically, the isomerization process probably involves a 1,4-addition followed by single-bond rotation and elimination.[10] This mechanism has also been suggested in more general cases of (*E*)/(*Z*) isomerization of α,β-unsaturated carboxylic acid derivatives when their mixed anhydrides were treated with DMAP in esterification reactions[12] or a Yamaguchi lactonization.[13] However, to the best of our knowledge, this is the first clear documentation of the importance of a proton source in addition to DMAP as nucleophile, and these findings may improve the scope of this conventional method for ester formation.

However, even with the reactive cetyl alcohol the yield for the carbodiimide coupling under conditions where minimum isomerization occurs (Table 1, Entries 2, 4 and 5) was considered inadequate. Employing a Mitsunobu reaction[14] to obtain cetyl esters of both all-(*E*)- and (13*Z*)-retinoic acid (Table 1, Entries 6 and 7) completely suppressed iso-

Table 1. Cetyl esters of retinoic acid.

Entry	Retinoic acid	Conditions[a]	(13*E*)/(13*Z*)[b]	Yield[c]
1	**2**	EDC·HCl (1.5 equiv.), DMAP (1.5 equiv.)	3:1	98%
2	**2**	DCC (1.5 equiv.), DMAP (1.5 equiv.)	1:0	80%
3	**2**	DCC (1.5 equiv.), DMAP (1.5 equiv.), pyr·HCl (1.5 equiv.)	3:1	69%
4	**2**	DCC (1.5 equiv.), DMAP (1.5 equiv.), TBACl (1.5 equiv.)	16:1	73%
5	**2**	EDC·HCl (2.0 equiv.)	16:1	53%
6	**2**	DIAD (1.5 equiv.), Ph_3P (1.5 equiv.)	1:0	99%
7	**3**	DIAD (1.5 equiv.), Ph_3P (1.5 equiv.)	0:1	81%

[a] Reactions performed with $C_{16}H_{33}OH$ (2.0 equiv.) in CH_2Cl_2 at 20 °C (Entries 1–5) or THF at 0 °C (Entries 6–7). [b] Determined by ^{1}H NMR spectroscopy. [c] Isolated yield after 24 h (48 h for Entry 5).

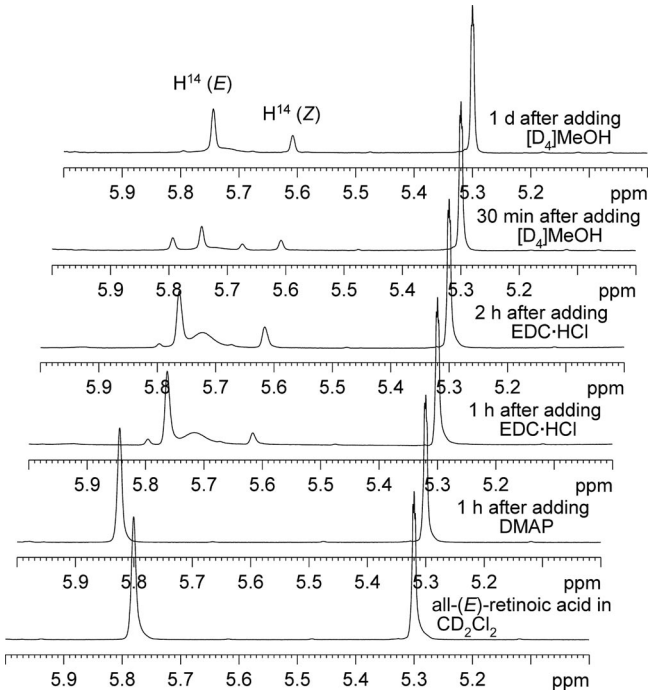
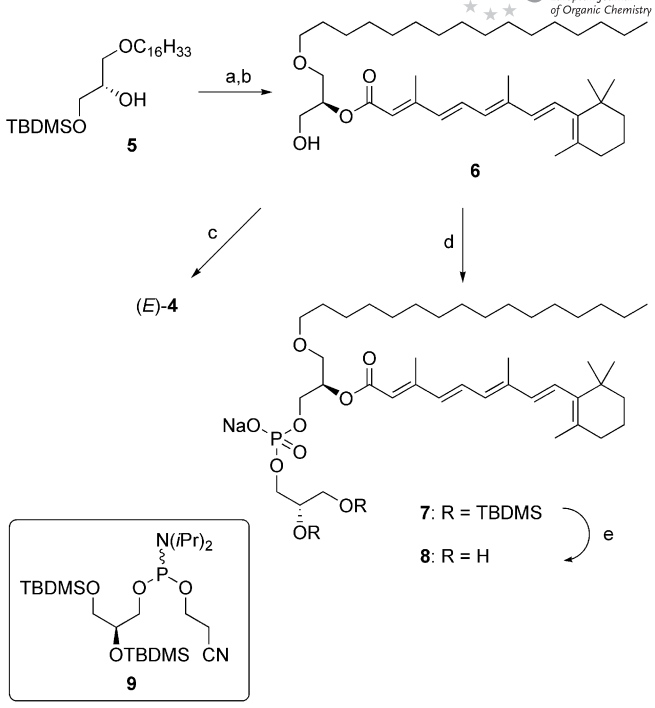


