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General Synthesis and Aggregation Behaviour of New Single-Chain Bolaphospholipids: Variations in Chain and Headgroup Structures

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Abstract: The chemical structures of polymethylene-1,ω-bis(phosphocholines) that self-assemble into nanofibres was modified on the one hand in the hydrophobic chain region, by introduction of sulfur and oxygen atoms, and on the other hand by variation of the polar headgroup structure with functionalised tertiary amines. The temperature-dependent self-assembly of these novel bolaphospholipids into nanofibres and spherical micelles was investigated by differential scanning calorimetry (DSC) and transmission electron microscopy (TEM). The thermal stabilities of the nanofibres strongly depend on the chemical compositions of the headgroups and of the hydrophobic chains. The insertion of new functionalities in the headgroup region by click chemistry makes these substances interesting for potential applications in bioscience and materials science.

Keywords: aggregation · amphiphiles · lipids · nanofibers · synthetic methods

Introduction

A bolalipid is composed of two hydrophilic headgroups attached to one or two hydrophobic spacers. The basic structure has its origin in the membrane lipids of certain species of archaebacteria.[1] The membrane-spanning—and thus membrane-stabilizing—properties of these bolalipids with two alkyl chains make these molecules attractive candidates for use in vesicular drug delivery systems or for the stabilisation of supported membrane biosensor devices.[2,3] Since well defined, natural bolaphospholipids are difficult to isolate from natural membranes, considerable efforts have been devoted to the synthesis of novel bipolar lipids.[4,5] Unexpectedly, the simplification of these model compounds to bipolar lipids with just one long alkyl chain gave rise to new aggregate structures, such as nanofibres and nanoparticles of self-assembled single-chain bolaphospholipids.[6,7]

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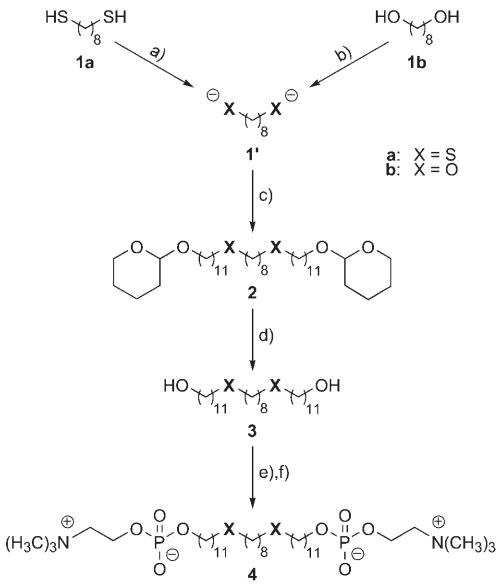
Recently, we reported the synthesis and temperature-dependent aggregation behaviour of symmetrical single-chain polymethylene-1,ω-bis(phosphocholines) with hydrocarbon chain lengths of 22 to 32 carbon atoms and two phosphocholine headgroups attached at both ends (PC-Cn-PC).[8] These bolalipids self-assemble at room temperature into long nanofibres that form a dense network, which gels water very efficiently. Within the fibres, the molecules are arranged side by side, but the bulky headgroups induce a twisted arrangement, causing a helical superstructure of the fibres.[9–11] Above a certain temperature, which depends on the length of the alkyl chain, the nanofibres reversibly transform into smaller aggregates such as spherical micelles or discs, and the gel character of the aqueous solution is lost.

The self-assembly process of polymethylene-1,ω-bis(phosphocholines) is exclusively driven by hydrophobic interactions of the long alkyl chains, because the phosphocholine headgroups cannot form intermolecular hydrogen bonds. The question arose of whether the substitution of methylene groups by sulfur or oxygen atoms would perturb this aggregation process as a result of increased polarity, together with the different binding angles and bond lengths relative to a methylene group in a hydrocarbon chain. We therefore inserted oxygen and sulfur at certain positions in the alkyl chain and investigated the influence of both atoms on the aggregation behaviour.

We have recently reported on bolaphospholipids with dimethylammonio headgroups that self-assemble into nanofi-

bres that display higher thermal stability due to the formation of intermolecular hydrogen bonds between the headgroups.[12] In this study we synthesised several bolaphospholipids with further variations in the polar headgroup structures. We modified the choline headgroups with several functional groups in order to scrutinise the size effect of the headgroup on the aggregation behaviour and also to find out the synthetic limits for the quarternisation reaction. In addition, the functionalised choline headgroups are suitable for further chemical modifications such as binding of fluorescence labels or low-molecular-weight proteins.

In this work we present a general synthetic approach to chain- and headgroup-modified dotriacontane-1,32-bis(phosphocholines). The temperature-dependent aggregation properties of these novel single-chain bolaphospholipids were investigated by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and dynamic light scattering (DLS).



Scheme 1. Synthesis of 1,ω-bis(phosphocholines) with modified alkyl chains. a) THF, potassium *tert*-butoxide, room temperature; b) THF, NaH, reflux, 3 h; c) THF, 2-(11-bromoundecyloxy)tetrahydro-2*H*-pyran, tetrabutylammonium iodide, reflux, 10–24 h; d) MeOH, pyridinium *p*-toluenesulfonate, reflux, 3 h; e) $CHCl_3$, bromoethylphosphoric acid dichloride, triethylamine, 24 h, room temperature; then THF, H_2O, 2 h; f) $CHCl_3$, CH_3CN, EtOH, $N(CH_3)_3$, 50 °C, 48 h.

Results and Discussion

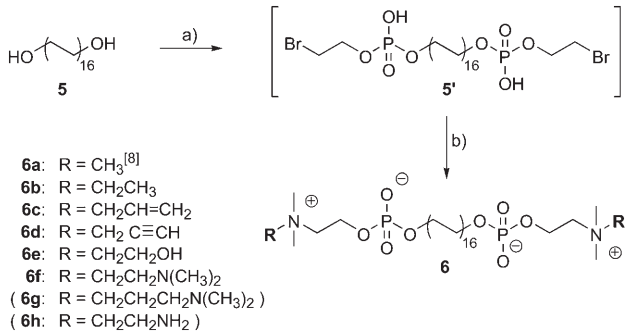
Synthetic methods: At first we prepared 1,ω-bis(phosphocholines) with modified alkyl chains of a total chain length of 32 atoms for direct comparison with the well investigated dotriacontane-1,32-diyl-bis[2-(trimethylammonio)ethylphosphate] **6a** (Scheme 2, below).[9,10] For the synthesis of the sulfur-containing compound **4a** (PC-C32SS-PC), commercially available octane-1,8-dithiol (**1a**) was deprotonated with potassium *tert*-butoxide in tetrahydrofuran (THF) at room temperature (Scheme 1). Treatment with 2-(11-bromoundecyloxy)tetrahydro-2*H*-pyran and catalytic amounts of tetrabutylammonium iodide in THF resulted in the formation of compound **2a** in high yield (up to 90%). After removal of the THP blocking groups under mild conditions the 12,21-dithiadotriacontane-1,32-diol (**3a**) was transformed into the corresponding 12,21-dithiadotriacontane-1,32-diyl-bis[2-(trimethylammonio)ethylphosphate] (**4a**) in a two-step procedure. For the phosphorylation reaction we used the common β-bromoethylphosphoric acid dichloride in combination with triethylamine (TEA) as basic additive. This reagent was more efficient than 2-chloro-1,3,2-dioxophospholane or other phosphites also used by others[13,14] in bolaphospholipid synthesis. Initial heating at the beginning of the phosphorylation reaction[8] was not necessary, because of the higher solubility of the thia-modified diol **3a** in chloroform in relation to the unmodified diol **5**. The final reaction step was quarternisation with a solution of trimethylamine in chloroform/acetonitrile/ethanol (Scheme 1).

