JOC Article

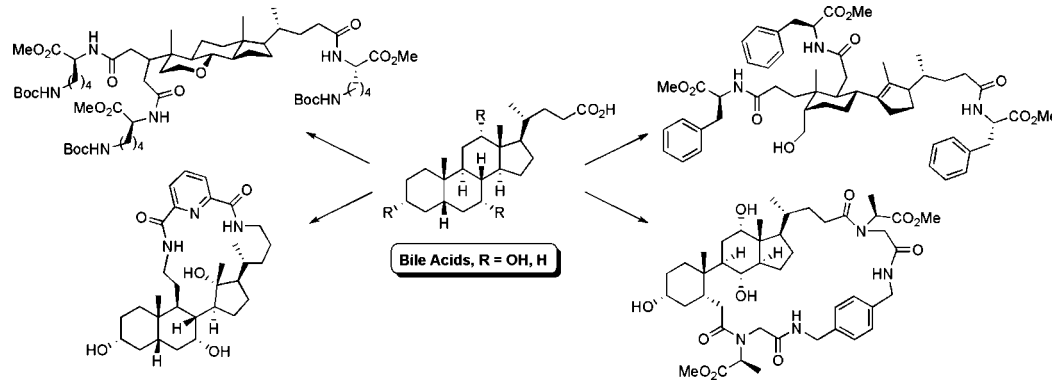
A Biomimetic Approach for Polyfunctional Secocholanes: Tuning Flexibility and Functionality on Peptidic and Macrocyclic Scaffolds Derived from Bile Acids

Daniel G. Rivera,*[,†,‡] Orlando Pando,[†] Rayser Bosch,[†] and Ludger A. Wessjohann*[,‡]

Center for Natural Products Study, Faculty of Chemistry, University of Havana, 10400 Havana, Cuba, and Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle/Saale, Germany

dgr@fq.uh.cu; wessjohann@ipb-halle.de

Received March 30, 2008



Bile acids are important scaffolds in medicinal and supramolecular chemistry. However, the use of seco bile acids, i.e., bile acids with opened rings, as cores or building blocks for the assembly of complex peptide conjugates or macrocycles has remained elusive so far. A biomimetic approach to secocholanes, based on an oxidative ring-expansion/ring-opening sequence, offers efficient access to novel structures with tunable flexibility and functionality. The process preserves selected portions of the original stereochemical and functional information of the steroid, while additional structural elements are incorporated in further (diversity-generating) steps. The potential of these building blocks for peptide and macrocycle chemistry is exemplified by the attachment of relevant α-amino acids and by the production of various complex macrocycles obtained by conventional (e.g., macrolactonization and macrolactamization) and multicomponent (e.g., Ugi four-component) macrocyclizations. This combination of secocholanic skeleton manipulation with, e.g., varied types of macrocyclization protocols, produces high levels of skeletal diversity and complexity. Therefore, this approach may have applicability either for the synthesis of biologically active ligands or as artificial receptors ("hosts").

Introduction

The steroid skeleton is a readily available source of rigidity, chirality, and lipophilicity combined in a single aliphatic system. These chemical features are the basis of many of the biological functions exhibited by steroidal compounds. Accordingly, (bio)synthetic manipulation of available substrates to vary these characteristics is a common approach employed by both nature and chemists to pursuit new biological and/or pharmacological properties. Rigidity and chirality arise from the distinctive fused-rings system known as the steroidal nucleus (the gonan core structure). This polycyclic skeleton (especially when it is highly substituted) not only possesses a high number of natural stereogenic centers but also can be easily functionalized in two different basic forms (i.e., α and β orientations) at each nonbridging carbon atom. Accordingly, the stereochemical information embedded into the steroid can be conserved, adjusted, or transferred into acyclic systems by a variety of ring-opening procedures.

[†] University of Havana.
[‡] Leibniz Institute of Plant Biochemistry.

10.1021/jo800708m CCC: $40.75 © 2008 American Chemical Society
Published on Web 07/22/2008

The partial loss of rigidity of ring-opened systems compared to the steroidal nucleus usually leads to the appearance of new biological functions, which frequently are quite different from those of the original steroid. Biologically active secosteroids (i.e., steroids with opened rings) commonly occur in nature, especially in animals and marine organisms.[1,2] In addition, it seems that some privileged substructural elements are common in biologically active natural products (analogues) structurally related to opened steroids.[3] Synthetic work on secosteroids has also traditionally rendered novel compounds displaying a wide variety of biological activities, ranging from hormone antagonists[4] to tyrosine phosphatase inhibitors.[5] Other types of interesting scaffolds derived from steroids are the macrocycles obtained by cleavage of the ABC ring system. Two distinct approaches rely on this methodology: the Winterfeldt approach toward ansa-steroids[6] and the fragmentation of endoperoxides that produces the "stereoklastanes".[7] These approaches have the advantage of conserving most of the stereochemical information of the original steroidal nucleus once the rings have been opened or the ring fusion has been cleaved. Nevertheless, further procedures that increase the number of functionalities during ring-opening process may be preferred as they lead to a higher number of diversity elements per synthetic operation.

A quite attractive type of steroidal nucleus is the cholanic skeleton of bile acids. These readily available compounds have been widely exploited for the construction of supramolecular receptors featuring macrocyclic scaffolds,[8,9] and to achieve rigid arrays of peptide strands with interesting biological, recognition and catalytic properties.[8,10] However, with the exception of a single example,[11] the use of seco bile acids as scaffolds to build up either peptide chains or relatively flexible macrocyclic structures has remained elusive so far.

Herein we report on a biomimetic approach to produce a variety of secocholanic scaffolds very amenable either for positioning multiple amino acids, and prospectively peptides, as well as for assembling diverse types of macrocyclic frameworks. The focus is posed mainly on producing skeletal diversity by enabling a tunable variation of the flexibility, stereochemistry, and functionality of the (originally) cholanic skeleton. This gives access to a wide variety of scaffolds that provide alternative topologies and functionalization patterns compared to the classical, fully conserved skeleton of the bile acids.

Results and Discussion

The partial cleavage of the steroidal nucleus to produce less rigid skeletons is a common approach in medicinal chemistry. This is mostly due to the fact that adjusting the conformational flexibility required for an appropriate binding to a biological target is a crucial issue in drug design. However, approaches that produce secosteroids having a large number of functionalities suitable for further derivatization and thus acting as amenable building blocks are very rare. Secosteroids have been usually considered as synthetic targets and not as substrates or building blocks or novel scaffolds that allow the introduction of binding or reactive motifs. Accordingly, we envisaged to implement this possibility through a simple and versatile approach that allows to tune the flexibility and functionality of the steroid-derived scaffolds while keeping most of the stereochemical information of the original nucleus.

The approach relies on a sequential ring-expansion/ring-opening procedure that ensures the production of multiple functional groups for subsequent derivatization. The Baeyer−Villiger reaction of ketocholanes was chosen for the ring expansion, as the process incorporates an additional oxygen atom and the resulting lactone ring can be submitted to standard ring-opening procedures. This method can be referred to as biomimetic, as both the Baeyer−Villiger and lactone ring-opening reactions are ubiquitous in biosynthetic pathways of many

(1) (a) Aiello, A.; Fattorusso, E.; Menna, M. *Steroids* **1999**, *64*, 687–714. (b) Morris, L. A.; Christie, E. M.; Jaspars, M.; van Ofwegen, L. P. *J. Nat. Prod.* **1998**, *61*, 538–541. (c) Seo, Y.; Cho, K. W.; Chung, H.; Lee, H. S.; Shin, J. *J. Nat. Prod.* **1998**, *61*, 1441–1443. (d) Rueda, A.; Zubia, E.; Ortega, M. J.; Carballo, J. L.; Salva, J. *J. Nat. Prod.* **1998**, *61*, 258–261. (e) Pika, J.; Andersen, R. J. *Tetrahedron* **1993**, *49*, 8757–8760. (f) Dopeso, J.; Quinoa, E.; Riguera, R.; Debitus, C.; Berquist, P. R. *Tetrahedron* **1994**, *50*, 3813–3828. (g) Kirson, I.; Zaretskii, V. I. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1244–1247.

(2) (a) Feldman, D.; Glorieux, F. H.; Pike, J. W. *Vitamin D*; Academic Press: New York, 1997. (b) Norman, A. W.; Bouillon, R.; Thomasset, M. *Vitamin D, a Pluripotent Steroid Hormone: Structural Studies, Molecular Endocrinology and Clinical Applications*; Walter de Gruyter: Berlin, 1994.

(3) (a) Arve, L.; Voigt, T.; Waldmann, H. *QSAR Comb. Sci.* **2006**, *25*, 449–456. (b) Dekker, F. J.; Koch, M. A.; Waldmann, H. *Curr. Opin. Chem. Biol.* **2005**, *9*, 232–239. (c) Koch, M. A.; Waldmann, H. *Drug Discovery Today* **2005**, *10*, 471–482. (d) Breinbauer, R.; Vetter, I. R.; Waldmann, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2878–2890.

(4) (a) Batzold, F. H.; Robinson, C. H. *J. Org. Chem.* **1976**, *41*, 313–317. (b) Smith, A. G.; Brooks, C. J. W. *Biochem. J.* **1977**, *167*, 121–129. (c) Zerhouni, N. A.; Maes, M.; Sultan, C.; Rothwell, S.; Migeon, C. J. *Steroids* **1979**, *33*, 277–285. (d) Penning, T. M.; Covey, D. F.; Talalay, P. *J. Biol. Chem.* **1981**, *256*, 6842–6850. (e) Penning, T. M. *Steroids* **1982**, *39*, 301–311. (f) Vazquez, M. H.; Tezon, J. G.; Blaquier, J. A. *J. Steroid Biochem.* **1987**, *28*, 227–231. (g) Hu, Y.; Covey, D. F. *J. Chem. Soc., Perkin Trans. 1* **1993**, 417–422. (h) Reich, I. L.; Lardy, H.; Wei, Y.; Marwah, P.; Kneer, N.; Powell, D. R.; Reich, H. J. *Steroids* **1998**, *63*, 542–553.

