Vesicle Formation from *N*-Alkylindoles: Implications for Tryptophan−Water Interactions

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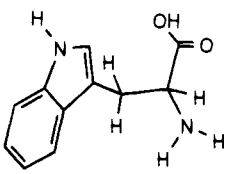
Abstract: Previously unreported indoles having hexyl (**2**), decyl (**3**), octadecyl (**4**), and cholestanylacetoxy (**5**) groups attached to nitrogen have been prepared. *N*-Methylindole (**1**) and **2** did not form aggregates detectable by laser light scattering but compounds **3**−**5** did so. Formation of vesicles was confirmed by size-exclusion chromatography, dye entrapment, and freeze-fracture electron microscopy. The surprising ability of indole to serve as a head group in single-chain, amphiphilic molecules is discussed relative to the indole residue of tryptophan and its role in the anchoring of membrane proteins.

Introduction

The structural model for most transmembrane proteins is based upon analogy with bacteriorhodopsin and the photosynthetic reaction center, the structures of which have been solved crystallographically.[1] By use of this model, membrane spanning segments of other proteins are predicted by hydrophobicity analysis[2] and they are presumed to be helical. Recent results raise questions about the mobility[3] as well as the helicity[4] of these putative transmembrane domains. Even so, it seems reasonable to assume that most of the amino acids in membrane-spanning segments should be hydrophobic. It occurred to us that the membrane stability of any transmembrane segment should be enhanced not only by hydrophobic sidechains in the nonpolar region of the membrane but also by one or more amino acid sidechains that could serve as "headgroups" or "anchors" at one or both of the membrane surface(s).

Indole, the sidechain of tryptophan, and its derivatives have been studied for many years in an effort to understand their solvent-induced fluorescence variations (solvatochromism).[5] These solvent interactions have implications for the essential amino acid tryptophan (trp, W, shown), a 3-substituted indole,[6] because tryptophan's ability to interact with water may not be adequately reflected in its free energy of transfer that forms the basis of polarity scales. Quite recently, computational studies have suggested that 3-methylindole is extensively solvated in water and that the solvent organization affects the compound's molecular dipole which, in turn, influences its fluorescence.[7] In light of this, one might expect an alkyl-substituted indole in water to exhibit amphipathic behavior in which the heterocyclic π-system constitutes the polar head group of an amphiphile. Indeed, it has been shown that the transmembrane segments and, in particular, the periplasmic loops connecting the transmembrane segments of the photosynthetic reaction center are unusually rich in tryptophan.[8] In several classes of membrane receptor proteins, conserved tryptophan residues have been found 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.[8,9]



Trp (W)

Further evidence for tryptophan as a membrane protein anchor derives from studies of the pentadecapeptide gramicidin which dimerizes in a "tail-to-tail" fashion to form a cation-conducting channel. An important feature of gramicidin which may have broad implications is the presence of a repeating, terminal tryptophan−leucine sequence at one end of the peptidic strand. It has been shown in calculations[10] and by NMR[11] in oriented bilayers that the four tryptophan residues of each gramicidin monomer form a symmetrical array of indoles in which the N−H bonds are directed away from the membranes. This suggests that at least some of the tryptophan indole residues may serve as "headgroups" to orient gramicidin for dimerization within the membrane.

In previous work, we have shown that stable vesicles can form from various amphiphiles. These include compounds that have crown ether or chemically-switchable headgroups,[12] struc-

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(5) (a) Truong, T. B. *J. Phys. Chem.* **1980**, *84*, 964−970. (b) Meech, S. R.; Phillips, D.; Lee, A. G. *Chem. Phys.* **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.

(6) Formally, tryptophan is 3-(2-glycinato)indole.

(7) Tubergen, M. J.; Cable, J. R.; Levy, D. H. *J. Chem. Phys.* **1989**, *92*, 51−60.

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

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(10) Etchebest, C.; Pullman, A. *J. Biomol. Struct. Dyn.* **1986**, *3*, 805−825.

