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Synthesis of Optically Pure Diglycerol Tetraether Model Lipids with Non-Natural Branching Pattern

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Three new, chain-modified, optically pure diglycerol tetraether lipids with one membrane-spanning chain have been synthesised. These lipids contain a different number and constitution of the methyl branches connected to the hydrophobic chains as compared with natural archaeal or other previously synthesised lipids. The correct chirality of the branched alkyl chain was introduced starting from commercially available (*S*)-citronellyl bromide. For chain elongation the Cu-catalysed Grignard coupling reaction was used. Suitable blocked glycerol ethers were condensed to the tetraether moieties by Grubbs metathesis. The insertion of two or four optically pure methyl branches at the 10- and/or 23-positions of the alkyl chains are sufficient to mimic the main properties of natural tetraether lipids. In this context, it has been shown that these lipids can form closed lipid vesicles.

Introduction

Diglycerol tetraether lipids, which are found in the cell membranes of methanogenic and thermoacidophilic archaebacteria, differ considerably in their chemical structure from common known lipids. They are composed of two glycerol-containing hydrophilic head-groups connected to one or two lipophilic, membrane-spanning alkyl chains with several methyl branches in an isoprenoid arrangement as well as a various number of cyclopentane rings. Furthermore, the glycerol-containing head-groups are connected to the alkyl chains by ether linkages in an *sn*-2,3-stereochemistry and are arranged on both sides of the cell membrane.[1] The resulting chemical, thermal and enzymatic stability as well as the tendency to form closed lipid vesicles make these tetraether lipids interesting for biotechnology, material science and pharmacy.[2] However, the isolation of archaebacterial lipids is laborious resulting generally in mixtures of tetraether lipids with different alkyl chain lengths. On the other hand, their total synthesis has enabled the preparation of tailor-made lipids.[3] Nevertheless, the assembly of branched isoprenoid alkyl chains and the macrocyclisation is a very time-consuming and expensive task yielding only small amounts of these lipids. Therefore most of the scientists interested in this field have focused their synthetic work on simpler model compounds that should mimic the properties of the natural archaebacterial lipids to a maximal extent.

Yamauchi et al.[4] and also others have prepared an unbranched model compound with only one membrane-spanning chain to avoid the challenge of macrocyclisation. When the C_{32} chain had no methyl branches, however, the compound[4] did not form closed liposomes, but flat extended sheets, and showed a high transition temperature (T_m) of 61 °C, which is not observed for archaebacterial lipids. The insertion of isoprenoid phytanyl residues into C_{16} chains led to a decrease in the value of T_m and allowed the preparation of lipid vesicles.[5] In the following years other researchers described the use of phytanyl[6] or (*R*)-citronellyl residues for even shorter chains.[7] Benvegnu and co-workers[6c] introduced a cyclopentane ring into the middle of the membrane-spanning chain to enhance the stability. In addition, non-symmetric compounds with different head-groups were prepared by using the same structural concept.[8] However, all model compounds with branched alkyl chains copied the isoprenoid pattern. In addition to our investigations on the synthesis of single-chain, bipolar phospholipids,[9] we were also interested in the synthesis of model compounds with a closer resemblance to the natural archaebacterial tetraether lipids. Our aim was to find out whether the isoprenoid arrangement of the methyl groups in the alkyl chains is necessary to form compounds with similar behaviour to the archaebacterial lipids or whether the insertion of only a few methyl branches at definite positions of the alkyl chains is sufficient to achieve the desired properties. Therefore we designed three different op-

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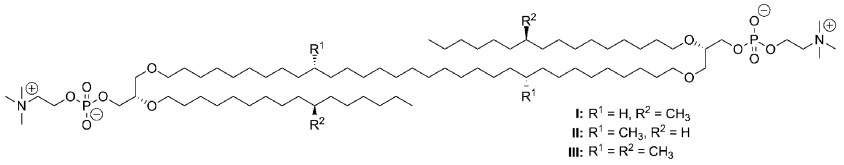


Figure 1. Structures of the synthesised archaebacterial model lipids **I**–**III**.

tically pure diglycerol tetraether lipids **I**–**III** with two or four methyl groups at certain positions in the alkyl chains (Figure 1).

As reference compounds we also synthesised bis(phosphocholines) consisting of a diglycerol tetraether backbone with one transmembrane dotriacontanyl and two shorter hexadecyl chains. The lyotropic behaviour of comparable compounds has been reported previously.[4,5]

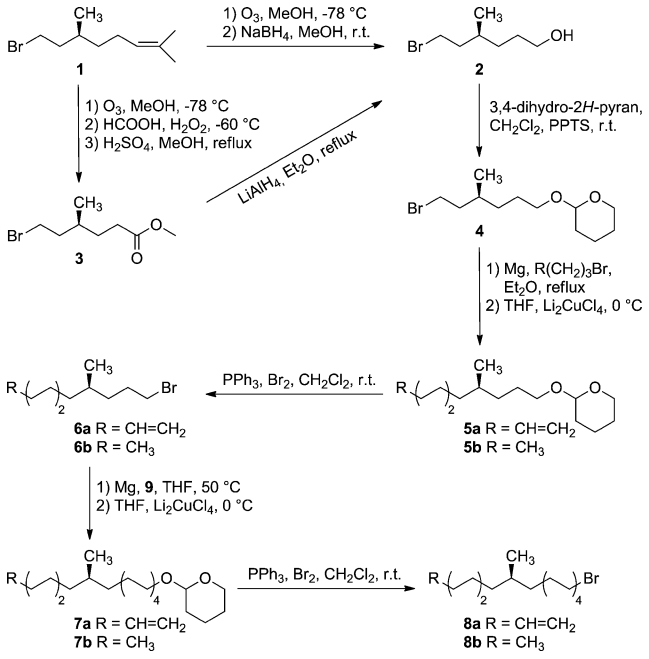
The methyl branches with *R* configuration were located at the 10-positions of the monopolar hexadecyl chains (**I**), at the 10,23-positions of the transmembrane chain (**II**) or in all of these positions (**III**; see Figure 1). We present herein the synthesis of the new, optically pure model lipids as well as the first investigations of the lyotropic behaviour of these compounds by differential scanning calorimetry (DSC) and electron microscopy (EM).

Results and Discussion

Synthetic Methods

Within our synthetic strategy, the correct *R* configuration of the methyl branching, which is also shown by natural archaeal membrane lipids, was obtained from a compound of the chiral pool. Kakinuma and co-workers constructed the isoprenoid-branched alkyl chains of the archaeal 36-membered macrocyclic diether lipid[3a] and also of the archaeal 72-membered tetraether lipid[3b] by using (*R*)-3-hydroxy-2-methylpropionate or (*R*)-citronellol as the starting material. After a multistep procedure, the authors isolated in both cases a selectively blocked octane-1,8-diol with two methyl branches in the correct stereoconfiguration, which were subsequently elongated to yield isoprenoid-branched alkyl chains. The synthesis starting from (*R*)-citronellol led to the blocking of the hydroxy group followed by ozonisation and reduction to the corresponding alcohol.

In accord with our final compounds **I**–**III** and C–C elongation strategy, respectively, the commercially available (*S*)-citronellyl bromide (**1**), already containing the bromo atom for the intended Grignard coupling, emerged as the starting material of choice. Thus, the bromide **1** was allowed to completely react with ozone at –78 °C in methanol. Afterwards, sodium borohydride was added portionwise over a period of 30 min accompanied by vigorous stirring. During this step the temperature increased to –30 °C. In contrast, the addition of all the sodium borohydride at –78 °C followed by slow warming[3a] led to an extreme exothermic reaction with a temperature increase up to 50 °C leading to the formation of byproducts. The resulting (4*S*)-6-bromo-4-methylhexan-1-ol (**2**) was isolated after purification by chromatography in 87% yield, in line with a similar ozonisation product prepared by Kakinuma and co-workers.[3a] We also investigated the oxidative work-up of the ozonides: instead of sodium borohydride, formic acid and hydrogen peroxide were added to the ozonide solution. After heating the solution at reflux for 2 h, the crude carboxylic acid was isolated, dissolved in methanol and heated again with catalytic amounts of sulfuric acid to yield the corresponding methyl ester **3**. But in this one-pot procedure, the amount of ester **3** isolated varied from 62% (chromatography) to 77% (distillation), depending on the purification process. Because the subsequent reduction of ester **3** to the bromo alcohol **2** gave only 70% yield, this oxidative synthetic strategy does not represent an alternative pathway to the reductive route using sodium borohydride (Scheme 1).



Scheme 1. Synthesis of optically pure methyl-branched alkyl bromides **8a**,**b** (PPTS: pyridinium *p*-toluenesulfonate).

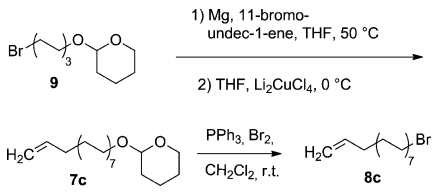
In the next step, the alcohol moiety of the bromo alcohol **2** was protected by 3,4-dihydro-2*H*-pyran and catalytic amounts of pyridinium *p*-toluenesulfonate (PPTS) in a nearly quantitative reaction. The resulting 2-{[(4*S*)-6-bromo-4-methylhexyl]oxy}tetrahydro-2*H*-pyran (**4**) was the optically pure starting compound for the chain elongation reaction. The frequently discussed problems associated with the formation of diastereomers with chiral centres and the tetrahydropyran (THP) ring did not play a role in the separation in our case because of the considerable distance be-

tween the two chiral centres. Only in the ^{13}C NMR spectra could we find two signals for relevant atoms near the optical centres indicating the formation of two diastereomers.

Then compound **4** was coupled with pent-4-enylmagnesium bromide or butylmagnesium bromide in a Grignard reaction under catalysis with dilithium tetrachlorocuprate-(II)[10] resulting in the THP-protected alcohols **5a** and **5b**, respectively (see Scheme 1). The insertion of the unsaturated residue is necessary for the final coupling (metathesis reaction) to the tetraether moieties whereas the saturated butylmagnesium bromide yielded the branched hexadecyl chain.

