



Synthesis of 6-azaprogesterone and 19-hydroxy-6-azasteroids

Mario D. Martínez, Valeria C. Edelsztejn, Fernando J. Durán, Pablo H. Di Chenna, Gerardo Burton*

Departamento de Química Orgánica and UMYMFOR (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, C1428EGA Buenos Aires, Argentina

ARTICLE INFO

Article history:

Received 26 August 2012

Received in revised form 29 September 2012

Accepted 10 October 2012

Available online 2 November 2012

Keywords:

Azasteroids

6-Azaprogesterone

19-Hydroxypregnane

Gestagenic activity

ABSTRACT

19-Hydroxy-6-azapregnanes were obtained from pregnenolone via a 7-azido-5-oxo-6-nor-5,7-seco-pregnane intermediate. The 6-azapregnane core was built in good yield in a straightforward way from the secosteroid, by means of a Staudinger (aza-Wittig) reaction. Finally the 19-hydroxy-6-azapregnane was transformed into 19-hydroxy-6-azaprogesterone (that cyclized spontaneously to the 19 → 3 hemiketal) and 6-azaprogesterone. The 6-azapregnanes lacked agonistic/antagonistic activity on the progesterone receptor.

© 2012 Elsevier Inc. All rights reserved.

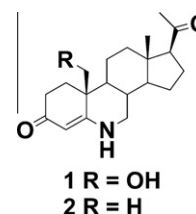
1. Introduction

The isosteric replacement of carbon atoms of the steroid nucleus by heteroatoms (e.g. O, S, N) gives rise to marked changes in the biological activity of steroidal derivatives [1,2]. These analogues mainly exhibit localized changes in hydrophobicity and hydrogen bonding capacity, without significant changes in overall conformation of the steroid nucleus or increased steric bulk. Among them, azasteroids have received much attention during the last decade due to their numerous biological activities. For example, 4-azasteroids and 6-azasteroids exhibit strong inhibition of human steroid 5 α -reductase [3] making them potential candidates for the treatment of benign prostatic hyperplasia [4]. Azasteroids can also exhibit other activities such as inhibition of the phosphatidylinositol phospholipase C [5], anti-parasitic [6] and antifungal [7] activities. Most of the therapeutically useful azasteroids reported in the literature are semisynthetic compounds prepared from naturally available steroids by the isosteric replacement of a C atom by a N atom [8] and 6-azasteroids are not the exception. They have been prepared mainly by a synthetic route that involves the following steps: (1) formation of a 6-carboxy-secosteroid from a 5,6 unsaturated steroidal precursor (for example by ozonization or oxidative epoxide cleavage), (2) preparation of the acid chloride, (3) transformation to the acyl azide and (4) a classical Curtius rearrangement followed by hydrolysis [9,10]. Another methodology involves preparation of the oxime of a 6-keto-7-norsteroid precursor followed by a Beckman rearrangement [11], even though these two steps are

synthetically efficient it is necessary to prepare the 7-nor-steroid from commercially available steroids.

In the search for steroids with novel and/or improved pharmacological effects and selectivity, we have focused our attention on the synthesis of 19-hydroxysteroids, key intermediates in the preparation of several analogues of biologically active steroids. For example, the 1,2-dehydrogenation of 19-hydroxysteroids is a highly efficient method for the preparation of estrogens [12]. In previous publications we have reported the synthesis of C(11)–C(19) [13], and C(2)–C(19) [14] oxygen bridged pregnanes and C(6)–C(19) sulfur bridged pregnanes, [15] from the corresponding 19-hydroxy precursors using an intramolecular remote functionalization and some of them have been shown to possess interesting activities. Moreover, the presence of the hydroxyl group at C-19 is by itself a starting point to prepare a wide variety of 19-functionalized steroids by simple chemical transformations as oxidations, substitutions or additions.

Prompted by the synthetic versatility that would confer the presence of a 19-hydroxyl group in a 6-azasteroid nucleus, we report the first direct route to 19-hydroxy-6-azasteroids from commercially available pregnenolone and its application to the synthesis of 19-hydroxy-6-aza-progesterone (**1**) and 6-aza-progesterone (**2**).



* Corresponding author. Tel./fax: +54 11 4576 3385.

E-mail address: burton@qo.fcen.uba.ar (G. Burton).

