

Bolaamphiphiles Form Ultrathin, Porous, and Unsymmetric Monolayer Lipid Membranes[†]

JÜRGEN-HINRICH FUHRHOP* and DETLEV FRITSCH

Institut für Organische Chemie der Freien Universität Berlin, 1000 Berlin 33, West Germany

Received November 18, 1985 (Revised Manuscript Received March 10, 1986)

Artificial bilayer lipid membranes (BLMs) have been known since 1854 when Virchow¹ observed "myelin figures" resembling biological cell membranes under the microscope. In the 1960s, the black lipid membranes² and vesicle membranes³ were developed. Several thousand publications evaluated the physical properties (e.g., ion transport, phase transitions) and biochemical applications (e.g. reconstitution of membrane proteins, transport of pharmaca) of these BLMs which were almost invariably composed of ill-defined biological mixtures or synthetic phospholipids.⁴ Such membranes serve very well as physical barriers between two water volumes, but they are not competitive with multilayered Langmuir-Blodgett films in the construction of organized systems.⁵

In the last 6 or 7 years, lipid membranes with three new features became known and attracted the curiosity of synthetic chemists: (i) the introduction of redox-active and photoactive head groups allows the buildup of functional vesicle membranes,⁶ (ii) polymerizable side chains in the hydrophilic head groups or hydrophobic tails react to extended, covalently connected domains,^{7,8} and (iii) the arrangement of monolayer membranes made from bolaamphiphiles can be stereochemically controlled⁹ and such membranes can be perforated with nonpolymeric organic compounds.^{10,11} This Account discusses the unique properties of bolaamphiphiles and their high molecular weight aggregates, the monolayer lipid membranes (MLMs).

The noun "bola" relates to the shape of a South American missile weapon. Its simplest form consists of two balls which are attached to both ends of a cord. The term "bolaform electrolyte" (short form: "bolyte" or "bolion") was introduced by Fuoss and Edelson¹² in 1951 for a chain of hydrophobic groups connecting two ionic groups.

For less water-soluble analogues, the name "bolaform amphiphiles"¹³ (short form: "bolaamphiphiles") is preferred. The hydrophilic head groups of bolaamphiphiles need not carry charges. We will use "bolyte" for water-soluble ions and "bolaamphiphile" or "bipolar lipid" for ions and molecules which aggregate in water (see Figure 1).

Jürgen-Hinrich Fuhrhop was born in Berlin in 1940 and received his Diplom, Dr. rer. nat., and Dr. habil. at the Technische Hochschule in Braunschweig under the direction of Professor H. H. Inhoffen. He has worked as a postdoctoral fellow in the Laboratories of Professors S. Granick and D. Mauzerall and for 9 years was a group leader in the Gesellschaft für Biotechnologische Forschung, Stockholm. In 1977 he joined the faculty of Chemistry at the Freie Universität Berlin, where he is now Professor of Organic Chemistry and Speaker of the Sonderforschungsbereich 312 "Gerichtete Membranprozesse". His research interests are in metalloporphyrins, synthetic membranes, and chiral gels.

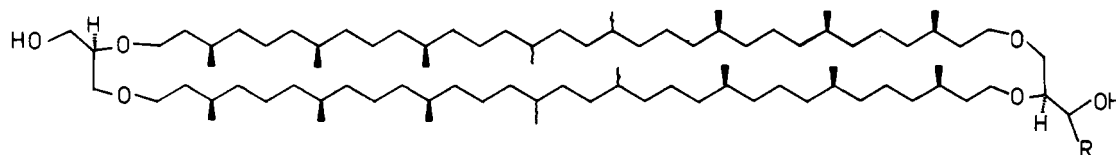
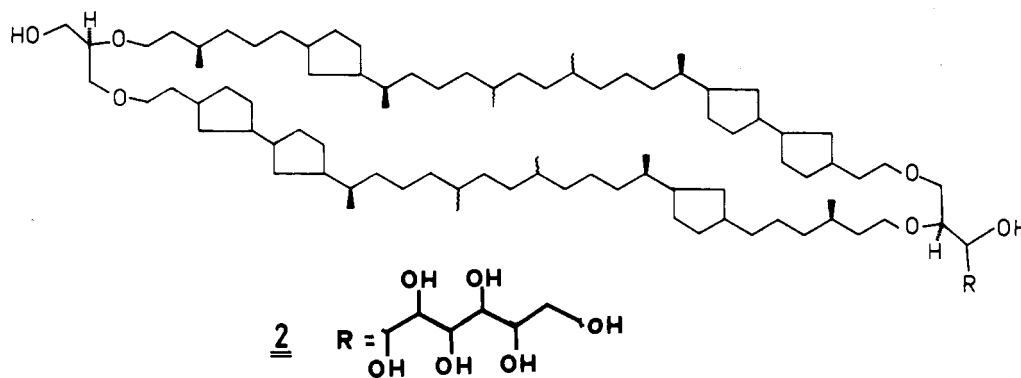
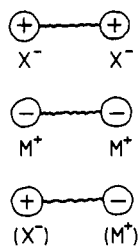
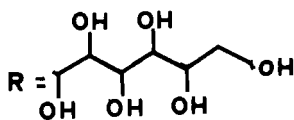
Detlev Fritsch worked as a technician at Merck, Darmstadt, from 1967 to 1972 before taking his Staatsexamen in chemistry and politics in 1981 and his Dr. rer. nat. in 1984 at the Freie Universität Berlin. He presently works as a postdoctoral fellow at the Freie Universität Berlin.

