Steroids 76 (2011) 1043–1050

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Azido analogs of neuroactive steroids

Lukáš Vidrna [a], Ivan Černý [a,*], Vladimír Pouzar [a], Jiřina Borovská [b], Vojtěch Vyklický [b], Ladislav Vyklický Jr. [b], Hana Chodounská [a]

[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., 166 10 Prague 6, Czech Republic
[b] Institute of Physiology, Academy of Sciences of the Czech Republic, v.v.i., 142 20 Prague 4, Czech Republic

ARTICLE INFO

Article history:
Received 27 January 2011
Received in revised form 4 April 2011
Accepted 8 April 2011
Available online 25 April 2011

Keywords:
Neuroactive steroids
Synthesis
Azides
NMDA receptor

ABSTRACT

Analogs of pregnanolone (3α-hydroxy-5β-pregnan-20-one), modified in position 17 were prepared. Compounds with 20-keto pregnane side chain replaced completely by azide (17α- and 17β-azido-5β-androstan-3α-ol), compounds with its part replaced (20-azido-21-nor-5β-pregnan-3α-ol), and compounds with keto group only replaced ((20*R*)- and (20*S*)-20-azido-5β-pregnan-3α-ol) were synthesized using tosylate displacements with sodium azide or Mitsunobu reaction with azoimide. All five azido steroids were converted into corresponding sulfates. Subsequent tests for inhibition of glutamate induced response on NMDA receptors revealed that modification of pregnanolone sulfate side chain with azide did not disturb the activity and some of sulfates tested were more active than parent compound.

1. Introduction

Steroidal azides are very often described in connection with preparation of corresponding amines [1–5]. Optical [6], thermal and photochemical properties [7–9] of steroidal azides were also studied. Recently, they were used in preparation of nitrogen heterocycles [10] or as intermediates for click chemistry [11].

In connection with broader project on neuroactive steroids we started with preparation of analogs of pregnanolone, modified in position 17. We designed analogs with the 20-keto pregnane side chain replaced with azido group only or with one or two carbon side chain bearing azido group. The rest of neurosteroid structure has to be conserved, so derivatives of 3α-hydroxy-5β-androstane, 21-nor-5β-pregnane or 5β-pregnane are to be synthesized.

In position 17 of androstane skeleton, 17α-azides are accessible by sulfonate substitution by sodium azide or Mitsunobu reaction with azoimide. The first approach was applied to 5α-androstane derivatives with 3-keto group [12], 3β-acetoxy group [13], or 3β-acetoxy-5-ene moiety [14]. On 3-keto-4-ene derivatives, effects of various solvents [15] or sulfonate leaving groups [16] were studied. So far mentioned examples covered substitution of pseudo-equatorial 17β-sulfonate with sodium azide to give 17α-azide. Also substitution in 14β-androstane series was described [17], where leaving 17α-sulfonate gave 17β-azide. The second approach, Mitsunobu reaction, was applied on 3-keto-4-ene derivatives and on 3-keto derivative with 5α-androstane skeleton and 17β-hydroxy group [18]. Nucleophilic substitution by sodium azide has been used also for preparation of 20-azidopregnane derivatives [19–21]. In this case, concurrent elimination reaction was noticed and the mechanism and solvent influence were studied [21].

2. Experimental

2.1. General

Melting points were determined on a Boetius micro melting point apparatus (Germany) and are uncorrected. Optical rotations were measured at 25 °C on a AUTOPOL IV polarimeter (Rudolph Research Analytical, USA), and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer. ^{1}H and ^{13}C NMR spectra were taken on Bruker AVANCE-400 instrument at 23 °C in deuterochloroform and referenced to tetramethylsilane as the internal standard. ^{13}C NMR spectra for pregnane derivatives **15a** and **15b** were measured on Bruker AVANCE-500 instrument under the above conditions, secondary referencing was performed using the solvent signal at position $\delta(CDCl_3) = 77.0$. In addition to 1D proton and carbon NMR spectra, homonuclear 2D-spectra (H,H-PFG-COSY, ROESY and J-resolved) together with heteronuclear 2D-spectra (H,C-PFG-HSQC) were used for complete structural assignment of signals (Table 1). Chemical shifts are given in ppm (δ-scale); coupling constants (J) and width of multiplets (W) are given in Hz.

* Corresponding author. Tel.: +420 220 183 385; fax: +420 220 183 385.
E-mail address: cerny@uochb.cas.cz (I. Černý).

0039-128X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2011.04.008

Table 1
Carbon-13 chemical shifts of selected azides in $CDCl_3$.

Carbon	3a	3b	4a	4b	9	10	15a	15b
1	37.12	37.00	35.47	35.40	37.06	35.43	35.29	35.32
2	37.18	37.14	30.60	30.55	37.18	30.59	30.48	30.54
3	212.91	212.84	71.80	71.74	213.02	71.81	71.78	71.84
4	42.31	42.29	36.47	36.43	42.35	36.49	36.38	36.38
5	44.30	44.20	42.08	42.05	44.29	42.12	41.98	42.03
6	26.53	26.46	27.09	27.01	26.69	27.14	27.09	27.11
7	26.08	25.50	26.73	26.13	25.86	26.77	26.34	26.44
8	35.84	35.69	36.16	35.98	35.42	35.72	35.56	35.74
9	40.55	40.92	40.29	40.59	41.06	40.77	40.38	40.47
10	34.99	34.99	34.66	34.68	34.99	34.69	34.56	34.58
11	20.74	20.77	20.36	20.37	20.91	20.51	20.53	20.62
12	32.71	37.34	32.79	37.44	38.14	38.22	39.24	38.74
13	45.95	44.58	45.99	44.57	42.25	42.22	42.21	42.70
14	49.50	52.18	49.64	52.23	55.68	49.86	56.22	55.80
15	24.57	23.55	24.64	23.59	24.54	24.59	24.09	24.34
16	28.66	26.99	28.65	27.00	26.58	26.51	26.86	26.52
17	71.51	71.27	71.62	71.38	49.80	55.74	56.07	55.22
18	17.65	12.31	17.67	12.27	12.51	12.50	12.31	12.40
19	22.63	22.64	23.32	23.33	22.66	23.36	23.32	23.35
20	–	–	–	–	52.80	52.91	61.12	59.09
21	–	–	–	–	–	–	19.54	18.84

Thin-layer chromatography (TLC) was performed on silica gel G (ICN Biochemicals), detection by spraying with concentrated sulfuric acid followed by heating. For column chromatography, Silica gel 60 (Merck, 63–100 μm) was used. Prior to evaporation on a rotary evaporator in vacuo (0.25 kPa, bath temperature 40 °C), solutions in organic solvents were dried over anhydrous sodium sulfate.

17β-Hydroxy-5β-androstan-3-one (**1a**), 17α-hydroxy-5β-androstan-3-one (**1b**), 3-oxo-21-nor-5β-pregnan-20-yl pivalate (**6**), and (20*R*)- and (20*S*)-20-hydroxy-5β-pregnan-3α-yl acetates (**12a** and **12b**) were prepared according to Refs. [22–25].

2.2. Chemical synthesis

2.2.1. 3-Oxo-5β-androstan-17β-yl tosylate (*2a*)

To a stirred solution of hydroxy derivative **1a** (3.0 g, 10.3 mmol) in pyridine (50 mL) cooled with ice-water bath, tosyl chloride (6.0 g, 31.5 mmol) was added in portions. After all reagent dissolved, the cooling bath was removed and the reaction mixture was left aside at room temperature overnight. Then the mixture was poured into water with ice (500 mL) and the product was filtered off and dried on air. After trituration with ethanol (30 mL), the crude product (4.2 g, 91%) was used further without purification. Analytical sample was crystallized from a mixture of chloroform/diethyl ether, m.p. 201–203 °C. ^{1}H NMR: 7.78 m, 2H (H-2′, H-6′); 7.32 m, 2H (H-3′, H-5′); 4.27 dd, 1H, J = 9.2, J' = 7.8 (H-17α); 2.62 dd, 1H, J = 15.0, J' = 13.6 (H-4α); 2.45 s, 3H (CH_3); 1.00 s, 3H (3× H-19); 0.82 s, 3H (3× H-18). Calcd. for $C_{26}H_{36}O_4S$ (444.6): C, 70.23; H, 8.16. Found: C, 70.50; H, 8.21.

