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Structure–property relationships in a series of diglycerol tetraether model lipids and their lyotropic assemblies: the effect of branching topology and chirality†

Thomas Markowski,[a] Simon Drescher,*[a] Annette Meister,[b] Alfred Blume[c] and Bodo Dobner*[a]

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Three novel diglycerol tetraether lipids with one membrane-spanning chain have been synthesized. These lipids contain only two or four *racemic* methyl branches at selected positions of the hydrophobic chains in contrast to natural lipids from archaebacterial membranes with an isoprenoid substitution pattern. The insertion of the methyl moieties was realized starting from either (*RS*)-citronellyl bromide or the inexpensive methyl malonic acid ethyl ester. For chain elongation the Cu-catalysed Grignard coupling reaction was used. The preparation of diglycerol tetraethers was either performed by condensing suitable blocked monoglycerol diethers by Grubbs metathesis or by reaction of the transmembrane C32-chain with blocked glycerols followed by further alkylation steps. Finally, we could show that the resulting lipids can form closed lipid vesicles comparable to the *optically pure* counterparts. Therefore, these much simpler lipids compared to the natural lipids from archaebacterial membranes are also suitable for preparation of stable tailored liposomes.

Introduction

The membrane lipids of archaebacteria are different with regard to common lipids found in *prokaryotes* and *eukaryotes*. This is due to the extreme living conditions under which such extremophiles exist. In detail, the hydrophobic alkyl chains are connected by ether linkages in the inverse *sn*-2,3-configuration to the glycerol backbone making these archaebacterial lipids resistant against enzymatic degradation. The fluidity and, hence, the adaptation to the milieu conditions are regulated by the insertion of a number of cyclopentane rings as well as by the insertion of several methyl branches in an isoprenoid substitution pattern resulting from the biosynthesis.[1,2]

Of special interest are the lipids of the *methanogens* and *thermoacidophiles* with one or two membrane-spanning (transmembrane) alkyl chains. These bipolar molecules are called bolaamphiphiles or bolalipids and they are mostly diglycerol tetraether lipids with a molecular length representing the thickness of the monolayer membrane formed by these lipids. This fact in combination with the stability of these bolalipids is therefore of great interest especially in terms of biotechnology, materials science, and pharmacy.[3–7] Since several phospholipids proved to be very suitable materials for the preparation of vesicles and also nanocontainers, bolalipids from archaebacteria should also be applicable for this purpose – especially with regard to their stability against decomposition enzymes. Liposomes composed of bolalipids are called archaeosomes.[8] To obtain such bolalipids in considerable amounts, isolation from natural sources is on the one hand a suitable method. However, this method yields only heterogeneous lipid mixtures with respect to the composition of the hydrophobic part of the lipids. On the other hand the total synthesis of an archaebacterial lipid with a diglycerol tetraether structure, exemplarily shown by Kakinuma *et al.*,[9] is not practicable on the gram scale. Based on the idea that such bolalipids are suitable for the preparation of tailored liposomes with outstanding possibilities and functionalities, many researchers interested in this field dealt with simpler model membranes and, hence, model lipids.

The main problem for the synthesis of archaebacterial model lipids is the macrocyclisation to the final cycle with 72

[a]*Martin-Luther-Universitaet Halle-Wittenberg, Institute of Pharmacy, Wolfgang-Langenbeck-Str. 4, 06120 Halle (Saale), Germany. E-mail: simon.drescher@pharmazie.uni-halle.de, bodo.dobner@pharmazie.uni-halle.de; Fax: +49 (0) 345 5527026, +49 (0) 345 5527018; Tel: +49 (0) 345 5525196, +49 (0) 345 5525120*
[b]*Center for Structure and Dynamics of Proteins (MZP) Biocenter, MLU Halle-Wittenberg, Weinbergweg 22, 06120 Halle (Saale), Germany*
[c]*MLU Halle-Wittenberg, Institute of Chemistry, von-Danckelmann-Platz 4, 06120 Halle (Saale), Germany*

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carbon atoms. Since tetraether lipids with only one membrane-spanning chain were also found in natural lipids it is reasonable for simpler model systems to copy this structural feature. The group of Yamauchi[10,11] and then especially Benvegnu and co-workers described an effective preparation procedure for such simpler compounds.[12,13] In a recent paper[14] we published the synthesis of diglycerol tetraethers with one membrane-spanning alkyl chain in the *sn*-3-position of the glycerol backbone. In contrast to other archaebacterial model lipids, we introduced only a discrete number of methyl branches at certain positions of the chain. We found that model lipids with four methyl branches show nearly the same physicochemical behaviour as the natural archaebacterial membrane lipids.[15] In addition, we could show that these lipids are able to form liposomes. Hence, these bolalipids represent a remarkable new model system of archaebacterial lipids applicable for liposomal drug delivery purposes.

Indeed, the preparation of the modified lipids is simpler than total synthesis of natural lipids from archaebacterial membranes, but the chiral methyl branching requires greater synthetic effort for the preparation of these lipids on gram scale. Whereas optically pure glycerol derivatives are nowadays comparatively low priced and simple to prepare, the optically pure methyl branches require more effort starting from chiral precursors. Therefore, we were interested in the influence of the chirality of the methyl branching at defined positions in the alkyl chain on the aggregation behaviour of the model lipids. The loss of the chirality of the methyl branches enabled us to use inexpensive starting materials and shorter reaction pathways.

In this paper, we present the synthesis of three bipolar phosphocholines (lipids **I–III**) with one membrane-spanning alkyl chain of 32 carbon atoms and two shorter alkyl chains with 16 carbon atoms in the *sn*-2-position of the glycerol backbone. The alkyl chains contain two or four *racemic* methyl branches, again at the 10-position of the hexadecyl chains and/or at the 10- and 10′-position of the transmembrane alkyl chain (Fig. 1). In addition, we present first investigations of the lyotropic behaviour of aqueous suspensions of these novel tetraether lipids in comparison with the *optically pure* derivatives[14] by differential scanning calorimetry (DSC), dynamic light scattering (DLS), and transmission electron microscopy (TEM).

Results and discussion

Synthetic methods

The synthetic strategy for the preparation of bolalipids **I–III** involves on the one hand the coupling of preformed glycerol triethers including one terminal double bond for the Grubbs coupling to the bipolar system. This synthetic pathway was already described previously[14] except that we now used a benzyl blocking group instead of the trityl residue. On the other hand, we developed a novel synthetic procedure for the preparation of lipids **I–III**, in which the whole membrane-spanning alkyl chain was bound to suitable blocked *sn*-glycerols. With respect to our aim to investigate the lipids with racemic methyl branches, the synthesis of the branched hexadecyl chains (**14a,b**; see Scheme 1) can also be performed by two different strategies: the *malonic ester* pathway or the *citronellyl bromide* pathway described previously in detail.[14]

The *malonic ester* pathway starts from a very inexpensive starting material: methyl malonic acid ethyl ester (**1**), which contains the necessary methyl group and can be easily transformed into the desired compounds by classic procedures (Scheme 1). The ester **1** was converted into its carbanion with sodium hydride in toluene and alkylated with 1-bromohexane and 7-bromohept-1-ene resulting in the formation of hexyl(methyl)malonic acid diethyl ether (**2a**) and hept-6-en-1-yl(methyl)malonic acid diethyl ether (**2b**). After the subsequently performed saponification with potassium hydroxide in an ethanol–water-mixture, hexyl(methyl)malonic acid (**3a**) and hept-6-en-1-yl(methyl)malonic acid (**3b**) were isolated. Then, the mono-decarboxylation of compounds **3** resulted in the formation of (2*RS*)-2-methyloctanoic acid (**4a**) and (2*RS*)-2-methylnon-8-enoic acid (**4b**), respectively. It is noteworthy that the decarboxylation of **3b** was performed in cumene with catalytic amounts of pyridine in order to avoid side reactions whereas compound **3a** was heated up to 180 °C in a pure manner to forfeit one acid moiety (see Scheme 1, left part).

The following conversion of the carboxylic acid into the hydroxy moiety can be performed either by reduction of the corresponding methyl ester (in the case of **4a**) or by direct reduction of the unsaturated acid **4b** with the use of lithium aluminium hydride finally resulting in the formation of (2*RS*)-2-methyloctan-1-ol (**5a**) and (2*RS*)-2-methylnon-8-en-1-ol (**5b**). In the next step, the hydroxy group has to be transformed into

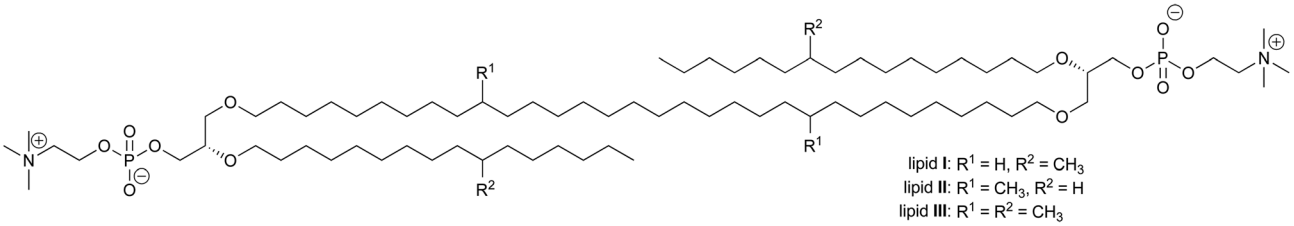
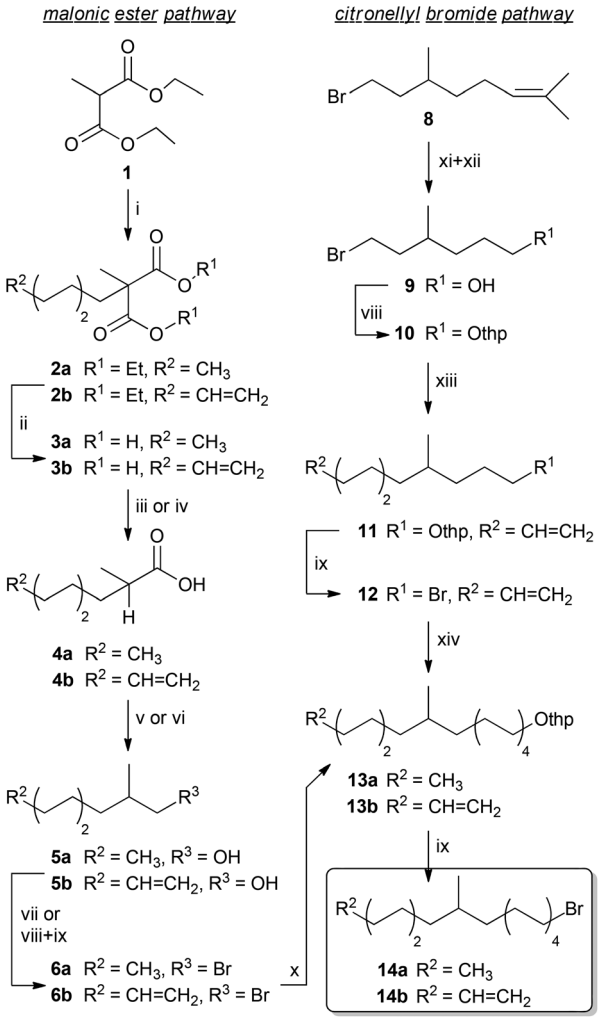


Fig. 1 Chemical structure of the synthesised archaebacterial model lipids I–III including racemic methyl branches.



Scheme 1 Reagents and conditions: (i) NaH, toluene, 1-bromohexane or 7-bromohept-1-ene, reflux; (ii) KOH, EtOH–H_2O; (iii) 180 °C; (iv) pyridine, cumene, reflux; (v), H_2SO_4, MeOH, reflux; then $LiAlH_4$, Et_2O, reflux; (vi) $LiAlH_4$, Et_2O, reflux; (vii) MesCl, $CHCl_3$, 0 °C; then LiBr, acetone, reflux; (viii) DHP, CH_2Cl_2, PPTS, r.t.; (ix) PPh_3, Br_2, CH_2Cl_2, r.t.; (x) Mg, Et_2O, reflux; then 2-[(8-bromooctyl)oxy]tetrahydro-2*H*-pyran (**7a**), Li_2CuCl_4, THF, 0 °C; (xi) O_3, MeOH, –78 °C; (xii) $NaBH_4$, MeOH, r.t.; (xiii) Mg, $R^2(CH_2)_3Br$, Et_2O, reflux; then Li_2CuCl_4, THF, 0 °C; (xiv) Mg, 2-[(6-bromohexyl)oxy]tetrahydro-2*H*-pyran (**7b**), 50 °C; then Li_2CuCl_4, THF, 0 °C.

a bromide. For this purpose two procedures are conceivable: (2*RS*)-1-bromo-2-methyloctane (**6a**) was prepared by a conventional two-step procedure including a reaction with methanesulfonyl chloride (MesCl) followed by a Finkelstein-replacement of the sulfonic ester with lithium bromide in anhydrous acetone. On the other hand, (8*RS*)-9-bromo-8-methylnon-1-ene (**6b**) was prepared using a procedure described by Schwarz *et al.*[16] Therefore, the alcohol **5b** was firstly converted into the appropriate acetal with 3,4-dihydro-2*H*-pyran (DHP) and a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS). Then, this compound was transformed into the favoured bromide **6b** using triphenylphosphoranediyl dibromide. The advantage of the last mentioned procedure is that both reaction steps nearly gave quantitative yields.

