Synthesis and Membrane Activity of a Bis(metacyclophane)bolaamphiphile

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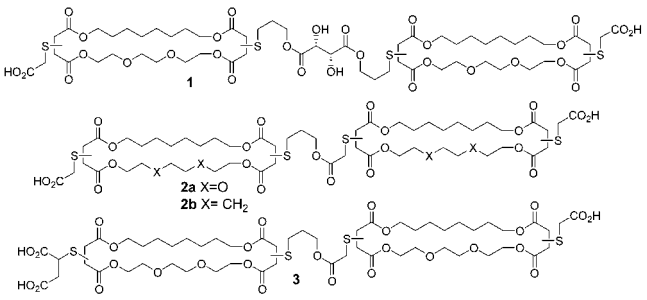
The synthesis of macrocyclic tetraesters from 5-substituted isophthalic acids and 1,8-octane diol gave macrocylic metacyclophanes with the axially symmetric positions differentially protected. Dimer bis(metacyclophane)bolaamphiphiles proved to be extremely insoluble, but one derivative was shown to have significant membrane activity. Very high continuous conductance and the formation of discrete single-ion channels were both observed, depending on how the compound was incorporated into the bilayer membrane.

Introduction

Synthetic compounds that insert into bilayer membranes and open channels for the transport of ionic species provide insights into the functional characteristics of natural channels and may lead to new technologies in sensors, separations, and signal propagation. Reports over the past decade[1−11] describe a wide range of compounds that are functionally similar in their ability to form ion channels, yet they are structurally disparate. Despite the broad range of active structural types, many of the classes of channel-forming compounds reported show clear indications that channel-formation within the class is closely controlled by structure.[12,13] The apparent dichotomy of structural disparity *between* classes of compounds and structural specificity *within* those classes is rationalized by an emerging view that membrane-spanning chains or pools of water form the channels.[14−16] In this view, each class of compound provides a general framework for water stabilization that can be further optimized through structural variations.

The synthesis of new channel-forming compounds typically starts from the selection of molecular building blocks bearing suitable functionality that are assembled using a few well-optimized reactions.[17] The main focus in this property-directed synthesis[18] is on the initial choice of building blocks, as these define the structural variations possible. Synthetic efficiency is often a secondary consideration. The reactions need to work well enough to produce the material, but a few micromoles of product is sufficient to assay the function of a putative channel-forming compound. Nonetheless, wasteful and inelegant syntheses eventually inhibit the optimization of the desired channel function. An example is given by our own work with bis(macrocyclic)bolaamphiphile pore-formers. The parent compound **1** was initially prepared as a structural control for a series of crown ether-based unimolecular channels.[19] The modest activity found for **1** was considerably enhanced through iterative optimization to give compounds such as **2ab** that form well-defined ion-selective channels.[20] The working hypothesis is that channels formed by **2ab** are membrane-spanning aggregates of a few molecules. This reasoning leads to **3**, which is capable of forming rectified ion channels.[21] At each stage of this evolution of structure, the activity and functional sophistication of the compounds increased. However, it is not their functional strengths but their synthetic weaknesses that are the issue here: despite its highly desirable properties as a voltage-gated channel, **3** will remain a laboratory curiosity as a result of its exceedingly tedious synthesis.



The synthesis of **3** begins with maleic anhydride, 1,8-octanediol, and triethylene glycol to give, in 6% yield, a

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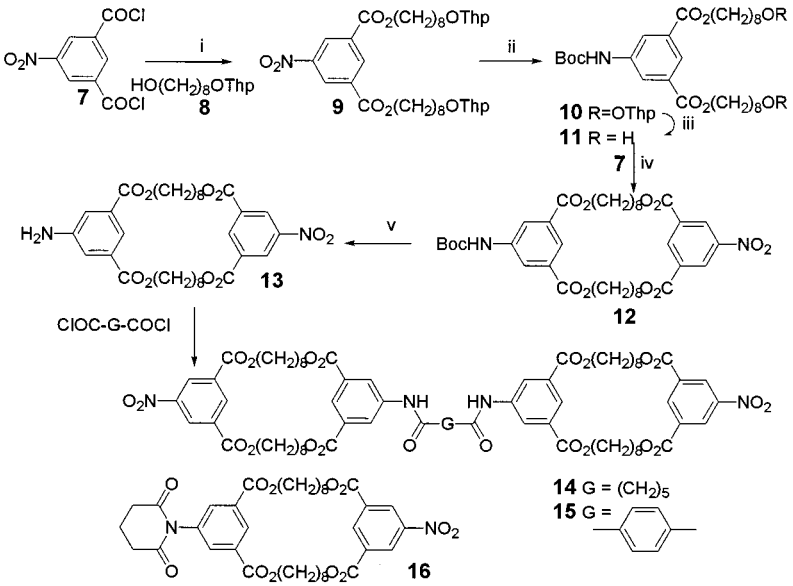
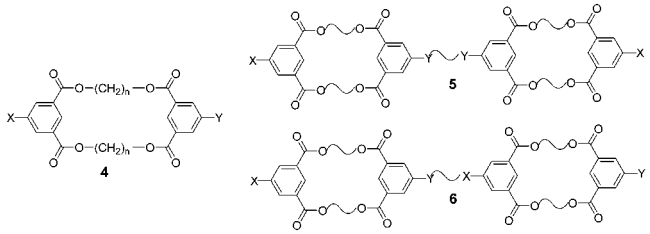
Scheme 1: Synthesis of Tetraester metacyclophanes[a]



