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Solid-Phase Synthesis of Oligoester Ion Channels

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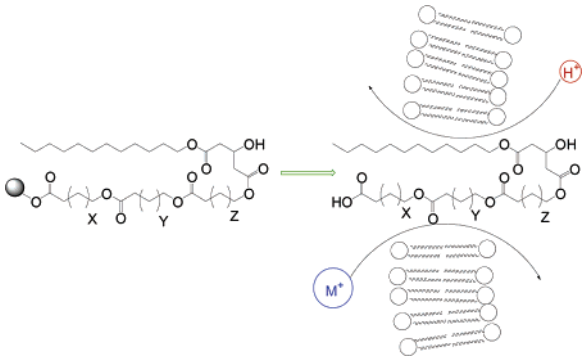

A simple synthesis of oligoesters from TBDMS-protected-β- and THP-protected-ω-hydroxycarboxylic acids using a solid-phase synthesis protocol is reported. Procedures were optimized for the efficient production of ion channel candidates in high purity with minimal purification. The product oligoesters were evaluated for ion transport activity using a fluorescent dye/vesicle assay. Oligoesters produced by this strategy show structure-dependent activity; the most active compounds are closely comparable to previously known oligoesters, but are available at a fraction of the synthetic effort.

Introduction

Ions in cells are transported from one side of the bilayer membrane to the other via an ion channel or transporter. Natural ion channels are complex membrane-spanning proteins and therefore are difficult to study and utilize. A variety of synthetic ion channels have been made that can mimic many functions of natural transporters, but these typically involve elaborate and lengthy solution-phase syntheses.[1] These extensive syntheses limit the number of potential ion channels produced for transport activity evaluation and optimization. As a result, structure−activity relationships in synthetic ion channels have only been explored with rather restricted numbers of structures. It would be desirable to generate a more diverse and extensive library of potential channel-forming compounds, both to provide an extensive dataset from which to infer mechanism, and to discover if preconceived design notions are supported by experiment. This suggests that a solid-phase approach to the synthesis would be a logical strategy for generating an array of potential active compounds.

A second motivation for this work is the requirement for dissymmetric channel-forming compounds as a necessary condition for the formation of voltage-gated channels. Previously, the macrocyclic tetraester **1** was shown to have voltage-gated ion transport ability due to the end-to-end dissymmetry that allowed preferential insertion of one end of **1** into a bilayer membrane;[2] the overall symmetry of **1** is illustrated schematically as **1a.** Recently our group reported that the nonmacrocyclic structural relative, **2**, forms an active ion channel as well.[3] Even though **2** is a structural analogue of **1**, the important difference is the higher symmetry of **2** (simplified in **2a**). Despite good activity and very simple synthesis, **2** is not suited for a voltage-gated channel synthesis. Rather an end-to-end synthesis as would occur in a solid-phase synthesis has the required dissymmetry within the synthesis plan. A suitable target is **3** in which all the ester units occur in the same orientation within the strand. In Figure 1, **3b** is drawn with two X and Y groups. An advantage

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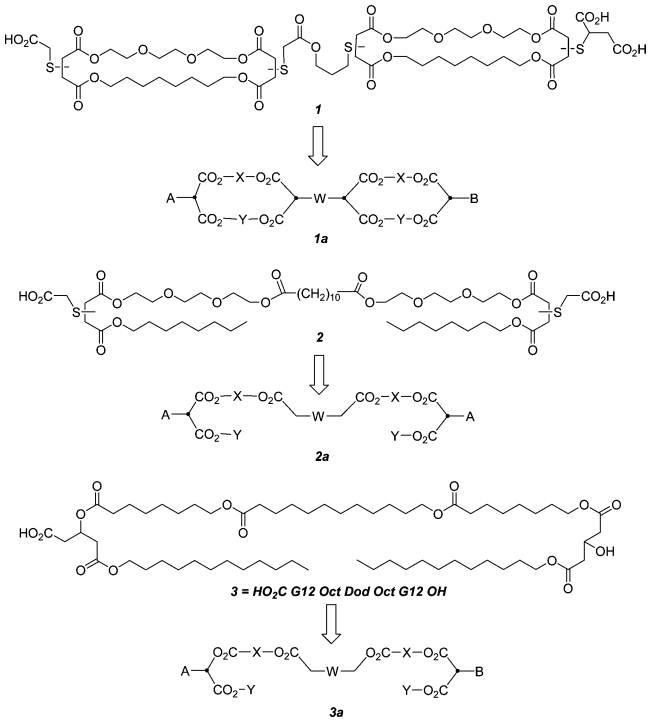

FIGURE 1. Design considerations in oligoester ion channels.

of solid-phase synthesis is that the compound is built sequentially with the possibility that the two X, Y groups need not be identical.

Merrifield's seminal work on the supported synthesis of a tetrapeptide[4] lead to the automated synthesis of oligonucleotides,[5] oligosaccharides,[6] polypeptides,[7] and nonoligomeric substrates such as heterocycles[8] on solid-supports.[9] We initially hoped that oligoamides linked using well-established procedures would lead to suitable channel-forming compounds. However, in a previous study, our group found that esters are favored over amides for channel formation within the series of compounds related to **2**.[3] Recently Kuisle et al. reported a general methodology for the solid-phase synthesis of depsides and depsipeptides using α-hydroxy and α-amino acids on Wang resin.[10] We therefore focused on the Kuisle approach to oligoester structures that might be suited as ion channels candidates in bilayer membranes.

