

Bolaamphiphiles Promote Phospholipid Translocation Across Vesicle Membranes

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Abstract: A series of membrane-spanning bolaamphiphiles (molecules with two hydrophilic end groups connected by a hydrophobic linker) were prepared by a modular synthetic method and evaluated for their abilities to affect the dynamics of a surrounding bilayer membrane. The goal was to determine if the bolaamphiphiles promote the translocation of phospholipids across vesicle membranes. The bolaamphiphiles were incorporated at low levels (up to 5 mol %) in vesicles composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). Inward translocation assays were performed using fluorescent, NBD-labeled phospholipid probes with phosphocholine (PC) or phosphoglycerol (PG) headgroups. The membrane-spanning bolaamphiphiles promote the translocation of both phospholipid probes in the order PG > PC, whereas shorter bolaamphiphiles (structures that must adopt a U-shape and keep both end groups in the same leaflet of the membrane), and regular amphiphiles with one hydrophilic end group, are inactive. These results are an exception to the rule-of-thumb that membrane-spanning bolaamphiphiles are inherently membrane-stabilizing molecules that inhibit all types of membrane transport.

Introduction

Bolaamphiphiles are surface-active molecules with two hydrophilic end groups connected by a hydrophobic spacer that has one, two, or three alkyl chains. Bolaamphiphiles often self-assemble in water to form monolayer lipid membranes that are more tightly packed and less permeable than analogous bilayer membranes.^{1–3} For example, the membranes of archaeobacteria are composed of approximately 90% tetraether isoprenoid-based bolaamphiphiles, and as a result, they are unusually stable at high temperature and low pH.^{4,5} Bolaamphiphiles are particularly promising building blocks for the fabrication of highly stable, supported lipid membranes to be used in various membrane mimetic devices.⁶

The molecular assembly under consideration here is not to a membrane composed predominantly of bolaamphiphiles but rather a fluid-phase bilayer membrane composed primarily of the common phospholipid, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), with a low concentration (up to 5 mol %) of added bolaamphiphile. The general goal of this work is

to ascertain if and how the bolaamphiphile alters the molecular dynamics of the surrounding bilayer. Any effect will, of course, depend on the bolaamphiphile's exact molecular structure. For example, a number of bolaamphiphiles have been *designed* to act as ion channels or membrane disruptors. These compounds have specific structural elements such as internal hydrophilic residues that promote a significant transport or disruption process.^{1,7} Much less attention has been paid to the ability of structurally simple bolaamphiphiles to alter bilayer membrane structure and function.⁸ We are particularly interested in the dynamic process of phospholipid translocation or flip-flop. In contrast to lateral membrane diffusion, which is rapid, translocation of a labeled phospholipid across a bilayer membrane is quite slow with a typical half-life in vesicles of many hours or even days (Figure 1).⁹ The activation barrier is due to the energetic penalty for moving the polar phospholipid headgroup through the lipophilic interior of the membrane, although acyl chain structure is also a significant factor.^{9,10} Recently, we and other, have demonstrated that phospholipid translocation can be accelerated by a number of low molecular weight organic

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- (2) Bolaamphiphiles self-assemble into a range of morphologies; for a discussion, see: Köhler, K.; Forster, G.; Hauser, A.; Dobner, B.; Heiser, U. F.; Ziethe, F.; Richter, W.; Steiniger, F.; Drechsler, M.; Stettin, H.; Blume, A. *J. Am. Chem. Soc.* **2004**, *126*, 16804–16813 and references therein.
- (3) Bolaamphiphiles can even form stable membranes in pure ethanol. Wang, X.; Liang, Y. *J. Colloid Interface Sci.* **2001**, *233*, 364–366.
- (4) De Rosa, M.; Gambacorta, A.; Gliozzi, A. *Microbiol. Rev.* **1986**, *50*, 70–80.
- (5) Fan, Q.; Relini, A.; Cassinadri, D.; Gambacorta, A.; Gliozzi, A. *Biochim. Biophys. Acta* **1995**, *1240*, 83–88.
- (6) Kai, T.; Sun, X.-L.; Faucher, K. M.; Apkarian, R. P.; Chaikof, E. L. *J. Org. Chem.* **2005**, *70*, 2606–2615. Halter, M.; Nogata, Y.; Dannenberger, O.; Sasaki, T.; Vogel, V. *Langmuir* **2004**, *20*, 2416–2423.

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- (8) Gu, Q.; Zou, A.; Yuan, C.; Guo, R. *J. Colloid Interface Sci.* **2003**, *266*, 442–447.
- (9) For recent measurements of translocation rates with unlabeled phospholipids, see: Liu, J.; Conboy, J. C. *Biophys. J.* **2005**, *89*, 2522–2532.
- (10) Phospholipid translocation is disfavored enthalpically, but it can be favored entropically, presumably due to the release of solvating water molecules. Homan, R.; Pownall, H. J. *Biochim. Biophys. Acta* **1988**, *938*, 155–166.

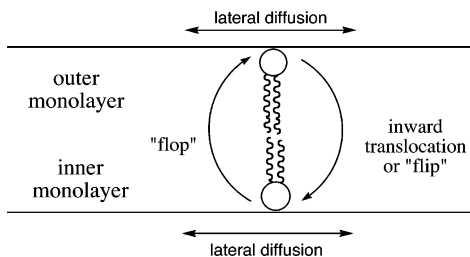


Figure 1. Definitions for phospholipid translocation.

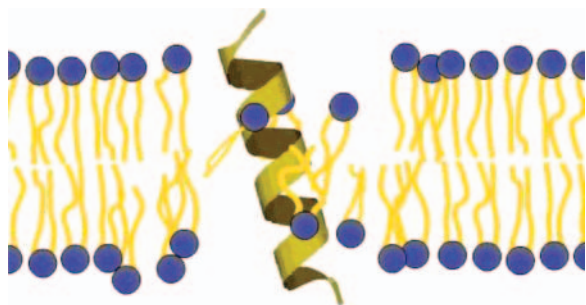
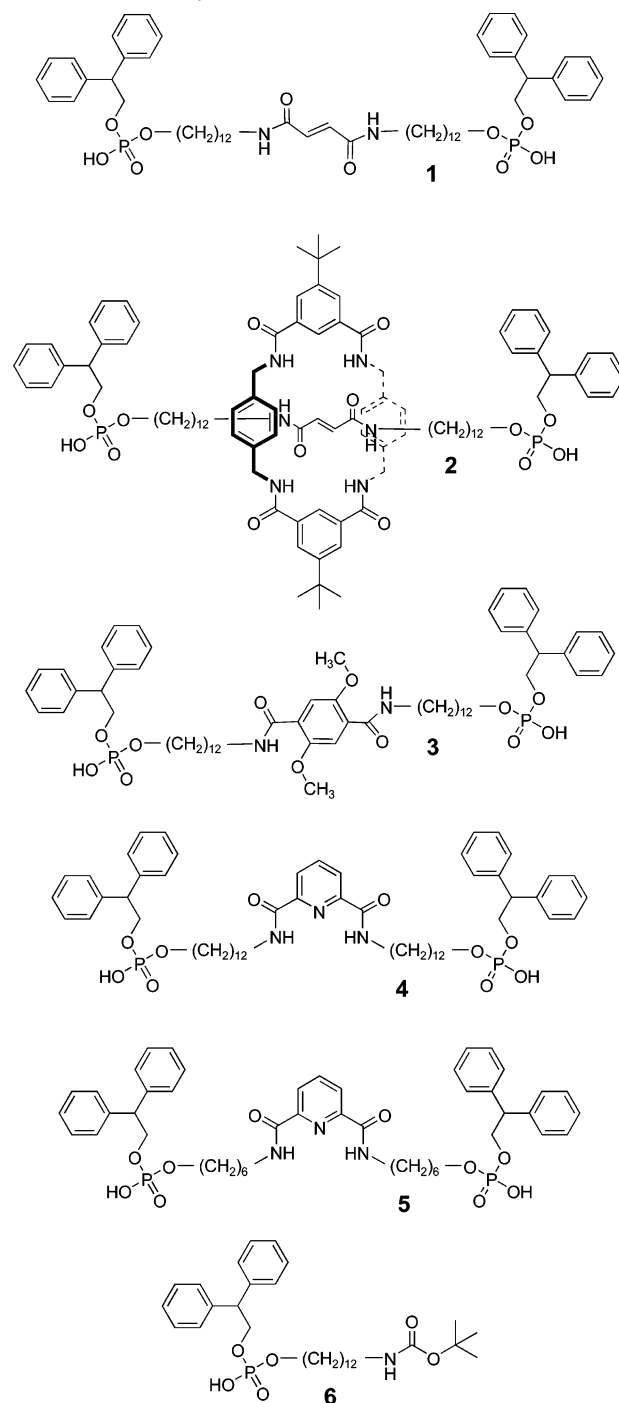


