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A New Class of Potent *N*-Methyl-D-Aspartate Receptor Inhibitors: Sulfated Neuroactive Steroids with Lipophilic D-Ring Modifications

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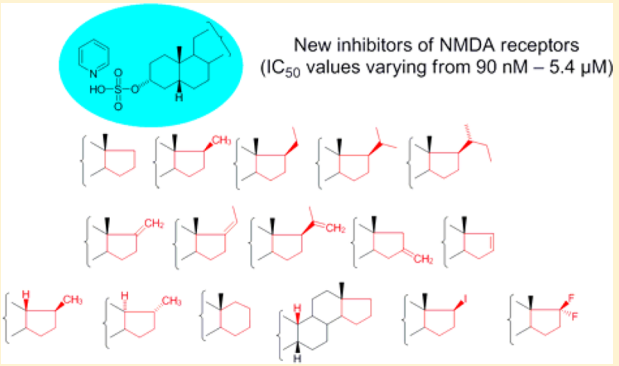
Supporting Information

ABSTRACT: *N*-Methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels that play a crucial role in excitatory synaptic transmission. However, the overactivation of NMDARs can lead to excitotoxic cell damage/death, and as such, they play a role in numerous neuropathological conditions. The activity of NMDARs is known to be influenced by a wide variety of allosteric modulators, including neurosteroids, which in turn makes them promising therapeutic targets. In this study, we describe a new class of neurosteroid analogues which possess structural modifications in the steroid D-ring region. These analogues were tested on recombinant GluN1/GluN2B receptors to evaluate the structure−activity relationship. Our results demonstrate that there is a strong correlation between this new structural feature and the in vitro activity, as all tested compounds were evaluated as more potent inhibitors of NMDA-induced currents (IC_{50} values varying from 90 nM to 5.4 μM) than the known endogeneous neurosteroid−pregnanolone sulfate (IC_{50} = 24.6 μM).



INTRODUCTION

N-Methyl-D-aspartate receptors (NMDARs) are glutamate-gated calcium permeable ion channels involved in excitatory synaptic transmission and synaptic plasticity.[1] However, their overactivation leads to excitotoxicity, a specific form of neuronal cell death that is thought to underlie various forms of neurodegeneration, such as Alzheimer's disease, ischemia, or traumatic brain injury.[2−5] The activity of NMDARs can be influenced by several allosteric modulators, including neurosteroids, neuroactive compounds synthesized in the nervous tissue from cholesterol, or steroidal precursors from peripheral sources.[6,7] Neurosteroids have both positive and negative effects on NMDARs, and such modulatory effects play a role in many physiological processes, including learning and development and certain neuropsychiatric disorders.[8,9]

20-Oxo-5β-pregnan-3α-yl sulfate (pregnanolone sulfate; 3α5βS, Figure 1) is an endogenous neurosteroid that inhibits responses of NMDARs.[10] Similarly to the open channel blockers, 3α5βS acts in a use-dependent manner (requiring receptor activation by agonists), but unlike them, its effect is voltage-independent.[10,11] Kinetic properties of steroid binding and inhibition are slow and not typical of a simple receptor−ligand interaction in an aqueous solution.[12] This indirectly suggests the importance of the plasma membrane as a compartment where the steroid accumulates to reach its binding site on NMDARs.

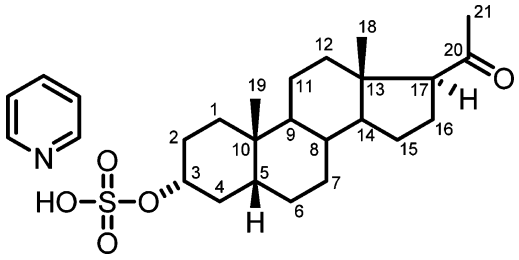


Figure 1. Structure of pregnanolone sulfate with steroid ring numbering system.

The inhibitory effect of neurosteroids on NMDARs is dependent upon the bent steroid ring structure that is associated with a 5β-stereochemistry, whereas the more planar arrangement of 5α-pregnanes favors potentiation.[13] In addition, the stereochemistry at position C-3 is also crucial to the inhibitory effect. As such, steroids having the combined 3α5β configuration were demonstrated as potent inhibitors of

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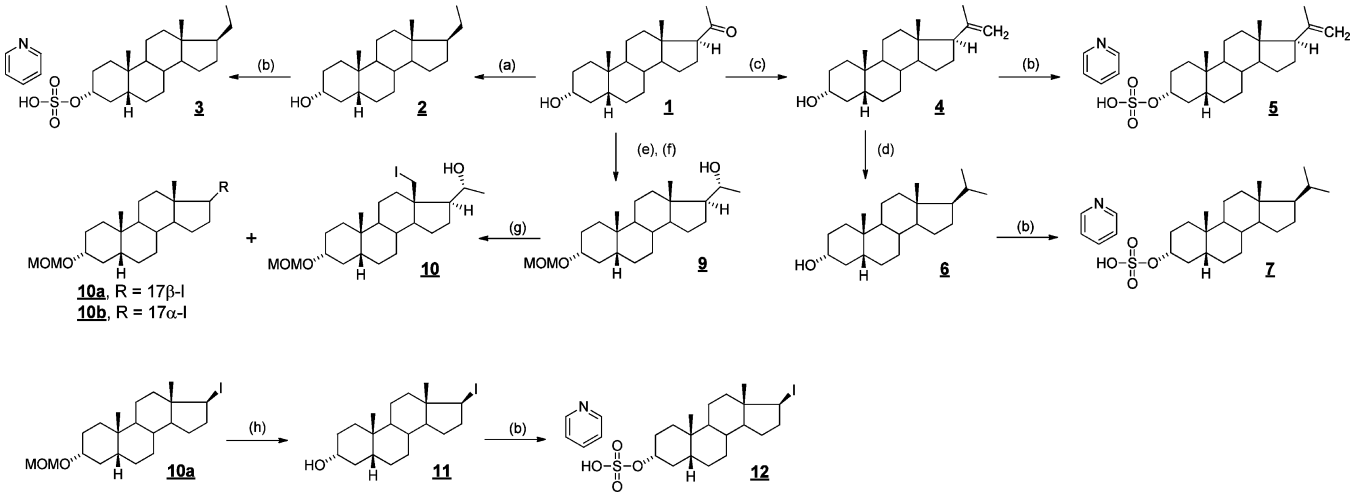
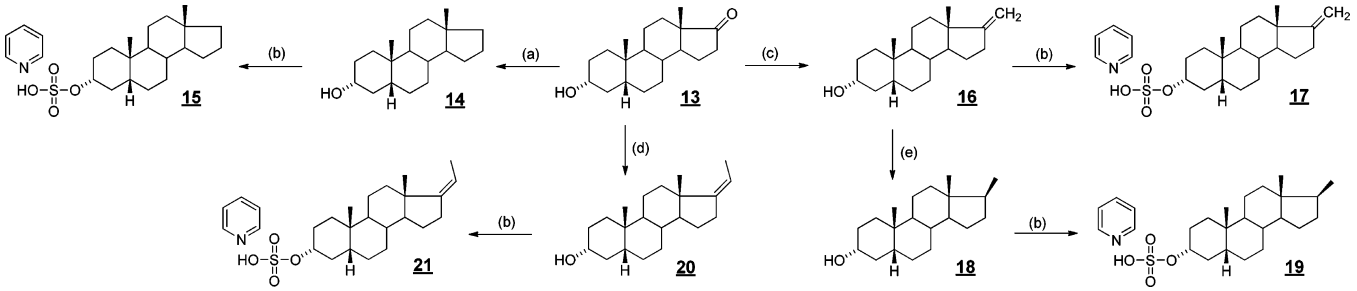
Scheme 1. Synthesis of D-Modified Compounds 3, 5, 7, and 12 Derived from Pregnanolone[a]



[a]Reagents and conditions: (a) diethylene glycol, NaOH, hydrazine hydrate; (b) py·SO_3, $CHCl_3$, pyridine; (c) $CH_3P(C_6H_5)_3I$, *n*-BuLi, THF; (d) H_2, Pd/$CaCO_3$, EtOAc, EtOH; (e) MOMCl, DIPEA, THF, reflux; (f) $NaBH_4$, MeOH, 0 °C; (g) $PhI(OAc)_2$, I_2, DCM, 500 W; (h) HCl, MeOH, H_2O.

Scheme 2. Synthesis of D-Modified Compounds 15, 17, 19, and 21[a]



[a]Reagents and conditions: (a) Zn, TMSCl, MeOH, DCM; (b) py·SO_3, $CHCl_3$, pyridine; (c) $CH_3P(C_6H_5)_3Br$, NaH, DMSO; (d) $CH_3CH_2P(C_6H_5)_3Br$, K^{t}OBu, THF; (e) H_2, Pd/$CaCO_3$, EtOAc, EtOH.

NMDA-induced currents.[13,14] To elucidate the effect of charge and chain length on modulatory action, a series of sulfate, hemiesters, permethylated amines, and carboxylic acids were synthesized (it has been established that C-3 substituent must bear charge to maintain the biological activity, while uncharged derivatives have no significant effect on NMDA modulation).[12,13] Our previous study on the structure–activity relationship of 3α5βS analogues also demonstrated that analogues having various substituents at the C-7 carbon may participate in steroid binding on the NMDA receptor[15] as well. The following paper describes the structure–activity relationship study of pregnanolone sulfate analogues with structural modifications on the steroidal D-ring that has not been previously reported. To elucidate the structure–activity relationship of the acetyl moiety of the pregnane skeleton and the relevance of the 20-oxo group, a series of nonpolar 3α5βS analogues were synthesized and their biological activity was evaluated on human embryonic kidney cells (HEK293) transfected with plasmids encoding GluN1-a/GluN2B/GFP genes. In addition, we have evaluated the action on recombinant (GluN1/Glu2A-D), native NMDA receptors expressed in hippocampal neurons, native AMPA/kainate, and native GABA receptors. A detailed understanding of the structure–activity relationships between neurosteroids and NMDA receptors is important for the development of drugs with potential therapeutic use. It has already been shown that a synthetic analogue of 3α5βS pregnanolone hemisuccinate ester has neuroprotective activity in both in vitro and in vivo models of neurodegeneration.[16–18]

RESULTS AND DISCUSSION

Chemistry. Compounds **3**, **5**, **7**, and **12** (Scheme 1) were prepared from the commercially available pregnanolone (**1**). 17-Ethyl-androstane sulfate **3** was prepared by a two-step reaction sequence: Huang–Minlon modification of Wolff–Kischner reduction,[19] followed by the treatment of the 3α-hydroxy derivative (**2**) with a sulfur trioxide pyridine complex in $CHCl_3$ to afford derivative **3** (57% yield). Compound **4** (20-exomethylene derivative) was prepared from pregnanolone (**1**) by Wittig reaction using methyltriphenylphosphonium iodide with *n*-BuLi in 76% yield. Then, sulfate **5** was prepared by sulfation (py·SO_3 complex in $CHCl_3$) in 78% yield. Compound **7** (17β-methyl derivative) was prepared from the previously described 20-methylene derivative (**4**): the double bond was reduced using Pd/$CaCO_3$ catalyzed hydrogenation in EtOAc and EtOH to give 20-methyl-5β-pregnane derivative **6** (76% yield). The 3α-hydroxy group was then transformed into the sulfate by sulfur trioxide pyridine complex in $CHCl_3$ and pyridine to afford 17-*iso*-propyl derivative **7** (93% yield). Compound **12** (17β-iodo-androstane) was prepared by a five-step sequence: compound **1** was first converted to MOM-derivative **8** (98%). Then, the 20-keto group was reduced by

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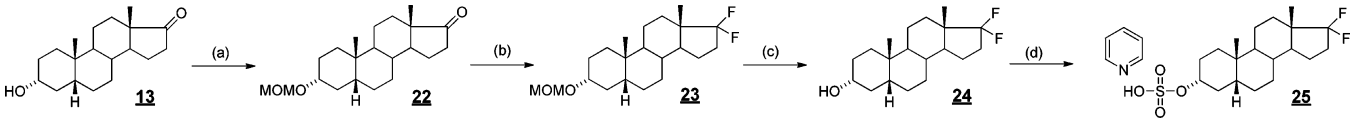
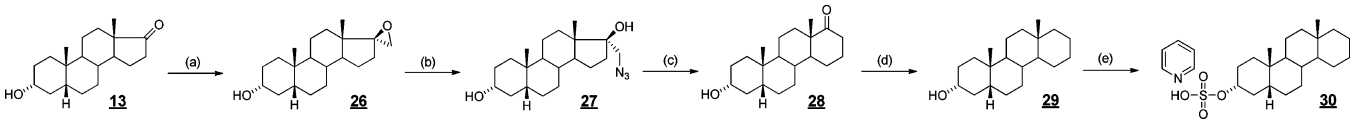
Scheme 3. Synthesis of D-Modified Compound 25[a]



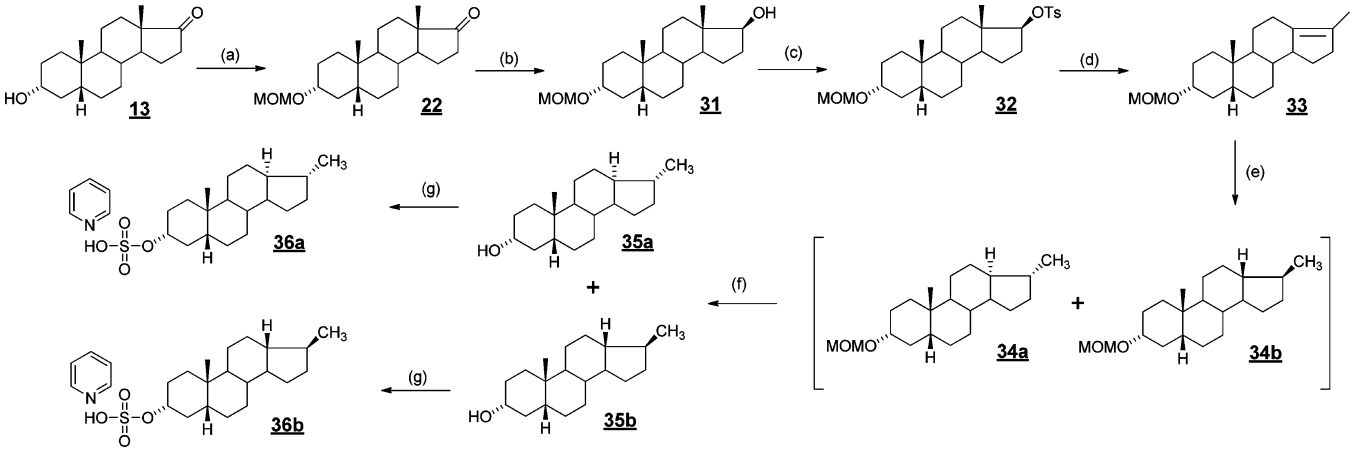
[a]Reagents and conditions: (a) MOMCl, DIPEA, DCM; (b) Bis(2-methoxyethyl)aminosulfur trifluoride, THF, 80 °C; (c) HCl, MeOH, H_2O; (d) py·SO_3, $CHCl_3$, pyridine.

Scheme 4. Synthesis of D-Modified Compound 30[a]



[a]Reagents and conditions: (a) $(CH_3)_3SI$, K^{t}OBu, DMF; (b) NaN_3, NH_4Cl, EtOH, H_2O, 90 °C; (c) NaI, TMSCl, acetonitrile; (d) Zn, TMSCl, MeOH, DCM; (e) py·SO_3, $CHCl_3$, pyridine.

Scheme 5. Synthesis of D-Modified Compounds 36a,36b[a]



[a]Reagents and conditions: (a) MOMCl, DIPEA, DCM; (b) $NaBH_4$, EtOH; (c) *p*-TsCl, DMAP, pyridine; (d) MeMgBr, 100 °C, toluene; (e) H_2, Pd/C, EtOAc, EtOH; (f) CH_3COCl, MeOH, benzene; (g) py·SO_3, $CHCl_3$, pyridine.

sodium borohydride to afford 20*R*-alcohol **9** as the major product (86% yield). Subsequently, compound **9** was treated with diacetoxyiodobenzene and iodine, and the reaction mixture was irradiated by halogen floodlight (500W). Compound **10**, having an iodine at position C-18, was isolated as the major product (58%). A mixture of compounds **10a** and **10b** was isolated as the byproduct. Attempts to isolate and purify compound **10b** on prep-TLC (30% ether in petroleum ether) failed due to the extreme similarity of R_f factors of compounds **10a** and **10b**. Therefore, only compound **10a** was isolated as a pure solid. Compound **10b** was isolated as a mixture with compound **10a**. Detailed spectroscopic data was obtained after acid hydrolysis of the MOM-protecting group, which afforded compound **11** (84% yield). The 3α-hydroxy group was then transformed into the sulfate by sulfur trioxide pyridine complex in $CHCl_3$ to afford derivative **12** (72% yield).

