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A Class of 4-Aza-lithocholic Acid-Derived Haptens for the Generation of Catalytic Antibodies with Steroid Synthase Capabilities

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Abstract—The syntheses of a class of three haptens derived from the same 4-aza-steroidal skeleton is described. The sequence begins with oxidative cleavage of ring A of commercially available, optically pure lithocholic acid. Insertion of nitrogen at position 4 and stereoselective hydrogenation of the resulting electron-rich enelactam under 600 psi H_2 yielded a system analogous to testosterone-5-alpha-reductase inhibitors. Upon exhaustive reduction of this compound with lithium aluminium hydride, a linker for bioconjugation was attached before the *N*-oxide key functionality is established in ring A. This functional group is believed to be a true transition-state mimic for the electronic nature of initiation of the cationic cyclization of 2,3-epoxy-squalene derivatives. In addition, it also holds promise for eliciting acidic residues as part of a bait-and-switch strategy. Remarkably, both *N*-oxide epimers obtained from *m*CPBA oxidation can be separated by column chromatography on a 60 mg scale and were used in enantiopure form for separate immunizations. Reliable configurative assignment was carried out by comparison studies with previously characterized and published systems. A catalytic antibody (HA8-25A10) was obtained from the immunization with the hapten bearing an amin-oxide oxygen in the beta position. Surprisingly, an inhibition study showed that the isomer with the inverted configuration at the *N*-oxide bound more strongly to this catalytic antibody.

Introduction

The enzymes responsible for the cyclization of squalene or its epoxy derivative are stunningly sophisticated tools developed by Nature for sterol biosynthesis. These isomerases not only generate tetracyclic (eucaryotic oxidosqualene-lanosterol synthase, OSC) or pentacyclic (bacterial squalene-hopene synthase, SHC) ring systems from linear poly-ene precursors, but in the course of the reaction cascade they also create six (OSC) to nine (SHC) new stereocenters in one chemical transformation. With the help of mechanistic studies[1] and a crystal structure at high resolution,[2] an increasingly refined picture emerges of how these cyclases function. Mutagenesis studies suggested a single aspartate (D456) to be the triggering unit in yeast OSC;[3] this was confirmed by the crystal structure of SHC that revealed the presence of an aspartate (D376) most likely responsible for initiating the cyclization. This three-dimensional structure showed how aromatic residues could be positioned for effective interaction of their quadrupoles with transient cationic species along the reaction coordinate. The dimensions of the catalytic cavity are larger than the cyclized product hopene and the aromatic residues, that are expected to stabilize the later cations, are distant from the initiation site. This is in accord with the notion that the linear substrate squalene, once coiled, needs a greater volume of space than the cyclized product. It was proposed that the substrate slides away from the aspartate thus reaching aromatic residues that can stabilize carbocations resulting from attack of the previous cationic center.[2] For termination, no basic amino acid has been discovered that may likely be responsible for deprotonation. Instead, a localized water molecule, found at a decisive location within the active site, has been proposed to play this role.

Catalytic antibodies are "currently the most successful enzyme mimics".[4] It is of particular interest to examine the possibility of catalyzing the multi-cyclization of a squalene-derived substrate with an antibody. This formidable task involves creating a catalytic site that can accomodate a large substrate while shielding it effectively from solvent molecules. In principle, antibodies should be able to achieve this, since they can provide a combining site of close to 2000 Å^3.[5] In addition, it

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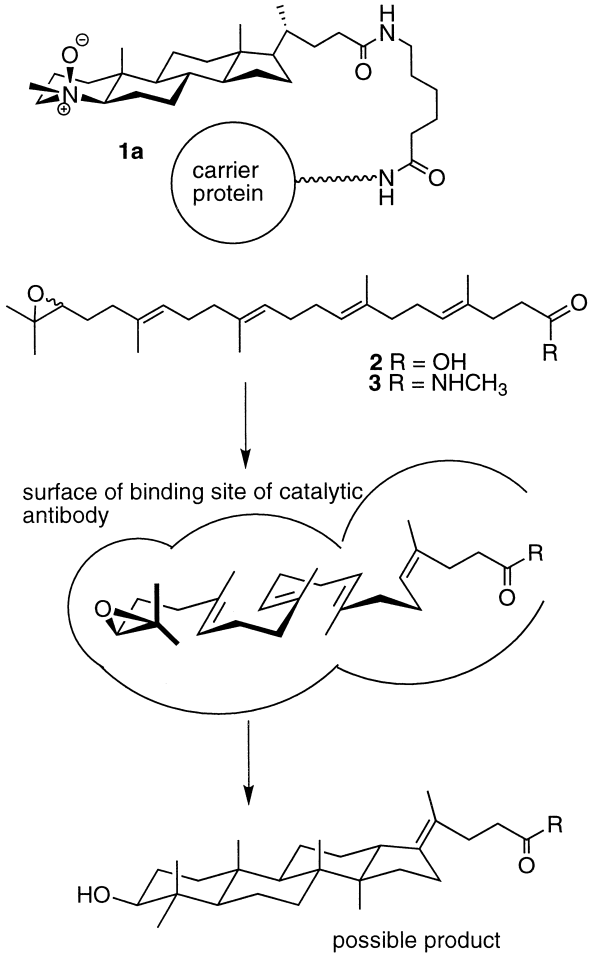
should be possible to elicit an acidic residue at the desired location within the active site by choosing a haptenic structure containing a counter-charged moiety.[6] An *N*-oxide moiety not only has this property[7] but it also displays a strong polarization as seen with the heterolytic cleavage of an oxygen–carbon bond in a potential epoxysqualene-derived substrate. *N*-oxide derivatives of squalene have shown superior inhibitory effects over their parent amines on natural OSCs.[8] This functional group may fulfill both roles, acting as a bait-and-switch tool[9] as well as a transition-state analogue. The extension of the cyclization cascade (propagation) consists of the catalyst enforcing a tight coiled conformation of the otherwise linear substrate, thus bringing double bonds and their respective carbocations into close proximity for barrier-less collapse.[10] This action by the catalyst may be installed into the antibody combining site by using a hapten where the cyclized structure has been preselected in form of a hydrophobic steroidal skeleton.

Steroidal antigens (progesterone, digoxin and oestrone) have been shown to elicit numerous aromatic residues for binding during immunization.[11,12] However, the precise constellation of aromatic quadrupoles for optimal stabilization and orientation of incipient carbocations cannot be elicited by any current methodology. Instead, the strategy here consists of the following: (a) creating diversity in combining site architecture (accomplished by action of the immune system itself) with the help of proper pre-synthesis selection of a haptenic structure; (b) Efficient post-synthesis selection from the obtained pool of antibodies with pre-determined specificity.[13]



Scheme 1.

