

Supplemental Information

Development of a Chemoenzymatic Process for Dehydroepiandrosterone

Acetate Synthesis

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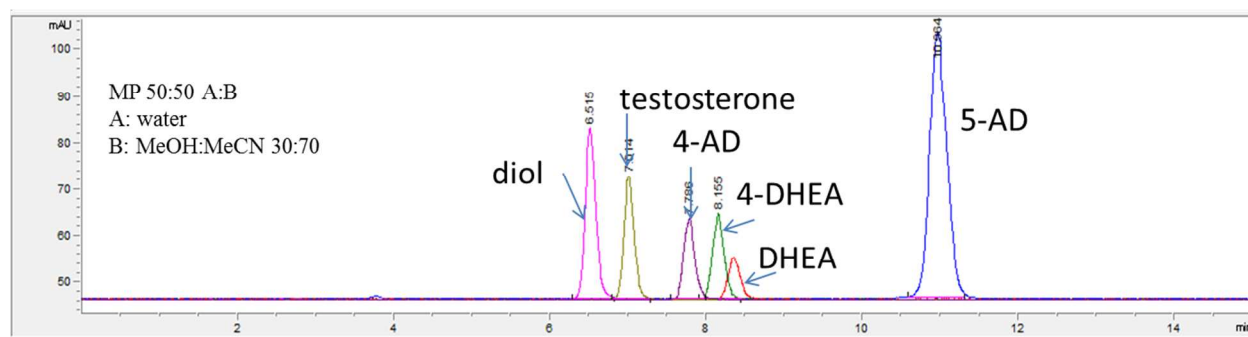
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Analytical methods

Method A: The isomerisation and the biotransformations were monitored by HPLC: ELSD and UV ($\lambda = 200$ nm) detectors with Luna C18 column 150×4.6 mm, column temp. 50°C , flow 1 mL/min, MP 50:50 A:B, A: water, B: MeOH:MeCN 30:70, inj. vol: 10 μL . The progress of the biotransformation reactions was also monitored by the base consumption as well as by ^1H NMR spectroscopy. LC-MS data was collected using ESI(+) ion detector and HPLC method A, where 0.1% v/v was added to water.

Figure S1 Overlaid chromatograms of steroid derivatives analysed by HPLC using Luna C18 column (Method A)

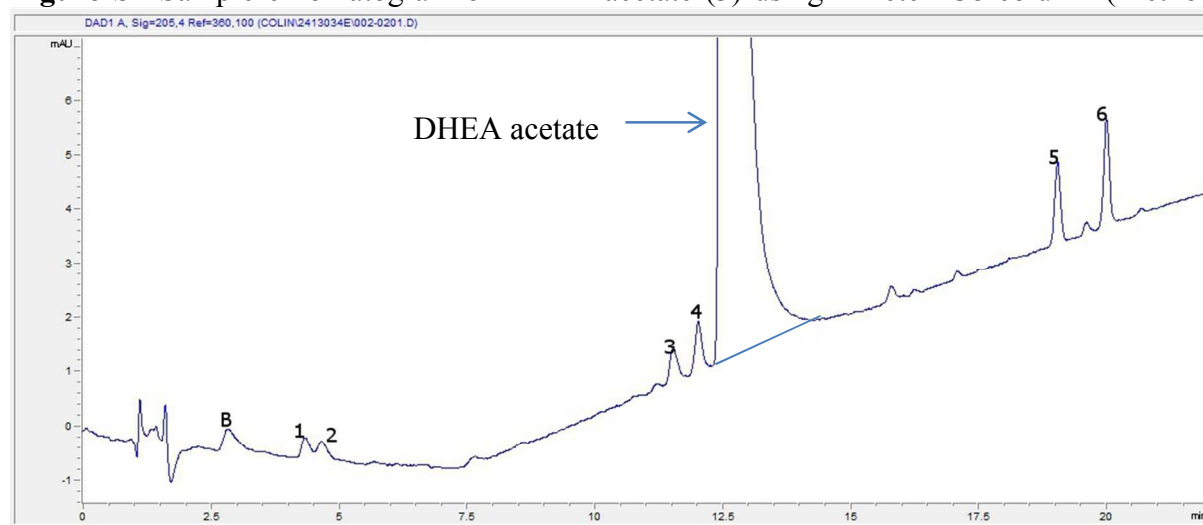


Method B: The purity of the DHEA acetate (**3**) was determined by HPLC **Method B** using UV detector ($\lambda = 205$ nm) and Kinetex C8 column $2.6 \mu\text{m}$ 100\AA $150 \times 4.6\text{mm}$, flow 1 mL/min, column temp. 40°C , using mobile phase gradient (A = 80:20 water:MeCN, B = MeCN, see **Table S1**), inj. vol: 10 μL . Retention time (**3**) – 12.5 min.

Table S1. Mobile phase gradient for HPLC analysis of DHEA acetate (**3**) using Kinetex C8 column (Method B).

Time (min)	0	5	20	25	35	50	55	60
Mobile phase A	65	65	35	10	5	5	65	65
Mobile phase B	35	35	65	90	95	95	35	35

Figure S2 Sample chromatogram of DHEA acetate (**3**) using Kinetex C8 column (Method B)



Main peak – DHEA acetate (**3**); 1-6 impurities (undisclosed).

Experimental - Biology

Construct

Synthetic ketoreductase was codon optimised for heterologous expression in *E. coli* and provided cloned into pET26b vector at NdeI/XhoI sites by GeneArt, Life Technologies. Standard protocols were used to transform plasmids into *E. coli* strains

Maintaining Strains and DNA stocks

Cloning strains of *E. coli* included Top 10, and expression *E. coli* strains included BL21(DE3) and BL21(DE3)T1. Typically strains were prepared from single colonies. Constructs in *E. coli* were selectively maintained using kanamycin at 50 µg/ml for LB agar plates or 30 µg/ml for LB liquid broths. Cultures were grown at 37 °C for 16 hours on selective LB plates, or in 50 ml selective LB liquid cultures in 250 ml baffled flasks at 37 °C and shaken at 250 rpm. Strains were stored in 10 % (V/V) glycerol at -80 °C.

DNA sequencing was carried out by Source Bioscience Cambridge. A typical sample concentration of 100 ng/µl was required.

Expression from pET26b ketoreductase constructs

The pET26b in *E. coli* BL21(DE3) were grown at 37 °C, 250 rpm overnight in 10 ml LB Kan (30 µg/ml) from glycerol stocks. The overnight broths were used as a 1% seed to inoculate 50 ml LB Kan (30 µg/ml) in disposable baffled flasks and grown at 37 °C, 250 rpm until the cells reached OD₆₀₀ 0.6 -1.0. 1 ml of broth was removed (t=0 sample) and the remaining 49 ml induced with IPTG to give a final concentration of 1 mM. The cells were grown at 25 °C, 250 rpm for a further 24 hours at which point they were harvested. The OD₆₀₀ was measured and volumes adjusted to normalise for OD₆₀₀ = 1.0. Cells were pelleted by centrifugation at 14,000 rpm for 5 min and the supernatant discarded. Cell pellets were frozen. Samples were lysed by the addition of 100 µL of Bugbuster (Merck), shaken at 800 rpm for 20 minutes and cell debris pelleted by centrifugation

at 14,000 rpm for 5 minutes. Cell free extract (cfe) was removed to a clean microcentrifuge tube. The pellet was re-suspended with another 100 µl of Bugbuster, shaken for 5 minutes before centrifugation at 14,000 rpm for 5 minutes. The supernatant was discarded and the pellet resuspended in 100 µl of water to give the pellet sample. The t=0 CFE extracts were prepared in a similar manner except the cell pellets were resuspended in 50 µl of Bugbuster. The level of enzyme expression was evaluated by SDS PAGE and ketoreductase activity assays.

Fermentation and downstream processing of 2nd generation enzyme.

The fermentation was performed in a 20 L Biostat Cplus SIP bioreactor. A glucose fed-batch fermentation method was used that had been specifically developed for heterologous protein production using *E. coli* BL21. Two 1 ml glycerol stocks of *E. coli* BL21 (DE3) expressing the relevant enzyme was thawed from -80 °C storage and inoculated into a 1L Erlenmeyer flask containing 200 mL vegetable peptone broth, 20 g/L glucose and 15 µg/mL kanamycin and cultivated for 6-7 hours at 37 °C, 250 rpm in a rotary shaker incubator. This preculture prepared in duplicate was used to seed a 20L fermentation vessel operating at 50% volume ensuring a starting optical density of 0.3 OD₆₀₀. The bioreactor was run with a glucose fed method; culturing the cells in a sugar free semi-defined base medium, controlled at pH 7.2, 30 °C, 20% dissolved oxygen and feeding at a pre-defined linear rate with base medium containing 400 g/L glucose from the point of inoculation. The culture was then induced when the cell population reached 100 OD₆₀₀ by the addition of 0.5mM IPTG final concentration based on initial fermenter volume and reduction in culture temperature to 25 °C. Following 24 hours elapsed fermentation time the feed rate was reduced to a pre-defined constant rate until cell harvest at 41-45 hours. The final cell population reached 176 OD₆₀₀, 84 g/L dry cell weight respectively.

At the end of fermentation the culture (17 L) was cooled to <12 °C, and a concentrate of 1 L of potassium phosphate buffer pH 6.3 was added to the culture, achieving a pH of 6.5. This material was then lysed by high pressure homogenization (Niro Soavi Pony) at 14,000 psi (single pass). Polyethyleneimine (PEI) from a 5% stock solution in water was added to the lysed cells (lysate) to a final working PEI amount of 0.35% while maintaining a pH of approximately 6.3 with concentrated HCl during addition, followed by gentle stirring for 1 hour. The resulting precipitate was removed by centrifugation (in multiple 1 L bottles at 15,900 x g for 20 minutes). The supernatant obtained was clarified by 0.65 µm filtration, and then concentrated by 10 kDa ultrafiltration to less than 5 L final volume. This concentrated enzyme solution was diafiltered against 5 volumes of 50 mM potassium phosphate buffer pH 6.3, and then 'sterile' filtered through a 0.2 µm filter to give 4.4 L of final liquid enzyme. Activity was 39 U/mL.

Fermentation and downstream processing of 3rd generation enzyme.

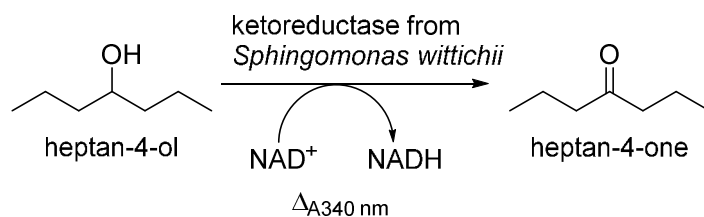
22 L fermentation of the *E. coli* strain expressing the ketoreductase was performed as described for the 2nd generation enzyme. At the end of fermentation the culture was cooled to <12°C, and a concentrate of 1 L of potassium phosphate buffer pH 6.3 was added to the culture, achieving a pH of 6.45. This material was then lysed by high pressure homogenization (Niro Soavi Pony) at 14,000 psi (single pass). Polyethyleneimine (PEI) from a 5% stock solution in water was added to the lysed cells (lysate) to a final working PEI amount of 0.6% while maintaining a pH of approximately 6.3 with concentrated HCl during addition, followed by gentle stirring for 1 hour. The resulting precipitate was removed by centrifugation (in multiple 1 L bottles at 15,900 × g for 20 minutes). The supernatant obtained was clarified by 0.65 µm filtration, and then concentrated

by 10 kDa ultrafiltration to less than 5 L final volume. This concentrated enzyme solution was ‘sterile’ filtered through a 0.2 µm filter to give 4.7 L of final liquid enzyme. This material was freeze dried to give a powdered form of the enzyme. Activity was 450 U/g.

Enzyme activity assay

The enzyme activity was determined using 4-heptanol as substrate, by following its oxidation spectrophotometrically at 340 nm, measuring the conversion of the NAD⁺ cofactor to NADH by monitoring an increase in absorbance at 340 nm.

Scheme S1: The principal of activity assay of ketoreductase from *Sphingomonas wittichii*



One unit of activity is defined as sufficient enzyme to catalyse the oxidation of 1 µmol/min of 4-heptanol to 4-heptanone.

Procedure: Ketoreductase powder was rehydrated in 0.1 M Tris HCl pH 8.5 to give a 10 mg/ml concentration (30 min, RT, roller mixer). Serial dilutions (1:1) of this were carried out with 0.1 M Tris HCl pH 8.5 buffer as diluent.

To a stock solution of 4-heptanol (450 µL) in DMSO (2.25 mL) was added Tris buffer (12.3 mL) containing NAD⁺ (187.5 mg). This cocktail was pre-incubated at 37 °C for 30 min.

