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ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 9960–9969

Steroid template associated peptides: design, synthesis and 2D NMR characterization of a novel protected 18-Phe,19-Gly-containing steroidal compound

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Received 8 May 2007; revised 3 July 2007; accepted 19 July 2007
Available online 26 July 2007

In memory of Professor Ljubinka Lorenc, deceased on February 25, 2007

Abstract—We report herein the synthesis of a novel modified steroid with two rigidly positioned amino acids in *C*- and *N*-protected forms (Gly–O^{t}Bu and *N*-Fmoc–L-Phe) at the angular positions (C-18 methylamino group and C-19 carboxylic function) of the steroid nucleus via amide bonds, starting from 18-cyanopregnenolone acetate over 10 steps. In an attempt to gain more insight into the structural and conformational features of this novel 18-Phe,19-Gly-containing steroidal compound, we describe the detailed 2D NMR spectral analysis. Despite the large size and the conformational flexibility of the amino acid units in this molecule, conformational analysis by NOESY connectivities showed the existence of mainly one conformation (~95%) in $CDCl_3$ solution with approximately parallel orientation of the phenylalanine and glycine moieties.

1. Introduction

One of the most fascinating challenges in modern organic chemistry is the design of strategies capable of providing structurally diverse and complex molecules, which are useful for the study of important biological processes. Peptidomimetics have emerged as an active field at the interface of bioorganic, organic, and medicinal chemistry.[1] This interest derives from the expectation that such molecules will have both better biostability and oral bioavailability than their peptide counterparts. The introduction of the 'TASP' concept (Template-Assembled Synthetic Protein)[2] has provided a novel, broadly applicable method for the construction of protein tertiary structures.[3,4] A wide spectrum of molecular frameworks ranging from simple glucose[5] to more complex polycyclic steroids have been used as scaffoldings to fix the conformation of otherwise flexible, linear peptides in a well-defined secondary structure. The steroid nucleus is one of the largest rigid units with multiple chiral centers most of which present two options for substitution (axial and equatorial). The biological importance of that structural entity is well documented.[6] A combination of the unique structural and functional properties has already led to a rich application of steroids in peptide[7,8] as well as taxoid mimicry,[9] in the preparation of receptors for various molecules,[10–14] and in the development of the antimicrobial agents.[15–19] These properties include: (i) a wide range of functionalized steroids are naturally and commercially available, and further functional group transformations have been worked out over the past decades; (ii) with their rigid tetracyclic frames, steroidal building blocks provide a high degree of preorganization to a recognition site; (iii) many steroids are drugs with excellent oral bioavailability; (iv) many steroids, in particular the natural and modified-bile acids, can be viewed as highly functionalized amphiphilic surfaces with a lipophilic β-face and a more polar α-face bearing functional groups that are converging in clefts or macrocyclic receptor frames for interaction with a bound substrate; (v) the functional groups on the α-face of A,B-*cis*- and A,B-*trans*-steroidal

Keywords: STAP concept; 18-Phe,19-Gly-containing steroidal compound; 2D NMR analysis; 18-Cyanopregnenolone acetate.
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0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.07.056

cores can be transformed into more expanded recognition elements such as oligopeptides in combinatorial receptor libraries.[10,11,20,21]

The conjugation of steroids to other chemically or biologically relevant molecules represents a valuable strategy to generate new properties in the resulting molecular hybrid. We decided to apply the TASP concept to the rigid framework of steroids as templates. The idea was called STAP (Steroid Template Associated Peptides).[†] One of the possibilities to generate STAP-molecules, which promise versatile applications, is represented in Figure 1.

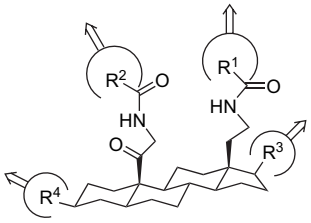


Figure 1.

In this case, one could attach different side-chains, or even better different amino acid units or peptidic sequences to the two angular methyl groups C(18) and C(19). With an appropriate substitution in position C(3) and/or C(17) additional amino acids or peptidic chains could be introduced or, the lipophility or solubility of the molecules could be modified. In addition, one could introduce four amino acids or peptidic residues, which could be connected or cyclized with the loop formation in a desired fashion as shown in the next schematic representation (Fig. 2).

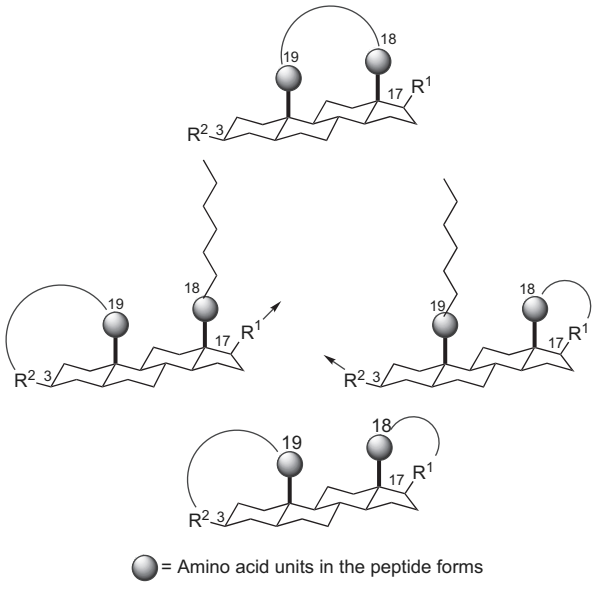


Figure 2.

[†] The concept has been in part discussed (Jaroslav Kalvoda) on September 10th 1999 at the 18th Conference on Isoprenoids, organized by the Academy of Sciences of the Czech Republic in Prachatice.

This article focuses on the introduction of α-amino acids (Phe and Gly) at the functionalized angular positions (C-18 and C-19) of the pregnane-3,20-diol diacetate skeleton. In the present work, in an attempt to obtain more insight into the structural and conformational features of this novel 18-Phe,19-Gly-containing steroidal compound, we describe the detailed 2D NMR (DQF-COSY, TOCSY, NOESY, HSQC, HMBC, INEPT edited HSQC) spectral analysis.