The high yield of this thia-modified 1,32-bis(phosphocholine) **4a** (up to 70%) relative to that obtained with the unmodified 1,32-bis(phosphocholine) **6a**[8] might also be due to the lack of any need for heating during the phosphorylation reaction.

The synthesis of the oxygen-containing analogue **4b** (PC-C32OO-PC) started from octane-1,8-diol (**1b**). However, the deprotonation of the two hydroxy groups required stronger conditions, so treatment with sodium hydride in THF at reflux and subsequent alkylation of **1b′** with 2-(11-bromoundecyloxy)tetrahydro-2*H*-pyran and catalytic amounts of tetrabutylammonium iodide gave compound **2b** only, not unexpectedly, in moderate yields. The subsequent steps in reaction were similar to the procedures described above (Scheme 1), and the 12,21-dioxadotriacontane-1,32-diyl-bis[2-(trimethylammonio)ethylphosphate] (**4b**) was obtained in moderate yield (45%) based on the diol **3b**.

Besides the chain modifications, our main attention was focused on the variation of the headgroup structure. The synthesis of dotriacontane-1,32-bis(phosphocholines) with modified headgroups (**6a**–**f**) started from dotriacontane-1,32-diol (**5**), which was esterified with the β-bromoethylphosphoric acid dichloride used above in combination with TEA to yield the corresponding bis(β-bromoethyl phosphoric acid ester) **5′**. The introduction of the quarternary nitrogen atom was carried out, in contrast to the preparation of compounds **4**, with various tertiary amines including two methyl groups and one larger residue (Scheme 2) in order to find out the synthetic limits for the quarternisation reaction. The reaction conditions and purification procedures were similar to those described previously.[8]

For the first quarternisation reactions we used larger, trialkylated amines such as *N*,*N*-dimethyl-*N*-ethylamine (**6b**), *N*-allyl-*N*,*N*-dimethylamine (**6c**) and *N*,*N*-dimethyl-*N*-prop-2-ynylamine (**6d**). Since these amines are commercially available as pure liquids and not as solutions in ethanol, a mixture of dry chloroform, acetonitrile and ethanol (3:3:1 *v*/



Scheme 2. Synthesis of dotriacontane-1,32-bis(phosphocholines) with modified headgroups. a) $CHCl_3$, bromoethylphosphoric acid dichloride, triethylamine, 60 °C; then 24 h at room temperature; then THF, H_2O, 2 h; b) $CHCl_3$, CH_3CN, EtOH, $(CH_3)_2NR$, 50 °C, 48 h.

v) was used instead of dry chloroform/acetonitrile[8] as solvent for this reaction. We also worked strictly under argon because of the sensitivity of the amines. The yields of the obtained 1,32-bis(phosphocholines) **6b–d** (45 to 55%) were smaller than that of compound **6a**.

Furthermore, we also used different heteroatom-substituted, trialkylated amines such as *N*,*N*-dimethylethanolamine, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, *N*,*N*,*N*′,*N*′-tetramethylpropylenediamine and *N*,*N*-dimethylethylenediamine. However, with increasing size of the amine the yields during the quarternisation reaction became rather low (see Table 1). Thus, for the quarternisation reaction with *N*,*N*,*N*′,*N*′-tetramethylethylenediamine we obtained a marginal yield of 25%, but for the reaction with *N*,*N*,*N*′,*N*′-tetramethylpropylenediamine we could detect the desired bis(phosphocholine) only by mass spectrometry, purification by MPLC not being worthwhile because of the very marginal yields.

In addition, quarternisation reactions with alkyl amines possessing two different reaction centres (*N*,*N*-dimethylethanolamine and *N*,*N*-dimethylethylenediamine) gave varying results. In the case of *N*,*N*-dimethylethanolamine no O-alkylation was observed during the quarternisation reaction. However, the use of *N*,*N*-dimethylethylenediamine led to a mixture of secondary and quarternary amines. The separation and purification of these single compounds by MPLC and a gradient technique was not possible due to the very similar chromatographic properties of the obtained products.

The functionalised choline headgroups of compounds **6c**, **d** and **e** were tested for coupling with different sulfur-containing residues suitable for complexation of gold nanoparticles. Such complexes are of great interest for electronic devices. Depending on the structures of **6c–e**, three methods—photoaddition, click reactions and esterification with appropriate sulfur-containing reagents—were tested for an effective binding reaction.

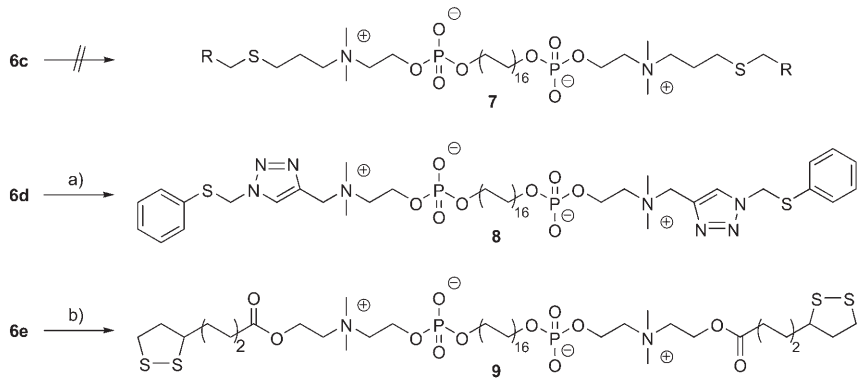
We first attempted to use the allyl groups in compound **6c** for UV-induced addition to diverse alkyl thiols as described in the literature[15,16] (Scheme 3, top). In our hands, however, the reaction failed to produce the corresponding thioethers **7**.

In another attempt the propynyl group in compound **6d** was used in a copper(I)-catalysed click reaction[17–19] with azidomethyl phenyl sulfide as one example. For this, **6d** was dissolved in a small amount of water/ethanol (2:1 *v*/*v*), and was then treated with the azide and catalytic amounts of a copper(I) salt to provide compound **8** (PTTPC-C32-PTTPC) in high yields (up to 75%). The catalyst was prepared in situ by reduction of copper(II) salts with sodium ascorbate (Scheme 3, middle). In addition, we found that the use of 20 mol% of copper(II) acetate[19] led to the best yields during this reaction, relative those obtained with other copper(II) salts such as copper sulfate.

Table 1. Yields (based on the diol **5**) of dotriacontane-1,32-bis(phosphocholines) **6** with modified headgroups.

Compound	Amine compound $N(CH_3)_2R$ with R =	Yield [%] [a]
6a (PC-C32-PC)	CH_3	60[8]
6b (EPC-C32-EPC)	CH_2CH_3	55
6c (APC-C32-APC)	$CH_2CH{=}CH_2$	52
6d (PPC-C32-PPC)	$CH_2C{\equiv}CH$	45
6e (HEPC-C32-HEPC)	CH_2CH_2OH	50
6f (DMAEPC-C32-DMAEPC)	$CH_2CH_2N(CH_3)_2$	25
6g (DMAPPC-C32-DMAPPC)	$CH_2CH_2CH_2N(CH_3)_2$	not determined[b]
6h (AEPC-C32-AEPC)	$CH_2CH_2NH_2$	not determined[c]

[a] Yield of isolated product. [b] Product was detectable by mass spectrometry but purification failed. [c] Reaction resulted in mixtures—purification was not possible.



