Chemical Switching of Vesicle Bilayer Membrane Disruption by Bis(crown ether) Bolaamphiphiles

T. M. Fyles* and B. Zeng

Department of Chemistry, University of Victoria, Victoria, B.C. Canada V8W 3V6

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Bis(crown ether) bolaamphiphiles derived from 18-crown-6 dicarboxylic acid were prepared, and their ability to release vesicle encapsulated 5[6]-carboxyfluorescein was determined. Bolaamphiphiles with a linear central spacer are poor membrane disrupting agents except in the presence of alkaline earth metal cations or ethylenediammonium cation. Divalent ion enhancement of membrane disruption is cation selective and can be used to determine the apparent association constant of the bolaamphiphile crown ether with the added cation. The cis-isomer of a thioindigo bis(crown ether) bolaamphiphile is an active membrane disrupting agent, but the trans-isomer is significantly less active. In homogeneous solution, cis-to-trans thermal and photochemical isomerization is retarded by added alkaline earth metal salts, indicating that cooperative ditopic binding of the cations occurs despite the inherent flexibility of the bolaamphiphile. The membrane disruption mechanism occurs via U-shaped conformations of the bolaamphiphile. All available data indicate that divalent cations accelerate membrane disruption by stabilization of the U-shaped conformation via cooperative interaction of the crown ethers with the dication. Thus the membrane disruption process is switched "on" by molecular recognition.

Semiochemistry is the chemistry of the generation, propagation, conversion, and detection of molecular signals.[1] Much of the current effort to develop chemical model systems which illustrate semiochemical principles is focused on switching phenomena, typically involving photochemical or redox processes.[2] Less attention has been paid to direct molecular or ionic switching, whereby a molecular recognition event controls another supramolecular process.[3] The potential of ionic switching is amply illustrated by the stunning array of biological signaling phenomena, from "simple" receptor-mediated cellular processes to the complex neurophysiological processes of thought and memory.[4] These processes are intimately associated with membranes: ionic signals are propagated along membranes, are transduced through membranes, and are amplified by release of vesicle encapsulated (ionic) signal molecules. Model systems which explore these phenomena must therefore be membrane-based and must be switched by the recognition of molecular signals near the membrane surface.

Interfacial molecular recognition has been demonstrated for a variety of systems (guanidinium−phosphate,[5a] melamine−barbiturate,[5b,c] other hydrogen bonding recognition[5d]) at a variety of interfaces (air−water,[5e] supported monolayer−water,[5f] vesicle bilayer[5g]). The selectivities and energetics of the molecular recognition are generally those expected from related recognition processes in homogeneous solutions.[5] However, the organization and structure of the interface imposed by the intermolecular organization of the amphiphiles can also play a role. In some cases this effect is reciprocal (binding influences organization[5d]) implying that the interfacial environment can be used to couple molecular recognition to larger scale supramolecular phenomena.

A significant goal in this area is to control bilayer membrane transport via ion channels using a molecular signal to switch from an inactive to an active state. We have investigated the design and characterization of candidate channels and have a mechanistic framework in which to evaluate switching.[6] This report examines a candidate switch based on molecular recognition in the absence of a defined ion channel.[7] In place of the channel we substitute the membrane-disruption process first defined by Regen.[8] This will permit the scope and

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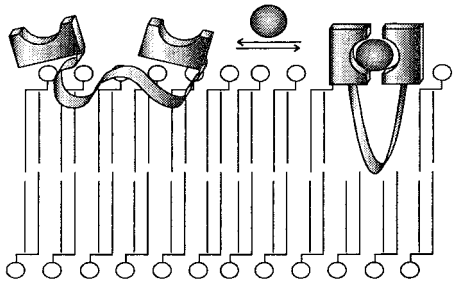
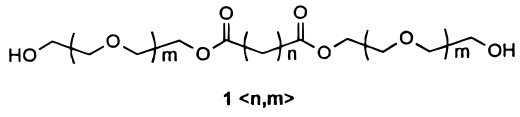
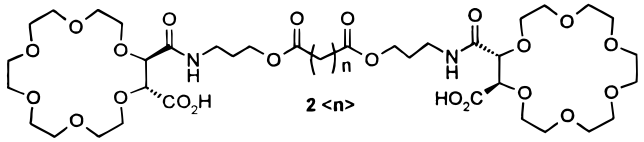


Figure 1. Schematic proposal for molecular recognition based membrane disruption. Binding of the substrate by a flexible ditopic receptor forms an active U-shaped segment within the bilayer membrane.

limitations of switching strategies to be evaluated in a simpler experimental context than a bilayer transport experiment. The membrane disruption process described by Regen is provoked by the formation of "hairpin" or "U-shaped" conformations of simple bolaamphiphiles such as **1**⟨*n*,*m*⟩ in a vesicle bilayer membrane. The depth



1 <n,m>

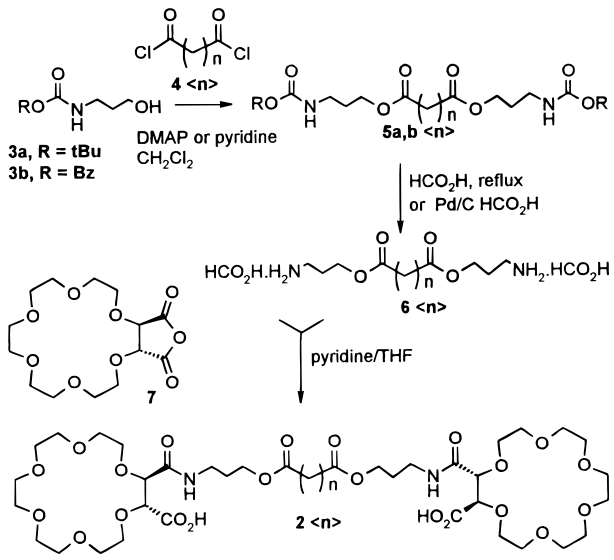


2 <n>

of penetration and the width of the U-shaped conformation controls the effectiveness of the membrane disruption.[8] In this context a bolaamphiphile ditopic receptor could provide a link between the molecular recognition of a substrate and the resultant conformation of the tethering segment. As illustrated in Figure 1, the cooperative binding of a substrate will bring the two headgroups into proximity, with the resultant deeper and narrower penetration of the U-shaped segment. Membrane disruption would therefore be enhanced by the recognition of the substrate and the system would be "switched" by the recognition process.[9]

The specific bolaamphiphiles chosen (**2**⟨*n*⟩) draw on the known complexing capabilities of carboxylate crown ethers[10] as both mono- and ditopic receptors. The ionic signal would be an alkaline earth metal cation capable of forming a neutral bis(carboxylate) complex. The selection of a divalent ion as the ionic signal is based on the following precedents: (1) The monoamides of crown ether dicarboxylic acids readily form 2:1 complexes with alkaline earth metal cations,[11a] and bis(crown ether) carboxylates form stable 1:1 complexes.[11b] (2) The complexes of alkaline earth metal cations with carboxylate crown ethers are substantially more stable than the

Scheme 1



competing alkali metal cation complexes as a result of greater electrostatic interaction with the higher charge density divalent ions.[10,11] (3) The electrostatic effect is enhanced in an interfacial environment where a favorable anionic surface potential can enrich divalent cations relative to a large excess of monovalent cations.[12] The divalent ion signal could therefore be expected to trigger membrane disruption in the presence of a large excess of competing monovalent cations. The divalent cation selectivity of the switch is expected to follow the selectivity of the parent carboxylate crown ethers on the basis of a balance of electrostatic and cation size effects. Either $Ba^{2+} > Sr^{2+} > Ca^{2+}$, or $Sr^{2+} > Ba^{2+} > Ca^{2+}$ would be consistent with previous examples.[10,11]

The membrane disruption assay involves release of encapsulated self-quenched carboxyfluorescein resulting in the enhancement of a fluorescence signal;[8,13] thus, the system envisaged would also demonstrate signal amplification and transduction. Extensions to applications in controlled release and drug delivery are implicit, but these other aspects are peripheral to the main purpose of this study, namely the synthesis and characterization of a molecular-recognition-based switch.

