### Membrane-Length Amphiphiles Exhibiting Structural Simplicity and Ion Channel Activity

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Abstract: A number of synthetic ion channels have been reported in recent years that incorporate unusual or sophisticated design elements. The present work demonstrates that extremely simple compounds can function as ion channels (insert in bilayers, exhibit open–close behavior) if they meet minimum criteria. A simple membrane spanning structure may function as a channel if 1) it possesses polar headgroups (is bolaamphiphilic), 2) possesses a "central relay," and 3) channel function (open-close behavior) must be detected after insertion of the amphiphile directly into the aqueous lipo-

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somal or cellular suspension. We show here compounds that are simple spans to which we have given the name "aplosspan" (from the Greek  $\alpha\pi\lambda\sigma\sigma$  + span) that meet these criteria. They are similar to, but simpler than, structures reported in the literatures that incorporate more complex design features.

### Introduction

Modern protein channels possess elaborate structures<sup>[1]</sup> and highly evolved functionality.<sup>[2]</sup> In short, modern protein channels transport cations, anions, or small molecules through a bilayer membrane. Typically, the transport process in nature is selective, unidirectional, and closely regulated. The earliest channels may not have used amino acids at all and presumably were less selective than the complex protein channels known today. Still, very simple structures can form effective channels. One example is the channel formed from poly(3-hydroxybutyrate) (PHB) and inorganic polyphosphate that functions in bacteria.<sup>[3]</sup> The structure of this nonprotein channel was proved by total synthesis and its efficacy was shown to be identical to the natural product.<sup>[4]</sup>

Unlike PHB, the complexity of protein channels currently exceeds the capability of organic total synthesis. Still, synthetic ion channels have been designed, prepared, and their activities characterized.<sup>[5]</sup> A number of these channels incorporate amino acids<sup>[6]</sup> although other structural elements are

[b] Prof. G. W. Gokel Departments of Chemistry & Biochemistry and Biology Center for Nanoscience, University of Missouri–Saint Louis One University Boulevard, Saint Louis, MO 63121 (USA) Fax. (+1)314-516-5321 E-mail: gokelg@umsl.edu often present. Many more channels or pore-forming structures contain no amino acid at all but still show channel function. These include crown ethers,<sup>[7]</sup> cyclodextrins,<sup>[8]</sup> steroids,<sup>[9]</sup> and recently open framework organometallic structures,<sup>[10]</sup> as well as others. Synthetic ion channels may function unimolecularly<sup>[11]</sup> or they may form pores.<sup>[12]</sup> Compounds that appear to be single spans of transmembrane length have also been prepared that transport protons faster than does gramicidin.<sup>[13]</sup> Of course, even membrane-length spans may function as carriers.<sup>[14]</sup>

Because structurally diverse molecules have been reported in recent years to form pores or channels or otherwise transport ions, we contemplated what structural components might be essential for pore formation. The thought experiment was to identify the minimum elements that might be required for a compound to insert into a bilayer and to permit transport by a pore or channel mechanism as evidenced by open–close behavior. It is clear that first and foremost, whatever compound is designed, it must be amphiphilic. This leads to the inevitable question of whether one has simply designed a new detergent. Triton X-100 (see below), for example, is often quoted as a simple amphiphile known to exhibit channel-like behavior in phospholipid bilayers.<sup>[15]</sup> The structure is not expected to form a pore as its





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hydrocarbon "tail" is barely 10 Å fully extended. Commercial material has an average PEG chain comprising ~9.5 ethylene oxide units. This polar element would have an extended length sufficient to span a bilayer but is not expected to have an affinity for the membrane's hydrocarbon regime.

It is also important to note that there is actually relatively little information available about the Triton "channel." The initial studies that showed open-close behavior were done by creating liposomes from a mixture of phospholipid and detergent and then fusing these with the planar bilayer membrane prior to voltage clamp studies. The high concentrations of detergent in the liposomes and the fusion method differ significantly from the direct injection experiments recorded here. The elaborate method required to obtain channel function shows that Triton detergent is not relevant to channels that form from amphiphiles present in aqueous suspension. Indeed, in a 1994 study, Rostostseva and co-workers stated that "bilayers of DPhPC, DOPC, or GMO, to which Triton was added only by injection into the aqueous medium, showed no channel activity."[16] Thus, a critical requirement for the compounds reported here was that they must exhibit channel function when added directly to an aqueous liposome suspension.

Given that amphiphilic character is essential for membrane activity, an additional design requirement was that the overall length of the compound (between the polar head groups) was sufficient to span the bilayer. The designed length, per se, does not ensure a transmembrane conformation, but it permits it. The absence of sufficient length precludes channel activity.<sup>[17]</sup> Finally, a central relay was required to mimic the "water and ion-filled capsule" identified first in the K<sup>+</sup> (KcsA) channel from *Streptomyces lividans*.<sup>[18]</sup>

We note that Fyles developed a number of structurally simple amphiphiles that he anticipated would be ion transporters.<sup>[19]</sup> For the most part, these compounds incorporated isophthalic acid headgroups and one or two "tails" of various descriptions. These compounds were not bolaamphiphiles, as were the Co<sup>2+</sup> transporters reported by Fuhrhop and co-workers,<sup>[20]</sup> and they were active in bilayers. The focus of the Fyles study was "well-behaved ion channel behavior rather than nonspecific detergent or membrane-disrupting activity." A twin alkyl chained isophthalic acid derivative reported by Fyles et al.<sup>[19]</sup> showed clear evidence of channel activity. Conductances of 9.2, 15.4, and 31 pS were observed for Na<sup>+</sup>, K<sup>+</sup>, and Cs<sup>+</sup>.

Commensurate with the structural and functional features outlined above, a key goal was to design compounds that would be structurally simple, modular, and synthetically accessible. In some recent cases, rather complex structures have been reported along with sophisticated design strategies. The novelty of the structures merited publication, but control experiments confirming the need for the required complexity were lacking. In keeping with the notion that the earliest channels must have been simple, rather than elaborate, structures, we have attempted to define in the work presented here, design minima for channel function. Overall, the five criteria that guided this work were 1) the channel must be of membrane-spanning length, 2) a residue that functions as a central relay must be present, 3) two head-groups of polarity greater than that of the spacer chains are required for amphiphilicity, 4) the synthetic approach should be straightforward and modular, and 5) channel function must be detected after insertion of the amphiphile into the aqueous suspension. In an attempt to distinguish these compounds both from "membrane disruptors" and more sophisticated and functional designs, we have suggested the name aplosspan from the Greek  $a\pi\lambda o\sigma + \text{span.}^{[21]}$ 

### **Results and Discussion**

**Compounds prepared for the present study**: Four compounds **1–4** and a previously reported control (5) were prepared for the present study. They are shown as structures **1–5**. Compounds **1–3** comprise a family in which the central relay<sup>[22]</sup> unit is *meta*-phenylenediamide, a residue that was used successfully in a structure reported by Kobuke et al.<sup>[9b]</sup> The polar headgroups of these three structures are diethanolamines. Compounds **1** and **2** differ from **3** in that the latter possesses two biphenyl units in each spacer chain. Compounds **1** and **2** contain a single biphenyl residue in each chain although their placement in the chains differs. In fact, **1** and **2** are isomers.

The spacer chains of **3** comprise two 4,4'-dihydroxybiphenyl units linked by an ethylene unit. Based only on the structural composition, we conclude that **3** is more rigid than either **1** or **2**. The concentration of arenes near the midpoint of **2** suggests that it may be more rigid than **1** but this assertion is not based on any quantitative test. Indeed, we are unaware of any experimental scale of rigidity. As noted above, **5** is a much studied and effective synthetic ion channel. It differs from **4** by the presence in the latter of a phenylenediamide central unit. Since **5** is a highly active Na<sup>+</sup> transporter, the function of the phenylenediamide unit would be confirmed if **4** were active. As shown below, replacement of the 4,13-diaza-[18]crown-6 central relay by the phenylenediamide significantly reduced, but did not eliminate, transport activity.

