Bolaamphiphiles and Monolayer Lipid Membranes Made from 1,6,19,24-Tetraoxa-3,21-cyclohexatriacontadiene-2,5,20,23-tetrone[⊥]

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Abstract: The title macrotetrolide contains two maleic diester units that add hydrophilic sulfur nucleophiles, e.g., bisulfite, thioacetic acid, and various thioglycosides, in high yield. Two-headed amphiphiles ("bolaamphiphiles" or "bolytes") are thus formed. They produce extremely thin (hydrophobic part ≃20 Å) monolayer lipid membrane (MLMs) vesicles, which have been characterized by electron microscopy. Vesicle MLMs produce well-resolved ^{1}H NMR spectra at room temperature, indicating fluidity. Such thin vesicle membranes can be effectively perforated with monensin pyromellitate to give water channels. They can be made unsymmetric; they resist fusion. The ester linkages are protected against acid- or base-catalyzed hydrolysis reactions within the membrane. Surface monolayer properties of some bolytes and the crystallization of a pure diastereomer of a sugar pentaacetate amphiphile are reported.

Two leather balls at the end of a string form a sling called "bola". Amphiphilic molecules with head groups at both ends of a hydrophobic core have been named "bolaform amphiphiles",[1] "bolaamphiphiles",[2] or "bolions".[3] Another convenient name for bolaform electrolytes is "bolyte". The ambiguous expression "bipolar lipids" is used by biochemists dealing with *archaebacterial* bolaamphiphiles.[4]

Water-insoluble bolaamphiphiles may aggregate in aqueous media to form monolayer lipid membranes (MLMs). MLMs provide at least four interesting new properties when compared with the usual bilayer lipid membranes (BLMs) from one-headed amphiphiles:

(i) MLMs may be thinner than BLMs. A thinness of 15–20 Å is possible. This permits the use of relatively short molecules for the formation of membrane pores, e.g., carotenoids and porphyrins for electrons, bile acids or ionophore derivatives for ions,[5] or organic molecules.

(ii) Bolytes with a large and a small head group may form unsymmetric vesicle membranes,[6] a starting point for syntheses of charge-separating systems.

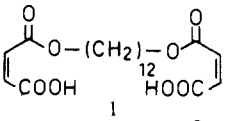
(iii) Bolytes with a positively charged and a neutral head group envelop anionic polyelectrolytes, e.g., nucleic acids, or cover negatively charged surfaces with a polar monomolecular layer.

(iv) MLM vesicles resist fusion.

This paper describes syntheses of macrocyclic bolaamphiphiles **3a–k** with several different head groups and a hydrophobic core that is less than 20 Å thick. Vesicle MLMs have been obtained from all of these compounds, and their unique properties have been evaluated, namely, thinness, fluidity, stability, and unsymmetry.

Results and Discussion

Syntheses. Treatment of maleic anhydride with 1,12-dodecanediol produced the diester diacid **1** in quantitative yield. **1** was cyclized with another mole of 1,12-dodecanediol to give **2** in 40%



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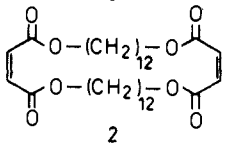

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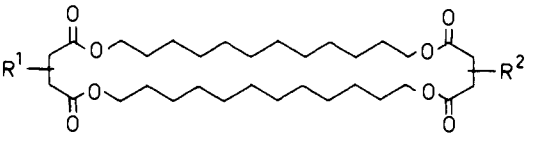
Chart I



3

a $R^1 = R^2 = SO_3^- Na^+$

b $R^1 = R^2 = S-CH_2-COO^- Na^+$

c $R^1 = R^2 = S-CH_2-CH_2OH$

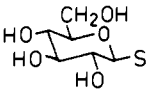
d $R^1 = R^2 = S-CHOH-CH_2OH$

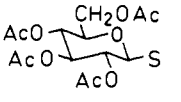
e $R^1 = R^2 = S-CH_2-CH_2-NH_3^+Cl^-$

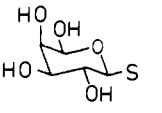
f $R^1 = R^2 = S-CH(CH_2-COO^- Na^+)-COO^- Na^+$

g $R^1 = SO_3^- Na^+$ $R^2 = S-CH(CH_2-COO^- Na^+)-COO^- Na^+$

h $R^1 = SO_3^- Na^+$ $R^2 = S-CH(CH_2-COOH)-COOH$

i $R^1 = R^2 =$ 

j $R^1 = R^2 =$ 

k $R^1 = R^2 =$ 

Results and Discussion

Syntheses. Treatment of maleic anhydride with 1,12-dodecanediol produced the diester diacid **1** in quantitative yield. **1** was cyclized with another mole of 1,12-dodecanediol to give **2** in 40% isolated yield. Gram quantities of **2** were produced in moderately dilute solutions (0.012 M) in one batch and isolated by a single

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[⊥] Dedicated to Professor Georg Mamecke on the occasion of his 70th birthday.

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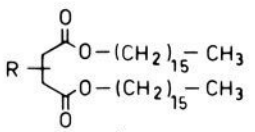
recrystallization. The macrolide **2** was mixed in 2-propanol solution with 2 equiv of a sulfur nucleophile, e.g., sodium bisulphite or thioacetic acid, and refluxed. Symmetric bolaamphiphiles **3a–k** (Chart I) were isolated in average yields of 70%. In some cases, amphiphiles with one head group and one unreacted maleic ester unit were also isolated. The unsymmetric bolaamphiphile **3g** was obtained by subsequent additions of thiosuccinic acid and sodium bisulfite.[6,7]

Thioglycosides **3i–k** were obtained from the appropriate β-glycosylmercaptides and the macrotetrolide **2** in 2-propanol acidic buffer (pH 2) mixtures. They produced glassy, amorphous powders that could not be crystallized. Although satisfactory elemental analyses were obtained from these materials, the ^{1}H NMR spectra were not resolved. The tetra-*O*-acetates were therefore prepared for analytical purposes. They yielded the expected ^{1}H NMR spectra as well as excellent C, H, and S combustion analyses.

The open-chain amphiphiles **4a–d** and **5a, b** were prepared by analogous Michael additions. In the case of the β-D-glucosyl **5a**, the separation of both diastereomers was achieved by fractionate crystallization.



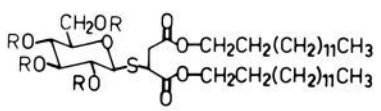
4

a R = $SO_3^-Na^+$

b R = $S-CH(CH_2-COO^-Na^+)-COO^-Na^+$

c R = $S-CH(CH_3)-COO^-Na^+$

d R = $S-CH_2-CH(OH)-CH_2OH$



5a R = H

5b R = $-C(=O)-CH_3$

Vesicle Formation and Membrane Thickness. The most regular and long-lived vesicles were formed from the bolytes **3b** and **3e** on ultrasonication of aqueous suspensions to constant turbidity at 50 °C. Vesicles were also produced when a solution of **3b** or **3f** in sodium hydroxide (pH 8.5) was acidified to pH 4. Once formed, the vesicles did not disintegrate when the pH was raised to 7. These observations are in analogy to earlier reports concerning the vesiculation of bipyridinium bolytes[8] and fatty acids.[9]

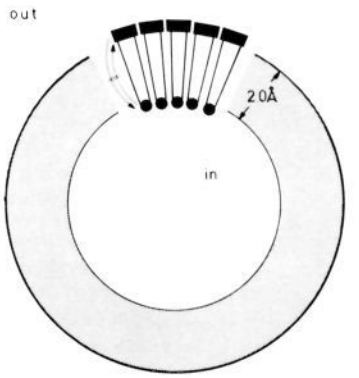


Figure 1. Scheme of an MLM vesicle that illustrates statements (i), (ii), and (iv).

