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"Flexiball" Toolkit: A Modular Approach to Self-Assembling Capsules

Brendan M. O'Leary,[†,‡] Tomas Szabo,[†] Niels Svenstrup,[†] Christoph A. Schalley,[†,§] Arne Lützen,[†,||] Mathias Schäfer,[⊥] and Julius Rebek, Jr.*[,†]

Contribution from The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, Kekulé-Institut für Organische Chemie und Biochemie der Universität, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany, Fachbereich Chemie der Universität Oldenburg, P.O. Box 2503, D-26111 Oldenburg, Germany, and Institut für Organische Chemie der Universität, Greinstr. 4, D-50939 Köln, Germany

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Abstract: We report the synthesis and characterization of new, self-assembling molecular capsules. The modular strategy makes use of glycoluril building blocks available in multigram amounts combined with aromatic spacer elements. The lengthy syntheses encountered with earlier generations of capsules are avoided, and several capsules of nanometer dimensions are now accessible. Single bond attachments between spacers and glycoluril modules result in monomers as dimeric capsules that are less rigid than their earlier counterparts. The host−guest properties of the homo- and heterodimeric capsules were studied using a combination of NMR and ESI-mass spectrometry. They show a less pronounced selectivity for guests of different sizes, and their increased flexibility prevents self-assembly when no rigidifying elements are present on the central spacer unit. Some of the new capsules bear inwardly directed, secondary amide N−H protons. These can be further functionalized, as shown by their methylation to give tertiary analogues. The structures hold broader implications for the placement of functional groups on concave molecular surfaces.

Introduction

Some might say that supramolecular systems rescued physical organic chemistry. The discovery of crown ethers gave the field new recognition: molecular recognition. At first, hosts were limited to ionic guests, but as the systems acquired sophistication, they became popular models for biological assembly processes;[1] they offered new ways to study reactions and interactions. For recent examples, reactive species such as cyclobutadiene have been stabilized in carcerands,[2] bimolecular reactions such as the Diels−Alder condensation have been accelerated and even catalyzed in hydrogen-bonded capsules,[3] and a variety of container molecules that act as selective synthetic receptors for small molecular targets have been developed.[4] If these applications are to expand, the synthetic ac-

[†] The Scripps Research Institute.
[‡] Massachusetts Institute of Technology.
[§] Kekulé-Institut für Organische Chemie und Biochemie der Universität.
[||] Fachbereich Chemie der Universität Oldenburg.
[⊥] Institut für Organische Chemie der Universität.

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cessibility of the receptors, especially with regard to larger capacities and more diverse shapes, must improve. Several research groups have adopted "modular" strategies that employ versatile molecular building blocks to simplify approaches to such structures,[5] and the present work was undertaken to apply this approach to host systems that self-assemble through hydrogen bonding. Here, we describe the development of new glycoluril building blocks.[6] Their use in the synthesis of capsules with endohedral functionality,[7] an aspect of supramolecular host systems receiving increased attention, is also included.[8]

The hydrogen-bonded complexes **1·1**[9] (the "tennis ball") and **2·2**[10] (the "jelly doughnut") illustrate the motivation for our current work (Figure 1). Each monomer consists of a planar spacer (benzene and triphenylene, respectively) fused to glycoluril units to provide self-complementary hydrogen-bonding sites and structural curvature. The resulting dimeric capsules possess modestly sized cavities with shapes resembling flattened spheres and inner volumes ranging from about 70 to 240 Å^3 that can be occupied by a guest molecule. While these sizes and shapes confer selectivity for small guests such as ethane and cyclohexane, respectively, larger guests with more complicated shapes or more than one guest could not be encapsulated.

Access to larger hosts was limited by several complications inherent in the syntheses of glycoluril-based capsules. For example, consider the last step in the synthesis of **1** (Scheme 1A). Under strongly basic conditions, treatment of brominated spacer **4** with excess glycoluril **3** results in the alkylation of two glycolurils. These units provide the curvature that imparts the shape of the desired C-shaped isomer *syn*-**1**, but the unavoidable formation of the S-shaped anti isomer lowers the theoretical yield of **1** to 50%.[11] In reality, the yields for this alkylation step are much lower. A large excess of glycoluril

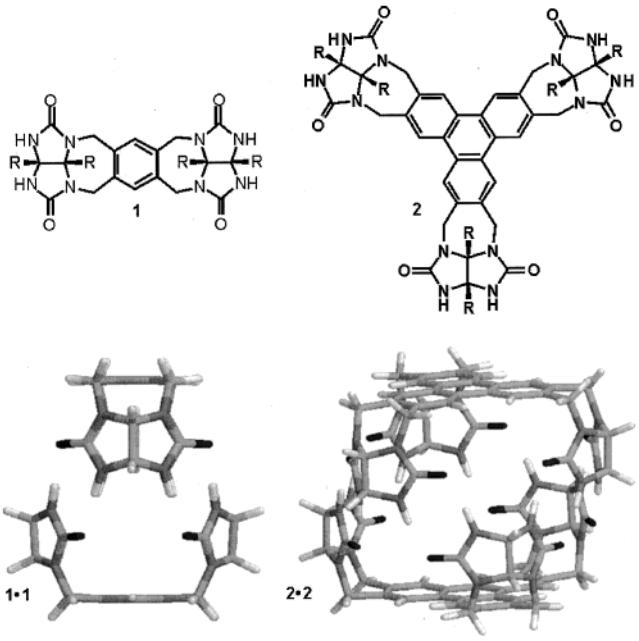
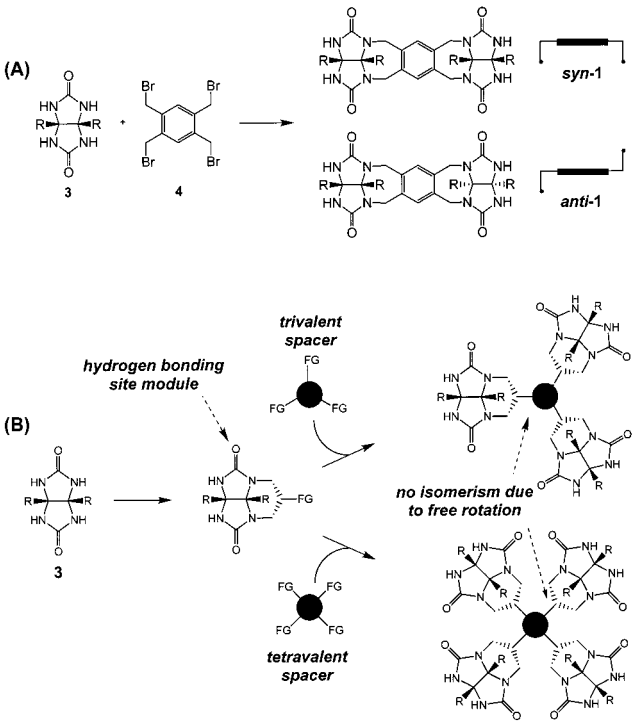


Figure 1. Monomers (top) dimerize via hydrogen bonds to give hollow capsules (bottom). **R** represents various solubilizing groups which have been omitted in the dimers for viewing clarity.

Scheme 1. (A) Final Step in the Synthesis of Monomer *syn*-**1**; Significant Amounts of the Undesired Isomer, *trans*-**1**, Also Form (B) Modular Strategy Offers Easier Syntheses, No Isomers after Condensation, and Capsules with Diverse Sizes and Shapes



complicates purification but is necessary in order to overcome poor solubility and reduce the undesired double alkylations. Furthermore, the brominated spacers decompose under the harsh alkylation conditions, as do the benzylamides.[12]

Scheme 1B outlines an alternative strategy. Condensation of a glycoluril with a "modular element" produces a fused six-

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membered ring, one that forms more easily than the seven-membered rings in **1** and **2**. This new heterocyclic system presents substituents in a well-defined orientation. For instance, an equatorial substituent at the 5-position could be connected to suitable spacers through a single, freely rotating bond (thereby interconverting C- and S-shaped conformations). Furthermore, the modular element can contribute greater size and shape diversity to the final capsules. Given the increased flexibility of contributing monomers, we term the resulting capsules "flexiballs". By choosing different central spacers, it is easy to envision a variety of flexiballs.

In addition to the simplicity of this synthetic approach, the functional groups of the 5-substituent can be directed to appear on the concave surface of the module. These groups would then be directed *into* the cavity of the assembled capsule. Such endohedral functionality is expected to alter the size, shape, and polarity of the cavity and may even equip it with hydrogen bond donors and acceptors. Introducing functional groups on concave surfaces remains a difficult challenge in supramolecular chemistry.[13] However, we will show how this modular approach offers promise for selective binding sites beyond those based on size and shape selection alone. As a future prospect, this approach will also allow the endohedral attachment of chiral groups, thus providing the basis for efficient chiral guest recognition.

Experimental Section

General Methods. The starting materials for the syntheses of the "flexiballs" were used as purchased. Compounds **12**,[14] **29**−**32**,[15] **39**,[16] and **41**[17] were prepared according to literature procedures. Solvents for synthesis were dried by passing them through columns of activated Al_2O_3. ^1H and ^{13}C NMR spectra were recorded on Bruker DRX-600 (600 and 151 MHz, respectively), Bruker DRX 500 (500 and 126 MHz, respectively), and AMX-300 (300 MHz) instruments with the solvent signals as internal standard. FAB positive ion mass spectra were obtained from NBA matrix on a VG ZAB-VSE double-focusing high-resolution mass spectrometer equipped with a cesium ion gun. For high resolution, CsI was added as a standard. MALDI mass spectra were performed on a PerSeptive Biosystems Voyager-STR mass spectrometer with DHB matrix. The purity of the target compounds was checked by HPLC, and it turned out that no further purification beyond the column chromatography and recrystallization steps below was necessary.

ESI-MS Experiments. The ESI-MS experiments were performed as reported according to our previously established protocol[18] on a single quadrupole Perkin-Elmer API-100 Sciex (mass range <3000 amu, "flexiballs" with dicationic guests) and a Finnigan MAT LCQ ion trap instrument (mass range <4000 amu, "flexiballs" with monocationic guests). Briefly, the samples were introduced as 50 μM solutions of the capsule monomers with 1.5 equiv of the guest salt in $CHCl_3$ (singly charged guest cations) or acetone (doubly charged guests) at flow rates of 4−6 μL/min. The ion intensities increased with the ion spray and the orifice potentials, which were set to 4−5 kV and 100−200 V, respectively. To improve the signal-to-noise ratio, 50−100 scans were accumulated. For guest competition experiments, 50 μM solutions of capsule in $CHCl_3$ with 2 equiv of each guest salt were prepared. These experiments were performed with the API-100 instrument (ion spray and orifice potentials set to 5000 and 150 V, respectively), and at least 100 scans were averaged. Additional ESI-mass spectra analyses of 25 μM solutions of the samples in acetone were performed with a Finnigan MAT 900 ST instrument (Finnigan MAT, Bremen, Germany) equipped with an EBT geometry and an ESI II ion source (Finnigan MAT, Bremen, Germany); spray voltage 3.6−3.7 kV, capillary temperature 120 °C; 200 scans/spectra were accumulated.

Glycoluril 5b. 4,4′-Di(*tert*-butylphenyl)benzil[19] (16.12 g, 50.0 mmol) was dissolved in benzene (200 mL), *N*-(4-methoxybenzyl)urea (21.63 g, 120.0 mmol) was added, followed by TFA (10 mL), and the resulting mixture was refluxed with azeotropic removal of water in a Dean−Stark apparatus for 24 h. The reaction mixture was evaporated in vacuo, and the resulting crystalline residue was purified by two recrystallizations from EtOH to give the product as a white crystalline solid. Yield 20.60 g (64%). ^1H NMR (DMSO-d_6, 600 MHz): δ (ppm) 8.23 (s, 2H), 7.15 (d, J = 8.6 Hz, 4H), 7.02 (d, J = 8.5 Hz, 2H), 6.92 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.6 Hz, 4H), 6.56 (d, J = 8.5 Hz, 2H), 4.33 (d, J = 16.5 Hz, 2H), 3.81 (d, J = 16.5 Hz, 2H), 3.72 (s, 6H), 1.12 (s, 9H), 1.09 (s, 9H). ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 161.14, 158.68, 151.96, 151.45, 135.07, 131.83, 131.08, 128.68, 128.38, 127.49, 125.29, 124.78, 114.28, 91.21, 80.16, 55.79, 45.04, 34.84, 31.78, 31.70. MS (FAB) calculated for $[M + H]^+$ $C_{40}H_{47}N_4O_4^+$, 647.3597; found, 647.3621.

Glycoluril 5c. 4,4′-Di(decanoxy)benzil (7.50 g, 14.3 mmol) was dissolved in benzene (70 mL), *N*-(4-methoxybenzyl)urea (5.2 g, 28.7 mmol) was added, followed by TFA (2.8 mL), and the resulting mixture was refluxed with azeotropic removal of water in a Dean−Stark apparatus for 24 h. After the mixture cooled to room temperature, saturated sodium bicarbonate solution was added, phases were separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over $MgSO_4$, filtrated, and concentrated. Column chromatography using 50−100% ethyl acetate/hexanes gave the product as a white foam. Yield 7.4 g (61%). ^1H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.09−6.47 (m, 16H), 5.90 (s, 2H), 4.35 (d, J = 16.3 Hz, 2H), 3.87 (d, J = 16.3 Hz, 2H), 3.79 (m, 4H), 3.75 (s, 6H), 1.68 (m, 4H), 1.37 (m, 4H), 1.26 (m, 28H), 0.87 (m, 6H). MS (FAB) calculated for $[M + Cs]^+$ $C_{52}H_{70}N_4O_6Cs^+$, 979.3450; found, 979.3488.

PMB (4-Methoxybenzyl)-Protected Hydroxy Module (PMB)$_2$7a. A mixture of glycoluril **5a (**5.57 g, 7.6 mmol) and $CsCO_3$ (5.45 g, 16.7 mmol) in 100 mL of acetonitrile was heated at reflux for 30 min. Epichlorohydrin (0.65 mL, 8.36 mmol) was then added and the mixture heated at reflux for 22 h. After cooling, the reaction mixture was quenched with water and partitioned between water and dichloromethane. The layers were separated, and the aqueous phase was extracted with two additional portions of dichloromethane. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated to provide the crude product. Column chromatography (50% EtOAc/hexanes) provided 1.58 g (26%) of the axial hydroxy module and 1.88 g (31%) of the equatorial hydroxy module.

PMB-Protected Equatorial Hydroxy Module (PMB)$_2$7a$_{eq}$. ^1H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.10−6.66 (m, 16H), 4.33 (m, 4H), 4.00 (m, 1H), 3.91 (d, J = 16.5 Hz, 2H), 3.78 (s, 6H), 2.77 (dd, J = 13.6, 10.8 Hz, 2H), 2.47 (d, 1H), 2.40 (m, 4H), 1.43 (m, 4H), 1.26 (m, 16H), 0.90 (t, J = 7.0 Hz, 3H), 0.89 (t, J = 7.1 Hz, 3H). ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 159.08, 158.22, 130.43, 130.15, 129.71, 129.13, 128.64, 128.43, 128.20, 128.03, 127.59, 127.25, 127.03, 113.74, 87.49, 80.22, 60.97, 55.01, 44.66, 44.51, 34.58, 34.43, 31.27, 31.23, 30.76, 30.65, 28.52, 28.49, 28.44, 28.37, 22.12, 22.08, 13.89, 13.86. MS (ESI): positive 788 $[M + H]^+$, 810 $[M + Na]^+$; negative 786 $[M - H]^-$.