The building blocks of the solid-phase product would ideally be commercially available or would be available from very simple syntheses. From the availability of potential building blocks and the design of the active compound **2**, the target **3** was selected as a test of the strategy. The ether oxygens have been removed in **3** relative to **1** or **2**, but this hydrophobic substitution on compound **1** did not significantly alter channel-forming activity.[11] Another design consideration is the choice of the β-hydroxyester units. Unlike previous oligoester channels, intramolecular depolymerization will be possible in linear oligoesters with the symmetry of **3a** and will be particularly rapid with γ- and δ-esters.[12] The 3-hydroxyglutarate half-esters chosen were chosen to minimize this potential decomposition reaction. The risk of this decomposition reaction also falls off with increasing $\alpha-\omega$ separation in the precursor hydroxyacids. There are many choices for ω-hydroxyacids, and the terminal functional groups (carboxylic acid and alcohol) will allow further functional group transformations that have the potential to lead to a wide diversity of structures. The structural features of these oligoester compounds is reflected by a sequence of three-letter codes starting with the carboxylic acid end and proceeding to the hydroxyl end, in the same directions as the synthesis. Within the simple choices used to demonstrate the strategy, the general ion channel candidate has two headgroups composed of a glutaric acid derivative with a lipophilic 12-carbon tail (G12) and three internal alkyl chains, formed from a combination of eight-carbon (Oct) or 12-carbon (Dod) chains. The syntheses reported in this study will address the methodological questions and uncover whether these compounds are suitably active. This background will serve as a basis for probing whether a nonmacrocyclic product can have voltage-gating properties due to the disymmetric structure implied by **3a** (to be reported separately).

Results and Discussion

Synthesis of Building Blocks. The building blocks of the previously reported synthesis were tetrahydropyranyl (THP)-protected α-hydroxy acids.[10] These were loaded onto the polymer support (Wang resin) using a diisopropylcarbodiimide (DIC) acylating reagent, and the THP was removed using a dilute acid solution. This general chemistry is acceptable, but we cannot use α-hydroxy acids because we need significantly longer products to span the lipid bilayer. Therefore, we chose ω-hydroxy acids of eight and twelve carbons long as having the spacing similar to the esters in **2**. The synthesis of the eight-carbon hydroxy acid (**6**) starts with the Baeyer−Villiger oxidation of cyclooctanone (**4**) to produce the eight-carbon lactone (**5**). Compound **5** is then hydrolyzed and protected with THP to yield **8.** Although most Baeyer−Villiger oxidations are performed at room temperature and for short periods due to the thermal instability of *m*-CPBA, we found that it was necessary to carefully heat the reaction for several days. Similar oxidation conditions are used for very large ketones.[13] The overall yield of **8** from **4** is 22%. The twelve-carbon hydroxy acid is commercially available, and the alcohol can be protected with THP to obtain **9** in 95% in a single step (Scheme 1).

The protected β-hydroxyacid building block (**12**) is synthesized from 12-dodecanol (**11**) and 3-(*tert*-butyldimethylsilyloxy)-glutaric anhydride (**10**). Both of these reagents are commercially available; the symmetric 3-(*tert*-butyldimethylsilyloxy)glutaric anhydride is an ideal reagent to use because it has a TBDMS-

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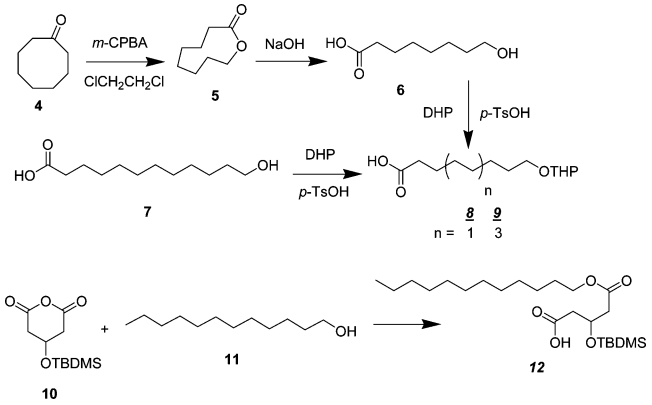
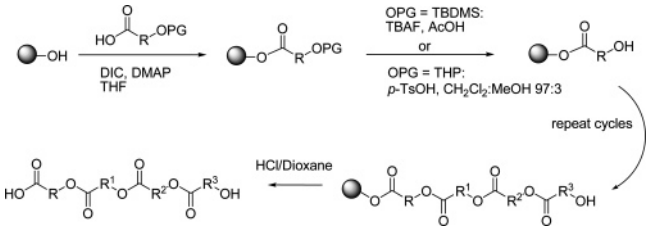
SCHEME 1. Synthesis of Building Blocks 8, 9, and 12



SCHEME 2. Solid-Phase Synthesis Methodology



protected alcohol, and the symmetry can be easily lowered though mono-reaction.

Solid-Phase Synthesis. The general scheme of the solid-phase synthesis is given in Scheme 2. Although this is similar to that reported by Kuisle et al.[10] for the synthesis of depsides, the use of the previously reported conditions did not produce oligoester products of sufficient purity. Each of the steps required specific optimization to achieve acceptable overall yields and purity. The extent of reaction was monitored by FTIR where possible and through analysis of cleavage product mixtures by NMR spectroscopy and/or HPLC. Much of the development work utilized phenylacetic acid as this compound coupled in high yield and provided a suitable spectroscopic probe for NMR spectroscopy or UV detection.

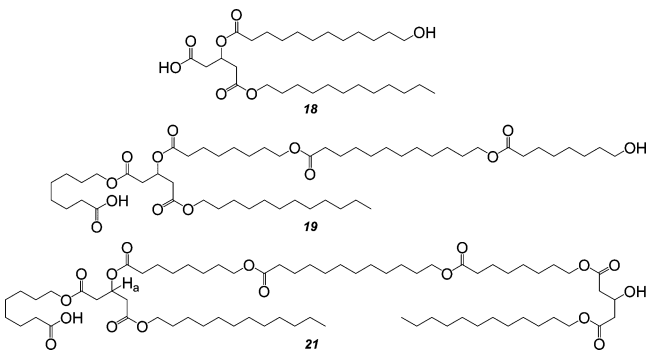
With respect to the coupling of activated acids to the solid support, or to partly elongated chains, longer times and more cycles were required relative to the previous report in order to drive the couplings to completion. This may simply reflect the slow reactions of the large units we require for our synthesis.