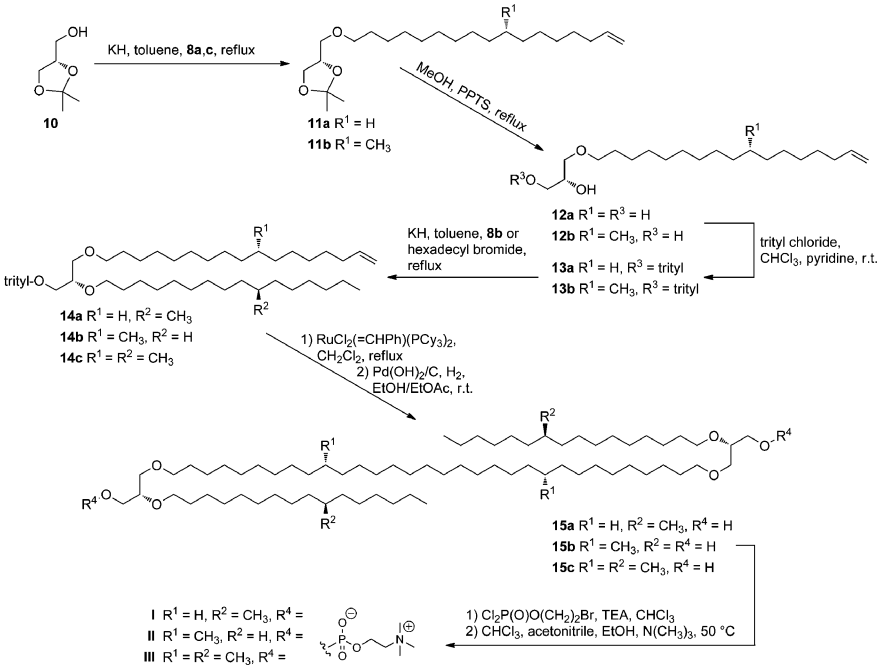
In addition to acting as a protecting group, the THP residue can be readily substituted in a high-yielding step to form bromides, necessary for further Grignard coupling reactions. According to the procedure described by Schwarz et al.,[11] compounds **5** were nearly quantitatively transformed into (8*R*)-11-bromo-8-methylundec-1-ene (**6a**) and (4*R*)-1-bromo-4-methyldecane (**6b**). In a second Grignard cross-coupling reaction of **6a**,**b** with 6-[(tetrahydro-2*H*-pyran-2-yl)oxy]hexyl bromide (**9**), the THP ethers **7a** and **7b**, with the alkyl chain lengths required for the precursors, were obtained. A second bromination[11] yielded (8*S*)-17-bromo-8-methylheptadec-1-ene (**8a**) and (10*R*)-1-bromo-10-methylhexadecane (**8b**), respectively, in an optically pure form and were used for the subsequent alkylation of the appropriately blocked glycerol derivative. The above-mentioned strategy has the advantage that, in addition to the methyl substitution pattern described herein, nearly all the desired positions of the methyl moiety are feasible by variation of the Grignard reagent.

17-Bromoheptadec-1-ene (**8c**), necessary for the metathesis reaction leading to the unbranched transmembrane alkyl chain of bolalipid **I**, was synthesised by the reaction of 11-bromoundec-1-ene with 2-[(6-bromohexyl)oxy]tetrahydro-2*H*-pyran (**9**) followed by transformation into the bromide as described above (Scheme 2).



Scheme 2. Synthesis of 17-bromoheptydec-1-ene (**8c**).

Our synthetic strategy was further pursued by connecting the hydrophobic, functionalized alkyl chains to appropriately blocked glycerol derivatives. According to the *sn*-2,3-stereochemistry of natural archaebacterial lipids of the glycerol backbone, the synthesis started with the commercially available (*S*)-1,2-*O*-isopropylideneglycerol (**10**; Scheme 3). In a first alkylation step, the terminal unsaturated bromides **8a** and **8c** were inserted after deprotonation of the alcohol component **10** by using potassium hydride leading to 1,2-*O*-isopropylidene-3-*O*-(heptadec-16-en-1-yl)-*sn*-glycerol (**11a**) and 1,2-*O*-isopropylidene-3-*O*-[(10*S*)-10-methylheptadec-16-en-1-yl]-*sn*-glycerol (**11b**), respectively. Performing the deprotonation with potassium hydride instead of sodium hydride required less time and gave higher yields. Subsequently, the isopropylidene blocking moiety of compounds **11a**,**b** was cleaved by using PPTS in methanol. Thereafter, the primary hydroxy function of the resulting glycerol derivatives **12a**,**b** was selectively blocked with trityl chloride in high yields (83–97%; see Scheme 3). Although other techniques are available to build up the glycerol backbone starting from optically pure glycide ether,[12] the



Scheme 3. Synthesis of the optically pure tetraether model lipids **I**–**III** (PPTS: pyridinium *p*-toluenesulfonate; TEA: triethylamine).

method described herein using (*S*)-1,2-*O*-isopropylideneglycerol was most appropriate for us.

The second alkylations of 3-*O*-(heptadec-16-en-1-yl)-1-*O*-trityl-*sn*-glycerol (**13a**) and 3-*O*-[(10*S*)-10-methylheptadec-16-en-1-yl]-1-*O*-trityl-*sn*-glycerol (**13b**), respectively, were carried out under nearly the same conditions as described above, although we had to heat the suspension of the glycerol derivatives **13a**,**b** with potassium hydride to 100 °C to attain a quantitative formation of the potassium salts. However, the yields of the 2,3-*O*,*O*-dialkyl-1-*O*-trityl-*sn*-glycerols **14a**–**c** were in the range of 55–68%, which has been attributed to steric hindrance.

In the final C–C bond formation reaction, the olefin metathesis with Grubbs first-generation catalyst[13] followed by hydrogenation using palladium hydroxide on carbon, enabling the detritylation as well as the hydrogenation of the double bonds in one step, provided the 3,3′-*O*-(alkane-1,1′-diyl)-bis(2-*O*-alkyl-*sn*-glycerols) **15a**–**c**. In the last step, the methyl-branched bis(phosphocholines) **I**–**III** were synthesised by the method of Eibl et al.,[14] that is, by esterification of the primary alcohol moiety of the glycerol using the classic 2-bromoethylphosphoric acid dichloride.[15] Attempts to use 2-chloro-1,3,2-dioxophospholane as the reactive phosphorylation reagent for the insertion of the phosphor ester moiety led to lower yields, in line with previous results.[16] The following quarternisation with trimethylamine in a mixture of chloroform, acetonitrile and ethanol provided the diglycerol tetraether lipids **I**–**III** in yields of 33–44% with respect to the glycerols **15a**–**c**.

Physicochemical Characterisation

The lyotropic phase behaviour of the novel, archaebacterial diglycerol tetraether phospholipid analogues **I**–**III** were characterized by differential scanning calorimetry (DSC). The final products were therefore suspended in aqueous solution (c = 1 mg mL^{-1}) and investigated in the temperature range between 2 and 95 °C. The DSC thermograms (Figure 2) of the lipids **I**–**III** show different peaks depending on the positions and the number of methyl

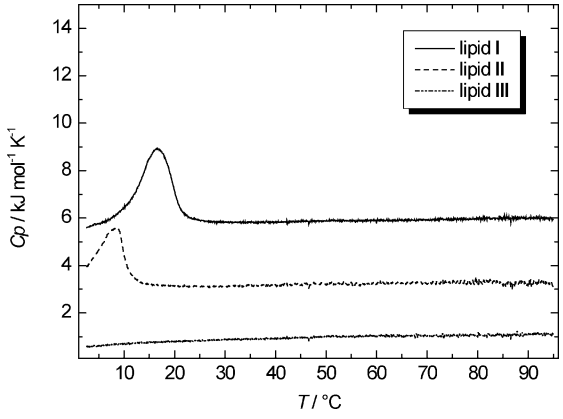


Figure 2. DSC heating curves of aqueous suspensions of lipids **I**–**III** (c = 1 mg mL^{-1}, heating rate = 20 K h^{-1}). The curves are shifted horizontally for clarity.

branches. Compared with the lipid with no methyl groups, the incorporation of two methyl groups into the shorter alkyl chains (lipid **I**) lowered the transition temperature from 61 °C for the unbranched tetraether lipid[4] to 17 °C for lipid **I**. When the methyl branches were located in the membrane-spanning chain (lipid **II**) the transition temperature decreased even further to 9 °C, which is nearly the same value as found for model systems with two phytanyl residues.[5,6a] For lipid **III** with four methyl branches in all the 10- and 23-positions of the alkyl chains, no phase transition above 2 °C could be detected. This behaviour is similar to that found for natural archaebacterial lipids.[17] In conclusion, not only the number but also the positions of the methyl branches within the alkyl chains are important for the aggregation and transition behaviour of the tetraether lipids. It seems that the van der Waals contacts of the longest unbranched segments of the alkyl chains determine the values of the transition temperature and transition enthalpy. Similar results have been found before for monopolar methyl-branched phospholipids.[18]

The ability of these new lipids to form closed lipid vesicles was studied by freeze-fracture replica electron microscopy (EM). The samples were prepared by the technique described by Bangham et al.[19] After treatment with ultrasound the liposomes were extruded through 200 nm poly-

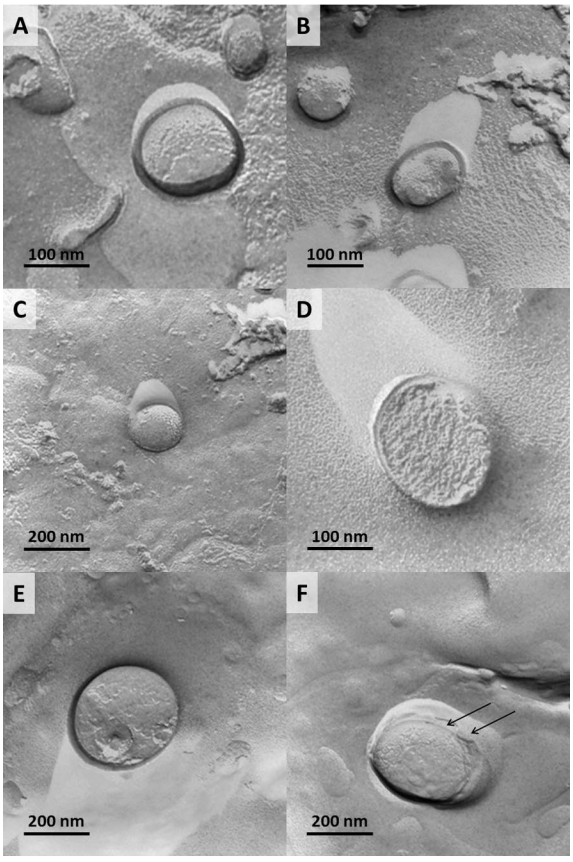


Figure 3. Electron microscopic images of freeze-fracture replicas of liposomes composed of lipids **I**–**III** (c = 15–20 mg mL^{-1}) prepared in water by using the film method of Bangham et al.[19] (A,B) Lipid **I**, (C,D) lipid **II** and (E,F) lipid **III**.

carbonate membranes. The resulting suspension was rapidly quenched from room temperature and freeze-fractured at –150 °C without etching. The surfaces were shadowed with platinum and subsequently with carbon to stabilize the ultra-thin metal films. Figure 3 shows the EM images, which indicate that all three tetraether lipids are capable of forming mostly unilamellar liposomes with diameters ranging from 80 to 200 nm. Major differences in liposome formation behaviour between the lipids **I**–**III** could not be detected. In some cases, however, we also found bi- and multilamellar liposomes (see arrows in part F of Figure 3), but this is normal as the extrusion procedure does not produce solely unilamellar vesicles, as known from other phospholipid systems. The fracture surface showed no distinct difference between the inner and outer side of the liposomes. Therefore one can conclude that cross-fracturing of the membranes occurred as no inner fracture faces could be observed as they appear when bilayer membranes are fractured. In the case of oligolamellar vesicles the fracture seems to run along the outer surface of the inner membrane before cross-fracturing occurs. Based on this observation we conclude that all three lipids are arranged in a membrane-spanning fashion and not in a U-shaped form with a bilayer arrangement, which would lead to a higher energy due to bending of the alkyl chain. The thickness of this lipid layer was determined to be 5–8 nm, which roughly corresponds to the length of one lipid molecule. Further investigations, including by X-ray diffraction, exploring the exact arrangement of the lipids are currently under way.