Results and Discussion

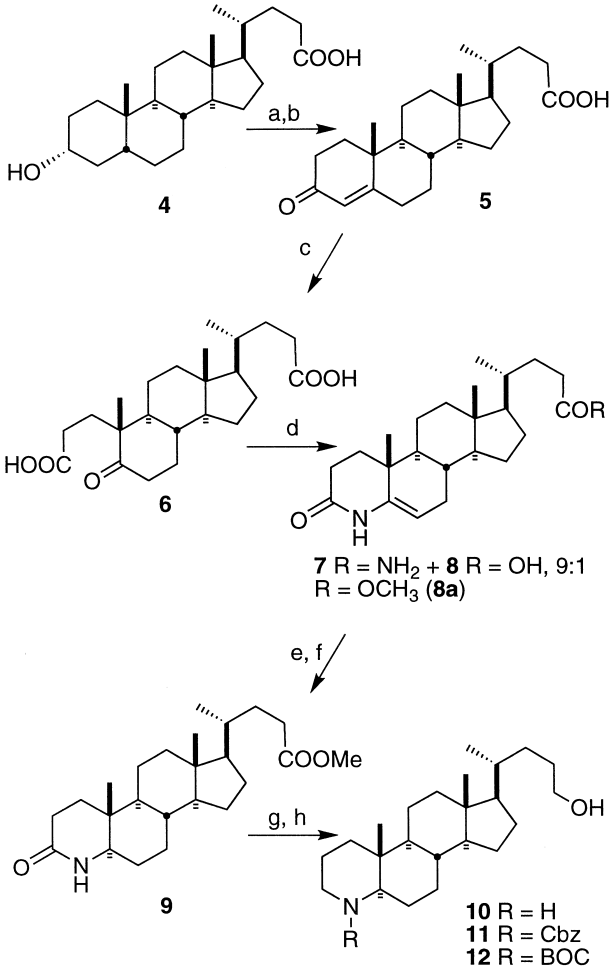
A dual-anchor design displaying functional groups at the head (epoxide) and the tail (carboxylic acid/-amide or 4-cyanobenzyl ether) of the otherwise hydrophobic squalene-derived substrate was chosen in this investigation (Scheme 1). These functionalized ends should give the antibody extra opportunities for specific molecular recognition in the form of hydrogen bonds. The development of this strategy has been supported by observations with an anti-oestrone antibody that particularly recognizes both functionalized 'ends' of the steroid hapten.[11] Thus, haptens **1a,b** and **20a,b** were targeted for the above considerations. They differ in the concept of linker attachment, one being conjugated through its head to carrier proteins (**20**) and the other through its tail (**1**). The cyanobenzyl ether moiety in **20** was chosen for convenient UV monitoring of substrate depletion and any formed products in future assays while leaving the opportunity to deprotect the hydroxyl group in any formed products.

4-Aza-steroids, to which **1a,b** and **20a,b** belong, are important targets as inhibitors of testosterone 5α-reductases and as antagonists of the androgen receptor.[14] The resulting 5α-dihydrotestosterone was shown to be the more potent intracellular hormone. This research has led to such widespread products as finasteride® and proscar® for the treatment of benign prostatic hypertrophy, acne and hair loss in males.[15] The long history of azasteroids dates back to the 1930s when Bolt investigated the introduction of nitrogen at the 4-position in the steroidal skeleton.[16] In this investigation, the starting material used was the oxocarboxylic acid obtained from ozonation or oxidation with potassium permanganate of cholestenone which resulted in the opening of the A ring.[17] Ring closure to form a piperidine system within the steroid framework was achieved by sodium/ethanol reduction of the corresponding oxime of this carboxylic acid. This classic approach has somewhat changed over the years to ensure selective formation of the isomer bearing a *trans* fusion between ring A and B. For this purpose, ring closure has to be enforced under non-reducing conditions to obtain an enamide moiety. This functionality is subsequently reduced stereoselectively to the corresponding 5-*trans*-lactam. More recently, numerous efforts have been made to accomplish complete diastereoselectivity. Here, the goal was to reduce costs caused by purification in industrial production. Catalytic hydrogenation appears to be the method of choice, yielding *trans*/*cis* ratios of up to 500:1 for specific substrates.[18] However, others could not obtain appreciable isomer ratios by catalytic hydrogenation

and reported better results with acid-catalyzed reduction by complex hydrides.[19]

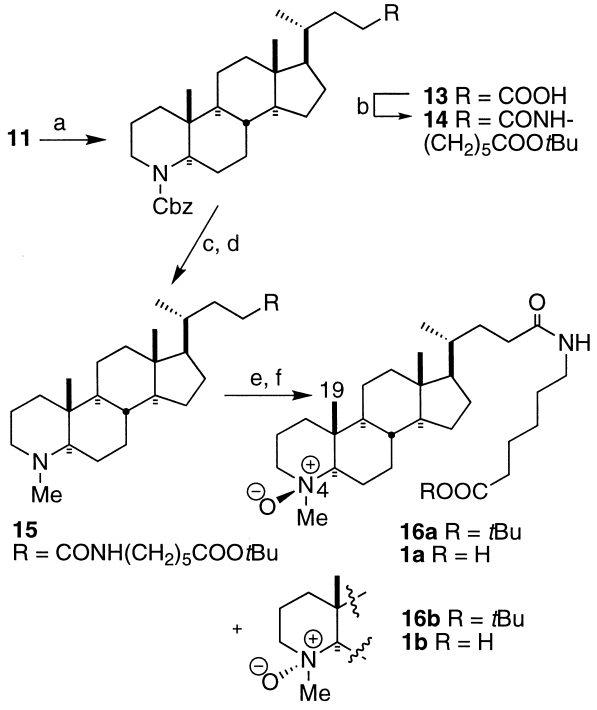
The synthesis of haptens **1a,b** and **20a,b** starts from commercially available lithocholic acid (Scheme 2). The *cis* configuration at C5 of this steroid is of no relevance since it is eliminated during the ring opening/closure procedure. The first sequence of steps involves oxidative bromination to yield the 4β-bromo ketone that is directly treated with lithium carbonate in DMF to furnish enone **5**.[20] Oxidative cleavage of this material with a sodium periodate/potassium permanganate system in refluxing *tert*-butanol affords ketodiacid **6** in high yields only, if 5 has been thoroughly purified.[21] The key step of nitrogen introduction involves bringing a solution of **6** in ethylene glycol close to its saturation point at 5 °C, quickly adding liquid ammonia, heating this mixture at 5 °C/min to 180 °C and finally cooling the solution. Thus, a 9:1 mixture of primary amide **7** and acid **8** is obtained in 83% yield.

This mixture is directly introduced into the next step, catalytic stereoselective hydrogenation with 10%Pd/C under 600 p.s.i. H_2 for 5 days. An extended reaction time of 5 days is necessary due to the electron-rich nature of the double bond and its steric congestion at the ring junction. A variety of conditions reported in the literature[18] employing more expensive rhodium or platinum catalysts were applied to **7**, however, they did not appreciably lower the percentages of the *cis*-fusion. The degree of stereoselectivity was determined by integration of the corresponding proton NMR signal for H5 (*trans*/*cis*: 10:1). This signal was also used to reliably monitor the progress of reduction since the R_f values for product and starting material were identical. The minor isomer was carried through the synthesis alongside the target compound and was chromatographically removed at a later stage. Subsequent transformation of the terminal carboxamide functionality into its methyl ester (acidic amberlyst 15 in refluxing methanol[22]) furnished **9**. This was then exhaustively reduced with $LiALH_4$ to amino alcohol **10**.[23] Selective protection of the nitrogen with either Cbz or Boc led into the two separate synthetic pathways for both haptens.



Scheme 2. Conditions: (a) NBS, dioxane/H_2O; (b) Li_2CO_3, DMF, 90 °C; (c) $KMnO_4$, $NaIO_4$, *t*BuOH reflux, 89%; (d) liquid NH_3, $HOCH_2CH_2OH$, 5–180 °C, 83%; (e) 600 p.s.i. (40 bar H_2, 10% Pd/C, glacial HOAc, PARR BOMB, 5 d; (f) amberlyst 15, MeOH, reflux, 75% over 2 steps; (g) $LiAlH_4$; (h) CbzCl (**9**- > **11**), 60–68% over 2 steps.