The THP deprotection step gave good results with the addition of a small amount of methanol as acceptor. Without added methanol, the degradation of the target oligoesters was detected. Kuisle et al. previously reported that the use of the TBDMS protecting group gave poor results.[10] We traced this problem to the buildup of tetrabutylammonium impurities within the resin (N analysis) that interfered with subsequent coupling steps. The solution to this problem was the addition of a washing step with NaI in aqueous acetone to generate the organic soluble tetrabutylammonium iodide salt. This controlled the buildup of this impurity and allowed subsequent steps to proceed correctly.

Finally, the use of TFA as the cleavage reagent as previously reported invariably gave mixtures containing the desired alcohols contaminated with the trifluoroacetate ester. The downfield shift of the methylene alpha to the hydroxyl group to $\delta = 4.32$ ppm ($J = 6.6$ Hz) is direct evidence of the ester formation. We were unsuccessful in finding simple extractive workup conditions that would hydrolyze these products rapidly without degradation of the oligoester targets, so we returned to the use of HCl in dioxane as a cleaner cleavage method.[14] The concentration of hydrochloric acid required is 5 M; at higher concentrations the Wang polymer will degrade upon prolonged exposure while 4 M is not sufficient to entirely release the oligoester product. The polymer degradation products can be removed by a size exclusion column followed by a short silica column. Products containing a primary alcohol, such as dimer **13** or trimer **14**, show evidence of oligomerization (low ratio of C*H*$_2$OH to C*H*$_2$-OCO by NMR; many high molecular weight ions in the MS) if the HCl concentration is below 5 M.

Even with optimized conditions, the pentameric product **3** cannot be made. We have explored this failure in considerable detail, using the series of compounds summarized in Table 1. The reagent sequence indicated is the order in which building blocks are added to produce the final structure.

Tetramer **20** can be isolated in good purity, but upon lengthening to make **3** by incorporating a final hydroxy-terminal unit from **12**, the product from the cleavage invariably lacked the carboxy-terminal **12** connection (as evident by ^{1}H NMR resonances) with the result that **17** was formed from all attempts to prepare **3.** Both the yield and purity of **17** were good, but **3** was undetected by NMR or MS. Since it is possible to synthesize **20**, the stability of the internal ester of the secondary alcohol from the glutarate block **12** is not inherently incompatible with the cleavage conditions. This is further confirmed by the isolation of the dimer **18**, and the pentamer **19** by direct synthesis. Rather, it appears that the cleavage reaction of the internal ester depends on the overall size of the oligoester product and the proximity of the secondary ester to the resin linker. A synthesis of a putative hexamer **21** (from **8** + **12** + **8** + **9** + **8** + **12**) gave only **17**. A different resin (Wang Tentagel) was also explored in an attempt to distance the product from the polystyrene backbone, but the synthesis of **3** failed on this support as well. The recovered resins showed the presence of a residual phenone−carbonyl stretching frequency consistent with acylation of the resin aromatic units under the cleavage conditions. Successful cleavage occurs when the diffusion of the liberated oligoester is faster than this competing acylation side-reaction.



Each of the products indicated in Table 1 was produced as the predominant species in the cleavage mixture, uncontaminated with lower homologues. The main impurities are small molecules from the reagents and the resin. These can be readily removed by a gel filtration. Some oligomeric impurities due to resin decomposition are occasionally found; these can be

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TABLE 1. Synthetic Sequences and Structures of Compounds

compound	reagent sequence	structure						
3	**12 + 8 + 9 + 8 + 12**	HO_2C	G12	Oct	Dod	Oct	G12	OH
13	**8 + 9**		HO_2C	Oct	Dod	OH		
14	**8 + 9 + 8**		HO_2C	Oct	Dod	Oct	OH	
15	**8 + 12**				HO_2C	Oct	G12	OH
16	**9 + 8 + 12**			HO_2C	Dod	Oct	G12	OH
17	**8 + 9 + 8 + 12**		HO_2C	Oct	Dod	Oct	G12	OH
18	**12 + 9**	HO_2C	G12	Dod	OH			
19	**8 + 12 + 8 + 9 + 8**	HO_2C	Oct	G12	Oct	Dod	Oct	OH
20	**12 + 8 + 9 + 8**	HO_2C	G12	Oct	Dod	Oct	OH	

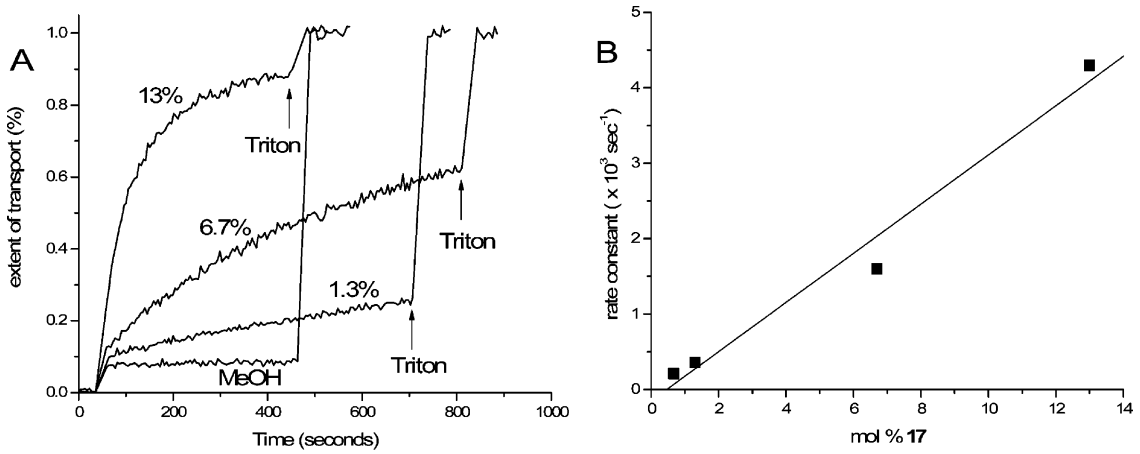

FIGURE 2. (A) Extent of transport as a function of time for added concentrations of **17**. (B) Rate as a function of the mol % of **17** in lipid.

removed through a short silica filtration. As a result the procedures and workup are very simple and permit parallel sequences to be completed simultaneously.