Conclusions

We have developed a new synthetic pathway for the preparation of diglycerol tetraether model lipids that enables the insertion of methyl branches into different positions and configurations. The Cu-catalysed Grignard coupling reaction has proved to be a suitable method for alkyl chain elongation. The coupling of glycerol diethers to form symmetric diglycerol tetraethers was successfully realised by using the Grubbs metathesis reaction. For selective glycerol alkylation and also for the insertion of the phosphocholine head-group, established and robust approaches were the methods of choice. Through the syntheses described herein we have shown that it is possible to reduce the number of methyl branches in archaebacterial model lipids without losing the main properties of the natural lipids. This fact is of particular interest with regard to a simpler and less expensive synthesis of such lipids and also in view of the preparation of stable liposomes. With the insertion of methyl branches into positions other than those described above, a fine-tuning of the physicochemical parameters seems to be feasible. This aspect and the preparation of model compounds with different head groups are under investigation.

Experimental Section

General: Apart from palladium hydroxide on carbon powder (20%; Acros Organics), all chemicals were purchased from Sigma Aldrich Co. and used without further purification. 2-Bromoethylphosphoric acid dichloride was prepared according to the literature.[14] All solvents were dried and distilled before use. The purity of all compounds was checked by thin-layer chromatography (TLC) using silica gel 60 F_{254} plates (Merck). The chromatograms were developed by using Bromothymol Blue. Silica gel (Merck, 0.063–0.200 mm) was used for the column chromatography of all products. Melting points were measured with a Boetius apparatus. Optical rotations were determined with a Polartronic E (Schmidt und Haensch) instrument and $[a]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Elemental analyses were carried out with a Leco CHNS-932 instrument. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini 2000 spectrometer at 400 and 100 MHz, respectively, using $CDCl_3$ or CD_3OD as internal standard. Mass spectrometric data were obtained with a Finnigan MAT SSQ 710 C (ESI-MS) or AMD 402 (70 eV) spectrometer (EI-MS). High-resolution mass spectra (HRMS) were recorded with a Thermo Fisher Scientific LTQ-Orbitrap mass spectrometer with static nano-electrospray ionisation. Analytical HPLC (Jasco) was performed with a Kromasil column (Si 100–5 μm, 250 × 4.6 mm), PU 980 Intelligent HPLC Pump and an LG-1580-02 Ternary Gradient Unit (Jasco) with an SEDEX 55 ELS detector (SEDERE, France) using the following solvents for elution: 5 min isocratic $CHCl_3$/MeOH/water (45:45:10), 5 min continuous increase to $CHCl_3$/MeOH/water (42:42:16), 10 min isocratic $CHCl_3$/MeOH/water (45:45:10); flow = 1 mL min^{-1}.

(4*S*)-6-Bromo-4-methylhexan-1-ol (2): A solution of (*S*)-citronellyl bromide (**1**; 10.96 g, 50 mmol) in methanol (30 mL) was cooled to –78 °C. At this temperature, a stream of ozone was passed through the solution for 10–11 h. After compound **1** had disappeared (TLC), the ozone was replaced by a stream of air for 30 min and the temperature was maintained at –78 °C. Afterwards, methanol (6 mL) and sodium borohydride (2.09 g, 0.056 mol) were added in portions and the solution was brought to –65 °C. A further portion of sodium borohydride (1.0 g, 27 mmol) was added within 15 min and the mixture was raised very slowly to room temperature. The solvent was evaporated and the residue was dissolved in methanol (40 mL). Sodium borohydride (0.75 g, 20 mmol) was added and the mixture was stirred overnight. Thereafter, water (50 mL) was added to the mixture, which was then acidified with hydrochloric acid, saturated with ammonium chloride and extracted with diethyl ether (2 × 50 mL). The ethereal solution was washed with water (50 mL), potassium hydroxide solution (50 mL, 5%), water (50 mL) and dried with sodium sulfate. After evaporation of the solvent the residue was purified by column chromatography; yield 8.52 g (87.3%). $[a]_D^{32}$ = +7.40 (c = 1.16 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, CH_3), 1.16–1.26 [m, 1 H, CHH-CH(CH$_2$)$_2$Br], 1.33–1.42 (m, 2 H, HOCH$_2$CH_2), 1.47–1.72 (m, 3 H, CHH-CH-CHH-CH$_2$Br), 1.82–1.91 (m, 1 H, CHH-CH$_2$Br), 3.35–3.48 (m, 2 H, CH_2Br), 3.62 (t, $^3J_{H,H}$ = 6.6 Hz, 2 H, HOCH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.04 (CH_3), 30.00, 31.58, 32.00, 32.55, 40.02 (CH_2CH$_2$Br), 63.29 (HOCH$_2$) ppm. MS (EI): m/z (%) = 176/178 (0.5) [M – H_2O]$^+$. $C_7H_{15}BrO$ (195.10): calcd. C 43.09, H 7.75; found C 43.15, H 7.72.

Methyl (4*S*)-6-Bromo-4-methylhexanoate (3): (*S*)-Citronellyl bromide (**1**; 21.9 g, 0.1 mol) was dissolved in dry methanol (100 mL) and the mixture was cooled to –78 °C. Afterwards, a stream of ozone gas was passed through the solution and the exothermic reaction led to a slight increase of temperature. After the reaction temperature had reached –78 °C again, the stream of ozone was stopped and the mixture was stirred for a further 1 h at this temperature. After the addition of formic acid (75 mL) at –60 °C and hydrogen peroxide (36 mL, 35%) at –40 °C, the mixture was brought to room temperature. After heating the solution for 2 h at

reflux, dry sodium acetate (10 g) was added at room temperature and the mixture was extracted with diethyl ether (2 × 100 mL). The combined ethereal extracts were dried with sodium sulfate, filtered, evaporated to dryness and the residue was dissolved in dry methanol (80 mL). After addition of concentrated sulfuric acid (2 mL) the solution was heated at reflux for 3 h. After removing the majority of the methanol under vacuum, water (50 mL) and diethyl ether (75 mL) were added and the organic layer was separated. The aqueous layer was extracted with diethyl ether (2 × 30 mL) and the collected ethereal phases were dried and the solvents evaporated. The residue was distilled or purified by column chromatography; yield 17.18 g (77% by distillation), 13.83 g (62% by chromatography), colourless liquid, b.p. 72 °C/0.3 kPa. $[\alpha]_D^{24}$ = +4.66 (*c* = 1.25 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.88 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, C*H*$_3$), 1.37–1.44 (m, 1 H, COCH$_2$C*H*$_2$), 1.56–1.68 (m, 3 H, CH*H*-C*H*-CH*H*-CH$_2$Br), 1.77–1.86 (m, 1 H, CH*H*-CH$_2$Br), 2.20–2.34 (m, 2 H, COC*H*$_2$), 3.31–3.43 (m, 2 H, C*H*$_2$Br), 3.61 (s, 3 H, *H*$_3$CO) ppm. MS (EI): *m*/*z* (%) = 223/225 (0.3) [M]$^+$. $C_8H_{15}BrO_2$ (223.10): calcd. C 43.07, H 6.78; found C 42.94, H 7.02. The data are in agreement with published values.[20]

(4*S*)-6-Bromo-4-methylhexan-1-ol (2) (via ester 3): Compound **3** (36.7 g, 0.165 mol), dissolved in dry diethyl ether (50 mL), was added very slowly whilst stirring to a suspension of lithium aluminium hydride (3.75 g, 0.1 mol) in dry diethyl ether (130 mL) at 0 °C. The mixture was stirred for a further 4 h at this temperature. The excess lithium aluminium hydride was destroyed with ice water and the white precipitate was dissolved with sulfuric acid (10%). Afterwards, the mixture was extracted with diethyl ether (3 × 60 mL). The combined ethereal phases were washed with water (100 mL) and brine (100 mL), and dried with sodium sulfate. After evaporation the crude alcohol **2** was purified by column chromatography (yield 22.53 g, 70%). The analytical data are in accordance with the data described above.

2-{[(4*S*)-6-Bromo-4-methylhexyl]oxy}tetrahydro-2*H*-pyran (4): Compound **2** (7.8 g, 0.04 mol) was dissolved in dichloromethane (50 mL), 3,4-dihydro-2*H*-pyran (5.04 g, 60 mmol) and PPTS (0.1 g) were added and the mixture was stirred for 18 h at room temperature. Afterwards, the solution was washed with water (50 mL), dried with sodium sulfate, evaporated and the residue was purified by column chromatography by using heptane/diethyl ether as eluent; yield 10.50 g (94%), colourless liquid. $[\alpha]_D^{22}$ = +3.26 (*c* = 1.17 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, C*H*$_3$), 1.16–1.26 [m, 1 H, CH*H*-CH(CH$_2$)$_2$Br], 1.32–1.43 [m, 1 H, CH*H*-CH(CH$_2$)$_2$Br], 1.47–1.73 [m, 9 H, 2 C*H*$_2$CH$_2$O, (C*H*$_2$)$_2$CHO, C*H*CH$_3$], 1.77–1.91 (m, 2 H, C*H*$_2$CH$_2$Br), 3.33–3.50 (m, 4 H, C*H*$_2$Br, 2 CHOC*H*H), 3.67–3.73 (m, 1 H, CHOCH*H*), 3.82–3.87 (m, 1 H, CHOCH*H*), 4.54–4.56 (m, 1 H, OC*H*O) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.04 and 19.05 (*C*H$_3$, diast.), 19.87, 25.67, 27.19, 30.93, 31.65, 31.69, 32.07, 33.01, 33.04, 40.09 and 40.11 (*C*H$_2$CH$_2$Br, diast.), 62.47 [(CH$_2$)$_3$*C*H$_2$O], 67.79 [C(CH$_3$)(CH$_2$)$_2$*C*H$_2$OCH], 98.91 and 98.96 (O*C*HO, diast.) ppm. MS (EI): *m*/*z* (%) = 277/279 (3.0) [M – H]$^+$. $C_{12}H_{23}BrO_2$ (279.21): calcd. C 51.62, H 8.30; found C 51.82, H 8.14.

