Pergamon

Tetrahedron, Vol. 53, No. 25, pp. 8421-8438, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00526-7

Synthesis of RNAse Active Site Model Systems Using a Steroid Template

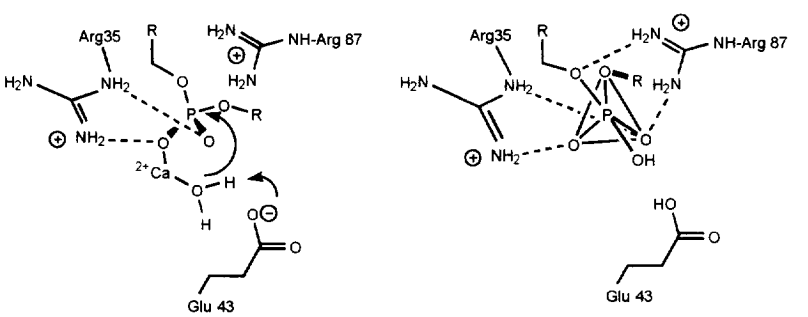
Thorsten Oost and Markus Kalesse*

Institut für Organische Chemie, Universität Hannover, Schneiderberg 1B, D-30167 Hannover (Germany)

Abstract: An RNAse active site model system is described. The rigid steroid backbone is used as the template on which guanidinium and imidazole moieties, necessary for the transesterification/cleavage, are assembled. By changing the stereochemistry at C11 of **2**, and varying the guanidinium side chains, active compounds **9**, **11**, **13**, **15** with different hydrolytic behavior are obtained. Comparison of the steroid compounds clearly demonstrates that changes in the geometry can influence the cleavage reaction of RNA analogs. Furthermore, an intramolecular base can enhance the cleavage rate. The pK_a values of the most active bis(guanidinium) compound **9** has been determined and the pH dependence of the cleavage reaction is discussed. © 1997 Elsevier Science Ltd.

INTRODUCTION

Bis(guanidinium) moieties have been used in the synthesis of artificial receptors in order to demonstrate the ability of these groups to bind to phosphates and to facilitate the cleavage of phosphodiesters. The use of guanidinium groups in the design of artificial ribonucleases has been stimulated by the fact that the active site of enzymes such as staphylococcal nuclease (SNase)[1] exhibit two essential arginine residues (Arg-35, Arg-87) (Scheme 1). On the basis of X-ray structural analysis, kinetic studies, and active site mutant investigation, the mechanism of SNase has been unraveled.[2] The mode of action involves augmenting the hydrogen bonding of the substrate by Arg-87 in the transition state. Staphylococcal nuclease is capable of hydrolyzing RNA and DNA. The nucleophile necessary for the hydrolysis of DNA is generated by water coordination to Ca^{2+} and deprotonation of the coordinated water by the carboxylate group at Glu-43.



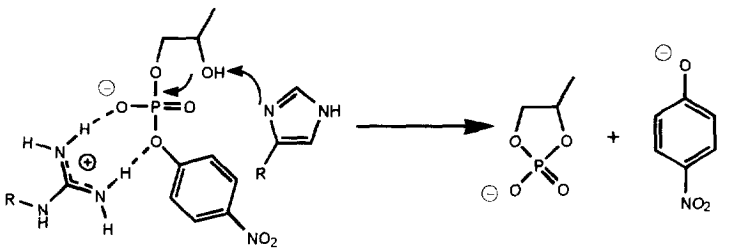
Scheme 1. Transition state of the staphylococcal nuclease catalyzed phosphodiesterase reaction.

Various approaches have been published in which the ability of basic polyamines[3] like poly(Arg-Leu) to coordinate to phosphates has been utilized to cleave RNA. The work published by Anslyn[4], Göbel[5], Hamilton[6] and Schmidtchen[7] clearly demonstrates the ability of various guanidinium receptors to bind to phosphates and by doing so accelerate the rate of transesterification / cleavage of RNA and RNA model compounds. Anslyn described the cleavage of RNA with a bis(guanidinium) receptor in water.[4b] Imidazole as the base was required for substantial cleavage. Hamilton synthesized a bis(guanidinium) receptor with an internal base, but the cleavage reactions were performed in acetonitrile as the solvent.[6c] Thus an appropriate receptor should be able to enhance the cleavage of RNA in water. In addition to binding experiments, hydrolysis and transesterification experiments on phosphodiesters have been performed and various systems have been published where bis(guanidinium) moieties were able to accelerate the hydrolysis of phosphodiesters more efficiently than mono-guanidinium groups. Additional rate enhancements have been observed when an internal base was used in combination with guanidinium groups.[6c] Based on the work that has been published by the above mentioned groups, we envisioned that cleavage of RNA can be achieved in aqueous media with a bis(guanidinium) compound employing an internal base. Our design of the bis(guanidinium) receptor was based on the fact that varying rates of cleavage have been reported depending on the receptor geometry.

Nevertheless, the precise mode of action for the transesterification/cleavage of RNA in the active site of arginine-containing ribonucleases is still unknown and efficient hydrolysis is probably due to the distinct arrangement of the amino acids in the active site of the ribonucleases.

Most of the reported bis(guanidinium) compounds were used in aprotic solvents but cleavage experiments in our laboratory with simple bis(guanidinium) compounds like 1,3-bis(guanidinium)propane sulfate or even imidazole pendant bis(guanidinium) compounds like 1,3-bis(guanidinium)-2-[2-(1H-imidazol-4-yl)-ethylamino]propane dinitrate gave almost no increase in the transesterification reaction above background hydrolysis. Our approach in synthesizing an active model system that is capable of cleaving RNA in water is based on the assumption that preorganization of functional groups with some degree of conformational flexibility can generate active compounds. The task of the steroid system was to provide a readily accessible backbone on which the reactive functionalities can be assembled and the configuration can be changed in order to find an active compound. Additionally, the steric bulk provided by the steroid restricts the conformational flexibility of the guanidinium groups.

As in the case of SNase, the substrate is coordinated to the guanidinium groups prior to the hydrolysis reaction. Tight binding of the intermediate in the pentacoordinated transition state requires a certain degree of conformational flexibility of the guanidinium groups. Since the phosphodiester to be cleaved is RNA, our model system did not require an external nucleophile but still an internal base in order to deliver the 2'-OH to the phosphodiester linkage (Scheme 2). Therefore we synthesized active site analogs employing both the rigidity of the steroid backbone, the configurational flexibility of substituents at the C11 position and an internal base.



1, HPNPP

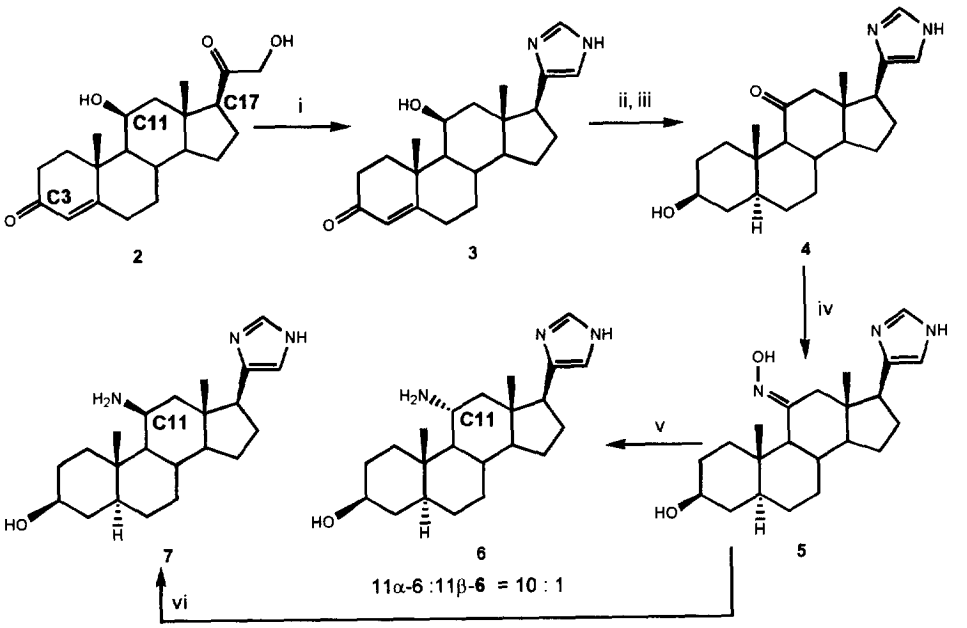
Scheme 2. Cleavage of the RNA analog HPNPP (**1**)[10] by steroid derivatives.

