Architectural Chemistry: Synthesis of Topologically Diverse Macromulticycles by Sequential Multiple Multicomponent Macrocyclizations

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Abstract: How can conformationally restricted polyvalent molecules be accessed rapidly? A sequential approach involving two *m*ultiple *m*ulticomponent *m*acrocyclizations *i*ncluding *b*ifunctional *b*uilding *b*locks (MiBs) with up to five Ugi-four-component reactions (Ugi-4CR) has been developed to produce nonsymmetric macromulticycles. Topologically diverse structures, such as nonsymmetric cryptands and clam- and igloo-shaped macromulticycles were obtained in reaction sequences that comprise the incorporation of up to 13 building blocks by forming 20 new bonds without purification of intermediates. Cryptands were produced by a sequential-MiB procedure in which the Ugi-type functional groups of the second MiB are attached to the peptoid backbones from the first multicomponent macrocyclization. These macrobicycles show two completely new features; i.e., three different tether chains can be obtained in one pot, and tertiary amide bonds are used as bridgeheads. Alternatively, the same reaction sequence, i.e., MiB/deprotection/MiB, can be used to produce clam-shaped macrobicycles, demonstrated with a tetrafunctional cholanic steroid as a hinge moiety. Macrotetracycles endowed with igloo-type topologies are accessible by an advanced protocol featuring consecutive double and 3-fold Ugi-4CR-based macrocyclizations. Other building blocks than cholanic steroids employed include aryl, heterocyclic, polyether, and other recognition motifs. The examples given are a first-generation demonstration of an "architectural chemistry" that allows to construct three-dimensional multimotif covalent molecular "buildings" of unprecedented complexity by design.

Introduction

The design and facile synthesis of macrocyclic compounds endowed with diverse topologies and functional domains is of dominant importance for the development of supramolecular chemistry and in some areas of nanotechnology and catalysis. Like supramolecular chemistry, bottom-up nanotechnology is based on the chemist's ability to construct large functional molecules from simple building blocks. Desirable are methods that allow moving away from the now common homooligomers, random self-assembly, or noncovalent networks toward the plannable construction of three-dimensional molecules of high complexity with covalent (and noncovalent) bonds formed in predefined manner. Ideally, the topological and functional possibilities of such molecules should be—in principle—comparable (but not identical) to those achieved by proteins. Architectural chemistry has the goal to devise design rules and synthesis tools for the fast and efficient assembly of complex three-dimensional molecules at will and from simple building blocks, similar to building planning and construction.

A variety of synthetic receptors, such as cryptands, cyclophanes, and cages, are early examples of rather simple mostly homo-oligomeric structures. They nevertheless demonstrated their applicability in coordination chemistry and molecular recognition due to their potential as encapsulating molecules.[1] Other compounds presenting more complex and unconventional topologies, such as interlocked and knotted molecules, have recently proved their potential as prototypical molecular devices.[2] Indeed, the advent of real applications for all these macrocycles has been only possible due to the progress in templated and nontemplated macrocyclization strategies leading to them.[1,3]

Recently, we have described the development of a novel synthetic methodology for the one-pot assembly of complex, nonrepetitive macrocycles.[4] The approach relies on the performance of *m*ultiple *m*ulticomponent *m*acrocyclizations *i*ncluding *b*ifunctional *b*uilding *b*locks (MiBs). Although several multicomponent reactions (MCRs) in principle work,[4b] the main focus was on the use of the Ugi-four-component reaction (Ugi-4CR) due to the tremendous capability of this process to generate molecular complexity and diversity. In addition, this methodol-

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(2) (a) Felder, T. Schalley, C. A. Artificial Rotary Motors Based on Rotaxanes. In *Highlights in Bioorganic Chemistry*; Wennemers, H., Schmuck, C., Eds.; Wiley-VCH: Weinheim, 2004; p 256. (b) Balsani, V.; Venturi, N.; Credi, A. *Molecular devices and machines: A journey into the nanoworld*; Wiley-VCH: Weinheim, 2003.

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ogy has proven to be suitable for the rapid construction of more challenging, peptidic macrobicycles such as cryptands, cryptophanes, and steroid-based cages.[5]

Cryptands are synthetic receptors capable of binding either ions or neutral molecules depending on the features of the macrobicyclic cavity.[6] In general, these and other macromulticycles are endowed with improved binding and inclusion properties compared to their macrocyclic analogues.[1] Most cryptands (e.g., benzene- and tren-bridgeheaded ones) are based on C_3-symmetric building blocks.[1,6] This feature renders their cores symmetric as a whole and makes it difficult to achieve selective recognition, for example of chiral guests, as well as further differentiation of the tether chains. Another important type of multicyclic receptors includes the so-called laterally nonsymmetric cryptands.[7] These are macrobicycles wherein the two bridgehead atoms are connected by one tether different from the other two bridging chains. The examples of this type reported in the literature are endowed with a typical dissymmetry arising from a symmetry plane running through the unlike tether.[7] Also, the peptidic macrobicycles obtained in our previous work[5] present either a C_3-symmetry or symmetry plane owing to the 3-fold character of the macrocyclization protocol as well as the symmetric nature of the trifunctional building blocks.

In view of an increasing interest in three-dimensional large molecules as well as in chiral recognition through encapsulation processes, we set out to develop an alternative approach capable

of producing genuine nonsymmetric cryptands[8] and other types of macromulticycles presenting hybrid peptidomimetic skeletons with varied molecular topologies. Such complex frameworks may include additional recognition or functional motifs (e.g., aromatic, heterocyclic, cholanic steroids, hydrogen bonding, etc.) to address the encapsulation capability of selected guests or for external binding domains suitable for supramolecular self-assembly. Herein we report on the sequential use of Ugi-MiB procedures to assemble nonsymmetric, peptoid-containing macromulticycles featuring cryptand-like as well as clam- and igloo-shaped architectures.

Results and Discussion

Scheme 1 is a schematic representation of the synthetic planning devised to implement two consecutive double Ugi-4CR-based macrocyclizations. Thus, cryptand and clam-type macrobicycles can be produced by performing a first MiB approach, followed by deprotection of further Ugi-reactive functional groups appended, and a subsequent MiB that may be or not of the same type as the former. Cryptands can be obtained when the functional groups taking part in the second macrocyclization are attached to the peptoid backbones resulting from the first MiB. In this case, the bridgehead cores of the final cryptands are tertiary amide bonds included in the peptoid skeletons arising from the first double Ugi-4CR-based macrocyclization (see Scheme 1A, cf. also Scheme 2). This structural feature is not found in any other type of macrobicyclic receptor and should influence the complexation properties of these unique macrobicyclic architectures.

Likewise, clam-shaped macrobicyclic compounds can be produced by utilizing tetrafunctional steroidal scaffolds by the same reaction sequence, i.e., MiB/deprotection/MiB (Scheme 1B, cf. also Scheme 4). The concave shape of cholanic scaffolds enables the precise geometrical positioning of each pair of Ugi-type functionalities to achieve the varied clam-type topologies. These are suitable to host two different guests in a defined proximity range to study their interaction. Of course, the clam design requires that two of the four functional groups originally positioned on the steroidal moiety remain protected during the first MiB process.

Very few types of ring-closing reactions are usually employed in supramolecular chemistry for the production of macrocyclic receptors.[1–3,6] This represents a marked limitation with regard to the structural diversity that is required for, e.g., screening of recognition and catalytic properties. Hence, most multiple macrocyclization protocols used in this field are based on the formation of imine and amide bonds by reaction of primary polyamines with aldehyhes and acyl chlorides (or active esters), respectively.[1–3,6] Another procedure widely used for the construction of synthetic receptors is the Richman–Atkins approach,[9] which involves the nucleophilic substitution of dihalides or their analogous tosylates and mesylates with disulfonylamides or diols in the presence of a base. All these procedures, besides serving as the ring-closing reaction, also incorporate binding functionalities into the final macrocyclic receptor. A different case is the ring-closing metathesis, which

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Scheme 1. Approach Towards Topologically Diverse Macrobicycles Based on Two Sequential Ugi-MiBs of the Same Bifunctional Combination[a]
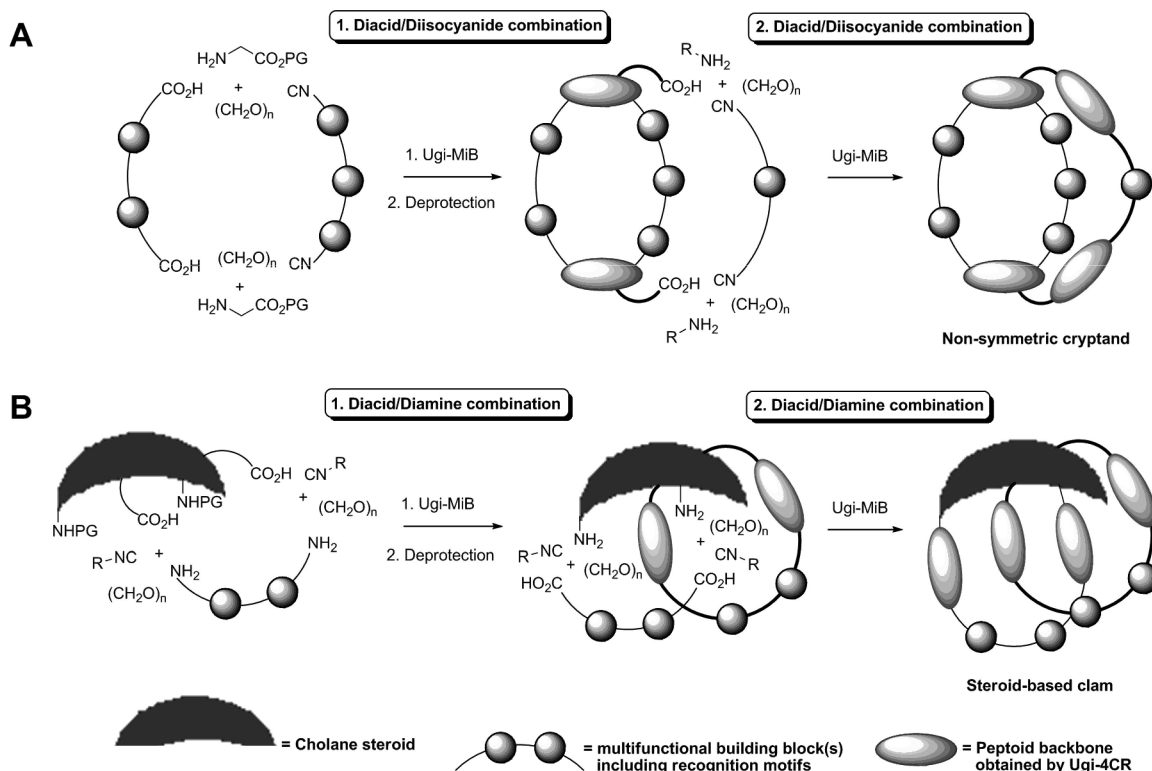
[a] See text for details; PG = Protective Group; A: Cryptands; B: Clams.

