Lipid Bilayer Fibers from Diastereomeric and Enantiomeric N-Octylaldonamides
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in methylene chloride (3 mL) and added dropwise to the cooled ethylideneisobacteriochlorin solution. After 20 min of stirring, the flask was transferred directly to a rotovap and evaporated to dryness. The crude product was purified on alumina (Brockman Grade III, eluting with 3–5% THF/methylene chloride), giving 6 mg (30% yield) of methyl phophonaphthorhodochlorin a, mp 216–218 °C (lit. mp 217–219 °C). 200–225 °C). Vis (relative absorbance): 412 nm (1.00), 506 (0.0879), 538 (0.0814), 608 (0.0744), 666 (0.339). NMR (360 MHz, CDCl3): 9.52 (s, 8-β-meso H); 9.40 (s, α-meso H); 8.55 (s, 6-β-meso H); 8.03 (X of ABX, 2a-H); 6.25 (AB of ABX, 2b- and 2b′-H); 5.20 (AB q, 10-CH2); 4.50 (q, 8-H); 4.33 (d, 7-H); 3.72 (q, 4-CH2); 3.70 (s, 5-Me); 3.53 (s, 7-OMe); 3.42 (s, 3-Me); 3.25 (s, 1-Me); 2.70, 2.58, 2.30 (each m, 7-CH2-CH3); 1.85 (d, 8-Me); 1.70 (t, 4a-Me).

Large-Scale Photoreduction in Benzene. Zinc(II) methyl phophonaphthorhodochlorin (7.85 mg) was dissolved in 1% ethanol/benzene (100 mL, previously saturated with nitrogen for 20 min). DABCO (1.4 g) and ascorbic acid (880 mg) were added, and the sealed Erlenmeyer flask was irradiated in a Rayonet light drum for a total of 18 h with intermittent monitoring of the visible spectrum. The product was poured into ether (200 mL) and water (100 mL) and separated. The organic layer was transferred directly to a rotovap and evaporated to dryness. The crude product was purified by preparative TLC (3% methanol/methylene chloride) using two plates. Crystallization from methylene chloride/n-hexane gave 45 mg of blue solid. NMR (360 MHz, CDCl3 and hexane) gave 45 mg of blue solid.

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Zinc(II) 2-ethylisobacteriochlorin (32).

Using the large-scale procedure 19 mg of zinc(II) mesorhodochlorin (30) was irradiated for 24 h with 800 mg of DABCO and 400 mg of ascorbic acid in 50 mL of 8% ethanol/pyridine. Purification of the reduced product was not possible as the product appeared to oxidize on silica gel. The crude product was obtained as a solid from n-hexane. Vis: 388 nm (ε 5000), 402 (6000), 518 (4900), 592 (300), 635 (7000). NMR (360 MHz, CDCl3 and pyridine-d5): 395 nm (1.00), 554 (0.160), 594 (0.443), 630 (0.109) (chlorin impurity?). NMR (360 MHz, CDCl3 and pyridine-d5): see Table I.

Zinc(II) 2-Vinyllisobacteriochlorin (27).

This reduction was done by the large-scale procedure using 22 mg of zinc(II) rhodochlorin (26), 700 mg of DABCO, and 400 mg of ascorbic acid in 50 mL of 10% ethanol/benzene. All the starting material had been completely consumed in 1 h, after which the reaction was worked up as in the previous reaction. Separation was achieved by preparative TLC (1.5% methanol/methylene chloride), and the product was obtained as a solid from n-hexane. Vis: 388 nm (ε 5000), 402 (6000), 518 (4900), 592 (300), 635 (7000). NMR (360 MHz, CDCl3 and pyridine-d5): 395 nm (1.00), 554 (0.160), 594 (0.443), 630 (0.109) (chlorin impurity?). NMR (360 MHz, CDCl3 and pyridine-d5): see Table I.

Vis: 382 nm (ε 5000), 394 (48 100), 608 (70 000). NMR (360 MHz, CDCl3 and pyridine-d5): see Table I.

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Vis: 388 nm (ε 5000), 402 (6000), 518 (4900), 592 (300), 635 (7000). NMR (360 MHz, CDCl3 and pyridine-d5): see Table I.

