Supporting information

The *in vivo* metabolism of *Jungle Warfare* in greyhounds

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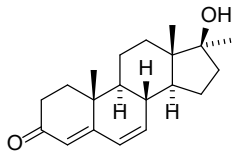
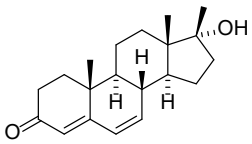
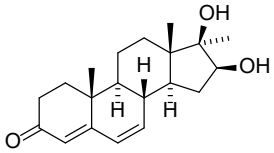
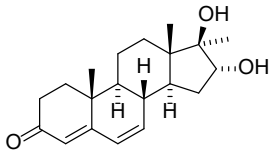
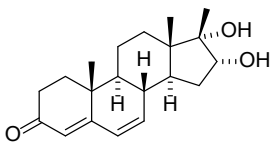
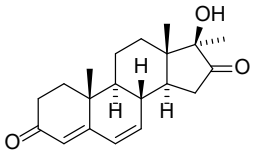
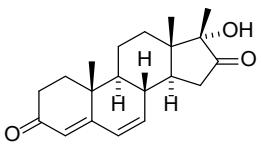
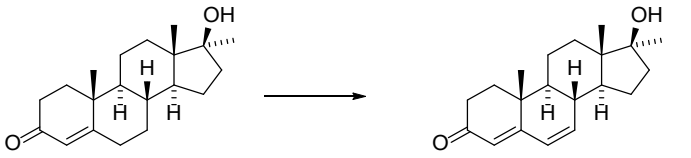
Reference materials

Structure	Name & abbreviation
	17β-hydroxy-17α-methylandrosta-4,6-dien-3-one (**JW**)
	17α-hydroxy-17β-methylandrosta-4,6-dien-3-one (**epiJW**)
	16β,17β-dihydroxy-17α-methylandrosta-4,6-dien-3-one (**16β-OH-JW**)
	16α,17β-dihydroxy-17α-methylandrosta-4,6-dien-3-one (**16α-OH-JW**)
	16α,17α-dihydroxy-17β-methylandrosta-4,6-dien-3-one (**16α-OH-epiJW**)
	17β-hydroxy-17α-methylandrosta-4,6-diene-3,16-dione (**16-keto-JW**)
	17α-hydroxy-17β-methylandrosta-4,6-diene-3,16-dione (**16-keto-epiJW**)

Chemical synthesis of reference materials

17β-Hydroxy-17α-methylandrosta-4,6-dien-3-one (JW) [1,2]

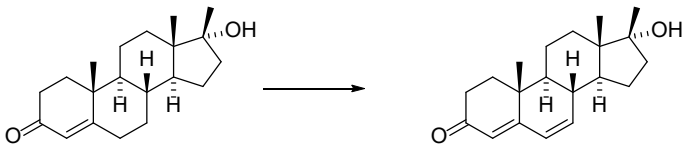


Chemical Formula: $C_{20}H_{30}O_2$
Molecular Weight: 302.4580

Chemical Formula: $C_{20}H_{28}O_2$
Molecular Weight: 300.4420

The reaction was conducted according to a literature method with modifications.[1] A solution of 17β-hydroxy-17α-methylandrost-4-en-3-one (80 mg, 0.26 mmol) in *tert*-butanol (6 mL) was treated with chloranil (322 mg, 1.31 mmol). The resulting slurry was heated to 100 ºC in a sealed tube and stirred for 2 h. Excess chloranil was filtered off and filtrate was extracted with dichloromethane (3 x 30 mL). The combined extract was washed with aqueous sodium hydroxide solution (1 M, 30 mL) and the aqueous fraction was re-extracted with dichloromethane (2 x 30 mL). The combined organics were washed with water (50 mL), then brine (50 mL), then dried with anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by column chromatography (2:3 ethyl acetate:n-hexane) to afford the title compound (52 mg, 65% yield) as a yellow solid. R_f 0.48 (2:3 ethyl acetate:n-hexane); mp 178-182 ºC (lit[2] 193-196 ºC); $[\alpha]^{25}_D$ +20º (*c* 1.0, $CHCl_3$) (lit[2] +35, *c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 6.15 (s, 2H, C6- & C7-H), 5.68 (s, 1H, C4-H), 2.57 (m, 1H, C2-H), 2.44 (m, 1H, C2-H), 2.28 (t, *J* = 10.3 Hz, 1H, C8-H), 2.02 (m, 1H), 1.90 (m, 1H), 1.93-1.56 (m, 5H), 1.51-1.30 (m, 4H), 1.25-1.15 (m, 1H), 1.23 (s, 3H, C20-H_3), 1.13 (s, 3H, C19-H_3), 0.96 (s, 3H, C18-H_3), OH not observed; ^{13}C NMR (101 MHz, $CDCl_3$): 199.7 (C3), 163.9 (C5), 140.8 (C7), 128.1 (C6), 123.8 (C4), 81.4 (C17), 50.9, 48.0, 46.5, 38.9, 38.5, 36.3, 34.1 (2C), 31.6, 26.0, 22.9, 20.5, 16.5 (C19), 13.9 (C18); IR (neat): 3436 (br, w, O-H), 3024-2872 (m, C-H), 1646 (s, C=O), 1615 (s, C=C); LRMS (+EI): *m/*z 300 (55%, $[C_{20}H_{28}O_2]^+$), 282 (60%), 267 (100%), 242 (45%), 171 (15%), 143 (15%), 131 (30%), 115 (35%), 91 (40%), 77 (20%), 43 (30%); HRMS (+EI): found 300.2099 $[C_{20}H_{28}O_2]^+$ requires 300.2089. ^{1}H and ^{13}C NMR data matched the literature reference.[1]

17α-Hydroxy-17β-methylandrosta-4,6-dien-3-one (epiJW)



Chemical Formula: $C_{20}H_{30}O_2$
Molecular Weight: 302.4580

Chemical Formula: $C_{20}H_{28}O_2$
Molecular Weight: 300.4420

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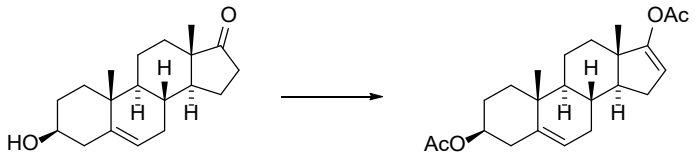
chloranil (322 mg, 1.31 mmol). The resulting slurry was heated to 100 °C in a sealed tube and stirred for 2 h. Excess chloranil was filtered off and filtrate was extracted with dichloromethane (3 x 30 mL). The combined extract was washed with aqueous sodium hydroxide solution (1 M, 30 mL) and the aqueous fraction was re-extracted with dichloromethane (2 x 30 mL). The combined organics were washed with water (50 mL), then brine (50 mL), then dried with anhydrous magnesium sulfate and concentrated under reduced pressure. The crude residue was purified by column chromatography (2:3 ethyl acetate:n-hexane) to afford the title compound (52 mg, 65% yield) as a yellow solid. R_f 0.48 (2:3 ethyl acetate:n-hexane); mp 155-160 °C; $[\alpha]^{25}_D$ -18.3° (*c* 0.3, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): 6.12 (m, 2H, C6- & C7-H), 5.66 (s, 1H, C4-H), 2.57 (m, 1H, C2-H), 2.42 (m, 1H, C2-H), 2.23 (t, *J* = 10.5 Hz, 1H, C8-H), 2.00 (m, 1H), 1.94-1.85 (m, 3H), 1.76-1.62 (m, 3H), 1.57-1.32 (m, 4H), 1.27-1.19 (m, 1H), 1.23 (s, 3H, C20-H_3), 1.12 (s, 3H, C19-H_3), 0.77 (s, 3H, C18-H_3), OH not observed; ^{13}C NMR (101 MHz, $CDCl_3$): 199.8 (C3), 164.1 (C5), 141.6 (C7), 127.9 (C6), 123.7 (C4), 81.9 (C17), 50.7, 47.6, 47.2, 38.5, 38.2, 36.3, 34.1 (2C), 29.8, 23.5, 22.9, 20.3, 16.5 (C19), 15.8 (C18); IR (neat): 3455 (br, w, O-H), 3027-2866 (m, C-H), 1645 (s, C=O), 1614 (s, C=C); LRMS (+EI): *m/*z 300 (1%, $[C_{20}H_{28}O_2]^+$), 282 (40%), 267 (100%), 173 (5%), 147 (10%), 131 (10%), 115 (10%), 91 (20%), 79 (10%), 43 (10%); HRMS (+EI): found 300.2083 $[C_{20}H_{28}O_2]^+$ requires 300.2089.

3β,17-Diacetoxyandrosta-5,16-diene [3–5]



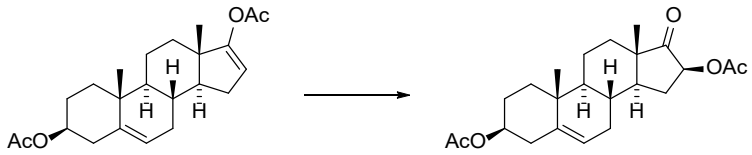
Chemical Formula: $C_{19}H_{28}O_2$
Molecular Weight: 288.4310

Chemical Formula: $C_{23}H_{32}O_4$
Molecular Weight: 372.5050

The reaction was conducted according to a literature method with modifications.[4] A solution of dehydroepiandrosterone (1.44 g, 4.99 mmol) in isopropenyl acetate (20 mL) was treated with concentrated sulfuric acid (5 drops) and heated to 110 °C for 5 h with stirring. Further isopropenyl acetate (10 mL) and concentrated sulfuric acid (5 drops) was added after 2 h and 4 h of heating. The reaction was then cooled to room temperature and diluted with diethyl ether (80 mL). The organic layer was washed with saturated aqueous sodium bicarbonate solution (2 x 50 mL), water (50 mL), followed by brine (50 mL). The organic layer was then dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (1:7 ethyl acetate:n-hexane) to afford the title compound (1.23 g, 3.30 mmol, 66% yield) as a white solid. R_f 0.63 (1:1 ethyl acetate:n-hexane); mp 143-146 °C (lit[5] mp 146-148 °C); $[\alpha]^{23}_D$ -40.5° (*c* 1.0, $CHCl_3$) (lit[3] $[\alpha]^{25}_D$ -47.7°, *c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 5.48 (dd, *J* = 3.0, 1.5 Hz, 1H, C16-H), 5.39 (d, *J* = 5.0 Hz, 1H, C6-H), 4.61 (m, 1H, C3-H),

2.38-2.28 (m, 2H), 2.21-2.13 (m, 2H), 2.15 (s, 3H), 2.03 (s, 3H), 2.04-1.82 (m, 4H), 1.74-1.38 (m, 7H), 1.19-1.03 (m, 2H), 1.05 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): 170.7, 169.0, 159.7, 140.2, 122.4, 111.4, 74.0, 54.2, 50.7, 44.8, 38.3, 37.0, 37.0, 33.5, 31.0, 30.2, 29.1, 27.9, 21.6, 21.3, 20.5, 19.4, 15.5; IR (neat): 2979-2836 (w, C-H), 1768 (m, C=O), 1725 (s, C=O), 1631 (m, C=C); LRMS (+ESI): 767 (20%, $[C_{46}H_{64}O_8Na]^+$, $[2M+Na]^+$), 1140 (100%, $[C_{69}H_{96}O_{12}Na]^+$, $[3M+Na]^+$); HRMS (+ESI): found 395.2203, $[C_{23}H_{32}O_4Na]^+$ requires 395.2198. ^{1}H NMR data matched the literature reference.[4,5]

3β,16β-Diacetoxyandrost-5-en-17-one (1) [5–7]

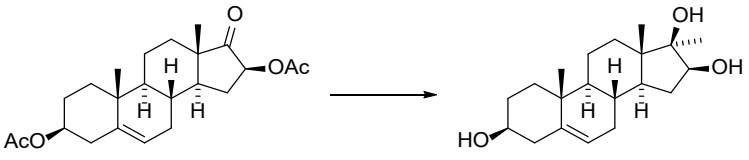


Chemical Formula: $C_{23}H_{32}O_4$
Molecular Weight: 372.5050

Chemical Formula: $C_{23}H_{32}O_5$
Molecular Weight: 388.5040

The reaction was conducted according to a literature method with modifications.[6] A solution of 3β,17-diacetoxyandrosta-5,16-diene (1.11 g, 2.98 mmol, 1.00 eq.) in acetic acid (24 mL) and acetic anhydride (4 mL) was treated with lead(IV) acetate (3.0 g, 6.8 mmol, 2.3 eq.) at room temperature and stirred in the dark for 23 h. The reaction was then diluted with diethyl ether (60 mL) and washed with saturated aqueous sodium bicarbonate solution (3 x 50 mL), followed by water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (1:7 ethyl acetate:n-hexane) to afford the title compound (0.92 g, 2.4 mmol, 80% yield) as a white solid. R_f 0.50 (1:1 ethyl acetate:n-hexane); mp 171-173 ºC (lit[7] mp 172-173 ºC); $[\alpha]^{23}_D$ +5.5º (*c* 1.0, $CHCl_3$) (lit[7] $[\alpha]^{27}_D$ +9º, *c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 5.41 (d, J = 5.3 Hz, 1H, C6-H), 5.00 (t, J = 8.7 Hz, 1H, C16-H), 4.61 (m, 1H, C3-H), 2.50 (m, 1H), 2.40-2.28 (m, 2H), 2.12 (s, 3H), 2.07-2.02 (m, 1H), 2.04 (s, 3H), 1.94-1.85 (m, 3H), 1.76-1.45 (m, 6H), 1.41-1.26 (m, 2H), 1.18-1.01 (m, 2H), 1.06 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): 214.6 (C17), 170.6, 170.4, 140.1, 121.8, 74.8, 73.8, 50.4, 47.0, 46.3, 38.2, 37.0, 37.0, 31.7, 31.0, 30.8, 29.5, 27.8, 21.6, 20.9, 20.2, 19.5, 14.4; IR (neat): 2978-2826 (w, C-H), 1754 (m, C=O), 1739 (s, C=O), 1722 (s, C=O); LRMS (+ESI): 799 (100%, $[C_{46}H_{64}O_{10}Na]^+$, $[2M+Na]^+$), 1188 (30%, $[C_{69}H_{96}O_{15}Na]^+$, $[3M+Na]^+$); HRMS (+ESI): found 411.2149, $[C_{23}H_{32}O_5Na]^+$ requires 411.2147. ^{1}H NMR, ^{13}C NMR, and IR data matched the literature reference.[5–7]

17α-Methylandrost-5-ene-3β,16β,17β-triol (2)

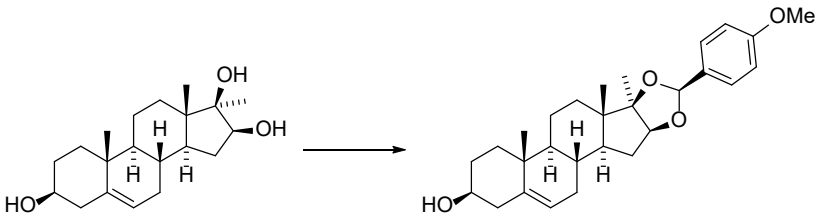


Chemical Formula: $C_{23}H_{32}O_5$
Molecular Weight: 388.5040

Chemical Formula: $C_{20}H_{32}O_3$
Molecular Weight: 320.4730

The reaction was conducted according to a literature method with modifications.[8] A solution of 3β,16β-diacetoxyandrost-5-en-17-one (871 mg, 2.24 mmol, 1.00 eq.) in dry tetrahydrofuran (32 mL) under nitrogen at room temperature was treated slowly over 4 min with a solution of methylmagnesium bromide in diethyl ether (3.0 M, 8.0 mL, 24 mmol, 11 eq.) and stirred for 18 h. The reaction was then diluted with ethyl acetate (80 mL) and washed with saturated aqueous ammonium chloride solution (3 x 50 mL), followed by water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (4:1 ethyl acetate:n-hexane) to afford the title compound (357 mg, 1.11 mmol, 50% yield) as a white solid. R_f 0.14 (1:1 ethyl acetate:n-hexane); mp 180-182 °C; $[\alpha]^{22}_D$ -19.8° (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, CD_3OD): 5.34 (d, *J* = 5.1 Hz, 1H, C6-H), 3.60 (m, 1H), 3.38 (m, 1H), 2.26-2.13 (m, 3H), 2.01 (m, 1H), 1.88 (m, 1H), 1.80 (m, 1H), 1.67-1.43 (m, 4H), 1.39-0.90 (m, 7H), 1.10 (s, 3H), 1.04 (s, 3H), 0.85 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD): 142.3, 122.2, 79.9, 78.4, 72.4, 51.8, 45.9, 43.0, 38.5, 37.8, 36.0, 33.6, 33.6, 33.0, 32.3, 24.3, 21.6, 19.9, 14.2, one peak overlapping or obscured; IR (neat): 3293 (br, m, O-H), 2967-2831 (m, C-H); LRMS (+ESI): 663 (100%, $[C_{40}H_{64}O_6Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 343.2249, $[C_{20}H_{32}O_3Na]^+$ requires 343.2249.

[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-5-en-3β-ol



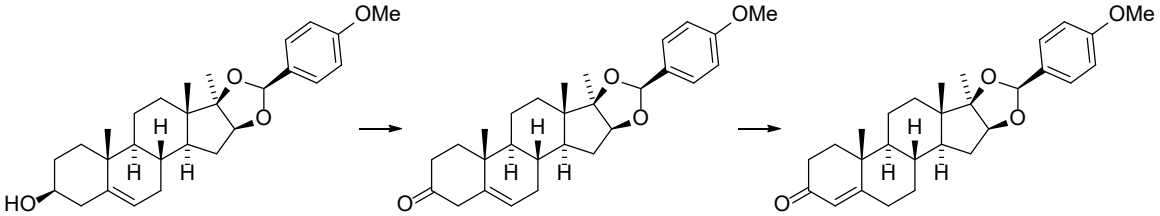
Chemical Formula: $C_{20}H_{32}O_3$
Molecular Weight: 320.4730

Chemical Formula: $C_{28}H_{38}O_4$
Molecular Weight: 438.6080

The reaction was conducted according to a literature method with modifications.[9] A solution of 17α-methylandrost-5-ene-3β,16β,17β-triol (200 mg, 0.624 mmol, 1.00 eq.) in dry dioxane:dichloromethane mixture (1:1, 4 mL total) under nitrogen at room temperature was treated with 3 Å molecular sieves and anisaldehyde dimethyl acetal (0.22 mL ~ 0.24 g, 1.3 mmol, 2.1 eq.). After stirring the mixture for 10 min, pyridinium *para*-toluenesulfonate (150 mg, 0.597 mmol, 0.957 eq.) was added.

After stirring the mixture for 4 h, the sieves were filtered and washed with ethyl acetate (50 mL). The filtrate was washed with water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (4:6 ethyl acetate:n-hexane) to afford the title compound (186 mg, 0.424 mmol, 68% yield) as a white solid. R_f 0.33 (1:1 ethyl acetate:n-hexane); mp 137-140 ºC; $[\alpha]^{24}_D$ -18.7º (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 7.49 (d, *J* = 8.3 Hz, 2H), 6.90 (d, *J* = 8.1 Hz, 2H), 5.91 (s, 1H), 5.34 (s, 1H), 4.13 (t, *J* = 6.7 Hz, 1H), 3.80 (s, 3H), 3.51 (m, 1H), 2.35-2.18 (m, 2H), 2.14-1.96 (m, 2H), 1.90-1.79 (m, 2H), 1.76-1.43 (m, 7H), 1.41-1.22 (m, 2H), 1.33 (s, 3H), 1.05 (s, 3H), 1.04 (s, 3H), 1.11-0.87 (m, 2H), 1 x OH not observed; ^{13}C NMR (101 MHz, $CDCl_3$): 160.55, 141.04, 128.96, 128.46 (2C), 121.26, 113.85 (2C), 105.70, 90.68, 88.41, 71.83, 55.43, 53.66, 49.98, 44.53, 42.37, 37.36, 36.73, 33.36, 32.19, 31.97, 31.75, 31.26, 20.89, 20.39, 19.54, 15.73; IR (neat): 3387 (br, w, O-H), 2957-2839 (m, C-H), 1616 (m, C=C); LRMS (+ESI): 439 (75%, $C_{28}H_{39}O_4$, $[M+H]^+$), 899 (100%, $[C_{56}H_{76}O_8Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 461.2660, $[C_{28}H_{38}O_4Na]^+$ requires 461.2668.

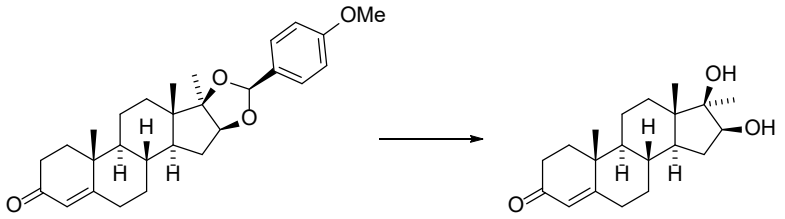
[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-4-en-3-one (3)



The reaction was conducted according to a literature method with modifications.[10] A stirred solution of dimethyl sulfoxide (0.30 mL ~ 0.33 g, 4.2 mmol, 11 eq.) in dry dichloromethane (2 mL) was cooled to -78 ºC before oxalyl chloride (0.20 mL ~ 0.30 g, 2.4 mmol, 6.2 eq.) was added dropwise. After stirring the mixture for 15 min, a solution of [(*S*)-16β,17β-(4-methoxyphenyl)methylenedioxy]-17α-methylandrost-5-en-3β-ol (170 mg, 0.388 mmol, 1.00 eq.) in dry dichloromethane (2 mL) was added dropwise and stirring was continued for 3 h at -78 ºC. The reaction mixture was quenched with triethylamine (0.60 mL ~ 0.44 g, 4.3 mmol, 11 eq.) and was left to warm to room temperature. The reaction was then diluted with ethyl acetate (50 mL) and washed with water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure to afford the crude intermediate [(*S*)-16β,17β-(4-methoxyphenyl) methylenedioxy]-17α-methylandrost-5-en-3-one as a pale-yellow solid, which was used in the next reaction without further purification.

A solution of crude [(*S*)-16β,17β-(4-methoxyphenyl)methylenedioxy]-17α-methylandrost-5-en-3-one (derived from 0.388 mmol of [(*S*)-16β,17β-(4-methoxyphenyl)methylenedioxy]-17α-methylandrost-5-en-3β-ol) in methanol (8 mL) was treated with potassium carbonate (150 mg, 2.01 mmol, 5.18 eq.) at room temperature and stirred for 15 h. The reaction was then diluted with ethyl acetate (50 mL) and washed with aqueous hydrochloric acid solution (1 M, 50 mL), followed by water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (4:6 ethyl acetate:n-hexane) to afford the title compound (91 mg, 0.208 mmol, 54% yield over 2 steps) as a white solid. R_f 0.40 (1:1 ethyl acetate:n-hexane); mp 207-210 ºC; $[\alpha]^{22}_D$ +76.9º (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 7.48 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 5.91 (s, 1H), 5.73 (s, 1H), 4.13 (dd, *J* = 7.6, 5.4 Hz, 1H), 3.81 (s, 3H), 2.48-2.25 (m, 4H), 2.11 (m, 1H), 2.03 (m, 1H), 1.86 (m, 1H), 1.82-1.44 (m, 6H), 1.40-1.23 (m, 2H), 1.33 (s, 3H), 1.21 (s, 3H), 1.08 (s, 3H), 1.06-0.86 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): 199.5 (C3), 170.9, 160.6, 128.9, 128.4 (2C), 124.1, 113.9 (2C), 105.7, 90.4, 88.2, 55.4, 53.7, 52.8, 44.6, 38.8, 35.9, 35.8, 34.1, 33.3, 32.8, 32.0, 31.1, 20.9, 20.4, 17.6, 15.8; IR (neat): 2981-2833 (m, C-H), 1674 (s, C=O), 1612 (m, C=C); LRMS (+ESI): 437 (80%, $C_{28}H_{37}O_4$, $[M+H]^+$), 491 (85%, $C_{29}H_{40}O_5Na$, $[M+Na+MeOH]^+$), 873 (85%, $C_{56}H_{73}O_8$, $[2M+H]^+$), 895 (100%, $C_{56}H_{72}O_8Na$, $[2M+Na]^+$); HRMS (+ESI): found 459.2508, $[C_{28}H_{36}O_4Na]^+$ requires 459.2511.

16β,17β-Dihydroxy-17α-methylandrost-4-en-3-one [11]



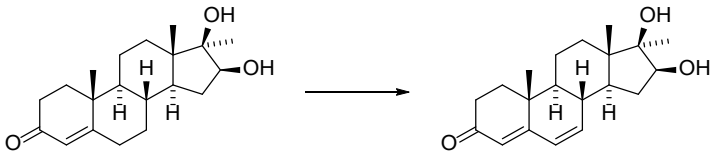
Chemical Formula: $C_{28}H_{36}O_4$
Molecular Weight: 436.5920

Chemical Formula: $C_{20}H_{30}O_3$
Molecular Weight: 318.4570

The reaction was conducted according to a literature method with modifications.[12] A solution of [(*S*)-16β,17β-(4-methoxyphenyl)methylenedioxy]-17α-methylandrost-4-en-3-one (73 mg, 0.17 mmol) in dichloromethane (0.5 mL) was treated with aqueous acetic acid solution (80% v/v, 2 mL) and heated to 40 ºC for 22 h with stirring. The reaction was then diluted with ethyl acetate (50 mL) and washed with saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), followed by brine (50 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (1:1 ethyl acetate:n-hexane) to afford the title compound (39 mg, 0.12 mmol, 71% yield) as a white solid. R_f 0.34 (100% ethyl acetate); mp 196-198 ºC (lit[11] mp 203-207 ºC); $[\alpha]^{22}_D$

+54.6° (*c* 1.0, dioxane) (lit[11] $[\alpha]^{22}_D$ +113°, 1%, dioxane); ^{1}H NMR (600 MHz, $CDCl_3$): 5.72 (s, 1H, C4-H), 3.68 (dd, *J* = 8.0, 5.4 Hz, 1H, C16-H), 2.49-2.17 (m, 4H), 2.03 (m, 1H), 1.86 (m, 1H), 1.74-1.41 (m, 5H), 1.36-1.21 (m, 3H), 1.20 (s, 3H), 1.13 (s, 3H), 1.07-0.84 (m, 3H), 0.91 (s, 3H), 2 x OH not observed; ^{13}C NMR (151 MHz, $CDCl_3$): 199.7 (C3), 171.1 (C5), 124.1 (C4), 79.3 (C17), 77.8 (C16), 54.1, 46.8, 44.9, 38.8, 36.1, 35.9, 34.9, 34.1, 32.9, 32.3, 32.0, 23.9, 20.6, 17.6, 13.6; IR (neat): 3335 (m, O-H), 2983-2854 (m, C-H), 1669 (s, C=O), 1618 (m, C=C); LRMS (+ESI): 319 (35%, $[C_{20}H_{31}O_3]^+$, $[M+H]^+$), 373 (45%, $[C_{21}H_{34}O_4Na]^+$, $[M+Na+MeOH]^+$), 637 (50%, $[C_{40}H_{61}O_6]^+$, $[2M+H]^+$), 659 (100%, $[C_{40}H_{60}O_6Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 341.2090, $[C_{20}H_{30}O_3Na]^+$ requires 341.2093.

16β,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one (16β-OH-JW)

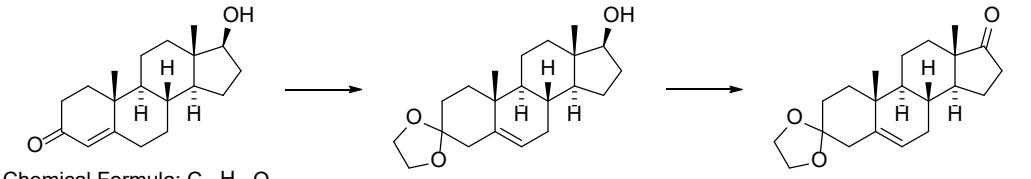


Chemical Formula: $C_{20}H_{30}O_3$
Molecular Weight: 318.4570

Chemical Formula: $C_{20}H_{28}O_3$
Molecular Weight: 316.4410

The reaction was conducted according to a literature method with modifications.[1] A solution of 16β,17β-dihydroxy-17α-methylandrost-4-en-3-one (10 mg, 0.031 mmol, 1.0 eq.) in *tert*-butanol:ethyl acetate mixture (1:1, 1.0 mL total) was treated with chloranil (20 mg, 0.081 mmol, 2.6 eq.) and pyridinium *para*-toluenesulfonate (10 mg, 0.040 mmol, 1.3 eq.) before heated to 100 °C for 4 h with stirring. The reaction was then allowed to cool to room temperature, diluted with ethyl acetate (20 mL) and washed with aqueous sodium hydroxide solution (1 M, 2 x 20 mL), water (20 mL), and brine (20 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (4:6 ethyl acetate:n-hexane) to afford the title compound (7 mg, 0.02 mmol, 65% yield) as a white solid. R_f 0.32 (100% ethyl acetate); mp 163-166 °C; $[\alpha]^{25}_D$ +15.0° (*c* 0.2, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): 6.10 (m, 2H, C6- & C7-H), 5.68 (s, 1H, C4-H), 3.73 (dd, *J* = 8.0, 5.4 Hz, 1H, C16-H), 2.58 (m, 1H), 2.46-2.37 (m, 2H), 2.32 (t, *J* = 10.8 Hz, 1H), 2.01 (ddd, *J* = 13.2, 5.4, 2.1 Hz, 1H), 1.71 (td, *J* = 13.8, 5.1 Hz, 1H), 1.65-1.60 (m, 2H), 1.48 (m, 1H), 1.41 (m, 1H), 1.34 (m, 1H), 1.19 (m, 2H), 1.15 (s, 3H), 1.13 (s, 3H), 0.96 (s, 3H), 2 x OH not observed; ^{13}C NMR (151 MHz, $CDCl_3$): 199.7 (C3), 163.6 (C5), 140.3 (C7), 128.3 (C6), 124.0 (C4), 79.1 (C17), 77.7 (C16), 51.1, 45.9, 44.6, 38.0, 36.3, 34.5, 34.1, 34.1, 32.3, 23.9, 20.2, 16.5, 13.5; IR (neat): 3397 (br, m, O-H), 2942-2871 (m, C-H), 1646 (s, C=O), 1615 (s, C=C); LRMS (+ESI): 317 (100%, $[C_{20}H_{29}O_3]^+$, $[M+H]^+$), 655 (15%, $[C_{40}H_{56}O_6Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 339.1937, $[C_{20}H_{28}O_3Na]^+$ requires 339.1936.

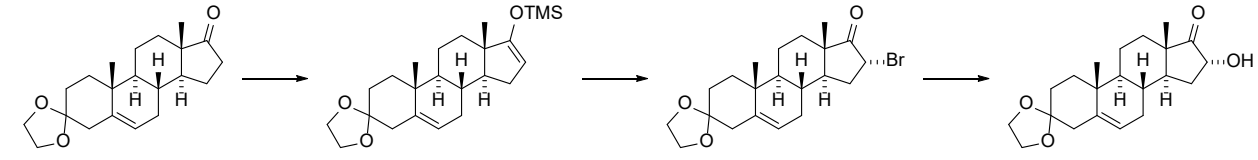
3,3-Ethylenedioxyandrost-5-en-17-one [13–16]



The reaction was conducted according to a literature method with modifications.[13] A solution of testosterone (1.0 g, 3.5 mmol, 1.0 eq.) in dry dichloromethane (13 mL) was treated with ethylene glycol (4.0 mL ~ 4.4 g, 71 mmol, 20 eq.), triethyl orthoformate (3.5 mL ~ 3.1 g, 21 mmol, 6.0 eq.), and *para*-toluenesulfonic acid monohydrate (20 mg, 0.11 mmol, 0.031 eq.). The reaction mixture was left stirring at room temperature for 16 h before it was quenched with triethylamine (3 drops). The reaction was then diluted with dichloromethane (50 mL) and washed with water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to afford the crude intermediate 3,3-ethylenedioxyandrost-5-en-17β-ol as a white solid, which was used in the next reaction without further purification.

The reaction was conducted according to a literature method with modifications.[13] A solution of crude 3,3-ethylenedioxyandrost-5-en-17β-ol (derived from 3.5 mmol of testosterone) in dichloromethane (60 mL) was treated with pyridinium chlorochromate (1.7 g, 7.9 mmol, 2.3 eq.) at room temperature and stirred for 3 h. The reaction was then diluted with dichloromethane (50 mL) and filtered through celite. The filtrate was washed with saturated aqueous sodium bicarbonate solution (2 x 50 mL), followed by water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (4:6 ethyl acetate:n-hexane) to afford the title compound (0.72 g, 2.2 mmol, 63% yield over 2 steps) as a white solid. R_f 0.42 (1:1 ethyl acetate:n-hexane); mp 195-197 ºC (lit[13] mp 197-199 ºC); $[\alpha]^{23}_D$ +16.9º (*c* 1.0, $CHCl_3$) (lit[14] $[\alpha]^{24}_D$ +15.4º, 1%, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 5.38 (dt, *J* = 5.5, 2.1 Hz, 1H, C6-H), 4.00-3.89 (m, 4H), 2.57 (m, 1H), 2.45 (m, 1H), 2.18-2.03 (m, 3H), 1.95 (m, 1H), 1.87-1.43 (m, 9H), 1.38-1.24 (m, 3H), 1.13 (m, 1H), 1.05 (s, 3H), 0.88 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): 221.3 (C17), 140.6, 121.6, 109.5, 64.6, 64.4, 51.9, 50.0, 47.7, 42.0, 36.9, 36.4, 36.0, 31.7, 31.6, 31.1, 30.8, 22.0, 20.5, 19.1, 13.7; IR (neat): 2974-2826 (m, C-H), 1734 (s, C=O); LRMS (+ESI): 353 (100%, $[C_{21}H_{30}O_3Na]^+$, $[M+Na]^+$), 683 (100%, $[C_{42}H_{60}O_6Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 353.2093, $[C_{21}H_{30}O_3Na]^+$ requires 353.2093. ^{1}H NMR, ^{13}C NMR, and IR data matched the literature reference.[13,15,16]

3,3-Ethylenedioxy-16α-hydroxyandrost-5-en-17-one (4)



Chemical Formula: $C_{21}H_{30}O_3$
Molecular Weight: 330.4680

Chemical Formula: $C_{24}H_{38}O_3Si$
Molecular Weight: 402.6500

Chemical Formula: $C_{21}H_{29}BrO_3$
Molecular Weight: 409.3640

Chemical Formula: $C_{21}H_{30}O_4$
Molecular Weight: 346.4670

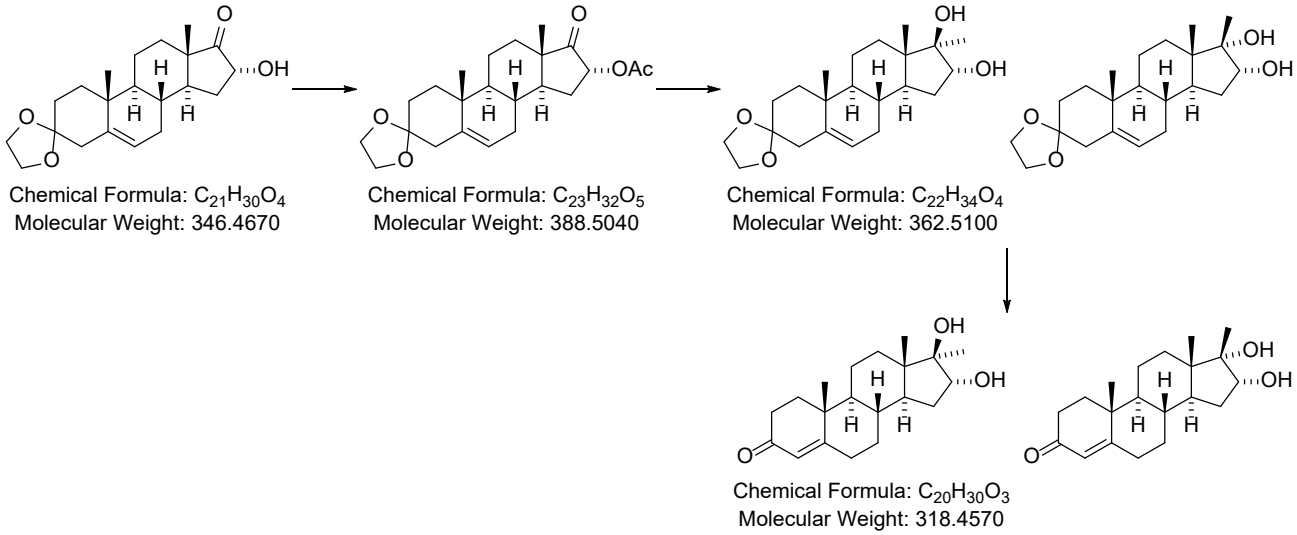
The reaction was conducted according to a literature method with modifications.[13] Under nitrogen, a solution of diisopropylamine (1.2 mL ~ 0.86 g, 8.5 mmol, 5.6 eq.) in dry tetrahydrofuran (14 mL) was cooled to -78 ºC and stirred for 5 min before *n*-butyllithium solution in cyclohexane (1.6 M actual concentration, 4.6 mL, 7.4 mmol, 4.9 eq.) was added dropwise over 2 min. After stirring the mixture for further 5 min, a solution of 3,3-ethylenedioxyandrost-5-en-17-one (500 mg, 1.51 mmol, 1.00 eq.) in dry tetrahydrofuran (9 mL) was added dropwise over 3 min and stirred for 10 min. The reaction mixture was then treated with trimethylsilyl chloride (0.7 mL ~ 0.6 g, 6 mmol, 4 eq.) and stirring was continued for 30 min at -78 ºC before the reaction was place in an ice bath and stirred for further 2 h. The reaction was then diluted with diethyl ether (50 mL) and washed with saturated aqueous ammonium chloride solution (2 x 50 mL), followed by water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to afford the crude intermediate 3,3-ethylenedioxyandrost-5,16-dien-17-yl trimethylsilyl ether as a white solid, which was used in the next reaction without further purification.

The reaction was conducted according to a literature method with modifications.[13] A solution of crude 3,3-ethylenedioxyandrost-5,16-dien-17-yl trimethylsilyl ether (derived from 1.51 mmol of 3,3-ethylenedioxyandrost-5-en-17-one) in dry dichloromethane (30 mL) was treated with *N*-bromosuccinimide (350 mg, 1.97 mmol, 1.30 eq.) under nitrogen at room temperature and stirred for 5 h. The reaction was then diluted with dichloromethane (50 mL) and washed with water (2 x 50 mL), followed by brine (50 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to afford the crude intermediate 16α-bromo-3,3-ethylenedioxyandrost-5-en-17-one as a yellow/orange oily residue. Flash silica column chromatography (4:6 ethyl acetate:n-hexane) was performed to remove minor impurities and afford 16α-bromo ketone intermediate as pale-yellow solid.

The reaction was conducted according to a literature method with modifications.[13] A solution of 16α-bromo-3,3-ethylenedioxyandrost-5-en-17-one (derived from 1.51 mmol of 3,3-ethylenedioxyandrost -5-en-17-one) in tetrahydrofuran (32 mL) was treated with aqueous potassium hydroxide solution (5 M, 6 mL, 0.03 mol, 20 eq.) at room temperature and stirred for 16 h. The reaction was then diluted with ethyl acetate (50 mL) and washed with water (2 x 50 mL), followed by brine (50 mL). The

organic layer was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (4:6 ethyl acetate:n-hexane) to afford the title compound (310 mg, 0.894 mmol, 59% yield over 3 steps) as a white solid. R_f 0.20 (1:1 ethyl acetate:n-hexane); mp 200-203 ºC; $[\alpha]^{24}_D$ +10.0º (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): 5.37 (m, 1H, C6-H), 4.38 (m, 1H, C16-H), 3.98-3.91(m, 4H), 2.57 (dq, *J* = 14.3, 2.9 Hz, 1H), 2.14 (dd, *J* = 14.2, 2.9 Hz, 1H), 2.04 (m, 1H), 1.99-1.61 (m, 8H), 1.57-1.23 (m, 5H), 1.14 (m, 1H), 1.05 (s, 3H), 0.98 (s, 3H), 1 x OH not observed; ^{13}C NMR (101 MHz, $CDCl_3$): 219.5 (C17), 140.5, 121.4, 109.4, 71.4, 64.6, 64.4, 49.8, 48.7, 47.6, 41.9, 36.9, 36.3, 31.6, 31.3, 31.1, 30.7, 30.4, 20.0, 19.1, 14.0; IR (neat): 3527 (w, O-H), 2931-2834 (m, C-H), 1737 (s, C=O); LRMS (+ESI): 369 (15%, $[C_{21}H_{30}O_4Na]^+$, $[M+Na]^+$), 401 (30%, $[C_{22}H_{34}O_5Na]^+$, $[M+Na+MeOH]^+$), 715 (100%, $[C_{42}H_{60}O_8Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 369.2053, $[C_{21}H_{30}O_4Na]^+$ requires 369.2042.

16α,17β-Dihydroxy-17α-methylandrost-4-en-3-one (5) [11] and 16α,17α-dihydroxy-17β-methylandrost-4-en-3-one (6) [17]



The reaction was conducted according to a literature method with modifications.[18] Acetic anhydride (11 mL ~ 12 g, 0.12 mol, 136 eq.) and pyridine (11 mL ~ 11 g, 0.14 mol, 159 eq.) was added to 3,3-ethylenedioxy-16α-hydroxyandrost-5-en-17-one (305 mg, 0.880 mmol, 1.00 eq.) at room temperature. The reaction mixture was left stirring at room temperature for 1.5 h before it was evaporated to dryness under reduced pressure. The crude residue was then re-dissolved with ethyl acetate (50 mL) and washed with aqueous hydrochloric acid solution (0.5 M, 50 mL), followed by saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated

under reduced pressure to afford the crude intermediate 16α-acetoxy-3,3-ethylenedioxyandrost-5-en-17-one as a white solid, which was used in the next reaction without further purification.

The reaction was conducted according to a literature method with modifications.[18] A solution of crude 16α-acetoxy-3,3-ethylenedioxyandrost-5-en-17-one (derived from 0.880 mmol of 3,3-ethylenedioxy-16α-hydroxyandrost-5-en-17-one) in dry diethyl ether (2 mL) under nitrogen at room temperature was treated slowly over 2 min with a solution of methylmagnesium bromide in diethyl ether (3.0 M, 4.0 mL, 12 mmol, 14 eq.) and stirred for 16 h. The reaction was then diluted with ethyl acetate (50 mL) and washed with saturated aqueous ammonium chloride solution (3 x 50 mL), followed by water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure to afford the crude intermediate mixture containing both 3,3-ethylenedioxy-17α-methylandrost-5-ene-16α,17β-diol (trans-diol, desired) and 3,3-ethylenedioxy-17β-methylandrost-5-ene-16α,17α-diol (cis-diol) as a white solid, which was used in the next reaction without further purification.

A solution of crude mixture 3,3-ethylenedioxy-17α-methylandrost-5-ene-16α,17β-diol (trans-diol, desired) and 3,3-ethylenedioxy-17β-methylandrost-5-ene-16α,17α-diol (cis-diol) (derived from 0.880 mmol of 3,3-ethylenedioxy-16α-hydroxyandrost-5-en-17-one) in methanol (40 mL) and water (2 mL) was treated with (-)-camphor-10-sulfonic acid (300 mg, 1.29 mmol, 1.47 eq.) at room temperature and stirred for 20 h. The reaction was then evaporated to dryness under reduced pressure, re-dissolved with ethyl acetate (50 mL), and washed with water (50 mL), then brine (50 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (6:4 ethyl acetate:n-hexane) to afford both 16α,17β-dihydroxy-17α-methylandrost-4-en-3-one (95 mg, 0.30 mmol, 34% yield over 3 steps) as a white solid, and 16α,17α-dihydroxy-17β-methylandrost-4-en-3-one (100 mg, 0.314 mmol, 36% yield over 3 steps) as a white solid.

Data for 16α,17β-dihydroxy-17α-methylandrost-4-en-3-one (5, trans-diol):

R_f 0.20 (100% ethyl acetate); mp 206-208 °C (lit[11] mp 220-222 °C); $[\alpha]^{23}_D$ +17.9° (*c* 1.0, dioxane) (lit[11] $[\alpha]^{22}_D$ +44.5°, 1%, dioxane); ^{1}H NMR (400 MHz, $CDCl_3$): 5.72 (s, 1H, C4-H), 4.31 (dd, *J* = 10.2, 3.3 Hz, 1H, C16-H), 2.46-2.24 (m, 4H), 2.02 (m, 1H), 1.92-1.78 (m, 3H), 1.72-1.23 (m, 7H), 1.19 (s, 3H), 1.18 (s, 3H), 1.06-0.88 (m, 2H), 0.92 (s, 3H), 2 x OH not observed; ^{13}C NMR (101 MHz, $CDCl_3$): 199.7 (C3), 171.2 (C5), 124.1 (C4), 84.2, 80.3, 53.8, 47.9, 46.1, 38.8, 36.1, 35.7, 34.1, 33.6, 32.9, 31.7, 31.7, 20.2, 18.2, 17.5, 14.4; IR (neat): 3381 (br, m, O-H), 2979-2850 (m, C-H), 1659 (s, C=O), 1612 (m, C=C); LRMS (+ESI): 341 (20%, $[C_{20}H_{30}O_3Na]^+$, $[M+Na]^+$), 659 (100%, $[C_{40}H_{60}O_6Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 341.2094, $[C_{20}H_{30}O_3Na]^+$ requires 341.2093.

Data for 16α,17α-dihydroxy-17β-methylandrost-4-en-3-one (6, cis-diol):

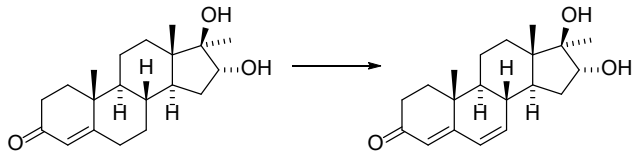
R_f 0.40 (100% ethyl acetate); mp 217-219 ºC (lit[17] mp 239 ºC); $[\alpha]^{23}_D$ +37.1º (*c* 0.8, EtOH) (lit[17] $[\alpha]_D$ +46º, *c* 0.85, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$): 5.72 (s, 1H, C4-H), 4.09 (dd, *J* = 9.0, 2.6 Hz, 1H, C16-H), 2.46-2.24 (m, 4H), 2.02 (m, 1H), 1.90-0.93 (m, 12H), 1.18 (s, 3H), 1.18 (s, 3H), 0.74 (s, 3H), 2 x OH not observed; ^{13}C NMR (101 MHz, $CDCl_3$): 199.8 (C3), 171.4 (C5), 124.0 (C4), 80.4, 77.4, 53.7, 47.7, 46.7, 38.8, 35.8, 35.7, 35.3, 34.1, 33.0, 32.2, 30.1, 20.5, 20.2, 17.5, 15.9; IR (neat): 3358 (br, m, O-H), 2934-2825 (m, C-H), 1641 (s, C=O), 1607 (m, C=C); LRMS (+ESI): 341 (10%, $[C_{20}H_{30}O_3Na]^+$, $[M+Na]^+$), 659 (100%, $[C_{40}H_{60}O_6Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 341.2097, $[C_{20}H_{30}O_3Na]^+$ requires 341.2093.

Note: comparing ^{1}H NMR spectra of the two epimers, 16α,17β-dihydroxy-17α-methylandrost-4-en-3-one (trans-diol) and 16α,17α-dihydroxy-17β-methylandrost-4-en-3-one (cis-diol), shows that chemical shifts of C18-H_3 of the two compounds has the biggest difference compared to C19-H_3 and C20-H_3. This is caused by the orientation of the hydroxyl group at C17. When the C17 hydroxyl group is at the β-position, same as the C18 methyl group, the C18-H_3 show a greater chemical shift than when the C17 hydroxyl group is at the α-position, opposite to the C18 methyl group. This pattern of shielding and deshielding was also observed for epimeric pairs **JW** and **epiJW**, **16α-OH-JW** and **16α-OH-epiJW** and **16-keto-JW** and **16-keto-epiJW** (Table S1). This observation is in accordance with previously published data by Schänzer *et al.*[19]

Table S1. ^{1}H NMR chemical shifts of C18, C19 and C20 methyl protons of synthesised steroids and their epimers with Δδ showing C18-H_3 chemical shift differences of the epimers ([δ 17α-methyl]-[δ 17β-methyl])

Steroid	C18	Δδ	C19	C20
17β-Hydroxy-17α-methylandrosta-4,6-dien-3-one (JW)	0.96	+0.19	1.13	1.23
17α-Hydroxy-17β-methylandrosta-4,6-dien-3-one (epiJW)	0.77		1.12	1.23
16α,17β-Dihydroxy-17α-methylandrost-4-en-3-one (trans-diol)	0.92	+0.18	1.18	1.19
16α,17α-Dihydroxy-17β-methylandrost-4-en-3-one (cis-diol)	0.74		1.18	1.18
16α,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one (16α-OH-JW)	0.98	+0.19	1.12	1.20
16α,17α-Dihydroxy-17β-methylandrosta-4,6-dien-3-one (16α-OH-epiJW)	0.79		1.11	1.21
17β-Hydroxy-17α-methylandrosta-4,6-diene-3,16-dione (16-keto-JW)	0.91	+0.09	1.16	1.20
17α-Hydroxy-17β-methylandrosta-4,6-diene-3,16-dione (16-keto-epiJW)	0.82		1.15	1.19

16α,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one (16α-OH-JW)

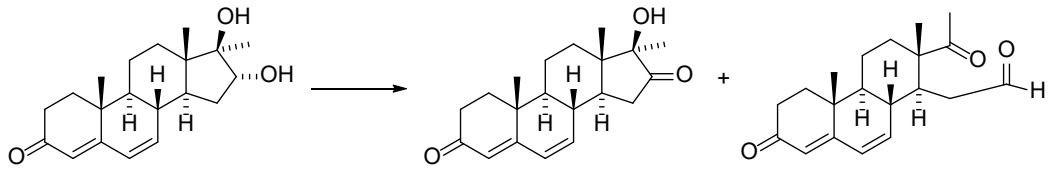


Chemical Formula: $C_{20}H_{30}O_3$
Exact Mass: 318.2195

Chemical Formula: $C_{20}H_{28}O_3$
Exact Mass: 316.2038

The reaction was conducted according to a literature method with modifications.[1] A solution of 16α,17β-dihydroxy-17α-methylandrost-4-en-3-one (32 mg, 0.10 mmol, 1.0 eq.) in *tert*-butanol:ethyl acetate mixture (1:1, 4 mL total) was treated with chloranil (70 mg, 0.28 mmol, 2.8 eq.) and pyridinium *para*-toluenesulfonate (25 mg, 0.099 mmol, 0.99 eq.) before heated to 100 °C for 4 h with stirring. The reaction was then let to cool down to room temperature, diluted with ethyl acetate (20 mL) and washed with aqueous sodium hydroxide solution (1 M, 2 x 20 mL), water (20 mL), followed by brine (20 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (6:4 ethyl acetate:n-hexane) to afford the title compound (15 mg, 0.047 mmol, 47% yield) as a white solid. R_f 0.15 (100% ethyl acetate); mp 173-176 °C; $[\alpha]^{26}_D$ -7.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 6.08 (m, 2H, C6- & C7-H), 5.68 (s, 1H, C4-H), 4.36 (m, 1H, C16-H), 2.57 (m, 1H), 2.44 (m, 1H), 2.24 (t, *J* = 10.3 Hz, 1H), 2.06-1.98 (m, 2H), 1.79-1.37 (m, 6H), 1.29-1.23 (m, 2H), 1.20 (s, 3H), 1.12 (s, 3H), 0.98 (s, 3H), 2 x OH not observed; ^{13}C NMR (101 MHz, $CDCl_3$): 199.8 (C3), 163.7 (C5), 140.3 (C7), 128.3 (C6), 123.9 (C4), 84.0 (C17), 80.2 (C16), 50.8, 47.0, 45.8, 38.0, 36.3, 34.1, 33.9, 33.2, 31.7, 20.0, 18.2, 16.5, 14.3; IR (neat): 3400 (br, m, O-H), 2926 (m, C-H), 2857 (m, C-H), 1644 (s, C=O), 1613 (s, C=C); LRMS (+ESI): 317 (100%, $[C_{20}H_{29}O_3]^+$, $[M+H]^+$), 339 (10%, $[C_{20}H_{28}O_3Na]^+$, $[M+Na]^+$), 655 (10%, $[C_{40}H_{56}O_6Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 339.1936, $[C_{20}H_{28}O_3Na]^+$ requires 339.1936.