Natural Products

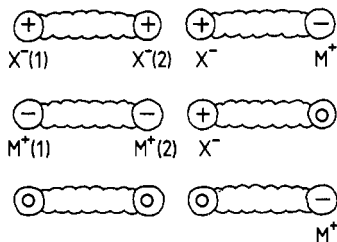
The most important natural bolaamphiphiles¹⁴⁻¹⁶ are the membrane lipids of thermophilic and acidophilic archaeobacteria, e.g., *Sulfolobus solfataricus*. This bacterium grows best at 85 °C and pH 2. After hydrolytic workup of the archaeobacterial lipids, mixtures of macrocyclic tetraethers, exemplified by the structures 1 and 2 were isolated. These bolaamphiphiles demonstrate the potentials of the "stereochemistry of MLMs" in a most splendid way. Their first and most important feature is that both head groups have different sizes and water solubilities. If these amphiphiles aggregate in water, the smaller water-insoluble glycerol head groups (one OH only) should conglomerate first, whereas the larger and more soluble nonitol groups should stay further apart on the other side of the aggregate. A highly unsymmetric distribution of the head groups in the MLM result in a curved structure. In solution, a vesicle would spontaneously form thus a black lipid membrane should be bent. Furthermore the chiral head groups would form a chiral monolayer which may react stereoselectively with fitting solutes. Finally, the sterical interactions of the eight methyl groups should lead to a twist of the hydrocarbon chain in analogy to isotactic polyisopropylene.¹⁷ Ideally a double helix would

[†] Dedicated to Professor H. H. Inhoffen on the occasion of his 80th birthday.

- (1) Virchow, R. *Virchows Arch. Pathol. Anat. Physiol.* 1854, 6, 571.
- (2) Tien, A. T. *Bilayer Lipid Membranes (BLM), Theory and Practice*, M. Dekker: New York, 1974.
- (3) Bangham, A. D.; Hill, M. W.; Miller, N. G. A. *Methods Membr. Biol.* 1974, 1, 1.
- (4) Eibl, H. *Angew. Chem.* 1984, 96, 247; *Angew. Chem., Int. Ed. Engl.* 1984, 23, 257.
- (5) (a) Kuhn, H.; Möbius, D. *Angew. Chem.* 1971, 83, 672; *Angew. Chem., Int. Ed. Engl.* 1971, 10, 620. (b) Kuhn, H. In *Modern Trends of Colloid Science in Chemistry and Biology*; Eicke, H.-F. Ed.; Birkhäuser: Basel, 1985; p 97ff.
- (6) Fuhrhop, J.-H.; Mathieu, J. *Angew. Chem.* 1984, 96, 124; *Angew. Chem., Int. Ed. Engl.* 1984, 23, 100.
- (7) Gros, L.; Ringsdorf, H.; Schupp, H. *Angew. Chem.* 1981, 93, 311; *Angew. Chem., Int. Ed. Engl.* 1981, 20, 305.
- (8) Fendler, J.-H.; Tundo, P. *Acc. Chem. Res.* 1984, 17, 3.
- (9) Fuhrhop, J.-H.; Mathieu, J. *J. Chem. Soc., Chem. Commun.* 1983, 144.
- (10) Fuhrhop, J.-H.; Liman, U. *J. Am. Chem. Soc.* 1984, 106, 4643.
- (11) Fuhrhop, J.-H.; Liman, U.; David, H. H. *Angew. Chem.* 1985, 97, 337; *Angew. Chem., Int. Ed. Engl.* 1985, 24, 339.
- (12) Fuoss, R. M.; Edelson, D. J. *J. Am. Chem. Soc.* 1951, 73, 269.
- (13) (a) Zana, R.; Yiv, S.; Kale, K. M. *J. Colloid Interface Sci.* 1980, 77, 456. (b) Menger, F. M.; Wrenn, S. *J. Phys. Chem.* 1974, 78, 1387. (c) Kachar, B.; Evans, D. F.; Ninham, B. W. *J. Colloid Interface Sci.* 1984, 100, 287.
- (14) Gliozzi, A.; Rolandi, R.; de Rosa, M.; Gambacorta, A. *J. Membr. Biol.* 1983, 75, 45.
- (15) Gliozzi, A.; Paoli, G.; Rolandi, R.; de Rosa, M.; Gambacorta, A. *J. Electroanal. Chem.* 1982, 141, 591.
- (16) Gulik, A.; Luzzati, V.; de Rosa, M.; Gambacorta, A. *J. Mol. Biol.* 1985, 182, 131.

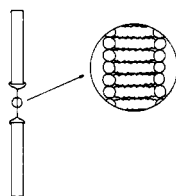
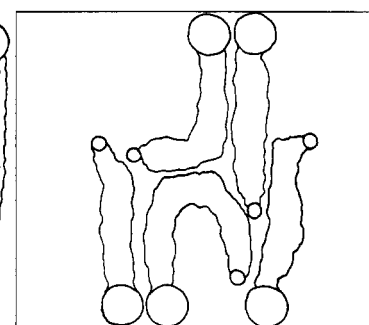
12

BOLYTES
(water soluble)

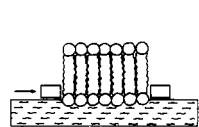


BOLAAMPHIPHILES
(aggregate in water)

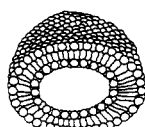
50Å

 ΔH 

BLACK MLM



SURFACE MLM



VESICLE MLM

Figure 1. Water-insoluble bolaamphiphiles form monolayered lipid membranes between two aqueous phases and on surfaces. X^- and M^+ indicate counterions.

be formed. The MLM could therefore be as short as 27 Å, as compared to the 38 Å predicted for nontwisted chains (Figure 2a).¹⁸

Experiments with black lipid membranes made from mixtures at 1 + 2 yielded asymmetric current voltage curves in the presence of the ionophore valinomycin¹⁴ and a dielectric thickness of 27 Å.¹⁵ Results from X-ray scattering on polymorphic liquid crystal phases of mixtures of 1 and 2, however, showed that only the nonitol side constitutes a polar head group region. The glycerol side was dispersed in the hydrocarbon matrix and U-form conformations prevailed (Figure 2b). At higher temperatures, the nonitol head groups are expected to segregate away from the glycerols to form a BLM at 80 Å thickness¹⁶ (Figure 2c). A membrane