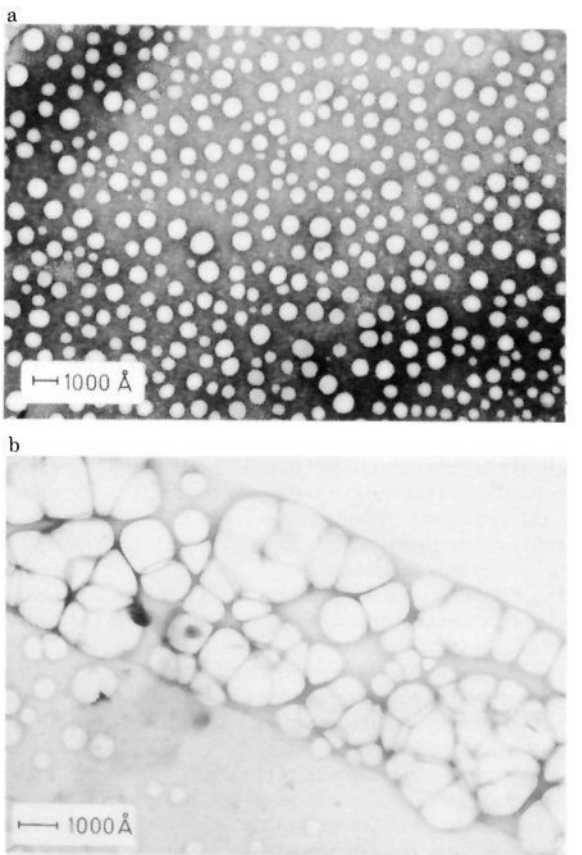


Figure 2. Electromicrographs (magnification 80 000) of negatively stained vesicles from the thioacetate bolyte **3b**: (a) vesicles prepared at pH 8; (b) at pH 4.

Typical electron micrographs of monolayered vesicles are given in Figure 2. The symmetric bolyte **3b** with two thioacetic acid head groups gives single vesicles at pH 7 and extended aggregates at pH 4.5. The vesicle's diameter in the nonaggregated state (pH >6) remains constant for very long periods of time. The vesicle size was still in the order of ≤1000 Å after a full year. At low pH, however, aggregation with subsequent fusion was observed. Charged head groups of bolytes do obviously not cross the hydrophobic membrane and form water passages to other vesicles. They do not fuse. Vesicles with uncharged bolaamphiphiles, however, fuse.

From Dreiding models, the length of the hydrophobic core of the bolaamphiphiles **3** (between succinate methine groups) can

(1) Jeffers, P. M.; Daen, J. *J. Phys. Chem.* **1965**, *69*, 2368–2373.
(2) Fuhrhop, J.-H; Mathieu, J. *Angew. Chem.* **1984**, *96*, 124–137; *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 100–113.
(3) Morawetz, H.; Kandaniem, A. Y. *J. Phys. Chem.* **1966**, *70*, 2995–3000.
(4) Gulik, A.; Luzzati, U.; de Rosa, M.; Gambacorta, A. *J. Mol. Biol.* **1985**, *182*, 131–149.
(5) Fuhrhop, J.-H.; Liman, U. *J. Am. Chem. Soc.* **1984**, *106*, 4643–4644.
(6) Fuhrhop, J.-H.; Mathieu, J. *J. Chem. Soc., Chem. Commun.* **1983**, 144–145.
(7) Fuhrhop, J.-H.; David, H. H.; Ellermann, K.; Mathieu, J. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 444–445.
(8) Fuhrhop, J.-H.; Fritsch, D.; Tesche, B.; Schmiady, H. *J. Am. Chem. Soc.* **1984**, *106*, 1998–2001.
(9) Hargreaves, W. R.; Deamer, D. W. *Biochemistry*, **1978**, *17*, 3759–3765.

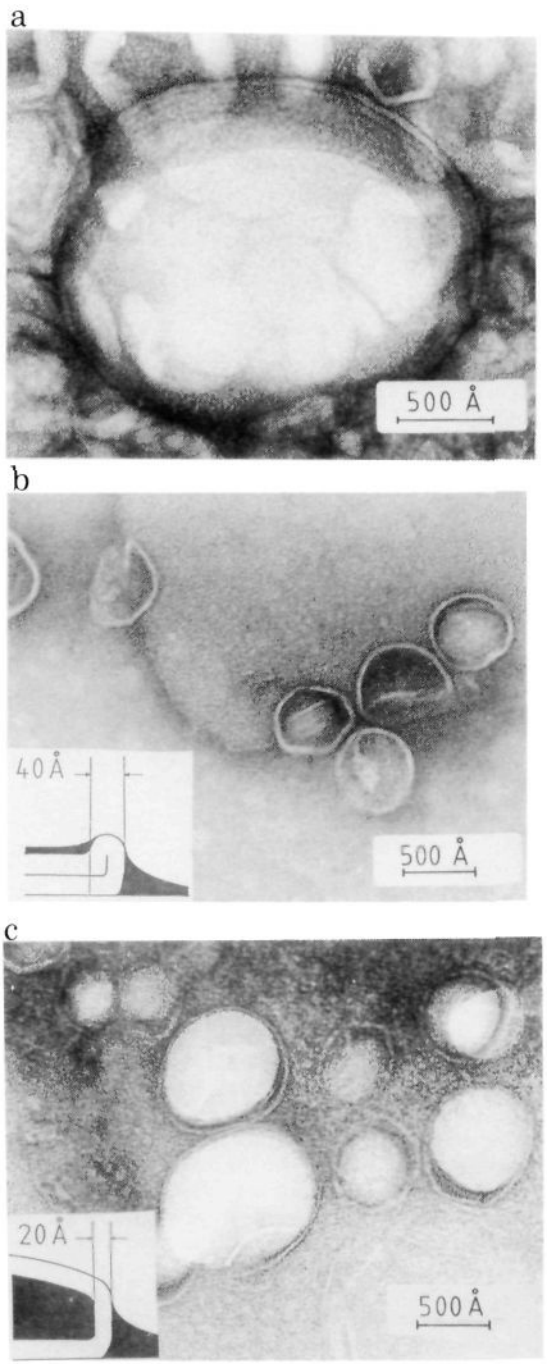


Figure 3. Electron micrographs of negatively stained disks obtained by collapse of vesicle membranes: (a) concentric BLMs from lecithin as a reference, 50-Å thickness; (b) a bulge of two MLMs from **3b** surrounded by uranyl acetate, 40-Å thickness; (c) a single MLM supported by a wolframate glass on both sides, 20-Å thickness.

be estimated to be approximately 21 Å. With both head groups included, all the bolaamphiphiles **3a–f** should be shorter than 30 Å.

We measured the thickness of the monolayer lipid membranes by electron microscopy. The hydrophobic membranes exclude metal salts, which are used as negative stains in electron microscopy.[10] We prepared vesicles filled with 0.02 M sodium tungstate(VI) by sonication. The vesicular dispersion was then placed on a carbon-coated copper grid, stained with uranyl acetate (1%), and dried. Under these conditions, the water evaporation led to a collapse of the vesicles and the membranes folded into

(10) Bangham, A. D. *Prog. Biphys. Mol. Biol.* **1968**, *18*, 29–95.

