A Stereoselective Inverting *sec*-Alkylsulfatase for the Deracemisation of *sec*-Alcohols

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General

Competent cells One Shot® TOP10 and One Shot® BL21 Star™ (DE3) were purchased from Invitrogen and transformed according to the manufacturer´s protocol. 1-Octyl sulfate (**1a**), 1-octanol (**1b**) and alcohols **2b-10b** were purchased from Sigma Aldrich and Alfa Aesar. $H_2{}^{18}O$ for the preparation of ^{18}O labelled buffer was purchased from Rotem (label >98%). NMR spectra were recorded on a Bruker spectrometer at 300 (^1H) and 75 (^{13}C) MHz. Shifts (δ) are given in ppm and coupling constants (*J*) are given in Hz.

Synthesis of alkyl sulfate esters

Racemic sulfate esters **2a-10a**, (*R*)- and (*S*)-(**2a**) were prepared from the corresponding alcohols **2b-10b**, (*R*)- and (*S*)-(**2b**) by using $NEt_3{*}SO_3$ following a known procedure[1] with the following modifications: Triethylamine-SO_3 complex was added in 0.9 eq. to avoid residual complex in the lyophilisate. Hence the re-crystallisation step in methanol was not required.

NMR Data and yields for alkyl sulfates:

***rac*-2-Octyl sulfate (2a):**

^1H NMR (300 MHz, D_2O): δ = 4.46-4.36 (m, 1H), 1.62-1.43 (m, 2H), 1.33-1.15 (m, 11H), 0.78 (t, 5.4 Hz, 3H); ^{13}C NMR (75 MHz, D_2O): δ = 78.8, 36.0, 31.0, 28.2, 24.4, 21.9, 20.0, 13.4; 82% yield.

***rac*-2-Heptyl sulfate (3a):**

^1H NMR (300 MHz, D_2O): δ = 4.45- 4.35 (m, 1H), 1.57-1.41 (m, 2H), 1.32-1.15 (m, 9H), 0.78 (t, 5.4 Hz, 3H). ^{13}C NMR (75 MHz, D_2O): δ = 78.8, 36.0, 30.8, 24.1, 21.8, 20.0, 13.3; 76% yield.

***rac*-2-Nonyl sulfate (4a):**

^1H NMR (300 MHz, D_2O): δ = 4.44-4.34 (m, 1H), 1.59-1.39 (m, 2H), 1.35-1.14 (m, 13H), 0.76 (t, 5.0 Hz, 3H); ^{13}C NMR (75 MHz, D_2O): δ = 78.8, 35.9, 31.0, 28.4, 28.3, 23.3, 22.0, 19.9, 13.4; 70% yield.

***rac*-3-Octyl sulfate (5a):**

^1H NMR (300 MHz, D_2O): δ = 4.29-4.21 (m, 1H), 1.70-1.47 (m, 4H), 1.28-1.13 (m, 6H), 0.85-0.75 (m, 6H); ^{13}C NMR (75 MHz, D_2O): δ = 83.6, 32.9, 30.9, 26.5, 23.9, 21.8, 13.3, 8.5; 83% yield.

***rac*-4-Octyl sulfate (6a):**

^1H NMR (300 MHz, D_2O): δ = 4.35-4.27 (m, 1H), 1.60-1.45 (m, 4H), 1.35-1.15 (m, 6H), 0.84-0.76 (m, 6H); ^{13}C NMR (75 MHz, D_2O): δ = 82.3, 35.7, 33.1, 26.4, 21.9, 17.6, 13.2; 70% yield.

***rac*-6-Methyl-5-hepten-2-yl sulfate (7a):**

^1H NMR (300 MHz, D_2O): δ = 5.14 (t, 35.5 Hz, 1H), 4.45-4.34 (m, 1H), 2.09-1.90 (m, 2H), 1.64-1.46 (m, 8H), 1.23 (d, 7.8 Hz, 3H); ^{13}C NMR (75 MHz, D_2O): δ = 133.9, 123.6, 78.2, 36.1, 24.8, 23.2, 19.9, 16.9; 74% yield.

***rac*-1-Phenylprop-2-yl sulfate (8a):**

^{1}H NMR (300 MHz, D_2O): δ = 7.33-7.20 (m, 5H), 4.66-4.60 (m, 1H), 2.94-2.81 (m, 2H), 1.20 (d, 7.6 Hz, 3H); the signal at 4.65-4.59 was occluded by the H□O-signal, but could be identified and annotated via 2D-NMR spectroscopy; ^{13}C NMR (75 MHz, D_2O): δ = 137.5, 129.9, 128.4, 126.6, 78.3, 41.9, 19.3; 80% yield.

***rac*-4-Phenylbut-2-yl sulfate (9a):**

^{1}H NMR (300 MHz, D_2O): δ = 7.30-7.12 (m, 5H), 4.45-4.35 (m, 1H), 2.72-2.53 (m, 2H), 1.86-1.71 (m, 2H), 1.25 (d, 7.5 Hz, 3H); ^{13}C NMR (75 MHz, D_2O): δ = 142.2, 128.6, 128.6, 126.0, 77.9, 37.9, 30.8, 19.9; 71% yield.

***rac*-1-Cyclohexylethyl sulfate (10a):**

^{1}H NMR (300 MHz, D_2O): δ = 4.23-4.15 (m, 1H), 1.72-1.35 (m, 6H), 1.24-0.83 (m, 8H); ^{13}C NMR (75 MHz, D_2O): δ = 82.6, 42.8, 28.0, 27.8, 26.0, 25.6, 16.9; 85% yield.

***rac*-1-Heptyn-3-yl sulfate (11a):**

^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.67-4.62 (m, 1H), 3.28 (d, 7.9 Hz, 1H), 1.70-1.53 (m, 2H), 1.42-1.21 (m, 4H), 0.86 (t, 5.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 84.2, 75.6, 66.0, 35.7, 27.0, 22.3, 14.4; 82% yield.

***rac*-1-Octyn-3-yl sulfate (12a):**

^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.67-4.62 (m, 1H), 3.24 (d, 6.8 Hz, 1H), 1.68-1.51 (m, 2H), 1.42-1.16 (m, 6H), 0.83 (t, 5.6 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 83.8, 75.7, 66.4, 35.8, 31.2, 24.3, 22.4, 14.3; 79% yield.

Cloning and expression of alkylsulfatase Pisa1 from *Pseudomonas sp.* DSM6611

Genomic DNA from *Pseudomonas sp.* DSM6611 was prepared using the PureLink™ Genomic DNA Mini Kit (Invitrogen). The open reading frame of the putative *Pseudomonas* alkylsulfatase was amplified by PCR using CTAGCTAGCATGTCCCGCTTCATTCGCGCCAG as forward and CCGCTCGAGGGGGTTCGACGATATTGAACTTCGGGCT as reverse primer. The PCR product was digested with *Nhe*I and *Xho*I (Fermentas) and cloned into the corresponding restriction sites of a modified pET-21a(+) vector (Novagen) using a T4 DNA ligase (Fermentas). The start codon present in the vectors *Nde*I restriction site had previously been removed by site-directed mutagenesis (ATG to CTG). The stop codon was omitted in order to obtain a protein with a C-terminal hexa-histidine tag. The ligated plasmid was first transformed into *E. coli* TOP10 cells. After plasmid preparation with a PureLink™ HiPure Plasmid Midiprep Kit (Invitrogen), the vector was further transformed into *E. coli* BL21 STAR cells according to the manual of the supplier. Ligation success was verified by restriction digest of the plasmid and subsequent agarose gel (1%) analysis. Cells were grown in LB- medium containing ampicillin (100 μg/mL) and $ZnSO_4$

(30 µg/mL) at 37° C and 120 rpm until the culture reached an OD_{600} of 0.7. After cooling to 20° C IPTG was added to a final concentration of 0.5 mM and Pisa1 was expressed at 20 ˚C and 120 rpm overnight. Cells were harvested at 4° C and 8000 rpm for 15 min. The cell pellet was washed once with sodium chloride (0.9%) and stored at -20 ˚C.

Cloning and expression of SdsA1 from *Pseudomonas aeruginosa*[2]

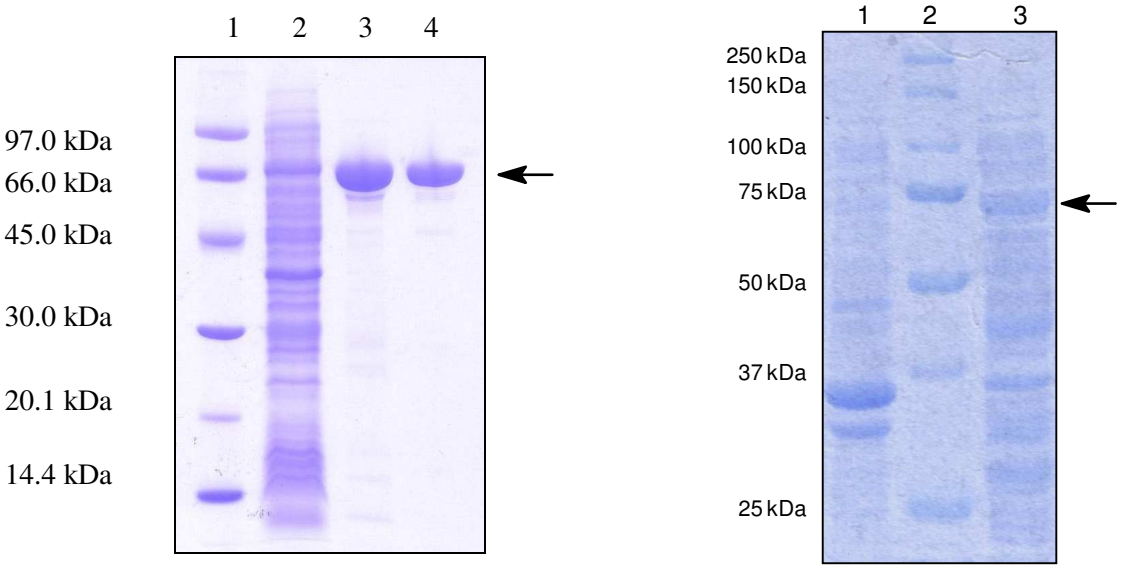
The gene *sdsa1* was synthesized by DNA 2.0 in a pJ201 vector with a kanamycin resistence. The lyophilized vector was treated as suggested by the supplier. Afterwards it was transferred into a pET-21a(+) vector (Novagen) using *Nde*I and *Xho*I restriction enzymes (Fermentas) following standard protocols. After transformation into competent *E. coli* BL21 (DE3) cells (as described for Pisa1), the protein was expressed with a C-terminal hexa-histidine tag. Expression was carried out as described for Pisa1.

Purification of His-tagged Pisa1 and SdsA1

After resuspension in phosphate buffer (50 mM, 300 mM NaCl, 10 mM Imidazol, pH 8.2) cells were disrupted by sonification using a Sonics & Materials Vibra Cell CV26, 13 mm tip, 30% amplitude, pulse 1s on, 2s off. SDS-PAGE analysis of the soluble and insoluble fraction showed that >80% of the protein was in the soluble fraction (Fig. S2, right). After centrifugation (4° C, 18000 rpm, 20 min) the supernatant was filtered through a MN 615 filter paper (Machery Nagel) and subjected to Ni-NTA affinity chromatography on a Bio-Rad DuoFlow FPLC system equipped with a Ni Sepharose 6 FF column employing standard procedures as described by the manufacturer (GE Healthcare). After SDS-PAGE selected fractions were exposed to dialysis in Tris-Cl (100 mM, pH 8.2) overnight (Fig. S2, left). The yield of this fraction is 18.5 mg protein/L of culture, it is >95% pure and can be used for biocatalytic transformations.

For protein crystallisation, the protein solution was concentrated and further purified with a MonoQ anion exchange 5/50 GL column (5 x 50 mm, GE Healthcare) previously equilibrated with Tris-Cl (20 mM, pH 8.2). The enzymes were eluted with a 10 mL linear gradient of 0-0.4 M NaCl in the same buffer at a flow rate of 1 mL/min and protein of >99% purity was dialysed in Tris-Cl (100 mM, pH 8.2) overnight. Final concentration was determined by the method of Bradford (as described) and purified proteins were flash-frozen and stored at -20° C. No significant loss of activity <10% was detected during several months.

Figure S2. SDS-PAGE of purified Pisa1 (left) and protein composition after cell disruption (right).



Left: Lane 1: LMW standard (GE Healthcare); lane 2: crude extract; lane 3: pool after Ni-NTA affinity chromatography; lane 4: pool after anion exchange chromatography.

Right: Lane 1: insoluble protein fraction (pellet); lane 2: MW standard (Bio-Rad Precision Plus Standard All Blue); lane 3: soluble protein fraction (supernatant).

Determination of protein concentrations

For determination of protein concentrations, Bradfords method was used: Protein solution (20 µL, diluted if necessary) was added to 980 µL of 1-fold Bio-Rad Bradford solution (diluted from a 5-fold concentrated stock solution) in a plastic cuvette. The reaction mixture was incubated at RT for 10 min and the extinction was measured at 595 nm against a blank containing Bradford solution and water. Protein concentrations were calculated with a calibration curve using bovine serum albumin.

$H_2{}^{18}O$ labelling experiments

Enzymatic assays of alkyl sulfates (**1a**, **2a,** 4 mg, 17 µmol) were conducted in unlabeled and ^{18}O-labeled Tris-HCl (250 µL, 100 mM, pH 8.2 for Pisa1, pH 7.5 for SdsA1). The reaction mixture was shaken at 30° C and 120 rpm for 24 h. After extraction with ethyl acetate (1 mL) the organic phase was dried over anhydrous sodium sulfate. Product **1b** was analyzed on a Agilent 7890A system equipped with an achiral HP-5 column and subsequently derivatized as acetate as described above. Product **2b** was directly derivatized. GC/MS spectra from ^{18}O-labelling experiments were recorded on an Agilent 7890A GC system equipped with an Agilent 5975C mass selective detector (electron impact, 70 eV). Measurements were carried out on an a)

Agilent HP-5-MS column (30 m x 0.25 mm x 0.25 µm film) or b) a Varian Chirasil Dex CB column (25 m x 0.32 mm x 0.25 µm film) using helium as carrier gas.

The following methods were used: a) injector temperature 200° C, flow: 0.62 mL/min; temperature program: 80° C, hold for 1.0 min, 15° C/min, to 110° C, 4° C/min, to 130° C, 10° C/min, to 180° C. b) injector temperature 250° C, flow: 0.5 mL/min; temperature program: 40° C, hold for 2.0 min, 20° C/min, to 180° C; c) injector temperature: 250° C, flow: 0.55 mL/min; temperature program: 100° C, hold for 0.5 min, 10° C/min, to 300° C.

