Organic & Biomolecular Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: K. Gruhle, S. Müller, A. Meister and S. Drescher, *Org. Biomol. Chem.*, 2018, DOI: 10.1039/C8OB00424B.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/obc

View Article Online
DOI: 10.1039/C8OB00424B

Organic & Biomolecular Chemistry

ARTICLE

Synthesis and aggregation behaviour of single-chain, 1,32-alkyl branched bis(phosphocholines): effect of lateral chain length†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

K. Gruhle,[a] S. Müller,[a] A. Meister,[b] and S. Drescher*[a]

Three novel single-chain bis(phosphocholines) bearing two lateral alkyl chains of variable length next to the headgroup have been synthesized as model lipids for natural occuring archaeal membrane lipids. The synthesis was realized using the Cu-catalyzed Grignard *bis*-coupling reaction of a primary bromide as side part and a 1,ω-dibromide as centre part. We could show that the aggregation behaviour of the resulting bolalipids strongly depends on the length of the lateral alkyl chain: the C3-branched bolalipid self-assembles into lamellar sheets, whereas the C6- and C9-analogue forms nanofibres. The lamella-forming bolalipids could be used in the future to prepare stable and tailored liposomes for oral drug delivery.

Introduction

Bolaamphiphiles (or bolalipids) consist of a long hydrophobic part, mainly one or two membrane-spanning (transmembrane) alkyl chains, and two hydrophilic headgroups bound to both ends of the alkyl spacer.[1] These bipolar lipids can be found in the membranes of certain species of archaea, *e.g.*, *thermoacidophiles*, where they are responsible for the great stability of archaea against extreme living conditions, such as high temperatures and/or low pH-values.[2-4] This stability is reasoned in the chemical structure of archaeal bolalipids that differs from the structure of membrane lipids from eukaryotes and bacteria in three important aspects:[5,6] first, the glycerol backbone is in the invers *sn*-2,3 configuration; second, the hydrophobic parts (fatty alcohols) are bound via ether bond to the glycerol; and third, the fatty alcohols contain isoprenoid-branched methyl groups and/or a variable number of 1,3-bound cyclopentane rings.[2,3,5] The main alcohols found in archaea are the *sn*-2,3-diphytanylglycerol diether (archaeol) and its dimer the *sn*-2,3-dibiphytanyldiglycerol tetraether (caldarchaeol).

The extraordinary stability of archaeal membranes against external stress, *e.g.* the presence of low pH-values, high temperatures, or hydrolysing enzymes, motivates researchers to use these membrane lipids in biotechnology,[7] material sciences,[8] and pharmacy.[9,10] Especially the possibility of bipolar lipids to incorporate into bilayers composed of conventional monopolar phospholipids is very attractive, since this approach led to stabilized liposomes, which can be used for drug delivery purposes. The applicability of liposomes prepared from either bolalipids alone, which are sometimes called archaeosomes,[11] or from bolalipid/phospholipid mixtures was already proved for a wide range of natural and artificial bipolar lipids.[12-18]

However, the main problem is to obtain such bolalipids in considerable amounts for practical applications. Isolation from natural sources is on the one hand a suitable method; but, this method yields mixtures of lipids with respect to the composition of the hydrophobic part of the bolalipid. On the other hand, the total synthesis of natural occurring archaeal membrane lipids, which was shown by Kakinuma *et al.*,[19-21] is not applicable for the production of large batches. Consequently, researchers in this field try to simplify the chemical structure of bolalipids by maintaining their stabilizing properties.[16,22-25] The simplest archaeal model lipid is, in our opinion, a single-chain bolalipid consisting of two phosphocholine (PC) headgroups connected by an unmodified C32 alkyl chain, the **PC-C32-PC** (Figure 1, top).[26]

When put into water, **PC-C32-PC** self-assembles into long nanofibres or micellar aggregates in dependence of the temperature.[27] The formation of vesicular structures (liposomes) was not observed. This surprising aggregation behaviour is caused by the chemical structure of this archaeal model lipid:

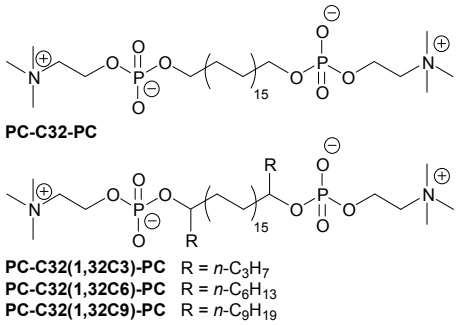


Figure 1 Chemical structure of the archaeal model lipid PC-C32-PC and its 1,32-alkyl branched derivatives investigated in this work.

[a] Institute of Pharmacy, Biophysical Pharmacy, Martin Luther University (MLU) Halle-Wittenberg, Wolfgang-Langenbeck-Strasse 4, 06120 Halle (Saale), Germany. E-mail: simon.drescher@pharmazie.uni-halle.de
[b] Institute of Biochemistry and Biotechnology, MLU Halle-Wittenberg, Kurt-Mothes-Strasse 3, 06120 Halle (Saale), Germany.

†Electronic Supplementary Information (ESI) available: DLS measurements; MS, ^{1}H and ^{13}C NMR spectra. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C8OB00424B

Due to the bulkiness of the PC headgroup in comparison the small cross-sectional area of its single alkyl chain, the self-assembly into lamellar structures is energetically unfavourable and the aggregation into fibres is preferred.[28] Within these nanofibres, the **PC-C32-PC** molecules are twisted relative to each other, which leads to a helical superstructure of the fibres.[29] However, **PC-C32-PC** cannot be used as stabilizer of phospholipid bilayers (liposomes).[30] The reason for that can be found again in the chemical structure of the **PC-C32-PC** molecule: if the bolalipid is inserted in a stretched, transmembrane manner into the 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) bilayer, void volume will be created in the hydrophobic region between both lipid species, which cannot be filled by either DPPC or **PC-C32-PC**. To circumvent these packing problems, the enlargement of the hydrophobic part of the bolalipid by expanding the cross-sectional area of the alkyl chain—to fill the void volume—is one possibility.

In recent years, we systematically variated the alkyl chain structure of single-chain bolalipids through the insertion of hetero atoms,[31,32] acetylene[33] or diacetylene[34] groups, phenyl- as well as biphenyl rings,[35-38] and also methyl moieties.[33] We could show that perturbations within the alkyl chain led to a decrease of the nanofibre stability and, in some cases, elongated micelles are formed instead. However, in mixing experiments of these modified bolalipids with classical phospholipids we could demonstrate that if a miscibility of both lipid species was observed, the liposomal structure is destroyed and small disk-like aggregates are found instead.[38,39] We think that small alterations in the bolalipid alkyl chain such as two methyl groups[39] are not sufficient to fill the void volume. Possibly, the insertion of longer alkyl (side)chains into the transmembrane C32 chain of the **PC-C32-PC** could result in a better miscibility of bolalipids with phospholipid by maintaining the vesicular structure.

In this paper, we present the synthesis of three novel alkyl-branched single-chain bis(phosphocholines) **PC-C32(1,32C*n*)-PC**. These bolalipids carry two side chains at the α,α'(1,32)-position of the long C32 chain with different chain lengths of *n* = 3, 6, or 9 carbon atoms (Figure 1, bottom). We will also discuss scopes and limits of the Grignard coupling reaction that is used for the preparation of the alkyl-branched diols. In the second part of the paper, we present first investigations of the lyotropic behaviour of these novel bolalipids in aqueous suspension by differential scanning calorimetry (DSC), turbidity measurements, and transmission electron microscopy (TEM).

Results and discussion

Synthetic methods

The preparation of single-chain bolalipids depends on an effective synthetic access to the corresponding diols. For the synthesis of 1,ω-diols with 22 or more carbon atoms, several procedures are known: (i) bis-acylation of cyclic enamines with dicarboxylic acid dichlorides, (ii) hydrolysis of enamino ketones and subsequent ring opening, (iii) Wolff-Kishner reduction of bis(oxoacids) and subsequent reduction with $LiAlH_4$, or (iv) double Wittig reaction with bis(phosphorylides) and ω-functionalized aldehydes.[40,41] In recent years, we established and elaborated the Grignard reaction to build up long-chain 1,ω-diols. This C-C-coupling reaction, which is catalyzed by Li_2CuCl_4,[42] can be designed as a *mono*-coupling[43] or *bis*-coupling[26,44] reaction (Figure 2).

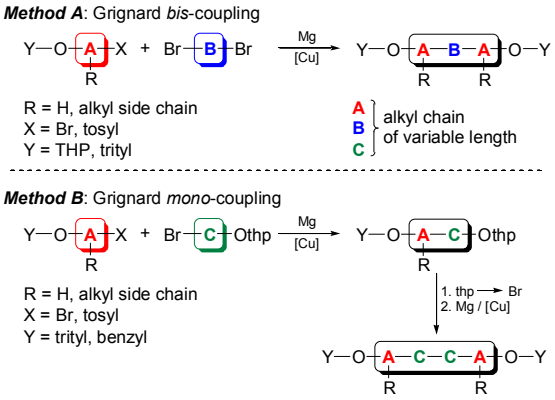


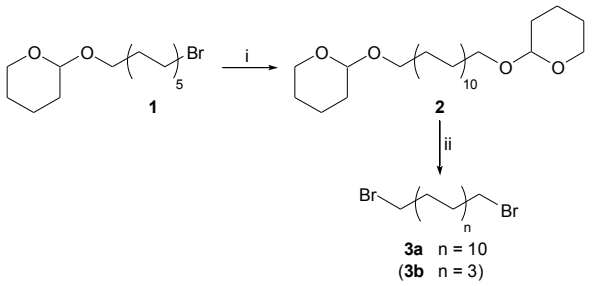
Figure 2 Schematic overview of Grignard coupling reactions. Top: Grignard *bis*-coupling of a side part **A** and a centre part **B** in a 2:1 ratio; bottom: Grignard *mono*-coupling consisting of two steps, a Grignard coupling of **A** + **C** and a dimerization of the **A–C** product. For both methods, either part **A** or part **B/C** can be transformed in the corresponding Grignard reagent.

The Grignard *bis*-coupling involves two simultaneously performed C-C-coupling reactions using a side part **A** and a centre part **B** in a molar ration of 2:1 (see Figure 2, method A). This reaction results in the formation of a **A-B-A** product. The main drawback of this approach is that due to a transmetallation reaction, firstly described by Tamura and Kochi,[42] an **A-A** product is formed (which we term *homo*-coupling product). In some cases, this by-product cannot be separated from the main product **A-B-A**.[36,44]

With the use of a Grignard *mono*-coupling of two different parts **A** and **C**, one can avoid this problem (see Figure 2, method B). Of course, this coupling reaction also leads to *homo*-coupling by-products **A-A** or **C-C**, but, due to a sufficient difference in chemical structures (polarity), both by-products can be fully separated from the main product **A-C** using column chromatography.[36] Finally, **A-C** is dimerized to the final product **A-C-C-A** using a second Grignard *homo*-coupling reaction. The increased effort (more synthetic steps) is sometimes necessary to avoid non-separable by-products.

In a first attempt, we used the Grignard *bis*-coupling reaction (method A). Since the total bolalipid chain length is set to 32 C-atoms and besides, commercially available δ-lactones (C5 building block) are used as starting material for the preparation of the side parts, a centre part of 22 C-atoms has to be synthesized at first. The 1,22-dibromodocosane (**3a**) was prepared from the tetrahydropyranyl(THP)-blocked 11-bromoundecanol (**1**).[44] After the Li_2CuCl_4-catalyzed Grignard *homo*-coupling of **1**,[45] the resulting bis-THP-ether **2** was converted into the favoured **3a** using triphenylphosphoranediyl dibromide and a procedure described by Schwarz *et al.* (Scheme 1).[46]

View Article Online
DOI: 10.1039/C8OB00424B

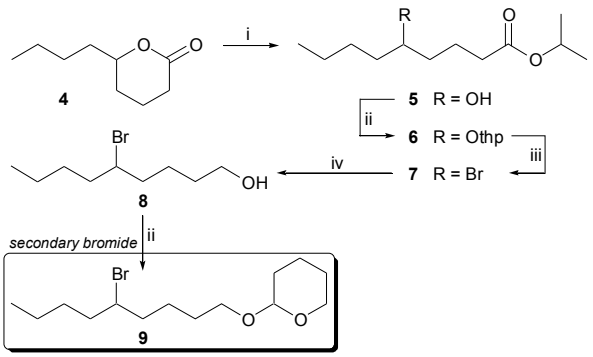


Scheme 1 Reagents and conditions for the preparation of the long-chain dibromide **3a**: (i) Mg, **1**, THF, 50 °C; then **1**, Li_2CuCl_4, THF, 0 °C; (ii) PPh_3, Br_2, CH_2Cl_2, r.t.; 1,8-dibromooctane (**3b**) is commercially available.

