Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Synthesis of stable isotope labelled steroid bis(sulfate) compounds and their behaviour in collision induced dissociation experiments

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S1 Supplementary Figures

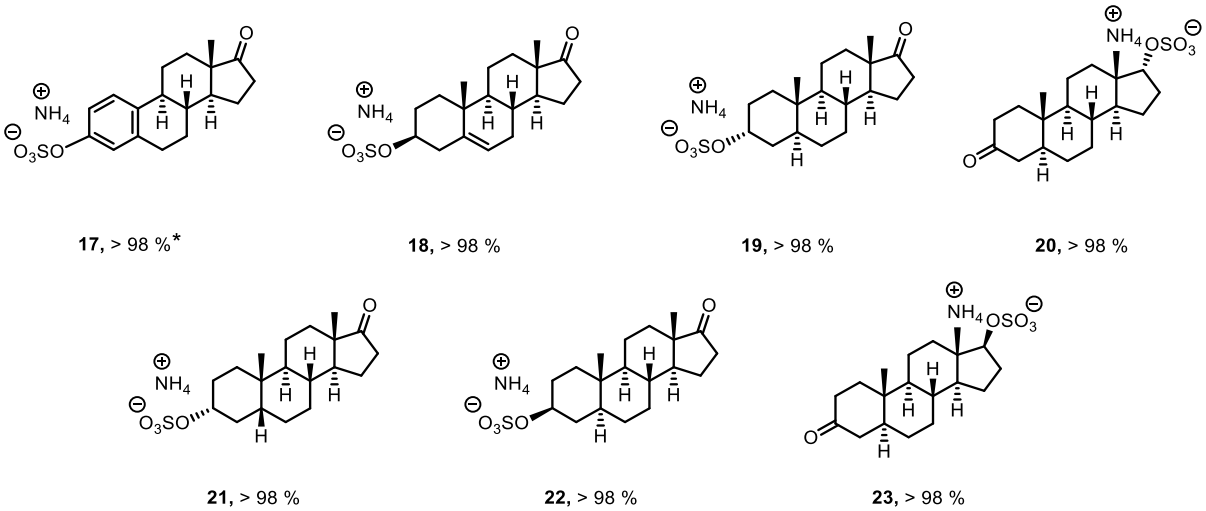


Figure S 1: Series of synthesised steroid mono-sulfates (**17**-**23**) using unlabelled sulfuric acid as the source of sulfate. *Denotes a different sulfate conjugation method was used with details available in synthesis procedures. Percentage conversion for the introduction of the sulfate ester as determined by ^{1}H NMR spectroscopy.

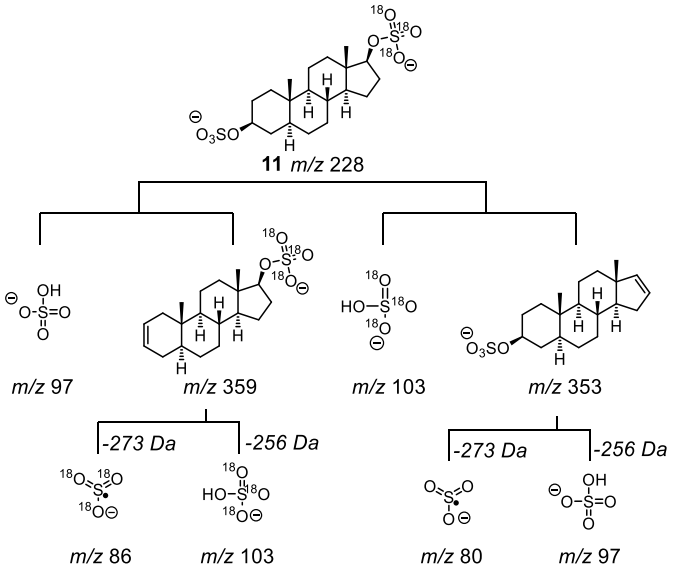


Figure S2: Fragmentation pathways for 5α-androstane-3β,17β-diol bis(sulfate) (**11**) based on MS/MS and pseudo MS^3 CID experiments.

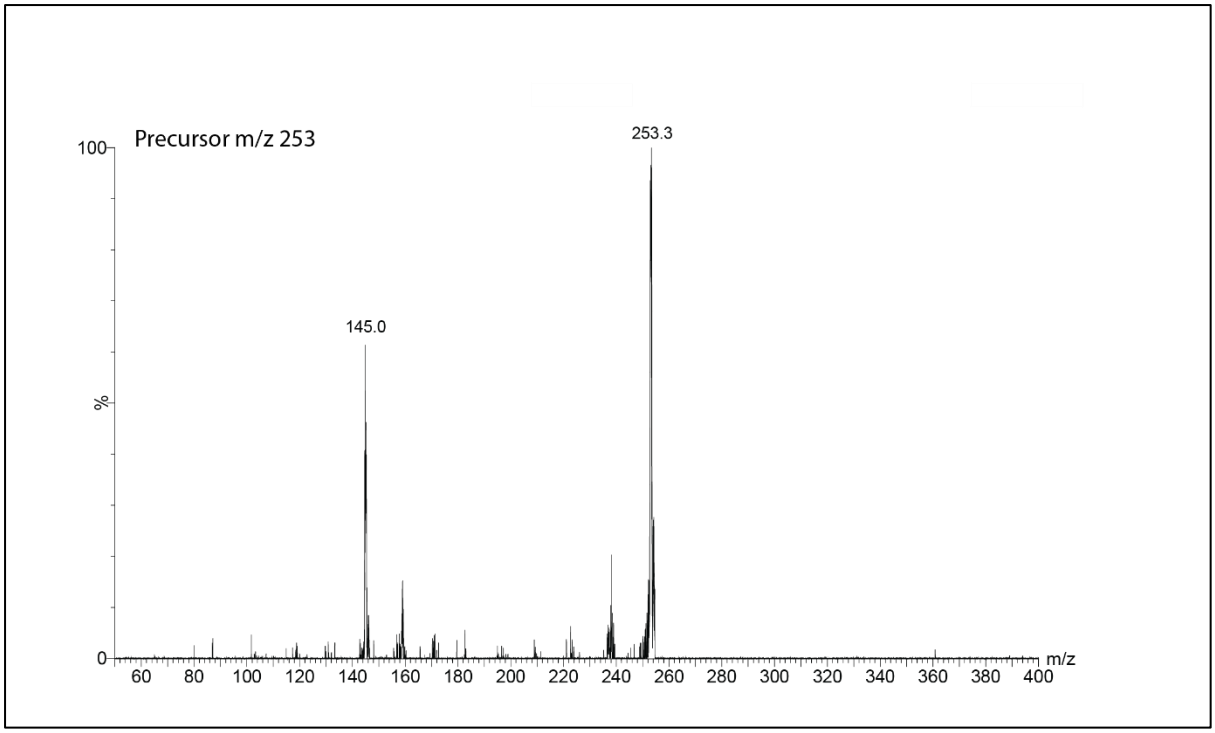


Figure S3: The CID product ion spectra of the pseudo MS^3 precursor *m/z* 253, showing *m/z* 145 as a product ion, at a collision energy of 20 eV.

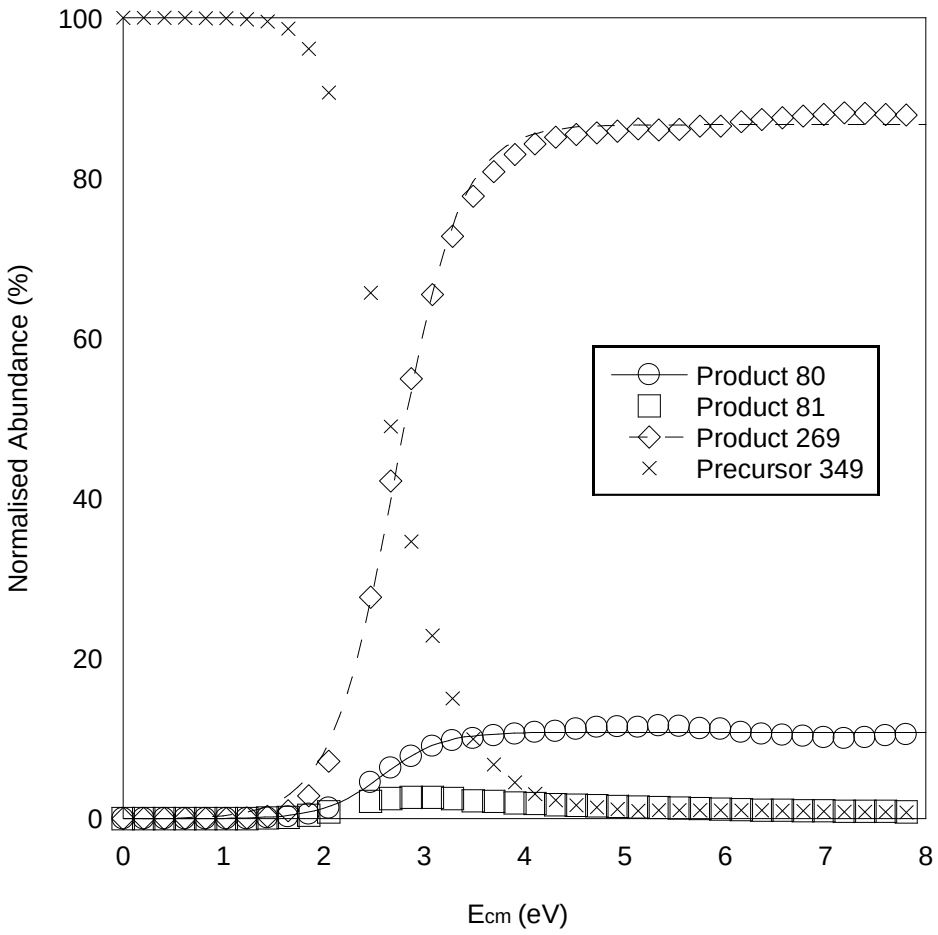


Figure S4: Normalised abundance (%) of estrone sulfate (**17,** *m/z* 349) and product ions over increasing collision energies (eV) in CID. Hydrogen sulfite (*m/z* 81) appears as a low energy product ion between E_{cm} 1-4.3 eV. Other product ions are sulfur trioxide radical anion (*m/z* 80, BR = 11) and the phenolate anion (*m/z* 269, BR = 87) that arises from neutral loss of sulfur trioxide.

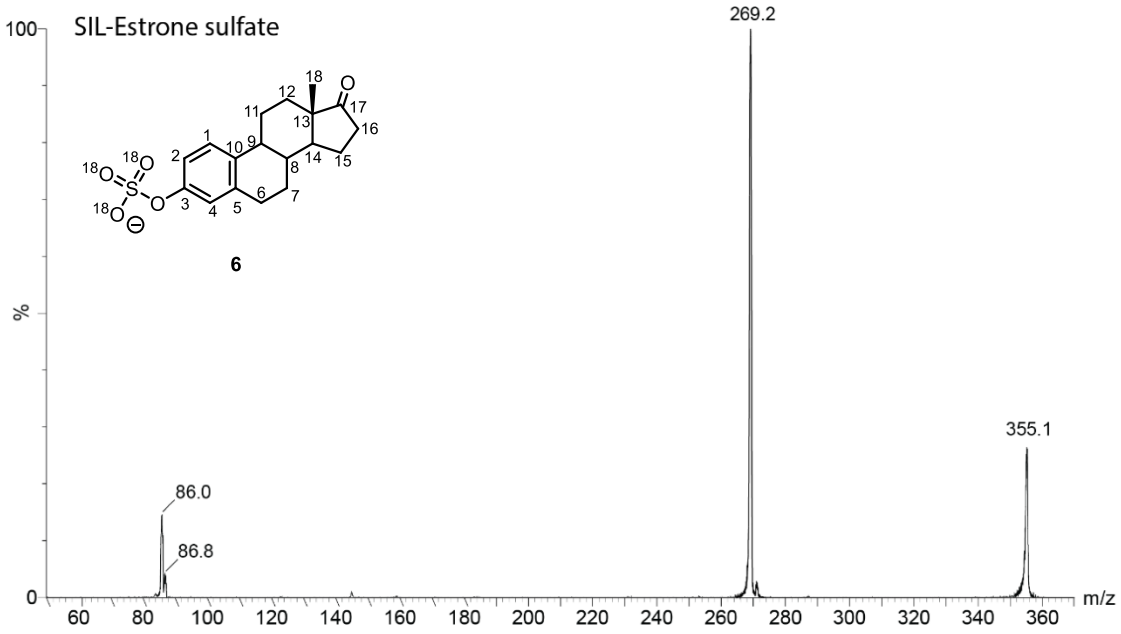


Figure S5: Product ion spectrum of SIL estrone sulfate (**6**, *m/z* 355), at a collision energy of 27 eV. This shows the formation of SIL labelled fragments *m/z* 86 ($^{\bullet}S[^{18}O_3]^-$) and *m/z* 87 ($HS[^{18}O_3]^-$) in addition to *m/z* 269.

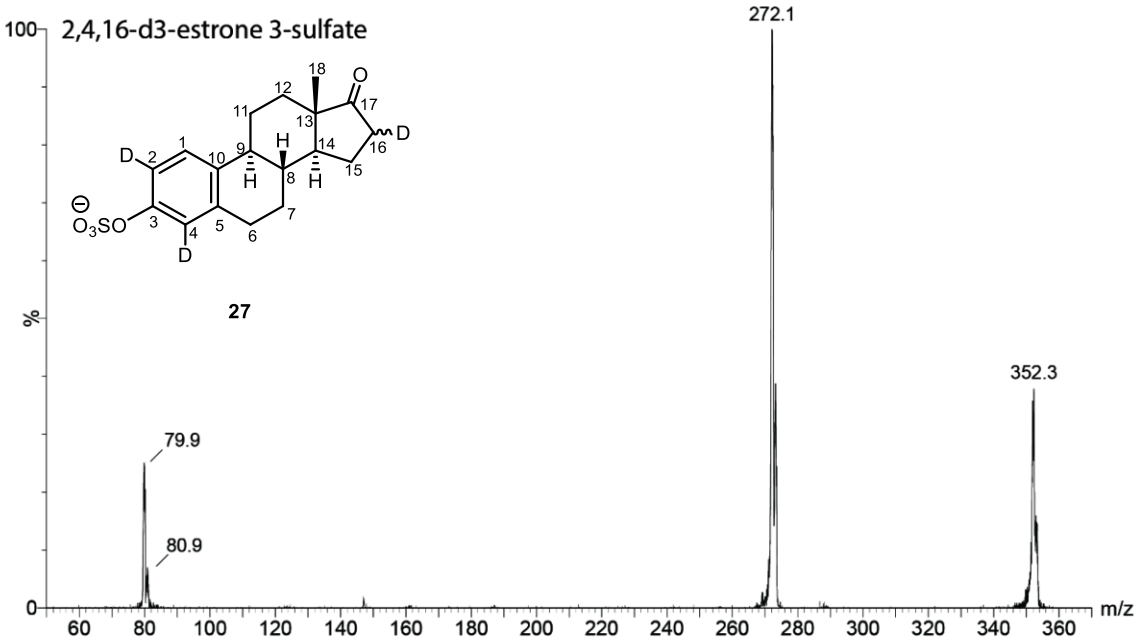


Figure S6: Product ion spectrum of 2,4,16-d_3-estrone 3-sulfate (**27**), at a collision energy of 27 eV. This shows the formation of fragments *m/z* 80 ($^{\bullet}SO_3^-$) and *m/z* 81 (HSO_3^-).

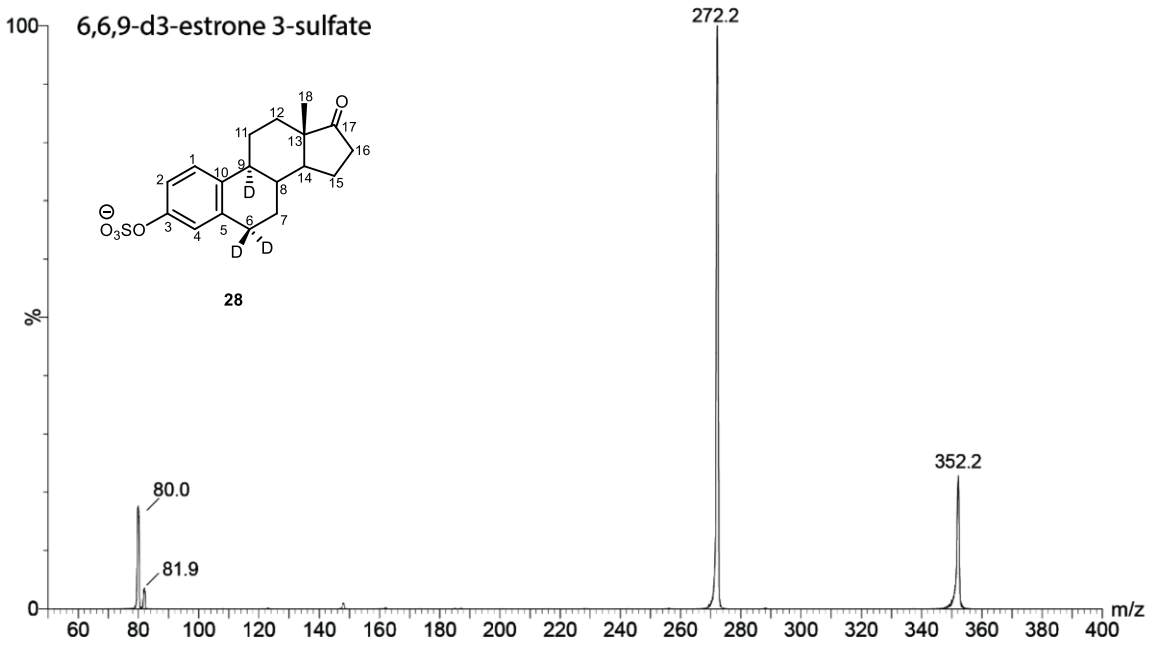


Figure S6: Product ion spectrum of 6,6,9-d_3-estrone sulfate(**28**), at a collision energy of 27 eV. This shows the formation of fragments *m/z* 80 ($^{\bullet}SO_3^-$) and *m/z* 82 (DSO_3^-).

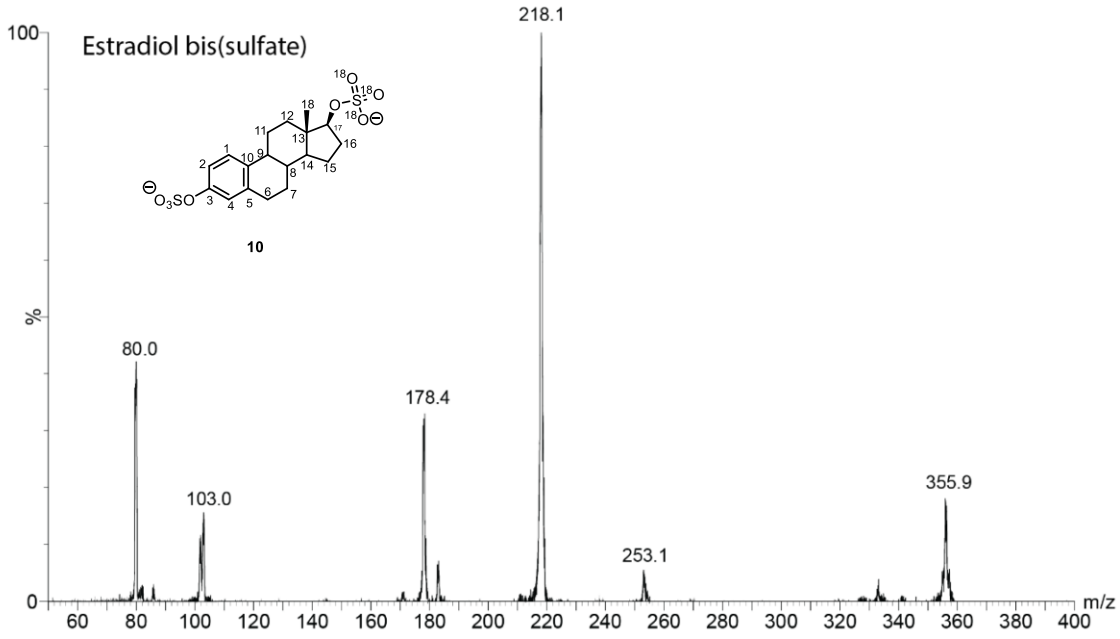
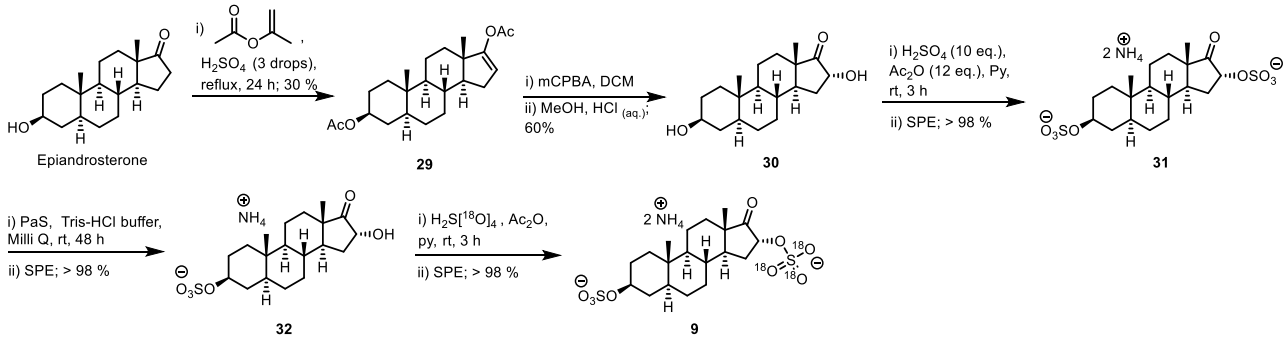


Figure S8: Product ion spectrum of estradiol 3,17[$^{18}O_3$]-bis(sulfate) (**10**), at a collision energy of 12 eV, shows different fragmentation behaviour to its mono sulfate counterpart, estrone sulfate (**6**). The *m/z* 87 ($HS[^{18}O_3]^-$) ion has low relative abundance.

S.2 Supplementary Schemes



Scheme S 1: Selective labelling for 3ß,16α-dihydroxy-5α-androstan-17-one 3,16[$^{18}O_3$]-bis(sulfate), ammonium salt (**9**). This commenced through the acetylation of epiandrosterone with isopropenyl acetate to form the corresponding diacetate (**29**). This was followed by epoxidation with *m*CPBA, to form the corresponding epoxide. Without further purification the epoxide was then subjected to acid hydrolysis to form the diol species (**30**).[1] This was followed by the formation of the unlabelled bis(sulfate) (**31**), using unlabelled sulfuric acid. The bis(sulfate) (**31**) was then subjected to selective cleavage of the 16-sulfate through the use of the *Pseudomonas aeruginosa* arylsulfatase (*PaS)* enzyme.[2,3] Hydrolysis at the 16 position was indicated by the up-field shift of the C16-H doublet, from δ 4.93 to 4.31, which has previously been reported.[2] This gave the diol mono-sulfate (**32**), which was subjected to sulfation using labelled sulfuric acid to give compound (**9**).

S3 Supplementary Tables

Table S1: Shows the extent of labelling of sulfates generated using labelled sulfuric acid. This was achieved by taking the percentage areas under the curve from extracted ion chromatograms for each *m/z* from full scan LC-MS spectra. ND denotes 'not detected'.

Compound	Area (%)			
	3 x ^{18}O	**2 x ^{18}O**	**1 x ^{18}O**	**0 x ^{18}O**
1	82	17	<1	ND
2	82	18	<1	ND
3	74	26	ND	ND
4	73	27	ND	ND
5	82	18	ND	ND
6	81	19	ND	ND
7	42	38	20	ND
8	53	46	<1	ND
9	72	28	ND	ND
10	76	24	ND	ND
11	85	15	ND	ND
12	85	15	ND	ND

Table S2: Mean threshold energies ($E_{5\%}$) with standand errors of the mean (SEM, n = 3) and branching ratios (BR) of various mono sulfates in CID experiments. Note the monosulfates are ordered from lowest to highest $E_{5\%}$.

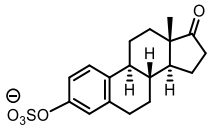
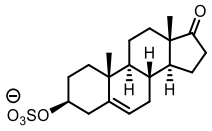
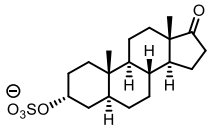
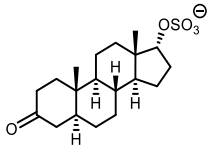
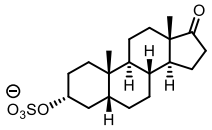
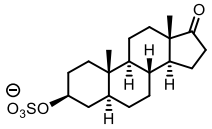
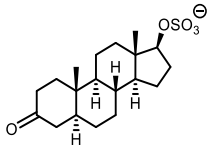
Mono sulfates					
Compound	**Structure**	**Reporter ion (*m/z*)**	**Mean $E_{5\%}$ (E_{cm})**	**SEM n = 3 (E_{cm})**	**BR**
Estrone 3-sulfate (**17**)		269	1.80	0.05	97 (*m/z* 80 = 10, *m/z* 269 = 87)
Dehydroepiandrosterone 3-sulfate (**18**)		97	1.78	0.04	98
Androsterone 3-sulfate (**19**)		97	2.12	0.05	98
Epidihydrotestosterone 17-sulfate (**20**)		97	2.12	0.01	97
Etiocholanolone 3-sulfate (**21)**		97	2.37	0.18	98
Epiandrosterone 3-sulfate (**22**)		97	2.50	0.11	96
Dihydrotestosterone 17-sulfate (**23**)		97	2.88	0.14	93

Table S3: Mean threshold energies ($E_{5\%}$) with standand errors of the mean (SEM, n = 3) for A-ring sulfates. Note results are ordered from lowest to highest $E_{5\%}$

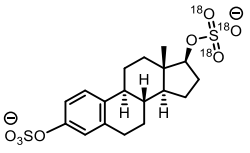
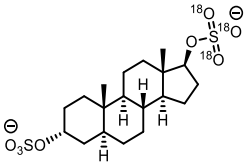
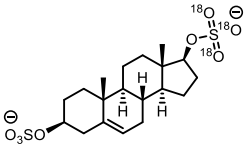
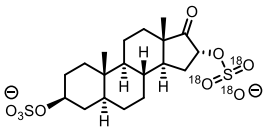
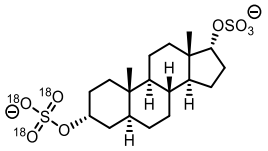
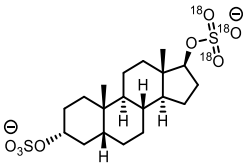
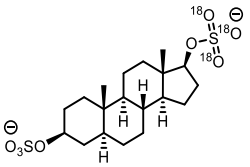
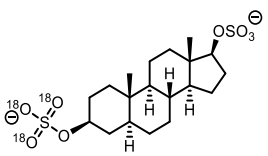
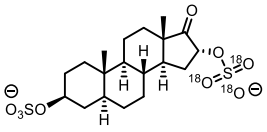
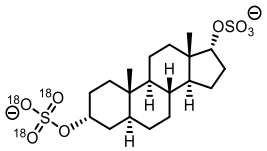
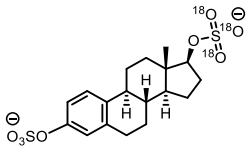
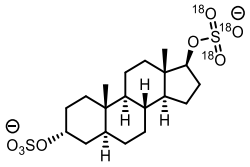
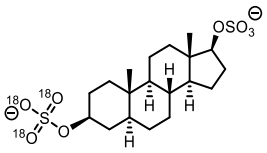
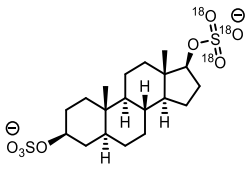
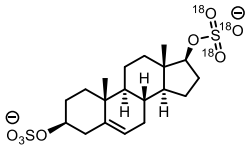
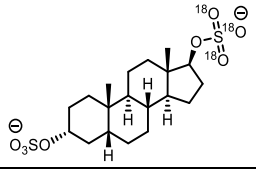
'A' ring positioned sulfate groups					
Compound	**Structure**	**Reporter ion (*m/z*)**	**Mean $E_{5\%}$ (E_{cm})**	**SEM n =3 (E_{cm})**	**BR_i**
estradiol 3,17[$^{18}O_3$]-bis(sulfate) (**10**)		80	2.40	0.04	40
		178	2.44	0.02	31
		combined	2.17	0.02	71
5α-androstane-3α,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**12**)		97	2.38	0.04	63
5-androstene-3β,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**14**)		97	2.43	0.06	60
3β,16α-dihydroxy-5α-androstan-17-one 3,16[$^{18}O_3$]-bis(sulfate) (**9**)		97	2.51	0.01	37
5α-androstane-3α,17α-diol 3[$^{18}O_3$],17-bis(sulfate) (**16**)		103	2.62	0.03	43
5β-androstane-3α,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**13**)		97	2.85	0.10	58
5α-androstane-3β,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**11**)		97	2.92	0.05	52
5α-androstane-3β,17β-diol 3[$^{18}O_3$],17-bis(sulfate) (**15**)		103	2.97	0.12	47

Table S4: Mean threshold energies ($E_{5\%}$) with standand errors of mean (SEM, n = 3) for D-ring sulfates. Note results are ordered from lowest to highest $E_{5\%}$.

'D' ring positioned sulfate groups					
Compound	**Structure**	**Reporter ion (*m/z*)**	**Mean $E_{5\%}$ (E_{cm})**	**SEM (E_{cm})**	**BR_i**
3β,16α-dihydroxy-5α-androstan-17-one 3,16[$^{18}O_3$]-bis(sulfate) (**9**)		86*	2.04	0.03	43
		87*	2.24	0.04	20
		Combined	1.89	0.01	61
5α-androstane-3α,17α-diol 3[$^{18}O_3$],17-bis(sulfate) (**16**)		97	2.56	0.04	54
estradiol 3,17[$^{18}O_3$]-bis(sulfate) (**10**)		103	2.90	0.07	49
5α-androstane-3α,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**12**)		103	3.09	0.07	37
5α-androstane-3β,17β-diol 3[$^{18}O_3$],17-bis(sulfate) (**15**)		97	3.18	0.08	50
5α-androstane-3β,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**11**)		103	3.19	0.09	46
androst-5-ene-3β,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**14**)		103	3.20	0.10	43
5β-androstane-3α,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**13**)		103	3.31	0.12	38

S4 Appearance energy (AE) and threshold energy ($E_{5\%}$) derivation

Breakdown curves were obtained by plotting the normalised abundance (intensity) of the product ion(s) of interest, against the energy in the centre-of-mass frame (E_{cm}), followed by least-squares fitting to the sigmoidal function of the type:

$$I_i(E_{cm}) = \frac{BR_i}{1+e^{(E_{1/2}-E_{cm})b_i}} \qquad \text{(Eq. 1)}$$

Where; I_i is the normalised abundance of the product ion of interest ($\Sigma I_i = 100$), BR_i is the branching ratio of the product ion, b_i describes the rise of the sigmodal curve, and $E_{1/2}$ is the energy at which the function has reached half of its maximum value.[4–7]

Where possible, the ions derived from secondary fragmentation were summed back to their primary product ion. However, this was not possible when secondary fragment ions corresponded to the primary product ions associated with alternative fragmentation pathways, as commonly observed for bis(sulfate) compounds.

The dissociation threshold energies ($E_{5\%}$) were derived from the calculated energy (E_{cm}) when $I_i = 5$.[5] Rearranging and substituting into Eq. 1 gives:

$$E_{5\%} = E_{1/2} - \frac{\ln\left(\frac{BR_i}{5}-1\right)}{b_i} \qquad \text{(Eq. 2)}$$

Alternatively, the appearance energies (AE) were obtained by linear extrapolation of the tangent to the sigmoidal curves at $E_{1/2}$ to the base line.[4,6,7] To do so, the derivative of Eq.1 was taken. This was performed using the online derivative calculator found at https://www.derivative-calculator.net/ to give the derivative:

$$\frac{dI}{dE_{cm}} = \frac{BR_i b_i e^{(E_{1/2}-E_{cm})b_i}}{(1+e^{(E_{1/2}-E_{cm})b_i})^2}$$

At $E_{cm} = E_{1/2}$:

$$E_{1/2} - E_{cm} = 0$$

Thus, giving the following expression for the gradient of the tangent of the sigmoidal curve at $E_{cm} = E_{1/2}$:

$$\frac{dI}{dE_{cm}} = \frac{BR_i b_i}{4}$$

Applying this as the slope m of the tangent to the curve in the form y = mx + c, gives:

$$y = \frac{BR_i b_i}{4} x + c$$

At $E_{cm} = E_{1/2}$ then $x = E_{1/2}$ and from Eq. 1, $y = BR_i/2$ giving:

$$\frac{BR_i}{2} = \frac{BR_i b_i E_{1/2}}{4} + c$$

Rearranging in terms of *c*, gives an expression for the y-intercept ($E_{cm} = 0$)*:*

$$c = \frac{BR_i(2 - b_i E_{1/2})}{4}$$

Applying this again to the equation of the tangent y = mx + c, gives:

$$y = \frac{BR_i b_i}{4} x + \frac{BR_i(2 - b_i E_{1/2})}{4}$$

At y = 0, and rearranging in terms of x, gives the following expression for the x-intercept or appearance energy AE:

$$AE = E_{1/2} - \frac{2}{b_i} \qquad \text{(Eq. 3)}$$

Dissociation thresholds are defined as the energy at which the product ion abundance is equal to 5% of the total ion intensity. This allows the qualitative onset of fragmentation and does not allow absolute quantitative comparison, this analysis was done in accordance to similar past studies.[5,8] Practically, threshold energies ($E_{5\%}$) were used as the main means of comparison in this study as calculations of AE were found to rely heavily on higher energy abundances of primary fragment ions of the modelled data near $E_{1/2}$, which were disproportionately affected by secondary fragmentations, an example of this is given below, Figures S10 & S11.

S5 Example energy resolved product ion spectra

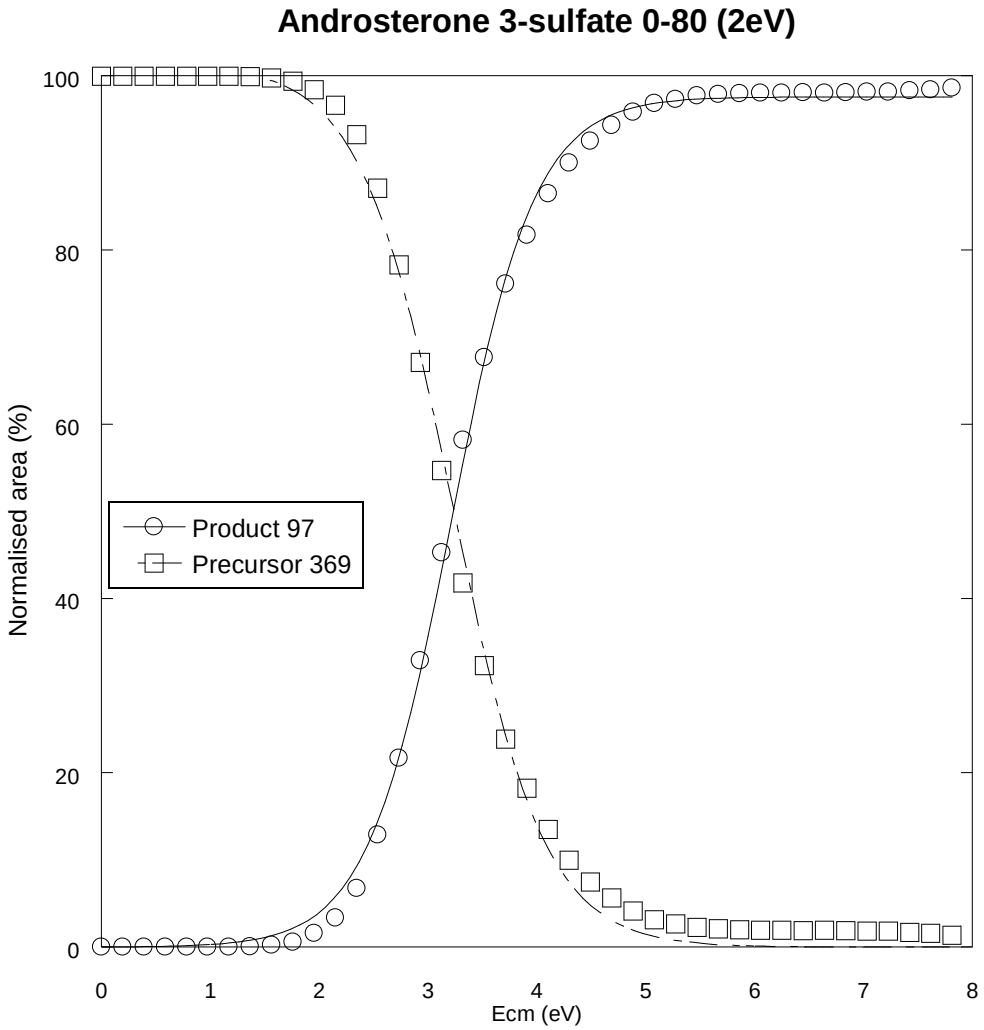
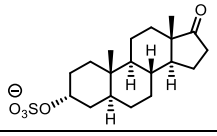


Figure S7: Breakdown curve modelled by Eq.1, for androsterone sulfate (**19**) (*m/z* 369), and the product ion hydrogen sulfate (*m/z* 97). The dissociation threshold energy ($E_{5\%}$) is calculated from the model described by Eq.1, as the energy E_{cm} when the normalised product ion area is equal to 5% (Eq. 2).

Table S 5: Fitted values for androsterone sulfate (**19**) of BR_i, $E_{1/2}$ and b_i, for the product ion *m/z* 97, as defined by Eq.1. From this model the threshold energy ($E_{5\%}$ = 2.12 eV) can be calculated as the energy E_{cm} when the normalised product ion area is equal to 5% (Eq. 2).

Compound	Structure	BR	$E_{1/2}$	b_i	Mean $E_{5\%}$ (E_{cm})	SEM (E_{cm})	χ^2
androsterone 3-sulfate (**19**)		98.5	3.10	2.85	2.12	0.05	9

Bis(sulfates) - Curve fitting to calculate AE and $E_{5\%}$

At higher energies, a loss of normalised abundance is seen for some product ions in some bis(sulfate) species. This is clearly seen for *m/z* 97 and is expected to arise due to secondary fragmentation of the corresponding monosulfate fragment ion ($[M\text{-}2NH_4\text{-}HSO_4]^-$) at higher energies, this is shown below for the bis(sulfate) **12**, Figure S10. Due to this, the modelling of bis(sulfate) compounds was constrained to medium fragmentation energies at around the maximum normalised abundance of the product ion curve to obtain a better fit to Eq. 1, Figure S11. A better fit to the modelled data was indicated by a reduction in Chi Squared values, e.g., *m/z* 97, χ^2 = 189 to 7, and *m/z* 103 χ^2 = 15 to 1, respectively. It should be noted that the absolute ion count reduces as fragmentation energy is increased, Figure S12. Note Chi squared values were calculated by using KaleidaGraph® 4.5 by Synergy Software

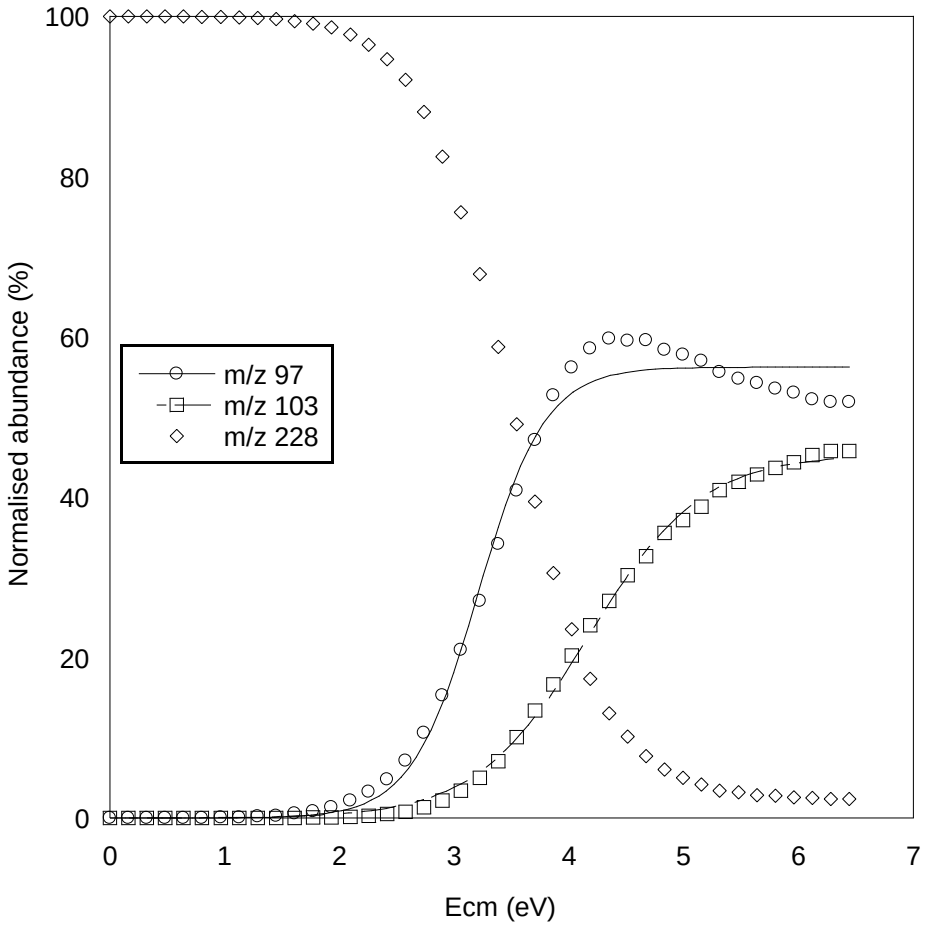


Figure S8: Breakdown curve for 5α-androstane-3α,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**12**) (*m/z* 228) and product ions hydrogen sulfate (*m/z* 97 and *m/z* 103). Fitting parameters, m/z 97 (BR = 56, Chi Squared, χ^2 = 189) and *m/z* 103 (BR = 45, Chi Squared, χ^2 = 15).

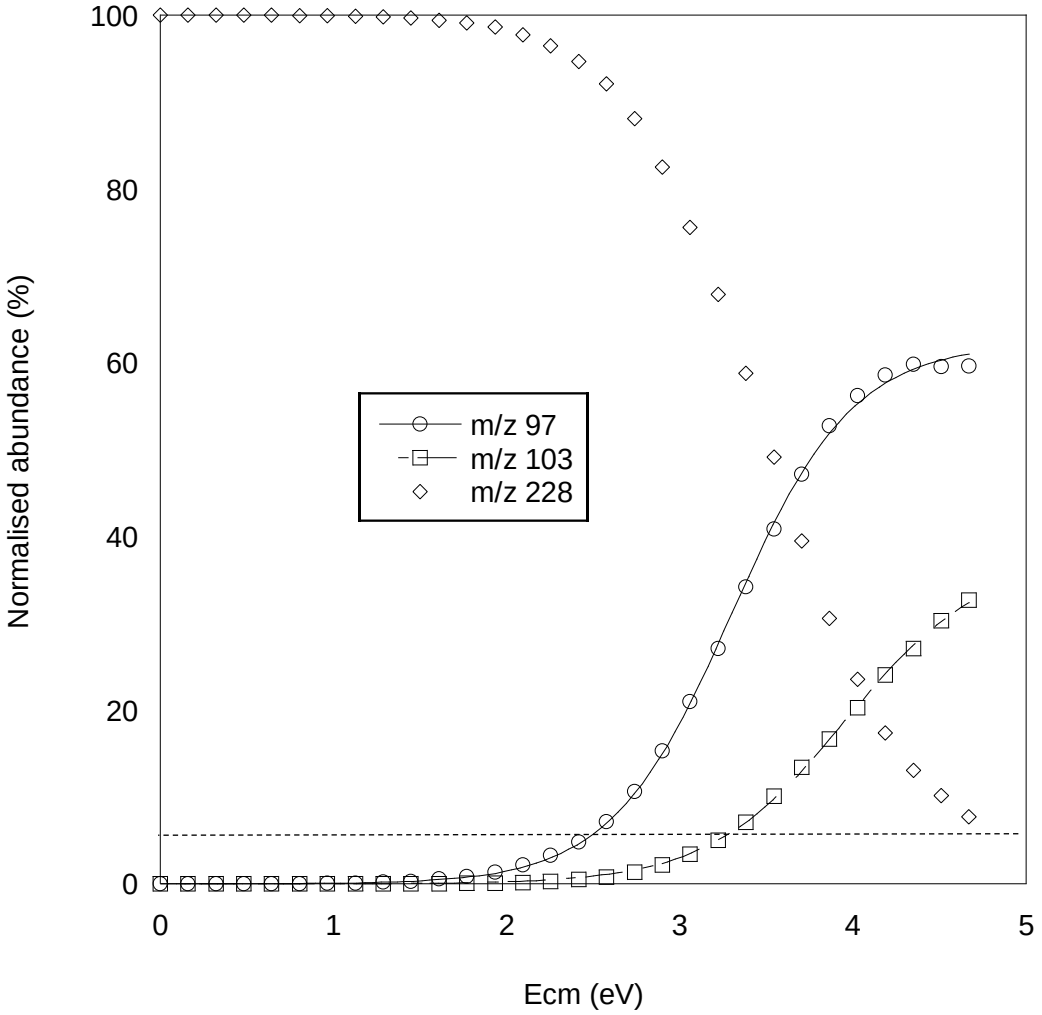
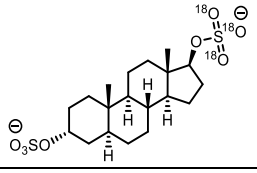


Figure S9: Breakdown curves modelled by Eq.1, for 5α-androstane-3α,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**12**) (*m/z* 228) and product ions hydrogen sulfate (*m/z* 97 and *m/z* 103). The dissociation threshold $E_{5\%}$ for each product ion is calculated from the model as described by Eq.1, as the energy E_{cm} when the normalised area is equal to 5% (Eq. 2). The $E_{5\%}$ displayed are provided for visual demonstration. Fitting parameters, m/z 97 (BR = 63, Chi Squared, χ^2 = 7) and *m/z* 103 (BR = 37, Chi Squared, χ^2 = 1). Line indicates 5% normalised abundance.

Table S 6: Fitted values for 5α-androstane-3α,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**12**) of Br_i, $E_{1/2}$ and b_i, for the product ions *m/z* 97 and 103, as defined by Eq.1. From this model the dissociation threshold $E_{5\%}$ can be calculated as the energy E_{cm} when the normalised product ion area is equal to 5% (Eq. 2). The appearance energy (AE) or x-intercept of the tangent to the curve at $E_{cm} = E_{1/2}$ was also calculated (Eq. 3).

Compound	Structure	Product ion	BR_i	$E_{1/2}$	b_i	$E_{5\%}$ (eV)	χ^2
5α-androstane-3α,17β-diol 3,17[$^{18}O_3$]-bis(sulfate) (**12**)		97	63	3.15	3.17	2.38	7
		103	37	3.85	2.53	3.09	1

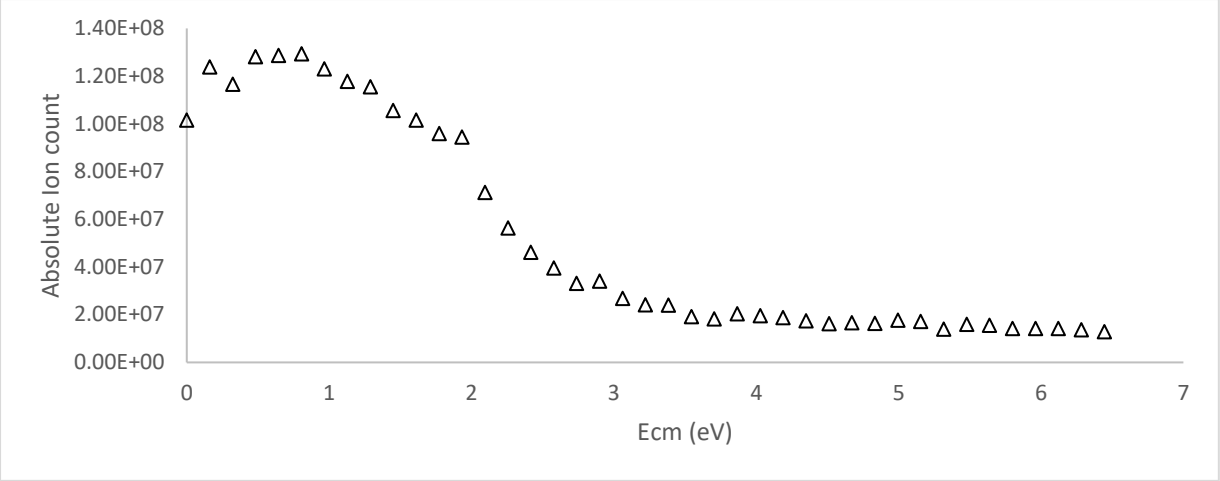


Figure S10: Sum of the absolute ion abundance of *m/z* 97, 103 & 228 over the energy ramp used in the CID experiments for compound (**12**). The abundance drops substantially going from low to high energy.

S6 Additional Methodology

Spectroscopic analysis and materials

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using either Bruker Ascend 400 MHz or Bruker Avance 400 MHz Spectrometers at 298 K using deuterated methanol solvent. Data is reported in parts per million (ppm), referenced to residual protons or ^{13}C in deuterated methanol solvent (CD_3OD: ^{1}H 3.31 ppm, ^{13}C 49.00 ppm) unless otherwise specified, with multiplicity assigned as follows: br = broad, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. Coupling constants 'J' are reported in Hertz (Hz).

Materials

Chemicals and solvents including ^{18}O-labelled sulfuric acid ($H_2S[^{18}O_4]$, 95 atom % in $H_2[^{18}O]$, 95 atom %), sodium borohydride ($NaBH_4$), dihydrotestosterone (17β-hydroxy-5α-androstan-3-one), estrone (3-hydroxyestra-1,3,5(10)-trien-17-one), and sulfur trioxide pyridine complex (SO_3•Py), were purchased from Sigma–Aldrich (Castle Hill, Australia). Alternatively, ^{18}O-labelled sulfuric acid ($H_2S[^{18}O_4]$, 95 atom % in $H_2[^{18}O]$, 95 atom %) was purchased from Icon Isotopes (Dexter MI, USA). Androsterone (3α-hydroxy-5α-androstan-17-one), etiocholanolone (3α-hydroxy-5β-androstan-17-one), epiandrosterone (3β-hydroxy-5α-androstan-17-one), and testosterone (17β-hydroxyandrost-4-en-3-one) were obtained from Steraloids (Newport RI, USA). Dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one) was obtained from BDH (Poole, UK). Epitestosterone (17α-hydroxyandrost-4-en-3-one) was synthesised from testosterone using literature methods.[1] Acetic anhydride was freshly distilled using literature methods.[2] MilliQ water was used in all aqueous solutions. Liquid chromatography (gradient) grade methanol was obtained from Merck (Kilsyth, Australia). Aqueous ammonia solution was obtained from ChemSupply (Gillman, Australia). Formic acid was obtained from Ajax Chemicals (Auburn, Australia). Solid-phase extraction (SPE) was performed using Waters (Rydalmere, Australia) Oasis weak anion exchange (WAX) 6 cc cartridges (186004647), Oasis WAX 3 cc cartridges (PN 186002492) or Sep-Pak Vac C18 3 cc cartridges (PN 186004619).

Instrumentation

For compound characterisation, ^{1}H NMR and 13C NMR spectra were recorded in deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), or deuterium monoxide (D_2O), using a Bruker Avance 400 MHz or 700 MHz spectrometer at 298 K. Chemical shifts are reported in parts per million (ppm) downfield shift from TMS (δ=0), as follows: chemical shift (δ) (multiplicity, coupling constant(s) J (Hz), relative integral, where multiplicity is defined as, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, or combinations of the foregoing. The signal due to the residual protonated solvent (i.e., $CHCl_3$) or ^{13}C was used as an internal reference. Low resolution mass spectrometry (LRMS) using negative or positive electrospray ionisation (ESI) was performed on a Micromass ZMD ESI-Quad. High resolution mass spectrometry (HRMS) was performed on a Waters LCT Premier XE mass spectrometer or a Thermo-Fischer Scientific Orbitrap Elite™ Hybrid Ion Trap-Orbitrap mass spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1800 Series FTIR spectrometer. Melting points were measured on an SRS Opti-melt MPA 100 automated melting point system and are uncorrected. Reactions were monitored by analytical thin layer chromatography (TLC) performed on aluminium-backed 0.2 mm thick silica gel 60 F254 plates as supplied by Merck. Eluted plates were visualised by staining using a solution of sulfuric acid: methanol (5% v/v), followed by heating. Flash chromatographic separations were carried out following protocols defined by Still et al.[3] with silica gel 60 (40–63 µm) as the stationary phase and analytical reagent (AR) or HPLC-grade solvents as indicated. Tetrahydrofuran (THF), methanol, and dichloromethane were dried using a glass contour solvent purification system based on technology originally described by Grubbs et al.[4] Optical rotations were performed on a Rudolph Research Analytical, Autopol I Automatic Polarimeter (589 nm fixed wavelength, 10 mm cell).

Synthesis characterisation

Melting points were determined using an SRS Optimelt MPA 100 melting point apparatus and are uncorrected. For synthesis characterization, low-resolution mass spectrometry (LRMS) and high-resolution mass spectrometry (HRMS) were performed using positive electron ionisation (+EI) on a Micromass VG Autospec mass spectrometer or negative electrospray ionization (–ESI) on a Micromass ZMD ESI-Quad, or a Waters LCT Premier XE mass spectrometer. Reactions were monitored by analytical thin layer chromatography (TLC) using Merck Silica gel 60 TLC plates (7:2:1 ethyl acetate: methanol: water, unless otherwise specified) and were visualised by staining with concentrated sulfuric acid in methanol (5% v/v), with heating as required.

General procedure 4 for determining conversion by ^{1}H NMR analysis

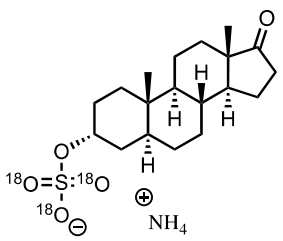
This step was used to calculate the ratio of steroid sulfate conjugate relative to free steroid or steroid diol remaining in the reaction mixture after a conjugation or small-scale reduction reaction. The procedure employed a modified WAX SPE procedure (general procedure 2), with, washing only performed with formic acid in water (2% v/v, 15 mL) and water (15 mL), followed by elution with aqueous ammonia solution in methanol (5% v/v, 15 mL). This fraction resulted in a mixture of product and any unreacted starting material. A ^{1}H NMR spectrum was obtained and integration of a suitable signal (typically C3-H or C17-H) from both the starting steroid and steroid product was used to determine the percent conversion of sulfation or reduction.

General procedure 5: C18 purification of steroid bis(sulfate) compounds. This was used to separate a steroid bis(sulfate) from any unreacted steroid mono-sulfates following a reaction or epimers in applicable cases. The procedure was adapted from the literature.[2] A C18 SPE cartridge (3 cc, Waters Oasis®) was preconditioned with methanol (3 mL) followed by water (3 mL), under positive pressure of nitrogen. The bis(sulfate)/mono-sulfate mixture (1 mg mL^{-1}, water) was then loaded onto the cartridge. The bis(sulfate) was then eluted with methanol:water (10-50 % v/v, 3 mL) and collected, the remaining mono-sulfate or epimer was eluted by methanol (3 mL). The methanol:water fraction was concentrated *in vacuo* to yield the steroid bis(sulfate) as the corresponding ammonium salt.

General procedure 6: for small scale reduction of steroid sulfates. This reduction was primarily used to reduce saturated ketones of mono-sulfates. The procedure adapted was from the literature.[2,9] Sodium borohydride (7.0 mg, 0.19 mmol) was added slowly (over one minute) to an ice cooled stirring solution of steroid conjugate (10 mg mL^{-1}, methanol). After the reaction subsided (no gas evolution observed) it was capped and stirred at room temperature for 2 hours. The reaction mixture was quenched by the addition of water (10 mL) and adjusted to pH 7 (universal indicator strips) by addition of aqueous hydrochloric acid (0.1 M, ≈ 2-3 mL). The resulting solution was subject to extraction by SPE as outlined in general procedures 2 and 3.

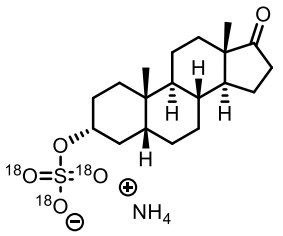
S7 Synthesis of $^{18}O_3$-labelled mono-sulfate compounds

Androsterone 3[$^{18}O_3$]-sulfate, ammonium salt (**2**)



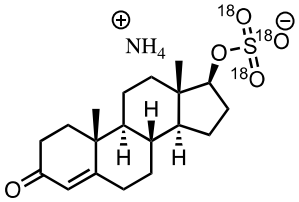
Androsterone (5.00 mg, 17.2 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This gave the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 4.59 (br s, 1H, C3-H), 2.43 (dd, J 19.2, 8.7 , 1H, C16-H), 2.13 – 1.90 (m, 3H), 1.88 – 1.45 (m, 10H), 1.40 – 1.17 (m, 6H), 1.06 (m, 1H), 0.87 (s, 3H, C18-H_3), 0.86 (s, 3H, C19-H_3), 0.81 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 224.2 (C3), 76.3 (C17), 55.9, 52.8, 40.8, 36.9, 36.7, 36.4, 34.6, 33.9, 32.8, 32.0, 29.3, 27.9, 22.7, 21.2, 14.2 (C18), 11.8 (C19), one carbon overlapping or obscured. LRMS (-ESI): *m/z* 375.3 (100%, $[C_{19}H_{29}[^{18}O_3]O_2S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{29}[^{18}O_3]O_2S]^-$ ($[M\text{-}NH_4]^-$) 375.1863, found 375.1867. Spectroscopic data and spectra matched the literature for the unlabelled compound.[10]

Etiocholanolone 3[$^{18}O_3$]-sulfate, ammonium salt (**3**)



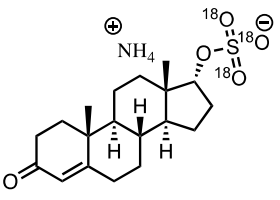
Etiocholanolone (5.00 mg, 17.2 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 4.29 (m, 1H, C3-H), 2.43 (dd, J 19.2, 8.6, 1H, C16-H), 2.09 (m, 1H, C16-H), 2.02 – 1.81 (m, 6H), 1.81 – 1.72 (m, 2H), 1.73 – 1.17 (m, 11H), 1.16 (m, 1H), 0.99 (s, 3H, C18-H_3), 0.86 (s, 3H, C19-H_3); ^{13}C NMR (101 MHz, CD_3OD) δ 224.1 (C17), 80.2 (C3), 52.6, 43.7, 42.1, 36.8, 36.7, 36.4, 35.8, 34.5, 32.9, 28.8, 28.0, 26.5, 23.7, 22.8, 21.2 (C18), 14.2 (C19), one carbon overlapping or obscured; LRMS (-ESI): m/z 375.3 (100%, $[C_{19}H_{29}[^{18}O_3]O_2S]^-$; HRMS (-ESI) m/z calcd. for $[C_{19}H_{29}[^{18}O_3]O_2S]^-$ ($[M\text{-}NH_4]^-$) 375.1863, found 375.1865. Spectroscopic data and spectra matched the literature for the unlabelled compound.[10]

Testosterone 17[$^{18}O_3$]-sulfate, ammonium salt (**4**)



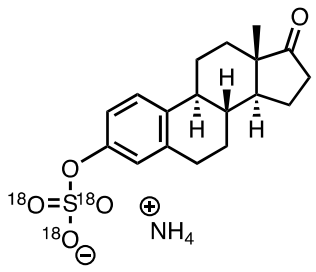
Testosterone (5.00 mg, 17.3 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 5.71 (d, J 1.7, 1H, C4-H), 4.23 (t, J 8.5, 1H, C17-H), 2.55 – 2.41 (m, 2H), 2.36 – 2.23 (m, 2H), 2.18 (m, 1H), 2.9 (m, 1H), 2.0 (m, 1H), 1.90 (m, 1H), 1.82 – 1.56 (m, 5H), 1.55 – 1.29 (m, 2H), 1.24 (s, 3H, C18-H_3), 1.19 (m, 1H), 1.12 – 0.93 (m, 3H), 0.87 (s, 3H, C19-H_3); ^{13}C NMR (101 MHz, CD_3OD) δ 202.4 (C3), 175.2 (C5), 124.1 (C4), 87.9 (C17), 55.4, 51.3, 43.8, 40.1, 37.7, 36.8, 34.7, 33.9, 32.8, 29.1, 24.3, 21.6, 17.7 (C18), 12.0 (C19), one carbon overlapping or obscured; LRMS (-ESI): m/z 373.2 (100%, $[C_{19}H_{27}[^{18}O_3]O_2S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{27}[^{18}O_3]O_2S]^-$ ($[M\text{-}NH_4]^-$) 373.1707, found 373.1706. Spectroscopic data and spectra matched the literature for the unlabelled compound.[10]

Epitestosterone 17[$^{18}O_3$]-sulfate, ammonium salt (**5**)



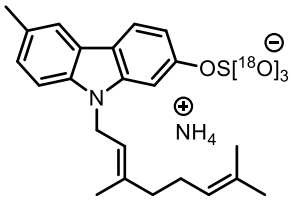
Epitestosterone (5.00 mg, 17.3 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This gave the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 5.71 (d, J 1.7, 1H, C4-H), 4.35 (d, J 5.8, 1H, C17-H), 2.56 – 2.44 (m, 2H), 2.36 – 2.25 (m, 2H), 2.25 – 2.06 (m, 2H), 2.02 – 1.90 (m, 2H), 1.86 – 1.36 (m, 8H), 1.29 (m, 1H), 1.24 (s, 3H, C18-H_3), 1.16 – 0.93 (m, 2H), 0.81 (s, 3H, C19-H_3); ^{13}C NMR (101 MHz, CD_3OD) δ 202.4 (C3), 175.3 (C5), 124.1 (C4), 87.7 (C17), 55.2, 50.5, 46.1, 40.1, 37.1, 36.8, 34.7, 34.0, 33.6, 32.7, 31.2, 25.5, 21.6, 17.7 (C18), 17.2 (C19); LRMS (-ESI): m/z 373.2 (100%, $[C_{19}H_{27}[^{18}O_3]O_2S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{27}[^{18}O_3]O_2S]^-$ ($[M\text{-}NH_4]^-$) 373.1707, found 373.1701. Spectroscopic data and spectra matched the literature for the unlabelled compound.[10]

Estrone 3[$^{18}O_3$]-sulfate, ammonium salt (**6**)



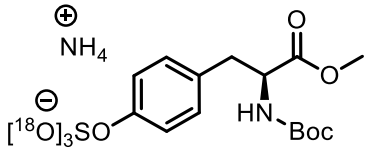
Estrone (5.00 mg, 17.3 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL), with stirring for 2 days. The final (SIL) sulfated product was separated from unreacted free steroid using general procedure 2 followed by general procedure 3. This gave the title compound as a colourless solid (70 % conversion). ^{1}H NMR (700 MHz, CD_3OD): δ 7.24 (d, J 8.5, 1H), 7.06 – 7.01 (m, 2H), 2.92 – 2.88 (m, 2H), 2.49 (dd, J 19.0, 8.7, 1H, C16-H), 2.46 – 2.41 (m, 1H), 2.32 – 2.27 (m, 1H), 2.19 – 2.12 (m, 1H, C16-H), 2.11 – 2.00 (m, 2H), 1.93 – 1.88 (m, 1H), 1.73 – 1.63 (m, 1H), 1.63 – 1.41 (m, 5H), 0.93 (s, 3H, C18-H_3); ^{13}C NMR (101 MHz, CD_3OD) δ 223.7 (C17), 151.8, 138.7, 137.5, 127.0, 122.5, 119.8, 51.7, 45.5, 39.7, 36.7, 32.8, 30.5, 27.6, 27.0, 22.5, 14.3 (C18), one peak overlapping or obscured; LRMS (-ESI): m/z 355.2 (100%, [$C_{18}H_{21}[^{18}O_3]O_2S]^-$); HRMS (-ESI) *m/z* calcd. for $[C_{18}H_{21}[^{18}O_3]O_2S]^-$ ([M-NH_4]$^-$) 355.1237, found 355.1236. Spectroscopic data and spectra matched the literature for the unlabelled compound.[10]

Karapinchamine A [$^{18}O_3$]-sulfate, ammonium salt (**7**)



Karapinchamine A.[11] (5.5 mg, 16.5 µmol**)** was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). The final (SIL) sulfated product was separated from unreacted material using general procedure 2 followed by general procedure 3. This gave the title compound as a colourless solid (> 96% conversion). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.44 (s, 1H), 7.27 (s, 1H), 7.09 (d, J 8.2, 1H), 7.05 (d, J 8.3, 1H), 6.98 (d, J 8.2, 1H), 4.97 (s, 1H), 4.79 (s, 1H), 4.46 (s, 2H), 2.35 (s, 3H), 1.84 – 1.77 (m, 2H), 1.73 – 1.68 (m, 2H), 1.58 (s, 3H), 1.47 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 150.2, 141.0, 139.3, 138.7, 131.5, 128.3, 126.7, 124.0, 122.8, 120.8, 120.4, 120.2, 119.7, 112.5, 109.0, 102.3, 41.1, 39.3, 26.3, 25.6, 21.4, 17.7, 16.4; LRMS (-ESI): m/z 418.2 (100%, $[C_{23}H_{26}N[^{18}O_3]OS]^-$); HRMS (-ESI) m/z calcd. for $[C_{23}H_{26}N[^{18}O_3]OS]^-$ ([M-NH_4]$^-$) 418.1721, found 418.1710.