2. Experimental

2.1. General

Melting points were taken on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded in thin films using KBr disks on a Nicolet Magna IR 550 FT-IR spectrometer. ^1H and ^{13}C NMR spectra were measured at 500.13 and 125.72 MHz respectively in a Bruker Avance II 500 NMR spectrometer in deuteriochloroform unless otherwise indicated, using TMS as internal standard. Exact mass spectra (ESI) were measured on an Agilent LCTOF, high resolution TOF analyzer with APCI/ESI ionization. Elemental analysis was performed on an EAI Exeter Analytical, Inc. CE-440 apparatus. Microwave assisted reactions were carried out in a CEM Discover reactor, mode Discover (closed vessel) Power max: on, with air cooling of the reaction vessel during irradiation. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel plates (Merck F254, 0.2 mm thick); compounds were visualized by staining with cerium molybdate (Hannessian's stain). Flash column chromatography was performed on silica gel Merck 9385 (0.0040–0.0063 mm). All solvents used were reagent grade. Solvents were evaporated at reduced pressure and ca. 40–50 °C. The homogeneity of all compounds was confirmed by TLC. Products obtained as solids or syrups were dried under high vacuum. 3 β -*tert*-Butyldimethylsilyloxy-19-formyloxy-7-iodo-6-nor-5,7-secopregnane-5,20-dione (**3**) was prepared from pregnenolone following a procedure previously described by us and was used without further purification [16].

2.2. 3 β -*tert*-Butyldimethylsilyloxy-7-azido-19-formyloxy-6-nor-5,7-secopregnane-5,20-dione (**4**)

To a solution of secosteroid **3** (0.86 g, 1.42 mmol) in DMF (20.0 mL) was added sodium azide (0.56 g, 8.70 mmol) dissolved in the minimum amount of water. The mixture was stirred at room temperature overnight. The reaction mixture was diluted with diethyl ether and washed with brine. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography (hexane/ethyl acetate 8:2) to give compound **4** as a white solid (0.574 g, 78%). IR ν_{max} (cm^{-1}) 2955, 2930, 2100, 1728, 1705 and 1175; ^1H NMR δ 0.03 (3H, s, CH_3Si), 0.07 (3H, s, CH_3Si), 0.62 (3H, s, H-18), 0.86 (9H, s, *t*-BuSi), 2.12 (3H, s, H-21), 2.41 (1H, dt, $J = 14.0$ and 2.7 Hz, H α -4), 2.56 (1H, t, $J = 9.3$, H-17), 3.22 (1H, dd, $J = 13.6$ and 3.6, H β -4), 3.26 (1H, dd, $J = 13.0$ and 2.0 Hz, Ha-7), 3.55 (1H, dd, 13.0 and 2.7, Hb-7), 4.28 (1H, d, $J = 12.0$, Ha-19), 4.44 (1H, d, $J = 12.0$, Hb-19), 4.44 (1H, m, H-3), 8.09 (1H, s, HCOO); ^{13}C NMR δ -5.0 (CH_3Si), -4.9 (CH_3Si), 13.0 (C-18), 18.0 ($(\text{CH}_3)_3\text{CSi}$), 22.6 (C-16), 23.2 (C-11), 24.5 (C-15), 25.6 ($(\text{CH}_3)_3\text{CSi}$), 27.4 (C-1), 28.5 (C-2), 31.3 (C-21), 38.3 (C-8), 39.0 (C-9), 39.0 (C-12), 43.5 (C-13), 47.1 (C-4), 51.3 (C-7), 52.2 (C-14), 53.8 (C-10), 63.4 (C-17), 65.1 (C-19), 70.4 (C-3), 160.9 (HCOO), 208.8 (C-20), 212.9 (C-5); HRMS (ESI) calcd. for $\text{C}_{27}\text{H}_{45}\text{N}_3\text{NaO}_5\text{Si}$ ($\text{M}+\text{Na}$) $^+$ 542.3021, found 542.2998.