17β-Hydroxy-17α-methylandrosta-4,6-diene-3,16-dione (16-keto-JW)



Chemical Formula: $C_{20}H_{28}O_3$
Exact Mass: 316.2038

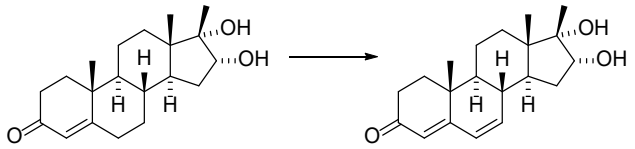
Chemical Formula: $C_{20}H_{26}O_3$
Exact Mass: 314.1882

Chemical Formula: $C_{20}H_{26}O_3$
Exact Mass: 314.1882

A solution of 16α,17β-dihydroxy-17α-methylandrosta-4,6-dien-3-one (10 mg, 0.032 mmol, 1.0 eq.) in dichloromethane (1 mL) was treated with pyridinium chlorochromate (10 mg, 0.046 mmol, 1.4 eq.) at room temperature and stirred for 40 mins. The reaction was then diluted with diethyl ether (20 mL)

and filtered through celite. The filtrate was washed with saturated aqueous sodium bicarbonate solution (20 mL), followed by water (20 mL), then brine (20 mL). The organic layer was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (1:1 ethyl acetate:n-hexane) to afford the title compound (7 mg, 0.02 mmol, 63% yield) as a white solid, mixed with 13% of side product based on ^{1}H NMR integration. The data provided is for the title compound. R_f 0.46 (100% ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$): 6.17 (m, 1H, C6- or C7-H), 6.01 (m, 1H, C6- or C7-H), 5.71 (s, 1H, C4-H), 2.65-2.39 (m, 4H), 2.17-2.01 (m, 3H), 1.84-1.50 (m, 5H), 1.40 (m, 1H), 1.20 (s, 3H), 1.16 (s, 3H), 0.91 (s, 3H), 1 x OH not observed; ^{13}C NMR (151 MHz, $CDCl_3$): 219.6, 199.4, 162.8, 139.2, 129.0, 124.4, 83.1, 51.0, 45.3, 42.6, 37.1, 36.4, 35.0, 34.0, 33.9, 30.9, 20.1, 19.4, 16.5, 13.9; IR (neat): 3455 (br, w, O-H), 2925 (s, C-H), 2856 (m, C-H), 1746 (s, C=O), 1660 (s, C=O), 1617 (s, C=C); LRMS (+ESI): 315 (100%, $[C_{20}H_{27}O_3]^+$, $[M+H]^+$), 337 (10%, $[C_{20}H_{26}O_3Na]^+$, $[M+Na]^+$); HRMS (+ESI): found 337.1779, $[C_{20}H_{26}O_3Na]^+$ requires 337.1780.

16α,17α-Dihydroxy-17β-methylandrosta-4,6-dien-3-one (16α-OH-epiJW)



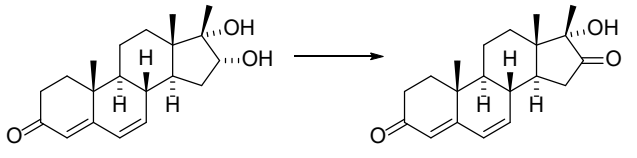
Chemical Formula: $C_{20}H_{30}O_3$
Exact Mass: 318.2195

Chemical Formula: $C_{20}H_{28}O_3$
Exact Mass: 316.2038

The reaction was conducted according to a literature method with modifications.[1] A solution of 16α,17α-dihydroxy-17β-methylandrost-4-en-3-one (60 mg, 0.19 mmol, 1.0 eq.) in *tert*-butanol:ethyl acetate mixture (1:1, 6 mL total) was treated with chloranil (100 mg, 0.407 mmol, 2.14 eq.) and pyridinium *para*-toluenesulfonate (50 mg, 0.20 mmol, 1.1 eq.) before heated to 100 ºC for 4 h with stirring. The reaction was then allowed to cool to room temperature, diluted with ethyl acetate (30 mL) and washed with aqueous sodium hydroxide solution (1 M, 2 x 30 mL), water (30 mL), and brine (30 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (6:4 ethyl acetate:n-hexane) to afford the title compound (40 mg, 0.13 mmol, 68% yield) as a white solid. R_f 0.35 (100% ethyl acetate); mp 162-164 ºC; $[\alpha]^{24}_D$ -11.5º (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 6.08 (m, 2H, C6- & C7-H), 5.67 (s, 1H, C4-H), 4.14 (m, 1H, C16-H), 2.57 (m, 1H), 2.44 (m, 1H), 2.17 (t, *J* = 10.7 Hz, 1H), 2.11-1.97 (m, 2H), 1.95-1.63 (m, 4H), 1.51-1.19 (m, 4H), 1.22 (s, 3H), 1.12 (s, 3H), 0.79 (s, 3H), 2 x OH not observed; ^{13}C NMR (101 MHz, $CDCl_3$): 199.8 (C3), 164.0 (C5), 141.2 (C7), 128.1 (C6), 123.8 (C4), 80.3 (C17), 77.2 (C16), 50.6, 47.7, 45.5, 37.6, 36.3, 34.8, 34.1, 34.0, 30.2, 20.6, 19.9, 16.5, 15.7; IR (neat): 3375 (br, m, O-H), 2940-2859 (m,

C-H), 1643 (s, C=O), 1612 (s, C=C); LRMS (+ESI): 317 (100%, $[C_{20}H_{29}O_3]^+$, $[M+H]^+$), 339 (10%, $[C_{20}H_{28}O_3Na]^+$, $[M+Na]^+$), 655 (65%, $[C_{40}H_{56}O_6Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 339.1944, $[C_{20}H_{28}O_3Na]^+$ requires 339.1936.

17α-Hydroxy-17β-methylandrosta-4,6-diene-3,16-dione (16-keto-epiJW)



Chemical Formula: $C_{20}H_{28}O_3$
Exact Mass: 316.2038

Chemical Formula: $C_{20}H_{26}O_3$
Exact Mass: 314.1882

The reaction was conducted according to a literature method with modifications.[10] A stirred solution of dimethyl sulfoxide (75 µL ~ 83 mg, 1.1 mmol, 12 eq.) in dry dichloromethane (0.45 mL) was cooled to -78 ºC before oxalyl chloride (50 µL ~ 75 mg, 0.59 mmol, 6.2 eq.) was added dropwise. After stirring the mixture for 15 min, a solution of 16α,17α-dihydroxy-17β-methylandrosta-4,6-dien-3-one (30 mg, 0.095 mmol, 1.0 eq.) in dry dichloromethane (0.6 mL) was added dropwise and stirring was continued for 4 h at -78 ºC. The reaction mixture was quenched with triethylamine (140 µL ~ 98 mg, 0.97 mmol, 10 eq.) and was let to warm up to room temperature. The reaction was then diluted with dichloromethane (20 mL), washed with water (20 mL), and then brine (20 mL). The organic layer was dried with anhydrous magnesium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude residue was subjected to silica column chromatography (1:1 ethyl acetate:n-hexane) to afford the title compound (10 mg, 0.032 mmol, 34% yield) as a white solid. R_f 0.54 (100% ethyl acetate); mp 180-182 ºC; $[\alpha]^{22}_D$ -101.3º (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 6.16 (m, 1H, C6- or C7-H), 6.02 (m, 1H, C6- or C7-H), 5.70 (s, 1H, C4-H), 2.63-2.27 (m, 5H), 2.06-1.98 (m, 2H), 1.88-1.64 (m, 3H), 1.53 (m, 1H), 1.42 (m, 1H), 1.25 (m, 1H), 1.19 (s, 3H), 1.15 (s, 3H), 0.82 (s, 3H), 1 x OH not observed; ^{13}C NMR (101 MHz, $CDCl_3$): 215.9, 199.6, 163.3, 140.0, 128.7, 124.2, 80.1, 50.6, 45.4, 42.4, 36.8, 36.3 (2C), 34.0, 33.8, 28.8, 19.9, 16.7, 16.5, 15.5, one peak overlapping or obscured; IR (neat): 3415 (br, w, O-H), 2944 (m, C-H), 2862 (w, C-H), 1745 (s, C=O), 1661 (s, C=O), 1615 (s, C=C); LRMS (+ESI): 315 (100%, $[C_{20}H_{27}O_3]^+$, $[M+H]^+$), 337 (10%, $[C_{20}H_{26}O_3Na]^+$, $[M+Na]^+$), 651 (25%, $[C_{40}H_{52}O_6Na]^+$, $[2M+Na]^+$); HRMS (+ESI): found 337.1783, $[C_{20}H_{26}O_3Na]^+$ requires 337.1780.

References

(1) Parr, M. K.; Fußhöller, G.; Schlörer, N.; Opfermann, G.; Geyer, H.; Rodchenkov, G.; Schänzer, W. Detection of Δ6-Methyltestosterone in a "Dietary Supplement" and GC–MS/MS Investigations on Its Urinary Metabolism. *Toxicol. Lett.* **2011**, *201* (2), 101–104. https://doi.org/10.1016/j.toxlet.2010.11.018.

(2) Campbell, J. A.; Babcock, J. C. The Synthesis of Some 7α- and 7β-Methyl Steroid Hormones[1]. *J. Am. Chem. Soc.* **1959**, *81* (15), 4069–4074. https://doi.org/10.1021/ja01524a063.

(3) Moffett, R. B.; Weisblat, D. I. $\Delta^{20(21)}$-Steroid Enol Acetates[1]. *J. Am. Chem. Soc.* **1952**, *74*, 2183.

(4) Pouzar, V.; Černý, I.; Lapčík, O.; Hill, M.; Hampl, R. Synthesis of Two New Haptens of 16α-Hydroxydehydroepiandrosterone (3β,16α-Dihydroxyandrost-5-En-17-One). *Steroids* **2003**, *68* (2), 149–158. https://doi.org/10.1016/S0039-128X(02)00176-9.

(5) Marwah, P.; Marwah, A.; Lardy, H. A.; Miyamoto, H.; Chang, C. C19-Steroids as Androgen Receptor Modulators: Design, Discovery, and Structure-Activity Relationship of New Steroidal Androgen Receptor Antagonists. *Bioorg. Med. Chem.* **2006**, *14* (17), 5933–5947. https://doi.org/10.1016/j.bmc.2006.05.022.

(6) Numazawa, M.; Shelangouski, M.; Nakakoshi, M. Production of 16β-(Acetoxy)Acetoxy Derivatives by Reaction of 17-Keto Steroid Enol Acetates with Lead (IV) Acetate. *Steroids* **2001**, *66* (10), 743–748. https://doi.org/10.1016/S0039-128X(01)00103-9.

(7) Aoki, T.; Yamamura, H.; Takei, K.; Mori, H. Synthesis of 16-Oxygenated Androst-5-en-3β-ol Derivatives. *Chem. Pharm. Bull. (Tokyo)* **1964**, *12* (7), 808–812. https://doi.org/10.1248/cpb.12.808.

(8) Takegoshi, T. Investigations on Steroids. XIII. Synthesis of the Compounds Related to the Metabolites of Furazabol (17β-Hydroxy-17α-Methyl-5α-Androstano [2, 3-c] Furazan). *Chem. Pharm. Bull. (Tokyo)* **1972**, *20* (6), 1260–1271. https://doi.org/10.1248/cpb.20.1260.

(9) Gladding, J. A.; Bacci, J. P.; Shaw, S. A.; Smith, A. B. Sporolide B: Synthetic Studies. *Tetrahedron* **2011**, *67* (35), 6697–6706. https://doi.org/10.1016/j.tet.2011.04.094.

(10) Morzycki, J. W.; Pérez-Díaz, J. O. H.; Santillan, R.; Wojtkielewicz, A. Unusual Oxidative Transformations of a Steroidal 16α,17α,22-Triol. *Steroids* **2010**, *75* (1), 70–76. https://doi.org/10.1016/j.steroids.2009.10.002.

(11) Modelli, R. Preparation of 17α-Methylandrostanes Oxygenated in C-16 Position. *Ann. Chim.* **1965**, *55* (3), 205–220.

(12) Takagi, C.; Sukeda, M.; Kim, H.-S.; Wataya, Y.; Yabe, S.; Kitade, Y.; Matsuda, A.; Shuto, S. Synthesis of 5′-Methylenearisteromycin and Its 2-Fluoro Derivative with Potent Antimalarial Activity Due to Inhibition of the Parasite S-Adenosylhomocysteine Hydrolase. *Org. Biomol. Chem.* **2005**, *3* (7), 1245–1251. https://doi.org/10.1039/B418829B.

(13) Liu, A.; Carlson, K. E.; Katzenellenbogen, J. A. Synthesis of High-Affinity Fluorine-Substituted Ligands for the Androgen Receptor. Potential Agents for Imaging Prostatic Cancer by Positron Emission Tomography. *J. Med. Chem.* **1992**, *35* (11), 2113–2129. https://doi.org/10.1021/jm00089a024.

(14) Dauben, H. J.; Löken, B.; Ringold, H. J. Preparation of Ethylenedioxy Derivatives of Ketosteroids by Exchange Dioxolanation. An Improved Synthesis of Testosterone from Δ^4-Androstene-3,17-Dione[1]. *J. Am. Chem. Soc.* **1954**, *76* (5), 1359–1363. https://doi.org/10.1021/ja01634a050.

(15) Ács, P.; Takács, A.; Kiss, M.; Pálinkás, N.; Mahó, S.; Kollár, L. Systematic Investigation on the Synthesis of Androstane-Based 3-, 11- and 17-Carboxamides via Palladium-Catalyzed Aminocarbonylation. *Steroids* **2011**, *76* (3), 280–290. https://doi.org/10.1016/j.steroids.2010.11.008.

(16) Zolottsev, V. A.; Ponomarev, G. V.; Taratynova, M. O.; Morozevich, G. E.; Novikov, R. A.; Timofeev, V. P.; Solyev, P. N.; Zavialova, M. G.; Zazulina, O. V.; Tkachev, Y. V.; Misharin, A. Y. Conjugates of 17-Substituted Testosterone and Epitestosterone with Pyropheophorbide a Differing in the Length of Linkers. *Steroids* **2018**, *138*, 82–90. https://doi.org/10.1016/j.steroids.2018.06.011.

(17) Bertin, D.; Nedelec, L. 17-Hydroxymethyltestosterones. *Bull. Soc. Chim. Fr.* **1964**, No. 9, 2140–2144.

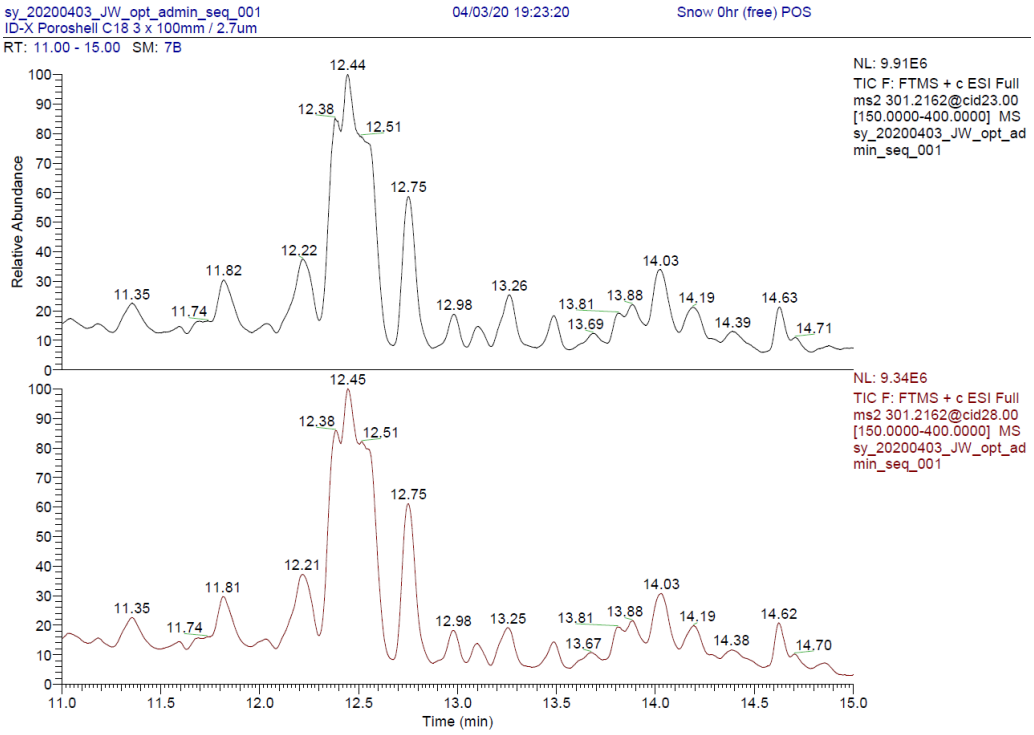
(18) McKinney, A. R.; Suann, C. J.; Stenhouse, A. M. A Stereochemical Examination of the Equine Metabolism of 17α-Methyltestosterone. *Anal. Chim. Acta* **2007**, *581* (2), 377–387. https://doi.org/10.1016/j.aca.2006.08.025.

(19) Schänzer, W.; Opfermann, G.; Donike, M. 17-Epimerization of 17α-Methyl Anabolic Steroids in Humans: Metabolism and Synthesis of 17α-Hydroxy-17β-Methyl Steroids. *Steroids* **1992**, *57* (11), 537–550. https://doi.org/10.1016/0039-128X(92)90023-3.

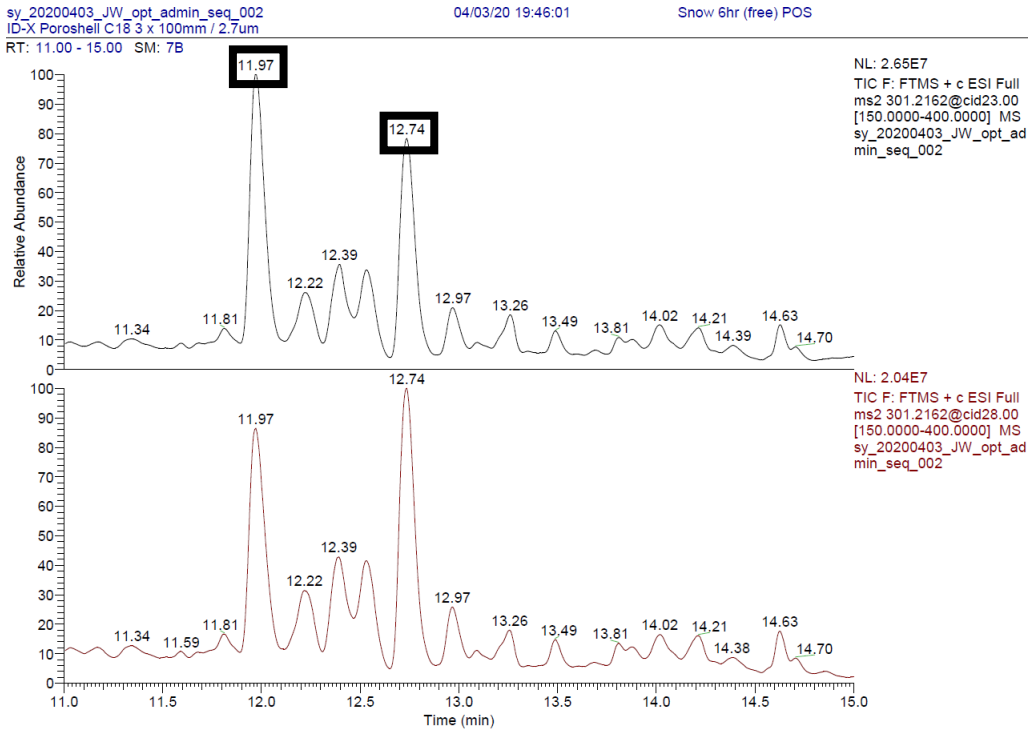
LC-MS figures – greyhound urine samples (free fraction from SPE with fractionation)

1. *Jungle Warfare* type (*m/z* $[M+H]^+$ = 301.2162)

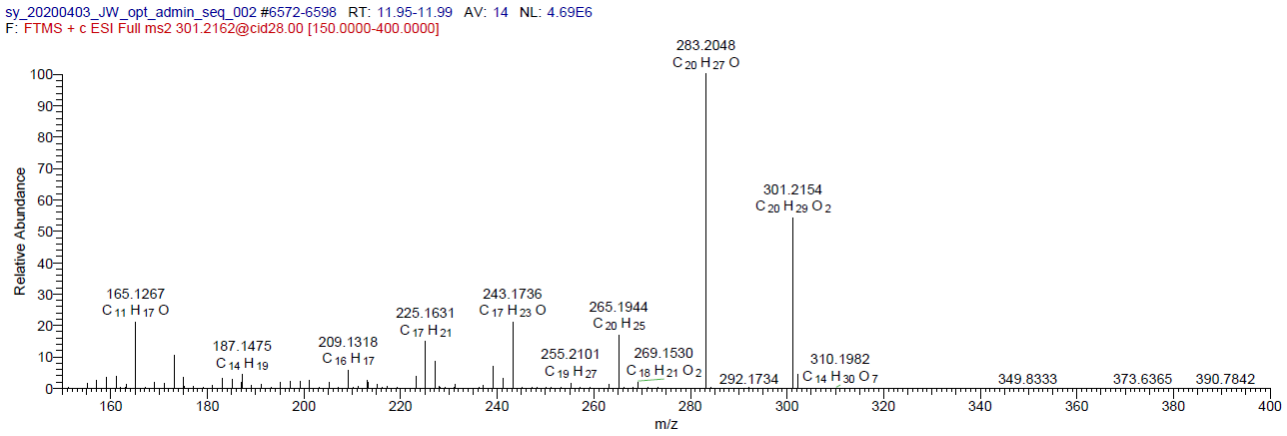
Dog 1, t = 0 h



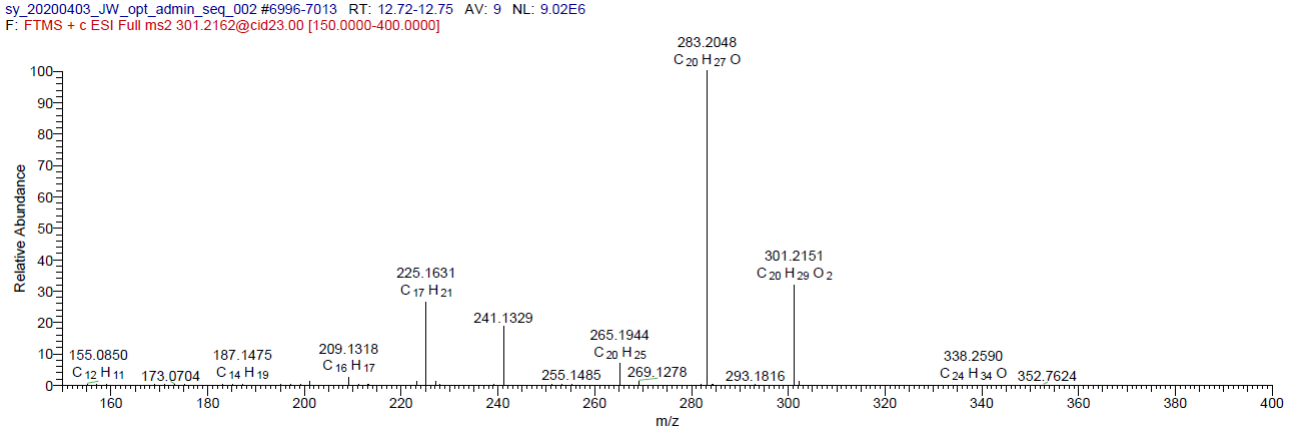
Dog 1, t = 6 h



MS/MS – 11.97 min (**JW**)



MS/MS – 12.74 min (**epiJW**)



2. Hydroxylated *Jungle Warfare* type (*m/z* $[M+H]^+$ = 317.2111)

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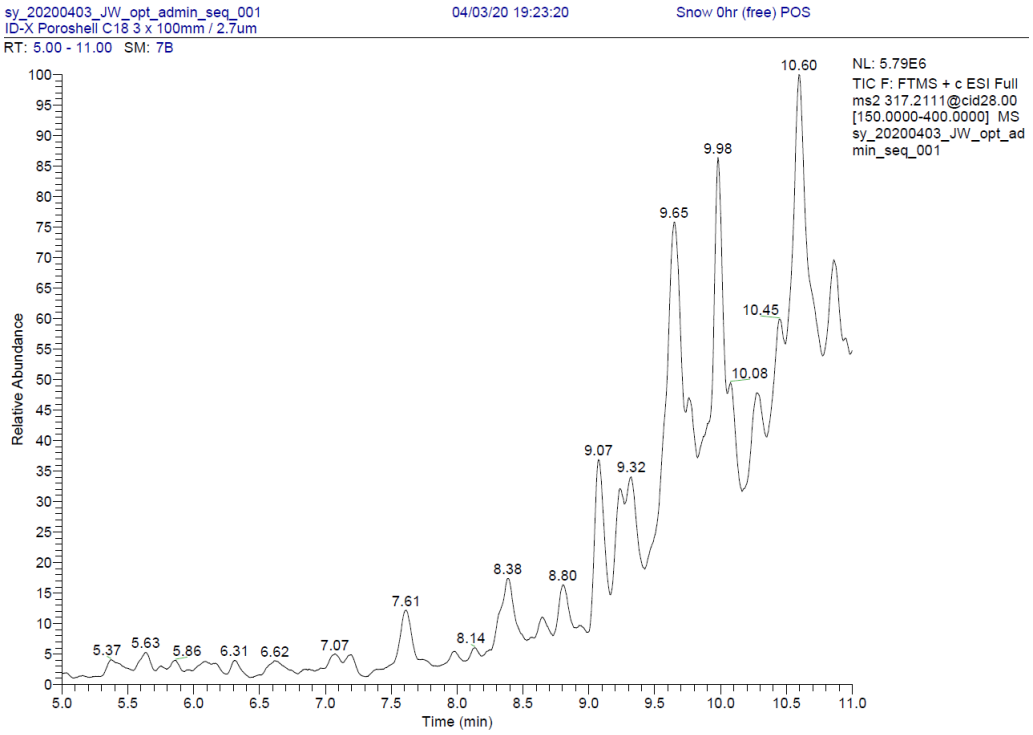
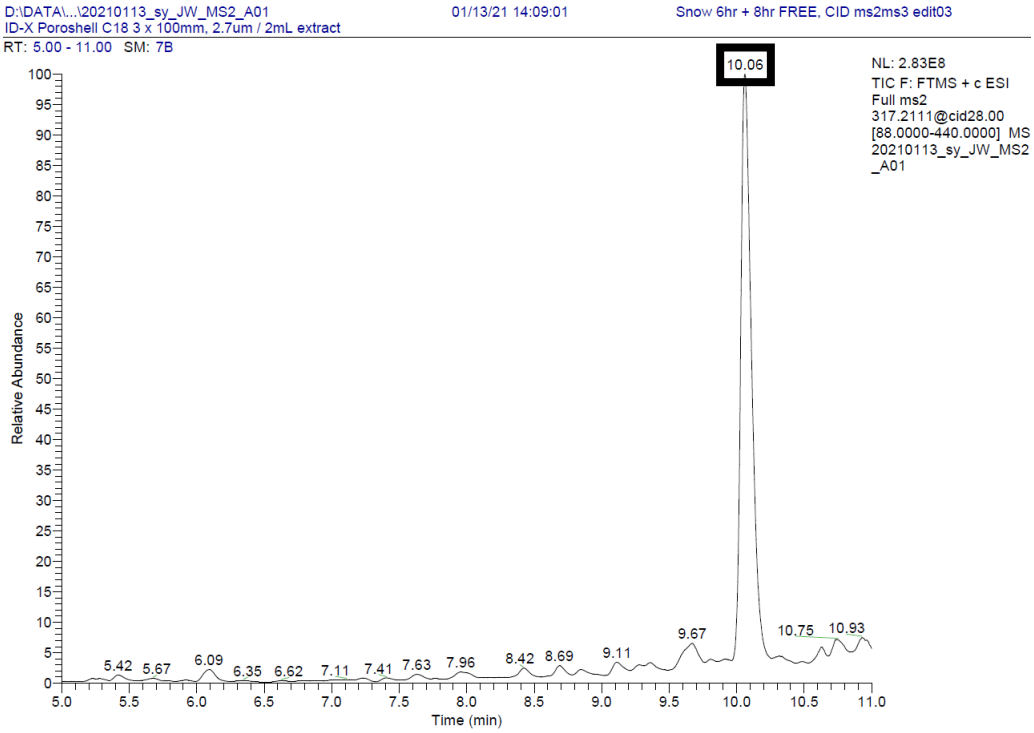
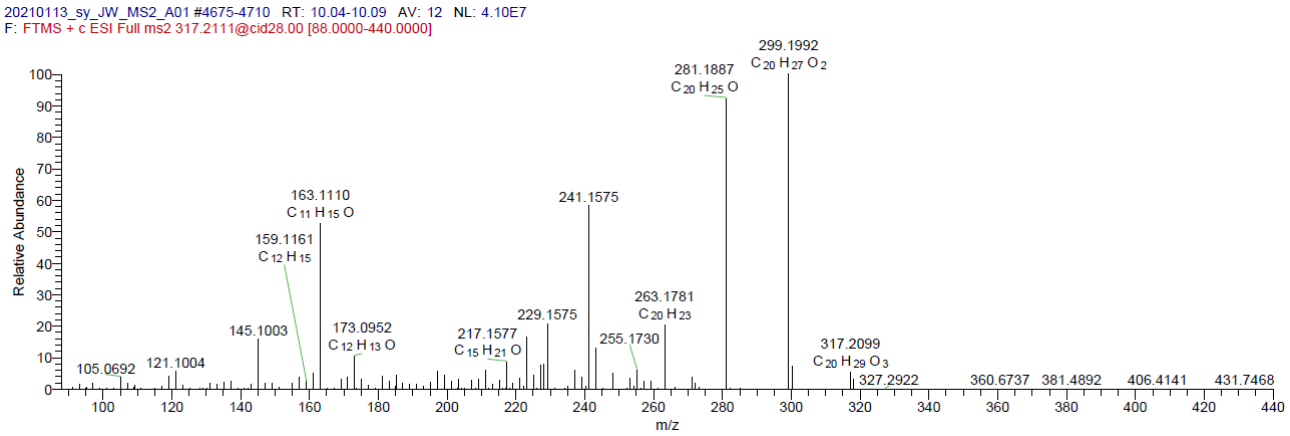


Dog 1, t = 6 + 8 h

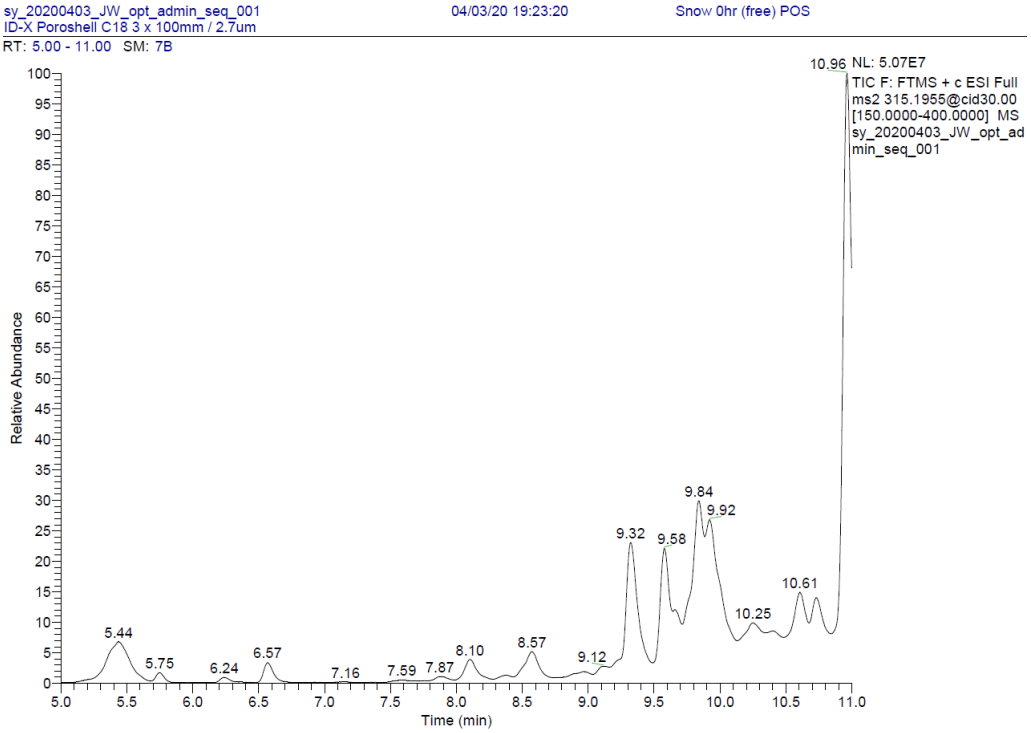


MS/MS – 10.06 min (**16α-OH-JW**)

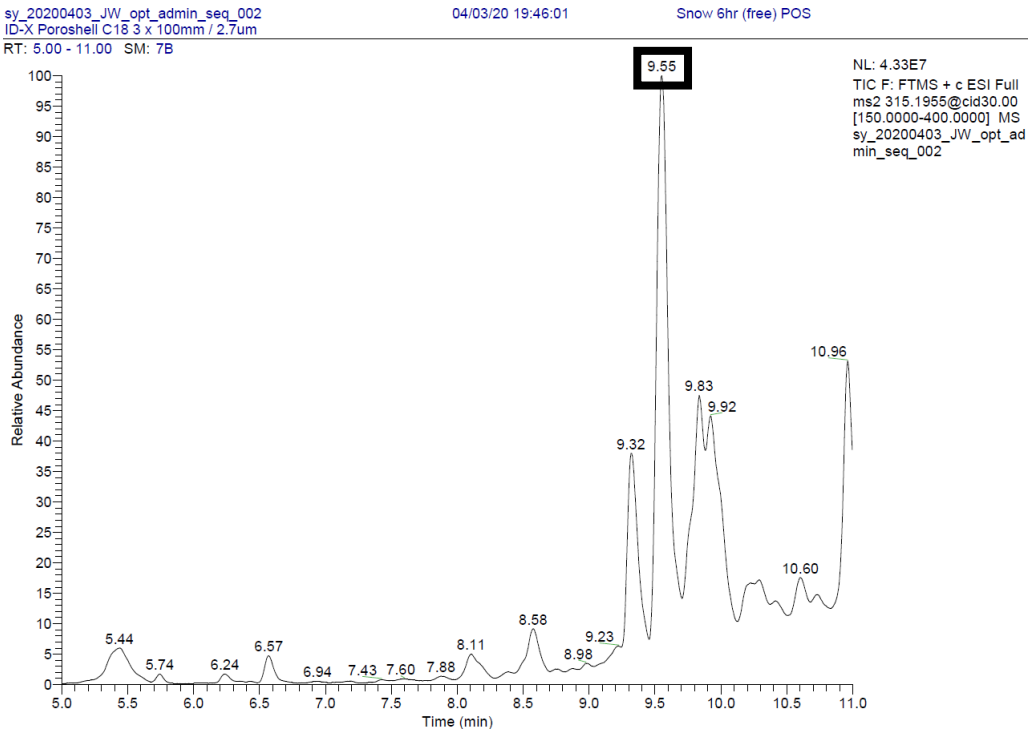


3. Hydroxylated and oxidised *Jungle Warfare* type (*m/z* $[M+H]^+$ = 315.1954)

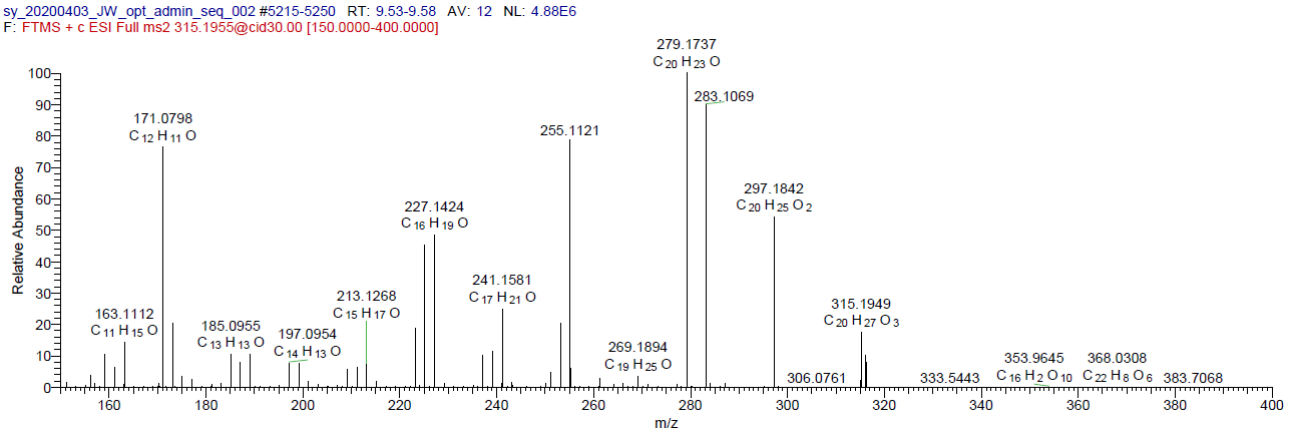
Dog 1, t = 0 h



Dog 1, t = 6 h

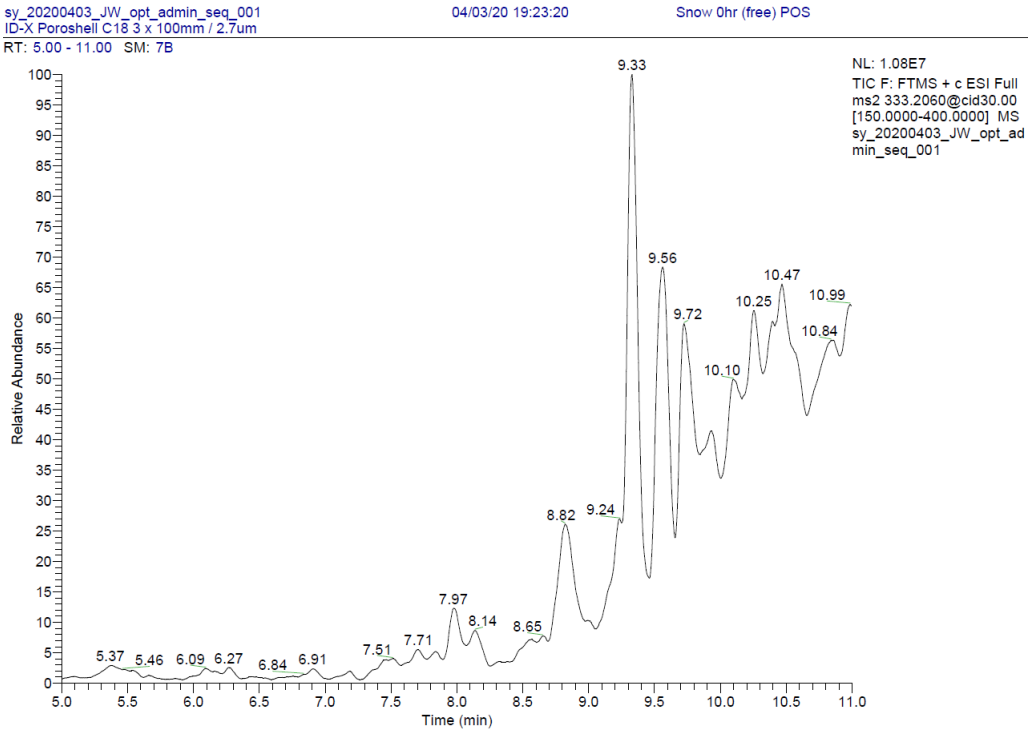


MS/MS – 9.55 min (unknown HOJW)

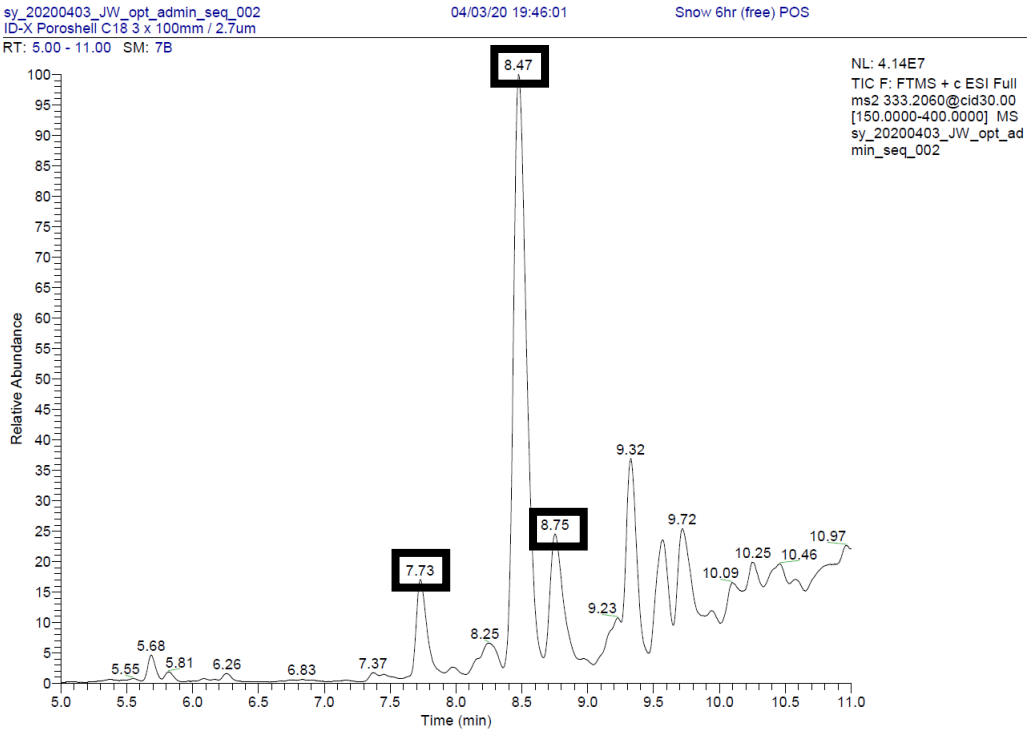


4. Dihydroxylated *Jungle Warfare* type (*m/z* $[M+H]^+$ = 333.2060)

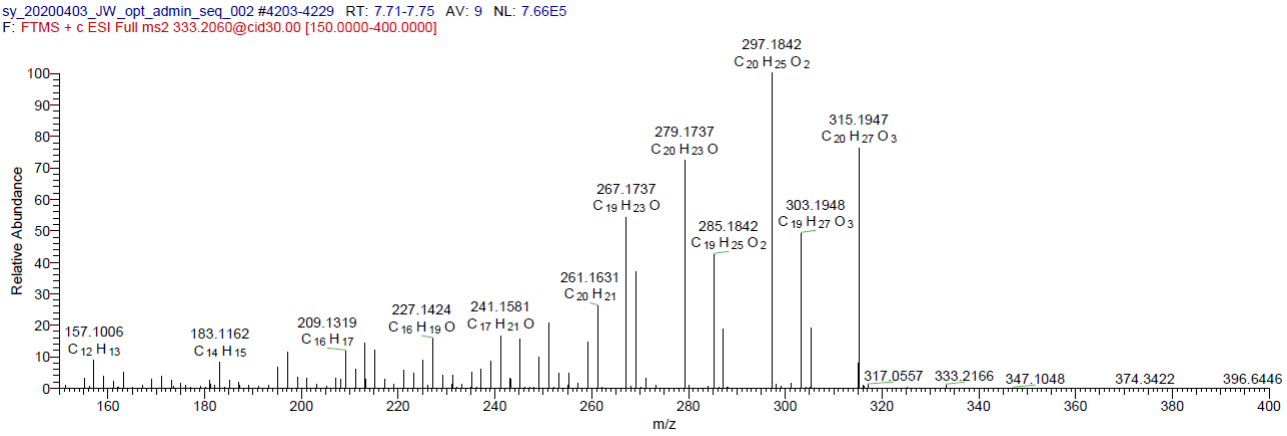
Dog 1, t = 0 h



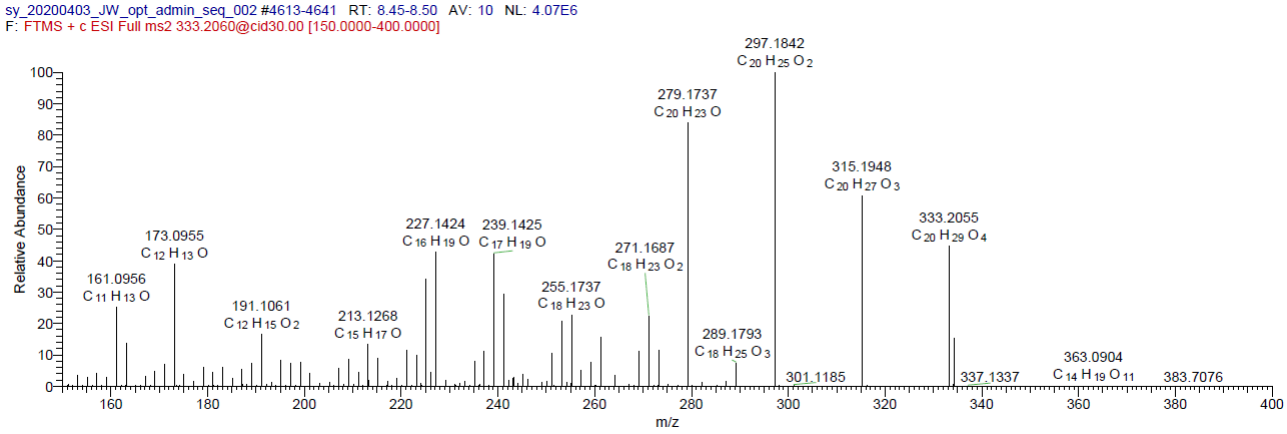
Dog 1, t = 6 h



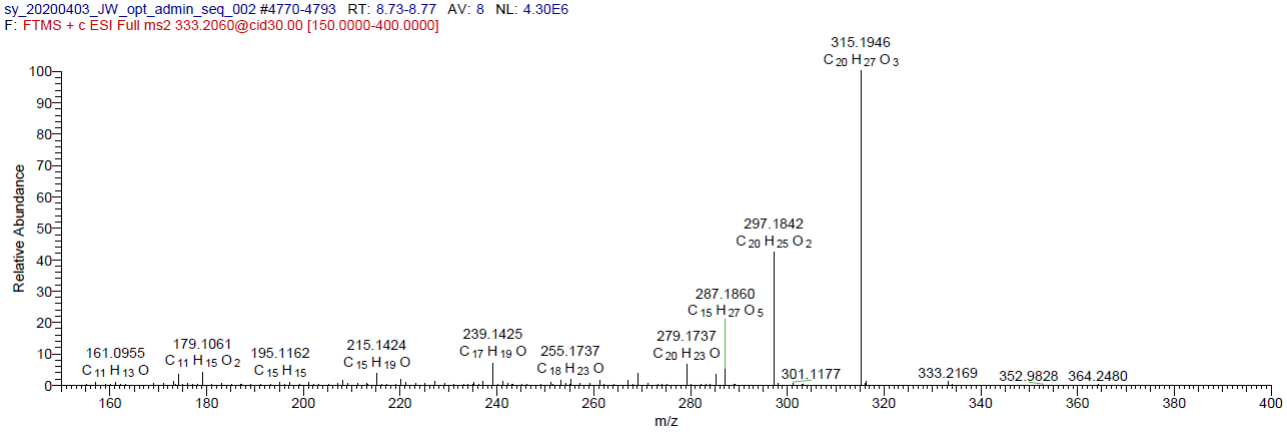
MS/MS – 7.73 min (unknown diHJW 1)



MS/MS – 8.47 min (unknown diHJW 2)



