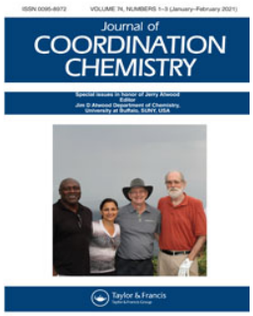

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REVIEW

Crown ethers having side arms: a diverse and versatile supramolecular chemistry

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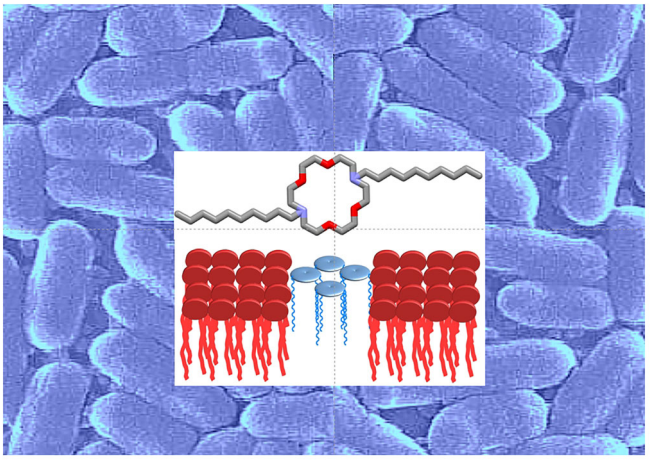
ABSTRACT

Lariat ethers are a variant of crown ethers that have pendant side arms. The original design for these molecules included donor groups in the side arms that were envisioned to enhance alkali metal cation binding without diminishing binding dynamics. Many of the examples that were prepared did fulfill this original vision. The compounds were used as cation binders and carriers. They were found to form membranes, other aggregates, and they were used as model systems to demonstrate alkali metal cation–pi interactions. When alkyl side arms were present, but secondary donors were absent, many of the structures proved to be antimicrobials. Several dialkyl lariat ethers proved to form aggregated pores or ion channels in bilayer membranes. These molecules also proved to be adjuvants that helped to overcome antimicrobial resistance in a range of bacteria and in fungi. The chemical behavior and biological activity are described in this review.



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Dedication

This review is dedicated to Professor Jerry L. Atwood, on the occasion of his retirement as Curators' Professor at the University of Missouri. Professor Atwood was once described to me by a successful inorganic chemist, who was at the time the chancellor of a major university, as "an icon." Dr. Atwood's contributions to inorganic, organometallic, inclusion, structural, and supramolecular chemistries can hardly be overstated. He has been one of the most innovative, productive, and highly cited chemists of our time. Indeed, despite his retirement, he continues to publish first class science. He has mentored numerous successful scientists who are both in academics and industry. Perhaps as important, he has organized meetings, founded book series and journals, served as editor of numerous journals, and has enthusiastically promoted the fields in which he has been involved.

In the 1980s, Jerry asked me to help him organize the scientific program for an inclusion phenomena meeting held in Orange Beach, Alabama. I knew him only as a professional colleague at that time. We worked together on the program inviting a majority of speakers who were young scientists on the cusp of great success. Many commented that it was their first major meeting invitation. It is this sort of mentoring of young people that has endeared him to a generation of well-known scientists. It was also the beginning of our decades-long friendship. Jerry is simply one of the finest people and scientists that it has ever been my privilege to know. I am honored to call him my friend.

Photo taken at a joint NSF-CNRS meeting held in Mavaleix, France in August, 1989. Clockwise from top: (the late) William Pirkle, Jerry Atwood, Andrew Hamilton, and George Gokel

Introduction

The discoveries and reports of crown ethers [1] and cryptands [2] in the 1960s engendered an explosion of new concepts [3], new structures [4], new applications [5], and

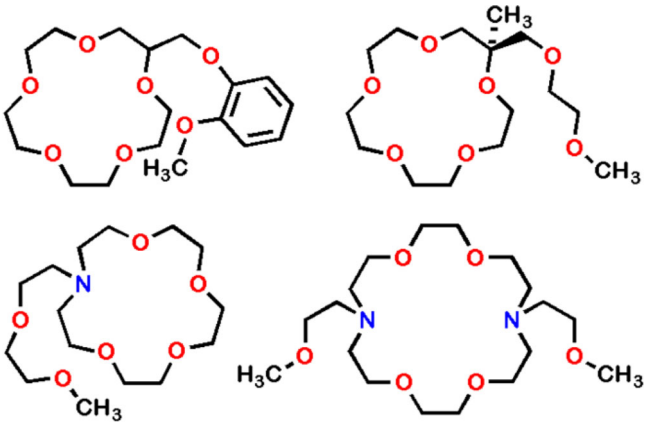

Figure 1. Top left: A carbon-pivot lariat ether. Top right: A carbon-pivot lariat ether having a geminal methyl group at the pivot atom. Bottom left: A nitrogen-pivot lariat ether. Bottom right: a bibracchial *N*-pivot lariat ether.

new chemical and biochemical insights. The diverse efforts in the areas of inclusion phenomena, host–guest chemistry, cation binding, and cation transport eventually merged into the broad designation supramolecular chemistry [6]. Today, this is a major and burgeoning area of study, which continues to grow and incorporate or merge with a range of subdisciplines.

The crown ether variant that is the subject of this review is the lariat ether class [7]. Crown ethers having flexible side arms were conceived in the late 1970s. Okahara and coworkers prepared macrocycles containing only oxygen heteroatoms in the ring and having various donor group containing side arms [8]. These were, in many respects, similar to those initially prepared in our own laboratory [9]. In both early cases, the side arms were attached at carbon (carbon-pivot lariat ethers). These compounds presented three issues that reduced their potential value. First, the carbon-to-carbon ring attachment permitted the side arm to interact with a ring-bound cation from only one side of the macrocycle. This position may also hinder access of a cation from the side arm face. This is especially problematic when more than one side arm is present. Second, when two sidearms (or possibly more) are present, obtaining a single constitutional isomer may be difficult. Third, the point of attachment is chiral. Considering the possibility of positional and stereoisomers, multiple compounds could result from the synthesis. It is worth noting that cation complexation strength in single side arm compounds was augmented by placing the side arm geminal to a methyl group. This *pseudo*(*gem*-dimethyl effect) diminished the mobility of the longer arm, leading to more favorable cation complexation. Examples of these early compounds are shown in Figure 1.

A more versatile family of compounds was developed in which the side arm(s) was attached to macroring nitrogen. The azacrown nitrogen atoms readily invert, obviating the side arm's flexibility problem presented by the all-oxygen crowns. We named these compounds lariat ethers [7]. The notion was that the macroring lassoed the cation and the side arm tied it up [10]. The analogy was certainly appropriate for those

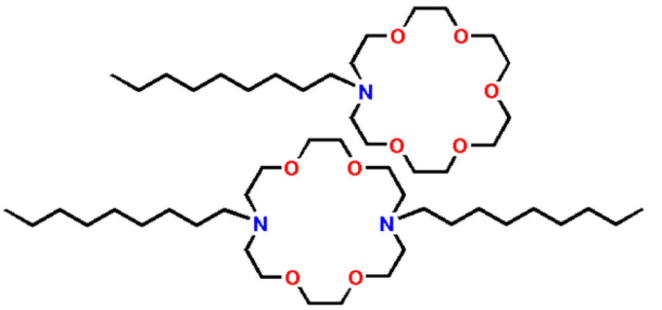

Figure 2. *N-n*-nonyl-4-aza-18-crown-6 (top) and *N,N'*-di-*n*-nonyl-4,13-diaza-18-crown-6.

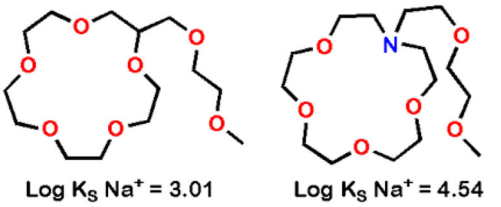

Figure 3. Comparison of sodium cation binding by side-armed *C*- and *N*-pivot lariat ethers having the same ring size and equal numbers of heteroatoms.

compounds having properly disposed donor groups on the side arms. The term lariat ether is now in general use and refers to any side-armed macrocycle whether or not there are donor groups present in the appendages. Figure 1 shows four lariat ethers. The two illustrated at the top of Figure 2 are carbon-pivot lariats. The bottom of the figure shows two nitrogen-pivot lariat ethers, one of which possesses two arms and is referred to as bibracchial (from Latin *bracchium* meaning arm).