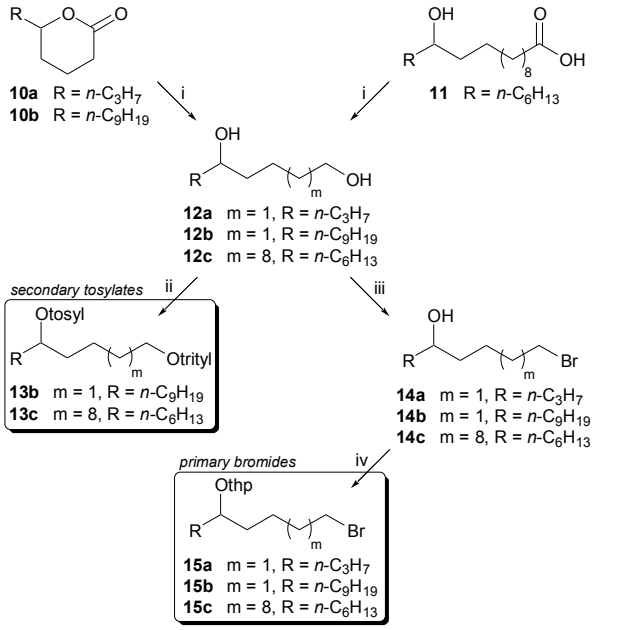
As mentioned above, commercially available racemic δ-nonalactone (**4**) was used as starting material for the preparation of the side part (Scheme 2). Compound **4** was converted into the isopropyl (5*RS*)-5-hydroxynonanoate (**5**) by a base-induced lactone ring opening followed by a esterification using 2-bromopropane.[47] The best yields could be obtained using *N*,*N*-dimethylformamide (DMF) and a sterically hindered alcohol (*tert*-butanol) as solvents. One can also apply an acid-catalyzed ring opening reaction by heating **4** in MeOH with catalytic amounts of *p*-toluenesulfonic acid.[44] However, this acidic transesterification led to a high amount of oligomer products. The next step was the conversion of the hydroxy group into a bromide. Here, we used again the procedure described by Schwarz *et al.*[46] and transferred the hydroxy ester **5** into the corresponding THP-ether **6**.[48] After bromination, the isopropyl (5*RS*)-5-bromononanoate (**7**) was obtained. The last step was the reduction of the ester function using $LiAlH_4$ in diethyl ether. This reaction has to be performed at −15 °C to prevent a possible reductive elimination of the secondary bromide. Finally, the primary alcohol function of the resulting (5*RS*)-5-bromononan-1-ol (**8**) was blocked via THP-ether yielding the 2-{[(5RS)-5-bromononyl]oxy}tetrahydro-2*H*-pyran (**9**).[48]

The first Grignard reactions were carried out in a *bis*-coupling manner (Figure 2, method A) using the dibromide **3a** as centre part and the secondary bromide **9** as side part according to the literature.[49] Now we have two options: We can transform either **3a** or **9** into the corresponding Grignard reagent. The first entry (see Table 1 on the next page) using **3a** failed. It was not possible to convert the dibromide into the organometallic compound, presumably due to the poor solubility of **3a** in tetrahydrofuran (THF). It was also not possible to convert the secondary bromide **9** into the Grignard reagent (entry 2), which might be due to steric hindrance. Also a prolonged reaction time or the addition of iodine or 1,2-dibromoethane to activate the magnesium did not work. *Yang et al.* suggested the addition of *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine (TMEDA), which act as a chelator and promote the formation of secondary organometallic compounds.[50] But, this attempt was not crowned with success, too (entry 3).

Since Grignard coupling reactions can also be carried out using pseudohalides such as mesylates or tosylates as coupling reagents,[50-52] we wanted to use secondary tosylates instead of bromides. Therefore, we synthesized two different tosylates starting from either racemic δ-lactones or hydroxy fatty acids (Scheme 3). Besides the base-induced lactone ring opening described above and the acid-catalyzed transesterification published elsewhere,[30] lactones **10** could also be cleaved by reduction using $LiAlH_4$ in diethyl ether. This reduction led to the diols (5*RS*)-octane-1,5-diol (**12a**) and (5*RS*)-tetradecane-1,5-diol (**12b**) in good yields including a primary as well as a secondary alcohol function. One can also start from the commercially available racemic 12-hydroxy-stearic acid (**11**). After the reduction of the carboxylic group, the (12*RS*)-octadecane-1,12-diol (**12c**) was obtained (Scheme 3). The next step is the selective protection of one hydroxy moiety of the diols **12**. We decided to use the trityl group to selectively block the primary alcohol moiety. Subsequently, the secondary hydroxy group was tosylated using tosyl chloride (TsCl). Both reactions can be performed as a one-pot synthesis using a chloroform/pyridine



Scheme 2 Reagents and conditions for the preparation of the secondary bromide **9**: (i) KOH, MeOH, r.t.; then 2-bromopropane, DMF, *tert*-butanol, reflux; (ii) DHP, CH_2Cl_2, PPTS, r.t.; (iii) PPh_3, Br_2, CH_2Cl_2, r.t.; (iv) $LiAlH_4$, Et_2O, −15 °C.



Scheme 3 Reagents and conditions for the preparation of secondary tosylates **13** and primary bromides **15**: (i) $LiAlH_4$, Et_2O, r.t.; (ii) TrCl, pyridine, $CHCl_3$, r.t.; then TsCl, pyridine, $CHCl_3$, r.t.; (iii) PPh_3, CBr_4, CH_2Cl_2, 10 °C; (iv) DHP, CH_2Cl_2, PPTS, r.t.

View Article Online
DOI: 10.1039/C8OB00424B

Table 1 Reagents and conditions for the Grignard coupling reactions performed in this work.[a]

Entry	Method (see Figure 2)	Educt 1 (Grignard reagent)	Yield[b] (%) of Grignard reagent	Educt 2 (coupling reagent)	Ratio Ed1 : Ed2	Cat. (10 mol%)[c]	Additive (20 mol%)	Temp. of coupling reaction[d]	Product	Yield[e] (%)
1	A	**3a** (1.78 mmol)	< 5	**9** (3.55 mmol)	1 : 2	Li_2CuCl_4	–	0 °C	–[g]	–
2	A	**9** (3.55 mmol)	< 5	**3a** (1.69 mmol)	2.1 : 1	Li_2CuCl_4	–	0 °C	–[g]	–
3	A	**9** (2.86 mmol)	< 5	**3a** (1.36 mmol)	2.1 : 1	Li_2CuCl_4	TMEDA[f]	0 °C	–[g]	–
4	B	**1** (4.92 mmol)	73	**13b** (4.36 mmol)	1.1 : 1	Li_2CuCl_4	–	0 °C	–[h]	–
5	B	**1** (4.56 mmol)	75	**13b** (3.51 mmol)	1.3 : 1	Li_2CuCl_4	–	0 °C → r.t.	–[h]	–
6	B	**1** (9.19 mmol)	76	**13b** (4.60 mmol)	2 : 1	CuI, LiOMe	TMEDA	0 °C → r.t.	–[h]	–
7	A	**3b** (1.98 mmol)	70	**13c** (4.36 mmol)	1 : 2.2	Li_2CuCl_4	–	0 °C	–[h]	–
8	A	**3b** (1.33 mmol)	75	**13c** (2.93 mmol)	1 : 2.2	CuI, LiOMe	TMEDA	0 °C → r.t.	–[h]	–
9	A	**15a** (26.77 mmol)	> 98	**3a** (9.16 mmol)	2.9 : 1	Li_2CuCl_4	–	0 °C → r.t.	**16a**	44
10	A	**3b** (3.93 mmol)	> 98	**15c** (8.71 mmol)	1 : 2.1	Li_2CuCl_4	–	0 °C	**16b**	14
11	A	**3b** (7.17 mmol)	> 98	**15c** (17.69 mmol)	1 : 2.5	Li_2CuCl_4	–	0 °C → r.t.	**16b**	59
12	A	**15b** (11.98 mmol)	89	**3a** (5.96 mmol)	2 : 1	Li_2CuCl_4	–	0 °C	**16c**	13

[a] All reactions were performed in THF. [b] Yields determined by weighing the Mg left after Grignard formation. [c] 10 mol% of Cu-catalyst with respect to the molar amount of Grignard reactions. [d] The formation of the Grignard (MgBr) reagent was done at 50 °C, whereas the subsequently performed coupling reaction was done at the temperature mentioned in the table (r.t. = room temperature). [e] Isolated yields after chromatography. [f] *N*,*N*,*N*',*N*'-tetramethylethane-1,2-diamine. [g] Reaction was not successful because no Grignard reagent was formed. [h] Reaction was not successful because no coupling reaction was observed.

mixture as solvent.[53] It has to be mentioned that in some cases a β-elimination of the secondary tosyl group was found, which resulted in lower yields. The obtained 1-[(4*RS*)-4-(trityloxy)-butyl]decyl 4-methylbenzenesulfonate (**13b**) and 1-hexyl-[(12*RS*)-12-(trityloxy)dodecyl] 4-methylbenzenesulfonate (**13c**) can be used for the subsequent Grignard reactions.

For the next set of Grignard reactions, we used the *mono*-coupling methodology (Figure 2, method B). As first part, we used the THP-blocked 11-bromoundecanol[44] (**1**), which was transferred into the corresponding Grignard reagent. The second part was the secondary tosylate **13b**. The reaction of both components should lead to a C16 building block with a THP- and a trityl-blocked hydroxy moiety. After a THP → bromine exchange,[46] this product could be dimerized to the alkyl-branched C32 diol. Unfortunately, all attempts (entries 4–6, Table 1) were not successful. Neither an increased temperature of the coupling reaction (entry 5) nor a change in the Cu-catalyst[50] (entry 6) resulted in a notable product formation. We then went back to the Grignard *bis*-coupling (Figure 2, method A) and used the short-chain 1,8-dibromooctane (**3b**) as centre part and the secondary tosylate **13c** as side part. After the formation of the Grignard reagent from the dibromide **3b**, we tested the Grignard coupling with **13c** using two different catalysts and temperatures (entries 7 and 8, Table 1). Again, no product formation was observed in both trials. That means Grignard coupling reactions using long-chain secondary bromides or tosylates are not feasible for the synthesis of long-chain alkyl-branched diols.

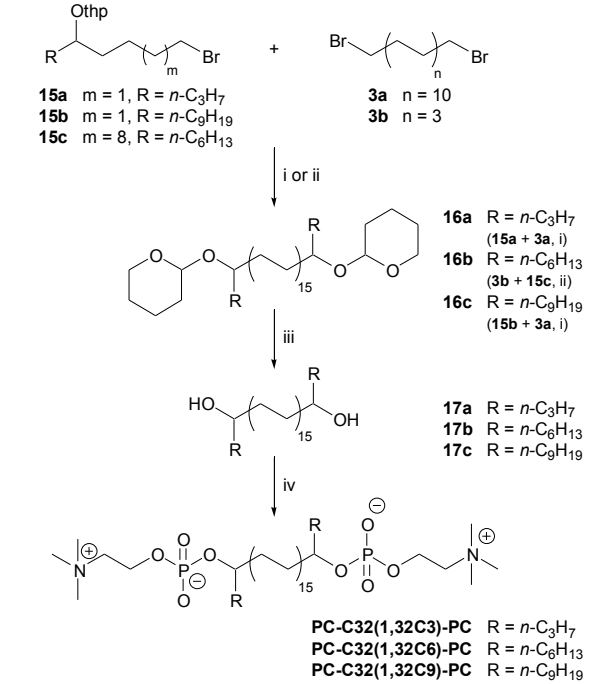
But, we did not get discouraged by these backlashes. Since we have very good experiences with Grignard coupling reactions of two primary bromides,[36,43,44] we synthesized a set of such primary bromides including a blocked secondary alcohol moiety (compounds **15**, Scheme 3). The preparation started from diols **12**, which we already had on hand. Now, the crucial step is the selective bromination of the primary hydroxy group. De Luca *et al.* suggested a reaction with 2,4,6-trichloro[1,3,5]triazine and DMF[54,55] to obtain a primary chloride, which could subsequently be substituted by bromine using LiBr. Unfortunately, the first step was not selective and both primary and secondary chlorides were obtained. Another possibility is the direct bromination using the Appel reaction.[56] For this purpose, the diols **12** were get into reaction with triphenylphosphane and tetrabromomethane in methylene chloride. The yields of this nucleophilic substitution reaction could be increased from 40% to about 70% by changing the reaction temperature from 0 °C to 10 °C. The selectivity of this reaction was proved by ^{1}H NMR experiments. In the last step, the secondary hydroxy group of the obtained racemic bromoalcohols

View Article Online
DOI: 10.1039/C8OB00424B

14 was protected as THP-ether.[48] The resulting racemic THP-blocked bromoalcohols **15** were obtained in very good yields (>95%) and can be used for the Grignard reaction.

