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Neurosteroid analogues: synthesis of 6-aza-allopregnanolone

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Abstract—An efficient synthesis of 6-azapregnane derivatives and their biological activity is described. The nitrogen was introduced into the B ring using Beckmann rearrangement of the (*E*)-oxime of 6-oxo-B-nor-5α-pregnane derivatives. The required 3α-hydroxyl was produced either by solvolysis of the corresponding 3β-mesyloxy group or by the Meerwein–Ponndorf–Verley reduction of the 3-oxo group; this reduction could be carried out selectively with an unprotected 3,20-dioxo derivative. The binding of the 6-aza-steroids to the γ-aminobutyric acid receptor ($GABA_A$) was measured using [^{35}S]-*tert*-butyl-bicyclo[2.2.2]phosphorothionate (TBPS) and [^{3}H]flunitrazepam. The only analogue to be slightly active was that lacking any oxygen function in position 3.

1. Introduction

In the search for biologically active analogues of natural hormones, substitution of a heteroatom for a carbon atom has often been successful. Total[1] or partial syntheses[2–4] have led to a number of steroids with a nitrogen atom in the skeleton. The substitution can modify the chemical nature of functionalities present in the molecule: for example, oxo derivatives are thus converted into lactams[5,6] (e.g., **1**, see Fig. 1) or their vinylogues[7] (e.g., **2**). The analogues could mimic an original hormone but not fulfil its functions[8,9] or behave as the original.[10–12] Recently, 6-aza-5α-cholestan-3β-ol (**3**) and its pregnane derivative **4** were found to be specific phosphatidylinositol phospholipase C inhibitors with antitumour activity.[13] Further, analogues with a heteroatom in the skeleton can also be useful for the study of interactions[14] between a ligand and its receptor.

Functional groups essential for neuronal activity of allopregnanolone (3α-hydroxy-5α-pregnan-20-one, **5**) comprise the 3α-hydroxy and 20-oxo groups. Since the B ring seems to be distant enough from these critical points, we envisaged that the introduction of a nitrogen atom into position 6 of allopregnanolone (**5**) would have no detrimental effect on its biological activity. In contrast, we hoped to obtain products with increased solubility, since low solubility of our earlier analogues in body liquids[15,16] often marred their biological activity and the usual ways of making compounds more soluble did not help: even though some quaternary ammonium salts (e.g., compounds **6** and **7**)[15] functioned well on isolated receptors, they were inactive in a living organism.

Several methods are known for the preparation of 6-azasteroids, all based on oxidation of 5-unsaturated steroids into 5-oxo-7-oic acids or their derivatives. This process[13] is less efficient in 3-substituted seco-steroids which easily lose the 3-substituents. The loss could be prevented by the use of 3-silylated intermediates[17] or exploited in a different route described by Sharp.[18] Here we present an alternative synthesis of 6-azapregnane derivatives (3α-hydroxy-6-aza-5α-pregnan-20-one, **8**, 3α-hydroxy-6-aza-5α-pregnane-7,20-dione, **9**): the loss of one carbon atom, required for the synthesis of the piperidine B ring, takes place during the preparation of the starting material—a 7-norsteroid derivative.

2. Results and discussion

2.1. Synthesis

The starting material, (20*R*)-7-norpregn-5-ene-3β,20-diyl acetate benzoate[19] (**10**, see Scheme 1) was converted into 6α-bromo-5β-alcohol **11** and then epoxide **12**. The 5β,6β-configuration of the epoxide was apparent from its ^{1}H NMR spectrum (a narrow multiplet of H-3α, typical[20] of 3β-hydroxy-5β-steroids). Lewis acid treatment of the epoxide **12** yielded the desired 6-ketone **13**. Although 6-oxosteroids of the normal series give a single oxime only, ketone **13** reacted with hydroxylamine to give two oximes (**14**, **15**). Their ^{1}H NMR spectra[21] were conspicuously different with

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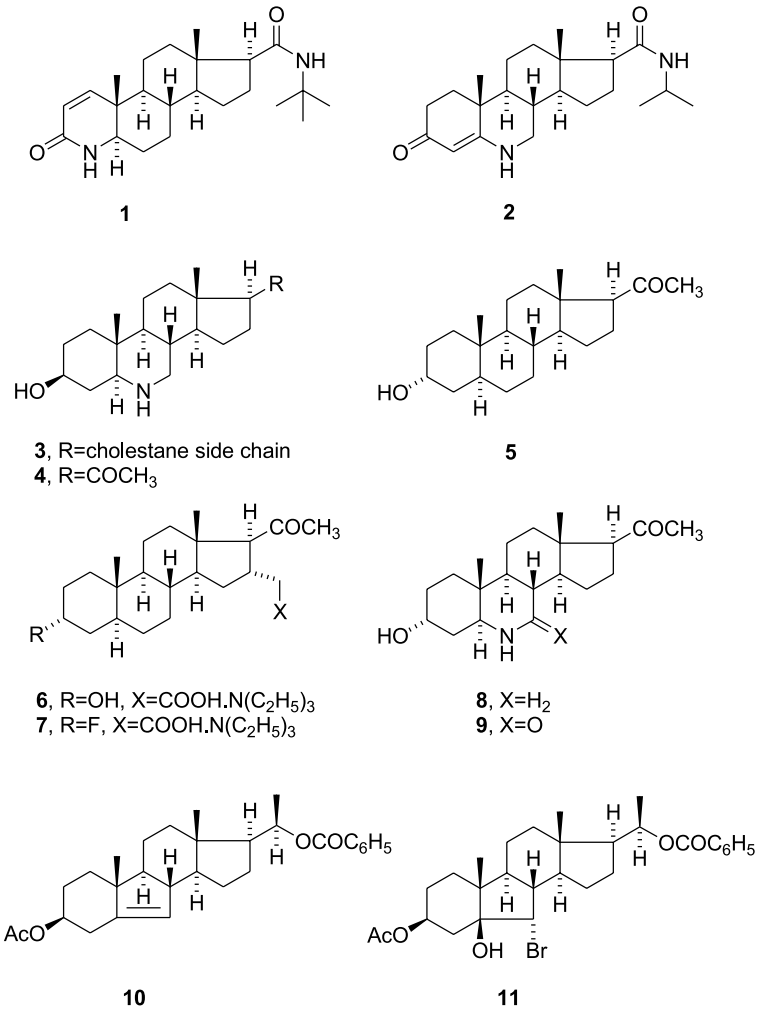
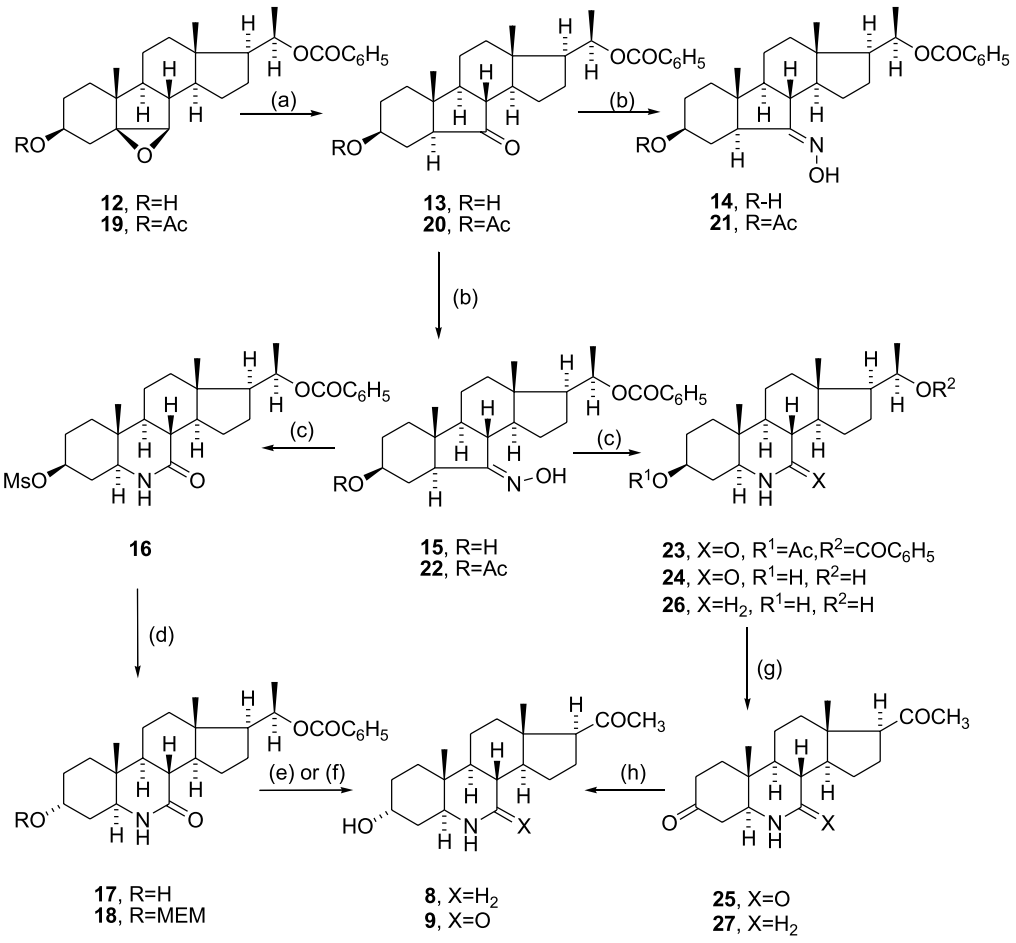