Figure 2. Representation of the “slip-pop” mechanism for phospholipid flip-flop. The inherent wobbling motion of a single-pass, membrane-spanning helical peptide causes local defects in the headgroup region of the bilayer, which allows neighboring phospholipids to slip into the hydrophobic center of the membrane and subsequently pop outside.¹⁹

compounds, including molecules that induce membrane pores,¹¹ or form hydrogen bonded complexes with the phospholipid headgroup.^{12–15} Membrane adsorbed polymers can also disrupt packing and induce phospholipid translocation.¹⁴

The specific goal of this study was to determine if structurally simple bolaamphiphiles promote the translocation of phospholipids across vesicle membranes. At first glance, this idea may seem to be counterintuitive because, as stated above, bolaamphiphiles are generally considered as inhibitors of membrane transport.^{1,8,16–18} Nonetheless, our curiosity was raised by a recent study by the group of de Kruijff and co-workers who observed that hydrophobic, single-pass, membrane-spanning helical peptides promote phospholipid flip-flop in vesicle membranes.¹⁹ They rationalize their results in terms of a “slip-pop” mechanism (Figure 2), which proposes that the inherent wobbling motion of the peptide causes local perturbations in the headgroup region of the membrane, allowing the polar headgroups of the adjacent phospholipids to slip into the hydrophobic center and, subsequently, pop out on either side. They also observed that single-pass helices promote phospholipid translocation more effectively than multiple-pass helices.¹⁹ Thus, we were interested in seeing if a bolaamphiphile, with a

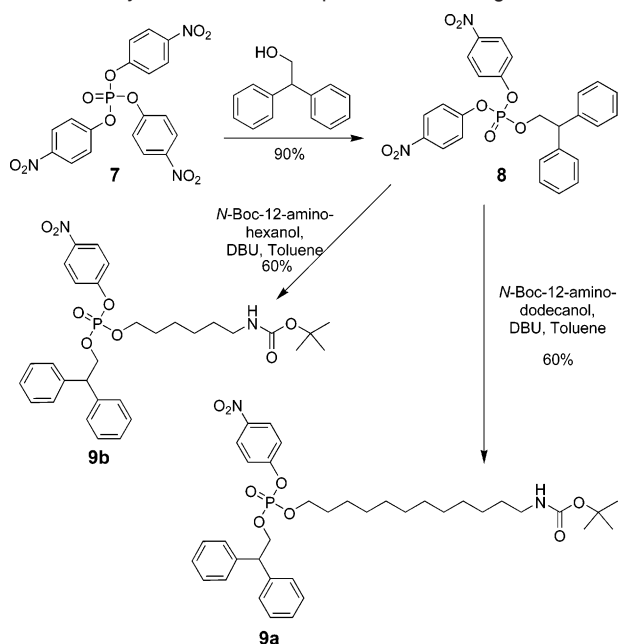
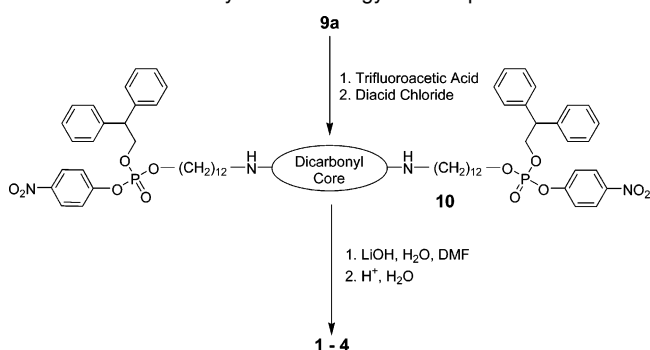
Scheme 1. Membrane-Spanning Bolaamphiphiles 1–4 and Shorter Control Compounds 5 and 6



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similar structural shape as a single-pass helix,²⁰ could also promote phospholipid translocation. We report the design and synthesis of a series of amphiphiles, 1–6, (Scheme 1) which are related by having the same phosphodiester end groups. The structures of bolaamphiphiles 1–4 are sufficiently long (30–32 heavy atoms) to span a vesicle membrane composed of POPC, whereas 5 and 6 are shorter control compounds. We find that the membrane-spanning bolaamphiphiles are indeed able to promote the translocation of fluorescently labeled

(20) A single-pass helical peptide is thicker than the single-chain bolaamphiphiles described in this paper, but their shapes are similar in that they are both relatively narrow rods that bisect the membrane and wobble.

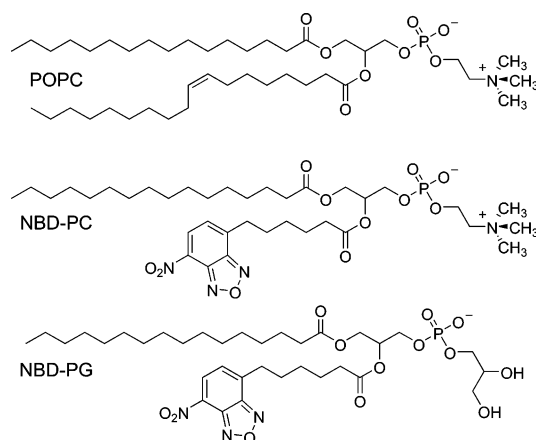
Scheme 2. Synthesis of the Phosphotriester Building Blocks**Scheme 3.** General Synthetic Strategy for Compounds 1–4

phospholipids across surface differentiated vesicle membranes, and the biological implications of our results are discussed.

Results

Synthesis. A modular synthetic strategy was employed to prepare the bolaamphiphiles. The six-carbon and twelve-carbon phosphotriester *N*-Boc-amines **9a** and **9b** were prepared as shown in Scheme 2. The *N*-Boc groups were removed by treatment with TFA, but prior to the next synthetic step, the trifluoroacetate counteranions were exchanged for chloride to prevent side reactions. The ammonium chloride salts were treated with the appropriate bis-acid chloride to give the appropriate phosphotriester compounds **1**_{PNP}–**4**_{PNP} (shown generically as **10** in Scheme 3), and subsequent hydrolysis with LiOH produced the phosphodiester **1**, **3**, and **4**. The six-carbon control bolaamphiphile **5** was prepared by an analogous pathway. The Leigh-type rotaxane **2** was made by treating the precursor thread compound **1**_{PNP} with 5-*tert*-butylisophthaloyl chloride and *p*-xylylenediamine.²¹ The rotaxane product, phosphotriester **2**_{PNP}, was hydrolyzed to give the rotaxane phosphodiester, **2**.