Completion of haptens **1a,b** were as follows (Scheme 3). Upon PDC oxidation of **11** to acid **13**,[24] 1,1-dimethylethyl 6-amino-caproate (generated from Cbz-protected 1,1-dimethylethyl 6-amino-caproate by standard hydrogenation) was attached by amide coupling to yield **14**. Selective deprotection of N4 led to a secondary amine that was transformed into methylamine **15** by reductive amination.[25] At this stage, the 5-*cis* isomer was removed by two rounds of flash chromatography on a 160 mg scale. Compound **15** was oxidized to an epimeric mixture of aminoxides (**16a,b**) with *m*CPBA at 0 °C. The mixture



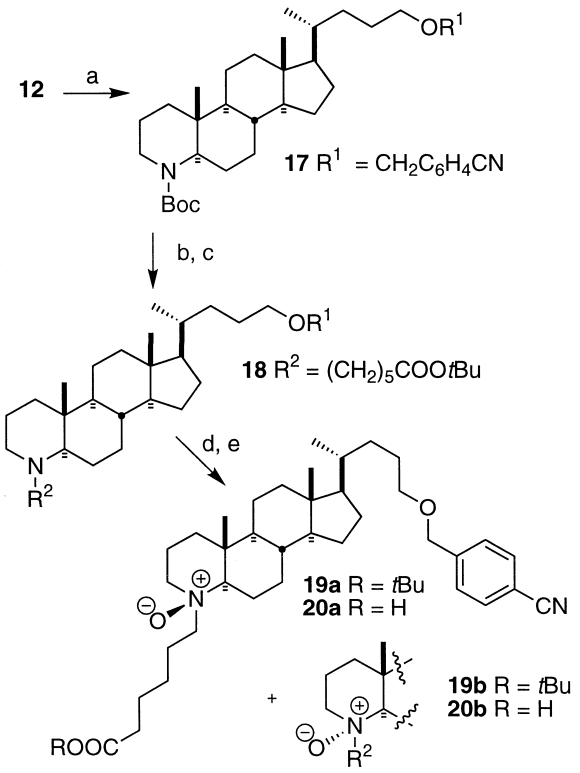
Scheme 3. Conditions: (a) pyridinium dichromate, 71%; (b) $H_2N(CH_2)_5COOtBu$, EDC, NEt_3, DMF, 91%; (c) H_2, 10% Pd/C; (d) 40% formalin, $NaBH_4$, 25 °C, 70% over 2 steps; (e)*m*CPBA, 61% separation of epimers; (f) TFA, 84%.

was separated by flash chromatography on a 60-mg scale and both isomers were characterized before deprotection to the target haptens **1a** and **1b**. The assignment of their absolute stereochemistry was made possible through the distinctive proton-NMR low-field shift (0.35 ppm) of the C19-methyl group in **16a** caused by its through-space interaction with the axial amin-oxide-oxygen. This behavior has been previously observed in a comparable system involving a perhydroquinoline N-oxide system where it was confirmed through ROESY spectroscopy as well as an X-ray structure.[26,27]

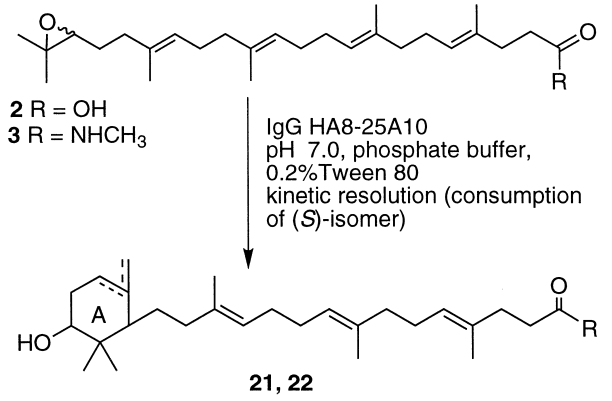
The synthesis of haptens **20a,b** continued with cyanobenzylation of **12** to form **17** (Scheme 4). This compound was deprotected at N4 before chromatographic removal of the 5-*cis* isomer. Alkylation of this isomerically pure secondary amine with 1,1-dimethylethyl-6-bromohexanoate afforded **18**.[28] Here too, N-oxidation with *m*CPBA led to a mixture of epimers (**19a,b**) that could be characterized separately after column chromatography on a small scale before deprotection to the target haptens **20a,b**.

Three separate immunizations with haptens **1a**, **1b** and a 1:1 mixture of **20a,b**, respectively, were carried out and to date, the immune responses to **1a** and **1b** were harvested according to standard hybridoma protocol. Screening of the obtained sets of monoclonal antibodies with substrates **2** and **3** demonstrated that hapten **1a** did indeed elicit a catalyst. This antibody, HA8-25A10, initiated the cyclization through epoxide opening and controlled the formation of ring A of the steroid nucleus (Scheme 5).[29] However, propagation to the desired multicyclic ring system with these particular substrates (**2** and **3**) was not observed. HA8-25A10 regioselectively produced two olefinic regioisomers (**21** and **22**) with a ratio of 93:7 while kinetically resolving racemic **2** and **3** (only (*S*)-isomer was consumed).

An inhibition study of IgG HA8-25A10 with the epimeric haptens **1a** and **1b** was performed to estimate the impact of a single inversion at an asymmetric center. Thus, we hoped to derive factors that may provide insight for the successful elicitation of a catalyst. Since both haptens were found to be tight-binding inhibitors, the 'classical' approach to K_i determination is not applicable.[30] Instead of studying the effect of inhibitor concentration on enzyme activity under conditions of varying substrate concentrations, a titration under a fixed substrate concentration can be conducted.[31] The dose–response plot constructed from this data furnishes the IC_{50} value for the corresponding inhibitor (Fig. 1). With the knowledge of the Michaelis–Menten parameters, a K_i value for **1a** of 9 μM has thus been determined. Surprisingly, the K_i for hapten **1b** has been found to be 0.5 μM! This result demonstrates that **1b** has the better fit to the active site of HA8-25A10.



Scheme 4. Conditions: (a) 4-NC-$C_6H_4CH_2Br$, NaH, 91%; (b) TFA, CH_2Cl_2, 1 h; (c) $Br(CH_2)_5COOtBu$, $NEtiPr_2$, DMF, 60 °C, 40 h, 57%; (d) *m*CPBA, CH_2Cl_2, −20 °C- > rt, 4 h, 99%, separation of epimers; (e) TFA, CH_2Cl_2, 4 h, 99%.



Scheme 5.

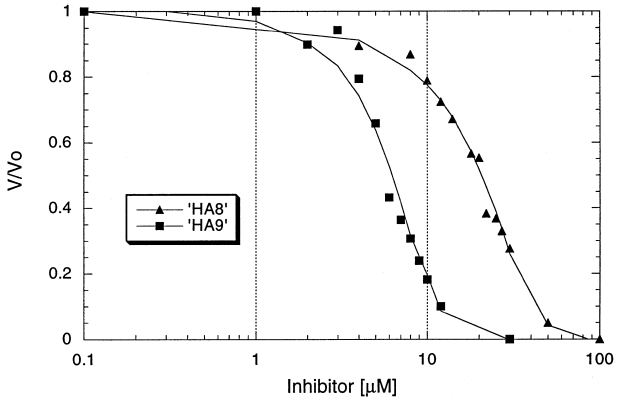


Figure 1. Dose–response plot of inhibition by **1a** (HA8) and **1b** (HA9). Conditions: 5 μM IgG HA8-25A10; 400 μM substrate **2**; 0.2% TWEEN 80; 50 mM phosphate buffer; pH 7.0; 37 °C. IC_{50} values derived from plots: 18.9 μM (**1a**), 5.7 μM (**1b**).

However, **1b** did not yet lead to the discovery of any antibody catalysts by conventional hybridoma technology. As to why **1b** did not lead to any catalytic antibodies, ultimately only a crystal structure determination of HA8-25A10 complexed with **1a** and **1b** will answer this intriguing question.