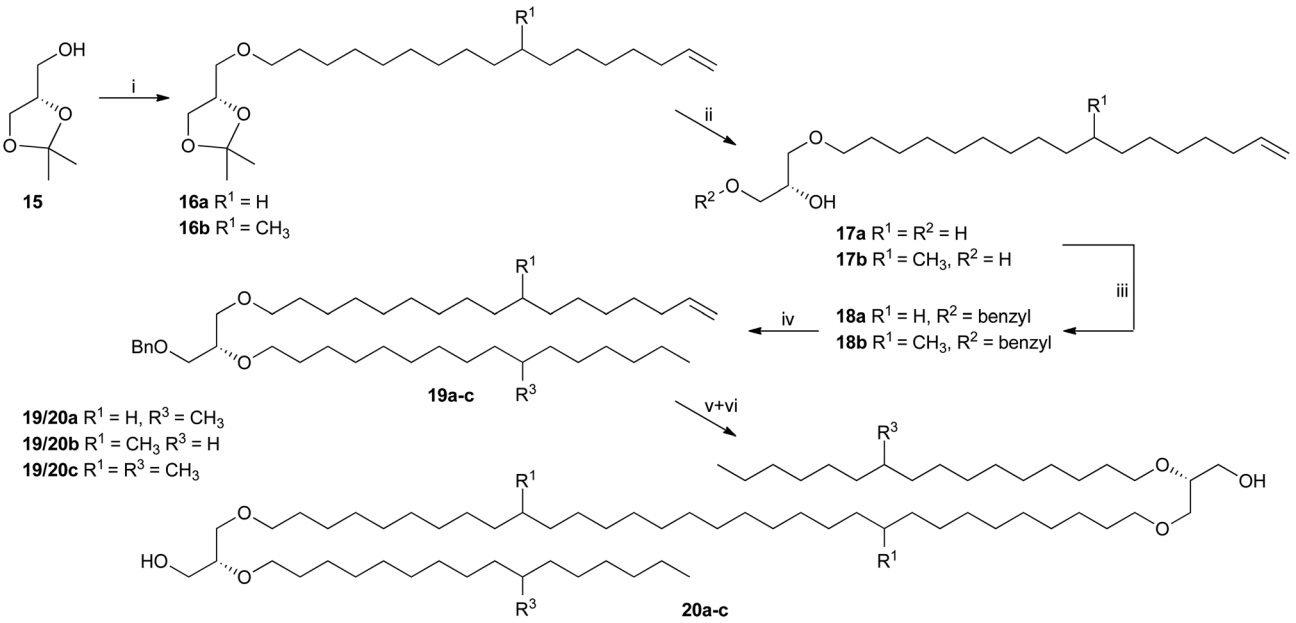
The bromides **6a,b** were further used for alkyl chain elongation reaction. For this purpose, compounds **6a,b** were transferred into the corresponding Grignard reagents in ethereal solution and coupled with 2-[(8-bromooctyl)oxy]tetrahydro-2*H*-pyran (**7a**) in THF under catalysis with dilithium tetrachlorocuprate-(II),[17] yielding the compounds **13a** and **13b**.

Both compounds (**13a,b**) can also be synthesised following the *citronellyl bromide* pathway (see Scheme 1, right part), which had been described previously in detail.[14] This reaction route is exemplarily shown for the preparation of compound **13b**. It starts from the commercially available (*RS*)-citronellyl bromide (**8**) that was allowed to react with ozone at −78 °C in methanol. After the reductive work-up using sodium borohydride, (4*RS*)-6-bromo-4-methylhexan-1-ol (**9**) can be isolated. After the protection of the hydroxy moiety using DHP and PPTS, the resulting compound **10** was coupled with pent-4-enylmagnesium bromide in a Grignard reaction under catalysis with dilithium tetrachlorocuprate-(II).[17] Then, the THP-alkane **11** was transformed into the corresponding bromide, again using the procedure described by Schwarz *et al.*[16] The (8*RS*)-11-bromo-8-methylundec-1-ene (**12**) obtained was used for the second chain elongation, again using the copper-catalysed Grignard reaction and **7b** finally resulting in compound **13b**.

Comparing the two synthetic routes – the *malonic ester* and the *citronellyl bromide* pathway – with compounds **13**, one can conclude that both pathways resulted in nearly the same overall yields: 27–58% *vs.* 35% (46–57%[14]) with a slight gain for the *malonic ester* route although it includes at least one synthetic step more. In addition, the purification of the products within the first two steps of the *malonic ester* pathway is not necessary, as shown for the synthesis of compound **5b** – an additional advantage for this route. Finally, both THP-blocked methyl-branched alcohols (**13a,b**) can be transferred into the bromides (10*RS*)-1-bromo-10-methylhexadecane (**14a**) and (8*RS*)-17-bromo-8-methylheptadec-1-ene (**14b**) using again the protocol of Schwarz *et al.*[16]

The synthesis of diglycerol tetraethers can be realised by two different synthetic strategies: *pathway* (*I*), one can use the olefin metathesis with the Grubbs first-generation catalyst[18] in order to connect pre-built monoglycerol diethers including a terminal double bond (see Scheme 2) or *pathway* (*II*), one can insert the transmembrane alkyl chain in the first step, followed by further alkylation steps (see Scheme 3). Realising the *sn*-2,3-stereochemistry of the glycerol backbone of natural archaebacterial lipids, both synthetic pathways have to start with the commercially available (*S*)-1,2-*O*-isopropylideneglycerol (**15**).

The reaction pathway I (Scheme 2) starts with a first alkylation step: compound **15** was deprotonated with potassium hydride in toluene and subsequently alkylated with terminal unsaturated bromides, namely **14b** and 17-bromoheptadec-1-ene,[14] yielding **16a** and **16b**. Afterwards, the isopropylidene (IP) blocking group of compounds **16a,b** was cleaved using PPTS in methanol leading to 3-*O*-alkyl-*sn*-glycerols **17**.



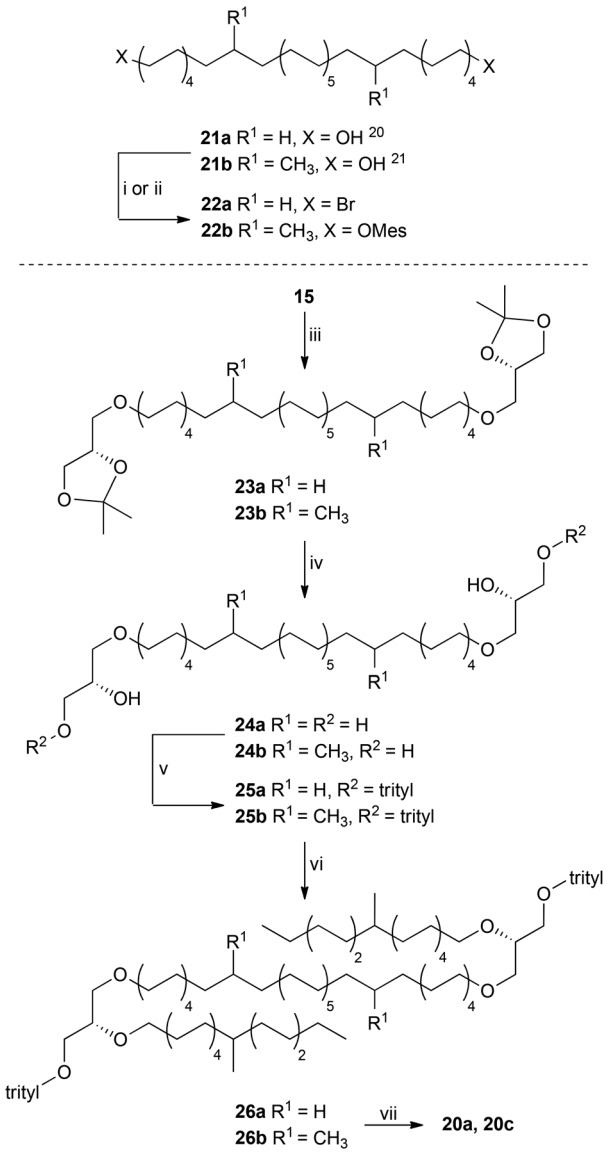
Scheme 2 Reagents and conditions: (i) KH, toluene, 17-bromoheptadec-1-ene or **14b**, reflux; (ii) PPTS, MeOH, reflux; (iii) Bu_2SnO, toluene–MeOH, reflux; then benzyl bromide, 1,2-dimethoxyethane, reflux; (iv) KH, toluene, **14a** or 1-bromohexadecane, reflux; (v) $RuCl_2(=CHPh)(PCy_3)_2$, CH_2Cl_2, reflux; (vi) $Pd(OH)_2/C$, H_2, EtOH–EtOAc, r.t.

In our previous work,[14] the selective introduction of the C16-chain in the *sn*-2-position of the glycerol was realised by regioselective tritylation of the primary hydroxyl group and subsequently performed deprotonation and alkylation with 1-bromohexadecane or the 10-methyl analogue. Since the trityl residue is very bulky, the yields during the deprotonation and alkylation steps are quite moderate and, hence, we used a regioselective benzylation of the dibutylstannylene derivatives of the 3-*O*-alkyl-*sn*-glycerols **17** instead.[19] However, beside the desired 3-*O*-alkyl-1-*O*-benzyl-*sn*-glycerols **18** the 3-*O*-alkyl-2-*O*-benzyl regioisomers were formed. But, both compounds could be fully separated by column chromatography using chloroform–diethyl ether as the eluent and the gradient technique. The subsequent second alkylations of glycerols **18a** and **18b** were carried out under nearly the same conditions described above using potassium hydride in toluene and (10*RS*)-1-bromo-10-methylhexadecane (**14a**) or 1-bromohexadecane. The yields of the 1-*O*-benzyl-2,3-*O*,*O*-dialkyl-*sn*-glycerols **19a–c** are slightly higher (67–74%) compared to the alkylation of the corresponding 3-*O*-alkyl-1-*O*-trityl-*sn*-glycerol derivatives (55–68%) described previously.[14] However, taking into account the more laborious synthesis of the pure 3-*O*-alkyl-1-*O*-benzyl-*sn*-glycerol **18** using the dibutylstannylene pathway, both methods benzylation and tritylation at the *sn*-1-position followed by *O*-alkylation at the *sn*-2-position of the glycerol moiety are comparable.

The synthesis of the final diglycerol tetraethers **20** based on compounds **19a–c** – following pathway I – was realised in two steps: as described previously,[14] the olefin metathesis with the Grubbs first-generation catalyst[18] followed by simultaneous hydrogenation of the double bonds and debenzylation using palladium hydroxide on carbon yielded 3,3′-*O*-(alkane-1,1′-diyl)-bis(2-*O*-alkyl-*sn*-glycerol)s **20** in very good yields (72–88% compared to **19**).

The reaction pathway II (Scheme 3) also starts from the glycerol compound **15**. In contrast to pathway I, the transmembrane alkyl chain was inserted in the first alkylation step. Therefore, the dotriacontane-1,32-diol (**21a**)[20] and the racemic (10*RS*,23*RS*)-10,23-dimethyldotriacontane-1,32-diol (**21b**)[21] were transferred into the corresponding dibromide **22a** and bis(methanesulfonate) (**22b**), respectively, by common chemical procedures and subsequently used to connect two molecules of compound **15** in a twofold *O*-alkylation reaction. Both products (**23a,b**) were obtained in acceptable yields (43–56%). After nearly quantitative cleavage of the IP blocking groups, resulting in bis(*sn*-glycerol)s **24a** and **24b**, the *sn*-1-position of the glycerol was blocked by tritylation. The reaction of compounds **24a,b** with trityl chloride was performed in pure pyridine with catalytic amounts of 4-dimethylaminopyridine (DMAP) at room temperature yielding **25a** and **25b**. It is noteworthy that despite the bulkiness of the trityl moiety we observed a tris- and tetrakis-tritylation of compounds **24a,b** (see ESI†) in a non-negligible amount, which prohibited a prolonged reaction time. However, the different tritylation products can be fully separated from each other by MPLC.

