Formation of Stable Vesicles from *N*- or 3-Alkylindoles: Possible Evidence for Tryptophan as a Membrane Anchor in Proteins

Ernesto Abel, Stephen L. De Wall, W. Barry Edwards, S. Lalitha, Douglas F. Covey, and George W. Gokel*

Bioorganic Chemistry Program and Department of Molecular Biology & Pharmacology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8103, St. Louis, Missouri 63110

ggokel@molecool.wustl.edu

Received January 12, 2000

Twelve indole derivatives have been prepared and studied. Five were 1-substituted: **1**, methyl; **2**, *n*-hexyl; **3**, *n*-octyl; **4**, *n*-octadecyl; and **5**, cholestanyloxycarbonylmethyl. Four were 3-substituted: **6**, methyl; **7**, *n*-hexyl; **8**, *n*-octyl; and **9**, *n*-octadecyl. Three were disubstituted as follows: **10**, 1-*n*-decyl-3- *n*-decyl; **11**, 1-methyl-3-*n*-decyl; and **12**, 1,3-bis(*n*-octadecyl)indole. Sonication of aqueous suspensions afforded stable aggregates from **3**−**5** and **8**−**12**. Laser light scattering, dye entrapment, and electron microscopy were used to characterize the aggregates. Aggregates formed from *N*-substituted indoles proved to be more robust than those formed from 3-alkylindoles. A stable monolayer formed from 3-*n*-octadecylindole but not from *N*- or 1,3-disubstituted analogues by using a Langmuir−Blodgett trough. The formation of aggregates was explained in terms of stacking by the relatively polar indole headgroup. In the monolayer experiment, this force was apparently overwhelmed by H-bonding interactions with the aqueous phase.

Introduction

The importance of peptides and proteins that span barrier membranes and permit the selective passage of ions and molecules can hardly be overstated. A good deal is currently known about the functions and selectivities of a number of transmembrane proteins,[1] but little is known about the detailed structural or mechanistic issues critical to their function.[2] The multispan α-helix model imputed to most transmembrane proteins has been based primarily upon the structures of bacteriorhodopsin and the photosynthetic reaction center, the structures of which are known from solid-state studies.[3] Based largely upon these models, the membrane spanning segments of the protein in question, presumably α-helices, are predicted by hydrophobicity analysis.[4] Questions about segment mobility[5] and the actual helicity[6] of these putative transmembrane domains have been raised. In the absence of evidence to the contrary, however, it seems reasonable that amino acids present in bilayer-spanning protein segments should be hydrophobic. Some of the uncertainty concerning channel structure has been laid to rest by the very recent solid-state structures of the KcsA channel of *Streptomyces lividans*[7] and the mechanosensitive ion channel homologue from *Mycobacterium tuberculosis*.[8]

If natural, protein channels are formed from multiple α-helical segments, it seems reasonable to suppose that some alignment of these chains would favor effective function. In previous work, we postulated that tryptophan, because of the well-established solvatochromism[9] of its indole side chain, played a key role in organization. It is known, for example, that the transmembrane segments, especially the periplasmic loops connecting the transmembrane segments of the photosynthetic reaction center, are unusually rich in tryptophan.[10] In certain membrane receptor proteins, tryptophan residues are conserved in all periplasmic loops, suggesting a "needle and thread" mechanism whereby tryptophan acts to guide protein segments through the membrane during translocation and subsequently interacts with the membrane surface and/or aqueous environment to anchor the protein.[11] Additionally, recent work by Cross and coworkers has shown that the tryptophan residues of the peptide channel model gramicidin are oriented toward the interface and appear to serve as anchors.[12] Perhaps

(1) Von Heijne, G. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 167−192.

(2) (a) Stein, W. D.; *Channels, Carriers, and Pumps: An Introduction to Membrane Transport*; Academic Press: San Diego, 1990. (b) Hille, B. *Ionic Channels of Excitable Membranes*, 2nd ed.; Sinauer Associates, Inc.: Sunderland, MA, 1992. (c) Nicholls, D. G. *Proteins, Transmitters, and Synapses*; Blackwell Science: Oxford, 1994.

(3) (a) Deisenhofer, J. *Annu. Rev. Biophys. Biophys. Chem.* **1991**, *20*, 247−266. (b) Henderson, R.; Baldwin, J. M.; Ceska, J. A.; Zemlin, F.; Beckmann, E.; *J. Mol. Biol.* **1990**, *213*, 899−929.

(4) Engelman, D. M.; Steitz, T. A.; Goldman, A.; *Annu. Rev. Biophys. Biophys. Chem.* ***1986***, *15*, 343.

(5) Slatin, S. L.; Qiu, X. Q.; Jakes, K. S.; Finkelstein, A. *Nature* **1994**, *371*, 158−161.

(6) Hucho, F.; Görne-Tschelnokow, U.; Strecker, A. *Trends Biochem. Sci.* **1994**, *19*, 383−387.

(7) Doyle, D. A.; Cabral, J. M.; Pfuetzner, R. A.; Kuo, A.; Gulbis, J. M.; Cohen, S. L.; Chait, B. T.; MacKinnon, R. *Science* **1998**, *280*, 69−77.

(8) Chang, G.; Spencer, R. H.; Lee, A. T.; Barclay, M. T.; Rees, D. C. *Science* **1998**, *282*, 2220−2226.

(9) (a) Truong, T. B.; *J. Phys. Chem.* **1980**, *84*, 964−970. (b) Meech, S. R.; Phillips, D.; Lee, A. G. *Chem. Phy.* **1983**, *80*, 317−328. (c) Lami, H.; Glasser, N. *J. Chem. Phys.* **1986**, *84*, 597−604. (d) Arnold, S.; Sulkes, M. *J. Chem. Phys.* **1992**, *96*, 4768−4778. (d) Serrano-Andres, L.; Fulscher, M. P.; Karlstrom, G. *Int. J. Quantum Chem.* **1997**, *65*, 167−181. (e) Borowicz, P.; Herbich, J.; Kapturkiewicz, A.; Nowacki, J. *Chem. Phys.* **1999**, *244*, 251−261. (f) Lombardi, J. R. *J. Phys. Chem. A* **1999**, *103*, 6335−6338.

(10) Schiffer, M.; Chang, C.-H.; Stevens, F. J. *Protein Eng.* **1992**, *5*, 213-214.

(11) Jacobs, R. E.; White, S. H. *Biochemistry* **1989**, *28*, 3421−3437.

10.1021/jo000040l CCC: $19.00
Published on Web 07/26/2000

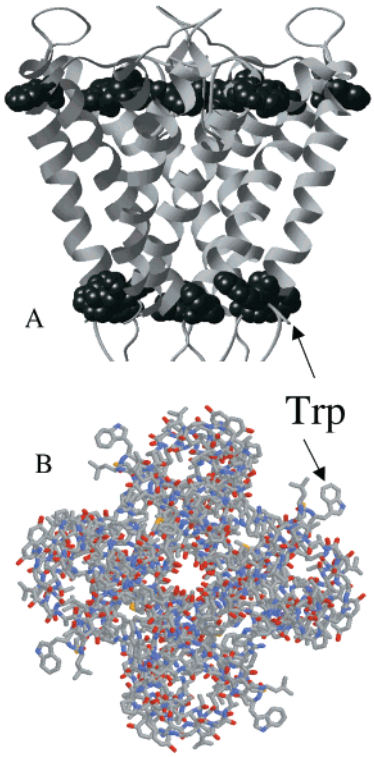
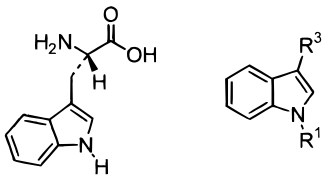


Figure 1. Ribbon (A) and tube (B) representations of the *Streptomyces lividans* KcsA potassium channel.

the most compelling evidence comes from the structure of the KcsA channel cited above. Figure 1 shows two views of the structure.[13] Panel a (top) is a ribbon diagram, but all of the Trp residues have been highlighted in the CPK metaphor. The two lines of Trp residues are apparent. A top view of the structure (tube format) shows the central channel and four symmetrically disposed indole residues from the upper ring of tryptophans. The Trp residues form two distinct bands that coincide with the midpolar regime of a phospholipid bilayer. This suggests an anchoring or organizing function of the tryptophan residues.