Ion Transport Activity. Ion transport activity was assessed using unilamellar vesicles (average diameter of about 340 nm) containing the encapsulated pH sensitive dye HPTS.[15] In the assay, a methanol solution of the putative channel-forming compound is added to the vesicle solution and allowed to equilibrate with the vesicles for about 30 s. Then a pH gradient of about one unit is induced via the injection of NaOH. The ion transport process is evident as a change in the fluorescence intensity of the dye; there is an increase in the signal from the deprotonated dye (excited at 460 nm) and a decrease in the signal from the protonated dye (excited at 403 nm). Finally the vesicles are lysed using Triton X-100. The ratio of the fluorescence intensities from the deprotonated and protonated species is used to calculate the rate of transport. Note that the ratiometric assay directly indicates that the dye remains encapsulated throughout. Were large and highly active holes to be formed, the response following mixing would be very fast.

The HPTS assay has a limited useable concentration range; if the concentration of the channel is too low, the proton leakage from the vesicles solely due to the methanol will be dominant whereas if the concentration of the channel is too high, then the rate becomes inconveniently fast. Each compound will fall into the optimum experimental window at a concentration related to its activity. We therefore surveyed a range of concentrations for each compound. Within the range, the rate constants were found to vary linearly with concentration. Experimental data for compound **17** is given in Figure 2. A survey of a range of compounds then consists of a series of individual concentration series, followed by the determination of the effective concentration required to produce a specific activity (rate of proton efflux).

TABLE 2. Concentrations of Compounds Required To Achieve a Rate Constant of 7×10^{-4} s^{-1}

compound	concentration (mol %)
dimer **13**	>8
trimer **14**	>10
dimer **15**	0.2
trimer **16**	1
tetramer **17**	2[a]
gramicidin D	2×10^{-3}
2	1

[a] Extrapolated assuming concentrations and rate constants have a linear relationship.

The weakness of any solid-phase synthesis is that it is prone to generate shorter analogues due to incomplete coupling or deprotection steps. These impurities are simple to detect by MS, but difficult to remove by simple chromatographic procedures. The compounds of Table 1 deliberately include some examples of "deletion sequences" to allow assessment of the ion transport capability of these compounds. Ideally, the deletion sequences not will be active transporters and the purification using size exclusion will prove to be sufficient. Some data to support this supposition are given in Table 2.

From Table 2 it is clear that the short fragments **13** and **14** require substantially higher concentrations to be active. This may be due to the overall lipophilic/ hydrophilic balance which controls partition into the membrane bilayer. The slightly longer fragments **15** and **16** are active at about the same concentration

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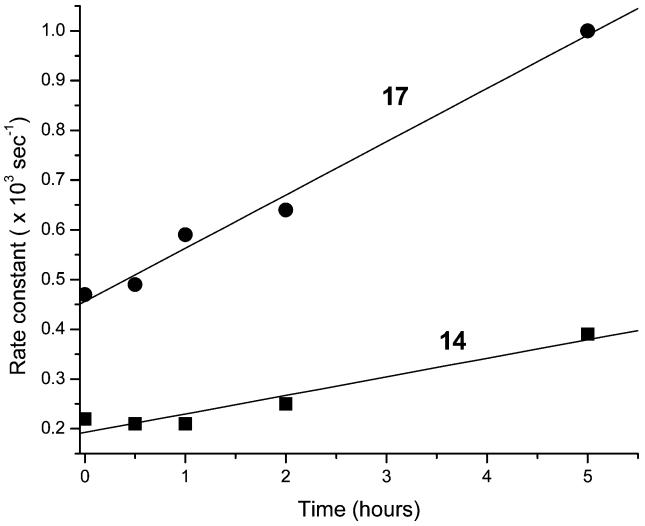

FIGURE 3. Comparison of transport activity as a function of equilibration time of channels trimer **14** and tetramer **17**.

as the tetramer **17.** Interestingly, dimer **15** is quite short compared to tetramer **17** but it has comparable ion transport activity. In fact all the active compounds (dimer **15**, trimer **16**, tetramer **17**) contain the G12 unit derived from **12**, so it is possible that even more lipophilic derivatives will show even higher activity.

The rates of transport for the oligoesters were compared to other known ion channels to bring into perspective how effective these compounds are as ion transporters. Gramicidin D is known to be an effective transporter and under our experimental conditions only a very small amount is needed to observe the standard transport rate (Table 2). Compound **2** was previously reported[3] as an ion channel and using the HPTS assay, the rate of transport is comparable to the oligoesters prepared. The expectation that removal of the ether oxygens from **2** would have a minor effect appears to be confirmed by these data.

The assays above were conducted with a standard 30 s equilibration of the channel with the vesicle. In many cases, this is sufficient for partitioning of the channel into the liposomes and to form an active structure. However, we noted in some cases that the apparent activity is time dependent as illustrated in Figure 3. Although the trimer **14** showed a minor increase in activity over the period of the experiment, the tetramer **17** showed quite a marked activity increase during the same period. This process is very slow, so it might reflect a slow equilibration of partition between the vesicles. This aspect will require more detailed analysis within the context of subsequent structure−activity studies based on a larger suite of compounds.

In conclusion, using the solid-phase methodology, we can envision the production of a library of compounds for ion channel structure−activity assessment. Even with the limitations described above, the methodology provides a rapid route to compounds of the complexity required for ion channel studies. The building blocks are simple to prepare in quantity, and the coupling and deprotection steps allow the synthesis of tetramers within a week. The product mixture contains small amounts of low molecular weight products that can be removed by a simple gel filtration step, and polymer decomposition impurities from the resin can be readily removed in a short silica filtration. The procedures can easily be done in parallel, making this a very rapid synthesis of members of this new class of membrane-active compounds.