[a] Reagents: (i) Et_3N, THF, 0 °C, 2 h, 80%; (ii) (1) H_2/PtO_2, EtOH, 1 equiv HCl, 25 psi, 2 h; (2) Boc_2O, THF, rt, 16 h, 88% (iii) HCl, MeOH, rt, 1 h, 70%; (iv) THF, rt, 16 h, 10−16%; (v) TFA, CH_2Cl_2, rt, 16 h, 93%; (iv) Et_3N, THF, rt, 50−90%.

tetraester diene macrocycle with equivalent alkenes. Monofunctionalization with mercaptopropanol, coupling in a second monofunctionalization process, followed by addition of the final headgroup completes the synthesis in an abysmal yield after several months' work.[20] The weaknesses of this synthetic plan are apparent: both the macrocyclization yield and the two monofunctionalization yields are low, and the regio- and stereoselectivity of the thiol addition reactions are completely random, leading to mixtures of isomers. These mixtures cannot be separated but behave as single compounds.[17] Some of the intermediates are contaminated as a competing retro-Michael reaction scrambles the thioacetate headgroups, leading to extensive chromatography at all stages.[17,19,20]

Many of these synthetic problems might be resolved through a redesign of the macrocyclic subunit. A simple alkyl strand is unlikely to be sufficient, as long-chain bolaamphiphiles are effective membrane-disrupting agents.[22] The macrocycle is expected to provide some rigidity and to provide a framework for functional-group substitutions that would influence channel properties.[23] If macrocycles are required, then the focus must be on high-yielding macrocyclization methods. To avoid diasteromeric mixtures, achiral or chirally pure macrocycles are required. To avoid regioisomers, axial symmetry will be required. To avoid the poor yields of monofunctionalization reactions, the "ends" of the macrocycle must be different or differentially protected. All these requirements lead to metacyclophanes as candidates. Tetraesters such as **4** would be available from high-dilution macrocyclization of a suitable diol with a diacid chloride to ensure end-to-end discrimination. Starting from commercially available 5-nitroisophthalic acid would give the X and Y groups via nitro reduction and amine protection. **4** could be converted to head-to-head (**5**) or head-to-tail (**6**) bis(macrocycles) through judicious choice of reaction sequence. This contribution reports synthetic explorations in this area and the eventual production of a new active channel-forming compound of type **5**.



Results and Discussion

Syntheses of Tetraester Cyclophanes. The synthesis proceeds as in Scheme 1, using the monotetrahydropyranyl derivative of 1,8-octanediol (**8**) coupled with the acid chloride of 5-nitroisophthalic acid (**7**) to give **9** in good yield. Reduction of the nitro group by hydrogenation over Adams' catalyst under neutral conditions gave an amine that was directly protected as the Boc derivative **10** in 88% yield. Selective removal of the Thp group gave diol **11**. The macrocyclization of **11** and **7** was achieved under high-dilution conditions in THF with excess Et_3N (final concentration $< 3 \times 10^{-4}$ M). The isolated yield of macrocycle **12** was only 16%, which appears to be fundamental, as the same coupling at a 10-fold higher final concentration still gave a 10% yield of **12**. Although **7** is a reactive acid chloride, **11** is likely to be relatively unreactive, hence a poor partner for high-dilution macrocyclization. Another feature of **12** that would prove problematic was its restricted solubility in many solvents.