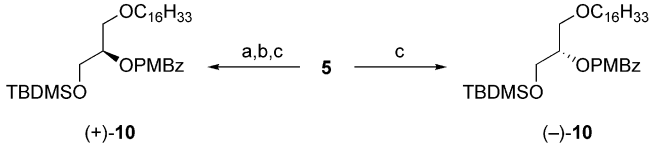
Figure 1. Successive NMR spectra showing H^{14} signals acquired during activation of all-(*E*)-retinoic acid by EDC·HCl followed by quenching with $[D_4]$MeOH in CD_2Cl_2 (solvent residue at δ = 5.3 ppm, indicated for reference of scale).



Scheme 4. Reagents and conditions: (a) **2**, Ph_3P, DIAD, THF; (b) aq. HF, MeCN (50%, 2 steps); (c) i: $POCl_3$, CH_2Cl_2, ii: choline tosylate, pyridine, iii: H_2O (40%, 3 steps); (d) i: **9**, 1*H*-tetrazole, MeCN, CH_2Cl_2, ii: *t*BuOOH, iii: DBU (89%, 3 steps); (e) aq. HF, MeCN (82%).

merization for both esters. For this reason and due to the difficulties of obtaining acylation precursors that were completely free of residual protons, we opted for this reaction for the preparation of the desired phospholipids (*E*)-**4** and **8** containing all-(*E*)-retinoic acid in the *sn*-2 position (Scheme 4). Thus, the known[15] alcohol **5**, obtained in 82% yield from L-1,2-isopropylidineglycerol in three steps, was esterified with all-(*E*)-retinoic acid by a Mitsunobu reaction. Coelution of excess diisopropyl azodicarboxylate (DIAD) with the product precluded isolation on a larger scale, and the ester was therefore deprotected immediately. Desilylation with TBAF in THF was hampered by acyl migration from the *sn*-2 to the *sn*-3 position. In contrast, the TBDMS group was removed cleanly by using aq. HF in acetonitrile and afforded **6** in a 50% yield over the two steps. The phosphatidylcholine headgroup was installed by treatment of **6** with excess phosphoryl trichloride followed by choline tosylate and then water to give **4** in a 40% yield. Alternatively, the phosphorylglycerol headgroup could be introduced to give **7** in 89% yield over three steps by standard amidite chemistry employing the TBDMS protected phosphoramidite **9**.[3] The amidite was activated with 1*H*-tetrazole, and subsequent phosphorus oxidation was achieved with careful sequential addition of *t*BuOOH in decane to avoid oxidation of the olefins.[16] The TBDMS groups were removed in 82% yield to give **8** again by employing aq. HF in acetonitrile, conditions tolerated by the sensitive unsaturated ester. Compared to the more conventional acetonide protection of the glycerol headgroup,[17] the milder deprotection conditions required for the silyl ethers make them very well suited for acid-sensitive lipids.