Figure 1.

the presence or absence of a signal at δ 3.04. To distinguish between the *E*- and *Z*-configuration of the oximes, the complete structural assignment of all proton and carbon signals in their ^{1}H and ^{13}C NMR spectra was carried out using homonuclear and heteronuclear 2D-COSY experiments (for data—see Table 1). The stereochemical assignment of methylene protons (in α- and β-position) was derived from 2D-ROESY spectra using mainly the NOE contacts of β-protons with the 18- and/or 19-Me group. The comparison of proton chemical shifts in both isomers shows a significant downfield shift (0.95 ppm) for H-4α in the minor and more lipophilic oxime **14**, while the major and less lipophilic oxime **15** shows a smaller downfield shift (0.37 ppm) for H-15α. The inspection of models indicates that H-4α is sterically closer to the oxime oxygen atom in the isomer with the *Z*-configuration and the H-15α appears very close to the oxime oxygen atom in the isomer with the *E*-configuration (H···O distances are ca. 2.4 and 2.6 Å, respectively); the van der Waals deshielding effect is presumably responsible for the observed downfield shifts. Analogous downfield shifts were observed also for the corresponding carbon atoms (1.99 ppm for C-4 and 1.59 ppm for C-15). Therefore, the *Z*-configuration could be assigned to oxime **14** and *E*-configuration to oxime **15**.

The major oxime **15** was submitted to the Beckman rearrangement, induced with mesyl chloride in pyridine. The ^{1}H NMR spectrum of the resulting 3β-mesyloxy lactam **16** confirmed the above assignment of configuration: the proton next to the nitrogen atom (i.e., the H-5) interacted strongly with H-4 hydrogens ($J=12.6$, 3.2 Hz). The solvolysis of the mesylate **16** in the presence of potassium nitrite in DMSO produced alcohol **17** with the required 3α-configuration. Before the subsequent transformations, its hydroxy group was protected by etherification: the 20-benzoyloxy group in the methoxyethoxymethoxy (MEM) ether **18** was hydrolysed, and oxidised; deprotection afforded the 6-aza analogue of 7-oxo-allopregnanolone **9**. Equally, the MEM-ether **18** was reduced with lithium aluminium hydride, oxidised and deprotected to yield the desired 6-aza analogue of allopregnanolone **8** (Table 2).

The low yield of the above solvolysis, however, failed to justify the orthogonal protection of both hydroxy groups in the starting material. Therefore, the hydroxy epoxide **12** was first acetylated to 3-acetate **19** and only then treated with boron trifluoride etherate. 3β-Acetoxy ketone **20** also yielded a mixture of two oximes (**21**, **22**), whose respective structures were assigned analogously to the earlier mentioned oximes **14** and **15**. The major and more polar oxime **22** was rearranged upon action of mesyl chloride in pyridine yielding cleanly the 3β-acetoxy lactam sought (**23**).



Scheme 1. Reagents and conditions: (a) $BF_3 \cdot Et_2O$, 64% or 94%; (b) $NH_2OH \cdot HCl$, $KHCO_3$, MeOH; 52%; (c) MsCl, py, 0 °C, 4 h; 97%; (d) KNO_2, DMSO, 115 °C; 26%; (e) KOH, then PCC, then HCl; 72%; (f) LAH, then CrO_3, then HCl, 20%; (g) CrO_3, 57%; (h) H_2IrCl_6, H_3PO_3, $(Me)_2CHOH$; 57 or 83%.

Table 1. Proton and carbon-13 chemical shifts of oximes **14**, **15**, **21** and **22** in $CDCl_3$

Proton	**14**	**15**	**21**	**22**	Carbon	**14**	**15**	**21**	**22**
1α	1.13	1.17	1.17	1.21	1	34.28	34.52	34.13	34.38
1β	1.65	1.68	1.67	1.70	2	30.49	30.88	26.63	26.88
2α	1.87	1.88	1.89	1.90	3	71.97	71.60	73.46	73.33
2β	1.57	1.54	1.60	1.60	4	33.07	31.08	29.36	27.35
3	3.71	3.69	4.80	4.77	5	53.86	53.46	53.53	53.12
4α	3.05	2.10	3.03	2.11	6	163.84	163.76	163.54	163.40
4β	1.65	1.35	1.74	1.43	8	44.88	44.01	44.77	43.88
5	2.13	2.05	2.15	2.09	9	57.00	58.92	56.94	58.90
8	2.35	2.46	2.34	2.46	10	40.67	40.48	40.57	40.37
9	1.00	1.11	1.02	1.13	11	21.01	21.16	20.99	21.13
11α	1.45	1.45	1.45	1.46	12	38.89	39.70	38.89	39.67
11β	1.28	1.28	1.29	1.27	13	44.76	45.28	44.73	45.28
12α	1.25	1.26	1.27	1.31	14	53.36	54.24	53.38	54.19
12β	1.90	1.94	1.92	1.94	15	25.81	27.39	25.11	27.37
14	1.53	1.59	1.54	1.61	16	25.07	25.50	25.77	25.50
15α	1.35	1.74	1.38	1.75	17	54.38	54.57	54.42	54.59
15β	1.81	1.82	1.97	1.84	18	13.07	13.40	13.09	13.40
16α	1.37	1.31	1.36	1.32	19	14.01	12.62	13.89	12.49
16β	1.95	1.78	1.81	1.77	20	73.17	73.36	73.18	73.33
17	1.79	1.82	1.81	1.82	21	20.04	19.98	20.04	19.97
18-Me	0.69	0.76	0.69	0.76	C═O	165.68	165.71	165.69	165.71
19-Me	0.82	0.68	0.84	0.69	Ac: C═O	—	—	170.47	170.52
20	5.15	5.17	5.15	5.17	CH_3	—	—	21.34	21.32
21-Me	1.28	1.27	1.28	1.28	C_6H_5: *i*-	130.75	130.79	130.80	130.82
N-OH	7.89	7.59	7.00	7.32	C_6H_5: *o*-	129.63	129.63	129.64	129.64
OAc	—	—	2.03	2.04	C_6H_5: *m*-	128.34	128.34	128.35	128.34
C_6H_5: *o*-	8.05	8.05	8.05	8.06	C_6H_5: *p*-	132.74	132.73	132.74	132.72
C_6H_5: *m*-	7.44	7.45	7.44	7.44					
C_6H_5: *p*-	7.56	7.56	7.55	7.56					

Table 2. Proton and carbon-13 chemical shifts of aza-derivatives **8**, **9**, **30**, **32** and **34** in $CDCl_3$