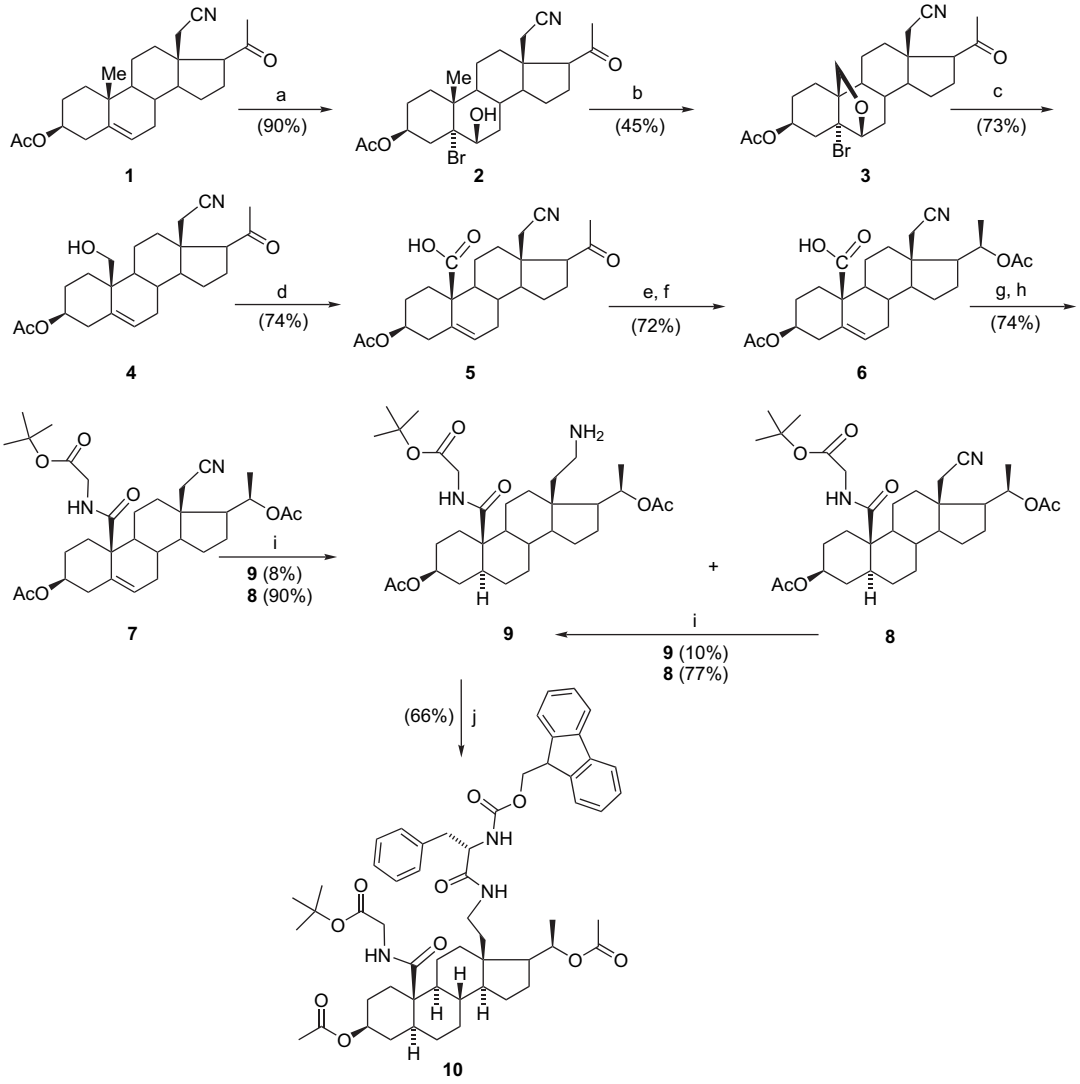
2. Results and discussion

We provide herein the first illustration of this concept, and report on the construction of a novel modified-steroidal compound with two rigidly positioned amino acids in *C*- and *N*-protected forms (Gly–O^{t}Bu and *N*-Fmoc–L-Phe) at the functionalized angular positions (C-18 methylamino group and C-19 carboxylic function) of the steroid nucleus via amide bonds. In this connection we describe the synthesis of the protected 18-Phe,19-Gly-containing steroid **10** starting from 18-cyano-pregnenolone acetate **1** that was obtained from commercially available pregnenolone acetate in ~55% yield over two steps (the preparation of 20-cyano-20-hydroxy derivative[22] and the oxidative cyanohydrin–cyanoketone rearrangement[23]).

Scheme 1 summarizes the synthesis of the key steroidal building block, 18-cyano-3β,20β-diacetoxypregn-5-en-19-oic acid (**6**) in 6 steps, which was finally transformed into the diamide **10** in 4 steps.

Addition of HOBr to the 5,6-double bond[24] of 18-cyano-pregnenolone acetate (**1**) gave the corresponding bromohydrin **2** in 90% yield. Compound **2** was identified as the desired 5α-bromo-6β-hydroxy derivative on the basis of its mass spectrum [*m*/*z* 480/482 (M^++1), 400 ((M^++1)−80/82), 322 ((M^++1)−80/82−18−60)], IR spectrum (presence of a hydroxyl band at 3481 cm^{-1}), and NMR data. The ^{1}H NMR spectrum of **2** showed a broad singlet at 4.20 ppm arising from proton adjacent to OH group (H-C(6)), while the down-field shift of the CH_3(19) group at 1.33 ppm suggested the 6β-configuration of the OH group. In the ^{13}C NMR spectrum, C(5) and C(6) show signals at 86.2 (s) and 75.0 ppm (d), respectively.

Application of the LTA-version of the hypoiodite reaction (HR)[25a,b] to the bromohydrin **2** produced the cyclic bromo ether **3** in 45% yield, which was subsequently reduced by zinc in acetic acid solution[24,25c] to the 19-hydroxy-18-cyano derivative **4** in 73% yield. In the IR spectrum of **3**, the 6β-hydroxyl band is missing, instead, a new absorption for an ether bond at 1034 cm^{-1} is observed. The ^{1}H NMR spectrum of epoxy-derivative **3** showed, instead of a s of CH_3(19), 2d at 3.69 and 3.97 ppm (*J* 8.8 Hz) of the CH_2(19) group. The structure confirmed by the ^{13}C NMR data (1s at 74.0 ppm, 1d at 81.7 ppm, and 1t at 67.3 ppm for C(5), C(6), and C(19), respectively). In the IR spectrum of **4**, the presence of a 19-hydroxyl group is evident from the absorption at 3480 cm^{-1}. In the ^{1}H NMR spectrum, the d of H-C(6) at 4.09 ppm in **3** is replaced by br s at 5.75 ppm, indicating the presence of the Δ^5-double bond. In addition, the ^{1}H spectrum showed an AB pattern at 3.58 and 3.92 ppm (*J* 11.4 Hz) attributable to the CH_2(19). The ^{13}C NMR spectrum of this



Scheme 1. Reagents: (a) NBA, dioxane/H_2O, $HClO_4$; (b) LTA/I_2, hν/Δ, CH_2Cl_2; (c) Zn/AcOH/H_2O; (d) CrO_3/H_2SO_4, acetone; (e) $NaBH_4$, MeOH; (f) Ac_2O/Py; (g) 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine, CH_2Cl_2; (h) Gly–O^{t}Bu HCl, Et_3N, CH_2Cl_2; (i) H_2/PtO_2/^{i}PrOH/$CHCl_3$/HCl; (j) *N*-Fmoc–L-Phe–OTcp, DMF, DIEA, HOBT, NMP.

compound contained a t at 62.6 ppm (C(19)), a s at 134.9 ppm (C(5)), and a d at 126.7 ppm (C(6)).

Oxidation of the primary 19-hydroxyl group of **4** with Jones' reagent in cold acetone solution gave the required carboxylic acid **5** in 74% yield. The absence of the 2d at 3.58 and 3.92 ppm for $H_2C(19)$ (in **4**) and the appearance of the br s at ~9 ppm in the ^1H NMR spectrum of **5** and very broad OH stretching absorption at 2500–3300 cm^{-1} in the IR spectrum indicated that this compound contains a carboxylic function at C(10). Instead of the ^{13}C signal for the $CH_2(19)$ group (t at 62.6 ppm in **4**) the ^{13}C NMR spectrum of **5** showed a s at 177.9 ppm (CO_2H).

The stereoselective reduction[26] of the C(20)-oxo group of compound **5** with sodium borohydride in methanol solution afforded the corresponding 3β,20β-diol, which was subsequently acetylated by the usual acetic anhydride/pyridine method to yield the 3β,20β-diacetate **6** in 72% yield. 3,20-Diacetoxy derivative **6** gives a new ^1H NMR methyl signal at 2.06 ppm (s) and a m at 4.70 ppm (H-C(20)). In the ^{13}C NMR spectrum a s at 209.0 ppm for the C(20)=O group in **5** replaced by a d at 72.6 ppm for H–C(20) and a s at 170.6 ppm for the carbonyl C atom of the new AcO group.

The next step in the synthesis included the following two-step process, involving the conversion of the acid **6** into the acyl chloride followed by the coupling reaction with glycine *tert*-butyl ester hydrochloride. The carboxylic group at C-10 in **6** was converted, by 1-chloro-*N*,*N*,2-trimethyl-1-propenylamine (Ghosez chlorinating agent),[27] into the corresponding acyl chloride, which was subsequently coupled with Gly–O^{t}Bu·HCl in CH_2Cl_2 solution in the presence of triethylamine[28] under Ar to give the 19-glycine derivative **7** in 74% yield. The structure of compound **7** was proved by the appearance of an AB pattern at 3.54 and 4.31 ppm (attributable to the CH_2(Gly), *J* 17.8 Hz), one d at 6.29 ppm (CONH, *J* 7.6 Hz), and a s at 1.47 ppm (^{t}Bu), characteristic for the Gly–O^{t}Bu moiety. The presence of the ^{t}Bu group is also evidenced by a s at 86 ppm and a q at 28.6 ppm in the ^{13}C NMR spectrum. The ^{13}C NMR spectrum of **7** also showed

four s at 170.0, 171.1, and 172.1 ppm characteristic of the C(19)=O, Gly(C=O), and two AcO groups.