For the last set of Grignard reactions (entries 9–12, Table 1) we used the *bis*-coupling methodology (Figure 2, method A) and the different THP-blocked bromoalcohols **15** as side parts and the 1,ω-dibromides **3** as centre part. Since the formation of the Grignard reagent from 1,22-dibromodocosane (**3a**) was not possible (see entry 1, Table 1, and description above), we used this compound as coupling partner (entries, 9 and 12). By contrast, we prepared the Grignard reagent from the 1,8-dibromooctane (**3b**) in entry 10 and 11. After the Grignard coupling reaction using Li_2CuCl_4 as catalyst, we obtained the corresponding alkyl-branched bis-THP-ethers **16** in moderate yields (Scheme 4). In contrast to previous work,[36,44] the yield could be significantly increased when the temperature of the coupling reaction is increased from 0 °C (entries 10, 12; 13–14%) to room temperature (entries 9, 11; 44–59%; Table 1). After cleavage of both THP protecting groups, the corresponding 1,32-alkyl-branched diols **17** were obtained in good yields of about 88 %.

Finally, the 1,32-alkyl-branched bis(phosphocholines) **PC-C32(1,32*n*)-PC** were prepared from the corresponding diols **17** by the method described by Eibl *et al.*[57] using β-bromoethylphosphoric acid dichloride[58] as phosphorylating reagent. The phosphorylation step has to be performed at higher temperatures for a prolonged period of time (about 24 h at 50 °C and up to 3 days at room temperature) in order to ensure a complete dissolution of the diols **17** in the chloroform/triethylamine (TEA) mixture. The subsequently performed quarternisation with trimethylamine in a chloroform/acetonitrile/ethanol mixture provided the bis(phosphocholines) **PC-C32(1,32*n*)-PC** in 11–33% yield after purification and with respect to the diols **17** (Scheme 4). The relatively low yields are due to the secondary alcohol function. It is conceivable the sterically hindrance reduces the yields during the phosphorylation reaction. For comparison, the yields for the phosphorylation/quarternisation reaction of unmodified long-chain 1,ω-diols are between 54 and 65%.



Scheme 4 Reagents and conditions for Grignard coupling reactions and final steps to the bolalipids: (i) Mg, **15a** or **15b**, THF, 50 °C; then **3a**, Li_2CuCl_4, THF, 0 °C or r.t.; (ii) Mg, **3b**, THF, 50 °C; then **15c**, Li_2CuCl_4, THF, 0 °C or r.t.; (iii) MeOH, PPTS, reflux; (iv) $Cl_2P(O)O(CH_2)_2Br$, TEA, $CHCl_3$, 50 °C; then THF, H_2O, r.t.; then $CHCl_3$, acetonitrile, EtOH, $N(CH_3)_3$, 40 °C.

Temperature-dependent aggregation behaviour

The lyotropic phase behaviour of the novel, single-chain alkyl-branched bolalipids **PC-C32(1,32*n*)-PC** was characterised by DSC, turbidity measurements, and TEM. The results were further compared to the unmodified **PC-C32-PC**. As already mentioned in the introduction, **PC-C32-PC** self-assembles in aqueous suspension into a dense network of long flexible, helical nanofibres. This leads to the formation of a clear and transparent hydrogel.[26] With increasing temperature, a transformation of the nanofibres into small micelles can be observed and the gel character is lost. This reversible gel/sol transformation is accompanied by an endothermic and cooperative transition at T_m1 = 48 °C, which can be followed by DSC.[27] At higher temperatures (T_m2 ≈ 73 °C), a second broad transition between two different types of micelles is observed (Figure 3, grey solid line).[27]

DSC and Turbidity. When put the new alkyl-branched bolalipids in water, a different behaviour can be observed. The C3-branched **PC-C32(1,32C3)-PC** forms a turbid suspension, which becomes completely clear when heated above 80 °C and changes into a turbid suspension again when cooled. In con-

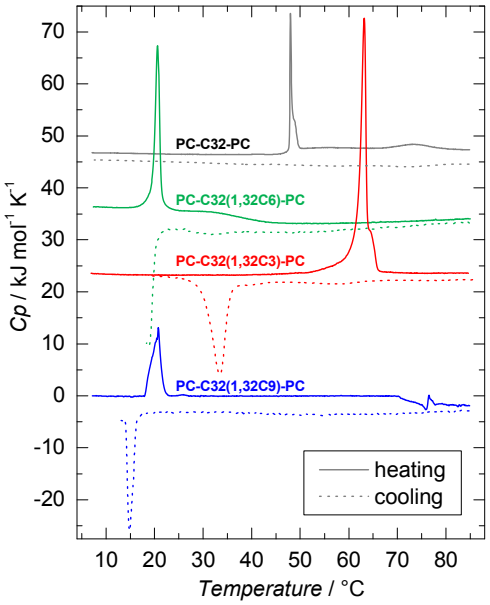


Figure 3 DSC heating (solid lines) and cooling (dotted lines) curves of aqueous suspensions of bolalipids **PC-C32(1,32C*n*)-PC** (c = 1 mg mL^{-1}, heating rate 60 K h^{-1}). The DSC curve of **PC-C32-PC** is shown for comparison. The curves are shifted vertically for clarity.

View Article Online
DOI: 10.1039/C8OB00424B

trast, the C6-branched **PC-C32(1,32C6)-PC** forms a transparent and gel-like suspension, which stays clear during heating. Finally, the C9-branched **PC-C32(1,32C9)-PC** also forms a transparent suspension, which is less gel-like compared to the C6-analogue. But, with increasing temperature, the **PC-C32(1,32C9)-PC** suspension turns into a turbid one and small particles start to precipitate.

The DSC heating curves of the alkyl-branched bolalipids in water (c = 1 mg mL^{-1}) are depicted in Figure 3. The **PC-C32(1,32C3)-PC**, with two *n*-propyl side chains, shows a cooperative transition at T_m = 63.2 °C including a small high-temperature shoulder (red solid line in Figure 3). A further elongation of the lateral side chain leads to a pronounced decrease in the transition temperature: **PC-C32(1,32C6)-PC** shows a cooperative, endotherm transition at T_m = 20.7 °C (green solid line in Figure 3). In addition, a stepwise decrease in the heat capacity is observed between 30–42 °C. This decrease is conceivably related to a metastable state that is reached after T_m. This kinetically stable state then transforms within the timescale of the DSC experiment into a thermodynamically ones, which is accompanied by an exothermic heat effect showing up as decrease in the C_p value. The bolalipid including the longest side chain, the **PC-C32(1,32C9)-PC**, shows a cooperative transition at T_m = 20.8 °C and a very small transition at T = 25.8 °C (blue solid line in Figure 3). Above 70 °C, the DSC signal becomes noisy, which might be due to precipitation of lipid within the DSC cell. The drastic decrease in T_m of the C6- and the C9-analogue, when compared to **PC-C32(1,32C3)-PC** as well as **PC-C32-PC**, is obviously due to the longer alkyl side chains. A comparable effect—although not as pronounced as in our case—was shown for alkyl-branched, monopolar phosphatidylcholines.[59] Here, the C3-branched 1,2-di(2C$_3$-16:0)PC depicts a main transition temperature at T_m = 29.4 °C, whereas the C6-analogue 1,2-di(2C$_6$-16:0)PC reveals T_m = 3.0 °C.

The corresponding cooling curves of the alkyl-branched bolalipids are shown in Figure 3 as dotted lines. The **PC-C32(1,32C3)-PC** shows a very broad transition between T = 65–50 °C and the main transition at T_m = 33.4 °C, indicating a pronounced hysteresis of about 30 K (Figure 3, red dotted line). The **PC-C32(1,32C6)-PC** depicts a main transition at T_m = 18.8 °C, which equals a very small hysteresis of about 2 K (Figure 3, green dotted line). Finally, the **PC-C32(1,32C9)-PC** shows a transition at T_m = 14.9 °C, indication a moderate hysteresis of ΔT = 6 K (Figure 3, blue dotted line). It might be conceivable that the short C3 side chain in the **PC-C32(1,32C3)-PC** leads to higher perturbation in the aggregate structure than the C6 and C9 ones, which results in a more hindered reorganisation and, hence, a larger hysteresis. Interestingly, the DSC cooling curve of **PC-C32-PC** shows only very broad and no sharp and cooperative transitions (Figure 3, grey dotted line). Probably, the additional alkyl side chains in the novel bolalipids trigger a faster (within the timescale of the DSC measurement) reorganisation from the high-temperature to low-temperature aggregate structures.

The variable and changing turbidity of the different bolalipid suspensions described above can also be followed by temperature-dependent turbidity measurements. The corresponding transmission *versus* temperature graphs are shown in Figure 4. For the **PC-C32(1,32C3)-PC**, we found an increase in the transmission from 60% at T = 20 °C to 90% at T = 70 °C (Figure 4A). This increase is most pronounced at T_m. The **PC-C32(1,32C6)-PC** stays more or less clear and transparent over the whole temperature range investigated with transmission values of about 90% (Figure 4B). Lastly, the **PC-C32(1,32C9)-PC** starts with a virtually clear suspension and a transmission value of about 75% at T = 10 °C. During heating, the transmission value drops down to 20% at temperatures between 20–24 °C, which corresponds to T_m. Above 25 °C, the suspension (supernatant) becomes clear again (transmission of 90% at T = 35 °C), which is due to precipitation of the lipid substance (Figure 4C).

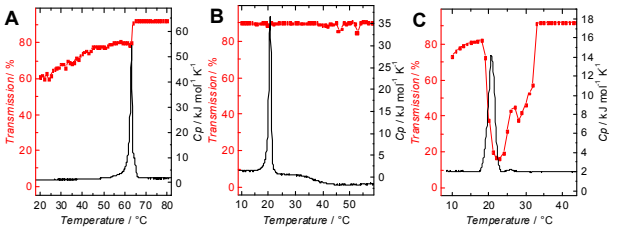


Figure 4 Temperature-dependent transmission measurements (red squares) of aqueous bolalipid suspensions (c = 1mg mL^{-1}): (A) **PC-C32(1,32C3)-PC**, (B) **PC-C32(1,32C6)-PC**, (C) **PC-C32(1,32C9)-PC**. The DSC heating curves (black solid line) of the corresponding bolalipid suspensions (c = 1 mg mL^{-1}; 60 K h^{-1}) are shown for comparison.

TEM. To get an idea about the shape of aggregates formed in water at different temperatures, *i.e.* below and above T_m, and in order to find an explanation for the different turbidity phenomena, TEM images were recorded from negatively stained samples (c = 0.05 mg mL^{-1}, Figure 5).

The **PC-C32(1,32C3)-PC** with the shortest alkyl side chain (C3) self-assembles at room temperature ($T \approx$ 22 °C), below T_m, into sheet-like aggregates of different size and irregular shape (Figure 5A, B). Closed lipid vesicles were not found. The insertion of two short (C3) alkyl side chains obviously prevents the formation of nanofibres as found for the unmodified **PC-C32-PC**.[26,27] Due to the high T_m-value of **PC-C32(1,32C3)-PC**, we were not able to get an EM image of the aggregates formed above T_m. Since the suspension becomes clear at temperatures above T_m (see Figure 4A), it is conceivable that the sheet-like aggregates transform into small micelles or nanofibres at these high temperatures. DLS measurements at T = 80 °C (see Figure S1, ESI†) reveal the presence of very small particles with a diameter between 10–14 nm, which can be attributed to micellar structures. However, also larger aggregates are present in the sample: a second population with diameters around 200 nm and a third population with very large particle sizes > 1 µm. Both could be attributed to fibrous aggregates, since the sample is virtually clear.