RESULTS

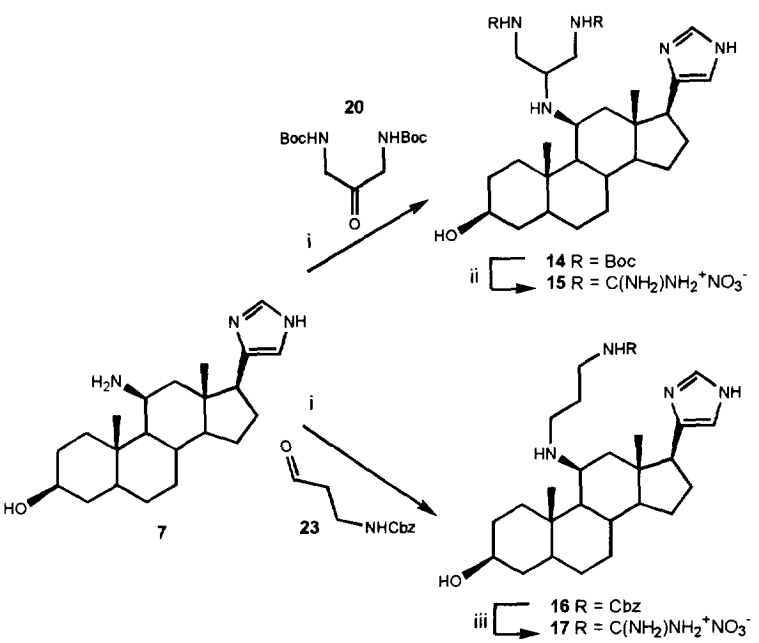
Our general idea was to search for a hydrolytically active steroid template by varying the configuration at C11 and the length of the guanidinium spacer with the imidazole group still in place. By doing so we envisioned to go through a process of fine-tuning and finding the most active SNase analog. Once we detect the most promising system we plan to attach this active site analog via the C3-position to shuttle-proteins[8] with RNA-specific recognition sites such as the tat-protein of the HIV-1 virus.[9]

Design of the Model System: In our search for a system that allows us the synthesis of rigid bis(guanidinium) compounds with some configurational flexibility, corticosterone (**2**) was chosen as the template on which the bis(guanidinium) subunit could be varied. It was our goal to bring mono- or bis(guanidinium) moieties and the imidazole ring into close proximity. In addition to configurational changes we were able to vary the size of the bis(guanidinium) scissors by using different diamine spacers **20**, **22** and **23**. As promising model systems we investigated the hydrolytic behavior of steroid derivatives **9**, **11**, **13**, **15** and **17** in aqueous solution with *para*-nitrophenyl-2-hydroxypropyl phosphate (**1**)[10] as the substrate. The hydrolytic behavior was measured at various pHs. To assess the potency of our steroid systems we compared the rates of cleavage to the hydrolytic behavior of the known compound **24**.

The Synthesis of the Steroid Derivatives 9, 11, 13, 15 and 17: The challenge of this approach was to find a synthetic strategy that allows selective functionalization of the C11 carbonyl group. This was achieved by utilizing a procedure published by Guthrie[11] (Scheme 3) that yielded oxime **5**, which can be then reduced to either the 11α-amine (**6**) or to the 11β-amine (**7**). Different side chains were attached to these two stereoisomers yielding five different guanidinium compounds. The C17 side chain of corticosterone (**2**) was transformed into an imidazole moiety by a Weidenhagen reaction.[12] Birch reduction and subsequent Jones oxidation gave ketone **4**. The 11-keto compound was transformed to the corresponding oxime **5**. By reducing **5** either with Na/*n*-PrOH[13] or by high-pressure hydrogenation with Pt/H_2 under acidic conditions we were able to obtain both diastereomeric amines in good diastereomeric purity. The β-**6**-isomer was the only detectable diastereomer during the reaction. The α-**6**-isomer was produced in a 10:1 ratio, but both diastereomers could be separated via flash chromatography.

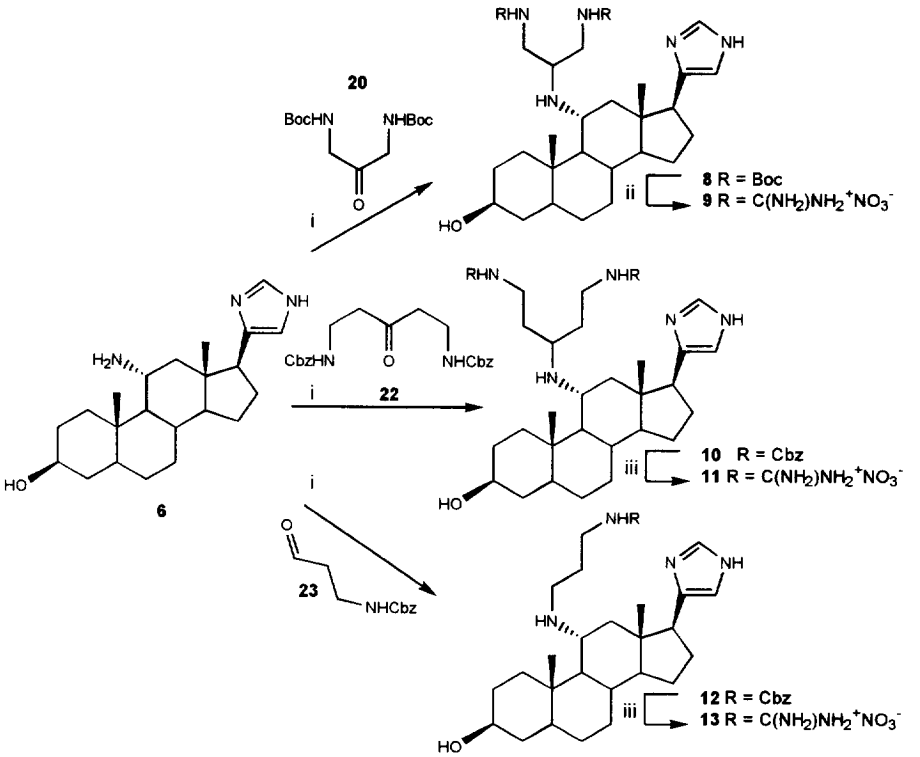


Scheme 3. i) $Cu(OAc)_2$, NH_3, CH_2O, EtOH, reflux, 83 %; ii) Li, NH_3 (l) / MeOH; iii) Jones Ox. (CrO_3, HOAc, H_2SO_4); iv) NH_2OH·HCl, pyridine reflux, 72 % (three steps); v) Na, *n*-PrOH reflux, 78 %; vi) H_2 (100 bar), Pt, HOAc / HCl, 76 %.



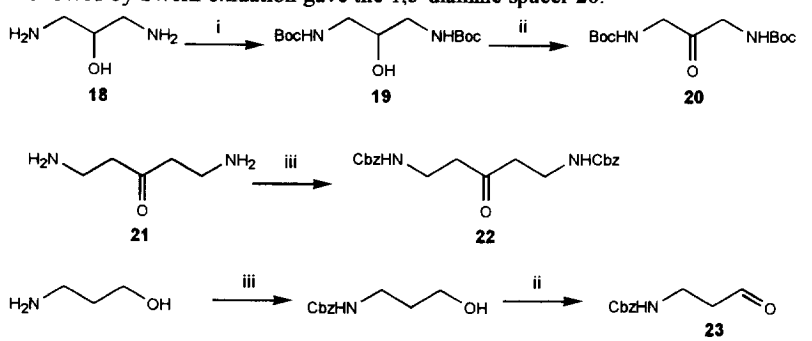
Scheme 4. i) $NaCNBH_3$, MeOH, pH = 7; ii) HCl (4 M), basic ion exchange resin, 3,5-dimethylpyrazole-1-carboxamidine nitrate, Hünig's base, DMF; iii) H_2, Pd /C, HCl, MeOH, basic ion exchange resin, 3,5-dimethylpyrazole-1-carboxamidine nitrate, Hünig's base, DMF.

Reductive amination conditions with $NaCNBH_3$ in MeOH were used to introduce the different guanidinium side chains. Deprotection and guanidation gave the desired guanidinium compounds **9**, **11**, **13**, **15** and **17** (Scheme 4 and 5). All guanidinium compounds were purified via reverse-phase chromatograhy[14] and fully characterized.



Scheme 5. i) $NaCNBH_3$, MeOH, pH = 7; ii) HCl (4 M), basic ion exchange resin, 3,5-dimethylpyrazole-1-carboxamidine nitrate, Hünig's base, DMF; iii) H_2, Pd / C, HCl, MeOH, basic ion exchange resin, 3,5-dimethylpyrazole-1-carboxamidine nitrate, Hünig's base, DMF.

The side chains **20**, **22** and **23** were synthesized from the corresponding amines (Scheme 6). Double Boc-protection of **18**[15] followed by Swern oxidation gave the 1,3-diamine spacer **20**.



Scheme 6. i) Boc_2O, NaOH, H_2O/dioxane; ii) Swern oxidation; iii) CbzCl, NaOH, H_2O.

The symmetric 1,5-diamino ketone **21**[16] was double Cbz-protected to generate 1,5-diamine spacer **22**. Monoamine spacer **23** was synthesized according to literature procedures.[17]

Cleavage experiments: The transesterification/cleavage of **1** (Scheme 2) was utilized in order to determine the rate constants of the various enzyme models. All experiments were recorded against background hydrolysis under our conditions and the reported values for the pseudo first order rate constants are the acceleration above background hydrolysis. Table 1 gives the values for the background hydrolysis at various pH.

Table 1. Rate constants for the background hydrolysis at 55 °C.

pH	**rate constant [s^{-1}]**
7.0	3.4×10^{-7}
7.4	12.5×10^{-7}
7.5	13.5×10^{-7}
8.0	41.8×10^{-7}
8.3	73.1×10^{-7}

In order to evaluate the Michaelis-Menten behavior of our steroid systems we investigated the hydrolytic activity at varying concentration of the catalyst and **1** but no saturation kinetics for any of the steroid systems were observed. As an example the rate constants for compound **9** are given in Table 2 and 3. The reason that we did not observe saturation kinetics for any of the steroid substrates is probably due to low associations constants of the steroids in water.

