Monatsh Chem
DOI 10.1007/s00706-012-0833-2

ORIGINAL PAPER

Synthesis of symmetrical, single-chain, phenylene/biphenylene-modified bolaamphiphiles

Simon Drescher · Stefan Sonnenberger · Annette Meister · Alfred Blume · Bodo Dobner

Received: 29 March 2012 / Accepted: 7 August 2012

Abstract Two new, symmetrical, phenylene- or biphenylene-modified bolaamphiphiles bearing two phosphocholine headgroups and an alkyl spacer chain length of 32 and 36 carbon atoms, respectively, have been synthesised. The key step was the Cu(II)-catalysed Grignard reaction used either as a simultaneous bis-coupling procedure or in a stepwise homo-coupling. Particularly with the use of the homo-coupling, we were able to separate the phenylene-free by-products from the desired products. This homo-coupling additionally offered the possibility of preparing unsymmetrical bolaamphiphiles. Conversion of the diols into bipolar phospholipids was achieved by bis-phosphorylation with β-bromoethylphosphoric acid dichloride and subsequent quarternisation with trimethylamine. Unlike previous studies with aliphatic bolaamphiphiles that formed flexible nanofibres in aqueous suspension, the bolaamphiphiles of the present study, containing phenylene- and biphenylene groups in the middle part of the alkyl spacer chain, formed small ellipsoidal aggregates at ambient temperature.

Keywords Grignard reactions · Lipids · Bolaamphiphiles · Nanostructures · Phosphates

Electronic supplementary material The online version of this article (doi:10.1007/s00706-012-0833-2) contains supplementary material, which is available to authorized users.

S. Drescher (✉) · S. Sonnenberger · B. Dobner
Institute of Pharmacy, Martin-Luther-University Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, 06120 Halle (Saale), Germany
e-mail: simon.drescher@pharmazie.uni-halle.de

A. Meister · A. Blume
Institute of Chemistry, Martin-Luther-University Halle-Wittenberg, von-Danckelmann-Platz 4, 06120 Halle (Saale), Germany

Introduction

Bipolar amphiphiles (bolaamphiphiles, BAs) [1] are naturally found in the cell membranes of certain species of archaebacteria, e.g. methanogenic and thermoacidophilic *Archaea*. These membrane lipids are the consequence of adaptations to the extreme living conditions of the *Archaea*, such as high temperatures or low pH values. Owing to their lipid composition, membranes of certain *Archaea* efficiently protect the cytoplasm while maintaining the fluid state that is important for transport and signal transduction. This stabilisation is achieved by virtue of the substantially different chemical structure of the membrane constituents compared to common phospholipids: they are composed of two hydrophilic headgroups, arranged on both sides of the cell membrane [2, 3], connected to one or two lipophilic membrane-spanning alkyl chains. Fluidisation of *Archaea*'s cell membranes is tuned by the addition of several methyl branches or various numbers of cyclopentane rings within the alkyl chain of the BAs. The resulting chemical and thermal stability as well as the tendency to form closed vesicles make this class of amphiphiles applicable in biotechnology, material science and pharmacy [4–9]; e.g. inherently stable archaeosomes are an alternative to conventional liposomes for use as drug delivery systems [10]. In addition, it has been shown that natural and synthetic BAs have a stabilizing effect on phospholipid vesicles [11]. Because of the laborious isolation procedures of archaebacterial lipids, generally resulting in mixtures of lipids with different alkyl chain lengths, and the time-consuming synthesis of natural BAs, less complex and well-defined model substances are required for systematic studies on the interactions of these BAs with conventional phospholipids.

Over the last decade, considerable efforts have been devoted to the synthesis of novel bipolar archaebacterial

Published online: 06 September 2012

model compounds [11–13]. Recently, we reported the synthesis and temperature-dependent aggregation behaviour of symmetrical, single-chain, polymethylene-1,ω-bis(phosphocholine)s (PC-Cn-PC)—a very simple archaebacterial model lipid with hydrocarbon chain lengths (n) of 22–32 carbon atoms and two phosphocholine (PC) headgroups attached at both ends [14]. It has been shown that this class of single-chain BAs self-assembles into well-defined long nanofibres and micelles, depending on temperature and concentration [14–16]. The thickness of the fibres corresponds roughly to the molecular length of the BAs. Within the fibres, the bola molecules are aligned side by side, but the bulky headgroups induce a twisted arrangement, causing a helical superstructure of the fibre, which could be imaged recently by atomic force microscopy (AFM) experiments [17].

Following the concept of archaebacterial lipids, we firstly tried to incorporate the single-chain BA PC-C32-PC into vesicles of common phospholipids, such as 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) or 1-palmitoyl-2-oleolyl-sn-glycero-3-phosphocholine (POPC). However, only a small amount of the bolalipids could be incorporated into bilayers [18]. The reasons for this behaviour are packing problems caused by the larger space requirement of the PC headgroup in comparison to the small cross-sectional area of the long alkyl chain [18]. Consequently, a membrane-spanning conformation of the PC-C32-PC in a lipid bilayer is energetically unfavourable, because of the resulting void volume which cannot be filled by the alkyl chains of the bilayer-forming lipid. To overcome these packing problems two structural changes of the BA are conceivable: (a) one can minimise the space requirement of the headgroup by a stepwise replacement of the methyl groups leading to phosphodimethylethanolamines (Me_2PE-Cn-Me_2PE), which form nanofibres [19, 20] or square lamellae [21], and the phosphomonomethylethanolamines (MePE-C32-MePE) which self-assemble into sheet-like structures [22]; (b) one can expand the cross-sectional area of the alkyl chains in order to fill the void volume. The concept of our work presented herein adapts the latter idea, namely the insertion of a phenylene and biphenylene ring system [23, 24], respectively, into the middle part of the long alkyl chain while keeping the headgroup unchanged and comparable to the headgroups of common phospholipids, such as 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), DPPC or POPC (except the presence of the glycerol moiety). Figure 1 depicts the target structures of the BAs synthesised in this work, which include an alkyl chain with an overall length of 36 or 32 carbon atoms and two phosphocholine headgroups. These spacer lengths were chosen in order to approximately span bilayer membranes comprising DSPC (two times C18) and DPPC (two times C16), respectively.

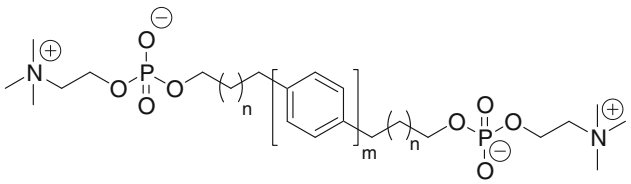


Fig. 1 Chemical structure of phenylene/biphenylene-modified bola-amphiphiles presented in this work (**1**, $m = 1$, $n = 14$; **2**, $m = 2$, $n = 10$)

We present herein two synthetic approaches to symmetrical, single-chain, phenylene/biphenylene-modified BAs (**1**, **2**), using a copper(II)-catalysed Grignard reaction as the key step as well as the first investigations into the aggregation behaviour of these compounds by transmission electron microscopy (TEM).

Results and discussion

The preparation of long-chain BAs depends on an effective synthetic approach for the corresponding diols which represent the hydrophobic part of the bola molecules. Unmodified 1,ω-diols with 22 or more carbon atoms have already been synthesised by well-known multistep procedures including (1) bis-acylation of cyclic enamines with dicarboxylic acid dichlorides, (2) hydrolysis of enamino ketones and subsequent ring opening, (3) Wolff–Kishner reduction of bis(oxoacid)s and subsequent reduction with $LiAlH_4$, or (4) double Wittig reaction with bis(phosphorylide)s and ω-functionalised aldehydes [25, 26]. Our synthetic approaches use the Grignard reaction under catalysis by Li_2CuCl_4 [27], which can be designed as a homo-coupling [28, 29] or bis-coupling reaction [14–16]. The latter ones use the Grignard reagent and 1,ω-dibromides in a molar ratio of 2:1 (Fig. 2a, b).