As shown by the transport results, ion channel activity can be achieved with very simple oligoesters which will further simplify the synthetic effort. The active dimer **15** is approximately the same molecular weight as other very simple channel forming compounds.[18−20] Our structure−activity exploration of an expanded class of these compounds, and our efforts to achieve voltage-gated channel activity from these very simple compounds, will be reported in due course.

Experimental Section

General Procedure for Solid-Phase Reactions. All reactions were conducted in standard fritted peptide synthesis vessels shaken at ambient temperature. The vessels were sealed under nitrogen during the reaction period. The standard conditions used roughly 0.2 g of Wang resin (0.75 mmol/g).

(A) Ester coupling conditions: A solution of DIC (3 equiv), DMAP (0.3 equiv), THP-protected hydroxy acid or **12** (3 equiv) in THF (5 mL) was shaken with resin for three cycles of 5 h, 5 h, and overnight duration. The resin was washed between reaction cycles by mixing with solvent for 1 min, followed by vacuum filtration. Three successive cycles of THF (10 mL), ether (10 mL), and CH_2Cl_2 (10 mL) were done.

(B) THP deprotection conditions: A solution of *p*-TsOH (5 mg) in CH_2Cl_2:MeOH (97:3, 10 mL) was shaken with resin for two cycles of 2 h each. The resin was washed between reaction cycles by mixing with solvent for 1 min, followed by vacuum filtration. Three successive cycles of THF (10 mL), ether (10 mL), and CH_2Cl_2 (10 mL) were done.

(C) TBDMS deprotection conditions: Acetic acid (100 uL) was added to TBAF (1 M in THF, 10 mL), and the resin was shaken in this mixture for two cycles of 2 h and 2.5 h. The resin was washed between reaction cycles by mixing with solvent for 1 min with THF (10 mL), followed by a mixture of acetone and 1.9 mM NaI (aq) (95:5 v/v; 10 mL) followed by vacuum filtration. The iodide treated resin was then using washed with three successive cycles of 10 mL THF (10 mL), ether (10 mL), and CH_2Cl_2 (10 mL).

(D) Cleavage from the support: A solution of HCl in dioxane solution was prepared for the cleavage by gently bubbling gaseous HCl into dioxane under an ice bath for 15 min. The HCl concentration varied between 5 and 7 M as determined by titration with 1.0 M NaOH. The freshly prepared HCl solution, diluted as appropriate to 5 M, was added to the solid support and shaken for 1 h. The filtrate was collected, and then the resin was washed with CH_2Cl_2 (10 mL). The washings were combined and concentrated *in vacuo*. The crude product was purified on a LH-20 column (23 cm × 1.8 cm ID) with $CHCl_3$:MeOH (3:4) as eluent. The samples were collected in 8 mL fractions. The solvents from the fractions were evaporated, and the resulting products were examined by NMR to identify the desired product. Products were typically found in fractions 8−10 dependent somewhat on the size of the product. Normally the first fraction containing the product also contained resin degradation products. These contaminants can be removed from the product by passing a chloroform solution through a short silica column (pipet size) and then washing the product immobilized on the column with about 3 mL of chloroform. The product was then released from the silica column by washing with methanol.

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8-(Tetrahydro-2*H*-pyran-2-yloxy)octanoic Acid (8). To a solution of cyclooctanone (65.42 g, 0.518 mol) and TFA (3 mL) in dichloroethane (2 L) was added *m*-CPBA (192.8 g, 1.117 mol). The mixture was heated at 70 °C for 2 days, and then the temperature was lowered to 60 °C for an additional 3 days. The mixture was allowed to cool to 50 °C at which point a saturated aqueous Na_2SO_3 solution (500 mL) was added. A solution of concentrated NaOH was added until the pH was about 12, which resulted in a biphasic separation (deep orange, brown aqueous layer and a yellow organic layer). The biphasic mixture was stirred overnight. CH_2Cl_2 (200 mL) was added and acidified with concentrated HCl until the pH was 1−2. The aqueous layer was removed, and then the organic layer was washed with $NaHCO_3$ (saturated) (3 × 1 L) and 1 M NaOH (3 × 1 L) to yield a yellow organic layer. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield a dark yellow oil (51.22 g) (partial ^{1}H NMR consistent with **5**: 4.27, t, J = 5.89 Hz; 2.38, t, J = 6.62 Hz). The crude product was dissolved in dioxane (200 mL), and a NaOH solution (300 mL, 2 M) was added with stirring and cooling with an ice bath. The mixture was stirred and warmed to ambient temperature over 5 h. The solution turned orange and was diluted with CH_2Cl_2 (300 mL). The aqueous layer was isolated and acidified to pH 1−2 with concentrated HCl and then extracted with 100 mL of CH_2Cl_2. The organic layer was washed with saturated NaCl (150 mL) and then dried over Na_2SO_4. The organic layer was concentrated *in vacuo* to yield a yellow oil (partial ^{1}H NMR consistent with **6**: 3.65, t, J = 6.62 Hz; 2.35, t, J = 7.72 Hz). The crude product was dissolved in THF (160 mL), and with stirring under an ice bath, a solution of DHP (32.9 mL, 360 mmol) and *p*-TsOH (3.766 g, 19.8 mmol) in THF (40 mL) was added slowly. The resulting solution was stirred for 3 h and then diluted with ether (600 mL) and washed with water (3 × 200 mL) and saturated NaCl (200 mL). The organic layer was then dried over Na_2SO_4. The crude product was purified on a silica gel column (380 g, 7 cm ID x 30 cm) with the eluent $CHCl_3$:MeOH (95:5) to yield a clear, colorless oil identified as **8** (28.26 g, 22% yield from **4**). ^{1}H NMR (300 MHz, $CDCl_3$): 1.10−1.85 (m, 16H), 2.19 (t, 2H, J = 7.4 Hz), 3.20−3.28 (m, 1H), 3.34−3.41 (m, 1H), 3.54−3.62 (m, 1H), 3.69−3.76 (m, 1H), 4.47 (t, 1H, J = 3.3 Hz), 10.9 (br s); ^{13}C NMR (75 MHz, $CDCl_3$): 19.8, 24.8, 25.7, 26.2, 29.17, 29.24, 29.8, 30.9, 34.2, 62.5, 67.8, 99.0, 179.9. IR: 1736 cm^{-1} (s), 1709 cm^{-1} (s). HRLSIMS: Calcd for $C_{13}H_{23}O_4^+$ [M − H$^+$]: 243.1596 Found: 243.1597.