Table 2. Rate constants at varying concentrations of steroid **9** (2 mM **1**, pH 7.5, 55 °C, I= 0.5 KCl).

concentration of steroid **9**	pseudo first order rate constant [s^{-1}]
20 mM	2.5×10^{-6}
10 mM	1.1×10^{-6}
5 mM	0.55×10^{-6}

Table 3. Rate constants at varying concentrations of **1** (10 mM steroid **9**, pH 7.5, 55 °C, I= 0.5 KCl).

concentration **1**	pseudo first order rate constant [s^{-1}]
1 mM	1.17×10^{-6}
2 mM	1.09×10^{-6}
3 mM	1.03×10^{-6}
4.mM	1.06×10^{-6}

The hydrolysis experiments were performed at 55°C in HEPES buffer with the pH ranging from pH = 7.0-8.0 (100 mM HEPES, I = 0.5 NaCl, 10 mM steroid compound, 2 mM **1**). The fact that the observed cleavage is actually a transesterification reaction as pointed out in Scheme 2 and not a hydrolysis reaction, was proven by ^{31}P NMR experiments. The only detectable ^{31}P NMR signals were observed at $\delta = -4.2$ for the substrate and at

δ = 18.8 for the cyclic phosphate. Figure 1 compares the cleavage rate constants for the five steroid-derived guanidinium compounds and compound **24**, used by Göbel et al..[5b] In both cases the 11α steroids were more active than the corresponding 11β compounds.[18]

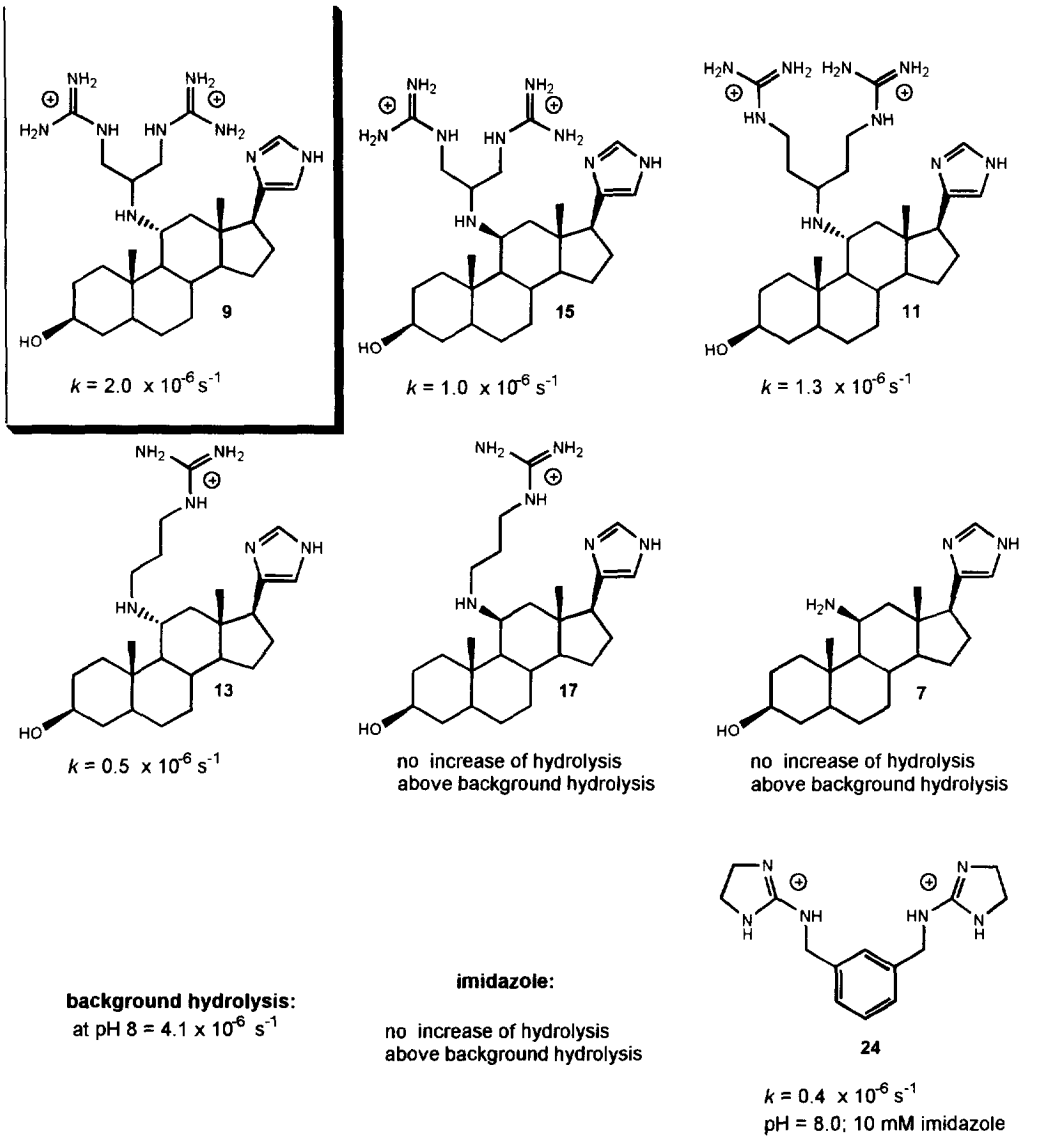


Figure 1. Rate constants for the steroid derivatives and compound **24**.[5b] Conditions: 100 mM HEPES; pH 8.00; I = 0.5 NaCl; 10 mM steroid; 2 mM **1**. The pseudo first order rate constants are the rate constants for the increase of hydrolysis above background hydrolysis.

We used compound **9** in hydrolysis experiments with UpU as the crucial probe whether such bis(guanidinium) compounds can be used for cleaving of non-activated phosphodiesters as in RNA. The hydrolysis experiments were performed in the presence of HEPES buffer (100 mM) to assure a pH of 7.5. Steroid **9** shows remarkable

hydrolysis of UpU at 60° C. The pseudo first order rate constant was 2.4 x 10^{-4} h^{-1} at a concentration of 10 mM steroid and 0.1 mM UpU (background hydrolysis: 9 x 10^{-5} h^{-1}). The 2',3'cyclic phosphate could be identified by HPLC analysis as well as the 2'- and the 3'-phosphate that were generated from hydrolysis of the 2',3'cyclic phosphate

DISCUSSION

With every steroid exhibiting a different rate constant we observed what we initially expected: Changes in the conformation can give changes in the kinetics. Nevertheless we did not expect steroid **11** to be less effective than **9**. The reason for the change in the reactivity can be seen in the increasing distance between the guanidinium groups and the steroid backbone by changing the 1,3 spacer into the 1,5 spacer. The effect of an internalized base is demonstrated by the fact that the very active bis(guanidinium) compound **24** is less active than the bis(guanidinium) steroids even in the presence of equimolar concentration of imidazole.

Even though we intended the internal imidazole to act as a base in the cleavage reaction we had to support that actually the imidazole accelerates the cleavage reaction by deprotonating the 2'-OH. Therefore the pH/rate constant profile for compound **9** was determined as shown in Figure 1. The rate constant increased from pH 7.3 to 7.65, and leveled out at pH 8.0.

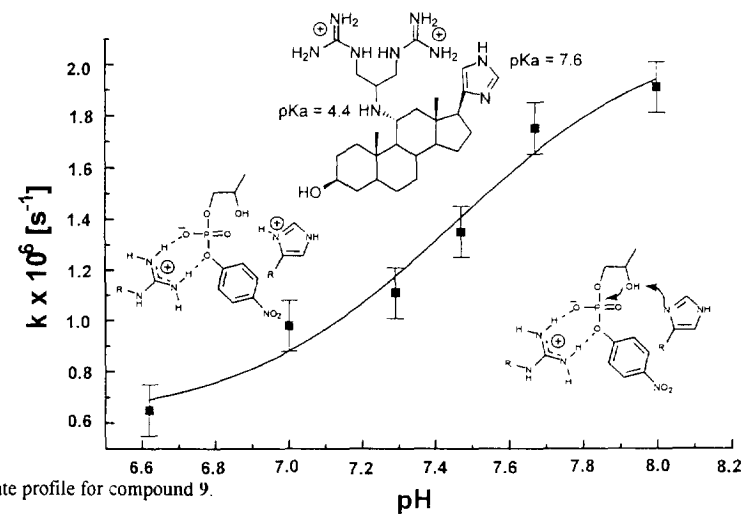


Figure 2. pH - rate profile for compound 9.

Then we identified the pK_a values for the basic nitrogen atoms in our most active compound **9**. The pK_a values of **9** were determined by potentiometric titration to be pK_a = 4.4 for the 11α-amine and pK_a = 7.6 for the imidazole. In the pH-range around 7.5 the basic nitrogen of the imidazole group is deprotonated and therefore act as an internal base. The low pK_a value for the secondary amine at C11 is probably due to electrostatic repulsion from the two guanidinium groups. These pK_a differences are known for poly-aza compounds in which the nitrogen atoms are close to each other. The pK_a values of the steroid nitrogens offer a reason for the increase in the hydrolytic activity observed for the RNA model system. The increase in the rate constant values can be

rationalized by increasing concentration of free imidazole (pK_a = 7.6) in these active site models. Even though the effective molarity for the imidazole moiety could not be determined since we did not perform the cleavage reactions with steroids lacking the imidazole moiety, the fact that the rate constant at pH 8.0 is only twice as high as the rate constant at pH 7.3 indicates a low effective molarity for the internal base. The slow or non-measurable cleavage rates in case for the mono-guanidinium compounds can be explained by the decreased electrostatic interaction of the phosphate anion with the positively charged guanidinium steroids. Another explanation can be the poor stabilization of the transition state by just one guanidinium group. Nevertheless, the hydrolytic behavior of all compounds depends on the presence of free base within the steroid model system and it seems likely that the two guanidinium groups are necessary for neutralizing the negative charge at the phosphate anion prior to the nucleophilic attack of the 2'-OH.

CONCLUSION

We established an efficient route to 11-substituted steroid-based *acitve site* analogs. The conformational differences due to the configurational change at C11 gives rise to different hydrolytic behavior of these SNase analogs. The comparatively small 1,3-spacer which is not able to coordinate to the phosphate via a fork-like geometry exhibits the highest hydrolytic activity of the guanidinium compounds reported in this work. The effect of a base present in the active site analog is demonstrated by pH-dependence of the hydrolysis reaction. Additionally, the increase of the hydrolysis can be rationalized by the pK_a of the imidazole moiety.

To sum, we developed a promising cleavage unit, which we plan to couple to a short peptide in order to achieve selective cleavage of RNA.