2-{[(4*R*)-4-Methylalk-1-yl]oxy}tetrahydro-2*H*-pyrans 5a and 5b: 5-Bromopent-1-ene or 1-bromobutane (50 mmol), respectively, dissolved in dry diethyl ether (45 mL), were slowly added to magnesium turnings (1.46 g, 60 mmol). The mixture was stirred for 2 h at reflux. The Grignard solution was decanted from excess magnesium under a stream of argon. After removing the diethyl ether in vacuo the oily residue was diluted in dry THF (50 mL) and cooled to –5 °C. Afterwards, compound **4** (7.0 g, 25 mmol), dissolved in dry THF (10 mL), was added in one portion followed by a freshly prepared dilithium tetrachlorocuprate solution (10 mL, 0.1 M). The mixture was stirred for 2–3 h at –5 to 0 °C. Afterwards, the mixture was poured into an ice-cold saturated solution of ammonium chloride. The organic layer was separated and the aqueous phase was extracted with diethyl ether (2 × 50 mL). The combined ethereal phases were washed with water and brine, dried with sodium sulfate and the solvents evaporated. The oily residues were purified by column chromatography.

2-{[(4*R*)-4-Methylundec-10-en-1-yl]oxy}tetrahydro-2*H*-pyran (5a): Yield 5.2 g (77%), colourless liquid. $[\alpha]_D^{22}$ = +0.27 (*c* = 0.95 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, C*H*$_3$), 1.06–1.40 [m, 11 H, C*H*$_2$C*H*(CH$_2$)$_4$], 1.48–1.62 (m, 6 H, 2 C*H*$_2$CH$_2$O, C*H*$_2$CH$_2$CHO), 1.67–1.73 (m, 1 H, CH*H*CHO), 1.77–1.85 (m, 1 H, CH*H*CHO), 1.99–2.04 (m, 2 H, C*H*$_2$CH=CH$_2$), 3.32–3.38 (m, 1 H, CHOCH*H*), 3.45–3.51 (m, 1 H, CHOCH*H*), 3.66–3.73 (m, 1 H, CHOCH*H*), 3.83–3.88 (m, 1 H, CHOCH*H*), 4.55–4.56 (m, 1 H, OC*H*O), 4.89–4.99 (m, 2 H, CH=CH$_2$), 5.74–5.84 (m, 1 H, C*H*=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.78 and 19.79 (*C*H$_3$, diast.), 19.87, 25.69, 27.01, 27.43, 27.45, 29.12, 29.59, 30.96, 32.76, 33.09, 33.51, 33.92, 37.02, 37.04, 62.41 [(CH$_2$)$_3$*C*H$_2$O], 68.09 and 68.12 [C(CH$_3$)(CH$_2$)$_2$*C*H$_2$OCH, diast.], 98.85 and 98.89 (O*C*HO, diast.), 114.07 (CH=*C*H$_2$), 139.14 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 267 (0.9) [M – H]$^+$. $C_{17}H_{32}O_2$ (268.43): calcd. C 76.06, H 12.02; found C 75.79, H 11.74.

2-{[(4*R*)-4-Methyldecyl]oxy}tetrahydro-2*H*-pyran (5b): Yield 5.5 g (86%), colourless liquid. $[\alpha]_D^{22}$ = +0.09 (*c* = 0.88 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, CHC*H*$_3$), 0.87 (t, $^3J_{H,H}$ = 6.8 Hz, 3 H, CH$_2$C*H*$_3$), 1.08–1.38 [m, 13 H, C*H*$_2$C*H*(CH$_2$)$_5$], 1.48–1.61 (m, 6 H, 2 C*H*$_2$CH$_2$O, C*H*$_2$CH$_2$CHO), 1.67–1.72 (m, 1 H, CH*H*-CHO), 1.80–1.83 (m, 1 H, CH*H*-CHO), 3.34–3.37 (m, 1 H, CHOCH*H*), 3.46–3.49 (m, 1 H, CHOCH*H*), 3.67–3.71 (m, 1 H, CHOCH*H*), 3.83–3.86 (m, 1 H, CHOCH*H*), 4.55–4.57 (m, 1 H, OC*H*O) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.23 (CH$_2$*C*H$_3$), 19.79 and 19.80 (*C*H$_3$, diast.), 19.87, 22.81 (*C*H$_2$CH$_3$), 25.70, 27.15, 27.44, 27.46, 29.78, 30.96, 32.06, 32.78, 33.53, 37.10, 37.12, 62.41 [(CH$_2$)$_3$*C*H$_2$O], 68.10 and 68.13 [C(CH$_3$)(CH$_2$)$_2$*C*H$_2$OCH, diast.], 98.84 and 98.89 (O*C*HO, diast.) ppm. MS (EI): *m*/*z* (%) = 255 (1.4) [M – H]$^+$. $C_{16}H_{32}O_2$ (256.42): calcd. C 74.94, H 12.58; found C 74.67, H 12.80.

Methyl–Branched Bromoalkanes 6a and 6b: Triphenylphosphane (7.87 g, 30 mmol) was dissolved in dry dichloromethane (70 mL) and bromine (2.4 g, 30 mmol), diluted in dichloromethane (10 mL), was added dropwise into the solution whilst stirring at 0 °C. Compound **5a** or **5b** (19 mmol) was added and the mixture was stirred overnight at room temperature. Afterwards, the organic layer was washed with water (100 mL) and the crude bromides **6a** and **6b** were purified by column chromatography with heptane as eluent.

(8*R*)-11-Bromo-8-methylundec-1-ene (6a): Yield 4.6 g (98%), colourless liquid. $[\alpha]_D^{22}$ = –2.06 (*c* = 0.98 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (d, $^3J_{H,H}$ = 6.6 Hz, 3 H, C*H*$_3$), 1.07–1.46 [m, 11 H, C*H*$_2$C*H*(CH$_2$)$_4$], 1.77–1.91 (m, 2 H, BrCH$_2$C*H*$_2$), 2.00–2.05 (m, 2 H, C*H*$_2$CH=CH$_2$), 3.35–3.39 (m, 2 H, BrC*H*$_2$), 4.90–5.01 (m, 2 H, CH=C*H*$_2$), 5.74–5.85 (m, 1 H, C*H*=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.34, 28.91, 29.11, 29.29, 29.57–29.78, 33.01, 33.93, 34.05, 114.04 (CH=*C*H$_2$), 139.19 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 246/248 (0.2) [M]$^+$. $C_{12}H_{23}Br$ (247.22): calcd. C 58.30, H 9.38; found C 57.96, H 9.21.

(4*R*)-1-Bromo-4-methyldecane (6b): Yield 4.16 g (93%), colourless liquid. $[\alpha]_D^{22}$ = –2.29 (*c* = 1.02 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.84–0.88 (m, 6 H, 2 C*H*$_3$), 1.06–1.44 [m, 13 H, C*H*$_2$C*H*(CH$_2$)$_5$], 1.77–1.89 (m, 2 H, BrCH$_2$C*H*$_2$), 3.35–3.39 (m, 2

H, BrCH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.23 (CH_2CH_3), 19.74 ($CHCH_3$), 22.82 (CH_2CH_3), 27.09, 29.74, 30.71, 32.04, 32.38, 34.39, 35.66, 37.01 ppm. MS (EI): *m*/*z* (%) = 234/236 (0.2) $[M]^+$. $C_{11}H_{23}Br$ (235.20): calcd. C 56.17, H 9.86; found C 56.38, H 9.96. The analytical data are comparable to the published values of the 4*S* enantiomer.[21]

2-[(10-Methylalk-1-yl)oxy]tetrahydro-2*H*-pyrans 7a and 7b: The Grignard reagent was prepared from magnesium (0.88 g, 36 mmol) and 2-[(6-bromohexyl)oxy]tetrahydro-2*H*-pyran (**9**; 8.0 g, 30 mmol) in dry THF (30 mL) according to the procedure described for compounds **5a**,**b**. The resulting Grignard solution was coupled with compound **6a** or **6b** (15 mmol) under catalytic conditions with dilithium tetrachlorocuprate (3 mL, 0.1 M) at 0 °C. After work-up as described for compounds **5a**,**b** the crude product was purified by column chromatography.

2-{[(10*S*)-10-Methylheptadec-16-en-1-yl]oxy}tetrahydro-2*H*-pyran (7a): Yield 4.39 g (83%), colourless liquid. $[\alpha]_D^{22}$ = +0.08 (*c* = 0.88 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, CH_3), 1.01–1.38 [m, 23 H, (CH_2)$_7$CH(CH_2)$_4$], 1.46–1.60 (m, 6 H, 2 CH_2CH$_2$O, CH_2CH$_2$CHO), 1.65–1.73 (m, 1 H, CHH-CHO), 1.78–1.85 (m, 1 H, CHH-CHO), 1.99–2.04 (m, 2 H, CH_2CH=CH$_2$), 3.33–3.39 (m, 1 H, CHOCHH), 3.45–3.50 (m, 1 H, CHOCHH), 3.67–3.73 (m, 1 H, CHOCHH), 3.82–3.87 (m, 1 H, CHOCHH), 4.54–4.56 (m, 1 H, OCHO), 4.89–5.00 (m, 2 H, CH=CH_2), 5.74–5.84 (m, 1 H, CH=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.86 (*C*H$_3$), 25.70, 26.40, 27.05, 27.21, 29.14, 29.63–30.13, 30.96, 32.89, 33.93, 37.16, 37.22, 62.39 [(CH$_2$)$_3$*C*H$_2$O], 67.77 [C(CH$_3$)(CH$_2$)$_8$*C*H$_2$OCH], 98.87 (O*C*HO), 114.03 (CH=*C*H$_2$), 139.18 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 351 (0.6) [M – H]$^+$. $C_{23}H_{44}O_2$ (352.59): calcd. C 78.35, H 12.58; found C 78.25, H 12.95.