Conclusion

This study has further paved the road towards finding answers how nature controls poly-ene cyclization. Concise hapten syntheses have been presented. A catalytic antibody (HA8-25A10) forming a monocyclic product has been elicited by hapten **1a**. However, routine production of monoclonal antibodies from immunization with epimer **1b** did not lead to any catalyst. A difference in binding energies with the catalyst HA8-25A10 has been observed between **1a** and its epimer **1b**. Surprisingly, **1b**, that was not present during immunization, bound stronger. Future studies should provide a clearer picture of how binding energy effects catalysis and how an increase in apparent congruency of alternate substrates to haptens **1a,b** effects product outcome.

Experimental

General procedures

If not stated otherwise, ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-500 instrument at 500 and 125 MHz, respectively. Only selected signals are quoted for the proton spectra. Generally, these include those for steroidal positions 3, 4, 5, 18, 19, 21, 23 and 24, and those caused by protons of the linkers, the *N*-methyl group, and the cyanobenzyl group. Chemical shifts (δ) are given in ppm relative to $CHCl_3$ in $CDCl_3$ (7.27 ppm, ^{1}H; 77.00 ppm, ^{13}C). Signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), o (octet) and m (multiplet). High-resolution mass spectra (HRMS) were recorded at The Scripps Research Institute on a VG ZAB-ZSE mass spectrometer under fast atom bombardment (FAB) or electrospray conditions.

All reactions were monitored by thin-layer chromatography (TLC), using 0.25 mM Merck Silicagel Glass Plates (60F-254), fractions being visualized by UV light, phosphomolybdic acid solution (or other staining solutions as indicated) with subsequent heat application. Column chromatography was carried out with Mallinckrodt SilicAR 60 silicagel (40–63 μM). Reagent grade solvents for chromatography were obtained from Fisher Scientific. Reagents and anhydrous solvents were obtained from Aldrich Chemical Co. and used without further purification. All reactions were carried out under anhydrous conditions and an atmosphere of argon, unless otherwise noted. Reported yields were determined after purification to homogenous material.

Keto-diacid (6). Enone **5**[20a] (7.18 g, 19.27 mmol) in 107 mL *t*-BuOH was treated with 3.00 g Na_2CO_3 in 14 mL H_2O. The mixture was brought to reflux under stirring with an oversized stirring bar, resulting in some precipitation. A heated solution of 28.5 g $NaIO_4$ and 0.21 g $KMnO_4$ in 74 mL H_2O (that had to be boiled 1/2 h to dissolve all salts) was added dropwise under vigorous stirring causing strong CO_2 evolution, further solid formation and a tan-like color. The mixture was refluxed an additional 10 min and cooled to room temperature. Filtration through Celite, extensive washing with 1 M Na_2CO_3, water, evaporation of *t*-BuOH, and acidification with 1 N HCl afforded a precipitate that was filtered and washed with water. The obtained solid was dissolved in CH_2Cl_2, extracted with H_2O and brine before being dried over $MgSO_4$. Silicagel chromatography (column conditioned with hexanes:ethyl acetate 5:1 plus 0.1% glacial HOAc, gradient 5:1→1:1) yielded 6.713 g of **6** (17.10 mmol, 89%, oil). (The high purity of the starting material is essential for this yield.) ^{1}H NMR ($CDCl_3$): δ 0.73 (3H, s), 0.93 (3H, d, J = 6.5 Hz), 1.12 (3H, s), 2.27 (3H, m), 2.38 (2H, m), 2.54 (1H, dt, J = 14.5, 6.0 Hz). ^{13}C NMR ($CDCl_3$): δ 11.9, 12.0, 18.1, 20.1, 21.4, 24.1, 27.9, 29.1, 30.6, 31.0, 31.3, 34.8, 35.2, 38.1, 39.2, 42.5, 47.8, 50.3, 55.6, 55.7, 179.9, 180.3. LRMS (FAB, NBA/NaI) calcd for $C_{23}H_{36}O_5$ ($M+Na^+$) 415; found 415.

Enelactam-amide and -acid (7,8). Very pure **6** (5.417 g, 13.8 mmol) was dissolved in 25.4 mL of warm ethylene glycol (1 L single-necked round-bottom flask with condenser) before cooling to 5 °C under stirring (Note: some precipitation may occur). Next, liquid ammonia (5.8 mL) were cautiously added in one portion causing only limited losses through evaporation. The suspension clears immediately and turns from colorless to light yellow (enamine formation). The solution in the open apparatus was heated slowly (3 °C/min) to 180 °C. At 160 °C a finely powdered solid precipitates and the mixture is kept at 180 °C for 15 min while turning grey-brown. Upon cooling to room temperature the mixture was diluted with 500 mL H_2O and acidified to pH 1.0 with concd HCl. The solid was removed by filtration and washed several times with water before being suspended in THF. Filtration and washing affords a completely pure white powder (**7**) (2.665 g, sparingly soluble in organic solvents except for DMSO). The recombined washings yield 1.63 g of a crude mixture of **7** and **8** (R_f = 0.18, CH_2Cl_2:MeOH 15:1). Combined yield: 4.29 g, 83%.

Enamide-methylester (8a). The crude mixture of **7** and **8** (1.63 g) in 20 mL THF was treated with an excess of a diazomethane solution and stirring was continued overnight. Evaporation of all the volatile components and silicagel chromatography on a short column (hexanes:ethyl acetate 3:1→1:2) afforded 674 mg **8a** as a white solid. Further elution with MeOH can afford additional enelactam-amide **7**. ^{1}H NMR ($CDCl_3$): δ 0.68 (3H, s), 0.91 (3H, d, J = 6.5 Hz), 1.06 (3H, s), 2.00 (1H, dt, J = 12.7, 3.5 Hz), 2.07 (1H, dt, J = 16.9, 5.0 Hz), 2.20 (1H, m), 2.34 (1H, m), 2.44 (2H, m), 3.64 (3H, s), 4.89 (1H, q, J = 2.5 Hz), 8.51 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.8, 18.2, 18.6, 20.8, 24.0, 28.0, 28.3, 29.6, 30.87, 30.94, 31.4, 31.5, 34.0, 35.2, 39.3, 42.4, 47.8, 51.4, 55.6, 56.3, 103.6, 139.8, 169.8, 174.6. HRMS (FAB,

NBA/NaI) calcd for $C_{24}H_{37}NO_3$ ($M+H^+$) 388.2852; found 388.2863.

Lactam-methylester (9). The mixture of **7** and **8** (3.088 g, 8.29 mmol) in 125 mL glacial HOAc was placed in the glass liner of a 300 mL steel pressure vessel (PARR Instr. Inc. Pt.-No. 4766/4316) equipped with a teflon stirring bar. Approximately 720 mg 10% Pd/C was added and the mixture was stirred under hydrogen (600 p.s.i./40 bar) for 5 days before being checked for starting material consumption by ^{1}H NMR (formation of H5 signal/decrease of olefinic H5 signal reveals 70% conversion). The mixture was next hydrogenated at 600 p.s.i. for an additional day at 50 °C (external oil bath) to achieve complete conversion. Filtration and evaporation afford a white solid (3.273 g, R_f=0.58, CH_2Cl_2: MeOH 5:1) that is introduced into the next step, directly; 45 mg Amberlyst 15 were washed 8 times with MeOH until the filtrate reached pH 6.0. This washed polymer is added to a solution of the above white solid (3.000 g, 8.01 mmol) in 120 mL of anhydrous MeOH and the mixture is refluxed for 14 h. After filtration, the polymer is applied to a column and eluted with at least 4 times the volume of the resin of MeOH:NEt_3 2:1. The combined filtrates are evaporated and the residue is subjected to silicagel chromatography (CH_2Cl_2: MeOH 30:1→10:1) to afford crystalline **9** (2.40 g, 6.193 mmol, 75% over 2 steps, R_f=0.42, CH_2Cl_2: MeOH 10:1), contaminated with 10% of the 5-*cis*-configurated epimer. ^{1}H NMR ($CDCl_3$): δ 0.63 (3H, s), 0.86 (3H, s), 0.88 (3H, d, J=6.5 Hz), 2.16–2.21 (1H, m), 2.28–2.38 (3H, m), 3.00 (1H, dd, J=12.5, 3.5 Hz), 3.63 (3H, s), 6.59 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.2, 12.0, 18.1, 21.0, 23.9, 27.1, 27.9, 29.5, 30.8, 30.9, 33.2, 34.9, 35.2, 35.5, 39.5, 42.6, 51.1, 51.4, 55.7, 55.8, 60.6, 172.5, 174.6. HRMS (FAB, NBA/NaI) calcd for $C_{24}H_{39}NO_3$ ($M+H^+$) 390.3008; found 390.3017.