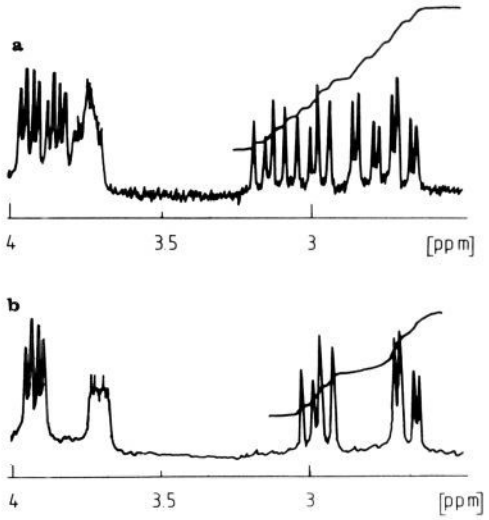


Figure 4. ^{1}H NMR spectrum (270 MHz) of D-thioglycoside pentaacetate thiosuccinate diastereomers in deuterated methanol: (a) mixture of two diastereomeric thiosuccinates **5b**; (b) pure *S*-thiosuccinate **5b**.

numerous forms. They show up in electron micrographs as white borders. If one measures the thickness of some of the well-defined white borders on photographs with a graduated magnifying glass, one finds that most of them have a thickness of either approximately 20 or 40 Å. A thickness of 20 Å is often found in vesicle membranes that are disrupted (Figure 3c). Other vesicle membranes, however, collapse in vacuo to a double-layered disk with an elevated edge (see Figure 3b). The result is a 40-Å hydrophobic layer made of two MLMs. The findings correspond well to the estimated 21 or 42 Å of one or two hydrophobic skeletons of the amphiphilic macrocycles **3a–k**. As a reference, we measured the thickness of the membranes in monolayer and multilayer (onion-like) lecithin vesicles filled with staining material (see Figure 3a). We found thicknesses of about 50 or 100 Å, which agree with the precise literature value of 50.3 Å[11] for a bilayer from X-ray diffraction measurements or 100.6 Å for a doubled bilayer.

^{1}H NMR Spectra: Chirality and Melting. The mixture of **4c** of four thiolactate diastereomers in deuterated acetic acid gives a ^{1}H NMR spectrum in which both methine protons neighboring the sulfur atom produce two multiplets separated by 0.1 ppm. The same separation is observed in the D-thioglucosyl acetate amphiphile **5b** (Figure 4a). When **5b** was recrystallized twice from methanol, the ^{1}H NMR spectrum of a pure diastereomer was observed (Figure 4b) with only one doublet for each of the α-CH protons. A comparison of the molar optical rotation of the isolated stereoisomer ([M] −393°) with the reported value for the corresponding dimethylester ([M] −402°)[12] shows that both compounds have the same absolute configuration in the aglycone, namely the *S* configuration.

The proton between the sulfide groups (or the corresponding sulfoxide) and the ester groups is exchangeable in deuterated aqueous media. Vesicles made from mixtures of diastereomers of amphiphiles such as **4c** or **5a** or the corresponding sulfoxides can therefore be used in studies of stereoselective rearrangements toward one single diastereomer on chiral guest vesicle surfaces. Studies along this line are in progress.

The ^{1}H NMR spectra of monolayer vesicles in D_2O made from the thioacetate **3b** gave nonresolved but relatively narrow signals for all protons. The width of the peaks was approximately the same at 30 and 90 °C (Figure 5a). In thermodifferential analysis (TDA) no transition point was observed. This is in contrast to analogous bilayer membranes made from the thiodiol **4d**, which produces sharp transition points around 40 °C in both TDA and ^{1}H NMR spectra (Figure 5b). This may be rationalized with the

(11) Stamatoff, J.; Bilash, T.; Ching, Y.; Eisenberger, P. *Biophys. J.* **1979**, *28*, 413.
(12) Michael, F.; Kriesten, W. *Chem. Ber.* **1968**, *101*, 3724–3728.

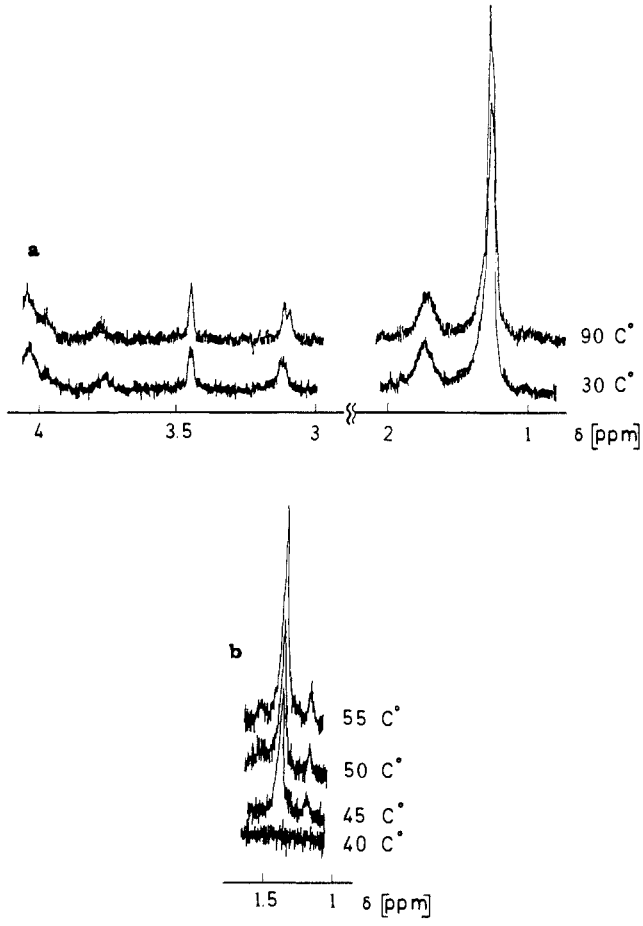


Figure 5. Temperature-dependent ^1H NMR spectra of vesicles in D_2O: (a) made from bolyte **3b**; (b) made from the single-head amphiphile **4d**.

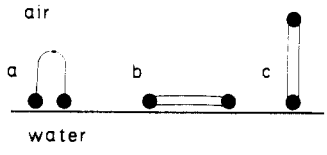


Figure 6. Most probable positions and conformations of bolaamphiphiles on aqueous surfaces: (a) single-chain bolaamphiphile; (b) macrolide bolaamphiphile at low surface pressure; (c) macrolide bolaamphiphiles at high surface pressure.

assumption that the average distance between bolaamphiphiles in a curved MLM (see Figure 1) is larger than in a BLM. Rotation about individual bonds within the oligomethylene chain is therefore favored. Black MLMs from archaebacterial terpenoid bolaamphiphiles, however, produce transition points close to 40 °C.[13] This difference may be caused by less curvature in the black as compared to vesicle membranes and/or by melting of chiral superstructures within the terpenoid membrane.

Surface Monolayers. Bolytes with only one oligomethylene chain, e.g., α,ω-dicarboxylic acids, have twice the molecular surface area as their one-headed counterparts.[1] Surface tensions are about the same in both cases. It is assumed that the head groups are anchored to the surface and that "wicket" conformations of the chains prevail in condensed layers (Figure 6a). A vertical looping of the hydrophobic chains becomes less probable in macrocycles such as **3** because the bending of the chains would have to occur cooperatively. We anticipated that **3** could therefore preferably lie parallel to the surface (Figure 6b). If the monolayer is compressed, the head groups may (Figure 6c) or may not (Figure 6b) be lifted from the aqueous surface. In the first case, the surface area of a molecule should be about 50 Å^2 or less and the surface

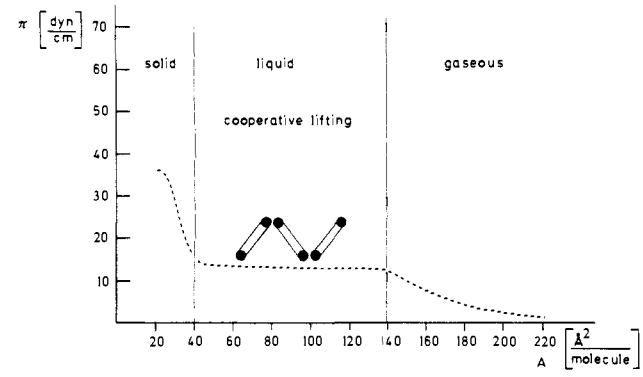


Figure 7. Pressure–area isotherms for the diol bolaamphiphile **3d** on water.