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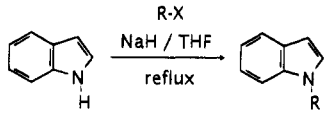
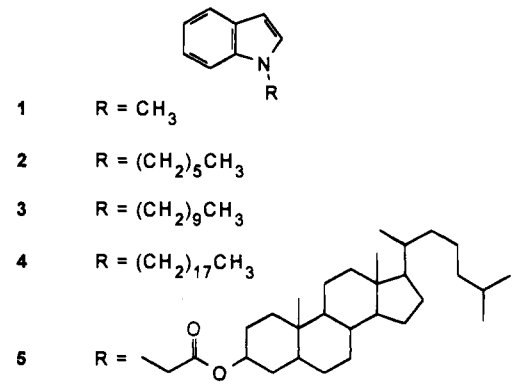
tures that possess a single, bulky tail,[13] or amphiphiles having two hydrocarbon tails.[14,15] It has also been demonstrated in a limited study that *N*-alkylindoles can aggregate.[16] In the present report, we demonstrate that indole is an effective headgroup for vesicle formation even though a single hydrocarbon chain comprises the amphiphile's apolar domain.

Results and Discussion

Syntheses. For our initial studies, we undertook the preparation of *N*-alkylated indoles rather than 3-alkylindoles. The advantage of this was ease of synthetic access. The disadvantage is that the failure of these studies would not necessarily have been indicative of tryptophan chemistry since the latter is 3-substituted and has a free N−H bond. Notwithstanding this potential limitation, we proceeded to bring to hand the requisite compounds. This was done by a straightforward alkylation of indole using NaH as base in THF solution.



Five compounds were used in the present study. *N*-Methylindole (**1**) was purchased commercially. *N*-*n*-Hexylindole (**2**), *N*-*n*-decylindole (**3**), *N*-*n*-octadecylindole (**4**), and cholestanyl *N*-indolylacetate (**5**)[17] were previously unreported and were prepared for the present study. *N*-*n*-Hexyl- (**2**) and *N*-*n*-decylindole (**3**) were obtained as light yellow oils in identical 55% yields after chromatography over silica gel. *N*-*n*-Octadecylindole (**4**) was prepared similarly and obtained as a white, pasty solid (mp 27−28 °C) in 61% yield. A similar procedure was used to prepare cholestanyl *N*-indolylacetate (**5**) from indole and cholestanyl 2-choloracetate.[18] The latter was obtained as a



white solid (mp 134−136 °C), also in 61% yield. It is possible to form the 3-isomer during the preparation of **1**−**5**. This possibility was dispelled by ^{1}H−NMR which confirmed the absence of an N−H proton. Further, none of the alkylindoles showed an N−H infrared band in the 3400 cm^{-1} region. Finally, *N*-*n*-decylindole was unchanged (TLC) after 1 h contact with acetic anhydride in pyridine at room temperature for 1 h.

Table 1. Laser Light Scattering Data for Aggregates Formed from *N*-Substituted Indoles

R	unimodal diameter (Å)	cumulative distribution (Å) by intensity	cumulative distribution (Å) by weight
CH_3 (**1**)[a]	*b*	*b*	*b*
hexyl (**2**)[a]	*b*	*b*	*b*
decyl (**3**)[a]	2720 ± 430	2840 ± 470	2900 ± 450
octadecyl (**4**)[a]	3130 ± 1000	3880 ± 2100	4550 ± 2200
octadecyl (**4**)[c]	1380[d]	1980 ± 1210	688[d]
cholestanyl (**5**)[c]	2180 ± 740	2600 ± 1000	2570 ± 1300

[a] Vesicles prepared by reverse-phase method. [b] No aggregates detected. [c] Vesicles prepared by lipid hydration method. [d] Standard deviation broad.

Aggregate Formation. Aggregates were formed by sonication after using either the lipid hydration[19] or reversed-phase[20] vesicle preparation methods. Vesicles were formed in aqueous suspension maintained at ice-bath temperature. The suspensions were irradiated with a tip sonicator operating at 40 MHz for 45 min except for **5** which required 2 h for the suspension to become distinctly cloudy. Sonication times as long as 4 h failed to afford any detectable aggregates of **1** or **2**. The size of the aggregates thus formed was then assessed by laser light scattering[21] (see Table 1). It is clear from the data that aggregates do, indeed, form from these extremely simple amphipathic monomers.

