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Short Synthesis of Orthogonally Protected 3α,12α-Diamino-5β-cholan-24-oic Acid, a Dipodal Steroid Scaffold for Combinatorial Chemistry

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A short, practical, multigram-scale synthesis of C3α-NHAlloc, C12α-NHBoc-diamino-5β-cholan-24-oic acid **2** was developed, applying a new, straightforward synthetic strategy. Key features are the conservation of the carboxyl moiety at C24 during oxime reduction, the late differentiation between the C3 and C12 amino groups and the gradual separation of diastereomers during the synthesis. This orthogonally protected diamino steroid derivative can be used as starting point for the generation of steroid based dipodal peptide and non-peptide combinatorial libraries.

Introduction

The steroid core has proven to be a versatile building block for the design of frameworks capable of ionic[1] and molecular recognition,[2] the preparation of antimicrobial agents[3] and novel amphiphiles.[4] Bile acid based systems can be further important in the understanding of the functioning of natural systems. Moreover, steroids have found extensive application as scaffold for the assembly of combinatorial libraries.[5] Therefore research on bile acids, their derivatives and potential applications is actively pursued.[6]

In order to attain these goals and to broaden the plethora of possible applications, a fast, easy and efficient access to these steroid building blocks is needed. Our earlier efforts in the area concerned the construction of heterodipodal peptidosteroid libraries of potential serine protease mimics and were based on the use of the C3α-NHAlloc-, C7α-OAc-, C12α-NHBoc-protected diamino steroid scaffold **1** (Figure 1).[5c,5e]

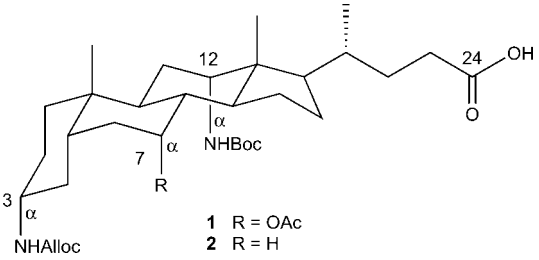

Figure 1. Structure of carboxylic acids **1** and **2**.

The synthesis of suitable quantities of scaffold starting material was a difficult and time-consuming task.[7] Starting from cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid), the large number of synthetic steps results from the sequential derivatization of the hydroxy functions, in order to guarantee the correct stereochemistry and differentiation of the different positions.

Previously, we incorporated up to three amino acids at the C3 and C12 positions of **1** in a combinatorial mix-and-split fashion.[5c,5e] However, using construct **1** for the synthesis of more elaborated peptidosteroid derivatives, the presence of the C7α-OAc proved problematic in some cases, due to partial hydrolysis leading to compound mixtures on solid phase.[8]

For the further construction of heterodipodal peptidosteroid derivatives for various purposes a more convenient preparation of gram quantities of orthogonally protected dipodal scaffold material was needed.

Results and Discussion

Recently, Davis et al. summarized existing preparations of di- and trifunctional amino steroid scaffolds.[9] Surprisingly, the preparation of the C7-deoxy analogue of **1**, orthogonally protected diamino carboxylic acid **2** has not been described before.

As for other suitable orthogonally protected dipodal derivatives, Still's C3α-NHAlloc-, C7α-NHBoc-protected diamino steroid, built around an A,B-*trans*-steroidal core, seemed an attractive alternative.[10] The synthesis of this compound, starting from chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid) is however again quite time-consuming (10 steps, overall yield < 30%), requiring chromatographic separation at various steps. We therefore decided to try and develop a shorter synthesis of scaffold **2** starting from deoxycholic acid (**3**) (3α,12α-dihydroxy-5β-cholan-24-oic acid), applying the straightforward strategy outlined in Scheme 1.