2.3. 3 β -*tert*-Butyldimethylsilyloxy-19-formyloxy-6-aza-5-pregnen-20-one (**5**)

To a solution of **4** (1.70 g, 3.27 mmol) in dry THF (20.0 mL) under argon was added triphenylphosphine (2.61 g, 9.81 mmol) and the mixture was heated under reflux for 2.5 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in dichloromethane and purified by flash chromatography (hexane/ethyl acetate/triethylamine 80:20:0.5) to give the imine **5** as a white solid (0.965 g, 62%). Mp 52–54 °C; IR ν_{max} (cm^{-1}) 2926, 2854, 1726, 1705, 1659 and 1097; ^1H NMR δ 0.08 (6H, s, $2 \times \text{CH}_3\text{Si}$), 0.66 (3H, s, H-18), 0.90 (9H, s, *t*-BuSi), 2.14

(3H, s, H-21), 2.51 (1H, m, H-17), 2.54 (2H, m, H-4), 2.95 (1H, ddd, $J = 18.0, 10.0$ and 3.0, H β -7), 3.73 (1H, m, H-3), 3.80 (1H, tdd, $J = 18.0, 5.0$ and 2.0, H α -7), 4.14 (1H, d, $J = 12.0$, Ha-19), 4.65 (1H, d, $J = 12.0$, Hb-19), 8.08 (1H, s, HCOO); ^{13}C NMR δ -4.7 (CH_3Si), -4.7 (CH_3Si), 13.5 (C-18), 21.0 (C-11), 18.1($(\text{CH}_3)_3\text{CSi}$), 23.1 (C-16), 23.9 (C-15), 25.8 ($(\text{CH}_3)_3\text{CSi}$), 31.5 (C-21), 31.5 (C-2), 32.2 (C-8), 32.3 (C-1), 38.6 (C-12), 41.3 (C-10), 44.1 (C-13), 46.1 (C-4), 47.5 (C-9), 54.8 (C-7), 55.1 (C-14), 63.1 (C-17), 63.8 (C-19), 71.2 (C-3), 160.4 (HCOO), 168.8 (C-5), 209.1 (C-20); HRMS (ESI) calcd. for $\text{C}_{27}\text{H}_{46}\text{NO}_4\text{Si}$ ($\text{M}+\text{H}$) $^+$ 476.3191, found 476.3188.

2.4. 3 β -*tert*-Butyldimethylsilyloxy-6-(*tert*-butoxycarbonyl)-19-formyloxy-6-aza-4-pregnen-20-one (**6**)

To a solution of **5** (0.20 g, 0.42 mmol) in THF (4.0 mL) in a microwave vessel, was added potassium carbonate (0.288 g, 1.57 mmol) and di-*tert*-butyl dicarbonate (0.343 g, 1.57 mmol). The reaction mixture was vigorously stirred under microwave irradiation at 300 W (120 °C) for 30 min. The reaction mixture was filtered and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography (hexane/ethyl acetate 9:1) to give compound **6** (0.153 g, 63%). Mp 52–53 °C; IR ν_{max} (cm^{-1}) 2955, 2931, 2856, 1728, 1701, 1389, 1157 and 1086; ^1H NMR δ 0.06 (3H, s, CH_3Si), 0.07 (3H, s, CH_3Si), 0.62 (3H, s, H-18), 0.87 (9H, s, *t*-BuSi), 1.40 (9H, s, $(\text{CH}_3)_3\text{CO}$), 2.09 (3H, s, H-21), 4.16 (1H, d, $J = 11.3$, Ha-19), 4.24 (2H, m, H-3 + H β -7), 4.43 (1H, d, $J = 11.3$, Hb-19), 5.45 (1H, s, H-4), 8.06 (1H, s, HCOO); ^{13}C NMR δ -4.7 (CH_3Si), -4.6 (CH_3Si), 13.6 (C-18), 18.1 ($(\text{CH}_3)_3\text{CSi}$), 21.3 (C-11), 23.1 (C-16), 23.7 (C-15), 25.8 ($(\text{CH}_3)_3\text{CSi}$), 28.2 ($(\text{CH}_3)_3\text{CO}$), 28.9 (C-2), 30.0 (C-1), 31.4 (C-21), 35.2 (C-8), 39.0 (C-12), 40.1 (C-10), 44.1 (C-13), 49.8 (C-7), 53.7, 54.0 (C-9 and C-14), 63.1 (C-17), 65.2 (C-19), 67.4 (C-3) 80.0 ($(\text{CH}_3)_3\text{COOC}$), 129.2 (C-4), 138.6 (C-5), 154.2 ($(\text{CH}_3)_3\text{COOC}$), 160.8 (HCOO), 209.0 (C-20); HRMS (ESI) calcd. for $\text{C}_{32}\text{H}_{53}\text{NNaO}_6\text{Si}$ ($\text{M}+\text{Na}$) $^+$ 598.3534, found 598.3525.