Head-to-head coupling to give bis(cyclophanes) of type **5** is most easily achieved from an amine with a bis(acid chloride). Thus, **12** was deprotected with TFA in CH_2Cl_2

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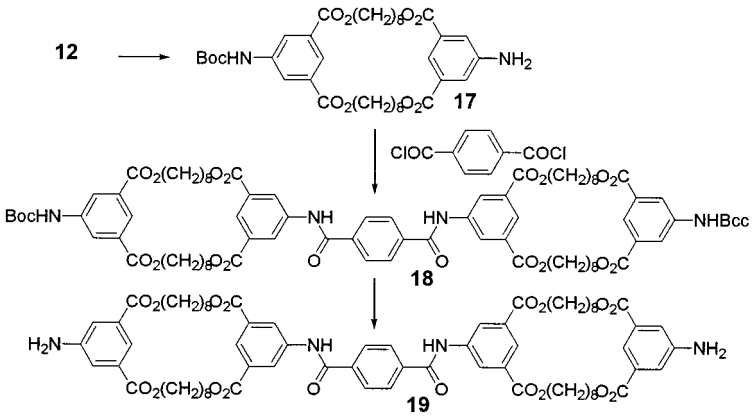
Scheme 2: Synthesis of Bis(metacyclophane) 19[a]



[a] Reagents: (i) H_2/PtO_2, EtOH, 1 equiv HCl, 25 psi, 2 h, 45%; (ii) Et_3N, THF, 0 °C, 2 h, 92%; (iii) TFA, CH_2Cl_2, rt, 16 h, 77%.

to give the nitro amine **13** and subsequently coupled with the diacid chlorides of glutaric, pimelic, and terephthalic acids. The glutaric acid system produced exclusively the imide **16**, indicating that cyclization competes efficiently with intermolecular dimer formation. The rigid terephthalic acid did give the expected diamide **15** as a very insoluble powder; the mass spectrum and elemental analysis were consistent with the structure, and the NMR spectrum was eventually obtained in DMSO at 150 °C. The product from pimelic acid **14** was similarly difficult to handle. Both **14** and **15** are well-characterized, but their very poor solubility effectively precludes further reactions.

An alternative pathway to the same target was completed as shown in Scheme 2. Reduction of the nitro group of **12** gave the monoprotected diamine **17**. This amine coupled with terephthaloyl chloride to produce the insoluble dimer **18**. Although only sparingly soluble in hot DMSO and apparently insoluble in methylene chloride, the Boc protecting groups could be removed by treatment with TFA in methylene chloride. This solid-to-solid transformation produced a material assigned as **19** on the basis of the expected molecular ion in the mass spectrum and on the ^{1}H NMR spectrum that showed only the expected resonances for the deprotected diamine. No *tert*-butyl signal was found in the ^{1}H NMR spectrum of the product. Like the other bis(macrocyclic) products, this material proved to be difficult to handle because of its poor solubility in anything but hot DMSO. A satisfactory ^{13}C NMR spectrum could not be obtained because of poor solubility, and the elemental analysis suggested that a small proportion of trifluoroacetic acid remained in the solid product (~0.25 equiv). Given that **18** was well-characterized and that the deprotection is apparently complete and preserves the core of the molecule the isolated material is assigned as **19** in a partly protonated form.

The synthesis of bis(metacyclophane) **19** of type **5** from 1,8-octanediol is formally complete in an overall yield of ca. 2% over eight steps. This synthesis is closely similar to that of **1**, a compound having the same centrosymmetry as cyclophanes of type **5**, which are produced in ~2% yield from the same starting diol. Although the intermediate **12** could give end-discriminated cyclophanes of type **6** more easily than was possible in the series that produced **2ab** and **3**, the poor macrocyclization yield and the poor solubility of the bis(cyclophanes) promise to plague any further exploration of this avenue.

Membrane Activity of 19. The transport activity of **19** was initially assessed in vesicle bilayer experiments of two types. The first method uses vesicle-entrapped carboxyfluorescein at a high concentration: any membrane disruption leading to large pores will release entrapped carboxyfluorescein and will result in an enhancement of fluorescence.[22,23] Vesicles were prepared in 0.1 M carboxyfluorescein sodium salt using a mixed-lipid system of phosphatidyl choline (PC), phosphatidic acid (PA), and cholesterol (Cho) (8:1:1 mole ratio). By this assay, **19** is a remarkably ineffective membrane-disrupting agent, with 50% release achieved only at millimolar concentration. Thus, large defects are formed only under conditions where the concentration of **19** exceeds the lipid concentration. The second assay method uses the titration of protons released from a vesicle-entrapped buffer (pH 6.6) into an unbuffered electrolyte held at pH 7.6 using a pH-stat titrimeter. Vesicles were prepared from PC/PA/Cho (8:1:1) by sonication and sized by extrusion through a 0.4 μm Nucleopore filter. This method has been used with other active transporters to determine transport rates, cation selectivities, and the apparent kinetic order of the transport process.[19,20] Addition of **19** as a DMSO solution always provokes an immediate consumption of basic titrant to maintain the set pH, which is proportional to the amount of **19** added. The release of protons occurs within 5 s of the addition of compound, but there is no further release during the following 500−1000 s. Addition of a detergent (Triton) results in the release of the expected amount of entrapped buffer. As little as a nanomole of **19** (less than 0.01 mol % with respect to the total lipid concentration) will provoke the initial response. The pH-stat technique measures the rate that new channels are initiated. Because **19** is virtually insoluble in water, injection of a DMSO solution probably results in the precipitation of the compound within the bulk solution and onto the surface of some of the vesicles. Presumably, some fraction of the vesicles will not have sufficient compound to form channels, so they are not detected until after the addition of Triton. The very low solubility of **19** would inhibit migration between vesicles, so no new channels would be initiated after the initial burst. Vesicle techniques, although suggestive, do not provide clear evidence of membrane activity.