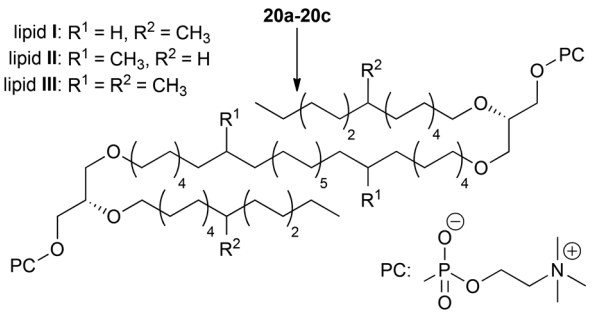
The second *O*-alkylation was performed using potassium hydride, toluene, and bromide **14a** in a comparable procedure as described above. Compounds **26a** and **26b** were obtained in very good yields (47–57%), particularly with regard to the *two* *O*-alkylation reactions performed in this synthetic step. The relatively high amount of the monoalkylated side-product (22–31%; see the ESI† for MS) is due to the steric shielding of the *sn*-2-position of the glycerol and it could not be reduced by either elongation of the reaction time or increasing the



Scheme 3 Reagents and conditions: (i) DHP, CH_2Cl_2, PPTS, r.t.; then PPh_3, Br_2, CH_2Cl_2, r.t.; (ii) MesCl, DMAP, $CHCl_3$, r.t.; (iii) KH, toluene, **15**; then **22a** or **22b**, reflux; (iv) PPTS, MeOH, reflux; (v) trityl chloride, pyridine, DMAP, r.t.; (vi) KH, toluene, **14a**, reflux; (vii) $BF_3{\cdot}Et_2O$, MeOH.

amount of bromide **14a**. The final cleavage of the trityl blocking groups of compounds **26a,b** was performed with $BF_3{\cdot}Et_2O$ in methanol in accordance with Hermetter and Paltauf[22] leading to the formation of compounds **20a** and **20c** in nearly quantitative yields (95%).

The comparison of the two synthetic pathways for the preparation of the diglycerol tetraethers **20** – starting from IP-glycerol **15** – shows that both routes gave nearly the same yields: pathway I, using the Grubbs metathesis reaction, provides 16–32% (previously 15–30%[14]) after 6 reaction steps whereas pathway II, which utilises the insertion of the transmembrane alkyl chain, provides 8–20% after 5 steps. Although the last route resulted in slightly lower overall yields, it is from the economical point of view our preferred pathway because it



Scheme 4 Reagents and conditions: $Cl_2P(O)O(CH_2)_2Br$, TEA, $CHCl_3$; then $CHCl_3$, acetonitrile, EtOH, $N(CH_3)_3$, 50 °C.

avoids the usage of expensive ruthenium catalysts and it is at least one step shorter.

Lastly, the methyl-branched bipolar phosphocholines **I–III** were prepared from the diglycerol tetraethers **20** by the method described by Eibl *et al.*[23] with the phosphorylating reagent of Hirt and Berchthold (Scheme 4).[24] The use of 2-bromoethylphosphoric acid dichloride in the phosphorylation step resulted in the highest yields in comparison with other phosphorylating agents, *e.g.* phospholanes or phosphoroxytrichloride.[25,26] The subsequently performed quarternisation with trimethylamine provided the diglycerol tetraether lipids **I–III** in 28–41% yield after purification and with respect to the glycerols **20.**

Temperature-dependent aggregation behaviour

The lyotropic phase behaviour of the novel, archaebacterial diglycerol tetraether phospholipid analogues **I–III** containing a different number and position of *racemic* methyl branches was characterised by differential scanning calorimetry (DSC) in the temperature range between 2 and 95 °C in comparison with the *optically pure* counterparts described previously.[14] Lipid **I** with two methyl groups in the shorter alkyl chain shows an endothermic transition at a temperature (T_m) of 19.1 °C

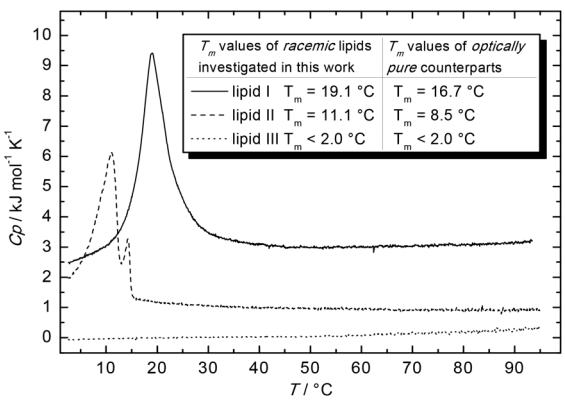


Fig. 2 DSC heating curves of aqueous suspensions of lipids **I–III** (c = 1 mg mL^{-1}, heating rate = 20 K h^{-1}). The curves are shifted vertically for clarity. The inset shows the corresponding transition temperatures (T_m values) of lipids investigated as well as the T_m values of the optically pure counterparts described previously.[14]

(Fig. 2), which is much lower compared to T_m of the unbranched tetraether lipid described by Yamauchi (T_m = 61 °C).[11] Compared to the optically pure counterpart of lipid **I**,[14] T_m has increased by 2.4 K. Lipid **II** with two methyl branches in the 10 and 10′-position of the transmembrane alkyl chain shows an endothermic transition at 11.1 °C (with a small shoulder at 14.3 °C), which is again 2.6 K higher compared to the optically pure analogue[14] but 8 K lower compared to lipid **I**. For lipid **III** with four methyl groups, no phase transition above 2 °C could be detected (Fig. 2), which is in line with the behaviour of natural archaebacterial lipids[15] and which was also found for the optically pure counterpart.[14]

It is obvious that the van der Waals contacts of the longest unbranched segment of the alkyl chains determine the value of the transition temperature. Similar results have been found before for monopolar methyl-branched phospholipids.[27,28] The differences in T_m values between tetraether lipids with either *racemic* or *optically pure* methyl branches could be related to small changes in the van der Waals interactions of the alkyl chains; due to the identical orientation of the methyl branches with respect to the long alkyl chain in an all-*trans* conformation in the *optically pure* tetraether lipids, close chain packing could be more difficult compared to the *racemic* lipids **I–III**, which as a consequence would lead to a further decrease of the transition temperature. In conclusion, not only the number and the positions but also the stereospecificity of the methyl branches within the alkyl chains are important for the aggregation and the lyotropic transitions of the tetraether lipids.

The ability of compounds **I–III** to form closed lipid vesicles was studied by transmission electron microscopy (TEM). Samples of lipid suspensions (c = 0.03–0.1 mg mL^{-1}) were stained with uranyl acetate, dried and then imaged. Fig. 3 shows the EM images of lipid samples prepared at two different temperatures. The images show that all three diglycerol tetraether lipids are capable of forming liposomes with diameters of about 100 nm (Fig. 3B and E) and up to 3 μm (giant vesicles; Fig. 3C). Major differences between the sample preparations at temperatures below the transition observed in DSC (Fig. 3A and B) and preparations at ambient temperature (Fig. 3C–F) could not be detected. For lipid **I** we observed large vesicles, which were disrupted (Fig. 3A) or collapsed and folded (Fig. 3C and D) during the drying process of sample preparation. Lipid **II** (Fig. 3B and E) showed the formation of smaller vesicles compared to lipid **I** and the aggregates seemed to be less structured. For lipid **III** we observed vesicles (Fig. 3F) with diameters ranging between 200 nm and 1 μm. The observations for these *racemic* tetraether lipids (**I–III**) are comparable to those found for the *optically pure* counterparts described previously.[14]

To further prove the capability of the tetraether lipids **I–III** to form liposomes stable against aggregation or fusion we performed dynamic light scattering (DLS) experiments (Fig. 4). Liposomes were prepared by the technique described by Bangham *et al.*[29] After treatment of sample suspensions (c = 1 mg mL^{-1}) with ultrasound, the liposomes were extruded through 100 nm polycarbonate membranes and examined by DLS. The hydrodynamic diameters obtained were in the range of 99–111 nm with a polydispersity index (PdI) between 0.08 and 0.12, which indicates a relatively narrow size distribution

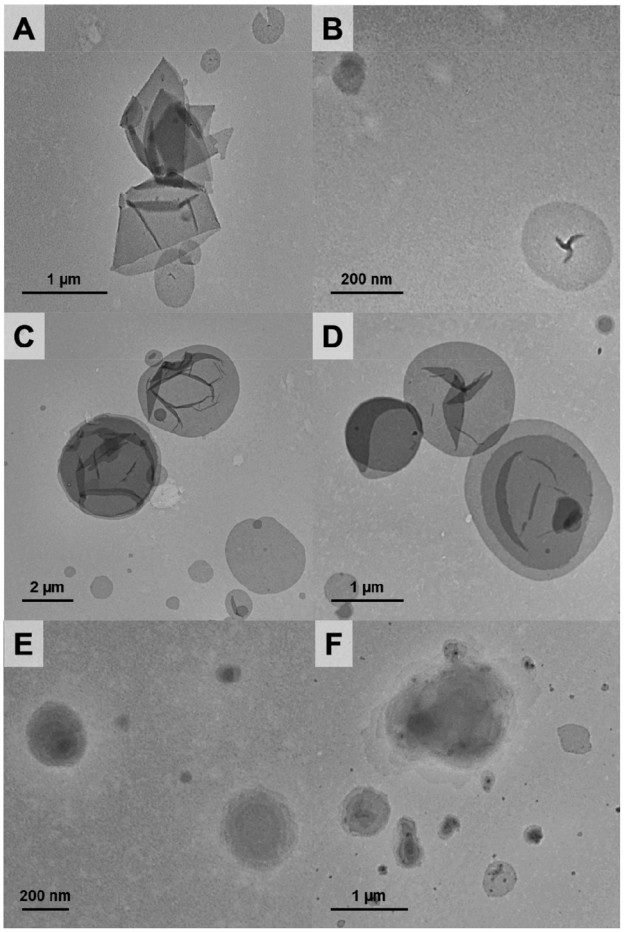


Fig. 3 TEM images of aqueous suspensions of lipids **I–III** (c = 0.03–0.1 mg mL^{-1}) prepared at different temperatures: (A, B) 4 °C, (C–F) 22 °C; (A, C, D) lipid **I**, (B, E) lipid **II**, and (F) lipid **III**. Samples were stained with uranyl acetate.

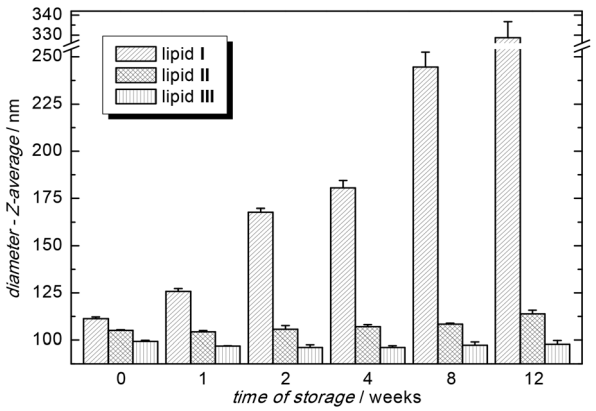


Fig. 4 DLS measurements of liposomes prepared from aqueous lipid suspensions (c = 1 mg mL^{-1}) using the technique described by Bangham *et al.*[29] after different times of storage (n = 3).

of the vesicles (see ESI, Fig. S1 and S2†). The DLS measurements were repeated after several weeks (1–12) of storage in order to check the stability of liposomes against aggregation or fusion. For lipid **I** with an unbranched transmembrane alkyl chain we observed a continuous increase of the *z*-average value up to 330 nm in diameter after 12 weeks of storage (Fig. 4). The corresponding PdI values also increased over time, indicating an increase in polydispersity (see ESI, Fig. S3†). In contrast, liposomes composed of lipids **II** and **III**, which contain two methyl groups within the long, transmembrane alkyl chain, are stable for at least 3 months. The reason for this behaviour remains unclear at this time, but it is conceivable that the additional methyl branches within the long, transmembrane alkyl chain induce a flexibility that is necessary for liposomal stability. The further enhanced stability of liposomes made from lipid **III** with two additional methyl branches in the short chains supports this assumption. This notion is also supported by experimental results on membranes composed of 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC), where each chain has 4 additional methyl groups. This compound is the lipid of choice for the formation of black lipid membranes (BLM) due to the high stability of the bilayer.[30]

Conclusions

We have developed several synthetic strategies for the preparation of diglycerol tetraether model lipids that enables the insertion of racemic methyl branches at selected positions within the alkyl chains. For the synthesis of the bromoalkane precursors we established a *malonic ester* pathway – besides the *citronellyl bromide* pathway described previously[14] – that improves the synthetic handling and the yields obtained. The subsequent preparation of the diglycerol tetraethers was realised using on the one hand the Grubbs metathesis reaction[14] (pathway I) and on the other hand the insertion of the transmembrane alkyl chain as a whole (pathway II). The latter ones further improve the synthetic economy circumventing the usage of ruthenium catalysts. For the selective glycerol alkylation reactions and also for the insertion of the phosphocholine headgroups, robust and established approaches were used.

