Electronic Supplementary Information

The *Escherichia coli* Glucuronylsynthase Promoted Synthesis of Steroid Glucuronides: Improved Practicality and Broader Scope.

*Paul Ma, Nicholas Kanizaj, Shu-Ann Chan, David L. Ollis & Malcolm D. McLeod**

Research School of Chemistry, Australian National University, Canberra ACT 0200, Australia

malcolm.mcleod@anu.edu.au

CONTENTS **PAGE**

General experimental

^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectra were obtained on a Mercury 400 (400 MHz), Bruker Avance 600 (600 MHz) or Bruker Avance 800 (800 MHz) at 300 K unless otherwise stated. Chemical shift data is expressed in ppm relative to δ_{TMS} = 0, using residual protons in deuterated solvent as an internal reference. The data is reported as chemical shift (δ), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants (J Hz), and assignment. Assignments, where reported, are deduced from the relevant COSY and HSQC experiments.

^{13}C Nuclear Magnetic Resonance (^{13}C NMR) spectra were obtained on a Mercury 400 (100 MHz), Bruker Avance 600 (150 MHz) or Bruker Avance 800 (200 MHz) at 300 K with complete proton decoupling unless otherwise stated. Chemical shift data is expressed in ppm relative to δ_{TMS} = 0, using deuterated solvent as an internal reference. The data is reported as chemical shift (δ) and assignment.

Low resolution mass spectrometry (LRMS) was recorded on a Micromass ZMD ESI-Quad mass spectrometer, using negative electrospray ionisation (–ESI). Data is expressed as mass to charge ratio (m/z) and assignment.

High resolution mass spectroscopy (HRMS) was recorded on a Waters LCT Premier XE mass spectrometer, using negative electrospray ionisation (–ESI).

Analytical thin layer chromatography (TLC) was performed using 0.2 mm thick, aluminium-backed, pre-coated silica gel plates (Merck Silica gel 60 F_{254}). Compounds were visualised by short and long wavelength ultra-violet fluorescence and by staining with 5% sulphuric acid in methanol with heating where required.

Solvent removal under reduced pressure refers to evaporation using a rotary evaporator connected to a vacuum pump. Removal of residual solvent when necessary was achieved by evacuation (0.1–0.2 mm Hg) with a high stage oil sealed vacuum pump.

Solid-phase extraction (SPE) was conducted with Waters Oasis WAX 60 mg, 3 cc (186002492) or 500 mg, 6 cc (186004647) SPE cartridges.

Enzyme expression and purification

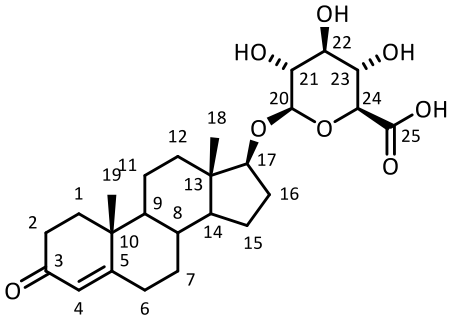
Literature procedures[1] were followed to obtain the E504G glucuronylsynthase mutant. Typical stock concentrations of enzyme used in this work ranged from 0.9–1.7 mg/mL.

Synthesis of α-D-glucuronyl fluoride 2

Literature procedures[1] were followed to obtain the α-D-glucuronyl fluoride, typically in gram quantities.

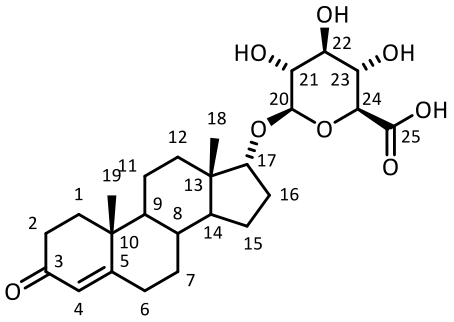
Synthesis of steroid glucuronides

Testosterone 17-glucuronide **3**[1]



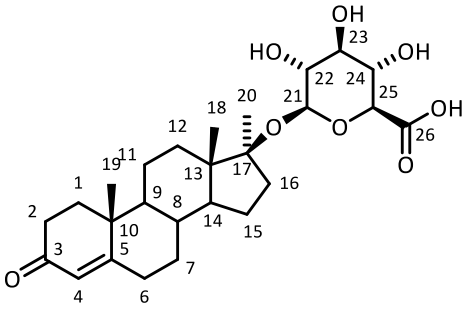
Testosterone 17-glucuronide **3** was prepared from testosterone (1.07 mg, 3.72 μmol) by method A with a 50% conversion as determined by 400 MHz ^{1}H NMR integration of the H17 protons. Re-purification by method B afforded pure testosterone 17-glucuronide **3**. The ^{1}H NMR data matched the literature.[1] A copy of the ^{1}H NMR spectrum is provided in the Electronic Supplementary Information (ESI). $\mathbf{R}_f$ 0.26 (7:2:1 EtOAc : MeOH : H_2O); **LRMS** (–ESI) *m/z* 463 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{25}H_{35}O_8$ ([M–H]$^-$) 463.2332, found 463.2331.

Epitestosterone 17-glucuronide **4**[2]



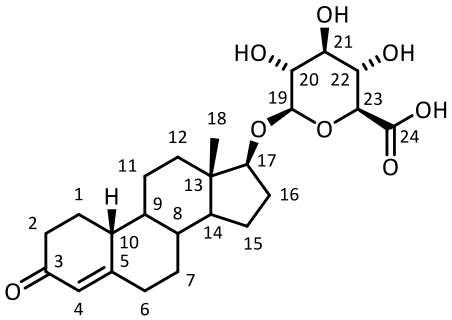
Epitestosterone 17-glucuronide **4** was prepared from epitestosterone (1.00 mg, 3.45 μmol) by method A with a 28% conversion as determined by 600 MHz ^{1}H NMR integration of the H17 protons. Re-purification by method B afforded pure epitestosterone 17-glucuronide **4**. A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.33 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 5.70 (1H, s, H4), 4.24 (1H, d, $J_{H20–H21}$ = 7.7 Hz, H20), 3.99 (1H, d, $J_{H17–H16}$ = 5.5 Hz, H17), 3.50–3.43 (2H, m), 3.37 (1H, t, $J_{H22–H23} \approx J_{H22–H21}$ = 8.8 Hz, H22), 3.18 (1H, t, $J_{H21–H22} \approx J_{H21–H20}$ = 7.2 Hz, H21), 2.52–2.44 (2H, m), 2.32–2.27 (2H, m), 2.10 (1H, m). 2.01 (1H, m), 1.93 (1H, m), 1.85–1.68 (4H, m), 1.65–1.56 (3H, m), 1.51 (1H, m), 1.26 (1H, m), 1.24 (3H, s, CH_3), 1.11 (1H, m), 1.02–0.87 (2H, m), 0.78 (3H, s, CH_3); **LRMS** (–ESI) *m/z* 463 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{25}H_{35}O_8$ ([M–H]$^-$) 463.2332, found 463.2332.

Methyltestosterone 17-glucuronide **5**[3]



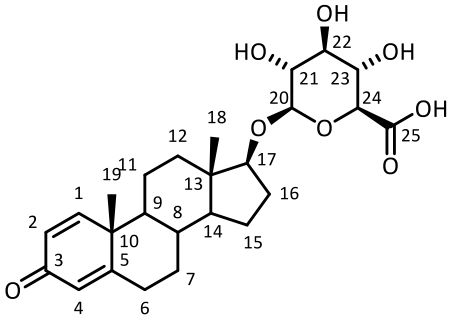
Methyltestosterone 17-glucuronide **5** was prepared from methyltestosterone (1.04 mg, 3.43 μmol) by method A with low conversion (< 5%) as determined by 400 MHz NMR analysis. Re-purification by method B afforded trace methyltestosterone 17-glucuronide **5**. Insufficient product was available for ^{1}H NMR analysis. **LRMS** (–ESI) *m/z* 477 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{26}H_{37}O_8$ ([M–H]$^-$) 477.2488, found 477.2488.

Nandrolone 17-glucuronide **6**[2]



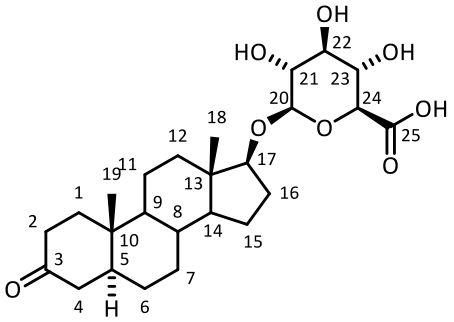
Nandrolone 17-glucuronide **6** was prepared from nandrolone (0.99 mg, 3.62 μmol) by method A with a 64% conversion as determined by 400 MHz ^{1}H NMR integration of the H17 protons. Re-purification by method B afforded pure nandrolone 17-glucuronide **6**. A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.26 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (400 MHz, CD_3OD) δ 5.80 (1H, s, H4), 4.36 (1H, d, $J_{H19–H20}$ = 8.0 Hz, H19), 3.84 (1H, t, $J_{H17–H16}$ = 8.6 Hz, H17), 3.52 (1H, d, $J_{H23–H22}$ = 9.2 Hz, H23), 3.43 (1H, t, $J_{H22–H23} \approx J_{H22–H21}$ = 9.0 Hz, H22) 3.36 (1H, t, $J_{H21–H22} \approx J_{H21–H20}$ = 8.8 Hz, H21), 3.20 (1H, t, $J_{H20–H21} \approx J_{H20–H19}$ = 8.8 Hz, H20), 2.50 (1H, m), 2.39–2.01 (7H, m), 1.89–1.84 (2H, m), 1.69–1.27 (7H, m), 1.12–1.04 (2H, m), 0.92 (3H, s, H18), 0.89–0.86 (1H, m); **LRMS** (–ESI) *m/z* 449 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{24}H_{33}O_8$ ([M–H]$^-$) 449.2175, found 449.2174.

Boldenone 17-glucuronide **7**[4]



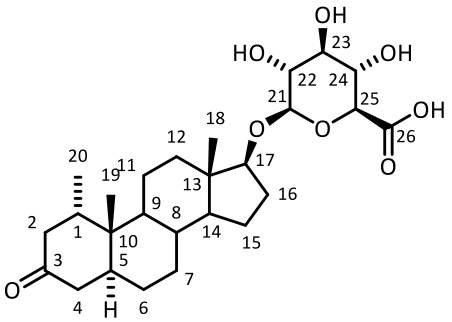
Boldenone 17-glucuronide **7** was prepared from boldenone (2.09 mg, 7.31 μmol) by method A with an 8% conversion as determined by 400 MHz ^{1}H NMR integration of the H17 protons. Re-purification by method B afforded pure boldenone 17-glucuronide **7**. A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.26 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 7.30 (1H, d, $J_{H1–H2}$ = 10.1 Hz, H1), 6.21 (1H, d, $J_{H2–H1}$ = 10.1 Hz, H2), 6.06 (1H, s, H4), 4.34 (1H, d, $J_{H20–H21}$ = 7.8 Hz, H20), 3.83 (1H, t, $J_{H17–H16}$ = 8.6 Hz, H17), 3.49 (1H, d, $J_{H24–H23}$ = 9.7 Hz, H24), 3.43 (1H, t, $J_{H23–H24} \approx J_{H23–H22}$ = 9.2 Hz, H23), 3.36 (1H, t, $J_{H22–H23} \approx J_{H22–H21}$ = 8.5 Hz, H22), 3.20 (1H, t, $J_{H21–H22} \approx J_{H21–H20}$ = 9.0 Hz, H21), 2.57 (1H, m), 2.41 (1H, m), 2.13–1.99 (3H, m), 1.80–1.72 (2H, m), 1.67 (1H, m), 1.59 (1H, m), 1.37–1.22 (8H, m), 1.09 (1H, m), 0.94 (3H, s, CH_3); **LRMS** (–ESI) *m/z* 461 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{25}H_{33}O_8$ ([M–H]$^-$) 461.2175, found 461.2175.

Androstanolone 17-glucuronide **8**[5]



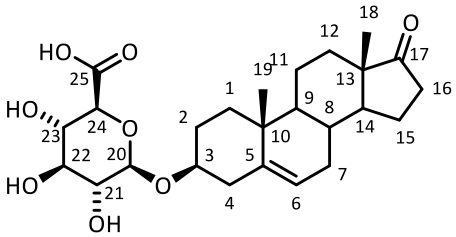
Androstanolone 17-glucuronide **8** was prepared from androstanolone (1.10 mg, 3.80 μmol) by method A with a 13% conversion as determined by 400 MHz ^{1}H NMR integration of the H17 protons. Re-purification by method B afforded pure androstanolone 17-glucuronide **8**. A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.52 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 4.35 (1H, d, $J_{H20–H21}$ = 7.8 Hz, H20), 3.84 (1H, t, $J_{H17–H16}$ = 8.7 Hz, H17), 3.49 (1H, d, $J_{H24–H23}$ = 9.6 Hz, H24), 3.43 (1H, t, $J_{H23–H24} \approx J_{H23–H22}$ = 9.3 Hz, H23), 3.36 (1H, t, $J_{H22–H23} \approx J_{H22–H21}$ = 9.0 Hz, H22), 3.19 (1H, t, $J_{H21–H22} \approx J_{H21–H20}$ = 8.5 Hz, H21), 2.48 (1H, m), 2.36 (1H, t, J = 14.5 Hz), 2.23–2.16 (2H, m), 2.12–1.98 (4H, m), 1.73 (1H, m), 1.63–1.41 (5H, m), 1.38–1.29 (3H, m), 1.27–1.22 (2H, m), 1.06 (3H, s, CH_3), 1.03 (1H, m), 0.95–0.89 (1H, m), 0.86 (3H, s, CH_3), 0.80 (1H, m); **LRMS** (–ESI) *m/z* 465 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{25}H_{37}O_8$ ([M–H]$^-$) 465.2488, found 465.2487.

Mesterolone 17-glucuronide **9**[2]



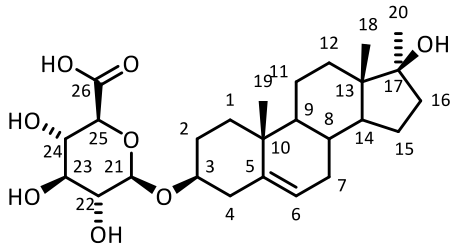
Mesterolone 17-glucuronide **9** was prepared from mesterolone (2.05 mg, 6.72 μmol) by method A with a 12% conversion as determined by 600 MHz ^{1}H NMR integration of the H17 protons. Re-purification by method B afforded pure mesterolone 17-glucuronide **9**. A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.20 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 4.35 (1H, d, $J_{H21–H22}$ = 7.8 Hz, H21), 3.85 (1H, t, $J_{H17–H16}$ = 8.6 Hz, H17), 3.49 (1H, d, $J_{H25–H24}$ = 9.7 Hz, H25), 3.43 (1H, t, $J_{H24–H25} \approx J_{H24–H23}$ = 9.2 Hz, H24), 3.36 (1H, t, $J_{H23–H24} \approx J_{H23–H22}$ = 9.0 Hz, H23), 3.20 (1H, t, $J_{H22–H23} \approx J_{H22–H21}$ = 8.5 Hz, H22), 2.82 (1H, m), 2.35 (1H, t, J = 14.2 Hz), 2.16 (1H, m), 2.09 (1H, m), 2.00–1.96 (2H, m), 1.80 (1H, m), 1.72–1.22 (11H, m), 1.17 (3H, s, CH_3), 1.07–1.00 (2H, m), 0.91–0.86 (7H, m); **LRMS** (–ESI) *m/z* 479 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{26}H_{39}O_8$ ([M–H]$^-$) 479.2645, found 479.2645.

Dehydroepiandrosterone 3-glucuronide **10**[1]



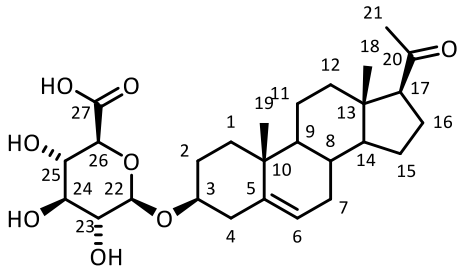
Dehydroepiandrosterone-3-glucuronide **10** was prepared from dehydroepiandrosterone (1.02 mg, 3.54 μmol) by method A with an 87% conversion as determined by 600 MHz ^{1}H NMR integration of the H6 protons. Synthesis by method B (5.03 mg of the parent steroid) afforded pure dehydroepiandrosterone 3-glucuronide **10** as a colourless solid (7.6 mg, 94%). The ^{1}H and ^{13}C NMR data matched the literature.[1] Copies of the ^{1}H and ^{13}C NMR spectra are provided in the ESI. $\mathbf{R}_f$ 0.26 (7:2:1 EtOAc : MeOH : H_2O); **LRMS** (–ESI) *m/z* 463 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{25}H_{35}O_8$ ([M–H]$^-$) 463.2332, found 463.2336.

