Synthesis of the neuroactive steroid antagonist 17-phenyl-5α-androst-16-en-3α-ol by a palladium-catalysed coupling reaction

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The neurosteroid antagonist, 3α,5α-17-phenylandrost-16-en-3-ol, which selectively antagonises the GABA-modulatory and GABA-mimetic effects of 3α,5α-tetrahydroprogesterone and related 5α-pregnanes, was synthesised by a new route using palladium-catalysed coupling reaction as a key step. Commercially available 3α-hydroxy-5α-androstan-17-one gave the desired product in 4 steps with an overall yield of 79.5%.

Keywords: neurosteroid antagonist, palladium-catalysed coupling reaction, vinyl triflate, 3α-hydroxy-5α-androstan-17-one, 17-phenyl-5α-androst-16-en-3α-ol

Neurosteroids are an important class of compounds[1] which possess hypnotic,[2] anticonvulsant,[3-5] anaesthetic,[6-8] or anxiolytic[9-11] activities *in vivo*. Early structure–activity relationship (SAR) studies showed that these steroidal positive allosteric $GABA_A$ receptor modulators must possess a 3α-hydroxyl group on ring A and a keto group at C20 in the 17β-acetyl side chain, as a hydrogen bond donor group at A ring and a hydrogen bond acceptor group at the steroid C-17 position. However, the optimal location in three-dimensional space for these hydrogen bonding groups has not been determined. Many important questions remain unanswered regarding steroid actions at the $GABA_A$ receptors. Despite concerted efforts, a steroid binding site on the $GABA_A$ receptor has not been identified. The known GABA-potentiating steroids form two distinct structural classes with either a 5α- or a 5β-hydrogen[12] (such as 3α-hydroxy-5α-pregnan-20-one (**1**) and 3α-hydroxy-5β-pregnan-20-one (**2**)). Although both classes produce $GABA_A$ responses with similar potency and efficacy, whether the two classes act at the same site or by the same mechanisms is unclear. Recently, the concept of a specific steroid binding site was given further credence by the development of a neurosteroid antagonist, 17-phenyl-5α-androst-16-en-3α-ol[13](**3**) (Scheme 1). This compound selectively antagonises the GABA-modulatory and GABA-mimetic effects of 3α,5α-tetrahydroprogesterone and related 5α-pregnanes.[13] Unexpectedly, this compound had little effect on the GABA-enhancing actions of 3α,5β-THPROG and related 5β-pregnanes, which indicates that these steroids might use distinct transduction mechanisms or binding sites.[13] This result presents the first evidence of a neurosteroid 'antagonist', which antagonises both the GABA-enhancing and anaesthetic actions of the 5α-pregnanes. This compound had no effect on GABA-evoked responses *per se*, or on the GABAmodulatory actions of barbiturates, benzodiazepines or 5β-pregnane steroids. These results further strengthen the concept of a neurosteroid binding site on the receptor protein.[14] Thus, a significant effort has been made to develop synthetic routes to the neurosteroid antagonist, 17-phenyl-5α-androst-16-en-3α-ol. Although a route to 17-phenyl-5α-androst-16-en-3α-ol (**3**) has been published,[13] there remains a need for a more efficient and practical route for its synthesis from a commercially available 17-ketosteroid precursor. Herein, we report a new synthesis of (**3**).

A significant drawback to the published route[13] to 17-phenyl-5α-androst-16-en-3α-ol, which proceeds via phenyllithium addition to 17-ketosteroid following dehydration, has been the low yield obtained for the phenyllithium addition reaction. Recent studies have shown that optically active vinyl triflates can easily be transformed by palladium-catalysed coupling reactions.[15] Among those reactions, an attractive method to make the substituted styrenes used the palladium-catalysed coupling of vinyl triflates and Grignard reagents, organozinc reagents or trimethylphenylsilicon. In this paper, we use the palladium-catalysed coupling to develop a new route. The route utilised commercially available 3α-hydroxy-5α-androstan-17-one as a starting material to give the desired product in 4 steps with an overall yield of 79.5%.