2.2.2. 3-Oxo-5β-androstan-17α-yl tosylate (*2b*)

Hydroxy derivative **1b** (2.0 g, 6.9 mmol), pyridine (13 mL), and tosyl chloride (4.0 g, 20.1 mmol) were processed according to procedure in Section 2.2.1 only at 5 °C for 24 h. The mixture was then poured into water with ice (400 mL) and the product was extracted with ethyl acetate (100 mL). The ethyl acetate extract was washed with 10% aqueous citric acid, saturated aqueous potassium hydrogen carbonate (2×), water and dried. Solvents were evaporated and the crude foamy tosylate **2b** (3.0 g, 98%) was immediately processed further. ^{1}H NMR: 7.78 m, 2H (H-2′, H-6′); 7.34 m, 2H (H-3′, H-5′); 4.49 d, 1H, J = 5.6 (H-17β); 2.68 bt, 1H, J = 14.1 (H-4α); 2.45 s, 3H (CH_3); 1.00 s, 3H (3× H-19); 0.70 s, 3H (3× H-18).

2.2.3. 17α-Azido-5β-androstan-3-one (*3a*)

A. Tosylate **2a** (4.0 g, 9.0 mmol) and sodium azide (4.0 g, 61.5 mmol) in hexamethylphosphoramide (25 mL) in argon atmosphere were stirred and heated to 90 °C for 60 h. The reaction mixture was diluted with ethyl acetate (200 mL) and washed successively with water, 10% sulfuric acid, saturated aqueous potassium hydrogen carbonate, and water again. After drying, the solvent was evaporated leaving crude oily product (2.9 g). Chromatography on a column of silica gel (70 mL) in benzene yielded 2.47 g (74%) of oily azide **3a**. After crystallization from ethanol, 1.55 g (55%) of product **3a** was obtained, m.p. 85–86 °C, $[\alpha]_D$ −23 (*c* 0.38, chloroform). IR: 2101 (N_3); 1708 (C=O); 1382 (CH_3). ^{1}H NMR: 3.54 dd, 1H, J = 0.6, J' = 6.6 (H-17β); 2.70 dd, 1H, J = 13.6, J' = 14.8 (H-4α); 1.02 s, 3H (3× H-19); 0.77 s, 3H (3× H-18). For ^{13}C NMR data see Table 1. Calcd. for $C_{19}H_{29}N_3O$ (315.5): C, 72.34; H, 9.27; N, 13.32. Found: C, 72.46; H, 9.14; N, 13.28.

B. The stirred solution of hydroxy derivative **1a** (1.0 g, 3.44 mmol) and triphenylphosphine (1.08 g, 4.12 mmol) in benzene (10 mL) under argon was cooled with ice bath and azoimide in benzene (3 mL of solution prepared from 650 mg sodium azide, 0.8 mL water, 4 mL benzene, and 0.28 mL conc. sulfuric acid, Ref. [26]) was added. Then, diethyl azodicarboxylate (40% solution in toluene, 1.81 mL, 3.98 mmol) was added dropwise and the reaction mixture was heated to 70 °C for 4 h. After cooling, the solvents were evaporated and chromatography on silica gel column (50 mL) in petroleum ether/diethyl ether (10:1) yielded 670 mg (62%) of oily azide **3a**, identical with the product from procedure A.

2.2.4. 17β-Azido-5β-androstan-3-one (*3b*)

A. Tosylate **2b** (2.3 g, 5.2 mmol), sodium azide (2.5 g, 38.5 mmol), and hexamethylphosphoramide (15 mL) were processed according to procedure in Section 2.2.3A Crude oily product (1.5 g) was chromatographed on a silica gel column (150 mL) in a mixture of petroleum ether/acetone (50:1). Firstly, a mixture of eliminated products was eluted (700 mg). Further elution with petroleum ether/acetone (20:1) yielded 450 mg (27%) of oily azide **3b**, which spontaneously crystallize. M.p. 67–68 °C, $[\alpha]_D$ +35 (*c* 0.35, chloroform). IR: 2101 (N_3); 1708 (C=O); 1388, 1381 (CH_3). ^{1}H NMR: 3.34 t, 1H, J = 9.0 (H-17α); 2.67 dd, 1H, J = 15.1, J' = 13.4 (H-4α); 1.03 s, 3H (3× H-19); 0.78 s, 3H (3× H-18). For ^{13}C NMR data see Table 1. Calcd. for $C_{19}H_{29}N_3O$ (315.5): C, 72.34; H, 9.27; N, 13.32. Found: C, 72.43; H, 9.15; N, 13.21.

B. Hydroxy derivative **1b** (100 g, 0.34 mmol), triphenylphosphine (108 mg, 0.41 mmol) in benzene (1 mL), azoimide in benzene (from 65 mg sodium azide), and diethyl azodicarboxylate (40% solution in toluene, 180 μL, 0.40 mmol) were processed according to procedure in Section 2.2.3B at 70 °C for 2 h. Chromatography on silica gel column (10 mL) in petroleum ether/diethyl ether (10:1) yielded 70 mg of a mixture of eliminated products and 20 mg (18%) of oily azide **3b**, identical with the product from procedure A.

*2.2.5. 17α-Azido-5β-androstan-3α-ol (**4a**)*

Azide **3a** (1.1 g, 3.49 mmol) was dissolved in a mixture of ethyl acetate (4 mL) and methanol (12 mL) and cerium(III) chloride heptahydrate (1.43 g, 3.84 mmol) was added to a stirred solution. After all the cerium(III) chloride was completely dissolved, sodium borohydride (132 mg, 3.49 mmol) was added in portions over 5 min at room temperature. The reaction mixture was stirred for 15 min and then poured into 5% hydrochloric acid and extracted with ethyl acetate (80 mL). The ethyl acetate extract was washed with saturated aqueous potassium hydrogen carbonate (2×), water and dried. Solvents were evaporated and the crude product (1.14 g) was crystallized from acetone. The yield of azide **4a** was 900 mg (81%), m.p. 135–137 °C, $[\alpha]_D$ −25 (*c* 0.34, chloroform). IR: 3609, 3448 (OH); 2100 (N_3); 1381 (CH_3); 1033 (C–OH). ^{1}H NMR: 3.62 tt, 1H, *J* = 4.8, *J*′ = 11.1 (H-3β); 3.50 dd, 1H, *J* = 0.7, *J*′ = 6.7 (H-17β); 0.92 s, 3H (3× H-19); 0.73 s, 3H (3× H-18). For ^{13}C NMR data see Table 1. Calcd. for $C_{19}H_{31}N_3O$ (317.5): C, 71.88; H, 9.84; N, 13.24. Found: C, 71.99; H, 10.02; N, 13.01.

*2.2.6. 17β-Azido-5β-androstan-3α-ol (**4b**)*

Azide **3b** (400 mg, 1.27 mmol), ethyl acetate (2 mL), methanol (6 mL), cerium(III) chloride heptahydrate (650 mg, 1.74 mmol), and sodium borohydride (60 mg, 1.59 mmol) were processed according to procedure in Section 2.2.5. Crude product (380 mg) was crystallized from acetone giving 245 mg (61%) of azide **4b**, m.p. 135–136 °C, $[\alpha]_D$ +27 (*c* 0.47, chloroform). IR: 3609, 3452 (OH); 2100 (N_3); 1387, 1378 (CH_3); 1033 (C–OH). ^{1}H NMR: 3.64 tt, 1H, *J* = 4.7, *J*′ = 11.0 (H-3β); 3.32 t, 1H, *J* = 9.0 (H-17α); 0.93 s, 3H (3× H-19); 0.73 s, 3H (3× H-18). For ^{13}C NMR data see Table 1. Calcd. for $C_{19}H_{31}N_3O$ (317.5): C, 71.88; H, 9.84; N, 13.24. Found: C, 72.09; H, 9.98; N, 13.26.