Table S3. GC/MS-measurements

Compund	Column	Method	Retention time [min]
1-octanol (**1b**)	HP-5	c)	4.0
1-octyl acetate (**1c**)	HP-5	b)	8.8
1-octyl acetate (**1c**)	Dex-CB	a)	5.5
(*R*)-2-octyl acetate [(*R*)-**2c**]	Dex-CB	a)	4.5
(*S*)-2-octyl acetate [(*S*)-**2c**])	Dex-CB	a)	4.0

Activity assays for Pisa1 and SdsA1

Alkyl sulfate [**1a**, *rac*-, (*R*)-**,** or (*S*)-**2a, 3a-10a**] (5 mg) was dissolved in Tris-HCl (0.98 mL, 100 mM, pH 8.2). An aliquot of Pisa1 enzyme solution (20 µL, 130 µg, 1.8 nmol) was added. The reaction mixture was shaken at 30° C and 120 rpm for 24 h. After extraction with ethyl acetate (1 mL) the organic phase was dried over anhydrous sodium sulfate, derivatized as acetate ester (see below) and analyzed by chiral GC. Biotransformations of alkyl sulfates (**1a**, **2a**, 5 mg) with SdsA1 were conducted with purified SdsA1 (20 µL, 130 µg, 1.8 nmol) as shown for Pisa1 but at pH 7.5. The identity and absolute configuration of samples was confirmed by comparison with reference samples.

Stereoselectivity assays for Pisa1

Step1, enzymatic hydrolysis:

Alkyl sulfate [**2a-10a**] (50 mg) was dissolved in Tris-HCl (9.8 mL, 100 mM, pH 8.2). Purified enzyme solution (200 µL, 1.3 mg, 17.7 nmol) was added. The reaction mixture was shaken at 30° C and 120 rpm for 6 h. Afterwards the aqueous solution was extracted twice with ethyl acetate (3 mL). An aliquot of the organic layer (1 mL) was dried over anhydrous sodium sulfate, derivatized to the corresponding acetate (**2c-10c**) as described below and analyzed by chiral GC. The remaining organic phase was discarded. The aqueous phase was lyophilized overnight.

Step 2, acidic hydrolysis:

The lyophilisate, *p*-toluenesulfonic acid monohydrate (350 mg, 1.8 mmol) and 1,4-dioxane (10 µL, 0.12 mmol) were dissolved in methyl *tert*-butyl ether/deionized water (20 mL, 97:3). The reaction was stirred under reflux at 40° C for 2 h. After cooling to room temperature saturated $NaHCO_3$ (5 mL) was added to stop the reaction. After extraction with ethyl acetate the organic phase was dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure, the residual alcohol was redissolved in ethyl acetate (1 mL), derivatized as described below and analyzed by chiral GC.

Derivatisation of alcohols

Acetic anhydride (100 µL) and DMAP (4-dimethylaminopyridine, cat.) were added to the dried organic phase containing the product alcohol (**1b**-**10b**). The reaction mixture was shaken at 30° C and 120 rpm for 18 h. The reaction was quenched with deionized water (300 µL) and the organic phase was dried over anhydrous sodium sulfate. The derivatives were analyzed on a Varian Chirasil Dex CB column (25 m x 0.32 mm x 0.25 µm film).

Determination of enantiomeric excess

Enantiomeric excess of alcohols (**2b-10b**) derivatised as acetates (**2c-10c**) was determined using an Agilent Technologies 7890A GC-FID system equipped with an Agilent Technologies 7683B autosampler and a Varian Chirasil Dex CB column (25 m x 0.32 mm x 0.25 µm film). Injector temperature: 200° C, flow: 2.0 mL/min, temperature program: 80° C, hold for 1.0 min, 15° C/min, to 110° C, 4° C/min, to 130° C, 10° C/min, to 180° C. Blank tests were performed in the absence of enzyme.

Method A: Injector temperature 200° C, flow 2.0 mL/min, temperature program: 80° C, hold for 1.0 min, 15° C/min to 110° C, 4° C/min to 130° C, 10° C/min to 180° C.

Method B: Injector temperature: 200° C, flow 2.0 mL/min, temperature program: 80° C, hold for 1.0 min, 3° C/min to 100° C, 15° C/min to 150° C.

Method C: Injector temperature: 200° C, flow 2.0 mL/min, temperature program: 80° C, hold for 1.0 min, 15° C/min to 140° C, 4° C/min to 160° C, 10° C/min to 180° C.

Table S2. GC-measurements.

Compound	Method	Retention time [min]	
1-octanol (**1b**)	A	6.3	
1-octyl acetate (**1c**)	A	7.0	
2-octyl acetate (**2c**)	A	5.3 (*S*)	5.9 (*R*)
2-heptyl acetate (**3c**)	B	5.8 (*S*)	7.1 (*R*)
2-nonyl acetate (**4c**)	A	7.1 (*S*)	7.8 (*R*)
3-octyl acetate (**5c**)	A	5.1 (*S*)	5.9 (*R*)
4-octyl acetate (**6c**)	A	4.6 (*S*)	4.8 (*R*)
6-methylhept-5-en-2-yl acetate (**7c**)	A	5.3 (*S*)	5.7 (*R*)
1-phenylprop-2-yl acetate (**8c**)	A	8.7 (*S*)	9.1 (*R*)
4-phenylbut-2-yl acetate (**9c**)	C	8.4 (*S*)	8.7 (*R*)
cyclohexylethyl acetate (**10c**)	A	6.9 (*S*)	7.5 (*R*)
1-heptyn-3-yl acetate (**11c**)	B	6.3 (*R*)*	7.3 (*S*)*
1-octyn-3-yl acetate (**12c**)	C	5.5 (*R*)*	5.9 (*S*)*

* Switch in CIP priority.

Determination of absolute configuration

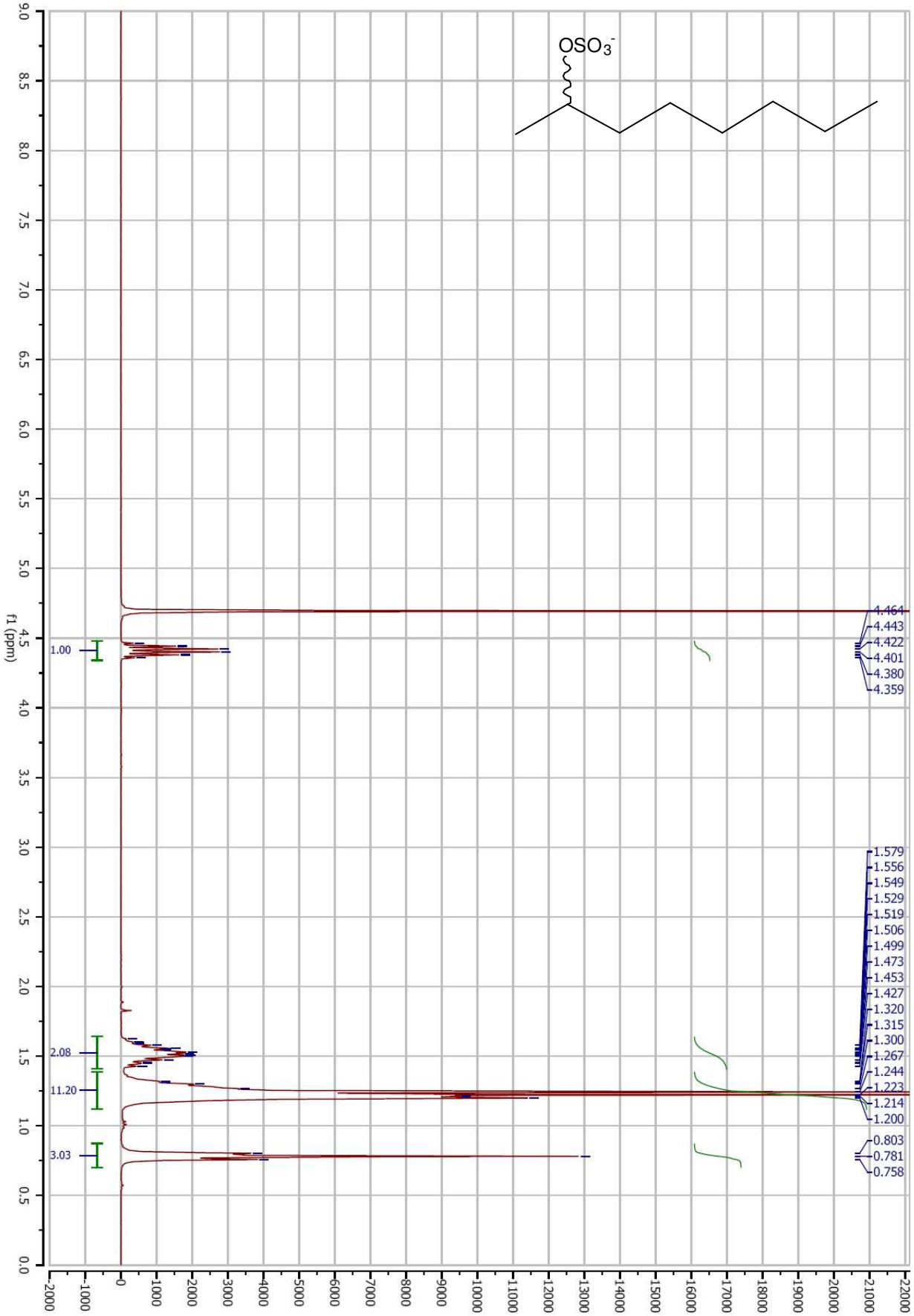
Absolute configuration was proven by comparison of the GC retention times with commercial reference samples of enantiopure alcohols (*R*)- and (*S*)-**2b**, (*R*)-**3b**, (*R*)-**4b**, (*S*)-**5b**, (*R*)- and (*S*)-**7b**, (*S*)-**8b,** (*S*)-**9b**, (*R*)- and (*S*)-**12b** after derivatisation. The absolute configuration of **10b** was determined to be (*S*) by comparison of GC retention times with a sample prepared according to literature.[3] A sample of (*S*)-**11c** was prepared according to literature.[4] Compound **6b** was determined to be (*S*) by optical rotation: $[\alpha]_D^{22}$ = +0.5° (c = 2, $CHCl_3$); ref.[5]:(*R*)-**6b** $[\alpha]_D^{22}$ = -0.65° (neat); ref.[6]: (*S*)-**6b** $[\alpha]_D^{25}$ = +0.75° (neat).

Preparative scale deracemisation

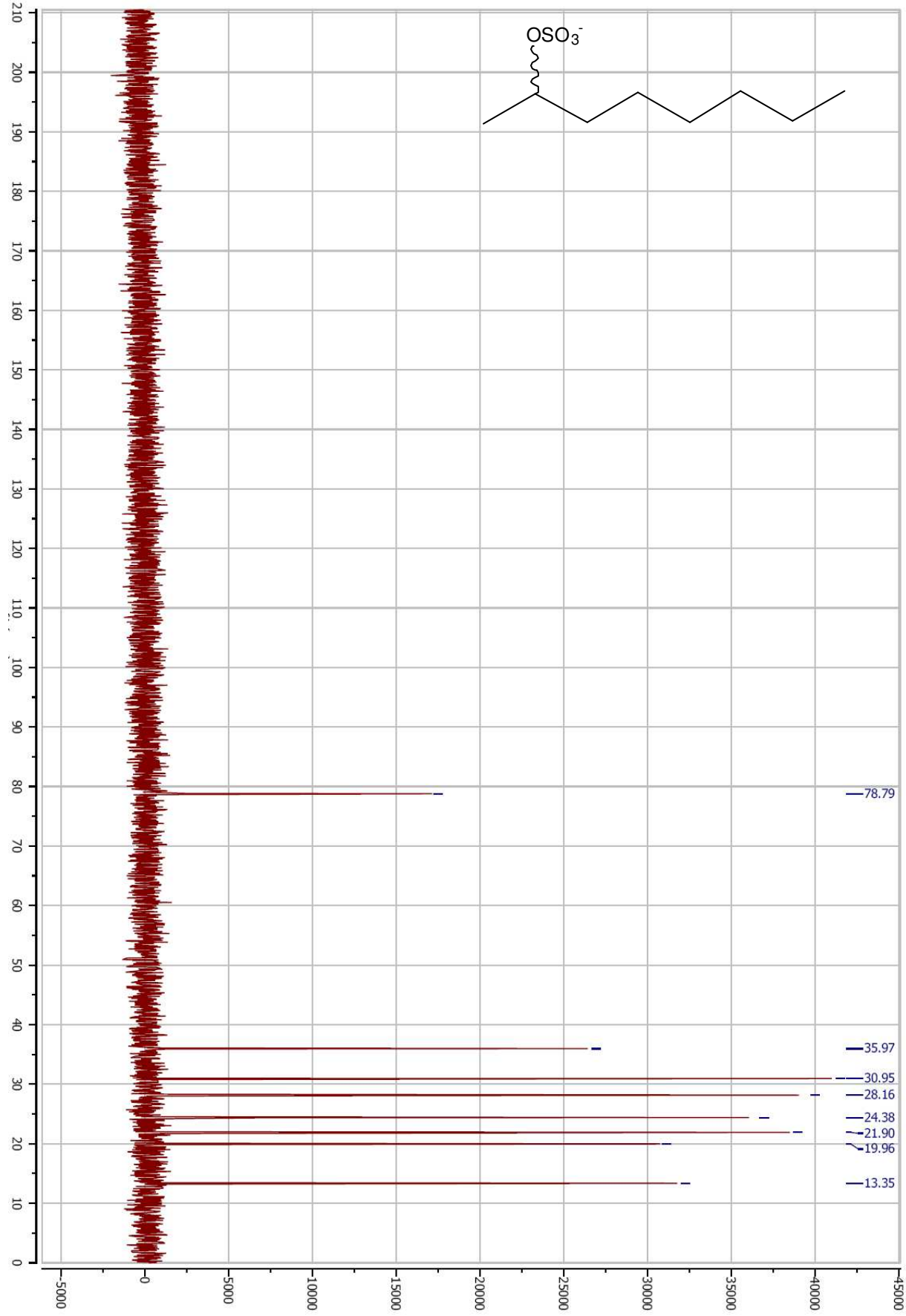
2-Octyl sulfate (*rac*-**2a**, 1 g, 4.3 mmol) was dissolved in Tris-HCl (99 mL, 100 mM, pH 8.2). Purified Pisa1 (1 mL, 6.5 mg, 88.2 nmol) was added. The reaction mixture was shaken at 30° C and 120 rpm for 24 h. The aqueous solution was then extracted with *t*BuOMe (3 x 100 mL). The combined organic phases were extracted with deionized water (100 mL) and saturated sodium chloride solution (100 mL). The organic layer was then dried with anhydrous sodium sulfate and filtered through a sintered glass filter. The solvent was evaporated under reduced pressure (250 mbar, RT). The product obtained was a clear yellow oil (248 mg, 1.9 mmol). Its purity was confirmed by NMR and GC/MS analysis. The aqueous phase was lyophilized

overnight. The lyophilisate, *p*-toluenesulfonic acid monohydrate (1.75 g, 9.2 mmol) and 1,4-dioxane (100 µL, 1.2 mmol) were dissolved in MTBE /deionized water (200 mL, 97:3). The reaction was stirred under reflux at 40° C for 5 h. After cooling to room temperature saturated sodium hydrogen carbonate (50 mL) was added to stop the reaction. The aqueous phase was extracted with MTBE (2x 50 mL). The combined organic phases were extracted with deionized water (100 mL) and saturated sodium chloride solution (100 mL) and dried with anhydrous sodium sulfate. After filtering through a sintered glass filter, the solvent was evaporated under reduced pressure (250 mbar, RT). The product was obtained as a clear yellow oil (243 mg, 1.9 mmol). (*S*)-**2b** was obtained in a total isolated yield of 87% (491 mg, 3.8 mmol) with an ee of >99%; $[\alpha]_D^{20}$ = +7.21, ($CHCl_3$, c = 2), lit.[7] (*S*)-**2b** $[\alpha]_D^{20}$ = 9.12. The purity of the product was proven by ^{1}H- and ^{13}C-NMR: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.86-3.75 (m, 1H), 1.55-1.17 (m, 13H), 0.93-0.86 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 68.2, 39.4, 31.8, 29.3, 25.7, 23.5, 22.6, 14.1.