Results and discussions

Firstly the hydroxyl group of starting material 4, was reacted with chloromethyl methyl ether to give the MOM derivative **5** in 95% yield (Scheme 1). The MOM protecting group was chosen since it is stable to the reagents and the basic conditions used in next steps of Scheme 1, and it is readily removed during the final deprotection. The reaction of compound **5** with $PhN(Tf)_2$ and KHMDS at –78°C gave triflate **6** (97%). Our studies also utilised Tf_2O and Et_3N at –20°C as reaction conditions for triflating of compound **5**. Under the optimised conditions, compound **5** was converted over 8 h to the desired triflate **6** (45% yield). Although Tf_2O and Et_3N are readily available and very cheap, compare to $PhN(Tf)_2$ and KHMDS, they gave a lower yield of triflate **6** than that obtained using $PhN(Tf)_2$ and KHMDS.

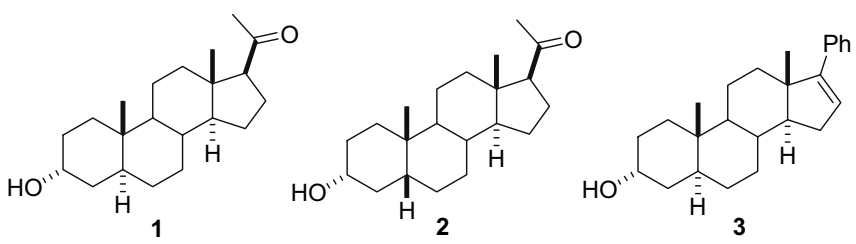

Fig. 1 Structures of steroid modulators of $GABA_A$ receptor function: 3α-hydroxy-5α-pregnan-20-one (**1**) and 3α-hydroxy-5β-pregnan-20-one (**2**); Structure of neurosteroid antagonist, 17-phenyl-5α-androst-16-en-3α-ol (**3**).

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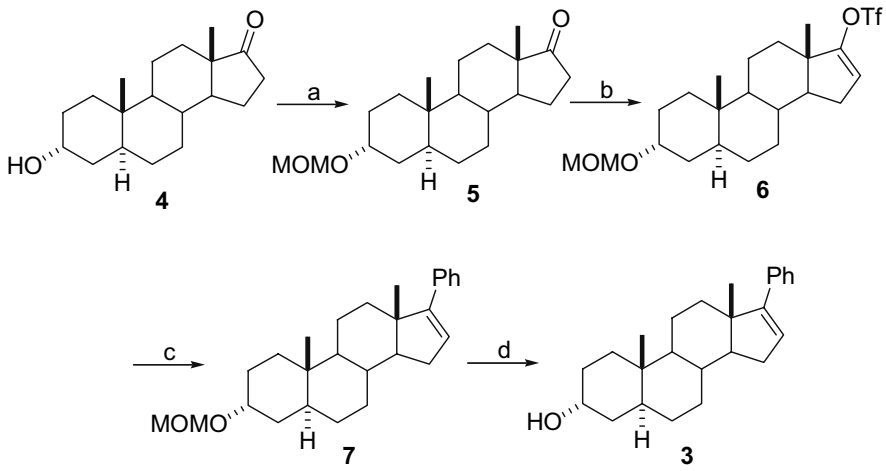

Scheme 1 Reaction conditions: a. MOMCl, diisopropylethylamine, DMAP, DCM, r.t. 2 h; b, $PhN(Tf)_2$, KHMDS (0.5 M in toluene), THF, –78°C, 2 h, 97%; c, $Pd(PPh_3)_4$, Ph MgBr (3 M in Et_2O), THF, 25–50°C, 12 h, 88%; d, 5% HCl, THF, room temperature, 12 h, 98%.