The voltage-clamp technique[21,23] indicates that **19** shows significant activity in planar bilayers, but the type

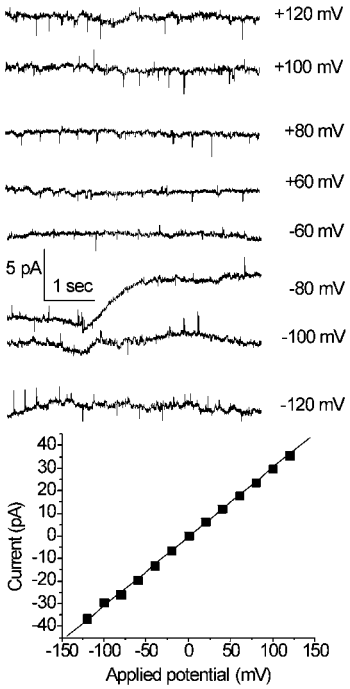


Figure 1. Persistent bilayer membrane conductance induced by **19** at various applied potentials (diphytanoyl phosphatidylcholine, 1 M KCl, pH 4.7). The individual records are consecutive 5 s snapshots separated by 15 s intervals in which the potential was adjusted and stabilized. Bottom: a current−voltage relationship constructed from the mean currents of the data shown (±40 and ±20 mV not shown).

of activity observed depends on the way the compound is introduced to the bilayer. Direct injection of DMSO solutions into the experimental system in the presence of a preformed bilayer results in visible precipitation of material in the electrolyte and no trace of membrane activity. However, injection of the DMSO solution such that the emerging drop contacts a preformed bilayer results in very significant membrane conductivity of the type shown in Figure 1. Incorporation by this method is haphazard at best, and usually 3−5 cycles of injection, sometimes resulting in membrane breakage, are required to generate activity. Incorporation by this technique has been achieved in diphytanoyl phosphatidylcholine membranes and in a PC/PA/Cho (8:1:1) mixed-lipid membrane. Incorporation by this method is apparently more efficient at slightly acidic pH (4.7) than at neutral pH.

As seen in Figure 1, discrete channel openings are not observed, but the current varies irregularly within a range of 1−2 pA up to 50 pA above the baseline. Although the general increase in bilayer conductance persists for hours, the current−voltage characteristics vary over a period of minutes. The current−voltage plot from the data of Figure 1 corresponds to a specific conductance of 310 ± 10 pS. Two minutes later, the specific conductance had shifted to 270 ± 10 pS ($i-V$ plot not shown). An example of how the shift occurs is given in the trace at −80 mV applied potential in Figure 1: over 1 s, the current at this potential decreases by 5 pA in a relatively smooth fashion. The enhanced conductance of the bilayer persists, following breakage and reformation of the bilayer. Throughout the experiment, the bilayer capacitance remains in the normal range for high-quality bilayers (200 pF in our system). Control experiments establish that DMSO alone is inactive up to a 10-fold higher bulk concentration than that of the highest levels explored with **19**.

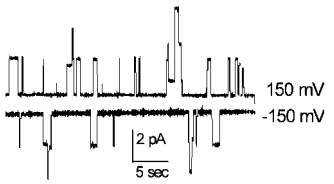


Figure 2. Single-channel conductance records of **19** at an applied potential of ±150 mV (PC/PA/Ch 8:1:1). The compound was incorporated as described in the text.

Discrete single channels can also be observed for **19**. Incorporation to produce single-channel events requires a mechanical transfer of material to a preformed bilayer held under a positive transmembrane potential. A 1−5 μL aliquot of a millimolar solution of **19** in hot DMSO (1−5 nmol) was added to a clean fine-camel-hair brush. The brush was then used to break and reform a bilayer. This method was successful in about half the attempts; failure resulted in no membrane activity rather than in the general increase in conductance illustrated in Figure 1. Examples of the type of single-channel openings observed are given in Figure 2: a main-step conductance change of about 2 pA dominates the population of openings at both potentials. At positive potentials, there is a smaller opening as well, and occasional double openings are seen at both potentials. The specific conductance of the major opening is 13.7 ± 1.7 pS for the experiment that included Figure 2 (1 M KCl electrolyte; pH 4.7). Independent experiments on different days gave the same specific conductance within experimental error. Single channels were observed only at pH 4.7 in membranes from mixed lipids (PC/PA/Ch).