*2.2.7. 17α-Azido-5β-androstan-3α-yl sulfate pyridinium salt (**5a**)*

To a solution of azide **4a** (200 mg, 0.63 mmol) in pyridine (5 mL) complex pyridine–sulfur trioxide (350 mg, 2.20 mmol) was added and the mixture was stirred in argon atmosphere and heated to 70 °C for 1 h. After cooling, the solvent was evaporated and the semisolid residue was shortly boiled with hot water (4 mL). The mixture was cooled in ice-bath and injected on to semi-preparative column of Lichrosorb RP-18 (75 mL). Polar impurities were washed out with water and the product was eluted with 95% aqueous methanol. The crude sulfate (280 mg) was crystallized from a mixture of methanol/diethyl ether. The yield of **5a** was 155 mg (52%), m.p. 165–169 °C, $[\alpha]_D$ −7 (*c* 0.42, chloroform). IR: 2535 (N^+–H); 2100 (N_3); 1637, 1549, 1490 (pyridine ring); 1381 (CH_3); 1262, 1172, 1049 (SO_3^-). ^{1}H NMR: 8.99 m, 2H (H-2′, H-6′); 8.50 tt, 1H, *J* = 1.5, *J*′ = 7.9 (H-4′); 8.03 m, 2H (H-3′, H-5′); 4.47 tt, 1H, *J* = 4.6, *J*′ = 11.3 (H-3β); 3.50 dd, 1H, *J* = 0.7, *J*′ = 6.7 (H-17β); 0.91 s, 3H (3× H-19); 0.72 s, 3H (3× H-18). Calcd. for $C_{24}H_{36}N_4O_4S$ (476.6): C, 60.48; H, 7.61; N, 11.75. Found: C, 60.65; H, 7.58; N, 11.57.

*2.2.8. 17β-Azido-5β-androstan-3α-yl sulfate pyridinium salt (**5b**)*

Azide **4b** (200 mg, 0.63 mmol) was processed according to procedure in Section 2.2.7. The crude sulfate (287 mg) was crystallized from a mixture of methanol/diethyl ether. The yield of **5b** was 175 mg (59%), m.p. 148–154 °C, $[\alpha]_D$ +35 (*c* 0.43, chloroform). IR: 2543 (N^+–H); 2100 (N_3); 1638, 1548, 1490 (pyridine ring); 1388, 1375 (CH_3); 1261, 1174, 1049 (SO_3^-). ^{1}H NMR: 8.98 m, 2H (H-2′, H-6′); 8.49 tt, 1H, *J* = 1.6, *J*′ = 7.8 (H-4′); 8.01 m, 2H (H-3′, H-5′); 4.47 tt, 1H, *J* = 4.9, *J*′ = 11.3 (H-3β); 3.31 t, 1H, *J* = 9.0 (H-17α); 0.92 s, 3H (3× H-19); 0.73 s, 3H (3× H-18). Calcd. for $C_{24}H_{36}N_4O_4S$ (476.6): C, 60.48; H, 7.61; N, 11.75. Found: C, 60.27; H, 7.62; N, 11.61.

*2.2.9. 20-Hydroxy-21-nor-5β-pregnan-3-one (**7**)*

3-Oxo-21-nor-5β-pregnan-20-yl pivalate (**6**, 1.36 g, 3.50 mmol) in benzene (17 mL) was refluxed in argon atmosphere with a solution of potassium hydroxide (3.5 g, 62.38 mmol) in 96% ethanol (70 mL) for 6 h and was left aside at room temperature overnight. The reaction mixture was poured into 5% hydrochloric acid (120 mL) with ice and extracted with ethyl acetate (120 mL). The extract was washed with aqueous saturated potassium hydrogen carbonate and water, dried, and the solvents were evaporated. Crude product (1 g) was chromatographed on a silica gel column (100 mL) in a mixture of petroleum ether/benzene/acetone (20:20:1) to give 720 mg (68%) of hydroxy derivative **7**. Analytical sample was crystallized from acetone, m.p. 166–168 °C, $[\alpha]_D$ +25 (*c* 0.34, chloroform). IR: 3623, 3474 (OH); 1707 (C=O); 1384 (CH_3). ^{1}H NMR: 3.73 ddd, 1H, *J* = 5.5, *J*′ = 6.7, *J*″ = 10.8 (H-20); 3.56 ddd, 1H, *J* = 4.3, *J*′ = 7.5, *J*″ = 10.8 (H-20′); 2.70 dd, 1H, *J* = 13.7, *J*′ = 14.7 (H-4α); 1.03 s, 3H (3× H-19); 0.68 s, 3H (3× H-18). Calcd. for $C_{20}H_{32}O_2$ (304.5): C, 78.90; H, 10.59. Found: C, 78.98; H, 10.80.

*2.2.10. 3-Oxo-21-nor-5β-pregnan-20-yl tosylate (**8**)*

Hydroxy derivative **7** (485 mg, 1.59 mmol) in pyridine (10 mL) with tosyl chloride (1.0 g, 5.0 mmol) was processed as described in Section 2.2.1. Then the mixture was poured into water with ice (100 mL) and the product was extracted with ethyl acetate (60 mL). The ethyl acetate extract was washed with 5% hydrochloric acid, saturated aqueous potassium hydrogen carbonate (2×), water and dried. Solvents were evaporated and the crude foamy tosylate **8** (700 g, 96%) was used without further purification. ^{1}H NMR: 7.79 m, 2H (H-2′, H-6′); 7.35 m, 2H (H-3′, H-5′); 4.06 dd, 1H, *J* = 7.6, *J*′ = 9.6 (H-20); 3.95 dd, 1H, *J* = 6.7, *J*′ = 9.6 (H-20′); 2.68 dd, 1H, *J* = 13.5, *J*′ = 14.8 (H-4α); 2.46 m, 3H (CH_3); 1.01 s, 3H (3× H-19); 0.59 s, 3H (3× H-18).

*2.2.11. 20-Azido-21-nor-5β-pregnan-3-one (**9**)*

Tosylate **8** (690 mg, 1.5 mmol), sodium azide (0.8 g, 12.3 mmol), and hexamethylphosphoramide (10 mL) were processed according to procedure in Section 2.2.3A, only reaction time was 4 h. Crude oily product (480 mg) was chromatographed on a silica gel column (100 mL) in a mixture of petroleum ether/benzene/acetone (50:50:1) to afford 440 mg (89%) of azide **9**. Crystallization from ethanol yielded 320 mg (65%), m.p. 85–87 °C, $[\alpha]_D$ +7 (*c* 0.26, chloroform). IR: 2098 (N_3); 1707 (C=O); 1390, 1381 (CH_3). ^{1}H NMR: 3.24 AB(ABX), 2H, J_{AB} = 12.2 (H-20, H-20′); 2.69 dd, 1H, *J* = 14.9, *J*′ = 13.6 (H-4α); 1.03 s, 3H (3× H-19); 0.66 s, 3H (3× H-18). For ^{13}C NMR data see Table 1. Calcd. for $C_{20}H_{31}N_3O$ (329.5): C, 72.91; H, 9.48; N, 12.75. Found: C, 73.00; H, 9.59; N, 12.54.

*2.2.12. 20-Azido-21-nor-5β-pregnan-3α-ol (**10**)*

Azide **9** (400 mg, 1.21 mmol), ethyl acetate (2 mL), methanol (6 mL), cerium(III) chloride heptahydrate (480 mg, 1.29 mmol), and sodium borohydride (46 mg, 1.21 mmol) were processed according to procedure in Section 2.2.5. Crude product (390 mg) was crystallized from acetone giving 240 mg (60%) of azide **10**, m.p. 118–119 °C, $[\alpha]_D$ +3 (*c* 0.24, chloroform). IR: 3609, 3448 (OH); 2097 (N_3); 1390, 1379 (CH_3); 1033 (C–OH). ^{1}H NMR: 3.63 ddt, 1H, *J* = 11.4, *J*′ = 15.8, *J*″ = 4.6 (H-3β); 3.22 AB(ABX), 2H, J_{AB} = 12.2 (H-20, H-20′); 0.93 s, 3H (3× H-19); 0.62 s, 3H (3× H-18). For ^{13}C NMR data see

Table 1. Calcd. for $C_{20}H_{33}N_3O$ (331.5): C, 72.46; H, 10.03; N, 12.68. Found: C, 72.59; H, 10.09; N, 12.56.