Through the syntheses described herein we have shown that, beside the usage of a reduced number of methyl branches,[14] the stereospecificity of those methyl moieties is not of substantial significance for the properties of our archaebacterial model lipids; the *optically pure*[14] and also the *racemic* model lipids – described herein – show an aggregation behaviour comparable to archaebacterial lipids found naturally, *i.e.*, the formation of stable liposomes. This fact is of particular interest for a simpler, less expensive, and a straight forward synthesis of such model lipids. With the insertion of a discrete number of methyl branches at positions other than those described above, fine-tuning of the aggregation behaviour and, hence, the formation of tailored liposomes seem to be feasible. This aspect and the preparation of model compounds with different headgroup structures are under investigation.

Experimental section

General

Apart from palladium hydroxide on carbon (20%, Acros Organics) all chemicals were purchased from Sigma Aldrich Co. and were used without further purification. 2-Bromoethylphosphoric acid dichloride was prepared according to the literature.[23] 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 means of bromothymol blue. Silica gel (Merck, 0.063–0.200 mm) was used for column chromatography of all products. Melting points were determined with Boetius apparatus. Optical rotation was quantified on a Polartronic E (Schmidt und Haensch) and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Elemental analyses were carried out on a Leco CHNS-932. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 spectrometer or a Varian Inova 500 using $CDCl_3$ or CD_3OD as the internal standard. Chemical shifts (δ) are reported in parts per million (ppm). The coupling constants (J) are reported in Hz. Mass spectrometric data were obtained with a Finnigan LCQ-Classic (ESI-MS) or were recorded on an AMD 402 (70 eV) spectrometer (EI-MS). High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific LTQ-Orbitrap mass spectrometer with static nano-electrospray ionisation. Analytical HPLC (Jasco) was performed on a Kromasil Si 100–5 μm–250 × 4.6 mm, a PU 980 Intelligent HPLC-Pump, and a LG-1580-02 Ternary Gradient Unit (Jasco) with a SEDEX 55 ELS detector (SEDERE France) using the following solvents: 5 min isocratic with $CHCl_3$–MeOH–water (45/45/10, v/v/v), 5 min continuous increase with $CHCl_3$–MeOH–water (42/42/16, v/v/v), 10 min isocratic with $CHCl_3$–MeOH–water (45/45/10, v/v/v); flow = 1 mL min^{-1}.

Synthesis of methyl-branched bromoalkanes – the malonic ester pathway

The synthetic procedures and analytical data of compounds **2a**, **3a**, **4a**, **5a**, and **6a** can be found in the ESI.†

Hept-6-en-1-yl(methyl)malonic acid diethyl ester (2b). The sodium salt of the methyl malonic acid ester was prepared from **1** (13.92 g, 0.08 mol) and sodium hydride (60%, 3.2 g, 0.08 mol) in dry toluene. Then, 7-bromohept-1-ene (10.0 g, 85 mmol) was added and the mixture was heated for 8 h under reflux. Water (20 mL) was added and the organic phase was separated and evaporated. The crude ester was used for the saponification without further purification.

Hept-6-en-1-yl(methyl)malonic acid (3b). The crude **2b** was taken up with EtOH (100 mL). Afterwards, KOH (11.2 g, 0.2 mol) dissolved in water (40 mL) was added and the mixture was refluxed for 8 h. Most of the EtOH was evaporated and the residue was diluted with water (100 mL). The solution was extracted two times with Et_2O (40 mL) and the alkaline water phase was cooled to 0 °C. At this temperature the water phase was acidified with ice-cold aqueous HCl and then extracted three times with Et_2O (75 mL). The collected ethereal layers

were washed with brine, dried and evaporated to dryness. The crude acid **3b** was used without purification.

(2*RS*)-2-Methylnon-8-enoic acid (4b). The crude hept-6-en-1-yl(methyl)malonic acid (**3b**) was dissolved in cumene (100 mL) and pyridine (1 mL). The mixture was heated for 1 h under reflux. The solvent was evaporated, the oily residue was dissolved in Et_2O (50 mL) and the solution was washed with diluted HCl and brine. After drying over Na_2SO_4, the Et_2O was removed under reduced pressure and the residue was purified by column chromatography yielding colourless oil (5.7 g, 82% with respect to compound **1**). $C_{10}H_{18}O_2$ requires C, 70.55; H, 10.65; found: C, 70.73; H, 10.88%; ^{1}H NMR (500 MHz; $CDCl_3$) δ 1.18 (d, J = 6.9 Hz, 3 H, CH_3), 1.22–1.49 (m, 7 H, CH_2), 1.65–1.72 (m, 1 H, CH_2CH), 2.00–2.06 (m, 2 H, H_2C=CHCH_2), 2.42–2.49 (m, 1 H, CHCH$_3$), 4.90–5.03 (m, 2 H, H_2C=CH), 5.80 (ddt, $^2J_{[Z]}$ = 16.9 Hz, $^2J_{[E]}$ = 10.2 Hz, ^3J = 6.7 Hz, 1 H, H_2C=CH); ^{13}C NMR (125 MHz; $CDCl_3$) δ 16.8 (*C*H$_3$), 26.9 (*C*H$_2$CH$_2$CHCH$_3$), 28.7 and 28.9 (H$_2$C=CHCH$_2$*C*H$_2$*C*H$_2$), 33.4, 33.7, 39.4 (*C*H), 114.2 (H$_2$*C*=CH), 138.9 (H$_2$C=*C*H), 183.6 (COOH); EI-MS m/z 170 (3%, M), 152 (10, M − H_2O).

(2*RS*)-2-Methylnon-8-en-1-ol (5b). To a slurry of lithium aluminium hydride (1.13 g, 30 mmol) in dry Et_2O (50 mL) was added dropwise a solution of compound **4b** (5.10 g, 30 mmol) in dry Et_2O (20 mL). The mixture was stirred for 6 h under reflux. Afterwards, the reaction mixture was hydrolysed with ice and acidified using 10% HCl. The layers were separated and the water phase was extracted two times with Et_2O (50 mL). The combined ethereal phase was washed with 5% KOH (50 mL), water (50 mL) and brine (50 mL). After drying over Na_2SO_4, the solvent was evaporated and the crude product was purified by column chromatography with heptane–Et_2O as the eluent using the gradient technique yielding colourless oil (4.4 g, 94%). $C_{10}H_{20}O$ requires C, 76.86; H, 12.90; found: C, 76.75; H, 12.98%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.91 (d, J = 6.7 Hz, 3 H, CHCH_3), 1.02–1.16 (m, 1 H, CH_2), 1.19–1.42 (m, 7 H, CH_2), 1.52–1.76 (m, 1 H, CH_2), 1.99–2.10 (m, 2 H, H_2C=CHCH_2), 3.37–3.55 (m, 2 H, CH_2OH), 4.88–5.01 (m, 2 H, H_2C=CH), 5.81 (ddt, $^2J_{[Z]}$ = 16.9 Hz, $^2J_{[E]}$ = 10.2 Hz, ^3J = 6.7 Hz, 1 H, H_2C=CH); ^{13}C NMR (100 MHz; $CDCl_3$) δ 16.5 (*C*H$_3$), 26.8 (*C*H$_2$CH$_2$CHCH$_3$), 28.9 and 29.4 (H$_2$C=CHCH$_2$*C*H$_2$*C*H$_2$), 33.1, 33.7, 35.7 (*C*H), 68.3 (*C*H$_2$OH), 114.2 (H$_2$*C*=CH), 139.1 (H$_2$C=*C*H); EI-MS m/z 156.2 (4%, M), 138.2 (0.4, M − H_2O).

(8*RS*)-9-Bromo-8-methylnon-1-ene (6b). Compound **5b** (4.06 g, 26 mmol) was dissolved in dry CH_2Cl_2 (30 mL). 3,4-Dihydro-2*H*-pyrane (3.78 g, 45 mmol) and PPTS (50 mg) were added and the mixture was allowed to stir overnight. After the reaction was complete (TLC control), the mixture was washed twice with water and the organic layer was dried over Na_2SO_4. The solvent was removed and the residue was filtered over a short column. The isolated and exhaustive dried 2-[(2-methylnon-8-en-1-yl)oxy]tetrahydro-2*H*-pyran was poured into a solution of triphenylphosphoranediyl dibromide prepared from Br_2 (9.2 g, 57 mmol) and triphenylphosphane (14.9 g, 57 mmol) in CH_2Cl_2 (160 mL) at 0 °C. The dark coloured solution was stirred overnight at r.t. The mixture was washed twice with water, dried over Na_2SO_4 and evaporated to dryness. For purification the crude product was run on a short column containing silica gel. Compound **6b** was eluted with pure heptane yielding colourless oil (5.36 g, 94%). $C_{10}H_{19}Br$ requires C, 54.80; H, 8.74; found: C, 54.96; H, 8.66%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.01 (d, J = 6.6 Hz, 3 H, CH_3), 1.15–1.51 (m, 8 H, CH_2), 1.70–1.86 (m, 1 H, CH), 1.99–2.14 (m, 2 H, H_2C=CHCH_2), 3.30–3.42 (m, 2 H, BrCH_2), 4.89–5.05 (m, 2 H, H_2C=CH), 5.81 (ddt, $^2J_{[Z]}$ = 17.0 Hz, $^2J_{[E]}$ = 10.2 Hz, ^3J = 6.7 Hz, 1 H, H_2C=CH); ^{13}C NMR (100 MHz; $CDCl_3$) δ 18.8 (*C*H$_3$), 26.7 (*C*H$_2$CH$_2$CHCH$_3$), 28.8 and 29.1 (H$_2$C=CHCH$_2$*C*H$_2$*C*H$_2$), 33.7, 34.8, 35.1 (*C*H), 41.5 (*C*H$_2$Br), 114.2 (H$_2$*C*=CH), 139.0 (H$_2$C=*C*H); EI-MS m/z 218/220 (2%, M).

Synthesis of methyl-branched bromoalkanes – the citronellyl bromide pathway

The synthetic procedures and analytical data of compounds **9–12**, **13a–b**, and **14a–b** can be found in the ESI.†

Synthesis of diglycerol tetraethers – reaction pathway I

The synthetic procedures and analytical data of compounds **16a–b** and **17a–b** can be found in the ESI.†

General procedure for the synthesis of 3-*O*-alkyl-1-*O*-benzyl-*sn*-glycerols 18

In a dried and argon-flashed flask were placed 3-*O*-alkyl-*sn*-glycerols **17** (10 mmol), a mixture of dry toluene–MeOH (40 mL; 10/1, v/v), a powdered molecular sieve (4 g) and dibutyl tin oxide (2.89 g, 11.6 mmol). The mixture was heated under reflux for 1 h and then filtered. The filtrate was evaporated to dryness and the residue was suspended in dry dimethoxyethane (40 mL). Benzyl bromide (1.99 mL, 13 mmol) dissolved in dry dimethoxyethane (30 mL) was dropped into the mixture with stirring. After 30 min at r.t., the mixture was stirred for 20 h under reflux. For work up the dimethoxyethane was removed *in vacuo* and substituted by dry toluene (60 mL). After cooling, the mixture was intensively stirred with ice (40 mL) and phosphate buffer (40 mL; 0.5 M, pH = 5). The organic phase was separated and the water layer was extracted two times with toluene (20 mL). The combined organic layers were dried over K_2CO_3, filtered and evaporated to dryness. The purification was done by column chromatography using heptane–Et_2O as the eluent and the gradient technique. The desired product was eluted with a polarity of 93/7 (v/v).