Results and Discussion

Synthesis. The synthesis of the bis(crown ether) bolaamphiphiles **2**⟨*n*⟩ follows simply from the structures as given in Scheme 1. The amino group of 3-amino-1-propanol was protected as the Boc[14a] or Cbz[14b] derivative (**3a,b**) and then coupled with long-chain diacid chlorides ($n = 14, 18, 20$) to afford the diesters **5a,b**⟨*n*⟩ in acceptable yields. The protecting groups were removed either by direct acid cleavage (Boc) or by catalytic transfer hydrogenolysis[15] (Cbz) in formic acid to give the stable formate salts **6**⟨*n*⟩. In basic solution, the free amines undergo self-

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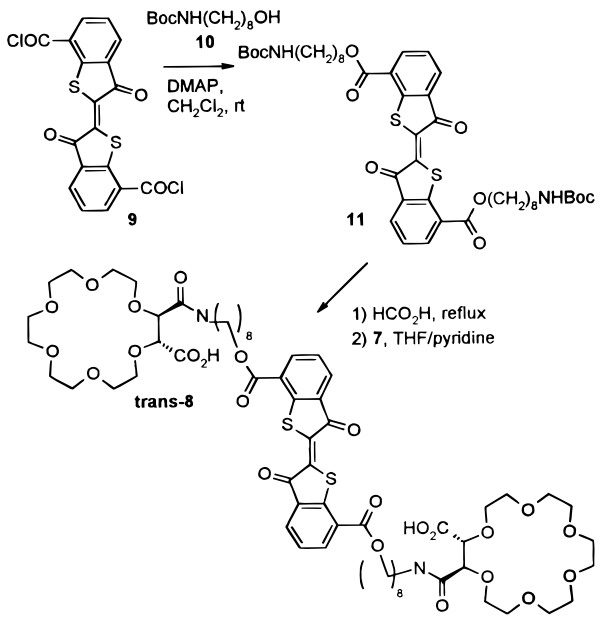
Scheme 2



aminolysis of the esters, but this process is slower than reaction with the crown ether anhydride **7**.[16] The desired bis(crown ethers) **2**⟨*n*⟩ (*n* = 14, 18, 20) were purified by gel permeation chromatography and gave the expected analytical and spectroscopic results.

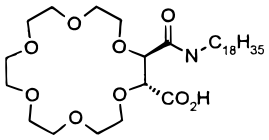
For reasons outlined in more detail below, we also prepared the thioindigo bis(crown ether) **8** by a directly analogous route (Scheme 2). The Boc-protected 8-amino-1-octanol[17] (**10**) reacted with 7,7′-thioindigo dicarboxylic acid chloride[18] (**9**) to afford the diester **11**. The protecting groups were removed in formic acid, and the corresponding diamine in pyridine was coupled with the anhydride **7** to give the desired thioindigo bis(crown ether) **8**. The isolation and purification of **8** by gel permeation and its characterization followed the same methods as for **2**⟨*n*⟩. The photophysical and photochemical characterization of **8** is discussed below.

Membrane Disruption. The membrane disruption assay is based on the release of vesicle-encapsulated 5[6]-carboxyfluorescien (CF).[8,13] Initially, the CF at a high concentration in the vesicle interior is self-quenched. Upon formation of a large enough defect, the CF can escape from the vesicle to be diluted in the external medium. Self-quenching no longer occurs, and the fluorescence of the mixture increases. Vesicles were prepared in 0.1 M CF (either the sodium or potassium salts), from the mixed lipid egg phosphatidyl choline, phosphatidic acid, cholesterol (egg PC, PA, chol; 8:1:1) using a sonication−freeze−thaw method.[19] The crude vesicle preparation was sized through 0.45 mm Nucleopore filters, and free CF was removed by gel permeation on Sephadex G-25 using NaCl or KCl (0.14 M) containing tris-HCl buffer (10 mM, pH 7.5) as eluent. As prepared, the vesicle solution was stable for some days; on a daily basis the minor amounts of external CF were removed by gel permeation chromatography as for the freshly prepared solution. In the membrane disruption assay, an aliquot of the vesicle solution was incubated with a known amount of amphiphile for 30 min at 23−25 °C, the mixture was diluted with additional KCl or NaCl tris-HCl buffer, and the fluorescence intensity at 515 nm (475 nm excitation) was recorded. The extent of CF release was calculated from

$$\% \text{ release} = (I_{obs} - I_{min})/(I_{max} - I_{min}) \qquad (1)$$

where I is the fluorescence intensity and the subscripts denote the values for the sample (obs), for a blank with no added amphiphile (min), and for a control sample fully disrupted by a high concentration of Triton X-100 (max).[8]

Figure 2 gives examples of the extent of membrane disruption (% release of CF) as a function of amphiphile concentration for some bolaamphiphiles, the detergent Triton X-100, and a control carboxylate crown ether 12.[16] The functional form follows a typical dose−response sigmoid curve, so the data were fitted to a 4-parameter logistic equation (minimum, maximum, inflection point, slope). The concentration at the inflection point of the sigmoid is a measure of the concentration effectiveness of amphiphile for membrane disruption. Not all dose−response curves achieve the maximum release of CF; therefore, Table 1 gives both the maximum release values and the concentration at the inflection point for the systems investigated as a general guide to the range of activities observed.



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Several points emerge from the data. In cases where the maximum of the dose−response is 100%, the concentration at the inflection point can be directly converted to the R_{50} formalism previously used by Regen[8] by division into the lipid concentration (0.8 mM). For cases where comparisons are possible, our values of R_{50} are generally higher than those reported by Regen, implying that our vesicle system is somewhat more susceptible to small amounts of membrane disrupting agents. This might reflect inherently lower vesicle stability in our case or might reflect more favorable partition of the amphiphiles to our mixed-lipid vesicle system. Table 1 also shows that vesicles in NaCl solution are less prone to disruption than those KCl medium. These are minor effects relative to the major differences between different amphiphiles.

Both **2**⟨14⟩ and **2**⟨18⟩ are rather poor membrane disrupting agents, and even at high concentrations only about 25% of the CF is released in 0.14 M KCl/vesicle solutions. However, addition of 1 mM $BaCl_2$ to the medium provokes a substantial increase in the extent of disruption (Figure 2B). The same increase with added $BaCl_2$ occurs with **2**⟨14⟩. The concentrations at the inflection points of the dose−response curves show **2**⟨18⟩ is about 1 order of magnitude more effective than **2**⟨14⟩.

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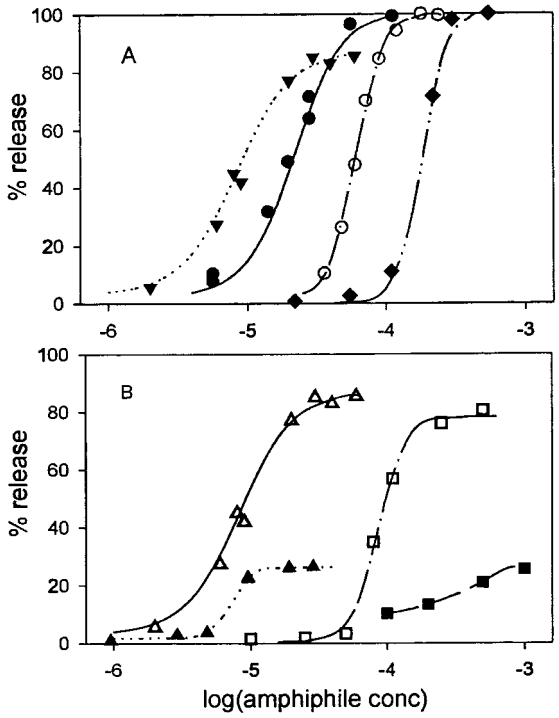

Figure 2. Release of vesicle-encapsulated CF (%) as a function of amphiphile concentration: (A) Triton X-100 (◆), **12** (○), **1**⟨18,5⟩ (●), and *cis*-**8** (▼) in KCl/KCF medium; (B) release in the absence (filled symbols) and presence (open symbols) of 1 mM added $BaCl_2$ of **2**⟨14⟩ (■, □) and **2**⟨18⟩ (▲, △).