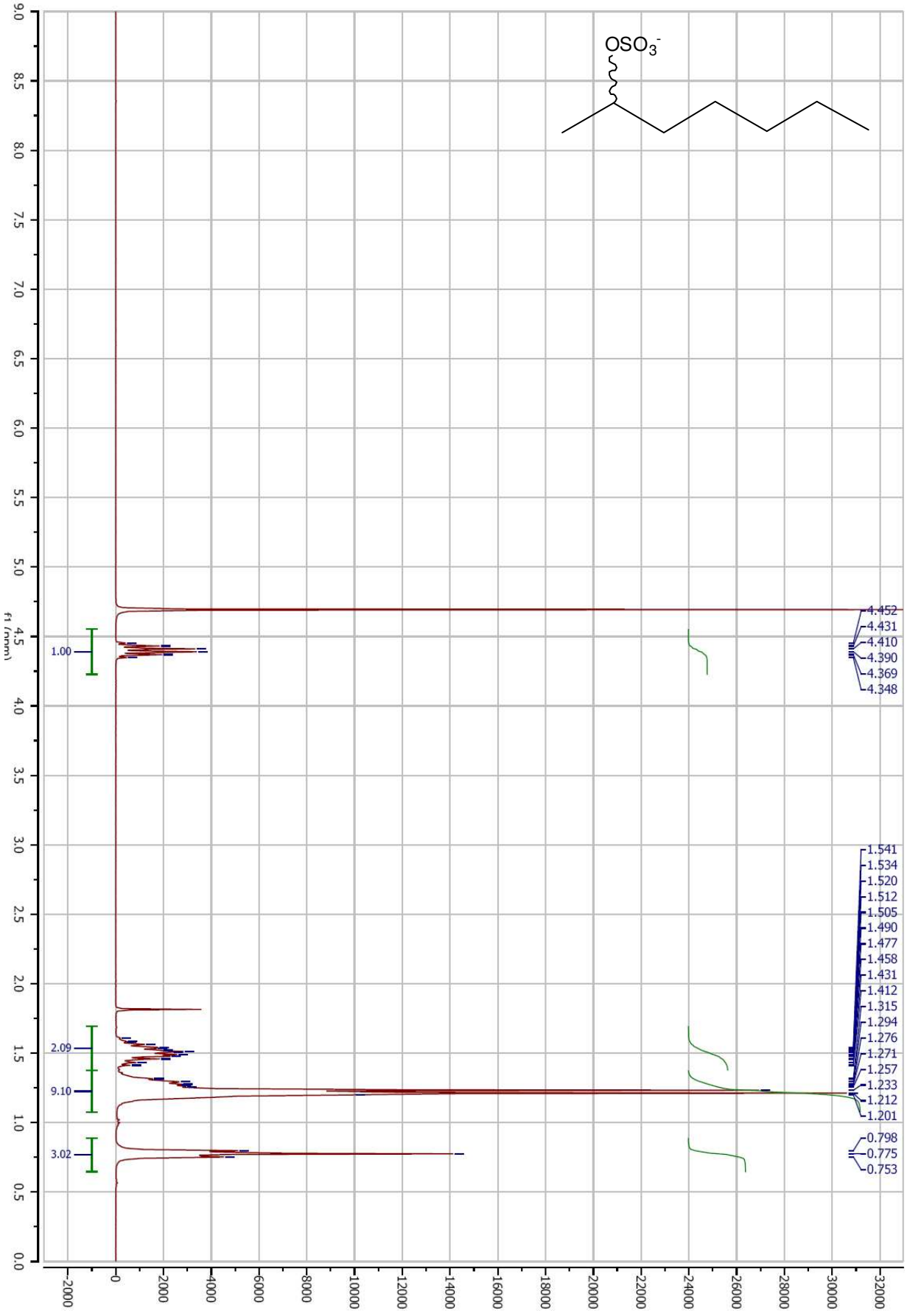
^{1}H-NMR (**2a**) *rac*-2-Octyl sulfate; D_2O



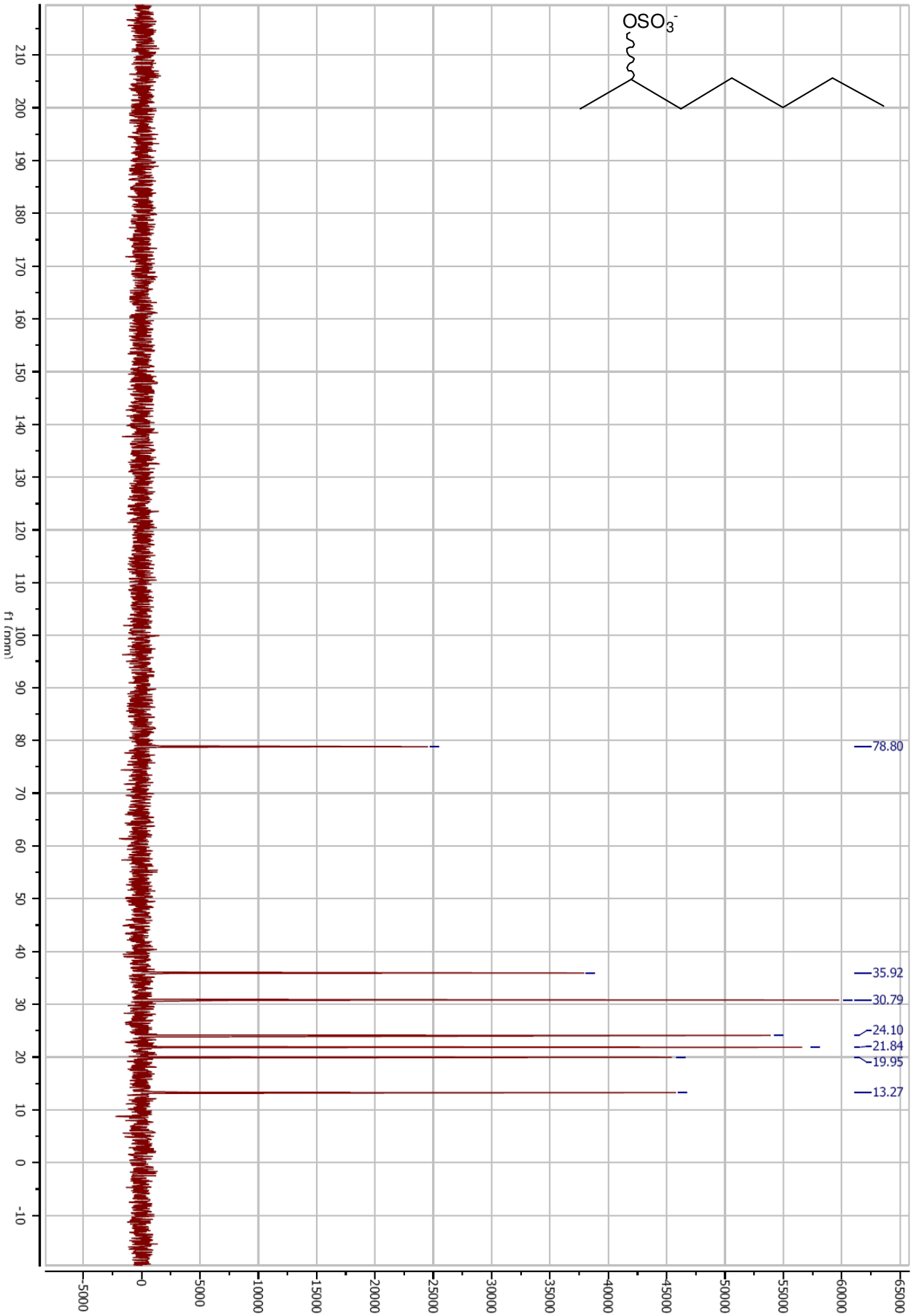
^{13}C-NMR (**2a**) *rac*-2-Octyl sulfate; D_2O



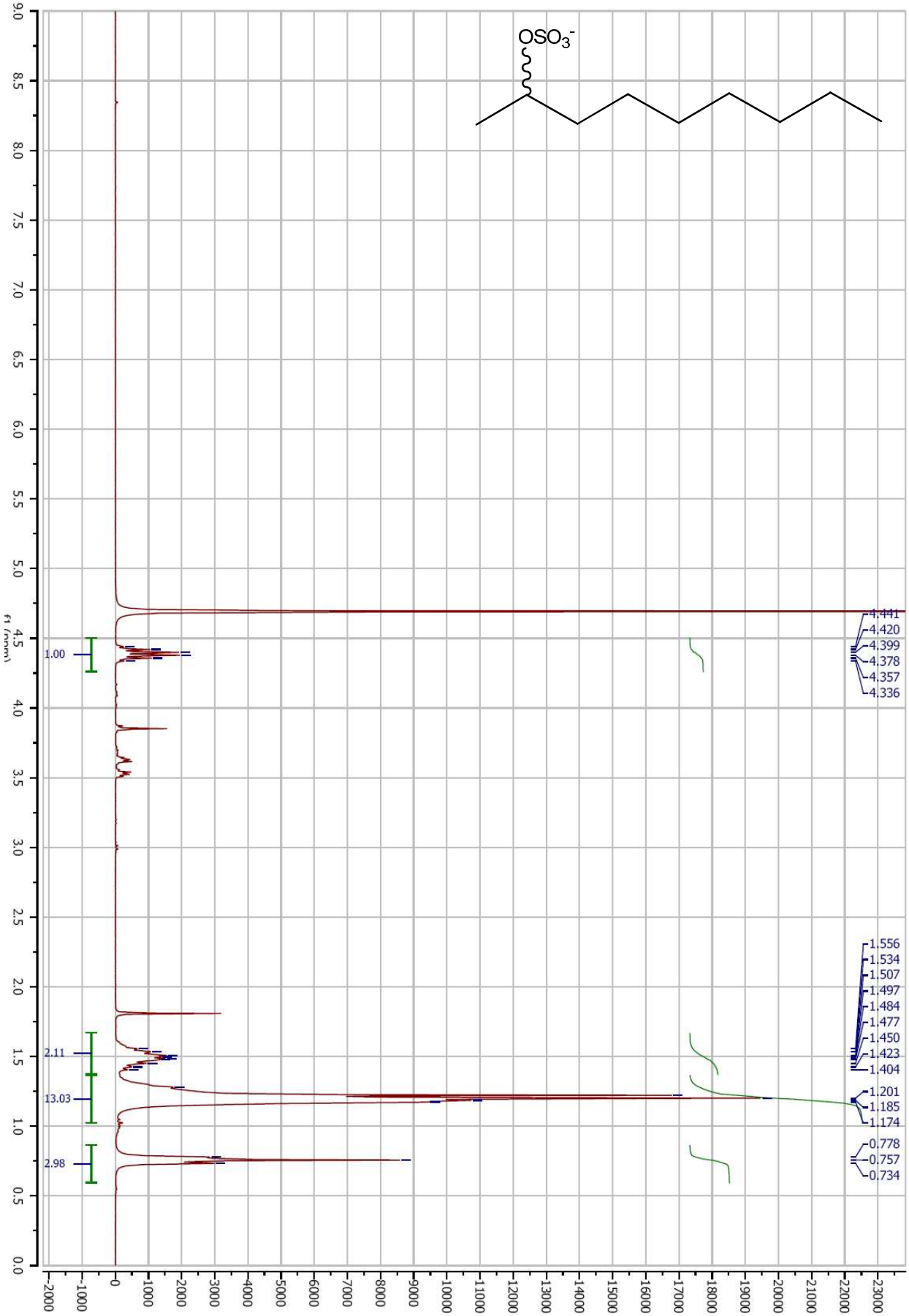
^{1}H-NMR (**3a**) *rac*-2-Heptyl sulfate; D_2O



