SUPPORTING INFORMATION

for

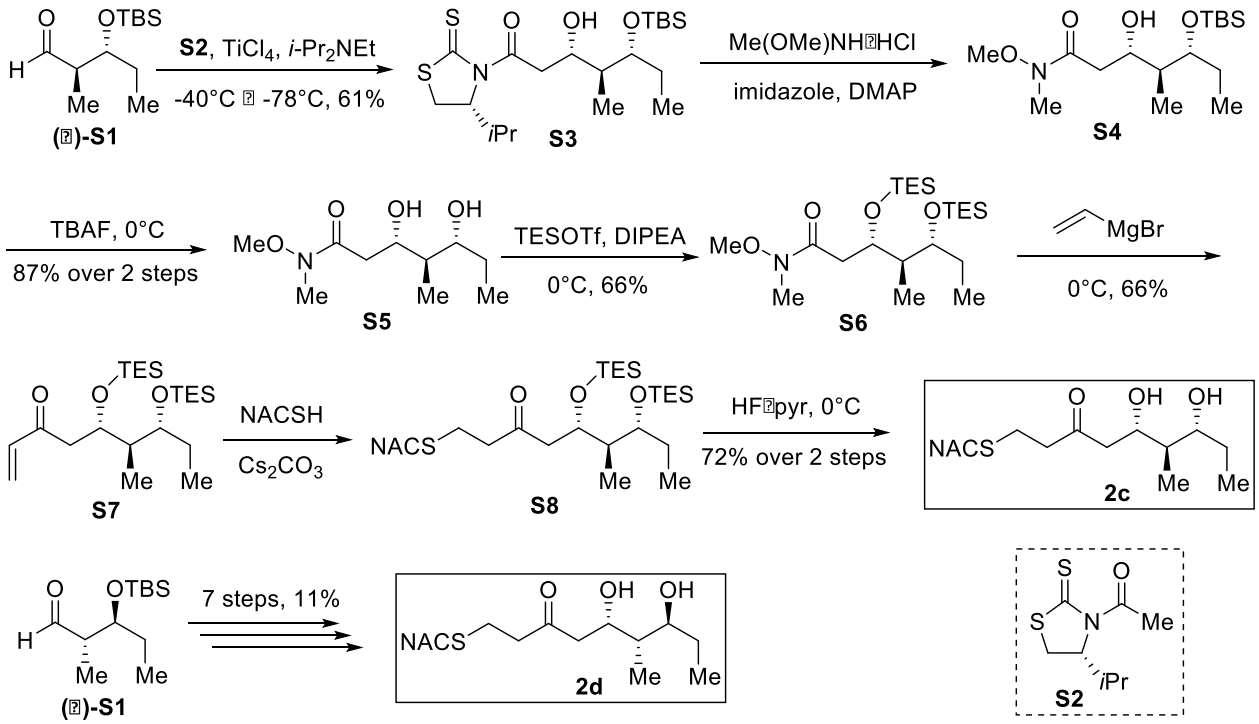
Functional Characterization of a Dehydratase Domain from the Pikromycin Polyketide Synthase

Yang Li,[1] Greg J. Dodge,[2] William D. Fiers, [1] Robert A. Fecik,[1] Janet L. Smith,[2] and Courtney C. Aldrich[1]*

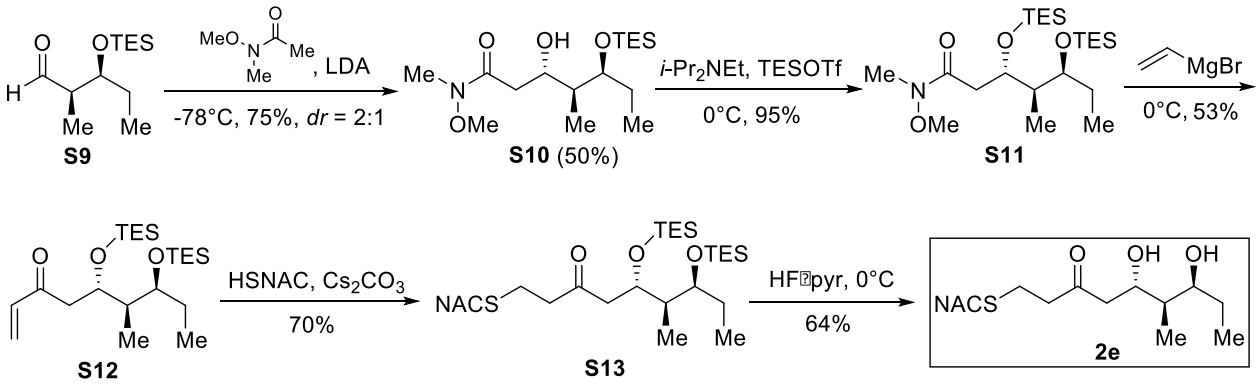
[1]Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455, United States

[2]Department of Biological Chemistry and Life Sciences Institute, University of Michigan, Ann Arbor, Michigan 48109, United States

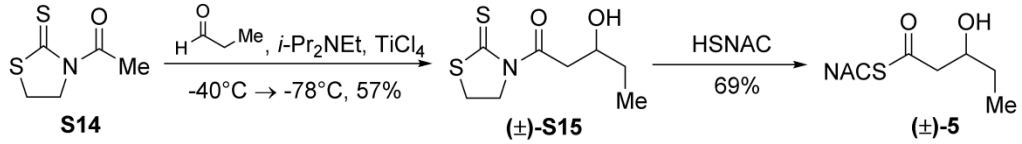
Table of Contents



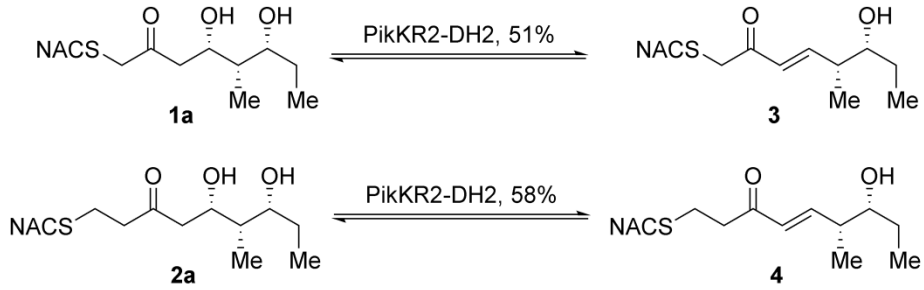
Scheme S1. Synthesis of substrate analogs **2c** and **2d**. Anti-aldol product (–)-**S1**, synthesized using Masamune's chiral auxalliary,[1,2] was submitted to an acetate aldol reaction with thiazolidinethione **S2**[3,4] to yield alcohol **S3** as the major diastereomer. The thiazolidinethione was displaced with *N*,*O*-dimethylhydroxylamine to afford Weinreb amide **S4**. The TBS protecting group stable in the previous aldol reaction was ultimately problematic in the final deprotection and was therefore replaced with a TES protecting group to facilitate its later removal. This was accomplished by treatment of **S4** with TBAF to furnish **S5** and subsequent silylation with TESOTf provided **S6**. Grinard reaction and Michael addition furnished the NAC-analog **S8**. A final deprotection under mild conditions furnished the substrate analog **2c**. Substrate analog **2d** was also synthesized in an analogous way by using aldehyde (+)-**S1**.



Scheme S2. Synthesis of substrate analog **2e**. The synthetic route commenced with the acetate aldol reaction of aldehyde **S9**[5] and *N*-methoxy-*N*-methylacetamide to give Weinreb amide **S10** as the major diastereomer (dr = 2:1, separated by chromatography). TES protection provided **S11** that was converted to vinyl ketone **S12** through Grignard addition of vinylmagnesium bromide. Sequential Michael addition of *N*-acetylcysteamine and silyl deprotection provided substrate analog **2e.**



Scheme S3. Synthesis of substrate analog (±)-**5**. Thiazolidinethione **S14**[5,6] was subjected to an acetate aldol reaction with propionaldehyde to provide a racemic mixture of **S15**, followed by displacement with *N*-acetylcysteamine to afford NAC thioester (±)–**5**.



Scheme S4. Chemoenzymatic synthesis of enones **3** and **4** in Tris buffer (pH 8.0) at 25 °C.

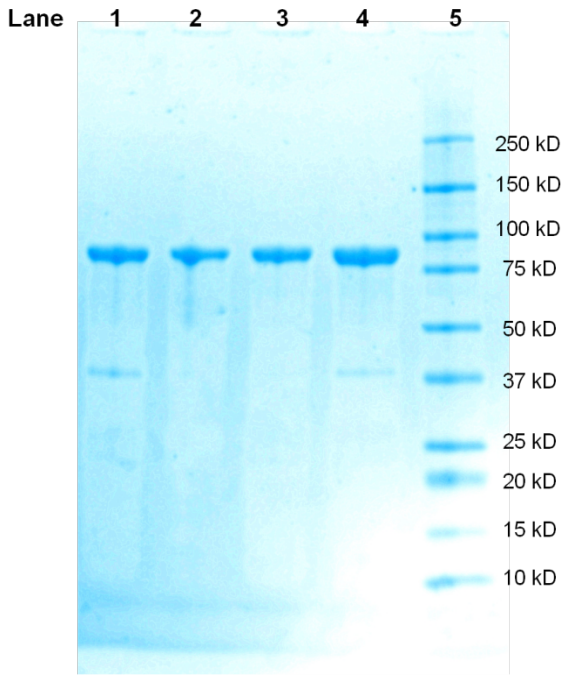


Figure S1. SDS-PAGE gel electrophoresis of purified PikDH2-KR2 and mutants on BIO-RAD precast gels (Mini-PROTEAN® TGX™ gels) stained by Bio-Safe™ Coomassie G-250 Stain; Lane 1 was loaded with PikDH2-KR2 wild type; Lane 2 was loaded with PikDH2-KR2 F3746L; Lane 3 was loaded with PikDH2-KR2 F3750L; Land 4 was loaded with PikDH2-KR2 F3750Y. Lane 5 was loaded with Kaleidoscope Precision Plus Protein Standards (BIO-RAD).

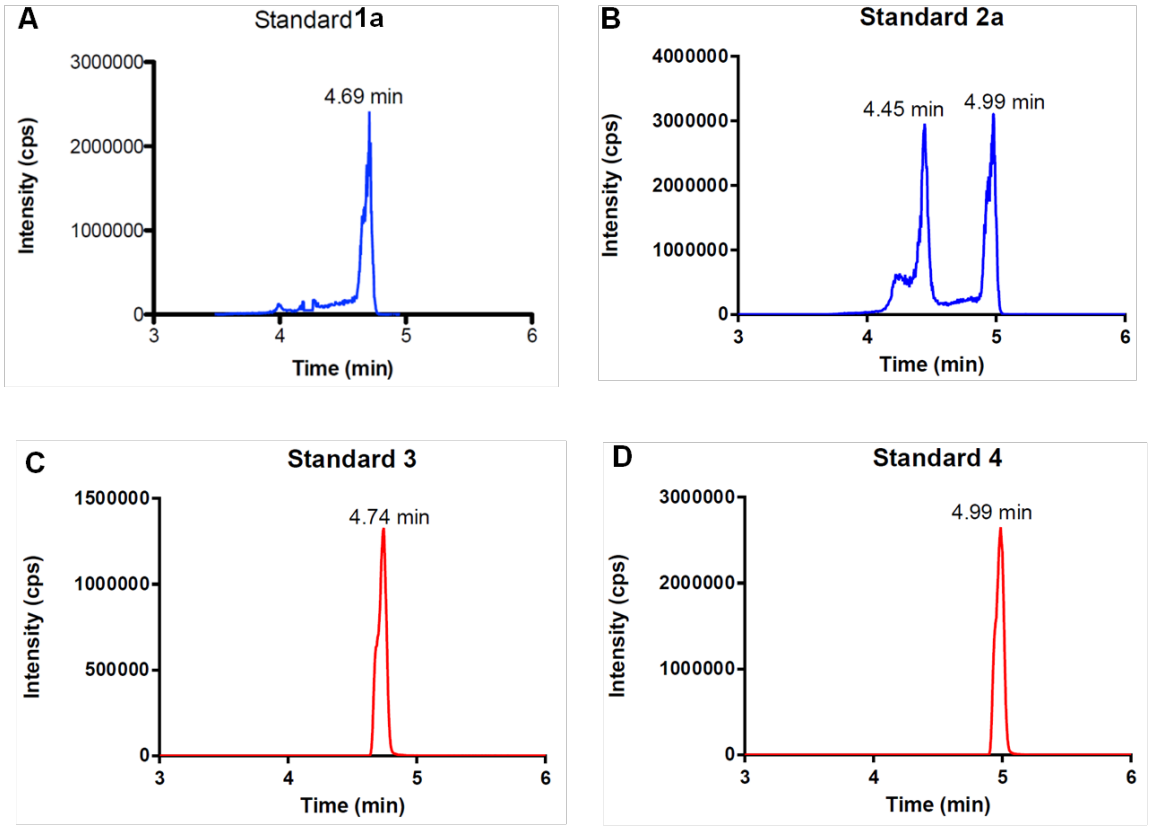


Figure S2. LC-MS/MS analysis of standards. (**A**) **1a** (MRM *m*/*z* 314→198); (**B**) **2a** (MRM *m*/*z* 328→212); (**C**) **3** (MRM *m*/*z* 274→216); (**D**) **4** (MRM *m*/*z* 288→120).

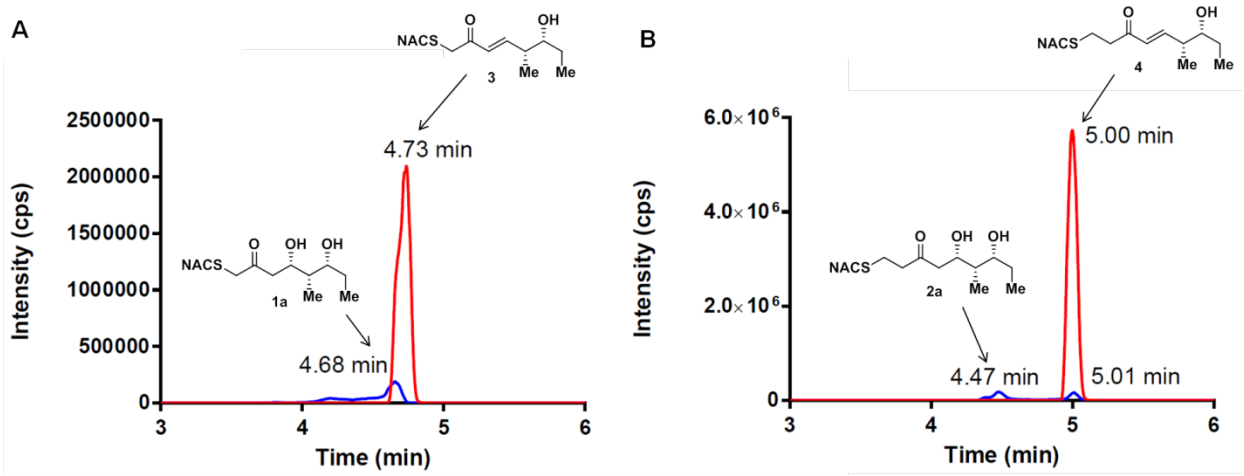


Figure S3. LC-MS/MS traces for hydration reactions of substrate **3** (**A**) and substrate **4** (**B**) by PikKR2-DH2.

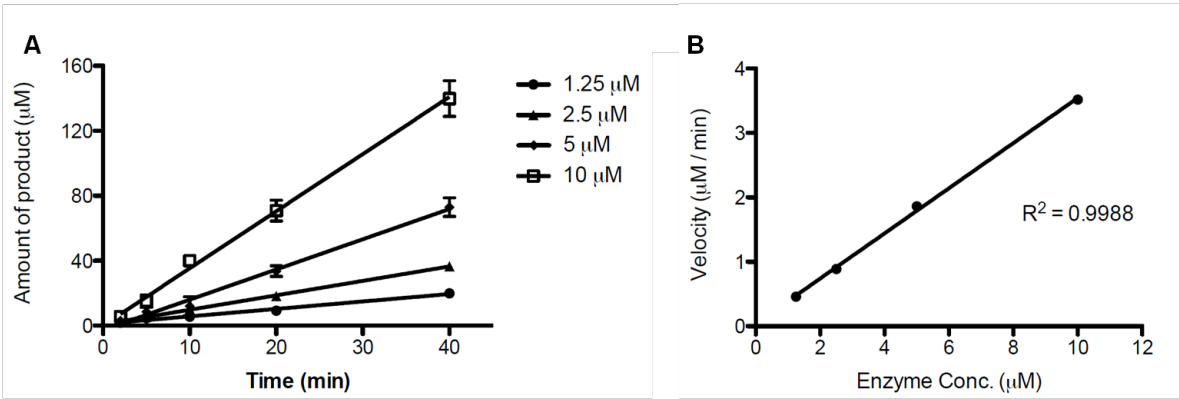


Figure S4. Measurement of initial velocity. **(A)** Progress curve of PikDH2 dehydration reaction at varying enzyme concentrations (1.25–10 μM); **(B)** Plot of initial velocity *vs*. enzyme concentration.

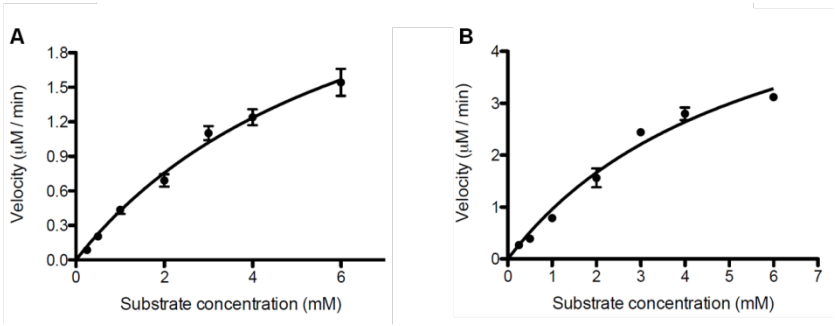


Figure S5. Michaelis-Menton curves of PikDH2. **(A)** with substrates **1a**; **(B)** with substrates **2a**.

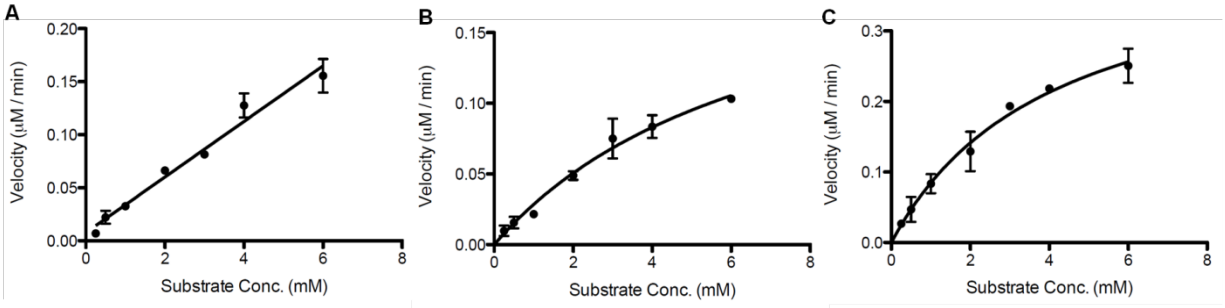


Figure S6. Michaelis-Menten and linear curves of PikDH2 mutants with substrate **2a**. **(A)** F3746L; **(B)** F3750L; **(C)** F3750Y.

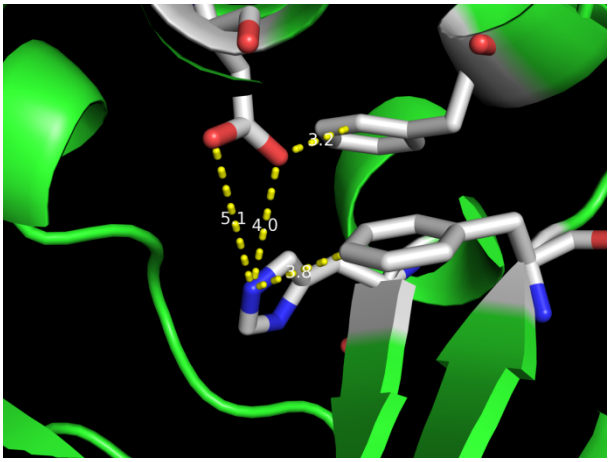


Figure S7. Active site of PikDH2 homology model. Catalytic residues Asp3800 and His3611 as well as hydrophobic residues Phe3746 and Phe3750 have been depicted in detail.

Table S1. Characterization of PikKR2-DH2 and mutants; including calculated molecular weight, monomeric molecular weight (ESI mass spectrometry), and overexpression yield.

Protein	Calculated MW (Da)	Monomeric MW (Da)	Yield (mg/L)
Wild-type	83009	83012	36
F3746L	82975	82975	66
F3750Y	83025	83028	29
F3750L	82975	82978	46

Table S2. V_{max}/K_M values at varying hydrogen ion concentrations.

pH	V_{max}/K_M ($\times 10^{-3}$ min^{-1})
6.6	0.2606 ± 0.0157
6.7	0.4571 ± 0.0276
7.0	0.6014 ± 0.0147
7.2	0.6675 ± 0.0504
7.5	0.9206 ±0.0345
8.0	0.9154 ± 0.0376
8.5	0.9479 ± 0.0413
9.0	1.112 ± 0.0428

General biology procedures. All chemical reagents were purchased from Sigma-Aldrich and were used directly without further purification. *E. coli* BL21(DE3) cells were from New England BioLabs. IPTG was acquired through Gold Biotechnology. His60 Ni Superflow resin was purchased from Clontech Laboratories, Inc. OD_{600} was measured on an Eppendorf BioPhotometer. Sonication was carried out by Branson Sonifier 450. Gel filtration purification was performed on HiLoad 16/600 Superdex 200 pg column (GE). The protein mass spectra data was obtained by Bruker BioTOF II mass spectrometer. LC–MS/MS was conducted with AB Sciex QTRAP 5500 mass spectrometer and Shimadzu LC system.

Cloning and mutagenesis. The PikKR2-DH2 didomain was cloned from the cosmid pLZ51 into the expression vector pMCSG7[7] using ligation independent cloning (LIC). PikKR2-DH2 forward primer: 5'-**TACTTCCAATCCAATGCC**AGCCGCGTCGGCGGG-3'; PikKR2-DH2 reverse primer: 5'-**TTATCCACTTCCAATGCTA**CGGCCGGGCCCGG-3' (LIC-overhangs in **bold**; inserted stop codon underlined). The insert was confirmed via sequencing. Primers for the F3746L, F3750L, and F3750Y variants were designed via the QuickChange primer design tool website (Agilent) (Figure S3). Mutagenesis was run using the standard protocol provided with the QuickChange Lightning Site Directed Mutagenesis kit (Agilent). Mutated codons are underlined below. All mutations were confirmed via sequencing.

Table S3. Primers of mutants F3746L, F3750L, and F3750Y.

Mutant	Primers (5' – 3')
F3746L	gaacagcggacctaaggcgaggccgtt aacggcctcgccttaggtccgctgttc
F3750L	cagcccctgtaacagcggaccgaagg ccttcggtccgctgttacaggggctg
F3750Y	gcgttcagcccctgatacagcggaccgaag cttcggtccgctgtatcaggggctgaacgc

Protein expression and purification. Competent *E. coli* BL21 (DE3) cells were transformed with pMCSG7 containing PikKR2-DH2 and grown in Terrific Broth (TB) media with 100 μg/mL ampicillin at 37 °C until OD_{600} reached to 1.2. The cultures were cooled to 20 °C and 200 μM IPTG was added to induce protein expression. After overnight expression, cells were harvested by centrifugation at 6,000*g* and 4 °C for 10 min. The resulting cell pellet was frozen at –80 °C for 10 min, resuspended in lysis buffer (50 mM HEPES, 300 mM NaCl, 10 mM imidazole, pH 8.0) and lysed by sonication. After centrifuging at 50,000*g* and 4 °C for 10 min, the cleared lysate was incubated with His-60 Ni superflow resin (2 mL) at 4 °C for 1 h and then loaded onto a gravity column. The column was washed with 14 mL of wash buffer (10 mM imidazole, 50 mM HEPES, 300 mM NaCl, pH 8.0) and eluted with 2.5 mL elution buffer (500 mM imidazole, 50 mM HEPES, 300 mM NaCl, pH 8.0). The protein was further purified via size exclusion chromatography on a Superdex 16/600 200pg gel filtration column eluting at 0.5 mL min^{-1} with 50 mM sodium phosphate (pH 7.1) and 150 mM NaCl. Purified protein was stored in storage buffer (50 mM Tris, 150 mM NaCl, 10% (v/v) glycerol, pH 8.0) at –80 °C. Protein concentration was determined using the Bio-Rad protein assay kit with bovine serum albumin as the standard. Exact molecular weight of the purified monomeric protein was determined by ESI mass

spectrometry. All the mutants were expressed and purified in an analogous way (Figure S1 and Table S1).

Determination of initial velocity conditions. Substrate **2a** (6 mM) was incubated with PikDH2-KR2 (1.25–10 μM) and reaction buffer (50 mM Tris, 150 mM NaCl, pH 8.0) in a total volume of 50 μL at 25 °C. At 2, 5, 10, 20 and 40 min time points, 5 μL of the reaction mixture was added to 495 μL of 1:1 MeCN–reaction buffer (100-fold dilution) to quench the reaction. The resulting solution was vortexed, centrifuged. 60 μL of the diluted reaction solution was added to a HPLC vial with 10 μL of internal standard **3** (320 nM) and analyzed by LC-MS/MS (Table S4) employing a Kinetix reverse-phase C_{18} column (50 mm × 2.1 mm, 2.6 μm, Phenomenex) operated at 0.4 mL min^{-1} with a gradient between mobile phase A (15 mM ammonium acetate in H_2O) and mobile phase B (MeCN). The gradient program was 0 min, 5% B; 2 min, 5% B; 7 min, 55% B; 8 min, 95% B; 9 min, 95% B; 9.5 min, 5% B; 12 min, 5% B. Standard curve of enzymatic product **4** was generated by injecting the authentic standard at varying concentrations with a fixed concentration of an internal standard **3**. The amount of enzymatic product formation at each time point was calculated by plotting the area ratio (analyte/internal standard) into the standard curve. Each reaction was performed in duplicate. The progress curve at varying enzyme concentrations was generated and the initial velocity at each enzyme concentration was obtained (Figure S4).

Table S4. LC-MS/MS analysis of analytes **1a**, **2a**, **3** and **4**.

Analyte	HPLC retention time (min)	Transition	Internal standard
1a	4.69	314→198	**2a**
2a	4.45, 4.99	328→212	**1a**
3	4.74	274→216	**4**
4	4.99	288→120	**3**

Reversibility of the dehydration reaction. Compounds **1a**, **2a**, **3** and **4** (1 mM) were individually added to the reaction buffer (50 mM Tris, 150 mM NaCl, pH 8.0) with PikDH2-KR2 (10 μM) in a total volume of 100 μL, respectively. After 20 h incubation, 5 μL of the reaction mixture was quenched by adding to 495 μL of 1:1 MeCN–reaction buffer (100-fold dilution). 10 μL of the diluted reaction mixture was added to 90 μL of 1:1 MeCN–reaction buffer (10-fold dilution, total 1000-fold dilution). The identities of the enzymatic products were determined by authentic standards (Figure S2 and Figure S3) and total amount was calculated by standard curve with internal standard added in. Each reaction was performed in duplicate.

Kinetic analysis of dehydration reaction by LC-MS/MS. The enzymatic reactions were carried out in a total volume of 50 μL under initial velocity conditions containing PikDH2-KR2 (5 μM), reaction buffer (50 mM Tris, 150 mM NaCl, pH 8.0) and substrates **1a** or **2a** at variable concentrations (0.25, 0.5, 1, 2, 3, 4, 6 mM). The final DMSO concentration was held constant at 3%. After incubation at 25 °C for 15 min, 5 μL of the reaction mixture was added to 495 μL of 1:1 MeCN–reaction buffer (100-fold dilution). The resulting solution was vortexed, centrifuged and analyzed by LC-MS/MS as described above. Control reactions for each concentration of substrate were performed without the addition of enzyme. Each reaction was performed in duplicate. Apparent steady-state kinetic parameters were determined by fitting the normalized v_0 vs [*S*] plots to the Michaelis–Menten equation by nonlinear regression analysis using GraphPad Prism 5.0 (Figure S5). Kinetic parameters of PikDH2-KR2 mutants were acquired in an analogous way except in some cases saturation was not achieved and the curve was fit by linear regression to provide specificity constant (Figure S6).

