

A Short Stereoselective Synthesis of Prepiscibactin using a SmI₂-Mediated Reformatsky Reaction and Zn²⁺-Induced Asymmetric Thiazolidine Formation

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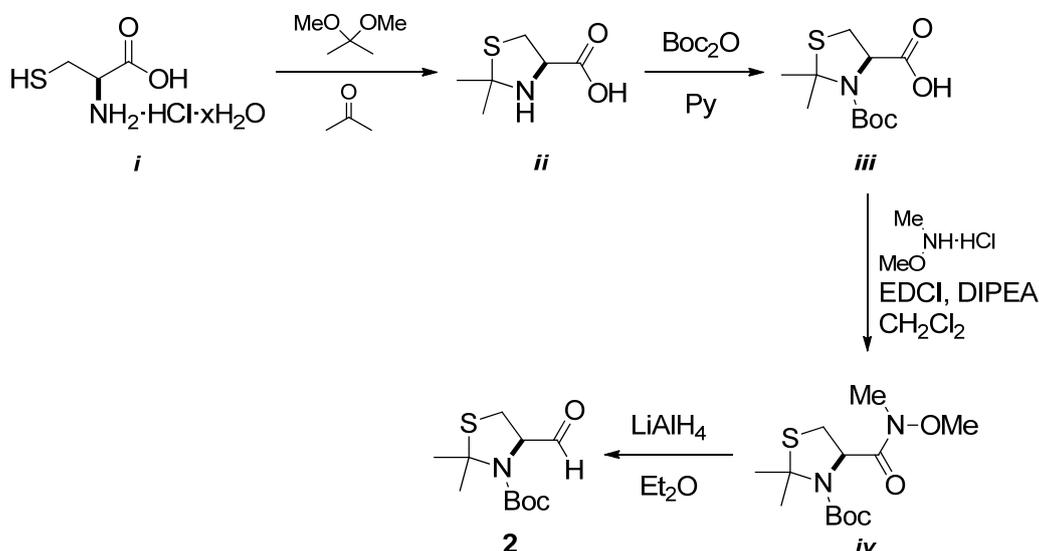
I. General information and procedures.

Nuclear magnetic resonance spectra (proton and carbon) were recorded on Bruker AC200 F, and 300 or 500 Advance spectrometers at the University of A Coruña, using CDCl_3 , D_2O and CD_3OD as the solvents and internal standards. Multiplicities of ^{13}C signals were obtained by DEPT. Medium-pressure chromatographic separations were carried out on silica gel 60 (230–400 mesh). Optical rotations were determined on a JASCO DIP-1000 polarimeter, with Na (589 nm) lamp and filter. LREIMS and HRESIMS were measured on Applied Biosystems QSTAR Elite. HPLC separations were carried out on an Agilent HP1100 liquid chromatography system equipped with a solvent degasser, quaternary pump and an UV detector (Agilent Technologies, Waldbronn, Germany). In the HPLC separations a Discovery® column HS F5 (100x4.6 mm, 5 μm) and an Eclipse® column XDB-C18 (150x4.6 mm, 5 μm) were used.

All moisture-sensitive reactions were carried out under an atmosphere of argon in flame-dried glassware closed by rubber septum, unless otherwise noted. Solvents were distilled prior to use under argon atmosphere and dried according to standard procedures using the following desiccants: Na/benzophenone for THF and Et_2O , CaH_2 for dichloromethane, pyridine and triethylamine; magnesium for methanol and anhydrous CaSO_4 for acetone. DMF was distilled over CaH_2 and was kept over molecular Sieves 4Å under argon atmosphere. Solutions and solvents were added via syringe or cannula. Thin layer chromatography was performed using silica gel GF-254 Merck, spots were revealed employing UV light (254 nm) and/or by heating the plate pretreated with an ethanolic solution of phosphomolibdic acid, a solution of cerium sulphate or a solution of ninhydrine in $\text{BuOH-AcOH-H}_2\text{O}$. CRYOCOOL apparatus was used for low-temperature reactions.

II. Synthesis of compounds 1-15.

Synthesis of compound 2.



L-cysteine hydrochloride polyhydrated (*i*) (5 g, 28.31 mmol) was suspended on a mixture of acetone (100 mL) and 2,2-dimethoxypropane (17 mL, 141.5 mmol) and refluxed during 4 days. Then the mixture was filtered and dried *in vacuo* to afford (R)-2,2-dimethylthiazolidine-4-carboxylic acid hydrochloride (*ii*) and unreacted L-cysteine (*i*) in a 8:1 ratio as a white and crystalline solid (4.7 g, quantitative yield). This material was directly used for subsequent reaction without any further purification.

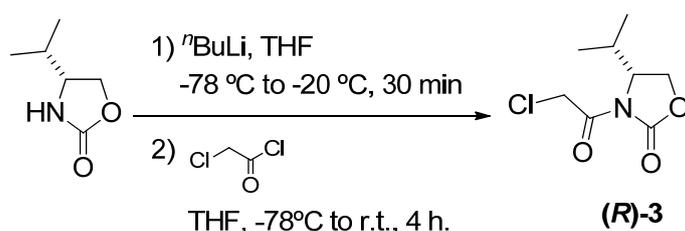
The carboxylic acid *ii* (4g, 24.81 mmol) was dissolved in pyridine (24 mL), a Boc_2O (5.95 g, 27.29 mmol) was added at 0 °C and the solution was stirred at room temperature overnight. Then the reaction was partitioned between EtOAc and HCl(aq) 5% and the organic layer was washed with water and brine, dried over MgSO_4 , filtered and finally concentrated under reduced pressure to afford *iii* as a white crystalline solid (3.2 g, 49% yield). This material was directly used for subsequent reaction without any further purification.

The carboxylic acid *iii* (1 g, 3.82 mmol) was dissolved in CH_2Cl_2 (100 mL) at 0 °C and then, EDCI (804 mg, 4.20 mmol) and a solution of *N,O*-dimethylhydroxylamine hydrochloride (448 mg, 4.60 mmol) and DIPEA (0.8 mL, 4.60 mmol) in CH_2Cl_2 (90 mL) were added. After stirring overnight at room temperature, the mixture was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography (EtOAc:hexane 1:2) affording the Weinreb amide *iv* as a white and crystalline solid (446 mg, 35% yield).

The Weinreb amide *iv* (400 mg, 1.32 mmol) was dissolved in Et_2O (10 mL) at 0 °C, LiAlH_4 (150 mg, 3.90 mmol) was added and the resulting mixture was stirred at 0 °C during 45 min. After that

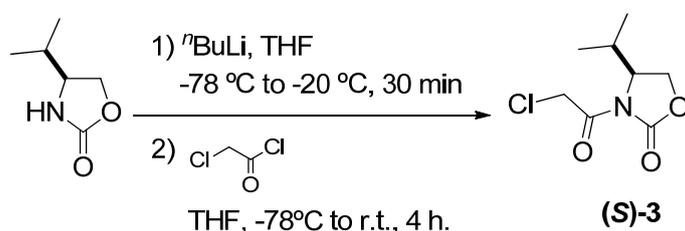
time, H₂SO₄ 5%, MeOH and NH₄Cl aqueous saturated solution were added, stirring during 15 min. and the resulting mixture was partitioned between water and EtOAc. The organic layer was washed with brine, dried using MgSO₄ and the solvent was removed under reduced pressure, to give a crude material that was purified by silica gel column chromatography (EtOAc:hexane 1:1) affording the aldehyde **2** as a white crystalline solid (300 mg, 92% yield). The spectral data and optical rotation were identical to the reported compound (ref. 15 in the manuscript).

Synthesis of compound (*R*)-3.



ⁿBuLi (0.81 mL, 2.10 M, 1.70 mmol) in hexane was added dropwise to a previously prepared solution of (*R*)-4-isopropylloxazolidin-2-one (200 mg, 1.55 mmol) in THF (8 mL) at -78 °C, and the resulting mixture was stirred at -20 °C during 30 min. After that time, the reaction was cooled at -78 °C and a previously prepared solution of chloroacetyl chloride (freshly distilled, 0.12 mL, 1.55 mmol) in THF (1 mL) was added dropwise and the reaction was allowed to reach room temperature slowly. The reaction was quenched with phosphate buffer pH 7, and extracted with Et₂O twice. The organic layers were combined, dried and the solvent was removed under reduced pressure. The crude material was purified by silica gel chromatography (Et₂O:hexane 1:1) affording (*R*)-3 (515 mg, 81% yield). The spectral data and optical rotation were identical to the reported compound (ref. 16 in the manuscript).

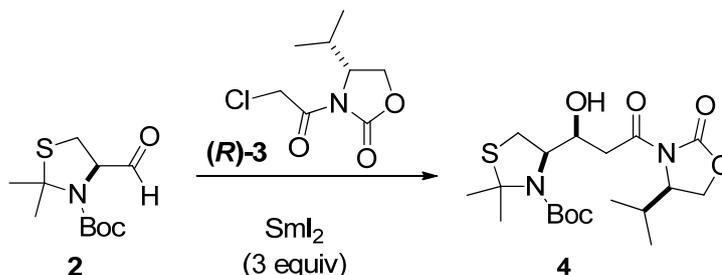
Synthesis of compound (*S*)-3.



ⁿBuLi (0.20 mL, 2.10 M, 0.42 mmol) in hexane was added dropwise to a previously prepared solution of (*S*)-4-isopropylloxazolidin-2-one (50 mg, 0.39 mmol) in THF (2 mL) at -78 °C, and the resulting mixture was stirred at -20 °C during 30 min. After that time, the reaction was cooled at -78 °C and a previously prepared solution of chloroacetyl chloride (freshly distilled, 0.03 mL, 0.39 mmol) in

THF (1 mL) was added dropwise and the reaction was allowed to reach room temperature slowly. The reaction was quenched with phosphate buffer pH 7, and extracted with Et₂O twice. The organic layers were combined, dried and the solvent was removed under reduced pressure. The crude material was purified by silica gel chromatography (Et₂O:hexane 1:1) affording (**S**)-**3** (103 mg, 65% yield). The spectral data and optical rotation were identical to the reported compound (ref. 16 in the manuscript).

Synthesis of compound 4.



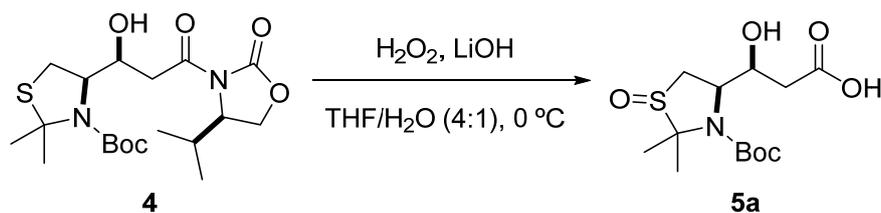
Preparation of Sml_2 in THF 0.1 M

A suspension of samarium metal (845 mg, 5.63 mmol) and I₂ (1134 mg, 4.5 mmol) in THF (45 mL) was prepared, carefully flushing with argon the reaction vessel and keeping it away from light. After stirring at room temperature for two hours, the reaction turned from brown to dark blue, indicating the complete formation of Sml_2 . The solution thus prepared must be used immediately.