Methandriol 3-glucuronide **11**



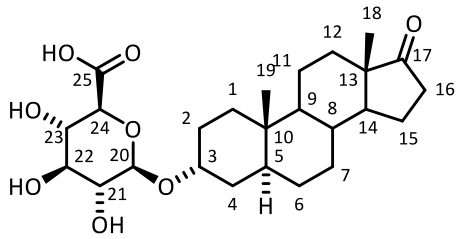
Methandriol 3-glucuronide **11** was prepared from methandriol (1.01 mg, 3.33 μmol) by method A with a 32% conversion as determined by 600 MHz ^{1}H NMR integration of the H6 protons. Synthesis by method B (10.0 mg of the parent steroid) afforded pure methandriol 3-glucuronide **11** as a colourless solid (2.4 mg, 15%). Copies of the ^{1}H and ^{13}C NMR spectra are provided in the ESI. $\mathbf{R}_f$ 0.20 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 5.38 (1H, d, $J_{H6–H7}$ = 5.2 Hz, H6), 4.40 (1H, d, $J_{H21–H22}$ = 7.8 Hz, H21), 3.65 (1H, m, H3), 3.56 (1H, d, $J_{H25–H24}$ = 9.4 Hz, H25), 3.43 (1H, t, $J_{H24–H25} \approx J_{H24–H23}$ = 9.1 Hz, H24), 3.40 (1H, t, $J_{H23–H24} \approx J_{H23–H22}$ = 8.9 Hz, H23), 3.19 (1H, t, $J_{H22–H23} \approx J_{H22–H21}$ = 8.4 Hz, H22), 2.43 (1H, m), 2.25 (1H, m), 2.02–1.97 (2H, m), 1.90–1.84 (2H, m), 1.68–1.47 (8H, m), 1.35–1.22 (3H, m), 1.19 (3H, s, CH_3), 1.12 (1H, m), 1.05 (3H, s, CH_3), 0.95 (1H, m), 0.86 (3H, s, CH_3); **^{13}C NMR** (150 MHz, CD_3OD) δ 176.9 (C26), 142.1 (C5), 122.5 (C6), 102.2 (C21), 82.3 (C17), 79.5 (C3), 77.9 (C23), 76.3 (C25), 75.0 (C22), 73.8 (C24), 52.5, 51.8, 46.6, 39.6, 39.3, 38.6, 38.0, 34.2, 32.9, 32.8, 30.6, 26.1 (CH_3), 24.4, 21.9, 19.9 (CH_3), 14.5 (CH_3); **LRMS** (–ESI) *m/z* 479 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{26}H_{39}O_8$ ([M–H]$^-$) 479.2645, found 479.2646.

Pregnenolone 3-glucuronide **12**[6]



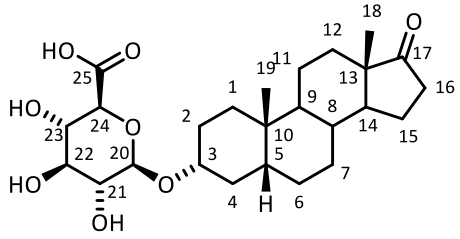
Pregnenolone 3-glucuronide **12** was prepared from pregnenolone (1.04 mg, 3.28 μmol) by method A with a 36% conversion as determined by 600 MHz ^{1}H NMR integration of the H6 protons. Synthesis by method B (5.02 mg of the parent steroid) afforded pure pregnenolone 3-glucuronide **12** as a colourless solid (2.0 mg, 26%). Copies of the ^{1}H and ^{13}C NMR spectra are provided in the ESI. $\mathbf{R}_f$ 0.36 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 5.38 (1H, d, $J_{H6–H7}$ = 5.0 Hz, H6), 4.41 (1H, d, $J_{H22–H23}$ = 7.8 Hz, H22), 3.65 (1H, m, H3), 3.57 (1H, d, $J_{H26–H25}$ = 9.2 Hz, H26), 3.43 (1H, t, $J_{H25–H26} \approx J_{H25–H24}$ = 9.1 Hz, H25), 3.38 (1H, t, $J_{H24–H25} \approx J_{H24–H23}$ = 8.9 Hz, H24), 3.19 (1H, t, $J_{H23–H24} \approx J_{H23–H22}$ = 8.3 Hz, H23), 2.65 (1H, t, $J_{H17–H16}$ = 9.0 Hz, H17), 2.44 (1H, m), 2.25 (1H, m), 2.17–2.12 (4H, m), 2.07 (1H, m), 2.02–1.95 (2H, m), 1.89 (1H, m), 1.72–1.48 (8H, m), 1.26–1.22 (2H, m), 1.13 (1H, m), 1.04–1.00 (4H, m), 0.63 (3H, s, CH_3); **^{13}C NMR** (150 MHz, CD_3OD) δ 212.4 (C20), 175.9 (C27), 142.1 (C5), 122.5 (C6), 102.4 (C22), 79.6 (C3), 77.8 (C24), 76.4 (C26), 75.0 (C23), 73.6 (C25), 64.7 (C17), 58.1, 51.5, 45.1, 39.9, 39.6, 38.5, 37.9, 33.2, 32.9, 31.6, 30.6, 25.5, 23.8, 22.2, 19.8 (CH_3), 13.6 (CH_3); **LRMS** (–ESI) *m/z* 491 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{27}H_{39}O_8$ ([M–H]$^-$) 491.2645, found 491.2649.

Androsterone 3-glucuronide **14**[7]



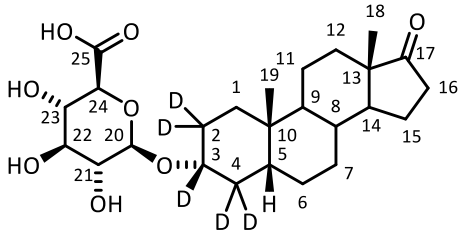
Androsterone 3-glucuronide **14** was prepared from androsterone (0.96 mg, 3.31 μmol) by method A with low conversion (< 5%) as determined by 400 MHz NMR analysis. Re-purification by method B afforded trace androsterone 3-glucuronide **14**. Insufficient product was available for ^{1}H NMR analysis. $\mathbf{R}_f$ 0.59 (7:2:1 EtOAc : MeOH : H_2O); **LRMS** (–ESI) *m/z* 465 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{25}H_{37}O_8$ ([M–H]$^-$) 465.2488, found 465.2488.

Etiocholanolone 3-glucuronide **15**[8]



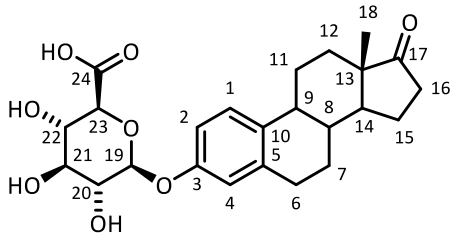
Etiocholanolone 3-glucuronide **15** was prepared from etiocholanolone (2.07 mg, 7.14 μmol) by method A with a 25% conversion as determined by 600 MHz ^{1}H NMR of the H3 protons. Synthesis by method B (5.10 mg of the parent steroid) afforded pure etiocholanolone 3-glucuronide **15** as a colourless solid (1.6 mg, 20%). A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.44 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 4.42 (1H, d, $J_{H20–H21}$ = 7.8 Hz, H20), 3.81 (1H, m, H3), 3.56 (1H, d, $J_{H24–H23}$ = 9.3 Hz, H24), 3.43 (1H, t, $J_{H23–H24} \approx J_{H23–H22}$ = 9.0 Hz, H23), 3.40 (1H, t, $J_{H22–H23} \approx J_{H22–H21}$ = 8.9 Hz, H22), 3.18 (1H, t, $J_{H21–H22} \approx J_{H21–H20}$ = 8.3 Hz, H21), 2.44 (1H, m), 2.08 (1H, m), 1.98–1.91 (2H, m), 1.90–1.83 (2H, m), 1.78 (1H, m), 1.66–1.64 (2H, m), 1.59–1.52 (4H, m), 1.46 (1H, m), 1.41–1.23 (7H, m), 1.04–0.98 (4H, m), 0.87 (3H, s, CH_3); **LRMS** (–ESI) *m/z* 465 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{25}H_{37}O_8$ ([M–H]$^-$) 465.2488, found 465.2488.

d_5-Etiocholanolone 3-glucuronide **21**



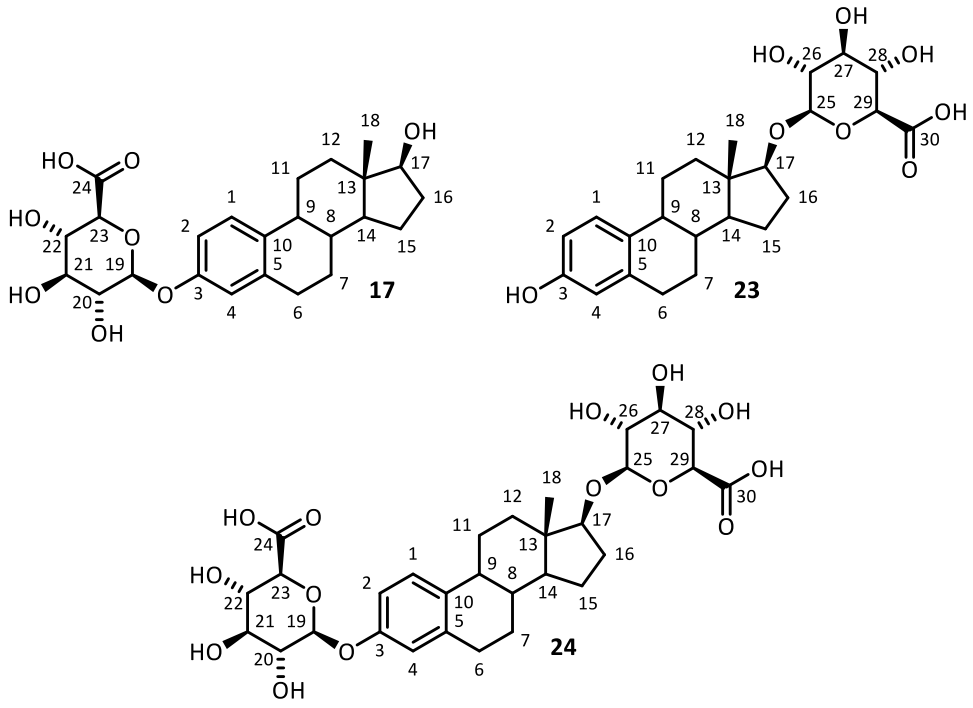
d_5-Etiocholanolone 3-glucuronide **21** was prepared from d_5-etiocholanolone **22** (1.00 mg, 3.38 μmol) by method B as a colourless solid. A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.41 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 4.41 (1H, d, $J_{H20–H21}$ = 7.8 Hz, H20), 3.55 (1H, d, $J_{H24–H23}$ = 9.4 Hz, H24), 3.43 (1H, t, $J_{H23–H22} \approx J_{H23–H24}$ = 9.2 Hz, H23), 3.40 (1H, t $J_{H22–H21} \approx J_{H22–H23}$ = 8.8 Hz, H22), 3.18 (1H, t, $J_{H21–H20} \approx J_{H21–H22}$ = 8.5 Hz, H21), 2.44 (1H, dd, J = 19.4, 8.4 Hz), 2.08 (1H, m), 1.98–1.90 (2H, m), 1.83 (1H, d, J = 14.3 Hz), 1.76 (1H, m), 1.66 (1H, m), 1.59–1.51 (4H, m), 1.44 (1H, m), 1.41–1.22 (6H, m), 0.98 (3H, s, CH_3), 0.87 (3H, s CH_3); **LRMS** (–ESI) *m/z* 470 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{25}H_{32}D_5O_8$ ([M–H]$^-$) 470.2802, found 470.2800.

Estrone 3-glucuronide **16**[9]



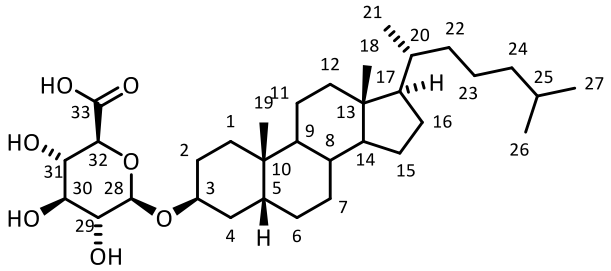
Estrone-3-glucuronide **16** was prepared from estrone (1.00 mg, 3.68 μmol) by method A with a 33% conversion as determined by 600 MHz ^{1}H NMR integration of the H2 and H4 protons. Re-purification by method B afforded pure estrone 3-glucuronide **16**. The ^{1}H NMR data matched the literature.[9] A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.38 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD): δ 7.19 (1H, d, $J_{H1–H2}$ = 8.6 Hz, H1), 6.89 (1H, d, $J_{H2–H1}$ = 8.6 Hz, H2), 6.84 (1H, s, H4), 3.73 (1H, d, $J_{H23–H22}$ = 9.5 Hz, H23), 3.55–3.47 (3H, m), 2.90–2.86 (2H, m), 2.49 (1H, dd, J = 18.9, 8.7 Hz), 2.42 (1H, m), 2.27 (1H, m), 2.14 (1H, m), 2.09–2.01 (2H, m), 1.90 (1H, m), 1.67 (1H, m), 1.62–1.56 (2H, m), 1.54–1.49 (3H, m), 0.93 (3H, s, CH_3), H19 obscured by CD_3OH δ 4.84; **LRMS** (–ESI) *m/z* 445 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{24}H_{29}O_8$ ([M–H]$^-$) 445.1862, found 445.1862.

Estradiol 3-glucuronide **17**,[9] *estradiol 17-glucuronide* **23**[10] and *estradiol 3,17-bis-glucuronide* **24**



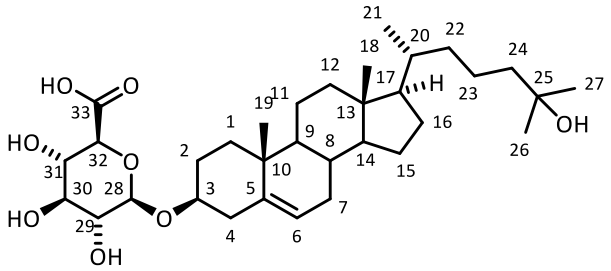
A mixture of estradiol 3-glucuronide **17**, estradiol 17-glucuronide **23** and estradiol 3,17-bis-glucuronide **24** was prepared from estradiol (1.04 mg, 3.82 μmol) by method A with a 88% conversion as determined by 600 MHz ^{1}H NMR integration of the H17 protons. Re-purification by method B afforded a mixture of estradiol 3-glucuronide **17**, estradiol 17-glucuronide **23** and estradiol 3,17-bis-glucuronide **24** in a 1.0:1.6:1.1 ratio as determined by 600 MHz ^{1}H NMR integration of the H17, H19 and H23 protons. A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.38 and 0.00 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD, *key signals only*) δ 7.18 (d, J_{H1-H2} = 8.6 Hz, **17** and **24** H1), 7.07 (d, J_{H1-H2} = 8.5 Hz, **23** H1), 6.88 (d, J_{H2-H1} = 8.6 Hz, **17** and **24** H2), 6.81 (s, **17** and **24** H4), 6.53 (d, J_{H2-H1} = 8.4 Hz, **23** H2), 6.47 (s, **23** H4), 4.40 (d, $J_{H25-H26}$ = 7.8 Hz, **23** and **24** H25), 3.91 (t, $J_{H17-H16}$ = 8.5 Hz, **23** and **24** H17), 3.72 (d, $J_{H23-H22}$ = 9.2 Hz, **17** and **24** H23), 3.66 (t, $J_{H17-H16}$ = 8.6 Hz, **17** H17), 0.89 (s, **23** and **24** CH_3), 0.78 (s, **17** CH_3), H19 for **17** and **24** obscured by CD_3OH δ 4.82; **LRMS** (–ESI) *m/z* 623 (42%) ([M–H]$^-$, **24**), 447 (100%) ([M–H]$^-$, **17** and **23**); **HRMS** (–ESI) *m/z* calcd. for $C_{30}H_{39}O_{14}$ ([M–H]$^-$, **24**) 623.2340, found 623.2343; calcd. for $C_{24}H_{31}O_8$ ([M–H]$^-$, **17** and **23**) 447.2019, found 447.2020.

Coprostanol 3-glucuronide **18**[11]



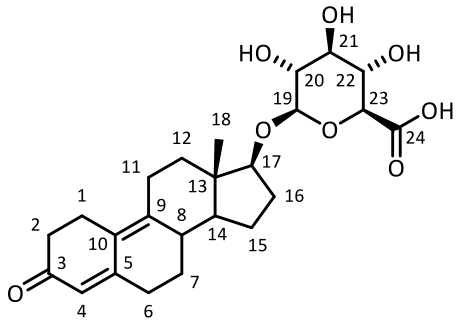
Coprostanol 3-glucuronide **18** was prepared from coprostanol (0.98 mg, 2.52 μmol) by method A with low conversion (< 5%) as determined by 400 MHz NMR analysis. Re-purification by method B afforded trace coprostanol 3-glucuronide **18**. Insufficient product was available for ^{1}H NMR analysis. $\mathbf{R}_f$ 0.50 (7:2:1 EtOAc : MeOH : H_2O); **LRMS** (–ESI) *m/z* 563 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{33}H_{55}O_7$ ([M–H]$^-$) 563.3948, found 563.3948.