12-(Tetrahydro-2*H*-pyran-2-yloxy)dodecanoic Acid (9). To a stirred suspension of 12-hydroxydodecanoic acid (9.180 g, 42.4 mmol) in THF (75 mL) under an ice bath was added a solution of DHP (5.4 mL, 59 mmol) and *p*-TsOH (0.263 g, 1.24 mmol) in THF (10 mL) dropwise. The resulting solution was stirred for an additional 4.5 h at 5 °C. The solution was diluted with ether (120 mL) and washed with water (3 × 40 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give a crude product. The crude product was purified on a silica gel column (1.1 kg, 10 cm ID x 90 cm) using $CHCl_3$ as eluent to afford a clear colorless oil identified as **9** (12.104 g, 95% yield). ^{1}H NMR (300 MHz, $CDCl_3$): 1.20−1.90 (m, 25H), 2.31 (t, 2H, J = 7.7 Hz), 3.31−3.39 (m, 1H), 3.45−3.51 (m, 1H), 3.66−3.74 (m, 1H), 3.81−3.88 (m, 1H), 4.56 (t, 1H, J = 3.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 19.8, 24.9, 25.7, 26.4, 29.2, 29.4, 29.57, 29.65, 29.68, 29.71, 29.9, 30.9, 34.3, 62.5, 67.9, 98.99, 180.02. IR: 1736 cm^{-1} (s), 1709 cm^{-1} (s). HREIMS: Calcd for $C_{17}H_{31}O_4^+$ [M − H$^+$]: 299.222 Found: 299.222. Anal. Calcd for $C_{17}H_{32}O_4$: C, 67.96; H, 10.74; O, 21.30. Found: C, 67.82; H, 10.80; O, 21.33.

3-(*tert*-Butyldimethylsiloxy)-5-oxo-5-(dodecyloxy)pentanoic Acid (12). To a stirred toluene solution (20 mL) of 3-(*tert*-butyldimethylsilyloxy)glutaric anhydride (1.9 g, 7.79 mmol) was added 1-dodecanol (1.40 g, 7.49 mmol). The solution was stirred at reflux overnight, and the solvent was removed under reduced pressure to give a crude product. The crude product was dissolved in pentane (80 mL) and cooled in a dry ice/ethanol bath for 10 min before filtration. The filtrate was cooled again in a dry ice/ethanol bath for 10 min and then filtered. This crystallization was repeated until no more solids were produced. The filtrate was concentrated under reduced pressure to afford a colorless oil identified as **12** (3.18 g, quantitative yield). ^{1}H NMR (300 MHz, $CDCl_3$): 0.05 and 0.06 (2 singlets, 6H), 0.82−0.88 (m, 12H), 1.24 (br, 18H), 1.50−1.63 (m, 2H), 2.51−2.66 (m, 4H), 3.95−4.10 (m, 2H), 4.52 (quintet, 1H, J = 6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): −4.8, −4.7, 14.3, 18.1, 22.9, 25.8, 26.1, 28.8, 29.5, 29.6, 29.71, 29.77, 29.84, 32.1, 42.4, 42.6, 65.1, 66.3, 171.2, 177.0. IR: 1738 cm^{-1} (s), 1713 cm^{-1} (s). HRLSIMS: Calcd for $C_{23}H_{47}O_5Si^+$ [M + H$^+$]: 431.319 Found: 431.320.

Dimer 13 was prepared using the standard conditions in the sequence given in Table 1, on Wang resin (0.259 g, 0.19 mmol) to produce **13** (8 mg; 0.022 mmol, yield 12%). ^{1}H NMR (500 MHz, $CDCl_3$): 1.20−1.65 (m, 28H), 2.27 (t, J = 7.5 Hz, 2H), 2.33 (t, J = 7.5, 2H), 3.62 (t, J = 6.6 Hz, 2H), 4.04 (t, J = 6.6 Hz, 2H). ^{13}C NMR (125.75 MHz, $CDCl_3$): 24.9, 25.1, 25.8, 26.1, 28.8, 29.1, 29.22, 29.24, 29.3, 29.37, 29.45, 29.54, 29.60, 29.63, 29.7, 32.85, 32.91, 33.9, 34.6, 63.2, 64.5, 64.7, 174.2, 177.9. HRLSIMS: Calcd for $NaC_{20}H_{38}O_5^+$ [M + Na$^+$]: 381.2617 Found: 381.2523.

Trimer 14 was prepared using the standard conditions in the sequence given in Table 1, on Wang resin (0.225 g, 0.17 mmol) to produce **14** (19 mg; 0.038 mmol, yield 23%). ^{1}H NMR (500 MHz, $CDCl_3$): 1.20−1.40 (m, 26H), 1.50−1.70 (m, 12H), 2.25−2.32 (m, 6H), 3.61 (t, J = 6.6 Hz, 2H), 4.03 (t, J = 6.6 Hz, 4H). ^{13}C NMR (125.75 MHz, $CDCl_3$): 24.9, 25.1, 25.2, 25.7, 26.0, 26.1, 28.79, 28.84, 29.08, 29.15, 29.2, 29.28, 29.34, 29.42, 29.44, 29.6, 29.7, 32.8, 34.57, 34.59, 63.2, 64.5, 64.7, 174.20, 174.23, 174.3, 178.9. HRLSIMS: Calcd for $NaC_{28}H_{52}O_7^+$ [M + Na$^+$]: 523.3611 Found: 523.3606.