Then, compounds **2** (215 mg, 0.878 mmol) and (**R**)-**3** (150 mg, 0.732 mmol) were dissolved together in THF (2.25 mL) and poured dropwise over a 0.1M Sml_2 solution (22 mL, see procedure above) previously cooled at -78 °C. After 10 minutes of stirring, air was bubbled inside the solution until the reaction turns to a brown-greenish colour. After reaching room temperature, a saturated aqueous solution of NH₄Cl was added and stirred for 20 min. Then, the mixture was extracted with Et₂O, the organic layer was washed with Na₂S₂O₃ 10% (w/w) aqueous solution, dried, filtered and the solvent was removed under reduced pressure. The crude material was purified by HPLC using an Eclipse® XDB-C18 (150x4.6 mm, 5 μm) column with a mobile phase consisting on an isocratic 60% CH₃CN in H₂O (v/v) at a flow rate of 1.0 mL/min, affording compound **4** (*t_R* = 5.8 min) (231 mg, 65%).

¹H-NMR (300 MHz, CDCl₃), δ_H ppm: 4.54 (t, *J* = 6.5 Hz, 1H), 4.43 (ddd, *J* = 7.6, 3.6, 3.5 Hz, 1H), 4.38 (m, 1H), 4.27 (dd, *J* = 9.1, 8.1 Hz, 1H), 4.21 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.25 (dd, *J* = 15.8, 3.2 Hz), 3.19 (dd, *J* = 12.5, 6.4 Hz), 3.12 (dd, *J* = 15.8, 9.1 Hz), 2.83 (d, *J* = 12.5 Hz), 2.38 (m, 1H), 1.79 (s, 3H) 1.77 (s, 3H), 1.48 (s, 9H), 0.90 (d, *J* = 7.0, 6H). **¹³C-NMR (75 MHz, CDCl₃), δ_C ppm:** 171.5/171.1 (CO), 154.1 (CO), 81.2 (C), 70.6 (CH), 67.2 (CH), 63.5 (CH₂), 58.4 (CH), 40.8 (CH₂), 30.3 (CH), 29.2 (CH₂), 28.4 (3xCH₃, *t*Bu), 17.9 (CH₃), 14.7 (CH₃). **(+)-HR-ESIMS *m/z*:** 417.2046 [M+H]⁺ (calcd. 417.2053 for C₁₉H₃₃N₂O₆S). **[α]_D²⁵** = -66.0 (c = 1.25, CHCl₃).

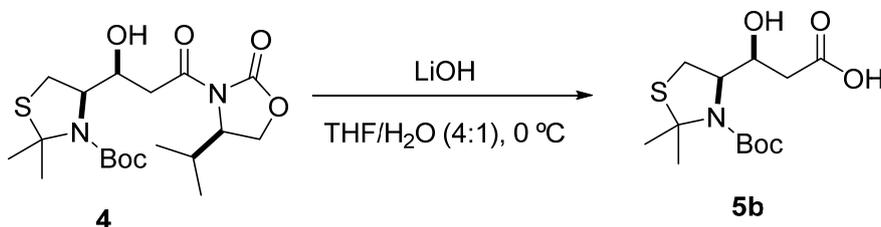
Synthesis of compound 5a.



To a stirred solution of compound **4** (85 mg, 0.21 mmol) in 1.5 mL of a 4:1 (v/v) mixture of tetrahydrofuran and water at 0°C, was added dropwise 50% aqueous H₂O₂ (0.065 mL, 0.756 mmol) via syringe. An 1.2 M LiOH aqueous solution (0.237 mL) was added and the resulting solution was stirred at 0°C for 3 h, until all the starting material was consumed which was checked *via* TLC. A solution of Na₂SO₃ (74 mg) in H₂O (0.350 mL, 1.46 M) was added, and the mixture was stirred at room temperature overnight. The mixture was concentrated *in vacuo* and the residue was dissolved in dichloromethane (8 mL), which was extracted with a 15% aqueous solution of sodium bicarbonate (8 mL). The organic phase was dried over MgSO₄ and concentrated *in vacuo* to recover the crystalline homochiral auxiliary. The aqueous layer was acidified to pH ~2 with 37% aqueous HCl and extracted with EtOAc (2 x 8 mL). The combined EtOAc phases were dried over MgSO₄, and taken to dryness *in vacuo* to afford the acid **5a** (58 mg, 0.18 mmol, yield: 86%) as a colorless oil.

¹H-NMR (300 MHz, CDCl₃), δ_H ppm: 7.29 (bs, 2H) 4.54 (bt, 1H), 4.42 (bs, 1H), 3.20 (dd, *J* = 12.6, 6.9 Hz, 1H), 2.78–2.65 (m, 2H), 2.58 (dd, *J* = 15.8, 8.4 Hz, 1H), 1.77 (s, 6H), 1.48 (s, 9H). **¹³C-NMR- (75 MHz, CDCl₃), δ_C ppm:** 171.6 (CO), 144.0 (CO), 98.5 (C), 82.2 (C), 70.6 (CH), 68.2 (CH), 60.7(CH₂), 38.9 (CH₂), 28.7 (3xCH₃, *t*Bu), 28.2 (2xCH₃). **(+)-HR-ESIMS *m/z*:** 322.1373 [M+H]⁺ (calcd. 322.1281 for C₁₃H₂₄NO₆S).

Synthesis of compound 5b.

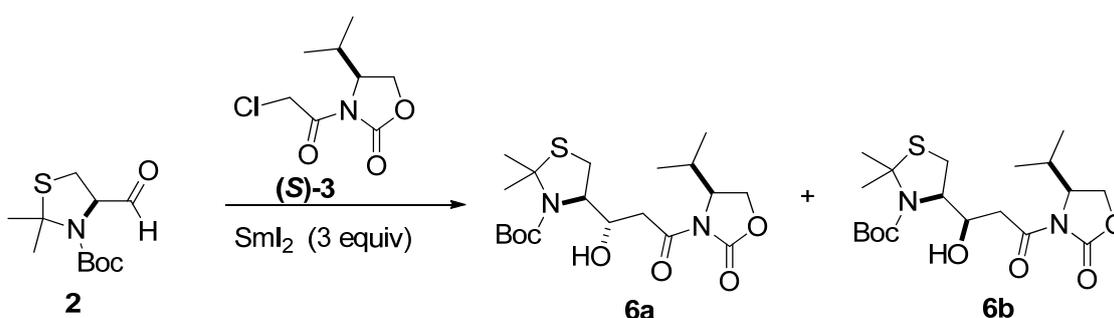


To a stirred solution of compound **4** (200 mg, 0.48 mmol) in 5 mL of a 4:1 (v/v) mixture of tetrahydrofuran and water at 0°C, was added an 1.2 M LiOH aqueous solution (0.750 mL) and the resulting mixture was stirred at 0°C for 90 min, until all the starting material was consumed, which was checked *via* TLC. The mixture was concentrated *in vacuo* and the residue was dissolved in

dichloromethane (10 mL). A 15% aqueous solution of sodium bicarbonate (10 mL) was added and then the organic phase was dried over MgSO₄ and concentrated *in vacuo* to recover the crystalline homochiral auxiliary. The aqueous layer was acidified to pH ~2 with 37% aqueous HCl and extracted with EtOAc (2 x 10 mL). The combined EtOAc phases were dried over MgSO₄, and concentrated to dryness *in vacuo* to afford the acid **5b** (140 mg, 0.46 mmol, yield: 96%) as a colorless oil.

¹H-NMR (300 MHz, CD₃OD), δ_H ppm: 4.52 (ddd, *J* = 9.8, 5.9, 3.0 Hz, 1H), 4.42 (dd, *J* = 6.9, 5.9 Hz, 1H), 3.22 (dd, *J* = 12.6, 6.9 Hz, 1H), 2.96 (bd, *J* = 12.6 Hz, 1H), 2.68 (dd, *J* = 15.6, 3.0 Hz, 1H), 2.49 (dd, *J* = 15.6, 9.8 Hz, 1H), 1.79 (s, 3H), 1.77 (s, 3H), 1.51 (s, 9H). **¹³C-NMR (75 MHz, CD₃OD), δ_C ppm:** 175.8 (CO), 155.0 (CO), 82.0 (C), 72.2 (C), 69.6 (CH), 68.6 (CH), 39.2 (CH₂), 28.9 (CH₂), 28.7 (3xCH₃, *t*Bu), 28.2 (2xCH₃). **(+)-HR-ESIMS *m/z*:** 306.1373 [M+H]⁺ (calcd. 306.1369 for C₁₃H₂₄NO₅S); 328.1192 [M+Na]⁺ (calcd. 328.1189 for C₁₃H₂₃NO₅NaS). **[α]_D²⁵ = -15.4** (c = 0.63, MeOH)

Synthesis of compounds **6a** and **6b**.



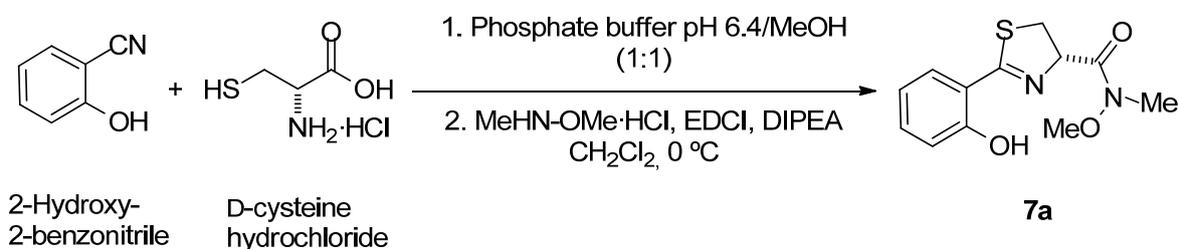
A solution of 0.1 M Sml₂ in THF was prepared using the same procedure as described before. Then, compounds **2** (100 mg, 0.41 mmol) and **(S)-3** (70 mg, 0.34 mmol) were dissolved together in THF (1 mL) and poured dropwise over a 0.1 M Sml₂ solution (10 mL) previously cooled at -78 °C. After 10 minutes stirring, air was bubbled inside the solution until the reaction turns to a brown-greenish colour. After reaching the room temperature, a saturated aqueous solution of NH₄Cl was added, stirring was maintained along 20 minutes. Then the mixture was extracted with Et₂O, the organic layer was washed with Na₂S₂O₃ 10% (w/w) aqueous solution; the organic layer was dried, filtered and the solvent was removed under reduced pressure. The crude material thus obtained was purified by HPLC using an Eclipse® XDB-C18 (150x4.6 mm, 5 μm) column with a mobile phase consisting on an isocratic 60% CH₃CN in H₂O (v/v) at a flow rate of 1.0 mL/min, affording **6a** (*t_R* = 3.8 min, 37 mg, 26 % yield) and **6b** (*t_R* = 4.1 min, 40 mg, 29% yield). HPLC chromatogram of the crude of the reaction show a 1:1.1 ratio between **6a** and **6b**. (Total 77 mg, 55% global yield)

