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# A Steroidal Cyclopeptide, Synthesis and Shape of the Cavity

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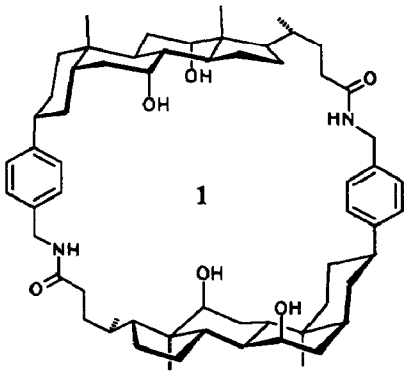
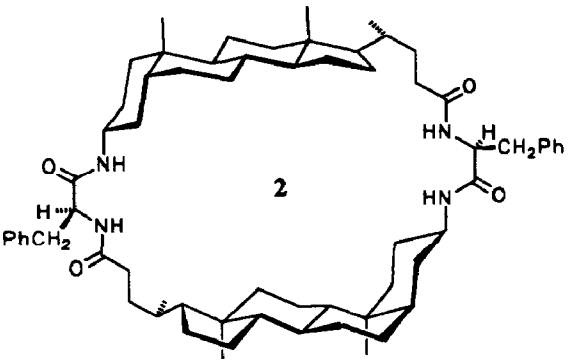
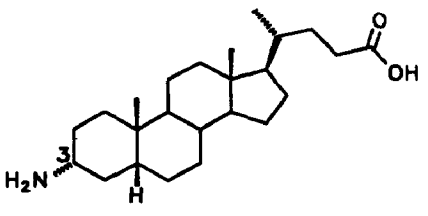
***Abstract***: Cyclo-[3α-(phenylalaninylamino)-5β-cholanate]$_2$ **2** was synthesized from lithocholic acid and (S)-phenylalanine. The synthesis requires three transformations: i) stereoselective conversion of the 3α-hydroxy group to the 3α-amino group; ii) preparation of the linear steroidal peptide; iii) cyclodimerisation. NMR measurements and MM3 calculations support a conformation of **2** with a lipophilic cavity.

The rigid concave shape of cholic acids makes them ideal building blocks for the construction of synthetic host molecules.[1] Advanced systems elaborated so far are cholaphanes of the type **1**[2] and cyclocholates developed by Bonar-Law.[3] Although the phenyl spacer in **1** gives the cavity an extended shape, the functionalization of the phenyl groups is limited and the size of the cavity is fixed. We report here the synthesis and characterization of the first chola-cyclopeptide **2** where two molecules of the pseudoamino acid **3** (3α-amino-lithocholic acid) form with two phenylalanines a cyclopeptide with a lipophilic steroid cavity.



