aldehyde **30** (0.22 g, 0.58 mmol) and iodoform (0.60 g, 1.55 mmol) in dry THF (4.0 mL) at 0 °C under N_2 was added a solution of 95% $CrCl_2$ (0.57 g, 4.65 mmol) in dry THF (4 mL) via cannula. The reaction mixture was stirred for 3 h before it was quenched with H_2O and extracted with Et_2O (3 × 10 mL). The combined ethereal extracts were dried over Na_2SO_4 and concentrated in vacuo. Purification of the resulting dark yellow oil by silica gel chromatography (5:1 hexane–ether) afforded 0.23 g (80%) of the vinyl iodide **46** as a 98:2 mixture of (*E*)- and (*Z*)-olefin isomers: R_f 0.48 (1:1 hexane–ether); $[\alpha]^{26}_D$ −24.0° (*c* = 0.2, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 5 H, aromatic), 6.50 (dd, *J* = 10.1, 1.0 Hz, 1 H), 6.44 (dd, *J* = 14.9, 7.7 Hz, 1 H), 6.29 (d, *J* = 14.9 Hz, 1 H), 4.91 (dd, *J* = 7.9, 5.2 Hz, 1 H), 4.54 (A of AB, J_{AB} = 11.8 Hz, 1 H), 4.31 (B of AB, J_{BA} = 11.8 Hz, 1 H), 3.71 (s, 3 H, ester methyl), 3.69 (m, 1 H), 2.92 (m, 1 H), 2.06 (m, 1 H), 2.01 (s, 3 H), 1.74 (d, *J* = 1.8 Hz, 3 H), 0.93 (d, *J* = 6.6 Hz, 3 H), 0.89 (d, *J* = 7.0 Hz, 3 H); IR (neat) 1730, 1715, 1650, 1605 cm^{-1}; MS *m/z* 393 ($M^+ - C_7H_7O$); HRMS for $C_{15}H_{22}O_4{}^{127}I$ ($M^+ - C_7H_7O$) calcd 393.0580, found 393.0579; HRMS for $C_{22}H_{29}O_5$ ($M^+ - {}^{127}I$) calcd 373.2036, found 373.2032.

Methyl (*E*,*E*,*E*,*E*)-(4*S*,5*R*,6*S*,7*S*)-5-Acetoxy-7-(benzyloxy)-14-hydroxy-2,4,6,12-tetramethyltetradeca-2,8,10,12-tetraenoate (25). To a 25 °C solution of vinylboronic acid **36** (25 mg, 0.16 mmol) and 10% TlOH (0.395 mL) in anhydrous THF (0.4 mL) under N_2 was added a premixed solution of vinyl iodide **46** (62 mg, 0.124 mmol) and $Pd(PPh_3)_4$ (32 mg, 0.025 mmol) in degassed THF (0.5 mL). After 4 min the light yellow reaction mixture was diluted with Et_2O (10 mL) and dried over anhydrous $MgSO_4$. The solution was filtered and concentrated in vacuo to give a crude product that was purified by silica gel chromatography (2:1 ether–hexane as eluent). In this way 44 mg (76%) of tetraene **25** was obtained: R_f 0.25 (1:1 hexane–EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 5 H, aromatic), 6.54 (dd, *J* = 10.1, 1.2 Hz, 1 H), 6.25 (m, 3 H), 5.69 (t, 1 H), 5.56 (dd, *J* = 14.5, 8.4 Hz, 1 H), 4.94 (dd, *J* = 7.2, 5.6 Hz, 1 H), 4.54 (A of AB, J_{AB} = 11.8 Hz, 1 H), 4.30 (m, 3 H, includes B of AB), 3.71 (m, 4 H), 2.97 (m, 1 H), 2.07 (m, 1 H), 2.00 (s, 3 H), 1.81 (s, 3 H), 1.77 (d, *J* = 1.2 Hz, 3 H), 1.33 (m, 1 H), 0.94 (d, *J* = 6.8 Hz, 3 H), 0.88 (d, *J* = 7.1 Hz, 3 H); MS *m/z* 361 ($M^+ - C_7H_7$).

Intramolecular Diels–Alder Reaction of Tetraene 25. A solution of **25** (44 mg, 0.094 mmol) and BHT (1.0 mg, 0.006 mmol) in anhydrous degassed toluene (3 mL) was transferred to a resealable Carius tube, sealed under N_2, and heated at 150 °C for 16 h. ^{1}H NMR analysis of the crude product showed a 90:10 mixture of two cycloadducts. Separation of this mixture by silica gel chromatography (2:1 hexane–ethyl acetate as eluent) provided 28 mg (64%) of cycloadduct **47** (64%) and 4 mg of a ca. 3:1 mixture of cycloadducts **47** and **48** (32 mg total; 73% combined yield). The minor cycloadduct **48** was isolated by preparative HPLC using a Waters system (1 mL/min) with a Magnum 9 Partisil (10 μm) silica column eluted with degassed 1.5:1 hexane–ethyl acetate. Retention times under these conditions were 67 (trans isomer **47**) and 79 min (cis isomer **48**).

Data for trans-fused cycloadduct **47**: R_f 0.23 (1:1 hexane–ethyl acetate); $[\alpha]^{26}_D$ −27.8° (*c* = 1.01, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (m, 5 H, aromatic), 6.11 (d, 10.3 Hz, 1 H), 5.46 (ddd, *J* = 10.3, 4.0,2.7 Hz, 1 H), 5.37 (t, 1 H), 4.69 (dd, *J* = 10.6 Hz, 1 H), 4.64 (A of AB, J_{AB} = 11.4 Hz, 1 H), 4.39 (B of AB, J_{BA} = 11.4 Hz, 1 H), 4.13 (m, 2 H), 3.51 (s, 3 H, ester methyl), 3.37 (dd, *J* = 11.0, 4.9 Hz, 1 H), 2.80 (m, 1 H), 2.62 (dd, *J* = 2.0, 2.0 Hz, 1 H), 2.13 (ddd, *J* = 11.0, 10.5, 4.0 Hz, 1 H), 2.08 (s, 3 H), 1.83 (m, 2 H), 1.56 (s, 3 H), 1.23 (s, 3 H), 0.99 (d, *J* = 7.0 Hz, 3 H), 0.68 (d, *J* = 6.2 Hz, 3 H); IR (neat) 3450, 1735, 1725, 1650 cm^{-1}; MS *m/z* 453 ($M^+ - H_2O$); HRMS for $C_{28}H_{36}O_5$ ($M^+ - H_2O$) calcd 453.2632, found 453.2635. Anal. Calcd for $C_{28}H_{38}O_6$: C, 71.46; H, 8.14. Found: C, 71.79; H, 7.91.

Data for cis-fused cycloadduct **48**: R_f 0.24 (1:1 hexane–ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 5 H, aromatic), 5.99 (ddd, *J* = 10.0, 3.0, 2.9 Hz, 1 H), 5.67 (dd, *J* = 10.0, 3.1, 2.5 Hz, 1 H), 5.42 (t, 1 H), 4.66 (A of AB, J_{AB} = 11.7 Hz, 1 H), 4.63 (B of AB, J_{BA} = 11.7 Hz, 1 H), 4.18 (m, 2 H), 3.65 (s, 3 H, ester methyl), 3.59 (dd, *J* = 4.0, 4.0 Hz, 1 H), 3.35 (m, 1 H), 2.56 (m, 1 H), 2.29 (dd, *J* = 6.9, 6.0 Hz, 1 H), 2.22 (m, 1 H), 2.05 (s, 3 H), 1.91 (m, 1 H), 1.84 (t, 1 H), 1.65 (s, 3 H), 1.19 (s, 3 H), 0.98 (d, *J* = 7.1 Hz, 3 H), 0.96 (d, *J* = 7.0 Hz, 3 H); IR ($CHCl_3$) 3440, 1730, 1725 cm^{-1}; HRMS for $C_{28}H_{38}O_6$ (M^+) calcd 470.2660, found 470.2663.

Conversion of Cycloadduct 47 to Diol 39. Cycloadduct **47** (3 mg, 0.006 mmol) was dissolved in anhydrous MeOH (0.5 mL) and treated with K_2CO_3 (0.1 mg) for 30 min at room temperature. Standard workup provided diol **39** that was identical to the material previously prepared from cycloadduct **37**.

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Supplementary Material Available: Copies of ^{1}H NMR spectra of **7**, **25**, **26**, **34**, **36**, the *N*-methyldiethanolamine complex of **36**, **38**, **39**, **44**, **45**, **46**, and **48** (11 pages). This material is contained in many 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.

Molecular Harpoons. Membrane-Disruptive Surfactants That Can Recognize Osmotic Stress in Phospholipid Bilayers[1]

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Abstract: A series of wedge-shaped, nonionic surfactant molecules (molecular harpoons) have been synthesized and used to disrupt large unilamellar vesicles derived from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), POPC/cholesterol (2/1), and POPC/cholesterol (55/45), under isotonic and hypotonic (osmotically stressed) conditions. The activity of each surfactant has been defined by measuring its ability to release vesicle-encapsulated 5(6)-carboxyfluorescein (CF). Comparative studies have also been carried out, using Triton X-100 as the disruptive agent. The principal results of this study establish that it is possible for a disruptive surfactant to distinguish between osmotically stressed and nonstressed membranes and that such recognition is a sensitive function of the surfactant's composition, structure, and oligomeric state, as well as the compactness of the target membrane and its degree of osmotic stress. The implications of these findings for the rational design of membrane-disrupting antimicrobial agents are briefly discussed.