6a. ¹H-NMR (500 MHz, CDCl₃), δ_H ppm: 4.58 (bs, 1H), 4.47 (ddd, *J* = 7.6, 7.4, 3.4 Hz, 1H), 4.42 (dd, *J* = 12.5, 6.5 Hz, 1H), 4.30 (dd, *J* = 7.6, 7.6 Hz, 1H), 4.23 (dd, *J* = 8.9, 3.4 Hz, 1H), 3.22 (m, 3H), 2.86 (d, *J* = 12.3, 1H), 2.43 (m, 1H), 1.82 (s, 3H), 1.80 (s, 3H), 1.50 (s, 9H), 0.92 (m, 6H). **¹³C-NMR**

(125 MHz, CDCl₃), δ_c ppm: 171.7 (CO), 154.2 (CO), 81.2 (C), 81.0 (CH), 70.5 (CH), 69.5 (CH), 67.2 (CH), 63.5 (CH₂), 58.6 (CH), 40.5 (CH₂), 29.8 (CH₂), 28.4 (3xCH₃, tBu), 28.3 (2xCH₃), 18.0 (CH₃), 14.7 (CH₃). (+)-HR-ESIMS m/z : 417.2046 [M+H]⁺ (calcd. 417.2053 for C₁₉H₃₃N₂O₆S).

6b. ¹H-NMR (500 MHz, CDCl₃), δ_H ppm: 4.55 (m, 1H), 4.45 (ddd, J = 8.1, 7.2, 3.4 Hz, 1H), 4.41 (ddd, J = 10.3, 7.4, 3.3 Hz, 1H), 4.28 (dd, J = 8.1, 8.1 Hz, 1H), 4.22 (dd, J = 8.1, 3.4 Hz, 1H), 3.26 (dd, J = 16.0, 3.3 Hz, 1H), 3.21 (dd, J = 12.3, 6.4 Hz, 1H), 3.14 (m, 1H), 2.85 (d, J = 12.3 Hz, 1H), 2.40 (m, 1H), 1.81 (s, 3H), 1.79 (s, 3H), 1.49 (s, 9H), 0.92 (d, 3H), 0.91 (d, 3H) ¹³C-NMR (125 MHz, CDCl₃), δ_c ppm: 171.6 (CO), 154.1 (CO), 81.2 (C), 70.7 (CH), 68.5 (CH), 66.7 (CH), 63.5 (CH₂), 58.6 (CH), 40.8 (CH₂), 29.7 (CH₂), 29.3 (3xCH₃, tBu), 28.4 (2xCH₃), 18.0 (CH₃), 14.80 (CH₃). (+)-HR-ESIMS m/z : 439.1895 [M+Na]⁺ (calcd. 439.1873 for C₁₉H₃₂N₂O₆NaS).

Synthesis of compound 7a.



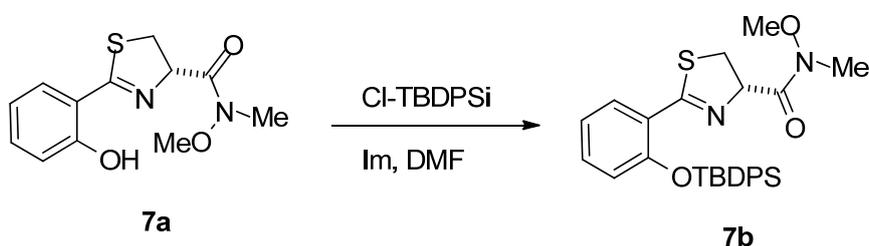
2-Hydroxy-2-benzonitrile (1.0 g, 8.4 mmol) and D-cysteine hydrochloride (2.640 g, 16.8 mmol) were dissolved in 80 mL of a 1:1 (v/v) mixture of methanol and phosphate buffer 0.1 M pH 6.4. The solution was adjusted to pH 6.4 by addition of solid K₂CO₃ and the reaction mixture was stirred at 60 °C overnight. The mixture was concentrated under reduced pressure, and the yellow crude material was diluted with water (50 mL). The solution was adjusted to pH 2-3 by addition of an aqueous solution of HCl (5%). After extraction with CH₂Cl₂ (3 x 50 mL), the organic layers were collected, dried over MgSO₄ and filtered. The solvent was removed under reduced pressure to give the expected thiazoline which was isolated as a yellow powder (1.9 g, 8.5 mmol, quantitative yield). It was directly used for subsequent reaction without any further purification.

To a solution of thiazoline (0.3 g, 1.34 mmol) and EDCI (0.285 g, 1.48 mmol) in CH₂Cl₂ (15 mL), cooled down at 0 °C, was added a solution of *N,O*-dimethylhydroxylamine hydrochloride (0.157 g, 1.61 mmol) and DIPEA (0.280 mL, 1.61 mmol) in CH₂Cl₂ (15 mL). The solution was allowed to warm up to room temperature and was stirred during 2 h. The mixture was washed with an aqueous solution of HCl (5%) and the resulting organic phase was dried over MgSO₄ and finally filtered. The solvent was removed under reduced pressure to give a brownish oily residue. This crude material was adsorbed on silica gel and then chromatographed on a silica gel column (hexane/Et₂O 1:1 to 1:2) to

give the expected Weinreb amide **7a** which was isolated as a yellow solid (0.152 g, 0.57 mmol, yield : 57%).

¹H-NMR (300 MHz, CDCl₃), δ_H ppm: 7.42 (dd, *J* = 7.8 Hz, 1.6 Hz, 1H), 7.35 (ddd, *J* = 8.3, 7.3, 1.6 Hz, 1H), 6.98 (ddd, *J* = 8.3, 1.2, 0.5 Hz, 1H), 6.86 (ddd, *J* = 7.8, 7.3, 1.2 Hz, 1H), 5.70 (t, *J* = 9.0 Hz, 1H), 3.83 (s, 3H), 3.79 (bt, *J* = 9.7 Hz, 1H), 3.49 (dd, *J* = 11.0, 9.2 Hz, 1H), 3.30 (s, 3H). **¹³C-NMR (75 MHz, CDCl₃), δ_C ppm:** 174.08 (CO), 169.7 (C), 158.9 (C), 133.3 (CH), 130.8 (CH), 118.9 (CH), 117.0 (CH), 116.2 (C), 74.6 (CH), 61.8 (CH₃), 32.8 (CH₂), 32.5 (CH₃). **(+)-HR-ESIMS *m/z*:** 267.0796 [M+H]⁺ (calcd. 267.0797 for C₁₂H₁₅N₂O₃S) [α]_D²⁶ = -19.6 (c = 0.65, CHCl₃)

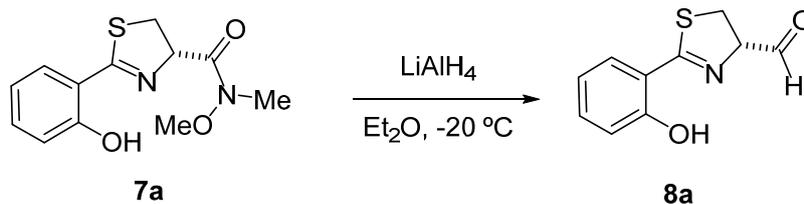
Synthesis of compound **7b**.



To an ice-cold solution of **7a** (50 mg, 0.19 mmol) in DMF (1.5 ml) were added 0.102 mL of TBDPSCI (0.39 mmol) and imidazole (55 mg, 0.82 mmol). After stirring at room temperature for 24 h, the mixture was diluted with EtOAc and washed with water and a saturated solution of aqueous NaCl, dried over MgSO₄. Evaporation of the solvent under reduced pressure afforded a yellow solid. This crude material was purified by silica gel column chromatography (hexane/ethyl acetate 3:1) to afford **7b** as a yellow amorphous solid (0.060 g, 0.12 mmol, yield: 62%).

¹H-NMR (300 MHz, CDCl₃), δ_H ppm: 7.81 – 7.65 (m, 5H), 7.51 – 7.28 (m, 6H), 6.96 – 6.79 (m, 2H), 6.41 (dd, *J* = 7.8, 1.5 Hz, 1H), 5.58 (t, *J* = 9.0 Hz, 1H), 3.87 (s, 3H), 3.81 – 3.71 (m, 1H), 3.47 (dd, *J* = 11.0, 9.2 Hz, 1H), 3.32 (bs, 3H), 1.12 (s, 9H). **¹³C-NMR (75 MHz, CDCl₃), δ_C ppm:** 167.8 (C), 153.6 (q), 135.4 (CH), 133.3 (C), 133.2 (C), 132.3 (CH), 132.2 (CH), 130.9 (CH), 130.4 (CH), 130.0 (CH), 129.9 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 124.4 (C), 120.7 (CH), 120.1 (CH), 74.8 (CH), 60.3 (CH₃), 32.6 (CH₂), 26.4 (2xCH₃, tBu), 21.0 (CH₃), 19.4 (CH₃), 14.2 (C). **(+)-HR-ESIMS *m/z*:** 505.1952 [M+H]⁺ (calcd. 505.1975 for C₂₈H₃₃N₂O₃SiS).

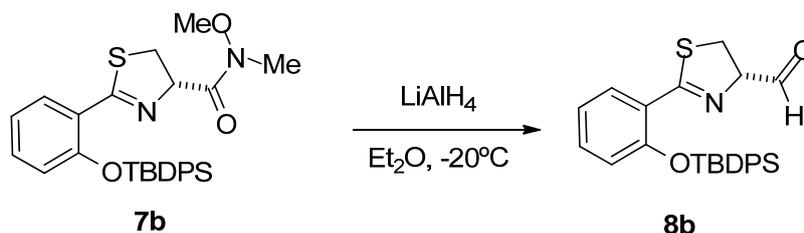
Synthesis of compound 8a.



To a solution of Weinreb amide **7a** (50 mg, 0.19 mmol) in diethyl ether (3 mL) at -20°C , was added 20 mg (0.53 mmol) of lithium aluminium hydride. The reaction mixture was stirred at -20°C for 15 min and then quenched by successive additions of methanol (0.5 mL), an aqueous solution of saturated NH_4Cl (4 mL) and a 5% (v/v) aqueous solution of H_2SO_4 (4 mL). The mixture was vigorously stirred and allowed to warm up to room temperature until two phases were formed. After partition and extraction with CH_2Cl_2 ($2 \times 10\text{mL}$), the organic layers were collected, dried over MgSO_4 and filtered before being evaporated under reduced pressure. The crude aldehyde **8a** (37 mg, 0.18 mmol, yield: 95%), very sensitive, was obtained as a yellow foam and it was directly used for subsequent reaction without any further purification.