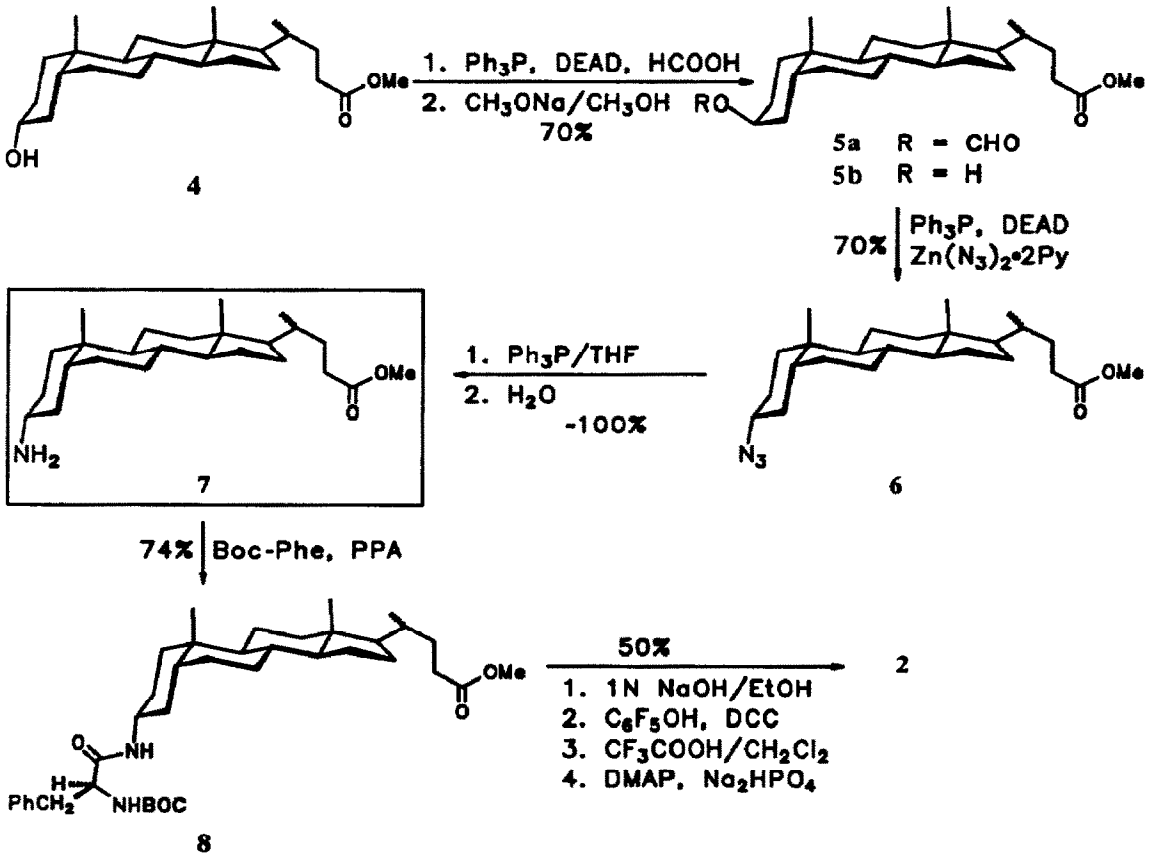




To achieve the synthesis of **3**, the 3α-OH group of lithocholic acid has to be replaced by an amino group with retention of the configuration. The triphenylphosphine - diethylazodicarboxylate - reagent[4] was the key to this transformation. It proved to be suitable for a twofold inversion of the configuration at the C3 atom. Therefore, the lithocholic acid was first converted into the corresponding methylester **4**.




**3**

Methyl-3β-formyloxy-5β-cholanate **5a** was then prepared by nucleophilic substitution under inversion of the 3α-OH group activated with the $PPh_3$/DEAD-system. The formyl derivative **5a** was subsequently cleaved to the 3β-hydroxyester **5b** with 10% sodium methoxide in methanol.[5,6] The configurational homogeneity is proved by the different chemical shifts of the C3-protons in equatorial and axial position ($\delta(H3_{eq}$ in **5b**) = 4.10 ppm; $\delta$ ($H3_{ax}$ in **4**) = 3.64 ppm) and by their different coupling constants, which lead to an narrow multiplet for the 3β-position and a wide triplet of triplets for the 3α-position.[7] The return to the initial configuration was achieved with a second Mitsunobu substitution using azide as nucleophilic amine equivalent.[8,9] Because of its stability the zinc azide-bis-pyridine complex was used instead of the uncomfortable hydrazoic acid. The azide group in **6** was reduced by triphenylphosphine[10] to the corresponding iminophosphorane which hydrolyzes to the amino derivative **7** in an overall yield of 49% based on **4**.[11]




N-BOC-protected amino acids are easily attached to **7** using propane phosphonic acid anhydride.[12,13] The phenylalanine derivative **8** is converted to the pentafluorophenolester which cyclizes to the cholapeptide **2** after removal of the BOC group.[14,15] The identity of the macrocycle **2** follows from the DCI-mass spectrum.[16] The $^1$H-NMR spectrum shown in figure 1 reflects the purity and C2 symmetry of **2**.