Cholest-5-ene-3β,25-diol 3-glucuronide **19**



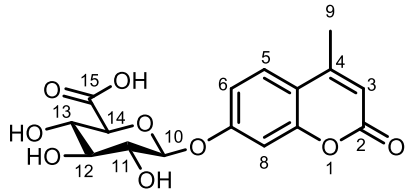
Cholest-5-ene-3β,25-diol 3-glucuronide **19** was prepared from cholest-5-ene-3β,25-diol (1.06 mg, 2.63 μmol) by method A with a 20% conversion as determined by 600 MHz ^{1}H NMR integration of the H3 protons. Synthesis by method B (10.0 mg of the parent steroid) afforded pure cholest-5-ene-3β,25-diol 3-glucuronide **19** as a colourless solid (1.2 mg, 8%). Copies of the ^{1}H and ^{13}C NMR spectra are provided in the ESI. $\mathbf{R}_f$ 0.40 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 5.37 (1H, d, $J_{H6–H7}$ = 5.1 Hz, H6), 4.40 (1H, d, $J_{H28–H29}$ = 7.8 Hz, H28), 3.65 (1H, m, H3), 3.55 (1H, d, $J_{H32–H31}$ = 9.5 Hz, H32), 3.44 (1H, t, $J_{H31–H32}$ ≈ $J_{H31–H30}$ = 9.1 Hz, H31), 3.40 (1H, t, $J_{H30–H31}$ ≈ $J_{H30–H29}$ = 8.9 Hz, H30), 3.19 (1H, t, $J_{H29–H30}$ ≈ $J_{H29–H28}$ = 8.3 Hz, H29), 2.43 (1H, m), 2.25 (1H, m), 2.05 (1H, m), 1.99–1.96 (2H, m), 1.88–1.85 (2H, m), 1.64–1.60 (2H, m), 1.58–1.22 (13H, m), 1.19 (6H, s, 2 × CH_3), 1.14–1.10 (2H, m), 1.08–1.02 (5H, m), 0.97–0.94 (4H, m), 0.72 (3H, s, CH_3); **^{13}C NMR** (150 MHz, CD_3OD) δ 176.8 (C33), 141.9 (C5), 122.5 (C6), 102.1 (C28), 79.3, 77.8, 76.2, 74.9, 73.7, 71.4, 58.1, 57.5, 51.6, 45.2, 43.4, 41.1, 39.5, 38.5, 37.8, 37.7, 37.0, 33.2, 33.0, 30.5, 29.2, 29.1, 29.0, 25.2, 22.1, 21.8, 19.8, 19.1, 12.2; **LRMS** (–ESI) *m/z* 577 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for ([M–H]$^-$) $C_{33}H_{53}O_8$ 577.3740, found 577.3740.

Trenazone 17-glucuronide **20**



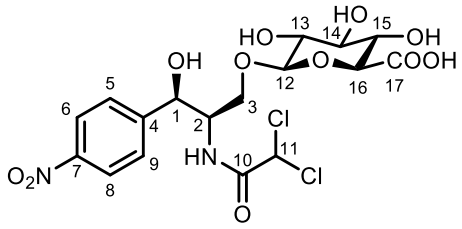
Trenazone 17-glucuronide **20** was prepared from trenazone (1.00 mg, 3.04 μmol) by method A with a 21% conversion as determined by 600 MHz ^{1}H NMR integration of the H17 protons. Synthesis by method B (9.4 mg of the parent steroid) afforded pure trenazone 17-glucuronide **20** as a colourless solid (5.3 mg, 34%). Copies of the ^{1}H and ^{13}C NMR spectra are provided in the ESI. $\mathbf{R}_f$ 0.45 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (600 MHz, CD_3OD) δ 5.64 (1H, s, H4), 4.37 (1H, d, $J_{H19–H20}$ = 7.8 Hz, H19), 3.85 (1H, t, $J_{H17–H16}$ = 9.0 Hz, H17), 3.53 (1H, d, $J_{H23–H22}$ = 9.3 Hz, H23), 3.42 (1H, t, $J_{H22–H23} \approx J_{H22–H21}$ = 9.1 Hz, H22), 3.38 (1H, t, $J_{H21–H22} \approx J_{H21–H20}$ = 8.9 Hz, H21), 3.21 (1H, t, $J_{H20–H21} \approx J_{H20–H19}$ = 8.5 Hz, H20), 2.95 (1H, m), 2.85 (1H, m), 2.55 (1H, m), 2.47–2.37 (4H, m), 2.31 (1H, m), 2.20 (1H, m), 2.15–2.09 (2H, m), 1.94 (1H, m), 1.73 (1H, m), 1.64 (1H, m), 1.42–1.34 (2H, m), 1.29–1.16 (2H, m), 1.02 (3H, s, H18); **^{13}C NMR** (150 MHz, CD_3OD) δ 202.5 (C3), 176.8, 160.9, 149.3, 126.2, 122.0, 104.4, 88.8, 77.8, 76.5, 75.2, 73.7, 52.4, 44.1, 40.4, 38.5, 37.8, 31.7, 29.6, 28.1, 26.7, 24.1, 11.2 (CH_3), one carbon overlapping or obscured; **LRMS** (–ESI) *m/z* 447 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for $C_{24}H_{31}O_8$ ([M–H]$^-$) 447.2019, found 447.2020.

4-Methylumbelliferone 7-glucuronide **23**[12]



4-Methylumbelliferone 7-glucuronide **23** was prepared from 4-methylumbelliferone (2.10 mg, 11.9 μmol) by method A with a 53% conversion as determined by 400 MHz ^{1}H NMR integration of the H5 protons. Re-purification by method B afforded 4-methylumbelliferone 7-glucuronide **23**. A copy of the ^{1}H NMR spectrum is provided in the ESI. $\mathbf{R}_f$ 0.04 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (400 MHz, CD_3OD): δ 7.71 (1H, d, $J_{H5–H6}$ = 8.8 Hz, H5), 7.15 (1H, dd, $J_{H6–H5}$ = 8.8, $J_{H6–H8}$ 2.1 Hz, H6), 7.09 (1H, s, H8), 6.20 (1H, s, H3), 5.07 (1H, d, $J_{H10–H11}$ = 5.3 Hz, H10), 3.83 (1H, d, $J_{H14–H13}$ 7.7, H14), 3.54 (3H, s, H11, H12, H13), 2.46 (3H, s, H9); **LRMS** (–ESI) *m/z* 351 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for ([M–H]$^-$) $C_{16}H_{15}O_9$ 351.0716, found 351.0717.

Chloramphenicol 3-glucuronide **2**[13]



Chloramphenicol 3-glucuronide **24** was prepared from chloramphenicol (1.09 mg, 3.37 μmol) by method A with a 11% conversion as determined by 800 MHz ^{1}H NMR integration of the H3 protons. Re-purification by method B afforded chloramphenicol 3-glucuronide **24**. A copy of the ^{1}H NMR and 2D HSQC and HMBC spectra are provided in the ESI. $\mathbf{R}_f$ 0.37 (7:2:1 EtOAc : MeOH : H_2O); **^{1}H NMR** (800 MHz, CD_3OD): δ 8.17 (2H, m, H6, H8), 7.70 (2H, m, H5, H9), 6.32 (1H, s, H11), 5.28 (1H, d, $J_{H1–H2}$ = 3.3 Hz, H1), 4.34 (1H, d, $J_{H12–H13}$ = 7.9 Hz, H12), 4.28 (1H, m, H2), 4.01 (1H, dd, $J_{H3a–H3b}$ = 10.4, $J_{H3a–H2}$ = 7.9 Hz, H3a), 3.71 (1H, dd, $J_{H3b–H3a}$ = 10.4 $J_{H3b–H2}$ = 4.8 Hz, H3b), 3.61 (1H, d, $J_{H16–H15}$ = 9.6 Hz, H16), 3.46 (1H, t, $J_{H15–H16} \approx J_{H15–H14}$ = 9.2 Hz, H15), 3.43 (1H, t, $J_{H14–H15} \approx J_{H14–H13}$ = 8.8 Hz, H14), H13 obscured by solvent; **^{13}C NMR** (CD_3OD, derived from 800 MHz HSQC and HMBC spectra): δ 176.8 (C17), 151.2 (C7), 148.2 (C4), 128.4 (C6, C8), 123.8 (C5, C9), 104.0 (C12), 77.5 (C14), 75.0 (C13), 74.9 (C16), 73.5 (C15), 70.3 (C1), 69.4 (C3), 66.5 (C11), 56.6 (C2), amide carbonyl not observed; **LRMS** (–ESI) *m/z* 497 ([M–H]$^-$); **HRMS** (–ESI) *m/z* calcd. for ([M–H]$^-$) $C_{17}H_{19}N_2O_{11}{}^{35}Cl_2$ 497.0366, found 497.0366; $C_{17}H_{19}N_2O_{11}{}^{35}Cl^{37}Cl$ 499.0336, found 499.0336; $C_{17}H_{19}N_2O_{11}{}^{37}Cl_2$ 501.0307, found 501.0317.

References

(1) Wilkinson, S. M.; Watson, M. A.; Willis, A. C.; McLeod, M. D. *J. Org. Chem.* **2011**, *76*, 1992–2000.

(2) Thevis, M.; Opfermann, G.; Schmickler, H.; Schänzer, W. *J. Mass Spectrom.* **2001**, *36*, 998–1012.

(3) Amarasinghe, K.; Chu, P.-S.; Evans, E.; Reimschuessel, R.; Hasbrouck, N.; Jayasuriya, H. *J. Agric. Food Chem.* **2012**, *60*, 5084–5088.

(4) Casati, S.; Ottria, R.; Ciuffreda, P. *Steroids* **2009**, *74*, 250–255.

(5) Murai, T.; Samata, N.; Iwabuchi, H.; Ikeda, T. *Drug Metab. Dispos.* **2006**, *34*, 1102–1108.

(6) Wotiz, H. H.; Smakula, E.; Lichtin, N. N.; Leftin, J. H. *J. Am. Chem. Soc.* **1959**, *81*, 1704–1708.

(7) Harding, J. R.; King, C. D.; Perrie, J. A.; Sinnott, D.; Stachulski, A. V. *Org. Biomol. Chem.* **2005**, *3*, 1501–1507.

(8) Becker, J. F. *Biochim. Biophys. Acta* **1965**, *100*, 574–581.

(9) Werschkun, B.; Gorziza, K.; Thiem, J. *J. Carbohydr. Chem.* **1999**, *18*, 629–637.

(10) Kashima, Y.; Kitade, T.; Kashima, Y.; Okabayashi, Y. *Chem. Pharm. Bull.* **2010**, *58*, 354–358.

(11) Goto, J.; Suzaki, K.; Nambara, T. *Chem. Pharm. Bull.* **1979**, *27*, 1926–1931.

(12) S. Park and I. Shin, *Org. Lett.*, 2007, **9**, 619–622.

(13) M. Chen, D. Howe, B. Leduc, S. Kerr and D. A. Williams, *Xenobiotica*, 2007, **37**, 954–971.

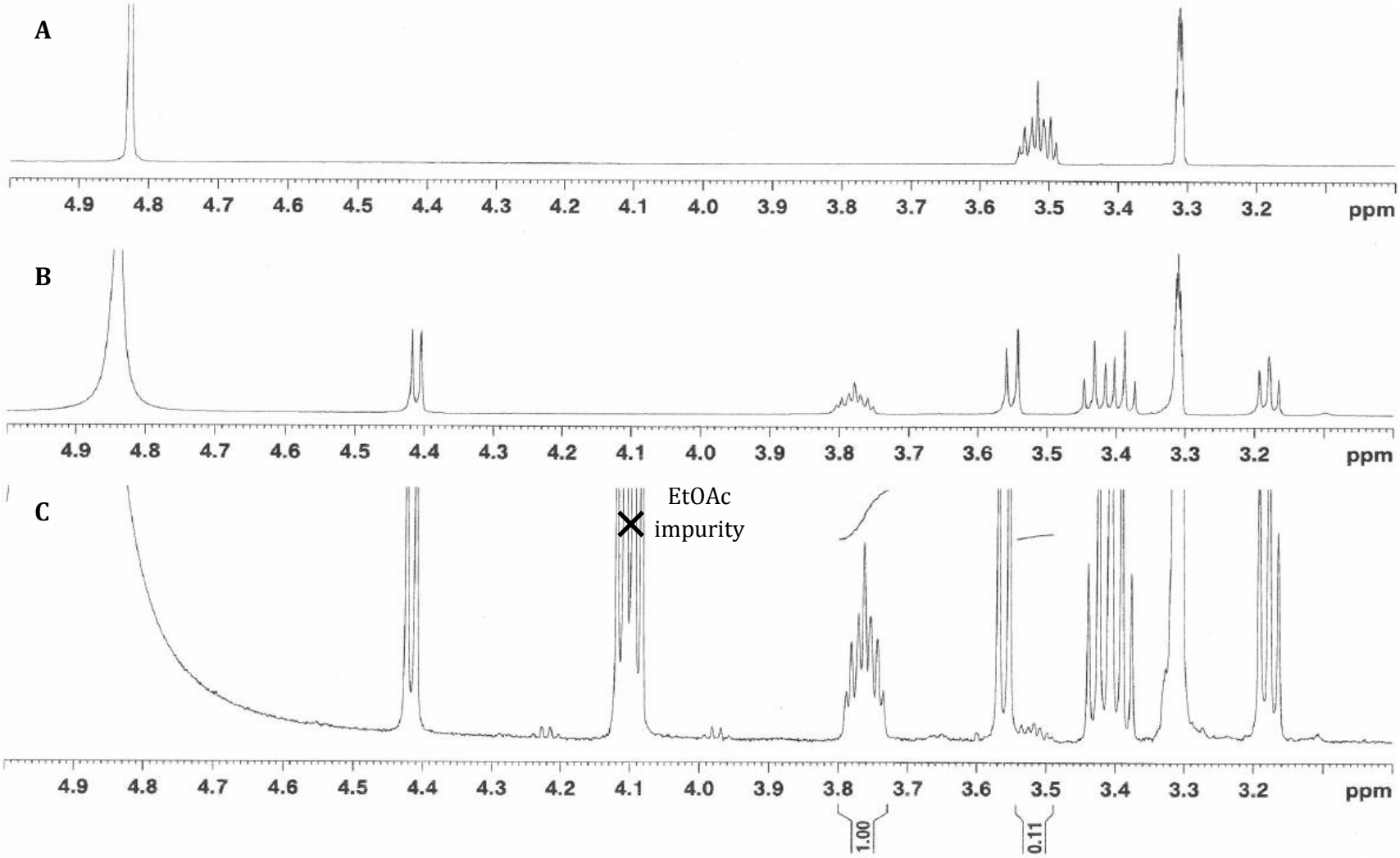
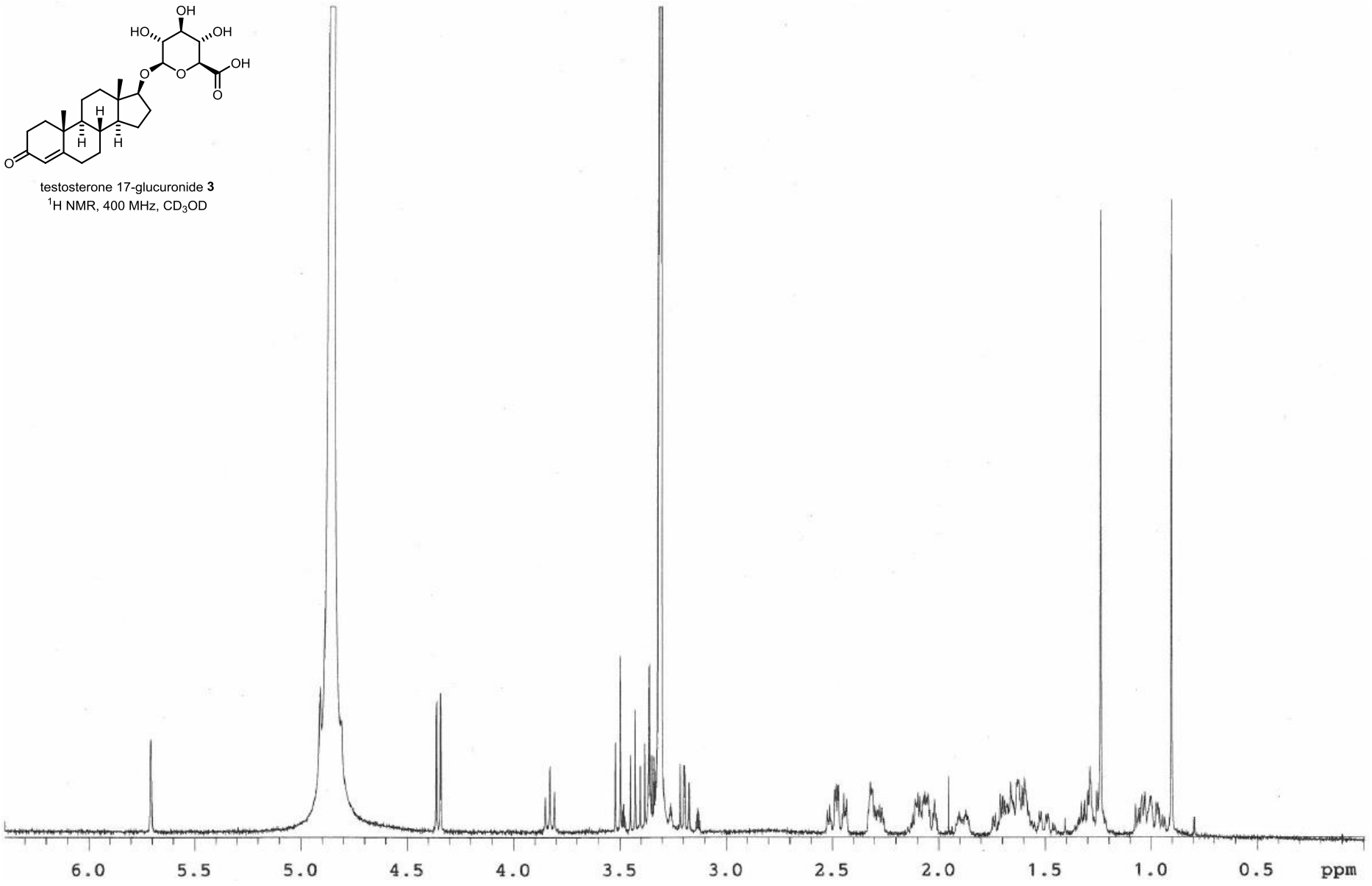
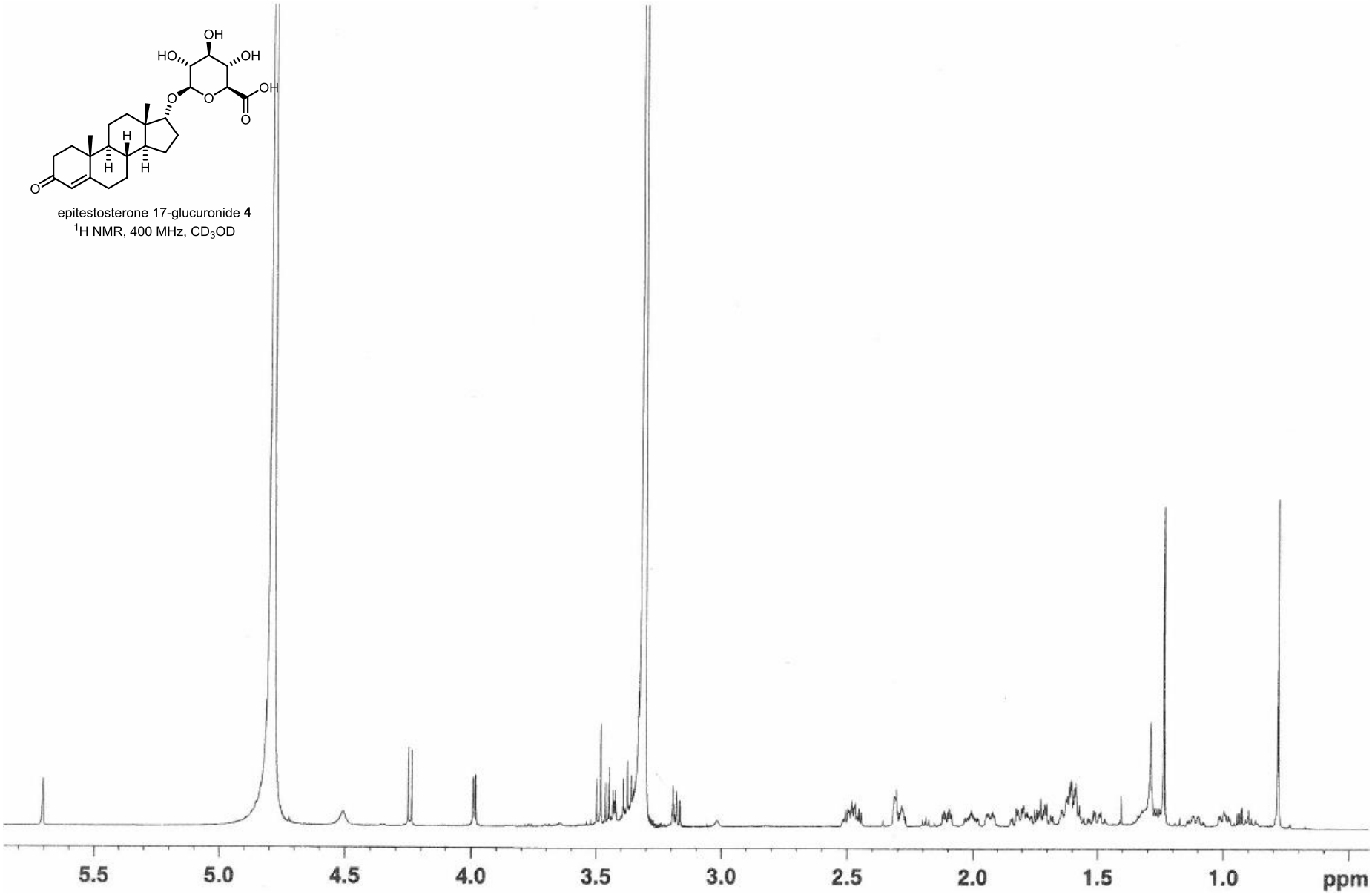