Scheme 3. a) H_2O, EtOH, **6d**, azidomethyl phenyl sulfide, sodium ascorbate, copper(II) acetate, room temperature, 8–24 h; b) CCl_4, (±)-α-lipoic acid, DCC, 30 min, room temperature; then $CHCl_3$, DMSO, **6e**, DMAP, 50 °C, 48 h.

Furthermore, the additional hydroxy group in the choline region of compound **6e** was also suitable for reactions with a broad spectrum of activated acids, such as lipoic acid. Compound **6e** was therefore converted into the lipid **9** (LAPC-C32-LAPC) by treatment with racemic lipoic acid, *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) as catalyst (Scheme 3, bottom). This reaction led to bolaphospholipids with ester-modified headgroups. The yield in that case was rather moderate, however, but compound **9** was successfully tested for the fixation of gold nanoparticles.[11]

By comparison of the yields and reaction procedures of the functionalisation reactions discussed above we can conclude that the click chemistry entry is the method of choice for coupling of various residues at the headgroup structure of the bolaphosphocholines.

Temperature-dependent aggregation: The conformational consequences of substitution of two methylene groups with oxygen or sulfur at positions C12 and C21 in the PC-C32-PC chain are presented in the inset of Figure 1. These energy-optimised structures were calculated with the aid of the COMPASS force field in the Discover module from Materials Studio (Accelrys, Inc.).

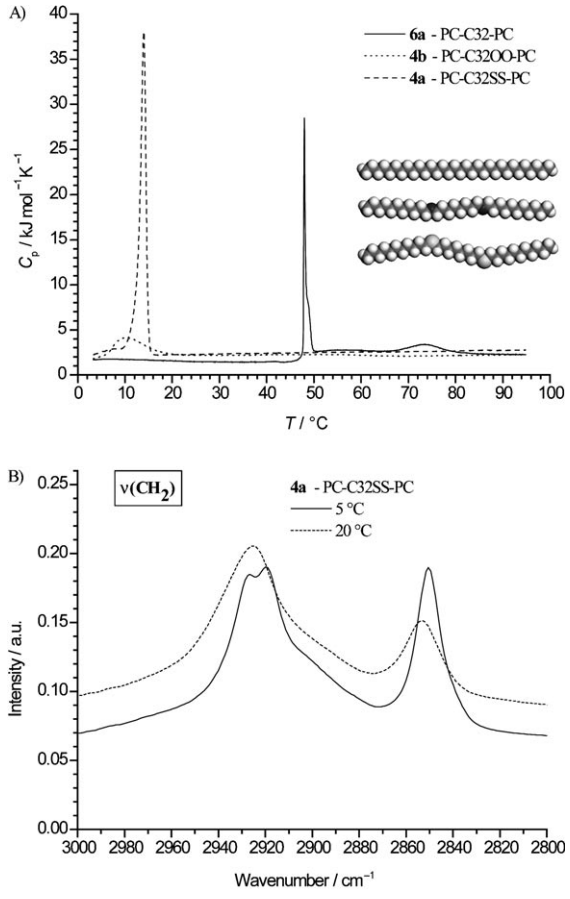


Figure 1. A) DSC curves of aqueous suspensions of polymethylene-1,ω-bis(phosphocholines) with modified alkyl chains. The inset shows the hydrophobic chains (without headgroups) of the bolaphospholipids PC-C32-PC (top), **4b** with oxygen atoms in dark grey (middle), and **4a** with sulfur atoms in light grey (bottom). B) FTIR curves of an aqueous suspension of **4a** at 5 and 20 °C in the CH_2-stretching vibrational region.

Whereas the oxygen atoms induce only a minor perturbation in the all-*trans* conformation (C–O 0.142 nm and COC 113.5°, relative to C–C 0.153 nm and CCC 113.3°), the substitution with sulfur atoms leads to two pronounced kinks (C–S 0.181 nm and CSC 96.1°) in the chain. In addition, the introduction of oxygen atoms increases the polarity in the chain region to a greater extent than the introduction of sulfur atoms.

The temperature-dependent aggregation behaviour of the novel bolaphospholipids was investigated by differential scanning calorimetry (DSC), transmission electron microscopy (TEM) and dynamic light scattering (DLS). Figure 1A shows the DSC curve of PC-C32-PC, with two endothermic transitions, one at 48 °C, associated with the breakdown of the nanofibres, and a high-temperature transition at about 73 °C, which occurs inside the stability range of nanoparticles.[8] Figure 1A also shows the DSC curves of the corresponding bolaphospholipids with oxygen- and sulfur-containing chains (**4a**, **4b**) for comparison. These two components each exhibit only one endothermic peak at much lower temperature: namely at 10 °C (**4b**) and 14 °C (**4a**). We presumed that this transition was also due to a breakdown of nanofibres and the formation of micelle-like aggregates, and show later by TEM and DLS that this is indeed the case. We observed a pronounced exothermic peak in the cooling curve of **4a** (data not shown), which indicates a reformation of the fibres with almost no hysteresis.

Figure 1B presents the CH_2-stretching vibrational bands of **4a** below (5 °C) and above (20 °C) the transition temperature. The wavenumbers of the symmetric and antisymmetric CH_2-stretching vibrations provide a sensitive measure for the conformational order of the chain. As a result of the interruption of the long chain by S atoms, the continuous CH_2 segments are much shorter than in the case of PC-C32-PC. At 5 °C the symmetric stretching vibrational band shows a wavenumber of 2850 cm^{-1}, which is characteristic for an almost all-*trans* conformation of the alkyl chain. Obviously, the sulfur-containing chains can achieve a relatively dense packing arrangement, despite the two pronounced kinks in the chain separating the all-*trans* CH_2-segments.

At 20 °C the symmetric stretching vibrational band has shifted to 2853 cm^{-1}, indicating that above the transition temperature a significant proportion of *gauche* conformers is present.

The DSC curve of the oxygen-containing bolaphospholipid **4b** shows one small, broad endothermic peak in the heating curve. Since the disturbance of the all-*trans* conformation of the chain by the oxygen atoms is smaller than in the case of the sulfur atoms, their hydrophilic character obviously has a stronger influence on the aggregation behaviour. One could assume that water penetrates into the chain region, disturbing the self-assembly of **4b** into nanofibres and limiting their low-temperature stability.

Chemical modification of the phosphocholine headgroup with ethyl, allyl, propynyl and 2-(dimethylamino)ethyl moi-

eties caused significantly lower transition temperatures than in the case of PC-C32-PC (48 °C), which shows the strong influence of the headgroup size on the aggregation behaviour (see Figure 2A). These bolaphospholipids are exclusively stabilised by hydrophobic interactions of the alkyl chains, and with increasing headgroup diameter from ethyl to 2-(dimethylamino)ethyl moieties the transition temperatures are shifted down to 47 and 42.5 °C, respectively. In contrast, the transition temperature is shifted upwards (51.5 °C) for compound **6e** with 2-hydroxyethyl-modified headgroups. The stabilizing effect of these headgroups on the fibres could be due to the possibility of forming intermolecular hydrogen bonds between the headgroups.