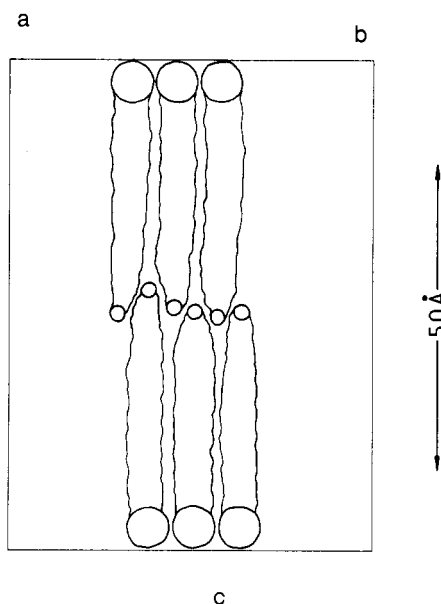


Figure 2. Possible orientations and membrane thicknesses in MLMs made of bolaamphiphiles with a highly hydrophilic (o) and a hydroxyl (o) head group such as 1 or 2. Possible formation of double helices in terpenoid membranes is also indicated.

made from compounds such as 1 and 2 may therefore have any thickness from 27 to 80 Å, depending on the presence of helices and the position of the glycerol's

(17) Natta, G.; Allegra, G. *Tetrahedron* 1974, 30, 1987.

(18) Fuhrhop, J.-H.; Fritsch, D. *System. Appl. Microbiol.*, in press.

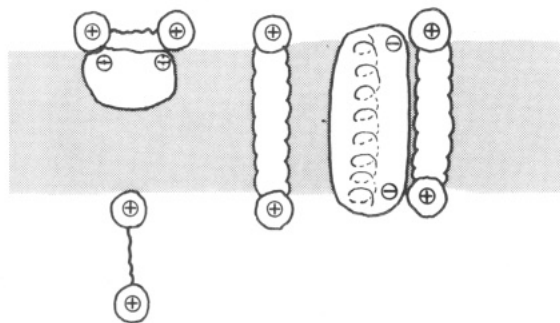


Figure 3. Possible orientations of dicationic bolytes and bolaamphiphiles in biological membranes containing proteins.

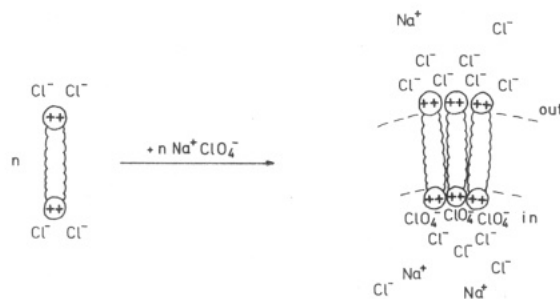
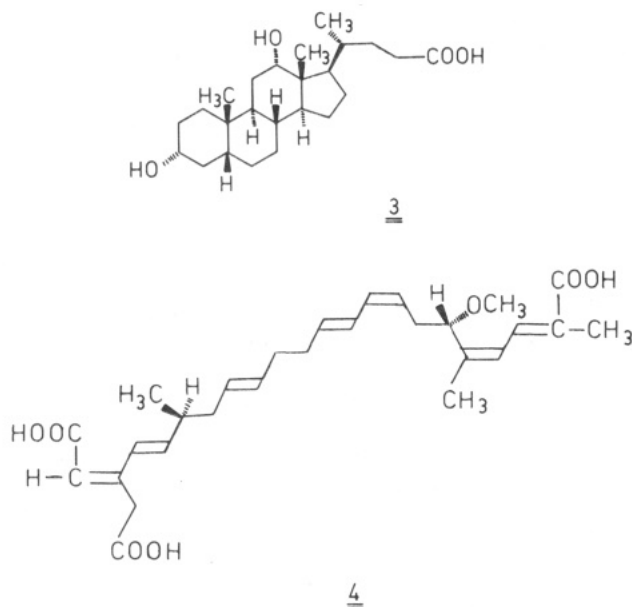


Figure 4. Spontaneous vesiculation by "one-sided aggregation" of a bolaamphiphile.

OH-group. The idealized structure of Figure 2a is certainly more an aim for synthetic chemists than a reality for the biochemist.

Several other bolaamphiphiles are known to act in biological systems. Their role always seems to be related to self-aggregation or to interaction with membrane proteins. Deoxycholic acid **3** aggregates in



aqueous media and entraps fatty acids or hydrocarbons in 2:1, 4:1, 6:1, or 8:1 molecular complexes (choleic acids), depending on the length of the "guest" molecule. It serves as a highly efficient biological detergent with several distinct critical micellar concentrations. The size of the "micelle" may be as small as two molecules.¹⁹

(19) (a) Herndon, W. C. *J. Chem. Ed.* **1967**, *44*, 724. (b) Small, D. M. In *The Bile Acids*; Chemistry, Nair, P. D.; Kritchevsky, D. Eds.; Plenum Press: New York, London, 1971; Vol. 1 p 324ff.