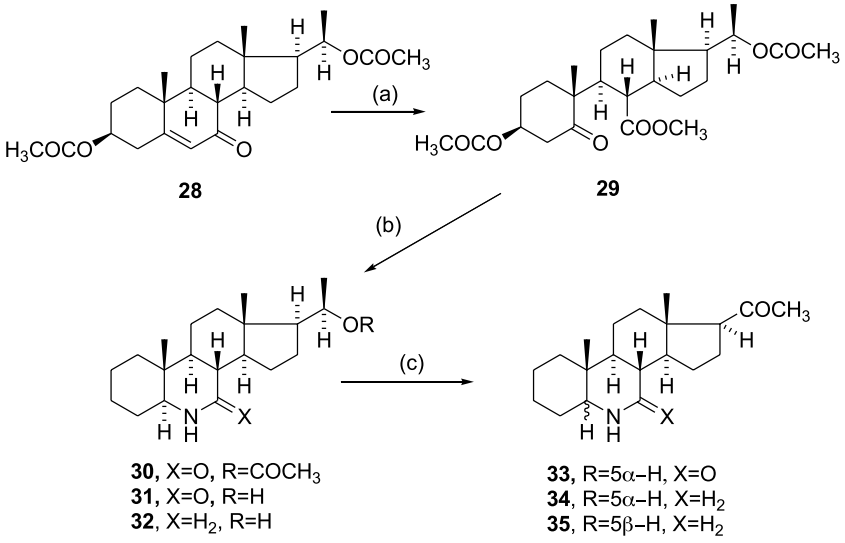
Proton	**8**	**9**	**30**	**32**	**34**	Carbon	**8**	**9**	**30**	**32**	**34**
1α	1.34	~1.46	0.99	0.89	0.90	1	30.32	28.89	34.45	36.53	36.50
1β	1.50	~1.46	1.65	1.66	1.66	2	28.56	28.46	21.01	21.53	21.47
2α	~1.65	~1.76	1.56	1.46	1.48	3	66.73	65.76	24.48	25.64	25.56
2β	~1.65	~1.70	1.52	1.39	1.42	4	35.67	34.16	27.34	28.70	28.53
3α	—	—	1.31	1.29	1.31	5	58.66	55.04	61.72	65.67	65.59
3β	4.15	4.19	1.76	1.71	1.73	7	52.44	173.52	173.43	52.64	52.36
4α	~1.58	~1.60	1.37	~1.36	~1.39	8	35.96	42.35	42.32	35.83	35.83
4β	~1.58	~1.60	1.42	~1.36	~1.39	9	53.67	51.55	51.60	54.16	53.98
5	2.69	3.59	3.01	2.18	2.20	10	36.36	35.33	35.36	36.42	36.40
7α	2.36	—	—	2.30	2.32	11	20.41	20.69	20.73	20.29	20.41
7β	3.02	—	—	2.99	3.01	12	38.91	38.44	38.92	40.02	38.94
8	1.57	2.20	2.18	1.57	1.57	13	44.34	45.66	44.27	42.68	44.34
9	0.90	1.40	1.49	0.78	0.81	14	53.95	52.24	52.02	53.24	53.98
11α	1.62	1.67	1.57	1.50	1.60	15	23.87	26.25	25.74	25.76	23.86
11β	1.31	1.32	1.25	1.28	1.31	16	22.97	23.51	26.05	23.93	22.95
12α	1.43	1.39	1.20	1.24	1.42	17	63.50	62.59	54.13	58.34	63.54
12β	2.03	2.06	1.84	2.05	2.02	18	13.50	13.48	12.68	12.62	13.49
14	1.17	1.58	1.28	1.03	1.16	19	10.94	9.42	10.35	12.08	12.03
15α	~1.63	2.21	1.73	1.65	1.62	20	209.48	209.77	72.95	70.55	209.50
15β	1.20	1.75	1.26	1.16	1.20	21	31.52	31.55	19.92	23.65	31.50
16α	1.66	1.73	1.67	1.09	1.63	OAc: C=O	—	—	170.38	—	—
16β	2.17	2.14	2.15	1.54	2.15	CH_3	—	—	21.54	—	—
17	2.52	2.49	1.59	1.32	2.51						
18-Me	0.62	0.69	0.68	0.76	0.62						
19-Me	0.86	0.87	0.86	0.88	0.88						
20	—	—	4.86	3.72	—						
21-Me	2.12	2.12	1.16	1.13	2.12						
OAc	—	—	2.02	—	—						
NH	[a]	5.38	5.22	[a]	[a]						

[a] Position of NH signal was not determined.

Hydrolysis and oxidation converted the lactam **23** into 3β,20-dihydroxy- and 3,20-dioxo lactams **24** and **25**. The Henbest[22] reaction of the latter (i.e., partial reduction of the 3-oxo group with 2-propanol catalysed with hexachloroiridic acid in the presence of phosphorous acids) produced a monohydroxy ketone identical with the above 3α-hydroxy lactam **9**.

Analogously, the lactam **23** was reduced with lithium aluminium hydride in dioxane yielding dihydroxy amine **26**. Oxidation with chromic acid afforded dioxo amine **27**, the Henbest reduction of which produced a monohydroxy ketone identical with the above 3α-hydroxy-6-aza-5α-pregnan-20-one (**8**).

For comparison, a few 3-deoxy analogues were prepared from (20*R*)-7-oxopregn-5-ene-3β,20-diyl diacetate[23] (**28**, see Scheme 2): oxidation according to a literature protocol[24] yielded crude 5,7-seco-6-nor acid **29** which was treated with ammonia, and hydrogenated: ^{13}C and ^{1}H NMR spectra of lactam **30** proved the loss of the substituent in position 3. The lactams **30** and **31** were converted to lactam **33** and amines **32** and **34** by routine reactions (see Scheme 2). Since the last compound was not found identical with recently



Scheme 2. (a) HIO_4, $KMnO_4$, K_2CO_3, $(CH_3)_3COH$, 45 °C, 6 h; then MeOH, CH_2N_2; (b) NH_3, MeOH, 55 °C, 20 h, then H_2/Pt; 14% of **30** from compound **28**.

published[13] '6-aza-pregnan-20-one' (**35**), additional ^{1}H NMR experiments were carried out to establish the C-5 configuration. The NOE contacts observed in 2D-H,H-ROESY spectrum allowed us to distinguish protons on the α- and β-side of the steroid skeleton: the absence of NOE contact between H-5 proton at δ 2.20 and 19-methyl protons on one side and the observed NOE contact of H-5 with axial protons H-1α, H-3α, H-7α and H-9α on the other side indicate unequivocally the 5α-configuration for our 6-aza-pregnan-20-one **34**. Thus the earlier produced '6-aza-pregnan-20-one' should be given the 5β-configuration (**35**).

2.2. Activity evaluation

Neuronal activity of 6-aza-allopregnanolone (**8**) and its 7-oxo derivative **9** was routinely checked by in vitro tests using [^{3}H]muscimol and [^{35}S]*tert*-butylbicyclo[2.2.2]-phosporothionate (TBPS) and [^{3}H]flunitrazepam as radiolabelled ligands to γ-aminobutyric acid receptors ($GABA_A$). The former two tests utilised receptors isolated from male rat brain and binding of the ligands was measured in the absence and presence of the tested compounds. For the last test, primary neuronal culture, obtained from young rat brains, was used and the binding of [^{3}H]flunitrazepam in neurones was measured in the presence of the varying concentration of the tested compounds. Allopregnanolone was used as a standard to check the viability of the methods. Preliminary results revealed that neither amine **8** nor lactam **9** exerted any binding in the three tests. Surprisingly, low activity only was found in a 3-deoxy analogue **34**. Complete biological results will be published in a separate paper dealing primarily with the testing methods.

3. Conclusions

While the synthesis proceeded according to expectation, no neuronal activity mediated through the γ-aminobutyric acid receptor was found in allopregnanolone analogues **8** and **9**. Nevertheless, even these results can have their value: they may demonstrate that the steroid binding site of the $GABA_A$ receptor requires a steroid compound having no electronegative substitution in the B ring. The earlier report on inactivity of 6-oxa-allopregnanolone[25] points to the same conclusion. Thus the structure–activity consideration should concentrate not only on the presence of polar groups in position 3 and 20, but should also reflect the lipophilic region at the B ring. Hydrophobic interactions between the steroid and the $GABA_A$ receptor apparently have a much greater role than hitherto taken for granted and any replacement of the hydrophobic B ring may lead to the loss of activity. The low activity of the 3-deoxy amine **34** may be explained by the presence of a different binding site within the receptor.