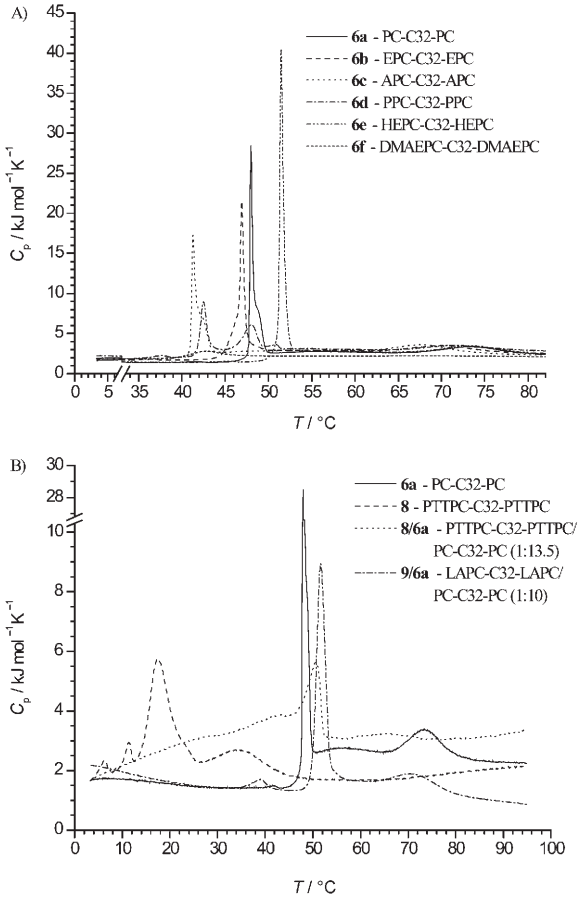


Figure 2. DSC curves of aqueous suspensions of: A) dotriacontane-1,32-bis(phosphocholines) with modified headgroups (**6a–f**) and B) bis(phosphocholines) **8**, **9** and mixtures of both lipids with PC-C32-PC (**6a**).

Figure 2B shows the DSC curves of pure PTTPC-C32-PTTPC (**8**) and of a mixture with PC-C32-PC (**6a**). The strong shift in the transition temperature to lower values for the pure compound is due to the increased size of the headgroup. In contrast, the **8**/**6a** mixture with a low amount of **8** exhibits a higher transition temperature than pure **6a**, indicating a stabilizing effect of **8** on the nanofibres of **6a**.

The lipoic acid-modified bolalipid **9** could only be investigated in a mixture with PC-C32-PC in a 1:10 ratio because of the insolubility of this compound in water. For this mixture the breakdown of the nanofibres occurred at a higher temperature than with **6a**, indicating a stabilizing effect of **9**.

In the next step, TEM and DLS were used to characterise the shapes of the formed aggregates. The TEM image of stained (uranyl acetate) suspensions of the sulfur-modified bolaphospholipid **4a** shows nanofibres with non-uniform diameters from 4 up to 10 nm when the sample was prepared below the transition temperature of 14 °C (see Figure 3A). The TEM image reflects the problems encountered by this compound in achieving dense packing of the sulfur-contain-

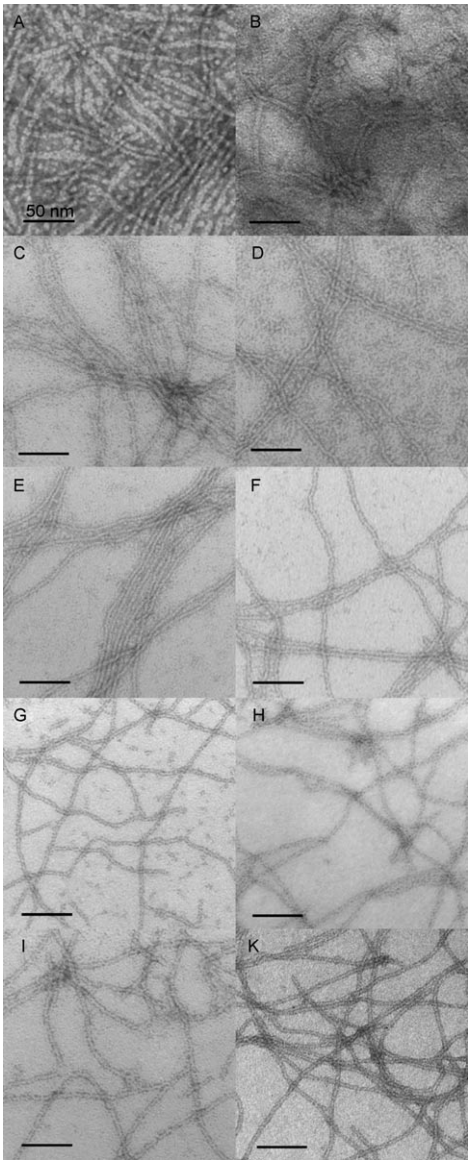


Figure 3. TEM images of aqueous stained (uranyl acetate) suspensions (1 mg mL^{-1}) of: A) PC-C32SS-PC (**4a**); B) PC-C32OO-PC (**4b**); C) EPC-C32-EPC (**6b**); D) APC-C32-APC (**6c**); E) PPC-C32-PPC (**6d**); F) HEPC-C32-HEPC (**6e**); G) DMAEPC-C32-DMAEPC (**6f**); H) PTTPC-C32-PTTPC (**8**); I) PTTPC-C32-PTTPC/PC-C32-PC (**8**/**6a**) (1:10); K LAPC-C32-LAPC/PC-C32-PC (**9**/**6a**) (1:10). Samples were prepared at 20 °C, except for A) at 5 °C, and B), H) and I) at 2 °C. The bar corresponds to 50 nm.

ing chains. As a consequence, the diameter of the nanofibres can exceed the molecular length of about 5 nm. This would mean that the headgroups are not in register any more.

The oxygen-modified bolaphospholipid **4b** shows the formation of short nanofibres of uniform diameter, indicating a uniform packing of the chains at 2 °C (see Figure 3B).

For the bolaphospholipids **6b–f** and **8**, the formation of long and flexible nanofibres was observed (see Figure 3C–H). The bolaphospholipid mixtures **8**/**6a** and **9**/**6a** also show long and flexible nanofibres, indicating that PC-C32-PC is able to accommodate the larger headgroups of **8** and **9** (see Figure 3I and K).

Above its first (or only) transition temperature, each investigated bolaphospholipid shows a nanofibre to micelle transformation. The diameters of the formed spherical micelles were determined by DLS. An example of an intensity correlation function and the number-weighted size distribution of the formed aggregates is given in Figure 4 for compound **6d**.

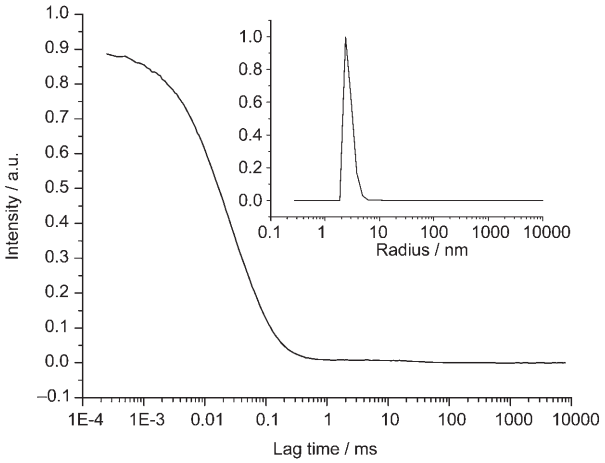


Figure 4. Intensity correlation function of an aqueous suspension (1 mg mL^{-1}) of PPC-C32-PPC (**6d**) at 25 °C. The inset shows the number-weighted size distribution.

The radii of the micellar-like aggregates of the bolaphospholipids are summarised in Table 2.

Table 2. Radii of micelles, determined by DLS, of aqueous suspensions (1 mg mL^{-1}) of bolaphospholipids above the transition temperature.