**Compound syntheses:** Compound **5** has been extensively studied and details of its synthesis have been reported.<sup>[24]</sup> Compound **4** is identical to benzyl hydraphile **5** except that the central relay element (diaza-[18]crown-6 in **5**) has been replaced by a 1,3-phenylenediamide, consistent with aplosspans **1–3**. Compounds **1** and **2** are isomers that differ only in the placement of the 4,4'-dihydroxybiphenyl unit. Compound **3** is similar to **1** and **2** but a second biphenylene unit replaces part of the aliphatic chain. The two biphenylenes in each spacer chain of **3** are linked by an ethylenedioxy residue. Ionophores **1–3** all contain the same central relay and headgroups and they have similar spacer chains. Scheme **1** summarizes the preparation of the modules required in the syntheses.

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Isomers 1 and 2 were prepared in a similar fashion. Generally, diethanolamine was protected (Scheme 1) as the bis-(tert-butyldimethylsilyl ether), which was then linked with the incipient spacer chain. For compound 2, this involved converting 12-bromododecanoic acid into 12-bromododecanoyl chloride, which acylated the protected amine. The bromoamide was treated with 4,4'-dihydroxybiphenyl to give  $HO-C_6H_5-C_6H_5-O-(CH_2)_{11}-CON(CH_2CH_2O-TBDMS)_2$ . The central relay (ClCH<sub>2</sub>CONH-C<sub>6</sub>H<sub>4</sub>-NHCOCH<sub>2</sub>Cl) was prepared (Scheme 1) by treating *m*-phenylenediamine with chloroacetyl chloride to afford the diamide. Two equivalents of the protected phenol shown above were condensed with the m-phenylenediamide to produce silvlated 2. Desilvlation was accomplished by using HCl in EtOH, yielding 2 as a slightly yellow solid. The preparation of 2 is summarized in Scheme 2.

The preparation of **3** required 12 steps. In some of these, modules prepared as shown in Scheme 1 were used as required for the preparation of compound **3**. Diethanolamine was protected by treatment with *tert*-butyldimethylsilyl chloride (74% yield) and then coupled with chloroacetyl chloride to give ClCH<sub>2</sub>CON(CH<sub>2</sub>CH<sub>2</sub>O-TBDMS)<sub>2</sub> (**B**, 74% yield). The acetylated central unit, ClCH<sub>2</sub>CONH-C<sub>6</sub>H<sub>4</sub>-NHCOCH<sub>2</sub>Cl (**C**) was prepared by direct reaction of 1,3-diaminobenzene with ClCOCH<sub>2</sub>Cl, albeit in only 16% yield. 4,4'-Dihydroxybiphenyl was monofunctionalized by treatment with benzyl bromide to give HO-C<sub>6</sub>H<sub>4</sub>-O<sub>6</sub>H<sub>4</sub>-OCH<sub>2</sub>Ph (**D**, 54% yield). Phenolic ether **D** was treated with 1,2-dibromoethane in a two-phase reaction mixture (aq. KOH, Bu<sub>4</sub>NOH) to afford bromoethyl ether **E** in 85% yield.

The individual modules were then assembled as described below (see Scheme 3). 4,4'-Dihydroxybiphenyl was coupled



headgroup

Scheme 1. Preparation of modular ionophore elements.

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Scheme 2. Synthesis of 2.

with **B** to give alkylated phenol **F** in 55% yield. Compounds **D** (Scheme 1) and **F** were coupled to give **L** in 86% yield. Hydrogenolysis debenzylated quaterphenyl **L** but left the incipient headgroup protected (**M**, 85%). 1,3-Di(chloroaceta-mido)benzene (**E**, Scheme 1) was then coupled with two malaxular of **M** affording res

molecules of M affording protected 3 (N) in 45% yield. Deprotection of N with tetrabutylammonium fluoride in THF gave 3 as a light brown solid in 66% yield.

Control compound 4 was prepared as follows. 12-Bromododecanoic acid was converted  $[(ClCO)_2]$  to the acid chloride, which was then treated with 1,3-diaminobenzene. The product. Br(CH<sub>2</sub>)<sub>11</sub>CONH-C<sub>6</sub>H<sub>4</sub>- $NHCO(CH_2)_{11}Br$ , was condensed with N-benzyl-4,13diaza-[18]crown-6 (Na<sub>2</sub>CO<sub>3</sub>, KI,  $C_3H_7CN$ ,  $\Delta$ ) to give (after chromatography) 4 in 13% yield as an oil, the structure of which was confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR, and FAB-MS. Hydraphile 5 has been previously reported.[23]

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**Ion transport**: The functional success of these designs was assayed in several different ways. The compound of interest may be added to an aqueous suspension of liposomes loaded with a particular ion. If the amphiphile inserts in the liposomal bilayer and releases the entrapped ion, the increase in ion concentration in the external solution may be monitored by using an ion-selective electrode (ISE). Alternately, a fluorescent material such as carboxyfluorescein or lucigenin may be entrapped within the liposomes. Carboxyfluorescein within vesicles self-quenches but it may be detected and quantitated by fluorimetry when released from the liposomes. Lucigenin is fluorescent even within liposomes, but it is quenched by chloride. Thus chloride transfer from the suspending solution into the vesicular interior is detected by a reduction in lucigenin fluorescence over time.

Ion selectivity can be estimated by comparing the results of individual ion transport experiments but planar bilayer conductance studies give this information directly. The latter experiments require instrumentation uncommon in organic chemistry laboratories and considerable expertise so liposomal experiments are more common. We present both types of experiments here.

**Ion-selective electrode (ISE) studies—solvent and solubility limitations**: The compounds prepared for the present study have two to four biphenyl residues in each spacer chain and a fifth arene as the central relay. The use of these elements simplifies construction but the products generally exhibit poor solubility. Compound **3**, which incorporates nine benzene rings, required dimethylsulfoxide (DMSO) as a solvent to achieve measurable solubility. Low concentrations of DMSO in contact with a bilayer are known to increase





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membrane permeability.<sup>[24]</sup> The solvent is reported to be a membrane penetration enhancer and a pore former. A recent computational study suggests that DMSO inserts rapidly into the bilayer, resides predominantly below the phospholipid headgroups, and facilitates membrane fusion. The authors state that "DMSO makes the membrane significantly floppy."<sup>[25]</sup>

In the studies reported here, DMSO concentrations were lower than those involved in the studies noted above. Still, the effect of DMSO in the experiments reported below was noticeable. A survey of the transport data obtained clearly revealed that the release of Na<sup>+</sup> mediated by 1-3 was influenced by the amount of DMSO in the final solvent mixture. An example of the solvent effect was apparent when 3 was used to mediate the transport of Na<sup>+</sup>. Identical amounts of 3 were added to the aqueous liposomal suspension using 1 mm or 4 mm stock solutions. When 3 was added from the more dilute (1 mm) stock solution, more DMSO was added to the suspending medium. Although the amounts of 3 were the same and the concentrations of 3 in the suspending medium were similar, the total sodium release changed from ~10% at 1500 s (1 mm) to ~5% (4 mm) under otherwise identical experimental conditions. Similarly, when solutions of 2 or 1 more dilute than 1 mm were used, greater amounts of DMSO were introduced into the aqueous suspensions, and high Na<sup>+</sup> release was observed. Control experiments in which corresponding amounts of DMSO were added in the absence of 1, 2, or 3, failed to mediate sodium release from the liposomes. The absence of simple leakage in the control experiments that lacked 1-3 suggests that DMSO is playing a cooperative role in the transport but the mechanism of interaction with 1-3 remains obscure. In any event, direct observation of Cl<sup>-</sup> release mediated by 1-3 was ineffective.

It should be noted that in all of the ion release experiments reported here, at least three data sets were acquired for each trace presented. The data sets are superimposed. The traces are not only data-rich but the range of all points is included. Only in Figure 4 (see below) was the data spread for each trace large enough that mathematical smoothing was required to distinguish trends.