4. Experimental

4.1. General methods and equipment

Melting points were determined on a Koefler melting point micro apparatus Boetius (Germany) and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C /100 Pa. Optical rotations were measured in chloroform using an Autopol IV (Rudolf Research Analytical, Flanders, USA, $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}). IR spectra were recorded on a Bruker IFS 88 spectrometer in chloroform solutions, wave-numbers are given in cm^{-1}. Detailed NMR study of selected compounds was performed on Bruker AVANCE-500 instruments (^{1}H at 500.13 MHz; ^{13}C at 125.77 MHz). Proton NMR spectra of other compounds were measured on Varian UNITY-200 (at 200 MHz) and/or Bruker AVANCE-400 spectrometer (at 400 MHz) in $CDCl_3$ with tetramethylsilane as internal reference. Chemical shifts are given in ppm (δ-scale) and coupling constants in Hz. Unless otherwise stated, the data were interpreted as the first-order spectra. Thin-layer chromatography (TLC) was performed on silica gel (ICN Biochemicals). Preparative TLC (PLC) was carried out on 200×200 mm plates coated with a 0.7-mm thick layer of the same material. For column chromatography, 60–120 μ silica gel was used. Whenever aqueous solutions of hydrochloric acid, potassium hydrogencarbonate or carbonate were used, their concentration was 5%. Solvents were evaporated on a rotary evaporator in vacuo (0.2 kPa, bath temperature 40 °C).

The [^{3}H]flunitrazepam test of binding of the products was carried out by using neurones in culture.[26] The TBPS and muscimol test was done with $GABA_A$ receptors.[15]

4.1.1. (20*R*)-6α-Bromo-5-hydroxy-7-nor-5β-pregnane-3β,20-diyl acetate benzoate (11). A solution of olefin **10** (3.9 g, 8.66 mmol) in dioxane (40 mL) was treated with perchloric acid (10%, 2 mL) and N-bromo acetamide (1.8 g, 13.8 mmol) at 15 °C. After 1 h, the mixture was poured into a cold solution of potassium hydrogen sulfite (7%, 100 mL). The precipitate formed was filtered off, the product was dissolved in chloroform (100 mL) and washed with water (30 mL). The solution was dried over sodium sulfate and filtered through a layer of silica gel (10 g) and the solvent was evaporated in vacuo to give the title compound **11** (4.7 g, 99%) as a colourless oil. A small sample was purified by thin layer chromatography; [found: C, 63.1; H, 7.4; Br, 14.1. $C_{29}H_{39}BrO_5$ requires C, 63.32; H, 7.18; Br, 14.61%]. δ_H (200 MHz, $CDCl_3$) 8.04 (2H, m, *ortho*-Ar*H*), 7.60 (1H, m, *meta*-Ar*H*), 7.45 (2H, t, *para*-Ar*H*), 5.35–5.10 (1H, m, *H*-3), 5.21–5.10 (1H, m, *H*-20), 4.34 (1H, d, J=6.2 Hz, *H*-6), 2.06 (3H, s, *Me*CO), 1.28 (3H, d, J=6.0 Hz, *H*-21), 0.90 (3H, s, *H*-19), 0.72 (3H, s, *H*-18).

4.1.2. (20*R*)-5,6β-Epoxy-3β-hydroxy-7-nor-5β-pregnan-20-yl benzoate (12). A solution of bromohydrin **11** (2.5 g, 4.57 mmol) in methanol (40 mL) was treated with the solution of potassium carbonate (1.5 g, 10.9 mmol) in water (10 mL) under stirring at laboratory temperature. After 20 h, the solution was concentrated in vacuo to a quarter of its volume and the product was precipitated on addition of brine (50 mL). The product was filtered off, dissolved in methylene chloride (10 mL), washed with water (2× 40 mL) and dried. The solvent was evaporated in vacuo to give the title compound as a white amorphous solid **12** (1.88 g, 97%), mp 88–90 °C; [found: C, 76.2; H, 8.6. $C_{27}H_{36}O_4$ requires C, 76.38; H, 8.55%]; $[\alpha]_D$=−37.1 (*c* 0.3, $CHCl_3$); ν_{max} ($CHCl_3$) 3609, 1728, 1707, 1451, 1284, 1050, 714; δ_H (200 MHz, $CDCl_3$) 8.04 (2H, m, *ortho*-Ar*H*),

7.60 (1H, m, *meta*-Ar*H*), 7.45 (2H, t, *para*-Ar*H*), 5.22–5.04 (1H, m, *H*-20), 4.0–3.84 (1H, m, *H*-3), 3.19 (1H, s, *H*-6), 2.37–2.25 (1H, m, *H*-16), 1.27 (3H, d, $J=6.0$ Hz, *H*-21), 0.84 (3H, s, *H*-19), 0.64 (3H, s, *H*-18).

4.1.3. (20*R*)-3β-Hydroxy-6-oxo-7-nor-5α-pregnan-20-yl benzoate (13). To a solution of epoxide **12** (1.880 g, 4.23 mmol) in tetrahydrofuran (50 mL) was added a solution of boron trifluoride etherate (0.3 mL, 2.37 mmol) in ether (50 mL) under stirring at laboratory temperature. After 20 h, the solution was diluted with ether (150 mL), washed with the solution of potassium hydrogen carbonate (50 mL) and brine (50 mL). The solvents were removed in vacuo and the product was purified by chromatography on silica (80 g, toluene–ether 5:1). The major component (1.21 g, 64%) consisted of the title compound **13**. Mp 174–176 °C (acetone–heptane); [found: C, 76.1; H, 8.6. $C_{27}H_{36}O_4$ requires C, 76.38; H, 8.55%]; $[\alpha]_D=+47.9$ (*c* 0.3, $CHCl_3$); ν_{max} ($CHCl_3$) 3609, 1729; 1707, 1284, 1274, 1050, 960, 714; δ_H (200 MHz, $CDCl_3$) 8.06 (2H, m, *ortho*-Ar*H*), 7.57 (1H, m, *meta*-Ar*H*), 7.45 (2H, t, *para*-Ar*H*), 5.24–5.08 (1H, m, *H*-20), 3.71–3.54 (1H, m, *H*-3), 1.28 (3H, d, $J=6.0$ Hz, *H*-21), 0.84 (3H, s, *H*-19), 0.64 (3H, s, *H*-18).

4.1.4. (*Z*,20*R*)-3β-Hydroxy-6-oximino-7-nor-5α-pregnan-20-yl benzoate (14). A solution of ketone **13** (200 mg, 0.47 mmol) in methanol (10 mL) was stirred with potassium hydrogen carbonate (280 mg, 2.8 mmol) and hydroxylamine hydrochloride (200 mg, 1.76 mmol) at reflux temperature. After 5 h, the mixture was diluted with brine (60 mL) and cooled in a refrigerator. The precipitate was dissolved in methylene chloride (50 mL) and washed with the potassium hydrogen carbonate solution (2× 20 mL). The solvent was removed in vacuo and the product applied on a column of silica (50 mL). A mixture of ethyl acetate and toluene (3:1) eluted the title compound **14** (62 mg, 29%) as a colourless solid. Mp 164–167 °C (toluene); [found: C, 73.8; H, 8.4; N, 2.8. $C_{27}H_{37}NO_4$ requires C, 73.77; H, 8.48; 3.19% N]; $[\alpha]_D=+38.2$ (*c* 0.2, $CHCl_3$); ν_{max} ($CHCl_3$) 3604, 3297, 3169, 1707, 1666, 1452, 1282, 1052, 965, 941, 714; δ_H (200 MHz, $CDCl_3$) 8.06 (2H, m, *ortho*-Ar*H*), 7.56 (1H, m, *meta*-Ar*H*), 7.44 (2H, t, *para*-Ar*H*), 5.25–5.07 (1H, m, *H*-20), 3.82–3.61 (1H, m, *H*-3), 3.05 (1H, bd, $J=12.0$ Hz, *H*-4α), 1.28 (3H, d, $J=6.0$ Hz, *H*-21), 0.82 (3H, s, *H*-19), 0.69 (3H, s, *H*-18).