Dimer 15 was prepared using the standard conditions in the sequence given in Table 1, on Wang resin (0.202 g, 0.15 mmol) to produce **15** (10 mg; 0.022 mmol, yield 15%). ^{1}H NMR (500 MHz, $CDCl_3$): 0.86 (t, J = 7 Hz, 3H), 1.19−1.70 (m, 30H), 2.20−2.35 (m, 2H), 2.50−2.60 (m, 4H), 4.08 (t, J = 6.8 Hz, 4H), 4.41−4.46 (m, 1H).^{13}C NMR (125.75 MHz, $CDCl_3$): 14.3, 22.9, 25.0, 25.8, 26.1, 28.6, 28.8, 29.0, 29.2, 29.5, 29.6, 29.7, 29.8, 29.85, 29.86, 32.1, 40.9, 40.9, 65.0, 65.1, 65.3, 172.3. HRLSIMS: Calcd for $NaC_{25}H_{46}O_7^+$ [M + Na$^+$]: 481.3141 Found: 481.3139.

Trimer 16 was prepared using the standard conditions in the sequence given in Table 1, on Wang resin (0.202 g, 0.15 mmol) to produce **16** (44 mg; 0.067 mmol, yield 45%). ^{1}H NMR (500 MHz, $CDCl_3$): 0.84 (t, J = 7.0, 3H), 1.20−1.40 (m, 40H), 1.51−1.65 (m, 10H), 2.26 (t, J = 7.5 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.51−2.53 (m, 4H), 4.01−4.08 (m, 6H), 4.40−4.45 (m, 1H). ^{13}C NMR (125.75 MHz, $CDCl_3$): 14.3, 22.9, 24.9, 25.0, 25.86, 25.93, 26.05, 26.08, 28.6, 28.7, 28.76, 28.81, 29.0, 29.1, 29.15, 29.19, 29.22, 29.4, 29.5, 29.55, 29.59, 29.63, 29.68, 29.74, 29.80, 29.81, 32.1, 34.2, 34.5, 40.9, 64.6, 65.0, 65.1, 65.2, 172.07, 172.11, 174.1, 179.4. HRLSIMS: Calcd for $NaC_{37}H_{68}O_9^+$: 679.4761 Found: 679.4781.

Tetramer 17 was prepared using the standard conditions in the sequence given in Table 1, on Wang resin (0.227 g, 0.17 mmol) to produce **17** (66 mg; 0.083 mmol, yield 49%). ^{1}H NMR (500 MHz, $CDCl_3$): 0.85 (t, J = 6.9 Hz, 3H), 1.20−1.65 (m, 60H), 2.26 (t, J = 7.5 Hz, 4H), 2.32 (t, J = 7.5 Hz, 2H), 2.52 (d, J = 6.6 Hz, 4H), 4.03 (t, J = 6.7 Hz, 4H), 4.07 (t, J = 6.7 Hz, 4H), 4.43 (quintet, J = 6.3 Hz, 1H). ^{13}C NMR (125.75 MHz, $CDCl_3$): 14.3, 22.9, 24.8, 25.05, 25.08, 25.2, 25.88, 25.94, 26.07, 26.11, 28.65, 28.73, 28.77, 28.83, 29.0, 29.06, 29.11, 29.16, 29.20, 29.3, 29.43, 29.44, 29.5, 29.6, 29.7, 29.75, 29.81, 29.82, 32.1, 34.0, 34.5, 34.6, 40.9, 64.47, 64.51, 64.7, 65.0, 65.1, 65.2, 172.09, 172.11, 174.1, 174.2, 179.0, 179.1. HRLSIMS: Calcd for $NaC_{45}H_{82}O_{11}^+$ [M + Na$^+$]: 821.5755 Found: 821.5760.

Dimer 18 was prepared using the standard conditions in the sequence given in Table 1, on Wang resin (0.234 g, 0.18 mmol) to produce **18** (14 mg; 0.027 mmol, yield 15%). ^{1}H NMR (500 MHz, $CDCl_3$): 0.86 (t, J = 7.0 Hz, 3H), 1.20−1.65 (m, 41H), 2.25 (t, J = 7.5 Hz, 2H), 2.67−2.78 (m, 4H), 3.64 (t, J = 6.5 Hz, 2H), 4.05 (t, J = 6.8 Hz, 2H), 5.48 (quintet, J = 6.0 Hz, 1H). ^{13}C NMR

(125.75 MHz, $CDCl_3$): 14.3, 22.9, 25.0, 25.8, 26.1, 28.8, 29.1, 29.3, 29.4, 29.47, 29.52, 29.6, 29.7, 29.80, 29.84, 29.9, 32.1, 32.8, 34.5, 38.1, 38.6, 63.3, 65.3, 66.7, 170.2, 173.0, 173.7. HRLSIMS: Calcd for $NaC_{29}H_{54}O_7^+$ [M + Na^+]: 537.3767 Found: 537.3764.