Phospholipid Translocation and Leakage Assays. Phospholipid translocation was monitored via the well-established

Scheme 4: Molecular Structure of POPC and the Fluorescent Probes NBD–PG and NBD–PC

7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)/dithionite quenching assay, which uses a phospholipid probe containing an NBD group in one of its acyl chains (all phospholipid structures are presented in Scheme 4).¹⁴ Exo-labeled vesicles were prepared by addition of a small aliquot of NBD–lipid (0.5 mol % of total phospholipid) in ethanol to a dispersion of vesicles composed of a mixture POPC and bolaamphiphile. The NBD–lipid readily inserts into the outer monolayer of the fluid-phase vesicles. Upon treatment with sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$), the NBD fluorescence is quenched due to reduction of the NBD nitro group. Vesicle membranes are effectively impermeable to dithionite; therefore, only NBD–phospholipid that is located in the outer leaflet is chemically quenched. At any given time, the fraction of probe located in the outer monolayer can be determined from the drop in fluorescence intensity when a portion of the vesicles are subjected to dithionite quenching.

All translocation measurements were conducted in 5 mM TES/100 mM NaCl buffer at pH 7.4 and 25 °C with unilamellar vesicles that were prepared by repeated extrusion through polycarbonate filters with 100-nm pores. The inward translocation of NBD–PG or NBD–PC across vesicle membranes composed of POPC and 4 or 5 mol % of amphiphiles **1**–**6** is shown in Figures 3 and 4. The experiments start with an initial value of 100% exo NBD–lipid and progress to an equilibrium value of around 60% exo NBD–lipid.¹³ The half-lives for these equilibration processes are listed in Table 1, along with the longer half-lives that were observed when the vesicles also contained 30% cholesterol. Inward translocation of NBD–PS was also investigated with the more active amphiphiles, but no significant translocation was observed (data not shown). In the case of active bolaamphiphiles **1** and **3**, the rate of NBD–PC translocation was measured over the rather narrow range of 2–6 mol % and a linear dependency was observed (uncertainty in the translocation rate measurement increases substantially outside of this concentration range). The fact that the NBD probes become protected from the externally added dithionite is strong evidence that vesicles do not allow passage of hydrophilic ions into the vesicles. This conclusion was independently confirmed by a carboxyfluorescein leakage experiment. The water-soluble fluorescent marker 5(6)-carboxyfluorescein was encapsulated inside vesicles composed of 95% POPC and 5% bolaamphiphile **2**, and no significant leakage was observed over several hours.

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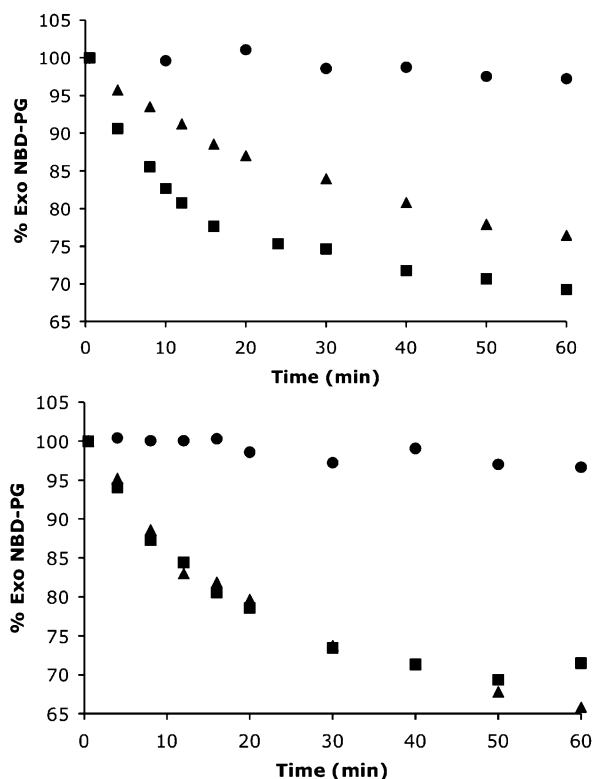


Figure 3. Change in the fraction of NBD-PG in the membrane outer monolayer (% Exo NBD-PG). Inward translocation induced at $t = 0$ min by adding $0.125 \mu\text{M}$ of NBD-PG to POPC vesicles ($25 \mu\text{M}$) that also contained (Top) 5 mol % of **1** (■), **2** (▲), or **6** (●); (Bottom) 4 mol % of **3** (■), **4** (▲), or **5** (●).

Discussion

Factors that were considered when designing the bolaamphiphiles for this study were synthetic accessibility, modular variation of the building blocks, and limited flexibility in the center of linking chain. In the case of **1–4**, the end groups are monoanionic phosphodiester (with terminal diphenylethanes^{22,23}) that are connected by dodecyl chains to a central dicarbonyl core. The two most likely orientations of a bolaamphiphile in a bilayer membrane are illustrated in Figure 5. Depending on the length and flexibility of the linking chain, the bolaamphiphile can extend completely across the membrane or otherwise form a U-shaped conformation in a single membrane leaflet. Previous work by Moss and co-workers has shown that bolaamphiphiles with linking chains that have been stiffened by the inclusion of central aromatic rings strongly prefer extended, membrane-spanning conformations.²⁴ Furthermore, the translocation rate for stiffened bolaamphiphiles is extremely slow. Therefore,

(22) A phosphodiester end group with a single phenyl ring is not large enough to prevent unthreading of the macrocycle/thread components in rotaxane **2**.

(23) Polar aromatic groups such as the side chains of tryptophan and tyrosine are known to prefer the interfacial regions of a bilayer membrane, but phenyl groups tend to bury into the membrane interior. Stahelin, R. V.; Cho, W. *Biochemistry* **2001**, *40*, 472–4678. Nonetheless, preorganized hosts with multiple aromatic rings are known to have moderate affinities for the quaternary ammonium cation in the phosphocholine headgroup. Lambert, T. N.; Smith, B. D. *Coord. Chem. Rev.* **2003**, *240*, 129–141. Therefore, it is hard to predict the preferred membrane locations of the diphenylethane end groups in these bolaamphiphiles.

(24) Moss, R. A.; Li, J. M. *J. Am. Chem. Soc.* **1992**, *114*, 9227–9229. For additional discussion of the factors controlling translocation of bolaamphiphiles, see: Moss, R. A.; Li, G.; Li, J.-M. *J. Am. Chem. Soc.* **1994**, *116*, 805–806.

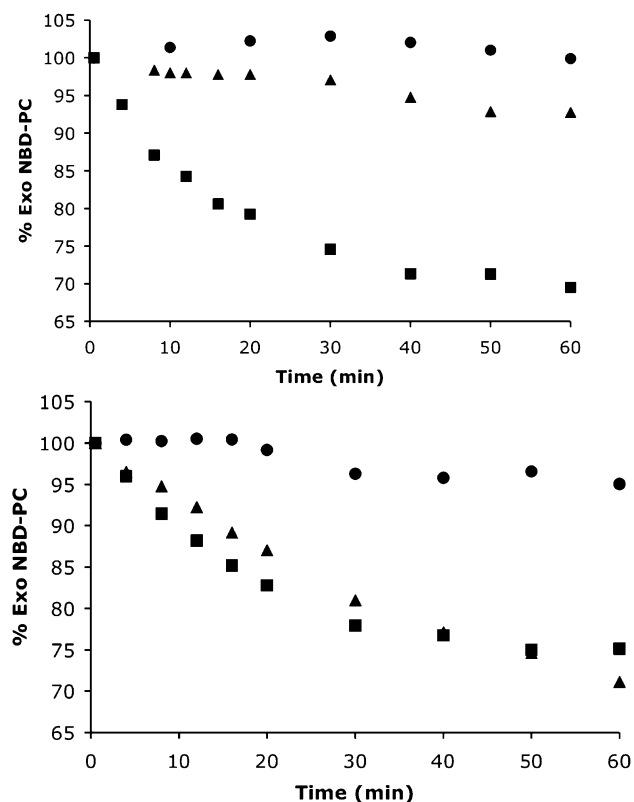


Figure 4. Change in the fraction of NBD-PC in the membrane outer monolayer (% Exo NBD-PC). Inward translocation induced at $t = 0$ min by adding $0.125 \mu\text{M}$ of NBD-PC to POPC vesicles ($25 \mu\text{M}$) that also contained (Top) 5 mol % of **1** (■), **2** (▲), or **6** (●); (Bottom) 4 mol % of **3** (■), **4** (▲), or **5** (●).