PMB-Protected Axial Hydroxy Module (PMB)$_2$7a$_{ax}$. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.18−6.82 (m, 16H), 4.70 (d, 1H), 4.68 (d, J = 16.2 Hz, 2H), 4.13−4.02 (m, 2H), 3.95 (d, J = 16.2 Hz, 2H),

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3.90 (m, 1 H), 3.78 (s, 6H), 3.73 (m, 1 H), 3.40 (dd, J = 11.8, 8.2 Hz, 1H), 2.39 (t, J = 7.5 Hz, 4H), 1.41 (m, 4H), 1.24 (m, 16H), 0.88 (m, 6H). MS (ESI): positive 788 $[M + H]^+$, 810 $[M + Na]^+$.

Hydroxy Module 7a. A solution of the PMB-protected equatorial hydroxy module **(PMB)$_2$7a$_{eq}$** (1.47 g, 1.9 mmol) in $CH_3CN/THF/H_2O$ (4:2:1) was treated with CAN (ammonium cerium(IV) nitrate) (8.19 g, 14.9 mmol) and stirred at room temperature. When the starting material was consumed, the reaction mixture was concentrated and partitioned between water and dichloromethane. The layers were separated, and the aqueous phase was extracted with two additional portions of dichloromethane. The combined organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated to provide the crude product. Trituration with diethyl ether provided 0.56 g (55%) of hydroxy module **7a**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.83 (s, 1H), 7.19−6.83 (m, 8H), 4.03 (m, J = 12.2, >1 Hz, 2H), 3.84 (m, 1H), 2.57 (dd, J = 12.2, 11.3 Hz, 2H), 2.40 (m, 4H), 1.65 (bs, 2H), 1.41 (m, 4H), 1.23 (m, 16H), 0.88 (m, 6H). MS (ESI): positive 547 $[M + H]^+$; negative 545 $[M - H]^-$.

PMB-Protected Alkene 8a. A mixture of glycoluril **5a** (2.50 g, 3.4 mmol), Cs_2CO_3 (2.23 g, 6.8 mmol), and 45 mL CH_3CN was stirred at reflux for 30 min to give a uniform suspension. After the mixture cooled somewhat, methallyl dichloride (593 μL, 5.1 mmol) was added dropwise over 1 min with stirring. The mixture was returned to reflux, and after 18 h, TLC indicated complete consumption of **5**. After cooling to room temperature, the reaction mixture was poured into 200 mL of 1 M HCl, followed by extraction with 2 × 100 mL of diethyl ether. The organic layer was then washed with 2 × 100 mL H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a slightly crude, white foam. Yield 2.42 g (91%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.07 (d, J = 8.4 Hz, 4H), 6.93 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.3 Hz, 2H), 6.81 (m, 4H), 6.75 (d, J = 8.3 Hz, 2H), 6.68 (d, J = 8.4 Hz, 2H), 5.12 (s, 2H), 4.58 (d, J = 15.1 Hz, 2H), 4.34 (d, J = 16.2 Hz, 2H), 3.89 (d, J = 16.2 Hz, 2H), 3.76 (s, 6H), 3.58 (d, J = 15.1 Hz, 2H), 2.41 (m, 4H), 1.42 (m, 4H), 1.25 (m, 16H), 0.88 (m, 6H).

Alkene 9a. Alkene **8a** (2.42 g, 3.1 mmol) was dissolved in 30 mL of a mixture of $CH_3CN/H_2O/THF$ (7:1.5:1). To this clear, colorless solution was added CAN (7.45 g, 13.6 mmol), and the yellow solution was allowed to stir overnight at room temperature. Upon pouring the reaction mixture into 200 mL of H_2O, a precipitate formed which was collected by filtration. This precipitate was taken up in 100 mL of CH_2Cl_2, washed with 2 × 75 mL of 1 N KOH, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting residue was taken up in 75 mL of refluxing ether, and a clear solution was obtained upon the addition of a few drops of MeOH. A slightly crude, white precipitate was collected after cooling by filtration. Yield 0.93 g (56%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.09 (d, J = 8.1 Hz, 2H), 7.00 (d, J = 8.1 Hz, 2H), 6.90 (d, J = 8.0 Hz, 2H), 6.87 (d, J = 8.0 Hz, 2H), 5.70 (s, 2H), 5.00 (s, 2H), 4.48 (d, J = 14.7 Hz, 2H), 3.51 (d, J = 14.7 Hz, 2H,), 2.41 (m, 4H), 1.42 (m, 4H), 1.24 (m, 16H), 0.88 (m, 6H).

Benzyl 2-(Bromomethyl)acrylate 14. Under anhydrous conditions, alcohol **22** (8.01 g, 41.7 mmol) was dissolved in ether (50 mL) and cooled in an ice/salt bath to −5 °C. PBr_3 (1.98 mL, 20.8 mmol) was dissolved in ether (7 mL) and added dropwise over 5 min to the chilled reaction mixture. After complete addition, the mixture was allowed to stir at room temperature for 6 h. The reaction flask was cooled to 0 °C, and H_2O (50 mL) was added slowly with stirring. The mixture was then diluted with hexane (150 mL) and washed with H_2O (2 × 100 mL). Drying with Na_2SO_4, filtration, and rotary evaporation provided an oil which was further purified by flash chromatography (0−5% MeOH/CH_2Cl_2) to give a clear oil. Yield 9.60 g (90%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.37 (m, 5H), 6.37 (bs, 1H), 5.95 (bs, 1H), 5.24 (s, 2H), 4.18 (bs, 2H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 166.35, 139.59, 135.84, 128.78, 128.50, 128.28, 126.15, 66.61, 62.11. IR ($CDCl_3$): 3057.26, 3033.70, 2955.13, 1724.01, 1328.23, 1306.12, 1221.23, 1174.15, 1115.41 cm^{-1}. HRMS (FAB) calcd for $[M + Na]^+$ $C_{11}H_{11}O_2Br{\cdot}Na^+$, 276.9840; found, 276.9831.

PMB-Protected Acid Module 15a via Hydrogenation. Ester **17a** (13.58 g, 15.0 mmol) and 5% palladium on carbon (1.36 g) were mixed in EtOH (300 mL). The mixture was efficiently stirred under H_2 (1 atm) for 3 h using a standard balloon setup. Catalyst was removed by filtration through a pad of Celite and the filtrate evaporated to give a white foam. Yield 11.62 g (95%).

PMB-Protected Acid Module 15a via Saponification. Ester **16a** (5.55 g, 6.7 mmol) and LiI·3H_2O (2.52 g, 13.4 mmol) were dissolved in 150 mL of 2,6-lutidine (Aldrich Sure-Seal) under N_2 atmosphere. After 16 h, TLC (50 EtOAc/hexanes) indicated complete disappearance of **16a**. The mixture was cooled to room temperature, poured into 400 mL of EtOAc, and washed with 4 × 200 mL of 1 M HCl. The organic layer was dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was further purified by flash chromatography (10% MeOH/CH_2Cl_2) to give an off-white foam. Yield 4.66 g (85%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 12.92 (s, 1H), 7.10 (d, J = 8.6 Hz, 4H), 6.91 (d, J = 8.2 Hz, 2H), 6.86 (d, J = 8.6 Hz, 4H), 6.83 (d, J = 8.2 Hz, 2H), 6.76 (d, J = 8.2 Hz, 2H), 6.10 (d, J = 8.2 Hz, 2H), 4.33 (d, J = 16.6 Hz, 2H), 4.21 (dd, J = 14.0, 4.5 Hz, 2H), 3.81 (d, J = 16.6 Hz, 2H), 3.72 (s, 6H), 2.87 (t, J = 13.0 Hz, 2H), 2.61 (m, 1H), 2.38 (t, J = 7.6 Hz, 2H), 2.35 (t, J = 7.6 Hz, 2H), 1.37 (m, 4H), 1.17 (m, 16H), 0.85 (m, 6H). ^{13}C (DMSO-d_6, 151 MHz): δ (ppm) 171.86, 158.70, 158.23, 143.42, 143.14, 130.32, 129.97, 129.55, 128.49, 128.20, 128.06, 127.98, 127.23, 113.78, 87.54, 55.02, 44.55, 37.55, 34.61, 34.44, 31.29, 31.24, 30.77, 30.67, 28.55, 28.50, 28.47, 28.38, 22.14, 22.09, 13.92, 13.88. IR ($CDCl_3$): 3435.49, 2926.78, 2854.85, 1722.68, 1715.10, 1612.84, 1513.41, 1465.89, 1246.94, 1177.69, 889.34 cm^{-1}. HRMS (FAB) calcd for $[M + Cs^+]$ $C_{50}H_{62}N_4O_6{\cdot}Cs^+$, 947.3724; found, 947.3691.

PMB-Protected Methyl Ester 16a. Glycoluril **5a** (12.57 g, 17.2 mmol) and Cs_2CO_3 (23.53 g, 72.2 mmol) were mixed in 250 mL of CH_3CN with vigorous stirring and mild heating to give a fine, white suspension. Methyl 2-(bromomethyl)acrylate **13** (2.95 mL, 20.6 mmol) was added slowly with stirring over 2 min, and then the mixture was refluxed overnight. After cooling, the mixture was poured into 300 mL of 1 M HCl, and extraction was accomplished with 2 × 300 mL of ether. The combined organics were washed with 2 × 200 mL of 1 M HCl and 200 mL of brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (30−70% EtOAc in hexanes) separated the equatorial ester isomer **16a$_{eq}$** (1st spot by TLC) from the axial isomer **16a$_{ax}$** (2nd spot). Eluent concentration gave white foams. Combined yield 10.59 g (72%).

PMB-Protected Equatorial Ester 16a$_{eq}$. Yield 8.14 g (57%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.08 (d, J = 8.6 Hz, 4H), 6.82 (m, 8H), 6.66 (d, J = 8.3 Hz, 2H), 6.62 (d, J = 8.3 Hz, 2H), 4.45 (dd, J = 14.3, 4.6 Hz, 2H), 4.35 (d, J = 16.2 Hz, 2H), 3.91 (d, J = 16.2 Hz, 2H), 3.78 (s, 6H), 3.68 (s, 3H), 3.07 (m, 2H), 2.87 (m, 1H), 2.40 (m, 4H), 1.41 (m, 4H), 1.24 (m, 16H), 0.88 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 171.37, 159.65, 158.96, 144.38, 144.10, 130.21, 130.12, 129.48, 129.01, 128.94, 128.73, 128.42, 128.35, 127.68, 114.21, 114.06, 88.63, 80.97, 55.39, 52.24, 45.90, 40.34, 38.24, 35.52, 35.38, 31.95, 31.92, 31.47, 31.37, 29.25, 29.13, 22.79, 14.19. HRMS (FAB) calcd for $[M + Cs]^+$ $C_{51}H_{64}N_4O_6{\cdot}Cs^+$, 961.3880; found, 961.3915.

PMB-Protected Axial Ester 16a$_{ax}$. Yield 2.08 g (15%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.19 (d, J = 8.6 Hz, 4H), 6.83 (m, 8H), 6.71 (d, J = 8.3 Hz, 2H), 6.65 (d, J = 8.3 Hz, 2H), 4.59 (d, J = 14.5 Hz, 2H), 4.28 (d, J = 16.3 Hz, 2H), 4.00 (d, J = 16.3 Hz, 2H), 3.79 (s, 3H), 3.78 (s, 6H), 3.19 (dd, 2H, J = 14.5, 4.3 Hz), 2.37 (m, 5H), 1.39 (m, 4H), 1.25 (m, 16H), 0.88 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 172.94, 159.84, 158.86, 144.06, 143.84, 130.78, 130.56, 130.18, 129.32, 128.97, 128.60, 128.21, 127.86, 113.97, 88.82, 80.66, 55.39, 52.56, 46.29, 39.44, 36.13, 35.48, 35.36, 31.95, 31.91, 31.48, 31.38, 29.26, 29.23, 29.15, 22.80, 22.78, 14.20, 14.18. HRMS (FAB) calcd for $[M + Cs]^+$ $C_{51}H_{64}N_4O_6{\cdot}Cs^+$, 961.3880; found, 961.3861.

PMB-Protected Module 16b. Glycoluril **5b** (10.08 g, 15.6 mmol) and Cs_2CO_3 (21.53 g, 66.1 mmol) were suspended in MeCN (175 mL) and heated to 50 °C with vigorous stirring. 3-Bromo-2-(bromomethyl)-propanoic acid methyl ester (3.0 mL) was added, and the reaction was refluxed for 20 h. The resulting reaction mixture was cooled to rt, poured into 1 M HCl (300 mL), and extracted with Et_2O (2 × 300 mL). The combined organic extracts were washed with 1 M HCl (300 mL) and brine (2 × 300 mL), dried (Na_2SO_4), and evaporated. The resulting colorless oil was subjected to flash column chromatography (SiO_2, eluent EtOAc−hexane 1:3) to give the product as a white solid after evaporation of the solvent. Yield 6.38 g (55%). Only the equatorial

isomer was isolated and characterized from this reaction; the axial isomer was present in trace amounts. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.09 (d, J = 8.5 Hz, 4H), 7.01 (d, J = 8.6 Hz, 2H), 6.91 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.5 Hz, 2H), 6.82 (d, J = 8.7 Hz, 4H), 6.68 (d, J = 8.5 Hz, 2H), 4.45 (dd, J = 14.2 Hz, 4.4 Hz, 2H), 4.37 (d, J = 16.3 Hz, 2H), 3.91 (d, J = 16.3 Hz, 2H), 4.78 (s, 6H), 3.67 (s, 3H), 3.08 (m, 2H), 2.86 (m, 1H), 1.16 (s, 9H), 1.14 (s, 9H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 170.99, 159.27, 158.55, 152.27, 151.87, 129.84, 129.55, 128.95, 128.62, 127.97, 127.10, 125.15, 124.90, 113.90, 88.32, 80.67, 55.19, 52.04, 45.71, 40.16, 38.08, 34.34, 31.10. HRMS (FAB) calcd for $[M + Na]^+$ $C_{45}H_{52}N_4O_6{\cdot}Na^+$, 767.3784; found, 767.3755.

PMB-Protected Module 16c. Glycoluril **5c** (2.00 g, 2.36 mmol) and Cs_2CO_3 (1.69 g, 5.19 mmol) were suspended in MeCN (30 mL). After the reaction was refluxed for 30 min, 3-bromo-2-(bromomethyl)-propanoic acid methyl ester (0.37 mL, 2.60 mmol) was added, and the reaction was refluxed for 20 h. The resulting reaction mixture was cooled to rt, poured into 100 mL of water, and partioned between water and dichloromethane (100 mL). After separation of the phases, the aqueous layer was further extracted with dichloromethane (2 × 100 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. Flash column chromatography (SiO_2, eluent EtOAc−hexane 1:3) gave the desired diastereomeric products in a combined yield of 630 mg (28%).

PMB-Protected Equatorial Ester 16c$_{eq}$. Yield 380 mg (17%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.08−6.46 (m, 16H), 4.43 (dd, J = 13.8 Hz, 4.4 Hz, 2H), 4.32 (d, J = 16.2 Hz, 2H), 3.88 (d, J = 16.2 Hz, 2H), 3.80 (m, 4H), 1.64 (m, 4H), 3.75 (s, 6H), 3.68 (s, 3H), 3.04 (dd, J = 13.8 Hz, 11.7 Hz, 2H), 2.84 (m, 1H), 1.26 (m, 28H), 0.87 (t, J = 6.4 Hz, 6H). HRMS (FAB) calcd for $[M + Cs]^+$ $C_{57}H_{76}N_4O_8{\cdot}Cs^+$, 1077.4717; found, 1077.4676

PMB-Protected Equatorial Ester 16c$_{ax}$. Yield 250 mg (11%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.20−6.27 (m, 16H), 4.57 (d, J = 14.2 Hz, 2H), 4.25 (d, J = 16.2 Hz, 2H), 3.97 (d, J = 16.2 Hz, 2H), 3.79 (s, 6H), 3.78 (s, 3H), 3.75 (m, 1H), 3.75 (m, 4H), 3.16 (dd, J = 14.2 Hz, 4.1 Hz, 2H), 1.67 (m, 4H), 1.26 (m, 28H), 0.87 (t, J = 6.4 Hz, 6H). HRMS (FAB) calcd for $[M + Cs]^+$ $C_{57}H_{76}N_4O_8{\cdot}Cs^+$, 1077.4717; found, 1077.4674.