1-*O*-Benzyl-3-*O*-(heptadec-16-en-1-yl)-*sn*-glycerol (18a). Following the general procedure, **17a** (3.29 g) gave **18a** (2.47 g, 58%), a white crystalline solid. M.p. 28–30 °C; $[\alpha]_{22}^{D}$ −1.5 (c 0.10 g mL^{-1}, $CHCl_3$); $C_{27}H_{46}O_3$ requires C, 77.46; H, 11.07; found: C, 77.08; H, 11.18%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.25–1.38 (m, 24 H, (CH_2)$_{12}$), 1.52–1.57 (m, 2 H, CH_2CH$_2$O), 2.00–2.05 (m, 2 H, CH_2CH=CH$_2$), 2.45 (bs, 1 H, OH), 3.36–3.62 (m, 6 H, 3× CH_2O), 3.94–3.99 (m, 1 H, CHOH), 4.54 (s, 2 H, CH_2C$_6$H$_5$), 4.90–5.01 (m, 2 H, CH=CH_2), 5.75–5.85 (m, 1 H, CH=CH$_2$), 7.12–7.35 (m, 5 H, C$_6H_5$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 26.18, 29.03, 29.23, 29.55–29.74, 33.88, 69.60, 71.50, 71.73, 71.87, 73.49, 114.09, 127.70, 128.40, 138.08, 139.21; ESI-MS m/z 436.9 (M + NH_4).

1-*O*-Benzyl-3-*O*-[(10*RS*)-10-methylheptadec-16-en-1-yl]-*sn*-glycerol (18b). Following the general procedure, **17b** (3.43 g) gave **18b** (3.3 g, 76%), slight yellow oil. $[\alpha]_{22}^{D}$ −2.0 (*c* 0.04 g mL^{-1}, $CHCl_3$); $C_{28}H_{48}O_3$ requires C, 77.72; H, 11.18; found: C, 77.39; H, 11.28%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.82 (d, *J* = 6.6 Hz, 3 H, CH_3), 1.03–1.38 (m, 23 H, (CH_2)$_7$CH(CH_2)$_4$), 1.51–1.58 (m, 2 H, CH_2CH$_2$O), 2.00–2.06 (m, 2 H, CH_2CH=CH$_2$), 2.44 (d, *J* = 4.2 Hz, 1 H, –OH). 3.41–3.56 (m, 6 H, 3× CH_2O), 3.94–4.00 (m, 1 H, CHOH), 4.55 (s, 2 H, CH_2C$_6$H$_5$), 4.89–5.00 (m, 2 H, CH=CH_2), 5.75–5.85 (m, 1 H, CH=CH$_2$), 7.19–7.35 (m, 5 H, C$_6H_5$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 19.77, 26.17, 26.97, 27.13, 29.05, 29.55–29.71, 30.07, 32.81, 33.87, 37.08, 37.15, 69.61, 71.47, 71.74, 71.83, 73.50, 114.07, 127.69, 128.40, 138.07, 139.23; EI-MS *m*/*z* 432.2 (1%, M − H).

General procedure for the synthesis of 1-*O*-benzyl-2,3-*O*,*O*-dialkyl-*sn*-glycerols 19

Compounds **19a–c** were synthesised according to the preparation of compounds **16a,b**. KH (2.45 mmol, 0.3 mL 30% suspension in mineral oil) was separated from the oil and suspended under argon in dry toluene (5 mL). A solution of **18a** or **18b** (2.45 mmol) in dry toluene (15 mL) was added slowly at r.t. and the mixture was stirred for 20 h at this temperature. Afterwards, the bromide **14a** and 1-bromohexadecane (7.6 mmol), respectively, dissolved in dry toluene (10 mL), were added and the mixture was refluxed for 12 h. Subsequently, the cooled reaction mixture was stirred vigorously with water (30 mL). Then, brine (20 mL) was added and the organic layer was separated. The water phase was extracted two times with toluene (20 mL) and the combined organic layers were dried over Na_2SO_4 and evaporated. The crude products were purified by column chromatography using heptane–$CHCl_3$ as the solvent and the gradient technique.

1-*O*-Benzyl-3-*O*-(heptadec-16-en-1-yl)-2-*O*-[(10*RS*)-10-methylhexadecyl]-*sn*-glycerol (19a). Following the general procedure, **18a** (1.02 g) and **14a** (2.43 g) gave **19a** (1.0 g, 62%), colourless oil. $[\alpha]_{22}^{D}$ +0.4 (*c* 0.094 g mL^{-1}, $CHCl_3$); $C_{44}H_{80}O_3$ requires C, 80.42; H, 12.27; found: C, 80.29; H, 12.11%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.82 (d, *J* = 6.4 Hz, 3 H, CHCH_3), 0.87 (t, *J* = 7.0 Hz, 3 H, CH$_2$CH_3), 1.05–1.38 (m, 49 H, (CH_2)$_{12}$, (CH_2)$_7$CH-(CH_2)$_5$), 1.51–1.64 (m, 4 H, 2× CH_2CH$_2$O), 1.99–2.05 (m, 2 H, CH_2CH=CH$_2$), 3.41 (t, *J* = 6.6 Hz, 2 H, CH_2O), 3.44–3.64 (m, 7 H, 3× CH_2O, CHO). 4.55 (s, 2 H, CH_2C$_6$H$_5$), 4.89–5.00 (m, 2 H, CH=CH_2), 5.75–5.85 (m, 1 H, CH=CH$_2$), 7.25–7.32 (m, 5 H, C$_6H_5$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.29, 19.89, 22.87, 26.29, 26.31, 27.22, 27.26, 29.13, 29.32, 29.68–29.85, 30.20, 30.29, 32.12, 32.93, 33.96, 37.27, 70.47, 70.70, 70.88, 71.75, 73.45, 78.05, 114.07, 127.45, 127.54, 128.26, 138.46, 139.17; EI-MS *m*/*z* 656 (1%, M − H).

1-*O*-Benzyl-2-*O*-hexadecyl-3-*O*-[(10*RS*)-10-methylheptadec-16-en-1-yl]-*sn*-glycerol (19b). Following the general procedure, **18b** (1.06 g) and 1-bromohexadecane (2.32 g) gave **19b** (1.19 g, 74%), slight yellow oil. $[\alpha]_{22}^{D}$ +1.1 (*c* 0.070 g mL^{-1}, $CHCl_3$). $C_{44}H_{80}O_3$ requires C, 80.42; H, 12.27; found: C, 80.11; H, 12.12%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.82 (d, *J* = 6.4 Hz, 3 H, CCH_3), 0.87 (t, *J* = 7.0 Hz, 3 H, CH$_2$CH_3), 1.05–1.38 (m, 49 H, (CH_2)$_7$CH(CH_2)$_4$, (CH_2)$_{13}$CH$_3$), 1.51–1.59 (m, 4 H, 2× CH_2CH$_2$O), 1.99–2.03 (m, 2 H, CH_2CH=CH$_2$), 3.41 (t, *J* = 6.6 Hz, 2 H, CH_2O), 3.47–3.59 (m, 7 H, 3× CH_2O, CHO), 4.54 (s, 2 H, CH_2C$_6$H$_5$), 4.89–4.99 (m, 2 H, CH=CH_2), 5.75–5.85 (m, 1 H, CH=CH$_2$), 7.24–7.32 (m, 5 H, C$_6H_5$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.26, 19.87, 22.84, 26.28, 26.31, 27.07, 27.25, 29.15, 29.51–29.85, 30.19, 30.29, 32.07, 32.90, 33.96, 37.17, 37.25, 70.49, 70.69, 70.89, 71.75, 73.45, 78.06, 114.07, 127.44, 127.53, 128.26, 138.48, 139.16; EI-MS *m*/*z* 656 (2%, M − H).

1-*O*-Benzyl-3-*O*-[(10*RS*)-10-methylheptadec-16-en-1-yl]-2-*O*-[(10*RS*)-methylhexadecyl]-*sn*-glycerol (19c). Following the general procedure, **18b** (1.06 g) and **14a** (2.43 g) gave **19c** (1.10 g, 67%), colourless oil. $[\alpha]_{22}^{D}$ −0.6 (*c* 0.1 g mL^{-1}, $CHCl_3$). $C_{45}H_{82}O_3$ requires C, 80.53; H, 12.32; found: C, 80.24; H, 12.40%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.82 (d, *J* = 6.4 Hz, 6 H, 2× CHCH_3), 0.87 (t, *J* = 6.8 Hz, 3 H, CH$_2$CH_3), 1.05–1.38 (m, 48 H, (CH_2)$_7$CH(CH_2)$_4$, (CH_2)$_7$CH(CH_2)$_5$CH$_3$), 1.51–1.63 (m, 4 H, 2× CH_2CH$_2$O), 2.00–2.05 (m, 2 H, CH_2CH=CH$_2$), 3.41 (t, *J* = 6.6 Hz, 2 H, CH_2O), 3.44–3.61 (m, 7 H, 3× CH_2O, CHO), 4.54 (s, 2 H, CH_2C$_6$H$_5$), 4.89–5.00 (m, 2 H, CH=CH_2), 5.75–5.85 (m, 1 H, CH=CH$_2$), 7.23–7.32 (m, 5 H, C$_6H_5$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.29, 19.88, 19.90, 22.87, 26.30, 26.32, 27.08, 27.23, 27.26, 29.16, 29.66–29.85, 30.20, 30.30, 32.12, 32.91, 32.93, 33.97, 37.19, 37.26, 37.28, 70.47, 70.70, 70.88, 71.76, 73.45, 78.06, 114.08, 127.45, 127.54, 128.27, 138.47, 139.17; EI-MS *m*/*z* 670 (5%, M − H).

General procedure for the synthesis of 3,3′-*O*,*O*-(alkane-1,1′-diyl)bis(2-*O*-alkyl-*sn*-glycerols) 20

The olefins **19a–c** (1 mmol) were dissolved in dry CH_2Cl_2 (30 mL) under an argon atmosphere. Then a solution of Grubbs first-generation catalyst {[RuCl$_2$(=CHPh)(PCy$_3$)$_2$], 29 mol%, 0.18 g} in dry CH_2Cl_2 (25 mL) was added dropwise. The mixture was stirred for 24 h under reflux. Afterwards, the solvent was removed under reduced pressure and the crude residue was purified by column chromatography using a heptane–$CHCl_3$ gradient. The subsequently performed hydrogenation of the double bonds and the removal of the benzyl blocking groups were realised in one step. Therefore, the olefins were dissolved in EtOH–EtOAc (50 mL, 1/1, v/v). After the addition of $Pd(OH)_2$ (33 mol%, 20% on carbon) the mixture was stirred under hydrogen (2 atm) at r.t. for 18 h. The catalyst was removed by filtration and washed with $CHCl_3$ several times. The combined organic solutions were evaporated. The residue was purified by column chromatography using the gradient technique and $CHCl_3$–Et_2O as the eluent to give the methyl-branched diols **20a–c**.

3,3′-*O*,*O*-(Dotriacontane-1,32-diyl)bis{2-*O*-[(10*RS*)-10-methylhexadecyl]-*sn*-glycerol} (20a). Following the general procedure, **19a** (0.66 g) gave **20a** (0.49 g, 88%), a white solid. M.p. 49 °C; $[\alpha]_{22}^{D}$ +5.5 (*c* 0.110 g mL^{-1}, $CHCl_3$). $C_{72}H_{146}O_6$ requires C, 78.05; H, 13.28; found: C, 78.34; H, 13.26%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.81 (d, *J* = 6.4 Hz, 6 H, 2× CHCH_3), 0.86 (t, *J* = 7.0 Hz, 6 H, 2× CH$_2$CH_3), 1.04–1.35 (m, 106 H, (CH_2)$_{28}$, 2× (CH_2)$_7$CH-(CH_2)$_5$CH$_3$), 1.50–1.57 (m, 8 H, 4× CH_2CH$_2$O), 2.14 (bs, 2 H, 2× OH), 3.39–3.71 (m, 18 H, 8× CH_2O, 2× CHO); ^{13}C NMR

(100 MHz; $CDCl_3$) δ 14.12, 19.74, 22.72, 26.15, 27.08, 27.11, 29.50–29.73, 30.04, 30.13, 31.97, 32.79, 37.13, 63.10, 70.39, 70.92, 71.84, 78.32; ESI-MS *m*/*z* 1130.3 (M + Na).