Compounds **15**, **17**, **19**, **21**, **25**, **30**, **36a**, **36b**, **43**, and **45** were prepared from 3α-hydroxy-5β-androstan-17-one (**13**). Compound **15** was prepared by a two-step reaction sequence (Scheme 2): Zn/TMSCl mediated Clemmensen reduction[20] of the 17-oxo group (79% yield), followed by treatment of 3α-hydroxy derivative **14** with a sulfur trioxide pyridine complex in $CHCl_3$ and pyridine to afford derivative **15** (67% yield). Compound **16** was prepared from compound **13** by Wittig reaction, using methyltriphenylphosphonium bromide with NaH, in 90% yield. 17-Exomethylene sulfate **17** (Scheme 2) was prepared by sulfation of **16** (py·SO_3 complex) in 81% yield. 17-Methylene derivative **16** was used for the synthesis of 17-methyl sulfate **19** (Scheme 2); catalytic hydrogenation using $Pd/CaCO_3$ gave 17-methyl derivative **18** (75% yield), which was converted into sulfate **19** (py·SO_3 complex, 78% yield). 17-Ethylidene-androstane sulfate **21** was prepared from compound **13** by a two-step reaction sequence: a Wittig reaction using ethyltriphenylphosphonium bromide,[21] followed by sulfation (py.SO_3 complex) to afford sulfate **21** (63% yield, Scheme 2).

17-Difluoro-androstane sulfate **25** (Scheme 3) was prepared by a four-step sequence: the 3α-hydroxy group of starting material **13** was protected as MOM-ether **22** in 93% yield. Then, the 17-ketone was treated with bis(2-methoxyethyl)-aminosulfur trifluoride in a sealed glass tube under an inert atmosphere, to afford difluoro derivative **23** (27%). The MOM group was removed under acidic conditions using HCl in MeOH in 91% yield. Sulfate **25** was prepared by treatment with sulfur trioxide pyridine complex (73% yield).

D-Homoandrostane sulfate **30** (Scheme 4) was prepared by modified Möller procedure for the conversion of a five-membered D-ring into a D-homo moiety:[22] the reaction of compound **13** with trimethylsulfonium iodide and potassium

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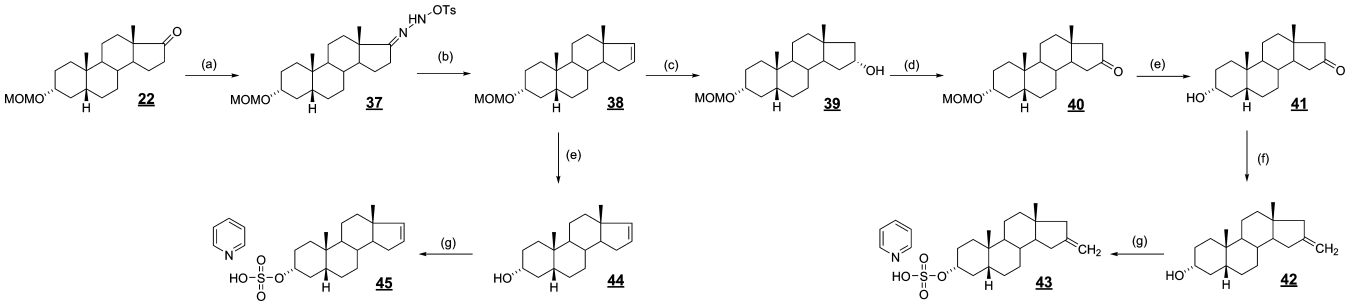
Scheme 6. Synthesis of D-Modified Compounds 43 and 45[a]



[a]Reagents and conditions: (a) $CH_3C_6H_4SO_2NHNH_2$, MeOH, reflux; (b) MeI, THF; (c) 9-BBN, THF, 0 °C; (d) PCC, DCM, pyridine; (e) HCl, MeOH, H_2O; (f) $CH_3P(C_6H_5)_3Br$, NaH, DMSO; (g) py·SO_3, $CHCl_3$, pyridine.

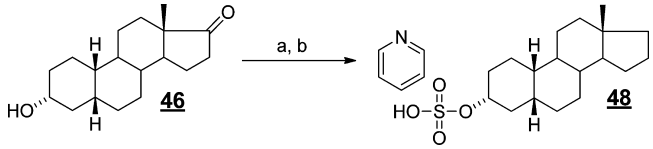
tert-butoxide in DMF afforded oxirane derivative **26** (78%), which was opened with sodium azide and ammonium chloride in an aqueous EtOH (**27**, 78% yield) and then cyclized with sodium iodide and trimethylsilyl iodide to afford D-homo derivative **28** in 73% yield. Finally, Zn/TMSCl modified Clemmensen reduction[20] of the 17a-oxo group (**29**, 65% yield), followed by treatment of the 3α-hydroxy group with a sulfur trioxide pyridine complex in $CHCl_3$ and pyridine, gave sulfate **30** (93% yield).

For the synthesis of isomeric 18-nor sulfates **36a** and **36b** (Scheme 5), we used our previously reported[23] methodology, utilizing the Kägi–Miescher rearrangement (1,2 shift of the C-18 methyl group): treatment of compound **13** with MOMCl in the presence of DIPEA gave derivative **22** in 93% yield. Reduction using $NaBH_4$ in EtOH gave 17β-alcohol derivative **31** in 94% yield. Compound **31** was then treated with *p*-toluenesulfonyl chloride in the presence of DMAP in pyridine to give 17β-tosylate **32** in 81% yield. The Kägi–Miescher rearrangement (1,2 shift of the C-18 methyl group-already described two sentences prior) was accomplished by refluxing 17β-tosylate **32** in toluene with methylmagnesium bromide to afford the desired $\Delta^{13(17)}$-ene derivative (**33**) in 63% yield. Reduction of the double bond using catalytic hydrogenation with Pd/C in EtOAc and EtOH gave a mixture of stereoisomers (**34a** and **34b**, 83%, stereoisomers were not separated). Acidic deprotection of the MOM group afforded a mixture of stereoisomers **35a** and **35b** (86%). HPLC separation gave compound **35a** and **35b** as a pure material. The homonuclear 2D-H,H-COSY, 2D-H,H-ROESY and heteronuclear 2D-H,C-HSQC and 2D-H,C-HMBC spectra were used for the structural assignment of the carbon and proton signals of compounds **35a** and **35b** (for details, see Supporting Information Table S1). Sulfates **36a** and **36b** were prepared by treatment with sulfur trioxide pyridine complex in 85% and 96% yield, respectively.

16-Exomethylene-androstane sulfate **43** and androst-16,17-ene sulfate **45** (Scheme 6) were prepared from common intermediate **38**: compound **13** was protected as MOM-ether **22** and then treated with tosylhydrazide in methanol under reflux to afford hydrazone **37** in 67% yield. Shapiro reaction of tosylhydrazone **37** with *n*-BuLi gave Δ^{16}-ene derivative **38** in 98% yield. Hydroxylation of the double bond of compound **38** using 9-BBN afforded 16-hydroxy derivative **39** (60% yield); position C-16 was functionalized by hydroboration. Preliminary experiments with borane in tetrahydrofuran afforded a mixture of 16- and 17-OH derivatives. Hydroboration with the bulkier hydroborating agent 9-borabicyclo(3.3.1)nonane (9-BBN) gave almost exclusively the 16α-derivative. Compound **39** was then oxidized by PCC in pyridine to afford 16-ketone derivative **40** in 95% yield. Deprotection of the MOM group using HCl in MeOH (**41**, 93% yield), Wittig reaction with methyltriphenylphosphonium bromide (**42**, 95% yield), and further treatment with pyridine sulfur trioxide pyridine complex gave sulfate **43** (56% yield). Direct deprotection of the MOM group of compound **38** using HCl in MeOH (**44**, 63% yield) and sulfation (py.SO_3 complex) gave sulfate **45** in 52% yield.

19-Nor-androstane derivative **47** (96% yield) was prepared by Zn/TMSCl modified Clemmensen reduction[20] of the 17-oxo group of compound **46**, which was prepared according to the literature.[24] Then, sulfate **48** was prepared by treatment of the 3α-hydroxy group with a sulfur trioxide pyridine complex in $CHCl_3$ and pyridine (35% yield, Scheme 7).

Scheme 7. Synthesis of D-Modified Compound 48 Derived from 3α-Hydroxy-5β-estran-17-one[a]

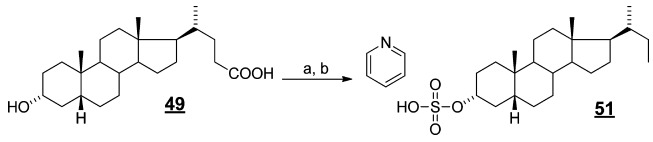


[a]Reagents and conditions: (a) Zn, TMSCl, MeOH, DCM; (b) py·SO_3, $CHCl_3$, pyridine.

Decarboxylation of commertially available lithocholic acid (**49**) with silver nitrate in DMSO afforded the desired 24-nor-5β-cholestane derivative (**50**) in 36% yield. Physical data were identical with literature.[25] Then, the 3α-hydroxy group was converted into sulfate **51** by treatment with a sulfur trioxide pyridine complex in $CHCl_3$ and pyridine in 83% yield (Scheme 8).

Biological Activity. To investigate the activity of pregnanolone sulfate and its analogues on NMDA receptors, cDNA encoding for the GluN1 and GluN2B subunits were

Scheme 8. Synthesis of D-Modified Compound 51 Derived from Lithocholic Acid[a]



[a]Reagents and conditions: (a) $AgNO_3$, DMSO, 80 °C; (c) py.SO_3, $CHCl_3$, pyridine.

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cotransfected into HEK293 cells. Figure 2 shows control responses of the transfected cells to a fast application of

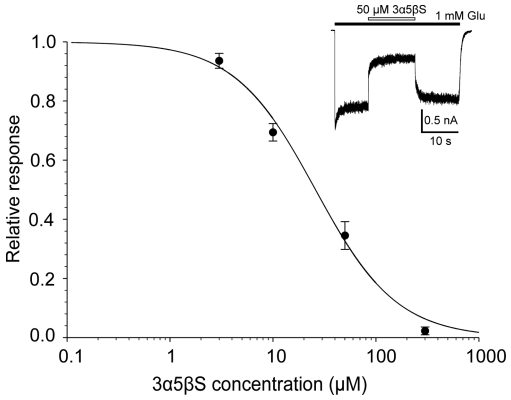

Figure 2. Effect of 3α5βS at GluN1/Glu2B receptors. Concentration–response curve for the 3α5βS effect at GluN1/Glu2B receptors. Data points are averaged values of normalized responses from five HEK293 cells. Error bars represent SD. The relative glutamate induced responses (I) recorded in the presence of 3α5βS (3–300 μM) and determined in individual cells were fit to the following logistic equation: $I = 1/(1 + ([3\alpha5\beta S]/IC_{50})^h)$, where IC_{50} is the concentration of 3α5βS that produces a 50% inhibition of agonist-evoked current, [3α5βS] is the 3α5βS concentration, and h is the apparent Hill coefficient. Inset show example of trace obtained from HEK293 cells transfected with cDNAs encoding GluN1/Glu2B receptors. 3α5βS (50 μM) was applied simultaneously with 1 mM glutamate (duration of 3α5βS and glutamate application is indicated by filled and open bars, respectively).

glutamate (1 mM) recorded in the presence of 10 μM glycine and no added Mg^{2+} from a cell voltage-clamped at a holding potential of −60 mV. The responses to a coapplication of 100 μM 3α5βS and glutamate made after the onset of the response to glutamate were inhibited in a dose-dependent manner. The results of the dose–response analysis indicate 100% inhibition at saturating steroid concentration with IC_{50} = 23.4 ± 1.6 μM and Hill coefficient 1.2 ± 0.1 (n = 5).

We have recently shown that the action of 3α5βS at the NMDA receptors involve several steps such as interaction of the steroid in its agglomerate form (the dominant form present in the water solution) with the cytoplasmic membrane and its subsequent binding in its single molecular form at the extracellular mouth of the channel vestibule to inhibit the NMDAR.[26] The value of IC_{50} therefore reflects total steroid concentration (sum of the single and agglomerate form) with predicted complex relation to the single molecular form present at the surface of the cytoplasmic membrane[26] (for details see Supporting Information). The amphipathic character of the D-modified steroids predicts that they may form solution similarly to 3α5βS. We have used light scattering analysis of compound **19** (as a model molecule) to infer on the form it exists in the water solution. The results show that compound **19** at concentrations ranging from 0.1 to 30 μM is present in the extracellular solution in three particle sizes (see Supporting Information, Figure S1; 3α5βS at a concentration relevant for NMDAR inhibition exists in two particle sizes (~2.5 and ~500 nm)).[26] At high steroid concentrations, the dose–response curve for some newly synthesized steroids deviated from that predicted by the logistic equation. We suspect that this was due to a greater proportion of the steroid being present in the form of large particle size(s) at higher steroid concentrations and the relatively lower contribution of the large particles to the steroid inhibitory effect. To avoid misinterpretation of the data, the IC_{50} for the newly synthesized steroids was determined from a single dose of the steroid using the following formula:

$$IC_{50} = [\text{compound}] \times \sqrt[h]{\frac{1 - I_I}{I_I}}$$

where I_I is the relative degree of inhibition, [compound] is the steroid concentration used, and h (fixed at 1.2). The IC_{50} value was determined for minimum of two steroid doses differing 3-fold in the concentration range. If the difference in the IC_{50} values was <10%, than the mean steroid IC_{50} was calculated from the steroid concentration most proximal to that inducing 50% inhibition. If the difference in the IC_{50} values determined for two steroid doses was >10%, then the dose–response analysis was determined at lower steroid concentrations to reach the formal criterion. In accordance with previous results, calculations of IC_{50} were made by assuming 100% inhibition at saturating steroid concentration[10,12] (see also Figure 2). Table 1 summarizes the IC_{50} determined for D-modified pregnanolone analogues.

Table 1. Effects of Pregnanolone Sulfate and its D-Modified Analogues on Responses of GluN1/GluN2B Receptors in HEK293 Cells to Glutamate

compound[a]	IC_{50} (μM) (h = 1.2), (n)	steroid concentration (μM)
pregnanolone sulfate[b]	24.6 ± 5.3 (n = 5)	100
3 (17β-CH_2CH_3)	0.4 ± 0.1 (n = 4)	1
5 (C_{20}=CH_2)	0.5 ± 0.1 (n = 5)	1
7 (17β-$CH(CH_3)_2$)	0.17 ± 0.03 (n = 3)	0.3
12 (17β-I)	0.6 ± 0.1 (n = 3)	0.3
15 (17β-H,17α-H)	1.2 ± 0.2 (n = 11)	3
17 (C_{17}=CH_2)	1.6 ± 0.3 (n = 6)	3
19 (17β-CH_3)	0.6 ± 0.1 (n = 5)	1
21 (C_{17}=$CHCH_3$)	0.8 ± 0.1 (n = 4)	1
25 (17β-F,17α-F)	7.0 ± 1.6 (n = 5)	10
30 (D-homo)	1.1 ± 0.3 (n = 4)	1
36a (17α-CH_3,18α-H)	0.9 ± 0.1 (n = 6)	3
36b (17β-CH_3,18β-H)	0.7 ± 0.1 (n = 4)	0.3
43 (C_{16}=CH_2)	2.2 ± 0.3 (n = 5)	3
45[c] ($\Delta^{16,17}$)	N/A	1, 3, 10, 50
48 (17α-H,17β-H,19β-H)	5.4 ± 1.5 (n = 6)	10
51 (17β-$CH(CH_3)CH_2CH_3$)	0.09 ± 0.01 (n = 3)	0.3

[a]The abbreviation of modification on the D-ring is added in the parentheses. [b]See the ref 12. [c]No effect at 1, 3, 10 μM, not soluble at 50 μM.

Effect of Pregnane 20-Oxo Group/Acetyl Moiety on Modulation of NMDARs. Comparison of the IC_{50} values of pregnanolone sulfate (IC_{50} = 24.6 ± 5.3 μM) with the deoxygenated analogue (compound **3**, IC_{50} = 0.4 ± 0.1 μM) shows more than a 60-fold decrease in the IC_{50} value, indicating that the 20-oxo group on the acetyl pregnane moiety can be removed while maintaining the ability of the neurosteroid to inhibit the NMDA induced currents. Additionally, changing the 20-oxo group to a methylene group (compound **5**) showed a ~50-fold decrease in IC_{50} values, in favor of steroid **5** (IC_{50} = 0.5 ± 0.1 μM). Moreover, interchange of the 20-methylene group (compound **5**) with a 20-methyl group (compound **7**) gave a ~3-fold decrease of IC_{50} values (IC_{50} = 0.16 ± 0.02 μM)

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in favor of compound **7**. Therefrom, we conclude that the 20-oxo group/acetyl moiety of pregnanolone sulfate (3α5βs) is not crucial for the ability of the neurosteroid to inhibit NMDA-induced currents.

Effect of Chain Length of Substituent at C-17 on Modulation of NMDARs. Comparison of alkyl modification on the D-ring of compounds **3**, **7**, **19**, and **51** with acetyl moiety of pregnanolone sulfate shows that such structural modification leads to significant decrease of IC_{50} values as compared with 3α5βS: the replacement of the 17-acetyl moiety of pregnanolone sulfate with hydrogens resulted in more potent NMDAR inhibitor (compound **15**, IC_{50} = 1.2 ± 0.21 μM). Compounds **3**, **7**, **19**, and **51** have alkyl substituents of increasing length on C-17. Comparison of the IC_{50} values of compound **19** (C-17-methyl, IC_{50} = 0.6 ± 0.1 μM), compound **3** (C-17-ethyl, IC_{50} = 0.4 ± 0.1 μM), compound **7** (C-17-*i*-propyl, IC_{50} = 0.16 ± 0.02 μM), and compound **51** (C-17-*i*-butyl, IC_{50} = 0.09 ± 0.01 μM) display anywhere from a 41- to a ~273-fold decrease in IC_{50} values, with respect to pregnanolone sulfate. These results clearly indicate that the replacement of the pregnanolone acetyl moiety with an alkyl substituent has a positive effect on the ability of the neurosteroid to inhibit NMDA-induced currents.