PMB-Protected Benzyl Ester 17a. Glycoluril **5a** (29.24 g, 40.0 mmol), Cs_2CO_3 (19.55 g, 60.0 mmol) and benzyl acrylate **14** (4.71 g, 18.5 mmol) were mixed in CH_3CN (400 mL), and the resulting reaction mixture was heated at reflux for 5 h. After cooling to room temperature, the mixture was partitioned between CH_2Cl_2 (800 mL) and 1 M HCl (400 mL). Drying over Na_2SO_4, filtration, and evaporation of the solvent gave an off-white foam. This residue was subjected to flash chromatography (25−50% EtOAc/hexanes) to separate the equatorial ester isomer **10c** (faster eluting isomer) from the axial isomer (slower eluting isomer). Each was isolated as a white foam. Combined yield 31.08 g (86%).

PMB-Protected Equatorial Benzyl Ester 17a$_{eq}$. Yield 22.12 g (61%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.35 (m, 5H), 7.08 (m, 4H), 6.82 (m, 8H), 6.73 (m, 2H), 6.65 (m, 2H), 5.10 (bs, 2H), 4.87 (dd, J = 14.2, 4.7 Hz, 2H), 4.34 (d, J = 16.3 Hz, 2H), 3.90 (d, J = 16.3 Hz, 2H), 3.77 (s, 6H), 3.08 (m, 2H), 2.89 (m, 1H), 2.38 (m, 4H), 1.41 (m, 4H), 1.24 (m, 16H), 0.88 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 170.75, 159.65, 158.96, 144.38, 144.10, 135.52, 130.21, 130.08, 129.47, 128.96, 128.81, 128.75, 128.67, 128.42, 128.35, 127.67, 114.21, 88.65, 80.97, 67.02, 55.39, 45.90, 40.31, 38.19, 35.52, 35.38, 31.95, 31.92, 31.47, 31.37, 29.26, 29.25, 29.13, 22.80, 14.20. IR ($CDCl_3$): 2927.16, 2854.96, 1722.64, 1710.81, 1612.81, 1513.36, 1460.06, 1247.14, 1175.94 cm^{-1}. MS (FAB) calcd for $[M + Cs]^+$ $C_{57}H_{68}N_4O_6{\cdot}Cs^+$, 1037; found, 1037.

PMB-Protected Axial Benzyl Ester 17a$_{ax}$. Yield 8.96 g (25%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.50 (m, 2H), 7.36 (m, 3H), 7.22 (d, J = 8.6 Hz, 4H), 6.85 (m, 8H), 6.72 (d, J = 8.3 Hz, 2H), 6.67 (d, J = 8.3 Hz, 2H), 5.20 (s, 2H), 4.64 (d, J = 14.3 Hz, 2H), 4.31 (d, J = 16.3 Hz, 2H), 4.02 (d, J = 16.3 Hz, 2H), 3.77 (s, 6H), 3.19 (dd, J = 14.3, 4.3 Hz, 2H), 2.39 (m, 5H), 1.40 (m, 4H), 1.25 (m, 16H), 0.88 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 172.32, 159.87, 158.84, 144.05, 143.83, 136.01, 130.72, 130.54, 129.27, 129.25, 128.93, 128.82, 128.61, 128.59, 128.20, 127.81, 113.96, 88.82, 80.66, 67.54, 55.36, 46.25, 39.45, 36.21, 35.45, 35.34, 31.92, 31.88, 31.44, 31.36, 29.23, 29.20, 29.13, 22.77, 22.75, 14.17, 14.15. IR ($CDCl_3$): 2927.04, 2855.03, 1735.84, 1710.15, 1612.81, 1513.21, 1458.05, 1417.67, 1284.28, 1246.83, 1175.69 cm^{-1}. HRMS (FAB) calcd for $[M + Cs^+]$ $C_{57}H_{68}N_4O_6{\cdot}Cs^+$, 1037.4193; found, 1037.4248.

Methyl Ester 18a. Glycoluril **16a** (8.14 g, 9.8 mmol) was dissolved in 230 mL of CH_3CN. While heating gently, water (45 mL) was added, and the solution was allowed to cool to room temperature slowly. CAN (23.68 g, 43.2 mmol) was added to the slightly cloudy solution, and this mixture was allowed to stir overnight. When TLC indicated complete disappearance of **7a**, the flask's contents were poured into 600 mL of EtOAc. This phase was washed with 1 M HCl (3 × 100 mL), 0.5 M KOH (2 × 100 mL), saturated $NaHCO_3$ solution (100 mL), and brine (2 × 100 mL). The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved in a minimum amount of hot ether, and after refrigeration overnight, a white precipitate was collected by filtration. Yield 1.33 g (23%); mp 160 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.09 (d, J = 8.3 Hz, 2H), 6.95 (d, J = 8.3 Hz, 2H), 6.88 (m, 4H), 5.91 (s, 2H), 4.35 (dd, J = 14.4, 4.6 Hz, 2H), 3.64 (s, 3H), 2.98 (m, 2H), 2.72 (m, 1H), 2.41 (m, 4H), 1.42 (m, 4H), 1.24 (m, 16H), 0.88 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 171.20, 159.20, 144.36, 144.27, 133.37, 129.94, 128.85, 128.46, 127.68, 127.40, 83.52, 78.67, 52.22, 39.89, 37.85, 35.47, 35.39, 31.94, 31.40, 29.24, 29.22, 29.16, 29.12, 22.81, 22.79, 14.20. IR ($CDCl_3$): 3255.88, 2951.54, 2925.14, 2854.42, 1736.43, 1691.82, 1465.60, 1432.07 cm^{-1}. HRMS (FAB) calcd for $[M + H]^+$ $C_{35}H_{48}N_4O_4$, 589.3754; found, 589.3738.

Module 18b. PMB-protected glycoluril **16b** (6.32 g, 8.5 mmol) was dissolved in MeCN (200 mL) and heated to 50 °C, whereupon H_2O (40 mL) was added. The resulting clear solution was allowed to cool to room temperature with stirring, causing the solution to become turbid. Ceric ammonium nitrate (20.55 g, 37.5 mmol) was added in one portion, and the reaction was stirred at room temperature for 24 h, after which it was poured into EtOAc (600 mL). The resulting solution was washed with 1 M HCl (3 × 300 mL), $NaHCO_3$ (2 × 300 mL), and brine (2 × 300 mL). The organic phase was dried (Na_2SO_4) and evaporated, and the resulting oil was subjected to flash column chromatography (2.5%−7.5% MeOH in CH_2Cl_2). Evaporation of the solvent gave the product as a white powder, yield 2.59 g (61%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.06 (m, 6H), 6.94 (dd, J = 7.0 Hz, 1.9 Hz, 2H), 6.40 (s, 2H), 4.33 (dd, J = 14.5 Hz, 4.6 Hz, 2H), 3.62 (s, 3H), 2.98 (m, 2H), 2.71 (m, 1H), 1.14 (s, 9H), 1.13 (s, 9H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 171.26, 159.59, 152.45, 133.32, 129.88, 127.56, 127.35, 125.63, 125.23, 83.62, 78.94, 52.41, 40.12, 38.10, 34.74, 31.49. HRMS (FAB) calcd for $[M + Na]^+$ $C_{29}H_{36}N_4O_4{\cdot}Na^+$, 527.2634; found, 527.2634.

Module 18c. PMB-protected glycoluril **16c$_{eq}$** (380 mg, 0.40 mmol) was dissolved in acetonitrile/THF/water (4/2/1, 15 mL), and cerium ammonium nitrate (1.76 g, 3.22 mmol) was added. The reaction was stirred at room temperature for 24 h, after which it was concentrated and partioned between water and dichloromethane. The aqueous layer was further extracted with dichloromethane. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. Trituration of residue with diethyl ether gave the desired equatorial methyl ester. Yield 125 mg (44%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.12−6.59 (m, 8H), 5.51 (s, 2H), 4.35 (dd, J = 14.6 Hz, 4.1 Hz, 2H), 3.81 (m. 2H), 3.64 (s, 3H), 2.97 (dd, J = 14.6 Hz, 11.6 Hz, 2H), 2.72 (m, 1H), 1.69 (m, 4H), 1.26 (m, 28 H, 0.87 (t, J = 7.2 Hz, 6H). HRMS (FAB) calcd for $[M + H]^+$ $C_{41}H_{61}N_4O_4{\cdot}Na^+$, 705.4591; found, 705.4622.

Benzyl Ester 19a. In a 2-L Erlenmeyer flask, glycoluril **17a** (18.10 g, 20.0 mmol) was dissolved in CH_3CN (670 mL). H_2O (130 mL) was added which caused turbidity. After the reaction mixture had reached room temperature, CAN (43.86 g, 80.0 mmol) was added and the resulting reaction mixture stirred overnight, ~16 h. The reaction mixture was diluted with H_2O (800 mL) and extracted with CH_2Cl_2 (800 mL). Drying of the organic layer over Na_2SO_4, filtration, and evaporation of the solvent afforded a yellow oil. Silica gel flash chromatography (50% EtOAc/hexanes) afforded the target molecule as a white foam. Yield 8.03 g (60%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.28 (m, 5H), 7.06 (d, J = 8.1 Hz, 2H), 6.91 (d, J = 8.1 Hz, 2H), 6.86 (m, 4H),

6.47 (s, 2H), 5.05 (bs, 2H), 4.32 (dd, J = 14.2, 4.5 Hz, 2H), 2.93 (m, 2H), 2.71 (m, 1H), 2.39 (m, 4H), 1.26 (m, 16H), 0.89 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 170.62, 159.50, 144.14, 144.12, 135.47, 133.46, 130.01, 128.89, 128.80, 128.76, 128.72, 128.57, 128.32, 127.67, 127.44, 83.42, 78.84, 66.93, 39.79, 37.80, 35.46, 35.38, 31.93, 31.38, 31.36, 29.21, 29.16, 29.14, 22.78, 14.19. IR ($CDCl_3$): 3258.19, 2926.24, 2854.85, 1734.66, 1696.24, 1465.30, 1381.65, 1161.71 cm^{-1}. HRMS (FAB): calcd for $[M + H]^+$ $C_{41}H_{53}N_4O_4^+$, 665.4067; found, 665.4041.

Acid Module 20a via Demethylation. Ester **18a** (1.30 g, 2.2 mmol) and LiI·3H_2O (0.88 g, 4.4 mmol) were dissolved in 75 mL of 2,6-lutidine (Aldrich Sure-Seal) under N_2 atmosphere. The yellow solution was heated at reflux in the dark. After 16 h, TLC (5% MeOH/CH_2Cl_2) indicated complete disappearance of **18a**. The mixture was cooled to room temperature, poured into 400 mL of EtOAc, and washed with 4 × 200 mL of 1 M HCl. The organic layer was dried with Na_2SO_4, filtered, and concentrated in vacuo to give an off-white foam. Yield 1.21 g (95%).

Acid Module 20c via Demethylation. Ester **18c** (120 mg, 0.17 mmol) and LiI·3H_2O (64 mg, 0.34 mmol) were mixed together, and 10 mL of 2,6-lutidine (Aldrich Sure-Seal) was added under N_2 atmosphere. The solids dissolved upon heating to a gentle reflux which was maintained for 15 h. The solution was cooled to room temperature and poured into 20 mL of 1 N hydrochloric acid. The solution was extracted with ethyl acetate three times, and the combined ethyl acetate solutions were washed several times with 20 mL portions of 1 M hydrochloric acid, dried over $MgSO_4$, decolorized with charcoal, and concentrated to give the desired acid. Yield 112 mg (95%).

Acid Module 20a via Hydrogenolysis. Ester **19a** (7.98 g, 12.0 mmol) and 10% palladium on carbon (0.80 g) were mixed in EtOH (240 mL). The mixture was efficiently stirred under H_2 (1 atm) for 3 h using a standard balloon setup. Catalyst was removed by filtration through a pad of Celite and the filtrate evaporated to give a white powdery solid. Yield 6.73 g (98%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 12.79 (s, 1H), 8.37 (s, 2H), 6.98 (d, J = 8.3 Hz, 2H), 6.92 (d, J = 8.3 Hz, 2H), 6.86 (m, 4H), 4.10 (dd, J = 14.2, 4.6 Hz, 2H), 2.71 (t, J = 13.0 Hz, 2H), 2.36 (m, 5H), 1.37 (m, 4H), 1.20 (m, 16H), 0.84 (m, 6H). ^{13}C (DMSO-d_6, 151 MHz): δ (ppm) 172.30, 158.83, 142.61, 142.35, 134.57, 131.15, 128.30, 127.47, 127.44, 127.33, 81.70, 78.25, 37.37, 34.58, 34.51, 31.30, 31.27, 30.88, 30.73, 28.53, 28.40, 28.35, 22.13, 13.91. HRMS (FAB) calcd for [(M − H^+ + 2Cs^+]: $C_{34}H_{45}N_4O_4$·Cs_2^+, 839.1549; found, 839.1520.

Module 20b. Acid module ester **18b** (1.305 g, 2.6 mmol) and LiI·3H_2O (1.22 g, 6.1 mmol) were dissolved in dry 2,6-lutidine (65 mL) and heated at reflux for 30 h in the dark. After cooling to rt, the reaction mixture was partitioned between EtOAc (400 mL) and 1 M HCl (400 mL). The aqueous phase was made acidic to pH = 1 with concentrated HCl and extracted with EtOAc (200 mL). The combined organic extracts were washed with 1 M HCl (3 × 200 mL) and dried (Na_2SO_4). Evaporation of the solvent gave the product as a yellowish microcrystalline solid. Yield 1.27 g (>99%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 12.76 (br s, 1H), 8.37 (s, 2H), 7.10 (d, J = 8.5 Hz, 2H), 7.04 (d, J = 8.5 Hz, 2H), 6.96 (d, J = 8.5 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H), 4.11 (dd, J = 14.2 Hz, 4.6 Hz, 2H), 2.75 (m, 2H), 2.35 (m, 1H), 1.11 (s, 9H), 1.09 (s, 9H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 172.81, 159.42, 151.61, 151.37, 134.90, 131.61, 127.91, 127.76, 125.68, 124.77, 82.39, 78.94, 38.26, 34.85, 31.73, 23.24. HRMS (FAB) calcd for $[M + Na]^+$ $C_{29}H_{36}N_4O_4$·Na^+, 527.2634; found, 527.2634.

Benzyl *O,O*-Diethylphosphonoacetate 21.[20] Benzyl 2-bromoacetate (23.76 mL, 150 mmol) and triethyl phosphite (28.29 mL, 165 mmol) were mixed and heated gradually to distill off ethylbromide (bp ≈ 42 °C). After the distillation was complete, the mixture was heated to 200 °C for 1 h and then allowed to cool to room temperature. Fractional vacuum distillation provided the crude product (bp = 130 °C, 0.1 mm Hg). Further purification was accomplished through a second distillation to give the desired product as a clear oil. Yield 37.35 g (87%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.37 (m, 5H), 5.18 (s, 2H), 4.13 (m, 4H), 3.03 (s, 1H), 3.00 (s, 1H), 1.30 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 166.04 (d, $J_{C,P}$ = 6.3 Hz), 135.628, 128.85, 128.71, 67.46, 62.90 (d, $J_{C,P}$ = 6.2 Hz), 34.93, 34.04, 16.36 (d, $J_{C,P}$ = 6.3 Hz). ^{31}P NMR ($CDCl_3$, 225 MHz): δ (ppm) 20.69 (m). HRMS (FAB) calcd for $[M + H]^+$ $C_{13}H_{20}O_5P^+$, 287.1048; found, 287.1041.