3,3′-*O*,*O*-[(10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diyl]-bis(2-*O*-hexadecyl-*sn*-glycerol) (20b). Following the general procedure, **19b** (0.66 g) gave **20b** (0.45 g, 82%), a white solid. M.p. 25–26 °C; $[\alpha]_{22}^{D}$ +7.2 (*c* 0.110 g mL^{-1}, $CHCl_3$); $C_{72}H_{146}O_6$ requires C, 78.05; H, 13.28; found: C, 77.71; H 13.18%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.82 (d, *J* = 6.4 Hz, 6 H, 2× $CHCH_3$), 0.87 (t, *J* = 6.8 Hz, 6 H, 2× CH_2CH_3), 1.02–1.36 (m, 106 H, $(CH_2)_7CH(CH_2)_{12}CH(CH_2)_7$, 2× $(CH_2)_{13}CH_3$), 1.51–1.58 (m, 8 H, 4× CH_2CH_2O), 1.73 (bs, 2 H, 2× O*H*), 3.40–3.73 (m, 18 H, 8× CH_2O, 2× C*H*O); ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.26, 19.86, 22.84, 26.26, 27.25, 29.50–29.89, 30.17, 30.20, 30.24, 32.07, 32.92, 37.26, 63.16, 70.47, 70.99, 71.92, 78.40; ESI-MS *m*/*z* 1130.6 (M + Na).

3,3′-*O*,*O*-[(10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diyl]-bis{2-*O*-[(10*RS*)-10-methylhexadecyl]-*sn*-glycerol} (20c). Following the general procedure, **19c** (0.67 g) gave **20c** (0.41 g, 72%), colourless oil. $[\alpha]_{22}^{D}$ +4.1 (*c* 0.025 g mL^{-1}, $CHCl_3$). $C_{74}H_{150}O_6$ requires C, 78.24; H, 13.31; found: C, 78.01; H, 13.08%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.82 (d, *J* = 6.4 Hz, 12 H, 4× $CHCH_3$), 0.87 (t, *J* = 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× $(CH_2)_7CH(CH_2)_5CH_3$), 1.52–1.57 (m, 8 H, 4× CH_2CH_2O), 3.40–3.72 (m, 18 H, 8× CH_2O, 2× C*H*O); ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.10, 19.71, 22.69, 26.12, 27.06, 27.10, 29.49, 29.63–30.10, 31.96, 32.77, 37.12, 63.01, 70.37, 70.88, 71.80, 78.36; ESI-MS *m*/*z* 1158.1 (M + Na).

Synthesis of diglycerol tetraethers – reaction pathway II

1,32-Dibromodotriacontane (22a). Compound **22a** was synthesised according to the preparation of **6b** from dotriacontane-1,32-diol (**21a**)[20] using **21a** (2.0 g, 4.1 mmol), 3,4-dihydro-2*H*-pyrane (1.25 g, 14.9 mmol) and PPTS. The crude and dried bis(tetrahydro-2*H*-pyranyl) ethers (85% yield) were subsequently reacted with triphenylphosphoranediyl dibromide prepared from Br_2 (1.8 g, 11.3 mmol) and triphenylphosphane (2.96 g, 11.3 mmol). The crude product was purified by column chromatography and heptane as the eluent yielding **22a** (2.04 g, 81% compared to **21a**, 95% compared to bis(thp) ether) as a white solid. M.p. 85–86 °C; $C_{32}H_{64}Br_2$ requires C, 63.14; H, 10.60; Br, 26.26; found: C, 62.89; H, 10.50; Br, 24.25%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.24–1.32 (m, 52 H, CH_2), 1.37–1.44 (m, 4 H, $BrCH_2CH_2CH_2$), 1.80–1.87 (m, 4 H, $BrCH_2CH_2$), 3.39 (t, *J* = 6.9 Hz, 4 H, $BrCH_2$f); ^{13}C NMR (100 MHz; $CDCl_3$) δ 28.19 ($Br(CH_2)_2CH_2$), 28.78 ($Br(CH_2)_3CH_2$), 29.45, 29.55, 29.62, 29.66, 29.68, 29.69 and 29.70 (CH_2), 32.86 ($BrCH_2CH_2$), 34.03 ($BrCH_2$).

(10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diylbismethanesulfonate (22b). (10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diol[21] (**21b**, 1.0 g, 1.9 mmol) was dissolved in dry $CHCl_3$ (50 mL). Methanesulfonyl chloride (0.89 g, 7.8 mmol) and DMAP (0.358 g, 7.8 mmol) were added and the mixture was stirred at r.t. After complete conversion of compound **21b**, water was added and the organic phase was separated, washed with water, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using heptane–$CHCl_3$ as the eluent giving compound **22b** (1.2 g, 97%) as a white solid. M.p. 57–58 °C; $C_{36}H_{74}O_6S_2$ requires C, 64.82; H, 11.18; S, 9.61; found: C, 64.85; H, 11.00; S, 9.48%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.81 (d, *J* = 6.6 Hz, 6 H, 2× $CHCH_3$), 1.02–1.38 (m, 54 H, CH_2 and C*H*), 1.69–1.76 (m, 4 H, CH_2CH_2O), 2.98 (s, 6 H, SCH_3), 4.20 (t, *J* = 6.6 Hz, 4 H, CH_2O); ^{13}C NMR (100 MHz; $CDCl_3$) δ 19.71 ($CHCH_3$), 25.42 ($CH_2(CH_2)_2O$), 27.06 and 27.10 ($CH_2CH_2CH(CH_3)CH_2CH_2$), 29.04, 29.14, 29.44, 29.56, 29.71, 29.74, 29.96 and 30.05 (CH_2), 32.77 (*C*H), 37.10 and 37.11 ($CH_2CH(CH_3)CH_2$), 37.38 (SCH_3), 63.09 (CH_2O); ESI-MS *m*/*z* 689.43 (M + Na), 1355.19 (2M + Na).

General procedure for the synthesis of 3,3′-*O*,*O*-(alkane-1,1′-diyl)bis(1,2-*O*-isopropylidene-*sn*-glycerols) 23

The suspension of KH (10 mmol, 30%) was separated from paraffin oil by washing with dry toluene under argon. The residue of KH was suspended in dry toluene (10 mL). A solution of 1,2-*O*-isopropylidene-*sn*-glycerol (**15**; 1.32 g, 10 mmol) in dry toluene (10 mL) was dropped into the slurry whilst stirring. The mixture was stirred for a further 18 h at r.t. until the K-salt formation was complete. Afterwards, compounds **22** (2.5 mmol), dissolved in dry toluene (10 mL), were added and the mixture was heated for 20 h under reflux. After cooling to r.t., the suspension was poured into a cold saturated NH_4Cl solution (30 mL). The organic layer was separated and the aqueous phase was extracted two times with $CHCl_3$ (20 mL). The combined organic layers were dried over Na_2SO_4, evaporated and purified by middle pressure liquid chromatography (MPLC) using $CHCl_3$–Et_2O as the eluent and the gradient technique to give the compounds **23a** and **23b**.

3,3′-*O*,*O*-(Dotriacontane-1,32-diyl)bis(1,2-isopropylidene-*sn*-glycerol) (23a). Following the general procedure, **15** (1.32 g) and **22a** (1.52 g) gave **23a** (1.0 g, 56%), a white solid. M.p. 74–76 °C; $C_{44}H_{86}O_6$ requires C, 74.31; H, 12.19; found: C, 73.77; H, 12.19%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.25–1.33 (m, 56 H, $O(CH_2)_2(CH_2)_{28}(CH_2)_2O$), 1.36 and 1.42 (2 s, 2× 6 H, CH_3), 1.53–1.60 (m, 4 H, $OCH_2CH_2(CH_2)_{28}CH_2CH_2O$), 3.39–3.53 (m, 8 H, $CH_2OCH_2(CH_2)_{30}CH_2OCH_2$), 3.73 (dd, ^2J = 8.2 Hz, ^3J = 6.4 Hz, 2 H, 2× OCH_2CH), 4.06 (dd, ^2J = 8.2 Hz, ^3J = 6.4 Hz, 2 H, 2× OCH_2CH), 4.23–4.29 (m, 2 H, C*H*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 25.43, 26.06 and 26.78 (CH_3, $O(CH_2)_2CH_2$), 29.47, 29.56, 29.60, 29.61, 29.67, 29.69 and 29.70 (CH_2), 66.96 (CH_2CHCH_2O-alkyl), 71.82 and 71.89 ($CH_2OCH_2(CH_2)_{30}CH_2OCH_2$), 74.79 (*C*H), 109.34 (*C*); ESI-MS *m*/*z* 733.51 (M + Na), 693.50 (M – IP + Na), 653.49 (M – 2IP + Na).

3,3′-*O*,*O*-[(10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diyl]-bis(1,2-isopropylidene-*sn*-glycerol) (23b). Following the general procedure, **15** (1.32 g) and **22b** (1.67 g) gave **23b** (0.79 g, 43%), pale yellow oil. $C_{46}H_{90}O_6$ requires C, 74.74; H, 12.27; found: C, 74.58; H, 12.21%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.81 (d, ^3J = 6.8 Hz, 6 H, 2× $CHCH_3$), 1.01–1.10 and 1.20–1.31 (2 m, 54 H, CH_2, $CHCH_3$), 1.34 and 1.40 (2 s, 2× 6 H, 4× CCH_3), 1.51–1.58 (m, 4 H, OCH_2CH_2-alkyl-CH_2CH_2O), 3.37–3.51 (m, 8 H, CH_2OCH_2-alkyl-CH_2OCH_2), 3.70 (dd, ^2J = 8.4 Hz, ^3J = 6.8 Hz, 2 H, 2× OCH_2CH), 4.03 (dd, ^2J = 8.4 Hz, ^3J = 6.8 Hz, 2 H,

2× OCH_2CH), 4.21–4.27 (m, 2 H, OCH); ^{13}C NMR (100 MHz; $CDCl_3$) δ 19.71 (CHCH$_3$), 25.43, 26.06 and 26.77 (CCH$_3$, O(CH$_2$)$_2$$CH_2$), 27.08 and 27.10 ($CH_2CH_2$CH(CH$_3$)CH$_2$$CH_2$), 29.47, 29.56, 29.59, 29.60, 29.65, 29.71, 29.74, 30.01 and 30.04 (CH$_2$), 32.76 (CHCH$_3$), 37.10 (CH$_2$CH(CH$_3$)CH$_2$), 66.96 (CH$_2$CHCH$_2$O-alkyl-OCH$_2$CHCH$_2$), 71.82 and 71.89 (CH$_2$OCH$_2$-alkyl-CH$_2$OCH$_2$), 74.76 (OCH), 109.32 (C); ESI-MS m/z 761.69 (M + Na).

General procedure for the synthesis of 3,3′-*O*,*O*-(alkane-1,1′-diyl)bis(*sn*-glycerols) 24

The IP-protected glycerols **23** and catalytic amounts of PPTS were dissolved in dry MeOH (50–100 mL) and heated for 3 h at reflux. The products crystallised while cooling the suspension. After filtration, compounds **24** were purified by recrystallization from heptane–MeOH.

3,3′-*O*,*O*-(Dotriacontane-1,32-diyl)bis(*sn*-glycerol) (24a). Following the general procedure, **23a** (1.23 g) gave **24a** (1.02 g, 93%), a white solid. M.p. 117–119 °C; $C_{38}H_{78}O_6$ requires C, 72.33; H, 12.46; found: C, 72.34; H, 12.82%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.23–1.29 (m, 56 H, O(CH$_2$)$_2$(CH$_2$)$_{28}$(CH$_2$)$_2$O), 1.45–1.58 (m, 4 H, OCH$_2$CH_2(CH$_2$)$_{28}$CH_2CH$_2$O), 3.43–3.54 (m, 8 H, CH_2OCH_2(CH$_2$)$_{30}$CH_2OCH_2), 3.63 (dd, ^2J = 11.4 Hz, ^3J = 5.4 Hz, 2 H, 2× HOCH_2CH), 3.70 (dd, ^2J = 11.4 Hz, ^3J = 4.0 Hz, 2 H, 2× HOCH_2CH), 3.81–3.86 (m, 2 H, CH); ESI-MS m/z 653.55 (M + Na).

3,3′-*O*,*O*-[(10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diyl]-bis(*sn*-glycerol) (24b). Following the general procedure, **23b** (480 mg) gave **24b** (360 mg, 85%), a white solid. M.p. 50–51 °C; $C_{40}H_{82}O_6$ requires C, 72.89; H, 12.54; found: C, 72.41; H, 12.87%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.81 (d, J = 6.8 Hz, 6 H, 2× CHCH_3), 1.02–1.10 and 1.18–1.33 (2 m, 54 H, CH_2, CHCH$_3$), 1.52–1.59 (m, 4 H, OCH$_2$CH_2-alkyl-CH_2CH$_2$O), 3.42–3.55 (m, 8 H, CH_2OCH_2-alkyl-CH_2OCH_2), 3.63 (dd, ^2J = 11.4 Hz, ^3J = 5.2 Hz, 2 H, 2× HOCH_2CH), 3.70 (dd, ^2J = 11.4 Hz, ^3J = 3.6 Hz, 2 H, 2× HOCH_2CH), 3.82–3.86 (m, 2 H, OCH); ESI-MS m/z 657.70 (M – H), 659.52 (M + H), 681.61 (M + Na).