Enzyme solutions (0.1 mL, serial dilutions) were added to a spectrophotometric 96-well plate. The plate was incubated at 37 °C for 30 minutes, followed by addition of the substrate solution (0.1 mL). The change in absorbance was monitored using Tecan Sunrise UV Plate Reader Spectrophotometer for 5 min at 340 nm and 37 °C.

The activity was calculated using formula below. All the readings were the blank-corrected for absorbance change for each enzyme dilution.

$$\text{Enzyme Activity (Units/ml)} = \frac{A \times TV \times DF}{6.22 \times EV \times PL}$$

Where:

A – Absorbance change (Abs/min)

TV – Total Volume of reaction (cm³)

DF – Dilution Factor of Enzyme

6.22 – Extinction coefficient (ε) for NADH at 340 nm (6.22 mM⁻¹cm⁻¹)

EV – Enzyme Volume (cm³)

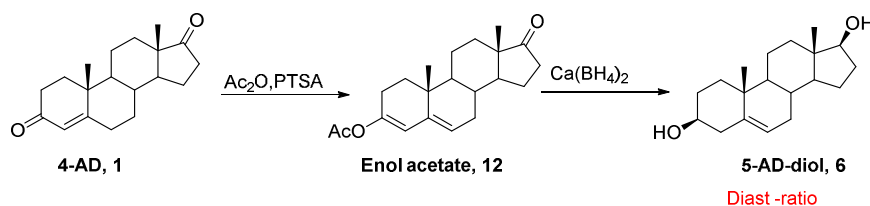
PL – Path Length

Synthesis of reference materials

5-Androsten-3,17-diol (6)

5-AD-diol (**6**) was obtained from 4-AD (**1**) in two steps *via* respective enol acetate (**12**) and $\text{Ca}(\text{BH}_4)_2$ -catalysed reduction (Scheme).

Scheme S2 Synthesis of 5-AD-diol (**6**) from 4-AD (**1**)



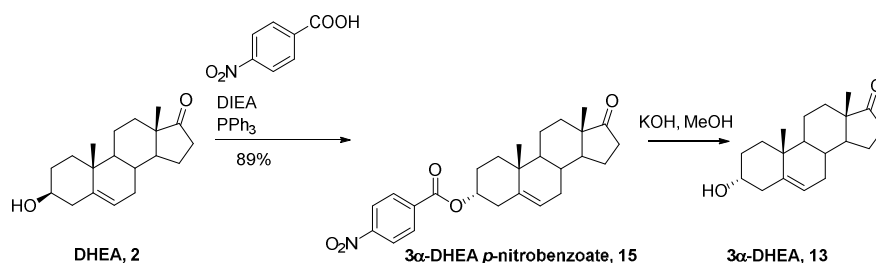
Stage-1: To a stirred solution of 4-Androstene-3,17-dione (40.0 g, 0.14 mol) and acetic anhydride (1.5 L) was added *p*-toluene sulfonic acid monohydrate (39.8 g, 0.209 mol). The mixture was heated in an oil bath at 65°C for 1 hr 30 minutes. The reaction was complete as indicated by TLC. The reaction mixture was cooled to room temperature, gradually poured into water (8.0 L) and stirred for 30 min. The precipitated solid was filtered and washed with water. The wet solid was dissolved in dichloromethane (400 mL) and dried over anhydrous sodium sulphate. Dichloromethane solution was filtered to remove sodium sulphate and then distilled under reduced pressure. The residue was crystallized from hexanes (100 mL) to give solid enol acetate **12** (35.0 g, 76.0%). ^1H NMR (400 MHz, CDCl_3): δ 5.71 – 5.70 (d, $J = 4.0$ Hz, 1H), 5.43 – 5.41 (t, $J = 4.0$ Hz, 1H), 2.51 – 2.41 (m, 2H), 2.33 – 2.26 (m, 1H), 2.18 – 1.71 (m, 10H), 1.62 – 1.43 (m, 3H), 1.38 – 1.28 (m, 3H), 1.25 – 1.09 (m, 4H), 0.91 (s, 3H).

Stage-2: A calcium borohydride solution was prepared by addition of a solution of sodium borohydride (2.24 g, 0.06 mol) in ethanol (80 mL) to a solution of anhydrous calcium chloride (3.88 g, 0.035 mol) in ethanol (80 mL) at -15°C under nitrogen. A solution of enol acetate (5.0 g, 0.015 mol) in dichloromethane (31.5 mL) was added with vigorous stirring to the calcium chloride solution at -15°C. The mixture was stirred at that temperature for 3.0 h, then warmed to room temperature and stirred for 2.0 h (monitored by TLC). The reaction mixture was concentrated under reduced pressure until a slurry was obtained. The semisolid upon cooling with ice water was acidified with a cold aqueous HCl solution (0.5 M, 100 mL). The solution was stirred for 1 h and the precipitate was filtered and washed with water (50 mL). The wet product was dissolved in dichloromethane (100 mL), dried over sodium sulphate and the solvent removed under reduced pressure to give 5-AD-diol **6** (3.80 g, 80.0%) as a colorless solid. ^1H NMR (400 MHz, d_6 -DMSO): δ 5.26 – 5.25 (d, $J = 4.0$ Hz, 1H), 4.59 – 4.57 (d, $J = 8.0$ Hz, 1H), 4.43 – 4.41 (d, $J = 8.0$ Hz, 1H), 3.45 – 3.39 (m, 1H), 3.28 – 3.28 (m, 1H), 2.16 – 2.05 (m, 2H), 1.93 – 1.65 (m, 5H), 1.52 – 1.30 (m, 7H), 1.21 – 1.11 (m, 1H), 0.99 – 0.81 (m, 7H), 0.64 (s, 3H).

5-Androsten-3 α -ol-17-one (3 α -DHEA (**13**))

5-androsten-3 α -ol-17-one (**13**) was synthesised from DHEA (**2**) using a Mitsunobu reaction sequence according to a previously reported method,³ as depicted in Scheme S3.

Scheme S3 Synthesis of 3 α -DHEA (**13**) from 3 β -DHEA (**2**)



DHEA (**2**, 607 mg, 2.104 mmol), *p*-nitrobenzoic acid (398 mg, 2.373 mmol) and PPh₃ (608 mg, 2.318 mmol) were dissolved in THF_{anh.} (20 mL) under nitrogen and cooled using an ice bath to 5 °C. Diisopropyl azodicarboxylate (500 μ L, 2.412 mmol) was added dropwise over 20 min. The mixture was warmed up to ambient temperature and stirred overnight. The reaction mixture was evaporated to dryness and re-dissolved in EtOAc (50 mL), washed with 18% Na₂CO₃, 1 M HCl and brine, then dried over MgSO₄ and evaporated to give a crude reaction mixture as a pale yellow solid (1.3 g). The product was recrystallised from EtOAc/MeOH to give white crystals of 3 α -DHEA nitrobenzoate (**15**, 180 mg, 89% yield). ¹H NMR (400 MHz, δ) 8.25 (d, 2H), 8.15 (d, 2H), 5.37 (d, 1H), 5.30 (t, 1H), 2.61-2.66 (m, 1H), 2.36-2.46 (m, 2H), 0.89-2.20 (m, 16H), 1.11 (s, 3H), 0.91 (s, 3H) – NMR agrees with literature.³ 3 α -DHEA nitrobenzoate (**15**, 170 mg) was dissolved in MeOH (10 mL) and water (2 mL) and solid KOH (100 mg) was added. The mixture was sonicated in a vial for 1h after which time the suspension turned into a clear solution. The solvent was evaporated under vacuum and 10 mL of water was added to precipitate the product. The white crystals were isolated by filtration to give 5-androsten-3 α -ol-17-one (3 α -DHEA, **13**, 85 mg, 72%). ¹H NMR (CDCl₃, 400 MHz) δ 5.45 (m, 1H), 5.04 (quintet, 1H), 2.56-2.61 (m, 1H), 2.46 (dd, 1H), 1.10-2.20 (m, 18H), 1.04 (s, 3H), 0.84 (s, 3H). The spectrum agreed with literature³ and with a reference sample obtained from Bujno Synthesis, Poland.

Characterisation of the oxygenated impurities

The impurities **19-23** were formed in large quantities during a biotransformation performed under the forcing conditions below:

In a Multimax reactor vessel 5-AD (**5**, 400 mg, 94% pure, 2% 4-AD, 4% oxygenated impurities **19-23**) was pre-dissolved in MTBE (20 mL). Buffer cocktail containing ketoreductase (3 mg, 1% w/w, 17 U/mg), glucose (460 mg, 2.56 mmol, 1.8 eq), NAD⁺ and NAD(P)⁺ (7 mg each) and glucose dehydrogenase (CDX-901, 3 mg, 66 U/mg) in phosphate buffer (50 mM, 20 mL) was added and the reaction mixture stirred at 750 rpm for 65h at 45 °C. The reaction pH of 6.5 was maintained by autotitration with 2M NaOH. The reaction mixture was extracted into MTBE (2 × 50 mL), the organic phase was dried over magnesium sulphate and evaporated to give a crude product (400 mg). The sample was analysed by HPLC using a Luna C18 column and it contained 21% of impurities **22** and **23** (*m/z* = 303); 33% of impurities **20-21** (*m/z* = 319); 16% of impurity **19** (*m/z* = 301); 3% of 4-AD (**1**), 6% of DHEA (**4**) and 17% of unreacted 5-AD – see Figure S3. 60 mg sample was loaded onto preparative TLC (Whatman PTLX plate, 20 cm×20 cm, 10000 μm, cat # 4859-840) and developed in heptane:EtOAc 6:4 (repeated 2×) to five fractions, which eluted in the order (top→bottom) as shown in Table S2.

Table S2 Preparative TLC results

Fraction	Amount	Compound
Fraction 1	20 mg	1+4
Fraction 2	9 mg	19
Fraction 3	9 mg	20^{maj}+21^{min}
Fraction 4	8 mg	22
Fraction 5	4 mg	23

Analytical data for compounds 19-23

Fraction 2: androst-4-ene-3,6,17-trione (19) – LC-MS (+) *m/z* 301 [M+H]⁺; ¹H NMR (700 MHz, Chloroform-*d*) δ 6.22 (d, *J* = 0.8 Hz, 1H), 2.84 – 2.77 (m, 1H), 2.60 – 2.45 (m, 3H), 2.22 – 2.07 (m, 4H), 2.03 – 1.78 (m, 4H), 1.70 – 1.33 (m, 5H), 1.21 (s, 3H), 0.94 (s, 3H). ¹H NMR agrees with literature.⁴

Fraction 3: 6β-perhydroxyandrost-4-ene-3,17-dione (20) – LC-MS (+) *m/z* 319 [M+H]⁺; ¹H NMR (700 MHz, Chloroform-*d*) δ 8.22 (s, 1H), 5.91 (s, 1H), 4.49 (dd, *J* = 3.7, 2.5 Hz, 1H), 2.59 – 2.39 (m, 3H), 2.28 – 2.23 (m, 1H), 2.13 – 2.10 (m, 1H), 2.07 (ddd, *J* = 11.3, 5.1, 2.5 Hz, 1H), 2.01 – 1.91 (m, 2H), 1.88 (ddd, *J* = 13.1, 4.1, 2.8 Hz, 1H), 1.75 (td, *J* = 14.5, 4.4 Hz, 1H), 1.69 (ddd, *J* = 13.7, 6.9, 4.1 Hz, 1H), 1.60 – 1.55 (m, 1H), 1.49 (td, *J* = 13.1, 4.2 Hz, 1H), 1.40 – 1.35 (m, 1H), 1.36 (s, 3H), 1.31 – 1.19 (m, 2H), 0.99 (ddd, *J* = 12.2, 10.9, 4.1 Hz, 1H), 0.92 (s, 3H).