4.1.5. (*E*,20*R*)-3β-Hydroxy-6-oximino-7-nor-5α-pregnan-20-yl benzoate (15). The more polar eluate of the above chromatography yielded the title compound **15** (107 mg, 52%) as white crystals, mp 155–157 °C (methanol–ether); [found: C, 73.8; H, 8.4; N, 2.9. $C_{27}H_{37}NO_4$ requires C, 73.77; H, 8.48; N, 3.19%]; $[\alpha]_D=+27.8$ (*c* 0.1, $CHCl_3$); ν_{max} ($CHCl_3$) 3610, 3589, 3295, 1706, 1667, 1603, 1586, 1277, 1049, 969; δ_H (200 MHz, $CDCl_3$) 8.06 (2H, m, *ortho*-Ar*H*), 7.56 (1H, m, *meta*-Ar*H*), 7.44 (2H, t, *para*-Ar*H*), 5.26–5.08 (1H, m, *H*-20), 3.79–3.60 (1H, m, *H*-3), 2.47 (1H, t, $J=10.2$ Hz, H-8), 1.27 (3H, d, $J=6.0$ Hz, *H*-21), 0.77 (3H, s, *H*-19), 0.68 (3H, s, *H*-18).

4.1.6. (20*R*)-6-Aza-7-oxo-5α-pregnane-3β,20-diyl mesylate benzoate (16). Mesyl chloride (0.2 mL, 2.6 mmol) was dripped into a solution of oxime **15** (140 mg, 0.32 mmol) in pyridine (1 mL) at 0 °C under stirring. After 4 h, the reagent was destroyed with crushed ice (10 g) and the precipitate was filtered off. The product was dissolved in methylene chloride (40 mL) and washed with the solution of hydrochloric acid (10 mL), water (5 mL) and potassium hydrogen carbonate (10 mL). The extract was dried over sodium sulfate and the solvent was evaporated to give the title compound **16** (160 mg, 97%) mp 201–202 °C (CH_2Cl_2 and ether);[found: C, 65.3; H, 7.3; N, 3.0. $C_{28}H_{39}NO_6S$ requires C, 64.96; H, 7.59; N, 2.71%]; $[\alpha]_D=-17.2$ (*c* 0.3, $CHCl_3$); ν_{max} ($CHCl_3$) 3390, 1706, 1657, 1603, 1585, 1344, 1358, 1175, 714, 533; δ_H (200 MHz, $CDCl_3$) 8.05 (2H, m, *ortho*-Ar*H*), 7.56 (1H, m, *meta*-Ar*H*), 7.44 (2H, t, *para*-Ar*H*), 5.34 (1H, s, *H*–N), 5.24–5.09 (1H, m, *H*-20), 4.77–4.56 (1H, m, *H*-3), 3.03 (3H, s, *Me*OSO_2), 1.28 (3H, d, $J=6.0$ Hz, *H*-21), 0.90 (3H, s, *H*-19), 0.72 (3H, s, *H*-18).

4.1.7. (20*R*)-3α-Hydroxy-6-aza-7-oxo-5α-pregnan-20-yl benzoate (17). A solution of mesylate **16** (80 mg, 0.15 mmol) in DMSO (2 mL) was stirred at 115 °C with potassium nitrite (250 mg, 2.94 mmol) under nitrogen. Brine (5 mL) was added and the mixture cooled in a refrigerator. The precipitate was filtered off, washed with water (25 mL) and dried. The product purified by PLC (ethyl acetate). The most polar component was identified the title compound **17** (18 mg, 26%), mp 153–154 and then 233–235 °C (acetone–heptane); [found: C, 73.6; H, 8.4; N, 3.0. $C_{27}H_{37}NO_4$ requires: C, 73.77; H, 8.48; N, 3.19% N]; $[\alpha]_D=-10.0$ (*c* 0.3, $CHCl_3$); ν_{max} ($CHCl_3$) 3614, 3392, 1706, 1651, 1603, 1281, 1586, 1006, 714; δ_H (200 MHz, $CDCl_3$) 8.05 (2H, m, *ortho*-Ar*H*), 7.56 (1H, m, *meta*-Ar*H*), 7.44 (2H, t, *para*-Ar*H*), 5.25–5.08 (1H, m, *H*-20), 4.78–4.68 (2H, m, OCH_2O), 4.26–4.16 (1H, m, *H*-3), 3.58 (1H, dd, $J=4.6$, 11.6 Hz, *H*-5), 2.19 (1H, t, $J=10.6$ Hz, *H*-16), 1.28 (3H, d, $J=6.0$ Hz, *H*-21), 0.82 (3H, s, *H*-19), 0.72 (3H, s, *H*-18).

4.1.8. (20*R*)-3α-(2′-Methoxyethoxy)methoxy-6-aza-7-oxo-5α-pregnan-20-yl benzoate (18). A solution of 3-alcohol **17** (260 mg, 0.59 mmol) in dichloromethane (3 mL) and *N,N*-diisopropylethylamine (0.5 mL, 3.1 mmol) was treated with (2-methoxyethoxy)methyl chloride (0.3 mL, 2.6 mmol) at laboratory temperature. After 18 h, the mixture was diluted with chloroform (30 mL), washed with an aqueous solution of citric acid (5%, 10 mL) and water (10 mL), and dried. The solvent was evaporated in vacuo to yield the title compound **18** (312 mg, 100%) as colourless oil. $[\alpha]_D=-10.3$ (*c* 0.3, $CHCl_3$). ν_{max} ($CHCl_3$) 3392, 2824, 1707, 1651, 1282, 1177, 989, 714; δ_H (400 MHz, $CDCl_3$) 8.05 (2H, m, *ortho*-Ar*H*), 7.56 (1H, m, *meta*-Ar*H*), 7.44 (2H, t, *para*-Ar*H*), 5.20–5.11 (1H, m, *H*-20), 3.97–3.92 (1H, m, *H*-3), 3.50 (1H, dd, $J=11.8$, 4.8 Hz, *H*-5), 3.40 (3H, s, O*Me*), 2.22 (1H, t, $J=10.6$ Hz, *H*-16), 1.27 (3H, d, $J=6.0$ Hz, *H*-21), 0.83 (3H, s, *H*-19), 0.72 (3H, s, *H*-18).

4.1.9. 3α-Hydroxy-6-aza-5α-pregnane-7,20-dione (9). (a) *By modification of the side chain*. Benzoate **18** (200 mg, 0.38 mmol) was dissolved in methanol (50 mL) and heated to boiling point with a solution of potassium hydroxide (500 mg, 8.9 mmol) in water (15 drops). After 8 h, the solution was concentrated in vacuo to a quarter of its volume, diluted with brine (50 mL) and placed in a refrigerator. The precipitate formed was filtered off, washed

with water (15 mL), dried with sodium sulfate, and concentrated in vacuo to yield (20*R*)-20-hydroxy-3α-(2′-methoxyethoxy)methoxy-6-aza-5α-pregnan-7-one (152 mg, 95%)—as a colourless solid. This crude material was dissolved in methylene chloride (2 mL) and stirred with pyridinium chlorochromate (500 mg, 2.3 mmol) and the suspension of potassium acetate (350 mg, 3.6 mmol) in methylene chloride (3 mL). After 18 h, the mixture was filtered through a column of Celite (5 mL), which was then washed with additional methylene chloride. The combined filtrate and eluate was evaporated in vacuo. The product was deprotected using hydrochloric acid (0.2 mL) in tetrahydrofuran (4 mL). After 18 h, the mixture was diluted with toluene (10 mL), partly evaporated and purified by TLC (ethyl acetate). Elution of the major zone with a mixture of acetone in chloroform (1:6) and evaporation afforded the title compound **9** (91 mg, 72%) as white crystals; mp 231–233 °C (acetone); [found: C, 70.2; H, 8.9; N, 3.8. $C_{20}H_{31}NO_3 \cdot 0.5H_2O$ requires: C, 70.14; H, 9.42; N, 4.09%]; $[\alpha]_D = +39.7$ (*c* 0.2, $CHCl_3$). ν_{max} ($CHCl_3$) 3614, 3392, 1652, 1359, 1007, 1701; δ_H (400 MHz, $CDCl_3$) 5.45–5.39 (1H, m, *H*–N), 4.22–4.15 (1H, m, *H*-3), 3.59 (1H, dd, $J=12.4$, 4.0 Hz, *H*-5α), 2.50 (1H, t, $J=9.3$ Hz, *H*-17), 2.13 (3H, s, *Me*CO), 0.86 (3H, s, *H*-19), 0.69 (3H, s, *H*-18). (b) *From the diketone* **25**. The reagent was prepared from hydrogen hexachloroiridate (50 mg, 0.12 mmol), phosphorous acid (400 mg, 4.9 mmol), 2-propanol (10 mL, 130.5 mmol) and water (2 mL). Part of this solution (1 mL) was added to a test tube containing 3,20-diketone **25** (40 mg, 0.12 mmol). The test tube was sealed and kept in a bath at 85 °C for 18 h. After cooling, the mixture was diluted with ethyl acetate (15 mL), the solution was washed with the solution of potassium hydrogen carbonate and water, dried over sodium sulfate and concentrated in vacuo. The product was purified by TLC (chloroform, acetone 6:1) to yield the title compound **9** (23 mg, 57%), mp 231–233 °C (acetone), identical with the sample prepared above.

