Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Synthesis of pharmaceutically relevant 17-α-amino steroids using an ω-transaminase

Nina Richter, Robert C. Simon, Wolfgang Kroutil, John M. Ward, Helen C. Hailes

Email: h.c.hailes@ucl.ac.uk

Content

I Experimental section

1 General Information

All starting materials were obtained from commercial suppliers and used as received unless otherwise stated. Solvents were dried and purified by conventional methods prior to use. Preparative chromatographic separations were performed by flash column chromatography on Merck silica gel 60 (0.063–0.200 mm). TLC analysis was carried out using pre-coated aluminium sheets (TLC Silica gel 60 F_{254}, Merck) with detection by UV (254 nm) and/or by staining with *p*-anisaldehyde or cerium molybdate solution. Optical rotation was measured at the temperature indicated on a Perkin–Elmer Polarimeter 341 in the solvent stated. GC-MS spectra were recorded with a Agilent 7890A GC-system, equipped with an Agilent 5975C mass selective detector and a HP-5 MS column (30 m × 0.25 mm × 0.25 μm; helium as carrier gas [flow = 0.55 mL/min]). ^{1}H and ^{13}C NMR spectra were recorded at 20 °C on a Bruker Avance 600 MHz NMR machine; chemical shifts are given in ppm relative to Me_4Si (^{1}H: Me_4Si = 0.0 ppm) or relative to the resonance of the solvent (^{1}H: CD_3OD = 3.31 ppm; ^{13}C: CD_3OD = 49.0 ppm). LC-MS data was acquired on a Waters Acquity uPLC SQD using HPLC grade water and acetonitrile (both with 0.1% formic acid) as the solvents.

2 Cloning, heterologous expression and preparation of the ω-transaminase

The previously reported *Arthrobacter* sp. variant (ArRMut11)[1] was designed as codon-optimised gene (DNA 2.0, U.S.A.), which was subsequently cloned into the expression vector pET29a (Invitrogen, Germany) using the restriction sites *Nde*I and *Xho*I. The ω-transaminase from *Vibrio fluvialis* (Vf-TAm)[2] was designed as a codon optimised synthetic gene (from Eurofins MWG Operon) and subcloned into pET29a, while the cloning of the *Chromobaterium violaceum* ω-transaminase (CV-TAm) has been previously reported.[3]

All ω-transaminases were expressed in *E. coli* BL21(DE3)pLysS in lysogeny broth medium containing kanamycin (20 μg mL^{-1}). Cultures were grown at 37 °C until an OD_{600} of 0.5-0.7 was reached. Enzyme expression was induced by addition of isopropyl-β-D-thiogalactopyranoside (0.5 mM), and the temperature was reduced to 25 °C. After 16-20 h cells were harvested by centrifugation and stored at -20 °C. To prepare cell free crude extract cells (20% v/w) were suspended in HEPES buffer (100 mM, pH 7.5) containing pyridoxal-5-phosphate (0.5 mM), and disrupted by ultra sonification (2 × 1 min, 40% output). The crude extract was cleared by centrifugation (20 min, 16.000 × g), and either directly used or freeze dried and stored at -20 °C.

3 Transaminase screening

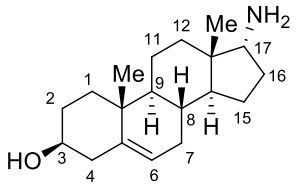
For the HPLC-based transaminase screening (*R*)- or (*S*)-α-methylbenzylamine (25 mM) and pyridoxal-5-phosphate (0.5 mM) were dissolved in HEPES buffer (100 mM, pH 8). 12.5 μL of *E. coli* crude extract containing the overexpressed ω-TAm were added to 212.5 μL of this solution. The reaction was started by the addition of 25 μL of substrate solved in DMSO (final concentration 10 mM, 10 vol%). After an incubation for 21 h at 30 °C and 180 rpm the reaction was stopped by the addition of 250 μL of acetonitrile containing 0.2% TFA. Denaturated protein was removed by centrifugation, and the supernatant was analysed by HPLC (Agilent) using a Discovery®Bio Wide Pore C18 column (Supelco, 25 x 4.6 mm, 10 μm beads) with UV detection at 254 nm. Concentrations of acetophenone were determined using a linear gradient: 30% - 60% B over 10 min (A = water, B = acetonitrile, both containing 0.1% TFA). The produced acetophenone eluted at a retention time of 8.6 min.

4 Isopropylamine transamination system

Transamination reactions using the isopropylamine donor-system were performed on 1 mL scale as follows: isopropylamine (0.2, 0.5 or 1 M final concentration) and pyridoxal-5'-phosphate (1 mM final concentration), were solved in distilled water and the pH was adjusted to 8 or 10. The corresponding steroid (10 mM) was dissolved in a co-colvent [1,2-dimethoxyethane (DME) or dimethylformamide (DMF)] and added to the solution (25-50 vol% final concentration). The reaction was started by the addition of 10 mg of freeze dried *E. coli* crude extract containing the overexpressed ω-TAm. The mixture was incubated at 45 °C and 800 rpm in a thermoshaker (Eppendorf, Germany). Samples were taken at different time points, and the reaction was stopped by addition of 10 vol% of $NaHCO_3$, and extracted with ethyl acetate (2 × 500 μL). The organic phase was analysed by gas chromatography (Agilent 7890 A system with a FID detector) equipped with an Agilent DB-1701 column (30 m, 0.25 mm, 0.25 μm) using the following temperature programmes for **4**: 180 °C, hold for 2 min, 5 °C min^{-1} to 205 °C, 40 °C min^{-1} to 280 °C, retention time: 8a-methyl-3,4,8,8a-tetrahydro-1,6(2*H*,7*H*)-naphtalenedione (**4**) 5.3 min. For **5-8**: 260 °C, hold for 10 min, 40 °C min^{-1} to 280 °C, hold for 2 min, retention times: 17α-amino-3β-hydroxyandrost-5-ene (**5a**) 5.1 min, *trans*-dehydroandrosterone (**5**) 6.1 min, 17α-amino-1,3,5(10)-estratrien-3-ol (**6a**) 7.6 min, estrone (**6**) 9.1 min, 17α-amino-5α-androstan-3β-ol (**7a**) 5.2 min, *trans*-androsterone (**7**) 6.4 min and testosterone (**8**) 9.2 min.