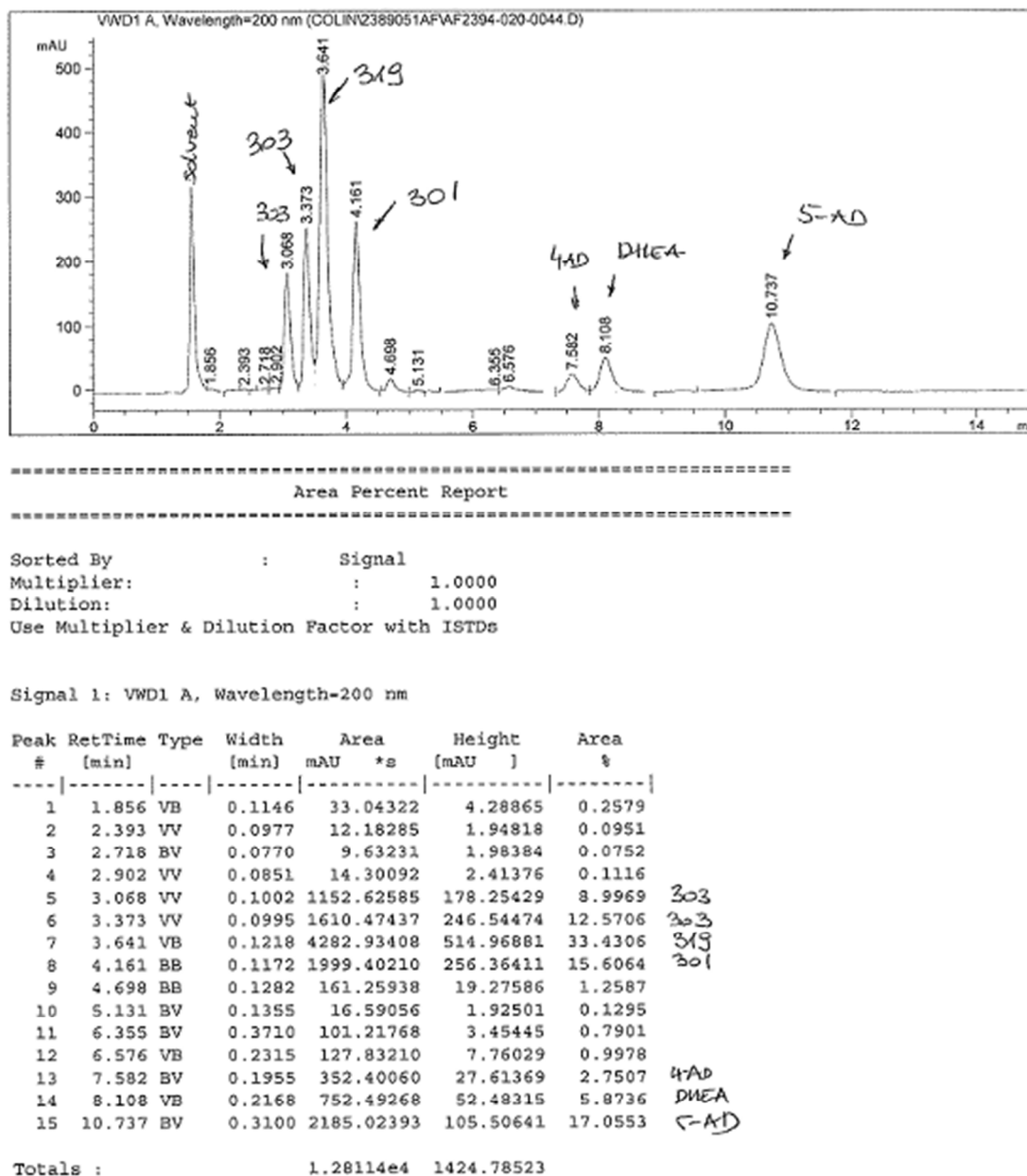
Proposed 6α-perhydroxy-androst-4-ene-3,17-dione (21) – Formation was observed in the NMR tube, during the analysis of peroxide **20**, LC-MS (+) *m/z* 319 [M+H]⁺; diagnostic peaks

observed in ^1H NMR (700 MHz, δ) 6.21 (d, $J = 1.0$ Hz, 1H), 4.65 (ddd, $J = 12.5, 5.2, 1.8$ Hz, 1H), further assignment was not possible due to spectral overlap with the main species 6 β -perhydroxy- Δ^4 -androstene-3,17-dione (**20**).

Fraction 4: 6 β -hydroxyandrost-4-ene-3,17-dione (22) – LC-MS (+) m/z 303 $[\text{M}+\text{H}]^+$; ^1H NMR (700 MHz, Chloroform- d) δ 5.84 (s, 1H), 4.41 (t, $J = 2.8$ Hz, 1H), 2.54 – 2.47 (m, 2H), 2.41 (dt, $J = 17.3, 3.2$ Hz, 1H), 2.20 – 2.05 (m, 4H), 2.00 (ddd, $J = 14.3, 8.3, 6.1$ Hz, 1H), 1.89 (dt, $J = 13.1, 3.4$ Hz, 1H), 1.77 – 1.58 (m, 3H), 1.52 (qd, $J = 13.4, 4.2$ Hz, 1H), 1.41 (s, 3H), 1.36 – 1.25 (m, 3H), 0.98 (m, 1H), 0.95 (s, 3H). ^1H NMR agrees with literature.⁵

Fraction 5: 6 α -hydroxyandrost-4-ene-3,17-dione (23) – LC-MS (+) m/z 303 $[\text{M}+\text{H}]^+$; ^1H NMR (700 MHz, Chloroform- d) δ 6.20 (dd, $J = 1.8, 0.6$ Hz, 1H), 4.39 (ddd, $J = 12.2, 5.5, 1.9$ Hz, 1H), 2.50 (dd, $J = 19.5, 8.2$ Hz, 1H), 2.45 – 2.41 (m, 1H), 2.38 (dt, $J = 16.8, 4.1$ Hz, 1H), 2.30 (ddd, $J = 11.9, 5.5, 3.5$ Hz, 1H), 2.17 – 1.96 (m, 3H), 1.91 – 1.85 (m, 1H), 1.85 – 1.68 (m, 3H), 1.61 (ddd, $J = 12.5, 9.2, 3.3$ Hz, 1H), 1.45 (ddd, $J = 27.2, 13.9, 4.7$ Hz, 1H), 1.39 – 1.24 (m, 2H), 1.22 (s, 3H), 1.20 – 1.14 (m, 1H), 1.00 (ddd, $J = 12.4, 11.0, 4.1$ Hz, 1H), 0.93 (s, 3H). ^1H NMR agrees with literature.⁵

Figure S3 Chromatogram of bioreduction crude mixture after stirring for 65h at 45 °C, pH 6.5 (Method A)



The oxygenated impurities (fractions 2-5) characterised by MS (ESI-MS, (+)) and 700 and 500 MHz NMR (^1H , ^{13}C and homo/heteronuclear 2D NMR) and their structures **19-23** are presented in Figure S4.

Figure S4 Structures of oxygenated derivatives of androstenedione. In **blue** – diagnostic ^1H NMR peaks; in **red** – mass of the molecular ions observed by MS.

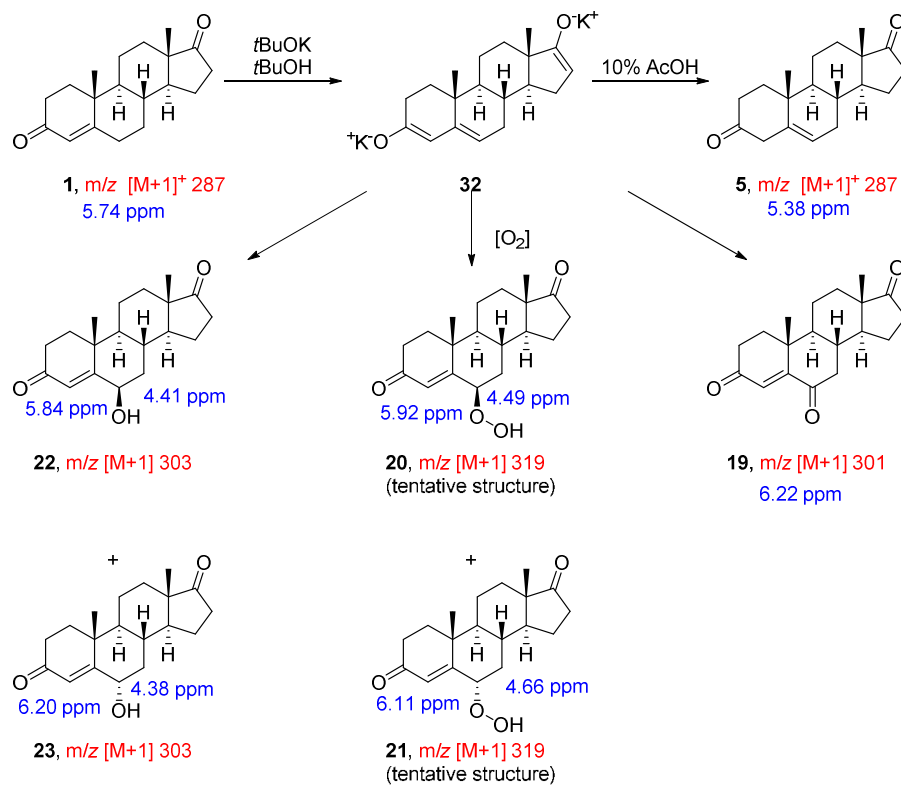
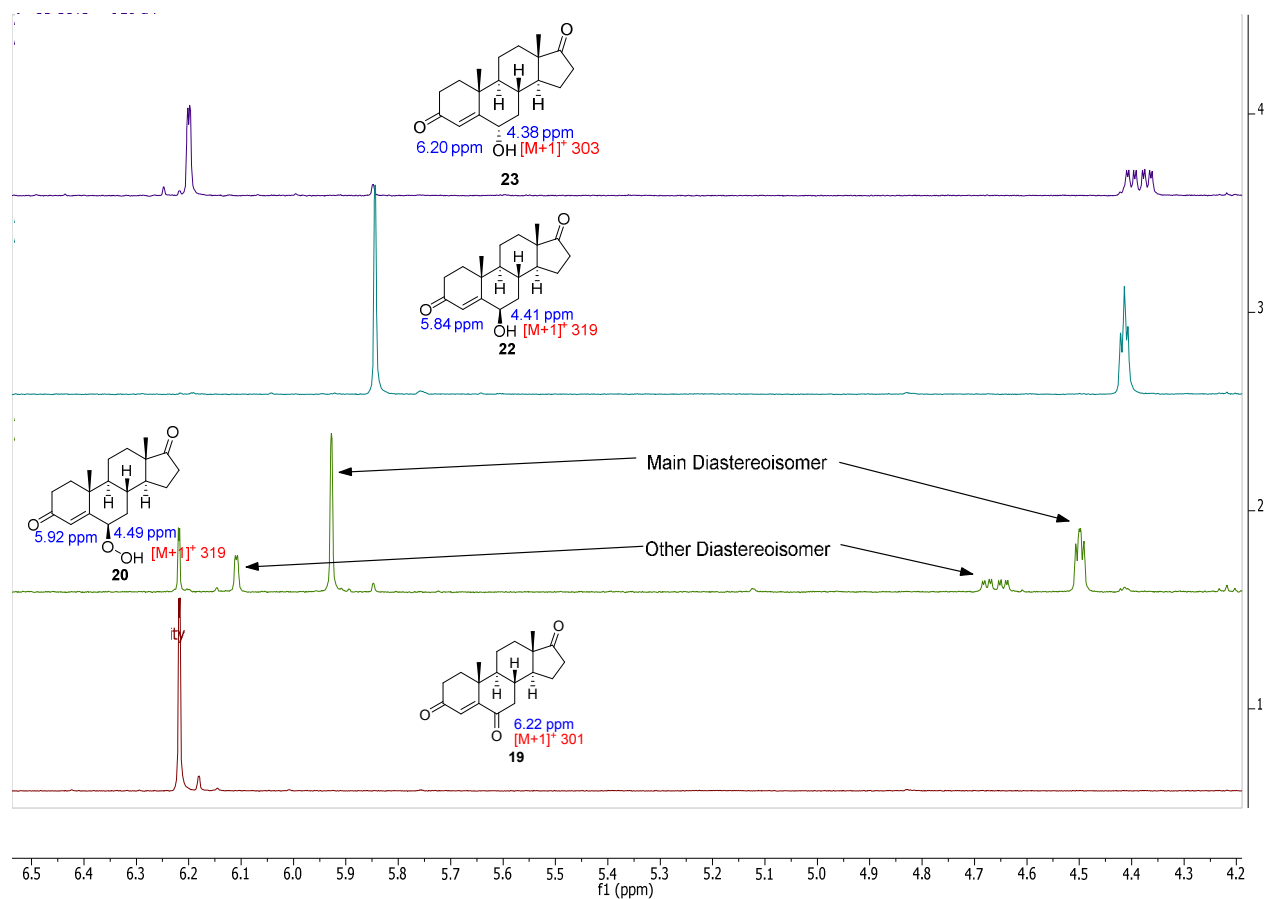


Figure S5 Diagnostic ^1H NMR signals of impurities **19-23** formed as degradants of 5-AD (**5**)

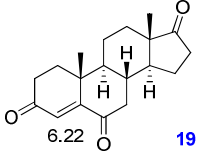
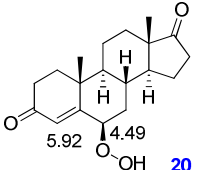
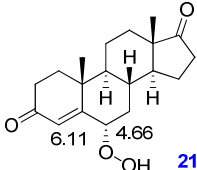
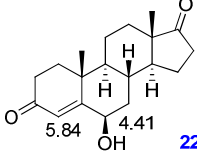
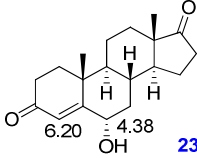


Top panel (purple) – compound **23**; 2nd panel (teal) – compound **22**; 3rd panel (green) – compounds **20** plus degradants **21** and **19**; bottom panel (red) – compound **19**.