Steroids **36a** and **36b** are close structural analogues of steroid **15** (the 18-methyl group on C-13 is substituted by hydrogen), however, the conformation at C-13 and C-17 is identical for compound **36b**. Conversely, the conformation of C-13 and C-17 on compound **36a** is isomeric to **36b**. Comparison of the IC_{50} of steroids **36a** and **36b** (IC_{50} = 0.9 ± 0.1 and 0.7 ± 0.1 μM, respectively) with compound **15** (IC_{50} = 1.2 ± 0.2 μM) indicates that methyl group at C-13 does not have a large effect on the ability of the neurosteroid to inhibit NMDA-induced currents. Surprisingly, 19-nor derivative **48** shows a ~7-fold increase in its IC_{50} value as compared with compounds **36a** and **36b**.

Effect of D-Ring on Modulation of NMDARs. Comparing the IC_{50} of five-membered D-ring steroid **15** with six-membered steroid **30** (IC_{50}= 1.2 ± 0.2 and 1.1 ± 0.3 μM, respectively) indicates that expansion of the ring and the presence of the 17a-carbon does not affect the IC_{50} value.

Effect of Double Bond on Modulation of NMDARs. Three compounds bearing a methylene double bond were prepared: a 16-methylene (**43**, IC_{50} = 2.2 ± 0.3 μM), 17-methylene (**17**, IC_{50} = 1.6 ± 0.3 μM), and 20-methylene (**5**, IC_{50} = 0.5 ± 0.1 μM) derivative. Comparison of the IC_{50} values of these compounds indicates that the position of methylene moiety does affect the IC_{50} value, displaying a preference for location in place of the former carbonyl moiety. This was also found to be the case with the similarly located double bond of compound **21** (IC_{50} = 0.8 ± 0.08 μM), which gave a comparable IC_{50} value to that of compound **5**. Surprisingly, compound **45**, wherein the Δ^{16}-double bond was located within the ring, had no effect on NMDA-induced currents.

Effect of Halogen on Modulation of NMDARs. Comparison of the IC_{50} values of steroids **12** (IC_{50} = 0.6 ± 0.2 μM) and **15** (IC_{50} = 1.2 ± 0.2 μM) indicates that substitution of the C-17 hydrogen with an iodine has a pronounced effect on inhibition; compound **12** showing a 2-fold decrease in IC_{50} value in favor of the halogenated analogue. Conversely, substitution of both hydrogen atoms at C-17 by fluorine leads to an increase in the IC_{50} value (steroid **25**, IC_{50} = 7.0 ± 1.6 μM).

Effect of 17-Methyl-5β-Androstane 3-Sulfate (Compound 19) on Native and Recombinant Ionotropic Receptors. To investigate the influence of the subunit composition of the NMDA receptor on the effect of compound **19**, cDNAs encoding for the GluN1 and GluN2A-D subunits were cotransfected into HEK293 cells. Comparison of the IC_{50} values of the steroid **19** at GluN1/GluN2A-D show no significant differences (ANOVA; P = 0.215) and indicate that the action of the steroid is not dependent on the receptor subunit composition (Figure 3 and Table 2). Similarly no

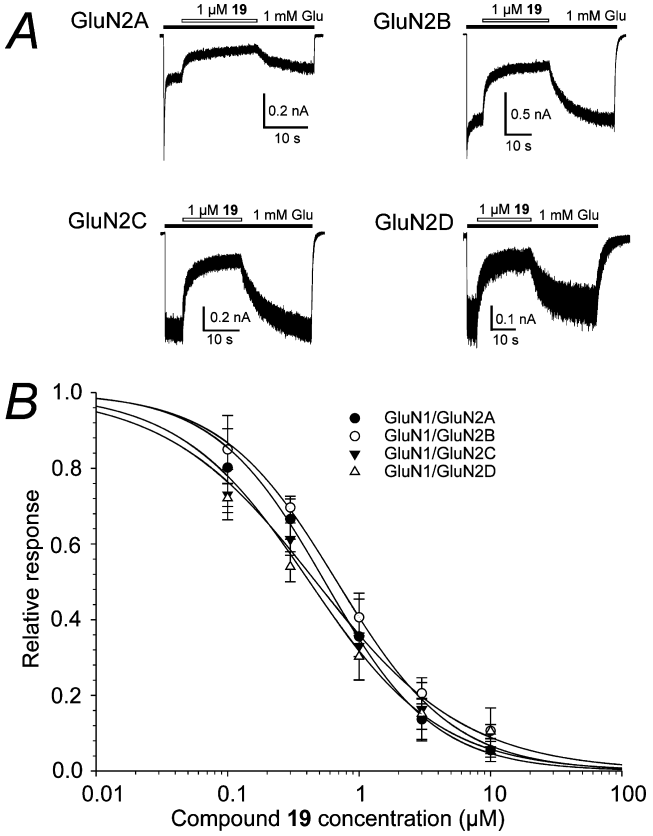

Figure 3. Effect of compound **19** at GluN1/Glu2A-D receptors. (A) Example of trace obtained from HEK293 cells transfected with cDNAs encoding GluN1/Glu2A (GluN2A), GluN1/Glu2B (GluN2B), GluN1/Glu2C (GluN2C), and GluN1/Glu2D (GluN2D) receptors. Compound **19** (1 μM) was applied simultaneously with 1 mM glutamate (duration of steroid and glutamate application is indicated by filled and open bars, respectively). (B) Concentration–response curves for the compound **19** effect at GluN1/Glu2A-D receptors. Data points are averaged values of normalized responses from five HEK293 cells. Error bars represent SD. The relative glutamate induced responses (I) recorded in the presence of the steroid (0.1–10 μM) and determined in individual cells were fit to the following logistic equation: $I = 1/(1 + ([\text{steroid}]/IC_{50})^h)$, where IC_{50} is the concentration of compound **19** that produces a 50% inhibition of agonist-evoked current, [steroid] is the compound **19** concentration, and h is the apparent Hill coefficient.

significant differences were found between native NMDA receptors expressed in cultured hippocampal neurons and recombinant GluN1/GluN2A-B receptors expected to be expressed in these cells.[27] In contrast to NMDA receptor response, the responses induced by 5 μM α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) recorded in the presence of 10 μM cyclothiazide to block the receptor

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Table 2. Effect of Compound 19 on Responses of Induced in GluN1/GluN2A-D Receptors in HEK293 Cells to Glutamate

	IC_{50} ± SD (μM)	h ± SD	n
GluN1/GluN2A	0.6 ± 0.2	1.0 ± 0.3	5
GluN1/GluN2B	0.7 ± 0.1	1.0 ± 0.3	5
GluN1/GluN2C	0.4 ± 0.1	0.9 ± 0.3	5
GluN1/GluN2D	0.5 ± 0.3	0.8 ± 0.1	5

desensitization[28] were significantly less sensitive to the compound **19**. The responses induced by 5 μM GABA were inhibited by compound **19**, with IC_{50} not significantly different from that determined for NMDA receptor responses (Figure 4 and Table 3).

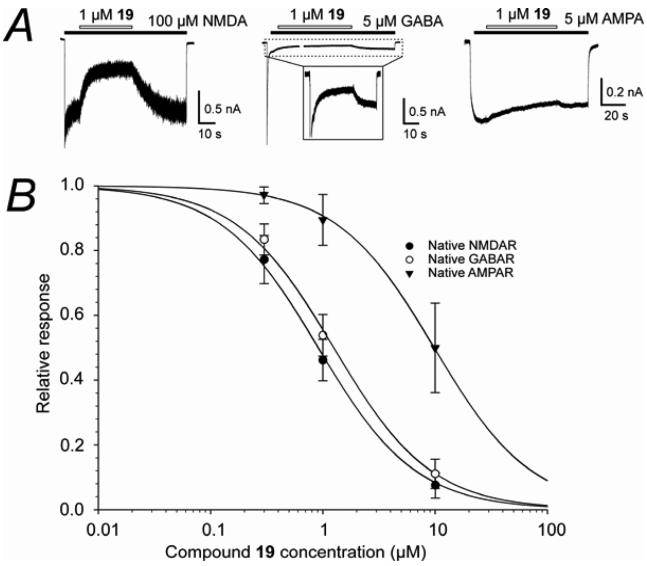

Figure 4. Effect of compound **19** at native AMPA/kainate, NMDA, and GABA receptors. (A) Example of trace obtained from cultured hippocampal neurons. Compound **19** (1 μM) was applied simultaneously with AMPA (5 μM), NMDA (100 μM), and GABA (5 μM) (duration of steroid and agonist application is indicated by filled and open bars, respectively). (B) Concentration–response curves for the compound **19** effect at AMPA, NMDA, and GABA receptors. Data points are averaged values of normalized responses from minimum of five cultured hippocampal neurons. Error bars represent SD. The relative agonist induced responses (I) recorded in the presence of the compound **19** (0.3–10 μM) and determined in individual cells were fit to the following logistic equation: $I = 1/(1 + ([\text{steroid}]/IC_{50})^h)$, where IC_{50} is the concentration of steroid that produces a 50% inhibition of agonist-evoked current, [steroid] is the steroid concentration, and h is the apparent Hill coefficient.

Table 3. Effect of Compound 19 on NMDA, AMPA, and GABA Receptor Responses in Hippocampal Neurons

	IC_{50} ± SD (μM)	h ± SD	n
NMDA R	0.9 ± 0.3	1.1 ± 0.3	6
AMPA R	9.5 ± 2.1	1.2[a]	5
GABA R	1.2 ± 0.2	1.1 ± 0.3	6

[a]Hill coefficient was fixed at 1.2.

The Computational Estimate of Thermodynamic Properties of Sulfated Neuroactive Steroids. In this computational analysis, we have investigated lipophilic qualities of the studied inhibitors in connection with the inhibitory effect. The relevant physicochemical properties of these neuroactive steroids (**3**, **5**, **7**, **12**, **15**, **17**, **19**, **21**, **25**, **30**, **36a**, **36b**, **43**, **48**, and **51**) were calculated by quantum mechanics (QM) computational methods and by physicochemical properties predictor.

The computational results are summarized in the Table 4. The calculated data of the studied compounds correlate well with the experimental ΔG_{exp} data. The correlation between ΔG_{exp} and the values estimated by the accurate QM computational methods (ΔG_{solv}, log P) is higher than between the values obtained by the physicochemical properties predictor (log P, log D), which is in agreement with the theory. The difference in the accuracy of both methods is plotted for log P values in Figure 5, where is $R^2 = 0.92$ for QM computations and $R^2 = 0.79$ for predictor, see below). Compound **12**, the inhibitor with iodine moiety at C-17, is the outlier among the values, which were computed by QM methods and therefore was not included in the correlation. The description of iodine by QM computational methods is nontrivial and less reliable. As the results show, the lipophilic character of this iodine compound is significantly underestimated, its log P is even less than log P of 3α5βS, while is more potent inhibitor.

The high correlation between ΔG_{exp} and ΔG_{solv} (in the case of water/n-octanol phase $R^2 = 0.94$ for neutral systems and $R^2 = 0.92$ for charged systems; in the case of vacuum/water phase $R^2 = 0.71$ for neutral systems and $R^2 = 0.74$ for charged systems) indicate that the (de)solvation free energy plays an important part in an inhibitory effect. The similar results for log P ($R^2 = 0.92$) and log D ($R^2 = 0.78$) show that the inhibition activity of this group of neuroactive steroids closely relates with their lipophilicity. These results are in accordance with expectations and can be used as valid parameters for further structural predictions: comparison of log P values (QM computation) vary from 3.62 to 5.83 for this structural group. However, the inhibitory effect of neurosteroid is multiparameter nature and, therefore, for the purpose of design of new compounds, especially drug candidate, other aspects should considered, e.g., enthalpy, entropy, solubility, permeability data, Lipinski rule of five, etc.

CONCLUSIONS

In this study, we were able to show that the pregnane acetyl group can be substituted with a variety of nonpolar substituents while still maintaining biological activity. In turn, this discovery has led to the development of NMDA receptor inhibitors that are even more potent than the endogeneous ligand–pregnanolone sulfate. In addition, we have shown that nonpolar substitution did not lead to NMDA subtype selectivity and we have shown that there is higher potency to inhibit NMDA receptor responses than AMPA responses.

Our computational data also revealed a correlation between the lipophilicity and the IC_{50} values, even throughout the relatively broad degree of structural variations. This supports our theory that lipophilic modification, and thus conveniently log P, could offer some guidance in the design of new neurosteroid inhibitors. The finding that the increase in the steroid potency was correlated with the lipophilicity also highlights the importance of the plasmatic membrane as a route of the steroid access to the receptor.[12,26]

The results of our previous experiments show that steroids have behavioral effects associated with altered brain function (neuroprotective effect)[18] and importantly, do not induce psychotomimetic symptoms, which can be the result of combined effect of the steroid on NMDA, AMPA, and

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Table 4. Summary of Computational Values of Physicochemical Properties of Sulfated Neuroactive Steroids[a]

			ΔG_{solv} [kcal/mol] (SMD) transfer:						
			from vacuum to water		from *n*-octanol to water		QM computations	physicochemical properties predictor	
steroids	IC_{50} [μmol/L]	ΔG_{exp} [kcal/mol]	neutral	charged	neutral	charged	log *P*	log *P*	log *D*
3α5βS	29.60	−6.20	−21.43	−75.18	1.78	−5.53	2.93	4.04	1.67
3	0.40	−8.77	−13.49	−68.48	4.46	−3.02	4.94	5.22	2.84
5	0.50	−8.63	−14.06	−68.71	4.23	−3.20	4.77	5.16	2.78
7	0.16	−9.31	−13.04	−67.98	4.87	−2.68	5.26	5.51	3.13
12	0.60	−8.52	−17.86	−71.21	1.83	−5.46	2.49	5.13	2.75
15	1.20	−8.11	−14.00	−68.80	3.53	−3.92	4.07	4.49	2.11
17	1.60	−7.94	−14.59	−69.40	3.52	−3.98	3.88	4.43	2.05
19	0.60	−8.52	−13.56	−68.48	4.00	−3.52	4.46	4.78	2.40
21	0.80	−8.35	−14.56	−69.54	4.10	−3.37	4.65	4.82	2.44
25	7.00	−7.06	−17.41	−71.52	3.06	−4.42	3.70	4.36	1.98
30	1.10	−8.16	−13.97	−68.92	3.96	−3.49	4.46	4.93	2.56
36a	0.90	−8.28	−13.86	−68.85	3.76	−3.77	4.19	4.48	2.10
36b	0.70	−8.43	−13.65	−68.53	3.93	−3.57	4.38	4.48	2.10
43	2.20	−7.75	−14.73	−69.05	3.53	−3.79	4.04	4.43	2.05
45	N/A	N/A	−15.05	−69.73	3.10	−4.25	3.62	4.13	1.75
48	5.40	−7.22	−14.24	−69.45	3.43	−3.95	3.90	4.19	1.81
51	0.09	−9.69	−12.91	−67.69	5.42	−2.13	5.83	5.95	3.58

[a]The negative values of ΔG_{solv} signify the energy gained and the positive values energy required during the transfer from the first phase to the second phase.

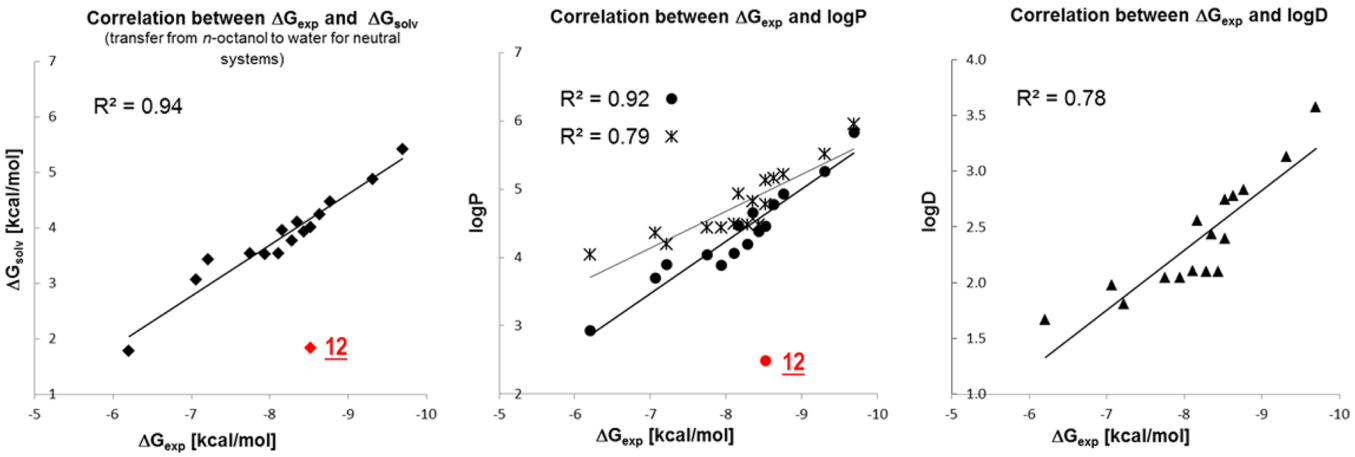

Figure 5. Correlation between the experimental ΔG_{exp} values and ΔG_{solv} (transfer from *n*-octanol to water for neutral systems), log *P* and log *D* values for synthesized neuroactive steroids (**3**, **5**, **7**, **12**, **15**, **17**, **19**, **21**, **25**, **30**, **36a**, **36b**, **43**, **48**, and **51**).