Introduction

The recent emergence of life-threatening microorganisms such as HIV and *Mycobacterium tuberculosis* and the growing problem of drug resistance provide considerable impetus for devising fundamentally new approaches toward drug design.[2-6] We believe that membrane-disrupting drugs are ideally suited as therapeutic agents because microbes should be less able to develop resistance

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to them, compared with drugs that function at the nuclear or cytoplasmic level. Specifically, we believe that *drugs that are not required to enter the cytoplasm of a microbe should be less susceptible toward enzymatic degradation, and that they should also be insensitive toward export mechanisms of drug resistance, i.e., processes by which the drug is transported out of the microbe.* Data that have been obtained for the polyene macrolide antibiotic amphotericin B (AmpB) strongly support this view.[8-12] We note, in particular, that the development of drug resistance toward AmpB during its therapeutic use is extremely rare.[7] While AmpB, like most other membrane-disrupting drugs, shows only a modest preference for destroying microbes over mammalian cells, it has become the drug of choice for the treatment of the disseminated fungal infections found in immunocompromised AIDS and cancer patients.[13]

We have begun a program that is focusing on the design and synthesis of novel membrane-disrupting agents.[14-20] Our specific aim is to exploit differences in composition and packing between the plasma membrane of mammalian cells and the lipid membrane of fungi, bacteria, and enveloped viruses. Our ultimate goal is to create new classes of antimicrobial agents that have efficacies that are equal to or greater than that of AmpB. Recently, we have begun to explore the possibility of designing a disruptive agent that can "recognize" osmotic stress.[19] The fact that gram-positive and gram-negative bacteria are under considerable osmotic stress (i.e., 15–20 and 0.8–5 atm, respectively), together with the fact that the plasma membrane of mammalian cells is relatively stress-free, suggests to us that osmotic stress can serve as a definable target for the selective destruction of bacteria.[21] In addition, we have theorized that if enveloped viruses are also under stress (the osmotic state of enveloped viruses remains to be defined), then they too should be susceptible to "stress-seeking" drugs. It should be noted that unlike bacteria, which have lipid membranes that are cholesterol-free, enveloped viruses are rich in cholesterol.[22,23] Whether or not the presence of this sterol can affect the recognizability of a stressed membrane remains to be established. Because osmotically stressed membranes have packing densities that are less than those of stress-free analogues, penetration into their hydrophobic interior by a disruptive surfactant should be relatively easy. We further reasoned that this difference in accessibility would also depend on the size of the hydrophobic portion of the surfactant; i.e., the larger the size, the greater the difference.

With these ideas in mind, we sought (i) to establish whether or not membrane-disruptive agents could be created that recognize osmotic stress and (ii) to define the sensitivity of such recognition toward the presence of cholesterol.

Results and Discussion

Membrane Targets. Membrane targets that were chosen for this investigation consisted of virus-size, large unilamellar vesicles (1000 Å) of varying phosphatidylcholine/cholesterol composition. In contrast to structurally complex biological membranes, such model systems allow us to study osmotic stress recognition in a well-defined and systematic manner. When large unilamellar vesicles are first prepared via freezing–thawing and extrusion, the concentration of ionic solute within their aqueous interior equals that of the external phase.[24,25] The membrane is thus formed under isotonic conditions, and its compactness is maximized through hydrophobic interactions.[26] Because water diffuses across lipid bilayers much faster than ionic solutes, dilution of such dispersions with aqueous solutions of lower tonicity transforms the vesicles into a swollen state and places the bilayer under osmotic stress.[12] Under such conditions, water from the hypertonic side pushes against the inner leaflet, thereby increasing hydrocarbon exposure at the membrane–hypotonic interface; i.e., the area that is occupied/lipid molecule increases due to osmotic stress. Alternatively, if these vesicles are exposed to a hypertonic solution, they tend to shrink due to egress of water from within their interior. The driving force for both osmotic swelling and shrinking derives from the system's tendency to eliminate osmolarity (and osmotic presure) gradients.

In order to create osmotically stressed vesicular membranes and, at the same time, to use such membranes for defining the recognition properties of our synthetic surfactants, we have chosen to encapsulate *varying concentrations of the ionic fluorophore* 5(6)-carboxyfluorescein (CF). Specifically, we take advantage of both the fluorescent features of this salt and its ability to alter the osmotic state within the vesicle interior. At high intravesicular concentrations (≥50 mM), CF has negligible fluorescence due to efficient self-quenching. As the fluorophore is diluted, by being released into the external phase, the fluorescence intensity of the dispersion increases.[27,28] This increase can then be used to estimate the percentage of CF that is released, according to eq 1.

$$I\ (\%) = 100(I_a - I_b)/(I_x - I_b) \qquad (1)$$

Here, I_x refers to the fluorescence intensity that is associated with the release of 100% of the dye (determined via addition of excess Triton X-100); I_b and I_a represent the fluorescence intensities before and after incubation with a given disruptive surfactant, respectively, when there is incomplete release.[27]

The specific membrane targets that we have selected for this investigation were derived from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), POPC/cholesterol (2/1), and POPC/cholesterol (55/45). At room temperature, POPC bilayers exist in the physiologically relevant fluid phase.[29] Addition of 45 mol % cholesterol significantly increases the compactness of such a membrane and creates an overall phospholipid/cholesterol content that is similar to that which is present in human red blood cells and in the HIV viral envelope.[22]

Osmotic Responsiveness of the Vesicular Targets. In order to define the osmotic responsiveness of these targets, we have measured their specific internal volume as a function of an applied osmotic gradient. Experimentally, such gradients can be imposed via an appropriate adjustment in the external salt concentration. The maximum osmotic stress that can be introduced into the lipid bilayer depends on the initial osmolarity gradient and on the ability of the vesicle to behave as an osmometer. One may view this

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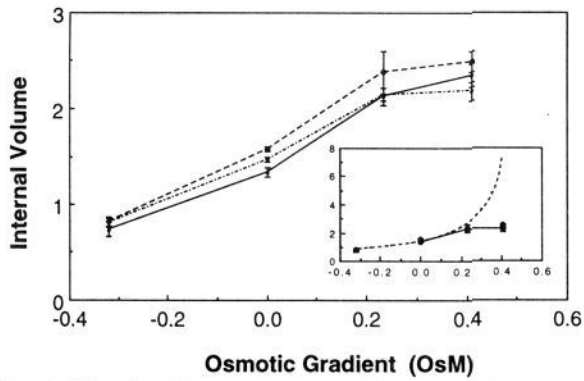


Figure 1. Plot of specific internal volume (L/mol of phospholipid) as a function of applied osmotic gradient for vesicles made from POPC (----), POPC/cholesterol (2/1) (—), and POPC/cholesterol 55/45) (---). The insert shows osmotic curves over a wider range of internal volumes (—) and a plot of internal volume of hypothetical vesicles exhibiting ideal osmotic responsiveness (---).

maximum stress either in terms of the initial osmolarity gradient or as the initial internal osmotic pressure, P. Here, $P = RT(C_v - C_b)$, and C_v and C_b represent the initial osmolarity within the vesicles and in the external buffer, respectively. If the vesicles were to behave as ideal osmometers, then their internal volume should increase in response to a hypotonic solution, such that the influx of pure water reduces the osmotic pressure gradient to 0. Here, all of the osmotic pressure is thus converted into osmotic stress. Alternatively, addition of a hypertonic solution should eliminate the pressure gradient by driving water out of the vesicles, thereby causing them to shrink in size. Specific internal vesicular volumes were determined by measurement of the intravesicular concentrations of CF, using appropriate calibration curves.[30,31] The latter were constructed from plots of the fluorescence self-quenching efficiency of CF as a function of its intravesicular concentration, under isotonic conditions (see the Experimental Section).

Figure 1 shows that vesicles made from POPC, POPC/cholesterol (2/1), and POPC/cholesterol (55/45) are osmotically responsive, but that they can deviate from ideality. This deviation is particularly significant when the gradient exceeds ca. +234 mosM. Here, we define an osmotic gradient as being positive when the external phase is hypotonic. Thus, when an osmolarity gradient of +234 mosM (equalling +5.7 atm) is applied to these vesicles, a residual osmotic pressure of ca. +2 atm persists. However, when the initial gradient is +552 mosM (+13.8 atm), the residual internal pressure is ca. +6 atm. Thus, a smaller percentage of the initial osmotic pressure is converted into osmotic stress when higher osmolarity gradients are employed. This nonideality is a likely consequence of membrane curvature plus strong van der Waals forces that limit the exposure of the hydrophobic portion of the bilayer to the aqueous phase. Figure 1 further shows that the presence of cholesterol does not significantly affect the osmotic behavior of these targets over the range of osmolarity that was employed.

In order to judge the ability of these targets to maintain osmotic stress, we have measured the internal volume of a stressed POPC/cholesterol (2/1) membrane (initial gradient of +234 mosM) as a function of time. Figure 2 shows that this gradient can, in fact, be maintained for more than 5 h. Moreover, readjustment of the external phase to isotonic conditions returns the vesicles to their original unstressed state (Figure 3). Thus, the osmotic responsiveness of these membranes is reversible.

Molecular Harpoons. Based on current supramolecular theory, wedge-shaped surfactant molecules are expected to pack poorly with cylindrically shaped phospholipids in the bilayer state.[32,33]

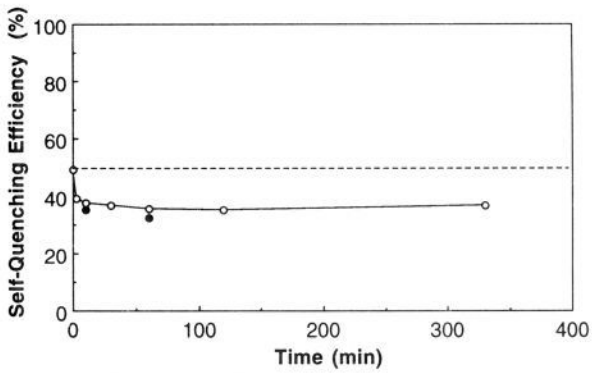


Figure 2. Self-quenching efficiency of 10 mM CF within POPC/cholesterol (2/1) vesicles that have been exposed to an osmotic gradient of +234 mosM, as a function of time: open circles (○) represent data obtained directly; closed circles (●), data obtained after first subjecting the vesicles to gel filtration in order to ensure re.noval of nonentrapped CF.