Aminoalcohol (10). Compound **9** (2.40 g, 6.193 mmol) was dissolved in 40 mL anhydrous THF for enhanced solubility before being diluted with 280 mL anhydrous ethyl ether and treated cautiously with 5.5 g lithium aluminum hydride. After 1 h of reflux an additional 280 mL of ether was added before reflux is continued for 2 days. The mixture is cooled to 0 °C and 200 mL H_2O are added cautiously before extraction with CH_2Cl_2. Filtration of both phases was necessary to achieve complete phase separation. Washing with H_2O and brine followed by drying over $MgSO_4$ afforded 2.272 g crude **10** as an oil (only one detectable spot on tlc, R_f=0.50, CH_2Cl_2:MeOH 3:1) that was introduced into the next step directly.

Cbz-protected alcohol (11). 1.704 g crude amino alcohol **10** in 30 mL CH_2Cl_2 were treated with benzyl chloroformate (920 mg, 5.39 mmol) in the presence of 1.7 mL Hünig's base and stirred overnight. Extraction with 1 M citric acid, H_2O and brine followed by drying over $MgSO_4$ afforded crude **11** that was purified by silicagel chromatography (hexanes:ethyl acetate 10:1→1:1, R_f=0.31 (3:1)). Yield: 1.522 g (3.16 mmol, 68% over 2 steps, R_f=0.21 (3:1), white solid). ^{1}H NMR ($CDCl_3$): δ 0.65 (3H, s), 0.87 (3H, s), 0.92 (3H, d, J=6.5 Hz), 2.41 (1H, qd, J=13.2, 3.4 Hz), 2.64 (1H, td, J=13.0, 2.4 Hz), 2.83 (1H, dd, J=12.5, 3.0 Hz), 3.61 (2H, o, J=5.0 Hz), 4.32 (1H, dm, J=13.0 Hz), 5.07 (1H, d, J=13.0 Hz), 5.11 (1H, d, J=13.0 Hz), 7.31 (1H, m), 7.35 (4H, m). ^{13}C NMR ($CDCl_3$): δ 12.0, 12.7, 18.6, 21.0, 22.7, 24.1, 27.5, 28.1, 29.3, 31.8, 32.0, 35.2, 35.5, 38.6, 39.1, 39.9, 42.5, 49.3, 53.8, 56.0, 56.1, 63.6, 66.3, 69.1, 127.7, 127.8, 128.4, 137.2, 155.5. HRMS (FAB, NBA/CsI) calcd for $C_{31}H_{47}NO_3$ ($M+Cs^+$) 614.2610; found 614.2628.

BOC-protected alcohol (12). 568 mg crude aminoalcohol **10** in 4 mL dioxane was treated with $(BOC)_2O$ (375 mg, 1.716 mmol) in the presence of 0.57 mL Hünig's base and stirred overnight. Work-up and chromatography as for **11** afforded 411 mg (0.918 mmol, 60% over 2 steps) crystalline **12**. ^{1}H NMR ($CDCl_3$): δ 0.66 (3H, s), 0.88 (3H, s), 0.92 (3H, d, J=6.5 Hz), 1.44 (9H, m), 2.33 (1H, qd, J=13.2, 3.4 Hz), 2.57 (1H, td, J=13.0, 2.4 Hz), 2.78 (1H, dd, J=12.5, 3.0 Hz), 3.61 (2H, o, J=5.0 Hz), 4.23 (1H, dm, J=13.0 Hz). ^{13}C NMR ($CDCl_3$): δ 12.0, 12.7, 18.6, 21.0, 21.1, 22.6, 24.1, 27.8, 28.0, 28.5, 29.3, 31.8, 32.2, 35.3, 35.5, 39.3, 39.9, 42.5, 49.3, 53.9, 56.0, 56.1, 63.6, 68.8, 79.0, 155.4.

Cbz-protected carboxylic acid (13). Compound **11** (491 mg, 1.019 mmol) in 6.5 mL DMF was treated with 1.58 g pyridinium dichromate (PDC, 4.19 mmol) in one portion at room temperature and stirred overnight. Addition of water (6.5 mL), exhaustive extraction with CH_2Cl_2, re-extraction with water, brine and drying over $MgSO_4$ afforded material that was purified by silicagel chromatography (hexanes:ethyl acetate 7:1→1:1, R_f= 0.45 (1:1)) to yield 361 mg (0.728 mmol, 71%) **13**. It should be noted that a significant unidentified side product (44 mg, R_f=0.74, not an aldehyde!) was also isolated. ^{1}H NMR ($CDCl_3$): δ 0.66 (3H, s), 0.87 (3H, s), 0.92 (3H, d, J=6.5 Hz), 2.25 (1H, m), 2.39 (2H, m), 2.66 (1H, td, J=13.0, 2.4 Hz), 2.83 (1H, dd, J=12.5, 3.0 Hz), 4.32 (1H, dm, J=13.0 Hz), 5.08 (1H, d, J=12.5 Hz), 5.11 (1H, d, J=12.5 Hz), 7.31 (1H, m), 7.35 (4H, m). HRMS (FAB, NBA/CsI) calcd for $C_{31}H_{45}NO_4$ ($M+Cs^+$) 628.2403; found 628.2378.

Cbz-protected *t*Butyl ester (14). A mixture of **13** (97 mg, 0.196 mmol) and 1,1-dimethylethyl 6-amino-caproate (generated from Cbz-protected 1,1-dimethylethyl 6-amino-caproate by standard hydrogenation procedure) in 0.5 mL CH_2Cl_2 was treated with bis(2-oxo-3oxazolidinyl)phosphinic chloride (BOP-Cl) (51 mg, 0.200 mmol). Then, ethyldiisopropylamine (Hünig's base) (52 mg, 0.400 mmol) in 0.2 mL CH_2Cl_2 was added over a period of at least 30 min. After 1 h of stirring the mixture was washed with 1 M citric acid, H_2O, brine and dried over $MgSO_4$. Silicagel chromatography with hexanes:ethyl acetate 7:1→1:1 yielded first 35 mg recovered starting material **13** and then 76 mg **14** (0.114 mmol, 91% based on converted material, R_f=0.41 with hexanes:ethyl acetate 1:1). ^{1}H NMR ($CDCl_3$): δ 0.64 (3H, s), 0.86 (3H, s), 0.91 (3H, d, J=6.5 Hz), 1.44 (9H, s), 2.21 (2H, t, J=7.0 Hz), 2.41 (1H, q, J=13.0 Hz), 2.65 (1H, t, J= 13.0 Hz), 2.80 (1H, dd, J=12.5, 3.0 Hz), 3.23 (2H, q), 4.31 (1H, dm, J=13.0 Hz), 5.09 (2H, q, J=13.0 Hz), 5.61 (1H, m), 7.30 (1H, m), 7.34 (4H, m). ^{13}C NMR

($CDCl_3$): δ 12.0, 12.7, 18.3, 20.9, 22.7, 24.0, 24.5, 26.2, 27.5, 28.1, 29.2, 31.8, 32.0, 33.6, 35.1, 35.2, 35.5, 39.0, 39.1, 39.9, 42.5, 49.2, 53.7, 55.87, 55.93, 66.3, 69.1, 80.1, 127.7, 127.8, 128.3, 137.2, 155.5, 173.0, 173.5. HRMS (FAB, NBA/CsI) calcd for $C_{41}H_{64}N_2O_5$ ($M+Cs^+$) 797.3870; found 797.3846.