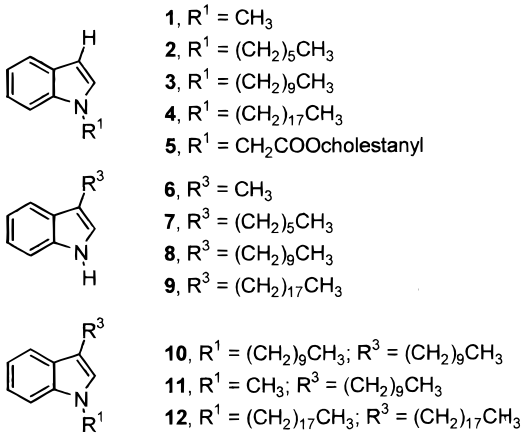
In addition to the studies cited above, considerable recent biochemical work has focused on or impinged this issue. Much contemporary work has used the intrinsic fluorescence[14] of trypophan's indole as a probe in concert with site-directed mutagenesis.[15] In addition, a recent study has employed phosphatidylcholine lipids and hydrophobic α-helical peptides incorporating either tryptophan or lysine as putative anchor residues.[16]

In the first stage of the present effort, the strategy was to discern if vesicles could be formed from single-strand alkanes[17] terminated with a headgroup corresponding to the indole side chain of tryptophan. Although hydropathy scales give information about the relative polarity of these residues, we felt that the feasibility of tryptophan[18] to serve as an "anchor" would be more convincingly demonstrated if it was possible to form stable vesicles from such indole-based amphiphiles.[19] In a previous study, limited in scope to *N*-alkylindoles, we were able to demonstrate that stable vesicles could be formed from *N*-alkylindoles when the hydrocarbon chain was of sufficient length.[20] The *N*-alkylated derivatives rather than the more biologically relevant 3-alkylated derivatives were studied because we felt that they constituted a more severe test of the concept. We fully anticipated that if the *N*-alkylated compounds formed stable vesicles, the 3-alkyl derivatives would do so readily since they retained the free NH group of indole. We have now expanded our study of indolyl amphiphiles to include the 3-substituted derivatives along with selected dialkylated derivatives.



Results and Discussion

Compound Classes. The compounds synthesized for this study fall into three groups. The first are the *N*-alkylindoles. As noted above, these structures were prepared as a "severe" test of indole's ability to serve as a headgroup. It was anticipated that indole would be most effective as a headgroup if the >NH groups were available to form hydrogen bonds[21] with water or other components in the medium. Compounds **6**−**9** were synthesized by analogy with **1**−**4** and because they were expected to more closely mimic the behavior of tryptophan, which has a free NH group and is substituted in the 3-position. After it was established that an *N*-alkylated indole could serve as a headgroup,[16] amphiphile



(12) Hu, W.; Lee, K. C.; Cross, T. A. *Biochemistry* **1993**, *32*, 7035−7047

(13) Protein database code 1bl8.pdf.

(14) Vecsey-Semjen, B.; Lesieur, C.; Moellby, R., & van der Goot, F. G. *J. Biol. Chem.* **1997**, *272*, 5709−5717.

(15) (a) Perisic, O.; Paterson, H. F.; Mosedale, G.; Lara-Gonzalez, S.; Williams, R. L. *J. Biol. Chem.* **1999**, *274*, 14979−14987. (b) Tory, M. C.; Merrill, A. R. *J. Biol. Chem.* **1999**, *274*, 24539−24549.

(16) DePlanque, M. R. R.; Kruijtzer, J. A. W.; Liskamp, R. M. J.; Marsh, D.; Greathouse, D. V.; Koeppe II, R. E.; De Kruijff, B.; Killian, J. A. *J. Biol. Chem.* **1999**, *274*, 20839−20846.

(17) Kunitake, T.; Okahata, Y.; Shimomura, M.; Yasunami, S.; Takarabe, K. *J. Am. Chem. Soc.* **1981**, *103*, 5401−5413.

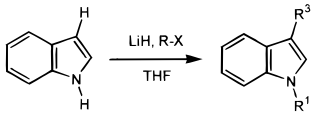
(18) (a) Jacobs, R. E.; White, S. H. *Biochemistry* **1986**, *25*, 2605−2612. (b) Jacobs, R. E.; White, S. H. *Biochemistry* **1989**, *28*, 3421−3437.

(19) (a) Wimley, W. C.; White, S. H. *Biochemistry* **1992**, *31*, 12813−12818. (b) Ladokhin, A. S.; Holloway, P. W. *Biophys. J.* **1995**, *69*, 506−517.

(20) Abel, E.; Fedders, M. F.; Gokel, G. W. *J. Am. Chem. Soc.* **1995**, *117*, 1265−1270.

(21) Mons, M.; Dimicoli, I.; Tardivel, B.; Piuzzi, F.; Brenner, V.; Millié, P. *J. Phys. Chem. A* **1999**, *103*, 9958.

Scheme 1



9 was prepared so that its behavior could be compared to that of **3** and **8**. Finally, structures **11** and **12**, the analogues of **8** and **9**, were synthesized to assess the effect of a single chain in the 3-substituted series when the possibility of hydrogen bonding was obviated.

Syntheses of Indoles. The structures of tryptophan and a disubstituted indole are shown in the illustration. Indole is the heterocyclic component of the tryptophan side chain. Its 1- (*N*) and 3-positions are indicated by the R^1 and R^3 groups, respectively. In the first phase of this study, we prepared a series of *N*-alkylindoles with the following side arms: CH_3 (**1**), *n*-hexyl (**2**), *n*-decyl (**3**), *n*-octadecyl (**4**), and cholestanyloxyacetyl (**5**). These compounds were prepared by alkylation of indole with an alkyl halide using NaH as base in THF. This treatment gave predominantly the 1-isomer. Yields for this process were in the 50−60% range; the major byproduct was presumably the 3-isomer (not isolated). To obtain the 3-isomer, indole was treated with LiH and the alkylating agent. The thought was that Li^+ would be more tightly bound to nitrogen and a greater percentage of the ambident anion would alkylate at the 3-position. The alkylation of pyrrole and, by extension, indole was studied extensively.[22] Alkylation was found to occur either at C(3) or N(1) depending upon how effectively the anion was solvated. Experimental conditions were thus adjusted to obtain either of the desired monomers. 3-Methylindole was obtained commercially, but compounds **6**−**9** were prepared by treatment of indole with lithium hydride in THF followed by addition of the alkylating agent. This approach did, in fact, afford the desired compounds, but yields were only on the order of 20−40%. The major byproducts in this process were the *N*-substituted isomer and the disubstituted compound. Compound **10**, a bis(alkylated) indole, was obtained as a byproduct in the synthesis of **9**. Compound **11** was prepared by methylation of **8**, and **12** was obtained by dialkylation of indole. The previously unknown materials **6**−**12** were obtained either as oils or low-melting solids (see Experimental Section).

