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Neurosteroids: 7-aza-allopregnanolone—a poor substitute for allopregnanolone

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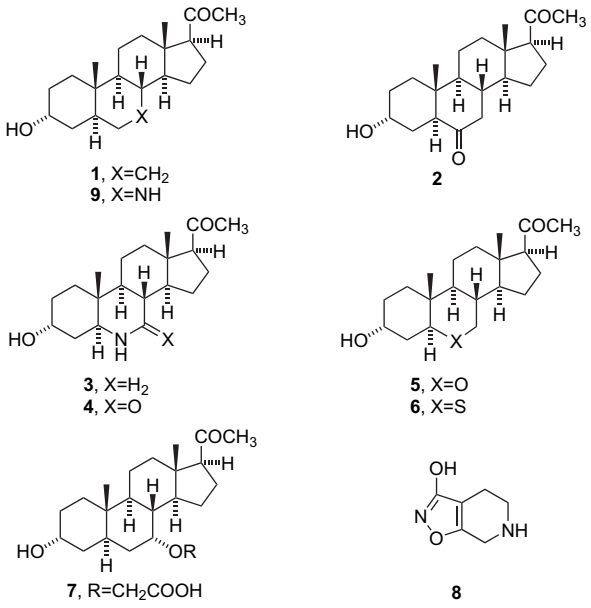
Abstract—7-Nor-20-oxopregn-5-en-3β-yl acetate was converted into (20*R*)-5β,6β-epoxy-7-nor-5β-pregnane-3β,20-diyl diacetate in three steps. Stereospecific migration of the 6α-hydride ion led to a 6-oxo derivative with a 5α-configuration. The (*Z*)-oxime of this ketone underwent Beckmann rearrangement to yield a lactam with the nitrogen in position 7. Lithium aluminium hydride reduction yielded the dihydroxy amine, which was either oxidised or Boc-protected and then oxidised to 7-aza-5α-pregnane-3,20-dione. Its regioselective reduction produced 7-aza-3α-hydroxy-5α-pregnan-20-one—a poor inhibitor for the binding of [^{35}S]TBPS to the $GABA_A$ receptor. The corresponding lactam—7-aza-3α-hydroxy-5α-pregnane-6,20-dione—was inactive.

1. Introduction

Steroid chemistry—once the golden egg of the pharmaceutical sciences—was several times considered outdated and still it repeatedly enjoyed a renaissance. The structures of the steroid hormones have been emulated by organic chemists many times, and various types of modification have resulted. New types of substitution of the classical steroid skeleton have been examined[1] or the skeleton itself was modified by increasing[2] or decreasing[3–5] the flexibility of the molecule or even reversing its chirality.[6,7] Various changes were made to the steroid side chain (e.g., Refs. 8 and 9). Nature itself has many times shown that a nitrogen atom[10,11] in an appropriate position often leads to pronounced biological effects. The nitrogen atom can be part of a substituent[12–14] or part of the steroid skeleton thus producing azasteroids with a skeletal heteroatom in most positions of steroid hormones.[1,15–34]

One of the classes of steroids, which have enjoyed renewed interest recently are the neurosteroids.[35,36] They can act as modulators of neurotransmitter receptors.[37] Thus, steroid enhancement of submaximal γ-aminobutyric acid (GABA) receptor currently occurs through an increase in both ion channel open frequency and open duration.[38,39] The fundamental neurosteroid[40,41] acting via the GABA receptor ($GABA_A$)-3α-hydroxy-5α-pregnan-20-one ('allopregnanolone', **1**, Scheme 1) has also been investigated many times in order to produce even a better neuro active product applicable in medicine.[35,42,43] However, such modifications at position 6 have sometimes turned out to be ineffective.[30,44] Thus, 6-oxo and 6-aza derivatives **2**–**4** were void of any neural activity when measured with GABA receptor tests using [^3H]flunitrazepam and [^{35}S]TBPS as ligands.[4,43] On the other hand, 6-thia- and some 7-oxygenated allopregnanolone derivatives, compounds **6** and **7**, interacted strongly with the $GABA_A$ receptor.[45] This result as well as reports on the neuronal activity of a non-steroidal piperidine derivative—gaboxadol (**8**) (e.g., Refs. 46 and 47)—prompted us to prepare a corresponding 7-aza analogue of allopregnanolone (compound **9**). The synthesis of **9** and its corresponding bioactivity are described in this paper.



Scheme 1. Neurosteroid **1** and some of its analogues.

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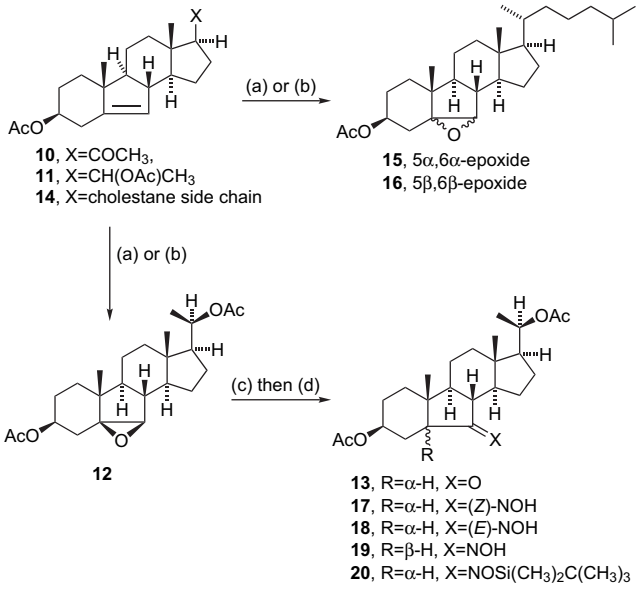
2. Results and discussion

7-Azasteroids have been previously prepared by Morrisawa who reported the synthesis of 7-aza cholestane and androstane derivatives using Beckmann rearrangement of the corresponding 6-oximino-7-nor steroids.[48,49] The starting material for our synthesis was also a B-nor derivative—20-oxo-7-norpregn-5-en-3β-yl acetate[50] (**10**, Scheme 2). It was reduced into the intermediate (20*R*)-20-alcohol, which was immediately protected against oxidation by acetylation to afford diacetate **11**; no effort was employed[30] to secure orthogonal protection[51] of the two hydroxy groups in positions 3 and 20.



