

Solid-state bilayer formation from a dialkyl-substituted lariat ether that forms stable vesicles in aqueous suspension

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Received 14 November 2000; revised 16 November 2000; accepted 4 December 2000

ABSTRACT: Previously established aggregate formation by alkyl-substituted crown ethers is considered with respect to the solid state structure of *N,N'*-didodecyldiaza-18-crown-6 (**7**), which was determined as its NaI complex (**7**·NaI·H₂O). The complex forms a bilayer structure that was used as a benchmark for understanding the formation of aggregates and their inclusion volumes. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: solid-state bilayer formation; dialkyl-substituted lariat ether; aggregate formation; inclusion volume.

INTRODUCTION

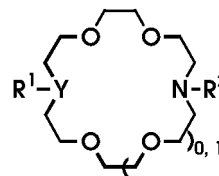
More than a decade ago, we initiated a study of substituted crown ether compounds as possible amphiphiles. Our interest in the ability of crowns to function as amphiphiles was driven by our desire to develop a synthetic transmembrane channel.¹ Crown ethers offered an obvious advantage as transient cation complexing agents if they could be adapted as headgroups. At the time this effort began, we were aware of studies conducted in the groups of Okahara² and Kuwamura.³ Their early efforts, focused on single-chain alkyl-substituted azacrowns, suggested that these compounds afforded micelles in preference to vesicles (liposomes).

Our channel design strategy,¹ in which crown ethers serve as headgroups, required that the crown's side-chains align with the bilayer lipids. We felt that the presence of either twin alkyl strands or a steroid would be effective in this application. The first channel compound we prepared used two dodecyl chains. The first lariat ether compound to afford stable liposomes⁴ incorporated a steroid side-arm. The presence of the steroid was apparently sufficient for these alkyl crowns to form stable liposomes in preference to the more dynamic micellar structures. We extended the studies to twin-tailed crowns⁵ and to bolaform crown amphiphiles⁶ as these were the desired functional subunits within the hydrophile channels.⁷

Three facts concerning the diazacrown amphiphiles were both intriguing and perplexing. First, the vesicles that formed from dialkyl crown monomers in aqueous suspension were typically in the 2000–3000 Å range almost irrespective of side-chain length. Second, azacrowns bearing a single steroidal side-chain afforded stable liposomes. Alkyl-substituted azacrowns did not. Third, remarkably short side-arms still afforded stable aggregates when two or three of them were present on a diaza- or triaza-18-crown-6 scaffold. In a recent collaborative effort, we found that apparently equivalent side-chains did not produce stable vesicles when phenolic hydroxyl groups were proximate to the macroring.⁸ In order to gain an insight into the organization of the crown–amphiphile aggregates, we undertook the studies that are described here.

RESULTS AND DISCUSSION

The compounds that are the focus of this study are 15- or 18-membered ring azacrowns of the form shown.



For the monoazacrown derivatives, Y-R¹ is O. When two chains are present, R¹ = R² and Y = N. Compound **10** is a triaza-18-crown-6 derivative similar to the structure shown but in which three ring oxygen atoms alternate

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Contract/grant sponsor: NIH; Contract/grant number: GM 36262.

Contract/grant sponsor: NSF; Contract/grant number: CHE-9805840.

Table 1. Aggregate sizes of alkyl-substituted crown ethers determined by laser light scattering

No.	Ring	Chain	Unimodal (Å)	Ref.
1	Aza-15-crown-5	CH ₂ CO-cholestanyl	340 ± 130	4
2	Aza-15-crown-5	CH ₂ CO-cholesteryl	350 ± 110	4
3	Aza-18-crown-6	CH ₂ CO-cholestanyl	180 ± 60	4
4	Aza-18-crown-6	Tetradecyl	(119) ^a	5
5	Diaza-18-crown-6	Butyl	2350 ± 860	5
6	Diaza-18-crown-6	Nonyl	4550 ± 1300	5
7	Diaza-18-crown-6	Dodecyl	2970 ± 710	5
8	Diaza-18-crown-6	Tetradecyl	2510 ± 800	5
9	Diaza-18-crown-6	Octadecyl	2200 ^a	5
10	Triaza-18-crown-6	Hexyl	2850 ± 690	5
11	Bis(aza-15-crown-5)	Dodecamethylene	1960 ± 620	6
12	Bis(diaza-18-crown-6)	Dodecamethylene	1200 ^b	6

^a Standard deviation is broad.^b Standard deviation is ±30%.

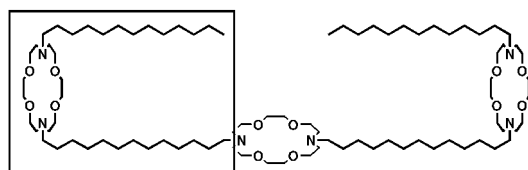
with *N*-*n*-hexyl groups. The compounds of interest in the present study are recorded in Table 1.

