Bioorganic & Medicinal Chemistry Letters 20 (2010) 6680–6684

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Neurosteroid analogues. 15. A comparative study of the anesthetic and GABAergic actions of alphaxalone, Δ^{16}-alphaxalone and their corresponding 17-carbonitrile analogues

Achintya K. Bandyopadhyaya [a], Brad D. Manion [b], Ann Benz [c], Amanda Taylor [c], Nigam P. Rath [e], Alex S. Evers [a,b], Charles F. Zorumski [c,d], Steven Mennerick [c,d], Douglas F. Covey [a,*]

[a] Department of Developmental Biology, Campus Box 8103, Washington University in St. Louis, School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, USA
[b] Department of Anesthesiology, Washington University in St. Louis, School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, USA
[c] Department of Psychiatry, Washington University in St. Louis, School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, USA
[d] Department of Anatomy and Neurobiology, Washington University in St. Louis, School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110, USA
[e] Department of Chemistry and Biochemistry and Center for Nanoscience, University of Missouri St. Louis, One University Boulevard, St. Louis, MO 63121, USA

ARTICLE INFO

Article history:
Received 4 August 2010
Accepted 1 September 2010
Available online 15 September 2010

Keywords:
Alphaxalone
Anesthetic steroid
Delta-16-alphaxalone
$GABA_A$ receptor
TBPS binding
Tadpole anesthesia

ABSTRACT

Alphaxalone, a neuroactive steroid containing a 17β-acetyl group, has potent anesthetic activity in humans. This pharmacological activity is attributed to this steroid's enhancement of γ-amino butyric acid-mediated chloride currents at γ-amino butyric acid type A receptors. The conversion of alphaxalone into Δ^{16}-alphaxalone produces an analogue that lacks anesthetic activity in humans and that has greatly diminished receptor actions. By contrast, the corresponding 17β-carbonitrile analogue of alphaxalone and the Δ^{16}-17-carbonitrile analogue both have potent anesthetic and receptor actions. The differential effect of the Δ^{16}-double bond on the actions of alphaxalone and the 17β-carbonitrile analogue is accounted for by a differential effect on the orientation of the 17-acetyl and 17-carbonitrile substituents.

Alphaxalone (**1a**, (3α,5α)-3-hydroxypregnane-11,20-dione, Chart 1) is a steroid that has potent anesthetic activity.[1,2] By contrast, Δ^{16}-alphaxalone (**2a**) does not.[3,4] Explanations for the dramatic influence of the Δ^{16}-double bond on anesthetic activity were initially focused on how this structural modification altered the behavior of these two steroids in lipids.[5–19]

Although the different behaviors of steroids **1a** and **2a** in lipids are likely factors contributing to the difference in the anesthetic activity of the two compounds, it has been hypothesized that a pharmacophore-based differential interaction of the two compounds with $GABA_A$ receptors is also important.[20] The Δ^{16} double bond found in steroid **2a** has effects on both the conformation of the steroid D-ring and the free rotation of the 17-acetyl group.[10,20] A study of analogues of steroids **1a** and **2a** without the 11-ketone group and the 17-acetyl group (**1b** and **2b**) found that these analogues share similarly weak potency as $GABA_A$ receptor modulators. Thus, it was proposed that the effect of the Δ^{16} double bond on the steroid D-ring conformation is not important. Instead, this structural modification was hypothesized to fix the orientation of the C-17 acetyl group in a position that is not favorable for its

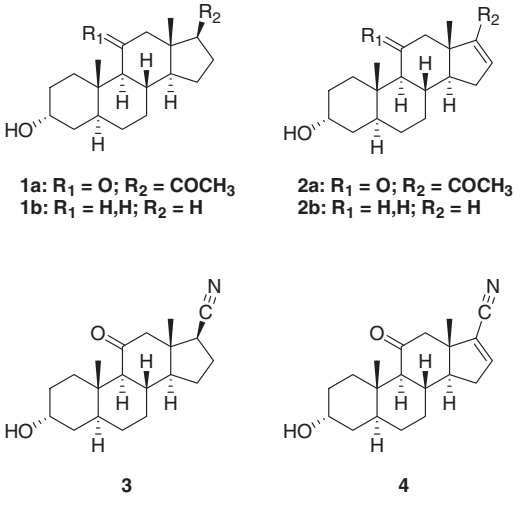


Chart 1.

Abbreviations: GABA, γ-amino butyric acid; $GABA_A$, γ-amino butyric acid receptor type A; [^{35}S]-TBPS, [^{35}S]-*t*-butylbicyclophosphorothionate; LRR, loss of righting reflex; LSR, loss of swimming reflex.

* Corresponding author. Tel.: +1 314 362 1726; fax: +1 314 362 7058.
E-mail address: dcovey@wustl.edu (D.F. Covey).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.09.008

interaction with the receptor thus explaining the diminished activities of steroid **2a**.[20] However, steroids **1b** and **2b** do not have a C-17 substituent and therefore do not directly address the effect that the Δ^{16} double bond has on the orientation of a C-17 substituent in three-dimensional space. Additionally, steroids **1b** and **2b** lack the 11-ketone group found in steroids **1a** and **2a** and any effect that this substituent might have on anesthetic activity is not addressed by these analogues.