Table 1. Activity of Membrane Disrupting Amphiphiles[a]

amphiphile	inflection concn (μM)	max disruption (%)
1⟨18,5⟩	22	100
1⟨18,5⟩[b]	42	99
2⟨14⟩	>300	25
2⟨14⟩[b]	≫1700	(4)[c]
2⟨14⟩ + Ba^{2+}	94	81
2⟨18⟩	7	27
2⟨18⟩[b]	≫1600	(17)[c]
2⟨18⟩ + Ba^{2+}	7	86
cis-**8**	4	83
trans-**8**	45	14
12	61	100
Triton X-100	180	100
melittin	16	85
melittin[d]	17	100

[a] Determined for a KCl/KCF vesicle system by incubation of aliquots. Data from 6−10 points fitted to a 4-parameter logistic equation. Inflection concentration and maximum extent taken from the fitted data except where noted. Reproducibility in log(inflection concentration) = ±0.2, in extent of disruption ±5%. Cases with added Ba^{2+} used 1 mM $BaCl_2$. [b] Sodium used in place of potassium. [c] Activity too poorly defined to fit; maximum extent of disruption observed. [d] Determined by a titration method in which melittin was progressively added to vesicles with withdrawals for analysis between additions.

The inflection concentration for **2**⟨18⟩ is unchanged on addition of $BaCl_2$, suggesting that the amphiphile is partitioned to the vesicles to about the same extent but is more efficient in disruption when Ba^{2+} ions are present. Controls establish that the $BaCl_2$ has no effect on the inherent extent of CF leakage up to a concentration of 10 mM, nor do the added divalent ion salts alter the vesicle size distribution. Similarly, added bolaamphiphiles up to 4 mol % of lipid do not alter the vesicle size distribution. We therefore draw the preliminary conclusion that the enhancements in membrane disruption can be attributed to the expected cation switch, operating as proposed in Figure 1.

Why do **2**⟨*n*⟩ bolaamphiphiles not achieve 100% disruption even when barium ions are present? We relate the extent of disruption to the fraction of unilamellar vesicles in the solution by the following sequence of experiments. The peptide melittin disrupts vesicle bilayer membranes solely via partition to the outermost bilayer leaflet, and this property is used as an assay for the unilamellar fraction.[20] The assay involves two stages: a titration by aliquot addition to establish the total amount of melittin required to fully disrupt all vesicles and a second bulk addition of that total amount to determine what fraction of the vesicle contents can be released via disruption of a single bilayer leaflet. The membrane disruption assay of Figure 2 and Table 1 is akin to the second stage of the melittin experiment in that a fixed amount of amphiphile is added at one time. As noted in Table 1, melittin under these conditions releases 85% of the entrapped CF, indicating the vesicles are approximately 85% unilamellar. A titration experiment like the first stage of the melittin assay confirms that melittin added in portions can release 100% of the entrapped CF (Supporting Information). Thus, the disruption assay indirectly reports the unilamellar fraction for amphiphiles which, like melittin, act one bilayer at a time. The vesicle preparation procedure can be altered to produce different vesicle samples with different unilamellar fractions. For vesicles which were only 62 ± 5% unilamellar (melittin assay), the maximum extent of disruption by **2**⟨18⟩ was 60% but the concentration at the inflection point was unchanged at 7 μM. We conclude that **2**⟨*n*⟩ bolaamphiphiles behave like melittin in their ability to selectively disrupt only the outermost bilayer leaflet, and it is this propensity which leads to incomplete membrane disruption even at high amphiphile concentrations.

If the enhancements observed with added barium ions are based on molecular recognition, then there ought to be ion selectivity among divalent cations. The situation is complicated by the problem that the apparent extent of enhancement is a function of the amphiphile concentration. For compounds such as **2**⟨18⟩ which show a clear plateau for the case of KCl/KCF alone, it will be sufficient to demonstrate a selective enhancement from this plateau value. For compounds such as **1**⟨18,5⟩, the amphiphile concentration chosen must lie below the inflection concentration to allow observation of any enhancement which might occur. As discussed in more detail below, the concentration of the divalent ion also plays a role, so a high concentration relative to the amphiphile is required. The net result is that there can be no standard experimental protocol to explore the ion selectivity of the extent of enhancement. However, a selection of data is presented in Table 2 which provides some useful comparisons.

All three bolaamphiphiles **2**⟨*n*⟩ show the same rank order among the alkaline earth metal cations (Ba^{2+} > Sr^{2+} > Ca^{2+}) as expected for a constant recognition site. Like other oxygen-based crown ethers,[21] the parent

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Table 2. Influence of Added Divalent Ions on Membrane Disruption[a]

entry	amphiphile	concn (mM)	% release	divalent ion	concn (mM)	% release (divalent)	factor[b]
1	**1**⟨18,5⟩	0.01	17.7	Ba	5.0	18.2	1.0
2	**1**⟨18,5⟩	0.01	16.4	Sr	1.1	15.1	0.9
3	**2**⟨14⟩	0.11	11.0	Ca	1.0	13.9	1.3
4	**2**⟨14⟩	0.11	11.0	Sr	1.0	19.8	1.8
5	**2**⟨14⟩	0.11	11.0	Ba	1.0	56.9	5.2
6	**2**⟨14⟩	0.51	9.4	Mn	7.5	7.7	0.8
7	**2**⟨18⟩	0.48	20.5	Ca	2.5	33.1	1.6
8	**2**⟨18⟩	0.48	20.5	Sr	2.5	57.2	2.8
9	**2**⟨18⟩	0.12	21.9	Ba	1.0	91.9	4.2
10	**2**⟨18⟩	0.48	20.5	Ba	2.5	78.9	3.8
11	**2**⟨18⟩	0.48	16.6	en[d]	5.0	44.5	2.7
13	**2**⟨18⟩[c]	0.25	6.2	Sr	1.0	78.0	12.5
14	**2**⟨18⟩[c]	0.25	8.2	Ba	1.0	77.8	9.5
15	**2**⟨20⟩	0.11	33.7	Ca	1.0	34.3	1.0
16	**2**⟨20⟩	0.11	33.7	Sr	1.0	67.1	2.0
17	**2**⟨20⟩	0.11	33.7	Ba	1.0	84.6	2.5
18	*cis*-**8**	0.06	39.5	Ba	1.25	53.7	1.3
19	*trans*-**8**	0.30	21.2	Ba	1.25	49.5	2.3
20	**12**	0.06	49.1	Ba	5	48.0	1.0

[a] Determined in a KCl/KCF system in the absence (column 4) and the presence (column 7) of added divalent ions (chloride salts). [b] Factor by which the membrane disruption is enhanced by the addition of divalent ions i.e. column 7/column 4. [c] Sodium used in place of potassium. [d] Ethylenediammonium.

monoamide carboxylate crown ethers have very low affinity for transition metal cations,[11] and Mn^{2+} fails to produce an enhancement (entry 6). Conversely, oxygen crown ethers do bind primary ammonium cations,[21] and ethylenediammonium produces a significant enhancement (entry 11). The oligoethylene glycol segment of **1**⟨18,5⟩ would be expected to have low affinity for alkaline earth metal cations and does not show any selectivity between Ba^{2+} and Sr^{2+} (entries 1 and 2). In contrast, the crown ether 12 does have inherent ability to recognize Ba^{2+} in preference to K^+,[11a] yet this is insufficient on its own to result in enhancement of disruption (entry 20). The enhancement factors observed in NaCl media are larger than in KCl media. This is consistent with an expected lower affinity of the crown ether for Na^+ relative to K^+,[10] but it is also consistent with the generally lower extents of disruption observed in the NaCl /NaCF system on its own relative to the KCl/KCF system. Overall, the data of Table 2 are consistent with the hypothesis sketched in Figure 1, in which a molecular recognition is a required element of the membrane disruption process.