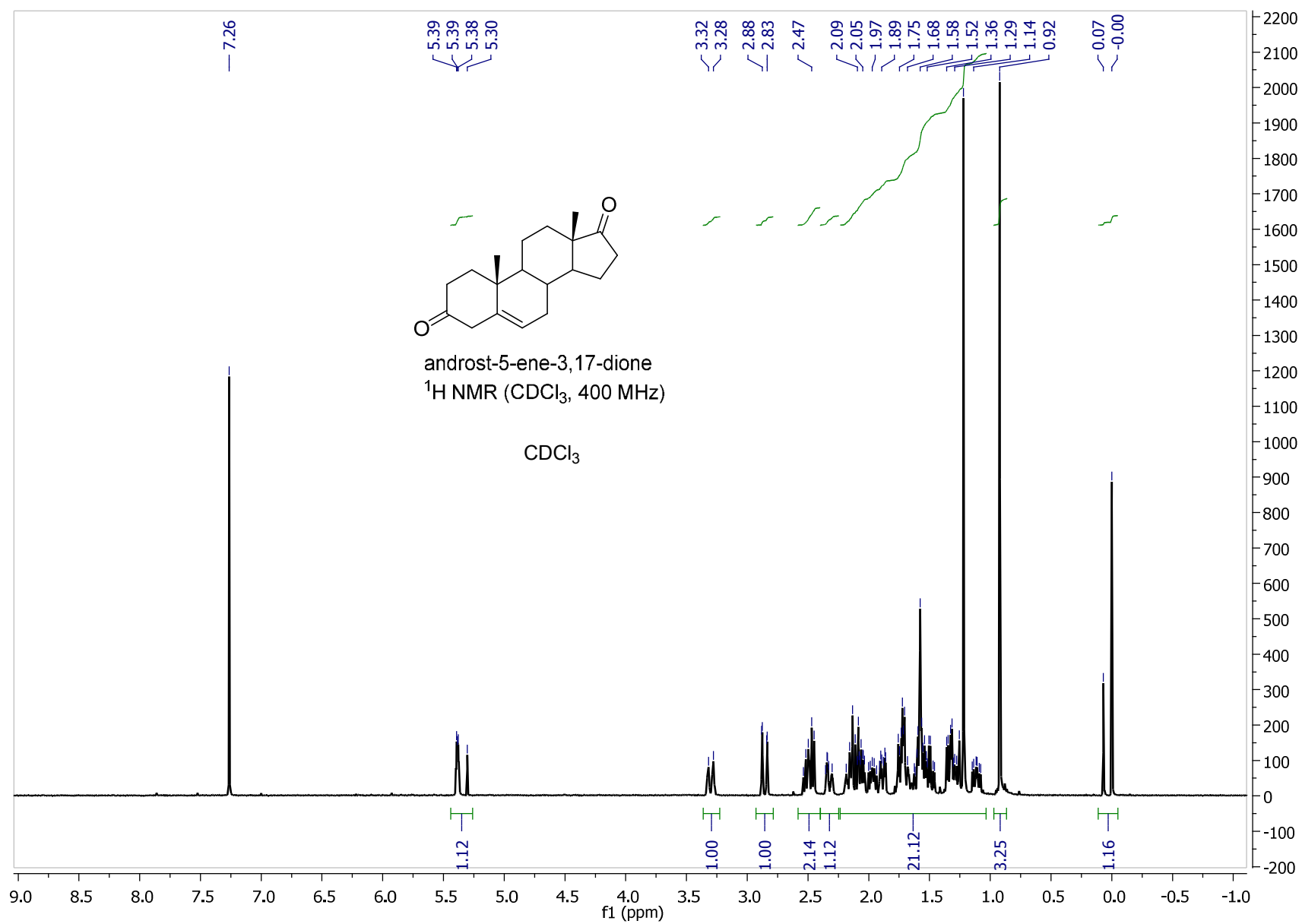
Diagnostic ^1H NMR data and references for steroid characterisation

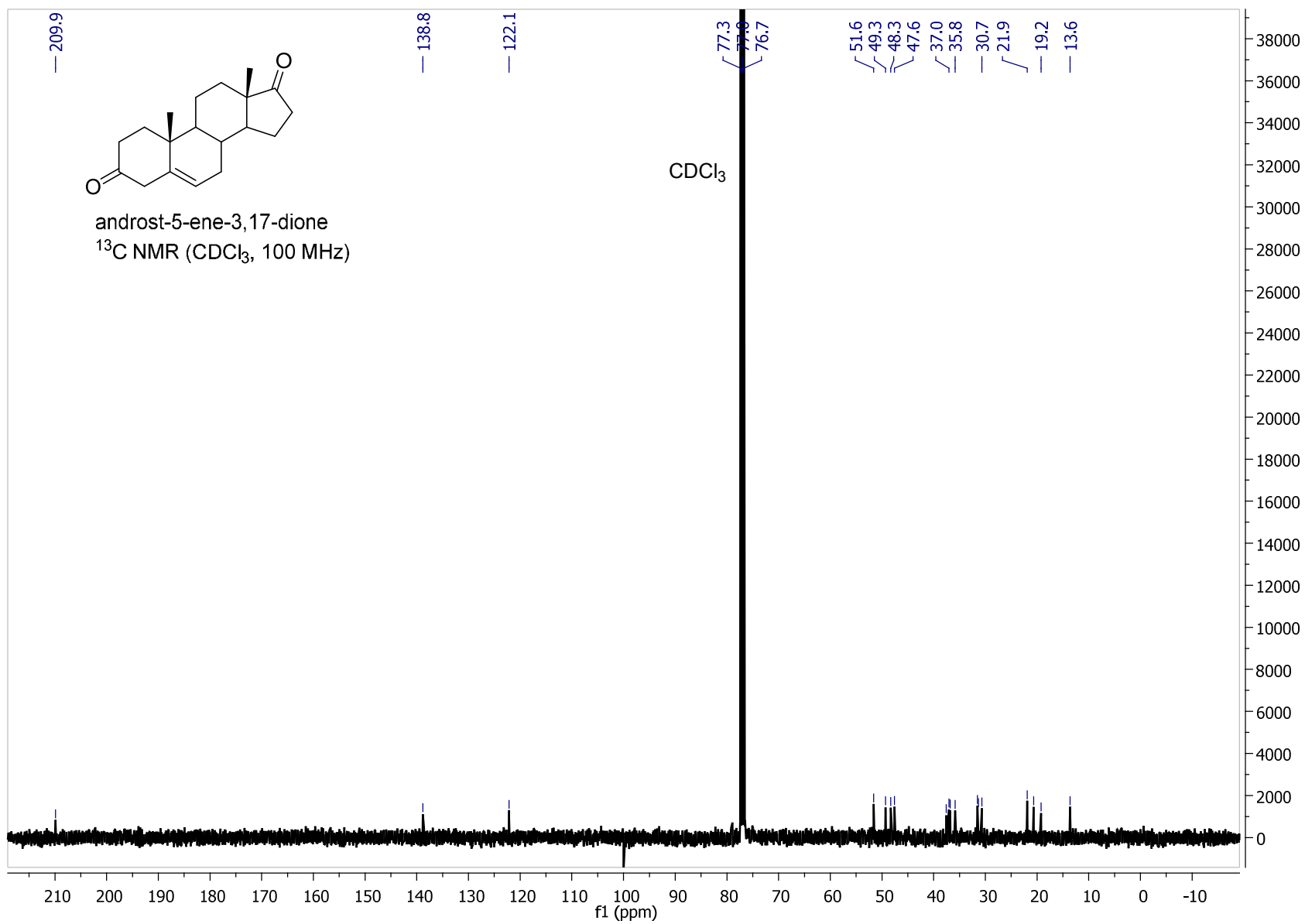
Table S3 ^1H NMR shifts of the substrates, products and impurities

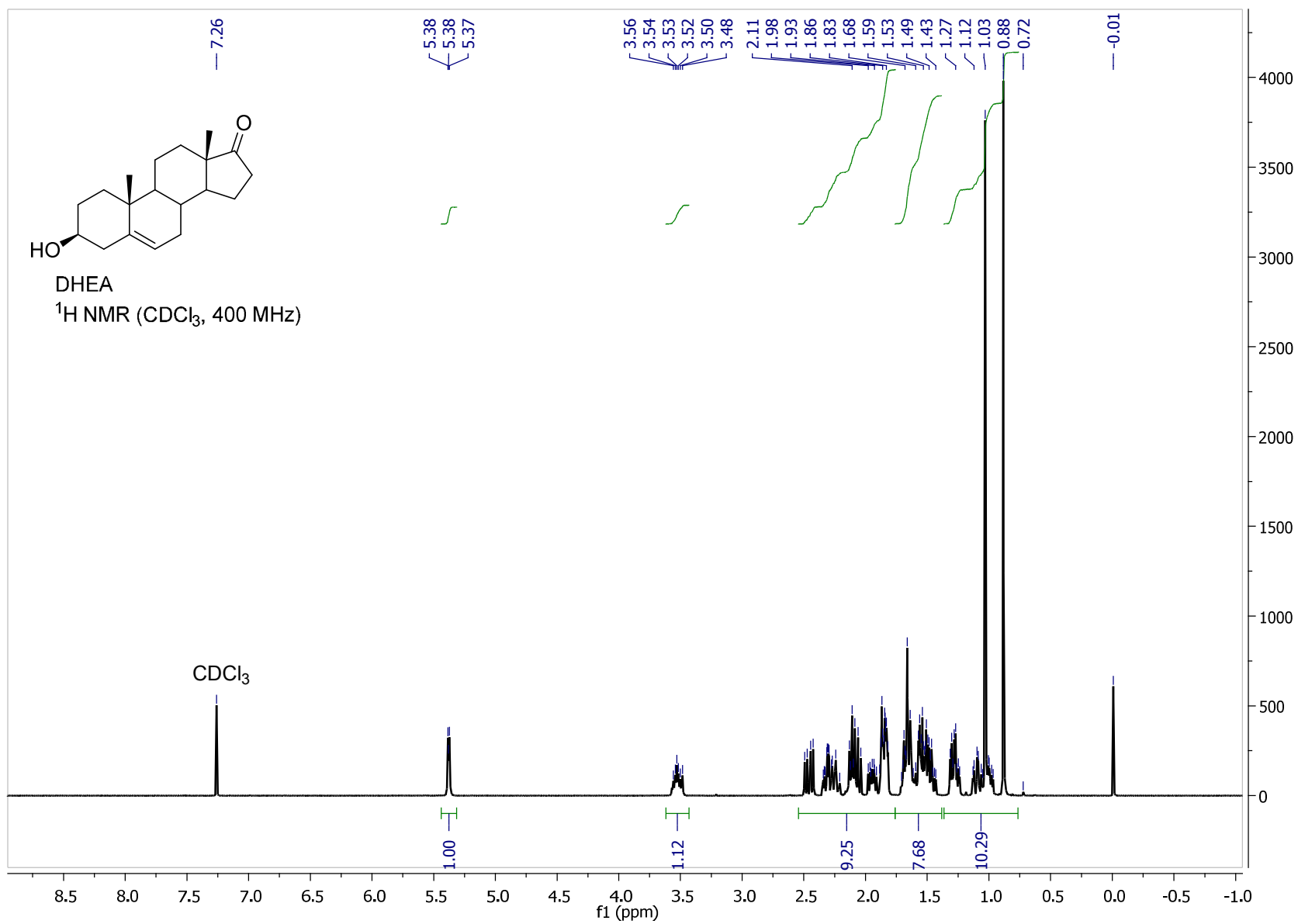
No	Compound	Name	Abbreviation	δ C=CH (ppm)	δ CHOH (or CHOAc) (ppm)	δ other characteristic peaks (ppm)
1		Androst-4-ene-3,17-dione	4-AD	5.76		
2		Dehydroepiandrosterone	DHEA	5.37	3.53	
3		Dehydroepiandrosterone acetate (5-androsten-3 β -ol-17-one acetate)	DHEA acetate	5.41	4.61	
5		Androst-5-ene-3,17-dione	5-AD	5.39		3.30 (d) 2.83 (d)
6		Androst-5-ene-3,17-diol	5-AD-diol	5.35	3.65 (C17) 3.53 (C3)	
9		Testosterone (17 β)		5.73	3.65	
10		Epitestosterone (17 α)		5.74	3.77	
12		3-(acetyloxy)-androsta-3,5-dien-17-one	Enol acetate	5.71, 5.43		
13		5-androsten-3 α -ol-17-one	3 α -DHEA	5.45	4.04	

