

Diversity Oriented One-Pot Synthesis of Complex Macrocycles: Very Large Steroid–Peptoid Hybrids from Multiple Multicomponent Reactions Including Bifunctional Building Blocks**

Ludger A. Wessjohann,* Brunhilde Voigt, and Daniel G. Rivera

Dedicated to Professor Armin de Meijere on the occasion of his 65th birthday

One of the most fascinating challenges in modern organic chemistry is the design of strategies capable of providing structurally diverse and complex molecules, which are useful either for the study of important biological processes^[1] or for the development of supra- and nanomolecular systems.^[2] The chemical genomics approach especially has focused efforts towards the rapid generation of molecules able to modulate protein functions, with the hope of thereby achieving a better understanding of the role of these molecules in many cellular pathways as well as providing new targets for drug development.^[3] Frank in his recent review on chemical genomics concludes:^[1e] “A general concept for the construction of protease-resistant (protein-like) synthetics for the interference of such (protein–protein) interactions is not yet available.” Also, in contrast to small classic enzyme inhibitors, molecules to study protein–protein interactions are believed to require much larger interaction surfaces with an interplay of lipophilic and polar interaction areas. A promising solution could be the one-pot construction of extended, highly complex structures in which spatially separated recognition motifs combine to achieve overall binding. For this purpose, macrocyclic skeletons are considered to be an especially remarkable class of target scaffolds, as they can combine conformational preorganization with flexibility and biological stability.^[4]

A useful prospect has been the development of diverse routes towards synthetic and natural-product-like macrocycles.^[5,6] However, most (large) macrocycles utilized by the chemical community so far are either of the repetitive type (for example, the homooligomeric cyclodextrins and calixarenes) or have to be made by multistep, complex synthetic routes, which allow the generation of a single specimen but do not easily provide the diverse libraries required for holistic or evolutionary approaches and activity/property screenings.

[*] Prof. Dr. L. A. Wessjohann, Dr. B. Voigt, D. G. Rivera
Department of Bioorganic Chemistry
Leibniz Institute of Plant Biochemistry
Weinberg 3, 06120 Halle/Saale (Germany)
Fax: (+49) 345-5582-1309
E-mail: wessjohann@ipb-halle.de

[**] We are grateful to Dr. Jürgen Schmidt for the HRMS spectra and Angela Schaks for experimental assistance. We also gratefully acknowledge the Deutsche Forschungsgemeinschaft for financial support (Grant GRK/894).

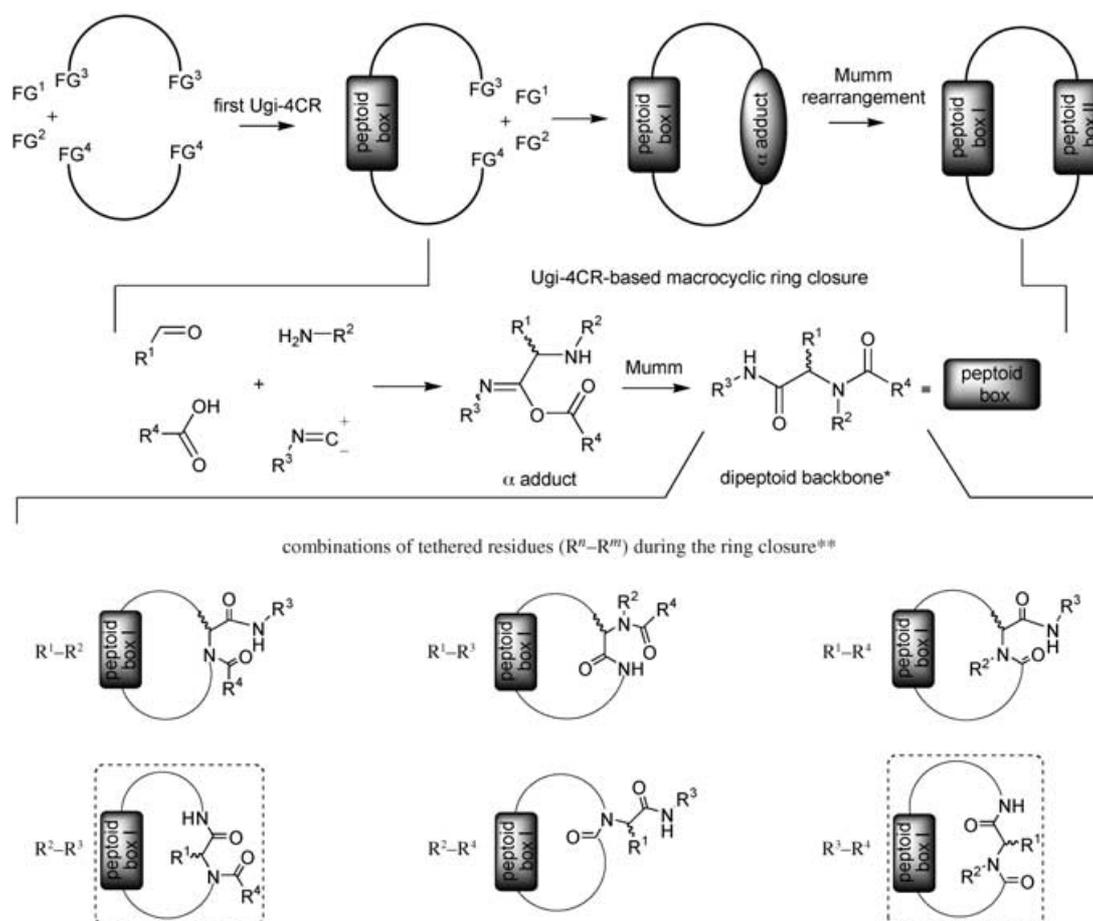
Naturally occurring macrocycles are usually endowed with unique structural complexity^[4] which, for example, enables the disruption of protein–protein interactions or binding to specific protein domains. Whilst diversity-oriented approaches have been devised to create the crucial complexity and diversity,^[7] improvements are still required for macrocyclization strategies to rapidly and readily access diversity and to simultaneously introduce, for example, protein binding motifs in a unified task in the macrocyclization step itself.