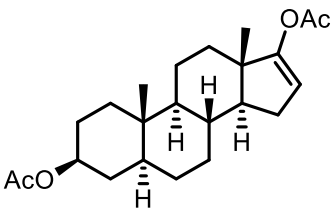
N-(Boc)-L-tyrosine methyl ester sulfate**,** ammonium salt (**8**)



N-(Boc)-L-tyrosine methyl ester (6 mg, 20.3 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). The final (SIL) sulfated product was separated from unreacted material using general procedure 2 followed by general procedure 3. This gave the title compound as a colourless solid (> 89% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 7.23 (m, 2H), 7.17 (m, 2H), 4.33 (dd, J 8.8, 5.5, 1H), 3.69 (s, 3H), 3.07 (dd, J 13.9, 5.6, 1H), 2.91 (dd, J 13.9, 8.8, 1H), 1.39 (s, 9H); ^{13}C NMR (176 MHz, MeOD) δ 174.2, 157.8, 152.9, 134.8, 130.9, 122.5, 80.7, 56.6, 52.6, 37.9, 28.7, four peaks obscured or overlapping); LRMS (-ESI): m/z 380.2 (100%, $[C_{15}H_{20}N[^{18}O_3]O_5S]^-$); HRMS (-ESI) m/z calcd. for $[C_{15}H_{20}N[^{18}O_3]O_5S]^-$ ([M-NH_4]$^-$) 380.1037, found 380.1031. Spectroscopic data and spectra matched the literature for the unlabelled compound.[12]

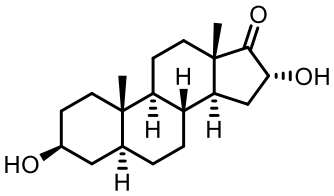
S8 Synthesis of $^{18}O_3$- labelled steroid bis(conjugates)

3ß,17-Diacetoxy-5α-androstan-16-ene (**29**)



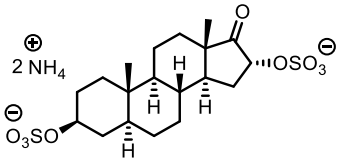
The procedure was modified from literature.[1] To a solution of epiandrosterone (1.02 g, 3.51 mol) in isopropenyl acetate (25 mL), concentrated sulfuric acid (three drops) was added. The solution was heated at reflux (110 ˚ C) for 24 h. A further amount of isopropenyl acetate (2 x 3 mL) was added at 4 h and 8 h of reflux respectively. Upon cooling, the reaction mixture was then diluted with diethyl ether (25 mL) and the organic layer was washed with saturated aqueous sodium bicarbonate solution (3 x 10 mL) followed by brine (25 mL). The organic layer was concentrated under reduced pressure to give a brown crude oil. The crude then was subjected to column chromatography (silica, 9:1 n-hexane:EtOAc) to give the title compound as a colourless solid (0.389 g, 30.0 % yield): R_f = 0.29 (9:1 n-hexane:EtOAc), m.p. 85-90 ˚C (lit.[1] 89-90 ˚ C); $[\alpha]_{24}^{D}$ +17.4 (c 0.5, $CHCl_3$) [lit.[1] $[\alpha]_{20}^{D}$ +75.3 (c 1.0, $CHCl_3$]; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.46 (dd, J 3.3, 1.6, 1H), 4.69 (tt, J 11.4, 4.9, 1H), 2.14 (s, 3H), 2.02 (s, 3H), 1.43 (m, 20H), 0.88 (s, 3H), 0.85 (s, 3H); LRMS (+ESI): m/z 397.3 (100%, $[C_{23}H_{34}O_4Na]^+$). HRMS (+ESI) m/z calcd. for $[C_{29}H_{35}O_4]^+$ ($[M+H]^+$) 375.2524, found 375.2530. Spectroscopic data and spectra was found to match the literature for the compound.[1]

3ß,16α-Dihydroxy-5α-androstan-17-one (**30**)



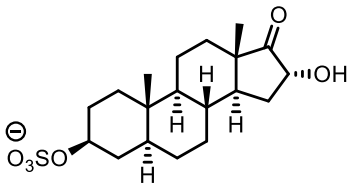
The procedure was modified from the literature.[13] *m*CPBA (167 mg, 0.968 mmol) was added to a stirring solution of 3ß,17-diacetoxy-5α-androstan-16-ene (**29**) (121 mg, 0.323 mmol) in dichloromethane (3 mL).The reaction was stirred at room temperature for 2 h, at which point the consumption of starting material was indicated by TLC (9:1 n-hexane:EtOAc). The reaction mixture was then diluted with dichloromethane (10 mL) and then washed with aqueous sodium thiosulfate (5 % w/w, 10 mL), which was followed by aqueous sodium carbonate (1 M, 10 mL). The organic layer was then dried using anhydrous magnesium sulfate and concentrated under reduced pressure, giving the crude epoxide as a colourless solid, which was used without purification in the next step. The crude epoxide was dissolved in stirring methanol (1.40 mL), 1-4-dioxane (1.40 mL) and aqueous sulfuric acid (3 M, 3 mL). The reaction was left to stir for 12 h, at which point the full consumption of the crude epoxide was determined by TLC (9:1 n-hexane:EtOAc). The reaction mixture was then diluted with ethyl acetate (10 mL) and washed with sodium hydroxide (1 M, 3 x 3 mL). The organic layer was dried with anhydrous $MgSO_4$, and concentrated under reduced pressure to give the title compound as a colourless solid (59.5 mg, 60 %). R_f = 0.30 (9:1 n-hexane:EtOAc); m.p. 180-183 ˚C (lit.[13] 182-183 ˚ C); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.36 (d, J 7.8, 1H), 3.62 (m, 1H), 2.05 (s, 1H), 1.99 – 1.10 (m, 18H), 0.96 (s, 3H), 0.83 (s, 3H), 0.70 (m 1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 219.6, 71.5, 71.3, 54.5, 48.4, 47.8, 44.9, 38.2, 37.0, 35.8, 35.2, 31.5, 31.5, 30.7, 28.5, 20.3, 14.3, 12.5, one peak overlapping or obscured; LRMS (+ESI): m/z 329.2 ($[C_{19}H_{30}O_3Na]^+$); HRMS (+ESI) m/z calcd. for $[C_{19}H_{30}O_3Na]^+$ ($[M+Na]^+$) 329.2092, found 329.2093. Spectroscopic data and spectra was found to match the literature for the compound.[13]

3ß,16α-Dihydroxy-5α-androstan-17-one 3,16-bis(sulfate), ammonium salt (**31**)



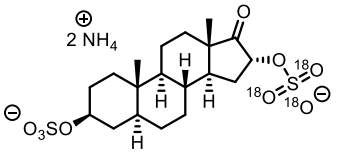
3ß,16α-Dihydroxy-5α-androstan-17-one (**30**) (5.00 mg, 15.2 µmol), was reacted according to general procedure 1 for sulfation using unlabelled sulfuric acid. This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 4.93 (d, J 8.2, 1H, C16-H), 4.26 (m, 1H, C3-H), 2.25 (m, 1H, C15-H), 2.09 – 1.92 (m, 3H), 1.90 – 1.16 (m, 13H), 1.09 – 0.99 (m, 2H), 0.95 (s, 3H, C18-H_3), 0.88 (s, 3H, C19-H_3), 0.77 (m, 1H);^{13}C NMR (101 MHz, CD_3OD) δ 216.3 (C17), 79.5, 77.7, 55.6, 55.1, 50.0, 46.2, 38.0, 36.6, 36.3, 36.2, 32.8, 31.8, 30.7, 29.7, 29.5, 21.3, 14.6 (C18), 12.6 (C19); LRMS (-ESI): m/z 232.2 (50%, $[C_{19}H_{28}O_9S_2]^{2-}$). HRMS (-ESI) m/z calcd. for $[C_{19}H_{29}O_9S_2]^-$ ($[M-2NH_4+H]^-$) 465.1253, found 465.1261.

3ß,16α-Dihydroxy-5α-androstan-17-one 3-sulfate, ammonium salt (**32**)



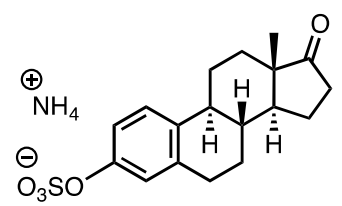
The procedure was adapted from the literature.[9] 3ß,16α-Dihydroxy-5α-androstan-17-one 3,16-bis(sulfate), ammonium salt (**31**) (5.00 mg, 10.6 µmol), PaS wild type enzyme (100 µL, 60 mg mL^{-1}), Tris-HCl buffer (500 µL, 1M, pH 8.2), Milli-Q water (9.30 mL) was added to a falcon tube and left to stand at room temperature overnight (approximately 16 h). The reaction mixture was then subjected to SPE purification as outlined in the general procedures 2 and 3, respectively. This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 4.31 (d, J 8.5, 1H, C16-H), 4.25 (m, 1H, C3-H), 2.05 – 1.96 (m, 2H), 1.88 – 1.16 (m, 15H), 1.10 – 0.99 (m, 2H), 0.93 (s, 3H, C18-H_3), 0.87 (s, 3H, C19-H_3), 0.76 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 221.0, 79.5, 72.3, 55.8, 55.1, 46.2, 38.1, 36.7, 36.3, 36.2, 32.8, 32.5, 31.8, 29.7, 29.5, 21.2, 14.7 (C18), 12.6 (C19), one peak overlapping or obscured; LRMS (-ESI): *m/z* 385.1 (100%, $[C_{19}H_{29}O_6S]^-$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{29}O_6S_1]^-$ ($[M-NH_4]^-$) 385.1693, found 385.1685.

3ß,16α-Dihydroxy-5α-androstan-17-one 3,16[$^{18}O_3$]-bis(sulfate), ammonium salt (**9**)



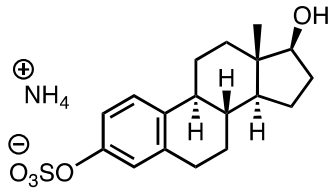
3ß,16α-Dihydroxy-5α-androstan-17-one 3-sulfate, ammonium salt (**32**) (5.00 mg, 12.3 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 4.93 (d, *J* 8.3, 1H, C16-H), 4.26 (m, 1H, C3-H), 2.26 (dd, *J* 14.2, 6.6, 1H, C15-H), 2.09 – 1.92 (m, 2H), 1.88 – 1.15 (m, 14H), 1.11 – 0.96 (m, 2H), 0.95 (s, 3H, C18-H_3), 0.88 (s, 3H, C19-H_3), 0.80 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 216.1 (C17), 79.5, 77.7, 55.6, 50.0, 46.2, 38.0, 36.7, 36.3, 36.2, 32.8, 31.8, 30.8, 29.7, 29.5, 21.3, 14.6 (C18), 12.6 (C19), one peak overlapping or obscured; LRMS (-ESI): *m/z* 235.2 (50%, $[C_{19}H_{28}[^{18}O_3]O_5S_2]^{2-}$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{29}[^{18}O_3]O_5S_2]^-$ ($[M-2NH_4+H]^-$) 471.1380, found 471.1379.

Estrone-3-sulfate, ammonium salt (**17**)



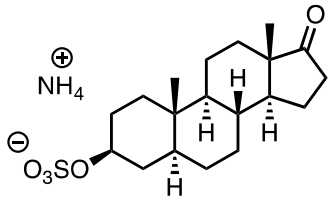
The procedure was modified from the literature.[10] Estrone (5.00 mg, 18.4 µmol) was added to a stirring solution of pyridine-sulfur trioxide complex (50.0 mg, 0.472 mmol, 17.1 eq.) in DMF (1 mL). The reaction was capped and stirred at room temperature for 2 h. The reaction was then quenched with water (7.50 mL) and subjected to SPE purification according to the general procedures 2 and 3 for purification by SPE. This gave the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD): δ 7.25 (d, J 8.3, 1H), 7.08 – 6.98 (m, 2H), 2.93 – 2.82 (m, 2H), 2.55 – 2.38 (m, 2H), 2.33 – 2.25 (m, 1H), 2.21 – 1.99 (m, 3H), 1.93 – 1.86 (m, 1H), 1.73 – 1.40 (m, 6H), 0.93 (s, 3H, C18-H); LRMS (-ESI): m/z 349.2 (100%, $[C_{18}H_{21}O_5S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{21}O_5S]^-$ ($[M-NH_4]^-$) 349.1110, found 349.1111. Spectroscopic data and spectra was found to match the literature for the compound.[2,10]

Estradiol 3-sulfate, ammonium salt (**33**)



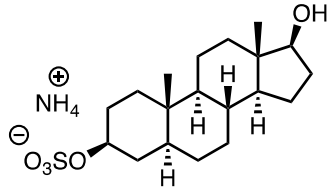
Estrone 3-sulfate, ammonium salt (**17**) (5.00 mg, 13.7 µmol) was reacted according to the general procedure 6 for small scale reduction. This gave the title compound as a colourless powder (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD): δ 7.24 (d, J 8.5, 1H), 7.08 – 6.92 (m, 2H), 3.67 (t, J 8.6, 1H), 2.88 – 2.79 (m, 2H), 2.39 – 2.30 (m, 1H), 2.24 – 2.13 (m, 1H), 2.08 – 1.85 (m, 3H), 1.79 – 1.63 (m, 1H), 1.59 – 1.14 (m, 8H), 0.78 (s, 3H, C18-H_3); CNMR δ 151.6, 138.8, 138.2, 127.0, 122.5, 119.7, 82.5, 51.4, 45.5, 44.3, 40.2, 38.0, 30.7, 30.6, 28.4, 27.5, 24.0, 11.7; LRMS (-ESI): m/z 351.2 (100%, $[C_{18}H_{23}O_5S]^-$); HRMS m/z calcd. for $[C_{18}H_{23}O_5S]^-$ ($[M\text{-}NH_4]^-$) 351.1266, found 351.1266. Spectroscopic data and spectra was found to match the literature for the compound.[2,9]

Epiandrosterone 3-sulfate (**22**)



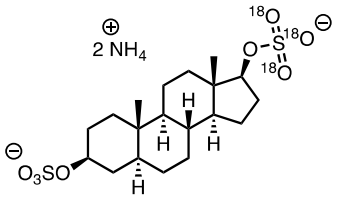
Epiandrosterone (5.00 mg, 17.2 µmol) was reacted according to general procedure 1 for sulfation using unlabelled sulfuric acid (non-SIL). This yielded the title compound as a white solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD): δ 4.25 (m, 1H, C3-H), 2.42 (m, 1H,C16-H), 2.11 – 1.91 (m, 3H), 1.85 – 1.16 (m, 15H), 1.14 – 1.00 (m, 2H), 0.88 (s, 3H, C18-H_3), 0.87 (s, 3H, C19-H_3), 0.75 (m, 1H); LRMS (-ESI): m/z 369.3 (100%, $[C_{19}H_{29}O_5S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{29}O_5S]^-$ ($[M\text{-}NH_4]^-$) 369.1738, found 369.1730. Spectroscopic data and spectra was found to match the literature for the compound.[10]

5α-Androstane-3β,17β-diol 3-sulfate, ammonium salt (**25**)



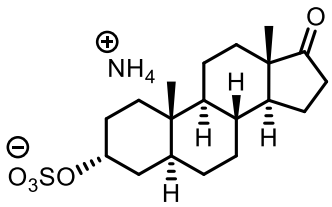
Epiandrosterone 3-sulfate, ammonium salt (**22**) (5.00 mg, 13.6 µmol) was reacted according to general procedure 6 for small scale reduction. This gave the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD): δ 4.25 (m, 1H, C3-H), 3.56 (t, J 8.6, 1H, C17-H), 2.05 – 1.90 (m, 2H), 1.87 – 1.65 (m, 4H), 1.64 – 1.11 (m, 11H), 1.10 – 0.85 (m, 4H), 0.86 (s, 3H, C18-H_3), 0.72 (s, 3H C19-H_3), 0.67 (m, 1H); ^{13}C NMR (101 MHz, CD_3OD) δ 82.5 (C3), 79.7, 55.9, 52.4, 46.4, 44.1, 38.3, 38.1, 36.9, 36.6, 36.4, 32.8, 30.7, 29.8, 24.3, 21.9, 12.7 (C18), 11.7 (C19), one peak overlapping or obscured; LRMS (-ESI): *m/z* 371.3 (100%, $[C_{19}H_{31}O_5S]^-$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{31}O_5S]^-$ ($[M\text{-}NH_4]^-$) 371.1899, found 371.1892.

5α-Androstane-3β,17β[$^{18}O_3$]-diol bis(sulfate) (**11**)



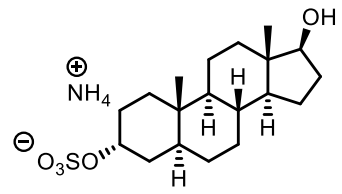
5α-Androstane-3β,17β-diol 3-sulfate, ammonium *salt* (**25**) (5.00 mg, 13.5 µmol), was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (700 MHz, CD_3OD): δ 4.27 – 4.18 (m, 2H, C3-H & C17-H), 2.19 – 2.11 (m, 1H), 2.07 – 1.99 (m, 1H), 1.98 – 1.90 (m, 1H), 1.84 – 1.67 (m, 4H), 1.64 – 1.49 (m, 3H), 1.49 – 1.38 (m, 2H), 1.35 – 1.24 (m, 4H), 1.21 – 1.12 (m, 2H), 1.09 – 0.98 (m, 2H), 0.98 – 0.89 (m, 1H), 0.86 (s, 3H, C18-H3), 0.80 (s, 3H, C19-H3), 0.73 – 0.66 (m, 1H); ^{13}C NMR (151 MHz, CD_3OD) δ 88.2, 79.7, 55.8, 51.8, 46.3, 44.0, 38.2, 38.0, 36.8, 36.6, 36.4, 32.8, 29.8, 29.2, 24.4, 21.8, 12.7 (C18), 12.2 (C19), one peak overlapping or obscured; LRMS (-ESI): m/z 228.2 (100%, $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$ ($[M\text{-}2NH_4]^{2-}$) 228.07602, found 228.07650. NMR spectra matched the literature for the unlabelled compound.[9]

Androsterone 3-sulfate, ammonium salt (**19**)



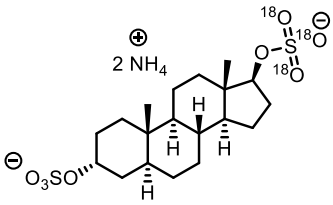
Androsterone (5.00 mg, 17.2 µmol) was reacted according to general procedure 1 for sulfation using unlabelled sulfuric acid (non-SIL). This gave the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 4.62 (m, 1H,C3-H), 2.44 (m, 1H, C16-H), 2.12 – 1.91 (m, 3H), 1.86 – 1.79 (m, 1H), 1.80 – 1.43 (m, 9H), 1.43 – 1.17 (m, 6H), 1.12 – 1.00 (m, 1H), 0.87 (s, 3H, C18-H_3), 0.86 (s, 3H, C19-H_3), 0.85-0.79 (m, 1H); LRMS (-ESI): m/z 369.3 (100%, $[C_{19}H_{29}O_5S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{29}O_5S]^-$ ($[M\text{-}NH_4]^-$) 369.1738, found 369.1730. Spectroscopic data and spectra was found to match the literature for the compound.[10]

5α-Androstane-3α,17β-diol 3-sulfate, ammonium salt (**34**)



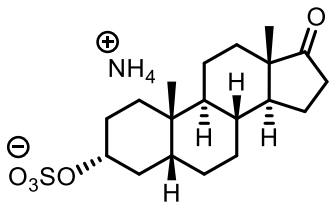
Androsterone 3-sulfate, ammonium salt (**19**) (5.00 mg, 13.6 µmol) was reacted according to general procedure 6 for small scale reduction. This gave the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD): δ 4.59 (m, 1H, C3-H), 3.57 (t, J 8.7, 1H, C17-H), 2.00 – 1.92 (m, 2H), 1.83 (m, 1H), 1.76 – 1.54 (m, 5H), 1.52 – 1.17 (m, 10H), 1.09 – 0.90 (m, 3H), 0.84 (s, 3H, C18-H_3), 0.75 (m, 1H), 0.72 (s, 3H, C19-H_3); ^{13}C NMR (101 MHz, CD_3OD) δ 82.5 (C17), 79.7, 55.9, 52.3, 46.4, 44.1, 38.3, 38.1, 36.9, 36.6, 36.4, 32.8, 30.7, 29.8, 24.3, 21.9, 12.7 (C18), 11.7 (C19), one peak obscured or overlapping; LRMS (-ESI): m/z 371.3 (100%, $[C_{19}H_{31}O_5S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{31}O_5S]^-$ ($[M\text{-}NH_4]^-$) 371.18977, found 371.18892.

5α-Androstane-3α,17β[$^{18}O_3$]-diol bis(sulfate) (**12**)



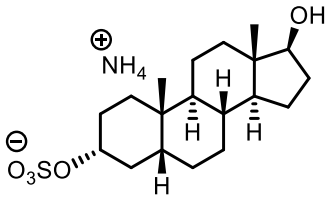
5α-Androstane-3α,17β-diol 3-sulfate, ammonium salt (**34**) (5.00 mg, 13.5 µmol), was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (700 MHz, CD_3OD): δ 4.59 (m, 1H, C3-H), 4.22 (m, 1H, C17-H), 2.22-2.08 (m, 2H), 2.03-1.89 (m, 2H), 1.78-1.54 (m, 6H), 1.53-1.41 (m, 3H), 1.38-1.11 (m, 6H), 1.10-0.89 (m, 2H), 0.84 (s, 3H, C18-H_3), 0.80 (s, 3H, C19-H_3), 0.76 (m, 1H); ^{13}C NMR (176 MHz, CD_3OD) δ 88.3, 76.4, 55.9, 51.9, 44.0, 40.8, 38.1, 36.8, 34.7, 33.9, 32.8, 29.5, 29.2, 27.9, 24.4, 21.4, 12.2, 11.9, one peak obscured or overlapping; LRMS (-ESI): *m/z* 228.2 (100%, $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$ ($[M\text{-}2NH_4]^{2-}$) 228.0755, found 228.0760. Spectra matched the literature for the unlabelled compound.[9]

Etiocholanolone 3-sulfate, ammonium salt (**21**)



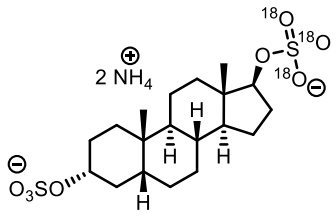
Etiocholanolone (5.00 mg, 17.2 µmol) was reacted according to general procedure 1 for sulfation using unlabelled sulfuric acid (non-SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 4.29 (m, 1H, C3-H), 2.43 (dd, J = 19.2, 8.6 Hz, 1H), 2.14 – 2.02 (m, 1H, C16-H), 2.02 – 1.81 (m, 5H), 1.77 (ddt, J = 11.6, 5.4, 2.5 Hz, 2H), 1.71 – 1.20 (m, 12H), 1.10 – 1.01 (m, 1H), 0.99 (s, 3H, C18-H_3), 0.87 (s, 3H,C19-H_3); LRMS (-ESI): m/z 369.3 (100%, $[C_{19}H_{29}O_5S]^-$; HRMS (-ESI) m/z calcd. for $[C_{19}H_{29}O_5S]^-$ ($[M\text{-}NH_4]^-$), 369.1737, found 369.1730. Spectroscopic data and spectra was found to match the literature for the compound.[10]

5β-Androstane-3α,17β-diol 3-sulfate, ammonium salt (**35**)



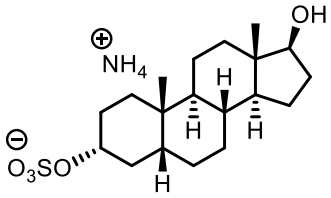
Etiocholanolone 3-sulfate, ammonium salt (**21**) (5.00 mg, 13.6 µmol) was reacted according to general procedure 6 for small scale reduction. This gave the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (700 MHz, CD_3OD) δ 4.28 (m, 1H), 3.59 (t, J=8.6, 1H), 2.04 – 1.80 (m, 6H), 1.76 (m, 1H), 1.59 (m, 1H), 1.52 – 1.39 (m, 8H), 1.36 – 1.19 (m, 3H), 1.15 – 0.98 (m, 3H), 0.96 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (176 MHz, CD_3OD) δ 82.5, 80.4, 52.3, 44.2, 43.7, 42.1, 38.2, 37.3, 36.5, 35.7, 34.6, 30.7, 28.9, 28.1, 27.2, 24.3, 23.8, 21.5, 11.7; LRMS (-ESI): *m/z* 371.2 (100%, $[C_{19}H_{31}O_5S]^-$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{31}O_5S]^-$ ($[M\text{-}NH_4]^-$), 371.18977, found 371.1889.

5β-Androstane-3α,17β[$^{18}O_3$]-diol bis(sulfate), ammonium salt (**13**)



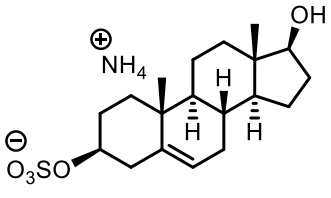
5β-Androstane-3α,17β-diol 3-sulfate, ammonium salt (**35**) (5.00 mg, 13.5 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (700 MHz, CD_3OD) δ 4.32 – 4.21 (m, 2H), 2.17 (m, 1H), 1.97 – 1.84 (m, 5H), 1.78 – 1.68 (m, 2H), 1.62 (m, 1H), 1.51 – 1.41 (m, 6H), 1.33 – 1.24 (m, 3H), 1.24 – 1.08 (m, 3H), 1.04 (m, 1H), 0.96 (s, 3H), 0.79 (s, 3H); ^{13}C NMR (176 MHz, CD_3OD) δ 88.2, 80.4, 51.8, 49.0, 44.1, 43.7, 42.0, 38.2, 37.1, 36.5, 34.6, 29.3, 28.9, 28.2, 27.1, 24.4, 23.8, 21.4, 12.1; LRMS (-ESI): *m/z* 228.2 (100%, $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$ ($[M\text{-}2NH_4]^{2-}$) 228.0755, found 228.07545. Spectra matched the literature for the unlabelled compound.[9]

Dehydroepiandrosterone 3-sulfate, ammonium salt (**18**)



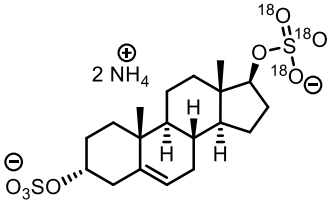
Dehydroepiandrosterone (5.00 mg, 17.3 µmol) was reacted according to general procedure for sulfation 1 using unlabelled sulfuric acid (non-SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 5.44 (m, 1H), 4.14 (m, 1H), 2.56 (m, 1H), 2.45 (m, 1H), 2.37 (m, 1H), 2.18 – 2.04 (m, 3H), 2.02 – 1.90 (m, 2H), 1.80 (m, 1H), 1.74 – 1.50 (m, 6H), 1.39 – 1.25 (m, 2H), 1.13 (m, 1H), 1.07 (s, 3H), 1.05 (m, 1H), 0.90 (s, 3H); LRMS (-ESI): m/z 367.1 (100%, $[C_{19}H_{27}O_5S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{27}O_5S]^-$ ($[M\text{-}NH_4]^-$) 367.15737, found 367.15780. Spectroscopic data and spectra was found to match the literature for the compound.[10]

5-Androstene-3β,17β-diol 3-sulfate, ammonium salt (**36**)



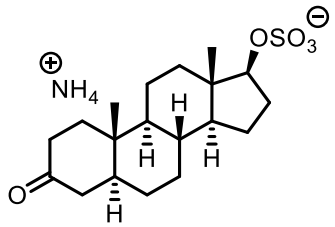
Dehydroepiandrosterone 3-sulfate, ammonium salt (**18**) (5.00 mg, 13.6 µmol) was reacted according to general procedure 6 for small scale reduction. This gave the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (700 MHz, CD_3OD) δ 5.39 (m, 1H), 4.13 (m, 1H), 3.58 (t, J 8.6 Hz, 1H), 2.54 (m, 1H), 2.35 (m, 1H), 2.07 (m, 1H), 2.03 – 1.96 (m, 2H), 1.95 – 1.83 (m, 2H), 1.69 – 1.44 (m, 8H), 1.28 (m, 1H), 1.16 - 1.08 (m, 2H), 1.05 (s, 3H), 1.02 – 0.94 (m, 2H), 0.76 (s, 3H); ^{13}C NMR (176 MHz, CD_3OD) δ 141.7, 123.1, 82.5, 79.8, 52.7, 51.8, 43.9, 40.4, 38.5, 37.9, 37.8, 33.3, 32.6, 30.6, 30.0, 24.4, 21.8, 19.8, 11.5; LRMS (-ESI): m/z 369.2 (100%, $[C_{19}H_{29}O_5S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{29}O_5S]^-$ ($[M\text{-}NH_4]^-$) 369.17412, found 369.17334.

5-Androstene-3β,17β[$^{18}O_3$]-diol bis(sulfate), ammonium salt (**14**)



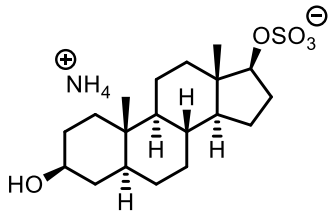
5-Androstene-3β,17β-diol 3-sulfate, ammonium salt (**36**) (5.00 mg, 13.5 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This yielded the title compound as a colourless solid with > 98% conversion. ^{1}H NMR (700 MHz, CD_3OD) δ 5.40 (m, 1H), 4.23 (m, 1H), 4.14 (m, 1H), 2.54 (m, 1H), 2.35 (m, 1H), 2.17 (m, 1H), 2.09 - 2.05 (m, 1H), 2.03 - 1.97 (m, 2H), 1.92 (m, 1H), 1.75 (m, 1H), 1.69 - 1.45 (m, 6H), 1.32 (m, 1H), 1.24 - 1.09 (m, 2H), 1.05 (s, 3H), 1.04 - 0.95 (m, 2H), 0.83 (s, 3H); ^{13}C NMR (176 MHz, CD_3OD) δ 141.7, 123.0, 88.1, 79.8, 52.1, 51.7, 43.8, 40.4, 38.5, 37.8, 37.8, 33.2, 32.6, 30.0, 29.2, 24.4, 21.7, 19.8, 12.0; LRMS (-ESI): *m/z* 227.2 (100%, $[C_{19}H_{28}[^{18}O_3]O_5S_2]^{2-}$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{28}[^{18}O_3]O_5S_2]^{2-}$ ($[M\text{-}2NH_4]^{2-}$) 227.0676, found 227.0685. Spectra matched the literature for the unlabelled compound.[9]

Dihydrotestosterone 3-sulfate, ammonium salt (**23**)



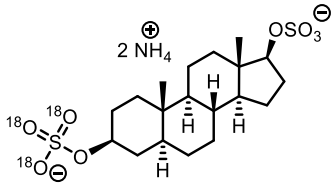
Dihydrotestosterone (5.00 mg, 17.2 µmol) was reacted according to general procedure for sulfation 1 using unlabelled sulfuric acid (non-SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 4.22 (t, J = 8.5 Hz, 1H), 2.49 (m, 1H), 2.35 (m, 1H), 2.28 – 1.92 (m, 5H), 1.80 – 1.12 (m, 13H), 1.07 (s, 3H), 1.07 (m, 1H), 0.83 (s, 3H), 0.77 (m, 1H); LRMS (-ESI): m/z 369.3 (100%, $[C_{19}H_{29}O_5S]^-$; HRMS (-ESI) m/z calcd. for $[C_{19}H_{29}O_5S]^-$ ($[M\text{-}NH_4]^-$) 369.17412, found 369.17353. Spectroscopic data and spectra was found to match the literature for the compound.[10]

5α-Androstane-3β,17β-diol 17-sulfate, ammonium salt (**37**)



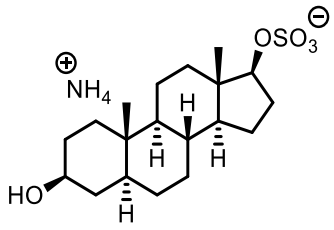
Dihydrotestosterone 3-sulfate, ammonium salt (**23**)(5.00 mg, 13.6 µmol) was reacted according to general procedure 6 for small scale reduction. This gave the title compound as a colourless solid (> 98% conversion as a mixture of 3α-OH : 3β-OH in a 3:10 ratio). Data reported for major epimer. ^{1}H NMR (600 MHz, CD_3OD) δ 4.21 (m, 1H), 3.51 (m, 1H), 2.15 (m, 1H), 1.94 (m, 1H), 1.78 – 1.67 (m, 4H), 1.65 – 1.49 (m, 4H), 1.48 – 1.09 (m, 10H), 1.06 – 0.88 (m, 2H), 0.84 (s, 3H), 0.79 (s, 3H), 0.68 (m, 1H); ^{13}C NMR (151 MHz, CD_3OD) δ 141.7, 123.0, 88.1, 79.8, 52.1, 51.7, 43.8, 40.4, 38.5, 37.8, 37.8, 33.2, 32.6, 30.0, 29.2, 24.4, 21.7, 19.8, 12.0; LRMS (-ESI): *m/z* 371.2 (100%, $[C_{19}H_{31}O_5S]^-$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{31}O_5S]^-$ ($[M\text{-}NH_4]^-$), 371.18977, found 371.18908.

5α-Androstane-3β[$^{18}O_3$],17β-diol bis(sulfate), ammonium salt (**15**)



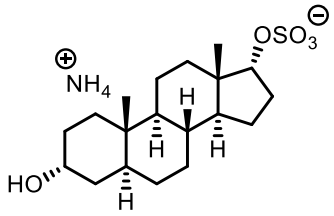
5α-Androstane-3β,17β-diol 17-sulfate, ammonium salt (**37**) (5.00 mg, 13.5 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This yielded the title compound as a colourless solid (> 98% conversion as a mixture of 3α-OH : 3β-OH in a 7 : 100 ratio). Data reported for major epimer**.** ^{1}H NMR (600 MHz, CD_3OD) δ 4.30 – 4.18 (m, 2H), 2.16 (m, 1H), 2.06 – 1.91 (m, 2H), 1.83 – 1.67 (m, 4H), 1.66 – 1.50 (m, 3H), 1.48 – 1.38 (m, 2H), 1.38 – 1.24 (m, 4H), 1.20 – 1.13 (m, 2H), 1.08 – 0.89 (m, 3H), 0.86 (s, 3H), 0.80 (s, 3H), 0.69 (m, 1H); ^{13}C NMR (176 MHz, CD_3OD) δ 88.2, 79.7, 55.8, 51.8, 46.3, 44.0, 38.2, 38.0, 36.8, 36.6, 36.4, 32.8, 29.8, 29.2, 24.4, 21.8, 12.7, 12.2, one peak obscured or overlapping; LRMS (-ESI): *m/z* 228.2 (100%, $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$ ($[M\text{-}2NH_4]^{2-}$) 228.0755, found 228.0760.

Epidihydrotestosterone 3-sulfate, ammonium salt (**20**)



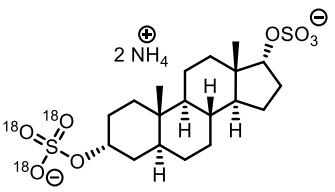
Epidihydrotestosterone (5.00 mg, 17.2 µmol) was reacted according to general procedure for sulfation 1 using unlabelled sulfuric acid (non-SIL). This yielded the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD) δ 4.33 (d, J = 5.7 Hz, 1H), 2.49 (m, 1H), 2.37 (m, 1H), 2.28 – 1.90 (m, 5H), 1.81 – 1.63 (m, 4H), 1.61 – 1.31 (m, 8H), 1.22 (m, 1H), 1.07 (s, 3H), 1.01 (m, 1H), 0.82 (m, 1H), 0.77 (s, 3H); LRMS (-ESI): m/z 369.2 (100%, $[C_{19}H_{29}O_5S]^-$; HRMS (-ESI) m/z calcd. for $[C_{19}H_{29}O_5S]^-$ ($[M\text{-}NH_4]^-$) 369.17412, found 369.17353. Spectroscopic data and spectra was found to match the literature for the compound.[10]

5α -Androstane-3α,17α-diol 17-sulfate, ammonium salt (**38**)



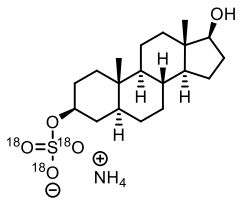
L-Selectride® was added dropwise to a stirring solution of dihydrotestosterone 3-sulfate, ammonium salt (**20**) (5.00 mg, 13.6 µmol) in anhydrous THF (0.5 mL) cooled at -78 °C. The reaction mixture was stirred for 2h and allowed to warm to room temperature. The reaction was then quenched with the addition of water (7-8 mL) and adjusted to a pH of 7 with aqueous hydrochloric acid (0.1 M, 2-3 mL). The reaction was then purified by SPE according to the general procedures 2 and 3. This gave the title compound as a colourless solid (96 % conversion as a mixture of 3α-OH : 3β-OH in a 10:3 ratio). Data reported for major epimer**.** ^{1}H NMR (600 MHz, CD_3OD) δ 4.32 (m, 1H), 3.95 (m, 1H), 2.14 (m, 1H), 1.95 (m, 1H), 1.79 – 1.72 (m, 3H), 1.70 – 1.14 (m, 12H), 0.93 – 0.85 (m, 6H), 0.82 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (151 MHz, CD_3OD) δ 88.0, 67.3, 55.7, 51.1, 46.2, 40.3, 37.2, 36.8, 33.7, 32.9, 31.2, 29.8, 27.5, 25.6, 21.3, 17.3, 15.8, 14.0, 11.7; LRMS (-ESI): m/z 371.2 (100%, $[C_{19}H_{31}O_5S]^-$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{31}O_5S]^-$ ($[M\text{-}NH_4]^-$), 371.18977, found 371.18902.

5α-Androstane-3β[$^{18}O_3$],17β-diol bis(sulfate), ammonium salt (**16**)



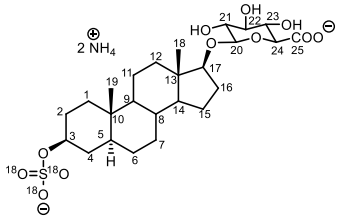
5α-Androstane-3β,17β-diol 17-sulfate, ammonium salt (**38**) (5.00 mg, 13.5 µmol) was reacted according to general procedure 1 for sulfation with enriched sulfuric acid (SIL). This yielded the title compound as a colourless solid (> 98% conversion as a mixture of 3α-OH : 3β-OH in a 10 : 3 ratio). Separation of the two epimers was achieved using general procedure 4. Specifically, three fractions of 3 mL were collected with an eluent of 25 % methanol in water was used. With the desired product being obtained in the second of these fractions. Data reported for major epimer**.** ^{1}H NMR (600 MHz, CD_3OD) δ 4.30 – 4.18 (m, 2H), 2.16 (m, 1H), 2.06 – 1.91 (m, 2H), 1.83 – 1.67 (m, 4H), 1.66 – 1.50 (m, 3H), 1.48 – 1.38 (m, 2H), 1.38 – 1.24 (m, 4H), 1.20 – 1.13 (m, 2H), 1.08 – 0.89 (m, 3H), 0.86 (s, 3H), 0.80 (s, 3H), 0.69 (m, 1H); ^{13}C NMR (176 MHz, CD_3OD) δ 88.0, 76.4, 55.5, 51.0, 46.2, 40.8, 37.2, 36.8, 34.7, 34.0, 33.6, 32.9, 31.3, 29.6, 27.9, 25.6, 21.3, 17.4, 11.9; LRMS (-ESI): m/z 228.2 (100%, $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$); HRMS (-ESI) m/z calcd. for $[C_{19}H_{30}[^{18}O_3]O_5S_2]^{2-}$ ($[M\text{-}2NH_4]^{2-}$) 228.0755, found 228.07561.

5α-Androstane-3β,17β-diol 3β[$S^{18}O_3$]-sulfate, ammonium salt (**26**)



Epiandrosterone 3β[$S^{18}O_3$]-sulfate, ammonium salt (**1**) (5.00 mg, 13.3 µmol) was reacted according to general procedure 6 for small scale reduction. This gave the title compound as a colourless powder with > 98% conversion. ^{1}H NMR (400 MHz, CD_3OD): δ 4.25 (m, 1H, C3-H), 3.56 (t, *J* 8.7, 1H, C17-H), 2.04 – 1.93 (m, 2H), 1.84 – 1.73 (m, 3H), 1.72 – 1.66 (m, 1H), 1.63 – 1.49 (m, 3H), 1.49 – 1.37 (m, 3H), 1.36 – 1.14 (m, 5H), 1.08 – 0.99 (m, 2H), 0.99 – 0.88 (m, 2H), 0.86 (s, 3H, C18-H_3), 0.72 (s, 3H, C19-H_3), 0.67 (m, 1H); ^{13}C NMR (176 MHz, CD_3OD) δ 82.53 (C17), 79.64, 55.92, 52.34, 46.37, 44.12, 38.27, 38.05, 36.92, 36.57, 36.35, 32.83, 30.64, 29.76, 24.31, 21.94, 12.68 (C18), 11.68 (C19), one peak obscured or overlapping; LRMS (-ESI): *m/z* 377.3 (100%, $[C_{19}H_{31}O_2[^{18}O_3]S]^-$); HRMS (-ESI) *m/z* calcd. for $[C_{19}H_{31}O_2[^{18}O_3]S]^-$ ($[M\text{-}NH_4]^-$) 377.2021, found 377.2020. NMR spectra matched the literature for the unlabelled compound.[10]

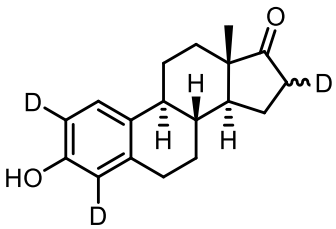
5α-Androstane-3β,17β-diol 3[$^{18}O_3$]-sulfate 17β-glucuronide, ammonium salt (**24**)



5α-Androstane-3β,17β-diol 3[$^{18}O_3$]-sulfate, ammonium salt (**26**) (3.00 mg, 7.59 µmol) was reacted according to the following procedure adapted from literature.[3] The steroid (0.7 mM) was dissolved in tert-butanol (10% v/v), and sodium phosphate buffer (50 mM, pH 7.5, ≈ 80% v/v), followed by *E. coli* E504G glucuronylsynthase. Following this, α-D-glucuronyl fluoride (5.0 eq. dissolved in sodium phosphate buffer 50 mM, pH 7.5) was added and the reaction incubated without agitation at 37 ˚C for 2 days. This gave the title compound as a colourless powder with 20% conversion. Isolation of the bis(conjugate) was achieved with 20% MeOH/H_2O eluent using general procedure 2.4.5. ^{1}H NMR (700 MHz, CD_3OD); δ 4.35 (d, $J_{H20\text{-}H21}$ = 7.8 Hz, 1H, C20-H), 4.27 – 4.22 (m, 1H, C3-H), 3.78 (t, J 8.7, 1H, C17-H), 3.54 (d, $J_{H23\text{-}H24}$ = 9.7 Hz, 1H, C24-H), 3.43 (app. t, J 9.3, 1H), 3.36 – 3.33 (m, 1H), 3.19 (app.t, J 8.5, 1H, C21-H), 2.09 – 1.92 (m, 2H), 1.84 – 1.49 (m, 7H), 1.48 – 1.12 (m, 9H), 1.05 – 0.87 (m, 3H), 0.85 (s, 3H,C18-H_3), 0.83 (s, 3H,C19-H_3), 0.69 (m, 1H); ^{13}C NMR (176 MHz, CD_3OD) δ 104.68, 89.75, 79.65, 75.32, 73.75, 55.80, 52.22, 49.00, 46.32, 44.37, 38.85, 38.25, 36.76, 36.56, 36.37, 32.77, 30.67, 29.78, 29.64, 24.27, 21.96, 12.67 (C18), 12.12 (C19) (COOH not observed); LRMS (-ESI): *m/z* 276.4 (100%, $[C_{25}H_{38}O_8[^{18}O_3]S]^{2-}$); HRMS (-ESI) *m/z* calcd. for $[C_{25}H_{39}O_8[^{18}O_3]S]^-$ ($[M\text{-}2NH_4\text{+}H]^-$) 553.2342, found 553.2335.

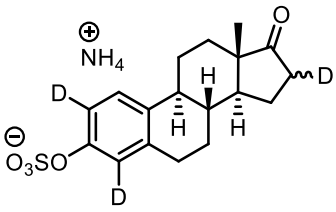
S9 Synthesis of deuterated derivatives of estrone sulfate

2,4,16-d_3-Estrone (**38**)



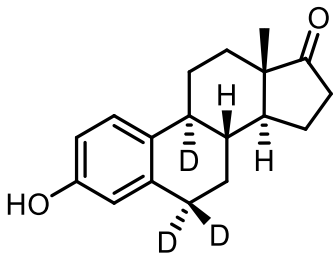
Estrone (20.0 mg, 74 µmol), and CF_3COOD (0.57 mL, 200 eq.) was added to a microwave reactor tube and sealed. The tube was heated for 30 seconds at 300 W in the microwave reactor. After irradiation the solution was evaporated to dryness under reduced pressure to give a colourless solid. This was followed by solvation of the solid in ethanol (5 mL) to allow –OD/-OH back-exchange. The crude product was then dissolved in ethyl acetate and filtered through silica to give the title compound as a colourless solid in a quantitative yield (20.0 mg, 73 µmol, 99 %).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15 (s, 1H), 4.51 (br s, 1H, OH), 2.92 – 2.83 (m, 2H), 2.51 – 2.44 (m, 1H), 2.41 – 2.33 (m, 1H), 2.31 – 2.19 (m, 1H), 2.10 – 1.91 (m, 2H), 1.72 – 1.36 (m, 7H), 0.91 (s, 3H, C18-H_3); LRMS (+ESI): *m/z* 296.3 (100 %, $[C_{18}H_{19}O_2D_3Na]^+$); HRMS (+ESI) *m/z* calcd. for $[C_{18}H_{20}O_2D_3]^+$ ($[M+H]^+$) 274.1884 , found 274.1886. Spectroscopic data was found to match the literature for the compound.[14]

2,4,16-d_3-Estrone 3-sufate (**27**)



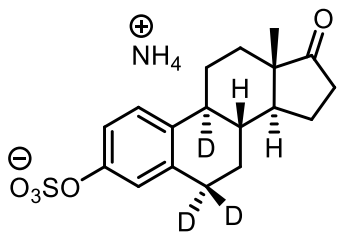
The procedure was modified from the literature.[10] *2,4,16-d_3-estrone* (**38**) (5 mg, 18.2 µmol) was added to a stirring solution of pyridine-sulfur trioxide complex (50.0 mg, 0.472 mmol, 17.1 equiv.) in dimethylformamide (1 mL). The reaction mixture was then capped and stirred at room temperature for 2 h. The reaction mixture was then quenched with water (7.50 mL) and subjected to SPE according to the general procedures 2 and 3. This gave the title compound as a colourless solid (98% conversion, 2% of un-deuterated estrone 3-sulfate observed by ^{1}H NMR). ^{1}H NMR (400 MHz, CD_3OD): δ 7.24 (s, 1H, C1-H), 2.93 – 2.86 (m, 2H), 2.50 – 2.40 (m, 2H), 2.34 – 2.26 (m, 1H), 2.09 – 2.02 (m, 1H), 1.93 – 1.88 (m, 1H), 1.70 – 1.40 (m, 7H), 0.93 (s, 3H, C18-H_3); ^{13}C NMR δ 223.7 (C17), 151.7, 138.6, 137.5, 126.8, 51.7, 45.5, 39.7, 36.7, 32.8, 30.4, 27.6, 27.0, 22.5, 22.4, 14.3 (C18), two carbons obscured or overlapping; LRMS (-ESI): m/z 352.2 (100%, $[C_{18}H_{18}D_3O_5S]^-$); HRMS (-ESI) m/z calcd. for $[C_{18}H_{18}D_3O_5S]^-$ ($[M-NH_4]^-$) 352.1298, found 352.1283.

6,6,9-d_3-Estrone (**39**)



A suspension of estrone (50 mg, 184 µmol), Pd/C (20 mg), THF (0.5 mL) and D_2O (0.5 mL) was stirred at 50 °C under an atmosphere of hydrogen for 2 d. The mixture was then diluted with diethyl ether (20 mL), and then filtered through cilite to remove the catalyst. The filtrate was then washed with water (20 mL), and the aqueous layer extracted with diethyl ether (3 x 10 mL). The combined organic layers were then washed with brine (30 mL), dried over MgSO4, filtered, and then concentrated under reduced pressure. The crude was then recrystallised in 70 % EtOH in water (5 mL per 10 mg of crude), this gave the title compound as a colourless solid (20.5 mg, 75 µmol, 40.8 %). ^{1}H NMR (400 MHz, CD_3OD): δ 7.09 (d, J = 8.5 Hz, 1H), 6.57 – 6.53 (m, 1H), 6.50 (s, 1H), 2.53 – 2.46 (m, 1H), 2.41 – 2.36 (m, 1H), 2.17 – 2.10 (m, 1H), 2.10 – 2.04 (m, 1H), 2.03 – 1.97 (m, 1H), 1.91 – 1.85 (m, 1H), 1.71 – 1.63 (m, 1H), 1.58 – 1.52 (m, 2H), 1.52 – 1.35 (m, 3H), 0.92 (s, 3H, C18-H_3); LRMS (+ESI): m/z 296.2 (100 %, $[C_{18}H_{19}O_2D_3Na]^+$); HRMS (+ESI) m/z calcd. for $[C_{18}H_{19}O_2D_3Na]^+$ ($[M+Na]^+$) 296.1706 , found 296.1704. Spectroscopic data was found to match the literature for the compound.[15]

6,6,9-d_3-Estrone 3-sufate (**28**)



The procedure was modified from the literature.[10] *6,6,9-d_3-estrone* (**39**) (5 mg, 18.2 µmol) was added to a stirring solution of pyridine-sulfur trioxide complex (50.0 mg, 0.472 mmol, 17.1 equiv.) in dimethylformamide (1 mL). The reaction mixture was then capped and stirred at room temperature for 2 h. The reaction mixture was then quenched with water (7.50 mL) and subjected to SPE according to the general procedures 2 and 3. This gave the title compound as a colourless solid (> 98% conversion). ^{1}H NMR (400 MHz, CD_3OD): δ 7.24 (m, 1H), 7.06 – 7.01 (m, 2H), 2.54 – 2.36 (m, 2H), 2.20 – 1.99 (m, 3H), 1.94 – 1.87 (m, 1H), 1.75 – 1.38 (m, 6H), 0.93 (s, 3H, C18-H3); 13C NMR (176 MHz, CD_3OD) δ 223.7, 151.83, 138.7, 138.6, 137.2, 127.0, 122.5, 121.4, 119.8, 51.7, 39.5, 36.8, 32.8, 27.4, 26.9, 22.5, 14.3 (C18), one peak overlapping or obscured; LRMS (-ESI): m/z 352.2 (100%, $[C_{18}H_{18}D_3O_5S]$-); HRMS (-ESI) m/z calcd. for $[C_{18}H_{18}D_3O_5S]$- ($[M\text{-}NH_4]$-) 352.1298, found 352.1284.

S10 References

(1) McKinney, A. R.; Cawley, A. T.; Young, E. B.; Kerwick, C. M.; Cunnington, K.; Stewart, R. T.; Ambrus, J. I.; Willis, A. C.; McLeod, M. D. *Bioanalysis* **2013**, *5* (7), 769–781.

(2) Pranata, A.; Fitzgerald, C. C.; Khymenets, O.; Westley, E.; Anderson, N. J.; Ma, P.; Pozo, O. J.; McLeod, M. D. *Steroids* **2019**, *143*, 25–40.

(3) Ma, P.; Kanizaj, N.; Chan, S.-A.; Ollis, D. L.; McLeod, M. D. *Org. Biomol. Chem.* **2014**, *12* (32), 6208–6214.

(4) Bouchoux, G.; Buisson, D.-A. *Int. J. Mass Spectrom.* **2006**, *249–250*, 412–419.

(5) Marshall, D. L.; Gryn'ova, G.; Coote, M. L.; Barker, P. J.; Blanksby, S. J. *Int. J. Mass Spectrom.* **2015**, *378*, 38–47.

(6) Schröder, D.; Engeser, M.; Schwarz, H.; Rosenthal, E. C. E.; Döbler, J.; Sauer, J. *Inorg. Chem.* **2006**, *45* (16), 6235–6245.

(7) Schröder, D.; Engeser, M.; Brönstrup, M.; Daniel, C.; Spandl, J.; Hartl, H. *Int. J. Mass Spectrom.* **2003**, *228* (2–3), 743–757.

(8) Zins, E. L.; Pepe, C.; Schröder, D. *J. Mass Spectrom.* **2010**, *45* (11), 1253–1260.

(9) McLeod, M. D.; Waller, C. C.; Esquivel, A.; Balcells, G.; Ventura, R.; Segura, J.; Pozo, Ó. J. *Anal. Chem.* **2017**, *89* (3), 1602–1609.

(10) Waller, C. C.; McLeod, M. D. *Steroids* **2014**, *92*, 74–80.

(11) Yan, Q.; Gin, E.; Wasinska-Kalwa, M.; Banwell, M. G.; Carr, P. D. *J. Org. Chem.* **2017**, *82* (8), 4148–4159.

(12) Vo, Y.; Schwartz, B. D.; Onagi, H.; Ward, J. S.; Gardiner, M. G.; Banwell, M. G.; Nelms, K.; Malins, L. R. *Chem. – A Eur. J.* **2021**, *27* (38), 9830–9838.

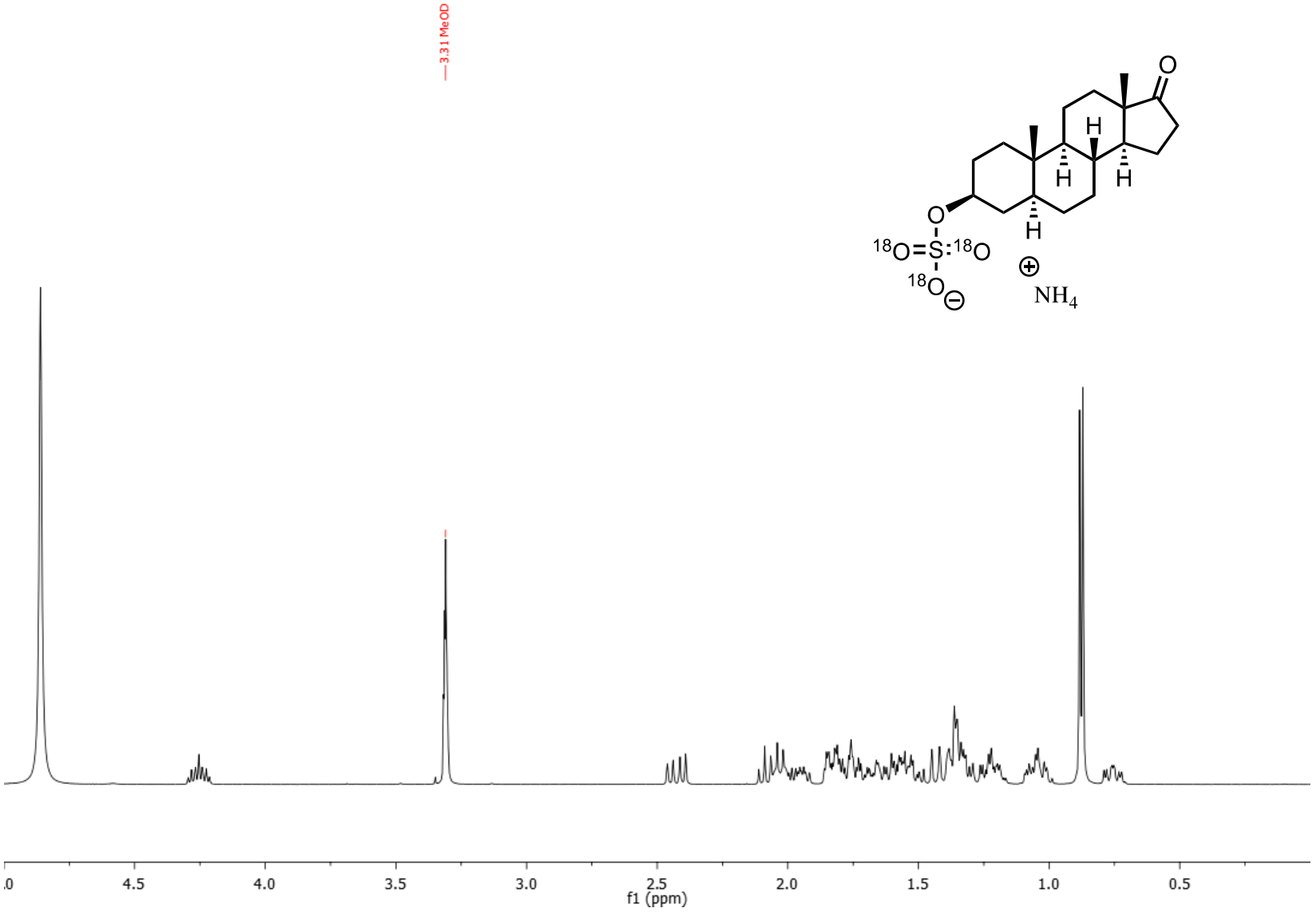
(13) Tokimatsu, K.; Yoshimi, Y.; Ariizumi, K. *Chem. Pharm. Bull.* **1990**, *30* (3), 153–158.

(14) Kiuru, P. S.; Wähälä, K. *Tetrahedron Lett.* **2002**, *43* (18), 3411–3412.

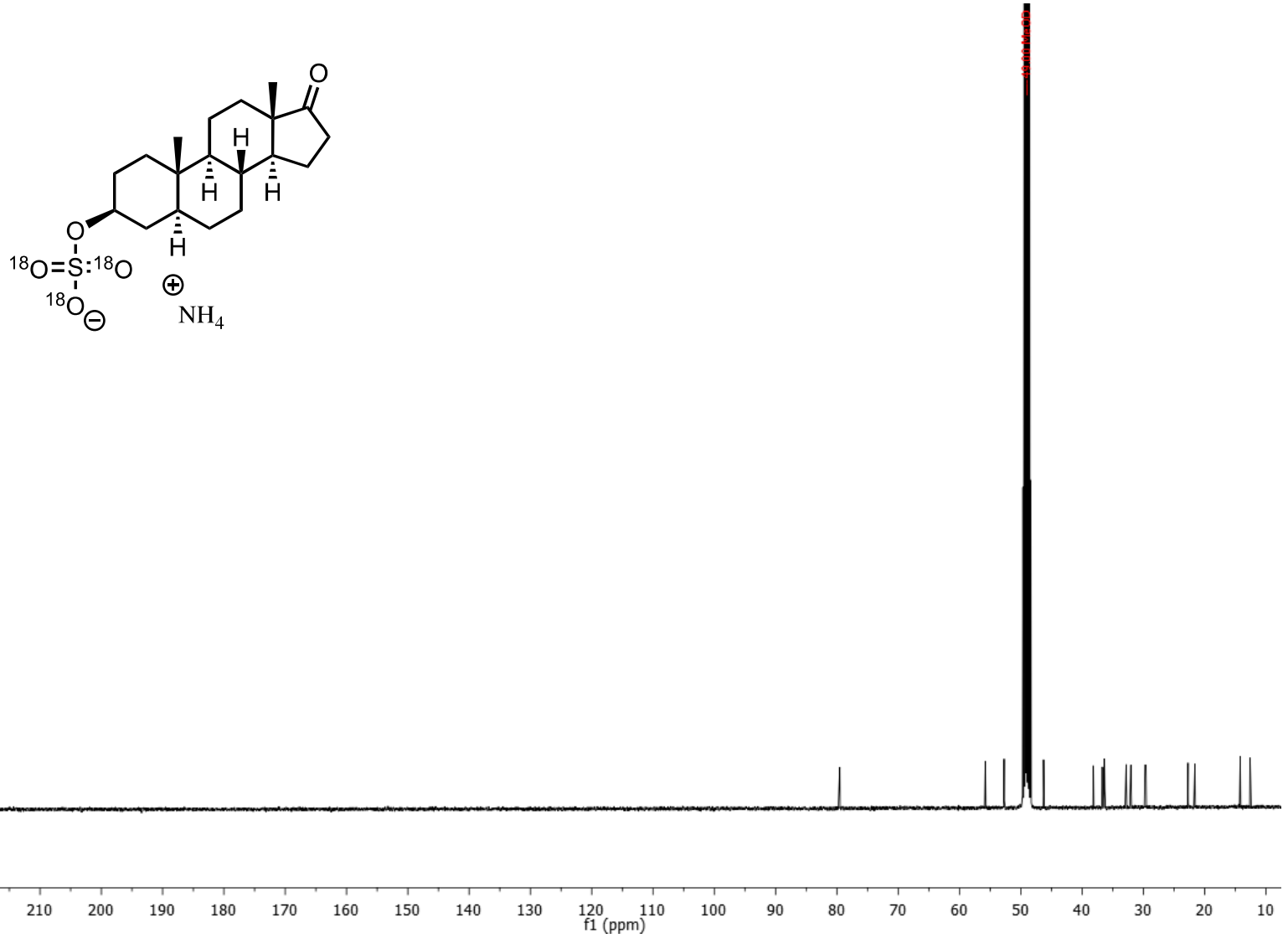
(15) Kurita, T.; Hattori, K.; Seki, S.; Mizumoto, T.; Aoki, F.; Yamada, Y.; Ikawa, K.; Maegawa, T.; Monguchi, Y.; Sajiki, H. *Chem. - A Eur. J.* **2008**, *14* (2), 664–673.