GABA receptors. However, the impact of the steroid lipophilicity to cross the blood−brain barrier has to be established.

Furthermore, this structural flexibility offers new prospects for the further modification and optimization of the pharmacological and pharmacokinetic properties of these new neuroactive steroids. On the other hand, this data revealed a new avenue of investigation, steroid lipophilicity, and how it correlates with plasma membrane interaction. For instance, it is the lower IC_{50} value caused by the steroid's high affinity toward the NMDA receptor or by its high concentration near the receptor?[26]

Finally, as the sulfate moiety at position C-3 is susceptible to hydrolysis by sulfatases, the synthesis of a hemiester or carboxylic acid derivative with a nonpolar substituent on the steroidal D-ring may offer a more metabolically stable compound, one which could penetrate into the CNS without succumbing to metabolic degradation.

EXPERIMENTAL SECTION

Chemistry. *General.* Melting points were determined on a micromelting point apparatus Hund/Wetzlar (Germany) and are uncorrected. Optical rotations were measured in chloroform using an Autopol IV (Rudolf Research Analytical, Flanders, USA). $[\alpha]_D$ values are given in deg (10^{-1} deg cm^2 g^{-1}). IR spectra were recorded on a Bruker IFS 55 spectrometer (wavenumbers in cm^{-1}). Proton and carbon NMR spectra were measured on a FT NMR spectrometer Bruker AVANCE-400 (400 MHz, 101 MHz) in $CDCl_3$ with tetramethylsilane as the internal standard; NMR spectra of compounds **35a** and **35b** were measured on Bruker AVANCE-600 (^{1}H at 600.13 MHz and ^{13}C at 150.9 MHz frequency) in $CDCl_3$. Chemical shifts are given in ppm (δ scale). Coupling constants (*J*) and width of multiplets (*W*) are given in Hz. High resolution MS spectra were performed with a Q-Tof microspectrometer (Waters). Thin layer chromatography (TLC) was performed on silica gel (ICN Biochemicals). Preparative TLC (prep-TLC) was carried out on 200 mm × 200 mm plates coated with a 0.4 mm thick layer of the same material. For column chromatography, neutral silica gel 60 μm (Merck) was used. Analytical

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samples were dried over phosphorus pentoxide at 50 °C/100 Pa. Anhydrous THF was prepared by distillation with benzophenone/Na immediately prior to use. Jones reagent has been prepared from chromium trioxide (67 g, 0.67 mmol) and a solution of sulfuric acid (58 mL of H_2SO_4 in 100 mL of water), and the mixture was diluted to 250 mL with water. The HPLC system consisted of high pressure pump (model 361, Gilson), Rheodyne injection valve . Preparative column (10 mm × 250 mm) with silica gel filling (Biospher PSI 200, 7 μm; Labio) and preparative ELSD detector (Gilson) connected with PC (software Trilution LC, Gilson). The purity of the final compounds was assessed by a combination of NMR and on the basis of analysis LC-HR-MS, and the results were greater than 95%.

General Procedure for Sulfation. A mixture of the hydroxy derivative (0.15 mmol) and sulfur trioxide pyridine complex (0.31 mmol) was dried in vacuo (25 °C, 100 Pa) for 30 min. Then, freshly dried chloroform (5 mL/50 mg) and dry pyridine (1 drop) were added, and the reaction mixture was stirred at room temperature under inert atmosphere. The progress of the reaction was checked by TLC. Then, the reaction mixture was allowed to stand at −5 °C for 2 h and solids were filtered off through a plug of cotton wool in a glass Pasteur pipet. The filtrate was evaporated and dried (25 °C, 100 Pa) for 1 h to afford a mix of sulfate and sulfur trioxide pyridine complex. The crude material was dissolved in a minimal amount of freshly dried chloroform, allowed to stand at −5 °C for 2 h, and the solids were filtered off through a plug of cotton wool in a glass Pasteur pipet. The filtrate was evaporated and dried (25 °C, 100 Pa) for 1 h to afford the corresponding steroidal sulfate.

Computational Section. *Preparation of Structures.* The compounds were obtained by modeling the ligand as taken from the crystal structure (PDB code 3CAV[29]) using PyMOL program[30] and were relaxed by the RI-DFT/B-LYP/SVP method with the Turbomole program.[31] The empirical dispersion correction (D)[32] and COSMO continuum solvation model[33] were applied on the gradient optimization. The most stable local minima of the compounds were obtained by the quenched molecular dynamics simulation method with PM6-D3H4X.[34] The resulting structures were reoptimized by the RI-DFT-D3/B-LYP/TZVPP//COSMO method.[35]

Computational Methods. The solvation free energy (ΔG_{solv}) of the neuroactive steroids was calculated in the SMD continuum solvation model (the transfer from vacuum to water and from *n*-octanol to water) at the HF/6-31G* level with the Gaussian program.[36] The partition coefficients (log *P*) of water/*n*-octanol phase were calculated at the M06-2X/6-31G* level in SMD with the Gaussian program as the difference between the total energies in water and in *n*-octanol. The iodine compound **12** was described by using cc-pVDZ-PP basis set, which is defined for iodine atom. The log *P* and distribution-coefficients (log *D*) of water/*n*-octanol phase were predicted by the MarvinSketch program.[37] The calculated values ΔG_{solv}, log *P*, and log *D* were compared with the experimental free energies (ΔG_{exp}) expressed from the IC_{50} values via the equation $\Delta G_{exp} = RT \ln(IC_{50})$. The linear regression was used for description of the relationship between ΔG_{exp} and the calculated data. The coefficient of determination (R^2) shows how well the calculated data fit the experimental data. The range of R^2 is from 0 to 1, where 0 indicates impossibility and 1 indicates certainty.

Biological Activity. Electrophysiological experiments were performed on human embryonic kidney cells (HEK293) transfected with plasmids encoding GluN1-a/GluN2A-D/GFP genes and cultured hippocampal neurons as described previously.[10,38,39] Glutamate-induced responses were voltage-clamped at a holding potential of −60 mV. Whole-cell voltage clamp recordings were made with a patch-clamp amplifier (Axopatch 200B; Axon Instruments. Inc., Foster City, CA) after a serial resistance (<10 MΩ) and capacitance compensation of 80−90%. For the application of test and control solutions, a microprocessor controlled multibarrel fast-perfusion system was used, with a time constant of solution exchange around cells of ∼10 ms. Agonist-induced responses were low-pass filtered at 2 kHz, digitally sampled at 5 kHz, and analyzed with pClamp software version 9.2 (Molecular Devices). Patch pipettes (3−5 MΩ) pulled from borosilicate glass were filled with Cs^+-based intracellular solution (ICS) containing the following (in mM): 120 gluconic acid, 15 CsCl, 10 BAPTA, 10 HEPES, 3 $MgCl_2$, 1 $CaCl_2$ and 2 ATP-Mg salt (pH-adjusted to 7.2 with CsOH). The extracellular solution (ECS) contained the following (in mM): 160 NaCl, 2.5 KCl, 10 HEPES, 10 glucose, (pH-adjusted to 7.3 with NaOH). NMDA receptor responses were induced by 1 mM glutamate (in recombinant receptors) and 100 μM NMDA (native receptors) and the ECS contained 0.2 mM EDTA, 0.7 mM CaCl2 10 μM glycine plus 10 μM CNQX, 10 μM bicuculline and 0.5 μM TTX for native receptors. AMPA receptors were induced by 5 μM AMPA and the ECS contained 50 μM D-AP5, 10 μM bicuculline, 0.5 μM TTX, and 10 μM cyclothiazide (citaceece Vyklicky J Neurosci). GABA receptor responses were induced by 5 μM GABA and the ECS contained 50 μM D-AP5, 10 μM CNQX, 0.5 μM TTX. Steroid solutions were prepared fresh, as a stock solution of either 5 or 20 mM in dimethyl sulfoxide (DMSO) before each experiment (1% DMSO final concentration). The same concentration of DMSO was added in all extracellular solutions. Experiments were performed at room temperature (21−25 °C).

Light Scattering Analysis. Light scattering (Zetasizer Nano ZS, Malvern Instruments, United Kingdom) was used to characterize particle size of compound **19** in ECS.[26]

Experimental Data for Compounds 2−9, 10a, 11, 12, 14, 15, 17−34, 35b, 36a, 36b, 37−45, 47, 48, 50, and 51. *5β-Pregnan-3α-ol (2).* Compound **2** was prepared according to the literature:[19] mp 150−152 °C (lit.[19] 148−149 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.54 (3H, s, H-18), 0.87 (3H, t, *J* = 7.3, H-21), 0.93 (1H, s, H-19), 3.63 (1H, m, H-3).

5β-Pregnan-3α-yl 3-Sulfate Pyridinium Salt (3). Compound **3** was prepared according to General Procedure for Sulfation. Starting from compound **2** (60 mg, 0.2 mmol), compound **3** (52 mg, 57%) was obtained as a white solid: mp 182−184 °C; $[\alpha]_D$ +61.5 (*c* 0.26, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.53 (3H, s, H-18), 0.86 (3H, t, *J* = 7.3, H-21), 0.92 (1H, s, H-19), 4.47 (1H, tt, J_1 = 11.3, J_2 = 4.9, H-3), 8.10−8.31 (2H, m, H-2′ and H-4′, pyridinium), 8.48 (1H, t, *J* = 7.9, H-3′, pyridinium), 8.96 (2H, d, *J* = 7.9, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 145.7 (C-1′ and C-5′, pyridinium), 142.4 (C-3′, pyridinium), 127.2 (C-2′ and C-4′, pyridinium), 79.8 (C-3), 56.2, 53.1, 42.4, 42.4, 41.1, 38.5, 36.0, 35.5, 34.7, 33.5, 28.4, 27.9, 27.2, 26.7, 24.7, 23.5, 23.2, 20.7, 13.5, 12.7. IR spectrum ($CHCl_3$): 1264, 1173, 970, 947 (OSO_3). MS ESI: *m*/*z* 383.1 (100%, M − pyH). HR-MS (ESI) *m*/*z*: For $C_{21}H_{35}O_4S$ [M − pyH] calcd, 383.2261; found, 383.2258.

20-Methylene-5β-pregnan-3α-ol (4). *n*-Butyllithium (1.6 M in hexanes, 5.8 mL, 9.4 mmol) was added dropwise to a solution of methyltriphenylphosphonium iodide (3.9 g, 9.7 mmol) in anhydrous tetrahydrofuran (35 mL) under inert atmosphere, and the reaction mixture was stirred at room temperature for 1 h. Then, a solution of **1** (1 g, 3.13 mmol) in anhydrous tetrahydrofuran (10 mL) was added. After 48 h of stirring at 80 °C, an aqueous solution of ammonium chloride was added. The product was extracted with chloroform (2 × 70 mL), and combined organic extracts were washed with brine and dried. Solvents were evaporated, and the residue was purified by chromatography on silica gel (10% ethyl acetate in petroleum ether) to afford compound **4** (760 mg, 76%): mp 149−151 °C (acetone/*n*-heptane); $[\alpha]_D$ +16.6 (*c* 0.39, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.54 (3H, s, H-18), 0.91 (3H, s, H-19), 1.75 (3H, s, H-21), 2.02 (1H, t, *J* = 9.1, H-17), 3.62 (1H, m, H-3), 4.69 (1H, s, H-22a), 4.83 (1H, s, H-22b). ^{13}C NMR (101 MHz, $CDCl_3$): δ 145.8 (C-20), 110.7 (=CH_2), 71.9 (C-3), 57.5 (C-17), 56.4 (C-14), 43.5, 42.3, 40.8, 39.2, 36.6, 36.3, 35.5, 34.8, 30.7, 27.3, 26.5, 25.6, 24.7, 24.3, 23.5, 21.0, 13.0. IR spectrum ($CHCl_3$): 3609, 3447, 1033 (OH); 3085, 1639 (=CH_2); 1376 (CH_3). MS (ESI) *m*/*z*: 339.2 (100%, M + Na). For $C_{22}H_{36}O$ (316.5) calcd: 83.48%, C; 11.46%, H. Found: 83.49%, C; 11.55%, H.

20-Methylene-5β-pregnan-3α-yl 3-Sulfate Pyridinium Salt (5). Compound **5** was prepared according to General Procedure for Sulfation. Starting from compound **4** (50 mg, 0.15 mmol), compound **5** (59 mg, 78%) was obtained as a white solid: mp 170−172 °C; $[\alpha]_D$ +42.0 (*c* 0.17, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.52 (3H, s, H-18), 0.90 (3H, s, H-19), 1.73 (3H, s, H-21), 4.45 (1H, m, H-3), 4.67 (1H, s, H-22a), 4.82 (1H, s, H-22b), 7.47 (2H, ddd, J_1 = 7.7, J_2 = 4.7,

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J_3 = 1.3, H-2′ and H-4′, pyridinium), 7.88 (1H, tt, J_1 = 7.7, J_2 = 1.7, H-3′, pyridinium), 8.70 (2H, d, J = 4.5, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 147.8 (C-1′ and C-5′, pyridinium), 145.8 (C-20), 138.6 (C-3′, pyridinium), 124.7 (C-2′ and C-4′, pyridinium), 110.7 (=CH_2), 79.6 (C-3), 57.4, 56.4, 43.5, 42.2, 40.6, 39.2, 36.2, 35.4, 34.6, 33.4, 27.8, 27.1, 26.4, 25.6, 24.8, 24.3, 23.4, 20.9, 12.9. IR spectrum ($CHCl_3$): 3140, 3073, 1683, 1490 (pyridinium); 3085, 1639 (=CH_2), 1262, 1172, 1046 (SO_3). MS: ESI m/z 395.2 (100%, M − pyH). HR-MS (ESI) m/z for $C_{22}H_{35}O_4S$ [M − pyH] calcd, 395.2261; found, 395.2259.

*20-Methyl-5β-pregnan-3α-ol (**6**).* Compound **4** (200 mg, 0.63 mmol) was dissolved in ethyl acetate (2 mL) and ethanol (8 mL). To this, Pd/$CaCO_3$ (5%, 40 mg) was added. The reaction mixture was hydrogenated under slight pressure at room temperature for 7 h. Then, the reaction mixture was filtered through a short column of silica gel to remove the catalyst by washing with chloroform (30 mL). Solvents were removed under vacuo. A mixture of products was purified by column chromatography on silica gel (10% ethyl acetate in petroleum ether) to afford 174 mg (86%) of compound **6**: mp 195−195.5 °C (acetone/*n*-heptane); $[\alpha]_D$ +17.0 (*c* 0.29, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.63 (3H, s, H-18), 0.83 (3H, d, J = 6.6, H-22a), 0.91 (3H, s, H-19), 0.92 (3H, d, J = 6.5, H-22b), 3.62 (1H, m, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 72.0 (C-3), 58.5 (C-17), 56.6 (C-14), 42.8, 42.3, 40.6, 40.2, 36.6, 36.0, 35.5, 34.7, 31.1, 30.7, 28.6, 27.4, 26.6, 24.4, 23.5, 23.3, 22.6, 20.9, 12.3. IR spectrum ($CHCl_3$): 3606, 3480, 1033 (OH); 1383, 1366 (*i*-propyl); 1377 (CH_3). MS ESI: m/z 341.4 (100%, M + Na). For $C_{22}H_{38}O$ (318.5) calcd: 82.95%, C; 12.02%, H. Found: 82.74%, C; 11.77%, H.

*20-Methyl-5β-pregnan-3α-yl 3-Sulfate Pyridinium Salt (**7**).* Compound 7 was prepared according to General Procedure for Sulfation. Starting from compound **6** (50 mg, 0.15 mmol), compound **7** (69 mg, 93%) was obtained as a white solid: mp 190−194 °C; $[\alpha]_D$ +26.2 (*c* 0.29, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.62 (3H, s, H-18), 0.82 (3H, d, J = 6.6, H-22a), 0.90 (3H, s, H-19), 0.91 (3H, d, J = 6.3, H-22b), 4.46 (1H, m, H-3), 8.01 (2H, dd, J_1 = 7.7, J_2 = 6.7, H-2′ and H-4′, pyridinium), 8.48 (1H, tt, J_1 = 7.9, J_2 = 1.4, H-3′, pyridinium), 8.98 (2H, d, J = 5.4, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 145.8 (C-1′ and C-5′, pyridinium), 142.3 (C-3′, pyridinium), 127.3 (C-2′ and C-4′, pyridinium), 80.0 (C-3), 58.4 (C-17), 56.6 (C-14), 42.8, 42.3, 40.5, 40.2, 35.9, 35.4, 34.6, 33.4, 31.1, 28.6, 27.8, 27.2, 26.5, 24.3, 23.4, 23.3, 22.6, 20.9, 12.2. IR spectrum ($CHCl_3$): 3140, 3100, 3073, 1637 (pyH); 1376 (*i*-propyl), 1263, 1171, 1046 (SO_3). MS: ESI m/z 397.2 (100%, M − pyH). HR-MS (ESI) m/z: For $C_{22}H_{37}O_4S$ [M− pyH] calcd, 397.2418; found, 397.2415.