(5) (a) Bäurle, S.; Blume, T.; Günther, J.; Henschel, D.; Hilling, R. C.; Husemann, M.; Mengel, A.; Parchmann, C.; Schmid, E.; Skuballa, W. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1673–1677. (b) Peng, H.; Xie, W.; Otterness, D. M.; Cogswell, J. P.; McConnell, R. T.; Carter, H. L.; Powis, G.; Abraham, R. T.; Zalkow, L. H. *J. Med. Chem.* **2001**, *44*, 834–848. (c) Peng, H.; Otterness, D. M.; Abraham, R. T.; Zalkow, L. H. *Tetrahedron* **2001**, *57*, 1891–1896. (d) Peng, H.; Xie, W.; Kim, D.; Zalkow, L. H.; Powis, G.; Otterness, D. M.; Abraham, R. T. *Bioorg. Med. Chem.* **2000**, *8*, 299–306. (e) Pestell, K. E.; Ducruet, A. P.; Wipf, P.; Lazo, J. S. *Oncogene* **2000**, *19*, 6607–6612. (f) Peng, H.; Zalkow, L. H.; Abraham, R. T.; Powis, G. *J. Med. Chem.* **1998**, *41*, 4677–4680.

(6) (a) Bäurle, S.; Blume, T.; Mengel, A.; Parchmann, C.; Skuballa, W.; Bäsler, S.; Schäfer, M.; Sülzle, D.; Wrona-Metzinger, H.-P. *Angew. Chem., Int. Ed.* **2003**, *42*, 3961–3964. (b) Winterfeldt, E. *Chimia* **1993**, *47*, 39–45. (c) Prelle, A.; Winterfeldt, E. *Heterocycles* **1989**, *28*, 333–346. (d) Chowdhury, P.-K.; Prelle, A.; Schomburg, D.; Thielmann, M.; Winterfeldt, E. *Liebigs Ann. Chem.* **1987**, 1095–1099. (e) Schomburg, D.; Thielmann, M.; Winterfeldt, E. *Tetrahedron Lett.* **1985**, *26*, 1705–1706.

(7) (a) Lorenc, L.; Bondarenko, L.; Pavlovic, V.; Fuhrer, H.; Rihs, G.; Kalvoda, J.; Mihailovic, M. L. *Helv. Chim. Acta* **1989**, *72*, 608–623. (b) Mihailovic, M. L.; Lorenc, L.; Pavlovic, V.; Bondarenko-Gheorghiu, L. *J. Serb. Chem. Soc.* **1989**, *54*, 459–464. (c) Lorenc, L.; Bondarenko-Gheorghiu, L.; Pavlovic, V.; Fuhrer, H.; Kalvoda, J.; Mihailovic, M. L. *Helv. Chim. Acta* **1992**, *75*, 203–209.

(8) For recent reviews, see: (a) Davis, A. P. *Molecules* **2007**, *12*, 2106–2122. (b) Davis, A. P. *Coord. Chem. Rev.* **2006**, *250*, 2939–2951. (c) Virtanen, E.; Kolehmainen, E. *Eur. J. Org. Chem.* **2004**, 3385–3399. (d) Davis, A. P.; Joss, J.-B. *Coord. Chem. Rev.* **2003**, *240*, 143–156.

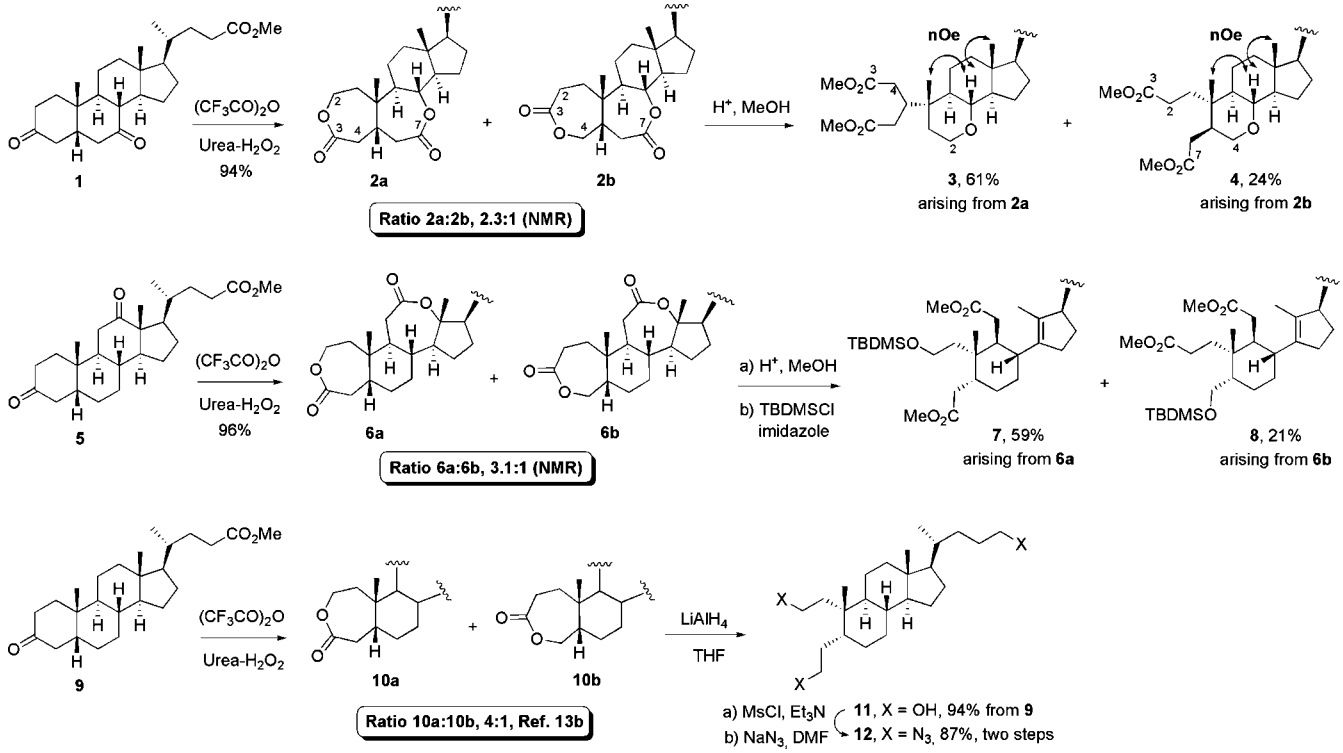
(9) For recent examples, see: (a) Rivera, D. G.; Wessjohann, L. A. *Molecules* **2007**, *12*, 1890–1899. (b) Feigel, M.; Ladeberg, R.; Engels, S.; Herbst-Irmer, R.; Fróhlich, R. *Angew. Chew., Int. Ed.* **2006**, *45*, 5698–5702. (c) Feigel, M.; Landberg, R.; Winter, M.; Bláaser, D.; Boese, R. *Eur. J. Org. Chem.* **2006**, 371–377. (d) Sisson, A. L.; Clare, A. P. *Chem. Commun.* **2005**, 5263–5265. (e) Averin, A. D.; Ranyuk, E. R.; Beletskaya, I. P. *Chem. Eur. J.* **2005**, *11*, 7030–7039.

(10) For examples, see: (a) Ding, B.; Taotofa, U.; Orsak, T.; Chadwell, M.; Savage, P. B. *Org. Lett.* **2004**, *6*, 3433–3436. (b) Madder, A.; Li, L.; De Muynck, H.; Van Haver, D.; Fant, F.; Vanhoenacker, G.; Sandra, P.; Davis, A. P.; De Clercq, P. J. *J. Comb. Chem.* **2002**, *4*, 552–562. (c) De Muynck, H.; Madder, A.; Farcy, N.; De Clercq, P. J.; Pérez-Payń, M. N.; Oḧberg, L. M.; Davis, A. P. *Angew. Chew., Int. Ed.* **2000**, *39*, 145–148. (d) Li, H.; Wang, L.-X. *Org. Biol. Chem.* **2003**, *1*, 3507–3513. (e) Cheng, Y.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 1813–1814. (f) Boyce, R.; Li, G.; Nestler, H. P.; Suenaga, T.; Still, W. *J. Am. Chem. Soc.* **1994**, *116*, 7955–7956.

(11) Wessjohann, L. A.; Voigt, B.; Rivera, D. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 4785–4790.

(12) (a) Sakurai, A.; Yokota, T.; Clouse, S. D. *Brassinosteroids. Steroidal Plant Hormones*; Springer-Verlag: Tokyo, 1999. (b) Khripach, V. A.; Zhabinskii, V. N.; de Groot, A. E. *Brassinosteroids. A New Class of Plant Hormones*; Academic Press: San Diego, 1999.

SCHEME 1. Biomimetic Approach towards Polyfunctional Secocholanes Based on the Baeyer−Villiger Oxidation/Lactone Ring-Opening Sequence of Different Keto Bile Acid Derivatives



natural products, including biologically active steroids.[12] Other advantages of the Baeyer−Villiger reaction are the complete retention of the configuration and its known regioselectivity for ketosteroids. Both issues have been studied in detail before,[13] thus offering possibilities for a reliable synthetic design.