2.2.13. 20-Azido-21-nor-5β-pregnan-3α-yl sulfate pyridinium salt (**11**)

Azide **10** (100 mg, 0.30 mmol), pyridine (2.5 mL), and complex pyridine–sulfur trioxide (175 mg, 1.10 mmol) were processed according to procedure in Section 2.2.7. The crude sulfate (130 mg) was crystallized from a mixture of methanol/diethyl ether. The yield of **11** was 85 mg (57%), m.p. 166–170 °C, $[\alpha]_D$ +35 (*c* 0.43, chloroform). IR: 2543, 2022 (N^+–H); 2097 (N_3); 1628, 1549, 1490 (pyridine ring); 1379 (CH_3); 1262, 1173, 1048 (SO_3^-). ^{1}H NMR: 8.97 m, 2H (H-2′, H-6′); 8.49 tt, 1H, *J* = 1.5, *J*′ = 7.9 (H-4′); 8.00 m, 2H (H-3′, H-5′); 4.47 tt, 1H, *J* = 4.8, *J*′ = 11.3 (H-3β); 3.21 AB(ABX), 2H, J_{AB} = 12.2 (H-20, H-20′); 0.92 s, 3H (3× H-19); 0.61 s, 3H (3× H-18). Calcd. for $C_{25}H_{38}N_4O_4S$ (490.7): C, 61.20; H, 7.81; N, 11.21. Found: C, 61.35; H, 7.86; N, 11.21.

2.2.14. (20R)-5β-Pregnan-3α,20-diyl 3-acetate 20-tosylate (**13a**)

(20*R*)-Alcohol **12a** (2.4 g, 6.62 mmol), pyridine (40 mL), and tosyl chloride (4.0 g, 21.0 mmol) were processed according to procedure in Section 2.2.1. The reaction mixture was then poured into water with ice (400 mL) and the product was extracted with chloroform (3× 50 mL). The extract was washed with 5% hydrochloric acid, saturated aqueous sodium hydrogen carbonate (2×), water and dried. Solvents were evaporated to afford (20*R*)-tosylate **13a** (3.29 g, 96%): m.p. 148–150 °C. IR: 1721 (C=O); 1382, 1362 (CH_3); 1258, 1251 (C–O); 1175 (SO_2). ^{1}H NMR: 7.79 m, 2H (H-2′, H-6′); 7.32 m, 2H (H-3′, H-5′); 4.74 dq, 1H, *J* = 9.8, *J*′ = 6.3 (H-20); 4.72 tt, 1H, *J* = 4.9, *J*′ = 11.3 (H-3); 2.44 s, 3H (CH_3); 2.03 s, 3H, (CH_3CO), 1.18 d, 3H, *J* = 6.1 (3× H-21); 0.92 s, 3H (3× H-19); 0.66 s, 3H (3× H-18). Calcd. for $C_{30}H_{44}O_5S$ (516.7): C, 69.73; H, 8.58. Found: C, 70.03; H, 8.76.

2.2.15. (20S)-5β-Pregnan-3α,20-diyl 3-acetate 20-tosylate (**13b**)

(20*S*)-Alcohol **12b** (1.2 g, 3.31 mmol), tosyl chloride (1.28 g, 6.71 mmol), and pyridine (20 mL) were processed according to procedure in Section 2.2.1 to afford (20*S*)-tosylate **13b** (1.58 g, 92%), m.p. 134–136 °C (Ref. [27] 124–126 °C). IR: 1722 (C=O); 1381, 1362 (CH_3); 1256, 1028 (C–O); 1175 (SO_2). ^{1}H NMR: 7.78 m, 2H (H-2′, H-6′); 7.32 m, 2H (H-3′, H-5′); 4.71 tt, 1H, *J* = 4.9, *J*′ = 11.6 (H-3); 4.67 dq, 1H, *J* = 9.6, *J*′ = 6.2 (H-20); 2.44 s, 3H (CH_3); 2.02 s, 3H, (CH_3CO), 1.31 d, 3H, *J* = 6.2 (3× H-21); 0.91 s, 3H (3× H-19); 0.62 s, 3H (3× H-18). Calcd. for $C_{30}H_{44}O_5S$ (516.7): C, 69.73; H, 8.58. Found: C, 70.02; H, 8.87.

2.2.16. (20S)-20-Azido-5β-pregnan-3α-yl acetate (**14a**)

Tosylate **13a** (1.02 g, 1.97 mmol), sodium azide (1.29 g, 19.84 mmol), and hexamethylphosphoramide (10 mL) were processed according to procedure in Section 2.2.3A at 50 °C for 3 h. Crude oily product (750 mg) was chromatographed on a silica gel column (100 mL) in a mixture of petroleum ether/diethyl ether (100:1). Less polar fractions contained products of elimination (300 mg) and further elution afforded 420 mg (55%) of azide **14a**. Analytical sample was crystallized from acetone, m.p. 109–110 °C, $[\alpha]_D$ +62 (*c* 0.43, chloroform). IR: 2103 (N_3); 1722 (C=O); 1380, 1364 (CH_3); 1257, 1027 (C–O). ^{1}H NMR: 4.72 tt, 1H, *J* = 4.8, *J*′ = 11.4 (H-3); 3.27 dq, 1H, *J* = 9.2, *J*′ = 6.4 (H-20); 2.03 s, 3H (CH_3CO); 1.32 d, 3H, *J* = 6.5 (3× H-21); 0.93 s, 3H (3× H-19); 0.66 s, 3H (3× H-18). Calcd. for $C_{23}H_{37}N_3O_2$ (387.6): C, 71.28; H, 9.62; N, 10.84. Found: C, 71.56; H, 9.75; N, 10.54.

2.2.17. (20R)-20-Azido-5β-pregnan-3α-yl acetate (**14b**)

Tosylate **13b** (500 mg, 0.97 mmol), sodium azide (0.65 g, 10.0 mmol), and hexamethylphosphoramide (5 mL) were processed according to procedure in Section 2.2.3A to afford 115 mg (31%) of azide **14b**. Analytical sample was crystallized from acetone/heptane, m.p. 107–108 °C, $[\alpha]_D$ −11 (*c* 0.14, chloroform). IR: 2105 (N_3); 1721 (C=O); 1379, 1364 (CH_3); 1257, 1028 (C–O). ^{1}H NMR: 4.72 tt, 1H, *J* = 4.8, *J*′ = 11.3 (H-3); 3.13 dq, 1H, *J* = 10.8, *J*′ = 6.4 (H-20); 2.03 s, 3H (CH_3CO); 1.26 d, 3H, *J* = 6.4 (3× H-21); 0.94 s, 3H (3× H-19); 0.68 s, 3H (3× H-18). Calcd. for $C_{23}H_{37}N_3O_2$ (387.6): C, 71.28; H, 9.62; N, 10.84. Found: C, 71.59; H, 9.85; N, 10.50.

2.2.18. (20S)-20-Azido-5β-pregnan-3α-ol (**15a**)

Azide **14a** (135 mg, 0.35 mmol) in ethanol (27 mL) was stirred with a solution of sodium hydroxide (21 mg, 0.53 mmol) in methanol (1 mL) at 40 °C for 2 h. Then, the reaction mixture was poured into water (100 mL) and extracted with ethyl acetate (3× 50 mL). Collected organic phase was washed with water (100 mL), 5% hydrochloric acid (2× 50 mL), saturated aqueous sodium hydrogen carbonate (2× 50 mL), water (100 mL), and dried. Solvent was evaporated to give alcohol **15a** (114 mg, 95%): m.p. 120–123 °C, $[\alpha]_D$ +51 (*c* 0.10, chloroform). IR: 3609 (OH); 2103 (N_3); 1378 (CH_3); 1031 (C–O). ^{1}H NMR: 3.63 tt, 1H, *J* = 4.6, *J*′ = 11.0 (H-3); 3.26 dq, 1H, *J* = 8.9, *J*′ = 6.4 (H-20); 1.32 d, 3H, *J* = 6.5 (3× H-21); 0.92 s, 3H (3× H-19); 0.66 s, 3H (3× H-18). Calcd. for $C_{21}H_{35}N_3O$ (345.5): C, 73.00; H, 10.21; N, 12.16. Found: C, 73.10; H, 10.36; N, 11.49.