*3α-Methoxymethoxy-5β-pregnan-20-one (**8**).* *N*,*N*-Diisopropylethylamine (13.7 mL, 78.5 mmol) was added to a solution of compound **1** (10.0 g, 31.4 mmol) in dry dichloromethane (70 mL). The solution was cooled to 0 °C, methoxymethyl bromide (1.4 mL, 17.2 mmol) was added, and the mixture was stirred under inert atmosphere at room temperature. After 30 h, chloroform (140 mL) was added and the organic layer was washed with the aqueous citric acid (5%, 2 × 30 mL), a saturated solution of sodium bicarbonate, and brine, and then dried. Solvents were evaporated to afford compound **8** as brownish oil (11.15 g, 98%), which was crystallized from ethyl acetate/*n*-heptane: mp 72−73 °C (ethyl acetate/*n*-heptane); $[\alpha]_D$ +105.8 (*c* 0.25, $CHCl_3$). ^{1}H NMR (400 MHz, CDCl3): δ 0.59 (3H, s, H-18), 0.92 (3H, s, H-19), 2.11 (3H, s, H-21), 2.53 (1H, t, J = 9.0, H-17), 3.38 (3H, s, OCH_3), 3.54 (1H, tt, J_1 = 11.2, J_2 = 4.7, H-3), 4.69 (2H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 209.7 (C-20), 94.5 (OCH_2O), 76.7 (C-3), 63.8, 56.7, 55.1 (CH_3O), 44.3, 42.0, 40.3, 39.2, 35.8, 35.3, 34.7, 33.5, 31.5, 27.7, 27.0, 26.3, 24.4, 23.3, 22.8, 20.7, 13.4. IR spectrum ($CHCl_3$): 2940, 2889, 2869 (OCH_2O); 1689 (C=O); 1385, 1373, 1659 (CH_3); 1145, 1103, 1047, 1039 (MOM). MS ESI: m/z 385.4 (100%, M + Na), 747.7 (7%, 2 M + Na). For $C_{23}H_{38}O_3$ calcd: 76.20%, C; 10.56%, H. Found: 76.12%, C; 10.52%, H.

*(20R)-3α-Methoxymethoxy-5β-pregnan-20-ol (**9**).* Ketone **8** (11.15 g, 30.8 mmol) was dissolved in methanol (250 mL), and the solution was cooled to 0 °C. Sodium borohydride (1.78 g, 47.05 mmol) was added slowly while stirring, After 2 h, the reaction mixture was partly evaporated (to $^1/_3$ of volume) and then poured into a solution of citric acid (5%, 100 mL). Product was extracted with ether (3 × 50 mL), combined organic extracts were washed with a saturated solution of sodium bicarbonate (100 mL) and brine (100 mL), and then dried with magnesium sulfate. Evaporation of solvents afforded mixture of stereoisomers, which were separated by column chromatography (10% ethyl acetate in hexanes) to afford compound **9** as colorless oil (9.84 g, 86%) and the minor *S*-isomer as white crystals (619 mg, 7%): $[\alpha]_D$ +17.9 (*c* 0.19, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, H-18), 0.92 (3H, s, H-19), 1.13 (3H, d, J = 6.1. H-21), 3.37 (3H, s, OCH_3), 3.53 (1 H, tt, J_1 = 11.2, J_2 = 4.5. H-3), 3.72 (1H, dq, J_1 = 9.5, J_2 = 6.0, H-20), 4.62 (2H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 94.5, 76.8, 70.6, 58.6, 56.0, 55.1, 42.6, 42.1, 40.4, 40.3, 35.7, 35.4, 34.7, 33.6, 27.7, 27.2, 26.4, 25.7, 24.5, 23.6, 23.4, 20.7, 12.5. IR spectrum ($CHCl_3$): 3611 (OH); 2942, 2933, 2890, 2869 (OCH_2O); 1450, 1377 (CH_3); 1040 (C−OH). MS ESI: m/z 387.3 (67%, M + Na), 751.6 (100%, 2 M + Na). For $C_{23}H_{40}O_3$ calcd: 75.77%, C; 11.06%, H. Found: 75.77%, C; 11.08%, H.

*17β-Iodo-3α-methoxymethoxy-5β-androstane (**10a**).* Alcohol **9** (9.85 g, 27.0 mmol), iodine (6.90 g, 27.2 mmol), and diacetoxyiodobenzene (9.95 g, 30.9 mmol) were dissolved in dichloromethane (750 mL) in a 500 mL jacketed flask. The reaction mixture was cooled to 15 °C, and the reaction vessel was irradiated by halogen floodlight (500 W) for 3 h. Then it was quenched with an aqueous solution (200 mL) containing sodium thiosulfate (20 g) and sodium bicarbonate (10 g). The colorless organic phase was washed with brine (200 mL), dried with $MgSO_4$, and concentrated in vacuo. The resulting oil was dissolved in a minimum amount of hexanes and crystallized at 5 °C to yield 4.19 g of colorless crystals of (20*R*)-18-iodo-3α-methoxymethoxy-5β-pregnan-20-ol (**10**). The mother liquor was concentrated in vacuo, adsorbed on a column of Florisil (4 × 30 cm), and eluted with 5−15% ethyl acetate in hexanes to afford mixture of 17α and 17β-iodo derivatives **10a** and **10b** (304 mg, 1:1 according to ^{1}H NMR, 2.5%) as white crystalline solid, followed by another portion of **10** (3.49 g, 58% together with crystalline fraction) as an off-white solid.

*Compound **10**.* Melting point 124−125.5 °C (hexanes); $[\alpha]_D$ +9.4 (*c* 0.23, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (3H, s, H-19), 1.14 (3H, d, J = 6.2. H-21), 2.25 (1H, d, J = 4.2), 2.39 (1H, dt, J_1 = 13.4, J_2 = 3.0. CH-12β), 3.13 (1H, dd, J_1 = 10.6, J_2 = 1.1, CH-18b), 3.32 (1H, dd, J_1 = 10.6, J_2 = 1.3, CH-18a), 3.37 (3H, s, OCH_3), 3.53 (1H, tt, J_1 = 11.2, J_2 = 4.6, H-3), 4.06 (1H, tt, J_1 = 10.1, J_2 = 6.1, H-20), 4.69 (2H, d, J = 1.1, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 94.6 (OCH_2O), 76.7 (C-3), 68.3 (C-20), 58.8 (C-17), 56.2 (C-14), 55.1 (CH_3O), 44.4, 41.8, 40.8, 40.3, 36.4, 35.3, 34.7, 33.5, 27.6, 27.1, 26.2, 25.4, 24.3, 23.3, 22.2, 20.2, 11.2. IR spectrum ($CHCl_3$): 3600 (OH); 2944, 2932, 2887 (OCH_2O); 1423 (CH_2I); 1376 (CH_3); 1145, 1102 (OCH_2O); 1045 (C−OH). MS ESI: m/z 513.2 (98%, M + Na), 1003.5 (100%, 2 M + Na). HR-MS (ESI) m/z: for $C_{23}H_{39}INaO_3$ [M + Na] calcd, 513.1836; found, 513.1835.

*Compound **10a**.* $[\alpha]_D$ +88.5 (*c* 0.287, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79 (3H, s, H-18), 0.93 (3H, s, H-19), 3.36 (3H, s, OCH_3), 3.48−3.58 (1H, m, H-3), 3.75 (1H, t, J = 9.4, H-17), 4.68 (2H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 94.7, 76.9, 55.3, 50.1, 44.3, 42.3, 42.1, 40.7, 37.4, 37.1, 35.5, 34.9, 34.4, 33.7, 27.8, 27.2, 26.4, 25.5, 23.5, 20.5, 17.1. IR spectrum ($CHCl_3$): 2940, 2869 (CH_2); 1145, 1047, 1036, 1102, 915 (COCOC). MS ESI: m/z 469.3 (80%, M + Na). HR-MS (ESI) m/z: for $C_{21}H_{35}INaO_2$ [M + Na] calcd, 469.1573; found, 469.1573.

*17β-Iodo-5β-androstan-3α-ol (**11**).* Compound **10a** (250 mg, 0.56 mmol) was dissolved in methanol (10 mL), and concentrated HCl (two drops) and triethylamine (one drop) were added to the stirred solution. After 48 h, the reaction mixture was quenched with a saturated solution of sodium bicarbonate (10 mL). Solvents were partially evaporated (to $^1/_2$ of volume), and the product was extracted with ethyl acetate (3 × 10 mL), the combined organic extracts were washed with brine and dried over magnesium sulfate, and the solvents were evaporated to afford compound **11** (190 mg, 84%): 167−169 °C (acetone/*n*-heptane); $[\alpha]_D$ +63.1 (*c* 0.28, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79 (3H, s, H-18), 0.93 (3H, s, H-19), 3.63 (1H, tt, J_1 = 11.0, J_2 = 4.7, H-3), 3.76 (1H, t, J = 9.5, H-17α). ^{13}C NMR (101

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MHz, $CDCl_3$): δ 71.8 (C-3), 50.1, 44.2 (C-17), 42.3, 42.1, 40.8, 37.4, 37.1, 36.5, 35.5, 34.8, 34.4, 30.6, 27.1, 26.5, 25.5, 23.4, 20.6, 17.1. IR spectrum ($CHCl_3$): 3609 (OH); 1031 (C−O); 606 (C−I). MS ESI: m/z 425.1 (25%, M + Na). HR-MS (ESI) m/z: for $C_{19}H_{31}$ OINa [M + Na] calcd, 425.1322; found, 425.1306.

*17β-Iodo-5β-androstan-3α-yl 3-Sulfate Pyridinium Salt (**12**).* Compound **12** was prepared according to General Procedure for Sulfation. Starting from compound **11** (255 mg, 0.63 mmol), compound **12** (298 mg, 72%) was obtained as a white solid: mp 118−120 °C; $[\alpha]_D$ +58.5 (*c* 0.39, $CHCl_3$/MeOH 1.94:0.20). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79 (3H, s, H-18), 0.92 (3H, s, H-19), 3.76 (1H, t, J = 9.4, H-17α), 4.39 (1H, tt, J_1 = 11.0, J_2 = 5.0. H-3), 7.32 (2H, ddd, J_1 = 7.6, J_2 = 4.3, J_3 = 1.5. H-2′ and H-4′, pyridinium), 7.72 (1H, tt, J_1 = 7.7, J_2 = 1.8, H-3′, pyridinium), 8.64 (2H, dt, J_1 = 4.6, J_2 = 1.7, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 149.6 (C-1′ and C-5′, pyridinium), 136.4 (C-3′, pyridinium), 128.6 (C-2′ and C-4′, pyridinium), 78.8, 50.1, 46.4, 44.2, 42.3, 42.1, 40.6, 37.2, 35.5, 34.7, 34.4, 33.4, 27.9, 27.0, 26.4, 25.5, 23.1, 20.5, 17.0. IR spectrum ($CHCl_3$): 3434, 3608 (OH); 1385 (CH_3); 1027, 1034 (C−O). MS (ESI): m/z 481.2 (100%, M − pyH). HR-MS (ESI) m/z: for $C_{19}H_{30}O_4IS$ [M-pyH] calcd, 481.0904; found, 481.0908.

*5β-Androstan-3α-ol (**14**).* Trimethylsilyl chloride (84 mL, 0.65 mol) was added dropwise to a stirred solution of compound **13** (6 g, 0.02 mol) and zinc powder (42 g, 0.38 mol) in dichloromethane and methanol (1:1, 180 mL) at 0 °C. The reaction mixture was stirred at room temperature. After 4 h, heterogeneous zinc pellets were removed by filtration and to the filtrate was added a saturated solution of sodium bicarbonate (to pH 7). To clear the white emulsion, chloroform (3 × 50 mL) and an aqueous solution of hydrochloric acid (5%, 30 mL) was added. The now transparent organic and aqueous layers were separated, and the organic phase was separated, washed with brine, and dried. Solvents were evaporated, and the residue was purified by column chromatography (4% of acetone in petroleum ether) to afford white solids of **14** (4.5 g, 79%): mp 143−144 °C (acetone/*n*-heptane); $[\alpha]_D$ +10.9 (*c* 0.27, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.68 (3H, s, H-18), 0.92 (3H, s, H-19), 3.58−3.67 (1H, m, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 72.0 (C-3), 54.7, 42.2, 41.0, 40.9, 40.6, 39.2, 36.6, 36.3, 35.6, 34.8, 30.7, 27.3, 26.9, 25.7, 23.5, 20.9, 20.7, 17.6. IR spectrum ($CHCl_3$): 3608, 3446 (OH); 2972, 2887, 1377 (CH_3); 2935, 2865, 1450 (CH_2); 1081, 1065, 1034 (CO). For $C_{19}H_{32}O$ (276.2) calcd: 82.55%, C; 11.67%, H. Found: 82.31% C, 11.82% H.

*5β-Androstan-3α-yl 3-Sulfate Pyridinium Salt (**15**).* Compound **15** was prepared according to General Procedure for Sulfation. Starting from compound **14** (200 mg, 0.72 mmol), compound **15** (211 mg, 67%) was obtained as a white solid: mp 180−182 °C (chloroform); $[\alpha]_D$ +16.0 (*c* 0.28, $CHCl_3$/MeOH, 2:0.1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (3H, s, H-18), 0.92 (1H, s. H-19), 4.48 (1H, tt, J_1 = 11.3, J_2 = 4.9, H-3), 7.94−8.04 (2H, m, H-2′ and H-4′, pyridinium), 8.47 (1H, tt, J_1 = 7.9, J_2 = 1.6, H-3′, pyridinium), 8.97 (2H, dt, J_1 = 5.6, J_2 = 1.5, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$/CD_3OD): δ 145.5 (C-1′ and C-5′, pyridinium), 142.3 (C-3′, pyridinium), 127.0 (C-2′ and C-4′, pyridinium), 79.7 (C-3), 54.6, 42.2, 40.9, 40.7, 40.5, 39.1, 36.2, 35.4, 34.6, 33.3, 27.8, 27.1, 26.8, 25.5, 23.3, 20.8, 20.6, 17.5. IR spectrum ($CHCl_3$): 1260, 1178, 1050, 970 (OSO_3). MS (EI): m/z 355.2 (100%, M − pyH). HR-MS (ESI) m/z: for $C_{19}H_{31}O_4S$ [M − pyH] calcd, 355.1949; found, 355.1949.

*17-Methylene-5β-androstan-3α-ol (**16**).* Sodium hydride (50% in parafine oil, 80 mg, 1.70 mmol) was added to a solution of methyltriphenylphosphonium bromide (619 mg, 1.73 mmol) in dry DMSO (4 mL) under inert atmosphere, and the reaction mixture was stirred at room temperature for 1 h. Then, a solution of **13** (100 mg, 0.34 mmol) in dry DMSO (3 mL) was added. After 1.5 h of stirring at 70 °C, an aqueous solution of ammonium chloride was added. The product was extracted with chloroform (2 × 20 mL), and the combined organic extracts were washed with brine and dried. Solvents were evaporated, and the residue was purified by chromatography on pre-TLC plates (40% ether in petroleum ether) to afford compound **16** (90 mg, 90%): mp 147−149 °C (acetone/*n*-heptane); $[\alpha]_D$ +30.5 (*c* 0.22, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (3H, s, H-18), 0.93 (3H, s, H-19), 2.22 (1H, dtt, J_1 = 17.7, J_2 = 8.8, J_3 = 2, H-16a), 2.47 (1H, dddd, J_1 = 16.9, J_2 = 10, J_3 = 4.4, J_4 = 2.2. H-16b), 3.62 (1H, m, H-3), 4.50−4.62 (2H, m, =CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.0 (C-17), 100.7 (C-20), 71.9 (C-3), 54.6, 44.3, 42.3, 40.8, 36.5, 36.0, 35.9, 35.5, 34.8, 30.6, 29.6, 27.2, 26.5, 24.3, 23.5, 20.8, 18.6. IR spectrum ($CHCl_3$): 3609, 3451, 1031 (OH); 1653 (C=C). MS (ESI): m/z 311.3 (100%, M + Na). HR-MS (ESI) m/z: for $C_{20}H_{32}ONa$ [M + Na] calcd, 311.2345; found, 311.2344.

*17-Methylene-5β-androstan-3α-yl 3-Sulfate Pyridinium Salt (**17**).* Compound **17** was prepared according to General Procedure for Sulfation. Starting from compound **16** (50 mg, 0.17 mmol), compound **17** (63 mg, 81%) was obtained as a white solid: mp 155−159 °C; $[\alpha]_D$ +41.6 (*c* 0.25, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, H-18), 0.91 (3H, s, H-19), 2.22 (1H, dt, J_1 = 17.3, J_2 = 8.7, H-16a), 2.47 (1H, m, H-16b), 4.44 (1H, m, H-3), 4.59 (2H, m, =CH_2), 7.51 (2H, ddd, J_1 = 7.7, J_2 = 4.7, J_3 = 1.4, H-2′ and H-4′, pyridinium), 7.93 (1H, tt, J_1 = 7.7, J_2 = 1.7, H-3′, pyridinium), 8.74 (2H, d, J = 4.5, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.0 (C-17), 147.4 (C-1′ and C-5′, pyridinium), 139.2 (C-3′, pyridinium), 124.9 (C-2′ and C-4′, pyridinium), 100.7 (C-20), 79.5 (C-3), 54.6, 44.3, 42.3, 40.8, 36.0, 35.8, 35.4, 34.7, 33.4, 29.5, 27.8, 27.0, 26.4, 24.2, 23.4, 20.8, 18.6. IR spectrum ($CHCl_3$): 3140, 3093, 1490, 826 (pyridinium); 1653 (C=C). MS ESI: m/z 367.1 (100%, M − pyH). HR-MS (ESI) m/z: for $C_{20}H_{32}O_4S$ [M − pyH] calcd, 367.1948; found, 367.1946.