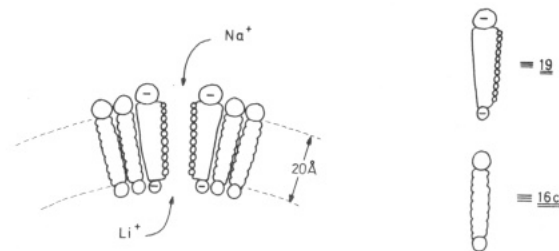
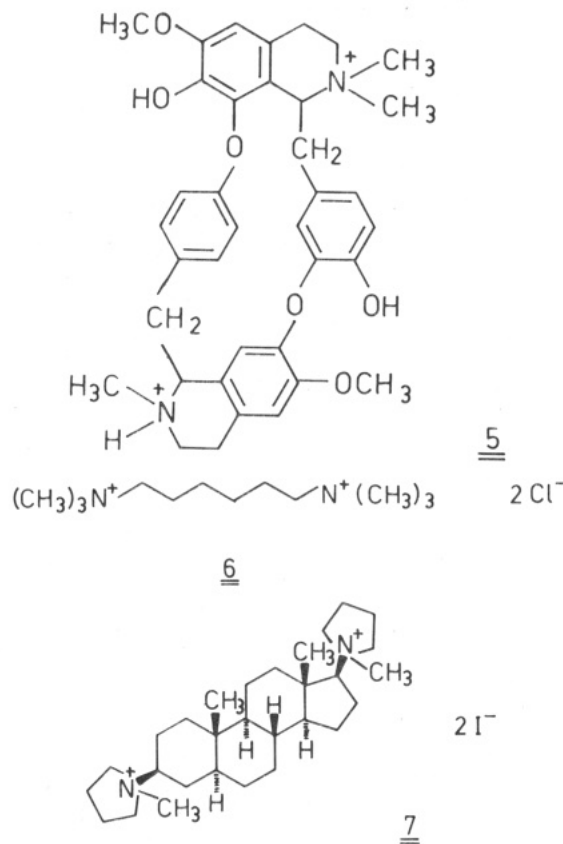


Figure 5. Pore formation by bolaamphiphilic ionophores.

Other biologically active bolaamphiphiles are localized in the cell membranes. Bongkreikic acid **4** is an antibiotic, which prevents the transport of ATP through biological membranes.²⁰ It strongly binds to electro-positive, lysine-containing ATP receptor sites on ADP/ATP carriers.²⁰

The reverse binding pattern is typical for numerous bolaamphiphiles, which act as antagonists for neural or muscular receptor proteins. Well-known examples are the acetylcholine receptor antagonists D-tubocurarine **5**, the South American arrow poison, and its synthetic



analogue hexamethonium **6**.²¹ Closely related steroid diammonium salts, such as dipyrandium **7**, recognize kinks in the double helix of DNA and bind to such sites.²² The utility of such bolaamphiphiles is based on their (i) solubility in biological membranes or hydrophobic pockets of polymers and (ii) on the 2-fold charge-charge interaction with biological polyelectro-

(20) Bogner, W.; Aquila, H.; Klingenberg, M. *Dev. Bioenerg. Biomembr.* **1983**, *6*, 145.

(21) Hucho, F. *Einführung in die Neurochemie*; Verlag Chemie: Weinheim, 1982; p 164ff.

(22) (a) Patel, D. J. *Acc. Chem. Res.* **1979**, *12*, 118. (b) Patel, D. J.; Kozłowski, A.; Suggs, J. W.; Cox, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4063.

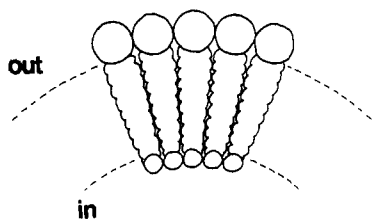
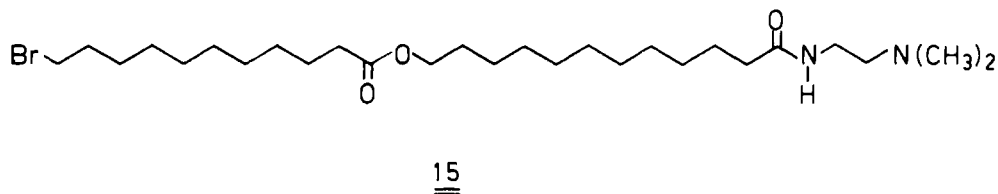
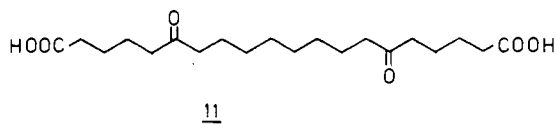
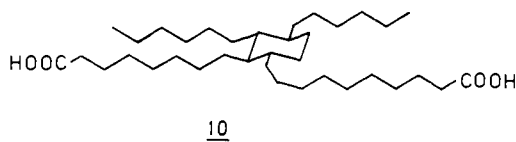
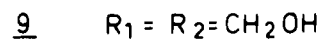
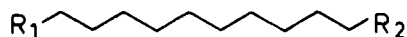


Figure 6. Bolaamphiphiles with a large and small head group can form unsymmetric membranes.

lytes. The effect is usually a change of the conformation of the biopolymer and its inactivation (see Figure 3).

Syntheses

We have prepared more than a hundred bolaamphiphiles. The most useful starting materials are the commercial products 8–10. Dimer acid 11, which is a



mixture of closely related isomers, is inexpensive.²³ Noncommercial long-chain α,ω -dicarboxylic acids were obtained from shorter α,ω -diacid dichlorides and cyclohexanone.^{24,25} Diketones, e.g., 11, are useful intermediates in this synthesis. Such central carbonyl groups have, for example, been used to introduce spin labels into BLMs.²⁶

The natural products, aleuritic acid 12 and bixin 13 are currently applied in syntheses of chiral or electron-conducting bolaamphiphiles. With these starting materials, we usually first prepare the hydrophobic core of the desired bolaamphiphiles, e.g., the macrolide 14²⁷