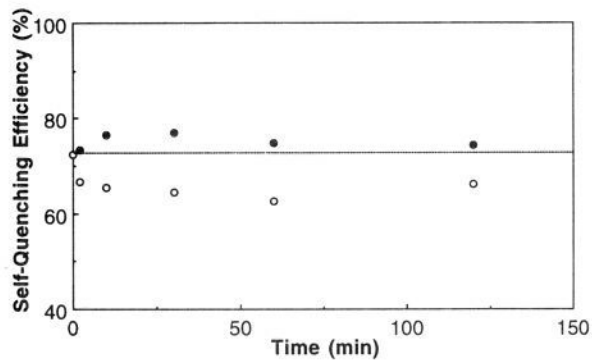
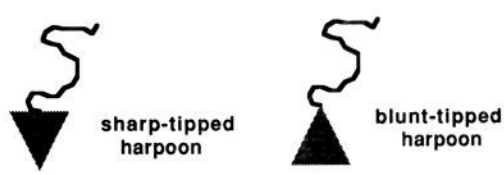


Figure 3. Self-quenching efficiency of 20 mM CF within POPC/cholesterol (2/1) vesicles that have been exposed to an osmotic gradient of +234 mosM (○) and exposed to an osmotic gradient of +234 mosM and then readjusted to isotonic conditions just prior to measurement (●), plotted as a function of time.

In principle, one may envision that a membrane-disruptive surfactant, comprising a *rigid, wedge-shaped hydrophobic unit* attached to a hydrophilic chain, might be able to recognize osmotic stress in lipid bilayers. Specifically, we reasoned that a hydrophobic wedge might favor insertion into a hypotonic leaflet over an isotonic analogue due to reduced steric hindrance and greater access to the hydrophobic region of the bilayer. On the basis of their overall molecular shape and their expected sensitivity toward osmotic stress, we viewed such molecules as "molecular harpoons". Whether or not the orientation of the hydrophobic tip would be an important factor in osmotic stress recognition, however (i.e., sharp versus blunt tips), was not obvious.



Specific surfactants that were chosen as prototypes for investigation are shown as structures **1**–**5**. Harpoons **1a** and **2a**, bearing *tert*-butyl and phenyl groups in the 2- and 6-positions, respectively, were considered as sharp-tipped harpoons; isomers **1b** and **2b** were considered as "blunt-tipped" analogues. Harpoons **3** and **4** bear a resemblance to **1a** and **1b**, except that they contain a hydrophobic spacer between the tip and the hydrophilic chain and also an ester moiety. In addition, **3** bears a methyl group in the para position which also increases its hydrophobicity and sharpness. It is

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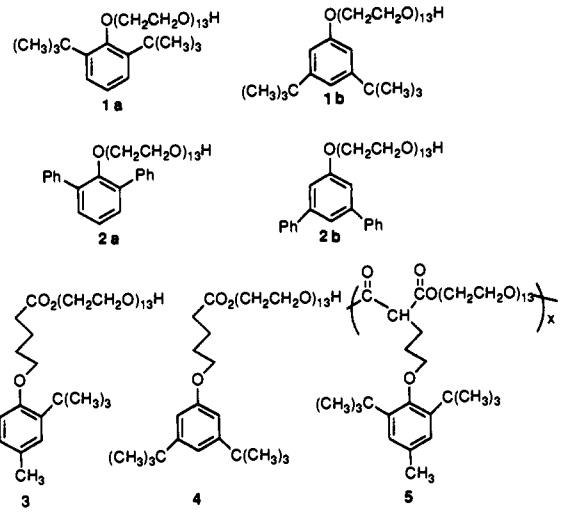
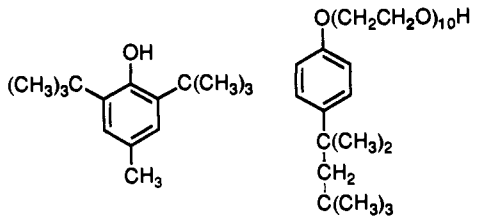
Harpoons



noteworthy that the parent phenol, 2,6-di-*tert*-butyl-4-methylphenol (BHT), is a potent inactivator of certain enveloped viruses.[34] It is also noteworthy that the antiviral activity of BHT has been attributed to its membrane-disrupting properties.[34-36] Harpoon **5** represents an oligomeric form of **3**. Finally, Triton X-100, which has a compositional but not a geometrical similarity to **1a** and **1b** (CPK suggest a "boxing glove" type of geometry), was also examined because of its extensive use as a membrane-disrupting agent. Thus, surfactants **1–5** were synthesized according to the synthetic routes outlined in Schemes I–III.



BHT Triton X-100

Membrane-Disrupting Selectivity. In order to compare the stress-recognition properties of each harpoon, we have measured the activity of the harpoons in inducing the release of CF from target vesicles under isotonic and hypotonic conditions.[15-19,37] Here, we define membrane-disrupting activity as R_{50} values, where R_{50} represents the ratio of phospholipid/surfactant (or repeat unit) that is needed to induce the release of 50% of the entrapped CF from a 3.5 μM (phospholipid) dispersion of vesicles after 30 min at 25 °C.[19] From an operational standpoint, the higher the R_{50} value, the greater the membrane-disrupting activity. In this work, each harpoon was investigated below its critical micelle concentration (cmc) in order to ensure that the observed recognition features reflect those of an attacking *monomer*. Specific cmc values that have been determined for **1a**, **1b**, **2a**, **2b**, **3**, **4**, and **5** were 0.82, 1.1, 0.57, 1.0, 0.09, 0.21, and 0.014 mM, respectively.

In Table I, we summarize the recognizability of pure POPC targets by harpoons **1–5** and also by Triton X-100. In each case, the activity of the surfactant was found to be greater against the osmotically stressed target; i.e., the ratio of R_{50} (hypotonic)/R_{50} (isotonic) was greater than 1.0. With the exception of **2b**, the

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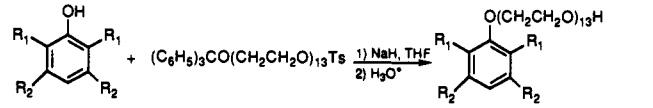
Scheme I



1a, R_1=C(CH3)3; R_2=H
b, R_1=H; R_2=C(CH3)3
2a, R_1=C6H5; R_2=H
b, R_1=H; R_2=C6H5

Table I. Harpoon Recognition of Osmotic Stress in POPC Targets

harpoon	$R_{50\text{-iso}}$[a]	$R_{50\text{-hypo}}$[b]	$R_{50\text{-hypo}}/R_{50\text{-iso}}$
1a	0.010 ± 0.001	0.016 ± 0.001	1.6
1b	0.0075 ± 0.0006	0.016 ± 0.0004	2.1
2a	0.016 ± 0.003	0.047 ± 0.011	2.9
2b	0.02 ± 0.01	0.11 ± 0.01	6
3			
4[c]	0.043	0.056	1.3
5[c]	0.90	2.7	3
Triton X-100[c]	0.038	0.047	1.2

[a] R_{50} value under isotonic conditions; average of data from two independent series of experiments ±1 SD. [b] R_{50} value under hypotonic conditions (initial osmolarity gradient of +552 mosM); average of data from two independent series of experiments ±1 SD. [c] Data from a single set of experiments.

Table II. Harpoon Recognition of Osmotic Stress in POPC/Cholesterol (2/1) Targets

harpoon	$R_{50\text{-iso}}$[a]	$R_{50\text{-hypo}}$[b]	$R_{50\text{-hypo}}/R_{50\text{-iso}}$
1a	0.0041 ± 0.0011	0.018 ± 0.001	4.4
1b	0.002 ± 0.001	0.011 ± 0.002	5.5
2a	<0.001	0.029 ± 0.002	>29
2b	<0.001	0.069 ± 0.004	>69
3	0.063 ± 0.008	0.12 ± 0.01	1.9
4	<0.001	0.11 ± 0.01	>110
5	<0.005	0.63 ± 0.18	>126
Triton X-100	0.018 ± 0.001	0.040 ± 0.003	2.2

[a] R_{50} value under isotonic conditions; average of data from two independent series of experiments ±1 SD. [b] R_{50} value under hypotonic conditions (initial osmolarity gradient of +552 mosM); average of data from two independent series of experiments ±1 SD.

Table III. Harpoon Recognition of Osmotic Stress in POPC/Cholesterol (55/45) Targets

harpoon	$R_{50\text{-iso}}$[a]	$R_{50\text{-hypo}}$[b]	$R_{50\text{-hypo}}/R_{50\text{-iso}}$
1a	<0.001	0.009 ± 0.001	>9
1b	<0.001	0.007 ± 0.002	>7
2a	<0.001	0.025 ± 0.003	>25
2b	<0.001	0.045 ± 0.007	>45
3	<0.005	0.11 ± 0.01	>22
4	<0.001	0.025 ± 0.004	>25
5	<0.005	0.94 ± 0.61	>188
Triton X-100	0.064 ± 0.002	0.061 ± 0.007	0.95

[a] R_{50} value under isotonic conditions; average of data from two independent series of experiments ±1 SD. [b] R_{50} value under hypotonic conditions (initial osmolarity gradient of +552 mosM); average of data from two independent series of experiments ±1 SD.