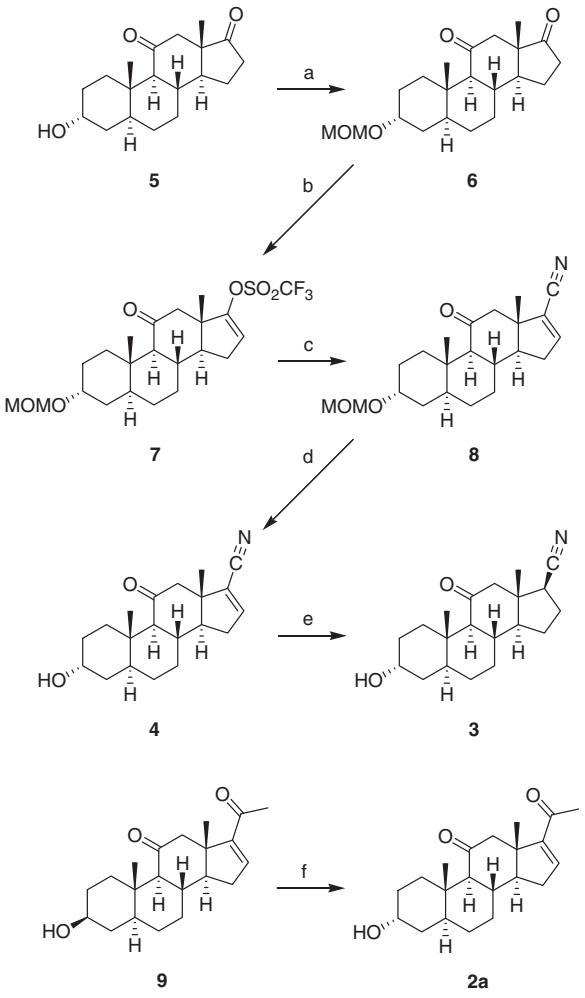
We have prepared the C-17 carbonitrile analogues (**3** and **4**; Chart 1) of steroid **1a** and Δ^{16}-steroid **2a** and compared the actions of compounds **1a**, **2a**, **3** and **4** on $GABA_A$ receptor function. Unlike the previously prepared compounds **1b** and **2b**, which are both relatively weak modulators of $GABA_A$ receptors because they lack a hydrogen bond acceptor group at C-17, steroids **3** and **4** are both strong modulators. Steroids **3** and **4** also contain the 11-ketone group which is present in steroids **1a** and **2a**, but not in steroids **1b** and **2b**, so that any potentially confounding effect caused by the absence of this group is avoided. We report that the conversion of steroid **3** into steroid **4** results in only a slight loss of potency for enhancement of GABA-mediated currents at $GABA_A$ receptors, a slight increase in the IC_{50} value for allosteric displacement of [^{35}S]-TBPS from the picrotoxin site on $GABA_A$ receptors and a slight decrease in anesthetic potency as measured by LRR and LSR in tadpoles. These results further refine the previous hypothesis regarding the effect that a Δ^{16} double bond has on the activity of steroids that modulate $GABA_A$ receptors, and identify a Δ^{16} analogue with high activity at these receptors.

The preparation of compounds is shown in Scheme 1. The 3α-hydroxyl group of commercially available steroid **5** was protected as the MOM derivative yielding steroid **6** in quantitative yield (Scheme 1). Conversion of steroid **6** into the Δ^{16}-17-carbonitrile **8** was achieved by cyanation of the intermediate Δ^{16}-17-triflate **7** in 89% yield. Removal of the MOM group under acidic conditions gave Δ^{16}-steroid **4** in an isolated yield of 93%. Catalytic hydrogenation of Δ^{16}-steroid **4** gave a quantitative yield of steroid **3** which was prepared previously by a different route as described in the patent literature.[21] Steroid **2a** (Chart 1) was prepared in 32% yield from its commercially available 3β-hydroxysteroid epimer **9** by a Mitsunobu reaction.[22]

The crystal structures of compounds **1a** and **2a** were previously unreported and were determined in this study. The conformations of these compounds in the solid state are shown in Figure 1 and are consistent with the solution conformations deduced from previous NMR studies[10] and molecular mechanics calculations.[20]