We have recently addressed this quest and devised a new strategy for developing macrocycle diversity, in which the proper application of multiple multicomponent macrocyclizations including bifunctional building blocks (MiBs) allows rapid access to libraries of constitutionally defined peptoid-containing macrocycles.^[6] Herein we describe the first applications of this concept for the straightforward synthesis of very large macrocycles (up to 60-membered rings), in which up to 4 multicomponent reactions (including the macrocyclization step) incorporate 12 components in 1 pot.

The Ugi four-component reaction (Ugi-4CR) has evolved as one of the most useful reactions in diversity-oriented approaches toward drug discovery.^[8] The only by-product of this (atom) economic process is water. Moreover, bifunctional building blocks have been appropriately utilized to access a

variety of structurally diverse cyclic scaffolds.^[9] However, the multicomponent reaction itself has previously not been exploited for recognition-motif generation or, with some exceptions,^[6,10] as a ring-closing reaction in macrocyclization strategies.

A simple survey of the skeletal (peptoid-core) diversity achieved by employing different symmetrically bifunctionalized (Ugi) components in a bidirectional macrocyclization is presented in Scheme 1. The ring size of the final macrocycle depends on the chosen combination of bifunctional building blocks. As either endo- or exocyclic amide bonds can be formed, higher or lower flexibility can be generated for both the ring and the differently tethered residues. However, it is important to note that even in the simplest double Ugi-4CR based approach, the two Ugi-4CRs do not occur simultaneously because a linear macrocycle precursor is formed initially. To understand the proper design of macrocycles, the final cyclization step is crucial to assess the ring size (multiplicity) and efficiency (yield), and thereby the result of the overall process.^[5a,g] In the case of Ugi-type reactions, the analysis should not only be directed to the strain of the final macrocycle but also to the cyclic precursor α -adduct, as well as the possibly strained 1,3-*ansa*-like intermediate of the Mumm rearrangement, both resulting from the various



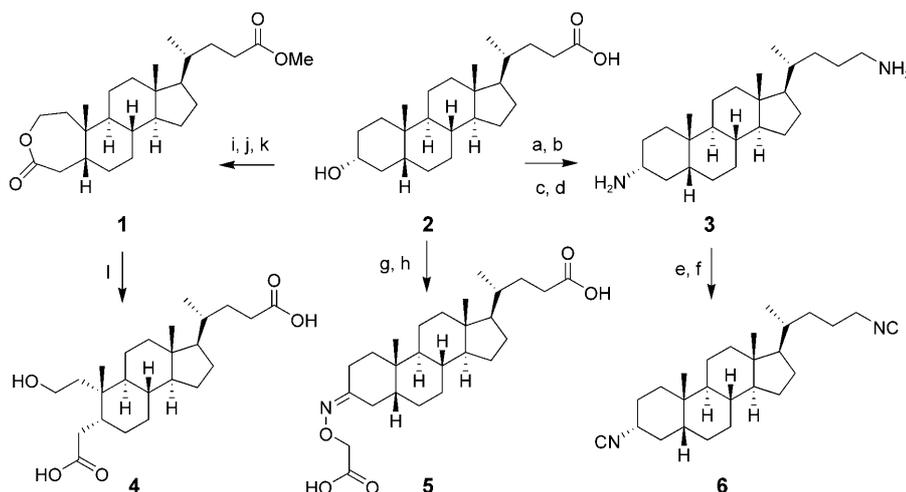
Scheme 1. Skeletal diversity of the peptoid backbones that can be obtained in a multiple multicomponent macrocyclization including bifunctional building blocks (MiBs) of the bidirectional Ugi type. *: The C→N terminus of box I runs parallel to that of box II (bidirectional peptoids), that is, even if the same FG¹ and FG² residues are included, the boxes are not identical owing to the asymmetry of the steroid tether.^[6] **: The different *endo/exo* peptoid cores available by the MiBs. Dashed boxes indicate examples used herein.