To confirm complete inversion in the Mitsunobu reaction, a sample of the ester was reductively cleaved with diisobutylaluminum hydride and subsequently converted into the *para*-methoxybenzoate for analytical convenience to give (+)-**10** (Scheme 5). Comparison with the enantiomer (–)-**10** by chiral HPLC (chiralcel OD-H column) showed clean inversion with retention times of 6.0 min for (–)-**10** and 6.5 min for (+)-**10** (see Supporting Information for details).



Scheme 5. Reagents and conditions: (a) **2**, Ph_3P, DIAD, THF, 0 → 20 °C; (b) DIBAL-H, CH_2Cl_2, –78 °C; (c) PMBzCl, DMAP, Et_3N, CH_2Cl_2, 0 → 20 °C.

To asses whether the phospholipid **8** was a substrate for secretory phospholipase A_2, the lipid was suspended in a stirred emulsion of 10 mM HEPES buffer (pH = 7.5, 0.03 mM $CaCl_2$) and Et_2O (5:2) and treated with snake (*Naja mossambica mossambica*) venom phospholipase A_2.[18] Evidently, neither TLC nor MALDI-TOF MS of the ethereal phase showed hydrolytic cleavage after 2 d of incubation, whereas the natural substrate dipalmitoylphosphatidylglycerol (DPPG) was cleaved quantitatively under the same conditions in less than 2 h. Likewise,

liposomes formulated from **8** with an average diameter of 96 nm as determined by dynamic light scattering (DLS) were not degraded by $sPLA_2$, neither from *Naja mossambica mossambica* nor *Agkistrodon piscivorus piscivorus* (see Supporting Information). Similarly, phosphatidylcholine lipid (*E*)-**4** was not hydrolyzed by $sPLA_2$ (data not shown). Since it has been reported[19] that retinoids can inhibit PLA_2 activity, we decided to investigate the hydrolysis of DPPG in the presence of ATRA. Our experiments ruled out that the lack of hydrolysis was due to inhibition by ATRA, since DPPG was completely hydrolyzed by PLA_2 within 24 h in the presence of both 0.1 and 1 equiv. of ATRA (see Supporting Information).

Conclusions

We have shown the importance of a proton source and a nucleophile such as DMAP in the isomerization of all-(*E*)-retinoic acid to its (13*Z*) isomer when activated with carbodiimide reagents. To the best of our knowledge, this represents a new aspect of this isomerization and may have consequences for acylations with α,β-unsaturated acids in general. The enzyme experiments show that ATRA–lipid conjugates are not hydrolyzed by PLA_2. Comparison to the natural substrates and our previous investigation of chlorambucil–lipid conjugates suggest that the rigid and sterically demanding structure of ATRA is responsible for the inertness of these compounds to PLA_2 degradation.

Experimental Section

General: All commercial reagents were used as supplied. Unless otherwise stated reactions were performed under nitrogen and anhydrous solvents used. Flash-column chromatography was carried out by using Merck silica gel 60 (particle size 0.040–0.063 mm). Mixtures were stirred by using a magnetic stir-bar and the reactions monitored by TLC on aluminum plates, pre-coated with silica gel 60 and spots visualized by dipping in an ethanolic solution of phosphomolybdic acid (12 g/250 mL) followed by heating. Optical rotations were measured with a Perkin–Elmer 241 polarimeter, and IR spectra were recorded with a Bruker Alpha FT-IR spectrometer. NMR spectral characterization was carried out with either a Varian Mercury 300 MHz instrument or a Varian Unity Inova 500 MHz instrument as indicated in the text. High-resolution mass spectra were recorded with an Ionspec Ultima Fourier Transform mass spectrometer at the Department of Physics and Chemistry, University of Southern Denmark.

Example for Entry 2 (Table 1): Acid **2** (100 mg, 0.33 mmol), DMAP (61 mg, 0.50 mmol) and cetyl alcohol (161 mg, 0.66 mmol) were dissolved in CH_2Cl_2 (3 mL), and DCC (1.0 M in CH_2Cl_2, 0.50 mL, 0.50 mmol) was added dropwise. The mixture was stirred for 24 h before flash chromatography on silica (CH_2Cl_2/hexane, 2:3) afforded the product. ^1H NMR spectra of the products in Entries 1, 6 and 7 are enclosed in the Supporting Information.