EXPERIMENTAL

General Information. All reagents were of analytical grade quality. Solvents were distilled in glas. All experiments in aqueous solutions were performed in Millipore water. Anhydrous reactions were performed under nitrogen in flame dried (under vacuum) glassware. Melting points (not corrected): Gallenkamp capillary melting-point apparatus. - IR: Perkin Elmer FT 1710. - NMR: Bruker WP 200, AM 400. ^{1}H NMR spectra in D_2O were referenced to the HDO signal (δ = 4.65). ^{13}C NMR spectra were measured with ^{1}H-broadband decoupling. The signal multiplicities were determined by means of the DEPT 135 (CH or CH_3 give positive signals (+), while CH_2 gives negative signals) or APT technique (CH and CH_3 give negative signals (–), while quaternary C and CH_2 give positive signals (+)).^{31}P NMR sectra were obtained at 162 MHz. The external reference was 85 % phosphoric acid, and chemical shifts downfield of the standard are positive. - Low-resolution mass spectra: Finnigan MAT 312. High resolution and FAB mass spectra: VG Autospec. - Thin layer chromatography (TLC): Merck TLC Aluminium sheets Silica gel 60 F_{254} pre-coated. - Flash chromatography: J. T. Baker silica gel (0.03 - 0.06 mm). - Elemental Analyses: Heraeus CHN-Rapid (Institut für Organische Chemie der Universität Hannover). - Anhydrous solvents: Dimethyl sulfoxide (DMSO), Dimethylformamide (DMF),

dichloromethane (CH_2Cl_2) , Hünig's base (diisopropylethylamine) and triethylamine were distilled from CaH_2 under nitrogen. Methanol (MeOH) was dried with magnesium and subsequently distilled under nitrogen.

The following compounds were prepared according to literature procedures: HPNPP[10], 3-(benzyloxycarbonyl)-aminopropionaldehyde (**23**)[17], 1,5-diamino-3-pentanone dihydrochloride (**21**)[16], 11β-amino-17β–(4(5)-imidazolyl)-5α-androstan-3β-ol (**6**)[11] and compound **24**.[5b]

Kinetic studies: HEPES is the commercial name for *N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid and was purchased from Biomol. The pH value of HEPES buffer solutions was adjusted with Metrohm titrator/pH meter (Metrohm 716 DMS Titrino) at room temperature. Molar extinction coefficients of *p*-nitrophenol were determined under hydrolysis conditions over a pH range from 5.2 to 10.2. Hydrolysis experiments were performed in teflon sealed quarz kuvettes (1 cm path length) which were incubated in an water bath at 55°C ± 0.2 °C. Additionally, in every run a reference kuvette was incubated which only contained buffer and **1**. Absorbance of *p*-nitrophenolate was measured at 400 nm against background hydrolysis with a Shimadzu UV-visible UV-1601 dual-beam spectrophotometer fitted with a thermostated cuvette holder. For each rate constant, at least six points over a period of 120 min were collected (conversion < 5 %, correlation coefficient of rate plots > 0.995). After each hydrolysis experiment the pH of the sample and reference solution was checked (differences ≤ 0.03). The observed first order k' values were converted to the corresponding second order rate constants (dc/dt = k [steroid] [phosphate] = k' [phosphate]; [steroid] = const.). Rate constants were reproducible to ± 10 %.

Potentiometric titrations of steroid compounds: The Millipore water used for the titrations was refluxed and stored under argon. Titrations were performed with an automatic Metrohm titrator/pH-meter (Metrohm 716 DMS Titrino) at 20°C. All titrations were performed under argon to avoid absorption of CO_2. The steroid compound was dissolved in 15 ml of 0.01 M HCl (I = 0.5 NaCl) to yield a final concentration of 0.0025 M. To this solution (initial pH = 2.3) was added 0.1 M NaOH (0.05 ml steps) and the pH after each addition was recorded. The pK_a values were determined by an internal computing program.

1,3-Bis[(*tert*-butoxycarbonyl)amino]-2-propanol (19): A solution of 1,3-diaminopropanol (901 mg, 10.0 mmol) in a mixture of dioxane (20 ml) and 1 N NaOH (20 ml, 20 mmol) was stirred and cooled in an ice-water bath. Di-*tert*-butyl dicarbonate (4.80 g, 22 mmol) was added and stirring was continued at room temperature for 18 h. The solution was concentrated *in vacuo* to about 10 ml and the resulting paste was diluted with water (50 ml). The mixture was then extracted with EtOAc (3 x 50 ml) and the combined organic layer was dried (Na_2SO_4). Evaporation *in vacuo* yielded 2.83 g (98 %) of a white solid, which was used for the next step without purification.

^{1}H NMR (400 MHz, $CDCl_3$, 20°C, TMS): δ = 5.25 (s, br, 2H; NH), 3.88 (s, br,1H; OH), 3.74 (m, 1H; C*H*OH), 3.23 (m, 4H; CH_2), 1.44 [s, 18H; $C(CH_3)_3$]; ^{13}C NMR (100 MHz, $CDCl_3$, 20°C, TMS): δ = 157.3 (C_q; C=O), 79.8 [C_q; *C*$(CH_3)_3$], 71.0 (+; CHOH), 43.5 (–; CH_2), 28.4 (+; CH_3); IR ($CHCl_3$): ν = 3456, 2980, 2932, 1704,

1508, 1392, 1368, 1248, 1164 cm $^{-1}$; MS (70 eV, EI): *m/z* (%): 290 (1.4) [M^+], 234 (3) [$M^+ - C_4H_8$], 178 (38) [$M^+ - 2\ C_4H_8$], 160 (44) [$M^+ - 2\ C_4H_8 - H_2O$].

1,3-Bis[(*tert*-butoxycarbonyl)amino]-2-propanone (20): Oxalyl chloride (0.77 ml, 9.0 mmol) was dissolved in CH_2Cl_2 (20 ml) and cooled to −78°C. Anhydrous DMSO (1.3 ml, 18.3 mmol) in CH_2Cl_2 (4 ml) was then added. After 2 min at −78°C alcohol **19** (2.23 g, 7.69 mmol) in CH_2Cl_2 (8 ml) was added dropwise over 10 min, keeping the temperature below −50°C at all times. After the addition was complete, the turbid reaction mixture was stirred for 15 min before triethylamine (5.4 ml, 39 mmol) was added. After 5 min the reaction was allowed to warm to room temperature. The mixture was diluted with CH_2Cl_2 (50 ml) and washed with water. The organic layer was separated, dried (Na_2SO_4) and the solvent was evaporated *in vacuo*. The yellow residue was then recrystallized (hexanes-EtOAc) to give 1.82 g (82 %) of colorless crystals.

M.p. 116°C; ^{1}H NMR (400 MHz, $CDCl_3$, 20°C, TMS): δ = 5.31 (s, br, 2H; NH), 4.06 (d, ^3J = 5.0 Hz, 4H; CH_2), 1.45 [s, 18H; $C(CH_3)_3$]; ^{13}C NMR (100 MHz, $CDCl_3$, 20°C, TMS): δ = 202.5 (C_q; ketone C=O), 155.7 (C_q; amide C=O), 80.2 [C_q; $C(CH_3)_3$], 48.3 (−; CH_2), 28.3 (+; CH_3); IR ($CHCl_3$): ν = 3440, 3008, 2980, 2932, 1708, 1500, 1368, 1248, 1160 cm $^{-1}$; MS (70 eV, EI): *m/z* (%): 232 (18) [$M^+ - C_4H_8$], 176 (57) [$M^+ - 2\ C_4H_8$], 159 (87); MS (FAB): *m/z* (%): 289 (25) [M^+ +1], 233 (27), 177 (100); $C_{13}H_{24}O_5N_2$ (288.3): calcd C 54.15, H 8.39, N 9.72; found C 54.05, H 8.33, N 9.60.

1,5-Bis[(benzyloxycarbonyl)amino]-3-pentanone (22): Crude **21** (1.11 g, 5.85 mmol) was dissolved in ice-cold 1 N NaOH (11.7 ml, 11.7 mmol) with stirring. To the cooled solution benzyl chloroformate (1.8 ml, 12.9 mmol) and 1 N NaOH (11.7 ml, 11.7 mmol) were added, in a few portions, alternatingly. After the addition was completed vigorous stirring of the suspension was continued for 18 h at room temperature. The resulting solid material was collected by vacuum filtration and purified by recrystallization (hexanes-EtOAc) to give 1.18 g (53 %) of colorless crystals.

M.p. 114° C; ^{1}H NMR (400 MHz, $CDCl_3$, 20°C, TMS): δ = 7.30 (s, br, 10H; aromatic H), 5.27 (s, br, 2H; NH), 5.06 (s, 4H, benzylic H), 3.40 (q, br, ^3J = 5.6 Hz, 4H; NH-CH_2), 2.63 (t, br, ^3J = 5.3 Hz, 4H; CO-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, 20 °C, TMS): δ = 209.1 (C_q; ketone C=O), 156.3 (C_q; amide C=O), 136.4 (C_q; aromatic), 128.5, 128.1, 128.1 (+; aromatic CH), 66.7 (−; benzylic CH_2), 42.6 (−; CH_2), 35.5 (−; CH_2); IR ($CHCl_3$): ν = 3452, 3000, 2952, 1716, 1512, 1236 cm $^{-1}$; MS (70 eV, EI): *m/z* (%): 293 (24) [$M^+ - C_7H_7$], 249 (10) [$M^+ - C_7H_7 - CO_2$], 108 (81), 91 (100); MS (FAB): *m/z* (%): 385 (100) [M^+ +1]; $C_{21}H_{24}N_2O_5$ (384.4): calcd C 65.61, H 6.29, N 7.29; found C 65.63, H 6.26, N 7.43.