bifunctional building block combinations (Scheme 1; for example, aldehyde/acid building blocks bridging R¹ and R⁴).^[6]

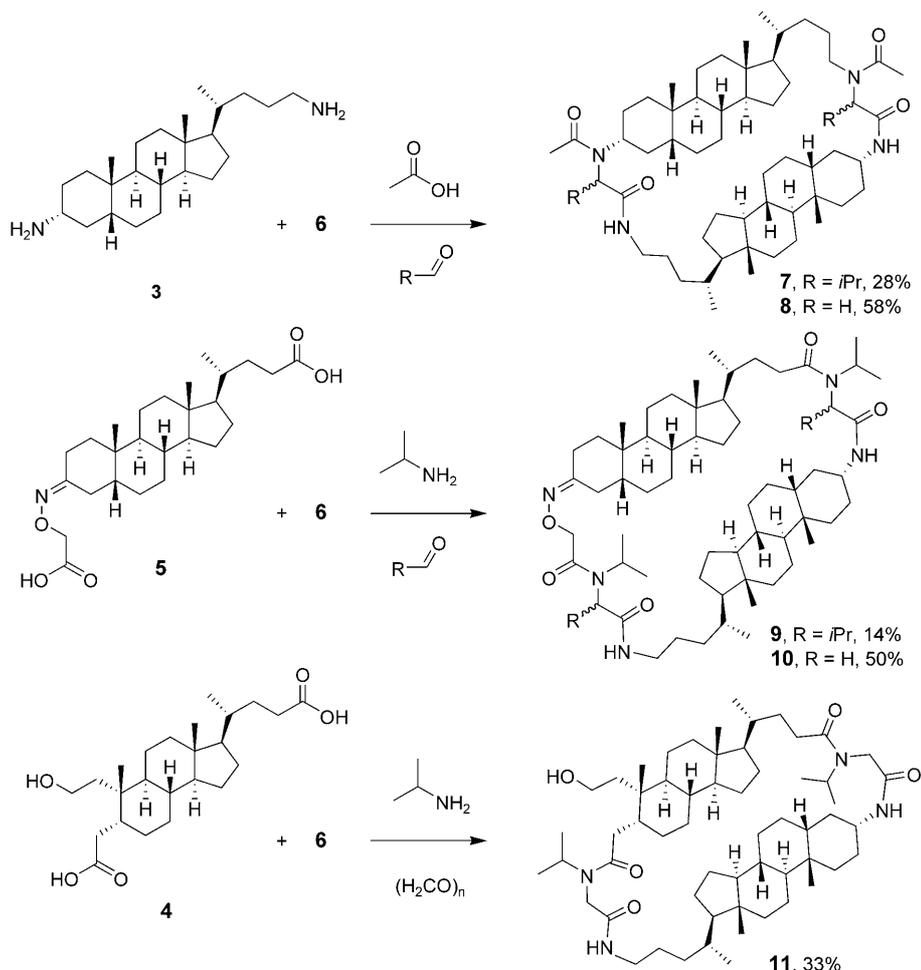
With a view to applying this concept in the work described herein, we concentrated on a straightforward strategy towards steroid-peptoid hybrid macrocycles through a one-pot multiple Ugi-4CR of steroidal bifunctional building blocks. Steroids combine several structural features that render them suitable architectural components in macrocycle synthesis. They present one of the few extended, readily available, chiral units that can be additionally functionalized by a large set of established procedures. The natural stereochemical diversity and rigid array of concave-directed differentiable functionalities in the steroidal nucleus have been previously used in the (target-oriented) design of macrocyclic frameworks for biomimetic and molecular recognition applications.^[11]

Scheme 2 summarizes the synthesis of the steroidal bifunctional building blocks from lithocholic acid (**2**). Synthesis of diamine **3** and diisonitrile **6** proceeded by a double Mitsunobu reaction to the corresponding reduced acid, followed by azide displacement,^[12a] reduction, and subsequent isonitrile formation.^[12b] Steroidal dicarboxylic acids are a source of additional skeletal diversity. They are most rapidly obtained from the 3-oxo derivative through lactone formation by a Baeyer–Villiger reaction^[13] and consecutive ring-opening saponification or alternatively by condensation with *O*-(carboxymethyl)hydroxylamine; these reactions afforded the dicarboxylic acids **4** and **5**, respectively.