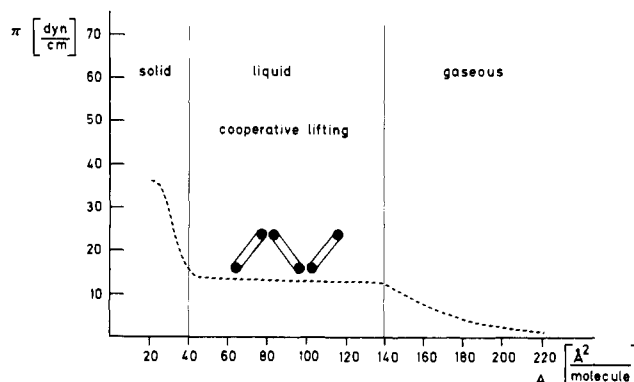
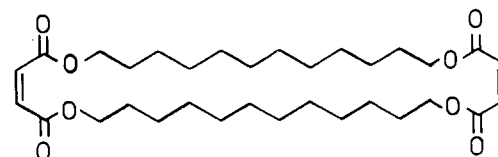
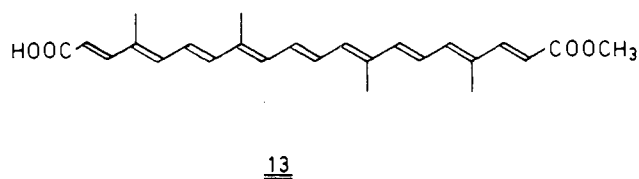
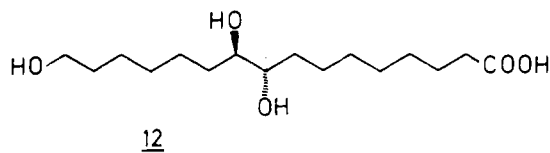


Figure 7. Typical pressure–area isotherm of a bolaamphiphile with one electroneutral head group. The solid phase is ill-defined.



or the open-chain, tertiary amine 15.²⁸ Macrocycles such as 14 are obtained in high yields, when reversible, acid-catalyzed reactions are applied. Irreversible nucleophilic substitutions, e.g., with amines or alkoxides and dibromides, give poor yields. The isolated products are soluble in common solvents. They can usually be chromatographed on silica gel and/or crystallize readily.

The polar head groups are only introduced in the last steps. The macrolide 14, for example, reacts in 2-propanol–water mixtures with thioglucoside to give 16a²⁹ or, first with sodium bisulfite, then thiosuccinate

(23) Fuhrhop, J.-H.; Kaufmann, W.; Schambil, F. *Langmuir* **1985**, *1*, 387.

(24) Hünig, S.; Lendle, W., *Chem. Ber.* **1960**, *93*, 913.

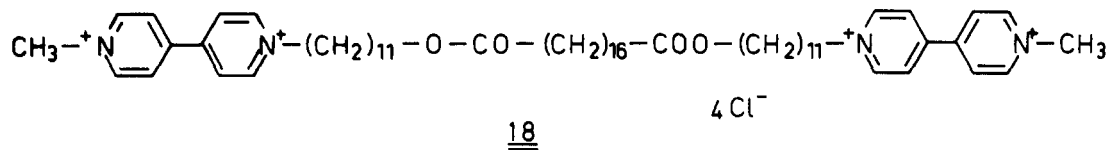
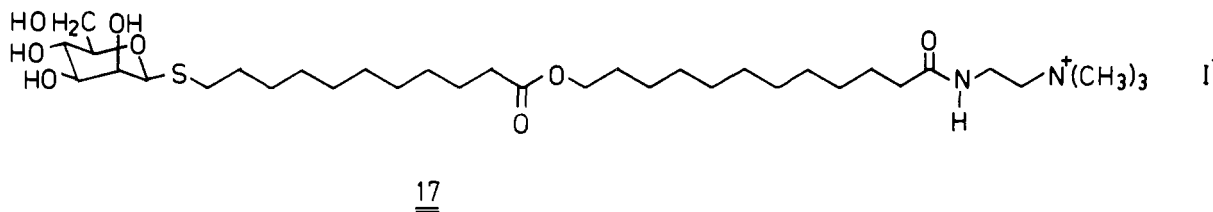
(25) *Organic Syntheses*, Wiley: New York, 1973; Collect. Vol. V, p 533.

(26) (a) Seelig, J.; Limacher, H.; Bader, P. *J. Am. Chem. Soc.* **1972**, *94*, 6364. (b) Gaffney, B. J.; Willingham, G. L.; Schepp, R. S. *Biochemistry* **1983**, *22*, 881.

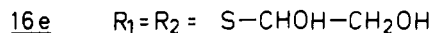
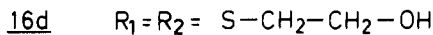
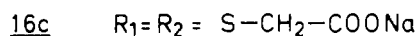
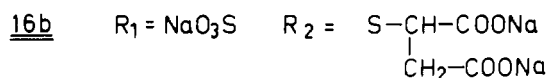
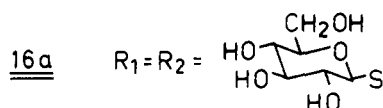
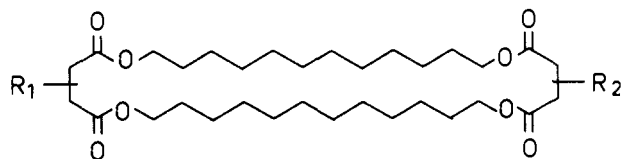
(27) Fuhrhop, J.-H.; Ellermann, K.; David, H. H.; Mathieu, J.; *Angew. Chem.* **1982**, *94*, 444; *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 440; *Angew. Chem. Suppl.* **1982**, 980.

(28) Fuhrhop, J.-H.; Tank, H., to be published.

(29) (a) Fuhrhop, J.-H.; David, H. H.; Mathieu, J.; Liman, U.; Boekema, E. J.; Winter, H. J. *J. Am. Chem. Soc.*, in press. (b) Mathieu, J. Ph.D. Dissertation, Berlin, 1984.



to give unsymmetric **16b** by two subsequent Michael addition reactions.^{9,29} The tertiary amine **15** was first



methyated with methyl iodide and then reacted with thiogalactoside to give **17**.²⁸ It is very important that these final steps in the syntheses of bolaamphiphiles (the introduction of the polar head groups) occur in yields >95%. Purification procedures should involve only precipitation, e.g., by change of pH (for carboxylic acids) or addition of less polar solvents (for carbohydrates) and final recrystallization. We found it extremely difficult to separate pure bolaamphiphiles from amphiphile mixtures in a gram scale.