11α-Amino-17β-(4(5)-imidazolyl)-5α-androstan-3β-ol (6): Oxime **5** (1.20 g, 3.23 mmol) was dissolved in refluxing *n*-propanol (180 ml). Sodium (6.0 g, 0.26 mol) was added in portions. After the sodium was consumed, TLC showed no remaining oxime. The solution was cooled, concentrated to about 50 ml and then poured into brine (200 ml). The resulting suspension was extracted with ethyl acetate (3 × 100 ml). The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. The crude oil, which also contained traces of 11β amine, was

purified by flash chromatography ($CHCl_3$/MeOH 2:1 with 1 % of 25 % aqueous ammonia) to give 0.90 g (78 %) of an white foam.

M.p. 177°C; $[\alpha]_D^{20}$ = + 5.0 (c = 1.0 in MeOH); ^{1}H NMR (400 MHz, [D_4]MeOH, 20°C, TMS): δ = 7.64 (d, 4J = 0.9 Hz, 1H; imidazole H), 6.87 (s, 1H; imidazole H), 3.54 (tt, $^3J_{ax\text{-}ax}$ = 10.9 Hz, $^3J_{ax\text{-}eq}$ = 5.0 Hz, 1H; C_3HOH), 3.45 (td, $^3J_{ax\text{-}ax}$ = 11.0 Hz, $^3J_{ax\text{-}eq}$ = 5.0 Hz, 1H; $C_{11}HNH_2$), 2.76 (t, J = 10.0 Hz, 1H), 2.13 (dd, J = 12.3 Hz, J = 5.2 Hz, 1H), 2.08-0.95 (m, 22H), 1.00 (s, 3H, CH_3), 0.51 (s, 3H, CH_3); ^{13}C NMR (100 MHz, [D_4]MeOH, 20°C, TMS): δ = 138.3 (C_q; imidazole), 135.9, 118.0 (+; imidazole CH), 70.8 (+; C_3HOH), 58.2 (+), 55.0 (+), 50.3 (+), 50.0 (+), 46.5 (–), 46.2 (+), 44.1 (C_q), 39.3 (–), 39.1 (–), 38.9 (C_q), 37.3 (+), 33.2 (–), 32.2 (–), 30.2 (–), 27.3 (–), 25.6 (–), 13.8, 12.8 (+; CH_3); IR (KBr): ν = 3336, 3128, 2924, 2856, 1560, 1400, 1040; MS (70 eV, EI): *m/z* (%): 357 (4) [M^+]; HRMS calcd for $C_{22}H_{35}N_3O$: 357.2780, found 357.2770.

11α–{1′,3′-Bis[(*tert*-butoxycarbonyl)amino]-2′-propyl}amino-17β-(4(5)-imidazolyl)-5α–androstan-3β–ol (8): 11α Amine **6** (100 mg, 0.280 mmol) was dissolved in anhydrous MeOH (3.0 ml). Ketone **20** (121 mg, 0.420 mmol, 1.5 eq) was added, followed by $NaCNBH_3$ (18 mg, 0.280 mmol, 1.0 eq) and anhydrous acetic acid (17 µl, 0.280 mmol, 1.0 eq). The solution was stirred at room temperature for 48 h. The reaction mixture was poured into 2 % aqueous NaOH (50 ml) and the resulting suspension was extracted with $CHCl_3$ (3 × 25 ml) The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by flash chromatography ($CHCl_3$/MeOH gradient 15:1 → 5:1) to give 142 mg (81 %) of a white foam.

M.p. 154°C; $[\alpha]_D^{20}$ = + 6.2 (c = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 20°C, TMS): δ = 7.59 (s, 1H; imidazole H), 6.77 (s, 1H; imidazole H), 5.58 (s, br, 1H; amide NH), 4.92 (s, br, 1H; amide NH), 3.58 (tt, $^3J_{ax\text{-}ax}$ = 11.0 Hz, $^3J_{ax\text{-}eq}$ = 5.5 Hz, 1H; C_3HOH), 3.24 (m, br, 2H), 2.93 (m, br, 2H), 2.70 (m, br, 3H), 2.60 (m, 2H), 2.10-0.88 (m, 41H), 1.47 [s, 9H; $C(CH_3)_3$], 1.44 [s, 9H; $C(CH_3)_3$], 0.90 (s, 3H, CH_3), 0.69 (t, J = 9.7 Hz, 1H), 0.43 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 20°C, TMS): δ = 156.9 (C_q; C=O), 135.3 (+; imidazole CH), 79.5 [C_q; $C(CH_3)_3$], 70.7 (+; C_3HOH), 59.3 (+), 55.5 (+), 54.5(+), 53.6(+), 48.0 (–), 45.6 (+), 43.2 (C_q), 42.6 (–), 41.7 (–), 39.6 (–), 39.0 (–), 38.1 (C_q), 36.3 (+), 32.5 (–), 32.0 (–), 29.5 (–), 28.5, 28.4 [+; $C(CH_3)_3$], 25.7 (–), 24.8 (–), 13.7, 13.0 (+; CH_3); IR ($CHCl_3$): ν = 3456, 3368, 2980, 2932, 2860, 1696, 1504, 1368, 1252, 1164; MS (FAB): *m/z* (%): 630 (100) [M^+ +1], 499 (22) [M^+ – CH_2NHBoc], 399 (10), 341 (21) [M^+ – side chain]; HRMS calcd for $C_{29}H_{47}N_4O_3$ (M – CH_2NHBoc) 499.3648, found 499.3656.

11α–{1′,5′-Bis[(benzyloxycarbonyl)amino]-3′-pentyl}amino-17β-(4(5)-imidazolyl)-5α–androstan-3β–ol (10): 11α Amine **6** (179 mg, 0.501 mmol) was dissolved in anhydrous MeOH (2.0 ml). Ketone **22** (288 mg, 0.75 mmol, 1.5 eq) was added, followed by $NaCNBH_3$ (38 mg, 0.60 mmol, 1.2 eq) and anhydrous acetic acid (30 µl, 0.50 mmol, 1.0 eq). The solution was stirred at room temperature for 6 d. The reaction mixture was poured into 2 % aqueous NaOH (50 ml) and extracted with $CHCl_3$ (3 × 25 ml). The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by flash chromatography ($CHCl_3$/MeOH gradient 15:1 → 2:1) to give 185 mg (51 %) of a white foam. 48 mg (27 %) of amine **5** could be recovered.

M.p. 87-92°C; $[\alpha]_D^{20}$ = + 30.0 (c = 1.0 in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 20°C, TMS): δ = 7.42 (s, 1H; imidazole H), 7.39-7.27 (m, 10H; aromatic H), 6.73 (s, 1H; imidazole H), 5.84 (s, br, 1H; amide NH), 5.17-4.15 (m, 5H; benzylic / amide H), 3.51 (tt, $^3J_{ax\text{-}ax}$ = 10.5 Hz, $^3J_{ax\text{-}eq}$ = 5.5 Hz, 1H; C_3HOH), 3.35-3.05 (m, 4H), 2.82-2.60 (m, 3H), 2.55 (t, *J* = 9.7 Hz, 1H), 2.39 (d, *J* = 9.2 Hz, 1H), 2.10-0.80 (m, 27H), 0.89 (s, 3H; CH_3), 0.68 (t, *J* = 9.8 Hz, 1H), 0.39 (s, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 20°C, TMS): δ = 156.9, 156.5 (C_q, C=O), 136.6, 136.5 (C_q; aromatic), 134.9 (+; imidazole CH), 128.6, 128.5, 128.2, 128.1, 128.0 (+;aromatic CH), 70.7 (+; C_3HOH), 66.7, 66.6 (–; benzylic CH_2), 59.2 (+), 54.7 (+), 52.1 (+), 50.5 (+), 48.6 (+), 47.4 (–), 45.6 (+), 43.1 (C_q), 39.5 (–), 38.9 (–), 38.4 (–), 38.2 (C_q), 38.2 (–), 36.4 (+), 35.2 (–), 34.8 (–), 32.5 (–), 31.9 (–), 29.5 (–), 25.9 (–), 24.7(–), 13.9, 13.0 (+; CH_3); IR ($CHCl_3$): ν = 3608, 3452, 3328, 2932, 2860, 1712, 1516, 1248, 1132, 1032; MS (FAB): *m/z* (%): 726 (100) [M^+ +1].

11α–[3′-(Benzyloxycarbonyl)amino-1′-propyl]amino-17β-(4(5)-imidazolyl)-5α–androstan-3β–ol (12): 11α amine **6** (120 mg, 0.336 mmol) was dissolved in anhydrous MeOH (3.0 ml). Aldehyde **23** (84 mg, 0.40 mmol, 1.2 eq) was added, followed by $NaCNBH_3$ (25 mg, 0.40 mmol, 1.2 eq) and anhydrous acetic acid (20 µl, 0.34 mmol, 1.0 eq). The solution was stirred at room temperature for 3 d. The reaction mixture was poured into 2 % aqueous NaOH (50 ml) and extracted with $CHCl_3$ (3 × 25 ml). The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by flash chromatography ($CHCl_3$/MeOH gradient 4:1 → 1:1) to yield 115 mg (63 %) of a white foam.