Homology model of PikDH2. The PikDH2 homology model (residues 1-293 of the PikKR2-DH2 didomain) was built in LOMETS[8] using the CurK dehydratase from the curacin pathway as

a template (PDB 3KG9) (Figure S7).[9] The PikDH2 model has an RMSD of 0.290 Å compared to the template, with a Z-score of 27.3 and 22% sequence identity.

pH dependency profile of PikDH2. The effect of pH on kinetic parameters was obtained by using HEPES (pH 6.6–7.5), Tris (pH 8.0), and bicine (pH 8.5–9.0). At desired pH values, enzymatic reactions were conducted with substrate **2a** (0–3 mM) under the conditions described above. Kinetic parameters V_{max}/K_M at different pH were obtained via fitting the v_0 vs [S] plots to by linear regression analysis. The pK_a value of the ionizable group was obtained through fitting V_{max}/K_M to eq 1:

$$\log \frac{V_{max}}{K_M} = \log \frac{C}{1-[H]^+/K_a} \quad (1)$$

where, C is the pH-independent plateau value, $[H]^+$ is the hydrogen ion concentration, and K_a is the dissociation constant of the acid.[10]

Inhibition assay with mechanism-based inhibitor 6. Inhibitor **6** (10–40 μM) was incubated with substrate **2a** (5 mM), PikDH2-KR2 (5 μM), reaction buffer (50 mM Tris, 150 mM NaCl, pH 8.0) in a total volume of 50 μL at 25 °C. At 2, 4, 8, 16 min time points, 5 μL of the reaction mixture was added to 495 μL of 1:1 MeCN–reaction buffer (100-fold dilution). The resulting solution was vortexed, centrifuged and analyzed by LC-MS/MS to detect the enzymatic product formation. Each reaction was conducted in duplicate. Inhibitor **6** showed time-dependent inhibition of PikDH2. The progress curve (Figure 4B) was fit to eq 2 and eq 3 for tight binding time-dependent inhibition:

$$[P] = v_s t + \frac{(1-\gamma)(v_i-v_s)}{\gamma k_{obs}} \ln \frac{1-\exp(-k_{obs}t)}{1-\gamma} \quad (2)$$

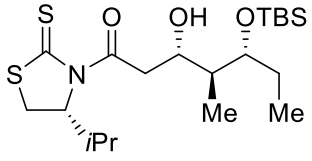
$$\gamma = \frac{[E]}{[I]}\left(1 - \frac{v_s}{v_i}\right)^2 \quad (3)$$

where [*P*] is the concentration of enzymatic product, *t* is the reaction time, v_i is the initial velocity, v_s is the steady state velocity, and k_{obs} is the observed rate constant of inhibition. Since mechanism-based inhibition is irreversible, the v_s value is equal to 0, thus the γ value is equal to the ratio of enzyme concentration to inhibitor concentration. The secondary plot of $1/k_{obs}$ versus 1/[I] was fit to a Kitz and Wilson plot (Figure 4C) to provide $K_{I,app}$ and k_{inact} values. The true K_I value was deduced from eq 4:

$$K_{I,app} = \frac{K_I}{1+[S]/K_M} \quad (4)$$

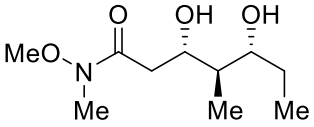
where [*S*] is the substrate concentration, and K_M is the Michaelis-Menten constant for the substrate.

General chemistry procedures. All commercial reagents were used as provided unless otherwise indicated. Substrate analogs **1a**[5], **2a**[5], **1b**[5], **2b**[5], intermediates **S1**[1,2], **S2**[3,4], **S9**[5] and **S14**[5,6] were prepared by the published procedures. THF and CH_2Cl_2 were purified by passage through alumina columns. All reactions were performed under an inert atmosphere of dry N_2 in oven-dried (150 ºC) glassware. Flash chromatography was conducted on silica gel (230–400 mesh) using the indicated solvent systems. TLC was performed on 250 μm, F_{254} silica gel plates, and visualized by UV and *p*-anisaldehyde stain. Optical rotations were determined on a Rudolph Autopol III polarimeter using the sodium D line (λ = 589 nm) at the temperature indicated and are reported as follows: $[\alpha]_D^{temp}$, concentration (*c* = g/100 mL), and solvent. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer at 400 Hz for ^1H NMR and at 100 Hz for ^{13}C NMR. Chemical shifts are reported in ppm from an internal standard of residual $CHCl_3$ (7.26 ppm for ^1H NMR and 77.00 for ^{13}C NMR). Proton chemical data are reported as follows: chemical shift (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sext = sextet, m = multiplet, br = broad), coupling constant (Hz), and integration. High resolution mass spectra were obtained on a Bruker BioTOF II ESI-TOF/MS using either PEG or PPG standards as high resolution calibrants.



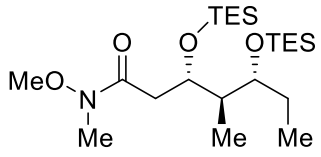
(3*S*,4*S*,5*R*)-5-[(*tert*-Butyldimethylsilyl)oxy]-3-hydroxy-1-[(*R*)-4-isopropyl-2-thioxothiazolidin-3-yl]-4-methylheptan-1-one (S3). To a solution of thiazolidinethione **S2** (172 mg, 0.846 mmol, 1.60 equiv) in CH_2Cl_2 (4.2 mL) at –40 °C was added $TiCl_4$ (99 μL, 0.90 mmol, 1.7 equiv). An aliquot of *i*-Pr_2NEt (0.16 mL, 0.90 mmol, 1.7 equiv) was added after stirring at –40 °C for 30 min. The reaction mixture was stirred at –40 °C an additional 2 h, and then cooled to –78 °C. Aldehyde **S1** (122 mg, 0.529 mmol, 1.00 equiv) was added and rinsed with CH_2Cl_2 (3 × 0.3 mL). The reaction

was stirred at –78 °C for 80 min, quenched by addition of saturated aqueous NH_4Cl (20 mL) and allowed to warm to room temperature. The organics were extracted with CH_2Cl_2 (3 × 20 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash chromatography (20% EtOAc/hexanes) afforded the title compound (140 mg, 61%) as a yellow oil: R_f= 0.34 (20% EtOAc/hexanes); $[\alpha]_D^{22}$ = –258 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.16 (t, *J* = 6.8 Hz 1H), 4.19–4.11 (m, 1H), 3.75 (q, *J* = 6.0 Hz, 1H), 3.64 (dd, *J* = 17.7, 2.0 Hz, 1H), 3.51 (dd, *J* = 11.5, 8.0 Hz, 1H), 3.15 (dd, *J* = 17.7, 9.9 Hz, 1H), 3.02 (d, *J* = 11.5 Hz, 1H), 2.36 (dq, *J* = 13.5, 6.8 Hz, 1H), 1.85 (sext, *J* = 7.0 Hz, 1H), 1.58–1.40 (m, 2H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.93–0.83 (m, 15H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.0, 173.4, 75.1, 71.4, 69.4, 43.0, 42.5, 30.8, 30.6, 25.9, 25.6, 19.1, 18.1, 17.8, 11.4, 9.2, -4.4, -4.6; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{20}H_{39}NO_3S_2SiNa$ 456.2033, found 456.2057.



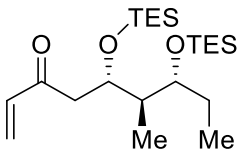
(3*S*,4*R*,5*R*)-3,5-Dihydroxy-*N*-methoxy-*N*,4-dimethylheptanamide (S5). To a solution of thiazolidinethione **S3** (136 mg, 0.314 mmol, 1.00 equiv) in CH_2Cl_2 (5 mL) at room temperature was added *N*,*O*-dimethylhydroxylamine hydrochloride (123 mg, 1.26 mmol, 4.00 equiv) and imidazole (171 mg, 2.51 mmol, 8.00 equiv), followed by the addition of DMAP (7.7 mg, 0.063 mmol, 0.20 equiv). The reaction mixture was stirred at room temperature until the bright yellow color faded (24 h), then quenched with water. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (20 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford the crude **S4** as a colorless oil, which was used directly in the next reaction without further purification. R_f = 0.41 (30% EtOAc/hexanes).

To a solution of silyl ether **S4** prepared above (105 mg, 0.314 mmol, 1.00 equiv) in THF (10 mL) at 0 °C was added TBAF (1.0 M in THF, 0.94 mL, 0.94 mmol, 3.0 equiv). The reaction mixture was stirred at 0 °C for 7 h, then quenched by addition of saturated aqueous NH_4Cl (10 mL). The reaction mixture was extracted with EtOAc (3 × 10 mL), and the combined organic layers were washed with saturated aqueous NaCl (20 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded the title compound (60 mg, 87% over 2 steps) as a colorless oil: R_f = 0.30 (5% MeOH/CH_2Cl_2); $[\alpha]_D^{21}$ = –43.4 (*c* 0.650, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.07–3.99 (m, 1H), 3.69 (s, 3H), 3.59 (td, *J* = 7.9, 2.9 Hz, 1H), 3.19 (s, 3H), 2.80 (d, *J* = 16.5 Hz, 1H), 2.51 (dd, *J* = 16.5, 9.8 Hz, 1H), 1.71–1.60 (m, 2H), 1.41 (dquin, *J* = 14.7, 7.4 Hz, 1H), 0.97 (t, *J* = 7.4 Hz, 3H), 0.82 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.9, 76.3, 72.9, 61.3, 42.8, 36.0, 31.9, 27.1, 12.9, 9.3; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{10}H_{21}NO_4Na$ 242.1363, found 242.1368.

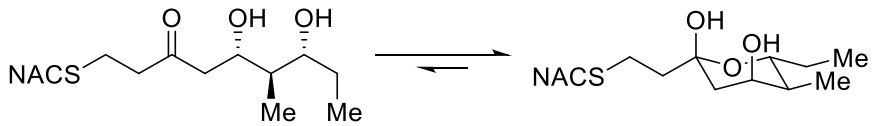


(3*S*,4*R*,5*R*)-*N*-Methoxy-*N*,4-dimethyl-3,5-bis[(triethylsilyl)oxy]heptanamide (S6). To a solution of alcohol **S5** (54 mg, 0.25 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) at 0 °C was added *i*-Pr_2NEt (0.15 mL, 0.89 mmol, 3.6 equiv) and TESOTf (0.18 mL, 0.79 mmol, 3.2 equiv). After stirring at 0 °C for 60 min, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ (20 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (50 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash chromatography (10% EtOAc/hexanes) afforded the title compound (73 mg, 66%) as a colorless oil: R_f = 0.44 (10% EtOAc/hexanes); $[\alpha]_D^{22}$ = –31.0 (*c* 1.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ

4.50–4.43 (m, 1H), 3.68 (s, 3H), 3.60 (q, J = 5.7 Hz, 1H), 3.16 (s, 3H), 2.76–2.65 (m, 1H), 2.36 (dd, J = 15.0, 2.9 Hz, 1H), 1.85–1.77 (m, 1H), 1.58–1.44 (m, 2H), 0.99–0.83 (m, 24H), 0.64–0.52 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1, 74.5, 69.5, 61.2, 43.7, 35.5, 32.0, 26.4, 10.3, 8.8, 7.0, 6.9, 5.2, 5.0; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{22}H_{49}NO_4Si_2Na$ 470.3092, found 470.3097.



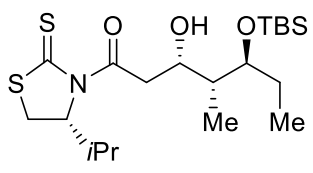
(5*S*,6*R*,7*R*)-6-Methyl-5,7-bis[(triethylsilyl)oxy]non-1-en-3-one (S7). To a solution of Weinreb amide **S6** (73 mg, 0.16 mmol, 1.0 equiv) in THF (5 mL) at 0 °C was added vinyl magnesium bromide (1.0 M in THF, 0.48 mL, 0.48 mmol, 3.0 equiv). After stirring at 0 °C for 55 min, the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (20 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash chromatography (10% EtOAc/hexanes) afforded the title compound (45 mg, 66%) as a colorless oil: R_f = 0.60 (10% EtOAc/hexanes); $[\alpha]_D^{21}$ = –34.6 (*c* 0.800, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.38 (dd, J = 17.6, 10.5 Hz, 1H), 6.20 (dd, J = 17.6, 1.0 Hz, 1H), 5.79 (dd, J = 10.5, 1.1 Hz, 1H), 4.51–4.45 (m, 1H), 3.58 (q, J = 5.3 Hz, 1H), 2.77 (q, J = 8.8 Hz, 1H), 2.59 (dd, J = 15.5, 2.9 Hz, 1H), 1.84–1.76 (m, 1H), 1.57–1.45 (m, 2H), 1.00–0.82 (m, 24H), 0.57 (app. dq, J = 19.0, 7.8 Hz, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.0, 137.5, 127.8, 74.6, 69.1, 43.52, 43.46, 26.5, 10.3, 8.6, 7.0, 6.9, 5.2, 5.0; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{22}H_{46}O_3Si_2Na$ 437.2878, found 437.2854.



(5*S*,6*R*,7*S*)-5,7-Dihydroxy-6-methyl-1-[*N*-(2-acetamidoethyl)thio]nonan-3-one (2c). To a solution of vinyl

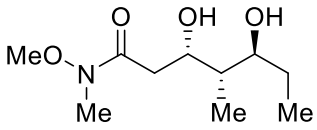
ketone **S7** (32 mg, 0.078 mmol, 1.0 equiv) in THF (5 mL) was added *N*-acetylcysteamine (12 μL, 0.12 mmol, 1.5 equiv) and a catalytic amount of Cs_2CO_3. The reaction was stirred at room temperature for 40 min, before quenching with saturated aqueous NH_4Cl (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (40 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure to yield crude **S8** (35 mg) as a colorless oil, which was used in the next reaction without further purification: R_f = 0.36 (5% MeOH/CH_2Cl_2).

To a solution of crude silyl ether **S8** prepared above (35 mg, 0.066 mmol, 1.0 equiv) in THF (6 mL) at 0 °C was added a solution of 70% HF-pyridine:pyridine:THF (1:2:8, 3 mL). The reaction was stirred at 0 °C for 3.5 h, then quenched with saturated aqueous $NaHCO_3$ to bring the reaction to pH 7, and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (40 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded the title compound (17 mg, 72% over 2 steps) as a colorless oil: R_f = 0.20 (5% MeOH/CH_2Cl_2); $[\alpha]_D^{21}$ = 41.1 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.10 (s, 1H), 4.98 (s, 1H), 3.97–3.85 (m, 1H), 3.75–3.68 (m, 1H), 3.50–3.36 (m, 3H), 2.78–2.61 (m, 4H), 2.04–1.96 (m, 4H), 1.90–1.81 (m, 2H), 1.71–1.59 (m, 2H), 1.50–1.32 (m, 2H), 0.96–0.90 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4, 97.1, 70.4, 70.2, 41.4, 39.9, 38.3 (overlap), 31.5, 25.4, 25.0, 23.2, 13.9, 9.3; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{14}H_{27}NO_4SNa$ 328.1553, found 328.1551.



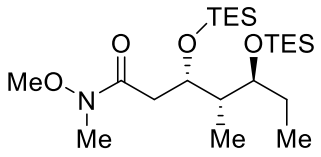
(3*S*,4*R*,5*S*)-5-[(*tert*-Butyldimethylsilyl)oxy]-3-hydroxy-1-[(*R*)-4-isopropyl-2-thioxothiazolidin-3-yl]-4-methylheptan-1-one (4,5-di-*epi*-S3). The title compound was prepared using otherwise identical conditions as described for the preparation of aldol product **S3** except for the use of aldehyde (+)–

S1 (111 mg, 0.482 mmol, 1.00 equiv). Purification by flash chromatography (20% EtOAc/hexanes) afforded the title compound (129 mg, 62%) as a yellow oil: R_f = 0.46 (20% EtOAc/hexanes); $[\alpha]_D^{21}$ = –211 (*c* 2.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.19–5.13 (m, 1H), 4.66 (d, *J* = 9.4 Hz, 1H), 3.72–3.65 (m, 2H), 3.51 (dd, *J* = 11.4, 8.0 Hz, 1H), 3.42 (dd, *J* = 17.1, 9.3 Hz, 1H), 3.27 (dd, *J* = 17.1, 2.0 Hz, 1H), 3.01 (d, *J* = 11.5 Hz, 1H), 2.40 (dq, *J* = 13.5, 6.7 Hz, 1H), 1.82–1.59 (m, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 1.01 (d, *J* = 7.1 Hz, 3H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.88 (s, 9H), 0.87 (t, *J* = 7.5 Hz), 0.093 (s, 3H), 0.085 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.0, 172.2, 79.3, 71.6, 66.7, 43.8, 38.0, 30.9, 30.5, 27.5, 25.8, 19.1, 17.9, 17.7, 11.7, 9.5, -4.4, -4.9; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{20}H_{39}NO_3S_2SiNa$ 456.2033, found 456.2052.

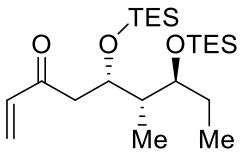


(3*S*,4*S*,5*S*)-3,5-Dihydroxy-*N*-methoxy-*N*,4-dimethylheptanamide (4,5-di-*epi*-S5). Identical reaction conditions employed for the synthesis of weinreb amide **S4** were used, except for the use of thiazolidinethione **4,5-di-*epi*-S3** (129 mg, 0.297 mmol, 1.00 equiv). The crude product **4,5-di-*epi*-S4** was used to carry out the next reaction without further purification: R_f = 0.34 (30% EtOAc/hexanes).

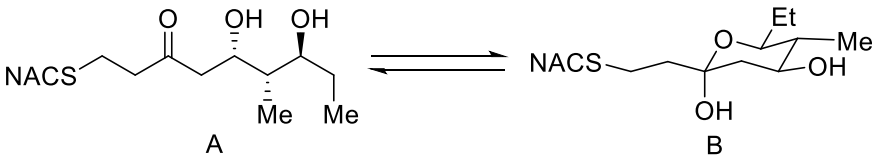
The title compound was prepared using otherwise identical conditions as described for the preparation of diol **S5**, except for the use of of silyl ether **4,5-di-*epi*-S4** prepared above (99 mg, 0.297 mmol, 1.00 equiv). Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded the title compound (45 mg, 69% over 2 steps) as a colorless oil: R_f = 0.29 (5% MeOH/CH_2Cl_2); $[\alpha]_D^{22}$ = –40.4 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.39 (dt, *J* = 9.8, 2.8 Hz, 1H), 3.69 (s, 4H), 3.55–3.48 (m, 1H), 3.22–3.14 (m, 4H), 2.68–2.49 (m, 2H), 1.72–1.65 (m, 1H), 1.63–1.44 (m, 2H), 1.00–0.91 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.9, 76.1, 69.4, 61.3, 40.6, 34.7, 31.9, 28.1, 11.9, 9.8; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{10}H_{21}NO_4Na$ 242.1363, found 242.1366.



(3*S*,4*S*,5*S*)-*N*-Methoxy-*N*,4-dimethyl-3,5-bis[(triethylsilyl)oxy]heptanamide (4,5-di-*epi*-S6). The title compound was prepared using otherwise identical conditions as described for the preparation of silylether **S6**, except for the use of alcohol **4,5-di-*epi*-S5** (42 mg, 0.19 mmol, 1.0 equiv). Purification by flash chromatography (10% EtOAc/hexanes) afforded the title compound (57 mg, 66%) as a colorless oil: R_f = 0.45 (10% EtOAc/hexanes); $[\alpha]_D^{21}$ = –26.3 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.25 (q, *J* = 5.6 Hz, 1H), 3.68 (s, 3H), 3.57 (dt, *J* = 7.9, 3.6 Hz, 1H), 3.16 (s, 3H), 2.66 (dd, *J* = 15.0, 6.7 Hz, 1H), 2.57 (dd, *J* = 15.0, 5.6 Hz, 1H), 1.73–1.65 (m, 1H), 1.55–1.44 (m, 1H), 1.43–1.35 (m, 1H), 1.00–0.85 (m, 24H), 0.60 (app. dq, *J* = 7.9, 3.5 Hz, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4, 75.9, 70.8, 61.2, 45.0, 38.8, 32.0, 25.5, 10.4, 9.6, 7.0, 5.2; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{22}H_{49}NO_4Si_2Na$ 470.3092, found 470.3088.

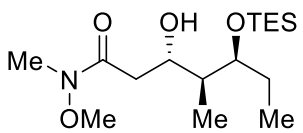


(5*S*,6*S*,7*S*)-6-Methyl-5,7-bis[(triethylsilyl)oxy]non-1-en-3-one (6,7-di-*epi*-S7). The title compound was prepared using otherwise identical conditions as described for the preparation of enone **S7**, except for the use Weinreb amide **4,5-di-*epi*-S6** (42 mg, 0.094 mmol, 1.0 equiv). Purification by flash chromatography (5% EtOAc/hexanes) afforded the title compound (28 mg, 72%) as a colorless oil: R_f = 0.68 (10% EtOAc/hexanes); $[\alpha]_D^{21}$ = –27.0 (*c* 0.650, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.37 (dd, *J* = 17.6, 10.5 Hz, 1H), 6.21 (d, *J* = 17.6 Hz, 1H), 5.83 (d, *J* = 10.5 Hz, 1H), 4.28 (q, *J* = 5.7 Hz, 1H), 3.55 (dt, *J* = 7.8, 4.0 Hz, 1H), 2.84–2.70 (m, 2H), 1.69–1.61 (m, 1H), 1.53–1.34 (m, 2H), 0.99–0.84 (m, 24H), 0.58 (app. dq, *J* = 7.9, 4.0 Hz, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.1, 137.1, 128.3, 75.6, 70.2, 46.5, 44.9, 25.6, 10.1, 9.7, 7.00, 5.2; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{22}H_{46}O_3Si_2Na$ 437.2878, found 437.2847.



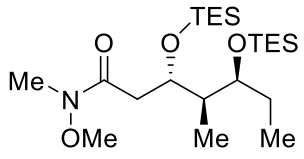
(5*S*,6*S*,7*S*)-5,7-Dihydroxy-6-methyl-1-[*N*-(2-acetamidoethyl)thio]nonan-3-one (2d). Identical reaction conditions as reported for the synthesis of thioether **S8** were employed, except for the use of vinyl ketone **6,7-di-*epi*-S7** (14 mg, 0.034 mmol, 1.0 equiv). The crude product **6,7-di-*epi*-S8** was used to carry out the next reaction without further purification: R_f = 0.45 (5% MeOH/CH_2Cl_2).

The title compound was prepared using otherwise identical conditions as described for the preparation of thioether **2c**, except for the use of silyl ether **6,7-di-*epi*-S8** prepared above (18 mg, 0.034 mmol, 1.0 equiv). Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded the title compound (5.8 mg, 56% over 2 steps) as a colorless oil: R_f = 0.27 (5% MeOH/CH_2Cl_2); Due to the instability of the compound the optical rotation could not be obtained; ^{1}H NMR ($CDCl_3$, 400 MHz, approximately 3:17 mixture of A:B where the integrations have been normalized) δ 6.03 (s, 0.15H), 5.89 (s, 0.85H), 4.45 (dt, *J* = 10.1, 2.4 Hz, 0.15H), 3.69 (dt, *J* = 10.7, 4.8 Hz, 0.85H), 3.54–3.36 (m, 3.15H), 3.20 (s, 0.85H), 2.83–2.62 (m, 4.45H), 2.46 (dd, *J* = 16.4, 2.7 Hz, 0.15H), 2.11 (dd, *J* = 12.3, 4.8 Hz, 0.85H), 1.99 (s, 3H), 1.95–1.89 (m, 1.7H), 1.76–1.68 (m, 1H), 1.46–1.30 (m, 1.85H), 1.28–1.21 (m, 1H), 1.01–0.87 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz, major isomer B) δ 170.2, 97.3, 74.6, 70.0, 43.2, 43.0, 41.1, 38.2, 31.4, 25.3, 25.2, 23.3, 12.6, 9.6; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{14}H_{27}NO_4SNa$ 328.1553, found 328.1560.



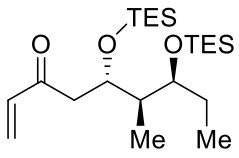
(3*S*,4*S*,5*S*)-3-Hydroxy-*N*-methoxy-*N*,4-dimethyl-5-[(triethylsilyl)oxy]heptanamide (S10). To a solution of freshly distilled *i*-Pr_2NH (0.21 mL, 1.5 mmol, 1.5 equiv) in THF (30 mL) at –78 °C was added *n*-BuLi

(1.2 M in THF, 1.2 mL, 1.4 mmol, 1.4 equiv). After stirring at –78 °C for 30 min, *N*-methoxy-*N*-methylacetamide (0.15 mL, 1.4 mmol, 1.4 equiv) was added. The reaction mixture was stirred at –78 °C for an additional 30 min, followed by the dropwise addition of a solution of aldehyde **S9** (233 mg, 1.01 mmol, 1.00 equiv) in THF (0.5 mL). The reaction mixture was stirred at –78 °C for an additional 1 h, then quenched by the addition of saturated aqueous NH_4Cl (20 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash chromatography (40% EtOAc/hexanes) afforded the title compound **S10** (169 mg, 50%) as a colorless oil (total yield = 75%, dr = 2: 1): $[\alpha]_D^{22}$ = –32.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.16 (d, *J* = 2.9 Hz, 1H), 4.01 (tt, *J* = 9.0, 3.0 Hz, 1H), 3.95 (dt, *J* = 6.8, 2.2 Hz, 1H), 3.70 (s, 3H), 3.20 (s, 3H), 2.66 (d, *J* = 14.3 Hz, 1H), 2.52 (dd, *J* = 15.7, 9.2 Hz, 1H), 1.74–1.63 (m, 1H), 1.60–1.46 (m, 2H), 0.96 (t, *J* = 7.9 Hz, 9H), 0.87 (t, *J* = 7.5 Hz, 3H), 0.81 (d, *J* = 7.0 Hz, 3H), 0.62 (q, *J* = 7.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7, 75.1, 70.0, 61.2, 41.8, 36.9, 31.9, 26.6, 10.7, 10.6, 6.9, 5.2; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{16}H_{35}NO_4SiNa$ 356.2228, found 356.2226.

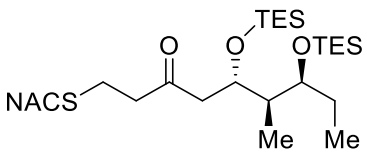


(3*S*,4*R*,5*S*)-*N*-Methoxy-*N*,4-dimethyl-3,5-bis[(triethylsilyl)oxy]heptanamide (S11). To a solution of alcohol **S10** (122 mg, 0.366 mmol, 1.00 equiv) in CH_2Cl_2 (10 mL) at 0 °C was added *i*-Pr_2NEt (0.11 mL, 0.66 mmol, 1.8 equiv) and TESOTf (0.13 mL, 0.59 mmol, 1.6 equiv). After stirring at 0 °C for 1 h, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ (10 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (20 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash

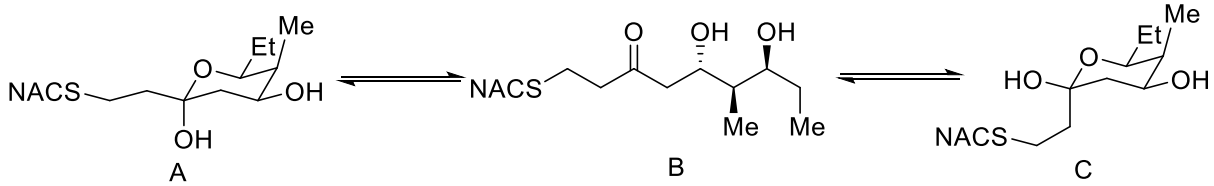
chromatography (20% EtOAc/hexanes) afforded the title compound (155 mg, 95%) as a colorless oil: R_f = 0.65 (20% EtOAc/hexanes); $[\alpha]_D^{22}$ = –28.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.30 (dt, *J* = 9.7, 3.0 Hz, 1H), 3.70 (s, 3H), 3.52 (q, *J* = 5.6 Hz, 1H), 3.17 (s, 3H), 2.87–2.72 (m, 1H), 2.20 (dd, *J* = 14.9, 2.0 Hz, 1H), 1.86–1.74 (m, 1H), 1.61–1.49 (m, 2H), 0.98–0.85 (m, 24H), 0.63–0.54 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1, 75.2, 71.1, 61.2, 42.9, 34.8, 32.0, 27.7, 8.9, 8.8, 7.0, 6.8, 5.4, 4.9; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{22}H_{49}NO_4Si_2Na$ 470.3092, found 470.3093.



(5*S*,6*R*,7*S*)-6-Methyl-5,7-bis[(triethylsilyl)oxy]non-1-en-3-one (S12). To a solution of Weinreb amide **S11** (155 mg, 0.346 mmol, 1.00 equiv) in THF (15 mL) at 0 °C was added vinyl magnesium bromide (1.0 M in THF, 1.04 mL, 1.04 mmol, 3.00 equiv). The reaction mixture was stirred at 0 °C for 70 min, quenched by the addition of saturated aqueous NH_4Cl (10 mL), and allowed to warm up to room temperature. The layers were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with saturated aqueous NaCl (30 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash chromatography (10% EtOAc/hexanes) afforded the title compound (77 mg, 53%) as a colorless oil: R_f = 0.64 (10% EtOAc/hexanes); $[\alpha]_D^{23}$ = –34.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.36 (dd, *J* = 17.6, 10.5 Hz, 1H), 6.20 (dd, *J* = 17.6, 1.1 Hz, 1H), 5.81 (dd, *J* = 10.5, 1.1 Hz, 1H), 4.30 (ddd, *J* = 9.2, 3.5, 2.3 Hz, 1H), 3.58–3.50 (m, 1H), 2.86 (dd, *J* = 15.6, 9.3 Hz, 1H), 2.49 (dd, *J* = 15.6, 2.3 Hz, 1H), 1.83–1.73 (m, 1H), 1.57–1.47 (m, 2H), 0.99–0.84 (m, 24H), 0.57 (q, *J* = 7.8 Hz, 6H), 0.54 (q, *J* = 7.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.2, 137.6, 128.1, 75.3, 71.1, 42.82, 42.77, 27.8, 9.2, 8.3, 7.0, 6.9, 5.4, 5.0; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{22}H_{46}O_3Si_2Na$ 437.2878, found 437.2868.