2.5. 3 β ,19-Dihydroxy-6-(*tert*-butoxycarbonyl)-6-aza-4-pregnen-20-one (**7**)

To a solution of **6** (0.62 g, 1.08 mmol) in THF (20.0 mL) was added tetrabutylammonium fluoride (0.845 g, 3.23 mmol) and the mixture was stirred at room temperature for 2.5 h. The solution was concentrated under reduced pressure. The residue was dissolved in methanol (20.0 mL) and a saturated solution of K_2CO_3 (2.3 mL) added. The mixture was stirred at room temperature for 0.5 h and concentrated under reduced pressure. The residue was partitioned with ethyl acetate and brine, the organic layer was dried with sodium sulfate and the solvent evaporated under reduced pressure. The resulting solid was used in the next step without further purification. An analytical sample of compound **7** was purified (preparative TLC) for characterization. IR ν_{max} (cm^{-1}) 3446, 2966, 2935, 1684, 1655, 1394 and 1159; ^1H NMR δ 0.62 (3H, s, H-18), 1.46 (9H, s, $(\text{CH}_3)_3\text{CO}$), 2.10 (3H, s, H-21), 2.43 (1H, t, $J = 12$, H-7 α), 3.43 (1H, d, $J = 12$, Ha-19), 3.79 (1H, d, $J = 12$, Hb-19), 4.12 (1H, m, H-7 β), 4.24 (1H, m, H-3), 5.84 (1H, s, H-4); ^{13}C NMR δ 13.7 (C-18), 20.8 (C-11), 23.0 (C-16), 24.2 (C-15), 28.3 ($(\text{CH}_3)_3\text{CO}$), 29.5 (C-8), 30.1 (C-1), 31.5 (C-21), 35.6 (C-2), 38.9 (C-7), 39.0 (C-12), 43.0 (C-13), 44.2 (C-10), 53.0, 53.2 (C-9, C-14), 63.1 (C-17), 65.2 (C-19), 66.4 (C-3), 81.2 ($(\text{CH}_3)_3\text{COOC}$), 130.7 (C-4), 139.8 (C-5), 154.6 ($(\text{CH}_3)_3\text{COOC}$), 209.0 (C-20); HRMS (ESI) calcd. for $\text{C}_{25}\text{H}_{39}\text{NNaO}_5$ ($\text{M}+\text{Na}$) $^+$ 456.2720, found 456.2729.

2.6. 19-Hydroxy-6-(*tert*-butoxycarbonyl)-6-aza-4-pregnene-3,20-dione (**8**)

Compound **7** from the previous step (0.89 g) was dissolved in CH_2Cl_2 (42.0 mL) and manganese dioxide (3.28 g, 37.7 mmol)

added. The reaction mixture was stirred at room temperature for 2 h and filtered through a Celite layer. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (hexane/ethyl acetate 8:2) to give **8** (0.393 g, 85% from compound **6**). Mp 60–62 °C; IR ν_{\max} (cm⁻¹) 3425, 2943, 1699, 1666, 1641, 1367 and 1155; ¹H NMR δ 0.67 (3H, s, H-18), 1.47 (9H, s, (H₃C)₃CO), 2.11 (3H, s, H-21), 2.16 (bd, J 11.4, 19-OH), 2.38 (1H, m, H α -2), 2.40 (1H, m, H β -1), 2.51 (1H, t, J 9.0, H-17), 2.57 (1H, t, J 12.7, H α -7), 2.99 (1H, ddd, J 17.4, 15.5 and 6.5, H β -2), 3.76 (1H, t, J 11.4, Ha-19), 3.95 (1H, d, J 11.4, Hb-19), 4.23 (1H, dd, J 12.7 and 4.5, H β -7), 5.97 (1H, s, H-4); ¹³C NMR δ 13.5 (C-18), 20.7 (C-11), 23.0 (C-16), 23.6 (C-15), 28.1 ((CH₃)₃CO), 31.5 (C-21), 33.0 (C-1), 35.0 (C-2), 35.1 (C-8), 38.7 (C-12), 44.0 (C-10), 44.1 (C13), 49.9 (C-7), 52.8 (C-9), 52.9 (C-14), 63.0 (C-17), 66.1 (C-19), 82.6 ((CH₃)₃COOC), 126.8 (C-4), 153.3 (C-5), 158.8 ((CH₃)₃COOC), 199.8 (C-3), 208.8 (C-20); HRMS (ESI) calcd. for C₂₅H₃₇NNaO₅ (M+Na)⁺ 454.2564, found 454.2567.