Grignard bis-coupling

For the preparation of the phenylene-modified diols we adapted the Grignard bis-coupling [14] replacing the 1,ω-dibromoalkanes by phenylene- or biphenylene dihalides. The synthesis started from tetrahydropyranyl (thp)-protected ω-bromoalcohols **3**, which were transformed into the corresponding Grignard reagent by treatment with magnesium in dry THF at 50 °C. After removing the excess of magnesium the Grignard solution reacts with 1,4-bis(chloromethyl)benzene (**4a**), 1,4-bis(bromomethyl)-benzene (**4b**) and 4,4′-bis(chloromethyl)-1,1′-biphenyl (**4c**), respectively, under Li_2CuC_4 catalysis yielding either 2,2′-[1,4-phenylenebis(hexadecane-16,1-diyloxy)]bis(tetrahydro-2*H*-pyran) (**5a**) or 2,2′-[biphenyl-4,4′-diylbis(dodecane-12,1-diyloxy)]bis(tetrahydro-2*H*-pyran) (**5b**; Scheme 1).

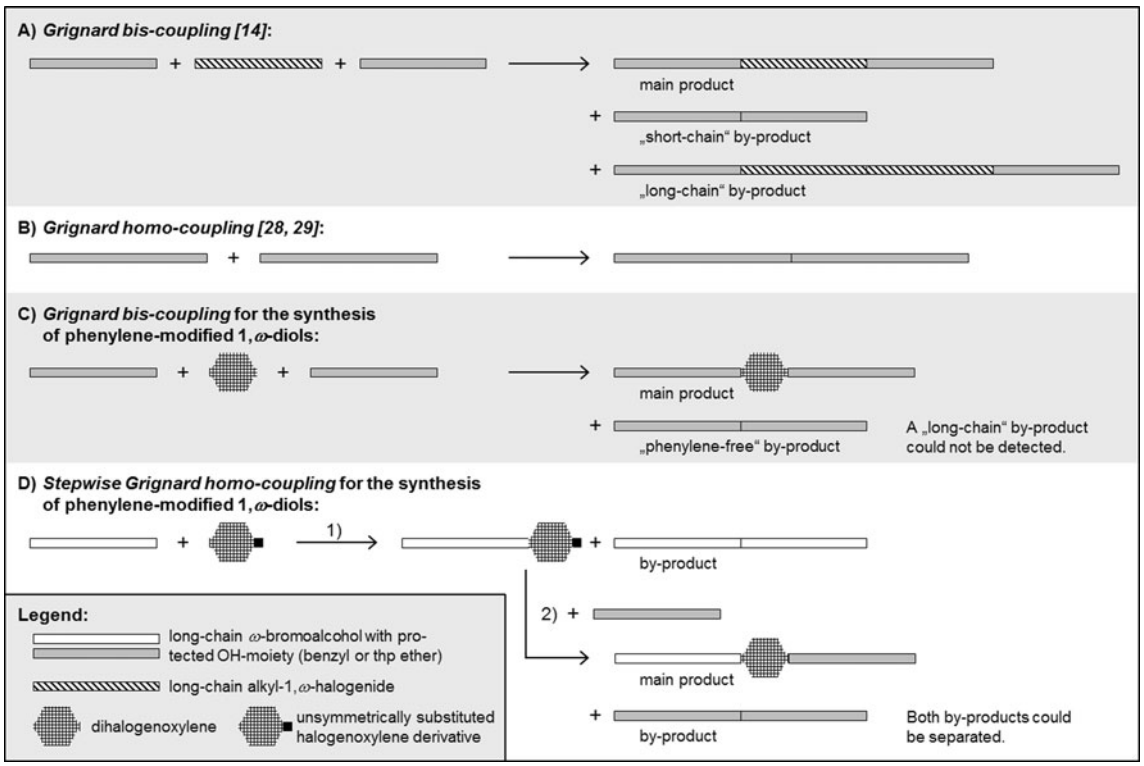
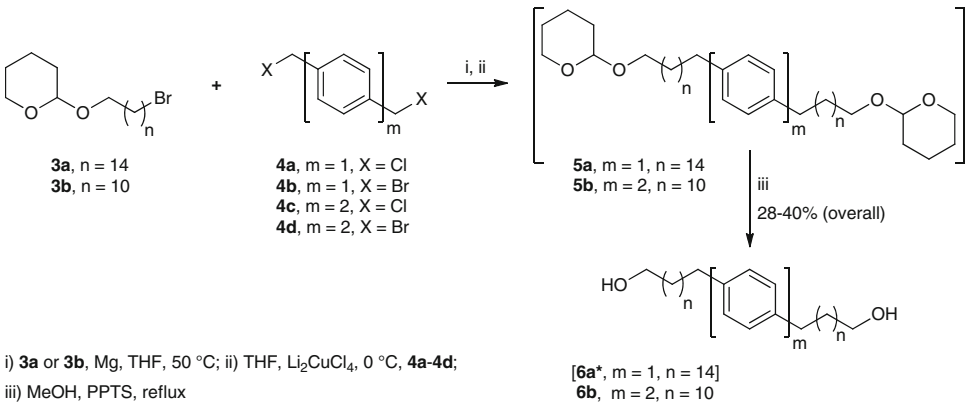


Fig. 2 Schematic overview of Grignard coupling reactions: **a** Grignard bis-coupling [14] and **b** Grignard homo-coupling [28, 29] of long-chain alkyl bromides; **c** Grignard bis-coupling and **d** stepwise Grignard homo-coupling for the synthesis of phenylene/biphenylene-modified bolaamphiphiles presented in this work

Scheme 1



One general drawback of these bis-coupling reactions is the formation of 'short-chain' by-products (Fig. 2a) resulting from the reaction of the Grignard reagent of compound **3a**, **3b** with unreacted bromide **3a**, **3b** [14]. In the present case, we obtained the bis(tetrahydro-2*H*-pyran)s of triacontane-1,30-diol and docosane-1,22-diol, respectively, depending on the bromides used. The separation of these by-products from the target compounds **5** failed because of their very similar chromatographic properties and, hence, we used the crude bis(tetrahydro-2*H*-pyran)s **5** without further purification. Unexpectedly, we did not detect the formation of 'long-chain' by-products in any case. These 'long-chain' by-products (Fig. 2a) result from dimerization of the 1:1 product of the Grignard reagent and the dihalide [14] by transmetallation, as described in the work by Tamura and Kochi [27]. One can hypothesise that the transfer of magnesium from the alkyl Grignard reagent to the aryl dihalide occurred as well, but the subsequent reaction of this aryl Grignard reagent with other bromides was suppressed because the aryl Grignard reagent is less reactive compared to the alkyl counterpart. Moreover, this transmetallation might also explain the large amount of 'short-chain' by-product as the transmetallation once again generated thp-protected ω-bromoalcohols (**3**), which produced further

Table 1 Experimental conditions for Grignard bis-coupling reactions

Entry	Bromide	Dihalide	Li_2CuCl_4	Temp./°C
1	**3a** (3.13 g, 8 mmol)	**4a** (0.61 g, 3.5 mmol)	+[a]	−45 to −35
2	**3a** (3.13 g, 8 mmol)	**4b** (0.61 g, 3.5 mmol)	+[a]	−10 to −5
3	**3a** (3.13 g, 8 mmol)	**4b** (0.61 g, 3.5 mmol)	+[b]	−45 to −35
4	**3a** (12.9 g, 33 mmol)	**4b** (3.96 g, 15 mmol)	−	−45 to −35
5	**3b** (6.0 g, 18 mmol)	**4c** (2.0 g, 8 mmol)	+[a]	−10 to −5

[a] Addition of the catalyst after the addition of the dihalide

[b] Incubation of the Grignard reagent with the catalyst prior to the addition of the dihalide

'short-chain' by-products as described above (Fig. 2c). Several attempts were made to reduce or, at best, to avoid this side reaction. Table 1 summarises the different experimental conditions of the Grignard bis-coupling reactions.

All attempts explored, e.g. reaction at lower temperatures, preliminary incubation of the Grignard reagent with the catalyst, reaction without copper(II) catalyst, or a changed sequence of addition of the reactants, had no effect on the final product composition. We detected the formation of the 'short-chain' by-product in all cases and, hence, we had to use the crude bis(tetrahydro-2*H*-pyran)s **5** for subsequent reactions. In addition, the overall Grignard yields (including compound **5** and the 'short-chain' by-product) are slightly higher using the bromide **4b** instead of the chlorides **4a**, **4c**.