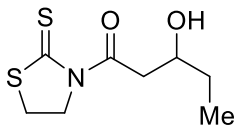


(5*S*,6*R*,7*S*)-6-Methyl-1-[*N*-(2-acetamidoethyl)thio]-5,7-bis[(triethylsilyl)oxy]nonan-3-one (S13). To a solution of vinyl ketone **S12** (42 mg, 0.10 mmol, 1.0 equiv) in THF (8 mL) was added *N*-acetylcysteamine (16 μL, 0.15 mmol, 1.5 equiv) and a catalytic amount of Cs_2CO_3. The reaction was stirred at room temperature for 1.5 h, then quenched by the addition of saturated aqueous NH_4Cl (10 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with saturated aqueous NaCl (40 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded the title compound (38 mg, 70%) as a colorless oil: R_f = 0.37 (5% MeOH/CH_2Cl_2); $[\alpha]_D^{23}$ = –28.4 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.07 (s, 1H), 4.31–4.20 (m, 1H), 3.54–3.43 (m, 3H), 2.83–2.58 (m, 7H), 2.40 (dd, *J* = 15.5, 1.9 Hz, 1H), 2.01 (s, 3H), 1.78–1.70 (m, 1H), 1.57–1.42 (m, 2H), 1.01–0.81 (m, 24H), 0.56 (app. quin, *J* = 8.0 Hz, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 208.3, 170.1, 75.2, 71.3, 46.1, 44.4, 42.5, 38.4, 32.3, 27.7, 25.0, 23.2, 9.2, 8.0, 7.0, 6.9, 5.4, 5.0; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{26}H_{55}NO_3SSi_2Na$ 556.3283, found 556.3291.



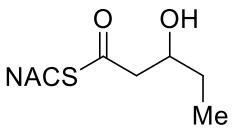
(5*S*,6*R*,7*S*)-5,7-Dihydroxy-6-methyl-1-[*N*-(2-acetamidoethyl)thio]nonan-3-one (2e). To a solution of silyl ether **S13** (44 mg, 0.082 mmol, 1.0 equiv) in THF (6 mL) at 0 °C was added a solution of 70% HF-pyridine:pyridine:THF (1:2:8, 3 mL). The reaction was stirred at 0 °C for 4.5 h, then quenched by the addition of saturated aqueous $NaHCO_3$ to bring the reaction to pH 7, and extracted with *n*-butanol (5 × 20 mL). The combined organic layers were washed with saturated aqueous NaCl (40 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure.

Purification by flash chromatography (10% MeOH/EtOAc) afforded the title compound (16 mg, 64%) as a colorless oil: R_f = 0.36 (10% MeOH/EtOAc); $[\alpha]_D^{22}$ = –35.5 (*c* 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz, approximately 6:6:1 mixture of A:B:C where the integrations have been normalized) δ 6.61 (s, 0.08H), 6.08 (s, 0.46H), 5.98 (s, 0.46H), 4.20 (dt, *J* = 11.9, 4.8 Hz, 0.46H), 4.17–4.08 (m, 0.46H), 4.07–4.03 (m, 0.08H), 3.85–3.77 (m, 1H), 3.75–3.69 (m, 0.16H), 3.53–3.34 (m, 1.84H), 3.22–3.13 (m, 0.32H), 2.86–2.58 (m, 5.52H), 2.00–1.98 (m, 3H), 1.95–1.84 (m, 1.38H), 1.81 (dd, *J* =4.8, 12.5 Hz, 0.46H), 1.65–1.34 (m, 3H), 1.09 (d, *J* = 7.2 Hz, 0.24H), 1.05 (t, *J* = 7.3 Hz, 0.24H), 0.98–0.87 (m, 4.14H), 0.80 (d, *J* = 6.9 Hz, 1.38H); ^{13}C NMR ($CDCl_3$, 100 MHz, major isomers A and B) δ 209.9, 170.4, 170.3, 97.5, 73.8, 72.9, 71.6, 67.5, 48.1, 43.4, 41.5, 41.3, 38.6, 38.3, 37.3, 37.1, 31.9, 31.4, 26.7, 25.2, 25.1, 25.0, 23.23, 23.21, 10.9, 10.8, 10.3, 3.6; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{14}H_{27}NO_4SNa$ 328.1553, found 328.1560.

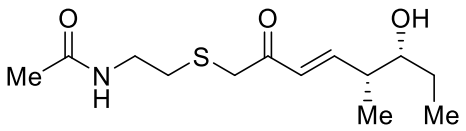


(±)-3-Hydroxy-1-(2-thioxothiazolidin-3-yl)pentan-1-one [(±)-S15]. To a solution of thiazolidinethione **S14** (1.06 g, 6.55 mmol, 1.00 equiv) in CH_2Cl_2 (30 mL) at –40 °C was added $TiCl_4$ (0.79 mL, 7.2 mmol, 1.1 equiv). Hünig's base (*i*-Pr_2NEt) (1.37 mL, 7.86 mmol, 1.20 equiv) was added after stirring at –40 °C for 30 min. The reaction mixture was stirred at –40 °C for another 2 h, and then cooled to –78 °C. Freshly distilled propionaldehyde (0.851 mL, 11.8 mmol, 1.80 equiv) was added. The reaction was stirred at –78 °C for 3 h, quenched by addition of saturated aqueous NH_4Cl (40 mL) and allowed to warm up to room temperature. The organics were extracted with CH_2Cl_2 (3 × 40 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification by flash chromatography (40% EtOAc/hexanes) afforded the title compound (825 mg, 57%) as a yellow oil: R_f = 0.14 (30% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.66–4.55 (m, 2H), 4.08–3.95 (m, 1H), 3.53 (dd, *J* = 17.6, 2.3 Hz, 1H), 3.35–3.24 (m, 3H), 2.87 (d, *J* = 3.9 Hz, 1H), 1.66–1.49 (m, 2H), 0.98 (t, *J* =

7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.9, 174.3, 69.5, 55.7, 45.4, 29.4, 28.3, 9.9; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_8H_{13}NO_2S_2Na$ 242.0280, found 242.0281.

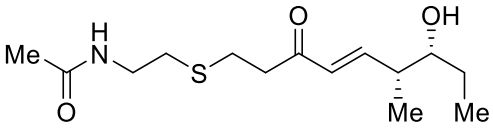


(±)-*S*-(2-Acetamidoethyl)-3-hydroxypentanethioate [(±)-5]. To a solution of thiazolidinethione (±)-**S15** (821 mg, 3.74 mmol, 1.00 equiv) in CH_2Cl_2 (30 mL) at room temperature was added imidazole (763 mg, 11.2 mmol, 3.00 equiv) and *N*-acetylcysteamine (0.44 mL, 4.1 mmol, 1.1 equiv). The reaction was stirred at room temperature for 12 h, then quenched by the addition of saturated aqueous NH_4Cl (30 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with saturated aqueous NaCl (40 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded the title compound (566 mg, 69%) as a colorless oil: R_f = 0.24 (5% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.79 (s, 1H), 4.04–3.96 (m, 1H), 3.53–3.40 (m, 2H), 3.11–2.98 (m, 2H), 2.77 (dd, *J* = 15.4, 3.3 Hz, 1H), 2.68 (dd, *J* = 15.4, 8.7 Hz, 1H), 2.61 (s, 1H), 1.97 (s, 3H), 1.61–1.45 (m, 2H), 0.97 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.6, 170.4, 70.1, 50.6, 39.3, 29.6, 28.9, 23.2, 9.8; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_9H_{17}NO_3SNa$ 242.0821, found 242.0827.



(5*R*,6*R*,*E*)-6-Hydroxy-5-methyl-1-[*N*-(2-acetamidoethyl)thio]oct-3-en-2-one (3). The enzymatic reaction was carried out with 4 mM diol **1a** (5.8 mg, 0.020 mmol, 1.0 equiv, dissolved in 200 μL 1: 1 DMSO: H_2O) in 10% glycerol, 1.5 M NaCl, 0.5 M Tris (pH 8.0) and 40 μM PikKR2-DH2 (16.6 mg, 0.2 μmol, 1 mol%) in a total volume of 5 mL. The reaction mixture was shaken at room temperature at a speed of 150 rpm for 24 h and then extracted with EtOAc (5 × 10 mL). The organic layers were combined, washed with saturated aqueous NaCl (20 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Since the enzymatic product and starting

material had the same R_f values, seperation of enzymatic product was accomplished by converting the starting material to its acetonide. The enzymatic reaction residue was dissolved in CH_2Cl_2 (2 mL) and *O,O*-dimethoxypropane (25 μL) and PPTS (cat.) were added. After stirring at room temperature for 3 h, the reaction was quenched by the addition of saturated aqueous NH_4Cl (2 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with saturated aqueous NaCl (10 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded the title compound (2.8 mg, 51%) as colorless oil: R_f = 0.23 (5% MeOH/CH_2Cl_2); $[\alpha]_D^{22}$ = 20.0 (*c* 0.0900, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.97 (dd, *J* = 15.9, 7.6 Hz, 1H), 6.28 (dd, *J* = 15.9, 1.3 Hz, 1H), 6.04 (s, 1H), 3.58–3.51 (m, 1H), 3.48–3.36 (m, 4H), 2.66 (t, *J* = 6.3 Hz, 2H), 2.55–2.42 (m, 1H), 1.99 (s, 3H), 1.73 (s, 1H), 1.57–1.49 (m, 1H), 1.48–1.38 (m, 1H), 1.11 (d, *J* = 6.8 Hz, 3H), 0.98 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.8, 170.2, 151.7, 127.9, 75.8, 42.4, 39.0, 38.4, 32.2, 27.4, 23.3, 13.6, 10.4; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{13}H_{23}NO_3SNa$ 296.1291, found 296.1292.



(6*R*,7*R*,*E*)-7-Hydroxy-6-methyl-1-[*N*-(2-acetamidoethyl)thio]non-4-en-3-one (4). The enzymatic reaction was carried out with 4 mM diol **2a** (6.1 mg, 0.020 mmol, 1.0 equiv, dissolved in 200 μL 1: 1 DMSO: H_2O) in 10% glycerol, 1.5 M NaCl, 0.5 M Tris (pH 8.0) and 40 μM PikKR2-DH2 (16.6 mg, 0.2 μmol, 1 mol%) in a total volume of 5 mL. The reaction mixture was shaken at room temperature at a speed of 150 rpm for 24 h. The enzymatic product was extracted with EtOAc (5 × 10 mL) and the combined organic layers were washed with saturated aqueous NaCl (20 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification by flash chromatography (5% MeOH/CH_2Cl_2) afforded the title compound

(3.3 mg, 58%) as colorless oil: R_f = 0.21 (5% MeOH/CH_2Cl_2); $[\alpha]_D^{22}$ = 22.0 (*c* 0.100, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.94 (dd, *J* = 16.1, 7.6 Hz, 1H), 6.13 (dd, *J* = 16.1, 1.2 Hz, 1H), 6.02 (s, 1H), 3.58–3.50 (m, 1H), 3.45 (q, *J* = 6.3 Hz, 2H), 2.93–2.87 (m, 2H), 2.84–2.78 (m, 2H), 2.67 (t, *J* = 6.5 Hz, 2H), 2.47 (sext, *J* = 6.9 Hz, 1H), 2.02–1.97 (m, 4H), 1.59–1.47 (m, 1H), 1.46–1.35 (m, 1H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.98 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 198.5, 170.4, 150.7, 129.8, 75.8, 42.3, 39.7, 38.7, 31.9, 27.3, 25.8, 23.3, 13.5, 10.4; HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ calcd for $C_{14}H_{25}NO_3SNa$ 310.1447, found 310.1438.

References

(1) Abiko, A.; Liu, J.-F.; Masamune, S. *J. Am. Chem. Soc.* **1997**, *119*, 2586.

(2) Barbier, J.; Jansen, R.; Irschik, H.; Benson, S.; Gerth, K.; Bohlendorf, B.; Hofle, G.; Reichenbach, H.; Wegner, J.; Zeilinger, C.; Kirschning, A.; Muller, R. *Angew. Chem. Int. Ed.* **2012**, *51*, 1256.

(3) Nagao, Y.; Hagiwara, Y.; Kumagai, T.; Ochiai, M.; Inoue, T.; Hashimoto, K.; Fujita, E. *J. Org. Chem.* **1986**, *51*, 2391.

(4) Nagao, Y.; Dai, W. M.; Ochiai, M.; Tsukagoshi, S.; Fujita, E. *J. Org. Chem.* **1990**, *55*, 1148.

(5) Li, Y.; Fiers, W. D.; Bernard, S. M.; Smith, J. L.; Aldrich, C. C.; Fecik, R. A. *ACS Chem. Biol.* **2014**, *9*, 2914.

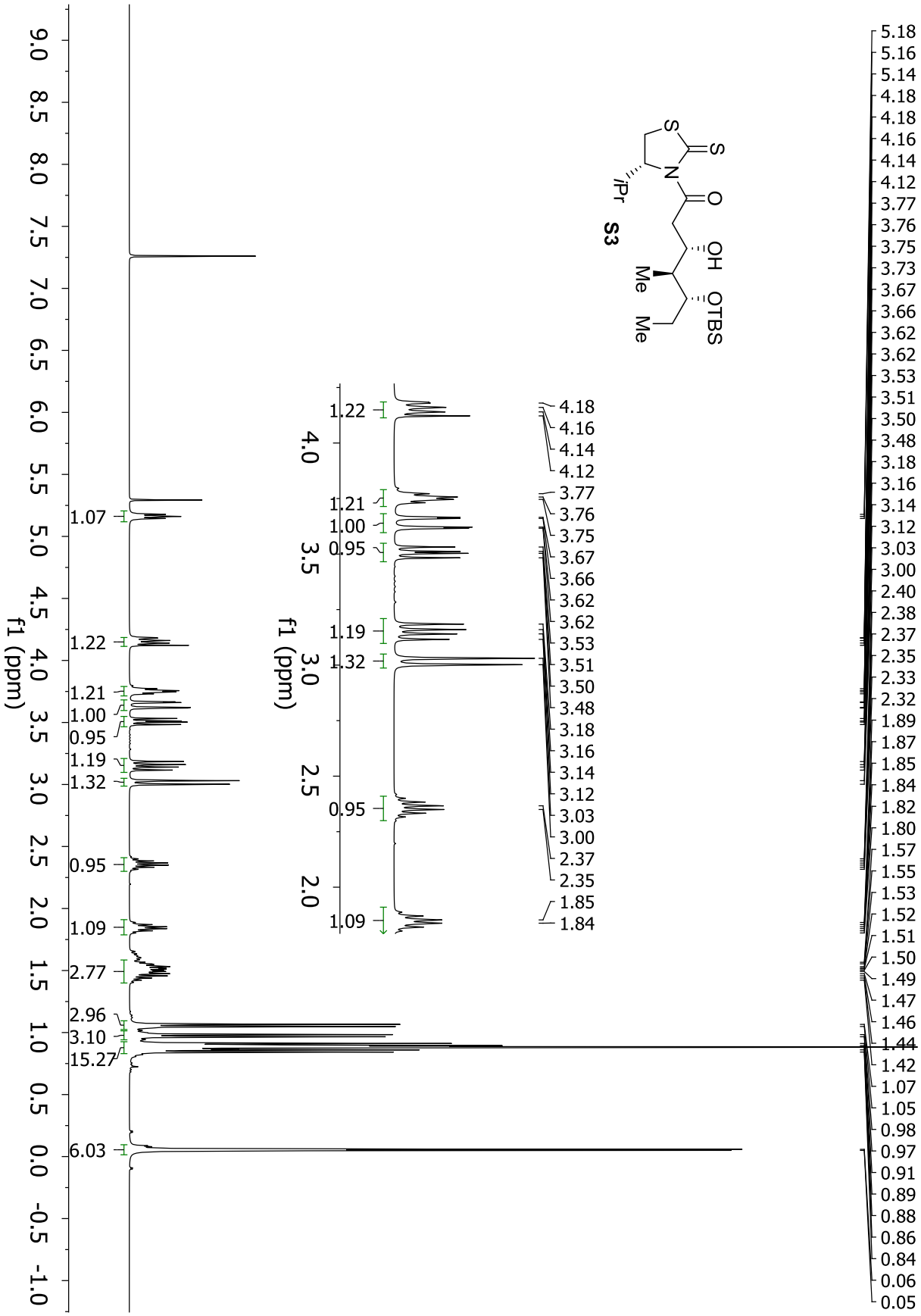
(6) Chung-hsi, L.; Yuen-hwa, Y.; Yao, L.; Yong-jun, L.; Ai-hsueh, C.; Chi-yi, H. *Tett. Lett.* **1981**, *22*, 3467.

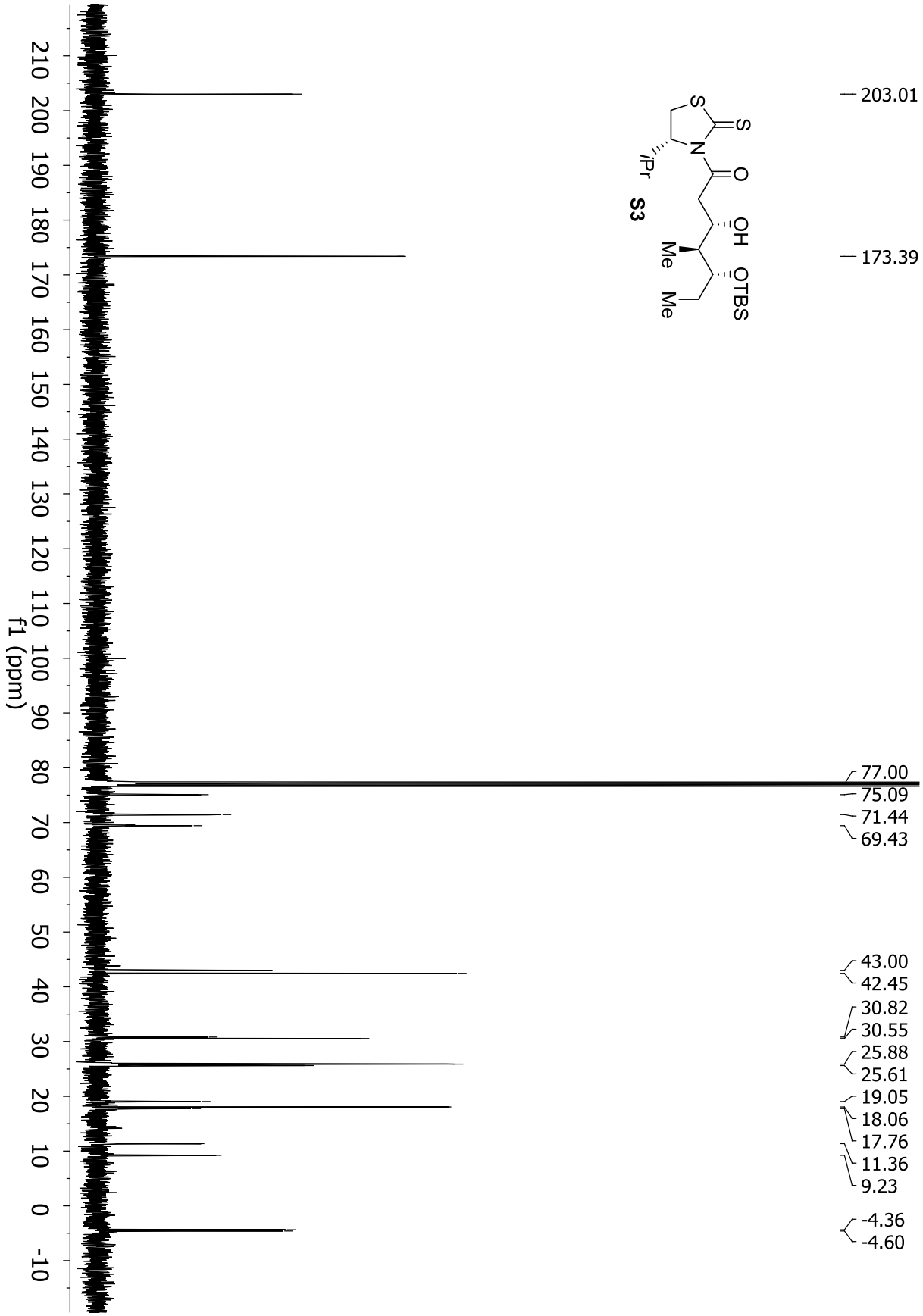
(7) Stols, L.; Gu, M.; Dieckman, L.; Raffen, R.; Collart, F. R.; Donnelly, M. I. *Protein Expr. Purif.* **2002**, *25*, 8.

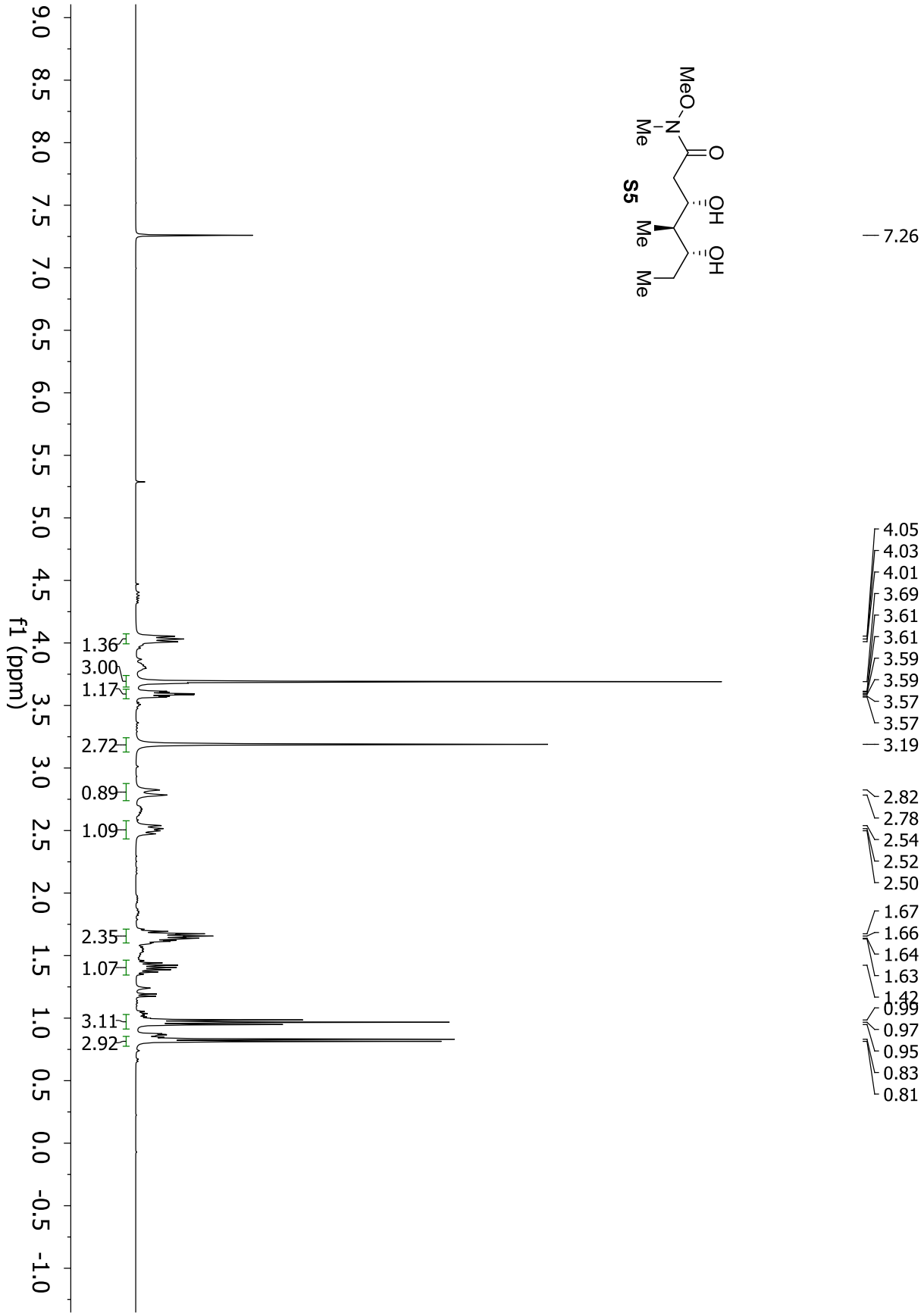
(8) Wu, S.; Zhang, Y. *Nucleic Acids Res.* **2007**, *35*, 3375.

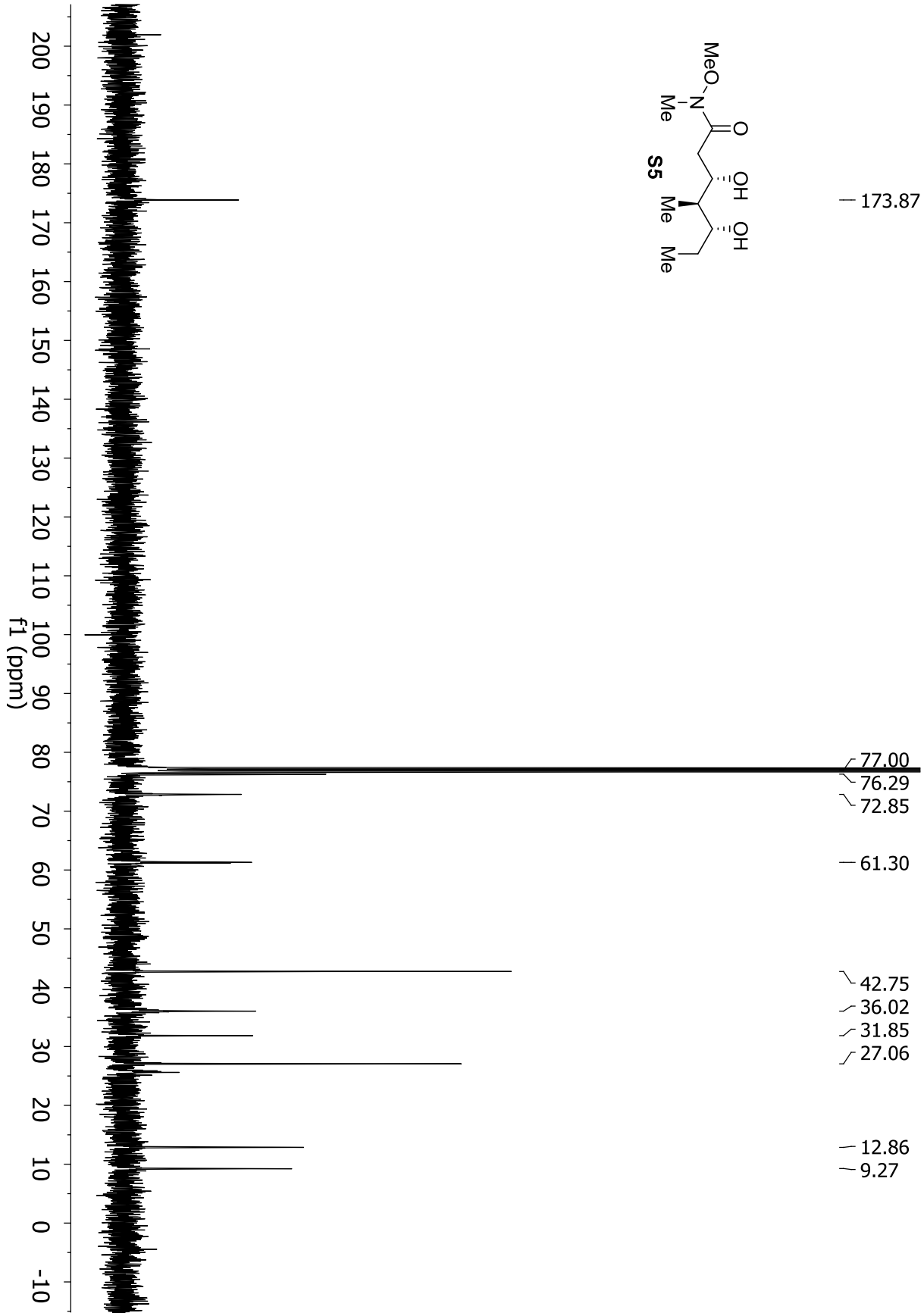
(9) Akey, D. L.; Razelun, J. R.; Tehranisa, J.; Sherman, D. H.; Gerwick, W. H.; Smith, J. L. *Structure* **2010**, *18*, 94.