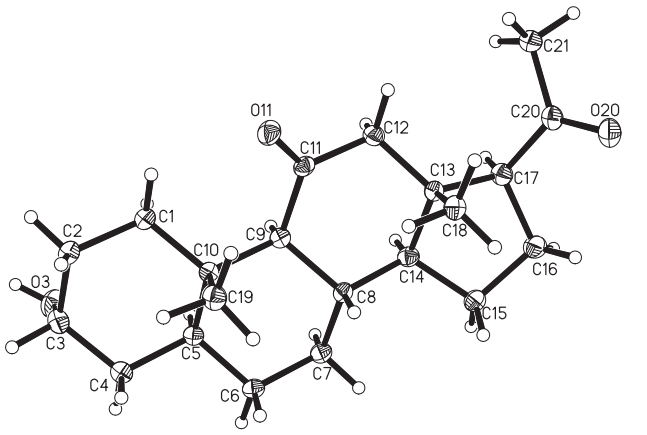
The potency of compounds **1a**, **2a**, **3** and **4** for allosteric displacement of [^{35}S]-TBPS from the picrotoxin binding site on $GABA_A$ receptors is reported in Table 1. The IC_{50} values measured for steroids **1a** and **2a** (226 ± 24 and 2220 ± 260 nM, respectively) are similar to literature values[20] (**1a**, 303 ± 37 nM; **2a**, 2956 ± 239 nM). Steroid **3** displaces [^{35}S]-TBPS with essentially the same potency as steroid **1a**. This is as expected since a 17β-carbonitrile group has previously been shown to produce neurosteroid analogues with high potency for [^{35}S]-TBPS displacement.[23] The Δ^{16}-steroid **4** is a weaker displacer of [^{35}S]-TBPS by a factor of two. This is in marked contrast to the tenfold loss of potency observed when steroid **1a** was converted into Δ^{16}-steroid **2a**.

The effects of compounds **1a**, **2a**, **3** and **4** on the GABA-mediated chloride currents of rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus laevis* oocytes are reported in Table 2. There is a close correlation of electrophysiology results with the [^{35}S]-TBPS binding results. Steroid **1a** produces a concentration-dependent increase in chloride current that at a steroid concentration of 10 μM is about 20-fold higher than the control response in the absence of steroid **1a**. By contrast, the Δ^{16}-steroid **2a** produces only about twofold concentration-dependent maximum enhancement of GABA responses. Steroid **3** produced an electrophysiological response that was essentially equal to that of steroid **1a**. The Δ^{16}-steroid **4** also gave a response that was essentially the same as that of steroid **1a** and, more significantly, much greater than the response of Δ^{16}-steroid **2a**. Steroids **1a**, **3** and **4** directly gated a small but significant chloride current in the absence of added GABA. The current directly gated by steroid **2a** was not significant. To further reduce any variation in responses due to the fact that different preparations of oocytes were used in the electrophysiological experiments for the different analogues, all four steroids were tested at the same concentration on the same ooctyes (Fig. 2). When evaluated in this way, steroid **4** did give a somewhat lower increase in chloride current than steroid **3**. However, the effect of steroid **4** continued to be far greater than the effect of steroid **2a**. The anesthetic effects of compounds **1a**, **2a**, **3** and **4** are summarized in Table 3. The potency of the anesthetic effects closely correlated with the effects found in the previous two bioassays. Steroids **1a** and **3** as well as Δ^{16}-steroid **4** all had similar EC_{50} values for tadpole LRR (EC_{50} ~1 μM) and LSR (EC_{50} ~5.5 μM). The Δ^{16}-steroid **2a** was not effective (EC_{50} >10 μM) in causing either LRR or LSR.

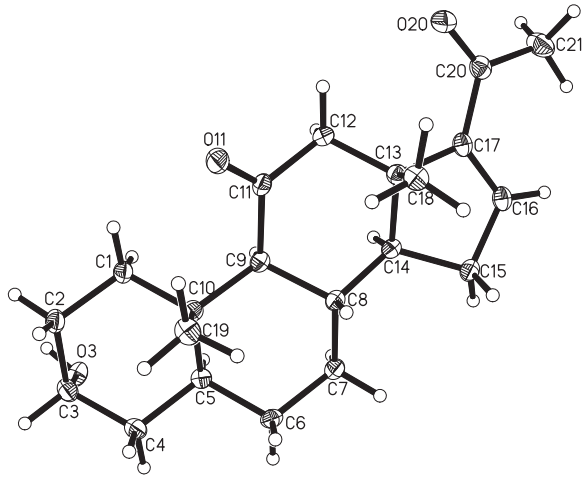


Scheme 1. Reagents and conditions: (a) MOMCl, Hunig's base, CH_2Cl_2, room temperature; (b) $PhN(SO_2CF_3)_2$, KHMDS, THF, −78 °C; (c) NaCN, CuI, $Pd(PPh_3)_4$, MeCN; (d) $MeOH/CH_2Cl_2$ (4:1), concd HCl, room temperature; (e) H_2 (30 psi), Pd/C (5%), EtOAc; (f) (i) DEAD, PPh_3, TFA, $PhCO_2Na$, THF; (ii) $NaHCO_3$, aqueous MeOH.

This study was performed to gain a better understanding of how anesthetic steroid analogues containing a Δ^{16} double bond interact with $GABA_A$ receptors. As mentioned previously, the presence of the Δ^{16} double bond in steroid **2a** has multiple structural effects.