1-Hexadecyl-2-[all-(*E*)-retinoyl]-*sn*-glycerol (6): Acid **2** (0.30 g, 1.0 mmol), Ph_3P (0.44 g, 2.0 mmol) and **5** (0.36 g, 0.84 mmol) were dissolved in THF (10 mL) and cooled to 0 °C. Diisopropyl azodicarboxylate (0.20 mL, 1.0 mmol) was added slowly over 10 min. The mixture was stirred for 2 h before another portion of Ph_3P, **2** and DIAD was added. The mixture was stirred for 3 h, concentrated in vacuo, and the residue was chromatographed (CH_2Cl_2/hexane, 1:1) to give an oil, which was suspended in MeCN (9.0 mL) and cooled to 0 °C before 40% aq. HF (1.0 mL) was added dropwise. Cooling was then removed and the mixture stirred for 9 h before it was poured into satd. aq. $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was dried (Na_2SO_4) and concentrated in vacuo before flash chromatography on silica (EtOAc/heptane, 1:9 → 1:1) gave **6** as a gum (0.25 g, 50%). $[\alpha]_D^{20}$ = +12.2 (*c* = 2.4, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 3446, 2924, 2853, 1710, 1609, 1584, 1458, 1358, 1239, 1152, 1050, 966 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.01 (dd, $^3J_{H,H}$ = 11.4, 15.0 Hz, 1 H, 11-H), 6.30–6.11 (m, 4 H, 12-H, 10-H, 8-H, 7-H), 5.82 (s, 1 H, 14-H), 5.04 (q, $^3J_{H,H}$ = 4.7 Hz, 1 H, *sn*-2-H), 3.84 (m, 2 H, *sn*-3-H), 3.65 (m, 2 H, *sn*-1-H), 3.47 (m, 2 H, 1′-H), 2.37 (d, $^3J_{H,H}$ = 1.0 Hz, 3 H, 13-Me), 2.00 (m, 5 H, 9-Me, 3-H), 1.71 (s, 3 H, 2-Me), 1.63–1.44 (m, 6 H, 4-H, 2′-H, 5-H), 1.24 (br., 26 H, 3′-H to 15′-H), 1.02 (br., 6 H, 2 6-Me), 0.87 (t, $^3J_{H,H}$ = 6.6 Hz, 3 H, 16′-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.77, 153.94, 139.91, 137.62, 137.18, 134.87, 131.39, 130.07, 129.40, 128.82, 117.88, 72.27, 71.88, 70.12, 63.20, 39.54, 34.22, 33.08, 31.90, 29.68–29.61 (8 C), 29.52, 29.44, 29.35, 28.93 (2 C), 26.02, 22.68, 21.73, 19.17, 14.12, 13.94, 12.90 ppm. HRMS (ESI): calcd. for $C_{39}H_{66}O_4Na$ $[M + Na]^+$ 621.4854; found 621.4848.