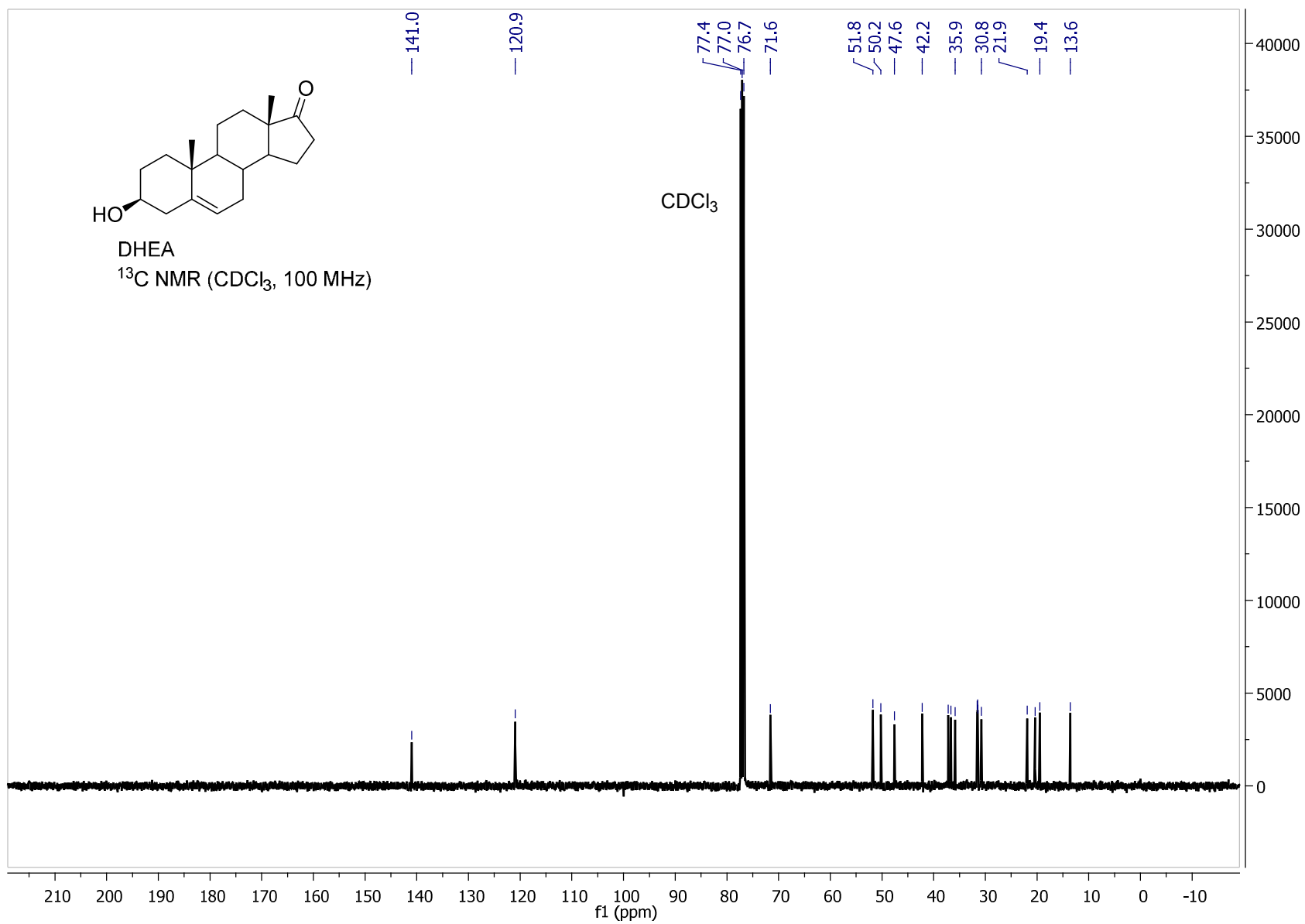
19	 6.22 19	Androst-4-ene-3,6,17-trione	trione	6.22		
20	 5.92 4.49 20	6β-perhydroxyandrost-4-ene-3,17-dione	6β-AD-OOH	5.92		
21	 6.11 4.66 21	6α-perhydroxyandrost-4-ene-3,17-dione	6α-AD-OOH	6.11		
22	 5.84 4.41 22	6β-hydroxyandrost-4-ene-3,17-dione	6β-AD-OH			
23	 6.20 4.38 23	6α-hydroxyandrost-4-ene-3,17-dione	6α-AD-OH	6.20		