4.1.10. 3α-Hydroxy-6-aza-5α-pregnan-20-one (8). (a) *From the 3α-alkoxy derivative* **18**. A solution of compound **18** (300 mg, 0.57 mmol) and lithium aluminium hydride (ca. 200 mg, 5.2 mmol) in dioxane (10 mL) was heated to boiling point under argon. After 5 h, the excess of the reagent was destroyed with wet ether (about 20 mL) and then an aqueous solution of Na_2SO_4 (about 5 mL). The mixture was saturated with anhydrous Na_2SO_4, inorganic material was filtered off and washed with ethyl acetate (60 mL). The filtrate was concentrated in vacuo. The remainder (230 mg, 99%, 0.56 mmol) was dissolved in methylene chloride (3 mL) and added to a suspension of pyridinium chlorochromate (700 mg, 3.25 mmol) and potassium acetate (350 mg, 3.56 mmol) in methylene chloride (4 mL). The mixture was stirred for 18 h at room temperature and then filtered through a layer of Celite (5 mL). The combined filtrate and eluate was evaporated and the remainder was dissolved in tetrahydrofuran (6 mL) containing hydrochloric acid (0.3 mL). After 4 h, the solution was made alkaline with ammonia, partly concentrated in vacuo, and extracted with chloroform (40 mL). The solution was dried over Na_2SO_4, and evaporated. The remainder (110 mg) was purified by chromatography on a column of silica gel (10 mL) in ammoniacal chloroform. Elution of the major zone afforded the title compound **8** (36 mg, 20%), mp 253–255 °C (aqueous methanol); [found: C, 74.9; H, 10.4; N, 4.3. $C_{20}H_{33}NO_2$ requires: C, 75.19; H, 10.41; N, 4.38%]; $[\alpha]_D = +56.7$ (*c* 0.18, $CHCl_3$). ν_{max} ($CHCl_3$) 3615, 3320, 1699, 1386, 1358, 1005; δ_H (400 MHz, $CDCl_3$) 4.17–4.11 (1H, m, *H*-3), 3.02 (1H, dd, $J=11.7$, 4.4 Hz, *H*-7β), 2.69 (1H, dd, $J=12.3$, 4.3 Hz, *H*-5α), 2.52 (1H, t, $J=8.8$ Hz, *H*-17), 2.36 (1H, t, $J=11.7$ Hz, *H*-7α), 2.12 (3H, s, *Me*CO), 0.86 (3H, s, H-19), 0.63 (3H, s, H-18); *m/z* (EI) 319 (M^+, 82), 304 (37), 276 (10), 246 (100%). (b) *From diketone* **27**. Compound **27** (160 mg, 0.50 mmol), hydrogen hexachloroiridate (31 mg, 0.08 mmol), and phosphorous acid (220 mg, 2.68 mmol) were put into a test tube and 2-propanol (3.5 mL, 45.7 mmol) and water (0.7 mL) were added. The tube was sealed and heated at 95 °C. The black mixture turned into a pale solution within the first half an hour. After 18 h, the mixture was diluted with ethyl acetate (50 mL) and transferred into a flask. Potassium carbonate (370 mg, 2.68 mmol) was added and organic solvents were evaporated. The content of the flask was made alkaline with ammonia (5 mL) and steroid products were extracted with chloroform (3×20 mL). The extract was washed with water (10 mL), dried and concentrated in vacuo. The remainder was purified by chromatography on a column of silica gel (25 g). Ammoniacal chloroform with 2% of methanol eluted the title compound **8** (133 mg, 83%) identical with the above sample.

4.1.11. (20*R*)-5,6β-Epoxy-7-nor-5β-pregnane-3β,20-diyl acetate benzoate (19). Epoxide **12** (450 mg, 1.1 mmol) was acetylated with acetic anhydride (0.4 mL) in pyridine (1 mL) at laboratory temperature. After 20 h, the mixture was poured into brine (10 mL), the precipitate formed was extracted with dichloromethane (3×20 mL), washed with the solution of potassium hydrogencarbonate (2×10 mL) and water, and dried. Evaporation of solvents in vacuo was repeated after dilution with toluene (10 mL) in order to remove pyridine from the title compound **19** (450 mg, 91%); the colourless oil failed to crystallise from usual solvents;[found: C, 74.4; H, 8.3. $C_{29}H_{38}O_5$ requires: C, 74.65; 8.21% H]; $[\alpha]_D$ −4.3° (*c* 0.3, $CHCl_3$). ν_{max} ($CHCl_3$) 1728, 1709, 1255, 1071, 1037, 1027, 961, 714; δ_H (200 MHz, $CDCl_3$) 8.04 (2H, m, *ortho*-Ar*H*), 7.55 (1H, m, *meta*-Ar*H*), 7.43 (2H, t, *para*-Ar*H*), 5.23–5.07 (1H, m, *H*-20), 5.04–4.88 (1H, m, *H*-3), 3.19 (1H, s, *H*-6), 2.04 (3H, s, *Me*CO), 1.28 (3H, d, $J=6.0$ Hz, *H*-21), 0.84 (3H, s, *H*-19), 0.63 (3H, s, *H*-18); *m/z* (EI) 406 (16), 344 (6), 284 (13), 209 (14), 149 (14), 105 (46), 91 (100%).

4.1.12. (20*R*)-7-Nor-6-oxo-5α-pregnane-3β,20-diyl acetate benzoate (20). In analogy with the preparation of ketone **13**, epoxide **19** (17.8 g, 38.1 mmol) was treated with a solution of boron trifluoride etherate (3.0 mL, 23.67 mmol) in a mixture of ether (900 mL) and tetrahydrofuran (400 mL). After 20 h, the reaction mixture was worked up as above and the product was purified by chromatography a column of silica (400 g). Ether in toluene (1:30) eluted the title compound **20** (16.8 g, 94%), mp 145–146 °C (ether–heptane); [found: C, 74.7; H, 8.5. $C_{29}H_{38}O_5$ requires C, 74.65; H, 8.21% H]; $[\alpha]_D = +48.1$ (*c* 0.28, $CHCl_3$); ν_{max} ($CHCl_3$) 1730, 1709, 1451, 1283, 1271, 1259, 1044, 994; δ_H (200 MHz, $CDCl_3$) 8.06 (2H, m, *ortho*-Ar*H*), 7.60 (1H, m, *meta*-Ar*H*), 7.45 (2H, t, *para*-Ar*H*), 5.25–5.09 (1H, m, *H*-20), 4.70–4.60 (1H, m, *H*-3), 2.23 (1H, bd, $J=10.8$ Hz,

H-5), 2.04 (3H, s, *Me*CO), 1.28 (3H, d, $J=6.0$ Hz, *H*-21), 0.85 (3H, s, *H*-19), 0.66 (3H, s, *H*-18).