2.2.19. (20R)-20-Azido-5β-pregnan-3α-ol (**15b**)

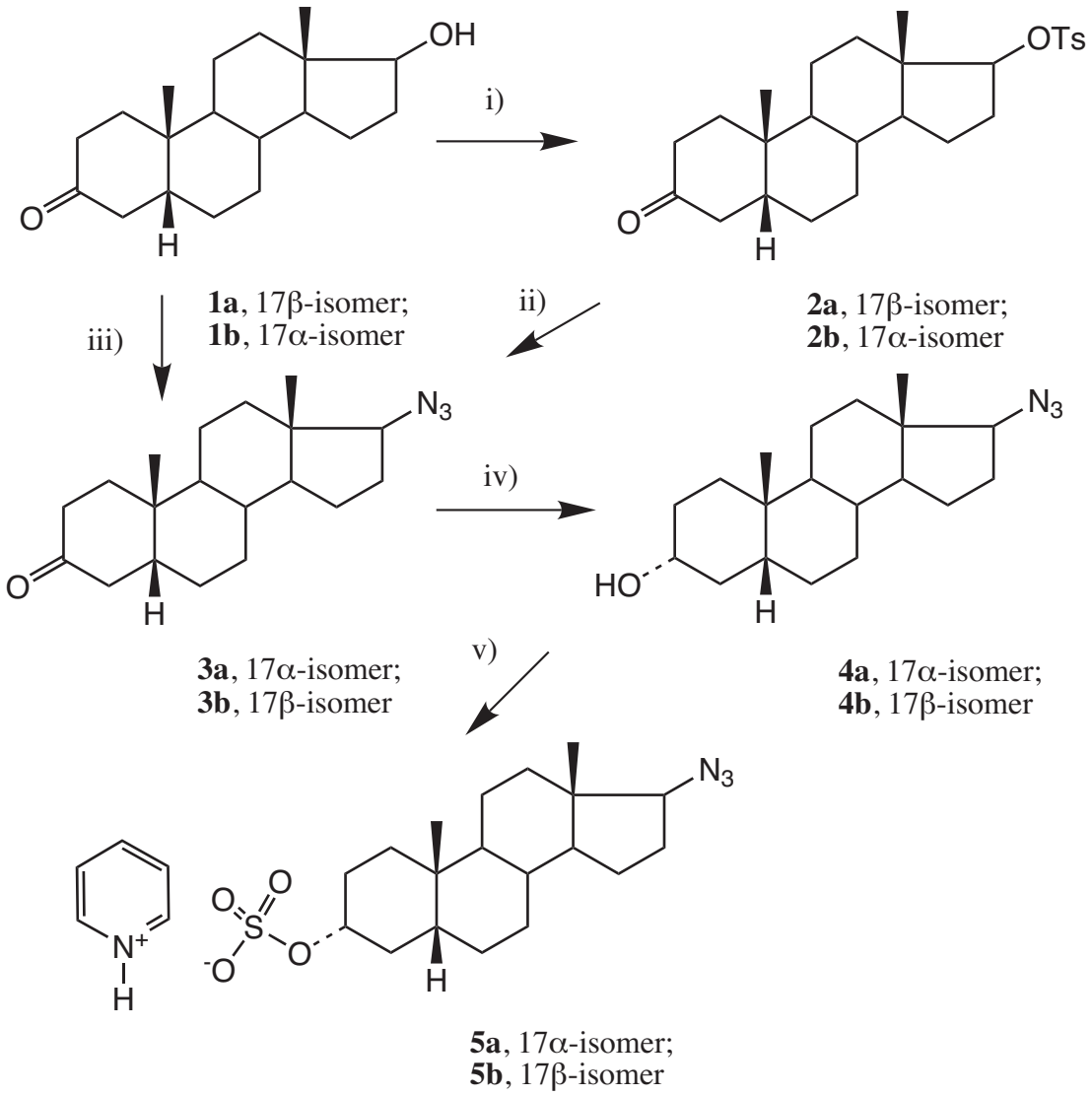
Azide **14b** (154 mg, 0.40 mmol) in ethanol (31 mL) and sodium hydroxide (24 mg, 0.60 mmol) in methanol (1 mL) were processed according to procedure in Section 2.2.18 to give alcohol **15b** (128 mg, 93%): m.p. 147–149 °C, $[\alpha]_D$ −29 (*c* 0.16, chloroform). IR: 3609 (OH); 2105 (N_3); 1378 (CH_3); 1031 (C–O). ^{1}H NMR: 3.63 tt, 1H, *J* = 4.6, *J*′ = 10.9 (H-3); 3.13 dq, 1H, *J* = 10.7, *J*′ = 6.4 (H-20); 1.26 d, 3H, *J* = 6.5 (3× H-21); 0.93 s, 3H (3× H-19); 0.68 s, 3H (3× H-18). Calcd. for $C_{21}H_{35}N_3O$ (345.5): C, 73.00; H, 10.21; N, 12.16. Found: C, 73.01; H, 10.36; N, 11.87.

2.2.20. (20S)-20-Azido-5β-pregnan-3α-yl sulfate pyridinium salt (**16a**)

A mixture of azide **15a** (100 mg, 0.29 mmol) and sulfur trioxide–pyridine complex (200 mg, 1.26 mmol) was stirred in freshly dried (phosphorus pentoxide) chloroform (5 mL) at room temperature for 6 h. After overnight-standing at −20 °C, the undissolved sulfur trioxide–pyridine complex was quickly filtered off and the filtrate was evaporated to afford sulfate **16a** (87 mg, 60%): m.p. 194–196 °C, $[\alpha]_D$ +36 (*c* 0.27, chloroform). IR: 2800–2200 (N^+–H); 2103 (N_3); 1637, 1548, 1489 (pyridine ring); 1378 (CH_3); 1257, 1174, 1048 (SO_3). ^{1}H NMR: 8.98 m, 2H (H-2′, H-6′); 8.48 tt, 1H, *J* = 1.5, *J*′ = 7.8 (H-4′); 8.00 m, 2H (H-3′, H-5′); 4.47 tt, 1H, *J* = 4.8, *J*′ = 11.2 (H-3); 3.26 dq, 1H, *J* = 9.5, *J*′ = 6.5 (H-20); 1.31 d, 3H, *J* = 6.5 (3× H-21); 0.91 s, 3H (3× H-19); 0.65 s, 3H (3× H-18). Calcd. for $C_{26}H_{40}N_4O_4S$ (504.7): C, 61.88; H, 7.99; N, 11.10. Found: C, 61.58; H, 8.10; N, 10.86.

2.2.21. (20R)-20-Azido-5β-pregnan-3α-yl sulfate pyridinium salt (**16b**)

Azide **15b** (50 mg, 0.14 mmol), sulfur trioxide–pyridine complex (200 mg, 1.26 mmol) and chloroform (5 mL) were processed according to procedure in Section 2.2.20 to give sulfate **16b** (40 mg, 55%): m.p. 172–174 °C, $[\alpha]_D$ +16 (*c* 0.20, chloroform). IR: 2800–2200 (N^+–H); 2105 (N_3); 1628, 1549, 1490 (pyridine ring); 1379 (CH_3); 1255, 1175, 1050 (SO_3). ^{1}H NMR: 8.96 m, 2H (H-2′, H-6′); 8.45 tt, 1H, *J* = 1.5, *J*′ = 7.8 (H-4′); 7.96 m, 2H (H-3′, H-5′); 4.47 tt, 1H, *J* = 4.8, *J*′ = 11.2 (H-3); 3.13 dq, 1H, *J* = 10.8, *J*′ = 6.3 (H-20); 1.25 d, 3H, *J* = 6.4 (3× H-21); 0.92 s, 3H (3× H-19); 0.67 s, 3H (3× H-18). Calcd. for $C_{26}H_{40}N_4O_4S$ (504.7): C, 61.88; H, 7.99; N, 11.10. Found: C, 61.60; H, 8.11; N, 10.97.



Scheme 1. Syntheses of 17-azido 5β-androstanes. (i) TsCl/Py, r.t. (5 °C), o.n. (24 h); (ii) NaN_3/HMPA, 90 °C, 60 h (16 h); (iii) Ph_3P, HN_3, DEAD/benzene, 70 °C, 4 h (2 h); (iv) $NaBH_4$, $CeCl_3 \cdot 7H_2O$/EtOAc, MeOH, r.t., 15 min; and (v) SO_3-Py/Py, 70 °C, 1 h. Temperatures or times for 17β-series if different are given in parentheses.