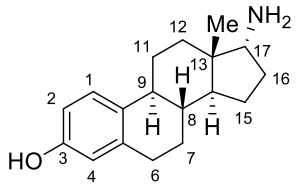
5 Biocatalytic production of 17-α-amino steroids

5.1 17α-amino-3β-hydroxyandrost-5-ene (5a)[3]



Pyridoxal-5'-phosphate (4.25 mg, 0.017 mmol) and isopropylamine (1.46 mL, 17 mmol) were dissolved in distilled water (9.6 mL) and the pH was adjusted to pH 10. *Trans*-dehydroandrosterone (**5**) (50 mg, 0.17 mmol) dissolved in DMF (5.95 mL, 35 vol%) was added. The reaction was started by the addition of 250 mg of freeze dried *E. coli* crude extract containing the overexpressed ω-TAm and was shaken at 45 °C for four days. The reaction was stopped by the addition of aq. 2 N HCl (5.1 mL) and extraction with EtOAc (2 x 10 mL). The remaining aqueous phase was treated with NaOH (10 N, 1 mL) and was again extracted with EtOAc (4 x 10 mL). The combined organic phases were dried over Na_2SO_4, and filtered and concentrated under reduced pressure to give a brownish solid which was purified by flash chromatography (eluent: CH_2Cl_2/MeOH/NEt_3 90:9:1). The amino-steroid **5a** was obtained as colourless powder (41.3 mg, 83%). Mp 192–195 °C (MeOH), lit. 191–193 °C (ether);[4] $[\alpha]^{20}_D$ –75.5 (*c* 1.0, MeOH), lit. $[\alpha]_D$ –93 ($CHCl_3$);[5] ^{1}H NMR (600 MHz; CD_3OD) δ_H 0.89 (3H, s, Me at C-13), 1.01–1.06 (1H, m), 1.06 (3H, s, Me at C-10), 1.08–1.14 (1H, td, *J* = 13.0 and 4.0 Hz) 1.28–1.38 (4H, m), 1.48–1.76 (7H, m), 1.80 (1H, m), 1.85–1.93 (2H, m), 2.07 (1H, m), 2.18–2.29 (2H, m, 4-H_2), 2.37 (1H, m, 16-H_a), 3.23 (1H, dd, *J* = 8.0 and 1.7 Hz, 17-H), 3.40 (1H, m, 3-H), 5.37 (1H, app. d, *J* = 5.1 Hz, 6-H); ^{13}C NMR (151 MHz; CD_3OD) δ_C 18.2 (Me at C-13), 19.8 (Me at C-10), 21.6, 26.0 (C-15), 29.3 (C-16), 32.2, 33.1, 33.2, 33.3, 37.7 (C_{quat}), 38.5, 42.9 (C-4), 44.5 (C_{quat}), 51.1, 51.2, 60.9 (C-17), 72.3 (C-3), 122.0 (C-6), 142-3 (C-5); IR (ATR-film)/cm^{-1} 1462, 1527, 1631, 2915, 3217; *m/z* [GC-MS (EI, 70 eV)] 289 (M^+, 2%), 274 (M^+-Me, 100), 56 ($C_3H_6N^+$, 32); HRMS-CI found MH^+ 290.2479, calcd. for $C_{19}H_{32}NO$ 290.2484.

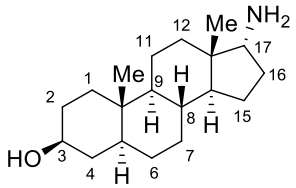
5.2 17α-amino-1,3,5(10)-estratrien-3-ol (6a)



Pyridoxal-5'-phosphate (5 mg, 0.02 mmol) and isopropylamine (1.72 mL, 20.1 mmol) were solved in distilled water (11.2 mL) and the pH was adjusted to pH = 10. Estrone (**6**) (50 mg, 0.18 mmol) in 7 mL of DMF (35 vol%) was added. The reaction was started by the addition of 500 mg of freeze dried *E.*

coli whole cells containing the overexpressed ω-TAm, and was shaken at 45 °C for three days. The reaction was stopped by the addition of aq. 2 N HCl (6 mL) and extraction with EtOAc (2 x 10 mL). The remaining aqueous phase was treated with NaOH (10 N, 1 mL) and was again extracted with EtOAc (4 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a brownish solid which was purified by flash chromatography (eluent: CH_2Cl_2/MeOH/NEt_3 90:9:1). The amino steroid **6a** was obtained as colourless amorphous solid (40.7 mg, 85%). Mp (decomp.) 220-230 °C, lit. (decomp.) 226-227 °C;[5] $[\alpha]^{25}_D$ 33.3 (*c* 1.0, MeOH); lit. $[\alpha]_D$ 50.9 (MeOH);[6] ^{1}H NMR (600 MHz; CD_3OD) δ_H 0.85 (3H, s, Me at C-13), 1.27–1.60 (9H, m), 1.73 (1H, m), 1.88-1.96 (2H, m), 2.20 (1H, m), 2.30–2.37 (1H, m), 2.38–2.42 (1H, m), 2.75–2.85 (2H, m, 6-H_2), 3.11 (1H, app. dd, *J* = 7.6 and 1.3 Hz, 17-H), 6.49 (1H, d, *J* = 2.7 Hz, 4-H), 6.55 (1H, dd, *J* = 8.5 and 2.7 Hz, 2-H), 7.09 (1H, d, *J* = 8.5 Hz, 1-H); ^{13}C NMR (151 MHz; CD_3OD) δ_C 18.9 (Me at C-13), 25.6, 27.4, 29.4, 30.70, 30.73 (C-6), 33.4, 40.7, 44.9, 45.4 (C_{quat}), 49.4, 61.2 (C-17), 113.8 (C-2), 116.1 (C-4), 127.2 (C-1), 132.1 (C-10), 138.7 (C-5), 156.1 (C-3); IR (ATR-film)/cm^{-1} 1452, 1497, 1585, 1609, 2857, 2924, 3230; m/z [GC-MS (EI, 70 eV)] 271 (M^+, 35%), 254 (M^+-Me, 23), 213 ($C_{15}H_{17}O^+$, 30), 56 ($C_3H_6N^+$, 100); HRMS-EI found M^+ 271.1941, calcd. for $C_{18}H_{25}NO$ 271.1936.