has been widely employed as the final bond-forming reaction in the synthesis of interlocked molecules, but it does not introduce a dominant recognition motif.[3b−d]

The MiB methodology was developed to accomplish several tasks in one step, e.g., to serve as multiple and simultaneous ring-closing reactions of polyfunctional building blocks and to incorporate several binding motifs with impressively low synthetic effort.[4,5] As demonstrated in this paper, the sequential MiBs allow a remarkable increment of skeletal diversity of macromulticyclic scaffolds by simply tuning the nature of the building blocks and the combination of reacting functional groups employed in each MiB. All MiB−deprotection−MiB protocols can be performed in one pot, i.e., without the necessity to isolate the intermediate macrocycle in protected or deprotected form. Ugi-4CRs can be performed in a wide variety of solvents and in the presence of, e.g., water (as used for protective group hydrolysis), mineral or Lewis acids, salts, or air.

Scheme 2 summarizes three different combinations of consecutive MiBs from all the possible permutations accessible by this sequential procedure. Because of the four components taking part in the Ugi-4CR[10] (i.e., a primary amine, an oxo compound, a carboxylic acid, and an isocyanide), six different combinations of bifunctional building blocks are possible: (i) diacid/diamine, (ii) diacid/diisocyanide, (iii) diacid/dialdehyde, (iv) diisocyanide/diamine, (v) diisocyanide/dialdehyde, and (vi) diamine/dialdehyde.[4] Accordingly, up to 36 dissimilar macrobicycles can be produced just by repeating two macrocyclization steps in a sequential manner. Indeed, the synthetic scope with regard to the accessible diversity of nonsymmetric cryptands is truly

remarkable when considering the great amount of readily available bifunctional building blocks containing Ugi-reactive functional groups. MiB approaches including dialdehyde building blocks have not been included in the present work as they can lead to the formation of stereoisomers and thus cause analytical complications. For the same reason, paraformaldehyde was employed as an oxo-component, although other aliphatic aldehydes usually give better yields. The resulting partially N-substituted oligoglycine moieties (i.e., peptoids) in the cryptand cores have no stereocenters, and thus single isomers result.

As depicted in Scheme 2, one of the building blocks taking part in the first Ugi-MiB must contain a protected, or initially unreactive, Ugi-reactive functional group to be subsequently activated for the next macrocyclization. The formation of several amide bonds during the sequential MiBs is one of the key features for any potential application of Ugi-based multimacrocycles in supramolecular chemistry. Amide-containing macrobicycles are one of the most exploited types of receptors due to their remarkable binding capability toward a variety of guests.[1,3j,6b,11] Moreover, the presence of both substituted and nonsubstituted amides alongside the macromulticyclic scaffold opens up possibilities either for the coordination of cations and anions or for the recognition of neutral guests based on hydrogen bonding.[11]

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Scheme 2. Synthesis of Nonsymmetric Cryptands by Two Sequential Double Ugi-4CR-Based Macrocyclizations: (A) Diacid/Diisonitrile−Diacid/Diisonitrile Combination; (B) Diacid/Diisonitrile−Diamine/Diisonitrile; (C) Diamine/Diisonitrile−Diamine/Diacid, With Further Orthogonally Protected Diamine Available
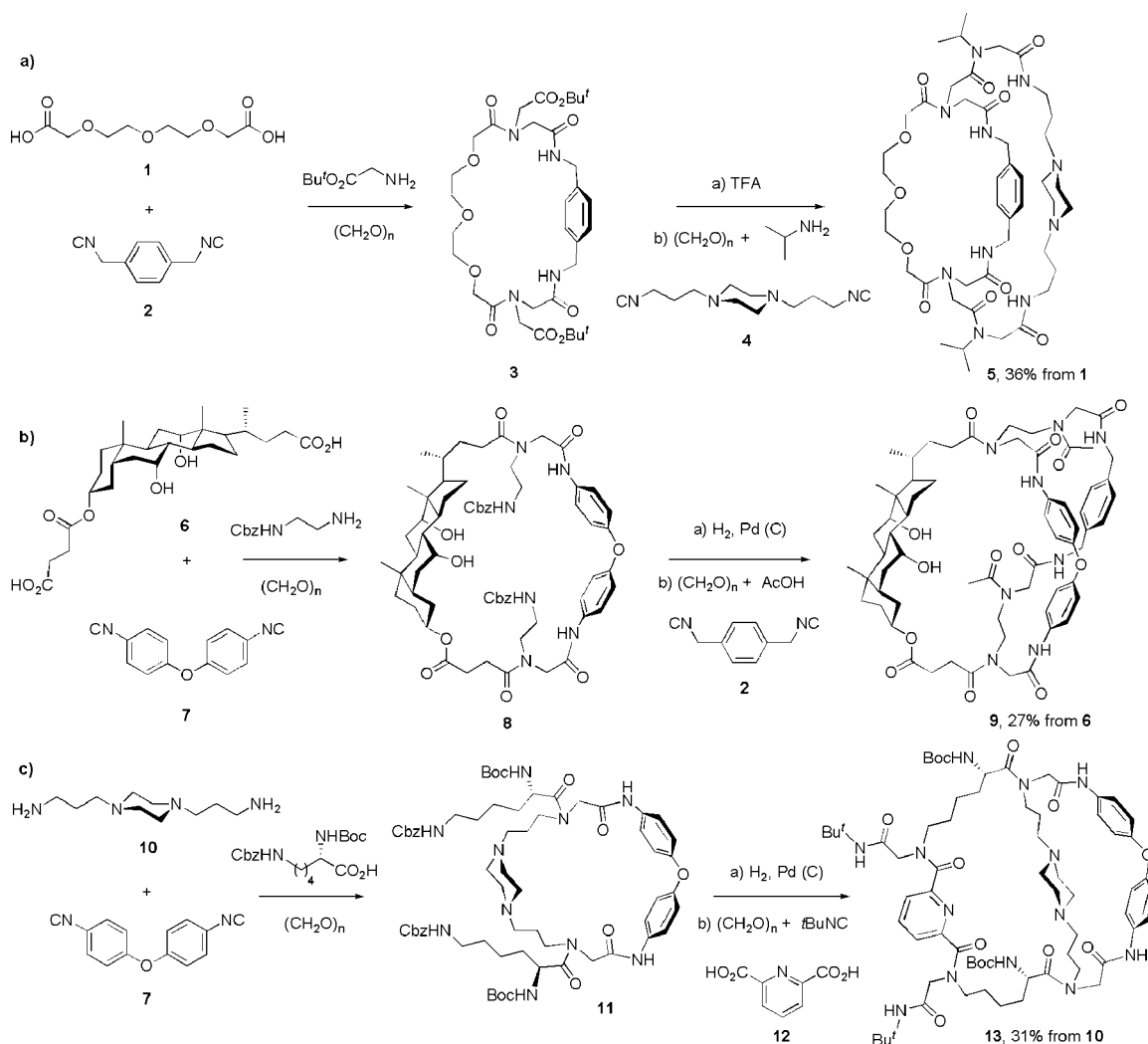

Rather than covering all possible combinations, our aim is to demonstrate the significant molecular complexity that can be reached in a very simple reaction sequence which involves a "sandwich" of two macrocyclizations and a simple deprotection step. All macrocyclization reactions were performed under pseudo-high-dilution conditions, implemented by slowly adding the bifunctional building blocks to a stirred solution of the other components. These experimental conditions have been previously standardized to give high macrocyclization yields (up to ca. 80%, usually ca. 45−55%) even in the absence of any template,[4] albeit such high yields may require reaction times as long as 5−7 days. Templating can give even higher yields and faster reaction without pseudo-high-dilution.[5c] In addition to the desired macrocycles, typical Ugi-MiBs also may furnish certain amounts of uncyclized product,[4c] which if required can be easily removed together with any excess of a monofuncional component by flash chromatography or by consecutive acidic and basic washings. Following this protocol, the first-generation macrocycles were not purified by chromatography for detailed characterization but only identified by ESI-MS, then deprotected, and the resulting crude intermediates were subjected to the next double Ugi-4CR-based macrocyclization. Hence, the yields given in Schemes 2, 4, and 5 refer to the overall process and can be considered as excellent regarding the great structural

complexity and the high number of bonds formed during the sequences. For example, 16 bonds are formed in the reactions of scheme 2 including two macrocyclizations. The simplicity and reliability of protection/deprotection processes available for the Ugi-reactive amino, carboxylic acid, and oxo functionalities, mainly as carbamate, ester, or ketal, respectively, contribute to the success of the sequential MiB approaches. In this regard, the more challenging combination that involves an isocyanide functionality not reacting during the first MiB to be later activated for the second one may be regarded as a future option. This appears possible due to the known differential reactivity of, e.g., aliphatic isocyanides versus aromatic ones,[4c] or simply by forming the isocyanide group from its precursor (i.e., amine or formamide) once the first-generation macrocycle has been produced.