### Chloride quenching of lucigenin in the presence of DMSO:

The Cl<sup>-</sup>-induced fluorescence quenching of lucigenin has been reported as a means to monitor transport.<sup>[26]</sup> In an earlier study, we found Cl<sup>-</sup> release assayed by a Cl<sup>-</sup>-selective electrode or by the lucigenin quenching method were generally similar.<sup>[27]</sup> In the experiments conducted for this report, we prepared liposomes from 1,2-dioleoyl-3-phosphocholine (DOPC, 0.4 mM) that contained within them 2 mM lucigenin and 225 mM NaNO<sub>3</sub>. The suspending solution contained 190 mM NaCl and 225 mM NaNO<sub>3</sub>. Compounds **1–4** were studied in separate experiments but in each case DMSO was used to prepare the stock solution. Figure 1 shows the results of these studies, in which the greatest fluorescence quenching corresponds to the most effective Cl<sup>-</sup> transport. Compound **4** is clearly a better transporter than **1**, **2**, or **3**. The control experiment in which only DMSO (and no other ionophore) was present showed significant  $Cl^-$  transport. Further, at the 1000 s time point, chloride release mediated by **1**, **2**, or **3** was identical. We presume that the activity of DMSO in this system levels the  $Cl^-$  transport effect but the efficacy of **4** compared to **1–3** is clear.



Figure 1. Chloride quenching of lucigenin fluorescence mediated by **1–4**. Liposomes (DOPC, 0.4 mM), internal buffer (2 mM lucigenin, 225 mM NaNO<sub>3</sub>), external buffer (190 mM NaCl, 225 mM NaNO<sub>3</sub>), compound concentrations: 12 mM in system; stock solution: 4 mM in DMSO.

**Sodium release from liposomes**: The transport efficacies of **1–4** were assayed by using a sodium ion selective electrode to detect sodium release from liposomes. Compound **5** has been studied and the results reported previously.<sup>[24]</sup> As above, DMSO was used to dissolve the transporter. The effect of the solvent was not discernible in these experiments, possibly because the ISE uses a sodium-selective glass rather than a membrane as an ion sensor. The transport efficacy of  $1^{[21]}$  was assayed in liposomes prepared from DOPC (0.4 mM) in 2 mL of buffer (salt solution and HEPES) solution. The Na<sup>+</sup> release data for **2** are shown in (Figure 2). We note that once a functional pore forms, ion release from an individual liposome is rapid<sup>[28]</sup> so the data reflect a combination of insertion and pore formation dynamics.



Figure 2. Fractional sodium release from liposomes (DOPC, 0.4 mm) mediated by **2**. External buffer (750 mm choline chloride, 15 mm HEPES, pH 7.0), internal buffer (750 mm NaCl, 15 mm HEPES, pH 7.0).

The data presented in Figure 2 show that Na<sup>+</sup> release is dependent on [2]. Although the release data reveal neither how much of the available amphiphile inserts in the bliayer nor how many inserted monomers actually contribute to transport, an increase in the amount of 2 added increases Na<sup>+</sup> transport. When the Na<sup>+</sup> release values observed at

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1500 s<sup>-1</sup> were plotted as a function of concentration, a linear relationship was observed with  $r^2 = 0.96$  for the four points (graph not shown).

Fractional ion release is the amount of Na<sup>+</sup> detected normalized to the total amount of ion measured after detergent-induced vesicular lysis. At a solubility-limited concentration of 108  $\mu$ m for **2**, only about a third of the available Na<sup>+</sup> was released. These experiments do not reveal if the release profile results from the insertion rate of **2** in the bilayer, the rate of pore formation, or both. Planar bilayer voltage clamp studies, reported below, shed some light on this issue.

Sodium ion release data are presented in Figure 3 for compound 3. The presence of more aromatic rings in 3 than in either 1 or 2 suggested that 3 would be less conformationally flexible (i.e., more rigid) than 1 or 2. Compound 3 is generally less soluble than either 1 or 2, as expected for a more rigid structure. The data shown in Figures 2 and 3 show that Na<sup>+</sup> release mediated by 1, 2, or 3 is similar. A difference was that 3 showed little increase in ion release at concentrations  $\geq 60 \,\mu\text{M}$ . Maximal release for this membrane and ionophore combination was ~25% at 1500 s. These results may reflect the poor solubility of 3, which required the use of considerable DMSO and the attendant problems described above.



Figure 3. Fractional sodium release from liposomes (DOPC, 0.4 mm) mediated by **3**. External buffer (750 mm choline chloride, 15 mm HEPES, pH 7.0), internal buffer (750 mm NaCl, 15 mm HEPES, pH 7.0).

The solubility issue frustrates a simple comparison of 1–4 as Na<sup>+</sup> transporters. Moreover, compound 3 aggregates in cool DMSO at concentrations as low as 1 mM while solutions of 1 and 2 remain clear at least to  $\leq 4$  mM. The comparative data shown in Figure 4 were obtained using 1 mM DMSO stock solutions of 1–4. The poor ionophore concentrations give low ion transport results. The combination makes the experiments challenging but the data are reproducible and the direct comparison of 1–4 is valid.

The release data shown in Figure 4 are informative. Isomers 1 and 2 show some variation in initial rates but exhibit similar transport efficacies. Aplosspan 3 consistently exhibits the highest Na<sup>+</sup>-transport activity despite having the poorest solubility. The reduced solubility likely results from the more rigid spacer chains of 3 compared to 1 and 2 but this



Figure 4. Fractional sodium release from liposomes (DOPC, 0.4 mM) mediated by **1–4**. External buffer (750 mM choline chloride, 15 mM HEPES, pH 7.0), internal buffer (750 mM NaCl, 15 mM HEPES, pH 7.0), compound concentration: 12  $\mu$ M in system; stock solution: 1 mM in DMSO. The data for three replicates have been mathematically smoothed.

rigidity may well enhance the insertion dynamics and the overall stability of the conductance conformation.

Compound **4** was designed to be a control. The threemacrocycle arrangement of **5** is well known to transport Na<sup>+</sup> ion. Compound **5** has been represented by the abbreviation<sup>[29]</sup> PhCH<sub>2</sub>(N18N)(CH<sub>2</sub>)<sub>12</sub>(N18N)(CH<sub>2</sub>)<sub>12</sub>(N18N)CH<sub>2</sub>Ph, in which  $\langle N18N \rangle$  represented 4,13-diaza-[18]crown-6. We note that replacing the two methylene groups on either side of the medial macrocycle with amide residues diminishes fractional Na<sup>+</sup> transport. Thus, transport by **5** is 0.27 and by PhCH<sub>2</sub>(N18N)(CH<sub>2</sub>)<sub>11</sub>CO(N18N)CO(CH<sub>2</sub>)<sub>12</sub>(N18N)CH<sub>2</sub>Ph, it is 0.10.<sup>[30]</sup> We conclude that the *m*-phenylenediamide module is likely less effective as a "central relay"<sup>[24]</sup> than is the macrocycle. It was used for structural simplicity and has served that purpose.

Compound 4 is the link that permits 1–3 and 5 to be compared. We note that the transport of Na<sup>+</sup> mediated by 5 exceeds (~10-fold)<sup>[24]</sup> that of 4. This suggests that 1–3 would be more active if the *m*-phenylenediamide central relay were replaced by a crown. This was not done as the point of the study was to probe the limits of structural simplicity. Still, the aralkyl spacer chains in combination with simple dihydroxyethylamine headgroups appear to be quite effective in mediating ion transport.

**Carboxyfluorescein (CF) release from liposomes**: Carboxyfluorescein self-quenches when it is contained within a liposome and its fluorescence is detected when the anion is released to the surrounding medium. Studies were conducted at an internal CF concentration of 20 mM (100 mM KCl, 10 mM HEPES buffer, pH 7.0) in DOPC liposomes. Fluorescence was monitored over time beginning immediately after addition of an ionophore to the aqueous suspension and final fluorescence intensity was determined after detergentinduced vesicular lysis.