S11 Spectra for synthesised materials

Epiandrosterone 3[$^{18}O_3$]-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD

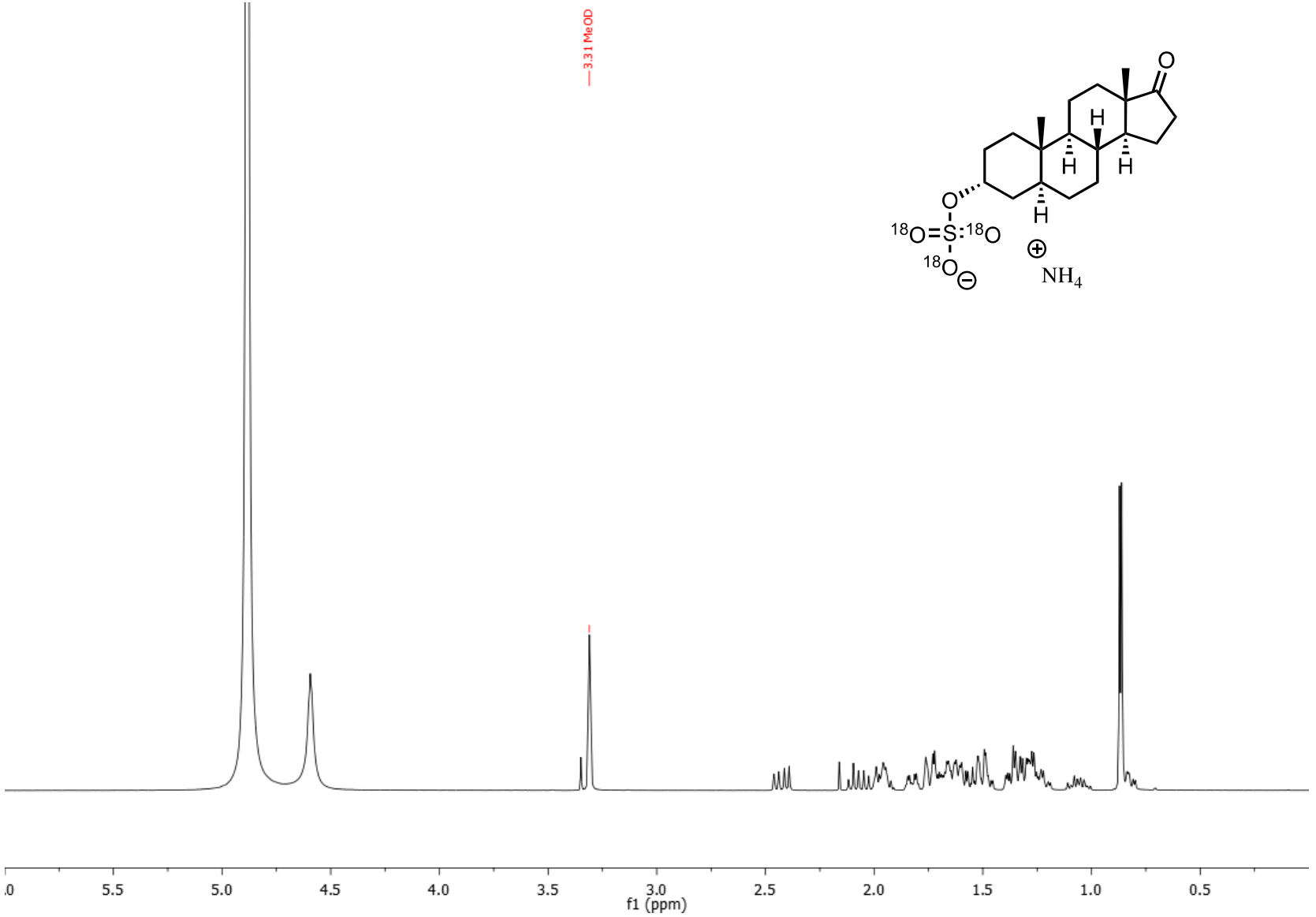


Epiandrosterone 3[$^{18}O_3$]-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD

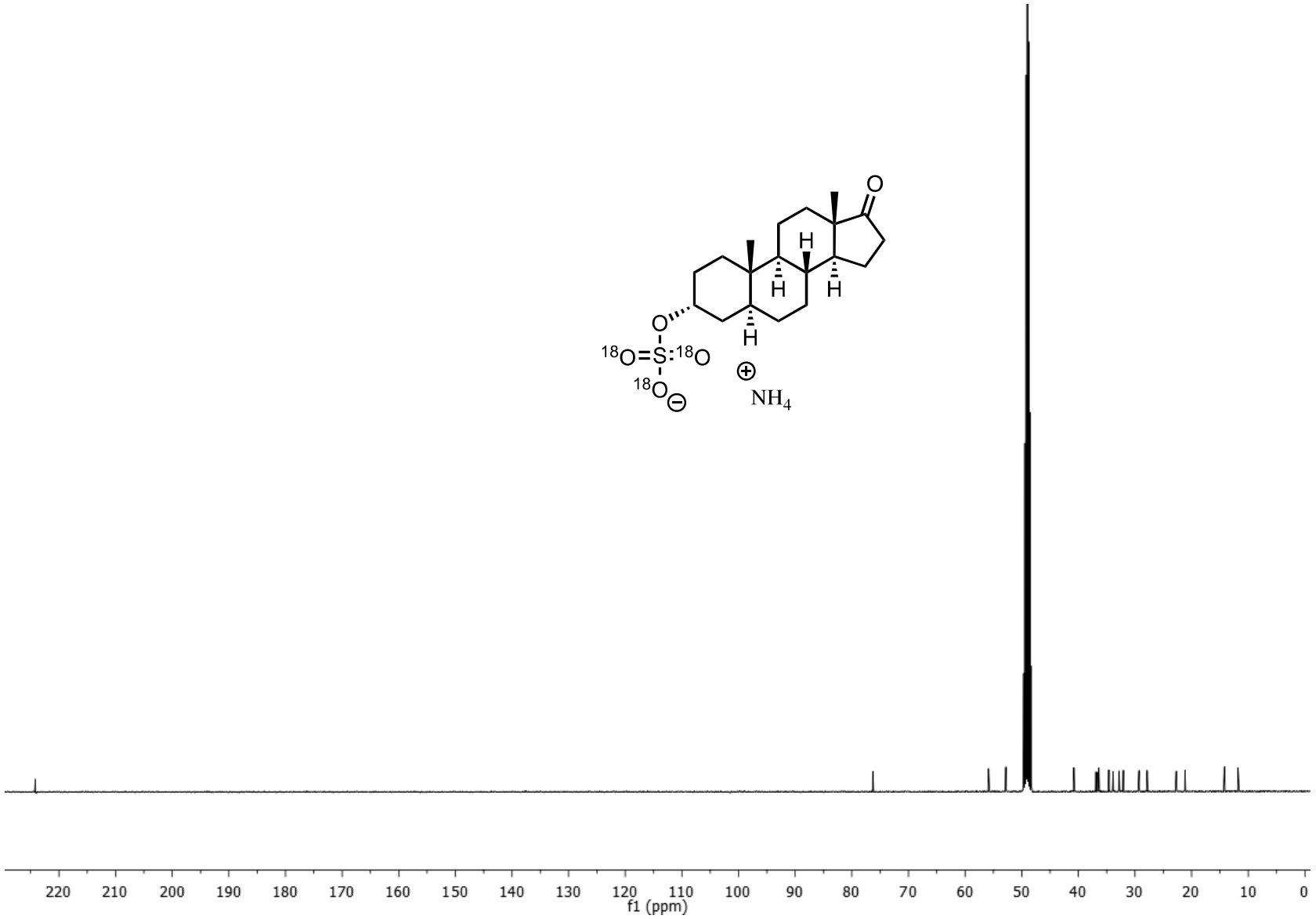


Epiandrosterone 3[$^{18}O_3$]-sulfate, ammonium salt LRMS

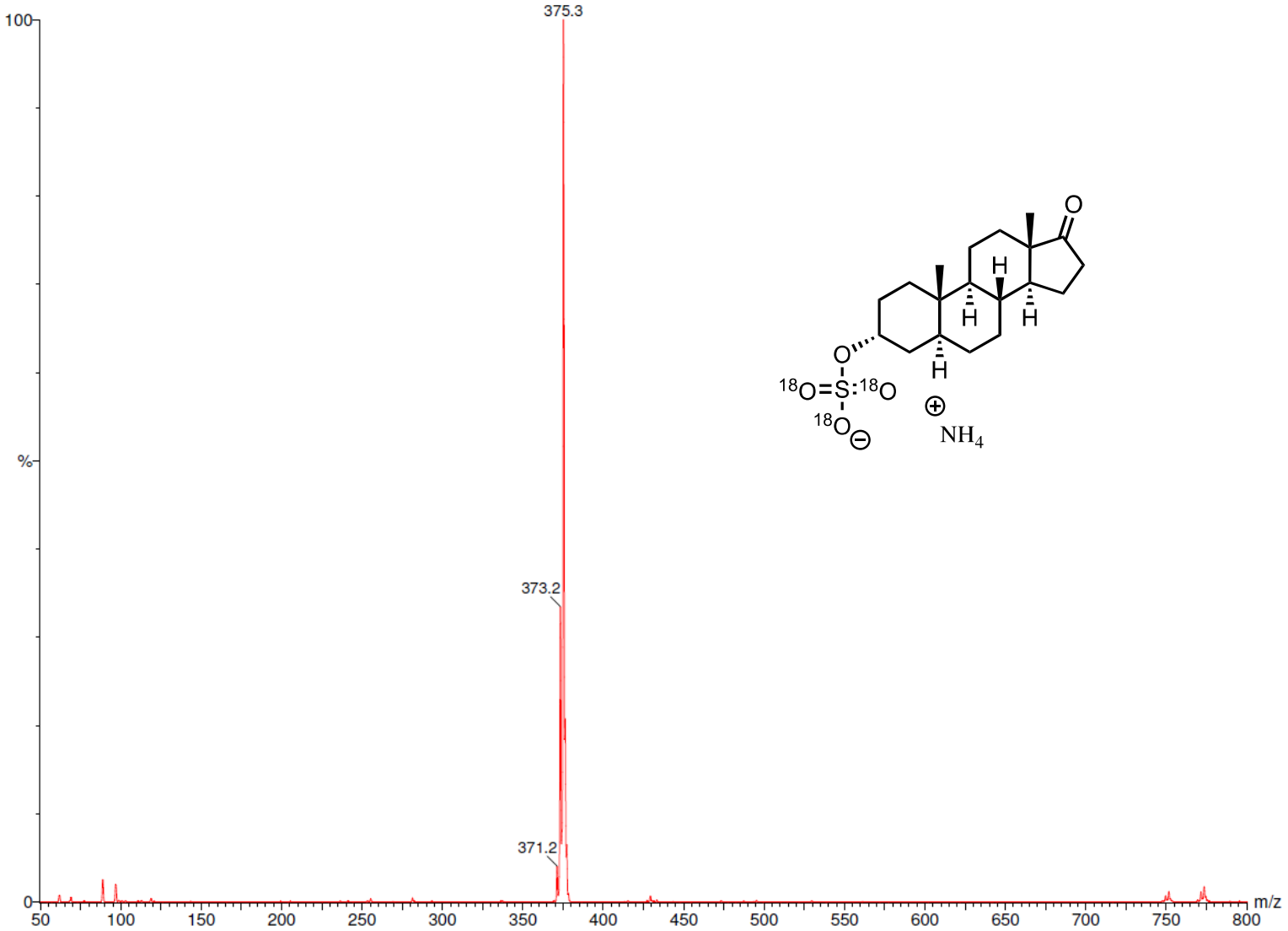
Androsterone 3[$^{18}O_3$]-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



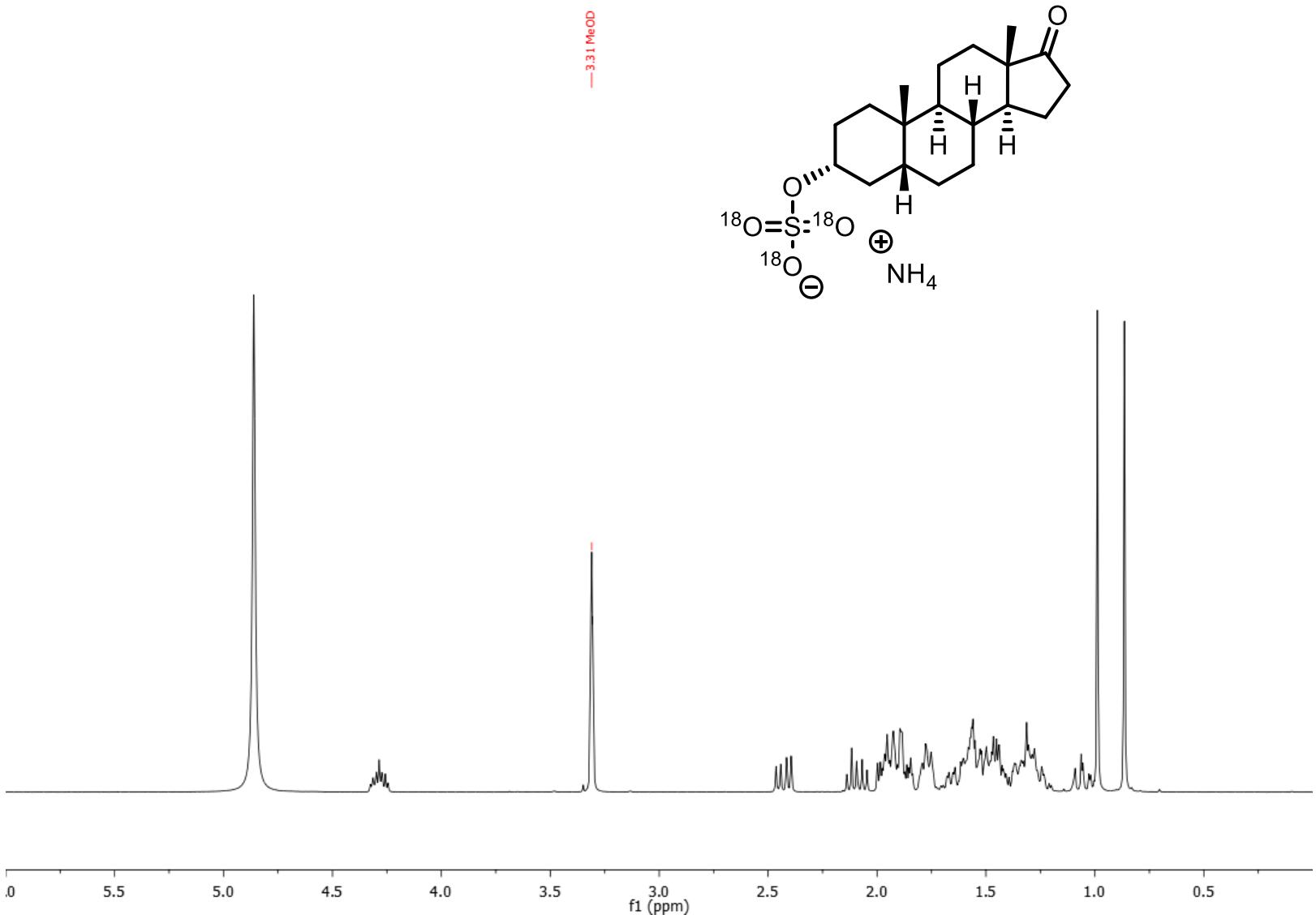
Androsterone 3[$^{18}O_3$]-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD



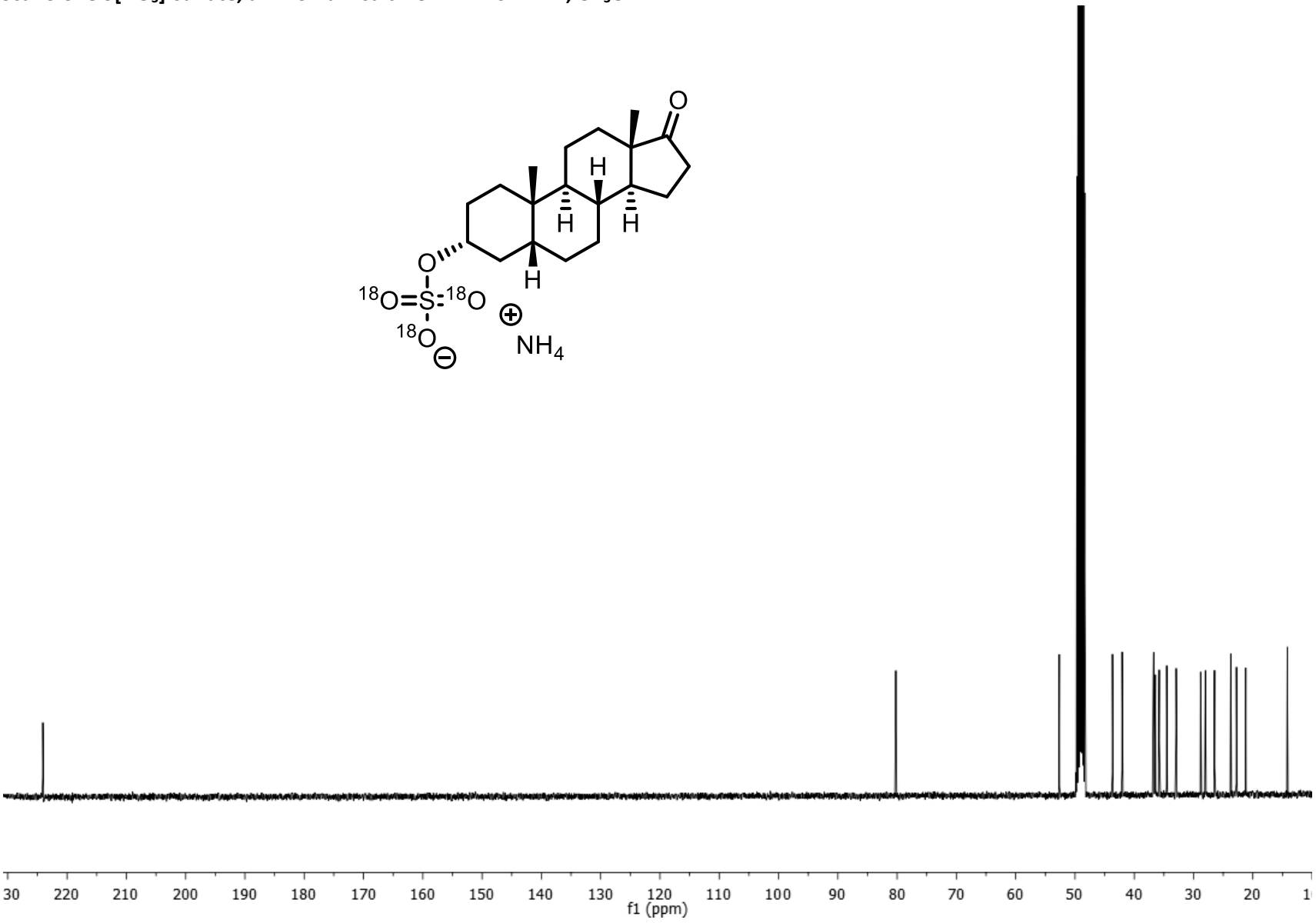
Androsterone 3[$^{18}O_3$]-sulfate, ammonium salt LRMS



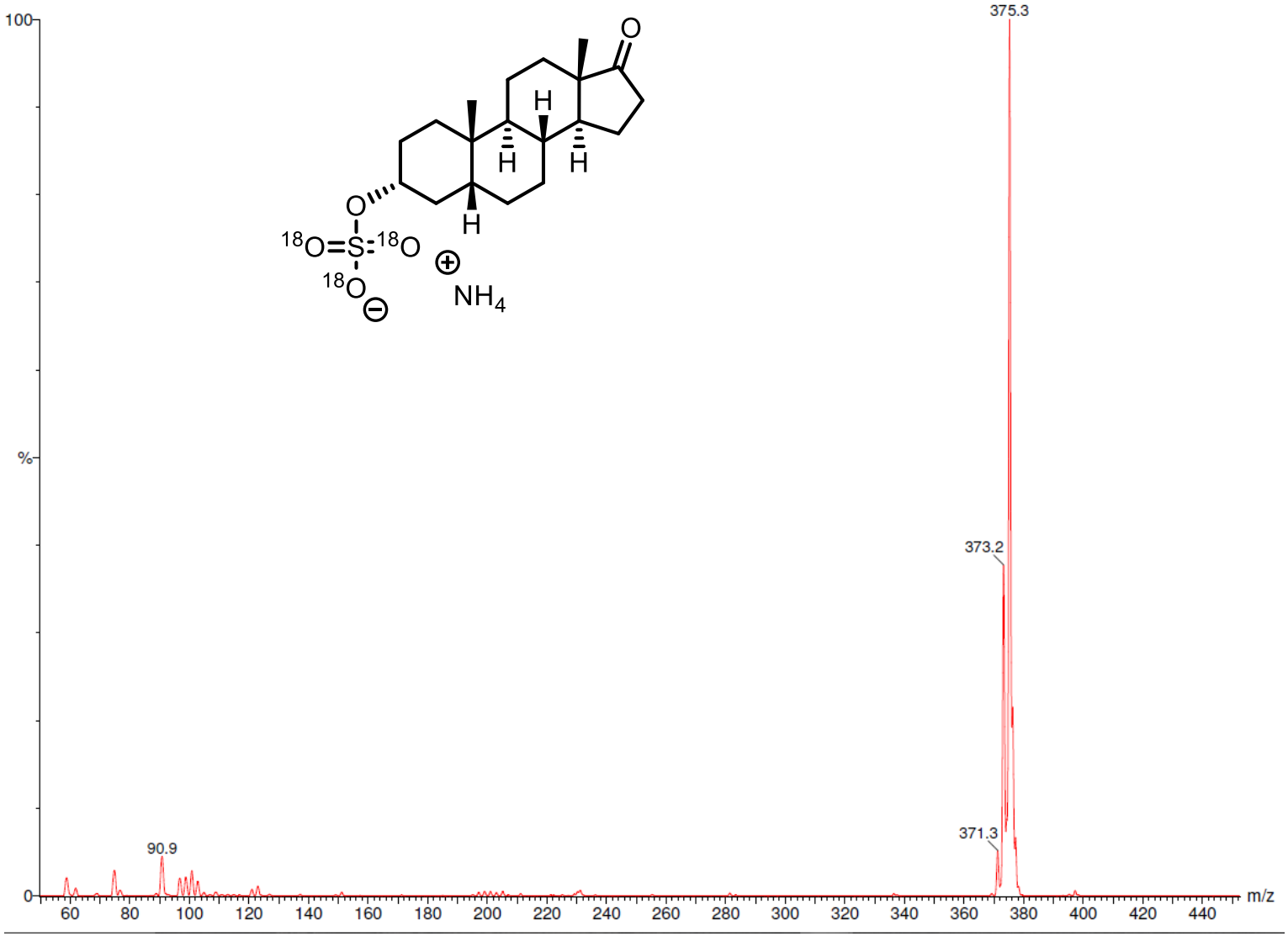
Etiochocanolone 3[$^{18}O_3$]-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



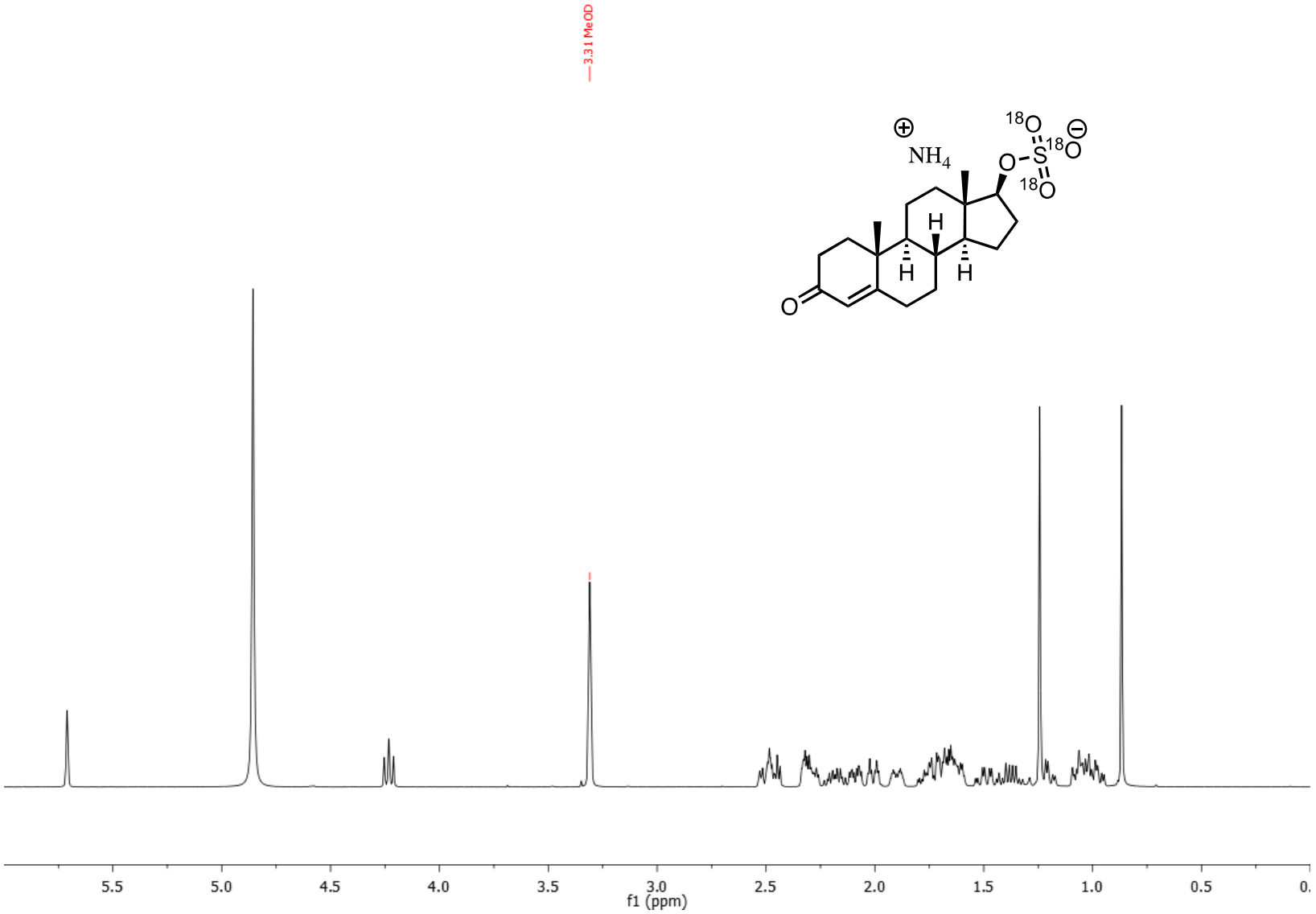
Etiochocanolone 3[$^{18}O_3$]-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD



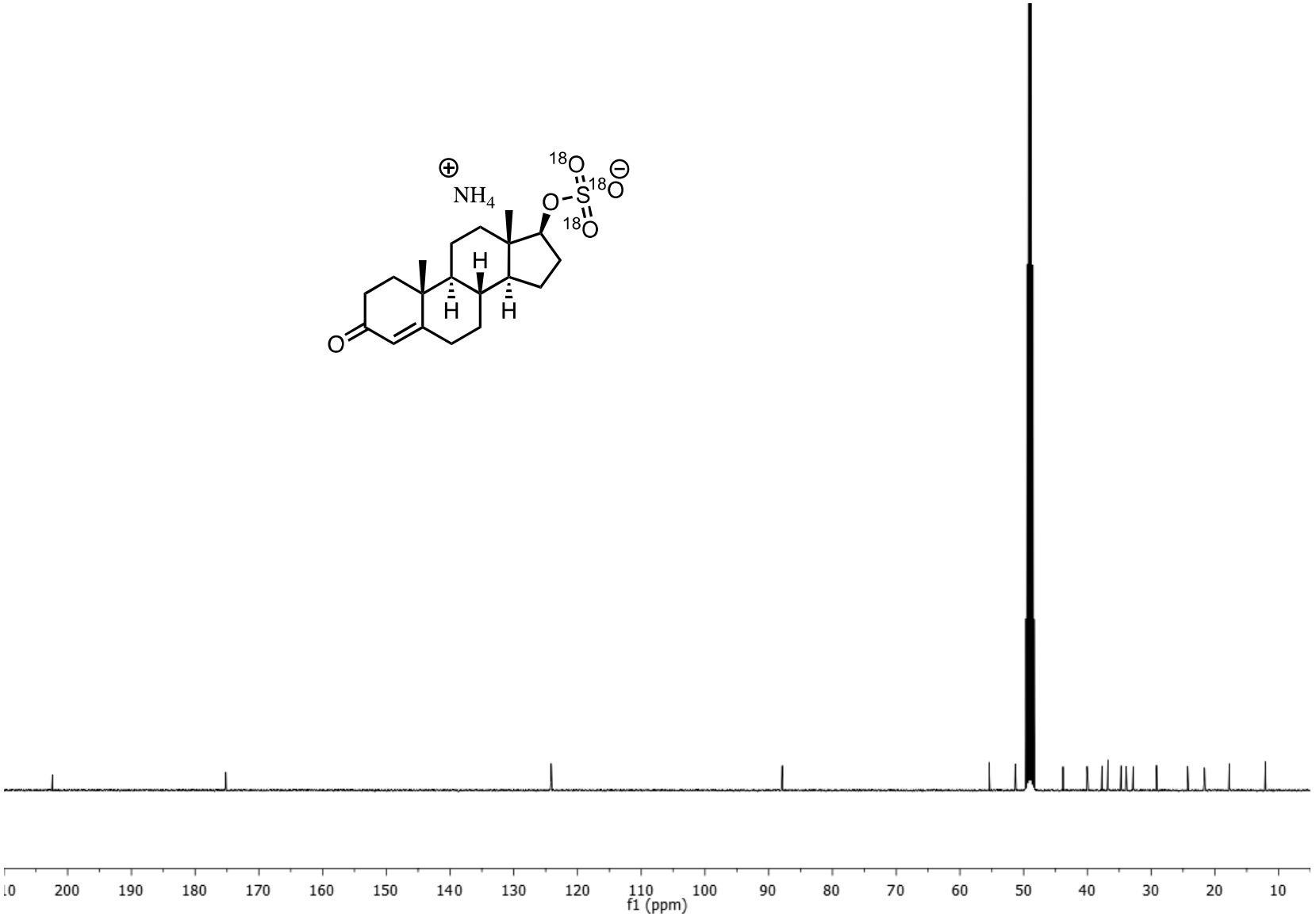
Etiochocanolone 3[$^{18}O_3$]-sulfate, ammonium salt LRMS



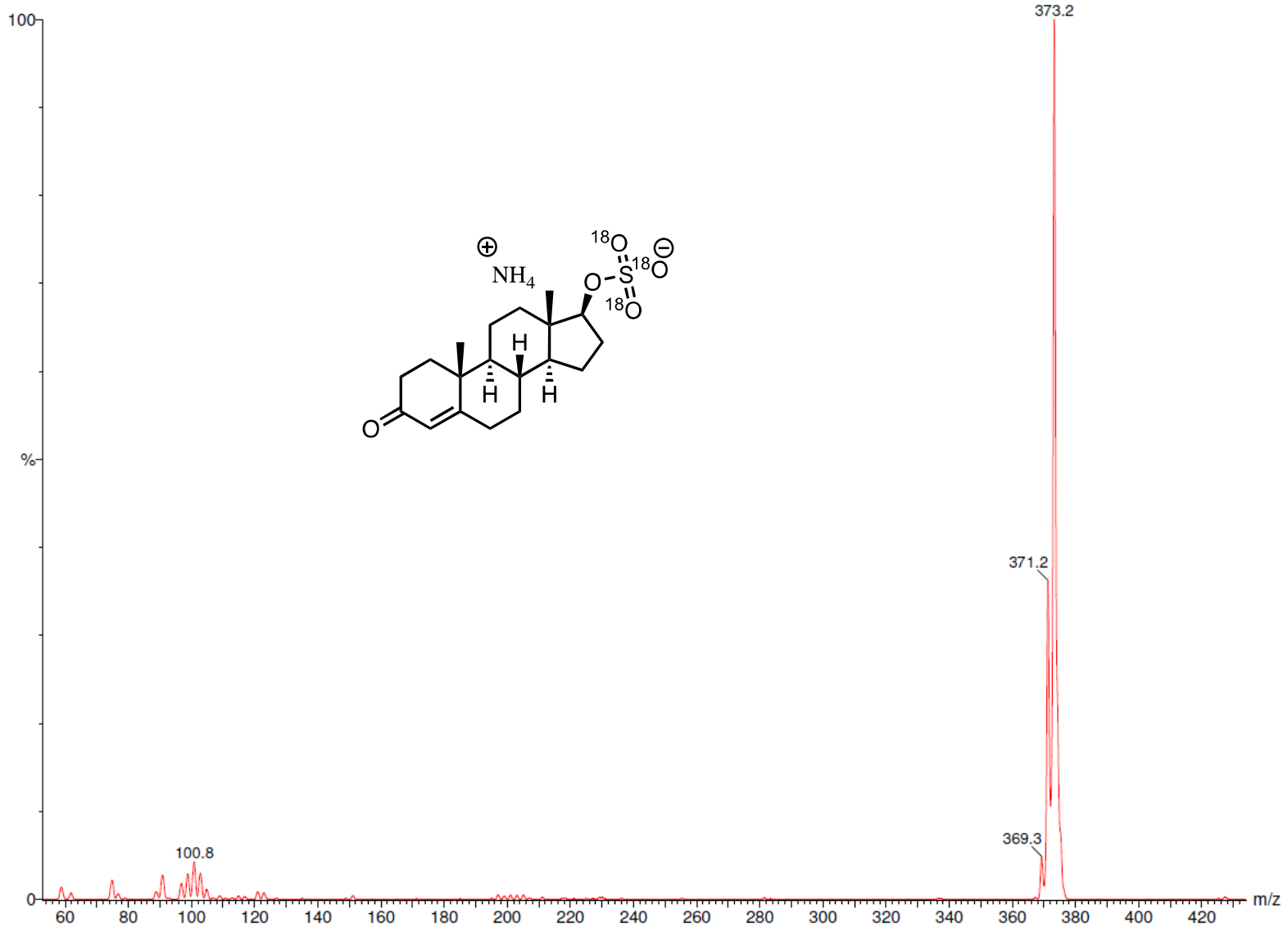
Testosterone 17[$^{18}O_3$]-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



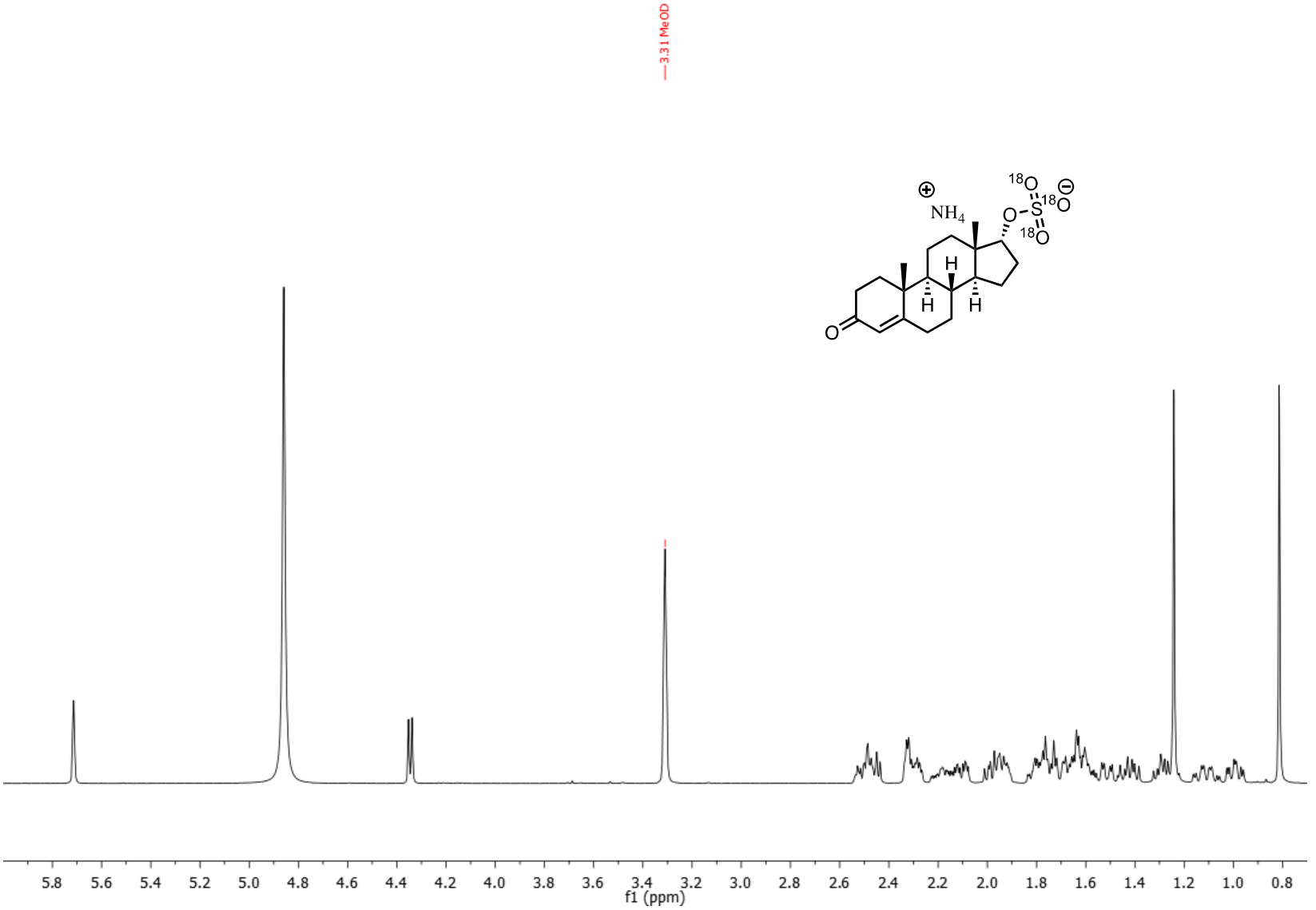
Testosterone 17[$^{18}O_3$]-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD



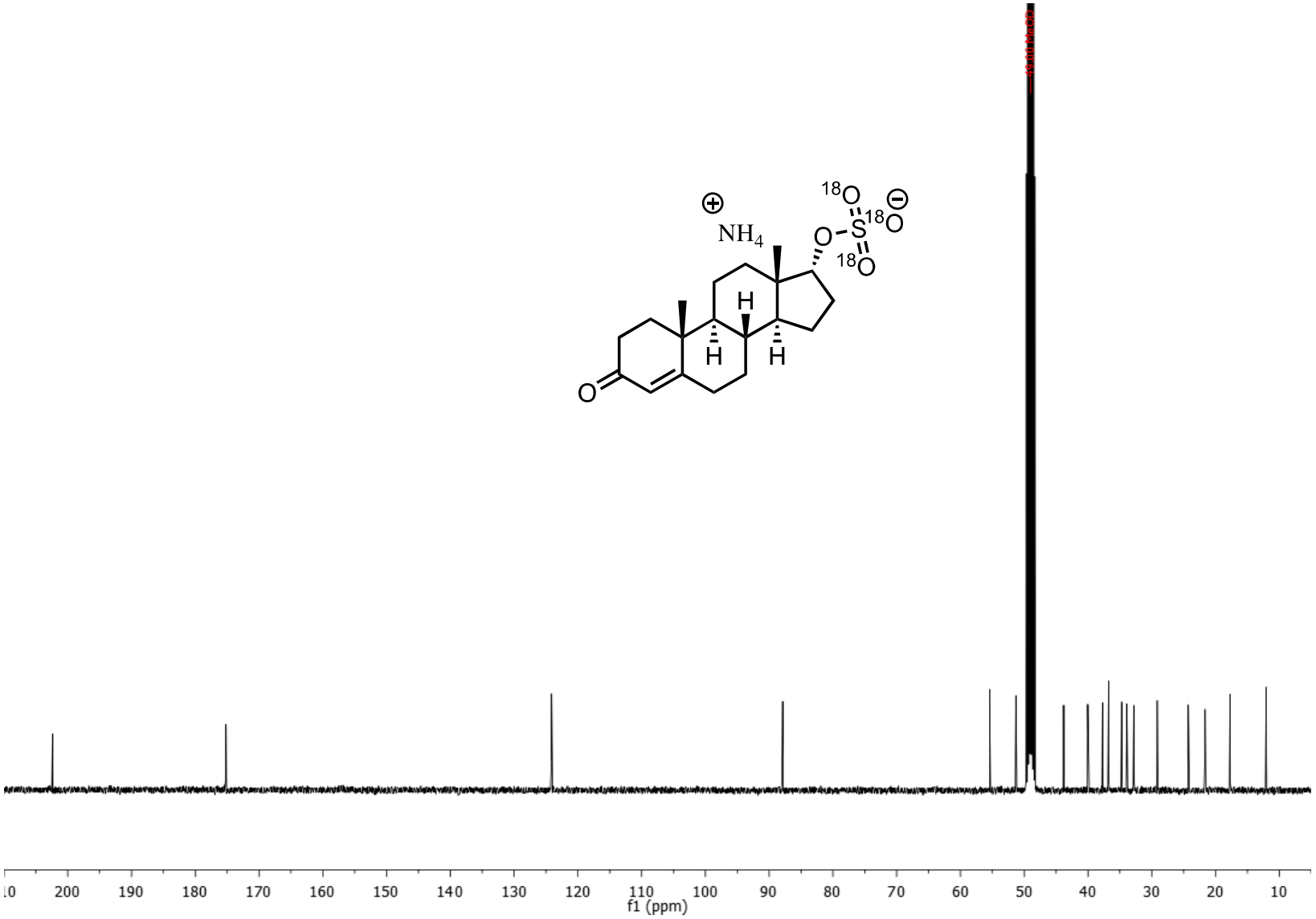
Testosterone 17[$^{18}O_3$]-sulfate, ammonium salt LRMS



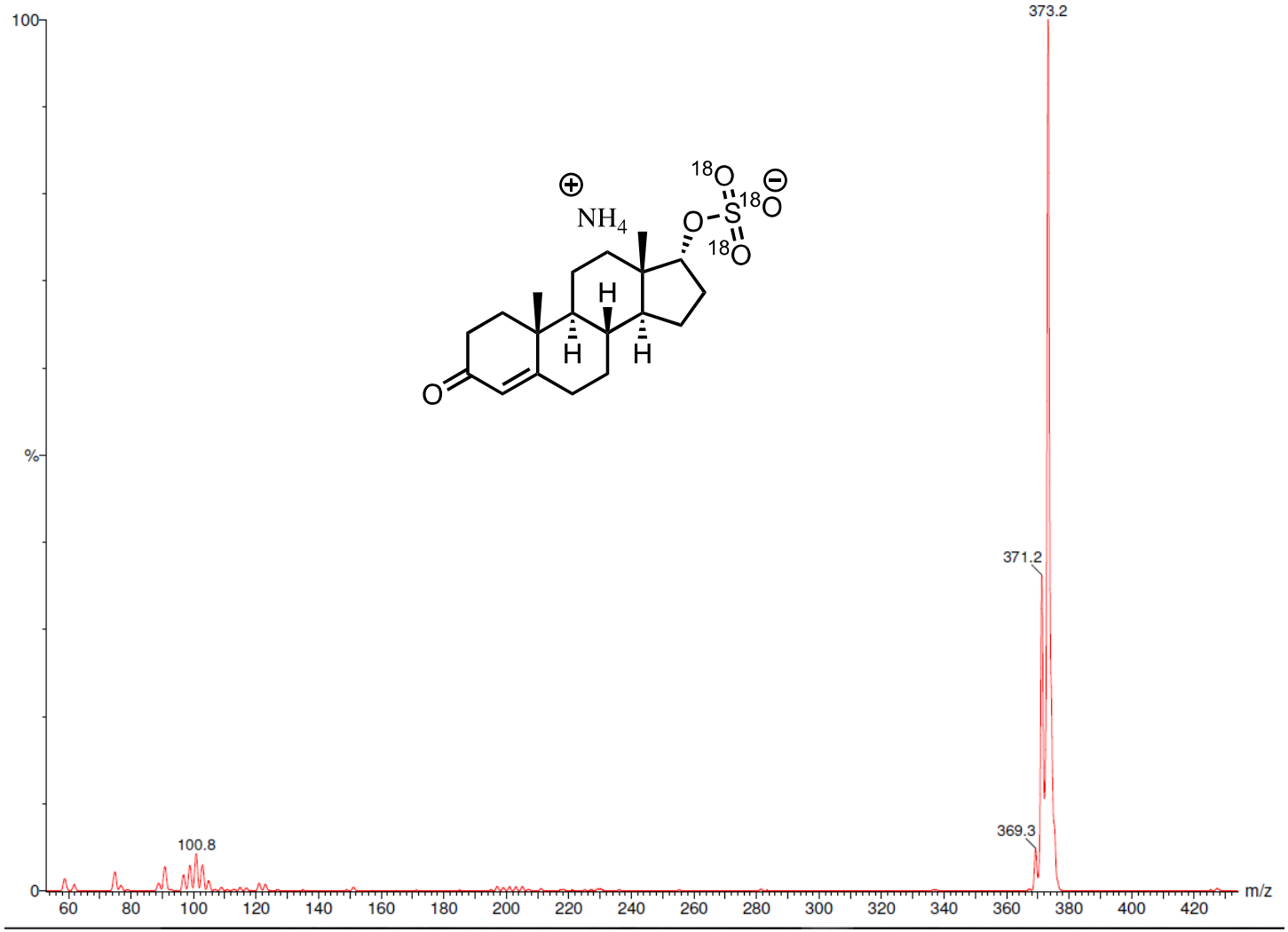
Epitestosterone 17[$^{18}O_3$]-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



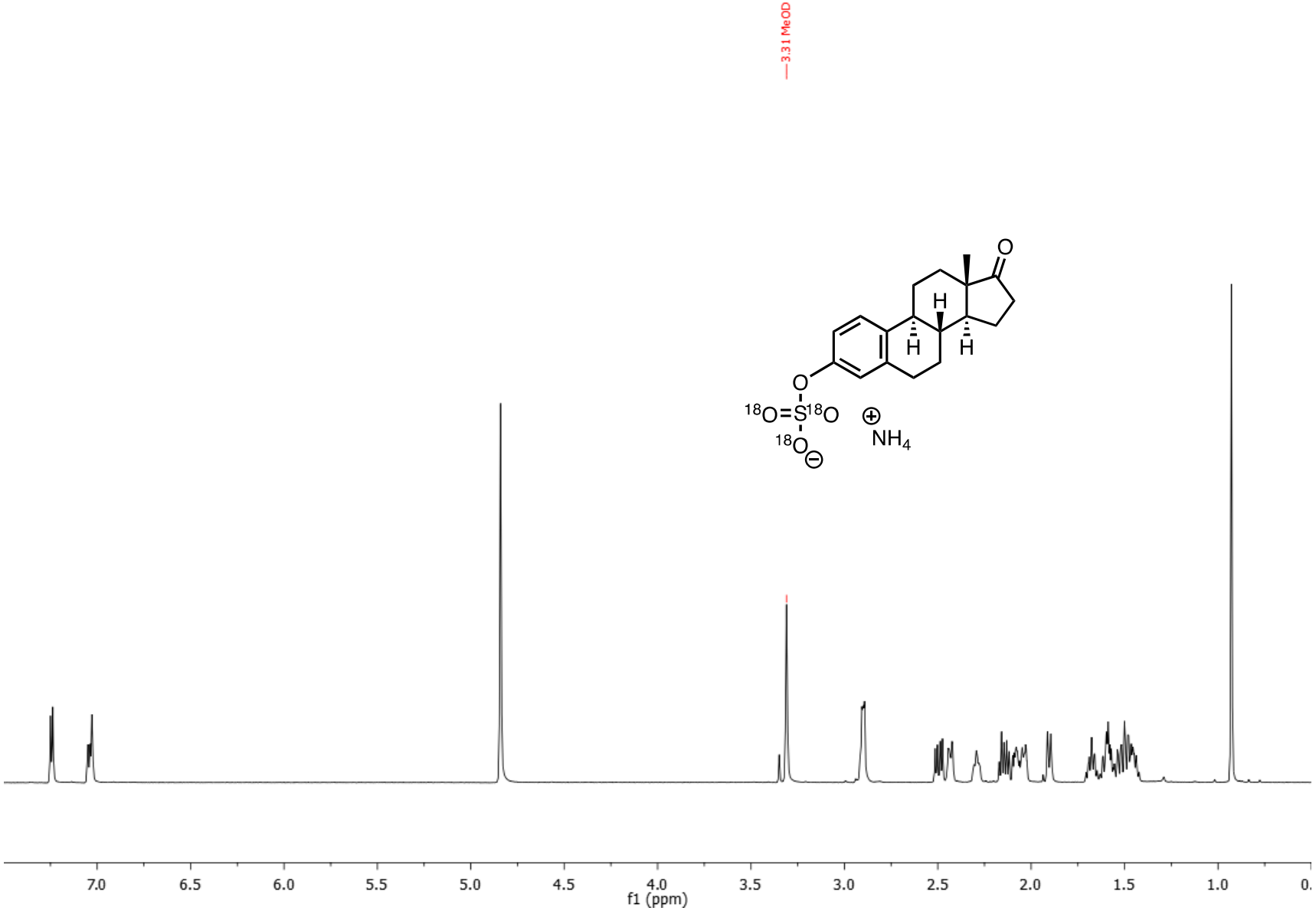
Epitestosterone 17[$^{18}O_3$]-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD



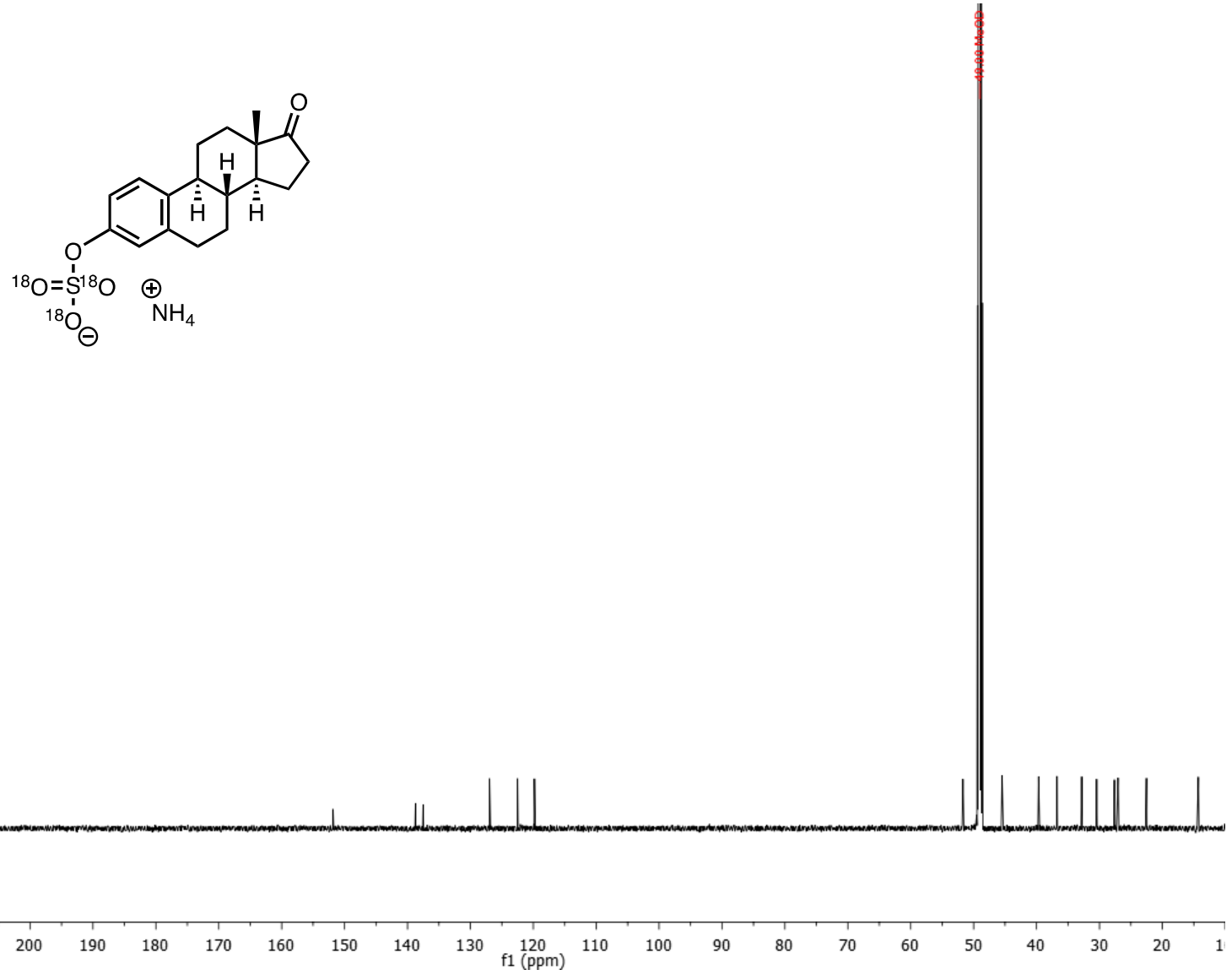
Epitestosterone 17[$^{18}O_3$]-sulfate, ammonium salt LRMS



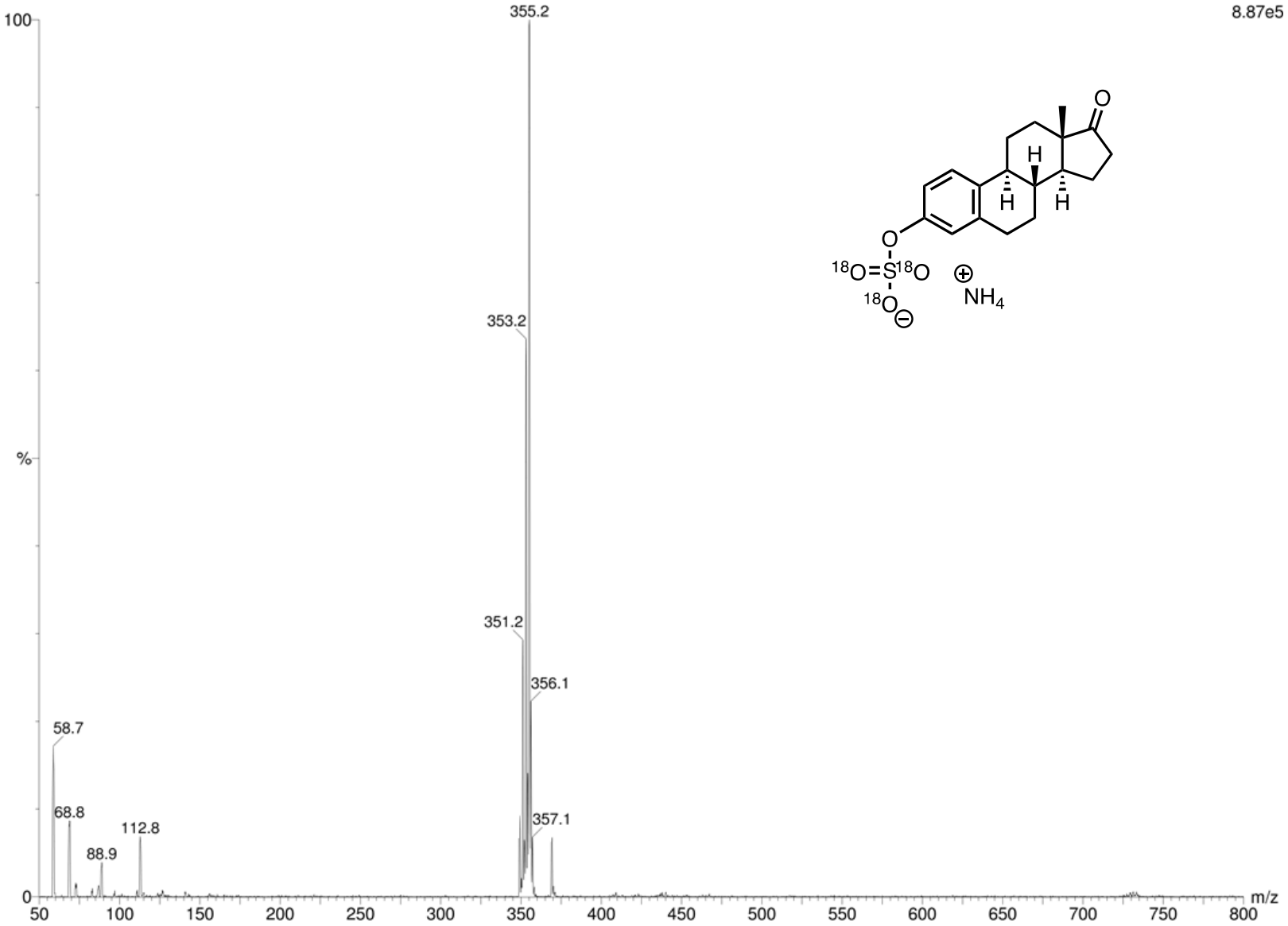
Estrone 3[$^{18}O_3$]-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



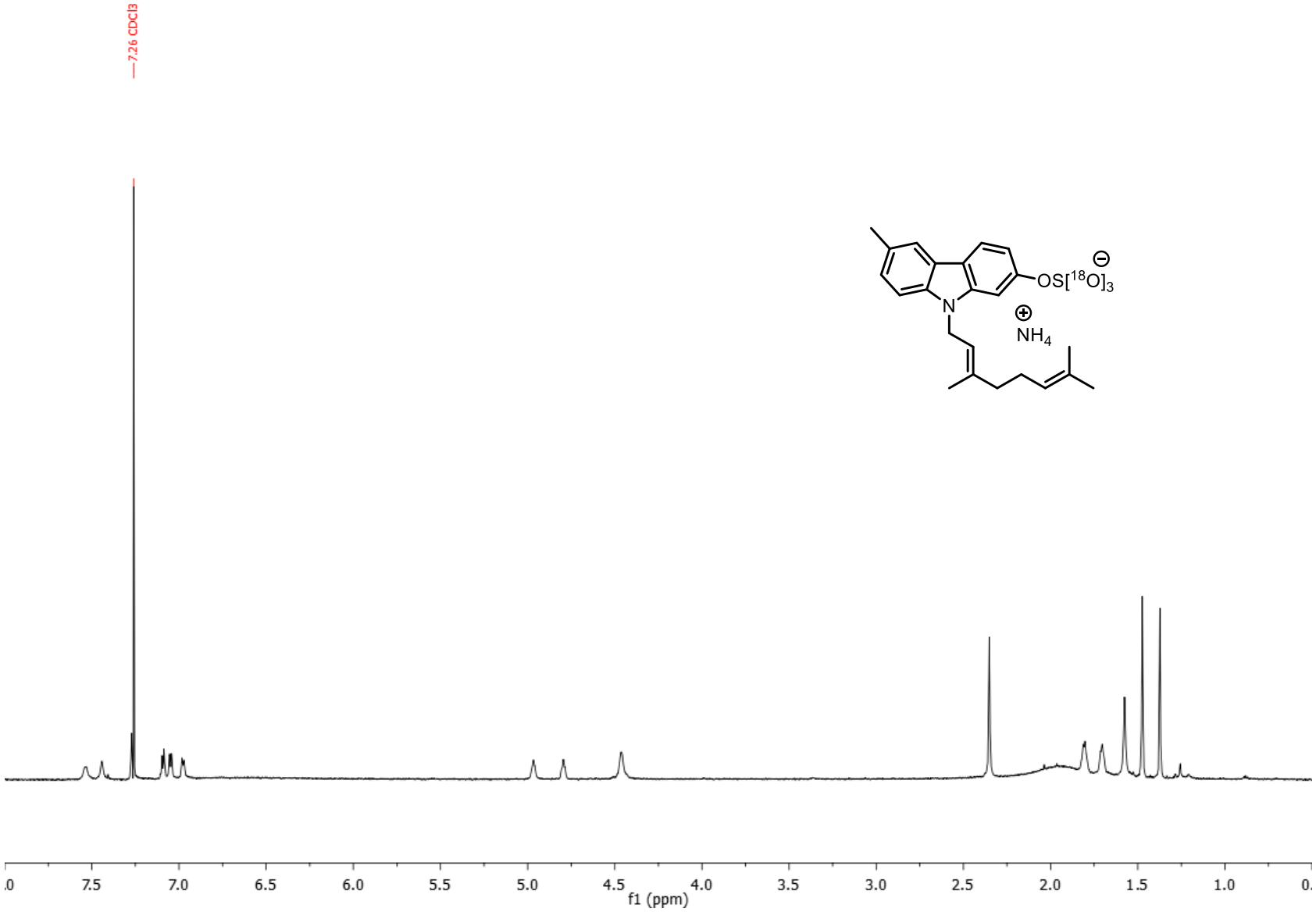
Estrone 3[$^{18}O_3$]-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD



Estrone 3[$^{18}O_3$]-sulfate, ammonium salt LRMS



Karapinchamine A [$^{18}O_3$]-sulfate, ammonium salt ^{1}H NMR 700 MHz, $CDCl_3$



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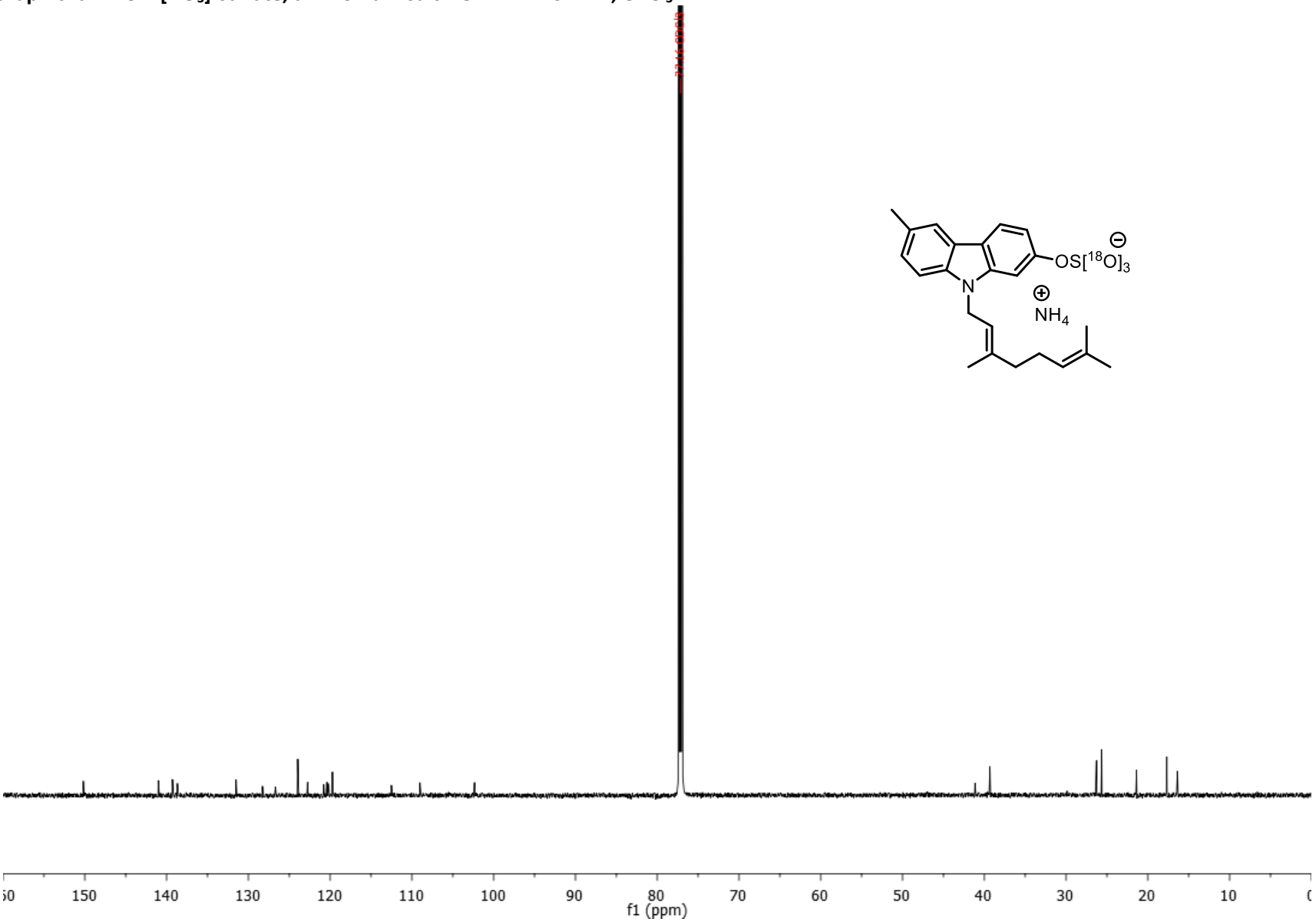
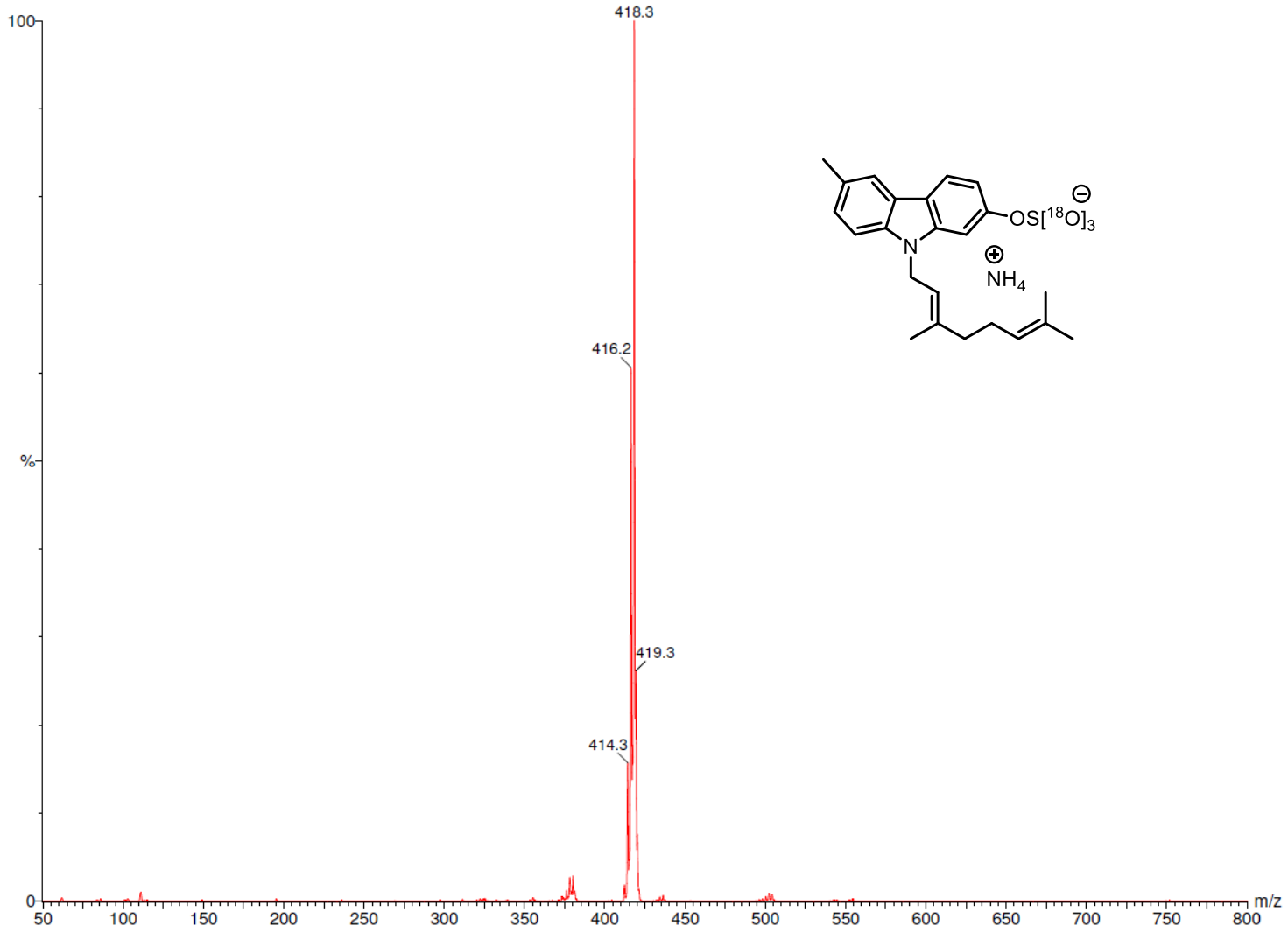
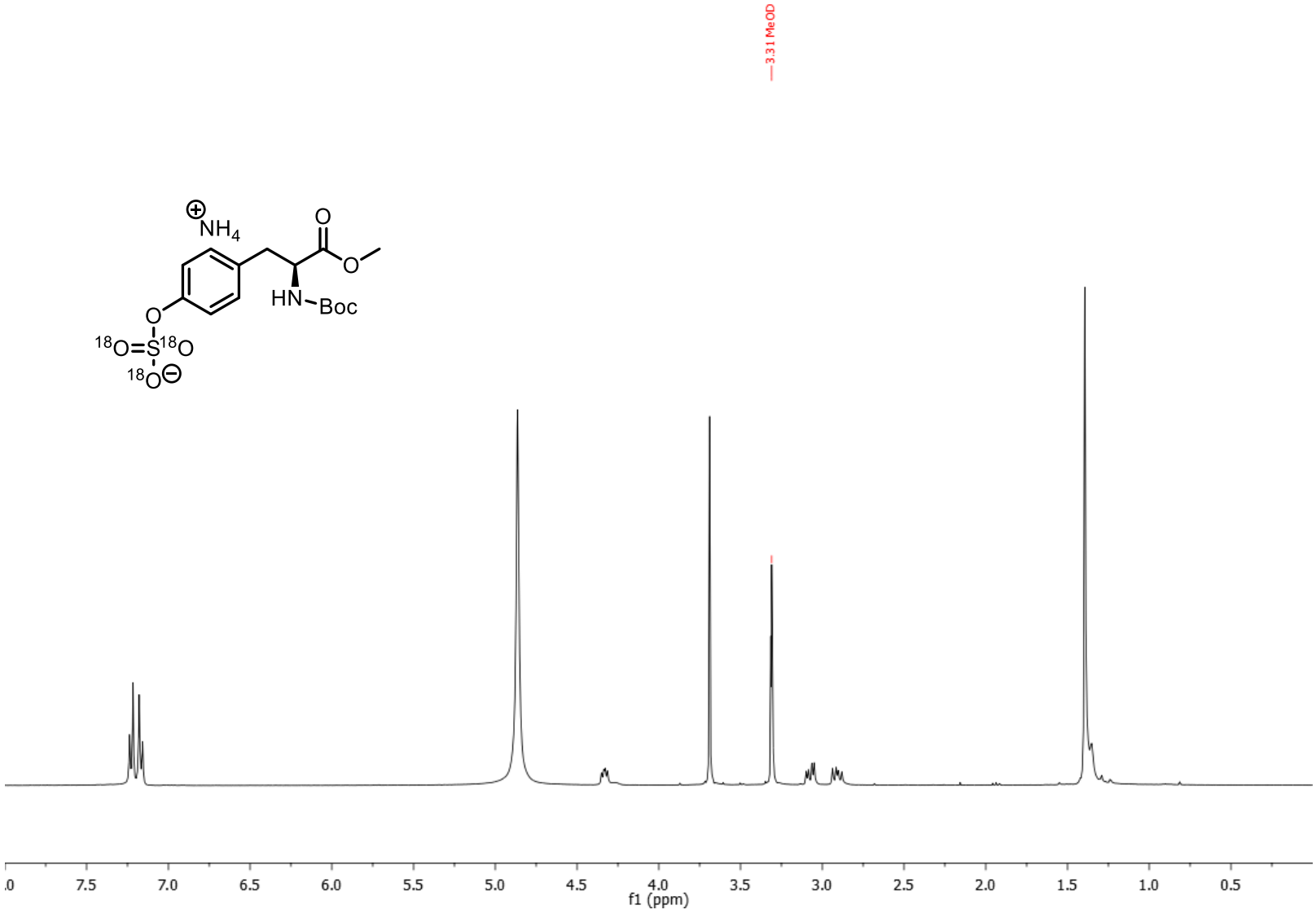