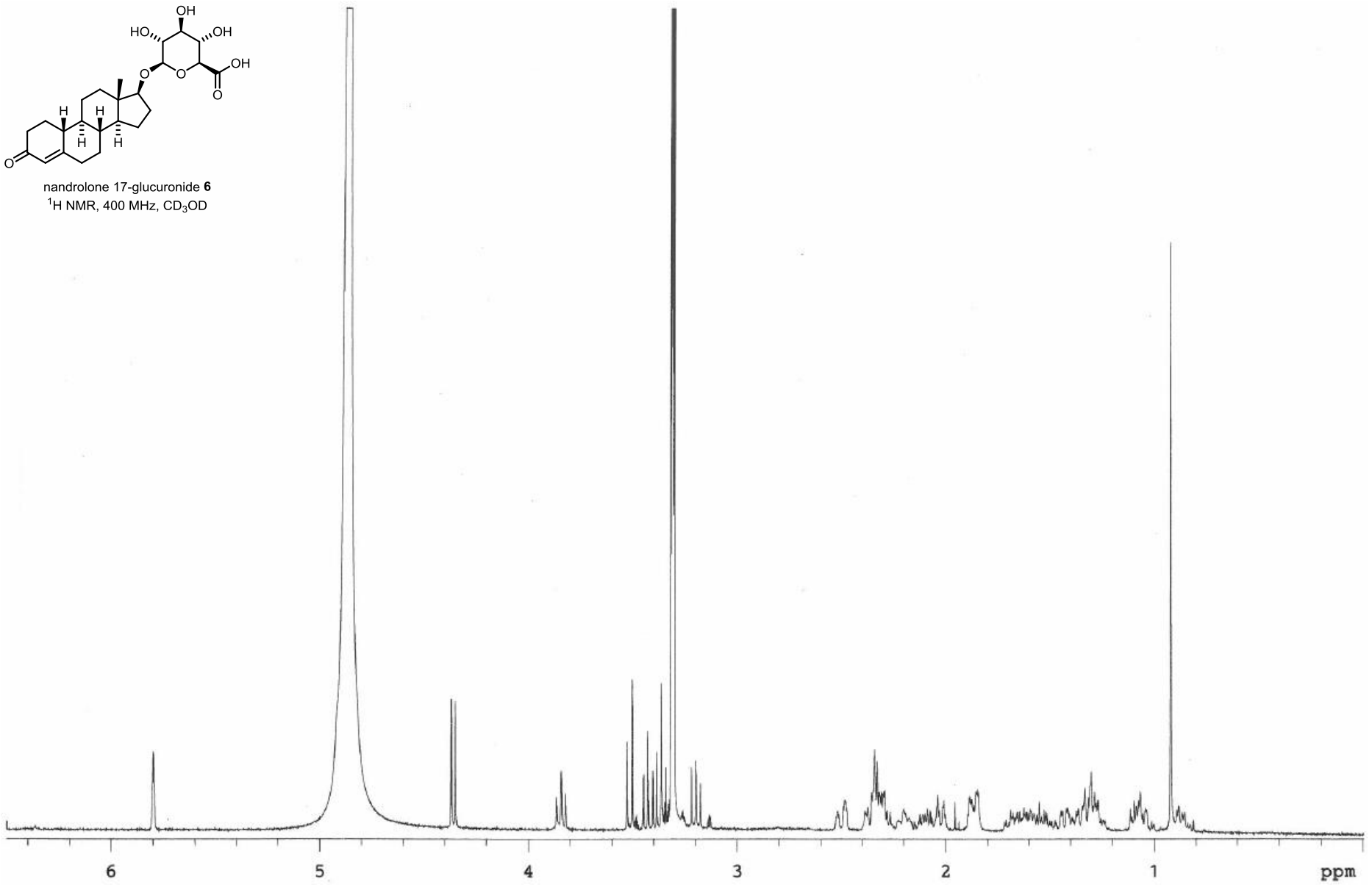
Figure S1. ^{1}H NMR conversion for epiandrosterone 3-glucuronide: **A** epiandrosterone, **B** epiandrosterone 3-glucuronide, and **C** mixed epiandrosterone and epiandrosterone 3-glucuronide.



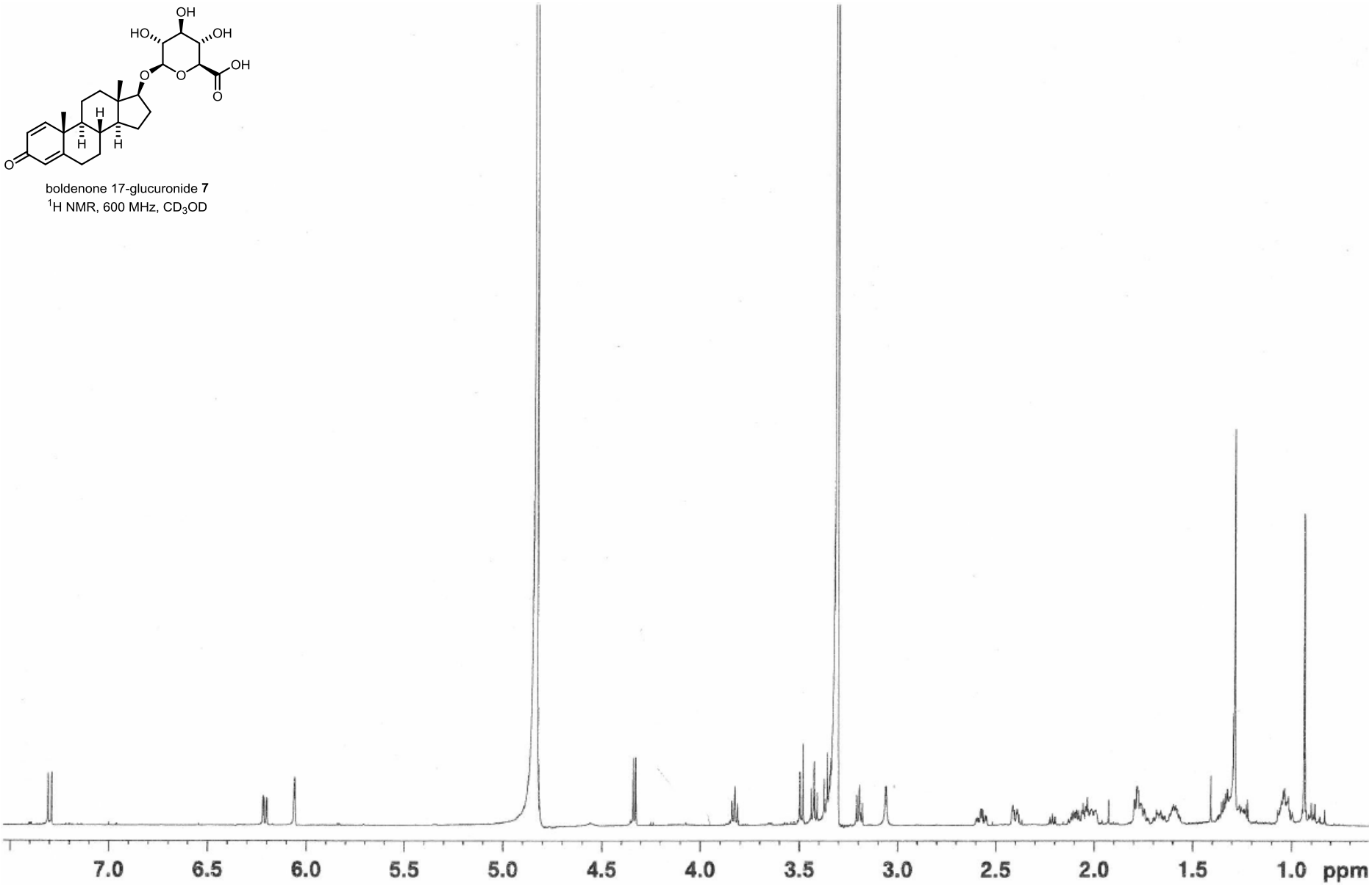
testosterone 17-glucuronide **3**
^{1}H NMR, 400 MHz, CD_3OD



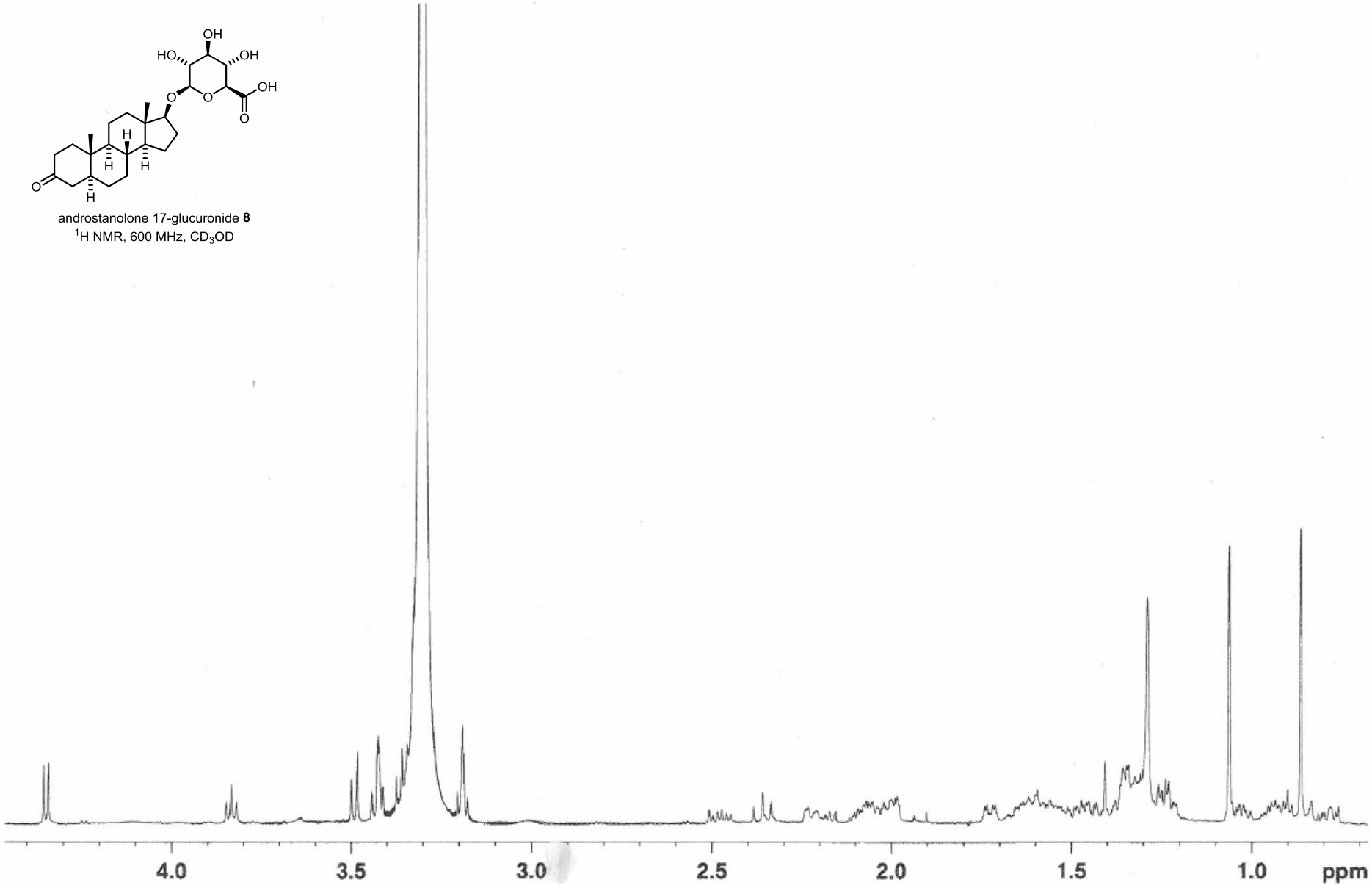
epitestosterone 17-glucuronide **4**
^{1}H NMR, 400 MHz, CD_3OD