$^1\text{H-NMR}$ (300 MHz, CDCl_3), δ_{H} ppm: 9.89 (s, 1H), 7.50 – 7.40 (m, 2H), 7.05 (dd, $J = 8.4, 1.1$ Hz, 1H), 6.98 (d, $J = 8.4$ Hz, 1H), 5.31 (dd, $J = 9.8, 6.1$ Hz, 1H), 3.77 (dd, $J = 11.2, 6.1$ Hz, 1H), 3.49 (dd, $J = 11.2, 9.8$ Hz, 1H). **$^{13}\text{C-NMR}$ (75 MHz, CDCl_3), δ_{C} ppm:** 196.4 (COH), 182.5 (C), 161.4 (C), 133.8 (CH), 131.9 (CH), 129.8 (CH), 126.5 (CH), 124.1 (CH), 82.2 (CH), 29.5 (CH_2). **(+)-HR-ESIMS m/z :** 208.0427 $[\text{M}+\text{H}]^+$ (calcd. 208.0426 for $\text{C}_{10}\text{H}_{10}\text{NO}_2\text{S}$).

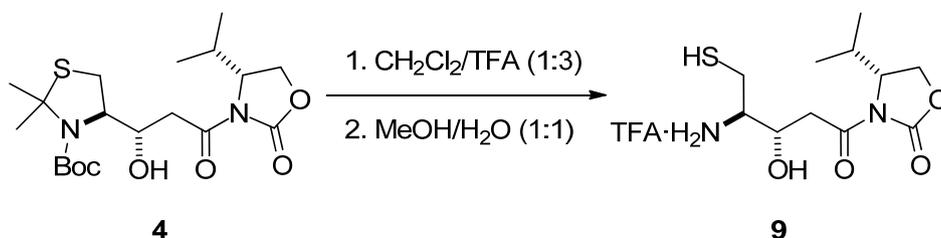
Synthesis of compound 8b.



To a solution of Weinreb amide **7b** (120 mg, 0.24 mmol) in diethyl ether (4.5 mL) at -20°C , was added 27 mg (0.71 mmol) of lithium aluminium hydride. The reaction mixture was stirred at -20°C for 15 min and then quenched by successive additions of methanol (1 mL), an aqueous solution of saturated NH_4Cl (8 mL) and a 5% (v/v) aqueous solution of H_2SO_4 (4 mL). The mixture was vigorously stirred and allowed to warm up to room temperature until two phases were formed. After partition and extraction with CH_2Cl_2 ($2 \times 15\text{mL}$), the organic layers were collected, dried over MgSO_4 and filtered

before being evaporated under reduced pressure. The crude aldehyde **8b** (0.097 g, 0.22 mmol, yield: 92%), very sensitive, was obtained as a yellow foam and it was directly used for subsequent reaction without any further purification.

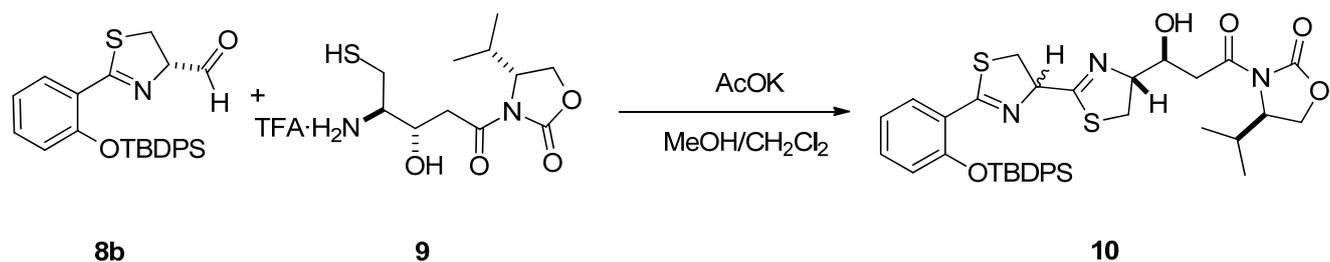
Synthesis of compound **9**.



A solution of **4** (95 mg, 0.23 mmol) in 6 mL of a 1:3 (v/v) mixture of dichloromethane and trifluoroacetic acid was stirred at room temperature for two days. The solution was then evaporated under reduced pressure and the crude acetamide was dissolved in a 1:1 (v/v) mixture of methanol and water (10 mL). The solvents were removed under reduced pressure. This crude material was purified by HPLC using a Discovery® HS F5 column (25 cm x 10 mm, 5 μm) with a mobile phase consisting of an isocratic 30% CH₃CN in H₂O (v/v, each containing 0.1% TFA) at a flow rate of 1.5 mL/min (*t_R* = 20.1 min) to give 78 mg (0.21 mmol, yield: 90%) of **9** as a white amorphous solid.

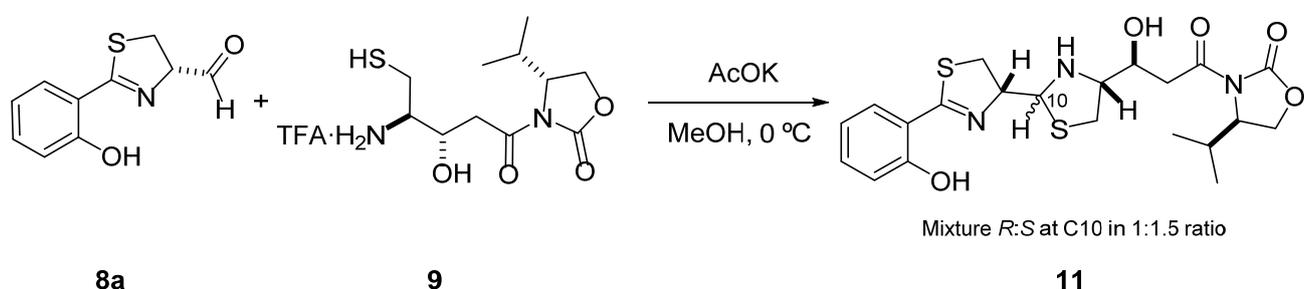
¹H-NMR (300 MHz, D₂O), δ_H ppm: 4.36 (m, 1H), 4.2 (m, 3H), 3.25 (m, 2H), 2.93 (dd, *J* = 16.2, 9.3 Hz, 1H), 2.83 (dd, *J* = 14.9, 5.1 Hz, 1H), 2.64 (dd, *J* = 14.9, 7.2 Hz, 1H), 2.08 (m, 1H), 0.74 (d, *J* = 7.0 Hz, 3H), 0.69 (d, *J* = 7.0 Hz, 3H). **¹³C-NMR (75 MHz, D₂O), δ_C ppm:** 171.4 (CO), 155.8 (CO), 65.7 (CH), 64.9 (CH₂), 58.9 (CH), 57.3 (CH), 39.6 (CH₂), 28.5 (CH), 23.4 (CH₂), 16.8 (CH₃), 14.1 (CH₃). **(+)-HR-ESIMS *m/z*:** 277.1207 [M+H]⁺ (calcd. 277.1216 for C₁₁H₂₁N₂O₄S). **[α]_D²⁵ = -45.6** (c = 0.9, MeOH).

Synthesis of compound 10.



To a stirred solution of **9** (29 mg, 0.1 mmol) and potassium acetate (69 mg, 0.7 mmol) in methanol (0.9 mL), a solution of freshly synthesized aldehyde **8b** (0.030 mg, 0.07 mmol) in dichloromethane (1 mL) was added. After stirring at room temperature for 24 h, a saturated aqueous solution of NH_4Cl was added and extracted with EtOAc. The organic layer was washed with a saturated solution of aqueous NaCl, dried over MgSO_4 and the solvent was evaporated under reduced pressure to give 63 mg (0.09 mmol, yield: 90%) of **10** as a yellow foam. This structure was confirmed by high resolution mass spectrum. **(+)-HR-ESIMS m/z** : 464.1296 ($[\text{M}-\text{C}_{16}\text{H}_{19}\text{Si}]^+$) (calcd. 464.1308 for $\text{C}_{21}\text{H}_{26}\text{N}_3\text{O}_5\text{S}_2$).

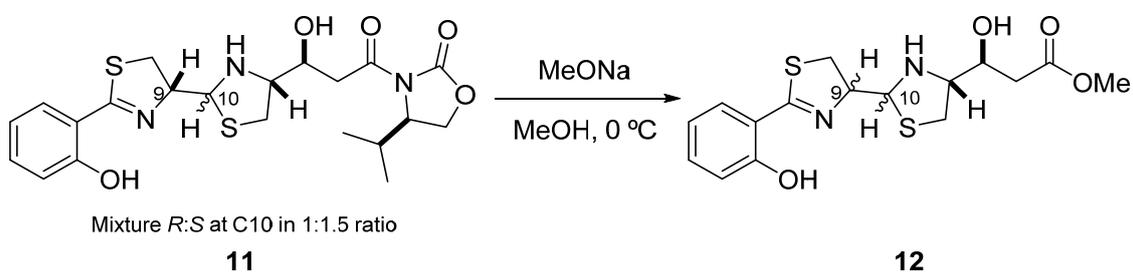
Synthesis of compound 11.



To a stirred solution of compound **9** (66 mg, 0.24 mmol) and potassium acetate (24 mg, 0.24 mmol) in methanol (1 mL), a solution of freshly synthesized aldehyde **8a** (50 mg, 0.24 mmol) in methanol (1 mL) was added at 0°C . After stirring at 0°C for 4 h, the solvent was evaporated under reduced pressure. The crude product was washed with a saturated solution of aqueous NaCl (10 mL) and extracted three times with CH_2Cl_2 (3×15 mL). Evaporation of the solvent under reduced pressure afforded a crude material as yellow foam. The thiazolidine **11**, very sensitive, was obtained as a mixture (105 mg, 0.23 mmol, yield: 96%) of the two diastereoisomers **11a** (9*S*, 10*R*, 12*R*, 13*S*) and **11b** (9*S*, 10*S*, 12*R*, 13*S*) in 1/1.5 ratio and it was directly used for subsequent reaction without any further purification.