General procedure for the synthesis of 3,3′-*O*,*O*-(alkane-1,1′-diyl)bis(1-*O*-trityl-*sn*-glycerols) 25

Trityl chloride (2.5 equiv.), glycerol **24a** or **24b** (1 equiv.), and DMAP (10 mol%) were suspended in dry pyridine (7–20 mL). The suspension was stirred for at least 3 d at r.t. After complete conversion of the glycerol compounds, water (10–20 mL) was added to hydrolyse the excess trityl chloride. After the addition of further water (~20 mL), the organic layer was separated, the aqueous phase was extracted with $CHCl_3$ and the combined organic layers were washed with brine. After drying with Na_2SO_4 and removing the solvent, the raw product was purified by MPLC using $CHCl_3$–heptane and 0.5% TEA as the eluent and the gradient technique to give compounds **25a** and **25b**.

3,3′-*O*,*O*-(Dotriacontane-1,32-diyl)bis(1-*O*-trityl-*sn*-glycerol) (25a). Following the general procedure, **24a** (0.92 g, 1.46 mmol), trityl chloride (1.02 g, 3.64 mmol), and DMAP (18 mg) in pyridine (20 mL) gave **25a** (0.81 g, 50%), a white solid. M.p. 68–70 °C; $C_{76}H_{106}O_6$ requires C, 81.82; H, 9.58; found: C, 81.96; H, 9.38%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.24–1.31 (m, 56 H, CH_2), 1.49–1.56 (m, 4 H, OCH$_2$CH_2(CH$_2$)$_{28}$CH_2CH$_2$O), 2.39–2.40 (bm, 2 H, 2× OH), 3.14–3.21 (m, 4 H, 2× CH_2O), 3.37–3.53 (m, 8 H, 4× CH_2O), 3.89–3.96 (m, 2 H, OCH), 7.19–7.30 and 7.40–7.43 (2 m, 30 H, 6× C$_6$H$_5$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 26.11 (O(CH$_2$)$_2$$CH_2$), 29.51, 29.61, 29.64, 29.67, 29.69, 29.70 and 29.72 (CH$_2$), 31.88 (OCH$_2$$CH_2$), 64.64 ($CH_2$OTr), 69.87, 71.65 and 72.04 (CHCH$_2$OCH$_2$), 86.64 (C), 127.03 (C-4, C_6H$_5$), 127.81 and 128.67 (C-2, C-3, C-5, C-6, C_6H$_5$), 143.89 (C-1, C_6H$_5$); ESI-MS m/z 1113.79 (M – H), 1137.78 (M + Na).

3,3′-*O*,*O*-[(10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diyl]-bis(1-*O*-trityl-*sn*-glycerol) (25b). Following the general procedure, **24b** (0.20 g, 0.30 mmol), trityl chloride (0.21 g, 0.76 mmol), and DMAP (5 mg) in pyridine (7 mL) gave **25b** (0.24 g, 70%), colourless oil. $C_{78}H_{110}O_6$ requires C, 81.91; H, 9.695; found: C, 81.84; H, 9.71%; ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.82 (d, J = 6.6 Hz, 6 H, 2× CHCH_3), 1.02–1.10 and 1.17–1.38 (2 m, 54 H, CH_2, CHCH$_3$), 1.49–1.58 (m, 4 H, OCH$_2$CH_2-alkyl-CH_2CH$_2$O), 2.38–2.40 (bm, 2 H, 2× OH), 3.14–3.21 (m, 4 H, 2× CH_2O), 3.38–3.53 (m, 8 H, 4× CH_2O), 3.89–3.96 (m, 2 H, OCH), 7.19–7.31 and 7.40–7.43 (2 m, 30 H, 6× C$_6$H$_5$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 19.72 (CHCH$_3$), 26.11 (O(CH$_2$)$_2$$CH_2$), 27.10 ($CH_2CH_2$CH(CH$_3$)CH$_2$$CH_2$), 29.47, 29.52, 29.60, 29.62, 29.66, 29.67, 29.69, 29.72, 29.75, 30.02, 30.04 and 30.06 (CH$_2$), 32.78 (CH(CH$_3$)), 37.13 (CH$_2$CH(CH$_3$)CH$_2$), 64.64 (CH$_2$OTr), 69.87, 71.65 and 72.05 (CHCH$_2$OCH$_2$), 86.64 (C), 127.03 (C-4, C_6H$_5$), 127.81 and 128.67 (C-2, C-3, C-5, C-6, C_6H$_5$), 143.89 (C-1, C_6H$_5$); ESI-MS m/z 1141.88 (M – H), 1166.15 (M + Na).

General procedure for the synthesis of 3,3′-*O*,*O*-(alkane-1,1′-diyl)bis(2-*O*-alkyl-1-*O*-trityl-*sn*-glycerols) 26

Compounds **26a,b** were synthesised according to the synthesis of compounds **16a,b** – a second *O*-alkylation on the glycerol moiety. KH (2.5 fold excess, 30% suspension in mineral oil) was separated from the oil and suspended under argon in dry toluene (2 mL). A solution of **25a** or **25b** in dry toluene (10 mL) was added at r.t. and the mixture was stirred for 20 h at this temperature and, in addition, for 2 h at 40 °C. Afterwards, the bromide **14a** (2.5 fold excess), dissolved in dry toluene (2 mL), was added and the mixture was refluxed for 12 h. Subsequently, saturated NH_4Cl solution (15 mL) was added and the organic layer was separated. The water phase was extracted two times with Et_2O (15 mL) and the combined organic layers were dried over Na_2SO_4 and evaporated. The crude products were purified by MPLC using heptane as the solvent to give compounds **26a** and **26b**.

3,3′-*O*,*O*-(Dotriacontane-1,32-diyl)bis{2-*O*-[(10*RS*)-10-methylhexadecyl]-1-*O*-trityl-*sn*-glycerol} (26a). Following the general procedure, **25a** (150 mg, 0.134 mmol), KH (13.4 mg, 0.335 mmol, 50 μL), and **14a** (107 mg, 0.335 mmol) gave **26a** (100 mg, 47%), colourless oil. $C_{110}H_{174}O_6$ requires C, 82.96; H, 11.01; found: C, 82.87; H, 10.96%; ^{1}H NMR (500 MHz; $CDCl_3$) δ 0.86 (d, J = 6.5 Hz, 6 H, 2× CHCH_3), 0.90 (t, J = 6.9 Hz, 6 H, 2× CH$_2$CH_3), 1.07–1.13 and 1.23–1.37 (2 m, 106 H, CH_2, CH), 1.49–1.61 (m, 8 H, 4× OCH$_2$CH_2-alkyl), 3.17–3.22, 3.40–3.43

and 3.50–3.60 (3 m, 18 H, CH_2O, OCH), 7.22–7.31 and 7.47–7.49 (2 m, 30 H, 6× C$_6H_5$); ^{13}C NMR (125 MHz; $CDCl_3$) δ 14.12 (CH_2CH_3), 19.73 ($CHCH_3$), 22.71 (CH_2CH_3), 26.13 and 26.19 ($O(CH_2)_2CH_2$-alkyl-$CH_2(CH_2)_2O$), 27.07 and 27.11 ($O(CH_2)_2CH_2(CH_2)_6CH(CH_3)(CH_2)_5CH_3$), 29.54, 29.57, 29.65, 29.68, 29.70 and 29.74 (CH_2), 30.03 and 30.17 ($OCH_2CH_2(CH_2)_7$ $CH(CH_3)(CH_2)_5CH_3$), 31.97 (OCH_2CH_2-alkyl-CH_2CH_2O), 32.78 ($CHCH_3$), 37.13 ($CH_2CH(CH_3)CH_2$), 63.66 (CH_2OTr), 70.71, 71.22 and 71.62 (CH_2O), 78.34 (CHO), 86.52 (C), 126.87 (C-4, C_6H_5), 127.70 and 128.77 (C-2, C-3, C-5, C-6, C_6H_5), 144.19 (C-1, C_6H_5); ESI-MS m/z 818.87 (M + 2Na), 1615.15 (M + Na).

3,3′-*O*,*O*-[(10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diyl]-bis{2-*O*-[(10*RS*)-10-methylhexadecyl]-1-*O*-trityl-*sn*-glycerol} (26b). Following the general procedure, **26a** (210 mg, 0.184 mmol), KH (18.4 mg, 0.459 mmol, 60 μL), and **14a** (147 mg, 0.459 mmol) gave **26b** (170 mg, 57%), colourless oil. $C_{112}H_{178}O_6$ requires C, 83.00; H, 11.07; found: C, 82.67; H, 11.20%; ^{1}H NMR (500 MHz; $CDCl_3$) δ 0.88 (d, J = 6.5 Hz, 12 H, 4× $CHCH_3$), 0.92 (t, J = 6.9 Hz, 6 H, 2× CH_2CH_3), 1.09–1.13 and 1.24–1.39 (2 m, 104 H, CH_2, CH), 1.52–1.63 (m, 8 H, 4× OCH_2CH_2-alkyl), 3.18–3.24, 3.40–3.47 and 3.51–3.62 (3 m, 18 H, CH_2O, OCH), 7.23–7.32 and 7.49–7.51 (2 m, 30 H, 6× C$_6H_5$); ^{13}C NMR (125 MHz; $CDCl_3$) δ 14.14 (CH_2CH_3), 19.74 and 19.75 ($CHCH_3$), 22.72 (CH_2CH_3), 26.15 and 26.20 ($O(CH_2)_2CH_2$-alkyl-$CH_2(CH_2)_2O$), 27.08 and 27.13 ($O(CH_2)_2CH_2(CH_2)_6CH(CH_3)$ $(CH_2)_5CH_3$), 29.57, 29.59, 29.68, 29.71, 29.73, 29.75 and 29.79 (CH_2), 30.08 and 30.18 ($OCH_2CH_2(CH_2)_7CH(CH_3)(CH_2)_5CH_3$), 31.99 ($OCH_2CH_2$-alkyl-$CH_2CH_2O$), 32.80 and 32.81 ($CHCH_3$), 37.15 and 37.17 ($CH_2CH(CH_3)CH_2$), 63.68 ($CH_2$OTr), 70.72, 71.23 and 71.63 (CH_2O), 78.36 (CHO), 86.54 (C), 126.88 (C-4, C_6H_5), 127.71 and 128.78 (C-2, C-3, C-5, C-6, C_6H_5), 144.21 (C-1, C_6H_5); ESI-MS m/z 832.91 (M + 2Na), 1643.18 (M + Na).

General procedure for detritylation

The detritylation reaction is based on a procedure described by Hermetter and Paltauf:[22] the trityl blocked compounds were suspended in dry MeOH. After the addition of methanolic solution of BF_3–Et_2O, the mixture was stirred at r.t. until the educt disappeared (TLC). For the work-up, Et_2O and water were added, the aqueous phase was neutralised, and the organic phase was separated, washed with water, dried over Na_2SO_4 and evaporated. The crude products were purified by column chromatography using heptane–$CHCl_3$ as the eluent and the gradient technique.

Following the general procedure, compounds **20a** and **20c** were synthesised from **26a** and **26b** in a quantitative manner (>95% yield). The analytical data of **20a** and **20c** are in accordance with data described above.

General procedure for the synthesis of bis(phosphocholines) I–III

For the synthesis of the final bis(phosphocholines) **I–III** we used phosphorylation of the diols **20** (0.15 mmol) with 2-bromoethylphosphoric acid dichloride (1.2 mmol) and a subsequent quarternisation with trimethylamine (1.5 mmol) as described previously in detail.[14,20] The crude bis(phosphocholines) were purified by column chromatography using $CHCl_3$–MeOH–water as the eluent and the gradient technique to give lipids **I–III**.