In the preliminary report of this work,<sup>[29]</sup> a Hill plot of CF release from DOPC liposomes mediated by **1** gave a slope of 1.5, suggesting that transport involves monomer aggregation. The CF release behavior for **2** and **3** were similar to that previously reported for **1**. For each data set, Hill plots were prepared (data not shown). The data for **2** or **3** gave a

slope of ~2 and  $r^2$  for the lines were typically  $\ge 0.9$ . We infer that **2** and **3** form pores that have a stoichiometry of at least two monomer units.

The graph of Figure 5 summarizes the data for CF release mediated by 1, 2, and 3 under identical experimental conditions. In order to better show the differences in their CF release efficacies, the CF vesicle concentration was reduced to  $3 \,\mu$ M. The CF release mediated by  $0.5 \,\mu$ M 3 at 1000 s is ~50%. The same concentration of 2 led to 36% release of total CF at 1000 s. Only 25% of total CF was released at 1000 s by 1. This result accords with the observations from sodium release experiments that the order of aplosspan activity is  $3 > 2 \geq 1$ . We note that isomeric 2 and 3 differ somewhat in their CF transport rates and the aggregation numbers but, as was the case for Na<sup>+</sup> transport, 3 is a superior ionophore to both. Although the apparent stoichiometries for pore formation by isomers 1 and 2 differed, the transport efficacy trend persisted.



Figure 5. Carboxyfluorescein release from liposomes (DOPC, 3 μM) mediated by 0.5 μM of 2–4. Internal buffer (20 mM CF, 100 mM KCl, 10 mM HEPES, pH 7.0), External buffer (100 mM KCl, 10 mM HEPES, pH 7.0)

Assay of selectivity: Our effort to determine the ion selectivity of 1–3 by planar bilayer voltage clamp (BLM) methods was limited by their solubility. Data for individual compounds have been obtained by the BLM method<sup>[31]</sup> (see 0.3 pA below, additional data in reference [21]). We reported in the latter reference that compound 1 has a K<sup>+</sup>/Cl<sup>-</sup> selectivity of 2.3. Significant gating behavior (see below) was observed for 2 but the data obtained were limited. Thus, comparisons were made by using various methods to assay the passage of ions through liposomal bilayers. We estimate from the liposomal sodium and chloride transport experiments that 2 and 3 show similar or greater selectivity (K<sup>+</sup>/Cl<sup>-</sup>) than observed for 1, since 2 and 3 show similar Cl<sup>-</sup> transport activity and 1 is the least active compound in sodium release experiments.

A different approach was taken to obtain some indication of selectivity. Thus, pyranine (8-hydroxy-l,3,6-pyrenetrisulfonate, HPTS), a pH-sensitive dye, was used in an effort to measure the selectivity of compounds among cations and anions.<sup>[32]</sup> The pyranine dye experiments were successful for 2 and 3 but 1 surprisingly caused dye leakage from the vesicles. Thus, 1 could not be compared directly with 2 and 3. Figure 6 shows comparative data obtained for 3. The top panel compares transport of Na<sup>+</sup> and K<sup>+</sup> with a common chloride anion. The lower panel compares the transport of  $Cl^-$  and  $Br^-$  with a common sodium cation. Compound **2** shows similar, if modest, selectivity (data not shown).



Figure 6. Assay of cation (upper panel) and anion (lower panel) selectivity of **3** by monitoring pyranine (HTPS) fluorescence. Liposomes were prepared from DOPC ( $0.4 \mu M$ ).

**Planar bilayer conductance experiments**: Planar bilayer (BLM) conductance experiments permit a direct measurement of conductance and a determination of ion selectivity. Our initial study of **1** showed a  $Na^+/Cl^-$  selectivity of 2.3. The poor solubility of **3** prohibited a BLM study but Figure 7 shows data for both **1** and **2** at negative applied voltages. The open–close behavior of these pores is apparent and reproducible.



Figure 7. Current-time records showing channel gating behavior for both 1 (top, applied potential -40 mV) and 2 (bottom, applied potential -50 mV) at the indicated voltages (asolectin, 1  $\mu$ M, 60 mV). Buffer: 450 mM KCl, 10 mM HEPES, pH 7.0. The x-axis is 70 s; the minor divisions are 0.2 min (12 s).

### Conclusions

Three new synthetic compounds deliberately designed to be structurally simple ion channels (1–3) referred to here as "aplosspans," have been synthesized, purified, and characterized. In addition, a derivative of a previously known tris(ma-

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crocycle) was prepared (4) in which the central relay was identical to that in 1-3. The ability of these compounds to transport cations and anions was assessed in both liposomal bilayer membranes (liposome) and planar bilayer membranes. All assays indicated that despite the simple structure of aplosspans, they transport ions. The planar bilayer voltage clamp experiments demonstrate open-close behavior for 1 and 2, which is a characteristic of channel function. Experiments conducted in liposomes suggested that transport involved approximately two monomers. The formation of an oligomeric pore is not surprising but the efficacy of these extremely simple structures is remarkable.

We note that ion flux measurements using 2 and using HTPS showed better ion transport for NaCl compared to KCl and better transport of NaCl compared to NaBr. Planar bilayer voltage clamp studies showed that 1 was also selective for  $K^+$  over Cl<sup>-</sup>, albeit by less than three-fold. We conclude that many structural modules may be successfully incorporated into membrane-length bolaamphiphiles to afford ion transporters. Because such simple structures show both functionality and selectivity, it is critical that attribution of special properties to unusual or complex headgroups, spacers, or central relay units, must be confirmed by appropriate control experiments.

### **Experimental Section**

**General:** <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on a Gemini 300 spectrometer at 300 MHz and 75 MHz and were reported in ppm ( $\delta$ ) downfield from internal (CH<sub>3</sub>)<sub>4</sub>Si unless otherwise stated. Melting points were determined on a Thomas Hoover apparatus in open capillaries and are uncorrected. Thin layer chromatography analyses were performed on silica gel 60-F-254 (0.2 mm thickness). Preparative chromatography columns were packed with silica gel (Kieselgel 60, 70–230 mesh or Merck grade 9385, 230–240 mesh, 60 Å). All reactions were conducted under dry N<sub>2</sub> unless otherwise stated. All reagents were the best (non-LC) grade commercially available and were distilled, recrystallized, or used without further purification, as appropriate. Fast atom bombardment (FAB) mass spectra were obtained using a JEOL Mstation (JMS-700) mass spectrometer.

**Vesicle preparation**: Vesicles were prepared from 1,2-dioleoyl-3-phosphocholine (DOPC) using the reverse phase procedure of Szoka and Papahadjopoulos.<sup>[33]</sup> The vesicles were passed through a Sephadex G25 column. Vesicle size was confirmed with a Brookhaven ZetaPALS zeta potential analyzer (dynamic light scattering). Data were collected by Axoscope 7.0 using a Digidata 1322 series interface. Addition of 10% aqueous *n*-octylglucoside (200  $\mu$ L) induced vesicular lysis and data were normalized to this value. OriginPro 7 software was used for all data analyses. Final lipid concentration was determined using the previously reported analytical method.<sup>[17]</sup>

**Sodium release experiments**: The internal buffer was 750 mM NaCl, 15 mM HEPES, pH 7.0 (pH adjusted with  $(CH_3)_4$ NOH). Sodium transport was measured using a Micro-Combination pH/Na<sup>+</sup> electrode in aqueous sodium-free buffer (750 mM cholineCl/15 mM HEPES, pH 7.0). The lipid concentration (0.4 mM, total volume 2.0 mL) was measured as reported.<sup>[17]</sup> A saturated DMSO solution of aplosspan was injected directly into the suspending solution and ion release was monitored for 25 min.