This review is focused primarily on lariat ether compounds that have hydrocarbon side arms, although the side arm donor family will be discussed for comparison. For the most part the structures described have *n*-alkyl chains, but may incorporate aromatic or other ring structures. In most of the examples discussed herein, the compounds are in the *N*-pivot family. The ring sizes vary as do the side chain lengths. The dialkyl-substituted lariat ethers behave quite differently from their donor group containing siblings.

Results and discussion

Enhanced binding of N*-pivot lariat ethers*

As noted above, when the lariat ether's side arms were attached at nitrogen, greater flexibility/invertibility of the structure was engendered compared to attachment at carbon. In addition, binding constants were higher. For example, a two-oxygen side arm on a 15-membered ring macrocycle binds Na^+ more strongly when the scaffold contains nitrogen as the pivot atom than when attachment of the arm is at carbon. Figure 3 compares the stability constants for binding Na^+ Cl^- in methanol solution. Although there are seven heteroatoms in each compound, one contains a macroring

nitrogen pivot atom. Log K_S for Na^+ in CH_3OH for 15-crown-5 is 3.27 (1860) compared to aza-15-crown-5, for which log K_S is 1.70 (50). The conformational flexibility of the two molecules is also a critical variable. Even for short alkyl substitutions there is notable variability: *N*-methyl- and *N*-butylaza-15-crown-5 have log K_S values of 3.39 (2450) and 3.02 (1050), respectively.

Flexibility and conformation significantly influence lariat ether binding strengths. This is clear from a comparison of 15-crown-5, (2-methoxyethoxy)methyl-15-crown-5 and *N*-(2-methoxyethoxy)ethyl-4-aza-15-crown-5, the last two compounds are shown in Figure 3. The equilibrium binding constant (log K_S) for Na^+ with aza-15-crown-5 is 1.70 (∼50), while the side-chained derivative shown in Figure 3 has log K_S of 4.54 (∼35,000). A similar comparison between 15-crown-5 and the side-chained derivative shown in Figure 3 is 3.27 (1860) *versus* 3.01 (1023). Although the increase from the unsubstituted to substituted compound in both cases involves an increase in donor atoms from 5 to 7, the *C*-pivot compound is a poorer Na^+ binder than its side arm free counterpart. To our knowledge, no solid state structure of the (2-methoxyethoxy)-methyl-15-crown-5 is available. Molecular models suggest that neither side arm oxygen is in an ideal position for supplemental binding. Compared to the *N*-pivot compound, the macrocycle is also not equally accessible to an entering cation from both sides.

An interesting trend was observed with the *N*-pivot lariat ethers having side arm donor groups [11]. When the number of total heteroatoms was the same, similar binding constants were observed regardless of ring size and side arm length. Thus, the 15-membered azacrown having a two-oxygen side arm and the 18-membered ring having a single oxygen side arm had log K_S values of 4.54 and 4.58 respectively. As discussed below, there is considerably less variation in binding strengths as the lariat ether chain lengths increase.

Solid state evidence for cation envelopment

An important model for the original lariat ethers was the cyclododecadepsipeptide called valinomycin [12]. Its 36-membered ring is too large to bind any alkali metal cation, but it is selective for K^+ over Na^+ by more than three powers of ten. It binds in a so-called "tennis ball seam" arrangement. The large and enveloping ring affords both strong binding and good binding dynamics. 18-Crown-6 binds and releases cations rapidly, but its binding strength is only modest in water. [2.2.2]Cryptand is a far stronger cation binder, but its binding rate is low and its cation release rate is extremely low. It is, therefore, a poor carrier molecule. Lariat ethers combine the envelopment of a cryptand with the higher binding dynamics of crowns or valinomycin [13]. The solid state structures of the K^+ complexes of valinomycin and [2.2.2] cryptand are shown in Figure 4.

Evidence for lariat ether solution binding dynamics was obtained by Kaifer, Echegoyen, and their coworkers. Carbon-13 NMR relaxation times for both *C*- and *N*-pivot lariat ethers were measured [14]. These reflected mobilities within the various structures. Clear evidence was obtained for Na^+ complexation as indicated by reduced mobility of macroring and sidearm. Additional evidence was obtained from an

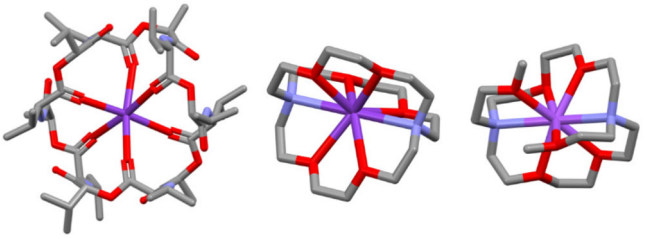

Figure 4. Solid state structures of valinomycin (left, CSD: VALINK), [2.2.2]cryptand (CSD: KCRYPT10) each complexing K^+, and *N,N'*-bis(2-methoxyethyl)-4,13-diaza-18-crown-6•NaI (right, CSD: FIRYAG). Hydrogens have been omitted for clarity.

ytterbium (Yb) NMR shift reagent study [13]. These observations were confirmed by solid state structure determinations as discussed below.

An especially interesting observation resulting from the ^{13}C-NMR relaxation time study was the difference between *N*-methyl- and *N*-butylaza-15-crown-5. As noted above, the binding constants (log K_S) in CH_3OH for *N*-methyl- and *N*-butylaza-15-crown-5 are 3.39 and 3.02, respectively. Confirmation of the greater rigidity of the *n*-butyl azacrown complex compared to the methyl complex analog was obtained by determining the ^{23}Na-NMR exchange rates. This difference is especially notable because no side arm donor group is available in either alkyl group.

Solid state structures were obtained of various lariat ethers and their cation complexes. Side arm donor groups included ester and $(ethyleneoxy)_n$ groups of various lengths. Three examples are shown in Figure 5. At the top of Figure 5, the donor group is ester carbonyl. The structures shown in the middle and bottom both contain six oxygens in one or two arms and bind KI in an enveloping complex.

Cation–pi interactions

Beginning in the mid-1970s, we made an effort to obtain evidence for cation–pi interactions by using the 4,13-diaza-18-crown-6 scaffold [15]. The side arms chosen for study were propyl, allyl, propargyl, cyanomethyl, and benzyl. The first three had side arms of the same length, but increasing unsaturation. Cyanomethyl was chosen as a propargyl isostere and control (Figure 6). The benzyl residue was simply a different type of potential pi-donor. The Na^+ and K^+ cation binding constants were measured for each ligand and increased with increased unsaturation. Initially, X-ray confirmation was lacking, so a van't Hoff analysis was used in an attempt to confirm the cation–pi interaction. The increase in binding constants reflected ΔG, but any enhanced cation–pi interaction was expected also to be detected in increased ΔH and decreased $T\Delta S$. The expected thermodynamic profile was not observed. Instead the cyanomethyl compound showed significantly different binding strengths compared to propargyl, but, within experimental error, values for ΔH were identical. Of course, $T\Delta S$ was substantially different for the two complexes as reflected in ΔG.

Eventually, solid state structures were obtained for the compounds noted above. One example is the structure shown in Figure 7. The KBF_4 complex of the propargyl derivative shows the side arms extended and not participating at all in complexation.

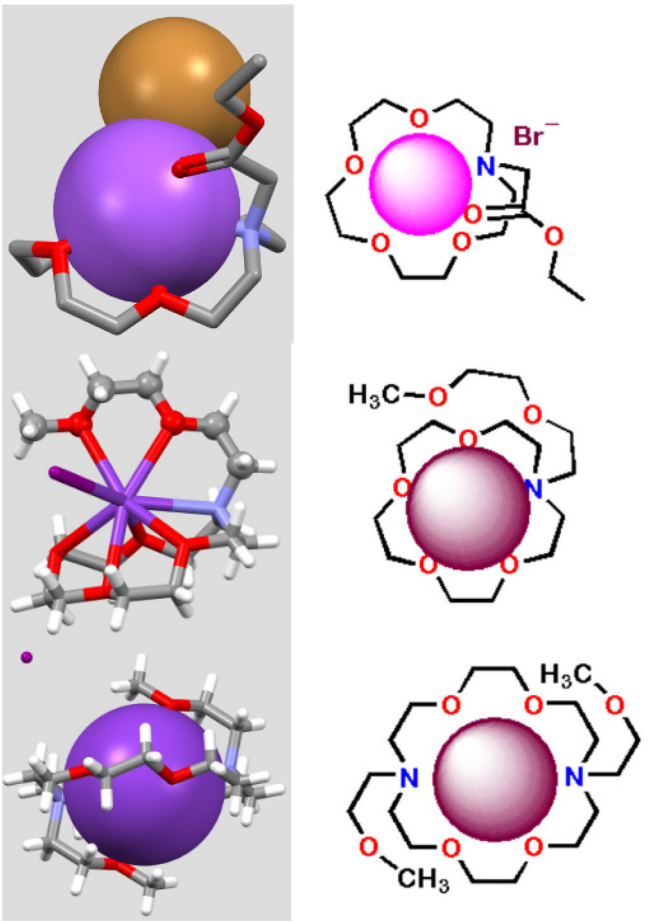

Figure 5. Solid state structures of lariat ether complexes confirming sidearm participation. The crystal structure is shown at the left and the structure is shown schematically at the right. Top: Aza-15-crown-5, $CH_2COOCH_2CH_3$ side arm; NaBr, CSD: CORNEC. Middle: Aza-15-crown-5, $CH_2CH_2OCH_2CH_2OCH_3$ side arm, KI, CSD: DUGHUI. Bottom: 4,13-diaza-18-crown-6, $CH_2CH_2OCH_3$ side arm; KI CSD: DUGHIW.