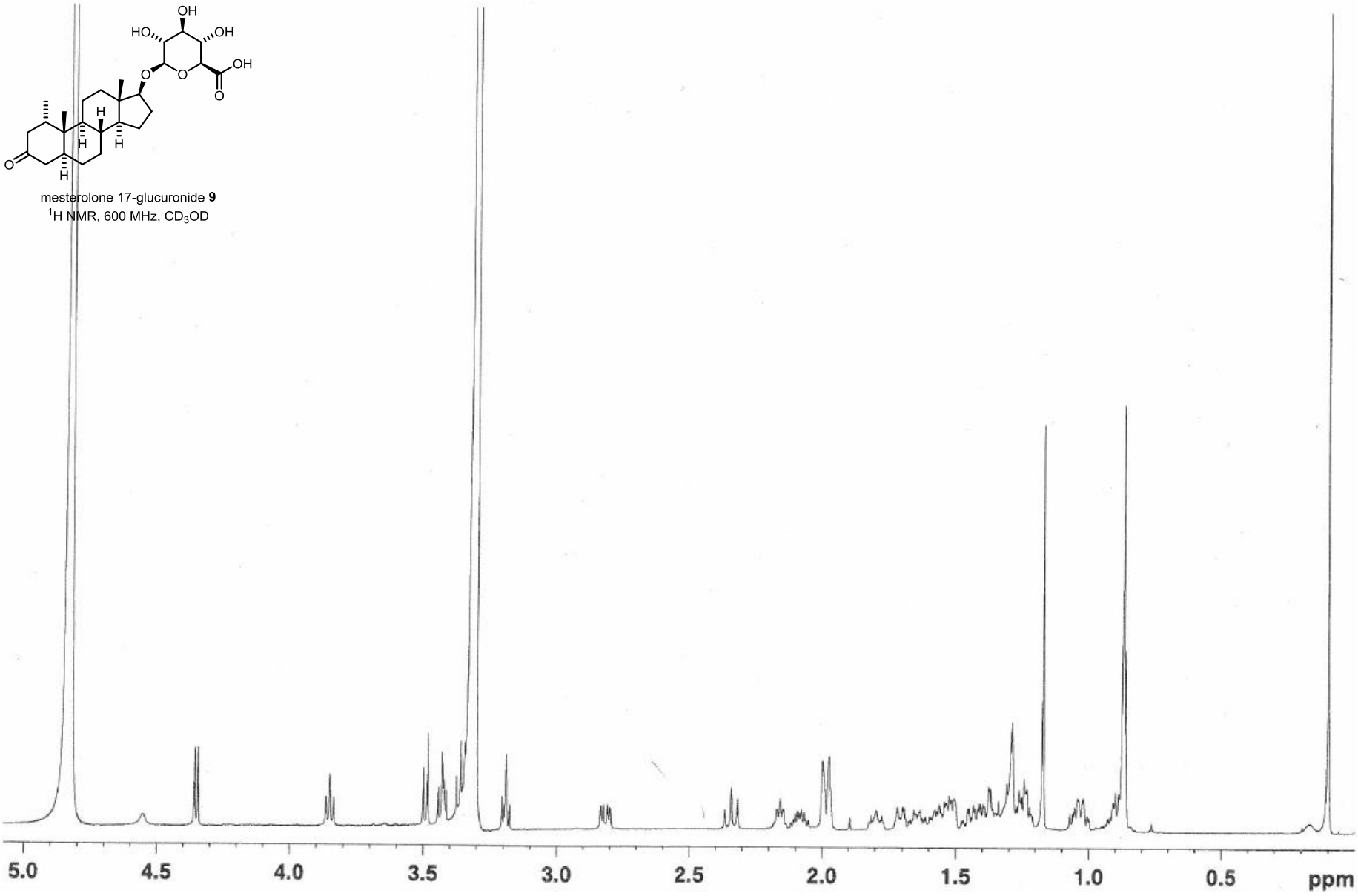
nandrolone 17-glucuronide **6**
^{1}H NMR, 400 MHz, CD_3OD



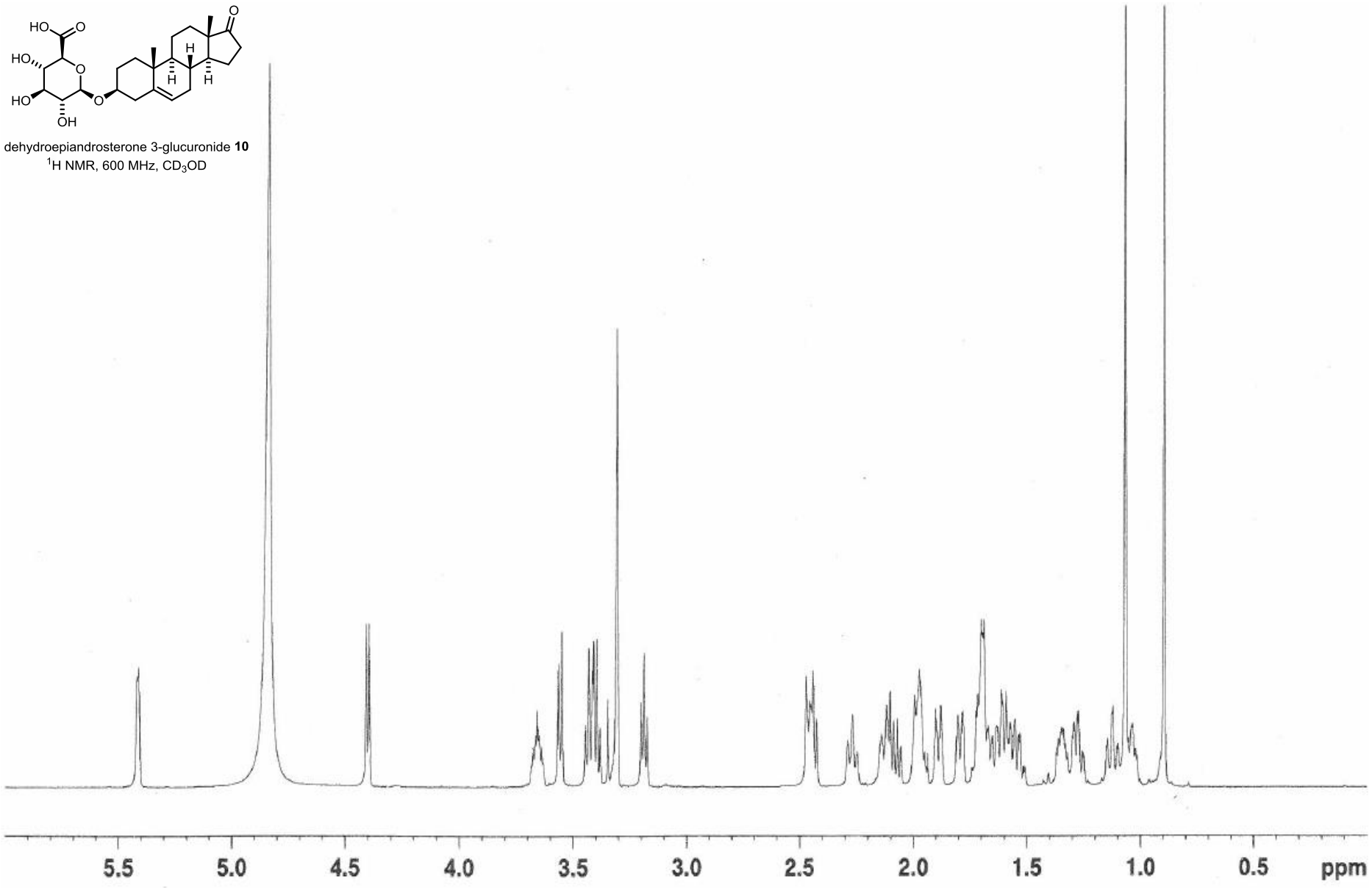
boldenone 17-glucuronide **7**
^{1}H NMR, 600 MHz, CD_3OD



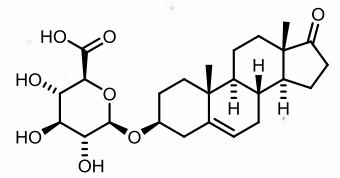
androstanolone 17-glucuronide **8**
^{1}H NMR, 600 MHz, CD_3OD



mesterolone 17-glucuronide **9**
^{1}H NMR, 600 MHz, CD_3OD

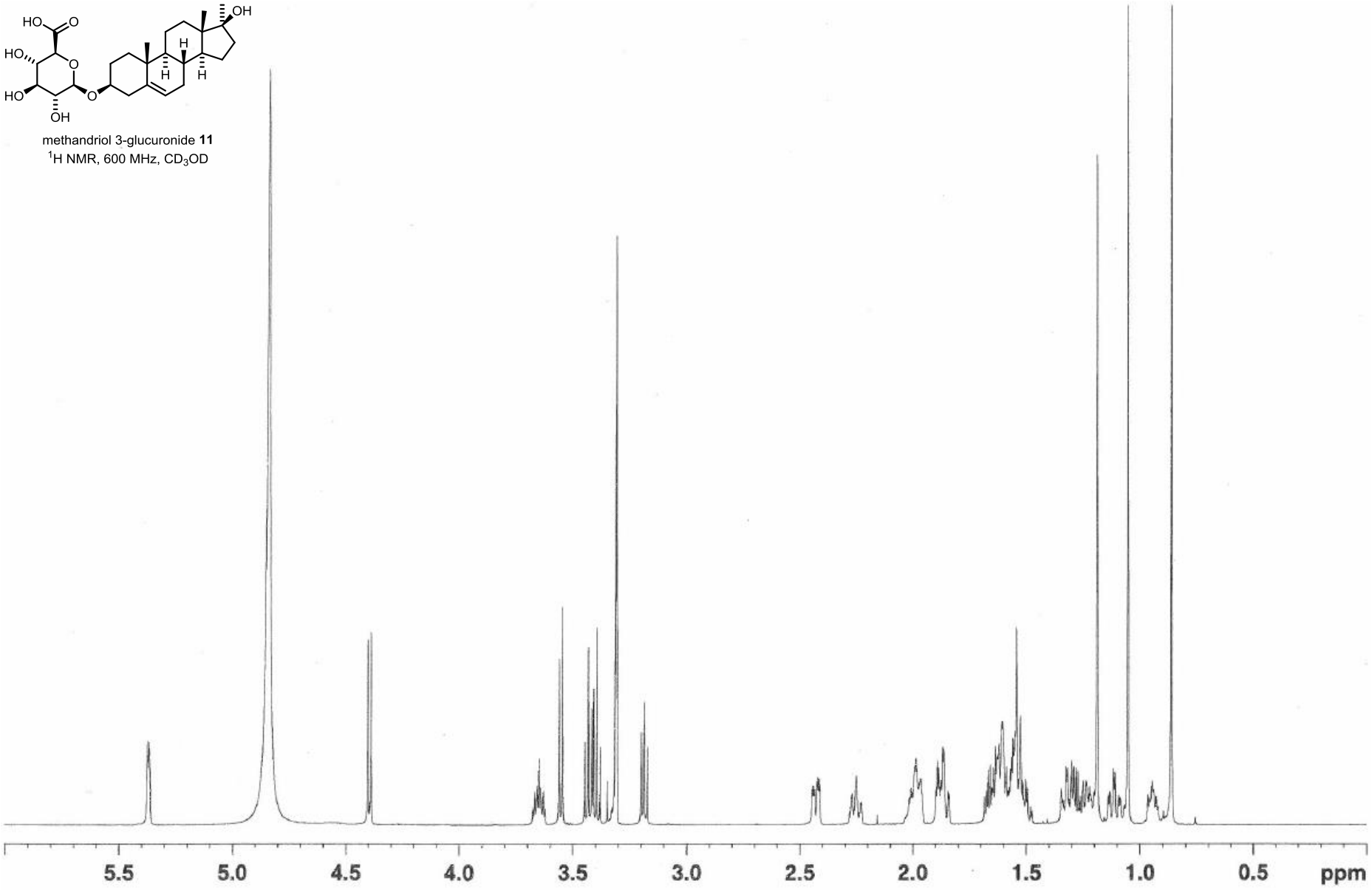


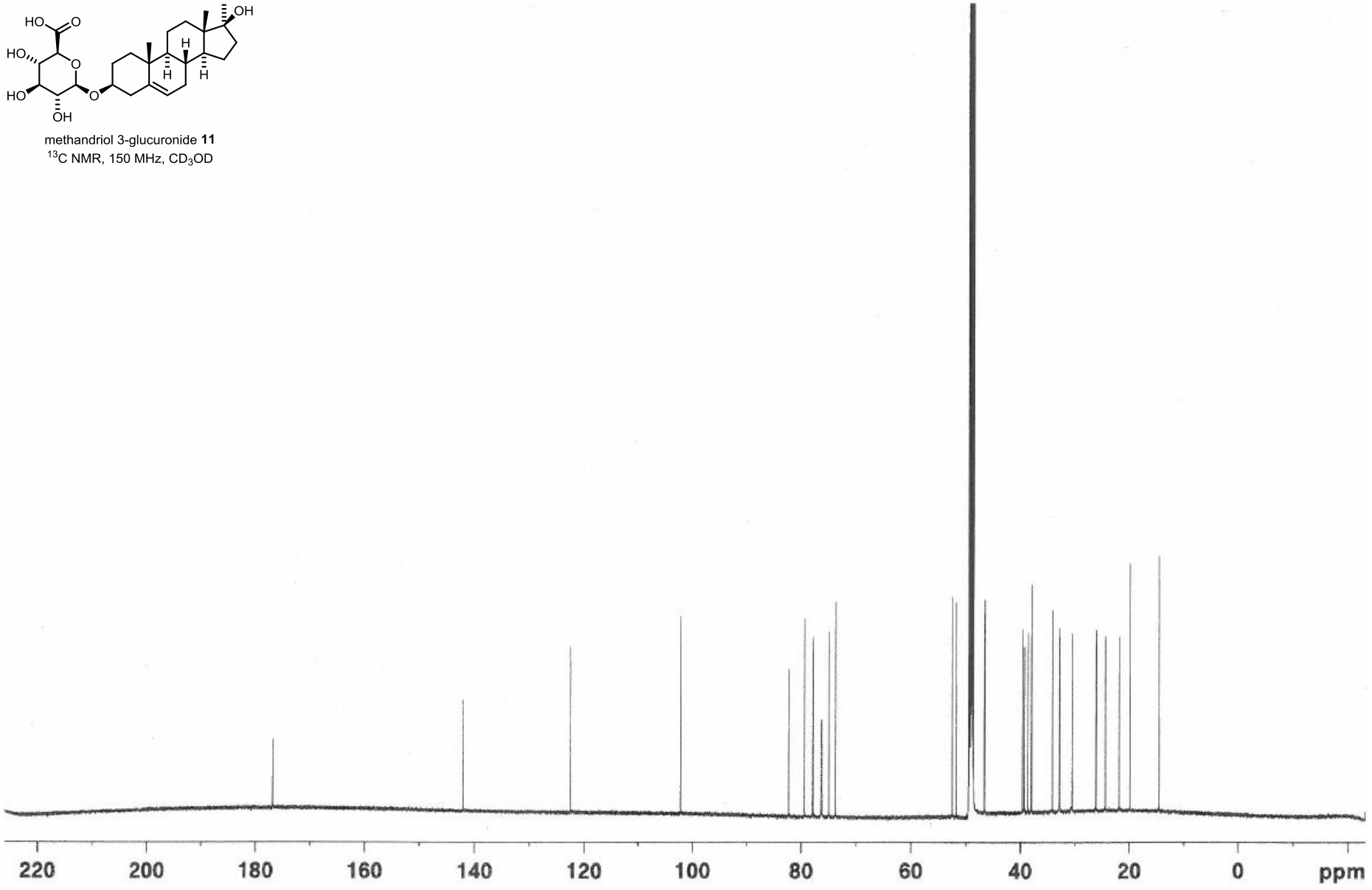
dehydroepiandrosterone 3-glucuronide **10**
^{1}H NMR, 600 MHz, CD_3OD