Methylamine, *t*Bu-ester (15). A solution of **14** (168 mg, 0.253 mmol) in 3.5 mL EtOH containing 90 mg 10% Pd/C was stirred under hydrogen for 4 h. After filtration and evaporation the remaining free secondary amine was dissolved in 2.4 mL THF and then treated with a mixture of 0.11 mL 37% formaldehyde and 1.1 mL CH_3CN. Subsequent addition of 30 mg $NaBH_3CN$, followed by two drops of glacial acetic acid after 10 min. and further stirring for 1 h resulted in complete consumption of the starting material. Evaporation of solvents, redissolution in CH_2Cl_2, extraction with 1 N NaOH and brine precedes column chromatography (2.5×10 cm) with ethyl acetate:MeOH 20:1→5:1 (every 200 mL of solvent containing 0.5 mL NH_4OH). Three fractions were obtained: 6 mg pure 5-*cis*-configurated isomer, 31 mg mixed fraction and 59 mg pure 5-*trans*-isomer **15**. Total yield: 96 mg (0.176 mmol, 70%). Water solubility of **15** (!) accounts for losses in yield during work-up. (R_f = 0.53 for 5-*cis* isomer and 0.50 for **15** with CH_2Cl_2:MeOH 5:1 + 4 drops of NH_4OH, both stain brown with ninhydrine). ^{1}H NMR ($CDCl_3$): δ 0.63 (3H, s), 0.90 (3H, d, J = 6.5 Hz), 0.92 (3H, s), 1.43 (9H, s), 2.02 (1H, m), 2.14 (3H, s), 2.20 (2H, t, J = 7.1 Hz), 2.21 (1H, m), 2.83 (1H, dm, J = 11.0 Hz), 3.23 (2H, q, J = 7.0 Hz), 5.59 (1H, t, J = 5.0 Hz). ^{13}C NMR ($CDCl_3$): δ 12.0, 18.3, 20.8, 22.1, 24.0, 24.5, 24.7, 26.2, 28.1, 29.2, 30.7, 31.8, 33.7, 34.5, 35.3, 35.5, 37.3, 39.1, 39.9, 42.4, 34.5, 52.7, 55.9, 56.3, 58.8, 73.2, 80.1, 173.0, 173.5. HRMS (FAB, NBA/CsI) calcd for $C_{34}H_{60}N_2O_3$ ($M+H^+$) 545.4682; found 545.4666.

N(O)Me, *t*Bu-ester (16a,b). Compound **15** (59 mg, 0.108 mmol) in 1 mL CH_2Cl_2 at −20 °C was treated with *m*CPBA (33 mg, 0.189 mmol, >57% activity = > at least 1 equiv) and the mixture is slowly warmed up to room temperature overnight under stirring. Now, the mixture was chromatographed on a short silicagel column, conditioned with $CHCl_3$:MeOH:NH_4OH 125:25:1, yielding 12 mg of a first fraction enriched in the minor isomer **16b** (ratio ca. 2:1) and 25 mg of pure major isomer **16a** (total yield 37 mg, 66 μmol, 61%). The mixed fraction is reapplied to a new silicagel column and eluted with $CHCl_3$:MeOH 20:1→3:1 to obtain 4.5 mg pure minor isomer **16b** (HRMS (FAB, NBA/CsI) calcd for $C_{34}H_{60}N_2O_4$ ($M+H^+$) 561.4631; found 561.4647), 1.7 mg slightly contaminated **16a** and 2.2 mg pure major isomer **16a** (R_f = 0.5 for minor isomer **16b** and 0.44 for major isomer **16a**, stains brown with ninhydrine). ^{1}H NMR (**16a**, $CDCl_3$): δ 0.62 (3H, s), 0.88 (3H, d, J = 6.5 Hz), 1.36 (3H, s), 1.41 (9H, s), 2.19 (3H, t, J = 7.5 Hz), 2.51 (1H, q, J = 13.5 Hz), 2.58 (1H, dm, J = 9.5 Hz), 3.09 (3H, s), 3.17 (1H, t, J = 12.0 Hz), 3.21 (2H, q, J = 6.0 Hz), 3.37 (1H, d, J = 10.5 Hz), 5.76 (1H, t). ^{13}C NMR (**16a**, $CDCl_3$): δ 12.0, 14.1, 17.1, 18.2, 20.0, 20.7, 23.9, 24.5, 26.2, 28.0, 29.2, 30.7, 31.7, 33.6, 34.2, 35.2, 35.4, 36.4, 39.1, 39.5, 42.4, 54.6, 55.7, 56.1, 59.5, 69.8, 79.8, 80.0, 173.0, 173.4. ^{1}H NMR (**16b**, $CDCl_3$): δ 0.64 (3H, s), 0.92 (3H, d, J = 6.5 Hz), 1.01 (3H, s), 1.45 (9H, s), 2.22 (2H, t, J = 7.5 Hz), 2.77 (1H, dm, J = 11.0 Hz), 3.00 (1H, m), 3.01 (3H, s), 3.25 (2H, q, J = 7.0 Hz), 3.34 (1H, m), 3.62 (1H, m), 5.51 (1H, t, J = 4.0 Hz).

Haptens HA8 (1a) and HA9 (1b). 16a or 16b. (4.5 mg, 8.03 μmol) in 1 mL CH_2Cl_2 was treated with 1 mL TFA and stirred for 3.5 h at room temperature, respectively. Evaporation of all volatile components and subsequent rinsing of the remaining oily residue with 0.8 mL $CHCl_3$ yielded very pure **1a** (HA8) or **1b** (HA9) (3.4 mg, 6.74 μmol, 84%), respectively (R_f = 0.23 for **1a** and 0.26 for **1b** with acetonitrile:water 2:1, both stain brown with ninhydrine). ^{1}H NMR (**1a**, CD_3OD, 400 MHz): δ 0.70 (3H, s), 0.95 (3H, d, J = 6.5 Hz), 1.20 (3H, s), 1.61 (2H, p J = 7.6 Hz) 2.28 (2H, t, J = 7.4 Hz), 3.15 (2H, t, J = 7.0 Hz), 3.30 (1H, m), 3.44 (3H, s), 3.62 (1H, t, J = 10.0 Hz), 3.73 (1H, d, J = 12.8 Hz). ^{1}H NMR (1b, CD_3 OD, 400 MHz): δ 0.70 (3H, s), 0.95 (3H, d, J = 6.5 Hz), 1.12 (3H, s), 1.61 (2H, p, J = 7.6 Hz), 2.28 (2H, t, J = 7.4 Hz), 3.15 (t, J = 7.6 Hz), 3.30 (3H, s), 3.40 (1H, dd, J = 12.4, 2.4 Hz), 3.59 (1H, tm, J = 14.1 Hz), 3.90 (1H, d, J = 12.1 Hz). HRMS (FAB, NBA/CsI) calcd for $C_{30}H_{52}N_2O_4$ ($M+H^+$) 505.4005; found 505.4023 (for **1b**).