Formation of Aggregates. The indolyl amphiphiles formed aggregates in aqueous suspension by using either the reversed-phase[23] or the lipid hydration[24] vesicle preparation method (Scheme 1). The *N*-alkylindole suspensions were sonicated using either a bath or tip sonicator (40 MHz) at ice-bath temperature, and the 3-alkylindoles were irradiated at 40 °C. Sonication was maintained for 20−45 min, with shorter times being required for the 3-alkylindoles. Aggregate size was assessed by using standard laser light scattering methods[25] and negative stain electron microscopy. The results of the turbidimetry studies are shown in Table 1.

The parallel between the results obtained for *N*- and 3-alkylindoles is obvious from the data shown in Table 1. Neither methyl (**1** and **6**) nor *n*-hexylindoles (**2** and **7**) produced any detectable aggregates whether the alkyl chain was substituted on nitrogen-1 or carbon-3 of indole. Vesicle preparation was attempted from the *N*-alkylindoles using the reversed-phase method, and the lipid hydration protocol was used for the 3-substituted indoles. In neither case were amphisomes expected to form from amphiphiles having 1- or 6-carbon tails. When the alkyl chain was either 10 or 18 carbons long, stable vesicles formed when the chain was attached at either position.

The vesicles were generally in the 2−3000 Å range and exhibited typical 15−30% size distributions. It is interesting to note that when vesicles were formed from *N*-octadecylindole, **4**, the resulting aggregate size differed. The vesicle sizes observed for **4** were ∼3000 Å when the reversed-phase method was used and only about 1400 Å when the lipid hydration technique was applied. Vesicle formation was not attempted from 3-octadecylindole, **9**, using the reversed-phase protocol but the aggregates obtained from the lipid hydration method were nearly identical in size (∼1400 Å) to those formed in the same way from the 1-isomer.

It is, of course, possible that none of these indole derivatives can behave as an amphiphile in the absence of protonation. We considered this possibility, but protonation of these compounds is not expected because the pK_A for protonation of indole is −2.3.[26] It is therefore unreasonable to expect these monomers to be protonated to any appreciable extent in aqueous solution at or near neutral pH. It should be noted that the pK_A of indole itself (indole $\rightleftharpoons$ indole$^-$ + H^+) is reported to be 16.97.[27]

Vesicle Characterization by Microscopy. In previous work, we obtained a freeze fracture electron micrograph of vesicles formed from *N*-decylindole, **3**. The spherical vesicles were obvious in that photograph, and sizes corresponded well to those determined by dynamic turbidimetry. In the present case, we used negative stain electron microscopy to produce the photomicrographs shown as panels A−D of Figure 2. Panels A and C show vesicles produced in deionized water from sonicated suspensions of **8** (3-*n*-decylindole) and **11** (1-methyl-3-decylindole), respectively. The magnification in panel A is 15 000 and only 10 000 in panel C, but the spherical nature of the aggregates is obvious in either case. Indeed, the dark boundary layer probably attributable to a bilayer is more apparent in panel C.

The vesicles formed from 3-decylindole, **8**, proved to be exceptionally frail. When the structures shown in panel A were treated with 0.2% uranyl acetate, only the unorganized structures shown in panel B could be identified. The fragility of vesicles formed from 3-alkylindoles was critical to the effort to characterize the vesicles by inclusion or entrapment of other substances

(22) (a) Shirley, D. A.; Roussel, P. A. *J. Am. Chem. Soc.* **1953**, *75*, 375. (b) Plieninger, H. *Chem. Ber.* **1954**, *87*, 127. (c) Cardillo, B.; Casnati, G.; Pochini, A.; Ricca, A. *Tetrahedron* **1967**, *23*, 3771. (d) Reinecke, M. G.; Sebastian, J. F.; Johnson, H. W., Jr.; Pyun, C. *J. Org. Chem.* **1972**, *37*, 3066−8. (e) Reutov, O. A.; Beletskaya, I. P.; Kurts, A. L. *Ambident Anions*; Consultants Bureau, Plenum Publishing Co.: New York, 1983; pp 251−257. (f) Appleton, J. E.; Dack, K. N.; Green, A. D.; Steele, J. *Tetrahedron Lett.* **1993**, *34*, 1529−32.

(23) Szoka, F.; Papahadjoupoulos, D. *Proc. Nat. Acad. Sci. U.S.A.* **1978**, *75*, 4194−4198.

(24) Saunders, L.; Perrin, J.; Gammock, D. B. *J. Pharm. Pharmacol.* **1962**, *14*, 567.

(25) The dynamic light scattering measurements were carried out by using a Coulter model N4MD spectrophotometer equipped with a 4 mW helium−neon laser source, operated at 632.8 nm. The detection angle was fixed at 90°.

(26) The pK_a values for protonation of 1-methylindole and ethylindole are −2.32 and −2.30, respectively: Hinman, R. L.; Lang, J. *J. Am. Chem. Soc.* **1964**, *86*, 3796−3806.

(27) Yagil, G. *Tetrahedron* **1967**, *23*, 2855.

Table 1. Particle Distribution for Alkylindole Vesicles

	position			particle size (Å)		
					cumulant distribution	
compd	1 (N)	3	prep.	uni. diameter	by intensity	by weight
1	methyl	H	rp	no aggregate		
2	hexyl	H	rp	no aggregate		
3	decyl	H	rp	2720 ± 430	2840 ± 470	2900 ± 450
4	octadecyl	H	rp	3130 ± 1000	3880 ± 2100	4550 ± 2200
4	octadecyl	H	lh	1380[a]	1980 ± 1210	688 ± 750
5	cholestanyl-oxyacetyl	H	lh	2180 ± 740	2600 ± 1000	2570 ± 1300
6	H	methyl	lh	no aggregate		
7	H	hexyl	lh	no aggregate		
8	H	decyl	lh	3400 ± 930	3550 ± 970	3730 ± 1000
9	H	octadecyl	lh	1420 ± 340	1460 ± 400	1270 ± 350
10	decyl	decyl	lh	2180 ± 580	2460 ± 860	2420 ± 1000
11	methyl	decyl	lh	2480 ± 780	2840 ± 700	2860 ± 690
12	octadecyl	octadecyl	lh	2100[b]	2900 ± 1800	1410[b]

[a] Narrow size distribution. [b] Standard deviation reported as "broad".

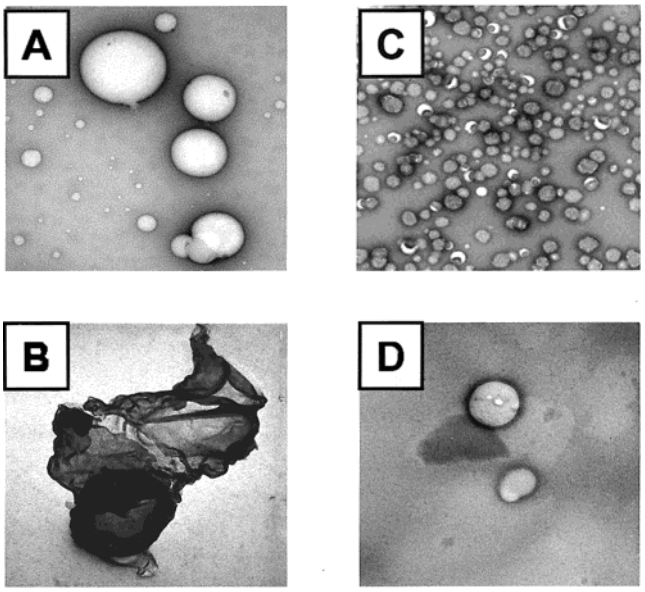


Figure 2. Electron micrographs of aggregates. (A) Aggregates formed from **8** suspended in deionized water. (B) Aggregates as in A, after treatment with 0.2% uranyl acetate. (C) Aggregates formed from **11** suspended in deionized water. (D) Aggregates as in C, after treatment with 0.2% uranyl acetate.