The examples shown in Scheme 2 illustrate the production of large cryptands by using diacids, diisocyanides, and diamines containing a wide variety of recognition motifs, e.g., steroidal, aromatic, polyether, and heterocyclic ones. Cryptand **5** was obtained by a sequential diacid/diisocyanide−diacid/diisocyanide combination which takes advantage of the bifunctional character of α-amino acids. Glycine *tert*-butyl ester hydrochloride was used as the amino component to provide, after cleavage of the ester groups, the two carboxylic functionalities required

Scheme 3. Synthesis of Tetrafunctional Cholanic Building Blocks
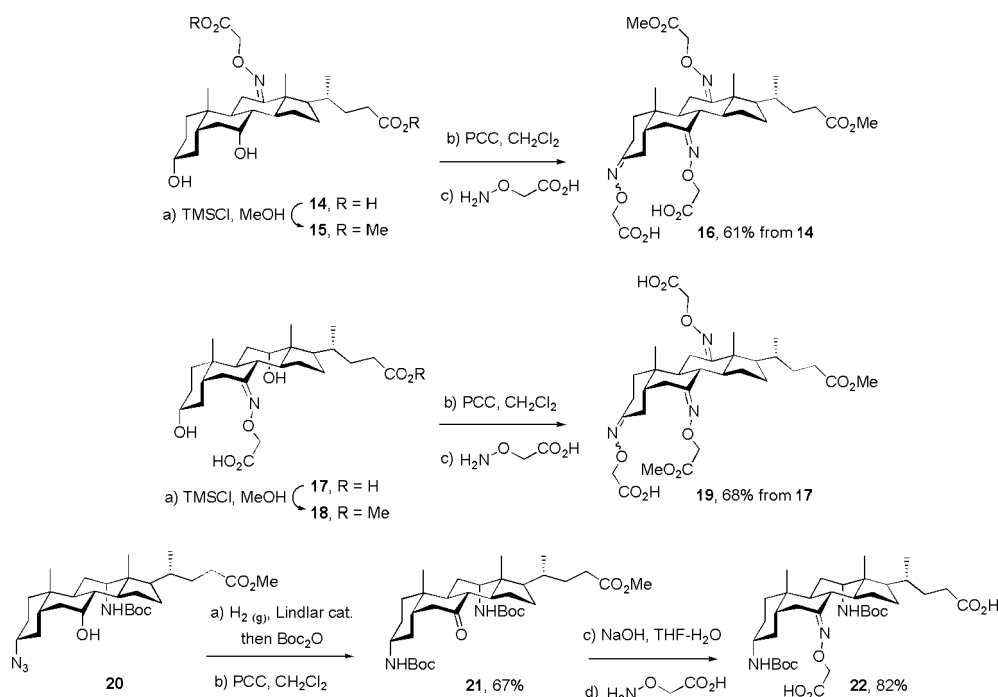
for the second MiB. Mild or orthogonal deprotection procedures are important to not affect either the formed peptoid backbones or other groups within the first-generation macrocycles. For example, partial hydrolysis of the hemisuccinate moiety has been noticed in analogues of macrocycle **8** during the deprotection of methyl ester groups attached to the peptoid backbone. Therefore, as outlined in Scheme 2b, protected amines rather than esters were chosen in this sequence to avoid partial destruction of the macrocyclic intermediate. Variation 2b illustrates the successful use of a monoprotected diamine to accomplish a sequential diacid/diisocyanide−diamine/diisocyanide combination. Cryptand **9** was produced via simple hydrogenolysis of the Cbz-protecting groups in intermediate **8** followed by the second MiB, thereby featuring a perfect orthogonality among all reaction steps.

Both C- and N-protected α-amino acids are well behaved as either an amino or acid component, respectively, in single MiBs.[4,5] The sequential MiB procedure shown in Scheme 2c may be considered especially interesting. It features a diamine/diisocyanide−diamine/diacid combination which employs the orthogonally biprotected α-amino acid L-lysine. The polyfunctional character of lysine allows that, for example, the final cryptand **13** may still behave as an intermediate for a third-generation MiB considering the presence of the additionally appended amino groups protected as carbamates.

As illustrated in Figure 1, due to the presence of three different tether chains in cryptands **5**, **9**, and **13**, a pair of (enantiomeric) topological stereoisomers can appear for each structure. Since the bridgeheads are planar tertiary amides (peptoids), this does not coincide with a bridgehead stereocenter like in carbon bridgeheads. Additionally, in compounds **9** and **13**, one or more of the tether building blocks is chiral itself (i.e., the cholanic steroid and L-lysine), and the two possible topo-stereomers would be topological diastereomers and not enantiomers. Interestingly, HPLC and NMR analyses of compounds **9** and **13** (^{1}H NMR spectra recorded at room and higher temperatures) do not show evidence of diastereomeric mixtures

but of only a single isomer (see the Supporting Information), which may be reasoned by rapid peptoid bridgehead s-cis/s-trans conversions or through the preferential stabilization or−unlikely−formation of one diastereomer. Alternatively, the chiroptical activity of these compounds was studied by circular dichroism (CD).

Figure 2 shows the CD spectra of cryptands **5**, **9**, and **13**, all revealing the occurrence of a single stereoisomer for this type of macrobicycles. It must be noted that cryptands **9** and **13** contain a chiral moiety in the tether chain (**9**) or in the periphery (**13**), while in cryptand **5** the three tethers and all appendages are achiral, so it is a typical dissymmetric structure. The curves observed in the CD spectra of compounds **9** and **13** exhibit multiple Cotton effects that demonstrate their chiroptical activities.[12] Thus, the CD spectrum of compound **9** displays a positive couplet with the maximum at 240 nm and two negative couplets with minima at 210 and 266 nm. Likewise, the CD spectrum of compound **13** shows a negative couplet with the minimum at 238 nm and a positive one with the maximum at 260 nm. However, the CD spectrum of compound **5** lacks any positive or negative Cotton effect over 200 nm. This result indicates that this type of macrobicyclic scaffold does not exhibit chiroptical activity derived either from the $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ transitions. The presence of aromatic and carbonyl groups in the structure of **5** advises that, in the case of the existence of a Cotton effect derived from the topological chirality, the couplets should appear in the region over 200 nm.[12] Therefore, the lack of any positive or negative couplet suggests either the absence of topological chirality in this type of skeleton or its impossibility to be detected by CD spectroscopy. This means that interconversion between rotamers does not comprise the occurrence of topological stereoisomers. In contrast to this, the Cotton effects observed for cryptands **9** and **13** arise from the

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Scheme 4. Synthesis of Clam-Shaped Macrobicycles by Sequential Double Ugi-4CR-Based Macrocyclizations: (a) Diacid/Diisonitrile−Diacid/Diisonitrile; (b) Diacid/Diisonitrile−Diacid/Diamine; (c) Diacid/Diamine−Diamine/Diacid, With Further Orthogonally Protected Tetraacid Available[a]
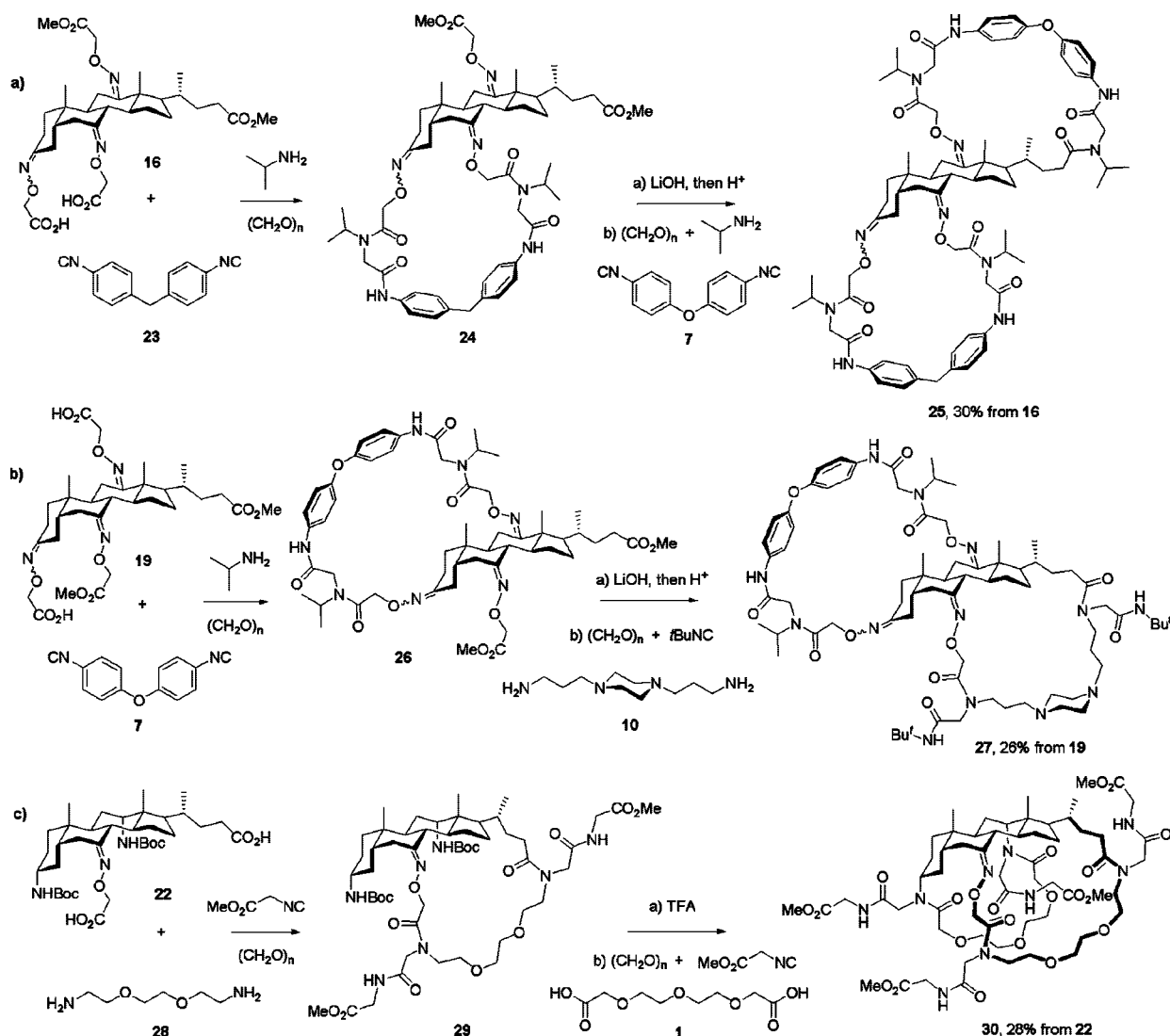
[a] Please note that for **25** and **27** the substituents at the central steroid attachment points are pointing into the concave α-side but that for clarity the macrocyclic rings are drawn at opposite sides of the steroid hinge ("open clam").