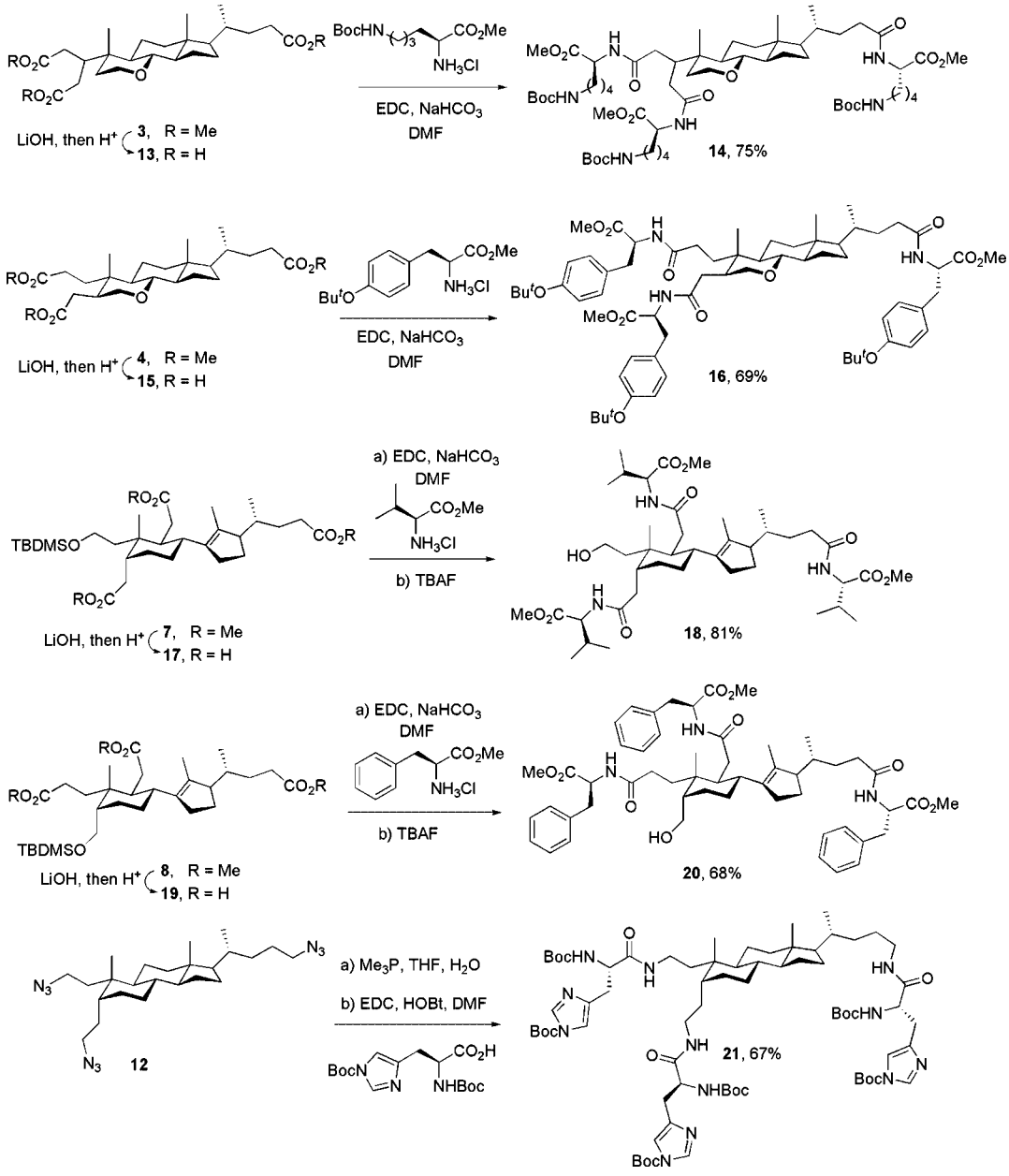
As depicted in Scheme 1, the procedure can be implemented either for producing protected tricarboxylic acids or a triazido secocholane, which can be subsequently converted into a triamino compound. The first two entries rely on a double lactonization reaction followed by acid-catalyzed lactone ring opening to afford two different polycarboxylic scaffolds. The regioisomeric mixtures of lactones **2** and **6** are a consequence of the unselective nature of the Baeyer−Villiger reaction on 3-ketosteroids. The resulting regioisomeric ratios are in agreement with previous results that explain the more favorable migration of C-2 compared to C-4, thus affording the 3-oxa-4-keto-4a-homocholanes **2a** and **6a** as the main products of the lactonization of diketones **1** and **5**, respectively. The regioisomeric lactones were difficult to separate by column chromatography; thus the mixtures were submitted to ring opening by refluxing in an acidic methanolic solution, and the resulting secocholanic esters were easily separated by flash chromatography. The harsh conditions used comprise the concomitant dehydration of the forming secondary and tertiary hydroxyl groups, which helps shifting the process toward the opened compounds. Interestingly, esters **3** and **4** were formed by a stereospecific nucleophilic attack of the primary hydroxyl group to the incipient carbocation at the α-face at C-8. The stereochemistry of the newly formed stereocenter at C-8 was revealed in NOESY experiments by confirming the cross-peaks between H-8 and the methyl groups 18 and 19 (see Supporting Information). For compounds **7** and **8**, protection of the primary hydroxyl group as *tert*-butyldimethylsilyl ether (TBDMS) was required to avoid renewed lactone ring closing during the subsequent deprotection of the methyl esters.

Synthesis of the secocholanic triazide **12** also started with the Baeyer−Villiger oxidation of ketosteroid **9** to furnish the regioisomeric mixture of lactones **10**,[13b] which was subjected to reductive opening of both ester functionalities to yield triol **11** as a single product. Subsequently, 3-fold mesylation and substitution by azide according to standard procedures furnished triazide **12**, which can be subsequently converted into the corresponding triamino secocholane-based scaffold. The great value of the general approach based on a ring-expansion/ring-opening sequence relies on the close control of the core's flexibility and stereochemistry and location of the functional groups. Of course, the approaches employed for polycarboxylic secocholanes can be easily adapted to produce polyamino skeletons.

To illustrate the scope of this idea in the production of multivalent peptidic scaffolds, we focused on the introduction of multiple α-amino acids into the different secocholanic skeletons. As shown in Scheme 2, esters **3**, **4**, **7**, and **8** were deprotected and subsequently submitted to a 3-fold coupling to C-protected α-amino acids by standard coupling protocols. The peptido-secosteroidal conjugates **14**, **16**, **18**, and **20** were thus obtained in good to excellent yields, considering the 3-fold character of the coupling processes and the additional cleavage of the TBDMS groups required to afford compounds **18** and **20**. Alternatively, triazide **12** was reduced by 3-fold Staudinger reaction giving rise to the corresponding triamine, which was identified by ESI-MS and subjected to peptide coupling without further purification and characterization. Accordingly, the trihistidinic secocholanic skeleton **21** was produced in 67% yield via the standard EDC/HOBt protocol and without affecting the

(13) (a) Takatsuto, S.; Ikekawa, N. *Tetrahedron Lett.* **1983**, *24*, 917–920. (b) Rivera, D. G.; Pando, O.; Suardiaz, R.; Coll, F. *Steroids* **2007**, *72*, 467–473, and references therein.

SCHEME 2. Synthesis of Trivalent Peptidic Compounds with a Polyfunctional Secocholane Core

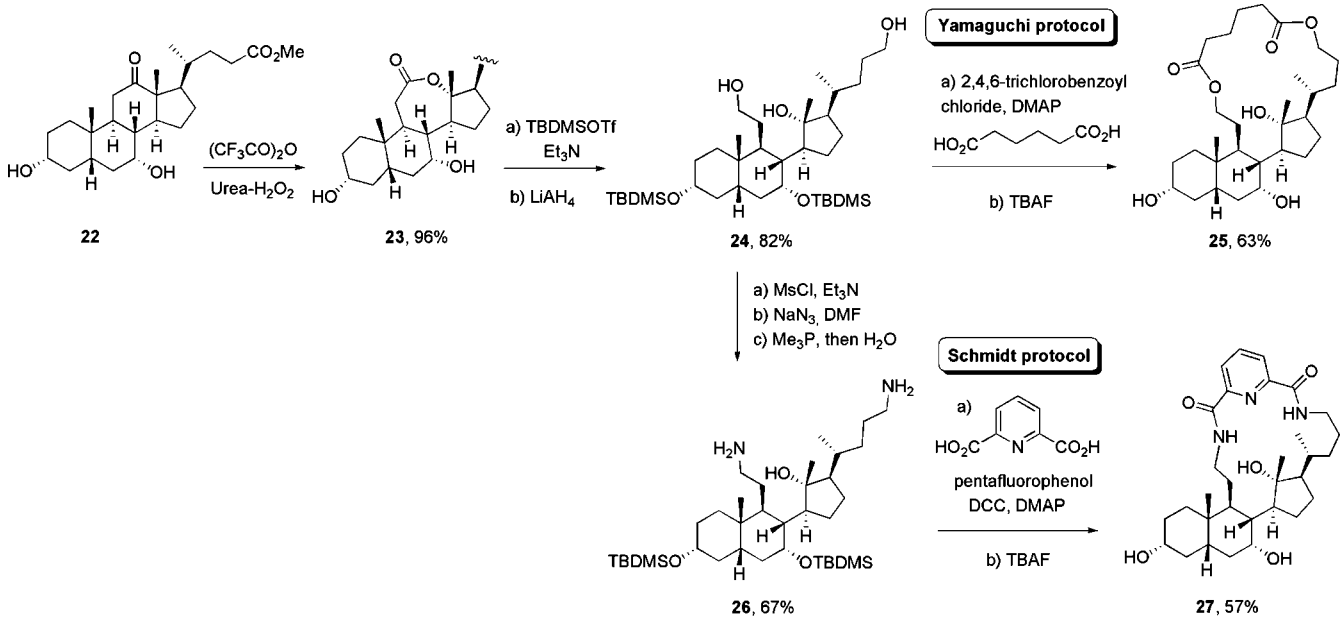


configuration of the di-Boc-L-histidine building block. It might be noticed that α-amino acids bearing bulky and functional side chains were utilized in most procedures in order to probe the reactivity of these novel polyfunctional skeletons and also to explore the possible assembly of biologically relevant, larger peptide strands. In addition, the variable character of the topology, directionality of functional groups and flexibility of these secocholanic scaffolds shows promise toward aligned, multivalent clusters of oligosaccharides.

As mentioned before, the rigid steroidal nucleus of bile acids has been one of the most exploited options for imposing preorganization and to align multiple recognition motifs within macrocyclic scaffolds.[8,9] To probe the more flexible secocholanes in macrocyclizations, we turned to the separate cleavage of rings B and C that produces bifunctional building blocks suitable to assemble macrocycles of varied cavity size, shape and flexibility. Also, to show applicability toward accessing chemical entities of either biological or supramolecular interest, three different and powerful macrocyclization protocols were tested, i.e., macrolactonization, macrolactamization, and multicomponent macrocyclization.

Scheme 3 illustrates the route toward C-secocholanes and their subsequent use in the synthesis of a macrobislactone and a macrobislactam by Yamaguchi and Schmidt macrocyclizations, respectively. Hence, Baeyer−Villiger oxidation of ketocholane **22** furnished lactone **23** as the single regiosiomer, as a result of the higher migration aptitude of the quaternary C-13 compared to the secondary C-11. To gain access to the selectively functionalized polyhydroxylic secocholane **24**, simultaneous protection of the secondary hydroxyl groups at C-3 and C-7 was needed prior to the reductive opening of the lactone ring. Because of the hindered character of the axial 7α-OH, the use of *tert*-butyldimethylsilyl triflate (TBDMSOTf) was required to accomplish the silylation process. Next, the simultaneous reduction of both the lactone moiety on ring C and the ester function at C-24 afforded the diprotected pentahydroxy-

SCHEME 3. Synthesis of a Macrobislactone and a Macrobislactam Including Secocholanic Skeletons



secocholane **24** in 82% yield. The two primary hydroxyls of **24** were converted into amino groups by consecutive mesylation, substitution by azide, and Staudinger reduction to afford the secocholanic diamine **26** in 67% overall yield. The bifunctional secocholanes **24** and **26** were next subjected to macrocyclizations by using different dicarboxylic acids as counterparts in ester and amide bond formation reactions, respectively.