The available data support the qualitative conclusion that **19** can insert into bilayer membranes and thereby enhance ion conduction. The discrete channels of Figure 2 are similar in specific conductance to channels formed by **2ab**, another bis(macrocyclic) bolaamphiphile. In the case of **2ab**, the active structure is postulated to be a membrane-spanning dimer or trimer on the basis of a combination of vesicle and planar bilayer results. The same type of structure may be responsible for the single channels shown in Figure 2. A systematic study as was done with **2ab** is precluded by the very low solubility of **19**: the mechanical transfer of material to the bilayer is simply too ineffective to allow a broad analysis. The high conductive states related to the behavior shown in Figure 1 are even harder to study, as the specific conductance is variable within an experiment and from day to day. It is possible that the Figure 1 data arises from domains of **19** incorporated in the bilayer. Variable conductance might then be related to variable domain size.

In conclusion, the successful synthesis and novel membrane activity seen with **19** confirm the design suggestions leading to structures of types **5** and **6**. At the same time, the compounds prepared are very intractable and are produced in yields that are no improvement over those leading to compounds **2ab**. As with the previous bis(macrocycle) systems, poor synthetic efficiency, coupled in this case with undesirable physical properties, effectively truncates further development of the very interesting membrane activity of the compounds.

Experimental Section

Vesicle and planar bilayer procedures have been described previously.[20,21]

Bis(8-tetrahydropyranyloxyoctyl)-5-nitroisophthalate (9). The mono Thp ether of 1,8-octanediol (**8**, 4.81 g, 20.9 mmol) was dissolved in freshly distilled ice-cold THF (200 mL), and 5-nitroisophthaloyl chloride (**7**, 9.95 mmol) in THF (50 mL) was added dropwise simultaneously with triethylamine (2.11 g, 20.9 mmol). The reaction was stirred on ice for 2 h. Triethylamine hydrochloride was removed by filtration, and the filtrate was evaporated to a yellow oil that was chromatographed on silica using 3:1 hexanes/Et_2O as the eluent to give **9** as a clear oil (5.18 g, 82%). ^{1}H NMR ($CDCl_3$, δ): 1.25−1.77 (m, 36H), 3.26−3.82 (m, 8 H), 4.33 (t, 4 H), 4.47−4.49 (m, 2 H), 8.89 (s, 1 H), 8.92 (d, 2 H, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$, δ): 19.6, 25.4, 25.8, 26.1, 28.5, 29.1, 29.3, 29.6, 30.7, 62.3, 66.3, 67.5, 98.7, 127.9, 132.7, 135.7, 148.4, 163.7; MS (+LSIMS, mNBA): 634.4 (M − 1).

***N-tert*-Butoxycarbonyl-bis(8-tetrahydropyranyloxyoctyl)-5-aminoisophthalate (10).** The nitro compound **9** (1.65 g, 2.6 mmol) was placed in a Parr hydrogenator with 95% EtOH (∼100 mL/0.5 g of nitro compound), PtO_2 (20 mol %), HCl (1 equiv) and hydrogenated at 25 psi for 2 h with shaking. When the hydrogenation was complete, the solution was clear except for the catalyst. This black particulate was gravity-filtered, and the solution was evaporated to yield the crude ammonium salt that was used without further purification. The free amines were generated by CH_2Cl_2-extraction of a basic solution of the ammonium salt, dried over $MgSO_4$, and used immediately upon solvent removal. ^{13}C NMR ($CDCl_3$, δ): 19.7, 25.5, 25.9, 26.2, 28.6, 29.2, 29.3, 29.7, 30.8, 62.4, 65.3, 67.6, 98.9, 119.6, 120.6, 131.8, 146.6, 166.1; MS (+LSIMS, mNBA) 606.4 (M + 1)). The amine was protected as the Boc derivative by overnight stirring with di-*tert*-butyl carbonate (1 equiv based on starting nitro) in 10 mL of freshly distilled THF. Solvent removal and purification by centrifugal chromatography on silica (10% MeOH in ether as eluent) gave the Boc-protected product **10** in 88% overall yield. ^{1}H NMR ($CDCl_3$, δ): 1.27−1.82 (m, 45H), 3.31−3.87 (m, 8H), 4.29 (t, 4H), 4.55 (t, 2H), 6.82 (s, 1H, N−H), 8.20 (d, 2H, J = 1.5 Hz), 8.31 (t, 1H, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$, δ): 19.7, 25.5, 25.9, 26.1, 28.3, 28.6, 29.2, 29.3, 29.7, 30.8, 62.3, 65.5, 67.6, 81.1, 98.8, 123.4, 125.0, 131.7, 138.6, 139.0, 152.4, 165.7.