2a- and 4a-CH2); 3.27 (s, 5-Me); 27.6 (s, 3-Me); 22.5 (m, 7-CH2CH3, impurity?); 1.50 (m, 1- and 8-Me, 4b-Me).

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Lipid Bilayer Fibers from Diastereomeric and Enantiomeric N-Octylandnamides

Jürgen-Hinrich Fuhrhop,** Peter Schmieder,*,1 Egbert Boekema,*, and Wolfgang Helfrich†

Contribution from the Institut für Organische Chemie der Freien Universität Berlin.

Abstract: The aggregation behavior of eight diastereomeric and enantiomeric N-octylandnamides, three enantiomers (galacton, mannon, glucuron), and corresponding racemates was investigated mainly by electron microscopy. Head groups with a sterically undisturbed all-anti conformation (galacton, mannon) lead to "whisker"-type aggregates, which appear as rolled up, bilayer sheets in both aqueous and 1,2-xylene gels. One pair of 1,3-syn-positioned OH groups in the all-anti conformation neighboring on the amide group, lead to extremely thin helical whiskers of high curvature in water (glucuron) or 1,2-xylene (talon). If the outer OH groups are in syn positions in the all-anti chain conformations, the N-octylandnamides become highly water-soluble (allon, altron, idon) and form rolled up, bilayer sheets in 1,2-xylene (guion). The length-to-diameter ratios in the aggregates are often higher than 100. The fibers are stabilized by amide hydrogen bonds and/or the hydrophobic effect. They can be conceived as models for prebiotic assemblies, which may lead to condensation biopolymers in aqueous media.

Hydrophobic bilayers with well-defined morphologies may serve (i) as structural models for biological membranes and (ii) as scaffolding systems for synthetic functional systems. So far, spherical vesicles1 and fibers2,3 have been obtained, the latter being either ribbons or tubes. If composed of chiral molecules, the ribbons are usually twisted or helically wound.4 A first survey on relationships between molecular structures of several synthetic amphiphiles and their nonspherical aggregates, Kunitake singled out the interaction of linear and bent aromatic rigid segments as the most important elements.2,5 We explained the helicity of elongated bilayers made from chiral molecules by involving spontaneous torsion of the edges6 and traced fibrous aggregates7


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from N-alkyl-D- and L-gluconamides back to formation of chains of amide hydrogen bonds. They can be regarded as substitutes of the rigid aromatic components of Kunitake's compounds. It was also shown that the racemic compound formed platelets rather than micellar fibers ("chiral bilayer effect"). We report here on eight diastereomeric N-octyl-D-aldonamides which produce "whisker-like" helices, tubes, or ribbons or do not aggregate at all. Simple relationships between the head group's stereochemistry and the aggregate morphologies have been found.

**Results**

The eight diastereomeric aldonamides 1-8 and three enantiomers 9-11 listed in Chart I were obtained by electrolytic oxidation of the corresponding hexoses to the lactones and amidation with octylamine.\(^9\)\(^,\)\(^10\)

Galactonamides 1 and 9 were the least soluble of the eleven compounds. Saturated aqueous solutions (0.4-0.5% w/v) were obtained by refluxing the D-enantiomer. On cooling to room temperature, the solution solidified to a gel. Fibers with diameters of about 100 nm and a length of several μm or more were observed on electron micrographs (Figure 1a), whereby the solution was cooled rapidly. The narrow fibers made from pure D- and L-enantiomers appeared as ribbons and were sometimes twisted. The D-enantiomer produced a left-handed twist and the L-enantiomer a right-handed twist (Figure 1 (parts b and c)). Slow cooling resulted not in fibers but in ill-defined microcrystallites. At branching points, the rapidly grown fibers often lay orthogonal to each other (Figure 1a).

The racemic mixture of 1 + 9 was even less soluble than the pure enantiomers. It also formed fibers but without branchings which would be expected for fast growing dendritic crystals.\(^11\) There is obviously never enough material in the dilute solutions to allow the formation of ramifications. The racemic fibers are usually wider by a factor of 10 and appear to be collapsed tubes (Figure 1d).

All gels made from galactonamides were of low tensile strength because of the low fiber concentration and were stable for several weeks. Over several months precipitation occurred. No organogels were formed in 1,2-xylene, since the lactonamide is insoluble in this solvent even after prolonged refluxing.