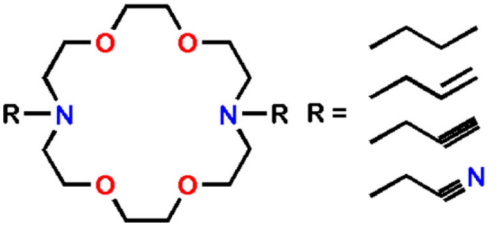

Figure 6. *N,N'*-disubstituted-diaza-18-crown-6 derivatives used to study possible cation–pi interactions.

Remarkably, the "non-nucleophilic" BF_4\bar ion serves as an apical donor. Two BF_4\bar anions interact with each complex forming an infinite chain (i.e. $^{\bullet\bullet\bullet}$H-BF_3\bar$^{\bullet}K^{+\bullet}$H-BF_3\bar$^{\bullet}K^{+\bullet}$H-BF_3\bar$^{\bullet\bullet\bullet}$).

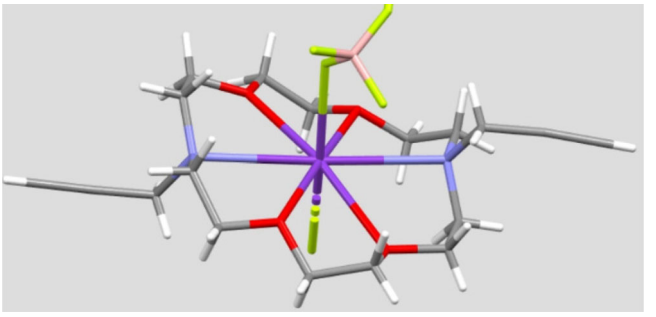

Figure 7. Solid state structure of *N,N'*-dipropargyl-4,13-diaza-18-crown-6■KBF_4.

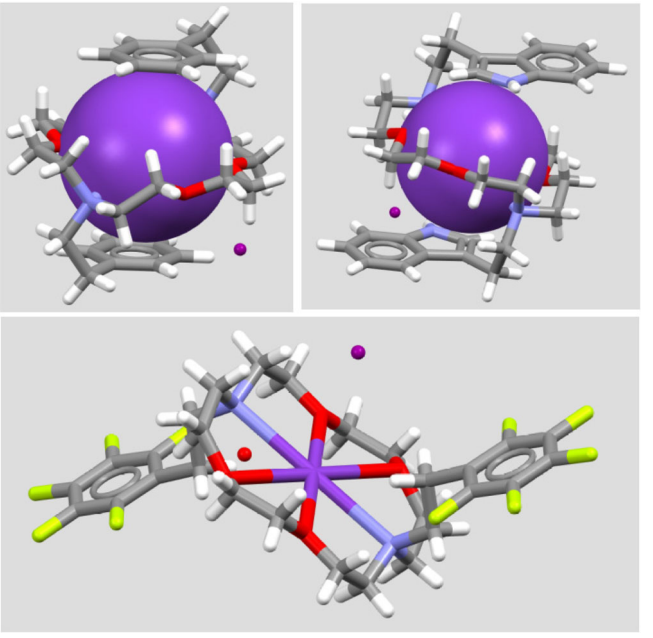

Figure 8. Three complexes of KI. Top left: Cation–pi complex of *N,N'*-di(2-phenethyl)-4,13-diaza-18-crown-6•KI. Top right: Cation–pi complex of *N,N'*-di(2-(3-indolyl))-4,13-diaza-18-crown-6•KI. Bottom: Non-pi complex of *N,N'*-di(2-pentafluorophenethyl)-4,13-diaza-18-crown-6•KI.

Some years after this failed attempt, two graduate students, now doctoral scientists, Steve DeWall and Eric Meadows, discussed the previous effort with me. They pointed out that Nature uses one more carbon in amino acids than we used in our earlier model system. In order to allay my skepticism, they prepared diaza-18-crown-6 derivatives having 2-phenylethyl-, 2-(4-hydroxyphenyl)ethyl-, and 2-(3-indolyl)ethyl side arms. These were analogs of the amino acid side chains in phenylalanine, tyrosine, and tryptophan. Dr. Len Barbour was then working with Jerry Atwood and was interested in solving the structures. Figure 8 shows a few of the many results [16] that were obtained that unequivocally confirmed the cation–pi interaction between arenes, double, and triple bonds with Na^+ and K^+. At the top of the figure are shown KI complexes having 2-phenylethyl (left) and indolylethyl side arms. Their orientation above and below the ring-bound cation is obvious. In the lower panel is shown the KI

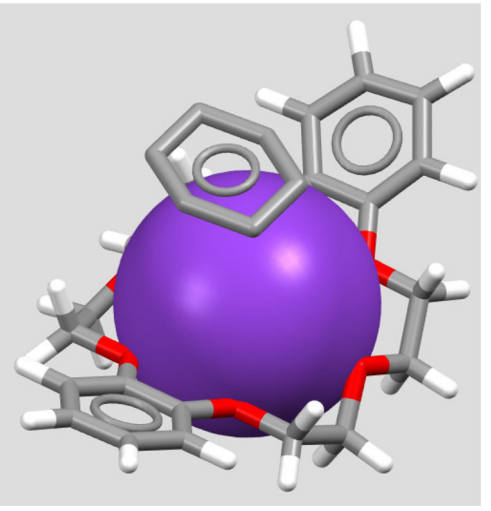

Figure 9. Solid state structure of dibenzo-18-crown-6 complexing K^+ in which the benzene participates in a cation–pi complex. The counter anion (not shown) is (μ2-dioxygen)-bis(trimethylaluminum). An additional, noncomplexed benzene is present within the lattice.

complex of the 2-pentafluorophenylethyl side arm KI complex. The electron deficient fluorine atoms prevent a cation–pi interaction. Instead, an extended chain of $^{\bullet\bullet\bullet}$I\bar$^{\bullet}$K$^{+\bullet}$I\bar$^{\bullet}$K$^{+\bullet}$I\bar$^{\bullet\bullet\bullet}$ contacts satisfies the apical positions in the complexes.

The structures of many other pi complexes were obtained with both sodium and potassium cations. These included $CH_2CH_2CH=CH_2$, $CH_2CH_2C{\equiv}CH$, and 4-HO-$C_6H_4CH_2CH_2$ attached alone or together or mixed in 15- or 18-crowns [16]. When the side arm was CH_2CH_2imidazole, a sigma complex formed in which the heterocycle coordinated to the cation through nitrogen rather than through the pi system. Efforts to obtain divalent calcium–pi complexes in this model system were unsuccessful [17].

It is important to note in this review dedicated to Professor Atwood, that he reported what to our knowledge was the earliest crown-cation-pi structure [18]. A rendering of the crystal structure (CSD: BACTUU10] is shown in Figure 9. The structure may be described as a benzene solvate of the dibenzo-18-crown-6 potassium complex, but there is no doubt that the interaction is cation–pi. The counterion is (μ2-dioxygen)-*bis*(trimethylaluminum) and a second benzene ring that does not interact with the ring-bound cation is also present in the matrix. It is also appropriate to acknowledge other work, some structural and some computational, that presaged the cation-pi structural work discussed above. Kebarle [19], Castleman [20], Dunbar [21], Meot-Ner [22], Hay [23], Petsko [24], Dougherty [25], Tilley [26], and their many coworkers all contributed to our understanding of cation–pi interactions.