The situation changes when the lateral chain is elongated. The C6-analogue **PC-C32(1,32C6)-PC** shows the formation of a dense network of nanofibres (Figure 5C), when the sample is prepared below T_m. These fibres appear as single fibre strands

View Article Online
DOI: 10.1039/C8OB00424B

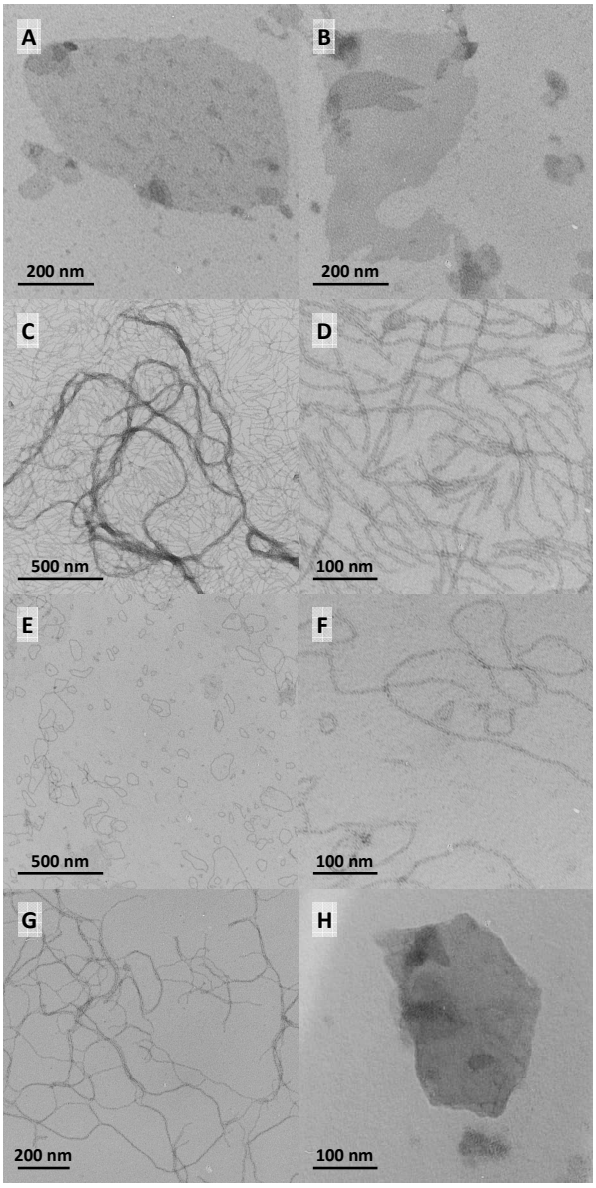


Figure 5 TEM images of aqueous suspensions of **PC-C32(1,32C*n*)-PC** (c = 0.05 mg mL^{-1}) prepared at different temperatures: (A, B) **PC-C32(1,32C3)-PC**, (C–F) **PC-C32(1,32C6)-PC**, (G, H) **PC-C32(1,32C9)-PC**; (A, B) 22 °C, (C, D, G) 4–7 °C, (E, F, H) 35 °C. Samples were stained with uranyl acetate before drying.

or as bundles of several fibres. A closer inspection reveals that a single fibre has a diameter of 5–6 nm (Figure 5D), which roughly corresponds to the length of a bolalipid molecule. The entanglement of these fibres leads to a gelation of the bolalipid suspension. A comparable behaviour was previously found for the single-chain unmodified bis(phosphocholine) **PC-C32-PC**.[26,27] That means the insertion of two lateral C6 alkyl chains at the 1,32-position of the long alkyl chain does not alter the aggregation behaviour and the formation of fibres is still possible—in contrast to **PC-C32(1,32C3)-PC**. A temperature increase above T_m leads to a change in the aggregation behaviour: although fibrillar aggregates are still present (Figure 5E, F; samples prepared at T = 35 °C), these nanofibres seems to form circular structures. This aggregations behaviour was observed in several independently prepared samples, thus preparation artefacts can be ruled out. Similar structures—although not as pronounced as in this case—have been observed for an asymmetrical single-chain bolalipid named DMAPPC-C32-POH in acetate buffer at pH 5.[60] The reason for this transformation as well as the inner structure of these circular aggregates are not understood up to now and are part of ongoing research.

The bolalipid bearing the longest (C9) lateral alkyl chains, **PC-C32(1,32C9)-PC**, self-assembles again into long and flexible nanofibres below T_m (Figure 5G), which lead to a gelation of the bolalipid suspension. These nanofibres are comparable to the fibres found for **PC-C32(1,32C6)-PC** and **PC-C32-PC**. Upon heating, the fibres transform into lamellar aggregates of irregular shape (Figure 5H; sample prepared at T = 35 °C), which is accompanied by an increase in turbidity (see Figure 4C) and a loss of the gel properties.

Geometrical considerations. By comparing the three alkyl-branched bolalipids **PC-C32(1,32C*n*)-PC** investigated in this work, one can conclude that all of them show a different aggregation behaviour in dependence of temperature—summarized in Figure 6. The bolalipids only differ in the length of the lateral alkyl chain. First temperature-dependent infrared(IR)-spectroscopic measurements of aqueous bolalipid suspensions reveal that the transition found in the DSC is accompanied with an increased number of *gauche* conformers within the alkyl chains of the bolalipids (data not shown). Hence at T_m, the bolalipids undergo a transition from gel phase into a liquid-crystalline phase. This fluidization is connected with a shortening of the alkyl chains. One can now speculate that the interaction of the two factors, one the one hand the length of the lateral alkyl chain and on the other hand the reduction of the overall chain length upon heating, determine the shape of aggregates found in aqueous suspension. However, further investigations are mandatory to prove this assumption.

Conclusions

In this work, we have developed a synthetic strategy for the preparation of single-chain alkyl-branched bis(phosphocholines). For the synthesis of 1,ω-diols bearing lateral alkyl

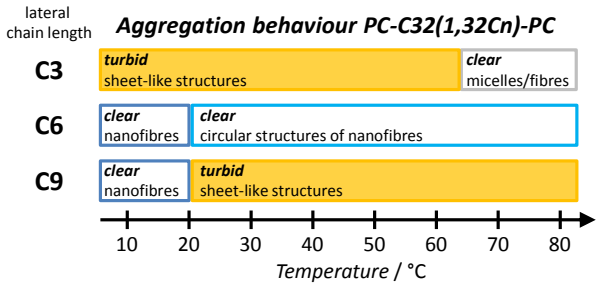


Figure 6 Comparison of the aggregation behaviour of aqueous suspension of the alkyl-branched **PC-C32(1,32C*n*)-PC** (c = 1 mg mL^{-1}).

View Article Online
DOI: 10.1039/C8OB00424B

chains of variable length, we finally used a Li_2CuCl_4-catalyzed Grignard *bis*-coupling reaction of primary bromides as side parts—including a THP-blocked secondary alcohol—and 1,ω-dibromides as centre part. This coupling reaction should be carried out at ambient temperatures instead of 0 °C to increase the yield. The corresponding primary bromides can be prepared in three steps from inexpensive alkyl-branched δ-lactones or hydroxy fatty acids. Other attempts using secondary bromides or tosylates for the Grignard coupling reaction as well as the use of additives such as TMEDA did not work in our case. For the final insertion of the PC headgroups, robust and established procedures were used. The synthetic strategy presented herein is also applicable for the preparation of bolalipids bearing other lengths of alkyl side chains.

The lyotropic phase behaviour of the novel single-chain alkyl-branched bolalipids **PC-C32(1,32C*n*)-PC** (*n* = 3, 6, or 9) was investigated using DSC and TEM. We could show that the length of the lateral alkyl chain has a great impact on the structure of aggregates formed in aqueous suspension as well as their stability upon heating. Whereas **PC-C32(1,32C3)-PC** self-assembles into sheet-like structures at ambient temperature, which are stable up to T_m = 63 °C, the C6- and the C9-analogue form a network of nanofibres below T_m leading to a gelation of the bolalipid suspension. With increasing temperature, the long fibres of **PC-C32(1,32C6)-PC** transform into circular structures at T_m, whereas the fibrous aggregates of **PC-C32(1,32C9)-PC** convert into lamellar sheet-like structures. It is conceivable that the nanofibres of **PC-C32(1,32C*n*)-PC** are composed of bola molecules that are arranged side by side. By contrast, the lamellar aggregates of **PC-C32(1,32C*n*)-PC** are probably composed of a bolalipid monolayer. The detailed molecular arrangement of the bolalipid molecules within the different aggregates as well as the reason for the transformation between different aggregate types are not understood up to now and are part of ongoing research.

Our investigations show that the aggregation behaviour of single-chain alkyl-branched bolalipids can be tuned by varying the length of the lateral alkyl chain. This fact is of particular interest since lamella-forming bolalipids, which can also be synthesized in larger scale, could be used in the future to stabilise phospholipid liposomes applicable for oral drug delivery. Investigations regarding the mixing behaviour of our novel bolalipids with classical phospholipids are currently under way.

Experimental section

General

Apart from 12-hydroxyoctadecanoic acid (**11**; Fluorochem Ldt, Hadfield, UK) and δ-octalactone (**10a**; Alfa Aesar, Ward Hill, USA), all chemicals were purchased from Sigma Aldrich Co. (Steinheim, Germany) and were used without further purification. All solvents were dried and distilled before use. The purity of all compounds was checked by thin-layer chromatography (TLC) using silica gel 60 F254 plates (Macherey-Nagel GmbH & Co KG, Düren, Germany). The chromatograms were developed by means of bromothymol blue. Purification of all products was carried out by recrystallization or by column chromatography using a middle pressure liquid chromatography (MPLC) system by Büchi (Essen, Germany) and silica gel 60 (Merck, 0.063–0.200 mm). Melting points were determined with Boetius apparatus. Elemental analyses were carried out on an Elemental Vario EL (Elementar Analysensysteme GmbH, Langenselbold, Germany). ^{1}H and ^{13}C NMR spectra were recorded on an Agilent Technologies 400 MHz VNMRS spectrometer (400/100 MHz) or an Agilent Technologies 500 MHz DD2 spectrometer (500/125 MHz) with the use of $CDCl_3$ or CD_3OD as 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; Thermo Separation Products, San José, USA) or an expression^S compact mass spectrometer (APCI-MS; Advion Inc., Ithaca, USA). High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific LTQ-Orbitrap mass spectrometer (Waltham, USA) with static nano-electrospray ionization. Analytical HPLC was performed on a Lichrospher Si 60–5 µm–125 × 4 mm column, using a PU 980 Intelligent HPLC-pump and a LG-1580-02 Ternary Gradient Unit (Jasco) and a SEDEX 55 ELS detector (SEDERE, France). The following eluents were used: solvent A = $CHCl_3$/MeOH/H_2O (42/42/16, v/v/v), B = $CHCl_3$/MeOH/H_2O (45/45/10, v/v/v); gradient: 14 min 0 → 100% A, 9 min 100 % A, 6 min 100 → 0% A, 5 min 0% A; flow 1 mL min^{-1}.

Synthesis of the long-chain 1,ω-dibromide 3a

2,2'-[Docosane-1,22-diylbis(oxy)]bis(tetrahydro-2*H*-pyran) (2).[45] Magnesium turnings (1.6 g, 65.8 mmol) were placed in a 100 mL round-bottomed flask under argon atmosphere. A solution **1** (14.56 g, 43.5 mmol) in dry THF (20 mL) was added dropwise. After the exothermic reaction had subsided, the mixture was stirred at 55 °C for 3 h. Afterwards, the excess magnesium was removed under argon atmosphere and the Grignard solution was cooled to 0 °C. A freshly prepared solution of dilithium tetrachlorocuprate(II) (0.1 M in THF, 7.0 mL) was added with stirring. After a solution of **1** (11.93 g, 35.6 mmol) in dry THF (70 mL) was added in one portion, stirring was continued for 20 h at 0 °C. For work-up, diethyl ether (150 mL) was added and the resulting mixture was poured into an ice-cold saturated solution of ammonium chloride (150 mL). The organic layer was separated and the aqueous phase was extracted several times with diethyl ether. The combined ethereal phases were washed with brine, dried over sodium sulphate and concentrated to dryness under reduced pressure. The crude product was purified by column chromatography using heptane/triethylamine/diethyl ether (98/0.5/1.5, v/v/v) as eluent yielding **2** as white solid (11.55 g, 64%). ^{1}H NMR (500 MHz, $CDCl_3$, 27 °C) δ 1.22–1.63 (m, 48 H, CH_2), 1.66–1.75 (m, 2 H, 2× C*H*HCHO), 1.78–1.88 (m, 2 H, 2× CH*H*CHO), 3.38 (dt, *J* = 9.6, 6.7 Hz, 2 H, 2× CH*H*Othp), 3.46–3.54 (m, 2 H, 2× CH*H*OCHOC$_{22}$H$_{44}$), 3.73 (dt, *J* = 9.6, 6.9 Hz, 2 H, 2× C*H*HOthp), 3.83–3.91 (m, 2 H, 2× C*H*HOCHOC$_{22}$H$_{44}$), 4.57 (dd, *J* = 4.5, 2.8 Hz, 2 H, 2× -C*H*O-); ESI-MS *m/z* 533.42 (M + Na). Analytical data are in accordance to data described previously.[45]

View Article Online
DOI: 10.1039/C8OB00424B

1,22-Dibromodocosane (3a).[45] Triphenylphosphane (26.08 g, 99.4 mmol) was dissolved in dry dichloromethane (200 mL) and bromine (15.89 g, 99.4 mmol), diluted in dichloromethane (10 mL), was added dropwise into the solution whilst stirring at 0 °C. Compound **2** (11.55 g, 22.6 mmol), dissolved in 100 mL dichloromethane, was added and the mixture was stirred overnight at room temperature. Afterwards, the organic layer was washed with water (400 mL) and the crude dibromide **3a** was purified by column chromatography with heptane as eluent yielding **3a** as white crystals (9.0 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 1.22–1.48 (m, 36 H, (CH_2)$_{18}$), 1.79–1.91 (m, 4 H, 2× CH_2CH_2Br), 3.41 (t, J = 6.9 Hz, 4 H, 2× CH_2Br); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 28.17 ($CH_2(CH_2)_2$Br), 28.75 ($CH_2(CH_2)_3$Br), 29.42, 29.52, 29.60, 29.63 and 29.67 (CH_2), 32.84 (CH_2CH_2Br), 34.01 (CH_2Br). Analytical data are in accordance to data described previously.[45]

Synthesis of the secondary bromide 9 from the corresponding lactone

The synthetic procedures and analytical data of compounds **5–8** can be found in the Electronic Supplementary Material (ESI†).