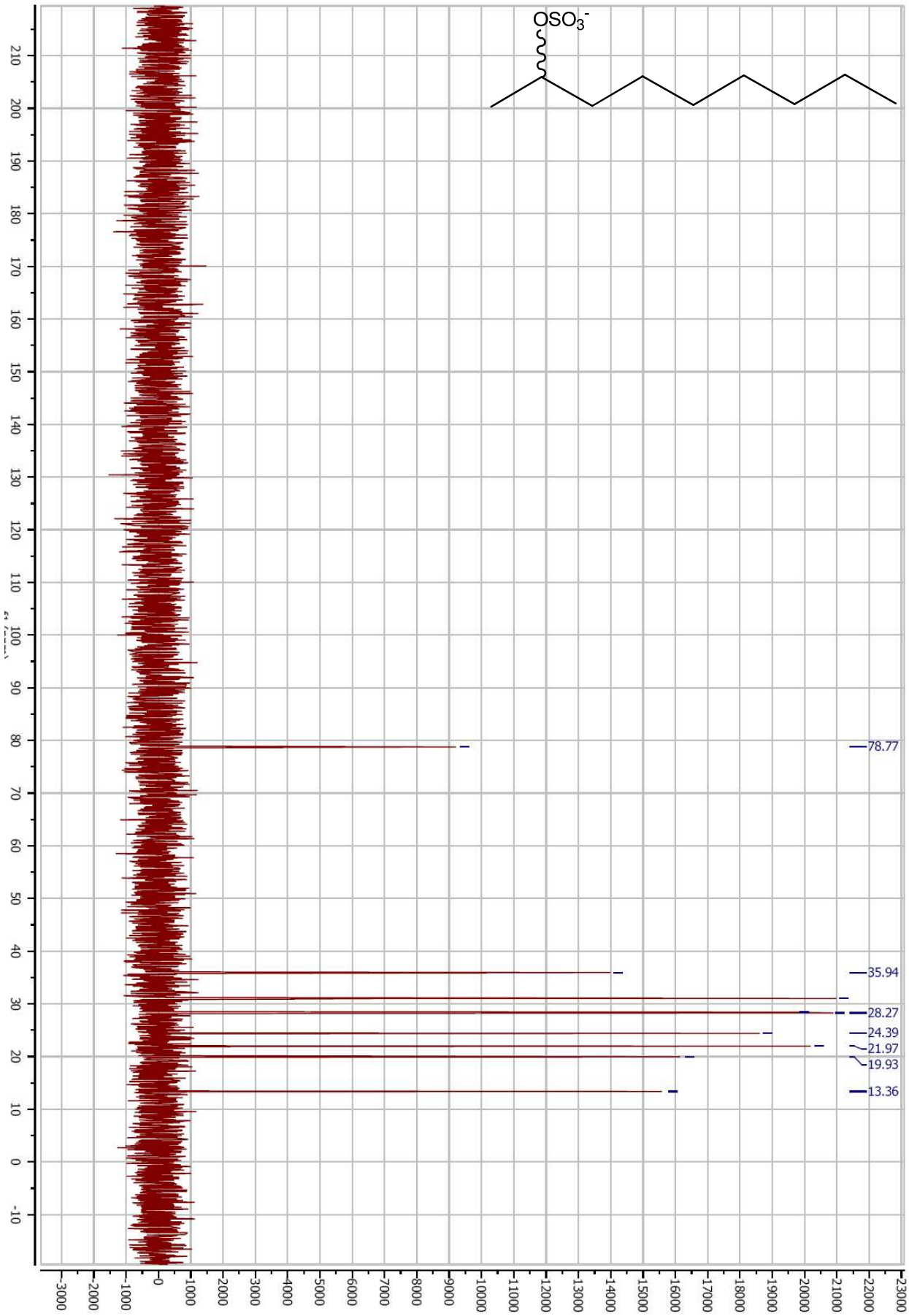
^{13}C-NMR (**3a**) *rac*-2-Heptyl sulfate; D_2O



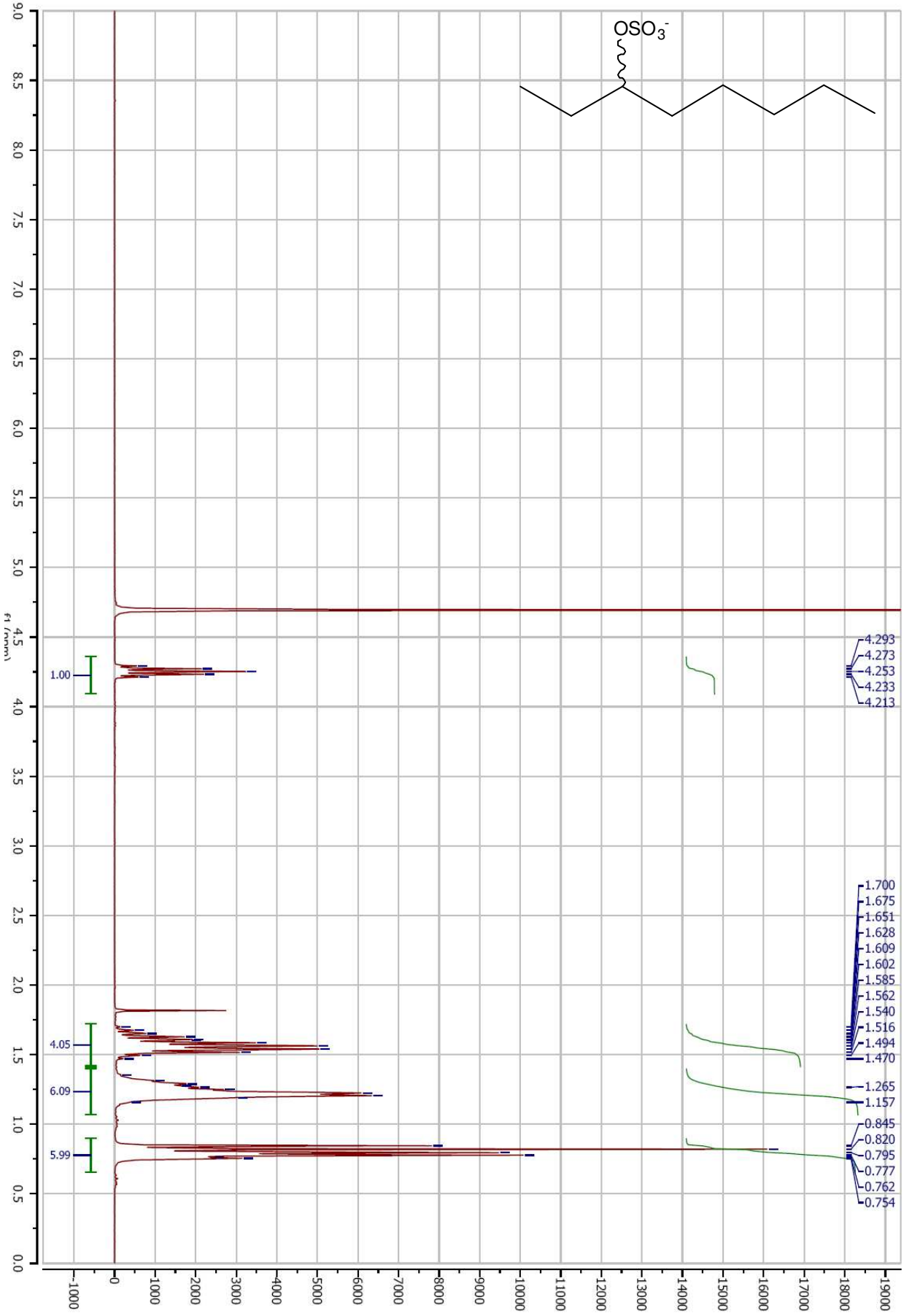
^{1}H-NMR (**4a**) *rac*-2-Nonyl sulfate; D_2O



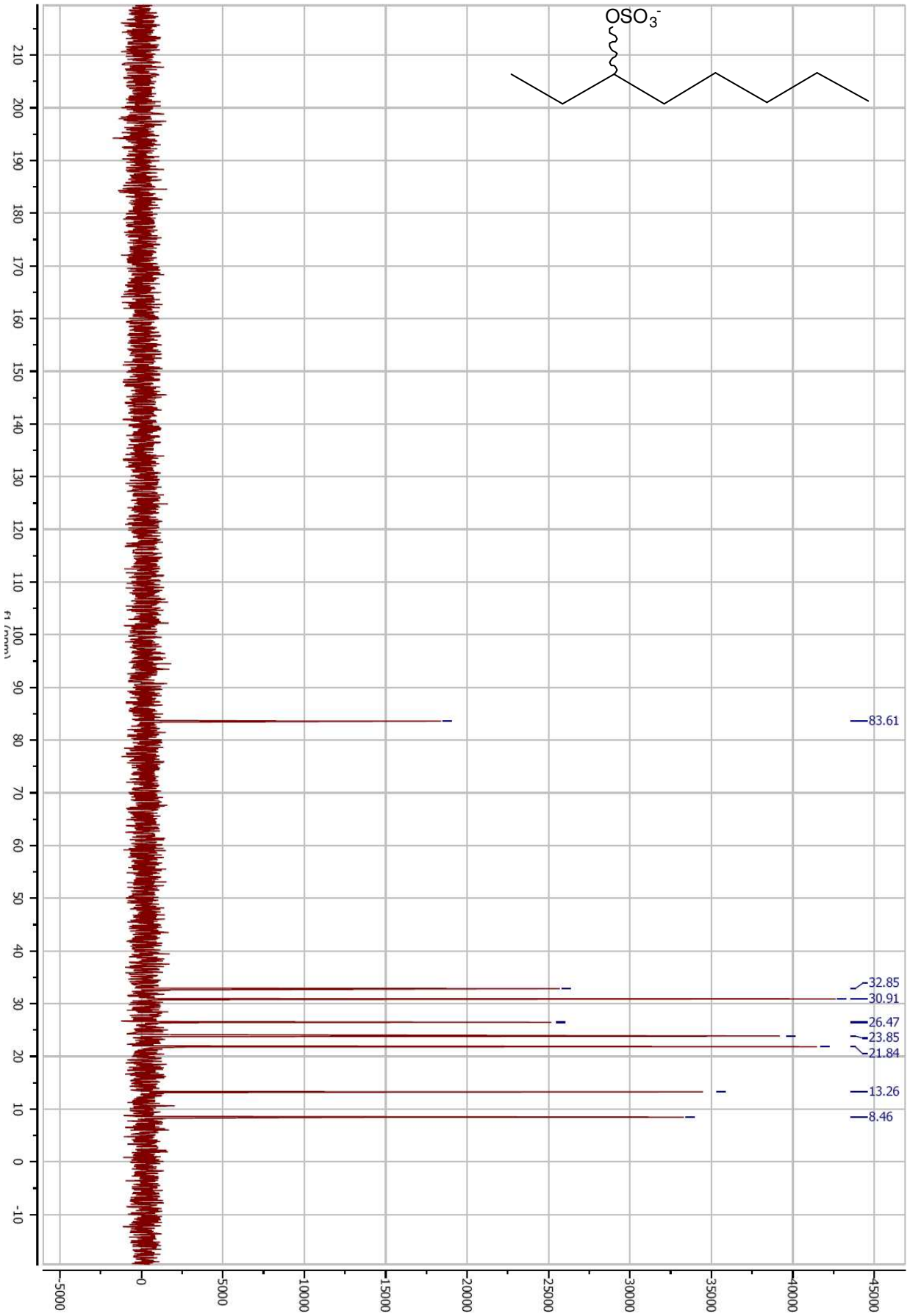
^{13}C-NMR (**4a**) *rac*-2-Nonyl sulfate; D_2O



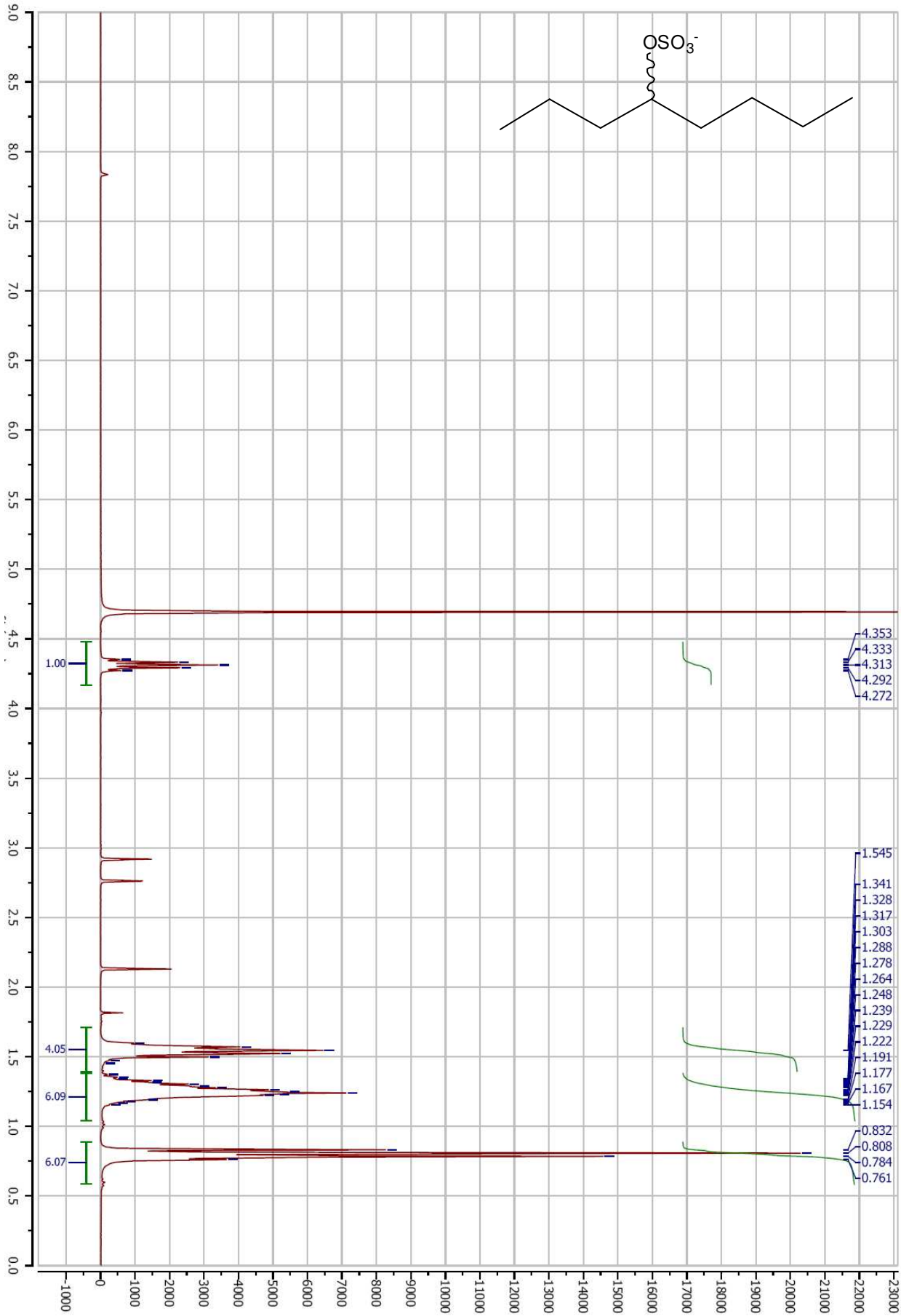
^{1}H-NMR (**5a**) *rac*-3-Octyl sulfate; D_2O