2.3. Biological assays

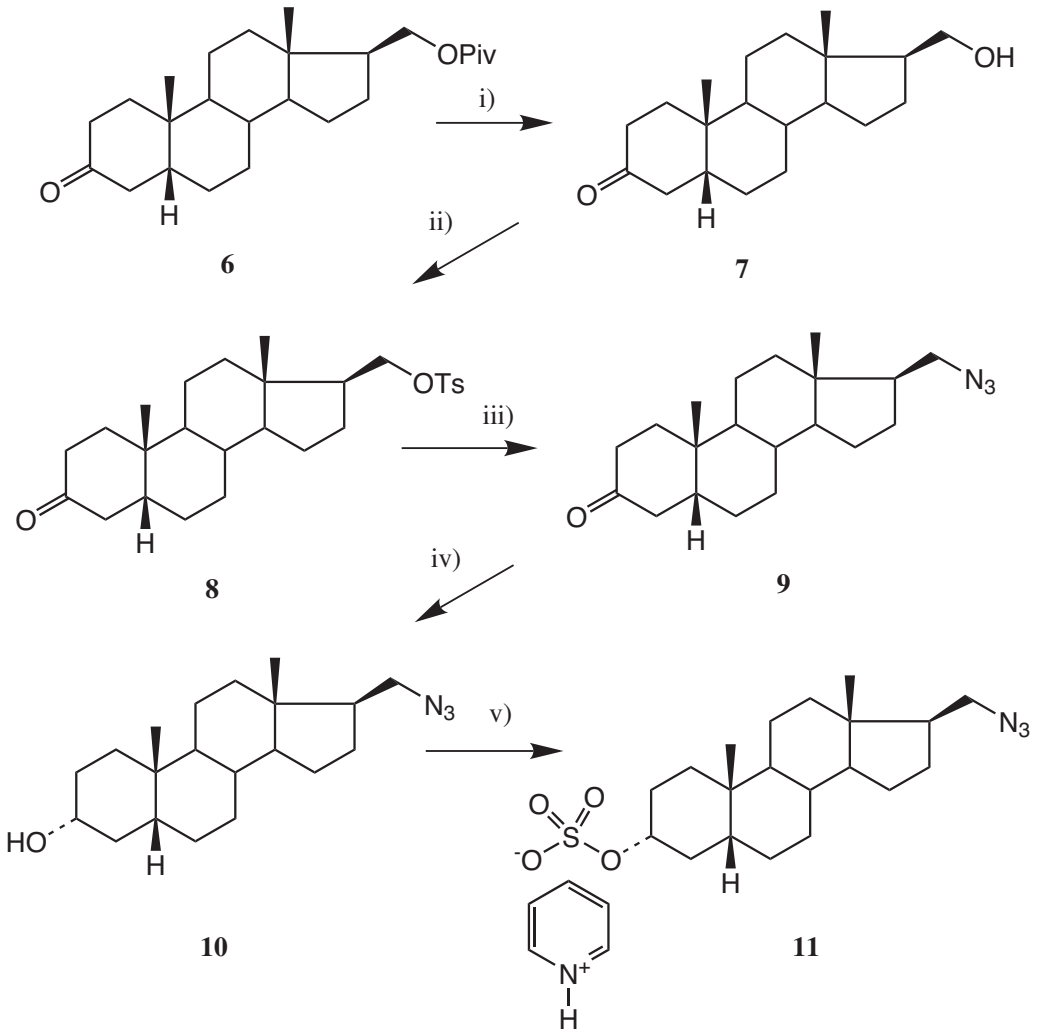
For the electrophysiological experiments on NMDA receptors, human embryonic kidney cells (HEK293) were transfected with NR1-1a/NR2B/GFP plasmids as described previously [28]. Experiments were performed 24–48 h after the end of transfection. Receptor cells were voltage-clamped at a holding potential of −60 mV and currents elicited were recorded. Whole-cell voltage-clamp recordings were made with a patch-clamp amplifier (Axopatch 1D; Axon Instruments, Inc., Foster City, CA). Patch pipettes (3–5 MΩ) pulled from borosilicate glass were filled with Cs^+-based intracellular solution (Cs-ICS) containing the following (in mM): 125 gluconic acid, 15 CsCl, 5 EGTA, 10 HEPES, 3 $MgCl_2$, 0.5 $CaCl_2$, and 2 ATP-Mg salt (pH-adjusted to 7.2 with CsOH). Extracellular solution (ECS) contained the following: (in mM): 160 NaCl, 2.5 KCl, 10 HEPES, 10 glucose, 0.2 EDTA, and 0.7 $CaCl_2$ (pH-adjusted to 7.3 with NaOH). Glycine (10 μM), a NMDA receptor co-agonist, was present in the control and test solutions. All steroids solutions were made from freshly prepared 20 mM stock in dimethyl sulfoxide (DMSO). The same concentration of DMSO was added in all extracellular solutions (see [29] for details).

3. Results and discussion

3.1. 17-Azido 5β-androstanes

For the preparation of steroidal azides in sterically hindered position 17, corresponding tosylates were treated with sodium azide in hexamethylphosphoramide (HMPA). From both isomers, 17α-azide is more easily available (Scheme 1). Starting from 17β-hydroxy-5β-androstan-3-one (**1a**), available from testosterone [22], tosylate **2a** was prepared. The nucleophilic substitution with sodium azide took 60 h at 90 °C for completion. Resulting 17α-azido-5β-androstan-3-one (**3a**) was obtained in 74% yield. In this case, the orientation of 17β-hydroxy group in **1a** is pseudo-equatorial, so Mitsunobu reaction with azoimide was used for comparison. After 4 h at 70 °C, azide **3a**, identical with that originated from tosylate **2a**, was obtained in 62% yield.

The preparation of 17β-azido derivative is more complicated. Firstly, starting 17α-hydroxy derivative **1b** (Scheme 1) is to be prepared from 17β-isomer **1a** by the reaction of corresponding tosylate with sodium nitrite [23] or by the Mitsunobu reaction with 4-nitrobenzoic acid [30,31], and both approaches has by-products



Scheme 2. Syntheses of 20-azido 21-nor-5β-pregnanes. (i) KOH/benzene, EtOH, rfl., 6 h; (ii) TsCl/Py, r.t., o.n.; (iii) NaN_3/HMPA, 90 °C, 4 h; (iv) $NaBH_4$,$CeCl_3 \cdot 7H_2O$/EtOAc, MeOH, r.t., 15 min; and (v) SO_3-Py/Py, 70 °C, 1 h.

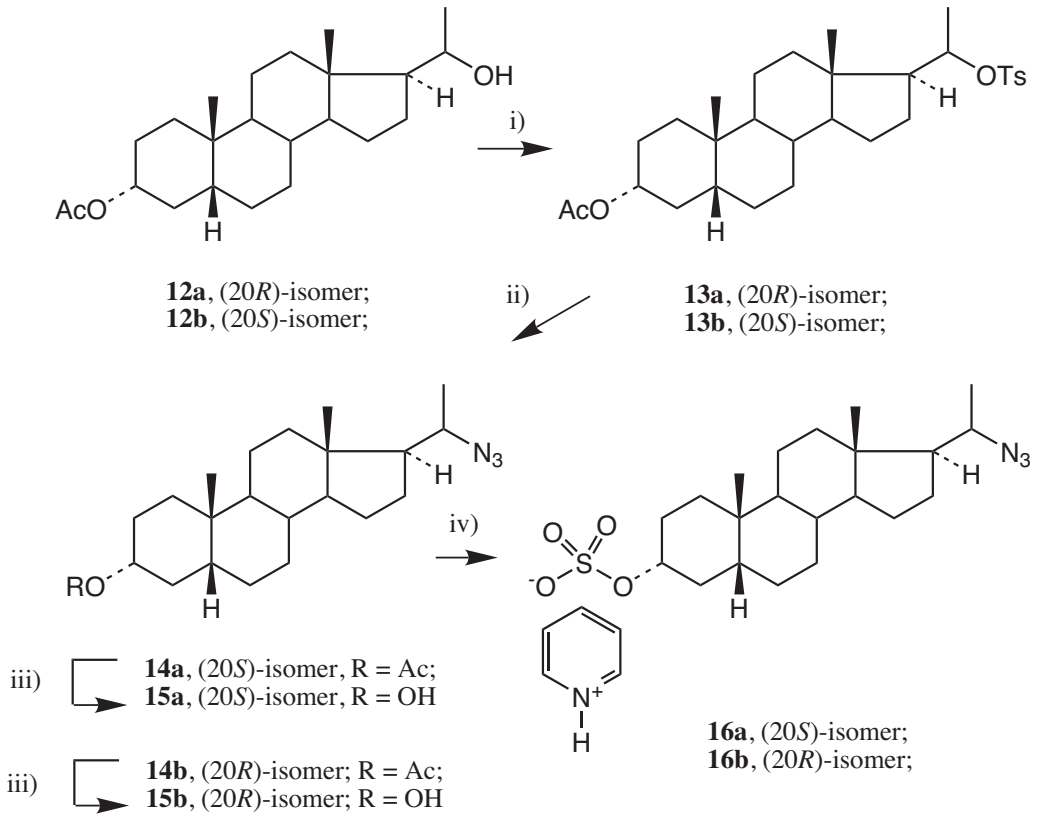
and gave only moderate yields. Tosyl derivative **2b** was susceptible to elimination reactions and it is better to keep it at lower temperature and use gentle and quick work-up. Also treatment of **2b** with sodium azide is accompanied by elimination and rearrangement reactions. Less polar fraction is formed by a mixture which was not separated and contained eliminated products (for similar side products on testosterone series see Refs. [16,32]). Desired 17β-azide **3b** was obtained in 27% yield. For comparison, Mitsunobu reaction with azoimide was performed, and regardless of pseudo-axial orientation of hydroxy group in starting **1b**, 18% of azide **3b** was obtained beside excess of eliminated products.