M.p. 108°C; $[\alpha]_D^{20}$ = –6.5 (c = 1.0 in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 20°C, TMS): δ = 7.53 (s, 1H; imidazole H), 7.36-7.30 (m, 5H; aromatic H), 6.77 (s, 1H; imidazole H), 5.38 (s, br, 1H; amide NH), 5.09 (s, 2H; benzylic H), 3.53 (tt, $J_{ax\text{-}ax}$ = 10.6 Hz, $J_{ax\text{-}eq}$ = 6.0 Hz, 1H; C_3HOH), 3.23 (q, *J* = 6.2 Hz, 2H), 2.72 (m, 1H), 2.60 (m, 2H), 2.41 (m, 2H), 2.24 (dd, *J* = 11.6 Hz, *J* = 4.1 Hz, 1H), 2.10-0.85 (m, 25H), 0.89 (s, 3H; CH_3), 0.75 (t, *J* = 10.1 Hz, 1H), 0.45 (s, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 20°C, TMS): δ = 156.6 (C_q; C=O), 136.6 (C_q; aromatic), 134.5 (+; imidazole CH), 128.6, 128.1, 128.0 (+; aromatic CH), 70.7 (+; C_3HOH), 66.6 (–; benzylic CH_2), 58.1 (+), 54.5 (+), 54.4 (+), 48.9 (+), 47.0 (–), 45.5 (+), 44.6 (–), 43.4 (C_q), 39.8 (–), 38.9(–), 38.8(–), 38.2 (C_q), 36.4 (+), 32.4 (–), 32.0 (–), 30.9 (–), 29.5 (–), 26.3 (–), 24.7 (–), 13.9, 13.0 (+; CH_3); IR ($CHCl_3$): ν = 3464, 3308, 2928, 2856, 1716, 1516, 1236; MS (FAB): *m/z* (%): 549 (100) [M^+ +1], HRMS calcd for $C_{33}H_{48}N_4O_3$ 548.3726, found 548.3736.

11β–{1′,3′-Bis[(*tert*-butoxycarbonyl)amino]-2′-propyl}amino-17β-(4(5)-imidazolyl)-5α–androstan-3β–ol (14): 11β amine **7** (179 mg, 0.501 mmol) was dissolved in anhydrous MeOH (2.0 ml). Ketone **20** (288 mg, 1.00 mmol, 2.0 eq) was added, followed by $NaCNBH_3$ (38 mg, 0.60 mmol, 1.2 eq) and anhydrous acetic acid (30 µl, 0.50 mmol, 1.0 eq). The solution was stirred at room temperature for 3 d. The reaction mixture was poured into 2 % aqueous NaOH (50 ml) and extracted with $CHCl_3$ (3 × 25 ml). The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by flash chromatography ($CHCl_3$/MeOH gradient 15:1 → 2:1) to give 120 mg (38 %) of a white foam. 63 mg (35 %) of amine **7** could be recovered.

M.p. 131°C; $[\alpha]_D^{20}$ = + 25.3 (c = 1.0 in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 20°C, TMS): δ = 7.58 (s, 1H; imidazole H), 6.78 (s, 1H; imidazole H), 5.21 (s, br, 1H; amide NH), 5.11 (s, br, 1H; amide NH), 3.58 (tt, $^3J_{ax\text{-}ax}$ = 11.0 Hz, $^3J_{ax\text{-}eq}$ = 5.5 Hz, 1H; C_3HOH), 3.29 (m, br, 2H), 3.19 (m, br, 2H), 2.95 (m, br, 1H), 2.83 (m, br, 1H), 2.74 (s, br, 1H), 2.57 (t, J = 9.5 Hz, 1H), 2.36 (d, J = 13.4 Hz, 1H), 2.10-0.80 (m, 42H), 1.44 [s, 9H; $C(CH_3)_3$], 1.41 [s, 9H; $C(CH_3)_3$], 1.04 (s, 3H, CH_3), 0.71 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 20°C, TMS): δ = 156.8 (C_q; C=O), 134.7 (+; imidazole CH), 79.4, 79.3 [C_q; $C(CH_3)_3$], 70.9 (+; C_3HOH), 58.4 (+), 58.3 (+), 53.7 (+), 50.6 (+), 49.1 (+), 46.3 (+), 42.8 (C_q), 42.4 (–), 39.3 (–), 37.8 (–), 36.7 (–), 35.7 (C_q), 32.7 (–), 31.9 (+), 31.2 (–), 28.5, 28.4, 28.4 [+; $C(CH_3)_3$], 28.0 (–), 25.9 (–), 24.2 (–), 16.6, 15.3 (+; CH_3); IR ($CHCl_3$): ν = 3456, 2980, 2932, 2860, 1700, 1504, 1164; MS (70 eV, EI): *m/z* (%): 498 (55) [M^+ – CH_2NHBoc], 399 (100), 341 (98) [M^+ – side chain]; MS (FAB): *m/z* (%): 630 (100) [M^+ +1], 499 (11), 341 (29); HRMS calcd for $C_{29}H_{47}N_4O_3$ (M – CH_2NHBoc) 499.3648, found 499.3643.

11β–[3′-(Benzyloxycarbonly)amino-1′-propyl]amino-17β-(4(5)-imidazolyl)-5α–androstan-3β–ol (16): 11β amine **7** (120 mg, 0.336 mmol) was dissolved in anhydrous MeOH (3.0 ml). Aldehyde **23** (84 mg, 0.40 mmol, 1.2 eq) was added, followed by $NaCNBH_3$ (25 mg, 0.40 mmol, 1.2 eq) and anhydrous acetic acid (20 µl, 0.34 mmol, 1.0 eq). The solution was stirred at room temperature for 24 h. The reaction mixture was poured into 2 % aqueous NaOH (50 ml) and extracted with $CHCl_3$ (3 × 25 ml). The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo*. The crude product was purified by flash chromatography ($CHCl_3$/MeOH gradient 4:1 → 1:1) to give 140 mg (76 %) of a white solid.

M.p. 160°C; $[\alpha]_D^{20}$ = + 12.3 (c = 1.0 in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$, 20°C, TMS): δ = 7.49 (s, 1H; imidazole H), 7.35-7.30 (m, 5H; aromatic H), 6.73 (s, 1H; imidazole H), 5.58 (s, br, 1H; amide NH), 5.06 (s, 2H; benzylic H), 3.54 (tt, $^3J_{ax\text{-}ax}$ = 11.0 Hz, $^3J_{ax\text{-}eq}$ = 5.5 Hz, 1H; C_3HOH), 3.34 (s, br, 2H), 3.28-3.09 (m, 2H), 3.05 (s, br, 1H), 2.67 (dt, J = 11.0 Hz, J = 5.5 Hz, 1H), 2.56 (t, J = 9.7 Hz, 1H), 2.31 (dt, J = 11.2 Hz, J = 5.6 Hz, 1H), 2.15 (d, J = 13.1 Hz, 1H), 2.07-0.83 (m, 25H), 1.02 (s, 3H; CH_3), 0.70 (s, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 20°C, TMS): δ = 156.7 (C_q; C=O), 136.7 (C_q; aromatic), 135.8 (C_q; imidazole), 134.3 (+; imidazole CH), 128.5, 128.1, 128.0 (+; aromatic CH), 119.5 (+; imidazole CH), 70.8 (+; C_3HOH), 66.6 (–; benzylic CH_2), 58.6 (+), 58.2 (+), 55.3 (+), 49.5 (+), 46.4 (+), 45.9 (–), 43.0 (C_q), 40.0 (–), 39.4 (–), 37.6 (–), 36.9 (–), 35.7 (C_q), 32.7 (–), 32.3 (+), 31.0 (–), 30.5 (–), 28.1 (–), 26.2 (–), 24.3 (–), 16.5, 15.1 (+; CH_3); IR ($CHCl_3$): ν = 3464, 2928, 2860, 1716, 1516, 1264; MS (FAB): *m/z* (%): 549 (100) [M^+ +1]; HRMS calcd for $C_{33}H_{48}N_4O_3$ 548.3726 found 548.3731.

11α–[1′,3′-Bis(guanidino)-2′-propyl]amino-17β-(4(5)-imidazolyl)-5α–androstan-3β–ol dihydronitrate (9): Compound **8** (195 mg, 0.310 mmol) was stirred with 4 M HCl (2 ml) for 3 h. The resulting solution was lyophilized and the remaining hydrochloride was converted to the free amine by the following general procedure: Amberlite IRA-400 strongly basic ion-exchange resin (OH^- form, approximately 15 ml) was packed in a column (1 cm diameter) and washed to neutrality with water. The hydrochloride was dissolved in a minimum volume of

water and loaded on the top of the column. The column was eluted with MeOH / water 1:1. The basic effluent was collected and evaporated *in vacuo* to give 112 mg (84 %) of the free amine as a colorless oil. The free amines were used for guanidation without further purification.

The amine (95 mg, 0.221 mmol) was dissolved in anhydrous DMF (250 µl). 3,5-Dimethylpyrazole-1-carboxamidine nitrate (89 mg, 0.442 mmol, 2.0 eq) and diisopropylethylamine (75 µl, 0.442 mmol, 2.0 eq) were added. The solution was stirred at room temperature for 3 d. After dilution with EtOH (1 ml) the solution was poured into well stirred Et_2O (20 ml) to give a white precipitate, which was separated by centrifugation. The crude product was dissolved in water and purified by reverse-phase chromatography on Waters Sep-Pak® C18 Cartridges[14]. Elution with water was followed by evaporation of the product containing fractions *in vacuo*. The resulting colorless oil was dissolved in a minimal volume of EtOH and the solution poured into well stirred Et_2O (20 ml) to give a white precipitate, which was separated by centrifugation. Yield after drying *in vacuo*: 100 mg (71 %) of a white powder.

M.p. 168°C; $[\alpha]_D^{20} = -21.0$ (c = 0.50 in MeOH); ^{1}H NMR (400 MHz, D_2O, 20°C): δ = 7.79 (s, 1H; imidazole H), 6.77 (s, 1H; imidazole H), 3.42 (s, br, 1H; C_3*H*OH), 3.20-2.98 (m, 5H), 2.65 (td, J = 9.5 Hz, J = 4.0 Hz, 1H), 2 53 (t, J = 9.8 Hz, 1H), 2.19 (d, J = 12.1 Hz, 1H), 2.00-0.70 (m, 21H), 0.74 (s, 3H, CH_3), 0.64 (t, J = 9,7 Hz, 1H), 0.32 (s, 3H, CH_3); ^{13}C NMR (100 MHz, D_2O, 20°C, dioxane): δ = 157.7, 157.5 (C_q; guanidinium), 136.2 (C_q; imidazole), 135.1, 117.8 (+; imidazole CH), 70.6 (+; C_3HOH), 58.8 (+), 54.6 (+), 52.8 (+), 52.5 (+), 48.7 (+), 46.2 (–), 45.5 (+), 44.2 (–), 43.5 (C_q), 42.8 (–), 39.2 (–), 38.2 (–), 38.2 (C_q), 36.7 (+), 32.8 (–), 31.0 (–), 29.8 (–), 26.7 (–), 25.2 (–), 13.8, 12.7 (+; CH_3); IR (KBr): ν = 3348, 3184, 2924, 2856, 1668, 1384, 1036; MS (FAB): *m/z* (%): 514 (100) [M^+ +1].