In the next step the thp-protecting groups of the bis(tetrahydro-2*H*-pyran)s **5** were cleaved in dry MeOH with catalytic amounts of pyridinium *p*-toluenesulfonate (PPTS) to yield the diols **6**. But, the 16,16′-(1,4-phenylene)bis(hexadecan-1-ol) (**6a***) still contained small amounts of the by-product triacontane-1,30-diol ($M_W = 454.81$ g/mol, see Fig. S1, Electronic Supplementary Material), which could not be separated by recrystallization or column chromatography (the asterisk indicates contamination with by-product). NMR measurements of the mixture of **6a*** and its by-product (entry 3 in Table 1) and the comparison of the peak areas of the signals of the methylene groups next to the hydroxy group and to the phenyl ring, respectively, allow for a rough estimation of the composition: 64 % of compound **6a*** is accompanied by 36 % of the by-product (Fig. S2, Electronic Supplementary Material). Since the yield of the Grignard reagent of compound **3a** was determined to be 93 %, the yield of the 'short-chain' by-product should not exceed 14 % and, hence, the high percentage of the by-product is caused by transmetallation during the coupling reaction. This phenomenon is a general drawback of Grignard bis-coupling reactions. Nevertheless, the corresponding biphenylene-modified diol **6b** could be purified in several steps of recrystallization and chromatography.

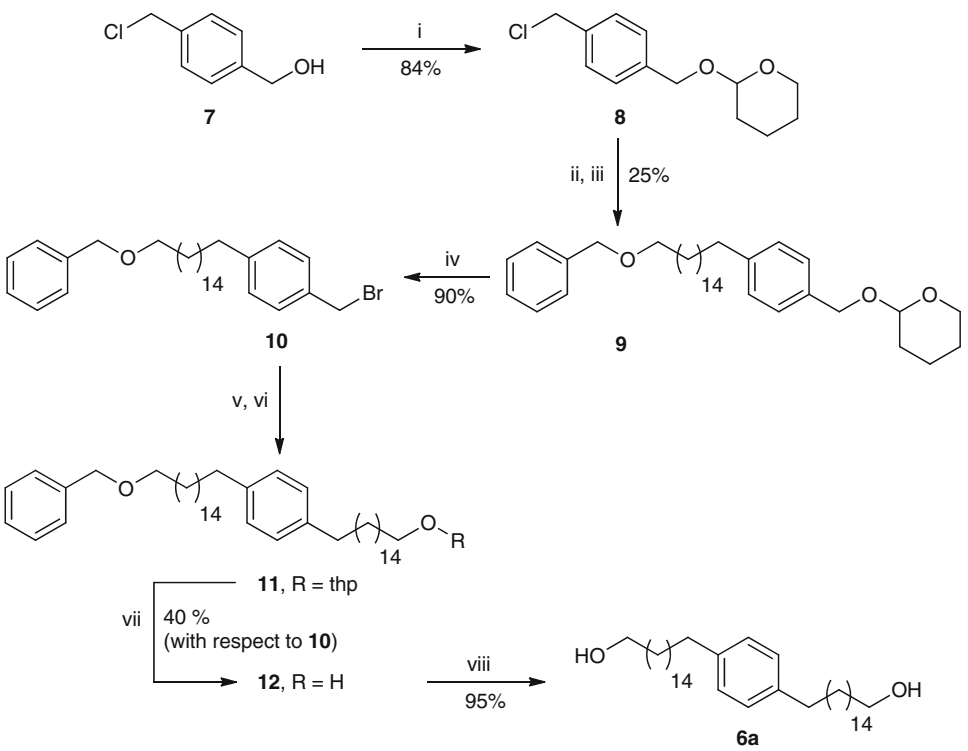
Comparing the yields of the Grignard bis-coupling reactions one might hypothesise that dibromides react better than dichlorides. Therefore, we decided to use the 4,4′-bis(bromomethyl)-1,1′-biphenyl (**4d**) instead of its chlorine counterpart **4c** to enhance the yield of **6b**. The synthesis of **4d**, starting from biphenyl-4,4′-dicarboxylic acid, and the analytical data of the intermediates are described in detail in the Electronic Supplementary Information. Finally, the dibromide **4d** was used for the Grignard bis-coupling reaction with **3b** as pointed out in Scheme 1. The resulting yield of the diol **6b** is slightly higher while using **4d** instead of **4c** (40 vs. 28 %).

Stepwise Grignard homo-coupling

To circumvent the unfeasible purification process of the phenylene-modified diol **6a*** a stepwise Grignard coupling using an unsymmetrical xylene derivative as starting material was applied (Fig. 2d). In a first attempt, we used benzene-1,4-dicarboxylic acid, which was esterified with MeOH and reduced to the corresponding 1,4-phenylenedimethanol. The following protection of only one hydroxy moiety using 3,4-dihydro-2*H*-pyran (DHP) and PPTS failed because of purification problems. Secondly, we attempted to reduce the commercially available 4-(bromomethyl)benzoic acid methyl ester by treating with $LiAlH_4$ in Et_2O at −10 °C. However, this reaction failed owing to the elimination of the bromine atom during the reduction, in line observations by Reuter et al. [30]. The third attempt started from [4-(chloromethyl)phenyl]methanol (**7**), which already contains the halogen atom required for the intended Grignard coupling and a hydroxy moiety. Firstly, the alcohol group was protected as its thp ether using DHP and PPTS. The resulting 2-[[4-(chloromethyl)benzyl]oxy]tetrahydro-2*H*-pyran (**8**) was obtained in 84 % yield after column chromatography. The simple and high-yielding substitution into bromides, necessary for the further Grignard coupling, is an advantage of this thp residue.

In the next step, the first elongation via Grignard coupling was performed. Therefore, the benzyl 15-bromopentadecyl ether was transformed into the corresponding Grignard reagent by treatment with magnesium in dry THF. The subsequent copper(II)-catalysed homo-coupling with compound **8** resulted in the formation of 2-[[4-[16-(benzyloxy)-hexadecyl]benzyl]oxy]tetrahydro-2*H*-pyran (**9**; Scheme 2). The marginal yields of 25 % after column chromatography are caused by the lower reactivity of the chlorine atom in Grignard reactions. Attempts to convert the chloro compound **8** (or its precursor compound **7**) into the analogous bromo derivative by heating with LiBr in dry acetone failed owing to

Scheme 2



i) DHP, CH_2Cl_2, PPTS, rt; ii) benzyl 15-bromopentadecyl ether, Mg, THF, 50 °C; iii) THF, $LiCuCl_4$, 0 °C, **8**; iv) PPh_3, Br_2, CH_2Cl_2, rt; v) **3a**, Mg, THF, 50 °C; vi) THF, Li_2CuCl_4, 0 °C, **10**; vii) PPTS, MeOH, reflux; viii) H_2, Pd/C (10%), heptane-EtOH-ethyl acetate

the instability of the thp residue and the hydroxy moiety against LiBr, and 1-(bromomethyl)-4-(chloromethyl)benzene and 1,4-bis(bromomethyl)benzene (**4b**) are formed instead.

In the following reaction the thp residue of compound **9** was substituted by bromide [31] quantitatively resulting in 1-[16-(benzyloxy)hexadecyl]-4-(bromomethyl)benzene (**10**). Afterwards, this bromide can be used for the second Grignard coupling: compound **3a** was converted to the Grignard reagent and subsequently coupled with compound **10** to yield 2-[[16-[4-[16-(benzyloxy)hexadecyl]phenyl]hexadecyl]oxy]tetrahydro-2*H*-pyran (**11**), a phenylene-modified long-chain 1,ω-diol with orthogonally cleavable protecting groups. Since the chromatographic properties of the desired compound **11** and the by-product, the bis(tetrahydro-2*H*-pyran) of the triacontane-1,30-diol, are very similar, a failure in purification was anticipated and we decided to use the crude product of **11** for further synthesis instead. The cleavage of the thp residue using the procedure mentioned above gave the mono-protected 16-[4-[16-(benzyloxy)hexadecyl]phenyl]hexadecan-1-ol (**12**) in an acceptable yield of 40 % with respect to bromide **10** after chromatography. Finally, the benzyl moiety of compound **12** was removed by hydrogenolysis using palladium on carbon as catalyst and a mixture of heptane, EtOH, and ethyl acetate as eluent to yield 16,16′-(1,4-phenylene)bis(hexadecan-1-ol) (**6a**) without any by-products.