2-{[(10*R*)-10-Methylhexadecyl]oxy}tetrahydro-2*H*-pyran (7b): Yield 4.04 g (79%), colourless liquid. $[\alpha]_D^{22}$ = –0.12 (*c* = 0.84 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H, CHCH_3), 0.85–0.88 (t, $^3J_{H,H}$ = 7.1 Hz, 3 H, CH$_2$CH_3), 1.02–1.35 [m, 25 H, (CH_2)$_7$CH(CH_2)$_5$], 1.48–1.73 (m, 7 H, 2 CH_2CH$_2$O, CH_2-CHH-CHO), 1.77–1.85 (m, 1 H, CHH-CHO), 3.33–3.39 (m, 1 H, CHOCHH), 3.45–3.51 (m, 1 H, CHOCHH), 3.68–3.74 (m, 1 H, CHOCHH), 3.82–3.88 (m, 1 H, CHOCHH), 4.55–4.56 (m, 1 H, OCHO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.24 (CH$_2$*C*H$_3$), 19.86, 19.87 (CH*C*H$_3$), 22.84 (*C*H$_2$CH$_3$), 25.70, 26.40, 27.19, 27.22, 29.64–30.96, 32.09, 32.91, 37.24, 62.39 [(CH$_2$)$_3$*C*H$_2$O], 67.77 [C(CH$_3$)(CH$_2$)$_8$*C*H$_2$OCH], 98.86 (O*C*HO) ppm. MS (EI): *m*/*z* (%) = 339 (4.5) [M – H]$^+$. $C_{22}H_{44}O_2$ (340.58): calcd. C 77.58, H 13.02; found C 77.45, H 13.02.

2-(Heptadec-16-en-1-yloxy)tetrahydro-2*H*-pyran (7c): The Grignard reagent was prepared from magnesium (0.88 g, 36 mmol) and 11-bromoundec-1-ene (7.0 g, 30 mmol) in dry THF (30 mL) according the procedure described for compounds **5a**,**b**. A solution of 2-[(6-bromohexyl)oxy]tetrahydro-2*H*-pyran (**9**; 5.3 g, 20 mmol) in dry THF (10 mL) and a freshly prepared dilithium tetrachlorocuprate solution (5 mL, 0.1 M) were added and the mixture was stirred for 3 h at temperatures between –5 and 0 °C. After work-up as described for compounds **5a**,**b**, the crude product was purified by column chromatography; yield 3.71 g (73%), colourless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.24–1.38 [m, 24 H, (CH_2)$_{12}$-CH$_2$CH=], 1.48–1.60 (m, 6 H, 2 CH_2CH$_2$O, CH_2CH$_2$CHO), 1.67–1.72 (m, 1 H, CHH-CHO), 1.79–1.84 (m, 1 H, CHH-CHO), 2.00–2.04 (m, 2 H, CH_2CH=CH$_2$), 3.34–3.38 (m, 1 H, CHOCHH), 3.46–3.50 (m, 1 H, CHOCHH), 3.68–3.73 (m, 1 H, CHOCHH), 3.83–3.87 (m, 1 H, CHOCHH), 4.55–4.56 (m, 1 H, OCHO), 4.89–4.99 (m, 2 H, CH=CH_2); 5.75–5.83 (m, 1 H, CH=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.86, 25.70, 26.40, 29.11, 29.29, 29.64–29.92, 30.96, 33.93, 62.39 [(CH$_2$)$_3$*C*H$_2$O], 67.77 [(CH$_2$)$_{14}$*C*H$_2$OCH], 98.86 (O*C*HO), 114.03 (CH=*C*H$_2$), 139.19 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 337 (4.3) [M – H]$^+$. $C_{22}H_{44}O_2$ (338.57): calcd. C 78.05, H 12.50; found C 77.98, H 12.67.

Methyl-Branched Bromoalkanes 8a–c: The bromides **8a**–**c** were prepared from triphenylphosphoranediyl dibromide (14.55 g, 25.40 mmol) and compounds **7a**–**c** (12.0 mmol) according to the method described above for compounds **6a**,**b**.

(8*S*)-17-Bromo-8-methylheptadec-1-ene (8a): Yield 3.61 g (91%), colourless liquid. $[\alpha]_D^{22}$ = +0.03 (*c* = 0.98 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, $^3J_{H,H}$ = 6.7 Hz, 3 H, CH_3), 1.05–1.42 [m, 23 H, (CH_2)$_7$CH(CH_2)$_4$], 1.84 (quint., $^3J_{H,H}$ = 7.0 Hz, 2 H, BrCH$_2$CH_2), 2.00–2.04 (m, 2 H, CH_2CH=CH$_2$), 3.39 (t, $^3J_{H,H}$ = 7.0 Hz, 2 H, BrCH_2), 4.90–4.99 (m, 2 H, CH=CH_2), 5.76–5.84 (m, 1 H, CH=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.86 (*C*H$_3$), 27.05, 27.19, 28.34, 28.92, 29.14–30.09, 32.88, 33.01, 33.94, 34.06, 37.15, 37.20, 114.05 (CH=*C*H$_2$), 139.19 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 330/332 (1.8) $[M]^+$. $C_{18}H_{35}Br$ (331.37): calcd. C 65.24, H 10.65; found C 65.41, H 10.80.

(10*R*)-1-Bromo-10-methylhexadecane (8b): Yield 3.64 g (95%), colourless liquid. $[\alpha]_D^{22}$ = –0.19 (*c* = 0.94 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, $^3J_{H,H}$ = 6.6 Hz, 3 H, CHCH_3), 0.87 (t, $^3J_{H,H}$ = 7.0 Hz, 3 H, CH$_2$CH_3), 1.05–1.42 [m, 25 H, (CH_2)$_7$-CH(CH_2)$_5$], 1.84 (quint., $^3J_{H,H}$ = 7.0 Hz, 2 H, BrCH$_2$CH_2), 3.37–3.40 (m, 2 H, BrCH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.25 (CH$_2$*C*H$_3$), 19.87 (CH*C*H$_3$), 22.84 (*C*H$_2$CH$_3$), 27.19, 28.34, 28.92, 29.58–30.09, 32.09, 33.01, 34.06, 37.22, 37.24 ppm. MS (EI): *m*/*z* (%) = 318/320 (0.6) $[M]^+$. $C_{17}H_{35}Br$ (319.36): calcd. C 63.93, H 11.05; found C 63.95, H 11.36.

17-Bromoheptadec-1-ene (8c): Yield 3.77 g (99%), colourless liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.24–1.43 [m, 24 H, Br(CH$_2$)$_2$-(CH_2)$_{12}$CH$_2$], 1.84 (quint., $^3J_{H,H}$ = 7.0 Hz, 2 H, BrCH$_2$CH_2), 2.00–2.04 (m, 2 H, CH_2CH=CH$_2$), 3.39 (t, $^3J_{H,H}$ = 7.0 Hz, 2 H, BrCH_2), 4.90–5.00 (m, 2 H, CH=CH_2), 5.76–5.84 (m, 1 H, CH=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.73, 26.95, 29.09, 29.54, 30.69, 32.35, 33.91, 34.38, 35.63, 36.93, 114.12 (CH=*C*H$_2$), 139.10 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 316/318 (10) $[M]^+$. $C_{17}H_{33}Br$ (317.35): calcd. C 64.34, H 10.48; found C 64.55, H 10.67. The data are in agreement with published values.[22]

3-*O*-Alkyl-1,2-*O*-isopropylidene-*sn*-glycerols 11a and 11b: Potassium hydride (14.7 mmol, 1.7 mL, 30% suspension in paraffin) was separated from the oil and suspended under argon in dry toluene (5 mL). A solution of 1,2-*O*-isopropylidene-*sn*-glycerol (**10**; 1.94 g, 14.7 mmol) in dry toluene (15 mL) was added slowly at room temperature and the mixture was stirred for 18 h at this temperature. The alkyl bromides **8a**,**c** (9.8 mmol) were dissolved in dry toluene (10 mL), added and the mixture was stirred for 10 h at reflux. Thereafter, water (30 mL) was added at room temperature and the mixture was stirred vigorously. After phase separation, the organic layer was washed with water (50 mL), saturated ammonium chloride solution (50 mL), dried with sodium sulfate, evaporated to dryness and purified by chromatography using heptane/diethyl ether as eluent and the gradient technique.

1,2-*O*-Isopropylidene-3-*O*-(heptadec-16-en-1-yl)-*sn*-glycerol (11a): Yield 2.6 g (72%), colourless oil. $[\alpha]_D^{22}$ = +12.82 (*c* = 0.86 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.23–1.32 [m, 24 H, (CH_2)$_{12}$], 1.34 (s, 3 H, CH_3), 1.40 (s, 3 H, CH_3), 1.55 (quint., $^3J_{H,H}$ = 7.0 Hz, 2 H, CH_2CH$_2$O), 1.99–2.04 (m, 2 H, CH_2CH=CH$_2$), 3.37–3.51 (m, 4 H, 2 CH_2O), 3.68–3.72 [m, 1 H, CHH-OC-(CH$_3$)$_2$O], 4.01–4.05 [m, 1 H, CHH-OC(CH$_3$)$_2$O], 4.23 (quint.,

$^3J_{H,H}$ = 6.0 Hz, 1 H, C*H*O), 4.88–4.99 (m, 2 H, CH=CH_2), 5.74–5.84 (m, 1 H, C*H*=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.89, 26.22, 26.94, 29.10, 29.30, 29.60–29.80, 33.94, 67.07 [*C*H$_2$OC(CH$_3$)$_2$O], 71.91 (*C*H$_2$O), 71.96 (*C*H$_2$O), 74.85 (O*C*H), 109.32 [O*C*(CH$_3$)$_2$O], 114.05 (CH=*C*H$_2$), 139.18 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 368 (8.6) [M]$^+$. $C_{23}H_{44}O_3$ (368.59): calcd. C 74.95, H 12.03; found C 75.07, H 12.24.

1,2-*O*-Isopropylidene-3-*O*-[(10*S*)-10-methylheptadec-16-en-1-yl]-*sn*-glycerol (11b): Yield 2.66 g (71%), colourless oil. $[a]_D^{22}$ = +11.4 (*c* = 0.84 g mL^{-1}, pure). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, CHCH_3), 1.05–1.38 [m, 23 H, (CH_2)$_7$C*H*-(CH_2)$_4$], 1.34 (s, 3 H, CCH_3), 1.40 (s, 3 H, CCH_3), 1.52–1.59 (m, 2 H, CH_2CH$_2$O), 1.99–2.05 (m, 2 H, CH_2CH=CH$_2$), 3.38–3.52 (m, 4 H, 2 CH_2O), 3.69–3.73 [m, 1 H, C*H*H-OC(CH$_3$)$_2$O], 4.02–4.05 [m, 1 H, CH*H*-OC(CH$_3$)$_2$O], 4.24 (quint., $^3J_{H,H}$ = 6.2 Hz, 1 H, C*H*O), 4.89–4.99 (m, 2 H, CH=CH_2), 5.74–5.84 (m, 1 H, C*H*=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.77 (CH*C*H$_3$), 25.50, 26.13, 26.84, 26.97, 27.13, 29.05, 29.53–30.06, 31.93, 32.79, 33.87, 37.06, 37.12, 66.67 [*C*H$_2$OC(CH$_3$)$_2$O], 71.82 (*C*H$_2$O), 71.86 (*C*H$_2$O), 74.75 (O*C*H), 109.22 [O*C*(CH$_3$)$_2$O], 113.96 (CH=*C*H$_2$), 139.05 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 382 (5) [M]$^+$. $C_{24}H_{46}O_3$ (382.62): calcd. C 75.34, H 12.12; found C 75.25, H 12.20.