1a



2a

Figure 1. X-ray crystal structures of steroids **1a** and **2a**. The conformation of the 17-acetyl group in each steroid in the solid state is the same as deduced from solution NMR experiments[10] and molecular mechanics calculations.[20]

Table 1
Inhibition of [^{35}S]-TBPS binding by steroids **1a**, **2a**, **3** and **4**

Compound	IC_{50}[a] (nM)	n_{Hill}
1a	226 ± 24	1.10 ± 0.11
2a	2,220 ± 260	1.24 ± 0.14
3	190 ± 18	1.14 ± 0.11
4	361 ± 58	1.00 ± 0.14

[a] Results are from duplicate experiments performed in triplicate. Error limits are calculated as standard error of the mean. Methods were as reported previously.[27]

It affects the conformation of the steroid D-ring, eliminates free rotation of the 17-acetyl group about the C-17, C-20 bond and reorients this group in three-dimensional space. By contrast, a Δ^{16} double bond has fewer structural effects when the C-17 substituent is a carbonitrile group. Although the effect on the conformation of the steroid D-ring caused by a Δ^{16} double bond is the same for both the 17-acetyl and 17-carbonitrile groups, loss of free rotation of the substituent is only a factor for the 17-acetyl group. Additionally, the orientation of the 17-carbonitrile group along a vector passing midway through the C-14, C-15 bond and through

Table 2
Modulation of rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptor function by steroids **1a**, **2a**, **3** and **4**

Compound	Oocyte electrophysiology[a]			
	0.1 μM	1 μM	10 μM	(gating) 10 μM
1a	2.91 ± 0.57	4.70 ± 1.11	19.64 ± 4.04	0.11 ± 0.02
2a	0.94 ± 0.04	0.97 ± 0.05	1.87 ± 0.14	0.08 ± 0.07
3	1.12 ± 0.03	4.59 ± 0.42	21.14 ± 2.14	0.14 ± 0.03
4	1.49 ± 0.44	4.07 ± 1.09	23.75 ± 3.61	0.21 ± 0.04

[a] The GABA concentration used for the control response was 2 μM. Each compound was evaluated on at least four different oocytes at the concentrations indicated, and the results reported are the ratio of currents measured in the presence/absence of added compound. Gating represents direct current gated by 10 μM compound in the absence of GABA, and this current is reported as the ratio of compound only current/2 μM GABA current. Error limits are calculated as standard error of the mean ($N \geqslant 4$). Methods were as reported previously.[27]

C-17 (Fig. 3) is not affected for the 17-carbonitrile group as it is for the 17-acetyl group.

Although it appears that the 17-acetyl substituent of steroid **2a** does not interact favorably with the $GABA_A$ receptor, it is not clear what conformation the 17β-acetyl substituent has when steroid **1a** is bound to the $GABA_A$ receptor. This substituent may not be in the minimum energy conformation found in calculations, solution and the solid state (Fig. 1). Indeed, in a different study that utilized steroids containing ring constrained C-17 substituents, evidence for the importance of placing a hydrogen bond acceptor group above C-17 and along the vector shown in Figure 3 for obtaining high activity was described. [24] Rotation of the 17β-acetyl group of steroid **1a** would allow this group to obtain such an orientation. A 17β-carbonitrile group also fulfills this structural requirement and introduction of a Δ^{16} double bond does not greatly displace this group to either side of the vector shown in Figure 3, although it does place the carbonitrile group in a vertical position that is intermediate between that of 17α and 17β substituents. Apparently, this intermediate positioning of the 17-carbonitrile group is of only minor significance since steroids **3** and **4** have similar biological activities. A somewhat larger loss of activity would not have been too surprising since steroids having a 17α-carbonitrile group are ineffective as modulators of $GABA_A$ receptor function.[25]

In conclusion, we have found that the potent GABAergic actions of steroids containing a 17β-carbonitrile instead of a 17β-acetyl group are not greatly affected by introduction of a Δ^{16} double bond. These results, obtained with analogues that are more similar to alphaxalone and Δ^{16}-alphaxalone than those studied previously, support and refine the earlier hypothesis which proposed that the loss of activity for Δ^{16}-alphaxalone is likely due to the negative consequences that the Δ^{16} double bond has on the positioning of the 17-acetyl group, not to conformational effects on the steroid D-ring.[20]

Experimental section. *General methods*. Solvents were either used as purchased or dried and purified by standard methodology. Extraction solvents were dried with anhydrous Na_2SO_4 and after filtration, removed on a rotary evaporator. Flash chromatography was performed using silica gel (32–63 μm) purchased from Scientific Adsorbents (Atlanta, GA). Melting points were determined on a Kofler micro hot stage and are uncorrected. FT-IR spectra were recorded as films on a NaCl plate. NMR spectra were recorded in $CDCl_3$ at ambient temperature at 300 MHz (^{1}H) or 74 MHz (^{13}C). Purity was determined by TLC on 250 μm thick Uniplates™ from Analtech (Newark, DE). All pure compounds (purity >95%) gave a single spot on TLC. Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ). Steroids **1**, **5** and **9** were purchased from Steraloids (Newport, RI).