central chirality of the steroidal and the lysine moieties, which make the cryptands chiral themselves.

Steroid-based macrocycles have proven their efficiency as supramolecular receptors for a wide variety of guests, ranging from anions to biomolecules like carbohydrates and peptides.[13] An important reason for their common use is the intrinsic preorganization and rigidity, which usually leads to excellent (and sometimes unexpected) macrocyclization results compared to other, more flexible building blocks.[13,14] In addition, the concave shape of cholanic steroids enables the creation of topologically interesting molecules such as the clam-shaped macrobicycles presented in Scheme 3. These latter macrobicycles are endowed with two (not interlocked) macrocyclic rings joined at the steroidal nucleus (cf. Scheme 1).

As shown in Scheme 3, the cholanic derivatives **14** and **17**, previously obtained from cholic acid,[4d] were functionalized to produce the tetracarboxylic acids **16** and **19** with two of their carboxylic groups protected as methyl esters. In this and all further approaches involving cholanic building blocks, the oxime bond formation from ketosteroids was preferred for the installation of carboxylic functionalities as the connection point of the steroidal nucleus. This choice is based on two arguments, the resistance of this junction to ester cleavage under basic

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Scheme 5. Synthesis of Igloo-Shaped Macrotetracycles by Sequential Double and Threefold Ugi-4CR-Based Macrocyclizations: (a, b) Diacid/Diisonitrile−Triacid/Triisonitrile; (c) Diacid/Diamine−Triacid/Triisonitrile Combinations
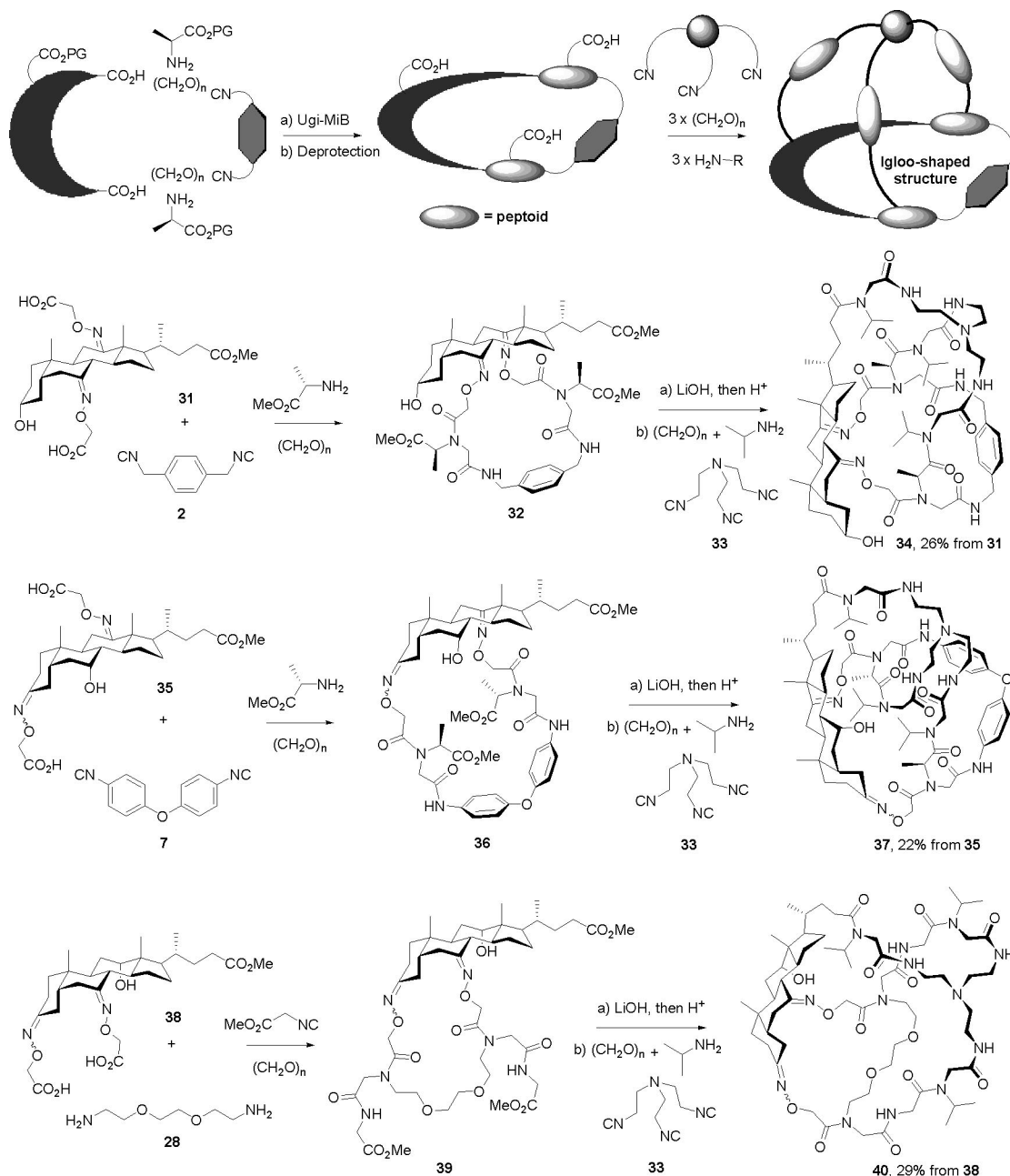

conditions and the difficulty to incorporate other types of functionalized arms (e.g., succinate) through acylation of the axial hydroxyl groups at positions 7 and 12. Another possibility to obtain tetrafunctional steroidal scaffolds is the introduction
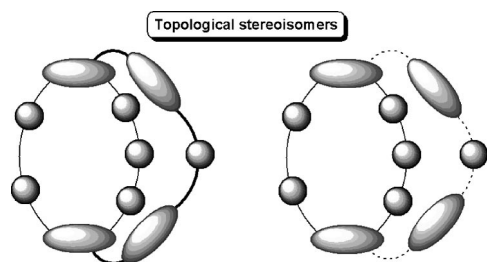
Figure 1. Pair of possible topological stereoisomers derived from the asymmetry of the macrobicycles.

of amino groups directly attached to the steroidal nucleus and not as functionalized arms. For example, the steroidal azide **20**, synthesized according to a procedure reported by Davis and co-workers,[15] is amenable for executing alternative combinations to those allowed by the steroidal tetracarboxylic acids. Thus, compound **20** was reduced to the corresponding amine, followed by protection as carbamate and consecutive PCC oxidation of hydroxyl 7α to furnish the intermediate **21**. Finally, cleavage of the methyl ester at C-24 and condensation of the ketone with *O*-(carboxymethyl)hydroxylamine afforded the diacid **22** in 55% overall yield from **20**. Although oximes in theory can react in

(15) Barry, J. F.; Davis, A. P.; Pérez-Payán, M. N. *Tetrahedron Lett.* **1999**, *40*, 2849–2852.
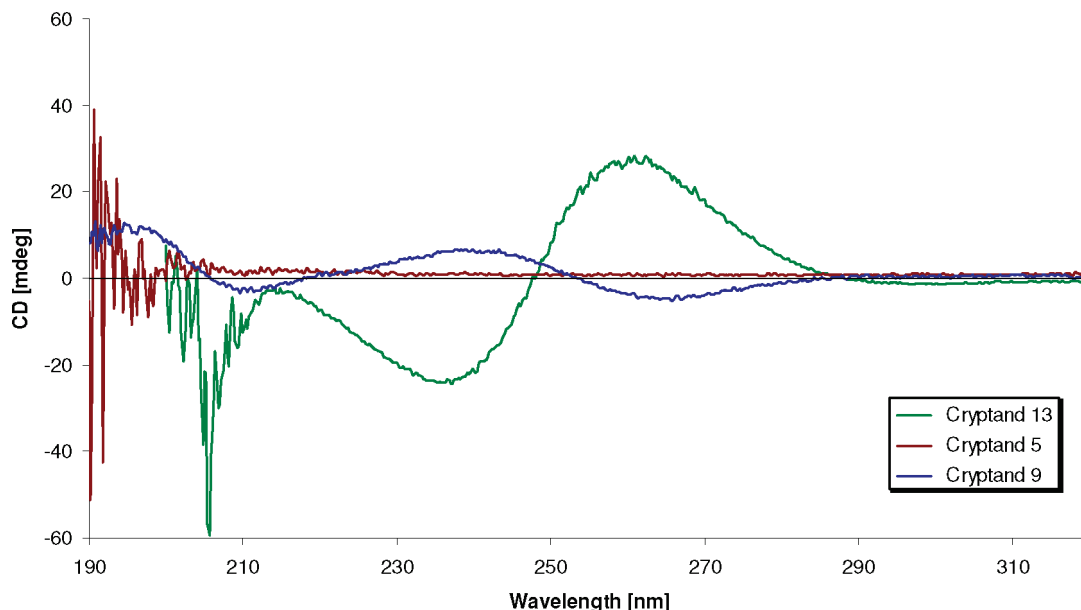
Figure 2. CD spectra of cryptands **5**, **9**, and **13** in methanol.