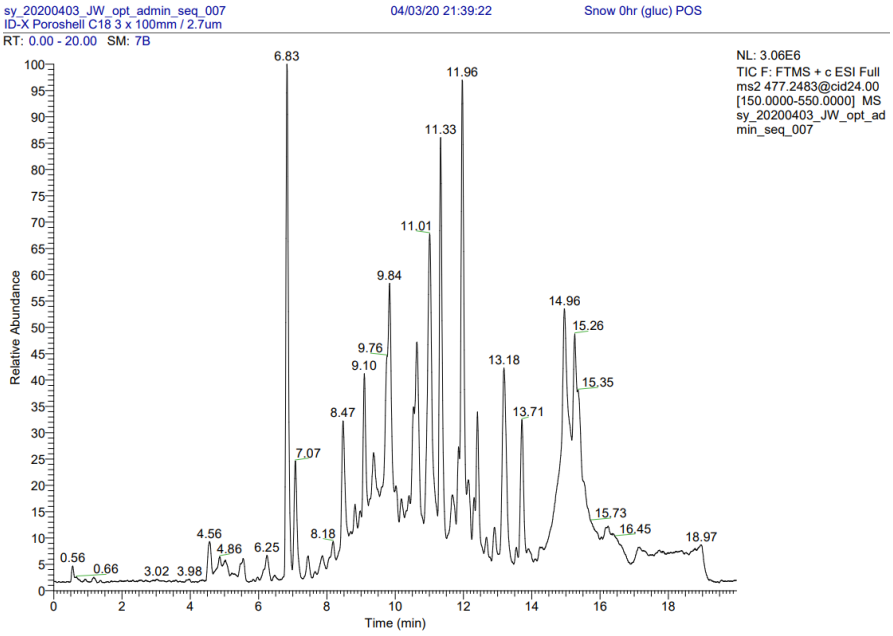
MS/MS – 8.75 min (unknown diHJW 3)



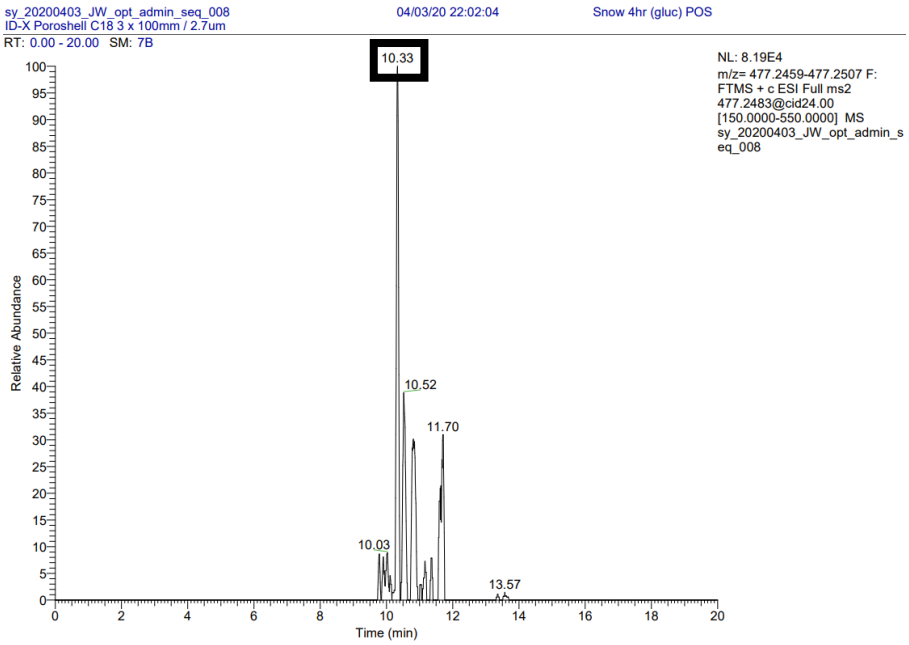
LC-MS figures – greyhound urine samples (gluc fraction from SPE with fractionation)

1. *Jungle Warfare* type (*m/z* $[M+H]^+$ = 477.2483 (gluc) or 301.2162 (hydrolysed))

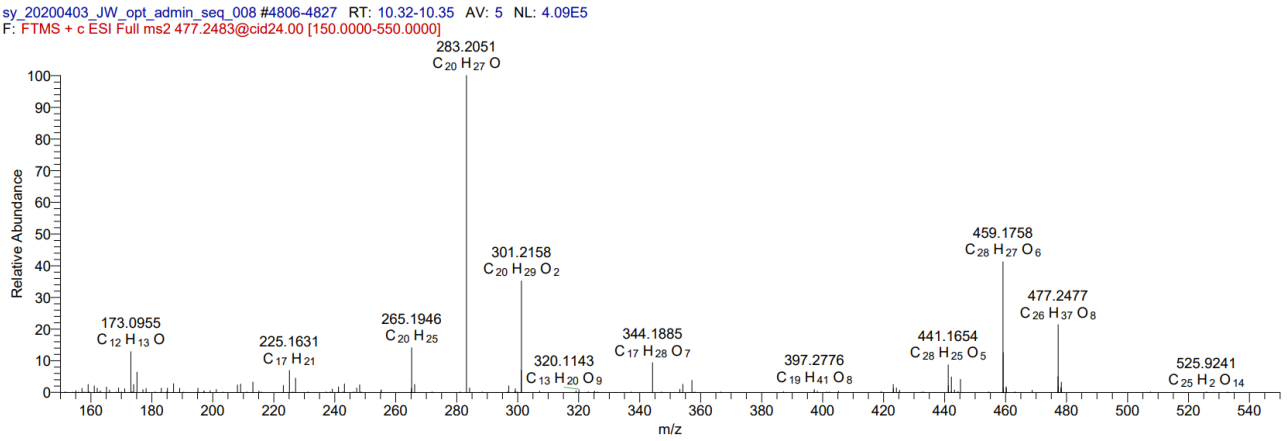
Dog 1, t = 0 h



Dog 1, t = 4 h

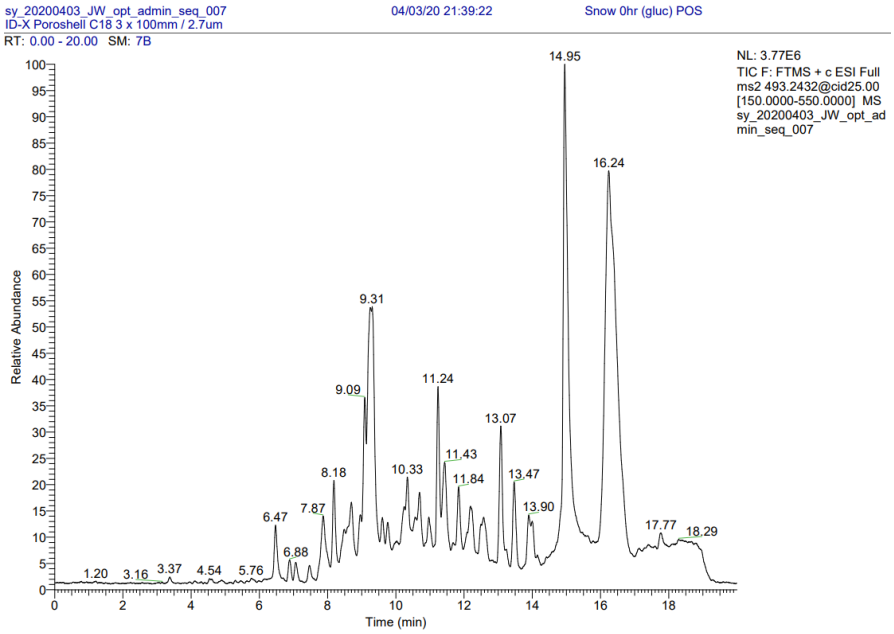


MS/MS – 10.33 min (unknown JW gluc)

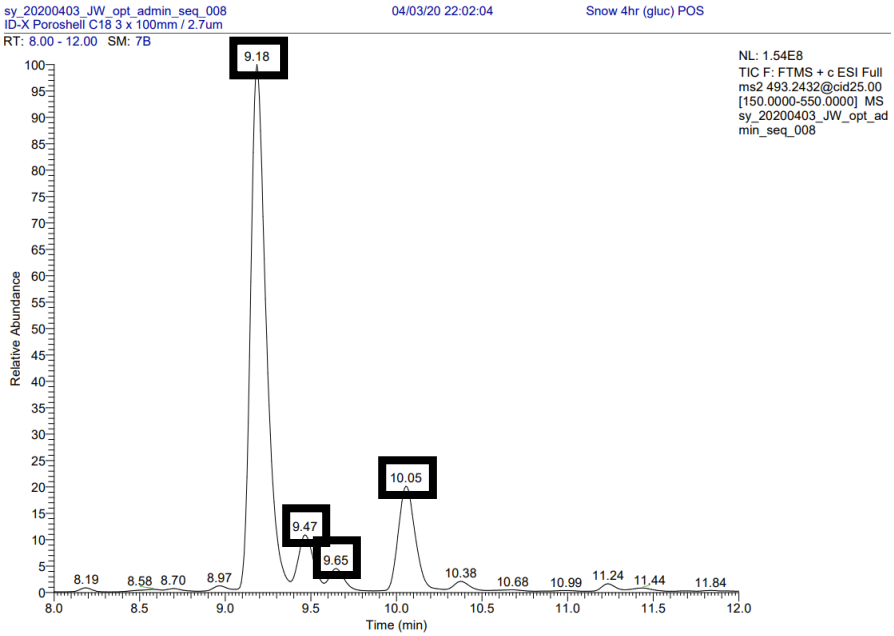


2. Hydroxylated *Jungle Warfare* type (*m/z* $[M+H]^+$ = 493.2432 (gluc) or 317.2111 (hydrolysed))

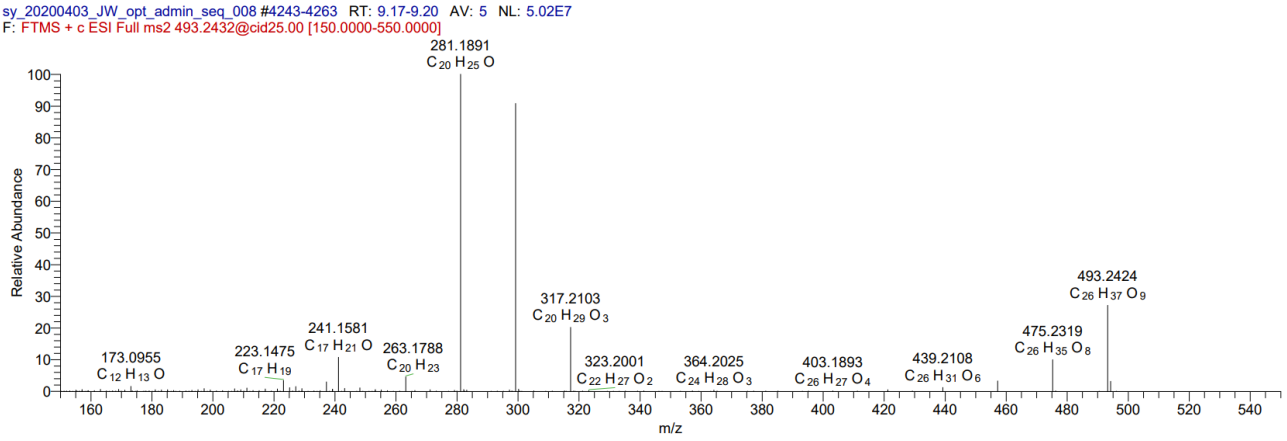
Dog 1, t = 0 h



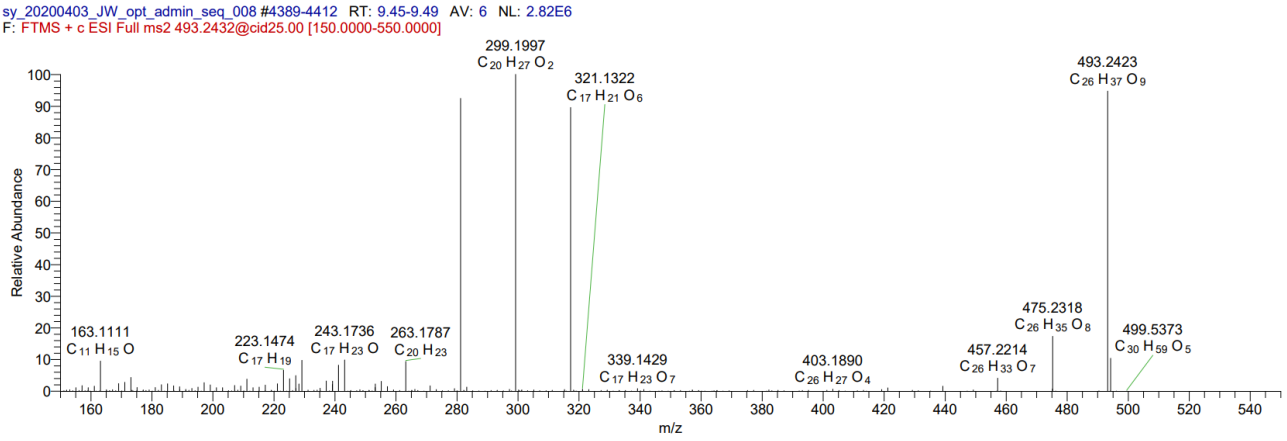
Dog 1, t = 4 h



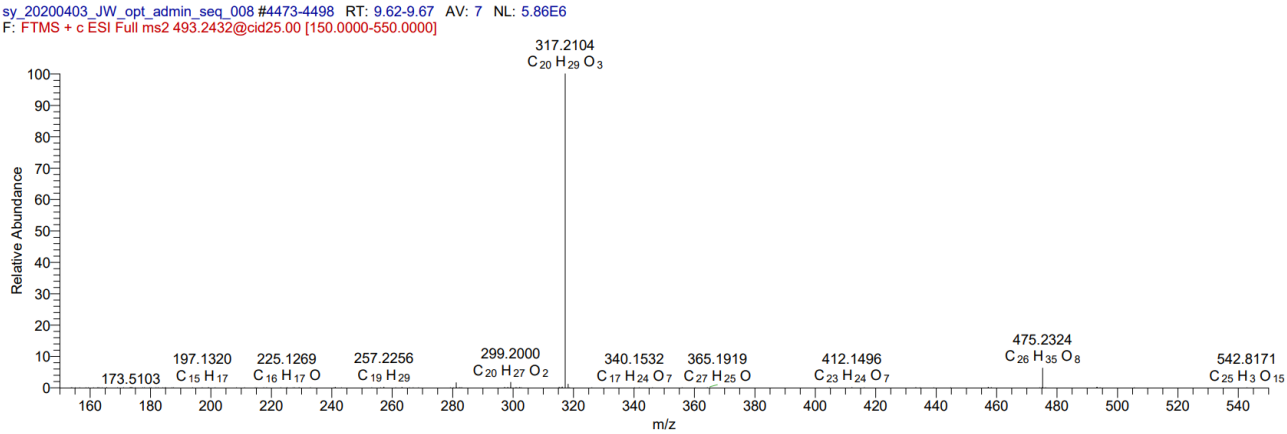
MS/MS – 9.18 min (unknown HJW gluc 1)



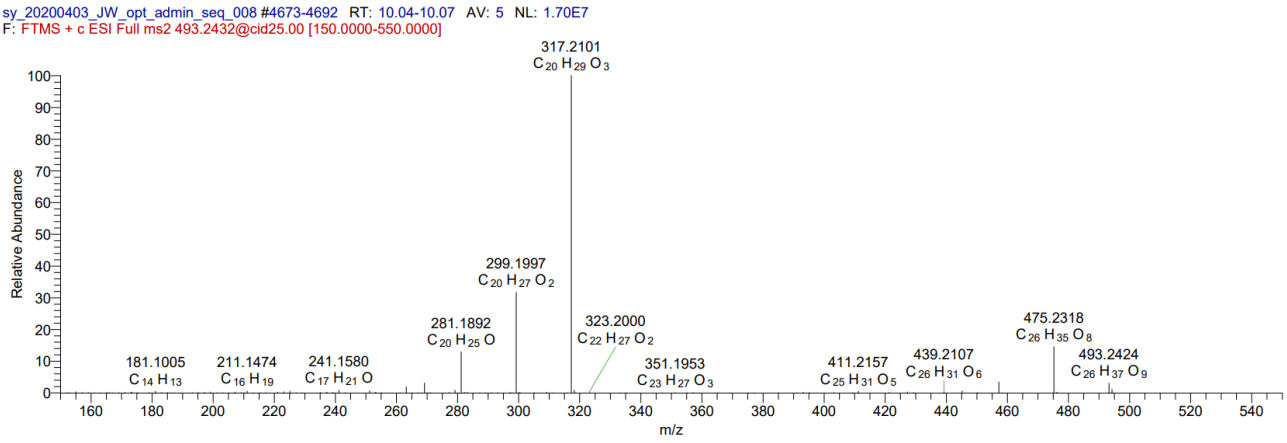
MS/MS – 9.47 min (unknown HJW gluc 2)



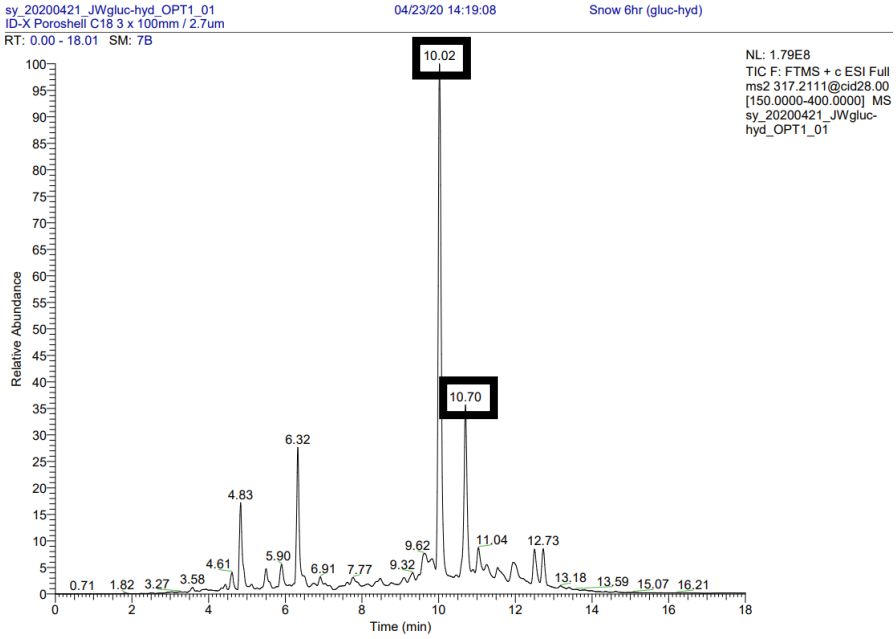
MS/MS – 9.65 min (unknown HJW gluc 3)



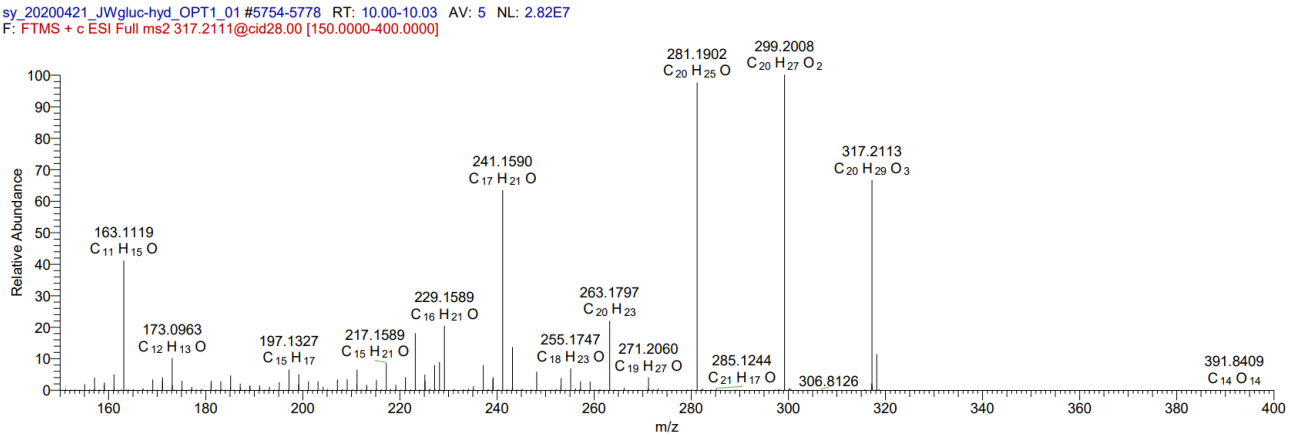
MS/MS – 10.05 min (unknown HJW gluc 4)



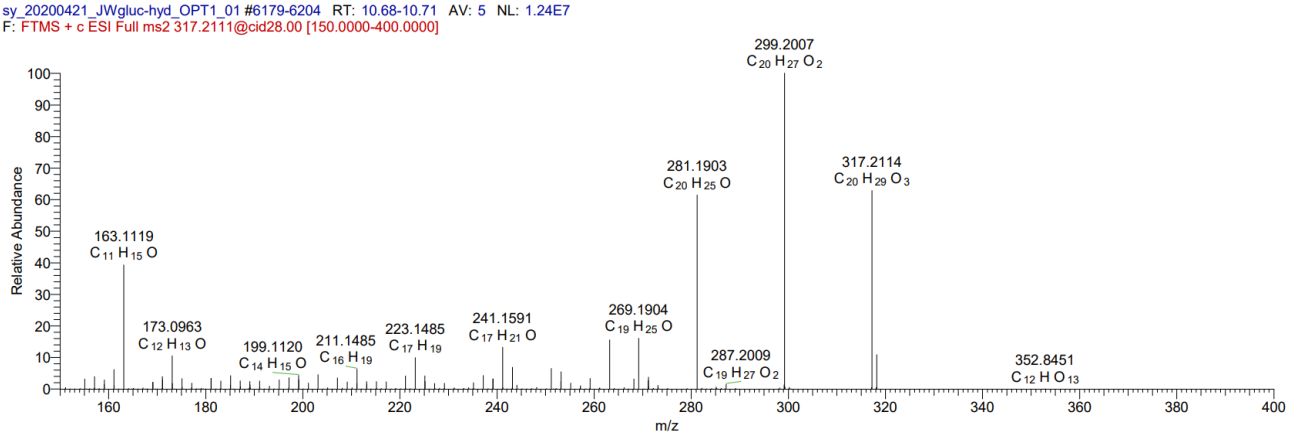
Dog 1, t = 6 h (after *E. coli* β-glucuronidase hydrolysis)



MS/MS – 10.02 min (**16α-OH-JW**)



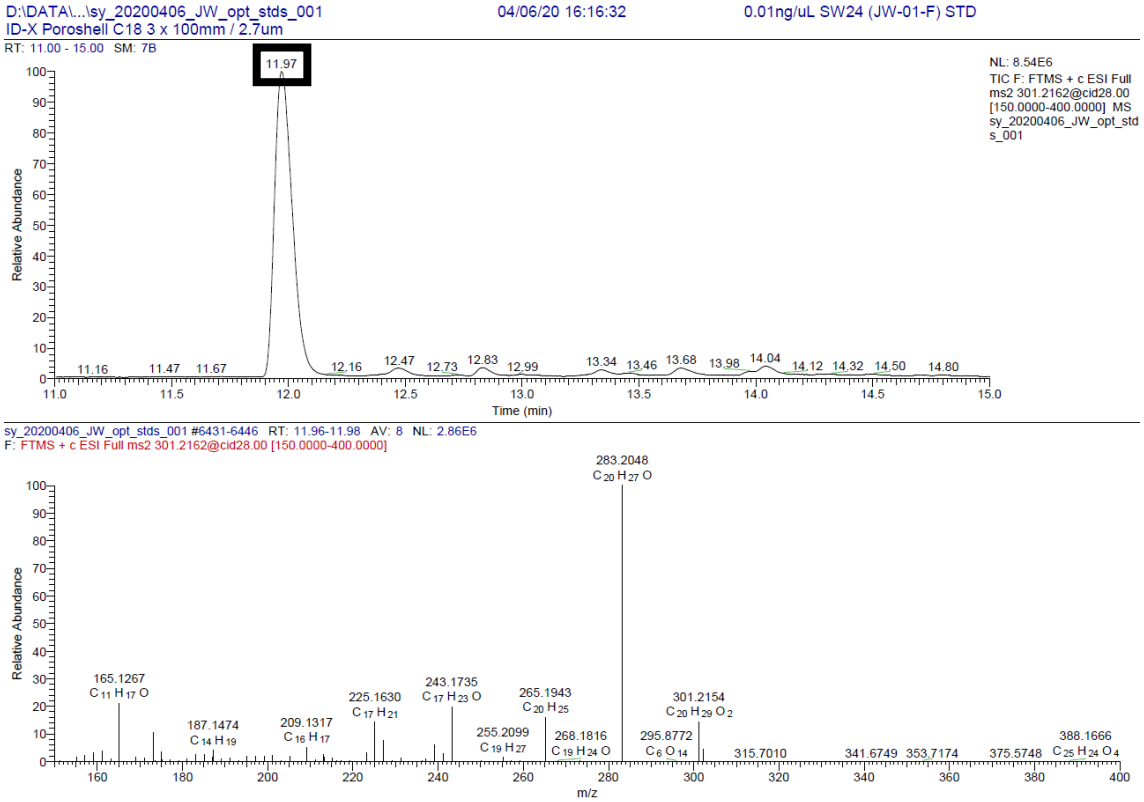
MS/MS – 10.70 min (unknown HJW)



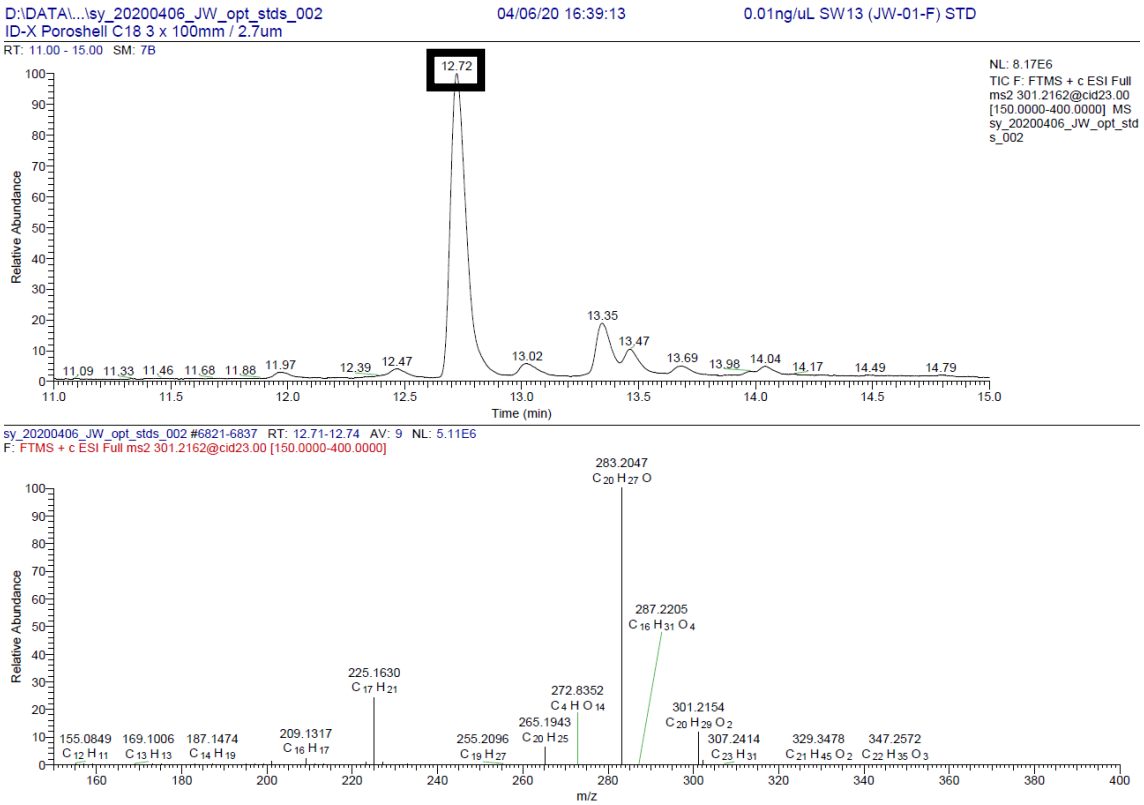
LC-MS figures – synthesised reference materials

1. *Jungle Warfare* type (*m/z* $[M+H]^+$ = 301.2162)

MS/MS – 11.97 min (**JW**)

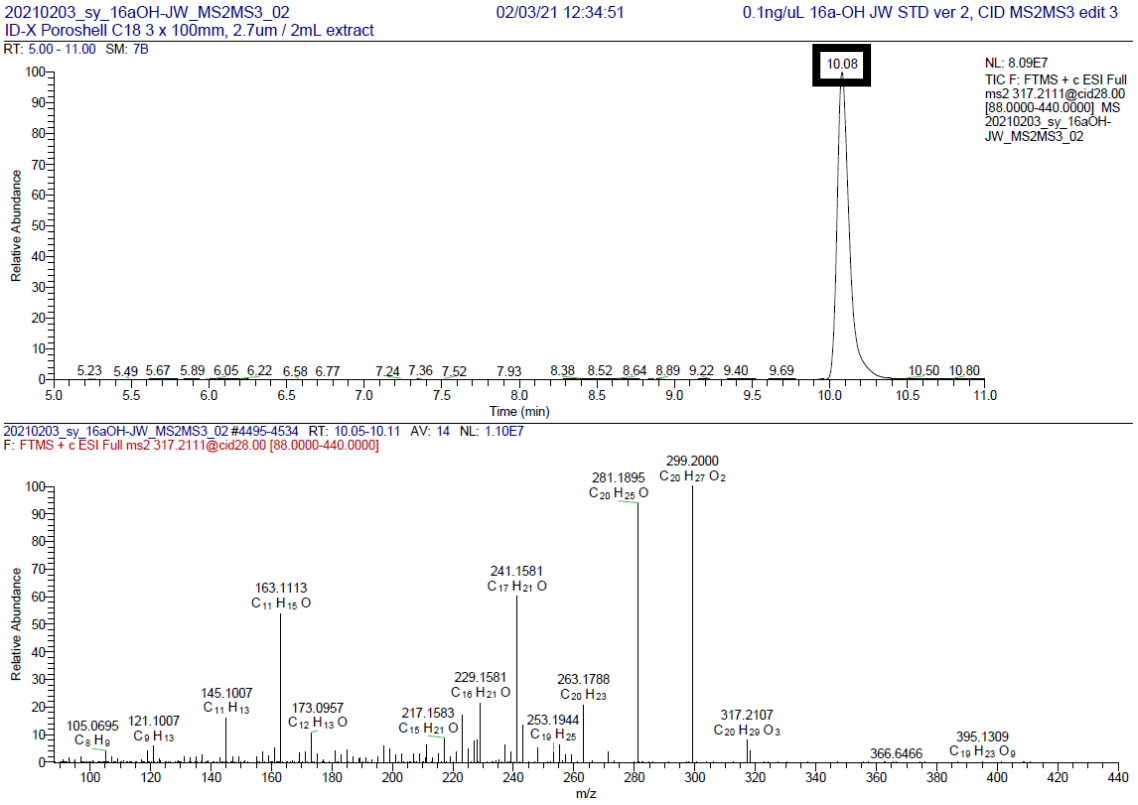


MS/MS – 12.72 min (**epiJW**)

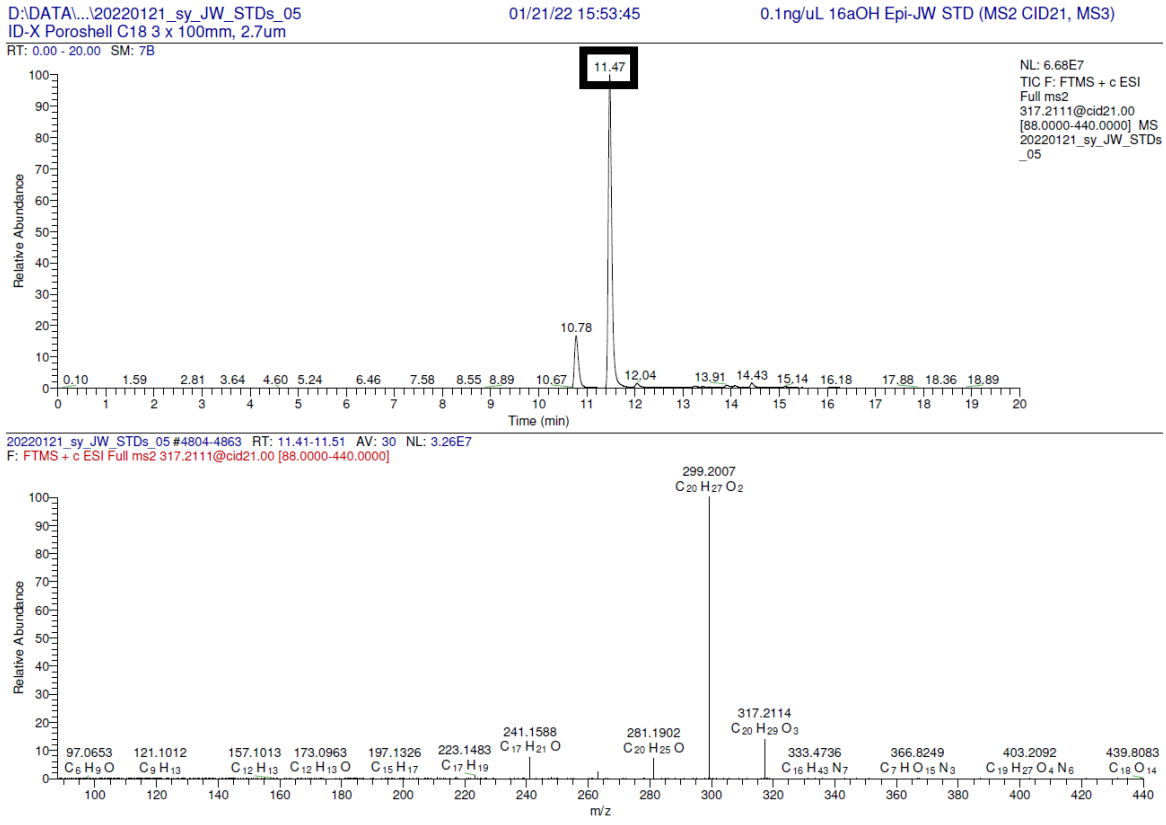


2. Hydroxylated *Jungle Warfare* type (*m/z* $[M+H]^+$ = 317.2111)

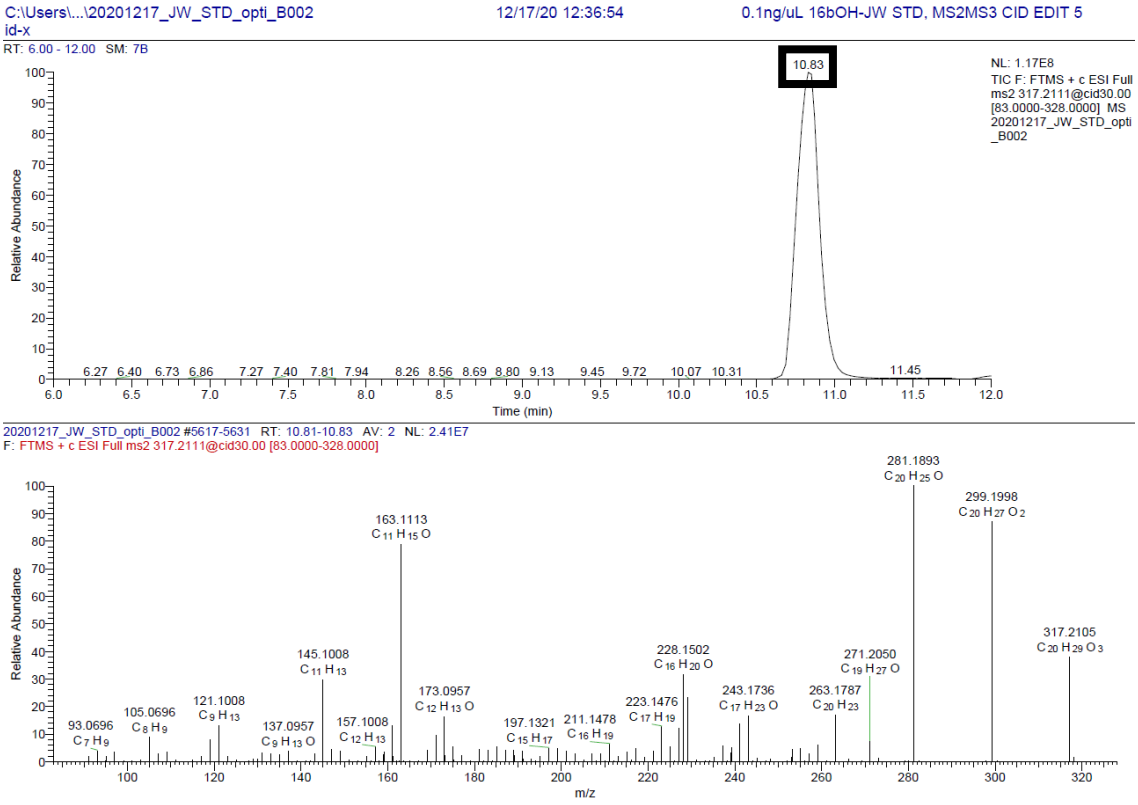
MS/MS – 10.08 min (**16α-OH-JW**)



MS/MS – 11.47 min (**16α-OH-epiJW**)

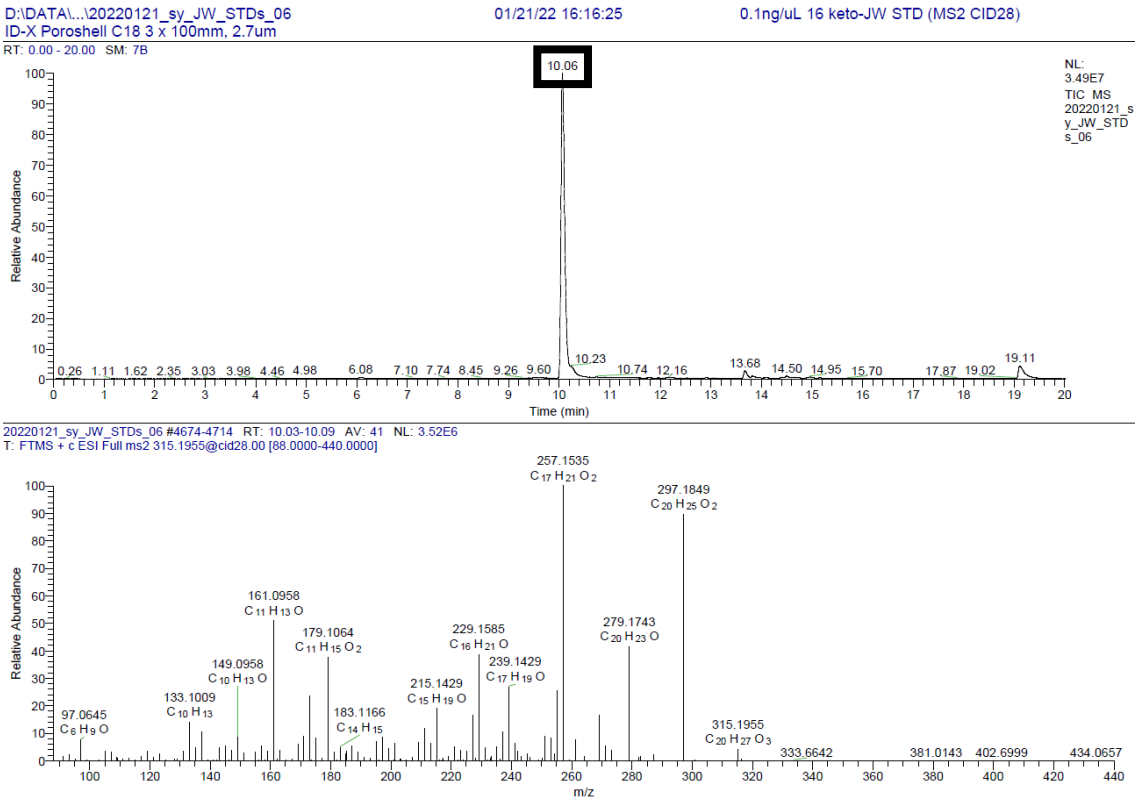


MS/MS – 10.83 min (**16β-OH-JW**)

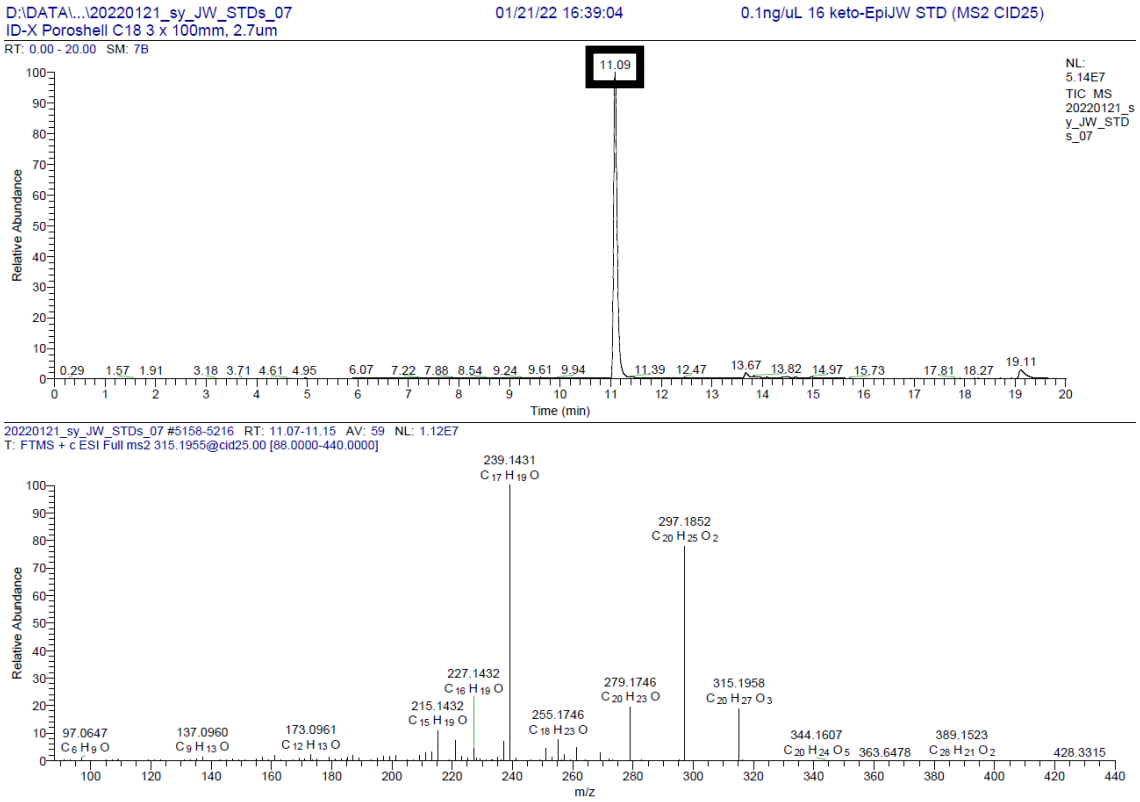


3. Hydroxylated and oxidised *Jungle Warfare* type (*m/z* $[M+H]^+$ = 315.1954)

MS/MS – 10.06 min (**16-keto-JW**)



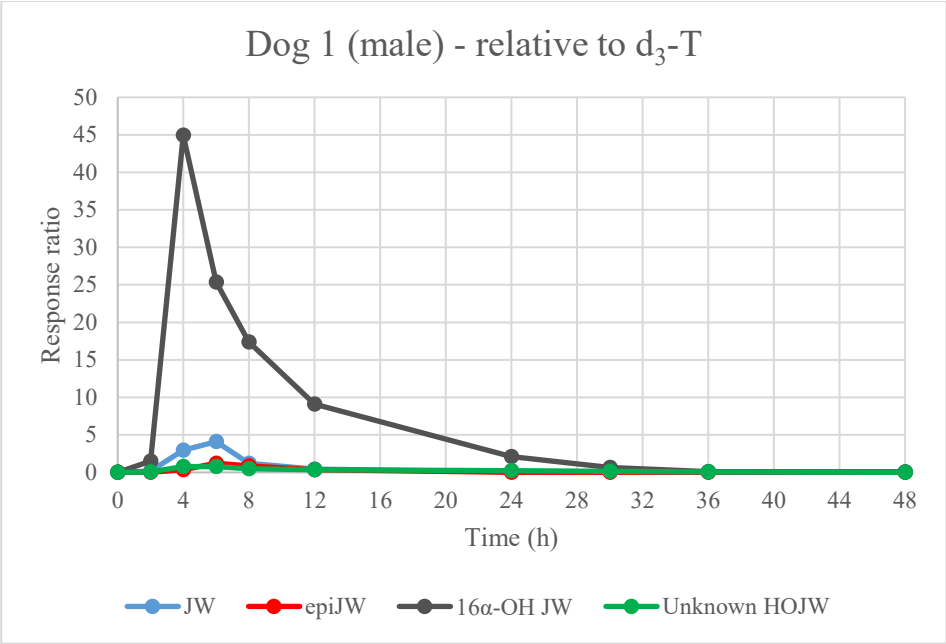
MS/MS – 11.09 min (**16-keto-epiJW**)



LOD and extraction recovery tables and figures

Table S2. Limit of detection (LOD) and extraction recovery.

Analyte	Precursor ion $[M+H]^+$	Retention time (min)	LOD (ng/mL)	Linear regression equation	Correlation coefficient (R^2)	Extraction recovery (%)
Δ6-Methyltestosterone **(JW)**	301.2162	11.97	0.50	y = 0.1486x + 0.0265	0.9925	80
17-Epi-Δ6-methyltestosterone **(epiJW)**	301.2162	12.72	0.40	y = 0.0698x - 0.0059	0.9953	57
16α,17β-Dihydroxy-17α-methyl-androsta-4,6-dien-3-one **(16α-OH-JW)**	317.2111	10.08	0.99	y = 0.0137x + 0.0025	0.9708	30



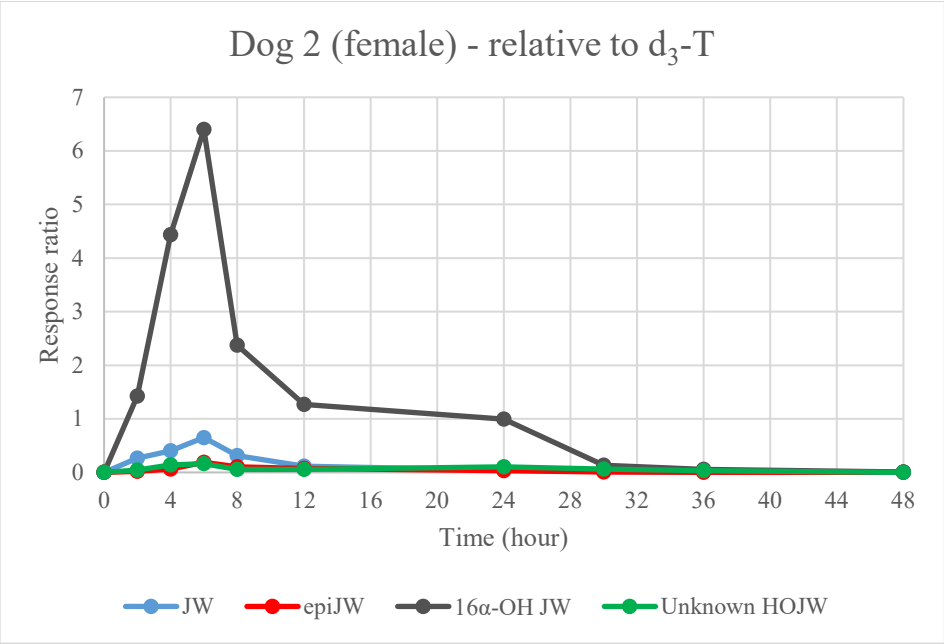
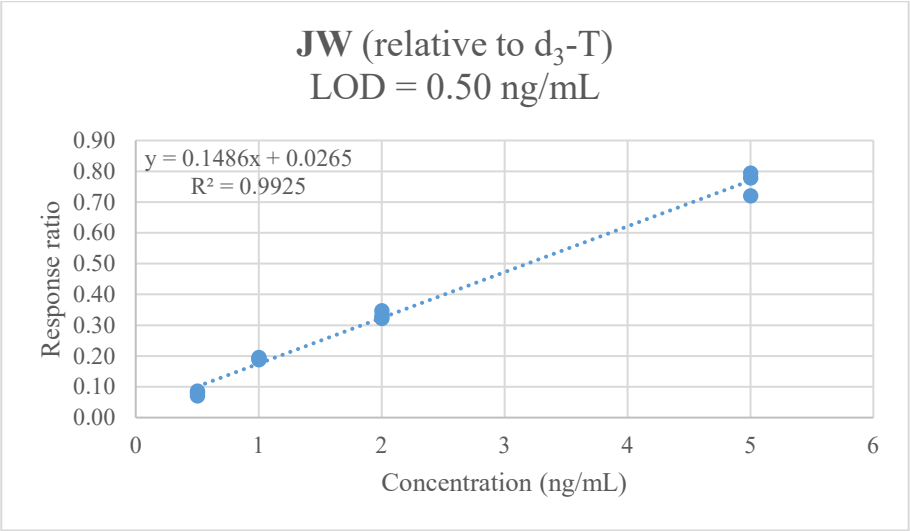
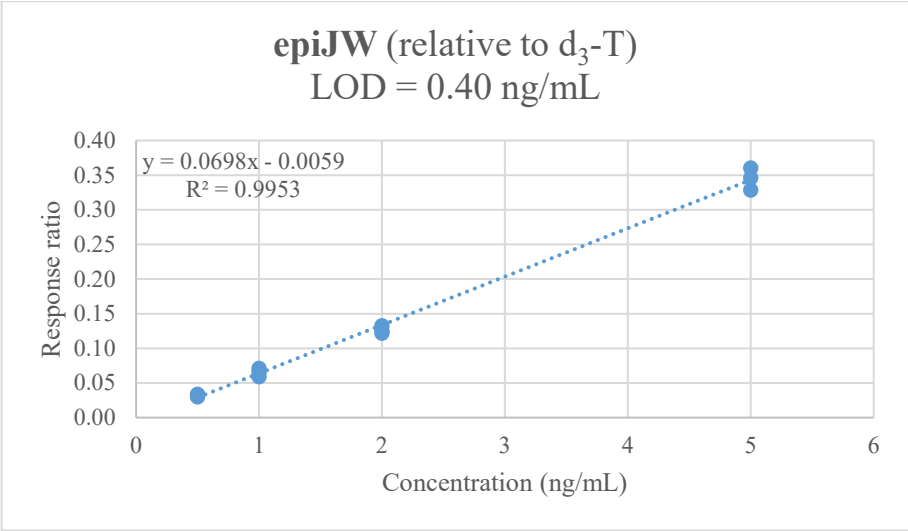


Figure S1. Excretion profile for jungle warfare metabolites in both dogs.