Vesicle preparation

In all cases, vesicles were prepared in the standard way by sonicating an aqueous suspension of the crown, which was present in low micromolar concentration. The vesicles were characterized by laser light scattering, electron microscopy and dye entrapment. Not all methods were applied to each compound but the vesicles may be considered appropriately characterized as described in previous papers.

Structure of *N,N*-didodecyldiaza-18-crown-6 (**7**)

The basic subunit of the first hydrophile channel is *N,N'*-didodecyldiaza-18-crown-6 (**7**), in which the 12-position of one chain is attached to the 'central relay.' The structure of this hydrophile channel is illustrated as 'dodecyl channel' and the subunit corresponding to **7** is shown enclosed in a box. Compound **7** is also known to form stable vesicles on its own (see Table 1). Thus, it was hoped that an examination of the solid-state structure would help in understanding the aggregation behavior of monomer **7**. In principle, this effort might also afford insight into the structure of the channel itself.



"dodecyl channel"

The alkyl-substituted aza- and diazacrowns we have

prepared thus far are all oils or waxy solids at room temperature. This precluded obtaining a crystal structure. We felt, however, that it might be possible to obtain the structure of an alkali metal complex of **7**. The dodecyl channel was extensively studied as a Na⁺ transporter so an ideal candidate would be **7**·Na⁺. We have also previously studied the aqueous aggregation behavior of **7** in the presence and absence of NaCl.⁵ Of course, this structure would be intrinsically interesting in any event, as there are relatively few dialkyldiazacrown crystal structures extant. The availability of the structure of **7** would also permit us to make size and distance estimates of the various molecular parameters.

A search of the Cambridge Structural Database (CSD) revealed only a handful of alkylcrown structures. Only one of these was an alkali metal complex. Czugler *et al.* reported the structure of [15,15-bis(dodecyloxymethyl)-16-crown-5]-isothiocyanato sodium complex in 1982.⁹ Soon afterwards, Weber and Sheldrick reported a clathrate involving *N,N'*-didecyl-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane tetradecakis(thiourea).¹⁰ A non-complexed bolaform structure was reported in 1989 in which two 13-crown-4 residues were symmetrically linked by a hexyl chain between their 3-carbon ring units [3,3'-hexamethylenebis(1,5,8,11-tetraoxacyclotridecane)].¹¹ Recently, a twin-chain thiocrown was reported [1,10-bis(4-*n*-dodecyloxybenzoyl)-1,10-diaza-4,7,13,16-tetrathiacyclooctadecane silver(I) hexafluorophosphate] in which silver cation was bound in the center of the macrocycle. In this structure, the macrocycle was canted with respect to the side-chains, the latter of which were parallel.¹²

Simultaneous with our effort to obtain the structure of **7**·NaI, a report appeared of the solid-state structure of **7**·NaClO₄.¹³ A number of features are similar between the reported structure and our own despite the difference in the salt complexed. Considerable information can be gleaned, however, from the association of monomers within the crystal and no related information is found in the published report for the **7**·NaClO₄ structure.

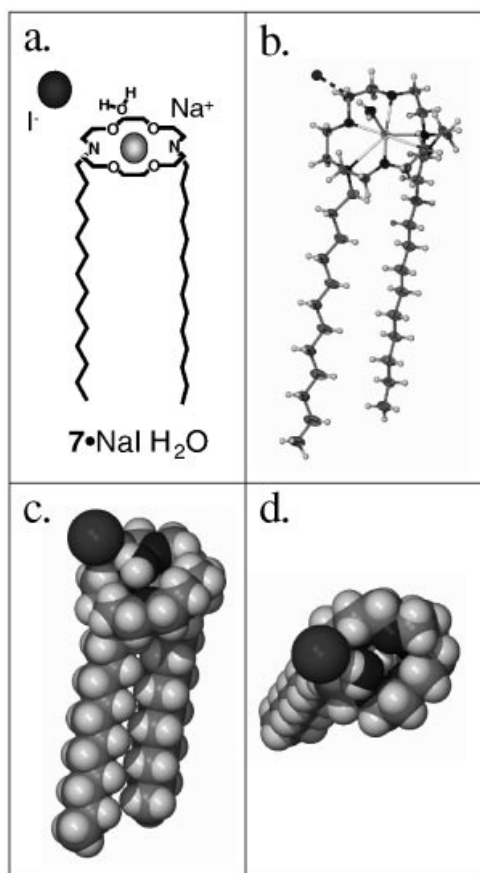


Figure 1. Schematic illustration and three views of the solid-state structure of $7 \cdot \text{NaI} \cdot \text{H}_2\text{O}$.

Solid $7 \cdot \text{NaI} \cdot \text{H}_2\text{O}$ crystallized from acetone–ethyl acetate (1:1, v/v). The complex was obtained as colorless crystals melting at 63–64 °C. The monomeric complex is shown schematically in Fig. 1(a) and as an ORTEP plot in Fig. 1(b). Figure 1(c) shows a similar view in the CPK metaphor. A ‘top view’ is shown in Fig. 1(d). The large sphere at the left in each structure is iodide anion.