(3α,5α)-3-Hydroxypregn-16-ene-11,17-dione (**2a**). Steroid **9** (75 mg, 0.23 mmol) in anhydrous THF (0.5 mL) was added to a stirred solution of DEAD (0.15 mL, 0.34 mmol, 40% in toluene) and at

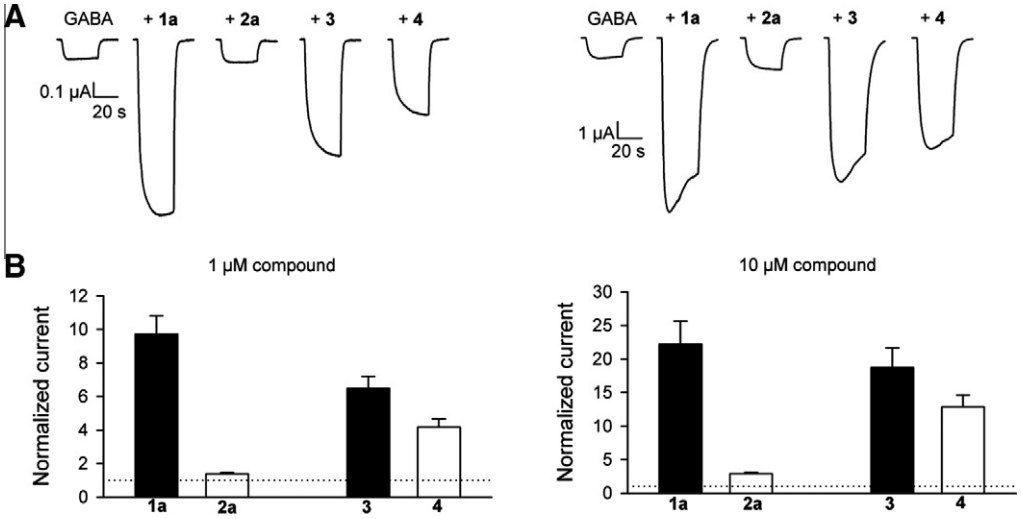


Figure 2. Direct comparison of the ability of steroids **1a**, **2a**, **3** and **4** to modulate $GABA_A$ receptor-mediated chloride currents at compound concentrations of 1 and 10 μM. The compounds were evaluated on the same oocytes expressing recombinant rat $\alpha_1\beta_2\gamma_{2L}$ receptors. (A) Sample currents from an oocyte clamped to −70 mV and exposed transiently to GABA alone and then GABA plus each of the steroids. (B) Summary of effects of steroids on GABA responses. The current mediated by GABA alone is set to one as the control value and indicated by the dotted line. Potentiation is calculated as R2/R1, where R2 is the response in the presence of a steroid and R1 is the response to GABA alone. Error limits are calculated as standard error of the mean for $n \geqslant 4$. Significance levels are as follows: steroids **1a** and **2a** at 1 and at 10 μM, $P < 0.005$; steroids **3** and **4** at 1 and at 10 μM, $P < 0.005$; steroids **1a** and **3** at 1 μM, $P < 0.05$ and at 10 μM, P not significant; steroids **2a** and **4**, at 1 and at 10 μM, $P < 0.005$.

Table 3
Effects of steroids **1a**, **2a**, **3** and **4** on tadpole righting and swimming reflexes

Compound	Tadpole LRR[a] EC_{50} (μM)	Tadpole LRR n_{Hill}	Tadpole LSR[a] EC_{50} (μM)	Tadpole LSR n_{Hill}
1a	1.12 ± 0.14	−3.38 ± 2.28	5.48 ± 0.11	−33 ± 0[c]
2a	>10	–	None[b]	—
3	0.72 ± 0.11	−1.49 ± 0.26	5.48 ± 0.12	−33 ± 0[c]
4	1.04 ± 0.14	−1.77 ± 0.38	5.48 ± 0.12	−33 ± 0[c]

[a] Error limits are calculated as standard error of the mean ($N = 10$ animals at each of five or more different concentrations). Methods were as reported previously.[27]
[b] None is defined as no loss of behavioral reflex at the highest concentration tested (10 μM).
[c] No tadpole had LSR at 3 μM and all tadpoles had LSR at 10 μM. Steep slopes for LSR dose–response curves are commonly observed for anesthetics in this bioassay.

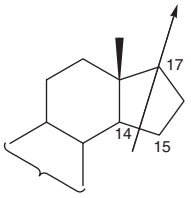


Figure 3. Partial structure of a steroid showing a vector that passes midway through the C-14, C-15 bond and C-17. A hydrogen bond acceptor group at C-17 oriented along this vector is predicted to have high activity. A significant displacement of the 17-acetyl carbonyl group to the left side of this vector occurs upon introduction of a Δ^{16} double bond into steroid **1a** (see Fig. 1), but a similar displacement does not occur for the 17-carbonitrile group of steroid **4**.