3-*O*-Alkyl-*sn*-glycerols 12a and 12b: Compound **11a** or **11b** (6.0 mmol) and PPTS (0.1 g) were poured into dry methanol (35 mL) and heated for 10 h at reflux. Afterwards, the solvent was evaporated and the residue was dissolved in chloroform (50 mL). After washing with water (50 mL), drying over sodium sulfate, the solvent was evaporated and the crude product was purified by column chromatography.

3-*O*-Heptadec-16-en-1-yl-*sn*-glycerol (12a): Yield 1.68 g (85%), white solid, m.p. 56 °C. $[a]_D^{22}$ = –0.6 (*c* = 0.1 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.19–1.37 [m, 24 H, (CH_2)$_{12}$], 1.52–1.59 (m, 2 H, CH_2CH$_2$O), 1.99–2.04 (m, 2 H, CH_2CH=CH$_2$), 2.29 (br., 2 H, 2 O*H*), 3.42–3.53 (m, 4 H, 2 CH_2O), 3.61–3.72 (m, 2 H, CH_2OH), 3.81–3.86 (m, 1 H, C*H*OH), 4.89–5.00 (m, 2 H, CH=CH_2), 5.74–5.84 (m, 1 H, C*H*=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.14, 29.01, 29.21, 29.52–29.71, 33.85, 64.27 (*C*H$_2$OH), 70.59 (*C*HOH), 71.88 (*C*H$_2$O), 72.46 (*C*H$_2$O), 114.05 (CH=*C*H$_2$), 139.20 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 328 (8.9) [M]$^+$. $C_{20}H_{40}O_3$ (328.53): calcd. C 73.12, H 12.27; found C 73.28, H 11.94.

3-*O*-[(10*S*)-10-Methylheptadec-16-en-1-yl]-*sn*-glycerol (12b): Yield 1.58 g (90%), colourless oil. $[a]_D^{22}$ = +0.2 (*c* = 0.18 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.81 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, CH_3), 1.02–1.37 [m, 23 H, (CH_2)$_7$C*H*(CH_2)$_4$], 1.51–1.58 (m, 2 H, CH_2CH$_2$O), 1.99–2.04 (m, 2 H, CH_2CH=CH$_2$), 2.30–2.51 (br., 2 H, 2 O*H*), 3.42–3.52 (m, 4 H, 2 CH_2O), 3.59–3.70 (m, 2 H, CH_2OH), 3.81–3.86 (m, 1 H, C*H*OH), 4.89–5.00 (m, 2 H, CH=CH_2), 5.74–5.84 (m, 1 H, C*H*=CH$_2$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.73 (*C*H$_3$), 26.11, 26.93, 27.09, 29.01, 29.48–29.66, 30.02, 32.76, 33.83, 37.04, 37.10, 64.29 (*C*H$_2$OH), 70.50 (*C*HOH), 71.86 (*C*H$_2$O), 72.48 (*C*H$_2$O), 114.03 (CH=*C*H$_2$), 139.18 (*C*H=CH$_2$) ppm. MS (EI): *m*/*z* (%) = 342 (5) [M]$^+$. $C_{21}H_{42}O_3$ (342.56): calcd. C 73.63, H 12.36; found C 73.41, H 12.39.

3-*O*-Alkyl-1-*O*-trityl-*sn*-glycerols 13a and 13b: Trityl chloride (1.34 g, 4.8 mmol) and the glycerol **12a** or **12b** (4.0 mmol) were dissolved in a mixture of dry chloroform and dry pyridine (30 mL, 1:1). The solution was stirred for 16 h at room temperature. Afterwards, water (20 mL) was added to hydrolyse the excess trityl chloride. After the addition of further water (20 mL), the organic phase was separated, the aqueous layer was extracted with chloroform (2× 50 mL) and the combined organic phases were washed with water (50 mL). After drying with sodium sulfate and removing the solvent, the residue was purified by column chromatography using chloroform/diethyl ether and 0.1% triethylamine as eluent.

3-*O*-(Heptadec-16-en-1-yl)-1-*O*-trityl-*sn*-glycerol (13a): Yield 2.21 g (97%), white waxy substance, m.p. 42–43 °C. $[a]_D^{22}$ = –1.9 (*c* = 0.064 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.20–1.38 [m, 24 H, (CH_2)$_{12}$], 1.48–1.56 (m, 2 H, CH_2CH$_2$O), 2.00–2.05 (m, 2 H, CH_2CH=CH$_2$), 2.40 (d, *J* = 3.6 Hz, 1 H, O*H*), 3.15–3.22 [m, 2 H, CH_2OC(C$_6$H$_5$)$_3$], 3.39–3.54 (m, 4 H, 2 CH_2O), 3.92–3.94 (m, 1 H, C*H*OH), 4.90–5.00 (m, 2 H, CH=CH_2), 5.77–5.84 (m, 1 H, C*H*=CH$_2$), 7.20–7.43 [m, 15 H, C(C$_6$*H*$_5$)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.20, 29.04, 29.24, 29.59–29.75, 33.88, 64.69, 69.89, 71.66, 72.06, 86.64 [*C*(C$_6$H$_5$)$_3$], 113.99 (CH=*C*H$_2$), 126.94, 127.15, 127.71, 127.83, 128.59, 139.15 (*C*H=CH$_2$), 143.79 ppm. MS (ESI): *m*/*z* = 593.6 [M + Na]$^+$. $C_{39}H_{54}O_3$ (570.70): calcd. C 82.06, H 9.53; found C 82.24, H 9.15.

3-*O*-[(10*S*)-10-Methylheptadec-16-en-1-yl]-1-*O*-trityl-*sn*-glycerol (13b): Yield 1.94 g (83%), colourless oil. $[a]_D^{22}$ = –2.5 (*c* = 0.26 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.83 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, CH_3), 1.04–1.39 [m, 23 H, (CH_2)$_7$C*H*-(CH_2)$_4$], 1.51–1.57 (m, 2 H, CH_2CH$_2$O), 1.99–2.04 (m, 2 H, CH_2CH=CH$_2$), 2.39 (d, *J* = 3.7 Hz, 1 H, O*H*), 3.15–3.22 [m, 2 H, CH_2OC(C$_6$H$_5$)$_3$], 3.37–3.53 (m, 4 H, 2 CH_2O), 3.92 (br. s, 1 H, C*H*OH), 4.90–5.00 (m, 2 H, CH=CH_2), 5.75–5.85 (m, 1 H, C*H*=CH$_2$), 7.16–7.43 [m, 15 H, C(C$_6$*H*$_5$)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.87 (*C*H$_3$), 26.27, 27.07, 27.23, 29.14, 29.65–29.82, 30.17, 32.89, 33.96, 37.17, 37.24, 64.77, 69.97, 71.73, 72.14, 86.71 [*C*(C$_6$H$_5$)$_3$], 114.07 (CH=*C*H$_2$), 127.01, 127.22, 127.78, 127.89, 128.67, 139.20 (*C*H=CH$_2$), 143.86 ppm. MS (ESI): *m*/*z* = 608.3 [M + Na]$^+$. $C_{40}H_{56}O_3$ (584.87): calcd. C 82.14, H 9.65; found C 81.76, H 10.02.

2,3-*O*,*O*-Dialkyl-1-*O*-trityl-*sn*-glycerols 14a–c: Compounds **14a**–**c** were synthesized according to the synthesis of compounds **11a**,**b**. Potassium hydride (1.51 mmol, 0.2 mL, 30% suspension in paraffin) was separated from the oil and suspended under argon in dry toluene (5 mL). A solution of **13a** or **13b** (1.51 mmol) in dry toluene (10 mL) was added slowly at room temperature. The mixture was stirred for 18 h at this temperature and then for 1 h at 100 °C. Afterwards, the alkyl bromide **8b** or hexadecyl bromide (4.51 mmol), dissolved in dry toluene (10 mL), was added and the mixture was stirred for 10 h at reflux. Thereafter, water (30 mL) was added at room temperature and the mixture was stirred vigorously. After phase separation, the organic layer was washed with water (30 mL) and ammonium chloride solution (30 mL). The organic solution was dried with sodium sulfate, evaporated and purified by chromatography using heptane/diethyl ether and the gradient technique.

3-*O*-(Heptadec-16-en-1-yl)-2-*O*-[(10*R*)-10-methylhexadecyl]-1-*O*-trityl-*sn*-glycerol (14a): Yield 0.83 g (68%), colourless oil. $[a]_D^{22}$ = –5.4 (*c* = 0.14 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82–0.84 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, CHCH_3), 0.88 (t, $^3J_{H,H}$ = 7.0 Hz, 3 H, CH$_2$CH_3), 1.08–1.35 [m, 49 H, (CH_2)$_{12}$, (CH_2)$_7$C*H*(CH_2)$_5$], 1.49–1.57 (m, 4 H, 2 CH_2CH_2O), 2.01–2.06 (m, 2 H, CH_2CH=CH$_2$), 3.13–3.20 [m, 2 H, CH_2OC(C$_6$H$_5$)$_3$], 3.34–3.41 (m, 2 H, CH_2O), 3.46–3.57 (m, 5 H, 2 CH_2O, C*H*O), 4.90–5.01 (m, 2 H, CH=CH_2), 5.74–5.86 (m, 1 H, C*H*=CH$_2$), 7.16–7.42 [m, 15 H, C(C$_6$*H*$_5$)$_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.18 (CH$_2$*C*H$_3$), 19.80 (CH*C*H$_3$), 22.77 (*C*H$_2$CH$_3$), 26.20, 26.26, 27.13, 27.18, 29.04, 29.23, 29.59–29.77, 30.12, 30.24, 32.04, 32.85, 33.88, 37.20, 63.74 (*C*H$_2$O), 70.75 (*C*H$_2$O), 71.28 (*C*H$_2$O), 71.67 (*C*H$_2$O),

78.39 (OCH), 86.57 [$C(C_6H_5)_3$], 114.08 (CH=CH_2), 126.87, 127.70, 128.78, 139.24 (CH=CH_2), 144.20 ppm. MS (ESI): *m*/*z* = 831.9 [M + Na]$^+$. $C_{56}H_{88}O_3$ (809.26): calcd. C 83.11, H 10.96; found C 83.32, H 11.27.