Palladium-promoted cross-coupling reactions have been successfully employed in Csp^2–Csp^2 bond formation and therefore we decided to explore palladium catalysed C–C bond formation methods to achieve an efficient synthesis of target compound **7**. In order to identify a suitable catalyst and standardise the reaction conditions the reaction of triflate **6** with phenylboronic acid,[16-20] tributylphenylstannane, phenylmagnesium bromide[21] or phenylzinc bromide[15] was studied with a variety of Pd catalysts under various experimental conditions. The choice of Pd catalysts was guided by the availability of compounds used in more conventional cross-coupling reactions, such as $PdCl_2$, $Pd(OAc)_2$, $Pd[(C_6H_5)_3P]_2Cl_2$ and $Pd[(C_6H_5)_3P]_4$. The results were summarised in Table 1. As evident from the results, the $Pd[(C_6H_5)_3P]_4$/Ph MgBr/THF system was found to produce the best results in terms of reaction time and yield. Long reaction times were not necessary.

After the successful optimisation of the preparation of compound **7**, acid-catalysed deprotection of the 3-MOM group of compound **7** gave compound **3** (98%).

In conclusion, we have developed an efficient method for the preparation of the neurosteroid antagonist: 17-phenyl-5α-androst-16-en-3α-ol using a palladium-catalysed coupling of a vinyl triflate and Grignard reagent and starting from commercially available 3α-hydroxy-5α-androstan-17-one.

Experimental

All melting points were determined on a Yanaco melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet FT-IR 5DX spectrometer as KBr pellets. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker ACF-300 spectrometer with TMS as internal reference. The *J* values are given in Hertz. Optical rotations were determined on a Perkin-Elmer 343 polarimeter. The elemental analyses were performed on a Perkin-Elmer 240C instrument.

3α-Methoxymethoxy-5α-androstan-17-one (**5**): To a mixture of (3α, 5α)-3-hydroxyandrostan-17-one (580 mg, 2.0 mmol), diisopropylethylamine (388 mg, 3.0 mmol) and a catalytic amount of 4-N-dimethylaminopyridine (25 mg, 0.2 mmol) in dried DCM (30 ml) was added chloromethyl methyl ether (241 mg, 3.0 mmol) at room temperature the resultant mixture was stirred at the same temperature for 2 h. The mixture was poured into ice water (15 ml, containing 5% sodium carbonate). The resulting mixture was extracted with DCM (2 × 15 ml) and the organic phase was washed with water and brine, and dried over Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography (silica gel, EtOAc/hexanes, 1/10) to give compound **5** (635 mg, 95%); compound **5** was obtained as a white crystals m.p. 110–112°C (hexane); $[\alpha]^{20}_D$ = + 79.25°(c = 0.480, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): 4.65 (m, 2H), 3.84 (m, 1H), 3.37 (s, 3H), 2.43 (m, 1H), 2.10 (m, 1H), 0.86 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 221.06, 94.52, 71.46, 55.05, 54.40, 51.48, 47.71, 39.68, 35.96, 35.73, 35.00, 33.57, 32.74, 31.54, 30.75, 28.25, 26.24, 21.66, 19.97, 13.75, 11.30; IR (film): 1746, 1043, 1033; Anal. Calcd for $C_{21}H_{34}O_3$: C, 75.4; H, 10.25; Found C, 75.6; H, 10.1%.

Table 1 The reaction conditions for the preparation of compound **7**

Entry	Reaction conditions	Yield/%
1	Ph MgBr/$Pd(OAc)_2$/PPh_3/^{i}PrNEt$_2$/THF/50°C/12 h	70
2	$PhB(OH)_2$/$Pd[(C_6H_5)_3P]_4$/tulene/reflux/12 h	84
3	$PhSn(Bu)_3$/$Pd[(C_6H_5)_3P]_4$/tulene/reflux/12 h	59
4	Ph MgBr/$PdCl_2$/PPh_3/^{i}PrNEt$_2$/THF/50°C/12 h	46
5	Ph MgBr/$Pd[(C_6H_5)_3P]_2Cl_2$/^{i}PrNEt$_2$/THF/50°C/12 h	58
6	Ph MgBr/$Pd[(C_6H_5)_3P]_4$/THF/50°C/12 h	88
7	Ph MgBr/$Pd[(C_6H_5)_3P]_4$/THF/50°C/8 h	79
8	Ph MgBr/$Pd[(C_6H_5)_3P]_4$/THF/50°C/16 h	87