5.3 17α-amino-5α-androstan-3β-ol (7a)



Pyridoxal-5'-phosphate (5 mg, 0.02 mmol) and isopropylamine (1.72 mL, 20.1 mmol) were solved in distilled water (11.2 mL) and the pH was adjusted to pH = 10. *Trans*-androsterone (**7**) (50 mg, 0.17 mmol) in 7 mL of DMF (35 vol%) was added. The reaction was started by the addition of 500 mg of freeze dried *E. coli* whole cells containing the overexpressed ω-TAm, and shaken at 45 °C for three days. The reaction was stopped by the addition of aq. 2 N HCl (6 mL) and extraction with EtOAc (2 x 10 mL). The remaining aqueous phase was treated with NaOH (10 N, 1 mL) and was again extracted with EtOAc (4 x 10 mL). Combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a brownish solid which was purified by flash chromatography (eluent: CH_2Cl_2/MeOH/NEt_3 90:9:1). The amino steroid **7a** was obtained as colourless amorphous solid (43.7 mg, 89%). Mp (decomp.) 220–230 °C; $[\alpha]^{20}_D$ − 12.5 (*c* 0.8, MeOH); ^{1}H NMR (600 MHz; CD_3OD) δ_H 0.75 (1H, td, *J* = 12.6 and 4.2 Hz, 9-H), 0.85 (3H, s, Me at C-13), 0.86 (3H, s, Me at C-10), 0.99–1.07 (2H, m), 1.11–1.18 (1H, m), 1.26–1.56 (11H, m), 1.66 (1H, m), 1.69–1.80 (4H, m), 1.81–

1.86 (1H, m), 2.33 (1H, m, 16-H_a), 3.16 (1H, app. dd, *J* = 7.8 and 1.8 Hz, 17-H), 3.51 (1H, dddd, *J* = 11.2, 9.5, 6.5, 4.7 Hz, 3-H); ^{13}C-NMR (151 MHz; CD_3OD) δ_C 12.7 (Me at C-10), 18.5 (Me at C-13), 21.8, 25.9, 29.5, 29.8, 32.1, 33.4, 33.5, 36.7 (C_{quat}), 37.1, 38.3, 38.8, 44.9 (C_{quat}), 46.1, 50.8 (C-14), 55.3 (C-9), 61.0 (C-17), 71.8 (C-3); IR (ATR-film)/cm^{-1} 1450, 1515, 1629, 2854, 2924, 3281; *m/z* [GC-MS (EI, 70 eV)] 291 (M^+, 15%), 276 (M^+-Me, 5), 56 ($C_3H_6N^+$, 100); HRMS-EI found M^+ 291.2573, calcd. for $C_{19}H_{33}NO$ 291.2562.

II Supplementary figures and tables

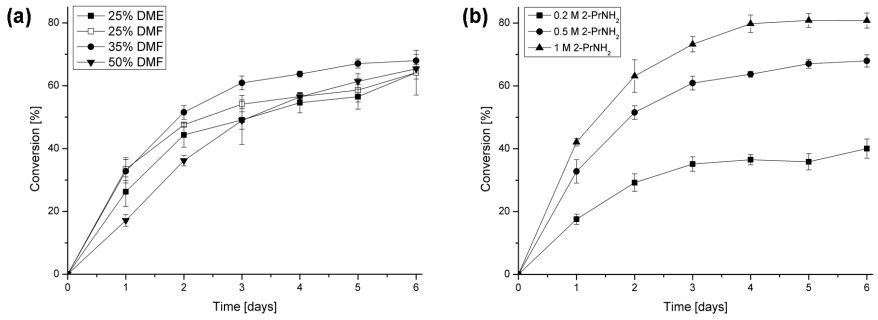
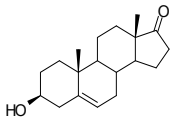
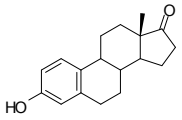
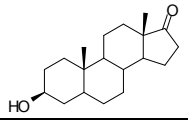


Fig. S1 Optimisation of the ω-TA reaction for the synthesis of **5a** with respect to co-solvent **(a)** and molar excess of 2-propylamine **(b)** used. Reactions were performed as described in section 4. In the co-solvent study **S1(a)** 0.5 M 2-propylamine and the co-solvent concentrations indicated were used. In the concentration of 2-propylamine study **S1(b)** the effect of different amounts of 2-propylamine were tested using a co-solvent concentration of 35% DMF.

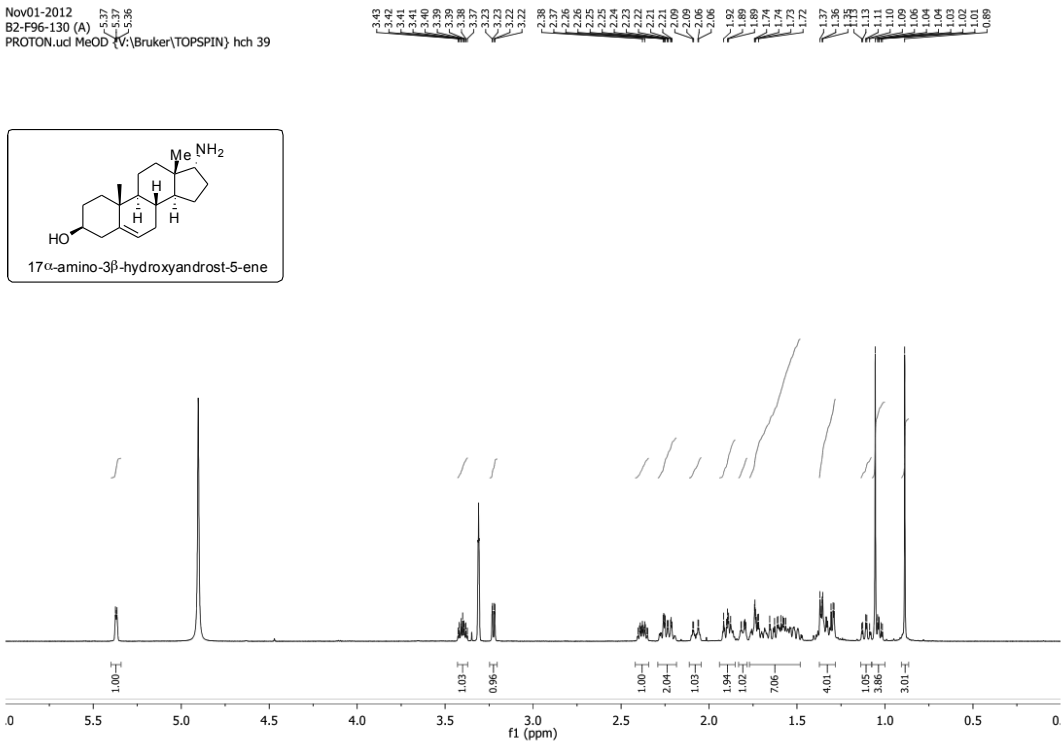
Table S1 ω-TA-catalysed asymmetric amination of steroids using the optimised reaction conditions

entry	substrate	time [days]	Product	conversion [%]
1		6	**5a**	81
2		6	**6a**	68
3		5	**7a**	71