4.1.13. (*Z*,20*R*)-6-Oximino-7-nor-5α-pregnane-3β,20-diyl acetate benzoate (21). A solution of ketone **20** (750 mg, 1.6 mmol) in methanol (10 mL) was stirred with potassium hydrogen carbonate (1 g, 9.99 mmol) and hydroxylamine hydrochloride (750 mg, 10.8 mmol) at reflux temperature. After 5 h, the mixture was worked up as in the preparation of oxime **14** and the product was applied on a column of silica (80 g). Ether in toluene (1:10) eluted the title compound **21** (176 mg, 23%), mp 193–195 °C (ether–heptane); [found: C, 72.3; H, 8.4; 2.8. $C_{29}H_{39}NO_5$ requires: C, 72.32; H, 8.16; N, 2.91%]; $[\alpha]_D=+40.2$ (*c* 0.2, $CHCl_3$); ν_{max} ($CHCl_3$) 3587, 3 298, 1729, 1708, 1452, 1281, 1271, 1262, 1246, 1047, 1033, 965, 941, 714. δ_H (200 MHz, $CDCl_3$) 8.05 (2H, m, *ortho*-Ar*H*), 7.56 (1H, m, *meta*-Ar*H*), 7.44 (2H, t, *para*-Ar*H*), 5.23–5.07 (1H, m, *H*-20), 4.90–4.70 (1H, m, *H*-3), 3.03 (1H, bd, $J=10.8$ Hz, *H*-4α), 2.03 (3H, s, *Me*CO), 1.28 (3H, d, $J=6.0$ Hz, *H*-21), 0.84 (3H, s, *H*-19), 0.69 (3H, s, *H*-18).

4.1.14. (*E*,20*R*)-6-Oximino-7-nor-5α-pregnane-3β,20-diyl acetate benzoate (22). A more polar fractions from the above chromatography yielded the title compound **22** (384 mg, 51%) as white crystals, mp 203–205 °C (ether–heptane); [found: C, 72.2; H, 8.3; N, 2.7. $C_{29}H_{39}NO_5$ requires C, 72.32; H, 8.16; N, 2.91%]; $[\alpha]_D=+30.2$ (*c* 0.2, $CHCl_3$); ν_{max} ($CHCl_3$) 3586, 3 296, 1728, 1708, 1667, 1451, 1282, 1274, 1259, 1049, 1033, 957, 924, 965, 714; δ_H (200 MHz, $CDCl_3$) 8.06 (2H, m, *ortho*-Ar*H*), 7.56 (1H, m, *meta*-Ar*H*), 7.44 (2H, t, *para*-Ar*H*), 5.26–5.09 (1H, m, *H*-20), 4.86–4.68 (1H, m, *H*-3), 2.46 (1H, bt, $J=9.9$ Hz, *H*-8β), 2.03 (3H, s, *Me*CO), 1.28 (3H, d, $J=6.0$ Hz, *H*-21), 0.76 (3H, s, *H*-19), 0.69 (3H, s, *H*-18).

4.1.15. (20*R*)-6-Aza-7-oxo-5α-pregnane-3β,20-diyl acetate benzoate (23). Oxime **22** (360 mg, 0.75 mmol) was dissolved in pyridine (1.0 mL) and cooled to 0 °C under stirring. Mesyl chloride (0.3 mL, 3.9 mmol) was dripped into the solution. After 2 h, the reagent was destroyed with crushed ice (10 g) and the precipitate was filtered off. The product was dissolved in methylene chloride (20 mL) and washed with the solution of hydrochloric acid, water and the potassium hydrogen carbonate solution. The extract was dried over sodium sulfate and the solvent was evaporated to yield the title compound **23** (360 mg, 100%) as white crystals. Mp 211–214 °C (278 mg, 77%, acetone–heptane); [found: C, 71.9; H, 8.3; N 2.80. $C_{29}H_{39}NO_5$ requires C, 72.32; H, 8.16; N, 2.91%]; $[\alpha]_D=-19.7$ (*c* 0.2, $CHCl_3$); ν_{max} ($CHCl_3$) 3392, 1731, 1709, 1654, 1273, 1252, 1036, 1027, 964, 714; δ_H (200 MHz, $CDCl_3$) 8.04 (2H, m, *ortho*-Ar*H*), 7.57 (1H, m, *meta*-Ar*H*), 7.44 (2H, t, *para*-Ar*H*), 5.24–5.09 (1H, m, *H*-20), 4.85–4.65 (1H, m, *H*-3), 4.16–3.62 (1H, m, *H*–N), 3.21 (1H, bd, $J=12.0$ Hz, *H*-5), 2.03 (3H, s, *Me*CO), 1.28 (3H, d, $J=6.0$ Hz, *H*-21), 0.88 (3H, s, *H*-19), 0.74 (3H, s, *H*-18); *m*/*z* (EI) 481 (M^+, 27), 359 (25), 344 (14), 224 (8), 105 (40), 65 (44), 43 (100%).

4.1.16. (20*R*)-3β,20-Dihydroxy-6-aza-5α-pregnan-7-one (24). Diester **23** (43 mg, 0.09 mmol) was treated with a boiling solution of potassium hydroxide (104 mg, 1.85 mmol) in aqueous methanol (2 drops of water, 10 mL of methanol). After 8 h, the solution was concentrated in vacuo, diluted with brine (5 mL) and placed in a refrigerator. The title compound **23** (29 mg, 100%) formed precipitate, which was filtered off and washed with water (15 mL); mp 274–275 °C (acetone); [found: C, 69.7; H, 9.9; N, 3.9. $C_{20}H_{33}NO_3\cdot 0.5H_2O$ requires C, 69.73; H, 9.95; N, 4.07%]; $[\alpha]_D=-10.1$ (*c* 0.35, $CHCl_3$). ν_{max} ($CHCl_3$) 3609, 3393, 1653, 1042; δ_H (200 MHz, $CDCl_3$) 5.27–5.19 (1H, m, *H*–N), 3.82–3.62 (2H, m, *H*-3, *H*-20), 3.05 (1H, dd, $J=12.2$, 3.6 Hz, *H*-5), 1.15 (3H, d, $J=6.1$ Hz, *H*-21), 0.92 (3H, s, *H*-19), 0.80 (3H, s, *H*-18).

4.1.17. (20*R*)-6-Aza-5α-pregnane-3β,20-diol (26). Dry lactam **23** (147 mg, 0.31 mmol) was put in a dripping funnel placed between a reflux condenser and a flask with a boiling solution of lithium aluminium hydride (ca. 200 mg, 5.27 mmol) in dioxane (10 mL). The substrate was gradually dissolved in the solvent condensed and washed into the solution. After 6 h, the solution was cooled, the excess of reagent was destroyed with ethyl acetate and a saturated, aqueous solution of sodium sulfate. Anhydrous sodium sulfate was added and the solution was filtered over sodium sulfate. The filter cake was washed with hot chloroform (3×30 mL). The solvent was evaporated in vacuo to yield the title compound **26** (69 mg, 70%), mp 189–192 °C (chloroform); [found: C, 67.2; H, 10.7; N, 3.5. $C_{20}H_{35}NO_2.2H_2O$ requires C, 67.19; H, 10.99; N, 3.92%]; $[\alpha]_D=-7.8$ (*c* 0.13, $CHCl_3$) ν_{max} ($CHCl_3$) 3614, 3394, 1045; δ_H (200 MHz, $CDCl_3$) 3.81–3.52 (2H, m, *H*-3, *H*-20), 3.13 (1H, dd, $J=12.4$, 4.8 Hz, *H*-5), 1.14 (3H, d, $J=6.2$ Hz, *H*-21), 0.90 (3H, s, *H*-19), 0.76 (3H, s, *H*-18).

4.1.18. 6-Aza-5α-pregnane-3,7,20-trione (25). Diol **24** (135 mg, 0.38 mmol) was dissolved in acetone (20 mL) and treated with Jones reagent at laboratory temperature. After 15 min, the reagent was decomposed with methanol (1 mL), the solution diluted with chloroform (100 mL) and its volume was reduced in vacuo to quarter of its volume. The mixture was washed with a solution of potassium hydrogen carbonate and brine. The mixture was dried over sodium sulfate and the solvent was evaporated. Purification of the crude product by PLC (ammoniacal chloroform with 10% of acetone) yielded the title compound **25** (77 mg, 58%), mp 249–250 °C (acetone–heptane); [found: C, 70.4; H, 8.6; N, 4.0. $C_{20}H_{29}NO_3\cdot 0.5H_2O$ requires C, 70.56; H, 8.88; N, 4.11%]; $[\alpha]_D=+37.4$ (*c* 0.36, $CHCl_3$); ν_{max} ($CHCl_3$) 3390, 1714, 1704, 1660, 1418, 1356, 1322; δ_H (200 MHz, $CDCl_3$) 5.34–5.27 (1H, s, *H*–N), 3.42 (1H, dd, $J=12.8$, 5.5 Hz, *H*-5), 2.14 (3H, s, *H*-21), 1.10 (3H, s, *H*-19), 0.72 (3H, s, H-18); *m*/*z* (EI) 317 (M^+, 6), 302 (5), 279 (3), 246 (100%).