![Figure 1. Electron micrographs of aggregate fibers in gels made from N-octylgalactonamides in water (Pt/C shadowing): (a) D-galactonamide 1; overall view; (b) left-handed twist in ribbons made from D-galactonamide 1; (c) right-handed twist in L-galactonamide 9 ribbons; and (d) tubes from racemic 1 + 9.](image-url)
produce fibers. The platelets which were found instead (Figure 2), probably consist of strongly hydrated bilayers, since the material is very soft.

The gluconamide 3 is well-soluble in boiling water (\(\sim 50\% \text{ w/v}\)). The hot aqueous solutions solidified to a gel on cooling, in the concentration range of 0.5–50\% (w/v). Electron micrographs of the fibers in the aqueous gels have already been published.\(^a\)\(^b\)\(^c\)\(^d\) Figure 3a gives a typical example of an aged gel which contains helices of unknown multiplicity. In the presence of phosphotungstate at pH 2 or 7, helical ropes of the minimal thickness of
Figure 3. Electron micrographs of fiber aggregates of gluconamide 3: (a) from water and (b) from xylene (Pt/C shadowing).

The aqueous gels and organogels from gluconamides were less stable than those made from galactonamides and mannonamides. Anhydrous crystals usually separated out within days. The crystal structure of the pure enantiomers showed head-to-tail arrangements. The racemate (3 + 11) again did not form fibers in water but precipitated quickly from hot solutions in the form of platelets without curvature. The X-ray structure of the racemate is not known.

The talonamide 4 has roughly the same solubility as the gluconamide diastereomer. It formed unstable gels in water, containing short and fragile fibers (Figure 4a). The fast grown organogel fibers were uniformly long and looked like well-developed whiskers (Figure 4b). In less rapidly cooled xylene gels, helical rods were sometimes detected (Figure 4c). The xylene gel was stable for several days, and from the aqueous gels, ill-defined crystals were rapidly formed.

The final compact gel was formed by d-gulonamide 5 in 1,2-xylene. Tubes from rolled up sheets were usually accompanied by platelets composed of bilayers (Figure 5). From hot aqueous solutions, 5 crystallized immediately on cooling.

The diastereomers 6-8 did not form gels or viscous solutions. They were all very soluble in cold water.

1H NMR spectra of the d-gluconamide 3 in hot deuterium oxide (275 °C) gave well-resolved signals which were indistinguishable from those of monomolecular methanolic solutions. Vesicles were therefore not present in the hot solutions. Galactonamides and mannonamides behaved similarly. Infrared spectra of 3 in D2O also showed the expected differences: the amide I band at 1460 cm⁻¹ was of much higher intensity at 25 °C (gel) than at 75 °C (solution), and the bands above 2500 cm⁻¹ are much broader in
the gel. The latter phenomenon has also been observed in gelatin sois and gels.13

Discussion

The fibrous aggregates are conceived as single crystals or sometimes polycrystals with high, length-to-diameter ratios. Such filaments are usually depicted as "whiskers" in the literature.14-16 The best known whiskers are made of ceramics or metals and are usually formed in electrolyses or condensation processes. Ribbon-structures and scroll-structures, which look similar to those in Figures 1 and 2 were, for example, obtained by sublimation of graphite at 3900 K and a pressure of 92 atmospheres of argon.14 Helical structures similar to those in Figures 3 and 4 were observed in metal whiskers which were made by reduction of metal halides at high temperatures.14a The high degree of crystal perfection found in the cylindrical graphite whiskers has been rationalized with the assumptions of near equilibrium conditions and molecular aggregates in the vapor phase at the high temperatures and pressures applied. Firstly, thin graphite sheets (±100 nm) were formed, cooled up to reduce surface energy and then thickenened by tangential growth in spiral fashion along screw dislocations.13 Similar sequences of events should occur in the formation of helical metal whiskers. The screw sense of the individual helices will be equally or righthanded.

The crystallization processes in the formation of micellar lipid fibers could also be similar to those proposed for ceramic and metal whiskers. We assume that the aldonamides occur as spherical micellar aggregates in hot aqueous solutions. Pfannemuller et al.8 determined a cmc at 90 °C (1.0·10-2 molar) for the gluconamides and talonamides in 1,2-xylene; and (iii) the chirality of the helices may be determined by the structure of the chiral monomers.