Preparation of guanidinium tetraphenylborates: Because nitrates of guanidinium compounds gave unsatisfactory combustion analysis, tetraphenylborates were prepared: The guanidinium nitrate was dissolved in water and then combined with sodiumtetraphenylborate also dissolved in water (1.1 eq. for mono-guanidinium, 2.2 eq. for bis-guanidinium compounds). A white precipitate formed immediately and the suspension was stirred overnight. The white solid was filtered off, washed with water and dried *in vacuo*.

$C_{75}H_{91}N_9O_2B_2$ (1172.2, ditetraphenylborate hydrate): calcd C 76.85, H 7.82, N 10.75; found C 77.12, H 7.78, N 10.01.

11α–[1′,5′-Bis(guanidino)-3′-pentyl]amino-17β-(4(5)-imidazolyl)-5α–androstan-3β–ol dihydronitrate (11): Compound **10** (167 mg, 0.230 mmol) was dissolved in MeOH (5 ml). Palladium on charcoal (25 mg of 10 % (w/w), 0.1 eq) and 12 M HCl (100 µl, 1.2 mmol) were added. The resulting suspension was stirred under an atmosphere of hydrogen for 48 h. The catalyst was removed by centrifugation, washed thoroughly with MeOH and the supernatant liquid was evaporated *in vacuo*. The remaining hydrochloride was converted to the free amine as described before. Yield: 93 mg (89 %) of the free amine as a colorless oil.

The amine (93 mg, 0.204 mmol) was dissolved in anhydrous DMF (200 µl). 3,5-Dimethylpyrazole-1-carboxamidine nitrate (82 mg, 0.408 mmol, 2.0 eq) and diisopropylethylamine (70 µl, 0.408 mmol, 2.0 eq) were

added. The solution was stirred at room temperature for 3 d. Product isolation was performed as described before. Yield: 100 mg (74 %) of a white powder.

M.p. 170°C; $[\alpha]_D^{20} = -15.6$ (ditetraphenylborate) (c = 1.0 in MeOH); ^{1}H NMR (400 MHz, D_2O, 20°C): δ = 7.73 (s, 1H; imidazole H), 6.76 (s, 1H; imidazole H), 3.43 (s, br, 1H; C_3HOH), 3.10 (m, 3H), 3.00 (m, 1H), 2.75 (s, br, 2H), 2.53 (t, *J* = 9.5 Hz, 1H), 1.95-0.70 (m, 27H), 0.78 (s, 3H, CH_3), 0.32 (s, 3H, CH_3); ^{13}C NMR (100 MHz, D_2O, 20°C, dioxane): δ = 157.2, 157.1 (C_q; guanidinium), 136.2 (C_q; imidazole), 135.1, 117.9 (+; imidazole CH), 70.4 (+; C_3HOH), 57.9 (+), 54.1 (+), 52.0 (+), 50.2 (+), 48.8 (+), 45.5 (–), 45.3 (+), 43.3 (C_q), 39.1 (–), 38.5 (–), 38.5 (C_q), 38.3 (–), 38.0 (–), 36.7 (+), 32.6 (–), 32.5 (–), 31.4 (–), 30.8 (–), 29.7 (–), 26.8 (–), 25.1 (–), 13.6, 12.8 (+; CH_3); IR (KBr): ν = 3348, 3180, 2924, 2856, 1664, 1384, 1036; MS (FAB): *m/z* (%): 542 (100) [M^+ +1]; $C_{77}H_{95}N_9O_2B_2$ (1200.3, ditetraphenylborate hydrate): calcd C 77.05, H 7.98, N 10.50; found C 77.31, H 7.76, N 10.26.

11α-(3′-Guanidino-1′-propyl)amino-17β-(4(5)-imidazolyl)-5α-androstan-3β-ol hydronitrate (13): Compound **12** (89 mg, 0.162 mmol) was dissolved in MeOH (2 ml). Palladium on charcoal (17 mg of 10 % (w/w), 0.1 eq) and 12 M HCl (50 µl, 0.6 mmol) were added. The resulting suspension was stirred under an atmosphere of hydrogen for 48 h. The catalyst was removed by centrifugation, washed thoroughly with MeOH and the supernatant liquid was evaporated *in vacuo*. The remaining hydrochloride was converted to the free amine as described before. Yield: 65 mg (97 %) of the free amine as a colorless oil.

The free amine (65 mg, 0.157 mmol) was dissolved in anhydrous DMF (150 µl). 3,5-Dimethylpyrazole-1-carboxamidine nitrate (32 mg, 0.157 mmol, 1.0 eq) and diisopropylethylamine (27 µl, 0.157 mmol, 1.0 eq) were added. The solution was stirred at room temperature for 48 h. Product isolation was performed as desribed before (elution gradient for reverse-phase chromatography: water → water / MeOH 1:1). Yield: 61 mg (75 %) of a white powder.

M.p. 170°C; $[\alpha]_D^{20} = -3.0$ (tetraphenylborate) (c = 1.0 in MeOH); ^{1}H NMR (400 MHz, D_2O, 20°C): δ = 7.51 (s, 1H; imidazole H), 6.62 (s, 1H; imidazole H), 3.43 (s, br, 1H; C_3HOH), 3.03 (t, *J* = 6.4 Hz, 2H), 2.76 (m, 1H), 2.65 (m, 1H), 2.50 (m, 2H), 1.93 (m, 1H), 1.87-0.65 (m, 24H), 0.73 (s, 3H, CH_3), 0.25 (s, 3H, CH_3); ^{13}C NMR (100 MHz, D_2O, 20°C, dioxane): δ = 157.9 (C_q; guanidinium), 136.5 (C_q; imidazole), 70.8 (+; C_3HOH), 56.7 (+), 55.4 (+), 54.3 (+), 49.9 (+), 45.7 (+), 44.8 (–), 43.8 (C_q), 43.4 (–), 40.0 (–), 39.0 (C_q), 38.7 (–), 37.2 (+), 33.1 (–), 31.4 (–), 30.4 (–), 28.4 (–), 27.2 (–) 25.7 (–), 14.4, 13.4 (+; CH_3); IR (KBr): ν = 3336, 3272, 3188, 2924, 2856, 1664, 1468, 1384, 1104, 1036; MS (FAB): *m/z* (%): 457 (100) [M^+ +1]; $C_{50}H_{65}N_6O_1B_1$ (776.9, tetraphenylborate): calcd C 77.30, H 8.43, N 10.82; found C 77.21, H 8.30, N 10.41.

11β-[1′,3′-Bis(guanidino)-2′-propyl]amino-17β-(4(5)-imidazolyl)-5α-androstan-3β-ol dihydronitrate (15): Compound **14** (100 mg, 0.159 mmol) was stirred with 4 M HCl (2 ml) for 3 h. The solution was lyophilized and the remaining hydrochloride was converted to the free amine as described before. Yield: 61 mg (89 %) of the free amine as a colorless oil.

The amine (61 mg, 0.142 mmol) was dissolved in anhydrous DMF (150 µl). 3,5-Dimethylpyrazole-1-carboxamidine nitrate (57 mg, 0.284 mmol, 2.0 eq) and diisopropylethylamine (49 µl, 0.284 mmol, 2.0 eq) were added. The solution was stirred at room temperature for 3 d. Product isolation was performed as desribed before (elution gradient for reverse-phase chromatography: water → water / MeOH 2:1). Yield: 60 mg (66 %) of a white powder.

M.p. 162°C; $[\alpha]_D^{20}$ = +13,5 (ditetraphenylborate) (c = 1.0 in MeOH); ^{1}H NMR (400 MHz, D_2O, 20°C): δ = 7.87 (s, 1H; imidazole H), 6.82 (s, 1H; imidazole H), 3.38 (s, br, 1H; C_3*H*OH), 3.27-2.95 (m, 6H), 2.44 (t, *J* = 9.5 Hz, 1H), 1.90 (d, *J* = 13.4 Hz, 1H), 1.85-0.73 (m, 22H), 0.89 (s, 3H, CH_3), 0.51 (s, 3H, CH_3); ^{13}C NMR (100 MHz, D_2O, 20°C, dioxane): δ = 157.5, 157.3 (C_q; guanidinium), 135.7 (C_q; imidazole), 134.8, 117.8 (+; imidazole CH), 70.9 (+; C_3HOH), 58.5 (+), 52.0 (+), 51.4 (+), 49.2 (+), 46.5 (+), 44.8 (–), 43.7 (–), 43.2 (C_q), 41.1 (–), 39.5 (–), 37.0 (–), 36.6 (–), 35.7 (C_q), 33.1 (–), 32.3 (+), 30.2 (–), 28.2 (–), 26.0 (–), 24.6 (–), 15.7, 14.9 (+; CH_3); IR (KBr): ν = 3348, 3180, 2924, 2856, 1668, 1384, 1040; MS (FAB): *m/z* (%): 514 (63) [M$^+$ +1], 329 (37), 176 (100); $C_{75}H_{91}N_9O_2B_2$ (1172.2, ditetraphenylborate hydrate): calcd C 76.85, H 7.82, N 10.75; found C 76.88, H 7.66, N 10.00.