It is generally observed in amphiphile chemistry that, irrespective of headgroup identity, longer hydrocarbon tails will foster aggregate formation whereas shorter chains may not. The absence of aggregates for the two shorter-chain compounds (**1** and **2**) is therefore expected within this context, suggesting that this series of amphiphiles behaves normally.

Quaternary ammonium salts of the type $CH_3(CH_2)_nN^+(CH_3)_3$ are well known to form aggregates (normally micelles). The possibility therefore exists that the alkylindoles are protonated in water, form quaternary salts, and in a sense behave normally. Simple protonation cannot account for the chemistry of these compounds as the pK_a of an alkylindole's protonated nitrogen is −2.3.[22] The extent of protonation for any of these compounds is therefore minimal in neutral, aqueous solution.

Vesicle Characterization. The internal volume and integrity of vesicular systems can be assessed by size exclusion chromatography and inclusion of a dye such as carboxyfluorescein (CF). The fluorescence behavior of the dye is both medium- and concentration-dependent. Determination of the dye's fluorescence spectrum for intact vesicles and again after lysis permits an evaluation of the inclusion volume. Two nearly identical spectra were obtained, each exhibiting a single, broad peak at 520 nm. Inclusion was evaluated using the following two equations: $F_1 = \epsilon\Phi f C_0 I_0$ and $F_2 = \epsilon\Phi f(C_0 + C_i)I_0$ in which ϵ = the extinction coefficient, Φ = quantum yield for carboxyfluorescein, f = fraction of the total emission that occurs at the

(12) (a) Echegoyen, L. E.; Hernandez, J. C.; Kaifer, A.; Gokel, G. W.; Echegoyen, L. *J. Chem. Soc., Chem. Commun.* **1988**, 836−837. (b) Muñoz, S.; Mallén, J.; Nakano, A.; Chen, Z.; Gay, I.; Echegoyen, L.; Gokel, G. W. *J. Am. Chem. Soc.* **1993**, *115*, 1705−1711.

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(21) 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°.

(22) The pK_a values for 1-methylindole and ethylindole are −2.32 and −2.30, respectively: Hinman, R. L.; Lang, J. *J. Am. Chem. Soc.* **1964**, *86*, 3796−3806. The pK_a of indole itself (indole → indole$^-$ + H$^+$) is reported to be 16.97: Yagil, G. *Tetrahedron* **1967**, *23*, 2855.

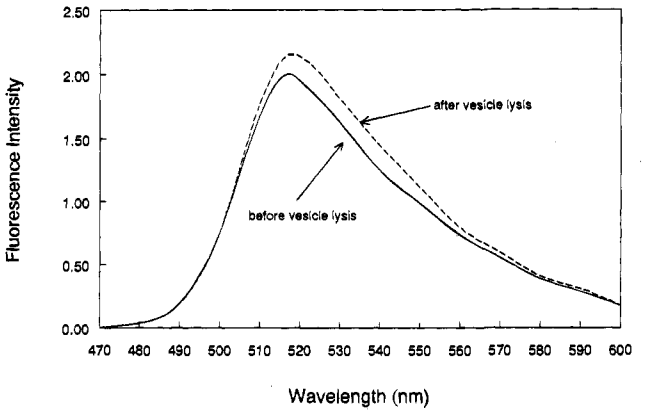


Figure 1. Fluorescence spectra of carboxyfluorescein in vesicles prepared from *N*-decylindole **3** before and after detergent-induced lysis.