Structural features of $7 \cdot \text{NaI} \cdot \text{H}_2\text{O}$

The key features of the $7 \cdot \text{Na}^+ \cdot \text{H}_2\text{O}$ monomer structure are as follows. The complexed cation is located in the crown macrocyclic ring and Na^+ is formally seven-coordinate. It is bound by the four macrocyclic oxygens, the two nitrogens and a water molecule. The sodium cation resides approximately at the center of the macrocycle, although the latter is slightly ‘puckered.’ Such a macrocyclic deformation is often observed in 18-crown-6 complexes of sodium ion, the ionic diameter of which is smaller than the crown’s cavity when the ring is flat. One donor group moves out of the plane in the puckered macrocyclic ring, leaving the remaining five heteroatoms to contract to the appropriate $\text{Na}^+ \cdots \text{O}$ distance of ~ 2.5 Å.

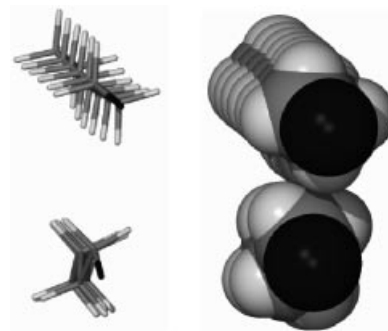


Figure 2. Partial structure of **7** showing the orientation and arrangement of the dodecyl chains

Despite the apparent asymmetry of the macrocyclic ring, the four $\text{Na}^+ \cdots \text{O}$ distances are similar to each other (2.53 ± 0.1 Å), as are the $\text{Na}^+ \cdots \text{N}$ distances (2.85 ± 0.02 Å). The seventh contact occurs between the cation and water; the $\text{Na}^+ \cdots \text{O}_{\text{water}}$ distance is 2.35 Å. The van der Waals radius for a seven-coordinate Na^+ is 1.12 Å.¹⁴

The reported structure of $7 \cdot \text{NaClO}_4$ shows similar puckering but the $\text{Na}^+ \cdots \text{O}$ distances for the macrocyclic donors are all in the range 2.37–2.45 Å. The $\text{Na}^+ \cdots \text{N}$ distances are likewise shorter, being 2.76 and 2.81 Å. A longer $\text{Na}^+ \cdots \text{O}$ distance, 2.47 Å, is observed for the $\text{Na}^+ \cdots \text{perchlorate}$ oxygen contact. These distances suggest that the cation is effectively six-coordinate and that perchlorate interacts only weakly.

The two alkyl chains of $7 \cdot \text{NaI} \cdot \text{H}_2\text{O}$ are aligned with each other and are canted with respect to the mean plane of the macrocycle by approximately 30°. Although the chain axes are nearly perfectly aligned, the planes containing the extended chains of carbon atoms are offset (angle $\approx 120^\circ$, see below and Fig. 5). End views of the chain alignment are shown in Fig. 2 in both the tube (left) and CPK metaphors. The dark circle at the top of each CPK model corresponds to the macrocyclic nitrogen atom. From the latter, it appears that the chains are oriented to accommodate the packing of the hydrogen atoms. Thus, the line of hydrogens on one chain bisects the hydrogens of the adjacent chain (it appears from Fig. 1 in Ref. 9 that the alkyl chains of $7 \cdot \text{NaClO}_4$ are similarly offset, but no data are provided). The overall length of each alkyl chain is identical (see Fig. 1): the $\text{N} \cdots \text{C}$ distance from the macrocyclic nitrogen to each methyl terminus is 15.3 Å.

The pseudo-apical water molecule serves as a donor and is, in turn, H-bonded to the iodide counterion. The $\text{O} \cdots \text{I}^-$ distance is 3.58 Å and the $\text{O} \cdots \text{H} \cdots \text{I}^-$ angle is 172.6°. As noted above, the reported macrocyclic to sodium donor group distances in the $7 \cdot \text{NaClO}_4$ structure are shorter (see above) than we observe. The seventh coordination site is occupied by a perchlorate oxygen atom. The perchlorate anion is thus in direct contact with

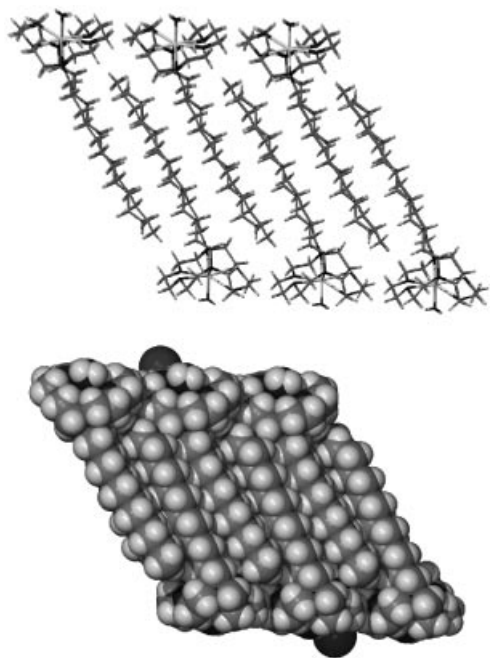


Figure 3. Crystal packing of **7**·NaI·H₂O shown in tube and CPK metaphors

sodium rather than H-bonded to a molecule of water as in the present case. The mean cavity radius¹⁵ reported for the perchlorate complex is 1.10 Å, which is similar to but slightly smaller than the 1.20 Å that we observe for **7**·NaI·H₂O.