Ketone in azides **3a** and **3b** was reduced with sodium borohydride in presence of cerium(III) chloride heptahydrate [25] to suppress formation of 3β-hydroxy derivative and 17α-azido- and 17β-azido-5β-androstan-3α-ols (**4a** and **4b**) were prepared in 81% and 61% yields, respectively. For the preparation of sulfates **5a** and **5b**, reaction with sulfur trioxide–pyridine complex in pyridine was used and resulting products were purified on preparative C-18 reverse phase column.

3.2. 20-Azido 21-nor-5β-pregnanes and 5β-pregnanes

Starting compound for a synthesis of 20-azido 21-nor-pregnane series (Scheme 2) was prepared by hydrolysis of protecting pivalate group from known [24] 3-oxo-21-nor-5β-pregnan-20-yl pivalate (**6**). So obtained 20-hydroxyderivative **7** was transformed into tosylate **8** and subjected to azide displacement with sodium azide in hexamethylphosphoramide. In this case, the reaction proceeded smoothly and after 4 h at 90 °C was complete and gave 89% of azide **9**. Reduction of 3-keto group was performed similarly to androstane series with sodium borohydride and cerium(III) chloride heptahydrate and 3α-hydroxy derivative **10** was prepared. Subsequent reaction with sulfur trioxide–pyridine complex in pyridine gave sulfate **11**.

In pregnane series, a little different approach was adopted. Acetates **12a** and **12b**, available by cerium(III) chloride mediated borohydride reductions [25], were used as starting compounds (Scheme 3). Tosylates **13a** and **13b** were prepared by standard method used previously, gentle work-up is necessary also in this case. Both tosylates are more reactive than 17-tosylates in androstane series and treating with sodium azide in hexamethylphosphoramide at 50 °C for 3 h gave azides **14a** and **14b** in 55% and 31% yields, respectively. In both cases, products from elimination side-reactions were observed. In agreement with literature [21], hexamethylphosphoramide gave better yields of desired azide: in preliminary experiments on preparation of **14a** dimethylsulfoxide and N,N-dimethylformamide were checked and lower yields were obtained (DMSO/90 °C/6 h: 36%; DMF/90 °C/3 h: 43%). Protecting acetyl group was removed from **14a** and **14b** with methanolic sodium hydroxide and hydroxy derivatives **15a** and **15b**



Scheme 3. Syntheses of 20-azido 5β-pregnanes. (i) TsCl/Py, r.t., o.n.; (ii) NaN_3/HMPA, 50 °C, 3 h; (iii) NaOH/MeOH, EtOH, 40 °C, 2 h; (iv) SO_3-Py/$CHCl_3$, r.t., 6 h.

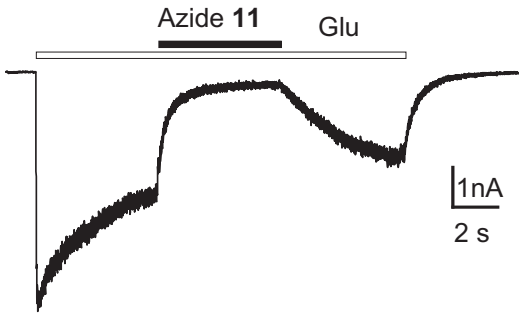


Fig. 1. Example of traces obtained from HEK293 cells transfected with NR1-1a/NR2B receptor. Azide **11** (15 μM) was applied simultaneously with 1 mM glutamate (duration of steroid and glutamate application is indicated by filled and open bars, respectively).

were obtained. Corresponding sulfates **16a** and **16b** were prepared with sulfur trioxide–pyridine complex in dry chloroform.

3.3. Biological activity

Synthetic analogs of pregnanolone sulfate were tested on responses mediated by NMDA receptors. Fig. 1 shows NR1-1a/NR2B receptor responses induced in HEK293 cells by 1 mM glutamate and that induced by glutamate co-application with azide sulfate **11**. At a concentration of 15 μM this steroid inhibited the responses by 83.9 ± 6.4% (n = 5) corresponding to IC_{50} = 3.0 ± 1.4 μM. The values of relative inhibition and estimated IC_{50} values of steroids **5a**, **5b**, **11**, **16a**, and **16b** are listed in Table 2. The data indicate that the value of IC_{50} varied for pregnanolone sulfate azido analogs 65-fold. The activity of pregnane derivatives **16a** and **16b** was comparable with previously published data for pregnanolone sulfate on the same receptors (IC_{50} = 44.4 ± 9.8 μM, Ref. [29]) and also on cultured hippocampal neurons (71.3 ± 5.0% inhibition, IC_{50} = 47.2 ± 9.9, Ref. [33]). Surprisingly the potency of compounds **5a**, **5b**, and **11** was approximately 10-fold higher.

Table 2
Inhibition of Glu-induced response in recombinant NMDA receptors cells by pregnanolone sulfate azido analogs.

Compound[a]	Conc. [μM]	% Inhib. ± S.D.[b]	IC_{50} (μM) ± S.D.	n[c]
17αN_3A **5a**	10	85.0 ± 2.9	2.4 ± 0.5	6
17βN_3A **5b**	5	68.8 ± 9.7	2.7 ± 1.0	6
20N_3nP **11**	15	83.9 ± 6.4	3.0 ± 1.4	5
20SN_3P **16a**	100	85.0 ± 7.6	24.0 ± 11.7	5
20RN_3P **16b**	100	37.5 ± 5.2	154.8 ± 21.9	6

[a] Skeleton and position/configuration of azido group were indicated (A – 5β-androstane, nP – 21-nor-5β-pregnane, P – 5β-pregnane).
[b] Relative degree of steroid inhibition of current responses induced in NR1-1a/NR2B receptors cells by fast application of 100 μM glutamate.
[c] Number of cells studied. Results are expressed as mean ± standard deviation (S.D.).

4. Conclusion

Syntheses of pregnanolone analogs with side chain in position 17 modified or replaced with azido group were developed. All five pregnanolone analogs were prepared by nucleophilic substitution of corresponding tosylates with sodium azide in hexamethylphosphoramide. In some cases, concurrent elimination reactions were observed so yields of this key reaction varied between 27% and 89%. For 17-azides **3a** and **3b** also alternative Mitsunobu reaction with azoimide was checked with comparable yields. Sulfates, intended for tests on NMDA receptor, were prepared by reactions with sulfur trioxide–pyridine complex in yields from 52% to 60%. All azido steroid sulfates tested showed inhibitory activity on responses induced by glutamate on NMDA receptor; in some cases higher

than that reported for pregnanolone sulfate [29]. Our results have shown that in case of pregnanolone sulfate, azido group can fully or partially replace side chain with oxygen function in position 17 without disturbing of inhibitory activity. These findings may contribute to the further study of NMDA receptor.