All multiple macrocyclizations by the Ugi-4CR presented here were carried out under pseudodilution conditions by adding the diisonitrile building block at a rate of 0.1 mL h⁻¹ (*c* = 0.05 mmol mL⁻¹) to a mixture of the other components. Competing oligomerization was successfully minimized and an almost equal mixture of head-to-head (H-H) and head-to-tail (H-T) isomers resulted in all cases with at least two bifunctional asymmetric building blocks.^[6,14] Three examples, highlighted in Scheme 3, illustrate the initial attempt to explore our strategy. By using the diamine **3** and the diacids **4** and **5** as counterparts of the common diisonitrile **6** in the Ugi-4CR, two different types of peptoid backbones are produced, according to the considerations summarized in Scheme 1. The diamine/diisonitrile combination is characterized by an



Scheme 2. Synthesis of the steroidal bifunctional building blocks. a) LiAlH₄, THF; b) DIAD, Ph₃P, MeSO₃H; c) NaN₃, DMPU; d) H₂, PtO₂; e) HCO₂Et, Δ; f) POCl₃, *i*Pr₂NEt; g) PCC, CH₂Cl₂; h) NH₂OCH₂CO₂H, Py; i) TMSCl, MeOH; j) PCC, CH₂Cl₂; k) urea/H₂O₂, (CF₃CO)₂O; l) KOH, MeOH. DIAD = diisopropylazodicarboxylate, DMPU = 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone, PCC = pyridinium chlorochromate, Py = pyridine, THF = tetrahydrofuran, TMS = trimethylsilyl.



Scheme 3. Double Ugi-4CR based macrocyclization of steroidal bifunctional building blocks (bidirectional MiBs of diamine/diisonitrile and diacid/diisonitrile type). Head-to-head (H-H) and head-to-tail (H-T) regiomers are formed in almost equal amounts. Yields refer to the mixture; however, for clarity, only the H-T isomer is shown.^[14]

exocyclic amide bond which shortens the tether chain but increases the flexibility of the peptoid portion.

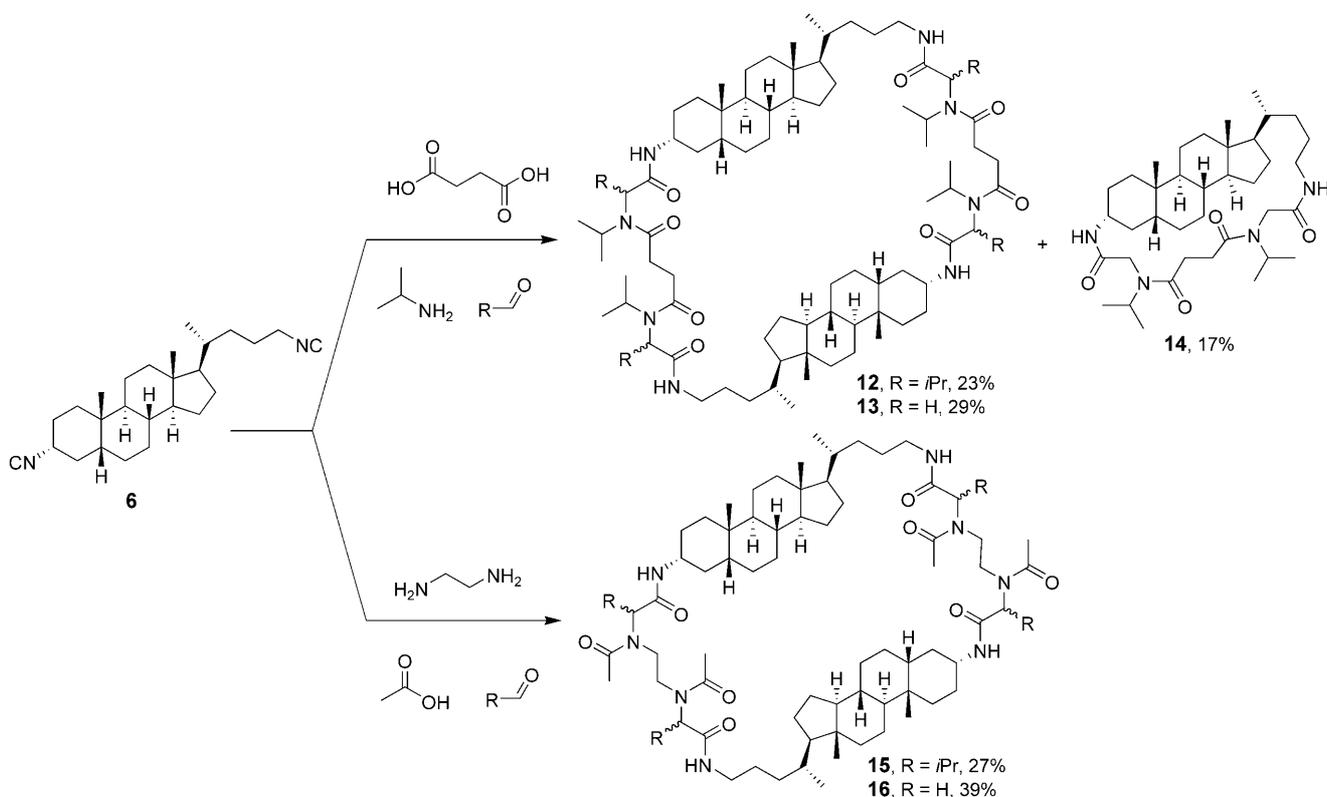
Of the various components that can be varied, the oxo component exhibits special influence on ring formation and stereochemical complexity. Thus, macrocycles **7–10** were isolated as almost equally distributed mixtures of diastereomers.^[15] The Ugi approach facilitates the rapid generation of stereochemical diversity that is much more difficult to access in a multistep synthetic strategy, although it could also be considered a drawback owing to the required (but often easy) separation and difficult characterization work. Furthermore, when isobutyraldehyde was replaced by paraformaldehyde there was a marked increase in the macrocyclization yields, which is probably the result of lower steric hindrance or a significantly modified prefolding bias.