Structural features of the **7**·NaI·H₂O bilayer

The parallel chains of **7** align to form a closely packed bilayer structure (see Fig. 3). Longitudinally, the chains are deeply interdigitated such that the overall bilayer thickness is 18.7 Å. The distance (Na ↔ Na = 18.7 Å) is a measure from sodium in one layer of complexes to the sodium bound in the interdigitated complex layer. Indeed, the interpenetration of the monomers is such that the methyl termini of the dodecyl chains are in contact with the bottom of the opposite macrocycle's headgroup. The figure shows both tube (top) and CPK representations of one stratum of the bilayer.

The closeness of the fit is apparently permitted in part by the fact that the side-chains are slanted with respect to the macroring plane. The chains are about 30° off a perpendicular to the macroring plane. This arrangement leaves little physical space between the chains and the headgroups fit compactly together at the interfaces. Figure 4 shows representations of the chains viewed from above. The dark spheres apparent at the top in both the tube and CPK representations are the macroring nitrogen atoms. The packing of the hydrogen atoms along the chains is especially clear in the top row of the CPK representation.

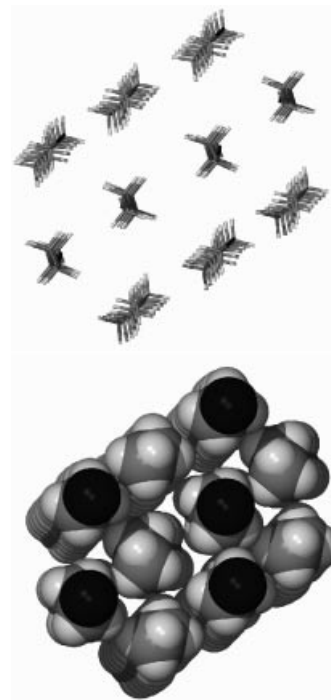


Figure 4. Top views showing alkyl chain packing for **7**·NaI·H₂O. The dark circles at the tops of several chains represent nitrogen

The headgroup packing arrangement is shown in Fig. 5. The crowns are viewed from above. Four macrocycles are arranged in a parallelogram having an acute angle of 59.1° and an obtuse angle of 120.9°.

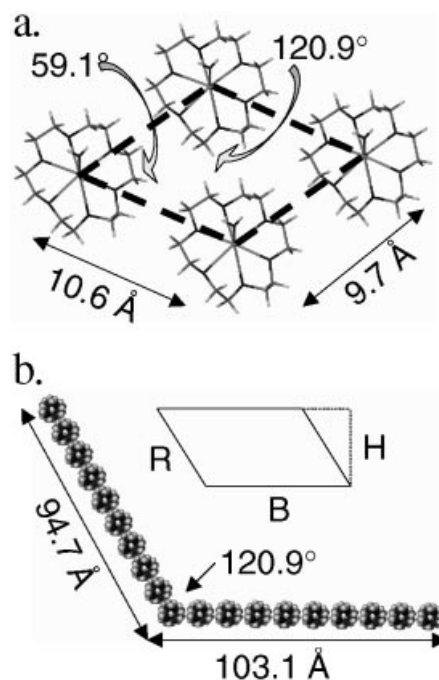


Figure 5. Packing of the macrorings of **7**·NaI·H₂O showing the repeating unit in the bilayer

Table 2. Calculated and measured surfactant parameters for **7**^a

Method	V (Å ³) ^b	L (Å) ^b	A (Å ²)	V/LA
Ninham and co-workers, ^{17–19}	700	16.7	78.5	0.53
From structure using Spartan	493	16	156.8	0.20
From monomer using X-Seed	395	16	50.1	0.49
From 10 × 10 block using X-Seed	395	16	83.8	0.29

^a Previously reported data for **7** are from Ref. 4.

The sides of the parallelogram are 10.6 and 9.7 Å, as measured using ring-bound Na⁺ as the approximate center of each macrocycle. The macrocyclic rings are essentially coplanar, although slightly puckered (see above). The water molecules coordinated to Na⁺ protrude almost perpendicularly to the approximate headgroup plane.

Theoretical considerations

An empirical model¹⁶ was developed by Ninham and co-workers^{17–19} to predict aggregate type based on the steric relationship of headgroup and sidearm size. A so-called surfactant parameter, V/LA , was proposed (A is headgroup surface area, V and L are the volume and length of the side-chains). A value $0.5 < V/LA < 1$ predicts vesicles and $V/LA < 0.5$ predicts micelle formation.