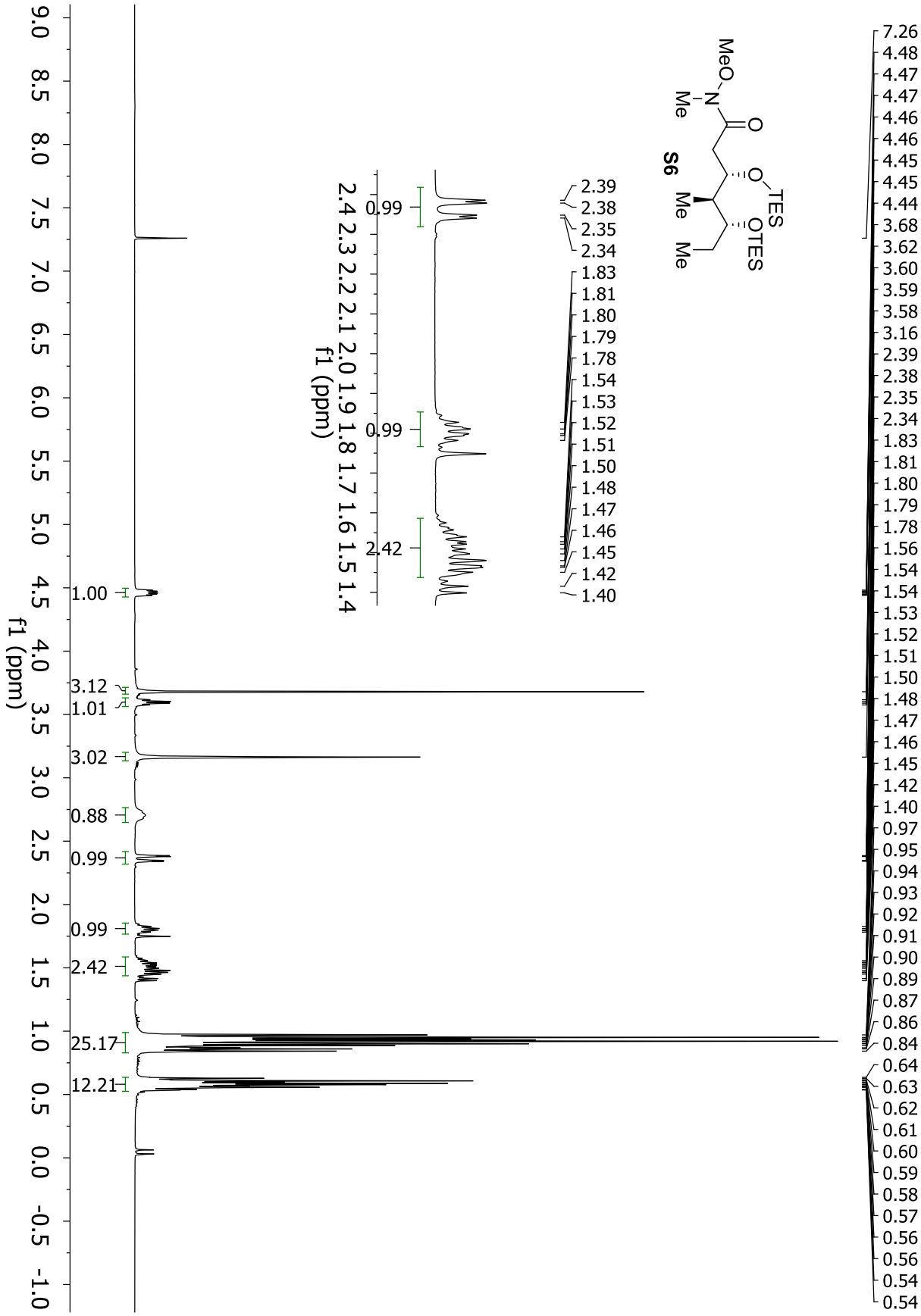
(10) Serrano, H.; Blanchard, J. S. *Biochemistry* **2013**, *52*, 5009.

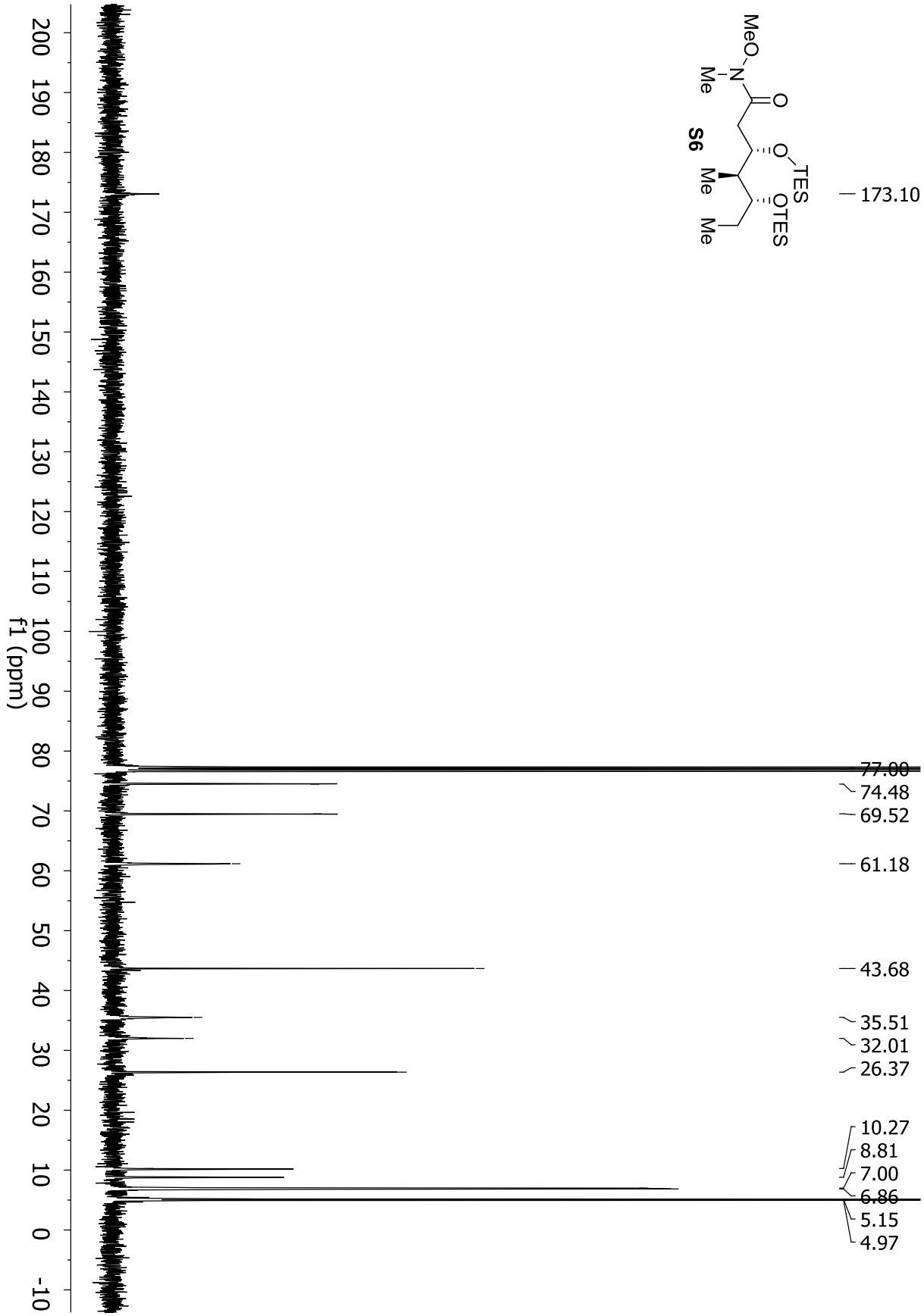

S3
iPr
S
S
N
O
OH
Me
OTBS
Me
f1 (ppm)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
1.07
1.22
1.21
1.00
0.95
1.19
1.32
0.95
1.09
2.77
2.96
3.10
15.27
6.03
4.0 3.5 3.0 2.5 2.0
1.22
1.21
1.00
0.95
1.19
1.32
0.95
1.09
4.18 4.16 4.14 4.12
3.77 3.76 3.75 3.67 3.66 3.62 3.62 3.53 3.51 3.50 3.48 3.18 3.16 3.14 3.12 3.03 3.00 2.37 2.35
1.85 1.84
5.18 5.16 5.14 4.18 4.18 4.16 4.14 4.12 3.77 3.76 3.75 3.73 3.67 3.66 3.62 3.62 3.53 3.51 3.50 3.48 3.18 3.16 3.14 3.12 3.03 3.00 2.40 2.38 2.37 2.35 2.33 2.32 1.89 1.87 1.85 1.84 1.82 1.80 1.57 1.55 1.53 1.52 1.51 1.50 1.49 1.47 1.46 1.44 1.42 1.07 1.05 0.98 0.97 0.91 0.89 0.88 0.86 0.84 0.06 0.05

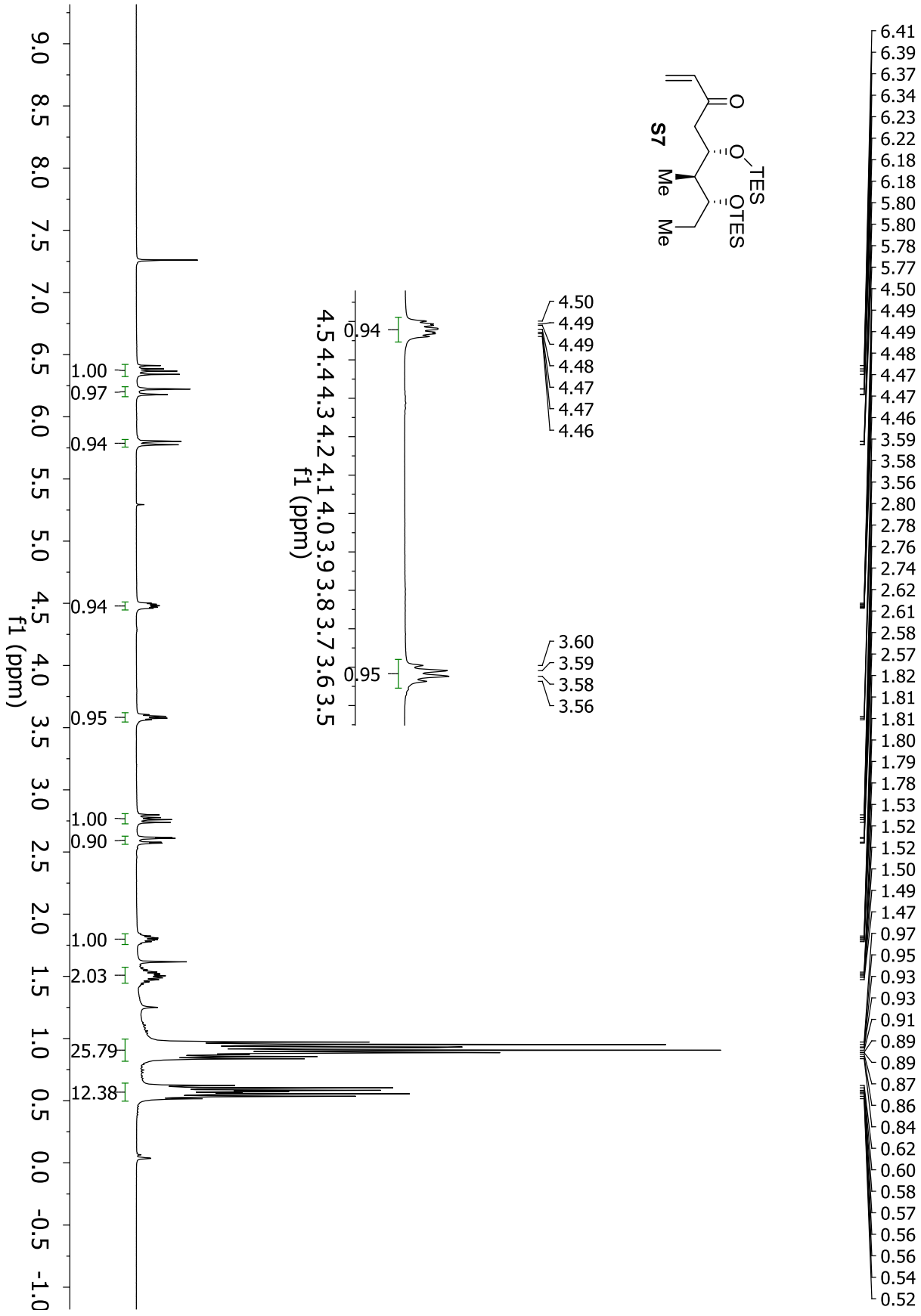

S3
iPr
Me
Me
OH
OTBS
203.01
173.39
77.00
75.09
71.44
69.43
43.00
42.45
30.82
30.55
25.88
25.61
19.05
18.06
17.76
11.36
9.23
-4.36
-4.60
f1 (ppm)

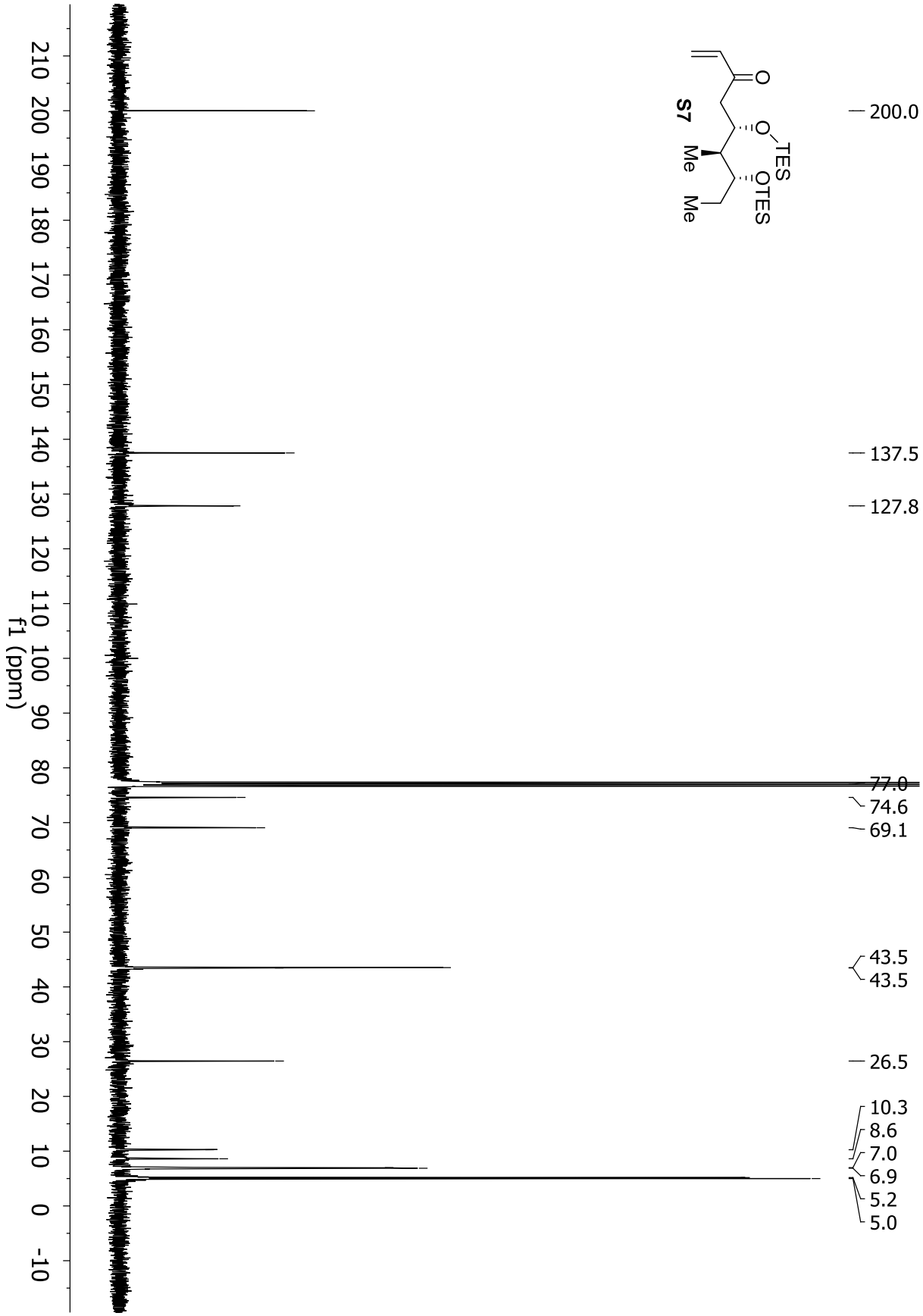

S5
MeO
N
Me
O
OH
OH
Me
Me
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
1.36
3.00
1.17
2.72
0.89
1.09
2.35
1.07
3.11
2.92
7.26
4.05
4.03
4.01
3.69
3.61
3.61
3.59
3.59
3.57
3.57
3.19
2.82
2.78
2.54
2.52
2.50
1.67
1.66
1.64
1.63
1.42
0.99
0.97
0.95
0.83
0.81

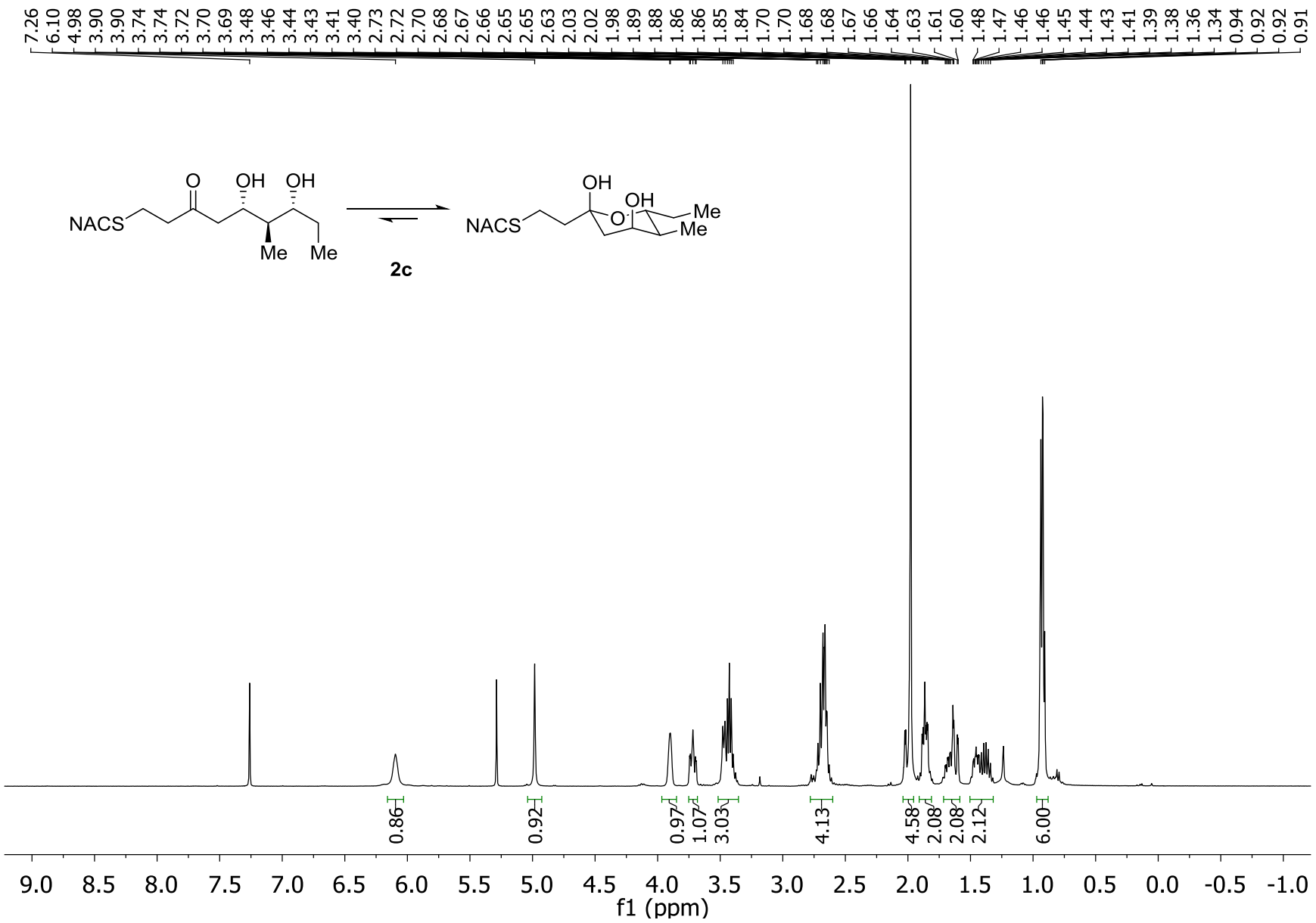

MeO
N
Me
O
OH
OH
Me
Me
Me
S5
173.87
77.00
76.29
72.85
61.30
42.75
36.02
31.85
27.06
12.86
9.27
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

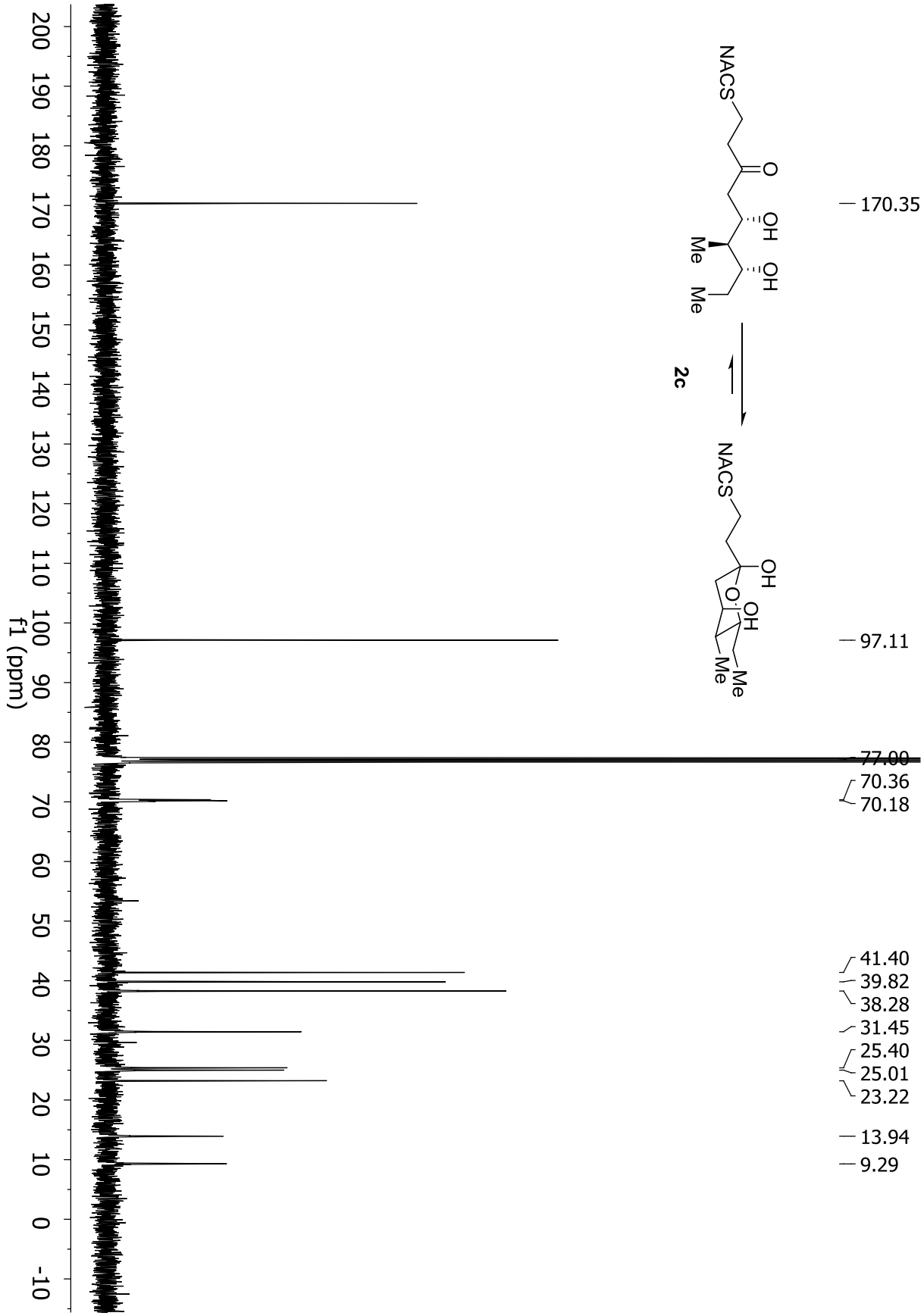

S6
MeO
N
Me
O
O
TES
OTES
Me
Me
f1 (ppm)
1.00
3.12
1.01
3.02
0.88
0.99
0.99
2.42
25.17
12.21
0.99
0.99
2.42
7.26
4.48
4.47
4.47
4.46
4.46
4.45
4.45
4.44
3.68
3.62
3.60
3.59
3.58
3.16
2.39
2.38
2.35
2.34
1.83
1.81
1.80
1.79
1.78
1.56
1.54
1.54
1.53
1.52
1.51
1.50
1.48
1.47
1.46
1.45
1.42
1.40
0.97
0.95
0.94
0.93
0.92
0.91
0.90
0.89
0.87
0.86
0.84
0.64
0.63
0.62
0.61
0.60
0.59
0.58
0.57
0.56
0.56
0.54
0.54

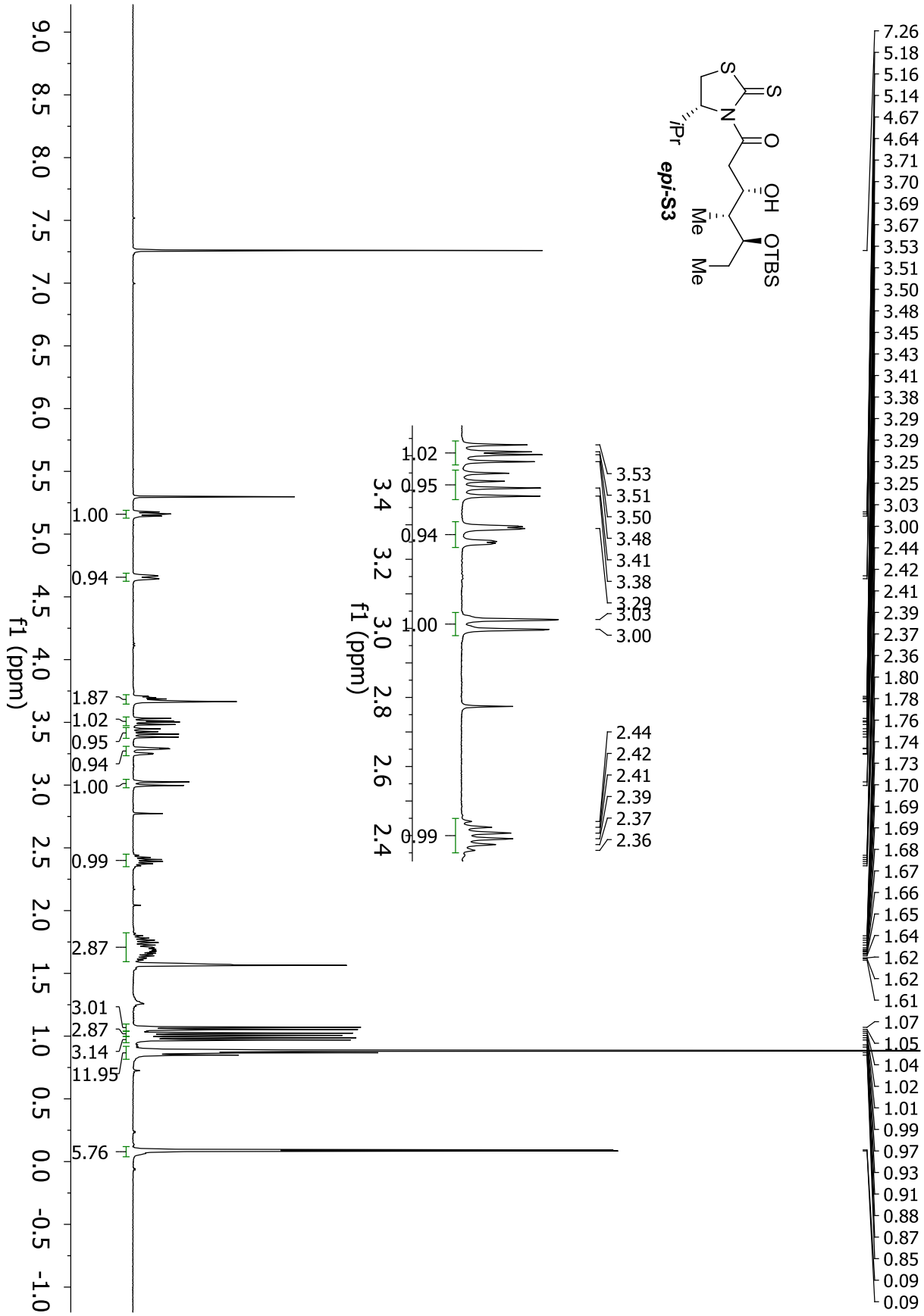

S6
MeO
N
Me
O
O
TES
OTES
Me
Me
173.10
77.00
74.48
69.52
61.18
43.68
35.51
32.01
26.37
10.27
8.81
7.00
6.86
5.15
4.97
f1 (ppm)