Scheme 2. Reagents and conditions: (a) $KMnO_4/FeSO_4$, H_2O, CH_2Cl_2, 20 °C, 2 h; (b) NBA/$HClO_4$, dioxane, 15 °C, then AcONa in EtOH, 80 °C; (c) $BF_3\cdot Et_2O$, THF, 20 °C, 20 h; (d) $NH_2OH\cdot HCl/KHCO_3$, boiling MeOH or EtOH, 0.25–5 h, or *O*-(*tert*-butyldimethylsilyl)hydroxylamine, toluene, 100 °C, 4 h, then Bu_4NF in THF.

For the stereoselective[52] synthesis of 5β,6β-epoxide **12**, ketone **13** was required. The direct oxygenation of olefins with potassium permanganate or ruthenium based catalysts was considered first.[53,54] The solid-phase oxidation of steroidal alkenes with potassium permanganate and metal salts was reported to yield almost exclusively the 5β,6β-epoxides.[53] First, we carried out a model experiment with 7-norcholesteryl acetate (**14**) because this system is known to behave differently to classical steroids: e.g., catalytic hydrogenation[55] affords mainly 5β-dihydro products, whereas 'normal' steroids with the six-membered B ring afford mainly 5α-isomers. Potassium permanganate oxidation of **14** led indeed to a 19:1 mixture of both epoxides **15** and **16**, unfortunately with the prevalence of the unwanted 5α,6α-epoxide **15**. The traditional pathway[56] was next employed. The addition of hypobromous acid to the double bond in **11** followed by alkaline (AcONa) treatment of the intermediate bromohydrin, a stereospecific process, gave the 5β,6β-epoxide **12**. Migration of the 6α-hydride ion to position 5α was enforced by the action of boron trifluoride etherate[57] to produce ketone **13**.

Oximation of B-nor-6-ketones affords a mixture of two isomeric oximes (**17** and **18**).[31,58] Simple ^1H NMR spectra allow us to distinguish both isomers: the presence of a broad doublet (J=12) at δ 3.04 corresponds to the 4α-proton signal of the (*Z*)-oxime **17**. The yield of the minor oxime **17** could not be increased by higher reaction temperatures, which only led to a more complicated reaction mixture with additional side products (e.g., 5β-derivative **19**). The use of a bulkier oximation reagent (*O*-*tert*-butyldimethylsilyl-hydroxylamine) also produced a mixture **20** with the same proportion of (*E*) and (*Z*)-oximes.

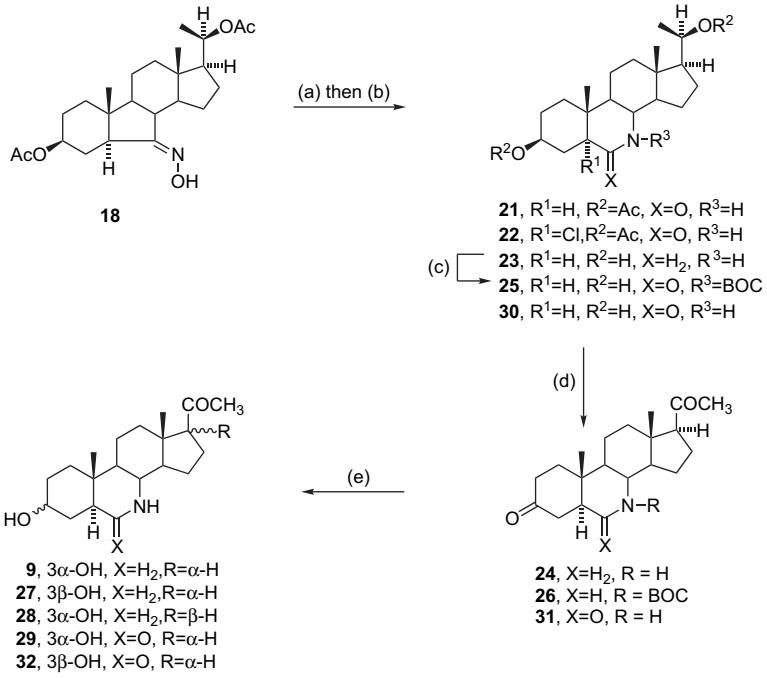
Beckmann rearrangement of the oxime **17** into lactam **21** (Scheme 3) was induced by treatment with mesyl chloride in pyridine; a short reaction time was required at low temperature,[58,59] otherwise a chlorine containing side product **22** was also formed. High-resolution mass spectroscopy (FAB) confirmed the presence of a chlorine atom in the molecule (see a signal of M^+ for $C_{24}H_{36}ClNO_5$ at 454.2377 *m*/*z*).

Lithium aluminium hydride reduced the lactam **21** to give a piperidine derivative **23**. Both hydroxy groups, liberated within the reduction, were oxidised with chromium trioxide. Since this reagent produced diketone **24** in unsatisfactory yields (53%), other reagents were explored. In our hands, the use of PCC, IBX[60] or Dess–Martin[61] reagent did not improve the yields. The best option was found in the protection of the amino group in diol **23**. Then the corresponding Boc derivative **25** was oxidised to dione **26** with Jones reagent almost quantitatively. Deprotection of **26** to diketone **24** was carried out using trifluoroacetic acid.

In diketone **24** the cyclohexanone moiety could be selectively reduced in the presence of 20-oxo group[62,63] to give the 7-aza-allopregnanolone (compound **9**). A more lipophilic side product was found to be its 3β-epimer **27** (10%), its H-3 proton was axial (see ^1H NMR spectroscopic data), on oxidation, afforded the starting diketone **24**. 17α-Epimer **28** was produced in 11% yield as a more polar fraction. The unnatural configuration at carbon 17 was proved by its ^1H NMR spectrum (H-17 is a doublet at δ 2.58, it is a triplet in normal 20-oxopregnanes). The formation of the above by-products **27** and **28** is in agreement with the results published[63] on the mechanism of the Henbest reduction. The yield of the 3β-alcohol **27** could not be restrained by varying the temperature (~70–120 °C), while that of the 17-isomer **28** was increased when the reaction was carried out at a higher temperature (120 °C) and/or for longer time (24 h).