To improve the size as well as recognition-motif diversity of the peptoid core of the macrocyclic skeleton, we set out to design a fourfold Ugi-4CR macrocyclization by using two equivalents of the steroidal diisonitrile **6** and another (at this stage simpler) bifunctional building block. This complementary and direct approach, which allows manipulation of the nature and length of the steroid bridging tethers, succeeded for the synthesis of the fourfold macrocycles **12–16** (Scheme 4) with ethylenediamine or succinic acid as the simpler bifunctional building blocks. Interestingly, in the case with succinic acid and with paraformaldehyde as the oxo component the smaller macrocycle **14** was also formed, although in lower yield, a result confirming that the succinate-bridged double dipeptoid chain is just long enough to span the distance between the two functional groups of the

steroidal skeleton. An alternative third pathway, isonitrile (imidate) induced condensation to succinic anhydride leading to the Ugi three-component product, was not observed, but it was also not specifically looked for, as the desired macrocycles were formed in typical combined yield.

The different outcomes in these macrocyclizations by using different aldehydes pinpoint the importance of understanding the effect of a bulky aldehyde side chain on the success and result of the overall process. In addition to pure steric effects, the favoring (or disfavoring) of double^[16] or fourfold Ugi-4CR based macrocyclizations is dependent on the length, flexibility, and prefolding characteristics of the acyclic precursor. The strain and strain change of the cyclic α -adduct are also crucial aspects which determine the formation and further ability to rearrange to the final macrocycle. Thus, the sterically demanding isopropyl groups might cause significant steric strain or incorrect prefolding in the formation of the cyclic α -adduct, thereby prohibiting the formation of the smaller macrocycle. Larger (for example, sixfold Ugi-4CR based) macrocycles, in contrast, could not be identified to any significant amount by ESI-FT-ICR mass spectrometry.

After reliable fourfold Ugi-4CR macrocyclizations were established and because of the intriguing result obtained with a diacid as the bridging building block, we extended our plan to cover other types of peptoid tethers for the steroids. This could be achieved with diacids that have a significant influence either on the molecular recognition behavior or on the internal folding of the obtained macrocycle. Terephthalic and cyclopropane-1,1-dicarboxylic acids were chosen as the counterparts, and consequently macrocycles **17–20** were



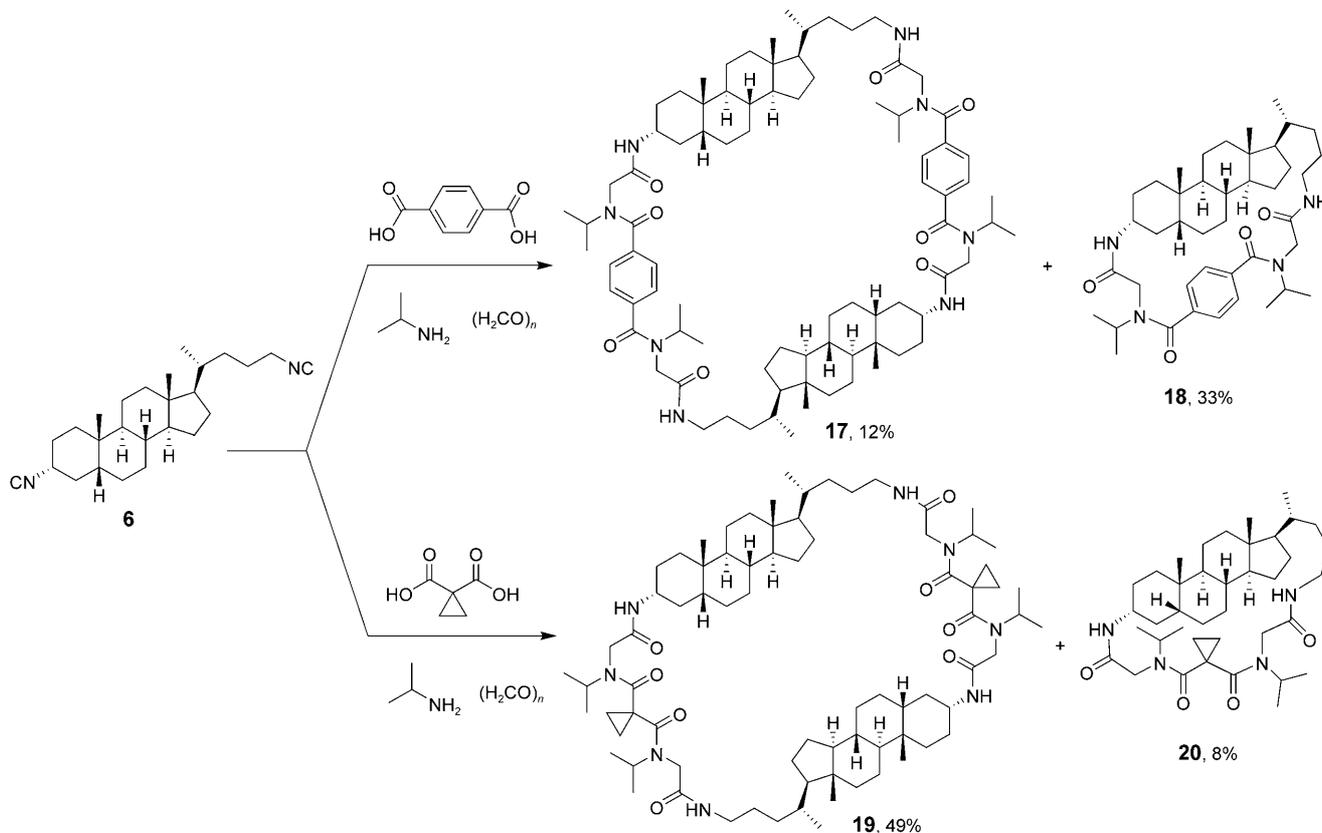
Scheme 4. Fourfold Ugi-4CR based macrocyclization of bifunctional building blocks (MiBs of diacid/diisonitrile and diamine/diisonitrile type). A mixture of H-H and H-T isomers is observed for fourfold reactions, (i.e., not for **14**).^[14,16]