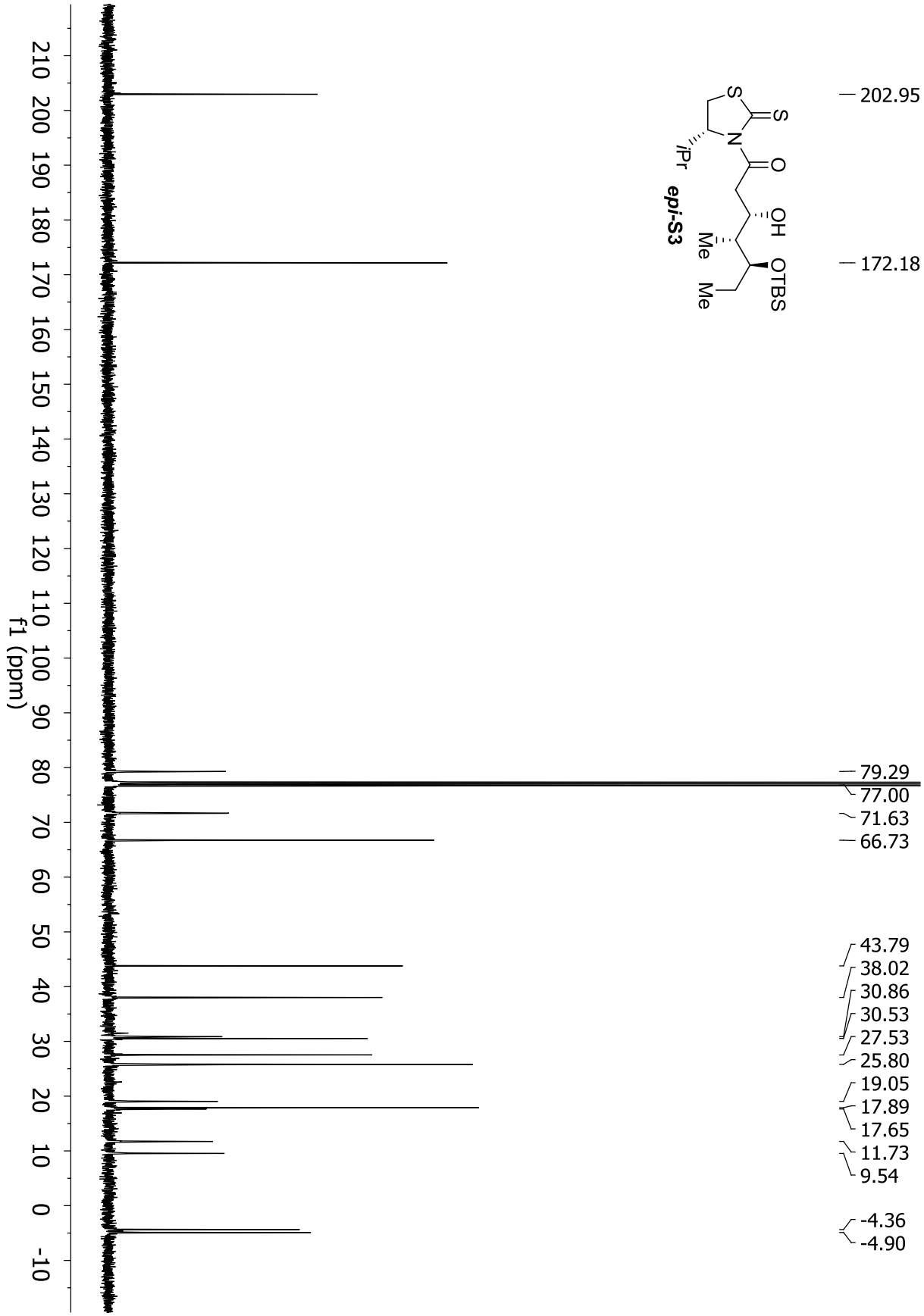

S7
Me
Me
TES
OTES
O
O
f1 (ppm)
1.00
0.97
0.94
0.94
0.95
1.00
0.90
1.00
2.03
25.79
12.38
0.94
0.95
4.50
4.49
4.49
4.48
4.47
4.47
4.46
3.60
3.59
3.58
3.56
6.41
6.39
6.37
6.34
6.23
6.22
6.18
6.18
5.80
5.80
5.78
5.77
4.50
4.49
4.49
4.48
4.47
4.47
4.46
3.59
3.58
3.56
2.80
2.78
2.76
2.74
2.62
2.61
2.58
2.57
1.82
1.81
1.81
1.80
1.79
1.78
1.53
1.52
1.52
1.50
1.49
1.47
0.97
0.95
0.93
0.93
0.91
0.89
0.89
0.87
0.86
0.84
0.62
0.60
0.58
0.57
0.56
0.56
0.54
0.52

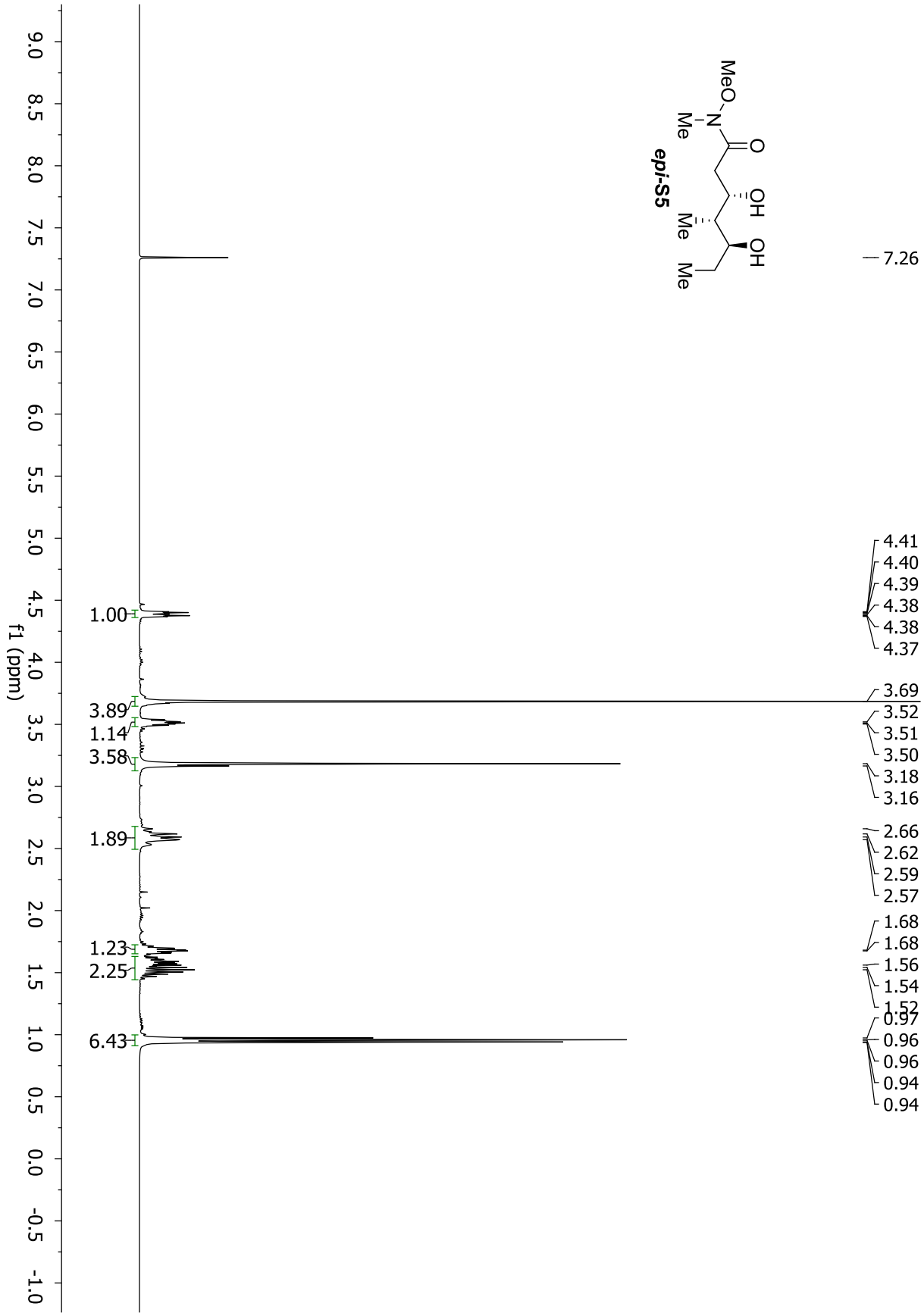

S7
O
O
TES
OTES
Me
Me
200.0
137.5
127.8
77.0
74.6
69.1
43.5
43.5
26.5
10.3
8.6
7.0
6.9
5.2
5.0
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

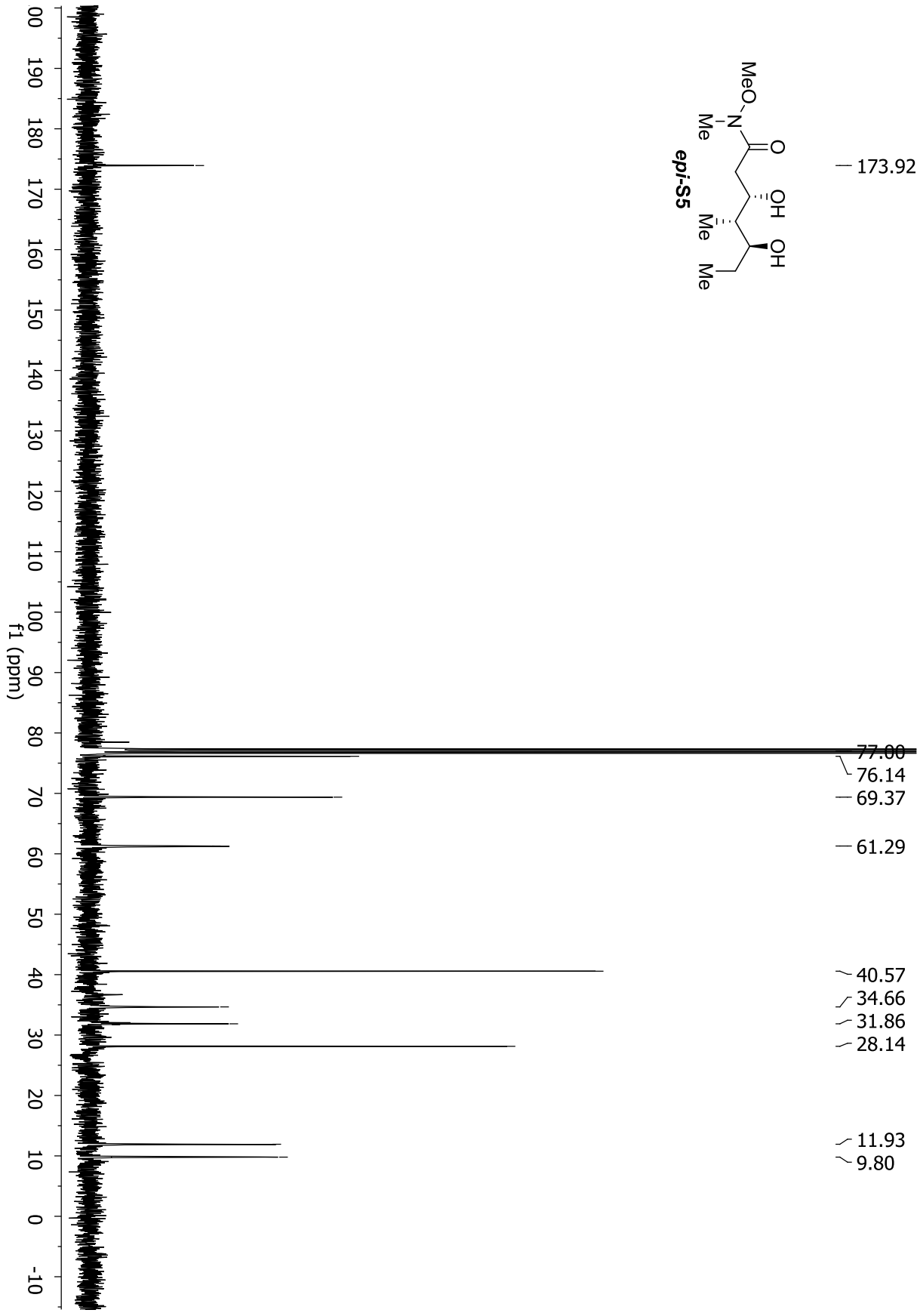

7.26
6.10
4.98
3.90
3.90
3.74
3.74
3.72
3.70
3.69
3.48
3.46
3.44
3.43
3.41
3.40
2.73
2.72
2.70
2.68
2.67
2.66
2.65
2.65
2.63
2.03
2.02
1.98
1.89
1.88
1.86
1.86
1.85
1.84
1.70
1.70
1.68
1.68
1.67
1.66
1.64
1.63
1.61
1.60
1.48
1.47
1.46
1.46
1.45
1.44
1.43
1.41
1.39
1.38
1.36
1.34
0.94
0.92
0.92
0.91
NACS
O
OH
OH
Me
Me
2c
NACS
OH
OH
O
Me
Me
0.86
0.92
0.97
1.07
3.03
4.13
4.58
2.08
2.08
2.12
6.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

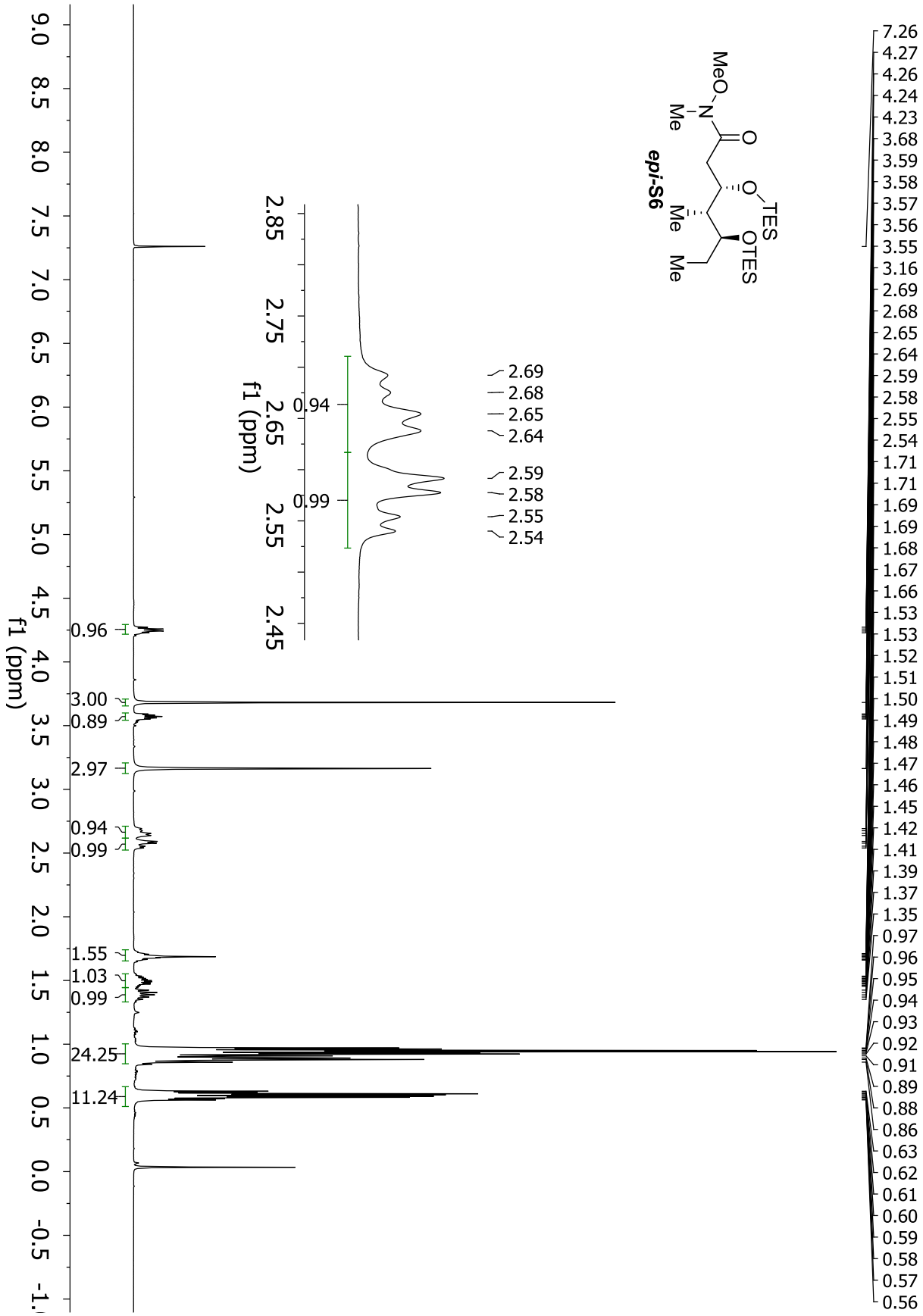

200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)
NACS
O
OH
OH
Me
Me
2c
NACS
OH
O
OH
Me
Me
170.35
97.11
77.00
70.36
70.18
41.40
39.82
38.28
31.45
25.40
25.01
23.22
13.94
9.29

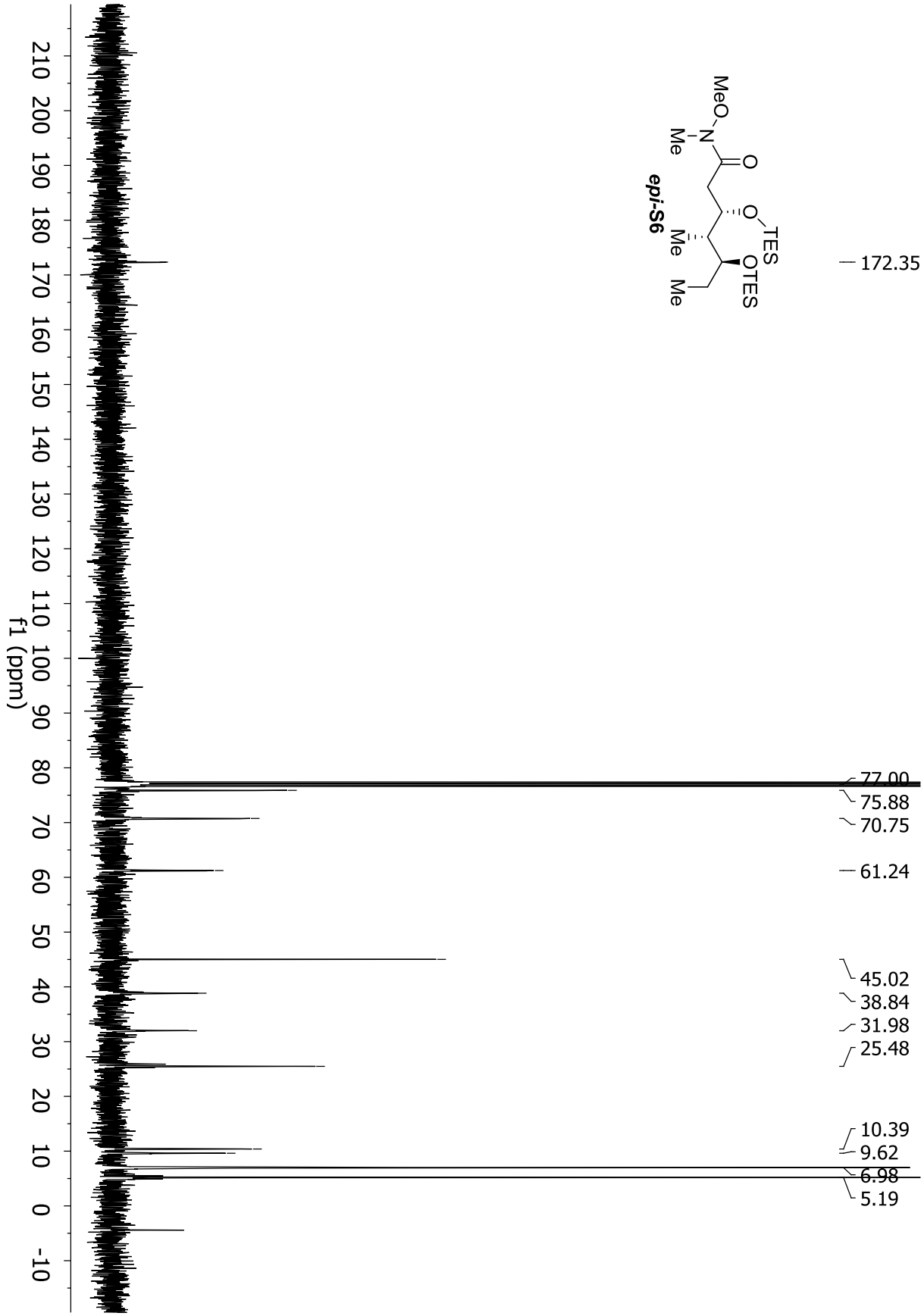

epi-S3
iPr
Me
Me
OH
OTBS
f1 (ppm)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
1.00
0.94
1.87
1.02
0.95
0.94
1.00
0.99
2.87
3.01
2.87
3.14
11.95
5.76
3.4 3.2 3.0 2.8 2.6 2.4
1.02
0.95
0.94
1.00
0.99
3.53
3.51
3.50
3.48
3.41
3.38
3.29
3.03
3.00
2.44
2.42
2.41
2.39
2.37
2.36
7.26
5.18
5.16
5.14
4.67
4.64
3.71
3.70
3.69
3.67
3.53
3.51
3.50
3.48
3.45
3.43
3.41
3.38
3.29
3.29
3.25
3.25
3.03
3.00
2.44
2.42
2.41
2.39
2.37
2.36
1.80
1.78
1.76
1.74
1.73
1.70
1.69
1.69
1.68
1.67
1.66
1.65
1.64
1.62
1.62
1.61
1.07
1.05
1.04
1.02
1.01
0.99
0.97
0.93
0.91
0.88
0.87
0.85
0.09
0.09

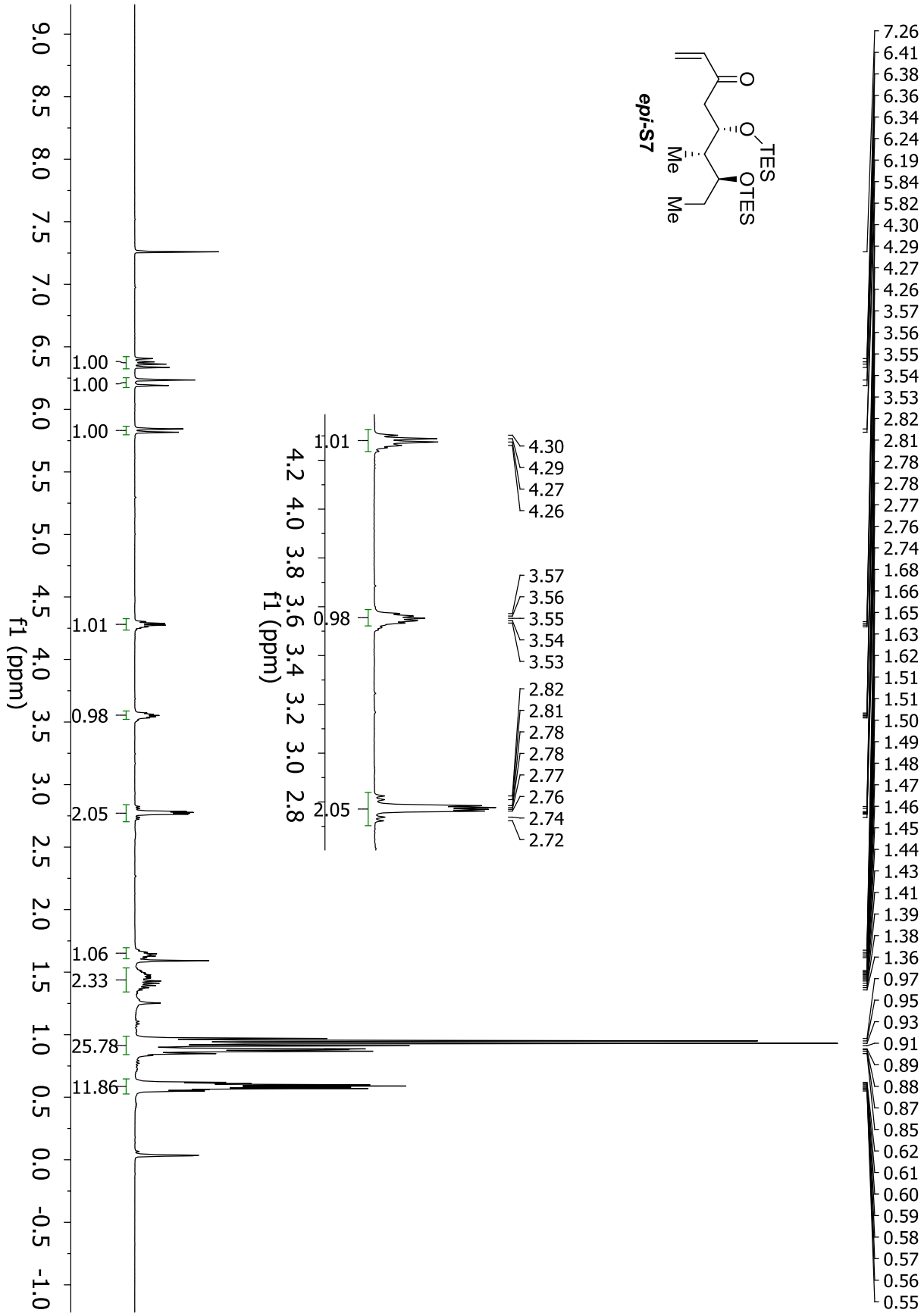

202.95
172.18
79.29
77.00
71.63
66.73
43.79
38.02
30.86
30.53
27.53
25.80
19.05
17.89
17.65
11.73
9.54
-4.36
-4.90
f1 (ppm)
epi-S3

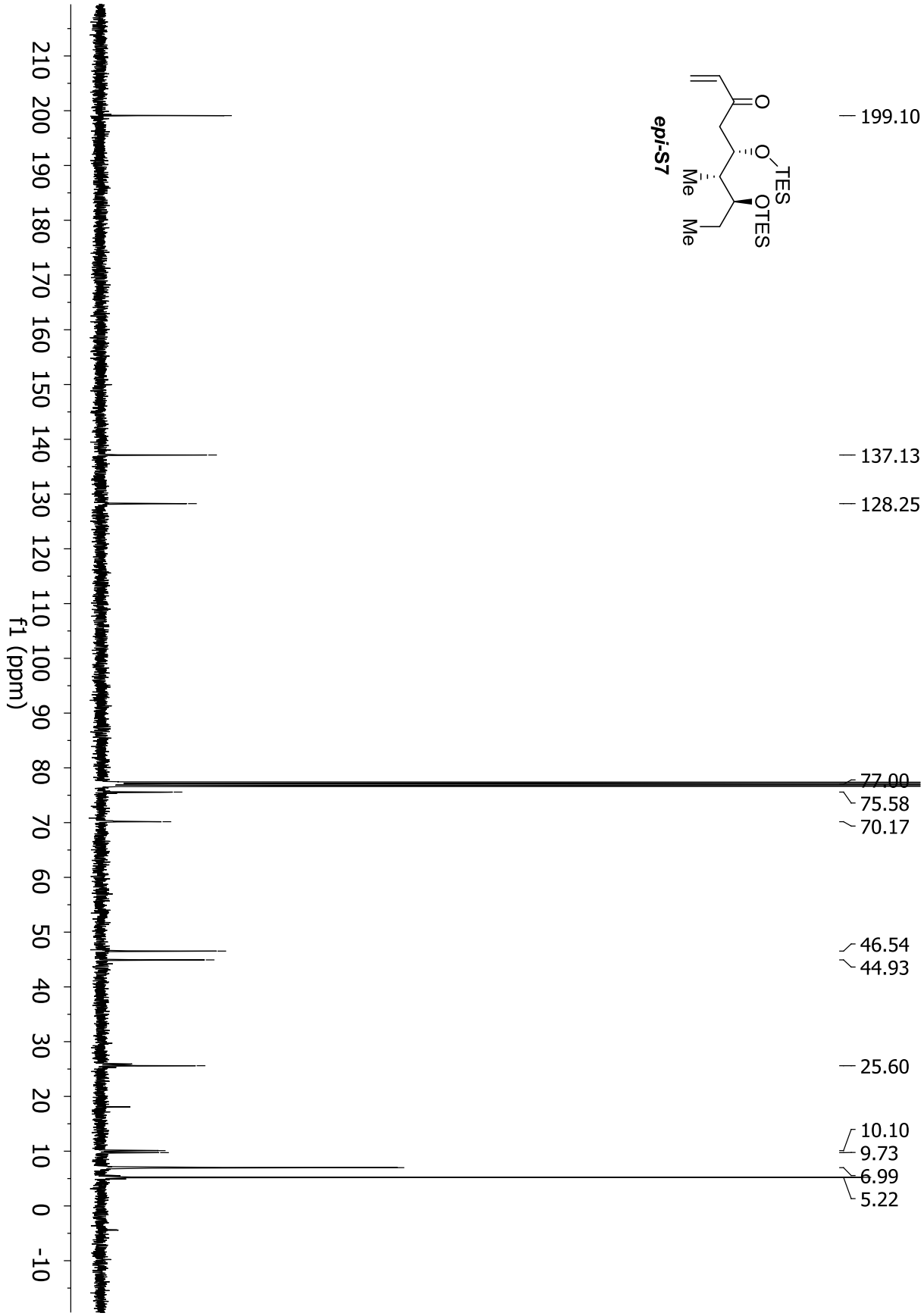

epi-S5
MeO
N
Me
O
OH
Me
OH
Me
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
1.00
3.89
1.14
3.58
1.89
1.23
2.25
6.43
7.26
4.41
4.40
4.39
4.38
4.38
4.37
3.69
3.52
3.51
3.50
3.18
3.16
2.66
2.62
2.59
2.57
1.68
1.68
1.56
1.54
1.52
0.97
0.96
0.96
0.94
0.94