The polarity of compound **9** and thus also its water solubility are due to the presence of the NH group: both are much higher than those of the carba analogue allopregnanolone **1**. Otherwise, the molecular conformation of both compounds **1** and **9**, torsion angles and interatomic distances within the molecule (e.g., between the hydroxy and oxo groups) are very close to each other. The other properties noticed for these compounds are also very similar: e.g., the specific rotations.

Diacetoxy lactam **21** was used for the synthesis of another allopregnanolone analogue **29**. Hydrolysis of the protective ester groups led to dihydroxy lactam **30**, which was oxidised to dioxo lactam **31**. Analogously, the Henbest reduction of the diketone **31** produced the expected 3α-hydroxy ketone **29**.



Scheme 3. Reagents and conditions: (a) MsCl, pyridine, 0 or 20 °C, 5 or 20 h; (b) LAH in boiling dioxane or KOH in MeOH; (c) $((CH_3)_3COCO)_2O$, pyridine; (d) CrO_3 in AcOH or Jones reagent or IBX or Dess–Martin reagent; (e) H_3PO_2 and H_2IrCl_6 in Me_2CHOH, 75 °C, 7 h.

3. Conclusion

7-Aza analogues of neurosteroid allopregnanolone, compounds **9** and **29**, were prepared in 12 or 9 steps (with or without protection of the amino group by Bocylation) in 6.3% and 4.2% respective overall yields from 20-oxo-7-norpregn-5-en-3β-yl acetate (**10**). Part of the process is a shorter modification of the preparation of 6-aza-allopregnanolone (**3**) in which no orthogonal protection[30] of hydroxyl groups in positions 3 and 20 was required. While Beckmann rearrangement of the (*E*)-oxime would produce 6-aza lactam (type **4**), the (*Z*)-oxime gave 7-aza lactam **21**. Direct chromium trioxide oxidation of 7-aza-diol **23** was less successful than in the case of the corresponding 6-aza-diol,[30] and the use of Boc-protection of the amino group in the oxidation step was required. Except for its basicity, properties of 7-aza-allopregnanolone (**9**) are close to those of its natural counterpart allopregnanolone (**1**).

The above analogues were tested for their effect on the γ-aminobutyric acid ($GABA_A$) receptor in experiments in vitro using rat brain membranes. The ligand used in the assay was [^{35}S]TBPS. As expected,[4] the 6-oxo derivative **29** was completely inactive. However, 7-aza-3α-hydroxy-5α-pregnan-20-one (**9**, 7-aza-allopregnanolone) exerted some activity at the $GABA_A$ receptor. Its effect was low (I_{max}=13% while allopregnanolone has I_{max}=79%). However, it even acted at a very low concentration (IC_{50}=1 nM; for allopregnanolone, IC_{50}=80 nM).

Thus the substitution of the carbon atom with the nitrogen atom does increase the polarity of the product and hence its solubility in blood in the desired way, unfortunately, it also reduces its neuronal activity.

4. Experimental

4.1. Chemistry

4.1.1. General. Melting points were determined on a Boetius micromelting point apparatus (Germany) and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Circular dichroism was recorded on a Mark V apparatus in CH_3OH, optical rotations, and IR spectra were measured in chloroformat at 20 °C. IR spectra (wave numbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer in $CHCl_3$ (if not specified otherwise). ^1H NMR spectra were measured on a Bruker *AVANCE*-400 (at 400 MHz) spectrometer at 23 °C in $CDCl_3$ with TMS as the internal standard. ^1H and ^{13}C NMR spectra of compounds **9** and **28** were measured on a Bruker *AVANCE*-500 spectrometer under same conditions. Chemical shifts are given in parts per million (δ scale), and coupling constants and widths of multiplets are given in hertz. Mass spectra were recorded on a VG Analytical ZAB-EQ spectrometer (energy of ionising electrons 70 eV, ion-source temperature 180–220 °C). Thin-layer chromatography (TLC) was performed on silica gel (ICN Biochemicals), and preparative TLC (PLC) was carried out on 200×200 mm plates coated with a 0.6 mm thick layer of the same material. For column chromatography, silica gel 60–120 μm was used.

Whenever aqueous solutions of hydrochloric acid, potassium hydrogen carbonate and potassium carbonate were used, their concentration was always 5%. Prior to evaporation on a rotary evaporator in vacuo (bath temperature 50 °C), solutions in organic solvents were dried over anhydrous sodium sulfate.

4.1.1.1. 7-Aza-3α-hydroxy-5α-pregnan-20-one (9). Hydrogen hexachloroiridate(IV) hydrate (22 mg, 0.05 mmol)

and hypophosphorous acid (a 50% aqueous solution, 0.5 mL, 4.8 mmol) were added to a solution of compound **24** (74 mg, 0.23 mmol) in 2-propanol (1.7 mL, 22.1 mmol) and the mixture was heated at 70–75 °C. The black mixture turned into a pale solution within the first half an hour. After 7 h, the mixture was diluted with AcOEt, potassium carbonate (180 mg, 1.3 mmol) was added and volatiles 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 PLC (three plates, ammoniacal chloroform with 10% of 2-propanol); the major zone was eluted with the same solvent yielding the title compound **9** (55 mg, 75%) as white crystals. Mp 162–163 °C (heptane). $[\alpha]_D$ +96 (*c* 0.1, $CHCl_3$). IR: 3616, 3187, 1004 (OH); 1700, 1359, 594 ($COCH_3$); 3383 (NH). ^{1}H NMR ($CDCl_3$) δ 0.64 (s, 3H, H-18), 0.87 (s, 3H, H-19), 2.12 (s, 3H, H-21), 2.59 (t, *J*=9.0, H-17), 2.63 (m, 2H, H-6), 2.72 (t, 1H, *J*=10.3, H-8), 4.08 (m, 1H, H-3). ^{13}C NMR ($CDCl_3$) δ 11.01 (C-19), 13.73 (C-18), 20.17 (C-11), 23.03 (C-16), 23.60 (C-15), 28.51 (C-2), 31.50 (C-21), 31.70 (C-4), 32.43 (C-1), 35.06 (C-10), 38.31 (C-12), 38.68 (C-5), 44.97 (C-13), 47.21 (C-6), 53.97 (C-9), 54.48 (C-8), 55.10 (C-14), 63.38 (C-17), 65.62 (C-3), 209.16 (C-20). Anal. Calcd for $C_{20}H_{33}NO_2$: 319.25113. Found: 319.249817.