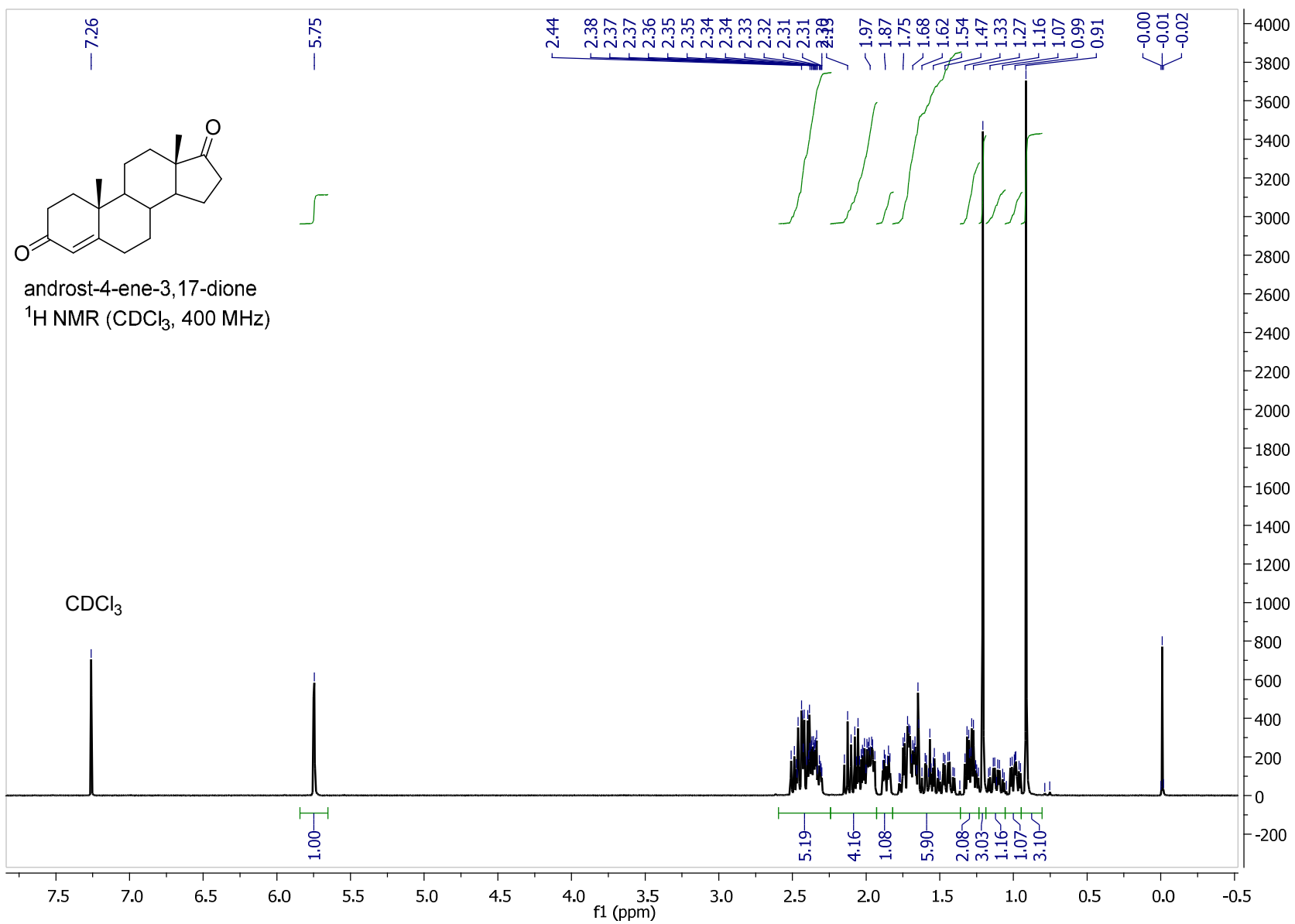
NMR Spectra



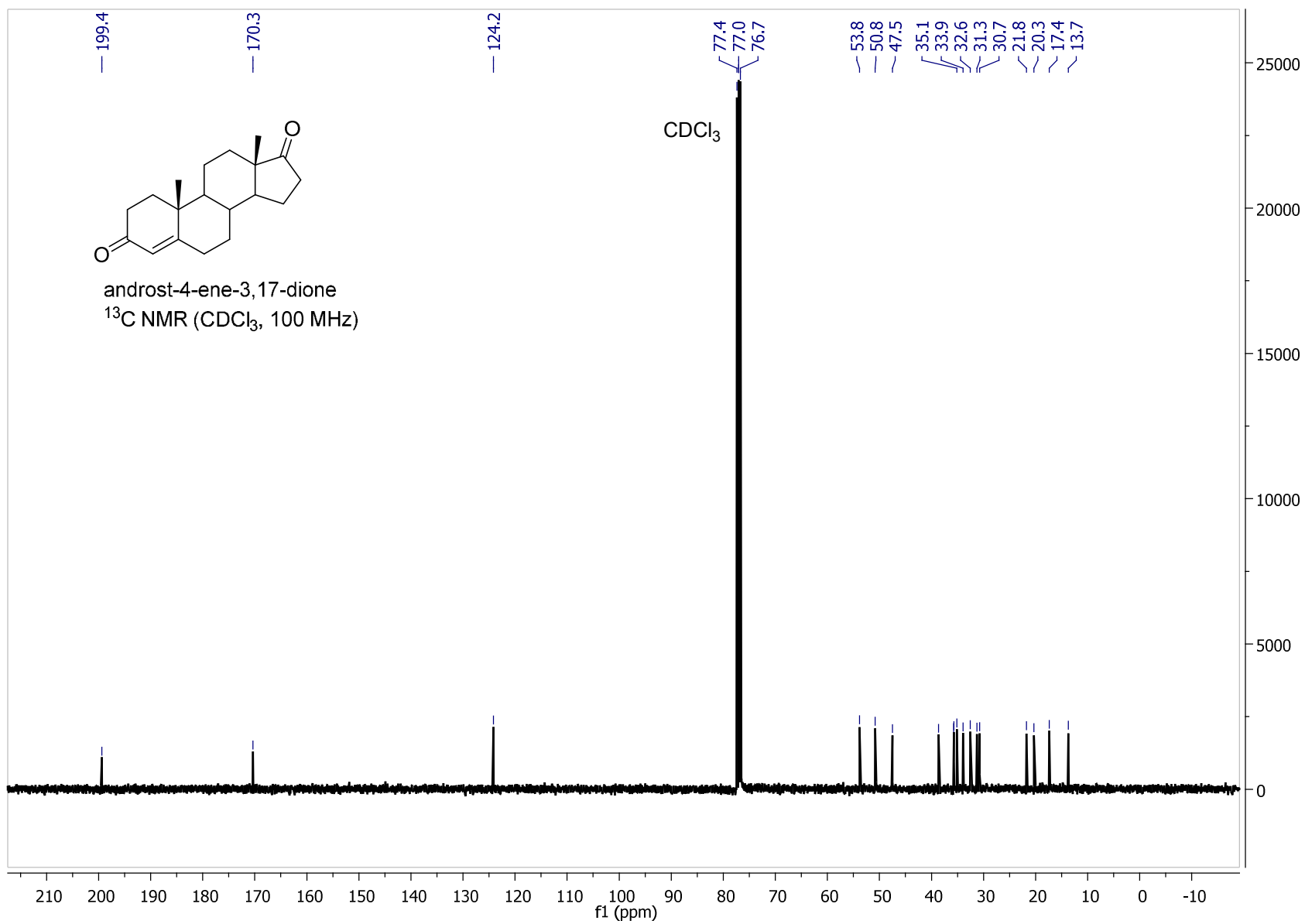


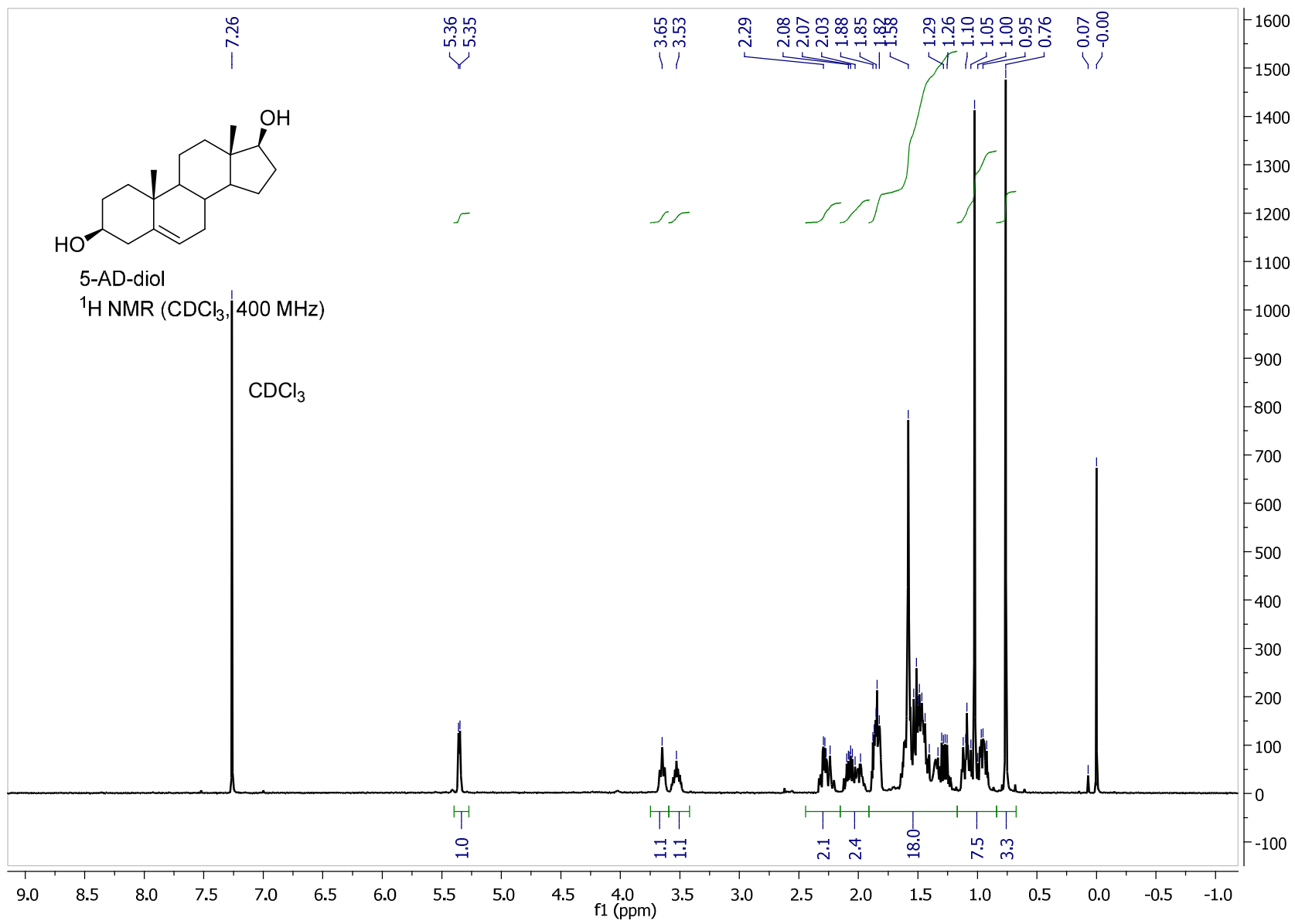


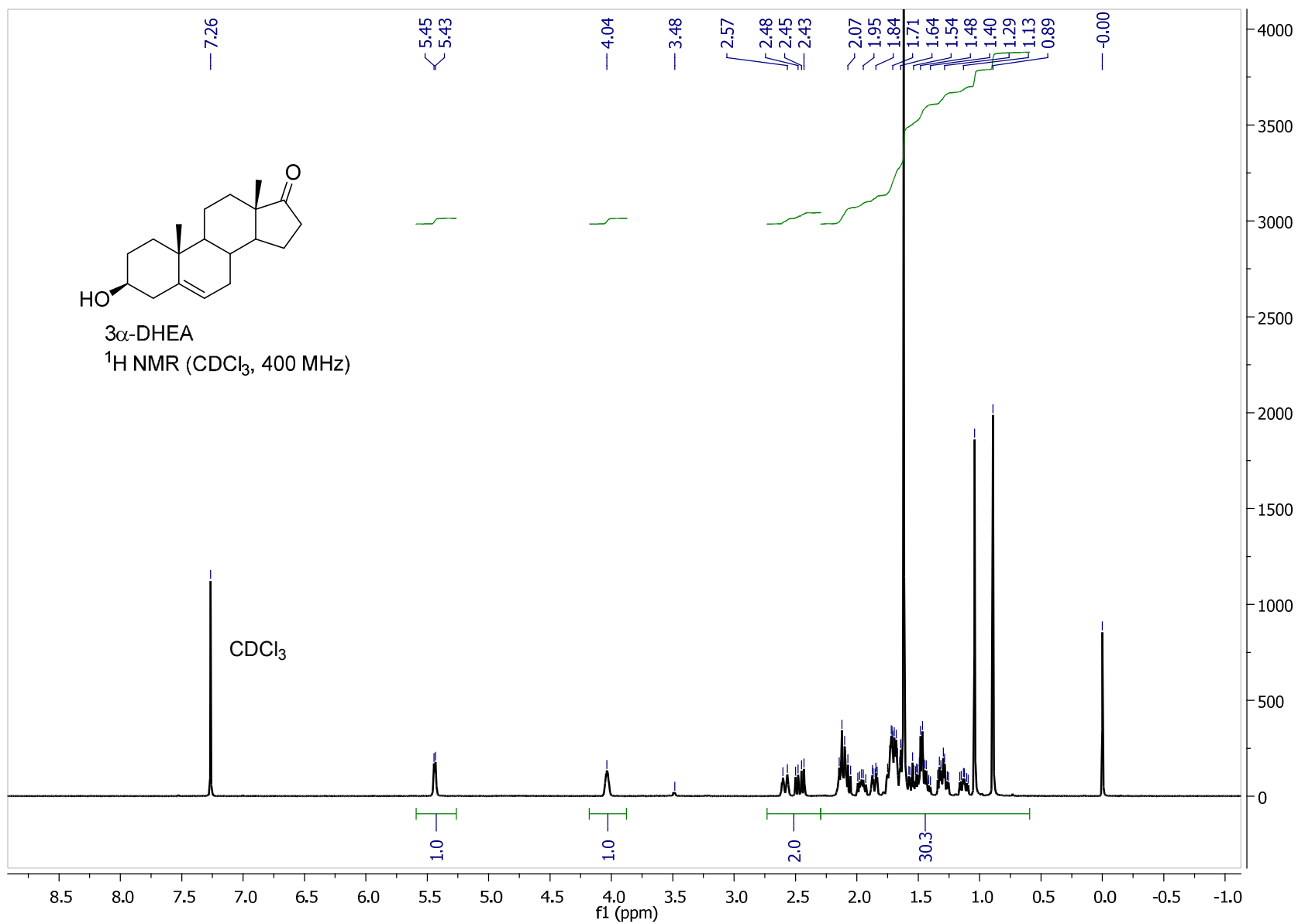