Vesicle Formation, Stability, and Fluidity

The archaeobacterial bolaamphiphiles **1** and **2** are not water-soluble enough to form vesicles. Only black lipid membranes have been reported so far. The known synthetic bolaamphiphiles, on the other hand, are too polar to dissolve in decane or squalane, which are used to spread black lipid membranes. Only vesicle MLMs (sizes between 300 and 5000 Å) could be prepared by the usual techniques such as ultrasonication, dialysis of micellar solutions, or swelling of thin layers. We have also developed a new technique, which may be specific for the formation of MLM vesicles, namely one-sided precipitation. If one dissolves for example, the sym-

metric bolaamphiphile **18** with two bipyridinium head groups and four chloride ions as counterions, one obtains clear solutions up to a concentration of about 10^{-1} M. After addition of 1 or 2 mol of sodium perchlorate per mol of **18**, however, the solution becomes turbid and contains only MLM vesicles. The one-sided "crystallization" of the bipyridinium head groups implies formation of a curved aggregate and finally vesiculation (for BLM analogues, see ref 13c). The same phenomenon can be obtained by partial photoreduction of **18** to the water-insoluble viologen radical.³⁰ In the case of symmetric α,ω -dicarboxylic acids, such as dithioacetic acid **16c**, the dicarboxylate may be water-soluble and vesiculate spontaneously on lowering the pH. The "one-sided precipitation" is by far the most gentle method to obtain small vesicles of uniform size (see Figure 4).

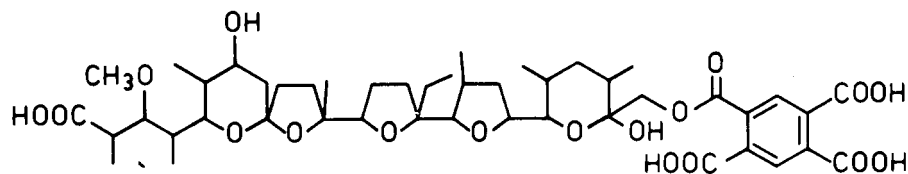
Vesicle surfaces with reactive head groups can also be used to form polymeric dyes by reversible aggregation. With benzidine the monolayer with bipyridinium head groups produces a blue charge-transfer complex ($\lambda_{\text{max}} = 650 \text{ nm}$, $\epsilon = 6500$) at 0°C , which decolorizes reversibly at 30°C . The blue polymeric complex is air-stable and does not contain free radicals.³¹ The analogous monomeric bipyridinium-benzidine CT-complex has a weak absorption band at 550 nm in ethanol-water 1:1.

In the case of vesicles made from **18** the localization of head groups is easy and accurate. Indeed, the outer bipyridiniums are reduced to colored radicals by sodium dithionite and their ^1H NMR signals are shifted by ferricyanide. The inner head groups are not reached by these ionic reagents.³⁰ Vesicles made of **18** have a diameter of $\leq 500 \text{ \AA}$ and a membrane thickness of 70 Å. This means that the outside surface is about twice as large as the inside surface. Nevertheless the number of outside bipyridinium groups equals exactly the number of the inner head groups. This is strong evidence for the correctness of the assumption of a single monolayer membrane, since in bilayer vesicle membranes the ratio of outer to inner head groups corresponds to the ratio of surface areas.

MLM vesicles with charged head groups resist fusion. This is presumably caused by the inability of charged head groups to cross the hydrophobic membrane. Thus it becomes impossible to form bridges and finally water passages between aggregated vesicles. Aggregation

(30) (a) Fuhrhop, J.-H.; Fritsch, D.; Tesche, B.; Schmiady, H., *J. Am. Chem. Soc.* **1984**, *106*, 1998-2001. (b) Fritsch, D. Ph.D. Dissertation, Berlin, 1984.

(31) Fuhrhop, J.-H.; Fritsch, D. *J. Am. Chem. Soc.* **1984**, *106*, 4287.



19

therefore remains a reversible process and we have found that some MLM-vesicle solutions remain unchanged for periods longer than a year.

It was also found that vesicle MLMs made from macrocycle **16c** are stable against acids down to pH 1. The ester linkages are not cleaved in vesicular solution, but are, of course, readily solvolyzed in methanolic solution. We assume that this membrane stability is caused by the reversibility of acid-catalyzed solvolytic reactions. In the MLM dodecanediol is not released, if one ester bond is cleaved;²⁹ it rather remains at the same place within the membrane. "Repair reactions" are therefore highly favored.

Another special property of vesicle MLMs is their fluidity above 10 °C. No sign of liquid crystal formation has, so far, been observed in any monolayer vesicle membrane. The ¹H NMR spectra of MLM vesicles produce relatively narrow, well-resolved signals at all temperatures above 10 °C. This is in sharp contrast to BLMs with analogous head groups and hydrophobic skeletons,²⁹ which are often "solid" at room temperature and "melt" above 35 °C. The fluidity of vesicle MLMs however, does not imply that they are leaky for metal ions. Lithium ions, for example, are not released to a measurable extent within a day.

Surprisingly, the hydroxyl group is suitable as head group for MLMs. Stable vesicles were made by sonication of the α,ω -diols **16d**, tetrols **16e**, and disaccharides such as **16a**. No BLM-formation, as implied by work on analogous archaeobacterial liquid crystals, was observed. The uncharged vesicles **16d,e**, however, all exhibited fusion. After several days, large vesicles have always been found on electron micrographs, although these were not present in fresh preparations.

Ultrathinness and Pores

The extreme thinness of vesicle MLMs made from macrolides **16** has been measured in liquid crystals by X-ray diffraction³² and in stained vesicles, by electron microscopy.²⁹ The repeating units were found to be 27.5 Å,³² while the hydrophobic, salt excluding part was 21 ± 2 Å²⁹ in thickness. These values are comparable to those of black lipid membranes made from interdigitizing glycerol monooleate³³ or archaeobacterial lipids.¹⁸

They are thinner by about a factor of two compared to the usual phospholipid BLMs.³⁴ As yet, it has not been possible to make MLMs less than 20 Å in thickness evidently because even electroneutral bolaamphiphiles **16a,d,e** become water-soluble if their hydrophobic chains become shorter than 2 × 12 methylene groups.