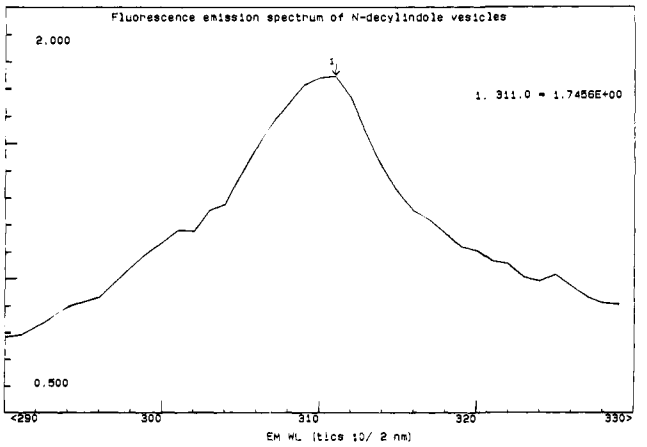


Figure 2. Fluorescence emission spectrum of *N*-decylindole (**3**) vesicles in water. λ_{max} (311 nm) is indicated by the arrow.

emission wavelength, C_0 = the concentration of carboxyfluorescein before lysis, $C_0 + C_i$ = the concentration after lysis of the vesicles, and I_0 = the absolute intensity of the exciting radiation.

After preparation of vesicles from *N*-*n*-decylindole (**3**) in a 0.1 M solution of carboxyfluorescein, 10 μL of the suspension was diluted 10000-fold and the fluorescence spectrum of CF was recorded. An additional 10-μL sample was taken from the original vesicle suspension and treated with 0.1 mL of methanol to ensure vesicle lysis. This system was then diluted as before and the fluorescence spectrum recorded. Evaluation of the difference in intensity from both spectra led to the conclusion that CF was entrapped to the extent of 8%.

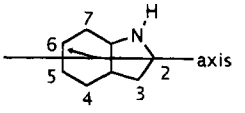
An aqueous vesicle suspension of *N*-*n*-decylindole was chromatographed over Sephadex G-50 using the spinning column method of Frye *et al.*[23] and was then analyzed by fluorescence spectroscopy. The emission spectrum of **3** showed a single broad peak with λ_{max} = 311 nm. The fluorescence spectrum of *N*-methylindole has been measured[24] in cyclohexane and water and found to have λ_{max} values of 311 and 352 nm, respectively. *N*-*n*-Hexadecylindole was also studied in cyclohexane and found to exhibit a maximum at 311 nm.[18] This suggests that the indole headgroup is quite deeply embedded in the vesicle surface. In other words, indole in the vesicles does not appear to be in a largely aqueous environment although strong interaction with water must occur for vesicles to form.

Vesicle Stability. The vesicles formed as described above proved to be stable. An aqueous suspension of *N*-*n*-decylindole vesicles was analyzed by laser light scattering after formation (see Table 1) and again 1 week later. During the interval, the sample was protected from light but was stored at room temperature. The observed unimodal distribution for the fresh sample was 2720 ± 430 Å. After 1 week, the unimodal diameter was found to be 3730 ± 1300 Å. Although the distribution of particles was broader, perhaps due to vesicle fusion, the aggregates persisted in suspension.

It is a common rule of thumb that amphiphiles having two hydrocarbon tails form vesicles while single-strand systems form micelles. Although uncommon, it is established that amphiphiles having single tails can form vesicles.[25] We noted above that when the *n*-alkyl group of indole was either methyl or hexyl, no vesicles formed even under vigorous conditions. In order to demonstrate our expectation that the longer-tail systems required a polar headgroup for vesicle formation, we obtained a sample of commercial *n*-decylbenzene.[26] No aggregate formation could be detected by laser light scattering on a sample treated in a fashion identical to that used to produce vesicles from **3**.

Additional confirmation of vesicle formation in the *N*-alkylindole system was obtained by analysis of a freeze-fractured[27] sample of *N*-*n*-decylindole. The latter compound was chosen for study because it is the shortest chain alkylindole prepared for this study that formed stable aggregates. Electron microscopic analysis of the fractured sample revealed domains in which one of two different sizes of vesicles dominated. Vesicle size was consistent throughout each domain, and the reason for the differences in sizes was not apparent. The sizes of the vesicles were determined by manual measurement of the electromicrographs. The smaller vesicles were found to be symmetrical and to have a size range of approximately 460–520 Å. The symmetrical, larger vesicles are shown in Figure 3. Direct measurement from the electromicrograph indicated that the vesicles were 2200 Å in diameter. This value compares with values of 2720–2900 Å obtained by light scattering and calculated using different methodologies (see Table 1).