The key step of this synthesis was a reduction of the 18-cyano group. After trying out several reaction conditions for hydrogenation, a low-pressure catalytic hydrogenation of the 18-cyano compound **7** in a Parr app. under 60 psi hydrogen pressure in the presence of PtO_2 activated with HCl[29] at room temperature for 20 h in the ^{i}PrOH/$CHCl_3$ solution resulted in the formation of two products, the major product 5α-saturated analogue **8** (90%) formed via stereoselective reduction of the olefinic double bond (Δ^5) and the desired 5α-saturated 18-aminomethyl compound **9** in a very low yield (8%), in which the both functional groups (olefinic and cyano groups) had undergone reduction. Attempts to increase the yield of the amine **9** using $NaBH_4/CoCl_2$ in CH_2Cl_2/CH_3OH, $NaBH_4/NiCl_2$ in EtOH, $PtO_2/CHCl_3$/60 psi, $PtO_2/^{i}$PrOH/$CHCl_3$/60 psi, and PtO_2/AcOH/90 atm, all proved less successful (the yields of **9** were less than 3%). In order to obtain the amino derivative **9** in larger amount (49% overall yield) the above procedure was applied to the 5α-saturated 18-cyano derivative **8** subsequently several times. Low yields obtained in the hydrogenation step could be attributed to the steric hindrance of the placed cyano group located on the β-face in the C-18 position. The NMR spectra of **8** proved the proposed structure, lacking the signal of the olefinic proton (H-C(6)) and of the two olefinic carbons (C(5) and C(6)). Comparison of the NMR data of **8** with those of **7**, indicate that **8** was hydrogenated from the α side. The ^{1}H NMR spectrum of **8** showed the following representative signals: two dd at 3.78 and 4.02 ppm attributable to CH_2(Gly) (*J* 17.8 Hz), one amide proton d at 6.18 ppm (*J* 5.3 Hz), and a s at 1.48 ppm (^{t}Bu). The molecular formula of derivative **9** was determined as $C_{32}H_{52}N_2O_7$ from its HRFABMS spectrum where the $[M+H]^+$ ion was observed at *m/z* 577.3873 ($C_{32}H_{53}N_2O_7$ requires 577.3853, Δ=+2.1 mmu). In the IR spectrum the typical CN band at 2245 cm^{-1} is replaced by absorption at 3429 cm^{-1} (broad) indicating the presence of the amino group. It was corroborated by the ^{1}H and ^{13}C NMR spectra, which displayed two br t at 2.73 and 2.93 ppm and a t at 35.4 ppm for the methylene $CH_2(18')$ group, adjacent to the amino group.

In addition, the IR and NMR spectra of the 18-cyano compounds **2–8** confirmed the absence of the CH_3(18) angular methyl group and, instead, the appearance of the 18-cyanomethyl CH_2CN group. In the IR spectra of these compounds, the corresponding absorption for CN group was found at ν_{max} 2238–2251 cm^{-1}. The ^{13}C NMR CH_2–CN signals appear in the expected positions, a t at 15.5–16.6 ppm (CH_2(18)) and a s at 117.9–119.3 ppm (CN) for all compounds **2–8**. ^{13}C Carbonyl C(20)=O (for **2–5**) and C(20)H–OAc (for **6–9**), as well as ^{1}H C(20)H–OAc (for **6–9**) give s, d, and m in the expected intervals, between 208.5–209.0, 72.6–74.2, and 4.5–5.2 ppm, respectively. For additional spectral data confirming these new structures see Table 1 and Section 4.

Coupling of the 18-aminomethyl derivative **9** with *N*-Fmoc–L-Phe was carried out using the more reactive ester activation

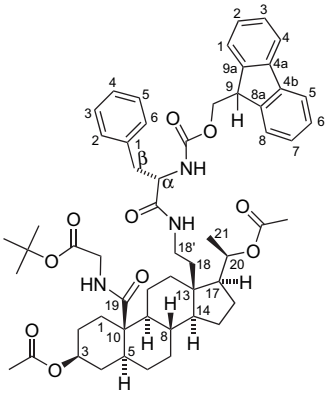
Table 1. Selected ^{1}H and ^{13}C NMR chemical shifts of compounds **2–9** in $CDCl_3$

Assignment	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
3	5.46 m	5.20 m	4.64 m	4.64 m	4.70 m[a]	4.5–4.82 m[b]	4.72 m[c]	4.73 m
	71.9 d	69.5 d	73.2 d	72.7 d	72.7 d	73.2 d	72.4 d	72.8 d
5	—	—	—	—	—	—	—[d]	—[d]
	86.2 s	74.0 s	134.9 s	133.6 s	133.6 s	136.0 s	45.2 d	45.5 d
6	4.20 br s	4.09 d	5.75 br s	5.72 d	5.71 d	5.82 br d		2.41 br d(Hα)
	75.0 d	81.7 d	126.7 d	125.3 d	125.2 d	127.7 d	28.8 t	28.9 t
17	2.75 t	2.69 t	2.73 t	2.4–2.80 m				
	61.8 d	61.6 d	61.6 d	61.7 d	54.3 d	55.1 d	54.4 d	55.6 d
18	—[d]	2.43 d[e]	2.20 d, 2.56 d	—[d]	2.35 d(1H)[e]	2.82 d, 2.17 d	2.58 d(1H)[e]	
	16.5 t	16.6 t	16.2 t	16.3 t	15.5 t	15.8 t	15.5 t	
CN	118.0 s	117.9 s	118.3 s	117.9 s	118.4 s	119.3 s	118.7 s	
CH_2NH_2								2.73 br t, 2.93 br t
								35.4 t
19	1.33 s	3.69 d, 3.97 d	3.58 d, 3.92 d	—	—	—	—	—
	17.8 q	67.3 t	62.6 t	177.9 s	178.2 s	172.1 s	173.0 s	173.6 s
20	—	—	—	—	4.70 m[a]	4.5-4.82 m[b]	4.72 m[c]	5.25 m
	208.9 s	208.5 s	208.9 s	209.0 s	72.6 d	73.2 d	72.7 d	74.2 d
21	2.30 s	2.29 s	2.30 s	2.29 s	1.24 d	1.24 d	1.23 d	1.15 d
	32.5 q	32.3 q	32.4 q	32.4 q	19.5 q	20.2 q	19.5 q	19.8 q
Gly(NH)						6.29 dd	6.18 d	6.16 br t
Gly(CH_2)						3.54 dd, 4.31 dd	3.78 dd, 4.02 dd	3.60 dd, 4.12 dd
						42.4 t	41.8 t	42.7 t
^{t}Bu						1.47 s	1.48 s	1.47 s
						28.6 q	27.9 q	28.2 q
						83.0 s	82.0 s	82.0 s
AcO(*C*=O) or	170.6 s	170.2 s	170.4 s	170.8 s	170.6 s	170.0 s	168.9 s	170.6 s
Gly(*C*=O)					170.7 s	171.1 s	170.3 s	170.7 s
							170.5 s	170.9 s

[a] Two overlapping signals.
[b] Two overlapping signals.
[c] Two overlapping signals.
[d] These signals are masked owing to overlap with other resonances.
[e] Obtained only from the 1H of CH_2(18).

procedure.[28] The synthesis of activated 2,4,6-trichlorophenyl ester of *N*-protected phenylalanine was accomplished via *N*-Fmoc–L-Phe–Cl as intermediate.[30] The esterification reaction was carried out using biphasic system containing CH_2Cl_2/aq $NaHCO_3$ solution at room temperature.[31] The *N*-Fmoc–L-Phe–OTcp was subjected to the peptide-coupling reaction with amine **9** following the standard solution-phase conditions using 1-hydroxybenzotriazole (HOBt) and *N*,*N*-diisopropylethylamine (DIEA) in *N*-methylpyrrolidone (NMP) to give the targeted diamide **10** in 66% yield.[28] The molecular formula of **10** was established as $C_{56}H_{71}N_3O_{10}$ on the basis of the HRFABMS data, *m*/*z* 968.5085 $[M+Na]^+$ (Δ=+4.8 mmu).