Ugi-4CRs, this potential side reaction is much too slow to compete with the standard components used for the macrocyclizations.

Scheme 4 illustrates three examples of steroid-based clams achieved by sequential MiBs. Compound **16** was reacted in a consecutive diacid/diisocyanide−diacid/diisocyanide combination to yield macrobicycle **25**, which contains two rims including two similar but different aryl moieties and fully endocyclic peptoid backbones. The very similar, but at the same time distinct diisocyanides **7** and **23**, were employed to allow NMR differentiation of the two rims in further structural and binding studies (see ^{1}H NMR spectrum in the Supporting Information). On the other hand, macrobicycle **27** was produced by a diacid/ diisocyanide−diacid/diamine combination, thereby affording a hybrid structure wherein one of the two rims contains partially exocyclic peptoid moieties. It is noteworthy that both types of macrobicycles, i.e., cryptands and clams, were obtained by incorporation of the same type of building blocks, e.g., steroidal, aryl, and piperidine moieties, into the final macrobicyclic cores. However, their dissimilar topologies arise from the different principles applied to the same synthetic planning, i.e., the cryptands by creating the bridgehead cores during the first MiB and the clams by employing a previously functionalized framework to which the two macrocyclic rings lay appended.

The example shown in Scheme 4c is remarkable considering its potential toward metal ion or ion pair recognition. Macrobicycle **30** was synthesized from steroid **22** by a consecutive diacid/diamine−diamine/diacid combination including the polyether diamine **28** and diacid **1**. This double polyether clam structure comprises all its peptoid backbones partially exocyclic, also containing four carboxylates suitably located for follow up MiBs−or metal ion binding. Compounds such as clam **30** and cryptand **13** perfectly show the potential of the sequential MiB approach toward even more complex multicycles, as they allow extending the sequential principle to further stages. This prospect may lead to new generations of amide-based polycyclic receptors due to the easy performance of the sequential MiBs and the high accessibility of Ugi-type tri- and tetrafunctional building blocks.

A similar approach including bifunctional building blocks has recently been employed for the construction of tetracyclic receptors for (oligo)saccharides, albeit relying on typical peptide coupling protocols.[16] The use of this simple sequential procedure to assemble complex functional receptors foresees important applications for novel macromulticycles, which should be more readily accessible by multiple Ugi-4CRs, and with even more intrinsic complexity if necessary.

A more demanding extension of the sequential MiB approach is the combination with Ugi-4CR-based macrocyclizations of higher multiplicity.[17] Some examples are illustrated in Scheme 5 with the synthesis of macrotetracyclic skeletons featuring igloo-shaped structures. These extremely complex architectures were assembled by the combination of double[4] and 3-fold[5a] Ugi-4CR-based macrocyclizations. The whole process occurs through incorporation of 13 building blocks, forming 20 new bonds in a very efficient reaction sequence to give cages of a defined constitution and with almost any desired degree of complexity within the tethers or on the appendages.

The use of C-protected L-alanine as the amino component in a first double Ugi-4CR-based macrocyclization featuring a diacid/diisocyanide combination led to the macrocyclic intermediates **32** and **36** (Scheme 5a,b). These were subjected to a mild cleavage of the ester groups to afford, upon acidification, the corresponding tricarboxylic acids. These triple Ugi-reactive macrocycles were employed in the assembly of macrotetracycles **34** and **37** by 3-fold Ugi-4CR-based macrocyclizations.

A slightly different route was used for the synthesis of macrotetracycle **40** (Scheme 5c). This cage target was also achieved by sequential double and 3-fold Ugi-4CR-based macrocyclizations, though with the first MiB featuring a diacid/ diamine combination that utilizes methyl isocyanoacetate as the isonitrile component. Accordingly, the macrocyclic intermediate **39** was generated in a triester form, which was consecutively

(16) (a) Klein, E.; Ferrand, Y.; Auty, E. K.; Davis, A. P. *Chem. Commun.* **2007**, *2390*, 2392. (b) Ferrand, Y.; Crump, M. P.; Davis, A. P. *Science* **2007**, *318*, 619–622.
(17) For a preliminary account on parts of the tetramacrocyclic work, see ref 5a.

subjected to deprotection and 3-fold Ugi-4CR-based macrocyclization to afford compound **40** in 29% overall yield, i.e., with some 95% yield for each bond formed, including three macrocyclizations.

The macrotetracycles illustrate the potential of multiple MCRs as a tool in architectural chemistry. The Ugi-MiBs allow combining the various possibilities inherent to MCRs to generate topological diversity by sequential processes of varied multiplicity. The Ugi reaction is an easy, fast, and reliable rivet for the rapid assembly of complex structures. A second requisite for success is access to a set of structurally varied building blocks with multiple functionalization sites (e.g., the steroidal diacids **31**, **35**, and **38**). The combination of bi- or multifunctional building blocks with an easy, efficient, and forgiving reaction lays the foundation for a second round, then going into the third dimension of structural diversity (i.e., topological).

Conclusions

Topologically diverse macromulticycles were produced by some selected examples of the many possible combinations allowed by the sequential use of multiple multicomponent macrocyclizations. The examples shown involve two multiple Ugi-4CR-based macrocyclizations that are performed according to typical macrocyclization protocols. A mild, ideally quantitative deprotection step is required before the second MiB is performed. A very high level of structural complexity can be reached due to the multicomponent nature of the two macrocyclization steps, which in addition allow the formation of several bonds per single operation. Usually no purification of intermediates is needed to achieve a good overall result. In several cases, the whole reaction sequence can be performed in one pot, but for new reactions the isolation of a crucial intermediate is advised for control purposes.

The scope of this methodology to assemble further types of large 3D structures, less or even more complex, in a combinatorial, parallel, or designed way is immense. Compounds devised from such molecular construction chemistry ("architectural chemistry", v.i.) can find applications in nanotechnology, complex biological interactions that require multiple binding domains,[18] or as highly selective supramolecular receptors, especially when considering: (i) the huge amount of readily available building blocks that can be used and (ii) the fast and efficient generation of structural diversity that is achieved in a very short reaction sequence. The building blocks to be incorporated into the final macromulticycles may have various binding elements and shapes, rigidity, or functions that will allow us to create topologically even better defined, more diverse, or multifunctional molecules in the future. The now possible (spatial) differentiation of several binding domains (differential tethers, bridgeheads, or appendages) within one molecule, as is available from a multiple MCR architectural chemistry approach, can have a remarkable influence on the specificity and selectivity of binding profiles, internally, e.g., for chiral guests, or externally for forming predefined supramolecular networks. We believe that the approach presented here opens new perspectives, not only for the synthesis and applications of macromulticycles which are well established in coordination chemistry, molecular recognition, and catalysis but also as a basis for the planned construction of very large but at the same time unique molecular ensembles of a nonrepetitve (nonsymmetrical) nature.

In a more general way, we termed such a design (cf. "blueprint") and three-dimensional construction of covalent molecular buildings like, e.g., the igloos, "architectural chemistry". In architectural chemistry, the chemical building blocks (cf. "stones", "prefab elements") are covalently and usually multiply connected by a limited reaction set or—like here—only one reliable reaction ("bolts" or "mortar") that is repeatedly applied to give the designed three-dimensional structure. Through their covalent bonds such structures differ from supramolecular assemblies or metal-organic-frameworks (MOFs). Also they can be absolutely nonrepetitve and irregular with respect to the building blocks, but nevertheless they have a defined sequence/3D-arrangement and thus constitution, to some extent comparable to (disulfide bridged) proteins that are basically also covalently bound molecules, nonhomooligomeric but linked by repetitve application of the same reaction(s), and which eventually form 3D structures.

Experimental Section

General. Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 399.94 and 100.57 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to the TMS (^{1}H) and to the solvent signal (^{13}C). High-resolution ESI mass spectra were obtained from a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, an RF-only hexapole ion guide and an external electrospray ion source. Flash column chromatography was carried out using silica gel (0.015−0.040 μm), and analytical thin layer chromatography (TLC) was performed using silica gel aluminum sheets. All commercially available chemicals were used without further purification. Diacid **6** was obtained from cholic acid as described in ref 19. Diacids **14**, **17**, **31**, **35**, and **38** were obtained as described in ref 4d. Diisocyanide **7** was obtained as described in ref 4c, and diisocyanide **2** and triisocyanide **33** were obtained as described in ref 5a. Azide **20** was prepared following the procedure described in ref 15.

General Procedure A: Ugi-4CR-based macrocyclizations of a diacid and a diisocyanide: A solution of the amine (1.0 mmol) and paraformaldehyde (1.0 mmol) in MeOH (100 mL) was stirred for 1 h at room temperature. Two solutions, one of the diacid (0.5 mmol) and another one of the diisocyanide (0.5 mmol), in 20 mL of MeOH each were simultaneously and slowly added to the reaction mixture using syringe pumps (flow rate 0.5 mL h^{-1}). After addition was completed, the reaction mixture was stirred for 8 h and concentrated under reduced pressure to give a crude product, which was redissolved in 100 mL of CHCl$_3$. The organic phase was washed sequentially with aqueous 10% HCl (2 × 50 mL), aqueous 10% NaHCO$_3$ (2 × 50 mL), and brine (50 mL) and then dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure.