11β-(3′-Guanidino-1′-propyl)amino-17β-(4(5)-imidazolyl)-5α-androstan-3β-ol hydronitrate (17): Compound **16** (103 mg, 0.188 mmol) was dissolved in MeOH (2 ml). Palladium on charcoal (20 mg of 10 % (w/w), 0.1 eq) and 12 M HCl (50 µl, 0.6 mmol) were added. The resulting suspension was stirred under an atmosphere of hydrogen for 48 h. The catalyst was removed by centrifugation, washed thoroughly with MeOH and the supernatant liquid was evaporated *in vacuo*. The remaining hydrochloride was converted to the free amine as described before. Yield: 73 mg (94 %) of the free amine as a colorless oil.

The amine (73 mg, 0.176 mmol) was dissolved in anhydrous DMF (300 µl) with warming. 3,5-Dimethylpyrazole-1-carboxamidine nitrate (36 mg, 0.176 mmol, 1.0 eq) and diisopropylethylamine (30 µl, 0.176 mmol, 1.0 eq) were added. The solution was stirred at room temperature for 48 h. Product isolation was performed as desribed before (elution gradient for reverse-phase chromatography: water → water / MeOH 1:1). Yield: 75 mg (82 %) of a white powder.

M.p. 218°C; $[\alpha]_D^{20}$ = + 6.2 (tetraphenylborate) (c = 1.0 in MeOH); ^{1}H NMR (400 MHz, [D_4]MeOH, 20°C, TMS): δ = 7.69 (s, 1H; imidazole H), 6.84 (s, 1H; imidazole H), 3.52 (tt, $^3J_{ax\text{-}ax}$ = 11.0 Hz, $^3J_{ax\text{-}eq}$ = 5.0 Hz, 1H; C_3*H*OH), 3.20 (m, 3H), 2.74 (dt, *J* = 11.6 Hz, *J* = 6.4 Hz, 1H), 2.61 (t, ^3J = 9.6 Hz, 1H; C_{17}H), 2.38 (dt, *J* = 11.6 Hz, *J* = 6.9 Hz, 1H), 2.19 (d, *J* = 12.3 Hz, 1H), 2.05- 0.90 (m, 24H), 1.10 (s, 3H, CH_3), 0.74 (s, 3H, CH_3); ^{13}C NMR (50 MHz, D_2O, 20°C, dioxane, APT): δ = 157.2 (+; C_q guanidinium), 133.6 (+; C_q imidazole), 70.4 (–; C_3HOH), 57.4 (–), 56.3 (–), 55.3 (–), 47.1 (–), 46.0 (–), 44.4 (+), 42.1 (C_q), 38.6 (+), 36.8 (+), 36.6 (+), 36.0 (+), 35.0 (C_q), 32.0 (+), 31.7 (–), 29.9 (+), 27.2 (+), 26.1 (+), 24.6 (+), 23.7 (+), 14.7, 13.8 (–; CH_3); IR (KBr): ν = 3348, 3272, 3188, 2924, 2856, 1664, 1384, 1040; MS (FAB): *m/z* (%): 457 (100) [M$^+$ +1]; $C_{50}H_{65}N_6O_1B_1$ (776.9, tetraphenylborate): calcd C 77.30, H 8.43, N 10.82; found C 77.39, H 8.38, N 10.41.

Acknowledgement: Thorsten Oost gratefully acknowledges a stipend by the Graduiertenkolleg "Chemische und Technische Grundlagen der Naturstofftransformation".

REFERENCES AND NOTES

1. (a) F. A. Cotton, E. E. Hazen, Jr., M. J. Legg, *Proc. Natal. Acad. Sci. USA* **1979**, *76*, 2551-2555; (b) E. H. Serpersu, D. Shortle, A. S. Mildvan, *Biochemistry* **1987**, *26*, 1289-1399; (c) P. J. Loll, E. E. Lattman, *Proteins* **1989**, *5*, 183; (d) J. Aqvist, A. Warshel, *Biochemistry* **1989**, *28*, 4680-4689; (e) E. H. Serpersu, D. W. Hilber,J. A. Gerlt, A. S. Mildvan, *Biochemistry* **1989**, *28*, 1539-1548; (f) D. J. Weber, E. H. Serpersu, D. Shoetle, A. S. Mildvan, *Biochemistry* **1990**, *29*, 8632-8642; (g) D. J. Weber, A. K. Meeker, A. S. Mildvan, *Biochemistry* **1991**, *30*, 6103-6114; (h) J. K. Judice, T. R. Gamble, E. C. Murphy, A. M. de Vos, P. G. Schultz, *Science* **1993**, *261*,1578-1581.
2. (a) D. W. Hibler,J. N. Stolowich, M. A. Reynolds, J. A. Gerlt, J. A. Wilde, P. H. Bolton, *Biochemistry* **1987**, *26*, 6278-6286; (b) E. H. Serpersu, D. Shortle, A. S. Mildvan, *Biochemistry* **1986**, *25*, 68-77; (c) E. H. Serpersu, J. McCracken, J. Peisach, A. S. Mildvan, *Biochemistry* **1988**, *27*, 8034-8044.
3. (a) B. Barbier, A: Brack, *J. Am. Chem. Soc.* **1988**, *110*, 6880-6882; (b) B. Barbier, A: Brack, *J. Am. Chem. Soc.* **1992**, *114*, 3511-3515.
4. (a) K. Ariga, E. V. Anslyn, *J. Org. Chem.* **1992**, *57*, 417-419; (b) J. Smith, K. Ariga, E. V. Anslyn, *J. Am. Chem. Soc.* **1993**, *115*, 362-364; (c) D. M. Kneeland, K. Ariga, V. M. Lynch, C.-Y. Huang, E. V. Anslyn, *J. Am. Chem. Soc.* **1993**, *115*, 10042-10055.
5. (a) M. W. Göbel, J. W. Bats, G. Dürner, *Angew. Chem.* **1992**, *104*, 217-218; M. W. Göbel, J. W. Bats, G. Dürner, *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 207-209; (b) R. Groß, G. Dürner, M. W. Göbel, *Liebigs Ann. Chem.* **1994**, 49-58; (c) R. Groß, J. W. Bats, M. W. Göbel, *Liebigs Ann. Chem.* **1994**, 205-210; (d) G. Müller, G. Dürner, J. W. Bats, M. W. Göbel, Liebigs Ann. Chem. 1994, 1075-1092.
6. (a) R. P. Dixon, S. J. Geib, A. D. Hamilton, *J. Am. Chem. Soc.* **1992**, *114*, 365-366; (b) V. Jubian, R. P. Dixon, A. D. Hamilton, *J. Am. Chem. Soc.* **1992**, *114*, 1120-1121; (c) V. Jubian, A. Veronese, R. P. Dixon, A. D: Hamilton, *Angew. Chem.* **1995**, *107*, 1343-1345; V. Jubian, A. Veronese, R. P. Dixon , A. D: Hamilton, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1237-1239. 9999
7. (a) F. P. Schmidtchen, *Tetrahedron Lett.* **1989**, *30*, 4493-4496; (b) P. Schiessl, F. P. Schmidtchen, *J. Org. Chem.* **1994**, *59*, 509-511.
8. (a) A. Frankel, C. Pabo, PCT Patent Appl. (1991) WO 9109958 (Appl. 454450, Filed 21 Dec. 1989). (b) J. G. Barsoum, S. E. Fawell, R. B. Pepinsky, PCT Patent Appl. (1994) WO 9404686 (Appl. US 07/934375, Filed 21 Aug. 1992).
9. (a) W.A. Haseltine; *FASEB J.* **1991**,2349-2360; (b) B.J. Calnan, B. Tidor, S. Biancalana, D. Hudson, A.D. Frankel, *Science* **1991**, *252*, 1167; (c) J.D. Puglisi, R. Tan, B.J. Calnan, A.D. Frankel, J.R. Williamson; *Science* **1992** *257*, 76; (d) E.P. Loret, P. Georgel, W.C. Johnson, Jr., P.S. Ho; *Proc. Natl. Acad. Sci. USA* **1992,** *89*, 9734-9738.
10. D. M. Brown, D. A. Usher, *J. Chem. Soc.* **1965**, 6558-6564.
11. J. P. Guthrie, *Can. J. Chem.* **1972**, *50*, 3993-3997.
12. (a) R. Weidenhagen, R. Herrmann, *Ber. Dtsch. Chem. Ges.* **1935**, *68*, 1953-1965; (b) R. Weidenhagen, R. Herrmann, H. Wegner, *Ber. Dtsch. Chem. Ges.* **1937**, *70*, 570-583.
13. G. H. Phillips, G. B. Ewan, (Glaxo, Glaxo Laboratories Ltd), DT 2715078 A,. **1997**, [*Chem. Abstr.* **1978**, *88*, 38078].
14. *Waters*, Sep-Pak® C18 cartridges.
15. (a) H. Brunner; H. Obermeier, R.-M. Szeimies, *Chem. Ber.* **1995**, *128*, 173-182; (b) K. Ramalingam, N. Raju, P. Nanjappan, D. P. Nowotnik, *Tetrahedron* **1995**, *51*, 2875-2894.
16. L. Macholán, *Collect. Czech. Chem. Commun.* **1974**, *39*, 653-661.
17. (a) A. Guggisberg, P. v. d. Broeck, M. Hesse, H. Schmid, *Helv. Chim. Acta* **1974**, *57*, 434-440; (b) J. E. Baldwin, R. M. Adlington, J. S. Bryans, A. O. Bringhen, J. B. Coates, N. P. Crouch, M. D. Lloyd, C. J. Schofield, S. W. Elson, K. H. Baggaley, R. Cassels, N. Nicholson, *Tetrahedron* **1991**, *47*, 4089-4100.
18. We were not able to obtain sufficient amounts of the 11β amino compound with the 1,5 spacer.

(*Received in Germany* 14 *March* 1997; *accepted* 9 *May* 1997)