If molecular recognition is essential to the membrane disruption process, then the extent of disruption should depend on the concentration of added divalent ion. Moreover, at some relatively high concentration of the added divalent ion, the available crown ethers will be completely complexed and no further enhancement of disruption will be observed. Thus the extent of disruption provoked by added divalent ion should behave like a binding isotherm. As for Table 2, a concentration range of divalent salt which shows a significant enhancement above a plateau value is required. At fixed concentration of 0.25 mM in **2**⟨18⟩, a variable concentration of $BaCl_2$ between 0.05 and 3.75 mM was suitable. Under these conditions, the observed extent of disruption ranges from 28% in the absence of divalent salt (Rel_{min}), to 85% disruption (Rel_{max}), equivalent to the unilamellar fraction. One can define a factor (F_{obs}) as the additional disruption produced by added Ba^{2+} above the level in the absence of Ba^{2+}:

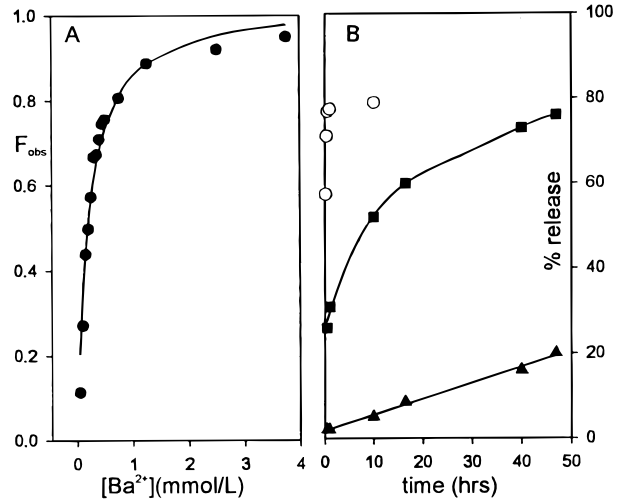

Figure 3. (A) Fraction of barium dependent release as a function of added $BaCl_2$. The solid line is the calculated curve for a 1:1 binding model with log $K = 3.7$. (B) Release of CF (%) as a function of time for **2**⟨18⟩ in the presence (○) and absence (■) of 1 mM added $BaCl_2$ in KCl/KCF medium. Slow leakage without added amphiphile is given for comparison (▲).

$$F_{obs} = (Rel_{obs} - Rel_{min})/(Rel_{max} - Rel_{min}) \quad (2)$$

Here Rel_{obs} is the observed release at some concentration of added Ba^{2+}. F_{obs} will take values between 0 and 1. As illustrated in Figure 3A, F_{obs} appears to follow a rectangular hyperbolic binding isotherm.[22] A fit to a 1:1 binding stoichiometry gives an apparent association constant for barium binding of log $K = 3.7 \pm 0.3$. Direct comparison of this result with other systems is difficult: **12** binds Sr^{2+} at a $CHCl_3-H_2O$ interface with an apparent log $K_i = 3.0 \pm 0.4$,[12] while the butyl homologue of **12** binds Ba^{2+} in a 1:1 complex in 90:10 methanol$-H_2O$ with a log $K = 5.5 \pm 0.2$.[11a] Neither of these comparisons is particularly apt, apart from establishing an approximate range for the expected value. The key point is that the disruption process behaves as expected if molecular recognition of barium were a required element in the process.

The Ba^{2+}/K^+ selectivity of 18-crown-6 monocarboxylates amounts to a factor of 10 in homogeneous solution,[10,11] so it is surprising that there is such a marked difference in disruption produced by added barium ions. The large excess of K^+ would be expected to overwhelm any thermodynamic selectivity for Ba^{2+}; thus, the origin of the selective disruption must also include some kinetic factor. The membrane disruption assay uses a standard incubation period of 30 min. Figure 3B shows data for disruption by **2**⟨18⟩ during incubation times extending over 2 days. The Ba^{2+}-containing system reaches its maximum disruption within 30 min, but the system without added Ba^{2+} continues to evolve over the following 40 h. This additional disruption is significantly above the blank leakage rate. Thus the switching illustrated in Figure 2B is basically a kinetic effect. The amphiphile is capable of membrane disruption at some slow rate which is accelerated by the binding of the divalent ion. This is consistent with a range of conformations in the

(22) F_{obs} = (maximum release × $[Ba^{2+}]$)/(K_{app} + $[Ba^{2+}]$), where K_{app} is the apparent *dissociation* constant of a 1:1 complex.

presence of K^+ which shift to favor a tight U-shaped conformation upon Ba^{2+} recognition.

The data above are consistent with an essential molecular recognition process which controls membrane disruption but do not compel the conclusion that the two crown ether groups act together as envisaged in Figure 1. Given the length of the hydrocarbon spacer, cooperative interactions might seem somewhat unlikely, even though the lipid bilayer environment must impose some constraints on the amphiphile conformation. The best evidence for cooperative action of the two crown ethers in disruption is the 1:1 binding model discussed above (Figure 3A). A reviewer suggests that a direct determination of the unbound Ba^{2+} would give the amount of membrane-bound Ba^{2+} and thence the Ba^{2+}/bis(crown ether) ratio in the membrane. This experiment would also require a knowledge of the partition of bis(crown ether) to the membrane over the range of cation concentrations. Since the **2**⟨*n*⟩ prepared lack a suitable chromophore, this experiment was not attempted. The same reviewer also suggests that electrospray ionization mass spectra could be used to show the formation 1:1 complexes. Such complexes are in fact readily observed, and we have explored this question in detail.[30] The short conclusion is that ESI-MS spectra do not directly reflect the solution composition in cases, like the ones of interest here, where complex ion fractionation and reequilibration can occur during the evaporation/ion production process.[30]

The key question is whether the type of restraints imposed by the bilayer membrane environment can promote cooperative interactions between remote groups. To approach this question we prepared **8**, a long-chain and conformationally mobile bis(crown ether) containing a photoisomerizable thioindigo. 7,7′-Thioindigo diesters undergo significant relative motion on photoisomerization from trans to cis, and this shape change has been exploited in a number of systems.[18,23] In compound **8**, the thioindigo is remote from the two crown ethers to probe whether a remote conformational change in a flexible chain can result in cooperative interactions between the crown ethers. The photoisomerization at the middle of the chain is akin to the role played by the bilayer in establishing a U-shaped conformation. The principal goal is to look for evidence of cooperative ditopic interactions between the crown ethers for *cis*-**8** in homogeneous solution which are absent in *trans*-**8**. As an additional check of the membrane disruption mechanism,

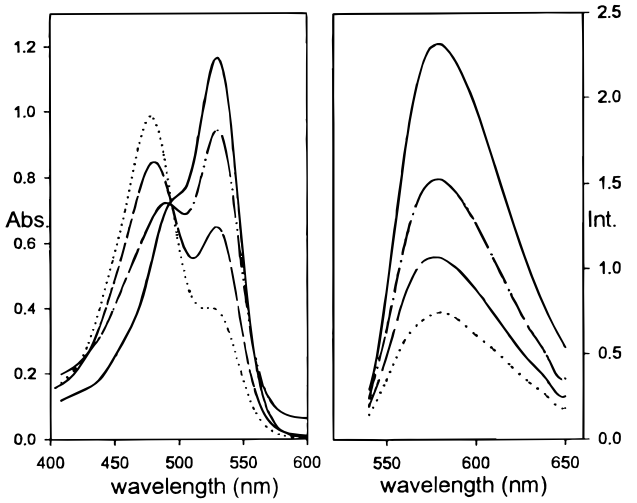

Figure 4. Changes in absorption (A) and fluorescence (B) spectra of **8** in acetone (0.3% water) as a function irradiation conditions: initial spectrum (—); irradiation at 530 nm (- - -); irradiation at 530 nm followed by 16h at 0 °C in the dark (— —); irradiation at 530 nm followed by 1 h in room temperature light (— - -).

cis-**8** should be a more effective membrane-disrupting agent than *trans*-**8** due to a predisposition toward a U-shaped conformation. Simple thioindigo diesters undergo cis-to-trans photoisomerization in bilayers,[24] but the reverse trans-to-cis photoisomerization is inhibited by the ordered environment.[24,25] Thus photoswitching of membrane disruption is not possible with **8**.