(see below). Somewhat greater success was experienced with 1-methyl-3-decylindole, aggregates of which were photographed in the presence of 0.2% uranyl acetate as shown in panel D of Figure 2. Notwithstanding the technical difficulties described below, the spherical nature of these aggregates is apparent in these and a number of other photomicrographs that are not shown.

The photomicrograph shown in panel A of Figure 2 is unusual and interesting. The camera has apparently captured the fusion of vesicles formed from 3-*n*-decylindole (**8**) in progress. Although fusion is not an unusual process, the extent apparent in this photo is remarkable. It is possible that this photo merely shows several vertical layers of vesicles and the overlap of these levels is an optical illusion that appears to be vesicular fusion. We do not think that this possibility is likely. First, we have obtained dozens of photomicrographs that characterize the aggregates reported in this paper and this is the only picture that shows such extensive apparent fusion. A simple optical illusion would probably be more common since all of the amphiphiles are closely related. Second,

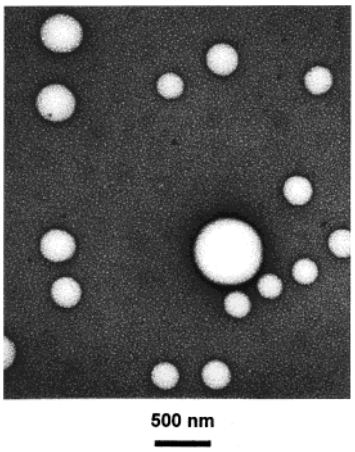


Figure 3. Negative stain electron micrographs of **8**.

the behavior of the vesicle suspension derived from **8** is different from that observed for **3**.

An aqueous suspension of 3-*n*-decylindole (**3**) was allowed to stand at room temperature in the dark for a week. During this period, the unimodal diameter was observed to increase from 2720 ± 430 to 3730 ± 1300 Å. No other alteration in the system could be detected. In contrast, an aqueous suspension of *N*-*n*-decylindole (**8**) was monitored weekly during 1 month. Aggregate size did not vary from the 3400 ± 930 Å value observed immediately after formation. However, during this period, a foamy substance deposited on the walls of the vessel and the fraction of very large particles[28] detected by the laser light scattering instrument increased from 0% to 7%. It thus appears that a significant amount of fusion[29] takes place in this system and that the larger aggregates precipitate (Figure 3).

Vesicle Characterization by Dye Entrapment. Size exclusion chromatography and guest inclusion[30] are standard methods for demonstrating the structural integrity of aggregates. The change in the fluorescence spectrum for a dye included within intact vesicles and again after lysis permits an assessment of the inclusion volume. Such a study was previously conducted on

(28) The light scattering instrument's internal calculation program reports large particles as "dust."

(29) Menger, F. M.; Angelova, M. I. *Acc. Chem. Res.* **1998**, *31*, 789−797.

(30) Gruber, H. J.; Wilmsen, H. U.; Schurga, A.; Pilger, A.; Schindler, H. *Biochim. Biophys. Acta* **1995**, *1240*, 266−276.

vesicles formed from 3-*n*-decylindole (**3**) using the fluorescent dye carboxyfluorescein (CF, 1 and 100 mM), and the inclusion volume was found to be 8%. When an identical study was attempted with **8**, the size of the aggregates tripled from 3400 to ~10 000Å during the process. The apparent inclusion was 3.5%, although the meaning of this number considering the apparent dynamics of the system is unclear (cf. panel C of Figure 2).

Because the entrapment of CF gave equivocal results at two different concentrations, we attempted to enclose methylene blue, which was introduced at a concentration of 1 mM. As in the CF case, very large aggregates of 3-*n*-decylindole (**8**) were obtained; these were difficult to characterize. The use of uranyl acetate (0.2%) with **8** resulted in aggregates that collapsed after filtration. One of the poorly organized structures was captured on film and is shown in panel C of Figure 1. An attempt to use methylumbellyferyl-D-glucopyranose (1 mM) as the entrapped substance failed to produce any detectable aggregates from **8**.

As an alternative to the approach of trapping an organic dye, we attempted to adapt the dynamic NMR technique of Riddell[31] to this problem. The idea was to prepare vesicles from **8** in the presence of 20 mM NaCl. These would then be treated with a dilute solution of Dy^{3+}, an NMR shift reagent, which would cause $^{23}Na^{+}$ inside the vesicles to be observed at a chemical shift different from the sodium ions present externally in the bulk medium. Although aggregates appeared to form, they collapsed after filtration, rendering this method ineffective.

Finally, a strategy similar to that described above was attempted in which $Na^{125}I$ replaced NaCl. In this case, the goal was to detect the iodide anion by residual radioactivity rather than the sodium cation by NMR. In this case, separation of the nonencapsulated radioisotopes from the vesicle suspension took over 48 h. Although both dialysis and centrifugal membrane filtration were tried, background radioactivity remained high and encapsulation could not be quantitated.

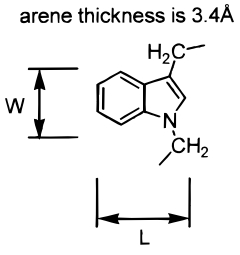
In summary, demonstrating the presence of an internal cavity in the *N*-substituted family of indolyl amphiphiles was straightforward. Attempts to demonstrate encapsulation for the corresponding 3-substituted derivatives proved unsuccessful despite the application of several different techniques. The fragility of vesicles formed from 3-alkylindoles, which retain a free NH, compared to the stability of the *N*-substituted indoles, was remarkable.

A Twin-Tailed, Indolic Amphiphile. 1,3-Bis(*n*-decyl)indole (**10**) was isolated as a byproduct from the reaction in which 3-decylindole was formed. There are two key differences between it and the corresponding *N*- (**3**) and 3-decylindoles (**8**). In **10**, two hydrocarbon chains are attached to indole, and the additional hydrophobicity is expected to favor vesicle formation. For example, the critical aggregate concentration (in water) for $C_{12}H_{25}N^{+}Me_3Cl^{-}$ is 19 mM whereas that for twin-chained $(C_{12}H_{25})_2N^{+}Me_2Cl^{-}$ is 0.15 mM.[32] On the other hand, the molecule is less polar as a result of the larger number of carbon atoms it contains. Vesicles were obtained from **10** and **11** by the lipid hydration method. They were found to be somewhat smaller (2180−2480 Å) than those formed from either **3** (2720 Å) or **8** (3400 Å), but their stability was comparable. Compound **12** gave similarly sized aggregates, but the apparent distributions were broad.

Theoretical Models. Why certain amphiphiles form vesicles and others form micellar aggregates is an interesting general question. Others have posed this question, and an empirical model based upon the relative sizes of headgroup and side chains has been developed by Israelachvilli,[32] Ninham,[33] and Evans.[34] More detailed theoretical treatments have been developed over the years, and these are described in the references. In the present work, we have chosen to apply the more empirical "surfactant parameter", V/LA, to assist in conceptualizing aggregate formation. This variable is useful for considering the structural relationships between headgroup size and tail length and volume. As defined, L is the length of the hydrocarbon chain and V is its volume. The value A is the headgroup area. According to the model, if $0.5 \leq V/LA \leq 1$, vesicles are anticipated. Below a value of 0.5, micelles are expected if aggregates form.