Molecular Shape. Molecular models (CPK) of *N*-*n*-decylindole (**3**) were constructed in an effort to understand how molecular packing might occur in this system. Lami and Glasser[5c] have suggested that indole's ground-state molecular dipole is oriented approximately as shown by the arrow in the figure. This would change to some extent in the presence of



water since the >N–H bond is certainly a strong donor. In our case, the N–H bond is absent, being replaced by an N–C bond. The orientation of an alkyl sidechain is apparent from the direction of the N–H bond. The angle subtended by the decyl sidechain with respect to the axis of pseudosymmetry is ≈144°. This assumes that the alkyl chain is extended (all gauche) and that the second methylene group is oriented toward the less hindered position 2 rather than the "peri" position (position 7, see figure). Exchange of the N–H bond for N–R will certainly alter the molecular dipole. Because of the relatively small angle (≈37°) between the long axis of indole

(23) Frye, D. W.; White, C.; Goldman, D. J. *Anal. Biochem.* **1978**, *90*, 809.

(24) Meech, S. R.; Phillips, D.; Lee, A. G. *Chem. Phys.* **1983**, *80*, 317.

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(26) 1-Decylbenzene (confirmed by NMR) was purchased from Sigma-Aldrich and used as received.

(27) Freeze fracture and electron microscopy were performed by Dr. Nancy Galvan, EM Lab, St. Louis University School of Medicine, 1402 S. Grand, St. Louis, MO 63104 (see Experimental Section).

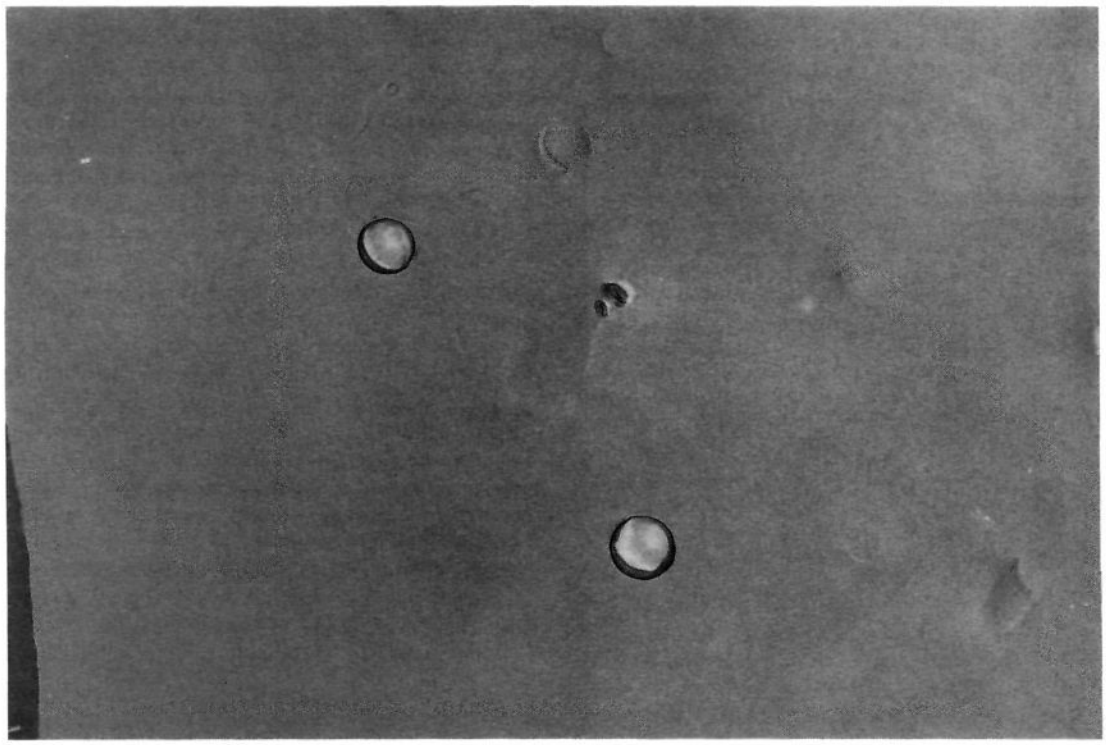

Figure 3. Electron micrograph of vesicles formed from *N*-*n*-decylindole by the reverse-phase method, obtained by the freeze fracture technique, magnification 78000×.