Table 1. Half-Life ($t_{1/2}$) for NBD-PG and NBD-PC Translocation Across Vesicles Containing Amphiphiles **1–6**

| vesicle composition | $t_{1/2}$ (min) ^a | |
|-------------------------------|------------------------------|--------|
| | NBD-PC | NBD-PG |
| POPC: 1 (95:5) | 15 | 12 |
| POPC: 2 (95:5) | 120 | 40 |
| POPC: 3 (96:4) | 25 | 15 |
| POPC: 4 (96:4) | 30 | 15 |
| POPC: 5 (96:4) | > 120 | > 120 |
| POPC: 6 (95:5) | > 120 | > 120 |
| POPC:Chol: 1 (66:29:5) | > 120 | 90 |
| POPC:Chol: 2 (66:29:5) | > 120 | > 120 |

^a 5 mM TES, 100 mM NaCl, pH 7.4, 25 °C; estimated uncertainty is $\pm 33\%$.

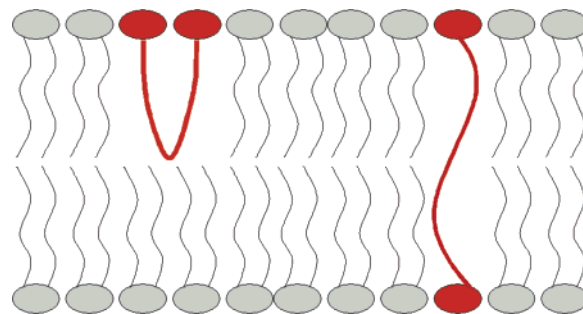


Figure 5. A graphical representation of a bilayer membrane containing a U-shape bolaamphiphile and an extended membrane-spanning bolaamphiphile.

bolaamphiphiles **1–3** with their rigid central regions are expected to adopt extended, transmembrane orientations.

The data in Figures 3 and 4 clearly show that bolaamphiphiles **1–4** can greatly accelerate inward phospholipid translocation across POPC membranes,²⁵ whereas the control amphiphile **6**, with only one phosphodiester end group, is unable to induce any significant translocation. Why are the bolaamphiphiles so effective at promoting phospholipid translocation?²⁶ In the case of bolaamphiphile **1**, we considered the possibility that the central fumaramide core may promote translocation by either forming transient hydrogen-bonded complexes with the phospholipid headgroups or self-associating to form phase-separated microdomains. Evidence against the first process is the efficient translocation induced by bolaamphiphiles **2** and **3**, which are analogues of **1** except that they are much less likely to form intermolecular hydrogen bonds in the middle of the membrane. The bolaamphiphile **2** is a rotaxane with an amide-containing macrocycle that encapsulates and protects the central fumaramide core. It is well-established that the macrocycle forms strong, internal hydrogen bonds with the fumaramide carbonyl oxygens,²¹ and this co-conformational preference was confirmed in the case of **2** by inspection of its ¹H NMR spectrum in organic solvent (the highly upfield chemical shift for the fumaramide olefin hydrogens indicates that they are located deep inside the macrocycle). Thus, the surrounding macrocycle in bolaamphiphile **2** prevents any hydrogen-bonded self-association of the fumaramide core. A similar situation occurs with bolaamphiphile **3**, which contains a 2,5-dimethoxyterephthalamide as the central core. This aromatic group forms strong, internal hydrogen bonds that will stiffen the central region of the bolaamphiphile and lower the propensity for intermolecular association.²⁷ The fact that both **2** and **3** promote the translocation of NBD-PG and NBD-PC almost as well as bolaamphiphile **1**, whereas amphiphile **6** is completely ineffective, indicates that intermolecular hydrogen bonding in the middle of the membrane is not a necessary structural feature for accelerated translocation.²⁶ Evidence against the second process, that flip-flop is accelerated by the presence of phase-separated microdomains,²⁸ was gained by measuring the translocation of NBD-PC across vesicle membranes composed of POPC:DPPC (55:45). This membrane composition is known to form a heterogeneous mixture of fluid and gel phase microdomains;²⁹ however, the rate of inward translocation of NBD-PC was as slow as that observed with pure POPC vesicles.

Another translocation pathway that must be considered is a small population of U-shaped orientations that are very effective at disturbing membrane integrity and promoting flip-flop.^{30,31} To explore the likelihood of this translocation mechanism, bolaamphiphiles **4** and **5** were prepared and evaluated. These two bolaamphiphiles have 2,6-pyridine dicarboxamide units as the central cores. This aromatic group is well-known to form internal hydrogen bonds, with both amide NH residues directed toward the pyridine nitrogen.³² Thus, these bolaamphiphiles are more likely to form a U-shaped conformation; however, an extended membrane-spanning orientation is still possible with version **4** and its two dodecyl chains (Scheme 1). The shorter version, **5**, has two hexyl chains and cannot span a bilayer; therefore, it must adopt a U-shaped orientation in one of the membrane leaflets. The data in Figures 3 and 4 show that the longer bolaamphiphile **4** is an efficient translocator, whereas the shorter version **5** is inactive. This latter result is evidence against the hypothesis that a bolaamphiphile in a U-shape orientation is especially active.

The above structure/activity results are consistent with the idea that phospholipid translocation is strongly promoted by bolaamphiphiles in a transmembrane orientation. Evidence that this occurs by some sort of slip-pop mechanism is the fact that the translocase activities of compounds **1** and **2** are greatly attenuated when the vesicles also include 29 mol % cholesterol (Table 1). The added cholesterol is known to increase order in the acyl chains and raise lateral pressure which is expected to decrease bolaamphiphile wobbling and inhibit the slip-pop pathway.^{19,31} The generality of this finding needs to be tested with further studies of alternative bolaamphiphile structures, especially compounds with different types of hydrophilic end groups and numbers of linking chains.³³

In summary, membrane-spanning bolaamphiphiles **1–4** are able to promote the translocation of monoanionic NBD-PG and to a lesser extent the translocation of zwitterionic NBD-PC across vesicle membranes.²⁵ The data are consistent with the slip-pop model for facilitated phospholipid translocation by membrane-spanning bolaamphiphilic structures.^{19,31} The membrane disruption effect is subtle and does not create defects that are large enough to allow the passage of small polar molecules. Our results are an exception to the rule of thumb that membrane-spanning bolaamphiphiles are inherently membrane stabilizing molecules that inhibit all types of membrane transport.^{8,18}

An intriguing, ongoing question in biomembrane research is the mechanism for accelerated phospholipid flip-flop across