***N-tert*-Butoxycarbonyl-bis(8-hydroxyoctyl)-5-aminoisophthalate (11).** The protected diol **10** (1.33 g, 1.9 mmol) was dissolved in MeOH (40 mL) and HCl (1 M, 20 mL), and the mixture was stirred for 1 h at rt. The acid was neutralized with 1 M NaOH, and the MeOH was allowed to evaporate. The residue was taken up in CH_2Cl_2 and washed with H_2O twice, and the organic layer was dried over $MgSO_4$ and evaporated. Centrifugal chromatography using Et_2O as the eluent afforded **11** as a white solid (0.71 g, 70%): mp 54 °C; ^{1}H NMR ($CDCl_3$, δ): 1.34−1.45 (m, 16H), 1.50 (s, 9H), 1.58 (m, 4H), 1.74 (dt, 4H), 3.61 (t, 4H, J = 6.6 Hz), 4.30 (t, 4H, J = 6.6 Hz), 7.01 (s, 1H), 8.21 (d, 2H, J = 1.5 Hz), 8.30 (t, 1H, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$, δ): 25.6, 25.8, 28.3, 28.6, 29.1, 29.2, 32.7, 63.0, 65.5, 123.4, 124.7, 131.7, 139.6, 152.8, 165.9; MS (mNBA, +LSIMS): 538.3 (M + 1); exact mass calculated for $C_{29}H_{48}NO_8$: 538.3380; found: 538.3394.

***N-tert*-Butoxycarbonyl-15-amino-33-nitro-2,11,20,29-tetraoxa-1,12,19,30-tetraoxo-[12$_2$]-metacyclophane (12).** 5-Nitroisophthaloyl dichloride **7** (0.20 g, 0.78 mmol) and the diol **11** (0.42 g, 0.78 mmol) were separately dissolved in THF (50 mL) and added dropwise overnight via a dual syringe pump into vigorously stirred, freshly distilled THF (3 L) and triethylamine (0.16 g, 1.56 mmol). The triethylammonium hydrochloride was removed by filtration, and the filtrate was evaporated to an off-white solid that was purified by centrifugal chromatography using Et_2O as the eluent to give **12** (0.09 g, 16% yield). An alternative reaction on the same scale using only 200 mL of THF gave the same product in 10% yield. ^{1}H NMR ($CDCl_3$, δ): 1.38−1.52 (m, 25H), 1.73−1.88 (m, 8H), 4.33 (t, 4H), 4.42 (t, 4H), 6.68 (s, 1H, N−H), 8.18 (2H), 8.25 (1H), 8.83 (1H), 8.89 (2H); ^{13}C NMR ($CDCl_3$, δ): 25.8, 28.3, 28.4, 28.5, 28.9, 65.4, 66.3, 81.2, 123.4, 124.5, 128.2, 131.5, 132.7, 135.3, 139.3, 148.5, 152.4, 163.7, 165.6; MS (−LSIMS, mNBA): 712.2 (M^-); exact mass (−LSIMS, mNBA) calculated for $C_{37}H_{48}N_2O_{12}$: 712.3208; found: 712.3183.

15-Amino-33-nitro-2,11,20,29-tetraoxa-1,12,19,30-tetraoxo-[12$_2$]-metacyclophane (13). 12 (0.47 g, 0.66 mmol) and 5.88 mL of TFA (trifloroacetic acid) were stirred at reflux in 95 mL of methylene chloride overnight. After cooling, saturated $NaHCO_3$(aq) was slowly added to the reaction mixture to neutralize TFA until the pH of the solution was above 7. The organic layer was washed twice with water and dried over Na_2SO_4. The methylene chloride was removed under reduced pressure, and the resulting off-white solid was sonicated in $CHCl_3$ (30 mL) for 20 min. This precipitate was collected by filtration and was identified as **13**. The $CHCl_3$ was removed under reduced pressure, and the residue was separated by centrifugal chromatography using THF/CH_2Cl_2 (1:3) as the eluent. The product from filtration was combined with the product from chromatography to give a total of 0.40 g (93%) of **13** as an off-white solid. ^{1}H NMR (360 MHz, DMSO-d_6, δ): 1.40−1.62 (m, 16H), 1.78 (m, 8H), 4.2 (t, 4H), 4.35 (t, 4H), 5.4 (d, 2H), 7.3 (s, 2H), 7.5 (s, 1H), 8.7 (m, 3H); ^{13}C NMR (90 MHz, DMSO-d_6, δ): 24.7, 24.8, 27.3, 27.5, 27.7, 64.1, 65.4, 116.3, 117.8, 117.8, 126.6, 130.7, 132.0, 133.8, 162.8, 165.0. Anal. Calcd for $C_{32}H_{40}N_2O_{10}$: C, 62.73; H, 6.58; N, 4.57. Found: C, 62.39; H, 6.52; N, 4.41; MS: 613.2 (M + H)$^+$.