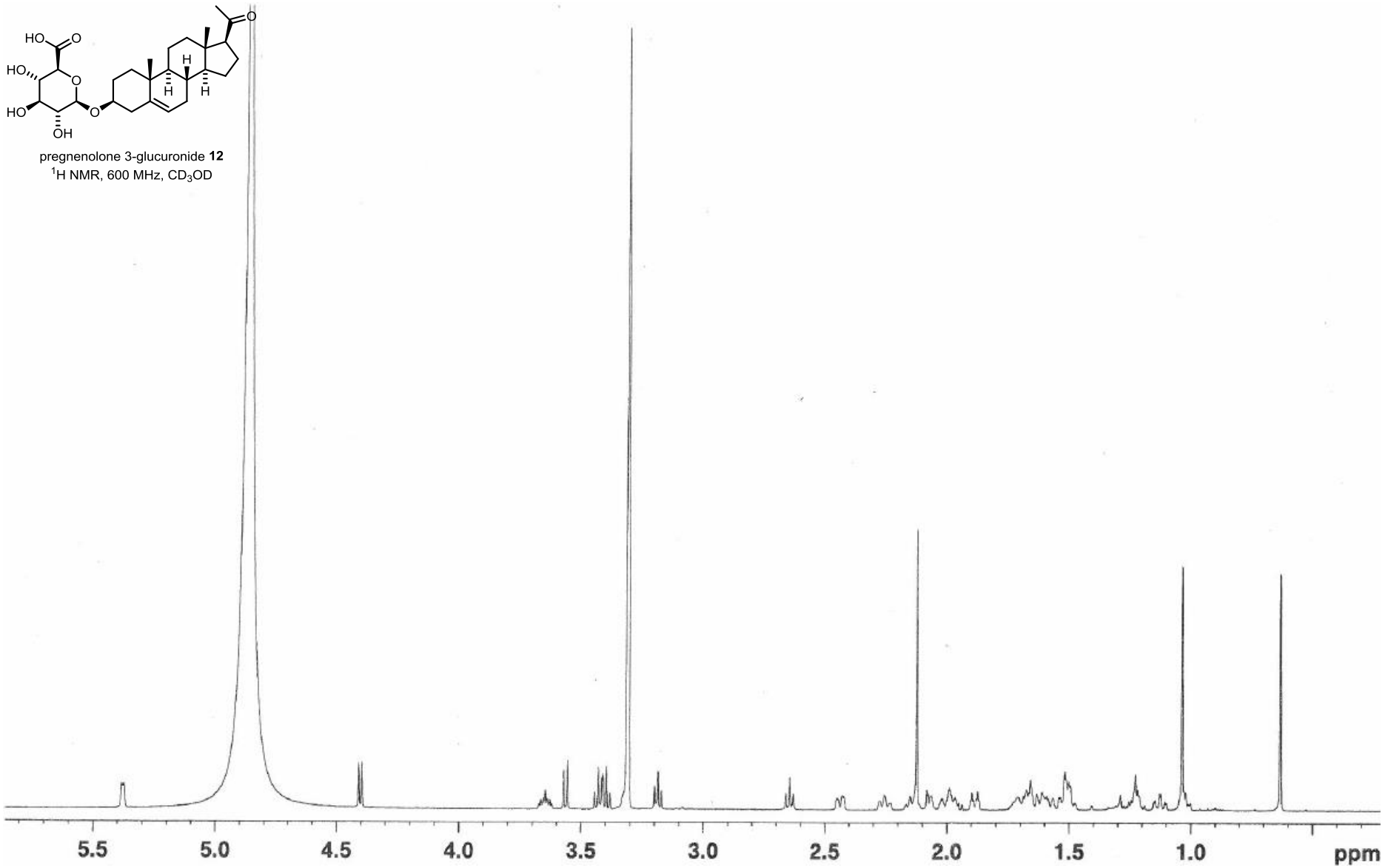
dehydroepiandrosterone 3-glucuronide **10**

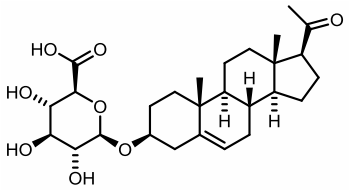
^{13}C NMR, 150 MHz, CD_3OD


HO
O
HO
O
HO
OH
O
OH
H
H
H
methandriol 3-glucuronide **11**
^{1}H NMR, 600 MHz, CD_3OD
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

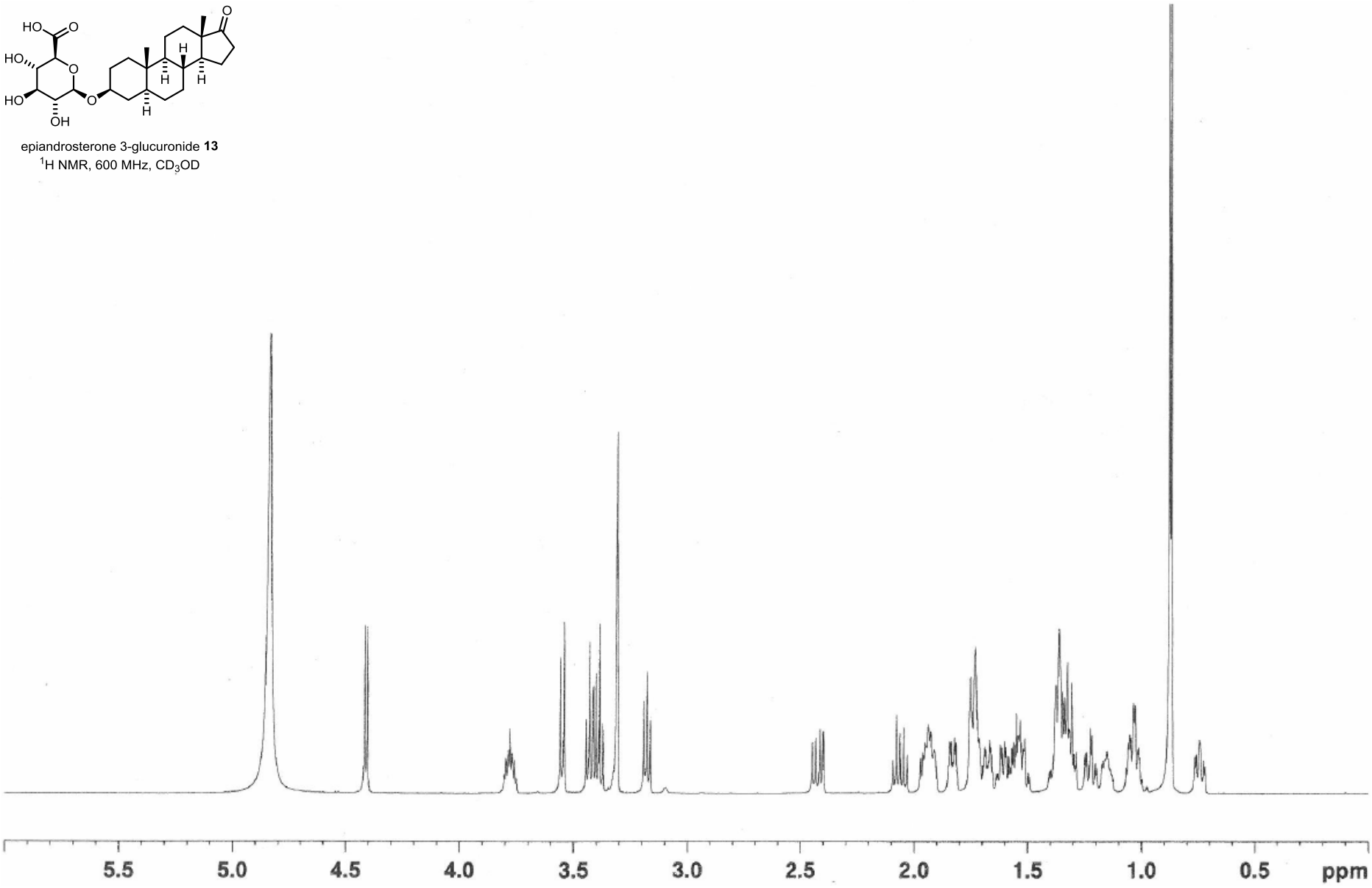


methandriol 3-glucuronide **11**
^{13}C NMR, 150 MHz, CD_3OD

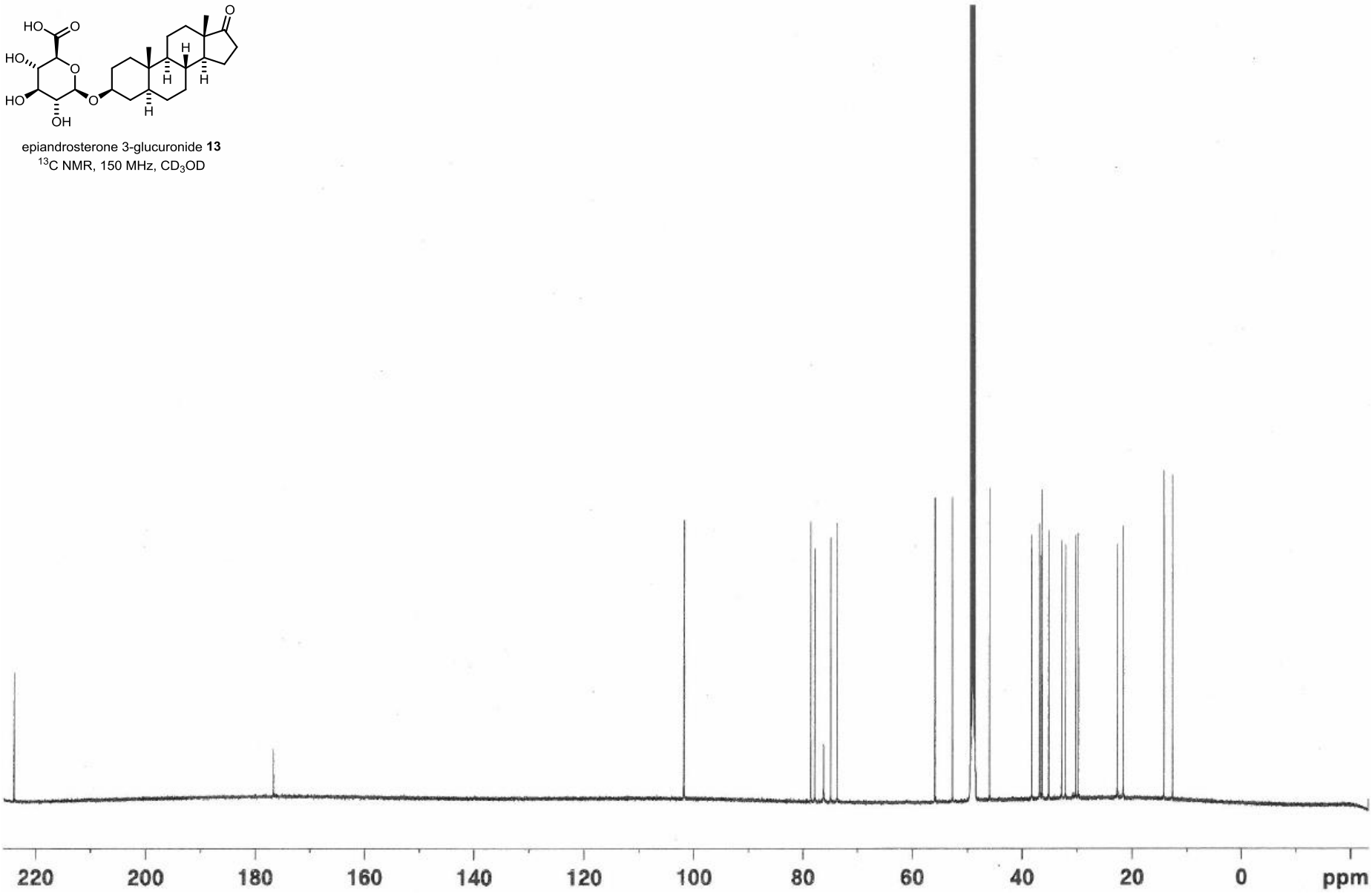

HO
O
HO
O
HO
OH
O
H
H
H
O
pregnenolone 3-glucuronide **12**
^{1}H NMR, 600 MHz, CD_3OD
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