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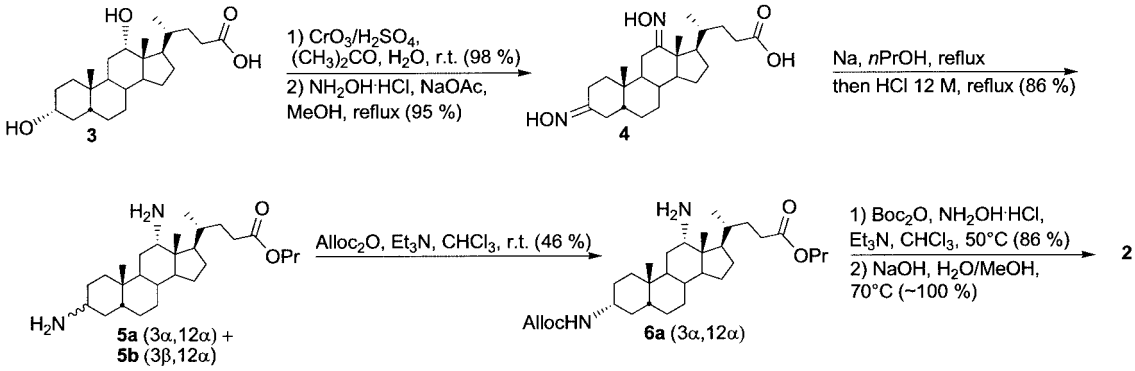
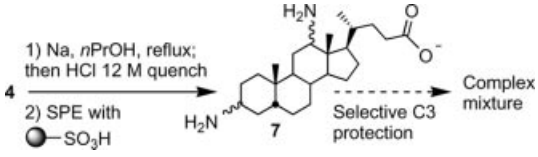


Scheme 1. Preparation of ester **6a** and carboxylic acid **2**.

In an attempt to significantly shorten and simplify the oxidation/oximation/reduction procedure reported by Burrows et al.,[11] deoxycholic acid was oxidized to the corresponding diketone[12] without prior reduction of the C24 carboxylic acid and protection of the corresponding primary alcohol with *tert*-butyldiphenylsilyl chloride. The resulting dioxo carboxylic acid was transformed without intermediate chromatographic purification into the desired dioximo carboxylic acid **4**, which was reduced by treatment with sodium in boiling *n*-propanol. The carboxylic acid moiety of **4** survives these reduction conditions, presumably due to prior conversion into the unreactive sodium salt under the conditions used.

Isolation of the formed diamino carboxylic acid **7** (Scheme 2) by conventional extraction proved problematic and only the use of an acidic ion-exchange resin allowed the isolation of diastereomeric **7** via a solid phase extraction (SPE) procedure.[13]



Scheme 2. SPE isolation of diamino carboxylic acid **7**.

However, all subsequent attempts to selectively protect the C3 amino group of the isolated diamino carboxylic acid with allyl chloroformate, diallyl pyrocarbonate ($Alloc_2O$),[14] di-*tert*-butyl dicarbonate (Boc_2O) or trifluoroacetic anhydride gave rise to complex mixtures, probably due to competitive activation of the carboxylic acid as mixed carbonate-anhydride.[15]

Following reduction of **4**, in situ conversion to the corresponding *n*-propyl ester by overnight heating of the HCl-quenched reaction mixture eventually solved these practical problems. Standard extraction procedures followed by flash chromatographic purification allows the isolation of the diastereomeric mixture **5a** + **5b** in 86% yield. It should be emphasized that in this manner we were able to conserve the carboxylic acid (protected as an ester) without the need for additional reduction or protection steps. In this way easy immobilization on solid support is possible via amide bond formation with classic amino-derivatized resins. We prefer to avoid the use of a more labile ester linkage[16] to the solid support as unwanted cleavage from support could occur during subsequent solid phase synthesis of larger peptides.

As for the analysis of the **5a/5b** reaction mixture, Burrows et al.[11] reported the isolation of pure 3α,12α-diamino-5β-cholan-24-ol in 62% yield obtained from reduction of 3,12-dioximo-5β-cholan-24-ol. Formation/isolation of diastereomers was not mentioned. In our case, although seemingly TLC-pure, careful analysis after flash chromatography by analytical HPLC using non-conventional TIC/SIC (Total Ion Count/Single Ion Count) detection (as described in the supporting information), revealed the presence of a diastereomeric compound that is believed to be the 3β,12α isomer **5b** (**5a/5b** ratio 85:15) (Figure 2, A).[17,18] Separation of the undesired isomer at this stage was very laborious and we therefore decided to proceed with C3 protection and separate at a later stage.