4.1.1.2. (20*R*)-7-Norpregn-5-ene-3β,20-diyl diacetate (11). To a solution of ketone **10** (5.1 g, 14.8 mmol) in a mixture of CH_2Cl_2 (10 mL), AcOEt (20 mL) and MeOH (20 mL) was added sodium borohydride (600 mg, 15.9 mmol) at 0 °C. After 3 h, the mixture was poured into water (300 mL). The precipitate formed was filtered off, washed with water and dried. The product was acetylated using acetic anhydride (20 mL) in pyridine (12 mL) at 20 °C. After 18 h, the mixture was diluted with brine (100 mL), the product was extracted with ether (3×70 mL) and washed with the solution of hydrochloric acid, water, $KHCO_3$ solution and water. The extract was dried and the solvent was evaporated in vacuo to give the title compound **11** (4.7 g, 82%) as white crystals. Mp 124–126 °C (acetone). $[\alpha]_D$ –57 (*c* 0.2, $CHCl_3$). IR: 1723, 1359, 1253, 1031 (AcO); 1658 (C=C). ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H, H-18), 0.89 (s, 3H, H-19), 1.16 (d, 3H, *J*=6.1, H-21), 2.02 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.63[64] (dd, 1H, *J*=13.4 and 2.4, H-4α), 4.63 (m, 1H, H-3), 4.86 (m, 1H, H-20), 5.38 (s, 1H, H-6). Anal. Calcd for $C_{24}H_{36}O_4$: C, 74.19; C, 9.34. Found: C, 73.89; C, 9.40.

4.1.1.3. (20*R*)-5β,6β-Epoxy-7-nor-5β-pregnane-3β,20-diyl diacetate (12). A solution of olefin **11** (2.1 g, 5.4 mmol) in dioxane (20 mL) was treated with $HClO_4$ (10%, 1 mL) and *N*-bromoacetamide (1.0 g, 7.25 mmol) at 15 °C. After 1 h, the mixture was poured into a cold solution of potassium hydrogen sulfite (7%, 50 mL). The precipitate formed was filtered off, the product was dissolved in chloroform (100 mL) and washed with water (50 mL). The solution was dried over sodium sulfate and filtered through a layer of silica gel (10 g). The solvent was evaporated in vacuo and the product was dissolved in EtOH (40 mL) and treated with AcONa (600 mg, 7.3 mmol) under reflux. After 90 min, the solution was concentrated in vacuo to a quarter of its volume and the product was precipitated on addition of brine (60 mL). The product was filtered off, dissolved in CH_2Cl_2 (25 mL), washed with water (2×20 mL) and dried. Evaporation of the solvent in vacuo produced the title compound **12** (2.0 g, 91%) as colourless crystals. Mp 95–97 °C (ether/heptane). $[\alpha]_D$ +17 (*c* 0.3, $CHCl_3$). IR: 1724, 1377, 1253, 1039, 1031 (AcO); 3025 (epoxide CH). ^{1}H NMR ($CDCl_3$) δ 0.59 (s, 3H, H-18), 0.95 (s, 3H, H-19), 1.16 (d, 3H, *J*=6.1, H-21), 2.08 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO), 3.40 (s, 1H, H-6), 4.84 (m, 1H, H-20), 5.00 (m, 1H, H-3). Anal. Calcd for $C_{24}H_{36}O_5$: C, 71.26; H, 8.97. Found: C, 71.30, H, 9.22.

4.1.1.4. (20*R*)-6-Oxo-7-nor-5α-pregnane-3β,20-diyl diacetate (13). A solution of $BF_3 \cdot Et_2O$ (0.2 mL, 1.62 mmol) in ether (30 mL) was added to a solution of epoxide **12** (929 mg, 2.3 mmol) in THF (25 mL) under stirring at 20 °C. After 20 h, the solution was diluted with ether (100 mL), and washed with the solution of $KHCO_3$ (30 mL) and brine (30 mL). The solvents were removed in vacuo and the product was purified by chromatography on silica (50 g, toluene/ether 5:1). The major component (620 mg, 67%) consisted of the title compound **13**. Mp 151–152 °C (ether/heptane). $[\alpha]_D$ +92 (*c* 0.2, $CHCl_3$). IR: 1727, 1378, 1257 (R_2CO+AcO). ^{1}H NMR ($CDCl_3$) δ 0.62 (s, 3H, H-18), 0.89 (s, 3H, H-19), 1.16 (d, 3H, *J*=6.1, H-21), 2.03 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.23 (m, 1H, H-5), 4.70 (m, 1H, H-3), 5.00 (m, 1H, H-20). Anal. Calcd for $C_{24}H_{36}O_5$: C, 71.26; H, 8.97. Found: C, 70.89; H, 8.94.