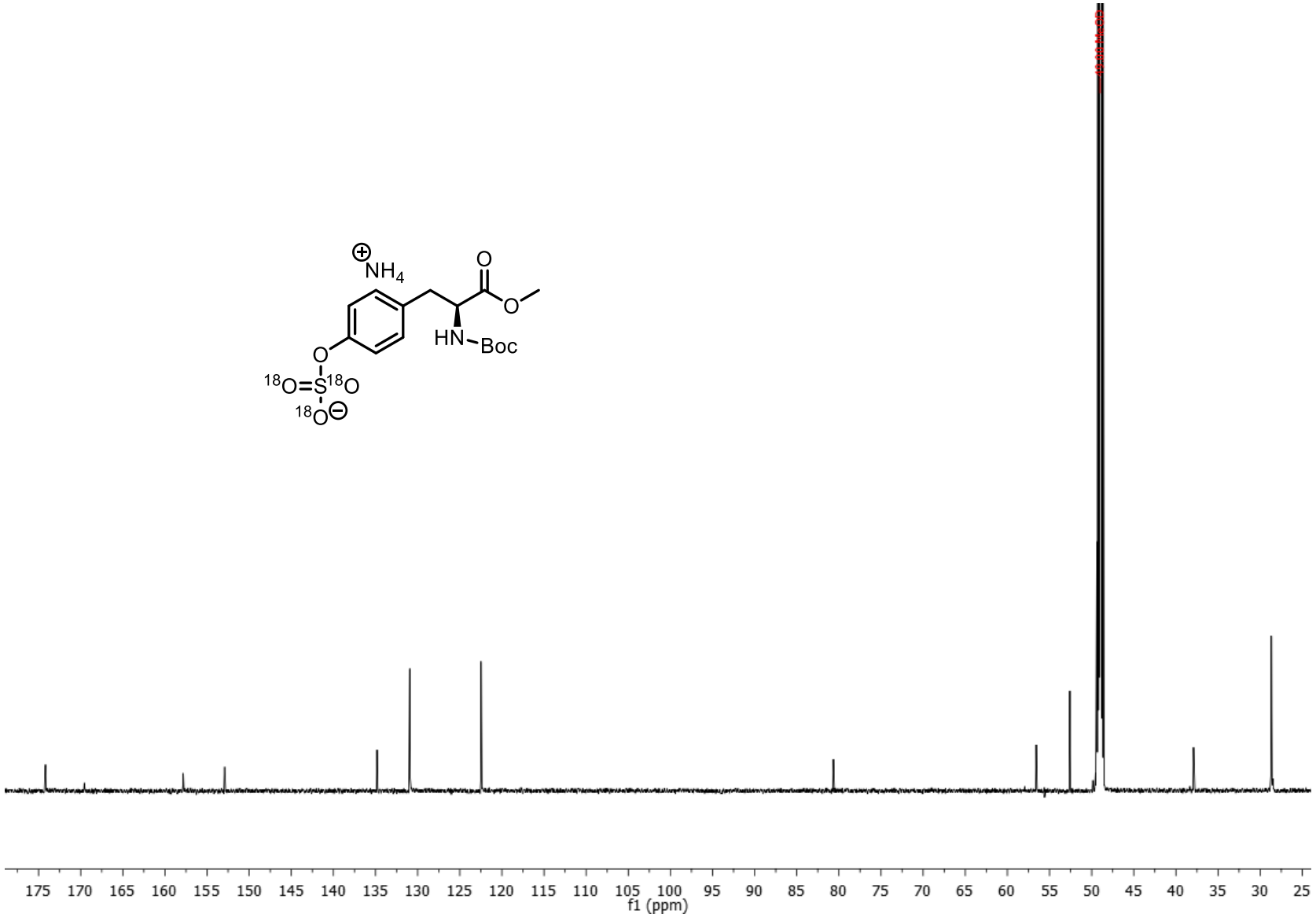
Karapinchamine A [$^{18}O_3$]-sulfate, ammonium salt LRMS



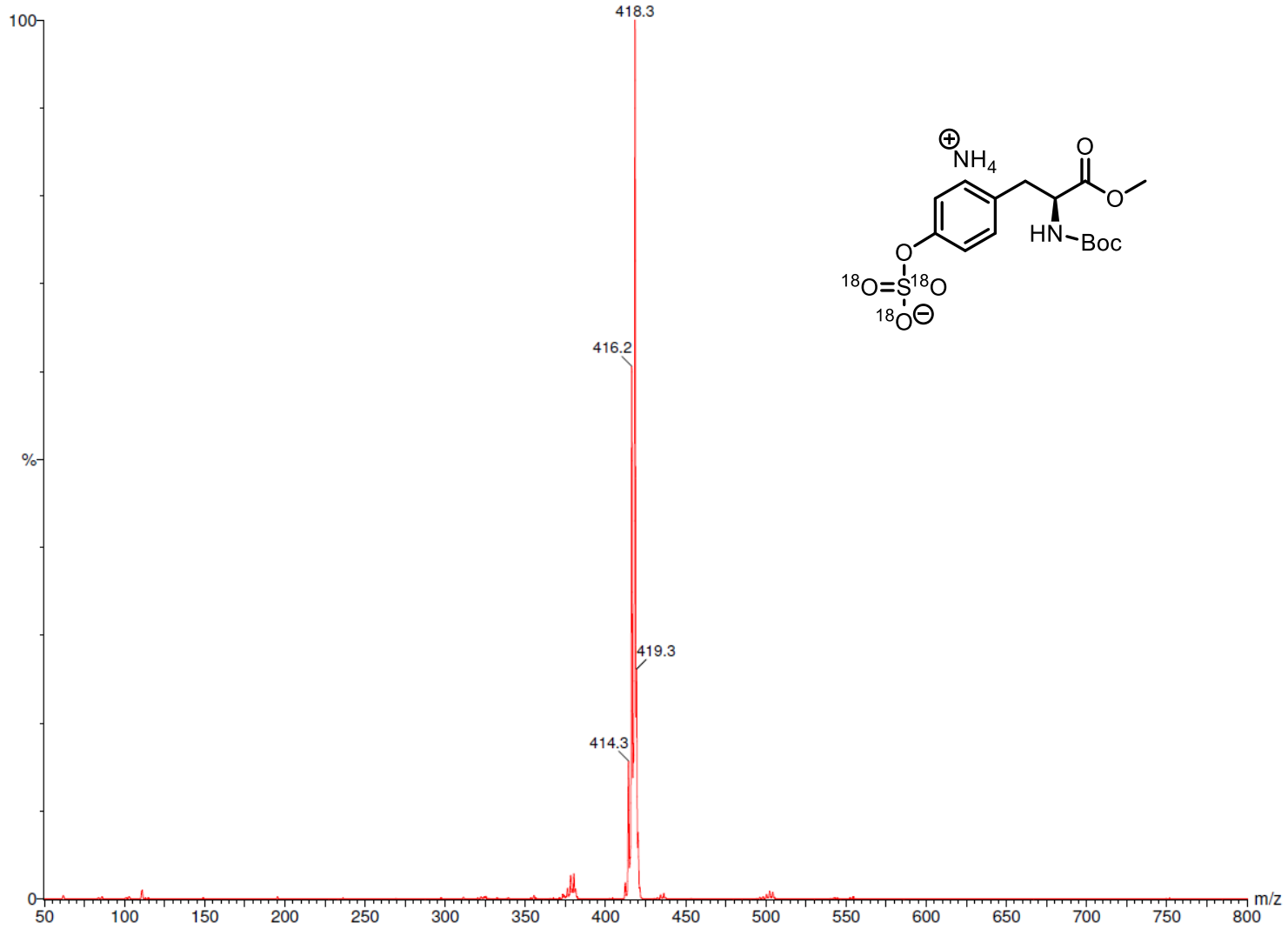
N-(*Boc*)-L-tyrosine methyl ester (phenyl)sulfate, ammonium salt ^{1}H NMR 700 MHz CD_3OD



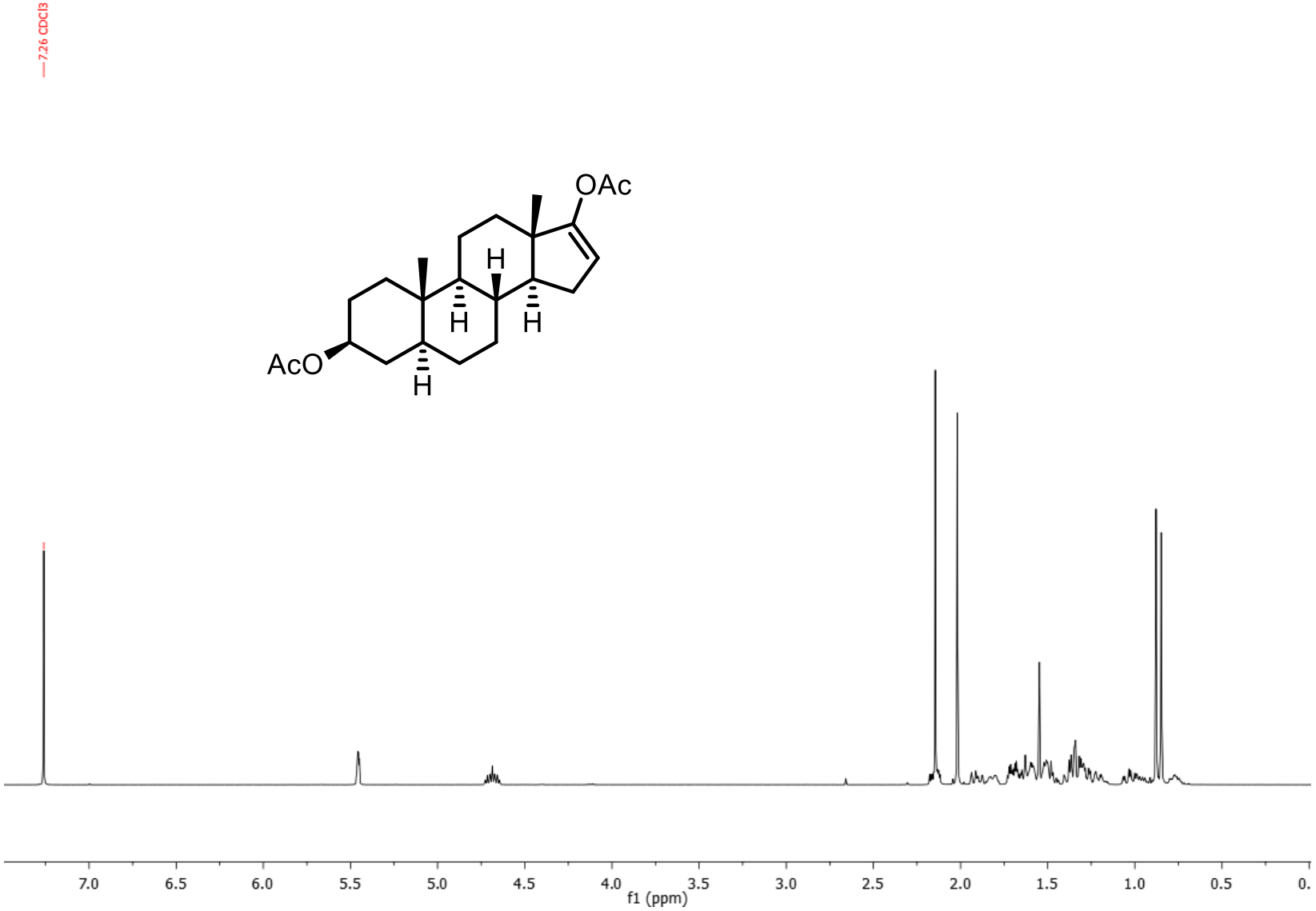
N-(*Boc*)-L-tyrosine methyl ester (phenyl)sulfate, ammonium salt ^{13}C NMR 176 MHz CD_3OD



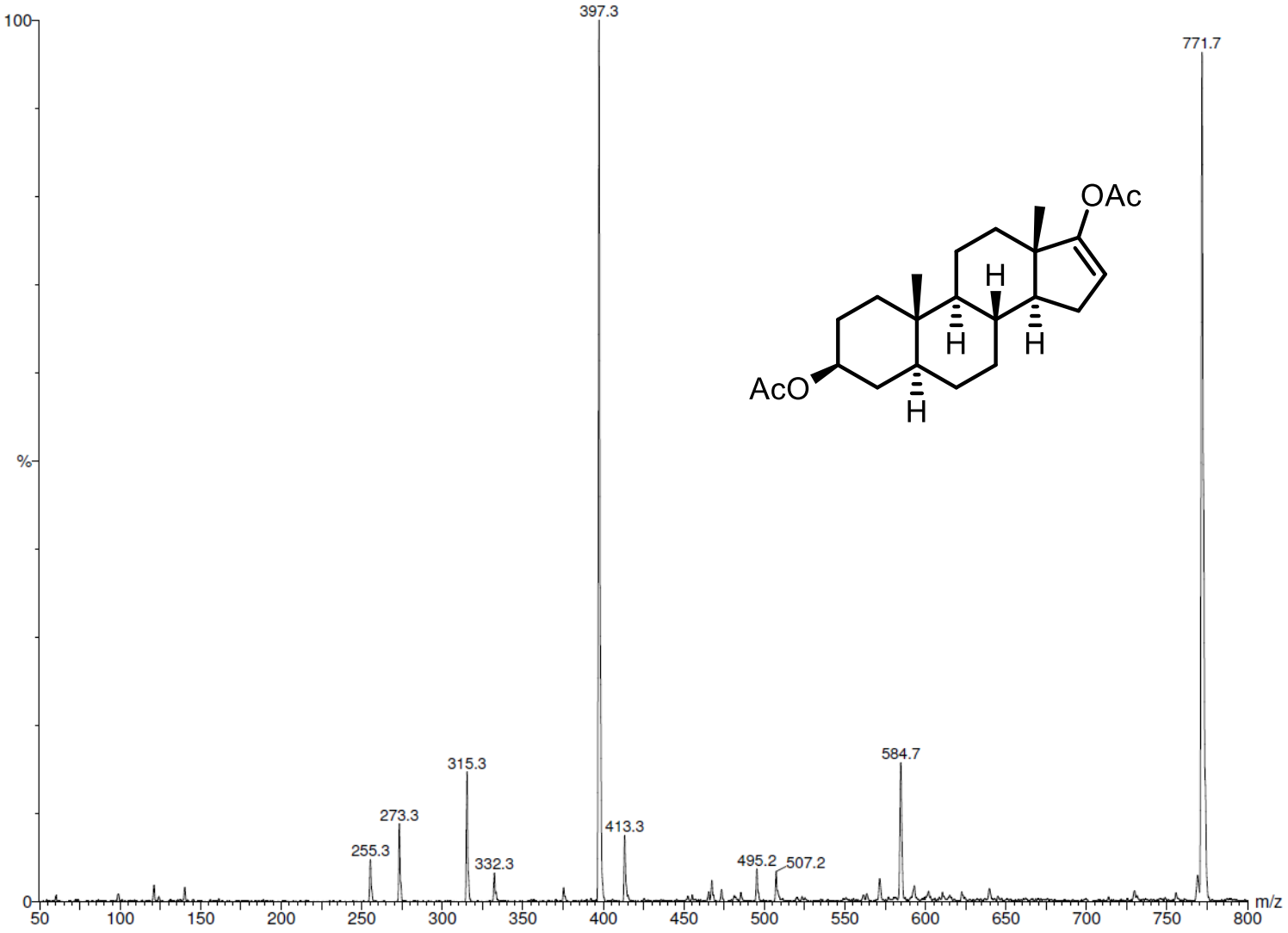
N-(Boc)-L-tyrosine methyl ester (phenyl)sulfate, ammonium salt LRMS



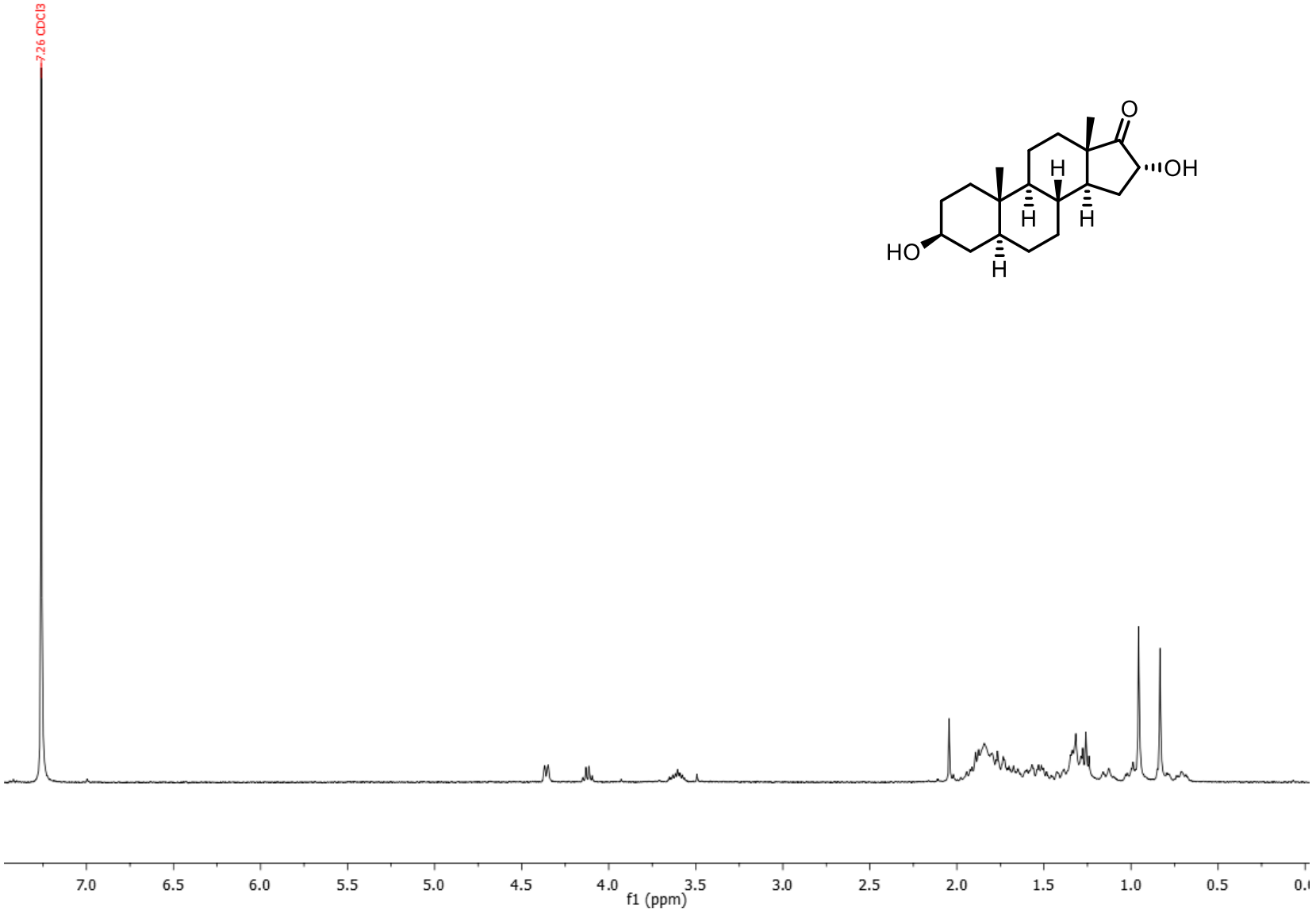
3ß,17-Diacetoxy-5α-androstan-16-ene ^{1}H NMR 400 MHz $CDCl_3$



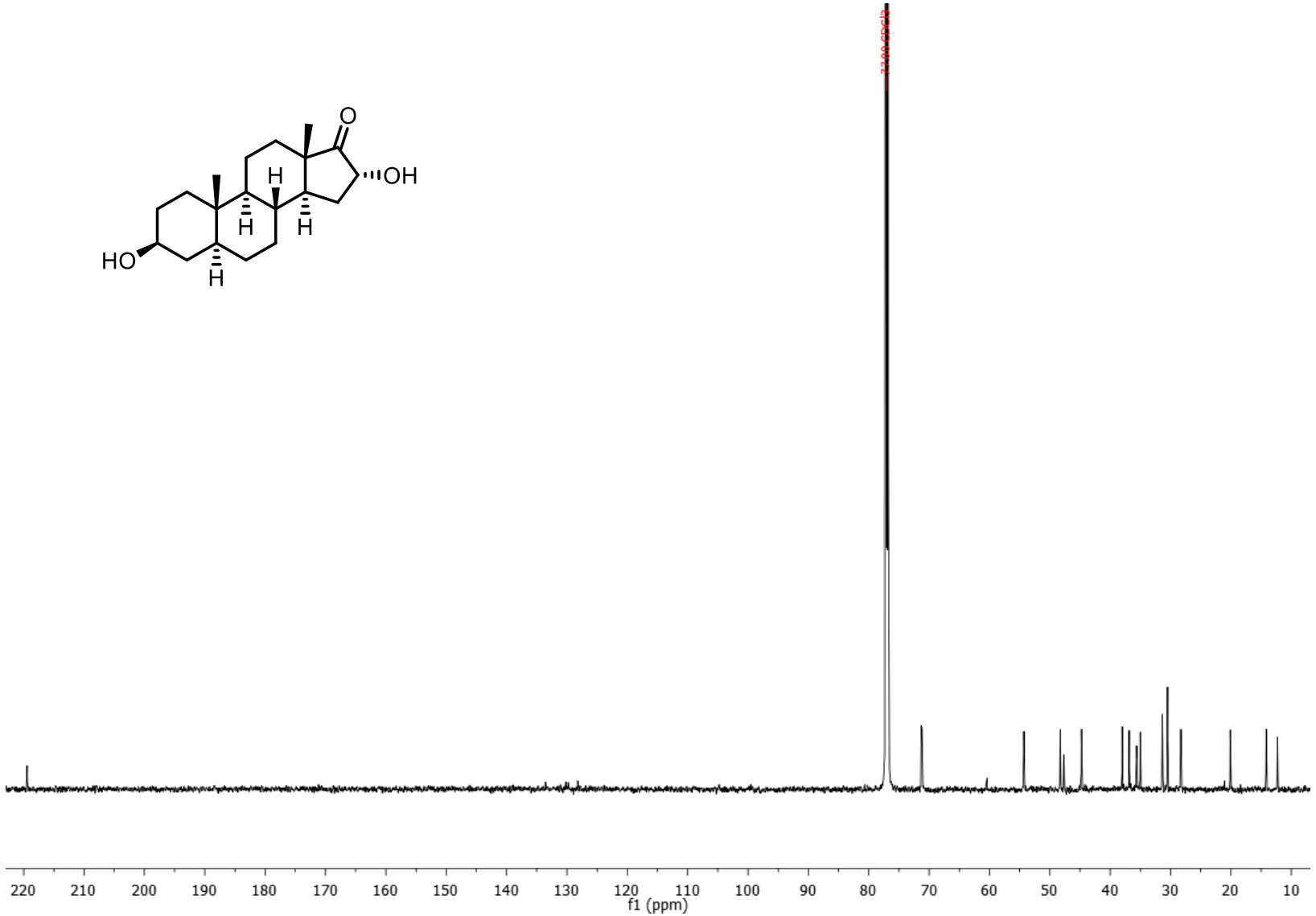
3ß,17-Diacetoxy-5α-androstan-16-ene LRMS



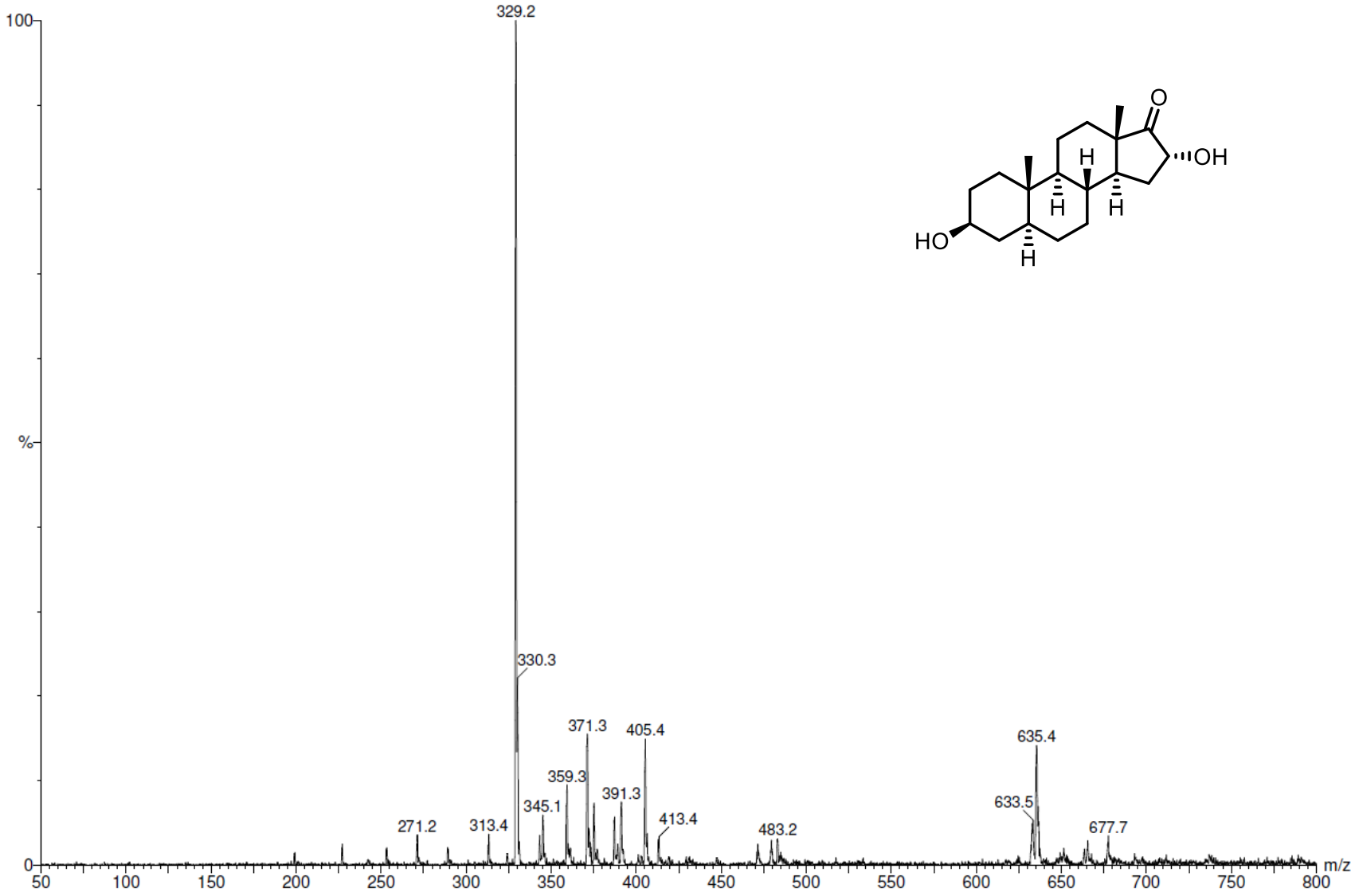
3ß,16α-Dihydroxy-5α-androstan-17-one ^{1}H NMR 400 MHz $CDCl_3$



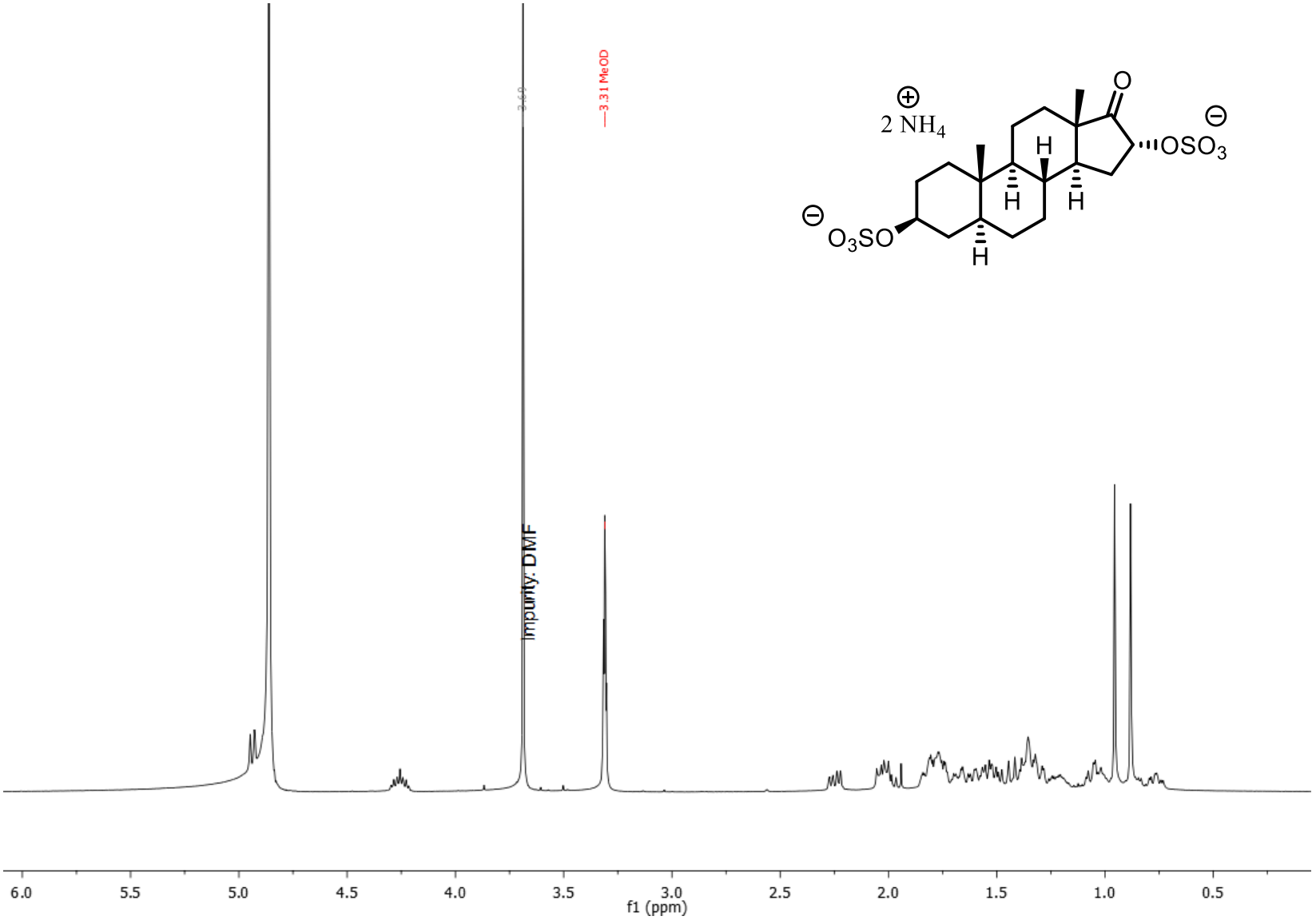
3ß,16α-Dihydroxy-5α-androstan-17-one ^{13}C NMR 101 MHz $CDCl_3$



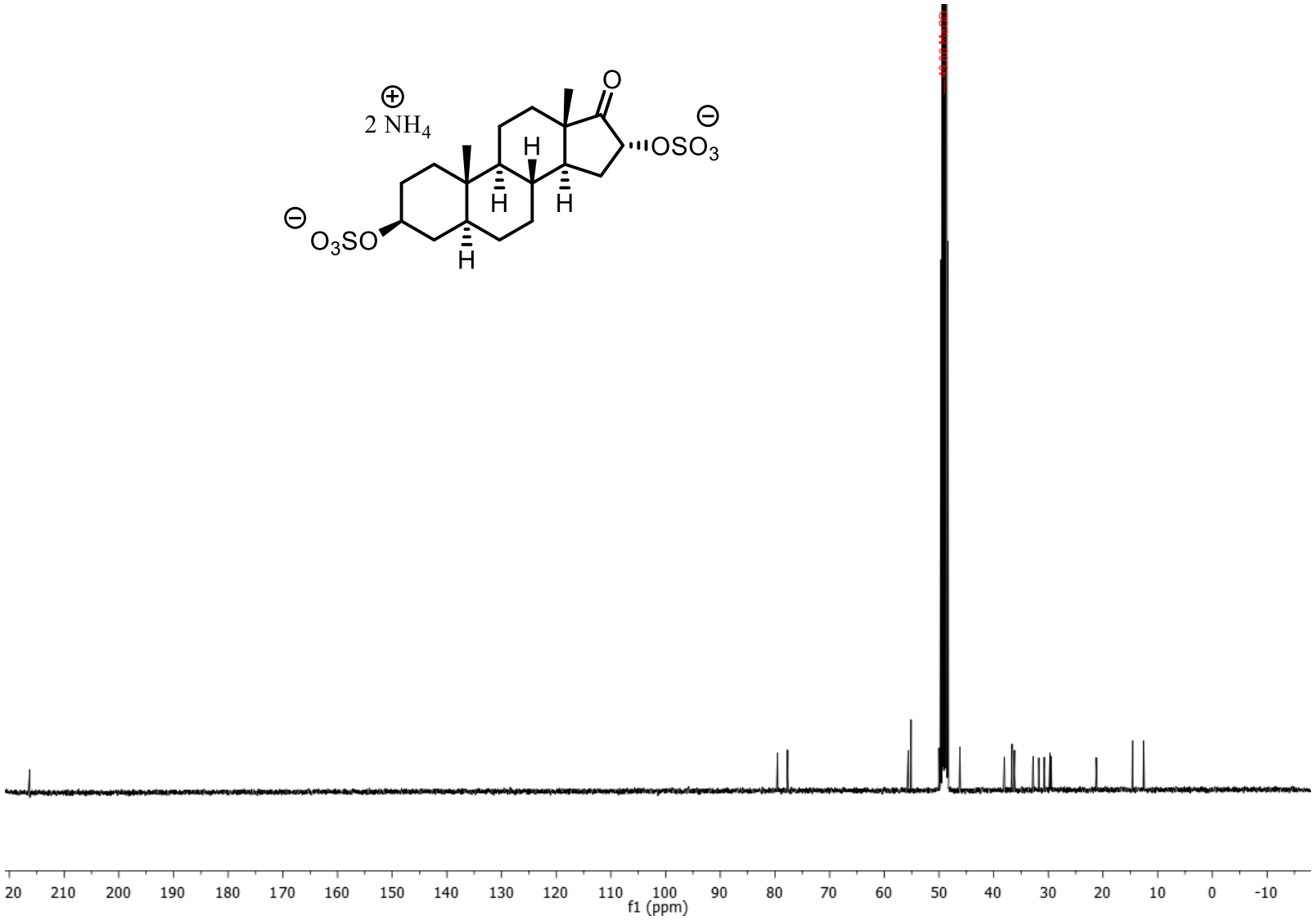
3ß,16α-Dihydroxy-5α-androstan-17-one LRMS



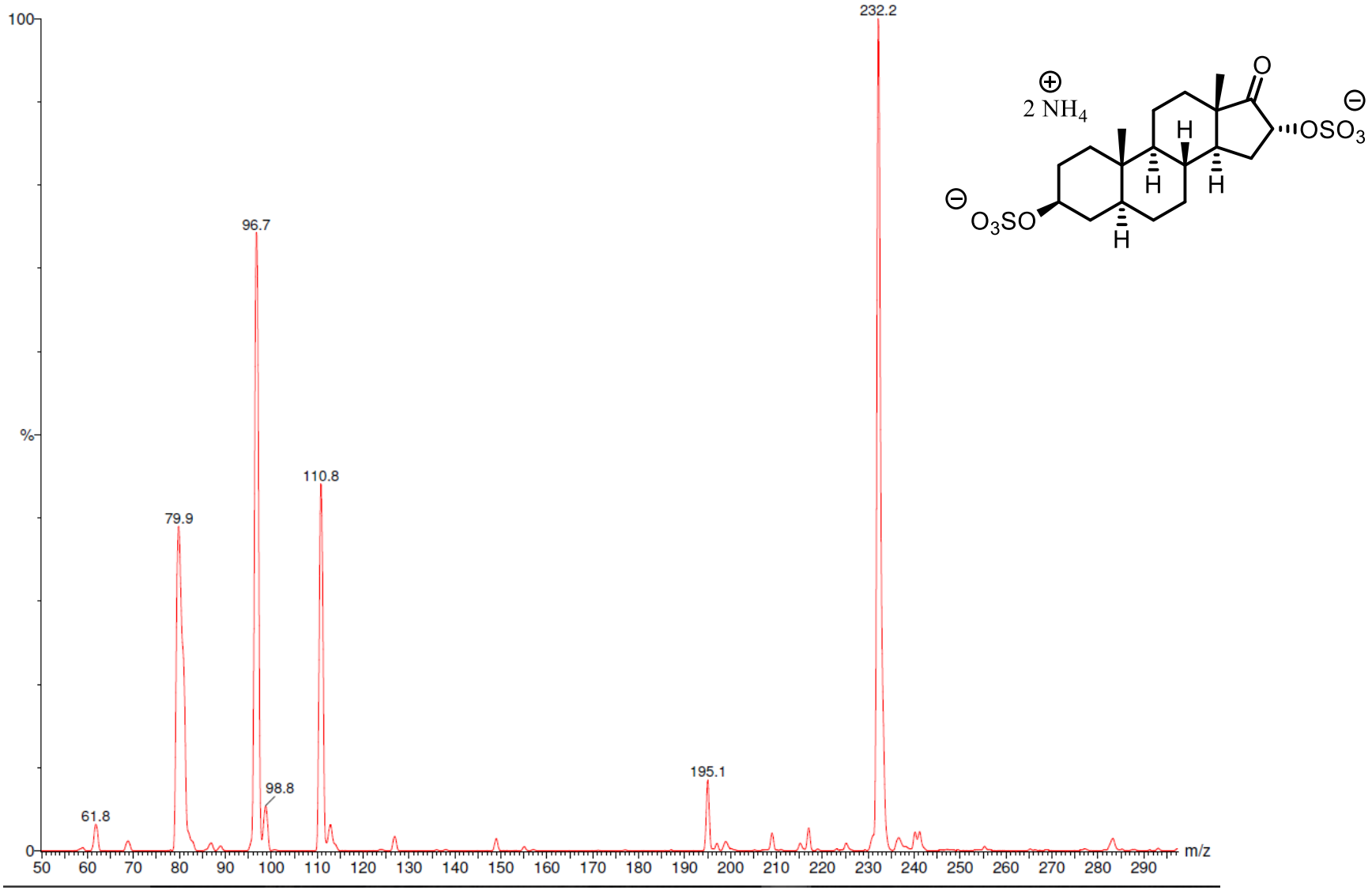
3ß,16α-Dihydroxy-5α-androstan-17-one 3,16-bis(sulfate), ammonium salt ^{1}H NMR 400 MHz, CD_3OD



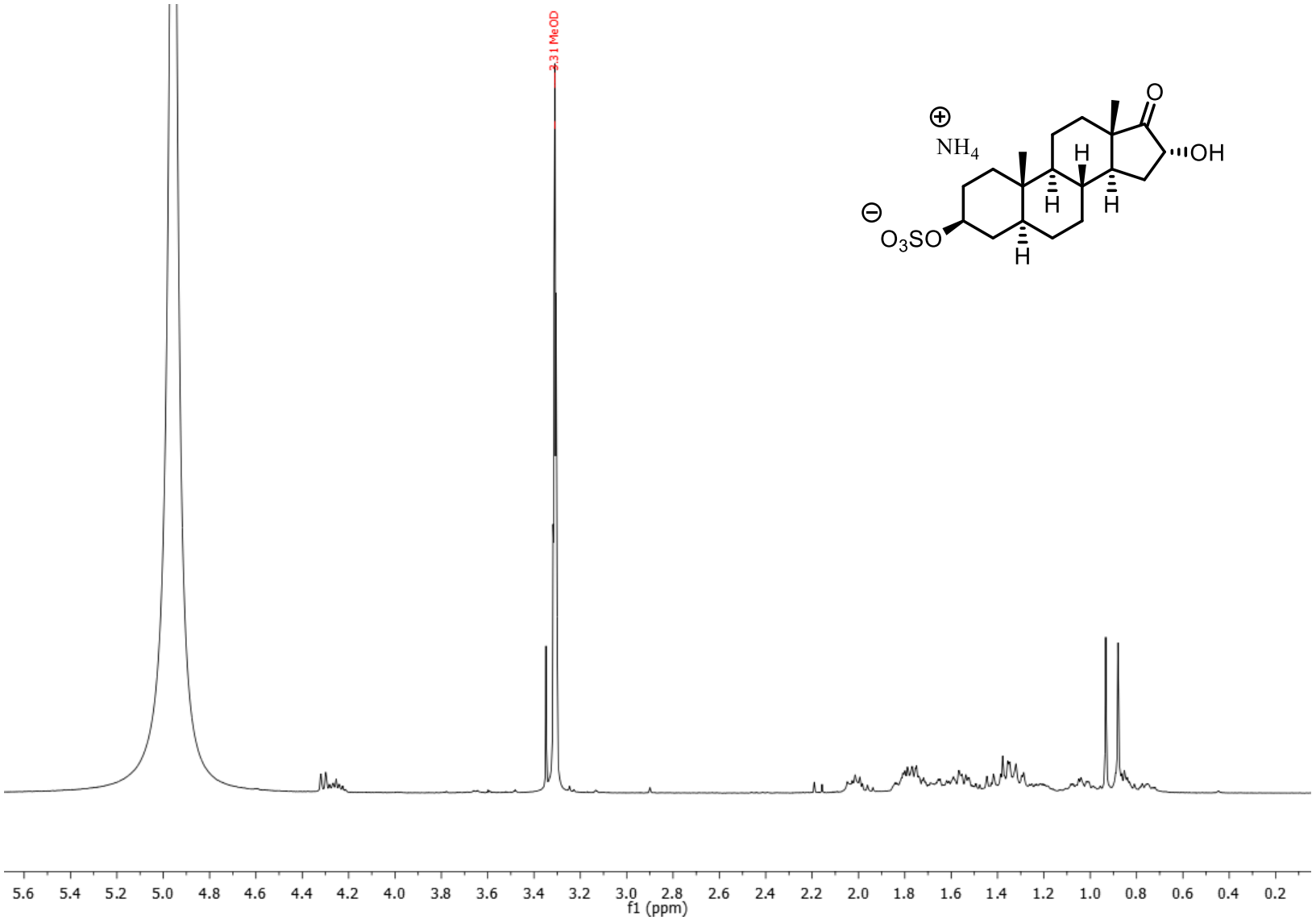
3ß,16α-Dihydroxy-5α-androstan-17-one 3,16-bis(sulfate), ammonium salt ^{13}C NMR 101 MHz, CD_3OD



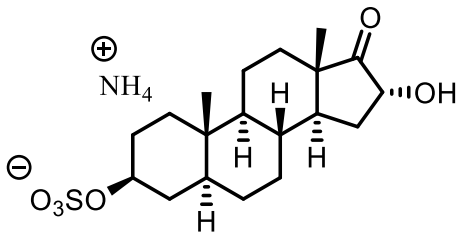
3ß,16α-Dihydroxy-5α-androstan-17-one 3,16-bis(sulfate), ammonium salt LRMS



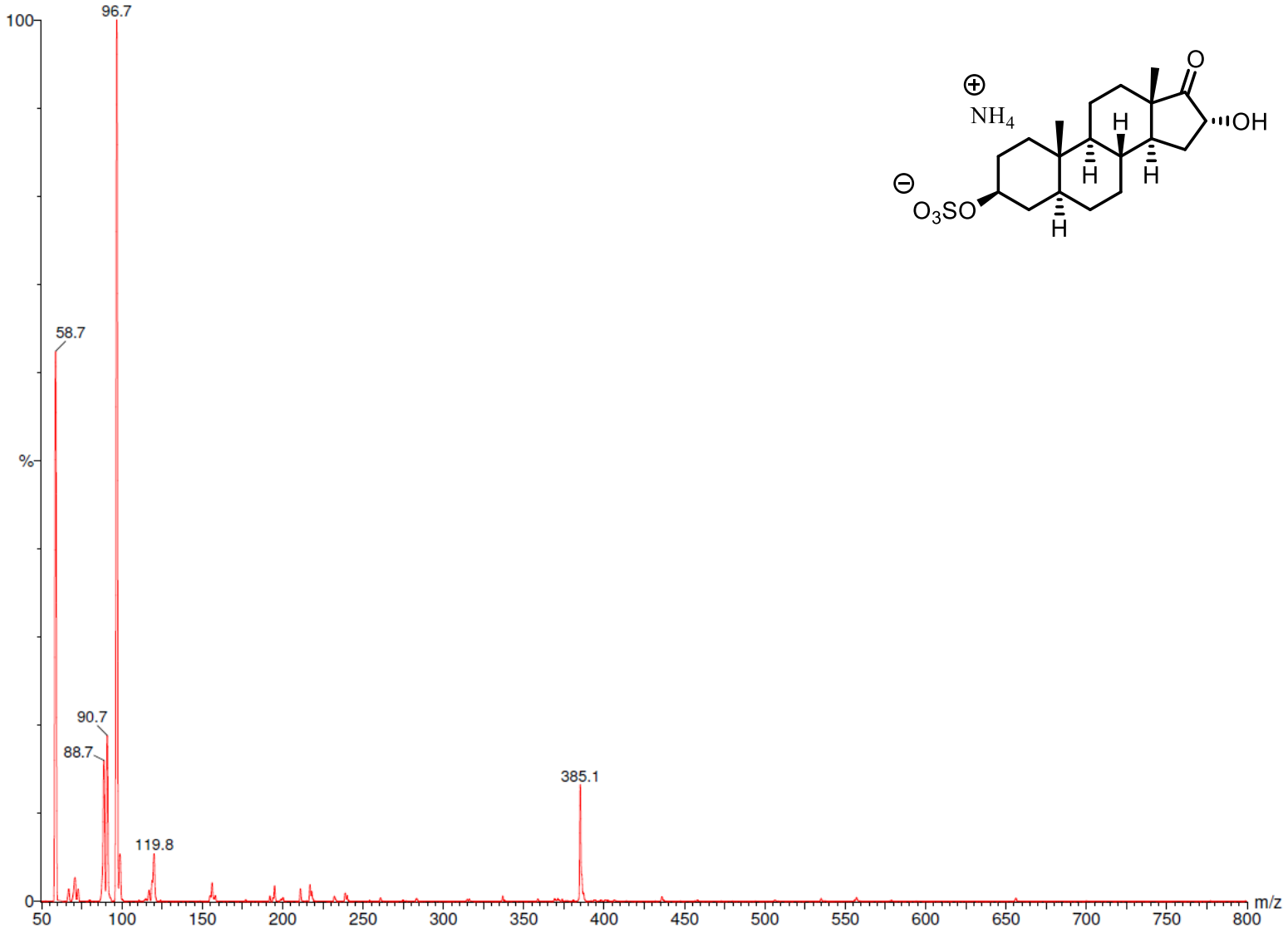
3ß,16α-Dihydroxy-5α-androstan-17-one 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



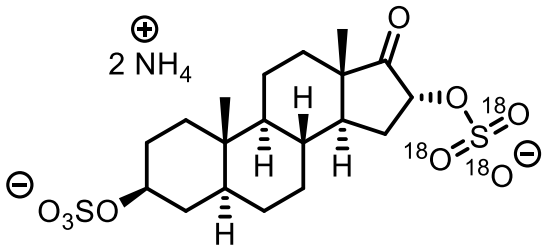
3ß,16α-Dihydroxy-5α-androstan-17-one 3-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD



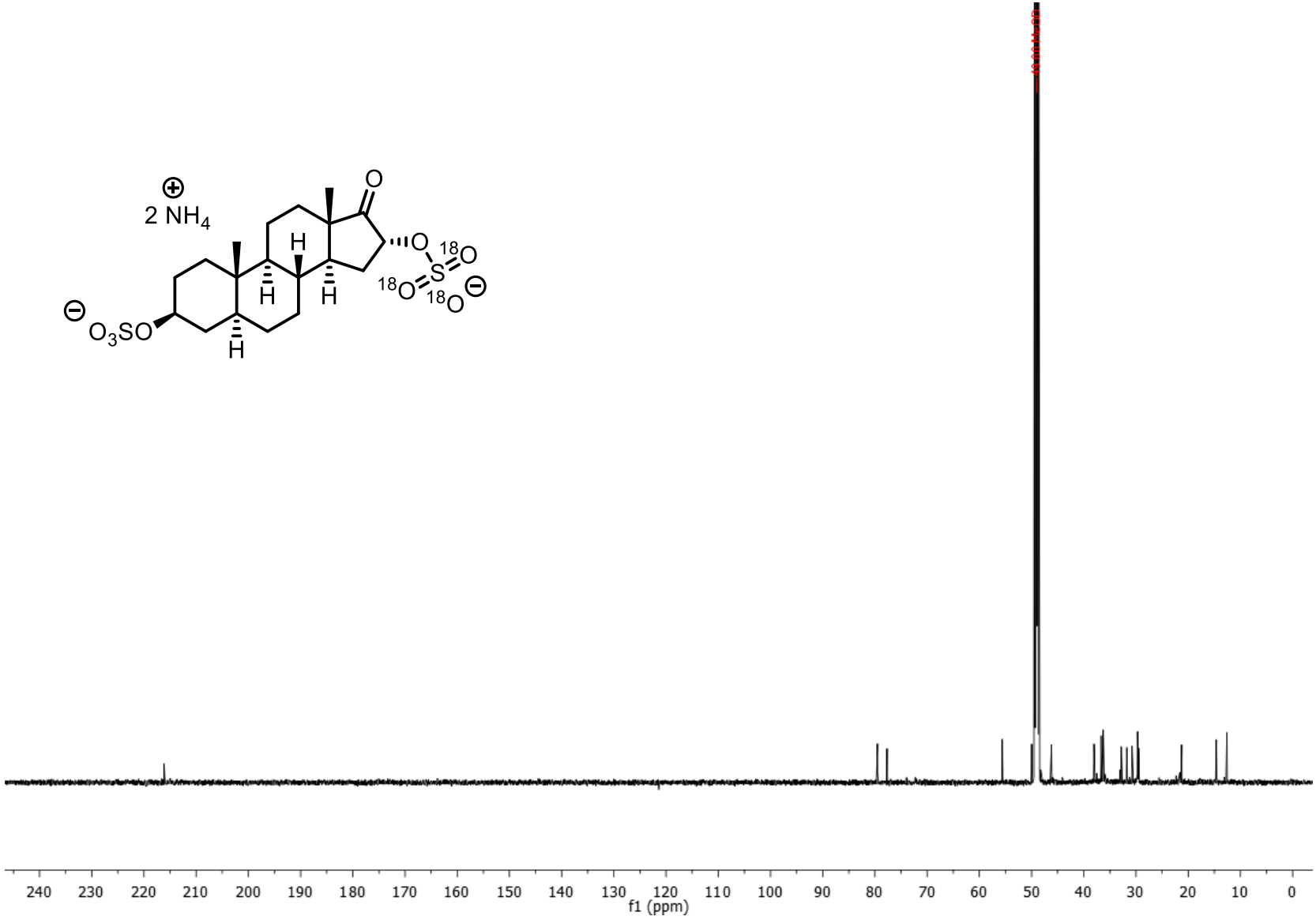
3ß,16α-Dihydroxy-5α-androstan-17-one 3-sulfate, ammonium salt LRMS



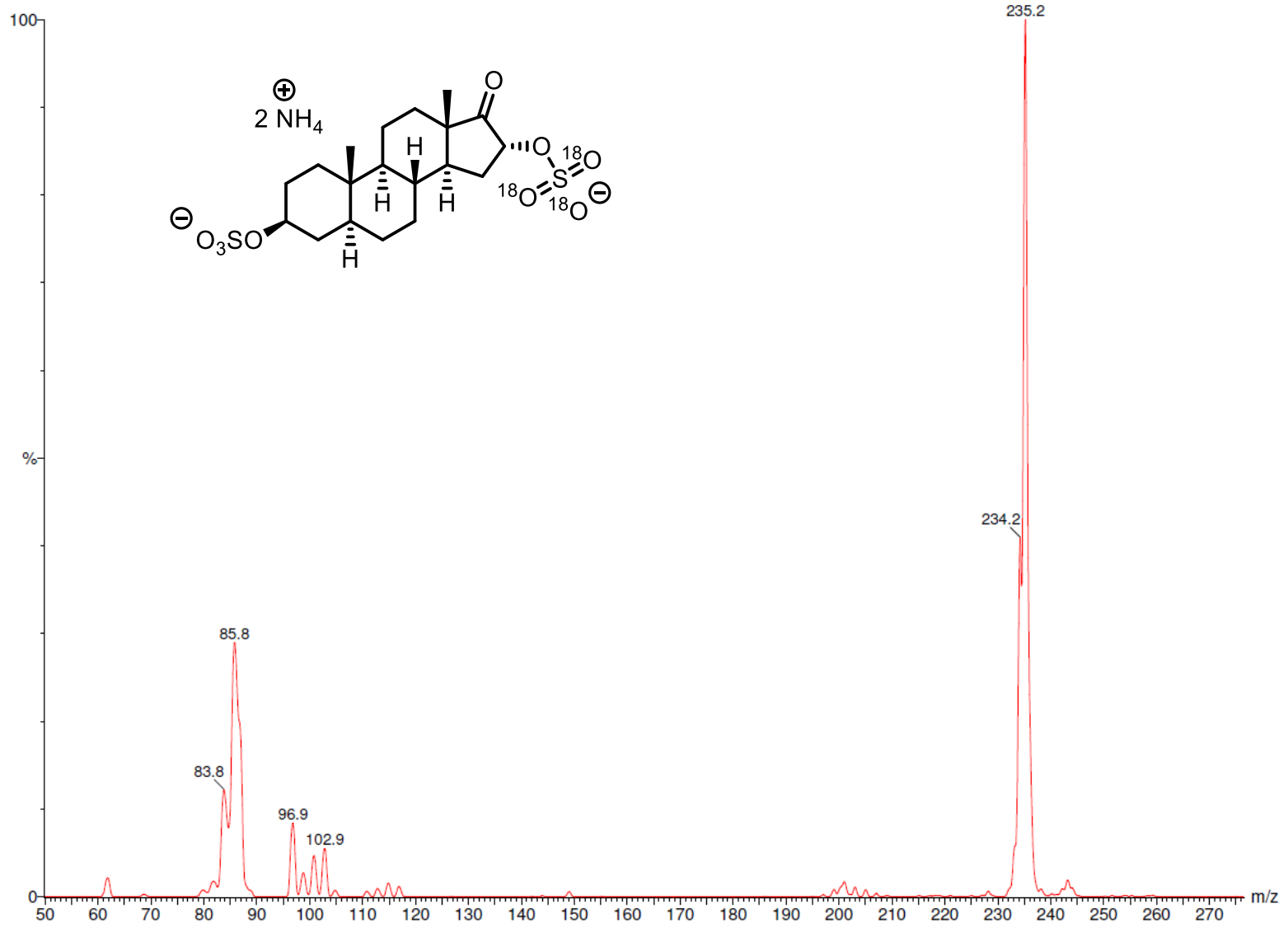
3ß,16α-Dihydroxy-5α-androstan-17-one 3,16[$^{18}O_3$]-bis(sulfate), ammonium salt ^{1}H NMR 400 MHz, CD_3OD



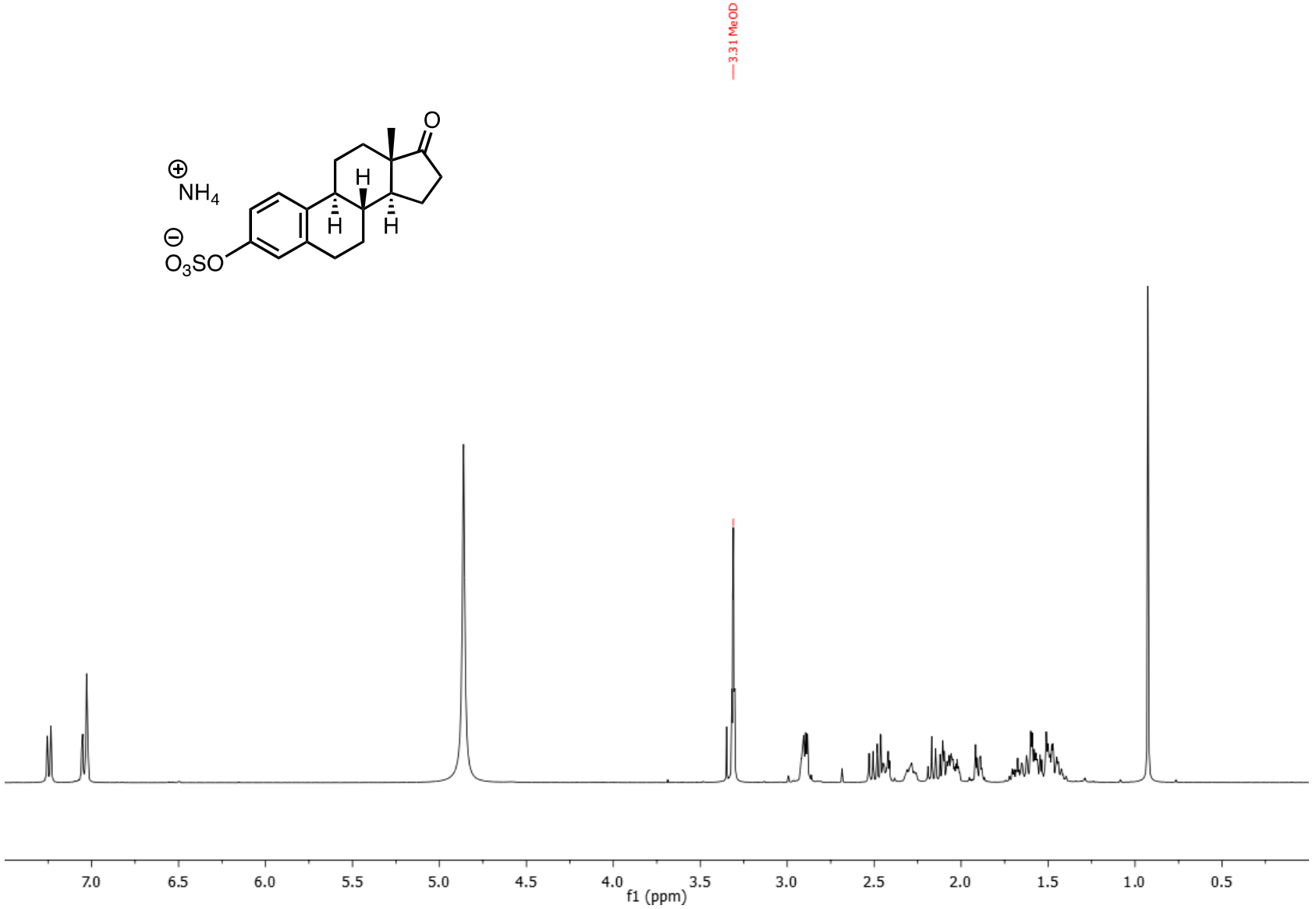
3ß,16α-Dihydroxy-5α-androstan-17-one 3,16[$^{18}O_3$]-bis(sulfate), ammonium salt ^{13}C NMR 101 MHz, CD_3OD



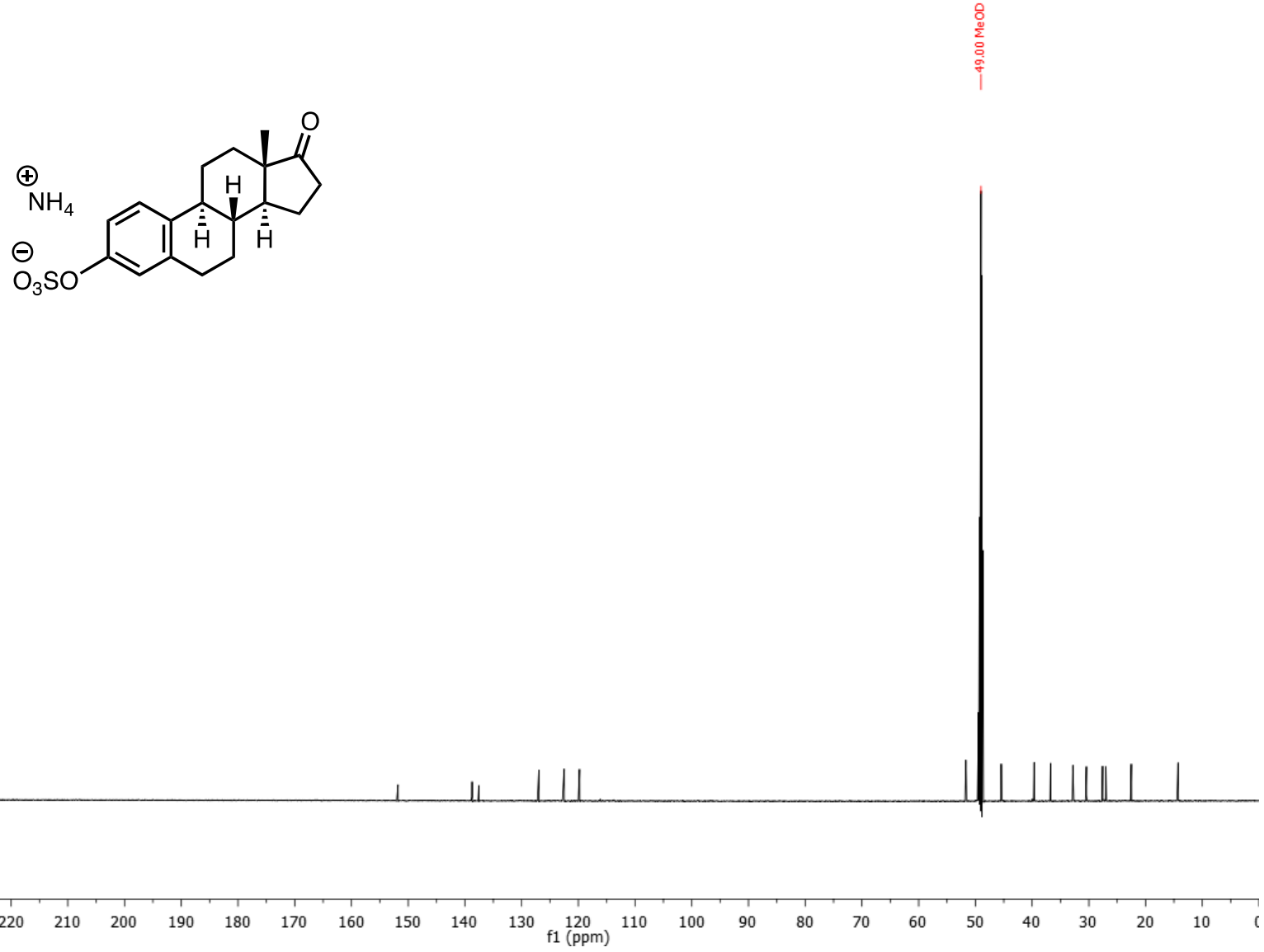
3ß,16α-Dihydroxy-5α-androstan-17-one 3,16[$^{18}O_3$]-bis(sulfate), ammonium salt LRMS