Compound	T [°C]	r [nm]
4a (PC-C32SS-PC)	25	3.3 ± 0.2
4b (PC-C32OO-PC)	25	3.3 ± 0.9
6b (EPC-C32-EPC)	55	1.7 ± 0.7
6c (APC-C32-APC)	45	2.2 ± 0.3
6d (PPC-C32-PPC)	45	2.5 ± 0.2
6d (PPC-C32-PPC)	55	2.1 ± 0.3
6e (HEPC-C32-HEPC)	55	2.5 ± 0.1
6f (DMAEPC-C32-DMAEPC)	55	2.2 ± 0.5

Conclusion

In this work we have presented a general synthetic approach to novel single-chain dotriacontane-1,32-bis(phosphocholines) with modified chain and headgroup structures. Oxygen and sulfur atoms were inserted at certain positions in the alkyl chain with the aid of bis-alkylation reactions of octane-1,8-diol or octane-1,8-dithiol with THP-protected 11-bromoundecanol. The oxygen- and sulfur-containing bolalipids self-assembled into nanofibres that are less stable than the unmodified bolalipid as a result of changes in the chain conformation and the decreased hydrophobic character of the chain.

The enlargement of the headgroup structures through the use of various tertiary amines for the quarternisation reaction was accompanied by some problems relating to the chemical yields. In two cases the corresponding product was not obtained in substantial amounts, due to the size or variability in the reaction centres of the amines used in the quarternisation reaction. In all other cases we were able to isolate the corresponding bolalipids in good yields. The physicochemical characterisation of these compounds showed either stabilizing or destabilizing effects of the modified headgroups on the nanofibre structure, depending on the size and the chemical composition of the attached moieties. In all cases micellar-like aggregates were observed at temperatures above the first endothermic transition.

The functionalised headgroup structures were successfully used for further chemical modifications. In particular, the functionalisation of the headgroup by click chemistry techniques offers new possibilities for the use of nanofibres as templates for the fixation of biological macromolecules or metal or semiconductor nanoparticles.

Experimental Section

General: The purities of all compounds were checked by thin layer chromatography (TLC, Merck) with common eluents. The purification of the final bolaamphiphiles was carried out by middle pressure liquid chromatography (MPLC, Büchi) on silica gel (Merck, 0.032–0.060 mm). Melting points were determined with a Boetius apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were performed on a Varian Inova 500 or a Varian Gemini 2000 NMR spectrometer with use of $CDCl_3$ or CD_3OD as internal standard. Mass spectrometric data were obtained with a Finnigan model MAT SSQ 710 C mass spectrometer (ESI-MS) or were recorded on an AMD 402 (70 eV) spectrometer. Elemental analyses were recorded on a Leco CHNS-932 instrument. All solvents were used purified and dried. Commercially available reagents were supplied by Aldrich, Co. and were used without further purification. β-Bromoethylphosphoric acid dichloride, 2-(11-bromoundecyloxy)tetrahydro-2*H*-pyran and dotriacontane-1,32-diol were prepared according to the literature.[8]

Synthesis of THP-blocked 1,32-diols with modified alkyl chains

2,2′-[(12,21-Dithiadotriacontane-1,32-diyl)oxy]bis(tetrahydro-2*H*-pyran) (2a): Octane-1,8-dithiol (**1a**, 0.5 g, 2.8 mmol) diluted with dry THF (25 mL) was placed in a round-bottomed flask under argon. Potassium *tert*-butoxide (0.63 g, 5.6 mmol) was added, and the mixture was stirred for 30 min at room temperature. A solution of 2-(11-bromoundecyloxy)tetrahydro-2*H*-pyran (1.88 g, 5.6 mmol) in dry THF (25 mL) was

added dropwise, and the mixture was heated under reflux for 10 h. For the workup, diethyl ether (100 mL) was added, and the resulting mixture was poured into a cold, saturated solution of sodium chloride (100 mL). The organic layer was separated, and the aqueous phase was extracted with diethyl ether (2×50 mL). The combined extracts were dried over sodium sulfate and concentrated to dryness under reduced pressure. The crude product was purified by MPLC by the gradient technique and with heptane/diethyl ether to give the title compound as a white solid (1.73 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$, 27°C): δ = 1.25–1.35 (m, 36H; 2×-$(CH_2)_7(CH_2)_2S(CH_2)_2(CH_2)_2$-), 1.48–1.59, 1.66–1.73, 1.77–1.85 (3×m, 24H; 2×-$CH(CH_2)_3CH_2$- and 2×-$OCH_2CH_2(CH_2)_7CH_2CH_2SCH_2CH_2(CH_2)_2$-), 2.46–2.50 (t, 8H; 2×-CH_2SCH_2-), 3.33–3.39, 3.68–3.74 (2×dt, 4H; 2×-$OCH_2(CH_2)_{10}S$-), 3.45–3.51, 3.83–3.88 (2×m, 4H; 2×-$CH_2OCHO(CH_2)_{11}S$-), 4.55–4.56 ppm (m, 2H; -CH-); ESI-MS: *m/z*: 709.8 $[M+Na]^+$; elemental analysis calcd (%) for $C_{40}H_{78}O_4S_2$ (687.14): C 69.91, H 11.44, S 9.33; found: C 69.90, H 11.20, S 9.58.

2,2′-[(12,21-Dioxadotriacontane-1,32-diyl)oxy]bis(tetrahydro-2*H*-pyran) (2b): Sodium hydride (0.84 g, 21 mmol) suspended in dry THF (15 mL) was placed in a round-bottomed flask under argon. A solution of octane-1,8-diol (**1b**, 1.5 g, 10.3 mmol) in dry THF (25 mL) was added slowly, and the mixture was stirred for 3 h under reflux. After the mixture had cooled down to room temperature, a solution of 2-(11-bromoundecyloxy)tetrahydro-2*H*-pyran (6.7 g, 20 mmol) in dry THF (10 mL), together with catalytic amounts of tetrabutylammonium iodide, were added, and the mixture was headed under reflux for at least 24 h. For the workup, diethyl ether (100 mL) was added, and the resulting mixture was poured into a cold, saturated solution of sodium chloride (50 mL). The organic layer was separated, and the aqueous phase was extracted with diethyl ether (2×50 mL). The combined extracts were dried over sodium sulfate and concentrated to dryness under reduced pressure. The crude product was purified by MPLC by the gradient technique and with heptane/ether as eluent to give **2b** as a white solid (0.67 g, 10%). ESI-MS: *m/z*: 672.4 $[M+NH_3]^+$; elemental analysis calcd (%) for $C_{40}H_{78}O_6$ (655.04): C 73.35, H 12.00; found: C 73.31, H 11.84.

Unmasking of hydroxy groups: A solution of a bis(tetrahydropyranyl) ether **2** (2 mmol) in dry methanol (100 mL) was heated under reflux for 3 h in the presence of catalytic amounts of pyridinium toluene-*p*-sulfonate. The hot suspension was filtered off, and the white residue was recrystallised from heptane to provide a pure diol **3** as white crystals.

12,21-Dithiadotriacontane-1,32-diol (3a): Yield: 0.93 g (90%); m.p. 94–95°C; ^{1}H NMR (400 MHz, $CDCl_3$, 27°C): δ = 1.26–1.37 (m, 36H; 2×HO-$(CH_2)_2(CH_2)_7(CH_2)_2S(CH_2)_2(CH_2)_2$-), 1.52–1.59 (m, 12H; 2×$HOCH_2CH_2$-$(CH_2)_7CH_2CH_2SCH_2CH_2(CH_2)_2$-), 2.46–2.50 (t, 8H; 2×-CH_2SCH_2-), 3.60–3.64 ppm (t, 4H; 2×$HOCH_2$-); ^{13}C NMR (100 MHz, $CDCl_3$, 27°C): δ = 25.85 (2×$HO(CH_2)_2CH_2(CH_2)_8S$-), 28.97, 29.04, 29.23, 29.34, 29.50, 29.60, 29.82, 29.86 (2×$HO(CH_2)_3(CH_2)_7CH_2SCH_2(CH_2)_3$-), 32.33, 32.35 (2×-$CH_2SCH_2$-), 32.93 (2×$HOCH_2CH_2(CH_2)_9S$-), 63.12 ppm (2×$HOCH_2$-); MS (70 eV): *m/z* (%): 518 (10) $[M]^+$, 347 (16) $[M-C_{11}H_{23}O]^+$, 315 (100) $[M-C_{11}H_{23}OS]^+$, 144 (80) $[M-C_{22}H_{46}O_2S]^+$; elemental analysis calcd (%) for $C_{30}H_{62}O_2S_2$ (518.91): C 69.43, H 12.04, S 12.36; found: C 69.29, H 11.91, S 12.42.