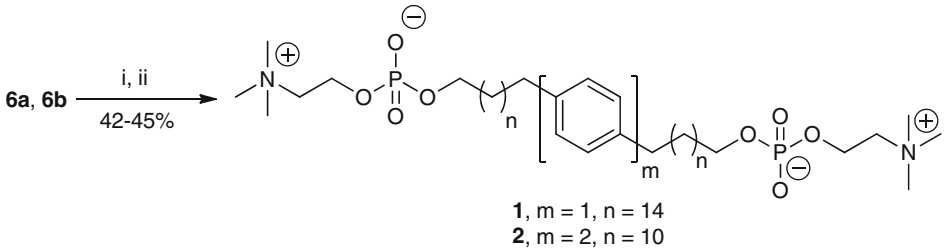
Although the procedure with two subsequently (stepwise) performed Grignard homo-coupling reactions required more synthetic steps, this pathway resulted in pure phenylene-modified 1,ω-diols without 'phenylene-free' by-products and, moreover, it can be used for the preparation of unsymmetrical 1,ω-diols and also BAs with different headgroups and/or unequal alkyl chain lengths substituted at the phenyl ring.

Phosphorylation and quarternisation

For the final phosphorylation step we used common reagents, such as β-bromoethylphosphoric acid dichloride, according to the method described by Eibl and Nicksch [32]. The subsequent quarternisation reaction was carried out with Me_3N in a solvent mixture of $CHCl_3$, CH_3CN, and EtOH to provide the bis(phosphocholine)s **1** and **2** (Scheme 3) in 42–45 % isolated yields.

Regarding the purification of the biphenylene-modified BA **2**, it is noteworthy that even if we used the 'short-chain' by-product-contaminated diol **6b** for the phosphorylation and quarternisation reaction, a separation of the

Scheme 3



i) $CHCl_3$, β-bromoethylphosphoric acid dichloride, 50 °C; then THF-H_2O, rt, 1.5 h; ii) $CHCl_3$, CH_3CN, EtOH, $N(CH_3)_3$, 50 °C, 24 h; then 3-4 days rt

resulting BA **2** from its 'biphenylene-free' by-product PC-C22-PC could be achieved. However, this purification failed if we used **6a*** for the phosphorylation and quarternisation reaction owing to the similar chromatographic properties of the phenylene-modified BA **1** and its 'phenylene-free' by-product PC-C30-PC. Regarding the latter phenomenon, we conclude that (1) the synthesis of phenylene-modified BAs is impossible using the Grignard bis-coupling reaction, hence, the stepwise Grignard homocoupling should be used and (2) the difference in the chain length of the bola molecules should be at least two phenyl rings, corresponding to eight carbon atoms, for a successful purification of the final BAs.

Aggregation behaviour

The aggregation behaviour of both novel phenylene- and biphenylene-modified BAs was studied in a preliminary investigation using TEM. As mentioned before, the BA without any modification (e.g. PC-C32-PC) aggregates in water into long and flexible nanofibres. To visualise the aggregates formed in suspension, TEM images were taken of samples prepared at 25 °C. Figure 3 shows the images of the negatively stained samples: Small worm-like aggregates are visible. In the case of BA **1** (Fig. 3a) the aggregates have a size of about 8–18 nm in length and 3–4 nm in width. Some of the aggregates are clustered together. The shape of the aggregates of BA **2** (Fig. 3b) is more irregular compared to BA **1**, but they also exhibit the form of small ellipsoidal micelles with slightly smaller dimensions. However, the formation of long fibrous aggregates, as was previously shown for PC-C32-PC suspensions [15, 16], was not observed for the samples at ambient temperatures. Since the self-assembly process of the PC-C32-PC into long nanofibres is exclusively driven by van der Waals interactions of the long alkyl chain [14–16], perturbations such as phenylene- or biphenylene rings within the polymethylene chain provoke decreased interactions of the long alkyl chains and, hence, resulted in a destabilization of the fibre aggregates [33]. This phenomenon is also observed if other chemical modifications, such as sulphur or oxygen atoms [34], diacetylene groups [35, 36] and methyl branches [33], respectively, were inserted into the alkyl chain.

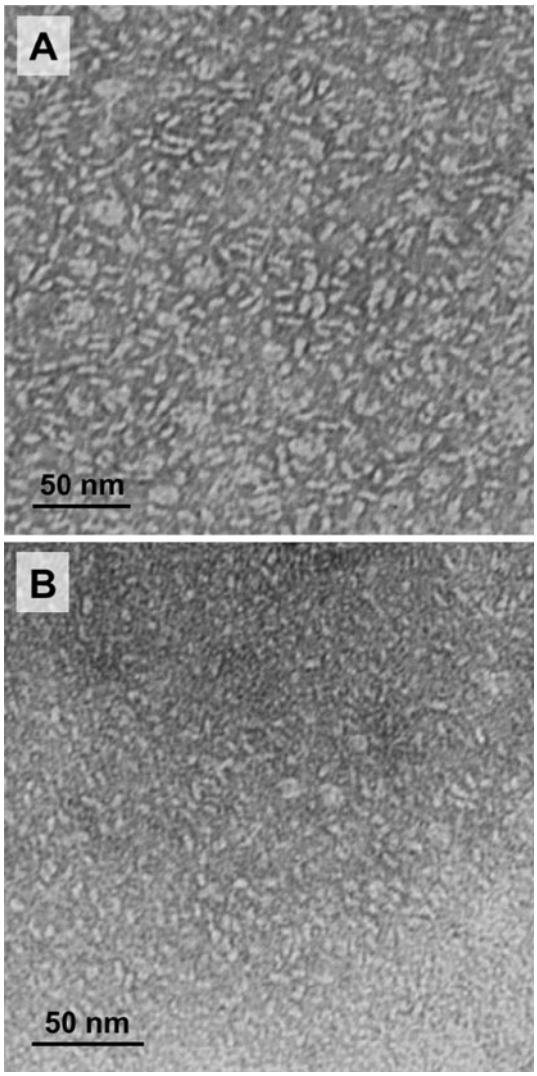


Fig. 3 TEM images of aqueous uranyl acetate stained suspensions of bolaamphiphiles **1** (**a**) and **2** (**b**) at a concentration of 0.05 mg/cm^3. The samples were prepared at 25 °C. The *bar* corresponds to 50 nm

Summary and outlook

The synthesis of biphenylene-modified BAs is feasible using the Grignard bis-coupling reaction. The preparation of the corresponding phenylene-modified BA failed because of purification problems of the resulting bola molecules. The alternative route using an unsymmetrical starting material and a stepwise Grignard homo-coupling led to the desired phenylene-modified BA in acceptable yields. During this homo-coupling, the 'short-chain' phenylene-free by-products, whose formation is a general drawback of the mentioned Grignard bis-coupling, can be successfully separated from the desired products. Moreover, the Grignard homo-coupling is also adaptable to the preparation of unsymmetrical bola molecules with unequal alkyl chain lengths substituted at the phenyl ring and/or different headgroups. The new phenylene/biphenylene-modified BAs do not self-assemble into long nanofibres in aqueous suspension as previously observed for bolalipids with long alkyl chains, but aggregate at room temperature into small ellipsoidal aggregates.

The preparation of phenylene-modified BAs with longer alkyl chains including other types of C–C coupling reactions, a detailed characterisation of the novel aggregates as well as investigations regarding the mixing behaviour of these new BAs with common phospholipids are underway. Preliminary experiments on the incorporation of phenylene-modified BAs into phospholipid bilayers showed promising results. The results of these investigations will be published in a separate paper.

Experimental

All chemicals were purchased from Sigma-Aldrich Co. and used without further purification. β-Bromoethylphosphoric acid dichloride was prepared according to the literature [37]. 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) and the following mobile phases: A = $CHCl_3$–heptane (3:2, v/v), B = $CHCl_3$–heptane (2:3, v/v), C = $CHCl_3$, D = $CHCl_3$–Et_2O (1:1, v/v), E = $CHCl_3$–MeOH–NH_3 (10:10:3, v/v/v). Silica gel (Merck, 0.063–0.200 mm) was used for column chromatography of the products. The purification of the final BAs was carried out by medium pressure liquid chromatography (MPLC, Büchi) on silica gel (Merck, 0.032–0.060 mm). Melting points were determined with a Boetius apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 spectrometer or a Varian Inova 500 with the use of $CDCl_3$ or CD_3OD as internal standard. Mass spectrometric data were obtained with a Finnigan MAT SSQ 710 C (ESI–MS) or were recorded on an AMD 402 (70 eV) spectrometer (EI–MS). High-resolution mass spectra (HR–MS) were recorded on a Thermo Fisher Scientific LTQ-Orbitrap mass spectrometer with static nano-electrospray ionisation. Elemental analyses (C, H, N) were conducted using a Leco CHNS-932; results were in good agreement (±0.4 %) with calculated values.