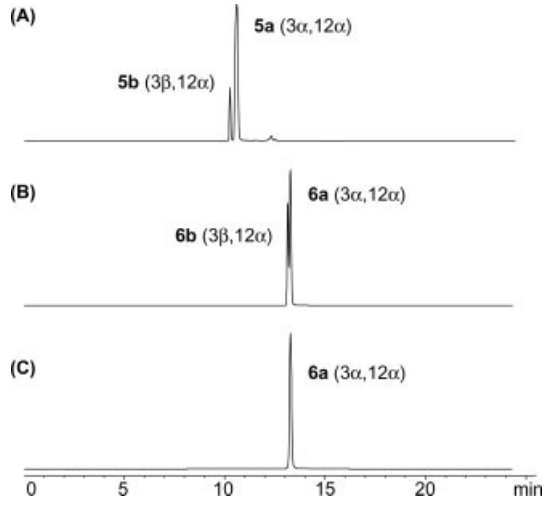


Figure 2. (A) Single Ion Chromatogram (SIC) of a purified mixture **5a** + **5b**; (B) SIC of crude reaction extract of the Alloc protection (Scheme 1, step 3); (C) SIC of purified **6a**.

Although it is generally accepted that the reactivity of the C3α–OH in cholic acid derivatives is significantly higher than that of the C12α–OH due to steric hindrance,[6,19] this selectivity did not apply for the acylation of the C3α–NH_2 vs. C12α–NH_2 group, probably because of the greater nu-

cleophilicity of the NH_2 groups. Complete C3 vs. C12 regioselectivity could not be obtained and yields are only moderate. Among the tested reagents, best results were obtained with $Alloc_2O$ in $CHCl_3/Et_3N$ at room temperature, yielding the desired compound **6a** in 46% yield on a 5-g scale.

Complete elimination of the undesired isomer could now be achieved by flash chromatography. It should be emphasized that in contrast to comparable literature, ^1H NMR spectroscopy alone proved insufficient to estimate the diastereomeric purity of compounds **5a** and **6a**. Only thorough analysis by LC-MS in conjunction with NMR experiments and literature data resulted in the unambiguous determination of the diastereomeric purity and 3α,12α identity of these products (Figure 2).[20] Considering the regio- and stereochemical challenges associated with this single synthetic step, yields remain satisfying. In an attempt to improve the selectivity, the benzotriazole-derived allyl 1-benzotriazolyl carbonate (AllocOBt) was applied. Earlier published procedures describe good C7 vs. C12 selectivity in *o*-nitrobenzenesulfonyl protection using the OBt-derived reagent.[9] In our case, however, no improvement in selectivity was observed.

Scaffold **6a** can immediately be used as a building block in solution, since the C12α–NH_2 group can be selectively functionalized. In order to obtain an orthogonally protected scaffold which can be immobilized on solid support, the remaining free amine had to be protected with a Boc group. Although this is a routine reaction, worth mentioning is the addition of $NH_2OH{\cdot}HCl$ during Boc protection, leading to a faster reaction and a higher, more reproducible yield.[21] Finally the *n*-propyl ester could be easily hydrolyzed to the carboxylic acid upon classic basic hydrolysis, yielding the desired scaffold **2** suitable for attachment to solid support. Both reactions moreover require no additional chromatographic purification.

Conclusions

In conclusion, through careful experimentation a very straightforward and short route towards the new dipodal scaffolds **6a** and **2** has been optimized. The resulting procedures can be easily applied on a large scale, while overall yields (37% and 32%, comparable to earlier published overall yields for related derivatives) are satisfying. In this way gram quantities of these building blocks are readily available.