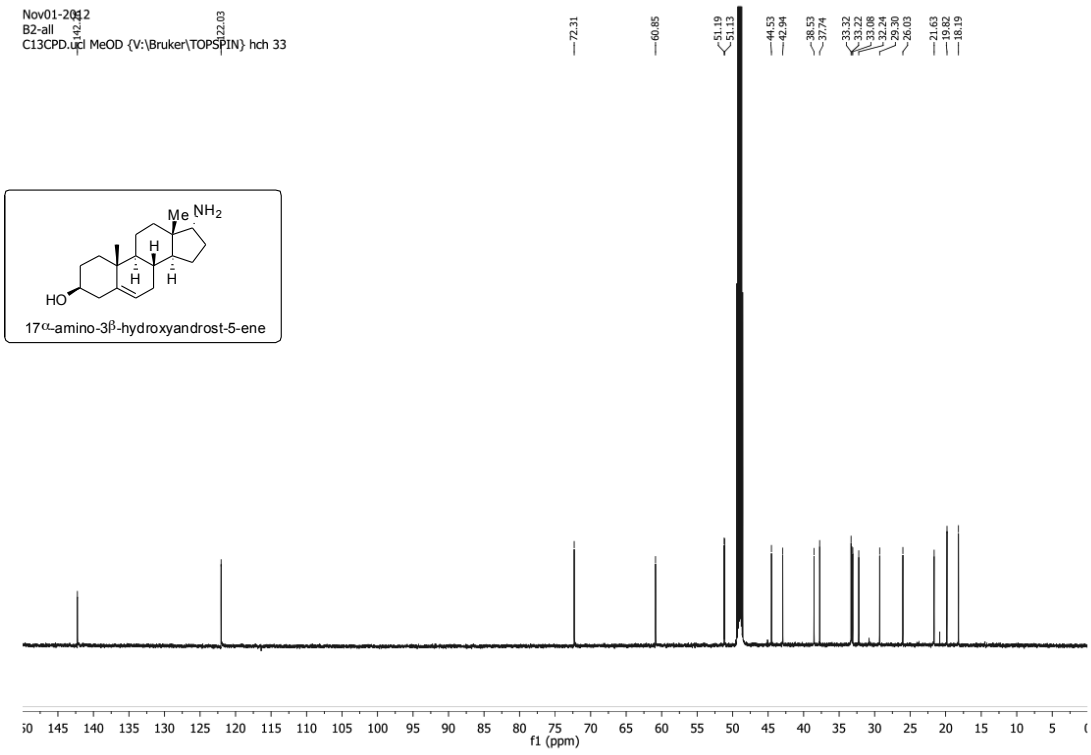
The reactions were performed as follows: 10 mM of substrate dissolved in DMF (final concentration 35 vol%); 1 mM of PLP, 1 M of 2-propylamine, 10 mg/mL TAm, and distilled water at a pH of 10. For further details see section 4.

III NMR Spectra

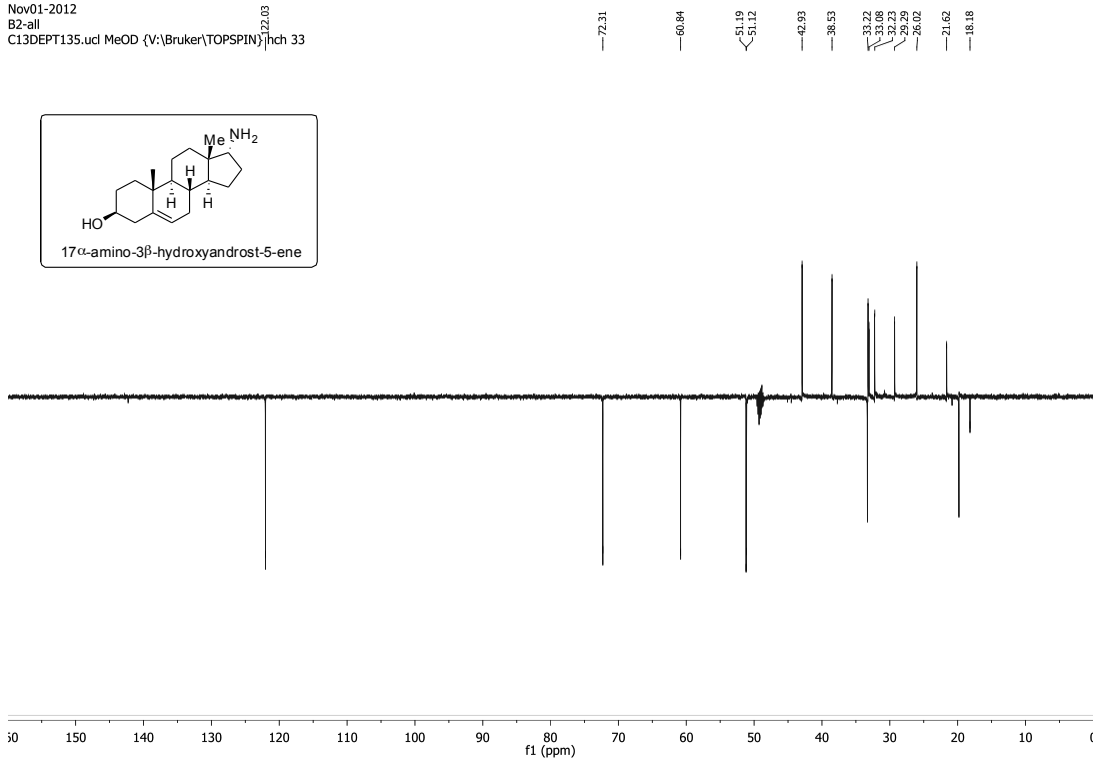
6.1.1 ^{1}H-NMR of 17α-amino-3β-hydroxyandrost-5-ene