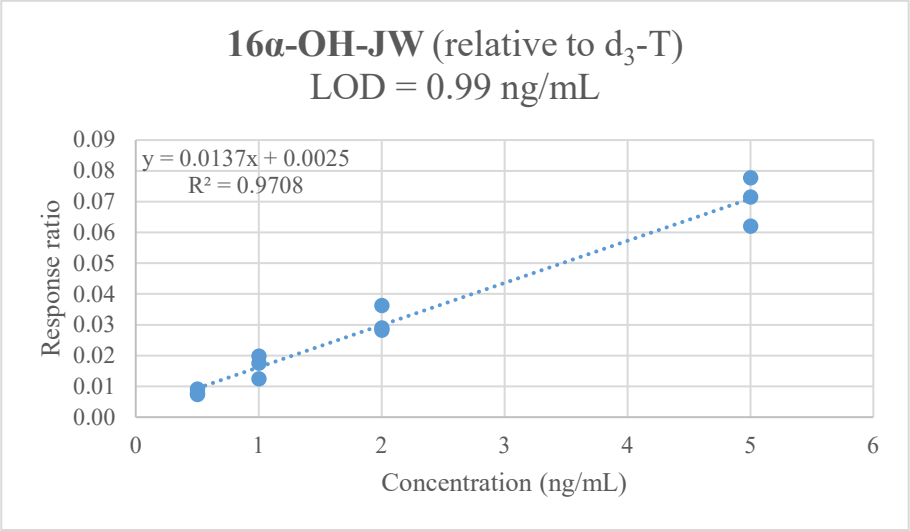
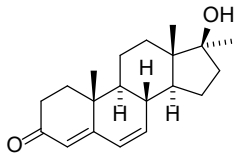
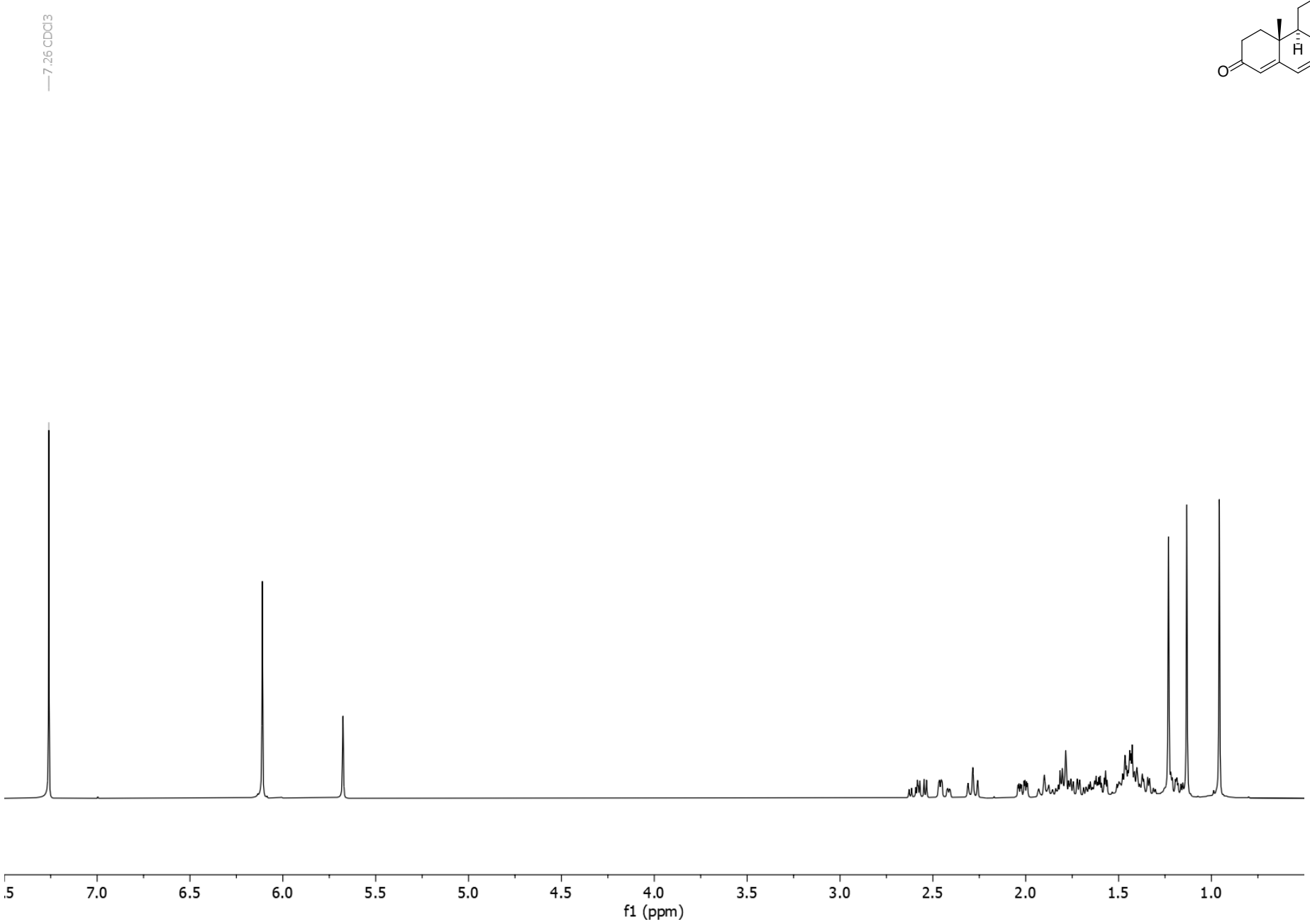


Figure S2. LOD curve for jungle warfare metabolites.

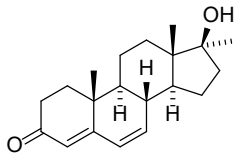
Copies of ^{1}H NMR, ^{13}C NMR, LRMS, and IR spectra

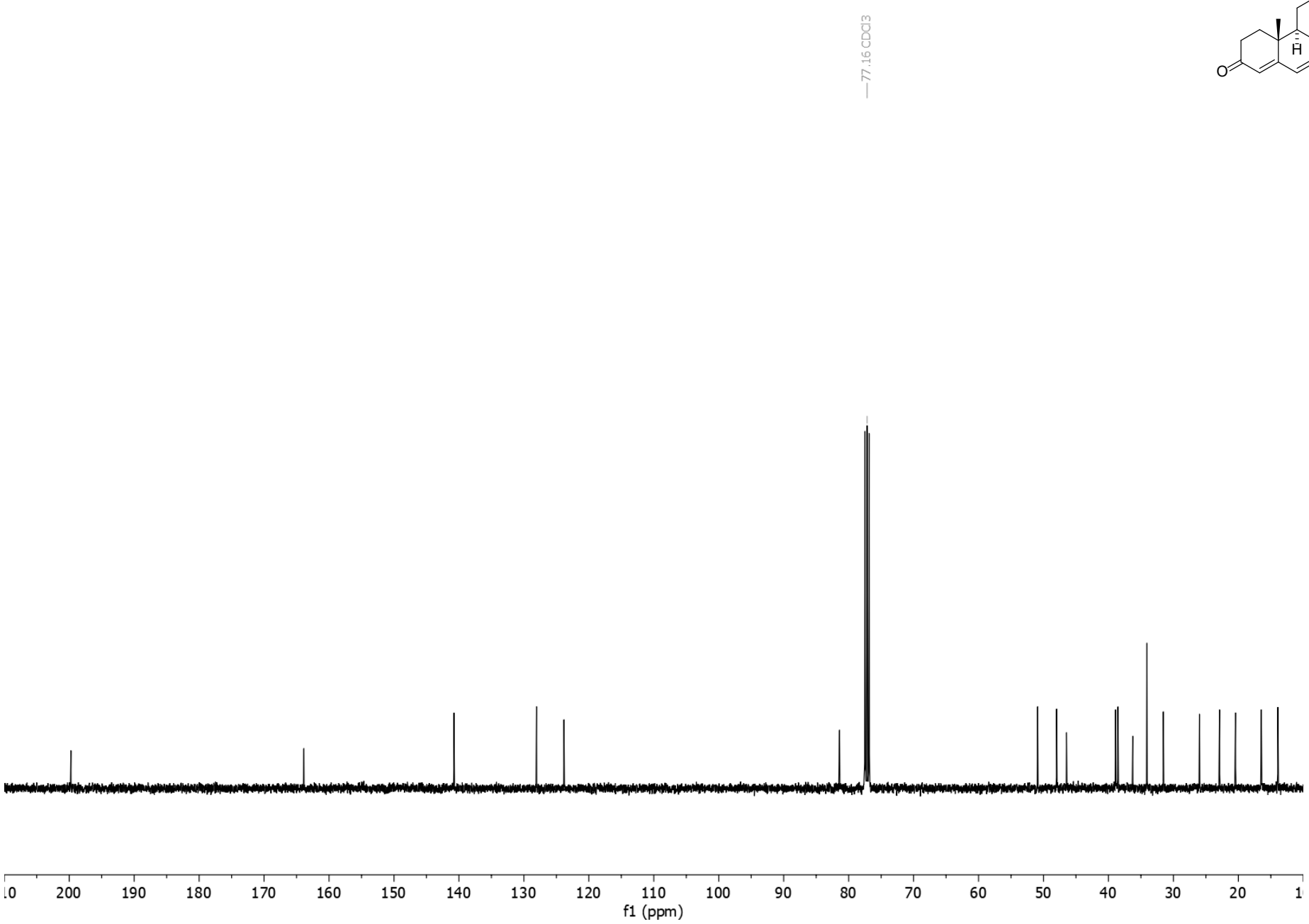
17β-Hydroxy-17α-methylandrosta-4,6-dien-3-one – ^{1}H NMR (400 MHz, $CDCl_3$)



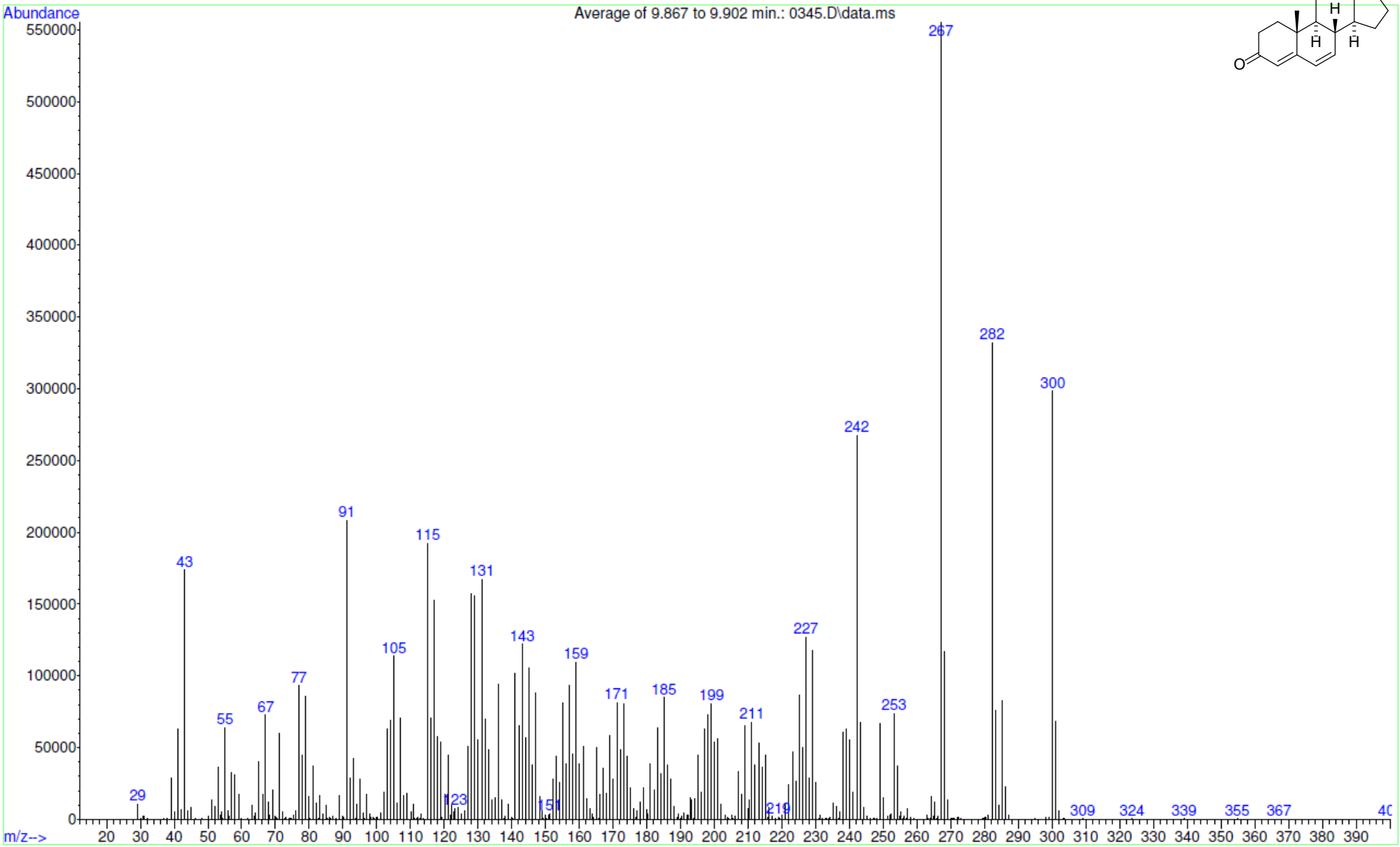


17β-Hydroxy-17α-methylandrosta-4,6-dien-3-one – ^{13}C NMR (101 MHz, $CDCl_3$)

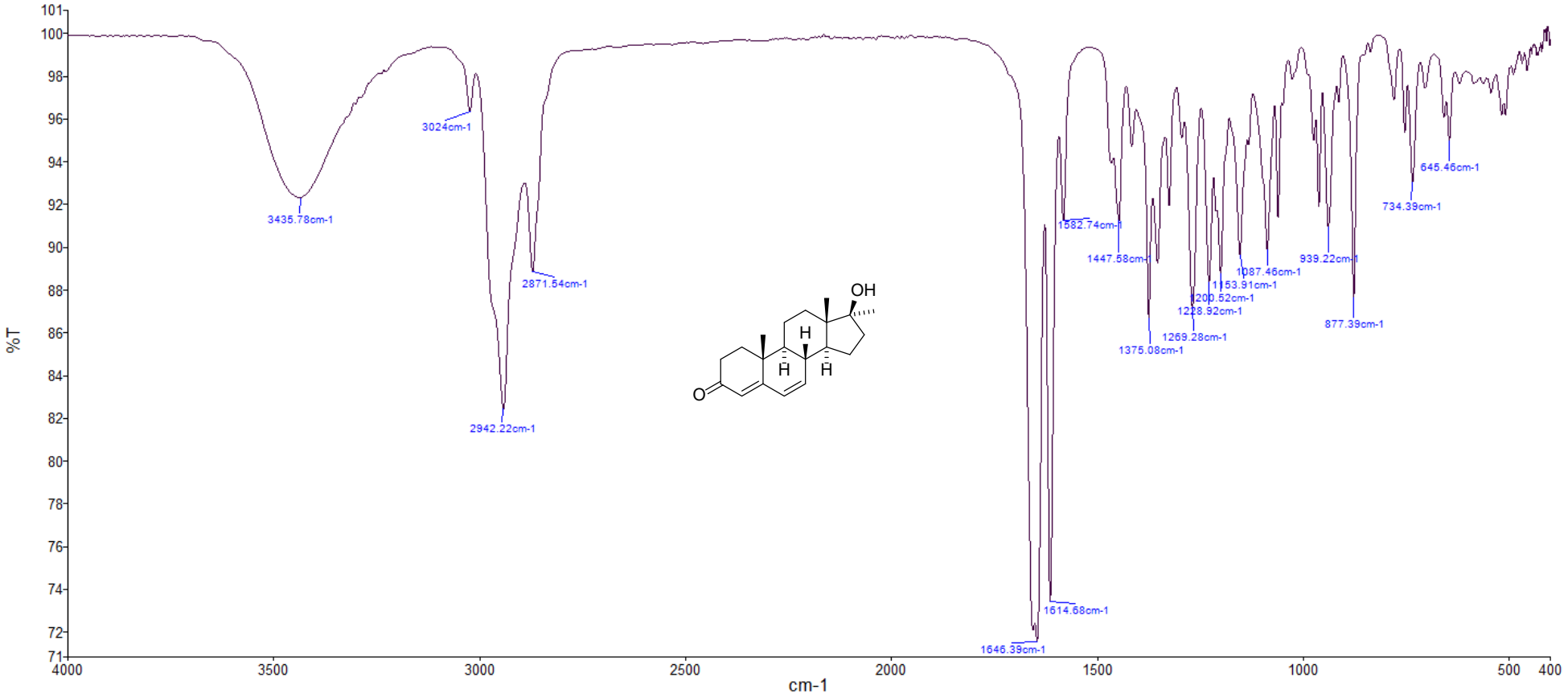




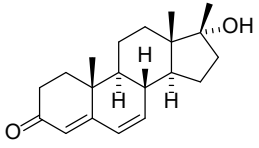
17β-Hydroxy-17α-methylandrosta-4,6-dien-3-one – LRMS (+EI)

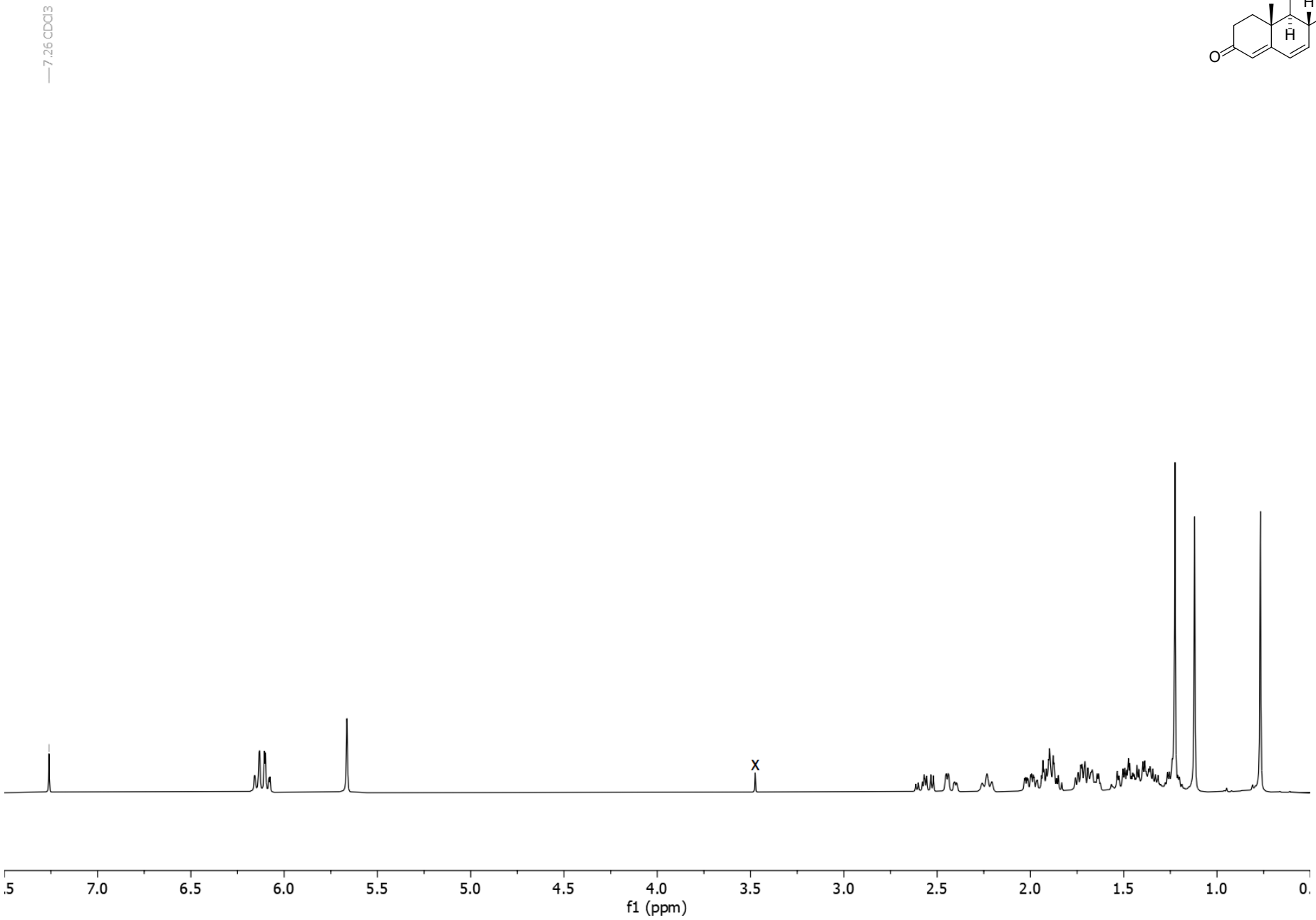


17β-Hydroxy-17α-methylandrosta-4,6-dien-3-one – IR (neat)

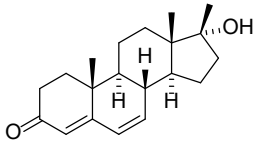


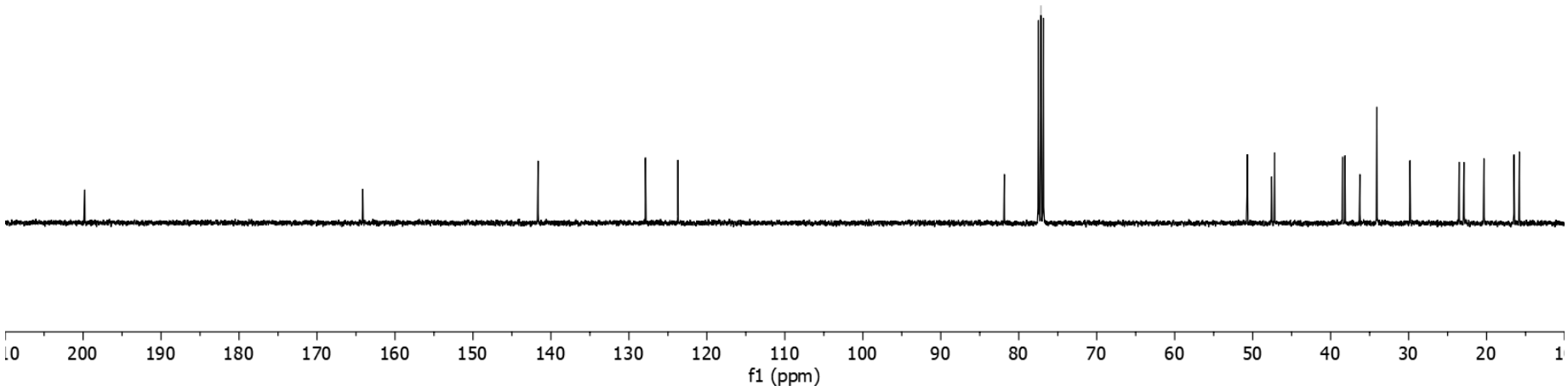
17α-Hydroxy-17β-methylandrosta-4,6-dien-3-one – ^{1}H NMR (400 MHz, $CDCl_3$)



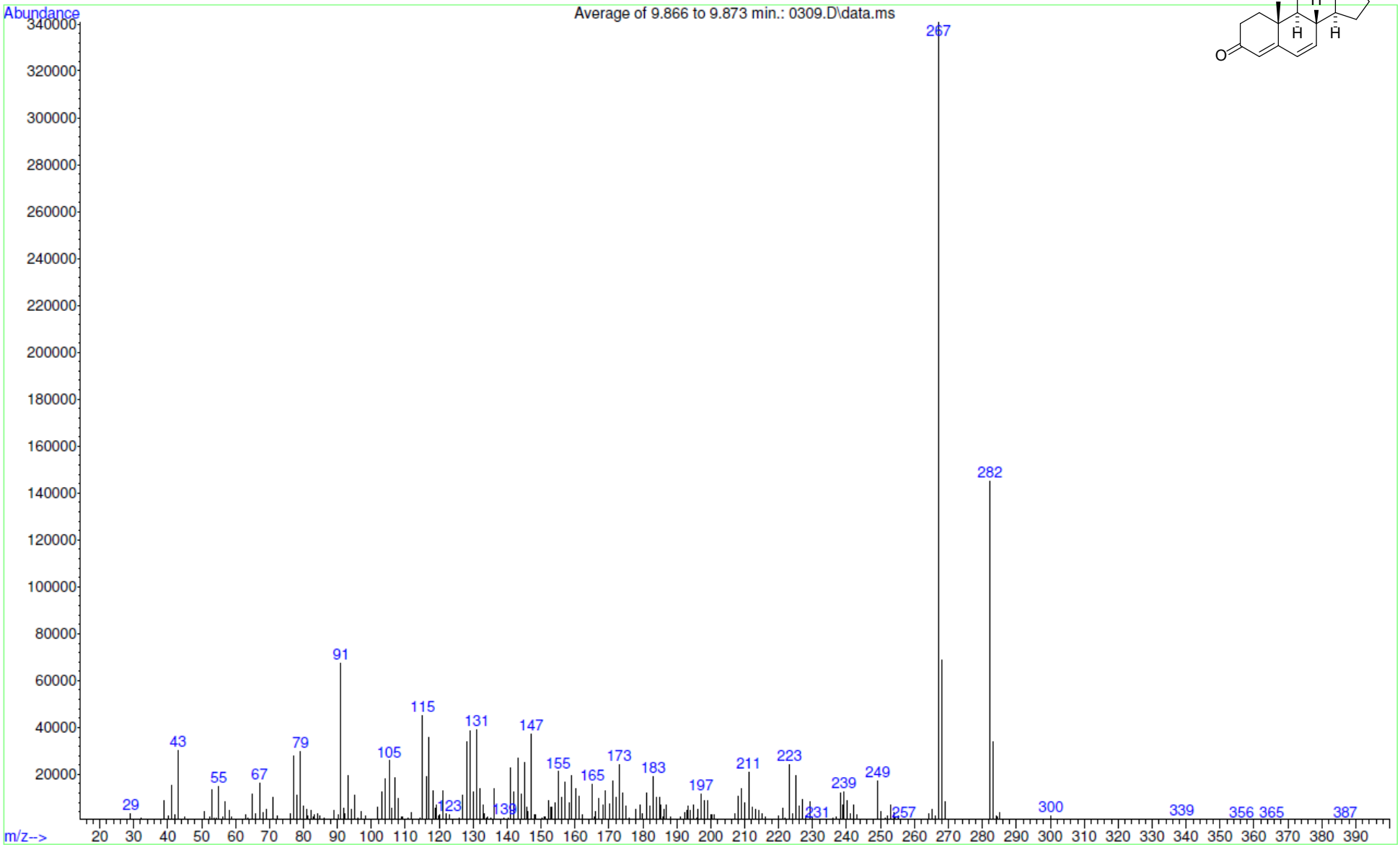


17α-Hydroxy-17β-methylandrosta-4,6-dien-3-one – ^{13}C NMR (101 MHz, $CDCl_3$)

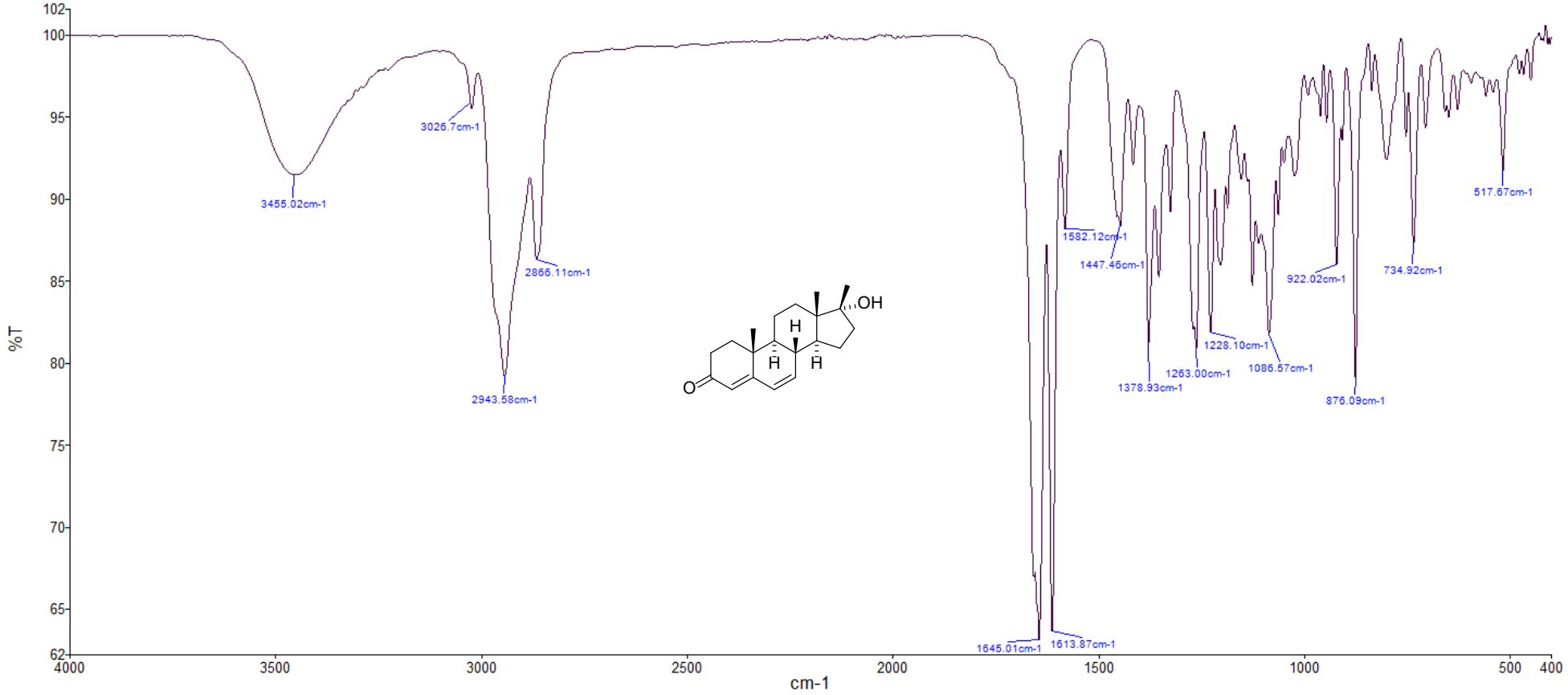




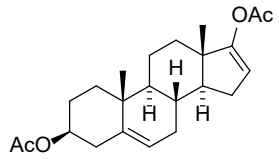
17α-Hydroxy-17β-methylandrosta-4,6-dien-3-one – LRMS (+EI)

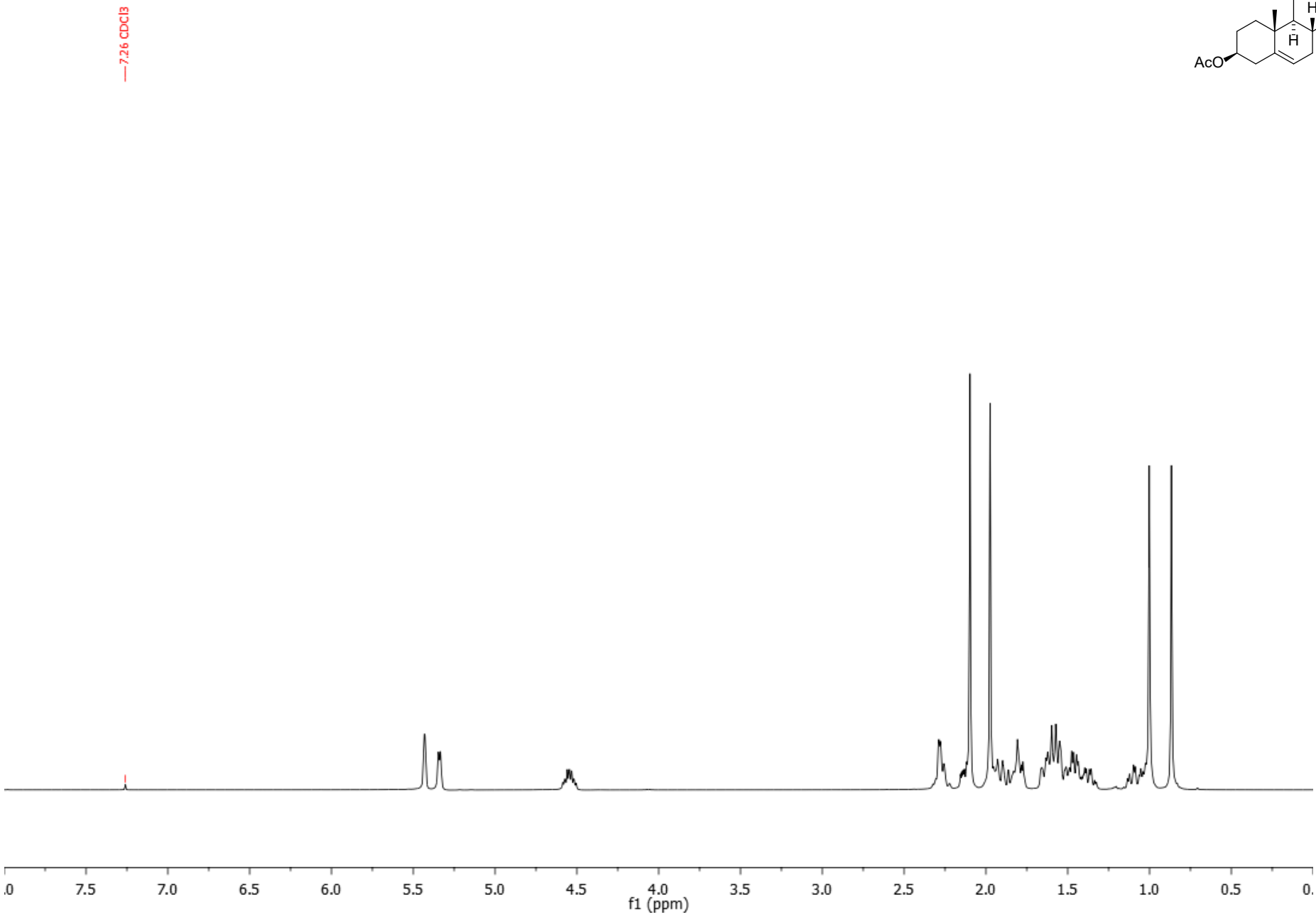


17α-Hydroxy-17β-methylandrosta-4,6-dien-3-one – IR (neat)

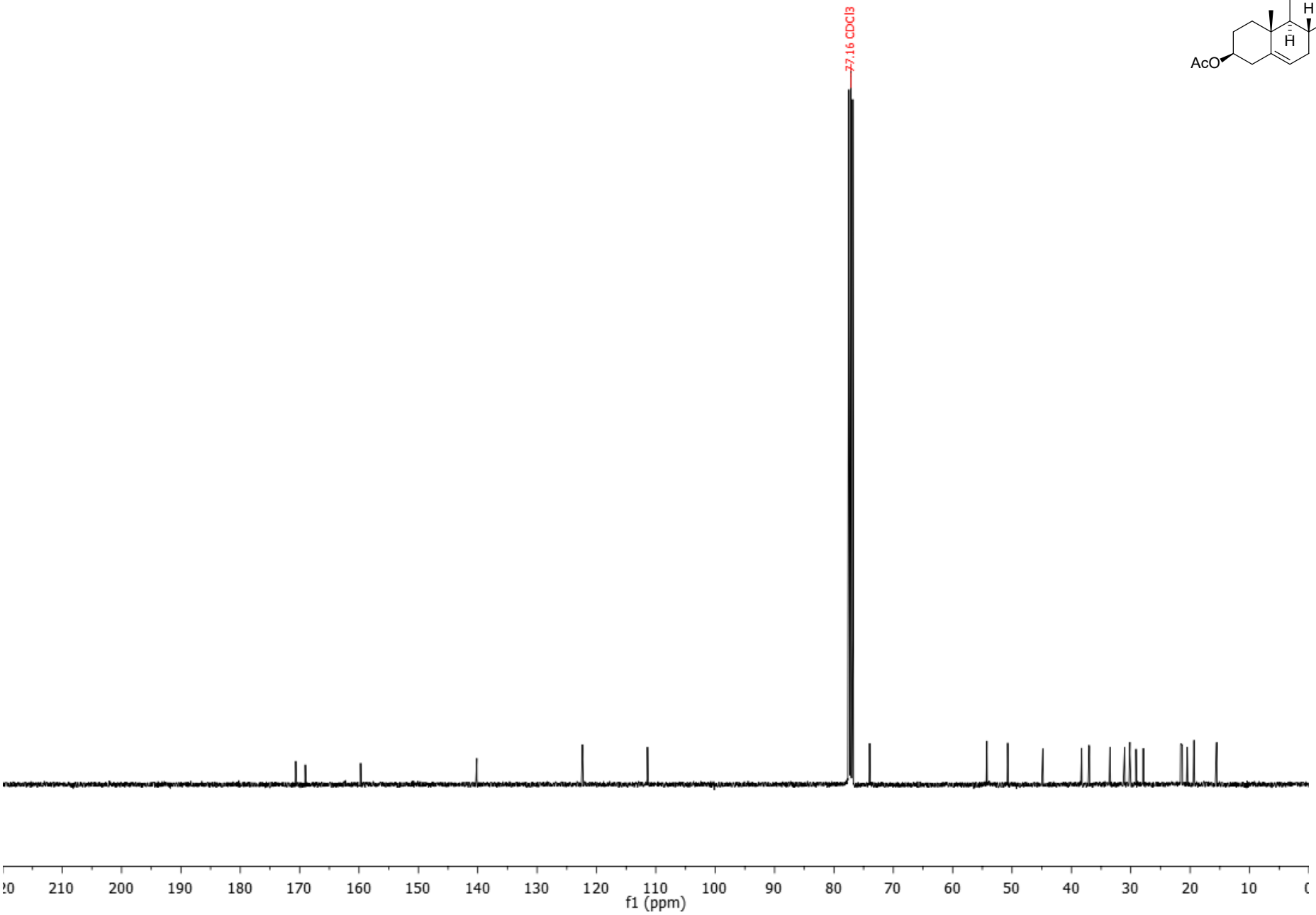


3β,17-Diacetoxyandrosta-5,16-diene – ^{1}H NMR (400 MHz, $CDCl_3$)

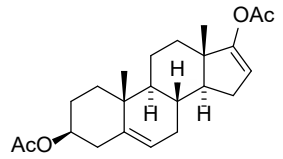




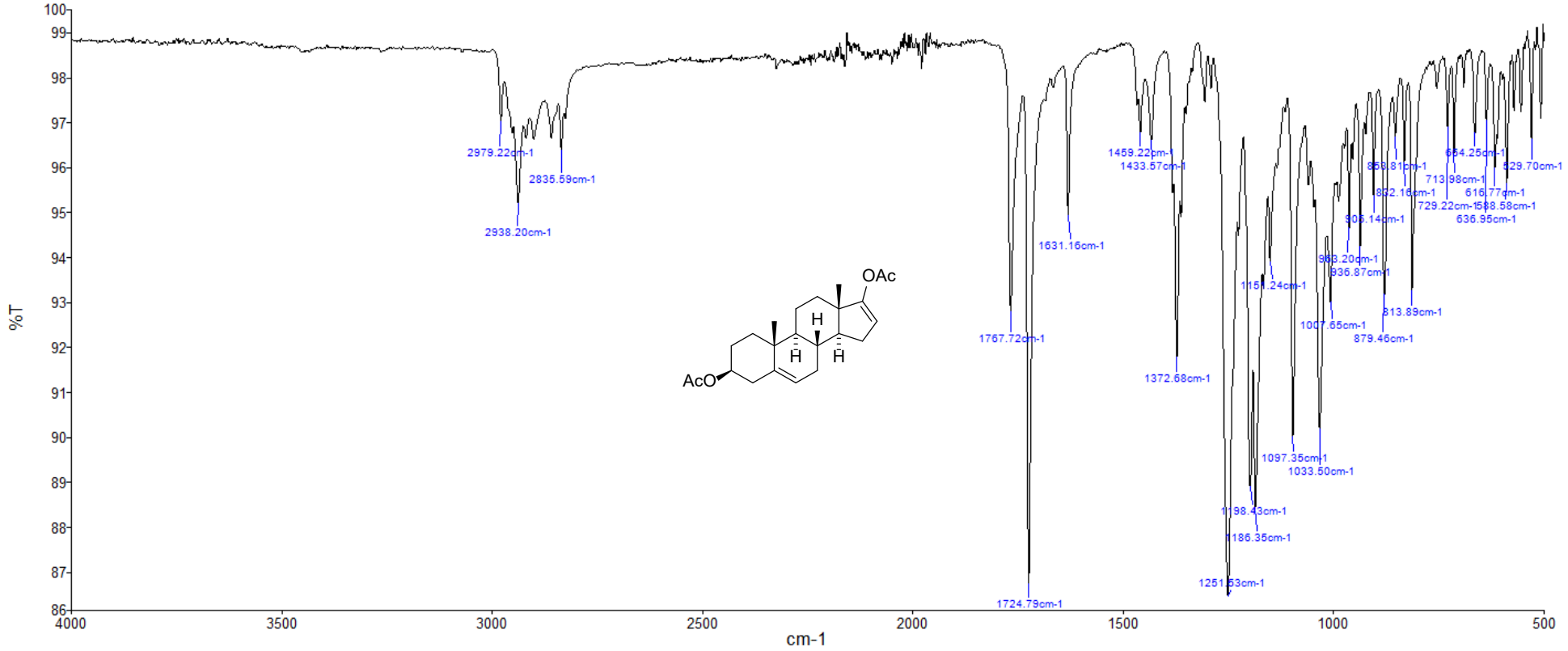
3β,17-Diacetoxyandrosta-5,16-diene – ^{13}C NMR (101 MHz, $CDCl_3$)



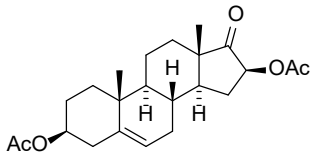
3β,17-Diacetoxyandrosta-5,16-diene – LRMS (+ESI)

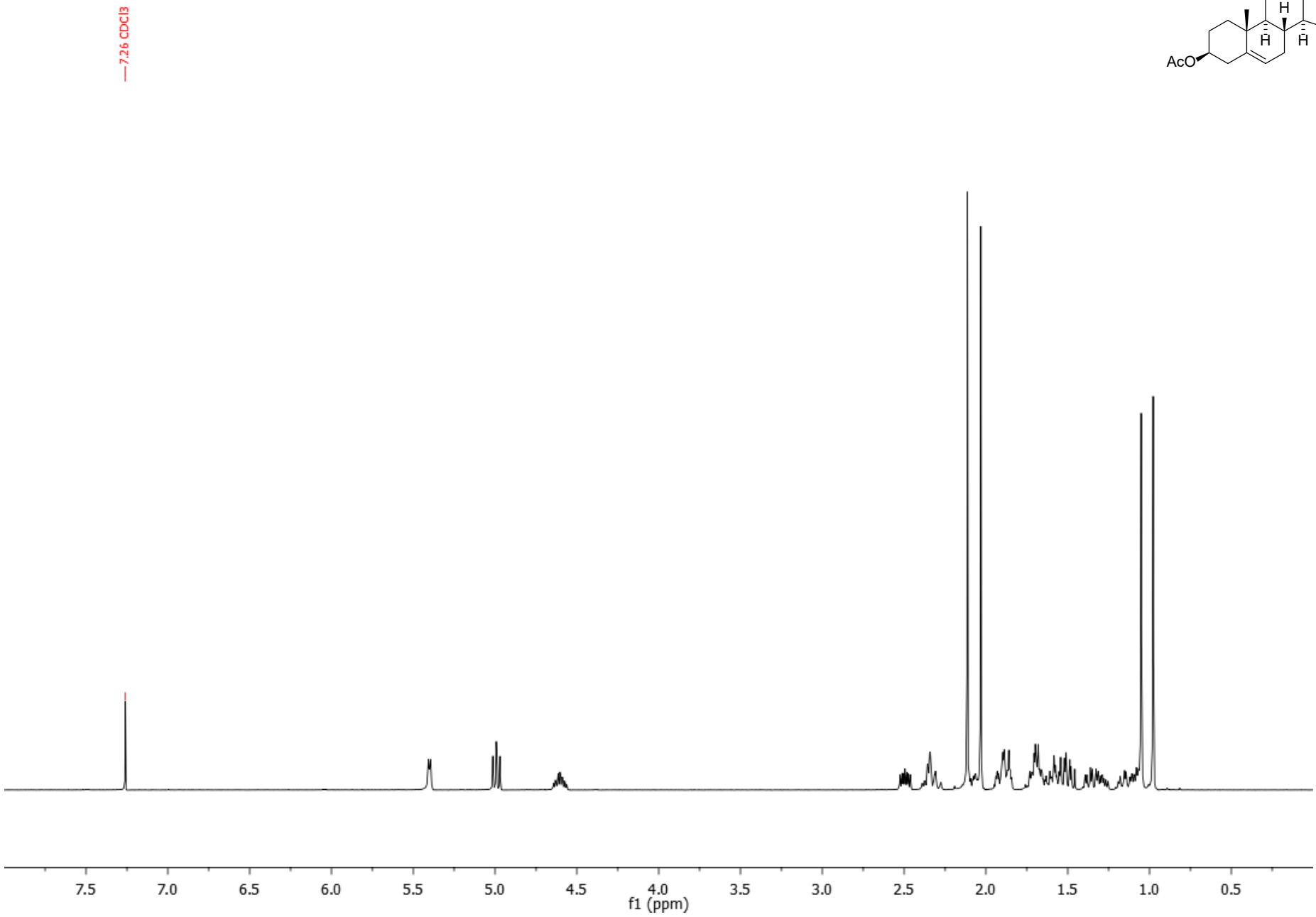


3β,17-Diacetoxyandrosta-5,16-diene – IR (neat)

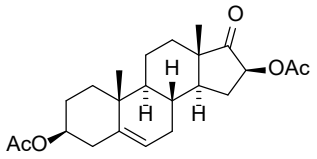


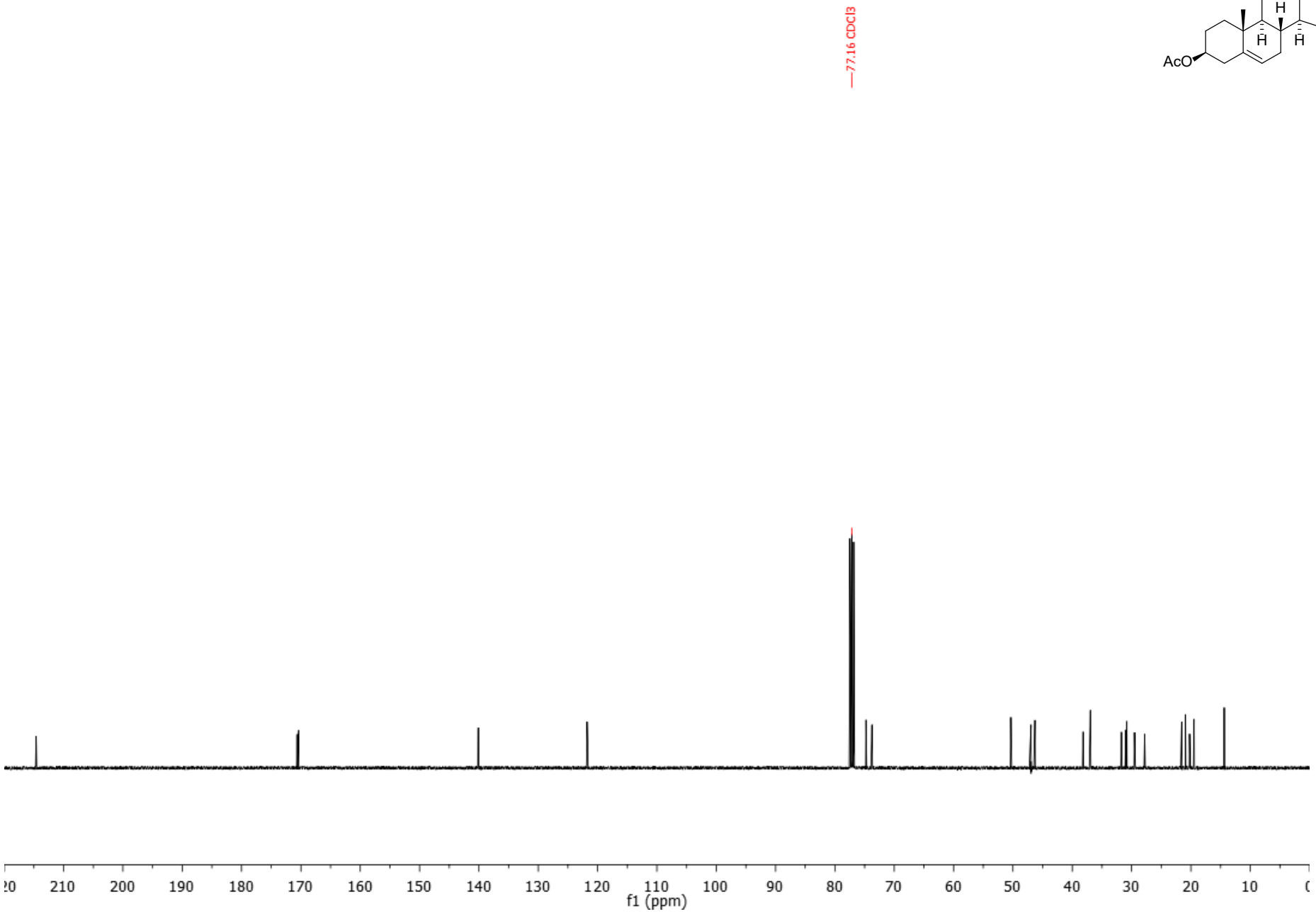
3β,16β-Diacetoxyandrost-5-en-17-one – ^{1}H NMR (400 MHz, $CDCl_3$)