Self-associating alkyl side-armed lariat ethers

Crown ethers are polar enough to function as amphiphile head groups in the presence of an appropriate hydrocarbon tail. A range of mono- and di-alkyl substituted 15- and 18-membered aza- and diazacrowns were studied. In some cases, critical micelle concentrations (CMCs) [27] were determined and in others laser light

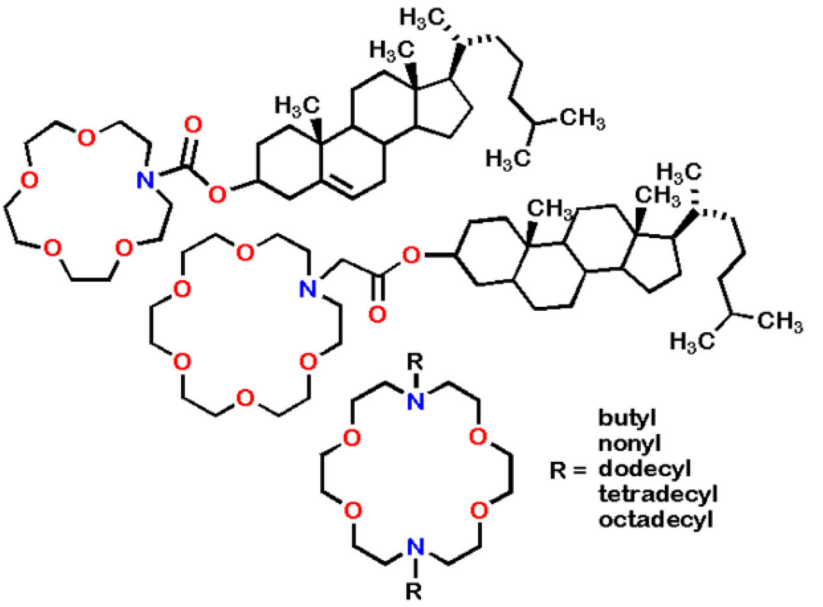

Figure 10. Examples of azacrown-based amphiphiles that form liposomes upon sonication in aqueous solution.

scattering reported aggregate sizes [28]. Examples of the compounds studied are shown in Figure 10. They include cholesteryl and cholestanyl derivatives as well as several di-*n*-alkyl-diazacrowns. The aggregation behavior of the saturated and unsaturated steroid derivatives studied showed results within experimental error of each other [29]. Similarly, the dialkyldiaza-18-crown-6 derivatives having nonyl or octadecyl side arms formed liposomes of apparent diameters (laser light scattering) of 2970 Å and 2200 Å (± experimental errors), respectively [28].

A solid state structure of the aza-15-crown-5 cholesteryl carbamate is shown in Figure 11. The cholesteryl lariat ether forms layers rather than a bilayer in the solid state. The layers are organized in alternating head-to-tail assemblies. This contrasts with the two reported structures of *N,N'*-didodecyl-4,13-diaza-18-crown-6 cation complexes [30, 31]. Both of these compounds crystallize in deeply interdigitated bilayers.

Aggregation of amphiphiles can occur in many ways. The two major assemblies of amphiphiles that form in aqueous solution are micelles and bilayers. Each of these general structural types can have variants. Fatty acid salts tend to form relatively small assemblies (100–300 monomers) [32] in which the hydrophobic tails are intertwined and the head groups may face outward and into the aqueous phase. Other monomers having diverse structures afforded different types of micelles, some that are multilayered and consist of many more monomers than do soap droplets.

One explanation for the formation of micelles rather than liposomes is that the head groups are typically larger than the single hydrocarbon tails (e.g. fatty acids). This forces the tails together in a more compact arrangement than seen with two-tailed amphiphiles. Bilayer formers such as natural phosphocholines have head groups that are more commensurate in size with the twin hydrocarbon chains. Of course,

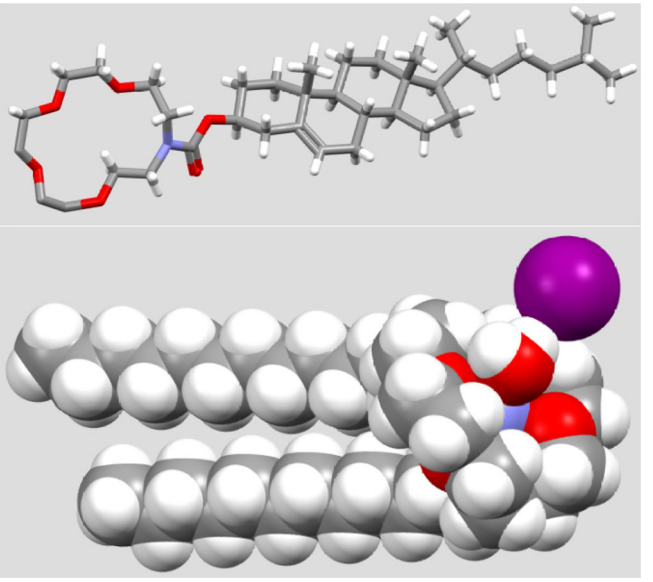

Figure 11. Solid state structures of (top) cholesteryl aza-15-crown-5 carbamate (CSD: FIXTEL) and *N,N′*-didodecyl-4,13-diaza-18-crown-6 sodium iodide complex (CSD: HUTGUY). In the latter complex, sodium cation is bound in the macroring and is solvated by a molecule of water that forms a hydrogen bond to iodide.

bilayers can form in concentric spheres (multilamellar) or as simple unilamellar liposomes. In either case, a wide range of sizes can result.

The formation of aggregates is often assessed by using laser light scattering to analyze particle size distribution. The steroidal crown analog of the compound shown in Figures 10 and 11 has the structure cholesteryl-O-CO-aza-18-crown-6. It formed aggregates of approximately 150 Å in water. When one equivalent of KCl was added, the aggregate size increased to approximately 230 Å. In the presence of 20 equivalents of KCl, the apparent aggregate size had increased to 330 ± 130 Å [27, 28].

Lariat ether bolaamphiphiles

A *bolas* is a weapon having heavy balls or weights connected by lines. It is thrown with the intent to ensnare animals. Fuhrhop coined the term "bolaamphiphile" to describe two-headed amphiphiles [33]. Compounds of this type are often abbreviated as "bolytes." As noted above, crown ethers are polar enough to function as amphiphile head groups. This capability was surveyed by preparing compounds in which two macrocycles were connected by polymethylene chains of varying lengths. Figure 12 shows the chemical structures of the families of compounds prepared in which the rings were either 15- or 18-membered azacrowns [34].

The compounds studied in the aza-15-crown-5 series had $n = 12$, 16, or 22. The dodecylene compound formed vesicles upon sonication in water, but the two longer chained analogs both formed micelles. In the aza-18-crown-6 series, $n = 10$, 12, 16, or 22. When $n = 10$ or 12, vesicles were formed, but the longer chained compounds formed micelles. 1,12-bis(aza-15-crown-5)-dodecane formed vesicles having an

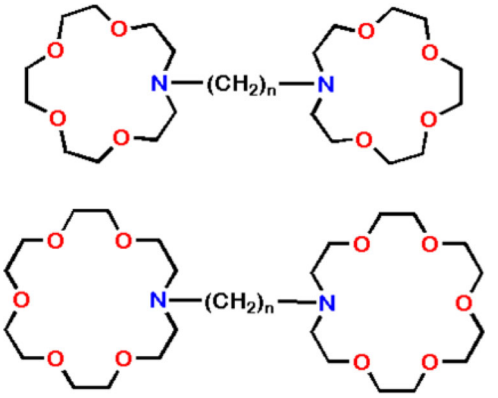

Figure 12. Bolaamphiphiles having azacrown head groups. Top: $n = 12$, 16, or 22. Bottom: $n = 10$, 12, 16, or 22.

Table 1. Complexation constants for dialkyldiaza-18-crown-6 derivatives.

	Log K_S (MeOH at 25 °C)	
Compound	NaCl	KCl
n-propyl	2.86	3.77
n-butyl	2.84	3.82
n-hexyl	2.89	3.78
n-nonyl	2.95	3.70
n-dodecyl	2.99	3.80
n-benzyl	2.68	3.38

apparent diameter of 730 Å. In contrast, the vesicles formed by 1,12-*bis*(Aza-18-crown-6)-dodecane were 1200 Å in diameter. These diameters were confirmed by using transmission electron microscopy [34]. It remains unclear why liposomes are favored by the dodecylene bolytes whereas longer and shorter chains lead to micelles. This may be the result of folding the connector chains. Even so, the ability of azacrowns to function as amphiphile head groups was confirmed.