Benzyl 2-(Hydroxymethyl)acrylate 22. Phosphonate **21** (37.35 g, 130.5 mmol) and formaldehyde [37% in H_2O] (48.90 mL, 652.4 mmol) were mixed with efficient stirring and cooled in an ice bath. A solution of K_2CO_3 (36.07 g, 261.0 mmol) in H_2O (45 mL) was added dropwise over 45 min and, after complete addition, the mixture allowed to stir at room temperature for 2 h. The mixture was poured into ether (300 mL) and washed with H_2O (3 × 200 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography of the residue (25% EtOAc/hexanes) gave a green oil which was further purified by vacuum distillation to give a clear oil (bp = 113 °C, 0.1 mmHg). Yield 8.01 g (32%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.32 (m, 5H), 6.29 (m, 1H), 5.85 (m, 1H), 5.19 (s, 2H), 4.32 (m, 2H), 2.94 (m, 1H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 166.35, 139.59, 135.84, 128.78, 128.50, 128.28, 126.15, 66.61, 62.11. HRMS (FAB) calcd for $[M + H]^+$ $C_{11}H_{13}O_3^+$, 193.0865; found, 193.0872.

PMB-Protected Carbamate 23a. Under anhydrous conditions, acid **15a** (4.63 g, 5.7 mmol), diphenylphosphoryl azide (DPPA) (1.47 mL, 6.8 mmol), and triethylamine (0.95 mL, 6.8 mmol) were mixed in dry toluene (60 mL) under N_2 for 30 min at 25 °C. Benzyl alcohol (0.82 mL, 8.0 mmol) was added and the mixture heated at reflux for 4 h. After cooling, the mixture was concentrated in vacuo to a brown residue which was triturated with EtOAc and filtered to provide a clear oil after rotary evaporation of the filtrate. Flash chromatography (35% EtOAc/hexanes) gave an off-white foam. Yield 4.13 g (79%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.35 (m, 5H), 7.08 (d, J = 8.6 Hz, 4H), 6.84 (m, 8H), 6.72 (d, J = 8.2 Hz, 2H), 6.64 (d, J = 6.6 Hz, 2H), 5.08 (s, 2H), 4.70 (s, 1H), 4.40 (dd, J = 13.7, 5.2 Hz, 2H), 4.34 (d, J = 16.2 Hz, 2H), 3.89 (m, 3H), 3.77 (s, 6H), 2.73 (t, J = 11.0 Hz, 2H), 2.38 (m, 4H), 1.40 (m, 4H), 1.24 (m, 16H), 0.89 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 159.62, 158.96, 155.49, 144.37, 144.13, 136.43, 130.23, 130.03, 129.55, 128.97, 128.89, 128.78, 128.54, 128.40, 127.98, 127.72, 127.30, 114.24, 88.61, 80.73, 67.24, 65.61, 55.41, 45.93, 43.69, 43.64, 42.96, 42.94, 35.52, 35.39, 31.95, 31.93, 31.47, 31.37, 29.25, 29.15, 22.80, 14.19. IR ($CDCl_3$): 2951.54, 2926.51, 2854.51, 1722.64, 1709.48, 1513.12, 1462.15, 1440.88, 1417.11, 1302.31, 1245.83, 1177.61, 1034.78, 890.44, 775.25 cm^{-1}. HRMS (FAB) calcd for $[M + Cs]^+$ $C_{57}H_{69}N_5O_6$·Cs^+, 1052.4302; found, 1052.4342.

Carbamate 24a. Carbamate **23a** (6.97 g, 7.6 mmol) was dissolved in 250 mL CH_3CN. Water (50 mL) was added slowly and the temperature maintained at 25 °C by use of a heat gun. To the clear solution was added CAN (18.28 g, 33.4 mmol), and the resulting orange solution was covered from light and stirred under N_2 for 16 h. The reaction mixture was poured into 1 M HCl and then extracted with 400 mL of EtOAc. The organic layer was washed again with 2 × 300 mL of 1 M HCl, dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was subjected to flash chromatography (2−10% MeOH/CH_2Cl_2) which gave a crude foam. Further purification was accomplished with ether trituration and filtration giving lustrous white crystals. Yield 3.67 g (71%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 8.30 (s, 2H), 7.30 (m, 5H), 7.21 (d, J = 8.1 Hz, 1H), 6.95 (d, J = 8.3 Hz, 2H), 6.90 (d, J = 8.1 Hz, 2H), 6.85 (d, J = 8.3 Hz, 2H), 6.83 (d, J = 8.3 Hz, 2H), 4.99 (s, 2H), 3.89 (dd, J = 13.3, 4.5 Hz, 2H), 3.33 (m, 1H), 2.51 (m, 2H), 2.34 (m, 4H), 1.35 (m, 4H), 1.19 (m, 12H), 1.12 (m, 4H), 0.83 (m, 6H). ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 159.01, 155.68, 142.58, 142.31, 137.04, 134.60, 131.24, 128.54, 128.27, 128.02, 127.95, 127.46, 127.40, 127.34, 81.64, 78.27, 65.59, 43.13, 41.64, 34.51, 34.48, 31.26, 30.84, 30.71, 28.50, 28.49, 28.39, 28.31, 22.11, 22.09, 13.88. IR ($CDCl_3$): 3271.56, 2942.73, 2926.03, 2854.97, 1727.04, 1694.25, 1465.40, 1441.84, 1248.67, 915.87 cm^{-1}. HRMS (FAB) calcd for $[M+Cs]^+$ $C_{41}H_{53}N_5O_4$·Cs^+, 812.3152; found, 812.3182.

Amine Module 25a. Carbamate **24a** (4.08 g, 6.0 mmol) and palladium (5%) on activated carbon (1.02 g) were mixed in 120 mL of an EtOAc/EtOH/AcOH mixture (49:49:2). The mixture was efficiently stirred under H_2 (1 atm) for 6 h using a standard balloon setup. Catalyst was removed by filtration through a pad of Celite and the filtrate evaporated to give the crude amine as a foam. Silica gel flash chromatography (10% MeOH/CH_2Cl_2) afforded the target molecule as a white, hygroscopic solid. Yield 2.97 g (91%). ^{1}H NMR ($CDCl_3$, 600

(20) Martin, D. J.; Griffin, C. E. *J. Org. Chem.* **1965**, *30*, 4034−4038.

MHz): δ (ppm) 7.08 (d, J = 8.0 Hz, 2H), 6.96 (d, J = 8.0 Hz, 2H), 6.88 (m, 4H), 5.85, (s, 2H), 4.12 (m, 2H), 2.99 (m, 1H), 2.52 (t, J = 12.0 Hz, 2H), 2.41 (m, 4H), 1.91 (s, 2H), 1.42 (m, 4H), 1.22 (m, 16H), 0.88 (m, 6H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 159.64, 144.25, 144.13, 133.56, 130.21, 128.77, 128.41, 127.74, 127.42, 83.57, 78.71, 77.71, 45.64, 44.21, 35.47, 35.40, 31.95, 31.94, 31.41, 29.24, 29.22, 29.15, 29.12, 22.81, 22.79, 14.21. IR ($CDCl_3$): 3347.32, 3217.04, 2956.18, 2925.00, 2854.04, 1681.63, 1467.64, 1441.27, 1102.17, 912.32 cm^{-1}. HRMS (FAB) calcd for $[M + H]^+$ $C_{33}H_{48}N_5O_2$, 546.3808; found, 546.3828.

Triamine Spacer 26.[21] Ground and oven-dried (150 °C, 3 d) 1,3,5-tricarboxamidobenzene (3.82 g, 18.4 mmol) was mixed with 140 mL of 1 M BH_3 in THF under anhydrous conditions (N_2) and then heated at reflux for 6 d. Dry THF was added occasionally to maintain solvent level. After this time, the mixture was cooled to 0 °C, and concentrated HCl (15 mL) was added slowly with stirring. The mixture was then brought to reflux for another 3 h followed by removal of the THF in vacuo. The residue was thoroughly triturated with H_2O and filtered followed by evaporation of the filtrate. This residue was diluted with MeOH (50 mL), the MeOH removed by evaporation, and the process repeated three more times to volatilize all borates. A minimum of MeOH (10 mL) was used to dissolve the crude product followed by addition of EtOH (25 mL) and ether (200 mL) which gave a white precipitate that was <90% pure by ^{1}H NMR. The precipitate was then dissolved in 3 N KOH (100 mL) and washed with EtOAc (3 × 100 mL), and the combined organic phases were concentrated to 50 mL. This solution was saturated with HCl gas and then left overnight in the refrigerator which produced white flakes isolated by filtration. Yield 0.51 g (10%). ^{1}H NMR (D_2O, 600 MHz): δ (ppm) 7.61 (s, 3H), 4.3 (s, 6H). ^{13}C NMR (D_2O, 151 MHz): δ (ppm) 134.74, 129.81, 42.04. HRMS (FAB) calcd for $[M + H]^+$ $C_9H_{16}N_3$, 166.1344; found, 166.1349.

Monomer 27a. Acid module **20a** (1.15 g, 2.0 mmol), triamine **26** (0.11 g, 0.4 mmol), PyBOP (1.04 g, 2.0 mmol), and NEt_3 (0.56 mL, 4.0 mmol) were mixed in 20 mL of DMF for 24 h at room temperature. The solvent was removed in vacuo and the residue taken up in EtOAc (40 mL). Washing this solution with 1 M HCl (2 × 40 mL), followed by drying (Na_2SO_4), filtering, and evaporation, gave a brown foam. Two purifications by flash chromatography (0−15% MeOH/CH_2Cl_2) gave an off-white foam which was triturated thoroughly with MeOH. Filtration provided a clean, white powder. Yield 0.27 g (36%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 8.75 (t, J = 5.7 Hz, 3H), 8.34 (s, 6H), 7.00 (d, J = 8.1 Hz, 6H), 6.93 (m, 9H), 6.90 (d, J = 8.2 Hz, 6H), 6.84 (d, J = 8.2 Hz, 6H), 4.17 (d, J = 5.3 Hz, 6H), 3.96 (dd, J = 13.6, 3.9 Hz, 6H), 2.84 (m, 6H), 2.40 (m, 15H), 1.38 (m, 12H), 1.24 (m, 36H), 1.14 (m, 12H), 0.85 (m, 18H). ^{13}C NMR (DMSO-d_6, 600 MHz): δ (ppm) 170.02, 158.65, 142.32, 142.05, 139.39, 134.63, 131.25, 128.08, 127.35, 127.15, 125.00, 81.56, 78.12, 41.89, 40.11, 40.05, 38.53, 34.65. 34.61, 31.32, 31.31, 30.99, 30.83, 28.62, 28.54, 28.46, 22.19, 22.14, 13.94. IR ($CDCl_3$): 3392.04, 3280.44, 2955.17, 2925.37, 2854.28, 1720.67, 1686.10, 1549.23, 1466.79, 1445.38, 1378.69, 1229.84, 1105.70 cm^{-1}. HRMS (FAB) calcd for $[M + Cs^+]$ $C_{111}H_{147}N_{15}O_9 \cdot Cs^+$, 1967.0561; found, 1967.0713.

Triacid Spacer 33. Trinitrile **32**[22] (0.28 g, 1.0 mmol) was mixed in concentrated HCl (10 mL) and glacial acetic acid (10 mL). The mixture was refluxed overnight and cooled to room temperature, and the volatiles were removed by rotary evaporation. The residue was triturated with acetone and the resulting white precipitate collected by filtration. Yield 0.31 g (92%). ^{1}H NMR (DMSO-d_6): δ (ppm) 12.32 (s, 3H), 3.62 (s, 6H), 2.57 (q, J = 7.5 Hz, 6H), 1.03 (t, J = 7.5 Hz, 9H). ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 173.34, 140.89, 129.44, 34.78, 23.06, 14.06. HRMS (FAB) calcd for $[M + Na^+]$ $C_{18}H_{24}O_6 \cdot Na^+$, 359.1471; found, 359.1466.

1,3,5-Triethyl-2,4,6-tris(methylaminomethyl)benzene 34. 1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene **29** (0.14 g, 0.3 mmol) was dissolved in dry THF (15 mL), and anhydrous monomethylamine (2 M solution in MeOH, 10 mL, 20.0 mmol) was added. The reaction was stirred at room temperature for 2 h, after which it was refluxed for 30 min (^{1}H NMR in $CDCl_3$ showed full conversion at this point). The solvent was evaporated, and the oily residue was partitioned between dichloromethane (75 mL) and 4 M NaOH (50 mL). The aqueous phase was extracted with dichloromethane (50 mL), and the combined organic extracts were washed with brine (50 mL). The organic phase was extracted with 4 M HCl (2 × 50 mL), and the aqueous extracts were washed with dichloromethane (50 mL). The organic phase was discarded, and the aqueous phase was made basic to pH = 12 by addition of solid NaOH. The resulting turbid aqueous phase was extracted with dichloromethane (2 × 50 mL), and the combined organic extracts were washed with brine (50 mL) and dried (Na_2SO_4). Evaporation of the solvent gave the product as a yellow oil, which slowly crystallized upon standing, yield 0.09 g (0.3 mol, 97%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 3.63 (s, 6H), 2.77 (q, J = 7.6 Hz, 6H), 2.53 (s, 9H), 1.25 (t, J = 7.6 Hz, 9H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 142.34, 134.09, 49.80, 37.25, 22.49, 16.83. HRMS(FAB) calcd for $[M + H]^+$ $C_{18}H_{33}N_3 \cdot H^+$, 292.2753; found, 292.2751.

Triamide Ball Monomer 35a. Triamine **31** (0.50 g, 0.2 mmol), acid module **20a** (0.57 g, 1.0 mmol), HOBt (1-hydroxybenzotriazole) (0.14 g, 1.0 mmol), and EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) (0.19 g, 1.0 mmol) were mixed in 20 mL of dry DMF under N_2 atmosphere. To this mixture was added NEt_3 (140 μL, 1.0 mmol), and the solution was stirred at room temperature for 6 h. The solvent was removed in vacuo and the filtrate taken up in EtOAc (50 mL). This solution was washed with 1 M HCl (2 × 15 mL), dried with Na_2SO_4, filtered, and evaporated to give an off-white foam. This residue was subjected to flash chromatography (0−5% MeOH/CH_2Cl_2) and, after isolation, further purified by precipitation from a minimum of $CHCl_3$ (3 mL) with MeOH (15 mL). Filtration gave a white powder. Yield 0.26 g (68%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 8.87 (s, 12H), 7.19 (d, J = 8.3 Hz, 12H), 6.98 (d, J = 8.1 Hz, 24H), 6.93 (d, J = 8.0 Hz, 12H), 5.47 (s, 6H), 4.21 (s, 12H), 4.13 (dd, J = 14.2, 3.4 Hz, 12H), 3.12 (t, J = 12.9 Hz, 12H), 2.48 (m, 42H), 1.51 (m, 24H), 1.30 (m, 96H), 1.06 (t, J = 7.6 Hz, 18H), 0.92 (m, 36H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 170.42, 161.03, 144.17, 143.87, 143.81, 133.89, 131.72, 130.97, 128.64, 128.36, 127.77, 127.58, 82.91, 80.17, 41.01, 40.89, 37.94, 35.57, 32.02, 31.53, 31.43, 29.84, 29.33, 29.25, 23.30, 22.87, 22.85, 16.35, 14.26. MS (MALDI) calcd for monomer $[M + H^+]$ $C_{117}H_{159}N_{15}O_9$, 1921; found, 1922; calcd for dimer $[2M + H^+]$ $C_{234}H_{318}N_{30}O_{18}$, 3841; found, 3842.