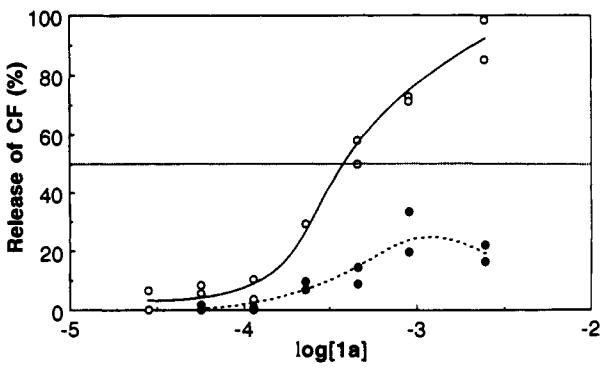
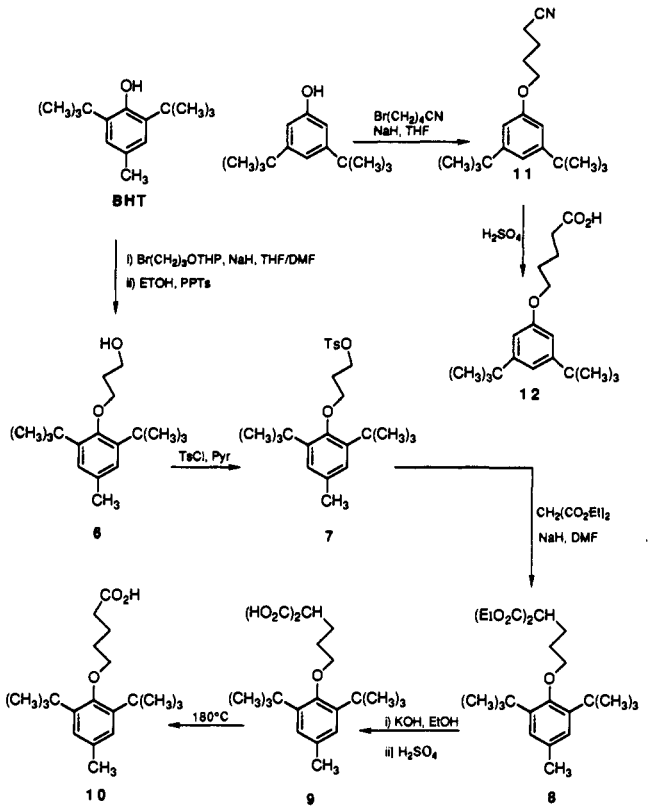
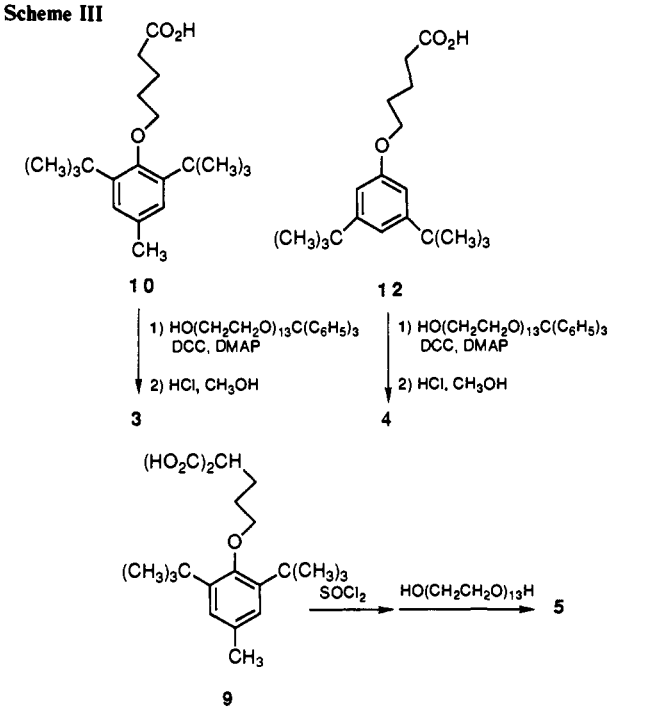


Figure 4. Plot of the percentage of CF released from POPC/cholesterol (55/45) vesicles as a function of concentration of **1a** under isotonic (●) and hypotonic (+552 mosM) conditions (○).

Scheme II



Scheme III



degree of recognition that was observed was modest. In contrast, analogous targets that contained 33 or 45 mol % cholesterol were significantly more recognizable by the harpoons; i.e., the difference in harpoon activity between stressed and unstressed bilayers was much greater (Tables II and III). Graphic illustrations of this recognition are presented in Figures 4 and 5 for **1a** and **1b** acting on POPC/cholesterol (55/45) targets, respectively. Here, the extent of release of CF (after 30 min) is plotted as a function of harpoon concentration under isotonic and hypotonic conditions.

The substantial increase in osmotic stress recognition that is observed upon inclusion of cholesterol is a direct consequence of increased stabilization of the isotonic over the hypotonic state.

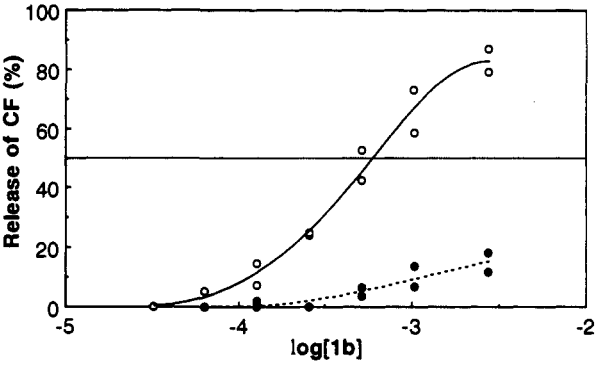


Figure 5. Plot of the percentage of CF released from POPC/cholesterol (55/45) vesicles as a function of concentration of **1b** under isotonic (●) and hypotonic (+552 mosM) conditions (○).

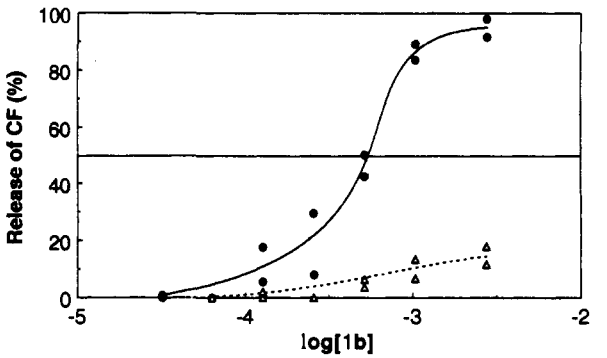


Figure 6. Plot of percentage of CF release from POPC (●) and POPC/cholesterol (55/45) vesicles (△) as a function of the concentration of **1b**, under isotonic conditions.

This conclusion is supported by a comparison of the R_{50} (isotonic) values in Tables I and III. A graphic illustration of this effect is presented in Figure 6. Exposure of cholesterol-rich targets to osmotic stress, however, leads to a significant increase in harpoon-lability (Figure 5). In striking contrast to the results that we have obtained with these harpoons, the nonwedge analogue (Trition X-100) has been found to exhibit a negligible degree of stress recognition (Tables I–III).

A plausible explanation that can account for the stress-recognition features of the harpoons, and also of Triton X-100, is based on the condensing effect that cholesterol has on the liquid-crystalline phase and the size and shape of the hydrophobic segments of these surfactants.[23] We hypothesize that the presence of cholesterol makes the penetration of the disruptive surfactants into the lipid bilayer difficult under isotonic conditions and especially difficult for the bulky harpoons. Molecular model (CPK) examination indicates that the hydrophobic wedges of **1** and **2** span a distance of 10.9 and 13.8 Å, respectively, at their broadest point. In contrast, Triton X-100 spans a maximum width of 6.5 Å. Thus, insertion of Triton X-100 into a compact fluid membrane should be much easier than insertion of these harpoons. When the cholesterol-rich membrane is placed under osmotic stress, however, its compactness is decreased and harpoon entry is made easier. It is noteworthy that the membrane-disrupting activity of all of the surfactants, acting on stressed and nonstressed POPC targets, shows an approximate relationship to the cmc values; i.e., the lower the cmc, the greater the R_{50} (Figure 7A). A similar correlation is also evident when osmotically stressed POPC/cholesterol (2/1) targets are used (Figure 7B). It appears, therefore, that when the hydrophobic region of the bilayer is readily accessible to a disruptive agent (either as a loosely packed fluid membrane or as an osmotically stressed cholesterol-rich membrane), the disruptive power of the surfactant depends more on its overall hydrophobicity than on its specific structure.

For the homologous series of surfactants **1a**, **1b**, **2a**, and **2b**, those harpoons bearing a wider tip exhibited a greater degree of stress recognition, regardless of tip orientation. Thus, **2a** and **2b**,

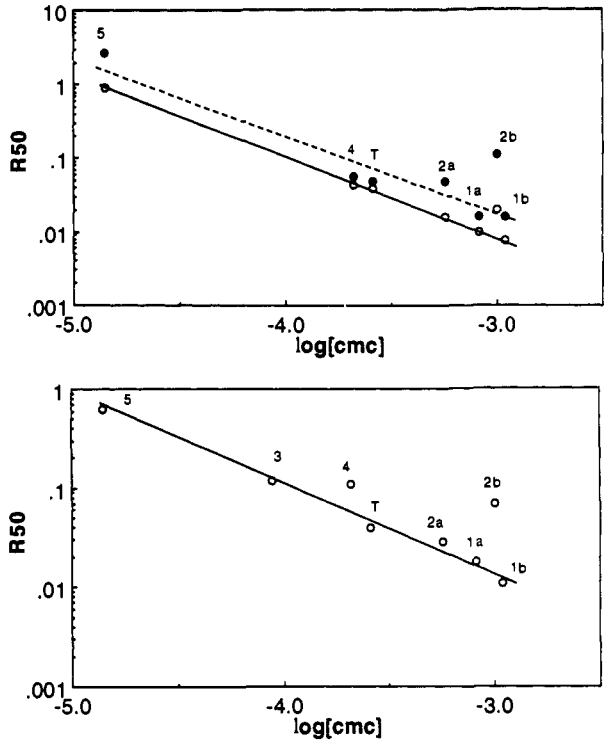


Figure 7. (A) Plot of R_{50} as a function of cmc for disruptive surfactants attacking POPC vesicles under isotonic (○) and hypotonic (+552 mosM) conditions) (●); isotonic data for each surfactant appear directly below the corresponding hypotonic data. (B) Plot of R_{50} as a function of cmc for disruptive surfactants attacking POPC/cholesterol (2/1) vesicles under hypotonic (+552 mosM) conditions (○).

which have phenyl groups placed either at the ortho or meta position, were more sensitive in recognizing osmotic stress than **1a** and **1b**, which have a *tert*-butyl substitution. These results are consistent with the large differences in stress recognition that were observed between the harpoons and Triton X-100. Comparison of **1a** with **1b**, and of **2a** with **2b**, further indicates that the recognition features of a harpoon are not greatly dependent on the orientation of the hydrophobic tip. Although **4** was found to be more sensitive to osmotic stress than **3**, this difference appears to be due to the greater hydrophobicity and disruptive power of the latter against the isotonic targets, as a consequence of the *p*-methyl group. The much higher degree of stress recognition that has been observed for the oligomeric surfactant, **5**, compared with its monomeric analogue, **3** (Table II), reflects its relatively high activity toward the stressed target and its low activity toward the nonstressed analogue. While the former may be rationalized in terms of cooperativity in membrane binding and/or pore formation among the covalently coupled repeat units, the reason for the lower activity against the stress-free membrane is not presently clear. Finally, we note that the degree of osmotic recognition that can be achieved is also dependent upon the degree of stress that is introduced into the bilayer. This conclusion is supported by the data that is presented in Table IV for surfactants **4**, **5**, and Triton X-100 acting on POPC/cholesterol (2/1) targets.