The efficient Yamaguchi macrolactonization protocol gave the macrobislactone **25** in 63% yield from compound **24** and adipic acid previously activated via the 2,4,6-trichlorobenzoyl mixed anhydride method.[14] Alternatively, the macrobislactam **27** was produced in 57% yield from diamine **26** and pyridine-2,6-dicarboxylic acid via the Schmidt macrolactamization protocol, which involves the carboxylate activation by formation of the pentafluorophenol ester.[15] As both macrocyclization protocols were originally designed for intramolecular cyclization under high dilution conditions, the synthetic procedures required adjustment for bifunctional building blocks. That is, one of the building blocks (activated if required) is slowly added to a ca. 10 mM solution of the other one. This provides a suitable high dilution condition for the acyclic intermediate formed in situ, which subsequently can cyclize via the second ester or amide bond forming reaction. Remarkably, both reactions proceeded well under these conditions with a variety of dicarboxylic acids of both aromatic and aliphatic origin. Compounds **25** and **27** are but examples that illustrate the possibility of obtaining macrocycles of either medicinal or supramolecular relevance, as both natural product- and receptor-type scaffolds can be mimicked with easiness. For example, macrocycle **27** seems to be especially suitable for anion binding upon protonation, as a convergent array of functionalities with hydrogen-donor character is provided within the cavity.

As depicted in Scheme 4, an alternative approach was carried out to synthesize the β-secocholanic dicaboxylic acid **30**, which was subsequently employed in two different multicomponent macrocyclization protocols. As expected, the Baeyer−Villiger oxidation of ketocholane **28** furnished exclusively the regiosiomer **29**, as a result of the favored migration of the tertiary C-8 compared to the secondary C-6. Consecutively, a reaction sequence consisted in base-catalyzed lactone ring opening, followed by TBDMS protection of all OH functionalities and finally selective deprotection of the silyl ester led to the secocholanic di-acid **30** in 68% overall yield. The choice of a base-catalyzed hydrolysis aims to preserve the full stereochemical information embedded in the original cholic acid skeleton, i.e., avoiding dehydration of the secondary hydroxyl at C-8 as it occurred previously when acid-catalyzed hydrolysis was utilized.

The resulting diacid **30** is an ideal building block for multiple multicomponent macrocyclizations, a recently developed methodology with great capacity to generate skeletal diversity in one step.[11,16] If the Ugi four-component reaction (Ugi-4CR) is employed, three different combinations could be accomplished with the use of diacid **30**.[11] As shown in Scheme 4, examples of the diacid/diisocyanide and diacid/diamine combinations were successfully implemented by conducting the double Ugi-4CR-based macrocyclizations under typical pseudodilution conditions.[17] Macrocycles **32** and **33** were thus obtained in 47% and 54% yield, respectively, after full deprotection of the hydroxyl groups under mild conditions that did not affect the integrity of the macrocyclic cavity. Whereas the overall yields are lower than those of macrocycles **25** and **27**, it must be noticed that

(14) (a) Dommerholt, F. J.; Thijs, L.; Zwanenburg, B. *Tetrahedron Lett.* **1991**, *32*, 1499–1502. (b) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.

(15) Grieβer, H.; Kroner, M.; Schmidt, U. *Synthesis* **1991**, 294–300.

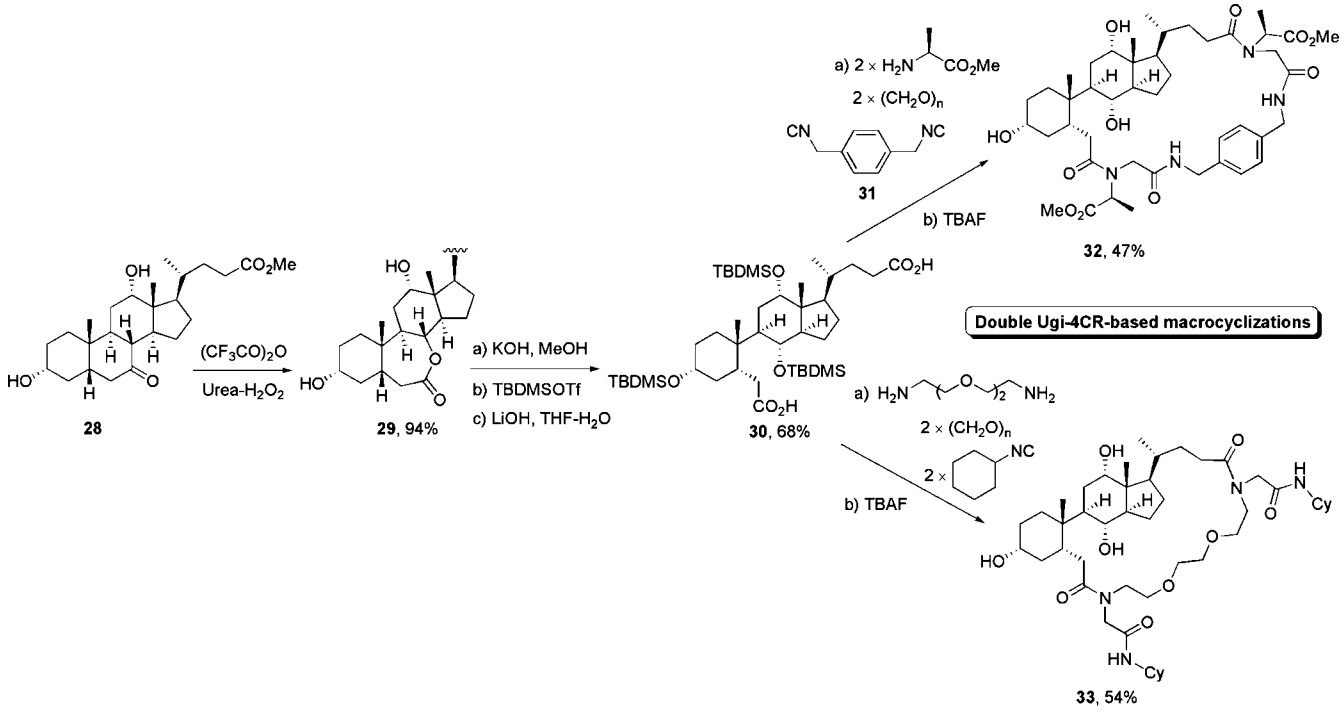
(16) For examples of multiple multicomponent macrocyclizations, see: (a) Wessjohann, L. A.; Ruijter, E. *Mol. Diversity* **2005**, *9*, 159–169. (b) León, F.; Rivera, D. G.; Wessjohann, L. A. *J. Org. Chem.* **2008**, *73*, 1762–1767. (c) Wessjohann, L. A.; Rivera, D. G.; Coll, F. *J. Org. Chem.* **2006**, *71*, 7521–7526. (d) Rivera, D. G.; Vercillo, O. E.; Wessjohann, L. A. *Synlett* **2007**, 308–312. (e) Michalik, D.; Schaks, A.; Wessjohann, L. A. *Eur. J. Org. Chem.* **2007**, 149–157. (f) Rivera, D. G.; Wessjohann, L. A. *J. Am. Chem. Soc.* **2006**, *128*, 7122–7123.

(17) The diacid/dialdehyde combination was not considered because of the poor availability and stability of dialdehydes. Also, due to the nonselective nature of the Ugi-4CR, mixtures of diastereoisomeric macrocycles are produced, thereby making the characterization process very difficult. For example, see: Wessjohann, L. A.; Rivera, D. G.; Leon, F. *Org. Lett.* **2007**, *9*, 4733−4336.

(18) Wessjohann, L. A.; Andrade, C. K. Z.; Vercillo, O. E.; Rivera, D. G. *Targets Heterocycl. Syst.* **2006**, *10*, 24–53.

(19) (a) Sui, Q.; Borchardt, D. L. *J. Am. Chem. Soc.* **2007**, *129*, 12042–12048. (b) Fischer, G. *Chem. Soc. Rev.* **2000**, *29*, 119–127.

SCHEME 4. Synthesis of Peptoid-Secocholane Hybrid Macrocycles by Ugi MiBs



eight new covalent bonds are formed during this type of multicomponent synthesis. In contrast, only two new bonds are formed during the "classical" synthesis of **25** and **27** by the Yamaguchi and Schmidt macrocyclizations, which are indeed much less "diversity-generating".

Interestingly, the NMR spectra of macrocycles **32** and **33** show broad sets of signals due to the occurrence of various conformers in solution. As previously discussed for this type of macrocyclic peptoids,[18] the N-substitution facilitates the amide bond isomerization by decreasing the energy barrier between the *s-cis* and *s-trans* configurations.[19] This intrinsic feature of peptoids, along with the presence of nonsymmetric structures, often leads to poorly resolved spectra. Fortunately, these macrocycles contain quasi-preorganized endocyclic moieties (i.e., fused aliphatic rings) capable to introduce certain conformational restrictions into the macrocyclic cavity. However, the "peptoid effect" is still significant since the NMR spectra of macrocycles **32** and **33** are poorly resolved as compared to, e.g., that of macrocycle **27** (see Supporting Information).

The prospect of combining the skeletal diversity arising from the wide variety of available secocholanes with that of the multiple peptoid moiety variations derived from MiBs is immense. For example, the diacid/diisocyanide combination used to obtain compound **32** produces only endocyclic amide bonds, while the diacid/diamine one used to obtain compound **33** produces peptoid backbones with an exocyclic amide bond. The tactic of varying the number of endo- and exocyclic amide bonds is strengthened by the easy incorporation of various functional groups at the secocholanic building blocks. As these latter skeletons are endocyclic, an appropriate adjustment of their preorganization/flexibility ratio is of high significance for tuning the overall conformational flexibility of the target macrocycles.