3α-Methoxymethoxy-5α-androst-16-en-17-yl trifluoromethanesulfonateone (**6**): To a mixture of compound **5** (167 mg, 0.5 mmol) and $PhN(Tf)_2$ (268 mg, 0.75 mmol) in dried THF (15 ml) was added KHMDS (0.5 M in toluene, 3 ml, 1.5 mmol) at –78°C and the resultant mixture was stirred at the same temperature for 2 h. The reaction was quenched by adding sat. NH_4Cl (aq), and the mixture was extracted with DCM (2 × 15 ml). The organic phase was washed with water and brine, and dried over Na_2SO_4. Removal of solvent, the residue was purified by flash chromatography (silica gel, EtOAc/hexanes, 1/20) to give product **6** (226 mg, 97%); Compound **6** was obtained as a white solid; m.p. 48–50°C (hexanes); $[\alpha]^{20}_D$ = + 15.82° (c = 0.158, $CHCl_3$); ^1H NMR ($CDCl_3$) 5.56 (m, 1H), 4.66 (m, 2H), 3.83 (m, 1H), 3.37 (s, 3H), 2.20 (m, 1H), 1.95 (m, 1H), 0.96 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 159.56, 118.73 (q, *J* = 319 Hz), 114.51, 94.72, 71.65, 55.22, 54.89, 54.50, 45.01, 40.06, 36.22, 33.76, 33.62, 32.88, 32.73, 30.89, 28.62, 28.40, 26.43, 20.22, 15.38, 11.42; IR (film): 3081, 1628, 1423, 1142, 1050, 601 cm^{-1}; Anal. Calcd for $C_{22}H_{33}F_3O_5S$: C, 56.6; H, 7.1; Found C, 56.3; H, 7.0%.

3α-Methoxymethoxy-17-phenyl-5α-androst-16-ene (**7**): To a mixture of compound **6** (466 mg, 1.0 mmol) and $Pd(PPh_3)_4$ (46.2 mg, 0.04 mmol) in dried THF (20 ml) was added Ph MgBr (3 M in Et_2O, 0.5 ml, 1.5 mmol) at room temperature under nitrogen. The resulting mixture was stirred at 50°C under nitrogen for 12 h. The mixture was added to saturated $NaHCO_3$ (5.0 ml) and extracted with EtOAc (2 × 15 ml). The organic phase was washed with water and brine and dried over Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography (silica gel, EtOAc/hexanes, 1/10) to give product **7** (347 mg, 88%) as white crystals; m.p. 134–135°C (hexanes); $[\alpha]^{20}_D$ = + 27.58°(c = 0.994, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): 7.37–7.33 (m, 2H), 7.30–7.17 (m, 3H), 5.88 (m, 1H), 4.65 (m, 1H), 3.83 (m, 1H), 3.37 (s, 3H), 2.23–2.15 (m, 1H), 2.06–1.93 (m, 2H), 1.01 (s, 3H), 0.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 155.16, 137.60, 128.17(2C), 127.28, 126.85(2C), 126.76, 94.73, 71.81, 57.86, 55.25, 54.86, 47.60, 40.13, 36.23, 35.76, 34.27, 33.86, 32.89, 32.01, 31.66, 28.78, 26.55, 20.98, 16.93, 11.57; IR (film): 3032, 3080, 1602, 1568, 1493, 1444, 1041, 795; Anal. Calcd for $C_{27}H_{38}O_2$: C, 82.2; H, 9.7; Found C, 82.0; H, 9.6%.