***N,N′*-bis[33-nitro-2,11,20,29-tetraoxa-1,12,19,30-tetraoxo-[12$_2$]-metacyclophane-15-yl]- octadioamide (14).** To a solution of **13** (0.15 g, 0.21 mmol) and Et_3N (0.025 g) in 25 mL THF was added pimeloyl chloride (0.0247 g, 0.21 mmol) as a solid. The reaction mixture was refluxed under a nitrogen atmosphere overnight. After cooling, the precipitate was collected by filtration and washed extensively with $CHCl_3$ to give **14** as a white solid (0.153 g, 90%). ^{1}H NMR (360 MHz, DMSO-d_6, 150 °C, δ): 1.48 (m, 34H), 1.78 (m, 20H), 4.3 (t, 8H), 4.4 (t, 8H), 8.1 (m, 2H), 8.37 (m, 4H), 8.74 (m, 6H); ^{13}C NMR (90 MHz, DMSO-d_6, 150 °C, δ): 23.8, 24.5, 27.1, 27.2, 27.4, 27.6, 35.6, 64.2, 65.2, 122.9, 123.1, 126.4, 130.5, 132.2, 133.5, 139.5, 149.0, 162.5, 164.2, 170.9. Anal. Calcd for $C_{71}H_{88}N_4O_{22}$: C, 63.19; H, 6.57; N, 4.15. Found: C, 62.80; H, 6.54; N, 4.20. MS: 1348.4 (M − H)$^-$.

***N,N′*-bis[33-nitro-2,11,20,29-tetraoxa-1,12,19,30-tetraoxo-[12$_2$]-metacyclophane-15-yl]- terephthalamide (15).** To a solution of **13** (0.194 g, 0.27 mmol) and Et_3N (0.032 g) in 25 mL THF was added terephthaloyl dichloride (0.032 g, 0.27 mmol) as a solid. The reaction mixture was refluxed under a nitrogen atmosphere overnight and then cooled, and the precipitate was collected by filtration. This residue was washed extensively with $CHCl_3$ to give **15** as a white solid (0.193 g, 90%). ^{1}H NMR (360 MHz, DMSO-d_6, 150 °C, δ): 1.43−1.59 (m, 32H), 1.75−1.85 (m, 16H), 4.35−4.45 (m, 16H), 8.13 (s, 4H), 8.23 (m, 2H), 8.65 (m, 4H), 8.7 (m, 6H), 10.3 (s, 2H); ^{13}C NMR (90 MHz, DMSO-d_6, 150 °C, δ): 24.5, 24.5, 27.1, 27.3, 27.4, 64.4, 65.2, 123.8, 124.3, 126.4, 127.0, 130.6, 132.0, 133.5, 136.6, 139.3, 148.0, 162.5, 164.2, 164.4. Anal. Calcd for $C_{72}H_{82}N_4O_{22}$: C, 63.80; H, 6.10; N, 4.13. Found: C, 63.53; H, 6.08; N, 4.06. MS: 1354.4 (M − H)$^-$.

15-(2,6-Dioxo-1-piperidinyl)-33-nitro-2,11,20,29-tetraoxa-1,12,19,30-tetraoxo-[12$_2$]- metacyclophane (16). Glutaryl dichloride (8 μL, 0.062 mmol) was added to **13** (0.077 g, 0.13 mmol) in THF (25 mL) containing Et_3N (0.5 mL). The mixture was refluxed overnight and cooled to room temperature, a white precipitate was removed by filtration, and the filtrate was concentrated under reduced pressure. The crude product was purified by centrifugal chromatography using $CHCl_3$ as the eluent to give **16** (0.022 g, 52%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.3−1.62 (m, 16H), 1.64−1.82 (m, 8H), 2.0−2.2 (m, 2H), 2.75−2.82 (t, 4H), 4.2−4.5 (m, 8H), 7.9 (s, 2H), 8.6 (s, 1H), 8.8 (s, 1H), 9.0 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 17.2, 25.9, 28.5, 28.6, 29.0, 32.9, 65.6, 66.3, 128.2, 130.3, 132.1, 132.8, 134.1, 135.3, 135.8, 148.6, 163.8, 164.9, 172.2; MS: 708.3 (M)$^-$. The structure of **16** was confrimed by reduction to the amine: **16** (0.141 g, 0.2 mmol) in THF (120 mL) was reduced over PtO_2 (0.1 g) under 30 psi of H_2. Following an overnight reaction, the catalyst was removed by