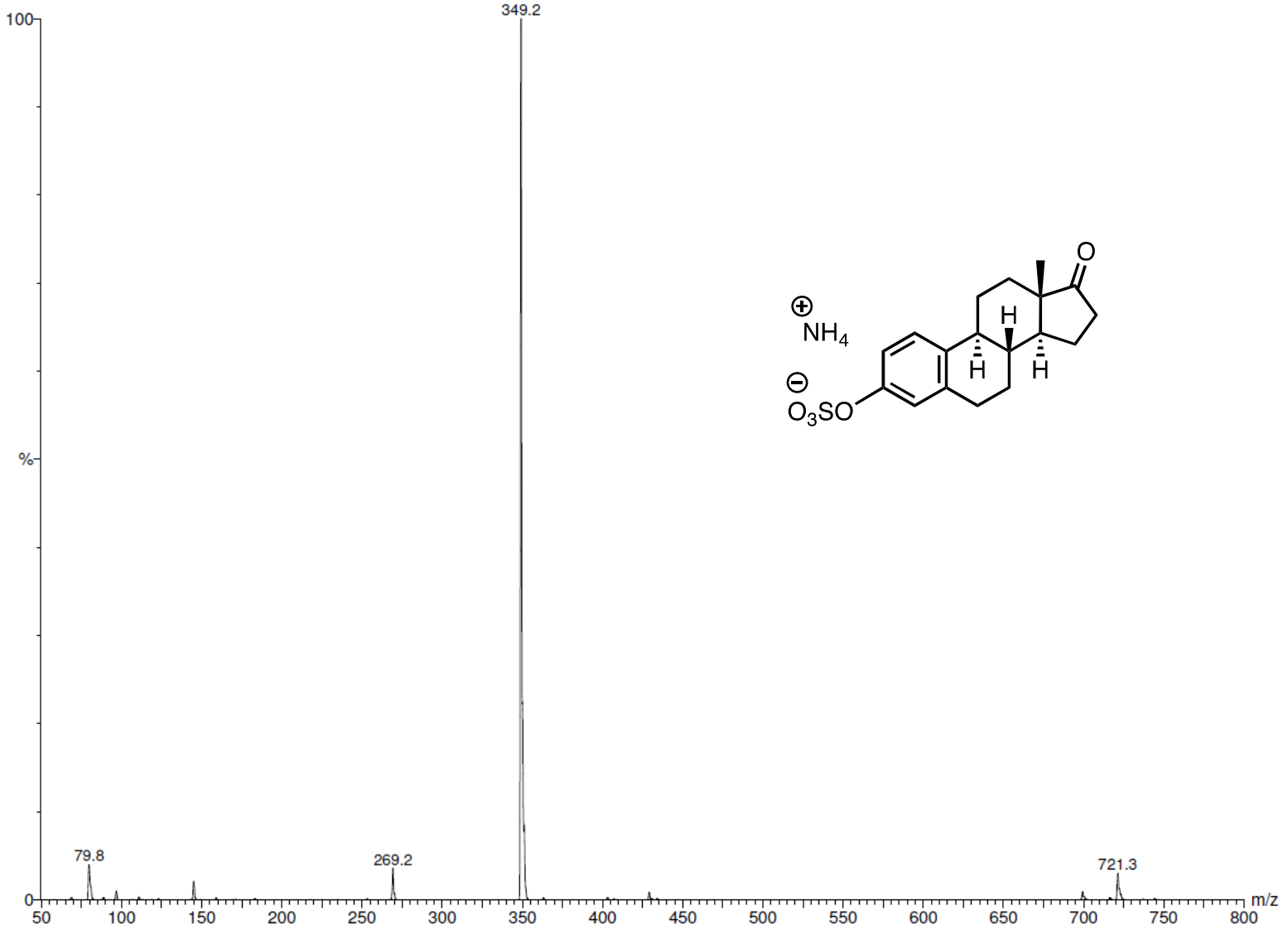
Estrone 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



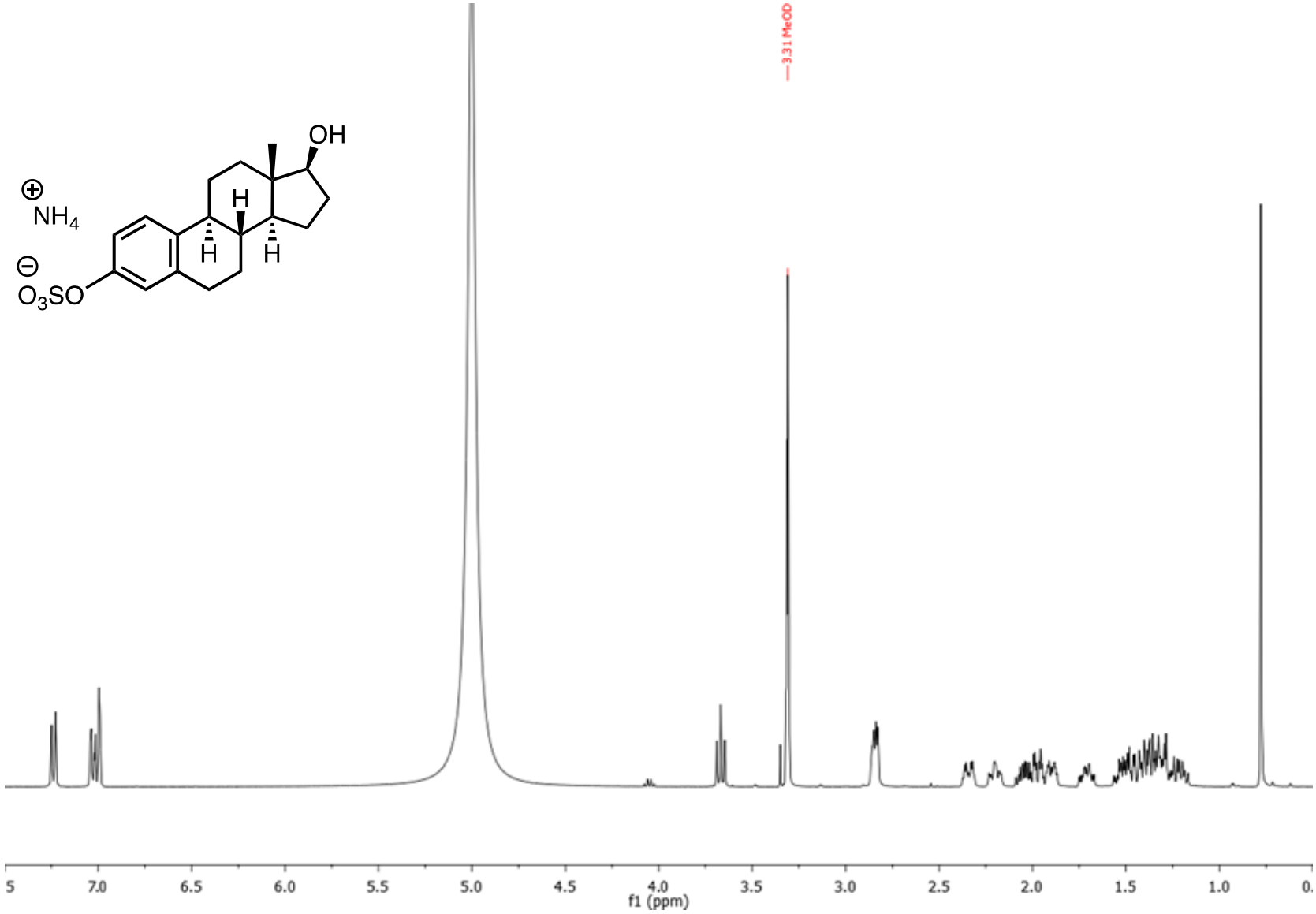
Estrone 3-sulfate, ammonium salt ^{13}C NMR 151 MHz, CD_3OD



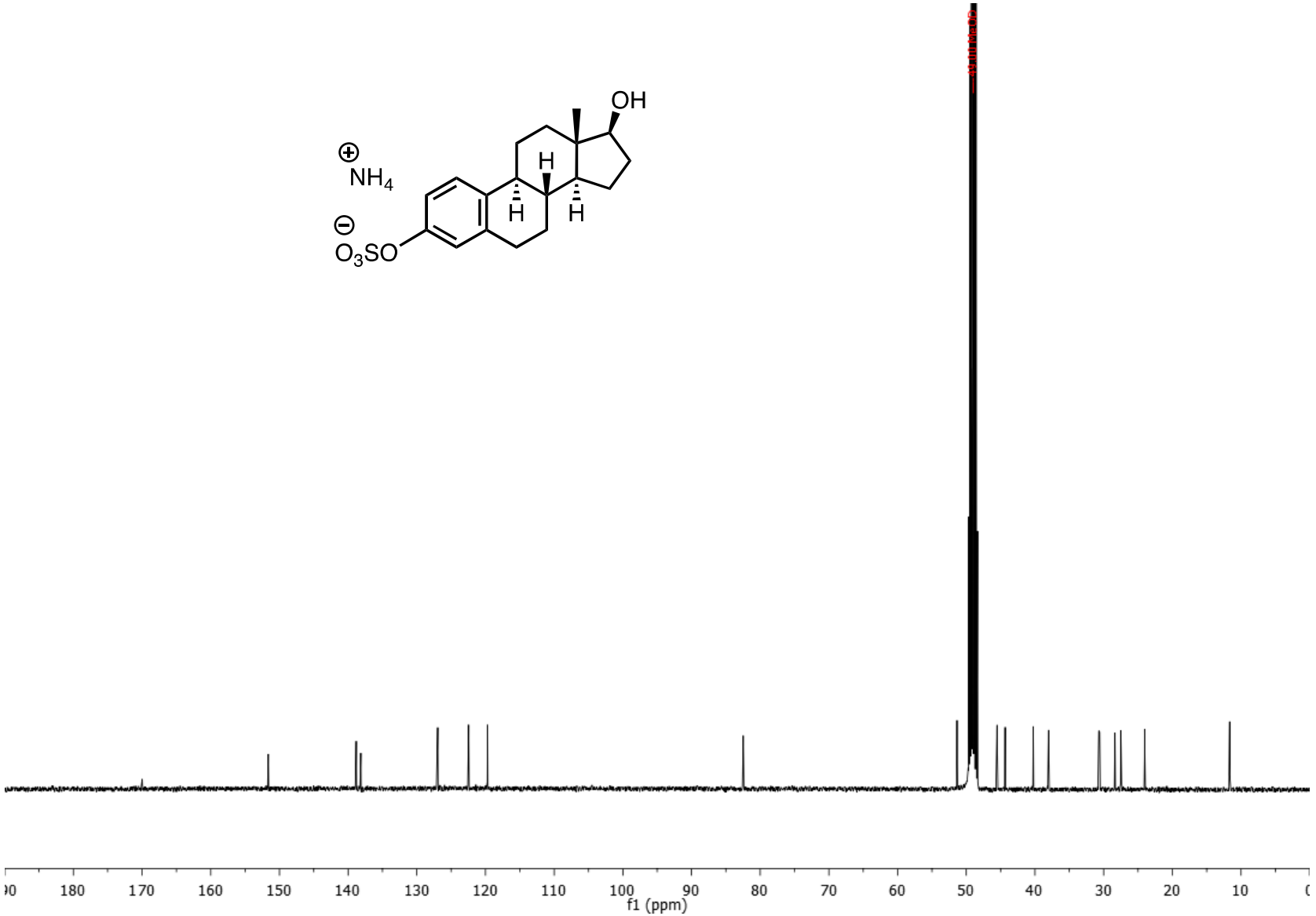
Estrone 3-sulfate, ammonium salt LRMS



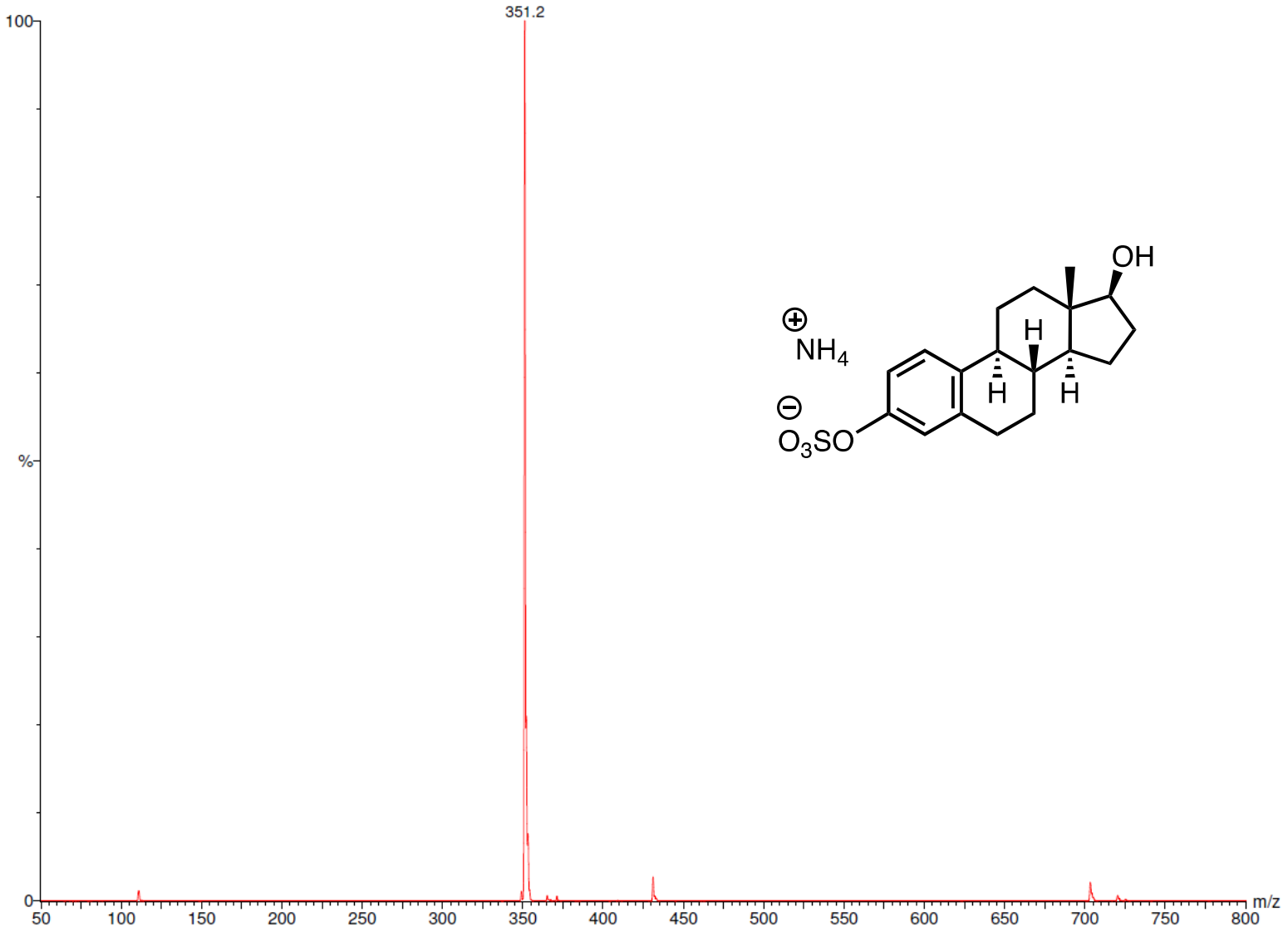
Estradiol 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



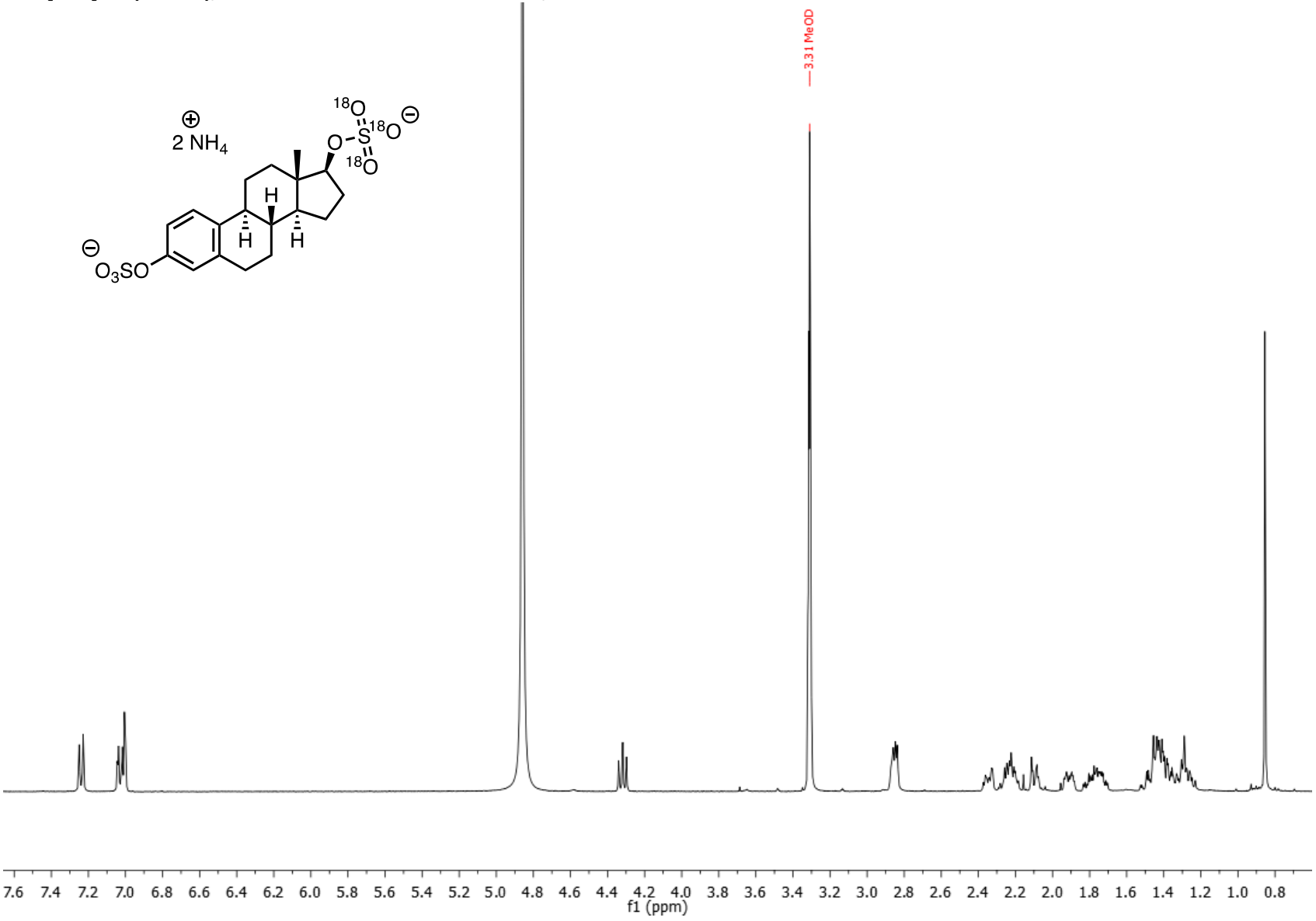
Estradiol 3-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD



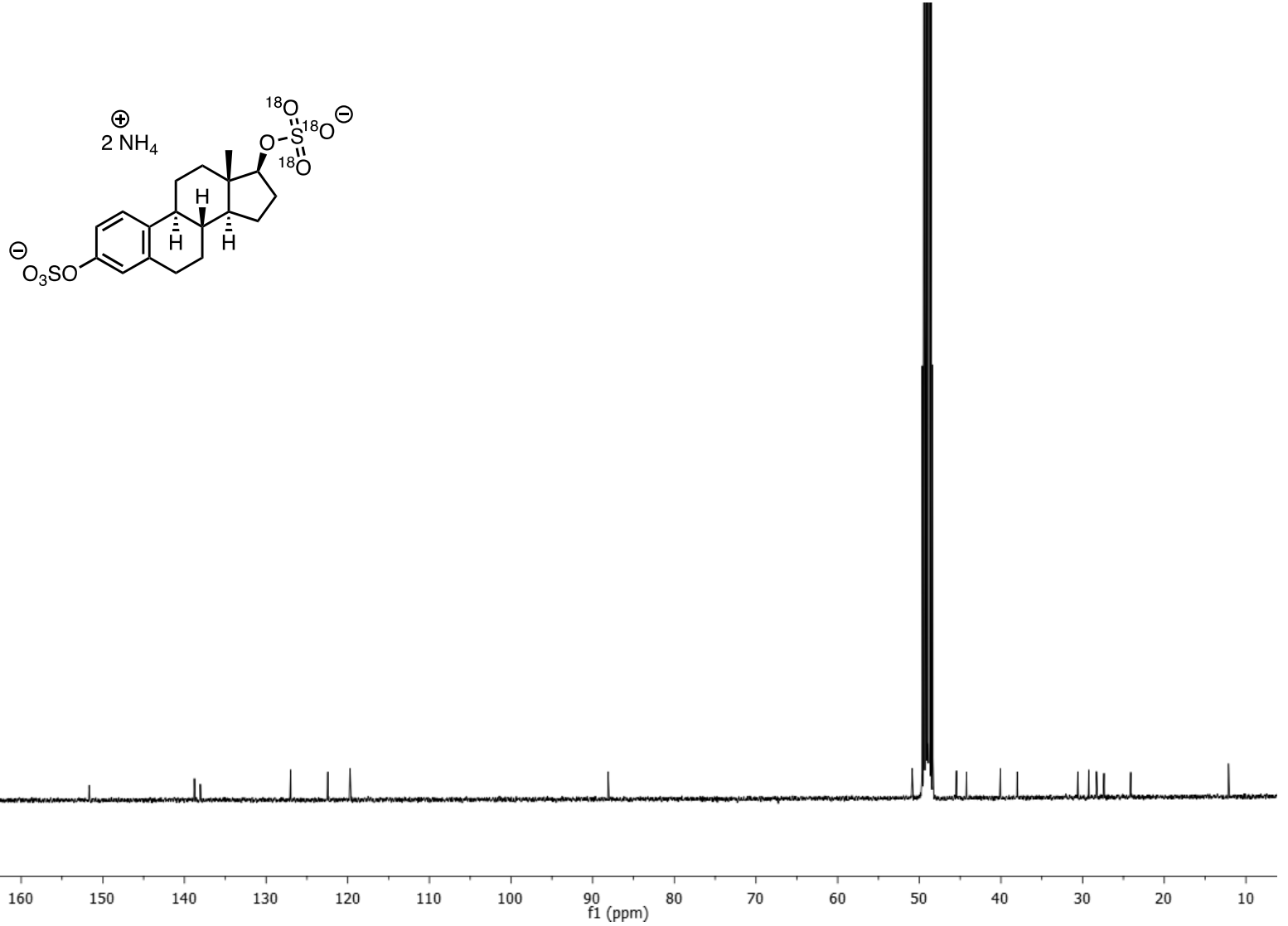
Estradiol 3-sulfate, ammonium salt LRMS



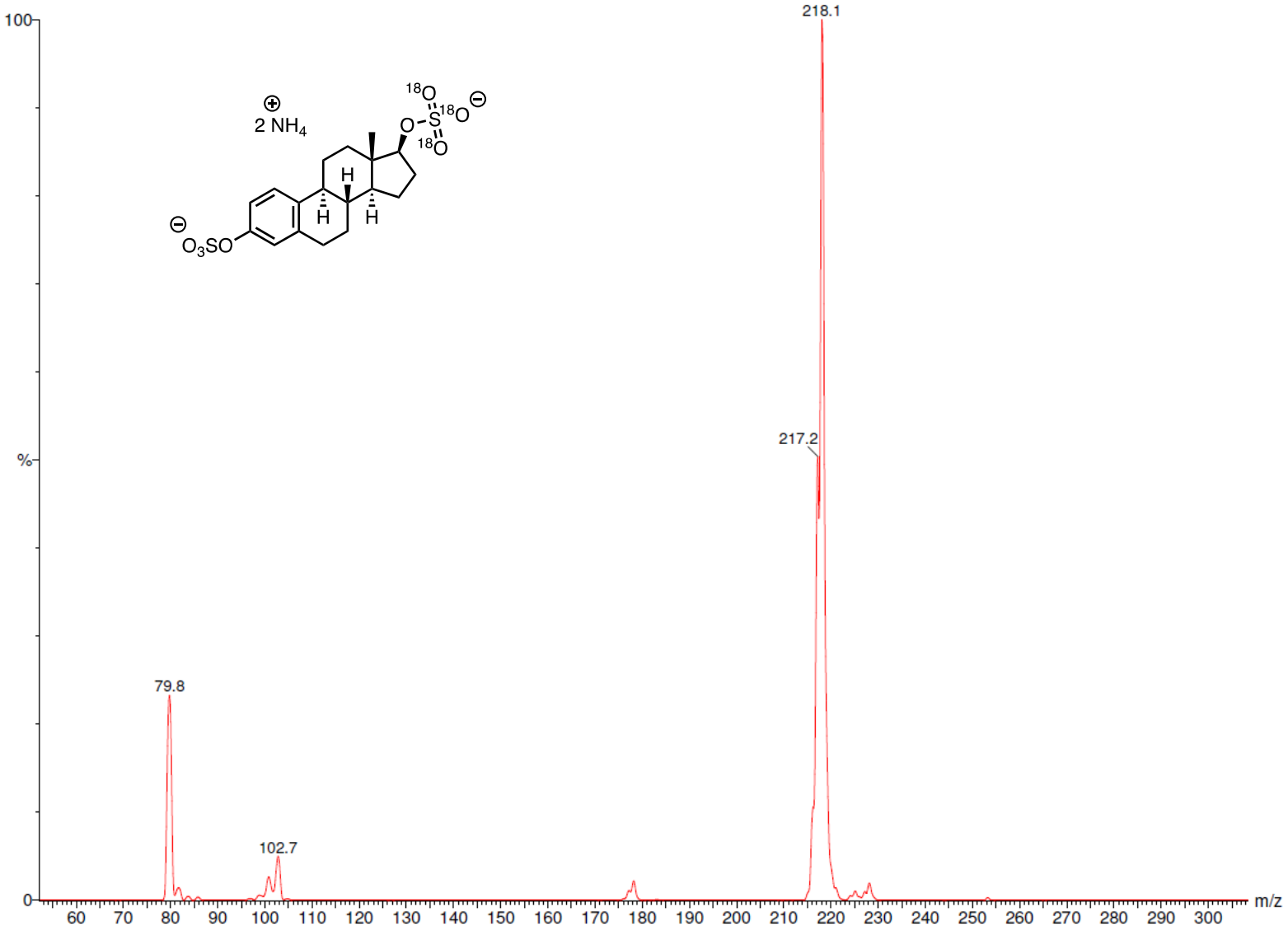
Estradiol 17[$^{18}O_3$]-bis(sulfate), ammonium salt ^{1}H NMR 400 MHz, CD_3OD



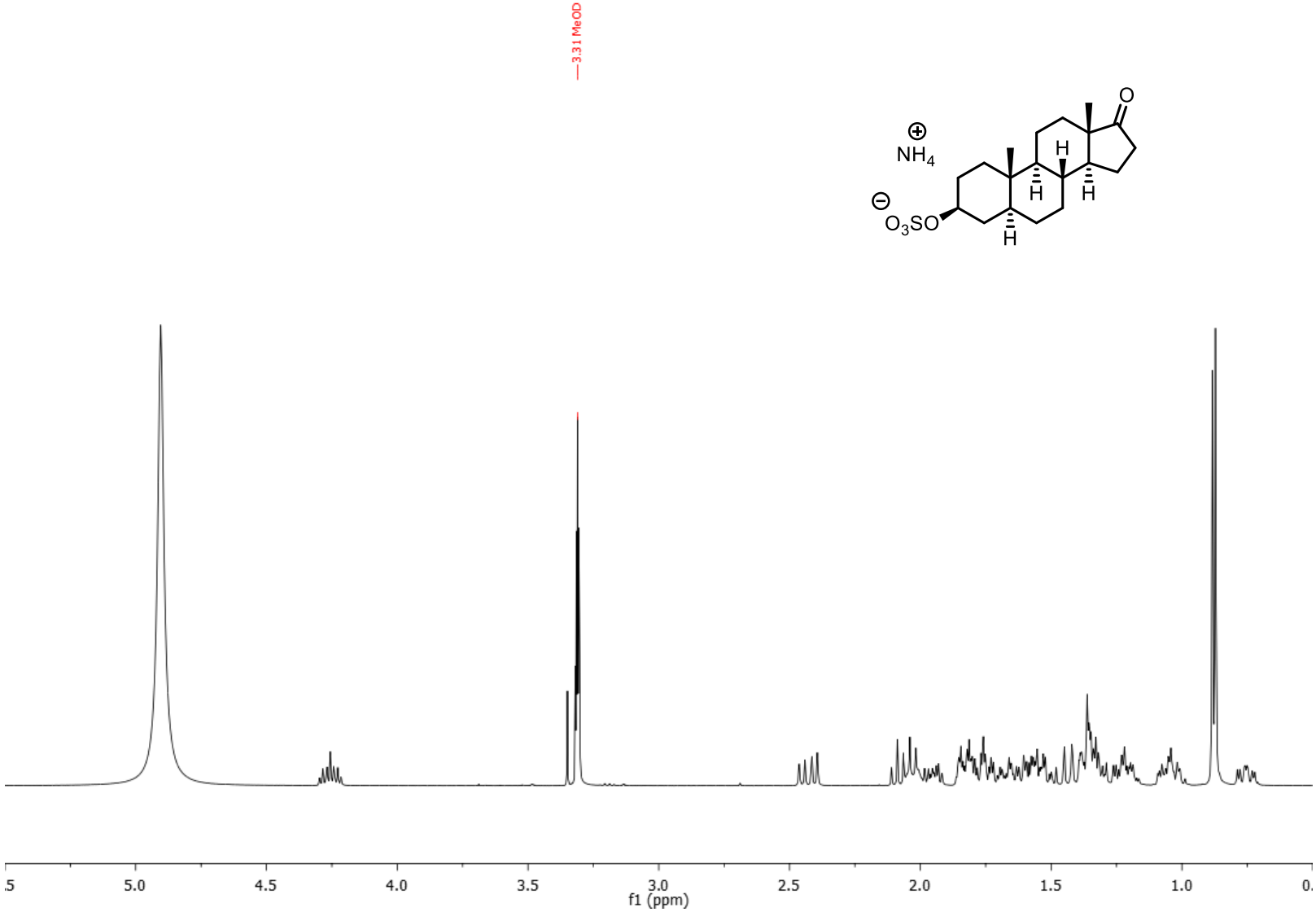
Estradiol 17[$^{18}O_3$]-bis(sulfate), ammonium salt ^{13}C NMR 101 MHz, CD_3OD



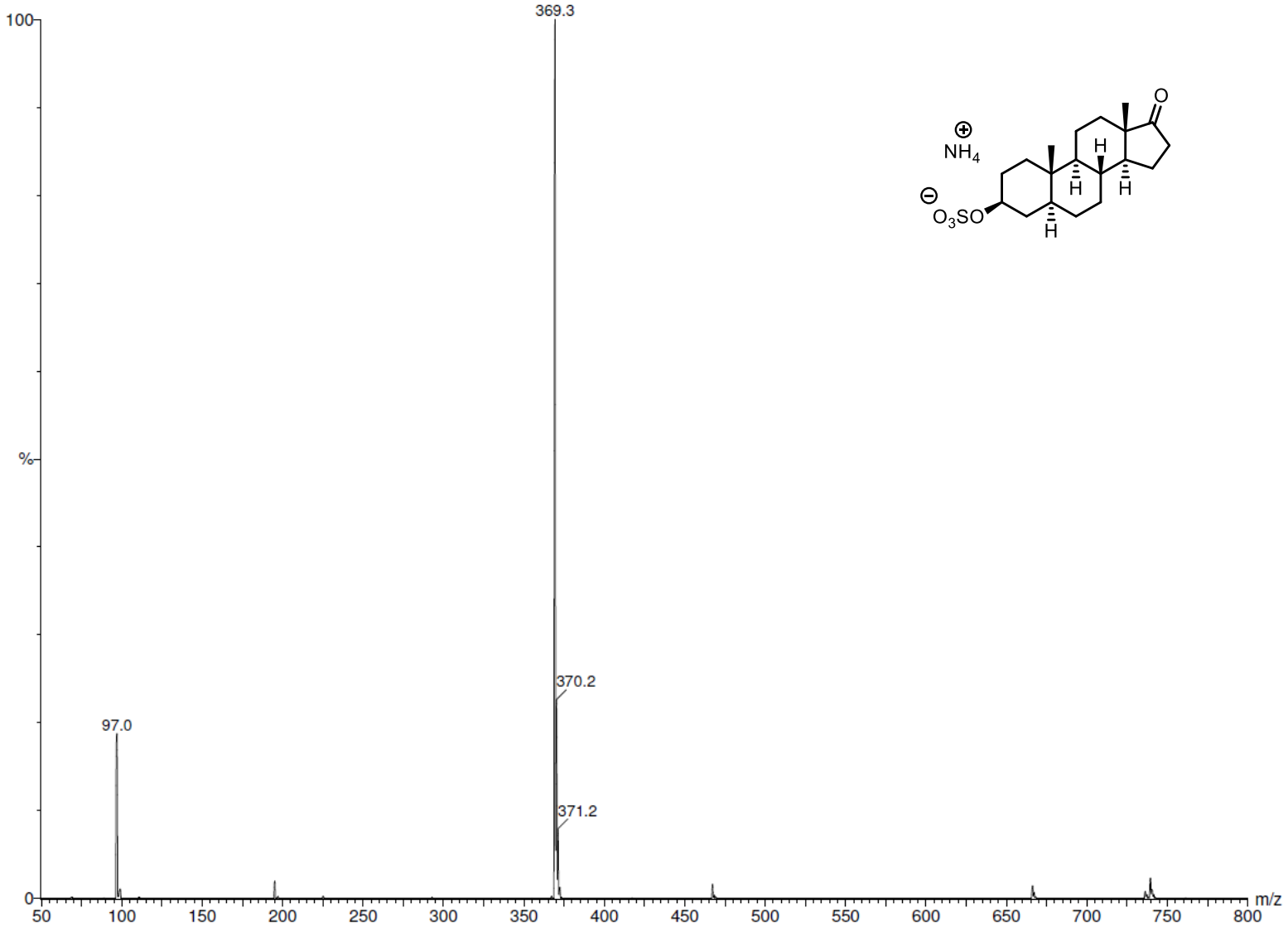
Estradiol 17[$^{18}O_3$]-bis(sulfate), ammonium salt LRMS



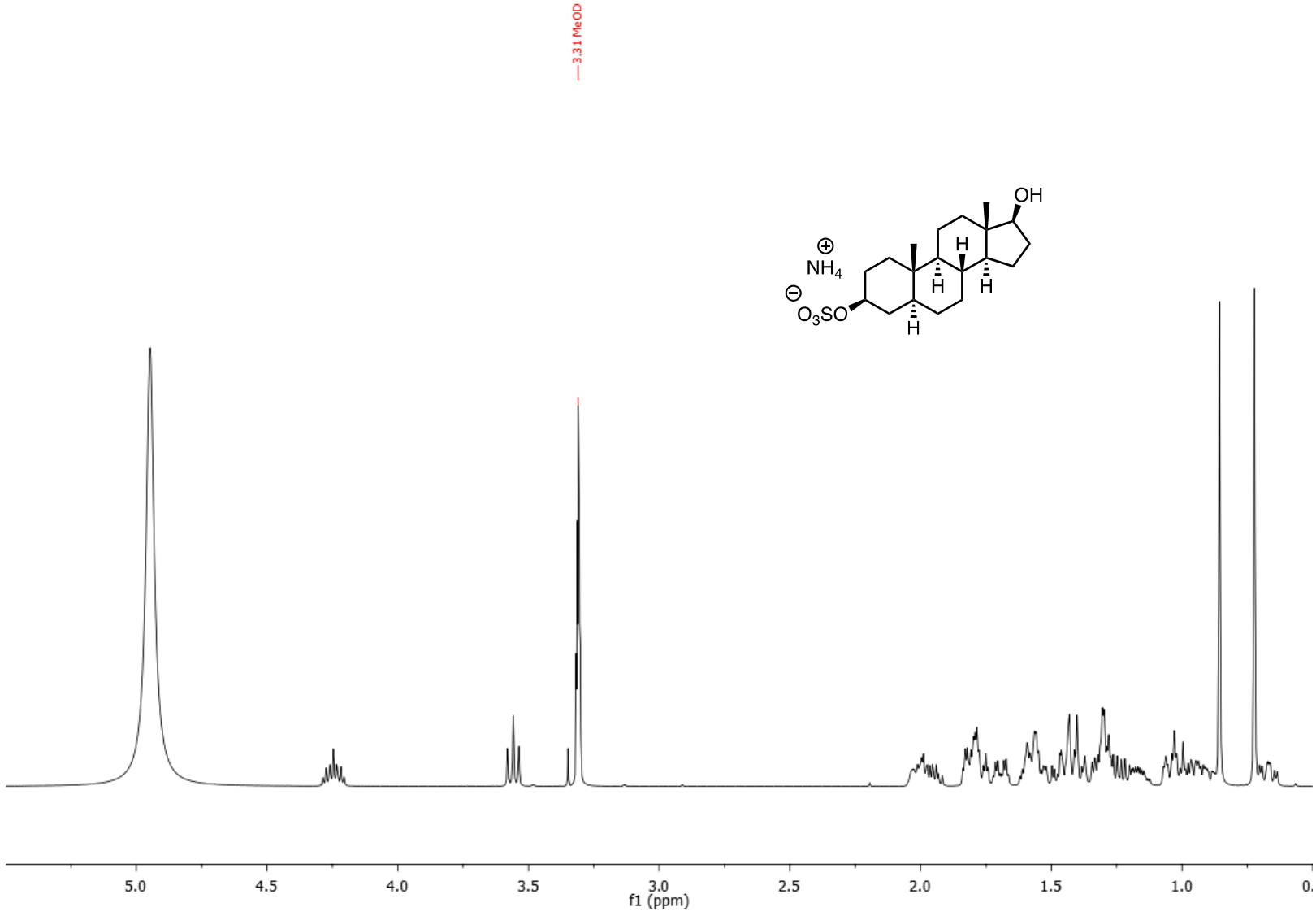
Epiandrosterone 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



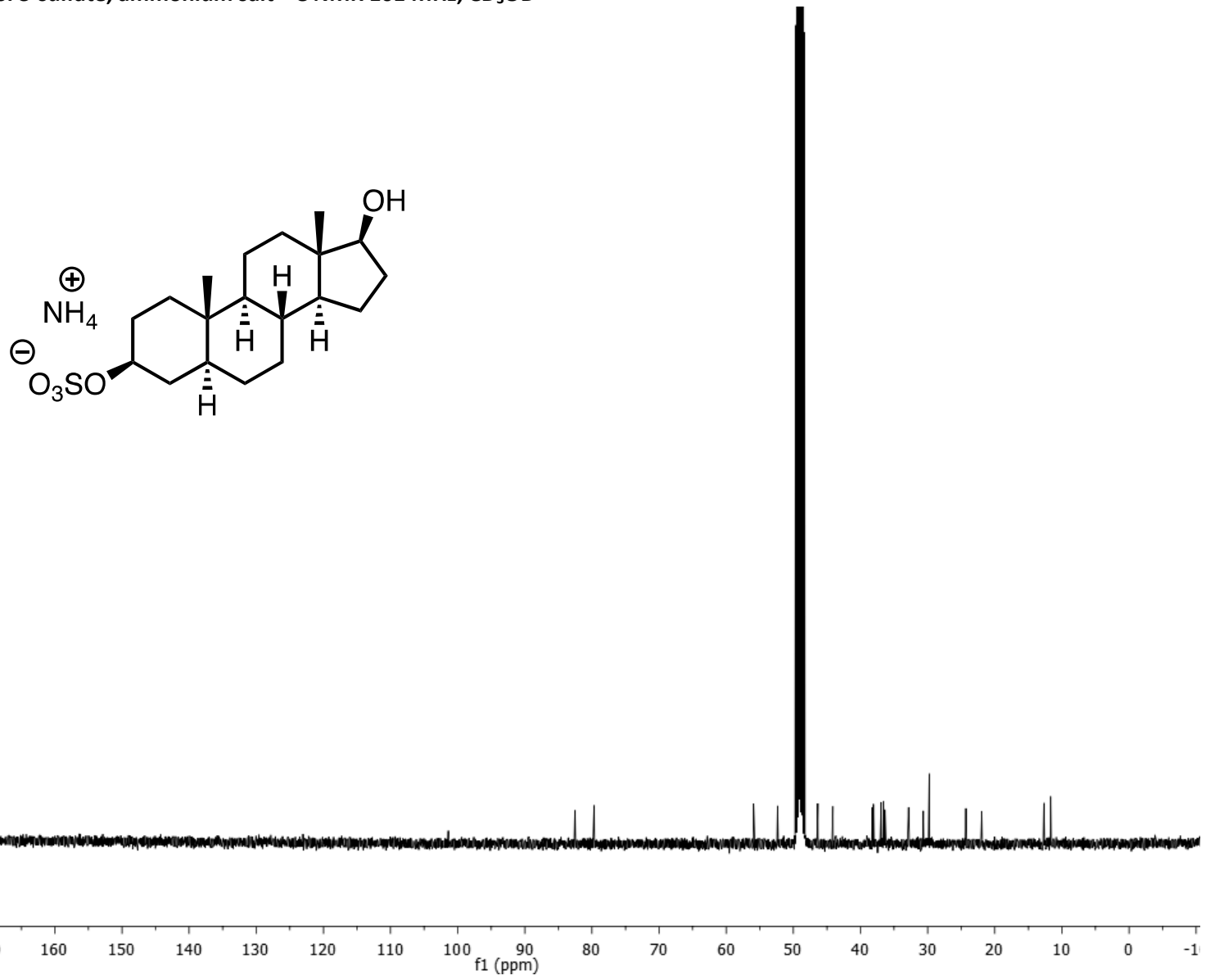
Epiandrosterone 3-sulfate, ammonium salt LRMS



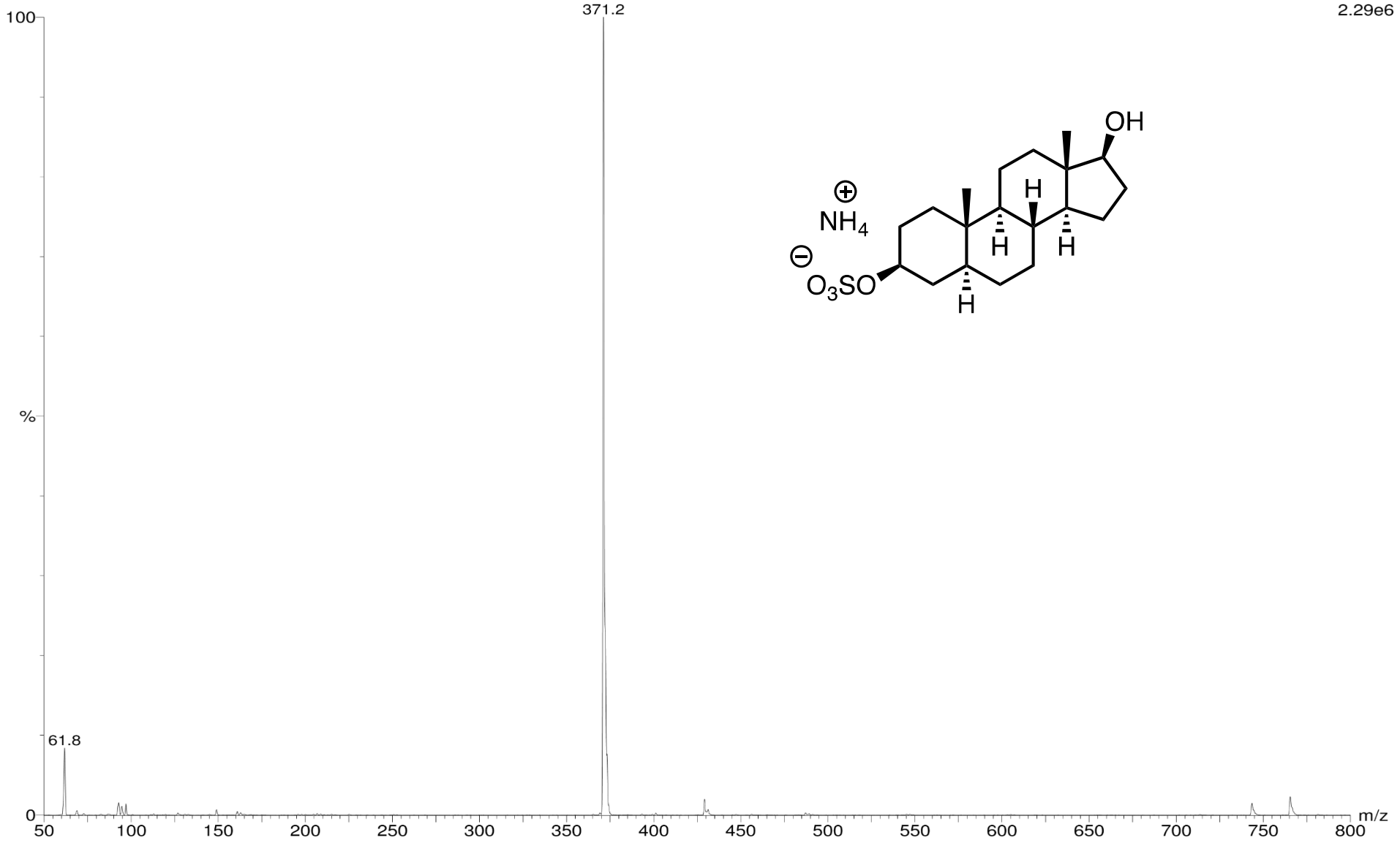
5α-Androstane-3β,17β-diol 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



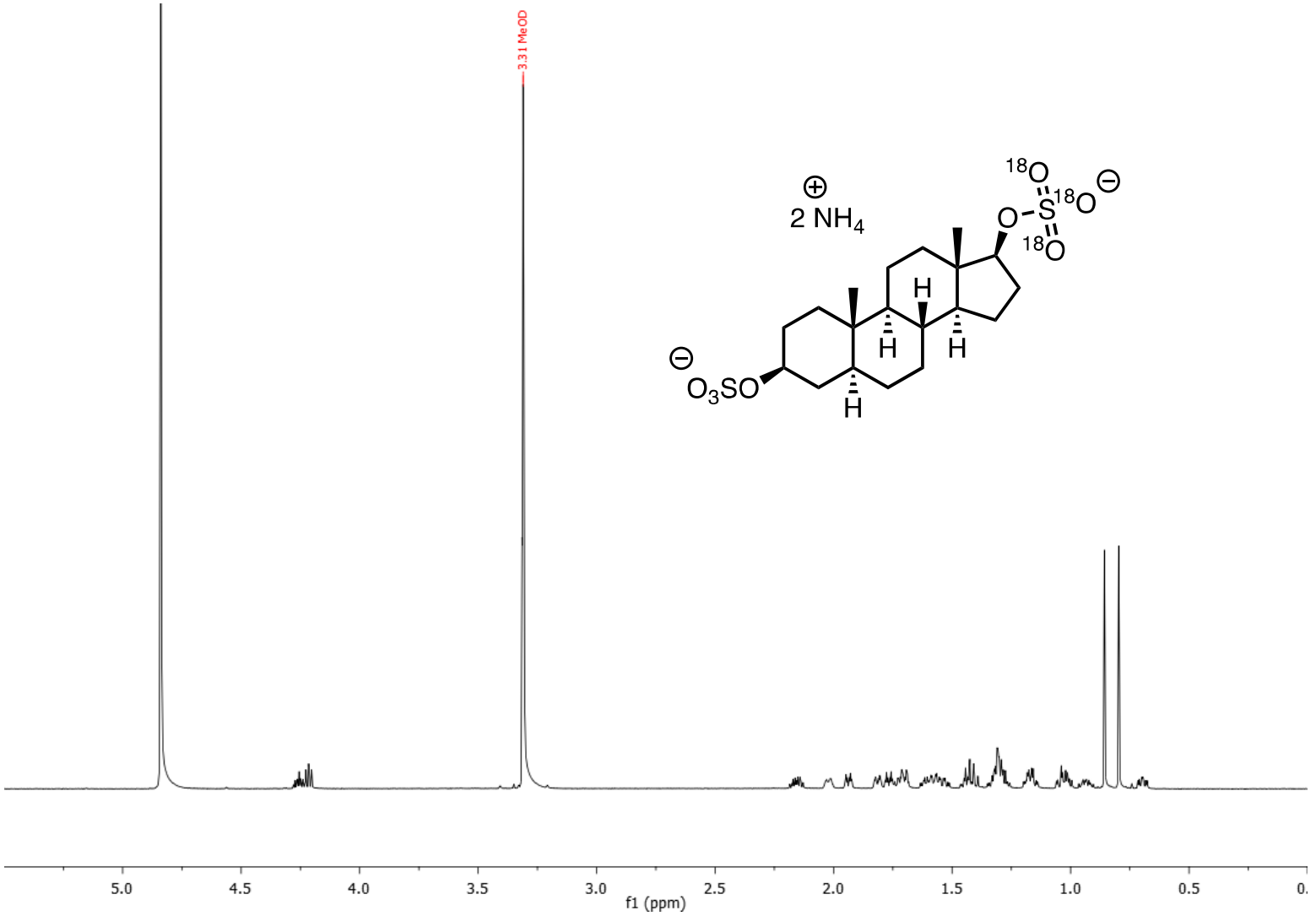
5α-Androstane-3β,17β-diol 3-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD



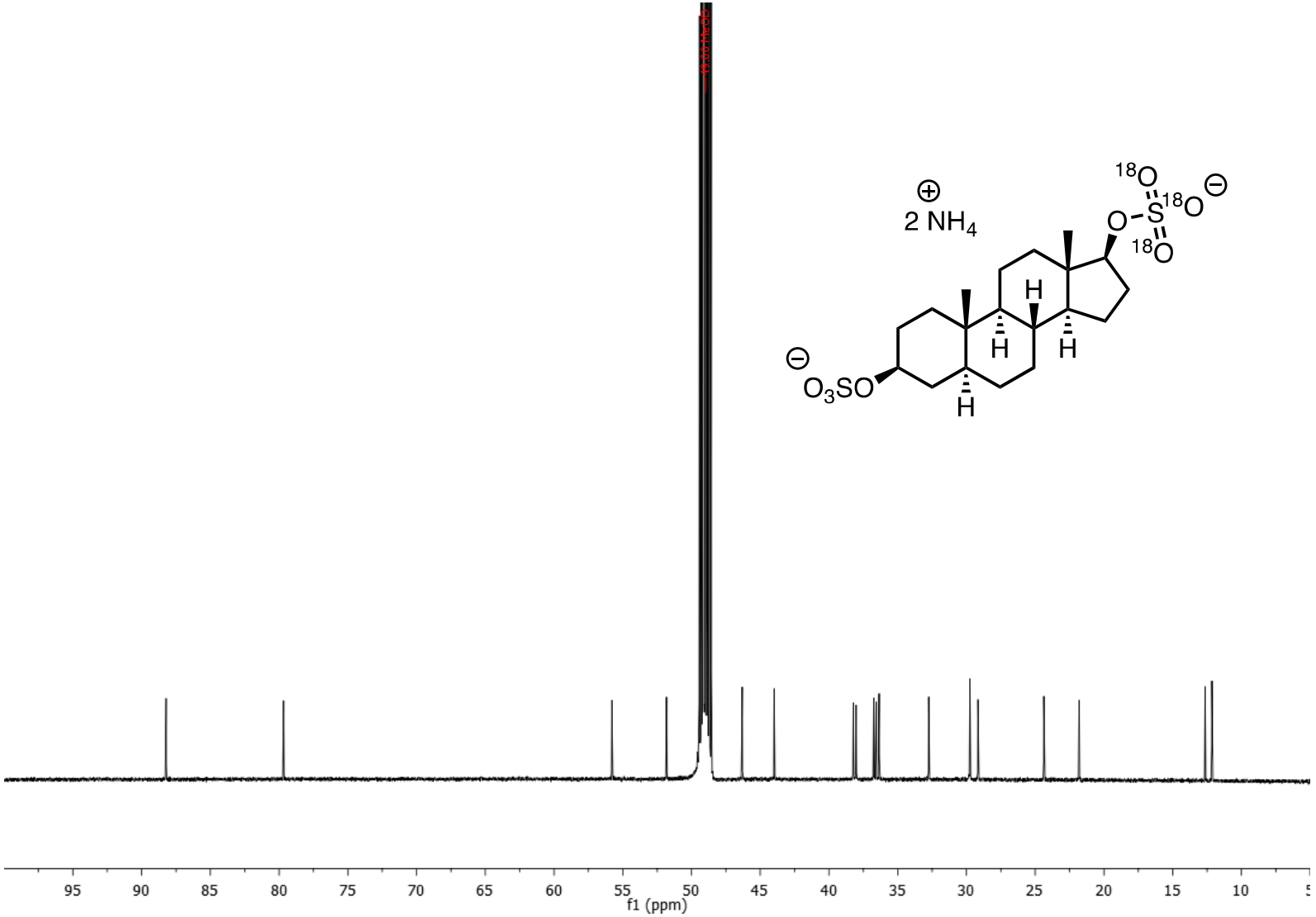
5α-Androstane-3β,17β-diol 3-sulfate, ammonium salt LRMS



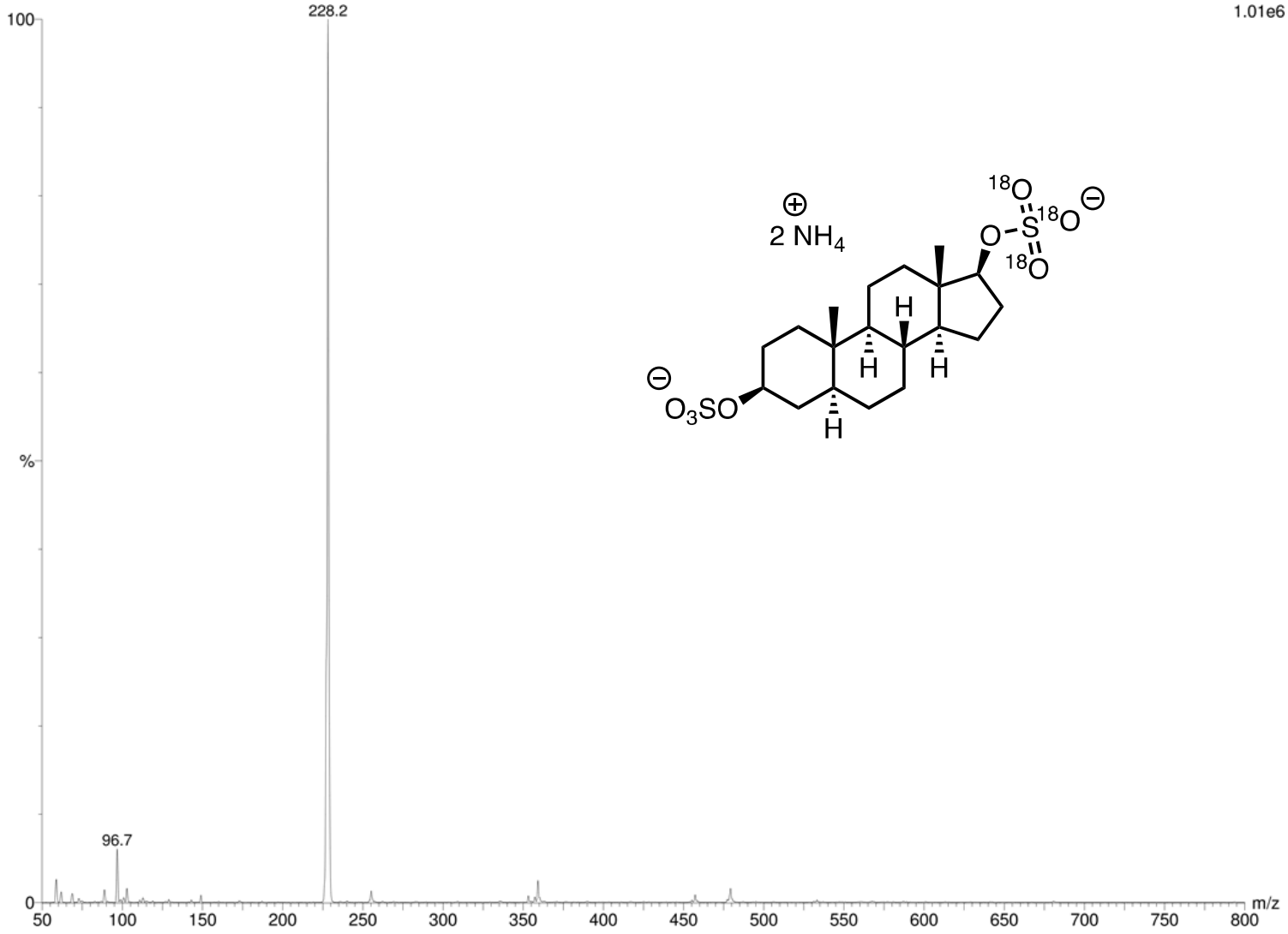
5α-Androstane-3β,17β[$^{18}O_3$]-diol bis(sulfate) ^{1}H NMR 400 MHz, CD_3OD



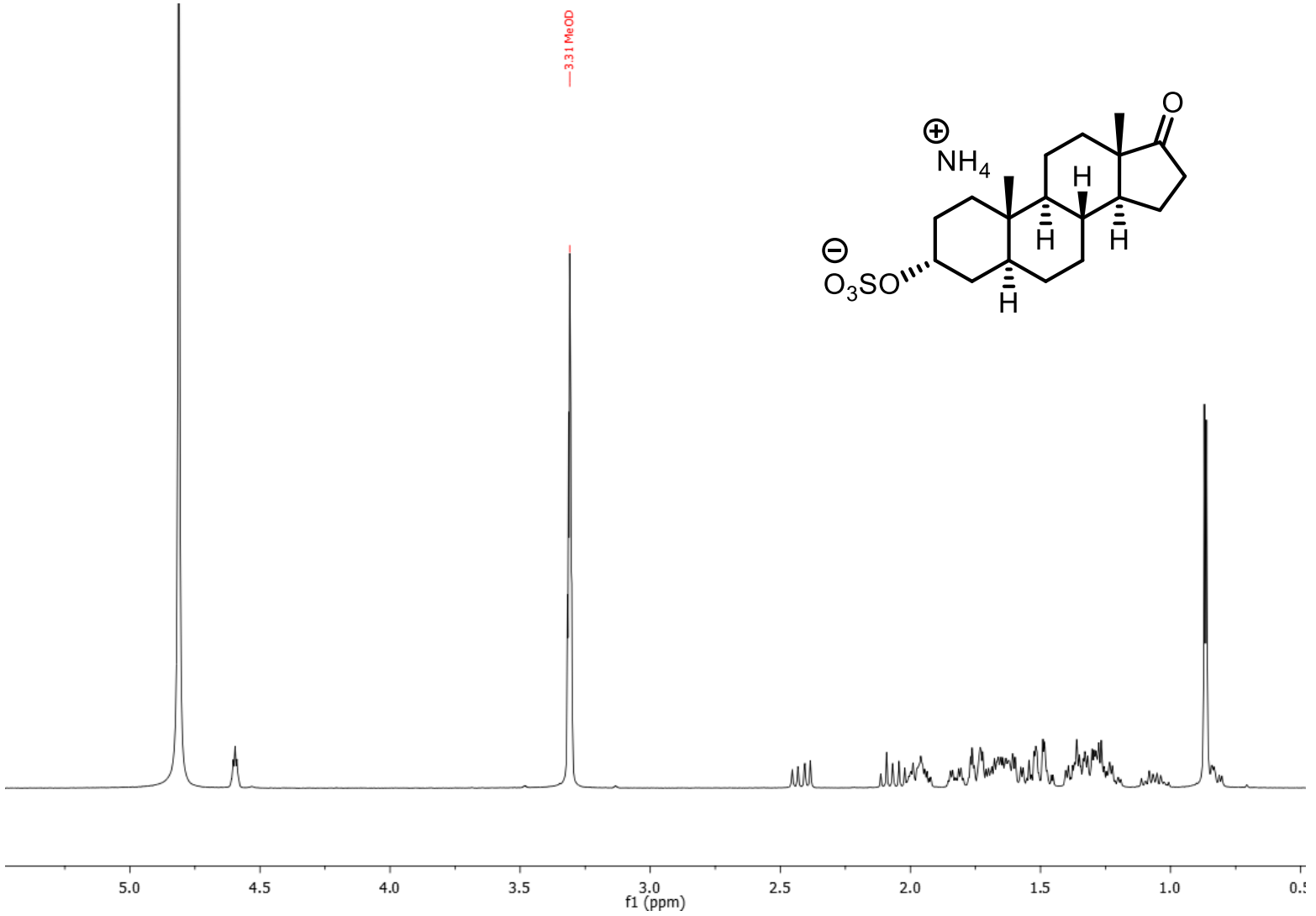
5α-Androstane-3β,17β[$^{18}O_3$]-diol bis(sulfate) ^{13}C NMR 151 MHz, CD_3OD