12,21-Dioxadotriacontane-1,32-diol (3b): Yield: 0.29 g (60%); ^{1}H NMR (400 MHz, $CDCl_3$, 27°C): δ = 1.26–1.35 (m, 36H; 2×$HO(CH_2)_2(CH_2)_7$-$(CH_2)_2O(CH_2)_2(CH_2)_2$-), 1.51–1.58 (m, 12H; 2×$HOCH_2CH_2$-$(CH_2)_7CH_2CH_2OCH_2CH_2(CH_2)_2$-), 3.35–3.38 (t, 8H; 2×-CH_2OCH_2-), 3.60–3.63 ppm (t, 4H; 2×$HOCH_2$-); MS (70 eV): *m/z* (%): 487 (3) $[M]^+$, 315 (40) $[M-C_{11}H_{23}O]^+$, 297 (76) $[M-C_{11}H_{25}O_2]^+$, 187 (42) $[M-C_{19}H_{39}O_2]^+$; ESI-MS: *m/z*: 488.2 $[M+H]^+$, 509.6 $[M+Na]^+$; elemental analysis calcd (%) for $C_{30}H_{62}O_4$ (486.81): C 74.02, H 12.84; found: C 73.91, H 12.69.

Phosphorylation and quarternisation: β-Bromoethylphosphoric acid dichloride (1.93 g, 8 mmol) was poured into dry chloroform (20 mL) with cooling (ice/water). A mixture of dry triethylamine (1.42 g, 14 mmol) and dry chloroform (20 mL) was added slowly with stirring, which was continued for 30 min at 0°C. A diol **3** (1 mmol) was added as a solid in one portion. The suspension was allowed to come to room temperature, and stirring was continued at this temperature for a further 24–48 h. After TLC (chloroform/ether 8:2) showed complete conversion of diol **3**, crushed ice (40 mL) was added to the solution, and the mixture was stirred for a further 2 h. The organic layer was separated, and the aqueous phase was diluted with cold saturated sodium chloride (50 mL) and then extracted twice with chloroform (50 mL). The combined organic phases were concentrated under reduced pressure, and the oily residue was dissolved in THF/water (9:1, 30 mL). After 1 h the solvent was evaporated, and the oily residue was transferred into a mixture of dry chloroform (25 mL), dry acetonitrile (25 mL) and an ethanolic solution of trimethylamine (10 mL, 4.2 M). The mixture was kept in a closed tube at 50°C for 48 h. Afterwards the mixture was concentrated by evaporation of the solvent, and the residue was purified by MPLC by the gradient technique and with chloroform/methanol/water as eluent to give a bis(phosphocholine) **4**, which was dried in vacuo over phosphorus pentoxide at room temperature for two days.

12,21-Dithiadotriacontane-1,32-diyl-bis[2-(trimethylammonio)ethylphosphate] (4a): Yield: 0.59 g (70%); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 27°C): δ = 1.20–1.32 (m, 36H; 2×-$(CH_2)_7(CH_2)_2S(CH_2)_2(CH_2)_2$-), 1.48–1.57 (m, 12H; 2×-$OCH_2CH_2(CH_2)_7CH_2CH_2SCH_2CH_2(CH_2)_2$-), 2.42–2.46 (t, 8H; 2×-CH_2SCH_2-), 3.19 (s, 18H; 6×-CH_3), 3.59–3.60 (m, 4H; 2×NCH_2CH_2O-), 3.77–3.82 (q, 4H; 2×-$OCH_2(CH_2)_{10}S$-), 4.16–4.22 ppm (m, 4H; 2×NCH_2CH_2O-); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 27°C): δ = 25.84 (2×-$O(CH_2)_2CH_2(CH_2)_8S$-), 28.74, 28.93, 29.00, 29.24, 29.40, 29.51, 29.54, 29.60, 29.66, 29.76 (2×-$O(CH_2)_3(CH_2)_7CH_2SCH_2(CH_2)_3$-), 30.86, 30.93 (2×-$OCH_2CH_2(CH_2)_9S$-), 32.16, 32.20 (2×-$CH_2SCH_2$-), 54.36–54.42 (6×-$CH_3$), 58.90, 58.95 (2×$NCH_2CH_2O$-), 65.72, 65.78 (2×-$OCH_2(CH_2)_{10}S$-), 66.49 ppm (2×$NCH_2CH_2O$-); ESI-MS: *m/z*: 849.4 $[M+H]^+$, 871.4 $[M+Na]^+$; elemental analysis calcd (%) for $C_{40}H_{86}N_2O_8P_2S_2\cdot 2H_2O$: C 54.27, H 10.25, N 3.16, S 7.24; found: C 54.22, H 10.41, N 3.13, S 7.17.

12,21-Dioxadotriacontane-1,32-diyl-bis[2-(trimethylammonio)ethylphosphate] (4b): Yield: 0.37 g (45%); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 27°C): δ = 1.09–1.14 (m, 36H; 2×-$(CH_2)_7(CH_2)_2O(CH_2)_2(CH_2)_2$-), 1.35–1.47 (m, 12H; 2×-$OCH_2CH_2(CH_2)_7CH_2CH_2OCH_2CH_2(CH_2)_2$-), 3.08 (s, 18H; 6×-$CH_3$), 3.21–3.24 (t, 8H; 2×-$CH_2OCH_2$-), 3.42–3.44 (m, 4H; 2×$NCH_2CH_2O$-), 3.64–3.69 (q, 4H; 2×-$OCH_2(CH_2)_{10}O$-) 3.99–4.09 ppm (m, 4H; 2×NCH_2CH_2O-); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 27°C): δ = 25.65, 25.88, 26.01 (6×-$O(CH_2)_2CH_2$-), 29.18, 29.29, 29.35, 29.45, 29.48, 29.53 (2×-$O(CH_2)_3(CH_2)_5CH_2CH_2CH_2OCH_2CH_2CH_2CH_2$-), 30.64, 30.70 (2×-$OCH_2CH_2(CH_2)_9O$-), 54.10–54.16 (6×-$CH_3$), 58.86, 58.89 (2×$NCH_2CH_2O$-), 65.81, 65.86 (2×-$OCH_2(CH_2)_{10}O$-), 66.33 (2×$NCH_2CH_2O$-), 70.75, 70.79 ppm (2×-CH_2OCH_2-); ESI-MS: *m/z*: 817.7 $[M+H]^+$, 839.6 $[M+Na]^+$; elemental analysis calcd (%) for $C_{40}H_{86}N_2O_{10}P_2\cdot 2H_2O$: C 56.31, H 10.64, N 3.28; found: C 56.12, H 10.86, N 3.21.