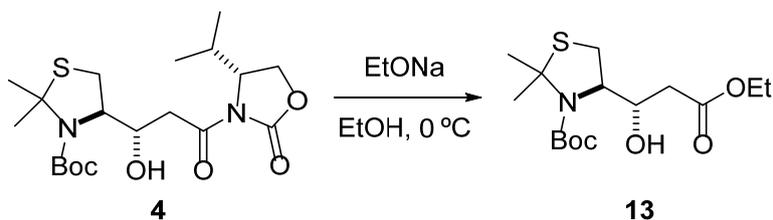
¹H-NMR (500 MHz, CDCl₃), δ_H ppm: 7.42 (2H), 7.35 (2H), 7.00 (2H), 6.89 (2H), 5.00 (2H), 4.92 (d, *J* = 5.4 Hz, 1H), 4.77 (d, *J* = 6.2 Hz, 1H), 4.46 (2H), 4.35 – 4.19 (m, 8H), 3.54 (dd, *J* = 11.2, 8.8 Hz, 1H), 3.46 (dd, *J* = 10.8, 8.6 Hz, 1H), 3.41 – 3.24 (m, 4H), 3.06 – 2.91 (m, 2H), 2.42-2.33 (m, 2H), 0.96 – 0.88 (m, 12H). **¹³C-NMR (125 MHz, CDCl₃), δ_C ppm:** 173.0 (CO), 172.4 (CO), 159.1 (C), 153.9 (CO), 133.2 (CH), 130.6 (CH), 118.9 (CH), 117.6 (CH), 116.1 (C), 79.9 (CH), 79.6 (CH), 72.4 (CH), 71.9 (CH), 68.3 (CH), 68.6 (CH), 67.7 (CH), 67.5 (CH), 63.6 (CH₂), 58.8 (CH), 41.4 (CH₂), 41.1 (CH₂), 35.8 (CH₂), 35.6 (CH₂), 34.6 (CH₂), 33.7 (CH₂), 18.0 (CH₃), 14.8 (CH₃). **(+)-HR-ESIMS *m/z*:** 488.1292 [M+Na]⁺ (calcd. 488.1289 for C₂₁H₂₇N₃O₅NaS₂).

Synthesis of compound 12.



To a stirring solution of thiazolidine **11** (30 mg, 0.06 mmol) in methanol (1 mL) at 0 °C was added a methanolic (0.5 mL) solution of sodium methoxide (4 mg, 0.07 mmol). The resulting solution was stirred at 0 °C for 2 h and then was quenched with an aqueous solution of saturated ammonium chloride and extracted with ethyl acetate (3 x 10 mL). The combined extracts were dried over MgSO₄, and the solvent was evaporated under reduced pressure to give the methyl ester **12** (9.5 mg, 0.026 mmol, yield: 65%) as a yellow foam. This structure was confirmed by high resolution mass spectrum. **(+)-HR-ESIMS *m/z*:** 369.0943 [M+H]⁺ (calcd. 369.0937 for C₁₆H₂₁N₂O₄S₂)

Synthesis of compound 13.

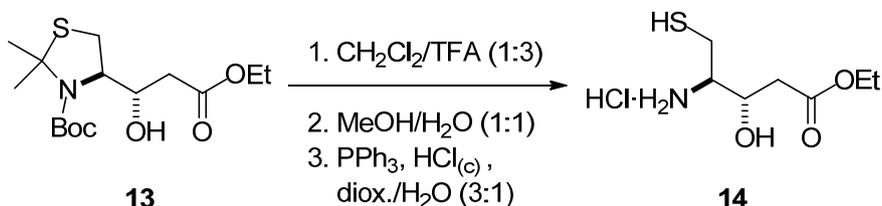


To a stirring solution of compound **4** (160 mg, 0.38 mmol) in ethanol (5 mL) at 0 °C was added 2 mL of an ethanolic solution of sodium ethoxide (55 mg, 0.77 mmol), the resulting solution was stirred for 15 min at 0 °C and then was quenched with an aqueous solution of saturated ammonium

chloride and extracted with diethyl ether (3 x 20 mL). The combined extracts were dried over MgSO₄, and the solvent was evaporated under reduced pressure to give a white solid. This crude material was purified by chromatography on silica gel column (hexane/ethyl acetate 2:1) to give the expected ester **13** isolated as a white crystalline solid (102 mg, 0.30 mmol, yield: 80%).

¹H-NMR (300 MHz, CDCl₃), δ_H ppm: 4.52 (t, *J* = 6.7 Hz, 1H), 4.37 (ddd, *J* = 8.5, 6.6, 3.8 Hz, 1H), 4.18 (bq, *J* = 7.2 Hz, 2H), 3.20 (dd, *J* = 12.5, 6.6 Hz, 1H), 2.76 (bd, *J* = 12.5 Hz, 1H), 2.67 – 2.46 (m, 2H), 1.79 (s, 3H), 1.78 (s, 3H), 1.49 (s, 9H), 1.28 (t, *J* = 7.2 Hz, 3H). **¹³C-NMR (75 MHz, CDCl₃), δ_C ppm:** 172.2 (CO), 154.2 (CO), 81.2 (C), 70.4 (C), 66.8 (CH), 65.9 (CH), 60.7 (CH₂), 37.8 (CH₂), 28.9 (CH₂), 27.4 (2xCH₃), 27.5 (3xCH₃, tBu), 14.2 (CH₃). **(+)-HR-ESIMS *m/z*:** 356.1511 [M+Na]⁺ (calcd. 356.1502 for C₁₅H₂₇NO₅NaS). **[α]_D²³** = -35.6 (c = 0.40, CHCl₃).

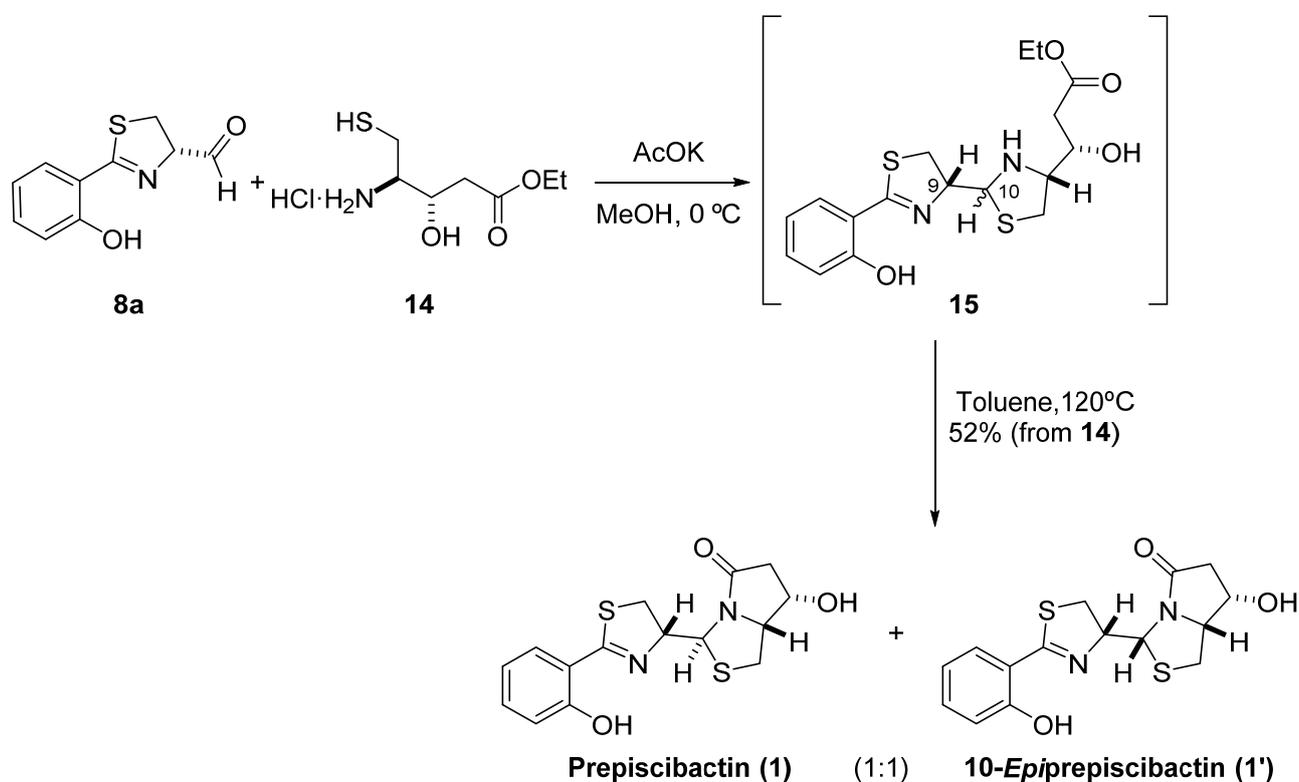
Synthesis of compound **14**.



A solution of **13** (80 mg, 0.24mmol) in 6 mL of a 1:3 (v/v) mixture of dichloromethane and trifluoroacetic acid was stirred at room temperature for two days. The solution was then evaporated under reduced pressure and the crude acetone was dissolved in a 1:1 (v/v) mixture of methanol and water (10 mL). The solvents were removed under reduced pressure to give a dimer. Cleavage of the disulfide bridge was performed by solving the dimer in 1.2 mL of dioxane:water 1:3 (v/v), then PPh₃ (92 mg, 0.35 mmol) was added and finally HCl_(c) (0.1 mL). After stirring at 40 °C for 4 hours, solvent was removed under reduced pressure. This crude material was redissolved in water and washed with hexane, the aqueous layer was concentrated under reduced pressure to give the crude material that was purified by HPLC using a Discovery® HS F5 column (25 cm x 10 mm, 5 μm) with a mobile phase consisting of an isocratic 25% CH₃CN in H₂O (v/v, each containing 0.1% TFA) at a flow rate of 1.5 mL/min (*t_R* = 14.1 min) to give 39 mg (0.17 mmol, yield: 71%) of **14** as a grayish crystalline solid.