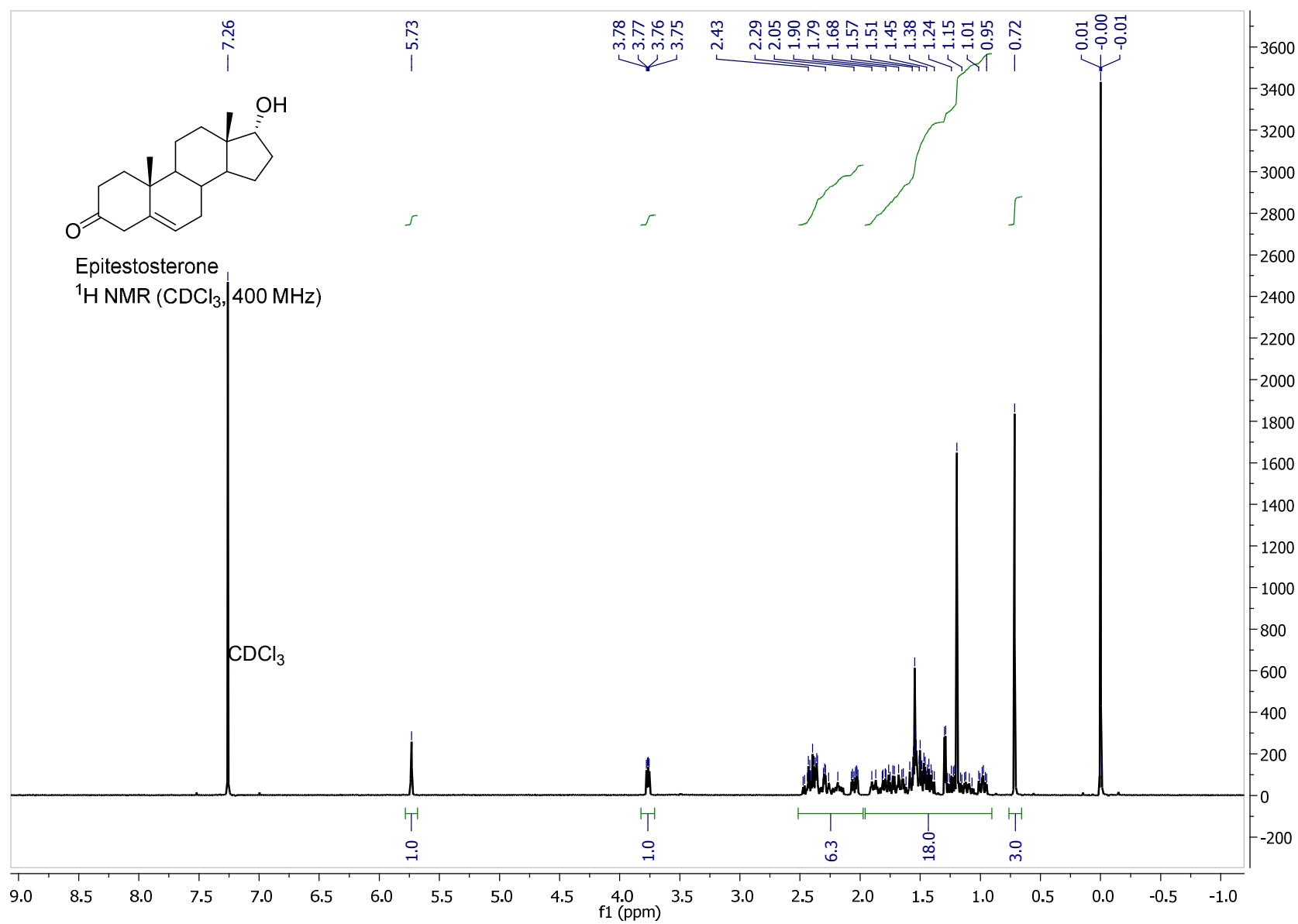


4-AD

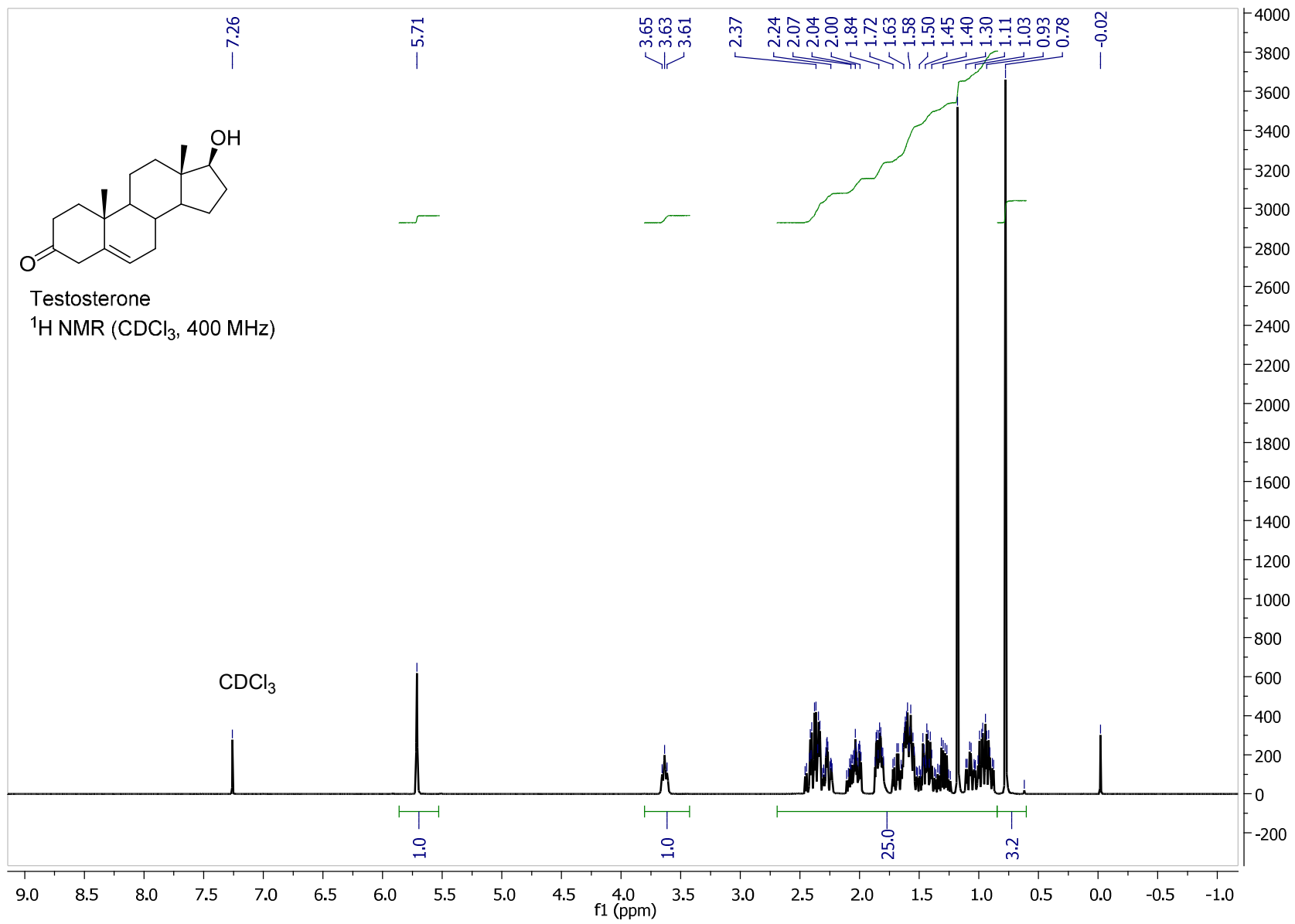


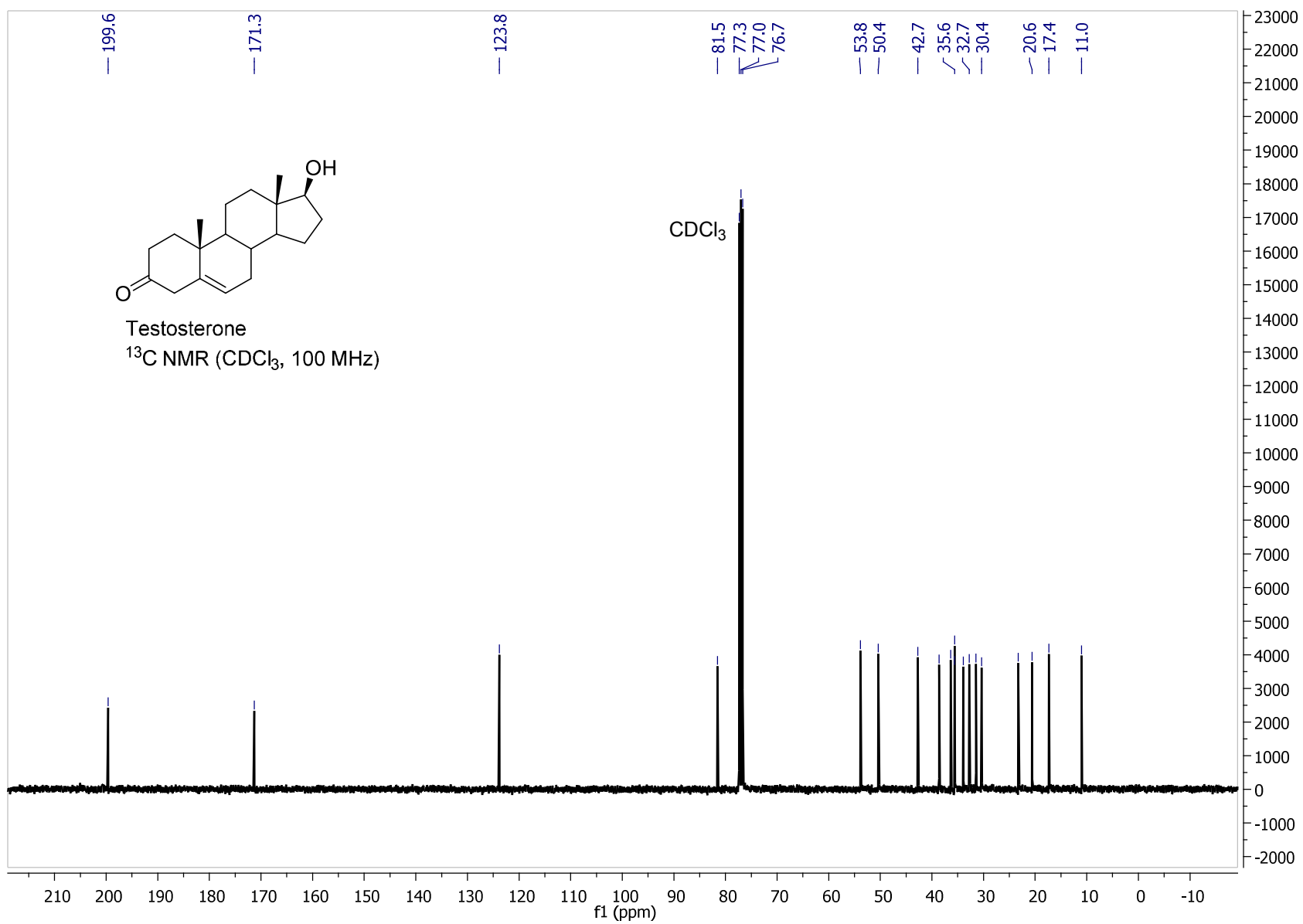


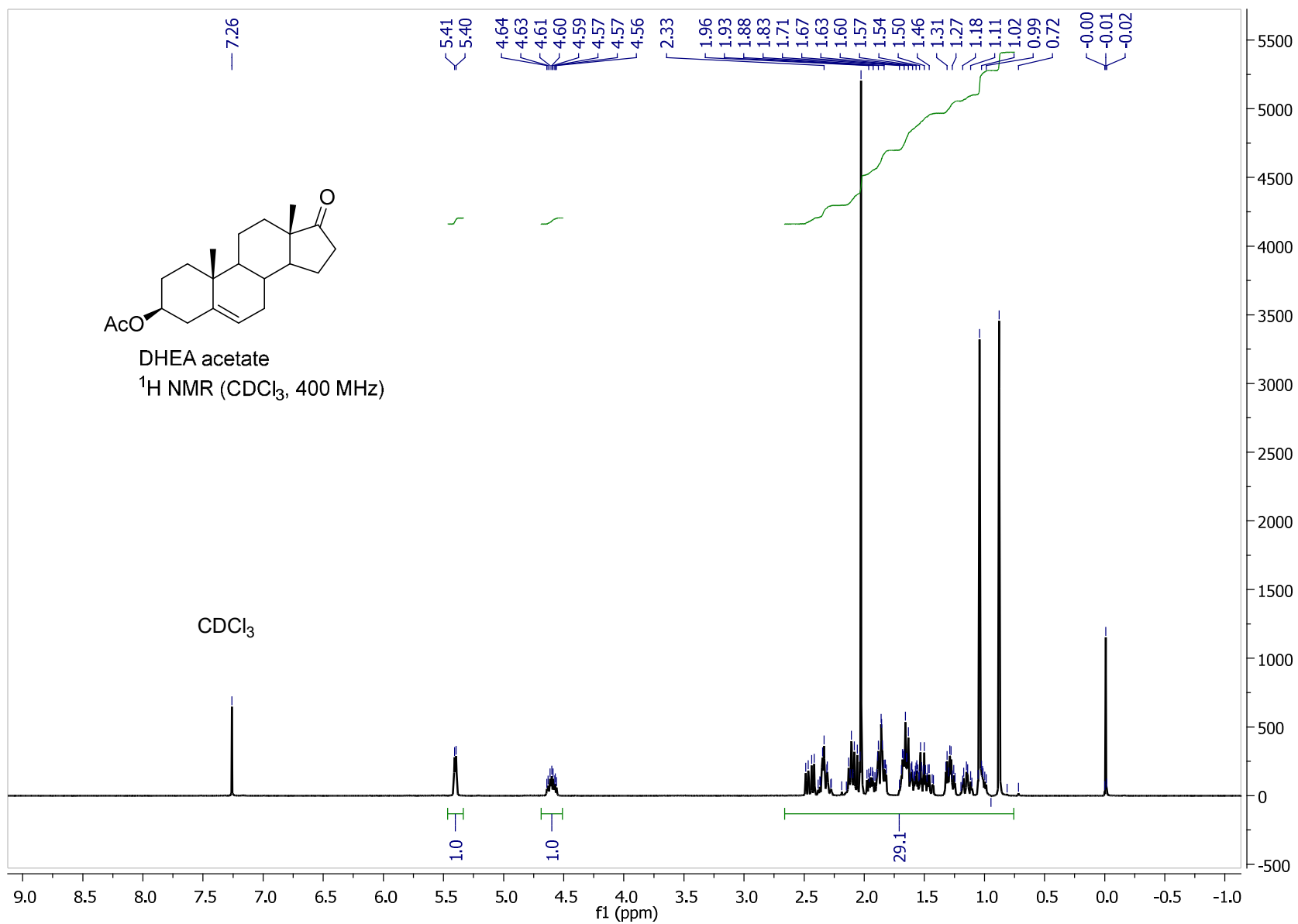


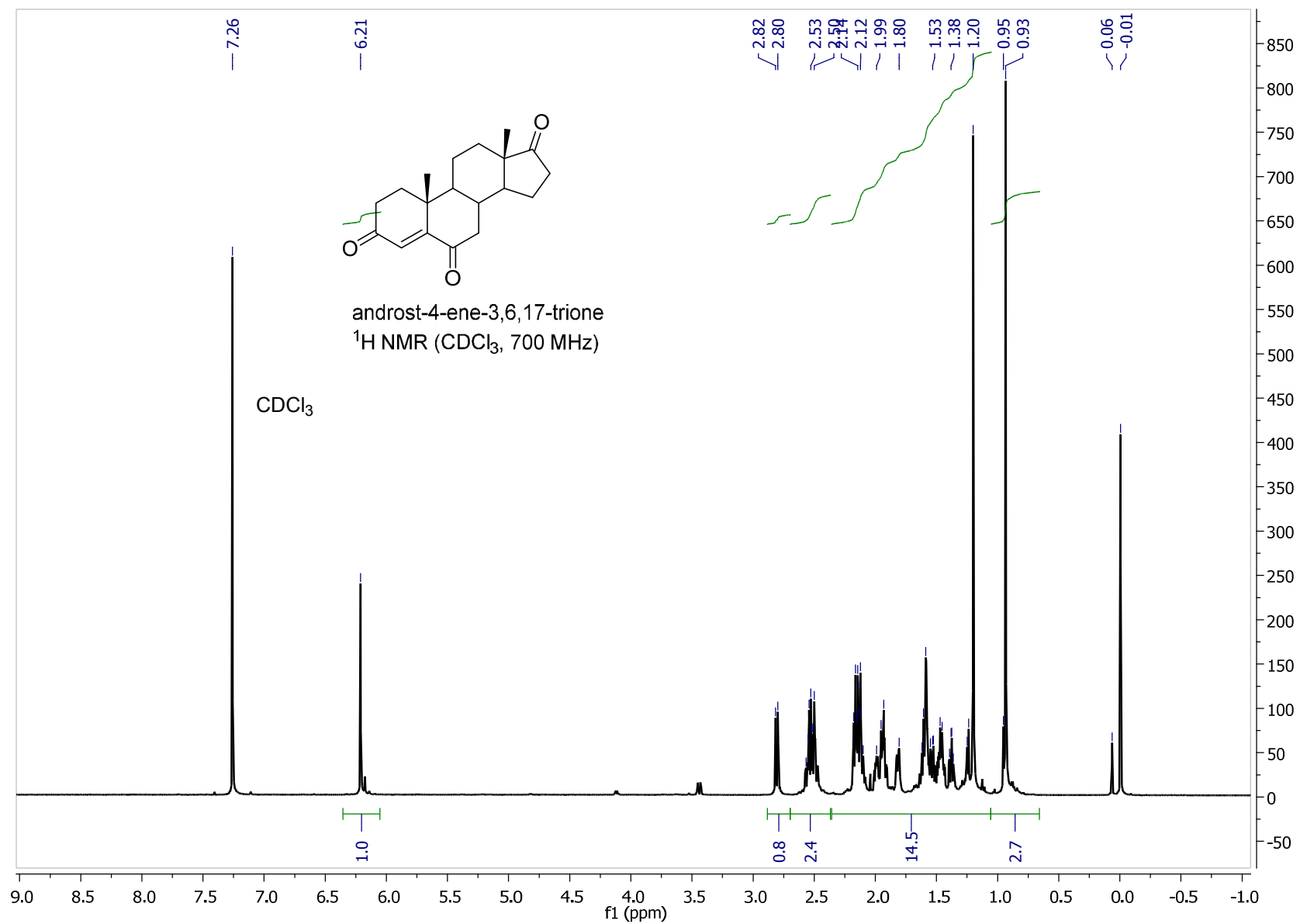