All NMR spectra for the diamide **10** were recorded by the use of *Brucker Avance* 600 MHz spectrometer equipped with cryo-probe. The homonuclear 2D spectra (DQF-COSY,[32] TOCSY,[33] NOESY[34]) and the heteronuclear 2D ^1H–^{13}C spectra (HSQC,[35] HMBC,[36] INEPT edited HSQC[37]) were recorded with the usual settings.[38] Combined use of the techniques mentioned above on compound **10** in $CDCl_3$ solution enabled its complete and unambiguous ^1H and ^{13}C NMR assignments. The NMR parameters for the characterization of **10** were: (a) assignment of the ^1H spectrum using COSY, TOCSY, and NOESY methods; (b) correlation via $^1J_{CH}$ to provide the ^{13}C assignments for protonated carbons; (c) correlation via $^{2-4}J_{CH}$ to provide assignments for non-protonated carbons and to independently verify ^1H and ^{13}C assignments made with the other methods; (d) the solvent dependence of the proton shifts of the amide resonances; and (e) the detailed analysis of the NOESY correlations allowed a determination of the dipolar interactions between proximal protons that provide evidence for the preferred conformations and conformational features of compound **10**.



10

Consistent with the molecular formula of **10**, 56 C signals comprising 12 quaternary C, 16 tertiary C, 14 secondary C, and 4 methyls were observed in the ^{13}C NMR spectrum (Table 2). Detailed spin system investigation and assignment of individual resonances were carried out using combination of the COSY, TOCSY, and NOESY spectra.

The ^{13}C NMR and DEPT spectra exhibited two doublets at 72.6 and 73.7 ppm, and three carbonyl carbons singlets at δ 169.9 (2C), 170.6 (2C), and 172.7 (1C). One of the carbonyl carbons at 172.7 ppm was attributed to the carbonyl of the 19-amide group. The differentiation of the two acetoxy carbonyl carbons at 170.6 and 169.9 ppm was based on their HMBC correlations. The acetoxy methyl singlets at 1.98 ppm and 1.96 ppm showed long-range correlations with carbonyl groups at 170.6 ppm and 169.9 ppm, as well as with C-3 and C-20 at 72.6 and 73.7, respectively (Table 2). Assignments of the carbonyl resonances of the Gly at 170.6 ppm and Phe at 169.9 ppm were established by comparison of the ^{13}C data with those of compounds **7**–**9**, and could be interchangeable.

In the first step, amino acid spin systems of **10** were readily identified from 2D COSY and TOCSY spectra, starting from amide protons in the region of 5–6.5 ppm and were confirmed by NOESY cross-peaks (Table 2 and Fig. 3). The down-field portion of the spectrum showed the two sets of exchangeable resonances for two amide protons at 6.11/6.50 ppm (br s, Gly) and 5.65/5.51 ppm (br s, $CH_2(18')$–NH) and one carbamate proton at 5.46/5.17 ppm (d, Fmoc–Phe), indicating that two conformational forms exist (from ^1H NMR spectrum by the peak areas of the amide protons, the ratio of the conformations ca. 95/5 was calculated). However, because of the weak population of the minor conformation, the characteristic NOESY connectivity was not observed and we were unable to analyze the minor conformation. In the TOCSY spectrum, three spin systems: 6.11 ppm (NH) and 4.03–4.19 ppm (2Hα); 5.46 ppm (NH), 4.25 ppm (Hα), 3.11 (Hβ) and 2.94 (Hβ); and 5.65 ppm (NH), 3.33 ppm (H-18′), 2.46 (H-18′), 1.18 ppm (H-18) and 0.70 (H-18), were assigned to Gly, Phe, and $CH_2(18)CH_2(18')$NH moieties. The NOESY correlations between the proton pairs of Gly(NH)/Gly(αH_2), Phe(NH)/Phe(αH), Phe(αH)/Phe(βH_2), Phe(αH)/Phe(2,6), and NH (at C-18′)/Phe(αH) were additional noteworthy features in the spectrum. All the aromatic proton assignments of the *N*-Fmoc–L-Phe unit were made using a combination of the COSY, HSQC, HMBC, and NOESY experiments (Table 2 and Fig. 3).

NOESY correlations between H_3-21, Hα-16, and Hα-17, provided evidence for the 20*R*-configuration. The H-20 proton at 4.56 ppm showed NOESY cross-peak with the H-18′ proton at 2.46 ppm and no correlations were observed with the second H-18′ proton at 3.33 ppm as well as with both H_2-18 protons, exhibiting the definite stereochemical arrangement of the $C(18')H_2$–$C(18)H_2$–C(13)–C(17)–C(20)H-moiety. This indicates that free rotation of the $CH_2(18)$–$CH_2(18')$ moiety is restricted and that it occupies a fixed position relative to steroid skeleton (see Figs. 3 and 4).

The down-field signals Hβ-1 (δ 2.23 ppm) and Hβ-6 (δ 2.48 ppm) indicated that these protons were close to the deshielding zone of the carbonyl C(19) amide group. These values are in a good agreement with the predicted effect of the carbonyl group on the protons lying in the plane of the trigonal atom implying the orientation of the C(19)=O carbonyl group (the plane defined by the C(19)=O group and C(6), and the carbonyl oxygen very close to the H_{ax} at C(6)).[39] Further evidence to support this orientation of the C(19) carbonyl group was obtained from the absence of the NOESY correlations between the NH proton from the Gly moiety and the axial protons at C(2), C(4), C(6), C(8), and C(11) of the steroidal part of the molecule (Figs. 3 and 4).

The most important NOESY cross-peaks for the conformational investigation were those involving the ^{t}Bu group.

Table 2. ^{1}H and ^{13}C NMR chemical shifts, NOESY and HMBC data for **10** (δ, mult., J in Hz)

Assignment	^{1}H	^{13}C	NOESY	^{1}H–^{13}C Long-range (^{2-4}J) correlations
1	β 2.23 d (12); α 1.18 br d (11)[a]	34.2 t	1β/1α; 1β/2β	C-2(^{2}J), C-3(^{3}J), C-10(^{2}J)
2	α 2.00 m; β 1.59 m[b]	28.9 t		
3	4.73 hept.	72.6 d	2α; 4α; 5α	
4	α 1.73 m; β 1.52 m[c]	35.3 t		
5	1.24 br d (13.9)	45.3 d	3α	
6	α 2.48 m[d]; β 1.33 br d (11)[e]	28.9 t	6α/6β	
7	β 1.77 m; α 0.91 dd (12.2, 3.5)	31.5 t		
8	1.55 m	35.0 d		
9	0.80 br t (11)	51.9 d		
10	—	49.0 s		
11	α 1.59 m[b]; β 1.33 br d (11)[e]	22.4 t		
12	β 2.03 m; α 0.98 m[f]	35.3 t		
13	—	43.6 s		
14	0.96 m[f]	57.7 d		
15	α 1.59 m[b]; β 0.97 m[f]	23.1 t		
16	α 1.65 m; β 1.14 m	25.2 t	16α/21	
17	1.52 q[c]	55.3 d	21	
18	1.18 br d (11)[a]; 0.70 br t (11)	25.8 t		
18′	3.33 m; 2.46 m[d]	35.0 t	18′; 20	
C(18′)–NH–	5.65 br s	—	Phe(αH)	
19	—	172.7 s		
20	4.56 m	73.7 d	18′(1H); 21	C-17(^{2}J)
21	1.11 d (5.9)	19.7 q	16α; 17α; 20	C-17(^{3}J), C-20(^{2}J)
AcO–C(3)	1.98 s	21.2 q		C=O(170.6)(^{2}J), C-3(^{4}J)
AcO–C(20)	1.96 s	21.4 q		C=O(169.9)(^{2}J), C-20(^{4}J)
AcO–C(3)	—	170.6 s		
AcO–C(20)	—	169.9 s		
Gly(CH_2)	4.03–4.19 m[g]	42.3 t	Gly(NH); ^{t}Bu	
Gly(NH)	6.11 br s	—	Gly(CH_2)	
^{t}Bu(Me)	1.44 s	28.0 q	Phe(αH, ArH, NH); Gly(αH_2), Fmoc(ArH-1/8, H-9)	C(81.9)(^{2}J)
^{t}Bu(C)	—	81.9 s		
Gly(CO)	—	170.6 s[h]		
Phe(C=O)	—	169.9 s[h]		
Phe(NH)	5.46 d (8)	—	Phe(αH); ^{t}Bu	
Phe(αCH)	4.24 q (7)	56.3 d	^{t}Bu; Phe(βH_2); Phe(NH); Phe(2,6); C(18′)-NH	
Phe(βCH_2)	3.11 br dd (4, 8); 2.95 br t (8)	39.2 t	Phe(α); Phe(2,6)	
Phe(1)	—	136.6 s		
Phe(2,6)	7.22 d (8)	128.6 d	Phe(αH); Phe(βH_2)	
Phe(3,5)	7.26 d (8)	129.4 d		
Phe(4)	7.19 t (8)	126.7 d		
Fmoc(C=O)	—	155.6 s		
Fmoc(9)	4.16 t (7)[g]	47.0 d	Fmoc(CH_2); Fmoc(1,8); ^{t}Bu	Fmoc(8a/9a)(^{2}J), Fmoc(CH_2)(^{2}J)
Fmoc(CH_2)	4.39 t (8); 4.30 t (7)	66.8 t	Fmoc(1,8); Fmoc(9)	Fmoc(9)(^{2}J)
Fmoc(1,8)	7.55 d (8); 7.53 br d (7)	125.0 d	Fmoc(2,7); Fmoc(9); Fmoc(CH_2); ^{t}Bu	Fmoc(9)(^{3}J), Fmoc(3/6)(^{3}J)
Fmoc(2,7)	7.28 t (7)	127.0 d	Fmoc(1,8); Fmoc(3,6)	Fmoc(9)(^{4}J)
Fmoc(3,6)	7.38 t (8)	127.6 d	Fmoc(2,7); Fmoc(4,5)	Fmoc(8a/9a)(^{4}J), Fmoc(4a/4b)(^{3}J), Fmoc(2/7)(^{2}J)
Fmoc(4,5)	7.75 d (8)	119.8 d	Fmoc(3,6)	Fmoc(8a/9a)(^{3}J), Fmoc(2/7)(^{3}J), Fmoc(3/6)(^{2}J)
Fmoc(8a,9a)	—	143.7 s		
Fmoc(4a,4b)	—	141.2 s		