Acknowledgments

We are indebted to Dr. M. Buděšínský for help with interpreting of NMR spectra and especially for full assignment of ^{13}C NMR spectra of pregnane azides **15a** and **15b**. We thank Mrs. L. Kloučková and Mrs. D. Hybšová for skillful technical assistance. Dr. S. Vašíčková was taking the IR spectra and Dr. P. Fiedler was interpreting them. Mass spectra were measured by the staff of the Laboratory of Mass Spectrometry (Dr. J. Cvačka, Head). Elemental analyses were carried out in the Analytical Laboratory (Dr. S. Matějková, Head). This work was carried out as a part of the CAS research projects Z4 055 0506 and Z5 011 0509, and supported by the projects LC06077, LC554, 1M0002375201 (MSMT) and 203/08/1498, 309/07/0271 (GACR).

References

[1] Campbell AC, Maidment MS, Pick JH, Woods GF. Aminosteroids. Part 8. Synthesis of the four isomeric 3,7-diamino-5α-pregnanes. J Chem Soc, Perkin Trans 1 1982:1783–9.
[2] Davis AP, Orchard MG. Stereocontrolled synthesis of cholic acid derivatives with N-protected 7β- and/or 12β-amino substituents. Tetrahedron Lett 1992;33:5111–2.
[3] Černý I, Pouzar V, Lapčík O, Hampl R. Addition of azoimide to unsaturated ketones in the steroid series. Synthesis of N-(17β-hydroxy-3-oxo-5α-androstan-15β-yl)succinamoic acid and its evaluation as hapten for dihydrotestosterone immunoanalysis. Collect Czech Chem Commun 1997;62:1931–9.
[4] Černý I, Buděšínský M, Pouzar V, Drašar P. Linear chaining of etienic acid derivatives with the amide bond. Synthesis of oligomeric steroids. Collect Czech Chem Commun 2001;66:933–46.
[5] Matyáš L, Kasal A, Rierab ZB, Sunol CE. Effects of 3α-amino-5α-pregnan-20-one on $GABA_A$ receptor: synthesis, activity and cytotoxicity. Collect Czech Chem Commun 2004;69:1506–16.
[6] Djerassi C, Moscowitz A, Ponsold K, Steiner G. Optical rotary dispersion studies CIX. An octant rule for the azide. J Am Chem Soc 1967;89:347–52.
[7] Edwards OE, Douglas JL, Horwell DC, Rank W, Sano T. Thermal and photochemical reactions of steroidal α-azido ketones. Can J Chem 1992;70:2405–12.
[8] Barton DHR, Morgan Jr LR. 117. Photochemical transformations. Part XII. The photolysis of azides. J Chem Soc 1962:622–31.
[9] Pancrazi A, Khuong-Huu Q. Photochimie d'azido-steroides. Tetrahedron 1975;31:2041–8.
[10] Wölfling J, Oravecz ÉA, Ondré D, Mernyák E, Schneider G, Tóth I, et al. Stereoselective synthesis of some 17β-dihydrooxazinyl steroids, as novel presumed inhibitors of 17α-hydroxylase-C17,20-lyase. Steroids 2006;71:809–16.
[11] Felici M, Nolte RJM, Feiters MC, Contreras-Carballada P, Williams RM, Smits JMM, et al. Cationic heteroleptic cyclometalated iridium(III) complexes containing phenyl-triazole and triazole-pyridine clicked ligands. Molecules 2010;15:2039–59.
[12] Glaser R, Gabbay EJ. Synthesis and characterization of C3 and C17 steroidal amines. J Org Chem 1970;35:2907–12.
[13] Merlani MI, Amiranashvili LS, Mulkidzhanyan KG, Shelar AR, Manvi FV. Synthesis and antituberculosis activity of certain steroidal derivatives of the 5α-series. Chem Nat Compd 2008;44:618–20.
[14] Robinson CH, Ermann C, Hollis DP. The synthesis of 17α-amino-5-androsten-3β-ol; N.M.R. spectra of 17-substituted androstanes. Steroids 1965;6:509–18.
[15] Henbest HB, ackson JWR. The use of aprotic solvents for nucleophilic substitution reactions at C(3) and C(17) in steroids. J Chem Soc 1962:954–9.
[16] Shimizu T, Ohzeki T, Hiramoto K, Hori N, Nakata T. Chloromethanesulfonate as an efficient leaving group: rearrangement of the carbon–carbon bond and conversion of alcohols into azides and nitriles. Synthesis 1999:1373–85.
[17] Nambara T, Shibata T, Mimura M, Hosoda H. Synthesis of N-(steroid-17-yl)-maleimide. Chem Pharm Bull 1971;19:954–8.
[18] Loibner H, Zbiral E. Reaktionen mit phosphororganischen Verbindungen XLII. Nucleophile Substitutionen an Hydroxysteroiden mit Hilfe von Triphenylphosphan/Azodicarbonsaureester. Helv Chim Acta 1977;60:417–25.
[19] Leboeuf M, Cave A, Goutarel R. Alcaloides steroidiques LIX. Influence de la nature des solvants sur la mecanisme et la stereochimie de l'azidolyse des tosyloxy-20 β steroides. Application a la synthese d'amino-20 α steroides. Bull Soc Chim France 1967;6:2100–4.
[20] Goutarel R, Conreur C, Djakouré L, Leboeuf M, Cavé A. Alcaloides stéroidiques – LXXVII: syntheses de mono- et di-amines stéroidiques. Alcaloides A (saracodine) et B (saracocine) du Sarcococca pruniformis lindl. Tetrahedron 1968;24:7013–26.
[21] Leboeuf M, Cave A, Goutarel R. Alcaloides steroidiques LXXXVI. Substitutions, eliminations et transpositions des tosyloxy-20 steroides. 1er partie: role de l'hexamethylphosphotriamide (HMPT) dans la substitution et l'elimination. Bull Soc Chim France 1969;5:1619–24.
[22] Gabbard RB, Segaloff A. Facile preparation of 17β-hydroxy-5β-androstan-3-one and its 17α- methyl derivatives. J Org Chem 1962;27:655–6.
[23] Kasal A, Fuksová K, Pouzar V. Preparation of unlabelled and [^3H]-labelled epitestosterone and its metabolites. Collect Czech Chem Commun 1993;58:600–11.
[24] Pouzar V, Černý I, Drašar P. Acrylate side chain derivatives of 5β-steroids. Collect Czech Chem Commun 1993;58:2963–76.
[25] Št'astná E, Černý I, Pouzar V, Chodounská H. Stereoselectivity of sodium borohydride reduction of saturated steroidal ketones utilizing conditions of Luche reduction. Steroids 2010;75:721–5.
[26] Fieser LF, Fieser M. Reagents for Organic. Synthesis 1967;1:446.
[27] Yoshizawa I, Oh'Uchi R, Nagata K, Itoh S, Kawahara N. Isolation and structural elucidation of the degradation products of pregnanediol disulfate obtained by hot acid hydrolysis. Chem Pharm Bull 1982;30:2474–86.
[28] Cais O, Sedlacek M, Horak M, Dittert I, Vyklicky Jr L. Temperature dependence of NR1/NR2B NMDA receptor channels. Neuroscience 2008;151:428–38.
[29] Petrovic M, Sedlacek M, Horak M, Chodounska H, Vyklicky Jr L. 20-Oxo-5β-pregnan-3α-yl sulfate is a use-dependent NMDA receptor inhibitor. J Neurosci 2005;25:8439–50.
[30] Dodge JA, Lugar III CW. Alcohol inversion of 17β-steroids. Bioorg Med Chem Lett 1996;6:1–2.
[31] Tapolcsányi P, Wölfling J, Mernyák E, Schneider G. The Mitsunobu inversion reaction of sterically hindered 17-hydroxy steroids. Monatsh Chem 2004;135:1129–36.
[32] Vatèle J-M. 2-(Prenyloxymethyl)benzoyl (POMB) group: a new temporary protecting group removable by intramolecular cyclization. Tetrahedron 2007;63:10921–9.
[33] Št'astná E, Chodounská H, Pouzar V, Kapras V, Borovská J, Cais O, et al. Synthesis of C3 C5, and C7 pregnane derivatives and their effect on NMDA receptor responses in cultured rat hippocampal neurons. Steroids 2009;74:256–63.