The visible and fluorescence spectra shown in Figure 4 establish that the expected photoisomerization occurs in homogeneous solution (acetone/3% methanol). As prepared (room light), **8** shows an intense absorption at 530 nm with a shoulder at 480 nm (Figure 4A). These bands are assigned to the trans and cis forms by analogy with other thioindigo spectra.[25,26] Irradiation at 530 nm results in a bleaching of the 530 nm band and increased intensity of the 480 nm band, consistent with the production of a cis-enriched sample. The trans isomer is slowly regenerated thermally and more rapidly regenerated in room light, to produce the isobestic point illustrated. Alternatively, the trans isomer can be regenerated by irradiation at 430 nm. Corresponding changes also occur in the fluorescence spectra (Figure 4B). In general, *cis*-thioindigo derivatives do not fluoresce;[26] thus, the residual fluorescence observed after long irradiation times at 530 nm indicates that only a cis-enriched photostationary state can be produced. Serial dilution of a cis-enriched sample showed that the intensity of the 480 nm absorption was a linear function of concentration, passing through the origin ($\log \epsilon = 3.81$). Thus the absorbance of the trans isomer at 480 nm is negligible. A similar dilution of a trans-enriched sample showed absorbance at 530 nm was a linear function of concentration ($\log \epsilon = 3.08$), but the best-fit linear regression did not pass through the origin. Thus there is some residual absorbance from the cis-isomer at 530 nm. From the residual fluorescence intensity, and the change in intensity of the 480 nm band upon prolonged irradiation at 530 nm, the cis-enriched photostationary state is estimated to be approximately 80 ± 5% *cis*-**8**, while *trans*-enriched samples are estimated to be 95 ± 5% *trans*-**8**. In vesicles without encapsulated CF, cis-to-trans photoisomerization was observed (irradiation at 480

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Table 3. Influence of Added Divalent Cations on the Thermal and Photochemical Conversion of *cis*-8 to *trans*-8[a]

cation added	concn (μM)	thermally regenerated (%)[b]	photochemically regenerated (%)[c]
none	0	65	98
Ba	8.3	51	88
Ba	16.7	42	70
Ba	25	29	48
Ca	83		20[d]
Sr	83		0[d]
Ba	83		39[d]

[a] In acetone containing 0.5 vol % water. A solution of 20 μM *trans*-**8** was irradiated at 530 nm for 1 min to generate a sample enriched in *cis*-**8**. Approximately 76% of the initial fluorescence intensity was bleached. [b] Extent of initial fluorescence intensity recovered after 8 min at 22 °C. [c] Extent of initial fluorescence intensity recovered after 8 min at 22 °C followed by 1 min irradiation at 430 nm. [d] Extent of initial fluorescence intensity recovered after 1 min irradiation at 430 nm.

nm, fluorescence increase observed), but the reverse trans-to-cis isomerization was not found. Thus **8** shows all the anticipated properties of a thioindigo diamide derivative.

The membrane disrupting ability of *cis*-**8** is significantly greater than *trans*-**8**. Like **2**⟨*n*⟩, *cis*-**8** apparently disrupts only the unilamellar fraction of the vesicle population, with an activity that is comparable to **2**⟨18⟩ in the presence of added $BaCl_2$ (Figure 2B, Table 1). The disrupting ability of *cis*-**8** is little enhanced by added barium ion (Table 2, entry 18), consistent with the proposal that *cis*-**8** already has a propensity toward a U-shaped conformation. The *trans*-**8** isomer is surprisingly inactive on its own but is markedly more active in the presence of additional barium (Table 2, entry 19). This is consistent with *trans*-**8** adopting a barium-stabilized U-conformation, although other rationalizations of this observation are possible. The simple conclusion is that *cis*-**8** is a reasonable model for the U-shaped state proposed in Figure 1.

The cis-to-trans isomerization of thioindigos proceeds via a twisted intermediate.[27] If the crown ethers act cooperatively in binding divalent ions in *cis*-**8**, then there will be an additional conformational restriction on the twisting process in the presence of added divalent ions. This should appear as slower rate of thermal cis-to-trans isomerization or as an altered extent of cis-to-trans photoisomerization for a short (constant) irradiation period (at 430 nm). The extent of isomerization is conveniently followed using the fluorescence intensity at 575 nm. The effect of added Ba^{2+} on the thermal and photochemical reactions in acetone/water (99.5:0.5) was determined by the following sequence: A *trans*-**8** solution (1.0×10^{-5} M) was irradiated at 530 nm for 1 min, a known variable amount of $BaCl_2$ was added, the sample was stored in the dark at 22 °C for 8 min, and it then was irradiated for 1 min at 430 nm. At each point in the sequence the emission intensity at 575 nm was determined. The initial photoisomerization to the *cis*-enriched state bleached the initial intensity to 24% of its original value. The extent of regeneration by the thermal reaction (8 min) and the photochemical reaction (subsequent 1 min) are summarized in Table 3. There was no change in fluorescence intensity following addition of Ba^{2+}, so the intensity differences observed are correctly assigned to incomplete regeneration of the trans-isomer as a result of the added cations. Note that the retarding effect on the regeneration processes is dependent upon the Ba^{2+} concentration. The divalent cation inhibition of the photochemical regeneration is more pronounced with Ca^{2+} and Sr^{2+}; the latter ion completely inhibits the cis-to-trans photochemical isomerization for short irradiation times. Control experiments establish that the thermal and photochemical isomerizations of dialkyl thioindigo diamides are unaffected by added divalent ion salts and that added $CaCl_2$, $SrCl_2$, or $BaCl_2$ do not quench the thioindigo fluorescence. In contrast, added divalent ions diminish the emission intensity of trans-enriched solutions in the order $Sr^{2+} > Ca^{2+} > Ba^{2+}$. This is the same order of inhibition found in Table 3 and is consistent with a cation induced trans-to-cis thermal isomerization. The expected corresponding changes in the visible absorption spectrum support this conclusion. Like other carboxylate crown ethers in nonpolar solvents, the selectivity sequence for this cooperative process probably reflects a combination of cation size and electrostatic effects,[10] but the position of Ba^{2+} in the sequence is unusual compared with the disruption sequence for **2**⟨*n*⟩ ($Ba^{2+} > Sr^{2+} > Ca^{2+}$) or the complex stability sequence in homogeneous solution of a butyl analogue of **12** ($Sr^{2+} > Ba^{2+} > Ca^{2+}$).[11] Barium chloride is significantly less soluble in the medium than the other two salts used; thus, we assume without further proof that the anomalous Ba^{2+} position is somehow related to a medium effect possibly involving the counterion.

The inhibition of thermal and photochemical reactions from *cis*-**8** and the acceleration of thermal reaction to *cis*-**8** are akin to related thermal and photochemical processes of azo-linked bis(crown ethers)[28] and anthraceno-crown glymes and ethers.[29] In the prior examples the photoisomerizable section is rigidly coupled to the ion binding sites, and there is a clear mechanical control over the binding site geometry. Our system is considerably more flexible and the binding site is remote from the photoswitched section, but the accumulated data support the conclusion that the crown ethers act in concert to bind divalent ions.