The parameters V and L may be calculated (i.e., V_C) for an alkyl chain as follows: $V_C = (27.4 + 26.9n)$ Å^3 and $L = (1.5 + 1.265n)$ Å per hydrocarbon chain, where V is the volume of the hydrocarbon chain(s), L is the fully extended length, and n is the number of carbon atoms. For the steroid, chain volume was estimated by considering the chain as a hydrocarbon attached to a steroid. Thus, CH_2CO was equated to a hydrocarbon of length $n = 2$. According to the formula $V_C = (27.4 + 26.9n)$ Å^3, the volume of this segment is 81.2 Å^3. The volume of cholesterol was estimated by considering it to be a rectangular box with dimensions $5 \times 7 \times 18$ Å $= 630$ Å^3. The total approximate volume is therefore 711 Å.^{3}

The area of indole is harder to estimate since the face, the edge, or some other aspect of the molecule may constitute the exposed surface. The volume of indole itself is calculated by Spartan 6.0[35] to be 137 Å^3. Considering indole as a rectangular box and dividing by the typical arene thickness of 3.4 Å, the surface area is 40.3 Å^2. As



shown in the diagram, indole is attached directly to two other carbon atoms. If the indole headgroups are stacked, the exposed surface is approximately a rectangle with a dimension (L) of about 7 Å. An arene is typically 3.4 Å thick, so the area is $7 \times 3.4 = 23.8$ Å^2. Considered in a side view, indole is ~7 Å $\times$ 5 Å $= 35$ Å^2 but this does not take into account the side chain methyl group. The

(31) (a) Riddell, F. G.; Hayer, M. K. *Biochim. Biophys. Acta* **1985**, *817*, 313−317. (b) Buster, D. C.; Hinton, J. F.; Millett, F. S.; Shungu, D. C. *Biophys. J.* **1988**, *53*, 145−152. (c) Riddell, F. G.; Arumugam, S.; Brophy, P. J.; Cox, B. G.; Payne, M. C. H.; Southon, T. E. *J. Am. Chem. Soc.* **1988**, *110*, 734−738. (d) Riddell, F. G.; Arumugam, S. *Biochim. Biophys. Acta* **1989**, *984*, 6−10. (e) Riddell, F. G.; Tompsett, S. J. *Biochim. Biophys. Acta* **1990**, *1024*, 193−197.

(32) Israelachvilli, J. N. *Intermolecular and Surface Forces*, 2nd ed.; Academic Press: New York, 1992.

(33) Mitchell, D. J.; Ninham, B. W. *J. Chem. Soc., Faraday Trans. 2* **1981**, *77*, 601.

(34) Evans, D. F.; Ninham, B. W. *J. Phys. Chem.* **1983**, *87*, 5025.

(35) Spartan version 6.0.

equation given above estimates the volume of a methyl side chain at 54.3 Å^3. From the volume, we may estimate the area as (54.3 Å^3)$^{2/3}$ = 14.3 Å^2. Added to the values above, the range is 38.1−49.3 Å^2.

The empirical treatment suggests that vesicles will form when $0 \leq V/LA \leq 1$. In those cases in which vesicles form, we may back-calculate to estimate the area of indole. This, in turn, will suggest the orientation of indole in the bilayer. For *N*-octadecylindole, *V* is 512 Å^3 and *L* is 24.3 Å. Note that the values for *V* and *L* are independent in this treatment of whether the hydrocarbon chain is attached at position 1 or 3. For either octadecylindole, *V*/*LA* should be greater than 0.5 to observe stable vesicles. Thus, $0.5 = V/LA_{max}$ and $A_{max} = V/0.5L$ = 512 Å^3/24.3 Å × 0.5 = 42.1 Å^2. Likewise, the minimum value of *A* will be achieved when $1 = V/LA_{min}$ or $A_{min} = V/L$ = 21.1 Å^2. Vesicle formation is therefore anticipated when *A* is in the range 21−42 Å^2. The estimates of area made above roughly correspond to this range: 23.8−49.3 Å^2. Unfortunately, these data do not permit us to distinguish between a flat or angular indolyl headgroup.

Studies Using a Langmuir−Blodgett (L−B) Trough. The difference in stability between C- and *N*-alkylated indoles was surprising to us. A possible explanation was that different forces dominated the formation of the liposomes. In the *N*-alkylated case, π-stacking could organize the "headgroups" and the tails would align in the way that is characteristic of lipid organization. A similar force was expected to be at work in the C-alkylated case, but this is a more complicated situation. When the alkyl group is attached at the 3-position, the hydrogen on the indolyl nitrogen remains free and available to interact with proximate water. Hydrogen bonding could, in principle, compete with the organizational force[36] available from π-stacking.[37] When vesicles form, a bilayer of lipid tails help to stabilize the overall structure. When there is no NH residue available for H-bonding, the hydrophobic forces are expected to dominate vesicular organization. We felt that it would be possible to probe these effects by studying the behavior of these monomers with a Langmuir−Blodgett (L−B) trough. In this way, only a single layer of alkyl chains should form and the influence of water could be assessed more directly. In addition, compression of the monolayer might indicate whether the indolyl residue preferred a flat or angled orientation with respect to the aqueous surface.

Three compounds were studied by using the L−B trough. The substituents in all cases were *n*-octadecyl. The substituents were placed as follows: **4**, *N*- or 1-octadecyl; **9**, 3-octadecyl; and **12**, 1,3-bis(*n*-octadecyl). The experimentally determined Π-A (Π = surface pressure) curves for compounds **4**, **9**, and **12** at 20 °C are shown in Figure 4. The two *N*-alkylated indoles, compounds **4** and **12**, did not give any significant isotherm. In contrast, the isotherm of 3-octadecylindole (**9**) revealed a phase transition typical of many known amphiphiles[38] going from an

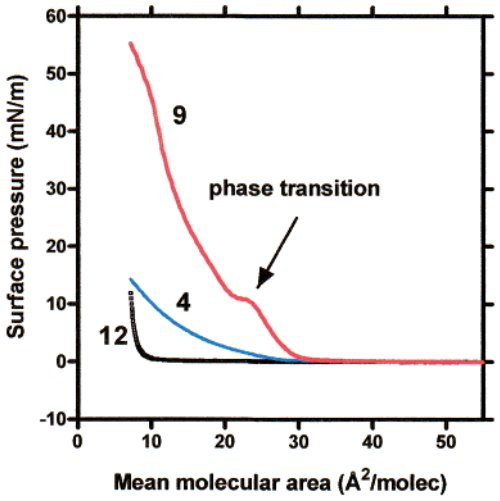


Figure 4. Pressure−area isotherms for compounds **4**, **9**, and **12** measured on a Langmuir−Blodgett trough.

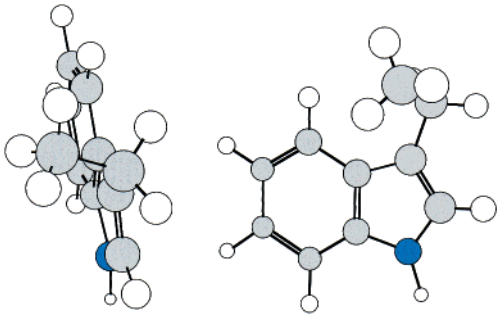


Figure 5. Calculated, ball-and-stick rendering of 3-methylindole.

expanded to a condensed state at 20 °C. Such behavior is observed when the monolayer is below its critical temperature. The phase transformation occurred at a surface pressure of the condensed phase, Π_c, of ∼12 milliNewtons/meter, (mN/m, formerly dynes/cm). The mean molecular area of the expanded phase at this pressure was 22.5 Å^2/molecule. The area of the condensed phase was obtained by extrapolating the condensed isotherm ($\Pi = \Pi_c$). The corresponding value is ∼20.5 Å^2/molecule. Unlike various other monolayer systems known in the literature,[32,39] the monolayers formed from **9** did not collapse. Instead, the surface pressure rose until the trough barriers could not further compress the layer.