**Carboxyfluorescein (CF) dequenching:** DOPC (15 mg) was dissolved in Et<sub>2</sub>O (0.35 mL) and CF (0.35 mL 20 mM CF in 100 mM KCl, 10 mM HEPES, pH 7.0) was added. The mixture was sonicated (1200 W,  $3 \times 20$  s, 20 °C) to produce a stable emulsion. The Et<sub>2</sub>O was removed (~30 mm

Hg, 30 °C) and an additional 20 mM CF solution (0.35 mL) was added. The mixture was filtered (5 ×, 200 nm filter) and the passed over a 1 × 20 cm Sephacryl G-25 column (Sigma) in 100 mM KCl:10 mM HEPES (pH 7.0). The liposome peak was collected (DLS=200 nm), the vesicles were diluted to 5 or 10  $\mu$ M (total volume =2 mL). The fluorescence [ $\lambda_{exc}$ = 497 nm,  $\lambda_{obs}$ =520 nm (2 nm bandpass)] was monitored at 25 °C. Compounds were added in DMSO (0.5 mM) to the indicated concentrations. Dequenching,  $F_{520}$ , was computed as the fraction of total release upon addition of 1 % Triton X100 using the equation  $F_{520} = (F - F_0)/(F_{\text{Triton}} - F_0)$ , where  $F_0$ =fluorescence at t=0, and  $F_{\text{Triton}}$ =maximal fluorescence after lysis. Dequenching data were fitted by nonlinear least squares (Levenberg–Marquardt algorithm. The number of individual trials generating the data set (degrees of freedom) was used to obtain the p value for the individual fits and kinetic constants.

Planar lipid bilayer clamp experiment: Experiments were performed at 25±1°C in a Faraday cage (from Warner Instruments, LLC). Sample was dissolved in CF<sub>3</sub>CH<sub>2</sub>OH to form a suspension (1 mM), which was used as a stock solution. The chamber and delrin cuvettes were filled with symmetric buffer solution (450 mM KCl, 10 mM HEPES, pH 7.00, 3 mL each). Membrane (asolectin from soybean extract dissolved in n-decane, 25 mgmL<sup>-1</sup>) was "painted" into the aperture. Formation of the membrane was confirmed by reading a capacitance >100 pF and an undistorted square wave displayed on the oscilloscope (HM305 from Hameg). The sample suspension  $(3\,\mu L)$  was added to the *cis* chamber to give a final concentration of 1 µm. The actual solubility might be lower owing to poor ionophore solubility. The mixture was stirred for 10 min and allowed to equilibrate for 5 min. The desired potential was applied (trans connected to ground) to record the currents, which were amplified (amplifier BC-525 D, from Warner Instruments, LLC), filtered with a 4-pole Bessel filter at 1 kHz, digitized by a Digidata 1322 A (Axon Instruments), sampled at the 100 Hz of amplifier filter frequency and collected by pClamp 9.2 and analyzed using Clampfit v. 9.2 (both from Axon Instruments).

Lucigenin quenching assay: Vesicles were prepared from a dry film of DOPC. Diethyl ether (375 µL) and 2 mM lucigenin/225 mM NaNO3  $(375 \,\mu\text{L})$  solution were added to the lipids  $(15 \,\text{mg})$  and then sonicated  $(2 \times 10 \text{ s})$ . The ether was subsequently evaporated under mild vacuum at 30°C. The resulting solution was extruded five times through a 200 nm membrane filter and then passed through a Sephadex column equilibrated with 225 mM NaNO3. The size of the collected vesicles were ~200 nm (Brookhaven ZetaPALS zeta potential analyzer). The concentration of the lipids in the final vesicle suspension was measured by using the colorimetric method previously described.<sup>[34]</sup> The vesicles were diluted to a 0.40 mm concentration and then a 2 mL aliquot was placed in a quartz cuvette to be used for the lucigenin quenching experiment. The excitation wavelength was set to 455 nm and the emission wavelength to 506 nm, with both slits set to 5 nm. After a brief initial equilibration phase, a 4M NaCl solution (100 µL) was added in order to create a chloride gradient between the outside (190  $\mu$ M) and the inside (0  $\mu$ ) of the vesicles. When the fluorescence reached a stable reading, a 4 mm solution (6 µL) of the desired compound in DMSO was added. At the end of each experiment the vesicles were lysed with a 2% Triton X-100 solution (100 µL). A calibration line for transforming fluorescence intensity into chloride concentration was obtained using the conditions of the described experiments. A 0.40 mm vesicles suspension (2 mL) in 225 mm NaNO3 were lysed with a 2% Triton X-100 solution (100 µL) and then titrated with aliquots of a 4M NaCl solution. The Stern-Volmer constant was found to be  $119.7 \text{ m}^{-1}$ .

**HPTS ion transport assay:** Vesicles were prepared from a dry film of DOPC. Diethyl ether (375  $\mu$ L) and internal buffer (375  $\mu$ L of 1 mm HPTS, 10 mm NaCl, pH 7.0) solution were added to the lipids (15 mg) and then sonicated (2×10 s). The ether was evaporated under mild vacuum at 30 °C. The resulting solution was extruded 5 times through a 200 nm membrane filter and then passed through a Sephadex column equilibrated with buffer solution (100 mm NaCl, 10 mm HEPES, pH 7.0). The size of the collected vesicles was found to be ~200 nm (Brookhaven ZetaPALS zeta potential analyzer). The lipid concentration in the final vesicle suspension was measured colorimetrically as previously de-

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scribed.<sup>[34]</sup> The vesicles were diluted to 0.40 mM by adding the external buffer (100 mM target salts, 10 mM HEPES, pH 7.0). A 2 mL aliquot was placed in a quartz cuvette to be used for the ion transport assay. The excitation wavelength (Perkin–Elmer Model LS 50B fluorescence spectrophotometer) was set to 450 nm and the emission wavelength to 510 nm, with both slits set to 5 nm.

After a brief initial equilibration phase, a 0.5 m NaOH solution (20 µL) was added in order to create a pH gradient between the outside and the inside of the vesicles. When the fluorescence emission reached a stable reading, a 0.5 mM solution (20 µL) of the desired compound in DMSO was added. At the end of each experiment the vesicles were lysed with a 2% Triton X-100 solution (100 µL). Fluorescence time courses were normalized to fractional emission intensity In using equation,  $I = (F_t - F_0)/(F_{\rm Triton} - F_0)$ , where  $F_0 =$  florescence at compound addition (t=0),  $F_{\rm Triton} =$  florescence at saturation after complete leakage. Then the the treference value of interest, the highest activity in a given series before addition of detergent, in our case the fractional florescence emission intensity of NaCl system at 200 s.

**Preparation of the protected diethanolamine (A): Bis[2-(***tert***-butyldimethylsilanyloxy)ethyl]amine: A solution of** *tert***-butyldimethylsilyl chloride (5.19 g, 34.4 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was added dropwise (0°C) to a solution of diethanolamine (1.65 g, 15.6 mmol), Et<sub>3</sub>N (3.80 g, 37.6 mmol) and DMAP (300 mg, 2.40 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (50 mL). The mixture stirred at RT overnight. Reaction system was washed with H<sub>2</sub>O (50 mL) and saturated aq. NaHCO<sub>3</sub> (50 mL). The organic phase was dried (MgSO<sub>4</sub>) and the solvent was evaporated (in vacuo) to afford crude product. The crude product was purified by distillation to give the target product as yellow oil (3.50 g, 74%). <sup>1</sup>H NMR: \delta = 0.016 (s, 12 H), 0.85 (s, 18 H), 2.66–2.70 (m, 4H), 3.67–3.71 ppm (m, 4H); <sup>13</sup>C NMR: \delta = 5.1, 18.5, 26.2, 52.0, 62.8 ppm.** 

Preparation of the protected headgroup module (B): *N*,*N*-Bis[2-(*tert*-bu-tyldimethylsilanyloxy)ethyl]-2-chloroacetamide: A solution of chloroacetyl chloride (1.50 g, 13.2 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added dropwise (0°C) to a solution of **A** (2.00 g, 3.30 mmol) and Et<sub>3</sub>N (2.00 g, 19.8 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The reaction was stirred at RT overnight. The mixture was washed with H<sub>2</sub>O (30 mL) and saturated aq. NaHCO<sub>3</sub> (30 mL). The organic phase was dried (MgSO<sub>4</sub>) and the solvent was evaporated (in vacuo) to afford the product as a yellow oil. Distillation afforded **B** as a yellow oil (1.92 g, 74%). <sup>1</sup>H NMR:  $\delta$  = 0.048–0.052 (d, 12 H, *J*=1 Hz), 0.88–0.89 (d, 18 H, *J*=1 Hz), 3.48 (t, 2 H, *J*=5 Hz), 3.62 (t, 2 H, *J*=5 Hz), 3.75–3.79 (m, 4 H), 4.24 ppm (s, 2 H); <sup>13</sup>C NMR:  $\delta$  = -5.4, -5.3, 18.4, 25.9, 26.00, 26.05, 41.7, 49.0, 51.8, 60.9, 61.5, 197.7 ppm.