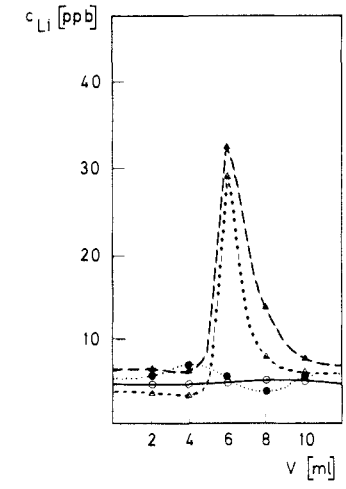


Figure 8. Elution diagram of the gel filtration (Sephacryl S 1000 1 × 20 cm; 0.03 M NaCl) of vesicles sonicated in 0.03 M LiCl: (△) vesicles made of **3b**; (○) vesicles made of **3b** with 0.1 mol % **6**; (▲) vesicles made of **3c**; (●) vesicles made of **3c** with 0.1 mol % **6**.

tension should remain relatively high.

The experimental surface pressure isotherms are in agreement with the predictions from the models in Figure 6b,c: The surface area per molecule is in all cases close to 40 Å^2 and the surface tension of macrocyclic bolytes is much higher (Π is lower) than with one-headed surfactants. An interesting effect was observed with the thioethanol **3c**. A distinct transition from the gaseous to the liquid state at molecular areas between 170 and 130 Å^2 was followed by an unexpected, highly cooperative compression of the liquid. The latter effect is probably caused by hydrogen bonding between the head groups (Figure 7). A similar model has been proposed by Kellner and Cadenhead for the 16-hydroxyhexadecanoic acid layer.[14]

Neither the sharp inflection nor the cooperativity occurs in the charged thioacetic acid analogus **3b**. We also examined the nonbolaamphiphiles **4**. The thiolactate **4c** produces no inflection at high molecular areas, occupies about the same molecular area as the bolytes (40 Å^2), but has a much higher tenside activity (15 dyn/cm instead of 65 dyn/cm). With electron microscopy, extended monomolecular layers of bolytes **3a–g** on carbon grids were observed several times. An example is seen in Figure 3b.

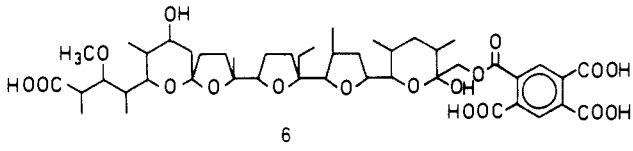
An important property of bolaform monolayers on water is their apparent instability. They collapse at relatively low pressures, e.g., 45 dyn/cm in the above example. The same behavior is observed if the aqueous subphase is a 3 M sodium chloride solution. The collapse is therefore not caused by a dissolution of the bolyte in the subphase[14] but rather by their piling up. This will, of course, be a problem in the preparation of Langmuir–Blodgett films, which are currently under investigation.

(13) Gliozzi, A.; Paoli, G.; de Rosa, M.; Gambacorta, A. *Biochim. Biophys. Acta* **1983**, *735*, 234–242.

(14) Kellner, M. J.; Cadenhead, D. A. *J. Colloid Interface Sci.* **1978**, *63*, 452–460.

Entrapment and Channel Formation. Vesicular solutions (10^{-3} M) made from bolytes **3b** (negatively charged) or **3c** (electroneutral) entrapped about 0.5% of a 0.03 M lithium chloride solution or of a 10^{-4} M pyranine[15] solution on ultrasonication. Gel filtration on Sephacryl S 1000 removed the salt or dye in the outer aqueous phase (Figure 8).

Addition of a bolyte with a hydrophobic and a hydrophilic side—namely, monensin pyromellitate **6**–perforated the monolayer vesicle membranes made from electronegative or electroneutral bolaamphiphiles **3b**, **c**.[5,16] Since **6** is only 25 Å long, this observation provides more evidence for the assumed ultrathinness of the MLMs.



6

Vesicular solutions of the bolytes also dissolved the highly hydrophobic magnesium octaethylporphyrin (MgOEP) on cosonication. Solutions (10^{-3} M) of the thioacetate **3b**, for example, dissolved 10 mol % of MgOEP. The resulting vesicles, however, were always by an order of magnitude larger (diameter ~5000 Å) than the corresponding vesicles without porphyrin. The large chromophore obviously prevents a high curvature of the thin, monolayer vesicle membrane. MgOEP dissolved in the unsymmetric vesicle membrane described below was not demetalated down to pH 2.

Unsymmetric Vesicle Membrane. The unsymmetric bolyte **3g** forms vesicle membranes in which all of the sulfonate head groups are at the inner surface. This has been proven by an application of the "metachromatic effect", which is briefly summarized here. Vesicles made from the sulfonate **4a** bear negative charges at the inside and outside surfaces. The anionic aggregates cause dimerization of acridine orange or methylene blue and short wavelength shifts ("metachromatic effect")[17] at pH 4 and 7. The thiosuccinate **4b** vesicle produces the same effect at pH 7 but no effect at pH 4. Mixtures of **4a** and **4b** also give a metachromatic effect at pH 4, which is clearly detectable down to concentrations of 2% of the sulfonate **4a**. Since vesicles from **3h** showed no metachromatic effect at pH 4, it was concluded that more than 98% of the *small* sulfonate groups must be located at the inner surface and more than 98% of the *large* thiosuccinate groups outside.[6] These results show clearly that wicket-type conformations do not occur in monolayer vesicle membranes. If they did, negatively charged sulfonate would appear on the outer surface. Earlier experiments with NMR shift reagents[8] led to the same conclusion: the bolaamphiphiles in monolayer vesicle membranes are in a stretched conformation to more than 95%.

Stability and MLM Vesicles. MLM vesicles with surface charges may form extended aggregates that precipitate from solution (see Figure 2b), but we have, so far, never observed fusion to large vesicles or to myelin figures[18] at room temperature or below. Only at temperatures above 80 °C a rearrangement of vesicles to noncurved monolayer membranes is sometimes found.[8] Vesicles with hydroxyl head groups, however, fuse within a few days. This applies to vesicles made from alcohols **3c**, **d** as well as to carbohydrate lipids **3i–k**.

Bolytes in monolayer vesicle membranes are exceptionally stable against hydrolysis reactions. Sodium hydroxide or hydrogen chloride solutions (1 M) did not release any (≤1%) 1,12-dodecanediol from **3b** or **3g** after 24 h at 70 °C. In homogeneous methanolic solutions of both **3b** and **3g**, rapid methanolyses were observed.

The unusual stability of the ester linkages may be caused by (i) their unaccessibility to ionic reagents, (ii) the reversibility of cleavage reactions within the organized membrane, and (iii) the improbability of cleaving two ester bonds on one diol molecule. The relative importance of the three "protection mechanisms" is unknown. It will be further investigated by comparisons of several monolayer and bilayer vesicle membranes.

Experimental Section

^{1}H NMR spectra were recorded on a Bruker WH 270-MHz instrument. Electron microscopy was carried out on a Philips EM 400 instrument at 100 kV or an Elmiskop 1139 (Siemens). Thermodifferential analyses were performed with a Perkin-Elmer Model DSC-2. For the monolayer studies a microprocesses-controlled film balance was used.[19]

Microanalyses were performed by the Mikrolabor des Instituts für Organische Chemie der Freien Universität Berlin. Sonications were performed by using a Heat System Model W 220 F with standard titanium horn. Atomic emission spectroscopy was carried out on a Varian Model AA 275. Specific rotations were determined on a Perkin-Elmer polarimeter Model 241. Sephacryl S 1000 was purchased from Pharmacia, pyranine from Serva. Chemicals for syntheses came from Aldrich or Fluka. They were used without further purification.