and that of the polymethylene chain, the headgroup size is not profoundly larger than the tail. This no doubt enhances the likelihood of aggregate formation. Some overlap of the six-membered rings could reduce the dead volume between alkyl chains and also alternate molecular dipoles.

Tryptophan as a Possible Gramicidin "Headgroup". Gramicidin is a pentadecapeptide isolated from *Bacillus brevis* having the structure HCO-L-Val-Gly-L-Ala-D-Lau-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2$-CH_2OH. It forms cation channels by dimerizing tail−to−tail at the ethanolamine end. This places the tryptophan residues at the cytosolic and extravesicular membrane boundaries. Calculations reported by Etchebest and Pullman[10] have resulted in the postulate of a left-handed helical dimer in which the tryptophan molecules occupy positions near the two opposite membrane surfaces. A recent NMR study of gramicidin in oriented bilayers conducted by Cross and co-workers[11] has placed the tryptophan (indole) residues in a nearly symmetrical, 4-fold array at each surface of the bilayer. Indeed, it appears from the illustration presented therein that the indole N−H is generally oriented toward the bulk aqueous phase surrounding the membrane. This clearly suggests that one or more of the indoles is functioning as the headgroup that holds gramicidin in place, orienting the opposite end of the pentadecapeptide for dimerization near the bilayer midplane. As noted above, the high incidence of tryptophan in putative transmembrane proteins suggests their apparent ability to aid in insertion and anchoring.

The ability of aromatic amino acid residues to mediate the passage of cations through ion channels has recently been postulated.[28] This suggestion seems very reasonable but the present results imply that the heterocycle may play a second role as well. If we approximate gramicidin's ethanolamine terminus by serine, the channel is equivalent to a 32 amino acid peptide. By use of the free energies of transfer[29] for the individual amino acids, the hydropathy index for a 20-residue window of gramicidin is, on average, 42. This exceeds the +20 kcal/mol criterion level required to assign a membrane-spanning role to a peptide segment. Thus the insertion of gramicidin in a membrane as it is well known to do is reasonable. On the other hand, if ethanolamine (≈serine) is the most polar residue, one might expect it to serve as the headgroup. This would prohibit its role in dimer formation. Thus, the tryptophan indole residues may possibly play two critical roles in the organization and function of the channel: as a headgroup and potentially as an arene relay.

Conclusion. We demonstrate here that *N*-alkylindoles having alkyl chains of sufficient length form stable vesicles. This is surprising in two senses. First, the vesicles are single chain and such systems are uncommon. Second, indole is not normally regarded as a polar function but clearly serves as the headgroup in these systems. These observations may have implications for the role of tryptophan, the indole-containing amino acid, in the insertion and anchoring of membrane-spanning proteins.

Experimental Section

Reagents and Chemicals. ^{1}H−NMR were recorded on a Gemini 300 spectrometer in $CDCl_3$ solvent and are reported in ppm (δ) downfield from internal $(CH_3)_4Si$. Infrared spectra were recorded on a Perkin−Elmer 1710 Fourier transform infrared spectrometer. Fluorescence analyses were done using an SLM 4000 sub-nanosecond spectrofluorometer. Melting points were determined on a Thomas Hoover apparatus in open capillaries and are uncorrected. Thin layer chromatography analyses 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

(28) Kumpf, R. A.; Dougherty, D. A. *Science* **1993**, *261*, 1708−1710.