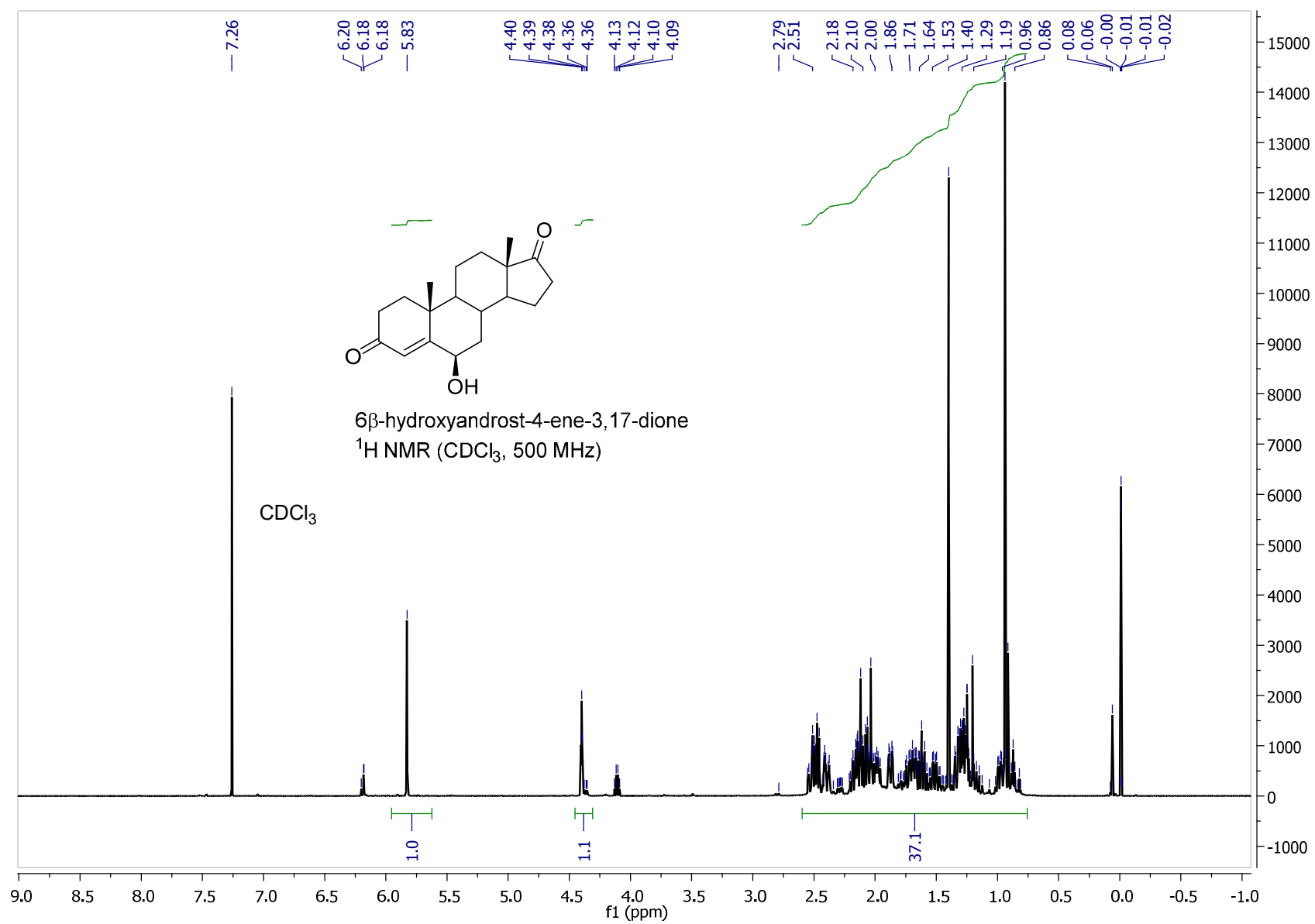
Testosterone

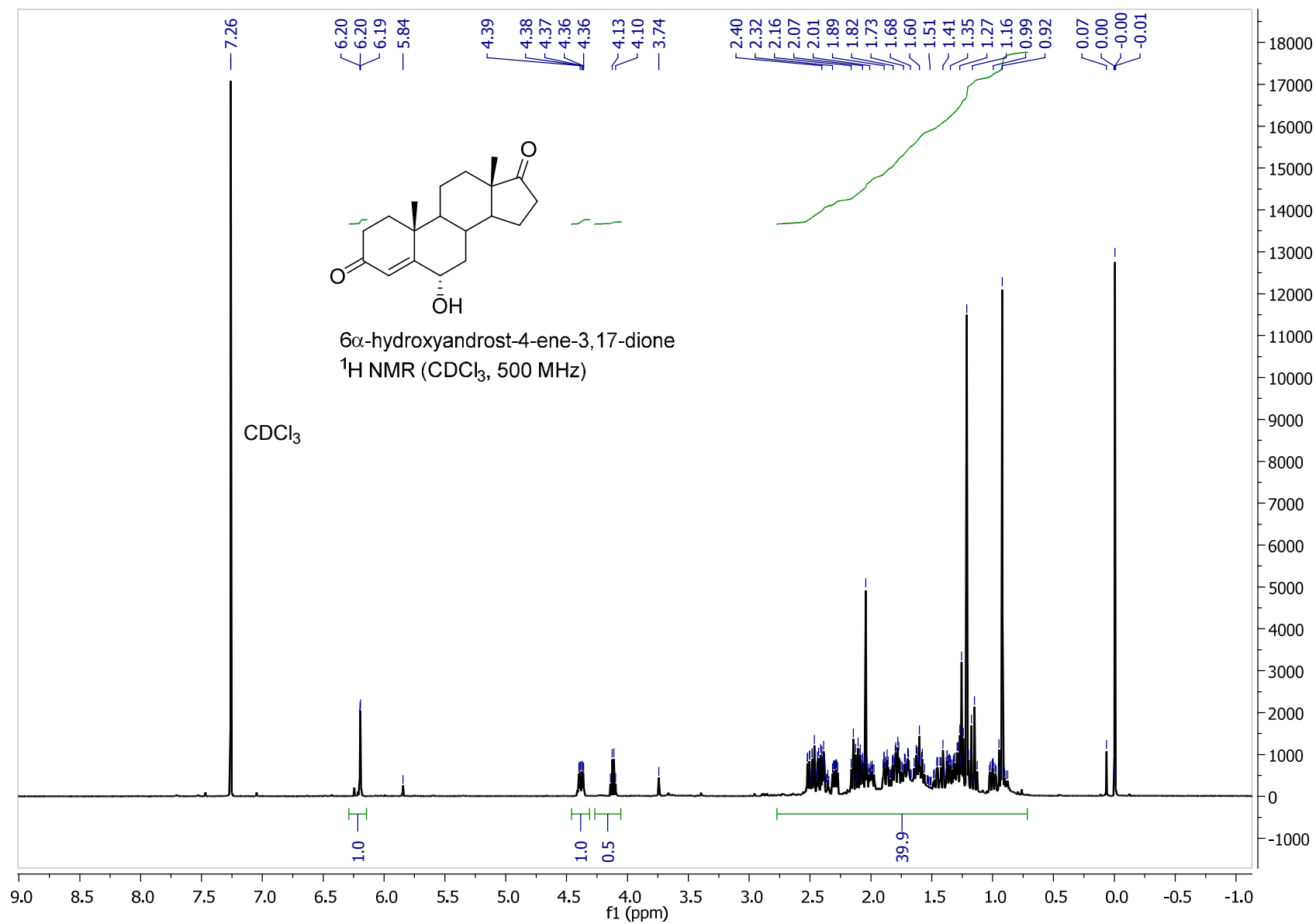


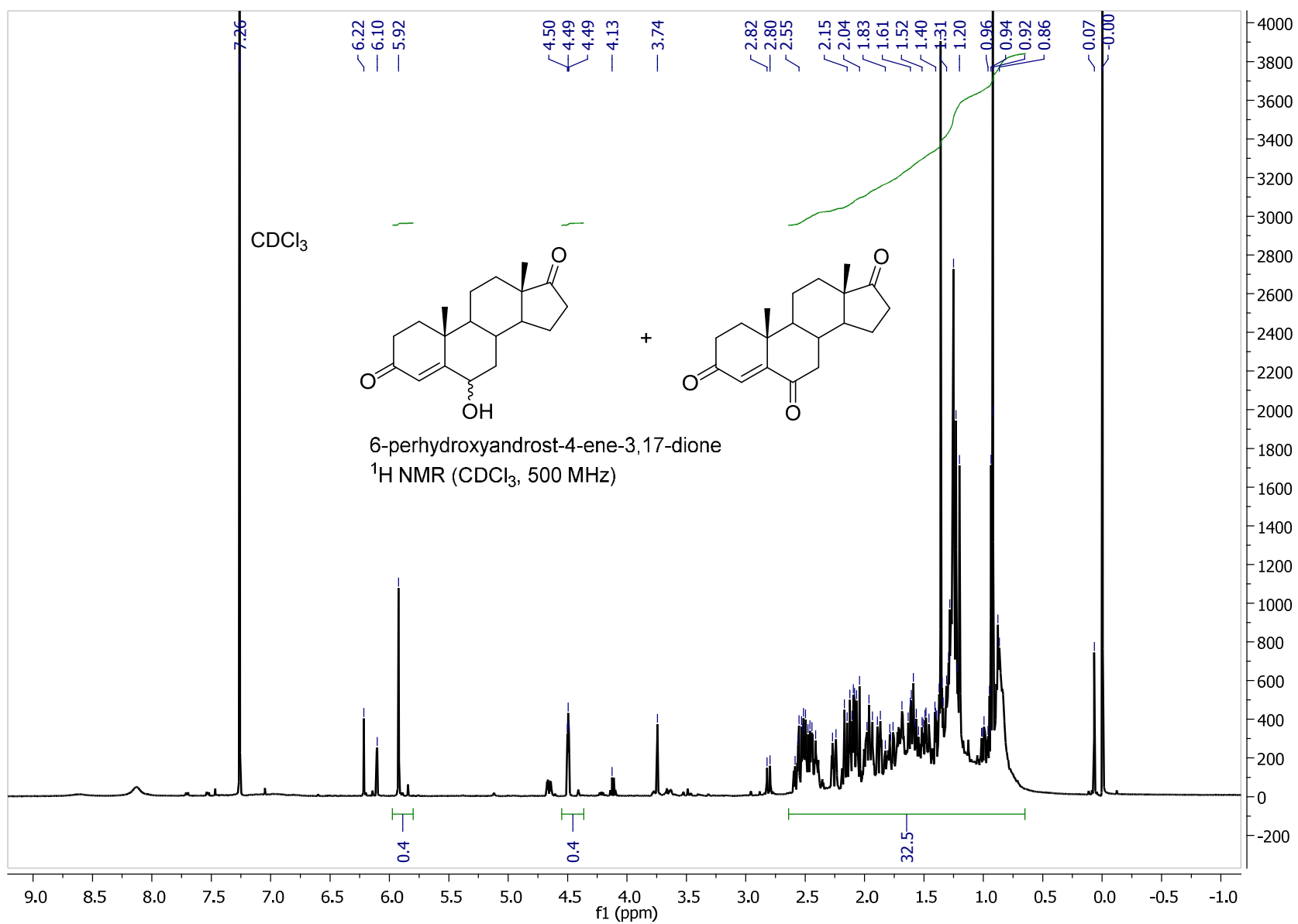












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