3,3′-*O*-(Dotriacontane-1,32-diyl)bis({2-*O*-[(10*RS*)-10-methylhexadecyl]-*sn*-glycer-1-yl}-2-(trimethylammonio)ethyl phosphate) (I). Following the general procedure, **20a** (166 mg) gave lipid **I** (88 mg, 41%), a white solid. M.p. 217–219 °C; $C_{82}H_{170}N_2O_{12}P_2 \times 2H_2O$ requires C, 66.81; H, 11.90; N 1.90; found: C, 66.47; H, 11.91; N, 1.89%; ^{1}H NMR (400 MHz; $CDCl_3$–CD_3OD) δ 0.79 (d, J = 6.6 Hz, 6 H, 2× $CHCH_3$), 0.83 (t, J = 7.0 Hz, 6 H, 2× CH_2CH_3), 1.00–1.33 (m, 106 H, $(CH_2)_{28}$, 2× $(CH_2)_7CH(CH_2)_5CH_3$), 1.45–1.52 (m, 8 H, 4× CH_2CH_2O), 3.21 (s, 18 H, 2× $N(CH_3)_3$), 3.35–3.57 (m, 14 H, 6× CH_2O, 2× CHO), 3.60–3.62 (m, 4 H, 2× NCH_2CH_2OP), 3.83–3.86 (m, 4 H, 2× $POCH_2CH$), 4.18–4.25 (m, 4 H, 2× NCH_2CH_2OP); ^{13}C NMR (100 MHz; $CDCl_3$–CD_3OD) δ 14.15, 19.75, 22.74, 26.14, 27.10, 27.18, 29.52–29.77, 30.12, 30.20, 31.99, 32.82, 37.16, 37.17, 54.52, 59.11, 59.15, 65.16, 65.21, 66.54, 70.54, 71.73, 77.94, 78.02; ESI-MS m/z 1438.0 (M + H), 1460.9 (M + Na); HRMS m/z calcd for $C_{82}H_{170}N_2O_{12}P_2Na_2$ (M + 2Na) 741.6007; found: 741.6034; HPLC t_R 3.92 min, purity 99.0%.

3,3′-*O*-[(10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diyl]-bis[(2-*O*-hexadecyl-*sn*-glycer-1-yl)-2-(trimethylammonio)ethyl phosphate] (II). Following the general procedure, **20b** (166 mg) gave lipid **II** (60 mg, 28%), a white waxy solid. M.p. 215–218 °C; ^{1}H NMR (400 MHz; $CDCl_3$–CD_3OD) δ 0.82 (d, J = 6.4 Hz, 6 H, 2× $CHCH_3$), 0.87 (t, J = 7.0 Hz, 6 H, 2× CH_2CH_3), 1.04–1.30 (m, 106 H, $(CH_2)_7CH(CH_2)_{12}CH(CH_2)_7$, 2× $(CH_2)_{13}CH_3$), 1.49–1.55 (m, 8 H, 4× CH_2CH_2O), 3.23 (s, 18 H, 2× $N(CH_3)_3$), 3.37–3.65 (m, 18 H, 6× CH_2O, 2× CHO, 2× NCH_2CH_2OP), 3.89–3.92 (m, 4 H, 2× $POCH_2CH$), 4.24–4.29 (m, 4 H, 2× NCH_2CH_2OP); ^{13}C NMR (100 MHz; $CDCl_3$–CD_3OD) δ 14.05, 19.74, 22.66, 26.08, 26.97, 27.05, 29.33, 29.54–30.08, 31.89, 32.67, 37.01, 54.38, 59.44, 65.42, 66.56, 70.40, 70.55, 71.70; 77.44; ESI-MS m/z 1438.1 (M + H); HRMS m/z calcd for $C_{82}H_{172}N_2O_{12}P_2$ (M + 2H) 719.6187; found: 719.6197, calcd for $C_{82}H_{171}N_2O_{12}P_2$ (M + H) 1438.2302; found: 1438.2336; HPLC t_R 3.92 min, purity 99.2%.

3,3′-*O*-[(10*RS*,23*RS*)-10,23-Dimethyldotriacontane-1,32-diyl]-bis({2-*O*-[(10*RS*)-10-methylhexadecyl]-*sn*-glycer-1-yl}-2-(trimethylammonio)ethyl phosphate) (III). Following the general procedure, **20c** (170 mg) gave lipid **III** (84 mg, 38%), a white waxy solid. M.p. 205–209 °C; ^{1}H NMR (400 MHz; $CDCl_3$–CD_3OD) δ 0.82 (d, J = 6.4 Hz, 12 H, 4× $CHCH_3$), 0.86 (t, J = 7.0 Hz, 6 H, 2× CH_2CH_3), 1.02–1.34 (m, 104 H, $(CH_2)_7CH(CH_2)_{12}CH(CH_2)_7$, 2× $(CH_2)_7CH(CH_2)_5CH_3$), 1.49–1.55 (m, 8 H, 4× CH_2CH_2O), 3.23 (s, 18 H, 2× $N(CH_3)_3$), 3.38–3.64 (m, 18 H, 6× CH_2O, 2× CHO, 2× NCH_2CH_2OP), 3.87–3.89 (m, 4 H, 2× $POCH_2CH$), 4.22–4.26 (m, 4 H, 2× NCH_2CH_2OP); ^{13}C NMR (100 MHz; $CDCl_3$–CD_3OD) δ 14.09, 19.70, 19.76, 22.70, 26.09, 26.12, 27.03, 27.07, 27.09, 27.14, 29.56–30.15, 31.97, 32.73, 32.80, 37.05, 37.08, 37.14, 37.15, 54.47, 58.91, 58.96, 65.06, 65.11, 66.59, 70.58, 70.62, 71.76, 78.00 78.08; ESI-MS m/z 1466.3 (M + H); HRMS m/z calcd for $C_{84}H_{176}N_2O_{12}P_2$

(M + 2H) 733.6344; found: 733.6356, calcd for $C_{84}H_{175}N_2O_{12}P_2$ (M + H) 1466.2615; found: 1466.2658; HPLC t_R 3.91 min, purity 97.7%.

DSC. DSC measurements were performed using a MicroCal VP-DSC differential scanning calorimeter (MicroCal Inc. Northampton, MA, USA). The samples were prepared by mixing 1 mg of lipids **I–III** and 1 mL of water (MilliQ), subsequent heating to 90 °C, vortexing and treatment with ultrasound. Before measurements, the sample suspension and the water reference were degassed under vacuum while stirring. A heating rate of 20 K h^{-1} was used, and the measurements were performed in the temperature interval from 2 to 95 °C. To check the reproducibility, three consecutive scans were recorded. The water–water baseline was subtracted from the thermogram of the sample and the DSC scans were evaluated using the MicroCal Origin 8.0 software.

TEM. The negative stained samples were prepared by spreading 5 μL of the lipid suspension (c 0.03–0.10 mg mL^{-1}) onto a copper grid coated with a Formvar film. After 1 min, excess liquid was blotted off with filter paper and 5 μL of 1% aqueous uranyl acetate solution were placed onto the grid and drained off again after 1 min. For the samples prepared below ambient temperature, all components were stored (for at least 24 h) and prepared in a cold room (4 °C). The samples were dried for 2 days at 4 °C and kept in an exsiccator at ambient temperature until the images were recorded. All specimens were examined with a Zeiss EM 900 transmission electron microscope (Carl Zeiss NTS GmbH, Oberkochen, Germany).

DLS. DLS experiments were carried out with a Zetasizer Nano-ZS ZEN3600 (Malvern Instruments Ltd., Worcestershire, GB) with a sample refractive index of 1.33 and a viscosity of 0.8872 MPa at 25 °C. The device was equipped with a 4 mW HeNe laser operating at a wavelength of 633 nm and a scattering angle of 173°. Liposomes were prepared from aqueous lipid suspensions (c 1 mg mL^{-1}) using the technique described by Bangham *et al.*[29] After treatment of sample suspensions with ultrasound the liposomes were extruded through 100 nm polycarbonate membranes. Before starting the measurement, each sample was equilibrated for 15 min. Three individual measurements were performed for each system in order to test the reproducibility with one measurement consisting of 10 runs of 10 seconds each. The experimental data were evaluated using the Zetasizer software (version 6.34, Malvern Instruments Ltd; z-average and PDI values) or with the aid of the ALV-correlator (ALV-5000/E, Langen, Germany) software taking into account the temperature correction of viscosity (see ESI†).

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References

1 T. A. Langworthy, *Biochim. Biophys. Acta, Lipids Lipid Metab.*, 1977, **487**, 37–50.
2 M. De Rosa, E. Esposito, A. Gambacorta, B. Nicolaus and J. D. Bu'Lock, *Phytochemistry*, 1980, **19**, 827–831.
3 B. A. Cornell, V. B. L. Braach-Maksvytis, L. G. King, P. D. Osmann, B. Raguse, L. Wieczorek and R. J. Pace, *Nature*, 1997, **387**, 580–583.
4 U. Bakowsky, U. Rothe, E. Antonopoulos, T. Martini, L. Henkel and H. J. Freisleben, *Chem. Phys. Lipids*, 2000, **105**, 31–42.
5 T. Benvegnu, G. Réthoré, M. Brard, W. Richter and D. Plusquellec, *Chem. Commun.*, 2005, 5536–5538.
6 G. P. Patel, A. Ponce, H. Zhou and W. Chen, *Int. J. Toxicol.*, 2008, **27**, 329–339.
7 D. A. Brown, B. Venegas, P. H. Cooke, V. English and P. L.-G. Chong, *Chem. Phys. Lipids*, 2009, **159**, 95–103.
8 L. Krishnan, L. Deschatelets, F. C. Stark, K. Gurnani and G. D. Sprott, *Clin. Dev. Immunol.*, 2010, **2010**, 1–13.
9 T. Eguchi, K. Ibaragi and K. Kakinuma, *J. Org. Chem.*, 1998, **63**, 2689–2698.
10 K. Yamauchi, Y. Sakamoto, A. Moriya, K. Yamada, T. Hosokawa, T. Higuchi and M. Kinoshitat, *J. Am. Chem. Soc.*, 1990, **112**, 3188–3191.
11 K. Yamauchi, A. Moriya and M. Kinoshita, *Biochim. Biophys. Acta*, 1989, **1003**, 151–160.
12 M. Brard, C. Lainé, G. Réthoré, I. Laurent, C. Neveu, L. Lemiègre and T. Benvegnu, *J. Org. Chem.*, 2007, **72**, 8267–8279.
13 M. Brard, W. Richter, T. Benvegnu and D. Plusquellec, *J. Am. Chem. Soc.*, 2004, **126**, 10003–10012.
14 T. Markowski, S. Drescher, A. Meister, G. Hause, A. Blume and B. Dobner, *Eur. J. Org. Chem.*, 2011, 5894–5904.
15 D. Blöcher, R. Gutermann, B. Henkel and K. Ring, *Biochim. Biophys. Acta*, 1984, **778**, 74–80.
16 M. Schwarz, J. E. Oliver and P. E. Sonnet, *J. Org. Chem.*, 1975, **40**, 2410–2411.
17 M. Tamura and J. Kochi, *Synthesis*, 1971, 303–305.
18 P. Schwab, M. B. France, J. W. Ziller and R. H. Grubbs, *Angew. Chem.*, 1995, **107**, 2179–2181.
19 F. Bauer, K.-P. Reuß and M. Liefländer, *Liebigs Ann. Chem.*, 1992, 47–50.
20 S. Drescher, A. Meister, A. Blume, G. Karlsson, M. Almgren and B. Dobner, *Chem. – Eur. J.*, 2007, **13**, 5300–5307.
21 U. F. Heiser, R. Wolf and B. Dobner, *Chem. Phys. Lipids*, 1997, **90**, 25–30.
22 A. Hermetter and F. Paltauf, *Chem. Phys. Lipids*, 1981, **29**, 191–195.

23 H. Eibl and A. Nicksch, *Ger. Offen.*, DE 2345057 A1 19750327, 1975 (CAN:83:9233).
24 G. Hirt and R. Berchthold, *Pharm. Acta Helv.*, 1958, **33**, 349–356.
25 O. Dannenmuller, K. Arakawa, T. Eguchi, K. Kakinuma, S. Blanc, A.-M. Albrecht, M. Schmutz, Y. Nakatani and G. Ourisson, *Chem. – Eur. J.*, 2000, **6**, 645–654.
26 K. Miyawaki, T. Takagi and M. Shibakami, *Synlett*, 2002, 1326–1328.
27 P. Nuhn, G. Brezesinski, B. Dobner, G. Förster, M. Gutheil and H.-D. Dörfler, *Chem. Phys. Lipids*, 1986, **39**, 221–236.
28 F. M. Menger, M. G. Wood, Q. Z. Zhou, H. P. Hopkins and J. Fumero, *J. Am. Chem. Soc.*, 1988, **110**, 6804–6810.
29 A. D. Bangham, M. M. Standish and J. C. Watkins, *J. Mol. Biol.*, 1965, **13**, 238–252.
30 W. R. Redwood, F. R. Pfeiffer, J. A. Weisbach and T. E. Thompson, *Biochim. Biophys. Acta*, 1971, **233**, 1–6.