An additional factor in the function of azacrowns as amphiphiles is the presence of nitrogen. The basic nitrogen is likely to be protonated in neutral aqueous solution, making the head groups ammonium salts. The pK_A values have been determined for both *N,N′-bis*(*n*-butyl)diaza-18-crown-6 and *N,N′-bis*(benzyl)diaza-18-crown-6. The protonation constants were as follows: dibutyl, $pK_1 = 9.40$, pK_2 7.97; dibenzyl: $pK_1 = 7.5$, $pK_2 = 6.83$ [35].

Alkyl side-armed lariat ethers

Three families of azalariat ethers have been prepared and studied. These are *N*-substituted azacrowns, *N,N′*-disubstituted diazacrowns, and *N,N′N″*-trisubstituted-4,10,16-triaza-18-crown-6. Among the three families, the *N,N′*-di-*n*-alkyl-4,13-diaza-18-crown-6 derivatives are the most investigated. For the most part, syntheses involved diacylation of the diazacrown with an acyl chloride followed by reduction of the resulting bis-amide.

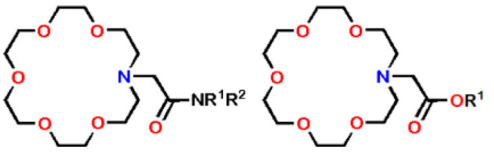

Figure 13. Aza-18-crown-6 derivatives having side chains of varied lipophilicities.

Table 2. Binding *vs*. transport in lariat ethers.

	Binding constant[a]	Transport rate
Sidearm	**Log K_S**	**Rate•10^{-7}[b]**
CH_2-CO-N(C_5H_{11})$_2$	4.61	4.62
CH_2-CO-N($C_{10}H_{21}$)$_2$	4.71	3.98
CH_2-CO-NH$C_{10}H_{21}$	3.63	0.30
CH_2-CO-O$C_{10}H_{21}$	4.48	2.40
CH_2-CO-N($C_{18}H_{37}$)$_2$	4.58	2.82
CH_2-CO-NH$C_{18}H_{37}$	3.64	0.33
CH_2-CO-O$C_{18}H_{37}$	4.61	1.44

[a]Determined in MeOH at 25 °C.
[b]In mol•h^{-1} for transport through a $CHCl_3$ bulk membrane.

In terms of aggregation, derivatives having *n*-butyl, *n*-nonyl, *n*-dodecyl, *n*-tetradecyl, and *n*-octadecyl side arms all formed liposomes [28]. The aggregates ranged in size from ≈2000 to 4000 Å as assessed by laser light scattering. As expected, each of the dialkyl lariat ethers showed cation binding behavior. Since the side arms did not incorporate any additional donor groups, cation binding was similar within the family. Selected binding data are shown in Table 1. The sodium cation binding appears to increase slightly with side arm chain length, but the dialkyl lariat ether binding values all fall within the range of experimental error: log $K_S = 2.9 \pm 0.1$.

Given the similarity of alkali metal binding constants for the dialkyldiaza-18-crown-6 compounds, it was of interest to see if ion transport correlated to side chain length. A suite of aza-18-crown-6 derivatives was prepared in which the side arms were varied in composition and length. The amides were either mono- or dialkyl substituted having *n*-pentyl, *n*-decyl, or *n*-octadecyl groups as R^1 or R^1 and R^2 (Figure 13).

Cation binding (KCl) for the compounds shown in Figure 13 and Table 2 was determined in CH_3OH at 25 °C by ion selective electrode methods. Transport of KNO_3 was conducted from one aqueous phase to a second through a bulk $CHCl_3$ membrane [36]. The secondary amides (R^1 = alkyl, R^2 = H) show low transport (Figure 14, solid line outliers). For the esters and dialkyl lariat ethers, transport rates increased with increasing side chain lipophilicity although the cation complexation strength remained nearly constant. This is apparent from the dotted line in the graph.

As noted above, the original intent of the lariat ether design was to enable enhanced complexation strength without sacrifice of the slow binding dynamics associated with cryptands. It was presumed that the lariat ethers would function as carriers as does valinomycin. A carrier complexes a cation on one side of a membrane, diffuses through it, decomplexes, and then diffuses back through the membrane. The process has been analogized to the function of a ferry boat. In contrast, a channel functions in a fashion similar to a tunnel through the membrane. The graph in Figure 14 shows that transport through a bulk liquid membrane is enhanced by increased lipophilicity. The absence of additional donor groups in the side arms means that binding of

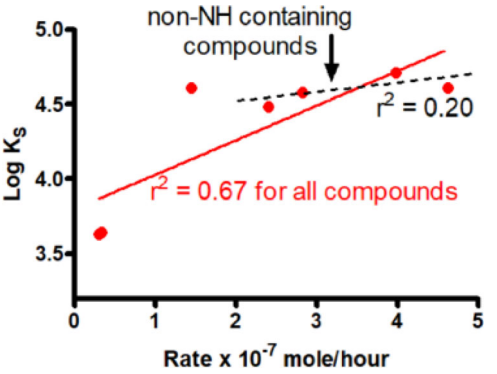

Figure 14. Table 2 and graph illustrating the correlation between lariat ether homogeneous binding constants in methanol and rates of ion transport (KNO_3) through a bulk chloroform membrane.

cations by the dialkyl lariat ethers was not significantly affected by any change in side arm length beyond the shortest chains. What eventually became apparent, however, was that the dialkyl lariat ethers did not function exclusively as carriers when the membrane was a bilayer, but formed aggregated channels instead of, or in addition to, functioning as carriers [37].

Much study of crown ether, lariat ether, and cryptand transport has been conducted in an apparatus in which two aqueous phases were separated by a solvent such as chloroform [38]. If the two water solutions were in the arms of a U-shaped tube, dense chloroform occupied the bend in the bottom of the tube and comprised the membrane. In such a system, carriers, but not channels, can function. A typical bilayer membrane is approximately 60–100 Å in overall thickness. The so-called hydrocarbon slab, which comprises the interdigitated alkyl chains, is estimated in a bilayer to be approximately 30–35 Å. A channel commensurate with this thickness can insert and transport ions or molecules. If the U-shaped bend is only 2 cm, the bulk membrane's thickness is 2×10^8 Å. It is obviously impossible for any channel to span this distance.

Early studies of lariat ether ion transport were undertaken in an apparatus similar in function to the U-tube device. Because of the huge distance – on a molecular scale – that was required to be traversed, it was impossible for the dialkyldiaza lariat ethers, whether or not side arm donors were present, to form channels in this experiment. When present in a phospholipid bilayer, however, the alkyl side chains could interact with the membrane's hydrocarbon side chains to form clusters. One might expect a stacked channel to form in which ions pass through a pore comprised of macrocycles.

When transport was studied in soybean asolectin membranes by using a planar bilayer apparatus, aggregated channels were observed [37]. Three types of pores were detected that corresponded to trimer, tetramer, and pentamer. In this case, the ions do not pass through the opening in the macrocycle, but rather through pores formed by the aggregated lariat ethers. An analogy is the barrel stave model [39] for the function of such natural channels as alamethicin [40] or melittin [41]. In retrospect, it is not unreasonable that aggregation should occur or that pore formation should follow.

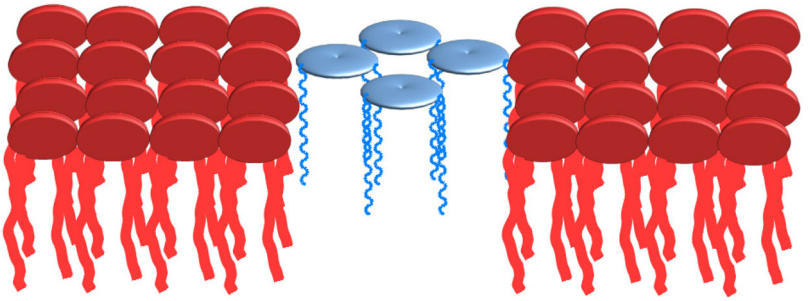

Figure 15. Schematic of intermolecular channel formation by a lariat ether tetramer within a phospholipid bilayer membrane.

After all, the known formation of micelles and liposomes by lariat ethers depends on amphiphile aggregation [42].