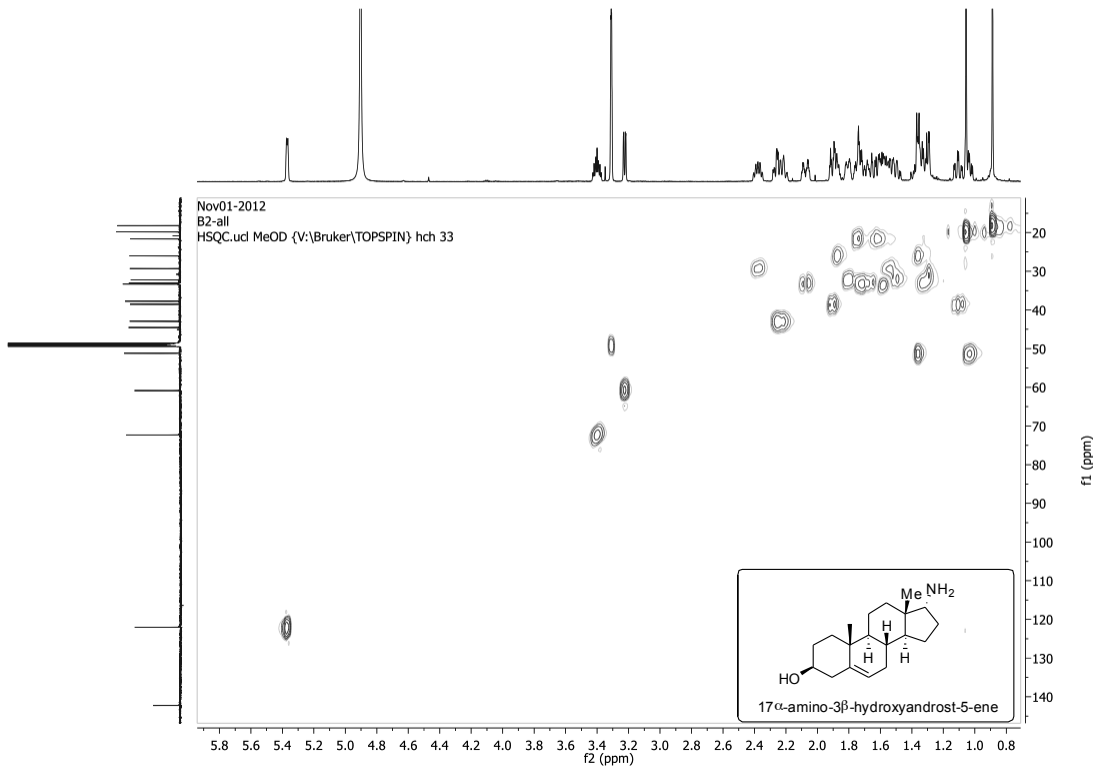
6.1.2 ^{13}C-NMR of 17α-amino-3β-hydroxyandrost-5-ene



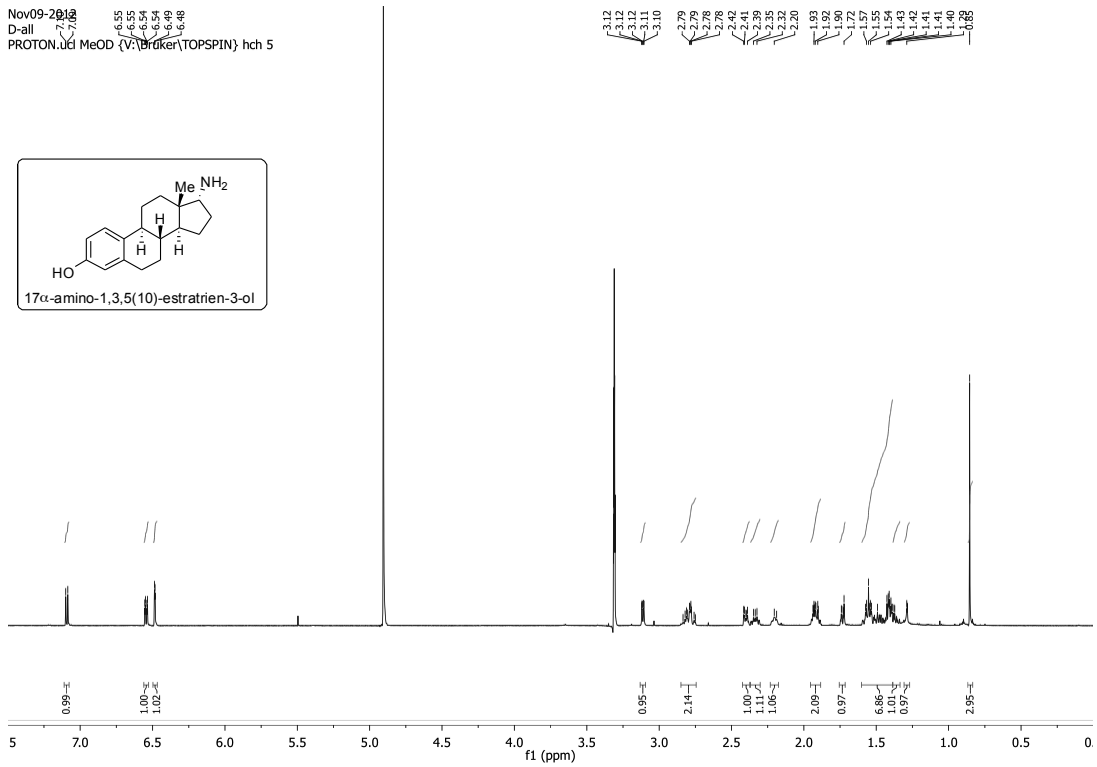
6.1.3 Dept-135 of 17α-amino-3β-hydroxyandrost-5-ene