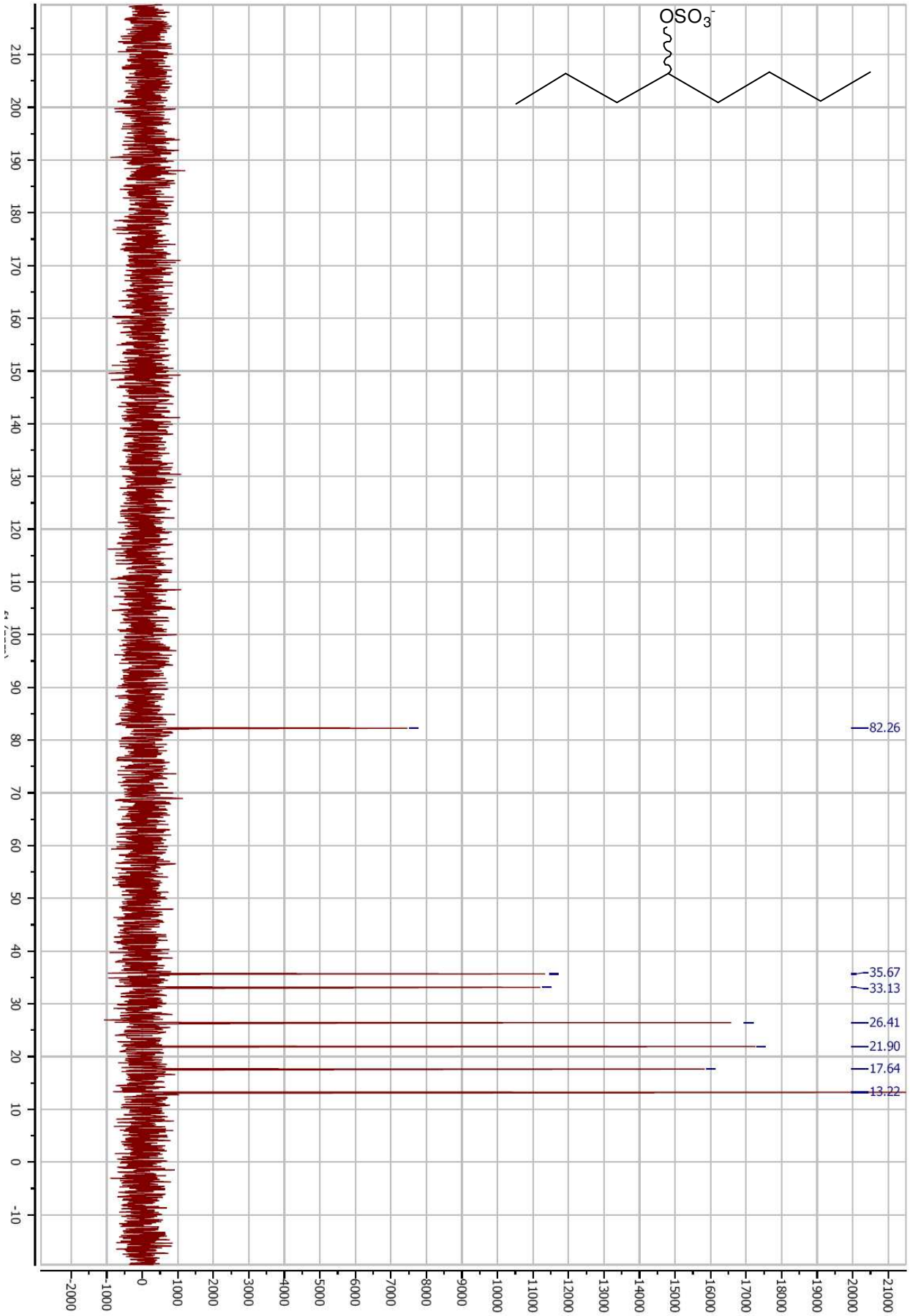
^{13}C-NMR (**5a**) *rac*-3-Octyl sulfate; D_2O



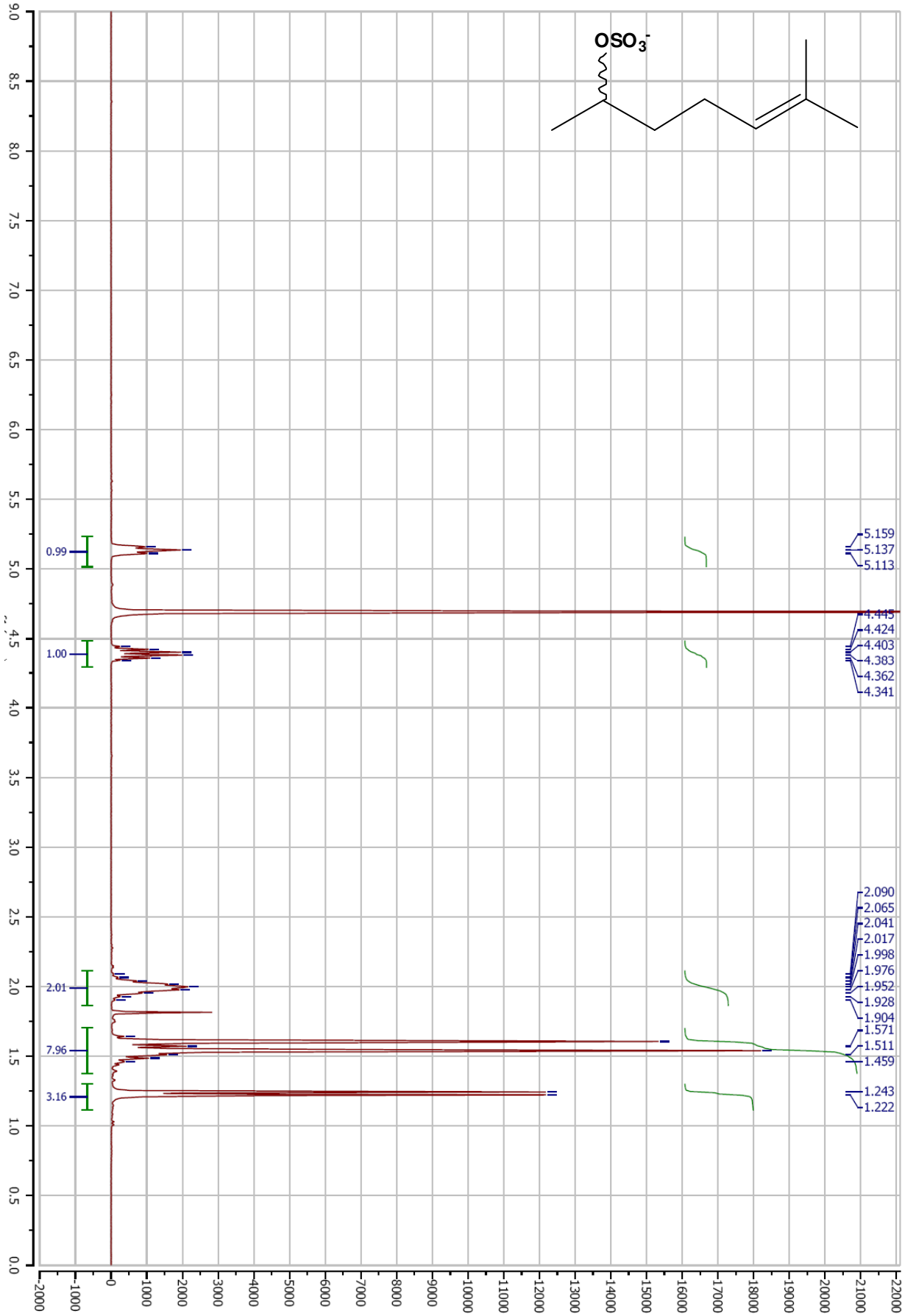
^{1}H-NMR (**6a**) *rac*-4-Octyl sulfate; D_2O



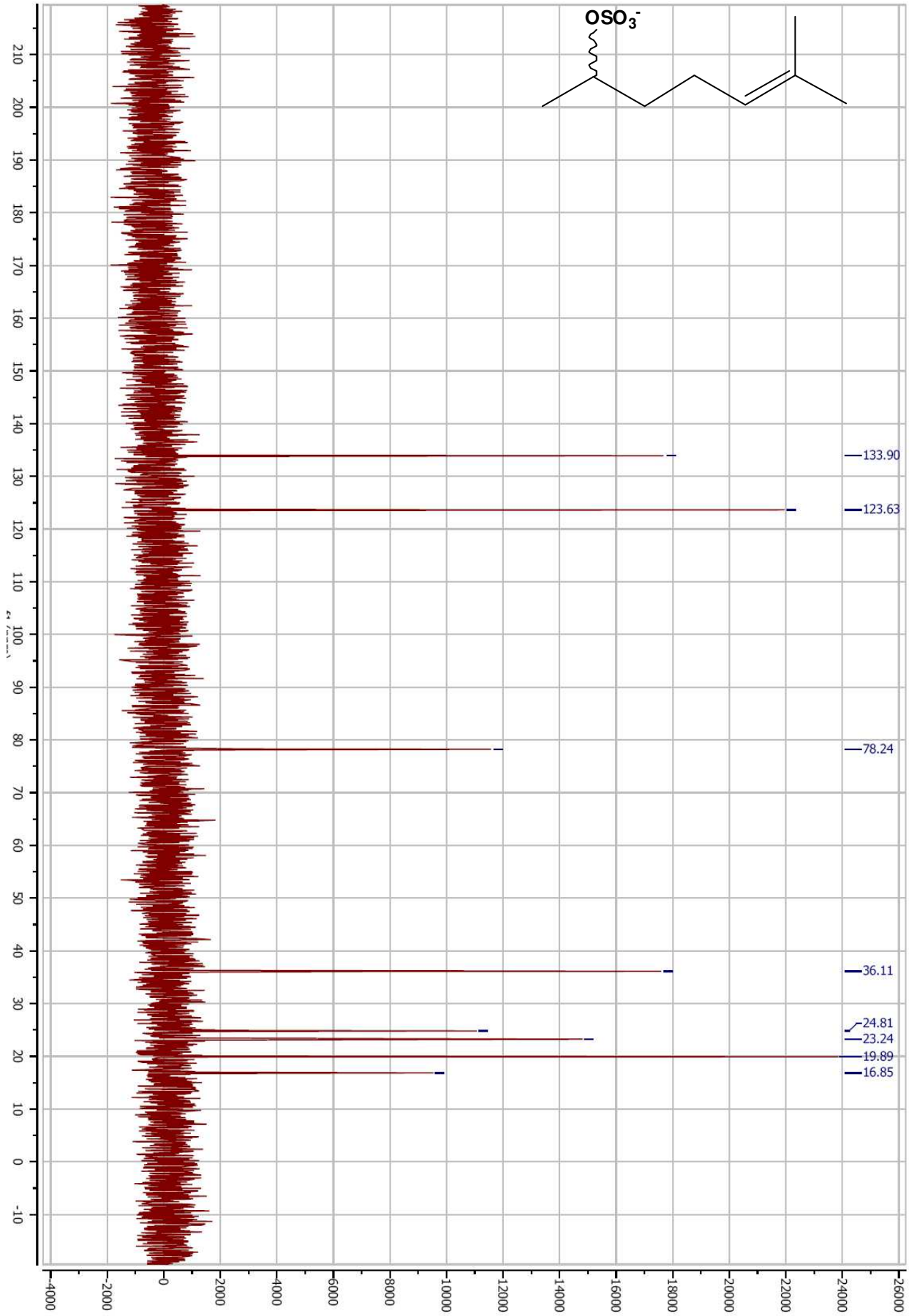
^{13}C-NMR (**6a**) *rac*-4-Octyl sulfate; D_2O



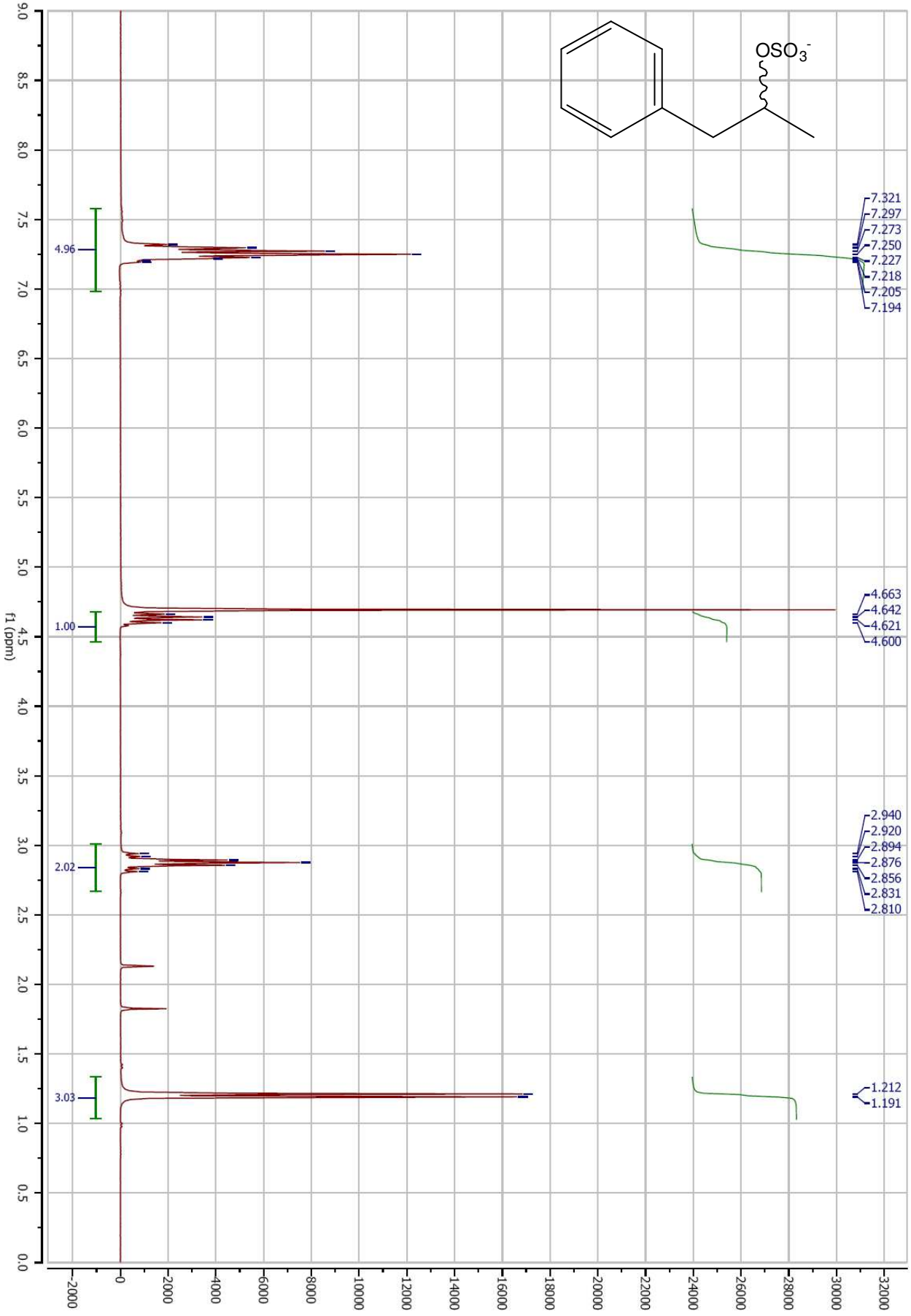
^{1}H-NMR (**7a**) *rac*-6-Methyl-5-hepten-2-yl sulfate; D_2O