4.1.1.5. 5α,6α-Epoxy-7-nor-5α-cholestan-3β-yl acetate (15). A mixture of $KMnO_4$ (1.0 g, 6.3 mmol) and $FeSO_4 \cdot 7H_20$ (0.5 g, 1.8 mmol) was ground to a fine powder. After addition of water (0.1 mL), the mixture was transferred into a reaction flask and stirred. A solution of olefin **14** (244 mg, 0.59 mmol) in CH_2C1_2 (5 mL) and 2-methylpropan-2-ol (0.5 mL) was added and the suspension was stirred at 20 °C for 2 h when the reaction was complete (TLC control on silica gel in benzene/ether, 1:1). The mixture was diluted with ether (10 mL) and filtered through a short column of sodium sulfate. The filtrate was washed with water and dried over anhydrous sodium sulfate. After evaporation of the solvent, white crystalline product was obtained (252 mg, 100%). Its ^{1}H NMR spectrum corresponded to a mixture of epoxides **15** and **16** in the ratio of 19.5:1. Crystallisation from MeOH produced the 5α,6α-epoxide **15** (152 mg, 60%) as white leaflets. Mp 112–113 °C. $[\alpha]_D$ −36 (*c* 0.2, $CHCl_3$).[65] ^{1}H NMR ($CDCl_3$) δ 0.63 (s, 3H, H-18), 0.86 (d, 3H, *J*=6.6, H-27), 0.87 (d, 3H, *J*=6.6, H-26), 0.90 (s, 3H, H-19), 0.90 (d, 3H, *J*=6.4, H-21), 2.03 (s, 3H, Ac), 2.18 (t, 1H, *J*=12, H-17), 3.26 (s, 1H, H-6), 4.98 (m, 1H, H-3).

4.1.1.6. 5β,6β-Epoxy-7-nor-5β-cholestan-3β-yl acetate (16). A sample produced earlier[56] was used for comparison. ^{1}H NMR ($CDCl_3$) δ 0.62 (s, 3H, H-18), 0.86 (d, 3H, *J*=6.7, H-27), 0.87 (d, 3H, *J*=6.7, H-26), 0.88 (s, 3H, H-19), 0.90 (d, 3H, *J*=6.4, H-21), 1.94 (t, 1H, *J*=10.2, H-17), 2.04 (s, 3H, Ac), 3.18 (s, 1H, H-6), 4.96 (m, 1H, H-3).

4.1.1.7. (*Z*,20*R*)-6-Oximino-7-nor-5α-pregnane-3β,20-diyl diacetate (17). A solution of ketone **13** (2.90 g, 7.17 mmol) in MeOH (100 mL) was stirred with the $KHCO_3$ solution (2.90 mg, 29.0 mmol) and hydroxylamine

hydrochloride (1.45 g, 21.2 mmol) at reflux temperature. After 5 h, the mixture was diluted with brine (500 mL) and cooled in a refrigerator. The precipitate was dissolved in CH_2Cl_2 (500 mL) and washed with the $KHCO_3$ solution (2×100 mL). The solvent was removed in vacuo and the product was purified by chromatography on a column of silica (500 mL). A mixture of AcOEt and toluene (3:1) eluted the title compound **17** (980 mg, 32.6%) as a colourless solid. Mp 190–191 °C (CH_2Cl_2/heptane). $[\alpha]_D$ +73 (*c* 0.2, $CHCl_3$). IR: 3587, 3299, 3151, 1668, 1475, 944, 910 (C═NOH); 1724, 1258, 1245, 1033, 1024, 610 (AcO). ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H, H-18), 0.87 (s, 3H, H-19), 1.16 (d, 3H, *J*=6.1, H-21), 2.02 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.34 (t, 1H, *J*=10.6, H-17), 3.04 (m, 1H, H-4α), 4.77–4.91 (m, 2H, H-3 and H-20), 7.32 (s, 1H, N–OH). Anal. Calcd for $C_{24}H_{37}NO_5$: C, 68.71; H, 8.89; N, 3.34. Found: C, 68.78; H, 9.13; N, 3.18.

4.1.1.8. (*E*,20*R*)-6-Oximino-7-nor-5α-pregnane-3β,20-diyl diacetate (18). The more polar eluate of the above chromatography yielded the title compound **18** (1.925 g, 64%) as white crystals. Mp 191–193 °C (CH_2Cl_2/heptane). $[\alpha]_D$ +67 (*c* 0.2, $CHCl_3$). IR: 3586, 3296, 3140, 1670, 1471, 935, 922 (C═NOH); 1723, 1258, 1032, 1025, 611 (AcO). ^{1}H NMR ($CDCl_3$) δ 0.72 (s, 6H, H-18 and H-19), 1.15 (d, 3H, *J*=6.1, H-21), 2.02 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.45 (t, 1H, *J*=10.9, H-17), 4.72–4.82 (m, 1H, H-3), 4.83–4.91 (m, 1H, H-20), 6.84 (s, 1H, H–ON). Anal. Calcd for $C_{24}H_{37}NO_5$: C, 68.71; H, 8.89; N, 3.34. Found: C, 68.56; H, 8.91; N, 3.12.

4.1.1.9. (*E*,20*R*)-6-Oximino-7-nor-5β-pregnane-3β,20-diyl diacetate (19). A boiling solution of ketone **13** (419 mg, 1.04 mmol) in EtOH (6 mL) was stirred with $KHCO_3$ (565 mg, 4.1 mmol) and hydroxylamine hydrochloride (400 mg, 5.8 mmol) for 15 min. The mixture was cooled and brine (25 mL) was added. The precipitate was extracted with methylene chloride, washed with water, dried over sodium sulfate and separated on a column of silica gel. Besides other fractions, white crystals of compound **19** (111 mg, 26%) were obtained. Mp 193–194 °C (CH_2Cl_2/heptane). $[\alpha]_D$ +42 (*c* 0.2, $CHCl_3$). IR: 3586, 3295, 3148, 1660, 1476, 962, 913 (C═NOH); 1723, 1255, 1034, 1022, 609 (AcO). ^{1}H NMR ($CDCl_3$) δ 0.65 (s, 3H, H-18), 0.89 (s, 3H, H-19), 1.16 (d, 3H, *J*=6.1, H-21), 2.02 (s, 3H, AcO), 2.08 (s, 3H, AcO), 2.36 (t, 1H, *J*=11.5, H-17), 2.92 (dd, 1H, *J*=12.4 and 6.3, H-5), 4.84 (m, 1H, H-20), 5.03 (m, 1H, H-3), 7.32 (s, 1H, N–OH). Anal. Calcd for $C_{24}H_{37}NO_5$: C, 68.71; H, 8.89; N, 3.34. Found: C, 68.78; H, 9.13; N, 3.18.