room temperature TFA (22 μL, 0.29 mmol) and then solid PPh_3 (90 mg, 0.34 mmol) were added. After stirring the reaction for 10 min $PhCO_2Na$ (50 mg, 0.35 mmol) was added and the reaction was stirred overnight. Since after this time a large amount of unreacted steroid **9** was detected by TLC, additional DEAD (60 μL, 0.14 mmol), PPh_3 (38 mg, 0.14 mmol) and $PhCO_2Na$ (22 mg, 0.15 mmol) were added. The reaction was stirred for another 20 h and volatiles were removed under reduced pressure. The product ester was separated from starting material by column chromatography on silica gel (40% EtOAc in hexanes). The inverted benzoate ester (50 mg) was then hydrolyzed by refluxing overnight with $NaHCO_3$ (60 mg, 0.71 mmol) in MeOH (10 mL). Volatiles were removed and the crude product was extracted with CH_2Cl_2. The combined organic layers were washed with water, then brine and dried. The crude product was further purified by column chromatography on silica gel (30–50% EtOAc in hexanes). Pure compound **2a** (24 mg, 32%) had: mp 253–54 °C; lit[26] mp 243–44 °C; $[\alpha]_D^{20}$ +71.2 (*c* 1.20, $CHCl_3$); IR 732, 919, 1000, 1368, 1434, 1666, 1703, 2857, 2923, 3407 cm^{-1}; ^1H NMR δ 0.82 (s, 3H), 1.02 (s, 3H), 2.27 (s, 3H), 3.02 (d, 1H, $J = 12.6$ Hz), 4.04 (br s, 1H), 6.75 (m, 1H); ^{13}C NMR δ 10.88, 17.27, 26.88, 27.76, 28.90, 30.87, 31.72, 32.39, 35.16, 35.29, 36.01, 39.07, 48.45, 53.99, 55.71, 65.71, 66.18, 144.40, 153.03, 195.93, 210.30.

(3α,5α,17β)-3-Hydroxy-11-oxoandrostane-17-carbonitrile (**3**). Steroid **4** (30 mg, 0.10 mmol) was dissolved in EtOAc (3 mL) and 5% Pd/C (10 mg, 0.08 mmol) was added. The hydrogenation flask was then evacuated and filled with H_2 gas three times. The compound was hydrogenated for 3 h at 30 psi. The catalyst was filtered through Celite® and washed with CH_2Cl_2. Product **3** was obtained in a quantitative yield (30 mg) and had: mp 175–76 °C; lit[21] mp 256–262 °C; $[\alpha]_D^{20}$ +71.5 (*c* 1.24, $CHCl_3$); IR 732, 917, 1044, 1166, 1447, 1705, 2238, 2859, 2924, 3491 cm^{-1}; ^1H NMR δ 0.88 (s, 3H), 1.01 (s, 3H), 2.03–2.33 (m, 3H), 2.46–2.58 (m, 2H), 4.05 (m, 1H); ^{13}C NMR δ 10.82, 15.12, 23.89, 26.89, 27.62, 28.79, 30.75, 32.54, 35.12, 35.73, 36.94, 38.78, 39.13, 47.26, 53.61, 54.66, 63.98, 66.07, 119.99, 208.34. Anal. Calcd for $C_{20}H_{29}NO_2$: C, 76.15; H, 9.27; N, 4.44. Found: C, 76.32; H, 9.06; N, 4.40.

(3α,5α)-3-Hydroxy-11-oxoandrost-16-ene-17-carbonitrile (**4**). Steroid **8** (43 mg, 0.12 mmol) was dissolved in CH_2Cl_2 (0.5 mL) and MeOH (2 mL). Concentrated HCl (0.5 mL) was then added and the reaction was stirred for 4 h at room temperature. The solvents were removed and CH_2Cl_2 was added to dissolve the residue. The CH_2Cl_2 was washed with Na_2CO_3 solution, brine and dried. The crude compound was purified by column chromatography (silica gel, 30–40% EtOAc in hexanes) to yield product **4** (35 mg, 93%) as a white solid: mp 174–76 °C; $[\alpha]_D^{20}$ +41.9 (*c* 1.30, $CHCl_3$); IR 732, 916, 1045, 1382, 1454, 1593, 1705, 2218, 2860, 2924, 3408 cm^{-1}; ^1H NMR δ 0.86 (s, 3H), 1.02 (s, 3H), 2.42–2.60 (m, 3H), 4.05 (br s, 1H), 6.70 (br s, 1H); ^{13}C NMR δ 10.87, 17.56, 27.59, 28.84, 30.81, 32.25, 32.36, 35.21, 35.40, 36.08, 38.96, 50.15, 52.96, 55.28,

65.78, 66.03, 114.83, 125.19, 147.82, 207.98. Anal. Calcd for $C_{20}H_{27}NO_2$: C, 76.64; H, 8.68; N, 4.47. Found: C, 76.82; H, 8.42; N, 4.47.