The precise structures of the complexes are not a critical issue. The key conclusion is that even very flexible bis(crown ethers) can be induced to act in a cooperative ditopic fashion as a result a generally U-shaped segment in the middle of the span. This certainly occurs for *cis*-**8** in homogeneous solution. In addition, membrane disruption by *cis*-**8** in vesicles is similar to **2**⟨*n*⟩; thus, the conclusion that they both act as indicated in Figure 1 is strongly supported. Although this system involves only a simple recognition process, the switching mechanism demonstrated here offers considerable generality. Extension to other recognition processes will be possible, as will adaptation to control ion transport via channels.

Experimental Section

General Procedure for the Preparation of Protected Diamines 5a,b⟨*n*⟩. A mixture of a 1,*n*+2-alkanedioic acid (0.5 g scale, 2 equiv) and $SOCl_2$ (excess) was refluxed for 3 h. The excess $SOCl_2$ was removed under reduced pressure, and the diacid chloride (**4**⟨*n*⟩) was dissolved in CH_2Cl_2 (15 mL). To this solution was added (dropwise) a solution of Boc[14a] or Cbz[14b] protected 3-amino-1-propanol (4 equiv) dissolved in CH_2Cl_2 (15 mL) and DMAP (6 equiv). After additional stirring (24 h) at room temperature, the organic phase was removed to yield brown sticky oil. The crude product was purified by silica gel

column chromatography (2.3 × 20 cm) using CH_2Cl_2 as eluent. The fractions with the same ^{1}H NMR were combined, and the solvents were removed to yield **5a,b**⟨*n*⟩ as white solids.

***N,N′*-Bis(*N-tert*-butoxycarbonyl)-1,30-diamino-4,27-dioxa-5,26-dioxotriacontane (5a⟨20⟩):** from docosanedioic acid and Boc-aminopropanol in 89% yield; mp 70−71 °C; ^{1}H NMR ($CDCl_3$, δ) 4.75 (br s, 2H), 4.08 (t, J = 7.0 Hz, 4H), 3.30−3.00 (m, 4H), 2.25 (t, J = 6.7 Hz, 4H), 1.90−1.70 (m, 4H), 1.65−1.50 (m, 4H), 1.20 (s, 18H), 1.32−1.16 (m, 32H); ^{13}C NMR ($CDCl_3$, δ) 173.9, 155.9, 79.1, 61.7, 37.4, 34.2, 29.6, 29.5, 29.4, 29.2, 29.1, 28.8, 28.3, 24.9.

***N,N′*-Bis(*N*-benzyloxycarbonyl)-1,24-diamino-4,21-dioxa-5,20-dioxotetracosane (5b⟨14⟩):** from hexadecanedioic acid and Cbz-aminopropanol in 58% yield; mp 106−107 °C; ^{1}H NMR ($CDCl_3$, δ) 7.4−7.2 (m, 10H), 5.06 (s, br, 6H), 4.11 (t, J = 6.0 Hz, 4H), 3.24 (m, 4H), 2.26 (t, J = 7.5 Hz), 1.81 (m, 4H), 1.77−1.15 (m, 24H); ^{13}C NMR ($CDCl_3$, δ) 173.9, 156.3, 136.5, 128.4, 128.0, 66.6, 61.5, 37.9, 34.2, 29.5, 29.4, 29.2, 29.0.

***N,N′*-Bis(*N*-benzyloxycarbonyl)-1,28-diamino-4,25-dioxa-5,24-dioxooctacosane (5b⟨18⟩):** from octadecanedioic acid and Cbz-aminopropanol in 59% yield; mp 109−110 °C; ^{1}H NMR ($CDCl_3$, δ) 7.4−7.25 (m, 10H), 5.07 (s, 4H), 4.95 (br, 2H), 4.12 (t, J = 6.0 Hz, 4H), 3.3−3.2 (m, 4H), 2.27 (t, J = 7.5 Hz, 4H), 1.9−1.7 (m, 4H), 1.6−1.1 (m, 32H); ^{13}C NMR ($CDCl_3$, δ) 174.0, 156.4, 136.6, 128.5, 128.1, 66.7, 61.6, 38.0, 34.3, 29.7, 29.5, 29.2, 29.2, 25.0.

1,30-Diamino-4,27-dioxa-5,26-dioxotriacontane Bis(hydrogen formate) (6⟨20⟩). Compound 5**a**⟨20⟩ (0.13 g, 0.2 mmol) was dissolved in 95% formic acid, and the solution was refluxed for 15 min. The solvent was removed by evaporation under reduced pressure to give a white solid [0.1 g, yield 99%; mp 108−110 °C; ^{1}H NMR (D_2O, δ): 8.3 (br s, 2H), 4.05 (t, J = 6.0 Hz, 4H), 3.04 (t, 7.1 Hz, 4H), 2.23 (t, J = 7.4 Hz, 4H), 1.95 (br s, 4H), 1.7−1.2 (m, 36H, aliphatic)], which was used without purification in the subsequent step.

1,24-Diamino-4,21-dioxa-5,20-dioxotetracosane Bis(hydrogen formate) (6⟨14⟩). Compound **5b**⟨14⟩ (0.6 g, 0.8 mmol) was dissolved in 1:1 formic acid/methanol (60 mL) and added to a round-bottom flask (100 mL) containing 1 equiv of palladium catalyst (10% Pd/C, 1.0 g, 0.9 mmol). The mixture was continuously stirred under reflux temperature for 24 h. The catalyst was removed by filtration and washed with an additional 10 mL of methanol. The combined solvents were removed by evaporation under reduced pressure to give a white solid (0.34 g, yield 81%, mp 96−98 °C). ^{1}H NMR (CD_3OD, δ): 4.16 (br, 4H), 3.02 (br, 4H), 2.33 (t, J = 7.4 Hz), 2.00 (br, 4H), 1.7−1.2 (m, 24H). ^{13}C NMR (CD_3OD, δ): 175.2, 167.8, 62.3, 38.0, 34.9, 30.7, 30.5, 30.3, 30.2, 27.9, 25.9. LSIMS (mNBA matrix) [*m/z* (relative intensity)]: 439.3 (M + K^+, 36), 423.3 (M + Na^+, 65), 401.4 (M + H^+, 90), 383.3 (M − OH, 79), 344.2 (60), 326.2 (100). This compound was used without further purification in the subsequent step.

1,28-Diamino-4,25-dioxa-5,24-dioxooctacosane Bis(hydrogen formate) (6⟨18⟩). This compound was prepared from **5b**⟨18⟩ (0.31 g, 0.4 mmol) using the procedure for **6**⟨14⟩ to give a white solid (0.19 g, 80%, mp 104−106 °C). ^{1}H NMR (CD_3OD, δ): 4.16 (t, J = 6.0 Hz, 4H), 3.02 (t, 7.1 Hz, 4H), 2.33 (t, J = 7.4 Hz, 4H), 1.98 (br, 4H), 1.7−1.2 (m, 32H). ^{13}C NMR (CD_3OD, δ): 175.3, 165.9, 62.3, 38.1, 34.9, 30.7, 30.5, 30.4, 30.2, 27.9, 25.9. LSIMS (mNBA matrix) [*m/z* (relative intensity)]: 495.4 (M + K^+, 9), 479.4 (M + Na^+, 23), 457.4 (M + H^+, 92), 439.4 (100), 400.3 (62), 382.3 (100). This compound was used without further purification in the subsequent step.

General Procedure for the Preparation of Bis(crown ether) Bolaamphiphiles from Bis(ammonium formates). A solution of the crown ether anhydride (**7**,[16] 0.26 g, 0.8 mmol) and excess dry pyridine (0.4 g) in 10 mL of THF was added to a THF solution (10 mL) containing the bis(ammonium formate) **6**⟨*n*⟩ (0.2 g, 0.4 mmol) at room temperature, and the solution was refluxed overnight. After cooling, the solvent was removed under reduced pressure. The oil was dissolved in chloroform (50 mL) and washed with 2 M HCl and water, respectively. The organic phase was dried over $MgSO_4$. The solvent was removed under reduced pressure to yield sticky oil. The products were isolated by gel permeation column chromatography (Sephadex LH 20, 1.2 × 120 cm) with 4:3 $CHCl_3/CH_3OH$ as eluent.