Conclusions

A biomimetic approach toward polyfunctional secocholanic skeletons suitable as a core component of multivalent peptidic and macrocyclic compounds has been developed. The method relies on the use of a ring-expansion/ring-opening sequence that utilizes the Baeyer−Villiger oxidation of ketosteroids as the ring-expansion step. This sequence allows a very straightforward and variable access to compounds with tunable flexibility, stereochemistry and functionality of the (subelements of) cholanic skeletons, thus creating structural diversity in very few steps. The potential of the polyfunctional secocholanes as core units of multivalent conjugates was proved by the synthesis of trivalent peptidic scaffolds. The polyfunctional building block character was proven by the synthesis of macrocycles of varied cavity shapes and sizes. The easy access to high levels of skeletal diversity and complexity with a concentration of biologically privileged structural elements, low synthetic cost, and the easy availability of the starting materials demonstrate the versatility of this diversity oriented approach. Finally, these results strengthen the use of renewable natural products such as steroids, not only for medicinal chemistry semisynthetic manipulation but also for the production of scaffolds featuring novel topologies and functionalization patterns. An appropriate selection of these functionalities ideally paired with a fixation of a suitable conformational flexibility can provide new applications in supramolecular chemistry or a biological tool.

Experimental Section

Methyl 4,7-Diketo-3,8-dioxa-4a,8a-dihomo-5β-cholan-24-oate (2a) and Methyl 3,7-Diketo-4,8-dioxa-4a,8a-dihomo-5β-cholan-24-oate (2b). A heterogenic mixture of trifluoroacetic anhydride (2.1 mL, 15 mmol, 3 equiv) and urea-H_2O_2 adduct (1.41 g, 15.0 mmol, 3 equiv) in 30 mL of CH_2Cl_2 was stirred at 0 °C for 10 min. A solution of diketone **1** (2.01 g, 5.0 mmol) in CH_2Cl_2 (30 mL) was then added dropwise, and the reaction mixture was stirred vigorously for 4 h at 0 °C. The mixture was diluted with CH_2Cl_2 (100 mL) and suction-filtered, and the filtrate was washed with aqueous 10% Na_2SO_3 (2 × 100 mL), aqueous 10% $NaHCO_3$ (2 × 100 mL) and brine (100 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. Flash

column chromatography purification (*n*-hexane//EtOAc 2:1) afforded the mixture of the regioisomeric lactones **2** (2.04 g, 94%, ratio 2a/2b, 2.3:1) as a white foam. R_f = 0.25 (*n*-hexane/EtOAc 1:1). IR (KBr, cm^{-1}): 3363, 2953, 1739, 1711, 1432, 1289, 1273, 1243, 1170, 1122, 1006. ^1H NMR ($CDCl_3$,*signals assigned to **2a**): δ 0.69 (s, 3H); 0.92 (d, 3H, J = 6.4 Hz); 1.18, 1.17* (s, 3H); 2.74, 2.88* (dd, 1H, J = 14.0/11.9 Hz); 3.08*, 3.02 (d, 1H, J = 13.8 Hz); 3.67 (s, 3H); 4.07−4.14* (m, 1H); 4.18−4.36 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 174.8, 174.4*, 173.8, 172.6 (*C*O); 79.4, 79.0* (*C*H); 66.2, 62.7 (CH_2); 55.6, 54.3 (*C*H); 51.5 (CH_3O); 45.1 (*C*H); 43.1 (*C*); 40.3, 40.25 (*C*H); 40.2 (CH_2); 39.5 (*C*); 38.2, 36.6 (CH_2); 34.9 (*C*H); 34.2, 34.0 30.9, 30.6, 27.6 (CH_2); 24.9 (CH_2); 24.9*, 24.5 (CH_3); 22.9 (CH_2); 18.1, 11.5 (CH_3). HRMS (ESI-FT-ICR) *m*/*z*: 457.2564 $[M + Na]^+$; calcd for $C_{25}H_{38}NaO_6$ 457.2567.

Methyl 3,13-Dioxa-4,12-diketo-4a,13a-dihomo-5β-cholan-24-oate (6a) and Methyl 3,12-diketo-4,13-dioxa-4a,13a-dihomo-5β-cholan-24-oate (6b). Diketone **5** (2.01 g, 5.0 mmol) was submitted to Baeyer−Villiger oxidation in a similar way as described in the synthesis of the mixture of lactones **2**. Flash column chromatography purification (CH_2Cl_2/EtOAc 3:1) afforded the mixture of the regioisomeric lactones **6** (2.04 g, 94%, ratio 6a/6b, 3.1:1) as a white foam. R_f = 0.41 (CH_2Cl_2/EtOAc 2:1). IR (KBr, cm^{-1}): 2968, 2955, 2880, 1739, 1733, 1436, 1379, 1250, 1111, 1053, 1026. ^1H NMR ($CDCl_3$,*signals corresponding to **6a**): δ 0.87 (s, 3H); 1.05 (d, 3H, J = 6.3 Hz); 1.26, 1.30* (s, 3H); 2.65*, 3.06 (m, 1H); 3.66 (s, 3H); 3.96* (d, J = 13.2 Hz); 4.04−4.20 (m, 1H), 4.65* (dd, 1H, J = 13.1/10.2 Hz). ^{13}C NMR ($CDCl_3$): δ 174.5, 174.0, 173.9, 173.5 (*C*O); 87.2 (*C*); 70.0, 64.7 (CH_2); 60.6, 54.7 (*C*H); 54.5 (CH_2); 51.5 (CH_3O); 50.9 (CH_2); 46.0 (*C*H); 42.8 (*C*); 44.2, 42.8 (*C*H); 37.8, 37.4 (CH_2); 36.8 (*C*H); 36.2, 34.2, 32.6, 30.7, 30.2, 29.0, 25.2, 24.1 (CH_2); 21.3, 18.7, 17.2, 14.5, 14.2 (CH_3). HRMS (ESI-FT-ICR) *m*/*z*: 457.2569 $[M + Na]^+$; calcd for $C_{25}H_{38}NaO_6$ 457.2567.

2,3-Seco-5β-cholane-2,3,24-triol (11). Ketone **9** (972 mg, 2.5 mmol) was submitted to Baeyer−Villiger as described in ref 13b to afford the regioisomeric mixture of lactones **10**. This crude product was dried in vacuo, dissolved in 50 mL of dry THF, and added dropwise under nitrogen to a stirred suspension of $LiAlH_4$ (576 mg, 15 mmol) in dry THF (100 mL) at 0 °C. Stirring was continued for 1 h at room temperature and finally for 2 h at reflux. The mixture was treated cautiously with aqueous 10% NaOH (80 mL), and the resulting white powder was filtered off and washed several times with EtOAc. The combined organic phase was washed with aqueous 10% HCl (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to dryness. Flash column chromatography purification (CH_2Cl_2/EtOAc 5:1) afforded the pure triol **11** (984 mg, 94%) as a white powder. R_f = 0.60 (CH_2Cl_2/MeOH 2:1). Mp (from *n*-hexane/CH_2Cl_2): 211−213 °C. IR (ATR, cm^{-1}): 2931, 2862, 1457, 1448, 1376, 1062, 1041, 1032. ^1H NMR ($CDCl_3$): δ 0.65 (s, 3H); 0.92 (d, 3H, J = 6.6 Hz); 0.95 (s, 3H); 3.69−3.64 (m, 4H); 3.65−3.69 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 63.6, 62.9, 62.4, 42.7 (CH_2); 40.1 (*C*); 39.9 (*C*H); 38.4, 37.8, 36.6 (CH_2); 36.3, 35.9, 35.6 (*C*H); 35.0 (CH_2); 34.6 (*C*); 31.8, 29.4, 28.3, 24.2 (CH_2); 23.0 (CH_3); 21.0 (CH_2); 18.7, 12.1 (CH_3). HRMS (ESI-FT-ICR) *m*/*z*: 403.3184 $[M + Na]^+$; calcd for $C_{24}H_{44}NaO_3$ 403.3186.

2,3-Seco-2,3,24-triazido-5β-cholane (12). A solution of triol **11** (807 mg, 2.0 mmol) in dry CH_2Cl_2 (60 mL) at 0 °C was treated with Et_3N (3.72 mL, 27.0 mmol) and mesyl chloride (1.08 mL, 9.0 mmol). The reaction mixture was stirred at 0 °C for 2 h, then diluted with 150 mL of CH_2Cl_2, and washed with brine (3 × 50 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduce pressure to dryness. The resulting crude product was dissolved in DMF (50 mL), and the solution was treated with NaN_3 (504 mg, 9.0 mmol). The reaction mixture was stirred vigorously under nitrogen atmosphere at 50 °C for 48 h and then diluted with 200 mL of Et_2O. The organic phase was washed with aqueous 10% HCl (2 × 50 mL) and brine (2 × 50 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 8:1) to give the pure triazide **12** (791 mg, 87%) as a white foam (**CAUTION**: Polyazido compounds may be explosive upon heating). R_f = 0.62 (*n*-hexane/EtOAc 5:1). IR (KBr, cm^{-1}): 2974, 2098, 2090, 2085, 1454, 1203, 1174. ^1H NMR ($CDCl_3$): δ 0.68 (s, 3H); 0.94 (d, 3H, J = 6.6 Hz); 0.95 (s, 3H); 3.07−3.25 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 58.9, 58.4, 57.9 (CH_2); 46.1, 44.3 (*C*H); 40.7 (*C*); 39.1, 38.0 (*C*H); 37.8 (CH_2) 36.5 (*C*H); 36.1, 35.8, 35.2 (CH_2); 34.6 (*C*); 33.5 (*C*H); 31.8, 29.4, 28.3 (CH_2); 28.0 (*C*H); 24.2 (CH_2); 23.0 (CH_3); 21.0 (CH_2); 18.7, 12.1 (CH_3). HRMS (ESI-FT-ICR) *m*/*z*: 456.3569 $[M + H]^+$; calcd for $C_{24}H_{42}N_9$: 456.3564.