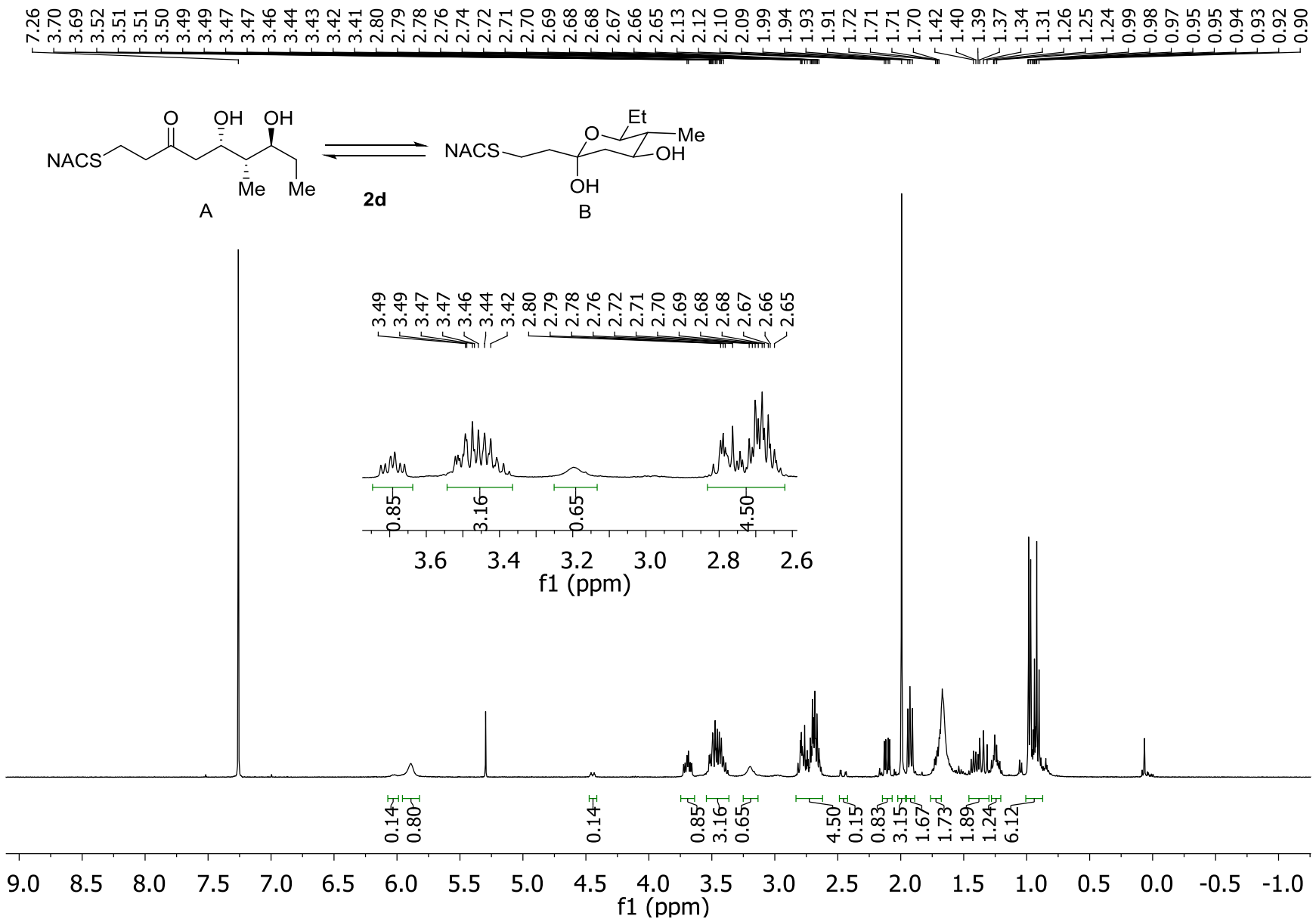

epi-S5
MeO
N
Me
O
OH
Me
OH
Me
173.92
77.00
76.14
69.37
61.29
40.57
34.66
31.86
28.14
11.93
9.80
f1 (ppm)

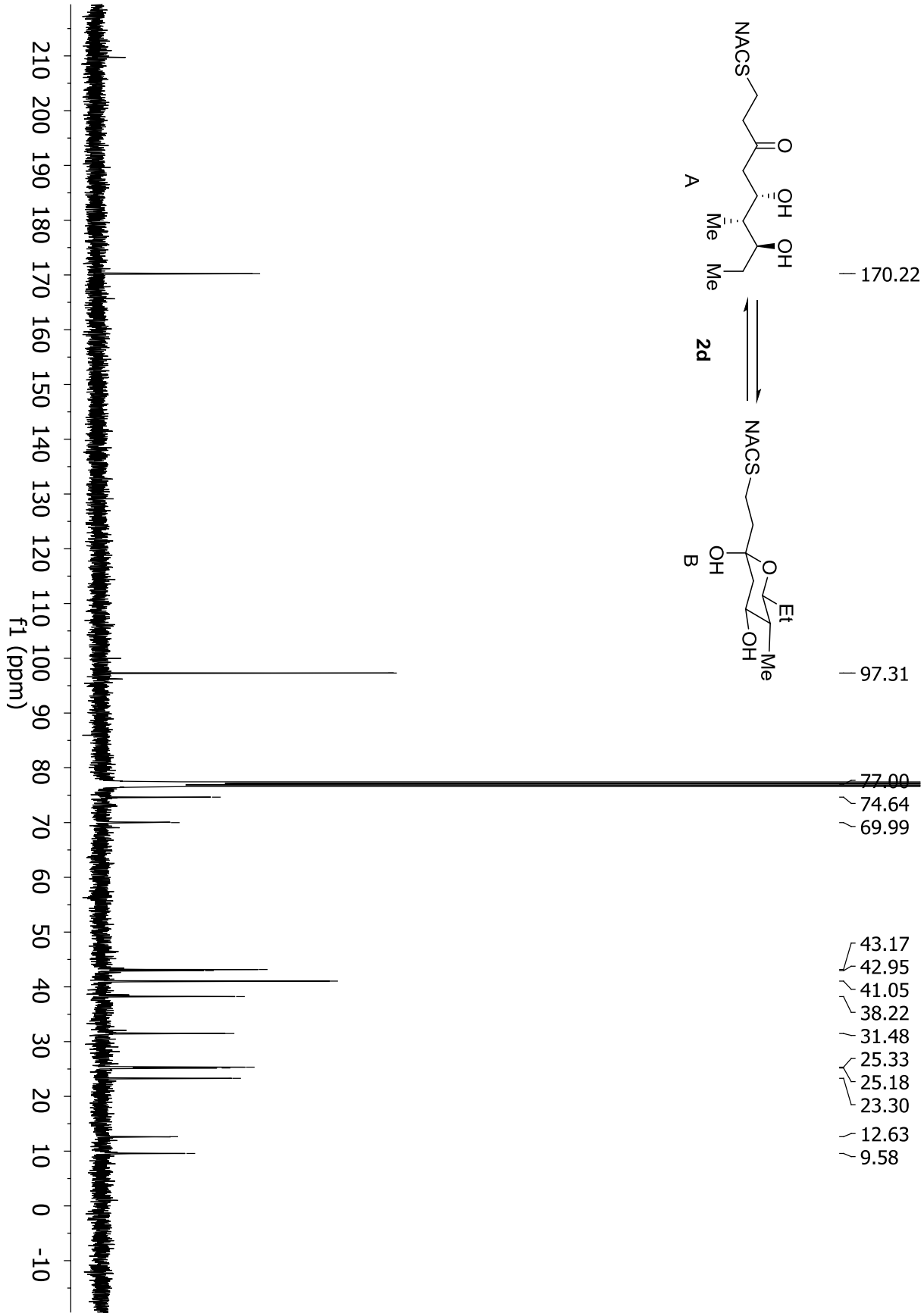

epi-S6
MeO
N
Me
O
O
TES
OTES
Me
Me
f1 (ppm)
0.96
3.00
0.89
2.97
0.94
0.99
1.55
1.03
0.99
24.25
11.24
2.69
2.68
2.65
2.64
2.59
2.58
2.55
2.54
0.94
0.99
7.26
4.27
4.26
4.24
4.23
3.68
3.59
3.58
3.57
3.56
3.55
3.16
2.69
2.68
2.65
2.64
2.59
2.58
2.55
2.54
1.71
1.71
1.69
1.69
1.68
1.67
1.66
1.53
1.53
1.52
1.51
1.50
1.49
1.48
1.47
1.46
1.45
1.42
1.41
1.39
1.37
1.35
0.97
0.96
0.95
0.94
0.93
0.92
0.91
0.89
0.88
0.86
0.63
0.62
0.61
0.60
0.59
0.58
0.57
0.56

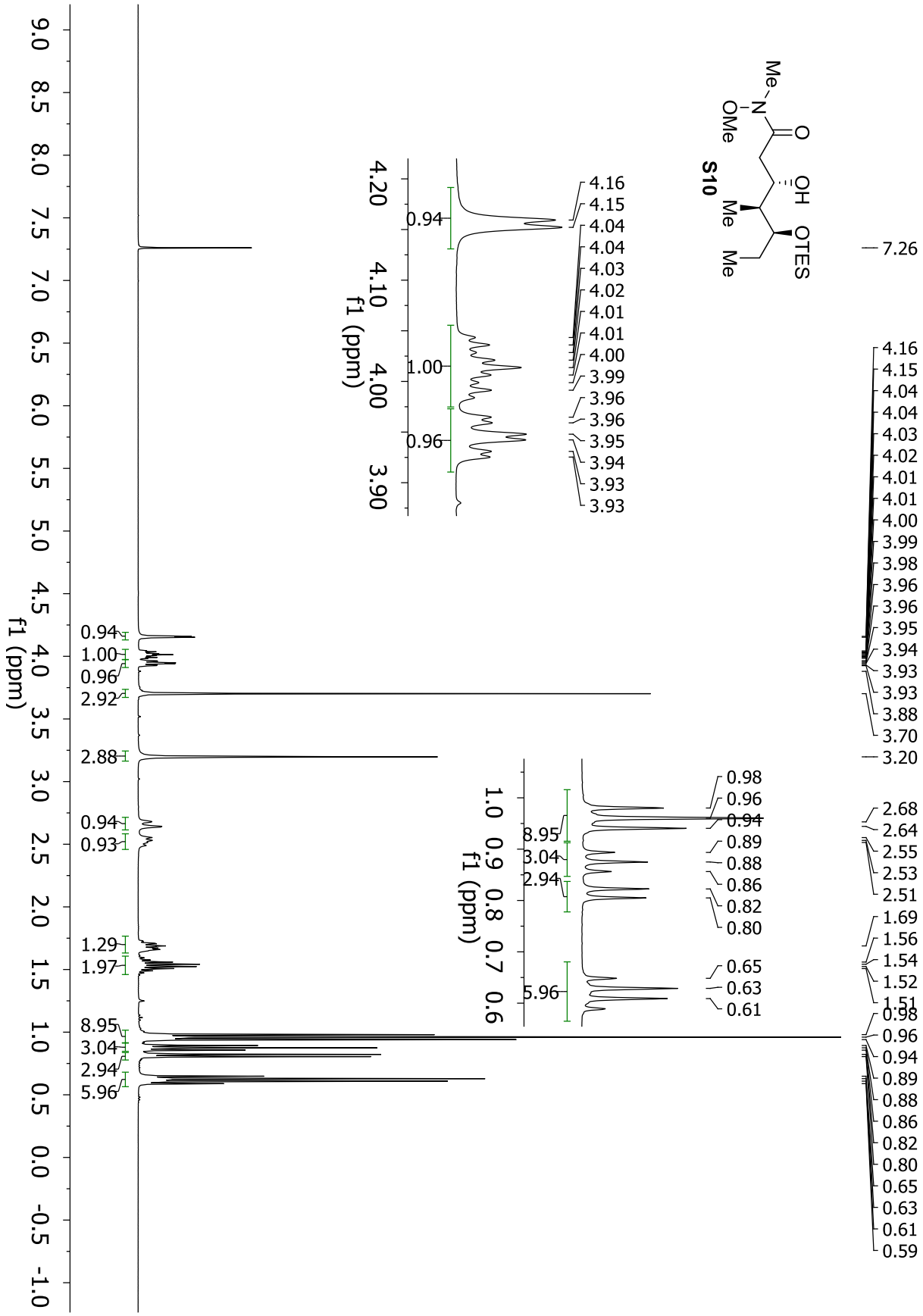

MeO
N
Me
O
O
TES
Me
OTES
Me
epi-S6
172.35
77.00
75.88
70.75
61.24
45.02
38.84
31.98
25.48
10.39
9.62
6.98
5.19
f1 (ppm)

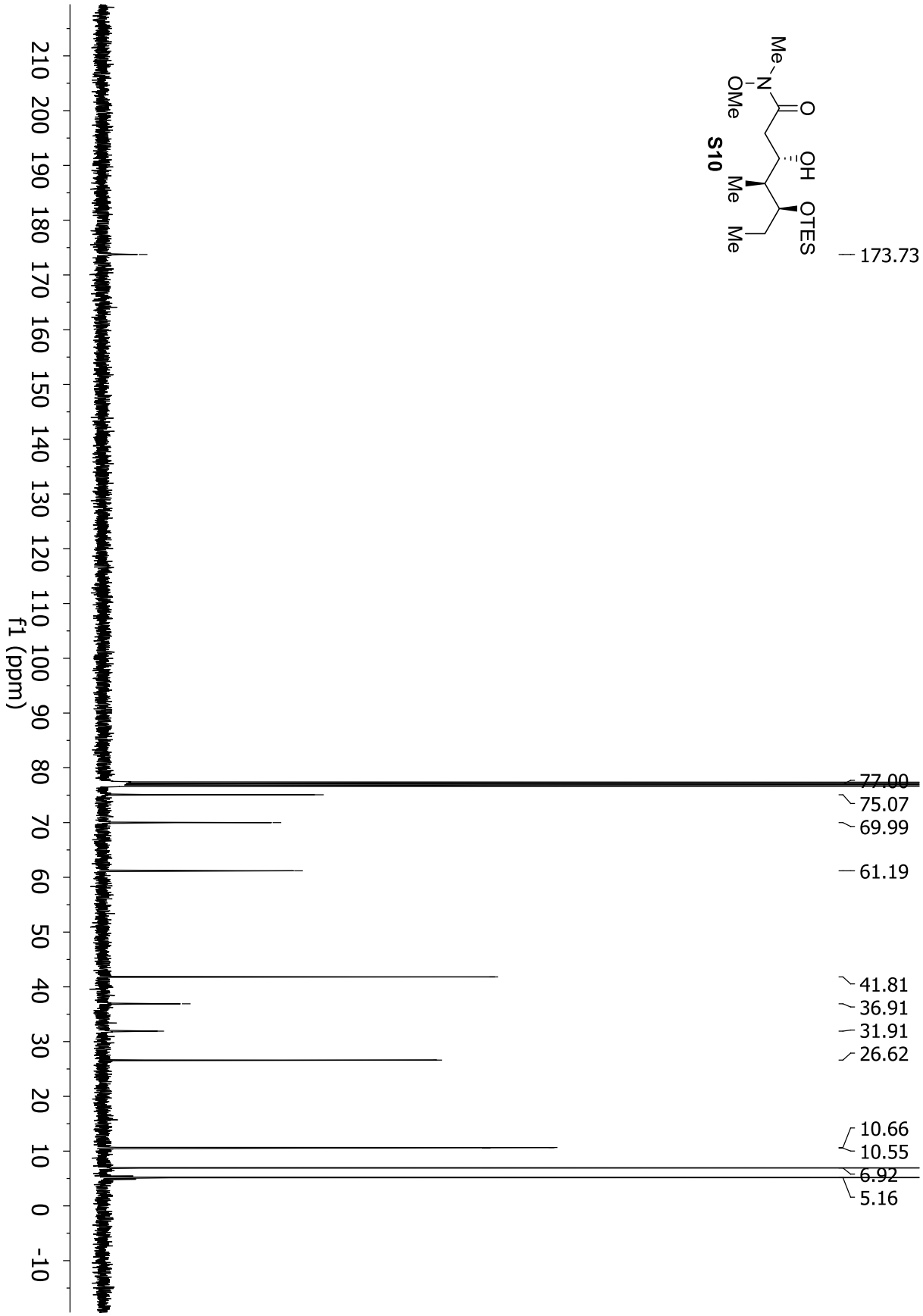

epi-S7
TES
OTES
Me
Me
f1 (ppm)
1.00
1.00
1.00
1.01
0.98
2.05
1.06
2.33
25.78
11.86
1.01
0.98
2.05
4.30
4.29
4.27
4.26
3.57
3.56
3.55
3.54
3.53
2.82
2.81
2.78
2.78
2.77
2.76
2.74
2.72
7.26
6.41
6.38
6.36
6.34
6.24
6.19
5.84
5.82
4.30
4.29
4.27
4.26
3.57
3.56
3.55
3.54
3.53
2.82
2.81
2.78
2.78
2.77
2.76
2.74
2.72
1.68
1.66
1.65
1.63
1.62
1.51
1.51
1.50
1.49
1.48
1.47
1.46
1.45
1.44
1.43
1.41
1.39
1.38
1.36
0.97
0.95
0.93
0.91
0.89
0.88
0.87
0.85
0.62
0.61
0.60
0.59
0.58
0.57
0.56
0.55

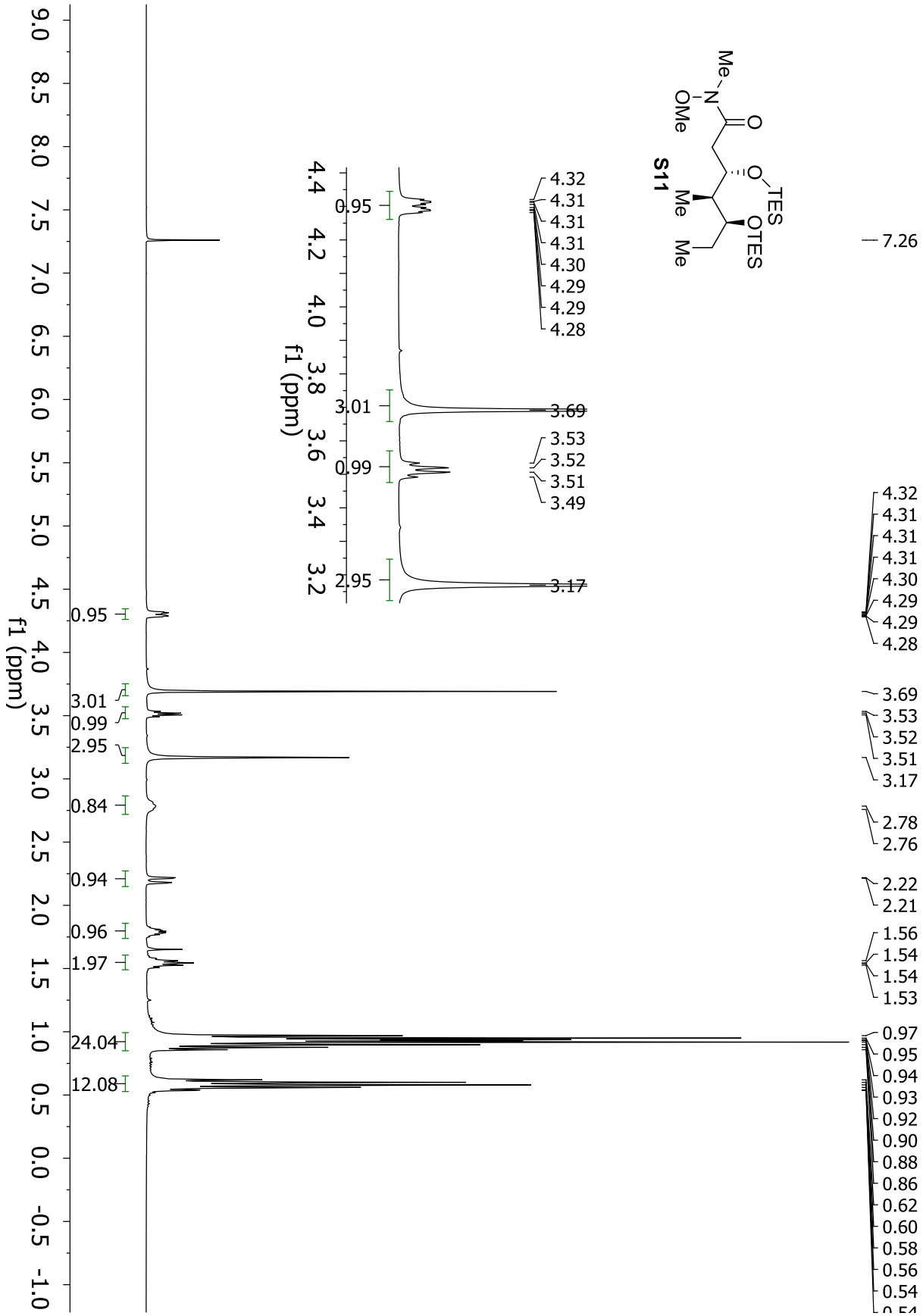

199.10
137.13
128.25
77.00
75.58
70.17
46.54
44.93
25.60
10.10
9.73
6.99
5.22
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
epi-S7
O
O
TES
OTES
Me
Me

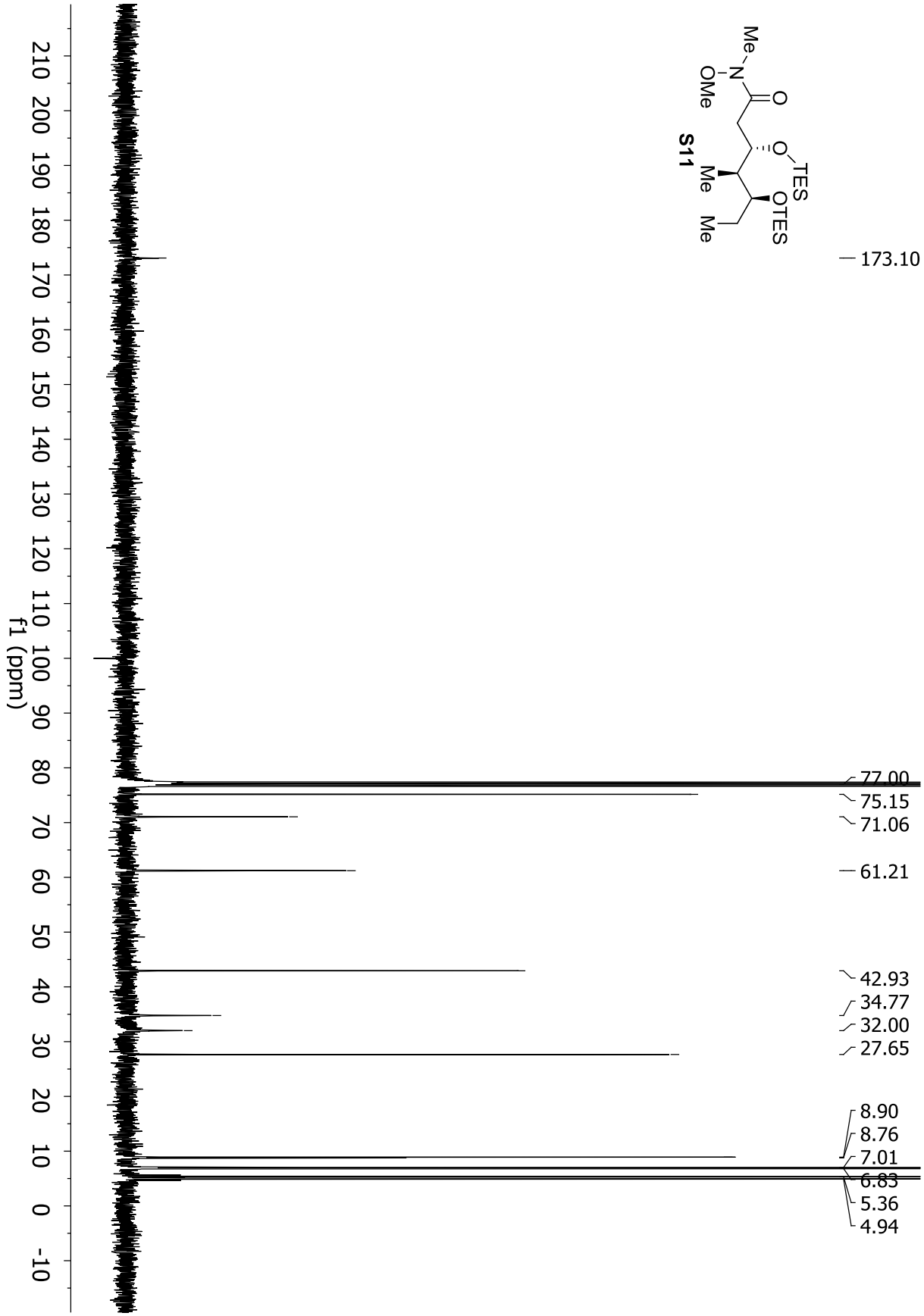

7.26 3.70 3.69 3.52 3.51 3.51 3.50 3.49 3.49 3.47 3.47 3.46 3.44 3.43 3.42 3.41 2.80 2.79 2.78 2.76 2.74 2.72 2.71 2.70 2.69 2.68 2.68 2.67 2.66 2.65 2.13 2.12 2.10 2.09 1.99 1.94 1.93 1.91 1.72 1.71 1.71 1.70 1.42 1.40 1.39 1.37 1.34 1.31 1.26 1.25 1.24 0.99 0.98 0.97 0.95 0.95 0.94 0.93 0.92 0.90
NACS
O
OH
OH
Me
Me
A
2d
NACS
Et
O
Me
OH
OH
B
3.49 3.49 3.47 3.47 3.46 3.44 3.42 2.80 2.79 2.78 2.76 2.72 2.71 2.70 2.69 2.68 2.68 2.67 2.66 2.65
0.85 3.16 0.65 4.50
3.6 3.4 3.2 3.0 2.8 2.6
f1 (ppm)
0.14 0.80 0.14 0.85 3.16 0.65 4.50 0.15 0.83 3.15 1.67 1.73 1.89 1.24 6.12
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

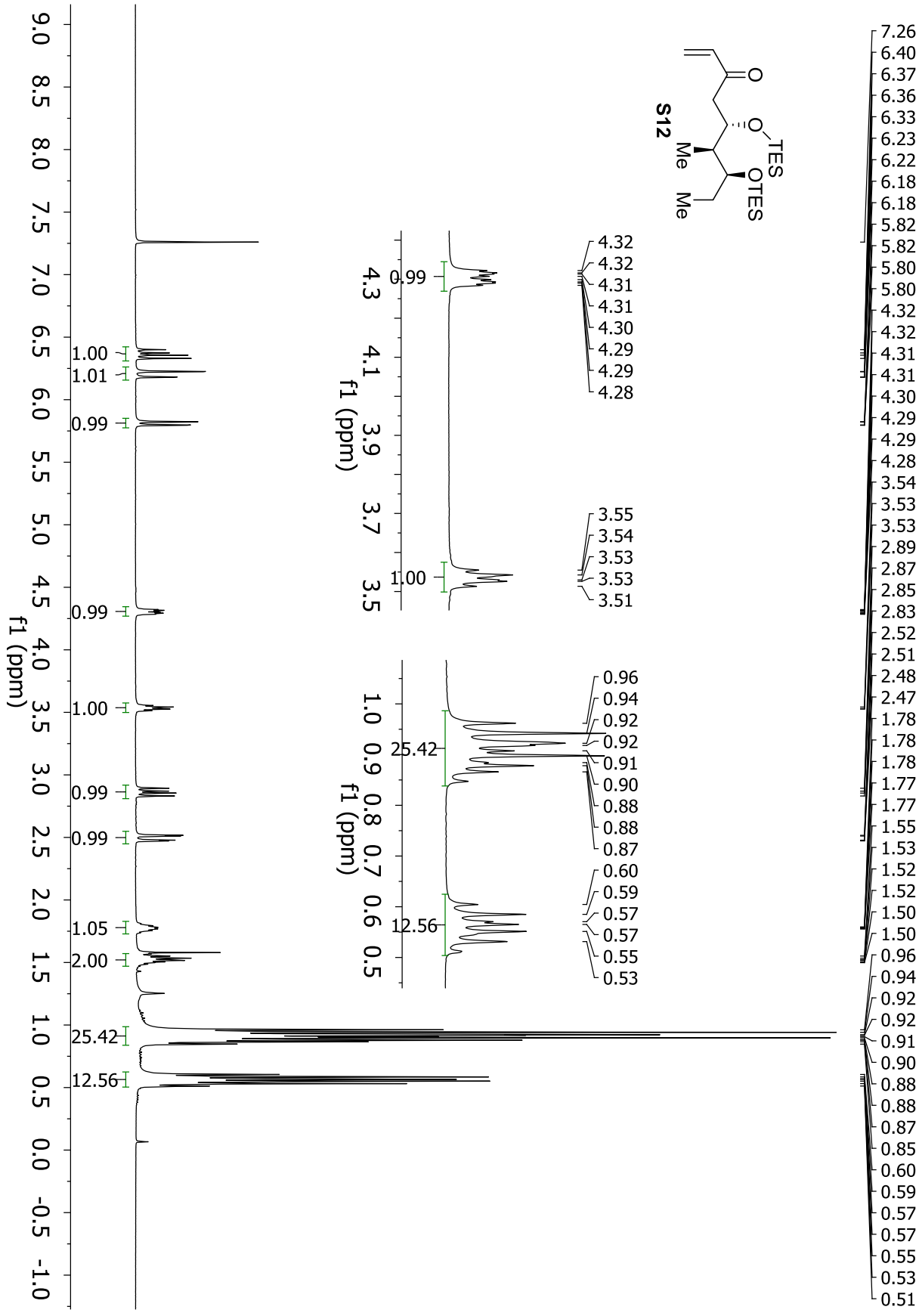

NACS
O
OH
OH
Me
Me
A
2d
NACS
OH
O
Et
Me
OH
B
170.22
97.31
77.00
74.64
69.99
43.17
42.95
41.05
38.22
31.48
25.33
25.18
23.30
12.63
9.58
f1 (ppm)