4.1.19. 6-Aza-5α-pregnane-3,20-dione (27). A solution of chromium trioxide (230 mg, 2.3 mmol) in water (8 drops) was added to a solution of diol **26** (286 mg, 0.89 mmol) in acetic acid (13 mL) under stirring at room temperature. After 24 h, the mixture was cooled with ice and made alkaline with ammonia (30 mL). The resulting precipitate was extracted with ether, the extract washed with water and dried. Chromatography on a silica column (16 g) in ammoniacal chloroform yielded the title compound **27** (162 mg, 56%), mp 202–204 °C (toluene–heptane); $[\alpha]_D=+79.5$ (*c* 0.3, $CHCl_3$); δ_H (200 MHz, $CDCl_3$) 3.05 (1H, dd, $J=11.7$, 4.3 Hz, *H*-5), 2.52 (1H, t, $J=8.7$ Hz,

H-17), 2.12 (3H, s, *H*-21), 1.07 (3H, s, *H*-19), 0.66 (3H, s, *H*-18); HRMS (EI) M^+ found 317.23539. $C_{20}H_{31}NO_2$ requires 317.23548.%H, 10.46%, 4.16% N.

4.1.20. (20*R*)-6-Aza-7-oxo-5α-pregnan-20-yl acetate (30). To a solution of (20*R*)-7-oxopregn-5-ene-3β,20-diyl diacetate (**28**, 8.0 g, 19.2 mmol) in 2-methyl-2-propanol (480 mL) were added[24] aqueous solutions of potassium carbonate (5.0 g, 36.2 mmol in 140 mL of water), potassium permanganate (70 mg, 0.44 mmol in 9 mL of water), and sodium periodate (7 g, 32.7 mmol in 80 mL of water). The mixture was stirred for 30 min at laboratory temperature and then an additional solution of sodium periodate (28.0 g, 130.9 mmol, in 350 mL of water) and several crystals of potassium permanganate were added. The solution was stirred for a further 6 h at 45 °C, then potassium hydrogen pyrosulfite was added until the solution became colourless. The solution was partially evaporated, sulfuric acid (5 mL, 93.1 mmol) was added and the organic substance was taken up into diethyl ether. The extract was washed with brine and concentrated in vacuo. Crude seco acid was dissolved in methanol (70 mL) and esterified with diazomethane (140 mL of ether solution). On evaporation, ester **29** was dissolved in methanol (16 mL), transferred into an autoclave and cooled to −60 °C. Liquid ammonia (ca. 20 mL) was added and the autoclave was sealed and heated to 55 °C for 20 h. The mixture was concentrated in vacuo and hydrogenated in acetic acid (50 mL) on Adam's catalyst (240 mg). Flash chromatography on a column of silica gel (toluene–ethyl acetate, 10:1) yielded the title compound **30** (940 mg, 14%); mp 280–282 °C (acetone); [found: C, 72.7; H, 10.2; N, 3.9. requires C, 73.09; H, 9.76; N, 3.87%]; $[\alpha]_D=+25.2$ (*c* 0.4, $CHCl_3$). ν_{max} ($CHCl_3$) 3392, 1723, 1651, 1258, 1050; δ_H (200 MHz, $CDCl_3$) 5.43–5.33 (1H, bs, *H*–N), 4.94–4.76 (1H, s, *H*-20), 3.01 (1H, dd, $J=10.0$, 5.0 Hz, *H*-5), 2.02 (3H, s, *Me*CO), 1.16 (1H, d, $J=6.2$ Hz, *H*-20), 0.87 (3H, s, *H*-19), 0.68 (3H, s, *H*-18); *m/z* (EI): 361 (M^+, 100), 333 (12), 318 (7), 302 (36), 286 (24), 206 (22), 192 (35%).

4.1.21. (20*R*)-20-Hydroxy-6-aza-5α-pregnan-7-one (31). Acetate **30** (860 mg, 2.38 mmol) was hydrolysed in a solution of hydrochloric acid (8.0 mL, 97.4 mmol) in chloroform (15 mL) and methanol (100 mL) at 50 °C for 44 h. A mixture was concentrated in vacuo to a quarter of its volume. After addition of brine (50 mL), the title compound **31** (585 mg, 77%) precipitated; mp 264–265 °C (toluene); [found: C, 75.3; H, 10.6; N,4.3. $C_{20}H_{33}NO_2$ requires C, 75.19; H, 10.41; 4.38%N]; $[\alpha]_D=-16.1$ (*c* 0.3, $CHCl_3$). ν_{max} ($CHCl_3$) 3609, 3392, 1648, 1102, 1093; Circular dichroism: $\Delta\varepsilon_{221}$ −1.2, $\Delta\varepsilon_{244}$ +0.3; δ_H (200 MHz, $CDCl_3$) 5.37–5.27 (1H, m, *H*–N), 3.84–3.66 (1H, m, *H*-20), 3.01 (1H, dd, $J=10.5$, 4.9 Hz, *H*-5), 1.15 (3H, d, $J=6.1$ Hz, *H*-21), 0.87 (3H, s, *H*-19), 0.80 (3H, s, *H*-18).

4.1.22. 6-Aza-5α-pregnan-7,20-dione (33). Alcohol **31** (100 mg, 0.33 mmol) was oxidised with Jones reagent in acetone (130 mL) at laboratory temperature. Excessive reagent was reduced with methanol, the solvent was partially removed on a rotary evaporator, and the product was precipitated with the solution of potassium hydrogen carbonate. Organics were extracted with chloroform, washed and dried. The solvent was evaporated in vacuo to yield the title compound **33** (88 mg, 89%); mp 247–251 °C (toluene); [found: C, 75.2; H, 9.8; N, 4.2. $C_{20}H_{31}NO_2$ requires C, 75.67; H, 9.84; N, 4.41%]; $[\alpha]_D=+48.5$ (*c* 0.1, $CHCl_3$). ν_{max} ($CHCl_3$) 3391, 1701, 1652, 1357; δ_H (200 MHz, $CDCl_3$) 5.37–5.27 (1H, m, *H*–N), 3.02 (1H, dd, $J=10.5$, 4.9 Hz, *H*-5), 2.13 (3H, s, *H*-21), 0.88 (3H, s, *H*-19), 0.69 (3H, s, *H*-18).

4.1.23. (20*R*)-6-Aza-5α-pregnan-20-ol (32). Lactam **31** (430 mg, 1.35 mmol) was reduced with lithium aluminium hydride as in Section 4.1.17. The chloroform extract was evaporated in vacuo to yield the title compound **32** (365 mg, 88%); mp 160–162 °C (acetone–heptane); [found: C, 78.4; H, 11.7; N, 4.4. $C_{20}H_{35}NO$ requires C, 78.63; H, 11.55; N, 4.58%]; $[\alpha]_D=-10.2$ (*c* 0.2, $CHCl_3$). δ_H (200 MHz, $CDCl_3$) 3.81–3.63 (1H, m, *H*-20), 1.14 (3H, d, $J=6.1$ Hz, *H*-21), 0.88 (3H, s, *H*-19), 0.76 (3H, s, *H*-18).

4.1.24. 6-Aza-5α-pregnan-20-one (34). Alcohol **32** (100 mg, 0.33 mmol) was oxidised as in Section 4.1.19. The solvent evaporated and the product was purified by PLC (ammoniacal $CHCl_3$ and 5% MeOH) to yield the title compound **34** (58 mg, 58%); mp 129–131 °C (ether); [found: C, 78.8; H, 11.1; N, 4.54. $C_{20}H_{33}NO$ requires C, 79.15; H, 10.96; N, 4.62%]; $[\alpha]_D=+81.0$ (*c* 0.1, $CHCl_3$). δ_H (200 MHz, $CDCl_3$) 3.02 (1H, dd, $J=11.6$, 4.4 Hz, *H*-7), 2.51 (1H, t, $J=8.4$ Hz, *H*-17), 2.11 (3H, s, *H*-21), 0.87 (3H, s, *H*-19), 0.62 (3H, s, *H*-18).

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