Experimental Section

General Information: All reagents were obtained either from Aldrich or ACROS Organics and were used without prior purification. Reactions were performed under argon atmosphere using HPLC grade solvents (obtained from Sigma–Aldrich or Fisher Scientific). MeOH was dried on Mg/I_2 and Et_3N on CaH_2. Optical rotations were recorded at room temperature on a Perkin–Elmer 241 polarimeter at room temperature. Melting points were obtained using an Electrothermal IA9000 Series Digital Melting Point Apparatus and are quoted uncorrected. Analytical TLC was carried out on glass plates precoated with silica gel (Macherey–Nagel, 60F254, 0.25 mm). Compounds were visualized by phosphomolybdic acid (PMA), $KMnO_4$, vanillin (4-hydroxy-3-methoxybenzaldehyde), ninhydrin (2,2-dihydroxy-1,3-indanedione) and chloranil (tetrachloro-1,4-benzoquinone, 2% solution in DMF). Due to large differences in polarity between most starting materials and reaction products, two different eluent systems were used to follow most reactions on TLC. Flash chromatography was performed on Kieselgel Merck Typ 9385 230–400 mesh, 60 Å. Semi-preparative normal-phase HPLC was carried out on a Bio-RAD Bio-Sil D 90–10 (250 mm × 10 mm) column with RI (Refractory Index) detection.

NMR spectra were recorded at room temperature on a Bruker Avance-500 spectrometer at 500 MHz for ^1H NMR and 125 MHz for ^{13}C spectra. Deuterated solvents $CDCl_3$ (99.8 atom% D) and [D_6]-DMSO (99.9 atom% D) were obtained from Aldrich. Chemical shifts (δ units) are expressed in parts per million (ppm) relative to tetramethylsilane (TMS) and the internal solvent peak was used for calibration. When peak multiplicities are reported, the following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Coupling constants (*J* values) are expressed in Hertz (Hz). The Attached Proton Test (APT) technique was used to assign ^{13}C peaks (C, CH, CH_2, CH_3). Assignment was aided by comparison with literature values for similar compounds.[9,11a,22] Infrared spectra were recorded on a Perkin–Elmer 1600 Series FTIR spectrometer. Bands were quoted in cm^{-1} and the following abbreviations are used: w, weak; m, medium; s, strong; br, broad. Only the diagnostive signals were listed.

ES-MS spectra were acquired on a quadrupole ion trap LC mass spectrometer (Thermo Finnigan MAT LCQ mass spectrometer) equipped with electrospray ionization. HR-MS values were measured on a Thermo Finnigan MAT95XP-Trap mass spectrometer. Reversed-phase LC-MS was performed on an Agilent 1100 series HPLC, using a Phenomenex Luna C18 (2) (250 mm × 4.6 mm) column coupled to an Agilent ESI-single quadrupole MS detector type VL. A gradient of 0–100% CH_3CN containing 0.1% formic acid over 15 min was applied.

3,12-Dioximo-5β-cholan-24-oic Acid (4): To a solution of 3,12-dioxo-5β-cholan-24-oic acid (19.01 g; 48.93 mmol) in dry MeOH (124 mL) were added $NH_2OH{\cdot}HCl$ (13.61 g, 195.8 mmol, 4 equiv.) and NaOAc (24.09 g, 293.6 mmol, 6 equiv.) to give a white suspension. The mixture was refluxed for 40 h. The mixture was cooled to room temperature, filtered and the solid was washed with MeOH and H_2O. The solid was suspended in H_2O, filtered again and washed with H_2O. The combined filtrates were concentrated and the resulting suspension filtered through the same filter and washed with H_2O. The collected white solid was initially dried on the filter at 60 °C and further dried under high vacuum. Dioximo carboxylic acid **4** (19.36 g, 46.26 mmol, 95%) was obtained as a white powder and proved pure enough to use in the next step. $[a]_D$ = +143.6 (*c* = 1.035, DMSO); R_f = 0.29 (cyclohexane/EtOAc, 1:1 + 1% HOAc; PMA). ^1H NMR (500 MHz, DMSO): δ = 10.09 (s, 1 H, NOH), 10.06 (s, 1 H, NOH), 2.92–3.21 (m, 2 H), 2.19–2.30 (m, 1 H), 1.93–2.16 (m, 4 H), 1.01–1.92 (series of m, 19 H), 0.98 (s, 3 H), 0.90 (d, 6.8 Hz, 3 H, 21-CH_3), 0.86 (s, 3 H). ^{13}C NMR (125 MHz, DMSO): δ = 174.9 (COOH), 162.9 (C=N), 157.5 (C=N), 58.6 (CH), 49.2 (C), 47.0 (CH), 41.7 (CH), 40.8 (CH), 37.0 (CH_2), 35.6 (C), 35.20 (CH), 35.15 (CH), 31.5 (CH_2), 30.5 (CH_2), 27.2 (CH_2), 26.4 (CH_2), 25.4 (CH_2), 24.5 (CH_2), 23.7 (CH_2), 22.4 (19-CH_3), 19.5 (21-CH_3), 12.1 (18-CH_3). IR (neat): $\tilde{\nu}_{max}$ = 3406 (s, br), 1696 (s), 1659 (m) cm^{-1}. ES-MS *m/z* (% rel. int.) 418.9 (100) $[M + H]^+$, 441.1 (28) $[M + Na]^+$, 858.8 (53) $[2M + Na]^+$.