Amphiphiles 1 and 2 with no 1,3-syn hydroxy groups favor a nondisturbed, all-anti conformation of the carbon chain. A stretched conformation is expected for these molecules and a noncurved bilayer aggregate should prevail. The very long and uniform ribbons displayed by these aldonamide aggregates are probably whiskers which grow with the screw dislocations which they enclose. Screw dislocations can occur even in a single bilayer between the monolayer lattices and are presumably caused by interlayer interactions of the hydroxyl groups. Ribbons could alternatively also result from a highly anisotropic energy per unit length of the bilayer edges. Such anisotropies can be caused in our crystals by amide hydrogen bonds, which should be arranged in straight or slightly curved parallel lines. However, the fact that irregular platelets were formed at low cooling rates instead of ribbons is more consistent with whiskers. The bending elasticity of the bilayers also appears to be isotropic as indicated by gradient angles of helically wound ribbons near 45°.8 Likely mechanisms of ribbon windings are summarized in Figure 7.

The micellar cylinders and helices produced by gluconamides and talonamide 4 are most probably caused by a bend in the head group region. This bend was found in crystal structures of corresponding polyols17-19 and is caused by 2,4-syn interactions of the hydroxyl groups leading to a relative broadening of the head group region. The estimated ratio of diameters head group to hydrophobic chain is about 1.5 (1.48) and is consistent with data for other micellar aggregates.15 The thickness of one micellar disk can be estimated from the volume of the enclosed methylene groups (24 X 17 X 8 Å3) and should be about 9-10 Å. The amphiphiles 5-8 which are highly water soluble and do not form aqueous gels all contain syn-oriented hydroxy groups on C (3) and C (5). The most stable conformation of these aldonamides is obviously bent in such a way that the formation of a regular amide hydrogen bond chain is prevented by extensive hydration. Figure 9 reproduces three typical crystal structures

References


Figure 6. 1H NMR spectrum of gluconamide 3 micelles4 at ≥75 °C in D2O.

Figure 7. Twisting (a) or rolling up (b and c) of planar bilayer sheets around corners (c) or edges (a and b) to yield helices, tubes, or cigarette scrolls.

of a nondisturbed straight chain polyl and a bent as well as a highly irregular diastereomere, which illustrate the argument.

Another important aspect in the stereochemistry of aldonamide aggregates is the presence of enantiomers. In all racemic mixtures tried, namely galactonamides 1-9, mannonamides 2+10, and gluconamides 3+11, the solubility in hot water was lowered by factors of about three to five, as compared to the pure enantiomers. The mannonamides and gluconamides precipitated, on the cooling of the hot solution, as platelets. No stable gel was formed. This behavior is rationalized with a "chiral bilayer effect", which leads to rapid formation of crystalline bilayer sheets in racemates, whereas pure enantiomers rearrange slowly to enantiopolar (or head to tail) sheets. The galactonamide racemate, however, gave stable gels which contained long tubes instead of platelets (Figure 1d). The explanation for the extraordinary stability of these racemic whiskers with the low solubility of the galactonamide in cold water is, that the rapidly formed whiskers presumably cannot rearrange to multilayered sheets because the equilibrium concentration of micelles in solution is too low.

In the 1,2-xylene gels, the stereochemical differences between the polar groups are not as important as in aqueous gels. All aldonamides are either insoluble or from bilayer scrolls. It is not known whether the aldon groups lie outside or within the bilayer sheets. The missing stereochemical differentiation in xylene, as compared to water, can be related to a lack of solvation of the polar groups. In xylene, the all-trans conformation of every aldonamide predominates in the same way as in water-free crystals of gluconamidmes.10,20 Only extensive hydration of the outer hydroxy groups which are attached to crystalline sheets of oligoglycener chains leads to disturbances of the all-trans conformations.