*7β-Methyl-5β-pregn-3α-ol (**18**).* Compound **18** was prepared in the same manner as compound **6**. Starting from compound **16** (100 mg, 0.34 mmol), a mixture of 17α- and 17β-isomers (1:10) was obtained. Further crystallization from acetone/*n*-heptane compound afforded 17β-methyl derivative **18** (75 mg, 75%): mp 151−153 °C (acetone/*n*-heptane); $[\alpha]_D$ +18.1 (*c* 0.21, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.52 (3H, s, H-18), 0.82 (3H, d, J = 6.8, 17-Me), 0.92 (3H, s, H-19), 3.62 (1H, m, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 72.0 (C-3), 56.0, 45.3, 42.4, 42.3, 41.0, 37.9, 36.7, 36.2, 35.6, 34.9, 30.7, 30.4, 27.4, 26.8, 24.9, 23.5, 20.7, 13.9, 12.1. IR spectrum ($CHCl_3$): 3610, 1034 (OH); 1379 (CH_3). MS (ESI): m/z 313.2 (100%, M + Na). For $C_{20}H_{34}O$ (290.5) calcd: 82.69%, C; 11.80%, H. Found: 82.62%, C; 11.37%, H.

*17β-Methyl-5β-pregn-3α-yl 3-Sulfate Pyridinium Salt (**19**).* Compound **19** was prepared according to General Procedure for Sulfation. Starting from compound **18** (50 mg, 0.17 mmol), compound **19** (66 mg, 85%) was obtained as a white solid: mp 174−175 °C; $[\alpha]_D$ +21.6 (*c* 0.18, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.51 (3H, s, H-18), 0.81 (3H, d, J = 6.8, 17-Me). 0.91 (3H, s, H-19), 4.46 (1H, m, H-3), 8.00 (2H, m, H-2′ and H-4′, pyridinium), 8.48 (1H, t, J = 7.8, H-3′, pyridinium), 8.97 (2H, d, J = 5.4, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 145.8 (C-1′ and C-5′, pyridinium), 142.3 (C-3′, pyridinium), 127.2 (C-2′ and C-4′, pyridinium), 79.9 (C-3), 56.0, 45.2, 42.3, 42.3, 40.9, 37.8, 36.1, 35.5, 34.7, 33.4, 30.3, 27.8, 27.1, 26.7, 24.8, 23.4, 20.6, 13.9, 12.1. IR spectrum ($CHCl_3$): 3140, 3100, 1490, 1028 (pyridinium); 1263, 1172 (SO_3); 1486, 1379 (CH_3). MS ESI: m/z 369.2 (100%, M − pyH). HR-MS (ESI) m/z: For $C_{20}H_{33}O_4S$ [M − pyH] calcd, 369.2105; found, 369.2103.

*(17Z)-5β-Pregn-17(20)-en-3α-ol (**20**).* Compound **20** was prepared according to the literature.[21] ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, s, H-18), 0.93 (3H, s, H-19), 1.64 (3H, dt, J_1 = 7.1, J_2 = 2.0, H-21), 3.62 (1H, bm, H-3), 5.11 (1H, qt. J_1 = 7.1, J_2 = 2.0, H-20). ^{13}C NMR (101 MHz, $CDCl_3$): δ 150.4 (C-20), 113.2 (C-20), 71.8 (C-3), 56.3, 44.4, 42.1, 40.5, 37.4, 36.4, 35.4, 35.3, 34.6, 31.5, 30.5, 27.1, 26.3, 24.4, 23.3, 21.0, 16.8, 13.0.

*(17Z)-5β-Pregn-17(20)-en-3α-yl 3-Sulfate Pyridinium Salt (**21**).* Compound **21** was prepared according to the General Procedure for Sulfation. Starting from compound **20** (100 mg, 0.33 mmol), compound **21** (95 mg, 63%) was obtained as a white solid: mp 171−173 °C (chloroform); $[\alpha]_D$ +35.1 (*c* 0.21, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, s, H-18), 0.91 (3H, s, H-19), 1.64 (3H, dt, J_1 = 7.1, J_2 = 2.0, H-21), 4.45 (1H, tt, J_1 = 10.9, J_2 = 4.9, H-3), 5.10 (1H, qt, J_1 = 7.1, J_2 = 2.1, H-20), 8.01 (2H, m, H-2′ and H-4′, pyridinium), 8.47 (1H, tt, J_1 = 7.9, J_2 = 1.6, H-3′, pyridinium), 8.99

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(2H, m, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 150.5 (C-20), 145.8 (C-1′ and C-5′, pyridinium), 142.4 (C-3′, pyridinium), 127.3 (C-2′ and C-4′, pyridinium), 113.3 (C-17), 79.7 (C-3), 56.4, 44.5, 42.2, 40.6, 37.5, 35.4, 35.4, 34.6, 33.4, 31.6, 27.9, 27.1, 26.3, 24.5, 23.4, 21.1, 16.9, 13.2. IR spectrum ($CHCl_3$): 3140, 3099, 3072 (pyridinium); 2943, 2868 (CH_3); 1665 (C═C); 1262, 1253, 1179 (OSO_3). MS (ESI): *m/z* 381.2 (100%, M + H − pyridine). HR-MS (ESI) *m/z*: For $C_{21}H_{33}O_4S$ [M + pyH] calcd, 381.2103; found, 381.2103.

*3α-Methoxymethoxy-5β-androstan-17-one (**22**).* The MOM-protecting group was introduced to compound **22** in the same manner as described for compound **8**. Starting from compound **13** (2 g, 6.88 mmol), compound **22** (2.14 g, 93%) was obtained as an oily product by column chromatography (10% ethyl acetate in petroleum ether): $[\alpha]_D$ +98.5 (*c* 0.13, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s, H-18), 0.92 (3H, s, H-19), 2.06 (1H, m, H-16a), 2.41 (1H, m, H-16b), 3.35 (3H, s, OCH_3), 3.51 (1H, m, H-3), 4.66 (2H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 221.3 (C-17), 94.7 (OCH_2O), 76.8 (C-3), 55.2 (CH_3O), 51.6, 47.9, 42.2, 40.8, 36.0, 35.5, 35.4, 35.0, 33.6, 31.8, 27.8, 27.0, 25.4, 23.4, 21.9, 20.2, 13.9. IR spectrum ($CHCl_3$): 2939, 2868, 2890 (OCH_2O); 1732 (C═O); 1045 (C−O). MS ESI: *m/z* 357.2 (100%, M + Na). HR-MS (ESI) *m/z*: for $C_{21}H_{34}O_3Na$ [M + Na] calcd, 357.2400; found, 357.2398.

*17,17-Difluoro-3α-methoxymethoxy-5β-androstane (**23**).* Bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-fluor, 50% solution in THF, 5 mL, 11.6 mmol) was added to compound **22** (820 mg, 2.45 mmol) in a glass tube. The tube was filled with argon and sealed, and the reaction mixture was stirred and heated to 80 °C for 48 h. Then, the tube was cooled to 0 °C and opened. The mixture was diluted with ethyl acetate (3 × 40 mL), washed with a saturated solution of potassium bicarbonate and water, and dried over $MgSO_4$. Solvents were evaporated, and the residue was purified by column chromatography (10% ether in petroleum ether) to afford oily product **23** (240 mg, 27%): $[\alpha]_D$ +8.7 (*c* 0.42, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, d, *J* = 2.0, H-18), 0.93 (3H, s, H-19), 3.37 (3H, s, OCH_3), 3.49−3.57 (1H, m, H-3), 4.68 (2H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 132.7 (C-17), 94.7 (OCH_2O), 76.9 (C-3), 55.3 (OCH_3), 49.6, 45.5, 42.2, 40.4, 35.9, 35.5, 34.9, 33.7, 33.1, 29.2, 27.8, 27.1, 25.7, 23.4, 22.4, 20.0, 13.5. IR spectrum ($CHCl_3$): 2825 (OCH_3); 1317, 1167 (CF_2); 1106 (MOM). MS ESI: *m/z* 379.1 (100%, M + Na). HR-MS (ESI) *m/z*: for $C_{21}H_{34}O_2F_2Na$ [M + Na] calcd, 379.2419; found, 379.2422.

*17,17-Difluoro-5β-androstan-3α-ol (**24**).* Starting from compound **23** (150 mg; 0.42 mmol), compound **24** (120 mg, 91%) was obtained by the hydrolysis of the MOM protecting group described for compound **11**, followed by crystallization: mp 130−132 °C (acetone/*n*-heptane); $[\alpha]_D$ +2.7 (*c* 0.29, $CHCl_3$). ^{1}H NMR (400 MHz, CDCl3): δ 0.85 (3H, d, *J* = 2.0, H-18), 0.93 (3H, s, H-19), 3.58−3.68 (1H, m, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 132.7 (C-17), 71.8 (C-3), 49.6, 45.5, 42.2, 40.5, 36.5, 35.9, 35.5, 34.8, 33.1, 30.7, 29.2, 27.0, 25.7, 23.4, 22.4, 20.0, 13.5. IR spectrum ($CHCl_3$): 3609 (OH); 1317, 1168 (CF_2); 1035 (C−OH). MS ESI: *m/z*: 335.2 (75%, M + Na), 313.2 (5%, M + 1). HR-MS (ESI) *m/z*: for $C_{19}H_{30}OF_2Na$ [M + Na] calcd, 335.2156; found, 335.2161.

*17.17-Difluoro-5β-androstan-3α-yl 3-Sulfate Pyridinium Salt (**25**).* Compound **25** was prepared according to General Procedure for Sulfation. Starting from compound **24** (86 mg, 0.28 mmol), compound **25** (96 mg, 73%) was obtained as a white solid: mp 185−187 °C; $[\alpha]_D$ +12.5 (*c* 0.28, $CHCl_3$/MeOH, 1.97:0.04). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, d, *J* = 2.0, H-18), 0.92 (3H, s, H-19), 4.46 (1H, tt, J_1 = 10.8, J_2 = 5.1, H-3), 8.09−7.99 (2H, m, H-2′ and H-4′, pyridinium), 8.44−8.51 (1H, m, H-3′, pyridinium), 7.96−8.02 (2H, m, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$/MeOH): δ 145.8 (C-1′ and C-5′, pyridinium), 142.4 (C-3′, pyridinium), 127.2 (C-2′ and C-4′, pyridinium), 79.5, 58.6, 49.6, 42.2, 40.4, 35.72, 35.4, 34.7, 33.4, 33.0, 29.2, 27.8, 26.8, 25.6, 23.3, 22.4, 20.0, 18.5, 13.4. IR spectrum ($CHCl_3$): 1350, 1121 (CF_2); 1247, 1168, 973, 947 (OSO_3). MS (ESI): *m/z* 391.2 (100%, M − pyH). HR-MS (ESI) *m/z*: for $C_{19}H_{29}F_2S$ [M − pyH] calcd, 391.1760; found, 391.1757.

*17β-Spiro-(5β-androstan-17.2′-oxiran)-3α-ol (**26**).* Potassium *tert*-butoxide (267 mg, 2.38 mmol) and trimethylsulfonium iodide (486 mg, 2.38 mmol) were added in one portion to a solution of compound **13** (346 mg, 1.19 mmol) in anhydrous *N,N′*-dimethylformamide (6 mL) under inert atmosphere. The reaction mixture was stirred at room temperature overnight. Then, brine was added and the product was extracted with chloroform (3 × 30 mL), combined organic extracts were dried, and solvent was evaporated. The residue was purified by column chromatography (0−15% ethyl acetate in petroleum ether) to afford compound **26** (284 mg, 78%):[40] mp 149−150 °C (ether/petroleum ether), mp 155−158 °C (ether/heptane).[40] ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, s, H-18), 0.92 (3H, s, H-19), 2.60 (1H, d, *J* = 5.1, H-20a), 2.89 (1H, d, *J* = 5.1, H-20b), 3.63 (1H, m, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 71.8 (C-3), 70.7 (C-17), 53.7 (C-20), 53.0 (C-14), 42.2, 40.7, 40.3, 36.5, 36.1, 35.5, 34.8, 34.3, 30.6, 29.2, 27.1, 26.0, 23.7, 23.4, 20.3, 14.4. HR-MS (ESI) *m/z*: For $C_{20}H_{32}O_2Na$ [M + Na] calcd, 327.2294; found, 327.2293.

*17α-Azidomethyl-3α-17β-dihydroxy-5β-androstane (**27**).* A solution of compound **26** (560 mg, 1.83 mmol), sodium azide (341 mg, 5.68 mmol), and ammonium chloride (341 mg, 6.37 mmol) in ethanol (28 mL) and water (5.6 mL) was heated at 90 °C overnight. Then, water was added, ethanol was evaporated, and the product was extracted with chloroform (2 × 50 mL), combined organic extracts were dried, and solvent was evaporated. The product was purified by column chromatography (30% ether in petroleum ether) to afford compound **27** (500 mg, 78%) as a white foam: $[\alpha]_D$ 0.0 (*c* 0.11, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (3H, s, H-18), 0.93 (3H, s, H-19), 3.26 (1H, d, *J* = 12, H_a-CH_2N_3), 3.54 (1H, d, *J* = 12, H_b-CH_2N_3), 3.63 (1H, m, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 83.6 (C-17), 71.8 (C-3), 58.5 (CH_2N_3), 51.5, 46.1, 42.1, 40.6, 36.6, 36.5, 35.4, 34.9, 34.8, 32.3, 30.6, 27.1, 26.34, 23.7, 23.4, 20.5, 14.3. IR spectrum ($CHCl_3$): 3613, 1037 (3α-OH); 3562, 1116 (17β-OH); 2106 (azide). MS ESI: *m/z* 370.2 (100%, M + Na). HR-MS (ESI) *m/z*: for $C_{20}H_{33}O_2N_3Na$ [M + Na] calcd, 370.2465; found, 370.2464.

*17a-Oxo-D-homo-5β-androstan-3α-ol (**28**).* Sodium iodide (948 mg, 6.3 mmol) was added to a solution of compound **27** (220 mg, 0.63 mmol) in dry acetonitrile (10 mL). Then, trimethylsilyl chloride (0.8 mL, 6.26 mmol) was added dropwise under inert atmosphere. The reaction mixture was stirred at room temperature, and the progress of the reaction was checked by TLC. An aqueous solution of hydrochloride acid (5%, 10 mL) was added, and the product was extracted with chloroform (2 × 40 mL), combined organic extracts were washed with sodium sulfite and brine and dried, and solvent was evaporated. Yellow solids were purified by column chromatography (30% ether in petroleum ether) to afford compound **28** (140 mg, 73%): mp 201−201 °C (ether/petroleum ether); $[\alpha]_D$ −23.6 (*c* 0.12, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91 (3H, s, H-18), 1.06 (3H, s, H-19), 2.04 (1H, m, H_a-16), 2.19 (1H, m, H_a −17), 2.61 (1H, td, J_1 = 14.0, J_2 = 6.8, H_b-17), 3.61 (1H, m, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 216.7 (C-17a), 71.8 (C-3), 51.7, 48.5, 41.7, 39.8, 37.3, 36.3, 35.6, 35.0, 35.0, 32.7, 30.7, 27.2, 26.1, 25.9, 23.49, 23.1, 19.8, 17.0. IR spectrum ($CHCl_3$): 3609, 1037 (OH); 1698 (C═O). MS ESI: *m/z* 327.2 (100%, M + Na), 631.5 (15%, 2 M + Na). For $C_{20}H_{32}O_2$ (304.4) Calcd: 78.90%, C; 10.59%, H. Found: 78.55%, C; 10.49%, H.

*D-Homo-5β-androstan-3α-ol (**29**).* Starting from compound **28** (110 mg, 0.36 mmol), compound **29** (68 mg, 65%) was obtained by the same deoxygenation procedure as compound **14** followed by column chromatography on silica gel (30% of ether in petroleum ether): mp 187−189 °C (acetone/*n*-heptane); $[\alpha]_D$ +17.1 (*c* 0.20, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78 (3H, s, H-18), 0.89 (3H, s, H-19), 3.62 (1H, m, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 72.0 (C-3), 51.2, 42.5, 42.4, 42.0, 40.7, 36.5, 36.0, 35.2, 35.0, 33.7, 30.7, 27.4, 27.3, 25.5, 24.2, 23.6, 21.6, 20.3, 17.0. IR spectrum ($CHCl_3$): 3609, 3447, 1031 (OH). MS ESI: *m/z* 313.3 (100%, M + Na). For $C_{20}H_{34}O$ (290.4) Calcd: 82.69%, C; 11.80%, H. Found: 82.42%, C; 11.71%, H.