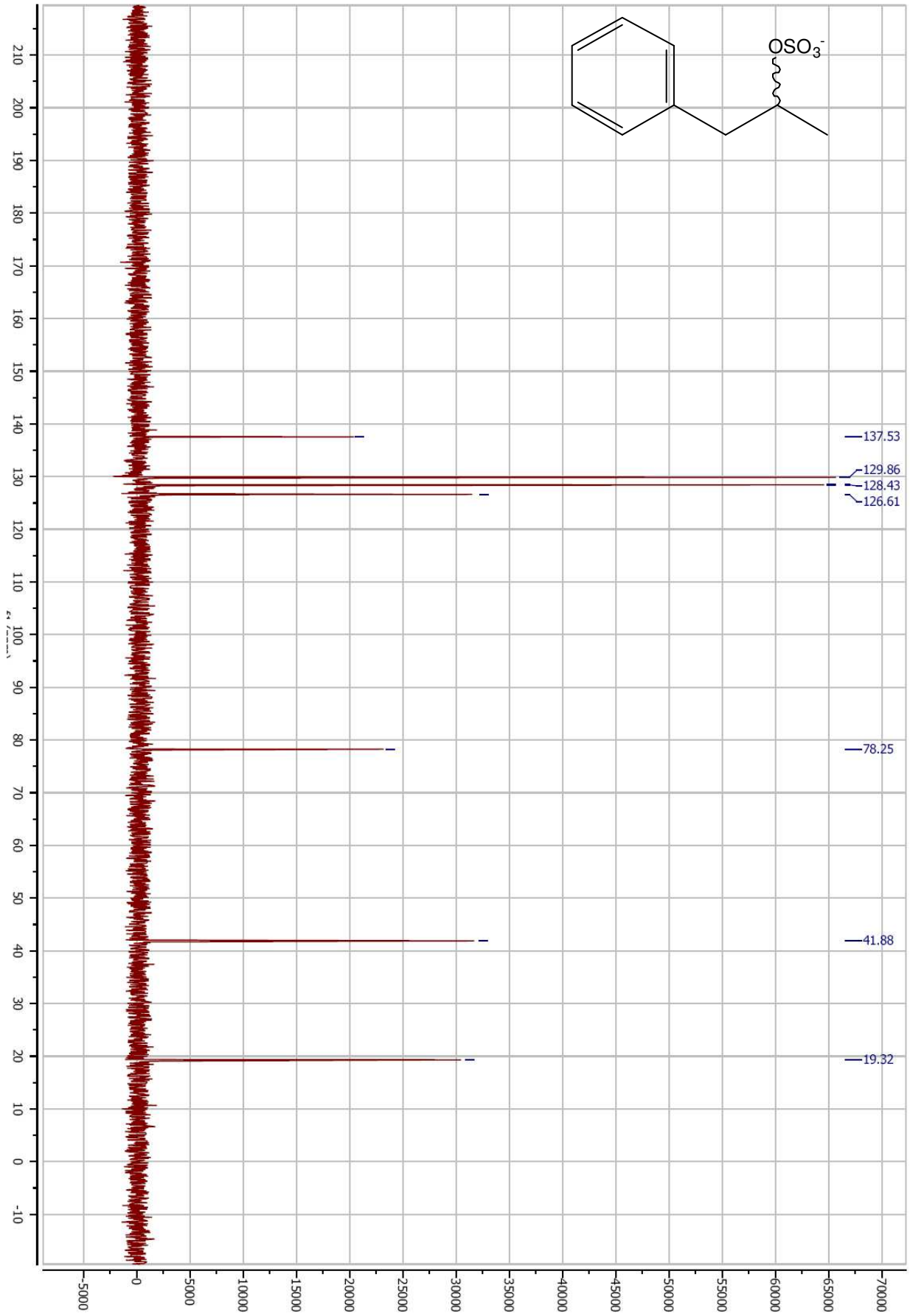
^{13}C-NMR (**7a**) *rac*-6-Methyl-5-hepten-2-yl sulfate; D_2O



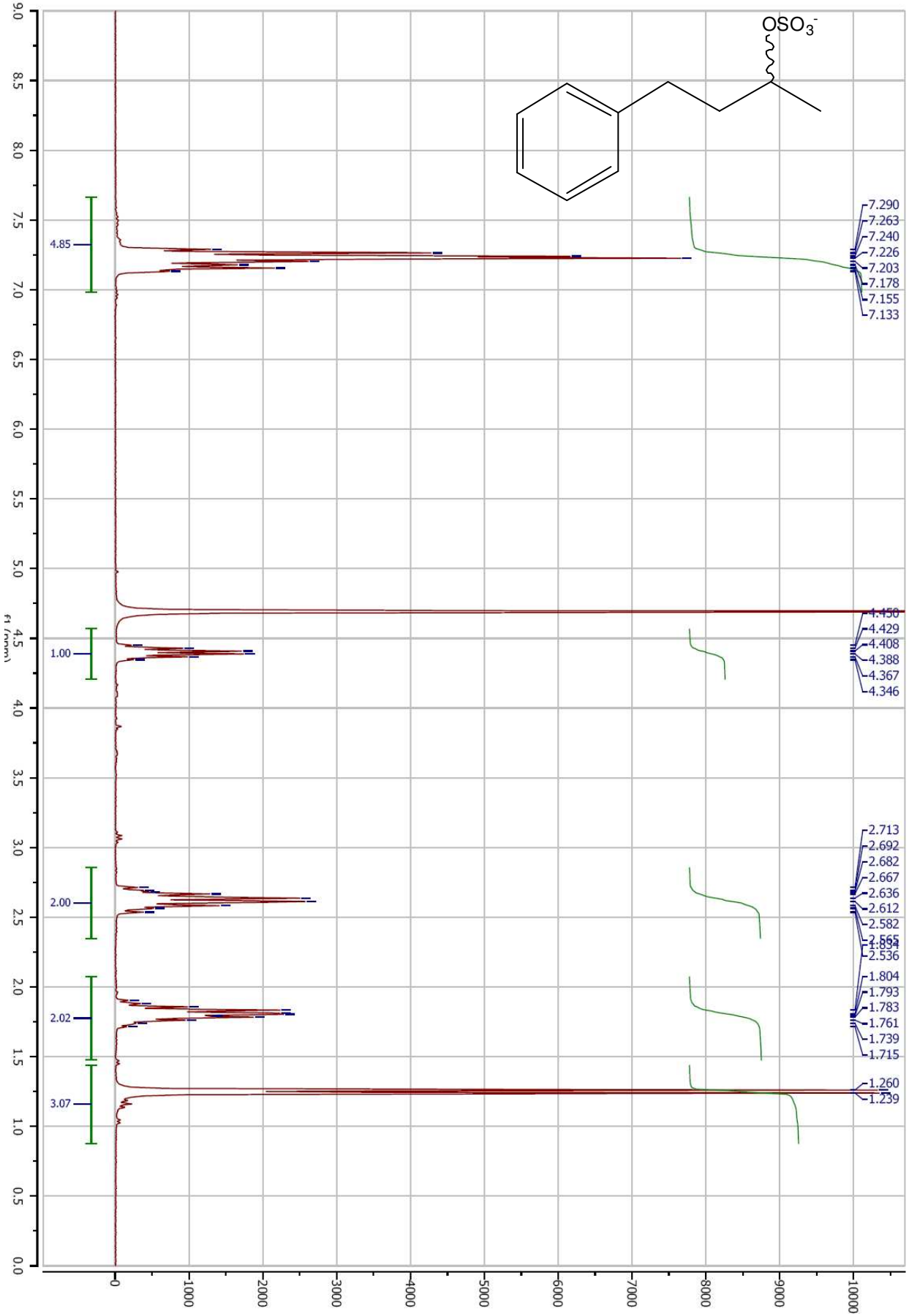
^{1}H-NMR (**8a**) *rac*-1-Phenylprop-2-yl sulfate; D_2O



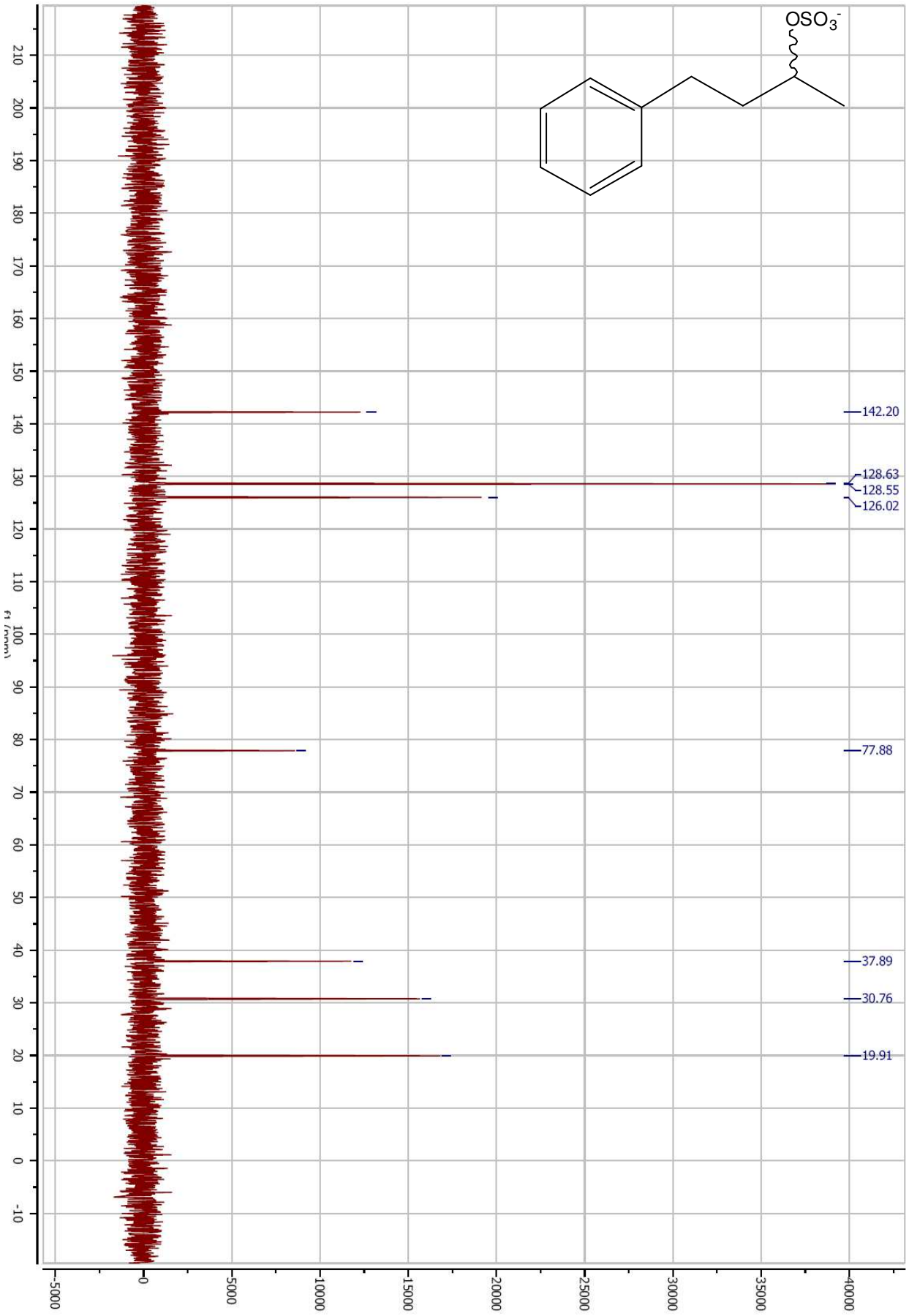
^{13}C-NMR (**8a**) *rac*-1-Phenylprop-2-yl sulfate; D_2O



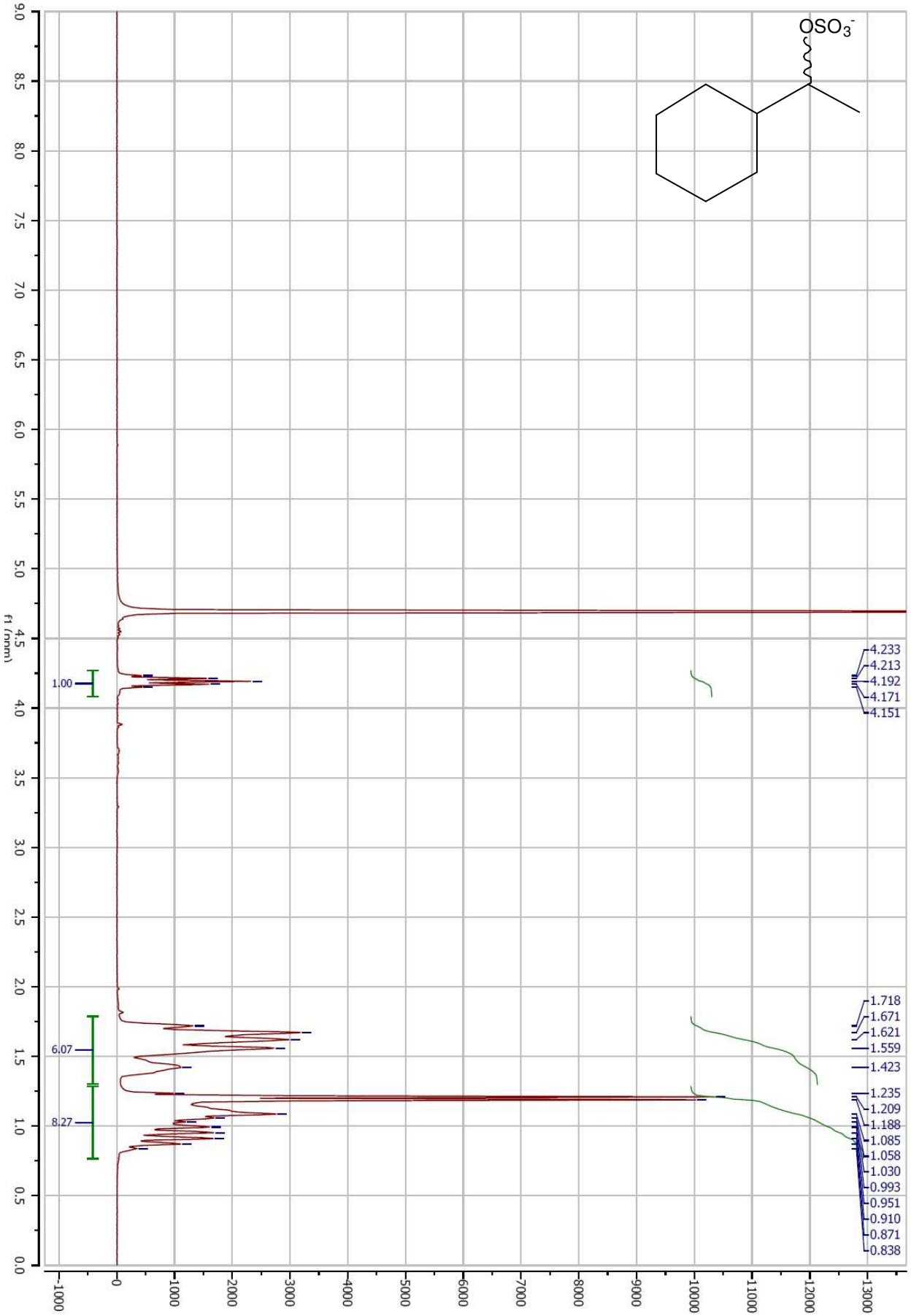
^{1}H-NMR (**9a**) *rac*-4-Phenylbut-2-yl sulfate; D_2O