**Preparation of benzyl-protected 4,4'-dihydroxybiphenyl (C): 4-Benzyloxy-4'-hydroxybiphenyl:** Sodium hydroxide (0.80 g, 20 mmol) was added to a suspension of 4,4'-biphenol (3.72 g, 20.0 mmol) in EtOH (30 mL) and heated under reflux until the solution turned dark green. Benzyl bromide (3.42 g, 20.0 mmol) was added all at once, reflux was continued for 3 h and then cooled to RT. The reaction mixture was acidified by addition of conc. HCl. The precipitate was collected by filtration, washed by EtOH and dried under high vacuum. The solids were dissolved in EtOH/ HOAc 1:1 and the undissolved disubstituted product was obtained by filtration. Recrystallization of the filtered solution at RT afforded **C** as white crystals (3.00 g, 54%). M.p. 217.5–219°C; <sup>1</sup>H NMR:  $\delta = 5.13$  (s, 21H), 6.79–6.82 (d, 2H, J=9 Hz), 7.02–7.06 (d, 2H, J=9 Hz), 7.40–7.50 (m, 9H), 9.45 ppm (s, 1H); <sup>13</sup>C NMR:  $\delta = 70.1$ , 115.1, 115.6, 127.4, 127.5, 127.7, 127.9, 128.5, 132.2, 134.0, 137.1, 156.1, 157.77 ppm.

**4-Benzyloxy-4'-(2-bromoethoxy)-biphenyl (D)**: Tetrabutylammonium hydroxide (using as 40% wt. solution in water) (0.187 g, 0.720 mmol) was added into a suspension solution of compound **C** (0.220 g, 0.720 mmol) and 1,2-dibromoethane (6.80 g, 36.2 mmol). Potassium hydroxide solid (0.213 g, 3.60 mmol) was added into the reaction system followed by water (1 mL). The mixture was stirred at 50 °C for 3 d. CHCl<sub>3</sub> (50 mL) was added into the reaction was washed with water (2×50 mL) and brine (2×50 mL), dried (MgSO<sub>4</sub>), the solvent was evaporated and the product was chromatographed (SiO<sub>2</sub>, CHCl<sub>3</sub>) to afford **D** as white a solid (0.26 g, 85%). M.p. 165–168 °C; <sup>1</sup>H NMR:  $\delta = 3.65$  (t,

2H, J=6 Hz), 4.32 (t,2H, J=6 Hz), 5.10 (s, 2H), 6.94–6.97 (d, 2H, J=9 Hz), 7.01–7.04 (d, 2H, J=9 Hz), 7.27–7.49 ppm (m, 9H); <sup>13</sup>C NMR:  $\delta$  = 29.3, 68.2, 70.3, 115.3, 115.4, 127.7, 128.0, 128.0, 128.2, 128.8, 133.8, 134.5, 137.2, 157.4, 158.2 ppm.

**2-Chloro-N-[3-(2-chloroacetylamino)phenyl]acetamide (E)**: A solution of chloroacetyl chloride (5.00 g, 44.0 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was added dropwise (0°C) to a suspension solution of Et<sub>3</sub>N (12 mL, excess), 1,3-phenylenediamine·2 HCl (2.00 g, 11.0 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The reaction warmed to RT during 20 min and was stirred at RT for 48 h. The solvent was evaporated (in vacuo) to afford crude product as a black residue. Chromatography (SiO<sub>2</sub>, 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub> to 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) and recrystallization from acetone afforded **C** as a white solid (0.68 g, 24%). M.p. 220–221 °C; <sup>1</sup>H NMR (CD<sub>3</sub>COCD<sub>3</sub>):  $\delta$  = 4.24 (s, 4H), 7.24 (m, 1H), 7.37–7.40 (m, 2H), 7.97 (m, 1H), 9.58 ppm (brs, 2H); <sup>13</sup>C NMR:  $\delta$  = 44.3, 111.8, 116.3, 130.1, 140.1, 167.2 ppm.

*N*,*N*-Bis[2-(*tert*-butyldimethylsilanyloxy)ethyl]-2-(4'-hydroxybiphenyl-4yloxy)-acetamide (F): 4,4'-Biphenol (0.564 g, 3.03 mmol) was added to a suspension of **B** (1.00 g, 2.52 mmol), Na<sub>2</sub>CO<sub>3</sub> (3.00 g, 28.3 mmol), KI (cat.) in butyronitrile (40 mL). The reaction mixture was stirred at reflux temperature for 5 h. The process was monitored by TLC (hexane/acetone 2:1). The reaction was cooled, filtered and the solvent was evaporated (in vacuo). The residue was chromatographed (SiO<sub>2</sub>, 1% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to afford **F** as a yellow oil (0.77 g, 55%). The crude product was used in the next step without further purification. <sup>1</sup>H NMR:  $\delta = 0.03$ –0.07 (m, 12H), 0.88–0.90 (m, 18H), 3.545 (m, 2H), 3.660 (m, 2H), 3.78–3.81 (m, 4H), 4.86 (s, 2H), 6.82–6.85 (d, 2H, J=9 Hz), 6.95–6.98 (d, 2H, J=9 Hz), 7.35–7.41 ppm (m, 4H).

#### Preparation of aplosspan 1

N,N'-(1,3-Phenylene)-bis(12-bromododecanamide) (G): Oxalyl chloride (0.91 g, 7.2 mmol) was added dropwise (0°C) to a solution of 12-bromododecanoic acid (0.50 g, 1.8 mmol) in CH2Cl2 (20 mL). The reaction mixture was stirred at RT for 2 h. Toluene (2×5 mL) was added into the residue and evaporated to ensure removal of residue (CICO)2. The residue was dissolved in dry CH2Cl2 (5 mL) and added dropwise (0°C) to a solution of 1,3-phenylenediamine·2 HCl (0.15 g, 0.83 mmol), Et<sub>3</sub>N (2 mL, excess) in dry CH2Cl2 (20 mL). The reaction warmed to RT during 20 min and was stirred overnight. Some product precipitated during the reaction. The solids were collected. Evaporation of the solvent gave additional product. The two portions of target product were combined to give a tan solid (0.44 g, 84%). M.p. 120–121 °C; <sup>1</sup>H NMR:  $\delta = 1.25$ –1.45 (m, 28H), 1.72-1.78 (m, 4H), 1.83-1.90 (m, 4H), 2.37 (t, 4H, J=7 Hz), 3.436 (t, 4H, J=7 Hz), 7.31–7.44 (m, 3H), 7.86 ppm (s, 1H); <sup>13</sup>C NMR:  $\delta$  = 25.8, 27.1, 28.4, 29.0, 29.1, 29.5, 29.6, 29.6, 29.7, 32.9, 33.0, 34.3, 38.1, 45.4, 111.1, 115.5, 129.7, 138.8, 171.8 ppm.