A comparison of the isotherms observed for compounds **4**, **9**, and **12** (Figure 4) showed that hydrogen bonding between the water subphase and the N−H of compound **9** was important for the formation of a stable monolayer. Compounds **4** and **12** lack a free NH and are incapable of H-bond donation.[40]

The mean molecular area measurements provide information concerning the interaction between the molecules in the monolayer, particularly the geometric arrangement of the molecules. Molecular modeling of 3-ethylindole (Figure 5) was done in Sybyl, using crystal structure data for the indole ring system.[41] The minimum

(36) Tucker. E. E.; Christian, S. D. *J. Phys. Chem.* **1979**, 83, 426.
(37) Hunter, C. A.; Sanders, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 5525.
(38) (a) MacRitchie, F. *Chemistry at Interfaces*; Academic Press: New York, 1990. (b) Phillips, M. C.; Chapman, D. *Biochim. Biophys. Acta* **1968**, *163*, 301. (c) Kim, M. W.; Cannell, D. S. *Phys. Rev. A* **1976**, *13*, 411. (d) Albrecht, O.; Gruler, H.; Sackmann, E. *J. Phys. Fr.* **1978**, *39*, 127. (e) Motomura, K.; Terazono, T.; Matuo, H.; Matuura, R. *J. Colloid Interface Sci.* **1976**, *57*, 52. (f) Cadenhead, D. A.; Muller-Landau, F.; Kellner B. M. J. *Ordering in two dimensions*; Sinha, S. K., Ed.; Elsevier N. Holland Publishing Co.: Amsterdam, 1980; p 73.
(39) Slotte, J. P. *Biochim. Biophys. Acta* **1992**, 326.
(40) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: Berlin, 1991.
(41) The size of 3-ethylindole was estimated by dissecting the solid structure of 2-(3-indolylmethyl)piperazine-3,6-dione: Morris, A. J.; Geddes, A. J.; Sheldrick, B *Cryst. Struct. Commun.* **1974**, *3*, 345.

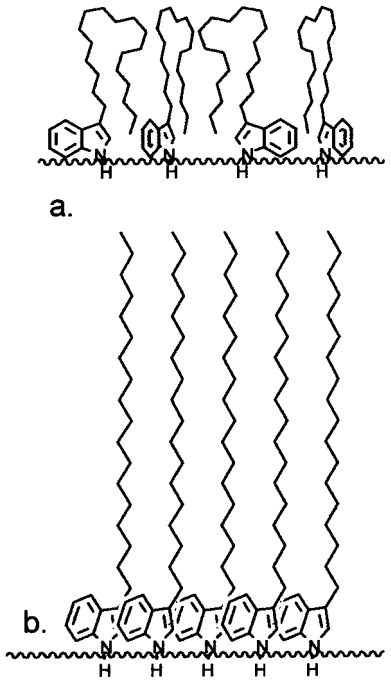
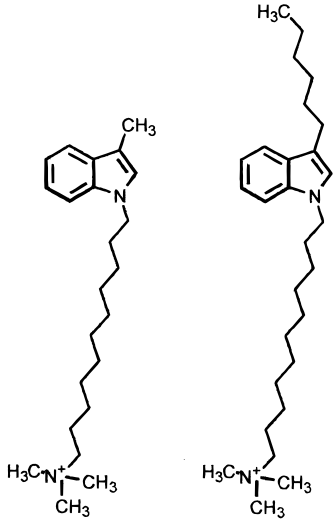


Figure 6. (A) Schematic of random organization of **9** at the air−water interface. (B) Schematic of **9** in a condensed phase monolayer.

width of the molecule, including the van der Waals surface, was found to be 5 Å (Figure 5a). If we consider each molecule of the monolayer to be a cylinder projecting perpendicular to the aqueous subphase, the area of the molecule exposed at the interface, r^2 , is 19.6 Å^2. The area of the condensed phase, 20.5 Å^2 /molecule, indicates that the molecule is in its minimum width orientation as shown in Figure 4a, with the long hydrocarbon chain projecting up, tightly packed with the adjacent molecules. When the molecule is laid in such a way that the six-membered ring is adjacent to the five-membered ring as shown in Figure 4b, the width is measured to be 6.4 Å and the area corresponding to this width is 32 Å^2. The area of the expanded phase, 22.5 Å^2/molecule, ensures that the molecule is arranged in an orientation in which the six-membered ring is above the five-membered ring in the air−water interface. During the phase transition the hydrocarbon chains go from a disordered (Figure 6a) to an ordered state (Figure 6b), hence the molecules pack more tightly. This is reflected in the decrease in mean molecular area of 2 Å^2/molecule as the molecules go from expanded to the condensed state.

Generalizations. Several things are clear from the evidence presented. First, and foremost, indole is capable of serving as a headgroup in the formation of stable vesicles. This is the case whether the alkyl chain is attached at nitrogen (N1) or at carbon (C3). No aggregates are observed for short-chained indole derivatives, irrespective of the point of attachment. When the alkyl chains are sufficiently long, the vesicles may be characterized using a variety of traditional techniques. These include laser light scattering, dye entrapment, freeze-fracture electron microscopy,[16] and negative stain electron microscopy. To be sure, different behavior is observed for the aggregates when the alkyl chains are attached at different positions, but this is understandable in terms of hydrogen bonding.

The fact that an indole can readily serve as a headgroup for the formation of stable aggregates is remarkable. It does not seem to be polar enough to do so, especially when the alkyl chain is attached at nitrogen, removing the H-bonding potential. The experimental evidence presented here removes any doubt that alkylindoles may form stable aggregates. Clearly differences are observed on the basis of chain length and the regiochemistry of the side chain. These differences are supported by studies using a Langmuir−Blodgett trough. Another interesting piece of evidence may be found in a study by Turro and Schore done more than 20 years ago.[42] In a study aimed at using fluorescence to probe the structure and environment of micelles, Schore and Turro prepared the two indole derivatives shown as **13** and **14**. They reasonably anticipated that the charged



13 **14**

trimethylammonium groups would continue to serve as headgroups and that the indole residues would constitute part of the nonpolar chain. They found that these amphiphiles having "an indole separated by 11 carbons from the trimethylammonium ion are solubilized no better than, and perhaps worse than, the other indoles in the host micelles!" The exclamation point suggests their surprise. This result is understandable in the sense that they had inadvertently prepared a two-headed amphiphile that no longer had the desired or expected head−tail definition. In contrast, the multiple indolyl headgroups (from tryptophan) of gramicidin are clearly focused to the aqueous boundary of the phospholipid bilayer. In combination, the results presented here show that the indolyl residue of tryptophan may function as an organizing element in membrane-bound peptide[43] and protein structures. As additional solid-state structures of membrane proteins are solved, it is anticipated that the rarest amino acid will be found in abundance at bilayer boundaries.

Experimental Section

Reagents and Chemicals. ^{1}H NMR were recorded at 300 HMz in $CDCl_3$ solvent and are reported in ppm (δ) downfield from internal $(CH_3)_4Si$. Thin-layer chromatography analyses

(42) Schore, N. E.; Turro, N. J. *J. Am. Chem. Soc.* **1975**, *97*, 2488−2497.

(43) Ladokhin, A. S.; Selsted, M. E.; White, S. H. *Biophys. J.* **1997**, *72*, 794−805.

were performed on aluminum oxide 60 F-254 neutral (type E) with a 0.2 mm thickness or on silica gel 60-F-254 with a 0.2 mm thickness. Preparative chromatography columns were packed with activated aluminum oxide (MCB 80−325 mesh, chromatographic grade, AX 611) or Kieselgel 60 (70−230 mesh). Flash chromatography columns were packed with silica gel Merck grade 9385, 230−400 mesh, 60 Å. Chromatotron chromatography was performed on a chromatotron using 4 mm, 2, or 1 mm thickness circular plates prepared from Kieselgel 60 PG-254.