2.7. 6-(tert-Butoxycarbonyl)-6-aza-4-pregnene-3,20-dione (**9**)

In a two necked flask equipped with a condenser and a septum under argon containing 1,1'-thiocarbonyldiimidazole (0.814 g, 4.56 mmol) and 4-dimethylaminopyridine (10 mg, 0.082 mmol) was added a solution of **8** (0.197 g, 0.46 mmol) in dry 1,2-dichloroethane (17.0 mL). The mixture was heated under reflux for 1.5 h and then the solvent was evaporated. The residue was purified by flash chromatography (hexane/ethyl acetate 4:6) to give the thioimidazole derivative as a white solid (0.183 g, 74%). The above compound and diphenylsilane (0.376 mL, 2.03 mmol) were dissolved in anhydrous toluene (4.8 mL) and heated to 115 °C under argon. Lauroyl peroxide in anhydrous toluene (0.112 g/mL, 1.35 mmol) was added in nine aliquots (0.53 mL each) at 20 min intervals. After the last portion was added, the mixture was heated under reflux for 2 h and then concentrated under reduced pressure. The residue was purified by flash chromatography (hexane/ethyl acetate 1:1) to give compound **9** (0.115 g, 61% yield from **8**). Mp 60–62 °C; IR ν_{\max} (cm⁻¹) 2979, 2948, 2879, 1710, 1669, 1613, 1374, 1297 and 1161; ¹H NMR δ 0.71 (3H, s, H-18), 1.22 (3H, s, H-19), 1.46 (9H, s, (H₃C)₃CO), 2.15 (3H, s, H-21), 2.54 (1H, t, J 8.8, H-17), 2.55 (1H, t, J 12.7, H α -7), 4.30 (1H, dd, J 12.7 and 4.7, H β -7), 5.83 (1H, s, H-4); ¹³C NMR δ 13.5 (C-18), 16.6 (C-19), 20.5 (C-11), 23.1 (C-16), 23.8 (C-15), 28.1 ((CH₃)₃CO), 31.5 (C-21), 33.5 (C-2), 33.6 (C-1), 34.0 (C-8), 38.3 (C-12), 38.4 (C-10), 44.0 (C-13), 49.9 (C-7), 52.1, 52.9 (C-9 and C-14), 63.1 (C-17), 81.7 ((CH₃)₃C)OOC), 121.3 (C-4), 153.6 ((CH₃)₃C)OOC), 163.5 (C-5), 198.9 (C-3), 208.9 (C-20); HRMS (ESI) calcd. for C₂₅H₃₈NO₄ (M+H)⁺ 416.2795, found 416.2799.

2.8. 6-Aza-4-pregnene-3,20-dione (6-azaprogestosterone, **2**)

To a solution of **9** (0.050 g, 0.12 mmol) in dry CH₂Cl₂ (2.5 mL) was added trifluoroacetic acid (0.287 mL, 2.52 mmol). The solution was stirred at room temperature overnight then aqueous NaHCO₃ (ss) was added and the mixture extracted with ethyl acetate. The organic layer was dried with sodium sulfate and the solvent evaporated under reduced pressure. The product was purified by preparative TLC (silica gel, ethyl acetate/methanol 9:1) to give **2** as a white, crystalline solid (0.033 g, 87%). Mp 215–216 °C; IR ν_{\max} (cm⁻¹) 3258, 2937, 2856, 1699, 1666, 1687, 1207 and 1134; ¹H NMR δ 0.68 (3H, s, H-18), 1.29 (3H, s, H-19), 2.14 (3H, s, H-21), 2.35–2.45 (2H, m, H-2), 2.54 (1H, t, J 9.0, H-17), 2.87 (1H, t, J 11.5, H α -7), 3.35 (1H, ddd, J 11.5, 6.0 and 2.5, H β -7), 5.12 (1H, s, H-4), 5.64 (1H, bs, NH); ¹³C NMR δ 13.3 (C-18), 19.2 (C-19), 20.2 (C-11), 23.0 (C-16), 23.8 (C-15), 31.5 (C-21), 31.9 (C-8), 32.7 (C-2), 33.8 (C-1), 36.1 (C-10), 38.2 (C-12), 43.8 (C-13), 46.2 (C-9), 47.7 (C-7), 53.9 (C-14), 63.0 (C-17), 99.2 (C-4), 173.5 (C-5), 194.8

(C-3), 208.9 (C-20); HRMS (ESI) calcd. for C₂₀H₂₉NNaO₂ (M+Na)⁺ 338.2091, found 338.2094. Analysis C₂₀H₂₉NO₂·H₂O: calcd. C, 72.04; H, 9.37; N, 4.20. Found C, 71.84; H, 9.26; N, 4.57.