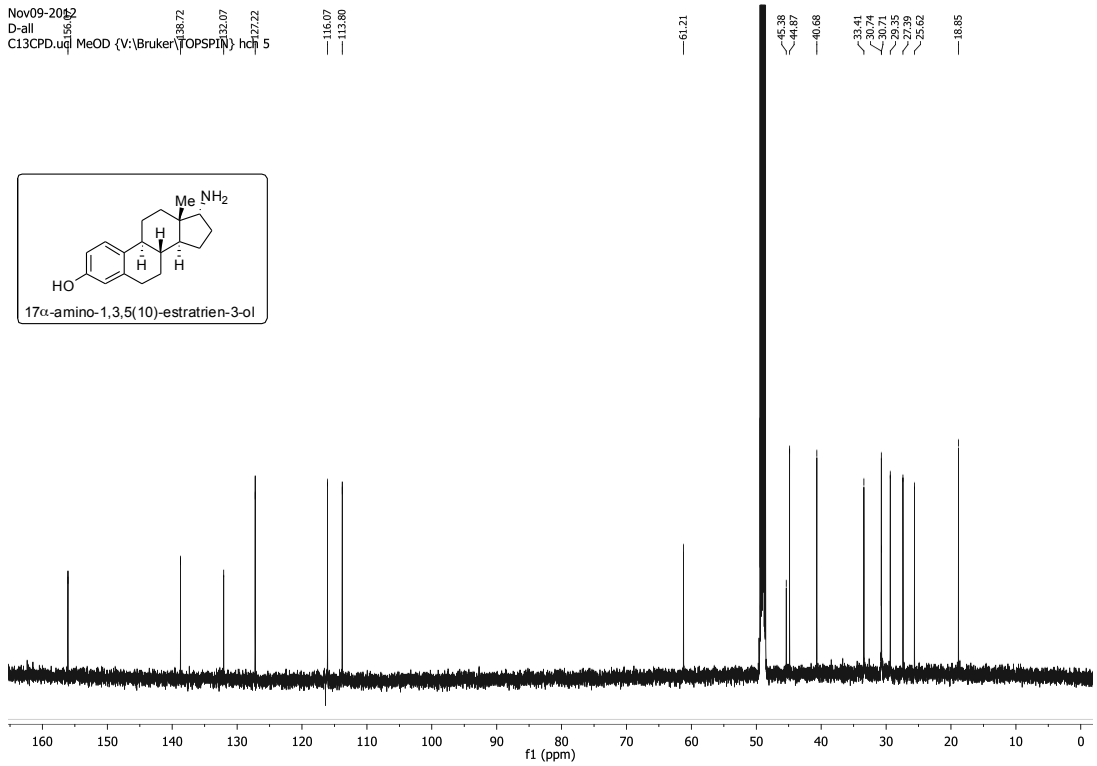
6.1.4 HSQC of 17α-amino-3β-hydroxyandrost-5-ene



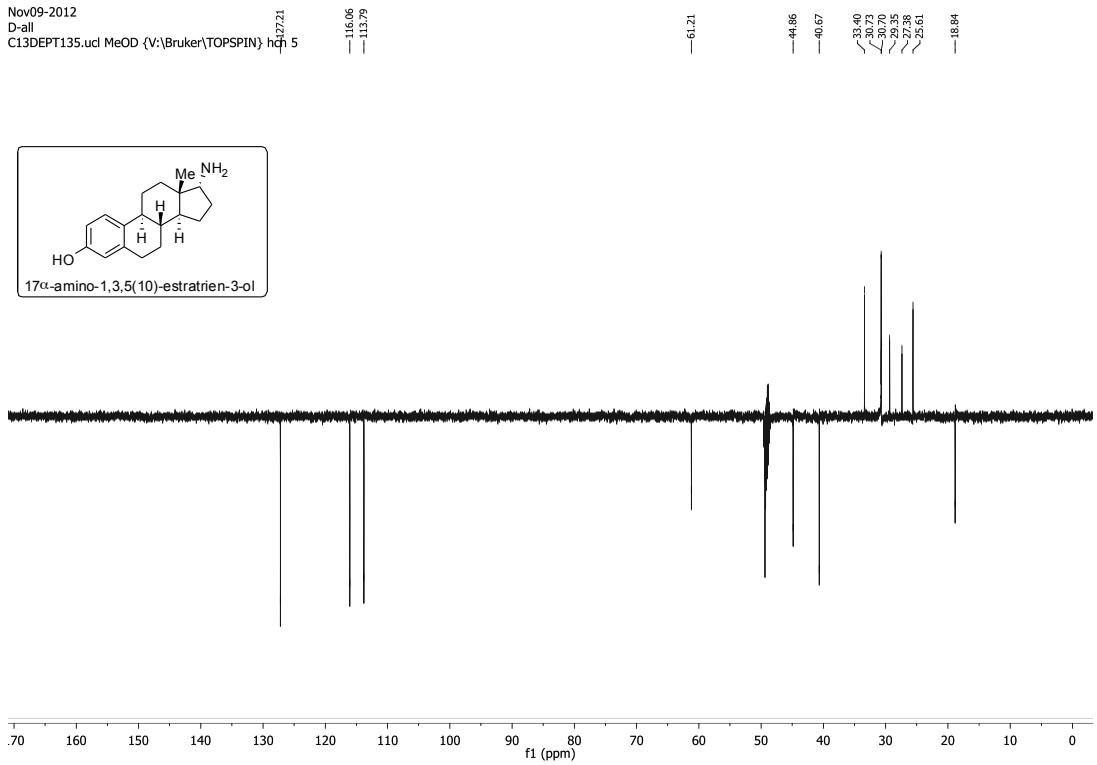
6.2.1 ^{1}H-NMR of 17α-amino-1,3,5(10)-estratrien-3-ol



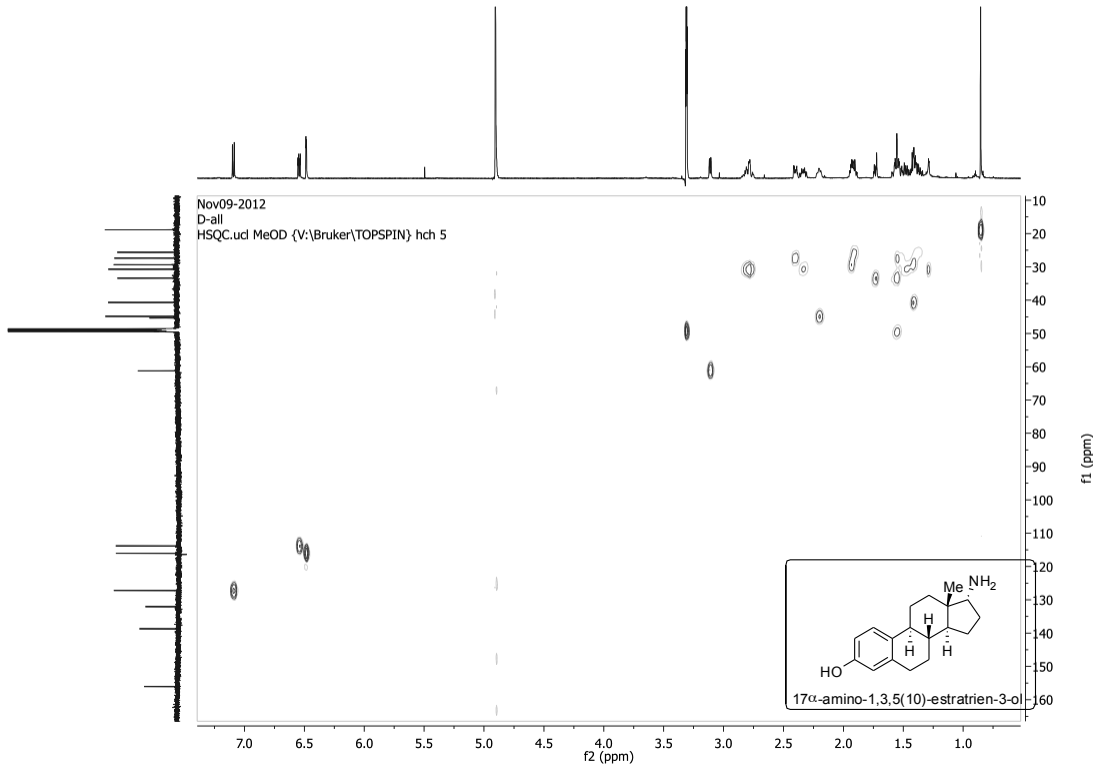
6.2.2 ^{13}C-NMR of 17α-amino-1,3,5(10)-estratrien-3-ol