2-{[(5*RS*)-5-Bromononyl]oxy}tetrahydro-2*H*-pyran (9). The alcohol **8** (0.84 g, 3.76 mmol) was dissolved in dry dichloromethane (100 mL) at room temperature, 3,4-dihydro-2*H*-pyran (0.62 g, 7.37 mmol) and pyridinium *p*-toluenesulfonate (0.95 g, 3.8 mmol) were added and the mixture was stirred for 20 h at room temperature. Afterwards, the organic solution was washed with water (150 mL), dried over sodium sulphate and concentrated to dryness under reduced pressure. The crude oil was purified by column chromatography using heptane/triethylamine/diethyl ether (98.5/0.5/1, v/v/v) yielding **9** as colourless oil (1.09 g, 94%). $C_{14}H_{27}BrO_2$ requires C, 54.72; H, 8.86; found: C, 54.14; H, 8.64; ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.91 (t, J = 7.2 Hz, 3 H, CH_3) 1.23–1.93 (m, 18 H, CH_2) 3.34–3.43 (m, 1 H, CH*H*Othp), 3.46–3.54 (m, 1 H, alkylOCHOCH*H*), 3.69–3.80 (m, 1 H, C*H*HOthp), 3.82–3.92 (m, 1 H, alkylOCHOC*H*H), 4.03 (quin, J = 6.4 Hz, 1 H, C*H*Br), 4.57 (t, J = 3.4 Hz, 1 H, OC*H*O); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 13.92 (*C*H$_3$), 19.64 and 19.66 (alkylOCHCH$_2$*C*H$_2$), 22.13 (CH$_3$*C*H$_2$), 24.34 (*C*H$_2$(CH$_2$)$_2$Othp), 25.44 and 25.47 (alkylOCHOCH$_2$*C*H$_2$), 29.12 and 29.14 (alkylOCH*C*H$_2$), 29.69 (CH$_3$CH$_2$*C*H$_2$), 30.68 and 30.74 (*C*H$_2$CH$_2$Othp), 38.85 (CH$_3$(CH$_2$)$_2$*C*H$_2$), 38.93 (alkylCHBr*C*H$_2$), 58.56 (*C*HBr), 62.32 and 62.35 (alkylOCHOC*H*$_2$), 67.26 and 67.29 (*C*H$_2$Othp), 98.84 and 98.90 (OCHO); ESI-MS *m/z* 329.56 (M + Na, ^{79}Br isotope), 331.76 (M + Na, ^{81}Br isotope).

General procedure for the synthesis of alkane-1,5-diols 12

Lithium aluminium hydride (1.1 equiv.) was suspended in dry diethyl ether (200 mL) in a 1 L round-bottomed flask under argon atmosphere. The suspension was cooled to −10 °C and stirred for 1 h. The corresponding lactone **10** or hydroxy fatty acid **11** (1 equiv.), dissolved in dry Et_2O (50 mL), was added very slowly. The ice bath was removed and the mixture was stirred for 20 h at room temperature. Afterwards, the reaction mixture was hydrolysed with ice and acidified using sulphuric acid (30%, 40 mL). The layers were separated and the aqueous phase was extracted three times with diethyl ether (200 mL). The combined ethereal phases were washed with brine (300 ml), dried over sodium sulphate and evaporated. The crude product was purified by column chromatography with the use of heptane/chloroform (2/8, v/v) as eluent yielding the diol as a white solid.

(5*RS*)-Octane-1,5-diol (12a).[61] Following the general procedure, **10a** (10.31 g, 72.5 mmol) and lithium aluminium hydride (3.20 g, 84.2 mmol) gave **12a** (7.64 g, 73%) as colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.85–0.98 (m, 3 H, CH_3), 1.24–1.66 (m, 10 H, CH_2), 1.96 (s, 2 H, 2× O*H*), 3.55–3.67 (m, 3 H, C*H*OH, CH_2OH); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.07 (*C*H$_3$), 18.81 (CH$_3$*C*H$_2$), 21.76 (*C*H$_2$(CH$_2$)$_2$OH), 32.51 (*C*H$_2$CH$_2$OH), 36.91 (*C*H$_2$(CH$_2$)$_3$OH), 39.70 (CH$_3$CH$_2$*C*H$_2$-), 62.57 (*C*H$_2$OH), 71.47 (*C*HOH); ESI-MS m/z 169.55 (M + Na). Analytical data are in accordance with data published previously.[61]

(5*RS*)-Tetradecane-1,5-diol (12b).[62] Following the general procedure, **10b** (25.33 g, 111.9 mmol) and lithium aluminium hydride (4.68 g, 123.2 mmol) gave **12b** (25.08 g, 97%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.88 (t, J = 6.9 Hz, 3 H, CH_3), 1.21–1.70 (m, 22 H, CH_2), 3.57–3.63 (m, 1 H, C*H*OH), 3.66 (t, J = 6.4 Hz, 2 H, CH_2OH); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.07 (*C*H$_3$), 21.81 (*C*H$_2$(CH$_2$)$_2$OH), 22.65 (CH$_3$*C*H$_2$), 25.64 (CH$_3$(CH$_2$)$_6$*C*H$_2$), 29.29–29.67 (*C*H$_2$), 31.87 (CH$_3$CH$_2$*C*H$_2$), 32.62 and 32.63 (*C*H$_2$CH$_2$OH), 36.99 and 37.00 (alkylCHOH*C*H$_2$), 37.54 (CH$_3$(CH$_2$)$_7$*C*H$_2$), 62.78 and 62.80 (*C*H$_2$OH), 71.86 (*C*HOH); ESI-MS *m/z* 253.69 (M + Na), 269.65 (M + K). Analytical data are in accordance with data published previously.[62]

(12*RS*)-Octadecane-1,12-diol (12c).[63] Following the general procedure, **11** (13.07 g, 43.5 mmol) and lithium aluminium hydride (1.98 g, 51.6 mmol) gave **12c** (8.16 g, 66%) as a white solid. M.p. 79–80 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.84–0.93 (m, 3 H, CH_3), 1.25–1.65 (m, 32 H, CH_2 and 2× -O*H*), 3.53–3.61 (m, 1 H, C*H*OH), 3.64 (t, J = 6.7 Hz, 2 H, CH_2OH); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.06 (*C*H$_3$), 22.60 (CH$_3$*C*H$_2$), 25.60 and 25.62 (*C*H$_2$CH$_2$(CHOH)CH$_2$*C*H$_2$), 25.71 (*C*H$_2$(CH$_2$)$_2$OH), 29.36– 29.67 (*C*H$_2$), 31.83 (*C*H$_2$CH$_2$OH), 32.80 (CH$_3$CH$_2$*C*H$_2$), 37.48 and 37.50 (*C*H$_2$(CHOH)*C*H$_2$), 63.08 (*C*H$_2$OH), 72.01 (*C*HOH); ESI-MS *m/z* 309.93 (M + Na). Analytical data are in accordance with data described previously.[63]

General procedure for the synthesis of secondary tosylates 13

The diol **12** (1 equiv. 21.70 mmol) was dissolved in dry chloroform (150 mL). Afterwards, pyridine (2.4 equiv.) and trityl chloride (1.2 equiv.) were added and the mixture was stirred for 24 h at room temperature. After TLC shows the complete conversion of the diol, tosyl chloride (1.2 equiv.) was added and the mixture was stirred for further 72 h. For work-up, water (100 mL) was added and the mixture was stirred for 1 h. Afterwards, the mixture was extracted for several times with chloroform. The combined organic phases were washed with brine, dried over sodium sulphate and evaporated. The resulting crude product was diluted in cold heptane and the insoluble trityl alcohol was separated by filtration. This procedure has to be repeated until a clear solution of the crude product

View Article Online
DOI: 10.1039/C8OB00424B

in heptane is obtained. Finally, the crude product was purified by MPLC using heptane and chloroform as eluents and the gradient technique.

1-[(4*RS*)-4-(Trityloxy)butyl]decyl 4-methylbenzenesulfonate (13b). Following the general procedure, **12b** (5.06 g, 22.0 mmol), trityl chloride (7.28 g, 26.1 mmol), tosyl chloride (4.97 g, 26.1 mmol), and pyridine (4.20 mL, 52.1 mmol) gave **13b** (10.49 g, 77%) as slight yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.91 (t, *J* = 6.9 Hz, 3 H, CH_2CH_3), 1.13–1.69 (m, 22 H, CH_2), 2.40 (s, 3 H, CH_3), 3.01 (t, *J* = 6.4 Hz, 2 H, CH_2O), 4.56 (quin, *J* = 6.0 Hz, 1 H, CHO), 7.19–7.47 and 7.74–7.83 (m, 19 H, 3× C_6H_5, C_6H_4); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.12 (CH_2CH_3), 21.57 (CH_3), 21.58 ($CH_2(CH_2)_2O$), 22.68 (CH_2CH_3), 24.63 ($CH_3(CH_2)_6CH_2$), 29.28–29.71 (CH_2), 31.88 ($CH_3CH_2CH_2$), 33.99 and 34.15 ($CH_2(CHO)CH_2$), 63.14 (CH_2O), 84.35 (CHO), 86.34 ($CH(C_6H_5)_3$), 126.85, 127.67, 127.70, 128.66, 129.60, 134.77, and 144.38 (CH); ESI-MS *m/z* 649.73 (M + Na).

1-Hexyl-[(12*RS*)-12-(trityloxy)dodecyl] 4-methylbenzenesulfonate (13c). Following the general procedure, **12c** (7.35 g, 25.7 mmol), trityl chloride (8.60 g, 30.9 mmol), tosyl chloride (5.95 g, 31.2 mmol), and pyridine (4.97 mL, 61.6 mmol) gave **13c** (2.96 g, 17%) as slight yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.85–0.89 (m, 3 H, CH_2CH_3), 1.11–1.72 (m, 30 H, CH_2), 2.43 (s, 3 H, CH_3), 3.05 (t, *J* = 6.6 Hz, 2 H, CH_2O), 4.54 (quin, *J* = 6.0 Hz, 1 H, CHO), 7.18–7.49 and 7.75–7.83 (m, 19 H, 3× C_6H_5, C_6H_4); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.02 (CH_2CH_3), 21.57 (CH_3), 22.47 (CH_2CH_3), 24.63 and 24.69 ($CH_2CH_2CHOCH_2CH_2$), 26.27 ($CH_2(CH_2)_2O$), 28.93–30.06 (CH_2), 31.57 ($CH_3CH_2CH_2$), 34.12 and 34.14 (CH_2CHOCH_2), 63.70 (CH_2O), 84.62 (CHO), 86.25 ($CH(C_6H_5)_3$), 126.75, 127.64, 127.70, 128.68, 129.57, 134.85 and 144.53 (CH); ESI-MS *m/z* 705.43 (M + Na).

General procedure for the synthesis of bromoalkanols 14

The diol **12** (1 equiv. 5.0 g, 21.7 mmol), placed in a 250 mL round-bottomed flask, was dissolved in dry CH_2Cl_2 (200 mL) and cooled to 10 °C. Triphenylphosphane (1.1 equiv.) and tetrabromomethane (1.1 equiv.) were added and the mixture was stirred for 20 h at 10 °C. Afterwards, the solvent was evaporated. The crude product was purified by column chromatography using heptane/diethyl ether (8/2, v/v) as eluent.

(4*RS*)-8-Bromooctan-4-ol (14a). Following the general procedure, **12a** (7.54 g, 51.6 mmol), triphenylphosphane (14.96 g, 57.0 mmol) and tetrabromomethane (18.91 g, 57.0 mmol) gave **14a** as colourless oil (6.21 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.83–0.96 (m, 3 H, CH_3), 1.23–1.64 (m, 8 H, CH_2), 1.78–1.94 (m, 3 H, CH_2CH_2Br, CHO*H*), 3.38 (m, 2 H, CH_2Br), 3.51–3.63 (m, 1 H, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.06 (CH_3), 18.77 (CH_2CH_3), 24.29 ($CH_2(CH_2)_2Br$), 32.76 (CH_2CH_2Br), 33.74 (CH_2Br), 36.40 ($CH_2CHOH(CH_2)_2CH_3$), 39.63 ($CH_2CH_2CH_3$), 71.32 and 71.33 (CH); APCI-MS *m/z* 191.0 (M – H_2O + H, ^{79}Br isotope), 193.0 (M – H_2O + H, ^{81}Br isotope).

(5*RS*)-1-Bromotetradecan-5-ol (14b). Following the general procedure, **12b** (5.0 g, 21.7 mmol), triphenylphosphane (5.87 g, 22.3 mmol) and tetrabromomethane (7.58 g, 22.9 mmol) gave **14b** (4.40 g, 70%) as white solid. M.p. 31–32 °C; $C_{14}H_{29}BrO$ requires C, 57.33; H, 9.97; found: C, 56.97; H, 9.87; ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.83–0.92 (m, 3 H, CH_3), 1.18–1.67 (m, 20 H, CH_2), 1.80–1.95 (m, 2 H, CH_2CH_2Br), 3.42 (t, *J* = 6.8 Hz, 2 H, CH_2Br), 3.55–3.65 (m, 1 H, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.08 (CH_3), 22.65 (CH_2CH_3), 24.31 ($CH_2(CH_2)_2Br$), 25.61 ($CH_2(CH_2)_6CH_3$), 29.29–29.65 (CH_2), 31.87 ($CH_2CH_2CH_3$), 32.77 (CH_2CH_2Br), 33.71 (CH_2Br), 36.43 and 37.53 ($CH_2CHOHCH_2$), 71.69 (CH); APCI-MS *m/z* 275.1 (M – H_2O + H, ^{79}Br isotope), 277.1 (M – H_2O + H, ^{81}Br isotope).