Bis(2-butenedicarboxylic acid) 1,12-Dodecanediyl Ester (1). Maleic anhydride (9.81 g, 0.1 mol) and 1,12-dodecanediol (10.17 g, 0.05 mol) were refluxed in 300 mL of dry benzene for 8 h. On standing at room temperature **1** crystallized from the solution and was recrystallized from methyl ethyl ketone: yield, 18.9 g (95%); mp 117–118 °C; R_f = 0.55 (silica gel, 90:25:4 benzenee–dioxane–acetic acid); IR (KBr) ν_{CO} 1725, $\nu_{C=C}$ 1648 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.3 (m, 16 H, CH_2), 1.70 (t, 4 H, OCH_2CH_2), 4.27 (t, 4 H, OCH_2), 6.39, 6.47 (2 d, 4 H, CH=CH), 10.50 (s, br, 2 H, COOH). Anal. Calcd for $C_{20}H_{30}O_8$ (348.46): C 60.29; H, 7.59. Found: C, 60.72; H, 7.83.

1,6,19,24-Tetraoxacyclohexatriaconta-3,21-diene-2,5,20,23-tetrone (2). Dicarboxylic acid **1** (3.98 g, 0.01 mol) was refluxed in benzene (700 mL) with 1,12-dodecanediol (2.02 g, 0.01 mol) and *p*-toluenesulfonic acid (1 g). The solvent was removed and the residue recrystallized once or twice from ethyl acetate: yield, 2.3 g (38%); mp 103.5 °C; IR (KBr) $\nu_{C=O}$ 1730, $\nu_{C=C}$ 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (m, 32 H, CH_2), 1.65 (m, 8 H, OCH_2CH_2), 4.26 (t, 8 H, OCH_2), 6.22 (s, 4 H, CH=CH); MS (70 ev) *m/e* 564 (100, M^+), 546 (50, M^+-H_2O). Anal. Calcd for $C_{32}H_{52}O_8$ (564.76): C, 68.06; H, 9.28. Found: C, 68.05; H, 9.36.

Disodium 2,5,20,23-Tetraoxo-1,6,19,24-tetraoxacyclohexatriacontane-3,21(22)-disulfonate (3a). Macrotetrolide **2** (0.9 g, 1.6 mmol) was dissolved in 100 mL of 4:1 2-propanol–water. Sodium disulfite (0.64 g, 3.4 mmol) in 2 mL of water and some sodium hydroxide were added (pH 8). The mixture was refluxed for 5 h under nitrogen, the solvent was removed, and the residue was extracted with hot methanol. The methanol was evaporated and the residue recrystallized from water: yield, 0.81 g (66%); mp 255–260 °C; R_f = 0.58 (silica gel, 4:8:10:8; water–ethyl acetate–2-propanol–concentrated ammonia); IR ν_{CO} 1730, ν_{SO_3} 1230, 1055 cm^{-1}; ^{1}H NMR (270 MHz, Me_2SO-d_6) δ 1.22 (2, 32 H, OH_2), 1.49 (m, 8 H, OCH_2CH_2), 2.77, 2.89, (2 dd, 4 HCH_2CHSO_2), 3.66 (dd, 2 H, $CHSO_3$), 3.91, 3.98 (2 m, 8 H, $COOCH_2$). Anal. Calcd for $C_{32}H_{54}Na_2S_2O_{14}$ (773.7): C, 49.73; H, 7.05; S, 8.50. Found: C, 49.76; H, 7.42; S, 8.26.

Disodium 2,5,20,23-Tetraoxo-1,6,19,24-tetraoxacyclohexatriacontan-3,21(22)-diylbis(thio)bis(acetate) (3b). Macrotetrolide **2** (2.0 g, 3.5 mmol) in 120 mL of 2-propanol was heated to 80 °C and mixed with 2-mercaptoacetic acid containing sodium hydroxide and a few drops of piperidine (final pH was 8). The mixture was refluxed for 1 h and cooled to room temperature. The crystalline product was filtered off and washed with chloroform. The residue was dried over calcium chloride and paraffin: yield, 2.07 g (74%); mp 215 °C (decomp); R_f = 0.15 (silica gel, 3:1 methanol–chloroform); IR ν_{CO} = 1610 cm^{-1}; ^{1}H NMR (270 MHz, CD_3COOD) δ 1.3 (s, 48 H, CH_2), 1.6 (s, 8 H, OCH_2CH_2), 2.88 (m, 4 H, CH_2CHS; AB-type), 3.88 (m, 2 H, CH_2S) 4.14 (m, 8 H, $COOCH_2$).

2,5,20,23-Tetraoxo-1,6,19,24-tetraoxacyclohexatriacontan-3,21(22)-diylbis(thio)bis(propanediol) (3d). Procedures were similar to those used for the preparation of **3b**: the isolated yield of **3c** was 65%; mp 82 °C; R_f = 0.28 (silica gel, 90:25:4 benzene–dioxane–acetic acid); IR ν_{OH} 3420, ν_{CO} 1740 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.3 (s, 32 H, CH_2), 1.62 (m, 8 H, OCH_2CH_2) 2.72, 2.82, 2.94, 3.56, 3.72, 3.86 (6 m. 20 H), 4.14 (m, 8 H, $COOCH_2$). Anal. Calcd for $C_{38}H_{68}O_2S_2$ (780.5): C, 58.46; H, 8.72: S, 8.21. Found: C, 58.29; H, 9.02; S, 8.17.

The dithioethanol **3c** was prepared analogously and gave the expected analytical data.[20]

3,21(22)-Bis((2-aminoethyl)thio)-1,6,19,24-tetraoxacyclohexatriacontan-2,5,20,23-tetrone Dihydrochloride (3e). A mixture of macro-

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tetrolide **2** (1 g, 1.77 mmol) and 2-aminoethanethiol hydrochloride (0.46 g, 4 mmol) in 150 mL of 7:3 2-propanol–water was brought to pH 8 with a few drops of triethylamine. The solution was stirred for 1 h at 70 °C under nitrogen, acidified with HCl (pH 4), and evaporated to a volume of about 50 mL. The precipitate was filtered and recrystallized from methanol: yield 1.1 g (79%); mp 153–160 °C; IR (KBr) ν_{NH} 3430 cm^{-1} (br); ^{1}H NMR (270 MHz, 1:1 $CDCl_3$ – CF_3COOD) δ 1.28 (s, 32 H, CH_2), 1.63 (t (br), 8 H, OCH_2CH_2) 4.07–4.14 (m, 8 H, OCH_2) 2.89, 3.03 (2 dd, 4 H, CH_2CO), 3.79 (dd, 2 H, CHS, ABM-type, J_{AB} = 18, J_{AM} = 6, J_{BM} = 8 Hz), 3.05, 3.19 (2 dt, 4 H, CH_2S, ABM_2-type, J_{AB} = 15, J_{AM} = J_{BM2} = 6 Hz), 3.45 (t (br), 4 H, CH_2NH_3); MS (FAB, Me_2SO–glycerol–H_2O): *m/e* 719 (100, M^+–HCl–Cl). Anal. Calcd for $C_{36}H_{68}Cl_2N_2O_8S_2$ (792.0): C, 54.69; H, 8.66; N, 3.54; S, 8.10; Cl, 8.95. Found: C, 54.52; H, 8.82; N, 3.53; S, 8.55; Cl, 9.19.