3α,β-Diastereomeric Mixture of *n*-Propyl 3,12α-Diamino-5β-cholan-24-oate (5a + 5b): Dioximo carboxylic acid **4** (17.04 g; 40.71 mmol) was suspended in *n*PrOH (3.5 L) and heated at reflux under argon. Sodium (56.44 g; 2.455 mol; 60.3 equiv.) was carefully added portionwise over a period of 3 h. After addition of the last piece of sodium the reaction was heated at reflux overnight to ensure complete reaction of the sodium before quenching. TLC indicated the complete consumption of starting material (acetone/MeOH, 7:3 + trace HOAc; chloranil). A 12 M HCl aqueous solution (210 mL) was added very slowly to the refluxing mixture, leading to the immediate precipitation of NaCl. 10 mL of a 12 M HCl aqueous solution was further added and the suspension was heated at reflux overnight (pH 2–1). TLC showed complete conversion (EtOAc/MeOH/NH_3 (aqu., 28.0–30.0%) 9:1:1; chloranil or $KMnO_4$), indicating four diastereomers. The mixture was cooled to room temperature and the solvent was co-evaporated with toluene. The off-white residue was transferred to a separation funnel with $CHCl_3$ and a saturated $NaHCO_3$ aqueous solution was added (≈ 1 L aqueous/≈ 1 L $CHCl_3$). The phases were separated and the H_2O was further extracted by two portions of $CHCl_3$ (700 and 300 mL). The organic phase was partially concentrated and washed with a saturated $NaHCO_3$ aqueous solution. The organic solvent was further evaporated to obtain an orange oil. The orange oil was carefully purified by flash chromatography (column diameter: 8 cm, length: 34 cm; the crude oil was dissolved in EtOAc, elution with EtOAc/MeOH/NH_3 (aqu., 28.0–30.0%) 28:3:1) to obtain the diamino ester as diastereomeric mixture **5a** + **5b** (diastereomeric ratio 85:15). The yellow oil solidifies upon freezing (15.16 g; 35.04 mmol; 86%). R_f = 0.26 (EtOAc/MeOH/NH_3 (aqu., 28.0–30.0%) 9:1:1; chloranil). ^{1}H NMR (500 MHz, $CDCl_3$, only indicative peaks are listed): δ = 4.01 (t, 6.5 Hz, 2 H, ester O–CH_2), 3.17 (br. s, 1 H, 12 βH), 2.73 (br. s, 1 H, 3 βH), 2.31–2.40 (m, 2 H), 2.18–2.26 (m, 2 H), 2.02 (s, 2 H), 0.99–1.94 (series of m), 0.88–0.99 (m, 12 H), 0.71 (s, 3 H). IR (neat): $\tilde{\nu}_{max}$ = 3382 (m), 3321 (m), 3188 (m, br), 1732 (s) cm^{-1}. ES-MS *m*/*z* (% rel. int.) 433.0 (100) [M + H]$^+$. HR-MS (ES) *m*/*z* calcd. for $C_{27}H_{48}N_2O_2$ + H 433.3789, found 433.3783.