General procedure for the preparation of phenylene/biphenylene-modified 1,ω-diols using Grignard bis-coupling

A solution of 3.13–12.90 g **3a** (8–33 mmol) or 6.0 g **3b** (18 mmol), respectively, in 20–100 cm^3 dry THF was added dropwise to 0.29–1.22 g magnesium turnings (12–50 mmol) under argon atmosphere while stirring. Afterwards, the mixture was heated to 50 °C for 2 h. The excess Mg was removed under argon atmosphere and the Grignard solution was cooled to 0 °C. Then 1–3 cm^3 Li_2CuCl_4 (0.1 M in THF) was added with stirring followed by a solution of 0.61 g **4a** (3.5 mmol), 0.92–3.96 g **4b** (3.5–15 mmol), 2.0 g **4c** (8 mmol), or 2.7 g **4d** (8 mmol) in 20–50 cm^3 dry THF. The stirring was continued for a further 3 h at 0 °C. For the work-up 100–200 cm^3 Et_2O was added and the resulting mixture was poured into 100–200 cm^3 of a cold saturated solution of NH_4Cl. The organic layer was separated and the aqueous phase was extracted with 100–200 cm^3 Et_2O (2×). The combined organic phases were washed with 100–200 cm^3 H_2O (2×), dried over Na_2SO_4 and concentrated to dryness in vacuo. The white and waxy crude products **5a** and **5b** were used for the cleavage of the thp-protecting group without further purification: the crude bis(tetrahydro-2*H*-pyran)s **5** were dissolved in 50 cm^3 dry MeOH and heated under reflux for 3 h with catalytic amounts of PPTS. The hot suspension was filtered and the white residue was recrystallized from heptane or purified by column chromatography using the gradient technique and $CHCl_3$–Et_2O as eluent.

16,16′-(1,4-Phenylene)bis(hexadecan-1-ol) (**6a***)
The product could not be separated from the by-product triacontane-1,30-diol using recrystallization or chromatography.

12,12′-(Biphenyl-4,4′-diyl)bis(dodecan-1-ol)
(**6b**, $C_{38}H_{58}O_2$)
Compound **6b** was obtained as a white solid in 28 % yield (1.17 g) from reaction of 6.0 g **3b** (18 mmol) with 2.0 g **4c** (8 mmol) and in 40 % yield from reaction of 6.0 g **3b** (18 mmol) with 2.7 g **4d** (8 mmol) according to the general procedure described above. M.p.: 126–128 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.25–1.31 (m, 32H, 2× –$(CH_2)_2(CH_2)_8(CH_2)_2$O–), 1.54–1.62 (m, 8H, 2× –$CH_2CH_2(CH_2)_8CH_2CH_2$O–), 2.61 (t, ^3J = 7.8 Hz, 4H,

2× –CH_2(CH$_2$)$_{11}$O–), 3.62 (t, ^3J = 6.6 Hz, 4H, 2× –(CH$_2$)$_{11}$CH_2O–), 7.20–7.48 (m, 8H, –C$_6H_4$C$_6H_4$–) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 45 °C): δ = 25.74 (–*C*H$_2$(CH$_2$)$_2$OH), 29.37, 29.54, 29.60, 31.35 (–*C*H$_2$CH$_2$C$_6$H$_4$C$_6$H$_4$CH$_2$*C*H$_2$–), 32.81 (–*C*H$_2$CH$_2$OH), 35.55 (–*C*H$_2$C$_6$H$_4$C$_6$H$_4$*C*H$_2$–), 63.05 (–*C*H$_2$OH), 126.71 (2,2′,6,6′-C, –*C*$_6$H$_4$*C*$_6$H$_4$–), 128.63 (3,3′, 5,5′-C, –*C*$_6$H$_4$*C*$_6$H$_4$–), 138.51 (1,1′-C, –*C*$_6$H$_4$*C*$_6$H$_4$–), 141.68 (4,4′-C, –*C*$_6$H$_4$*C*$_6$H$_4$–) ppm; MS (70 eV): *m*/*z* (%) = 522 (100) [M$^+$], 504 (5) [M$^+$−H$_2$O].

General procedure for the preparation of phenylene-modified 1,ω-diols using stepwise Grignard homo-coupling

2-[[4-(Chloromethyl)benzyl]oxy]tetrahydro-2H-pyran (**8**, $C_{13}H_{17}ClO_2$)

A solution of 5.0 g **7** (32 mmol) and 3.8 g DHP (45 mmol) in 100 cm^3 dry $CHCl_3$ was stirred at 25 °C for 24 h. Afterwards, 100 cm^3 water was added and the organic layer was separated. The aqueous residue was extracted with 50 cm^3 $CHCl_3$ (2×). The combined organic phases were washed with 50 cm^3 water, dried over Na_2SO_4, evaporated, and the residue was purified by column chromatography using the gradient technique and heptane–Et_2O and TEA (0.5 %) as eluent to afford 6.5 g (84 %) of **8** as colourless oil. R_f = 0.44 (solvent A); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.46–1.81 (m, 6H, –OCH(CH_2)$_3$CH$_2$O–), 3.43–3.49 (m, 1H, –OCH(CH$_2$)$_3$-CH_2O–), 3.78–3.86 (m, 1H, –OCH(CH$_2$)$_3$CH_2O–), 4.44 (d, ^2J = 12.2 Hz, 1H, –OCH_2C$_6$H$_4$–), 4.50 (s, 2H, –CH_2Cl), 4.63–4.64 (m, 1H, –OC*H*O–), 4.71 (d, ^2J = 12.2 Hz, 1H, –OCH_2C$_6$H$_4$–), 7.29 (s, 4H, –C$_6H_4$–) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.27 (CHCH$_2$*C*H$_2$(CH$_2$)$_2$O–), 25.43 (CH(CH$_2$)$_2$*C*H$_2$CH$_2$O–), 30.49 (CH*C*H$_2$(CH$_2$)$_3$O–), 45.92 (Cl*C*H$_2$–), 61.99 (CH(CH$_2$)$_3$*C*H$_2$O–), 68.25 (–O*C*H$_2$C$_6$H$_4$–), 97.69 (–O*C*HO–), 127.88 and 128.44 (2,3,5,6-C, –*C*$_6$H$_4$–), 136.54 and 138.60 (1,4-C, –*C*$_6$H$_4$–) ppm; MS (70 eV): *m*/*z* (%) = 240 (14) [M$^+$], 205 (7) [M$^+$−Cl], 139 (100) [M$^+$−C$_5$H$_9$O$_2$], 104 (59) [M$^+$−C$_5$H$_9$O$_2$Cl], 91 (15) [C$_7$H$_7^+$], 85 (39) [C$_5$H$_9$O$^+$].