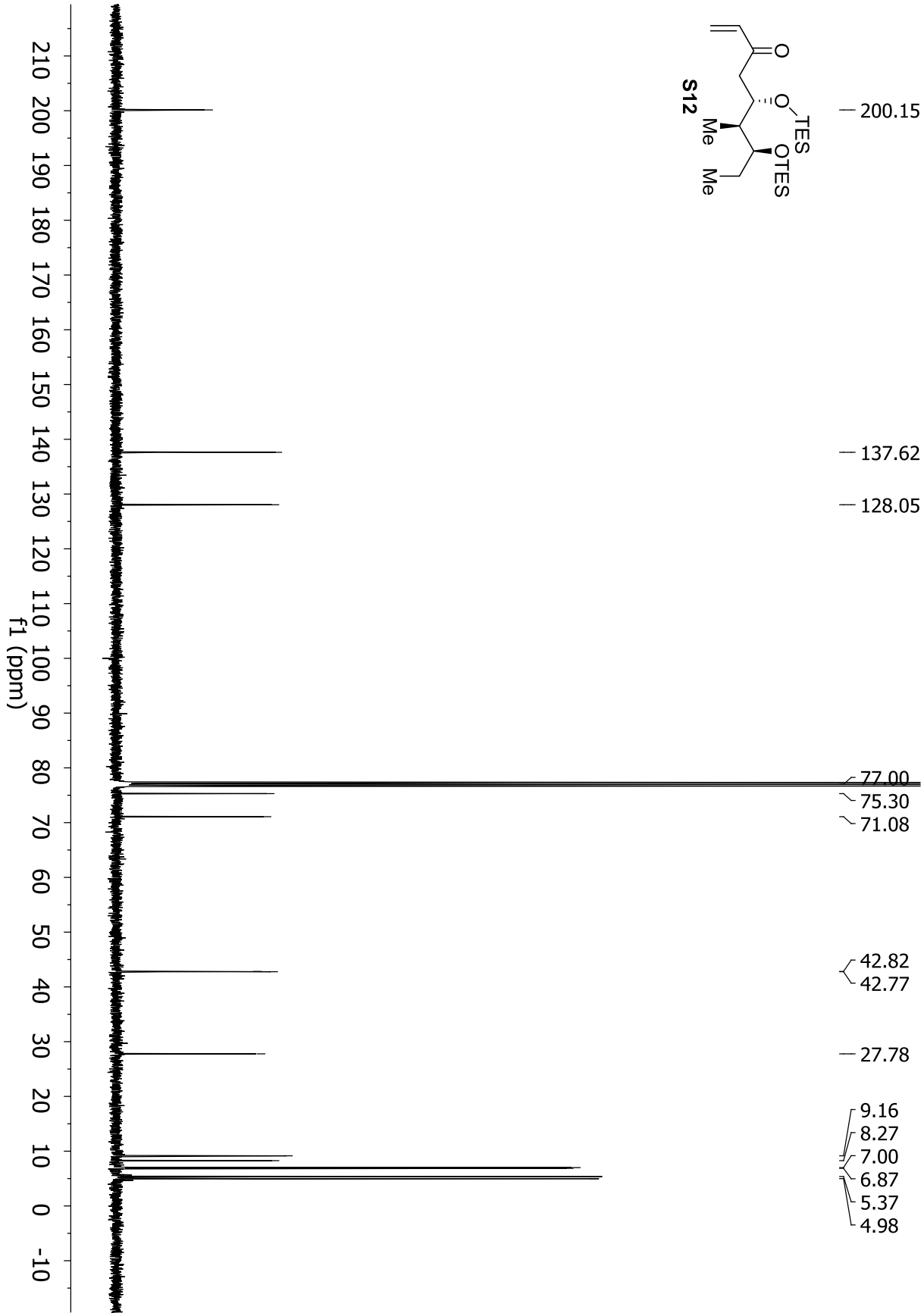

S10
Me
N
OMe
O
OH
OTES
Me
Me
f1 (ppm)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
0.94
1.00
0.96
2.92
2.88
0.94
0.93
1.29
1.97
8.95
3.04
2.94
5.96
4.20 4.10 4.00 3.90
0.94
1.00
0.96
4.16
4.15
4.04
4.04
4.03
4.02
4.01
4.01
4.00
3.99
3.96
3.96
3.95
3.94
3.93
3.93
1.0 0.9 0.8 0.7 0.6
8.95
3.04
2.94
5.96
0.98
0.96
0.94
0.89
0.88
0.86
0.82
0.80
0.65
0.63
0.61
7.26
4.16
4.15
4.04
4.04
4.03
4.02
4.01
4.01
4.00
3.99
3.98
3.96
3.96
3.95
3.94
3.93
3.93
3.88
3.70
3.20
2.68
2.64
2.55
2.53
2.51
1.69
1.56
1.54
1.52
1.51
0.98
0.96
0.94
0.89
0.88
0.86
0.82
0.80
0.65
0.63
0.61
0.59

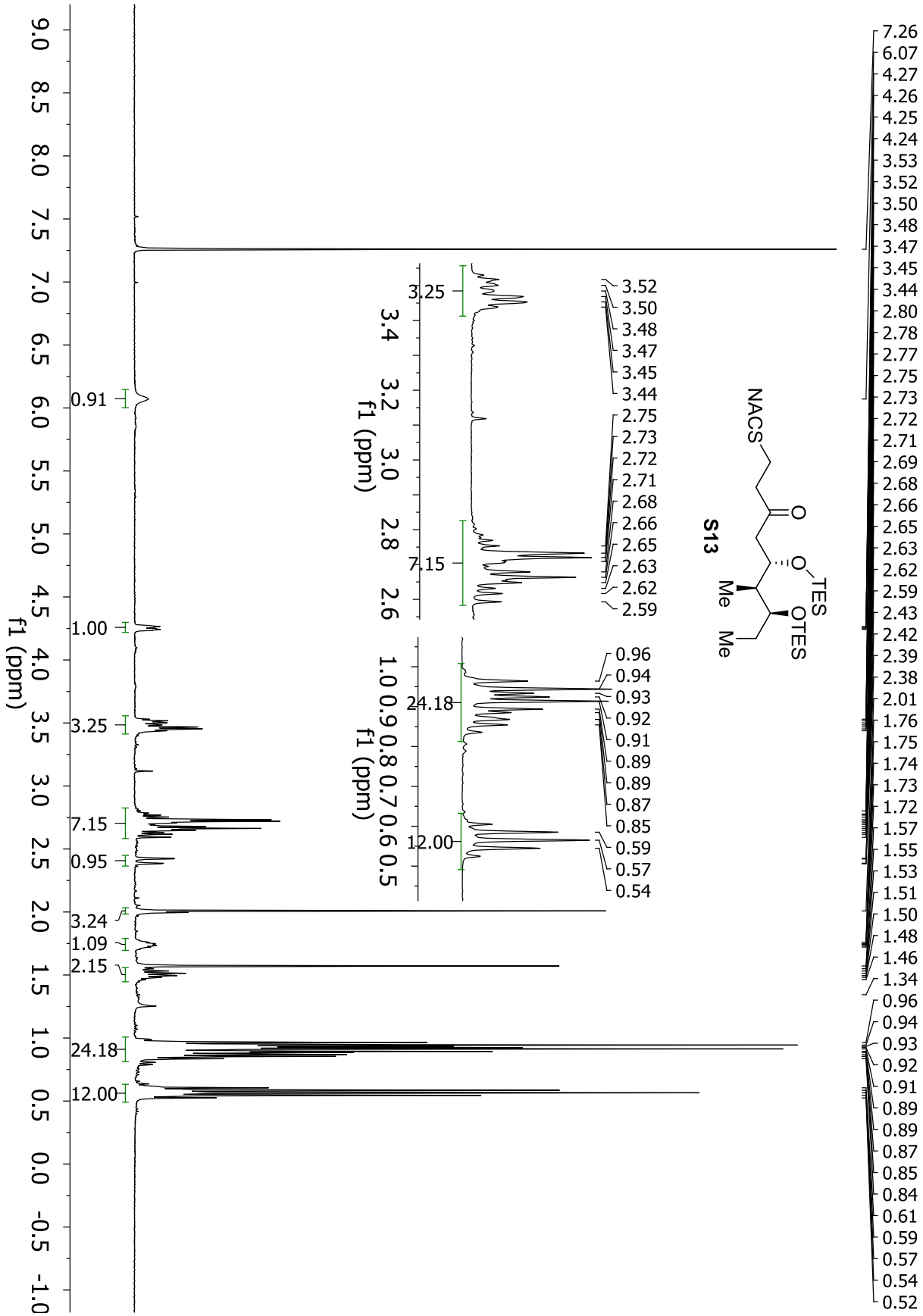

S10
Me
N
OMe
O
OH
OTES
Me
Me
Me
173.73
77.00
75.07
69.99
61.19
41.81
36.91
31.91
26.62
10.66
10.55
6.92
5.16
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

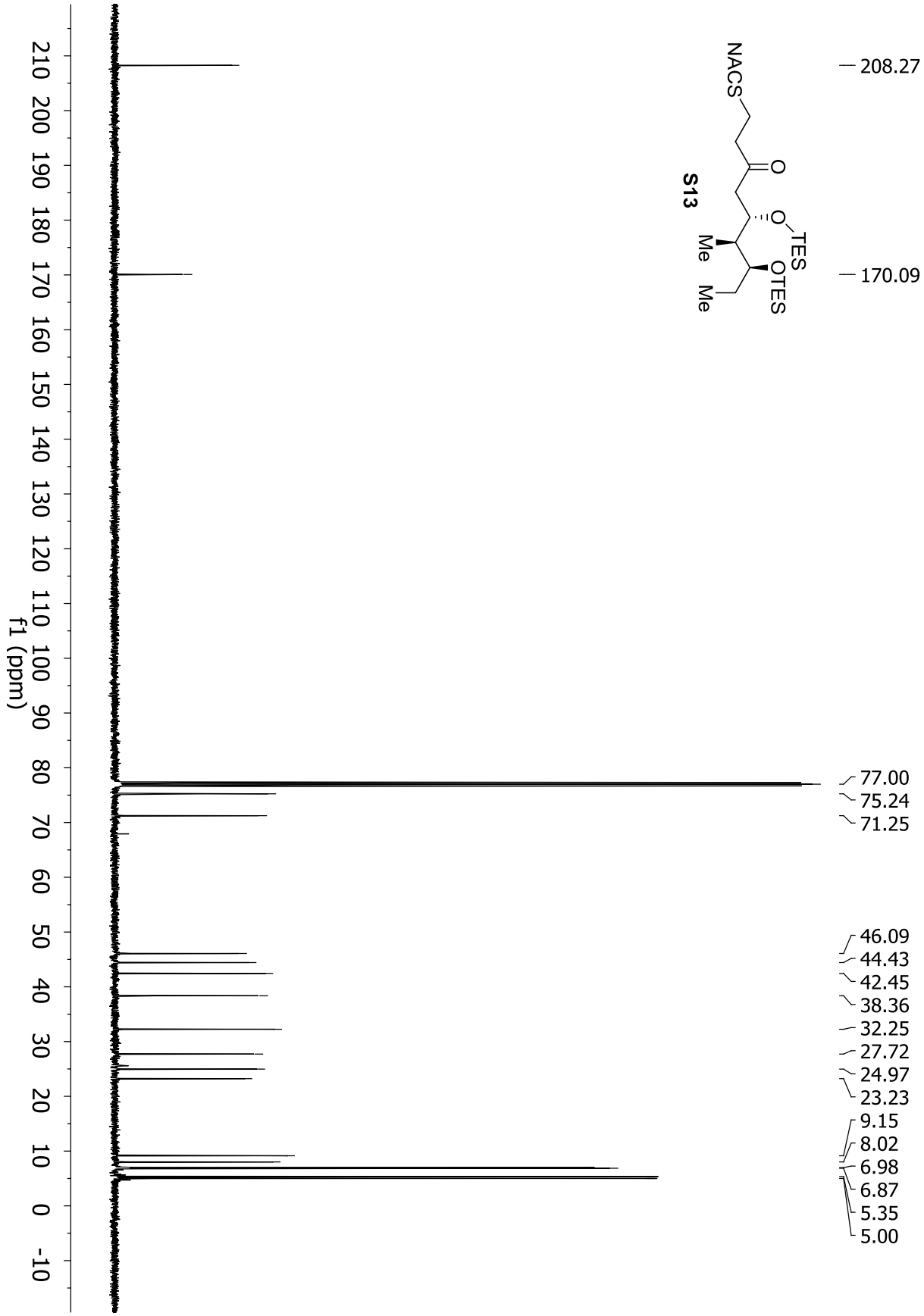

S11
Me
N
OMe
O
O
TES
OTES
Me
Me
f1 (ppm)
0.95
3.01
0.99
2.95
0.84
0.94
0.96
1.97
24.04
12.08
4.32
4.31
4.31
4.31
4.30
4.29
4.29
4.28
3.69
3.53
3.52
3.51
3.49
3.17
2.78
2.76
2.22
2.21
1.56
1.54
1.54
1.53
0.97
0.95
0.94
0.93
0.92
0.90
0.88
0.86
0.62
0.60
0.58
0.56
0.54
7.26

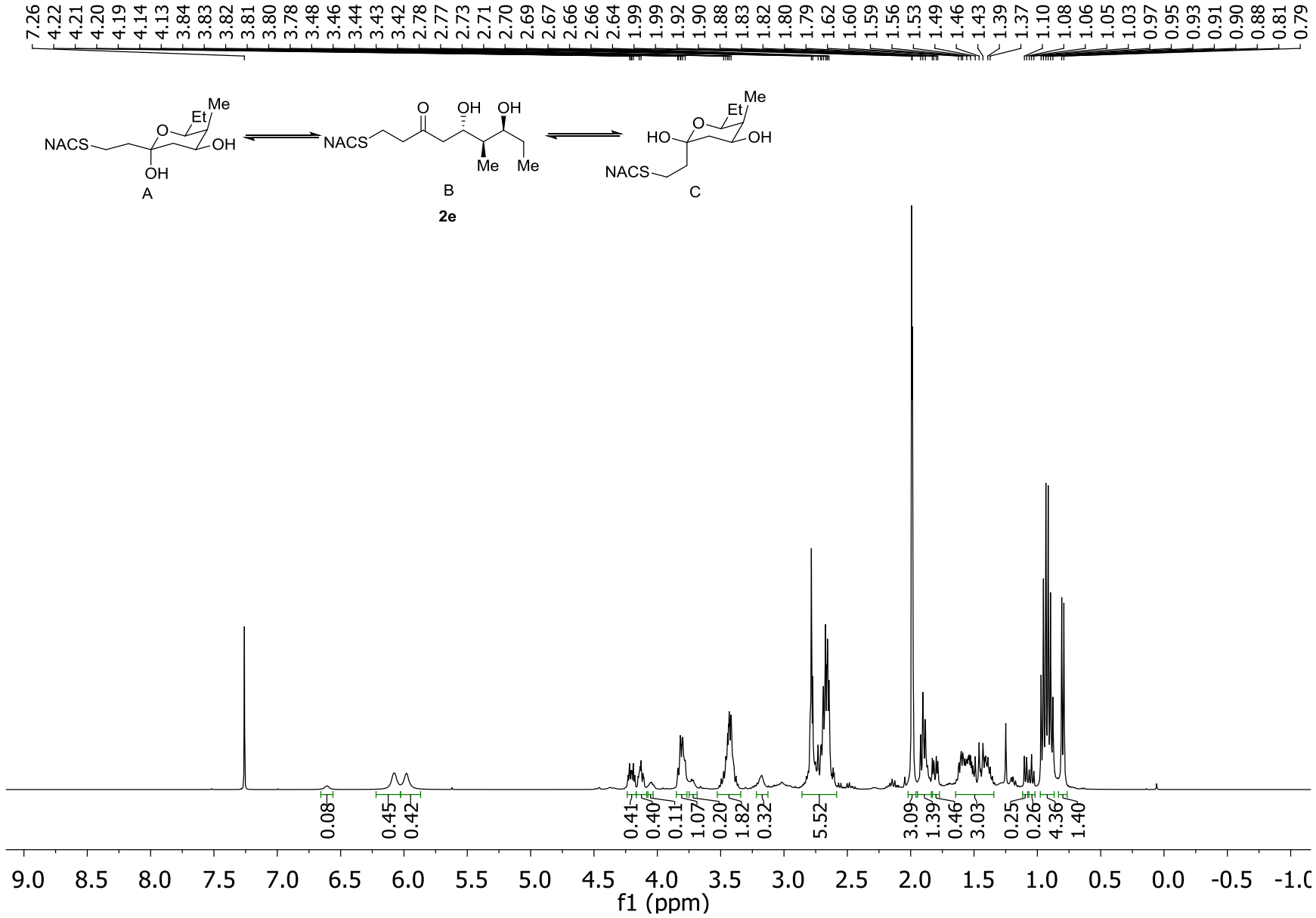

S11
Me
N
OMe
O
O
TES
OTES
Me
Me
173.10
77.00
75.15
71.06
61.21
42.93
34.77
32.00
27.65
8.90
8.76
7.01
6.83
5.36
4.94
f1 (ppm)

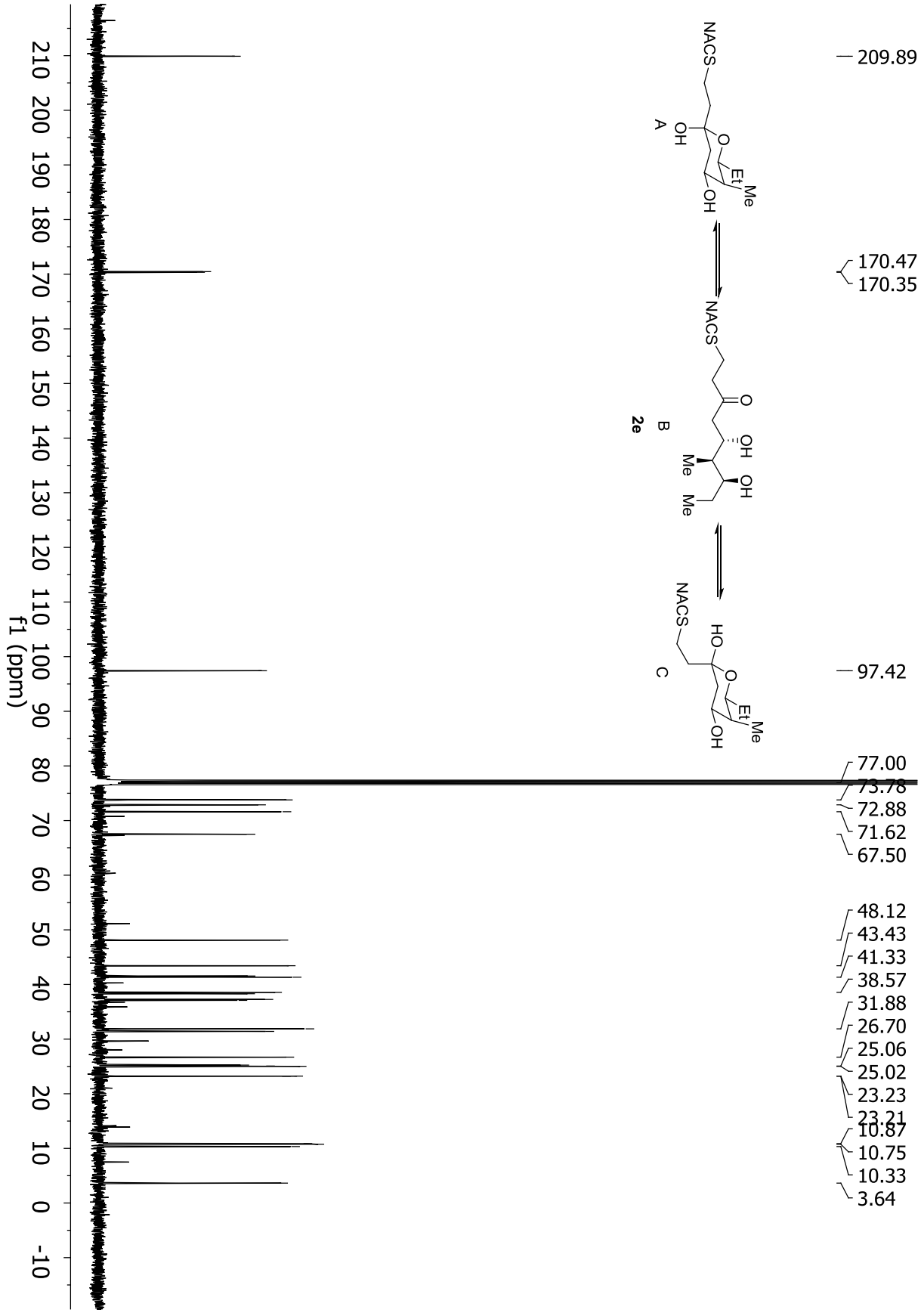

S12
O
O
TES
OTES
Me
Me
f1 (ppm)
1.00
1.01
0.99
0.99
1.00
0.99
0.99
1.05
2.00
25.42
12.56
0.99
1.00
25.42
12.56
4.32
4.32
4.31
4.31
4.30
4.29
4.29
4.28
3.55
3.54
3.53
3.53
3.51
0.96
0.94
0.92
0.92
0.91
0.90
0.88
0.88
0.87
0.60
0.59
0.57
0.57
0.55
0.53
7.26
6.40
6.37
6.36
6.33
6.23
6.22
6.18
6.18
5.82
5.82
5.80
5.80
4.32
4.32
4.31
4.31
4.30
4.29
4.29
4.28
3.54
3.53
3.53
2.89
2.87
2.85
2.83
2.52
2.51
2.48
2.47
1.78
1.78
1.78
1.77
1.77
1.55
1.53
1.52
1.52
1.50
1.50
0.96
0.94
0.92
0.92
0.91
0.90
0.88
0.88
0.87
0.85
0.60
0.59
0.57
0.57
0.55
0.53
0.51

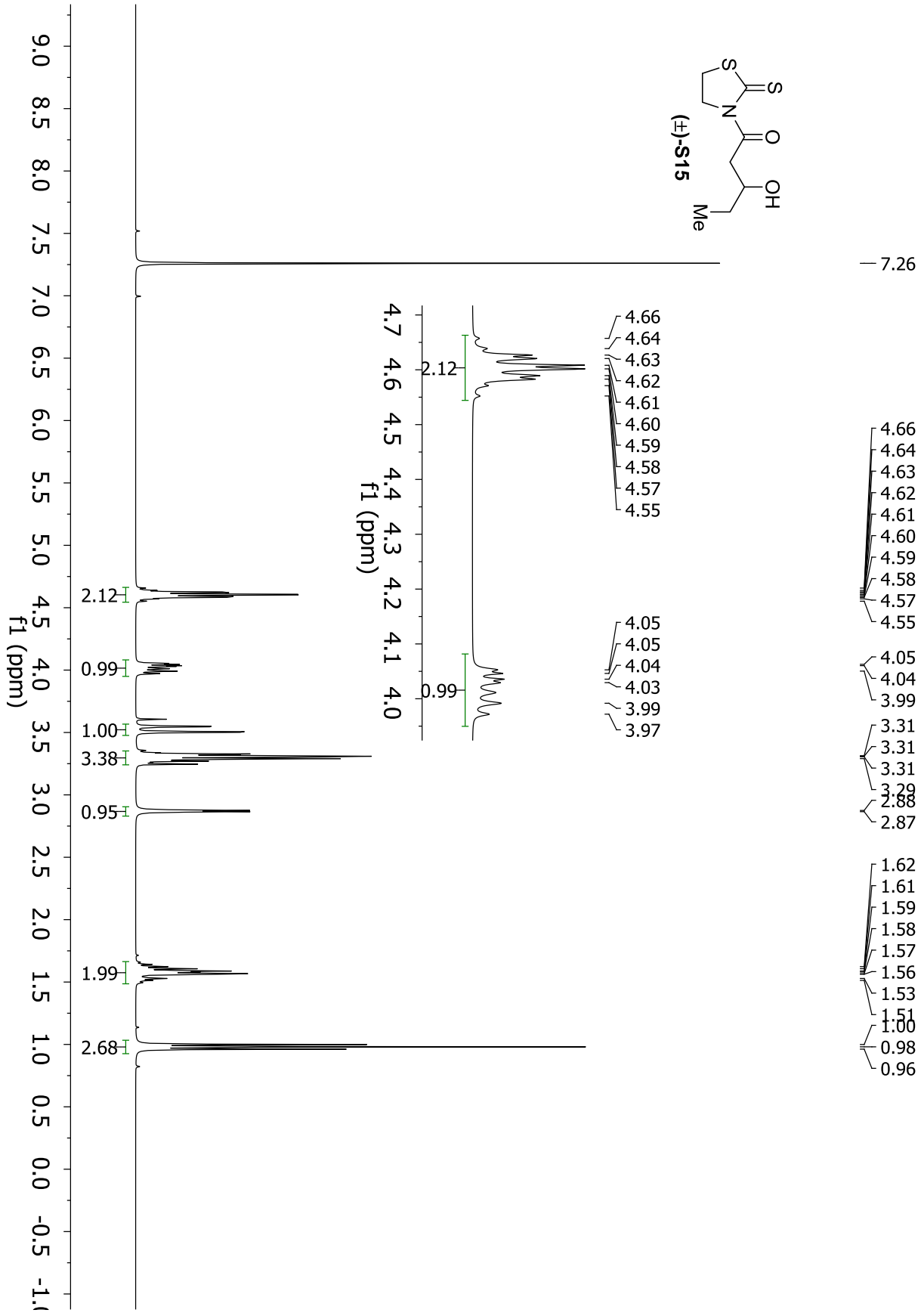

S12
O
O
TES
OTES
Me
Me
200.15
137.62
128.05
77.00
75.30
71.08
42.82
42.77
27.78
9.16
8.27
7.00
6.87
5.37
4.98
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