***n*-Propyl 3α-(Allyloxycarbonylamino),12α-amino-5β-cholan-24-oate (6a):** The diastereomeric mixture **5a** + **5b** (5.068 g; 11.71 mmol) was dissolved in $CHCl_3$ (500 mL) and dry Et_3N (3.4 mL, 24.39 mmol, 2.1 equiv.) was added. The yellow solution was stirred at room temperature and $Alloc_2O$ (1.75 mL, 10.54 mmol, 0.9 equiv.) was added in one portion and the mixture was reacted overnight. After TLC showed completeness of reaction (EtOAc/MeOH/NH_3 (aqu., 28.0–30.0%) 9:1:1 and CH_2Cl_2/MeOH, 9:1; chloranil and ninhydrin), the mixture was transferred to a separation funnel and a saturated $NaHCO_3$ aqueous solution (300 mL) was added. The phases were separated and the aqueous phase was extracted with $CHCl_3$ (300 mL). The combined organic phases were partially concentrated under reduced pressure and washed with a new portion of a saturated $NaHCO_3$ aqueous solution. The organic phase was concentrated under reduced pressure to obtain a viscous yellow oil. The oil was carefully purified by flash chromatography (column: diameter: 4.5 cm, length: 32 cm; crude oil dissolved in CH_2Cl_2, elution with CH_2Cl_2/MeOH, 98:2) to obtain diastereomerically pure **6a** as a yellow oil (2.801 g, 5.419 mmol, 46%). $[\alpha]_D$ = +96.7 (*c* = 0.770, EtOH); R_f = 0.66 (CH_2Cl_2/MeOH, 9:1; ninhydrin). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.86–5.97 (m, 1 H, Alloc), 5.29 (dd, 1.6 Hz; 17.3 Hz, 1 H, Alloc), 5.20 (dd, 1.3 Hz; 10.4 Hz, 1 H, Alloc), 4.59 (d, 6.7 Hz, 1 H, NH), 4.54 (d, 4.4 Hz, 2 H, Alloc), 4.02 (t, 6.7 Hz, 2 H, ester O-CH_2), 3.49 (br. s, 1 H, 3 βH), 3.17 (br. s, 1 H, 12 βH), 2.32–2.40 (m, 1 H), 2.18–2.27 (m, 1 H), 0.99–1.91 (series of m, 26 H), 0.90–0.99 (m, 9 H), 0.71 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.2 (COOR), 155.4 (Alloc C=O), 133.0 (Alloc *C*H=CH_2) 117.3 (Alloc CH=*C*H_2), 65.8 (ester O–CH_2), 65.1 (Alloc O–CH_2), 54.0 (CH), 50.9 (CH), 47.9 (CH), 47.8 (CH), 46.1 (C), 42.3 (CH), 36.2 (CH), 35.6 (CH_2), 35.1 (CH), 34.0 (C), 33.8 (CH_2), 33.5 (CH), 31.3 (CH_2), 30.9 (CH_2), 28.7 (CH_2), 28.1 (CH_2), 27.6 (CH_2), 26.9 (CH_2), 26.0 (CH_2), 23.7 (CH_2), 23.2 (19-CH_3), 21.9 (ester CH_2), 17.1 (21 – CH_3), 13.7 (18-CH_3), 10.3 (ester CH_3). IR (neat): $\tilde{\nu}_{max}$ = 3336 (w), 1724 (s) cm^{-1}. ES-MS *m*/*z* (% rel. int.) 517.5 (100) [M + H]$^+$. HR-MS (ES) *m*/*z* calcd. for $C_{31}H_{52}N_2O_4$ + H 517.4000, found 517.4020