- (25) The half-lives listed in Table 1 indicate that the monoanionic probe NBD-PG is always translocated faster than the zwitterionic NBD-PC, a preference that has been noted in previous studies.^{13,19} It is not clear if the NBD-PG translocates as an anion or as the neutral acid. Recent work indicates that organic anions may be more permeable than predicted by the pH-partition hypothesis. See: Thomae, A. V.; Wunderli-Allenspach, H.; Krämer, S. D. *Biophys. J.* **2005**, *89*, 1802–1811.
- (26) Previous studies by our group have evaluated small organic molecules that were specifically designed to promote phospholipid flip-flop. These compounds form hydrogen bonded complexes with the polar headgroup of the phospholipid and the complexes diffuse across the membrane. With these mobile transport carriers, the half-lives for NBD-PC and NBD-PG translocation are around 4–30 min and <1 min, respectively. Compared to the bolaamphiphiles reported here, these mobile transport carriers transport NBD-PC equally as well, but they transport NBD-PG almost 10 times better.
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- (30) Bolaamphiphiles that adopt U-shaped conformations tend to disrupt the membrane so much that salt transport occurs. See, for example: Fyles, T. M.; Zeng, B. *J. Org. Chem.* **1998**, *63*, 8337–8345; Fuhrhop, J. H.; Krull, M.; Schulz, A.; Möbius, D. *Langmuir* **1990**, *6*, 497–505.
- (31) Another bolaamphiphile motion that may promote phospholipid flip-flop is the interconversion between transmembrane and U-shaped bolaamphiphile orientation (Figure 5). Even if this bolaamphiphile interconversion process is quite slow, it may induce a local defect that lives long enough to permit the translocation of many phospholipids. Because transverse phospholipid diffusion is very rapid, a small but long-lived local defect may be an effective short-circuit point for large amounts of rapid transmembrane flip-flop.
- (32) Affeld, A.; Hübner, G. M.; Seel, C.; Schalley, C. A. *Eur. J. Org. Chem.* **2001**, 2877–2890.
- (33) Membrane additives that increase the lateral pressure at the membrane surface are known to increase the rate of phospholipid translocation (Bootsveld, A.; Degenhardt, R.; Kamp, D.; Haest, C. W. M. *Mol. Membr. Biol.* **2004**, *21*, 315–322). Thus, it is possible that bolaamphiphiles with large hydrophilic end groups (compared to the cross-sectional area of the linking chain) may lower the barrier for translocation. However, the inactivity of controls **5** and **6** suggests that this is not a dominant factor in the present case.

biogenic membranes (i.e., membranes that contain the enzymes responsible for phospholipid biosynthesis) such as the bacterial cytoplasmic membrane and the mammalian endoplasmic reticulum.^{19,34} Because phospholipid biosynthesis occurs only on one face of the membrane, there has to be a process to translocate the newly formed phospholipid and allow the membrane to grow. Indeed, phospholipid translocation across biogenic membranes is known to be rapid, and for some time, researchers have tried to identify the associated translocase protein(s). In the case of the endoplasmic reticulum, there is evidence for an energy-independent, bidirectional translocase that is equally effective with a range of phospholipid headgroups, however, the specific protein(s) has not yet been identified.³⁵ An alternative theory, proposed by de Kruijff and co-workers states that phospholipid translocation across biogenic membranes does not require a dedicated translocase because it can be promoted by simple single-pass helical peptides.¹⁹ Our results agree with the model that simple, membrane-spanning bolaamphiphilic structures can promote phospholipid translocation. The bolaamphiphiles reported here are about an order of magnitude less efficient than a single-pass helical peptide, but both systems show the same headgroup selectivity, that is, the translocation of monoanionic PG is faster than zwitterionic PC.²⁵ Because the fraction of PG in bacterial membranes (15–70%) is much higher than in mammalian membranes (<2%), it appears that the slip-pop mechanism is more likely to be kinetically significant in bacterial membranes.³⁶

Experimental Section

Phospholipid Translocation Assay. POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine), NBD-PC (1-palmitoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-*sn*-glycero-3-phosphocholine), and NBD-PG (1-palmitoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (ammonium salt) were purchased from Avanti Polar Lipids. Cholesterol was purchased from Sigma-Aldrich. An appropriate mixture of POPC (in CHCl₃), cholesterol (in CHCl₃), and/or bolaamphiphile (in 7:3 CHCl₃:CH₃OH) was dried in vacuo for 1 h. A stock solution of 10 mM vesicles was made by rehydration at room temperature with TES buffer (5 mM TES, 100 mM NaCl, pH 7.4). Multilamellar vesicles were extruded to form unilamellar vesicles with a Basic LiposoFast device purchased from Avestin, Incorporated. The vesicles were extruded 29 times through a 19-mm polycarbonate Nucleopore filter with 100-nm diameter pores. An aliquot of NBD-phospholipid (NBD-PC or NBD-PG in 140 μL of 200 proof ethanol, final concentration 0.125 μM) was added to the vesicles (45 mL, 25 μM) in TES buffer. Samples (3.0 mL) were withdrawn over time and assayed in the following way for % exo NBD-phospholipid. At assay time $t = 50$ s, sodium dithionite (180 μL of 60 mM solution in 1 M Tris, pH ~10) was added and at $t = 180$ s, Triton X-100 (20 μL of 20% v/v) was added. The amount of total NBD-PX that was translocated to the inside of the vesicles was calculated by the following equation: % Exo NBD-phospholipid = $((F_i - F_f)/F_i) \times 100$, where F_i and F_f are the fluorescence intensities (ex: 460 nm, em: 530 nm) just prior to the additions of sodium dithionite and Triton X-100. The half-lives reported are the averages of 2 or 3 independent experiments.

Vesicle Leakage Assay. A solution of **2** (5 mol %) and POPC (100 mol %) in CHCl₃:MeOH was evaporated and dried in vacuo for 1 h. A solution of 10 mM vesicles was made by rehydration at room temperature with 5(6)-carboxyfluorescein (CF) buffer (50 mM CF, 5 mM TES, 100 mM NaCl, pH 7.4). The vesicles were frozen and thawed 10 times and then extruded 29 times through a 19-mm polycarbonate Nucleopore filter with 100-nm diameter pores. Unencapsulated dye was removed from the vesicles by separation via a Sephadex-G50 column in TES buffer. The fluorescence intensity of a 3-mL sample of vesicles (25 μM in TES buffer) was monitored for 400 s then Triton X-100 (20 μL of 20% v/v) was added to lyse the vesicles.

Synthesis.³⁷ **N-Boc-12-aminododecanoic acid.** 12-Aminododecanoic acid (1.00 g, 4.65 mmol) was suspended in 15.0 mL of methanol with 0.775 mL (5.58 mmol) of triethylamine and 1.01 g (4.65 mmol) of dissolved di-*tert*-butyl-dicarbonate and heated under a reflux condenser to 60 °C overnight. The solvent was evaporated on a rotary evaporator, and the residue was redissolved in ethyl acetate. The mixture was then washed with 0.25 M HCl (2×), dried, and filtered, and the solvent evaporated to give a white solid. *N*-Boc-12-aminododecanoic acid (90%) was purified by recrystallization in hexanes. δ_{H} (500 MHz; CDCl₃; Me₄Si) 11.67 (1H, s, COOH), 4.55 (1H, s, Boc NH), 3.10 (2H, quar, $J = 6$ Hz, Dodec), 2.34 (2H, t, $J = 7$ Hz, Dodec), 1.62 (2H, quin, $J = 7$, Dodec), 1.44 (11H, s, Boc + Dodec), 1.27 (16H, s, Dodec). δ_{C} (125 MHz; CDCl₃; CDCl₃) 179.7, 156.2, 79.3, 41.9, 40.8, 34.3, 30.2, 29.63, 29.55, 29.45, 29.38, 29.22, 28.6, 27.0, 24.9.

N-Boc-12-aminododecanol. To a solution of *N*-Boc-12-aminododecanoic acid (4.22 g, 13.4 mmol) in 100 mL of dry THF was added Red-Al (12.3 mL, 40.2 mmol-65% weight in toluene) via a syringe over 30 min. Because of the extensive precipitation, a mechanical stirrer was used. The reaction was stirred for 30 min after the precipitate dissolved and then quenched with saturated sodium sulfate and stirred for another 1 h. The solids formed during the quenching process were then filtered, and the solution was concentrated to an oil on a rotary evaporator, redissolved in ethyl acetate, and washed with water, dried, filtered, and evaporated to give the desired product (3.39 g, 84%) as a white solid which was recrystallized in toluene and used without further purification. δ_{H} (500 MHz; CDCl₃; Me₄Si) 4.55 (1H, s, Boc NH), 3.64 (2H, t, $J = 7$ Hz, Dodec), 3.10 (2H, quar, $J = 7$ Hz, Dodec), 1.57 (2H, quin, $J = 7$ Hz, Dodec), 1.44 (11H, s, Boc + Dodec), 1.26 (16H, m, Dodec). δ_{C} (125 MHz; CDCl₃; CDCl₃) 156.2, 79.2, 63.2, 51.3, 40.8, 33.0, 30.2, 29.75, 29.71, 29.60, 29.46, 28.6, 27.0, 25.9.