1-Hexadecyl-2-[all-(*E*)-retinoyl]-*sn*-glycero-3-phosphorylcholine [(*E*)-4]: A solution of **6** (166 mg, 0.28 mmol) and Et_3N (50 μL, 0.36 mmol) in CH_2Cl_2 (4 mL) was dropwise added to a solution of $POCl_3$ (32 μL, 0.35 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C over 15 min. The solution was stirred at room temperature for 30 min, after which pyridine (180 μL, 2.23 mmol) and choline tosylate (153 mg, 0.55 mmol) were added. The solution was stirred at room temperature for 16.5 h. Water (0.2 mL) was added, and the mixture was stirred for 40 min. Continuous concentration with ethanol/toluene (1:1, 20 mL) gave a yellow residue, which was redissolved in THF/H_2O (9:1) and slowly passed through an MB-3 column. The solvent was removed by continuous concentration with ethanol/toluene (1:1, 20 mL). The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH, 4:1 → CH_2Cl_2/MeOH/H_2O, 15:10:1) to give (*E*)-**4** as a yellow oil (84 mg, 40%). $[\alpha]_D^{20}$ = –7.7 (*c* = 0.6, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2923, 2853, 2490, 1706, 1458, 1238, 1152, 1085, 967 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$/CD_3OD, 4:1): δ = 7.03 (dd, $^3J_{H,H}$ = 11.5, 15.0 Hz, 1 H, 11-H), 6.30 (d, $^3J_{H,H}$ = 16.0 Hz, 1 H, 7-H), 6.29 (d, $^3J_{H,H}$ = 15.0 Hz, 1 H, 12-H), 6.15 (d, $^3J_{H,H}$ = 16.0 Hz, 1 H, 8-H), 6.15 (d, $^3J_{H,H}$ = 11.5 Hz, 1 H, 10-H), 5.79 (s, 1 H, 14-H), 5.20–5.16 (m, 1 H, *sn*-2-H), 4.23 (br., 2 H, $CH_2CH_2NMe_3$), 4.09–4.02 (m, 2 H, *sn*-3-H), 3.67–3.62 (m, 2 H, *sn*-1-H), 3.58 (br., 2 H, $CH_2CH_2NMe_3$), 3.49–3.42 (m, 2 H, 1′-H), 3.20 (s, 9 H, NMe_3), 2.34 (s, 3 H, 13-Me), 2.05–2.00 (m, 5 H, 3-H), 1.72 (s, 3 H, 2-Me), 1.66–1.60 (m, 2 H, 4-H), 1.57–1.51 (m, 2 H, 3′-H), 1.49–1.47 (m, 2 H, 5-H) 1.26 (br., 26 H, 3′-H to 15′-H), 1.03 (s, 6 H, 2 6-Me), 0.88 (t, $^3J_{H,H}$ = 6.9 Hz, 3 H, 16′-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$/CD_3OD, 4:1): δ = 166.49, 153.48, 139.75, 137.30, 136.87, 134.36, 131.27, 129.74, 129.00, 128.63, 117.55, 71.37, 70.90 (d, $J_{C,P}$ = 9.0 Hz), 68.89, 66.15 (d, $J_{C,P}$ = 9.0 Hz), 63.90 (d, $J_{C,P}$ = 5.3 Hz), 58.53 (d, $J_{C,P}$ = 5.3 Hz), 53.79, 53.75, 53.70, 39.19, 33.86, 32.70, 31.55, 29.33–29.29 (8 C), 29.14, 29.13, 28.99, 28.47 (2 C), 25.64, 22.30, 21.24, 18.81, 13.60, 13.39, 12.41 ppm. HRMS (ESI): calcd. for $C_{44}H_{78}NO_7P$ $[M + Na]^+$ 786.5408; found 786.5422.

1-Hexadecyl-2-[all-(*E*)-retinoyl]-*sn*-glycero-3-phosphoryl-1′-[2′,3′-bis-(*tert*-butyldimethyl)-*sn*-glycerol] (7): Compound **6** (131 mg,