4.1.1.10. Oximation via *O*-*tert*-butyldimethylsilyloximes 20. A solution of ketone **13** (360 mg, 0.9 mmol) in toluene (8 mL) was treated with *O*-*tert*-butyldimethylsilylhydroxylamine (830 mg, 1.55 mmol) at 100 °C for 4 h. The reaction mixture was cooled, diluted with ethyl acetate (30 mL), washed with brine (10 mL) and dried. After evaporation of solvents, the remainder was purified using flash chromatography on silica gel (ether/toluene, 1:6). The product was treated with a solution of tetrabutylammonium fluoride in THF (1 M, 10 mL). After 1 h, the solution was diluted with chloroform (40 mL), washed with water and dried. After evaporation of the solvent, the remainder was separated by thin-layer chromatography, which yielded compounds **17** (90 mg, 24%) and **18** (259 mg, 69%). Their identity was proved by comparison with compounds prepared above (Sections 4.1.1.7 and 4.1.1.8).

4.1.1.11. (20*R*)-7-Aza-6-oxo-5α-pregnane-3β,20-diyl diacetate (21). A solution of mesyl chloride in toluene (50%, 5.0 mL, 32.3 mmol) was dripped into a solution of oxime **17** (620 mg, 1.48 mmol) in pyridine (12 mL) at 0 °C under stirring. After 5 h, the reagent was destroyed with crushed ice (20 g) and the precipitate was filtered off. The product was dissolved in CH_2Cl_2 (20 mL) and washed with the solution of hydrochloric acid (5 mL), water (5 mL) and $KHCO_3$ solution (2×5 mL). The extract was dried over sodium sulfate and the solvent was evaporated to give the title compound **21** (510 mg, 82%) as white crystals. Mp 245–247 °C (acetone/heptane). $[\alpha]_D$ +21 (*c* 0.2, $CHCl_3$). IR: 3393, 3273, 3211, 1657 (CONH); 1726, 1251, 1049, 1039, 610 (AcO). ^{1}H NMR ($CDCl_3$) δ 0.69 (s, 3H, H-18), 0.92 (s, 3H, H-19), 1.16 (d, 3H, *J*=6.2, H-21), 2.02 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.42 (br d, 1H, *J*=12.9, H-5), 3.20 (t, 1H, *J*=10.4, H-8), 4.71 (m, 1H, H-3), 4.84 (m, 1H, H-20), 5.37 (s, 1H, NH). MS: 419 (M^+, 96), 359 (49), 344 (24), 332 (13), 317 (11), 290 (17), 277 (9), 264 (6), 250 (35). HRMS calcd: 419.267174; found: 419.264417. Anal. Calcd for $C_{24}H_{37}NO_5$: C, 68.71; H, 8.89; N, 3.34. Found: C, 68.41; H, 8.92; N, 3.17.

4.1.1.12. (20*R*)-7-Aza-5-chloro-6-oxo-5α-pregnane-3β,20-diyl diacetate (22). The above reaction was carried out in a similar manner. After the initial hour at 0 °C, when TLC (benzene/ether 3:1) showed no apparent progress, the amount of mesyl chloride was doubled and the temperature was kept at 20 °C for 20 h. Thus, oxime **17** (500 mg, 1.2 mmol) yielded a complex mixture, the least polar component being the title compound **22** (83 mg, 15%). Mp 218–220 °C (acetone/heptane). $[\alpha]_D$ +41 (*c* 0.3, $CHCl_3$). IR: 3389, 3318, 3214, 3096, 1674, 1330, 1074 (CONH); 1726, 1370, 1251, 1048, 1022, 610 (AcO). ^{1}H NMR ($CDCl_3$) δ 0.68 (s, 3H, H-18), 1.11 (s, 3H, H-19), 1.16 (d, 3H, *J*=6.2, H-21), 2.01 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.22 (dt, 1H, *J*=11.1 and 3.2, H-17), 2.72 (dd, 1H, *J*=14.1, 5.0 and 1.6, H-4), 3.22 (t, 1H, *J*=10.8, H-8), 4.86 (m, 1H, H-20), 5.24 (s, 1H, H–N), 5.31 (m, 1H, H-3). Anal. Calcd for $C_{24}H_{36}NClO_5$: C, 63.17; H, 8.13; N, 3.21. Found: C, 63.49; H, 7.99; N, 3.09. HRMS (FAB) M^+ 454.2377, $C_{24}H_{36}ClNO_5$ requires 454.2360.

4.1.1.13. (20*R*)-7-Aza-5α-pregnane-3β,20-diol (23). Dry lactam **21** (280 mg, 0.67 mmol) was put in a dripping funnel placed between a reflux condenser and a flask with a boiling solution of $LiAlH_4$ (200 mg, 5.27 mmol) in dioxane (10 mL). The substrate was gradually dissolved in the solvent condensed and washed into the solution. After 4 h, the excess of reagent was destroyed with AcOEt and a saturated, aqueous solution of sodium sulfate. Anhydrous sodium sulfate was added and the solution was filtered and dried over sodium sulfate. The filter cake was washed with hot chloroform (3×100 mL). The solvent was evaporated in vacuo to yield the title compound **23** (169 mg, 79%), mp 189–192 °C (chloroform). Mp 214–216 °C (acetone/

heptane). $[\alpha]_D$ –34 (*c* 0.1, $CHCl_3$). IR: 3610, 3445, 1087, 1036 (OH). ^1H NMR ($CDCl_3$) δ 0.78 (s, 3H, H-18), 0.90 (s, 3H, H-19), 1.16 (d, 3H, *J*=6.2, H-21), 2.55–2.69 (m, 2H, N–CH), 3.62 (m, 1H, H-3), 3.72 (m, 1H, H-20). Anal. Calcd for $C_{20}H_{35}NO_2$: C, 74.72; H, 10.97; N 4.36. Found: C, 74.32; H, 11.0; N, 4.16.