Tetrasodium 2,5,20,23-Tetraoxo-1,6,19,24-tetraoxacyclohexatriacontan-3,21(22)-diylbis(thio)bis(succinate) (3f). Procedures were similar to those used for the preparation of **3b**; the isolated yield of **3d** was 65%: mp 270 °C; (decomp.); R_f = 0.07 (silica gel, 99:1 methanol–acetic acid; ^{1}H NMR (270 MHz, CD_3COOD) δ 2.84, 3.04 (2 m, 8 H, CH_2CHS), 3.56, 3.96 (2 m, 4 H, CHS), 4.14 (m, 8 H, $COOCH_2$). Anal. Calcd for $C_{40}H_{60}Na_4O_{16}S_2 \cdot 2H_2O$ (989.1): C, 48.58; H, 6.12. Found: C, 48.74; H, 6.05.

Trisodium (2,5,20,23-Tetraoxo-21(22)-sulfonato-1,6,19,24-tetraoxacyclohexatriacontan-3-ylthio)succinate (3g). 2-Mercaptosuccinic acid (0.39 g, 2.6 mmol) in dilute sodium hydroxide solution containing piperidine (final pH 7.2) was mixed with macrotetrolide **2** (1.65 g, 2.9 mmol) in 100 mL of 2-propanol. The solution was briefly refluxed until a homogeneous solution was formed (~10–15 min). The solvent was then quickly removed and the residue extracted twice with 100 mL of hot acetone. The acetone was removed and the residue was washed on a filter plate (03) with ice water and dried at 70 °C in vacuo. The isolated product (1.5 g, 70%) was disodium (2,5,20,23-tetraoxo-1,6,19,24-tetraoxacyclohexatriacont-3-en-21(22)-ylthio)succinate: mp 240 °C (decomp); R_f = 0.16 (conditions as for **3d**); ^{1}H NMR similar to **3d**, extra signal at δ 6.88 (dd, 2 H, CH=CH). Anal. Calcd for $C_{36}H_{56}Na_2O_{12}S$ (758): C, 56.99; H, 7.39; S, 4.22. Found: C, 56.82; H, 7.31; S, 4.18.

This maleic diester (1.52 g, 2 mmol) was dissolved in hot 2-propanol–water (4:1, 100 mL) and sodium bisulfite (0.19 g, 1 mmol) and 2 mL of water was added. The mixture was refluxed under nitrogen for 5 h and left overnight. The solvent was removed. The residue was extracted with hot methanol, which was again evaporated to dryness. The white residue (1.2 g, 70%) had a melting point of 290 °C (decomp) and was analytically pure. IR and ^{1}H NMR corresponded to a 1:1 mixture of **3a** and **3d**. Anal. Calcd for $C_{36}H_{57}Na_3O_{15}S_2$ (862): C, 50.12; H, 6.61; S, 7.42. Found: C, 49.92; H, 6.54; S, 7.36.

Identical **3e** was also obtained by starting with the sulfonation and subsequent addition of thiosuccinic acid. The yield was, however, less.

Thiosuccinic Acid 3h. The free thiosuccinic acid was obtained when the aqueous solution of **3e** was titrated with HCl to pH 4 (partial precipitation) and extracted with ethyl acetate: R_f = 0.25 (silica gel, 90:25:4 benzene–dioxane–acetic acid). Anal. Calcd for $C_{36}H_{59}NaO_{15}S_2$ (818): C, 52.81; H, 7.21; S, 7.82. Found: C, 52.64; H, 7.13; S, 7.74.

(*R*,*S*)-3,21(22)-Bis(β-D-glucopyranosylthio)-1,6,19,24-tetraoxacyclohexatriacontan-2,5,20,23-tetrone (3i) and the β-D-Galactopyranosyl Analogue 3k. The macrotetrolide **2** in 150 mL of 7:3 2-propanol–titrisol buffer (Merck, pH 2) was heated to 50 °C and mixed with 1 g (3.94 mmol) of solid sodium β-D-glucosylmercaptide.[21] The pH of the solution rose to about 10 and was brought to 8 with a few drops of acetic acid. The solution was stirred at 50 °C for another 3 h and evaporated to dryness. The resulting syrup was redissolved in 30 mL of water and reprecipitated with 40 mL of a saturated sodium chloride solution. The white precipitate was filtered off, redissolved in 50 mL of methanol, and again filtered. Toluene (50 mL) was added to the methanol solution, and both solvents were slowly removed in vacuo at 40 °C. The resulting syrup solidified to a glassy and hygroscopic powder. TLC on silica gel with 5:6:3:1 1-butanol–acetic acid–diethyl ether–water or 4:1 chloroform–ethanol revealed only one spot: yield, 1.3 g (77%); mp 60–130 °C; $d^{20}{}_D$ –27 in CH_3OH; MS (FAB, Me_2SO–glycerol)^D: *m/e* 965 (100, M^+–$2H_2O$ + 2 Na^+); IR(KBr) ν_{OH} 3420, ν_{CO} 1735 cm^{-1}; no resolved ^{1}H NMR spectra (see below, **3i**). Anal. Calcd for $C_{44}H_{76}O_{18}S_2 \cdot 4H_2O$ (1029,2): C, 51.35; H, 8.22. Found: C, 51.19; H, 7.81.

The same procedure was followed to obtain the galactopyranosyl derivative **3k**: yield 1.4 g (83%); mp 71–135 °C; $[\alpha]^{20}{}_D$ –35° (CH_3OH); IR and MS identical with data given for **3h**. Anal. Calcd for $C_{44}H_{76}O_{18}S_2 \cdot 2H_2O$ (993.2): C, 53.21; H, 8.21. Found: C, 52.68; H, 7.78.

(*R*,*S*)-21(22)-Bis(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosylthio)-1,6,19,24-tetraoxacyclohexatriacontan-2,5,20,23-tetrone (3j) and the β-D-Galactopyranosyl Analogue 3k. Acetic anhydride (3 g) and dry pyridine (4 g) were mixed with 500 mg (0.5 mmol) of the diglycosides **3h** (or **3k**). After 1 h a clear solution was formed and was left for 18 h at room temperature. The solvents were removed, and the residue was redissolved in chloroform, washed with water (2 × 10 mL), dried over sodium sulfate, and recrystallized twice from methanol: yield, 500 mg (80%); mp 105–115 °C; IR ν_{CO} 1755 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.29 (s, 32 H, CH_2), 1.56–1.72 (m, 8 H, OCH_2CH_3), 2.01, 2.02, 2.03, 2.04, 2.10, 2.12 (6 s, 24 H, CH_3CO) 2.70, 2.85, 2.97, 3.12, 3.85, 3.96 (6 dd, 6 H, 2 ABM systems, J_{AB} = 18, J_{AM} = 5, J_{BM} = 12 Hz, CH_2CO, CHSCO) 3.68–3.78 (m, 2 H, at C-5), 4.00–4.32 (m, 12 H, CH_2O), 4.77–4.87 (2 d, 2 H, CHS at C-1, J_{AA} = 10 Hz), 4.95, 4.98, 5.03, 5.09, 5.22, 5.25 (6 t, 6 H, CHOAc); MS (–FAB/+FAB, $CHCl_3$, TEG) *m/e* 1291 (20, M-H). Anal. Calcd for $C_{60}H_{32}O_{26}S_2$ (1293.5): C, 55.71; H, 7.17; S, 4.96. Found: C, 55.49; H, 7.15; S, 5.60.

For the galactosyl analogue **3l** the same procedure was applied: yield, 470 mg (70%); mp 57–67 °C; $[\alpha]^{20}{}_D$ –10° ($CHCl_3$); all spectra very similar to those of **3i**. The only significant difference was in ^{1}H NMR: δ 5.44 (t, 2 H, CHOAc on C-4 of galactose). Anal. Calcd for $C_{60}H_{32}O_{26}S_2$ (1293.5): C, 55.71; H, 7.17; S, 4.96. Found: C, 55.48; H, 7.26; S, 5.26.