2.9. 3 α -Hydroxy-3 β ,19-epoxy-6-aza-5-pregnen-20-one (**10**)

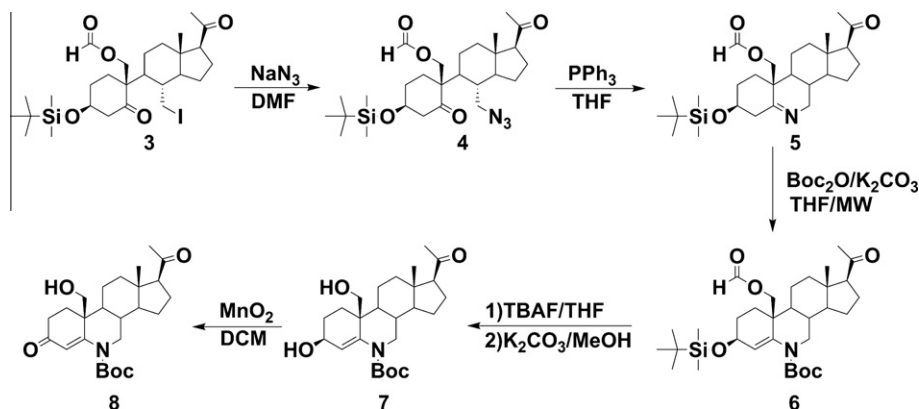
To a solution of **8** (0.080 g, 0.185 mmol) in dry CH₂Cl₂ (2.5 mL) was added trifluoroacetic acid (0.30 mL, 3.9 mmol). The mixture was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate and washed with aqueous NaHCO₃ (ss). The organic layer was dried with sodium sulfate and evaporated under reduced pressure. The residue was purified by preparative TLC (silica gel, ethyl acetate/methanol 9:1) to give **10** (0.052 g, 85%). Mp 203–205 °C; IR ν_{\max} (cm⁻¹) 3386, 2926, 2854, 1699, 1614, 1549, 1223, 1063 and 735; ¹H NMR (CDCl₃ + 10% CD₃OD) δ 0.655 (3H, s, H-18), 1.19 (2H, s, H-4), 2.08 (3H, s, H-21), 2.48 (1H, t, J 9.0, H-17), 2.87 (1H, dd, J 13.7 and 10.4, H α -7), 3.41 (1H, dd, J 13.7 and 6.3, H β -7), 3.76 (1H, d, J 11.7, Ha-19) and 3.83 (1H, d, J 11.7, Hb-19); ¹³C NMR (CDCl₃ + 10% CD₃OD) δ 13.2 (C-18), 20.5 (C-11), 22.8 (C-16), 23.4 (C-15), 29.3 (C-1), 29.4 (C-2), 29.5 (C-4), 31.2 (C-21), 31.3 (C-8), 38.3 (C-12), 41.2 (C-10), 43.9 (C-13), 45.0 (C-14), 46.9 (C-7), 54.7 (C-9), 62.8 (C-17), 64.3 (C-19), 114.4 (C-3), 175.6 (C-5) and 209.8 (C-20); HRMS (ESI) calcd. for C₂₀H₂₉NNaO₃ (M+Na)⁺ 354.2040, found 354.2040. Analysis C₂₀H₂₉NO₃: calcd. C, 72.47; H, 8.82; N, 4.23. Found C, 72.23; H, 9.11; N, 4.43.