Pentamer 19 was prepared using the standard conditions in the sequence given in Table 1, on Wang resin (0.227 g, 0.17 mmol) to produce **19** (64 mg; 0.068 mmol, yield 40%). ^{1}H NMR (500 MHz, $CDCl_3$): 0.82 (t, J = 6.5 Hz, 3H), 1.10−1.70 (m, 68H), 2.15−2.35 (m, 8H), 2.65 (d, J = 6.2 Hz, 4H), 3.58 (t, J = 6.4 Hz, 2H), 3.90−4.10 (m, 8H), 5.45 (quintet, J = 6.1 Hz, 1H), 5.5 (br s, 1H). ^{13}C NMR (125.75 MHz, $CDCl_3$): 14.2, 22.8, 24.73, 24.88, 24.94, 25.00, 25.03, 25.1, 25.7, 25.8, 25.9, 26.0, 26.1, 26.5, 28.4, 28.6, 28.7, 28.7, 28.8, 28.96, 29.03, 29.06, 29.09, 29.15, 29.20, 29.3, 29.4, 29.46, 29.54, 29.60, 29.64, 29.70, 29.74, 29.8, 31.0, 32.0, 32.7, 34.0, 34.1, 34.3, 34.4, 34.5, 38.6, 45.25, 51.6, 63.0, 64.5, 64.6, 65.0, 65.1, 66.9, 170.08, 170.10, 172.7, 174.1, 174.2, 178.5. HRLSIMS: Calcd for $NaC_{53}H_{96}O_{13}^+$ [M + Na^+]: 963.6749 Found: 963.6765.

Tetramer 20 was prepared using the standard conditions in the sequence given in Table 1, on Wang resin (0.304 g, 0.228 mmol) to produce **20** (67 mg; 0.084 mmol, yield 37%). ^{1}H NMR (300 MHz, $CDCl_3$): 0.84 (t, J = 6.6 Hz, 3H), 1.10−1.68 (m, 61H), 2.15−2.35 (m, 6H), 2.60−2.80 (m, 4H), 3.50−3.65 (m, 2H), 3.95−4.10 (m, 6H), 5.46 (quintet, J = 5.9 Hz, 1H), 5.2 (br s, 1H). ^{13}C NMR (125.75 MHz, $CDCl_3$): 29.1, 29.18, 29.21, 29.23, 29.3, 29.36, 29.41, 29.50, 29.55, 29.58, 29.6, 29.68, 29.75, 29.79, 29.80, 31.02, 31.06, 32.07, 32.69, 34.2, 34.4, 34.43, 34.52, 34.55, 38.2, 38.4, 38.5, 38.6, 45.3, 63.0, 63.1, 64.5, 64.53, 64.6, 65.2, 66.8, 66.9, 170.2, 172.9, 173.0, 174.1, 174.2, 174.3, 174.4. HRLSIMS: Calcd for $NaC_{45}H_{82}O_{11}^+$ [M + Na^+]: 821.5755 Found: 821.5745.

Vesicles and HPTS Assay. The general procedure for vesicle preparation and the HPTS fluorescence assay has been described elsewhere[16] our procedure employed minor modifications. A chloroform solution containing PC:PA:cholesterol (8:1:1 mole ratio) was dried *in vacuo* and then left on the vacuum line overnight. The approximately 60 mg of lipid film was hydrated with 1 mL of internal buffer solution (9.9 μM HPTS, 10.1 mM Na_3PO_4, 74.7 mM Na_2SO_4, pH adjusted to 6.4 using H_3PO_4), and transferred into a small test tube. The suspension was frozen under liquid nitrogen and subsequently thawed at room temperature over 10 min. This cycle was repeated three times. The mixture was then equilibrated in an ice bath and sonicated with a 13 mm tip probe for 20 s with 2 s pulses (at 50% duty cycle and 20% power output), the sonicator was switched off for 30 s and the sonication cycle was repeated until a total sonication time of two minutes was reached. The liposomes were then allowed to anneal overnight. The vesicle solution was then sized 19 times through a 400 nm polycarbonate nucleopore filter using a membrane extrusion apparatus and purified using an external buffer solution (10.1 mM Na_3PO_4, 74.7 mM Na_2SO_4, pH adjusted to 6.4 using H_3PO_4) equilibrated PD-10 Sephadex G-25 column. The first three cloudy drops were discarded but thereafter the cloudy fraction was collected and diluted to 5.00 mL using the external buffer solution. A typical preparation of this vesicle stock solution contained 340 nm diameter vesicles (dynamic light scattering) and a lipid concentration of typically 7 mg/mL as determined by the Barlett assay.[17] The vesicle solution was used within 24 h of preparation.

HPTS Ion Transport Assay. General Method. In a fluorimetric cell was added 2.00 mL of the external buffer solution, 100 μL of the stock vesicle solution, and 30 μL of a 0−4.3 mM MeOH solution of the oligoester compound. HPTS emission was monitored at 510 nm and excitation wavelengths of 403 and 460 nm were used concurrently. Raman emission artifacts were not an issue based on the examination of fluorescence spectra of vesicles made without the HPTS dye. After a 30 s equilibration time, 50 μL of 0.5 M NaOH was added (time = zero). After about 500 s, the vesicles were lysed with a 50 μL 5% Triton-X100 solution. The data were imported into a Microsoft Excel worksheet and all data after the first time of opening the lid of the fluorimeter was deleted. The relative intensity of $I_{460\ nm}/I_{403\ nm}$ was calculated. The vesicle internal pH (related to $[H^+]_{in}$) and external pH ($[H^+]_{ex}$, which is the fluorescence after vesicle lysis) was calculated using the equation $pH = 1.13 \times \log(I_{460\ nm}/I_{403\ nm}) + 7.25$ determined as described by Sidorov et al.[16] A graph of $\ln([H^+]_{in} - [H^+]_{ex})$ versus time was plotted and the slope of which gave the rate constants. The error in the rates assessed from triplicates was ± 20% within one batch of vesicles.

HPTS Ion Transport Assay. Equilibration Time. To 20.0 mL of external buffer was added 1.00 mL of the vesicle solution as well as 300 μL of the methanol solution of trimer **14** (0.858 mM) or tetramer **17** (0.403 mM)**.** Aliquots of 2.13 mL were taken at intervals of initial, 0.5 h, 1 h, 2 h, and 5 h from this mixture and injected with 50 μL of NaOH (0.5 M), and the fluorescence intensity ratios was monitored at 510 nm of the excitation at 403 and 460 nm. Finally the vesicles were lysed with 50 μL 5% Triton-X100.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all compounds are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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