5α-Androstane-3β,17β[$^{18}O_3$]-diol bis(sulfate) LRMS

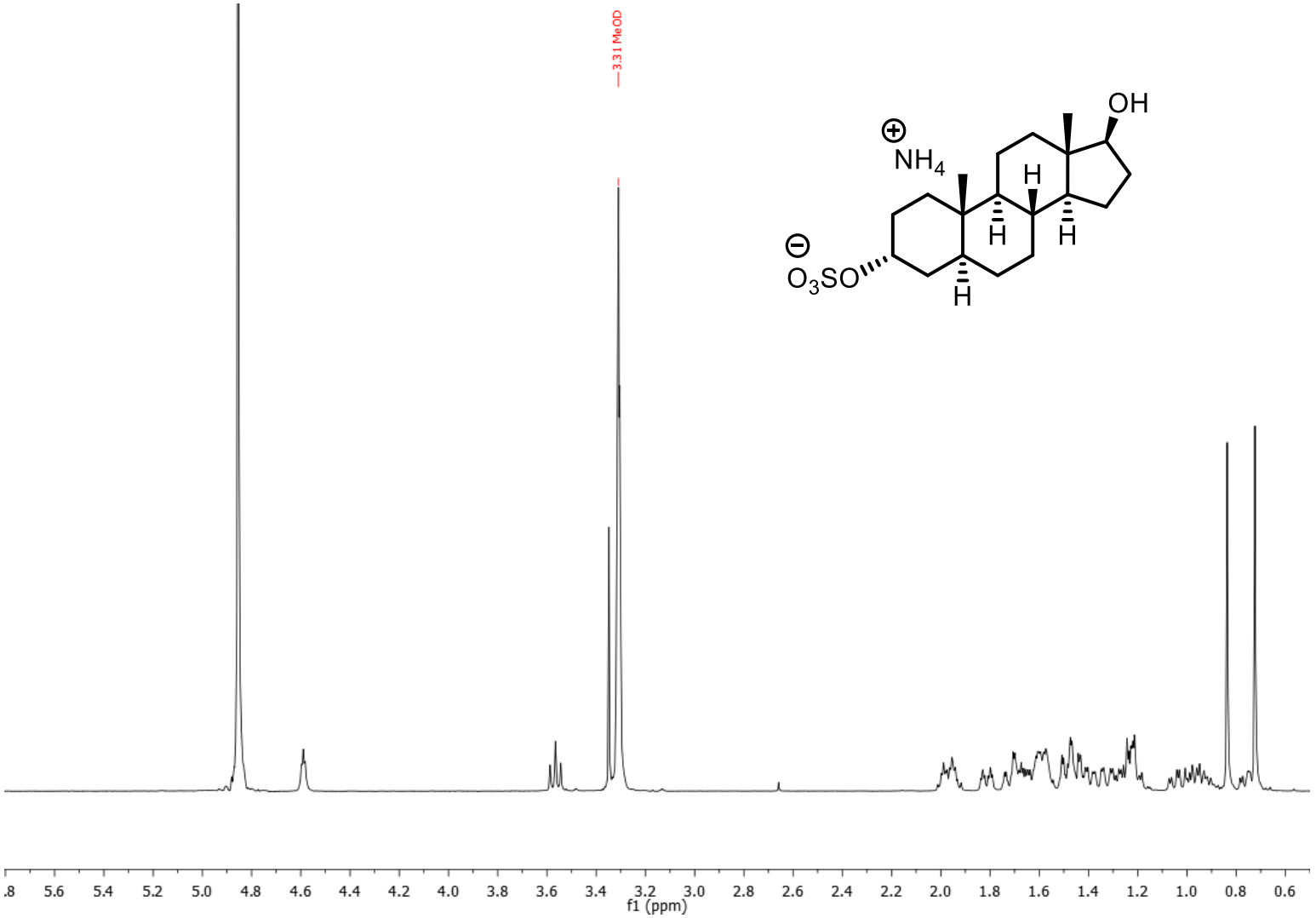


Androsterone 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD

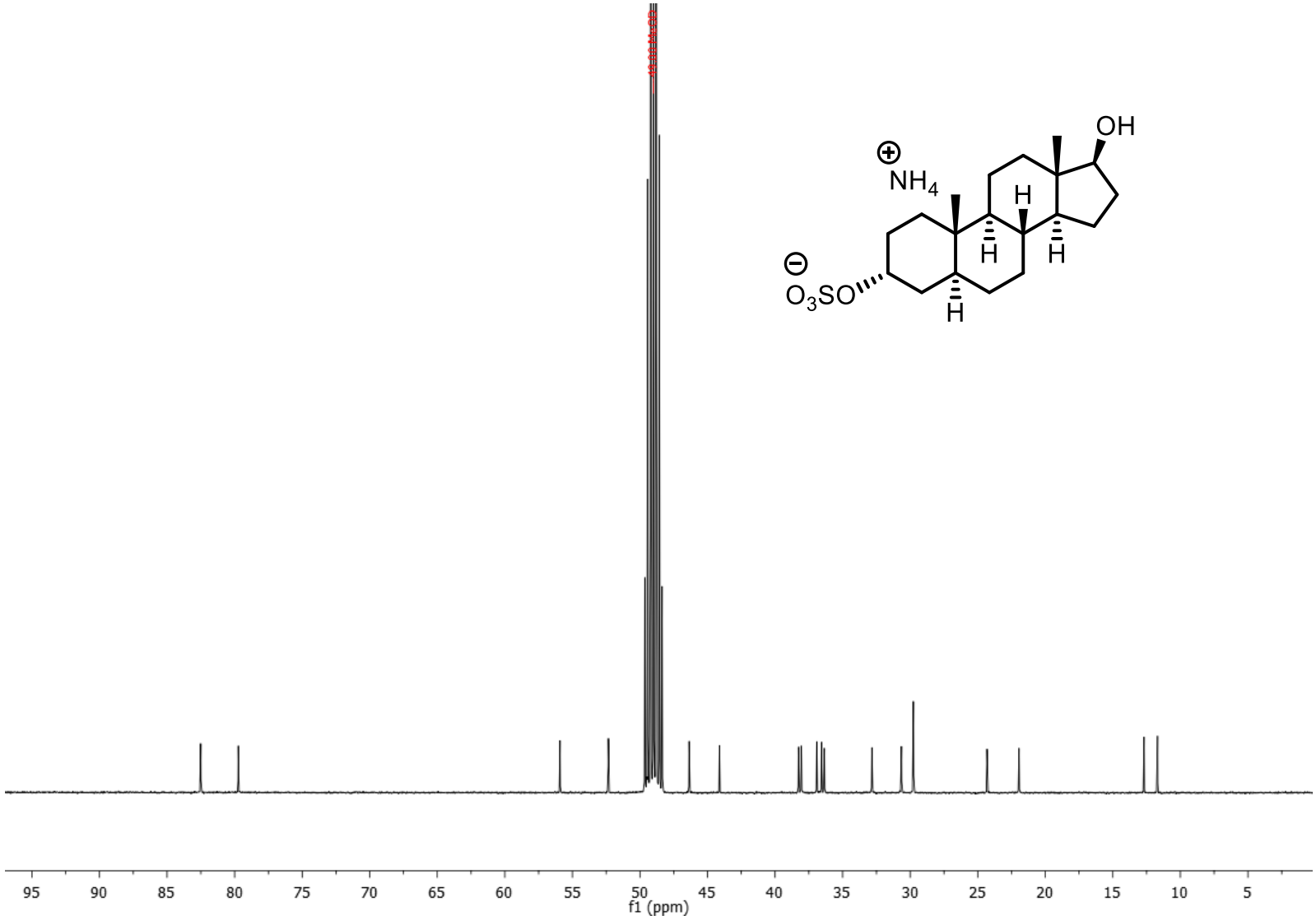


Androsterone 3-sulfate, ammonium salt LRMS

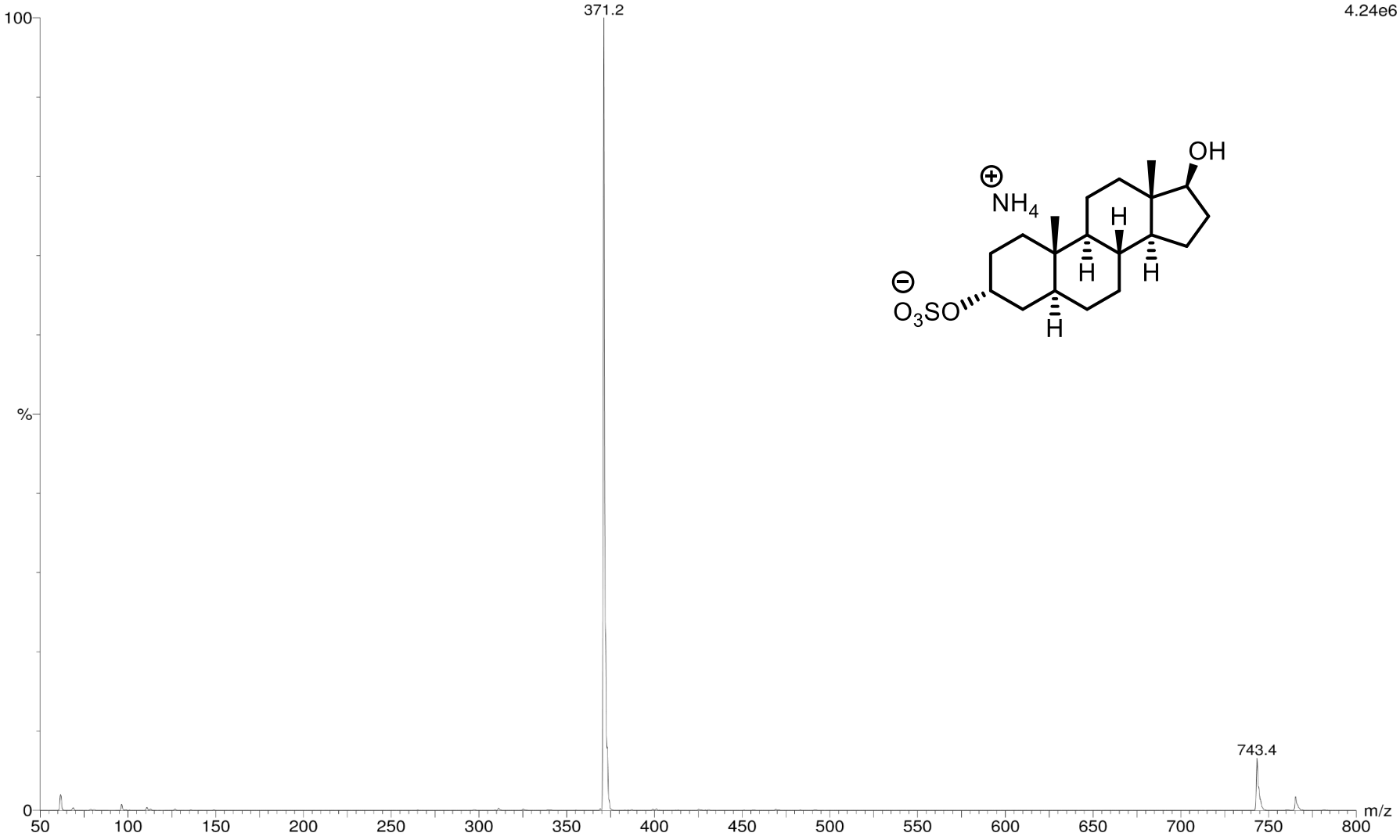
5α-Androstane-3α,17β-diol 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



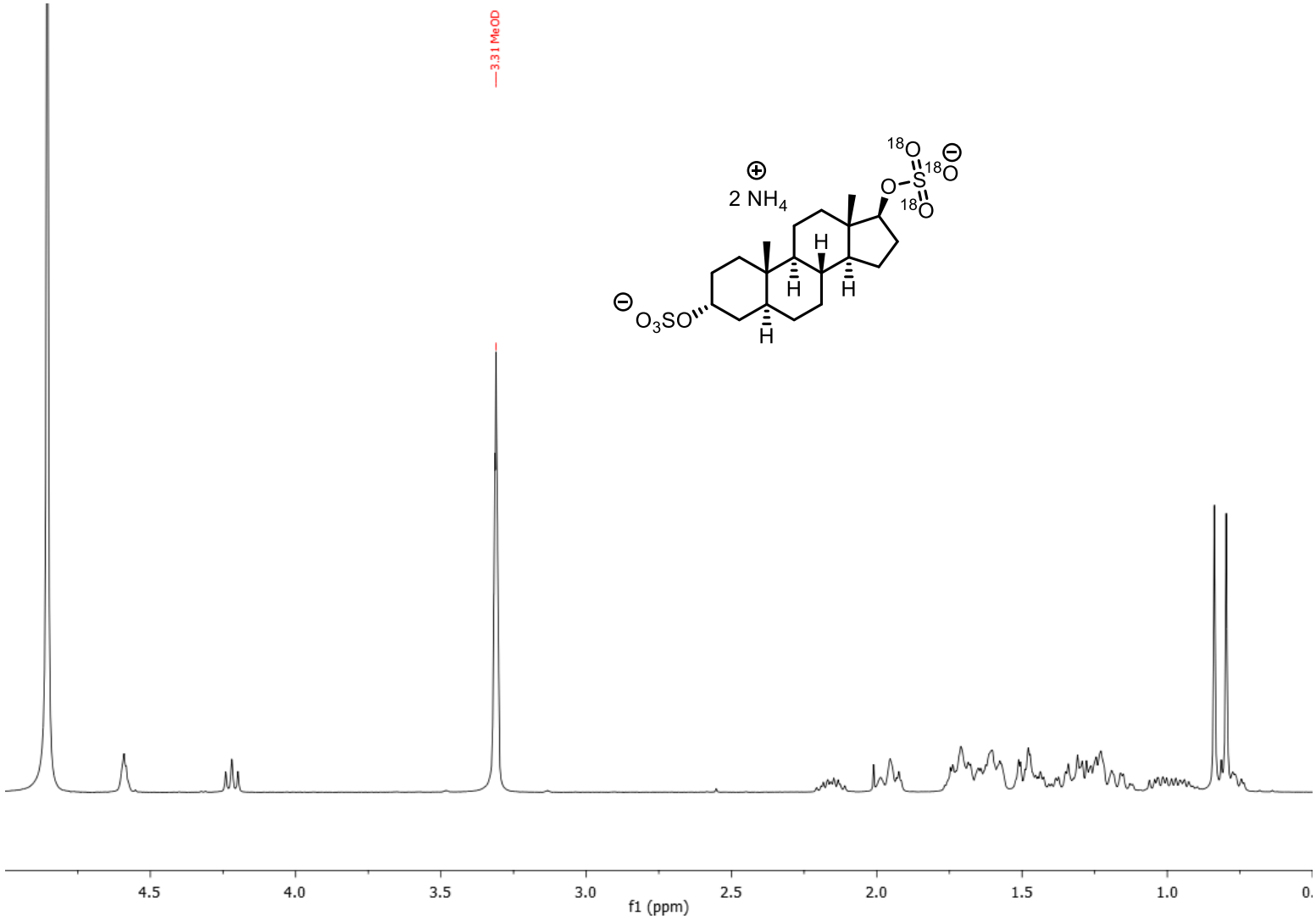
5α-Androstane-3α,17β-diol 3-sulfate, ammonium salt ^{13}C NMR 101 MHz, CD_3OD



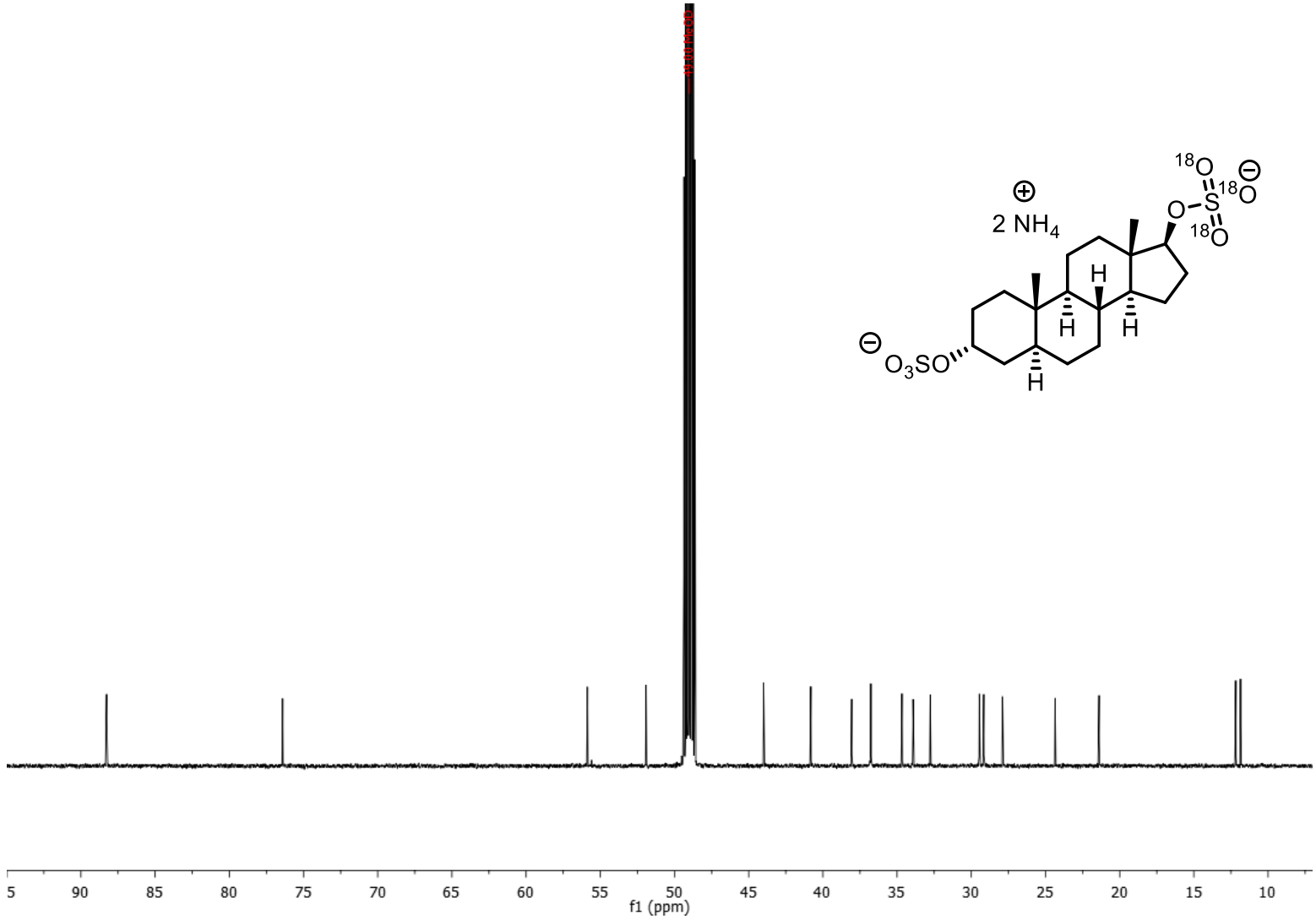
5α-Androstane-3α,17β-diol 3-sulfate, ammonium salt LRMS



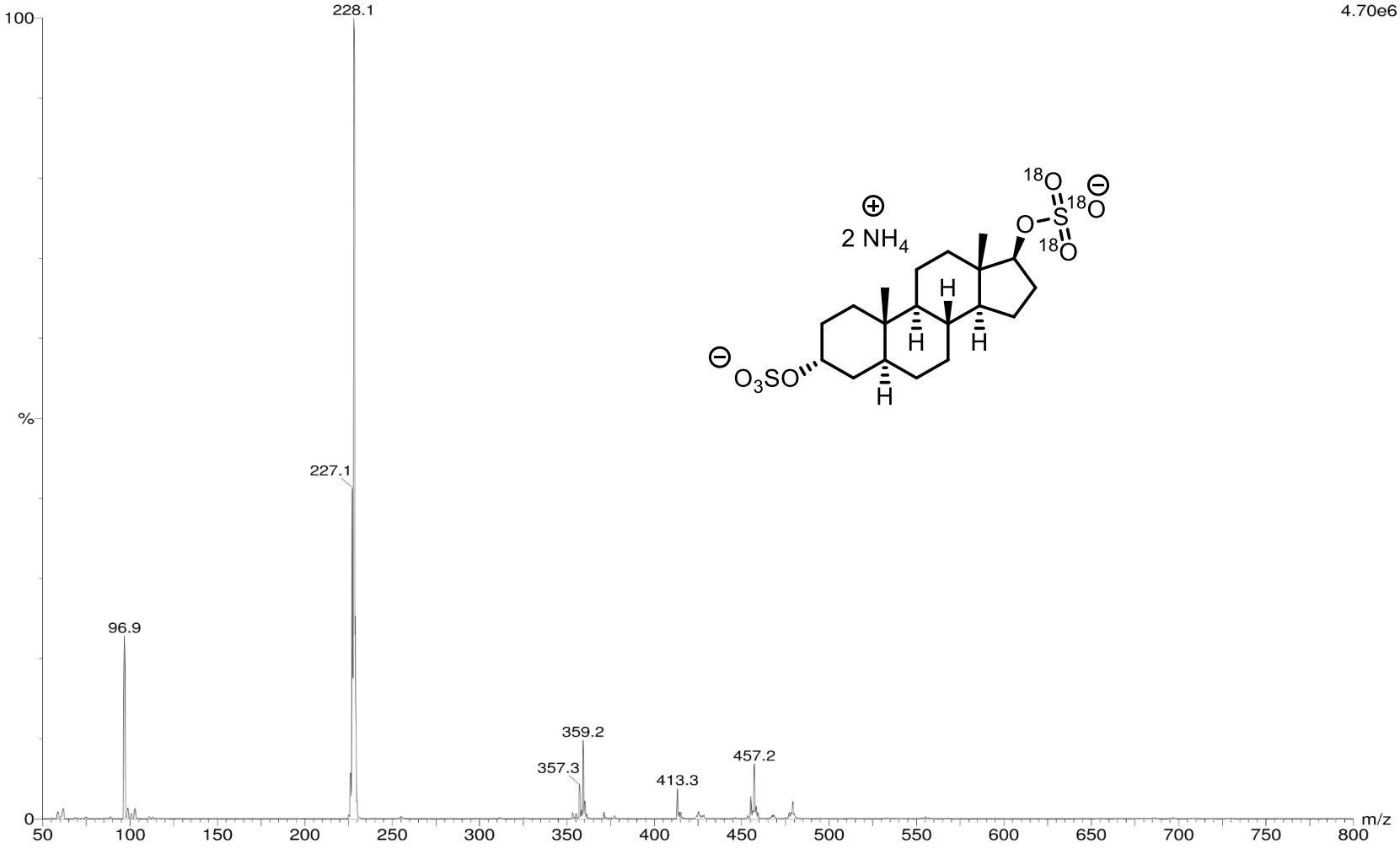
5α-Androstane-3α,17β[$^{18}O_3$]-diol bis(sulfate) ^{1}H NMR 400 MHz, CD_3OD



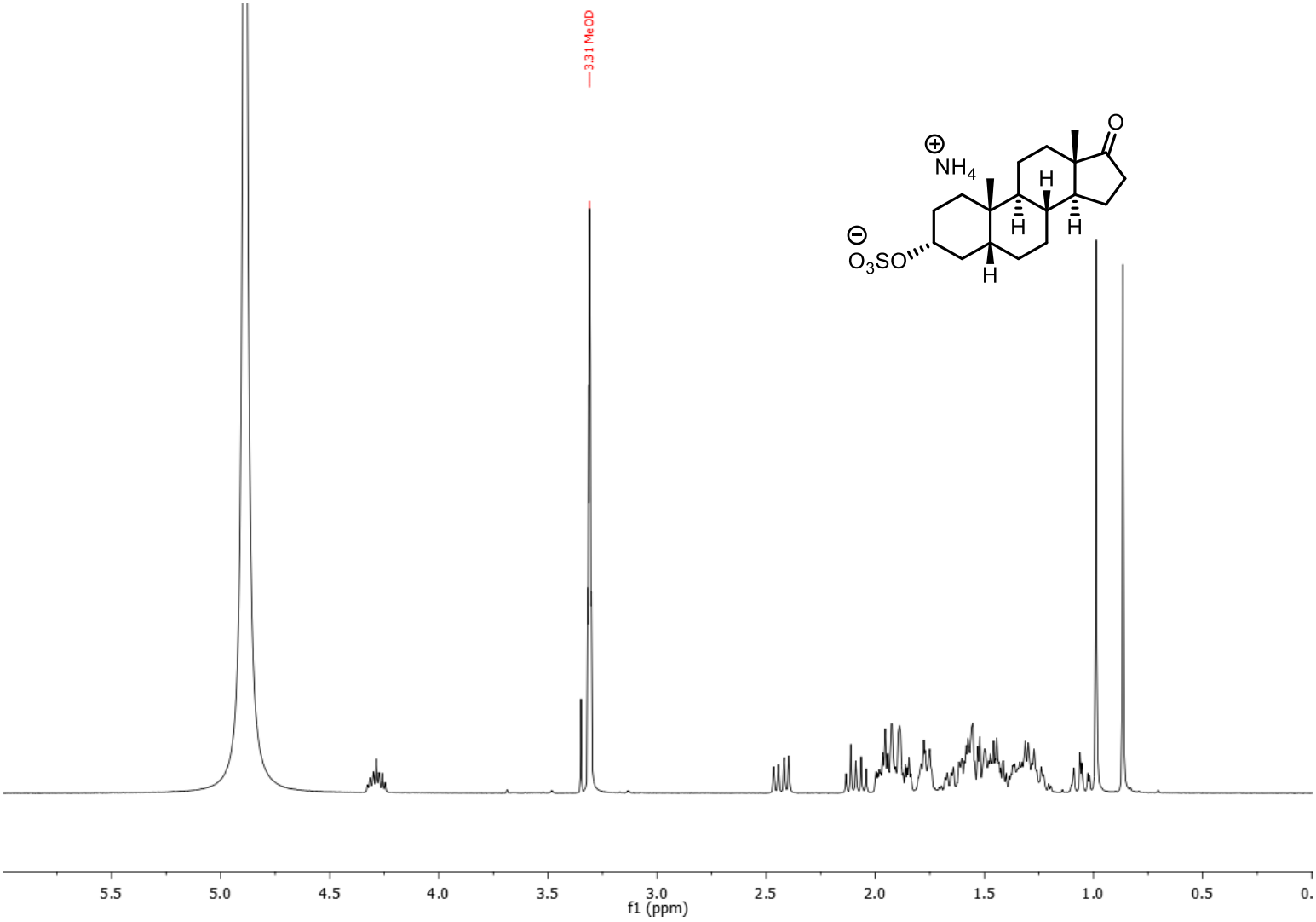
5α-Androstane-3α,17β[$^{18}O_3$]-diol bis(sulfate) ^{13}C NMR 176 MHz, CD_3OD



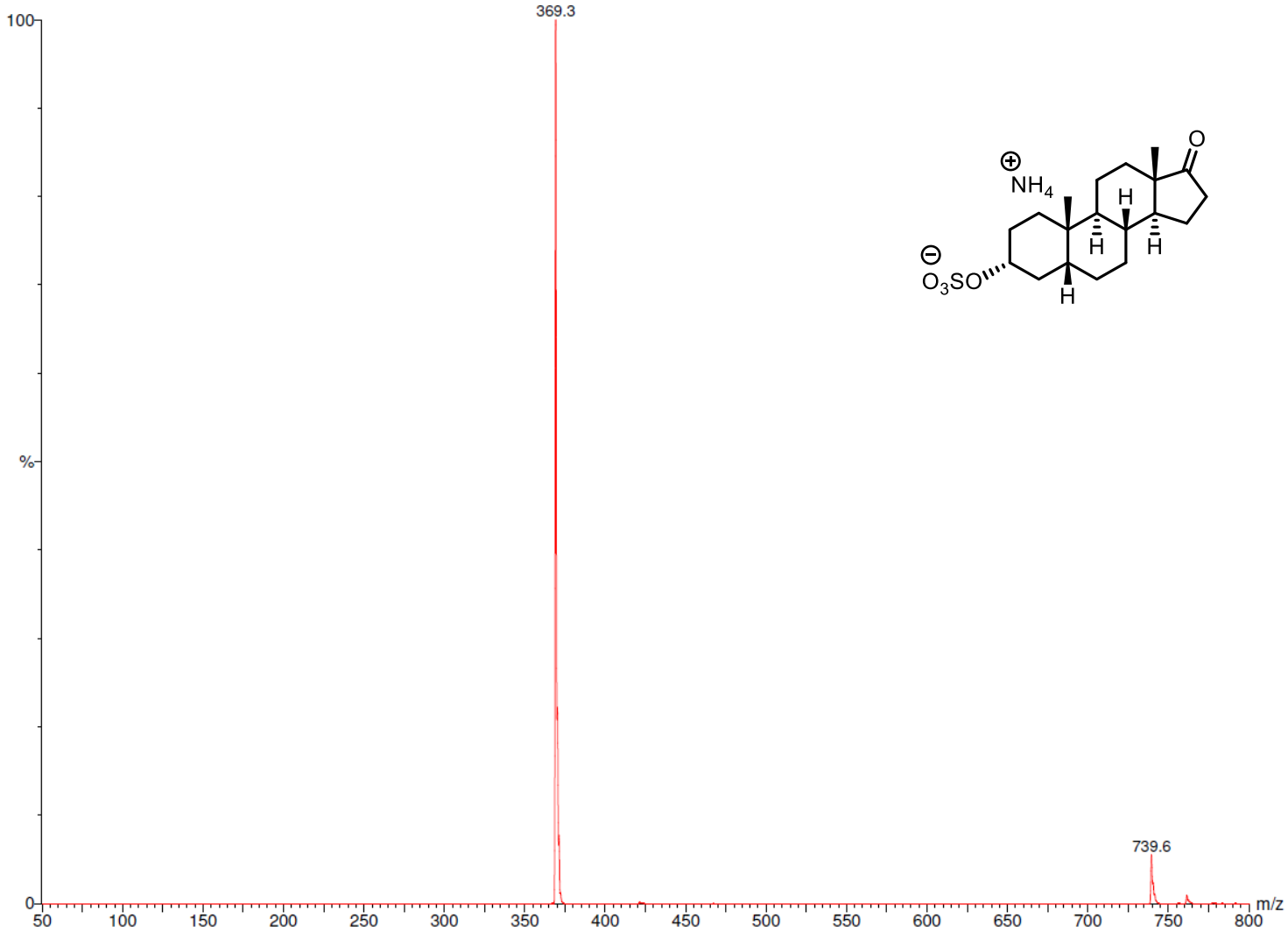
5α-Androstane-3α,17β[$^{18}O_3$]-diol bis(sulfate) LRMS



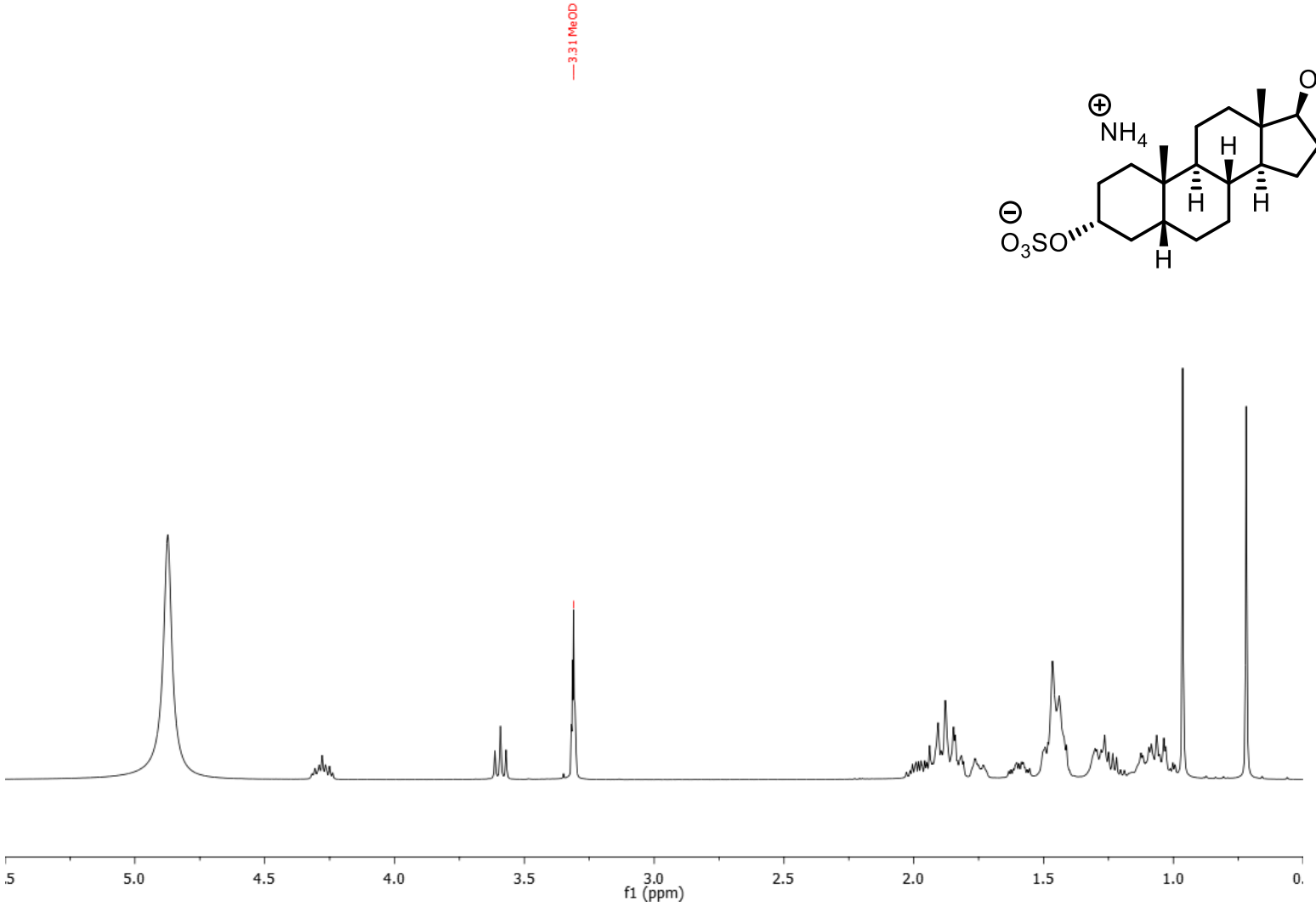
Etiocholanolone 3-sulfate, ammonium salt (3a) ^{1}H NMR 400 MHz, CD_3OD



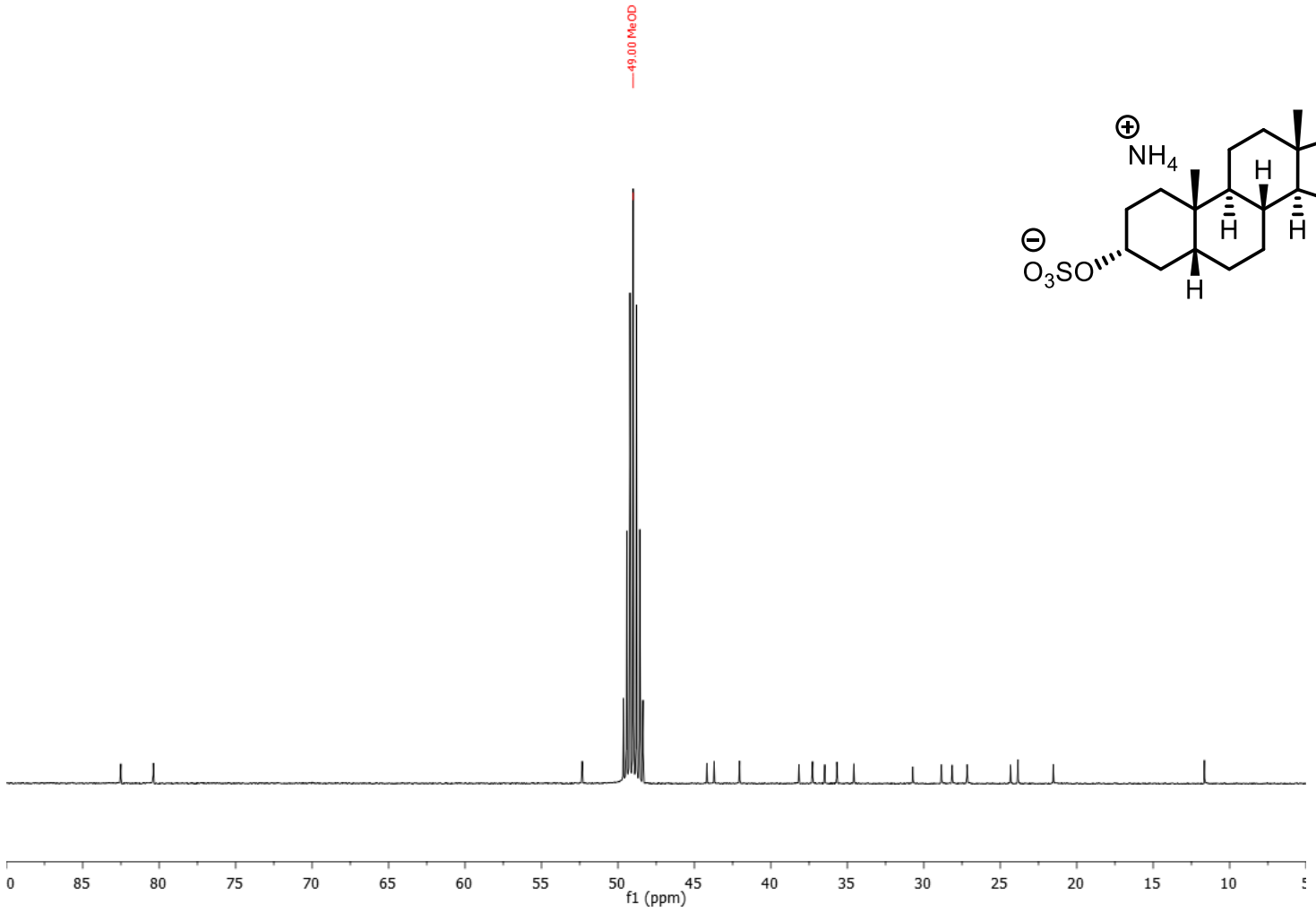
Etiocholanolone 3-sulfate, ammonium salt LRMS



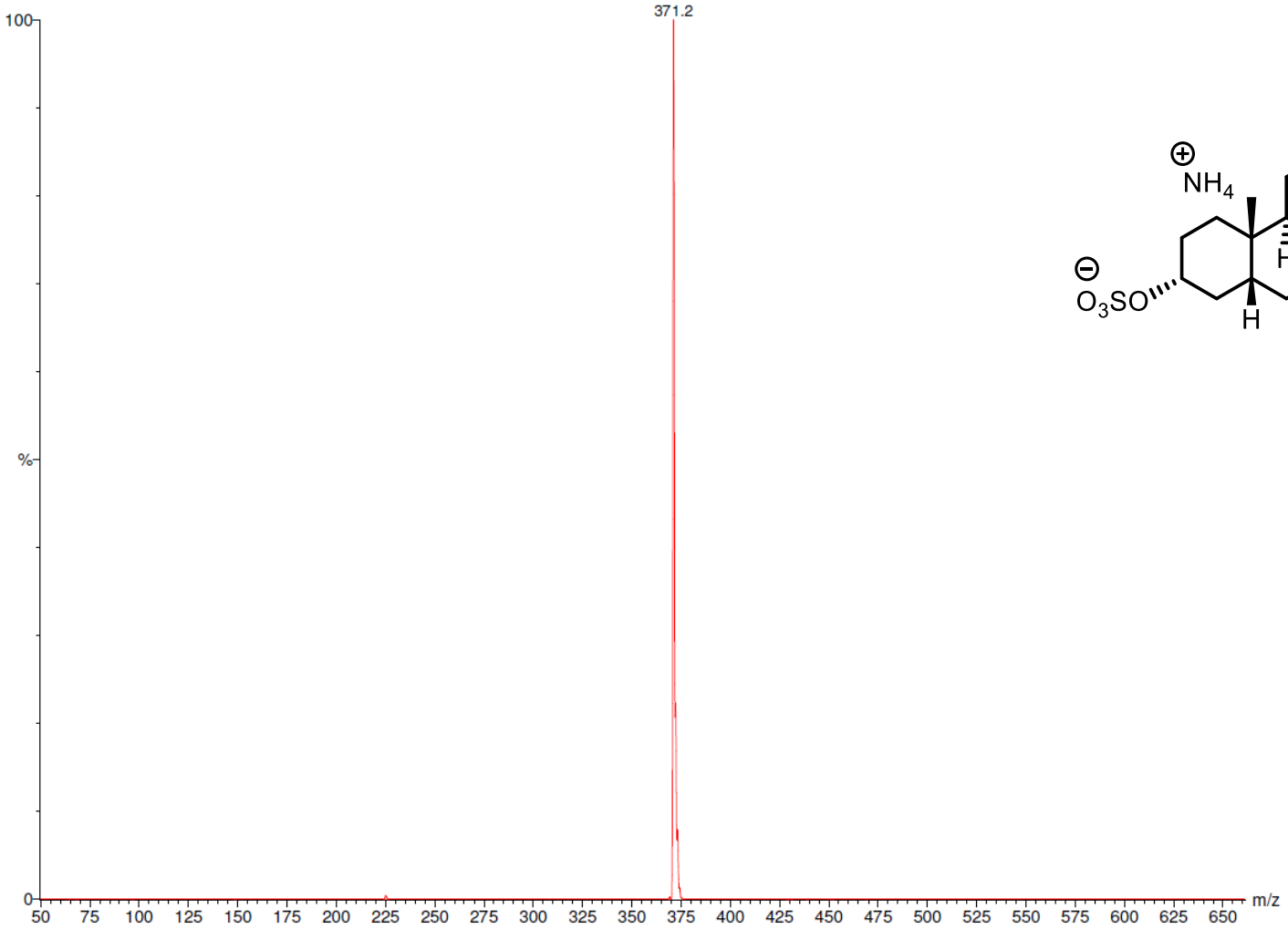
5β -Androstane-3α,17β-diol 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



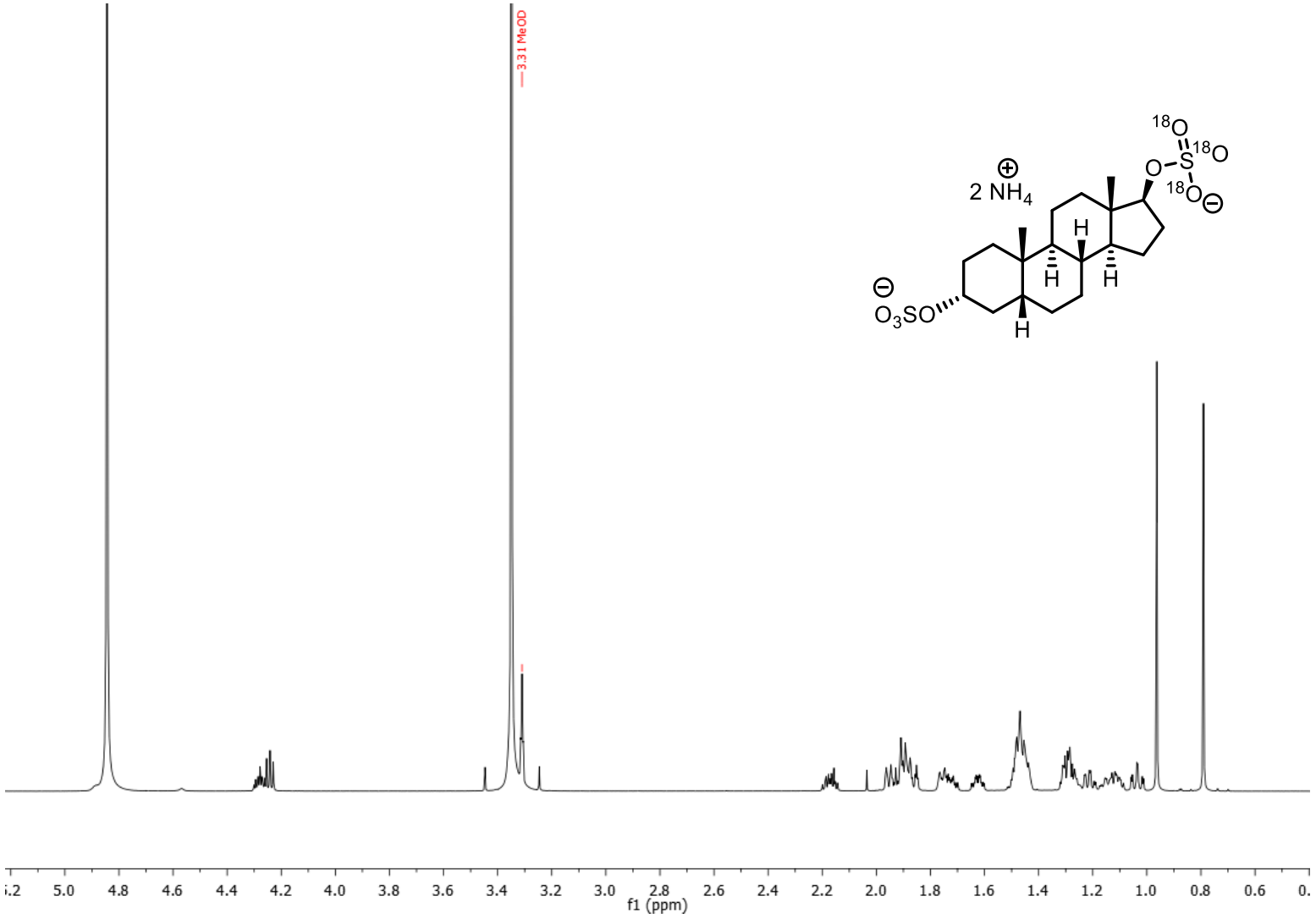
5β-Androstane-3α,17β-diol 3-sulfate, ammonium salt ^{13}C NMR 151 MHz, CD_3OD



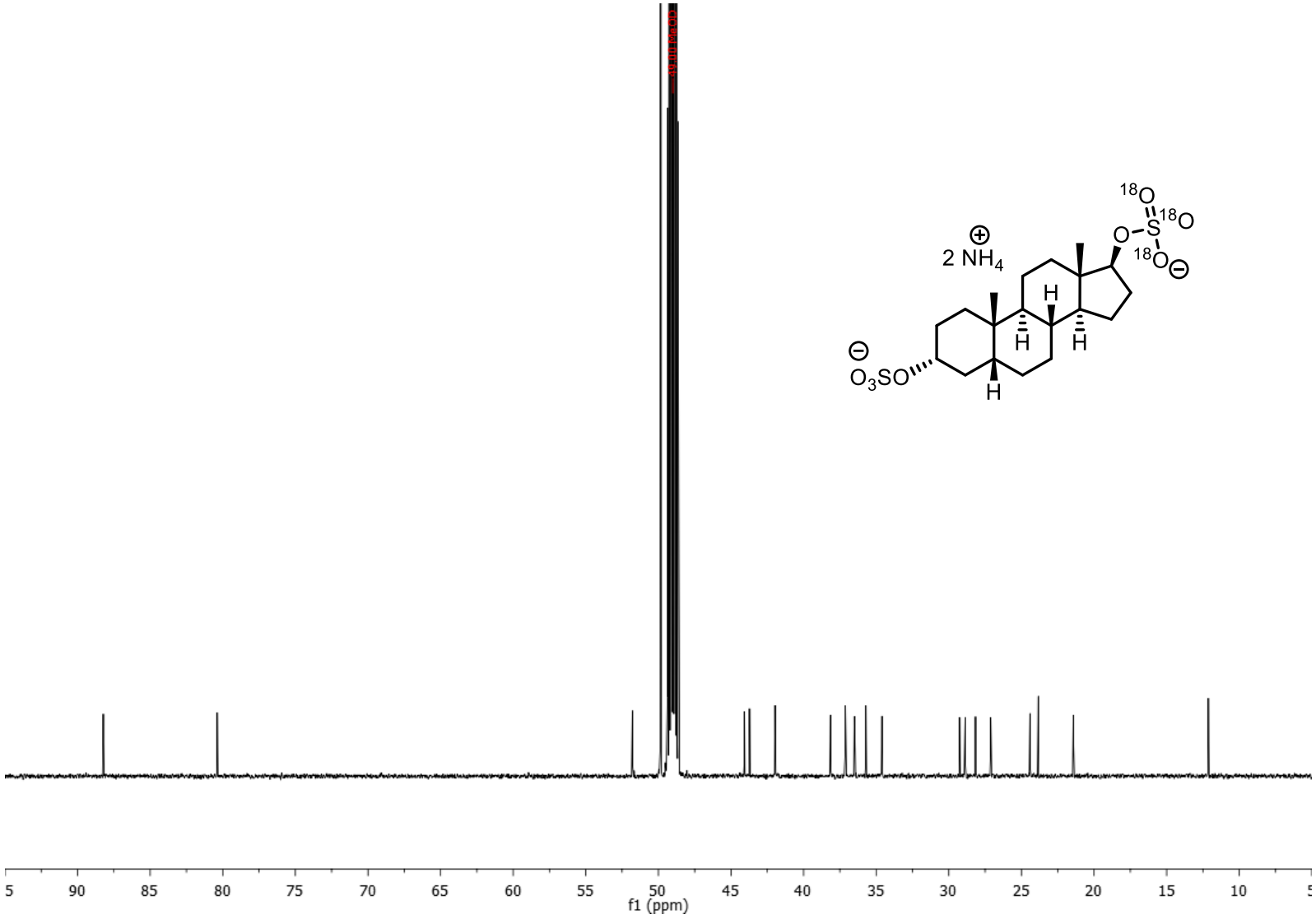
5β -Androstane-3α,17β-diol 3-sulfate, ammonium salt LRMS



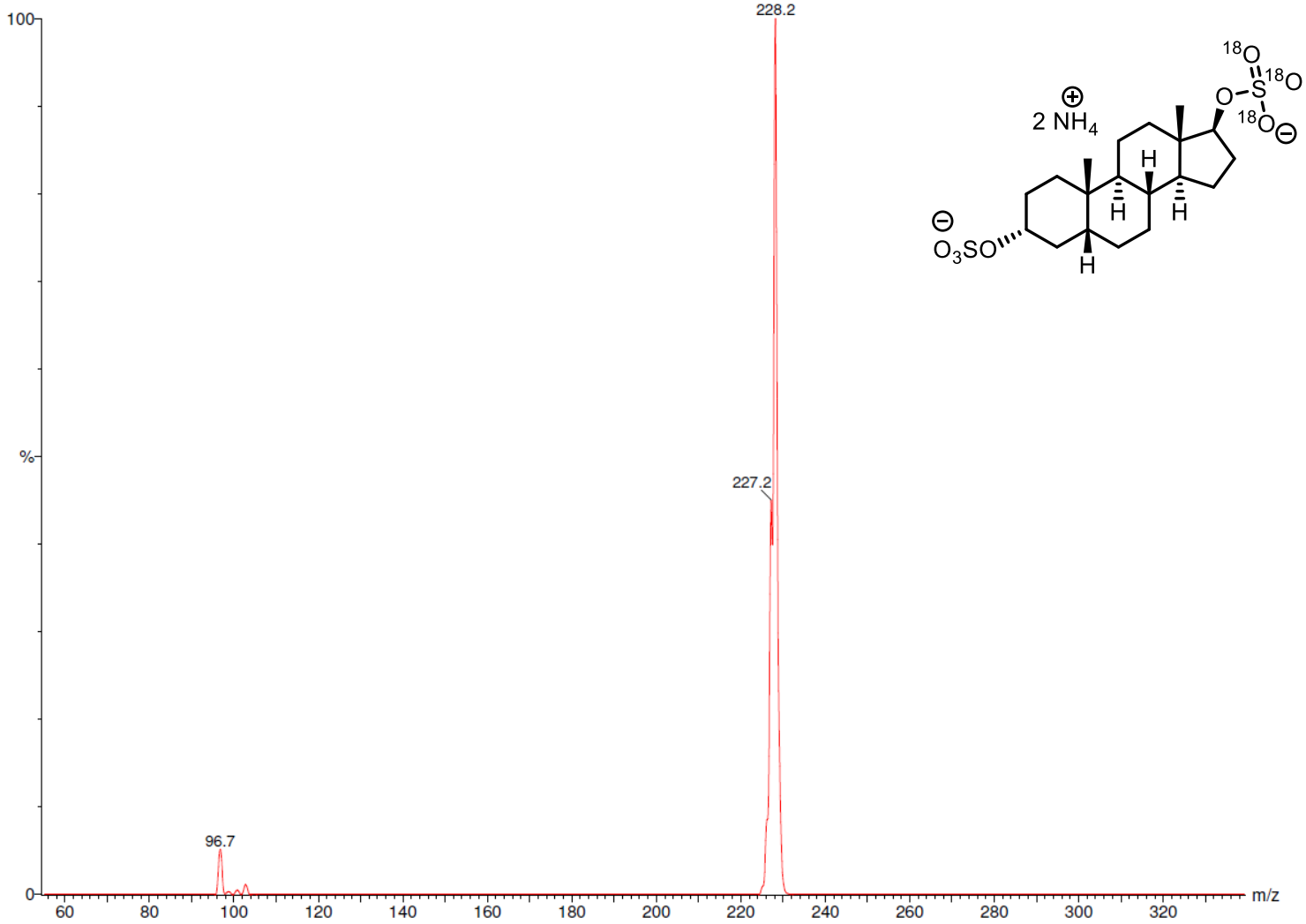
5β -Androstane-3α,17β[$^{18}O_3$]-diol bis(sulfate), ammonium salt ^{1}H NMR 400 MHz, CD_3OD



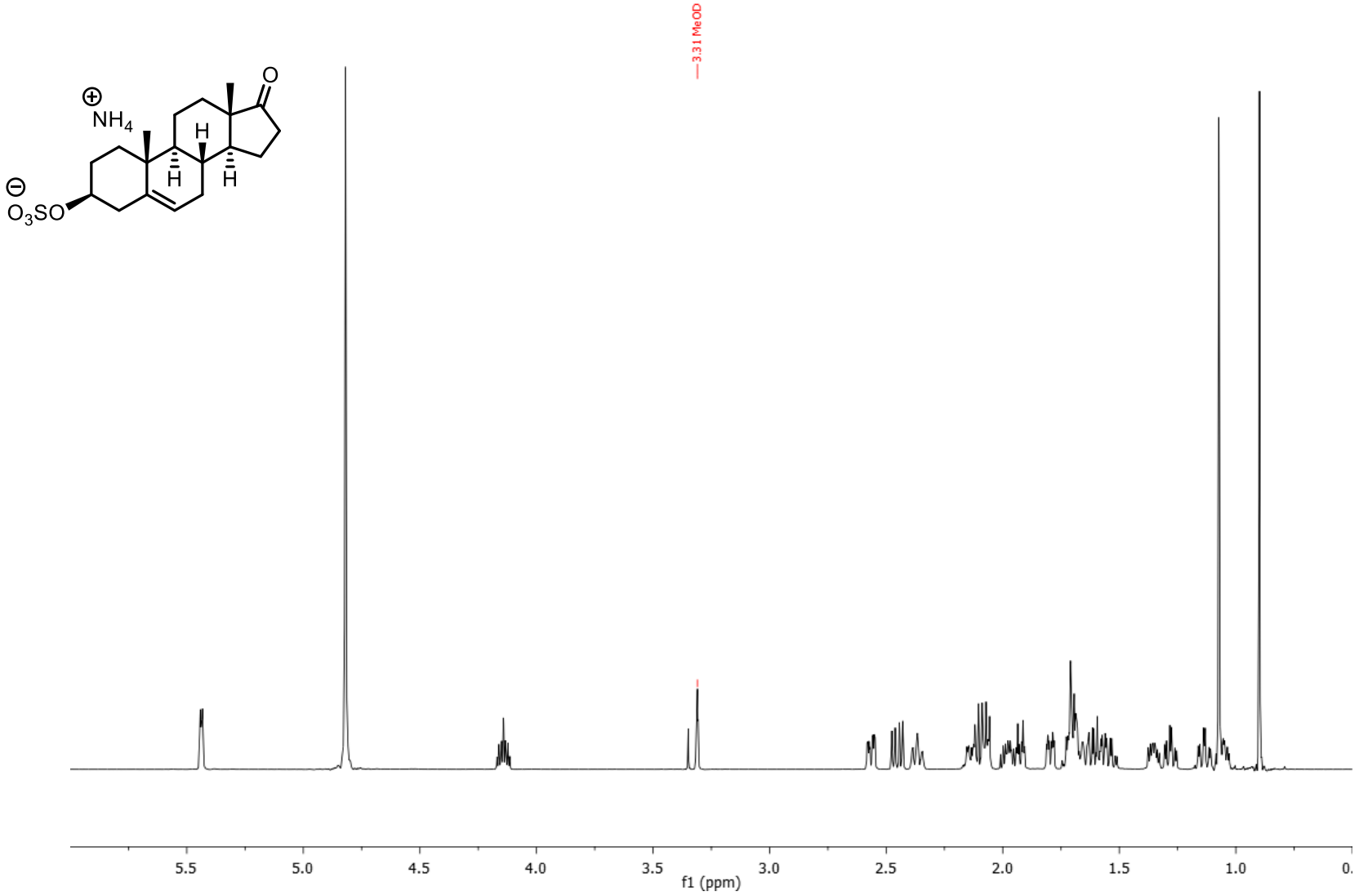
5β -Androstane-3α,17β[$^{18}O_3$]-diol bis(sulfate), ammonium salt ^{13}C NMR 151 MHz, CD_3OD



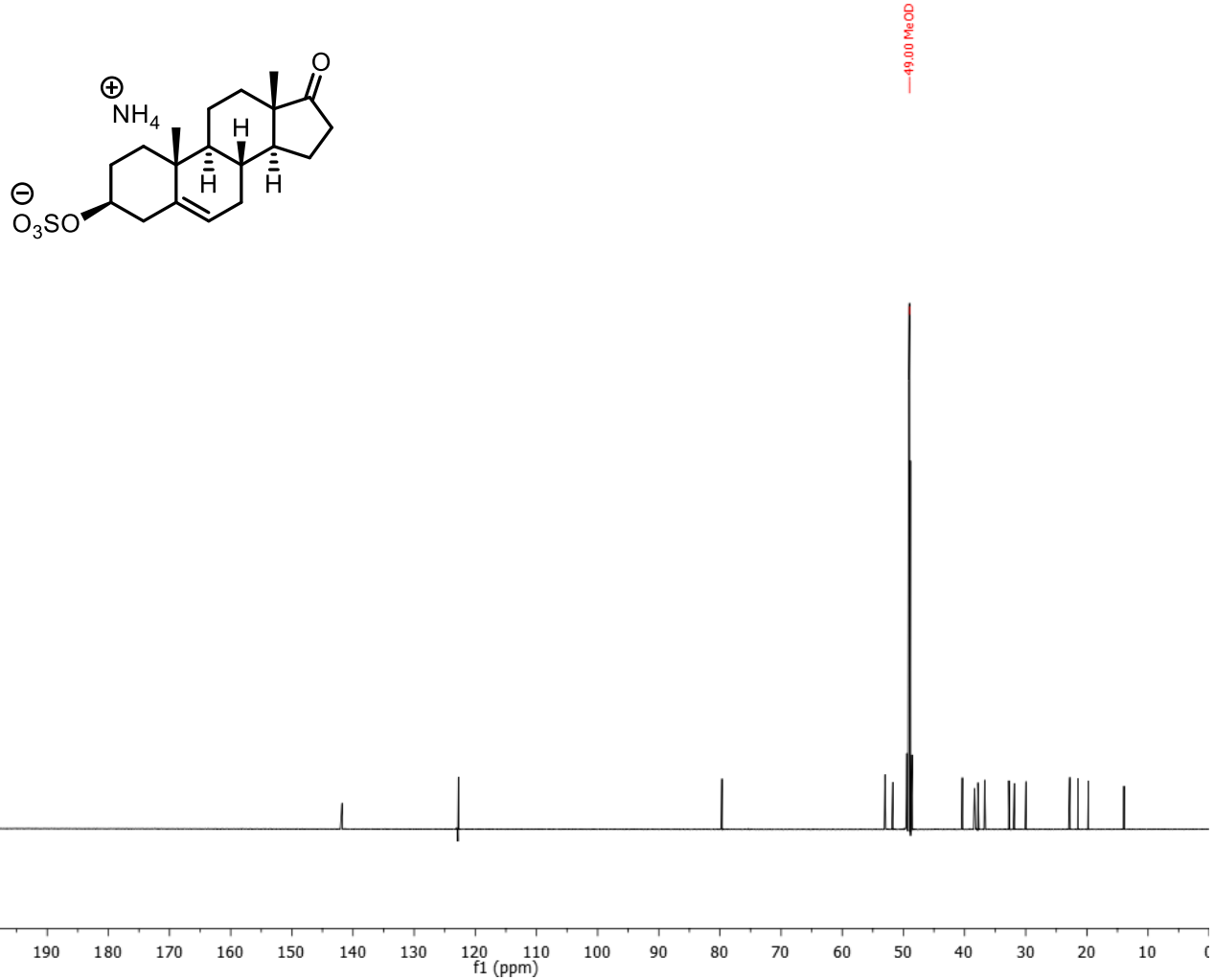
5β -Androstane-3α,17β[$^{18}O_3$]-diol bis(sulfate), ammonium salt LRMS



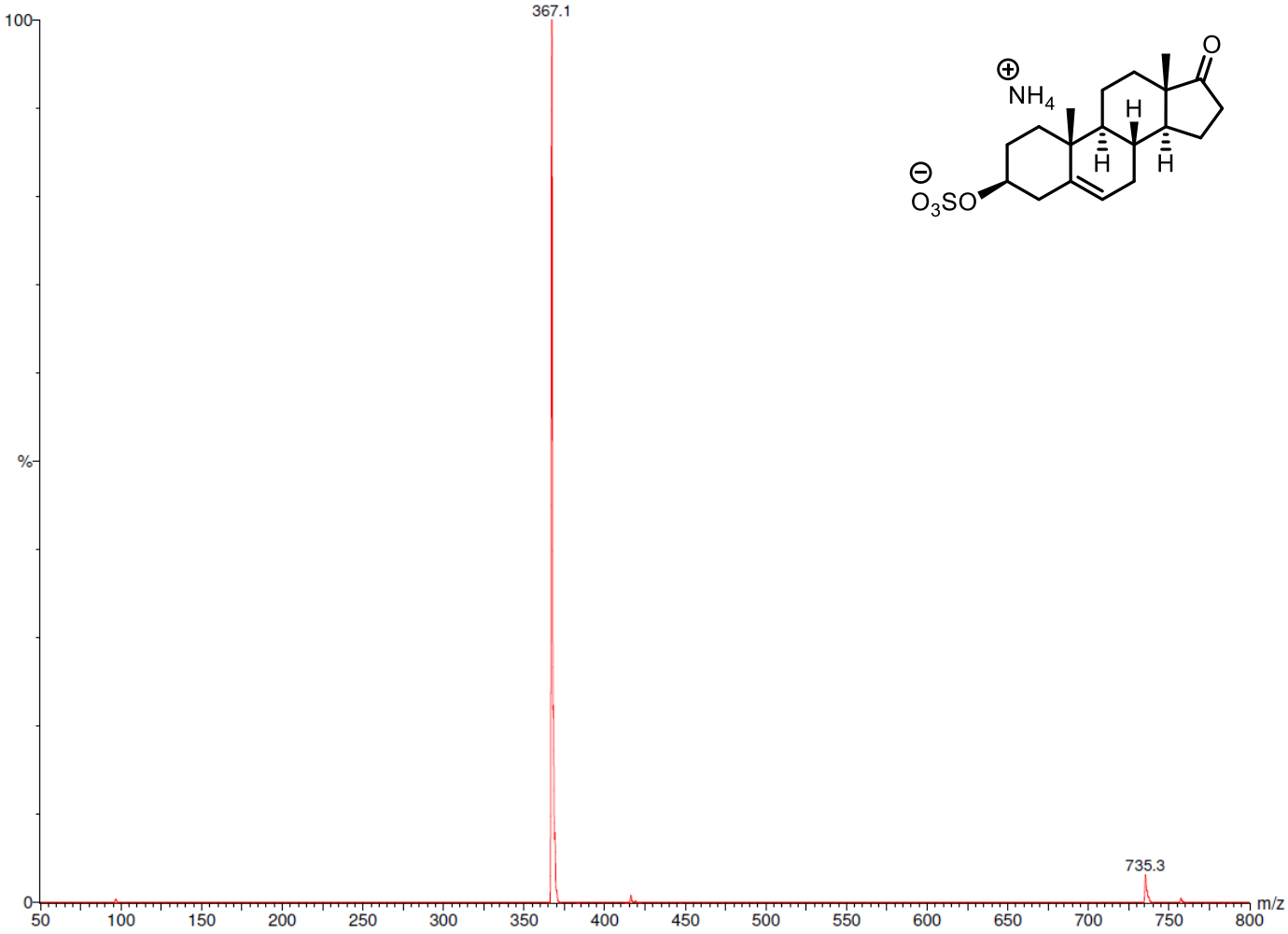
Dehydroepiandrosterone 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



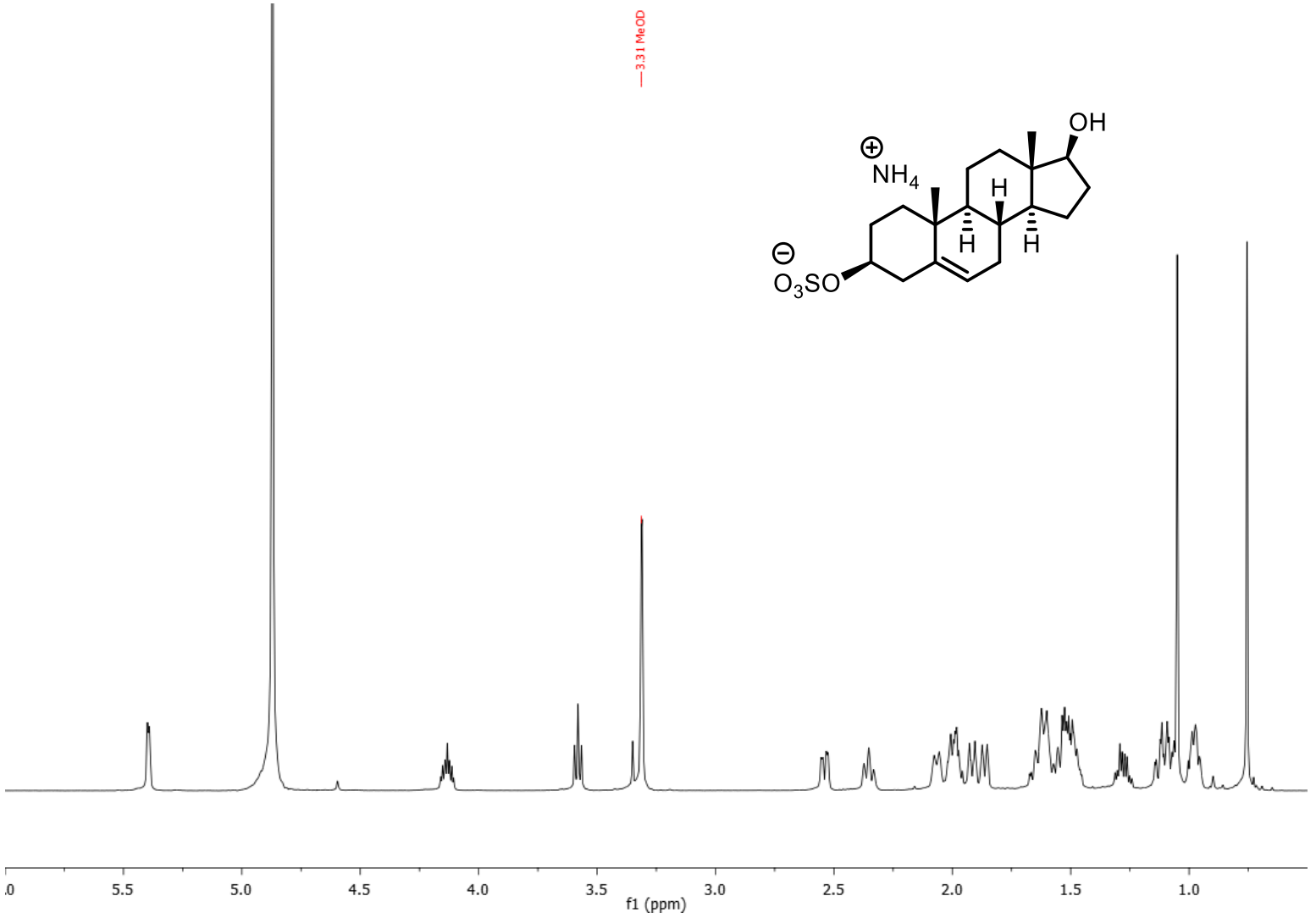
Dehydroepiandrosterone 3-sulfate, ammonium salt ^{13}C NMR 151 MHz, CD_3OD