Dihexadecyl 3-Sulfonatosuccinate (4a). Dihexadecyl maleate[12] (2.82 g, 5 mmol) in 2-propanol–water (4:1) was mixed with sodium bisulfite (0.91 g, 9.5 mmol) in water (100 mL) and refluxed under nitrogen for 5 h. On cooling, the product precipitated and was dried over P_2O_5: yield, 2.28 g (68%); mp 212 °C; R_f = 0.49 (silica gel, 8:10:4:3 ethyl acetate–2-propanol–water–20% ammonia); IR ν_{CO} 1710 $\nu_{SO_3^-}$ 1300 cm^{-1}. Anal. Calcd for $C_{36}H_{69}NaO_7S \cdot 1.5\ H_2O$ (667): C, 62.16; H, 10.36; S, 4.60. Found: C, 62.13; H, 10.31; S, 4.55.

Dihexadecyl 3-[(1,2-Dicarboxyethyl)thio]succinate Disodium Salt (4b). 2-Mercaptosuccinic acid (1.81 g, 12 mmol) in 100 mL of water containing NaOH (pH 7.2) was mixed with dihexadecyl maleate (6.9 g, 12 mmol) in ethanol (100 mL). The mixture was refluxed for 4 h and then cooled to room temperature. The white precipitate was recrystallized from 4:1 ethanol–water: yield, 8.1 g (88%); mp 220 °C (decomp); IR ν_{CO} 1738 cm^{-1}; ^{1}H NMR (270 MHz, CD_3COOD) δ 0.89 (t, 6 H, CH_3), 1.3 (s, 52 H, CH_2), 1.62 (m, 4 H, OCH_2CH_2), 2.82, 3.06 (m, 2 H each, AB-type, CH_2CO), 3.66, 3.98 (dd and m, 1 H each, CHS), 4.12 (m, 4 H, OCH_2). Anal. Calcd for $C_{40}H_{72}Na_2O_8S$ (758): C, 60.45; H, 9.57. Found: C, 60.58; H, 9.55.

Dihexadecyl 3-[(1-Carboxyethyl)thio]succinate Sodium Salt (4c). Same procedure as for **4b** with 0.58 g racemic thiolactic acid: yield, 2.5 g (75%); mp 200 °C (decomp); ^{1}H NMR 3.67, 3.76 (q, 1 H, $CHCH_3$); otherwise similar data as for **4b**. Anal. Calcd for $C_{39}H_{73}NaO_6S$ (692): C, 67.63; H, 10.54; S, 4.62. Found: C, 67.39; H, 10.48; S, 4.58.

Dihexadecyl 3-[(1,2-Hydroxypropyl)thio]succinate (4d). Same procedure as for **4b** with 0.6 g of 3-mercapto-1,2-propanediol: yield, 1.6 g (50%); mp 45–46 °C; R_f = 0.53 (silica gel, 5:1 chloroform–methanol). Anal. Calcd for $C_{39}H_{76}O_6S$ (672): C 69.64; H, 11.31; S, 4.76. Found: C, 68.98; H, 11.03; S, 4.69.

Ditetradecyl (*R*,*S*)-2-(β-D-Glucopyranosylthio)succinate (5a). Ditetradecyl maleate (1.7 g, 3.34 mmol) was dissolved in 100 mL of 4:1 2-propanol–citric acid buffer (0.2 mol, pH 2). Sodium β-glucopyranosylmercaptide (0.94 g, 3.70 mmol) was added. The pH was raised to a value of 9 and was brought to pH 8 with a few drops of acetic acid. The solution was then stirred for 1 h at 55 °C under nitrogen. The course of the reaction was followed by TLC (silica gel, 4:1 chloroform–ethanol). Product **5a** precipitated from the reaction mixture at 50 °C, was collected, and was recrystallized from methanol: yield 2 g (85%); mp 60–130 °C $[\alpha]^{20}{}_D$ –9° (*c* 0.1, $CHCl_3$); IR ν_{CO} = 1735 cm^{-1}; ^{1}H NMR (D_2O) broad signals; MS (Cl, isobutane, 130 eV, 110 °C) *m/e* 705 (M^+ + H, 3), 509 (M^+–thioglucoside, 100). Anal. Calcd for $C_{38}H_{72}O_9S$ (705.0): C, 64.74; H, 10.30; S, 4.55. Found: C, 64.47; H, 10.21; S, 5.05.

Ditetradecyl (*R*,*S*)-2-(2,3,4,6-Tetra-*o*-acetyl-β-D-glucopyranosylthio)succinate (5b). Ditetradecyl maleate (16.96 g, 33.3 mmol) and 12.13 g (33.3 mmol) of tetraacetylthioglucose[21] were dissolved in 20 mL of chloroform and mixed with 4.12 mL of triethylamine in 100 mL of 1-propanol. The course of the reaction was followed by TLC (silica gel, 4:1 benzene–ethyl acetate). The product precipitated at 50 °C from the reaction mixture and was crystallized from methanol: yield, 27 g (93%); mp 48–51 °C; $[\alpha]^{20}{}_D$ –19° (*c* 0.1, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.90 (t, 6 H, CH_3), 1.28 (s, 44 H, CH_2), 1.56–1.75 (m, 4 H, OM_2, CH_2), 2.02, 2.03, 2.04, 2.06, 2.12, 2.14 (6 s, 12 H, CH_3CO), 2.72, 2.84, 3.02, 3.16, 3.86, 3.95 (6 dd, 3 H, 2 ABM-systems, J_{AB} = 16, J_{AM} = 5,

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J_{BM} = 11 Hz, CH_2CO, SHCO), 3.69–3.81 (m, 1 H, H-5), 4.06–4.36 (m, 6 H, OCH_2-6), 4.80, 4.90 (2d, 1 H, J_{AA} = 10 Hz, H-1), 4.96–5.32 (m, 3 H, H-2,3,4), the spectrum shows two signals for all protons neighboring the chirality center in the aglycon; IR (KBr) ν_{CO} 1745 cm^{-1}, broad; MS (–FAB/+FAB, $CHCl_3$, TEG) *m/e* 871 (M^+ -H, 5). Anal. Calcd for $C_{46}H_{80}O_{13}S$ (873.2): C, 63.27; H, 9.23; S, 3.67. Found: C, 63.61; H, 9.34; S, 3.27.

Ditetradecyl (*S*)-2-(β-D-Glucopyranosylthio)succinate (5c). The above mixture of diastereomers (5 g) was recrystallized twice from methanol. Melting point (56–58 °C) and rotation ($[\alpha]^{20}_D$ –45.3° (*c* 0.1, $CHCl_3$)) did not change after a third crystallization.

^1H NMR (270 MHz, $CDCl_3$, only signals that differ significantly from the above mixture are given) δ 2.01, 2.04, 2.10 (3 s, 12 H, CH_3CO), 2.72, 3.02, 3.95 (3 dd, 3 H, ABM system J_{AB} = 17, J_{AM} = 5, J_{BM} = 11 Hz, CH_2CO, SCHO), 4.90 (d, 1 H, J_{AA} = 10 Hz, H-1), 4.99, 5.10, 5.24 (3 t, 3 H, H-2,3,4). Anal. Calcd for $C_{46}H_{80}O_{13}S$ (873.2): C, 63.27; H, 9.23; S, 3.67. Found: C, 63.41; H, 9.25; S, 3.38.