Taken as a whole, these results demonstrate that it is possible for a disruptive surfactant to distinguish between osmotically stressed and nonstressed membranes and that such recognition is a sensitive function of the surfactant's composition, structure, and oligomeric state, as well as the compactness of the target membrane and its degree of osmotic stress. These results not only are of interest from a theoretical standpoint but also support our hypothesis that osmotic stress may serve as a viable target in bacterial (and possibly viral) membranes. How much fine-tuning is possible via molecular design and how much osmotic stress recognition can be observed in biological targets are issues that we are currently addressing. The results of these efforts will be reported in due course.

Table IV. Pressure Sensitivity of Harpoons **4** and **5** and Triton X-100 toward POPC/Cholesterol (2/1)

internal vesicle pressure (atm)	R_{50}		
	4[a]	**5**[a]	Triton X-100[a]
0.0	<0.001	<0.005	0.018 ± 0.001
+5.7	0.023 ± 0.004	0.22 ± 0.16	0.030 ± 0.004
+13.8	0.11 ± 0.01	0.63 ± 0.18	0.040 ± 0.003

[a] Average of data from two independent series of experiments ±1 SD.

Experimental Section

General Methods. Unless stated otherwise, all reagents and chemicals were obtained from Aldrich Chemical Co. and used without further purification. Tetrahydrofuran (THF) and ethyl ether were distilled from sodium benzophenone ketyl. Methylene chloride was purified by distillation over $CaCl_2$. Chloroform and methanol that were used for chromatography were HPLC grade (Burdick & Jackson). Tridecaethylene glycol was prepared from tetraethylene glycol monotrityl ether and pentaethylene glycol di-*p*-toluenesulfonate, using procedures similar to those described previously.[16] 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was obtained from Avanti Polar Lipids (Birmingham, AL) as a chloroform solution and was used directly. 5(6)-Carboxyfluorescein (CF) was obtained from Eastman Kodak and purified according to literature methods.[27] Cholesterol was purchased from Fluka. House-deionized water was purified using a Millipore Milli-Q filtering system containing one carbon stage and two ion-exchange stages. Chromatographic separations were carried out by use of precoated Merck 0.25-mm silica gel 60 TLC plates (with fluorescent indicator) and Merck 70-230 ASTM silica gel. ^{1}H NMR, IR, and UV spectra were recorded on JEOL FX 90Q, Perkin-Elmer 283, and MILTON ROY Spectronic 1201 spectrometers, respectively. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Mass spectral analyses were performed at the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, NE. Vesicle extrusions were carried out using a 0.5-mL AVESTIN LiposoFast extruder (Ottawa, Canada). All fluorescence measurements were made using a Perkin-Elmer LS-50 luminesence spectrometer. Excitation of CF was at 491 nm; the observed emission was measured at 521 nm. Critical micelle concentrations were determined in 10 mM borate buffer (pH 7.4, 140 mM NaCl, 2 mM NaN_3) by means of surface tension measurements using a Nima Model ST tensiometer (Coventry, U.K.). Dynamic light scattering measurements were carried out by use of a Nicomp 270 submicrometer particle analyzer, equipped with a helium–neon laser (632.8 nm, scattering angle of 90°) and a computing autocorrelator. Phosphorus analyses were performed using methods described previously.[38] The osmolarity of CF and buffer solutions was measured by use of a Micro-Osmette osmometer (freezing point depression).

Tridecaethylene Glycol Monotrityl Ether. To a solution made from tridecaethylene glycol (809 mg, 1.37 mmol), triethylamine (140 mg, 1.4 mmol), and 5 mL of anhydrous CH_2Cl_2 was added trityl chloride (390 mg, 1.40 mmol). The resulting solution was stirred at ambient temperature for 3.5 h, followed by dilution with 15 mL of CH_2Cl_2, washing with water (2 × 10 mL), and drying ($MgSO_4$). Removal of solvent under reduced pressure left an oily residue, which was chromatographed (silica; $CHCl_3$ followed by $CHCl_3/CH_3OH$, 20:1, v/v), affording 383 mg (34%) of the monoprotected tridecaethylene glycol as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.92 (s, 1 H), 3.22 (t, 2 H), 3.68 (br s, 50 H), 7.15–7.5 (m, 15 H). Anal. Calcd for $C_{45}H_{68}O_{14}$: C, 64.88; H, 8.23. Found: C, 64.58; H, 8.23.

Tosyltridecaethylene Glycol Monotrityl Ether. Tridecaethylene glycol monotrityl ether (1.2 g, 1.4 mmol) was dissolved in a solution made from 10 mL of dry THF and 0.5 mL triethylamine. After addition of 0.4 g (2 mmol) of *p*-toluenesulfonyl chloride, the resulting mixture was stirred at room temperature for 91 h, filtered, and concentrated under reduced pressure. The residual oil was chromatographed on silica gel using 1% MeOH in CH_2Cl_2 followed by 4% MeOH in CH_2Cl_2 to give 1.1 g (80%) of tosyltridecaethylene glycol monotrityl ether as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3 H), 3.25 (t, 2 H), 3.65 (br s, 48 H), 4.16 (t, 2 H), 7.2–7.5 (m, 17 H), 7.80 (d, 2 H). Anal. Calcd for $C_{52}H_{74}O_{16}S$: C, 63.27; H, 7.56. Found: C, 62.99; H, 7.54.

3,5-Diphenylanisole. To a mixture of [1,2-bis(diphenylphosphino)-ethane]nickel(II) chloride (60 mg, 0.11 mmol), 3,5-dichloroanisole (1.0 g, 5.65 mmol), and 10 mL of dry Et_2O was added phenylmagnesium bromide (15 mmol in 5.0 mL of Et_2O) at 0 °C. The resulting mixture

(38) Regen, S. L.; Kirszenstejn, P.; Singh, A. *Macromolecules* **1983**, *16*, 335.

was refluxed for 54 h and hydrolyzed by addition of 10 mL of 10% hydrochloric acid. The organic layer was washed with water (3 × 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Chromatographic purification of the residue (silica gel; 2% $CHCl_3$ in *n*-hexane followed by 10% $CHCl_3$ in *n*-hexane) afforded 0.47 g (31%) of 3,5-diphenylanisole as a colorless solid. Recrystallization from *n*-hexane gave 0.32 g of product: mp 91–92 °C; ^1H NMR ($CDCl_3$) δ 3.90 (s, 3 H), 7.10 (d, 2 H), 7.3–7.7 (m, 11 H). Anal. Calcd for $C_{19}H_{16}O$: C, 87.66; H, 6.19. Found: C, 87.42; H, 6.42.

3,5-Diphenylphenol. A 10-mL round-bottomed flask, fitted with a No-Air septum and a magnetic stirring bar, was charged with 3,5-diphenylanisole (85 mg, 0.32 mmol) and 0.5 mL of dry CH_2Cl_2. Iodotrimethylsilane (0.2 mL, 1.4 mmol) was then added via syringe while a nitrogen atmosphere was maintained. The mixture was stirred at room temperature for 90 h and then diluted with 1.6 mL of methanol. After partial removal of solvent (ca. 0.4 mL by rotary evaporation), 4 mL of ether was added, and the solution was washed with sodium bisulfite and saturated sodium chloride and dried (Na_2SO_4). Subsequent concentration under reduced pressure followed by preparative TLC (silica; CH_2Cl_2) afforded an oily residue, which was recrystallized from *n*-hexane to give 59 mg (75%) of 3,5-diphenylphenol as white needlelike crystals: mp 94–95 °C (lit.[39] mp 93–94 °C); ^1H NMR ($CDCl_3$) δ 5.00 (br s, 1 H), 7.02 (d, 2 H), 7.3–7.7 (m, 11 H).

Tridecaethylene Glycol 2′,6′-Di-*tert*-butylphenyl Ether (1a). A 60% NaH dispersion in mineral oil (0.05 g, 1.2 mmol) was washed with *n*-hexane (2 × 1 mL) and then added to a solution made from 2,6-di-*tert*-butylphenol (22 mg, 0.11 mmol) and 1 mL of anhydrous THF. After the evolution of hydrogen had ceased, 1 mL of an anhydrous THF solution containing tosyltridecaethyleneglycol monotrityl ether (100 mg, 0.10 mmol) was added. The mixture was placed in an oil bath (90 °C), stirred under reflux conditions (under nitrogen) for 4 h, and cooled to room temperature. The product mixture was then concentrated under reduced pressure, diluted with 2 mL of $CHCl_3$, and washed with water (2 mL). The water phase was extracted with $CHCl_3$ (2 × 2 mL), and the combined organic phase was dried (Na_2SO_4). Removal of solvent under reduced pressure afforded a residue, which was purified by preparative TLC (silica; $CHCl_3$/MeOH, 20/1, v/v; R_f = 0.40). Deprotection was carried out by dissolving this product in a solution made from 2 mL of $CHCl_3$ and 1 mL of MeOH, adding one drop of concentrated HCl, and allowing the mixture to stir at room temperature for 1 h. Concentration under reduced pressure and purification by preparative TLC (silica; $CHCl_3$/MeOH, 20:1, v/v; R_f = 0.24) afforded 39 mg (50%) of **1a** as a pale yellow oil: ^1H NMR ($CDCl_3$) δ 1.32 (s, 18 H), 2.70 (br s, 1 H), 3.65 (br s, 50 H), 4.16 (t, 2 H), 6.78 (d, 2 H), 7.03 (t, 1 H). Anal. Calcd for $C_{40}H_{74}O_{14}$: C, 61.67; H, 9.57. Found: C, 61.59; H, 9.56.