(29) Free energies are for transfer of an amino acid from a membrane's interior (estimated $\epsilon = 2$) to water. For the present case, they are A, 1.6; G, 1.0; L, 2.8; V, 2.6; and W, 1.9. Serine, not actually represented in the structure but approximating ethanolamine, is 0.6.

chromatography columns were packed with silica gel Merck grade 9385, 230−400 mesh, 60 Å. Chromatotron chromatography was performed on a chromatotron using 4 or 2 mm thickness circular plates prepared from Kieselgel 60 PG-254.

All reagents were the best grade commercially available and were 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** was purchased from Sigma-Aldrich Chemical Co. Its purity was assessed by ^{1}H-NMR.

***N*-*n*-Hexylindole, 2.** NaH (0.240 g, 10.0 mmol) and 18-crown-6 were suspended in THF and a solution of indole (0.586 g, 5.00 mmol) in THF was added. A solution of 1-bromohexane (0.825 g, 5.00 mmol) was added dropwise and then stirred for 3 h. The residue was purified by column chromatography (silica, 1%, 2%, and 3% MeOH in hexane) to give a yellow oil (0.555 g, 55% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, CH_3), 1.27 (m, 6H, CH_2), 1.78 (t, 2H, indole−CH_2−CH_2), 4.04 (t, 2H, indole−CH_2), 6.46 (d, 1H, indole), 7.04−7.33 (m, 4H, indole), 7.61 (d, 1H, indole). IR (AgCl): 2934, 2857, 1511, 1463, 1317, 740, 426 cm^{-1}. Anal. Calcd for $C_{14}H_{19}N$: C, 83.53; H, 9.51; N, 6.96. Found: C, 83.45; H, 9.53; N, 6.85.

***N*-*n*-Decylindole, 3.** NaH (0.240 g, 10.0 mmol) and 18-crown-6 were suspended in THF and a solution of indole (0.600 g, 5.12 mmol) in THF was added. A solution of 1-bromodecane (0.945 g, 4.27 mmol) was added dropwise and then stirred for 15 min at room temperature. The residue was purified by column chromatography (silica, 2% MeOH in hexane) to give a yellow oil (0.604 g, 55% yield). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.87 (t, 3H, CH_3), 1.25 (m, 14H, CH_2), 1.81 (t, 2H, indole−CH_2−CH_2), 4.08 (t, 2H, In−CH_2), 6.48 (d, 1H, indole), 7.07−7.34 (m, 4H, indole), 7.62 (d, 1H, indole). IR (AgCl): 2926, 2854, 1511, 1464, 1316, 739, 426 cm^{-1}. Anal. Calcd for $C_{18}H_{27}N$: C, 83.99; H, 10.57; N, 5.44. Found: C, 83.72; H, 10.47; N, 5.37.

***N*-*n*-Octadecylindole, 4.** NaH (0.050 g, 2.1 mmol) was suspended in 5.0 mL of dry THF, together with (0.005 g, 0.02 mmol) of 18-crown-6. To this suspension, a solution of indole (0.175 g, 1.5 mmol) in 5.0 mL of THF was added dropwise and then stirred for 15 min at room temperature. A solution of 1-iodooctadecane (0.511 g, 1.5 mmol) in 10.0 mL of THF was added during 30 min. After the addition, the reaction mixture was stirred for 6 h. The reaction was then filtered and concentrated under reduced pressure to give a brown oily residue. The residue was then purified by flash chromatography (1%, 3%, and 5% MeOH in hexane) and Chromatotron chromatography (silica, 4 mm, 1% MeOH in hexane) to give a white pasty solid (0.3386 g, 61%), mp 27−28 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.87 (t, 3H, CH_3), 1.25 (m, 30H, CH_2), 1.83 (t, 2H, indole−CH_2−CH_2), 4.10 (t, 2H, In−CH_2), 6.48 (d, 1H, indole), 7.08−7.36 (m, 4H, indole), 7.63 (d, 1H, indole). IR (AgCl): 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.