Cyanobenzylated BOC-alcohol (17). Compound **11** (103 mg, 0.223 mmol) in 2 mL anhydrous THF was cooled to 0 °C before NaH (35 mg 60% dispersion in mineral oil) was cautiously added under stirring. The mixture was stirred at room temperature for another hour before the solution was decanted via syringe from the settled excess NaH and introduced into a fresh flame-dried flask (the excess NaH is washed once with 1 mL anhydrous THF). Next, α-bromo-p-tolunitrile (53 mg, 0.268 mmol) in 1 mL anhydrous THF was added and the mixture was refluxed for 24 h under nitrogen. The solvent was evaporated, the residue dissolved in ethyl acetate, washed with H_2O, brine, and dried over $MgSO_4$. Silicagel chromatography with hexanes:ethyl acetate 9:1→4:1 first yielded 76 mg **17** (R_f = 0.49 with hexanes:ethyl acetate 3:1, stains blue with anisaldehyde) and then 37 mg recovered starting material **12**. Yield, based on transformation: 91%. ^{1}H NMR ($CDCl_3$): δ 0.66 (3H, s), 0.88 (3H, s), 0.92 (3H, d, J = 6.5 Hz), 1.45 (9H, s), 1.95 (1H, dm, J = 12.7 Hz), 2.34 (1H, qd, J = 13.1, 3.3 Hz), 2.58 (1H, td, J = 13.0, 2.4 Hz), 2.79 1H, dd, J = 12.6, 3.3 Hz), 3.47 (2H, td, J = 6.5, 2.7 Hz), 4.24 (1H, d, J = 13.0 Hz), 4.55 (2H, s), 7.44 (2H, d, J = 8.0 Hz), 7.64 (2H, d, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ 12.0, 12.7, 18.5, 18.6, 21.0, 21.1, 22.6, 24.1, 26.2, 28.0, 28.2, 28.5, 28.6, 32.1, 32.2, 35.3, 35.5, 38.6, 39.3, 39.9, 42.5, 49.2, 53.9, 56.0, 68.7, 71.6, 71.8, 78.9, 111.1, 118.9, 127.6, 132.2, 144.4, 155.3. HRMS (FAB, NBA/CsI) calcd for $C_{36}H_{54}N_2O_3$ ($M+Cs^+$) 695.3189; found 695.3168.

***N*-Alkylated cyanobenzylether (18).** Compound **17** (124 mg, 0.220 mmol) in 2 mL CH_2Cl_2 was treated with 0.2 mL TFA for 1 h. The volatile components were evaporated and the residue in ethyl acetate was extracted

with 1 N NaOH, washed with H_2O, brine, and dried over $MgSO_4$. The free secondary amine in 4.9 mL anhydrous DMF was stirred with 1,1-dimethylethyl-6-bromohexanoate (160 mg, 0.637 mmol, 2.9 equiv), 142 μL Hünig's base and a catalytic amount of tetrabutylammonium iodide for 24 h at 50 °C and subsequently for 16 h at 70 °C. The cooled reaction mixture was partitioned twice between ethyl acetate and water. The combined organic phases were washed with brine, dried over $MgSO_4$ and the residue, upon evaporation, was subjected to silicagel chromatography ($CHCl_3$:MeOH 50:1→25:1, 11 mg of mixture of A/B *cis*- and *trans*-fused isomers (2:1) elute, 25:1→15:1, 68 mg of major *trans*-configured isomer **18** elute; $R_f = 0.54$ CH_2Cl_2:MeOH 6:1, stains brown with ninhydrine). Yield: 79 mg (0.125 mmol, 57%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.63 (3H, s), 0.91 (3H, d, $J = 6.5$ Hz), 1.02 (3H, s), 1.43 (9H, s), 1.96 (1H, dm, $J = 12.5$ Hz), 2.08 (1H, broad signal), 2.20 (2H, t, $J = 7.4$ Hz), 2.39 (1H, broad signal), 2.74 (2H, broad signal), 3.45 (2H, m), 4.53 (2H, s), 7.43 (2H, d, $J = 8.1$ Hz), 7.62 (2H, d, $J = 8.1$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.0, 13.7, 18.5, 20.7, 21.0 (broad), 22.5 (broad), 23.7 (broad), 23.9, 24.8, 26.2, 26.8, 28.0, 28.1, 30.6, 32.1, 34.3, 35.3, 35.4, 36.7 (broad), 37.1, 39.8, 42.4, 52.7, 53.0, 53.4, 55.9, 56.1, 69.5, 71.6, 71.8, 80.1, 111.0, 118.9, 127.6, 132.1, 144.3, 172.9. HRMS (FAB, NBA/CsI) calcd for $C_{41}H_{64}N_2O_3$ ($M + H^+$) 633.4995; found 633.4974.

Cyanobenzyl-N(O)COO*t*Bu (19a,b). In a Dewar-ice bath at −20 °C, **18** (68 mg, 0.107 mmol) in 1.2 mL CH_2Cl_2 was treated with *m*CPBA (32.5 mg, 0.188 mmol, >57% quality). The stirred mixture was warmed to room temperature overnight, concentrated and directly filtered through a short silicagel column that has previously been conditioned with $CHCl_3$:MeOH:NH_4OH (100:5:0.5) to remove the reagent. Yield nearly quantitative. In a second column chromatography using $CHCl_3$:EtOH (20:1→6:1) the two epimers **19b**, **19a** can be partially separated. ($R_f = 0.44$ (**19b**), 0.37 (**19a**) $CHCl_3$:EtOH 6:1, stains brown with ninhydrine).^{1}H NMR (**19b**, $CDCl_3$, 500 MHz): δ 0.64 (3H, s), 0.92 (3H, d, $J = 6.5$ Hz), 1.04 (3H, s), 1.45 (9H, s), 2.32 (2H, t, $J = 7.5$ Hz), 2.72 (1H, dm, $J = 9.5$ Hz), 2.89 (1H, t, $J = 8.5$ Hz), 3.12 (1H, m), 3.24 (1H, td, $J = 17.0$, 5.0 Hz), 3.47 (2H, t, $J = 6.5$ Hz), 3.74 (1H, d, $J = 13.5$ Hz), 4.55 (2H, s), 7.44 (2H, d, $J = 8.1$ Hz), 7.64 (2H, d, $J = 8.1$ Hz). ^{1}H NMR (**19a**, $CDCl_3$, 500 MHz): δ 0.65 (3H, s), 0.92 (3H, d, $J = 6.5$ Hz), 1.41 (3H, s), 1.44 (9H, s), 2.23 (2H, t, $J = 7.5$ Hz), 3.11 (1H, t, $J = 8.5$ Hz), 3.19 (1H, t, $J = 8.5$ Hz), 3.28 (1H, d, $J = 14.0$ Hz), 3.33 (1H, td, $J = 12.5$, 4.5 Hz), 3.46 (2H, o, $J = 4.0$ Hz), 4.55 (2H, s), 7.44 (2H, d, $J = 8.1$ Hz), 7.64 (2H, d, $J = 8.1$ Hz).

Cyanobenzyl-N(O)COOH, 2 epimers (1:1) (20a,b). A 1:1 mixture of **19a** and **19b** (8.0 mg, 12.3 μmol) was treated with 1 mL CH_2Cl_2 and 1 mL trifluoroacetic acid for 4 h at room temperature. The volatile components were removed and the residue is subjected to high vacuum to yield **20a,b** (7.5 mg, 99%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.64 (3 H, s, one epimer), 0.66 (3H, s, other epimer), 0.92 (6H, d, both epimers), 1.08 (3H, s, epimer I), 1.24 (3H, s, epimer II), 2.35–2.45 (4H, m, I + II), 2.45–2.5 (2H, m, one epimer), 2.83 (1H, d, $J = 11.5$ Hz, one epimer), 3.25 (1H, t, $J = 10.0$ Hz, one epimer), 3.34 (1H, m, one epimer), 3.47 (2H, t, $J = 6.5$ Hz, I + II), 3.72 (1H, m, one epimer), 3.81 (1H, m), 3.87 (1H, d, $J = 11.5$ Hz), 3.94 (1H, m), 4.05 (1H, m), 4.55 (4H, s, I + II), 7.45 (4H, d, $J = 8.0$ Hz, I + II), 7.64 (4H, d, $J = 8.0$ Hz, I + II). HRMS (FAB, NBA/CsI) calcd for $C_{37}H_{56}N_2O_4$ ($M + H^+$) 593.4318; found 593.4334.