(32) Büldt, G., unpublished results.

(33) (a) White, S. H., *Biophys. J.* 1978, 23, 337. (b) Dilger, J. P.; Benz, R. *J. Membr. Biol.* 1985, 85, 181.

(34) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York 1982, Chapter 6.

Membranes which have a thickness of less than 25 Å are potentially more useful in synthetic systems than the usual 40 Å BLMs, because several nonpolymeric natural compounds may span up to 25 Å, but not 40 Å. Such compounds could be used as building blocks for channels or pores through the membrane. β -Carotene and/or protoporphyrin IX may, for example, transfer electrons across such ultrathin membranes. Cholic acid or similar compounds with one hydrophilic and one hydrophobic molecular surface may form water channels. Such (selective) pores (see Figure 5) which perforate vesicle membranes make a basis for the construction of efficient molecular machines, capable of pumping ions into the vesicle, or releasing entrapped chemicals after a signal from the environment.³⁵

The first example of a pore perforating a vesicle MLM was verified with the monensin derivative **19**. Although monensin itself is an efficient ionophore, transporting sodium through any hydrophobic barrier, the highly charged pyromellitate **19** has no such effects; instead, it produces pores for lithium ions in vesicles made from **16c** or **16d**, if it is embedded in the vesicle MLM by ultrasonication. In (thicker) BLM vesicles, no lithium leakage is observed when **19** is added during the sonication procedure.¹⁰ The pore made of **19** bears negative charges at its entrance and exit. If one binds a positively charged bolaamphiphile to the pore, the water channel of the monensinpyromellitate aggregate is replaced by the hydrophobic chain of the bolcation, acting as a stopper of the membrane hole. No lithium can escape the vesicle's interior at pH 7. If, however, the pH is lowered to 3, the negative charges of the monensinpyromellitate are removed and the stopper is released together with the lithium content of the vesicle.¹¹ To our knowledge, these MLM vesicles with a diameter of 300 Å are the smallest possible containers capable of being opened. Reversible opening and re-sealing, will presumably be possible if the stopper can be covalently attached to the molecules which produce pores.

Unsymmetric Vesicle Membranes

Membranes of biological cells are unsymmetric in the sense that the inner membrane surface varies chemically from the outer surface. Typical examples are found in thylakoid membranes with an oxidizing inside and reducing outside³⁶ and in membranes of differentiated tissue cells, where the receptor glycoproteins are exclusively localized on the outer surface.³⁷ What is true for the polar surfaces of biological membranes also occurs in the hydrophobic centers. These are also highly organized sequences of reaction centers, mainly

(35) Reference 34, Chapters 13, 14.

(36) Witt, H. T., *Biochim. Biophys. Acta* 1979, 505, 355.

(37) (a) Block, M. C.; Flowers, H. In *The Glycoconjugates*; Horowitz, M.; Pigmann, W. Eds., Academic Press, New York 1978; Vol. II, p 338ff. (b) Dolowy, K. *Prog. Surf. Sci.* 1984, 15, 245.

based on the molecular complexes and associates of proteins.³⁸ Artificial vesicle membranes can, however, also be used as a framework for an organized system. One can, for example, localize solutes in the inner and outer water phases or regioselectively derivatize the outer head groups with water-soluble reagents which do not penetrate the hydrophobic membrane, or, one may adsorb reactive amphiphiles on the inner and outer surfaces.

The most elegant and efficient way of producing unsymmetric vesicle membranes is based on the correlation of the unsymmetry of bolaamphiphiles with two head groups of different size and the varying size of the inner and outer surfaces of small vesicles. In 300-Å diameter vesicles with a membrane thickness of 30 Å, for example, the outer surface is larger than the inner by a factor of 1.5. In the bolaamphiphile **16b** the succinic acid head group is about double the size of the sulfonate head group (see Figure 6). It has been anticipated that the self-organization of **16b** to a small vesicle would lead to an arrangement where all succinic acid groups were on the outer and all the sulfonate groups on the inner surface.

These expectations were fully realized in vesicle membranes made of **16b**. By means of charge-sensitive metachromatic effects, it was shown, that at least 99% of the outside head groups were indeed bulky succinates. Such an a priori unsymmetric vesicle membrane opens the pathway to systems where the host membrane's bolaamphiphiles and unsymmetric guest bolaamphiphiles can be aligned. Light-driven vectorial membrane processes may then be realized. So far, however, bolaamphiphile **16b** has produced the one and only fully unsymmetric MLM and this has no reactive sites.

Monolayers and Association with Polyelectrolytes

Pressure-area isotherms of various bolaamphiphilic monolayer films spread on water surfaces have been measured. In the gaseous state (without compression of the surface layer), bolaamphiphiles lie horizontally on the water surface. When a bolion monolayer is compressed, both head groups remain bound to the aqueous surface. The hydrocarbon chain assumes a "U" form or "wicket" conformation. However, the final diacid monolayers, for example, do not correspond to a stable ("solid") condensed phase and are, in fact, metastable.^{39,40} On the other hand corresponding diethyl esters do form solid films.^{41,42} Such results correspond with the "fluidity" of vesicle MLMs (see section 3). Similar observations have been made with bolaamphiphiles containing one polar carboxylic head group and one less polar hydroxylic or ester group. 16-Hydroxyhexadecanoic acid condenses to a molecular area of 70 Å², and then exhibits an inflection point.⁴⁰ Monomethyl-1,18-octadecanoate also has a similar inflection point between 80 and 100 Å².⁴³

(38) Jain, M. K.; Wagner, R. C. *Introduction to Biological Membranes*; Wiley: New York, 1980, Chapter 7.

(39) Jeffers, P. M.; Daen, J. J. *Phys. Chem.* **1963**, *69*, 2368.