General Procedure B: Ugi-4CR-based macrocyclizations of a diacid and a diamine: A solution of the diamine (0.5 mmol) and paraformaldehyde (1.0 mmol) in MeOH (150 mL) was stirred for 1 h at room temperature. Two solutions, one of the diacid (0.5 mmol) and one of the isocyanide (1.0 mmol), in 20 mL of MeOH each were simultaneously, but slowly, added to the reaction mixture using syringe pumps (flow rate 0.5 mL h^{-1}). After addition was completed, the reaction mixture was stirred for 8 h and concentrated under reduced pressure to give a crude product, which was redissolved in 100 mL of CHCl$_3$. The organic phase was washed sequentially with aqueous 10% HCl (2 × 50 mL), aqueous 10% NaHCO$_3$ (2 × 50 mL), and brine (50 mL) and then dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure.

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(19) Batta, A. K.; Aggarwal, S. K.; Salen, G.; Shefer, S. *J. Lipid Res.* **1991**, *32*, 977–983.

General Procedure C. Ugi-4CR-based macrocyclizations of a diisocyanide and a diamine: A solution of the diamine (0.5 mmol) and paraformaldehyde (1.0 mmol) in MeOH (150 mL) was stirred for 1 h at room temperature. The acid (1.0 mmol) was then added and the stirring continued for 30 min. A solution of the diisocyanide (0.5 mmol) in MeOH (20 mL) was slowly added to the reaction mixture using a syringe pump (flow rate 0.5 mL h^{-1}). After addition was completed, the reaction mixture was stirred for 8 h and concentrated under reduced pressure to give a crude product, which was redissolved in 100 mL of CHCl$_3$. The organic phase was washed sequentially with aqueous 10% HCl (2 × 50 mL), aqueous 10% NaHCO$_3$ (2 × 50 mL), and brine (50 mL) and then dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure.

Cryptand 5: Diacid **1** (111 mg, 0.5 mmol), diisocyanide **2** (78 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), glycine *tert*-butyl ester hydrochloride (167 mg, 1.0 mmol), and Et$_3$N (0.14 mL, 1.0 mmol) were reacted according to macrocyclization procedure A to give the macrocycle **3** (identified by ESI-MS). The crude product was dissolved in dry CH$_2$Cl$_2$ (20 mL) followed by trifluoroacetic acid (TFA, 10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 4 h, and the volatiles were evaporated under reduced pressure. TFA was removed completely by repetitive addition and evaporation of further CH$_2$Cl$_2$. The resulting crude diacid, diisocyanide **4** (110 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and *iso*-propylamine (0.085 mL, 1.0 mmol) were reacted according to macrocyclization procedure A. Flash column chromatography purification (CH$_2$Cl$_2$/MeOH 10:1) afforded cryptand **5** (167 mg, 36% from **1**) as a white solid. R_f = 0.43 (CH$_2$Cl$_2$/MeOH/Et$_3$N 5:1:0.1). mp (from EtOAc): 176−178 °C. IR (KBr): 3336, 2976, 2930, 1698, 1692, 1679, 1653, 1540, 1249, 1166. ^{1}H NMR (CD$_3$OD): δ = 7.39 (d, 1H, J = 8.0 Hz, Ar); 7.32−7.27 (m, 2H, Ar); 7.24 (d, 1H, J = 8.1 Hz, Ar); 4.50−4.44 (m, 4H); 4.42 (m, 4H); 4.33−4.26 (m, 4H); 4.21−4.19 (m, 4H); 4.15−4.10 (m, 4H); 4.08 (m, 1H); 4.03 (m, 1H); 3.91−3.82 (m, 4H); 3.72−3.65 (m, 4H); 3.60−3.48 (m, 8H); 3.24−3.19 (m, 4H); 4.50−4.44 (m, 4H); 3.07−2.99 (m, 4H); 1.94−1.83 (m, 4H); 1.27 (d, 6H, J = 6.1 Hz); 1.26 (d, 6H, J = 6.2 Hz). ^{13}C NMR (CD$_3$OD): δ = 174.4, 174.2, 173.3, 172.0, 171.7, 171.4, 170.5, 169.5, 139.2, 138.5, 129.2, 128.8, 127.9, 127.8, 72.2, 71.9, 71.6, 71.3, 70.6, 70.5, 70.3, 69.6, 69.3, 55.9, 55.5, 55.2, 54.7, 54.0, 52.5, 52.1, 51.8, 50.5, 45.8, 43.8, 43.3, 43.1, 37.1, 36.6, 25.9, 25.7, 25.6, 21.0, 20.9, 20.8. HRMS (ESI-FT-ICR) *m/z*: 915.5307 [M + H]$^+$; calcd. for C$_{44}$H$_{71}$O$_{11}$N$_{10}$: 915.5298.

Cryptand 9: Steroidal diacid **6** (254 mg, 0.5 mmol), diisocyanide **7** (110 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and mono-Cbz-ethylenediamine (97 mg, 1.0 mmol) were reacted according to macrocyclization procedure A to give the macrocycle **8** (identified by ESI-MS). The resulting product was dissolved in dry EtOH (100 mL), and 10% Pd(C) (200 mg) was added. The reaction mixture was treated successively with hydrogen and vacuum and finally stirred under hydrogen atmosphere for 48 h. The catalyst was removed by filtration, and the resulting solution was evaporated under reduced pressure. The resulting crude diamine, diisocyanide **2** (78 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and acetic acid (0.055 mL, 1.0 mmol) were reacted according to macrocyclization procedure C. Flash column chromatography purification (CH$_2$Cl$_2$/MeOH 18:1) afforded cryptand **9** (160 mg, 27% from **6**) as a white solid. R_f = 0.56 (CH$_2$Cl$_2$/MeOH 10:1). mp (from CH$_2$Cl$_2$/*n*-hexane): 247−250 °C. IR (KBr, cm^{-1}): 3521, 3334, 3087, 3064, 2924, 1728, 1716, 1698, 1684, 1666, 1641, 1506, 1251, 1206, 1168, 1157. ^{1}H NMR (CDCl$_3$): δ = 9.72 (br. s, 1H, N*H*); 9.47 (br. s, 1H, N*H*); 7.52 (d, 2H, J = 8.4 Hz, Ar); 7.44 (d, 2H, J = 8.4 Hz, Ar); 7.19 (m, 4H, Ar); 6.88 (m, 4H, Ar); 6.69 (m, 1H, N*H*); 6.48 (m, 1H, N*H*); 4.52 (m, 1H); 4.37 (d, 4H, J = 6.6 Hz); 4.19−4.12 (m, 8H); 3.83 (m, 1H); 3.75 (m, 1H); 2.02 (s, 6H); 1.17 (s, 3H, H-19); 1.02 (d, 3H, J = 6.4 Hz, H-21); 0.85 (s, 3H, H-18). ^{13}C NMR (CDCl$_3$): δ = 174.9, 172.0, 171.4, 170.3, 169.7, 167.1, 166.8, 154.5, 153.8, 137.5, 136.4, 133.2, 132.8, 128.7,

128.5, 128.0, 127.8, 121.9, 121.3, 120.2, 119.8, 118.7, 118.1, 75.0, 72.3, 67.7, 66.5, 66.4, 57.2, 52.5, 52.3, 51.2, 46.3, 46.1, 42.6, 42.2, 41.6, 41.2, 39.4, 34.9, 34.6, 34.2, 31.2, 30.3, 29.0, 28.2, 27.0, 26.5, 26.2, 23.0, 22.5, 22.3, 21.2, 17.5, 14.7, 12.8. HRMS (ESI-FT-ICR) *m/z*: 1211.6369 [M + Na]$^+$; calcd. for C$_{65}$H$_{88}$NaO$_{13}$N$_8$: 1211.6368.

Cryptand 13: Diamine **10** (100 mg, 0.5 mmol), diisocyanide **7** (110 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and N_ε-Cbz-N_α-Boc-L-lysine (380 mg, 1.0 mmol) were reacted according to macrocyclization procedure C to give the macrocycle **11** (identified by ESI-MS). The resulting product was dissolved in dry EtOH (90 mL), and 10% Pd(C) (250 mg) was added. The mixture was treated successively with hydrogen and vacuum and finally stirred under a hydrogen atmosphere for 48 h. The catalyst was removed by filtration, and the resulting solution was evaporated under reduced pressure. The resulting crude diamine, diacid **12** (84 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and *tert*-butylisocyanide (0.115 mL, 1.0 mmol) were reacted according to macrocyclization procedure B. Flash column chromatography purification (CH$_2$Cl$_2$/MeOH/Et$_3$N 20:1:0.2) afforded cryptand **13** (200 mg, 31% from **10**) as a pale yellow solid. R_f = 0.64 (CH$_2$Cl$_2$/MeOH/Et$_3$N 10:1:0.1). mp (from MeOH): 221−222 °C. IR (KBr, cm^{-1}): 3317, 3064, 3032, 2935, 2870, 1703, 1697, 1693, 1633, 1497, 1248, 1211, 1168. ^{1}H NMR (CDCl$_3$): δ = 9.76 (s, 1H, N*H*); 8.58 (s, 1H, N*H*); 7.66 (d, 2H, J = 8.6 Hz, Ar); 7.57 (d, 2H, J = 8.4 Hz, Ar); 7.48 (d, 2H, J = 7.4 Hz, Ar); 7.29 (t, 1H, J = 7.7 Hz, Ar); 6.94 (d, 2H, J = 8.8 Hz, Ar); 6.87 (d, 2H, J = 8.8 Hz, Ar); 5.48 (s, 1H, N*H*); 5.29 (s, 1H, N*H*); 4.95−4.89 (m, 2H); 4.60−4.56 (m, 2H); 4.43−4.38 (m, 4H); 4.27−4.24 (m, 2H); 1.43 (s, 9H, (C*H$_3$*)$_3$C); 1.41 (s, 9H, (C*H$_3$*)$_3$C); 1.37 (s, 9H, (C*H$_3$*)$_3$C); 1.35 (s, 9H, (C*H$_3$*)$_3$C). ^{13}C NMR (CDCl$_3$): δ = 173.8, 172.9, 172.6, 168.3, 168.0, 166.9, 156.3, 153.3, 153.1, 133.9, 134.7, 132.9, 130.5, 122.3, 121.7, 120.5, 120.1, 80.5, 80.3, 55.6, 55.4, 53.6, 52.2, 51.5, 50.6, 50.6, 40.0, 31.8, 29.6, 28.9, 28.5, 28.4, 28.2, 28.0, 22.6, 14.2. HRMS (ESI-FT-ICR) *m/z*: 1294.7561 [M + H]$^+$; calcd. for C$_{67}$H$_{100}$O$_{13}$N$_{13}$: 1294.7568.