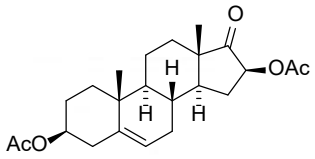


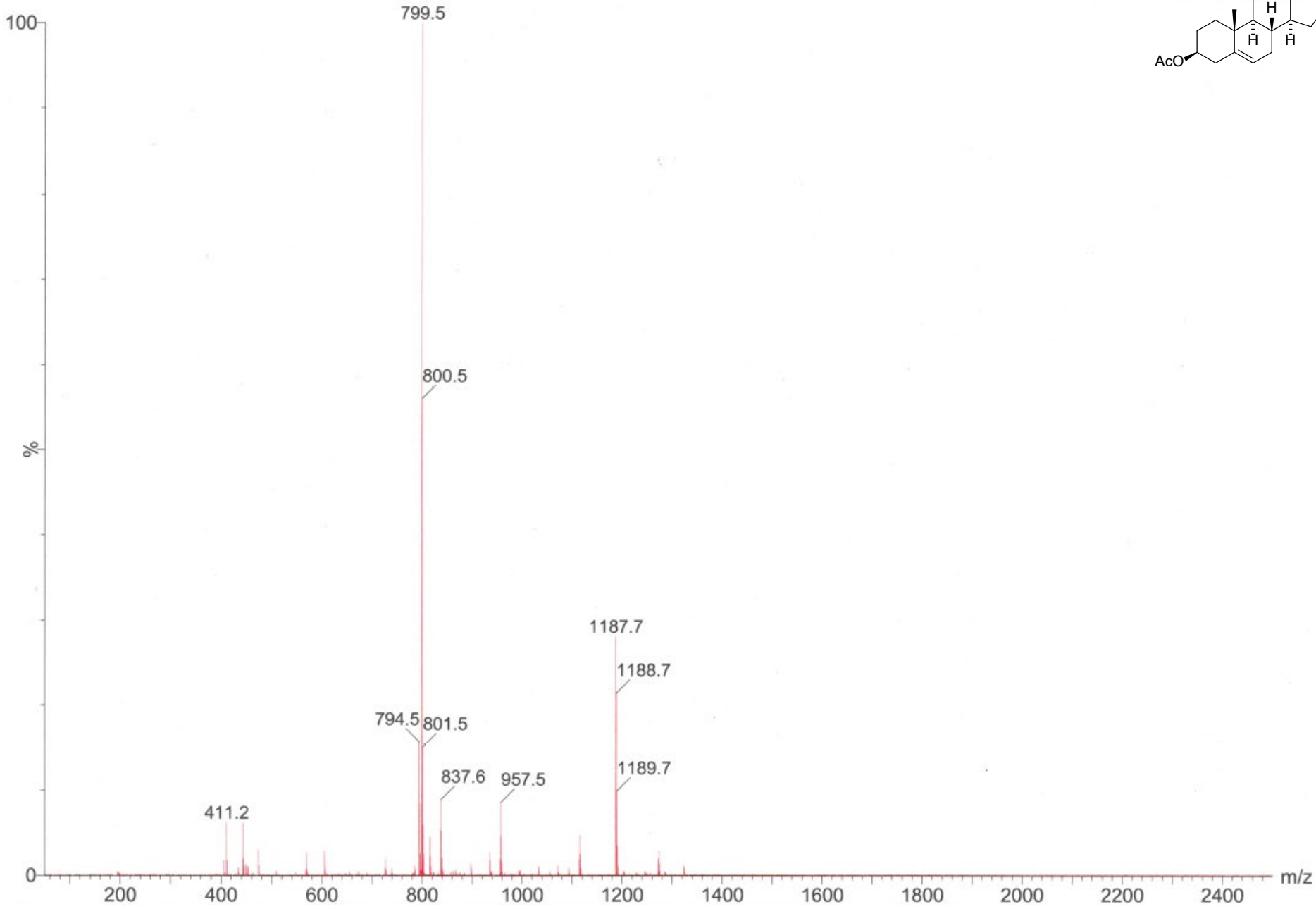
3β,16β-Diacetoxyandrost-5-en-17-one – ^{13}C NMR (101 MHz, $CDCl_3$)



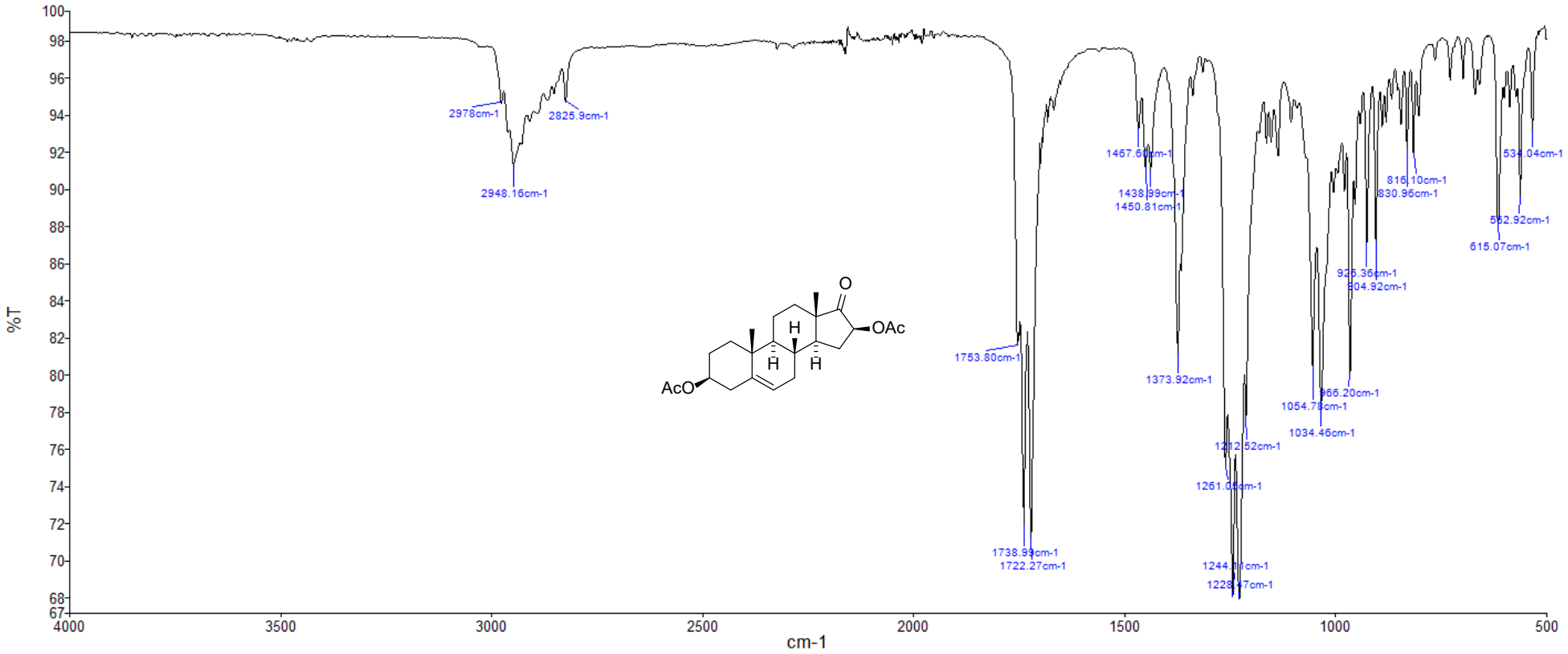


3β,16β-Diacetoxyandrost-5-en-17-one – LRMS (+ESI)

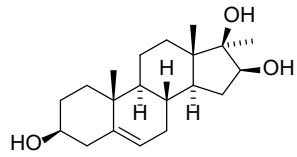


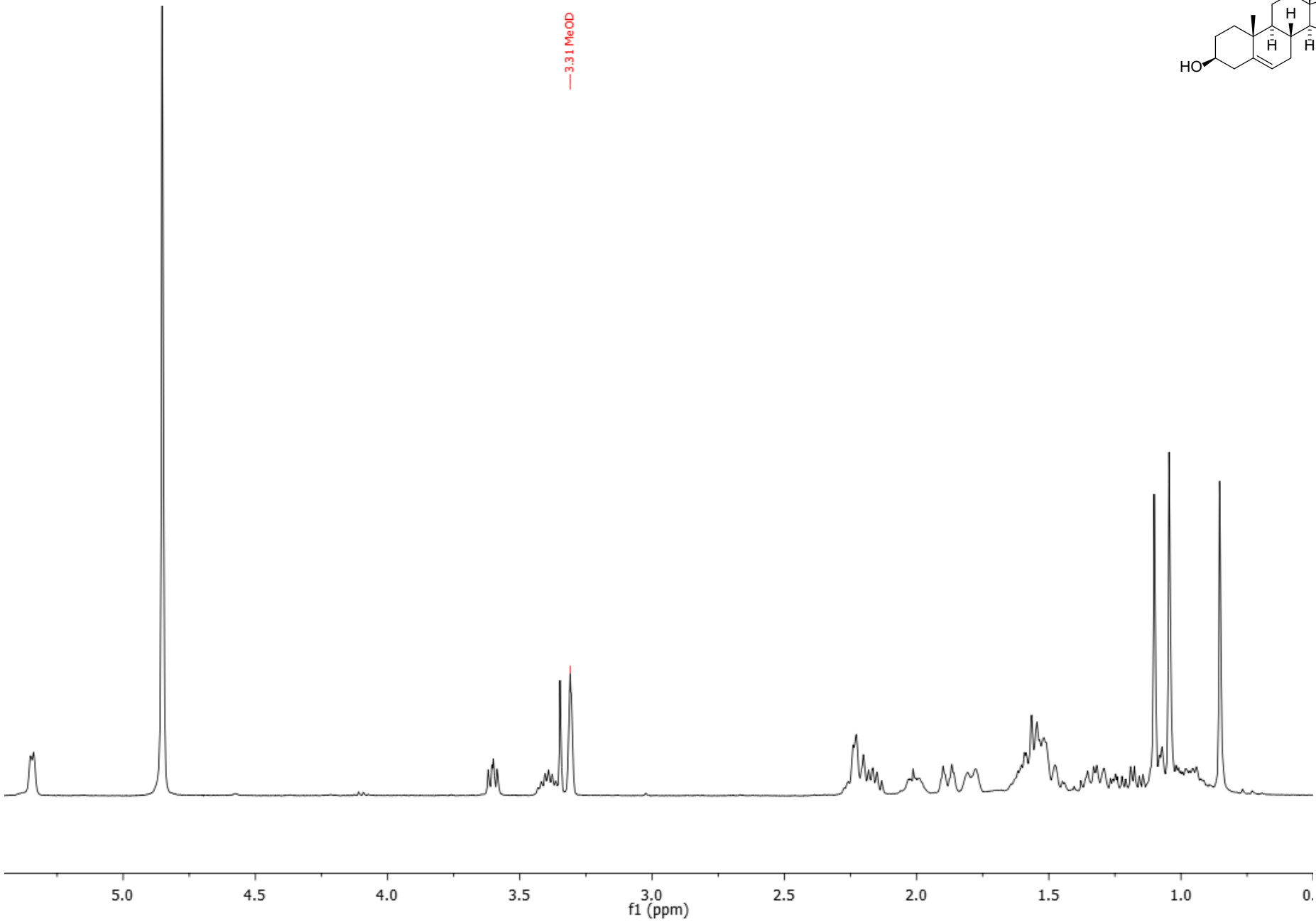


3β,16β-Diacetoxyandrost-5-en-17-one – IR (neat)

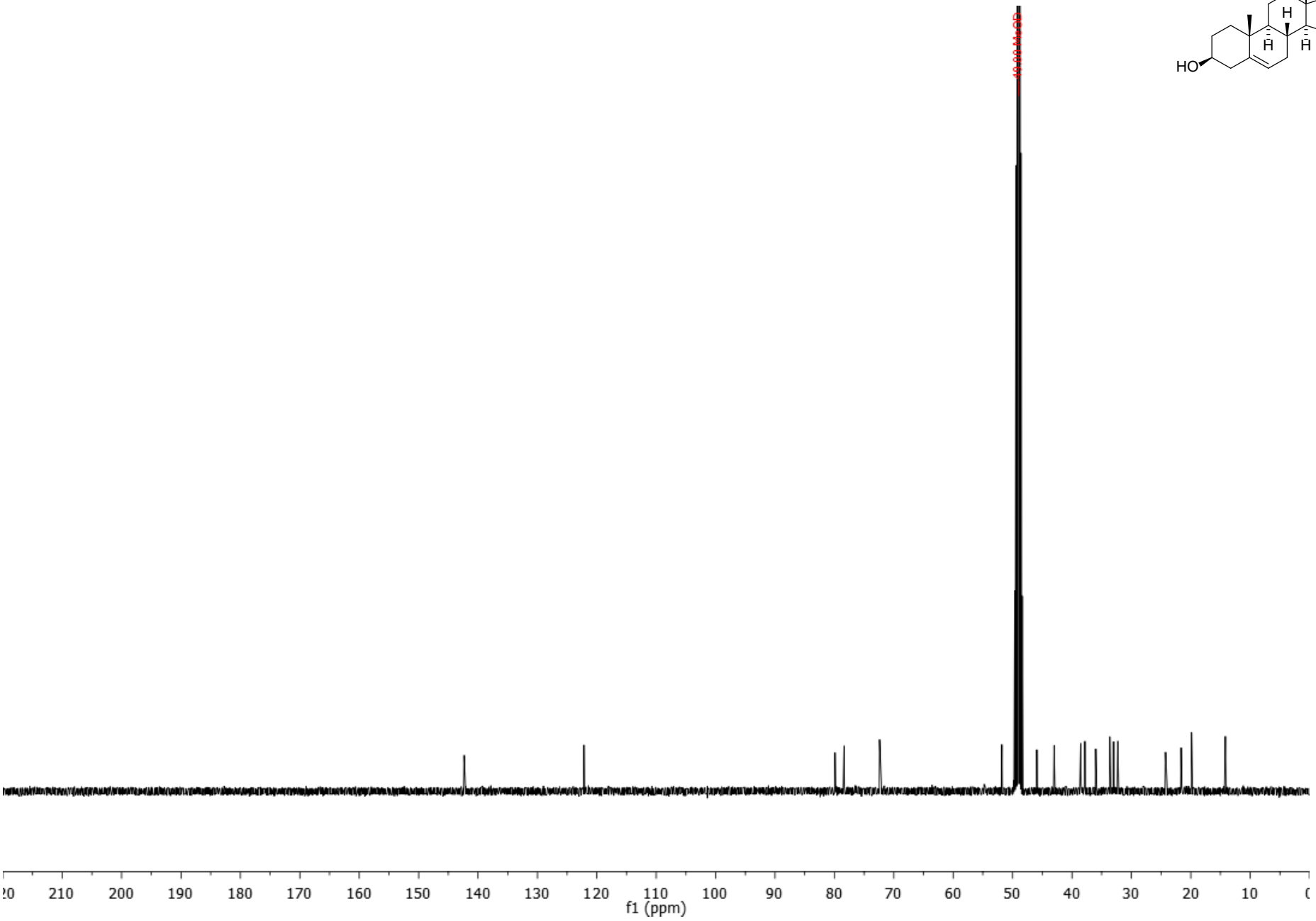


17α-Methylandrost-5-ene-3β,16β,17β-triol – ^{1}H NMR (400 MHz, CD_3OD)

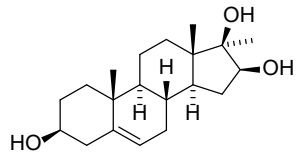


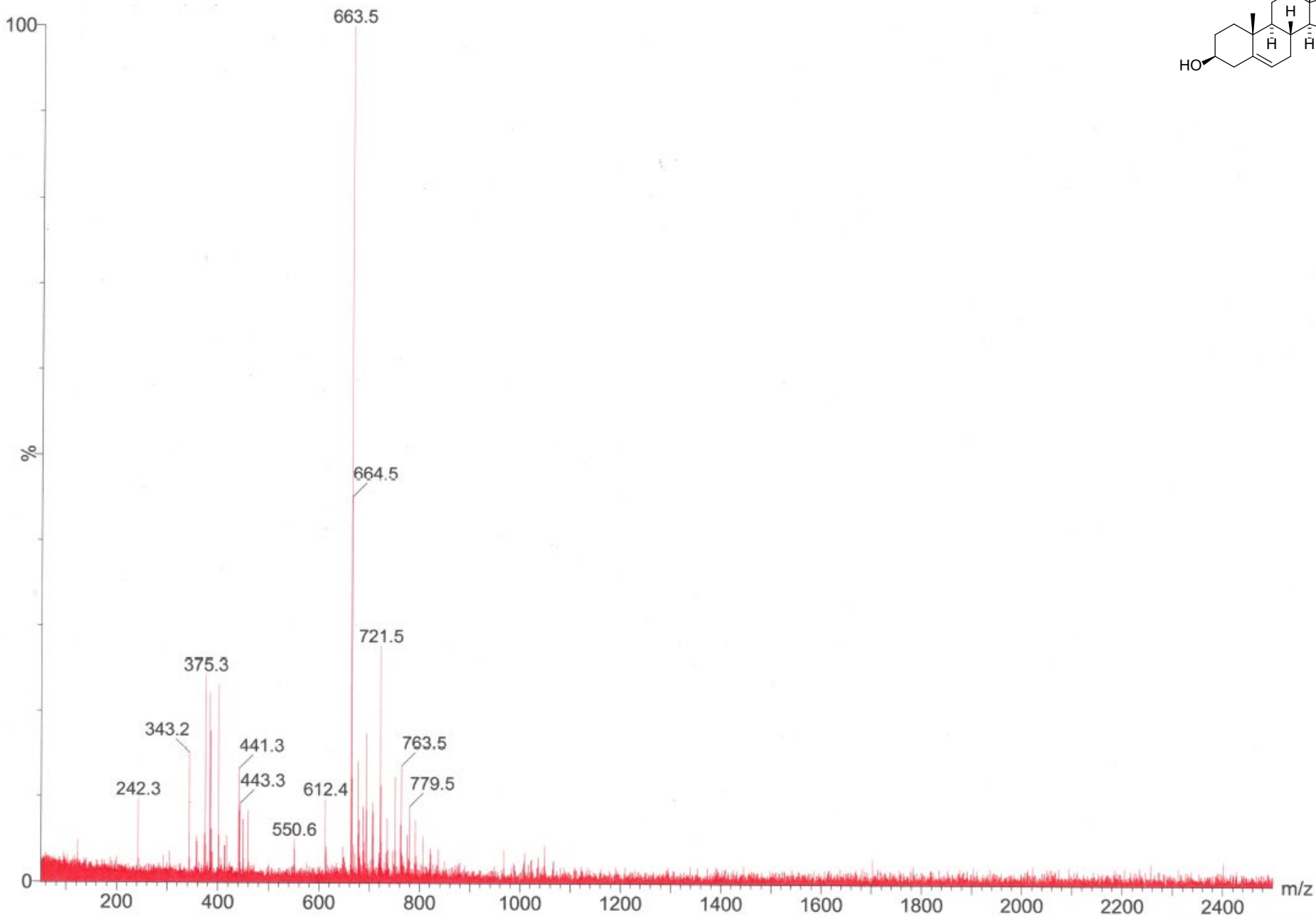


17α-Methylandrost-5-ene-3β,16β,17β-triol – ^{13}C NMR (101 MHz, CD_3OD)

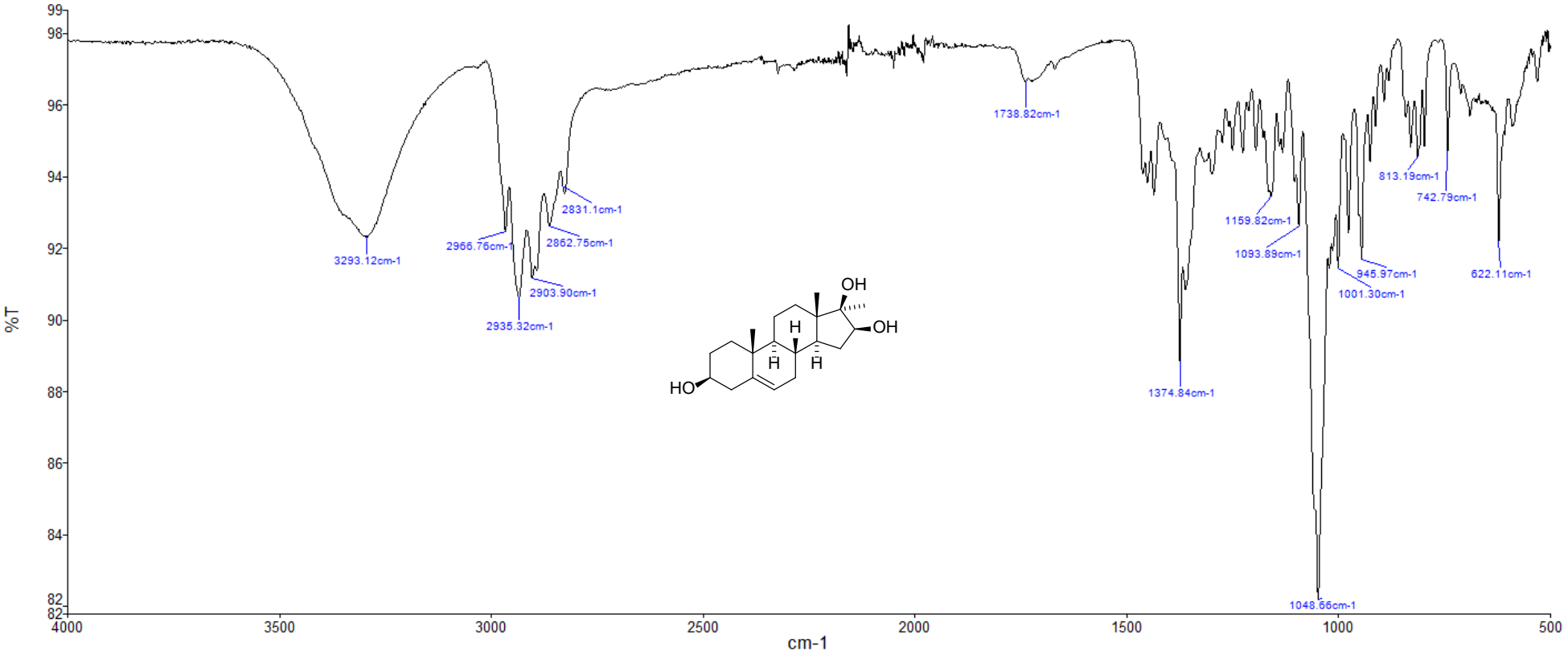


17α-Methylandrost-5-ene-3β,16β,17β-triol – LRMS (+ESI)

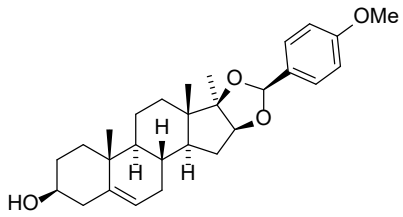




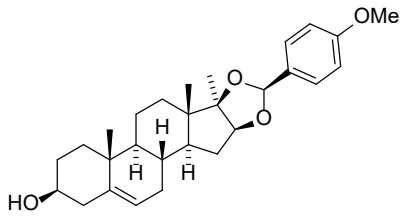
17α-Methylandrost-5-ene-3β,16β,17β-triol – IR (neat)



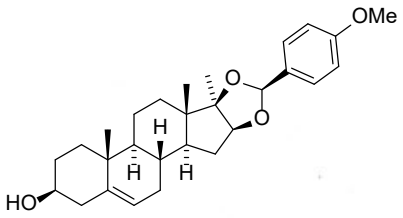
[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-5-en-3β-ol – ^{1}H NMR (400 MHz, $CDCl_3$)



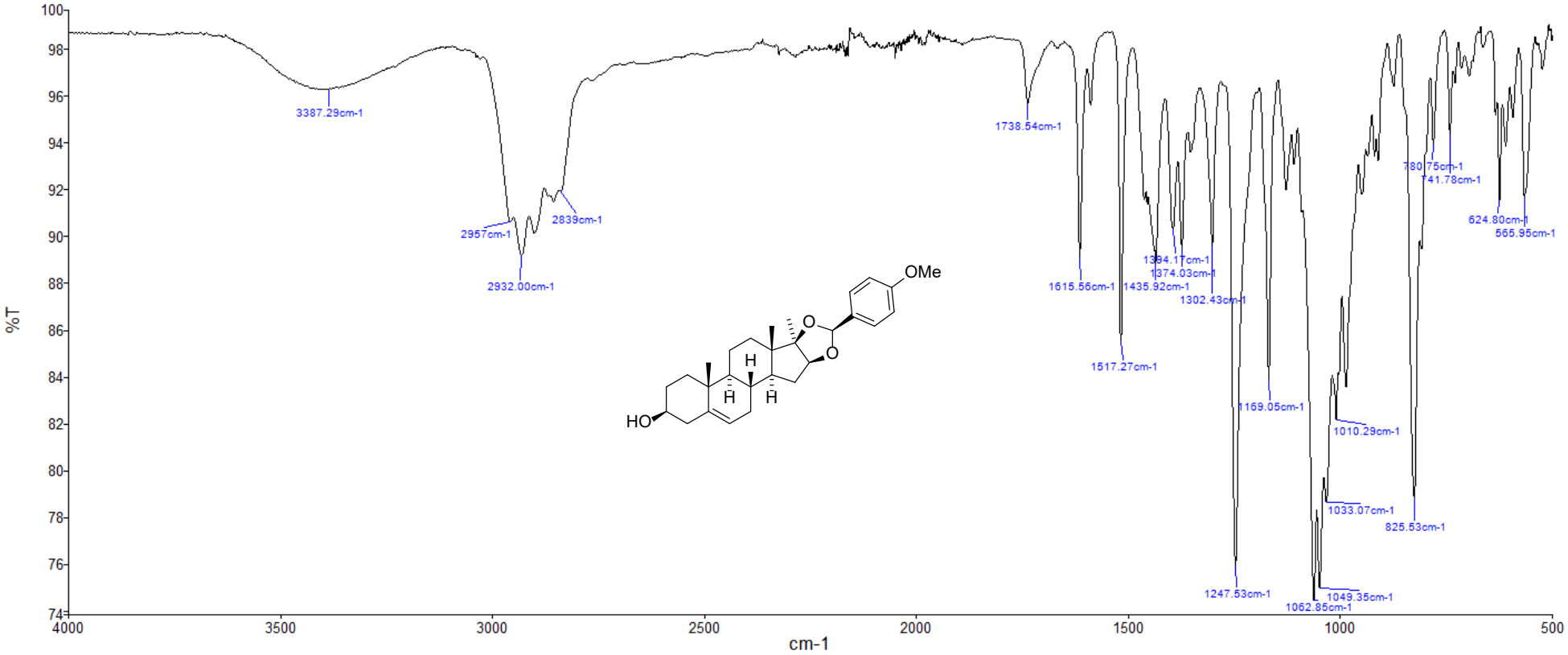
[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-5-en-3β-ol – ^{13}C NMR (101 MHz, $CDCl_3$)



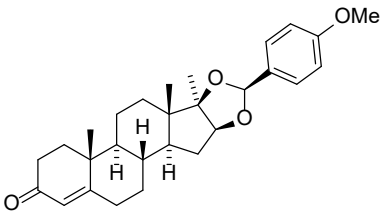
[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-5-en-3β-ol – LRMS (+ESI)



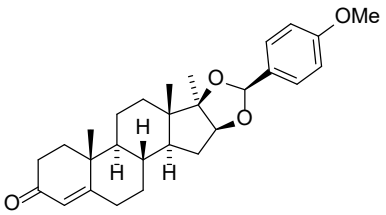
[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-5-en-3β-ol – IR (neat)



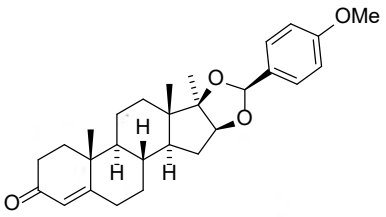
[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-4-en-3-one – ^{1}H NMR (400 MHz, $CDCl_3$)



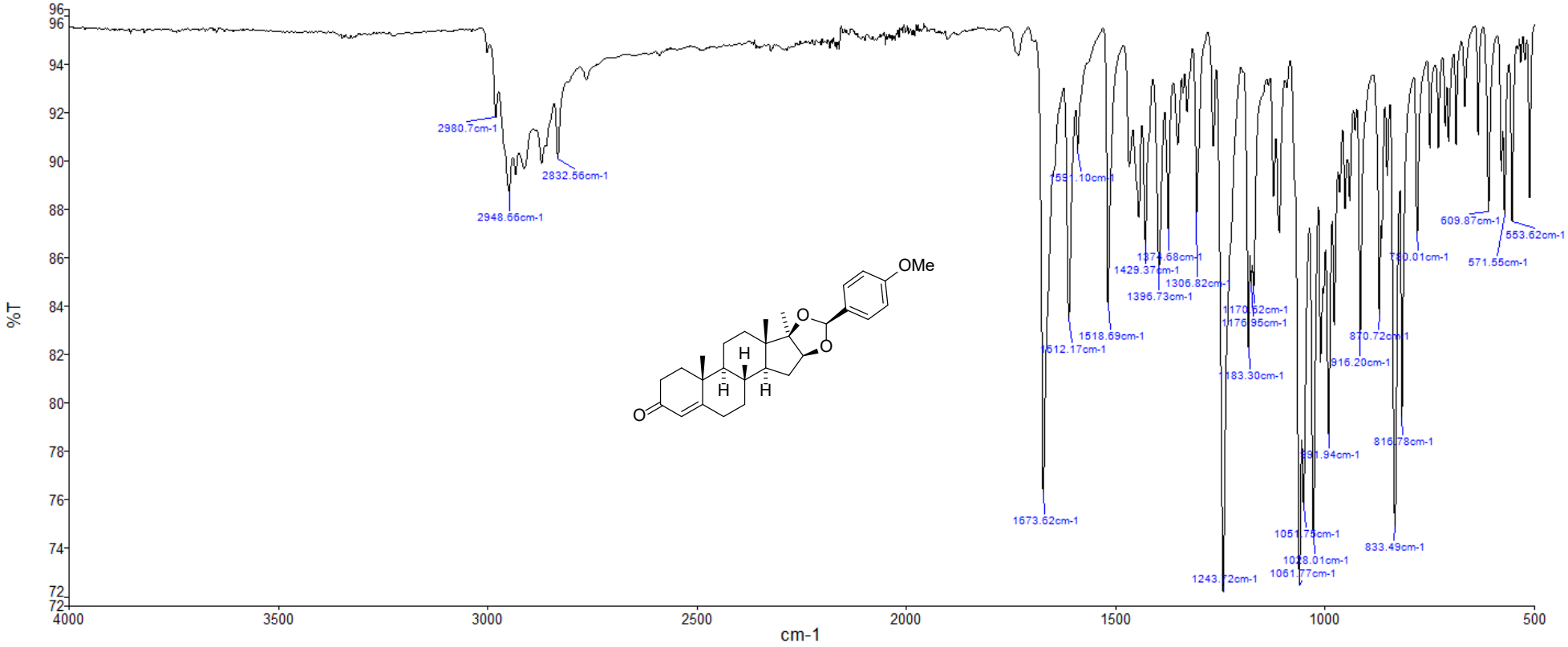
[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-4-en-3-one – ^{13}C NMR (151 MHz, $CDCl_3$)



[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-4-en-3-one – LRMS (+ESI)



[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-4-en-3-one – IR (neat)



[(*S*)-16β,17β-(4-Methoxyphenyl)methylenedioxy]-17α-methylandrost-4-en-3-one – crystal structure

Summary of Crystallographic Data

Compound	**3**
Formula	$C_{28}H_{36}O_4$
$D_{calc.}$/ g cm^{-3}	1.283
μ/mm^{-1}	0.665
Formula Weight	436.57
Colour	colourless
Shape	needle
Size/mm^3	0.15×0.13×0.09
T/K	150.00(10)
Crystal System	monoclinic
Flack Parameter	0.04(8)
Hooft Parameter	-0.07(3)
Space Group	$P2_1$
a/Å	7.77440(10)
b/Å	11.04020(10)
c/Å	13.1698(2)
α/°	90
β/°	90.8400(10)
γ/°	90
V/Å^3	1130.25(2)
Z	2
Z'	1
Wavelength/Å	1.54184
Radiation type	Cu K$_\alpha$
Θ_{min}/°	5.228
Θ_{max}/°	73.705
Measured Refl's.	8745
Indep't Refl's	8745
Refl's I≥2 σ(I)	8417
R_{int}	.
Parameters	294
Restraints	1
Largest Peak	0.164
Deepest Hole	-0.186
GooF	1.084
wR_2 (all data)	0.0891
wR_2	0.0885
R_1 (all data)	0.0343
R_1	0.0331

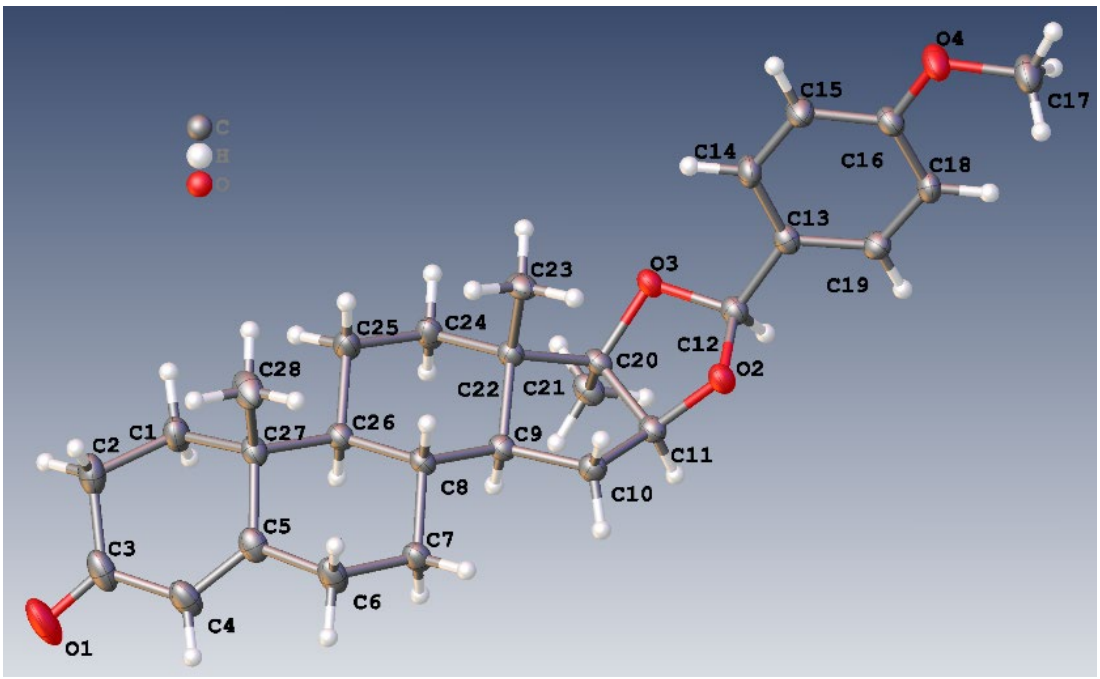
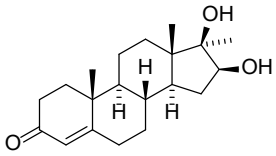
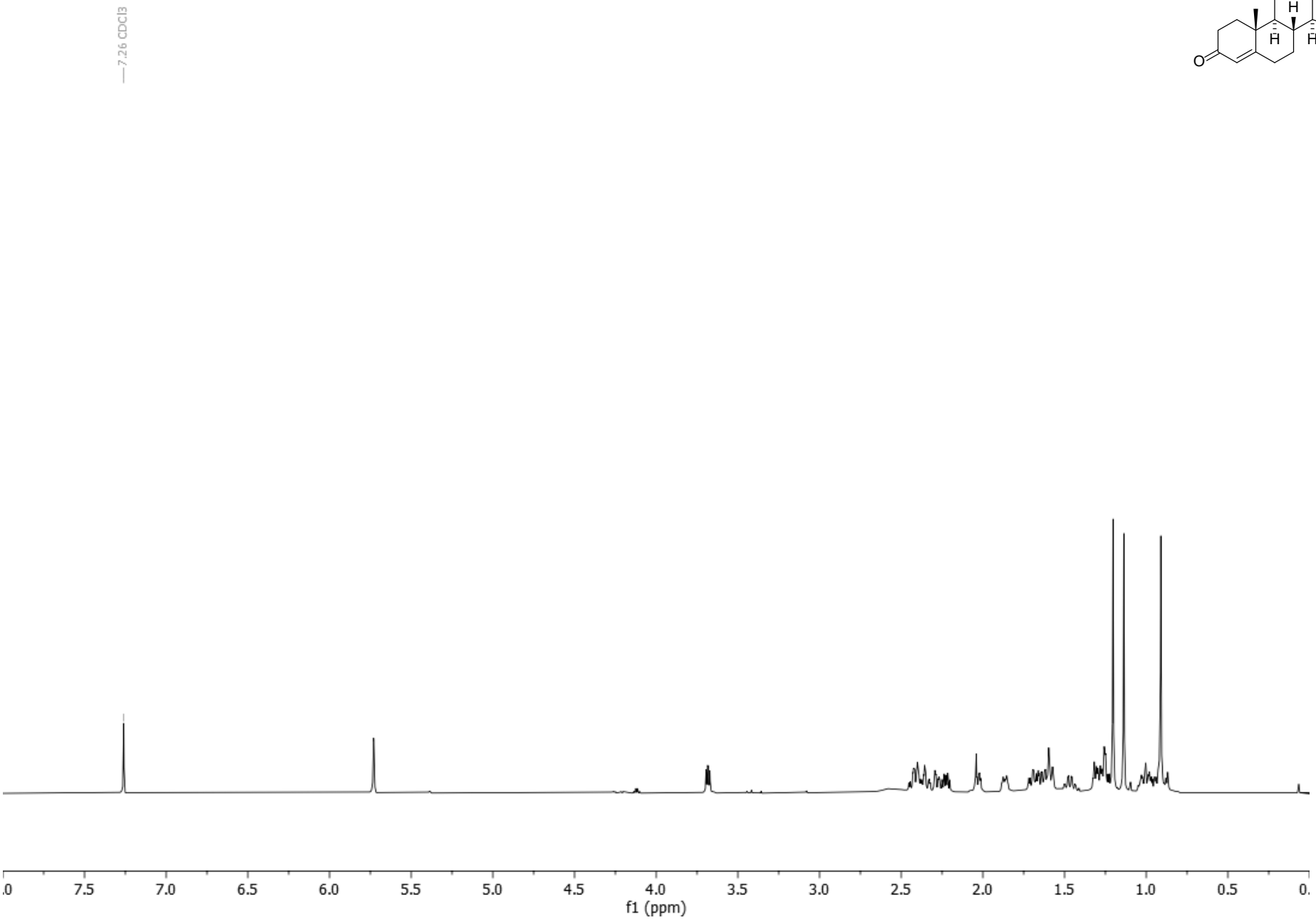


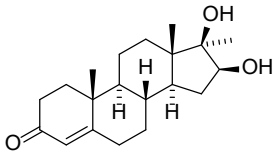
Figure S3. Molecular structure of **3** (atomic displacement parameters shown at 50% probability level).

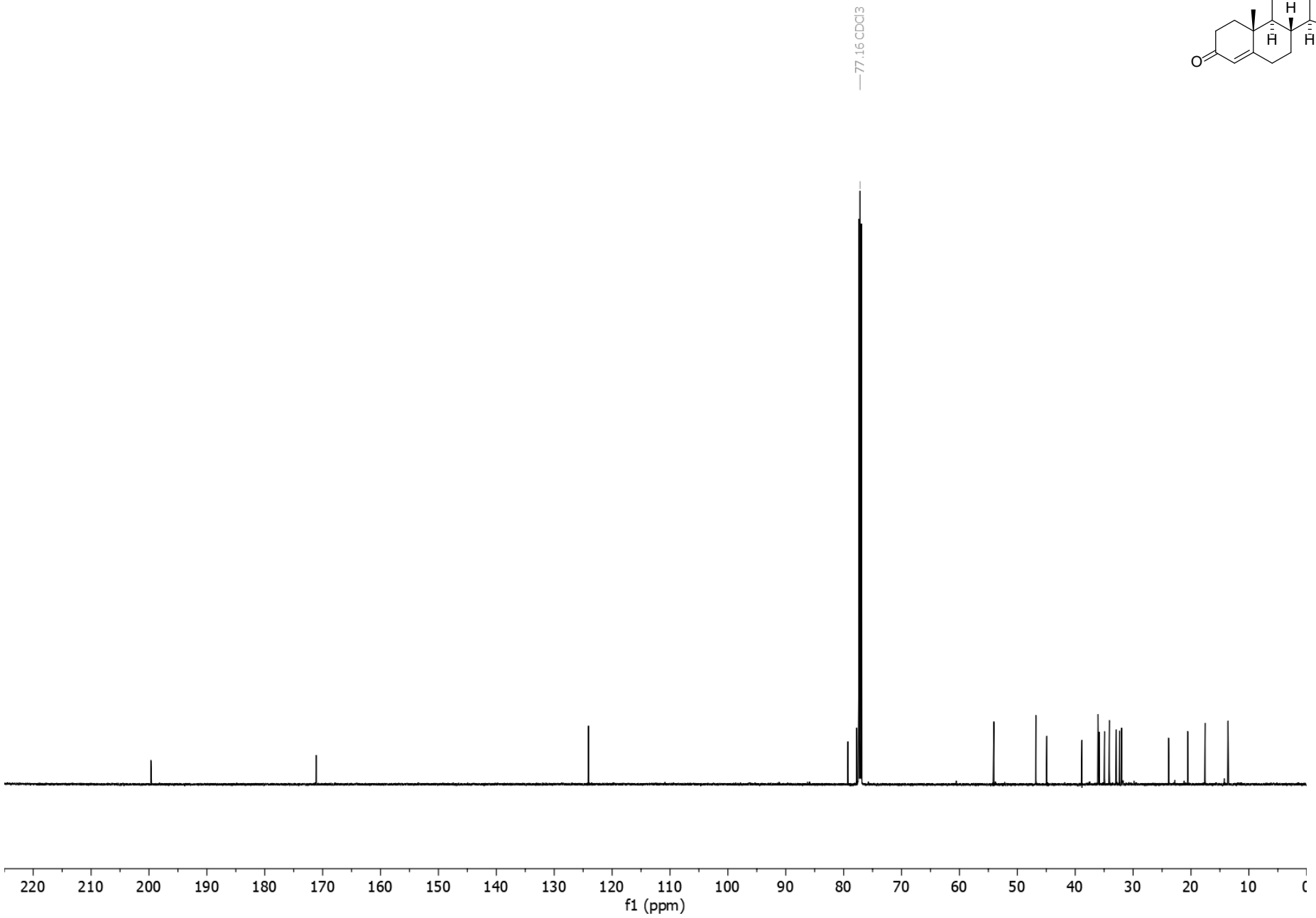
16β,17β-Dihydroxy-17α-methylandrost-4-en-3-one – ^{1}H NMR (600 MHz, $CDCl_3$)



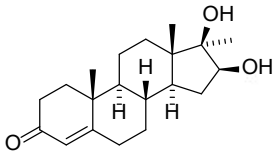


16β,17β-Dihydroxy-17α-methylandrost-4-en-3-one – ^{13}C NMR (151 MHz, $CDCl_3$)

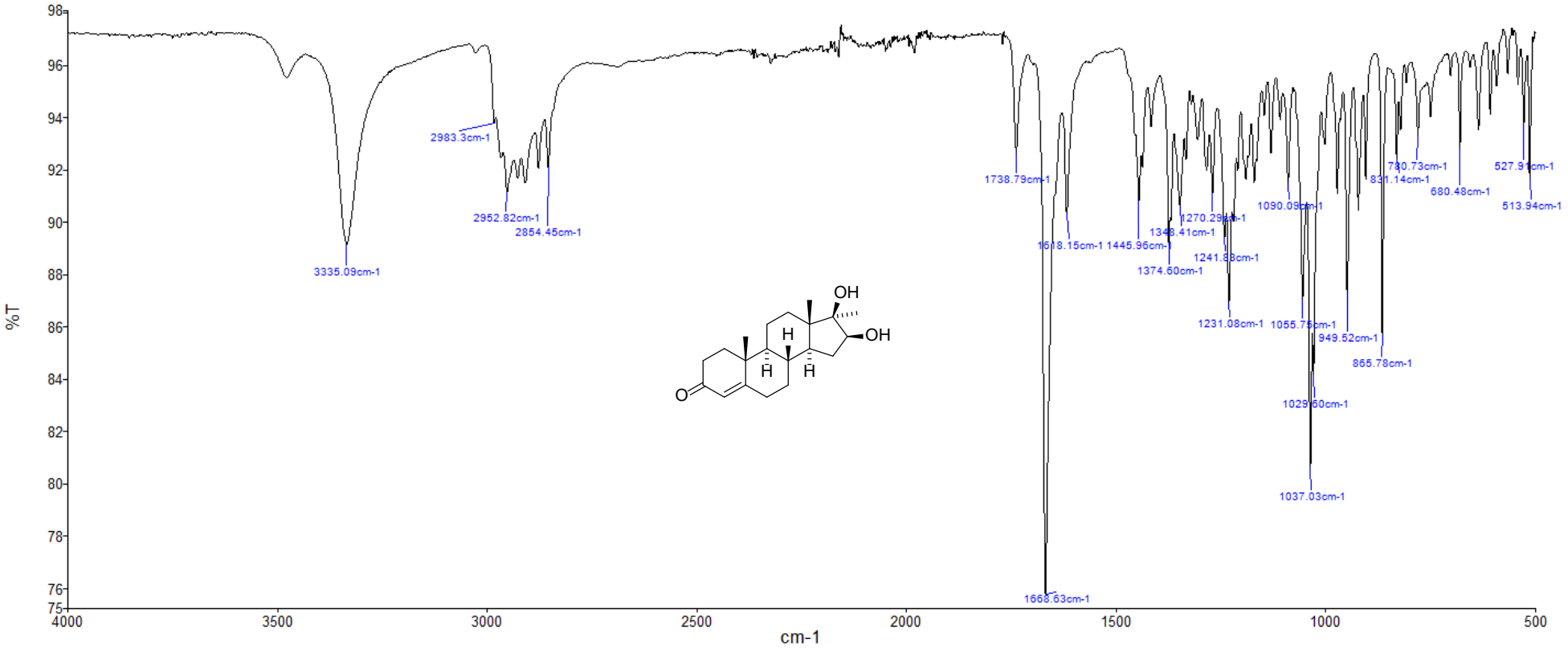




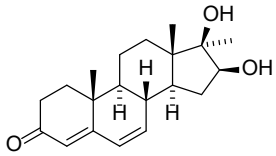
16β,17β-Dihydroxy-17α-methylandrost-4-en-3-one – LRMS (+ESI)

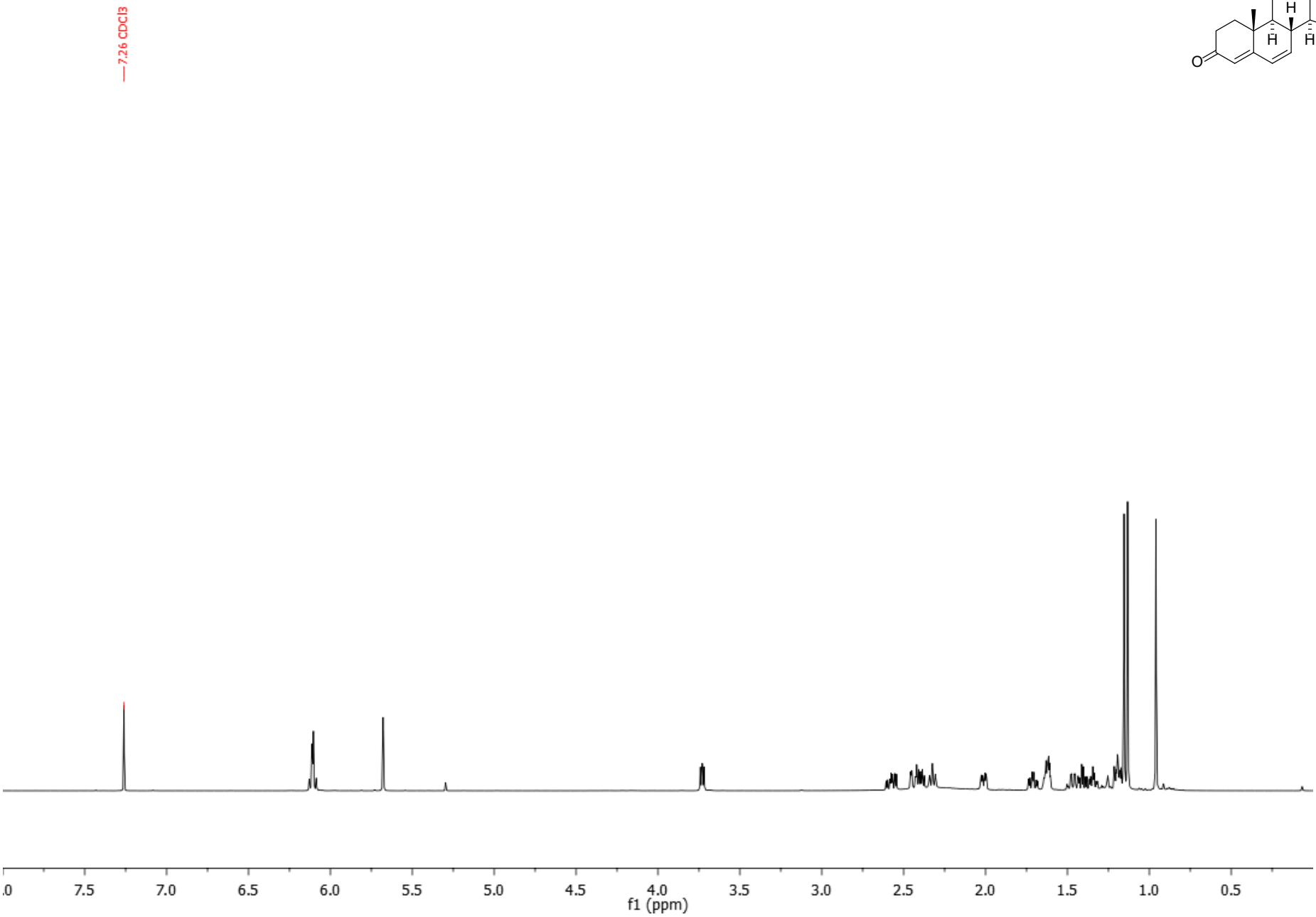


16β,17β-Dihydroxy-17α-methylandrost-4-en-3-one – IR (neat)