Several possible configurations were considered for the formation of channels. Barbiou, Fyles, and their coworkers [43] have demonstrated the formation of a stacked channel formed by a lariat ether having a single, urea-containing side arm. Channel formation in this case formed in a phospholipid bilayer, but a solid state structure suggested that the urea fostered side arm H-bonding and stacking. In the case of dialkyl lariat ethers [37], it was concluded from the planar bilayer conductance results that trimers, tetramers, and pentamers formed. Figure 15 shows a schematic representation of the upper leaflet of a phospholipid bilayer incorporating a dialkyl lariat ether tetramer. In such situations, the membrane likely condenses in thickness and phospholipid chains reorganize in the lower leaflet to continue the opening [44].

Biological activity of alkyl side-armed lariat ethers

Whether lariat ethers function as carriers or form ion channels, they conduct cations. Of course, channels are typically more efficient transporters than are carriers. If the lariat ethers insert into and conduct ions through membranes, the ion balance of a vital organism will be affected. Ion balances are closely regulated in all living systems. Disruption of the concentration gradients across the membranes is inimical to a cell's survival. Of course, crown ethers have long been known to be biologically active. Numerous studies have involved a wide range of bacteria and fungi and antimicrobial activity has been evaluated by using a range of assays [45].

A commonly used method to evaluate the toxicity to bacteria of a compound is the so-called Kirby–Bauer test [46]. Typically, a petri dish is prepared with growth media. Cellulose disks (filter paper circles) are dosed with the compounds to be tested and controls, if any, and placed on the medium. The bacterium to be tested is added to the plate. The plate is covered and the bacteria are allowed to replicate. If a compound is toxic to the microbe, a "halo" of no growth will be apparent surrounding the disk. (Figure 16) In a series of experiments, the width of the halo can be compared with those of other compounds and approximately quantitated.

A more quantitative method to assess antibacterial potency is to determine the minimum inhibitory concentration (MIC) [47]. In this experiment, suspensions of

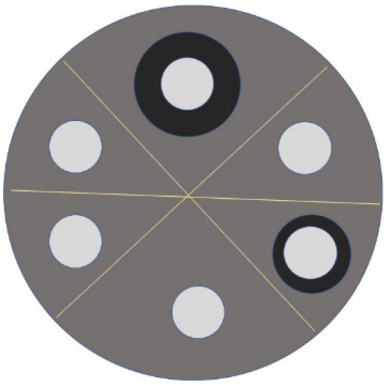

Figure 16. Typical results for a Kirby-Bauer experiment. The white circles represent cellulose disks to which various compounds have been applied. The hindrance of growth is indicated by the dark circles ("halos") surrounding active compounds. The thickness of the halo corresponds to the compound's potency. The absence of a halo indicates little or no activity.

bacteria are exposed to decreasing (usually by half) concentrations of the antimicrobial. At two adjacent concentrations, growth will be apparent at the lower concentration and not at the higher one. The higher concentration is assigned as the minimum inhibitory concentration. It may be expressed in concentration units (e.g. μM, nM, etc.) or in μg/mL. The lower the MIC, the greater the drug's potency.

The general toxicity of crown ethers was recognized by Pedersen [48], who is credited with inventing this class of compounds, and by Ts'o [49] who worked with cyclic oligomers of ethylene oxide. However, to put toxicity in perspective, Hendrixson and coworkers surveyed a number of common crowns to determine toxicity in white male mice [50]. They reported LD_{50} data [51] (in g/kg of body weight) for the "parent" compounds. Their values were 12-crown-4, 3.15 g/kg; 15-crown-5, 1.02 g/kg; and 18-crown-6, 0.70 g/kg. The average weight of a man in the United States is currently 195 lb and of a woman it is 165 lb. This gives an average value for a human of 180 lb, or 81.8 kg. At 0.70 g/kg the lethal dose for 50% of average humans would be 57.7 g. For comparison, we note that LD_{50} for aspirin to mice is 1.1 g/kg. For a mouse weighing 81.8 kg (not known so far), the amount of aspirin required to reach LD_{50} would be 90 g or 277×325 mg pills. Were the toxic dose 0.70 g/kg, it would still require 176 aspirin pills to kill the enormous mouse. A further comparison is widely prescribed alprazolam (Xanax®), for which LD_{50} is a little over 300 mg/kg of body weight in rats.

Okahara, Kato, and their coworkers [52], who had prepared numerous early examples of carbon-pivot and some nitrogen-pivot lariat ethers studied several alkyl-substituted crown ethers and *N*-alkyl substituted azacrowns. The intent was to establish antimicrobial potency, if any. Rather than depicting the numerous crowns studied, a shorthand was devised to represent crown structures as text. In this chemical shorthand, the ring size of a simple macrocycle is represented in angle brackets. A simple, cyclic ethyleneoxy compound such as 18-crown-6 would be represented as <18>. Aza-15-crown-5 is written as H<N15>. *N,N'*-Dibutyl-4,13-diaza-18-crown-6 is simplified to C_4<N18N>C_4. Of course, the system fails for more complicated structures, but it is applicable for many structures discussed herein.

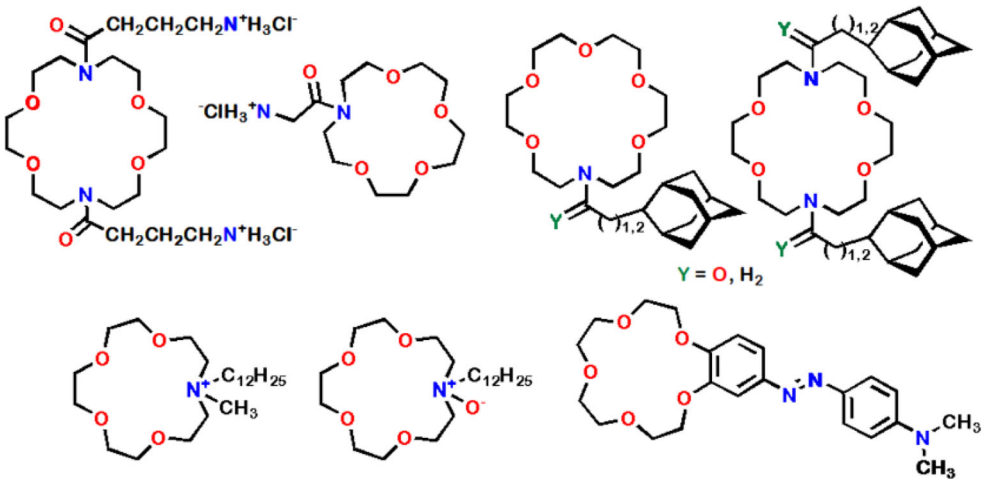

Figure 17. Side-armed macrocycles studied for physiologic effects.

Among the compounds that Okahara et al. studied were *n*-dodecyl-15-crown-5 ($<15>C_{12}H_{25}$) and $<18>C_{10}$. He observed the greatest potency against three Gram positive bacteria: *Bacillus subtilis, Bacillus cereus*, and *Staphylococcus aureus*. The MICs for these two compounds were both in the modest range of approximately 40 μM. Ideally, one seeks potencies in the low micromolar to nanomolar range, especially for Gram positive organisms, which are generally more susceptible to antibiotics than are Gram negative microbes.

In a study conducted by Kato [53], $<15>C_{10}$, $<18>C_{12}$, $<15N>C_{10}$, $<18N>C_{12}$, $<12>CH_2OC_{10}$, and $<12>CH_2OC_{12}$ showed a lag time in the normal growth curve observed for *B. subtilis*. The lag stage was longest within this group for *N*-*n*-alkyl substituted azacrowns. Tso et al. [54] also reported this lag phenomenon in a study of Gram negative *Escherichia coli* when either dicyclohexano-18-crown-6 or 18-crown-6 was present in the growth medium. In further studies, the presence of NaCl or KCl altered the growth curves, but the authors were not able to establish any clear pattern. As a result, they cited "interacting inhibitory factors," which seems to be a reasonable conclusion for such complex systems.

Several side-armed macrocycles have been prepared to study toxic or physiologic effects on mice or other mammals, but not strictly for antimicrobial function. The compounds shown in Figure 17 are some of the most potent. In general, these compounds have activities other than antimicrobial potencies such as effects on cellular processes, at least as reported.