synthesized in a similar fashion, with paraformaldehyde as the best oxo component for analytical purposes. Scheme 5 highlights the convincing results of our design. It also shows the effect of a more strained chain on the success of the fourfold Ugi-4CR macrocyclization versus the double reaction. With the long and straight terephthalic acid, the double Ugi-4CR based small macrocycle **18** was the main product, whereas the short, kinked 1,1-cyclopropane diacid led to the formation of the fourfold Ugi-4CR based large macrocycle **19** as the major product; this result shows that the formation of the cyclopropane-bridged double dipeptoid chain is disfavored owing to its significantly lower flexibility and shorter length to span the steroidal moiety. At this point it should be mentioned that, for example, a yield of 49% for isolated **19** corresponds to an approximately 96% calculated yield for each individual reaction/bond formed, *including* the macrocyclization.

Apart from the vast array of functionalities that could be placed inside the macrocyclic cavity by altering one of the differentiable faces of the steroidal moiety, the proven degree of complexity and diversity accessible through this one-pot synthetic process can be highlighted by considering four distinct issues: 1) additional binding elements or biologically or catalytically interesting motifs can be appended as side chains to some of the Ugi components, 2) the macrocyclization can be performed in a template-based manner to control the size of the cavity, 3) further skeletal diversity can be achieved by varying the nature of the building blocks, and 4) conformational diversity of the cavity can be designed either a) from geometrically varied building blocks (for

example, straight or kinked, as shown above) or b) by generating new stereogenic centers. While the first issues are being developed in connection with ongoing projects, an efficient access to diastereoselective Ugi reactions (4b) is the last problem that remains unsolved—with a few exceptions.^[17] Supported by the possible automation of the Ugi-4CR,^[5,6,8] the current strategy may be extended to independently modify the different access points of diversity generation in a parallel (or mixed) combinatorial fashion. Moreover, the whole system is suitable for evolutionary-based or holistic approaches, for the generation of sets of biologically relevant molecules or biomimetic supramolecular systems, or for any application that requires large, constitutionally defined complex molecules of a nonrepetitive or only partly repetitive nature.

In conclusion, we have presented a very straightforward strategy to generate a collection of chimeric peptoid macrocycles—here specifically with steroid moieties—of a size and structural complexity that bears no resemblance to any known natural product but that is potentially useful in chemical genomics approaches, as well as for artificial receptors for molecular recognition studies or as bottom-up building units for nanotechnology. To our knowledge, multi-component reactions have not so far been used to directly form macrocycles of this size and complexity that can easily incorporate and display chemical motifs previously identified as suitable, for example, for anion and carbohydrate recognition.^[18] We believe that no other macrocycle synthesis is known that better combines ease, versatility, functionality,



Scheme 5. Fourfold versus double Ugi-4CR based macrocyclizations of geometrically different bifunctional building blocks. A mixture of H-H and H-T isomers is observed for **17** and **19**.^[14]

size control, and speed in one pot than the process presented here. Finally, the principal concept of MiBs^[6] should not be limited to Ugi type multicomponent reactions only.