2-[[4-[16-(Benzyloxy)hexadecyl]benzyl]oxy]-tetrahydro-2H-pyran (**9**, $C_{32}H_{54}O_3$)

A solution of 2.66 g benzyl 15-bromopentadecyl ether (6.7 mmol) in 25 cm^3 dry THF was added dropwise to 0.24 g magnesium turnings (10 mmol) under argon atmosphere while stirring. Afterwards, the mixture was heated to 50 °C for 3 h. The excess magnesium was removed under argon atmosphere and the Grignard solution was cooled to 0 °C. Then 1.0 cm^3 Li_2CuCl_4 (0.1 M in THF) was added with stirring followed by a solution of 1.6 g **8** (6.6 mmol) in 10 cm^3 dry THF. The stirring was continued for a further 2 h at 0 °C. For the work-up 100 cm^3 Et_2O was added and the resulting mixture was poured into 100 cm^3 of a cold saturated solution of NH_4Cl. The organic layer was separated and the aqueous phase was extracted with 60 cm^3 Et_2O (2×). The combined organic phases were washed with 50 cm^3 brine and 50 cm^3 H_2O, dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was purified by column chromatography using the gradient technique and heptane–Et_2O and TEA (0.5 %) as eluent to afford 0.91 g (25 %) of **9** as white crystalline substance. R_f = 0.42 (solvent B); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.24–1.36 (m, 24H, –(CH$_2$)$_2$(CH_2)$_{12}$-(CH$_2$)$_2$O–), 1.50–1.89 (m, 10H, –CH$_2$CH_2(CH$_2$)$_{12}$-CH_2CH$_2$O–, –OCH(CH_2)$_3$CH$_2$O–), 2.57 (t, ^3J = 7.5 Hz, 2H, –CH_2C$_6$H$_4$CH$_2$O–), 3.45 (t, ^3J = 6.7 Hz, 2H, –CH_2OCH$_2$C$_6$H$_5$), 3.51–3.56 (m, 1H, –OCH(CH$_2$)$_3$-CH_2O–), 3.88–3.94 (m, 1H, –OCH(CH$_2$)$_3$CH_2O–), 4.49 (d, ^2J = 12.2 Hz, 1H, –CH$_2$C$_6$H$_4$CH_2O–), 4.57 (s, 2H, –OCH_2C$_6$H$_5$), 4.68–4.70 (m, 1H, –OC*H*O–), 4.77 (d, ^2J = 12.2 Hz, 1H, –CH$_2$C$_6$H$_4$CH_2O–), 7.13–7.15 (m, 2H, 2,6-H –C$_6H_4$–), 7.25–7.27 (m, 2H, 3,5-H –C$_6H_4$–), 7.32–7.35 (m, 5H, –C$_6H_5$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 19.32 (CHCH$_2$*C*H$_2$(CH$_2$)$_2$O–), 25.45 (CH(CH$_2$)$_2$*C*H$_2$CH$_2$O–), 26.19 (–*C*H$_2$(CH$_2$)$_2$OCH$_2$C$_6$H$_5$), 29.33, 29.43, 29.51, 29.59, 29.60, 29.65, 29.66, 29.77 (–*C*H$_2$–), 30.54 (CH*C*H$_2$(CH$_2$)$_3$O–), 31.51 (–*C*H$_2$CH$_2$C$_6$H$_4$CH$_2$O–), 35.69 (–*C*H$_2$C$_6$H$_4$CH$_2$O–), 62.07 (CH(CH$_2$)$_3$*C*H$_2$O–), 68.34 (–C$_6$H$_4$*C*H$_2$O–), 70.53 (–O*C*H$_2$C$_6$H$_5$), 72.83 (–*C*H$_2$OCH$_2$C$_6$H$_5$), 97.78 (–O*C*HO–), 127.41 (4-C, –C$_6$H$_5$), 127.58 (2,6-C, –C$_6$H$_5$), 127.89 (2,6-C, –C$_6$H$_4$–), 128.30 and 128.37 (3,5-C, –C$_6$H$_5$ and –C$_6$H$_4$–), 138.70 and 138.73 (1-C, –C$_6$H$_5$ and –C$_6$H$_4$–), 142.28 (4-C, –C$_6$H$_4$–) ppm; MS (70 eV): *m*/*z* (%) = 522 (49) [M$^+$].

1-[16-(Benzyloxy)hexadecyl]-4-(bromomethyl)benzene (**10**, $C_{30}H_{45}BrO$)

A solution of 0.19 cm^3 bromine (3.8 mmol), diluted in 5 cm^3 CH_2Cl_2, was added dropwise into a solution of 1.0 g triphenylphosphane (3.8 mmol) in 10 cm^3 dry CH_2Cl_2 whilst stirring at 0 °C. A solution of 0.9 g **9** (1.7 mmol), dissolved in 3 cm^3 CH_2Cl_2, was added and the mixture was stirred for 16 h at 25 °C. Afterwards, the organic layer was washed with 15 cm^3 brine (2×), dried over Na_2SO_4, evaporated, and the crude product was purified by column chromatography with heptane as eluent to afford 0.77 g (90 %) of **10** as white crystalline substance. R_f = 0.53 (solvent A); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.23–1.36 (m, 24H, –(CH$_2$)$_2$(CH_2)$_{12}$(CH$_2$)$_2$O–), 1.54–1.63 (m, 4H, –CH$_2$CH_2(CH$_2$)$_{12}$CH_2CH$_2$O–), 2.57 (t, ^3J = 7.8 Hz, 2H, –CH_2C$_6$H$_4$CH$_2$Br), 3.45 (t, ^3J = 6.7 Hz, 2H, –CH_2OCH$_2$C$_6$H$_5$), 4.47 (s, 2H, –CH_2Br), 4.48 (s, 2H, –OCH_2C$_6$H$_5$), 7.12–7.16 (m, 2H, 2,6-H –C$_6H_4$–), 7.25–7.29 (m, 2H, 3,5-H, –C$_6H_4$–), 7.32–7.33 (m, 5H, –C$_6H_5$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.20 (–*C*H$_2$(CH$_2$)$_2$OCH$_2$C$_6$H$_5$), 29.30, 29.31, 29.49, 29.57, 29.60, 29.61, 29.66, 29.67, 29.78

(–CH_2–), 31.38 (–$CH_2CH_2C_6H_4CH_2O$–), 33.79 (–CH_2Br), 35.67 (–$CH_2C_6H_4CH_2O$–), 70.54 (–$OCH_2C_6H_5$), 72.85 (–$CH_2OCH_2C_6H_5$), 127.43 (4-C, –C_6H_5), 127.59 (2,6-C, –C_6H_5), 128.32 (3,5-C, –C_6H_5), 128.52 (3,5-C, –C_6H_4–), 128.76 (2,6-C, –C_6H_4–), 134.96 (1-C, –C_6H_4–), 138.74 (1-C, –C_6H_5), 143.38 (4-C, –C_6H_4–) ppm; MS (70 eV): *m/z* (%) = 501 (2) [M^+], 421 (40) [M^+-Br], 329 (10) [$M^+-C_7H_7Br$], 91 (100) [$C_7H_7^+$].

16-[4-[16-(Benzyloxy)hexadecyl]phenyl]hexadecan-1-ol (**12**, $C_{45}H_{76}O_2$)

A solution of 1.1 g **3a** (2.7 mmol) in 6 cm^3 dry THF was added dropwise to 0.1 g magnesium turnings (4 mmol) under argon atmosphere while stirring. Afterwards, the mixture was heated to 50 °C for 3 h. The excess magnesium was removed under argon atmosphere and the Grignard solution was cooled to 0 °C. Then 0.5 cm^3 Li_2CuCl_4 (0.1 M in THF) was added with stirring followed by a solution of 0.5 g **10** (1 mmol) in 5 cm^3 dry THF. The stirring was continued for a further 3 h at 0 °C. For the work-up 15 cm^3 Et_2O was added and the resulting mixture was poured into 25 cm^3 of a cold saturated solution of NH_4Cl. The organic layer was separated and the aqueous phase was extracted again with 30 cm^3 Et_2O (2×). The combined organic phases were washed with 30 cm^3 brine and 30 cm^3 water, dried over Na_2SO_4 and concentrated to dryness in vacuo. The white and waxy crude product **11** was used for the cleavage of the thp-protecting groups without further purification. Therefore, compound **11** was dissolved in 20 cm^3 dry MeOH and heated under reflux for 2 h with catalytic amounts of PPTS. The reaction was then allowed to stand overnight at 25 °C. The cold suspension was filtered and the white residue was purified by column chromatography using $CHCl_3$–MeOH (95:5, v/v) as eluent to afford 0.26 g (40 % with respect to **10**) of **12** as white crystalline substance. M.p.: 82–83 °C; R_f = 0.12 (solvent A), 0.27 (solvent C), 0.57 (solvent D); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.24–1.35 (m, 48H, 2× –$(CH_2)_2(CH_2)_{12}(CH_2)_2O$–), 1.52–1.62 (m, 8H, 2× –$CH_2CH_2(CH_2)_{12}CH_2CH_2O$–), 2.54 (t, ^3J = 7.7 Hz, 4H, –$CH_2C_6H_4CH_2$–), 3.45 (t, ^3J = 6.6 Hz, 2H, –$CH_2OCH_2C_6H_5$), 3.62 (t, ^3J = 6.6 Hz, 2H, –CH_2OH), 4.48 (s, 2H, –$OCH_2C_6H_5$), 7.06 (s, 4H, –C_6H_4–), 7.25–7.33 (m, 5H, –C_6H_5) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.73 (–$CH_2(CH_2)_2OH$), 26.19 (–$CH_2(CH_2)_2OCH_2C_6H_5$), 29.39, 29.43, 29.48, 29.53, 29.60, 29.66, 29.77, 31.58 (–$CH_2CH_2C_6H_4CH_2CH_2$–), 32.82 (–CH_2CH_2OH), 35.56 (–$CH_2C_6H_4CH_2$–), 63.10 (–CH_2OH), 70.54 (–$CH_2C_6H_5$), 72.84 (–$CH_2OCH_2C_6H_5$), 127.43 (4-C, –C_6H_5), 127.60 (2,6-C, –C_6H_5), 128.20 (2,3,5,6-C, –C_6H_4–), 128.32 (3,5-C, –C_6H_5), 138.74 (1-C, –C_6H_5), 140.07 (1,4-C, –C_6H_4–) ppm; MS (70 eV): *m/z* (%) = 648 (4) [M^+], 630 (11) [M^+-H_2O], 557 (66) [$M^+-C_7H_7$], 539 (100) [$M^+-C_7H_9O$], 521 (74) [$M^+-C_7H_{11}O_2$].