**Conclusion**

Solubilities and aggregate structures of aldonamide 1-11 depend directly on the stereochemistry of the polyl head groups. Undisturbed all-anti chains lead to bimolecular sheets; a bend close to the amide group produces cylindrical micelles with high curvature, and a twist at the end of the chain causes water solubility of the amphiphiles and prevents fiber formation. The last result is in qualitative agreement with Kunitake's statement that "too much bending... is not advantageous for molecular ordering". A little bending, on the other hand, leads to ultrathin aggregates with high curvature and low tendency to sheets formation. A rigid segment is clearly not needed, in order to produce stable fibers.

The structural principles outlined in this paper for N-octyl-aldonamides need to be tested with other stereoisomeric fibers. Another example for the "chiral bilayer effect", namely D-polylysine and L-polylysine, has been published. Work on amphiphiles with cyclic carbohydrate head groups is in progress. It is also tempting to assume that the chiral fiber aggregates may be useful as substrates for condensation polymerization in aqueous media.

**Experimental Section**

**General Methods.** Electron microscopy was carried out with a Philips EM 300 at 80 K. Freeze etching was carried out with a Balzers freeze etching unit. $^1$H NMR spectra were recorded on a Bruker WH 270 MHz spectrometer. A Perkin-Elmer DSC-2C calorimeter and large pans were used for differential scanning calorimetry.

**Syntheses of N-1-Octylaldonamides.** Aldonic acid lactones were either purchased (Sigma) or prepared by indirect electrochemical oxidation of the corresponding hexoses in the presence of calcium bromide. Excess bromide was precipitated with silver carbonate and filtered off. The solution was then stirred with a strongly acidic ion exchange resin (Merck) and dehydrated by azeotropic distillation with 1-butanol. Amylase of the lactones (to give amides 1-11) was performed by heating

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Figure 9. Examples for an undisturbed straight-chain aldonamide, namely 2, an aldonamide with a bend (3), and a highly irregular chain conformation (8). The given conformations are based on crystal structures of the corresponding alditoles10,20 and related conformational studies in solution.11
Acidities of Radical Cations Derived from Remotely Substituted and Phenyl-Substituted Fluorenes

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Abstract: Estimates of the acidities of the radical cations derived from remotely substituted and phenyl-substituted fluorenes have been made from measurements of the acidities and oxidation potentials of the fluorenes and the oxidation potentials of their conjugate bases. The radical cation acidities of fluorene, 2-bromo-fluorene, and 4-aza-fluorene were all estimated to be ~1.7. The pKₐ,α⁺ values for fluorenes bearing remote donor substituents were as follows: 3-Me (-15); 2-Me (-15); 3-MeO (-13.5); 2-PhS (-12); 3,6-(MeO₂)₂ (-12); 2-MeO (-10); 2,7-(PhS)₂ (-9.5); 2,7-(MeO₂)₂ (-6); 2,3,6,7-(MeO₄)₄ (-2); 2-MeN⁺ (+1). A plot of Eₐ (HA) for the fluorenes vs the acidities of the corresponding radical cations, pKₐ,α⁺, is linear (slope = 0.93, R² = 0.995). The introduction of a phenyl or aryl group into the 9-position of fluorene, as in 9-Ph, 9-p-tolyl, 9-m-C₆H₄, or 9-mesitylfluorenes and fluoradene, increased the radical cation acidity (pKₐ,α⁺ = -21 to -23). A similar PH effect was observed on the acidities of 2,7- or 3,6-dimethoxyfluorene radical cations. These acidity increases are associated with decreases in the 9-C=H bond dissociation energies (BDEs) of 4-9 kcal/mol, relative to that of fluorene. On the other hand, fusion of a benzene ring onto the 1,2- or 2,3-positions lowers the acidity of fluorene by 6 and 5 pKₐ,α⁺ units, respectively, an effect which overshadows the small acid-strengthening effects caused by the ~1 kcal/mol lower 9-C=H BDEs.

The acidities of only a few radical cations have been determined because of the difficulty in measuring the position of an equilibrium involving two highly reactive radical species, such as those in eq 1. Nevertheless, acidities of about a dozen nitrogen and oxygen radical cation acids have been measured, including those for PhNH⁺,² Me₂NH⁺³, and PhOH⁺⁴, which have pKₐ,α⁺ values of 7, 6.5-7, and -2, respectively. Typically, the radical cations were generated by photolysis or pulse radiolysis in an