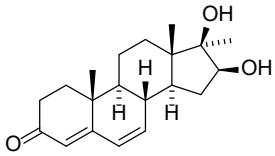


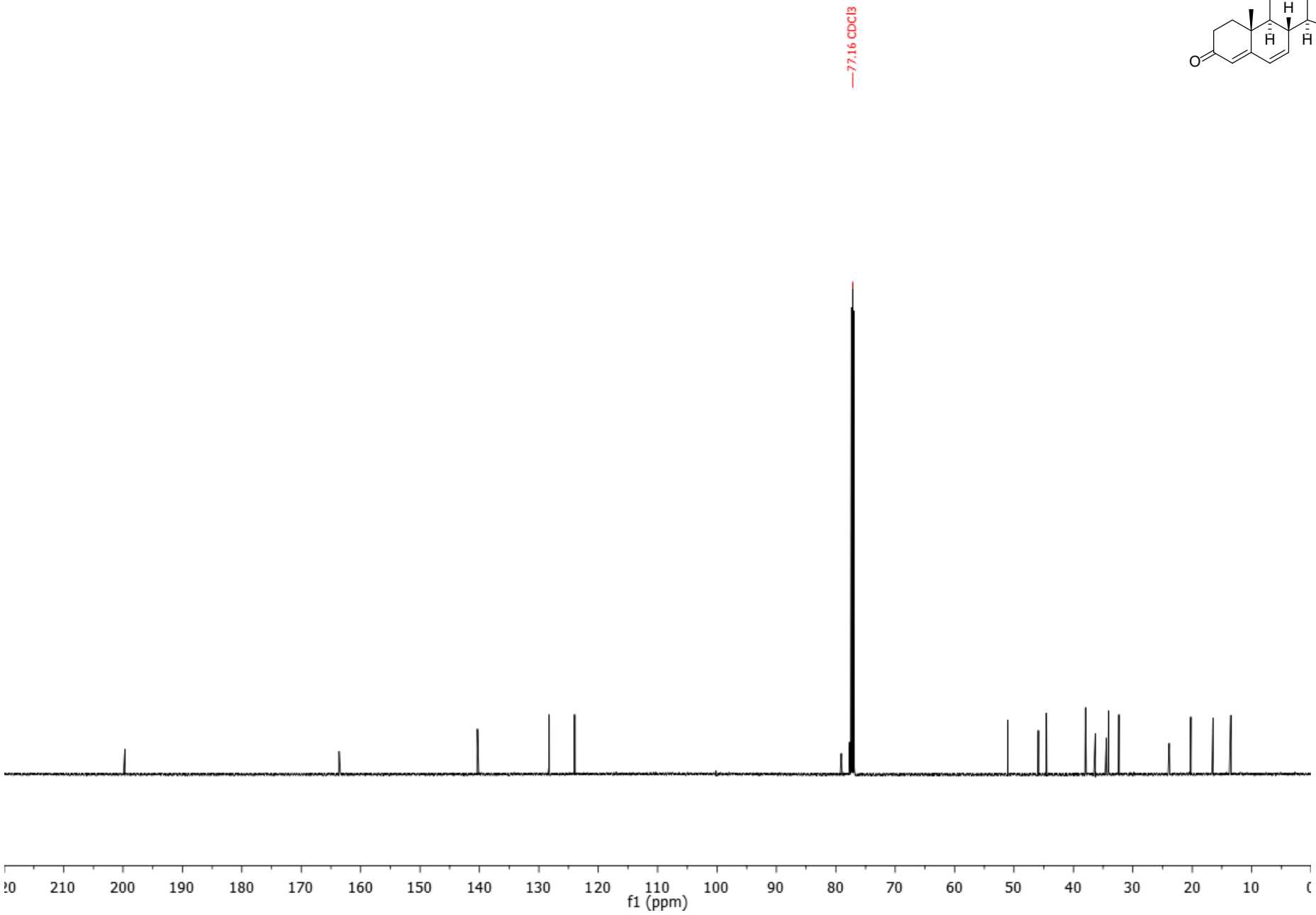
16β,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one – ^{1}H NMR (600 MHz, $CDCl_3$)



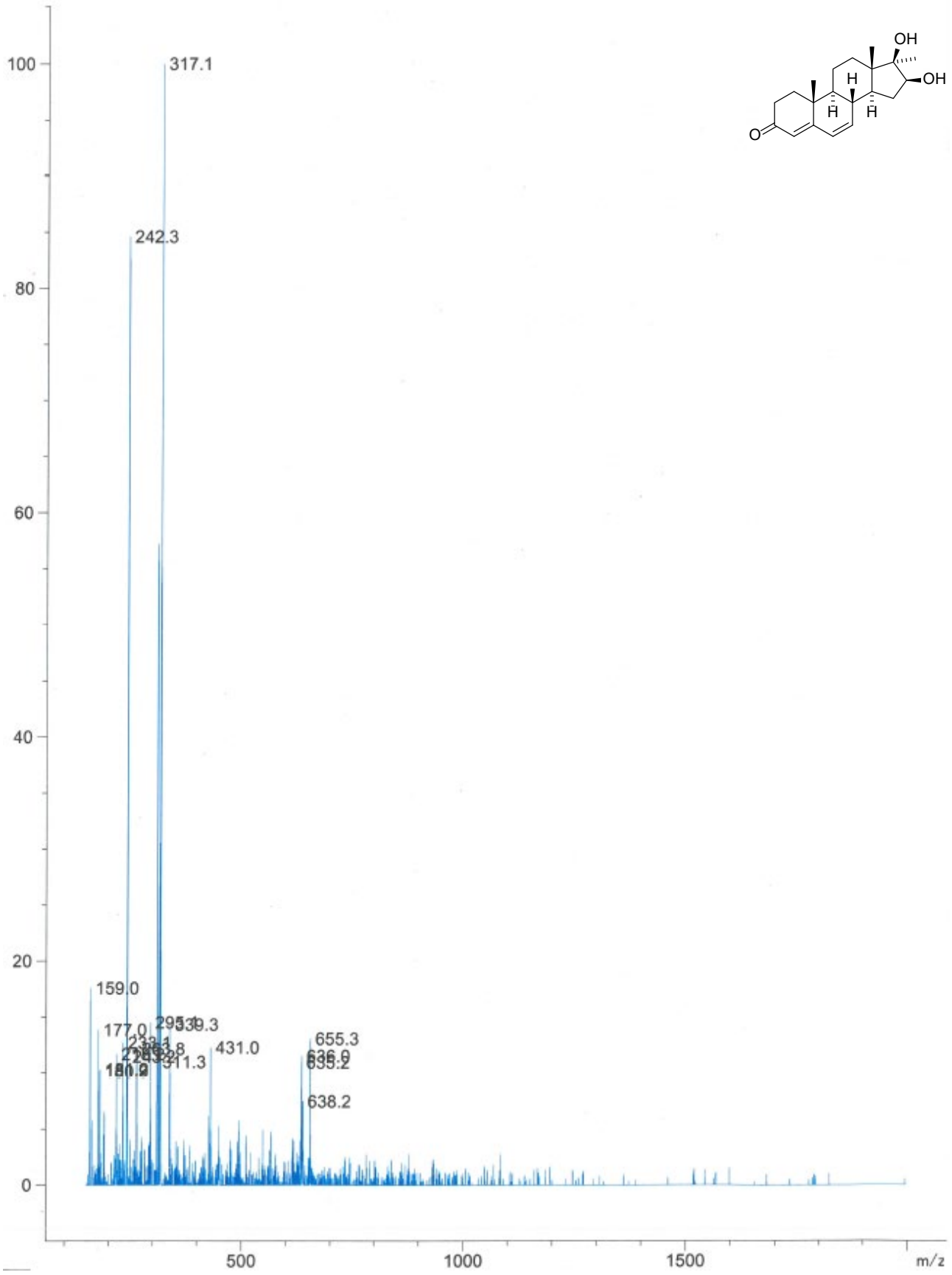


16β,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one – ^{13}C NMR (151 MHz, $CDCl_3$)

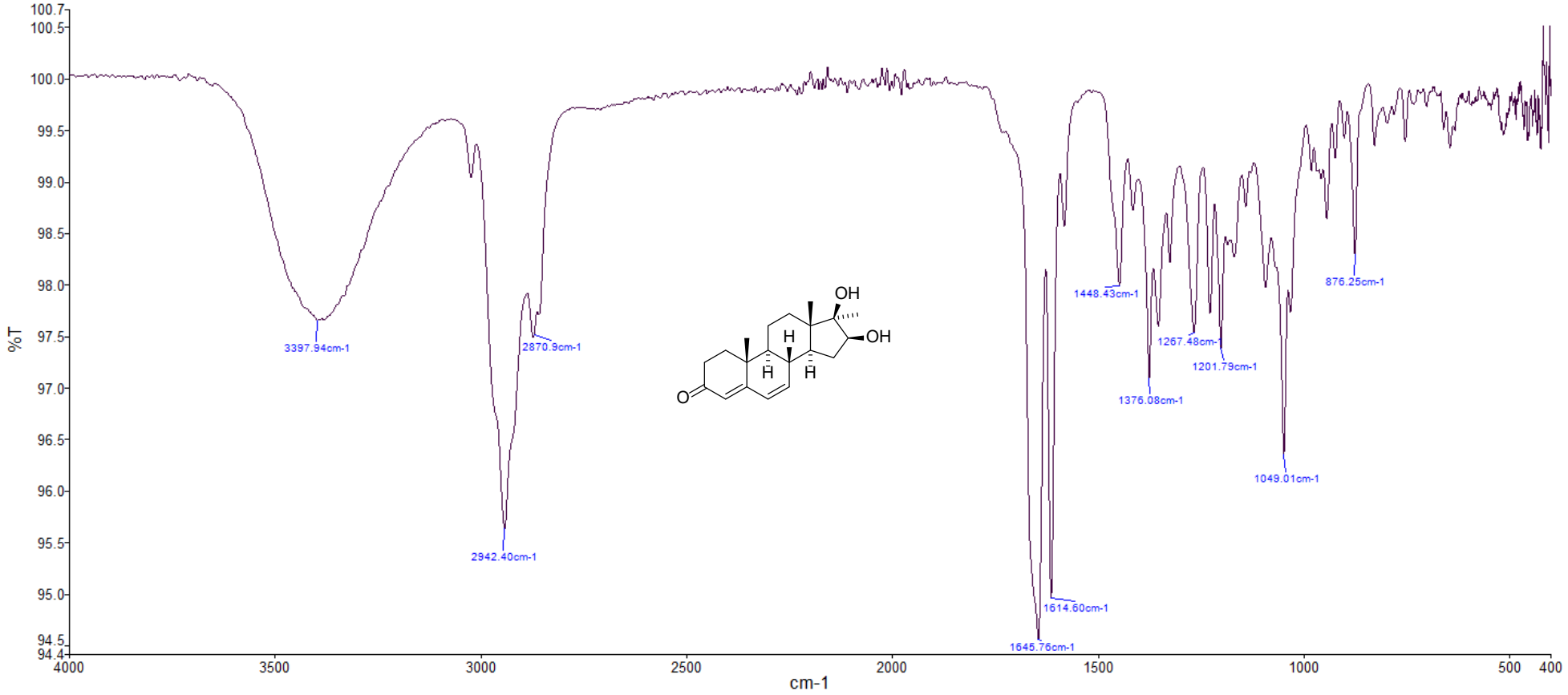




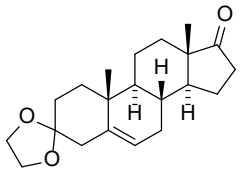
16β,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one – LRMS (+ESI)

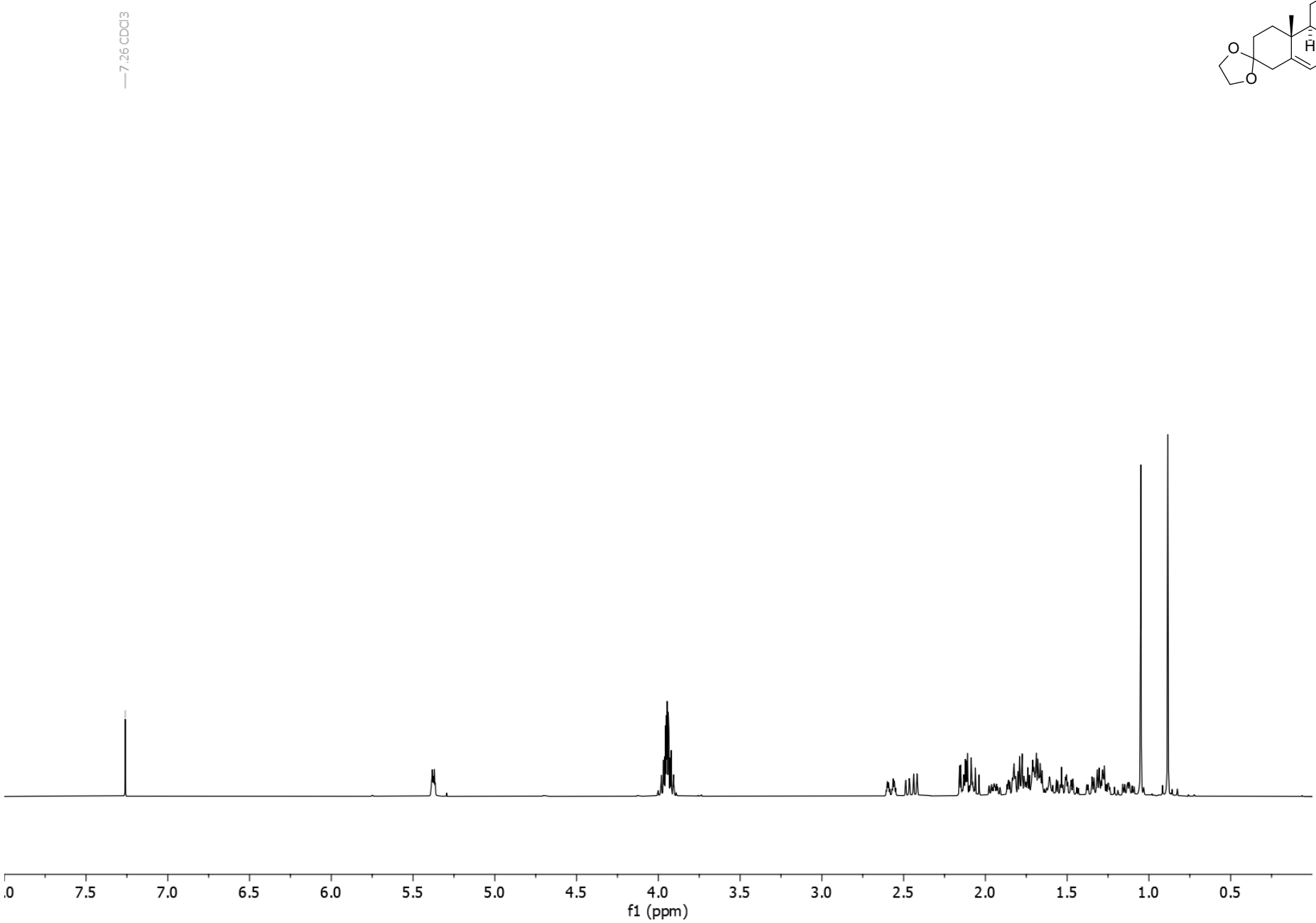


16β,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one – IR (neat)

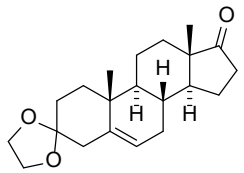


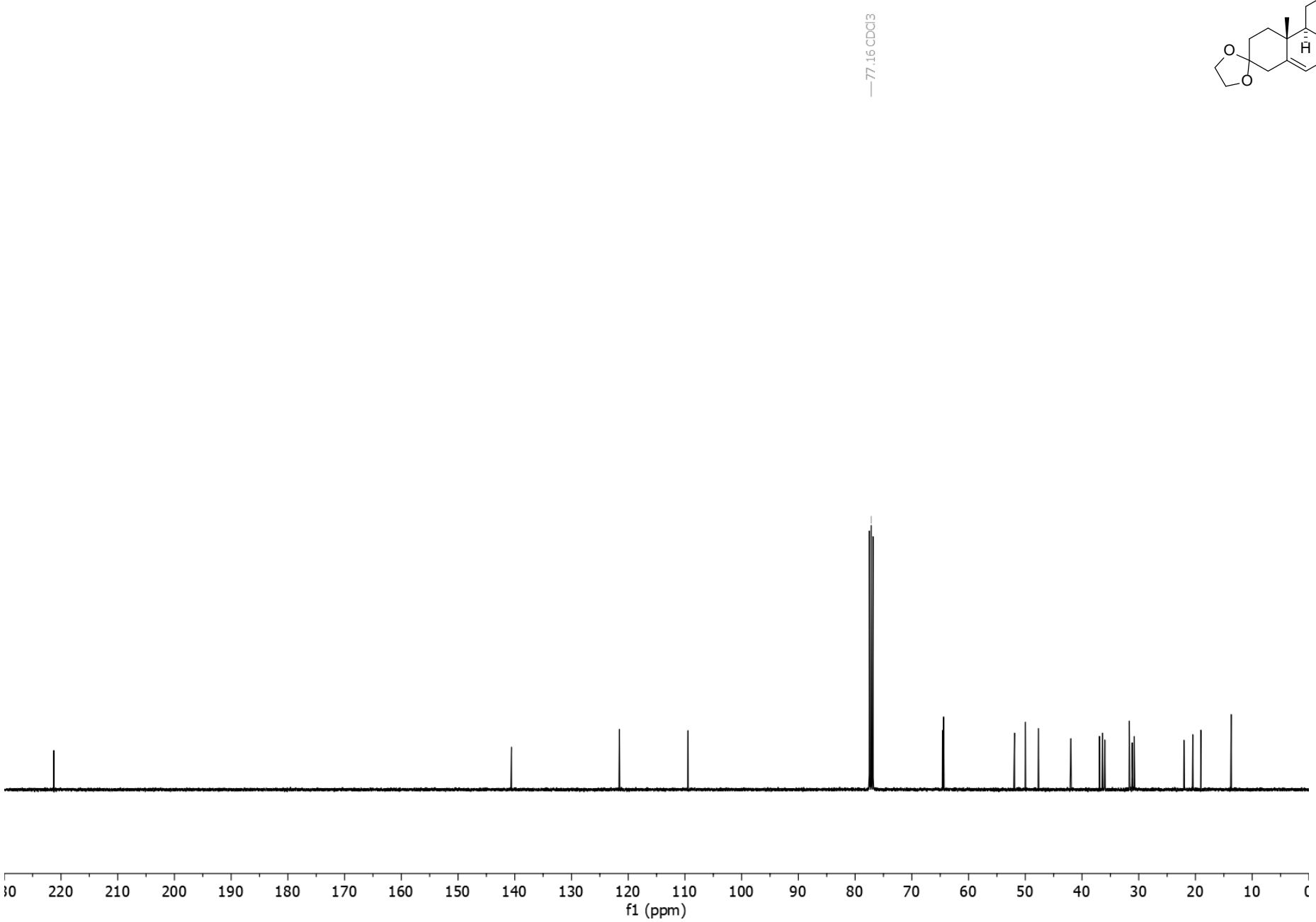
3,3-Ethylenedioxyandrost-5-en-17-one – ^{1}H NMR (400 MHz, $CDCl_3$)



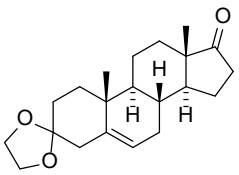


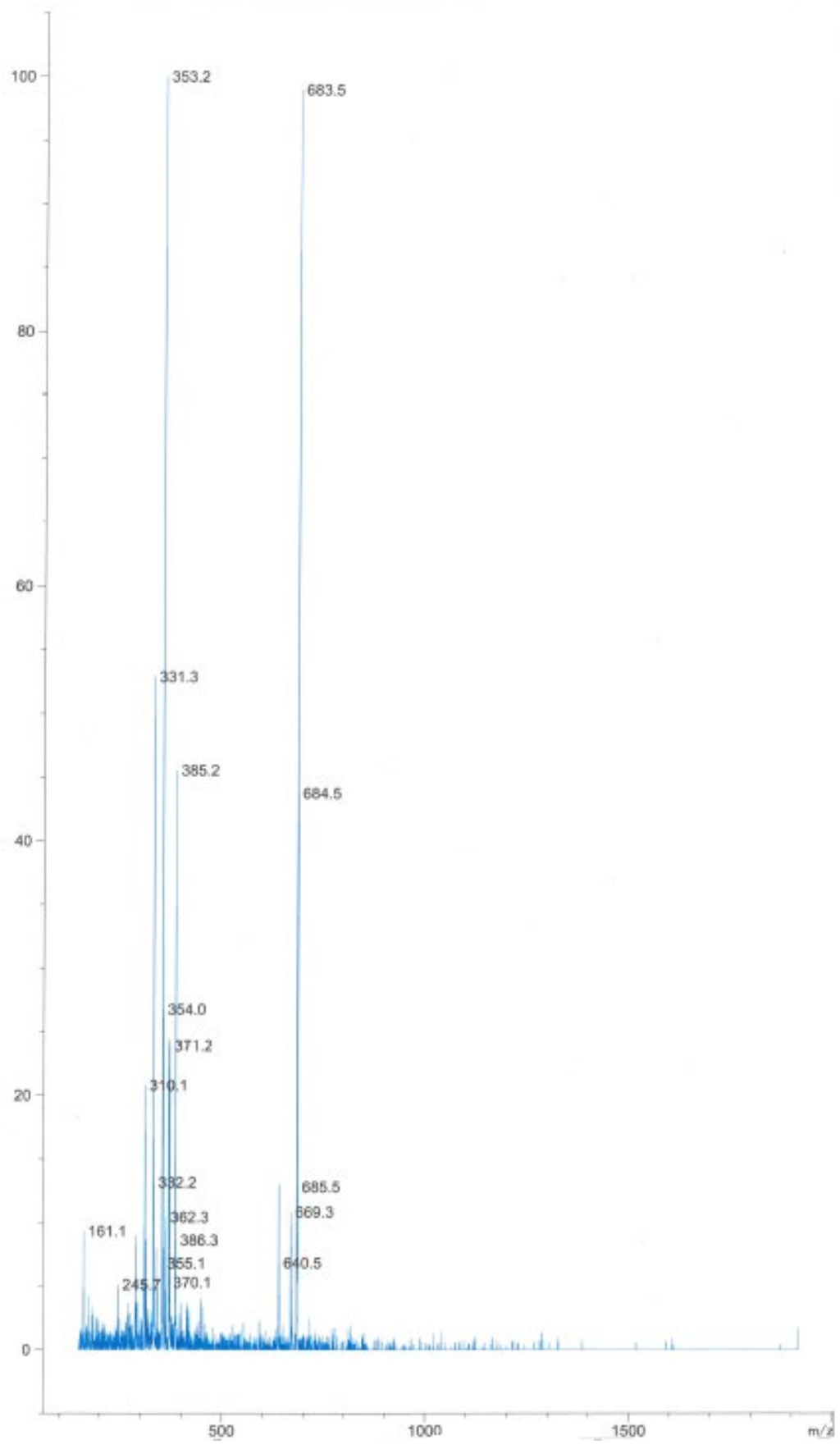
3,3-Ethylenedioxyandrost-5-en-17-one – ^{13}C NMR (101 MHz, $CDCl_3$)



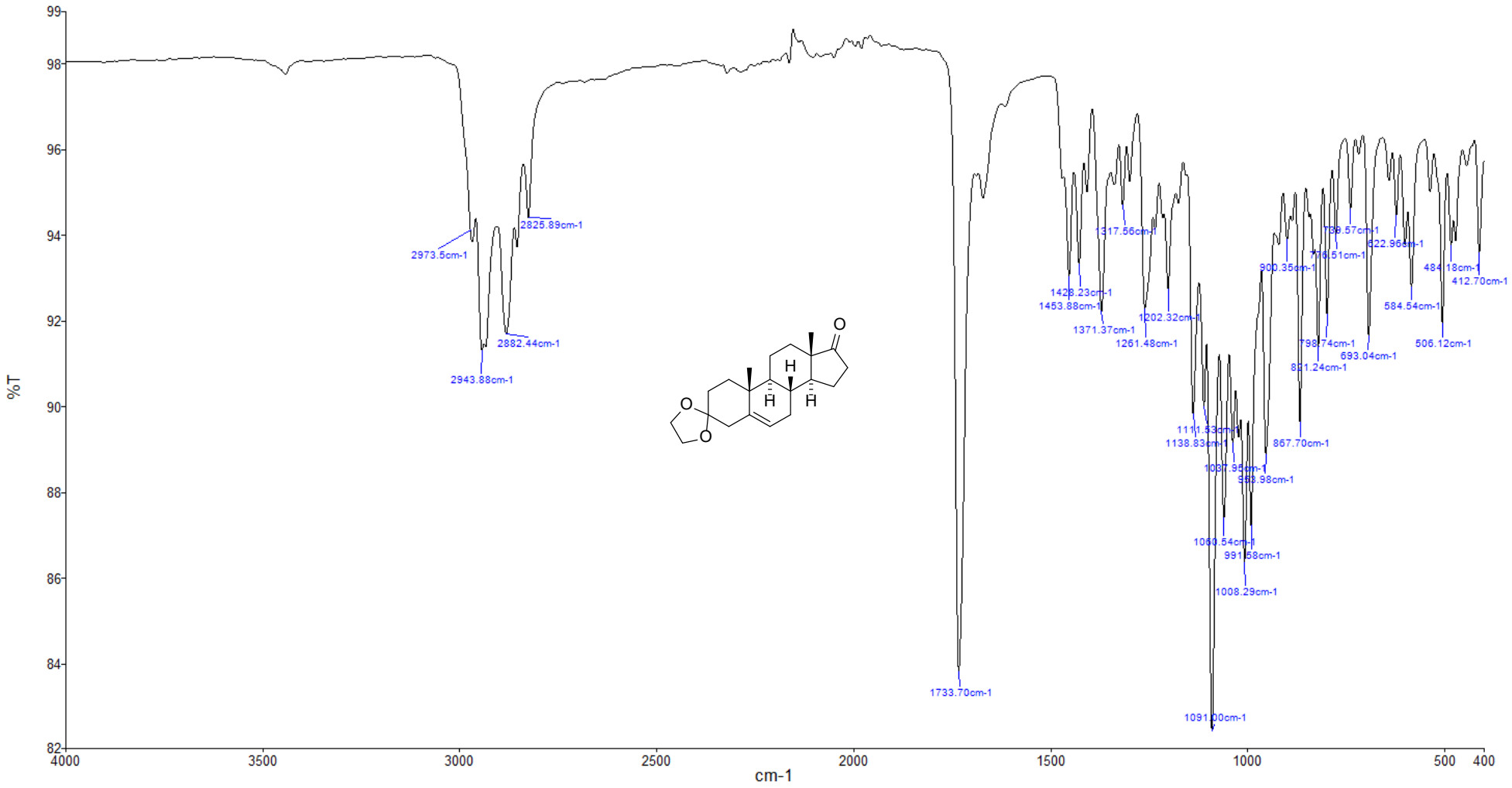


3,3-Ethylenedioxyandrost-5-en-17-one – LRMS (+ESI)

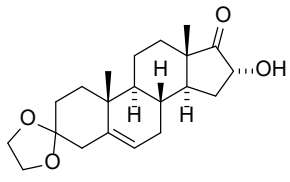


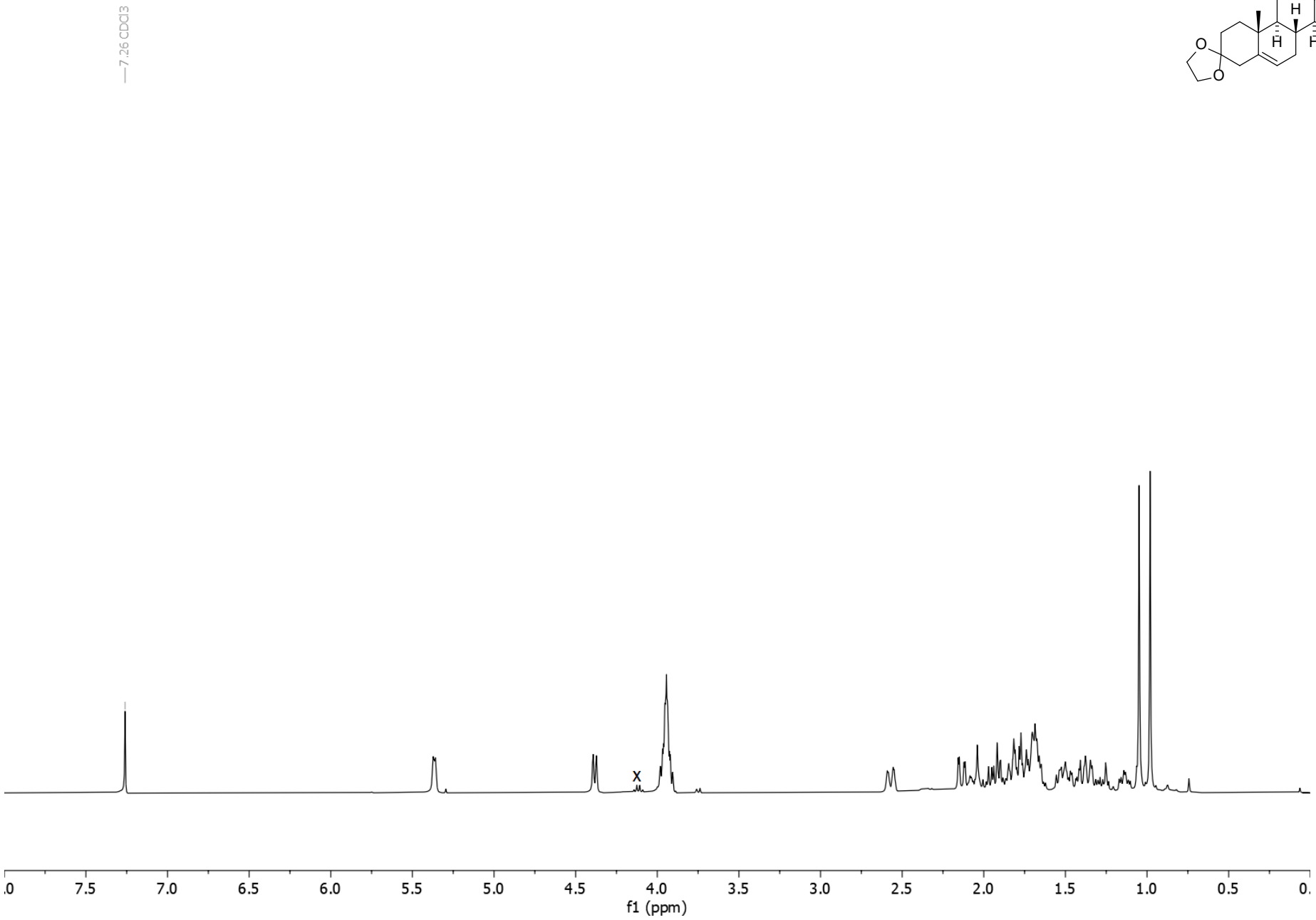


3,3-Ethylenedioxyandrost-5-en-17-one – IR (neat)

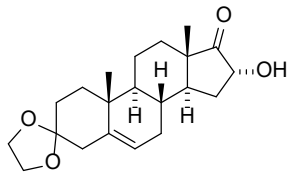


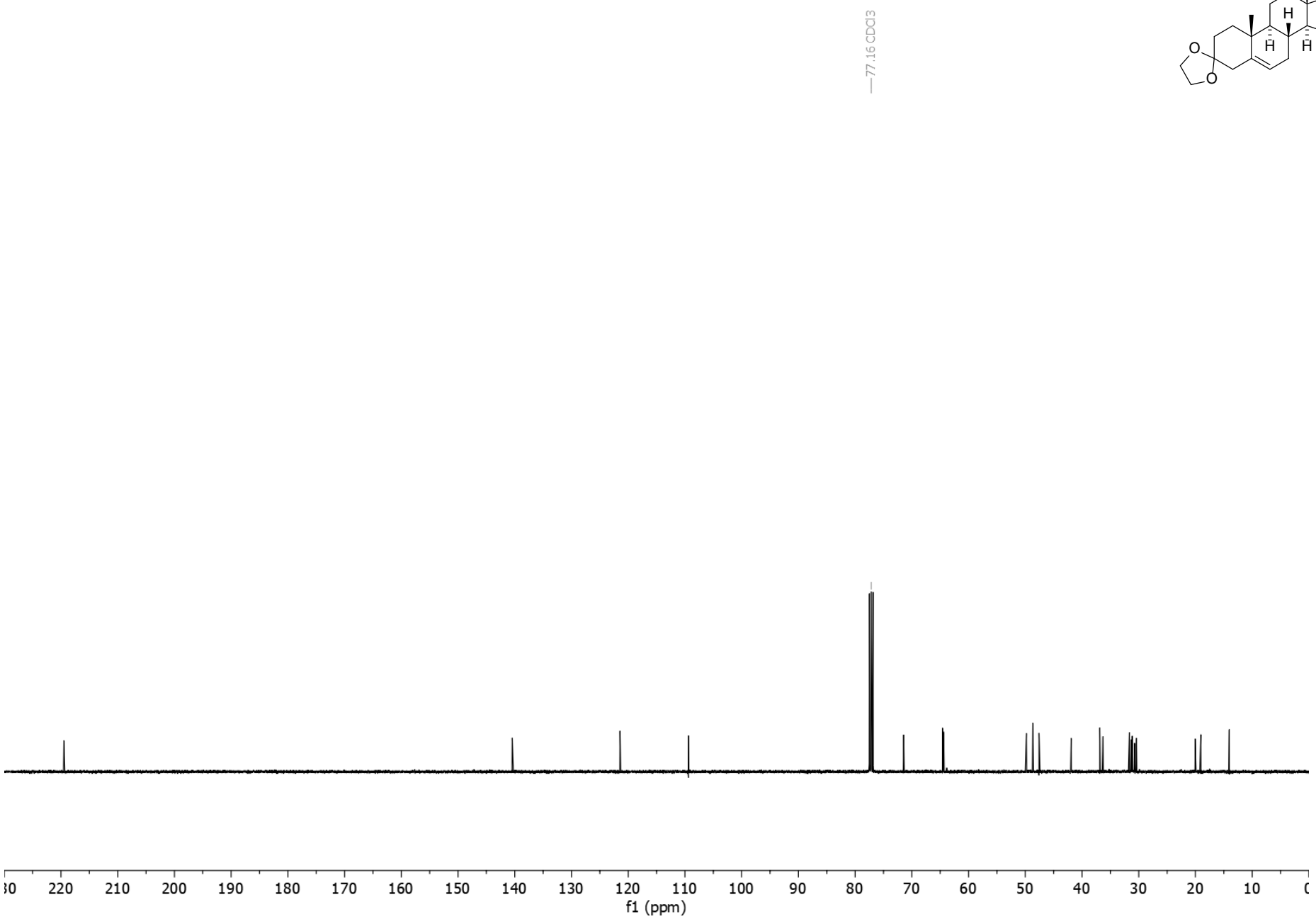
3,3-Ethylenedioxy-16α-hydroxyandrost-5-en-17-one – ^{1}H NMR (400 MHz, $CDCl_3$)



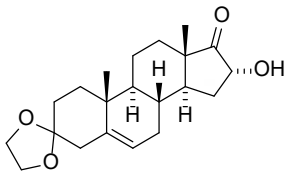


3,3-Ethylenedioxy-16α-hydroxyandrost-5-en-17-one – ^{13}C NMR (101 MHz, $CDCl_3$)

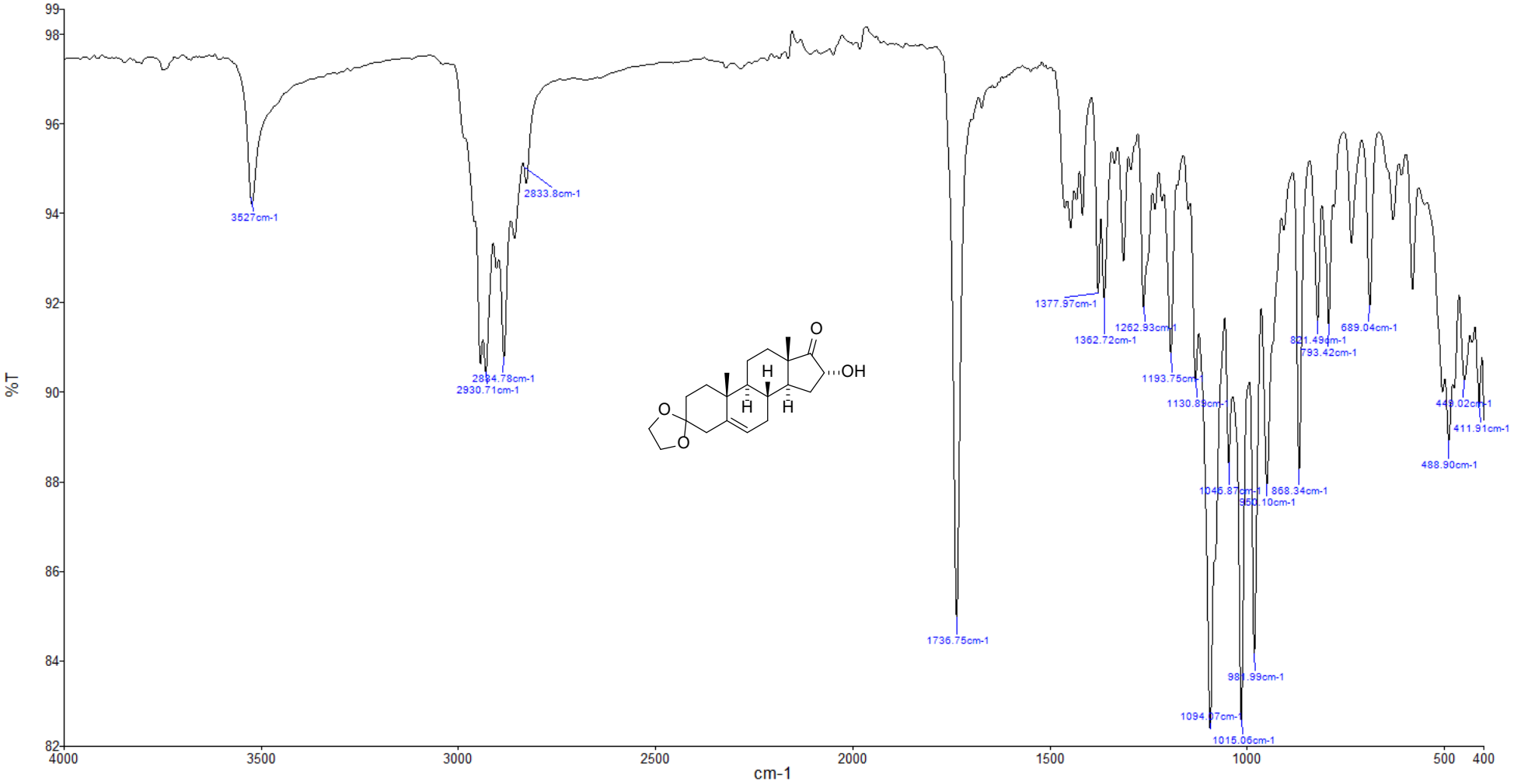




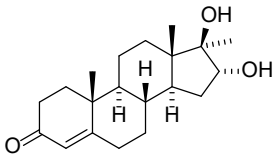
3,3-Ethylenedioxy-16α-hydroxyandrost-5-en-17-one – LRMS (+ESI)

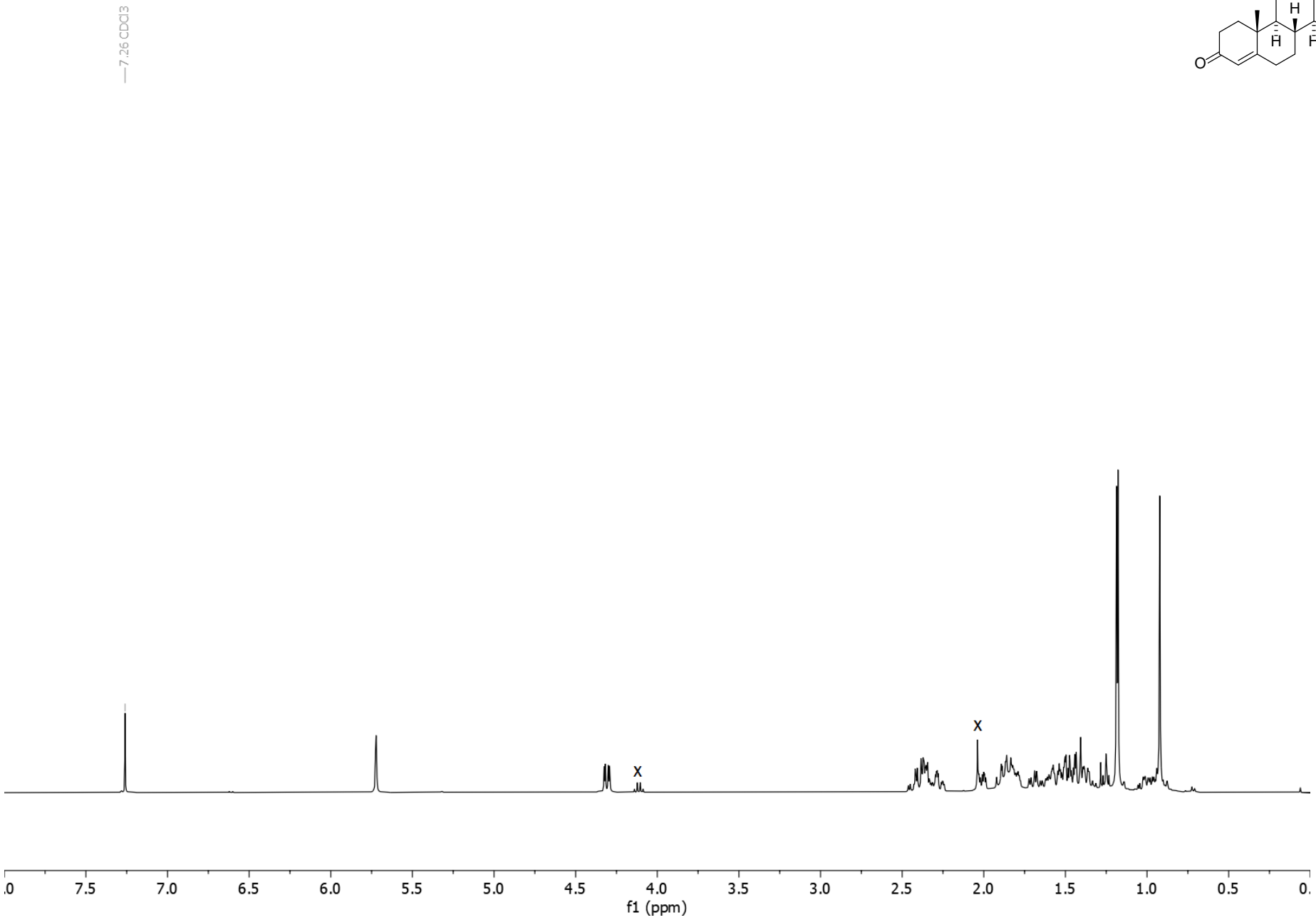


3,3-Ethylenedioxy-16α-hydroxyandrost-5-en-17-one – IR (neat)

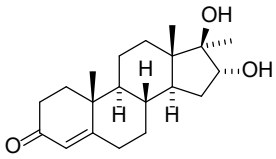


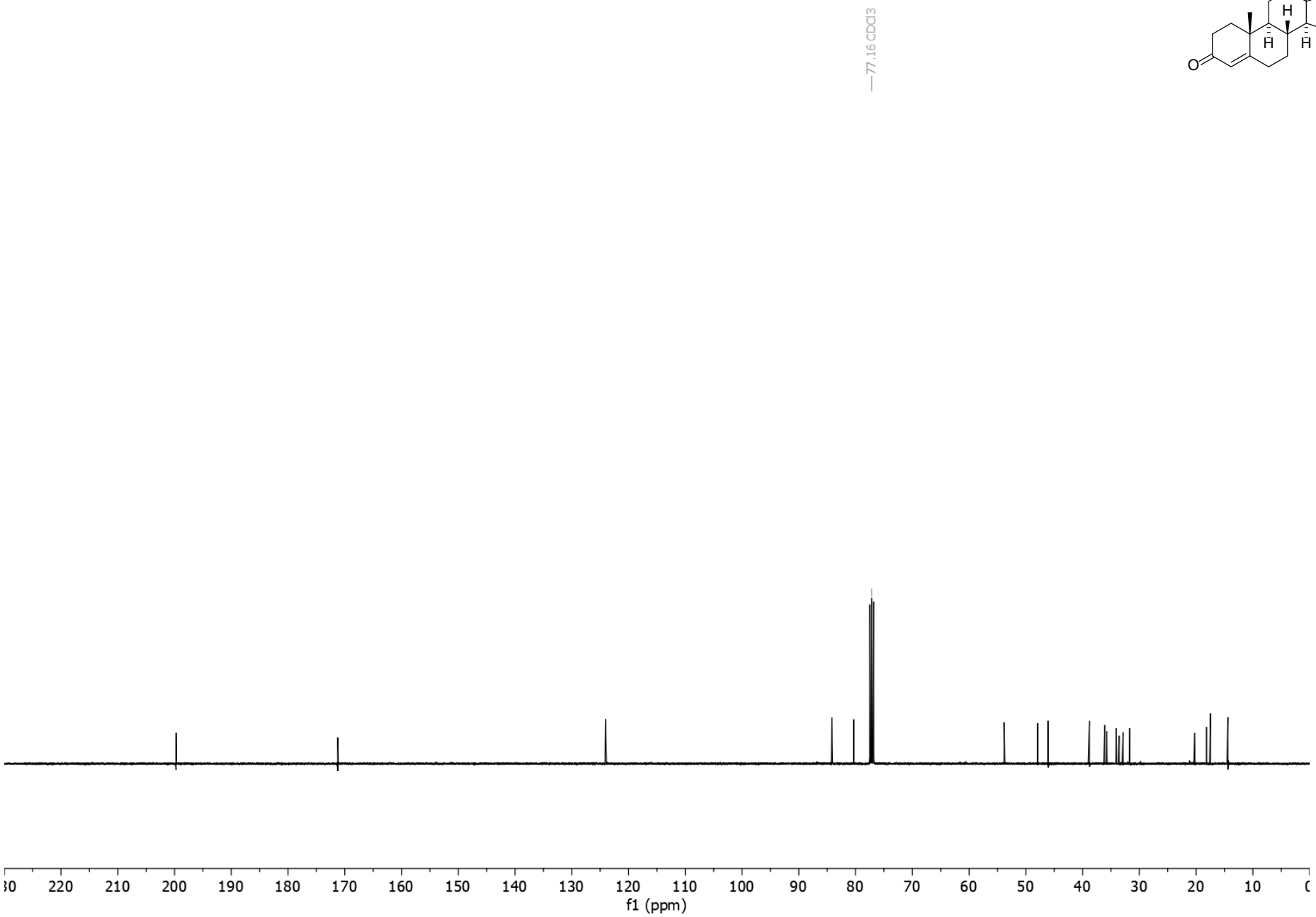
16α,17β-Dihydroxy-17α-methylandrost-4-en-3-one – ^{1}H NMR (400 MHz, $CDCl_3$)



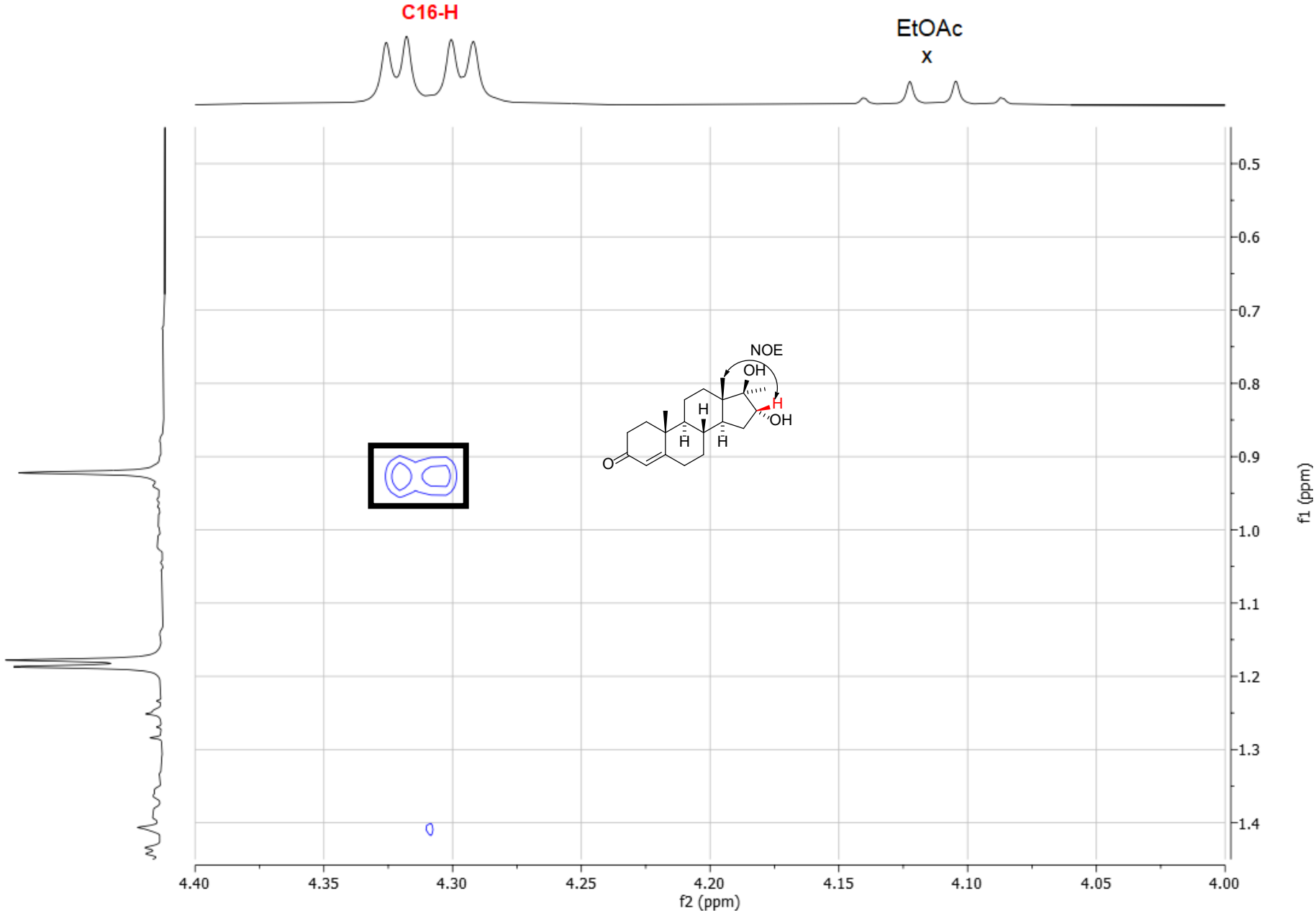


16α,17β-Dihydroxy-17α-methylandrost-4-en-3-one – ^{13}C NMR (101 MHz, $CDCl_3$)