Simple formulas have been developed that can be applied to calculate the volume (V) and length (L) of an unbranched alkyl chain: $V = (27.4 + 26.9n)$ Å³ ($= 350.2$ Å³ for $n = 12$) and $L = (1.5 + 1.265n)$ Å ($= 16.7$ Å for $n = 12$). The volume is doubled when two identical chains are present. Other approaches afford different results. For example, the volume calculated by Spartan (v. 5.0) (Wavefunction, Irvine, CA, USA²⁰) for two 12-carbon alkyl chains (2×246.3 Å) is 492.6 Å³. Clearly, this is only about 70% of the volume calculated by the above-specified (empirical) formula. Estimating the headgroup area presents an additional challenge. Measurements of CPK molecular models suggest a value of 78.5 Å² as the headgroup area of 18-crown-6. The Spartan-calculated value for the headgroup's solvent exposed (one side) surface is 156.8 Å².

A further means for obtaining these molecular parameters is to use the solid-state structure of **7**. Using the crystallographic coordinates for **7**·NaI·H₂O and the program X-Seed,²¹ we measured the cross-ring H—H distances directly. The measurements were limited to those hydrogen atoms as nearly opposite to each other as possible that were pseudo-equatorial. Six values were measured: 7.44, 7.88, 7.94, 8.16, 8.20 and 8.30 Å. The radius corresponds to half the average of these values: 3.99 Å. The area, $A = \pi r^2$, calculated using this radius is 50.1 Å².

Surface area of a fragment of the solid-state membrane

A final, alternative approach for assessing area was undertaken as follows. Using the crystal structure coordinates, X-Seed was used to 'grow' a stratum of the membrane [see Fig. 5(b)]. Each side of the fragment consisted of 10 monomers, resulting in a block (**7**·NaI·H₂O)₁₀ × (**7**·NaI·H₂O)₁₀ on a side. The block's dimensions were measured to be 94.7 × 103.1 Å. The angle between these two sides was 120.9°. The surface area of this block can be calculated by multiplying the base (B) by the height (H). The height (H) of the fragment was calculated from the trigonometric relationship $\sin(59.1^\circ) = H/94.7$; $H = 81.3$ Å. The product $B \times H$ (103.1×81.3 Å) gives an area of 8382 Å² for the block. The fragment contains 100 monomers, so the surface area of each monomer is 83.8 Å². The values estimated by these various methods are given in Table 2.

The surfactant parameter V/LA is an excellent tool but it is empirically derived and its extensibility is therefore limited. A consideration of steric relationships in amphiphiles is a valid approach in an effort to understand aggregation. Moreover, the exercise remains useful as a caveat for the application of simple models and modeling programs in understanding supramolecular forces.

Bolaform azacrown amphiphile

If the headgroup network of azacrown vesicles formed in aqueous solution is organized by hydrogen bonds and water bridge interactions,²² then the chain length should not be an important determinant of vesicular size. Previous work bears on this point. It was found that bolaamphiphiles formed from two aza-15-crown-5 ($<15N>C_{12}<N15>$, **11**) or two aza-18-crown-6 ($<18N>C_{12}<N18>$, **12**) headgroups separated by 12-carbon spacers formed stable lipid monolayers.⁶ Light scattering revealed that their aggregate sizes were 1960 and 1200 Å, respectively despite the identity of chain length. Of course, bolaform monolayer lipid membranes that form in aqueous solution cannot be compared directly with the solid-state bilayers discussed here, but the structures and the aggregates they form are clearly influenced by similar forces.

Analysis of liposomes based on the crystal structure of 7·NaI·H₂O

In the discussion that follows, we attempt to derive information about azacrown aggregates from previous data obtained in aqueous suspension and the solid-state data reported here. We recognize that liposome and solid-state bilayer membranes formed from **7** almost certainly differ in some respects. Still, the fact that a stable membrane forms from **7** in solution and the solid-state structure of 7·NaI·H₂O shows a close-packed bilayer is suggestive. We have therefore used the crystal structure of 7·NaI·H₂O as a model for the organization of the liposomes.

Previous studies show that **7** and related amphiphiles form bilayer vesicles that are 2000–3000 Å when sonicated in aqueous suspension. The solid-state structure of 7·NaI·H₂O shows that the membrane thickness is 18.7 Å and the exposed surface area of the headgroup is 83.8 Å². The bilayer thickness, chain interdigitation and headgroup packing observed in the solid state all seem reasonable if applied to vesicular aggregates. Of course, water and any hydrogen bonding it contributes would be lacking in the solid state.

We compare data obtained from vesicles formed from **8** with the solid-state data reported here for **7**. Compound **8** has two 14-methylene chains rather than the 12-carbon chains in **7**. In a previous study,⁵ we used dye entrapment (methylene blue, [**8**] = 5 mM, sample size = 10 ml) to verify the presence of an internal compartment in these aggregates. Aggregates formed from **8** entrapped dye to the extent of 13 ± 4%, which corresponds to a volume in this case of ~1.3 ml. We assumed that vesicles formed from **7** would show an inclusion volume similar to that observed for vesicles formed from **8**. Calculations of the entrapped volume were done using 3000 Å as the diameter of the vesicles, 18.7 Å as the thickness of the membrane and 83.8 Å² as the exposed surface area of one headgroup.