Macrobicycle 25: Steroidal diacid **16** (324 mg, 0.5 mmol), diisocyanide **23** (109 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and *iso*-propylamine (0.085 mL, 1.0 mmol) were reacted according to macrocyclization procedure A to give the macrocycle **24** (identified by ESI-MS). The crude product was dissolved in THF/H$_2$O (2:1, 100 mL), and LiOH (210 mg, 5.0 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 6 h and then acidified with aq. 10% NaHSO$_4$ to pH 3. The resulting phases were separated, and the aqueous phase was additionally extracted with EtOAc (2 × 100 mL). The combined organic phases were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting crude diacid, diisocyanide **7** (110 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and *iso*-propylamine (0.085 mL, 1.0 mmol) were reacted according to macrocyclization procedure A. Flash column chromatography purification (CH$_2$Cl$_2$/MeOH 20:1) afforded the macrobicycle **25** (202 mg, 30% from **16**). R_f = 0.47 (CH$_2$Cl$_2$/MeOH 10:1). mp (from MeOH): 286−288 °C. IR (KBr, cm^{-1}): 3126, 3064, 2974, 1711, 1702, 1698, 1677, 1665, 1633, 1511, 1498, 1208. ^{1}H NMR (CDCl$_3$): δ = 9.40 (br. s, 1H, N*H*); 9.25 (br. s, 1H, N*H*); 8.98 (br. s, 1H, N*H*); 8.86 (m, 1H, N*H*); 7.54−7.50 (m, 4H, Ar); 7.35−7.31 (m, 4H, Ar); 7.10 (m, 4H, Ar); 6.86−6.78 (m, 4H, Ar); 4.91 (m, 2H); 4.87 (m, 2H); 4.69 (m, 2H); 4.64 (m, 2H); 4.55−4.51 (m, 4H); 4.38−4.33 (m, 4H); 4.02−3.89 (m, 4H); 1.30−1.25 (m, 24H); 1.13 (s, 3H, H-19); 0.93 (s, 3H, H-18); 0.89 (d, 3H, J = 5.9 Hz, H-21). ^{13}C NMR (CDCl$_3$): δ = 176.5, 171.2, 169.3, 168.6, 168.0, 167.9, 159.2, 156.2, 155.5, 138.4, 135.7, 134.9, 134.4, 129.4, 129.0, 128.6, 121.1, 120.3, 121.1, 119.3, 72.0, 70.4, 69.6, 55.3, 50.6, 49.8, 47.9, 46.9, 46.7, 45.6, 42.2, 42.0, 40.8, 38.9, 36.7, 36.2, 33.4, 33.3, 32.8, 28.4, 27.9, 25.9, 22.5, 21.5, 21.2, 20.9, 20.4, 18.6, 12.0. HRMS (ESI-FT-ICR) *m/z*: 1344.7373 [M + H]$^+$; calcd. for C$_{75}$H$_{98}$N$_{11}$O$_{12}$: 1344.7390.

Macrobicycle 27: Steroidal diacid **19** (324 mg, 0.5 mmol), diisocyanide **7** (110 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and *iso*-propylamine (0.085 mL, 1.0 mmol) were reacted

according to macrocyclization procedure A to give the macrocycle **26** (identified by ESI-MS). The crude product was dissolved in THF/H$_2$O (2:1, 100 mL), and LiOH (210 mg, 5.0 mmol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 6 h and then acidified with aq. 10% NaHSO$_4$ to pH 3. The layers were separated, and the aqueous phase was additionally extracted with EtOAc (2 × 100 mL). The combined organic phases were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting crude diacid, diamine **10** (100 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and *tert*-butylisocyanide (0.115 mL, 1.0 mmol) were reacted according to macrocyclization procedure B. Flash column chromatography purification (CH$_2$Cl$_2$/MeOH 15:1) afforded the macrobicycle **27** (178 mg, 26% from **19**). R_f = 0.35 (CH$_2$Cl$_2$/MeOH 5:1). mp (from EtOAc/CH$_2$Cl$_2$): 232−233 °C. IR (KBr, cm^{-1}): 3434, 3337, 2938, 2872, 1738, 1733, 1696, 1684, 1675, 1653, 1540. ^{1}H NMR (CDCl$_3$): δ = 7.45 (m, 1H, N*H*); 7.42 (d, 2H, *J* = 8.8 Hz, Ar); 7.38 (d, 2H, *J* = 8.4 Hz, Ar); 6.92 (m, 1H, N*H*); 6.85 (d, 2H, *J* = 9.2 Hz, Ar); 6.81 (d, 2H, *J* = 8.8 Hz, Ar); 6.62 (s, 1H, N*H*); 6.19 (s, 1H, N*H*); 4.88−4.82 (m, 4H); 4.61 (m, 2H); 4.42 (m, 2H); 4.30−4.21 (m, 4H); 4.02 (m, 2H); 3.64−3.59 (m, 2H); 3.53−3.49 (m, 2H); 3.37−3.33 (m, 2H); 1.31 (s, 18H, 2 × (C*H$_3$*)$_3$C); 1.28−1.26 (m, 12H, 2 × (C*H$_3$*)$_2$C); 0.98 (d, 3H, *J* = 6.6 Hz, H-21); 0.91 (s, 3H, H-19); 0.77 (s, 3H, H-18). ^{13}C NMR (CDCl$_3$): δ = 174.8, 172.2, 171.0, 170.4, 168.9, 168.4, 168.1, 167.8, 166.0, 163.0, 162.8, 155.0, 154.2, 133.6, 133.4, 121.2, 120.5, 119.6, 119.3, 72.6, 71.1, 67.8, 56.7, 54.4, 53.3, 53.9, 52.0, 51.7, 51.5, 50.7, 46.6, 42.6, 41.5, 40.6, 40.0, 35.5, 34.3, 33.6, 32.0, 31.6, 30.1, 28.5, 27.8, 27.4, 26.7, 26.4, 26.1, 24.3, 24.0, 23.4, 23.1, 22.0, 20.9, 20.7, 20.6, 18.7, 12.0. HRMS (ESI-FT-ICR) *m/z*: 1374.8557 [M + H]$^+$; calcd. for C$_{74}$H$_{112}$N$_{13}$O$_{12}$: 1374.8553.

Macrobicycle 30: Steroidal diacid **22** (340 mg, 0.5 mmol), diamine **28** (74 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and methyl isocyanoacetate (0.12 mL, 1.0 mmol) were reacted according to macrocyclization procedure B to give the macrocycle **29** (identified by ESI-MS). The crude product was dissolved in dry CH$_2$Cl$_2$ (20 mL), and trifluoroacetic acid (TFA, 12 mL) was added to the solution at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 4 h. The solvent was evaporated under reduced pressure and the TFA removed by addition and evaporation of further CH$_2$Cl$_2$. The resulting trifluoroacetate salt was dissolved in CH$_2$Cl$_2$ (200 mL) and washed with sat. aq. NaHCO$_3$ (3 × 60 mL). The organic layer was examined for the absence of TFA by ESI-MS and then dried over anh. Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting crude diamine, diacid **1** (111 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and methyl isocyanoacetate (0.12 mL, 1.0 mmol) were reacted according to macrocyclization procedure B. Flash column chromatography purification (CH$_2$Cl$_2$/MeOH 20:1) afforded the macrobicycle **30** (185 mg, 28% from **22**). R_f = 0.29 (CH$_2$Cl$_2$/MeOH 10:1). mp (from CH$_2$Cl$_2$): 183−185 °C. IR (KBr, cm^{-1}): 3427, 3345, 2946, 1734, 1730, 1707, 1703, 1692, 1678, 1232, 1205, 1191, 1176. ^{1}H NMR (CDCl$_3$): δ = 6.55 (s, 1H, N*H*); 6.42 (s, 1H, N*H*); 6.24 (s, 1H, N*H*); 6.12 (s, 1H, N*H*); 4.50 (m, 2H); 4.37 (m, 2H); 4.28 (m, 2H); 4.13 (m, 2H); 3.99 (m, 1H); 3.90−3.82 (m, 4H); 3.72 (s, 3H, C*H$_3$*O); 3.70 (s, 3H, C*H$_3$*O); 3.66 (s, 6H, C*H$_3$*O); 3.56 (br. m, 1H); 3.46−3.37 (m, 4H); 3.29 (m, 4H); 3.15 (m, 2H); 3.08 (m, 2H); 1.15 (s, 3H, H-19); 1.03 (d, 3H, *J* = 5.8 Hz, H-21); 0.92 (s, 3H, H-18). ^{13}C NMR (CDCl$_3$): δ = 174.5, 173.2, 172.1, 168.4, 166.7, 166.0, 165.8, 74.2, 72.5, 70.1, 69.7, 68.4, 63.2, 62.9, 60.7, 59.5, 57.4, 56.8, 53.1, 51.8, 50.7, 49.1, 47.8, 46.2, 44.0, 42.9, 42.7, 40.9, 40.7, 40.3, 37.1, 36.4, 35.8, 35.3, 32.0, 31.6, 28.7, 26.9, 26.3, 24.4, 22.1, 21.3, 20.1, 18.8, 18.7, 12.1. HRMS (ESI-FT-ICR) *m/z*: 1314.6332 [M + H]$^+$; calcd. for C$_{60}$H$_{93}$NaN$_9$O$_{22}$: 1314.6338.