N-Boc-6-aminohexanol. 6-Aminohexanol (1.00 g, 8.50 mmol) was dissolved in 5.00 mL of THF and di-*tert*-butyl-dicarboxylate (1.86 g, 8.50 mmol) was added. The solution was allowed to stir at room temperature for 3 h, and solvent was removed with a rotary evaporator to give the desired product (99%) as a colorless oil, which crystallized after 2 weeks. The compound was used without further purification. δ_{H} (500 MHz; CDCl₃; Me₄Si) 4.65 (1H, s, Boc NH), 3.62 (2H, t, $J = 7$ Hz, Hexyl), 3.12 (2H, quar, $J = 7$ Hz, Hexyl), 2.09 (1H, s, OH), 1.57 (2H, quin, $J = 7$ Hz, Hexyl), 1.49 (2H, quin, $J = 7$ Hz, Hexyl), 1.44 (9H, s, Boc), 1.36 (4H, m, Hexyl). δ_{C} (125 MHz; CDCl₃; CDCl₃) 156.3, 79.2, 62.7, 40.5, 32.7, 30.2, 28.6, 26.5, 25.4.

2,5-Dimethoxyterephthaloyl Dichloride. This compound (a white solid) was synthesized by a literature method.³⁸ δ_{H} (500 MHz; CDCl₃; Me₄Si) 7.55 (2H, s, Ph), 3.96 (6H, s, Me). δ_{C} (125 MHz; CDCl₃; CDCl₃) 163.8, 152.0, 128.3, 116.6, 57.0.

12-(*tert*-Butylcarbamoyl)dodecyl-4-nitrophenyl-2,2-diphenylethyl Phosphate (9a). To a stirred solution of tris(*p*-nitrophenyl) phosphate (200.0 mg; 0.433 mmol) and 2,2-diphenyl ethanol (85.8 mg; 0.433 mmol) in 3 mL of dry chloroform (stabilized with amylenes) was added DBU (68 μL; 0.455 mmol), and the reaction was allowed to stir for 3

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(36) That is not to say that all translocase proteins in the bacterial membrane are single-pass helical structures. For example, the *wzx*E translocase is a large protein with multiple transmembrane domains. Rick, P. D.; Barr, K.; Sankaran, K.; Kajimura, J.; Rush, J. S.; Waechter, C. J. *J. Biol. Chem.* **2003**, *278*, 16534–16542.

(37) The NMR data includes magnetic field strength, solvent, and chemical shift reference. The ¹³C NMR signals are quoted to two decimal places only in spectral regions that are crowded.

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h. The reaction was then concentrated to an oil using a rotary evaporator and the residue was redissolved in 5.00 mL of dry toluene. *N*-Boc-12-aminododecanol powder (131 mg; 0.433 mmol) was added to the new solution followed by another 68 μ L of DBU, and the reaction was allowed to stir overnight. The mixture was then diluted with ethyl acetate and washed with 0.25 M HCl. Compound **9a** (60%) was purified as a clear viscous oil by column chromatography using chloroform as the eluent. δ_{H} (300 MHz; CDCl₃; Me₄Si) 8.08 (2H, d, $J = 9$ Hz, PNP), 7.22 (10H, m, DPE), 7.11 (2H, d, $J = 9$ Hz, PNP), 4.67 (2H, t, $J = 7$ Hz, DPE), 4.59 (1H, s-br, Boc NH), 4.38 (1H, t, $J = 8$ Hz, DPE), 3.90 (2H, quar, $J = 7$ Hz, Dodec), 3.07 (2H, quar, $J = 7$ Hz, Dodec), 1.58 (2H, quin, $J = 7$ Hz, Dodec), 1.42 (11H, s, Boc + Dodec), 1.22 (16 Hz, s-br, Dodec). δ_{C} (75 MHz; CDCl₃; CDCl₃) 156.1, 155.4 (d, $J = 7$ Hz), 144.6, 140.1, 128.8, 128.3, 127.2, 125.6, 120.5, 79.0, 70.7 (d, $J = 7$ Hz), 69.3 (d, $J = 7$ Hz), 51.3, 40.7, 30.2, 30.1, 29.59, 29.56, 29.52, 29.36, 29.08, 28.52, 26.9, 25.3

6-(tert-Butylcarbamoyl)hexyl-2,2-diphenylethyl-4-nitrophenyl phosphate (9b). To a stirred solution of tris(*p*-nitrophenyl) phosphate (200 mg; 0.433 mmol) and 2,2-diphenyl ethanol (85.8 mg; 0.433 mmol) in 3 mL of dry chloroform (stabilized with amylenes) was added DBU (68 μ L; 0.455 mmol), and the reaction was allowed to stir for 3 h. The reaction was then concentrated to an oil using a rotary evaporator and the residue was redissolved in dry toluene. *N*-Boc-6-aminohexanol (94.0 mg; 0.433 mmol) was added to the new solution followed by another 68 μ L of DBU and reaction was allowed to stir overnight. The mixture was then diluted with ethyl acetate and washed with acid. The product (50%) was purified as a clear viscous oil by column chromatography using chloroform as the eluent. δ_{H} (500 MHz; CDCl₃; Me₄Si) 8.08 (2H, d, $J = 9$ Hz, PNP), 7.25 (10H, m, Ph), 7.11 (2H, d, $J = 9$ Hz, PNP), 4.95 (1H, t, $J = 7$ Hz, PNP), 4.68 (2H, t, $J = 7$ Hz, Boc NH), 4.38 (1H, t, $J = 7$ Hz, DPE), 3.97 (2H, quar, $J = 7$ Hz, Hexyl), 3.15 (2H, quar, $J = 7$ Hz, Hexyl), 1.55 (4H, m, Hexyl) 1.41 (9H, s, Boc), 1.29 (4H, m, Hexyl).

General Procedure for the Synthesis of Compounds **1**_{PNP}–**5**_{PNP}.

The appropriate Boc-protected amine was dissolved in the minimal amount of dichloromethane and then diluted with trifluoroacetic acid to double the volume. The solution was stirred for 20 min after gas evolution stopped, and the solvent was removed with a rotary evaporator. The residue was then dissolved in methanol with 0.5 mL of added concentrated HCl and evaporated (achieving ion exchange). The residue was then evaporated twice with toluene and dissolved in dry chloroform. Added to this mixture was the corresponding bis-acid chloride (0.5 molar equivalents). Triethylamine (two molar equivalents with respect to the amine) was first diluted with chloroform and added to the reaction with a dropping funnel over 1 h. The reaction was stirred for 2 h after the last addition of base, and the product was purified by chromatography (all 5 compounds required 0–3% methanol in dichloromethane as the eluent).

Bis-*N,N*-(*p*-nitrophenyl-2,2-diphenylethyl-12-aminododecyl phosphate)-fumaramide (1**_{PNP}).** The product was a white solid (70%). δ_{H} (500 MHz; CDCl₃; Me₄Si) 8.10 (4H, d, $J = 9$ Hz, PNP), 7.45 (2H, t, $J = 6$ Hz, Fu NH), 7.23 (20H, m, Ph), 7.18 (2H, s, Fu CH), 7.12 (4H, d, $J = 9$ Hz, PNP), 4.69 (4H, t, $J = 7$ Hz, DPE), 4.40 (2H, t, $J = 7$ Hz, DPE), 3.99 (4H, quar, $J = 7$ Hz, Dodec), 3.33 (4H, quar, $J = 7$ Hz, Dodec), 1.58 (8H, quin, $J = 7$ Hz, Dodec), 1.23 (32H, m, Dodec). δ_{C} (125 MHz; CDCl₃; CDCl₃) 164.9, 155.4 (d, $J = 7$ Hz), 144.6, 140.1, 133.3, 128.8, 128.3, 127.2, 125.6, 120.6, 70.7 (d, 7 Hz), 69.3 (d, 7 Hz), 51.4, 40.0, 30.16, 30.11, 29.65, 29.62, 29.55, 29.51, 29.46, 29.1, 27.2, 25.4.