***N,N′*-Bis(1*R*,2*R*-3,6,9,12,15,18-hexaoxacycloocta-2-carboxyl-1-carbonyl)-1,24-diamino-4,21-dioxa-5,20-dioxotetracosane (2⟨14⟩):** from **6**⟨14⟩ as sticky colorless oil (0.21 g, yield 50%); ^{1}H NMR ($CDCl_3$, δ) 7.46 (t, J = 6.0 Hz, 2H), 4.36 (d, J = 2.5 Hz, 2H), 4.22 (d, J = 2.4 Hz, 2H), 4.07 (t, J = 6.4 Hz, 4H), 3.70−3.50 (m, 40H), 3.45−3.25 (m, 4H), 2.24 (t, J = 7.6 Hz, 4H), 1.88−1.77 (m, 4H), 1.60−1.48 (m, 4H), 1.30−1.10 (m, 20H); ^{13}C NMR ($CDCl_3$, δ) 173.8, 171.7, 169.2, 81.2, 80.5, 71.0, 70.9, 70.3, 70.2, 70.2, 70.0, 69.8, 69.7, 69.6, 69.5, 61.6, 35.9, 34.2, 29.6, 29.5, 29.4, 29.2, 29.1, 28.5, 24.9 (7 CH_2); ESI-MS (1:1 CH_3CN/H_2O with 1 equiv of K^+ added) [*m/z* (relative intensity)] 1145.7 (M + 2K^+ − H^+, 14), 1107.7 (M + K^+, 6), 573.3 (M + 2K^+, 100); LSIMS (*m*NBA as matrix) [*m/z* (relative intensity)] 1067.6 (M − H^+, 100). Observed and calculated isotope peak intensities of the molecular ion ([M − H]$^-$, $C_{50}H_{87}N_2O_{22}$) (obs, calcd): 1067.6 (100, 100), 1068.6 (57.6, 57.6), 1069.6 (20.6, 20.6), 1070.6 (5.5, 5.4), 1071.6 (0.02, 0.01). High-resolution MS calcd for $C_{50}H_{87}N_2O_{22}$, *m/e* 1067.5735; found, *m/e* 1067.5750.

***N,N′*-Bis(1*R*,2*R*-3,6,9,12,15,18-hexaoxacycloocta-2-carboxyl-1-carbonyl)-1,28-diamino-4,25-dioxa-5,24-dioxooctacosane (2⟨18⟩):** from **6**⟨18⟩ (0.12 g, 0.2 mmol), as sticky colorless oil (0.13 g, 55%); ^{1}H NMR ($CDCl_3$, δ) 7.48 (t, J = 5.8 Hz, 2H), 4.37 (d, J = 2.4 Hz, 2H), 4.22 (d, J = 2.4 Hz, 2H), 4.08 (t, J = 6.5 Hz, 4H), 3.71−3.50 (m, 40H), 3.45−3.25 (m, 4H), 2.26 (t, J = 7.6 Hz, 4H), 1.90−1.78 (m, 4H), 1.61−1.50 (m, 4H), 1.30−1.15 (m, 28H); ^{13}C NMR ($CDCl_3$, δ) 173.9, 171.8, 169.2, 81.3, 80.6, 70.9, 70.8, 70.3, 70.2, 70.1, 70.0, 70.8, 69.8, 69.7, 69.6, 61.6, 35.9, 34.2, 29.6, 29.6, 29.4, 29.2, 29.1, 28.6, 24.9; LSIMS (mNBA matrix) [*m/z* (relative intensity)] 1123.7 (M − H^+, 100). Observed and calculated isotope peak intensities of the molecular ion ([M − H]$^-$, $C_{50}H_{87}N_2O_{22}$) (obs, calcd): 1067.6 (100, 100), 1068.6 (60.9, 61.7), 1069.6 (23.0, 23.2), 1070.6 (6.5, 6.4), 1071.6 (0.02, 0.01). High-resolution MS: calcd for $C_{54}H_{95}N_2O_{22}$, *m/e* 1123.6376; found, *m/e* 1123.6357.

***N,N′*-Bis(1*R*,2*R*-3,6,9,12,15,18-hexaoxacycloocta-2-carboxyl-1-carbonyl)-1,30-diamino-4,27-dioxa-5,26-dioxotriacontane (2⟨20⟩):** from **6**⟨20⟩ (0.13 g, 0.2 mmol), as colorless sticky oil (0.15, yield 64%); ^{1}H NMR ($CDCl_3$, δ) 7.54 (m, 2H), 4.37 (d, J = 2.4 Hz, 2H), 4.20 (d, J = 2.4 Hz, 2H), 4.05 (t, J = 6.7 Hz, 4H), 3.80−3.42 (m, 40H), 3.40−3.25 (m, 4H), 2.22 (t, J = 7.6 Hz, 4H), 1.90−1.78 (m, 4H), 1.61−1.50 (m, 4H), 1.30−1.15 (m, 32H); ^{13}C NMR ($CDCl_3$, δ) 173.9, 171.7, 169.3, 81.2, 80.4, 71.0, 70.9, 70.3, 70.2, 70.0, 69.8, 69.6, 61.7, 36.0, 34.2, 29.6, 29.4, 29.2, 29.1, 28.6, 24.9; ESI-MS (1:1 CH_3CN/H_2O with 1 equiv of K^+ added) [*m/z* (relative intensity)] 1230.0 (M + 2K^+ − H^+, 12), 1191.8 (M + K^+, 9), 615.4 (M + 2K^+, 100). High-resolution MS: calcd for $C_{56}H_{99}N_2O_{22}$, *m/e* 1151.6689; found, *m/e* 1151.6583.

***N*-(*tert*-Butoxycarbonyl)-8-amino-1-octanol (10).** 8-Amino-1-octanol[17] (3.1 g, 21 mmol; see Supporting Information) was dissolved in CH_2Cl_2 (15 mL) in a 50 mL round-bottom flask equipped with a magnetic stirrer and a pressure-equalizing addition funnel. Di-*tert*-butyl dicarbonate (5 g, 23 mmol) in CH_2Cl_2 (15 mL) was added dropwise over 30 min. After the mixture was stirred for 12 h at room temperature, the solvent was removed and the crude product was dissolved with Et_2O (30 mL) and washed twice with phosphate buffer (0.5 M, pH 5.4, 2 × 15 mL), saturated $NaHCO_3$ (15 mL), and saturated brine (15 mL). The organic phase was dried over $MgSO_4$ and evaporated to give the crude product (5.3 g) as a pale yellow oil that rapidly solidified. The crude product (1.1 g) was purified by alumina column chromatography (2.3 × 17 cm) with $CHCl_3$ as eluent to remove unreactive Boc_2O, and the final product was eluted with 1% $CH_3OH/CHCl_3$ as white solid (0.55 g, yield 50%). ^{1}H NMR ($CDCl_3$, δ): 4.50 (br S, 1H), 3.58 (t, J = 6.8 Hz, 2H), 3.05 (t, J = 7.0 Hz, 2H), 1.90−1.05 (m, 21H). ^{13}C NMR ($CDCl_3$, δ): 156.0, 79.1, 62.8, 40.6, 32.7, 30.0, 29.3, 29.2, 28.4, 26.7, 25.6. This compound was used in the subsequent step without further purification.