Inverted Triamide Ball Monomer 36a. Triacid **33** (0.67 g, 0.2 mmol), amine module **25a** (0.55 g, 1.0 mmol), HOBt (0.14 g, 1.0 mmol), and EDC (0.19 g, 1.0 mmol) were mixed in 20 mL of dry DMF under N_2 atmosphere. To this mixture was added NEt_3 (140 μL, 1.0 mmol), and the solution was stirred at room temperature for 24 h. The solvent was removed in vacuo and the filtrate taken up in CH_2Cl_2 (50 mL). This solution was washed with 1 M HCl (2 × 15 mL), dried with Na_2SO_4, filtered, and evaporated to give an off-white foam. This residue was subjected to flash chromatography (0−7% MeOH/CH_2Cl_2) which gave a white powder after isolation. Yield 0.19 g (49%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 9.10 (s, 12H), 7.22 (d, J = 8.2 Hz, 12H), 7.00 (d, J = 8.1 Hz, 12H), 6.94 (d, J = 8.2 Hz, 12H), 6.89 (d, J = 8.1 Hz, 12H), 6.49 (s, 6H), 3.96 (m, 12H), 3.39 (m, 30H), 2.48 (t, J = 7.6 Hz, 12H), 2.43 (t, J = 7.7 Hz, 12H), 2.27 (m, 12H), 1.48 (m, 24H), 1.28 (m, 96H), 0.92 (m, 54H). MS (MALDI) calcd for monomer $[M + H^+]$ $C_{117}H_{159}N_{15}O_9$, 1921; found, 1922; calcd for dimer $[2M + H^+]$ $C_{234}H_{318}N_{30}O_{18}$, 3841; found, 3842.

Triester Ball Monomer 37a. Tribromide **29** (88 mg, 0.2 mmol), acid module **20a** (0.57 g, 1.0 mmol), Cs_2CO_3 (0.33 g, 1.0 mmol), CH_3CN (20 mL), and DMF (20 mL) were mixed at 100 °C for 2 h. After cooling, the solvents were removed by evaporation, and the residue was taken up in CH_2Cl_2/*t*-BuOH (4:1, 25 mL). Washing with 1 M HCl and drying with Na_2SO_4, followed by filtration and evaporation, gave a brown foam. This residue was subjected to flash chromatography (0−5% MeOH/CH_2Cl_2) and, after isolation, further purified by precipitation from a minimum of $CHCl_3$ (3 mL) with MeOH (15 mL). Filtration gave a white powder. Yield 0.20 g (52%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 8.90 (s, 12H), 7.23 (d, J = 8.1 Hz, 12H), 6.97 (m, 24H), 6.91 (d, J = 8.0 Hz, 12H), 4.99 (s, 12H), 4.27 (dd, J = 14.0, 3.8 Hz, 12H), 2.96 (t, J = 12.9 Hz, 12H), 2.84 (m, 6H), 2.50 (m, 36H), 1.50 (m, 24H), 1.30 (m, 96H), 1.05 (t, J = 7.7 Hz, 18H),

(21) Synthesis adapted from a literature preparation: Weitl, F. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2728−2731.

(22) Walsdorff, C.; Saak, W.; Pohl, S. *J. Chem. Res.,Synop.* **1996**, 282−283.

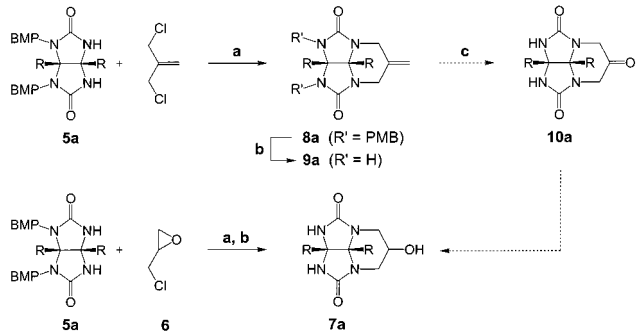
0.92 (m, 36H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 172.22, 160.55, 146.91, 143.60, 143.46, 134.64, 131.38, 129.48, 128.52, 128.24, 127.89, 127.70, 82.64, 79.90, 60.58, 40.15, 38.24, 35.58, 35.57, 32.01, 31.53, 31.47, 29.36, 29.33, 29.26, 23.17, 22.85, 16.32, 14.25. MS (MALDI) calcd for monomer [M + H$^+$] $C_{117}H_{156}N_{12}O_{12}$, 1924; found, 1925; calcd for dimer [2M + H$^+$] $C_{234}H_{312}N_{24}O_{24}$, 3847; found, 3848.

Me$_3$flexiball Monomer 38b. Acid module **20b** (0.44 g, 0.9 mmol) was dissolved in dry DMF (10 mL) and cooled to 0 °C, whereupon 1,3,5-triethyl-2,4,6-tris(methylaminomethyl)benzene **34** (0.06 g, 0.2 mmol) and PyBOP (0.42 mg, 0.9 mmol) were added. Finally, DIPEA (310 μL, 1.8 mmol) was added, and the reaction was stirred at 0 °C, then allowed to warm to room temperature, and stirred for 60 h. The solvent was evaporated, and the residue was redissolved in EtOAc (75 mL), washed with 1 M HCl (2 × 50 mL), dried (Na_2SO_4), and evaporated. The resulting brown oil was subjected to flash column chromatography (0−8% MeOH/CH_2Cl_2). Evaporation of the solvent gave the product as a white foam, yield 0.15 g (0.1 mmol, 50%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 8.37 (s, 6H), 7.13 (d, J = 8.4 Hz, 6H), 7.05 (d, J = 8.3 Hz, 6H), 6.99 (d, J = 8.3 Hz, 6H), 6.92 (d, J = 8.4 Hz, 6H), 4.57 (m, 6H), 3.99 (m, 6H), 2.86 (m, 9H), 2.73 (m, 6H), 2.69 (s, 6H), 2.38 (m, 3H), 1.13 (s, 27H), 1.10 (s, 27H), 0.90 (m, 9H). MS(MALDI) calcd for [M + Na]$^+$ $C_{102}H_{129}N_{15}O_9$•Na$^+$, 1732; found, 1732.

Calixarene Ball Monomer 40a. Tetraaminocalixarene **39** (0.10 g, 0.15 mmol), acid module **20a** (0.35 g, 0.61 mmol), and PyBOP (0.32 g, 0.61 mmol) were mixed in dry CH_2Cl_2 (50 mL), followed by addition of dry ^{i}Pr$_2$NEt (0.214 mL, 1.23 mmol) under anhydrous conditions (N_2). After the reaction was stirred for 18 h at room temperature, TLC (10% MeOH/CH_2Cl_2) indicated complete consumption of the tetraaminocalixarene. More CH_2Cl_2 (100 mL) was added and the solution washed with brine (100 mL), 1 M aq HCl (3 × 100 mL), and saturated aq $NaHCO_3$ (2 × 100 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated by evaporation. The resulting brown residue was sonicated thoroughly with MeOH and filtered, and the precipitate was collected. Dissolving the precipitate in a minimum of $CHCl_3$ (3 mL), precipitation with CH_3CN (25 mL), sonication, and filtration gave an off-white powder. Yield 0.23 g (52%). ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 9.88 (s, 4H), 8.34 (s, 8H), 7.03 (d, 8H), 6.93 (m, 24H), 6.87 (d, 8H, J = 8.3 Hz), 4.28 (d, 4H, J = 12.1 Hz), 3.99 (m, 8H), 3.73 (m, 8H), 3.00 (d, 4H, J = 11.3 Hz), 2.83 (t, 8H, J = 12.6 Hz), 2.43 (t, 8H, J = 7.5 Hz), 2.38 (t, 8H, J = 7.4 Hz), 1.88 (m, 8H), 1.40 (m, 16H), 1.22 (m, 64H), 0.92 (t, 12H, J = 7.4 Hz), 0.86 (m, 24H). ^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 168.58, 158.83, 152.18, 142.49, 142.26, 134.83, 134.19, 132.81, 131.36, 128.19, 127.51, 127.33, 120.07, 81.65, 78.15, 76.64, 34.63, 34.54, 31.35, 31.26, 30.90, 30.84, 28.63, 28.48, 28.39, 22.54, 22.16, 22.09, 13.88, 13.86, 10.03. MS (MALDI) calcd for [M_{av}] $C_{176}H_{228}N_{20}O_{16}$, 2878; found, 2878.

Tetraester Ball Monomer 42a. Tetrahydroxy cavitand **41** (0.60 g, 0.48 mmol) and acid module **20a** (1.11 g, 1.94 mmol) were both thoroughly dried in high vacuum. The reactants were mixed together with PyBOP (1.08 g, 1.94 mmol) and 540 μL triethylamine and dissolved in 120 mL of absolute dichloromethane. The mixture was stirred at room temperature for 12 h and then quenched with brine. Phases were separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with 2 N hydrochloric acid, twice with saturated sodium bicarbonate solution and brine, dried over $MgSO_4$, filtrated, and concentrated. The residue was subjected to column chromatography on silica using ethyl acetate/hexanes (80/20) to give 795 mg (48%) of the desired product as a white foam. ^{1}H NMR (DMSO-d_6/$CDCl_3$, 600 MHz): δ (ppm) 8.39 (s, 8H), 7.36 (s, 4H), 7.04 (d, J = 8.1 Hz, 8H), 6.94 (d, J = 8.1 Hz, 8H), 6.91 (d, J = 8.1 Hz, 8H), 6.84 (d, J = 8.1 Hz, 8H), 5.36 (d, J = 6.7 Hz, 4H), 4.42 (t, J = 7.8 Hz, 4H), 4.27 (m, 8H), 4.19 (m, 4H), 2.84 (m, 12H), 2.39 (m, 16H), 2.26 (m, 8H), 1.39 (m, 16H), 1.33−1.20 (m, 136 H), 0.85 (m, 12 H), 0.81 (m, 24 H). ^{13}C NMR ($CDCl_3$, 151 MHz): δ (ppm) 168.66, 160.55, 146.91, 144.01, 143.94, 139.32, 136.10, 134.68, 130.89, 128.78, 128.57, 127.97, 127.71, 117.35, 99.14, 83.17, 80.26, 77.88, 76.87, 69.91, 64.77, 54.14, 40.53, 37.11, 35.93, 32.34, 32.26, 30.05, 30.03, 29.97, 29.74, 29.70, 29.60, 29.58, 23.14, 23.12, 23.03, 14.53, 14.50, 14.47. MS (MALDI) calcd for monomer [M + H$^+$] $C_{212}H_{289}N_{16}O_{24}$, 3446; found, 3446.

Scheme 2. Synthesis of the Hydroxy Module[a−c]



[a] Cs_2CO_3, CH_3CN, reflux. [b] CAN, CH_3CN/H_2O (5:1). [c] OsO_4, $NaIO_4$, THF [**a**: R = 4-(1-heptyl)phenyl, PMB = 4-methoxybenzyl].

Tetraester Ball Monomer 42c. Tetrahydroxy cavitand **41** (46 mg, 0.04 mmol) and acid module **20c** (105 mg, 0.15 mmol) were both thoroughly dried in high vacuum. The reactants were mixed together with PyBOP (79 mg, 0.15 mmol) and 42 μL triethylamine and dissolved in 15 mL absolute dichloromethane. The mixture was stirred at room temperature for 12 h and then quenched with brine. Phases were separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed twice with 2 N hydrochloric acid, twice with saturated sodium bicarbonate solution and brine, dried over $MgSO_4$, filtrated, and concentrated. The residue was subjected to column chromatography on silica using ethyl acetate/hexanes (80/20) to give 41 mg (28%) of the desired product as a white foam. ^{1}H NMR (DMSO-d_6/$CDCl_3$, 600 MHz): δ (ppm) 8.33 (s, 8H), 7.35 (s, 4H), 7.06 (d, J = 8.6 Hz, 8H), 6.96 (d, J = 8.6 Hz, 8H), 6.66 (d, J = 8.7 Hz, 8H), 6.59 (d, J = 8.7 Hz, 8H), 5.52 (d, J = 6.2 Hz, 4H), 4.42 (t, J = 7.4 Hz, 4H), 4.26 (d, J = 11 Hz, 8H), 4.21 (m, 4H), 3.78 (m, 16H), 2.88 (m, 4H), 2.84 (m, 8H), 2.26 (m, 8H), 1.61 (m, 16H), 1.32 (m, 16 H), 1.23−1.18 (m, 168 H), 0.87−0.80 (m, 36 H). MS (MALDI) calcd for monomer [M + Na$^+$] $C_{236}H_{336}N_{16}O_{32}Na$, 3930; found, 3930; calcd for monomer [M + K$^+$] $C_{236}H_{336}N_{16}O_{32}K$, 3946; found, 3946.

Results and Discussion

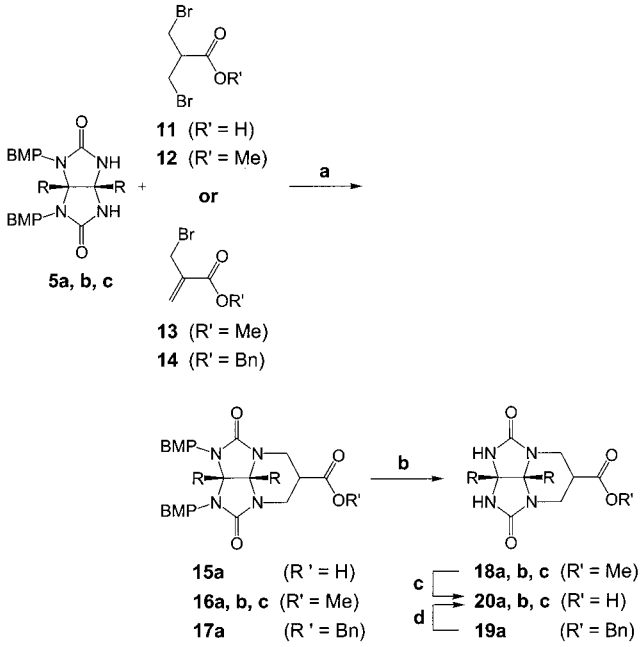
Syntheses. (a) Hydroxy Module. Our initial efforts focused on glycoluril module **7a** which bears an equatorial hydroxy group at the 5-position. Scheme 2 shows two routes to hydroxy module **7a**. Alkylation of the *cis*-protected glycoluril **5a**[23] with methallyl dichloride proceeded smoothly to give **8a** in high yield (for designation "**a**", R = 4-(1-heptyl)phenyl; for "**b**", R = 4-(1,1-dimethylethyl)phenyl; for "**c**", R = 4-(1-decanoxy)-phenyl[24]). However, following removal of the PMB-protecting groups using ammonium cerium(IV) nitrate (CAN), Lemieux−Johnson oxidation of alkene **9a** did not yield the desired ketone **10a**. Instead, a mixture of ketals was isolated which indicated that a carbonyl at the 5-position is destabilized, probably because of the inductive effects of the flanking amides. Dave et al. reported a similar synthesis in which they found ketones at the 5-position to exist as hydrates.[25] Although conversion to the ketone by azeotropic removal of water was described, we pursued a different route to **7a**.

(23) Rivera, J. M.; Martín, T.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 819−820. The *cis*-protected glycoluril **5** was chosen for increased solubility and to eliminate *trans* alkylations. This eliminated the need for excess glycoluril and tremendously simplified the reaction work-up.

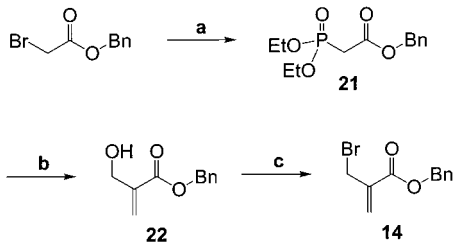
(24) While the 4-heptylphenyl and 4-decanoxyphenyl substituted glycolurils are the most soluble, both groups have a tendency to dominate the ^{1}H NMR spectra, sometimes obliterating other relevant signals. However, both groups provide glycoluril modules that significantly differ in their molecular masses, thus allowing easier detection in the heterodimerization ESI-MS experiments. The 4-(1,1-dimethylethyl)phenyl substituted analogue as an alternative, although by no means NMR-silent, opened a larger window in the ^{1}H NMR spectra for observing signals from guest molecules and has the added benefit of being more crystalline and easier to purify.