Bis-*N,N*-(*p*-nitrophenyl-2,2-diphenylethyl-12-aminododecyl phosphate)-2,5-dimethoxy-terephthalamide (3**_{PNP}).** The product was a white solid (75%). δ_{H} (500 MHz; CDCl₃; Me₄Si) 8.11 (4H, d, $J = 9$ Hz, PNP), 8.10 (2H, br s, Tera NH), 7.89 (2H, s, Tera Ph), 7.27 (20H, m, Ph), 7.13 (4H, d, $J = 9$ Hz, PNP), 4.69 (4H, t, $J = 7$ Hz, DPE), 4.40 (2H, t, $J = 7$ Hz, DPE), 4.00 (4H, quar, $J = 7$ Hz, Dodec), 3.99 (6H, s, Tera Me), 3.46 (4H, quar, $J = 7$ Hz, Dodec), 1.61 (8H, m,

Dodec), 1.37 (8H, m, Dodec), 1.24 (24H, m, Dodec). δ_{C} (125 MHz; CDCl₃; CDCl₃) 164.3, 155.5 (d, $J = 7$ Hz), 151.6, 144.7, 140.1, 128.9, 128.3, 127.3, 125.7, 124.7, 120.6, 115.4, 70.7 (d, $J = 7$ Hz), 69.3 (d, $J = 7$ Hz), 56.7, 51.42, 51.37, 40.1, 30.2, 30.2, 29.73, 29.67, 29.63, 29.5, 29.2, 27.3, 25.4.

Bis-*N,N*-(*p*-nitrophenyl-2,2-diphenylethyl-12-aminododecyl phosphate)-2,6-pyridine Dicarbamide (4**_{PNP}).** The product was a white solid (78%). δ_{H} (500 MHz; CDCl₃; Me₄Si) 8.33 (2H, d, $J = 8$ Hz, Py), 8.18 (2H, t, $J = 6$ Hz, Py NH), 8.11 (4H, d, $J = 9$ Hz, PNP), 7.98 (1H, t, $J = 8$ Hz, Py), 7.25 (20H, m, Ph), 7.11 (4H, d, $J = 9$ Hz, PNP), 4.69 (4H, t, $J = 7$ Hz, DPE), 4.40 (2H, t, $J = 7$ Hz, DPE), 3.98 (4H, quar, $J = 7$ Hz, Dodec), 3.41 (4H, quar, $J = 7$ Hz, Dodec), 1.59 (8H, m, Dodec), 1.24 (32H, m, Dodec).

Bis-*N,N*-(*p*-nitrophenyl-2,2-diphenylethyl-6-aminohexyl phosphate)-2,6-pyridine Dicarbamide (5**_{PNP}).** The product was a white solid (70%). δ_{H} (500 MHz; CDCl₃; Me₄Si) 8.34 (2H, d, $J = 8$ Hz, Py NH), 8.18 (2H, t, $J = 6$ Hz, Py), 8.10 (4H, d, $J = 9$ Hz, PNP), 8.00 (1H, t, $J = 8$ Hz, Py), 7.27 (8H, m, Ph), 7.22 (12H, m, Ph), 7.12 (4H, d, $J = 9$ Hz, PNP), 4.69 (4H, t, $J = 7$ Hz, DPE), 4.40 (2H, t, $J = 7$ Hz, DPE), 3.98 (4H, quar, $J = 7$ Hz, Hexyl), 3.41 (4H, quar, $J = 7$ Hz, Hexyl), 1.58 (8H, m, Dodec), 1.29 (8H, m, Hexyl). δ_{C} (125 MHz; CDCl₃; CDCl₃) 163.7, 155.3 (d, $J = 6$ Hz), 149.0, 144.6, 139.9, 139.0, 128.8, 128.2, 127.2, 125.6, 125.0, 120.5, 70.7 (d, $J = 6$ Hz), 68.9 (d, $J = 6$ Hz), 51.3, 39.5, 29.9, 29.8, 26.3, 24.9.

Bis-*N,N*-(2,2-diphenylethyl-12-aminododecyl phosphate)-fumaramide (1**).** Compound **1**_{PNP} (200 mg, 0.161 mmol) was dissolved in 10.0 mL of DMSO, and 1.0 mL of 1.0 M LiOH solution was added. Reaction was stirred for 1 h, and the DMSO solution was added to 50 mL of HCl (0.25 M). The pure product immediately precipitated out of solution as a white solid, was filtered, and was washed with water to give a yield of 50%. δ_{H} (500 MHz; CDCl₃/CD₃OD 1:1; Me₄Si) 7.26 (20H, m, Ph), 6.81 (2H, s, Fu CH), 4.51 (4H, t, $J = 7$ Hz, DPE), 4.37 (2H, t, obscured by solvent peak, DPE), 3.75 (4H, quar, $J = 7$ Hz, Dodec), 3.28 (4H, t, $J = 7$ Hz, Dodec), 1.54 (4H, m, Dodec), 1.25 (36H, m, Dodec). δ_{C} (125 MHz; CDCl₃/CD₃OD 1:1; CDCl₃) 165.3, 140.7, 132.5, 128.5, 128.2, 126.8, 69.0 (d, $J = 7$ Hz), 67.4 (d, $J = 7$ Hz), 51.2, 39.8, 30.1, 30.0, 29.44, 29.40, 29.22, 29.04, 28.99, 26.9, 25.3. FAB-MS (NBA matrix): m/z 1004 [M + H]⁺.

Bis-*N,N*-(2,2-diphenylethyl-12-aminododecyl phosphate)-2,5-dimethoxyterephthalamide (3**).** Compound **3**_{PNP} (200 mg, 0.148 mmol) was dissolved in 10 mL of DMF, and 1.0 mL of 1.0 M LiOH was added. The reaction was allowed to stir for 1 h, and compound **3** was purified by diluting the reaction mixture with water and washing with chloroform, separating and acidifying the organic layer, and then drying on a high vacuum line (65%). δ_{H} (500 MHz; CDCl₃; Me₄Si) 8.09 (2H, t, $J = 6$ Hz, Tera NH), 7.88 (2H, s, Tera CH), 7.23 (20H, m, Ph), 4.47 (4H, t, $J = 7$ Hz, DPE), 4.34 (2H, t, $J = 7$ Hz, DPE), 3.96 (6H, s, Tera Me), 3.72 (4H, quar, $J = 7$ Hz, Dodec), 3.47 (4H, quar, $J = 7$ Hz, Dodec), 1.61 (4H, quin, $J = 7$ Hz, Dodec), 1.47 (4H, m, Dodec), 1.25 (32H, m, Dodec). δ_{C} (125 MHz; CDCl₃; CDCl₃) 164.4, 151.6, 140.9, 128.7, 128.5, 127.0, 124.7, 115.5, 69.2, 67.7, 56.8, 51.4, 51.3, 40.0, 30.23, 30.17, 29.50, 29.46, 29.28, 29.14, 27.1, 25.4. FAB-MS (NBA matrix): m/z 1114 [M + H]⁺.