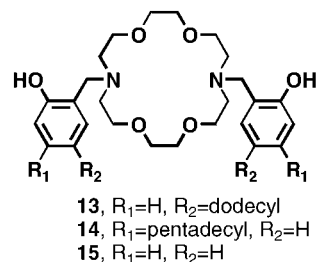
The surface area and volume of the vesicle were estimated using the formulas $4\pi r^2$ and $4\pi r^3/3$, respectively. Thus, the value of the external surface area is 2.83×10^7 Å² and the internal surface area is 2.76×10^7 Å². Dividing these values by the surface area of a single headgroup (83.8 Å²) gives the total number of amphiphiles in the outer (3.38×10^5) and inner leaflets (3.29×10^5) of the vesicle. The total number of amphiphiles in a single vesicle then is 6.67×10^5 . The sample contained 5×10^{-5} (0.005 M × 0.01 l) mol, and the total number of amphiphiles in the sample, 3.01×10^{19} , was determined by multiplying 5×10^{-5} times 6.022×10^{23} . The total number of vesicles in the sample is the total number of amphiphiles (3.01×10^{19}) divided by the number of amphiphiles in one vesicle (6.67×10^5) = 4.51×10^{13} vesicles. The internal volume of a single vesicle is calculated to be 1.36×10^{10} Å³, using the formula $4\pi r^3/3$ and a radius of 1500 Å. The

entrapped volume is then the internal volume of one vesicle (1.36×10^{10} Å³) times the total number of vesicles (4.51×10^{13}) = 6.1×10^{23} Å³.

The experimental value obtained from the dye entrapment study was $\sim 1.3 \pm 0.4$ ml. Applying the same error, 0.61 ± 0.4 ml places the two values in the same experimental range. Therefore, the calculation approximates the experimental value to a remarkable degree. Moreover, the fact that the vesicles exist as a distribution of sizes was not taken into account as the distribution is itself a calculated value. A further variable is the curvature of the vesicles, which is not exactly mimicked by the planar bilayer structure of (**7**)_n. Thus, the similarity between the observed and calculated values is striking given the inherent geometric differences in the planar structure observed in the crystal structure and the spherical structures that are present in solution. A final consideration is that water must permeate the liposomes and there is no way to take this into account using the solid state bilayer structure of **7**.

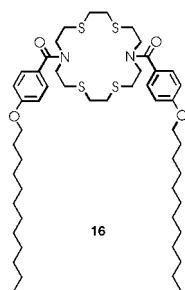
Phenolic diaza-18-crown-6 amphiphiles

In a recent collaborative effort with Bradshaw and co-workers, we experimentally assessed the ability of compounds **13** and **14** to form vesicles.⁸ The success of **7**, which has twin dodecyl chains, suggested that **13** and **14** would also form stable aggregates. The hydroxyl groups were expected to lend additional efficacy to the headgroup function. Despite these optimistic expectations, prolonged sonication of **13–15** resulted in aggregates that precipitated within 30–45 min after sonication ceased. This is in sharp contrast to aggregates that formed from the alkyl substituted crowns, some of which persisted for weeks.



We have previously postulated that protonated nitrogen on the macrocycles is critical to organizing the aggregates that form from aqueous suspension.²³ Several experiments were undertaken in an effort to understand the association of monomers in the aggregates that were observed. For example, fourfold isotonic dilution of aggregates formed from azacrown amphiphiles (bolaamphiphiles, in this case)⁶ did not change the observed hydrodynamic radius. Similar results were observed for eightfold dilution. Cooling from 20 to 10 °C an aqueous

suspension of vesicles prepared from crown ether bolaamphiphiles likewise had no effect on the hydrodynamic diameters. Evans and co-workers²⁴ demonstrated that temperature increases enhance the thermal motion of vesicular counterions, which causes surfactant headgroups to dissociate to a greater extent. Dissociation of the counteranions reduces their ability to share headgroup charge. The resulting increase in local curvature of the vesicular surface decreases aggregate size, ultimately lysing the aggregates (presumably to micelles). Above 25 °C, the critical micelle concentration (cmc) of charged surfactants normally increases with rising temperature,^{25,26} so the concentration of free surfactant tends also to increase. Heating to 35 °C or higher led to apparent irreversible vesicular fusion and/or the formation of large, multilamellar organizes. Similar studies conducted under acidic conditions (pH = 2) showed little change in aggregate size from 20 to 40 °C.