Cholestanyl 2-(1-indole)acetate, 5. NaH (0.048 g, 2.0 mmol) and 18-crown-6 (40 mg) were suspended in 5 mL of anhydrous THF and to this suspension was added dropwise a solution of indole (0.117 g, 1.0 mmol) in 3 mL of THF. After the addition was complete, the reaction mixture was refluxed for 15 min to ensure conversion of all indole to its salt. The mixture was then cooled in an ice bath (0−5 °C) and a solution of cholestanyl 2−chloroacetate (0.500 g, 1.07 mmol) in 10 mL of THF was slowly added. After 15 min, the reaction mixture was filtered. The filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography (10% acetone in hexane) and then by column chromatography (silica gel, CH_2Cl_2). The product was obtained as a white solid (0.302 g, 61% yield), mp 134−136 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.64 (s, cholestanol), 1.9−2 (m, cholestanol), 4.77 (s, O−CH_2−COO, 2H), 4.81 (m, COO−CH−, 1H), 6.56 (d, 3′, 1H), 7.13 (m, 4′,5′,6′, 3H), 7.24 (d, 7′, 1H), 7.63 (d, 2′, 1H). IR (KBr): 2933, 2850, 1750, 1467, 1212, 743 cm^{-1}. Anal. Calcd for $C_{37}H_{55}NO_2$: C, 81.42; H, 10.16; N, 2.57. Found: C, 81.44; H, 10.04; N, 2.50.

Vesicle Preparation (Lipid Hydration Method). In a 15-mL test tube 33 μmol of the indole derivative was dissolved in approximately 5 mL of chloroform, and this solution was concentrated slowly under reduced pressure at room temperature. For 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, until a cloudy suspension was obtained (2 h for the cholestanyl derivative, 45 min for the rest). The suspension was then centrifuged if necessary and filtered through a 1.0-μm membrane filter into a clean cuvette. The cuvette was placed for 10 min in a particle analyzer to equilibrate the temperature, and then a unimodal analysis was run for 10 min. This unimodal analysis was used to obtain a SDP (standard deviation polynomial) analysis that gave the distribution of the size of the particles.

Vesicle Preparation (Reverse-Phase Method). The indole derivative (33 μmol) was dissolved in 1.5 mL of chloroform or diethyl ether, and 10.0 mL of dialyzed water was added. The two phases were sonicated as above for 45 min. The resulting suspension was concentrated under reduced pressure to eliminate the organic phase: for chloroform 10 min at 0.5 atm (room temperature), 10 min at 0.25 atm (room temperature), 10 min at 0.1 atm (30 °C water bath), 35 min at 1.0 atm (45 °C water bath); for diethyl ether 10 min at 0.75 atm (room temperature), 10 min at 0.4 atm (room temperature), 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 was proceeded as above.

Fluorescence Analysis. 1. Dye entrapment. 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.

N-*n*-Decylindole 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 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 spectra were taken 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 spectra were taken at 520 nm. The difference between the intensities of the two spectra was used to determine the percent encapsulation.

2. Size Exclusion Chromatography and Fluorescence of *N*-Alkylindole Vesicles. Sephadex G-50 (10 g) was allowed to swell overnight in 125 mL of 0.9% NaCl. The plungers from six 5.0-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 disposed.

To each of the columns 1.0 mL of a *N*-*n*-decylindole vesicle suspension was applied 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 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 determination was made by exciting the indole at 280 nm. Only the first fraction showed emission fluorescence as a broad band with a maximum observed at 311 nm.

Electron Microscopy. Electron microscopy was conducted[27] according to the following procedure. Liposome solution (1 μL) was rapidly frozen by immersion in liquid N_2 and transferred to the precooled stage (−150 °C) of a Baizer's freeze-etching system (BAF 400T). Under high vacuum (10^{-6} Torr), samples were warmed to −110 °C and fractured with a stainless steel knife. Without etching, the fractured sample surface was rotary-shadowed with Pt/C at an angle of 35° and then coated with carbon. The Pt/C replicas were floated from the surface of the thawed sample onto distilled water and then transferred to 5% sodium hypochlorite followed by several changes of distilled water. Replicas were picked up on 300 mesh copper grids and viewed in a JEOL 100 CX transmission electron microscope.

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