[a] Two overlapping signals.
[b] Three overlapping signals.
[c] Two overlapping signals.
[d] Two overlapping signals.
[e] Two overlapping signals.
[f] Three overlapping signals.
[g] Two overlapping signals.
[h] Interchangeable.

Elucidation of the network of dipolar connectivities of the ^{t}Bu group was the key-point for the conformational analysis of the amino acid units. In particular, a conformationally dependent pattern of cross-peaks was observed for ^{t}Bu group, Phe(αH), Phe(ArH), Phe(NH), Fmoc(H9), Fmoc(1/8H), and Gly(2αH) indicating that the amino acid moieties of **10** assume specific conformations. Also, the NH(Phe) and NH (at C-18′) protons showed NOESY cross-peaks with the Hα(Phe) proton. The intensities of the NOESY interactions were found to be significantly different for these spin-pairs (NH (at C-18′)/Hα(Phe) is stronger), suggesting that NH (at C-18′) is directed toward the ^{t}Bu group (Figs. 3 and 4).

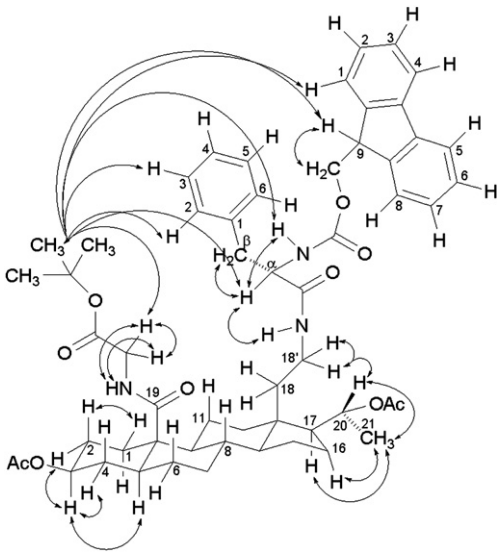


Figure 3. Selected NOESY correlations for compound **10**.

The NOESY cross-peaks between ^{t}Bu and Phe(αH), Phe(ArH), Phe(NH), Fmoc(H9), and Fmoc(1/8H), were crucial in the assignment of the orientations of amino acid units and confirmed the close proximity of the Phe and Gly moieties, which are lying approximately parallel to each other and perpendicular in respect to the steroid skeleton (see Fig. 4). Significantly, the arrangement of the amino acid units enables hydrogen bonds between the amide NH proton at C-18′ and both Gly CO and Fmoc CO, which may stabilize the solution conformation of **10**.

Further evidence for the involvement of the NH amide protons in intramolecular hydrogen bonding of the amino acid units was elucidated from their chemical shift differences in non-coordinating ($CDCl_3$ and C_6D_6) and coordinating (acetone-d_6 and DMSO-d_6) solvents. When DMSO-d_6 and acetone-d_6 were used as the solvent, the chemical shifts for all the amide hydrogens moved appreciably downfield with respect to apolar solvents ($CDCl_3$ and C_6D_6), indicating that the amide NH hydrogens were not involved in strong intramolecular hydrogen bonding. On that basis we cannot ascertain that the conformation of **10** is solely influenced by hydrogen bonding. The solvent dependence was determined at 10 mM concentrations of compound **10** and shown in Table 3.

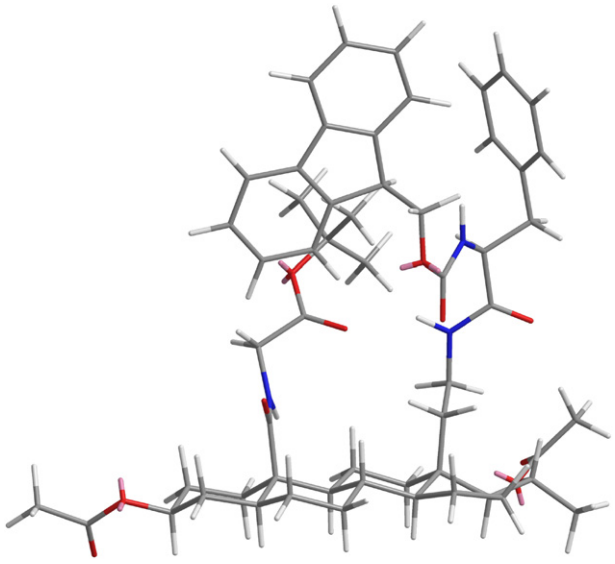


Figure 4. 3D representation of the proposed conformation of **10** on the basis NOESY connectivities.

Table 3. Solvent dependence of ^{1}H NMR amide proton chemical shifts for **10** in $CDCl_3$, C_6D_6, acetone-d_6 and DMSO-d_6

NH	$CDCl_3$	C_6D_6	Acetone-d_6	DMSO-d_6[a]
Gly	6.11 br s	6.10 br s	7.04 br s	7–8
Phe	5.46 d	5.41 d	6.55 d	7–8
At C(18′)	5.65 br s	7.04 br s	7.21 br s	7–8

[a] The signals fell in the range of the aromatic protons (7.2–8 ppm) but could not be located unambiguously.

Our NMR data indicate that the amino acids in **10** are not as flexible as might be expected and that observed NOESY correlations for the ^{t}Bu group of Gly–O^{t}Bu moiety confirm the existence of mainly one conformation in $CDCl_3$ solution, which is probably stabilized by contribution of CH/π (^{t}Bu/Phe, Phe/Fmoc) and π/π (Phe/Fmoc) interactions, together with hydrophobic interactions.

On the basis of the above data, the solution structure of **10** can be presented as shown in Figure 4.