0.22 mmol) and amidite **9** (171 mg, 0.33 mmol) were dissolved in CH_2Cl_2 (5 mL), and molecular sieves (3-Å, 250 mg) were added. After 30 min, 1*H*-tetrazole in MeCN (0.50 mL, 0.45 M, 0.23 mmol) was added, and the mixture was stirred for 1 h. *t*BuOOH in decane (60 μL, 5.5 M, 0.33 mmol) was added, and, after 1 h, another portion was added (30 μL, 0.17 mmol). The mixture was then stirred for 30 min before addition of DBU (90 μL, 0.60 mmol). After 30 min, the mixture was concentrated in vacuo. Flash chromatography on silica gel (EtOAc, then CH_2Cl_2/MeOH, 4:1) gave **7** (193 mg, 89%) as a gum. $[\alpha]_D^{20}$ = –68 (*c* = 5.2, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2926, 2855, 1709, 1610, 1582, 1463, 1360, 1250, 1152, 1105 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 6.85 (dd, $^3J_{H,H}$ = 11.4, 15.0 Hz, 1 H, 11-H), 6.13–5.95 (m, 4 H, 12-H, 10-H, 8-H, 7-H), 5.59 (s, 1 H, 14-H), 4.99 (q, $^3J_{H,H}$ = 5 Hz, 1 H, *sn*-2-H), 3.80 (m, 2 H, *sn*-3-H), 3.65 (m, 2 H, sn-1′-H), 3.58 (m, 1 H, *sn*-2′-H), 3.46–3.36 (m, 4 H, *sn*-1-H, *sn*-3′-H), 3.26 (m, 2 H, 1′-H), 2.16 (s, 3 H, 13-Me), 1.86–1.83 (m, 5 H, 9-Me, 3-H), 1.53 (s, 3 H, 2-Me), 1.46–1.28 (m, 6 H, 4-H, 2′-H, 5-H), 1.07 (br., 26 H, 3′-H to 15′-H), 0.85 (s, 6 H, 2 6-Me), 0.71 (s, 21 H, 2 $SiCMe_3$, 16′-H), –0.08 (s, 6 H, $SiMe_2$), –0.12 (s, 6 H, $SiMe_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 166.39, 153.61, 139.63, 137.30, 136.91, 134.43, 131.21, 129.64, 129.06, 128.53, 117.46, 72.37 (d, $J_{C,P}$ = 9.6 Hz), 71.36, 70.72 (d, $J_{C,P}$ = 8.7 Hz), 68.79, 66.47 (d, $J_{C,P}$ = 5.7 Hz), 64.64, 63.72 (d, $J_{C,P}$ = 4.9 Hz), 39.19, 33.84, 32.69, 31.56, 29.33–29.16 (10 C), 29.00, 28.48 (2 C), 25.65, 25.46 (3 C), 25.36 (3 C), 22.30, 21.25, 18.81, 17.94, 17.73, 13.62, 13.40, 12.40, –5.17, –5.24, –5.89, –5.94 ppm. ^{31}P NMR (202 MHz, $CDCl_3/CD_3OD$, 3:1): δ = –2.05 ppm. HRMS (ESI): calcd. for $C_{54}H_{100}O_9PSi_2Na_2$ $[M + Na]^+$ 1025.6434; found 1025.6443.

1-Hexadecyl-2-[all-(*E*)-retinoyl]-*sn*-glycero-3-phosphoryl-1′-*sn*-glycerol (8): Compound **7** (57 mg, 58 μmol) was dissolved in MeCN (1.8 mL), cooled to 0 °C, and 40% aq. HF (0.2 mL) was added. Cooling was removed, and the mixture was stirred vigorously for 5 h before it was poured into saturated aq. $NaHCO_3$ (5 mL). The mixture was diluted with brine (10 mL) and extracted with CH_2Cl_2 (5 × 10 mL) and then EtOAc (10 mL). The combined organic phases were dried (Na_2SO_4), concentrated in vacuo, and the residue was purified by flash chromatography on silica gel (CH_2Cl_2 → CH_2Cl_2/MeOH/H_2O, 65:25:4) to afford **8** (36 mg, 82%) after azeotropic removal of residual water with toluene at 40–50 °C in vacuo. $[\alpha]_D^{20}$ = –20 (*c* = 0.5, $CHCl_3$). IR (neat): $\tilde{\nu}$ = 2924, 2853, 1709, 1611, 1585, 1466, 1388, 1359, 1239, 1118, 1052 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 6.84 (dd, $^3J_{H,H}$ = 11.4, 15.0 Hz, 1 H, 11-H), 6.11–5.93 (m, 4 H, 12-H, 10-H, 8-H, 7-H), 5.60 (s, 1 H, 14-H), 4.99 (q, $^3J_{H,H}$ = 5 Hz, 1 H, *sn*-2-H), 3.82 (br., 2 H, *sn*-3-H), 3.72 (br., 2 H, sn-1′-H), 3.59 (br., 1 H, *sn*-2′-H), 3.43 (m, 4 H, *sn*-1-H, *sn*-3′-H), 3.26 (m, 2 H, 1′-H), 2.15 (s, 3 H, 13-Me), 1.83–1.82 (m, 5 H, 9-Me, 3-H), 1.52 (br., 3 H, 2-Me), 1.44 (m, 2 H, 4-H), 1.33 (m, 2 H, 2′-H), 1.29 (m, 2 H, 5-H), 1.05 (br., 26 H, 3′-H to 15′-H), 0.84 (s, 6 H, 2 6-Me), 0.68 (t, $^3J_{H,H}$ = 6.8 Hz, 3 H, 16′-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$, 3:1): δ = 166.51, 153.85, 139.81, 137.32, 136.89, 134.39, 131.40, 129.78, 129.07, 128.68, 117.40, 71.46, 70.78 (d, $J_{C,P}$ = 8.3 Hz), 70.56 (d, $J_{C,P}$ = 4.5 Hz), 68.80, 66.10 (d, $J_{C,P}$ = 3.8 Hz), 64.05, 61.87 (d, $J_{C,P}$ = 1.5 Hz), 39.24, 33.90, 32.75, 31.60, 29.39–29.22 (10 C), 29.04, 28.54 (2 C), 25.70, 22.35, 21.32, 18.85, 13.67, 13.47, 12.48 ppm. ^{31}P NMR (202 MHz, $CDCl_3/CD_3OD$, 3:1): δ = –0.79 ppm. HRMS (ESI): calcd. for $C_{42}H_{72}O_9PNa_2$ $[M + Na]^+$ 797.4704; found 797.4731.