4.1.1.14. 7-Aza-5α-pregnane-3,20-dione (24). (a) *From (20R)-7-aza-5α-pregnane-3β,20-diol*: part (8 drops) of a solution of chromium trioxide (380 mg, 3.8 mmol) in water (13 drops) was added to a solution of diol **23** (200 mg, 0.81 mmol) in acetic acid (13 mL, 227.3 mmol) under stirring at 20 °C. After 24 h, the mixture was cooled with ice and made alkaline with ammonia (30 mL) and anhydrous potassium carbonate (380 mg, 2.75 mmol). The resulting precipitate was extracted with chloroform, the extract washed with water and dried. Preparative TLC on 5 silica plates in ammoniacal chloroform yielded the title compound **24** (105 mg, 53%). Mp 194–196 °C (acetone/heptane). $[\alpha]_D$ +136 (*c* 0.3, $CHCl_3$). IR: 3328 (NH), 1704 (C=O), 1418 (CH_2). ^1H NMR ($CDCl_3$) δ 0.68 (s, 3H, H-18), 1.09 (s, 3H, H-19), 2.13 (s, 3H, H-21), 2.58 (t, 1H, *J*=9.3, H-17), 2.62–2.72 (m, 3H, N–CH). MS: 317 (M^+, 33), 302 (15), 246 (7), 178 (100). Anal. Calcd for $C_{20}H_{31}NO_2$: C, 75.67; H, 9.84; N, 4.41. Found: C, 75.32; H, 9.98; N, 4.29.

(b) *Using IBX*: diol **23** (24 mg, 0.07 mmol) was treated with iodosobenzoic acid (360 mg, 1.36 mmol) and tetrabutylammonium bromide (57 mg, 0.18 mmol) in chloroform (2.0 mL). The mixture was stirred at 70 °C for 6 h, then it was diluted with warm AcOEt (50 °C), washed with brine and dried. Lactam **30** was not detected in the mixture. PLC afforded diketone **24** (9 mg, 38%) identical with the above sample (TLC, IR and NMR).

(c) *Using Dess–Martin periodinane*: diol **23** (54 mg, 0.17 mmol) was treated with iodosobenzoic acid triacetate (Dess–Martin periodinane, 150 mg, 0.57 mmol) in chloroform (3.0 mL). The mixture was stirred at 20 °C for 6 h and then it was diluted with ammoniacal chloroform (50 mL). The solution was washed with brine, dried and concentrated in vacuo. Lactam **30** was not detected in the mixture. PLC afforded diketone **24** (13 mg, 24%) and hydroxy ketone **27** (15 mg, 28%), both identical with the above samples (TLC, IR and NMR).

(d) *From N-(tert-butyloxycarbonyl)-7-aza-5α-pregnane-3,20-dione*: compound **26** (85 mg, 0.2 mmol) was dissolved in CH_2Cl_2 (2.0 mL) and treated with trifluoroacetic acid (99%, 0.5 mL, 6.7 mmol) at 20 °C. After 1 h, the mixture was evaporated and the remainder was dissolved in ammoniacal chloroform (5.0 mL), washed with brine and dried. Evaporation of the solvent yielded the diketone **24** as white crystals (63 mg, 97%). The product was found to be identical with the above sample (Section 4.1.1.14a).

4.1.1.15. *N*-(*tert*-Butyloxycarbonyl)-aza-5α-pregnane-3,20-dione (26). 7-Aza diol **23** (294 mg, 0.91 mmol) was dissolved in pyridine (1.0 mL) and toluene (1.0 mL), and treated with a solution of di-*tert*-butyl dicarbonate (250 mg, 1.15 mmol) in toluene (1.0 mL) at 20 °C. After 15 min, the mixture was washed with the solution of $KHCO_3$ and water, and dried. After evaporation of the solvent, the product (**25**) was dissolved in acetone (2.5 mL) and oxidised with Jones reagent at 0 °C. After 3 min, the reaction was stopped with the solution of $KHCO_3$ and the product was extracted with ether, washed with water and dried. PLC yielded 310 mg (81%) of diketone **26** as white crystals. Mp 150–152 °C (acetone/heptane). IR: 1702, 1355 (R_2C=O); 1670, 1150 (N–C=O). ^1H NMR ($CDCl_3$) δ 0.63 (s, 3H, H-18), 1.13 (s, 3H, H-19), 1.43 (s, 9H, Boc), 2.13 (s, 3H, H-21), 2.61 (t, 1H, *J*=8.6, H-17α), 2.97 (t, 1H, *J*=11.2, H-8), 3.71 (dd, 1H, *J*=13.6, 3.1, H-6β). HRMS (FAB): calcd for $C_{25}H_{40}NO_4$: 418.295734. Found: 418.297652.

4.1.1.16. 7-Aza-3β-hydroxy-5α-pregnan-20-one (27). The lipophilic zone (see preparative thin-layer chromatography in Section 4.1.1.1) afforded compound **27** (7 mg, 10%) as white crystals. Mp 225–227 °C (acetone). ^1H NMR ($CDCl_3$) δ 0.64 (s, 3H, H-18), 0.89 (s, 3H, H-19), 2.12 (s, 3H, H-21), 2.57 (t, 1H, *J*=9.1, H-17), 3.65 (m, 1H, H-3). MS (*m*/*z*): 319 (M^+, 40%), 304 (21%), 276 (3%), 180 (100%), 152 (6%); Anal. Calcd for $C_{20}H_{33}NO_2$: 319.251130. Found: 319.25052.