16,16′-(1,4-Phenylene)bis(hexadecan-1-ol) (**6a**, $C_{38}H_{70}O_2$)

A mixture of 0.15 g **12** (0.23 mmol), dissolved in 50 cm^3 heptane–EtOH–ethyl acetate (3:1:1, v/v/v), and 35 mg palladium (10 % on carbon) was stirred under hydrogen (5 atm) at 25 °C for 18 h. The catalyst was removed by filtration and washed with warm $CHCl_3$ several times. The combined organic layers were evaporated and the white residue was recrystallized from heptane to afford 0.12 g (95 %) of **6a**. M.p.: 102–103 °C; R_f = 0.27 (solvent D); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.23–1.33 (m, 48H, 2× –$(CH_2)_2(CH_2)_{12}(CH_2)_2OH$), 1.51–1.60 (m, 8H, 2× –$CH_2CH_2(CH_2)_{12}CH_2CH_2OH$), 2.52 (t, ^3J = 7.7 Hz, 4H, –$CH_2C_6H_4CH_2$–), 3.62 (t, ^3J = 6.6 Hz, 4H, 2× –CH_2OH), 7.06 (s, 4H, –C_6H_4–) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.74 (–$CH_2(CH_2)_2OH$), 29.39, 29.43, 29.53, 29.59, 29.61, 29.66, 31.58 (–$CH_2CH_2C_6H_4CH_2CH_2$–), 32.82 (–CH_2CH_2OH), 35.57 (–$CH_2C_6H_4CH_2$–), 63.11 (–CH_2OH), 128.20 (2,3,5,6-C, –C_6H_4–), 140.07 (1,4-C, –C_6H_4–) ppm; MS (70 eV): *m/z* (%) = 558 (100) [M^+], 540 (58) [M^+-H_2O].

General procedure for phosphorylation and quarternisation reaction

The reaction procedure is based on the general synthesis of polymethylene-1,ω-diyl-bis(phosphocholines) described previously [14]: 0.96 g β-bromoethylphosphoric acid dichloride (4 mmol) was poured into 20 cm^3 dry $CHCl_3$ under cooling with ice–water. A mixture of 1.0 cm^3 dry TEA (7 mmol) in 20 cm^3 dry $CHCl_3$ was added slowly with stirring, which was continued for 30 min at 0 °C. The diols **6** (0.5 mmol, in solid form) were poured in one portion into the mixture. To completely dissolve the solid diols, the reaction mixture was gently warmed until a clear solution appeared. Afterwards, the mixture was stirred at 25 °C for 24 h. After the complete conversion of the diols, 40 cm^3 crushed ice was added to the solution and the mixture was stirred vigorously for a further 2 h. The organic layer was separated and the aqueous phase was extracted with 20 cm^3 $CHCl_3$ (3×). The combined organic layers were evaporated and the residue was dissolved in 10 cm^3 THF–H_2O (9:1, v/v). After 1.5 h, the solvent was evaporated and the crude bromoesters were transferred into a mixture of 10 cm^3 $CHCl_3$, 10 cm^3 CH_3CN and an ethanolic solution of Me_3N (2 mmol, 4.2 M). The mixture was kept in a closed tube at 50 °C for 24 h. Thereafter, the clear solution was allowed to stand for 3–4 days at 25 °C. Afterwards, the reaction mixture was evaporated to dryness and the residue was purified by MPLC using $CHCl_3$–MeOH–H_2O as eluent and the gradient technique to afford 0.18–0.20 g (42–45 %) of the phenylene/biphenylene-modified BAs **1** and **2**.

16,16′-(1,4-Phenylene)bis[hexadec-1-yl-[2-(trimethylammonio)ethyl-phosphate]] (**1**, $C_{48}H_{94}N_2O_8P_2$)

Compound **1** was obtained as a white solid in 45 % yield (0.20 g) from the phosphorylation reaction of 0.28 g **6a** (0.5 mmol) and subsequent quarternisation with Me_3N following the general procedure described above. $R_f = 0.26$ (solvent E); ^{1}H NMR (500 MHz, $CDCl_3$–CD_3OD): $\delta = 1.08$–1.20 (m, 48H, 2× –$(CH_2)_2(CH_2)_{12}$ $(CH_2)_2O$–), 1.38–1.47 (m, 8H, 2× –$CH_2CH_2(CH_2)_{12}$ CH_2CH_2O–), 2.38 (t, $^3J = 7.7$ Hz, 4H, –$CH_2C_6H_4CH_2$–), 3.03 (s, 18H, 6× –CH_3), 3.39–3.41 (m, 4H, 2× NCH_2CH_2O–), 3.68 (q, $^3J = 6.6$ Hz, 4H, 2× –$(CH_2)_{15}CH_2O$–), 4.02–4.06 (m, 4H, 2× NCH_2CH_2O–), 6.90 (s, 4H, –C_6H_4–) ppm; ^{13}C NMR (125 MHz, $CDCl_3$–CD_3OD): $\delta = 25.47$ (–$(CH_2)_{13}$ $CH_2(CH_2)_2O$–), 29.03, 29.10, 29.20, 29.28, 29.33, 29.35, 29.36, 29.38, 30.46 (d, $^3J_{C,P} = 7.7$ Hz, 2× –$(CH_2)_{14}$ CH_2CH_2O–), 31.28 (–$CH_2CH_2C_6H_4CH_2CH_2$–), 35.22 (–$CH_2C_6H_4CH_2$–), 53.88 (t, $J = 3.8$ Hz, 6× –CH_3), 58.46 (d, $^2J_{C,P} = 5.3$ Hz, 2× NCH_2CH_2O–), 65.65 (d, $^2J_{C,P} = 6.2$ Hz, 2× –$(CH_2)_{15}CH_2O$–), 66.27 (b, 2× NCH_2CH_2O–), 127.89 (2,3,5,6-C, –C_6H_4–), 139.77 (1,4-C, –C_6H_4–) ppm; ESI–MS: $m/z = 467.39$ [M^{2+}+2Na], 889.47 [M^++H], 911.36 [M^++Na], 923.42 [M^-+Cl]; HR-MS: calcd. for $C_{48}H_{95}N_2O_8P_2$ [M^++H]: 899.6558, found 889.6573; calcd. for $C_{48}H_{94}N_2O_8P_2Na$ [M^++Na]: 911.6378, found 911.6389.