Tridecaethylene Glycol 3′,5′-Di-*tert*-butylphenyl Ether (1b). Compound **1b** was prepared in 68% isolated yield (53 mg) from 3,5-di-*tert*-butylphenol (22 mg, 0.11 mmol) and tosyltridecaethylene glycol monotrityl ether (100 mg, 0.10 mmol), using procedures similar to those described for the preparation of **1a**. The ether was obtained as a pale yellow oil: ^1H NMR ($CDCl_3$) δ 1.32 (s, 18 H), 2.70 (br s, 1 H), 3.65 (br s, 50 H), 4.16 (t, 2 H), 6.78 (d, 2 H), 7.03 (t, 1 H). Anal. Calcd for $C_{40}H_{74}O_{14}$: C, 61.67; H, 9.57. Found: C, 61.22; H, 9.45.

Tridecaethylene Glycol 2′,6′-Diphenylphenyl Ether (2a). Compound **2a** (51 mg, 63%) was prepared from 2,6-diphenyl phenol (27 mg, 0.11 mmol) and tosyltridecaethylene glycol monotrityl ether (100 mg, 0.10 mmol) as a pale yellow oil: ^1H NMR ($CDCl_3$) δ 1.32 (s, 18 H), 2.70 (br s, 1 H), 3.65 (br s, 50 H), 4.16 (t, 2 H), 6.78 (d, 2 H), 7.03 (t, 1 H). Anal. Calcd for $C_{44}H_{66}O_{14}$: C, 64.53; H, 8.12. Found: C, 64.78; H, 8.34. Exact mass calcd for $C_{44}H_{66}O_{14}$ (M + H): 819.4532. Found: 819.4537.

Tridecaethylene Glycol 3′,5′-Diphenylphenyl Ether (2b). Compound **2b** (52 mg, 64%) was prepared from 3,5-diphenylphenol (27 mg, 0.11 mmol) and tosyltridecaethylene glycol monotrityl ether (100 mg, 0.10 mmol) as a pale yellow oil: ^1H NMR ($CDCl_3$) δ 1.32 (s, 18 H), 2.70 (br s, 1 H), 3.65 (br s, 50 H), 4.16 (t, 2 H), 6.78 (d, 2 H), 7.03 (t, 1 H). Anal. Calcd for $C_{44}H_{66}O_{14}$: C, 64.53; H, 8.12. Found: C, 64.35; H, 8.08. Exact mass calcd for $C_{44}H_{66}O_{14}$ (M + H): 819.4532. Found: 819.4558.

1-Bromo-3-[(2′-tetrahydropyranyl)oxy]propane. 3-Bromo-1-propanol (25.0 g, 0.17 mol) was dissolved in 100 mL of CH_2Cl_2, and the solution was cooled to 0 °C using an ice bath. To this solution was added, dropwise, 32 mL (0.35 mol) of 3,4-dihydro-2*H*-pyran, followed by addition of 200 mg (0.8 mol) of pyridinium *p*-toluenesulfonate (PPTS). The reaction mixture was then warmed to ambient temperature, stirred for 2 h, and concentrated to ca. one-half of its initial volume. After addition of diethyl ether (100 mL), the mixture was washed with water (3 × 50 mL) and saturated NaCl (1 × 50 mL) and dried over anhydrous Na_2CO_3. The solution was concentrated under reduced pressure at 50 °C, and the residue was distilled to give 31.5 g (83%) of 1-bromo-3-[(2′-tetrahydropyranyl)oxy]propane: bp 86–87 °C (3 mm Hg); lit.[40] bp 85–90 °C (4 mm Hg); ^1H NMR ($CDCl_3$) δ 1.6 (m, 6 H), 2.13 (quint, 2 H), 3.53 (t, 2 H), 3.38–4.0 (m, 4 H), 4.59 (m, 1 H).

3-[(2′,6′-Di-*tert*-butyl-4′-methyl)phenoxy]-1-propanol (6). A 60% NaH dispersion in mineral oil (1.05 g, 25 mmol) was washed with anhydrous benzene (2 × 5 mL) and suspended in 40 mL of anhydrous THF. BHT (4.40 g, 20.0 mmol) was gradually added during a 5-min period. After hydrogen evolution ceased, 10 mL of anhydrous DMF was added to the mixture, followed by the dropwise addition of 1-bromo-3-[(2-tetrahydropyranyl)oxy]propane (9.44 g, 42 mmol). The mixture was then placed in an oil bath (90 °C) and stirred at reflux conditions under a nitrogen atmosphere for 40 h (pale yellow NaBr precipitated). The mixture was then concentrated by partial solvent evaporation under reduced pressure and diluted with 100 mL of ethyl ether. The resulting solution was washed with water (4 × 50 mL) and saturated NaCl (2 × 25 mL) and dried ($MgSO_4$). The solvent was then removed under reduced pressure, and the residue (ca. 11 g) was dissolved in 50 mL of 95% EtOH. After addition of PPTS (250 mg, 1.0 mmol), the solution was stirred for 4 h at 65 °C. The product mixture was concentrated under reduced pressure, and the residue was dissolved in 100 mL of ethyl ether (resulting in the precipitation of PPTS). After being washed with water (5 × 30 mL) and saturated NaCl (2 × 20 mL) and dried ($MgSO_4$), the solution was concentrated under reduced pressure, and the desired product (**6**) was purified by column chromatography (silica; $CHCl_3$ followed by $CHCl_3$/CH_3OH, 30:1, v/v) to give 2.36 g (42%) of **6** as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.42 (s, 18 H), 2.08 (t, 2 H), 2.25 (s, 1 H), 2.28 (s, 3 H), 3.8 (t, 4 H), 7.04 (s, 2 H). Anal. Calcd for $C_{18}H_{30}O_2$: C, 77.65; H, 10.86; Found: C, 77.71; H, 10.90.

3-[(2′,6′-Di-*tert*-butyl-4′-methyl)phenoxy]-1-propyl *p*-Toluenesulfonate (7). *p*-Toluenesulfonyl chloride (1.08 g, 5.65 mmol) was dissolved in 5 mL of anhydrous pyridine, and the solution was stirred at ambient temperature for 0.5 h and cooled (0 °C) using an ice–water bath. A solution of 3-[(2′,6′-di-*tert*-butyl-4′-methyl)phenoxy]-1-propanol (754 mg, 2.70 mmol) in 5 mL of pyridine was then added, and the mixture was stirred for 0.5 h at 0 °C and then for 6 h at ambient temperature. The mixture was poured onto 60 mL of ice–water containing 15 mL of concentrated HCl and stirred for 20 min. The organic product was then extracted with 50 mL of ethyl ether, washed with water (2 × 20 mL) and saturated NaCl (1 × 20 mL), dried ($MgSO_4$), and concentrated under reduced pressure (<25 °C). The residue was chromatographed (silica; benzene) to give 715 mg (61%) of **7** as a colorless oil, which crystallized on standing. Recrystallization from petroleum ether gave **7**: mp 65.5–66.5 °C; ^1H NMR ($CDCl_3$) δ 1.35 (s, 18 H), 2.2 (m, 2 H), 2.27 (s, 3 H), 2.44 (s, 3 H), 3.73 (t, 2 H), 4.23 (t, 2 H), 7.02 (s, 2 H), 7.3–7.85 (dd, 4 H). Anal. Calcd for $C_{25}H_{36}O_4S$: C, 69.41; H, 8.39; S, 7.41. Found: C, 69.40; H, 8.45; S, 7.45.

Diethyl [3-[(2′,6′-Di-*tert*-butyl-4′-methyl)phenoxy]-1-propyl]malonate (8). A 60% NaH dispersion in mineral oil (120 mg, 3.0 mmol) was washed with anhydrous benzene (2 × 1.5 mL) and suspended in 3 mL of anhydrous DMF. The suspension was cooled to 0 °C using an ice–water bath. Diethylmalonate (480 mg, 3.0 mmol) was then carefully added dropwise via a syringe. After the evolution of hydrogen ceased, the mixture was allowed to remain at 0 °C for 15 min. A solution of 3-[(2′,6′-di-*tert*-butyl-4′-methyl)phenoxy]-1-propyl-*p*-toluenesulfonate (630 mg, 1.45 mmol) in 6 mL of DMF was then added, and the mixture was stirred at 70 °C under a nitrogen atmosphere for 7 h and cooled to room temperature. The mixture was then poured into 20 mL of cold water and extracted with ethyl ether (4 × 20 mL). The combined extract was washed with water (5 × 20 mL) and saturated NaCl (2 × 15 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by chromatography (silica; $CHCl_3$) to give 420 mg (67%) of **8** as a viscous oil: ^1H NMR ($CDCl_3$) δ 1.27 (t, 3 H), 1.38 (s, 18 H), 1.85–2.05 (m, 4 H), 2.29 (s, 3 H), 3.39 (t, 1 H), 3.70 (t, 2 H), 4.22 (q, 4 H), 7.02 (s, 2 H). Anal. Calcd for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59. Found: C, 71.95; H, 9.83.