Our initial effort involved *N,N'*-dialkyl-4,13-diaza-18-crown-6 derivatives in which the two side arm alkyl groups were identical and comprised octyl, decyl, dodecyl, tetradecyl, hexadecyl, and octadecyl [55]. Their biological activity was assayed against *Escherichia coli* (Gram-, DH5α), *Bacillus subtilis* (Gram+), and the fungus (yeast) *Saccharomyces cerevisiae*. The graph of Figure 18 shows the side arm length dependence of the toxicity in this family of structures. It is notable that when the side arms are 14–18 carbon atoms in length, no activity was seen against any of the three organisms. It is also notable that there is such a stark difference in activities that

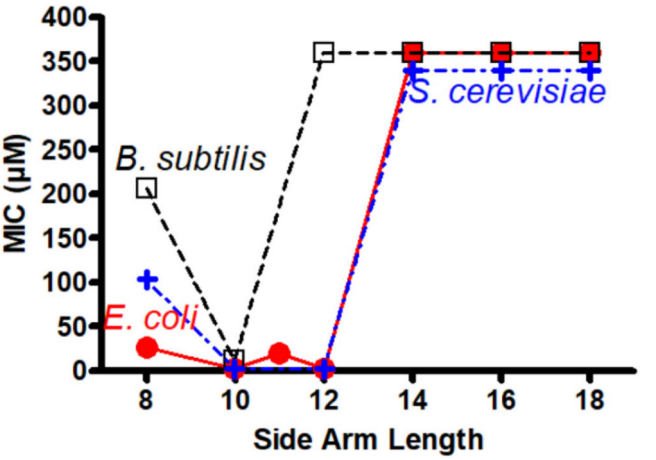

Figure 18. Graph showing the minimum inhibitory concentration (MIC) for toxicity against *E. coli*, *B. subtilis*, and *S. cerevisiae*. The lower the MIC, the more potent the antimicrobial effect. The top values are all >360 μM, but the *S. cerevisiae* data are offset slightly for clarity.

converge on the didecyl compound, although the didodecyl lariat is potent against both *B. subtilis* and *S. cerevisiae*.

It was surmised that the potency of these lariat ethers was the result of ion transport. The concentrations of ions are closely regulated in bacteria and disruption of the balance will affect any ion-dependent process. The effect of ion deregulation (disruption of ion homeostasis) is expected to be greater for channels than for carriers. The apparent discontinuity in biological activity between C_{10}<N18N>C_{10} and C_{12}<N18N>C_{12} is unclear, however, whether the carrier or the channel model is applied.

However ion homeostasis is disrupted by ion transport, it will affect any metabolic process that is dependent on ions. An example is efflux pump function [56]. Efflux pump proteins are used by bacteria to remove exogenous materials from the cytosol. Efflux pumps specific to an antibiotic can account for resistance development. In order to test this theory, we transformed a competent JM109 *E. coli* cell line and produced an *E. coli* that we call tet^R containing the Tet A efflux pump [37]. Its MIC against *E. coli* increased from the typical MIC of about 12 μM for laboratory strains to >900 μM. We treated this organism with hydraphiles of the form $PhCH_2$<N18N>C_n<N18N>C_n<N18N>CH_2Ph [57]. We abbreviate these hydraphiles as BC_nH, where "B" stands for benzyl and the carbon number is designated by "*n*." When $n = 12$, the hydraphile is 10-fold more potent as an efflux pump inhibitor than reserpine, the standard control compound. When $n = 14$ in the hydraphile structure shown, it is even more potent than $n = 12$ against the same model organism: *Staphylococcus aureus 1199B* [58].

The di-*n*-alkyl lariat ethers show antimicrobial potency when they are used as drugs *per se* [55]. The data summarized in the graph of Figure 18 illustrate the potency of LEs against the DH5α strain of *E. coli*. This strain is a non-pathogenic ("laboratory") strain. A more robust laboratory strain of *E. coli* is K12, against which the LEs are less potent. The MIC values for the di-*n*-alkyl diaza-18-crown-6 molecules against K12 in

μM are as follows: C_6LE, >512; C_8LE, 300; C_{10}LE, 12; and C_{11}LE, 24. Notwithstanding the differences, the trend is the same.

Lariat ethers in combination with other antimicrobials

The concept of antimicrobial combination therapy is well established in modern medicine. A very successful example is the product called Augmentin®. It is a combination of amoxicillin and potassium clavulanate, a β-lactamase inhibitor [59]. A major resistance mechanism for the penicillin family of antibiotics is cleavage of the β-lactam ring. Clavulanate inhibits this enzyme, allowing amoxicillin to realize its full potency. Other products include Neosporin ointment, a mixture of three antibiotics: zinc bacitracin, neomycin sulfate, and polymyxin B sulfate. A mixture of three antibiotics enhances the chance of one or two – perhaps all three – showing potency against the offending microbe. The mechanism of action is not like that in Augmentin® which uses an additive to prevent destruction of the active substance. Any mechanism by which two or three of the drugs in Neosporin interact to enhance potency is less clear.

As noted above, di-*n*-alkyl lariat ethers show antimicrobial effects against a number of different microbes. The formation of ion channels by the di-*n*-alkyl lariat ethers in phospholipid bilayers [37] suggests that they might do the same within microbial membranes. If so, their presence within a bacterial boundary layer would likely lead to disrupted ion regulation. This in turn, would lead to altered function of any enzymatic or other process that is regulated by ion balance. Efflux pump function is one process that might be disrupted. If, so the ability of the affected organism to efflux (remove) an antibiotic or other exogenous material [56]. If less antibiotic is expelled, more remains and potency is enhanced.

Four graphs are shown in Figure 19. The top two show the effect on DH5α *E. coli* of combinations of lariat ethers and tetracycline. The bottom two graphs refer to the same lariat ethers and organism with rifampicin. In each case, the ordinate shows the MIC in μM. The highest filled circle shows the MIC for the drug. In each case, the abscissa shows the MIC of the lariat ether on the far right. A simple combination effect resulting from equally contributed potencies should produce a straight line connecting the two individual MIC values. There is clearly a synergistic effect in all four cases. We note that evidence has been obtained of membrane activity for both C_8LE and C_{11}LE.

In order to evaluate the effect of lariat ethers on bacteria, we used the transformed tetracycline resistant *E. coli* strain mentioned above that we call tet^R. Its MIC against tetracycline is 900 μM. This poor susceptibility to tetracycline compares to a MIC value for tetracycline against normal *E. coli* of 12 μM and for minocycline of 18 μM. The two antimicrobials are quite similar in structure as shown in Figure 20. The MIC of 18 μM for minocycline is an expected value and compares with a MIC of 12 μM for tetracycline against DH5α *E. coli*.

Table 3 shows results for treatment of tet^R *E. coli* with various combinations of tetracycline and lariat ether. The MIC of each lariat ether as a drug *per se* against tet^R *E. coli* is shown in the second column. The amount of each LE used in the combination experiment is specified in the third column. The fourth column shows the

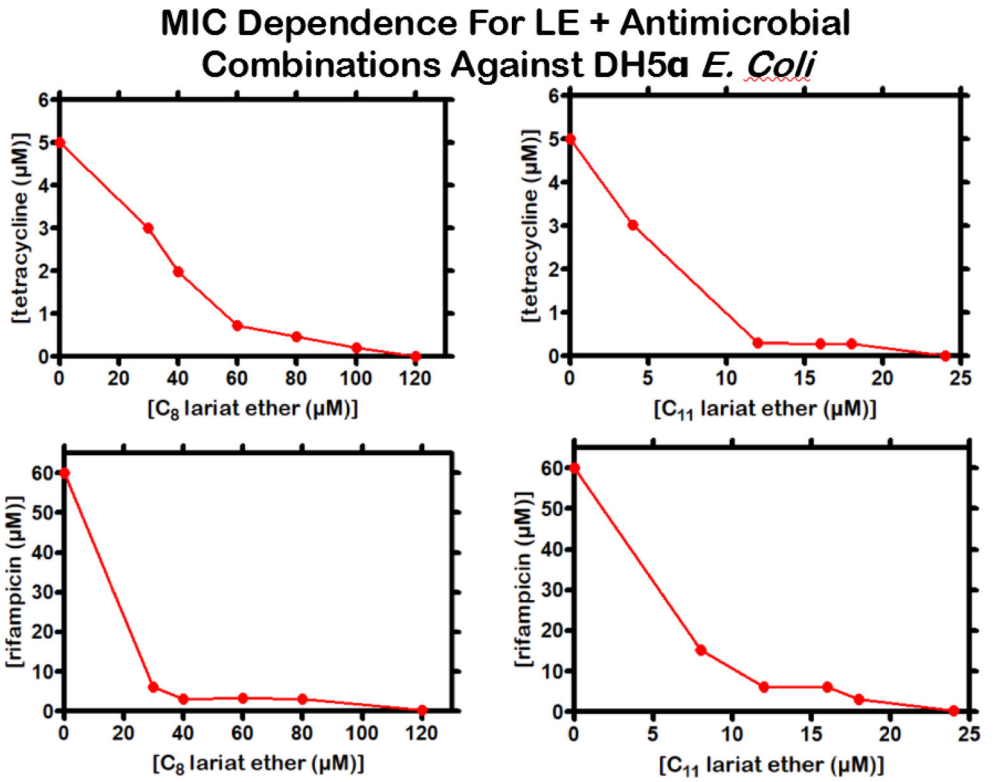

Figure 19. Synergy between lariat ethers and antimicrobials.