12,12′-(Biphenyl-4,4′-diyl)bis[dodec-1-yl-[2-(trimethylammonio)ethyl-phosphate]] (**2**, $C_{46}H_{82}N_2O_8P_2$)

Compound **2** was obtained as a white solid in 42 % yield (0.18 g) from the phosphorylation reaction of 0.26 g **6b** (0.5 mmol) and subsequent quarternisation with Me_3N following the general procedure described above. $R_f = 0.24$ (solvent E); ^{1}H NMR (500 MHz, $CDCl_3$–CD_3OD): $\delta = 1.19$–1.29 (m, 32H, 2× –$(CH_2)_2(CH_2)_8$ $(CH_2)_2O$–), 1.52–1.62 (m, 8H, 2× –$CH_2CH_2(CH_2)_8$ CH_2CH_2O–), 2.58 (t, $^3J = 7.6$ Hz, 4H, 2× –CH_2 $(CH_2)_{11}O$–), 3.17 (s, 18H, 6× CH_3), 3.56–3.57 (m, 4H, 2× NCH_2CH_2O–), 3.79 (q, $J = 6.6$ Hz, 4H, 2× –$(CH_2)_{11}$ CH_2O–), 4.15–4.20 (m, 4H, 2× NCH_2CH_2O–), 7.10–7.46 (m, 8H, –$C_6H_4C_6H_4$–) ppm; ^{13}C NMR (125 MHz, $CDCl_3$–CD_3OD): $\delta = 25.62$ (–$(CH_2)_9CH_2(CH_2)_2O$–), 28.87, 29.23, 29.34, 29.39, 29.43, 29.62, 30.68 (–$(CH_2)_{10}CH_2CH_2O$–), 31.19 (–$CH_2CH_2(CH_2)_{10}O$–), 35.37 (–$CH_2(CH_2)_{11}O$–), 54.35 (b, –CH_3), 58.80 (NCH_2CH_2O–), 64.09 (d, $^2J_{C,P} = 7.7$ Hz, –$(CH_2)_{11}CH_2O$–), 65.83 (b, NCH_2CH_2O–), 126.64 (2,2′,6,6′-C, –$C_6H_4C_6H_4$–), 128.65 and 128.77 (3,3′,5,5′-C, –C_6H_4 C_6H_4–), 138.32 (1,1′-C, –$C_6H_4C_6H_4$–), 141.64 (4,4′-C, –C_6H_4 C_6H_4–) ppm; ESI–MS: $m/z = 853.5$ [M^++H], 875.4 [M^++Na]; HR-MS: calcd. for $C_{46}H_{83}N_2O_8P_2$ [M^++H]: 853.5625, found 853.5622; calcd. for $C_{46}H_{82}N_2O_8P_2Na$ [M^++Na]: 875.5444, found 875.5441.

Sample preparation

The appropriate amount of bolalipid was suspended in water (ultrapure water from a Milli-Q A10 system, Millipore GmbH, Schwalbach, Germany) to a concentration of 1.0 mg/cm^3. To achieve a homogeneous suspension, the samples were heated above 80 °C three times and vortexed. Prior to the TEM measurements, the bola stock suspensions were diluted with ultrapure water to a concentration of 0.05 mg/cm^3 and allowed to stand at 25 °C for at least 24 h.

Transmission electron microscopy (TEM)

Samples were prepared by spreading 5 mm^3 of the BA suspension ($c = 0.05$ mg/cm^3) onto a Cu grid coated with a formvar film. After 1 min, excess liquid was blotted off with filter paper and 5 mm^3 of 1 % aqueous uranyl acetate solution was placed onto the grid and drained off after 1 min. The dried specimens were examined with a Zeiss EM 900 transmission electron microscope.

Acknowledgments This work was supported by grants from the Deutsche Forschungsgemeinschaft (projects Bl 182/19-3 and Do 463/4-2). We thank Prof. Andrea Sinz and Dr. Christian Ihling (Department of Pharmaceutical Chemistry and Bioanalytics, Martin-Luther-University Halle-Wittenberg) for the high resolution mass spectrometry analysis. The support of Dr. Gerd Hause (Biocenter, Martin-Luther-University Halle-Wittenberg) by providing us access to the electron microscope facility is greatly appreciated. Finally, S.D. and B.D. thank Dipl.-Pharm. Susann Althaus and Dr. Beate Stiebitz for the help in the synthesis.

References

1. Fuhrhop J-H, Wang T (2004) Chem Rev 104:2901
2. De Rosa M, Esposito E, Gambacorta A, Nicolaus B, Bu'Lock JD (1980) Phytochemistry 19:827
3. Langworthy TA (1977) Biochim Biophys Acta Lipids Lipid Metab 487:37
4. Bakowsky U, Rothe U, Antonopoulos E, Martini T, Henkel L, Freisleben HJ (2000) Chem Phys Lipids 105:31
5. Benvegnu T, Réthoré G, Brard M, Richter W, Plusquellec D (2005) Chem Commun 5536
6. Brown DA, Venegas B, Cooke PH, English V, Chong PL-G (2009) Chem Phys Lipids 159:95
7. Cornell BA, Braach-Maksvytis VBL, King LG, Osmann PDJ, Raguse B, Wieczorek L, Pace RJ (1997) Nature 387:580
8. Patel GP, Ponce A, Zhou H, Chen W (2008) Int J Toxicol 27:329
9. Réthoré G, Montier T, Le Gall T, Delépine P, Cammas-Marion S, Lemiègre L, Lehn P, Benvegnu T (2007) Chem Commun 2054
10. Sprott GD, Tolson DL, Patel GB (1997) FEMS Microbiol Lett 154:17
11. Benvegnu T, Brard M, Plusquellec D (2004) Curr Opin Colloid Interface Sci 8:469
12. Meister A, Blume A (2007) Curr Opin Colloid Interface Sci 12:138
13. Meister A, Blume A (2012) Adv Planar Lipid Bilayers Liposomes. doi:10.1016/B978-0-12-396534-9.00004-0

14. Drescher S, Meister A, Blume A, Karlsson G, Almgren M, Dobner B (2007) Chem Eur J 13:5300
15. Köhler K, Förster G, Hauser A, Dobner B, Heiser UF, Ziethe F, Richter W, Steiniger F, Drechsler M, Stettin H, Blume A (2004) J Am Chem Soc 126:16804
16. Köhler K, Förster G, Hauser A, Dobner B, Heiser UF, Ziethe F, Richter W, Steiniger F, Drechsler M, Stettin H, Blume A (2004) Angew Chem Int Ed 43:245
17. Meister A, Drescher S, Mey I, Wahab M, Graf G, Garamus VM, Hause G, Mögel H-J, Janshoff A, Dobner B, Blume A (2008) J Phys Chem B 112:4506
18. Meister A, Köhler K, Drescher S, Dobner B, Karlsson G, Edwards K, Hause G, Blume A (2007) Soft Matter 3:1025
19. Köhler K, Meister A, Förster G, Dobner B, Drescher S, Ziethe F, Richter W, Steiniger F, Drechsler M, Hause G, Blume A (2006) Soft Matter 2:77
20. Meister A, Bastrop M, Koschoreck S, Garamus VM, Sinemus T, Hempel G, Drescher S, Dobner B, Richtering W, Huber K, Blume A (2007) Langmuir 23:7715
21. Meister A, Drescher S, Karlsson G, Hause G, Baumeister U, Hempel G, Garamus VM, Dobner B, Blume A (2010) Soft Matter 6:1317
22. Meister A, Drescher S, Garamus VM, Karlsson G, Graf G, Dobner B, Blume A (2008) Langmuir 24:6238
23. Zou B, Wang M, Qiu D, Zhang X, Chi L, Fuchs H (2002) Chem Commun 1008
24. Wu G, Verwilst P, Xu J, Xu H, Wang R, Smet M, Dehaen W, Faul CFJ, Wang Z, Zhang X (2012) Langmuir 28:5023
25. Grechishnikova I, Johansson LB-A, Molotkovsky JG (1996) Chem Phys Lipids 81:87
26. McKiernan R, Gido SP, Penelle J (2002) Polymer 43:3007
27. Tamura M, Kochi J (1971) Synthesis 303
28. Heiser UF, Dobner B (1997) J Chem Soc Perkin Trans 1:809
29. Heiser UF, Dobner B (1996) Chem Commun 2025
30. Kathiresan M, Walder L, Ye F, Reuter H (2010) Tetrahedron Lett 51:2188
31. Schwarz M, Oliver JE, Sonnet PE (1975) J Org Chem 40:2410
32. Eibl H, Nicksch A (1975) Ger Offen DE 2345057 A1 19750327; (1975) Chem Abstr 83:27617
33. Blume A, Drescher S, Meister A, Graf G, Dobner B (2012) Faraday Disc. doi:10.1039/C2FD20102J
34. Drescher S, Meister A, Graf G, Hause G, Blume A, Dobner B (2008) Chem Eur J 14:6796
35. Drescher S, Helmis K, Langner A, Dobner B (2010) Monatsh Chem 141:339
36. Graf G (2011) Ph.D. thesis. Martin-Luther-University, Halle
37. Hirt G, Berechthold R (1958) Pharm Acta Helv 33:349