gravity filtration, and the filtrate was evaporated. The crude product was purified by a centrifugal chromatography plate using THF/CH_2Cl_2 (1:4) as the eluent. The major band was collected, and the solvent was evaporated to give an off-white solid. This solid was washed with ether (5 mL) to give **16** (0.081 g, 60%) as an off-white solid. ^{1}H NMR (300 MHz, CD_2Cl_2, δ): 1.25−1.9 (m, 24H), 2.0−2.2 (m, 2H), 2.8 (t, 4H), 4.1 (b, 2H), 4.4 (m, 8H), 7.5 (s, 2H), 8.0 (m, 3H), 8.7 (s, 1H); ^{13}C NMR (75 MHz, CD_2Cl_2, δ): 17.5, 26.4, 26.5, 28.9, 28.9, 29.4, 33.2, 65.6, 66.0, 119.7, 120.0, 130.1, 132.2, 132.5, 134.4, 136.6, 138.5, 147.7, 165.2, 166.3, 172.7. Anal. Calcd for $C_{37}H_{46}N_2O_{10}$: C, 65.47; H, 6.83; N, 4.13. Found: C, 65.28; H, 6.89; N, 3.98. MS: 679.2 $(M + H)^+$.

***tert*-Butyl 33-amino-2,11,20,29-tetraoxa-1,12,19,30-tetraoxo-[12$_2$]-metacyclophane-15- ylcarbamate (17).** Pt_2O (0.15 g) was added to a solution of **12** (0.23 g) in 400 mL of THF/EtOH (1:1) and shaken under 35 psi of H_2 overnight. The catalyst was removed by filtration, the solvent was removed under reduced pressure, and the crude product was purified by centrifugal chromatography using $CHCl_3$ as the eluent. The major band was **17** (0.10 g, 45%). ^{1}H NMR (300 MHz, THF-d_8, δ): 1.37 (m, 21H), 1.65 (m, 12H), 4.2 (m, 8H), 7.31 (s, 2H), 7.74 (s, 1H), 8.12 (s, 1H), 8.24 (s, 2H), 8.74 (s, 1H); ^{13}C NMR (300 MHz, THF-d_8, δ): 27.0, 28.5, 29.5, 30.1, 65.4, 65.7, 80.4, 118.8, 119.5, 123.5, 124.1, 132.4, 141.7, 153.5, 165.8, 166.4. Anal. Calcd for $C_{37}H_{50}N_2O_{10}$: C, 65.08; H, 7.38; N, 4.10. Found: C, 65.12; H, 7.35; N, 3.90. MS: 683.3 $(M + H)^+$.

***N*,*N*′-bis[33-(*tert*-butyloxycarbamoyl)-2,11,20,29-tetraoxa-1,12,19,30-tetraoxo-[122]- metacyclophane-15-yl]-terephthalamide (18).** To a solution of **17** (0.13 g, 0.19 mmol) and Et_3N (0.022 g) in THF (25 mL) was added terephthaloyl dichloride (0.019 g, 0.19 mmol) as a solid. The reaction mixture was refluxed under a nitrogen atmosphere overnight and cooled, and the precipitate was collected by filtration. The precipitate was washed with $CHCl_3$ to give **22d** as a white solid (0.12 g, 92%). ^{1}H NMR (360 MHz, DMSO-d_6, 120 °C, δ): 1.33−1.61 (m, 50H), 3.53−3.72 (m, 16H), 4.16−4.47 (m, 16H), 8.06−8.20 (d, 6H), 8.20−8.38 (d, 6H), 8.57−8.75 (d, 4H), 9.26−9.40 (s, 2H), 10.39−10.5 (s, 2H). Anal. Calcd for $C_{82}H_{102}N_4O_{22}$: C, 65.85; H, 6.87; N, 3.75. Found: 65.61; H, 6.94; N, 3.63. MS: 1493.7 $(M - H)^-$.

***N*,*N*′-bis[33-amino-2,11,20,29-tetraoxa-1,12,19,30-tetraoxo-[12$_2$]-metacyclophane-15-yl]- terephthalamide (19).** A mixture of **18** (0.075 g, 50 μmol) and TFA (0.9 mL) in CH_2Cl_2 (25 mL) was stirred at reflux overnight. After cooling, the white residue was collected by filtration and extensively washed with CH_2Cl_2 to give **19** as a white solid (0.050 g, 77%). ^{1}H NMR (360 MHz, DMSO-d_6, 120 °C, δ): 1.16−1.61 (m, 32H), 1.61−1.87 (m, 16H), 4.10−4.49 (m, 16H), 7.35−7.46 (d, 4H), 7.67−7.74 (t, 2H), 8.07−8.18 (s, 4H), 8.21−8.29 (t, 2H), 8.60−8.72 (d, 4H), 10.42−10.54 (s, 2H). Anal. Calcd for $C_{72}H_{86}N_4O_{18}$ + 0.25CF_3CO_2H: C, 65.78; H, 6.55; N, 4.23. Found: 65.66; H, 6.54; N, 4.24. MS: 1293.5 $(M - H)^-$.

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

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