*D-Homo-5β-androstan-3α-yl 3-Sulfate Pyridinium Salt (**30**).* Compound **30** was prepared according to General Procedure for Sulfation. Starting from compound **29** (30 mg, 0.10 mmol),

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compound **30** (43 mg, 93%) was obtained as a white solid: mp 173−174 °C; $[\alpha]_D$ +20.4 (*c* 0.27, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.76 (3H, s, H-18), 0.87 (3H, s, H-19), 4.45 (1H, m, H-3), 8.00 (2H, m, H-2′ and H-4′, pyridinium), 8.48 (1H, t, *J* = 7.8, H-3′, pyridinium), 8.98 (2H, d, *J* = 5.5, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 145.8 (C-1′and C-5′, pyridinium), 142.3 (C-3′, pyridinium), 127.3 (C-2′ and C-4′, pyridinium), 79.8 (C-3), 51.2, 42.5, 42.4, 42.0, 40.6, 35.9, 35.1, 34.9, 33.7, 33.3, 27.9, 27.3, 27.2, 25.4, 24.2, 23.5, 21.6, 20.4, 17.0. IR ($CHCl_3$): 3139, 3100, 1637, 1490 (pyH); 1263, 1238, 1235, 1043 (SO_3). MS ESI: *m*/*z* 369.3 (100%. M − pyH). HR-MS (ESI) *m*/*z*: for $C_{20}H_{33}O_4S$ [M − pyH] calcd, 369.2105; found, 369.2103.

*3α-Methoxymethoxy-5β-androstan-17β-ol (**31**).* Compound **31** was prepared in the same manner as compound **9**. Starting from compound **22** (600 mg, 1.79 mmol), compound **31** (568 mg, 94%) was obtained as oily product by introduction of the MOM protecting group described for derivative **9**, followed by column chromatography on silica gel (20−30% of ether in petroleum ether): $[\alpha]_D$ +24.6 (*c* 0.39, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.71 (3H, s, H-18), 0.91 (3H, s, H-19), 2.05 (1H, m, H-16a), 3.35 (3H, s, OCH_3), 3.52 (1H, m, H-3), 3.62 (1H, t, *J* = 8.6, H-17), 4.67 (2H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 94.6 (OCH_2O), 82.0 (C-17), 76.9 (C-3), 55.2 (OCH_3), 51.2, 43.2, 42.2, 40.7, 37.0, 36.0, 35.5, 34.9, 33.6, 30.7, 27.8, 27.2, 26.1, 23.5 (2 × C), 20.5, 11.2. IR spectrum ($CHCl_3$): 3614 (OH); 1450, 1042 (C−O). MS ESI: *m*/*z* 359.3 (100%, M + Na), 695.7 (15%, 2 M + Na). HR-MS (ESI) *m*/*z*: for $C_{21}H_{36}O_3Na$ [M + Na] calcd, 359.2556; found, 359.2556.

*3α-Methoxymethoxy-5β-androstan-17β-yl 17-Tosylate (**32**).* A solution of compound **31** (548 mg, 1.62 mmol), 4-dimethylaminopyridine (10 mg, 0.08 mmol), and *p*-TsCl (555 mg, 2.9 mmol) in anhydrous pyridine (15 mL) was heated at 45 °C for 35 h. Then, additional portions of 4-dimethylaminopyridine (10 mg, 0.08 mmol), and *p*-TsCl (92 mg, 0.48 mmol) were added and the reaction mixture was heated at 45 °C overnight. Then, it was poured into water; the product was extracted with chloroform (2 × 50 mL), and combined organic extracts were washed with aqueous hydrochloric acid (5%, 2 × 20 mL), sodium bicarbonate and brine and then dried. After solvent evaporation, the oily residue was purified by column chromatography (10% ether in petroleum ether) to give compound **32** (650 mg, 81%) as an oily product: $[\alpha]_D$ +5.5 (*c* 0.29, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.75 (3H, s, H-18), 0.87 (3H, s, H-19), 2.43 (3H, s, CH_3, tosyl), 3.34 (3H, s, OCH_3), 3.49 (1H, m, H-3), 4.21 (1H, t, *J* = 8.5, H-17), 4.65 (2H, s, OCH_2O), 7.31 (2H, m, H-3′ and H-5′, tosyl), 7.77 (2H, m, H-2′and H-6′, tosyl). ^{13}C NMR (101 MHz, $CDCl_3$): δ 144.4 (C-1′, tosyl), 134.3 (C-4′, tosyl), 129.7 (C-2′ and C-6′, tosyl), 127.9 (C-3′ and C-5′, tosyl), 94.6 (OCH_2O), 90.1 (C-17), 76.6 (C-3), 55.2 (OCH_3), 50.1, 43.2, 42.1, 40.4, 36.4, 35.6, 35.4, 34.8, 33.6, 27.8, 27.7, 27.0, 25.9, 23.4, 21.7, 20.1, 14.3, 11.8. IR spectrum ($CHCl_3$): 3020 (phenyl); 1600 (C=C); 1357, 1175 (SO_2); 1098 (C−O). MS ESI: *m*/*z* 513.1 (100%, M + Na), 1003.3 (55%. 2 M + Na). HR-MS (ESI) *m*/*z*: for $C_{28}H_{42}O_5NaS$ [M + Na] calcd, 513.2645; found, 513.2645.

*3α-Methoxymethoxy-10,17β-methyl-5β-gon-13(17)-ene (**33**).* Compound **32** (600 mg, 1.22 mmol) was dissolved in anhydrous toluene (30 mL) and heated at 100 °C under inert atmosphere. Then, methyl magnesium bromide (3.0 M in ether, 2 mL, 6.25 mmol) was added to the stirring hot solution under inert atmosphere as a white precipitate appeared. The reaction mixture was heated at 120 °C for 1 h. The flask was cooled, a few pieces of crushed ice were added, and the pH of the solution was adjusted to pH 2 by dropwise addition of 2 N HCl. The toluene layer was separated, and the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined extracts were washed with brine and dried and the solvents evaporated. Column chromatography on silica gel (5% ether in petroleum ether) gave compound **33** (247 mg, 63%) as an oily product: $[\alpha]_D$ −2.2 (*c* 0.13, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, s, H-19), 1.58 (3H, s, CH_3), 3.37 (3H, s, OCH_3), 3.54 (1H, m, H-3), 4.69 (2H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 136.7 (C-13), 127.7 (C-17), 94.7 (OCH_2O), 76.6 (C-3), 55.2 (OCH_3), 53.9, 45.8, 42.4, 38.9, 37.3, 35.4, 34.9, 33.6, 28.3, 28.1, 27.1, 26.6, 26.1, 25.7, 23.6, 13.5. IR spectrum ($CHCl_3$): 2824, 1145, 1043, 1102 (OCH_2O); 1375 (CH_3). MS ESI: *m*/*z* 341.2 (100%, M + Na), 659.5 (50%, 2 M + Na). HR-MS (ESI) *m*/*z*: for $C_{21}H_{34}O_2Na$ [M + Na] calcd, 341.2451; found, 341.2449.

*3α-Methoxymethoxy-10,17α-dimethyl-5β,13α-gonane and 3α-Methoxymethoxy-10,17β-dimethyl-5β-gonane, a mixture of isomers (**34**).* Compound **33** (119 mg, 0.37 mmol) was dissolved in ethyl acetate (2 mL) and ethanol (6 mL). To this, Pd/C (10%, 30 mg) was added. The reaction mixture was hydrogenated under slight pressure at room temperature for 7 h. Then, the reaction mixture was filtered through a short column of silica gel to remove the catalyst and washing with ethyl acetate (30 mL). Solvents were removed under vacuo. A mixture of products was purified by chromatography on preparative TLC (three plates, 15% ether in petroleum ether) to afford 99 mg (83%) of compound **34** as a mixture of two inseparable products. ^1H NMR (400 MHz, $CDCl_3$): δ 0.76 (3H, d, *J* = 7.2, H-13*β*, 17*β*-Me). 0.86 (6H, s, H-19), 0.92 (3H, d, *J* = 6.5, H-13*α*, 17*α*-Me), 3.36 (6H, s, OCH_3), 3.53 (2H, m, H-3), 4.68 (4H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 94.7 (OCH_2O), 55.2 (OCH_3), 53.4, 53.2, 49.1, 48.4, 43.1, 42.8, 42.4, 42.4, 39.9, 39.8, 39.3, 35.6, 34.9, 34.9, 34.4, 33.7, 32.6, 32.3, 30.0, 29.4, 28.0, 27.9, 27.6, 27.2, 26.9, 26.7, 25.8, 25.5, 23.6, 18.8, 17.8. IR spectrum ($CHCl_3$): 2826, 1145, 1043, 1103 (OCH_2O); 1375 (CH_3). MS ESI: *m*/*z* 343.2 (100%, M + Na). HR-MS (ESI) *m*/*z*: for $C_{21}H_{36}O_2Na$ [M + Na] calcd, 343.2607; found, 343.2608.

*10,17α-Dimethyl-5β,13α-gonan-3α-ol (**35a**) and 10,17β-Dimethyl-5β-gonan-3α-ol (**35b**).* Acyl chloride (0.4 mL, 5.6 mmol) was added dropwise to a solution of stereoisomers **34** (173 mg, 0.53 mmol) in methanol (5 mL) and benzene (1 mL), and the reaction mixture was stirred at room temperature for 7 h. Then, a saturated solution of sodium bicarbonate was added (up to pH 7), the products were extracted with chloroform (2 × 20 mL), combined organic extracts were washed with brine and dried, and solvents were-evaporated. The crude products were prepurified by chromatography on preparative TLC plates (three plates, 40% ether in petroleum ether) to afford a mixture of isomers (128 mg, 86%). Isomers were separated by HPLC (5% ethyl acetate in hexanes) to afford compounds **35a** (45 mg, 30%) and **35b** (46 mg, 30%) as pure products:

*Compound **35a**.* Melting point 83−86 °C (ether/petroleum ether); $[\alpha]_D$ −21.9 (*c* 0.11, $CHCl_3$). IR spectrum ($CHCl_3$): 3608, 1031 (OH); 1378, 1367 (CH_3). MS ESI: *m*/*z* 299.2 (45%, M + Na), 553.5 (15%, 2 M + 1). HR-MS (ESI) *m*/*z*: for $C_{19}H_{32}ONa$ [M + Na] calcd, 299.2345; found, 299.2344.

*Compound **35b**.* Melting point 110−113 °C (ether/petroleum ether); $[\alpha]_D$ +9.7 (*c* 0.12, $CHCl_3$). IR spectrum ($CHCl_3$): 3608, 1031 (OH); 1376, 1366 (CH_3). MS ESI: *m*/*z* 299.2 (30%, M + Na), 553.5 (20%, 2 M + 1). HR-MS (ESI) *m*/*z*: for $C_{19}H_{32}ONa$ [M + Na] calcd, 299.2345; found, 299.2340.

*10β,17α-Dimethyl-5β,13α-gonan-3α-yl 3-Sulfate Pyridinium Salt (**36a**).* Compound **36a** was prepared according to General Procedure for Sulfation. Starting from compound **35a** (34 mg, 0.12 mmol), compound **36a** (45 mg, 85%) was obtained as a white solid: mp 152−154 °C; $[\alpha]_D$ −0.5 (*c* 0.19, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.75 (3H, d, *J* = 7.2, 17-Me). 0.85 (3H, s, H-19), 4.46 (1H, m, H-3), 7.50 (2H, ddd, J_1 = 7.7. J_2 = 4.6, J_3 = 1.4, H-2′ and H-4′, pyridinium), 7.92 (1H, tt, J_1 = 7.7, J_2 = 1.7, H-3′, pyridinium), 8.73 (2H, d, *J* = 4.5, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 147.5 (C-1′ and C-5′, pyridinium), 139.1 (C-3′, pyridinium), 124.9 (C-2′ and C-4′, pyridinium), 79.7 (C-3), 49.0, 48.4, 43.0, 42.3, 39.7, 35.5, 34.6, 34.3, 33.3, 32.5, 29.4, 27.9, 27.6, 26.9, 26.8, 25.7, 23.4, 17.8. IR spectrum ($CHCl_3$): 3140, 3073, 1490 (pyridinium); 1263, 1172, 1046 (SO_3); 1475, 1377 (CH_3). MS ESI: *m*/*z* 355.2 (100%, M − pyH). HR-MS (ESI) *m*/*z*: for $C_{19}H_{31}O_4S$ [M − pyH] calcd, 355.1948; found, 355.1947.

*10,17β-Dimethyl-5β-gonan-3α-yl 3-Sulfate Pyridinium Salt (**36b**).* Compound **36b** was prepared according to General Procedure for Sulfation. Starting from compound **35b** (37 mg, 0.13 mmol), compound **36b** (56 mg, 96%) was obtained as a white solid: mp 155−157 °C; $[\alpha]_D$ +26.9 (*c* 0.18, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, s, H-19), 0.90 (3H, d, *J* = 7.2, 17-Me), 4.45 (1H, m, H-3), 7.37 (2H, ddd, J_1 = 7.7, J_2 = 4.5, J_3 = 1.4, H-2′ and H-4′, pyridinium),

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7.70 (1H, tt, J_1 = 7.7, J_2 = 1.8, H-3′, pyridinium), 8.65 (2H, d, J = 4.3, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 148.8 (C-1′ and C-5′, pyridinium), 137.4 (C-3′, pyridinium), 124.3 (C-2′ and C-4′, pyridinium), 79.7 (C-3), 53.3, 53.2, 42.7, 42.3, 39.8, 39.3, 35.7, 35.5, 34.6, 33.3, 32.2, 30.0, 27.9, 26.9, 26.6, 26.6, 26.5, 25.4, 23.4, 18.8. IR spectrum ($CHCl_3$): 3140, 3073, 1490 (pyH); 1263, 1172, 1046 (SO_3); 1464, 1374 (CH_3). MS ESI: m/z 355.2 (100%, M − pyH). HR-MS (ESI) m/z: for $C_{19}H_{31}O_4S$ [M − pyH] calcd, 355.1948; found, 355.1946.

*3α-Methoxymethoxy-5β-androstan-17-tosylhydrazone (**37**).* Molecular sieves (40 msh, 2 g) were added to a solution of ketone **22** (1 g, 3 mmol) and tosylhydrazide (1.63 g, 8.5 mmol) in dry methanol (70 mL), and the reaction mixture was refluxed for 72 h under inert atmosphere. The progress of the reaction was monitored by TLC. Then, the sieves were filtered off and the solvent was evaporated in vacuo. The residue was dissolved in toluene (100 mL), the precipitate was filtered off, and the solvent was evaporated. The residue was dissolved in ethyl acetate (2 × 50 mL), washed with a saturated solution of sodium bicarbonate (3 × 25 mL), a saturated solution of sodium chloride, and dried. Solvents were evaporated, and the residue was purified by column chromatography (15% of ethyl acetate in petroleum ether) to afford white solids of **37** (1.05g, 67%): mp 97.4−99.5 °C (benzene); $[\alpha]_D$ +44.2 (c 0.33, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, s, H-18), 0.92 (3H, s, H-19), 2.43 (3H, s, CH_3, tosyl), 3.36 (3H, s, OCH_3), 3.52 (1H, m, H-3), 4.67 (2H, s, OCH_2O) 7.29 (2H, m, H-2′ and H-4′, tosyl), 7.81 (2H, d, J = 8.3, H-1′ and H-5′, tosyl). ^{13}C NMR (101 MHz, $CDCl_3$): δ 161.0 (C-17), 144.3 (C-1′, tosyl), 129.4 (C-2′, tosyl), 128.6 (C-6′, tosyl), 128.0 (C-3′, tosyl), 125.9 (C-5′, tosyl), 94.6 (OCH_2O), 77.4 (C-3), 55.1 (OCH_3), 53.2, 41.9, 40.5, 35.3, 35.2, 34.8, 33.5, 31.7, 27.6, 26.9, 25.8, 25.3, 23.2, 21.8, 21.6, 20.1, 20.0, 16.6, 13.6. IR spectrum ($CHCl_3$): 2938 (CH_2); 1659 (C═N); 1167 (SO_3); 1045, 1036 (MOM). MS (ESI): m/z 503.2 (10%, M − CH_3). For $C_{28}H_{42}N_2O_5S$ (518.7) Calcd: 64.84%, C; 8.16%, H; 5.40%, N. Found: 65.17%, C; 8.30%, H; 5.18%, N.

*3α-Methoxymethoxy-5β-androst-16-ene (**38**).* Methyllithium (14 mL, 1.6 M in ether, 22.4 mmol) was added at 0 °C to a solution of tosylhydrazone **37** (1 g, 1.93 mmol) in dry tetrahydrofuran (30 mL) under inert atmosphere. Reaction mixture was stirred at 0 °C for 2 h. Then, the excess reagent was quenched with water (50 mL). The product was extracted with ethyl acetate (3 × 25 mL), combined organic extracts were washed with a solution of citric acid (3 × 25 mL), a saturated solution of sodium chloride, and dried, and solvents were evaporated. The residue was purified by column chromatography (1% of acetone in petroleum ether) to afford solids of **38** (606 mg, 98%) that did not crystallize: $[\alpha]_D$ +20.2 (c 0.35, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.74 (3H, s, H-18), 0.95 (3H, s, H-19), 3.37 (3H, s, OCH_3), 3.53 (1H, tt, J_1 = 11.2, J_2 = 4.7, H-3), 4.69 (2H, s, OCH_2O), 5.67 (1H, ddd, J_1= 5.8, J_2 = 3.0, J_3 = 1.5, H-17), 5.83 (1H, ddd, J_1= 5.8, J_2 = 2.5, J_3 = 1.1, H-16). ^{13}C NMR (101 MHz, $CDCl_3$): δ 144.0 (C-17), 129.4 (C-16), 94.7 (OCH_2O), 77.0 (C-3), 56.3 (C-14), 55.3 (OCH_3), 45.8, 42.4, 41.4, 36.2, 35.5, 35.2, 34.6, 33.8, 32.1, 27.8, 27.3, 26.7, 23.5, 20.8, 17.1. IR spectrum ($CHCl_3$): 3049 (C═C); 2935 (CH_2); 1047, 1036 (MOM). MS (ESI): m/z 341.4 (100%, M + Na). HR-MS (ESI) m/z: for $C_{21}H_{34}O_2Na$ [M + Na] calcd, 341.2451; found, 341.2452.