**Protected 1 (H):** Compound **F** (0.10 g, 0.16 mmol), compound **G** (0.20 g, 0.36 mmol), sodium carbonate (0.40 g, 3.7 mmol), KI (cat.) in butyronitrile (20 mL) was heated to reflux for 3 d. The reaction system was cooled, filtered and the solvent was evaporated (in vacuo) to afford crude product. The crude product was recrystallized in MeOH (freeze) to afford **H** as a light yellow powder (110 mg, 19%). M.p. 78–81 °C; <sup>1</sup>H NMR:  $\delta$  = 0.3–0.8 (m, 24H), 0.87–0.91 (m, 36H), 1.29–1.81 (m, 36H), 2.28–2.34 (m, 4H), 3.53–3.65 (m, 8H), 3.78–3.80 (m, 8H), 3.96–4.00 (m, 4H), 4.83 (s, 4H), 6.93–6.99 (m, 8H), 7.26–7.45 (m, 11H), 7.79 ppm (s, 1H); <sup>13</sup>C NMR: δ = -5.2, 18.4, 18.5, 25.8, 26.1, 29.5, 29.6, 29.7, 38.0, 48.9, 61.3, 61.7, 67.4, 68.3, 115.0, 115.2, 115.4, 127.9, 129.7, 133.4, 134.4, 138.8, 157.5, 158.5, 168.7, 171.8 ppm; MS-FAB: *m/z*: calcd for: 1609.9912, found: 1609.9950 [*M*+Na]<sup>+</sup>.

**Aplosspan 1**: Concentrated HCl (37.4%, 0.5 mL) was added dropwise to a solution of protected **1** (**H**, 30 mg, 0.019 mmol) in EtOH (5 mL). The solution was stirred at RT for 2 h. The target product (20 mg, 93%) precipitated from the reaction system as brown solid. M.p. ~189°C (decomp); <sup>1</sup>H NMR ([D<sub>6</sub>]DMSO):  $\delta$  = 1.31–1.61 (m, 36H), 2.29–2.32 (m, 4H), 3.46–3.63 (m, 16H), 4.01 (m, 4H), 4.95 (s, 4H), 6.97–7.02 (m, 8H), 7.20–7.31 (m, 3H), 7.51–7.56 (m, 8H), 7.96 (s, 1H), 9.88 ppm (s, 2H); <sup>13</sup>C NMR:  $\delta$  = 25.2, 25.3, 36.4, 47.9, 49.5, 58.6, 58.8, 65.7, 67.5, 110.2, 114.0, 114.9, 115.0, 127.0, 127.3, 128.7, 132.2, 132.5, 139.6, 157.4, 157.8, 167.7, 171.4 ppm.

Preparation of aplosspan 2

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12-Bromo-*N*,*N*-bis(2-(trimethylsilyloxy)ethyl)dodecanamide (I): A solution of 12-bromododecanoic acid (1.84 g, 6.59 mmol), protected diethanolamine **A** (2.00 g, 6.59 mmol), EDCI (1.50 g, 7.82 mmol) and DMAP (cat.) in dry CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was stirred at RT overnight. The reaction was washed with 5% aq. citric acid (2×20 mL) and brine (20 mL). The organic phase was dried (MgSO<sub>4</sub>) and the solvent was evaporated (in vacuo) to afford target product as viscous oil (3.24 g, 89%). <sup>1</sup>H NMR:  $\delta$  = 0.0058–0.0616 (s, 12 H), 0.84–0.89 (s, 8 H), 1.22–1.24 (m, 14 H), 1.57 (m, 2 H), 1.81 (m, 2 H), 2.33 (t, 2 H, *J*=7.5 Hz), 3.35–3.50 (m, 6 H), 3.65–3.71 ppm (m, 4 H); <sup>13</sup>C NMR:  $\delta$  = -5.2, 18.5, 25.6, 26.1, 26.1, 28.4, 29.0, 29.7, 29.9, 33.1, 33.5, 34.3, 49.0, 51.6, 61.4, 61.9, 174.0 ppm.

### 12-(4'-Hydroxybiphenyl-4-yloxy)-N,N-bis(2-(trimethylsilyloxy)ethyl)-do-

**decanamide (J)**: A mixture of compound **I** (1.00 g, 1.82 mmol), 4,4'-biphenol (0.35 g, 1.9 mmol), Na<sub>2</sub>CO<sub>3</sub> (2.00 g, 18.8 mmol), KI (cat.) in butyronitrile (20 mL) was heated to reflux overnight. The reaction system was cooled, filtered and the solvent was evaporated (in vacuo) to afford crude product as dark oil. The crude product was chromatographed (SiO<sub>2</sub>, CHCl<sub>3</sub>/MeOH 98:2) to afford **J** as a light yellow oil (0.38 g, 30%). <sup>1</sup>H NMR:  $\delta = 0.00-0.048$  (s, 12H), 0.84 (s, 18H), 1.24 (m, 18H), 2.40 (t, 2H, *J*=7.5 Hz), 3.44–3.50 (m, 4H), 3.68–3.72 (m, 4H), 3.92 (t, 2H, *J*=6 Hz), 6.85–9.91 (m, 4H), 7.35–7.42 ppm (m, 4H); <sup>13</sup>C NMR:  $\delta = -5.2$ , 18.4, 25.6, 26.1, 29.7, 33.5, 49.1, 51.7, 61.4, 61.9, 68.3, 115.0, 115.9, 127.9, 128.1, 133659, 155.3, 158.5, 174.0 ppm.

**Protected 2 (K)**: Compound **J** (0.26 g, 0.37 mmol), central unit **E** (0.040 g, 0.15 mmol), Na<sub>2</sub>CO<sub>3</sub> (0.40 g, 3.7 mmol), and KI (cat.) in butyronitrile (20 mL) were heated to reflux for 3 d. The reaction system was cooled, filtered and the solvent was evaporated (in vacuo) to afford crude product. The crude product was recrystallized from CH<sub>3</sub>Cl/MeOH to give protected **2 (K)** as a light yellow powder (25 mg, 10%). M.p. 92–96°C; <sup>1</sup>H NMR:  $\delta = 0.036$ –0.045 (m, 24H), 0.88 (s, 36H), 1.25–1.79 (m, 36H), 2.37 (t, 4H, *J*=7.5Hz), 3.46–3.52 (m, 8H), 3.69–3.75 (m, 8H), 3.98 (t, 4H, *J*=6 Hz), 4.64 (s, 4H), 6.94–7.06 (m, 8H), 7.45–7.54 (m, 11H), 8.01 (s, 1H), 8.38 ppm (s, 2H); <sup>13</sup>C NMR:  $\delta = -5.3$ , -5.2, 14.3, 18.4, 18.4, 22.9, 25.6, 26.1, 26.3, 29.5, 29.6, 29.7, 29.8, 29.9, 32.1, 33.5, 49.0, 51.6, 61.4, 61.9, 68.0, 68.3, 111.8, 115.1, 115.4, 116.6, 127.8, 128.0, 128.3, 130.0, 132.9, 135.7, 137.8, 156.2, 158.8, 166.6, 173.8 ppm; MS-FAB: *m/z*: calcd for: 1609.9912; found: 1609.9894 [*M*+Na]<sup>+</sup>.

**Aplosspan 2:** Concentrated HCl (37.4%, 0.5 mL) was added dropwise in a solution of **K** (30 mg, 0.019 mmol) in EtOH (5 mL). The reaction was stirred at RT for 2 h. The target product (12 mg, 56%) precipitated from the reaction system. M.p > 245 °C (decomp); <sup>1</sup>H NMR ([D<sub>6</sub>]DMSO):  $\delta$  = 1.34–1.49 (m, 32 H), 1.77–1.79 (m, 4 H), 2.40–2.42 (m, 4H), 3.09–3.74 (m, overlap with solvent peak), 4.03–4.07 (t, 4H, *J* = 6 Hz), 4.34 (m, 2H), 4.78 (s, 4H), 7.02–7.13 (m, 8H), 7.34–7.64 (m, 13 H), 8.13 (s, 1H), 10.27 ppm (s, 2H); <sup>13</sup>C NMR:  $\delta$  = 24.2, 24.9, 25.5, 28.5, 28.8, 29.0, 32.2, 33.2, 45.5, 49.1, 56.2, 59.1, 59.3, 67.2, 67.4, 114.5, 115.0, 127.2, 127.3, 128.9, 132.0, 133.0, 138.7, 156.9, 157.8, 166.6, 172.4, 172.7 ppm.