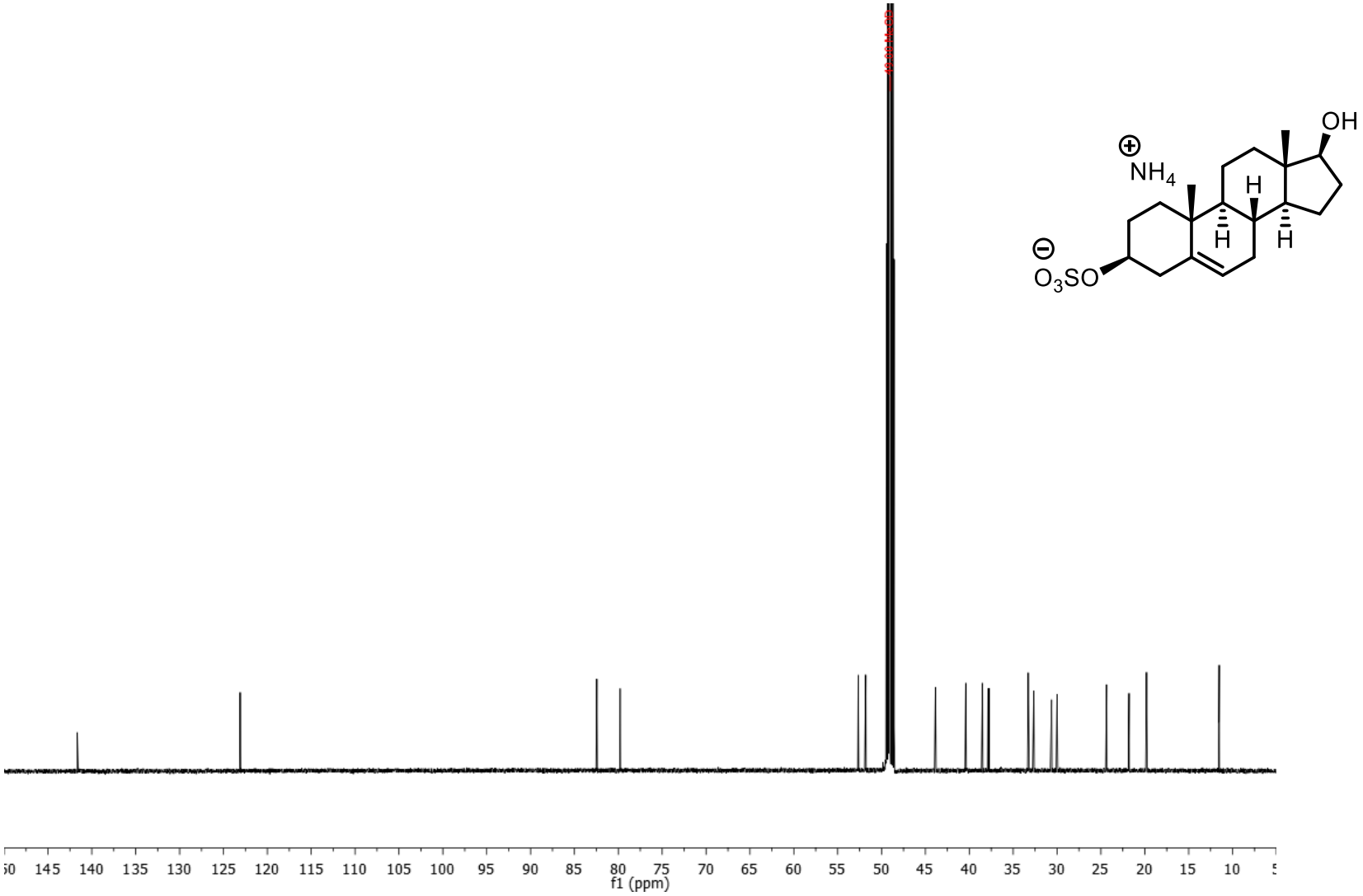
Dehydroepiandrosterone 3-sulfate, ammonium salt LRMS



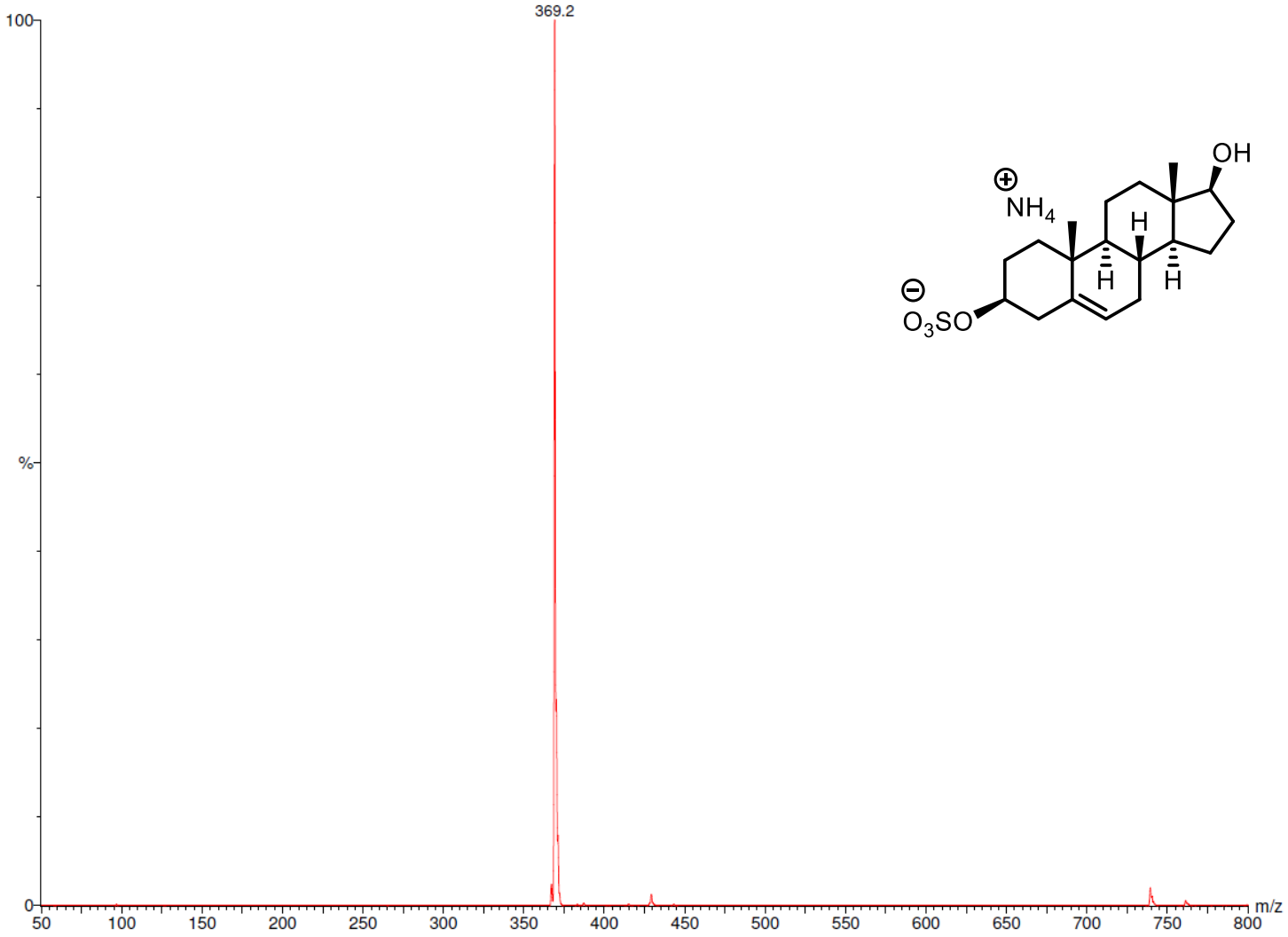
5-Androstene-3β,17β-diol 3-sulfate, ammonium salt ^{1}H NMR 400 MHz, CD_3OD



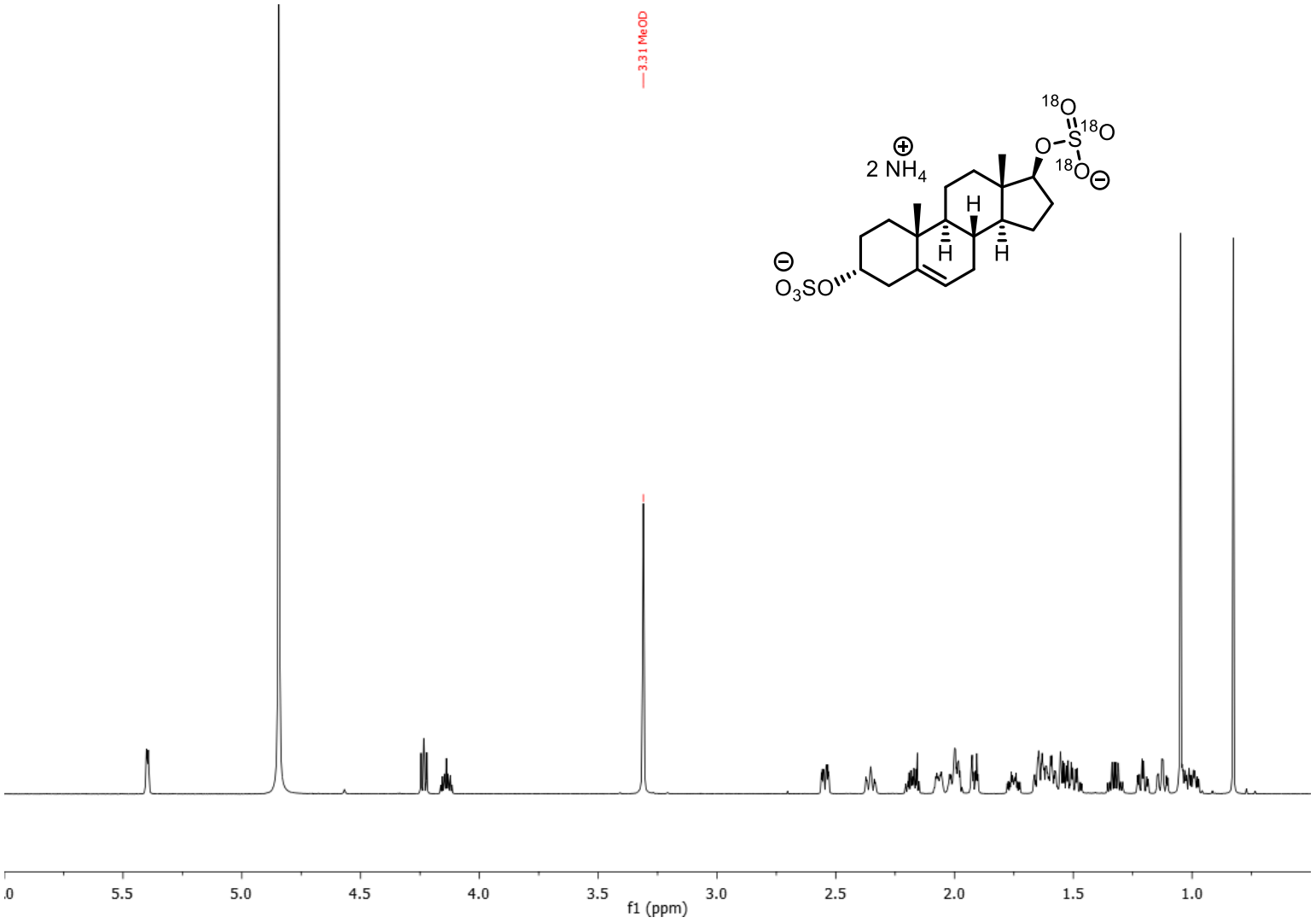
5-Androstene-3β,17β-diol 3-sulfate, ammonium salt ^{13}C NMR 151 MHz, CD_3OD



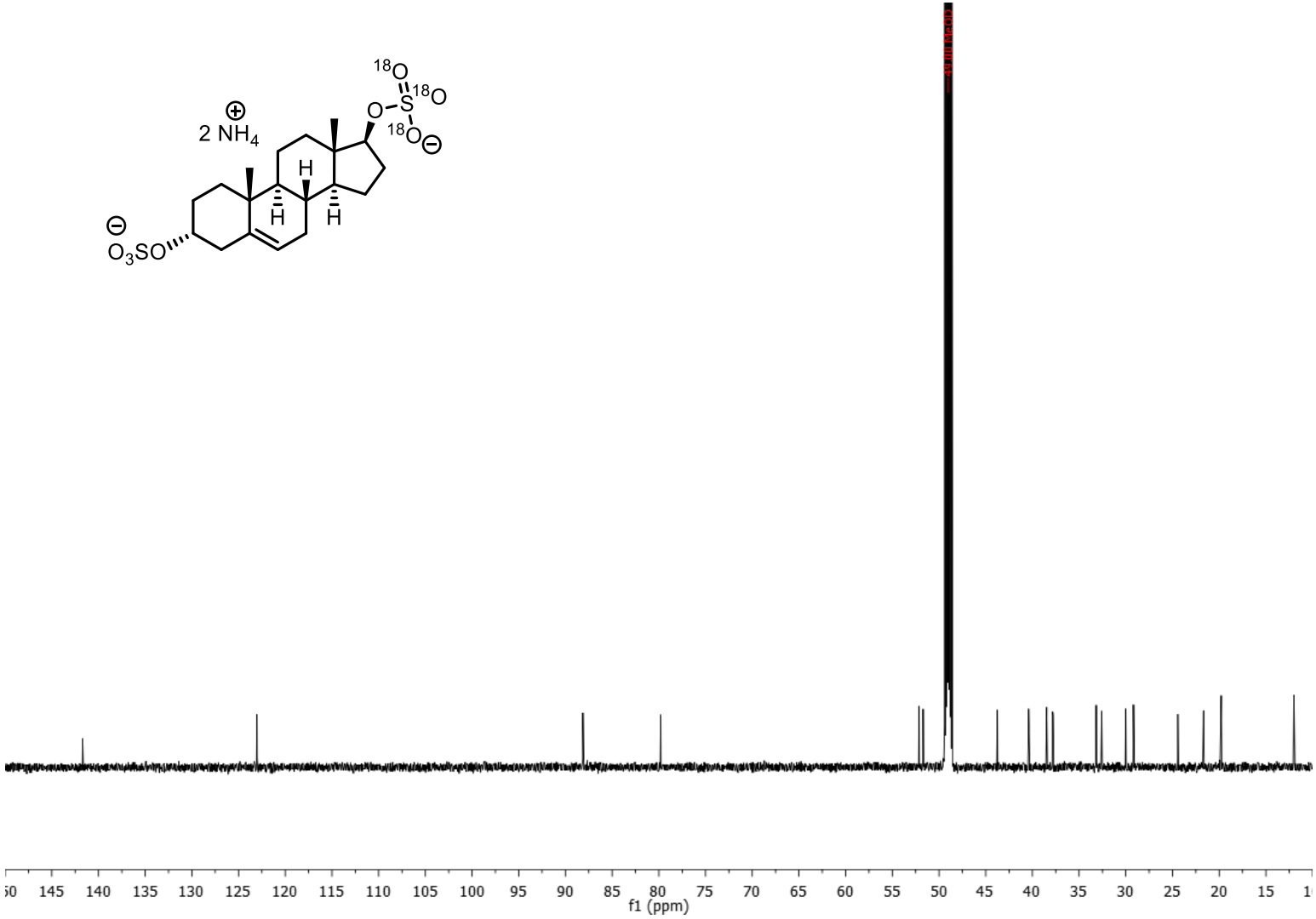
5-Androstene-3β,17β-diol 3-sulfate, ammonium salt LRMS



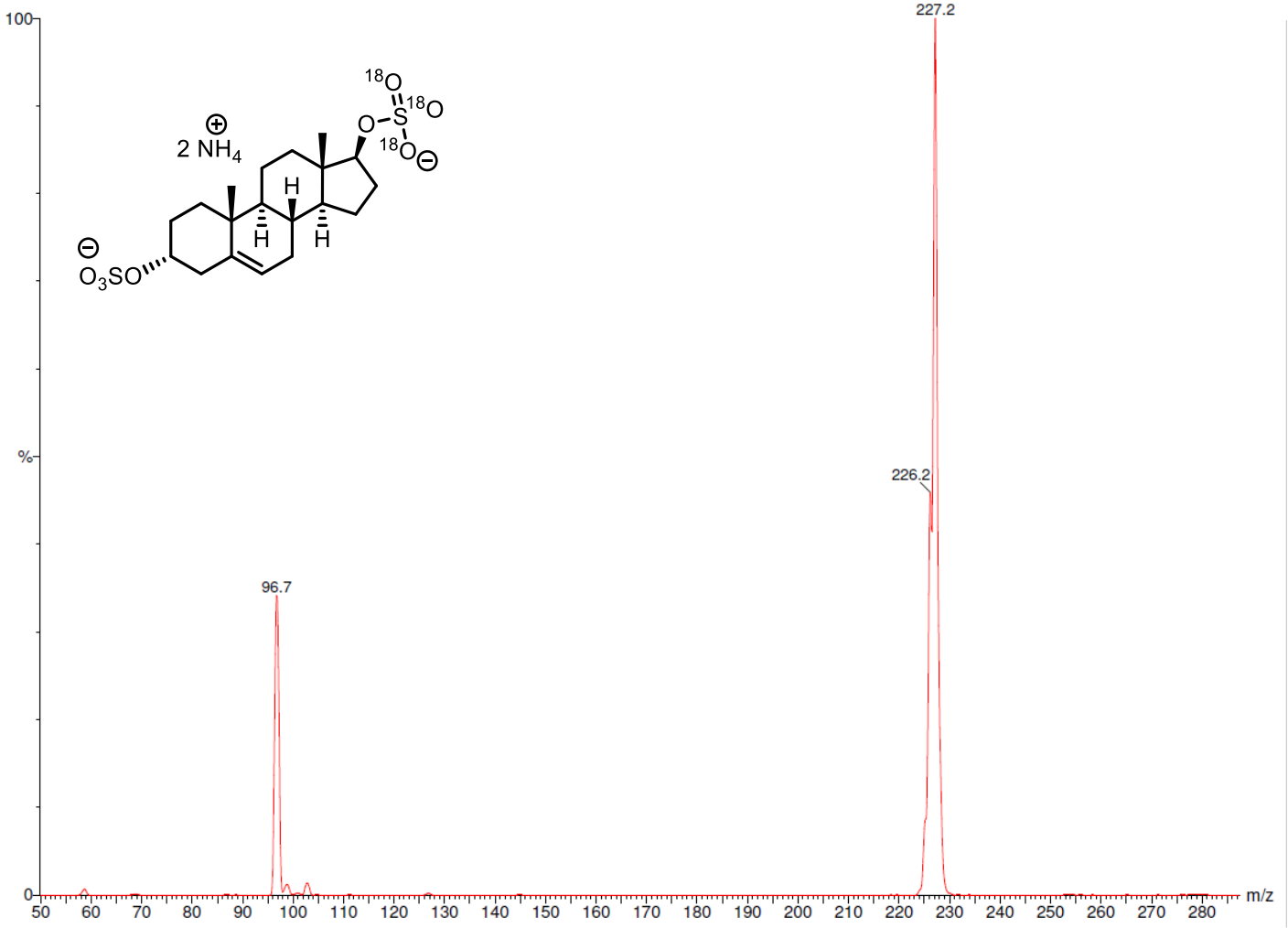
5-Androstene-3β,17β[$^{18}O_3$]-diol bis(sulfate), ammonium salt ^{1}H NMR 400 MHz, CD_3OD



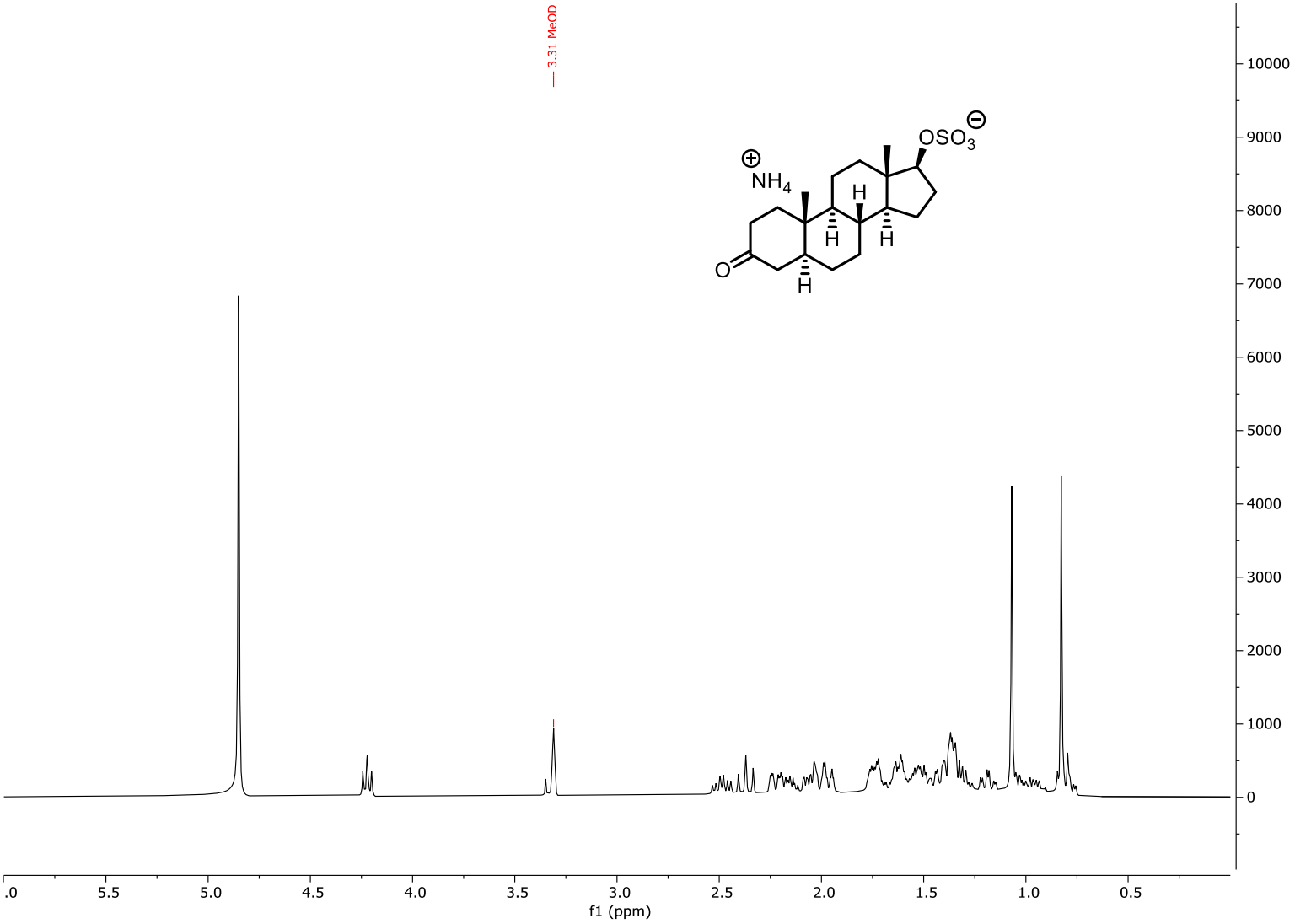
5-Androstene-3β,17β[$^{18}O_3$]-diol bis(sulfate), ammonium salt ^{13}C NMR 151 MHz, CD_3OD



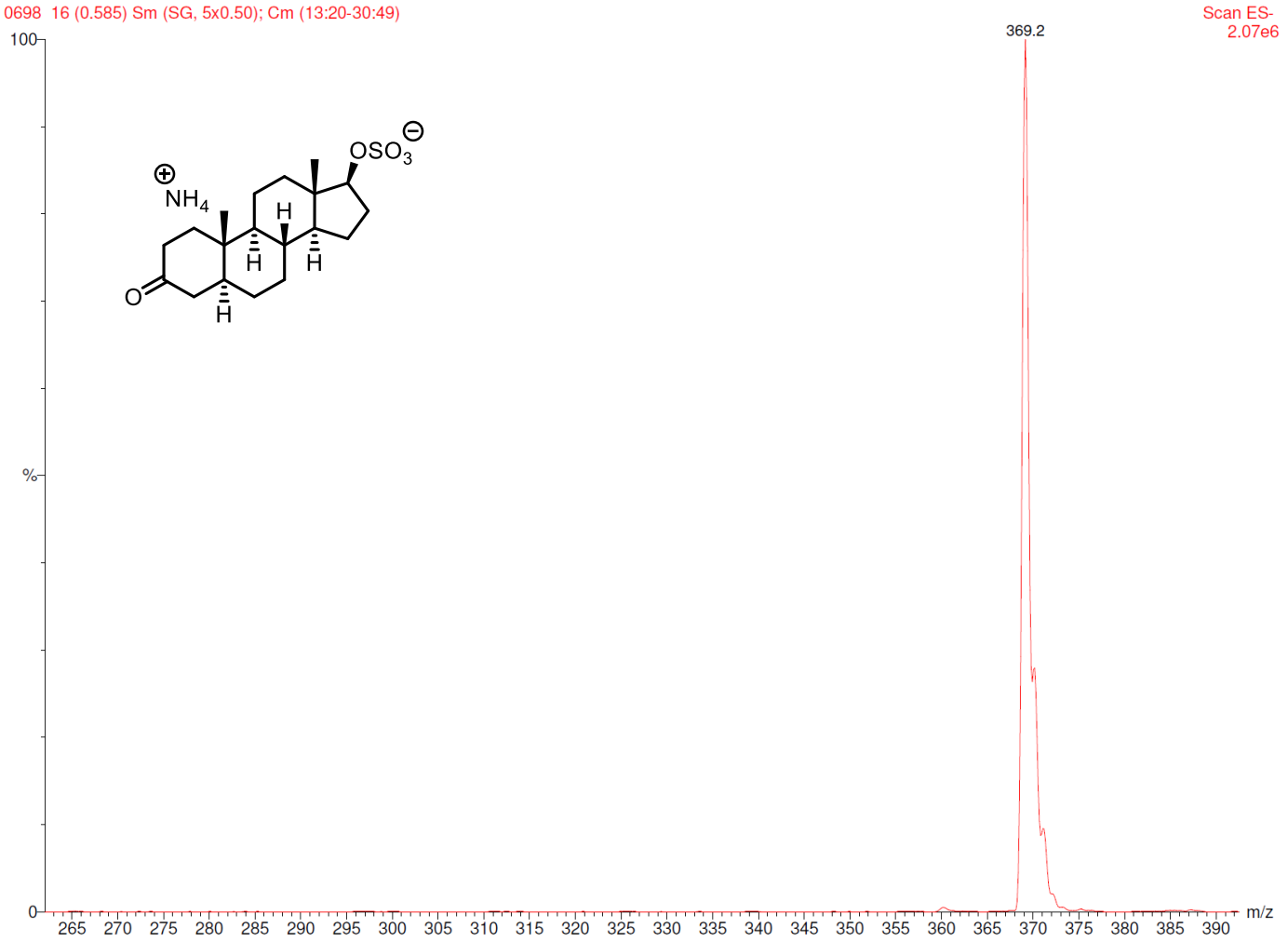
5-Androstene-3β,17β[$^{18}O_3$]-diol bis(sulfate), ammonium salt LRMS



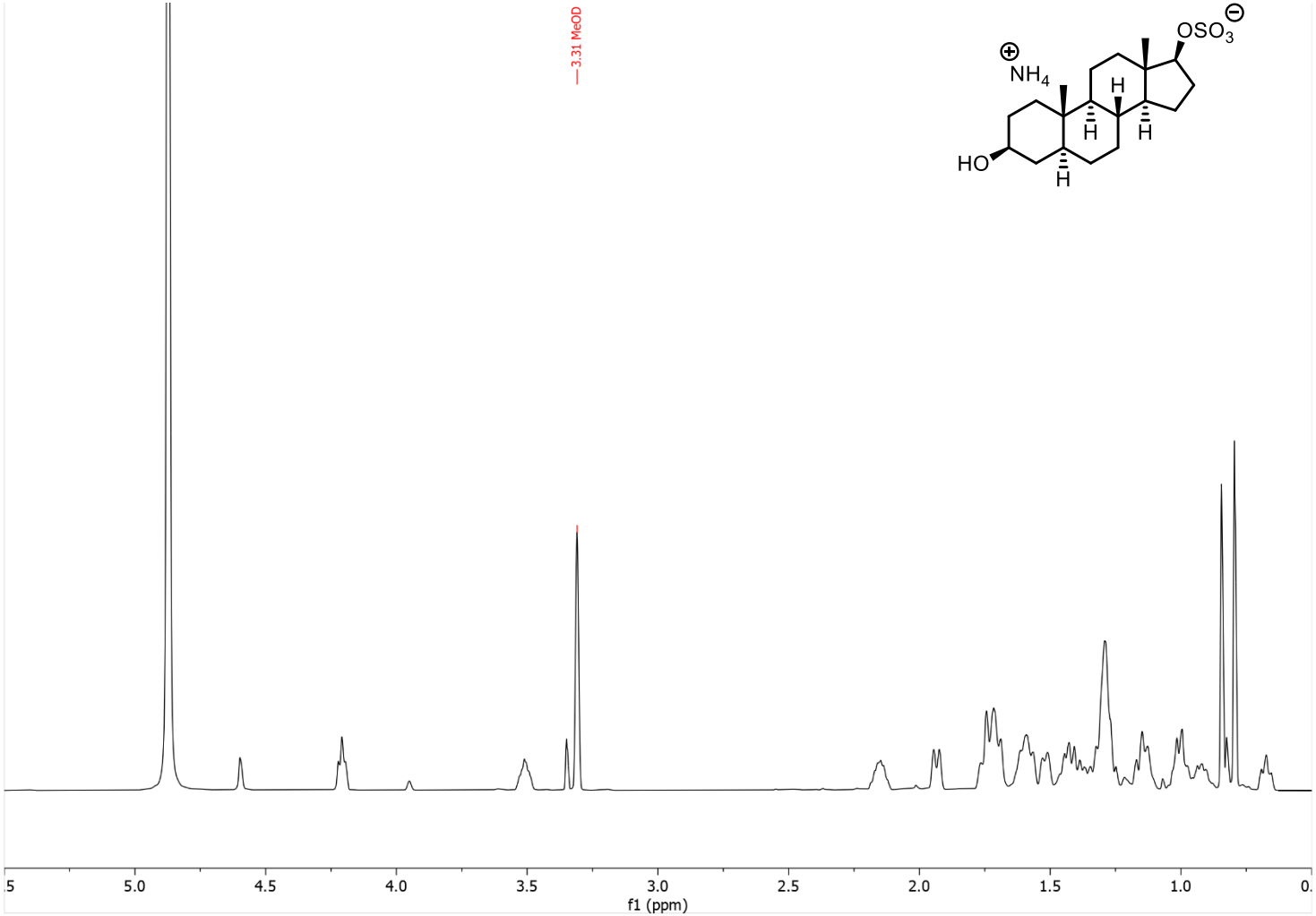
Dihydrotestosterone 3-sulfate, ammonium salt ^{1}H NMR 600MHz



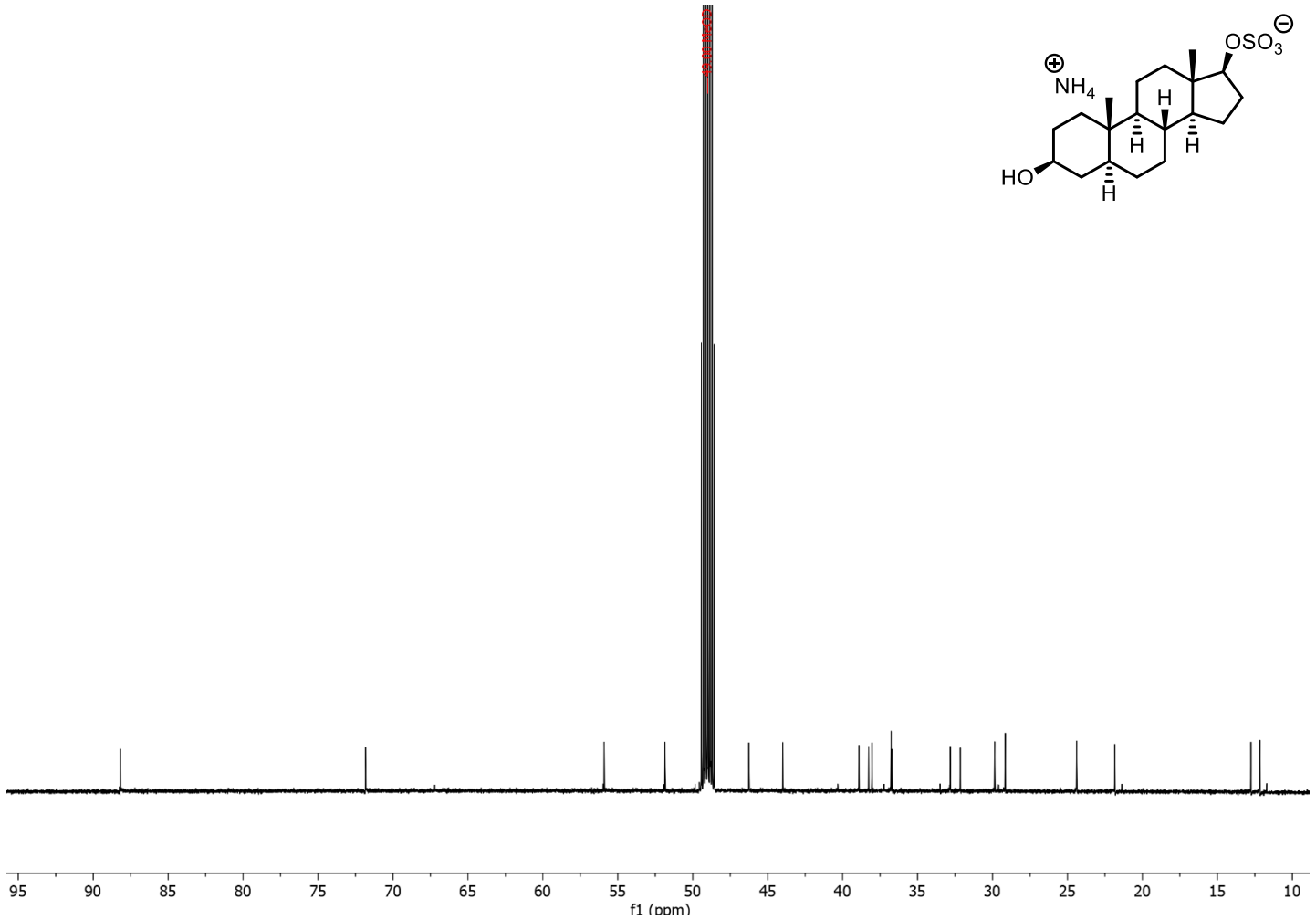
Dihydrotestosterone 3-sulfate, ammonium salt LRMS



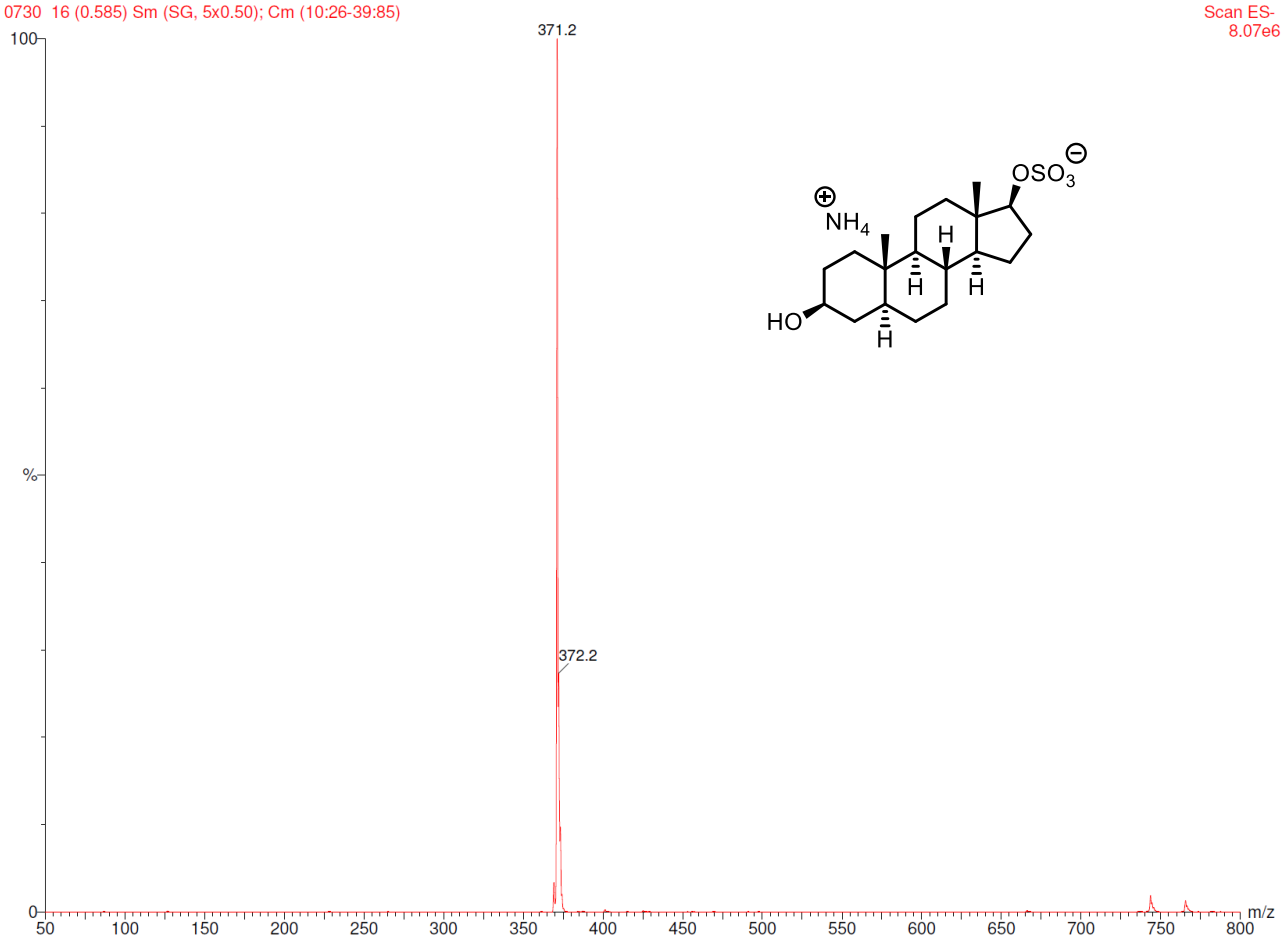
5α-Androstane-3β,17β-diol 17-sulfate, ammonium salt ^{1}H NMR 600 MHz



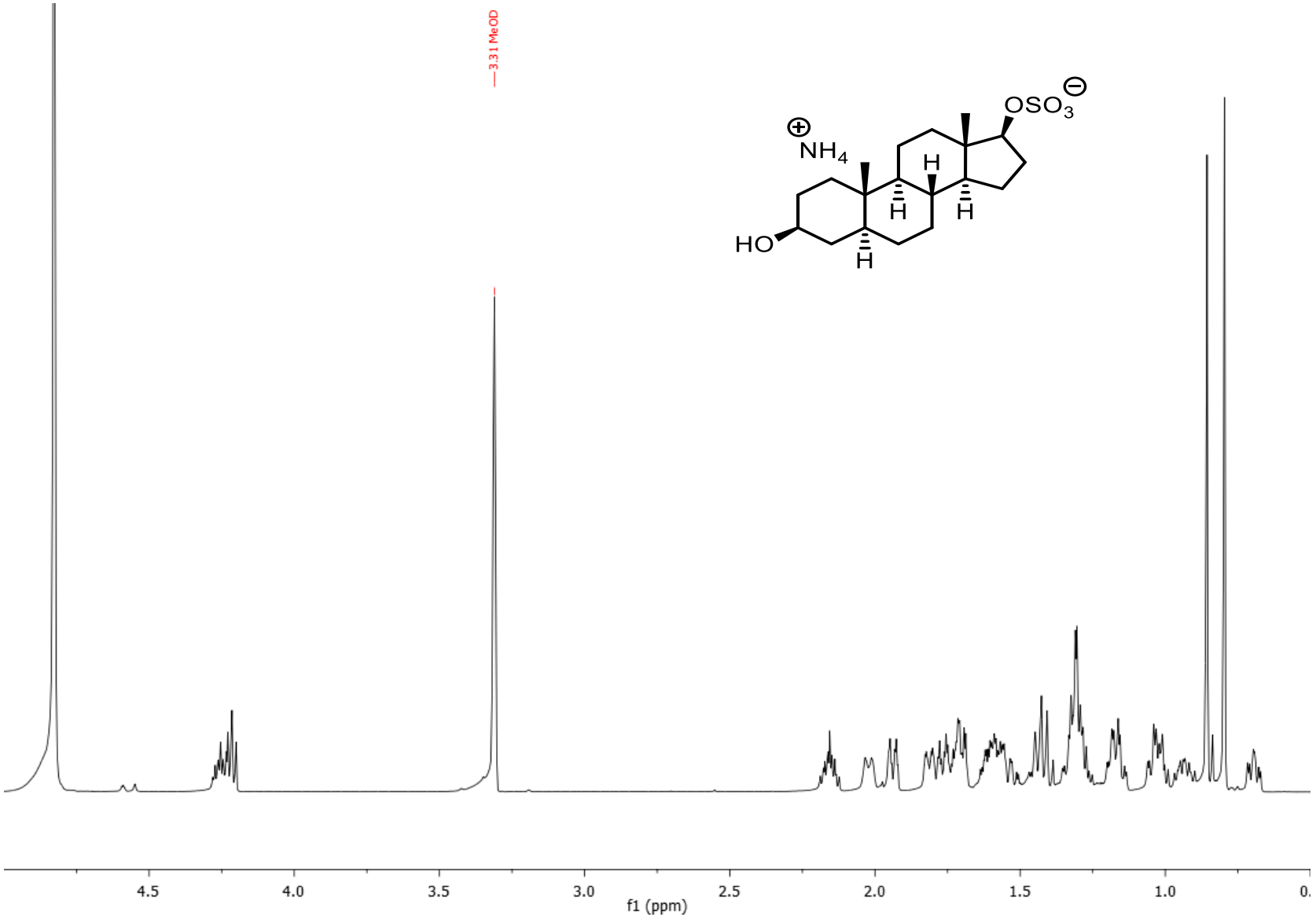
5α-Androstane-3β,17β-diol 17-sulfate, ammonium salt ^{13}C NMR 151 MHz



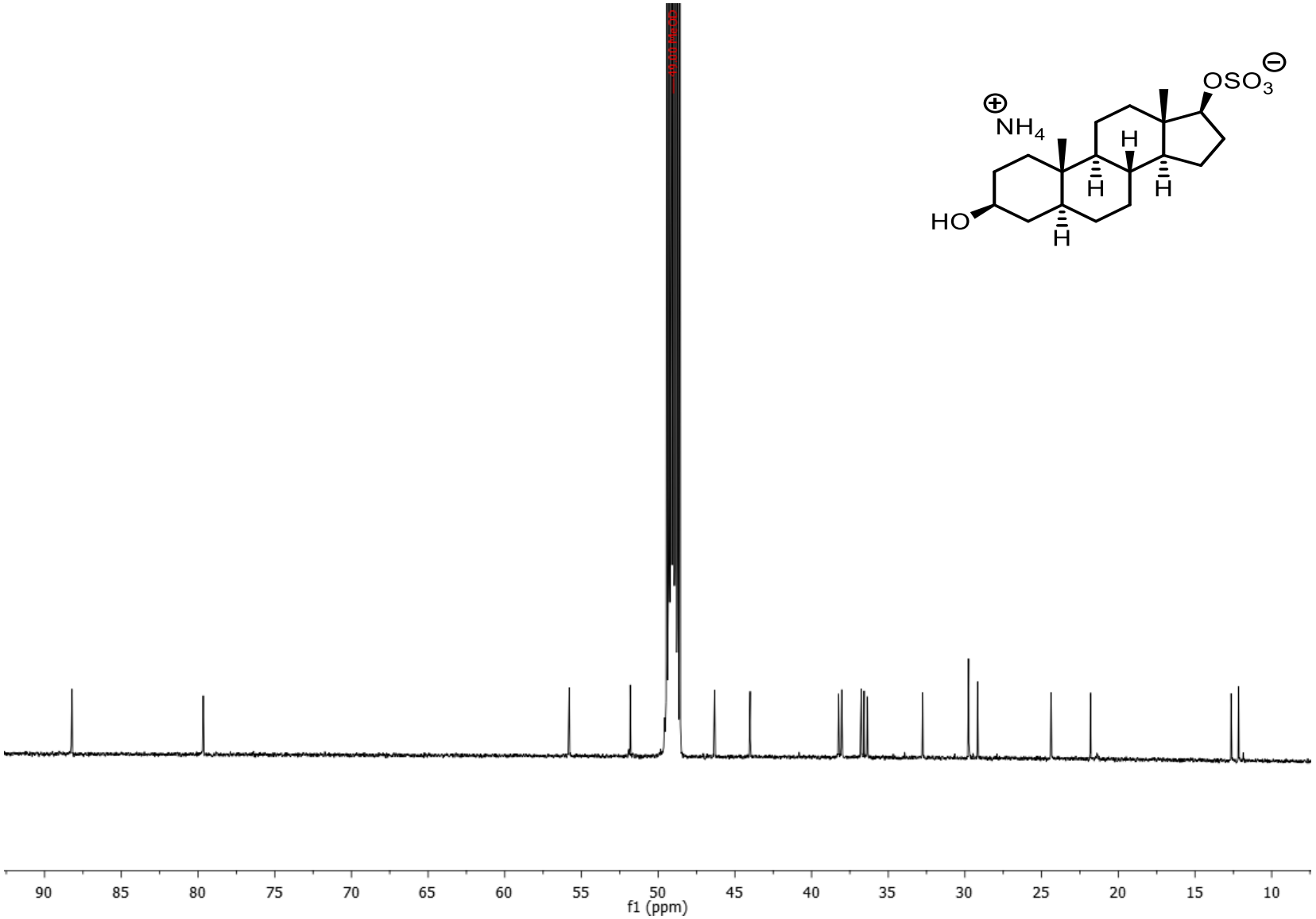
5α-Androstane-3β,17β-diol 17-sulfate, ammonium salt LRMS



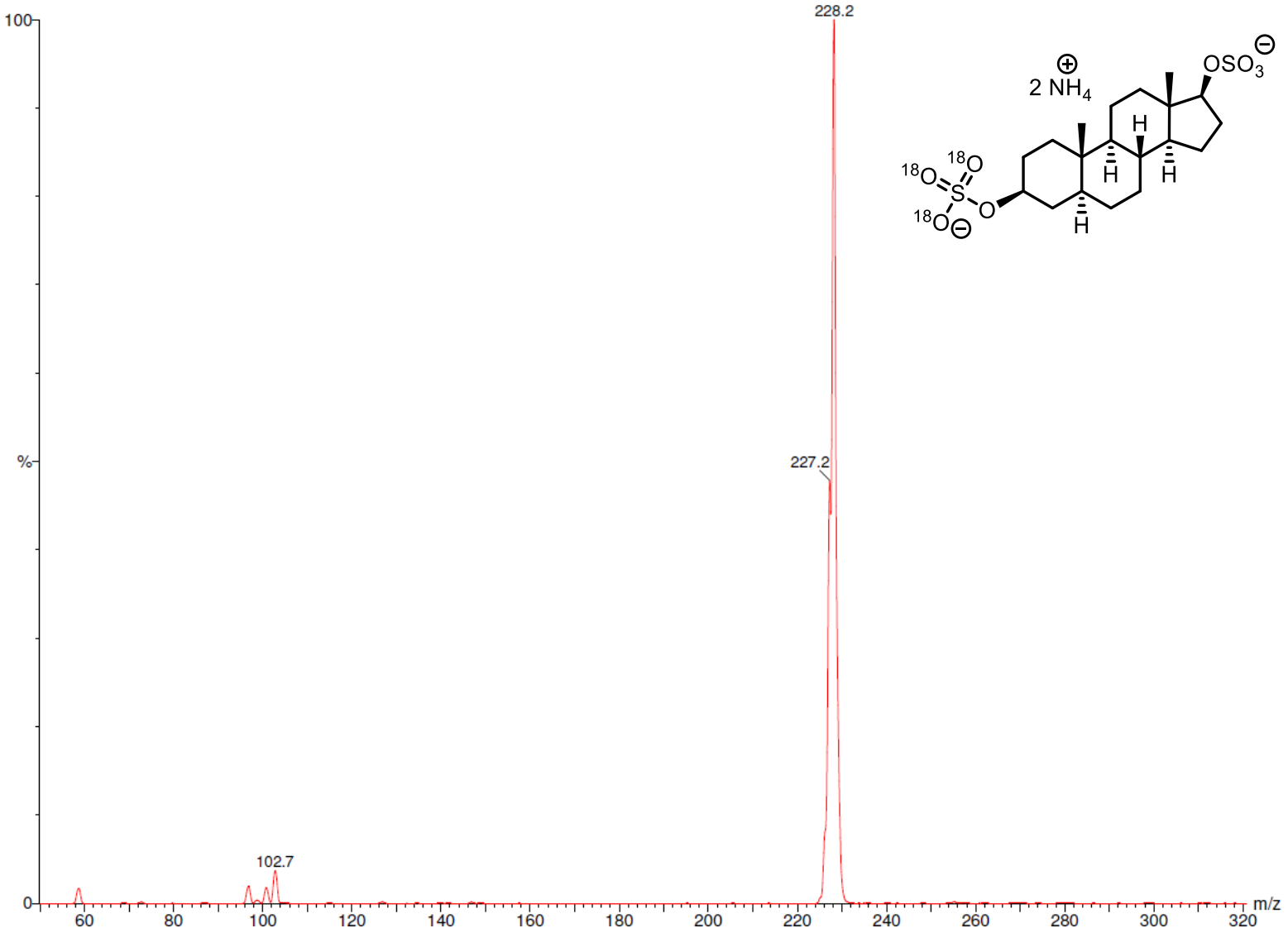
5α-Androstane-3β[$^{18}O_3$],17β-diol bis(sulfate), ammonium salt ^{1}H NMR 400 MHz, CD_3OD



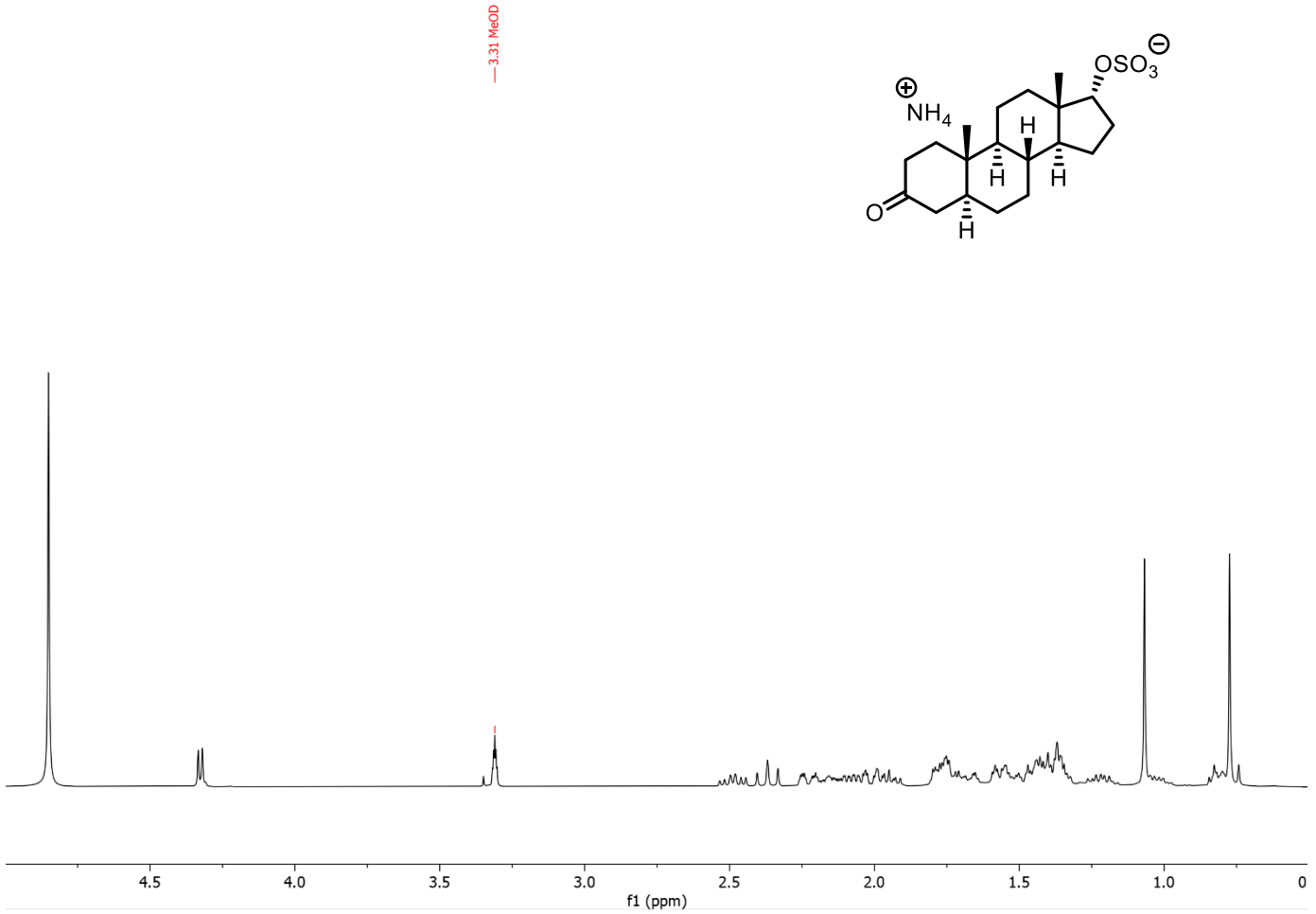
5α-Androstane-3β[$^{18}O_3$],17β-diol bis(sulfate), ammonium salt ^{13}C NMR 151 MHz, CD_3OD



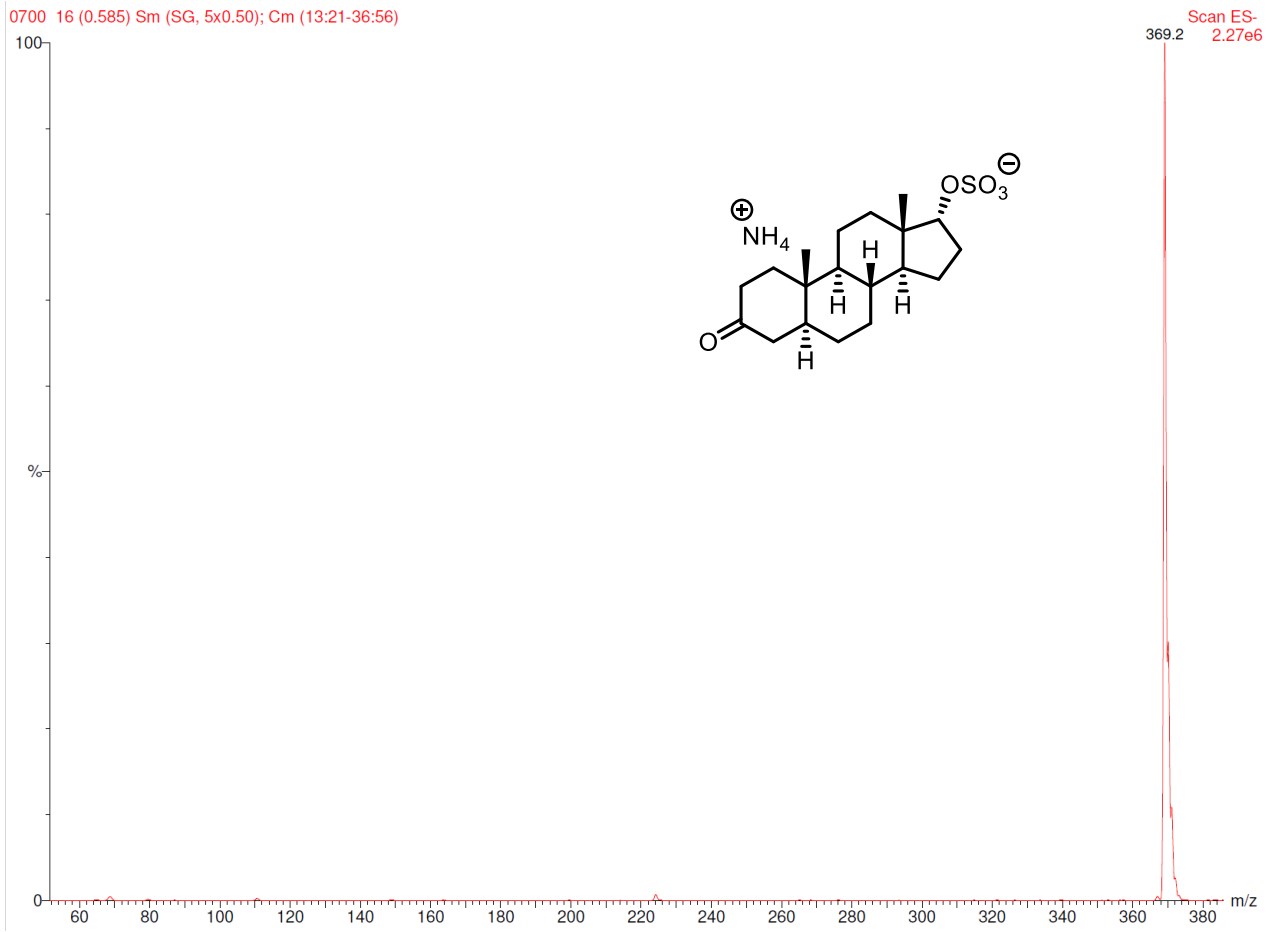
5α-Androstane-3β[$^{18}O_3$],17β-diol bis(sulfate), ammonium salt LRMS



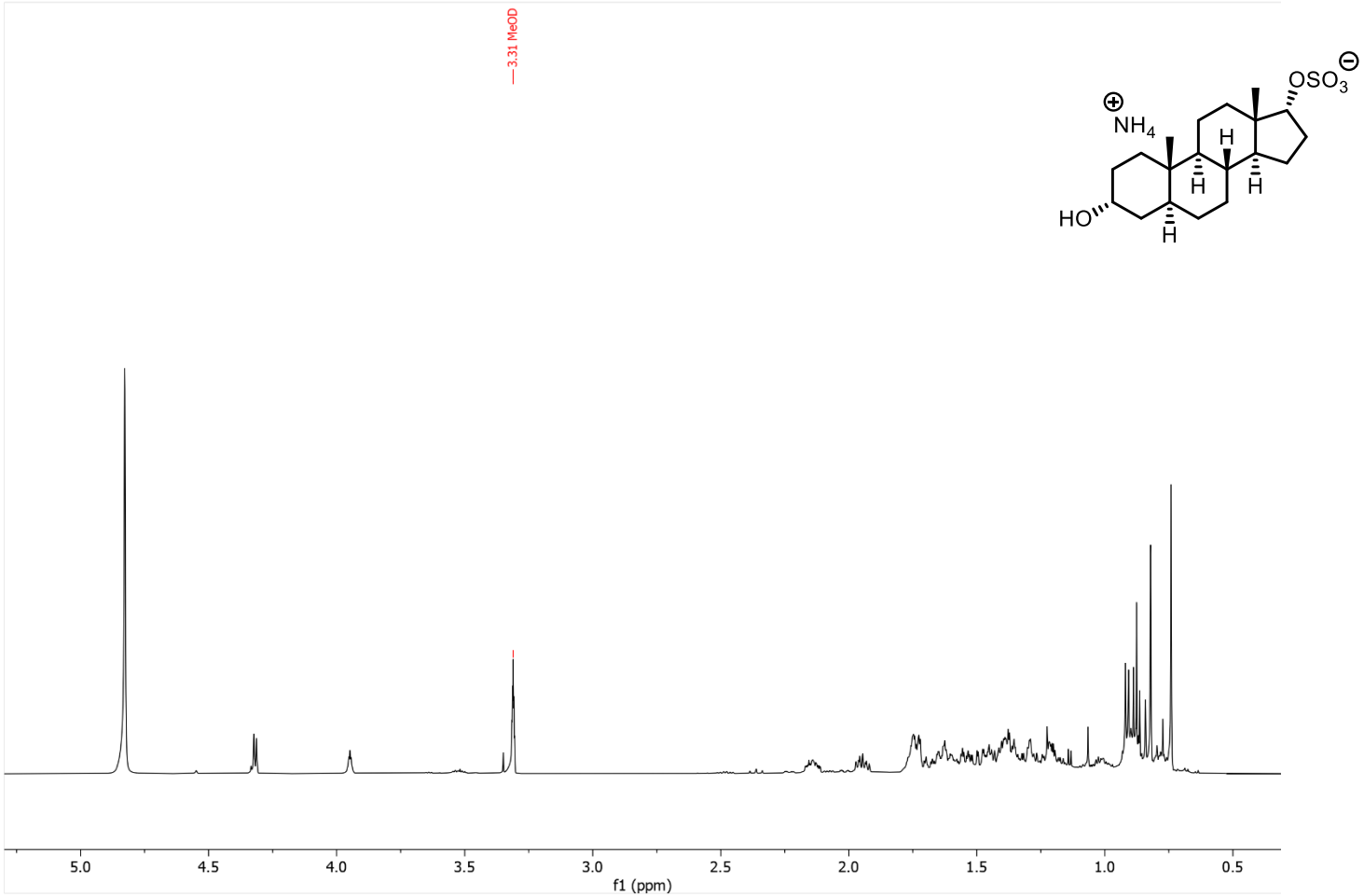
Epidihydrotestosterone 3-sulfate, ammonium salt ^{1}H NMR 600 MHz



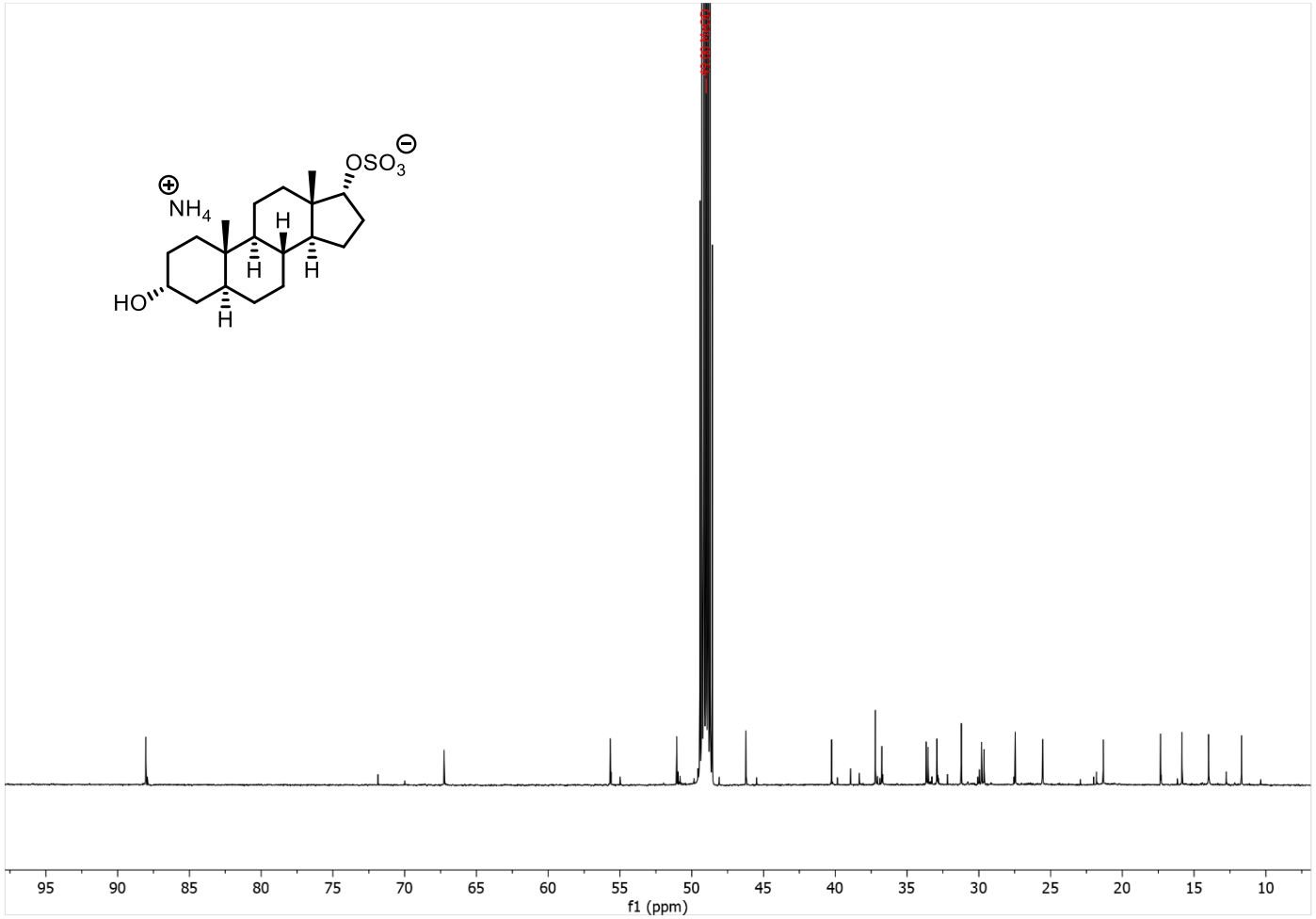
Epidihydrotestosterone 3-sulfate, ammonium salt ^{13}C NMR 151 MHz