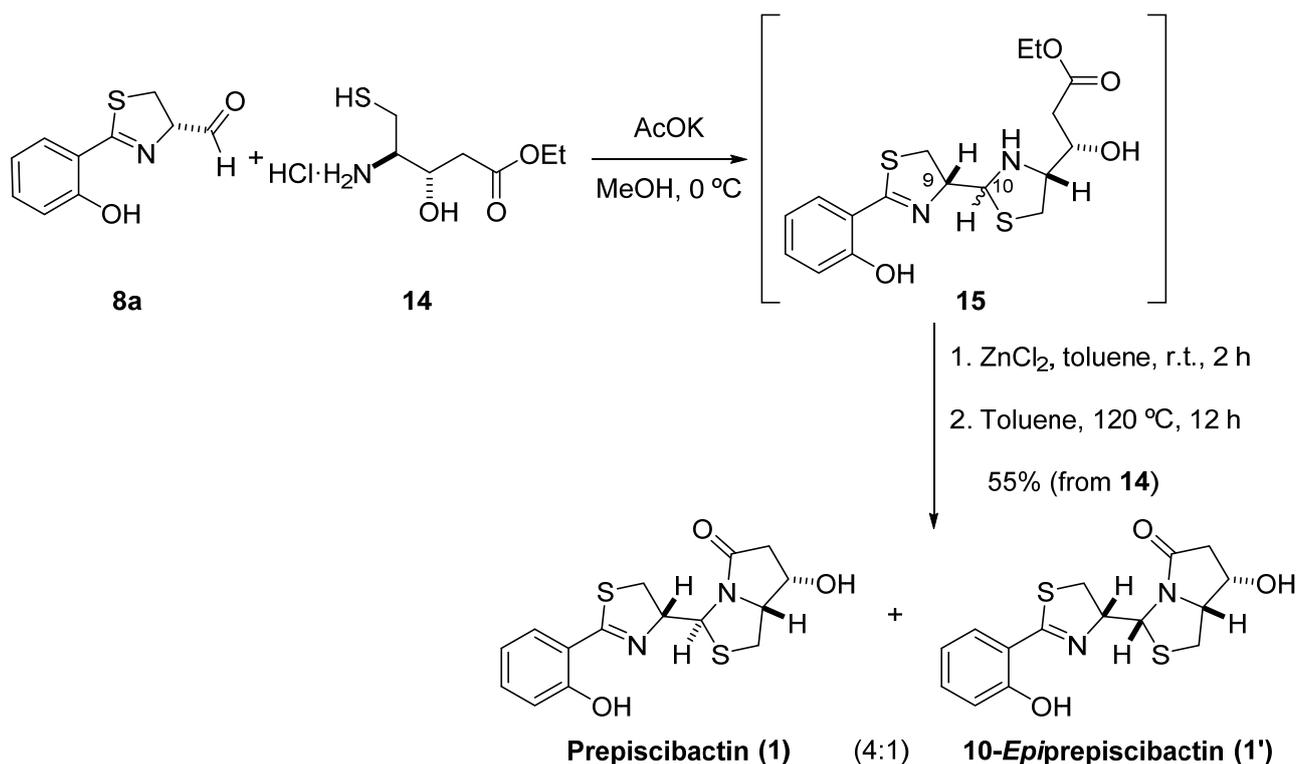
¹H-NMR (300 MHz, CDCl₃), δ_H ppm: 4.30 (dt, *J* = 7.7, 5.1 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.35 – 3.25 (m, 1H), 2.92 (dd, *J* = 14.5, 5.1 Hz, 1H), 2.83 – 2.55 (m, 3H), 1.28 (t, *J* = 7.1 Hz, 3H). **¹³C-NMR (75 MHz, CDCl₃), δ_C ppm:** 171.0 (CO), 65.5 (CH), 60.6 (CH₂), 57.5 (CH), 38.6 (CH₂), 23.6 (CH₂), 13.1 (CH₃). **(+)-HR-ESIMS *m/z*:** 194.0842 [M+H]⁺ (calcd. 194.0845 for C₇H₁₆NO₃S). **[α]_D²⁶** = -19.6 (c = 0.65, MeOH).

Synthesis of prepiscibactin (**1**) and 10-epiprepiscibactin (**1'**).



To a stirred solution of compound **14** (25 mg, 0.12 mmol) and potassium acetate (12 mg, 0.12 mmol) in methanol (1 mL), a solution of freshly synthesized aldehyde **8a** (25 mg, 0.12 mmol) in methanol (1 mL) was added at 0 °C. After stirring at 0 °C for 2 h, the solvent was evaporated under reduced pressure. The crude product was washed with a saturated solution of aqueous NaCl (5 mL) and extracted with CH₂Cl₂ (3×10 mL). The organic phase was dried over MgSO₄ and the solvent evaporated. The crude thiazolidine **15**, obtained as very sensitive yellow foam, was directly used for subsequent reaction without any further purification.

A solution of thiazolidine **15** (44 mg, 0.12 mmol) in toluene (3 mL) was stirred at 120 °C overnight. Evaporation of the solvent under reduced pressure afforded a mixture of the two diastereoisomers **1** (9*R*, 10*S*, 12*R*, 13*S*) and **1'** (9*R*, 10*R*, 12*R*, 13*S*) that were separated by HPLC using a Discovery® HS F5 (100×4.6 mm, 5 μm) column with a mobile phase consisting on this sequence at a flow rate of 1 mL/min (percentages are v/v, each containing 0.1% TFA): from 0 to 10 min, gradient from 10% to 30% CH₃CN in H₂O; from 10 to 13 min, isocratic 30% CH₃CN in H₂O; from 13 to 15 min, gradient from 30% to 100% CH₃CN in H₂O. Fractions containing 10-epiprepiscibactin (**1'**, *t_R* = 9.5 min, 10 mg, 26% yield) and prepiscibactin (**1**, *t_R* = 10.5 min, 10 mg, 26% yield) were pooled and dried *in vacuo*. HPLC chromatogram of the crude of the reaction showed an 1:1 ratio between **1** and **1'** (total 21 mg, 0.06 mmol, global yield: 52%).



To a stirred solution of compound **14** (25 mg, 0.12 mmol) and potassium acetate (12 mg, 0.12 mmol) in methanol (1 mL), a solution of freshly synthesized aldehyde **8a** (25 mg, 0.12 mmol) in methanol (1 mL) was added at 0 °C. After stirring at 0 °C for 2 h, the solvent was evaporated under reduced pressure. The crude product was washed with a saturated solution of aqueous NaCl (5 mL) and extracted with CH₂Cl₂ (3×10 mL). The organic phase was dried over MgSO₄ and the solvent evaporated. The crude thiazolidine **15**, obtained as very sensitive yellow foam, was directly used for subsequent reaction without any further purification.

To a solution of thiazolidine **15** (45 mg, 0.12 mmol) in toluene (10 mL) was added ZnCl₂ (40 mg, 0.3 mmol) and the mixture was stirred at room temperature for 2 h and then, at 120 °C overnight. The reaction mixture was diluted with EtOAc, washed with 5% aq. KHSO₄ and brine, dried over Na₂SO₄, then concentrated in vacuo to give a mixture of prepiscibactin (**1**) and 10-*epi*prepiscibactin (**1'**) (ca. 4:1 by HPLC). Two diastereoisomers **1** (9*R*, 10*S*, 12*R*, 13*S*) and **1'** (9*R*, 10*R*, 12*R*, 13*S*), were separated by HPLC using a Discovery® HS F5 (100×4.6 mm, 5 μm) column with a mobile phase consisting on this sequence at a flow rate of 1 mL/min (percentages are v/v, each containing 0.1% TFA): from 0 to 10 min, gradient from 10% to 30% CH₃CN in H₂O; from 10 to 13 min, isocratic 30% CH₃CN in H₂O; from 13 to 15 min, gradient from 30% to 100% CH₃CN in H₂O. Fractions containing 10-*epi*prepiscibactin (**1'**, *t_R* = 9.3 min, 4.2 mg, yield 11%) and prepiscibactin (**1**, *t_R* = 10.3 min, 17.5 mg, yield 44%) were pooled and dried *in vacuo*. Total 21.9 mg, 0.06 mmol, global yield: 55%.

Prepiscibactin (1): ¹H-NMR (500 MHz, CD₃OD), δ_H ppm: 7.46 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.41 (ddd, *J* = 8.4, 7.6, 1.7 Hz, 1H), 6.97 (dd, *J* = 8.4, 1.0 Hz, 1H), 6.93 (ddd, *J* = 7.8, 7.6, 1.0 Hz, 1H), 5.32 (d, *J* =

7.1 Hz, 1H), 5.00 (ddd, $J = 8.9, 7.3, 7.1$ Hz, 1H), 4.64 – 4.50 (m, 2H), 3.54 (dd, $J = 11.5, 8.9$ Hz, 1H), 3.39 (dd, $J = 11.5, 7.3$ Hz, 1H), 3.29 (dd, $J = 10.7, 8.4$ Hz, 1H), 3.06 (dd, $J = 17.2, 6.0$ Hz, 1H), 3.01 (dd, $J = 10.7, 6.5$ Hz, 1H), 2.44 (dd, $J = 17.2, 2.2$ Hz, 1H). **$^{13}\text{C-NMR}$ (125 MHz, CD_3OD), δ_{C} ppm:** 175.5 (CO), 175.2 (C), 160.2 (C), 134.5 (CH), 131.7 (CH), 120.2 (CH), 117.9 (CH), 117.3 (C), 82.5 (CH), 70.4 (CH), 66.7 (CH), 63.1 (CH), 43.9 (CH_2), 34.7 (CH_2), 30.4 (CH_2). **(+)-HR-ESIMS m/z :** 337.0664 $[\text{M}+\text{H}]^+$ (calcd. 337.0675 for $\text{C}_{15}\text{H}_{17}\text{N}_2\text{O}_3\text{S}_2$). $[\alpha]_{\text{D}}^{26} = -35.3$ ($c = 0.12$, MeOH).

10-Epiprepiscibactin (1'): **$^1\text{H-NMR}$ (500 MHz, CD_3OD), δ_{H} ppm:** 7.51 (dd, $J = 7.8, 1.7$ Hz, 1H), 7.46 (ddd, $J = 8.4, 7.6, 1.7$ Hz, 1H), 7.00 (dd, $J = 8.4, 1.1$ Hz, 1H), 6.96 (ddd, $J = 7.8, 7.6, 1.1$ Hz, 1H), 5.50 (d, $J = 4.8$ Hz, 1H), 5.03 (ddd, $J = 8.9, 8.2, 4.8$ Hz, 1H), 4.45 (ddd, $J = 5.9, 5.0, 1.8$ Hz, 1H), 4.40 (ddd, $J = 7.8, 7.4, 5.0$ Hz, 1H), 3.63 (dd, $J = 11.2, 8.9$ Hz, 1H), 3.53 (dd, $J = 11.2, 8.2$ Hz, 1H), 3.37 (dd, $J = 10.4, 7.8$ Hz, 1H), 3.13 (dd, $J = 10.4, 7.4$ Hz, 1H), 2.99 (dd, $J = 17.1, 5.9$ Hz, 1H), 2.39 (dd, $J = 17.1, 1.8$ Hz, 1H). **$^{13}\text{C-NMR}$ (125 MHz, CD_3OD), δ_{C} ppm:** 177.1 (CO), 175.4 (C), 160.3 (C), 135.6 (CH), 131.9 (CH), 120.5 (CH), 118.1 (CH), 116.6 (C), 80.8 (CH), 70.6 (CH), 67.2 (CH), 62.8 (CH), 44.3 (CH_2), 34.0 (CH_2), 30.2 (CH_2). **(+)-HR-ESIMS m/z :** 337.0677 $[\text{M}+\text{H}]^+$ (calcd. 337.0675 for $\text{C}_{15}\text{H}_{17}\text{N}_2\text{O}_3\text{S}_2$). $[\alpha]_{\text{D}}^{26} = -25.3$ ($c = 0.12$, MeOH).