3. Results and discussion

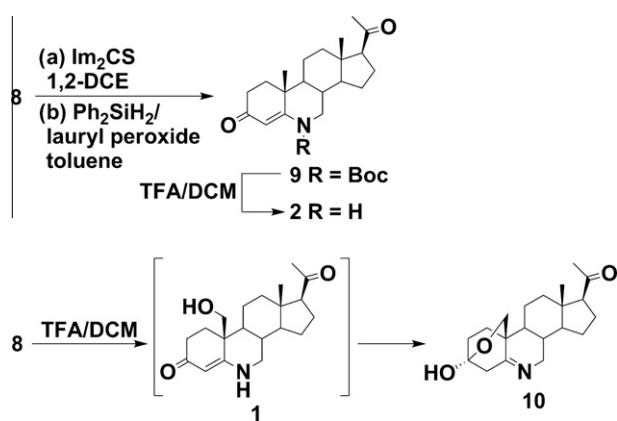
The synthesis of the 19-hydroxy-6-azapregnanone core was straightforward from the readily available secosteroid **3** (Scheme 1). This secosteroid may be easily prepared from pregnenolone in four steps and 42% yield following a synthetic route previously described by us [16]. Treatment of **3** with sodium azide in DMF gave the key 7-azido intermediate **4**. Reaction of the latter compound under Staudinger conditions with triphenylphosphine in THF gave the imine **5** as the only cyclization product in 62% yield. Acylation of the 6-nitrogen with migration of the double bond was performed by treatment with di-*tert*-butyl dicarbonate in THF/K₂CO₃, [17] the reaction was slow with conventional heating (12 h) but could be completed in 30 min under microwave heating, giving the *N*-Boc protected enamine **6** in 63% yield.

Treatment of **6** with tetrabutylammonium fluoride followed by deformylation with potassium carbonate in methanol gave the diol **7** that was selectively oxidized in good yield to *N*-Boc-19-hydroxy-6-azaprogestosterone (**8**) with manganese dioxide in dichloromethane at room temperature. The ¹H NMR spectrum of azasteroid **8** showed two mutually coupled signals, a doublet and a triplet at 3.95 and 3.76 ppm respectively, assigned to the C-19 hydrogens; at the same time the triplet showed coupling with the 19-hydroxyl at 2.16 ppm. Deoxygenation of the primary alcohol at C-19 was carried out with the Barton-Mc Combie procedure by formation of the 19-imidazolylthiocarbonate derivative and reduction with diphenylsilane to give *N*-Boc-6-azaprogestosterone (**9**) (Scheme 2). Removal of the protecting group with trifluoroacetic acid (TFA) gave 6-azaprogestosterone (**2**). Deprotection of the 19-hydroxylated analogue **8** with TFA gave the hemiketal **10** as the only product. Formation of the intramolecular hemiketal and migration of the double bond was evident from the NMR (1D and 2D) and mass spectra. Particularly diagnostic was the ¹³C NMR spectrum that lacked the δ^4 -3-keto system signals but showed signals at 175.6 ppm assigned to the imine carbon (C-5) and at 114.4 ppm corresponding to the hemiketetic carbon (C-3). The ¹H NMR spectrum showed two mutually coupled doublets at 3.76 and 3.83 ppm (*J* = 11.7 Hz), assigned to the hydrogens at position 19.

Preliminary activity results on the progesterone receptor (PR) were obtained using Cos-1 cells co-transfected with an expression



Scheme 1.



Scheme 2.

vector codifying the luciferase reporter gene under the control of the Mouse Mammary Tumor Virus Promoter (MMTV) and the vectors expressing the corresponding receptor [18]. 6-Azaprogesterone (**2**), hemiketal **10** and the *N*-Boc derivatives **8** and **9**, did not show significant progestagen activity when administered at 10^{-5} M and did not block the progesterone response (antiprogestagen activity). The lack of activity of compound **2** compared to the natural progestagen progesterone, indicates that the replacement of C-6 by a nitrogen atom negatively affects binding to the progesterone receptor. This is in line with previous SAR studies that indicate that polar substituents on the B ring are deleterious for progestagen activity [19].

Overall, we have developed a novel direct synthetic route for the preparation of 6-azapregnanes and their 19-hydroxylated derivatives from commercially available pregnenolone. We synthesized 6-azaprogesterone (**2**) and its *N*-protected 19-hydroxylated analogue (**8**) in a straightforward way with good yields. Although the predominance of the internal hemiketalic form is common in the case of 18-hydroxy-20-oxosteroids (e.g. 18-hydroxyprogesterone, 18-hydroxy-deoxycorticosterone), [20] this is not the case for 19-hydroxy-3-oxosteroids. However, at variance with 19-hydroxyprogesterone that is stable in its oxo form, 19-hydroxy-6-azaprogesterone cyclized spontaneously to the internal hemiketal **10**.