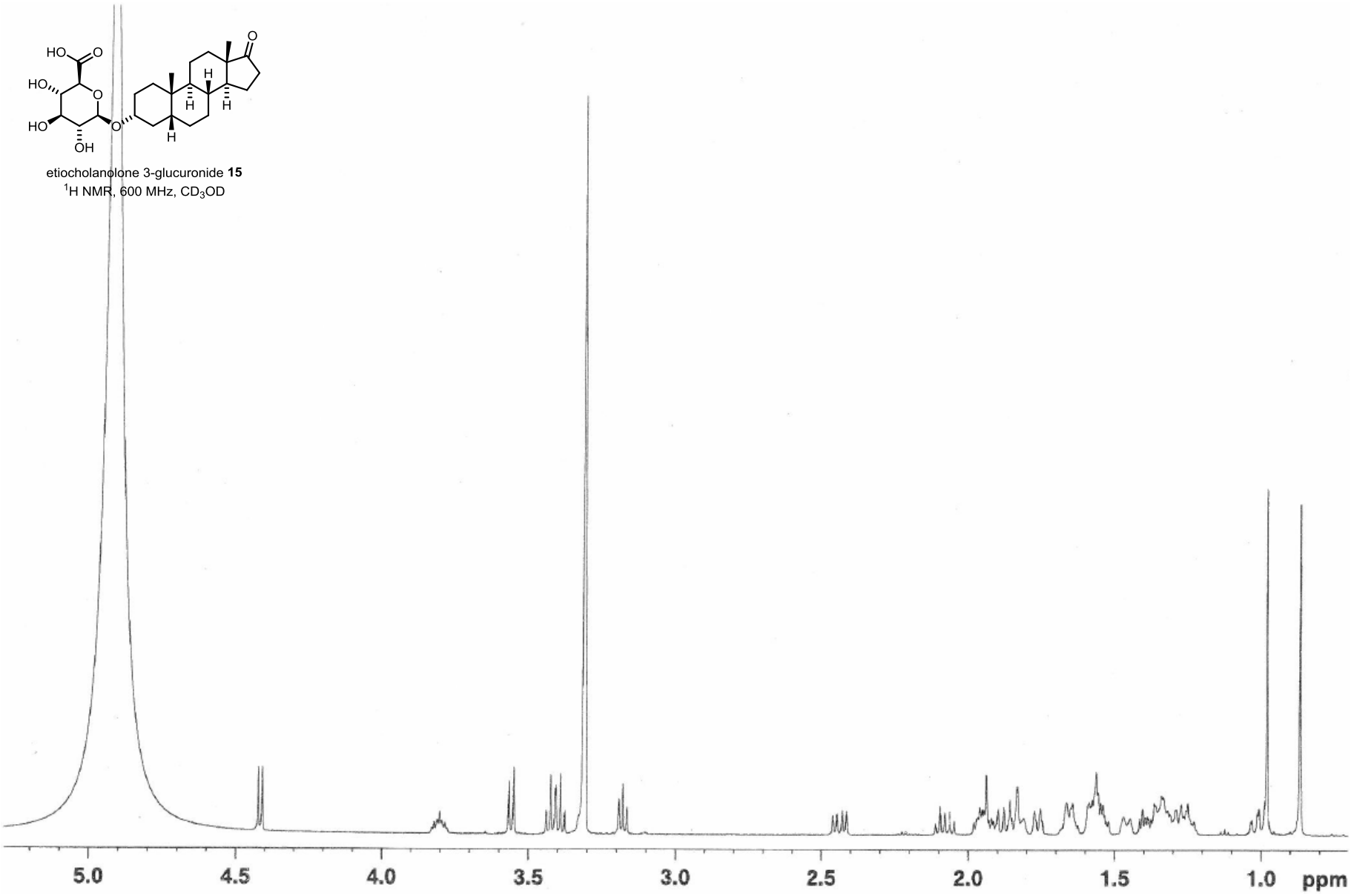


pregnenolone 3-glucuronide **12**
^{13}C NMR, 150 MHz, CD_3OD

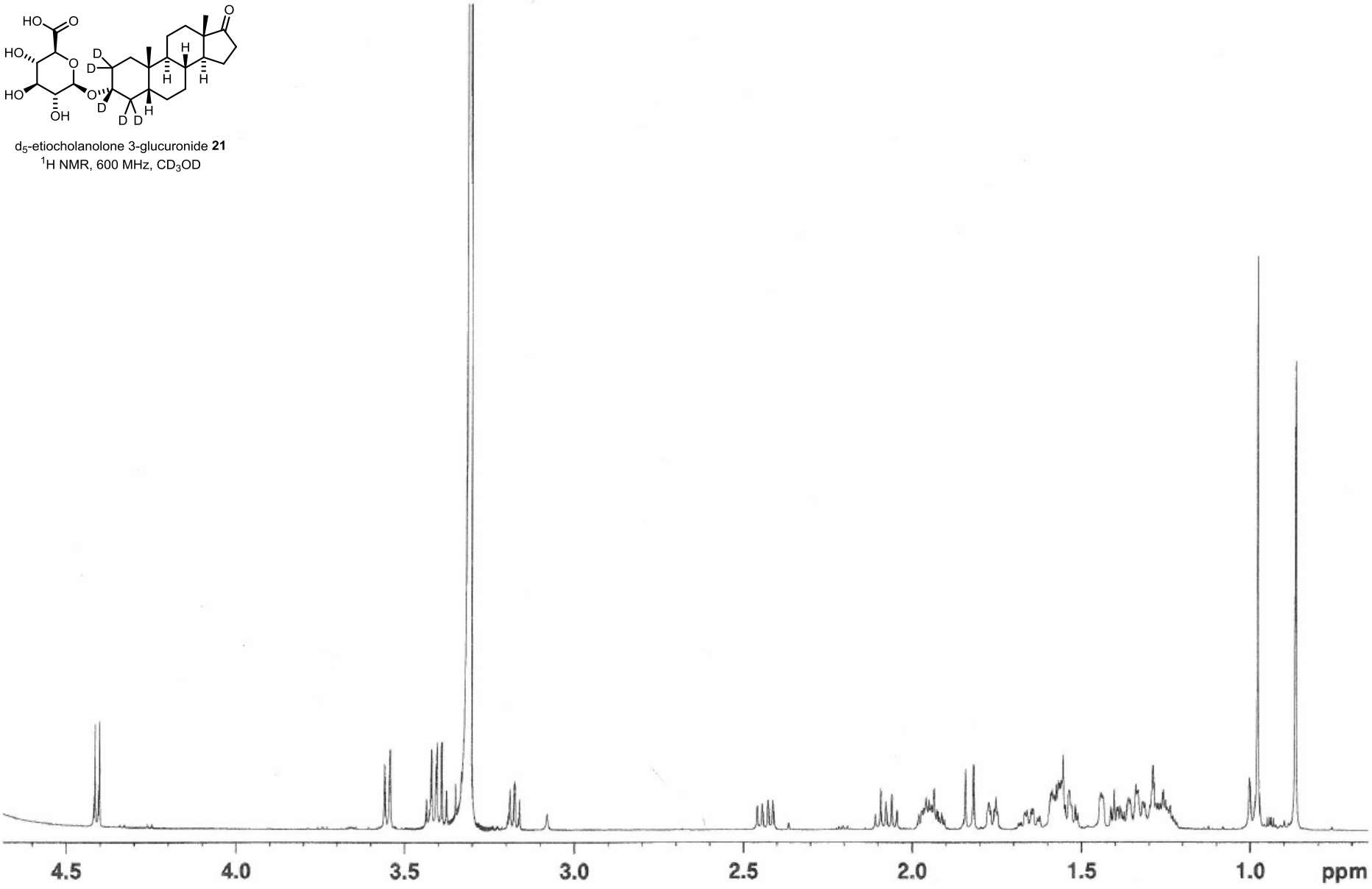


epiandrosterone 3-glucuronide **13**
^{1}H NMR, 600 MHz, CD_3OD

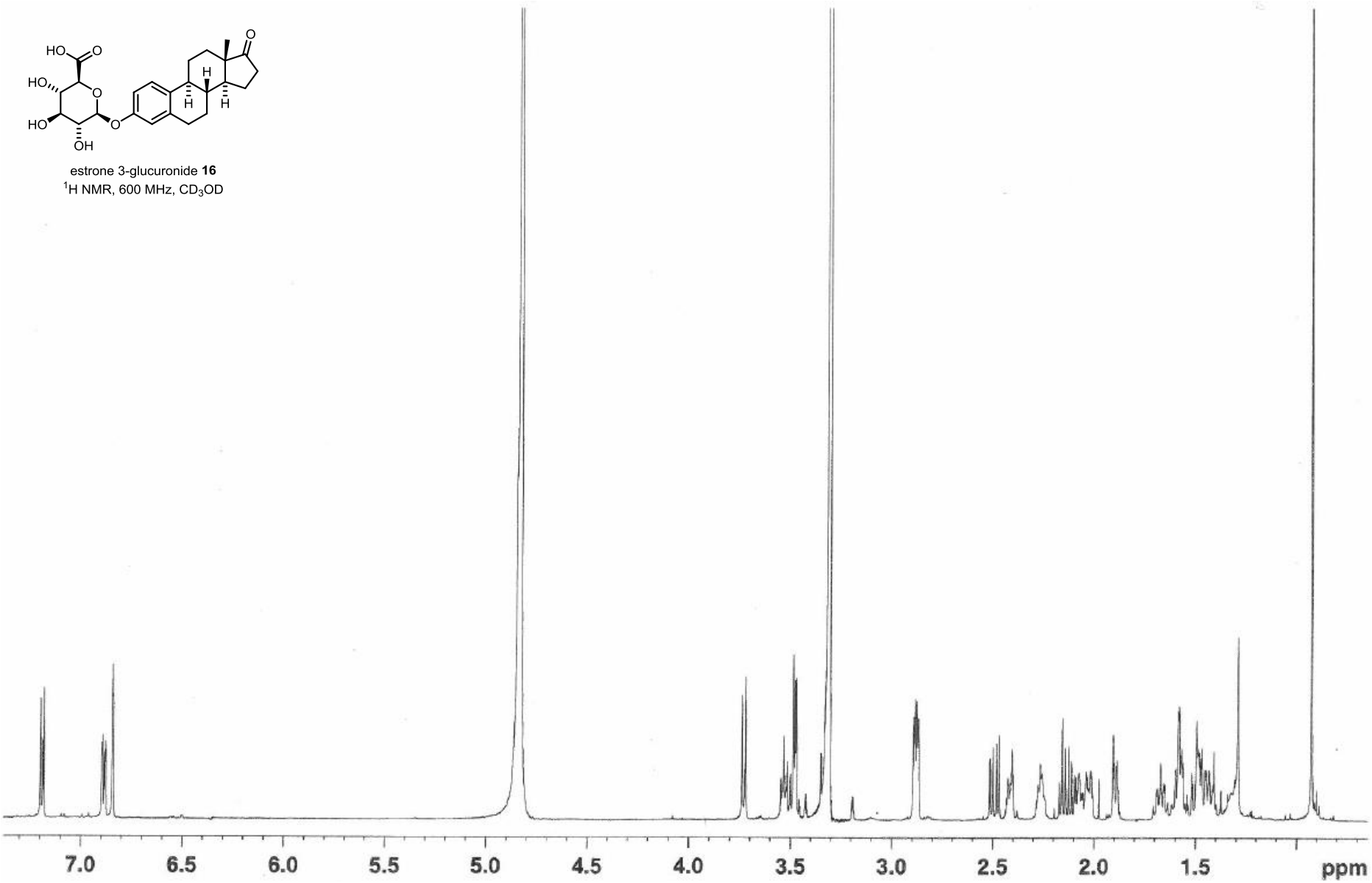

HO
O
HO
O
HO
O
OH
O
H
H
H
H
epiandrosterone 3-glucuronide 13
13C NMR, 150 MHz, CD3OD
220
200
180
160
140
120
100
80
60
40
20
0
ppm



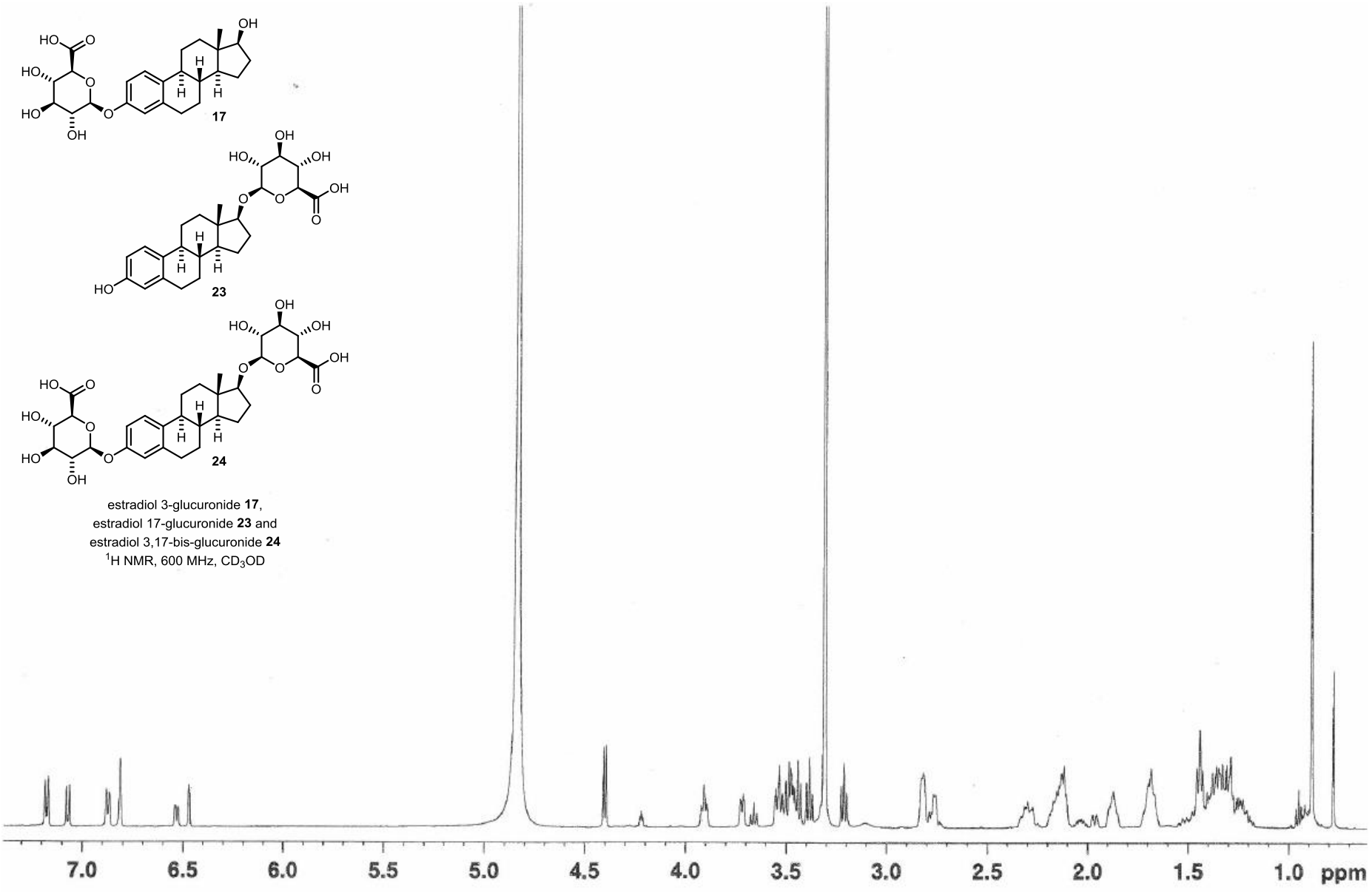
etiocholanolone 3-glucuronide **15**
^{1}H NMR, 600 MHz, CD_3OD



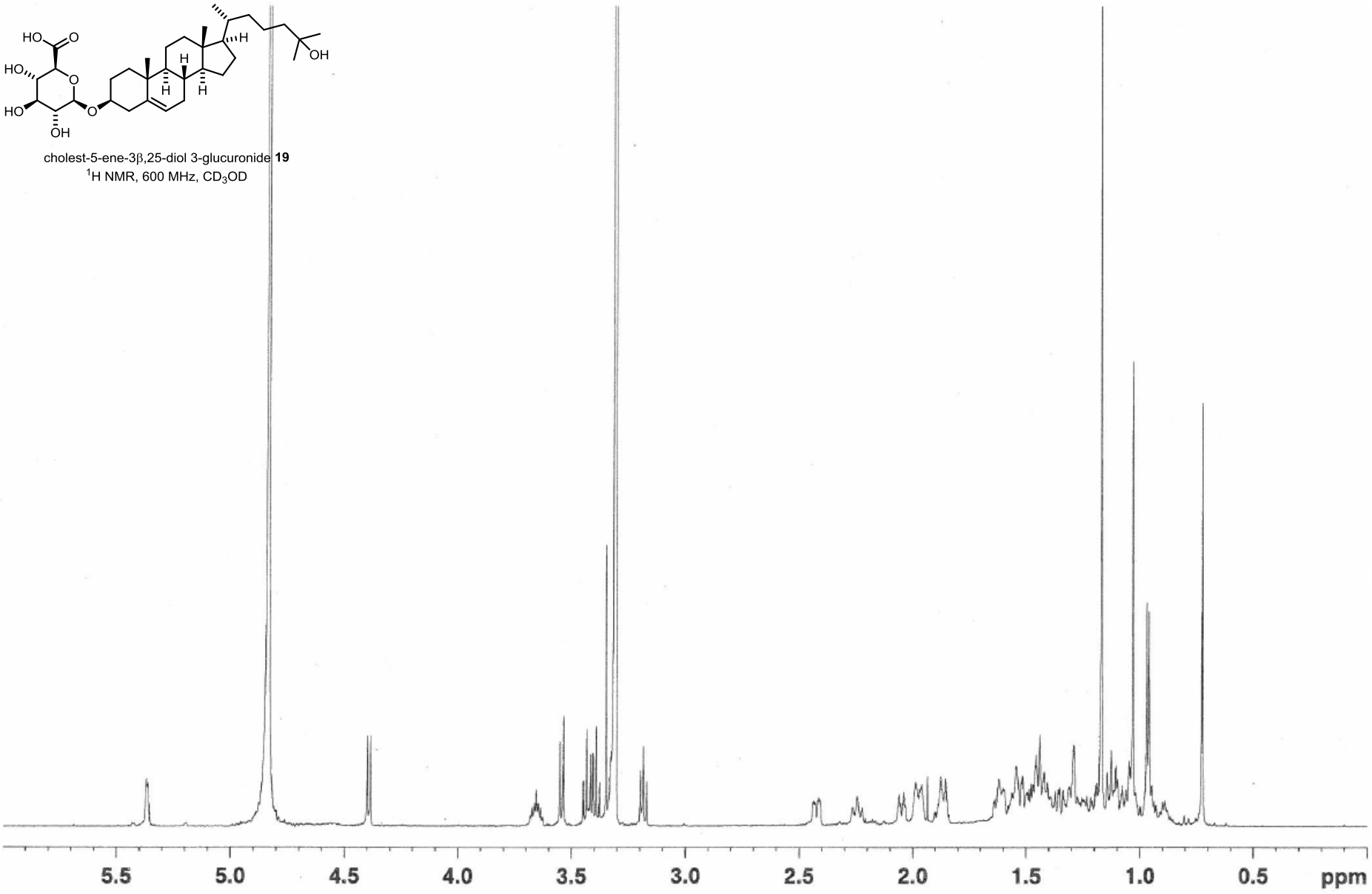
d_5-etiocholanolone 3-glucuronide **21**
^{1}H NMR, 600 MHz, CD_3OD



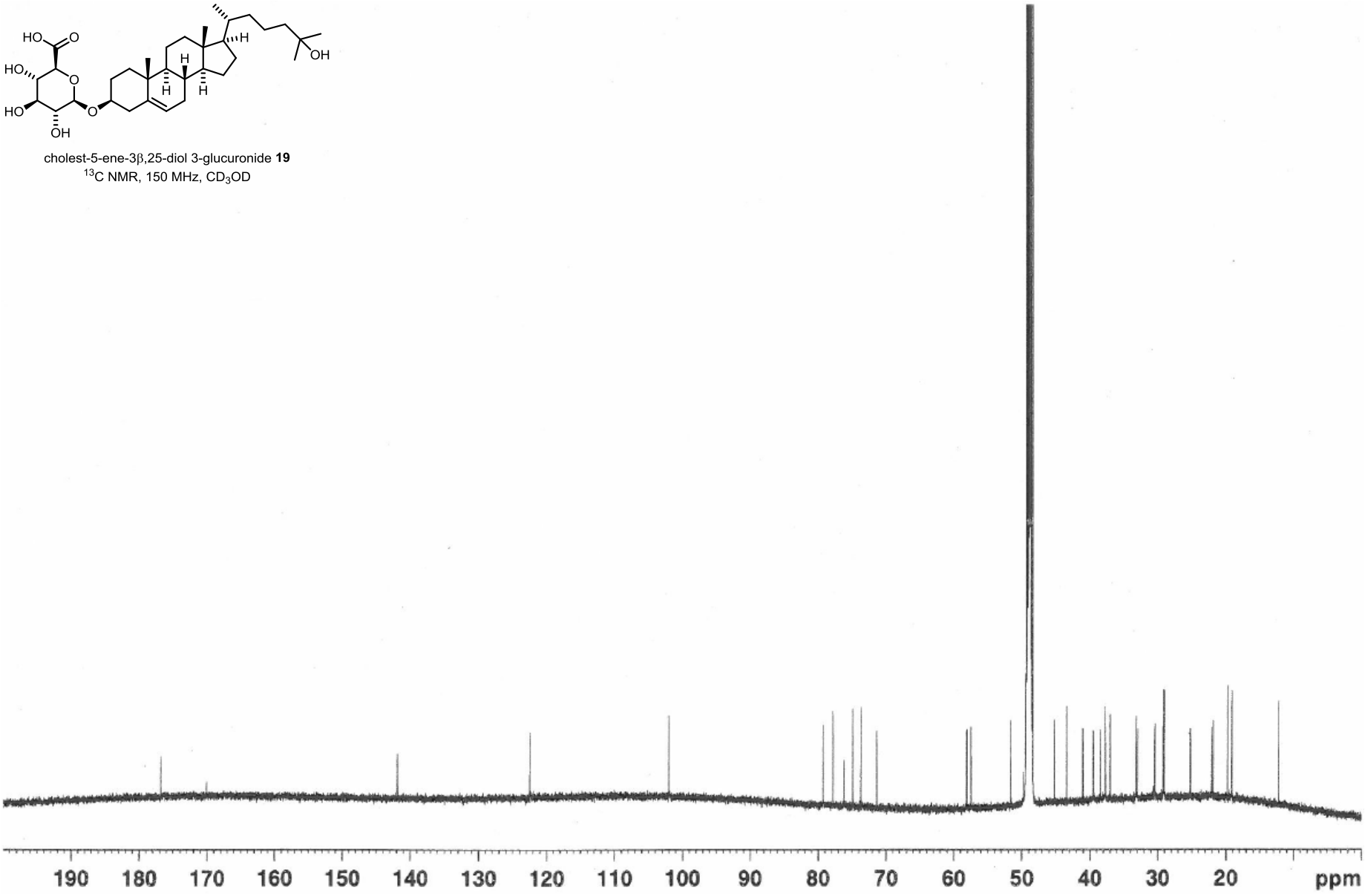
estrone 3-glucuronide **16**
^{1}H NMR, 600 MHz, CD_3OD