III. NMR spectra and HRESIMS of 1, 4-14

¹H-NMR (300 MHz, CDCl₃)

4.5528
4.5316
4.5107
4.4728
4.4613
4.4482
4.4337
4.4218
4.4108
4.4017
4.3925
4.3808
4.3701
4.3464
4.2937
4.2635
4.2365
4.2177
4.2068
4.1875
4.1765

3.2779
3.2669
3.2250
3.2217
3.2144
3.2002
3.1802
3.1591
3.1309
3.1083
3.0783
2.8458
2.8047

2.4068
2.3936
2.3835
2.3704
2.3602
2.3472

1.7873
1.7709

1.4697
1.4620

0.9173
0.8939
0.8709

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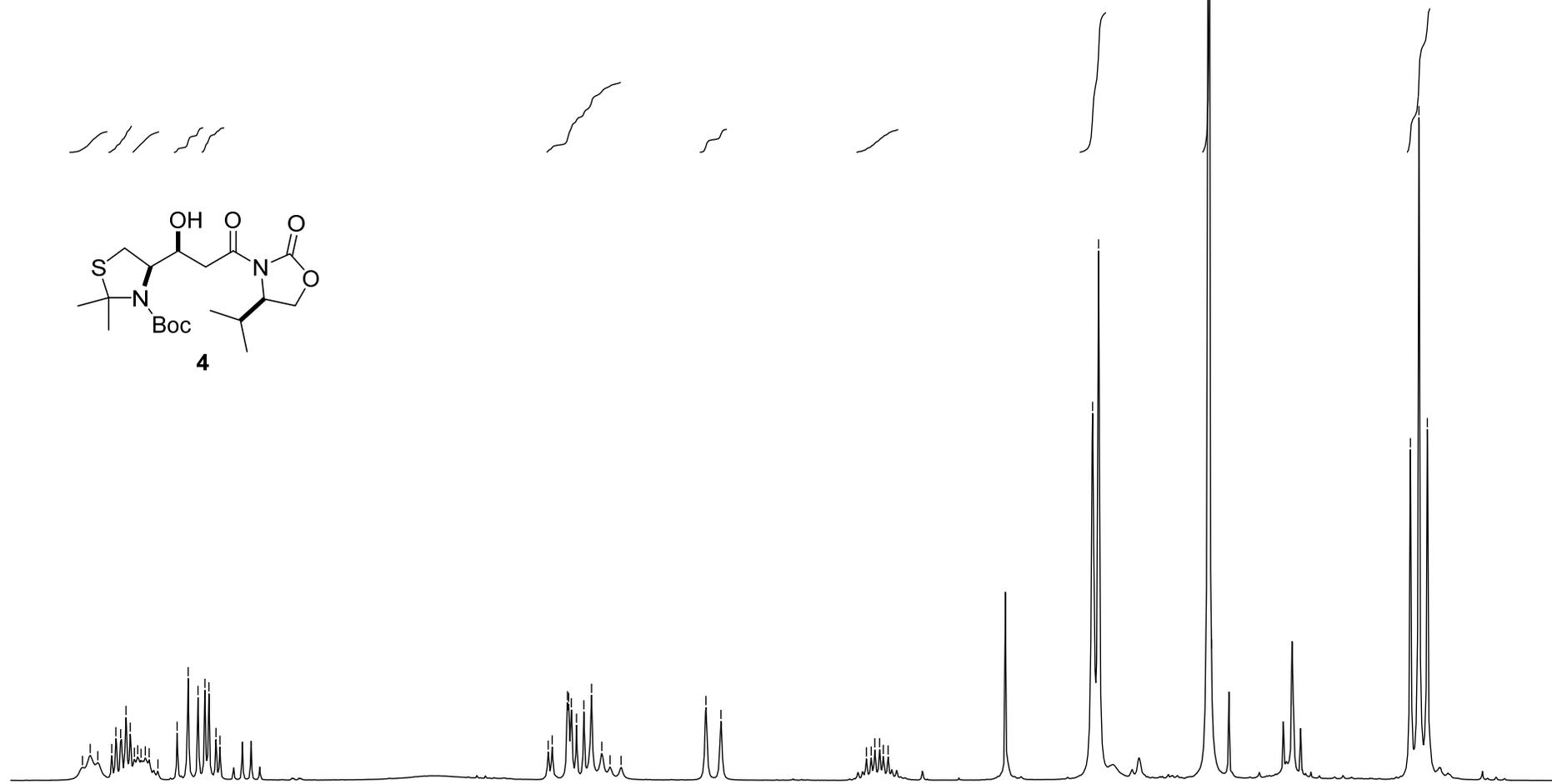
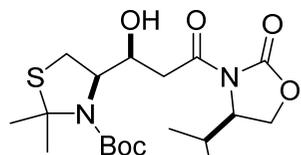
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0.9
1.1
0.9
1.1
1.1
1.1

3.1

1.0

1.0

6.1

8.9

6.3

4.5

4.0

3.5

3.0

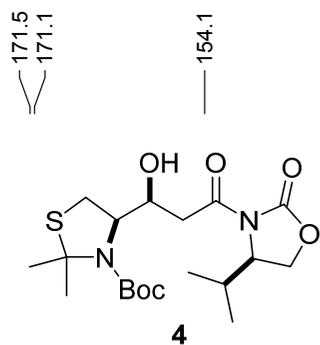
2.5
f1 (ppm)

2.0

1.5

1.0

$^{13}\text{C-NMR}$ (75 MHz, CDCl_3)