2-*O*-Hexadecyl-3-*O*-[(10*S*)-10-methylheptadec-16-en-1-yl]-1-*O*-trityl-*sn*-glycerol (14b): Yield 0.67 g (55%), colourless oil. $[\alpha]_D^{22}$ = –5.6 (*c* = 0.086 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.83 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H, CHCH_3), 0.87 (t, $^3J_{H,H}$ = 7.0 Hz, 3 H, CH_2CH_3), 1.06–1.39 [m, 49 H, $(CH_2)_7CH(CH_2)_4$, $(CH_2)_{13}CH_3$], 1.47–1.56 (m, 4 H, 2 CH_2CH_2O), 2.00–2.05 (m, 2 H, CH_2CH=CH_2), 3.14–3.16 [m, 2 H, CH_2OC$(C_6H_5)_3$], 3.36–3.40 (m, 2 H, CH_2O), 3.47–3.56 (m, 5 H, 2 CH_2O, C*H*O), 4.90–5.00 (m, 2 H, CH=CH_2), 5.75–5.83 (m, 1 H, C*H*=CH_2), 7.18–7.46 [m, 15 H, C$(C_6H_5)_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.21 (CH_2CH_3), 19.81 (CHCH_3), 22.78 (CH_2CH_3), 26.21, 26.26, 27.01, 27.19, 29.08, 29.44–29.79, 30.13, 30.24, 32.01, 32.84, 33.90, 37.11, 37.19, 63.70 (CH_2O), 70.72 (CH_2O), 71.24 (CH_2O), 71.64 (CH_2O), 78.35 (OCH), 86.52 [$C(C_6H_5)_3$], 114.00 (CH=CH_2), 126.78, 127.60, 128.68, 139.14 (CH=CH_2), 144.08 ppm. MS (ESI): *m*/*z* = 832.7 [M + Na]$^+$. $C_{56}H_{88}O_3$ (809.26): calcd. C 83.11, H 10.96; found C 83.20, H 11.30.

3-*O*-[(10*S*)-10-Methylheptadec-16-en-1-yl]-2-*O*-[(10*R*)-10-methylhexadecyl]-1-*O*-trityl-*sn*-glycerol (14c): Yield 0.72 g (59%), colourless oil. $[\alpha]_D^{22}$ = –4.6 (*c* = 0.06 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.83 (d, $^3J_{H,H}$ = 6.6 Hz, 6 H, 2 CHCH_3), 0.88 (t, $^3J_{H,H}$ = 6.8 Hz, 3 H, CH_2CH_3), 1.06–1.39 [m, 48 H, $(CH_2)_7CH(CH_2)_4$, $(CH_2)_7CH(CH_2)_5$], 1.48–1.57 (m, 4 H, 2 CH_2CH_2O), 2.01–2.04 (m, 2 H, CH_2CH=CH_2), 3.15–3.17 [m, 2 H, CH_2OC$(C_6H_5)_3$], 3.37–3.40 (m, 2 H, CH_2O), 3.47–3.55 (m, 5 H, 2 CH_2O, C*H*O), 4.90–5.00 (m, 2 H, CH=CH_2), 5.77–5.81 (m, 1 H, C*H*=CH_2), 7.18–7.46 [m, 15 H, C$(C_6H_5)_3$] ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.21 (CH_2CH_3), 19.81 (CHCH_3), 19.82 (CHCH_3), 22.79, 22.97, 26.22, 26.28, 26.44, 26.53, 27.01, 27.15, 27.20, 29.09, 29.59–29.82, 30.14, 30.25, 31.04, 31.96, 32.04, 32.84, 32.87, 33.89, 35.52, 37.12, 37.20, 63.73 (CH_2O), 70.73 (CH_2O), 71.26 (CH_2O), 71.64 (CH_2O), 78.36 (OCH), 86.53 [$C(C_6H_5)_3$], 114.00 (CH=CH_2), 126.78, 127.15, 127.60, 127.82, 128.69, 139.12 (CH=CH_2), 144.09 ppm. MS (ESI): *m*/*z* = 846.4 [M + Na]$^+$. $C_{57}H_{90}O_3$ (823.32): calcd. C 83.15, H 11.02; found C 83.03, H 11.24.

3,3′-*O*-(Alkane-1,1′-diyl)bis(2-*O*-alkyl-*sn*-1-glycerols) 15a–c: The olefins **14a**–**c** (0.75 mmol) were dissolved in dry dichloromethane (30 mL) under argon. A solution of Grubbs first-generation catalyst {$[RuCl_2(=CHPh)(PCy_3)_2]$, 0.18 g, 29 mol-%} in dry dichloromethane (18 mL) was added dropwise. The resulting mixture was heated at reflux for 24 h. The solvent was removed under reduced pressure and the crude residue was purified by silica gel column chromatography using a heptane/chloroform gradient. A mixture of the resulting light-brown oil in ethanol/ethyl acetate (50 mL, 1:1) and palladium(II) hydroxide (48 mg, 20% on carbon) was stirred under hydrogen (2 atm) at room temperature for 18 h. The catalyst was removed by filtration and washed with chloroform several times. The combined organic solutions were evaporated. The residue was passed through a silica gel column using the gradient technique and chloroform/diethyl ether as eluent to give the methyl-branched diols **15a**–**c**.

3,3′-*O*-(Dotriacontane-1,32-diyl)bis{2-*O*-[(10*R*)-10-methylhexadecyl]-*sn*-glycerol} (15a): Yield 0.258 g (31%), white solid, m.p. 49 °C. $[\alpha]_D^{22}$ = +10.7 (*c* = 0.023 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.82 (d, $^3J_{H,H}$ = 6.4 Hz, 6 H, 2 CHCH_3), 0.86 (t, $^3J_{H,H}$ = 6.8 Hz, 6 H, 2 CH_2CH_3), 1.02–1.32 [m, 106 H, $(CH_2)_{28}$, 2 $(CH_2)_7CH(CH_2)_5CH_3$], 1.50–1.57 (m, 8 H, 4 CH_2CH_2O), 1.89 (br., 2 H, 2 O*H*), 3.40–3.72 (m, 18 H, 8 CH_2O, 2 C*H*O) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.17 (CH_2CH_3), 19.78 (CHCH_3), 22.75 (CH_2CH_3), 26.17, 27.11, 27.14, 29.54–29.77, 30.73, 30.16, 32.02, 32.83, 37.17, 63.17 (CH_2OH), 70.44 (CH_2O), 70.97 (CH_2O), 71.90 (CH_2O), 78.31 (OCH) ppm. MS (ESI): *m*/*z* = 1108.7 [M + H]$^+$. $C_{72}H_{146}O_6$ (1107.93): calcd. C 78.05, H 13.28; found C 77.69, H 13.16.

3,3′-*O*-[(10*R*,23*R*)-10,23-Dimethyldotriacontane-1,32-diyl]bis(2-*O*-hexadecyl-*sn*-glycerol) (15b): Yield 0.224 g (27%), white waxy solid, m.p. 27 °C. $[\alpha]_D^{22}$ = +8.7 (*c* = 0.050 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.81 (d, $^3J_{H,H}$ = 6.6 Hz, 6 H, 2 CHCH_3), 0.86 (t, $^3J_{H,H}$ = 7.0 Hz, 6 H, 2 CH_2CH_3), 1.02–1.35 [m, 106 H, $(CH_2)_7CH(CH_2)_{12}CH(CH_2)_7$, 2 $(CH_2)_{13}CH_3$], 1.50–1.58 (m, 8 H, 4 CH_2CH_2O), 2.20 (br., 2 H, 2 O*H*), 3.37–3.77 (m, 18 H, 8 CH_2O, 2 C*H*O) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.09 (CH_2CH_3), 19.69 (CHCH_3), 22.67 (CH_2CH_3), 26.09, 27.08, 27.09, 29.35–30.07, 30.42, 31.91, 32.76, 37.11, 63.07 (CH_2OH), 70.39 (CH_2O), 70.89 (CH_2O), 71.84 (CH_2O), 78.28 (OCH) ppm. MS (ESI): *m*/*z* = 1108.0 [M]$^+$. $C_{72}H_{146}O_6$ (1107.93): calcd. C 78.05, H 13.28; found C 77.71, H 13.34.

3,3′-*O*-[(10*R*,23*R*)-10,23-Dimethyldotriacontane-1,32-diyl]bis{2-*O*-[(10*R*)-10-methylhexadecyl]-*sn*-glycerol} (15c): Yield 0.298 g (35%), colourless oil. $[\alpha]_D^{22}$ = +6.4 (*c* = 0.123 g mL^{-1}, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.81 (d, $^3J_{H,H}$ = 6.4 Hz, 12 H, 4 CHCH_3), 0.86 (t, $^3J_{H,H}$ = 7.0 Hz, 6 H, 2 CH_2CH_3), 1.05–1.38 [m, 104 H, $(CH_2)_7CH(CH_2)_{12}CH(CH_2)_7$, 2 $(CH_2)_7CH(CH_2)_5CH_3$], 1.52–1.56 (m, 8 H, 4 CH_2CH_2O), 2.24 (br., 2 H, 2 O*H*), 3.38–3.71 (m, 18 H, 8 CH_2O, 2 C*H*O) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.09 (CH_2CH_3), 19.68 (CHCH_3), 22.67 (CH_2CH_3), 25.74, 26.08, 27.03, 27.06, 27.07, 27.08, 29.34–30.06, 31.94, 32.73, 32.75, 37.09, 37.10, 63.03 (CH_2OH), 70.37 (CH_2O), 70.87 (CH_2O), 71.82 (CH_2O), 78.29 (OCH) ppm. MS (ESI): *m*/*z* = 1136.1 [M]$^+$. $C_{74}H_{150}O_6$ (1135.98): calcd. C 78.24, H 13.31; found C 78.53, H 13.15.