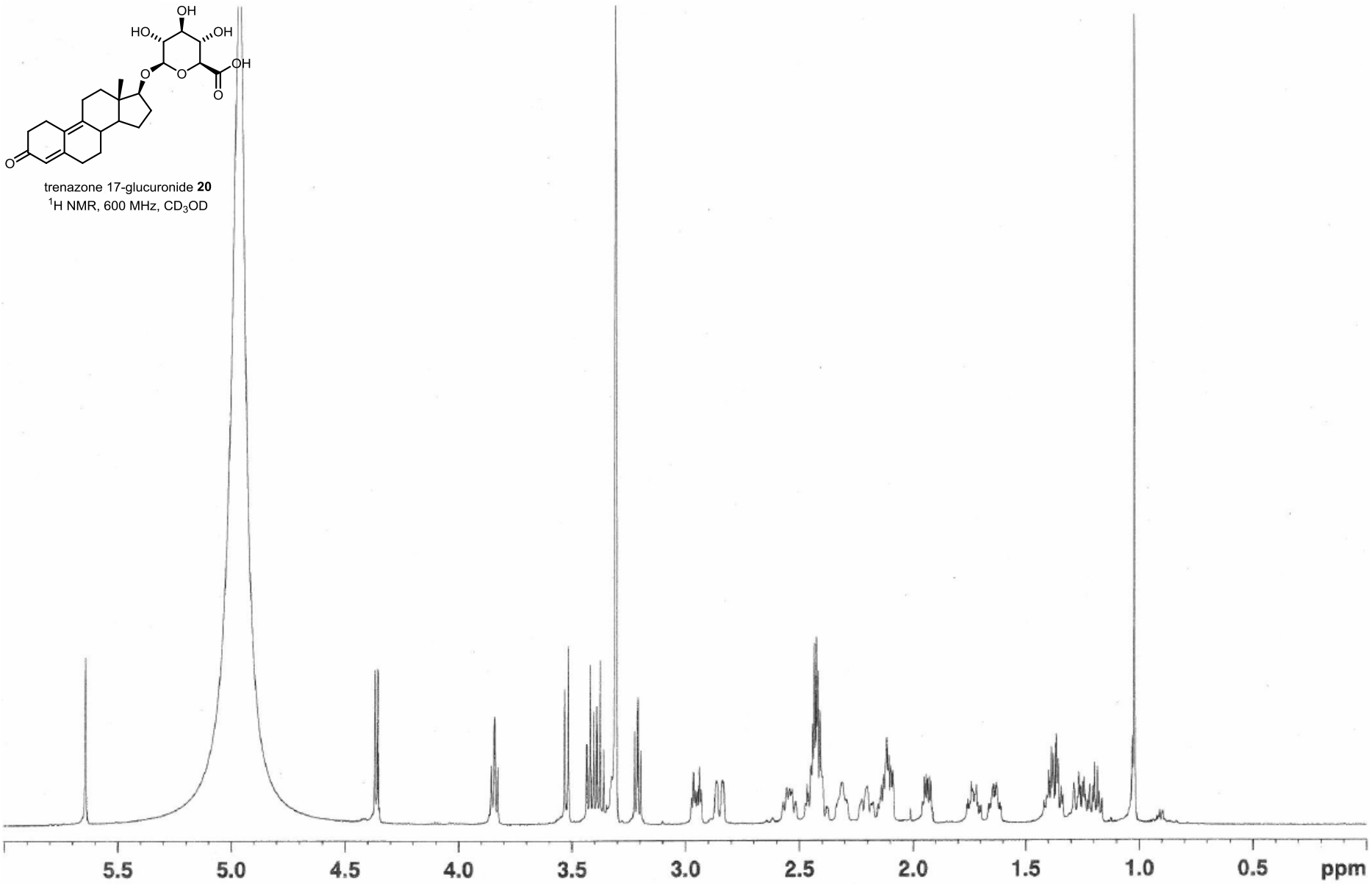
estradiol 3-glucuronide **17**,
estradiol 17-glucuronide **23** and
estradiol 3,17-bis-glucuronide **24**
^{1}H NMR, 600 MHz, CD_3OD

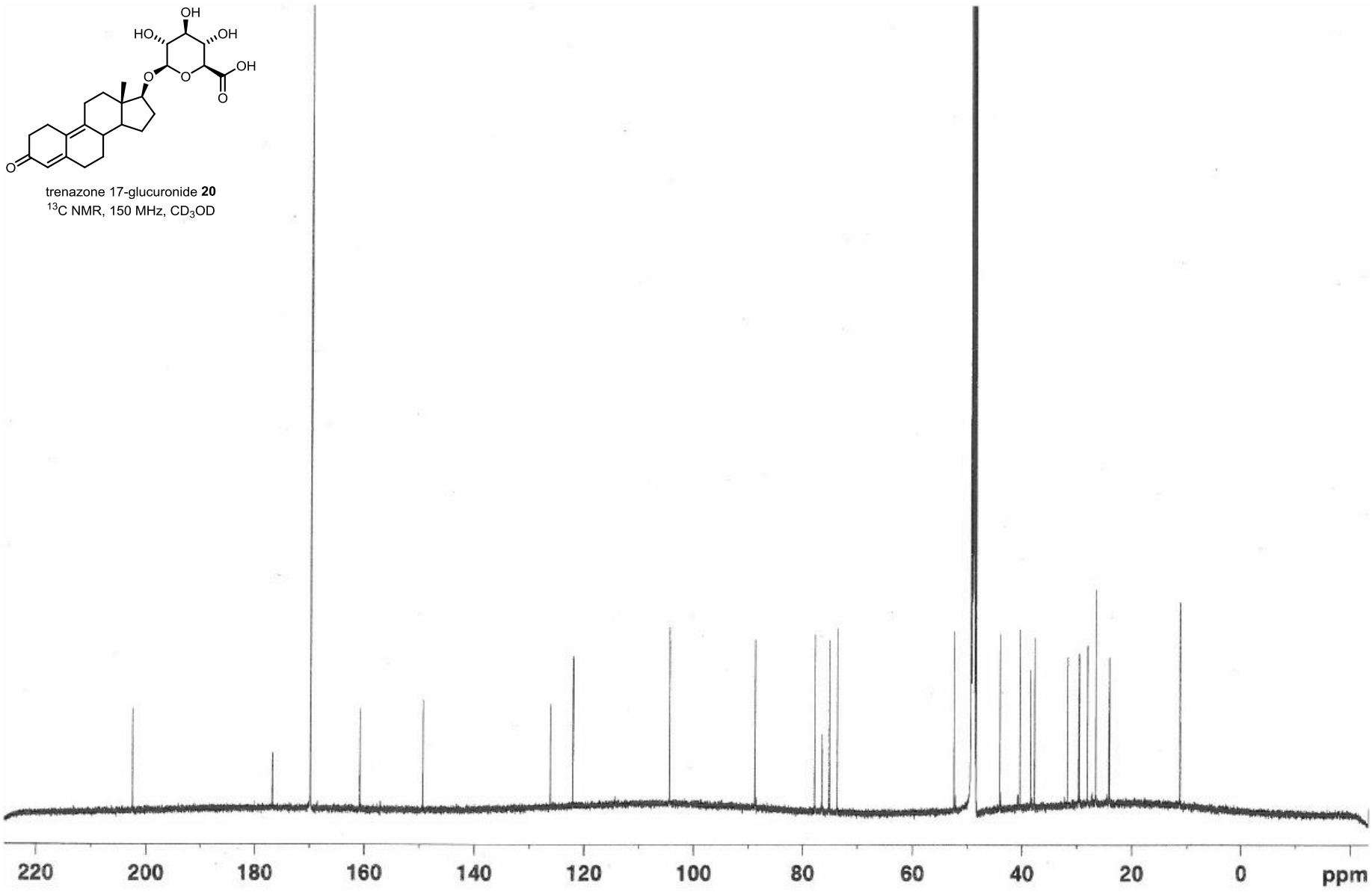


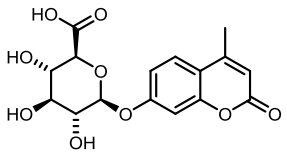
cholest-5-ene-3β,25-diol 3-glucuronide **19**
^{1}H NMR, 600 MHz, CD_3OD



cholest-5-ene-3β,25-diol 3-glucuronide **19**
^{13}C NMR, 150 MHz, CD_3OD

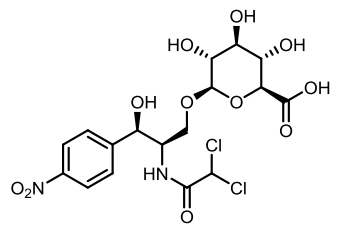

OH
HO
OH
OH
O
O
O
O
trenazone 17-glucuronide **20**
^{1}H NMR, 600 MHz, CD_3OD
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


OH
HO
OH
OH
O
O
O
O
trenazone 17-glucuronide **20**
^{13}C NMR, 150 MHz, CD_3OD
220
200
180
160
140
120
100
80
60
40
20
0
ppm

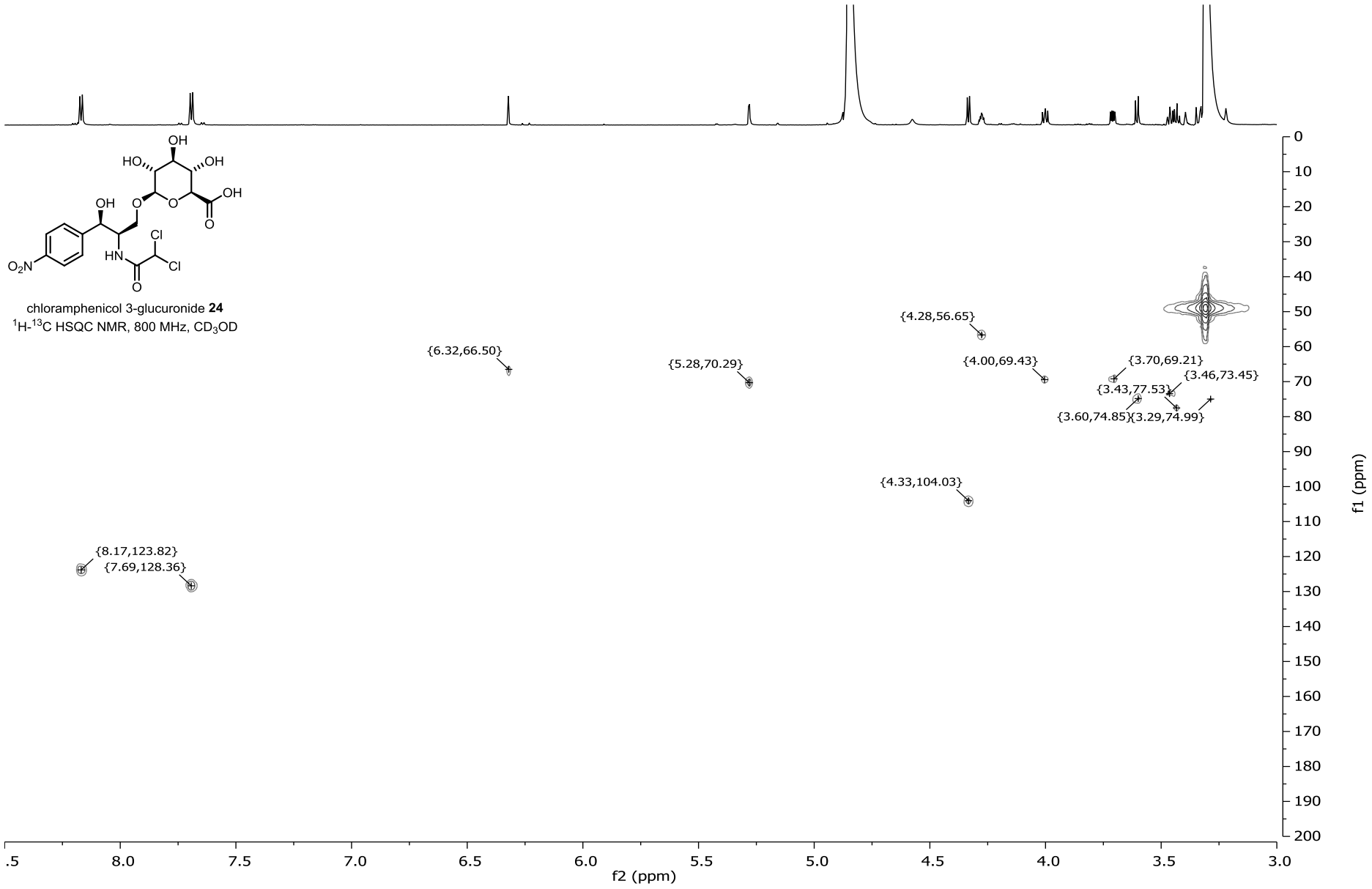


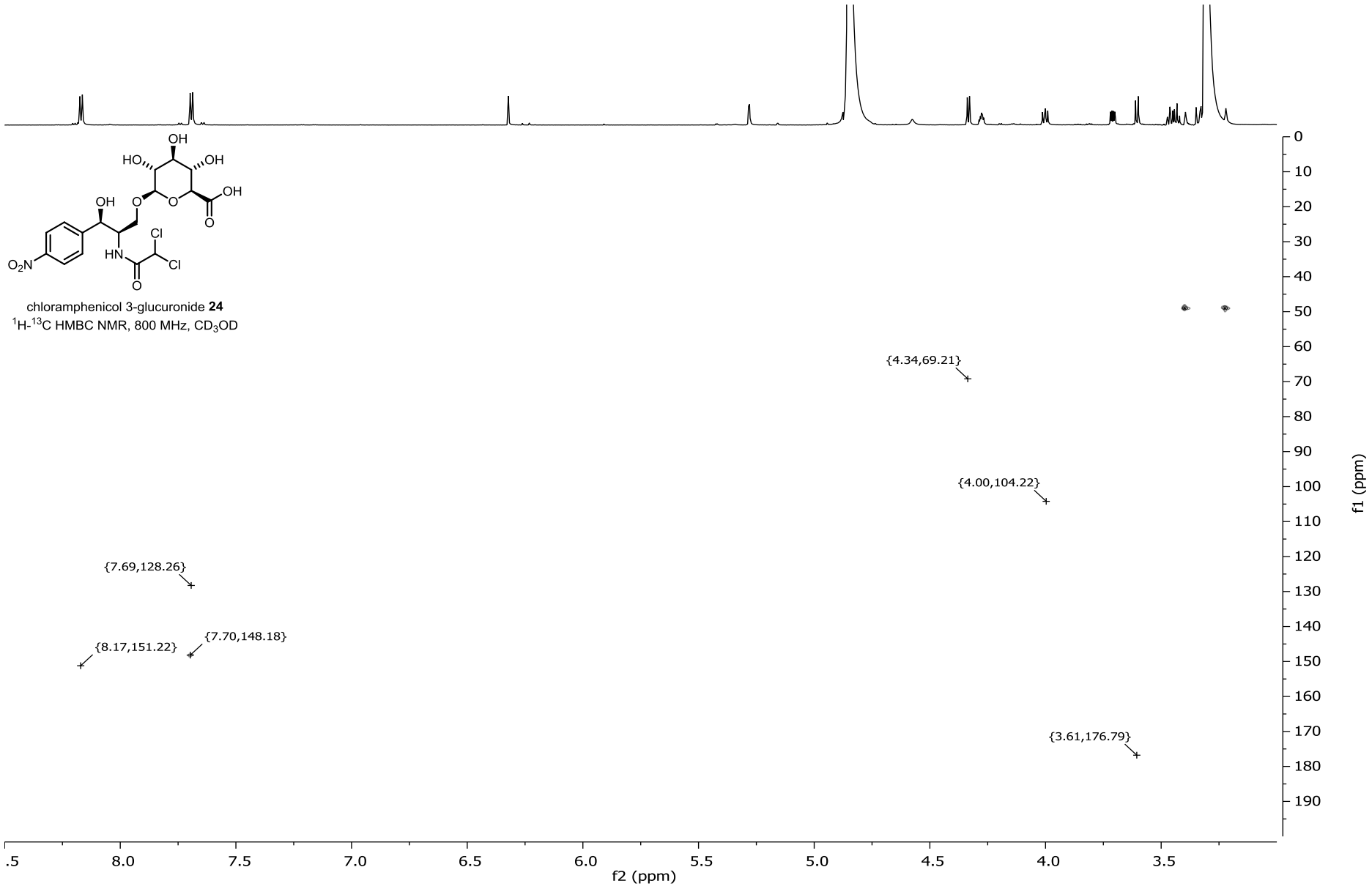
4-methylumbelliferone 7-glucuronide **23**

^{1}H NMR, 400 MHz, CD_3OD



chloramphenicol 3-glucuronide **24**
^{1}H NMR, 800 MHz, CD_3OD


chloramphenicol 3-glucuronide **24**
^{1}H-^{13}C HSQC NMR, 800 MHz, CD_3OD
{8.17,123.82}
{7.69,128.36}
{6.32,66.50}
{5.28,70.29}
{4.33,104.03}
{4.28,56.65}
{4.00,69.43}
{3.70,69.21}
{3.46,73.45}
{3.43,77.53}
{3.60,74.85}
{3.29,74.99}
f2 (ppm)
f1 (ppm)


chloramphenicol 3-glucuronide **24**
^{1}H-^{13}C HMBC NMR, 800 MHz, CD_3OD
{4.34,69.21}
{4.00,104.22}
{7.69,128.26}
{8.17,151.22}
{7.70,148.18}
{3.61,176.79}
f2 (ppm)
f1 (ppm)

CD_3OD blank
^1H NMR, 400 MHz