It is interesting to compare the recent structure of *N,N'*-bis(4-*n*-dodecyloxybenzoyl)-4,13-diaza-1,7,10,16-tetrathiacyclooctadecane silver(I) hexafluorophosphate (**16**) with the structure of **7**. This compound is related to **7** in that it is a diaza-18-crown-6 derivative having twin tails. It is related to **13** and **14** because benzene rings are an integral part of the tail and are present near the macrocyclic 'headgroup.' Clearly, the presence of amide links and sulfur atoms in the macrocyclic ring of **16** distinguish it from **7**. Moreover, it is a silver complex rather than a sodium complex. Still, **7** is clearly related in a number of ways to **16** and the latter's interesting features bear discussion.

Silver cation in **16**·AgPF₆ is bound essentially by the four sulfur atoms. The Ag—S distances are 2.47, 2.68, 2.49, and 2.65 Å (S1—S4, respectively). Contacts between macrocyclic nitrogen and silver, normally a very important interaction, are 3.45 and 3.64 Å. Of course, as amides, these nitrogen atoms are expected to be poor donors. The carbonyl groups do not compensate, however, being 3.39 and 4.45 Å distant. Unlike the **7**·Na⁺ complexes, the alkyl chains are parallel and their 'zig-zag' axes are aligned. This is apparent in Fig. 6, which shows the arrangement of the alkyl chains and the orientation of the methylene group hydrogen atoms.

Like **7**·NaI·H₂O, the alkyl tails of **16**·AgPF₆ interdigitate but the resulting structure appears less a bilayer than an offset stack. This is apparent in figure 7, which shows two pairs of interdigitated complexes. The tails of

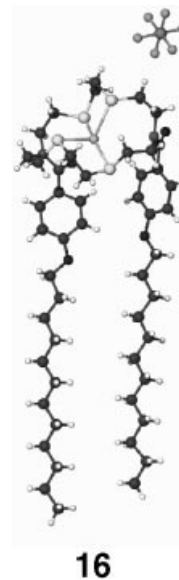


Figure 6. Solid-state structure of **16**·AgPF₆, shown in ball and stick metaphor

16 are longer than in **7**, being 20–21 Å in the former case and only ~16 Å in the latter. The cation–cation separation, by which we estimated the bilayer thickness (see above), is 36.8 (Ag ↔ Ag in **16**·Ag⁺) rather than 18.7 Å (Na ↔ Na in **7**·Na⁺).

The similarities observed in these structures reinforce the hypothesis that the solid state and vesicular organizations of alkylcrown amphiphiles are related. There is an obvious difference in the extent to which the side-chains of **7** and **16** interdigitate, leading to a significant difference in membrane thickness. Still, the side-chains of either compound pack in parallel axes in bilayers rather than any of the other arrangements one might easily envisage.

Previous evidence suggested that a hydrogen-bonded network of protonated macrocycles may define the 2000–3000 Å aggregate size typically observed for these systems. Protonation may also contribute to vesicle stability. Compounds **13** and **14** appear to possess all of the elements present in **5**–**10** required for assembly but fail to form the expected, stable liposomes. The instability of aggregates formed from **13** and **14** may be due to the formation of competing, intramolecular hydrogen bonds between the phenolic hydroxyl group and the macrocyclic nitrogen. Such an intramolecular H-bond would preclude the formation of the postulated intermolecular H-bond network discussed above. Indeed, exactly this type of intramolecular H-bond formation has been observed in the solid-state structure of *N,N'*-bis(*o*-hydroxybenzyl)diaza-18-crown-6 (**15**) and further inferred from solution studies.²⁷ Alternately, headgroups may be bridged by water molecules across the nitrogen and oxygen macrocyclic donors. Such bridges would help to stabilize the two-dimensional array of headgroups.

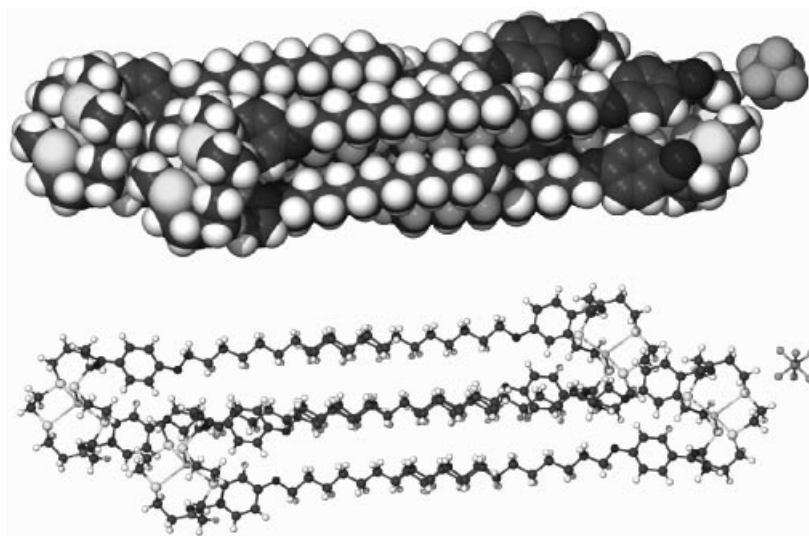


Figure 7. Solid-state structure of two pairs of **16**·AgPF₆, shown in CPK (top) and ball and stick metaphors

The recently reported solid-state structure of **15** is shown in Fig. 8.²⁸ The alkyl substituents that would be present in compounds **13** and **14** are indicated in Fig. 8 as circles labeled R₁ and R₂. In the solid state, the side-arms are positioned above and below the macrocycle. The proximity of the hydroxyl groups to the macrocyclic nitrogens allows for the formation of an intramolecular hydrogen bond that rigidifies the system and reduces the ability of the macrocycle to bind alkali metal cations. The observed O···N distance is 2.77 Å and the O—H···N angle is 145.4°.