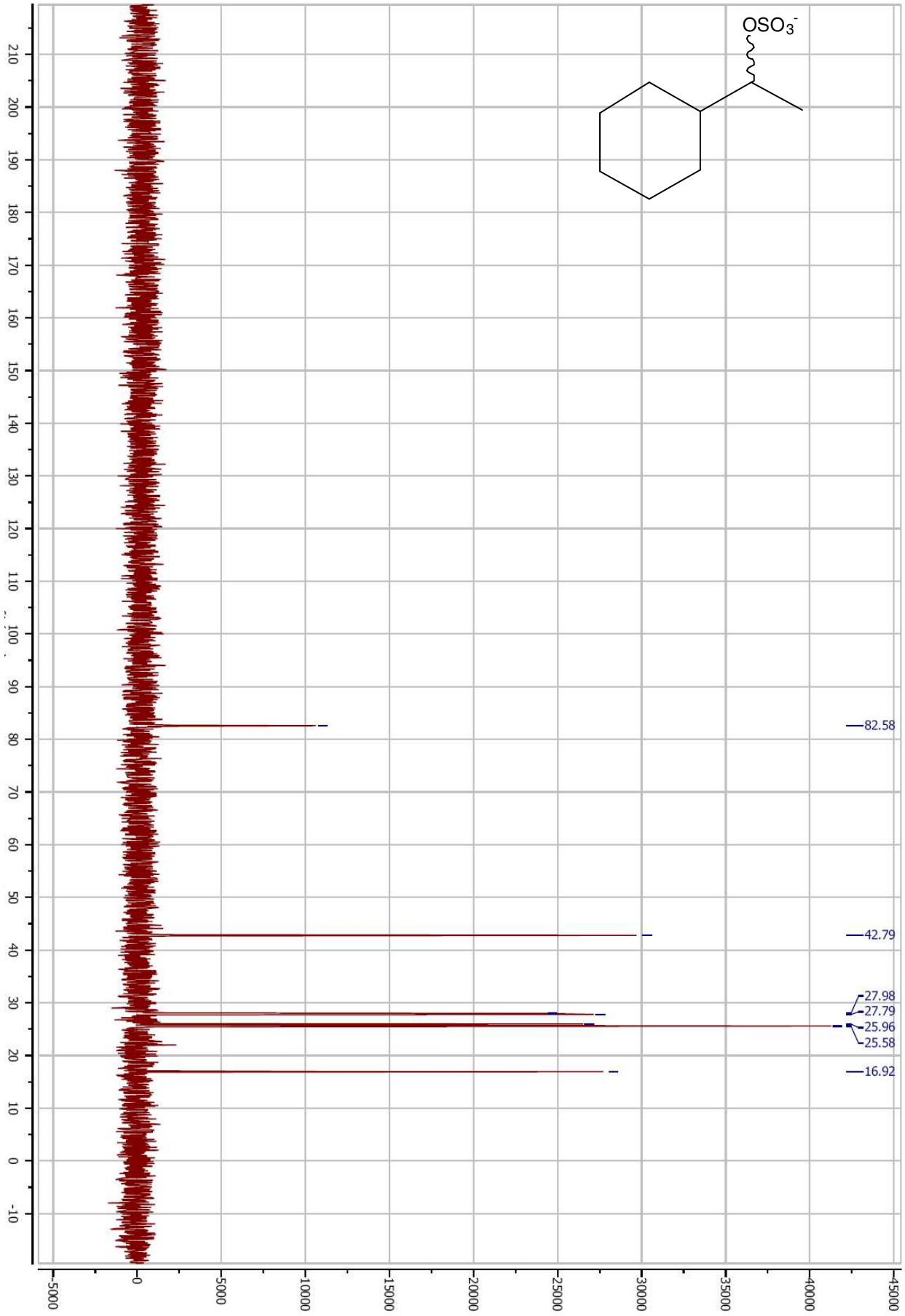
^{13}C-NMR (**9a**) *rac*-4-Phenylbut-2-yl sulfate; D_2O



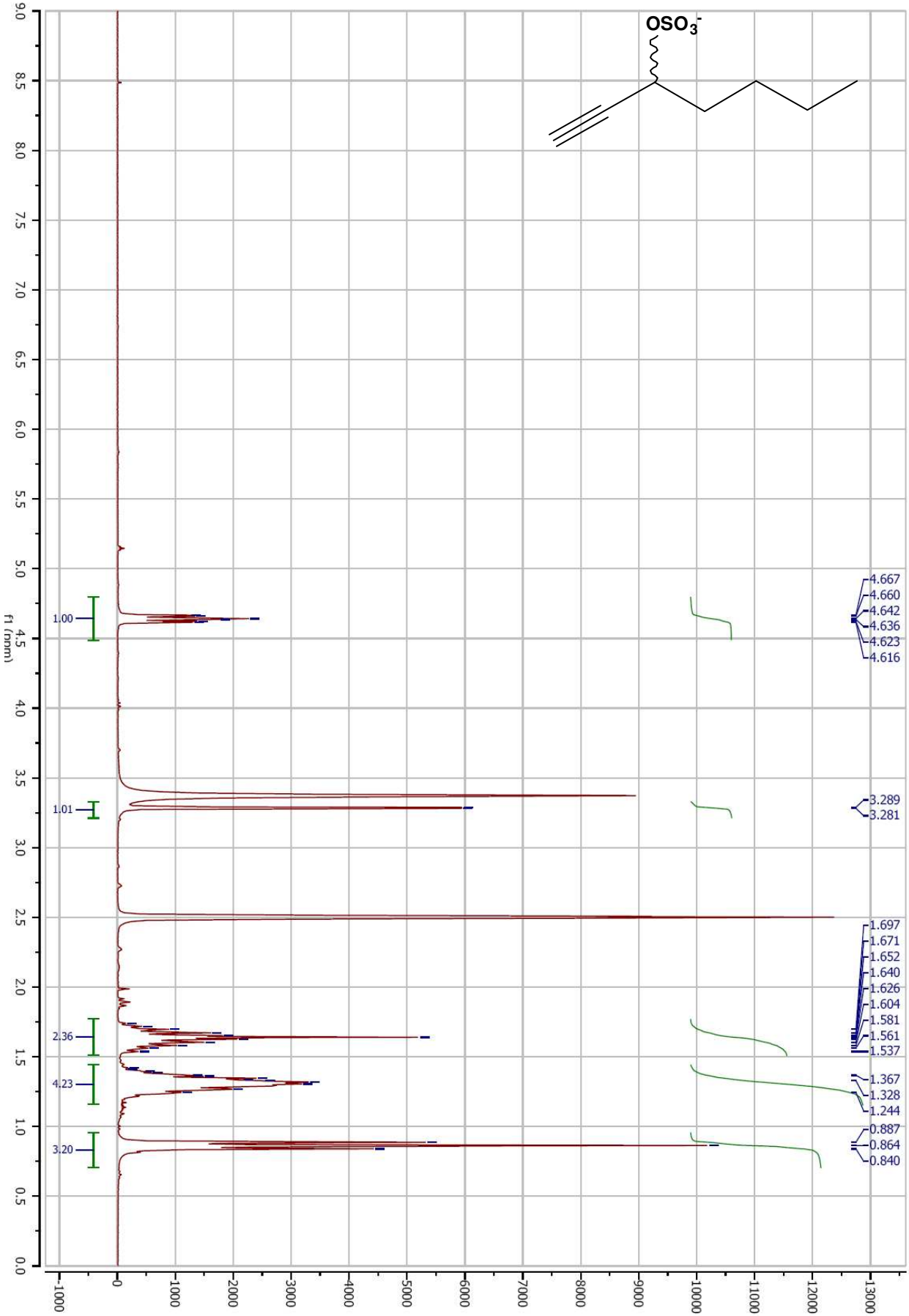
^{1}H-NMR (**10a**) *rac*-1-Cyclohexylethyl sulfate; D_2O



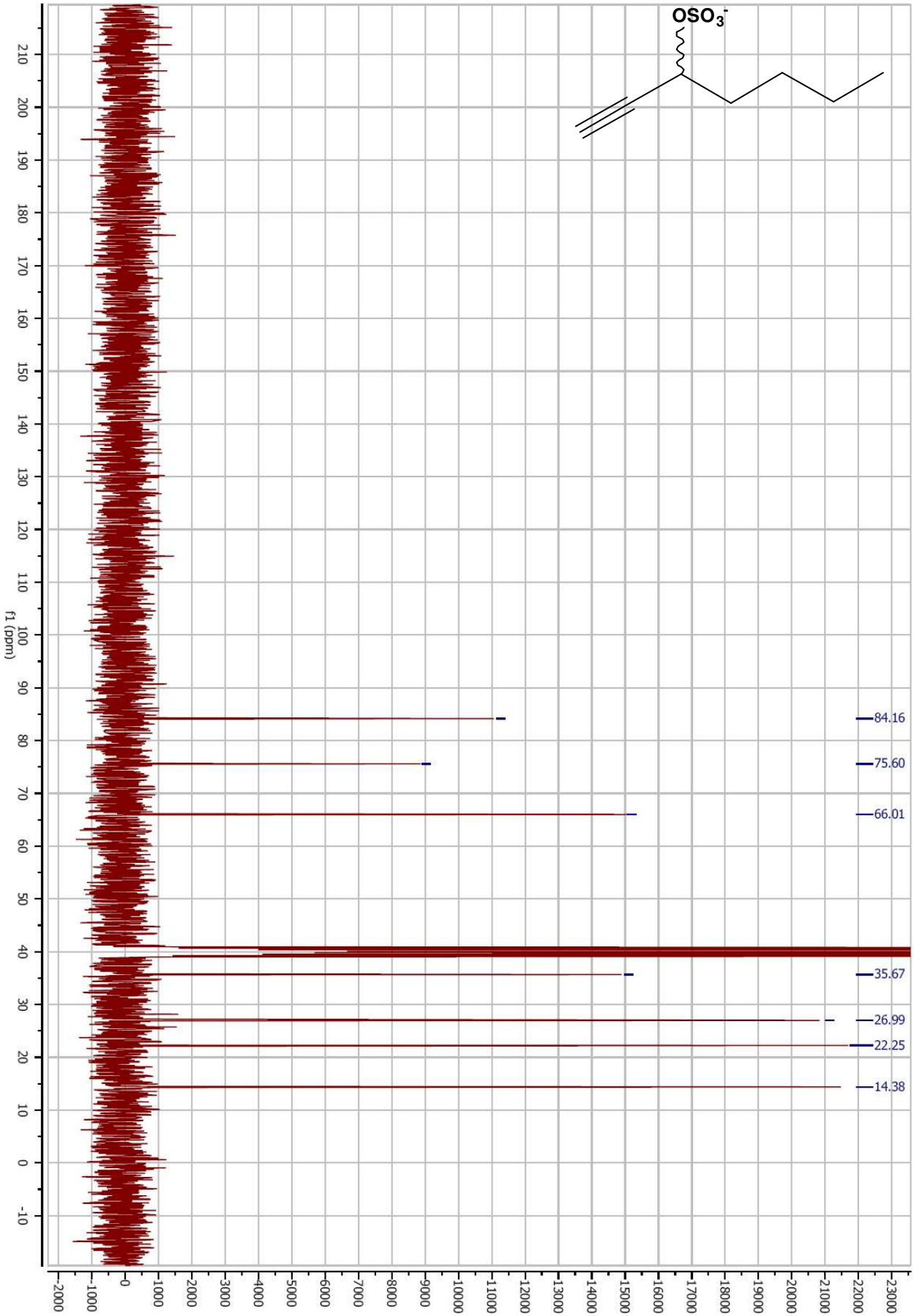
^{13}C-NMR (**10a**) *rac*-1-Cyclohexylethyl sulfate; D_2O