Bis[*N-tert*-butoxycarbonyl-8-amino-1-octanoyl]thioindigo-7,7′-dicarboxylate (11). DMAP (0.15 g, 1.2 mol) was

added to a solution in CH_2Cl_2 (15 mL) of **10** (0.10 g, 0.2 mmol) and thioindigo-7,7′-dicarboxylic acid chloride[18] (0.11 g, 0.44 mol), and the solution was refluxed overnight. After cooling, the solvent was removed and the crude product was purified by silica gel column chromatography (2.3 × 16 cm) with $CHCl_3$ as eluent. The colored fractions were combined, and the solvent was removed to yield **11** as red solid product (0.1 g, 60%). ^{1}H NMR ($CDCl_3$, δ): 8.22 (d, J = 6.9 Hz, 2H), 8.02 (d, J = 7.0 Hz, 2H), 7.20 (t, J = 6.9 Hz, 2H), 4.52 (br s, 2H), 4.40 (t, J = 7.0 Hz, 4H), 3.05 (t, J = 6.7 Hz, 4H), 2.00−1.10 (m, 42H). ^{13}C NMR ($CDCl_3$, δ): 189.7, 164.8, 156.0, 150.4, 138.9, 137.0, 135.0, 130.4, 126.0, 125.9, 79.0, 66.1, 40.6, 30.0, 29.2, 28.7, 28.4, 26.7, 26.0.

***N,N′*-Bis((1*R*,2*R*-3,6,9,12,15,18-hexaoxacycloocta-2-carboxyl-1-carbonyl)-8-amino-1-octyl)-thioindigo-7,7′-dioate) (8).** Compound **11** (0.1 g, 0.12 mmole) was deprotected as described above for **6**⟨20⟩ to give the bis(ammonium formate) as a red solid (0.09 g, yield 99%). ESI-MS (1:1 CH_3CN/H_2O) [m/z (relative intensity)]: 638.9 ($M + H^+$, 62), 320 ($M + 2H^+$, 100). Coupling with the crown ether followed the general procedure described above. Thus a solution of the crown ether anhydride (**7**, 0.14, 0.36 mmol) and excess dry pyridine (0.4 g) in 5 mL of THF was added into a solution (THF, 5 mL) containing the bis(ammonium formate) (0.09 g, 0.12 mmol) at room temperature, and the solution was refluxed overnight. After workup, the product was purified by gel permeation column (Sephadex LH 20, 1.2 × 120 cm) with 4:3 $CHCl_3$/CH_3OH as eluent. Colored fractions with the same ^{1}H NMR spectra were combined, and the solvent was evaporated to yield **8** as bright red solid (0.1 g, 0.08 mmol, yield 64%). ^{1}H NMR ($CDCl_3$, δ): 8.30 (d, J = 6.7 Hz, 2H), 8.13 (d, J = 6.9 Hz, 2H), 7.42 (t, J = 7.0 Hz, 2H), 7.30 (br s 2H), 4.42 (t, J = 7.0 Hz, 6H), 4.27 (br s, 2H), 3.9−3.4 (m, 40H), 3.40−3.20 (m, 4H), 1.95−1.70 (m, 4H), 1.65−1.20 (m, 20H). ^{13}C NMR ($CDCl_3$, δ): 189.8, 171.8, 164.9, 150.4, 137.1, 135.1, 130.5, 135.4, 126.1, 126.0, 80.7, 77.2, 70.8, 70.3, 70.1, 69.9, 69.8, 69.7, 66.1, 39.3, 29.5, 29.2, 28.7, 26.8, 26.0. ESI-MS (1:1 CH_3CN/H_2O containing 2 equiv of KCl and NaCl) [m/z (relative intensity)]: 1383.9 ($M + 2K^+ - H^+$, 12), 1367.9 ($M + Na^+ + K^+ - H^+$, 28), 1345.9 ($M + K^+$, 97), 1329.8 ($M + Na^+$, 100), 1307.8 ($M + H^+$, 16), 692.3 ($M + 2K^+$, 51), 684.3 ($M + Na^+ + K^+$, 97), 676.3 ($M + 2Na^+$, 58), 673.3 ($M + H^+ + K^+$, 23), 665.3 ($M + H^+ + Na^+$, 17). High-resolution negative LSIMS: calcd for $C_{62}H_{85}N_2O_{24}S_2$, m/e 1305.4934; found, m/e 1305.4973.

Vesicle Experiments. The 5(6)-carboxyfluorescein (Sigma, 99%) was suspended in distilled water and slowly titrated to dissolution at pH = 7.50 with 0.1 M NaOH or 0.1 M KOH, to give on evaporation the CF sodium or potassium salts. The CF salts were dissolved in an appropriate amount of 10 mM tris-HCl (pH = 7.5) to obtain a 0.100 M CF-salt buffer solution. A buffer of 10 mM tris-HCl with 0.14 M NaCl or KCl buffer solution (pH = 7.5) was used for the external solution. Amphiphiles (2−5 mM) and divalent ion salts (chlorides, 20−50 mM) were dissolved in the tris-HCl/NaCl or KCl buffer solution. Compounds **1**⟨18,5⟩[8] and **12**[17] were prepared by following literature procedures and had the published spectral properties.

Vesicle entrapped CF was prepared from a dried film of a lipid mixture containing 8:1:1 PC/PA/cholesterol (50 mg) and 1 mL of 0.100 M CF salt solution. The lipids were suspended by slow swirling, and the suspension was rapidly frozen (liquid nitrogen) and thawed over 15 min at room temperature. The freeze−thaw cycle was repeated three times. The mixed vesicle suspension was then sonicated (Branson probe sonicator, 2 min, 50% power, 50% duty cycle) and allowed to stand at room temperature for a minimum of 2 h. The vesicles were sized through a 0.45 μm Nucleopore filter using nitrogen gas pressure, and the external CF was removed by gel permeation (Sephadex G-25, PD-10) with 10 mM tris-HCl/NaCl or KCl buffer (pH 7.5) as the eluent. Vesicle encapsulated CF in the opaque orange front fractions was completely separated the bright green later fractions (free CF). The vesicles were kept at 0−3 °C to reduce leakage. Vesicles older than 24 h were rechromatographed to remove free CF. A typical vesicle preparation contained 4.0 mM phospholipid (phosphomolybdate analysis), showed a bimodal size distribution (88% 470−500 nm diameter, 12% 130−150 nm diameter; laser light scattering, a NICOMP model 370), and was 85 ± 5% unilamellar (melittin assay).

For the membrane disruption assay, 20 μL aliquots of the vesicle dispersion were added into each of a series of disposable culture tubes (6 × 50 mm, Kimble) that contained 80 μL of buffer plus a given concentration of surfactants and divalent cations if needed. The resulting suspension was immediately vortex-mixed for ca. 10 s. After the mixtures were allowed to incubate at 23 °C for 30 min, a 35 μL aliquot was withdrawn and diluted into a 5.00 mL volumetric flask with the buffer prior to measurement of the fluorescence (excitation, 475 nm; emission, 515 nm). A blank value was obtained by carrying out a similar experiment in the absence of surfactant. The total fluorescence intensity value was determined by the addition of Triton X-100. The percentage of released CF was calculated according to I (%) = $100[I_a - I_b]/[I_x - I_b]$, where I_x is the 100% fluorescence intensity value and I_a and I_b are the fluorescence intensities after incubation with and without surfactant, respectively. Only vesicles with high self-quenching efficiency and high entrapment capacity (i.e., $I_a/I_b > 10$) were used.

Photochemical Experiments. Preparative photoirradiation was performed using a super-high-pressure mercury lamp installed in a illuminator with a shutter control for timing and a variable-wavelength monochromator. Samples were placed in capped quartz cell (3 mL) at ca. 3 cm distance from the exit slit. For the fluorescence of thioindigo derivatives, excitations was at 530 nm; the observed emission was centered at 575 nm.

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Supporting Information Available: ^{1}H and ^{13}C spectra of **2**⟨n⟩ (n = 14, 18, 20) and of **8** (predominantly trans), procedures and spectral data for the precursors of **9** and **10**, and melittin vesicle disruption titration data (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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