4.1.1.17. 7-Aza-3α-hydroxy-5α,17α-pregnan-20-one (28). The polar PLC zone from Section 4.1.1.1 afforded compound **28** (8 mg, 11%), which had the following spectrum: ^1H NMR ($CDCl_3$) δ 0.88 (s, 3H, H-19), 0.92 (s, 3H, H-18), 2.12 (s, 3H, H-21), 2.65 (t, 1H, *J*=12.7, H-6β), 2.80 (dd, 1H, *J*=8.3 and 2.8, H-17), 2.84 (dd, 1H, *J*=12.7 and 4.0, H-6α), 2.87 (t, 1H, *J*=10.6, H-8), 4.12 (m, 1H, H-3). ^{13}C NMR ($CDCl_3$) δ 10.80 (C-19), 20.18 (C-11), 20.80 (C-18), 24.75 (C-16), 25.06 (C-15), 28.44 (C-2), 31.84 (C-4), 32.09 (C-1), 32.86 (C-21), 34.17 (C-12), 34.68 (C-10), 37.68 (C-5), 45.51 (C-6), 46.78 (C-13), 48.41 (C-14), 53.04 (C-9), 54.42 (C-8), 60.48 (C-17), 64.83 (C-3), 212.20 (C-20). Circular dichroism (EtOH): $\Delta\varepsilon_{232}$ +0.49; $\Delta\varepsilon_{288}$ −0.64.

4.1.1.18. 7-Aza-3α-hydroxy-5α-pregnane-6,20-dione (29). Henbest reduction was carried out as in the preparation of alcohol **9**: 3,20-dione **31** (36 mg, 0.11 mmol) was treated with 2-propanol (0.7 mL), hydrogen hexachloroiridate (6 mg, 0.014 mol) and the aqueous solution of hypophosphorous acid (50%, 0.3 mL, 2.9 mmol). After 9 h at 80 °C, the mixture was worked up as above. The major product was purified by TLC in chloroform yielding the title compound **29** (31 mg, 87%) as white crystals. Mp 294–295 °C (acetone). $[\alpha]_D$ +4 (*c* 0.1, $CHCl_3$). IR: 3612, 1007, 999 (OH); 3393, 1651 (CONH); 1702, 1359 (AcO). MS: 333 (16, M^+), 318 (20), 300 (15), 290 (4), 263 (26), 248 (34), 230 (4), 209 (7), 194 (46), 43 (100). ^1H NMR ($CDCl_3$) δ 0.67 (s, 3H, H-18), 0.88 (s, 3H, H-19), 2.14 (s, 3H, H-21), 2.60 (t, 1H, *J*=9.1, H-17α), 3.21 (t, 1H, *J*=10.0, H-8), 4.22 (m, 1H, H-3), 5.45 (s, 1H, N–H). Anal. Calcd for $C_{20}H_{31}NO_3$: 333.23039. Found: 333.23215.

4.1.1.19. (20*R*)-7-Aza-6-oxo-5α-pregnane-3β,20-diol (30). A solution of potassium hydroxide (540 mg, 9.6 mmol) in water (10 drops) was added to a boiling solution of diester **21** (520 mg, 1.24 mmol) in MeOH (50 mL). The reaction mixture was heated to reflux under nitrogen for 8 h. The solvent was partly removed and product was precipitated on addition of brine (100 mL). The precipitate

(amine **30**, 341 mg, 83%) was filtered off, washed with water and dried. Mp 283–285 °C (acetone). $[\alpha]_D$ −13 (*c* 0.27, $CHCl_3$). IR: 3609, 1056 (OH); 3395, 1653 (CONH). ^1H NMR ($CDCl_3$) δ 0.81 (s, 3H, H-18), 0.91 (s, 3H, H-19), 1.16 (d, 3H *J*=6.0, H-21), 3.23 (t, 1H, *J*=10.5, H-8), 3.62 (m, 1H, H-3), 3.74 (m, 1H, H-20), 5.37 (s, 1H, NH). Anal. Calcd for $C_{20}H_{33}NO_3$: C, 71.60; H, 9.91; N, 4.17. Found: C, 70.95; H, 10.11; N, 3.99.

4.1.1.20. 7-Aza-5α-pregnane-3,6,20-trione (31). Diol **30** (146 mg, 0.44 mmol) was dissolved in hot acetone (80 mL) and treated with Jones reagent. After 15 min, the excess of the reagent was destroyed with potassium hydrogen sulfite and the mixture was concentrated in vacuo to a quarter of its volume. Brine (20 mL) was added to the remainder and the product was extracted with chloroform. The extract was washed with a solution of $KHCO_3$ 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 **31** (104 mg, 72%) as white crystals. Mp 243–244 °C (acetone/heptane). $[\alpha]_D$ +58 (*c* 0.2, $CHCl_3$). IR: 3392, 1661, 1450, 1333, 1315, 1074 (CONH); 1707, 1419, 1358 (CO). ^1H NMR ($CDCl_3$) δ 0.71 (s, 3H, H-18), 1.11 (s, 3H, H-19), 2.14 (s, 3H, H-21), 2.60 (t, 1H, *J*=8.8, H-17), 2.89 (dd, 1H, *J*=11.4 and 5.7, H-5), 3.28 (t, 1H, *J*=10.5, H-8), 5.46 (s, 1H, NH). MS: 331 (66), 316 (40), 302 (40), 288 (30), 274 (10), 261 (20), 246 (32), 192 (100). Anal. Calcd for $C_{20}H_{29}NO_3$: 331.214744. Found: 331.213993. Circular dichroism (EtOH): $\Delta\varepsilon_{225}$ +1.12; $\Delta\varepsilon_{288}$ +0.50.

4.2. Biological evaluation

The binding of the above analogues to $GABA_A$ receptors was tested in vitro using neural membranes of male rat brains.[66,67] The specific steroid binding was detected by the decrease of the specific [^{35}S]-*tert*-butylbicyclo[2.2.2]-phosphorothionate (TBPS) binding after application of the tested compounds. Experiments with 2 nM TBPS were performed with the 100 nM concentration of steroids; for the well soluble analogues, IC_{50} and I_{max} were also measured. The results are related to the binding with DMSO and expressed in percentage. For compound **9**, I_{max}=13%, IC_{50}=1 nM (corresponding values for allopregnanolone **1** are 79% and 80 nM). Compound **29** was inactive ($I_{100\ nM}$ 92%).

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