Bis-*N,N*-(2,2-diphenylethyl-12-aminododecyl phosphate)-2,6-pyridine Dicarbamide (4**).** Compound **4**_{PNP} (300 mg, 0.231 mmol) was dissolved in 10 mL of DMF, and 1.0 mL of 1.0 M LiOH was added. The reaction was allowed to stir for 1 h, then 1 mL of 2.5 M HCl was added, and the resulting solution was concentrated on a rotary evaporator. The residue was washed with water (2 \times) and then washed with anhydrous diethyl ether (3 \times) to give **4** (50%). δ_{H} (500 MHz; CDCl₃/CD₃OD 3:1; Me₄Si) 8.28 (2H, d, $J = 8$ Hz, Py), 8.01 (1H, t, $J = 8$ Hz, Py), 7.22 (20H, m, Ph), 4.51 (4H, t, $J = 8$ Hz, DPE), 4.37 (2H, t, $J = 8$ Hz, DPE), 3.75 (4H, quar, $J = 7$ Hz, Dodec), 3.44 (4H, t, $J = 7$ Hz, Dodec), 1.66 (4H, quin, $J = 8$ Hz, Dodec), 1.53 (4H, quin, $J = 7$ Hz, Dodec), 1.35 (8H, m, Dodec), 1.24 (24H, m, Dodec). δ_{C} (125 MHz; CDCl₃/CD₃OD 3:1; CDCl₃) 164.2, 148.8, 140.7, 138.9, 128.4, 128.1,

126.7, 124.6, 69.0 (d, $J = 7$ Hz), 67.3 (d, $J = 7$ Hz), 51.2, 39.6, 30.1, 30.0, 29.53, 29.48, 29.42, 29.40, 29.31, 29.0, 27.0, 25.3. FAB-MS (NBA matrix): m/z 1055 $[M+H]^+$, 1077 $[M + Na]^+$.

Bis-*N,N*-(2,2-diphenylethyl-6-aminoethyl phosphate)-2,6-pyridine Dicarbamide (5). Compound **5**_{PNP} (200 mg, 0.177 mmol) was dissolved in 10 mL of DMF, and 1.0 mL of 1.0 M LiOH solution was added. The solution was allowed to stir at room temperature for 1 h and was then concentrated on a rotary evaporator. The residue was dissolved in the minimum amount of water, and the bis-lithium phosphate salt of the product was driven out of solution with acetone. The mother liquor was decanted, and the remaining oily residue was dissolved in methanol with 3 drops of concentrated HCl solution, and the solution evaporated to dryness. The product **5** was selectively extracted into 1:1 methanol/chloroform and regained as an off-white solid after filtration and solvent evaporation (40%). δ_H (500 MHz; CD₃OD; CHD₂-OD) 8.34 (2H, d, $J = 7$ Hz, Py), 8.21 (1H, t, $J = 7$ Hz, Py), 7.25 (20H, m, Ph), 4.45 (4H, t, $J = 7$ Hz, DPE), 4.33 (2H, t, $J = 7$ Hz, DPE), 3.75 (4H, quar, $J = 6$ Hz, Hexy), 3.50 (4H, t, $J = 6$ Hz, Hexyl), 1.67 (4H, s, Hexyl), 1.55 (4H, s, Hexyl), 1.37 (8H, s, Hexyl). δ_C (125 MHz; CD₃OD; CD₃OD) 166.4, 150.3, 142.4, 129.7, 129.5, 128.2, 128.0, 125.7, 70.4, 68.4, 52.8 (d, $J = 7$ Hz), 40.9, 31.1, 30.4, 27.4, 26.2. FAB-MS (NBA matrix): m/z 925 $[M + K]^+$.

12-(*tert*-Butylcarbonyl)dodecyl-2,2-diphenylethyl Phosphate (6). Compound **6**_{PNP} was dissolved in 10 mL of DMF, and 1.0 mL of 1.0 M LiOH was added. The reaction was allowed to stir for 1 h and was then concentrated on a rotary evaporator. The residue was dissolved in ethyl acetate and washed with 0.25 M NaOH (3 \times) and then with 0.25 M HCl (2 \times). The organic layer was dried with magnesium sulfate, filtered, and evaporated to give compound **6** as a clear oil (65%). δ_H (300 MHz; CDCl₃; Me₄Si) 7.76 (1H, br s, Phos OH), 7.20 (10H, m, Ph), 4.46 (2H, t, $J = 7$ Hz, DPE), 4.35, (1H, t, $J = 7$ Hz, DPE), 3.70 (2H, quar, $J = 7$ Hz, Dodec), 3.09 (2H, t, $J = 7$ Hz, Dodec), 1.47 (4H, m, Dodec), 1.44 (9H, s, Dodec), 1.22 (16H, m, Dodec). δ_C (75 MHz; CDCl₃; CDCl₃) 141.1, 128.7, 128.5, 126.9, 126.2, 69.1, 67.5, 51.4, 51.3, 40.8, 30.4, 30.3, 30.2, 29.7, 29.5, 29.4, 28.6, 27.0, 25.6, 25.1. FAB-MS (NBA matrix): m/z 460 $[M - Boc + H]^+$.

Rotaxane 2_{PNP}. To a solution of **1**_{PNP} and 10 equiv triethylamine in dry chloroform (stabilized with amylenes) was added simultaneously

using a dual syringe pump a chloroform solution of *p*-xylylene diamine and a chloroform solution of 5-*tert*-butyl-isophthaloyl dichloride. The resulting precipitate was filtered through Celite, and the solvent was evaporated using a rotary evaporator. The product was purified by chromatography using 3% methanol in dichloromethane as the mobile phase to give **2**_{PNP} as a white solid (15%). δ_H (300 MHz; CDCl₃; Me₄-Si) 8.45 (2H, s, Wh Iso), 8.21 (4H, s, Wh Iso), 8.06 (4H, d, $J = 9$ Hz, PNP), 7.69 (4H, t, $J = 5$ Hz, Wh NH), 7.25 (20H, m, Ph), 7.11 (4H, d, $J = 9$ Hz, PNP), 7.05 (8H, s, Wh Xy), 6.40 (2H, t, $J = 5$ Hz, Th NH), 5.72 (2H, s, Th alkene), 4.67 (4H, t, $J = 7$ Hz, DPE), 4.47, (8H, d, $J = 5$ Hz, Wh Benz), 4.38 (2H, t, $J = 7$ Hz, DPE), 3.98 (4H, quar, $J = 7$ Hz, Dodec), 3.14 (4H, quar, $J = 7$ Hz, Dodec), 1.51 (8H, m, Dodec), 1.37 (18H, s, *t*-Bu), 1.22 (32H, m, Dodec).

Rotaxane (2). Compound **2**_{PNP} (100 mg, 0.053 mmol) was dissolved in 10 mL of DMSO, and 1.0 mL of 1.0 M LiOH solution was added. The reaction was stirred for 1 h, and the DMSO solution was added to 50 mL of HCl (0.25 M). The product, compound **2**, precipitated out of solution as a white solid, was filtered, and was washed with water (3 \times) (50%). Yield = 50% ¹H NMR δ_H (500 MHz, 3:1 CDCl₃/CD₃OD) 8.60 (s, 2H, Wh Iso), 8.25 (s, 4H, Wh Iso), 7.26 (m, 16H, Ph), 7.20 (m, 4H, Ph), 7.04 (s, 8H, Wh Xy), 5.68 (s, 2H, Th alkene), 4.49 (t, 4H, $J = 7$ Hz, DPE), 4.45 (s, 8H, Wh CH₂), 4.36 (t, 2H, $J = 7$ Hz, DPE), 3.73 (quar, 4H, $J = 7$ Hz, Dodec), 3.11 (t, 4H, $J = 7$ Hz, Dodec), 1.51 (m, 4H, Dodec), 1.43 (s, 18H, *t*-Bu), 1.26 (m, 36H, Dodec). δ_C (125 MHz, CDCl₃/CD₃OD 3:1) δ 167.6, 165.6, 152.8, 140.9, 136.9, 133.4, 129.7, 128.9, 128.5, 128.4, 128.2, 126.7, 122.2, 68.8, 67.1, 51.3, 43.8, 39.8, 35.1, 31.1, 30.2, 30.1, 29.6, 29.5, 29.45, 29.44, 29.28, 29.09, 27.0, 25.4. FAB-MS (NBA matrix): m/z 1647 $[M + H]^+$, 1669 $[M + Na]^+$.

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Supporting Information Available: ¹H NMR spectra of synthetic amphiphiles **1–6**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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