3. Conclusion

In the described work, we realized the synthesis of a new modified-steroidal compound with two rigidly positioned amino acids at the angular positions (C-18 and C-19) of the steroid nucleus via amide bonds starting from 18-cyano-pregnenolone acetate **1**. The importance of the synthetic approach lies in its potential applicability toward a general conjunction strategy of varied types of molecules with steroids. The methodology reported here may be extended to the synthesis of a new class of different 18,19-functionalized peptidosteroidal derivatives that may play an important role in the design of biologically active molecules. Despite the large size and the conformational flexibility of amino acid units in molecule **10**, conformational analysis by NOESY connectivities showed the existence of mainly one conformation in $CDCl_3$ solution (~95%) with approximately parallel orientation of phenylalanine and glycine moieties.

4. Experimental

4.1. General

Column chromatography and TLC were carried out using Merck silica gel 0.04–0.063 mm and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were visualized under UV light and by spraying with aq 50% H_2SO_4 soln followed by heating. Analytical HPLC was carried out on *Agilent 1100* instrument (Zorbax SB C-18 column, 5 μm, 150×4.6 mm). Melting points were determined on *Boetius PMHK* apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ using *Autopol IV Automatic Polarimeter* at 20 °C. IR Spectra: *Perkin–Elmer-FTIR 1725X* spectrophotometer;

ν in cm^{-1}. NMR Spectra: *Varian Gemini 200* and *Brucker Avance 600* MHz; δ in parts per million, *J* in hertz. Mass spectra: *Finnigan-MAT 8230*; *m/z* (rel intensity in %); ionization energy 70 eV. Elemental analyses were determined on *Variol III*. FABHRMS: *JEOL JMS-SX 102A* spectrometer.

4.2. 5α-Bromo-18-cyano-20-oxopregnane-3β,6β-diyl 3-acetate (2)

To a stirred solution of **1** (30 g, 78 mmol) in dioxane (340 mL), H_2O (60 mL) and 6.5 mL $HClO_4$ (70%) was added portionwise *N*-bromoacetamide (NBA) (15.1 g, 109.4 mmol) for 15 min and the mixture was stirred at room temperature for 30 min. After cooling to 0 °C, H_2O (1 L) was added and then aq $Na_2S_2O_3$ soln, stirred for 15 min, filtered and the solid part suspended in water (1.5 L), filtered, dissolved in CH_2Cl_2, washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product (33.7 g, 90%) was used in the next step without further purification. Two-fold recrystallization from CH_2Cl_2/Et_2O yielded an analytically pure specimen, mp 160–161 °C. $[\alpha]_D$ 0 (*c* 1, $CHCl_3$); IR (KBr) ν_{max} 3481, 3456, 3437, 2249, 1732, 1705, 1244, 1028; ^1H NMR (200 MHz, $CDCl_3$) δ 1.33 (s, CH_3-19), 2.04 (s, AcO), 2.30 (s, CH_3-21), 2.50 (dd, *J*=10.7, 13.7 Hz, H_β-4), 2.75 (t, *J*=8.8 Hz, H_α-17), 4.20 (br s, H-6), 5.46 (m, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 208.9 (s, C-20), 170.6 (s, AcO), 118.0 (s, CN), 86.2 (s, C-5), 75.0 (d, C-6), 71.9 (d, C-3), 61.8 (d, C-17), 55.6 (d, C-14), 47.0 (d, C-9), 46.1 (s, C-13), 40.2 (s, C-10), 38.2 (t, C-12), 35.5 (t, C-7), 34.9 (t, C-4), 34.1 (t, C-1), 32.5 (q, C-21), 30.6 (d, C-8), 26.1 (t, C-2), 23.8 (t, C-15), 23.1 (t, C-16), 21.3 (q, AcO), 21.1 (t, C-11), 17.8 (q, C-19), 16.5 (t, C-18); CI-MS: 480/482 (M^++1), 400 [(M^++1)−80/82], 322 [(M^++1)−80/82−18−60, 100%]. Anal. Calcd for $C_{24}H_{34}BrNO_4$ (480.44): C, 60.00; H, 7.13; N, 2.92. Found: C, 59.76; H, 7.26; N, 3.17.

4.3. 5α-Bromo-18-cyano-6β,19-epoxy-20-oxopregnan-3β-yl acetate (3)

A stirred suspension of LTA (156.1 g) and $CaCO_3$ (70.4 g) in CH_2Cl_2 (2.1 L) was refluxed for 20 min (using as the source of heat a 250 W tungsten lamp), and I_2 (35.7 g) was added; the mixture was stirred under reflux for 15 min. The crude bromohydrin **2** (33.7 g) in CH_2Cl_2 (140 mL) was then added and the mixture refluxed under irradiation (250 W lamp) for 70 min. The solid was filtered, the filtrate washed successively with aq $Na_2S_2O_3$ soln, H_2O, dried (Na_2SO_4), and evaporated. The residue was recrystallized from CH_2Cl_2/Et_2O to give **3** (15.13 g, 45%), mp 256–257 °C. $[\alpha]_D$ +5 (*c* 1, $CHCl_3$). IR ν_{max} 2238, 1722, 1703, 1240, 1034; ^1H NMR (200 MHz, $CDCl_3$) δ 2.04 (s, AcO), 2.29 (s, CH_3-21), 2.43 (d, 1H, *J*=17.2 Hz, H_2-18), 2.50–2.65 (m, 1H, H_β-4), 2.69 (t, *J*=9 Hz, H_α-17), 3.69, 3.97 (2d, 2H, *J*=8.8 Hz, H_2-19), 4.09 (d, *J*=4 Hz, H-6), 5.20 (m, H-3); ^{13}C NMR (50 MHz, $CDCl_3$) δ 208.5 (s, C-20), 170.2 (s, AcO), 117.9 (s, CN), 81.7 (d, C-6), 74.0 (s, C-5), 69.5 (d, C-3), 67.3 (t, C-19), 61.6 (d, C-17), 54.3 (d, C-14), 48.1 (d, C-9), 46.2 (s, C-13), 45.6 (s, C-10), 40.9 (t, C-12), 35.5 (t, C-7), 33.3 (d, C-8), 32.4 (t, C-4), 32.3 (q, C-21), 26.5 (t, C-1), 23.2 (t, C-15), 23.0 (2t, C-2, C-16), 22.3 (t, C-11), 21.1 (q, AcO), 16.6 (t, C-18); MS: 478/480 (M^++1), 418/420 [(M^++1)−60], 338 [(M^++1)−80/82−60, 100%]. Anal. Calcd for $C_{24}H_{32}BrNO_4$ (478.42): C, 60.25; H, 6.74; N, 2.93. Found: C, 59.97; H, 6.66; N, 3.05.

4.4. 18-Cyano-20-oxopregn-5-en-3β,19-diyl 3-acetate (4)

Activated zinc powder (69.38 g, 1.045 mol) was added to a solution of **3** (10.0 g, 20.9 mmol) in AcOH (355 mL) and water (15.3 mL) and the mixture was heated with stirring at 50 °C for 1 h. The warm suspension was filtered and the residue thoroughly washed with CH_2Cl_2. Removal of the solvent by evaporation afforded the residue, which was dissolved in CH_2Cl_2, washed with H_2O, aq $NaHCO_3$ soln, H_2O, dried (Na_2SO_4), and evaporated. The crude product (9.70 g) was crystallized from CH_2Cl_2/Et_2O to give pure **4** (6.10 g, 73%), mp 139–141 °C. $[\alpha]_D$ +2.2 (*c* 1.267, $CHCl_3$). IR ν_{max} 3480, 2246, 1730, 1705, 1249, 1032; ^1H NMR (200 MHz, $CDCl_3$) δ 2.03 (s, AcO), 2.20 (br d, 1H, *J*=17.1 Hz, H_2-18), 2.30 (s, CH_3-21), 2.56 (d, 1H, *J*=17.1 Hz, H_2-18), 2.73 (t, *J*=8.9 Hz, H_α-17), 3.58, 3.92 (2d, 2H, *J*=11.4 Hz, H_2-19), 4.64 (m, H-3), 5.75 (br s, H-6); ^{13}C NMR (50 MHz, $CDCl_3$) δ 208.9 (s, C-20), 170.4 (s, AcO), 134.9 (s, C-5), 126.7 (d, C-6), 118.3 (s, CN), 73.2 (d, C-3), 62.6 (t, C-19), 61.6 (d, C-17), 57.0 (d, C-14), 49.5 (d, C-9), 46.0 (s, C-13), 41.1 (s, C-10), 37.8 (t, C-12), 35.6 (t, C-4), 33.3 (t, C-1), 33.1 (d, C-8), 32.4 (q, C-21), 30.7 (t, C-7), 27.7 (t, C-2), 23.8 (t, C-15), 23.0 (t, C-16), 21.2 (q, AcO), 21.1 (t, C-11), 16.2 (t, C-18); MS: 399 (M^+), 339 (M^+−60). Anal. Calcd for $C_{24}H_{33}NO_4$ (399.24): C, 72.15; H, 8.33; N, 3.51. Found: C, 71.91; H, 8.10; N, 3.59.