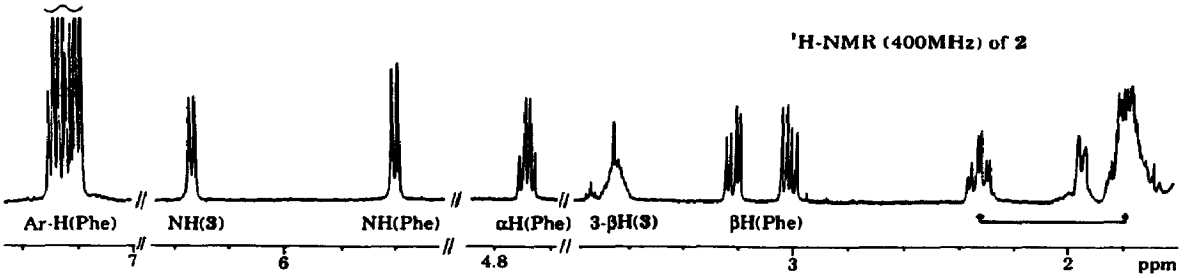



Figure 1 : Parts of the 400 MHz $^1$H-NMR spectrum of **2** in $CDCl_3$.

The conformation of **2** is manifested in NOE cross peaks found in a ROESY spectrum in $CDCl_3$. The strong NOE connectivities, drawn schematically in figure 2, confirm a trans-orientation of both types of peptide bonds. The alternating strong and weak connectivities suggest further that the peptide chain has an extended conformation. First molecular modelling studies on **2** using Still's systematic bond rotation approach[17] and the MM3 force field[18] result in the conformation of lowest energy shown in figure 3. The MM3 calculation of the isolated molecule optimizes all intramolecular attractions so the peptide part is folded to a γ-turn.[19] The cavity of the conformation is large enough to encapsulate small organic substrates.

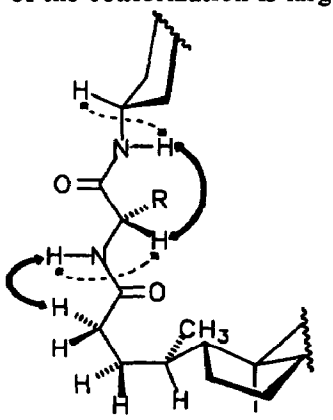


Figure 2 : Strong (solid) and weak (dotted) NOE connectivities in **2**.

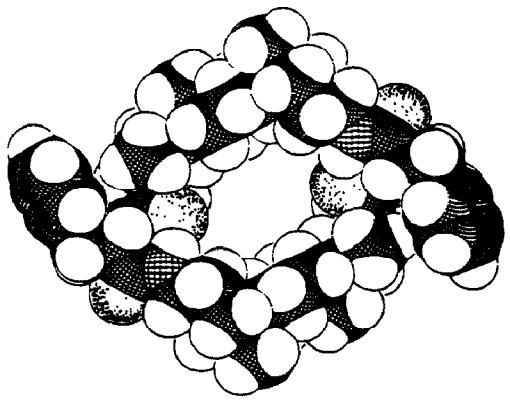


Figure 3 : Conformation of lowest energy of **2** (MM3).

The macrocycle **2** is the first member of a class of host compounds, where the rigid surface of a steroid is combined with amino acids having variable chemical functions and conformations.