General synthesis of headgroup-modified dotriacontane-1,32-bis(phosphocholines): β-Bromoethylphosphoric acid dichloride (1.93 g, 8 mmol) was poured into dry chloroform (20 mL) with cooling (ice/water). A mixture of dry triethylamine (1.42 g, 14 mmol) and dry chloroform (20 mL) was added slowly with stirring, which was continued at 0°C for 30 min. A diol **5** (0.48 g, 1 mmol) was added as a solid in one portion. The suspension was heated to 60°C until the diol was dissolved, then rapidly cooled to room temperature. Stirring at this temperature was continued for a further 24–48 h. After TLC (chloroform/ether 8:2) showed complete conversion of diol **6**, crushed ice (40 mL) was added, and the mixture was stirred for a further 2 h. The organic layer was separated, and the aqueous phase was diluted with cold saturated sodium chloride solution (50 mL) and then extracted twice with chloroform (50 mL). The combined organic phases were concentrated under reduced pressure, and the oily residue was dissolved in THF/water (9:1, 30 mL). After 1 h the solvent was evaporated, and the oily residue was transferred under argon into a mixture of dry chloroform (30 mL), dry acetonitrile (30 mL) and dry ethanol (10 mL). The pure amine (20 mmol) was added slowly, and the mixture was kept in a closed tube at 50°C for 48 h. Afterwards the mixture was concentrated by evaporation of the solvent, and the residue was purified by MPLC by the gradient technique and with chloroform/methanol/water as eluent to give the bis(phosphocholine) **6**, which was dried in vacuo over phosphorus pentoxide at room temperature for two days.

Dotriacontane-1,32-diyl-bis[2-(*N*,*N*-dimethyl-*N*-ethylammonio]ethylphosphate] (6b): Yield: 0.46 g (55%); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$,

27 °C): δ = 1.24–1.38 (m, 62H; 2×-CH_2CH_3, -$(CH_2)_2(CH_2)_{28}(CH_2)_2$-), 1.57–1.63 (m, 4H; -$CH_2CH_2(CH_2)_{28}CH_2CH_2$-), 3.19 (s, 12H; 4×-$CH_3$), 3.45–3.49 (q, 4H; 2×-$CH_2CH_3$), 3.52–3.54 (m, 4H; 2×$NCH_2CH_2O$-), 3.82–3.86 (q, 4H; -$OCH_2(CH_2)_{30}CH_2O$-), 4.17–4.21 ppm (m, 4H; 2×NCH_2CH_2O-); ESI-MS: *m/z*: 841.8 $[M+H]^+$, 863.6 $[M+Na]^+$; elemental analysis calcd (%) for $C_{44}H_{94}N_2O_8P_2{\cdot}4H_2O$: C 57.79, H 11.26, N 3.07; found: C 57.87, H 11.13, N 2.87.

Dotriacontane-1,32-diyl-bis[2-(*N*-allyl-*N*,*N*-dimethylammonio)ethylphosphate] (6c): Yield: 0.45 g (52%); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 27 °C): δ = 1.17–1.31 (m, 56H; -$(CH_2)_2(CH_2)_{28}(CH_2)_2$-), 1.53–1.60 (m, 4H; -$CH_2CH_2(CH_2)_{28}CH_2CH_2$-), 3.14 (s, 12H; 4×-$CH_3$), 3.61–3.63 (m, 4H; 2×$NCH_2CH_2O$-), 3.79–3.84 (q, 4H; -$OCH_2(CH_2)_{30}CH_2O$-), 4.03–4.05 (d, 4H; 2×$H_2C$=$CHCH_2N$), 4.21–4.27 (m, 4H; 2×$NCH_2CH_2O$-), 5.69–5.73 (dd, 4H; 2×H_2C=$CHCH_2N$), 5.89–6.00 ppm (m, 2H; 2×H_2C=$CHCH_2N$); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 27 °C): δ = 25.59 (2×-$O(CH_2)_2CH_2(CH_2)_{13}$-), 29.19 (2×-$O(CH_2)_{15}CH_2$-), 29.47 (2×-$O(CH_2)_3(CH_2)_{12}CH_2$-), 30.55, 30.62 (2×-$OCH_2CH_2(CH_2)_{14}$-), 50.67–50.75 (4×-CH_3), 58.26, 58.31 (2×NCH_2CH_2O-), 64.03 (2×H_2C=$CHCH_2N$), 65.64, 65.69 (2×-$OCH_2(CH_2)_{15}$-), 67.75–67.79 (2×NCH_2CH_2O-), 123.82 (H_2C=CH-), 129.46 ppm (H_2C=CH-); ESI-MS: *m/z*: 866.6 $[M+H]^+$, 887.8 $[M+Na]^+$; elemental analysis calcd (%) for $C_{46}H_{94}N_2O_8P_2{\cdot}4H_2O$: C 58.95, H 10.97, N 2.99; found: C 59.39, H 10.97, N 2.66.

Dotriacontane-1,32-diyl-bis[2-(*N*,*N*-dimethyl-*N*-propynylammonio)ethylphosphate] (6d): Yield: 0.39 g (45%); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 27 °C): δ = 1.11–1.21 (m, 56H; -$(CH_2)_2(CH_2)_{28}(CH_2)_2$-), 1.43–1.50 (m, 4H; -$CH_2CH_2(CH_2)_{28}CH_2CH_2$-), 2.91–2.92 (t, 2H; 2×$HC$≡$CCH_2N$), 3.11 (s, 12H; 4×-$CH_3$), 3.51–3.53 (m, 4H; 2×$NCH_2CH_2O$-), 3.68–3.73 (q, 4H; -$OCH_2(CH_2)_{30}CH_2O$-), 4.06–4.10 (m, 4H; 2×$NCH_2CH_2O$-), 4.22–4.23 ppm (d, 2H; 2×HC≡CCH_2N); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$, 27 °C): δ = 25.80 (2×-$O(CH_2)_2CH_2(CH_2)_{13}$-), 29.01 (2×-$O(CH_2)_{15}CH_2$-), 29.45–29.63 (2×-$O(CH_2)_3(CH_2)_{12}CH_2$-), 30.77, 30.84 (2×-$OCH_2CH_2(CH_2)_{14}$-), 51.49 (4×-CH_3), 55.36 (2×HC≡CCH_2N), 58.72, 58.76 (2×NCH_2CH_2O-), 64.35, 64.42 (2×-$OCH_2(CH_2)_{15}$-), 65.93 (2×NCH_2CH_2O-), 71.33 (HC≡C-), 81.20 ppm (HC≡C-); ESI-MS: *m/z*: 861.5 $[M+H]^+$, 883.5 $[M+Na]^+$; elemental analysis calcd (%) for $C_{46}H_{90}N_2O_8P_2{\cdot}4H_2O$: C 59.20, H 10.59, N 3.00; found: C 59.43, H 10.44, N 2.97.

Dotriacontane-1,32-diyl-bis{2-[*N*,*N*-dimethyl-*N*-(2-hydroxyethyl)ammonio]ethylphosphate} (6e): Yield: 0.44 g (50%); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 27 °C): δ = 0.93–1.05 (m, 56H; -$(CH_2)_2(CH_2)_{28}(CH_2)_2$-), 1.26–1.33 (m, 4H; -$CH_2CH_2(CH_2)_{28}CH_2CH_2$-), 2.91 (s, 12H; 4×-$CH_3$), 3.21–3.24 (m, 4H; 2×$NCH_2CH_2OP$-), 3.34–3.36 (m, 4H; 2×$HOCH_2CH_2N$), 3.50–3.55 (q, 4H; -$OCH_2(CH_2)_{30}CH_2O$-), 3.64–3.67 (m, 4H; 2×$NCH_2CH_2OP$-), 3.86–3.93 (m, 4H; 2×$HOCH_2CH_2N$); ESI-MS: *m/z*: 873.8 $[M+H]^+$, 896.0 $[M+Na]^+$; elemental analysis calcd (%) for $C_{44}H_{94}N_2O_{10}P_2{\cdot}2H_2O$: C 58.12, H 10.87, N 3.08; found: C 58.03, H 10.95, N 3.01.