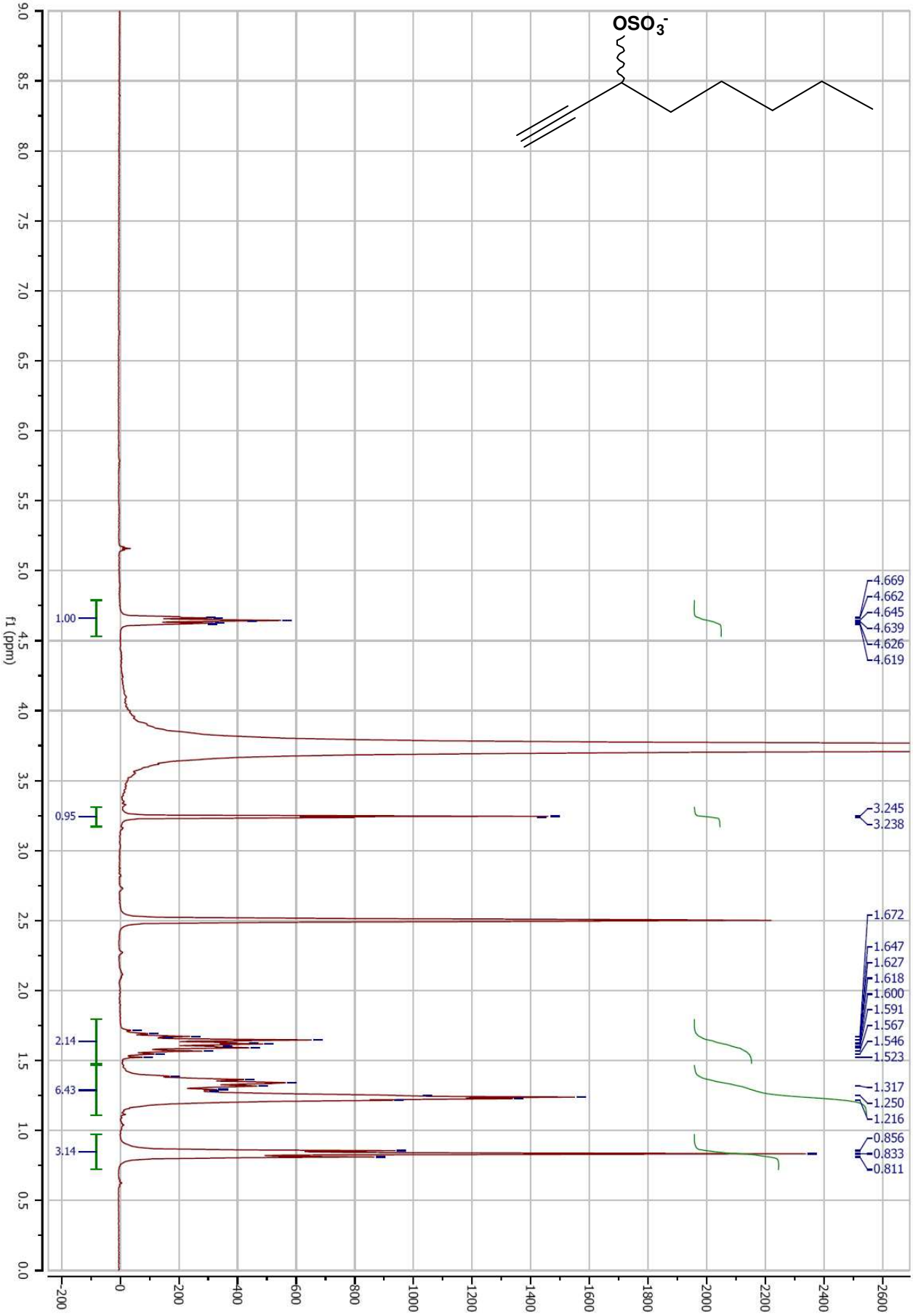
^{1}H-NMR (**11a**) *rac*-1-Heptyn-3-yl sulfate; DMSO-d_6



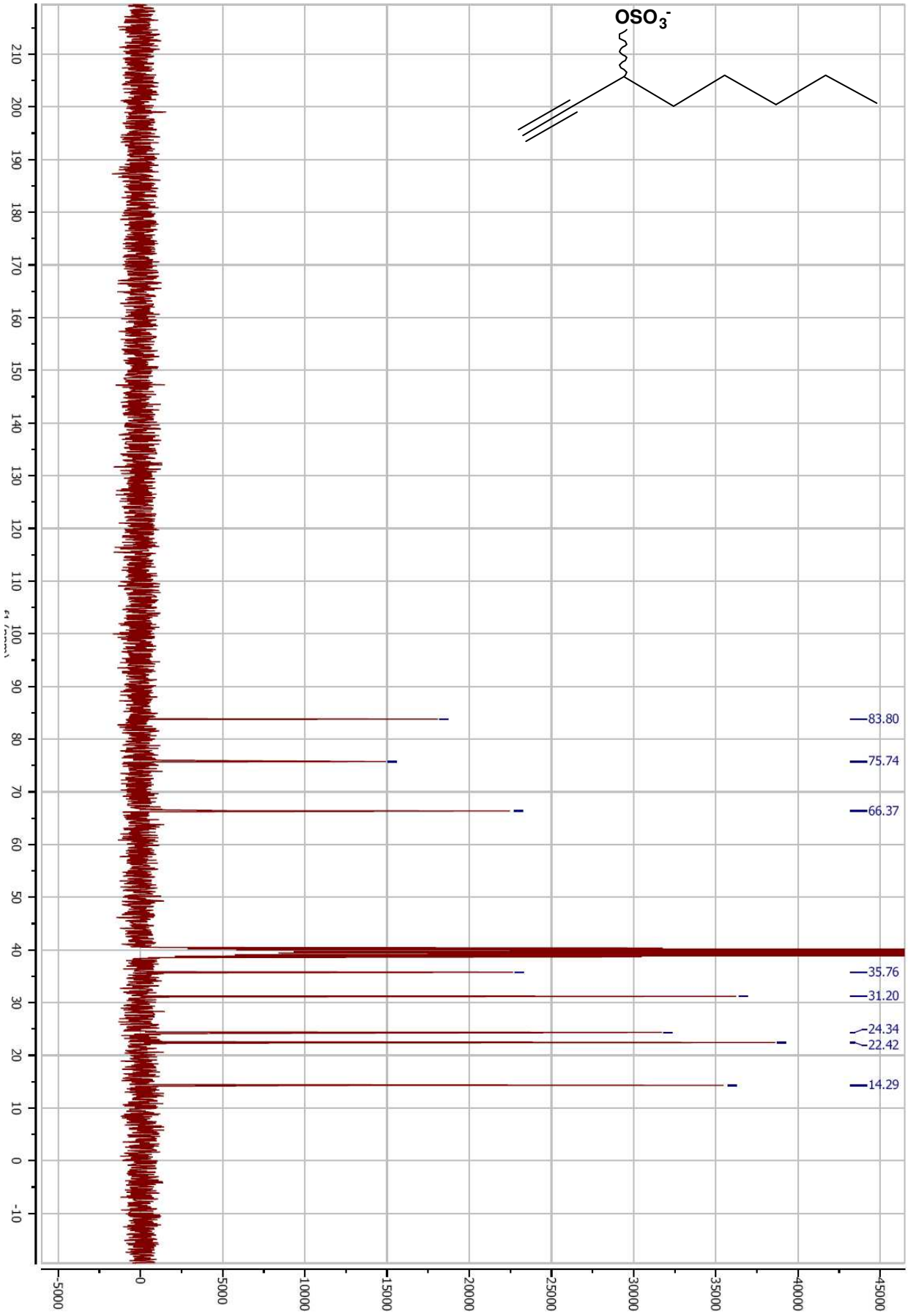
^{13}C-NMR (**11a**) *rac*-1-Heptyn-3-yl sulfate; DMSO-d_6



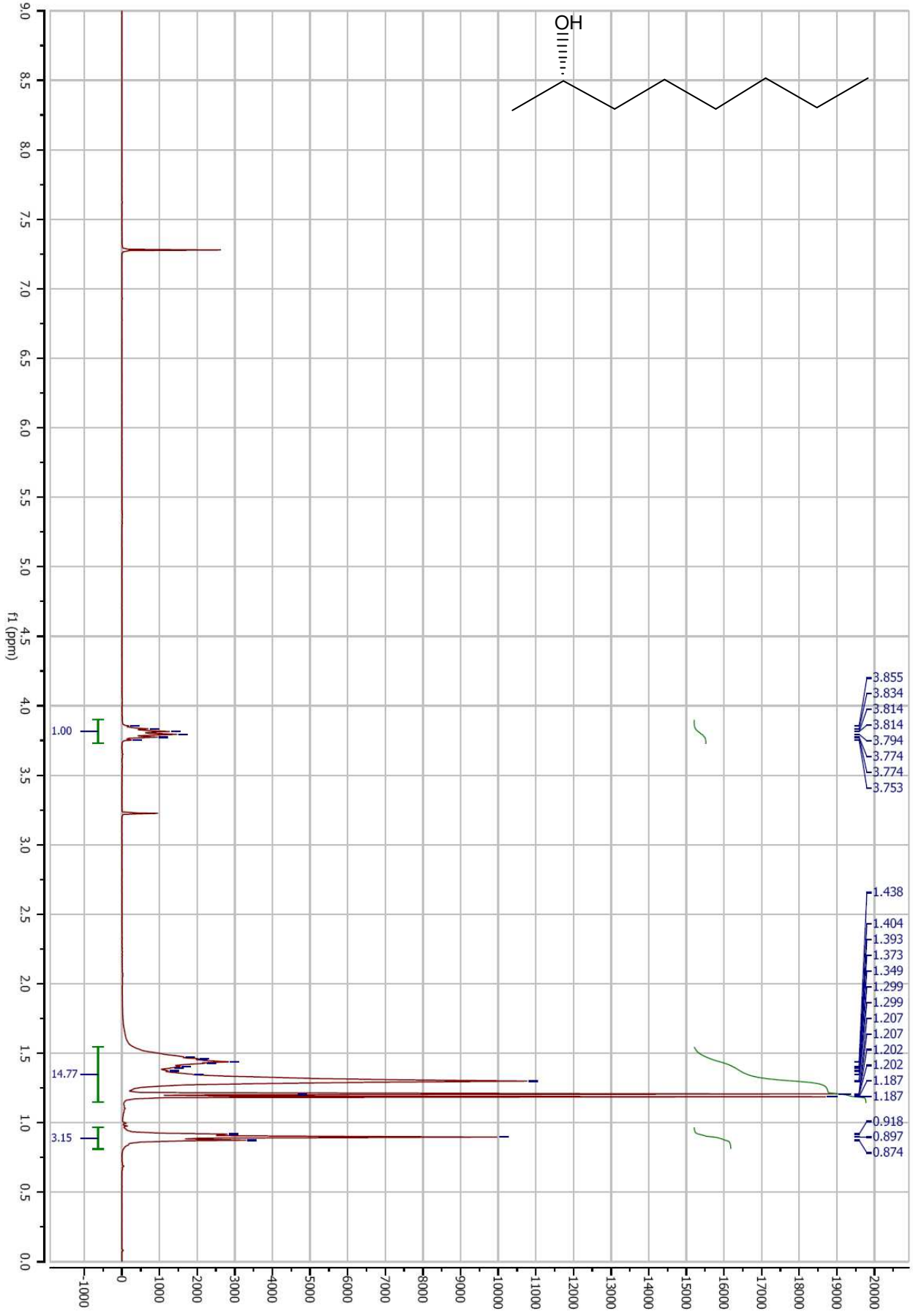
[1]H-NMR (**12a**) *rac*-1-Octyn-3-yl sulfate; DMSO-d_6



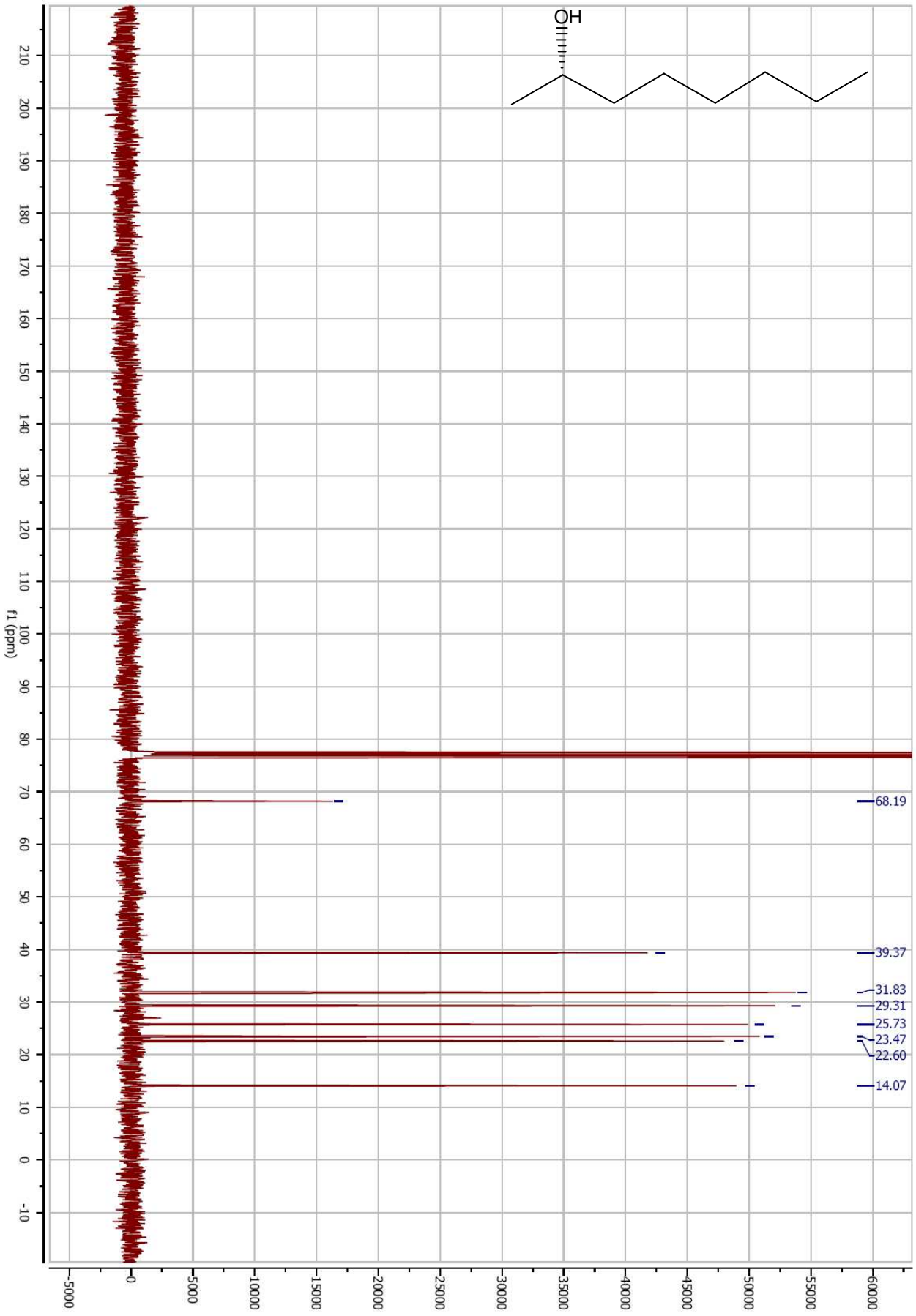
^{13}C-NMR (**12a**) *rac*-1-Octyn-3-yl sulfate; DMSO-d_6



^{1}H-NMR ((***S*)-2b**) (*S*)-2-Octanol; $CDCl_3$



^{13}C-NMR ((***S*)-2b**) (*S*)-2-Octanol; $CDCl_3$



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