(7*RS*)-18-Bromooctadecan-7-ol (14c). Following the general procedure, **12c** (8.16 g, 28.5 mmol), triphenylphosphane (7.81 g, 29.8 mmol) and tetrabromomethane (9.91 g, 29.9 mmol) gave **14c** (7.24 g, 73%) as white solid. M.p. 49–50 °C; $C_{18}H_{37}BrO$ requires C, 61.88; H, 10.67; found: C, 62.20; H, 10.75; ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.82–0.94 (m, 3 H, CH_3), 1.24–1.50 (m, 28 H, CH_2), 1.85 (m, 2 H, CH_2CH_2Br), 3.40 (t, *J* = 6.9 Hz, 2 H, CH_2Br), 3.52–3.66 (m, 1 H, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.06 (CH_3), 22.60 (CH_2CH_3), 25.60 and 25.63 ($CH_2CH_2CHOHCH_2CH_2$), 28.16 ($CH_2(CH_2)_2Br$), 28.74 ($CH_2(CH_2)_3Br$), 29.36–29.68 (CH_2), 31.83 (CH_2CH_2Br), 32.82 ($CH_2CH_2CH_3$), 34.01 (CH_2Br), 37.48 and 37.50 ($CH_2CHOHCH_2$), 72.00 (CH); APCI-MS *m/z* 331.3 (M – H_2O + H, ^{79}Br isotope), 333.2 (M – H_2O + H, ^{81}Br isotope).

General procedure for the synthesis of thp-protected bromoalkanols 15

The bromoalkanol **14** (1 equiv.) was dissolved in dry dichloromethane (100 mL) at room temperature. 3,4-Dihydro-2*H*-pyran (2.1 equiv.) and pyridinium *p*-toluenesulfonate (10 mol%) were added and the mixture was stirred for 20 h. Afterwards, the organic solution was washed with water (150 mL), dried over sodium sulphate and concentrated to dryness under reduced pressure. The crude oil was purified by column chromatography using heptane/triethylamine/diethyl ether (98.5/0.5/1, v/v/v) as eluent.

2-{[(1*RS*)-5-Bromo-1-propylpentyl]oxy}tetrahydro-2*H*-pyran (15a). Following the general procedure, **14a** (6.09 g, 29.1 mmol) and 3,4-dihydro-2*H*-pyran (4.57 g, 87.2 mmol) gave **15a** (8.19 g, 96%) as colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.86–0.96 (m, 3 H, CH_3), 1.22–1.94 (m, 16 H, CH_2), 3.41 (td, *J* = 6.8, 3.6 Hz, 2 H, CH_2Br), 3.44–3.52 (m, 1 H, OCHOCH*H*), 3.56–3.68 (m, 1 H, CHOthp), 3.85–3.97 (m, 1 H, OCHOC*H*H), 4.59–4.67 (m, 1 H, OC*H*O); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.23 and 14.31 (CH_3), 18.35 and 18.88 (CH_2CH_3), 19.95 and 20.11 ($OCHO(CH_2)_2CH_2$), 23.61 and 24.21 ($CH_2(CH_2)_2Br$), 25.51 and 25.52 ($OCHOCH_2CH_2$), 31.20–34.13 (CH_2), 35.92 and 37.09 ($CH_2CH_2CH_3$), 62.73 and 62.95 ($OCHOCH_2$), 76.15 and 76.36 (CHOthp), 97.56 and 97.91 (OCHO); ESI-MS m/z 315.30 (M + Na, ^{79}Br isotope), 317.22 (M + Na, ^{81}Br isotope).

2-{[(1*RS*)-1-(4-brombutyl)decyl]oxy}tetrahydro-2*H*-pyran (15b). Following the general procedure, **14b** (3.85 g, 13.1 mmol) and 3,4-dihydro-2*H*-pyran (2.37 g, 28.2 mmol) gave **15b** (4.71 g, 95%) as colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.79–0.90 (m, 3 H, CH_3), 1.22–1.94 (m, 28 H, CH_2), 3.35–3.42 (m, 2 H, CH_2Br), 3.43–3.51 (m, 1 H, OCHOCH*H*), 3.59 (quin, *J* = 4.6 Hz, 1H, CHOthp), 3.84–3.94 (m, 1 H, OCHOC*H*H), 4.58–4.66 (m, 1 H, OC*H*O); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.07 (CH_3), 19.91 and 20.08 ($OCHO(CH_2)_2CH_2$), 22.65 (CH_2CH_3), 23.60, 24.21, 25.03, 25.51, 25.53, 25.58 29.28, 29.30, 29.53,

View Article Online
DOI: 10.1039/C8OB00424B

29.57, 29.59, 29.79, 29.84, 31.18, 31.22, 31.86, 31.87, 32.49, 32.95, 32.96, 33.55, 33.66, 33.76, 34.06, 34.96, 62.65 and 62.90 (OCHOCH_2), 76.40 and 76.44 (*C*HOthp), 97.60 and 97.75 (O*C*HO); ESI-MS *m/z* 399.56 (M + Na, ^{79}Br isotope), 401.52 (M + Na, ^{81}Br isotope).

2-{[(1*RS*)-12-Bromo-1-hexyldodecyl]oxy}tetrahydro-2*H*-pyran (15c). Following the general procedure, **14c** (7.20 g, 20.6 mmol) and 3,4-dihydro-2*H*-pyran (3.28 g, 39.0 mmol) gave **15c** (8.61 g, 97%) as colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.80–0.89 (m, 3 H, CH_3), 1.22–1.73 (m, 34 H, CH_2), 1.76–1.86 (m, 2 H, CH_2CH$_2$Br), 3.36 (t, *J* = 6.9 Hz, 2 H, CH$_2$Br), 3.39–3.50 (m, 1 H, OCHOCH*H*), 3.56 (quin, *J* = 5.8 Hz, 1 H, C*H*Othp), 3.83–3.93 (m, 1 H, OCHOC*H*H), 4.59–4.65 (m, 1 H, OC*H*O); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.04 and 14.06 (*C*H$_3$), 19.91 and 19.94 (OCHO(CH$_2$)$_2$*C*H$_2$), 22.59 and 22.63 (*C*H$_2$CH$_3$), 24.97 and 24.98 (*C*H$_2$CH$_2$CHOthpCH$_2$*C*H$_2$), 25.75 (OCHOCH$_2$*C*H$_2$), 28.14 and 28.15 (*C*H$_2$CH$_2$CH$_2$Br), 28.73 (*C*H$_2$(CH$_2$)$_3$Br), 29.39, 29.48, 29.49, 29.51, 29.55, 29.79, 29.86, 31.20, 31.83, 32.81, 32.82, 33.46, 33.49, 33.79, 35.00, 35.02, 62.53 and 62.73 (OCHO*C*H$_2$), 76.62 and 76.65 (*C*HOthp), 97.36 and 97.42 (O*C*HO); ESI-MS m/z 455.90 (M + Na, ^{79}Br isotope), 458.03 (M + Na, ^{81}Br isotope).

General procedure for the synthesis of thp-protected 1,32-alkyl branched 1,32-diols 16 via Grignard bis-coupling reaction

Variation A: Magnesium turnings (1.5 equiv.) were placed in a 100 mL round-bottomed flask under argon atmosphere. A solution **15a** or **15b** (1 equiv.) in dry THF (20 mL) was added dropwise. After the exothermic reaction had subsided, the mixture was stirred at 55 °C for 3 h. Afterwards, the excess magnesium was removed under argon atmosphere and the Grignard solution was cooled to 0 °C. A freshly prepared solution of dilithium tetrachlorocuprate(II) (0.1 M in THF, 0.1 equiv.) was added with stirring. After a solution of **2a** (0.35–0.55 equiv.) in dry THF (70 mL) was added in one portion, stirring was continued for 20 h at room temperature or at 0 °C.

Variation B: Magnesium turnings (3 equiv.) were placed in a 100 mL round-bottomed flask under argon atmosphere. A solution **3b** (1 equiv.) in dry THF (20 mL) was added dropwise. After the exothermic reaction had subsided, the mixture was stirred at 55 °C for 3 h. Afterwards, the excess magnesium was removed under argon atmosphere and the Grignard solution was cooled to 0 °C. A freshly prepared solution of dilithium tetrachlorocuprate(II) (0.1 M in THF, 0.1 equiv.) was added with stirring. After a solution of **15c** (2.5 equiv.) in dry THF (70 mL) was added in one portion, stirring was continued for 20 h at room temperature or at 0 °C.

Both variations: For work-up, diethyl ether (150 mL) was added and the resulting mixture was poured into an ice-cold saturated solution of ammonium chloride (150 mL). The organic layer was separated and the aqueous phase was extracted several times with diethyl ether. The combined ethereal phases were washed with brine, dried over sodium sulphate and concentrated to dryness under reduced pressure. The crude product was purified by column chromatography using heptane/triethylamine/diethyl ether (98/0.5/1.5, v/v/v) as eluent yielding the bis(tetrahydropyranyl)ethers **16** as waxy substances.

2,2'-[(4*RS*,35*RS*)-Octatriacontane-4,35-diylbis(oxy)]bis-(tetrahydro-2*H*-pyran) (16a). Following the general procedure variation A, **15a** (7.85 g, 26.8 mmol), magnesium turnings (0.98 g, 40.3 mmol) and **3a** (4.29 g, 9.2 mmol) gave **16a** (2.95 g, 44% for the reaction at room temperature) as white solid. M.p. 51–52 °C; $C_{48}H_{94}O_4$ requires C, 78.41; H, 12.89; found: C, 78.38; H, 12.77; ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.86–0.96 (m, 6 H, 2× CH_3), 1.22–1.87 (m, 80 H, CH_2), 3.48 (dt, *J* = 10.7, 5.3 Hz, 2 H, 2× OCHOCH*H*), 3.60 (p, *J* = 5.4 Hz, 2 H, 2× C*H*Othp), 3.87–3.96 (m, 2 H, 2× OCHOC*H*H), 4.62–4.82 (m, 2 H, 2× OC*H*O); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.27 and 14.35 (*C*H$_3$), 18.32, 18.93, 19.97 and 19.99 (OCHO(CH$_2$)$_2$*C*H$_2$, *C*H$_2$CH$_3$), 25.01, 25.57 and 25.63 (OCHOCH$_2$*C*H$_2$, *C*H$_2$CH$_2$CHOthpCH$_2$*C*H$_2$), 29.60, 29.62, 29.65, 29.67, 29.69, 29.84, 29.61, 31.21 and 31.24 (OCHO(CH$_2$)$_3$*C*H$_2$), 33.52, 35.09, 35.87 and 37.28 (*C*H$_2$CHOthp*C*H$_2$) 62.66 and 62.68 (OCHO*C*H$_2$), 76.40 (*C*HOthp), 97.39 and 97.57 (O*C*HO); ESI-MS ***m/z*** 742.11 (M + Li), 757.82 (M + Na).

2,2'-[(7*RS*,38*RS*)-Tetratetracontane-7,38-diylbis(oxy)]bis-(tetrahydro-2*H*-pyran (16b). Following the general procedure variation B, **3b** (1.95 g, 7.2 mmol), magnesium turnings (0.52 g, 21.6 mmol) and **15c** (7.67 g, 17.7 mmol) gave **16b** (3.41 g, 59% for the reaction at room temperature) as white solid. M.p. 53–54 °C; $C_{54}H_{106}O_4$ requires C, 79.15; H, 13.04; found: C, 78.97; H, 13.35; ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.84–0.92 (m, 6 H, 2× CH_3), 1.23–1.90 (m, 92 H, CH_2), 3.42–3.51 (m, 2 H, 2× OCHOCH*H*), 3.59 (p, *J* = 5.8 Hz, 2 H, 2× C*H*Othp), 3.86–3.98 (m, 2 H, 2× OCHOC*H*H), 4.60–4.70 (m, 2 H, 2× OC*H*O); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.06 and 14.08 (*C*H$_3$), 19.95 (OCHO(CH$_2$)$_2$*C*H$_2$), 22.65 and 22.67 (*C*H$_2$CH$_3$), 24.99, 25.01, 25.58, 25.61 and 25.64 (OCHOCH$_2$*C*H$_2$, *C*H$_2$CH$_2$CHOthpCH$_2$*C*H$_2$), 29.51, 29.58, 29.61, 29.62, 29.65, 29.69, 29.84, 29.90, 31.22, 31.86 (*C*H$_2$CH$_2$CH$_3$), 33.49 (OCHO(CH$_2$)$_3$*C*H$_2$), 35.05 and 35.41 (*C*H$_2$CHOthp*C*H$_2$) 62.62 (OCHO*C*H$_2$), 76.71 (*C*HOthp), 97.43 (O*C*HO); ESI-MS *m/z* 826.18 (M + Li), 841.88 (M + Na).