(25) Dave, P. R.; Forohar, F. F.; Kaselj, M.; Gilardi, R.; Trivedi, N. *Tetrahedron Lett.* **1999**, *40*, 447−450.

Scheme 3. Acid Module Synthesis[a–d]



Scheme 4. Synthesis of Benzyl Acrylate **14**[a–c]



[a] $P(OEt)_3$, Δ. [b] HCOH, K_2CO_3. [c] PBr_3, Et_2O.

Substitution of epichlorohydrin **6** for methallyl dichloride gave the PMB-protected hydroxy module as a mixture of equatorial and axial isomers (1:1) in high yield. Column purification provided the desired equatorial isomer, which was then deprotected to give **7a**. Unfortunately, this module exhibited poor reactivity with a variety of electrophilic spacers (e.g., mesitoic acid chloride). The nucleophilicity of the secondary hydroxy group in **7a** may be reduced by electronic factors as well as steric interactions imposed by the proximal R group.

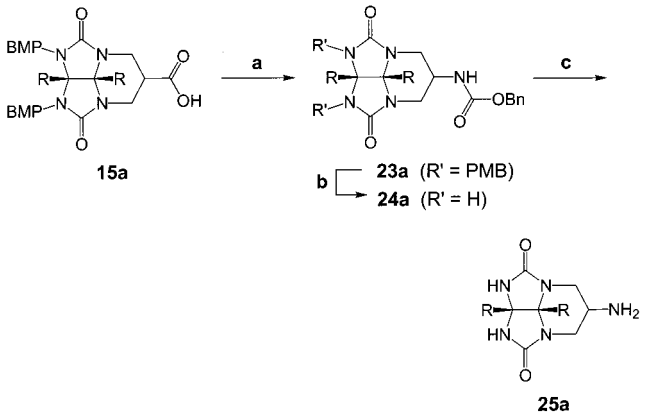
(b) Acid Module. Given the low reactivity of the hydroxy module, we sought other functions with electrophilic or enhanced nucleophilic character. A group capable of fulfilling both roles appeared in acid module **20**. Our first choice for the modular element was the commercially available acid **11**. The alkylation of the glycoluril proceeded in almost quantitative yield, but the stereoisomers proved resistant to separation (Scheme 3).

Fortunately, replacement of acid **11** with methyl ester **12** or **13** gave ester **16** as a mixture of easily separable isomers and in good yield. Deprotection (CAN) gave **18**, and then LiI-mediated demethylation[26] provided the equatorial acid **20** in high yield. An alternative modular element, benzyl acrylate **14**, was employed as well. The synthesis of **14**[27] is illustrated in Scheme 4.

(26) Because epimerization of the 5-position in the six-membered ring in **20** was possible, we chose demethylation conditions preventing isomerization during deprotection of the acid. See: Elsinger, F.; Schreiber, J.; Eschenmoser, A. *Helv. Chim. Acta* **1960**, *43*, 113−118.

(27) Prepared analogously to the ethyl ester, see: Villieras, J.; Rambaud, M. *Synthesis* **1982**, 924−926.

Scheme 5. Amine Module Synthesis[a–c]



[a] I. DPPA, toluene; II. BnOH, reflux. [b] CAN, CH_3CN/H_2O (5:1) [c] H_2, Pd/C, EtOH/EtOAc/AcOH (49:49:2) [**a**: R = 4-(1-heptyl)phenyl, PMB = 4-methoxybenzyl].

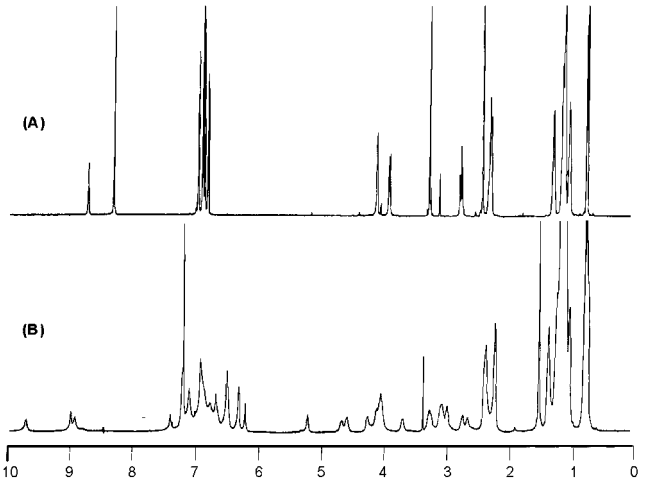


Figure 2. ^{1}H NMR spectra (600 MHz) of **27a** in (A) DMSO-d_6 and (B) $CDCl_3$.

Alkylation of **5a** with **14** gave **17a** as a mixture of isomers (eq:ax = 4:1) that were purified easily by chromatography. Standard CAN deprotection of **17a** gave ester **19a** which, after hydrogenolysis, gave **20a** in high yield with no epimerization at the α carbon.

(c) Amine Module. The synthesis of the amine module **25a** is outlined in Scheme 5. Ester **16a** was demethylated as before to provide acid **15a** and then converted to carbamate **23a** via a modified Curtius rearrangement.[28] Removal of the PMB groups followed by hydrogenolysis gave **25a** in good overall yield.

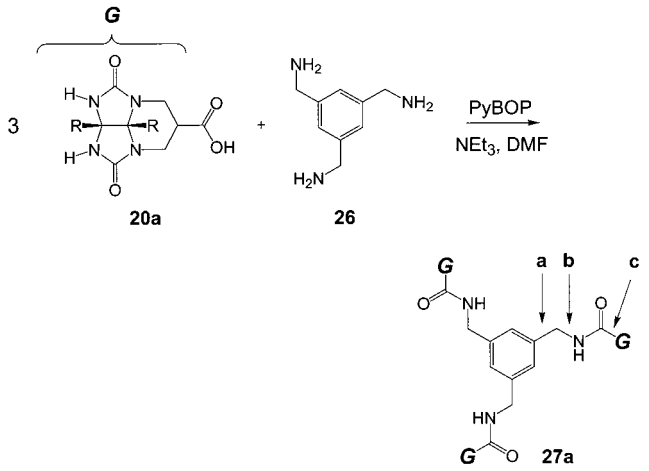
(d) Trivalent Monomers. With sizable amounts of the new glycoluril modules in hand, a variety of complementary spacers were investigated. Monomer **27a**, available from the condensation of acid module **20a** with triamine spacer **26**,[21] provided the first test (Scheme 6). While **27a** gave a first-order ^{1}H NMR spectrum in highly competitive solvents such as DMSO-d_6, the spectrum was broad and concentration dependent in $CDCl_3$ (Figure 2). The absence of sharp signals or a single downfield resonance for the glycoluril N*H*s suggested that **27a** exists as a disordered aggregate in noncompetitive solvents rather than as a discrete dimer or other finite assembly.[29]

Raymond and co-workers also used triamine **26** as a platform

(28) Shiori, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203−6205.

(29) For a discussion on the characterization of capsules, see: Rebek, J., Jr. *Chem. Soc. Rev.* **1996**, *25*, 255−264.

Scheme 6. Synthesis of Monomer **27a**[a]



[a] In the monomer, three bonds per arm (a−c) can rotate freely.

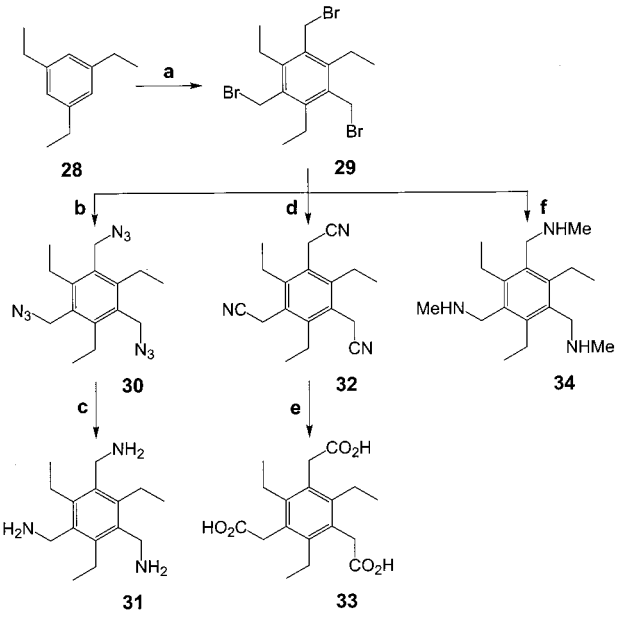
for an enterobactin analogue.[30] However, despite close structural similarities to the natural product, their mimic demonstrated a 10^6 lower affinity for ferric ion. They rationalized that greater rotational freedom in the mimic was responsible for the lower binding affinity. Introduction of ethyl groups at the 2, 4, and 6 positions of the spacer (as in **31a**) mitigated this deficit. Steric effects then enforced an *ababab* conformation with the ethyl groups all on one side of the aromatic rings and the hydrogen-bonding sites on the other by restricting rotation about the $C_{ar}-CH_2$ bond (bond a in Scheme 6). This preorganizing factor resulted in a 10^4 enhancement in binding affinity for the modified mimic above the initial one.

Locking rotation of the three a bonds can lower the entropic cost[31] of organizing **27a** into a 1,3,5-*cis* arrangement by an estimated 4.5 kcal/mol.[32] Accordingly, we used four hindered spacers (**29**, **31**, **33**, **34**) for coupling with the acid and amine modules (Scheme 7). Repetitive bromomethylation[33] of **28** gave spacer **29** and treatment of this tribromide with NaN_3 or NaCN provided triazide **30** or trinitrile **32**,[22] respectively. Reduction of **30** under Staudinger conditions gave the known triamine **31**,[15] and hydrolysis of **32** produced the new triacid **33**. For the purpose of preparing a methyl-substituted analogue of monomer **35**, we synthesized triamine **34** from **29** by treatment with anhydrous methylamine in a mixture of tetrahydrofuran and methanol heated at reflux.

Scheme 8 illustrates the condensation of the various spacers with the appropriate glycoluril modules. Using standard peptide coupling methods, we obtained triamide monomers **35a**, **36a**, and **38a**,**b** in good yields. The monomers **35a** and **36a** were prepared by EDC coupling, whereas PyBOP was employed for the synthesis of the more hindered monomer **38a**,**b**. In addition, triester **37a** was available from the reaction of tribromide **29** with the acid module under mildly alkaline conditions. All four monomers were evaluated for their dimerization and complexation abilities.

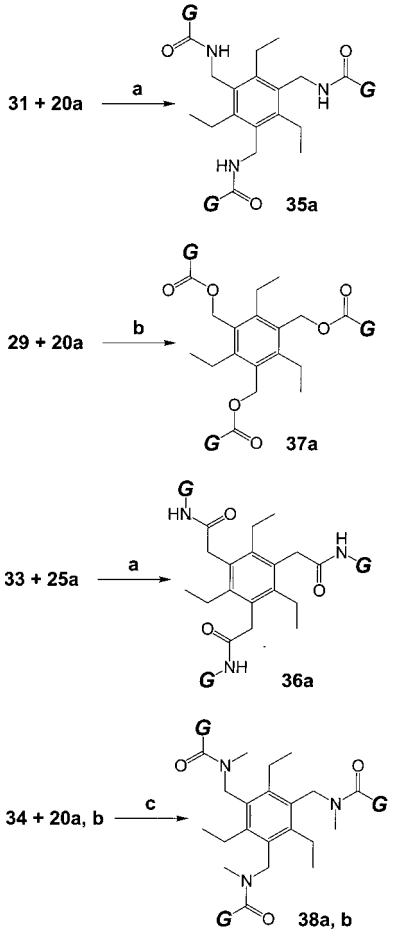
(e) Tetravalent Monomers. For access to larger capsules, we used spacers with more functional groups to connect to suitable glycoluril modules. We chose calix[4]arene and resorcin-

Scheme 7. Synthesis of Hindered Spacers[a−f]



[a] I. HBr, CH_2O, AcOH; II. CH_2O, KBr, H_2SO_4. [b] NaN_3, DMF. [c] PPh_3, THF, H_2O. [d] NaCN, DMF. [e] HCl (concentrated), AcOH. [f] $MeNH_2$, THF, MeOH.

Scheme 8. Flexiball Syntheses I[a−c]



[a] EDC, HOBt, NEt_3, DMF. [b] Cs_2CO_3, CH_3CN, DMF. [c] PyBOP, DIPEA, DMF. **G** = glycoluril unit (see Scheme 6).

[4]arene scaffolds. Their monomers feature self-complementary shapes and recognition surfaces capable of self-assembly to D_{4d} symmetric dimeric capsules. Simple PyBOP couplings of

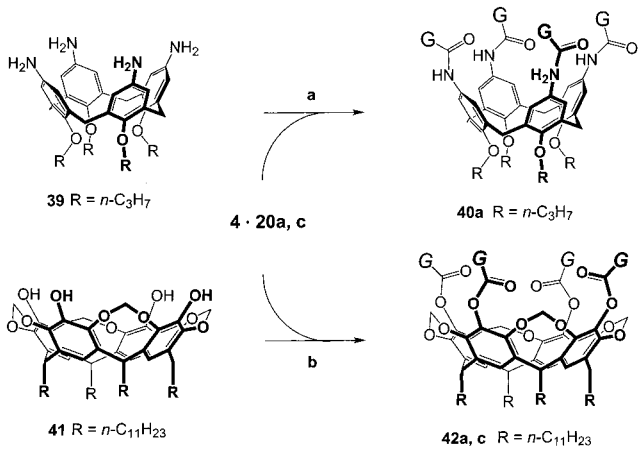
(30) Stack, T. D. P.; Hou, Z.; Raymond, K. N. *J. Am. Chem. Soc.* **1993**, *115*, 6466−6467.

(31) Mammen, M.; Shakhnovich, E. I.; Deutch, J. M.; Whitesides, G. M. *J. Org. Chem.* **1998**, *63*, 3821−3830.

(32) Page, M. I.; Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1678−1683.

(33) We are grateful to Prof. Eric Anslyn and Paul M. Thompson who provided us with the synthetic procedure for the triethylated benzene spacers.

Scheme 9. Flexiball Syntheses II[a,b]



[a] PyBOP, *i*-Pr_2NEt, CH_2Cl_2. [b] PyBOP, Et_3N, CH_2Cl_2. **G** = glycoluril unit (see Scheme 6).

centerpieces **39**[16] and **41**[17] with acid modules **20a**,**c**, respectively, led to the desired monomers **40a**, **42a**,[6b] and **42c** in good yields (Scheme 9). However, as in the case of monomer **27**, relatively flexible compound **40a** did not form discrete dimers (or any other identifiable assembly) in noncompetitive solvents. Apparently, **40** is trapped in the undesired C_{2v} (pinched cone) conformation reinforced by intramolecular hydrogen bonding, as indicated by NMR spectroscopy and molecular modeling.[34] The C_{4v} (cone) conformation, necessary for dimerization, is not its preferred conformer. The other more rigid monomers **35**, **36**, **37**, **38**, and **42** were evaluated for their dimerization and complexation abilities by two different and independent methods: ^{1}H NMR and electrospray ionization mass spectrometry.

NMR Studies of the Dimerization and Encapsulation Behavior of Trivalent Monomers. In contrast to monomer **27a**, the three new C_{3v} symmetric monomers **35a**−**37a** were shown to exist exclusively as discrete dimers in noncompetitive solvents.[35] For example, the ^{1}H NMR spectrum of **35a** in $CDCl_3$ (Figure 3A) shows only sharp, concentration-independent resonances (to the limit of detection in our 600 MHz instrument), including a far downfield shift for the glycoluril N*H*. Although twelve rotations (bonds b and c) per dimer must be restricted in the assemblies, the high binding enthalpy defrays the entropic costs and the capsules assemble.