#### Preparation of aplosspan 3

N,N-Bis(2-(*tert*-butyldimethylsilyloxy)ethyl)-2-(4'-hydroxybiphenyl-4-yloxy)acetamide (**F**) was prepared as described above.

**2-(4'-(2-(4'-(Benzyloxy)biphenyl-4-yloxy)ethoxy)biphenyl-4-yloxy)-***N*,*N***-bis(2-(***tert***-<b>butyldimethylsilyloxy)ethyl)acetamide (L)**: A mixture of **F** (0.27 g, 0.70 mmol), **D** (0.26 g, 0.46 mmol), Na<sub>2</sub>CO<sub>3</sub> (1.00 g, 9.43 mmol) and KI (cat.) in butyronitrile (20 mL) was stirred under reflux for 4 d. The mixture was cooled, filtered, the solvent was evaporated, and the resulting solid was recrystallized from MeOH/CHCl<sub>3</sub> to afford **L** as a white solid (0.13 g, 50%). M.p. 155–157°C; <sup>1</sup>H NMR:  $\delta = 0.039-0.066$  (d, 12H, J = 8 Hz), 0.88–0.89 (d, 18H, J = 3 Hz), 3.54 (m, 2H), 3.65 (m, 2H), 3.78–3.80 (m, 4H), 4.39 (s, 4H), 4.84 (s, 2H), 5.11 (s, 2H), 6.99–7.05 (m, 8H), 7.44–7.51 ppm (m, 13H); <sup>13</sup>C NMR:  $\delta = -5.2$ , 18.5, 26.1, 29.9, 49.0, 51.1, 61.4, 61.7, 67.0, 67.6, 70.4, 115.3, 115.4, 127.7, 128.0, 128.1, 128.2, 128.8, 133.9, 134.2, 134.4, 137.4, 157.7, 158.1, 158.3, 168.6 ppm.

*N,N*-Bis(2-(*tert*-butyldimethylsilyloxy)ethyl)-2-(4'-(2-(4'-hydroxybiphenyl-4-yloxy)ethoxy)biphenyl-4-yloxy)acetamide (M): Palladium on carbon (10%, 0.32 g) was added to a hot solution of L (0.29 g, 0.34 mmol) in EtOAc (10 mL) and shaken (Parr apparatus) at 60 psi H<sub>2</sub> for 40 h. The mixture was filtered hot and the solids were washed twice with CHCl<sub>3</sub>. The combined organic phases were evaporated to afford **M** as a white solid (0.20 g, 85 %). M.p. 191–193 °C; <sup>1</sup>H NMR:  $\delta = 0.031-0.067$  (d, 12 H, J=11 Hz), 0.88–0.89 (d, 18 H, J=5 Hz), 3.54 (t, 2 H, J=5 Hz), 3.66 (t, 2 H, J=5 Hz), 3.79 (m, 4 H), 4.37 (s, 4 H), 4.87 (s, 2 H), 6.82–6.84 (d, 2 H, J=8 Hz), 6.97–7.01 (m, 6 H), 7.35–7.48 ppm (m, 8 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>+CD<sub>3</sub>OD):  $\delta = -5.4$ , 18.4, 26.0, 48.9, 51.0, 61.2, 61.6, 66.9, 97.3, 115.2, 115.7, 127.8, 127.7, 134.0, 134.4, 157.5, 157.8, 158.0, 169.0 ppm.

**Protected 3 (N):** Compound **M** (0.19 g, 0.25 mmol), **C** (0.032 g, 0.12 mmol), Na<sub>2</sub>CO<sub>3</sub> (0.30 g, 2.8 mmol), and KI (cat.) were suspended in butyronitrile (20 mL). The mixture was stirred and heated under reflux for 3 d, cooled, filtered, and concentrated under vacuum. The residue was washed with hot MeOH/CHCl<sub>3</sub> to give the target compound as a brown powder (0.10 g, 45%). M.p. 158–162 °C; <sup>1</sup>H NMR:  $\delta = -0.040$ –0.013 (d, 24H, J = 8 Hz), 0.80–0.82 (d, 36H, J = 3 Hz), 3.46 (t, 4H, J = 5 Hz), 3.57 (t, 4H, J = 5 Hz), 3.69–3.73 (m, 8H), 4.31 (s, 8H), 4.58 (s, 4H), 4.76 (s, 4H), 6.91–7.0 (m, 16H), 7.28–7.48 (m, 19H), 7.89 (s, 11H), 8.30 ppm (s, 2H); <sup>13</sup>C NMR:  $\delta = -5.3$ , 18.4, 26.1, 29.9, 48.9, 51.0, 61.4, 61.7, 66.9, 67.6, 68.1, 111.8, 115.3, 115.4, 116.6, 128.0, 128.0, 128.1, 128.4, 130.0, 134.2, 137.8, 156.3, 157.7, 158.0, 158.3, 166.6, 168.6 ppm; MS-FAB: m/z: calcd for: 1753.8457, found: 1753.8483 [M+Na]<sup>+</sup>.

**Aplosspan 3**: To a solution of **I** (20 mg, 0.012 mmol) in THF (3.0 mL), a solution of Bu<sub>4</sub>NF (30 mg, 0.12 mmol) in THF (3 mL) was added dropwise at RT. The mixture was stirred at RT for 2.5 h. Water (3 mL) was added to quench the reaction. The solid was collected, washed with CHCl<sub>3</sub>, acetone, and water to afford **3** (9.73 mg, 66%). M.p. >200°C; <sup>1</sup>H NMR ([D<sub>6</sub>]DMSO, 50°C):  $\delta$  = 3.43–3.62 (m, 16H), 4.39 (s, 8H), 4.50 (brs, 2H), 4.73 (s, 4H), 4.80 (brs, 2H), 4.892 (s, 4H), 6.98–7.10 (m, 16H), 7.27 (t, 1H, *J*=8 Hz), 7.38–7.39 (m, 2H), 7.50–7.58 (m, 16H), 8.02 (s, 1H), 9.95 ppm (s, 2H).

**Preparation of hydraphile 4**: A mixture of *N*-benzyl-4,13-diaza-[18]crown-6 (0.12 g, 0.34 mmol), *N,N'*-(1,3-phenylene)-bis(12-bromododecanamide) (**G**; 0.10 g, 0.16 mmol, see preparation above for **1**), Na<sub>2</sub>CO<sub>3</sub> (0.50 g, 4.7 mmol), and KI (cat.) in butyronitrile (30 mL) was heated to reflux for 24 h. The reaction mixture was cooled, filtered and the solvent was evaporated (in vacuo). The residue was chromatographed (SiO<sub>2</sub>, EtOAc/Et<sub>3</sub>N 10:1) to afford the product as an oil (23 mg, 13%). <sup>1</sup>H NMR:  $\delta = 1.17$ –1.43 (m, 32H), 1.66–1.68 (m, 4H), 2.27–2.34 (m, 4H), 2.47–2.52 (m, 4H), 2.76–2.82 (m, 16H), 3.56–3.64 (m, 36H), 7.20– 7.34 (m, 13H), 7.79 ppm (m, 1H); <sup>13</sup>C NMR:  $\delta = 25.9$ , 27.1, 27.6, 29.4, 29.5, 29.6, 29.7, 38.0, 54.1, 54.2, 56.0, 60.1, 69.9, 70.2, 70.9, 115.4, 127.1, 128.4, 129.1, 129.7, 138.9, 139.8, 171.9 ppm; MS-FAB: *m/z*: calcd for: 1195.8338, found: 1195.8344 [*M*+Na]<sup>+</sup>.

**Preparation of hydraphile 5**: Compound **5** was reported in previous studies.<sup>[24]</sup>

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