2,2'-[(10*RS*,41*RS*)-Pentacontane-10,41-diylbis(oxy)]bis-(tetrahydro-2*H*-pyran) (16c). Following the general procedure variation A, **15b** (4.52 g, 12.0 mmol), magnesium turnings (0.44 g, 18.1 mmol) and **3a** (2.79 g, 6.0 mmol) gave **16c** (0.70 g, 13% for the reaction at 0 °C) as white solid. M.p. 56–58 °C; $C_{60}H_{118}O_4$ requires C, 79.75; H, 13.16; found: C, 79.39; H, 13.38; ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.84–0.92 (m, 6 H, 2× CH_3), 1.23–1.91 (m, 104 H, CH_2), 3.44–3.52 (m, 2 H, 2× OCHOCH*H*), 3.59 (p, *J* = 5.8 Hz, 2 H, 2× C*H*Othp), 3.86–3.98 (m, 2 H, 2× OCHOC*H*H), 4.65 (t, *J* = 3.7 Hz, 2 H, 2× OC*H*O); ^{13}C NMR (100 MHz, $CDCl_3$, 27 °C) δ 14.08 (*C*H$_3$), 19.96 (OCHO(CH$_2$)$_2$*C*H$_2$), 22.67 (*C*H$_2$CH$_3$), 25.01, 25.58 and 25.64 (OCHOCH$_2$*C*H, *C*H$_2$CH$_2$CHOthpCH$_2$*C*H$_2$), 29.31, 29.32, 29.60, 29.61, 29.62, 29.65, 29.69, 29.84, 29.90, 31.22, 31.89 (*C*H$_2$CH$_2$CH$_3$), 33.48 (OCHO(CH$_2$)$_3$*C*H$_2$), 35.03 (*C*H$_2$CHOthp*C*H$_2$) 62.62 (OCHO*C*H$_2$), 76.69 (*C*HOthp), 97.42 (O*C*HO); ESI-MS *m/z* 926.65 (M + Na).

General procedure for the synthesis of 1,32-alkyl branched 1,32-diols 17

View Article Online
DOI: 10.1039/C8OB00424B

The bis(tetrahydropyranyl)ether **16** and catalytic amounts of pyridinium *p*-toluenesulfonate were suspended in dry methanol (50 mL) and the mixture was heated under reflux for at least 3 h until a white precipitate appeared and no educt is detectable via TLC. The hot suspension was filtered off.

(4*RS*,35*RS*)-Octatriacontane-4,35-diol (17a). Following the general procedure, **16a** (2.95 g, 4.0 mmol) gave **17a** (2.01 g, 89%) as white solid. M.p. 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C) δ 0.92–0.99 (m, 6 H, 2× CH_3), 1.26–1.59 (m, 68 H, CH_2), 3.58–3.64 (m, 2 H, 2× C*H*OH); ^{13}C NMR (125 MHz, $CDCl_3$, 50 °C) δ 14.00 (*C*H_3), 18.75 (*C*H_2CH$_3$), 25.59 (*C*H_2CH$_2$CHOH(CH$_2$)$_2$CH$_3$), 29.55, 29.56, 29.59, 29.60, 29.62, 37.54 (*C*H_2CHOH(CH$_2$)$_2$CH$_3$), 39.71 (*C*H_2CH$_2$CH$_3$), 71.71 (*C*HOH); APCI-MS *m/z* 531.6 (M – 2× H_2O + H).

(7*RS*,38*RS*)-Tetratetracontane-7,38-diol (17b). Following the general procedure, **16b** (3.41 g, 4.2 mmol) gave **17b** (2.38 g, 88%) as white solid. M.p. 111–113 °C; ^{1}H NMR (500 MHz, $CDCl_3$, 50 °C) δ 0.90 (t, *J* = 6.5 Hz, 6 H, 2× CH_3), 1.22–1.54 (m, 80 H, CH_2), 3.55–3.70 (m, 2 H, 2× C*H*OH); ^{13}C NMR (125 MHz, $CDCl_3$, 50 °C) δ 13.92 (*C*H_3), 22.53 (*C*H_2CH$_3$), 25.55 and 25.60 (*C*H_2CH$_2$CHOHCH$_2$*C*H$_2$), 29.31, 29.55, 29.56, 29.59, 29.61, 29.62, 29.67, 31.79 (*C*H_2CH$_2$CH$_3$), 37.52 (*C*H_2CHOH*C*H$_2$), 71.99 (*C*HOH); APCI-MS *m/z* 615.7 (M – 2× H_2O + H).

(10*RS*,41*RS*)-Pentacontane-10,41-diol (17c). Following the general procedure, **16c** (0.70 g, 0.78 mmol) gave **17c** (0.50 g, 88%) as white solid. M.p. 115–116 °C; ^{1}H NMR (500 MHz, $CDCl_3$, 50 °C) δ 0.90 (t, *J* = 6.5 Hz, 6 H, 2× CH_3), 1.05–1.56 (m, 92 H, CH_2), 3.55–3.65 (m, 2 H, 2× C*H*OH); ^{13}C NMR (125 MHz, $CDCl_3$, 50 °C) δ 13.94 (*C*H_3), 22.58 (*C*H_2CH$_3$), 25.60 (*C*H_2CH$_2$CHOHCH$_2$*C*H$_2$), 29.24, 29.50, 29.56, 29.62, 29.67, 31.83 (*C*H_2CH$_2$CH$_3$), 37.51 (*C*H_2CHOH*C*H$_2$), 71.98 (*C*HOH); APCI-MS *m/z* 699.7 (M – 2× H_2O + H).

General procedure for the synthesis of bis(phosphocholines)

2-Bromoethylphosphoric acid dichloride (8 equiv.) was poured into dry chloroform (15 mL) whilst cooling with ice/water. A mixture of dry triethylamine (14 equiv.) in dry chloroform (15 mL) was added slowly with stirring, which was continued for further 30 min at 0 °C. Diol **17** (1 equiv.) was poured in one portion into the mixture. To dissolve the solid diol, the reaction mixture was slowly heated to 50 °C until a clear solution appeared and was stirred for 24 h at 50 °C. Afterwards, the mixture cooled to room temperature and stirred for further 72 h. After the complete conversion of the diol, crushed ice was added and the mixture was stirred vigorously for 2 h. The organic layer was separated and the aqueous phase was extracted several times with chloroform (3 x 50 mL). The combined organic phases were concentrated under reduced pressure, and the oily brownish residue was dissolved in THF/water (9/1, v/v, 20 mL). After 1 h of stirring, the solvent was evaporated and the crude bromo esters were transferred into a mixture of dry chloroform (30 mL), dry acetonitrile (30 mL). A ethanolic solution of trimethylamine (20 equiv.) was added slowly, and the mixture was kept in a closed tube while warming up to 50 °C for 48 h. The solution was allowed to stand for 7 days at room temperature. Afterwards, the reaction mixture was evaporated to dryness and the crude bolalipid was purified by MPLC using chloroform/methanol/water and the gradient technique.

(1*RS*,32*RS*)-1,32-Dipropyldotriacontane-1,32-diylbis[2-trimethylammonio)ethyl phosphate] (PC-C32(1,32C3)-PC). Following the general procedure, **17a** (1.00 g, 1.76 mmol) gave **PC-C32(1,32C3)-PC** (0.17 g, 11%). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, 27 °C) δ 0.80–0.91 (m, 6 H, 2×x CH_3), 1.16–1.57 (m, 68 H, CH_2), 3.13–3.18 (m, 18 H, 2× N(CH_3)$_3$), 3.46–3.56 (m, 4 H, 2× OCH$_2$CH_2N), 4.07–4.21 (m, 6 H, 2× OCH_2CH$_2$N, 2× C*H*O); ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD, 27 °C): δ 13.90 and 13.93 (*C*H_3), 18.10 (*C*H_2CH$_3$), 24.97 (*C*H_2CH$_2$CHO(CH$_2$)$_2$CH$_3$), 29.57, 29.60 and 29.79 (*C*H_2), 35.12 and 35.16 (*C*H_2CHO(CH$_2$)$_2$CH$_3$), 37.16 and 37.20 (*C*H_2CH$_2$CH$_3$), 54.09 (N(*C*H$_3$)$_3$), 58.58 and 58.63 (O*C*H$_2$CH$_2$N), 66.54 (OCH$_2$*C*H$_2$N), 76.76 and 76.83 (*C*HO); ESI-MS *m/z* 897.66 (M + H), 919.84 (M + Na); HRMS *m/z* calcd. for $C_{48}H_{104}N_2O_8P_2$ (M + 2H) 449.3629; found: 499.3648; calcd. for $C_{48}H_{103}N_2O_8P_2$ (M + H) 897.7184; found: 897.7223; HPLC t_R = 7.3 min, purity: 98.3 %.

(1*RS*,32*RS*)-1,32-Dihexyldotriacontane-1,32-diylbis[2-trimethylammonio)ethyl phosphate] (PC-C32(1,32C6)-PC). Following the general procedure, **17b** (0.20 g, 0.31 mmol) gave **PC-C32(1,32C6)-PC** (0.10 g, 33%). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD, 27 °C) δ 0.82–0.89 (m, 6 H, 2× CH_3), 1.20–1.62 (m, 80 H, CH_2), 3.18 (s, 18 H, 2× N(CH_3)$_3$), 3.53–3.59 (m, 4 H, 2× OCH$_2$CH_2N), 4.12–4.23 (m, 6 H, 2× OCH_2CH$_2$N, 2× C*H*O); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD, 27 °C): δ 13.83 and 13.87 (*C*H_3), 22.52 (*C*H_2CH$_3$), 24.86 and 24.92 (*C*H_2CH$_2$CHOCH$_2$*C*H$_2$), 29.38, 29.54, 29.60 and 29.76 (*C*H_2), 31.76 (*C*H_2CH$_2$CH$_3$), 34.96, 34.99 and 35.01 (*C*H_2CHO*C*H$_2$), 54.12 (N(*C*H$_3$)$_3$), 58.75 and 58.78 (O*C*H$_2$CH$_2$N), 66.50 and 66.51 (OCH$_2$*C*H$_2$N), 76.96, 77.21 and 77.47 (*C*HO); ESI-MS *m/z* 981.81 (M + H), 1003.78 (M + Na); HRMS *m/z* calcd for $C_{54}H_{115}N_2O_8P_2$ (M + H) 981.8123; found: 981.8111; HPLC t_R = 4.9 min, purity: 95.8 %.

(1*RS*,32*RS*)-1,32-Dinonyldotriacontane-1,32-diylbis[2-trimethylammonio)ethyl phosphate] (PC-C32(1,32C9)-PC). Following the general procedure, **17c** (0.50 g, 0.68 mmol) gave **PC-C32(1,32C9)-PC** (0.15 g, 21%). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD, 27 °C) δ 0.84 (t, *J* = 6.9 Hz, 6 H, 2× CH_3), 1.18–1.37 (m, 84 H, CH_2), 1.48–1.58 (m, 8 H, 2× CH_2CHOCH_2), 3.19 (s, 18 H, 2× N(CH_3)$_3$), 3.55–3.61 (m, 4 H, 2× OCH$_2$CH_2N), 4.11–4.21 (m, 6 H, 2× OCH_2CH$_2$N, 2× C*H*O); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD, 27 °C): δ 13.90 (*C*H_3), 22.56 (*C*H_2CH$_3$), 24.94 (*C*H_2CH$_2$CHOCH$_2$*C*H$_2$), 29.24, 29.55, 29.60, 29.62, 29.78 and 29.79 (*C*H_2), 31.81 (*C*H_2CH$_2$CH$_3$), 34.98 and 35.01 (*C*H_2CHO*C*H$_2$), 54.13 (N(*C*H$_3$)$_3$), 58.76 and 58.80 (O*C*H$_2$CH$_2$N), 66.49 (OCH$_2$*C*H$_2$N), 77.12 and 77.17 (*C*HO); ESI-MS *m/z* 1065.98 (M + H), 1087.96 (M + Na); HRMS *m/z* calcd for $C_{60}H_{128}N_2O_8P_2$ (M + 2H) 533.4568; found: 533.4564; calcd for $C_{60}H_{127}N_2O_8P_2$ (M + H) 1065.9062; found: 1065.9051; HPLC t_R = 3.4 min, purity: 96.4 %.

Physicochemical characterisation

Sample preparation. The bolalipid was suspended in H_2O (Milli-Q Millipore water). Homogeneous suspensions were obtained by heating and vortexing.

DSC. As described previously,[64] DSC measurements were performed using a MicroCal VP-DSC differential scanning calo-

View Article Online
DOI: 10.1039/C8OB00424B

rimeter (MicroCal Inc. Northampton, MA, USA). Before the measurements, the sample suspension (c = 1 mg mL^{-1}) and the water reference were degassed under vacuum while stirring. A heating rate of 60 K h^{-1} was used, and the measurements were performed in the temperature interval from 5 °C to 90 °C. To check the reproducibility, three consecutive scans were recorded for each sample. The water/water baseline was subtracted from the thermogram of the sample, and the DSC scans were evaluated using MicroCal Origin 8.0 software.