Experimental Section

General procedure for the Ugi-4CR based macrocyclizations: A solution of the oxo component (2 mmol) and the amino component (2 mmol) in MeOH (250 mL) was stirred for 1 h at room temperature. The diacid building block (0.5 mmol) was then added and the stirring was continued for another 30 min. A solution of diisocyanide **6** (0.5 mmol) in MeOH (10 mL) was added slowly to the reaction mixture by using a syringe pump (flow rate of 0.1 mL h⁻¹). After addition was complete, the reaction mixture was concentrated under decreased pressure and the crude material was purified by flash column chromatography or preparative HPLC to afford the corresponding macrocycles, usually as amorphous solids.

Received: January 4, 2005

Revised: April 4, 2005

Published online: June 29, 2005

Keywords: combinatorial chemistry · macrocycles · molecular recognition · multicomponent reactions · steroids

- [1] For reviews, see: a) G. Mitsopoulos, D. P. Walsh, Y.-T. Chang, *Curr. Opin. Chem. Biol.* **2004**, *8*, 26; b) R. S. Lokey, *Curr. Opin. Chem. Biol.* **2003**, *7*, 91; c) D. L. Boger, J. Desharnais, K. Capps, *Angew. Chem.* **2003**, *115*, 4270; *Angew. Chem. Int. Ed.* **2003**, *42*, 4138; d) T. Berg, *Angew. Chem.* **2003**, *115*, 2562; *Angew. Chem. Int. Ed.* **2003**, *42*, 2462; e) R. Frank, *BIOspektrum* **2002**, *8*, 474; f) P. Arya, R. Joseph, D. T. H. Chou, M.-G. Baek, *Angew. Chem.* **2001**, *113*, 351; *Angew. Chem. Int. Ed.* **2001**, *40*, 339; g) S. L. Schreiber, *Bioorg. Med. Chem.* **1998**, *6*, 1127.
- [2] a) H.-J. Schneider, A. Yatsimirsky, *Principles and Methods in Supramolecular Chemistry*, Wiley, Chichester, **2000**; b) C. A. Schalley, J. Rebek in *Stimulating Concepts in Chemistry* (Eds.: F. Vögtle, S. Stoddart, M. Shibasaki), Wiley-VCH, Weinheim, **2000**.
- [3] For selected reports, see: a) D. Barnes-Seeman, S. B. Park, A. N. Koehler, S. L. Schreiber, *Angew. Chem.* **2003**, *115*, 2478; *Angew. Chem. Int. Ed.* **2003**, *42*, 2376; b) S. M. Khersonsky, D. W. Jung, T. W. Kang, D. P. Walsh, H. S. Moon, H. Jo, E. M. Jacobson, V. Shetty, T. A. Neubert, Y. T. Chang, *J. Am. Chem. Soc.* **2003**, *125*, 11804; c) F. G. Kuruvilla, A. F. Shamji, S. M. Sternson, P. J. Hergenrother, S. L. Schreiber, *Nature* **2002**, *416*, 653; d) H. E. Pelish, N. J. Westwood, Y. Feng, T. Kirchhausen, M. D. Shair, *J. Am. Chem. Soc.* **2001**, *123*, 6740; e) T. U. Mayer, T. M. Kapoor, S. J. Haggarty, R. W. King, S. L. Schreiber, T. J. Mitchison, *Science* **1999**, *286*, 971.
- [4] a) L. A. Wessjohann, E. Ruijter, D. G. Rivera, W. Brandt, *Mol. Diversity* **2005**, *9*, 171; b) M. Feher, J. M. Schmidt, *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 218.
- [5] a) L. A. Wessjohann, E. Ruijter, *Top. Curr. Chem.* **2005**, *243*, 137; b) P. Cristau, J.-P. Vors, J. Zhu, *Tetrahedron* **2003**, *59*, 7859; c) B. Beck, G. Larbig, B. Bejat, M. Magnin-Lachaux, A. Picard, E. Herdtweck, A. Dömling, *Org. Lett.* **2003**, *5*, 1047; d) J. K. Sello, P. R. Andreana, D. Lee, S. L. Schreiber, *Org. Lett.* **2003**, *5*, 4125; e) L. A. Wessjohann, *Curr. Opin. Chem. Biol.* **2000**, *4*, 303; f) D. Lee, J. K. Sello, S. L. Schreiber, *J. Am. Chem. Soc.* **1999**, *121*, 10648; g) J. Blankenstein, J. Zhu, *Eur. J. Org. Chem.* **2005**, 1949.
- [6] L. A. Wessjohann, E. Ruijter, *Mol. Diversity* **2005**, *9*, 159.
- [7] a) M. A. Burke, S. L. Schreiber, *Angew. Chem.* **2004**, *116*, 48; *Angew. Chem. Int. Ed.* **2004**, *43*, 46; b) D. Lee, J. K. Sello, S. L. Schreiber, *Org. Lett.* **2000**, *2*, 709; c) S. L. Schreiber, *Science* **2000**, *287*, 1964.