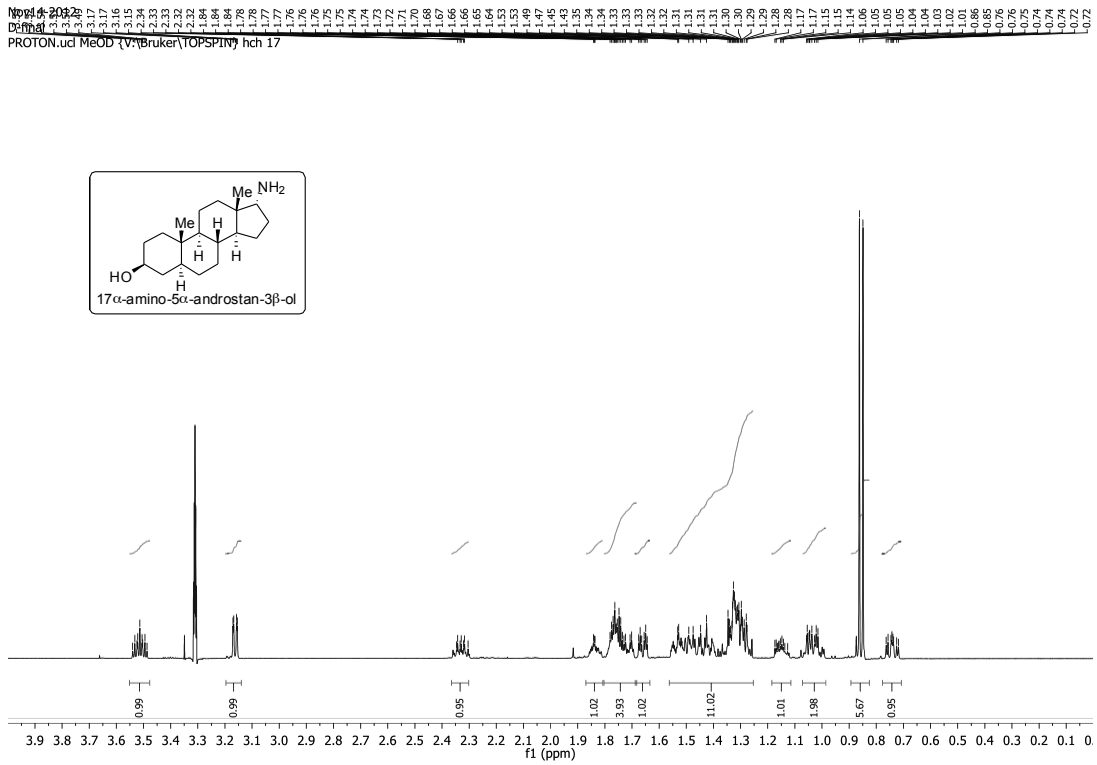
6.2.3 Dept135 of 17α-amino-1,3,5(10)-estratrien-3-ol



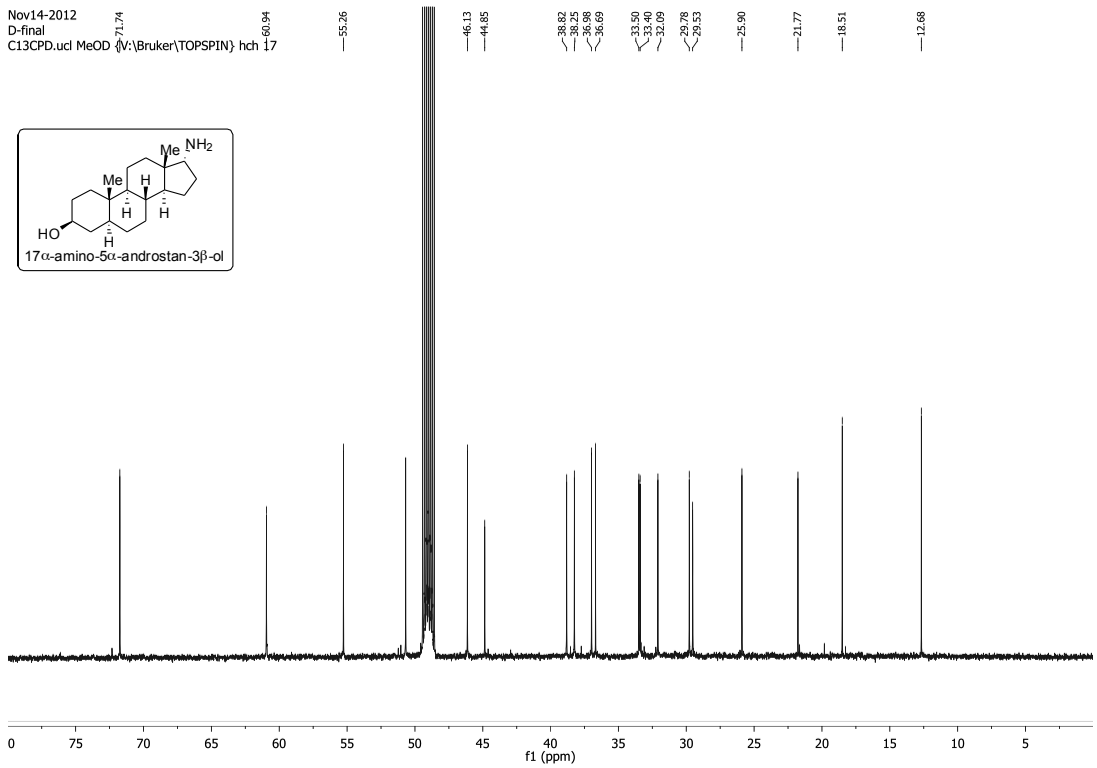
6.2.4 HSQC of 17α-amino-1,3,5(10)-estratrien-3-ol