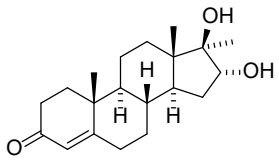


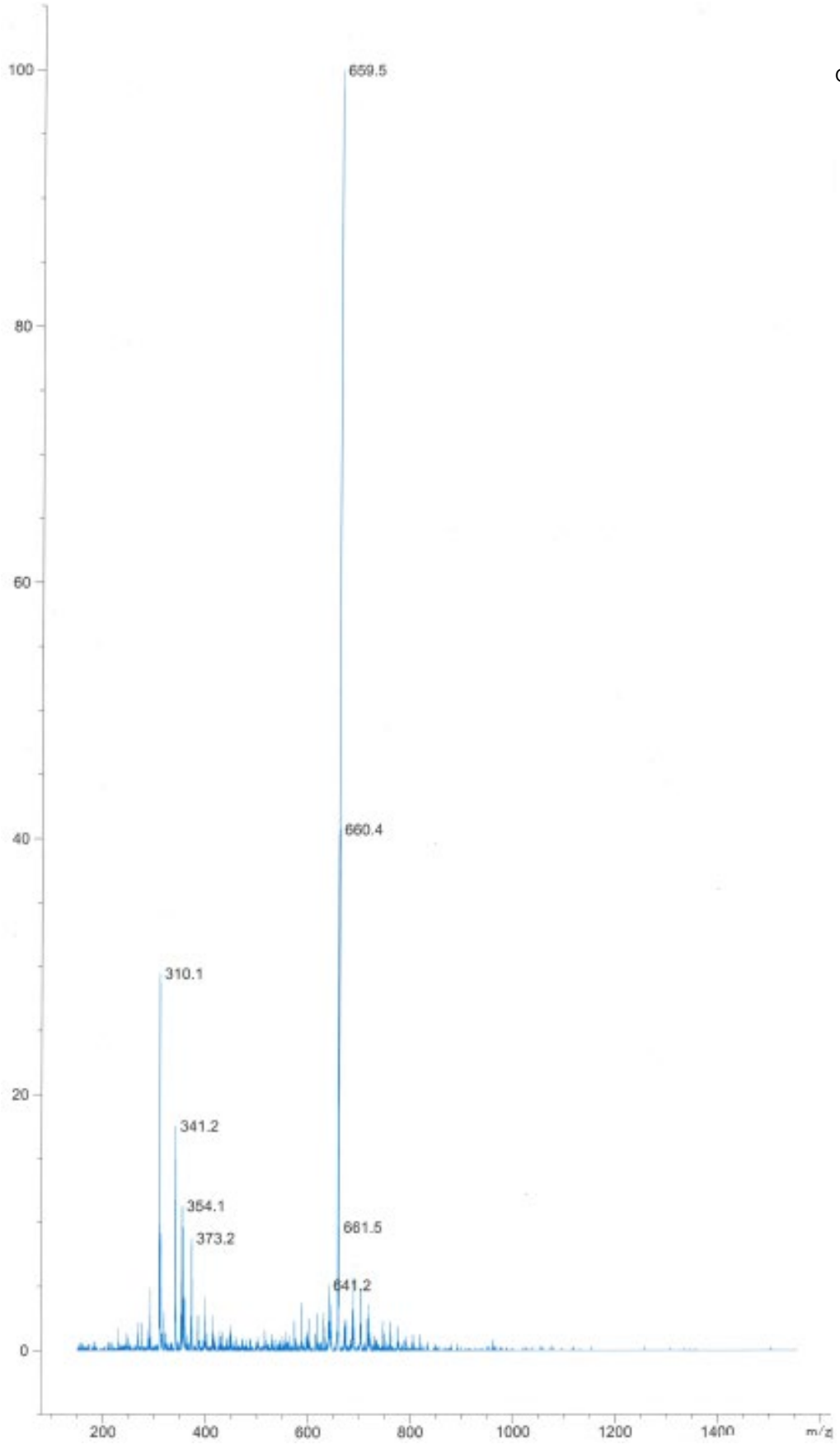


16α,17β-Dihydroxy-17α-methylandrost-4-en-3-one – ^{1}H-^{1}H NOE (400 MHz, $CDCl_3$)

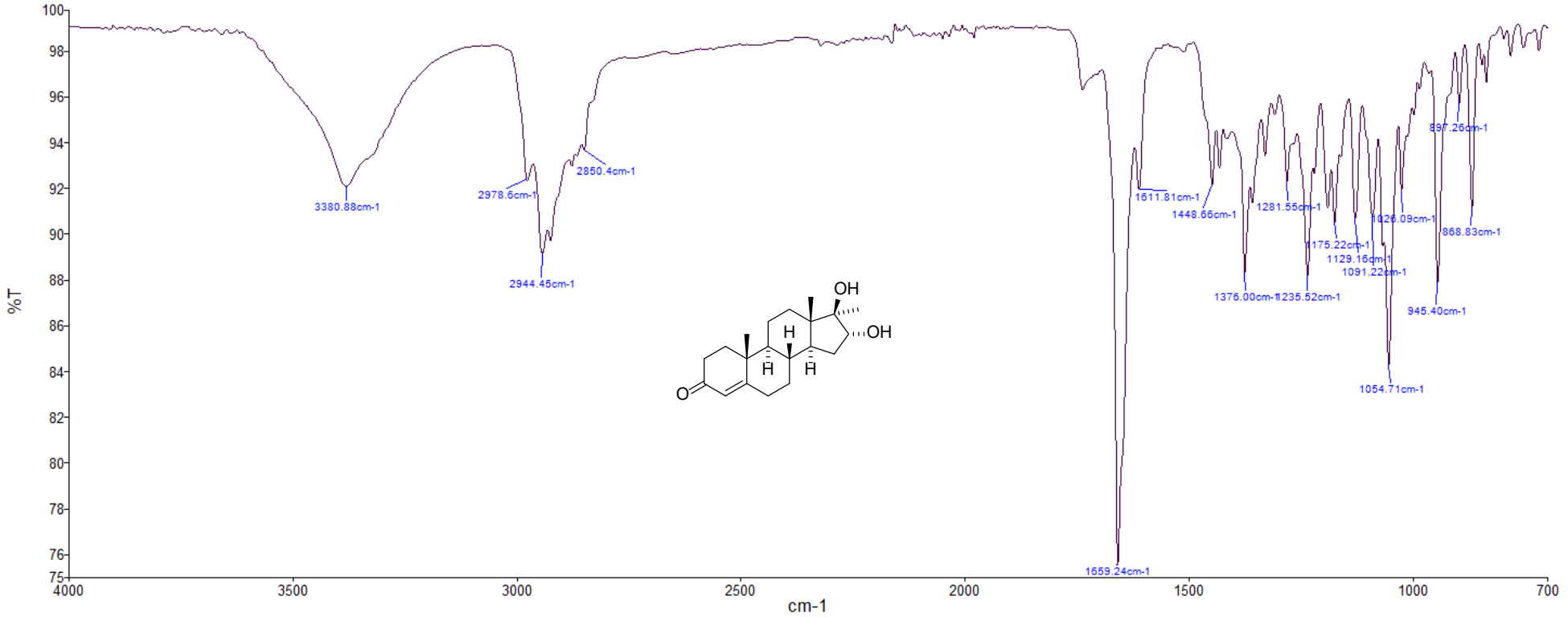


16α,17β-Dihydroxy-17α-methylandrost-4-en-3-one – LRMS (+ESI)

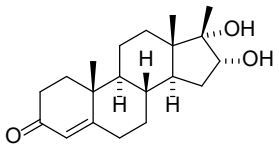


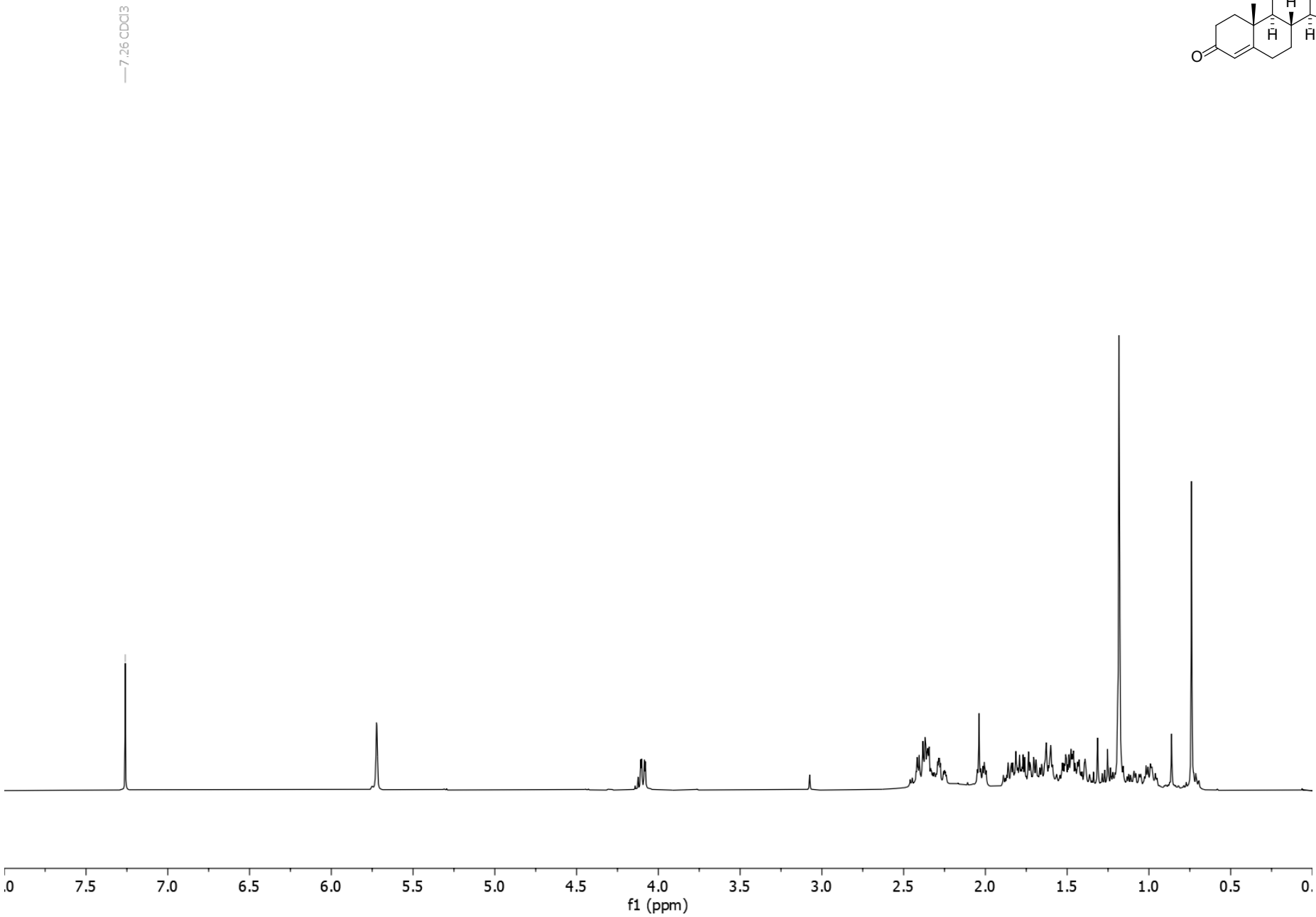


16α,17β-Dihydroxy-17α-methylandrost-4-en-3-one – IR (neat)

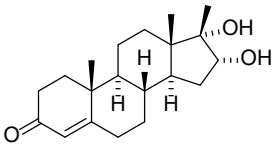


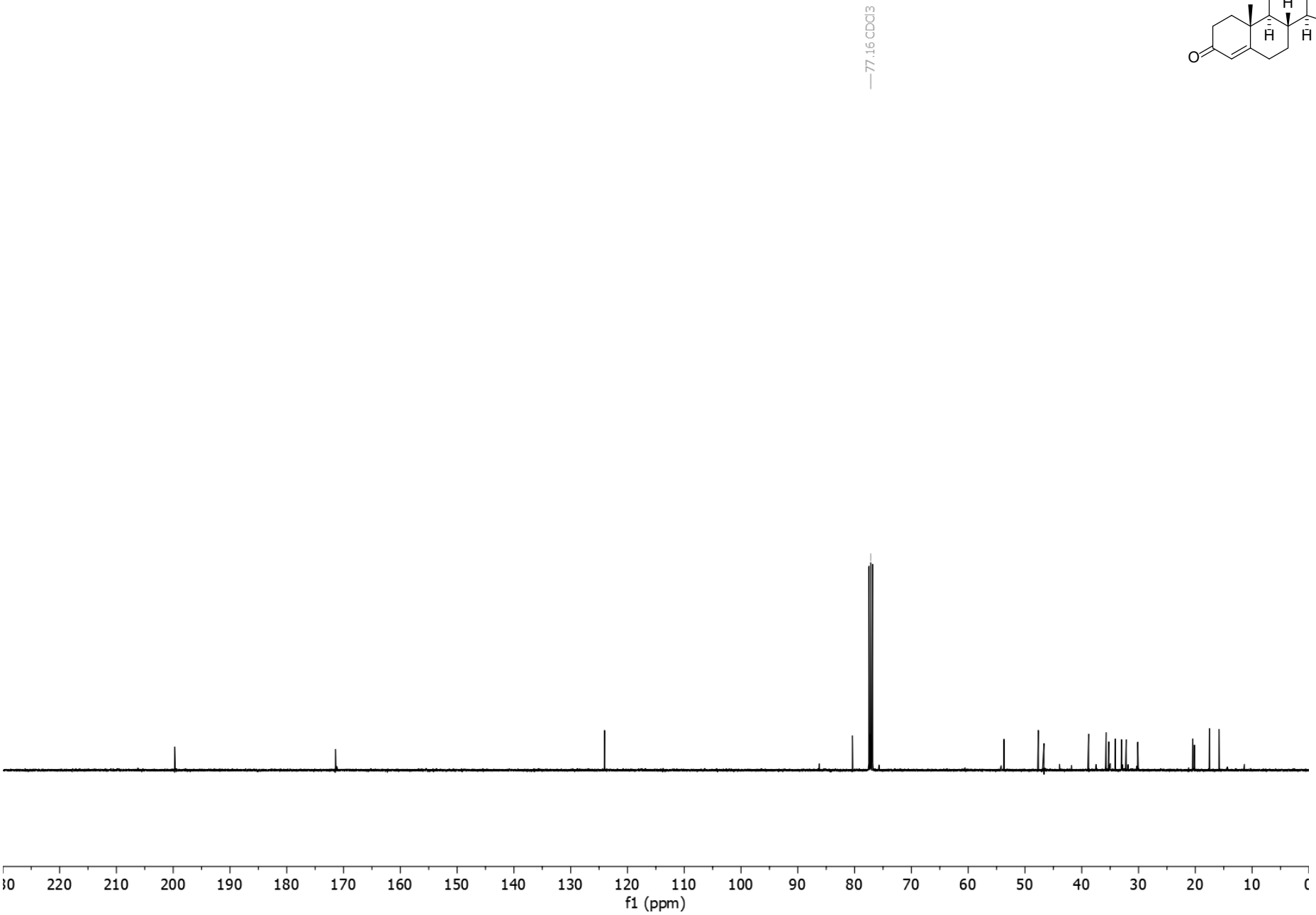
16α,17α-Dihydroxy-17β-methylandrost-4-en-3-one – ^{1}H NMR (400 MHz, $CDCl_3$)



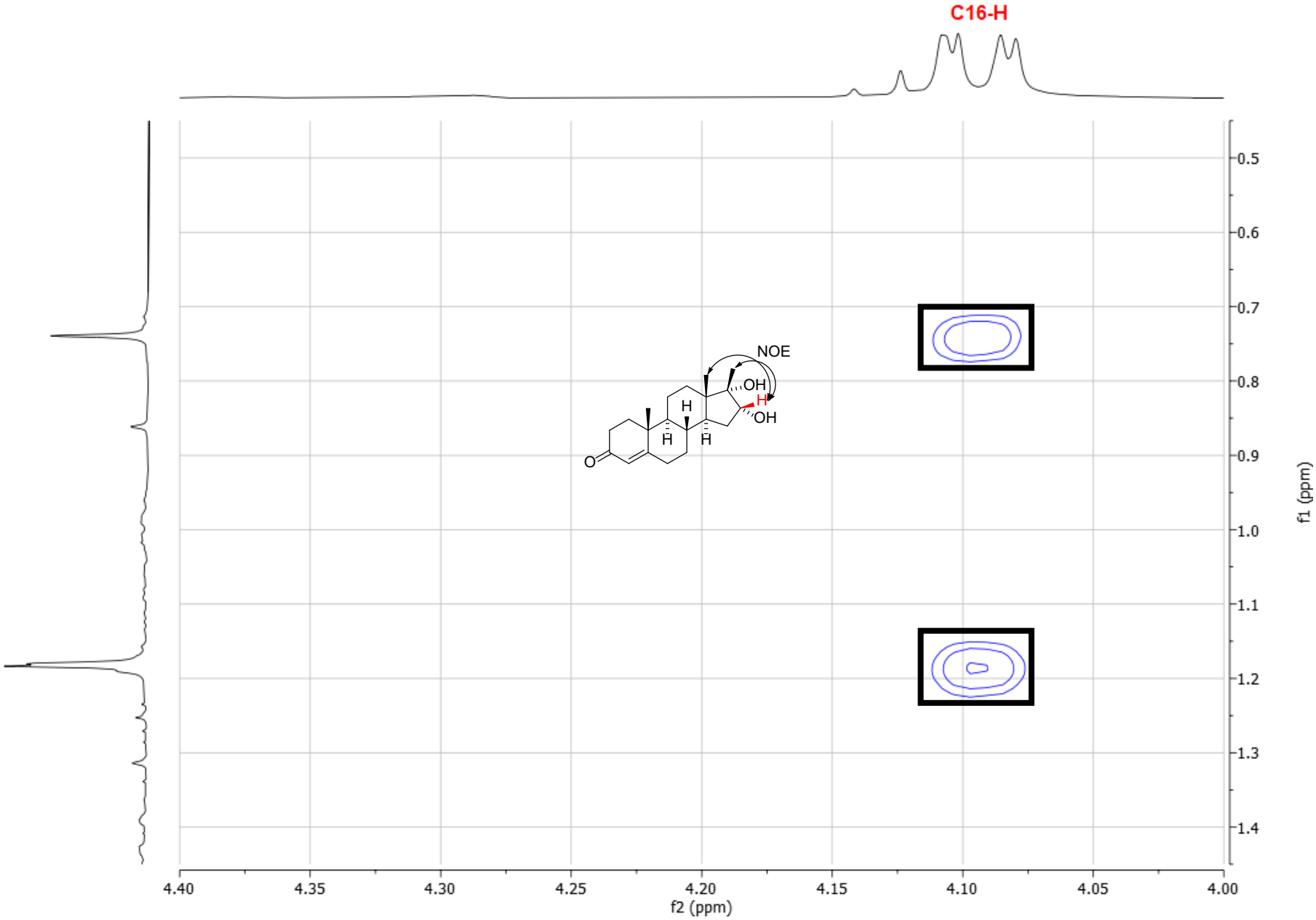


16α,17α-Dihydroxy-17β-methylandrost-4-en-3-one – ^{13}C NMR (101 MHz, $CDCl_3$)

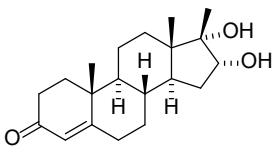


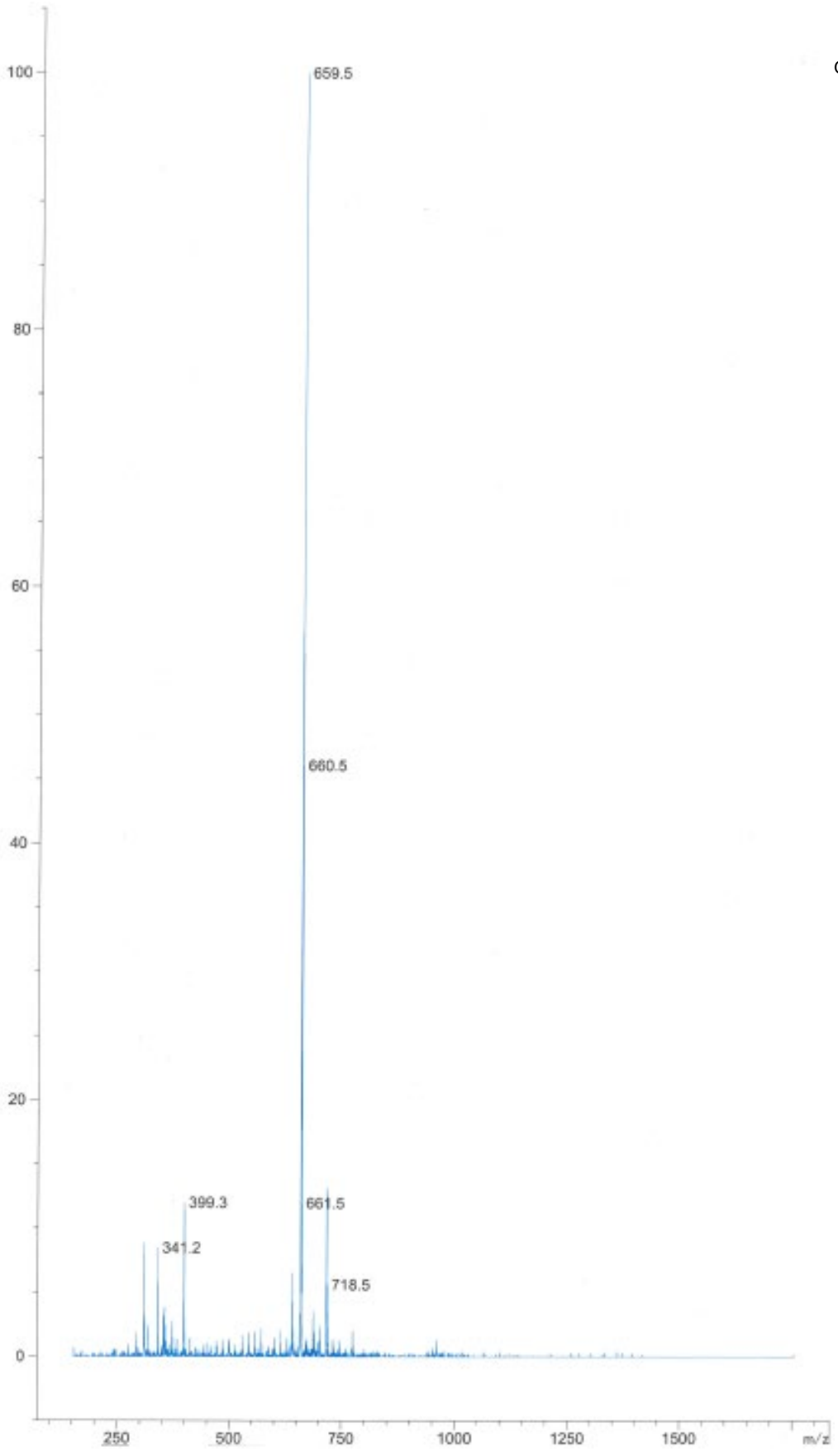


16α,17α-Dihydroxy-17β-methylandrost-4-en-3-one – ^{1}H-^{1}H NOE (400 MHz, $CDCl_3$)

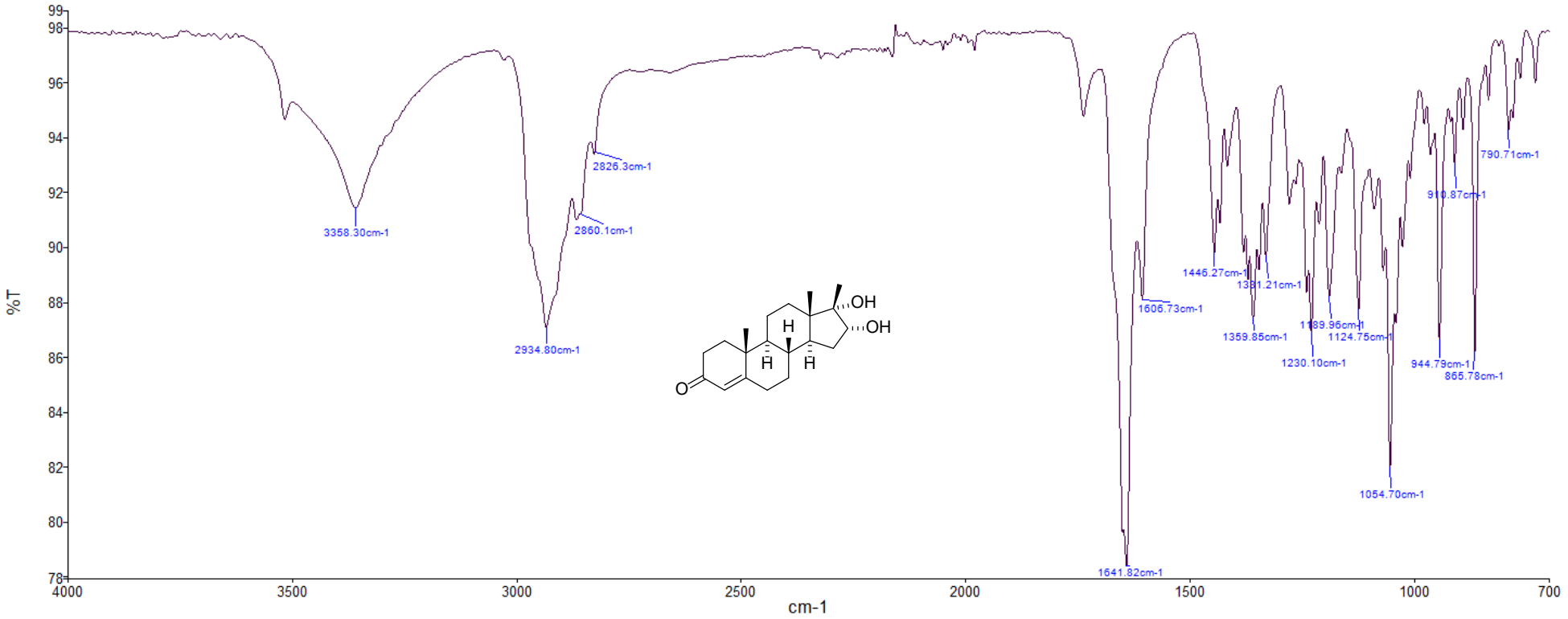


16α,17α-Dihydroxy-17β-methylandrost-4-en-3-one – LRMS (+ESI)

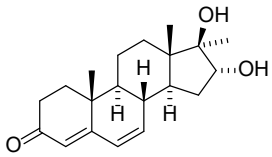


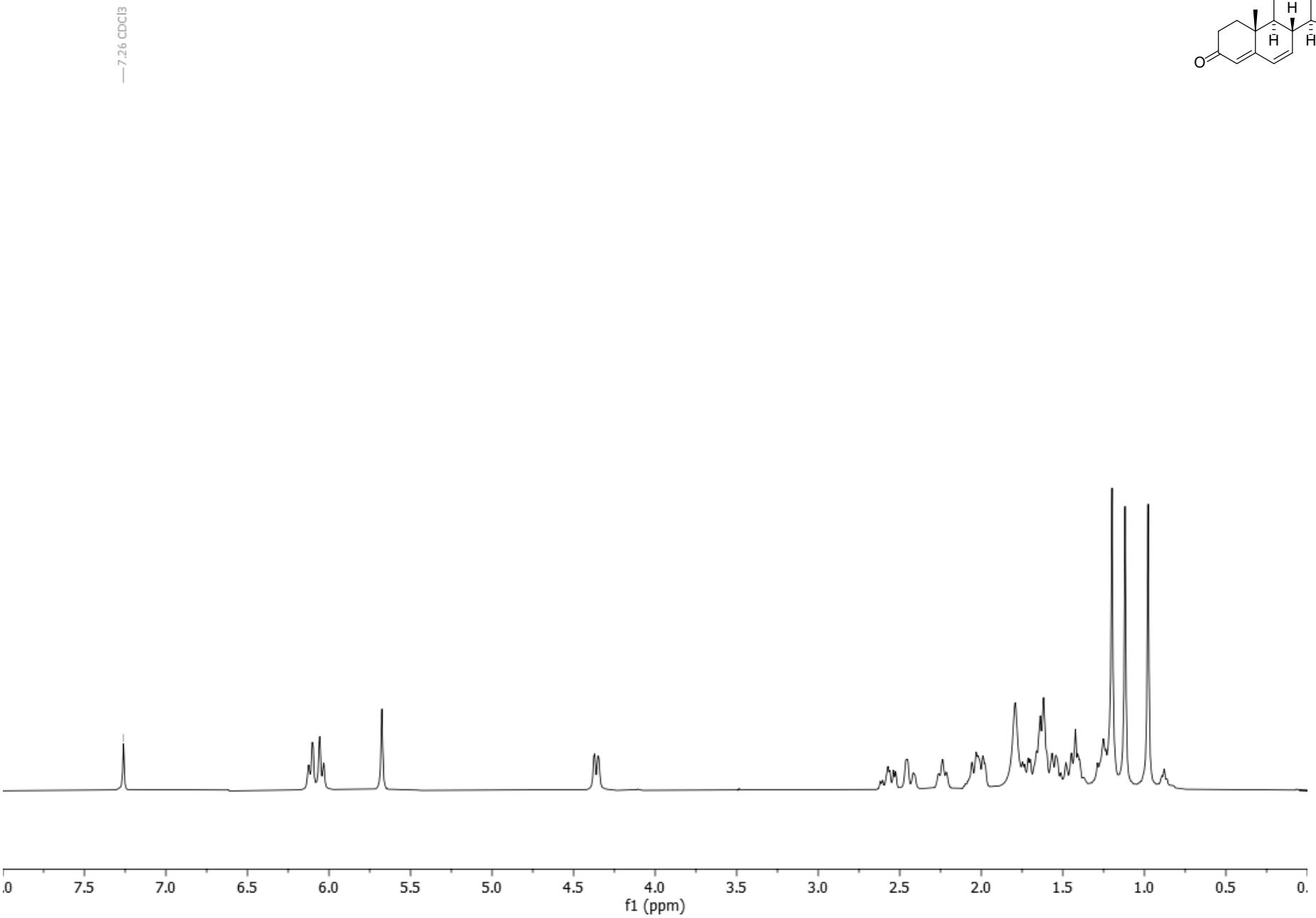


16α,17α-Dihydroxy-17β-methylandrost-4-en-3-one – IR (neat)

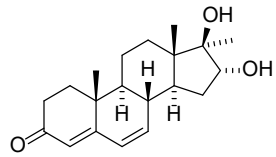


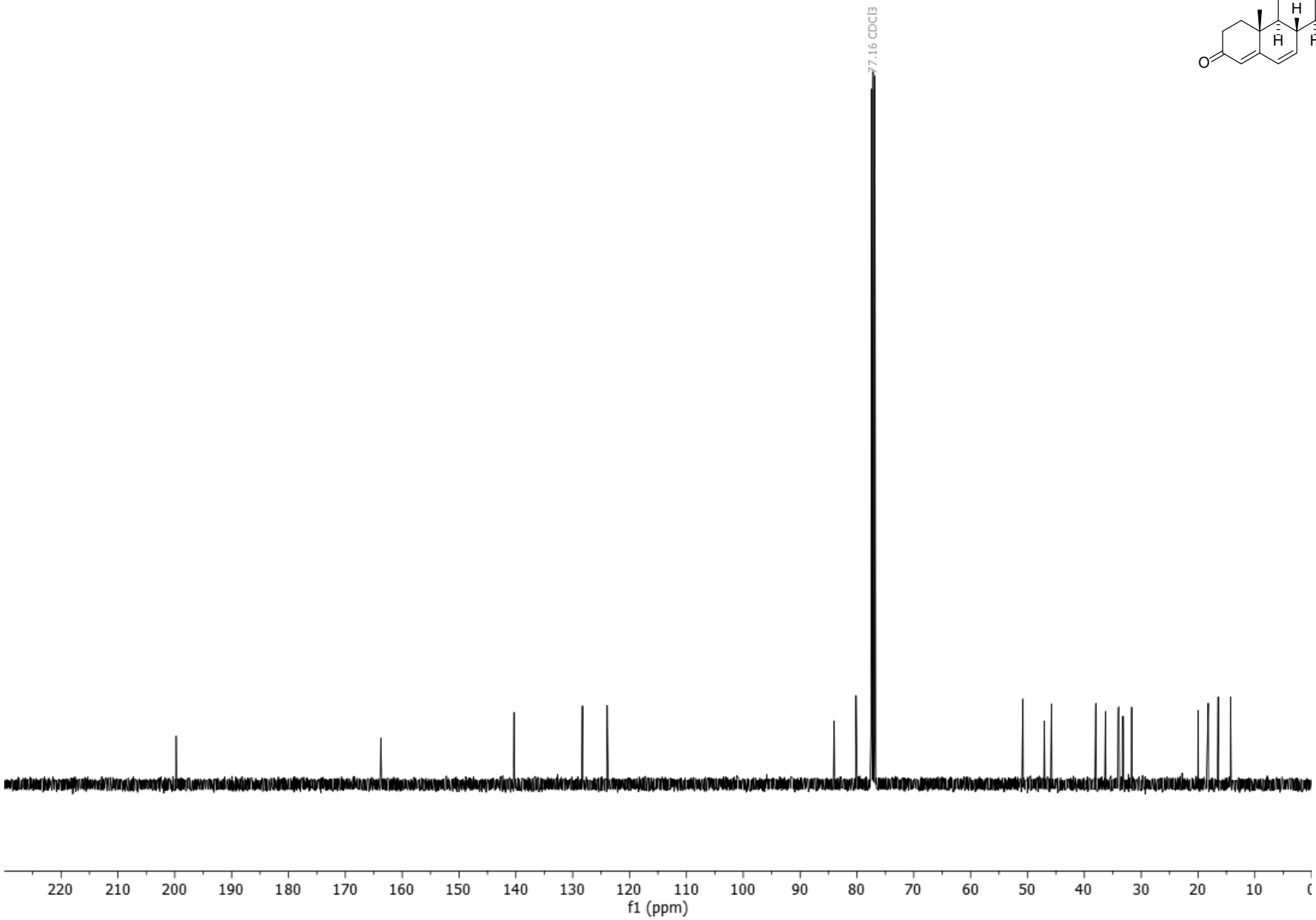
16α,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one – ^{1}H NMR (400 MHz, $CDCl_3$)



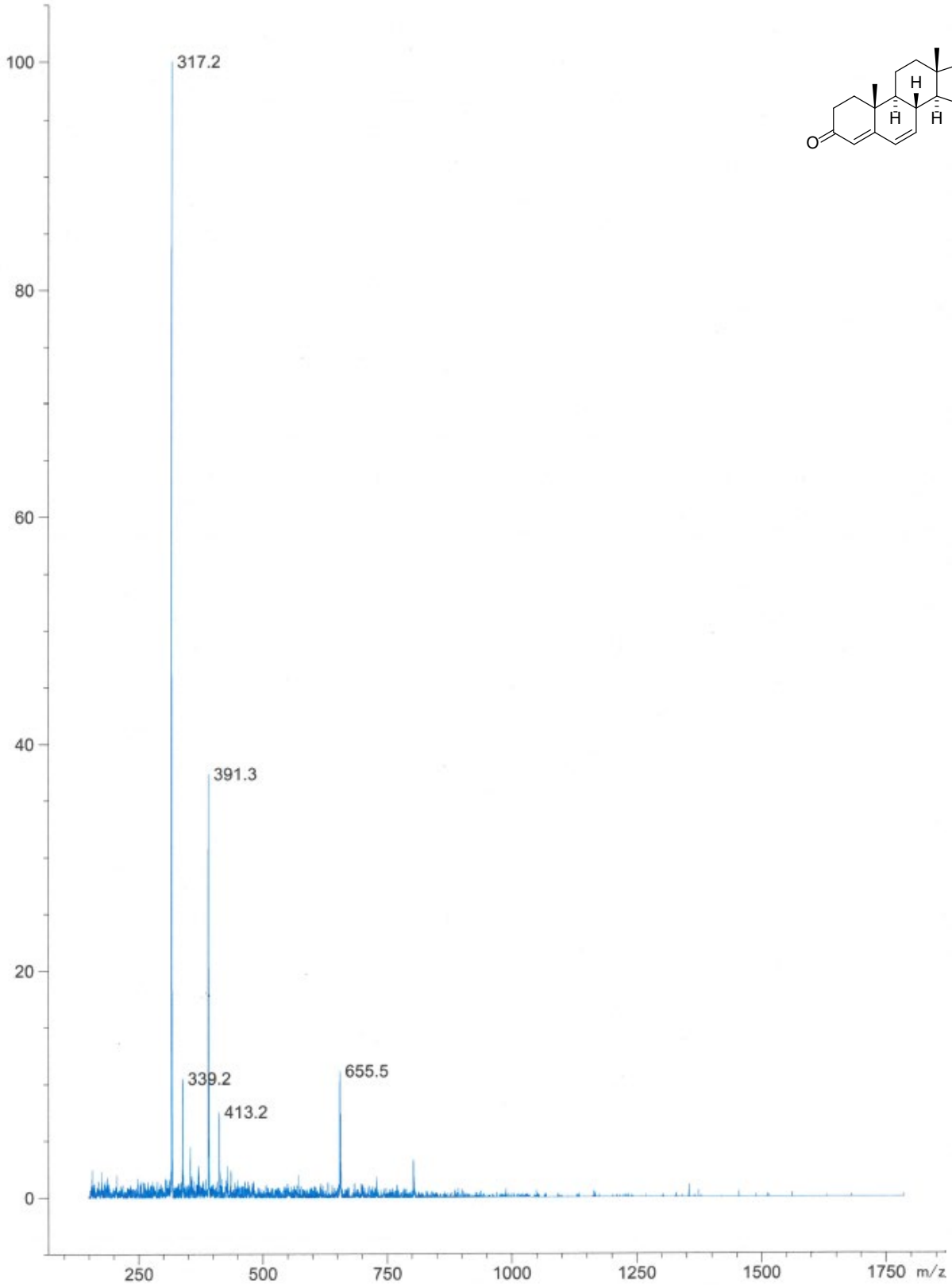


16α,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one – ^{13}C NMR (101 MHz, $CDCl_3$)

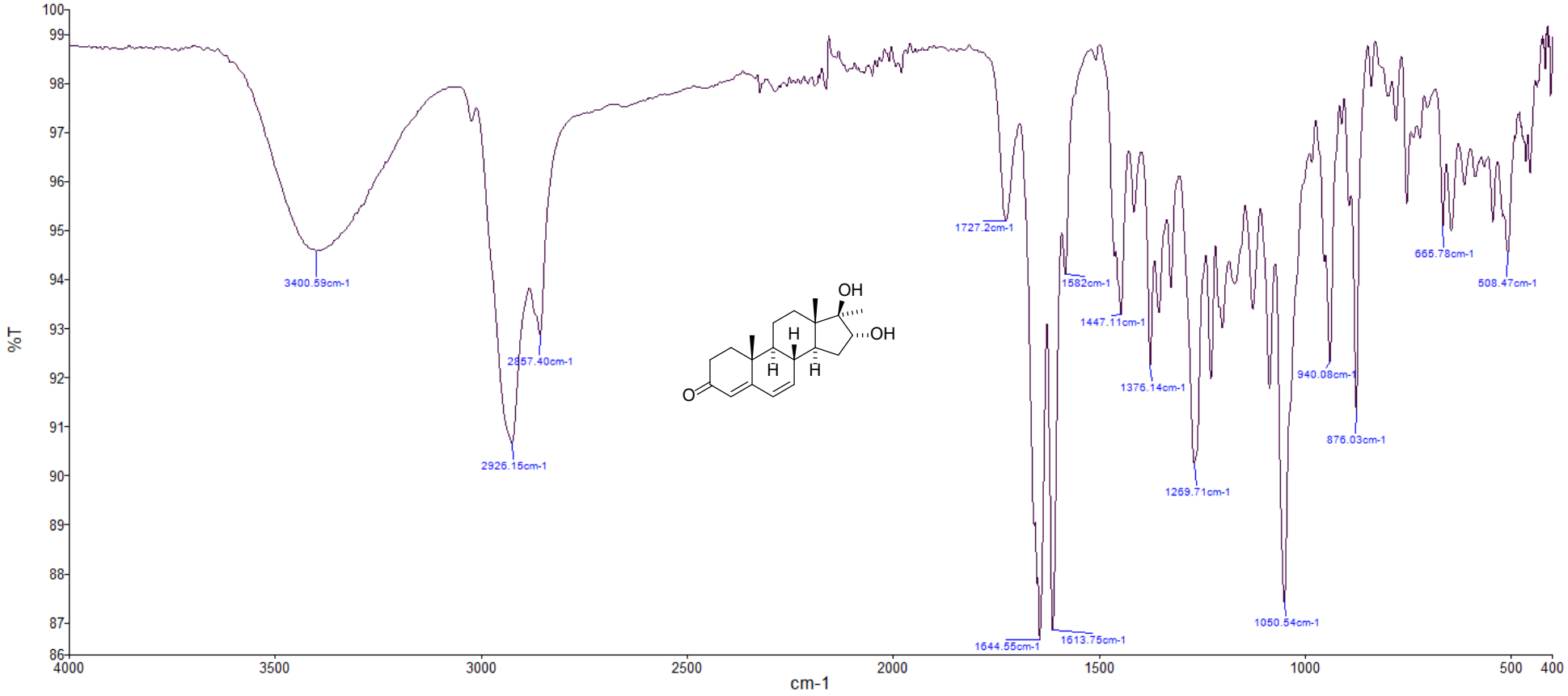




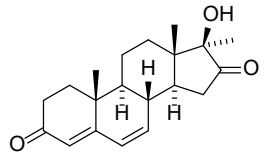
16α,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one – LRMS (+ESI)

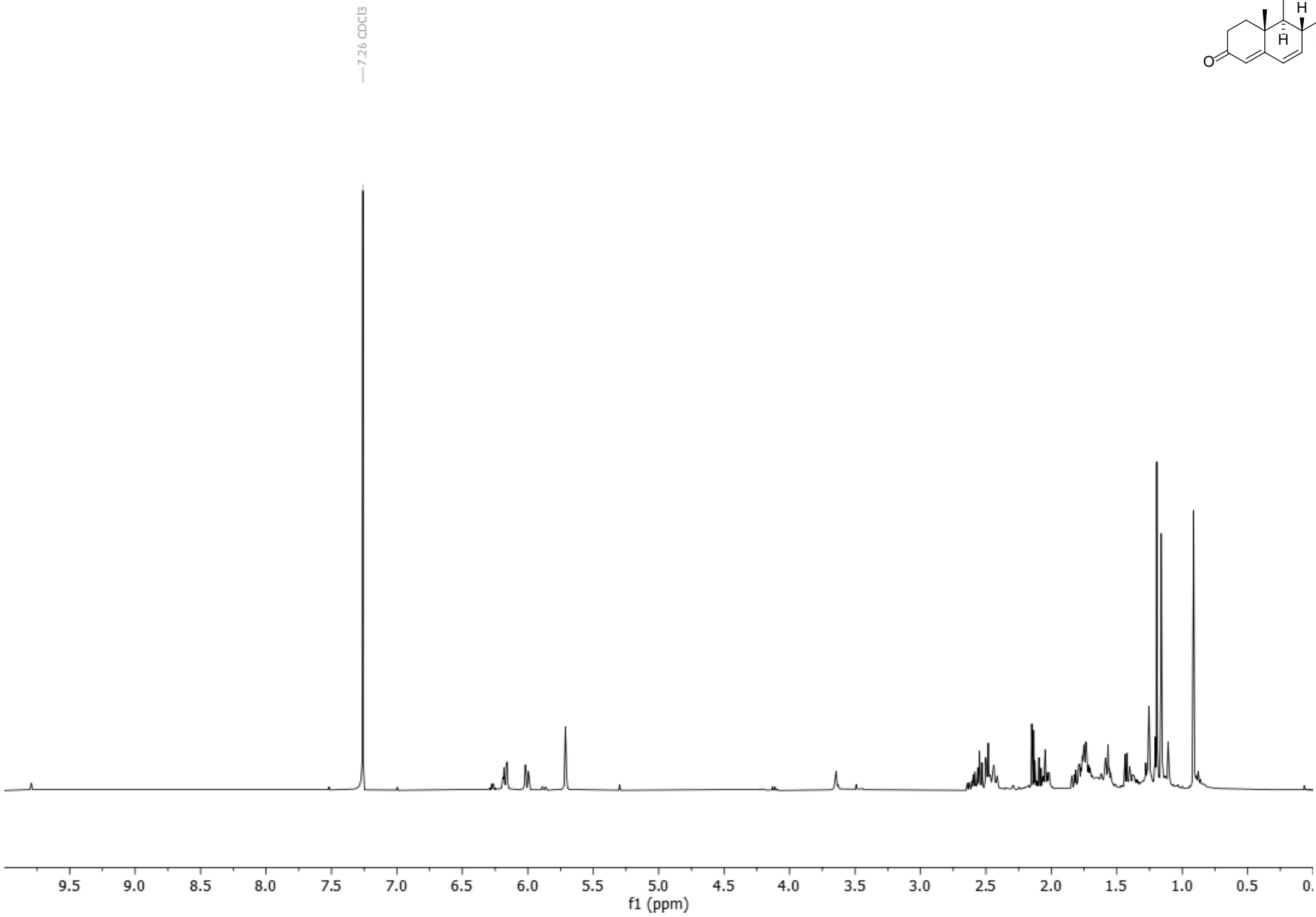


16α,17β-Dihydroxy-17α-methylandrosta-4,6-dien-3-one – IR (neat)

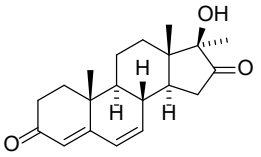


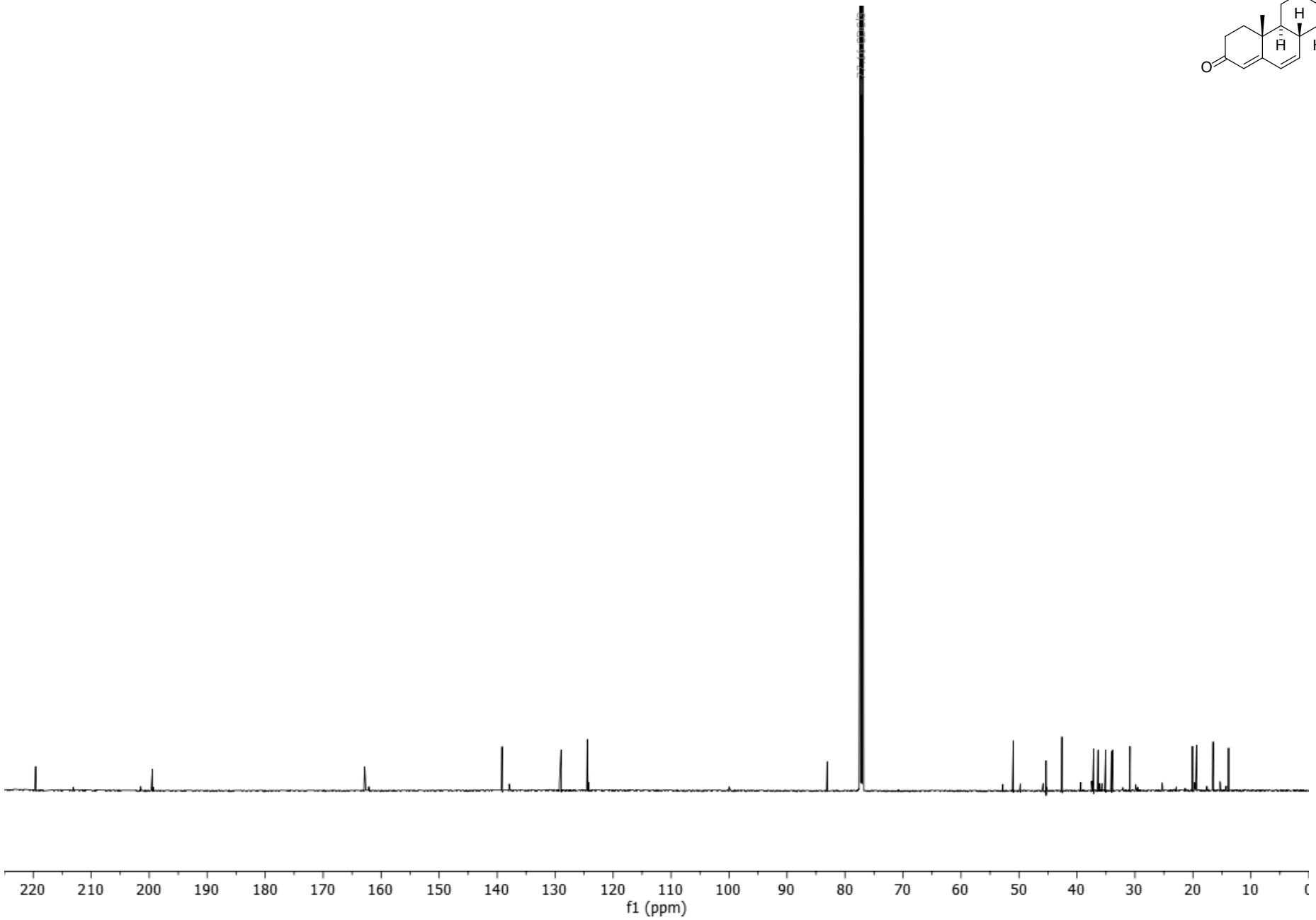
17β-Hydroxy-17α-methylandrosta-4,6-diene-3,16-dione – ^{1}H NMR (400 MHz, $CDCl_3$)