Every reagent was the best grade commercially available and was distilled, recrystallized, or used without further purification, as appropriate. THF was distilled from sodium metal. All reactions were performed under dry nitrogen unless otherwise specified. Molecular distillation temperatures refer to the oven temperature of a Kugelrohr apparatus. Combustion analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are reported as percents.

The dynamic light scattering measurements were carried out by using a Coulter model N4MD spectrophotometer equipped with a 4 mw helium−neon laser source, operated at 632.8 nm. The detection angle was fixed at 90°.

***N*-Methylindole**, **1**, was purchased from Sigma-Aldrich Chemical Co. Its purity was established by ^{1}H NMR.

***N*-*n*-Hexylindole**, **2**, was obtained by alkylation of indole with *n*-hexyl bromide as previously described.[16]

***N*-*n*-Decylindole**, **3**, was obtained by alkylation of indole with *n*-decyl bromide as previously described.[16]

***N*-*n*-Octadecylindole**, **4**, was obtained by alkylation of indole with *n*-octadecyl iodide as previously described.[16]

Cholestanyl 2-(1-Indole)acetate, 5. was obtained by alkylation of indole with cholestanyl 2-chloroacetate as previously described.[16]

3-*n*-Hexylindole, 7. Lithium hydride (0.08 g, 10 mmol) was suspended in dry THF (40 mL). A solution of indole (1.0 g, 8.5 mmol) in THF (40 mL) was added dropwise during 10 min. The mixture was stirred and heated (reflux, 24 h). A solution of 1-iodohexane (1.8 g, 8.5 mmol) in THF (50 mL) was added dropwise and reflux continued for 24 h. The reaction was quenched (5% HCl), and the reaction mixture was concentrated in vacuo to a pale yellow oil. The residue was purified by flash chromatography (silica, packed in hexane, eluted with 3.75% and then 5% THF in hexane) to give a light yellow oil (0.36, g, 21% yield). Anal. Calcd for $C_{14}H_{19}N$: C, 83.53; H, 9.51; N, 6.96. Found: C, 83.40; H, 9.54; N, 6.89.

3-*n*-Decylindole, 8. Lithium hydride (0.105 g, 12.5 mmol) was suspended in dry THF (50 mL), a solution of indole (1.18 g, 10 mmol) in THF (50 mL) was added dropwise, and the reaction mixture was heated under reflux for 24 h. A solution of 1-iododecane (2.68 g, 10 mmol) in THF (50 mL) was added dropwise to the hot mixture, and stirring at reflux was continued for 24 h. The reaction was quenched with 5% HCl (3 mL). The resulting yellow solution was dried over $MgSO_4$, filtered, concentrated in vacuo, and purified by column chromatography (silica, packed in hexane and eluted with 10% and 15% THF in hexane) to give **8** (0.39 g, 15%) as an orange-to-white, pasty solid, mp 38−40 °C. Anal. Calcd for $C_{18}H_{27}N$: C, 83.99; H, 10.57; N, 5.44. Found:, C, 83.88; H, 10.49; N, 5.39.

3-*n*-Octadecylindole, 9. Lithium hydride (0.15 g, 19 mmol) was suspended in 50 mL of dry THF. A solution of indole (2.0 g, 17 mmol) in 50 mL of THF was then added dropwise. The reaction mixture was heated to reflux (6 h). The resulting suspension was allowed to cool to room temperature, and a solution of 1-iodooctadecane (6.5 g, 17 mmol) in THF (50 mL) was added dropwise. The reaction was stirred overnight, quenched with 20 mL of a 5% HCl solution, and then brought to neutral pH with $NaHCO_3$. The reaction mixture was concentrated in vacuo to a brown solid that was dissolved in $CHCl_3$ and extracted with H_2O. The organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo to give a brown oil (8.7 g). The residue was purified by flash chromatography (silica, packed in hexane and eluted with 3%, 4%, and 7% THF in hexane) to give an off-white, pasty solid (1.94 g, 31%), mp 63.5−64 °C. Anal. Calcd for $C_{26}H_{43}N$: C, 84.49; H, 11.73; N, 3.79. Found: C, 84.39; H, 11.70; N, 3.70.

1,3-*n*-Decylindole, 10. Lithium hydride (0.80 g, 100 mmol) was suspended in dry THF (50 mL). A solution of indole (1.17 g, 10 mmol) in THF (50 mL) was then added dropwise. The reaction mixture was set to reflux for 5 h. A solution of 1-iododecane (8.05 g, 30 mmol) in 50 mL of THF was added dropwise. The reaction was left stirring overnight. The reaction mixture was then concentrated in vacuo to give an orange oil. The residue was purified by column chromatography (silica, eluted with 3% and 5% THF in hexane) to give a fraction containing *N*-decylindole (0.59 g, 15% yield) as a light yellow oil. TLC showed the presence of residual iododecane, but the quantity was insufficient to be detectable by NMR or combustion analysis. Anal. Calcd for $C_{28}H_{47}N$: C, 84.57; H, 11.91; N, 3.52; Found: C, 84.29; H, 11.86; N, 3.47.

Synthesis of 1-Methyl-3-*n*-decylindole, 11. NaH (0.0807 g, 3.36 mmol) was suspended in dry THF (10 mL), and a solution of 3-*n*-decylindole in THF (10 mL) was added dropwise. The mixture was heated at reflux for 18 h and allowed to cool, CH_3I (0.382 g, 2.69 mmol) was added dropwise, and stirring was continued for 4 h at room temperature. The reaction mixture was then filtered and concentrated in vacuo to give a yellow oil that was purified by column chromatography (silica, 10% THF in hexane (v/v)) to give a yellow oil (0.3213 g, 44.0%). Anal. Calcd for $C_{19}H_{29}N$: C, 84.07; H, 10.77; N, 5.16. Found: C, 84.26; H, 10.81; N, 4.92.

Single-Pot Synthesis of 1-Octadecylindole, 4, 3-Octadecylindole, 9, and 1,3-Octadecylindole, 12. NaH (0.615 g, 26 mmol) was suspended in dry THF (10 mL) at 0 °C, and a solution of indole (2.5 g, 21 mmol) in dry THF (10 mL) was added dropwise. The solution was stirred (room temperature, 1 h), and a solution of 1-iodooctadecane (7.99 g, 21 mmol) in dry THF (10 mL) was added dropwise during 15 min. The reaction mixture was stirred at ∼40 °C for 24 h, cooled, and quenched by dropwise addition of 5% HCl (v/v) until gas evolution ceased. The mixture was filtered and concentrated in vacuo to give a yellow oil which was dissolved in CH_2Cl_2 and washed with deionized H_2O. The organic phase was collected, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (silica, 2% EtOAc in hexanes (v/v)) gave **12** as a white solid (0.973 g, 7.5%), mp 61−62 °C. Anal. Calcd for $C_{44}H_{79}N$: C, 84.95; H, 12.80; N, 2.25. Found: C, 85.07; H, 12.79; N, 2.21. Compounds **4** and **9** were obtained by Chromatotron chromatography (silica, 4 mm, hexanes followed by 2% EtOAc in hexanes (v/v)). This gave **4** as a white solid (1.75 g, 22.3%), mp 36−37.5 °C, and **9** as a white solid (0.355 g, 4.6%), mp 70−71 °C. Analysis for **4**: ^{1}H NMR 0.88 (t, C*H*$_3$, 3H), 1.25 (s, -(C*H*$_2$)$_{15}$-, 30H), 1.81 (p, indole(1)-CH$_2$C*H*$_2$CH$_2$-, 2H), 4.08, t, indole(1)-CH$_2$-, 2H), 6.48 (d, indole-H2, 1H), 7.06−7.11 (m, indole, 2H), 7.19 (t, indole, 1H), 7.34 (d, indole, 1H), 7.63 (d, indole, 1H); IR (KBr) 2926, 2853, 2361, 1512, 1465, 1317, 738 cm^{-1}. Anal. Calcd for $C_{26}H_{43}N$: C, 84.49; H, 11.73; N, 3.79. Found: C, 84.26; H, 11.79; N, 3.70. Analysis for **9**: Anal. Calcd for $C_{26}H_{43}N$: C, 84.49; H, 11.73; N, 3.79. Found: C, 84.39; H, 11.70; N, 3.70.