(3α,5α)-(3-Methyloxymethyl)oxy-androstane-11,17-dione (**6**). To a stirred solution of steroid **5** (100 mg, 0.33 mmol) in anhydrous CH_2Cl_2 (4 mL), diisopropylethyl amine (0.10 mL, 0.57 mmol) was added in a N_2 atmosphere followed by dropwise addition of chloromethyl methyl ether (0.05 mL, 0.66 mmol). The reaction was stirred overnight at room temperature. Volatiles were removed and the product was purified by column chromatography (silica gel, 10–15% EtOAc in hexanes). Product **6** was isolated in a quantitative yield (114 mg) as a white crystalline solid: mp 135–36 °C; $[\alpha]_D^{20}$ +108.5 (*c* 1.18, $CHCl_3$); IR 1046, 1455, 1710, 1745, 2926 cm^{-1}; ^{1}H NMR δ 0.82 (s, 3H), 1.04 (s, 3H), 3.36 (s, 3H), 3.82–3.83 (m, 1H), 4.65 (q, 2H, J = 9.4 Hz, J = 6.6 Hz); ^{13}C NMR δ 11.12, 14.54, 21.43, 25.86, 27.56, 29.61, 31.25, 31.37, 33.13, 35.61, 35.99, 39.52, 50.33, 50.58, 50.60, 55.18, 64.81, 71.17, 94.45, 209.04, 217.55. Anal. Calcd for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.50; H, 9.06.

(3α,5α)-3-(Methyloxymethyl)oxy-17-(trifluoromethanesulfonyl-oxy)-androst-16-en-11-one (**7**). A solution of steroid **6** (100 mg, 0.29 mmol) in anhydrous THF (1.4 mL) was cooled to −78 °C and KHMDS (0.6 mL, 0.5 M in toluene, 0.3 mmol) was added dropwise. After stirring the reaction for 15 min, a solution of *N*-phenyltrifluoromethane sulfonimide (125 mg, 0.35 mmol) in THF (2.0 mL) was added dropwise and stirring at −78 °C was continued for 3 h. The reaction was quenched by adding satd NH_4Cl solution (1 mL). The product was extracted with hexanes and the combined extracts were washed with brine and dried. After solvent removal, the product was further purified by column chromatography (silica gel, 1–2.5% EtOAc in hexanes). Product **7** (91 mg, 91% based on recovery of steroid **6**, 27 mg) retained a trace of an aromatic impurity that could not be removed by column chromatography. Attempts to remove it by recrystallization also were not successful. Oily product **7** containing the trace impurity had: $[\alpha]_D^{20}$ +39.5 (*c* 1.24, $CHCl_3$); IR 1044, 1143, 1213, 1423, 1632, 1708, 2863, 2926 cm^{-1}; ^{1}H NMR δ 0.89 (s, 3H), 1.02 (s, 3H), 3.36 (s, 3H), 3.82–3.83 (m, 1H), 4.66 (q, 2H, J = 9.4 Hz, J = 6.9 Hz), 5.65–5.67 (m, 1H); ^{13}C NMR δ 11.08, 16.50, 25.92, 27.59, 28.09, 29.66, 31.31, 31.43, 33.26, 35.27, 35.87, 39.72, 46.99, 51.90, 53.60, 55.11, 66.01, 71.25, 94.49, 115.66, 118.48 (q, J_{CF} = 320.6 Hz), 156.13, 208.16.

(3α,5α)-3-(Methyloxymethyl)oxy-11-oxoandrost-16-ene-17-carbonitrile (**8**). Compound **7** (1.39 g, 2.89 mmol) was placed in a 100 mL round bottom flask and the flask was evacuated and filled with N_2. NaCN (193 mg, 1.36 mmol), tetrakis(triphenylphosphine) Pd(0) (239 mg, 0.21 mmol), and CuI (55 mg, 0.29 mmol) were added, followed by addition of anhydrous acetonitrile (40 mL). The yellowish solution was then heated to reflux for 3 h. The flask was cooled and the reaction mixture was filtered through a Celite® pad and washed with EtOAc. After solvent removal, the residue was redissolved in EtOAc and the EtOAc was washed with water, brine and dried. After solvent removal, the crude product was purified by column chromatography (silica gel, 5–10% EtOAc in hexanes) and purified product **8** (92 mg, 89%) was isolated as a white solid: mp 165–67 °C; $[\alpha]_D^{20}$ +47.3 (*c* 1.19, $CHCl_3$); IR 910, 1045, 1094, 1144, 1366, 1380, 1456, 1587, 1714, 2216, 2871, 2949 cm^{-1}; ^{1}H NMR δ 0.85 (s, 3H), 1.02 (s, 3H), 2.42–2.60 (m, 3H), 3.36 (s, 3H), 3.80–3.85 (m, 1H), 4.65 (q, 2H, J = 9.7 Hz, J = 6.9 Hz), 6.69 (q, 1H, J = 1.7 Hz); ^{13}C NMR δ 11.09, 17.58, 25.94, 27.67, 31.46, 32.24, 32.38, 33.26, 35.40, 35.87, 39.66, 50.18, 52.97, 55.14, 55.36, 65.81, 71.20, 94.52, 114.86, 125.24, 147.81, 207.93. Anal. Calcd for $C_{22}H_{31}NO_3$: C, 73.91; H, 8.74; N, 3.92. Found: C, 74.12; H, 8.53; N, 3.88.