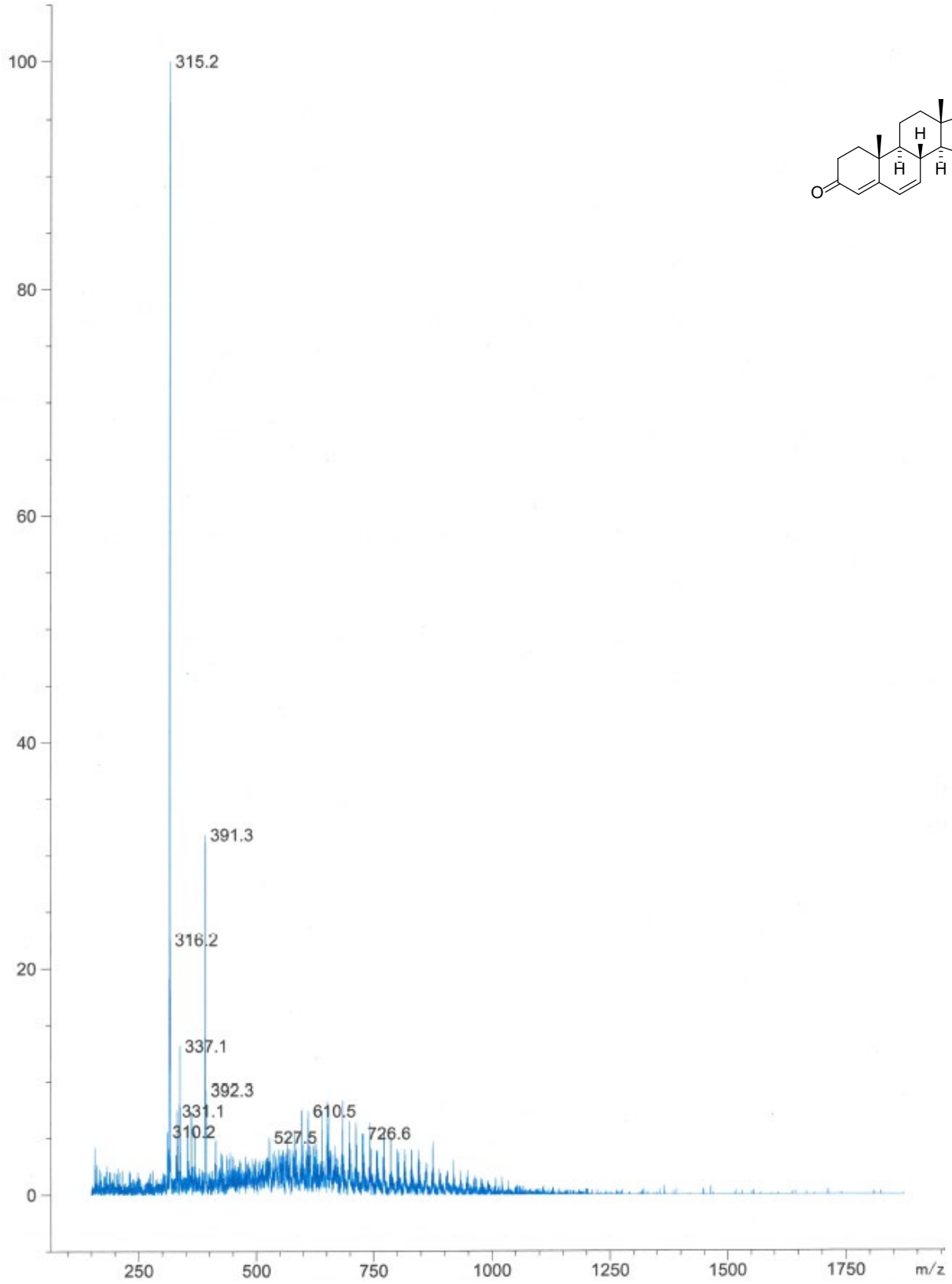


17β-Hydroxy-17α-methylandrosta-4,6-diene-3,16-dione – ^{13}C NMR (151 MHz, $CDCl_3$)

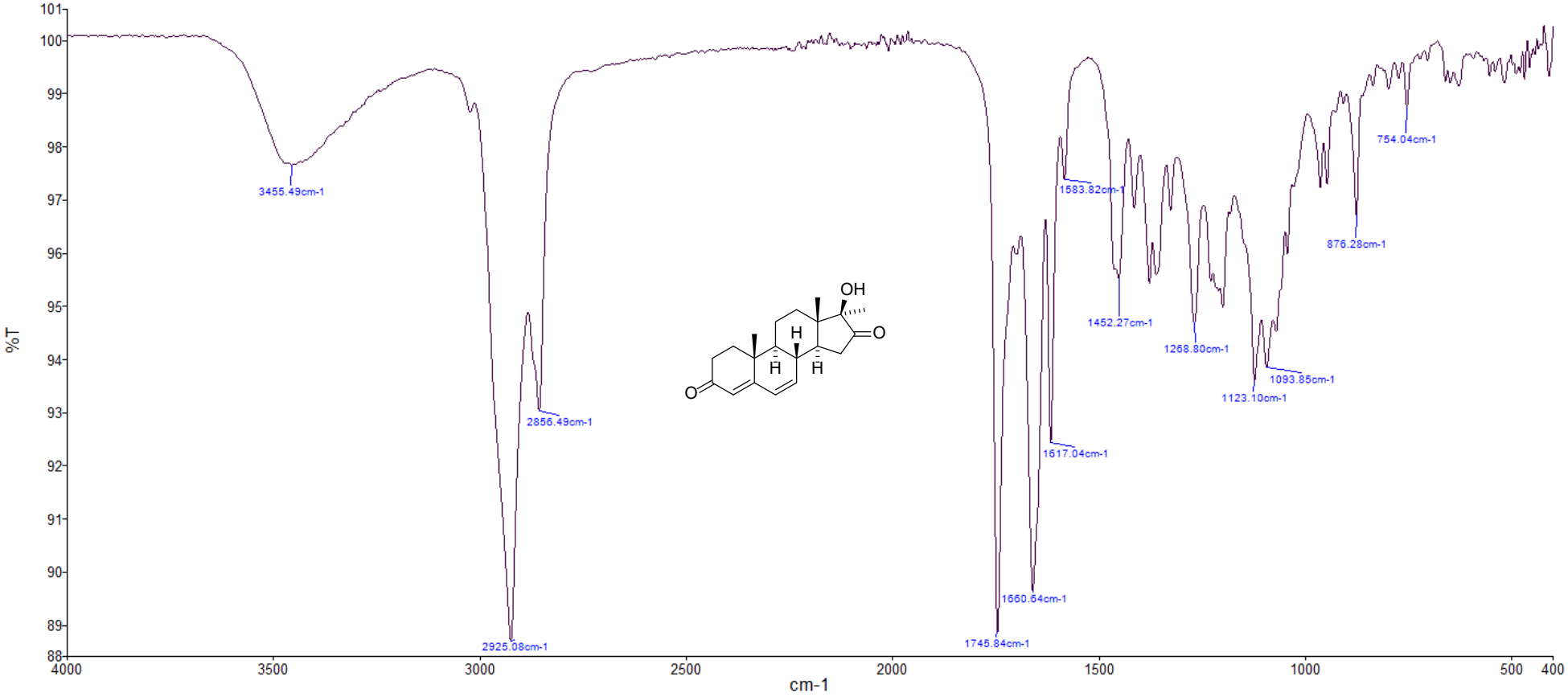




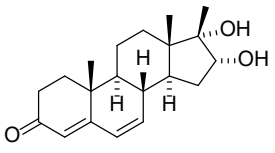
17β-Hydroxy-17α-methylandrosta-4,6-diene-3,16-dione – LRMS (+ESI)

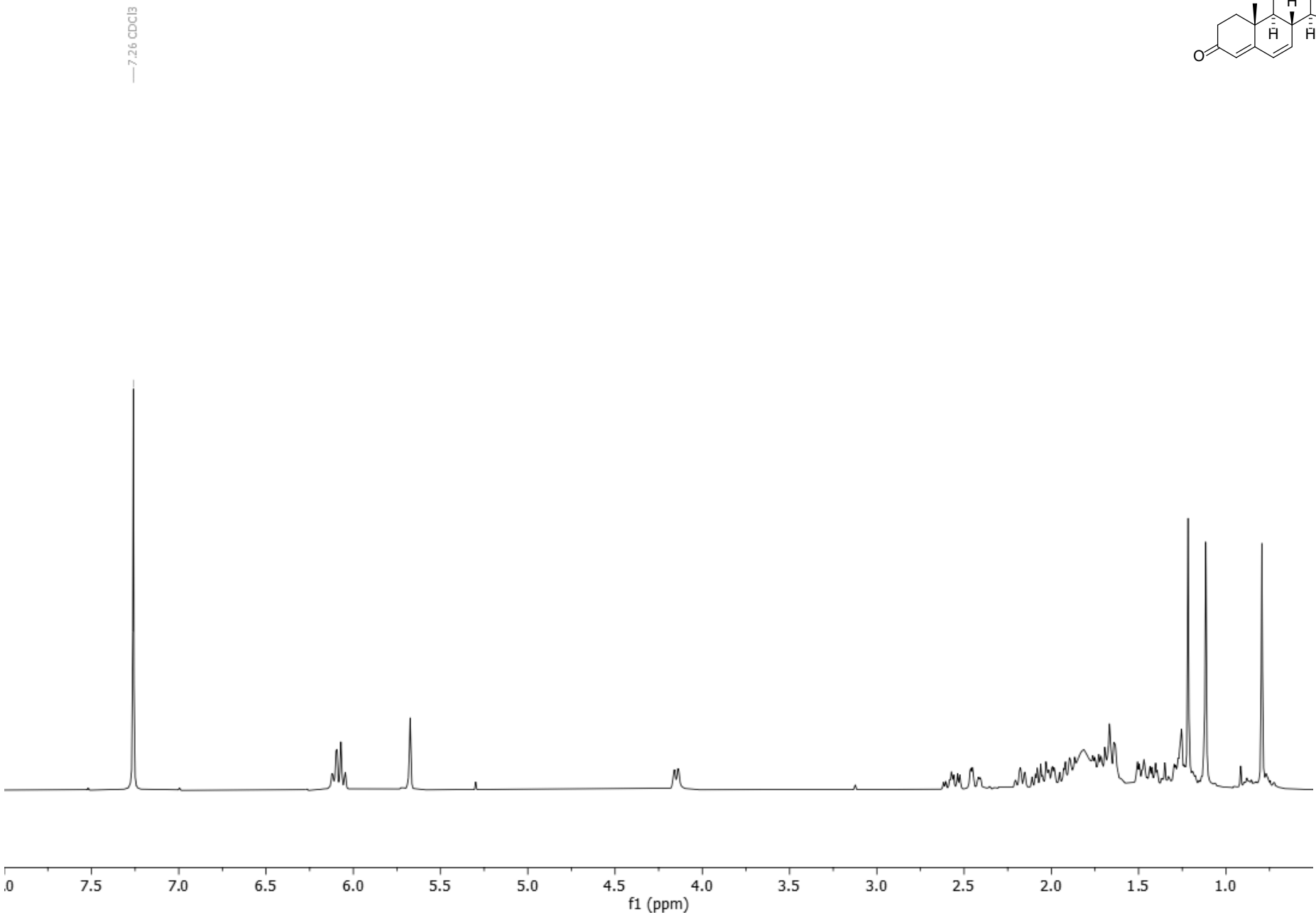


17β-Hydroxy-17α-methylandrosta-4,6-diene-3,16-dione – IR (neat)

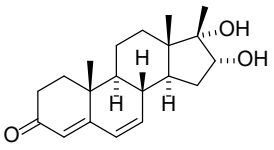


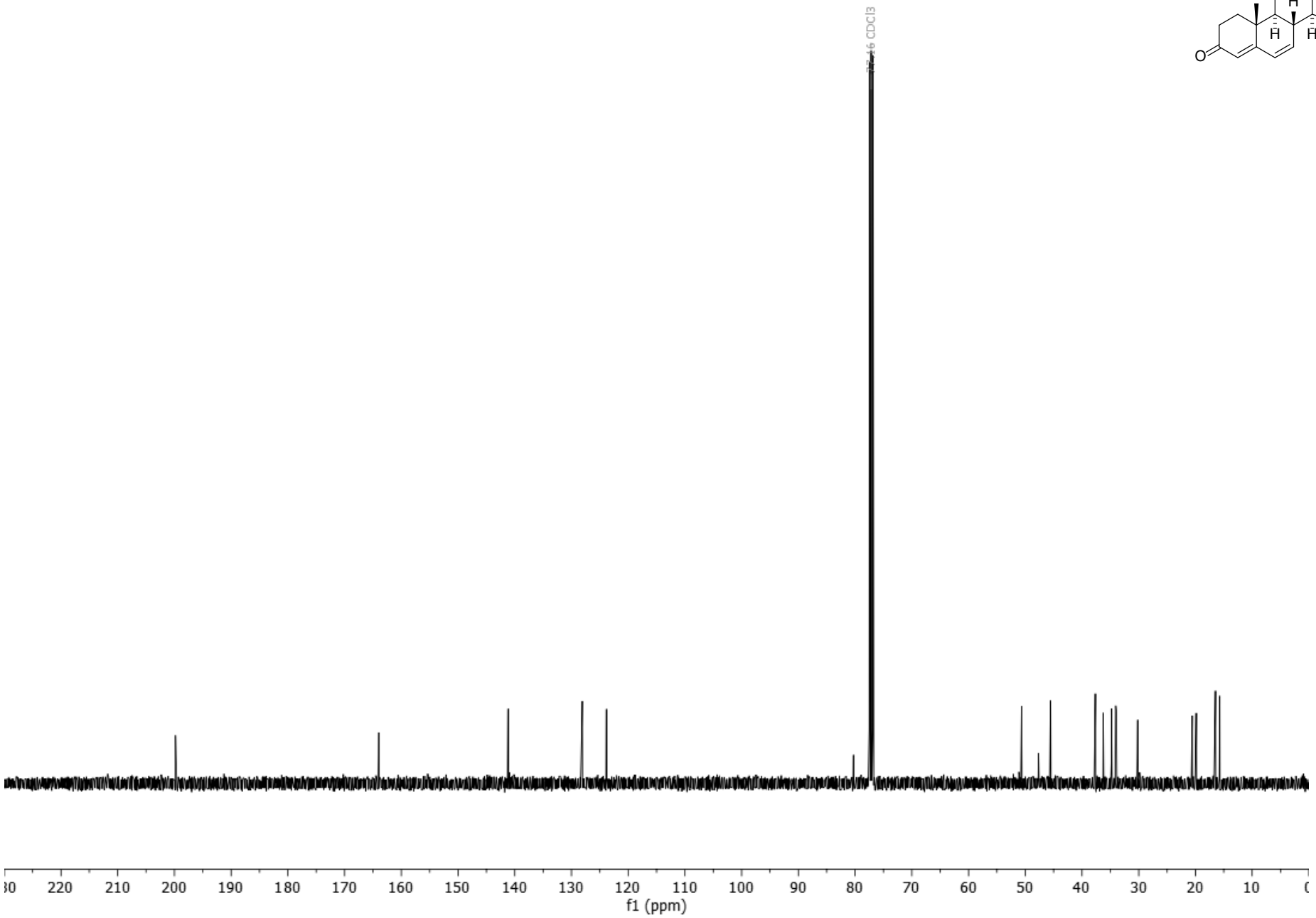
16α,17α-Dihydroxy-17β-methylandrosta-4,6-dien-3-one – ^{1}H NMR (400 MHz, $CDCl_3$)



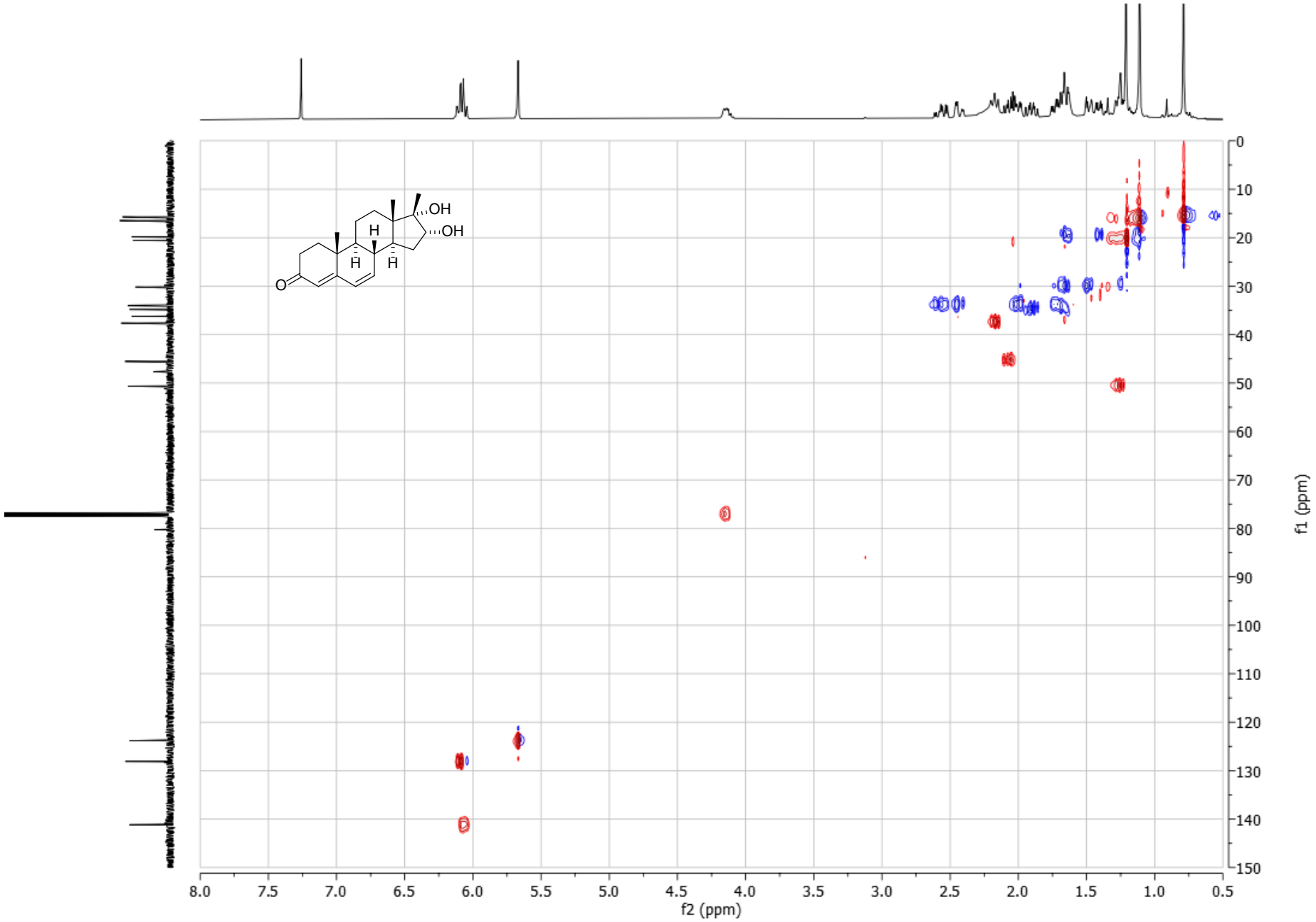


16α,17α-Dihydroxy-17β-methylandrosta-4,6-dien-3-one – ^{13}C NMR (101 MHz, $CDCl_3$)

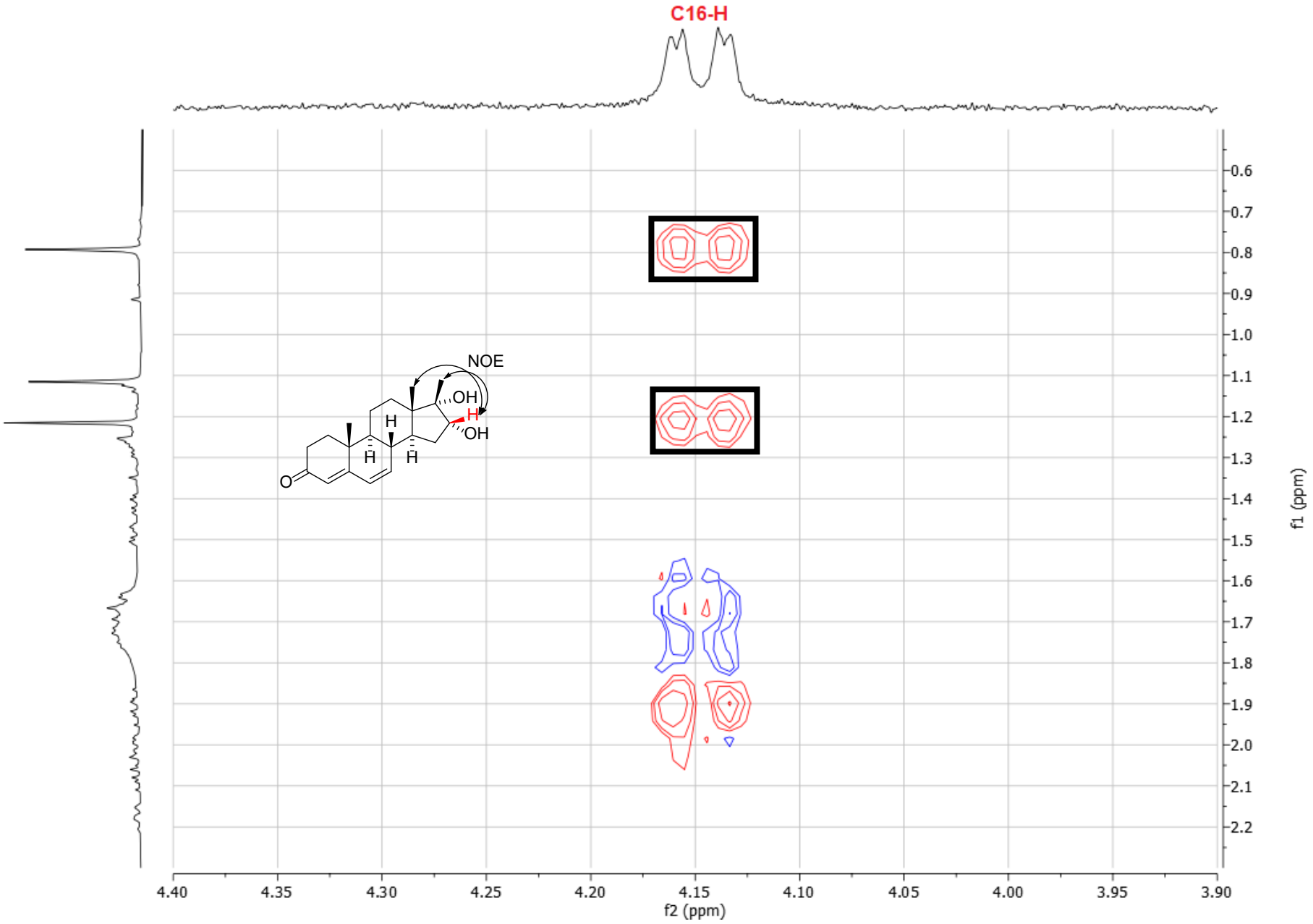




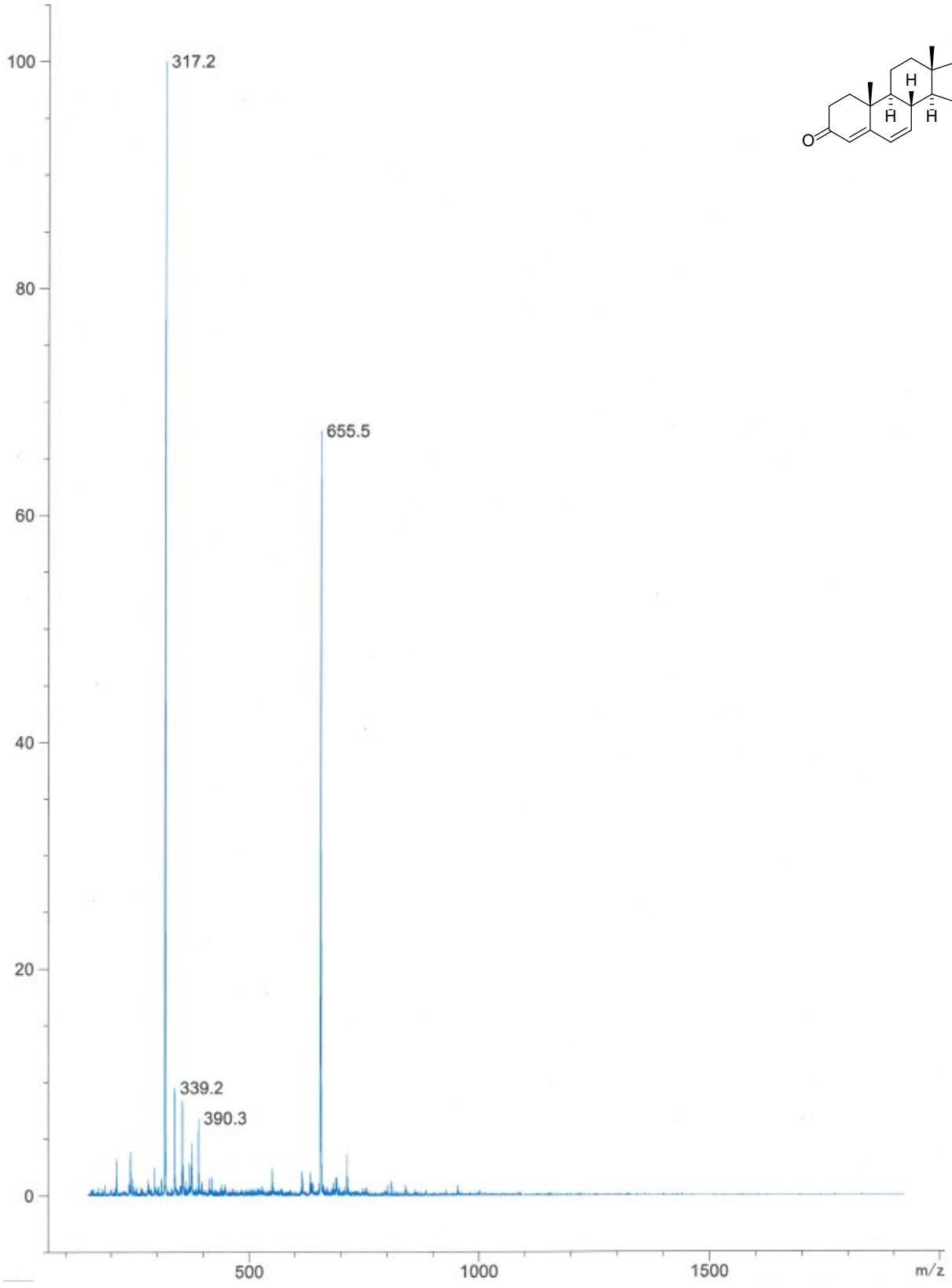
16α,17α-Dihydroxy-17β-methylandrosta-4,6-dien-3-one – ^{1}H-^{13}C HSQC (400 MHz, $CDCl_3$)



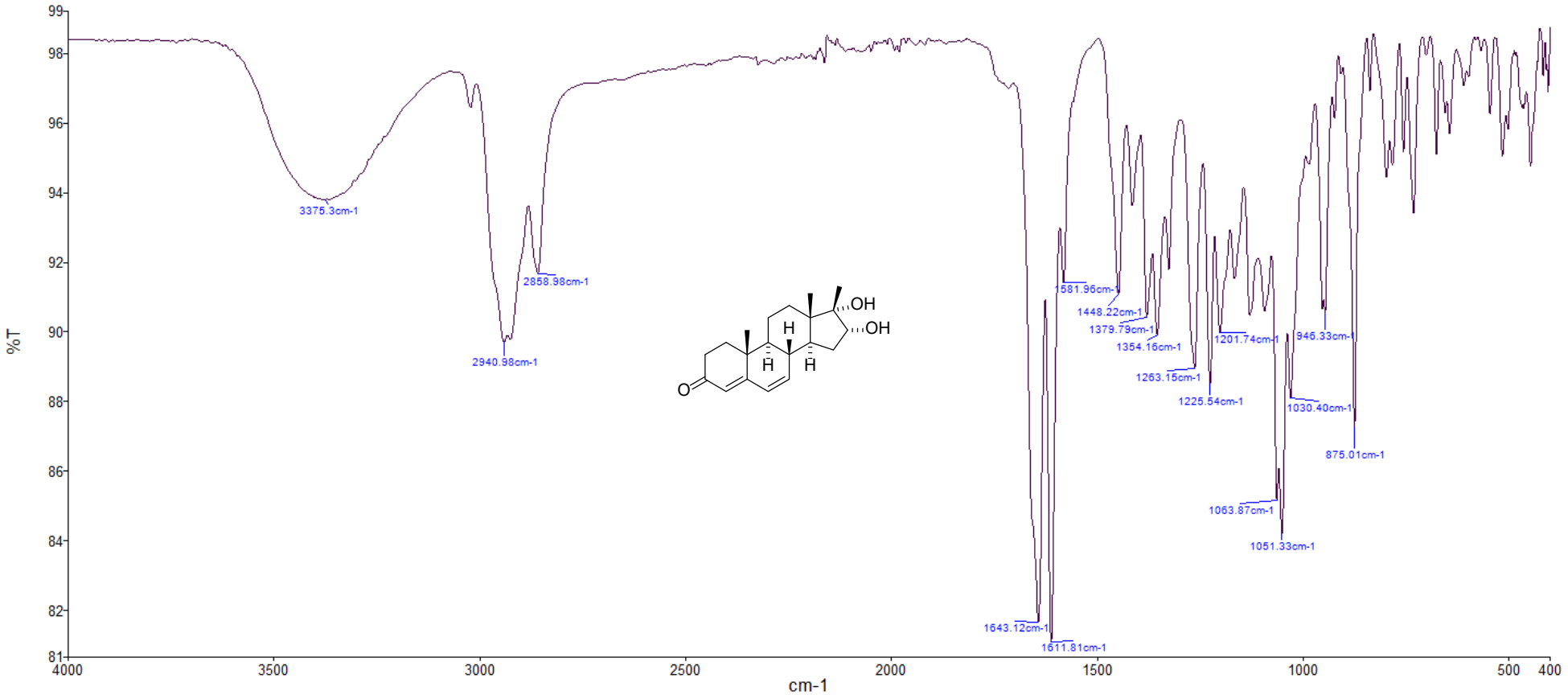
16α,17α-Dihydroxy-17β-methylandrosta-4,6-dien-3-one – ^{1}H-^{1}H NOE (400 MHz, $CDCl_3$)



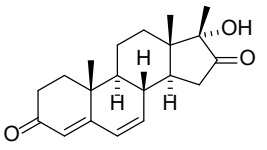
16α,17α-Dihydroxy-17β-methylandrosta-4,6-dien-3-one – LRMS (+ESI)

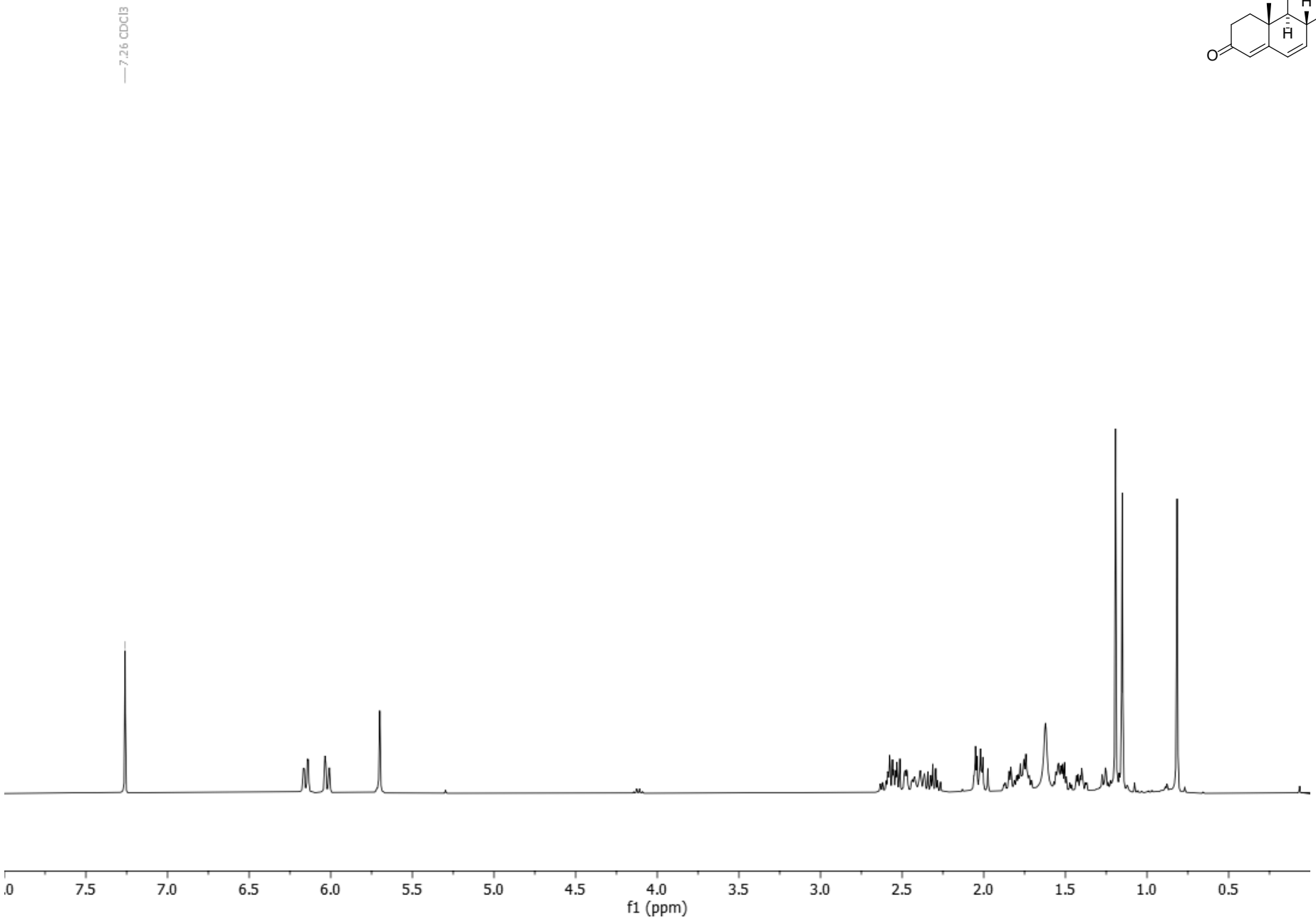


16α,17α-Dihydroxy-17β-methylandrosta-4,6-dien-3-one – IR (neat)

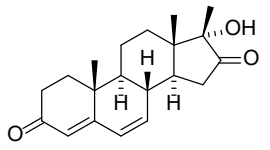


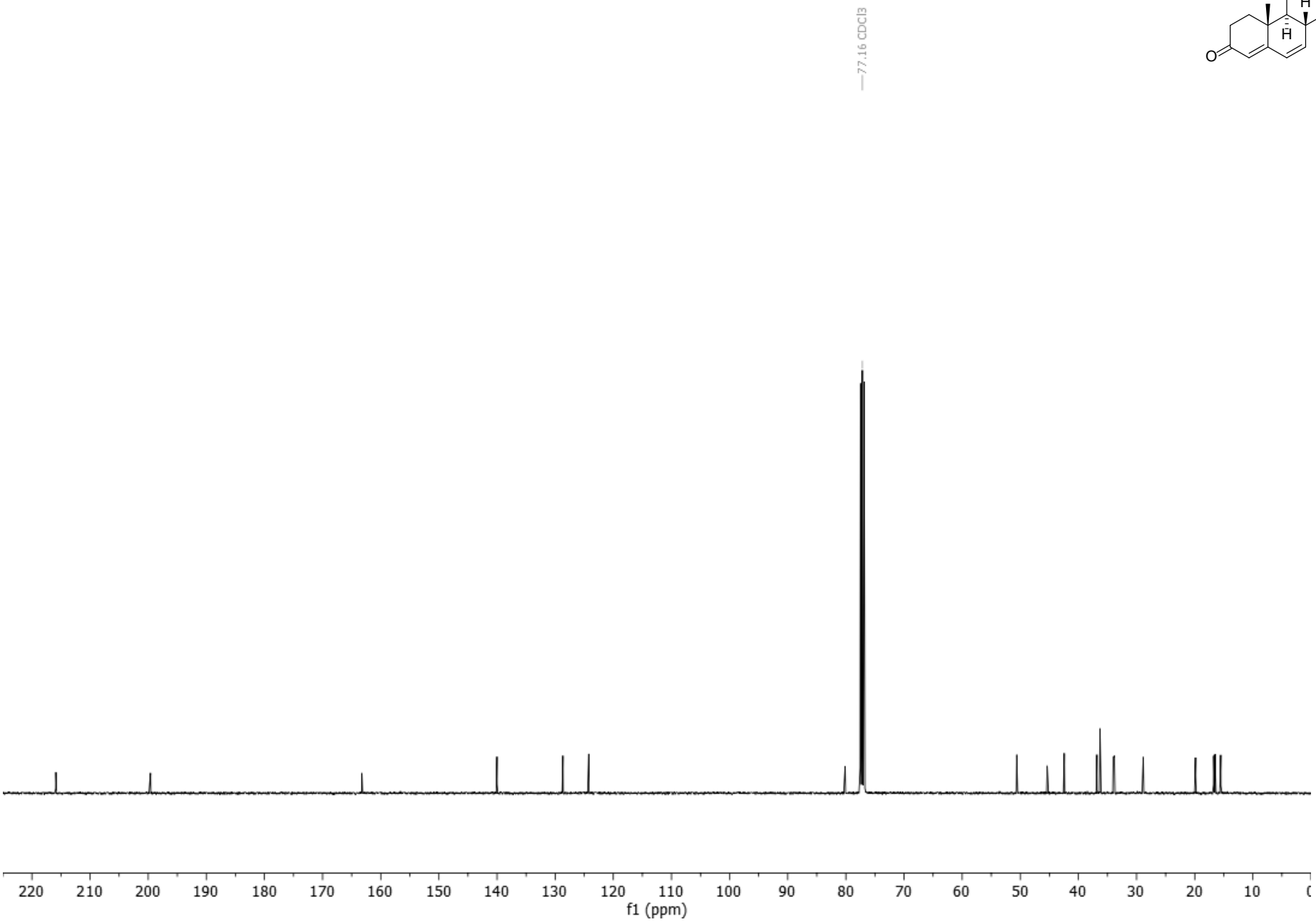
17α-Hydroxy-17β-methylandrosta-4,6-diene-3,16-dione – ^{1}H NMR (400 MHz, $CDCl_3$)



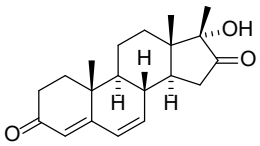


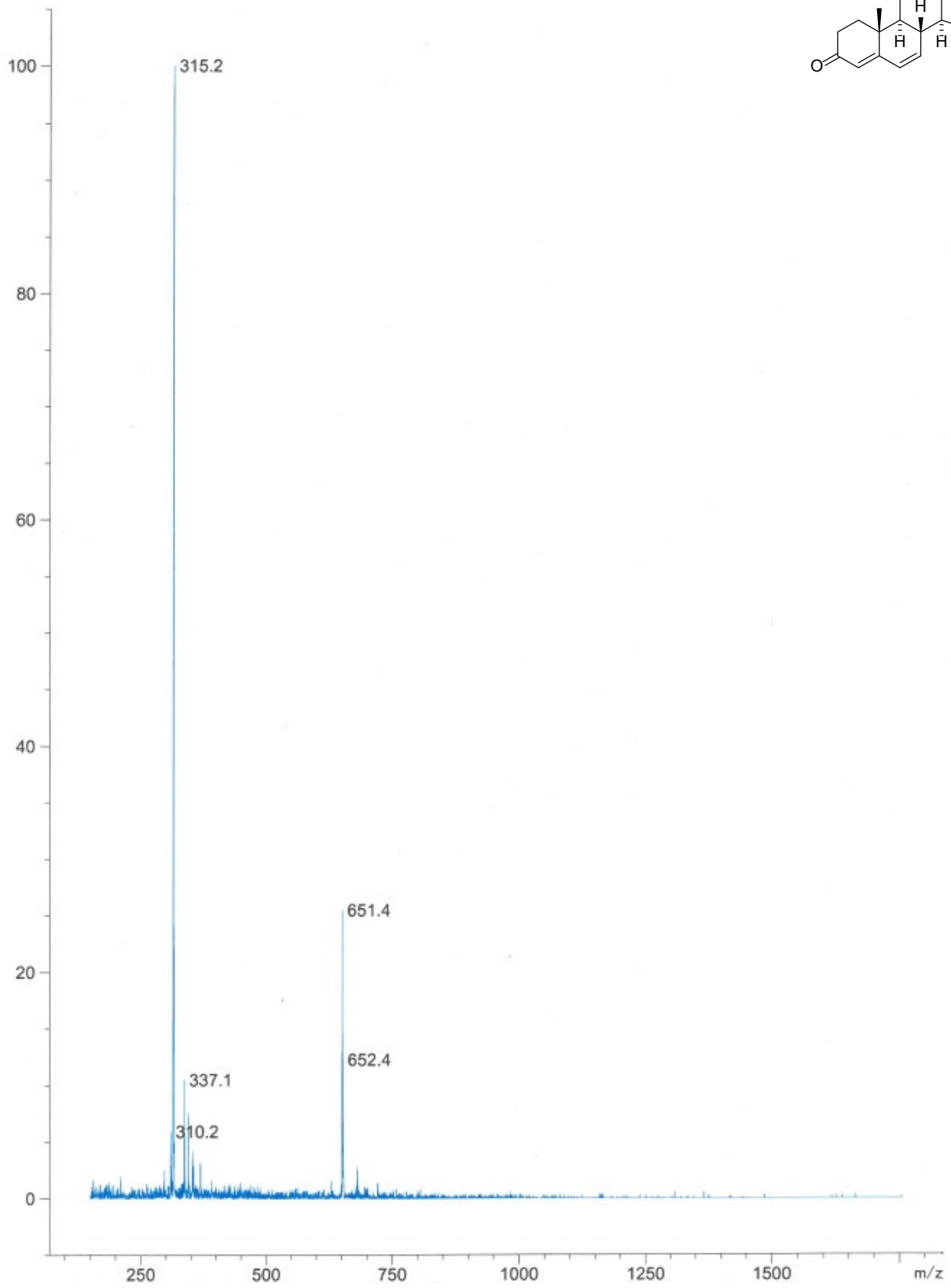
17α-Hydroxy-17β-methylandrosta-4,6-diene-3,16-dione – ^{13}C NMR (101 MHz, $CDCl_3$)



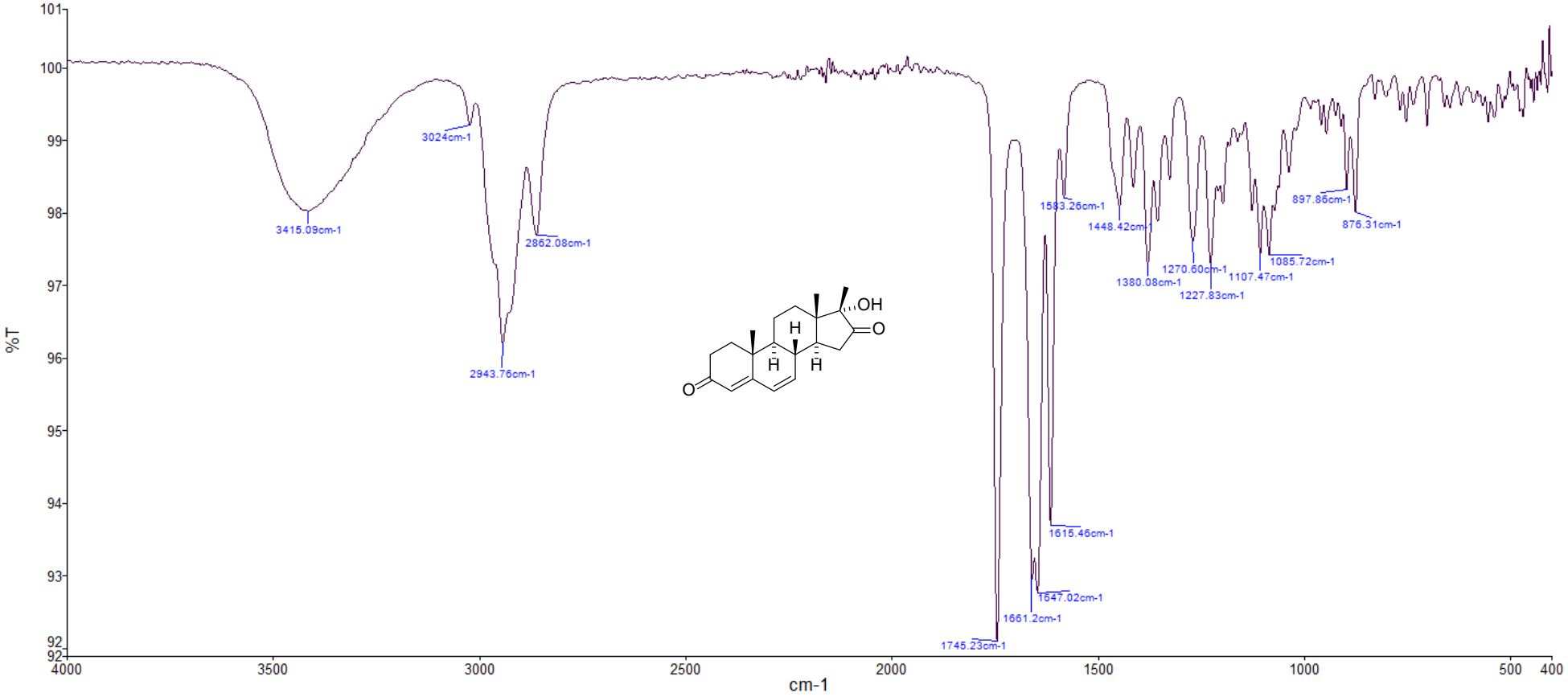


17α-Hydroxy-17β-methylandrosta-4,6-diene-3,16-dione – LRMS (+ESI)





17α-Hydroxy-17β-methylandrosta-4,6-diene-3,16-dione – IR (neat)



Summary of Crystallographic Data

Compound	**16-keto-epiJW**
Formula	$C_{20}H_{26}O_3$
$D_{calc.}$/ g cm^{-3}	1.261
μ/mm^{-1}	0.659
Formula Weight	314.41
Colour	colourless
Shape	needle
Size/mm^3	0.18×0.10×0.05
T/K	150.00(10)
Crystal System	orthorhombic
Flack Parameter	0.04(5)
Hooft Parameter	0.05(5)
Space Group	$P2_12_12_1$
a/Å	6.71350(10)
b/Å	11.4629(2)
c/Å	21.5142(2)
α/°	90
β/°	90
γ/°	90
V/Å^3	1655.65(4)
Z	4
Z'	1
Wavelength/Å	1.54184
Radiation type	Cu K$_\alpha$
Θ_{min}/°	4.110
Θ_{max}/°	73.170
Measured Refl's.	11762
Indep't Refl's	3310
Refl's I≥2 σ(I)	3226
R_{int}	0.0160
Parameters	215
Restraints	0
Largest Peak	0.153
Deepest Hole	-0.126
GooF	1.070
wR_2 (all data)	0.0719
wR_2	0.0712
R_1 (all data)	0.0272
R_1	0.0263

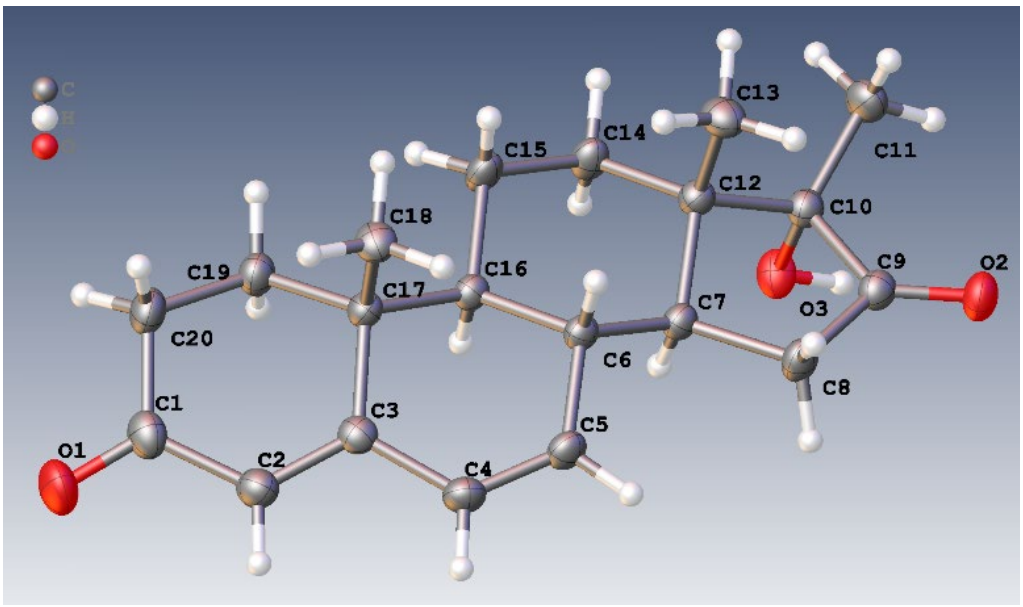


Figure S4. Molecular structure of **16-keto-epiJW** (atomic displacement parameters shown at 50% probability level). Molecules are associated into H-bonded strands in the solid state (omitted for clarity).

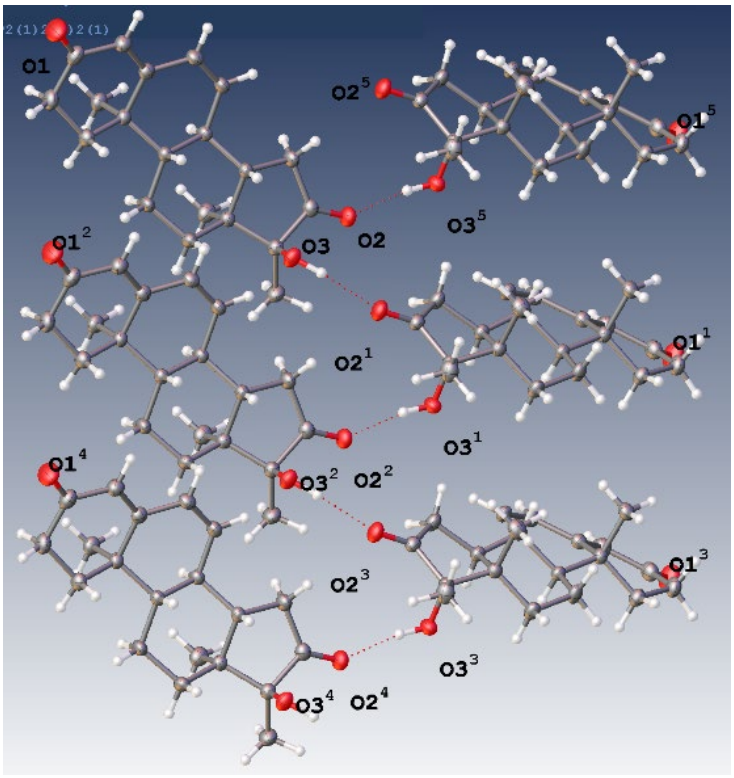


Figure S5. Molecular structure of **16-keto-epiJW** showing association into H-bonded strands in the solid state (atomic displacement parameters shown at 50% probability level). Symmetry operator [1] denotes ½+*x*, ½-*y*, 2-*z*, symmetry operator [2] denotes 1+*x*, *y*, *z*, symmetry operator [3] denotes 3/2+*x*, ½-*y*, 2-*z*, symmetry operator [4] denotes 2+*x*, *y*, *z*, Symmetry operator [5] -½+*x*, ½-*y*, 2-*z*.