Turbidity measurements. Transmission measurements were performed using a Litesizer 500 (Anton Paar GmbH, Graz, Austria). The sample suspension (c = 1 mg mL^{-1} in water) was set in a low volume quartz cuvette (Hellma Analytics, Müllheim, Germany) and the measurement was performed in the temperature range from 20 °C to 80 °C for **PC-C32(1,32C3)-PC**, from 10 °C to 60 °C in case of **PC-C32(1,32C6)-PC**, and from 10 °C to 50 °C for **PC-C32(1,32C9)-PC** with ΔT = 1 K. At each temperature, the system was allowed to equilibrate for 5 min. Afterwards, the transmission at λ = 658 nm was measured for 2 min and averaged. Pure water was used as reference.

TEM. In a similar manner to a procedure from an earlier publication,[65] samples were prepared by spreading 5 µL of the bolalipid suspension (c = 0.05 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 after 1 min. Specimens prepared below ambient temperature (T ≈ 7 °C) were dried for at least one day at this temperature and kept in a desiccator at room temperature. Specimens, which were prepared in a modified drying oven above ambient temperature, were further dried for 1 h at the appropriate temperature and finally kept in a desiccator at room temperature. All specimens were examined with a Zeiss EM 900 transmission electron microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was financially supported by grants from the Deutsche Forschungsgemeinschaft (DFG), project DR 1024/1-1 (to K.G. and S.D.) and by grants from the Phospholipid Research Center Heidelberg (to S.M.). The support of Dr. Gerd Hause (Biocenter, MLU Halle-Wittenberg) by providing us access to the electron microscope facility is greatly appreciated. The authors thank Annekatrin Rother (Institute of Biochemistry and Biotechnology, MLU Halle-Wittenberg) for technical assistance during electron microscopy. Finally, S.D. like to thank Prof. Dr. Bodo Dobner (Institute of Pharmacy, MLU) and Prof. Dr. Alfred Blume (Institute of Chemistry, MLU) for support and discussions.

Notes and references

1 J.-H. Fuhrhop and T. Wang, *Chem. Rev.*, 2004, **104**, 2901-2937.
2 T. A. Langworthy, *Biochim. Biophys. Acta, Lipids Lipid Metab.*, 1977, **487**, 37-50.
3 M. De Rosa, E. Esposito, A. Gambacorta, B. Nicolaus and J. D. Bu'Lock, *Phytochemistry*, 1980, **19**, 827-831.
4 A. Gambacorta, A. Gliozzi and M. Rosa, *World J. Microbiol. Biotechn.*, 1995, **11**, 115-131.
5 C. R. Woese, L. J. Magrum and G. E. Fox, *J. Mol. Evol.*, 1978, **11**, 245-252.
6 W. Baumeister and G. Lembcke, *J. Bioenerg. Biomemb.*, 1992, **24**, 567-575.
7 B. A. Cornell, V. B. L. Braach-Maksvytis, L. G. King, P. D. J. Osmann, B. Raguse, W. L. and R. J. Pace, *Nature*, 1997, **387**, 580-583.
8 N. Nuraje, H. Bai and K. Su, *Progress in Polymer Science*, 2013, **38**, 302-343.
9 D. A. Brown, B. Venegas, P. H. Cooke, V. English and P. L.-G. Chong, *Chem. Phys. Lipids*, 2009, **159**, 95-103.
10 N. Jain, Y. Arntz, V. r. Goldschmidt, G. Duportail, Y. Mély and A. S. Klymchenko, *Bioconjugate Chem.*, 2010, **21**, 2110-2118.
11 L. Krishnan, L. Deschatelets, F. C. Stark, K. Gurnani and G. D. Sprott, *Clinical and Developmental Immunology*, 2010, **2010**, 1-13.
12 A. Jacquemet, J. Barbeau, L. Lemiègre and T. Benvegnu, *Biochimie*, 2009, **91**, 711-717.
13 G. Mahmoud, J. Jedelska, B. Strehlow and U. Bakowsky, *Eur. J. Pharm. Biopharm.*, 2015, **95**, 88-98.
14 P. Uhl, F. Helm, G. Hofhaus, S. Brings, C. Kaufman, K. Leotta, S. Urban, U. Haberkorn, W. Mier and G. Fricker, *Eur. J. Pharm. Biopharm.*, 2016, **103**, 159-166.
15 A. C. Jacobsen, S. M. Jensen, G. Fricker, M. Brandl and A. H. Treusch, *Eur J Pharm Sci*, 2017, **108**, 101-110.
16 G. Leriche, J. L. Cifelli, K. C. Sibucao, J. P. Patterson, T. Koyanagi, N. C. Gianneschi and J. Yang, *Org. Biomol. Chem.*, 2017, **15**, 2157-2162.
17 V. Paolucci, G. Leriche, T. Koyanagi and J. Yang, *Bioorg. Med. Chem. Lett.*, 2017.
18 P. Uhl, S. Pantze, P. Storck, J. Parmentier, D. Witzigmann, G. Hofhaus, J. Huwyler, W. Mier and G. Fricker, *Eur. J. Pharm. Sci.*, 2017, **108**, 111-118.
19 T. Eguchi, K. Arakawa, T. Terachi and K. Kakinuma, *J. Org. Chem.*, 1997, **62**, 1924-1933.
20 T. Eguchi, K. Ibaragi and K. Kakinuma, *J. Org. Chem.*, 1998, **63**, 2689-2698.
21 K. Arakawa, T. Eguchi and K. Kakinuma, *J. Org. Chem.*, 1998, **63**, 4741-4745.
22 T. Benvegnu, M. Brard and D. Plusquellec, *Curr. Opin. Colloid Interface Sci.*, 2004, **8**, 469-479.
23 T. Markowski, S. Drescher, A. Meister, G. Hause, A. Blume and B. Dobner, *Eur. J. Org. Chem.*, 2011, 5894-5904.
24 A. Meister and A. Blume, *Curr. Opin. Colloid Interface Sci.*, 2007, **12**, 138-147.
25 T. Markowski, S. Drescher, A. Meister, A. Blume and B. Dobner, *Org. Biomol. Chem.*, 2014, **12**, 3649-3662.
26 K. Köhler, G. Förster, A. Hauser, B. Dobner, U. F. Heiser, F. Ziethe, W. Richter, F. Steiniger, M. Drechsler, H. Stettin and A. Blume, *Angew. Chem. Int. Ed.*, 2004, **43**, 245-247.
27 K. Köhler, G. Förster, A. Hauser, B. Dobner, U. F. Heiser, F. Ziethe, W. Richter, F. Steiniger, M. Drechsler, H. Stettin and A. Blume, *J. Am. Chem. Soc.*, 2004, **126**, 16804-16813.
28 M. Wahab, P. Schiller, R. Schmidt and H. J. Moegel, *Langmuir*, 2010, **26**, 2979-2982.
29 A. Meister, S. Drescher, I. Mey, M. Wahab, G. Graf, V. M. Garamus, G. Hause, H.-J. Mögel, A. Janshoff, B. Dobner and A. Blume, *J. Phys. Chem. B*, 2008, **112**, 4506-4511.

View Article Online
DOI: 10.1039/C8OB00424B

30 A. Meister, K. Köhler, S. Drescher, B. Dobner, G. Karlsson, K. Edwards, G. Hause and A. Blume, *Soft Matter*, 2007, **3**, 1025-1031.
31 S. Drescher, A. Meister, G. Graf, G. Hause, A. Blume and B. Dobner, *Chem. Eur. J.*, 2008, **14**, 6796-6804.
32 G. Graf, S. Drescher, A. Meister, V. M. Garamus, B. Dobner and A. Blume, *J. Coll. Interf. Sci.*, 2013, **393**, 143-150.
33 A. Blume, S. Drescher, A. Meister, G. Graf and B. Dobner, *Faraday Disc.*, 2013, **161**, 193-213.
34 S. Drescher, K. Helmis, A. Langner and B. Dobner, *Monatsh. Chem.*, 2010, **141**, 339-349.
35 S. Drescher, S. Becker, B. Dobner and A. Blume, *RSC Adv.*, 2012, **2**, 4052-4054.
36 S. Drescher, S. Sonnenberger, A. Meister, A. Blume and B. Dobner, *Monatsh. Chem.*, 2012, **143**, 1533-1543.
37 S. Drescher, B.-D. Lechner, V. M. Garamus, L. Almásy, A. Meister and A. Blume, *Langmuir*, 2014, **30**, 9273-9284.
38 S. Drescher, V. M. Garamus, C. J. Garvey, A. Meister and A. Blume, *Beilstein J. Org. Chem.*, 2017, **13**, 995-1007.
39 A. Blume, S. Drescher, G. Graf, K. Köhler and A. Meister, *Adv. Colloid Interf. Sci.*, 2014, **208**, 264-278.
40 I. Grechishnikova, L. B.-A. Johansson and J. G. Molotkovsky, *Chem. Phys. Lipids*, 1996, **81**, 87-98.
41 R. McKiernan, S. P. Gido and J. Penelle, *Polymer*, 2002, **43**, 3007-3017.
42 M. Tamura and J. Kochi, *Synthesis*, 1971, 303-305.
43 U. F. Heiser and B. Dobner, *J. Chem. Soc., Perkin Trans. 1*, 1997, 809-815.
44 S. Drescher, A. Meister, A. Blume, G. Karlsson, M. Almgren and B. Dobner, *Chem. Eur. J.*, 2007, **13**, 5300-5307.
45 W. Mohr, C. R. Horn, J. Stahl and J. A. Gladysz, *Synthesis*, 2003, 1279-1285.
46 M. Schwarz, J. E. Oliver and P. E. Sonnet, *J. Org. Chem.*, 1975, **40**, 2410-2411.
47 A. Mosandl and C. Günther, *Journal of Agricultural and Food Chemistry* 1989, **37**, 413-418.
48 M. Miyashita, A. Yoshikoshi and P. A. Grieco, *J. Org. Chem.*, 1977, **42**, 3772-3774.
49 G. Schill and C. Merkel, *Chem. Ber.*, 1978, **111**, 1446-1452.
50 C.-T. Yang, Z.-Q. Zhang, J. Liang, J.-H. Liu, X.-Y. Lu, H.-H. Chen and L. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 11124-11127.
51 G. Fouquet, M. Schlosser, W. Malisch and M. Kuhn, *Angew. Chem.*, 1974, **86**, 50-51.
52 M. Nakamura, K. Matsuo, S. Ito and E. Nakamura, *J. Am. Chem. Soc.*, 2004, **126**, 3686-3687.
53 V. Popsavin, O. Berić, M. Popsavin, L. Radić, J. Csanádi and V. Ćirin-Novta, *Tetrahedron*, 2000, **56**, 5929-5940.
54 L. De Luca, G. Giacomelli and A. Porcheddu, *J. Org. Chem.*, 2002, **67**, 5152-5155.
55 L. De Luca, G. Giacomelli and A. Porcheddu, *Org. Lett.*, 2002, **4**, 553-555.
56 T. Oishi, K. Ando, K. Inomiya, H. Sato, M. Iida and N. Chida, *Bulletin of the Chemical Society of Japan*, 2002, **75**, 1927-1947.
57 H. Eibl and A. Nicksch, Ger. Offen. DE 2345057 A1 19750327, 1975 (CAN:83:9233), 27.03.1975.
58 G. Hirt and R. Berchtold, *Pharm. Acta Helv.*, 1958, **33**, 349-356.
59 P. Nuhn, G. Brezesinski, B. Dobner, G. Förster, M. Gutheil and H.-D. Dörfler, *Chem. Phys. Lipids*, 1986, **39**, 221-236.
60 G. Graf, S. Drescher, A. Meister, V. M. Garamus, B. Dobner and A. Blume, *Soft Matter*, 2013, **9**, 9562-9571.
61 X.-H. Yang, K. Wang, S.-F. Zhu, J.-H. Xie and Q.-L. Zhou, *J. Am. Chem. Soc.*, 2014, **136**, 17426-17429.
62 I. A. Baser, *Fette, Seifen, Anstrichmittel*, 1972, **74**, 524-526.
63 P. S. G. Tassignon, D. de Wit, T. C. de Rijk and L. F. De Buyck, *Tetrahedron*, 1995, **51**, 11863-11872.
64 S. Lindner, K. Gruhle, R. Schmidt, V. M. Garamus, D. Ramsbeck, G. Hause, A. Meister, A. Sinz and S. Drescher, *Langmuir*, 2017, **33**, 4960-4973.
65 T. Markowski, S. Müller, B. Dobner, A. Meister, A. Blume and S. Drescher, *Polymers*, 2017, **9**, 573.

TOC-graphic

View Article Online
DOI: 10.1039/C8OB00424B

The syntheses of three single-chain, alkyl-branched bolalipids—using a Grignard bis-coupling reaction as key step—and first investigations of the lyotropic behaviour of these lipids are reported.