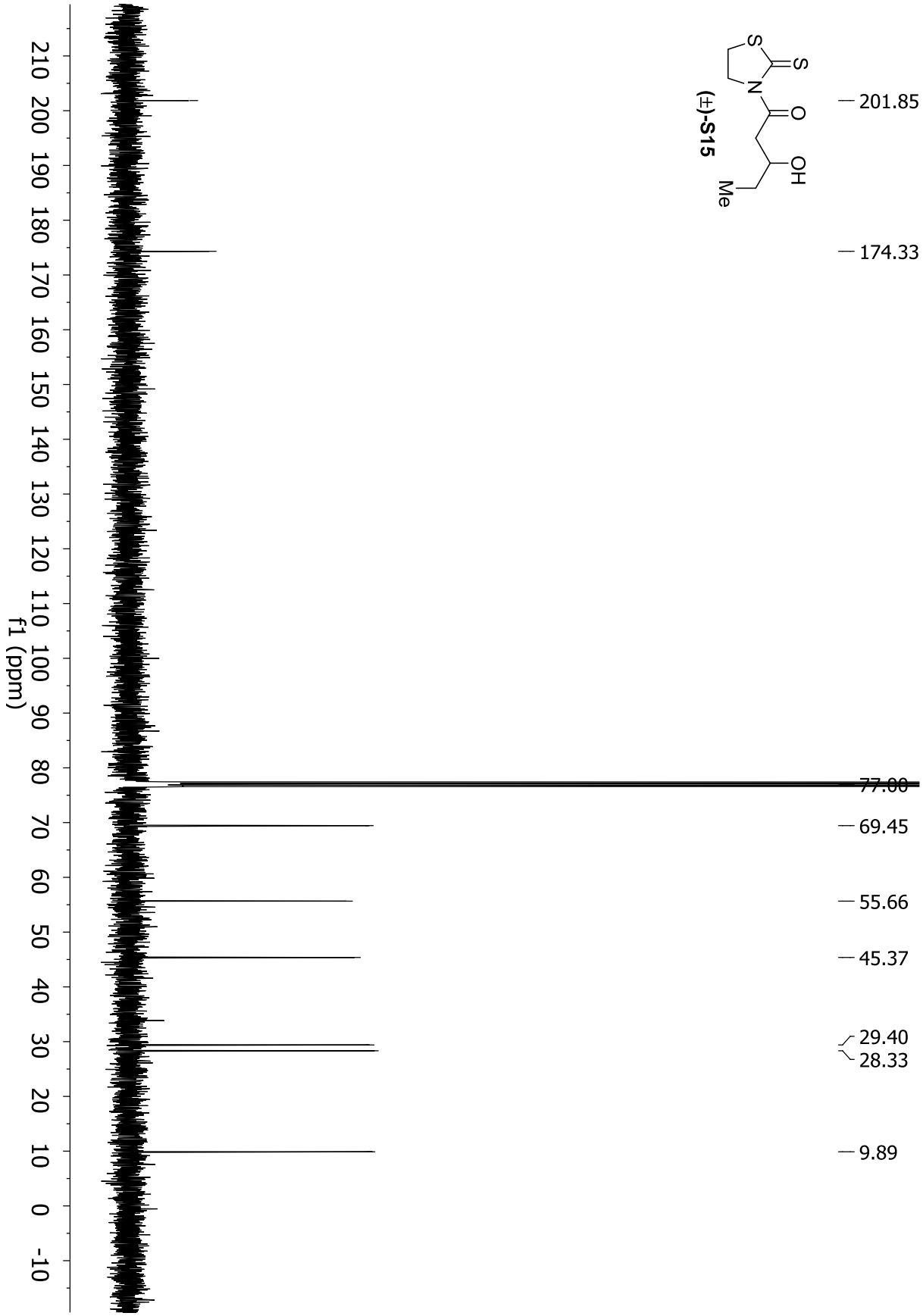

f1 (ppm)
0.91
1.00
3.25
7.15
0.95
3.24
1.09
2.15
24.18
12.00
NACS
O
O
TES
OTES
Me
Me
S13
7.26
6.07
4.27
4.26
4.25
4.24
3.53
3.52
3.50
3.48
3.47
3.45
3.44
2.80
2.78
2.77
2.75
2.73
2.72
2.71
2.69
2.68
2.66
2.65
2.63
2.62
2.59
2.43
2.42
2.39
2.38
2.01
1.76
1.75
1.74
1.73
1.72
1.57
1.55
1.53
1.51
1.50
1.48
1.46
1.34
0.96
0.94
0.93
0.92
0.91
0.89
0.89
0.87
0.85
0.84
0.61
0.59
0.57
0.54
0.52

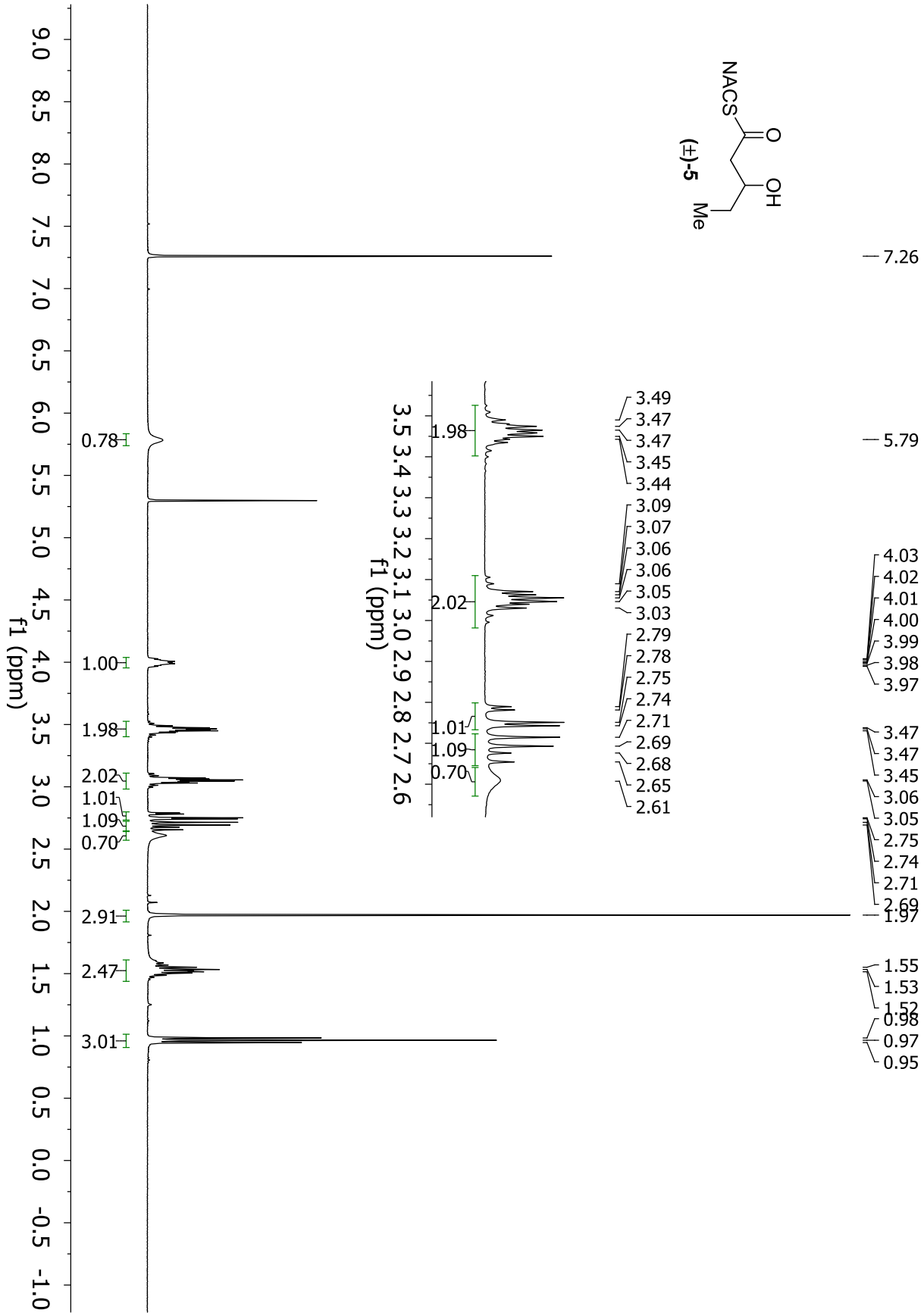

208.27
170.09
77.00
75.24
71.25
46.09
44.43
42.45
38.36
32.25
27.72
24.97
23.23
9.15
8.02
6.98
6.87
5.35
5.00
NACS
O
O
TES
OTES
Me
Me
S13
f1 (ppm)

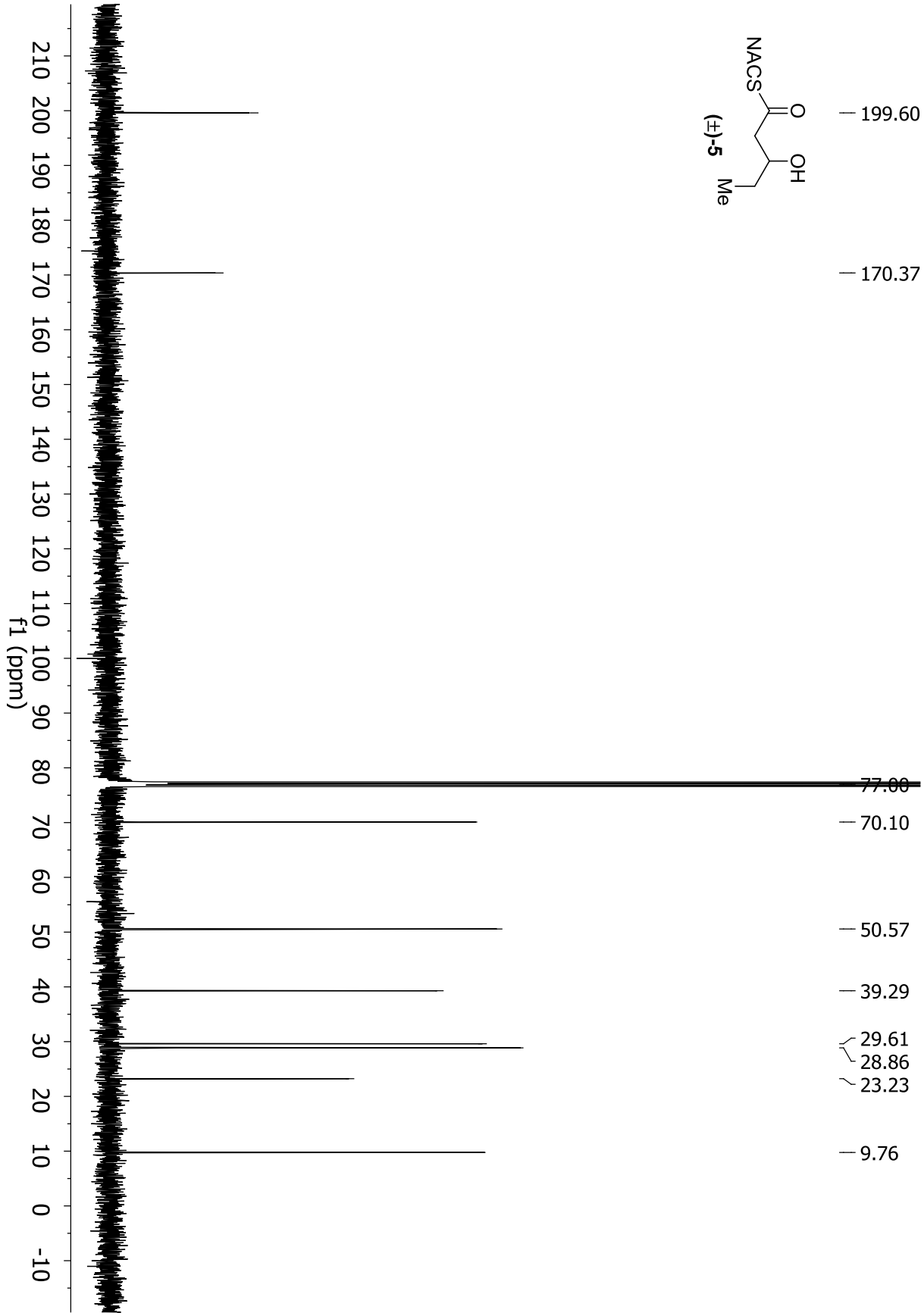

7.26 4.22 4.21 4.20 4.19 4.14 4.13 3.84 3.83 3.82 3.81 3.80 3.78 3.48 3.46 3.44 3.43 3.42 2.78 2.77 2.73 2.71 2.70 2.69 2.67 2.66 2.66 2.64 1.99 1.99 1.92 1.90 1.88 1.83 1.82 1.80 1.79 1.62 1.60 1.59 1.56 1.53 1.49 1.46 1.43 1.39 1.37 1.10 1.08 1.06 1.05 1.03 0.97 0.95 0.93 0.91 0.90 0.88 0.81 0.79
NACS
Me
Et
O
OH
OH
A
B
C
Me
Me
HO
2e
0.08 0.45 0.42 0.41 0.40 0.11 1.07 0.20 1.82 0.32 5.52 3.09 1.39 0.46 3.03 0.25 0.26 4.36 1.40
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

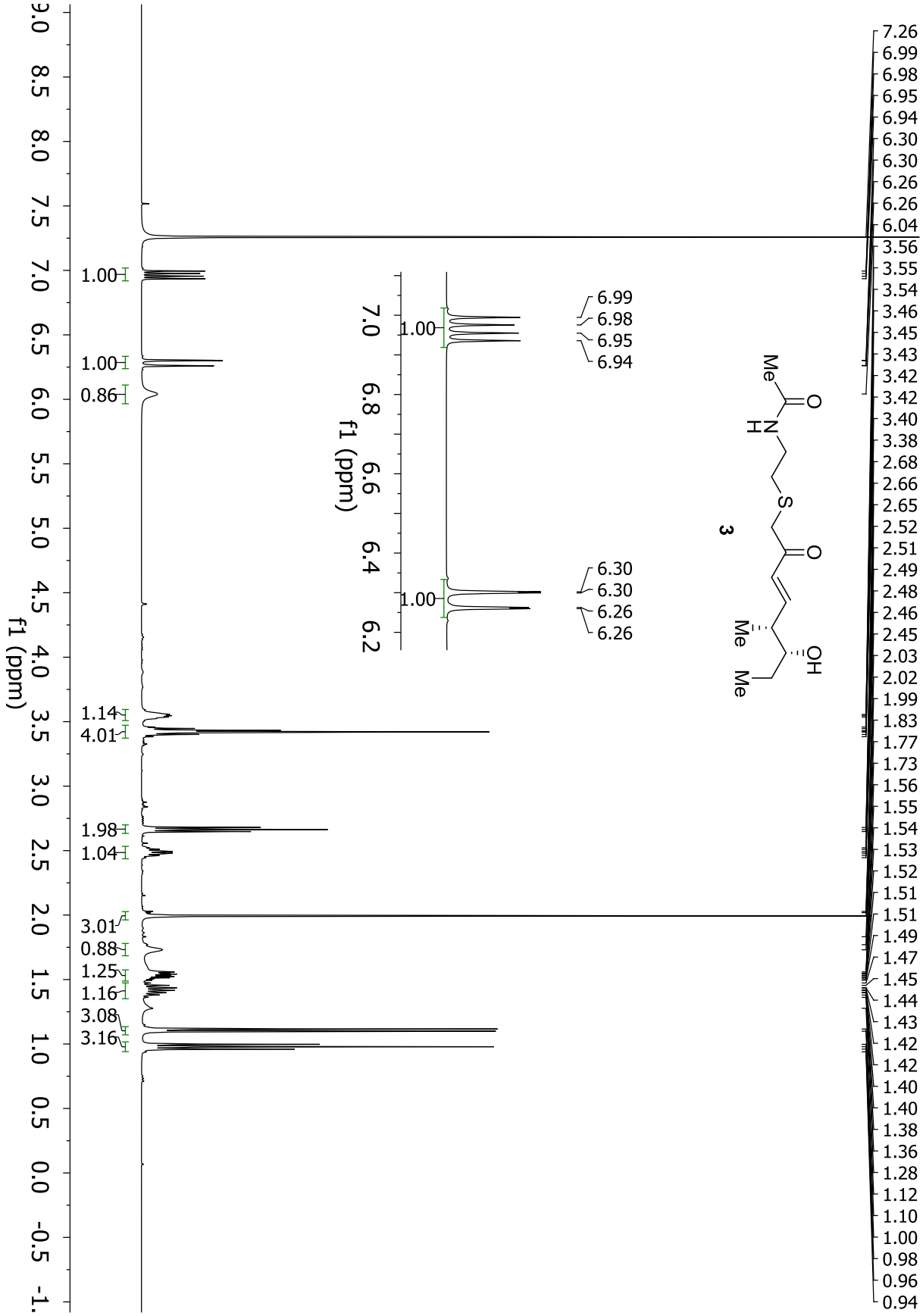

209.89
170.47
170.35
97.42
77.00
73.78
72.88
71.62
67.50
48.12
43.43
41.33
38.57
31.88
26.70
25.06
25.02
23.23
23.21
10.87
10.75
10.33
3.64
f1 (ppm)
2e
A
B
C

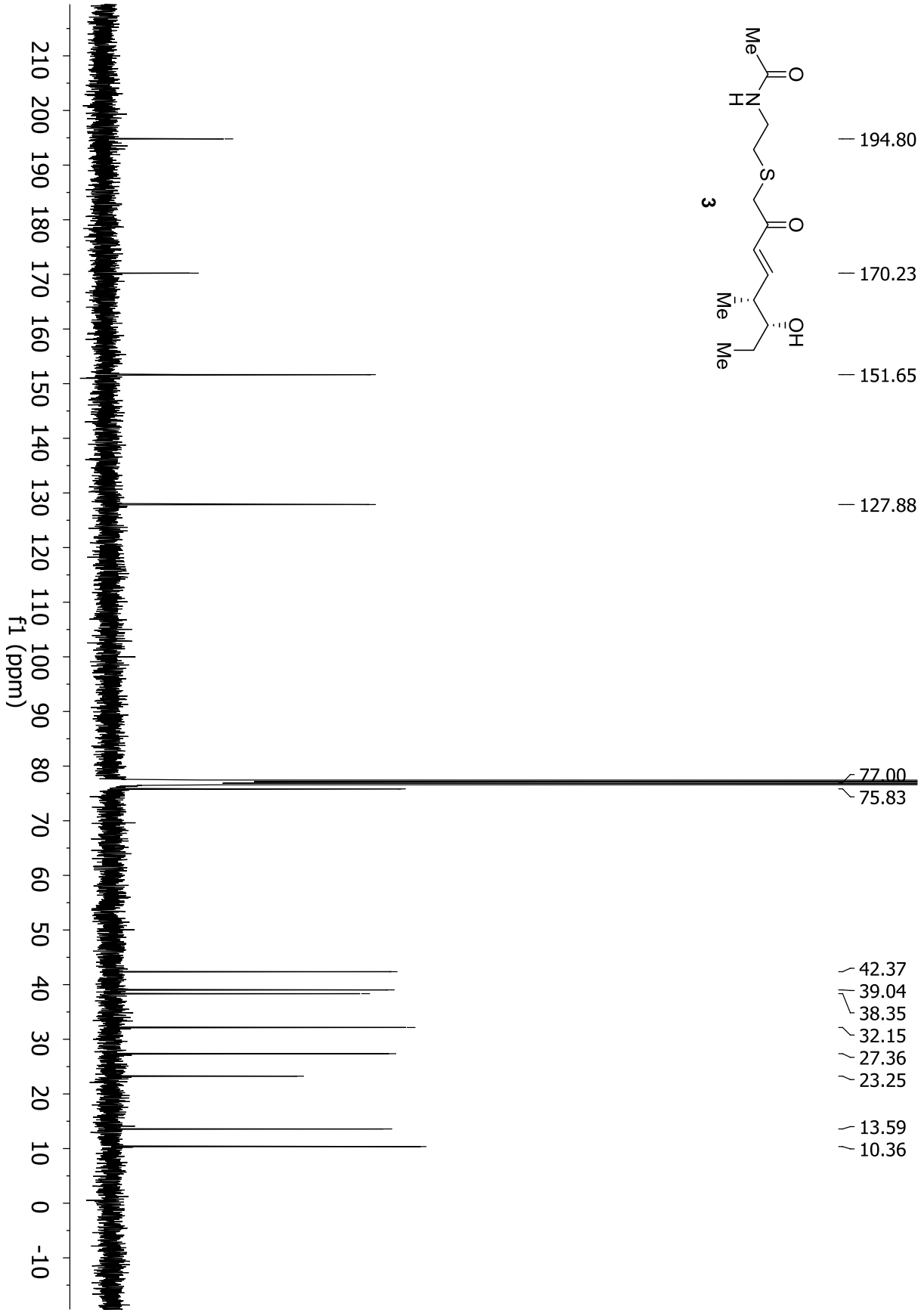

(±)-S15
S
S
N
O
OH
Me
f1 (ppm)
2.12
0.99
1.00
3.38
0.95
1.99
2.68
7.26
4.66
4.64
4.63
4.62
4.61
4.60
4.59
4.58
4.57
4.55
4.05
4.05
4.04
4.03
3.99
3.97
3.31
3.31
3.31
3.29
2.88
2.87
1.62
1.61
1.59
1.58
1.57
1.56
1.53
1.51
1.00
0.98
0.96

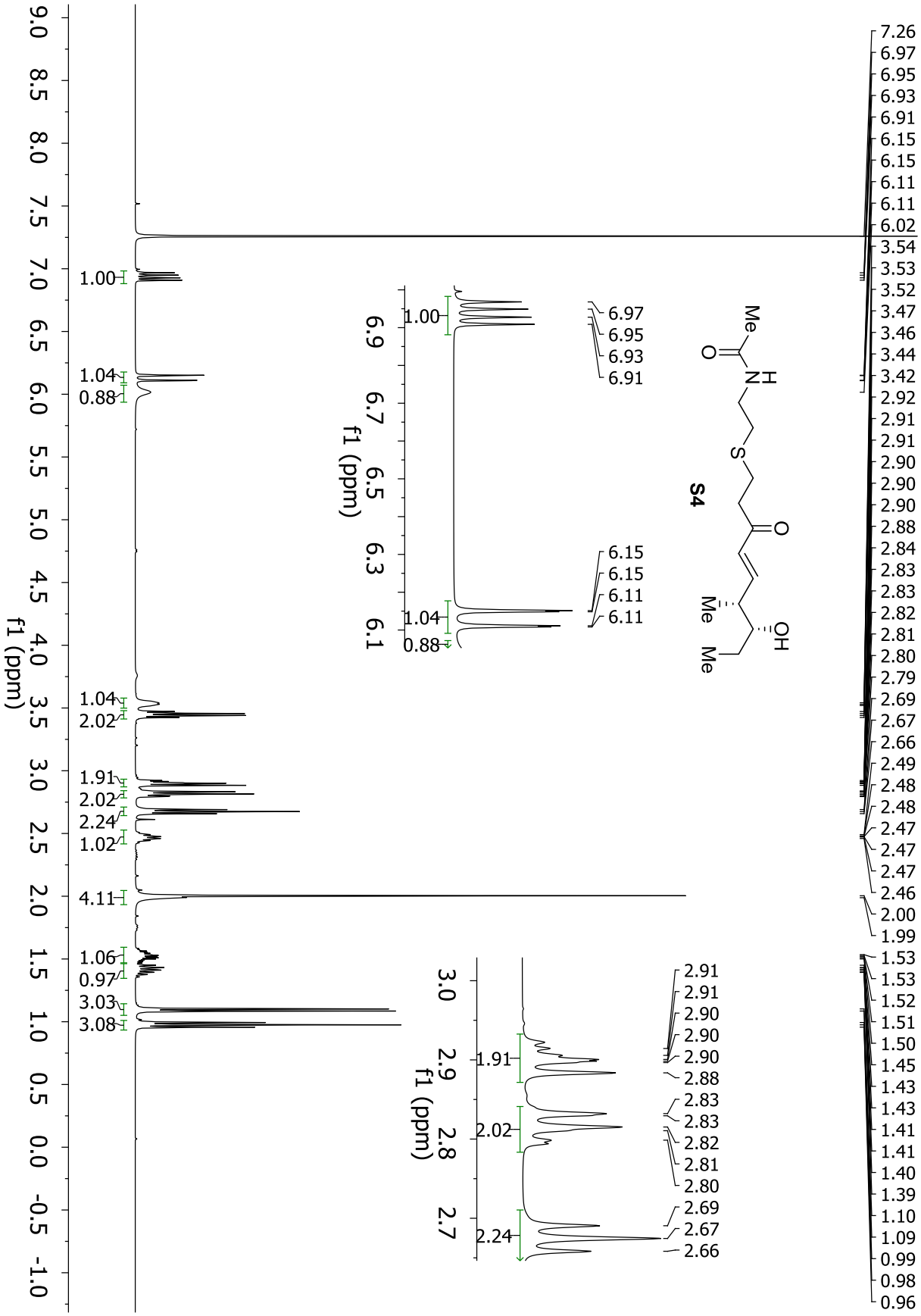

(±)-S15
201.85
174.33
77.00
69.45
55.66
45.37
29.40
28.33
9.89
f1 (ppm)

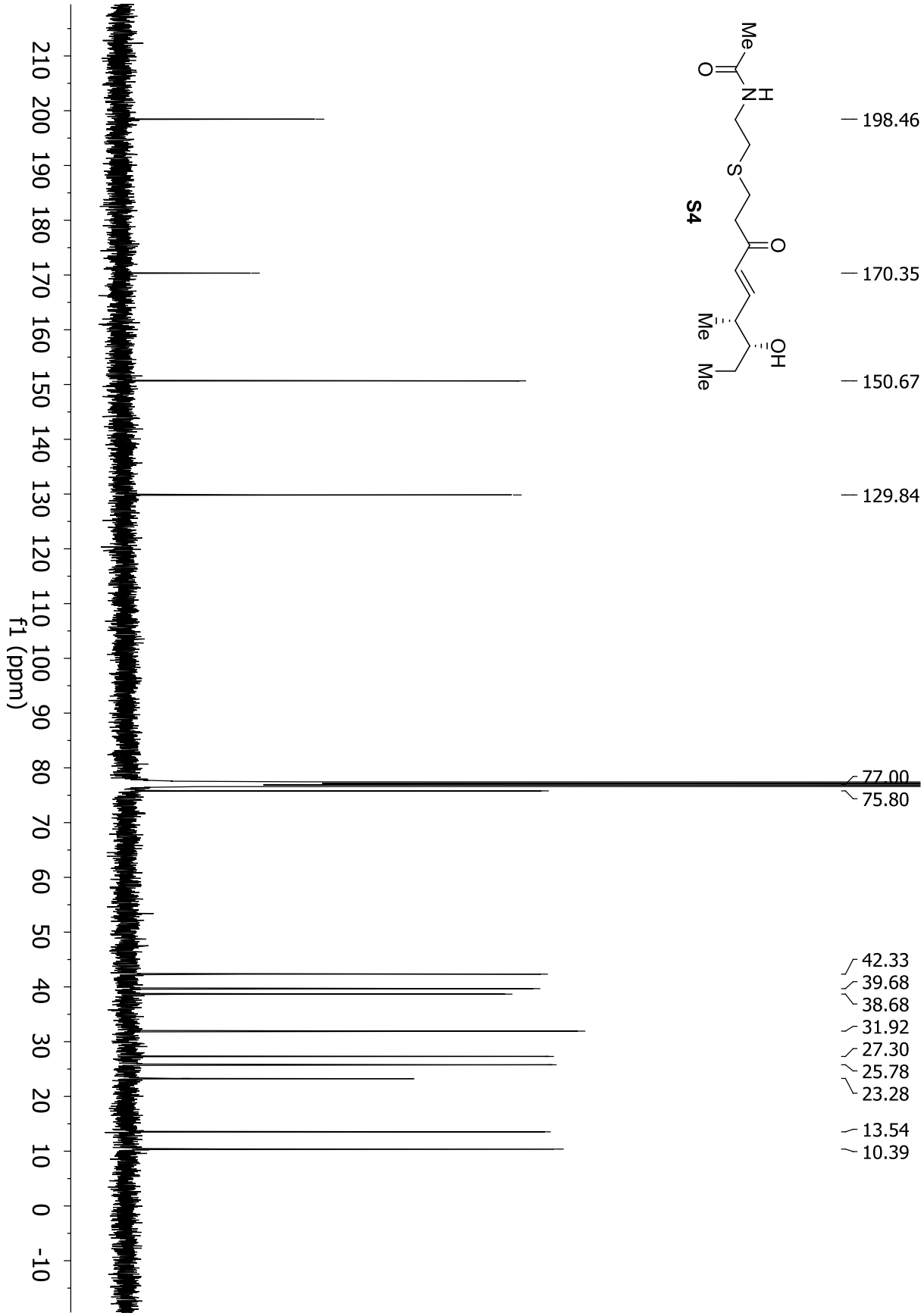

NACS
O
OH
Me
(±)-5
f1 (ppm)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
0.78
1.00
1.98
2.02
1.01
1.09
0.70
2.91
2.47
3.01
3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6
1.98
2.02
1.01
1.09
0.70
3.49
3.47
3.47
3.45
3.44
3.09
3.07
3.06
3.06
3.05
3.03
2.79
2.78
2.75
2.74
2.71
2.69
2.68
2.65
2.61
7.26
5.79
4.03
4.02
4.01
4.00
3.99
3.98
3.97
3.47
3.47
3.45
3.06
3.05
2.75
2.74
2.71
2.69
1.97
1.55
1.53
1.52
0.98
0.97
0.95


NACS
O
OH
Me
(±)-5
199.60
170.37
77.00
70.10
50.57
39.29
29.61
28.86
23.23
9.76
f1 (ppm)


f1 (ppm)
1.00
1.00
0.86
1.14
4.01
1.98
1.04
3.01
0.88
1.25
1.16
3.08
3.16
6.99
6.98
6.95
6.94
6.30
6.30
6.26
6.26
7.26
3
Me
N
H
S
OH
Me
Me


Me
O
N
H
S
O
3
Me
Me
OH
194.80
170.23
151.65
127.88
77.00
75.83
42.37
39.04
38.35
32.15
27.36
23.25
13.59
10.36
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10


f1 (ppm)
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
1.00
1.04
0.88
1.04
2.02
1.91
2.02
2.24
1.02
4.11
1.06
0.97
3.03
3.08
7.26
6.97
6.95
6.93
6.91
6.15
6.15
6.11
6.11
6.02
3.54
3.53
3.52
3.47
3.46
3.44
3.42
2.92
2.91
2.91
2.90
2.90
2.90
2.88
2.84
2.83
2.83
2.82
2.81
2.80
2.79
2.69
2.67
2.66
2.49
2.48
2.48
2.47
2.47
2.47
2.46
2.00
1.99
1.53
1.53
1.52
1.51
1.50
1.45
1.43
1.43
1.41
1.41
1.40
1.39
1.10
1.09
0.99
0.98
0.96
S4
Me
O
H
N
S
O
Me
Me
OH
6.9 6.7 6.5 6.3 6.1
f1 (ppm)
3.0 2.9 2.8 2.7
f1 (ppm)


Me
O
N
H
S
O
Me
Me
OH
S4
198.46
170.35
150.67
129.84
77.00
75.80
42.33
39.68
38.68
31.92
27.30
25.78
23.28
13.54
10.39
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10