Dotriacontane-1,32-diyl-bis{2-[*N*,*N*-dimethyl-*N*-(2-dimethylaminoethyl)ammonio]ethylphosphate} (6f): Yield: 0.23 g (25%); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 27 °C): δ = 1.06–1.17 (m, 56H; -$(CH_2)_2(CH_2)_{28}(CH_2)_2$-), 1.38–1.46 (m, 4H; -$CH_2CH_2(CH_2)_{28}CH_2CH_2$-), 2.10 (s, 12H; 2×$N(CH_2)_2N(CH_3)_2$), 2.54–2.57 (m, 4H; 2×$NCH_2CH_2N(CH_3)_2$), 3.03 (s, 12H; 2×-$(CH_2)_2N(CH_3)_2(CH_2)_2$-), 3.29–3.32 (t, 4H; 2×$NCH_2CH_2N(CH_3)_2$), 3.42–3.45 (m, 4H; 2×$NCH_2CH_2O$-), 3.63–3.68 (q, 4H; -$OCH_2(CH_2)_{30}CH_2O$-), 4.00–4.04 (m, 4H; 2×$NCH_2CH_2O$-); ESI-MS: *m/z*: 927.9 $[M+H]^+$, 949.9 $[M+Na]^+$; elemental analysis calcd (%) for $C_{48}H_{104}N_4O_8P_2{\cdot}4H_2O$: C 57.69, H 11.30, N 5.61; found: C, 57.95; H, 11.35; N, 5.42.

Further modifications of bis(phosphocholines)

Dotriacontane-1,32-diyl-bis[2-(*N*,*N*-dimethyl-*N*-{[1-(phenylthiomethyl)-1,2,3-triazol-4-yl]methyl}ammonio)ethylphosphate] (8): Propynylcholine (**6d**, 17.2 mg, 20 μmol) and azidomethyl phenyl sulfide (6.6 mg, 40 μmol) were suspended in a mixture of water and ethanol (2:1, 7.5 mL). Sodium ascorbate (8 μmol, 0.4 mL of freshly prepared 20 mM solution in water) was added, followed by copper(II) acetate monohydrate (4 μmol, 0.4 mL of 10 mM solution in water). The heterogeneous mixture was stirred vigorously for 8–24 h at room temperature. After TLC (chloroform/methanol/ammonia 5:5:1) showed complete conversion of **6d**, the mixture was concentrated by evaporation of the solvent, and the residue was purified by MPLC by the gradient technique and with chloroform/methanol/water as eluent to give **8** as a white powder (17.9 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$, 27 °C): δ = 1.21–1.31 (m, 56H; -$(CH_2)_2(CH_2)_{28}(CH_2)_2$-), 1.51–1.59 (m, 4H; -$CH_2CH_2(CH_2)_{28}CH_2CH_2$-), 3.16 (s, 12H; 4×-$CH_3$), 3.54–3.56 (m, 4H; 2×$NCH_2CH_2O$-), 3.81–3.86 (q, 4H; -$OCH_2(CH_2)_{30}CH_2O$-), 4.25–4.29 (m, 4H; 2×$NCH_2CH_2O$-), 4.76 (s, 4H; 2×$CCH_2N$), 5.70 (s, 4H; 2×$SCH_2N$), 7.26–7.35 (m, 10H; 2×$C_6H_5$), 8.51 ppm (s, 2H; 2×$CH$); ESI-MS: *m/z*: 1192.2 $[M+H]^+$, 1214.8 $[M+Na]^+$; elemental analysis calcd (%) for $C_{60}H_{104}N_8O_8P_2S_2{\cdot}4H_2O$: C 57.03, H 8.93, N 8.87, S 5.07; found: C 57.33, H 9.05, N 8.62, S 4.81.

Dotriacontane-1,32-diyl-bis{2-[*N*,*N*-dimethyl-*N*-(2-{[5-(1,2-dithiolan-3-yl)-1-oxopentyl]oxy}ethyl)ammonio]ethylphosphate} (9): (±)-α-Lipoic acid (0.24 g, 1.2 mmol) and DCC (0.12 g, 0.6 mmol), dissolved in dry tetrachloromethane (10 mL), were placed in a round-bottomed flask. The mixture was stirred for 30 minutes at room temperature. The precipitation was filtered off, and the solution was concentrated under reduced pressure. DMAP (0.12 g, 0.6 mmol), dissolved in dry chloroform (10 mL), DMSO (5 mL) and compound **6e** (50 mg, 57 μmol) were then added, and the mixture was kept in a closed tube at 50 °C for 48 h. The crude product was purified by MPLC by the gradient technique and with chloroform/methanol/water as eluent to give **9** as a white solid. Yield: 10.7 mg (15%); ESI-MS: *m/z*: 1249.5 $[M+H]^+$, 1271.5 $[M+Na]^+$.

Sample preparation: Homogenous dispersions of the bolalipids in water were achieved by heating the aqueous mixture to 80 °C and vortexing.

DSC: DSC measurements were performed with a MicroCal VP-DSC differential scanning calorimeter (MicroCal, Inc., Northampton, MA, USA). Before the measurements, the sample solution (1 mg mL^{-1}) and the water reference were degassed under vacuum while stirring. A heating rate of 20 K h^{-1} was used, and the measurements were performed in the temperature interval from 2 to 95 °C. To check the reproducibility, three consecutive scans of each sample were recorded. The reference thermogram (water/water baseline) was subtracted from the thermograms of the samples, and the DSC scans were evaluated with the MicroCal ORIGIN 7.0 program.

FTIR spectroscopy: Infrared spectra were measured on a Bruker Vector 22 Fourier transform spectrometer with a DTGS detector operating at 2 cm^{-1} resolution. The sample, with a concentration of 50 mg mL^{-1}, was placed between two BaF_2 windows, separated by a 56 μm spacer, for measurements in D_2O. IR spectra were measured at 5 and 20 °C. After an equilibration time of 8 min, 32 scans were recorded and accumulated. The corresponding spectra of the solvent were subtracted from the obtained sample spectra with the aid of the OPUS software supplied by Bruker.

TEM: The negatively stained samples were prepared by spreading the bolalipid dispersion (5 mL) onto a Cu grid coated with a formvar film. After 1 min of adsorption time, excess liquid was blotted off with filter paper, and aqueous uranyl acetate (1%, 5 mL) was placed on the grid and drained off after 1 min. The dried specimens were examined with a Zeiss EM 900 transmission electron microscope.

DLS: The DLS experiments were performed with an ALV-NIBS-HPPS particle sizer (ALV-Laser Vertriebsgesellschaft, mbH, Langen, Germany). The device was equipped with a 3 mW HeNe laser with a wavelength of 632.8 nm. Because of the principle of noninvasive back scattering the scattering angle was 173°. All samples (1 mg mL^{-1}) were freshly prepared and then filtered through a membrane filter of 0.2 μm pore size (at 80 °C) into quartz cuvettes (HELLMA GmbH & Co. KG, Muellheim, Germany). Three individual measurements were performed for each system to test the reproducibility. The experimental data were analysed with the aid of the ALV-5000/E software based on the modified CONTIN method,[20] with the temperature correction of the viscosity being taken into account.

Calculation of hydrophobic chain structures: The energy-optimised structures of hydrophobic chains with substituted oxygen and sulfur atoms were calculated with the aid of the COMPASS force field in the Materials Studio (Accelrys, Inc.) Discover module.

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