*3α-Methoxymethoxy-5β-androst-16α-ol (**39**).* A solution of compound **38** (2 g, 6.30 mmol) in tetrahydrofuran (42 mL) was added at 0 °C to a solution of dimer of 9-borabicyclo[3.3.1]nonane (102 mL, 51 mmol) under inert atmosphere. The reaction mixture was stirred at 0 °C for 4 h. Then, water (32.5 mL), an aqueous solution of sodium hydroxide (10%, 32.5 mL), and hydrogen peroxide (30%, 48.5 mL) were added and the mixture was stirred overnight at room temperature. Then, sodium sulfite (3.36 g), acetic acid (98%, 16.4 mL), water (81 mL), and an aqueous solution of citric acid (5%, 82 mL) were added. The product was extracted with ethyl acetate (3 × 80 mL), combined organic extracts were washed with brine (50 mL) and dried, and solvents were evaporated. The residue was purified by column chromatography (1−5% of acetone in petroleum ether) to afford solids of **39** (1.26 g, 60%): mp 98.7−100 °C (acetone/*n*-heptane); $[\alpha]_D$ +16.6 (c 0.33, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.69 (3H, s, H-18), 0.91 (3H, s, H-19), 3.37 (3H, s, OCH_3), 3.53 (1H, tt, J_1 = 11.2, J_2 = 4.7, H-3), 4.45 (1H, tdd, J_1= 7.6, J_2 = 6.0, J_3 = 1.6, H-16), 4.69 (2H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 94.7 (OCH_2O), 77.0 (C-3), 72.0 (C-16), 55.3 (OCH_3), 52.3, 52.2, 42.2, 42.1, 40.8, 39.0, 37.4, 35.8, 35.5, 35.0, 33.7, 27.8, 27.3, 26.8, 23.5, 20.5, 18.8. IR spectrum ($CHCl_3$): 3612 (OH); 2941 (CH_2); 1040 (MOM). MS (ESI): m/z 359.3 (100%, M + Na). HR-MS (ESI) m/z: for $C_{21}H_{35}O_3$ [M + H] calcd, 359.2558; found, 359.2558.

*3α-Methoxymethoxy-5β-androst-16-on (**40**).* Dry pyridine (10 mL) and pyridinium chlorochromate (16.7 g, 77.5 mmol) were added to a solution of compound **39** (3.17 g, 9.4 mmol) in freshly dried dichloromethane (150 mL), and the reaction mixture was stirred at room temperature for 2 h. Then, the solids were filtered off through a short column of silica gel (15 g), washing with ethyl acetate. Solvents were evaporated, the residue was dissolved in ethyl acetate (3 × 50 mL), washed with an aqueous solution of citric acid (5%, 2 × 40 mL), brine (50 mL), a saturated solution of sodium bicarbonate (2 × 30 mL), and brine, and then dried, and solvents were evaporated to afford compound **40** (2.59 g, 95%): mp 107.9−108 °C (acetone/*n*-heptane); $[\alpha]_D$ −134.7 (c 0.35, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (3H, s, H-18), 0.95 (3H, s, H-19), 3.37 (3H, s, OCH_3), 3.54 (1H, ddd, J_1 = 15.8, J_2 = 11.1, J_3 = 4.7, H-3), 4.69 (2H, s, OCH_2O). ^{13}C NMR (101 MHz, $CDCl_3$): δ 218.9 (C-16), 94.7 (OCH_2O), 77.0 (C-3), 56.1, 55.3 (OCH_3), 51.9, 42.0, 40.6, 39.4, 39.4, 38.5, 35.4, 35.2, 35.1, 33.7, 27.8, 27.1, 26.8, 23.4, 20.5, 18.2. IR spectrum ($CHCl_3$): 2937 (OCH_2O); 1737 (C═O); 1045, 1037 (MOM). MS (ESI): m/z 335.3 (32%, M + H) 273.2 (100%). For $C_{21}H_{34}O_3$ (334.5) Calcd: 75.41%, C; 10.25%, H. Found: 75.66%, C; 10.33%, H.

*16-Oxo-5β-androstan-3α-ol (**41**).* Compound **41** was prepared in the same manner as compound **11**. Starting from compound **40** (3.4 g, 10.2 mmol), compound **41** (3.17 g, 93%) was obtained by crystallization: mp 135.8−136.7 °C (acetone/*n*-heptane); $[\alpha]_D$ −167.4 (c 0.33, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (3H, s, H-18), 0.96 (3H, s, H-19), 3.65 (1H, tt, J_1 = 10.8, J_2 = 4.7, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 218.8 (C═O), 71.8 (C-3), 56.1, 51.9, 42.0, 40.7, 39.4, 39.4, 38.5, 36.5, 35.4, 35.2, 34.9, 30.6, 27.0, 26.9, 23.4, 20.5, 18.2. IR spectrum ($CHCl_3$): 3609, 1032 (OH); 2936 (CH_2); 1736 (C═O). MS (ESI): m/z 290.2 (100%, M). HR-MS (ESI) m/z: for $C_{19}H_{30}O_2Na$ [M + Na] calcd, 313.2138; found, 313.2137.

*16-Methylen-5β-androstan-3α-ol (**42**).* Compound **42** was prepared in the same manner as compound **16**. Starting from compound **41** (800 mg, 2.67 mmol), compound **42** (756 mg, 95%) was obtained by chromatography on preparative TLC plates (1−5% acetone in petroleum ether): mp 165.3−165.6 °C (acetone/*n*-heptane); $[\alpha]_D$ −69.8 (c 0.35, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.72 (3H, s, H-18), 0.93 (3H, s, H-19), 2.33 (1H, ddt, J_1 = 16.1, J_2 = 7.5, J_3 = 1.6, H-16a) . 3.63 (1H, tt, J_1 = 11.1, J_2 = 4.7, H-3), 4.88 (2H, ddt, J_1 = 4.4, J_2 = 3.0, J_2 = 1.6, ═CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ 151.3 (═CH_2), 107.0 (C-16), 71.9 (C-3), 54.3, 49.6, 42.2, 40.8, 40.7, 38.7, 36.6, 35.9, 35.5, 34.9, 33.5, 30.7, 27.2, 26.8, 23.5, 20.9, 17.7. IR spectrum ($CHCl_3$): 3609 (OH); 3015, 1658 (═CH_2); 2934 (CH_2). MS (ESI): m/z 311.3 (100%, M + Na). HR-MS (ESI) m/z: for $C_{20}H_{32}ONa$ [M + Na] calcd, 311.2345; found, 311.2344.

*16-Methylen-5β-androstan-3α-yl 3-Sulfate Pyridinium Salt (**43**).* Compound **43** was prepared according to General Procedure for Sulfation. Starting from compound **42** (83 mg, 0.29 mmol), compound **43** (66 mg, 56%) was obtained as a white solid: mp 180−182 °C (chloroform); $[\alpha]_D$ −41.9 (c 0.35, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.70 (3H, s, H-18), 0.91 (3H, s, H-19), 2.30 (1H, dd, J_1 = 16.0, J_2 = 7.7, H-16a), 4.45 (1H, tt, J_1 = 11.2, J_2 = 4.9, H-3), 4.87 (2H, m, ═CH_2), 8.02 (2H, m, H-2′ and H-4′, pyridinium), 8.48 (1H, t, J = 8.6, H-3′, pyridinium), 8.99 (2H, d, J = 5.2, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 151.3 (═CH_2), 145.8 (C-1′ and C-5′, pyridinium), 142.3 (C-3′, pyridinium), 127.3 (C-2′ and C-4′, pyridinium), 107.0 (C-16), 79.5 (C-3), 56.1, 51.9, 42.0, 40.6, 39.4, 39.3, 38.5, 35.4, 35.2, 34.8, 33.4, 27.8, 26.8, 26.8, 23.6, 20.4, 18.0. IR spectrum ($CHCl_3$): 1736 (C═O); 1656 (C═C); 1460 (═CH, pyridine); 1263, 1171, 969, 947 (OSO_3). MS (ESI): m/z 367.2 (100%, M − pyH). HR-MS (ESI) m/z: for $C_{20}H_{31}O_4S$ [M − pyH] calcd, 367.1946; found, 367.1945.

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*5β-Androst-16-ene-3α-ol (**44**).* Starting from compound **38** (1.76 g, 5.53 mmol), compound **44** (0.95 g, 63%) was obtained by hydrolysis of the MOM protecting group in the same manner as described for compound **11** followed by crystallization: mp 150−151 °C (ethyl acetate/*n*-heptane); $[\alpha]_D$ +17.6 (*c* 0.44, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.74 (3H, s, H-18), 0.96 (3H, s, H-19), 3.63 (1H, m, H-3), 5.69 (1H, ddd, J_1 = 5.6, J_2 = 2.9, J_3 = 1.4, H-17), 5.84 (1H, m, H-16). ^{13}C NMR (101 MHz, $CDCl_3$): δ 143.9 (C-16), 129.2 (C-15), 71.8 (C-3), 56.1, 45.6, 42.20, 41.3, 36.5, 36.1, 35.3, 34.9, 34.5, 32.0, 30.5, 27.1, 26.6, 23.3, 20.7, 17.0. IR spectrum ($CHCl_3$): 3609 (OH); 3046, 1588, 834 (=CH); 2934, 2865 (CH_3). For $C_{19}H_{30}O$ (274.4) Calcd: 83.15%, C; 11.02%, H. Found: 83.12%, C; 11.28%, H.

*5β-Androst-16-ene-3α-yl 3-Sulfate Pyridinium Salt (**45**).* Compound **45** was prepared according to General Procedure for Sulfation. Starting from compound **44** (17 mg, 0.06 mmol), compound **45** (14 mg, 52%) was obtained as a white solid: mp 163−165 °C (benzene); $[\alpha]_D$ +18.7 (*c* 0.17, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, H-18), 0.94 (3H, s, H-19), 4.45 (1H, tt, J_1 = 10.9, J_2 = 4.9, H-3), 5.67 (1H, ddd, J_1 = 5.9, J_2 = 2.3, J_3 = 1.0, H-17), 5.81 (1H, ddd, J_1 = 5.8, J_2 = 2.9, J_3 = 1.3, H-16), 8.0 (2H, t, *J* = 7.0, H-2′ and H-4′, pyridinium), 8.47 (1H, m, H-3′, pyridinium), 8.98 (2H, d, *J* = 5.7, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 144.0 (C-16), 145.8 (C-1′ and C-5′, pyridinium), 142.4 (C-3′, pyridinium), 129.4 (C-17), 127.3 (C-2′ and C-4′, pyridinium), 79.7 (C-3), 56.3, 45.1, 42.3, 41.3, 36.2, 35.4, 34.9, 34.6, 33.5, 32.1, 27.8, 27.1, 26.7, 23.4, 20.8, 18.0, 17.1. IR spectrum ($CHCl_3$): 3140, 3100, 3072 (pyridinium); 2971, 2937 (CH_3); 1604 (C=C); 1260, 1249, 1237 (OSO_3). MS (ESI): *m*/*z* 353.2 (100%, M − pyH). HR-MS (ESI) *m*/*z*: for $C_{19}H_{29}O_4S$ [M − pyH] calcd, 353.1789; found, 353.1789.

*3α-Hydroxy-5β-estrane (**47**).* Compound **47** was prepared in the same manner as compound **14**. Starting from compound **46** (90 mg, 0.33 mmol), compound **47** (82 mg, 96%) was obtained by the deoxygenation procedure described for derivative **14**, followed by column chromatography on silica gel (20% of ether in petroleum ether): mp 131−132 °C (chloroform); $[\alpha]_D$ +19.3 (*c* 0.23, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.70 (3H, s, H-18), 1.93 (1H, ddt, J_1 = 8.3, J_2 = 5.9, J_3 = 2.8), 3.63 (1H, tt, J_1 = 10.4, J_2 = 4.8, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 72.0 (C-3), 53.7, 42.4, 41.1, 40.8, 40.2, 39.1, 38.8, 36.6, 35.8, 31.7, 29.9, 26.8, 26.2, 25.9, 25.5, 20.6, 17.7. IR spectrum ($CHCl_3$): 3609, 3457, 1034 (OH); 2925, 2871 (CH_3). MS (EI): *m*/*z* 285.2 (100%, M + Na). For $C_{18}H_{30}O$ (262.4) Calcd: 82.38%, C; 11.52%, H. Found: 82.11%, C; 11.37%, H.

*5β-Estran-3α-yl 3-Sulfate Pyridinium Salt (**48**).* Compound **48** was prepared according to General Procedure for Sulfation. Starting from compound **47** (75 mg, 0.29 mmol), compound **48** (40 mg, 33%) was obtained as a white solid: mp 192−194 °C (chloroform); $[\alpha]_D$ +19.0 (*c* 0.22, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.66 (3H, s, H-18), 4.29−4.55 (1H, m, H-3), 7.98−8.06 (2H, m, H-2′ and H-4′, pyridinium), 8.48 (1H, tt, J_1 = 7.9, J_2 = 1.6, H-3′, pyridinium), 8.97−9.03 (2H, m, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 145.9 (C-1′ and C-5′, pyridinium), 142.4 (C-3′, pyridinium), 127.3 (C-2′ and C-4′, pyridinium), 79.7 (C-3), 53.7, 42.3, 41.1, 40.7, 40.0, 39.0, 38.7, 35.9, 33.6, 31.6, 27.0, 26.7, 26.2, 25.8, 25.4, 20.6, 17.6. IR spectrum ($CHCl_3$): 950 (COS); 1045, 1264 (SO_3); 2919 (CH_3). MS (EI): *m*/*z* 341.2 (100%, M − pyH). HR-MS (EI) *m*/*z*: for $C_{18}H_{29}O_4S$ [M − pyH] calcd, 341.1792; found, 341.1791.

*24-Nor-5β-cholan-3α-ol (**50**).* Silver nitrate was added (350 mg, 2.1 mmol) to a solution of lithocholic acid **49** (200 mg, 0.53 mmol) in dimethyl sulfoxide (15 mL), and the reaction mixture was heated at 80 ° C for 20 h. Then, the solids were removed by filtration. The filtrate was poured into water (20 mL), the product extracted with ethyl acetate (3 × 20 mL), combined organic extracts were washed with a solution of hydrochloric acid (5%, 20 mL), sodium bicarbonate, and brine, and then dried. Solvents were evaporated, and the residue was purified by column chromatography (3−10% acetone in petroleum ether). Recrystallization from ether afforded 63 mg (36%) of compound **50**:[24] mp 159−161 °C (ether); $[\alpha]_D$ +31.7 (*c* 0.12, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.64 (3H, s, H-18), 0.82 (3H, t, *J* = 7.4, H-23), 0.89 (3H, d, *J* = 6.5, H-21), 0.92 (3H, s, H-19), 3.62 (1H, m, H-3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 72.0 (C-3), 56.6, 55.9, 42.7, 42.2, 40.6, 40.3, 37.1, 36.6, 36.0, 35.5, 34.7, 30.7, 28.4, 28.3, 27.3, 26.6, 24.4, 23.5, 20.9, 18.1, 12.1, 10.5.

*24-Nor-5β-cholan-3α-yl 3-Sulfate Pyridinium Salt (**51**).* Compound **51** was prepared according to General Procedure for Sulfation. Starting from compound **50** (26 mg, 0.16 mmol), compound **51** (34 mg, 83%) was obtained as a white solid: mp 186−188 °C; $[\alpha]_D$ +13.8 (*c* 0.11, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 0.62 (3H, s, H-18), 0.81 (3H, t, *J* = 7.4, H-23), 0.88 (3H, d, *J* = 6.5, H-21), 0.90 (3H, s, H-19), 4.46 (1H, m, H-3), 7.79 (2H, m, H-2′ and H-4′, pyridinium), 8.19 (1H, t, *J* = 7.7, H-3′, pyridinium), 8.84 (2H, d, *J* = 4.9, H-1′ and H-5′, pyridinium). ^{13}C NMR (101 MHz, $CDCl_3$): δ 145.0 (C-1′ and C-5′, pyridinium), 142.3 (C-3′, pyridinium), 126.0 (C-2′ and C-4′, pyridinium), 79.8 (C-3), 56.7, 55.9, 42.8, 42.3, 40.5, 40.3, 37.1, 35.9, 35.4, 34.6, 33.5, 28.4, 28.3, 27.9, 27.2, 26.5, 24.4, 23.4, 20.9, 18.1, 12.1, 10.5. IR spectrum ($CHCl_3$): 3140, 3073, 1490 (pyridinium); 1263, 1170, 1043 (SO_3). MS ESI: *m*/*z* 411.4 (100%, M − pyH). For $C_{28}H_{45}NO_4S$ (491.7) Calcd: 68.39%, C; 9.22%, H; 2.85%, N. Found: 68.65%, C; 9.36%, H; 2.70%, N.

■ ASSOCIATED CONTENT

Supporting Information

^{13}C and ^1H NMR data (in $CDCl_3$) of compounds **35a** and **35b**, dependency of the particle size of compound **19** on concentration (PDF), and molecular strings file for target compounds (CSV). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00570.

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Author Contributions

‖The computational analysis was done by M.N.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

NMDA, *N*-methyl-D-aspartate; TLC, thin layer chromatography

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