3-(*tert*-Butyldimethylsilyl)-1-hexadecyl-2-(4-methoxybenzoyl)-*sn*-glycerol [(+)-10]: Compound **5** (168 mg, 0.24 mmol) and Ph_3P (0.23 g, 0.88 mmol) were dissolved in THF (10 mL) and cooled to 0 °C. Then **2** was added (0.18 g, 0.60 mmol) followed by portionwise addition of DIAD (0.20 mL, 1.0 mmol) over 30 min. The mixture was stirred for 24 h, concentrated in vacuo and purified by flash chromatography on silica gel (CH_2Cl_2/heptane, 1:1) to yield the ester (127 mg). ^1H NMR (300 MHz, $CDCl_3$): δ = 6.98 (dd, $^3J_{H,H}$ = 11.4, 15.0 Hz, 1 H, 11-H), 6.30–6.09 (m, 4 H, 12-H, 10-H, 8-H, 7-H), 5.79 (s, 1 H, 14-H), 5.04 (q, $^3J_{H,H}$ = 5 Hz, 1 H, *sn*-2-H), 3.80–3.70 (m, 2 H, *sn*-3-H), 3.63–3.54 (m, 2 H, *sn*-1-H), 3.50–3.37 (m, 2 H, 1′-H), 2.16 (s, 3 H, 13-Me), 2.03–1.99 (m, 5 H, 9-Me, 3-H), 1.70 (s, 3 H, 2-Me), 1.64–1.44 (m, 6 H, 4-H, 2′-H, 5-H), 1.24 (br., 26 H, 3′-H to 15′-H), 1.01 (s, 6 H, 2 6-Me), 0.87 (s, 12 H, $SiCMe_3$, 16′-H), 0.04 (s, 6 H, $SiMe_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.47, 152.97, 139.53, 137.64, 137.24, 135.14, 130.92, 129.95, 129.48, 128.59, 118.60, 72.36, 71.56, 68.89, 61.70, 39.55, 34.22, 33.07, 31.91, 30.91, 29.68–29.58 (8 C), 29.47, 29.35, 28.92 (2 C), 26.06, 25.79 (3 C), 22.68, 21.72, 19.18, 18.22, 14.11, 13.86, 12.87, –5.41, –5.43 ppm. The ester (127 mg, 0.18 mmol) was then dissolved in CH_2Cl_2 (5 mL) and cooled to –78 °C before DIBAL-H in hexane (1.0 M, 0.25 mL, 0.25 mmol) was added slowly over a 5 min period and the mixture then stirred for 1 h. Then, MeOH (0.2 mL) was added, the mixture stirred for 15 min and satd. aq. Rochelle's salt solution (20 mL) added before the mixture was left to warm up for 16 h. The mixture was diluted with water (10 mL) and extracted with CH_2Cl_2 (2 × 10 mL), dried (Na_2SO_4) and concentrated in vacuo. The crude mixture was then dissolved in CH_2Cl_2 (2 mL) containing Et_3N (0.2 mL) and cooled to 0 °C. PMBzCl (100 μL, 1.30 mmol) was added followed by a spatula tip of DMAP. The mixture was stirred for 24 h before water (0.1 mL) was added. The mixture was then stirred for 2 h, dried (Na_2SO_4) and purified by flash chromatography on silica gel (CH_2Cl_2/heptane, 1:1) to yield (+)-**10** (56 mg, 55%). $[\alpha]_D^{20}$ = +4.8 (*c* = 0.8, $CHCl_3$). HPLC (chiralcel OD-H column, *n*-hexane/*i*PrOH, 400:1, 1.00 mL min^{-1}): t_r = 6.5 min. IR (neat): $\tilde{\nu}$ = 2924, 2854, 1715, 1607, 1511, 1463, 1255, 1167, 1100, 1033, 836, 770 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 8.03–7.98 (m, 2 Ar-H), 6.93–6.88 (m, 2 Ar-H), 5.21 (q, $^3J_{H,H}$ = 5 Hz, 1 H, *sn*-2-H), 3.85–3.84 (m, 5 H, *sn*-3-H, OMe), 3.69–3.68 (m, 2 H, *sn*-1-H), 3.52–3.40 (m, 2 H, 1′-H), 1.58–1.49 [m, 2 H, overlap with residual water at δ = 1.56 (2′-H) ppm], 1.25 (br., 26 H, 3′-H to 15′-H), 0.87 (s, 12 H, $SiCMe_3$, 16′-H), 0.04 (s, 3 H, SiMe), 0.03 (s, 3 H, SiMe) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.79, 163.27, 131.71 (2 C), 122.80, 113.47 (2 C), 73.51, 71.60, 68.95, 61.75, 55.38, 31.91, 29.69–29.61 (9 C), 29.46, 29.35, 26.08, 25.78 (3 C), 22.68, 18.21, 14.12, –5.42, –5.44 ppm. HRMS (ESI): calcd. for $C_{33}H_{60}O_5SiNa$ $[M + Na]^+$ 587.4102; found 587.4076.