[3-[(2′,6′-Di-*tert*-butyl-4′-methyl)phenoxy]-1-propyl]malonic Acid (9). Diethyl [3-[(2′,6′-di-*tert*-butyl-4′-methyl)phenoxy]-1-propyl]malonate (415 mg, 0.95 mmol) was dissolved in 10 mL of ethanol/water (70/30, v/v) containing 2.5 g of KOH. The two-phase mixture was stirred under nitrogen at 80 °C for 8 h, cooled to 0 °C, and acidified with 40% H_2SO_4. Water (20 mL) was then added, and the mixture was extracted with ethyl ether (2 × 30 mL). The extract was washed with water (2 × 20 mL) and saturated sodium chloride (2 × 10 mL) and dried ($MgSO_4$). The dried extract was concentrated under reduced pressure, and the residue was chromatographed (silica; $CHCl_3$ followed by $CHCl_3$/HOAc, 20:1,

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v/v). Subsequent concentration under reduced pressure (50 °C), dissolution in 3 mL of ethyl ether, and dilution with 40 mL of light petroleum ether (20–40 °C) resulted in crystallization of **9** (260 mg, 72%) as a colorless solid: mp 142–143.5 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 18 H), 1.9–2.15 (m, 4 H), 2.27 (s, 3 H), 3.53 (t, 1 H), 3.73 (t, 2 H), 7.03 (s, 2 H), 10.2–10.4 (br s, 2 H). Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.07; H, 8.89.

5-[(2′,6′-Di-*tert*-butyl-4-methyl)phenoxy]pentanoic Acid (10). A 65-mg (0.17 mmol) sample of **10** was placed in a small dry flask and heated to 180 °C for 2.5 h under a nitrogen atmosphere. Evolution of CO_2 was visible during the first 0.5 h of heating. The monocarboxylic acid (**10**) was thus produced as a colorless oil (52 mg, 96%): ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 18 H), 1.6–2.2 (m, 4 H), 2.27 (s, 3 H), 2.43 (t, 2 H), 3.72 (t, 2 H), 7.03 (s, 2 H). Anal. Calcd for $C_{20}H_{32}O_3$: C, 74.96; H, 10.07. Found: C, 75.04; H, 10.18.

Tridecaethylene Glycol 5-[(2′,6′-Di-*tert*-butyl-4-methyl)phenoxy]pentanoate (3). To a solution made from **10** (52 mg, 0.16 mmol) and 2 mL of CH_2Cl_2 was added a solution made from tridecaethylene glycol monotrityl ether (133 mg, 0.16 mmol), 3 mL of CH_2Cl_2, dicyclohexylcarbodiimide (70 mg, 0.34 mmol), and 4-(dimethylamino)pyridine (DMAP) (22 mg, 0.18 mmol). After the resulting solution was stirred at ambient temperature for 2 h, dicyclohexylurea was removed by filtration, and the filtrate was concentrated under reduced pressure and applied to a silica column, using $CHCl_3/CH_3OH$ (20:1, v/v) as an eluting solvent. Fractions containing the monotritylated ester were combined, concentrated under reduced pressure, and redissolved in a solution made from 2 mL of CH_2Cl_2 and 1 mL of CH_3OH. To this solution was added 1 drop of concentrated HCl, and the mixture was stirred at ambient temperature for 1 h. The solution was then diluted with 15 mL of ethyl ether, washed with water (2 × 5 mL) and saturated NaCl (1 × 5 mL), and dried ($MgSO_4$). The dried solution was concentrated under reduced pressure, and the oily residue was purified by chromatography (silica; $CHCl_3/CH_3OH$, 20:1, v/v), affording 145 mg of **3** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 18 H), 1.6–1.9 (m, 4 H), 2.28 (s, 3 H), 2.40 (t, 2 H), 2.55 (br s, 1 H), 3.65 (br s, 52 H), 4.24 (t, 2 H), 7.02 (s, 2 H). Anal. Calcd for $C_{46}H_{84}O_{16}$: C, 61.86; H, 9.48. Found: C, 61.91; H, 9.67.

5-[(3′,5′-Di-*tert*-butyl)phenoxy]pentanenitrile (11). A 60% NaH dispersion in mineral oil (1.5 g, 35 mmol) was washed with anhydrous benzene (2 × 5 mL) and suspended in 50 mM of anhydrous THF. 3,5-Di-*tert*-butylphenol (**1**) (4.16 g, 20 mmol) was gradually added during a 5-min period, resulting in hydrogen evolution. When this evolution ceased, 5-bromovaleronitrile (4.44 g, 27 mmol) was added. The mixture was then placed in an oil bath (90 °C) and stirred at reflux conditions under a nitrogen atmosphere for 38 h (pale yellow NaBr precipitated). The mixture was then concentrated by solvent evaporation under reduced pressure and diluted with 100 mL of ethyl ether. The resulting solution was washed with water (5 × 40 mL) and saturated NaCl (2 × 25 mL) and dried (Na_2SO_4). The solvent was then removed under reduced pressure, and the residure was purified by column chromatography (silica; *n*-hexane/$CHCl_3$, 2:3, v/v, followed by $CHCl_3/CH_3OH$, 30:2, v/v) and by recrystallization from *n*-hexane (−15 °C) to give 2.25 g (39%) of **11** as a colorless crystal: mp 63.0–64.0 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 18 H), 1.90 (m, 4 H), 2.45 (t, 2 H), 4.00 (t, 2 H), 6.75 (d, 2 H), 7.05 (t, 1 H), IR (KBr, cm^{-1}) 2965 vs, 2870 s, 2252 w, 1591 s, 1304 s, 1049 s, 862 m, 717 m. Anal. Calcd for $C_{19}H_{29}ON$: C, 79.39; H, 10.17; N, 4.87. Found: C, 79.04; H, 10.03; N, 4.89.

5-[(3′,5′-Di-*tert*-butyl)phenoxy]pentanoic Acid (12). To 3.0 mL of water was added 2.5 mL of concentrated sulfuric acid followed by **11** (300 mg, 1.04 mmol). The mixture was refluxed and stirred for 4 h. The contents of the flask were cooled and poured into 30 mL of ice–water. The pale brown solid was collected and washed well with water (3 × 10 mL). The crude product was purified by recrystallization from *n*-hexane to give 227 mg (71%) of **12** as a colorless crystal: mp 112.5–114.0 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 18 H), 1.85 (m, 4 H) 2.48 (t, 2 H), 4.00 (t, 2 H), 6.75 (d, 2 H), 7.05 (t, 1 H); IR (KBr, cm^{-1}) 2965 vs, 2677 s, 1716 vs, 1597 s, 1429 s, 1303 s, 1211 s, 1060 s, 935 m, 711 m. Anal. Calcd for $C_{19}H_{30}O_3$: C, 74.47; H, 9.87. Found: C, 74.24; H, 9.84.

Tridecaethylene Glycol 5-[(3′,5′-Di-*tert*-butyl)phenoxy]pentanoate (4). A sample (51 mg, 0.16 mmol) of **12** was converted into **4** (52%) using procedures similar to those used for the preparation of **3**. ^{1}H NMR of ($CDCl_3$): δ 1.30 (s, 18 H), 1.7–1.9 (m, 4 H) 2.42 (t, 2 H), 2.60 (br s, 1 H), 3.6 (br s, 50 H), 3.98 (t, 2 H), 4.25 (t, 2 H), 6.74 (d, 2 H), 7.00 (t, 1 H). Anal. Calcd for $C_{45}H_{82}O_{16}$: C, 61.48; H, 9.40. Found: C, 61.63; H, 9.43.

Polyester 5. A solution of diacid **9** (109 mg, 0.3 mmol) and $SOCl_2$ (0.3 mL, 4.0 mmol) in 2 mL of benzene was refluxed under nitrogen for 2.5 h. After removal of solvent and excess $SOCl_2$ under reduced pressure, the oily residue was dissolved in 2.5 mL of CH_2Cl_2 and added to a solution of tridecaethylene glycol (177 mg, 0.3 mmol) made with 2.5 mL of CH_2Cl_2 and 0.5 mL of pyridine. The mixture was stirred at ambient temperature for 70 h and transferred with the use of 8 mL of additional CH_2Cl_2 into two test tubes, where it was washed with 2 M HCl (2 × 2 mL) and water (2 × 1.5 mL), with the aid of centrifugation for phase separation. The organic layer was dried ($MgSO_4$), concentrated under reduced pressure, and chromatographed (silica; $CHCl_3/CH_3OH$, 25:1, v/v) to give a polyester product as a yellow oil (187 mg, 68%) having a ratio of ethylene glycol protons (δ 3.63) to *tert*-butyl protons (δ 1.38) equalling 2.7:1.0. Analysis by gel permeation chromatography indicated M_n = 3709, M_w = 5214, and DP = 4.0.

Gel Permeation Chromatography. The isocratic liquid chromatograph which was used for all GPC measurements was a modular system consisting of a Waters Model 510 solvent delivery system, a Waters Model U6K injector, and a Waters Model 410 differential refractometer, that was interfaced with a Maxima 820 work station. Ultrastyragel columns (10^3 Å, 500 Å, and 100 Å) were obtained from Waters and used in series. The column temperature was maintained at 40 °C. The flow rate that was used in all cases was 1 mL/min. Polystyrene standards that were used were purchased from Showa Denko K.K., Japan, (MW: 6.60 × 10^4, 2.85 × 10^4, 9.24 × 10^3, 3.25 × 10^3, 1.25 × 10^3) and from Waters (MW: 776). Chromatography grade tetrahydrofuran was purchased from Burdick and Jackson and degassed before use. Calibration standards and test solutes were injected as dilute solutions in the eluant. Polystyrene standards were 0.1% (w/v), and the polyesters were 0.1–0.2% (w/v). The injection volume that was used in all cases was 100 μL. All molecular weights that are reported are polystyrene-equivalent molecular weights.