3α-(Allyloxycarbonylamino)-12α-(*tert*-butoxycarbonylamino)-5β-cholan-24-oic Acid (2). a) Protection of 6a with Di-*tert*-butyl Dicarbonate: To a solution of **6a** (1.000 g; 1.935 mmol) in $CHCl_3$ (80 mL) was added dry Et_3N (540 μL; 3.874 mmol; 2.0 equiv.). The reaction was stirred at room temperature and Boc_2O (1.35 mL; 1.3 g; 5.876 mmol; 3.0 equiv.) was added, followed by NH_2OH·HCl (134.5 mg; 1.936 mmol; 1.0 equiv.). The pale yellow solution was heated to 50 °C and stirred overnight. After TLC indicated complete consumption of starting material (CH_2Cl_2/MeOH, 9:1 and isooctane/EtOAc 2:1; ninhydrin and PMA) the reaction mixture was concentrated under reduced pressure. The residue was redissolved in CH_2Cl_2, filtered and washed with CH_2Cl_2 over a plug of silica. The silica was further washed with EtOAc, which was collected into a different flask. Finally the silica was washed with MeOH and the three phases were evaluated on TLC (isooctane/EtOAc 2:1; vanillin and PMA) which clearly indicated the separation of the excess Boc_2O (CH_2Cl_2 phase) and the pure product (EtOAc phase). Upon evaporation of EtOAc under reduced pressure, the residue was further dried under high vacuum to obtain the desired product as a yellow oil (1.037 g; 1.681 mmol; 87%). $[\alpha]_D$ = +86.8 (*c* = 0.505, EtOH); R_f = 0.49 (isooctane/EtOAc 2:1; PMA). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.87–5.97 (m, 1 H, Alloc), 5.29 (dd, 1.5 Hz; 17.3 Hz, 1 H, Alloc), 5.20 (br. d, 10.3 Hz, 1 H, Alloc), 4.74 (d, 6.7 Hz, 1 H, NH), 4.59 (br. s, 1 H, NH), 4.55 (br. s, 2 H, Alloc), 4.01 (t, 6.8 Hz, 2 H, ester O–CH_2), 3.91 (br. s, 1 H, 12 βH), 3.47 (br. s, 1 H, 3 βH), 0.98–1.91 (series of m, 37 H), 1.44 (s, 9 H, Boc CH_3), 0.86–0.96 (m, 9 H), 0.77 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.2 (COOR), 155.2 (Alloc C=O), 154.9 (Boc C=O), 132.9 (Alloc *C*H=CH_2), 117.4 (Alloc CH=*C*H_2), 78.9 (Boc C), 65.7 (ester O–CH_2), 65.1 (Alloc O–CH_2), 53.4 (CH), 50.9 (CH), 50.6 (CH), 48.4 (CH), 44.6 (C), 42.1 (CH), 35.8 (CH), 35.5 (CH_2), 34.8 (CH), 34.6 (CH), 34.0 (C), 33.8 (CH_2), 31.3 (CH_2), 30.8 (CH_2), 28.4 (Boc 3 x CH_3), 27.2 (CH_2), 26.7 (CH_2), 26.4 (CH_2), 26.0 (CH_2), 23.6 (CH_2), 23.3 (19-CH_3), 21.9 (ester CH_2), 17.0 (21-CH_3), 13.7 (18-CH_3), 10.3 (ester CH_3). IR (neat): $\tilde{\nu}_{max}$ = 3333 (m), 1713 (s) cm^{-1}. ES-MS (200 °C) *m*/*z* (% rel. int.) 517.1 (62) [M – Boc]$^+$, 616.5 (35) [M + H]$^+$, 639.1 (100) [M + Na]$^+$, 1254.5 (33) [2M + Na]$^+$. HR-MS (ES) *m*/*z* calcd. for $C_{36}H_{60}N_2O_6$ + H 617.4524, found 617.4525.

b) Basic Hydrolysis: 865.2 mg (1.403 mmol) of the product isolated in the previous step (which proved pure enough for this reaction) was suspended in a mixture of MeOH (70 mL) and a 2 M NaOH aqueous solution (18 mL). The mixture was heated at 70 °C for 2 h. TLC (isooctane/EtOAc, 2:1 and pentane/EtOAc, 1:1; PMA) showed complete conversion of starting material and the mixture was cooled to room temperature. MeOH was evaporated under reduced pressure and the residue was transferred to a separation funnel. The pH was lowered to 3–4 with a 0.12 M HCl aqueous solution and the product was extracted in EtOAc. The organic phase was dried on $MgSO_4$, filtered and concentrated. Drying of the product at high vacuum afforded carboxylic acid **2** as a white solid (805.8 mg; 1.402 mmol; ≈ 100%). An analytical sample was carefully purified by normal-phase HPLC (isooctane/EtOAc, 8:2 + 0.1% HOAc). $[\alpha]_D$ = +69.3 (*c* = 0.505, EtOH); m.p. 104.0–107.0;