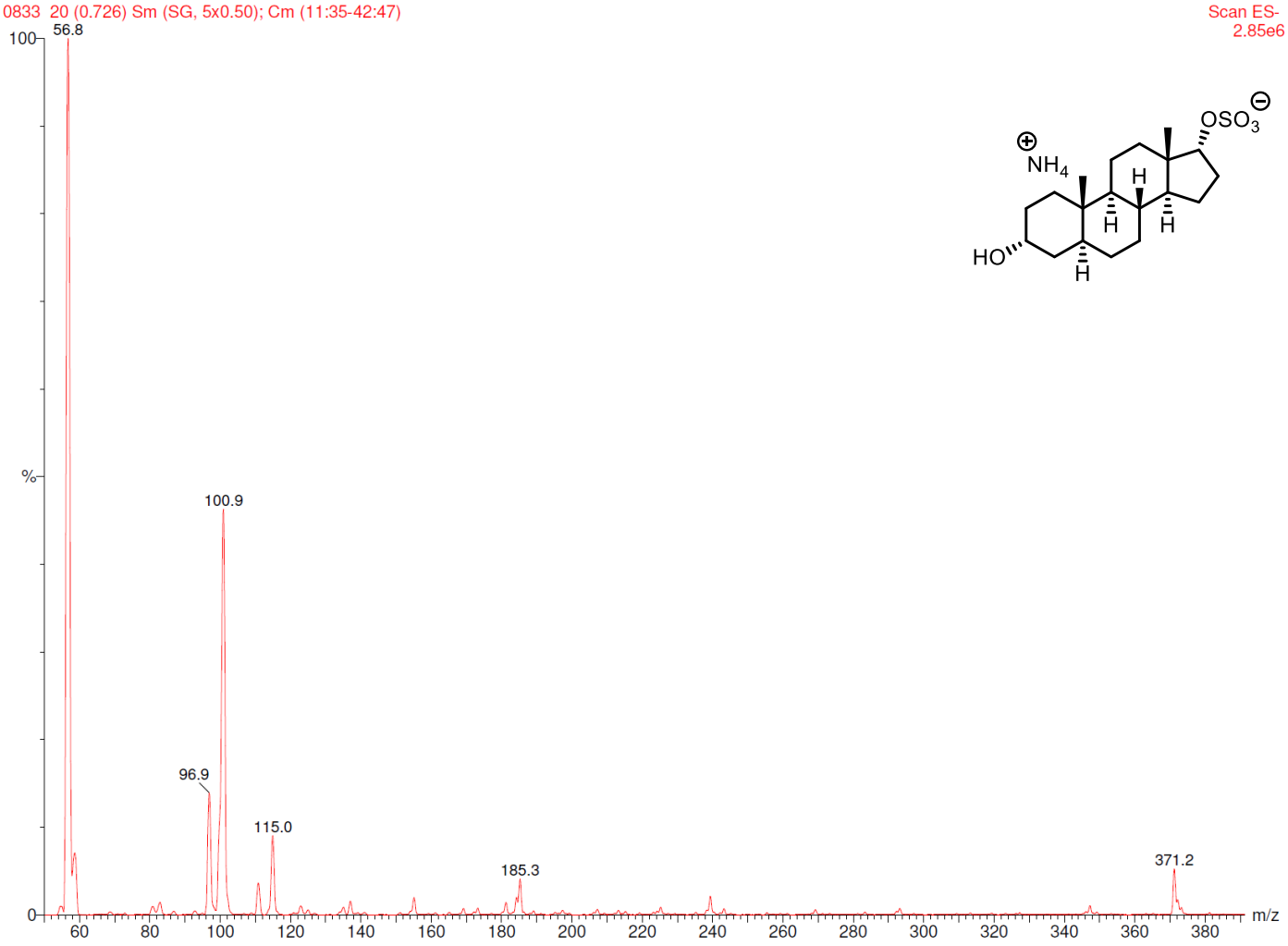
5α -Androstane-3α,17α-diol 17-sulfate, ammonium salt ^{1}H NMR 600 MHz



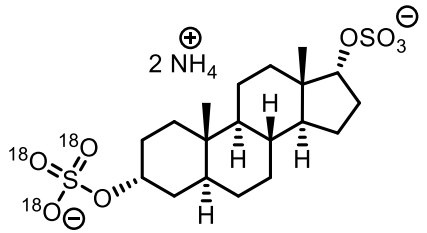
5α -Androstane-3α,17α-diol 17-sulfate, ammonium salt ^{13}C NMR 151 MHz

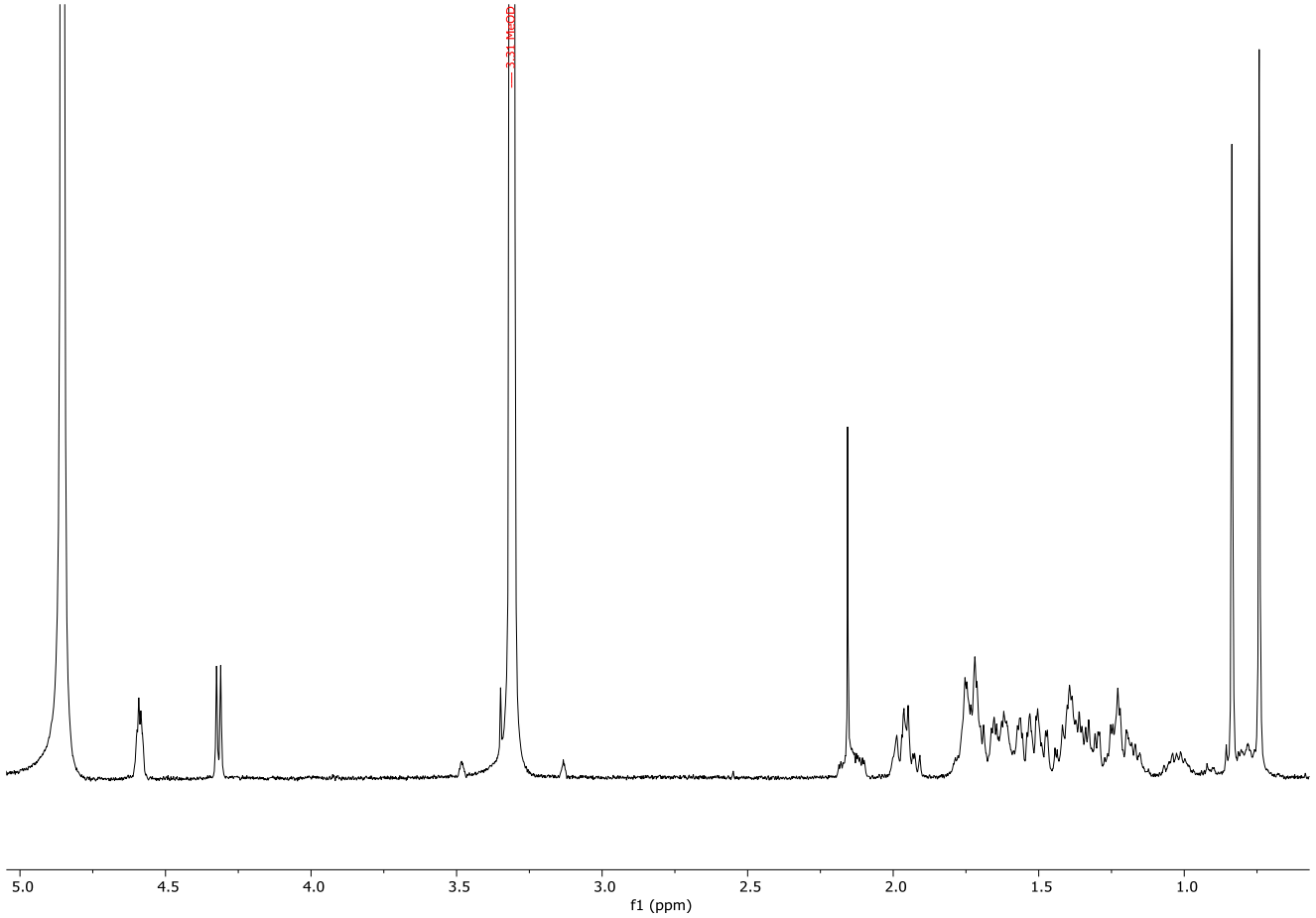


5α -Androstane-3α,17α-diol 17-sulfate, ammonium salt LRMS

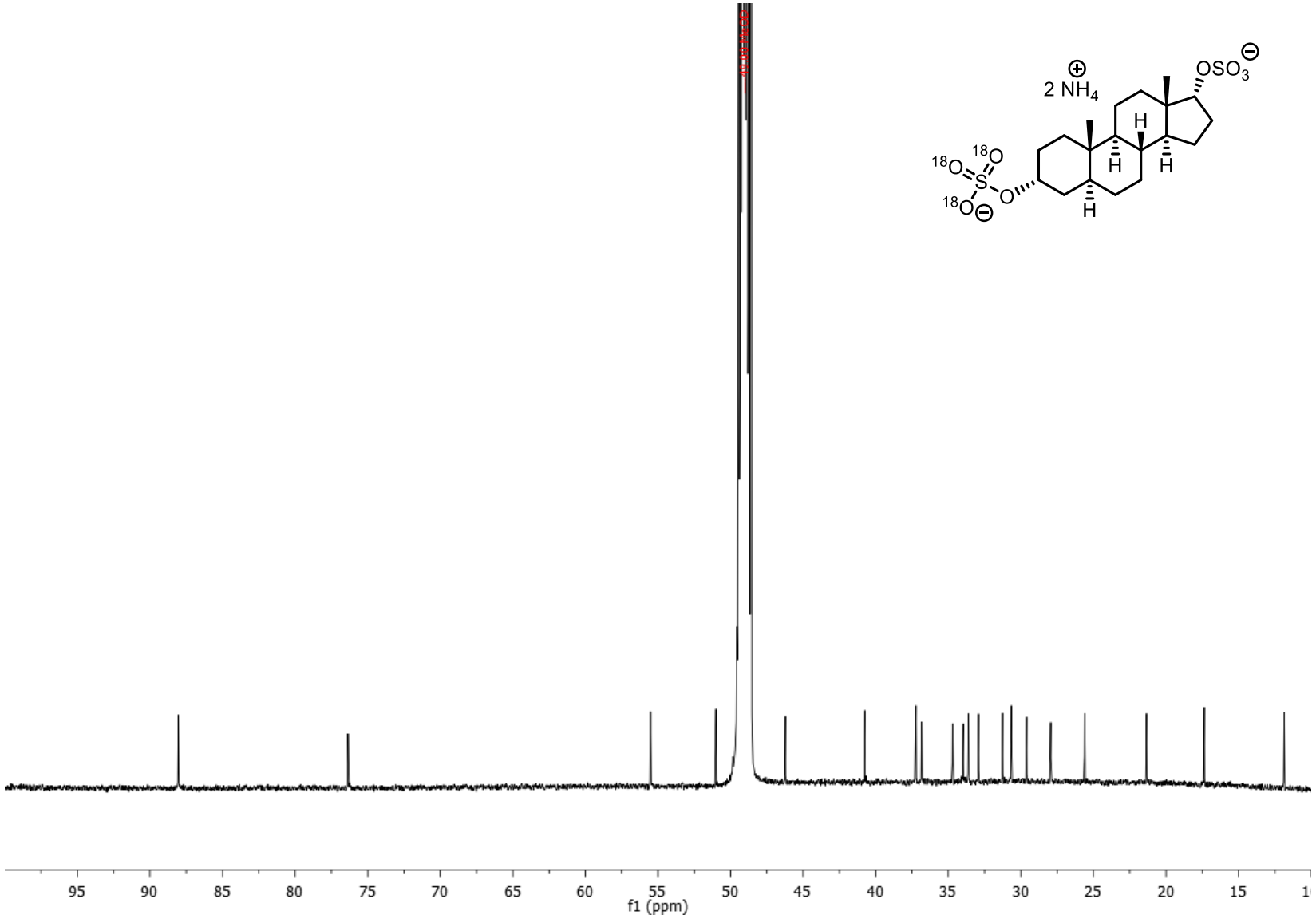


5α-Androstane-3β[$^{18}O_3$],17β-diol bis(sulfate), ammonium salt ^{1}H NMR 600 MHz

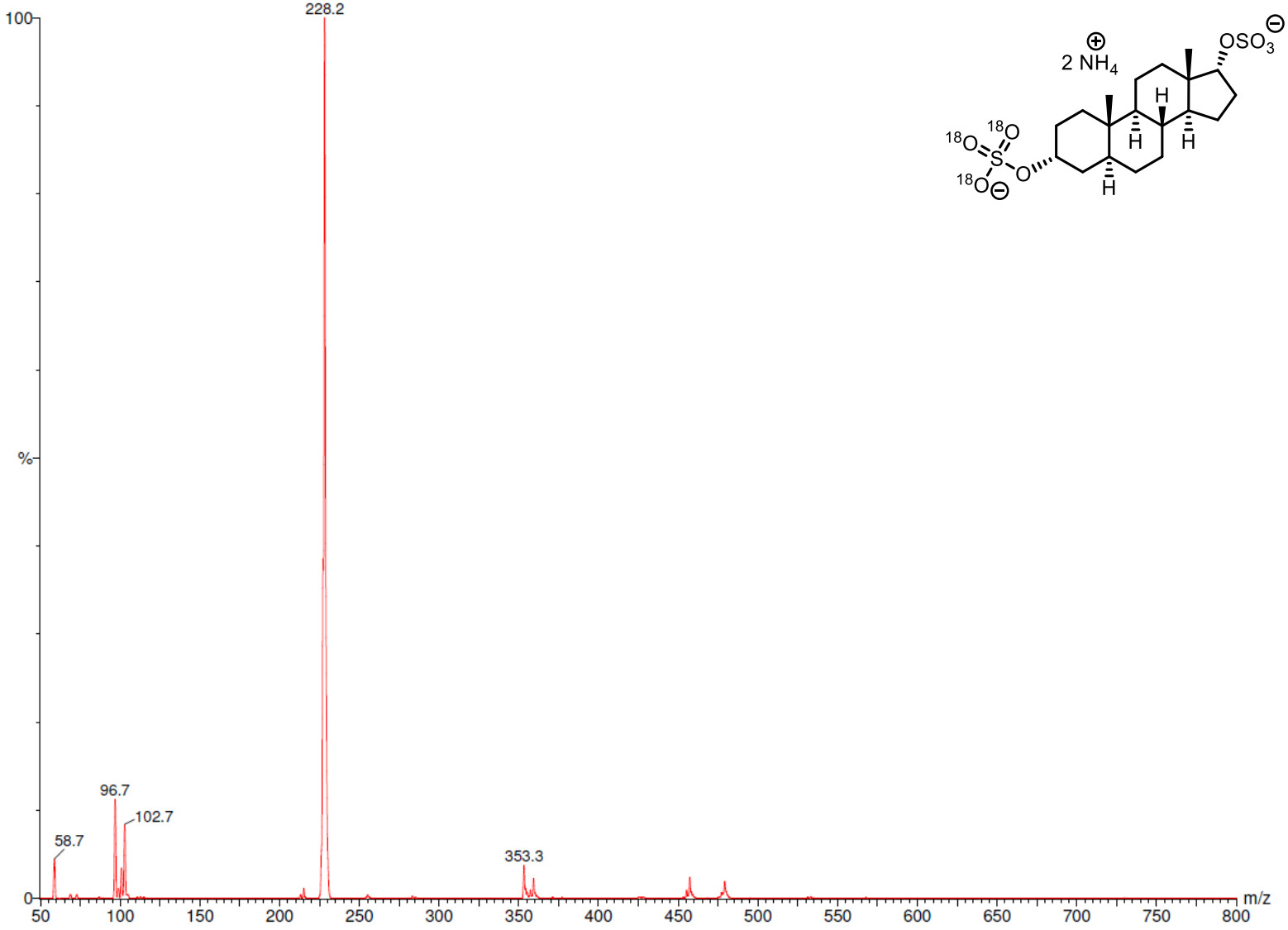




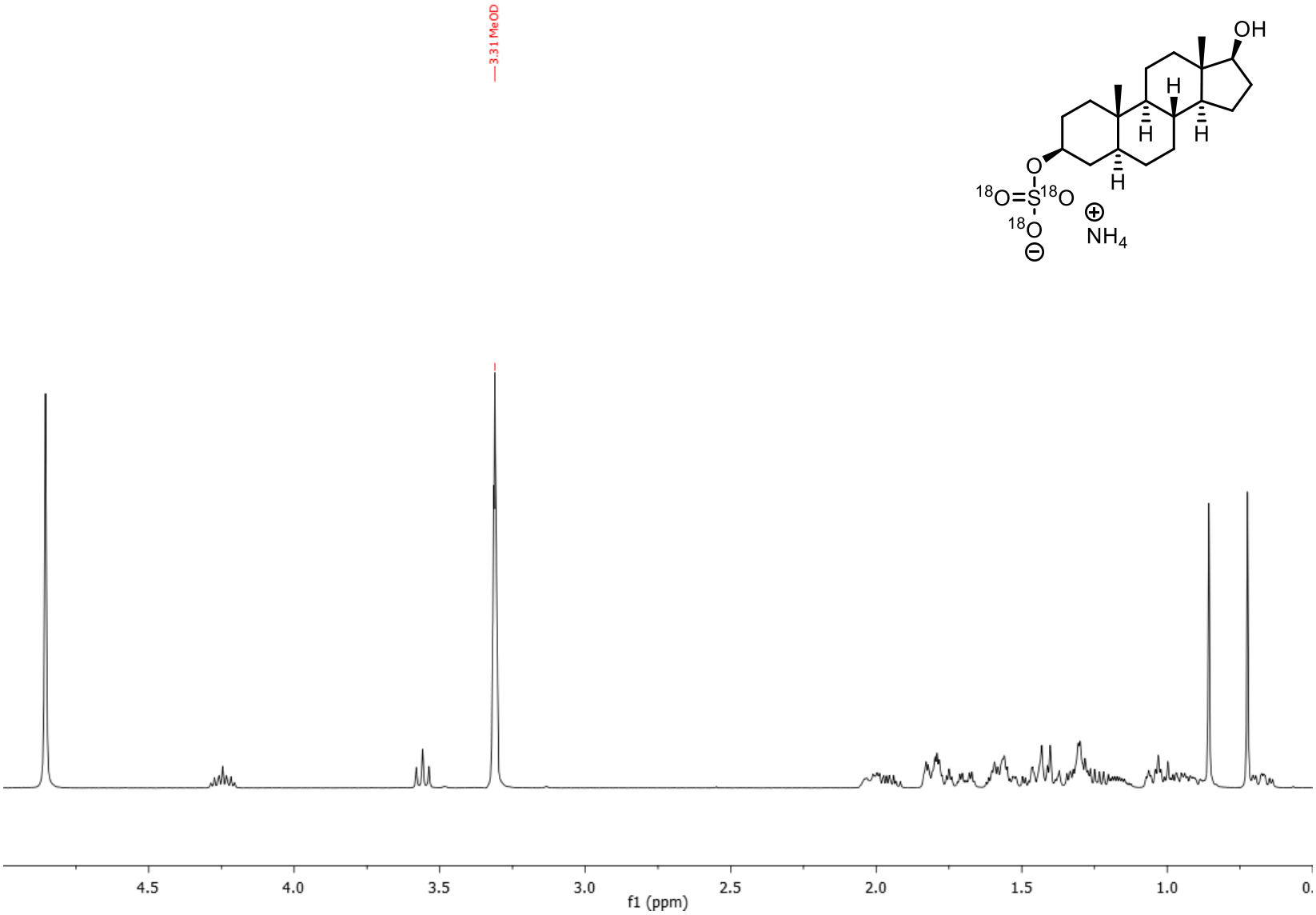
5α-Androstane-3β[$^{18}O_3$],17β-diol bis(sulfate), ammonium salt ^{13}C NMR 151 MHz, CD_3OD



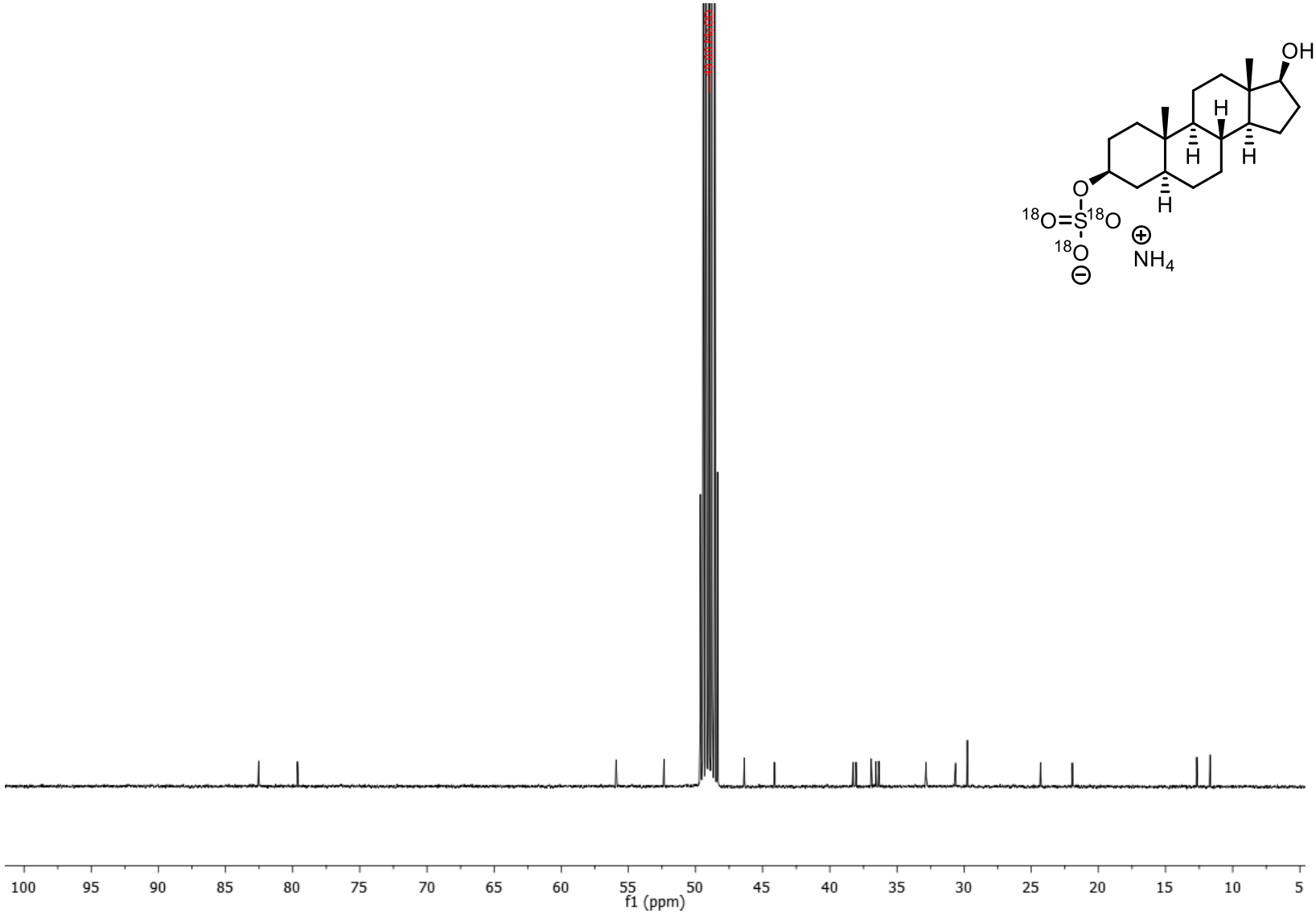
5α-Androstane-3β[$^{18}O_3$],17β-diol bis(sulfate), ammonium salt LRMS



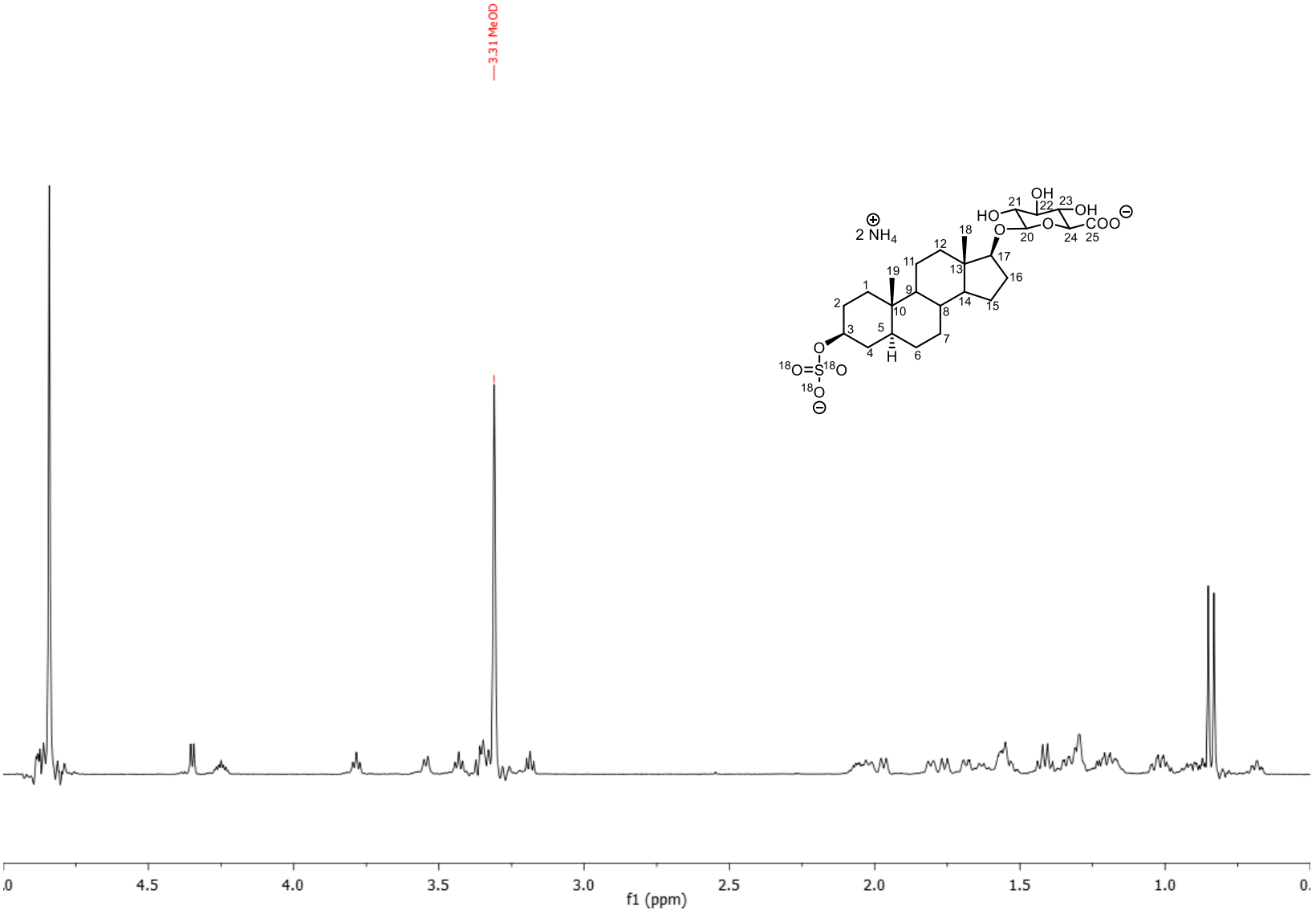
5α-Androstane-3β,17β-diol 3β[$S^{18}O_3$]-sulfate, ammonium salt ^1H NMR 400 MHz, CD_3OD



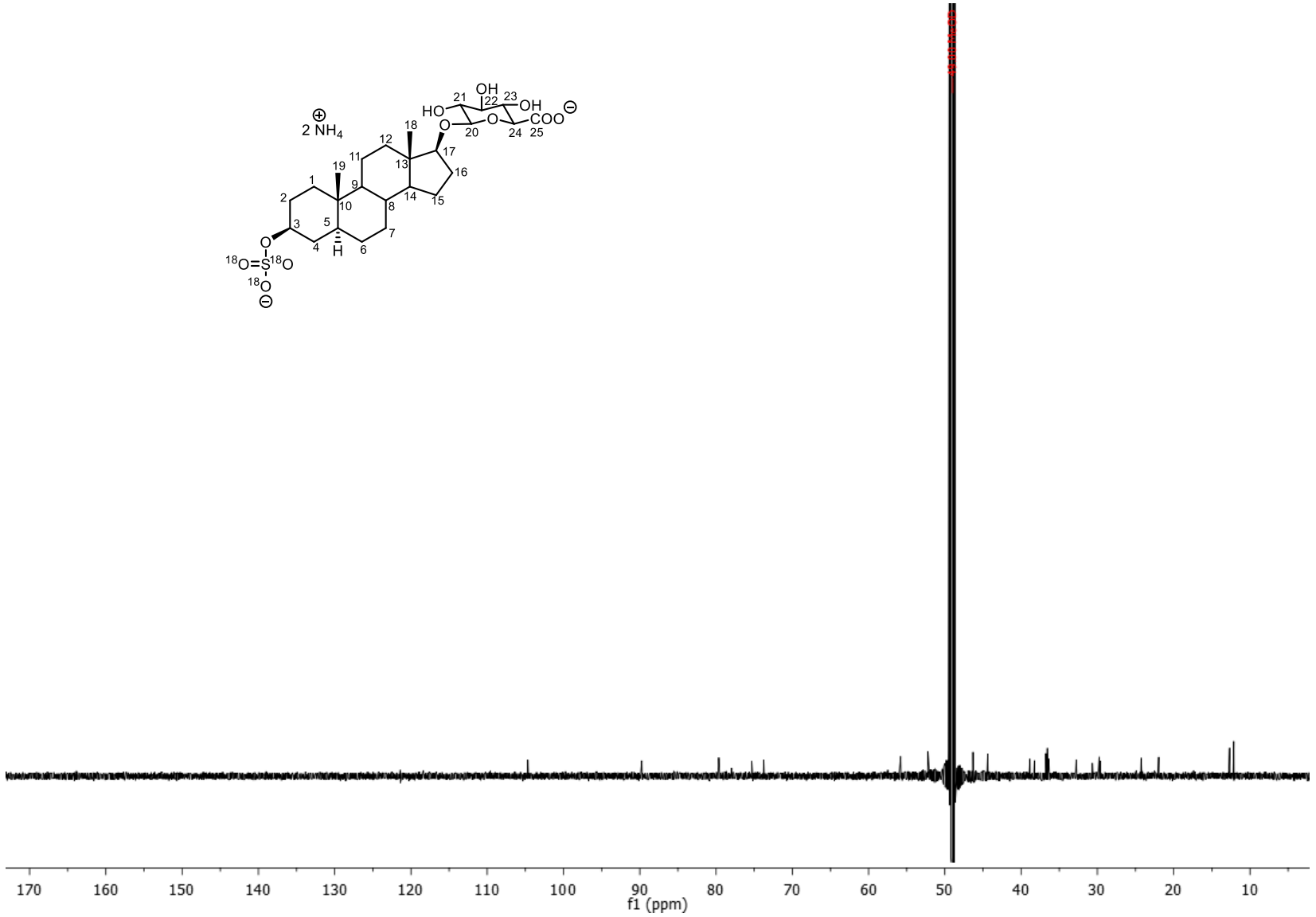
5α-Androstane-3β,17β-diol 3β[$S^{18}O_3$]-sulfate, ammonium salt ^{13}C NMR 176 MHz, CD_3OD



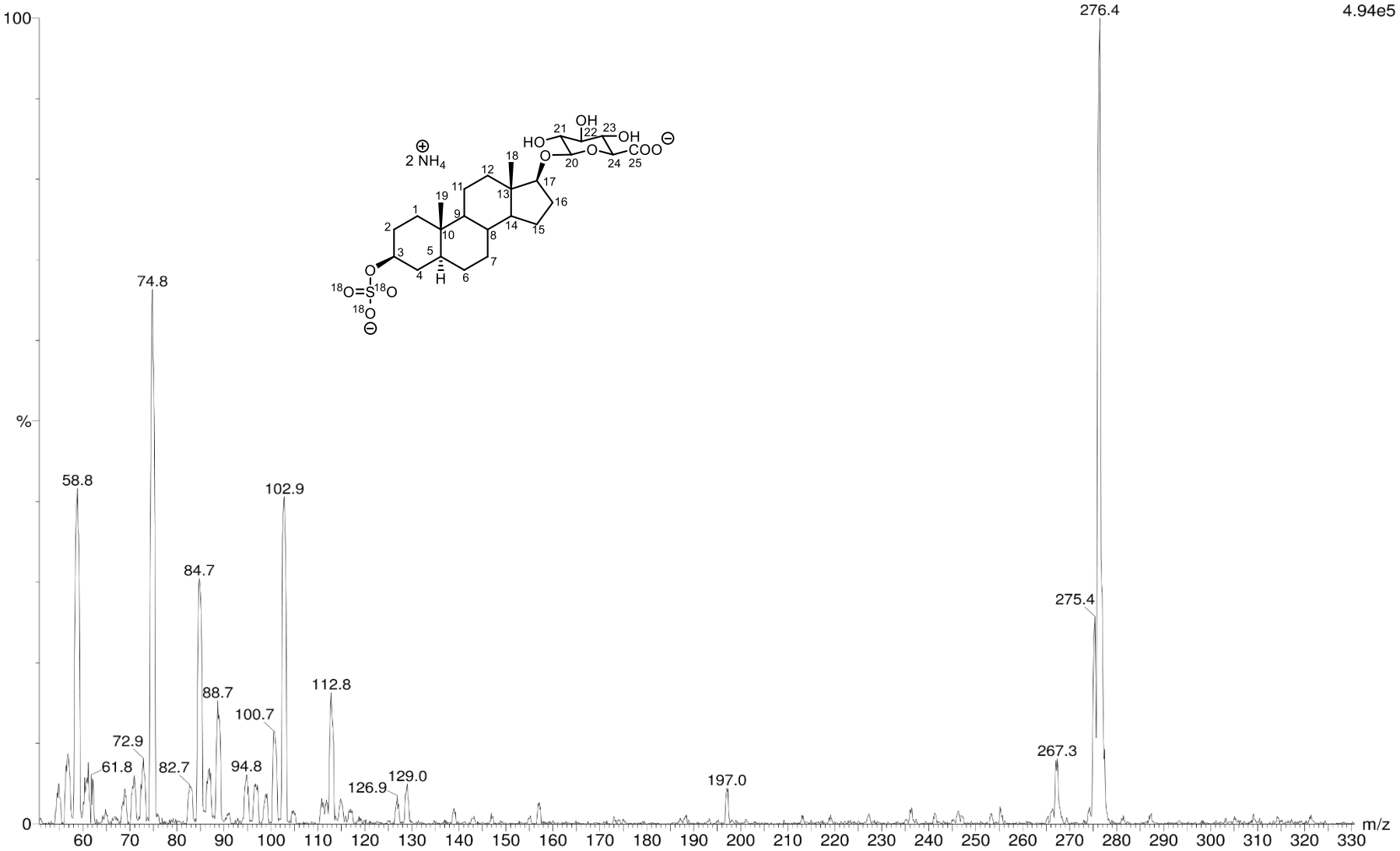
5α-Androstane-3β,17β-diol 3β[$S^{18}O_3$]-sulfate 17β-glucuronide, ammonium salt ^1H NMR 700 MHz, CD_3OD



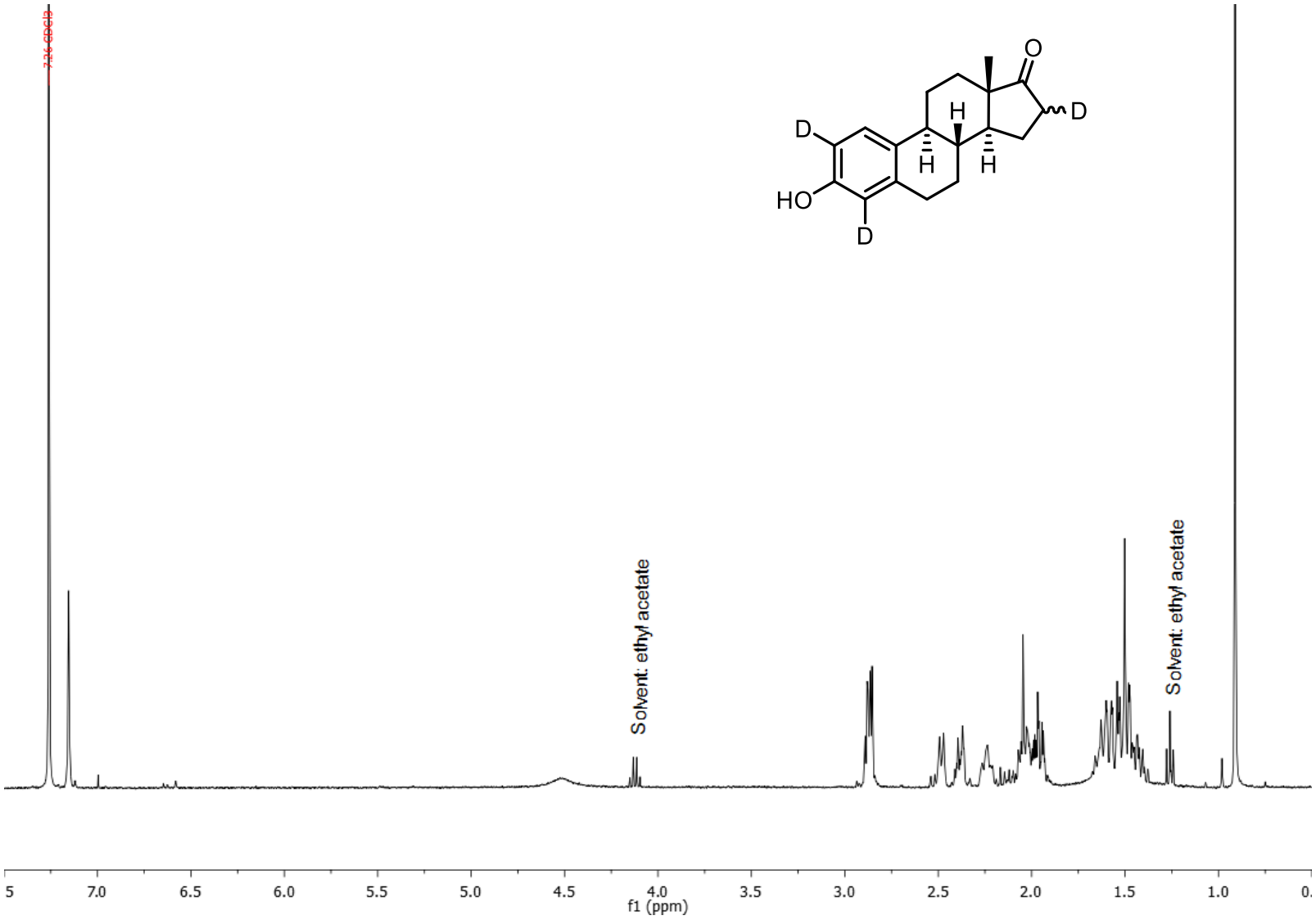
5α-Androstane-3β,17β-diol 3β[$S^{18}O_3$]-sulfate 17β-glucuronide, ammonium salt ^{13}C NMR 176 MHz, CD_3OD



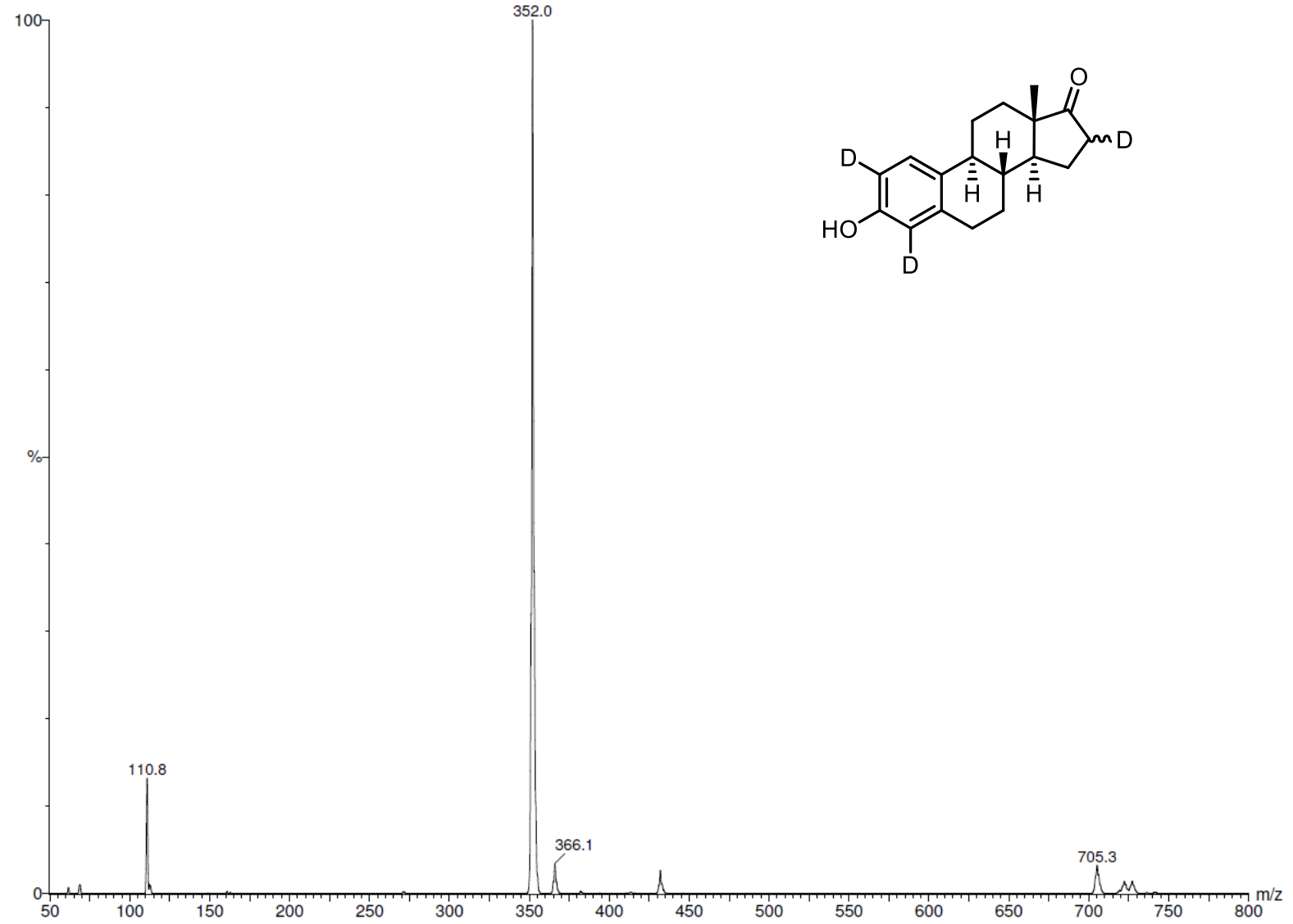
5α-Androstane-3β,17β-diol 3β[$S^{18}O_3$]-sulfate 17β-glucuronide, ammonium salt LRMS



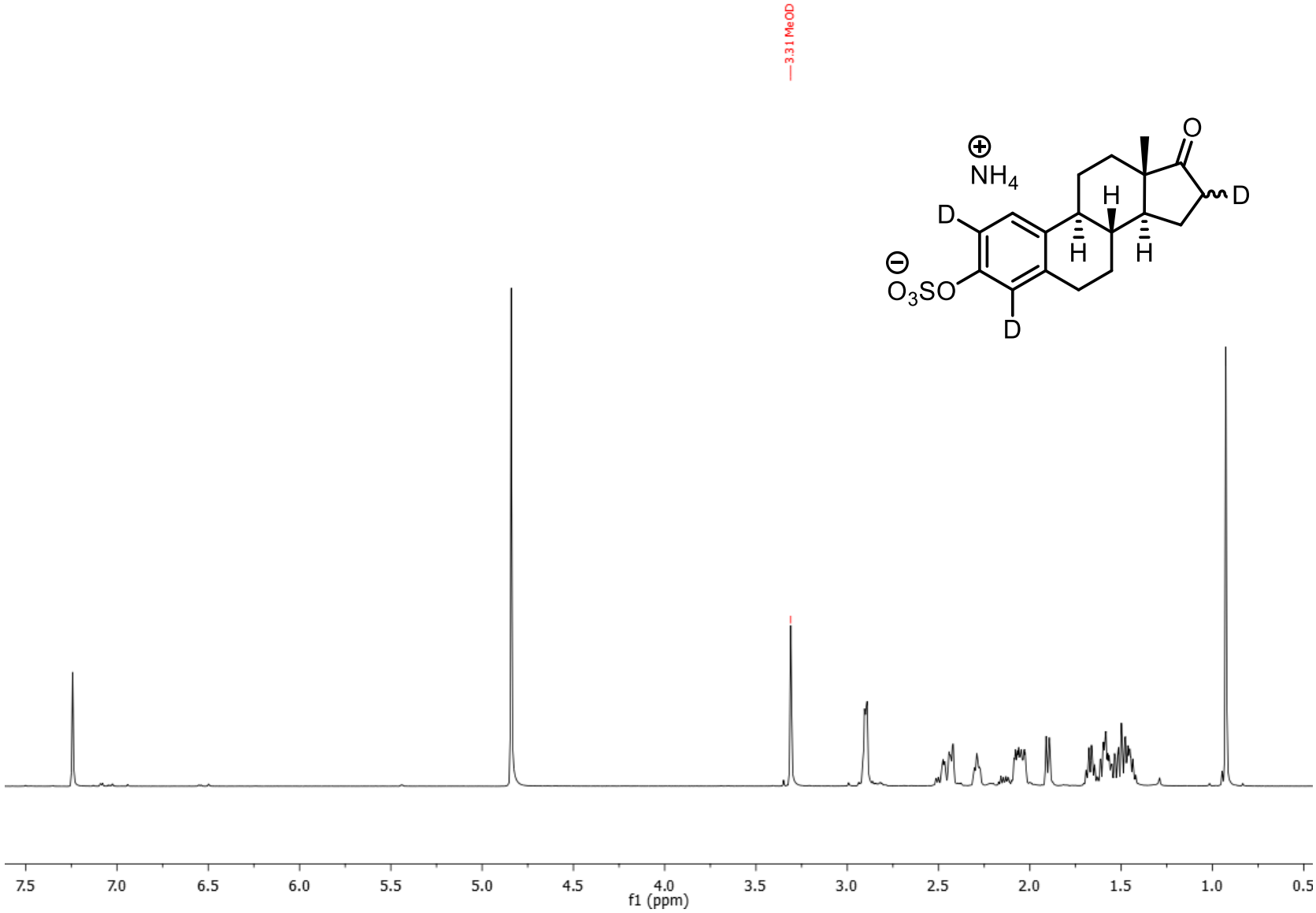
2,4,16-d_3-Estrone ^{1}H NMR 400 MHz, $CDCl_3$



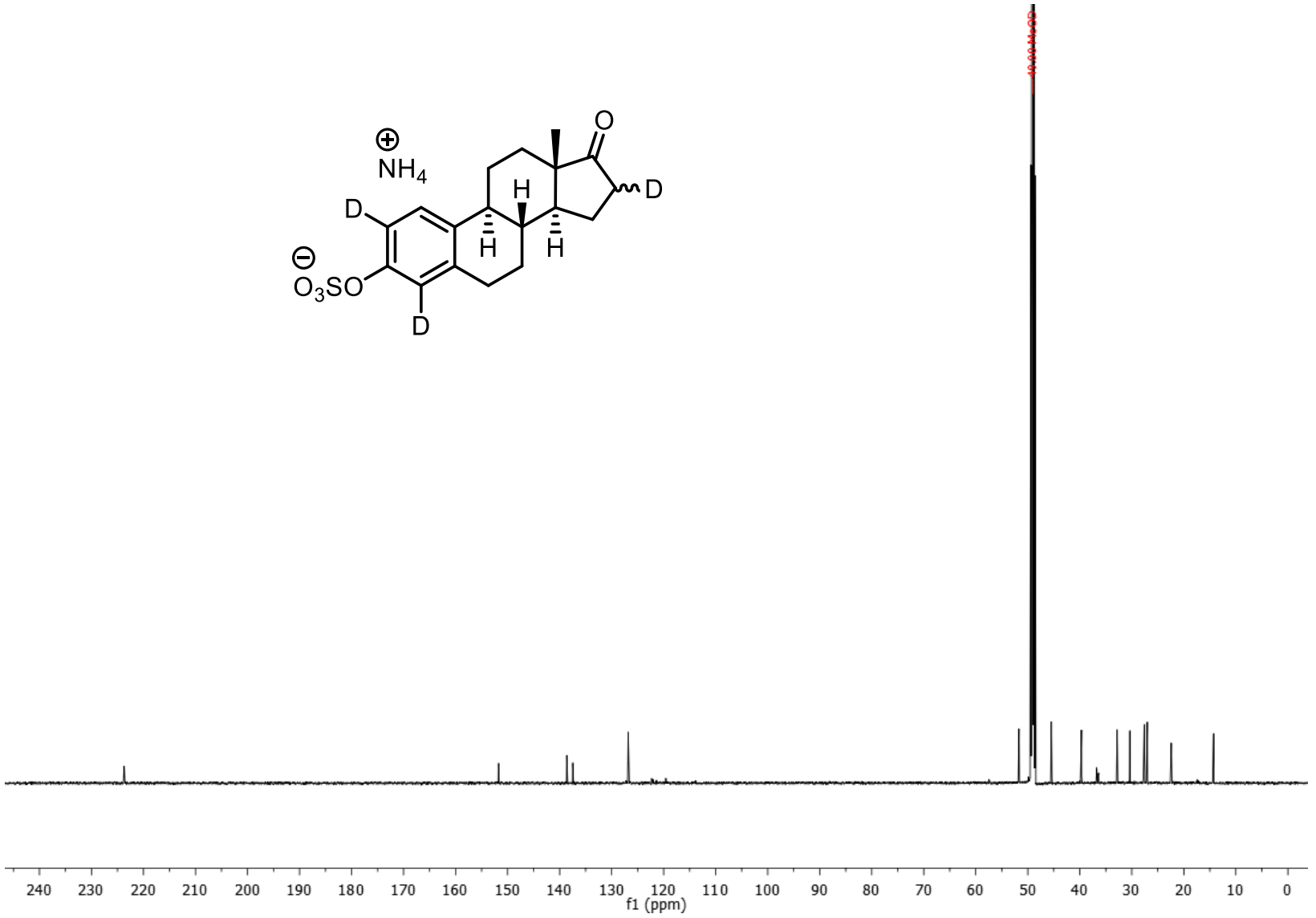
2,4,16-d_3-Estrone LRMS



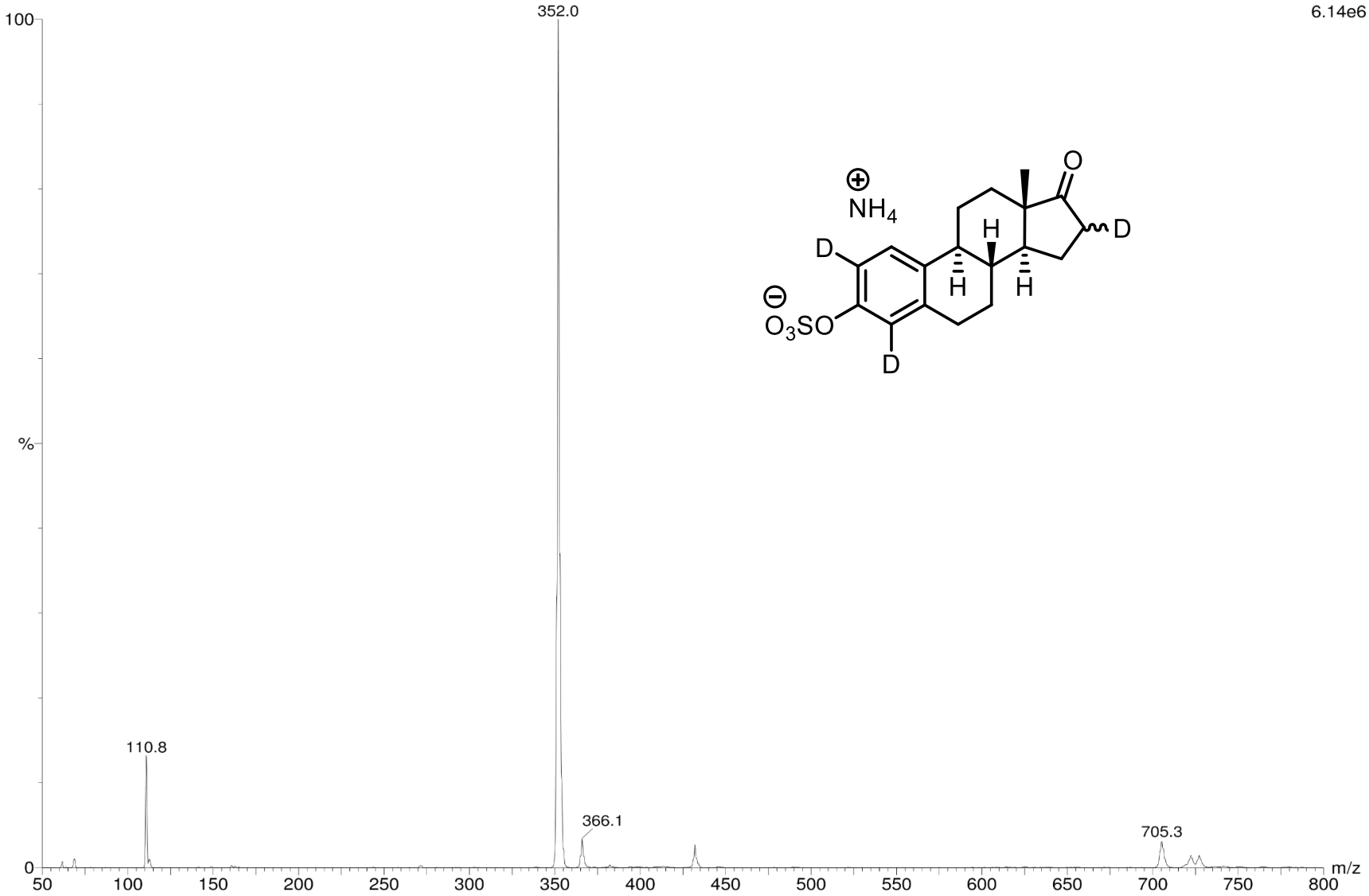
2,4,16-d_3-Estrone 3-sufate ^{1}H NMR 400 MHz, CD_3OD



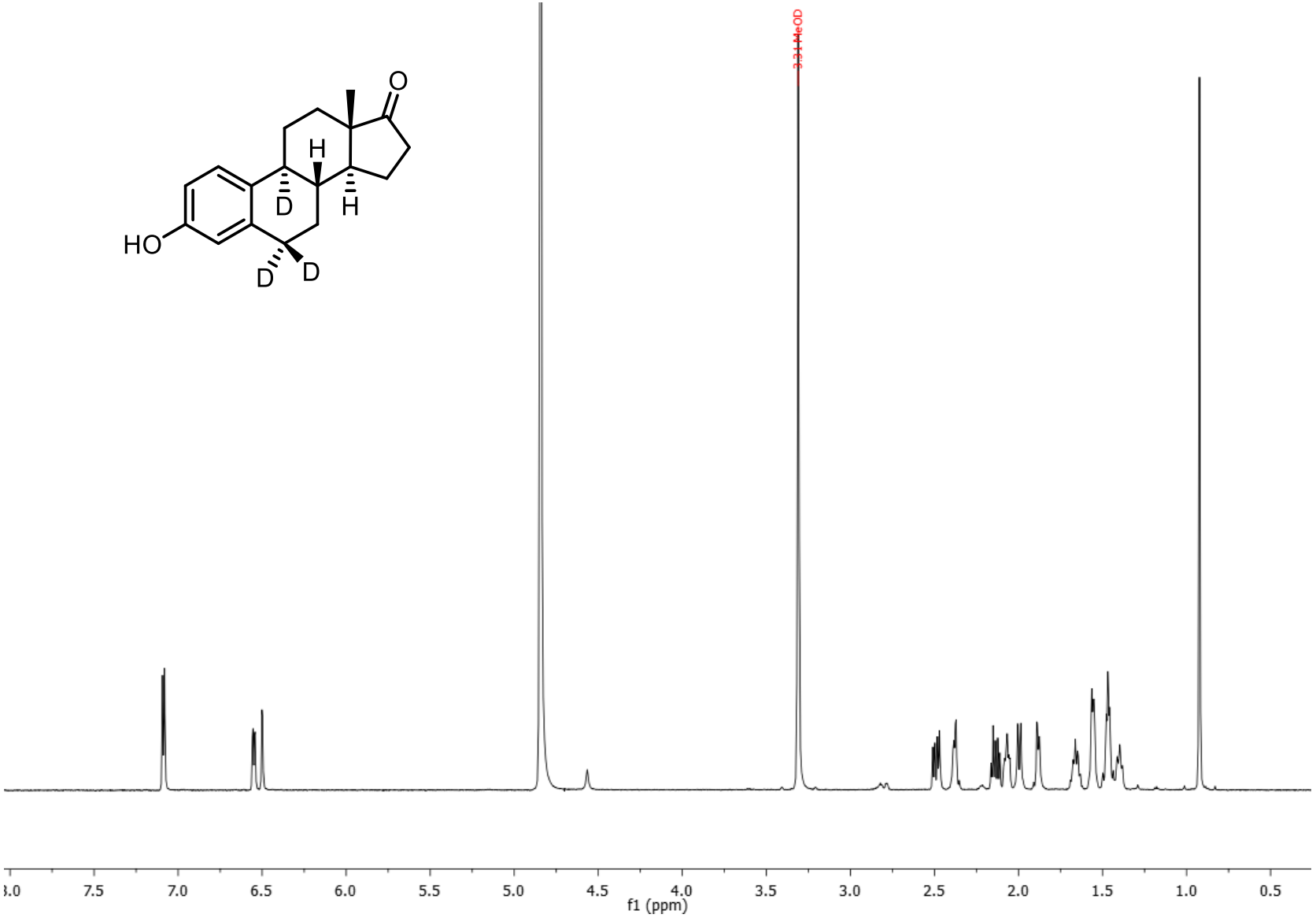
2,4,16-d_3-Estrone 3-sufate ^{13}C NMR 176 MHz, CD_3OD



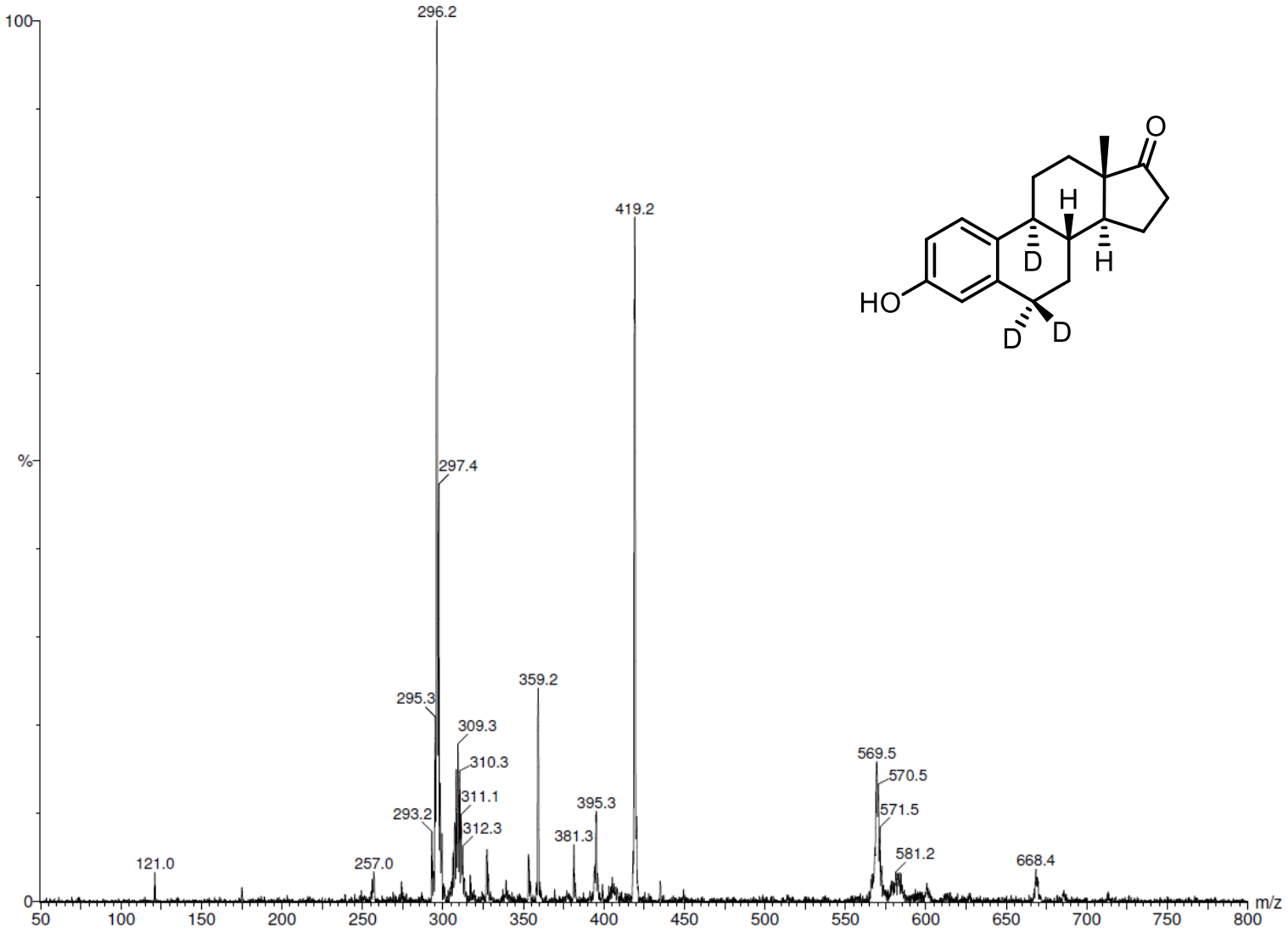
2,4,16-d_3-Estrone 3-sufate LRMS



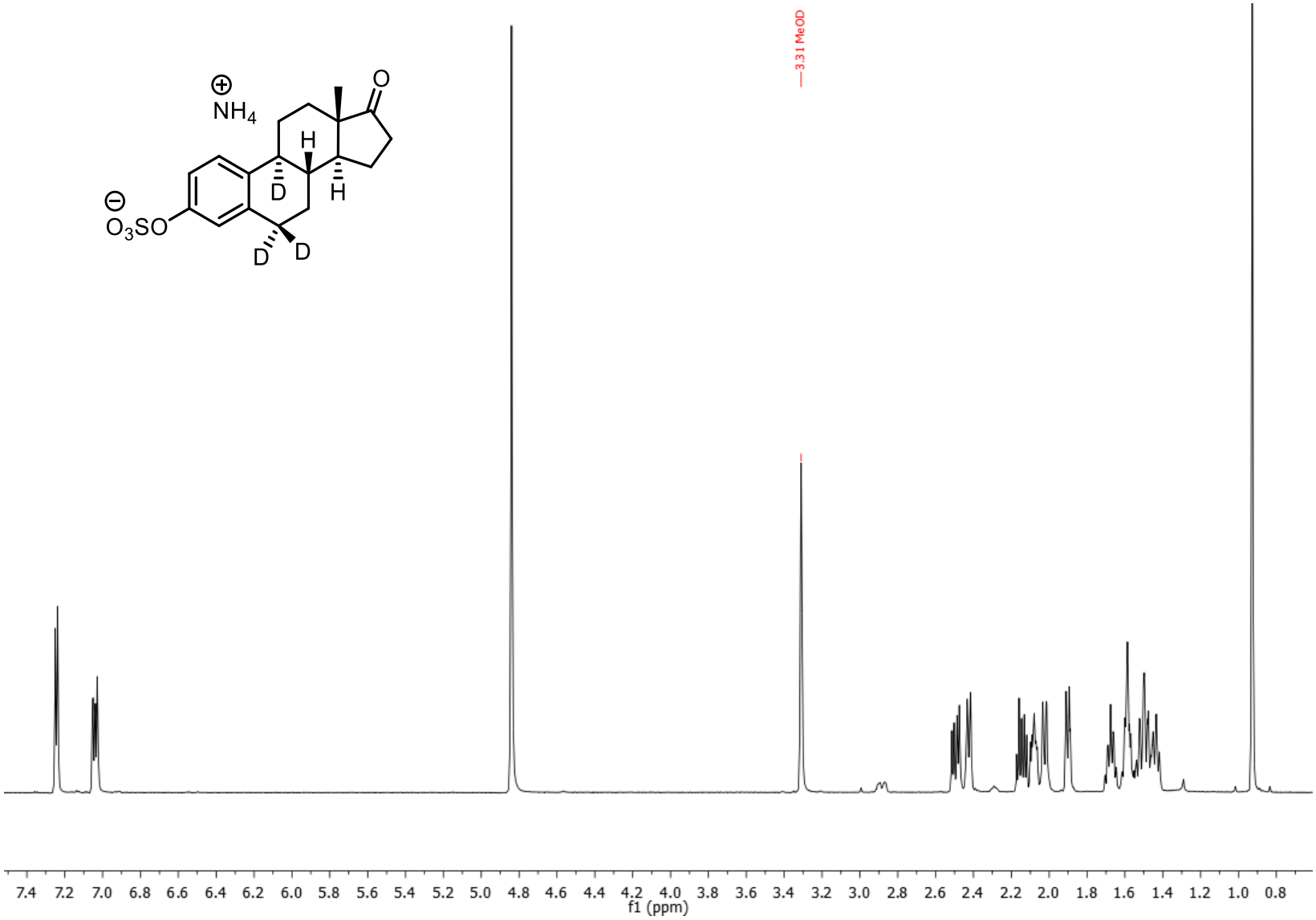
6,6,9-d_3-Estrone ^{1}H NMR 400 MHz, $CDCl_3$



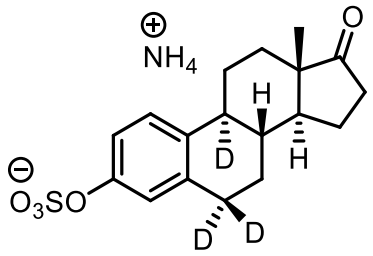
6,6,9-d_3-Estrone LRMS



6,6,9-d_3-Estrone 3-sufate ^{1}H NMR 400 MHz, CD_3OD



6,6,9-d_3-Estrone 3-sufate ^{13}C NMR 176 MHz, CD_3OD



6,6,9-d_3-Estrone 3-sufate LRMS