Macrotetracycle 34: Diacid **31** (283 mg, 0.5 mmol), diisocyanide **2** (78 mg, 0.5 mmol), paraformaldehyde (30 mg, 1 mmol), L-alanine methyl ester hydrochloride (139 mg, 1 mmol), and Et$_3$N (0.14 mL, 1 mmol) were reacted according to macrocyclization procedure A. The resulting macrocycle **32** (identified by ESI-MS)

was dissolved in THF/H$_2$O (2:1, 60 mL), and LiOH (158 mg, 2.5 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 6 h and then acidified with aq. 10% NaHSO$_4$ to pH 3. The resulting phases were separated, and the aqueous phase was additionally extracted with EtOAc (2 × 80 mL). The combined organic phases were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting crude triacid, triisocyanide **33** (88 mg, 0.5 mmol), paraformaldehyde (45 mg, 1.5 mmol), and *iso*-propylamine (0.125 mL, 1.5 mmol) were reacted according to macrocyclization procedure A. Flash column chromatography purification (CH$_2$Cl$_2$/MeOH 20:1) afforded **34** (169 mg, 26% from **31**) as a light yellow solid. R_f = 0.54 (CH$_2$Cl$_2$/MeOH 10:1). mp (from EtOAc): 213−217 °C. ^{1}H NMR (CDCl$_3$): δ = 7.35−7.32 (m, 4H, Ar); 6.32 (m, 1H, N*H*); 6.28 (m, 1H, N*H*); 5.72 (m, 1H, N*H*); 5.69 (m, 1H, N*H*); 4.64 (m, 2H); 4.61 (m, 2H); 4.30−4.02 (m, 14H); 3.54 (m, 1H); 1.24−1.16 (m, 18H, 2 × (C*H$_3$*)$_2$CH); 1.13 (s, 3H, H-19); 0.92 (s, 3H, H-18); 0.91 (d, 3H, *J* = 6.6 Hz, H-21). ^{13}C NMR (CDCl$_3$): δ = 171.9, 171.3, 170.8, 170.4, 169.8, 166.2, 161.6, 156.1, 136.5, 128.5, 128.3, 71.5, 70.9, 70.8, 54.8, 54.6, 54.4, 53.9, 50.6, 47.3, 45.4, 43.4, 43.1, 42.5, 42.3, 40.8, 40.6, 37.5, 37.4, 37.1, 35.4, 32.6, 31.9, 30.7, 29.6, 28.8, 26.2, 22.1, 22.0, 19.8, 16.1, 12.6. HRMS (ESI-FT-ICR) *m/z*: 1298.7839 [M + H]$^+$; calcd. for C$_{67}$H$_{104}$O$_{13}$N$_{13}$ 1298.7837.

Macrotetracycle 37: Diacid **35** (283 mg, 0.5 mmol), diisocyanide **7** (110 mg, 0.5 mmol), paraformaldehyde (30 mg, 1 mmol), L-alanine methyl ester hydrochloride (139 mg, 1 mmol), and Et$_3$N (0.14 mL, 2 mmol) were reacted according to macrocyclization procedure A. The resulting macrocycle **36** (identified by ESI-MS) was dissolved in THF/H$_2$O (2:1, 60 mL), and LiOH (158 mg, 2.5 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 6 h and then acidified with aq. 10% NaHSO$_4$ to pH 3. The resulting phases were separated, and the aqueous phase was additionally extracted with EtOAc (2 × 80 mL). The combined organic phases were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting crude triacid, triisocyanide **33** (88 mg, 0.5 mmol), paraformaldehyde (45 mg, 1.5 mmol), and *iso*-propylamine (0.125 mL, 1.5 mmol) were reacted according to macrocyclization procedure A. Flash column chromatography purification (CH$_2$Cl$_2$/MeOH 22:1) afforded **37** (149 mg, 22% from **35**) as a pale yellow solid. R_f = 0.48 (CH$_2$Cl$_2$/MeOH 10:1). mp (from MeOH): 241−244 °C. ^{1}H NMR (CDCl$_3$): δ = 8.65 (m, 1H, N*H*); 8.61 (m, 1H, N*H*); 7.42 (d, 2H, *J* = 8.8 Hz, Ar); 7.38 (d, 2H, *J* = 8.4 Hz, Ar); 7.04 (m, 1H, N*H*); 6.85 (d, 2H, *J* = 9.2 Hz, Ar); 6.81 (d, 2H, *J* = 8.8 Hz, Ar); 6.56 (m, 1H, N*H*); 6.49 (m, 1H, N*H*); 1.26−1.18 (m, 18H, 3 × (C*H$_3$*)$_2$CH); 1.12 (d, 3H, *J* = 6.4 Hz, H-21); 1.02 (s, 3H, H-19); 0.92 (s, 3H, H-18). ^{13}C NMR (CDCl$_3$): δ = 171.9, 171.0, 170.4, 169.8, 168.7, 168.1, 167.8, 166.1, 166.1, 162.8, 155.1, 154.0, 133.5, 133.3, 121.1, 120.8, 119.8, 119.2, 72.6, 71.1, 67.8, 55.0, 54.9, 54.6, 54.3, 52.8, 52.5, 52.1, 51.2, 50.5, 49.6, 49.1, 48.7, 47.5, 45.8, 44.4, 44.0, 42.1, 39.1, 37.0, 35.6, 34.2, 33.8, 31.6, 30.5, 29.2, 27.4, 26.9, 23.4, 22.1, 21.8, 21.7, 21.4, 20.9, 20.6, 20.4, 19.2, 12.3. HRMS (ESI-FT-ICR) *m/z*: 1362.7819 [M + H]$^+$; calcd. for C$_{71}$H$_{104}$O$_{16}$N$_{13}$ 1362.7813.

Macrotetracycle 40: Diacid **38** (283 mg, 0.5 mmol), diamine **28** (74 mg, 0.5 mmol), paraformaldehyde (30 mg, 1.0 mmol), and methyl isocyanoacetate (0.12 mL, 1.0 mmol) were reacted according to macrocyclization procedure B. The resulting macrocycle **39** (identified by ESI-MS) was dissolved in THF/H$_2$O (2:1, 50 mL), and LiOH (158 mg, 2.5 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 6 h and then acidified with aq. 10% NaHSO$_4$ to pH 3. The resulting phases were separated, and the aqueous phase was additionally extracted with EtOAc (2 × 80 mL). The combined organic phases were dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The resulting crude triacid, triisocyanide **33** (88 mg, 0.5 mmol), paraformaldehyde (45 mg, 1.5 mmol), and *iso*-propylamine (0.125 mL, 1.5 mmol) were reacted according to macrocyclization procedure A. Flash column chromatography purification (CH$_2$Cl$_2$/MeOH 18:1) afforded **40** (186 mg, 29% from **38**) as a pale yellow solid. R_f = 0.23 (CH$_2$Cl$_2$/MeOH 10:1). mp (from EtOAc): 217−219 °C. ^{1}H NMR (CDCl$_3$): δ = 7.68 (m, 1H,

N*H*); 7.14 (m, 1H, N*H*); 6.53 (m, 1H, N*H*); 5.01−4.91 (m, 2H); 4.84−4.76 (m, 2H); 4.71−4.64 (m, 2H); 4.61−4.53 (m, 2H); 4.32−4.25 (m, 2H); 4.22−3.98 (m, 8H); 3.98 (m, 1H); 3.62−3.56 (m, 4H); 1.29−1.24 (m, 12H, 2 × (C*H*$_3$)$_2$CH); 1.22 (m, 6H, 2 × (C*H*$_3$)$_2$CH); 1.13 (s, 3H, H-19); 1.07 (d, 3H, *J* = 6.1 Hz, H-21); 0.91 (s, 3H, H-18). ^{13}C NMR (CDCl$_3$): δ = 171.5, 171.2, 170.3, 170.8, 170.4, 170.3, 169.8, 168.6, 168.4, 167.7, 166.3, 165.7, 70.6, 70.3, 68.8, 61.5, 59.9, 54.6, 52.8, 52.3, 51.9, 51.1, 50.9, 50.7, 49.8, 48.7, 48.5, 48.4, 45.6, 44.3, 44.1, 43.9, 42.1, 41.9, 41.7, 40.8, 40.3, 40.1, 39.7, 39.2, 38.1, 42.1, 37.0, 36.2, 36.1, 35.7, 35.6, 34.9, 34.6, 34.5, 33.7, 31.3, 30.6, 30.5, 29.8, 28.7, 28.1, 27.6, 25.6, 25.2, 22.7, 22.4, 21.8, 21.7, 21.6, 21.4, 20.9, 20.8, 20.6, 18.1. HRMS (ESI-FT-ICR) *m/z*: 1304.7597 [M + Na]$^+$; calcd. for C$_{63}$H$_{103}$O$_{15}$N$_{13}$Na 1304.7594.

Supporting Information Available: Experimental procedures for the preparation of building blocks. NMR and HRMS spectra of final compounds and intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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