[^{35}S]-TBPS binding methods. The methods used were as described previously.[27]

Xenopus oocyte electrophysiological methods. Receptor expression and whole-cell recordings were carried out as described previously.[27]

Tadpole behavioral methods. The methods used were as described previously.[27]

Crystal structure methods. The methods used and structures obtained have been deposited at The Cambridge Crystallographic Data Centre (**1a**, CCDC 792719; **2a**, CCDC 792720).

Acknowledgments

This work was supported by NIH Grant GM47969 (D.F.C., A.S.E., C.F.Z.) and the Bantly Foundation. X-ray crystal structures were made possible by NSF Shared Instrument Grant No. CHE-042097.

References and notes

1. Gyermek, L.; Soyka, L. F. *Anesthesiology* **1975**, *42*, 331.
2. Phillipps, G. H. *J. Steroid Biochem.* **1975**, *6*, 607.
3. Atkinson, R. M.; Davis, B.; Pratt, M. A.; Sharpe, H. M.; Tomich, E. G. *J. Med. Chem.* **1965**, *8*, 426.
4. Phillipps, G. H. In *Molecular Mechanisms of General Anaesthesia*; Halsey, M. J., Millar, R. A., Sutton, J. A., Eds.; Churchill Livingstone: New York, 1974; pp 32–47.
5. Lawrence, D. K.; Gill, E. W. *Mol. Pharmacol.* **1975**, *11*, 280.
6. Lee, A. G. *Biochem. Pharmacol.* **1979**, *28*, 91.
7. Makriyannis, A.; Fesik, S. W. *J. Neurosci. Res.* **1980**, *5*, 25.
8. Makriyannis, A.; Fesik, S. *J. Med. Chem.* **1983**, *26*, 463.
9. O'Leary, T. J.; Ross, P. D.; Levin, I. W. *Biochemistry* **1984**, *23*, 4636.
10. Fesik, S. W.; Makriyannis, A. *Mol. Pharmacol.* **1985**, *27*, 624.
11. Makriyannis, A.; Siminovitch, D. J.; Das Gupta, S. K.; Griffin, R. G. *Biochim. Biophys. Acta* **1986**, *859*, 49.
12. Makriyannis, A.; Yang, D. P.; Mavromoustakos, T. *Ciba Found. Symp.* **1990**, *153*, 172. discussion 185.
13. Makriyannis, A.; DiMeglio, C. M.; Fesik, S. W. *J. Med. Chem.* **1991**, *34*, 1700.
14. Ueda, I.; Tatara, T.; Chiou, J. S.; Krishna, P. R.; Kamaya, H. *Anesth. Analg.* **1994**, *78*, 718.
15. Mavromoustakos, T.; Yang, D. P.; Makriyannis, A. *Biochim. Biophys. Acta* **1994**, *1194*, 69.
16. Mavromoustakos, T.; Yang, D. P.; Makriyannis, A. *Biochim. Biophys. Acta* **1995**, *1239*, 257.
17. Mavromoustakos, T.; Theodoropoulou, E.; Yang, D. P. *Biochim. Biophys. Acta* **1997**, *1328*, 65.
18. Mavromoustakos, T. *Epitheorese Klin. Farmakol. Farmakokinet. Int. Ed.* **1998**, *12*, 15.
19. Tatara, T.; Ueda, I. *Progr. Anesth. Mech.* **2000**, *6*, 603.
20. Bolger, M. B.; Wieland, S.; Hawkinson, J. E.; Xia, H.; Upasani, R.; Lan, N. C. *Pharm. Res.* **1996**, *13*, 1488.
21. Phillipps, G. H.; Lawrence, R.; Newall, C. E. Ger. Offen. DE 2162595, 1972; *Chem. Abstr.* **1972**, *77*, 114685.
22. Mitsunobu, O. *Synthesis* **1981**, 1.
23. Covey, D. F.; Nathan, D.; Kalkbrenner, M.; Nilsson, K. R.; Hu, Y.; Zorumski, C. F.; Evers, A. S. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 1009.
24. Anderson, A.; Boyd, A. C.; Clark, J. K.; Fielding, L.; Gemmell, D. K.; Hamilton, N. M.; Maidment, M. S.; May, V.; McGuire, R.; McPhail, P.; Sansbury, F. H.; Sundaram, H.; Taylor, R. *J. Med. Chem.* **2000**, *43*, 4118.
25. Purdy, R. H.; Morrow, A. L.; Blinn, J. R.; Paul, S. M. *J. Med. Chem.* **1990**, *33*, 1572.
26. Cook, M. C.; Lawrence, R.; Phillipps, G. H.; Hunter, A. C.; Newall, C. E.; Stephenson, L.; Weir, N. G. Ger. Offen. DE 2162555, 1972; *Chem. Abstr.* **1972**, *77*, 102039.
27. Jiang, X.; Manion, B. D.; Benz, A.; Rath, N. P.; Evers, A. S.; Zorumski, C. F.; Mennerick, S.; Covey, D. F. *J. Med. Chem.* **2003**, *46*, 5334.