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References and Notes

1. (a) Dang, T.; Abe, I.; Zheng, Y. F.; Prestwich, G. D. *Chem. Biol.* **1999**, *6*, 333. (b) Pale-Grosdemange, C.; Feil, C.; Rohmer, M.; Poralla, K. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2237. (c) Ceruti, M.; Rocco, F.; Viola, F.; Balliano, G.; Milla, P.; Arpicco, S.; Cattel, L. *J. Med. Chem.* **1998**, *41*, 540. (d) Sato, T.; Abe, T.; Hoshino, T. *Chem. Commun.* **1998**, 2617. (e) Corey, E. J.; Cheng, H., Baker, C. H.; Matsuda, S. P. T.; Li, D.; Song, X. *J. Am. Chem. Soc.* **1997**, *119*, 1289. (f) Zheng, Y. F.; Oehlschlager, A. C.; Georgopapadakou, N. H.; Hartmann, P. G.; Scheliga, P. *J. Am. Chem. Soc.* **1995**, *117*, 670. (g) Ourisson, G.; Nakatani, Y. *Chem. Biol.* **1994**, *1*, 11. (h) Kyler, K. S.; Novak, M. J. *NATO ASI Ser., Ser. C* **1992**, *381*, 3. (i) Hoshino, T.; Williams, H. J.; Chung, Y.; Scott, A. I. *Tetrahedron* **1991**, *47*, 5925–5932.

2. Wendt, K. U.; Lenhart, A.; Schulz, G. E. *J. Mol. Biol.* **1999**, *286*, 175.

3. Corey, E. J.; Cheng, H.; Baker, C. H.; Matsuda, S. P. T.; Li, D.; Song, X. *J. Am. Chem. Soc.* **1997**, *119*, 1277.

4. Kirby, A. J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 707.

5. Sinha, S. C.; Barbas, III, C. F.; Lerner, R. A. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 14603.

6. Janda, K. D.; Weinhouse, M. I.; Danon, T.; Pacelli, K. A.; Schloeder, D. M. *J. Am. Chem. Soc.* **1991**, *113*, 5427.

7. *N*-oxides are slightly less basic than their parent tertiary amines: Kruger, T. L.; White, W. N.; White, H.; Hartzell, S. L.; Kress, J. W.; Walter, N. *J. Org. Chem.* **1975**, *40*, 77.

8. Cerutti, M.; Delprino, L.; Cattel, L.; Bouvier-Nave, P.; Duriatti, A.; Schuber, F.; Benveniste, P. *J. Chem. Soc. Chem. Commun.* **1985**, 1054.

9. Janda, K. D.; Shevlin, C. G.; Lerner, R. A. *Science* **1993**, *259*, 490.

10. Jenson, C.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1997**, *119*, 10846.

11. Arevalo, J. H.; Hassig, C. A.; Stura, E. A.; Sims, M. J.; Taussig, M. J.; Wilson, I. A. *J. Mol. Biol.* **1994**, *241*, 663.

12. He, M.; Gani, M.; Livnah, O.; Stura, E. A.; Beale, D.; Coley, J.; Wilson, I. A.; Taussig, M. J. *Immunology* **1997**, *90*, 632.

13. Compare to: Eschenmoser, A. *Angew. Chem.* **1994**, *106*, 2455.

14. Li, X.; Singh, S. M.; Labrie, F. *J. Med. Chem.* **1995**, *38*, 1158.

15. Rasmusson, G. H.; Toney, J. H. *Annu. Rep. Med. Chem.* **1994**, *29*, 225.

16. Bolt, C. C. *Recl. Trav. Chim. Pays-Bas*. **1938**, *57*, 905.
17. Windaus, A., *Ber*., **1906**, *39*, 2008; *Ber*. **1917**, *50*, 133.
18. (a) Bakshi, R. K.; Patel, G. F.; Rasmussen, G. H.; Baginsky, W. F.; Cimis, G. ; Ellsworth, K.; Chang, B.; Bull, H.; Tolman, R. L.; Harris, G. S. *J. Med. Chem*. **1994**, *37*, 3871. (b) Miller, R. A.; Humphrey, G. R.; Thompson, A. S. *Tetrahedron Lett*. **1995**, *36*, 7949.
19. Morzycki, J. W.; Wilczewska, A. Z.; Zochowska, E.; Lotowski, Z. *Heterocycles* **1995**, *41*, 2729.
20. (a) Miyamoto, K.; Kubodera, N.; Mursyama, E.; Ochi, K.; Mori, T.; Matsunaga, I. *Synth. Commun*. **1986**, *16*, 513. (b) Demir, A. S.; Sabol, M. R.; Jeganathan, A.; Dolence, E. K.; Watt, D. S. *Org. Prep. Proc. Int*. **1987**, *19*, 197.
21. For application of this method to other steroidal systems: (a) Uskokovic, M.; Gut, M. *Helv. Chim. Acta* **1959**, *42*, 2258. (b) Rasmusson, G. H.; Reynolds, G. F.; Steinberg, N. G.; Walton, E.; Patel, G. F.; Liang, T.; Cascieri, M. A.; Cheung, A. H.; Brooks, J. R.; Berman, C. *J. Med. Chem*. **1986**, *29*, 2298. (c) Piniella, J. F.; Estape, J.; Lupon, P.; Merino, L.; Puig, M.; Bonet, J.-J.; Brianso, J. L.; Germain, G. *Bull. Chem. Soc. Jpn*. **1987** *60*, 3011. (d) Aboul-Enein, H. Y.; Doorenbos, N. J. *J. Heterocyclic Chem*. **1974**, *11*, 557. (e) Singh, H.; Paul, D.; Parashar, V. V. *J. Chem. Soc. Perkin Trans. 1* **1973**, 1204. (f) Singh, H.; Paul, D. *J. Chem. Soc. Perkin Trans. 1* **1974**, 1475. (g) Shoppe, C. W.; Killick, R. W.; Krüger, G. *J. Chem. Soc*. **1962**, 2275. (h) Gut, M.; Uskokovic, M. *J. Org. Chem*. **1961**, *26*, 1943. (i) Gupta, R.; Pathak, D.; Jindal, D. P. *Eur. J. Med. Chem*. **1996**, *31,* 241.
22. Greenlee, W. J.; Thorsett, E. D. *J. Org. Chem*. **1981**, *46*, 5351.
23. Singh, H.; Sharma, P. P.; Mathur, R. B. *Ind. J. Chem*. **1973**, *11*, 1254.
24. Corey, E. J.; Schmidt, G. *Tetrahedron Lett*. **1979**, 399.
25. Borch, R. F.; Hassid, A. I. *J. Org. Chem*. **1972**, *37*, 1673.
26. Hasserodt, J.; Janda, K. D. *Tetrahedron* **1997**, *53*, 11237.
27. Paschall, C.; Hasserodt, J.; Jones, T.; Lerner, R. A.; Janda, K. D.; Christianson, D. W. *Angew. Chem., Int. Ed. Engl*. **1999**, *38*, 1743.
28. Gomez-Parra, V.; Sanchez, F.; Torres, T. *Synthesis* **1985**, 282.
29. Hasserodt, J.; Janda, K. D.; Lerner, R. A. *J. Am. Chem. Soc*. **2000**, *122*, 40.
30. Henderson, P. J. F. *Biochem, J*. **1972**, *127*, 321.
31. Copeland, R. A.; Lombardo, D.; Giannaras, J.; Decicco, C. P. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 1947.