In summary, previous studies suggested that an H-bond network organizes liposomes formed from diazacrown amphiphiles. Phenolic amphiphiles **13** and **14** do not form stable liposomes even though they appear to possess the structural elements required to do so. The solid-state structure and solution studies of **15** show clear evidence

that intramolecular hydrogen bonds form between the phenolic hydroxyl groups and the adjacent macrocyclic nitrogen atoms. Because the lone pair electrons on nitrogen are involved in intramolecular H-bonds, they cannot form the intermolecular network that we believe is required for liposome formation. Thus, aggregates form transiently from **13** and **14** but quickly lose their structure.

CONCLUSIONS

The solid-state structure of **7** has proved to be both revealing and useful in understanding the formation of a bilayer structure from diaza-18-crown-6 compounds. Inferential evidence clearly supports the formation of a hydrogen bond network that contributes to the stability of aggregates formed from alkyl-substituted diaza-18-crown-6 derivatives. When competing H-bonds (viz. **13** and **14**) can form as in the known structure of **15**, aggregate formation is suppressed. The solid-state structure of **7**·NaI·H₂O does not show the putative H-bond network. Indeed, the N···N distance measured in the solid-state structure is 5.44 Å — too long for any reasonable H-bond. Of course, protonation would not be expected in a crystal obtained from organic solvents and dominated by the formation of a sodium complex. Even so, the bilayer formation, side-chain packing and head-group organization in this structure are revealing and the observations generally comport with data obtained by a variety of other methods.

EXPERIMENTAL

Compound 7. The preparation of **7** has been described previously.⁵

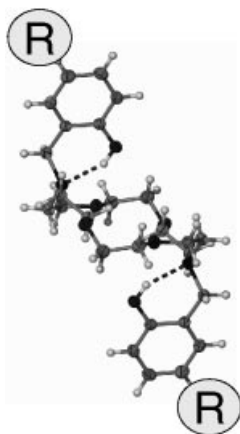


Figure 8. Solid-state structure of **15**. The circled R groups indicate the positions of alkyl chains in the related compounds **13** and **14** (see text)

Crystallization of 7·NaI·H₂O. Compound **7** (15 mg) and an equivalent amount of NaI were dissolved in 2 ml of acetone–ethyl acetate (1:1, v/v) at ambient temperature. Slow evaporation of the solvent over several weeks afforded colorless crystals suitable for x-ray analysis. The complex was isolated as colorless crystals (m.p. 63–64 °C).

X-ray crystallography. Intensity data for all crystals reported here were collected at 173(1) K on a Bruker SMART CCD diffractometer (ω scan mode, Mo K α radiation, $\lambda = 0.7107$ Å). Data were corrected for absorption using the program SADABS.²⁷ Structure solution was carried out by direct methods and structure refinement was accomplished with the SHELX-97 software²⁹ using the X-Seed²² interface. Direct methods yielded all non-hydrogen atoms of the asymmetric unit. These atoms were refined anisotropically (full-matrix least-squares method on F^2). Hydrogen atoms were placed in calculated positions with their isotropic thermal parameters riding on those of their parent atoms. The figures were prepared with X-Seed and POV-Ray.³⁰

Crystal Data for 7·NaI·H₂O. $M = 766.88$; colorless parallelepiped, $0.45 \times 0.25 \times 0.10$ mm; triclinic, $P - 1$; $a = 9.6900(13)$ Å, $b = 10.0245(13)$ Å, $c = 23.993(3)$ Å, $\alpha = 83.597(2)^\circ$, $\beta = 80.638(2)^\circ$, $\gamma = 64.900(2)^\circ$; $Z = 2$; $V = 2080.1(5)$ Å³; $D_c = 1.224$ g/cm³; $2\theta_{\max} = 54.32^\circ$, 16 695 reflections collected, 8991 unique [$R(\text{int}) = 0.0242$]; final $G_0F = 0.954$, $R1 = 0.0331$, $\omega R2 = 0.0798$, R indices based on 7383 reflections with $I > 2\sigma(I)$, $\mu = 0.818$ mm⁻¹, minimum transmission factor = 0.7097.

Crystal structure of 16·AgPF₆. Coordinates for the previously reported structure¹² were obtained from the Cambridge Structural Database (ZQYUL) and analyzed using X-Seed.²¹

Acknowledgements

We thank the NIH (GM 36262) and NSF (CHE-9805840) for grants that supported this work.

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