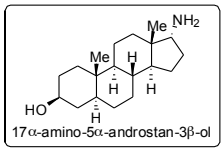
6.3.1 ^{1}H-NMR of 17α-amino-5α-androstan-3β-ol



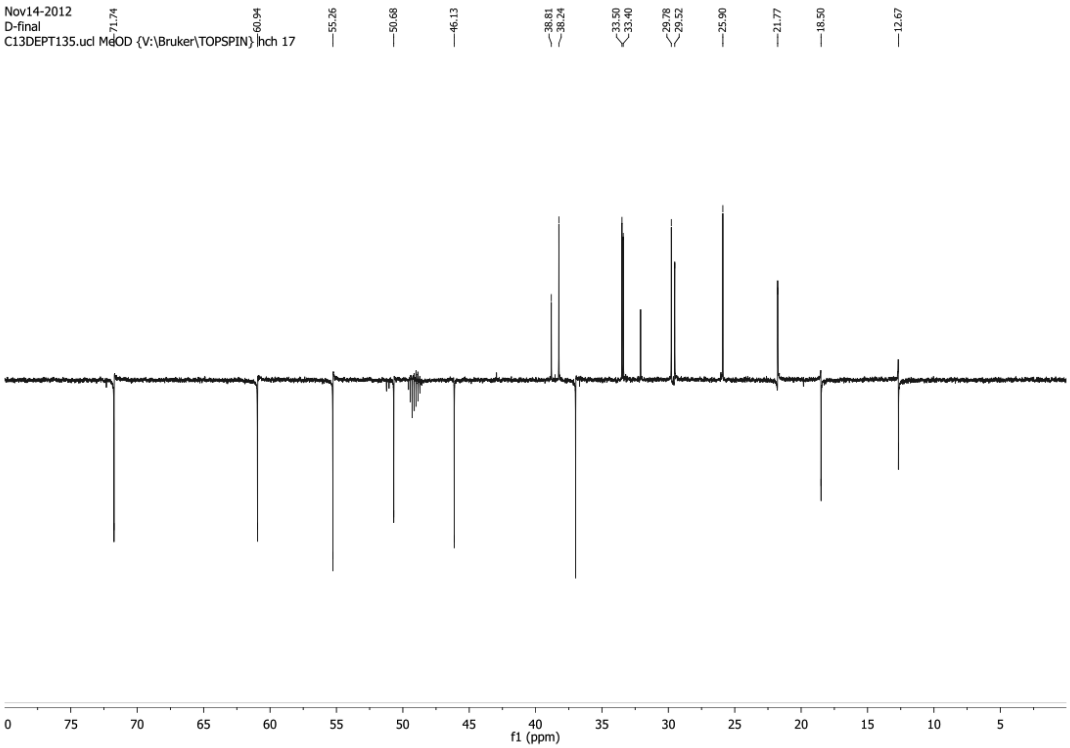
6.3.2 ^{13}C-NMR of 17α-amino-5α-androstan-3β-ol



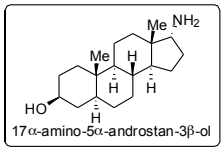
6.3.3 Dept135 of 17α-amino-5α-androstan-3β-ol



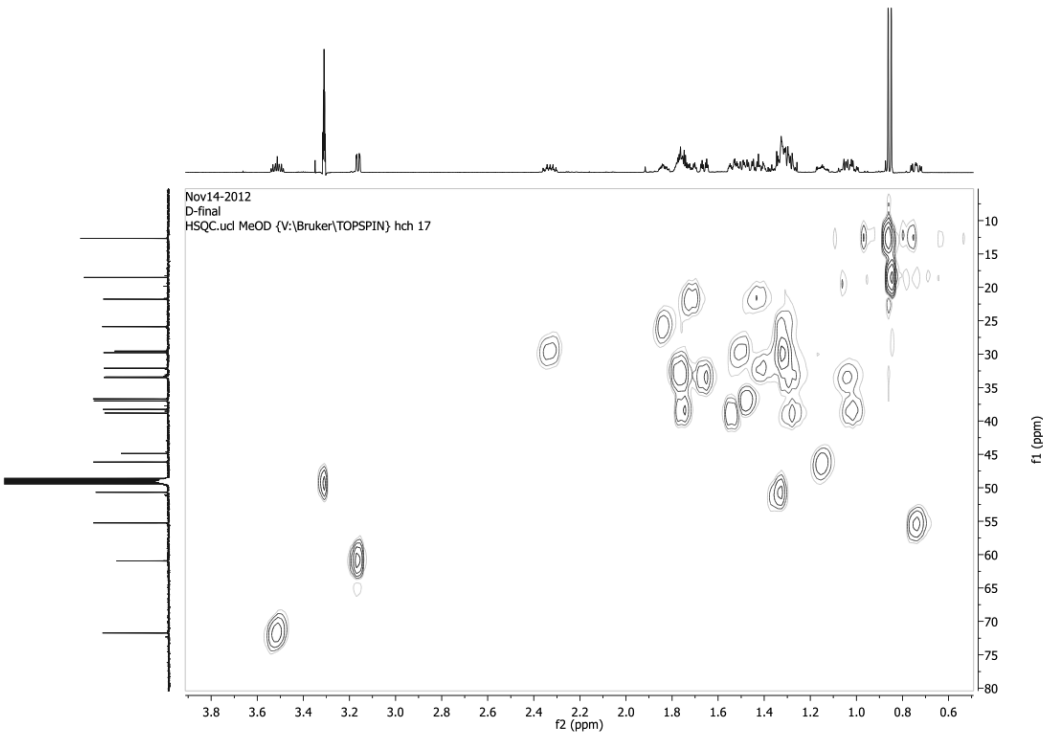
17α-amino-5α-androstan-3β-ol



6.3.4 HSQC of 17α-amino-5α-androstan-3β-ol



17α-amino-5α-androstan-3β-ol



IV References

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