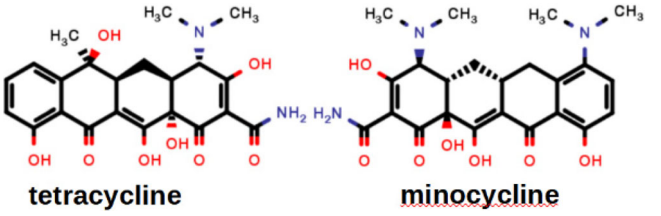

Figure 20. The structures of tetracycline and minocycline.

apparent MIC of the combination against the organism. The right-hand column shows the enhancement. For example, the MIC for C_{11}LE against tet^R is 24 μM. In the third line from the bottom of the table, 12 μM of the LE is used. This is half the MIC, although other fractions are clearly used in other entries. When C_{11}LE is used at $^1/_2$ MIC against tet^R, it is impotent. However, when present with tet^R, instead of 900 μM tetracycline being required, only a concentration of 87 μM is required to prevent growth. At a MIC for the combination of 87 μM, the potency of tetracycline has been enhanced by 900/87 = 10 (fold). As expected, and confirmed in the table, LEs having different alkyl chain lengths show different efficacies.

We attribute the potency mediated by the presence of lariat ethers to disruption of membrane integrity, and thus, of ion balance, often called ion homeostasis, within the organism. This, in turn, should disrupt efflux pump function. As noted above, evidence

Table 3. Fold recovery of tetracycline potency against tet^R *E. coli*.

Cpd.[a]	MIC (μM)	Used (μM)	Tetracycline[b] MIC used (μM)	Fold enhance-ment
C_6LE	>512	192	413	2
C_8LE	120	80	87	10
C_8LE	120	60	175	5
C_8LE	120	40	233	4
C_{10}LE	16	6	225	4
C_{10}LE	16	9	56	16
C_{11}LE	24	18	87	10
C_{11}LE	24	16	87	10
C_{11}LE	24	12	87	10
C_{11}LE	24	8	175	5
C_{12}LE	>512	192	450	2

[a]C_6LE refers to C_6<N18N>C_6, etc.
[b]*E. coli* used is tet^R, a transformed bacterium incorporating the Tet A efflux pump, MIC = 900 μM against tetracycline.

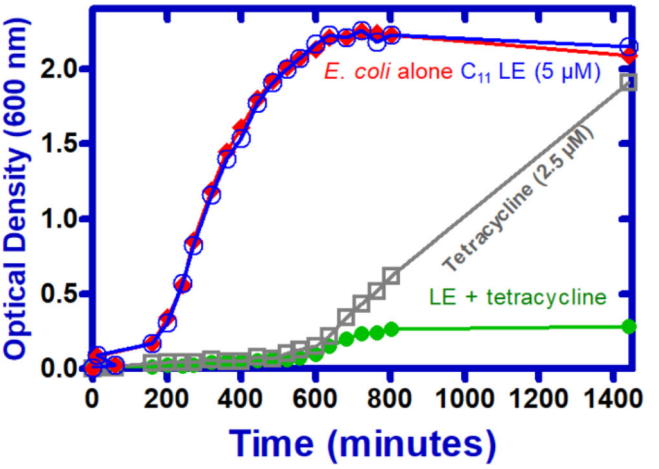

Figure 21. Growth curves for *E. coli* DH5α in the absence and presence of lariat ethers and tetracycline. The LE + tetracycline combination includes 5 μM and 2.5 μM of each, respectively.

for ion channel formation had been confirmed for LEs in planar phospholipid bilayers and bacterial membranes are far more complex. Even so, the LEs are carriers and can penetrate membranes and disrupt ion balance within a microbe, even if the disruption is less than would be engendered by channel formation. It should be noted that a recent report has suggested that the "biological activities of these lariat ethers are due to their membrane lytic activity, as opposed to the expected ion transport activity" [60].

A question related to the combination of lariat ethers and antimicrobials concerned whether or not the growth of the organism was affected by sub-MIC concentrations of the ionophore. The growth of *E. coli* was observed during nearly 24 h. At 5 μM, C_{11}LE showed growth that was indistinguishable from that of *E. coli* alone. When a low concentration of tetracycline (2.5 μM) was present in the growth media, there was an extended lag phase, but growth recovered almost to the level of *E. coli* alone. When both C_{11}LE (5 μM) and tetracycline (2.5 μM) were added to the growth media, bacterial growth was dramatically inhibited. The results of these experiments are

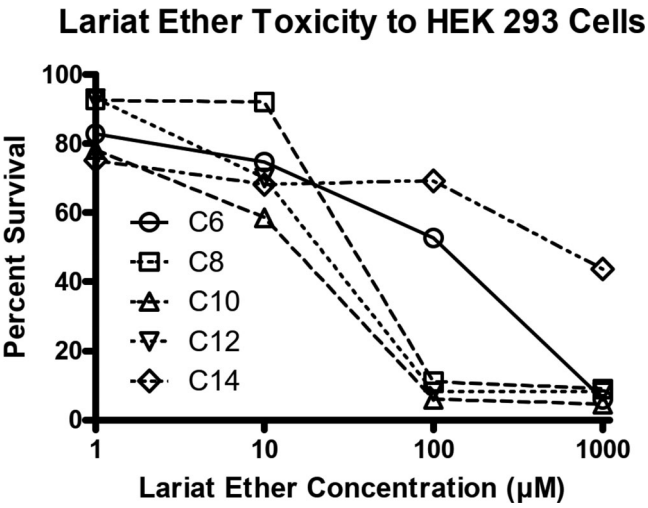

Figure 22. Graph showing the survival of human embryonic kidney (HEK-293) cells after exposure to di-*n*-alkyl-diaza-18-crown-6. The digit following the letter C indicates the number of carbons present in the linear alkyl chains.

shown graphically in Figure 21. Each data point represents the average of three separate experiments. Error bars are not shown to enhance clarity in the overlapping lines.

Whether LEs are used as drugs *per se* or in concert with an antimicrobial, the question of toxicity looms. Data for the toxicity of a series of di-*n*-alkyl diaza-18-crown-6 to human embryonic kidney cells are shown in the graph of Figure 22. The abscissa in the figure is logarithmic. The most toxic of the compounds is C_{10}LE, which is also the most active against bacteria and the most active as an adjuvant. When it is used as an adjuvant, it is administered at $^1/_2$ MIC, making the mammalian toxicity less of an issue. It is interesting that C_6LE and C_{14}LE, the compounds having the longest and the shortest alkyl chains, are the least toxic having concentrations of 100 μM or more.

Conclusions

The original design concept for the lariat ethers was that side arms having donor groups would make the crowns stronger alkali metal cation binders. It was anticipated that the side arms would encapsulate the ring-bound cation to mimic the envelopment known for cryptands. Because the side arms were not fixed in place, it was further anticipated that the dynamics of cation complexation would be greater than for cryptands. A range of experiments including dynamic NMR and X-ray structure determination confirmed these hypotheses. The confirmation of these concepts has permitted the side-armed crown ethers to be used as model systems to demonstrate, for example, cation–pi interactions.

When side arms were present, but donor groups were absent, the lariat ethers behaved differently. Such compounds as *N*,*N*′-di-undecyl-4,13-diaza-18-crown-6 formed aggregated ion-conducting channels in phospholipid membranes. Interestingly, it did not appear that the cations passed through the macrocycle, but rather aggregates of the lariat ethers that formed what might be called a “barrel-stave” pore. Of course, the

lariat ethers could still function as carriers, but the lack of side arm donor groups reduces their complexation strengths. To the extent that lariat ethers were present in membranes – synthetic or natural – they affect membrane permeability, ion transport, and ion balance.

Any alteration in membrane structure or channel formation caused by the presence of lariat ethers or hydraphiles alters ion balance. One effect of these compounds is to enhance the permeability of the membranes in which they insert. To the extent they alter membrane order or integrity, they compromise ion balance. This, in turn, adversely affects the function of enzymes that depend on ion regulation. It is the potential of the lariat ethers both as drugs *per se* and as adjuvants that is currently under vigorous investigation.

Disclosure statement

Several of the authors have a financial interest in a company that may benefit from some of the science recorded herein, all of which has previously been publicly disclosed.

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