Peptidosecosteroid 14. LiOH (158 mg, 3.75 mmol, 7.5 equiv) was added to a solution of ester **3** (240 mg, 0.5 mmol) in 50 mL of THF/H_2O (2:1, v/v) at 0 °C. The reaction mixture was stirred at 0 °C for 6 h and then acidified with aqueous 10% $NaHSO_4$ to pH 3. The layers were separated and the aqueous phase was extracted with EtOAc (2 × 80 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to dryness. The resulting tricarboxylic acid **13** was added to a stirred suspension of N_ϵ-Boc-L-lysine methyl ester hydrochloride (890 mg, 3.0 mmol, 6 equiv) and $NaHCO_3$ (252 mg, 3.0 mmol, 6 equiv) in dry DMF (30 mL) under nitrogen atmosphere. EDC (228 mg, 3.0 mmol, 6 equiv) was then added and the stirring was continued at room temperature for 24 h. The reaction mixture was diluted with EtOAc (200 mL), washed with aqueous 10% HCl (2 × 50 mL) and brine (50 mL), and then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2/EtOAc 3:1) to afford the peptidosecosteroid **14** (437 mg, 75%) as a white solid. R_f = 0.50 (EtOAc). Mp (from MeOH): 224−226 °C. IR (KBr, cm^{-1}): 3433, 2972, 2937, 1734, 1732, 1702, 1680, 1521, 1453, 1367, 1247, 1170, 1027. ^1H NMR ($CDCl_3$): δ 0.77 (s, 3H); 0.89 (d, 3H, J = 6.3 Hz); 1.00 (s, 3H); 1.44 (s, 27H); 2.48−5.57 (m, 3H); 2.80−2.74 (m, 2H); 3.10 (m, 7H); 3.48−3.37 (m, 2H); 3.73 (s, 3H); 3.76 (s, 6H); 4.80 (m, 2H); 4.54−4.61 (m, 6H); 6.05 (d, 1H, J = 7.5 Hz); 7.87 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 176.0, 175.8, 174.1, 173.1, 172.9, 172.45, 155.9, 155.7 (*C*O); 79.0 (*C*); 75.5 (*C*H); 64.5 (CH_2); 56.3, 55.8, 55.2 (*C*H); 52.9 (CH_3O); 52.4, 52.1, 51.8 (*C*H); 44.4 (*C*); 40.1, 40.0, 39.9, 39.4, 39.0, 35.7 (CH_2); 35.2, 34.9 (*C*H); 34.6 (*C*); 33.3, 32.1, 31.5, 31.0, 30.7, 29.5, 29.6 (CH_2); 28.45 (CH_3); 24.0 (CH_3); 23.8, 23.3, 23.2, 22.5, 22.4 (CH_2); 18.3, 12.55 (CH_3). HRMS (ESI-FT-ICR) *m*/*z*: 1187.7397 $[M + Na]^+$; calcd for $C_{60}H_{104}N_6NaO_{16}$: 1187.7401. Anal. Found: C 62.0 H 9.3 N 7.5; Calcd.: C 61.8 H 9.0 N 7.2.

Peptidosecosteroid 16. Ester **4** (240 mg, 0.5 mmol) was deprotected to tricarboxylic acid **15**, which was coupled with *O*-*t*-butyl-L-tyrosine methyl ester hydrochloride (863 mg, 3.0 mmol) in a similar way as described in the synthesis of **14**. Flash column chromatography purification (CH_2Cl_2/EtOAc 5:1) yielded the peptidosecosteroid **16** (440 mg, 69%) as a white foam. R_f = 0.55 (CH_2Cl_2/EtOAc 1:1). Mp (from acetone): 246−247 °C. IR (KBr, cm^{-1}): 3415, 3085, 3029, 2964, 2932, 1738, 1732, 1674, 1667, 1448, 1248, 1205, 1167, 1033. ^1H NMR ($CDCl_3$): δ 0.62 (s, 3H); 0.77 (s, 3H); 0.85 (d, 3H, J = 6.3 Hz); 1.30 (s, 27H); 2.91−2.97 (m, 2H); 3.02−3.07 (m, 4H); 3.23 (t, 1H, J = 9.7 Hz); 3.31 (t, 1H, J = 11.5 Hz); 3.63 (s, 3H); 3.64 (s, 3H); 3.67 (s, 3H); 3.72 (dd, 1H J = 11.5/4.5 Hz); 4.69−4.78 (m, 2H); 4.79−4.84 (m, 1H); 5.90 (d, 1H, J = 7.6 Hz); 6.50 (d, 1H, J = 7.6 Hz); 6.86−6.90 (m, 6H); 6.95 (d, 2H, J = 8.4 Hz); 6.99 (d, 2H, J = 8.6 Hz); 7.04 (d, 2H, J = 8.6 Hz). ^{13}C NMR ($CDCl_3$): δ 173.9, 172.8, 172.45, 172.4, 172.1 (*C*O); 131.2, 130.8, 130.3 (*C*); 128.7 (*C*H); 124.8 (*C*); 123.3 (*C*H); 78.3 (*C*); 74.5 (*C*H); 66.0 (CH_2); 55.15, 54.25, 52.9, 52.8 (*C*H); 52.3, 51.8, 51.6 (CH_3O); 50.2 (*C*); 46.0, 44.6 (*C*H); 37.9 (*C*); 38.4 (CH_2); 38.0 (*C*H); 36.6, 36.45, 36.4 (CH_2); 34.35 (*C*H); 32.6, 32.55, 31.3, 30.8, 29.0 (CH_2); 28.1 (CH_3); 27.7, 22.9, 19.85 (CH_2); 17.6, 14.8, 11.6 (CH_3). HRMS (ESI-FT-ICR) *m*/*z*: 1138.6942 $[M + H]^+$; calcd for $C_{66}H_{96}N_3O_{13}$ 1138.6938. Anal. Found: C 69.8, H 8.5, N 3.8. Calcd: C 69.6, H 8.4, N 3.7.

Peptidosecosteroid 18. Ester **7** (298 mg, 0.5 mmol) was deprotected to tricarboxylic acid **17**, which was coupled with L-valine methyl ester hydrochloride (503 mg, 3.0 mmol) in a similar way as described in the synthesis of **14**. The resulting crude product was dissolved in 30 mL of THF, and tetra-*n*-butylammonium fluoride trihydrate (357 mg, 1.0 mmol) was added. The reaction mixture was stirred at room temperature for 12 h and then diluted with 100 mL of EtOAc. The solution was washed with aqueous 10% $NaHCO_3$ (2 × 20 mL) and brine (30 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to dryness. Flash column chromatography purification (CH_2Cl_2/EtOAc 8:1) yielded the peptidosecosteroid **18** (315 mg, 81%) as a white foam. R_f = 0.26 (CH_2Cl_2/EtOAc 6:1). Mp (from MeOH): 217−218 °C. IR (KBr, cm^{-1}): 3314, 2954, 2930, 1741, 1650, 1527, 1463, 1435, 1373, 1257, 1202, 1153, 1074, 836. ^{1}H NMR ($CDCl_3$): δ 0.89 (m, 6H); 0.87, 0.88, 0.89, 0.90, 0.91, 0.92 (6 × d, 18H, J = 6.8 Hz); 3.69−3.71 (m, 2H); 3.72 (s, 3H); 3.73 (s, 6H); 4.48 (dd, 1H, J = 8.3/4.7 Hz); 4.52 (dd, 1H, J = 8.8/4.9 Hz); 4.56 (dd, 1H, J = 8.9/4.9 Hz); 5.81 (d, 1H, J = 8.5 Hz); 5.88 (d, 1H, J = 9.0 Hz); 5.92 (d, 1H, J = 9.0 Hz). ^{13}C NMR ($CDCl_3$): δ 173.2, 173.15, 172.8, 172.7, 172.6, 172.1 (*C*O); 139.7, 133.8 (*C*); 60.3 (*C*H$_2$); 56.8, 56.7, 55.6 (*C*H); 52.0 (*C*H$_3$O); 43.5, 39.4, 38.5 (*C*H); 37.6 (*C*); 36.1, 35.2 (*C*H$_2$); 33.9, 31.9, 31.3, 31.2 (*C*H); 30.6, 29.65, 26.2, 25.2, 22.7, 21.5 (*C*H$_2$); 18.7, 17.9, 17.8, 17.7, 12.8 (*C*H$_3$). HRMS (ESI-FT-ICR) *m/z*: 800.5068 [M + Na]$^+$; calcd for $C_{42}H_{71}N_3NaO_{10}$ 800.5037. Anal. Found: C 64.7, H 9.5, N 5.6. Calcd: C 64.8, H 9.2, N 5.4.

Peptidosecosteroid 20. Ester **8** (298 mg, 0.5 mmol) was deprotected to tricarboxylic acid **19**, which was coupled with L-phenylalanine methyl ester hydrochloride (647 mg, 3.0 mmol) in a similar way as described in the synthesis of **14**. The resulting crude product was dissolved in 45 mL of THF, and tetra-*n*-butylammonium fluoride trihydrate (357 mg, 1.0 mmol) was added. The reaction mixture was stirred at room temperature for 12 h and then diluted with 150 mL of EtOAc. The solution was washed with aqueous 10% $NaHCO_3$ (2 × 30 mL) and brine (30 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to dryness. Flash column chromatography purification (CH_2Cl_2/EtOAc 10:1) yielded the peptidosecosteroid **20** (314 mg, 68%) as a white foam. R_f = 0.42 (CH_2Cl_2/EtOAc 6:1). Mp (from acetone): 238−240 °C. IR (ATR, cm^{-1}): 3307, 3006, 2950, 2928, 1738, 1651, 1522, 1436, 1250, 1202, 1176, 835, 729, 700. ^{1}H NMR ($CDCl_3$): δ 0.82 (d, 3H, J = 6.8 Hz); 0.87 (s, 3H); 0.93 (s, 3H); 3.01−3.15 (m, 6H); 3.62−3.67 (m, 2H); 3.66 (s, 3H); 3.69 (s, 3H); 3.71 (s, 3H); 4.77−4.81 (m, 1H); 4.83−4.89 (m, 2H); 5.76 (d, 1H, J = 7.2 Hz); 5.84 (d, 1H, J = 7.6 Hz); 5.92 (d, 1H, J = 7.8 Hz); 7.06−7.09 (m, 5H); 7.23−7.31 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 173.4, 132.1, 172.9, 172.6, 172.2, 171.8 (*C*O); 139.8, 138.4, 136.1, 135.7 (*C*); 133.8 (*C*); 129.3, 129.2, 128.5, 128.45, 128.4, 127.1, 127.0, 126.9 (*C*H); 60.1 (*C*H$_2$); 57.0, 55.3, 55.1, 53.9 (*C*H); 52.25, 52.0, 52.0 (*C*H$_3$O); 44.3, 40.4, 39.4 (*C*H); 38.7 (*C*); 38.5, 38.3, 36.4, 35.4 (*C*H$_2$); 35.2 (*C*H); 34.6 (*C*H$_2$); 30.5 (*C*H); 26.2 (*C*H$_2$); 20.2, 18.4, 12.8 (*C*H$_3$). HRMS (ESI-FT-ICR) *m/z*: 944.5035 [M + Na]$^+$; calcd for $C_{54}H_{71}N_3NaO_{10}$ 944.5032. Anal. Found: C 70.5 H 7.5 N 4.2. Calcd: C 70.3 H 7.8 N 4.55.