Monensin 26-Pyromellitate (6). Monensin sodium salt (1.0 g, 1.44 mmol) (Sigma) in 5 mL of dry pyridine were mixed with 3.0 g (15.5 mmol) of pyromellitic anhydride in 30 mL of dry pyridine. The mixture was stirred for 15 min at room temperature. Chloroform (100 mL), water (100 mL), and approximately 50 mL of 5% aqueous HCl were added (pH ≃2). The aqueous phase was extracted twice with 50 mL of chloroform. The chloroform was removed, the residue was redissolved in 5 mL of methanol, and, thereafter, 50 mL of water was added. Addition of 100 mL of 1 M HCl precipitated **6**, which was filtered, washed with a few milliliters of water and dried in vacuo: yield 1.04 g (80%); R_f = 0.5, (silica gel, 4:1 $CHCl_3$–CH_3OH); UV(CH_3OH) λ_{max} 216 (ε 20 000), 291 nm (2400); ^1H NMR ($CDCl_3$) identical to monensin[23] with additional peaks at δ 8.59, 8.20 (2 H, pyromellitate); MS (FAB) *m/e* 928 (M^+ + Na). Anal. Calcd for $H_{46}H_{66}O_{18}\cdot 2H_2O$ (928): C, 58.59; H, 7.42. Found: C, 58.89; H, 7.68.

Vesicle Preparation. Typically 0.01 mmol of the amphiphile was dissolved in 1 mL of chloroform or methanol. The solvent was removed with the aid of a stream of nitrogen. Water or buffer (5 mL) was added and the mixture sonicated at 40–55 °C. The power of the sonicator was slowly raised from step 2 to 3.5. After 30–60 min the (slight) turbidity of the solution remained constant. These vesicular solutions contained 2×10^{-3} mol L^{-1} of amphiphile.

For the negatively stained vesicles, 0.02 M solutions of sodium tungstate in distilled water was used.

Dissolution of Magnesium Octaethylporphyrinate (MgOEP). Bolytes **3b** or **3g** (10 mg, 10^{-5} mol) and 1 mg of MgOEP (1.6×10^{-6} mol) were dissolved in 5:1 chloroform–methanol and evaporated to dryness. The red film of lipids was then sonicated with 10 mL of distilled water. Slightly turbid violet (MgOEP) or red solutions (H_2OEP) were obtained. On Sephadex G-25 the colored vesicles eluted with water as sharp zones.

Visible spectra: (a) **3a** vesicle at pH 6, λ_{max} 580, 543 nm (MgOEP), at pH 2, λ_{max} 575, 542 nm (no demetalation); (b) **3b** vesicle at pH 6, λ_{max} 624, 575, 542, 502 nm (H_2OEP complete demetalation).

Electron micrographs of **3b** vesicles showed giant vesicles with uniform diameters of about 5000 Å. This was found with both negative staining and freeze etching.

Acid Stability of a Vesicle MLM. The unsymmetric bolaamphiphile **3h** (10 mg) was sonicated in 10 mL of 1 N HCl for 20 min at 50 °C. The vesicular solution was placed in an oven and incubated for 48 h. Aliquots (1 mL) were taken after 1, 2, 4, 8, 16, 24, and 48 h, neutralized, and extracted with 3 × 1 mL of chloroform. The chloroform solution was then concentrated to 0.5 mL and examined by TLC (silica gel; dioxane; R_f(1,12-dodecanediol) = 0.53; spray reagent: Rhodamin B). The detection limit was less than 1% of the dodecanediol present in the probes. No trace of dodecanediol was, however, found.

Several probes were also examined by GC (R_Z = 12.53 on SE 30/19 and 1.97 on silicon rubber 17/6 for 1,12-dodecanediol). The detection limit was less than 1‰ of the dodecanediol present in the probe. Again no trace of the diol was found.

Entrapment of Lithium Ions and Pyranine. Vesicles containing lithium ions were prepared as follows. **3b** (7.67 mg), **3c** (7.31 mg), **6** (9.07 mg) was dissolved in 1 mL each of THF. In a 20-mL beaker the solvent was evaporated in a steam of argon. A 0.03 M LiCl solution (10 mL) was added. After ultrasonication at 50–60 °C for 15 min an opalescent dispersion was obtained. This vesicle (0.5 mL) solution from **3b** or **3c** was passed through a Sephacryl S 1000 column (1.0 × 20 cm) with 0.03 M NaCl as eluent. Vesicles with lithium were detected in the 4–6-mL fraction.

The 2-mL samples were directly sprayed into the flame of the atomic emission spectrometer. Lithium carbonate solutions containing 10, 30, 50, or 100 ppb of lithium were used as standards. A typical result was 30 ppb of lithium ions in the vesicle fraction.

Vesicles with pores were prepared by ultrasonication of 9.0 mL of the vesicle dispersion of **3b** or **3c** together with 1.0 mL of **6** in 0.03 M LiCl solution. Analysis for lithium was as described above. Less than 5 ppb lithium were found. This corresponded to the blank (Figure 8).

Pyranine–containing vesicles were prepared analogously by using 10^{-4} M pyranine in 0.03 M NaCl solution. Pyranine was detected in the vesicle fraction by fluorescence measurements at an excitation wavelength of 400 nm and emission wavelength of 510 nm at 20 °C.

^1H NMR Spectra of Vesicles and D_2O Exchange. A vesicular solution made from 10 mg of the bolaamphiphile **3b** in 5 mL of D_2O was measured at seven different temperatures. The line widths of the CH_2 band at δ 1.3 ppm changed from 16 (30 °C) to 12 Hz (90 °C) (see Figure 5a). Forty scans were accumulated each time. The signal at δ 3.98 lost intensity during the measurements and was less than 50% after a few hours at 50 °C. Attempts to exchange the proton neighboring the sulfur atom quantitatively with NaOD failed. In basic solution all signals broadened. Only the δ 1.3 ppm remained detectable.

The unsymmetric bolaamphiphile **3h** (10 mg in 10 mL of D_O) also produced a sharp ^1H NMR signal at δ 1.3 at temperatures between 20 (line width of 14 Hz) and 70 °C (10 Hz). The head-group proton signal from the thiosuccinic acids, however, were badly resolved.

The bilayer vesicle membrane from **4d** was obtained and measured in the same way as described for **3b**. Below 40 °C no signal was observed, from 45–55 °C the line width was 32 Hz (broader than MLMs) and, above 60 °C a broadening to 56 Hz was observed (Figure 5b).

Metachromatic Effects. Solution A. A total of 100 mL each of 3 mM solutions of the MLM vesicle **3b** (245 mg) and the BLM vesicles **4a** (200 mg) and **4b** (227 mg) were prepared in HCl (pH 2.0).

Solution B. Acridine orange (301 mg, 1 mmol) or 374 mg of methylene blue (1 mmol) were dissolved in 1 L of distilled water; 1 mL of the dye solution B was mixed with 1–10 mL of the vesicular solution B. The total volume was then brought to exactly 15.0 mL and the final pH to 2.0 by dilution with water and 0.1 M HCl. The acridine orange or methylene blue solutions gave identical spectra of the monomer in water and in vesicular solutions of **3b** and **4b**, all at pH 2. In the presence of **4a** a strong dimer peak was always predominant. If the BLM vesicular solutions of **4a** and **4b** were mixed in ratios of 5:95, 10:90, and 20:80 the monomer absorption of acridine orange decreased linearly. Also, 10% of the sulfonic acid **4a** added to the **3b**–MLM vesicle gave a strong decrease of the 450–500 nm absorption. Less than 3% of the sulfonic acid head groups of **3b** can point to the outer surface.

At pH 6 the thiosuccinic acid head groups produce the same metachromatic effect as the sulfonate head groups.

Entrapment experiments with acridine orange and **3b** vesicles gave no results because the UV spectrum of entrapped acridine orange could not be detected. Experiments with methylene blue, however, were successful. One part of solution A was cosonicated with one part of solution B containing methylene blue. The mixture was chromatographed on Amberlite IRC 50. The eluate only contained methylene blue absorbing at 608 nm (OD = 0.1). This corresponds to the dimer. The monomer absorbs at 670 nm. Addition of sodium dithionite had no effect on the electronic spectrum. After addition of 0.25 mL of a 1% solution of Triton X100, however, the solution decolorized. The experiments give strong qualitative evidence that the inside surface of the MLM vesicle made of **3b** is strongly negatively charged at pH 2 by sulfonate groups.

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