Surfactant-Induced Release of Liposome-Encapsulated CF. Large unilamellar vesicles (1000 Å diameter), containing 5(6)-carboxyfluorescein (CF), were prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocoline (POPC), or from POPC/cholesterol mixtures, using standard extrusion procedures.[18,24] Typically, 1 mL of a chloroform solution, containing 10 mg (0.013 mmol) of POPC, was placed in a test tube (13 × 100 mm), and the chloroform was evaporated under a stream of nitrogen. Cholesterol (4.1 mg, 0.011 mmol) was added directly to this tube, and the mixture was redissolved in 1 mL of chloroform. The chloroform was then removed under a stream of nitrogen. After further drying (12 h, 23 °C, 0.3 mm Hg), the resulting film was dispersed in 0.45 mL of a desired concentration of CF (i.e., 79, 150, or 250 mM, corresponding to 269, 503, and 821 mosM), via vortex mixing. The resulting multilamellar vesicle dispersion was allowed to equilibrate for 0.5 h, subjected to five freeze–thaw cycles (liquid nitrogen), and passed through a 0.1-μm polycarbonate filter (Nuclepore) 29 times. Nonentrapped CF was removed via gel filtration on a Sephadex G-50 column (1.2 × 40 cm), using an isotonic pH 7.4 borate buffer (10 mM borate, 140 mM NaCl, and 2 mM NaN_3 for 79 mM CF; 19 mM borate, 260 mM NaCl, and 3.8 mM NaN_3 for 150 mM CF; and 31 mM borate, 439 mM NaCl, and 6.3 mM NaN_3 for 250 mM CF) as the eluant. Vesicle fractions were collected, and the final volume was adjusted to 4 mL by adding additional buffer.

An aliquot (20 μL) of a given dispersion was diluted with 2 mL of 10 mM borate buffer (pH 7.4, 140 mM NaCl, 2 mM NaN_3) in order to adjust the tonicity of the external phase to 269 mosM. The dispersion was then incubated for 1 h at 25 ± 1 °C. Aliquots (6 μL) were then added to each of a series of test tubes (6 × 50 mm), which contained 54 μL of varying concentrations of a given surfactant in 269 mosM borate buffer, followed by vortex mixing for 10 s. Each tube was then mechanically shaken at 60 strokes/min. In all cases, the final phospholipid concentration was 3.3 μM. After the vesicle/surfactant mixture was allowed to incubate for 0.5 h at 25 ± 1 °C, 45-μL aliquots were withdrawn and diluted with 4 mL of borate buffer. A blank value was determined in every case by treating 6-μL vesicle aliquots with 54 μL of borate buffer in the absence of detergent. A total fluorescence value was determined by complete disruption of the vesicles, using 54 μL of a borate buffer solution which was 80 mM in 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 determined using an excess of Triton X-100; I_a and I_b are the fluorescence intensities after incubation with and without surfactant, respectively. Values of R_{50} represent the ratio of phospholipid/surfactant (or repeat unit) that is needed to release 50% of the entrapped CF from a 3.3 μM (phospholipid) dispersion of liposomes after 30 min.

Specific Internal Volumes. A stock solution of 150 mM (503 mosM) CF was prepared and diluted with varying volumes of 503 mosM borate buffer, such that the final concentrations of the fluorophore were 5, 7.5, 10, and 20 mM. Each of these four solutions was then used to prepare large unilamellar vesicles; nonentrapped CF was removed by use of a Sephadex G-50 column (1.2 × 40 cm) and a 503 mosM borate buffer. Vesicle fractions were collected, and the final volume was adjusted to 5 mL by addition of isotonic buffer. An aliquot (40 μL) of each vesicle type was then added to 2.0 mL of an isotonic borate buffer, and a portion

(0.5 mL) of the dispersion was then diluted with 3.0 mL isotonic borate buffer. The fluorescence intensities of these diluted suspensions were determined immediately. A total fluorescence value was determined by complete disruption of the vesicles, using 50 μL of a 80 mM Triton X-100 solution. Based on these measurements, a calibration curve was then constructed where the self-quenching efficiency was plotted as a function of the internal CF concentration. Self-quenching efficiency (Q) is defined as a percentage such that Q (%) = $100[1 - (I/I_o)]$, where I_o is the total fluorescence and I is the fluorescence intensity of the entrapped CF.

In order to measure the internal volume of an osmotically-stressed vesicle, a 40-μL dispersion (10 mM in CF and 503 mosM in overall tonicity) was diluted with 2 mL of an appropriate buffer solution, followed by incubation for 0.5 h at 25 ± 1 °C. A portion (0.5 mL) was then added to 2.0 mL of this same buffer, and the self-quenching efficiency was determined from fluorescence intensity measurements before (I) and after (I_o) addition of excess Triton X-100. The concentration of intravesicular CF was then estimated by the use of an appropriate calibration curve.

Stability of Osmotic Stress. A 100-μL aliquot of the POPC/cholesterol (2/1) vesicles that were prepared for calibration measurements (10 mM CF and 503 mosM tonicity) was added to 5.0 mL of 10 mM borate buffer (269 mosM), and the mixture was incubated at 25 ± 1 °C. Aliquots (0.5 mL) were withdrawn as a function of time and were immediately chromatographed using a Sephadex G-50 column (0.7 × 15 cm). The vesicles (1.6 mL) were collected and added to 2.0 mL of 269 mosM normal borate buffer, and the fluorescence intensity of the mixture was analyzed to give a value for the vesicle-entrapped CF. A total fluorescence value was then measured after complete disruption of the vesicles, via addition of 50 μL of Triton X-100 (80 mM).

Aliquots (0.5 mL) were also withdrawn as a function of time and diluted with 3.0 mL of 10 mM, 269 mosM borate buffer, and their fluorescence was analyzed and compared with the above results. Self-quenching efficiency values showed that there were no significant differences from those found after gel filtration. These results indicate that the observed increase in fluorescence intensity is not the result of leakage of CF but is due to a dilution of the fluorophore that is entrapped within the vesicles.

This conclusion is also supported by osmotic reversibility studies. An aliquot (50 μL) of the 2.2 mM POPC/cholesterol (2/1) vesicle dispersion encapsulating 20 mM CF (269 mosM) was added to 2.0 mL of a 1 mM borate buffer (14 mM NaCl, 0.2 mM NaN_3, 36 mosM) and then incubated at 25 ± 1 °C. Aliquots (50 μL) were withdrawn as a function of time and diluted with 4.0 mL of same borate buffer, and their fluorescence was analyzed. At the same time, aliquots (50 μL) were also diluted with 4.0 mL of isotonic buffer in order to return the vesicles to an isotonic state, prior to analysis.

Infrared Spectroscopic Study of the Photochemical Substitution and Oxidative Addition Reactions of $(\eta^5\text{-}C_5R_5)M(CO)_4$ Compounds of the Group 5 Metals: Characterization of the Products of Reaction with N_2, H_2, and $HSiEt_{3-x}Cl_x$ and the Kinetic Investigation of $(\eta^5\text{-}C_5R_5)M(CO)_3$ Intermediates

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Abstract: IR spectroscopy has been used to study the photochemical reactions of H_2 and N_2 with $CpM(CO)_4$ and $Cp^*V(CO)_4$ ($Cp = \eta^5\text{-}C_5H_5$, $Cp^* = \eta^5\text{-}C_5Me_5$; M = V, Nb, and Ta) and of $HSiEt_{3-x}Cl_x$ (x = 0, 2, and 3) with $CpV(CO)_4$. The reactions have been studied by FTIR in liquid xenon solution (lXe) at ca. −80 °C and by time-resolved IR spectroscopy (TRIR) in *n*-heptane solution at room temperature. In nearly all cases, UV irradiation leads to substitution of only *one* CO group. The only exceptions were the reactions of N_2 with $CpNb(CO)_4$ in lXe and with $CpV(CO)_4$ in solid matrices at 20 K, where $CpM(CO)_2(N_2)_2$ species were generated as secondary photoproducts. Reaction with H_2 led to formation of nonclassical dihydrogen complexes $CpV(CO)_3(\eta^2\text{-}H_2)$, $Cp^*V(CO)_3(\eta^2\text{-}H_2)$ and $CpNb(CO)_3(\eta^2\text{-}H_2)$, which was in thermal equilibrium with the classical dihydride $CpNb(CO)_3H_2$. ν(H–H) IR bands have been observed for all three dihydrogen complexes. Reaction of $CpTa(CO)_4$ with H_2 led to oxidative addition and formation of $CpTa(CO)_3H_2$, which decayed over a period of 30 min in supercritical xenon solution at room temperature. IR data were also obtained for reaction with D_2 and, in the case of V, with HD. IR spectra suggest that reaction of $CpV(CO)_4$ with $HSiEt_3$ results in the arrested oxidative addition to form a labile $CpV(CO)_3(\eta^2\text{-}H\text{–}SiEt_3)$ complex, the first example of this type of compound of a group 5 metal. By contrast, reaction with $HSiCl_3$ and $HSiEtCl_2$ led to full oxidative addition, $CpV(CO)_3(H)SiR_3$. TRIR measurements showed that formation of all of these $CpM(CO)_3L$ species proceeds via a dissociative mechanism with transient formation of $CpM(CO)_3$. $CpV(CO)_3$ is ca. 100 times more reactive than its Nb and Ta analogs and nearly 1000 times more reactive than $CpMn(CO)_2$ under similar conditions. $Cp^*V(CO)_3$ is even more reactive. Photoacoustic calorimetry (PAC) has been combined with TRIR to estimate V–(N_2) and V–($\eta^2\text{-}H_2$) bond dissociation enthalpies. The PAC results suggest that $CpV(CO)_3$ interacts with the *n*-heptane solvent and is probably more correctly formulated as $CpV(CO)_3\cdots(n\text{-heptane})$. Finally, the reactions of $V(CO)_6$ with N_2 and with H_2 were studied to compare the behavior of d^5 vanadium. IR evidence was found for the formation of $V(CO)_5(N_2)$ and $V(CO)_5(\eta^2\text{-}H_2)$ both in lXe and in *n*-heptane (TRIR). These compounds were significantly shorter lived than the corresponding $CpV(CO)_3L$ species under similar conditions. The photochemical formation of $V(CO)_5L$ occurred via $V(CO)_5$, detected by TRIR, which was significantly less reactive toward CO, N_2, and H_2 than was $CpV(CO)_3$.

Introduction

The mechanism of oxidative addition of small molecules to transition metal centers has long been of interest.[1] Over recent years, whole new classes of compounds have been found where the oxidative addition is arrested and ligands are coordinated to

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(1) See e.g.: Collman, J. P.; Hegedus, L. S.; Norton, J. R. *Principles and Applications of Organotransition Metal Chemistry*; University Science Books: Mill Valley, CA, 1987.

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