Peptidosecosteroid 21. Trimethylphosphine (1 M in THF, 1.8 mL, 1.8 mmol) was added to a solution of azide **12** (228 mg, 0.5 mmol) in dry THF (10 mL), and the reaction mixture was stirred at room temperature under nitrogen for 5 h. Then water (0.2 mL) was added, and stirring was continued for additional 8 h. The volatiles were evaporated under reduced pressure, and the crude product was dried by repeated addition of toluene and evaporation. The resulting triamine (identified by ESI-MS) in 15 mL of dry DMF was added to a stirred suspension of *N*,*N*-Di-Boc-L-histidine (1.07 g, 3.0 mmol) and HOBt (168 mg, 3.0 mmol) in dry DMF (20 mL) under nitrogen. The reaction mixture was then treated with EDC (233 mg, 3.0 mmol) and stirred at room temperature under nitrogen atmosphere for 24 h. The mixture was diluted with EtOAc (150 mL) and washed with aqueous 10% $NaHCO_3$ (2 × 40 mL), aqueous 10% HCl (40 mL), and brine (50 mL) and then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. The crude product was purified by flash column chromatography (CH_2Cl_2/EtOAc 10:1) to give the peptidosecosteroid **21** (465 mg, 67%) as a white foam. R_f = 0.37 (CH_2Cl_2/EtOAc 5:1). Mp (from MeOH/CH_2Cl_2): 233−235 °C. IR (KBr, cm^{-1}): 3411, 3065, 3033, 2939, 2871, 1727, 1672, 1659, 1583, 1451, 1247, 1050, 1027. ^{1}H NMR ($CDCl_3$): δ 0.67 (s, 3H); 0.92 (d, 3H, J = 6.4 Hz); 0.91 (s, 3H); 1.43 (s, 27H); 1.45 (s, 27H); 3.37−3.48 (m, 6H); 4.67−4.71 (m, 1H); 4.74−4.79 (m, 2H); 5.67 (m, 1H); 5.84 (m, 1H); 5.88 (m, 1H); 5.91−5.99 (m, 2H); 6.76−6.79 (m, 3H); 7.38−7.43 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 172.4, 172.1, 171.8, 155.8, 154.9 (*C*O); 136.0, 135.6, 135.3, 134.7, 133.6 (*C*H); 80.6, 80.1, 79.8 (*C*); 54.4, 54.3, 44.2, 43.7 (*C*H); 42.5, 42.2, 41.9 (*C*H$_2$); 40.0 (*C*); 39.6, 37.9 (*C*H); 37.3 (*C*); 36.2 (*C*H$_2$); 35.8 (*C*H); 35.1, 34.7 (*C*H$_2$); 33.5 (*C*H); 33.0, 32.7, 32.7, 32.6 (*C*H$_2$); 31.5, 29.4 (*C*H$_2$); 28.5, 28.4, 28.3 (*C*H$_3$); 28.1, 24.2, 21.9 (*C*H$_2$); 20.2 (*C*H$_3$); 17.6, 12.6 (*C*H$_3$). HRMS (ESI-FT-ICR) *m/z*: 1411.8590 [M + Na]$^+$; calcd for $C_{72}H_{116}N_{12}NaO_{15}$ 1411.8584. Anal. Found: C 62.4, H 8.65, N 12.3. Calcd: C 62.2, H 8.4, N 12.1.

3α,7α-Di(*tert*-butyldimethylsilyloxy)-12,13-seco-5β-cholane-12,13α,24-triol (24). TBDMSOTf (1.72 mL, 7.5 mmol, 3 equiv) was added to a stirred solution of lactone **23** (1.09 g, 2.5 mmol) and Et_3N (1.04 mL, 7.5 mmol) in dry CH_2Cl_2 (50 mL) under nitrogen atmosphere. Stirring was continue for 12 h, and then the solution was diluted with 200 mL of CH_2Cl_2 and washed sequentially with with aqueous 10% $NaHCO_3$ (2 × 50 mL), aqueous 10% HCl (50 mL), and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The resulting crude product was dried in vacuo, then dissolved in 50 mL of dry THF, and added dropwise to a stirred suspension of $LiAlH_4$ (576 mg, 15 mmol) in dry THF (100 mL). The reaction mixture was stirred at reflux under nitrogen atmosphere for 5 h and then treated cautiously with aqueous 10% NaOH (50 mL). The resulting white powder was filtered off and washed several times with EtOAc. The combined filtrate was washed with aqueous 10% HCl (50 mL) and brine (50 mL), then dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to dryness. Flash column chromatography purification (CH_2Cl_2/EtOAc 5:1) afforded the triol **24** (1.31 g, 82%) as a white powder. R_f = 0.22 (CH_2Cl_2/EtOAc 5:1). Mp (from EtOAc): 206−207 °C. IR (ATR, cm^{-1}): 3301, 1944, 1205, 1046, 1024, 829, 770. ^{1}H NMR ($CDCl_3$): δ 0.03 (s, 3H); 0.04 (s, 3H); 0.06 (s, 6H); 0.76 (s, 3H); 0.86 (s, 9H); 0.91 (s, 9H); 0.93 (d, 3H, J = 6.5 Hz); 0.94 (s, 3H); 3.38 (br. m, 1H); 3.72−3.66 (m, 4H); 3.90 (m, 1H). ^{13}C NMR ($CDCl_3$): δ −4.7, −4.5, −3.3, −3.2, 12.8, 13.3 (*C*H$_3$); 17.9, 18.0 (*C*); 23.4, 26.0, 26.2, 26.3, 26.4 (*C*H$_3$); 28.5, 30.0, 32.8 (*C*H$_2$); 33.3 (*C*H); 33.7, 34.9, 36.5 (*C*H$_2$); 37.9 (*C*); 39.4 (*C*H$_2$); 42.0, 42.9, 55.1 (*C*H); 62.8, 63.3 (*C*H$_2$); 72.8, 72.3 (*C*H); 78.4 (*C*). HRMS (ESI-FT-ICR) *m/z*: 663.4809 [M + Na]$^+$; calcd for $C_{36}H_{72}NaO_5Si_2$ 663.4810.

Macrocycle 25. To an ice-cold solution of adipic acid (73 mg, 0.5 mmol, 1.0 equiv) and Et_3N (0.165 mL, 1.2 mmol, 2.4 equiv) in 5 mL of dry THF was added 2,4,6-trichlorobenzoyl chloride (0.23 mL, 1.1 mmol, 2.2 equiv). The reaction mixture was allowed to reach room temperature, stirred for 2 h, and then diluted with 80 mL of dry toluene. This suspension was added dropwise over 8 h to a stirred solution of DMAP (430 mg, 3.5 mmol, 7.0 equiv) and diol **24** (320 mg, 0.5 mmol, 1.0 equiv) in dry toluene (50 mL) at reflux. After addition was completed, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The crude product was dissolved in 200 mL of Et_2O, and the solution was washed with aqueous 10% HCl (50 mL), aqueous 10% $NaHCO_3$ (2 × 50 mL), and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. The resulting crude product was dissolved in 50 mL of THF, and tetra-*n*-butylammonium fluoride trihydrate (356 mg, 1.0 mmol) was added. The reaction mixture was stirred at room temperature for 12 h and then diluted with 150 mL of EtOAc. The solution was washed with aqueous 10% $NaHCO_3$ (2 × 30 mL)

and brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to dryness. Flash column chromatography purification (CH_2Cl_2/EtOAc 10:1) yielded the macrocycle **25** (165 mg, 63%) as a colorless oil. IR (ATR, cm^{-1}): 2948, 2889, 1734, 1732, 1205, 1141, 1106, 1046. ^1H NMR ($CDCl_3$): δ 0.74 (s, 3H); 0.92 (d, 3H, $J = 6.4$ Hz); 1.04 (s, 3H); 3.33 (br. m, 1H); 3.78−3.72 (m, 4H); 3.92 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 12.6, 13.8, 17.4 (*C*H_3); 23.3, 24.0, 24.3 (*C*H_2); 24.7 (*C*H); 28.1, 29.6, 30.5, 33.3, 34.0 (*C*H_2); 35.3, 35.8 (*C*H); 37.9 (*C*); 39.7, 40.8 (*C*H); 54.7 (*C*H_2); 45.6 (*C*H); 48.5 (*C*); 49.0, 49.4 (*C*H); 58.9 (*C*H); 64.8, 65.2 (*C*H_2); 72.3, 73.8 (*C*H); 78.2 (*C*); 174.9, 175.8 (*C*O). HRMS (ESI-FT-ICR) *m*/*z*: 545.3559 $[M + Na]^+$; calcd for $C_{30}H_{50}NaO_7$ 545.3554.