4.5. 3β-Acetoxy-18-cyano-20-oxopregn-5-en-19-oic acid (5)

To a cooled (0–5 °C) and stirred solution of **4** (3.4 g, 8.51 mmol) in acetone (170 mL) a slight excess of Jones' reagent (17 mL) was added dropwise. After 24 h reaction mixture was diluted with ice-cold H_2O, cryst NaOAc (60 g) was added, and extracted with CH_2Cl_2. The organic layer was washed with H_2O, aq $NaHCO_3$ soln, H_2O, dried over Na_2SO_4, and evaporated to dryness, leaving 3.1 g of crude product, which was chromatographed on SiO_2 (150 g, R_f 0.55, 95/5 CH_2Cl_2/MeOH). Elution with CH_2Cl_2/MeOH (96/4) gave **5** as a white solid (2.6 g, 74%), mp 211–212 °C. $[\alpha]_D$ −3.2 (*c* 1.23, $CHCl_3$). IR ν_{max} 3600–2500, 2251, 1729, 1706, 1247, 1034; ^1H NMR (200 MHz, $CDCl_3$) δ 2.04 (s, AcO), 2.29 (s, CH_3-21), 2.40–2.80 (m, 3H, H_α-17, H_2-18), 4.64 (m, H-3), 5.72 (d, *J*=4.6, H-6), 9.2 (br s, COOH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 209.0 (s, C-20), 177.9 (s, C-19), 170.8 (s, AcO), 133.6 (s, C-5), 125.3 (d, C-6), 117.9 (s, CN), 72.7 (d, C-3), 61.7 (d, C-17), 56.1 (d, C-14), 50.1 (s, C-10), 48.1 (d, C-9), 45.8 (s, C-13), 39.8 (t, C-12), 35.5 (t, C-4), 33.3 (t, C-1), 32.4 (q, C-21), 31.7 (d, C-8), 30.5 (t, C-7), 28.8 (t, C-2), 23.8 (t, C-15), 23.1 (t, C-16), 22.8 (t, C-11), 21.2 (q, AcO), 16.3 (t, C-18); CI-MS: 395 (413(M^+)−18), 367 (413−46, 100%), 307 (413−60−46). Anal. Calcd for $C_{24}H_{31}NO_5$ (413.51): C, 69.71; H, 7.56; N, 3.39. Found: C, 69.45; H, 7.54; N, 3.32.

4.6. 18-Cyano-3β,20β-diacetoxypregn-5-en-19-oic acid (6)

Sodium borohydride (1.44 g) was added in small portions to a solution of 20-ketone **5** (2.50 g, 6.04 mmol) in MeOH

(130 mL). The reaction mixture was stirred at room temperature for 30 min. Workup was accomplished by addition of H_2O followed by removal of most of the solvent under reduced pressure. The residue was taken up in Et_2O and the organic layer was washed with H_2O, aq $NaHCO_3$ soln, and H_2O. After drying over Na_2SO_4, the solvent was removed under reduced pressure to give a crude 3β,20β-diol (2.52 g), which was immediately acetylated (Ac_2O/Py) (3 mL/3 mL). The usual work up of the reaction mixture gave the corresponding crude 3β,20β-diacetate **6** (2.72 g), which was purified by crystallization from acetone/MeOH (1.99 g, 72%), mp 129–130 °C. $[\alpha]_D$ −11.2 (*c* 0.426, $CHCl_3$). IR ν_{max} 3600–2500, 2242, 1730, 1239, 1036; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.24 (d, CH_3-21), 2.04, 2.06 (2s, 2AcO), 2.35 (d, 1H, *J*=17.6 Hz, H_2-18), 2.40–2.75 (m, 2H, H_2-4), 4.70 (m, 2H, H-3, H-20), 5.71 (d, *J*=3.8 Hz, H-6), 10.5 (br s, COOH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.2 (s, C-19), 170.7, 170.6 (2s, 2AcO), 133.6 (s, C-5), 125.2 (d, C-6), 118.4 (s, CN), 72.7 (d, C-3), 72.6 (d, C-20), 55.3 (d, C-14), 54.3 (d, C-17), 50.1 (s, C-10), 48.2 (d, C-9), 44.5 (s, C-13), 39.8 (t, C-12), 36.1 (t, C-4), 33.1 (t, C-1), 31.6 (d, C-8), 30.5 (t, C-7), 28.8 (t, C-2), 24.7 (t, C-16), 23.3 (t, C-15), 22.6 (t, C-11), 21.5, 21.2 (2q, 2AcO), 19.5 (q, C-21), 15.5 (t, C-18); MS: 351 (457(M^+)−46−60, 20%), 291 (351−60, 100%), 307 (413−60−46). Anal. Calcd for $C_{26}H_{35}NO_6$ (457.56): C, 68.25; H, 7.71; N, 3.06. Found: C, 68.01; H, 7.90; N, 3.09.

4.7. 19-Gly-containing steroidal compound 7

To a solution of **6** (630 mg, 1.38 mmol) in dry CH_2Cl_2 (6.5 mL) was added dropwise 1-chloro-*N*,*N*,2-trimethylpropenylamine (0.22 mL, 1.55 mmol) at 0–5 °C under Ar. After 2 h a small amount of starting material was still present (TLC) and another portion of chlorotrimethylpropenylamine (0.055 mL, 0.387 mmol) was added, and the reaction mixture stirred for another 2 h. The reaction mixture was poured on ice, diluted with CH_2Cl_2, washed with H_2O, aq $NaHCO_3$ soln, H_2O, dried (Na_2SO_4), and evaporated. Unpurified 'acyl chloride' (650 mg) was dissolved in CH_2Cl_2 (5 mL) and treated with Gly–O^{t}Bu·HCl (253 mg, 1.51 mmol) in CH_2Cl_2 (4 mL) and triethylamine (0.23 mL, 1.66 mmol) for 5 h under Ar. The reaction mixture diluted with CH_2Cl_2, washed with H_2O, dried (Na_2SO_4), and evaporated. Crystallization from acetone/*n*-hexane gave 580 mg (74%) crystalline compound **7**, mp 211–212 °C. $[\alpha]_D$ −4.3 (*c* 0.636, $CHCl_3$). IR ν_{max} 3360, 2239, 1739, 1650, 1370, 1242, 1158, 1032; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.24 (d, CH_3-21), 1.47 (s, 9H, ^{t}Bu(Me)), 2.01, 2.04 (2s, 2AcO), 2.82 (d, 1H, *J*=17 Hz, H_2-18), 2.17 (d, 1H, *J*=17 Hz, H_2-18), 2.43–2.85 (m, H_2-4), 3.54 (dd, *J*=4.4, 17.8 Hz, 1H, Gly(CH_2)), 4.31 (dd, *J*=7.7, 17.8 Hz, 1H, Gly(CH_2)), 4.50–4.82 (m, 2H, H-3, H-20), 5.82 (br d, *J*=5.2 Hz, H-6), 6.29 (dd, *J*=4.4, 7.6 Hz, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.1 (s, C-19), 171.1, 170.0 (3s, 2AcO, Gly(CO)), 136.0 (s, C-5), 127.7 (d, C-6), 119.3 (s, CN), 83.0 (s, ^{t}Bu(C)), 73.2 (2d, C-3, C-20), 56.7 (d, C-14), 55.1 (d, C-17), 50.9 (s, C-10), 49.2 (d, C-9), 45.3 (s, C-13), 42.4 (t, Gly(CH_2)), 40.5 (t, C-12), 37.1 (t, C-4), 33.6 (t, C-1), 32.5 (d, C-8), 31.2 (t, C-7), 29.6 (t, C-2), 28.6 (q, ^{t}Bu(Me)), 25.6 (t, C-16), 24.0 (t, C-15), 23.2 (t, C-11), 22.2, 21.9 (2q, 2AcO), 20.2 (q, C-21), 15.8 (t, C-18); MS: 570 (M^+), 497 (M^+−73), 454 (M^+−116), 413 (M^+−157), 353 (413−60), 293 (353−60, 100%). Anal. Calcd for $C_{32}H_{46}N_2O_7$ (570.72): C, 67.34; H, 8.12; N, 4.91. Found: C, 67.12; H, 8.02; N, 4.92.