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6. (a) Methyl-3β-formyloxy-5β-cholanate, **5a**: 10 mmol of formic acid and 10 mmol of DEAD were added to a solution of 5 mmol methylester **4** and 10 mmol triphenylphosphine in 60 ml of THF. Removal of the solvent after 14 h and chromatography on silica gel (benzol/hexane 60/40) yielded 1.5g of **5a** (3.5 mmol, 70%, TLC, Rf 0.4). M.p. 114-116°C; charact. $^1$H-NMR: 8.05 (s, 1H, HCO), 5.20 (s, 1H, CH-3). (b) Methyl-3β-hydroxy-5β-cholanate **5b**: Hydrolysis of **5a** with 2.5% sodium methoxide in methanol afforded **5b** in high yield (>98%). M.p. 107-109°C; charact. $^1$H-NMR: 4.1 (nm, 1H, CH-3).
7. For example the 3β-H in compound **4** has coupling constants $^3$JHH of 4.2, 4.4, 11.6 and 11.8 Hz while all four $^3$JHH of the 3α-H in **5b** are found between 4.0 and 4.4 Hz.
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9. Methyl-3α-azido-5β-cholanate, **6**: 2.6 mmol zinc azide-bis-pyridine complex[8] followed by 7 mmol DEAD were added to 3.5 mmol of **5b** and 7 mmol triphenylphosphine in 26 ml toluol. The solvent was removed; chromatography on silica gel (hexane/ethyl acetate 90/10) yielded 1.0g of **6** (2.45 mmol, 70%, TLC, Rf 0.8). M.p. 78-79°C; charact. $^1$H-NMR: 3.30 (tt, 1H, CH-3); $C_{25}H_{41}O_2N_3$ Calc. (Found) C, 72.26 (71.89), H, 9.91 (9.53), N, 10.12 (9.74).
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11. Methyl-3α-amino-5β-cholanate **7**: 2.45 mmol of **6** was stirred with triphenylphosphine in 15 ml THF for 24 h, 0.1 ml of water were added and the solvent was removed after additional 24 h. Chromatography of the residue on Florisil ($CH_2Cl_2$/MeOH 20/1 then with traces of AcOH) gives 0.95g (2.4 mmol, 98%) of **7**. M.p. 195°C (decomp.); charact. $^1$H-NMR: 3.00 (m, 1H, CH-3); $C_{25}H_{43}O_2N$ Calc. (Found) C, 77.09 (77.25), H, 11.09 (10.63), N, 3.60 (3.12).
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13. Methyl-3α-(phenylalaninylamino)-5β-cholanate **8**: A solution of 0.64 mmol of **7** and 0.64 mmol BOC-(S)-phenylalanine in 25 ml of dichloromethane was treated at -15°C with 3.7 mmol N-methylmorpholine and 2.5 mmol propane phosphonic acid anhydride. The mixture was stirred for 2 days at RT and the solvent was removed in vacuo. The residue was taken up in ethylacetate (120 ml), extracted with saturated aqueous $NaHCO_3$, 5% $NaHSO_4$ and brine. The organic phase was dried ($MgSO_4$) and evaporated yielding 300 mg (0.47 mmol 74%) of **8**. Charact. $^1$H-NMR: 7.33-7.17 (m, 5H, ar-H(Phe)), 5.41 (d, 1H, 3α-NH), 5.10 (d, 1H, NH(Phe)), 4.20 (m, 1H, α-CH(Phe)), 3.01 (ABX, 2H, CH2(Phe)).
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15. Cyclo-[3α-(phenylalaninylamino)-5β-cholanate]$_2$ **2**: 0.7 ml of 1N NaOH were added to a solution of 0.47 mmol of ester **8** in 3 ml of ethanol. The mixture was stirred for 2 h. The solvent was removed in vacuo, the residue taken up in water, acidified with dil. HCl to pH=1 and extracted with ethylacetate. The organic phase was dried ($MgSO_4$) the solvent removed and 0.6 mmol pentafluorophenol in 5 ml $CH_2Cl_2$ were added to the residue. The mixture was cooled to -20°C and 0.6 mmol dicyclohexylcarbodiimide were added. The solution was stirred overnight at RT, the precipitated urea was filtered and an equal volume of trifluoroacetic acid was added at 0°C. After 1 h the solvent was removed in vacuo, the residue taken up in 200 ml $CH_2Cl_2$ and 0.8 mmol of DMAP and 1.6 mmol $Na_2HPO_4$ were added. After 3 days of stirring at RT the mixture was filtered and evaporated to 150 mg of crude product (20% urea, yield 50%). Part of the material was further purified by chromatography on silica gel ($CH_2Cl_2$/MeOH 90/10, then $CH_2Cl_2$/MeOH/AcOH 100/20/1) and recrystallized from THF/MeOH/$H_2O$ (recovered material ca. 70%). M.p. 292 (decomp.); $^1$H-NMR see fig. 1; DCI-MS: 1010 ($MH^+$, base peak for masses > 300).
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