Macrocycle 27. Dicyclohexylcarbodiimide (260 mg, 1.25 mmol, 2.5 equiv) was added protionwise to a stirred solution of pyridine-2,6-dicarboxylic acid (84 mg, 0.5 mmol, 1.0 equiv) and pentafluorophenol (276 mg, 1.5 mmol, 3.0 equiv) in dry CH_2Cl_2 (20 mL) at 0 °C. Stirring was continued for 20 h at room temperature, and then the resulting precipitate was filtered off. The filtrate was diluted with CH_2Cl_2 (100 mL) and saturated aqueous $NaHCO_3$ (50 mL). A solution of diamine **26** (320 mg, 0.5 mmol, 1.0 equiv) in 20 mL of CH_2Cl_2 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 additional 24 h and diluted with 100 mL of CH_2Cl_2. The organic phase was washed with aqueous 10% $NaHCO_3$ (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting crude product was dissolved in 50 mL of THF, and tetra-*n*-butylammonium fluoride trihydrate (356 mg, 1.0 mmol) was added. The reaction mixture was stirred at room temperature for 12 h and then diluted with 150 mL of EtOAc. The solution was washed with aqueous 10% $NaHCO_3$ (2 × 50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 25:1) and then recrystallized from EtOAc to yield the macrocycle **27** (195 mg, 47%) as pale yellow solid. Mp (from EtOAc): 169−170 °C. IR (ATR, cm^{-1}): 3307, 3102, 3056, 2944, 1203, 1159, 1086, 1025. ^1H NMR ($CDCl_3$): δ 0.81 (s, 3H); 0.97 (d, 3H, $J = 6.5$ Hz); 1.05 (s, 3H); 2.54−2.69 (m, 4H); 2.92−3.04 (m, 2H); 3.11−3.20 (m, 2H); 3.35 (br. m, 1H); 3.94 (m, 1H); 7.61 (d, 1H, $J = 7.7$ Hz); 6.92 (m, 1H); 7.20 (m, 1H); 7.94 (t, 1H, $J = 7.7$ Hz); 8.08 (d, 1H, $J = 7.7$ Hz). ^{13}C NMR ($CDCl_3$): δ 12.8, 17.7, 18.2 (*C*H_3); 23.8, 24.0 (*C*H_2); 25.2 (*C*H); 28.2, 29.6, 33.7 (*C*H_2); 35.7, 36.3 (*C*H); 39.8 (*C*); 40.2, 40.8 (*C*H); 46.6 (*C*H); 48.9, 49.4 (*C*H); 52.4, 54.9 (*C*H_2); 72.1, 73.2 (*C*H); 78.5 (*C*); 155.4, 152.4 (*C*); 136.8, 123.7, 119.1 (*C*H); 171.1, 170.5 (*C*O). HRMS (ESI-FT-ICR) *m*/*z*: 542.3591 $[M + H]^+$; calcd for $C_{31}H_{48}N_3O_5$ 542.3597. Anal. Found: C 68.4, H 8.6, N 7.6. Calcd: C 68.7, H 8.7, N 7.8.

3α,8α,12α-Tri(*tert*-butyldimethylsilyloxy)-7,8-seco-5β-cholane-7,24-dioic Acid (30). KOH (0.55 g, 10 mmol) was added to a solution of lactone **29** (872 mg, 2.0 mmol) in 40 mL of the mixture MeOH/H_2O (4:1, v/v). The reaction mixture was stirred at reflux for 6 h, then cooled to room temperature, and poured into 100 mL of saturated aqueous NH_4Cl. The organic phase was extracted with EtOAc (4 × 50 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to dryness. The resulting crude product was dried in vacuo for 2 h and suspended in 80 mL of dry CH_2Cl_2 under nitrogen. The suspension was treated with Et_3N (1.3 mL, 12.0 mmol) and TBDMSOTf (2.75 mL, 12.0 mmol, 6 equiv) and stirred under nitrogen atmosphere for 48 h. The reaction mixture was diluted with 150 mL of CH_2Cl_2 and washed sequentially with saturated aqueous NH_4Cl (2 × 50 mL) and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. The crude product was then dissolved in 50 mL of the mixture THF/H_2O (2:1, v/v) and treated with LiOH (120 mg, 5 mmol) at 0 °C. The suspension was allowed to reach room temperature, stirred for 4 h, and then acidified with 10% aqueous $NaHSO_4$ to pH 3. EtOAc (200 mL) was added, and the organic phase was washed sequentially with aqueous 5% HCl (50 mL) and brine (50 mL), then dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. Flash column chromatography purification (CH_2Cl_2/EtOAc 1:1) afforded the dicarboxylic acid **30** (1.064 g, 68%) as pale brown oil. $R_f = 0.22$ (CH_2Cl_2/EtOAc 1:1). IR (ATR, cm^{-1}): 3446, 3332, 2934, 2876, 1732, 1454, 1436, 1245, 1171, 1067. ^1H NMR ($CDCl_3$/CD_3OD 95:5): δ 0.05 (s, 6H); 0.06 (s, 6H); 0.09 (s, 6H); 0.85 (s, 9H); 0.88 (s, 9H); 0.93 (s, 9H); 0.92 (d, 3H, $J = 6.5$ Hz); 0.67 (s, 3H); 1.02 (s, 3H); 3.61 (m, 1H); 3.63 (m, 1H); 3.92 (m, 1H). ^{13}C NMR ($CDCl_3$/CD_3OD 95:5): δ −5.05, −5.0, −4.7, −3.3, 12.7, 17.7 (*C*H_3); 17.8, 18.3 (*C*); 23.5, 25.7, 26.2 (*C*H_3); 28.1, 29.6, 30.1, 30.7, 31.3, 31.9 (*C*H_2); 35.9 (*C*H); 37.6 (*C*); 38.3, 46.5 (*C*H); 48.6 (*C*); 49.3 (*C*H); 66.9, 73.5, 73.9 (*C*H); 174.8, 175.4 (*C*O). HRMS (ESI-FT-ICR) *m*/*z*: 781.5295 $[M - H]^-$; calcd for $C_{42}H_{81}O_7Si_3$ 781.5291.

Macrocycle 32. A solution of L-alanine methyl ester hydrochloride (139.6 mg, 1.0 mmol), Et_3N (0.14 mL, 1.0 mmol) and paraformaldehyde (30 mg, 1.0 mmol) in MeOH (100 mL) was stirred at room temperature for 1 h. Two solutions, one of diacid **30** (392 mg, 0.5 mmol) and another of diisocyanide **31**[16f] (78 mg, 0.5 mmol) in 20 mL of MeOH each, were simultaneously, 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 dryness. The resulting crude product was dissolved in 50 mL of THF, and tetra-*n*-butylammonium fluoride trihydrate (535 mg, 1.5 mmol) was added. The reaction mixture was stirred at room temperature for 12 h and then diluted with 150 mL of EtOAc. The solution was washed with aqueous 10% $NaHCO_3$ (2 × 30 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to dryness. Flash column chromatography purification (CH_2Cl_2/MeOH 25:1) yielded the macrocycle **32** (195 mg, 47%) as pale yellow solid. $R_f = 0.62$ (CH_2Cl_2/MeOH 4:1). Mp (from CH_2Cl_2): 216−218 °C. IR (ATR, cm^{-1}): 3323, 2935, 2871, 1733, 1652, 1646, 1436, 1205, 1022. ^1H NMR ($CDCl_3$): δ 0.80 (d, 3H, $J = 6.8$ Hz); 0.82 (s, 3H); 0.90 (s, 3H); 1.50 (d, 3H, $J = 7.0$ Hz); 1.52 (d, 3H, $J = 7.2$ Hz); 2.80−3.40 (m, 6H); 3.52 (br. m, 1H); 3.67 (s, 3H); 3.70 (s, 3H); 3.92−3.98 (m, 2H); 4.12−4.24 (m, 4H); 4.34−4.47 (m, 2H); 4.61−4.82 (m, 4H); 7.04 (m, 1H); 7.22−7.31 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 174.7, 174.3, 172.8, 171.6, 169.7, 168.4 (*C*O); 139.8, 139.2 (*C*); 128.4, 128.2, 128.1, 128.0 (*C*H); 72.0, 71.8, 70.9 (*C*H); 58.1, 56.7 (*C*H_2); 55.4, 55.1, 53.2 (*C*H); 52.9, 52.7 (*C*H_3O); 51.4, 49.3 (*C*H); 43.2, 43.0 (*C*H_2); 42.2 (*C*); 41.7, 39.9 (*C*H_2); 39.7 (*C*); 36.3, 36.2 (*C*H_2); 36.0, 35.7 (*C*H); 34.9, 34.6 (*C*H_2); 34.4, 34. 1, 33.8 (*C*H); 32.7 (*C*H_2); 30.8, 30.5 (*C*H_3); 30.0, 29.6 (*C*H); 28.8, 23.7 (*C*H_2); 18.0, 16.4, 12.6 (*C*H_3). HRMS (ESI-FT-ICR) *m*/*z*: 849.4630 $[M + Na]^+$; calcd for $C_{44}H_{66}N_4NaO_{11}$ 849.4627. Anal. Found: C 64.1, H 8.3, N 7.0. Calcd: C 63.9, H 8.0, N 6.8.

Macrocycle 33. A solution of the 2,6-dioxa-1,8-diaminooctane (74 mg, 0.5 mmol) and paraformaldehyde (1.0 mmol) in MeOH (200 mL) was stirred at room temperature for 2 h. Two solutions, one of diacid **30** (392 mg, 0.5 mmol) and another of cyclohexylisocyanide (90 μL, 1.0 mmol) in 20 mL of MeOH each, were simultaneously, 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 dryness. The resulting crude product was dissolved in 50 mL of THF and tetra-*n*-butylammonium fluoride trihydrate (535 mg, 1.5 mmol) was added. The reaction mixture was stirred at room temperature for 12 h and then diluted with 150 mL of EtOAc. The solution was washed with aqueous 10% $NaHCO_3$ (2 × 30 mL) and brine (50 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. Flash column chromatography purification (CH_2Cl_2/MeOH 20:1) yielded the macrocycle **33** (224 mg, 54%) as a pale yellow oil. $R_f = 0.58$ (CH_2Cl_2/MeOH 4:1). IR (ATR, cm^{-1}): 3304, 2930, 2856, 1653, 1638, 1634, 1449, 1272, 1098, 1026. ^1H NMR ($CDCl_3$): δ 0.81 (s, 3H); 0.87 (s, 3H); 0.92 (d, 3H, $J = 6.6$ Hz); 3.44−3.78 (m, 21H); 3.84−4.17 (m, 4H); 6.21 (d, $J = 8.0$ Hz); 6.66 (d, $J = 8.0$ Hz). ^{13}C NMR ($CDCl_3$): δ 174.8, 172.7, 167.9, 167.7 (*C*O); 72.2, 72.0, 71.9 (*C*H); 70.8, 70.4, 69.2, 67.5, 59.6, 53.4 (*C*H_2); 52.0, 50.3, 48.8 (*C*H); 48.5, 48.1 (*C*H_2); 47.6, 41.7, (*C*H); 40.6 (*C*); 39.5, 36.9 (*C*H_2); 36.5 (*C*); 33.5, 33.2, 33.0, 32.8, 32.4 (*C*H_2); 31.5, 29.7 (*C*H); 25.5, 24.9, 24.8, 24.7,

22.8 (CH_2); 22.3, 18.9, 14.2 (CH_3). HRMS (ESI-FT-ICR) *m*/*z*: 853.5662 $[M + Na]^+$; calcd for $C_{46}H_{78}N_4NaO_9$ 853.5669.

Acknowledgment. We are grateful to the Deutsche Forschungsgemeinschaft for financial support (Programs GRK-894, CERC-3). We also thank Dr. Andrea Porzel for recording the NOESY spectra.

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

JO800708M