4.8. Catalytic hydrogenation of 7

A solution of 18-cyano derivative **7** (1.0 g) in ^{i}PrOH (99 mL), $CHCl_3$ (33 mL), and HCl (32%, 0.15 mL) was hydrogenated in the presence of PtO_2 (1 g) in a Parr reactor under 60 psi H_2 pressure at room temperature for 20 h. The catalyst was filtered off, and the filtrate evaporated in vacuo, the residue taken up in CH_2Cl_2, washed with aq NaOH soln, H_2O, dried (Na_2SO_4), and evaporated. The resulting mixture (1.02 g) separated by flash column chromatography (SiO_2, 30 g, 0.04–0.063 mm). Elution with CH_2Cl_2/MeOH (97:3) afforded 5α-saturated analogue **8** (908 mg, 90%), mp 208–208.5 °C. $[\alpha]_D$ −0.9 (*c* 1.74, $CHCl_3$). IR ν_{max} 3424, 2245, 1734, 1655, 1370, 1244, 1158, 1027; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.23 (d, CH_3-21), 1.48 (s, 9H, ^{t}Bu(Me)), 2.00, 2.04 (2s, 2AcO), 2.26 (br d, *J*=14.4 Hz, 1H, H_β-1), 2.40 (m, 1H, H_α-6), 2.58 (d, 1H, *J*=17.2 Hz, H_2-18), 3.78 (dd, *J*=5.2, 17.7 Hz, 1H, Gly(CH_2)), 4.02 (dd, *J*=5.5, 17.8 Hz, 1H, Gly(CH_2)), 4.72 (m, 2H, H-3, H-20), 6.18 (d, *J*=5.3 Hz, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.0 (s, C-19), 170.5, 170.3, 168.9 (3s, 2AcO, Gly(CO)), 118.7 (s, CN), 82.0 (s, ^{t}Bu), 72.7, 72.4 (2d, C-3, C-20), 55.8 (d, C-14), 54.4 (d, C-17), 51.6 (d, C-9), 49.0 (s, C-10), 45.2 (d, C-5), 44.5 (s, C-13), 41.8 (t, Gly(CH_2)), 36.3 (t, C-12), 35.2 (t, C-4), 35.0 (d, C-8), 33.9 (t, C-1), 31.3 (t, C-7), 28.8 (t, C-6), 28.6 (t, C-2), 27.9 (q, ^{t}Bu(Me)), 24.8 (t, C-16), 23.4 (t, C-15), 21.9 (t, C-11), 21.5, 21.2 (2q, 2AcO), 19.5 (q, C-21), 15.5 (t, C-18); MS: 572 (M^+), 516 (M^+−57), 457 (M^+−115), 415 (M^+−157), 294 (M^+−2×60−158). Anal. Calcd for $C_{32}H_{48}N_2O_7$ (572.73): C, 67.11; H, 8.45; N, 4.89. Found: C, 66.97; H, 8.25; N, 4.76. Elution with CH_2Cl_2/MeOH/NH_4OH (25%) (90:20:4) gave 5α-saturated 18-aminomethyl-derivative **9** (75.8 mg, 8%), mp 212–217 °C. $[\alpha]_D$ +1.0 (*c* 0.147, $CHCl_3$). IR ν_{max} 3429, 1727, 1646, 1372, 1250, 1159; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15 (d, CH_3-21), 1.47 (s, 9H, ^{t}Bu(Me)), 2.00, 2.04 (2s, 2AcO), 2.24 (br d, 1H, *J*=14.2 Hz, H_β-1), 2.41 (br d, 1H, *J*=10 Hz, H_α-6), 2.73 (br t, 1H, H_2-18′), 2.93 (br t, 1H, H_2-18′), 3.60 (dd, *J*=5.0, 16.8 Hz, 1H, Gly(CH_2)), 4.12 (dd, *J*=5.4, 17.2 Hz, 1H, Gly(CH_2)), 4.73 (m, H-3), 5.25 (m, H-20), 6.16 (br t, *J*=5.6 Hz, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.6 (s, C-19), 170.9, 170.7, 170.6 (3s, 2 AcO, Gly(CO)), 82.0 (s, ^{t}Bu), 74.2 (d, C-20), 72.8 (C-3), 57.6 (d, C-14), 55.6 (d, C-17), 51.5 (d, C-9), 49.1 (s, C-10), 45.5 (d, C-5), 43.8 (s, C-13), 42.7 (t, Gly(CH_2)), 37.2 (t, C-12), 35.4 (d, C-8), 35.4 (C-4, C-18, C-18′), 34.3 (t, C-1), 31.6 (t, C-7), 28.9 (t, C-6), 28.2 (t, C-2), 28.2 (q, ^{t}Bu(Me)), 24.9 (t, C-16), 23.3 (t, C-15), 22.3 (t, C-11), 21.6, 21.3 (2q, 2AcO), 19.8 (q, C-21). Anal. Calcd for $C_{32}H_{52}N_2O_7$ (576.76): C, 66.64; H, 9.09; N, 4.86. Found: C, 66.46; H, 9.13; N, 4.46. HRMS calcd for $C_{32}H_{53}N_2O_7$ [M+H]$^+$ 577.3853, found 577.3873.

4.9. Catalytic hydrogenation of 8

Treatment of saturated cyano derivative **8** (908 mg) in the same way as above afforded a mixture, which was chromatographed to give **8** (772 mg, 77%) and **9** (91.4 mg, 10%). This procedure was repeated several times (446 mg, 49% overall yield).

4.10. 18-Phe,19-Gly-containing steroid 10

To a solution of the amine **9** (102 mg, 0.177 mmol) in DMF (0.646 mL), *N*-Fmoc–L-Phe–OTcp (204.2 mg, 0.360 mmol), 1 M soln of 1-hydroxy-1*H*-benzotriazole (HOBT) in *N*-methylpyrrolidone (NMP) (0.361 mL, 0.360 mmol), and 1.5 M soln of diisopropylethylamine (DIEA) in NMP (0.120 mL, 0.176 mmol) were added and the reaction mixture stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc, washed with H_2O, aq $NaHCO_3$ soln, dried (Na_2SO_4), and evaporated to give 314 mg of the crude product. By flash column chromatography on SiO_2 (20 g, 0.04–0.063 mm) (toluene/EtOAc (8:2)) 119 mg (71%) of pure compound **10** was obtained. Crystallization from CH_2Cl_2/Et_2O/*n*-hexane mixture gave 110 mg (66%) crystalline product, mp 123–125 °C. $[\alpha]_D$ −0.6 (*c* 0.514, $CHCl_3$). The purity of **10** was checked by analytical HPLC. UV (MeOH): λ_{max}=196, 206, 264, 280, 300 nm. IR ν_{max} 3419, 1728, 1664, 1453, 1369, 1247, 1159, 1078. ^1H and ^{13}C NMR: see Tables 2 and 3 and Figure 3. Anal. Calcd for $C_{56}H_{71}N_3O_{10}$ (946.18): C, 71.09; H, 7.56; N, 4.44; Found: C, 70.83; H, 7.71; N, 4.16. FABHRMS calcd for $C_{56}H_{71}N_3O_{10}Na[M+Na]^+$ 968.5037, found 968.5085.

Acknowledgements

The authors acknowledge the financial support of the Ministry of Science and Environment of Serbia (part of the project 'Synthesis of different useful products from steroidal molecules'—Project No. 142033).

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