R_f = 0.66 (pentane/EtOAc, 1:1 + 1% HOAc; PMA). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.87–5.97 (m, 1 H, Alloc), 5.30 (dd, 1.3 Hz; 17.2 Hz, 1 H, Alloc), 5.20 (br. d, 10.3 Hz, 1 H, Alloc), 4.78 (br. s, 1 H, NH), 4.60 (br. s, 1 H, NH), 4.55 (br. s, 2 H, Alloc), 3.92 (br. s, 1 H, 12 βH), 3.48 (br. s, 1 H, 3 βH), 2.34–2.45 (m, 1 H), 2.18–2.29 (m, 1 H), 0.97–1.92 (series of m, 34 H), 1.44 (s, 9 H, Boc CH_3), 0.91 (s, 3 H), 0.90 (d, 6.4 Hz, 3 H, 21-CH_3), 0.78 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 178.3 (COOH), 155.4 (Alloc C=O), 155.0 (Boc C=O), 132.9 (Alloc *C*H=CH_2), 117.4 (Alloc CH=*C*H_2), 79.0 (Boc C), 65.2 (Alloc O–CH_2), 53.3 (CH), 50.9 (CH), 50.6 (CH), 48.4 (CH), 44.6 (C), 42.1 (CH), 35.8 (CH), 35.5 (CH_2), 34.8 (CH), 34.5 (CH), 33.9 (C), 33.8 (CH_2), 30.8 (CH_2), 30.5 (CH_2), 29.6 (CH_2), 28.4 (Boc 3 x CH_3), 27.3 (CH_2), 26.7 (CH_2), 26.4 (CH_2), 26.0 (CH_2), 23.6 (CH_2), 23.3 (19-CH_3), 17.0 (21-CH_3), 13.7 (18-CH_3). IR (neat): $\tilde{\nu}_{max}$ = 3333 (m, br), 1708 (s) cm^{-1}. ES-MS *m/z* (% rel. int.) 475.4 (42) $[M - Boc]^+$, 597.4 (100) $[M + Na]^+$, 1172.4 (43) $[2M + Na]^+$. HR-MS (ES) *m/z* calcd. for $C_{33}H_{54}N_2O_6$ + H 575.4055, found 575.4058.

Supporting Information (see also the footnote on the first page of this article): Integrated analytical approach for the determination of the diastereomeric purity and identity of **5a** and **6a** and copies of ^{13}C (APT) and ^{1}H NMR spectra.

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[12] 3,12-dioxo-5β-cholan-24-oic acid is also commercially available from Steraloids, Inc. (Newport, Rhode Island, USA).

[13] After quenching the reduction mixture with a 12 M HCl aqueous solution, solvents were evaporated under reduced pressure. The crude residue (2.6 g) was dissolved in MeOH (30 mL), Amberlyst 15 (wet) acidic ion-exchange resin (1.5 g) was added and the mixture was shaken for 1 h. The resin was filtered and washed with water and MeOH. After concentration under reduced pressure, the filtrate was treated similarly with a new batch of Amberlyst resin. Both batches of resin were suspended in a 7 M NH_3 solution in MeOH (30 mL) and the mixture was gently shaken for 45 min. Upon filtration and washing with MeOH, concentration of the filtrates under reduced pressure and drying under high vacuum yielded about 88 mg of diamino carboxylic acid **7** as an off-white solid. ES-MS (250 °C) *m/z* (% rel. int.) 391.4 (100) $[M + H]^+$, 782.4 (15) $[2M + H]^+$.

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[16] Savage and co-workers immobilize the C24 alcohol on an acid chloride polystyrene resin (see ref.[5d]).

[17] Literature suggests sodium reduction at the C12 position is nearly stereoselective (see ref.[5a,5d]). Other simultaneous reduction protocols of oximes (see ref.[11b]) give lower yields and/or are again mainly hampered by formation of isomers at the C3 position.

[18] Presumably chromatographic elimination of the minor diastereomer is easier in case of the more polar diamino alcohol compared to a diamino ester.

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