17-Phenyl-5α-androst-16-en-3α-ol (**3**):[13] The mixture of compound **7** (198 mg, 0.5 mmol) and HCl (3 M, 0.5 ml, 1.5 mmol) in THF (10 ml) was stirred at room temperature overnight. The mixture was neutralised with sat. Na_2CO_3, and the resultant mixture was extracted with CH_2Cl_2 (2 × 15 ml). The organic phase was washed with water and brine, and dried over Na_2SO_4. The solvent was removed and the residue was purified by flash chromatography (silica gel, EtOAc/hexanes, 1/4) to give product **3** (172 mg, 98%) as white crystals; m.p. 175–176°C (EtOAc/hexanes) (174.5–175.5°C[13]); $[\alpha]^{20}_D$ = + 24.69°(c = 0.397, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): 7.38–7.21 (m, 5H), 5.89 (s, 1H), 4.05 (m, 1H), 2.22–2.18 (m, 1H), 2.05–1.94 (m, 2H), 1.02 (s, 3H), 0.84 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 155.13, 137.64, 128.26(2C), 127.41, 126.90(2C), 126.84, 66.79, 57.86, 54.89, 47.63, 39.57, 36.51, 36.13, 35.73, 34.27, 32.26, 32.07, 31.71, 29.24, 28.73, 20.99, 16.97, 11.38; IR (film): 3302, 1493, 1444, 1041, 1002, 795; Anal. Calcd for $C_{25}H_{34}O$: C, 85.7; H, 9.8; Found C, 85.8; H, 9.6%.

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References

1 B.O. Dubrovsky, *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, 2005, **29**, 169.
2 W.B. Mendelson, J.V. Martin, M. Perlis, R. Wagner, M.D. Majewska and S.M. Paul, *Psychopharmacology*, 1987, **93**, 226.
3 D. Belelli, M.B. Bolger and K.W. Gee, *Eur. J. Pharmacol.*, 1989, **166**, 325
4 T.G. Kokate, B.J. Svensson and M.A. Rogawski, *J. Pharmacol. Exp. Ther.,* 1994, **270**, 1223.
5 A.S. Veleiro, R.E. Rosenstein, C.O. Jaliffa, M.L. Grilli, F. Speroni and G. Burton, *Bioorg. Med. Chem. Lett*., 2003, **13**, 343.
6 S.H. Mellon and L.D. Griffin, *Trends Endocrinol. Metab.*, 2002, **13,** 35.
7 C.F. Zorumski, S. Mennerick, K.E. Isenberg and D.F. Covey, *Curr. Opin. Invest. Drugs (Thomson Sci.)* 2000, **1**, 360.
8 W.M. Mok, S. Herschkowitz and N.R. Krieger, *J. Neurochem.*, 1991, **57**, 1296.
9 D. Bitran, R.J. Hilvers and C.K. Kellogg, *Brain Res*. 1991, **561**, 157.
10 M.D. Brot, Y. Akwa, R.H. Purdy, G.F. Koob and K.T. Britton, *Eur. J. Pharmacol.,* 1997, **325**, 1.
11 R.J. Rodgers and N.J.T. Johnson, *Pharmacol. Biochem. Behav.*, 1998, **59**, 221.
12 D.F. Covey, A.S. Evers, S. Mennerick, C.F. Zorumski and R.H. Purdy, *Brain Res. Brain Res. Rev.*, 2001, 3791.
13 S. Mennerick, Y. He, X. Jiang, B.D. Manion, M. Wang, A. Shute, A. Benz, A.S. Evers, D.F. Covey and C.F. Zorumski, *Mol. Pharmacol.*, 2004, **65**, 1191.
14 D. Belelli and J.J. Lambert, *Nature Rev. Neurosci.*, 2005, **6**, 565.
15 L.F. Tietze, H. Ila and H.P. Bell, *Chem. Rev*., 2004, **104**, 3453.
16 N. Miyaura, K. Yamada and A. Suzuki, *Tetrahedron Lett.*, 1979, **20**, 3437.
17 N. Miyaura and A. Suzuki, *Chem. Commun*., 1979, 866.
18 N. Miyaura and A. Suzuki, *Chem. Rev.* 1995, **95**, 2457.
19 E.-I. Negishi (ed.), *Handbook of organopalladium chemistry for organic synthesis*, Wiley- Interscience: New York, 2002.
20 N. Miyaura (ed.), *Organometallic reactions: a practical guide*, Topics in Current Chemistry Series 219; Springer-Verlag: New York, 2002.
21 R.M. Suarez, D. Pena, A.J. Minnaard and B.L. Feringa, *Org. Biomol. Chem.* 2005, **3**, 729.