- [8] For reviews, see: a) A. Dömling, I. Ugi, *Angew. Chem.* **2000**, *112*, 3300; *Angew. Chem. Int. Ed.* **2000**, *39*, 3168; b) C. Hulme, T. Nixey, *Curr. Opin. Drug Discovery Dev.* **2003**, *6*, 921; c) R. V. A. Orru, M. de Greef, *Synthesis* **2003**, 1471; d) L. Weber, *Curr. Med. Chem.* **2002**, *9*, 1241; e) L. Weber, *Drug Discovery Today* **2002**, *2*, 143; f) H. Bienaymé, C. Hulme, G. Oddon, P. Schmitt, *Chem. Eur. J.* **2000**, *6*, 3321; g) C. Hulme, V. Gore, *Curr. Med. Chem.* **2003**, *10*, 51.
- [9] B. Beck, A. Picard, E. Herdtweck, A. Dömling, *Org. Lett.* **2004**, *6*, 39, and references therein.
- [10] a) P. Janvier, M. Bois-Choussy, H. Bienaymé, J. Zhu, *Angew. Chem.* **2003**, *115*, 835; *Angew. Chem. Int. Ed.* **2003**, *42*, 811; b) A. Failli, H. Immer, M. D. Götz, *Can. J. Chem.* **1979**, *57*, 3257.
- [11] For reviews, see: a) A. P. Davis, R. P. Bonar-Law, J. K. M. Sanders in *Comprehensive Supramolecular Chemistry, Vol. 4* (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle), Elsevier, Oxford, **1996**, pp. 257–286; b) P. Wallimann, T. Marti, A. Furer, F. Diederich, *Chem. Rev.* **1997**, *97*, 1567; c) Y. Li, J. R. Dias, *Chem. Rev.* **1997**, *97*, 283; d) J. Tamminen, E. Kolehmainen, *Molecules* **2001**, *6*, 21; e) E. Virtanen, E. Kolehmainen, *Eur. J. Org. Chem.* **2004**, 3385.
- [12] a) A. P. Davis, S. Dresen, L. J. Lawless, *Tetrahedron Lett.* **1997**, *38*, 4305; b) R. Obrecht, R. Herrmann, I. Ugi, *Synthesis* **1985**, 400.
- [13] M. S. Cooper, H. Heaney, A. J. Newbold, W. R. Sanderson, *Synlett* **1990**, 533.
- [14] For bidirectional MiBs with at least two bifunctional asymmetric building blocks, see ref. [6]. For simplicity reasons only the head-to-tail (H-T) isomers are shown in all schemes. The yields refer to the mixture of both isomers, which often cannot be distinguished by the available structural determination techniques (HRMS, NMR) prior to chromatographic separation. This separation, however, is usually not problematic.
- [15] HPLC and LC-MS spectra showed the presence of all diastereomers in the mixture in close to equal amounts.
- [16] The double MiBs of Schemes 4 and 5 are different from those in Scheme 3 in that only one bifunctional building block is steroidal. This results in a different multiplicity bias (disfavoring the double MiBs) and the absence of regioisomers (H-H/H-T isomers).
- [17] For lead references for stereoselective Ugi and Passerini reactions, see: a) D. J. Ramón, M. Yus, *Angew. Chem.* **2005**, *117*, 1628; *Angew. Chem. Int. Ed.* **2005**, *44*, 1602; b) K. Oertel, G. Zech, H. Kunz, *Angew. Chem.* **2000**, *112*, 1489; *Angew. Chem. Int. Ed.* **2000**, *39*, 1431; c) H. Kunz, W. Pfrengle, *J. Am. Chem. Soc.* **1988**, *110*, 651; d) I. Ugi, K. Offermann, *Angew. Chem.* **1963**, *75*, 917; *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 624; e) P. R. Andreana, C. C. Liu, S. L. Schreiber, *Org. Lett.* **2004**, *6*, 4231; f) U. Kusebauch, B. Beck, K. Messer, E. Herdtweck, A. Dömling, *Org. Lett.* **2003**, *5*, 4021; g) G. Cuny, R. Gamez-Montano, J. Zhu, *Tetrahedron* **2004**, *60*, 4879; h) R. Frey, S. F. Galbraith, S. Guelfi, C. Lamberth, M. Zeller, *Synlett* **2003**, 1536; i) T. Ziegler, H.-J. Kaisers, R. Schlomer, C. Koch, *Tetrahedron* **1999**, *55*, 8397; j) S. E. Denmark, Y. Fan, *J. Am. Chem. Soc.* **2003**, *125*, 7825.
- [18] a) A. P. Davis, J. F. Gilmer, J. J. Perry, *Angew. Chem.* **1996**, *108*, 1410; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1312; b) R. P. Bonar-Law, A. P. Davis, B. A. Murray, *Angew. Chem.* **1990**, *102*, 1497; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1407; c) K. M. Bhattarai, R. P. Bonar-Law, A. P. Davis, B. A. Murray, *J. Chem. Soc. Chem. Commun.* **1992**, 752; d) A. P. Davis, R. S. Wareham, *Angew. Chem.* **1999**, *111*, 3160; *Angew. Chem. Int. Ed.* **1999**, *38*, 2979.