Acknowledgements

This work was supported by Grants from Universidad de Buenos Aires, ANPCyT and CONICET (Argentina). We thank Paola Y. Bertucci for performing the biological activity assays.

Appendix A. Supplementary data

^1H and ^{13}C NMR spectra of compounds **2**, **4**, **5**, **6**, **8** and **10**. Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.steroids.2012.10.012>.

References

- Ibrahim-Ouali M, Rocheblave L. Recent advances in azasteroids chemistry. *Steroids* 2008;73:375–407.
- Ibrahim-Ouali M. Recent advances in oxasteroids chemistry. *Steroids* 2007;72:475–508.
- Aggarwal S, Thareja S, Verma A, Raj Bhardwaj T, Kumar M. An overview on 5α -reductase inhibitors. *Steroids* 2010;75:109–53.
- Singh H, Jindal DP, Yadav MR, Kumar M. Heterosteroids and drug research. *Prog Med Chem* 1991;28:233–300.
- Xie W, Peng H, Kim DI, Kunkel M, Powis G, Zalkow LH. Structure-activity relationship of aza-steroids as PI-PLC inhibitors. *Bioorg Med Chem* 2001;9:1073–83.
- Gros L, Orenes Lorente S, Jimenez Jimenez C, Yardley V, Rattray L, Wharton H, Little S, Croft SL, Ruiz-Perez LM, Gonzalez-Pacanowska D, Gilbert IH. Evaluation of azasterols as anti-parasitics. *J Med Chem* 2006;49:6049–103.
- Burbiel J, Bracher F. Azasteroids as antifungals. *Steroids* 2003;68:587–94.
- Ibrahim-Ouali M, Rocheblave L. First synthesis of thia steroids from cholic acid. *Steroids* 2010;75:701–9.
- Aster SD, Von Langen DJ. 16-Substituted-6-aza-steroid 5- α -reductase inhibitors. *PCT Int Appl* 1997;WO 97/14418.
- del Rio RE, Wang S, Bohé L. Highly enantioselective oxidation of sulfides to sulfoxides by a new oxaziridinium salt. *Org Lett* 2007;9:2265–8.
- Kasal A, Matyas L, Budesinsky M. Neurosteroid analogues: synthesis of 6-aza-allopregnanolone. *Tetrahedron* 2005;61:2269–78.
- Jing X, Xu C-G, Ding K, Lin J-R, Jin R-H, Tian W-S. Protecting group effect on the 1,2-dehydrogenation of 19-hydroxysteroids: a highly efficient protocol for the synthesis of estrogens. *Tetrahedron Lett* 2010;51:3242–5.
- Galigniana MD, Vincent GP, Piwien-Pilipuk G, Burton G, Lantos CP. Mechanism of action of the potent sodium-retaining steroid 11,19-oxidoprogesterone. *Mol Pharmacol* 2000;58:58–70.
- Eduardo SL, Ghini AA, Burton G. Oxido-bridged neurosteroid analogues. Synthesis of 2,19-oxido-allopregnanolone. *Arkivoc* 2003:468–76.
- Veleiro AS, Pecci A, Monteserín MC, Baggio RF, Garland MT, Lantos CP, Burton G. 6,19-Sulfur-bridged progesterone analogues with antiimmunosuppressive activity. *J Med Chem* 2005;48:5675–83.
- Duran FJ, Ghini AA, Coirini H, Burton G. Synthesis of 6-thia analogs of the natural neurosteroid allopregnanolone. *Tetrahedron* 2006;62:4762–8.
- Schultz AG, Guzzo PR, Nowak DM. Asymmetric organic synthesis. Radical cyclizations of chiral enamides. *J Org Chem* 1995;60:8044–50.
- Alvarez LD, Dansey MV, Martí MA, Bertucci PY, Di Chenna PH, Pecci A, Burton G. Biological activity and ligand binding mode to the progesterone receptor of A-homo analogues of progesterone. *Bioorg Med Chem* 2011;19:1683–91.
- Bursi R, Groen MB. Application of (quantitative) structure-activity relationships to progestagens: from serendipity to structure-based design. *Eur J Med Chem* 2000;35:787–96.
- Weet JF, Lenz GR. Mineralocorticoid properties of potential metabolites of 18-hydroxydeoxycorticosterone and 18-hydroxyprogesterone. *J Med Chem* 1985;28:233–9.