1-(*tert*-Butyldimethylsilyl)-3-hexadecyl-2-(4-methoxybenzoyl)-*sn*-glycerol (–)-10: Compound **5** (75 mg, 0.11 mmol) and Et_3N (70 μL, 0.73 mmol) were dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. Then, PMBzCl (100 μL, 1.30 mmol) was added followed by a spatula tip of DMAP. The mixture was stirred for 24 h before water (0.1 mL) was added, then stirred for another 2 h, dried (Na_2SO_4) and purified by flash chromatography on silica gel (CH_2Cl_2/heptane, 1:1) to yield compound (–)-**10** (38 mg, 61%). $[\alpha]_D^{20}$ = –5.2 (*c* = 1.1, $CHCl_3$). HPLC (chiracel OD-H, *n*-hexane/*i*PrOH, 400:1, 1.00 mL min^{-1}): t_r = 6.0 min. IR, ^1H and ^{13}C NMR spectra are identical to those of (+)-**10**. HRMS (ESI): calcd. for $C_{33}H_{60}O_5SiNa$ $[M + Na]^+$ 587.4102; found 587.4086.

Supporting Information (see footnote on the first page of this article): ^1H, ^{13}C and ^{31}P NMR spectra of compound **6**, **7** and **8** and ^1H, ^{13}C NMR spectra of **4** and **10** together with NMR assignment tables for **4** and **8** with the applied atom numbering; HPLC chromatograms of compounds (+)-**10** and (–)-**10**, NMR experiment data, DLS measurements, liposome formulation and MALDI results for hydrolysis experiments.

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