Bis(phosphocholines) I–III: 2-Bromoethylphosphoric acid dichloride (0.38 g, 1.6 mmol) was poured into dry chloroform (10 mL) whilst cooling with ice/water. A mixture of dry triethylamine (0.38 mL, 2.8 mmol) in dry chloroform (10 mL) was added slowly with stirring, which was continued for 30 min at 0 °C. The glycerols **15a**,**b** (in solid form) or **15c** [dissolved in chloroform (5 mL)] (0.2 mmol) were poured in one portion into the mixture. To dissolve the solid glycerols **15a**,**b**, the reaction mixture was gently warmed until a clear solution disappeared. Afterwards, the mixture was stirred at room temperature for 24 h. After the complete conversion of the glycerols, crushed ice (20 mL) was added to the solution and the mixture was stirred vigorously for a further 2 h. The organic layer was separated and the aqueous phase was extracted with chloroform (3 × 20 mL). The combined organic layers were evaporated and the residue was dissolved in THF/water (10 mL, 1:1). After 1 h, ammonium chloride solution (10 mL, 5%) was added and the mixture was extracted with a chloroform/methanol solution (5:1, 3 × 25 mL). The chloroform fraction was evaporated to dryness. The crude bromo esters were transferred into a mixture of chloroform (10 mL), acetonitrile (10 mL), and an alcoholic solution of trimethylamine (2 mmol, 4.2 M). The mixture was kept in a closed tube while warming up to 50 °C for 10 h. The clear solution was allowed to stand 3–4 d at room temperature. Afterwards, the reaction mixture was evaporated to dryness and the residue was purified by chromatography using chloroform/methanol/water and the gradient technique.

3,3′-*O*-(Dotriacontane-1,32-diyl)bis({2-*O*-[(10*R*)-10-methylhexadecyl]-*sn*-glycer-1-yl}-2-(trimethylammonio)ethyl phosphate) (I): Yield 0.124 g (43%), white solid, m.p. 217–219 °C. $[\alpha]_D^{22}$ = –10 (*c* = 2 mg mL^{-1}, DMSO*). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD): δ =

0.82 (d, $^3J_{H,H}$ = 6.4 Hz, 6 H, 2 CHCH_3), 0.87 (t, $^3J_{H,H}$ = 6.9 Hz, 6 H, 2 CH_2CH_3), 1.03–1.31 [m, 106 H, $(CH_2)_{28}$, 2 $(CH_2)_7CH$-$(CH_2)_5CH_3$], 1.48–1.55 (m, 8 H, 4 CH_2CH_2O), 3.26 [s, 18 H, 2 $N(CH_3)_3$], 3.39–3.61 (m, 14 H, 6 CH_2O, 2 CHO), 3.66–3.69 (m, 4 H, 2 NCH_2CH_2OP), 3.87–3.90 (m, 4 H, 2 $POCH_2CH$), 4.25–4.30 (m, 4 H, 2 NCH_2CH_2OP) ppm. ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD): δ = 13.82 (CH_2CH_3), 19.46 (CHCH_3), 22.50 (CH_2CH_3), 25.89, 25.93, 26.87, 26.93, 29.36, 29.39, 29.50–29.53, 29.87, 29.91, 31.77, 32.60, 36.94, 54.05 [t, J = 3.7 Hz, $N(CH_3)_3$], 58.59 (d, $^2J_{C,P}$ = 4.6 Hz, NCH_2CH_2O), 64.90 (d, $^2J_{C,P}$ = 5.4 Hz, $POCH_2CH$), 66.41 (br., NCH_2CH_2O), 70.38 (CH_2O), 70.41 (CH_2O), 71.53 (CH_2O), 77.79 (d, $^3J_{C,P}$ = 8.1 Hz, $POCH_2CH$) ppm. HRMS: calcd. for $C_{82}H_{170}N_2O_{12}P_2$ $[M + 2H]^{2+}$ 719.6187; found 719.6200. HPLC: t_R = 3.65 min, purity: 99.0%.

3,3′-*O*-[(10*R*,23*R*)-10,23-Dimethyldotriacontane-1,32-diyl]bis[(2-*O*-hexadecyl-*sn*-glycer-1-yl)-2-(trimethylammonio)ethyl phosphate] (II): Yield 0.095 g (33%), white waxy solid, m.p. 212–214 °C. $[\alpha]_D^{22}$ = –10 (c = 2 mg mL^{-1}, DMSO*). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD): δ = 0.81 (d, $^3J_{H,H}$ = 6.6 Hz, 6 H, 2 CHCH_3), 0.86 (t, $^3J_{H,H}$ = 6.9 Hz, 6 H, 2 CH_2CH_3), 1.04–1.37 [m, 106 H, $(CH_2)_7$-$CH(CH_2)_{12}CH(CH_2)_7$, 2 $(CH_2)_{13}CH_3$], 1.49–1.55 (m, 8 H, 4 CH_2CH_2O), 3.20 [s, 18 H, 2 $N(CH_3)_3$], 3.35–3.56 (m, 14 H, 6 CH_2O, 2 CHO), 3.58–3.61 (m, 4 H, 2 NCH_2CH_2OP), 3.87–3.91 (m, 4 H, 2 $POCH_2CH$), 4.22–4.27 (m, 4 H, 2 NCH_2CH_2OP) ppm. ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD): δ = 14.02 (CH_2CH_3), 19.70 (CHCH_3), 22.65 (CH_2CH_3), 26.06, 26.09, 27.01, 27.06, 29.32, 29.51, 29.54, 29.58–29.68, 29.94, 30.00, 30.10, 31.89, 32.71, 37.04, 54.40 [t, J = 3.4 Hz, $N(CH_3)_3$], 58.75 (d, $^2J_{C,P}$ = 5.0 Hz, NCH_2CH_2O), 65.02 (d, $^2J_{C,P}$ = 5.8 Hz, $POCH_2CH$), 66.70 (br., NCH_2CH_2O), 70.50 (CH_2O), 70.60 (CH_2O), 71.67 (CH_2O), 77.95 (d, $^3J_{C,P}$ = 5.4 Hz, $POCH_2CH$) ppm. HRMS: calcd. for $C_{82}H_{170}N_2O_{12}P_2$ $[M + 2H]^{2+}$ 719.6187; found 719.6201. HPLC: t_R = 3.94 min, purity: 98.8%.

3,3′-*O*-[(10*R*,23*R*)-10,23-Dimethyldotriacontane-1,32-diyl]bis({2-*O*-[(10*R*)-10-methylhexadecyl]-*sn*-glycer-1-yl}-2-(trimethylammonio)ethyl phosphate) (III): Yield 0.129 g (44%), white waxy solid, m.p. 195–200 °C. $[\alpha]_D^{22}$ = –10 (c = 2 mg mL^{-1}, DMSO*). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD): δ = 0.82 (d, $^3J_{H,H}$ = 6.4 Hz, 12 H, 4 CHCH_3), 0.87 (t, $^3J_{H,H}$ = 6.9 Hz, 6 H, 2 CH_2CH_3), 1.07–1.25 [m, 104 H, $(CH_2)_7CH(CH_2)_{12}CH(CH_2)_7$, 2 $(CH_2)_7CH(CH_2)_5CH_3$], 1.46–1.56 (m, 8 H, 4 CH_2CH_2O), 3.26 [s, 18 H, 2 $N(CH_3)_3$], 3.38–3.60 (m, 14 H, 6 CH_2O, 2 CHO), 3.68–3.71 (m, 4 H, 2 NCH_2CH_2OP), 3.89–3.91 (m, 4 H, 2 $POCH_2CH$), 4.28–4.32 (m, 4 H, 2 NCH_2CH_2OP) ppm. ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD): δ = 14.00 (CH_2CH_3), 19.63 (CHCH_3), 19.65 (CHCH_3), 22.63 (CH_2CH_3), 26.02, 26.06, 27.00, 27.05, 27.07, 29.50, 29.52, 29.59–29.67, 29.94, 29.99, 30.01, 30.04, 31.89, 32.71, 32.73, 37.02, 37.05, 37.07, 37.08, 54.32 [t, J = 3.5 Hz, $N(CH_3)_3$], 58.79 (d, $^2J_{C,P}$ = 5.0 Hz, NCH_2CH_2O), 65.04 (d, $^2J_{C,P}$ = 5.0 Hz, $POCH_2CH$), 66.54 (br., NCH_2CH_2O), 70.46 (CH_2O), 70.52 (CH_2O), 71.68 (CH_2O), 77.90 (d, $^3J_{C,P}$ = 8.1 Hz, $POCH_2CH$) ppm. HRMS: calcd. for $C_{84}H_{176}O_{12}N_2P_2$ $[M + 2H]^{2+}$ 733.6344; found 733.6355. HPLC: t_R = 3.80 min, purity: 98.8%.

*For polarimetry it was not possible to obtain a clear and concentrated solution of the lipids **I**–**III** in chloroform/methanol/water mixtures. Even in dry DMSO only concentrations of 2 mg mL^{-1} could be achieved. Therefore, the stated $[\alpha]_D$ values only give an estimation of the optical rotation.

Differential Scanning Calorimetry: The DSC curves were recorded with a Microcal VP-DSC differential scanning calorimeter (Microcal Inc. Northampton, MA, USA). The samples were prepared by mixing 1 mg of the lipids **I**–**III** and 1 mL of water (Millipore Q) followed by warming, vortexing and treatment with ultrasound. The sample solution and the water reference were degassed under vacuum whilst stirring. The measurements were performed at a scan rate of 20 °C h^{-1} from 2 to 95 °C in three consecutive runs. To eliminate any variation due to the cells a water/water baseline was subtracted. The DSC scans were evaluated by using the MicroCal ORIGIN 8.0 software.

Electron Microscopy: The liposomes were prepared for electron microscopy by using the film method. The bolalipids **I**–**III** (15–20 mg) were dissolved in chloroform/methanol (1:1), the solvent was evaporated and the samples were dried overnight in vacuo. The lipid films were then incubated with water (1 mL) at 50 °C for 1 h, treated with ultrasound for 30 min at the same temperature and extruded 41 times through a polycarbonate membrane (200 nm). The liposomes were freeze-fixed by using a JFD 030 (BAL-TEC, Balzers, Lichtenstein) propane jet-freeze device. Thereafter, the samples were freeze-fractured at –150 °C without etching by using a BAF 060 (BAL-TEC, Balzers, Liechtenstein) freeze-fracture/freeze-etching system. The surfaces were shadowed with platinum to produce good topographic contrast (2 nm layer, shadowing angle 45°) and subsequently with carbon to stabilise the ultra-thin metal film (20 nm layer, shadowing angle 90°). The replicas were floated in sodium chloride (4%, Roth, Karlsruhe, Germany) for 30 min, rinsed in distilled water (10 min), washed in 30% acetone (Roth, Karlsruhe, Germany) for 30 min and rinsed again in distilled water (10 min). Thereafter the replicas were mounted on copper grids, coated with formvar film and observed with a transmission electron microscope (EM 900, Carl Zeiss SMT, Oberkochen, Germany) operating at 80 kV. Pictures were taken with a Variospeed SSCCD SM-1k-120 camera (TRS, Moorenweis, Germany).

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