Vesicle Preparation (Lipid Hydration Method). A 15 mL test tube was charged with 33 μmol of the indole derivative dissolved in $CHCl_3$ (∼5 mL), and the solution was concentrated slowly under reduced pressure (room temperature). For *n*-octadecylindole, the temperature of the bath was kept at 15 °C to avoid melting the compound. The vessel was left under high vacuum overnight. Dialyzed water (10.0 mL) was added to the test tube, and the system was sonicated at 40 MHz with a tip sonicator, in an ice bath (for 3-alkylindoles, t = 38−40 °C), until a cloudy suspension was obtained (2 h for the cholestanyl derivative, 45 min for the other *N*-alkylindoles, 20 min for all 3-alkylindoles). The suspension was then centrifuged if necessary and filtered through a 1.0 μm membrane filter into a clean cuvette. The cuvette was placed in a particle analyzer to equilibrate (10 min) and a unimodal analysis was run for 10 min. The unimodal analysis was used to obtain a SDP (standard deviation polynomial) analysis that gave the size distribution of the particles.

Vesicle Preparation (Reversed-Phase Method). The indole derivative (33 mmol) was dissolved in $CHCl_3$ or Et_2O (1.5 mL) and dialyzed H_2O (10 mL) was added. The two phases

were sonicated as above for 45 min. The resulting suspension was concentrated in vacuo to eliminate the organic phase as follows: $CHCl_3$, 10 min at 0.5 atm (rt), 10 min at 0.25 atm (rt), 10 min at 0.1 atm (30 °C water bath), 35 min at 1.0 atm (45 °C water bath); Et_2O, 10 min at 0.75 atm (rt), 10 min at 0.4 atm (rt), 10 min at 0.4 atm. (25 °C water bath), 10 min at 0.1 atm (25 °C water bath). If the suspension still contained ether, it was put in a water bath at 30 °C for 1 h. The solution was then filtered through a 1.0 μm membrane filter, and the analysis proceeded as above.

Fluorescence Analysis—Dye Entrapment. Fluorescence analyses were done using an SLM 4000 subnanosecond spectrofluorimeter. Carboxyfluorescein (CF) (3.76 g, 0.01 mol) was dissolved in 60 mL of a 0.5 M solution of NaOH and the pH adjusted to 7.5 by adding dropwise a 1.0 M solution of HCl. This solution was then placed in a 100 mL volumetric flask and diluted to the mark with deionized water. The final concentration of CF was 0.1 M and showed no fluorescence emission. Upon dilution of an aliquot of this solution to 10^{-5} M, a broad fluorescence emission band was detected at 520 nm.

Alkylindole vesicles were prepared as previously described but instead of using deionized water the 0.1 M CF solution was used. Of the resulting particle suspension, 10.0 μL was placed in a 100 mL volumetric flask and diluted to the mark with deionized water. The resulting solution was deoxygenated by bubbling dry N_2 through it for 2 min. From this solution 2.0 mL was placed in a cuvette and CF was excited at 450 nm. The fluorescence emission spectrum was recorded at 520 nm.

Another 10.0 μL of the vesicle suspension was placed in a 100 mL volumetric flask, and 0.1 mL of spectroscopic grade MeOH was added. This solution was stirred for 5 min to ensure that all vesicles were lysed and then the system was diluted to the mark with deionized water. The resulting solution was deoxygenated by bubbling dry nitrogen through it for 2 min. From this solution 2.0 mL was placed in a cuvette and CF was excited at 450 nm. The fluorescence emission spectrum was recorded at 520 nm. The difference in the intensity of the two spectra was used to determine the percent encapsulation.

Size Exclusion Chromatography and Fluorescence of *N*-Alkylindole Vesicles. Sephadex G-50 (10 g) was allowed to swell overnight in 125 mL of a 0.9% NaCl. The plungers from six 5 mL syringes were removed and the barrels plugged with glass wool. The barrels were then rested in 15 mL centrifuge tubes and filled to the top with the swelled gel. The tubes containing the columns were placed in a bench centrifuge and spun at 3200 rpm for 3 min until the columns were dry and the Sephadex came off the sides of the barrels. The saline solution expelled was discarded. To each of the columns was applied 1.0 mL of alkylindole vesicle suspension dropwise taking care that the sample did not trickle down the sides of the column bed. The columns were then spun for 3 min at 3200 rpm to expel the void volume containing the liposomes. All six eluants were mixed and set aside for analysis. Deionized water (1.0 mL) was then added to the columns and spun as before. All six eluants were mixed and set aside for analysis. More deionized water (0.5 mL) was applied to the columns, and they were spun as before. All six eluants were mixed and set aside for analysis. All three fractions were deoxygenated by bubbling dry nitrogen through them for 3 min.

Fluorescence analysis of the indole was done at 280 nm. Only the first fractions showed emission fluorescence as broad bands with maxima observed at 311 nm for *N*-decylindole and at 346 nm for 3-decylindole.

Monolayer Experiments—Pressure−Area Isotherms. Pressure−area isotherms were recorded using a KSV mini-trough−isotherm trough. The area of the Teflon trough was varied between approximately 32.5×7.5 cm^2 to 5×7.5 cm^2 by adjusting the position of the motor-driven, Delrin barriers. The subphase temperature was maintained constant at 20 °C by circulating water through the base plate on which the trough is mounted. The surface pressure measurements employed a Wilhelmy plate of roughened platinum, connected to a microelectronic feedback system. Isotherms, usually containing 800−900 points, were stored on disk for later graphical analysis.

At the beginning of each experiment the trough and the barriers were thoroughly cleaned with milli-Q water followed by hexane and methanol (both HPLC grade). The aqueous subphase was "ultrapure" water (carbon and Organex filters, Millipore Corp., Bedford, MA). The purity of the subphase was established by measuring the interfacial pressure while the barriers were brought together before spreading the monolayer. The increase in the surface pressure was always less than 0.3 mN/m. The stock solutions were prepared from pure samples of the compounds using HPLC grade chloroform as the spreading solvent. Spreading solutions were applied to the water surface with a Hamilton syringe. After spreading the monolayer at the air−water interface, the spreading solvent was allowed to evaporate for 20 min. The film was then compressed at a rate of approximately 1 Å^2 molecule·min. Compressing more slowly produced no significant differences in the isotherm. Compressing much faster introduced substantial noise into the measurement of surface pressure. A complete isotherm was obtained within about 30 min of commencing the compression. The experiments were repeated several times ($n \geq 6$). The mean molecular areas could be reproduced to ± 1 Å^2, and Π could be determined to within ± 0.4 mN/m.

Acknowledgment. We thank the NIH for grants (GM-36262 to G.W.G.) and (GM-47969 to D.F.C.) that supported this work.

JO000040L