(40) Kellner, B. M. J.; Cadenhead, D. A. *J. Colloid Interface Sci.* **1978**, *63*, 452.

(41) Adam, N. K.; Jessop, G. *Proc. R. Soc. London, A.* **1926**, *112*, 376.

(42) von Hahn, H.; Grün, F. *Helv. Chim. Acta* **1955**, *38*, 1803.

(43) (a) Vogel, V. Diplomarbeit, Frankfurt, a.M. 1984. (b) Vogel, V.; Möbius, D., to be published.

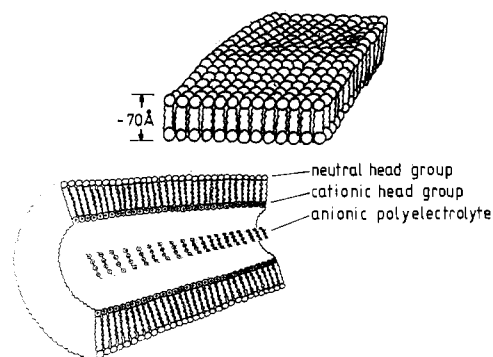


Figure 8. (a) Part of a noncurved bolaamphiphilic monolayer, which may (b) form a tube around a polyelectrolyte, e.g., DNA.

These molecular areas correspond to the molecules lying flat on the water surface. The inflection does not reflect a bending of the hydrocarbon chain, but instead, corresponds to the rising of the less polar hydroxyl or ester ends from the water phase. This process may be cooperative,⁴⁴ since its completion needs almost no external pressure. Close to a molecular area of 20 Å², a stable film may or may not have been formed, depending on external conditions such as temperature, salt concentration, and pH in the subphase.^{43,44}

The macrocyclic diol **16d** has two oligomethylene chains and a hydroxyl group on both ends. Its pressure-area isotherm (see Figure 7) shows the same behaviour as single chain ω -hydroxy acids, but the erect molecular layer collapses at relatively low pressures. Repeatedly a "solid phase" is not formed.²⁹

The observations of monomolecular films on water surfaces once again demonstrate the ambiguous character of hydroxyl groups: they perfectly play the role of a hydrated head group, but they may also strip off their water shell and bind only to neighboring OH groups or even dissolve in hydrophobic phases.

In the case of the tetracationic bolaamphiphile **18** with a very long (≈ 60 Å) hydrophobic chain and four perchlorate counterions, a stable monolayer could also be formed in water and deposited on a thin carbon film and observed by electron microscopy.³⁰ Obviously, the interactions between the monolayer and the carbon surface are much stronger than between two monolayers with positively charged head groups. So far, a multilayer has not been observed.

Attempts to wrap the bolcationic film of Figure 8 around polyanionic nucleic acids gave irreproducible results. Polymeric aggregates of ill-defined compositions were formed. This is in agreement with earlier reports on the interactions of bispyridinium bolions with polycarboxylic acids.⁴⁵⁻⁴⁷ The wrapping of a polynucleotide with a soluble coat presents, however, no problems if one uses a long-chain bolaamphiphile with a cationic ammonium head group on one end and an electroneutral carbohydrate on the other such as **17**. The usual intercalation of acridine orange or proflavin in DNA⁴⁸ is inhibited by MLM coats. The polyelec-

(44) Bell, G. M.; Wilson, G. L. *Physica A* **1982**, *115A*, 85.

(45) Ehrenpreis, S.; Fishman, M. M. *Biochim. Biophys. Acta* **1960**, *44*, 577.

(46) Ehrenpreis, S.; Kellock, M. G. *Biochim. Biophys. Acta* **1960**, *45*, 525.

(47) Morawetz, H.; Kanadianian, A. Y. *J. Phys. Chem.* **1966**, *70*, 2995.

(48) (a) Wille, H.; Pauluhn, J.; Zimmermann, H. W. *Z. Naturforsch. C: Biosci.* **1982**, *37C*, 413. (b) Armstrong, R. W.; Kurucsev, T.; Strauss, U. P. *J. Am. Chem. Soc.* **1970**, *92*, 3174.

trolyte becomes inaccessible for water-soluble compounds. Attempts to use such "sugar coatings" as vehicles for the transport of nucleotides through biological membranes and to cover potentially harmful biological cells with negative surface charges with artificial MLMs are among our current activities.

Concluding Remarks

We have tried to make a case that self-organization of membranes can be manipulated in the same way as syntheses of molecules. For monolayer lipid membranes, regioselectivity in the aggregation of head groups of different sizes, introduction of hydrophilic channels into the hydrophobic membrane, and the coverage of surfaces with membranes of defined surface charges have been achieved. These three "reactions" correspond to regioselective conversions of functional groups, oxidation of nonpolar groups, and dipole inversions ("umpolung") in conventional syntheses of molecules. Even stereoselective aggregations are pos-

sible if one takes advantage of the fact that racemates are sometimes much less soluble than the pure enantiomers.⁴⁹

The first synthetic monolayers were reported in 1979⁵⁰ and 1980.⁵¹ Few organic chemists have become interested since then in the development of these stable aggregates, which are potentially useful in the construction of any organized reaction system. It is hoped, that this account may attract more synthetic chemists to this fertile field.

Sincere gratitude is expressed to the graduate students and postdoctoral collaborators whose names are cited in the references. Generous support for this research has been provided by the Deutsche Forschungsgemeinschaft (SFB Gerichtete Membranprozesse), the Fonds der Chemischen Industrie, and the FNK of the Freie Universität Berlin.

(49) Brønsted, J. N. *Fresenius' Z. Anal. Chem.* **1903**, *42*, 15.

(50) Okahata, Y.; Kunitake, T. *J. Am. Chem. Soc.* **1979**, *101*, 5231.

(51) Baumgartner, E.; Fuhrhop, J.-H. *Angew. Chem.* **1980**, *92*, 564; *Angew. Chem., Int. Ed. Engl.* **1982**, *19*, 550.