An unexpected, but nonetheless welcome, dividend came from the use of **35a** as a rigid template: it complexed the less rigid monomer **27a** and formed heterodimer **27a·35a** (Figure 3B). Seemingly, rigidifying only one of the monomers already is sufficiently favorable to stabilize its capsules. Monomer **36a**, with inverted amide connectivity, behaved similarly to **35a·35a** in terms of dimerization but showed markedly different solubility and guest binding properties.[6] This monomer also formed a heterodimer (**35a·36a**) on mixing with **35a** as indicated by ^{1}H NMR (Figures 3C and 4).[36] Triester **37a** also dimerizes to form capsule **37a·37a**, although the broadened signal for the glycoluril NH protons (Figure 5a) indicates that the assembly is more dynamic than **35a·35a** or **36a·36a**.[37] This might well

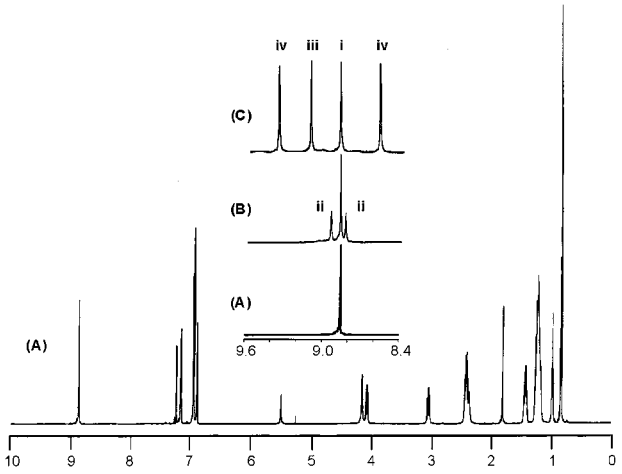


Figure 3. (A) Full ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **35a·35a**. The inset shows the glycoluril NH region for samples containing monomers (A) **35a** [dimer **35a·35a** = i], (B) **27a** and **35a** [heterodimer **27a·35a** = ii], and (C) **35a** and **36a** [dimer **36a·36a** = iii; heterodimer **35a·36a** = iv].

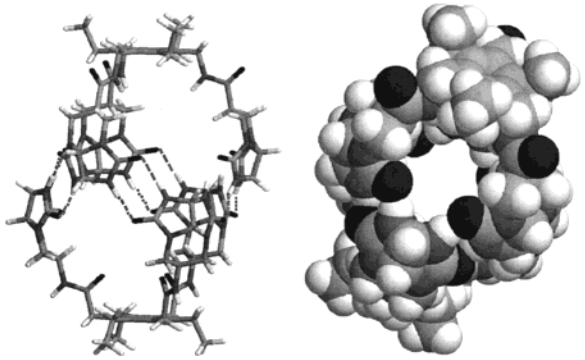


Figure 4. Computed polytube and CPK models of the heterodimer composed of **35**(top)·**36**(bottom), from which the structures of the corresponding homodimers may be inferred (side chains are omitted for viewing clarity).

reflect the lower rotational barrier about an ester bond when compared to an amide.

In contrast to the behavior of the monomers **35a**−**37a**, **38a** failed to dimerize to the flexiball **38a·38a** in noncompetitive solvents and displayed only very broad features in its ^{1}H NMR spectrum. In the competitive solvent DMSO-d_6, **38a** displayed a first order ^{1}H NMR spectrum. Compound **35a** exists almost exclusively as the more stable *Z*,*Z*,*Z* amide conformation typical of secondary amides. In contrast, the presence of the *N*-methyl groups of **38a** narrows the energy difference between the two amide conformers (*E* and *Z*) and results in a mixture of conformational isomers. Only one of the eight interconverting structures is preorganized for assembly, but its concentration is apparently too small to allow for efficient self-assembly to **38a·38a**. Instead, **38a** exists as a mixture of aggregates, much in the same way as **27a** does. The analogous monomer **38b**, which differs only with respect to the solubilizing side chains, displayed the same behavior.

Assembly of the monomers **38a**,**b** was eventually achieved by heterodimerization in chloroform solution with the flexiball monomer **35a** to give **35a·38a** and **35a·38b**. Titration of a solution of **38a**,**b** with **35a** resulted in pronounced sharpening of the ^{1}H NMR spectrum, which remained complex because of

(34) ^{1}H NMR spectra in noncompetitive solvents showed numerous signals for what should be equivalent protons (including several downfield glycoluril NH peaks) in a C_{4v} structure. This supports a C_{2v} pinched cone structure.

(35) If up to 4.5 kcal/mol are required to preorganize free monomer **27a** into an all *cis* conformation, each of the dimers arising from **35a**−**37a** will be ∼9 kcal/mol more stable than **27a·27a**.

(36) As calculated from peak integrations, the heterodimer and homodimers were statistically distributed upon mixing [**35a·35a**:**36a·36a**:**35a·36a** = 1:1:2].

(37) Ester flexiball **37a·37a** also forms heterodimers with the amide flexiballs upon mixing.

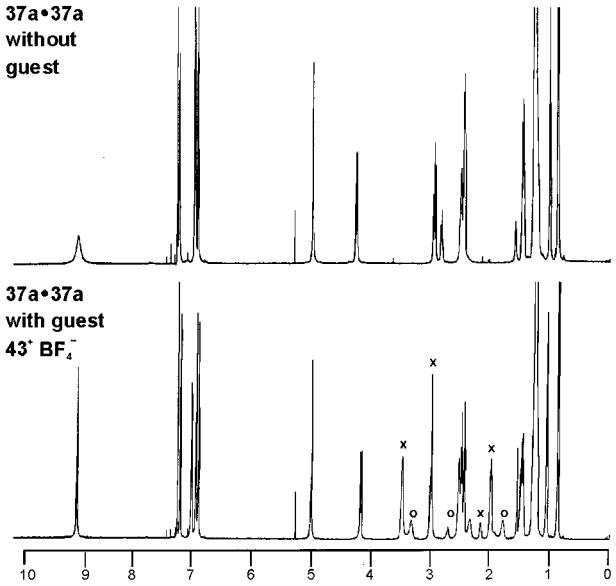
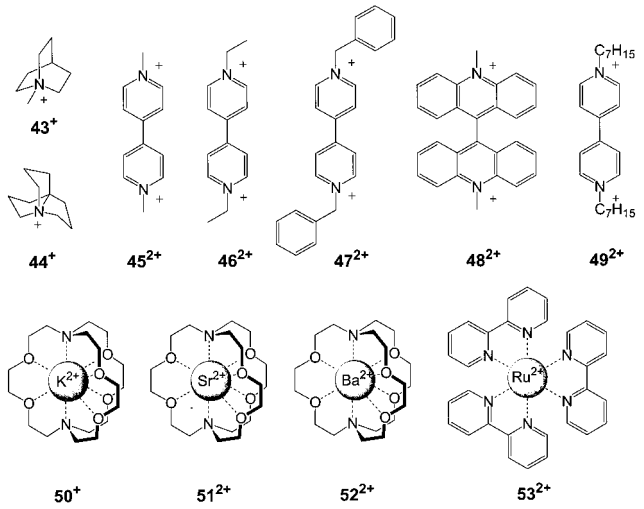


Figure 5. ^{1}H NMR (600 MHz, $CDCl_3$) of triester flexiball **37a·37a** with ~3 equivalents of added guest (bottom) and without guest (top). Some signals attributed to $\mathbf{43}^+BF_4^-$ are marked (× = free; ○ = encapsulated). Integration indicates a 1:1 capsule-to-guest ratio. The ^{19}F NMR spectra gave no direct evidence of BF_4^- encapsulation, although it is likely that both the cation and anion are encapsulated as ion pairs.

Chart 1. Cationic Guests Employed for Mass Spectrometric Studies



the very similar resonances in the two compounds. The downfield region of the spectrum displayed a broadened N−H signal at 9 ppm, which could not be resolved into the two lines that would be expected of a complex of this type (see Figure 3). We attribute this observation to the fact that **35a·38a** is probably a more loosely bound, dynamic assembly (similar to **37a·37a**). Clearer evidence for a heterodimer of **35a** and **38a** came from the ESI mass spectra (see in a following section).

The capsules possess large cavities with approximate volumes of 0.5 nm^3.[38] Sizable holes (~60 Å^2) in the capsule shell preclude slow exchange encapsulation of molecules the size of solvents such as $CDCl_3$. Larger guests with sizes and shapes complementary to the cavities give kinetically stable encapsulation complexes. For instance, the broad ^{1}H NMR resonances

(38) All structural models were created using MacroModel v.5.5. Calculations of cavity volume were performed as described previously (Mecozzi, S.; Rebek, J., Jr. *Chem.—Eur. J.* **1998**, *4*, 1016−1022).

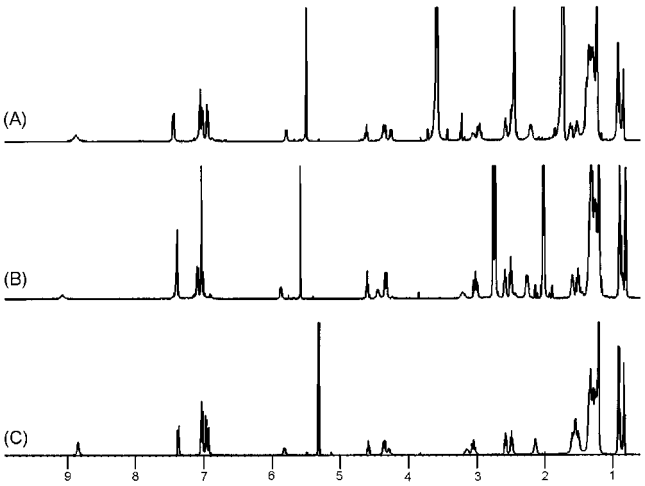


Figure 6. Full ^{1}H NMR spectra (500 MHz, 300 K) of **42a·42a** in (A) THF-d_8, (B) acetone-d_6, (C) and dichloromethane-d_2.

for ester flexiball **37a·37a** sharpen significantly upon encapsulating an appropriate guest such as salt $\mathbf{43}^+\cdot BF_4^-$ (Figure 5, Chart 1). Furthermore, two different sets of signals appear for the added guest, one for the free, uncomplexed guest, and the other one for an encapsulated guest. The latter signals integrate in a 1:1 ratio with those of the dimeric capsule. The differences in chemical shifts for signals of free and bound guests are somewhat smaller as compared to other capsules. However, the existence of two independent sets does not only provide evidence for encapsulation but also points to slow exchange on the NMR time scale.

The centerpiece using the resorcin[4]arene derivative **42** resulted in discrete dimer formation in typical noncompetitive solvents, for example, dichloromethane-d_2, benzene-d_6, toluene-d_8, xylene-d_{10}, or mesitylene-d_{12},[6b] but to our initial surprise, more competitive solvents such as acetone-d_6 and THF-d_8 were also accommodating (Figure 6).

Even so, our hope that **42a** would heterodimerize with the less rigid monomer **40** was not fulfilled. Geometrical differences between the two monomers and the intramolecular hydrogen bonding in **40** conspired to make heterodimerization unfavorable. Unfortunately, heterodimerization experiments with **42a** and **42c** also turned out to be difficult to interpret: evidently, the chemical and structural differences between the monomers are too small. In any event, the NMR spectra of equimolar mixtures of both compounds in acetone-d_6 or dichloromethane-d_2 did not unambiguously show the formation of the desired heterodimer **42a·42c**. The signal crowding with the resonances of the homodimers **42a·42a** and **42c·42c** left no room for clear interpretation.

The D_{4d} symmetric capsules **42·42** (Figure 7) feature a cavity of ~0.95 nm^3 (still the largest cavity known for a hydrogen-bonded dimeric self-assembly to date) with even larger holes than the smaller capsules.[38] Fast exchange with benzene, toluene, or *p*-xylene was observed in NMR experiments when the corresponding nondeuterated solvents were added: no signals for trapped solvent molecules could be detected. However, larger, geometrically and chemically complementary guests can be encapsulated in mesitylene-d_{12}. Specifically, dibenzo-24-crown-8, cryptand[2.2.2] and its alkali (e.g., $\mathbf{50}^+SCN^-$)[6b] and earth alkali metal inclusion complexes ($\mathbf{51}^{2+}(ClO_4^-)_2$ and $\mathbf{52}^{2+}(Cl^-)_2$) were encapsulated in this medium or in acetone-d_6. This was also the case for 2,2′-bipyridine complexes and 1,10-phenanthroline complexes (Figure 8).

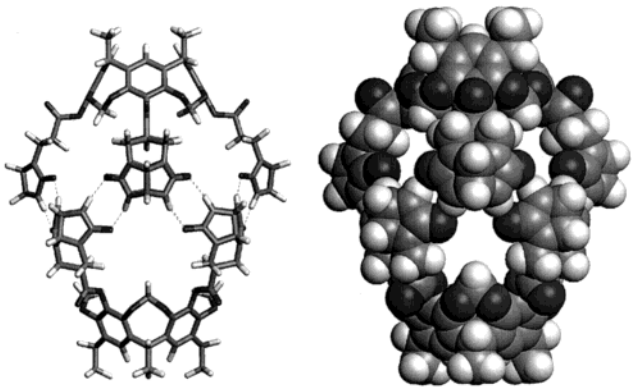


Figure 7. Computed polytube and CPK models of the homodimer **42a·42a** (side chains are omitted for viewing clarity).

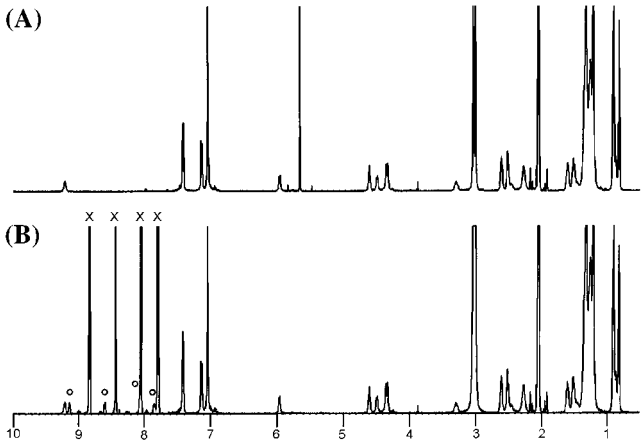


Figure 8. ^{1}H NMR (500 MHz, 273 K, acetone-d_6) of tetraester flexiball **42a·42a** (A) without guest and (B) with excess of added guest tris-(1,10-phenanthroline)-iron(II) hexafluorophosphate. Signals attributed to the (1,10-phenanthroline)-iron(II) coordination complex are marked ($\times$ = free; $\circ$ = encapsulated). Integration indicates a 1:1 capsule-to-guest ratio.

In summary, there are three unambiguous pieces of evidence for the formation of capsules rather than unspecific aggregates from ^{1}H NMR experiments. These hold for the trivalent as well as the tetravalent glycolurils: (i) sharp and concentration independent spectra indicating the formation of discrete species, (ii) the formation of heterodimeric assemblies, (iii) guest encapsulation.

ESI-MS Experiments. Earlier, we described an electrospray ionization mass spectrometric (ESI-MS)[39−41] protocol for the structural characterization of capsules containing quaternary ammonium ions as guests.[18a−d] These complexes are easily electrosprayed from noncompetitive solvents, such as chloroform, methylene chloride, or the moderately competitive acetone, and can be characterized by isotope pattern analysis, providing information about the elemental composition and the charge state. If isotopically labeled guests are used, characteristic mass shifts are observed from which the number of guests and, indirectly from the mass difference, the number of capsule monomers in the complex can be deduced. Experiments described here provide compelling evidence for the structures of these host−guest complexes in solution. Collision experiments confirm that the capsular structure is retained in the gas phase.

The capsules presented here differ from those examined earlier in several aspects that rendered mass spectral characterization more difficult. First, their cavity sizes far surpass those studied previously by MS and make the identification of suitable guests challenging. Second, the large molecular masses for these hosts (up to 3909 amu for monomer **42c**) approached or even exceeded the mass range of our instruments (m/z < 4000). Therefore, we chose dicationic guests to lower m/z ratios within our detection limit. These guests behaved exactly the same way monocationic guests do. Third, the modular strategy involves coupling modules to spacers through single bonds. As a consequence, these capsules possess larger holes in the capsule walls and greater flexibility in the binding sites than those in previous capsules. These features can hasten guest release in the gas phase and obscure collision experiments, which delivered additional compelling evidence for a capsular structure of the softballs even in the gas phase.[18a] Finally, **35a·35a** and **36a·36a** are isomeric species given their identical elemental composition, and **37a·37a** differs by only 6 amu from its amide analogues. These small mass differences could hinder the quantitative detection of heterodimers because of poor separation of capsular isotope patterns.

The mass spectrometric investigation was carried out with the guests **43**$^+$−**49**$^{2+}$, in the form of their BF_4^- salts, as well as **51**$^{2+}(ClO_4^-)_2$,[6b] **52**$^{2+}(Cl^-)_2$, and **53**$^{2+}(Cl^-)_2$ (Chart 1). As shown in Figure 9, m/z ratios corresponding to flexiballs **35a·35a**, **36a·36a**, and **37a·37a** encapsulating monocationic guest **44**$^+$ were detected at the far limit (Figure 9A−C). Similarly, **43**$^+$ gave clean mass spectra. In fact, the only intense signals correspond to 1:1 complexes of flexiballs to guest, that is, **[44**$^+$**@35a·35a]** (m/z = 3990), **[44**$^+$**@36a·36a]** (m/z = 3990), and **[44**$^+$**@37a·37a]** (m/z = 3996).[42] A very similar result is found for complexes containing doubly charged **49**$^{2+}$. Well within our detection window, these base peaks correspond to **[49**$^{2+}$**@35a·35a]** (m/z = 2097), **[49**$^{2+}$**@36a·36a]** (m/z = 2097), and **[49**$^{2+}$**@37a·37a]** (m/z = 2100). Some ions of low abundance are detected which correspond to the protonated monomers and monomer−guest complexes.

The dicationic guests **45**$^{2+}$−**49**$^{2+}$ are not soluble enough in $CHCl_3$ for use in these studies. Therefore, acetone was chosen as the solvent for their characterization. The measured isotope patterns of the capsule ions (insets in Figure 9) are not resolved into separate isotope peaks. These cannot be ions with only one

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(41) For examples of MS studies on hydrogen-bonded supramolecular complexes, see: (a) Russell, K. C.; Leize, E.; Van Dorsselaer, A.; Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 209. (b) Cheng, X.; Gao, Q.; Smith, R. D.; Simanek, E. E.; Mammen, M.; Whitesides, G. M. *J. Org. Chem.* **1996**, *61*, 2204. (c) Jolliffe, K. A.; Crego Calama, M.; Fokkens, R.; Nibbering, N. M. M.; Timmerman, P.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1247. (d) Scherer, M.; Sessler, J. L.; Moini, M.; Gebauer, A.; Lynch, V. *Chem.—Eur. J.* **1998**, *4*, 152. (e) Timmerman, P.; Jolliffe, K. A.; Crego Calama, M.; Weidman, J.-L.; Prins, L. J.; Cardullo, F.; Snellink-Ruël, B. H. M.; Fokkens, R. H.; Nibbering, N. M. M.; Shinkai, S.; Reinhoudt, D. N. *Chem.—Eur. J.* **2000**, *6*, 4104−4115.

(42) The following nomenclature has been employed: **[43**$^+$**@24·24]** means that guest ion **43**$^+$ is encapsulated (indicated by the "@" sign) within the dimer of flexiball **24·24**. In contrast, **[43**$^+$**·24]** indicates that **43**$^+$ and **24** form a complex with a structure which is not further specified.

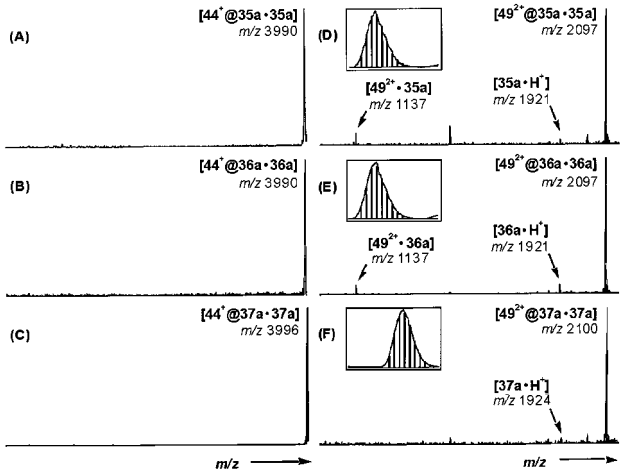

Figure 9. ESI mass spectra of $CHCl_3$ solutions of $\mathbf{44}^+$ BF_4^- (75 μM) as the guest salt with (A) **35a** (50 μM), (B) **36a** (50 μM), and (C) **37a** (50 μM) (m/z = 1900−4000 amu) and acetone solutions of $\mathbf{49}^+$ $(BF_4^-)_2$ (75 μM) as the guest salt with (D) **35a** (50 μM), (E) **36a** (50 μM), and (F) **37a** (50 μM) (m/z = 1000−2150 amu). The insets show the measured isotope patterns together with those calculated on the basis of natural abundances for the dicationic capsules (m/z = 2093−2108 amu).

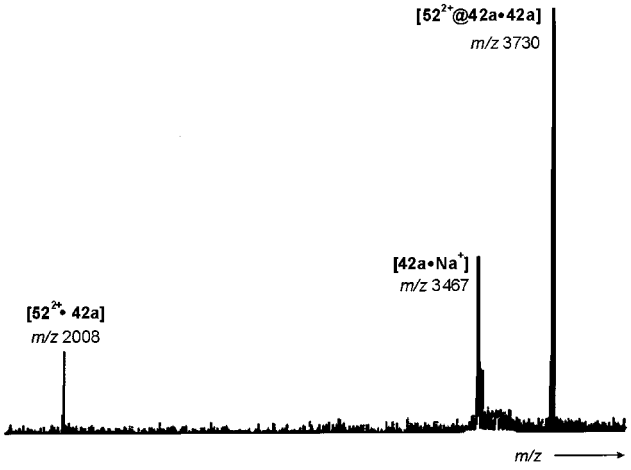

Figure 10. ESI mass spectra of acetone solutions of $\mathbf{53}^{2+}$ $(Cl^-)_2$ as the guest salt (7.5×10^{-5} M) (m/z = 1000−4000 amu). Guests $\mathbf{45}^{2+}$−$\mathbf{49}^{2+}$, $\mathbf{51}^{2+}$, and $\mathbf{52}^{2+}$ give similar spectra.

charge, because monocations in this mass range could easily be separated with the resolving power of $m/\Delta m \approx 2000$ provided by the instrument. In addition, the measured curve fits beautifully the intensities calculated for the doubly charged complexes on the basis of natural isotope abundances, an indication for the correct composition of the ions. Essentially the same results were obtained for the tetraester dimer **42a·42a**, where complexes containing doubly charged $\mathbf{45}^{2+}$, $\mathbf{48}^{2+}$, $\mathbf{49}^{2+}$, $\mathbf{51}^{2+}$, $\mathbf{52}^{2+}$, or $\mathbf{53}^{2+}$ could be observed (Figure 10).

Two experiments provide evidence of the hydrogen-bonded nature of these capsules. First, addition of highly competitive solvents to the sample solutions disrupts the assemblies. For example, upon addition of methanol, new signals appear for the protonated monomers and dimers at the expense of the capsule signals. The proton-bridged dimer does not contain a guest suggesting an unspecific structure other than capsular. Second, heterodimers can be detected by MS (Figure 11). Because of the small mass difference of $\Delta m/z = 3$ for doubly charged **[$\mathbf{49}^{2+}$@35a·35a]** or **[$\mathbf{49}^{2+}$@36a·36a]** as compared to **[$\mathbf{49}^{2+}$@37a·37a]**, the isotope patterns of the homodimers

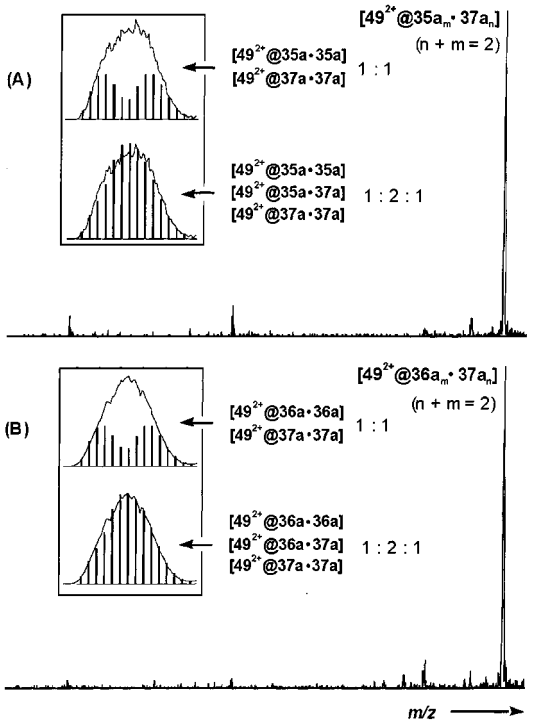

Figure 11. ESI mass spectra of acetone solutions of $\mathbf{49}^{2+}$ $(BF_4^-)_2$ (75 μM) as the guest salt with (A) **35a** (25 μM) and **37a** (25 μM) and (B) **36a** (25 μM) and **37a** (25 μM) (m/z = 1000−2150). The insets compare the measured isotope pattern with those calculated for either a 1:1 mixture of the two homodimers or a statistical 1:2:1 ratio of the homo- and heterodimers.

overlap. Furthermore, the heterodimer is expected to appear between the two homodimers and could complicate its detection. Comparison of the measured signal shapes of ions sprayed from acetone solutions containing equimolar amounts of **35a** and **37a** (Figure 7A) or **36a** and **37a** (Figure 7B), however, reveals a good fit with the isotope patterns calculated for a 1:2:1 ratio of the homo- and heterodimers. In contrast, the patterns calculated for the two homodimers alone do not fit at all. This provides qualitative evidence for the formation of heterodimers and is expected to reflect the statistical distribution found in solution[36] for these complexes. Similarly, in the mass spectrum of a mixture of **42a** and **42c** with guest $\mathbf{51}^{2+}$, the heterodimer **[$\mathbf{51}^{2+}$@42a·42c]** (m/z = 3910) could be seen. NMR experiments suggested, but did not reveal, clear-cut evidence for formation of a heterodimer from **35a** and **38a**. The mass spectra obtained from a mixture of these two monomers with guest $\mathbf{49}^{2+}BF_4^-$ clearly showed signals for the two expected assemblies **[$\mathbf{49}^{2+}$@35a·35a]** and **[$\mathbf{49}^{2+}$@35a·38a]**. As anticipated by the NMR experiments, only a very weak signal for **[$\mathbf{49}^{2+}$@38a·38a]** was detected. The destabilizing tendency caused by **38a** is also reflected in the much lower signal intensity for **[$\mathbf{49}^{2+}$@35a·38a]** as compared to the homodimer **[$\mathbf{49}^{2+}$@35a·35a]**.

To explore the nature of these host−guest complexes in solution, we performed competition experiments with doubly charged ammonium ions $\mathbf{45}^{2+}$−$\mathbf{49}^{2+}$ and their binding to ester flexiballs **37a·37a**. The structural similarity of these guests should result in similar properties if nonspecific binding occurs. However, selectivity in guest binding would indicate that the guests are encapsulated within the cavity of the capsules. While guest selectivity was observed for these systems in the order of $\mathbf{45}^{2+} < \mathbf{46}^{2+} < \mathbf{47}^{2+} < \mathbf{48}^{2+} < \mathbf{49}^{2+}$, it was less pronounced than that in previously studied capsules.[18] For instance, the best

guest, $\mathbf{49}^{2+}$, is only about 15 times better than $\mathbf{45}^{2+}$. In view of the flexibility of the capsule monomer, this result is not surprising. As supported by molecular modeling,[43] the capsule can adapt to the shape of guests and can accommodate even large guests such as $\mathbf{47}^{2+}$ or $\mathbf{48}^{2+}$ in a significantly deformed capsule. In the series of *n*-alkyl substituted paraquat salts, $\mathbf{45}^{2+}$ and $\mathbf{46}^{2+}$ are too small to be good guests alone and require solvent molecules to be coencapsulated with them. While they bear appropriate lengths, their small widths preclude sufficient van der Waals contacts with the capsule walls. Surprisingly, the long *n*-heptyl side chains featured by $\mathbf{49}^{2+}$ do not hamper the assembly. On the contrary, their presence seems to be quite favorable. Modeling suggests two possible scenarios for encapsulation of $\mathbf{49}^{2+}$. The side chains might fold in yielding a shape more congruent with that of the cavity than $\mathbf{45}^{2+}$ or $\mathbf{46}^{2+}$ and filling the space inside the cavity more favorably. However, several degrees of rotational freedom would be restricted, which makes this model less likely from an entropic standpoint. Alternatively, the side chains can easily fit through the large holes in the capsule walls. Regardless of which mode operates, the size selectivity observed in these experiments, although modest, points to a capsular structure and strongly supports the solution phase studies.

Like the NMR experiments, the mass spectrometric results strongly point to the formation of dimeric capsular complexes. One might argue that unspecific aggregates are often formed in electrospray ionization mass spectrometric experiments. Indeed, in the control experiments with methanol added to our sample solutions such unspecific dimers are indeed observed. However, the unspecific aggregates never carried a guest ion but rather were proton-bridged complexes. Furthermore, no dimer−guest complexes can be detected by mass spectrometry, which do not also assemble in solution: The mass spectrometric results exactly parallel the NMR data. The methods are complementary but independent and thus yield clear evidence for capsule formation.

Conclusions

The greater ease of synthesis, even on a gram scale, is one of the most advantageous properties of the flexiballs. The modular strategy rapidly provided a whole family of capsules, and it can be extended to various central spacers as long as they provide the necessary conformational rigidity. With these building blocks, there is a tool kit in hand that permits a greater range of cavity volumes and shapes to be fashioned. The cavity volumes of the capsules described in this paper range between 0.45 and 0.95 nm^3. The capsules have been thoroughly characterized by NMR and MS experiments, two independent but complementary methods. As reported before for other capsules with different hydrogen bonding patterns,[18] the results from both methods are in complete agreement.

A second aspect of note is the presence of functionality, for example, the secondary amide protons of flexiball **35a**, inside the cavity. The synthetic strategy offers some flexibility as to which functionality appears at what position. For example, monomer **35a** exposes hydrogen bond donors to the cavity while its inverted analogue **36a** provides acceptors. This may well be the reason for the striking differences in their guest selectivity.[6] Removal of these NH protons in ester flexiball **37a** and the modulation of the cavity volume by alkylating the amides with methyl groups further support the concept.

The flexiballs are also good examples of the contributions of entropy to self-assembly. Unspecific aggregation of **27a**·**27a** and the formation of heterodimeric capsules from **27a** and **35a** demonstrate how close these systems are to the thin borderline between ordered self-assembly and chaotic aggregation: Freezing only 3 out of 18 otherwise freely rotating single bonds is sufficient to ensure assembly. Similarly, the entropic influences of the rotational barrier of esters versus amides and the relative energies of the *E* and *Z* conformers of tertiary versus secondary amides are exposed through the NMR and mass spectra. An analogous effect has been observed for the calixarene- and resorcinarene-based flexiballs **40** and **42**. The calixarene scaffold of **40** is less restricted and can adopt a pinched cone conformation that hampers dimerization; the resorcinarene **42** has an enforced cone conformation and assembles nicely in solution.

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