171.5
171.1

154.1

81.2

70.6

67.2

63.5

58.4

40.8

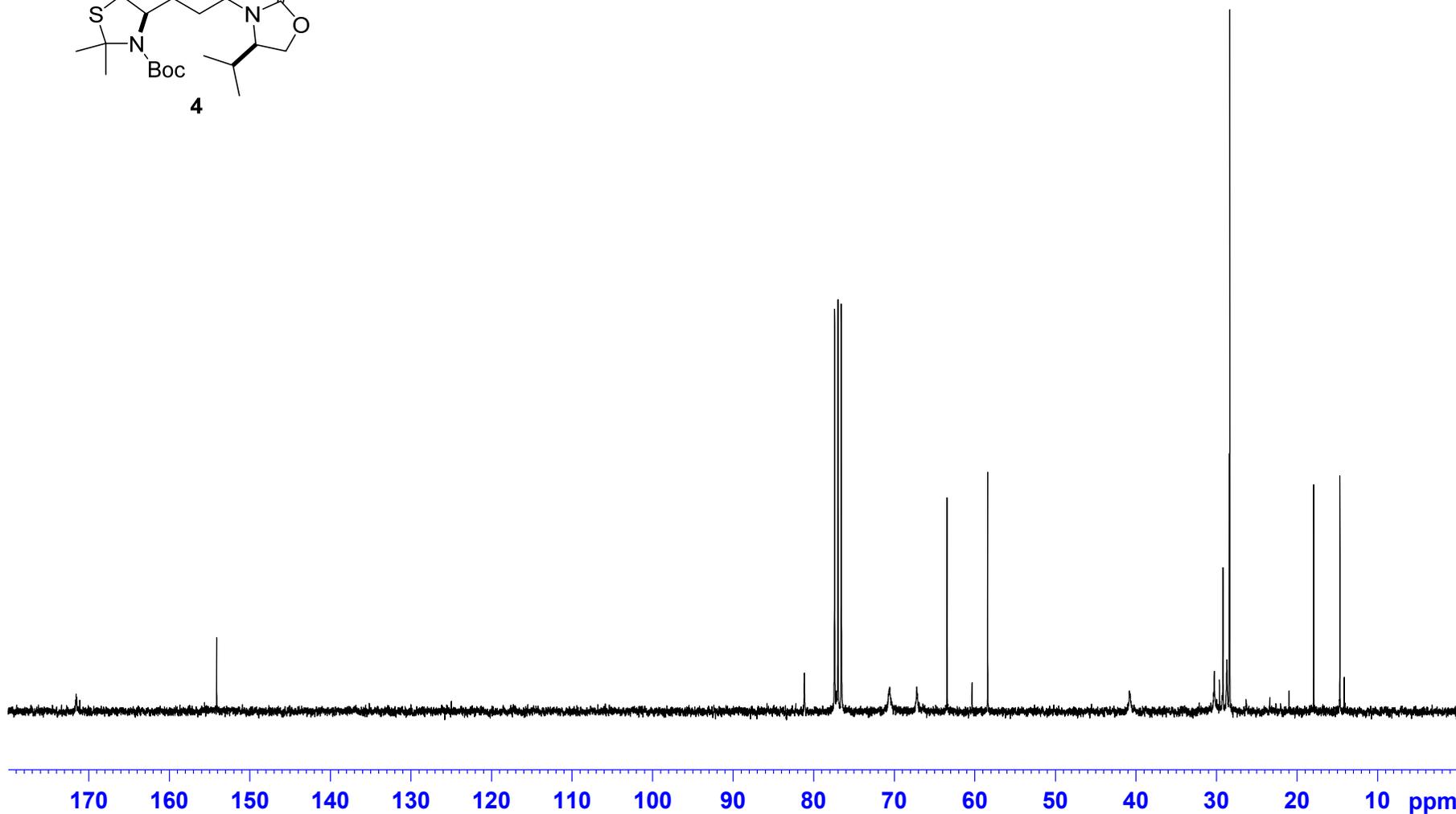
29.2

28.4

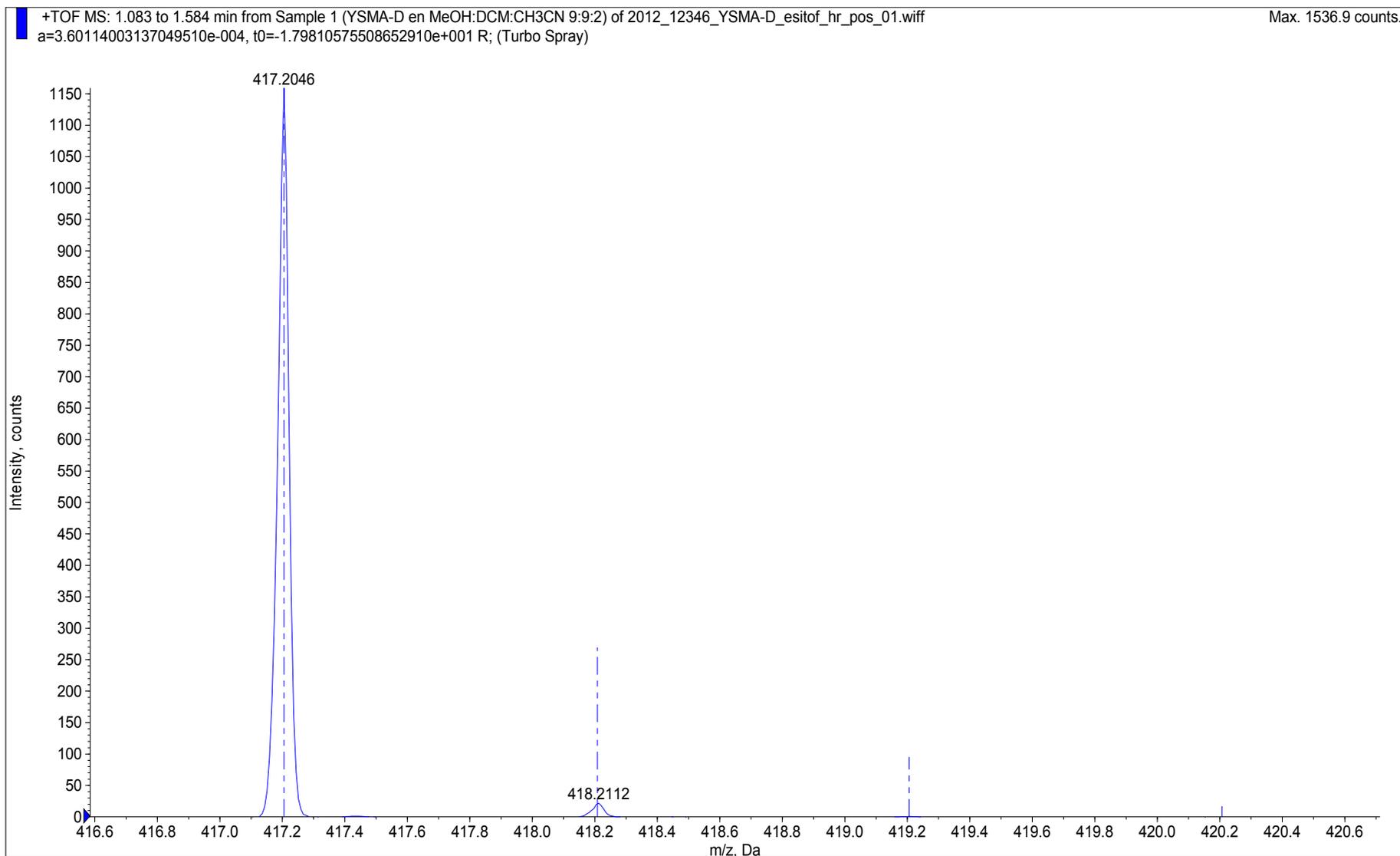
28.4

17.9

14.7

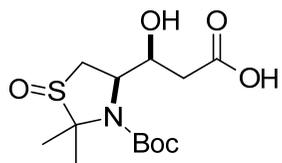


HRESIMS of 4: Ion: m/z 417.2046, ([M+H]⁺)

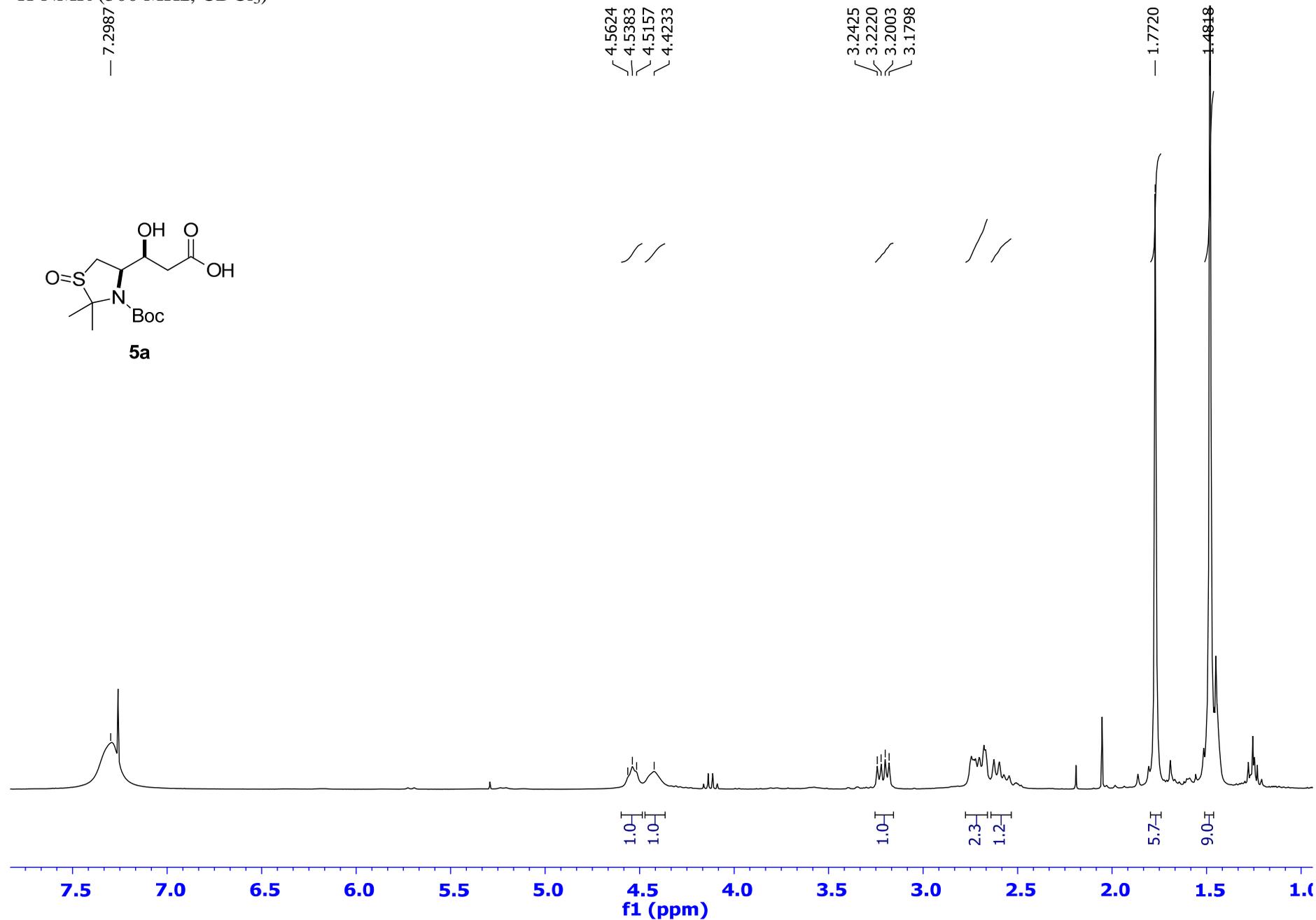


Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
C ₁₉ H ₃₃ N ₂ O ₆ S	417.2053	-0.7853	-1.8825	4.5

¹H-NMR (300 MHz, CDCl₃)

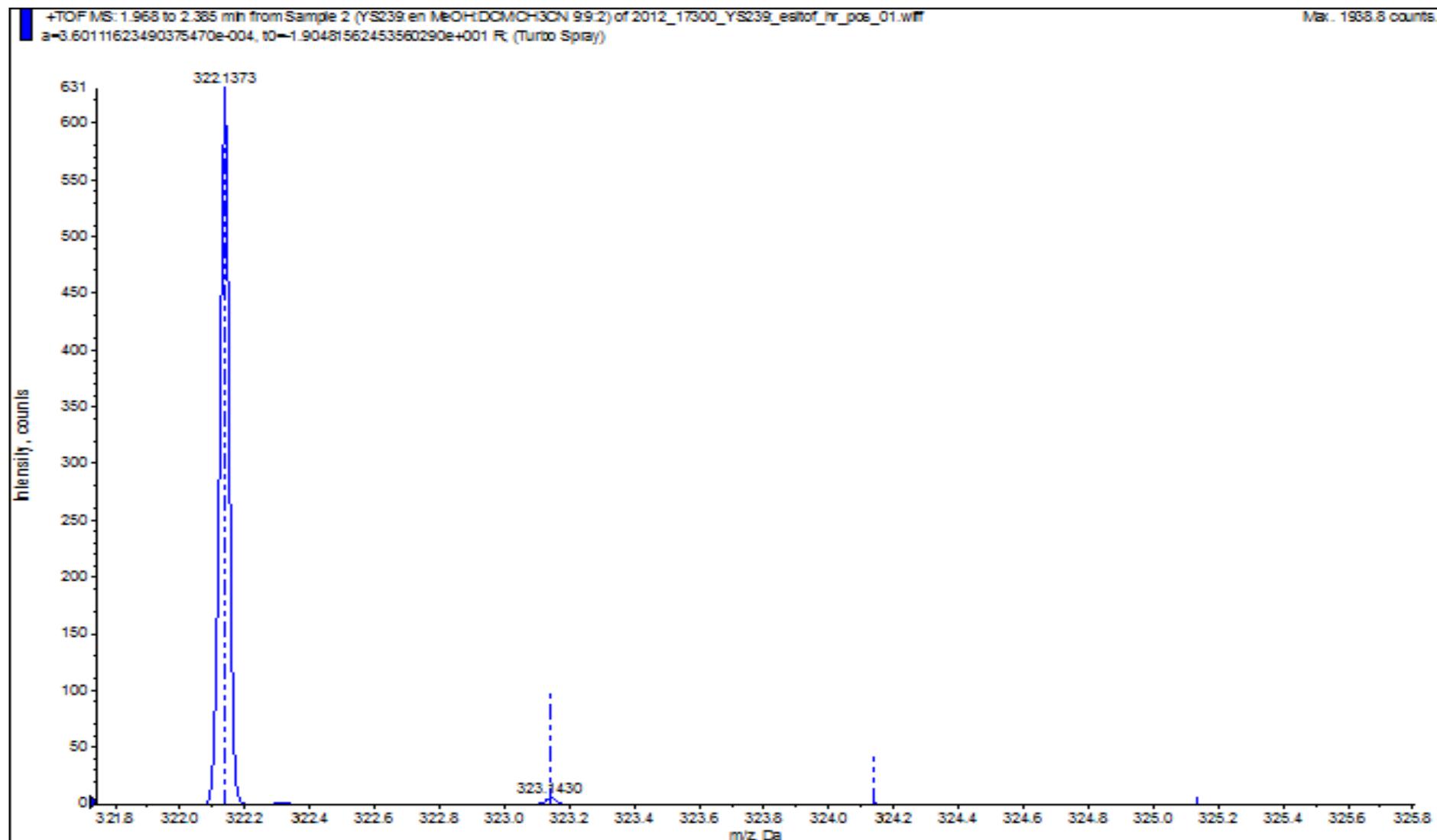


5a



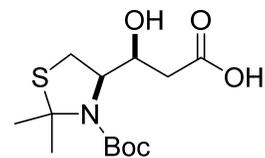
SI22

HR-ESIMS of 5a. Ion: m/z 322.1373, $([M+H]^+)$



Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
$C_{13}H_{24}NO_6S$	322.1281	0.3286	1.0734	2.5

¹H-NMR (500 MHz, CD₃OD) of 5b

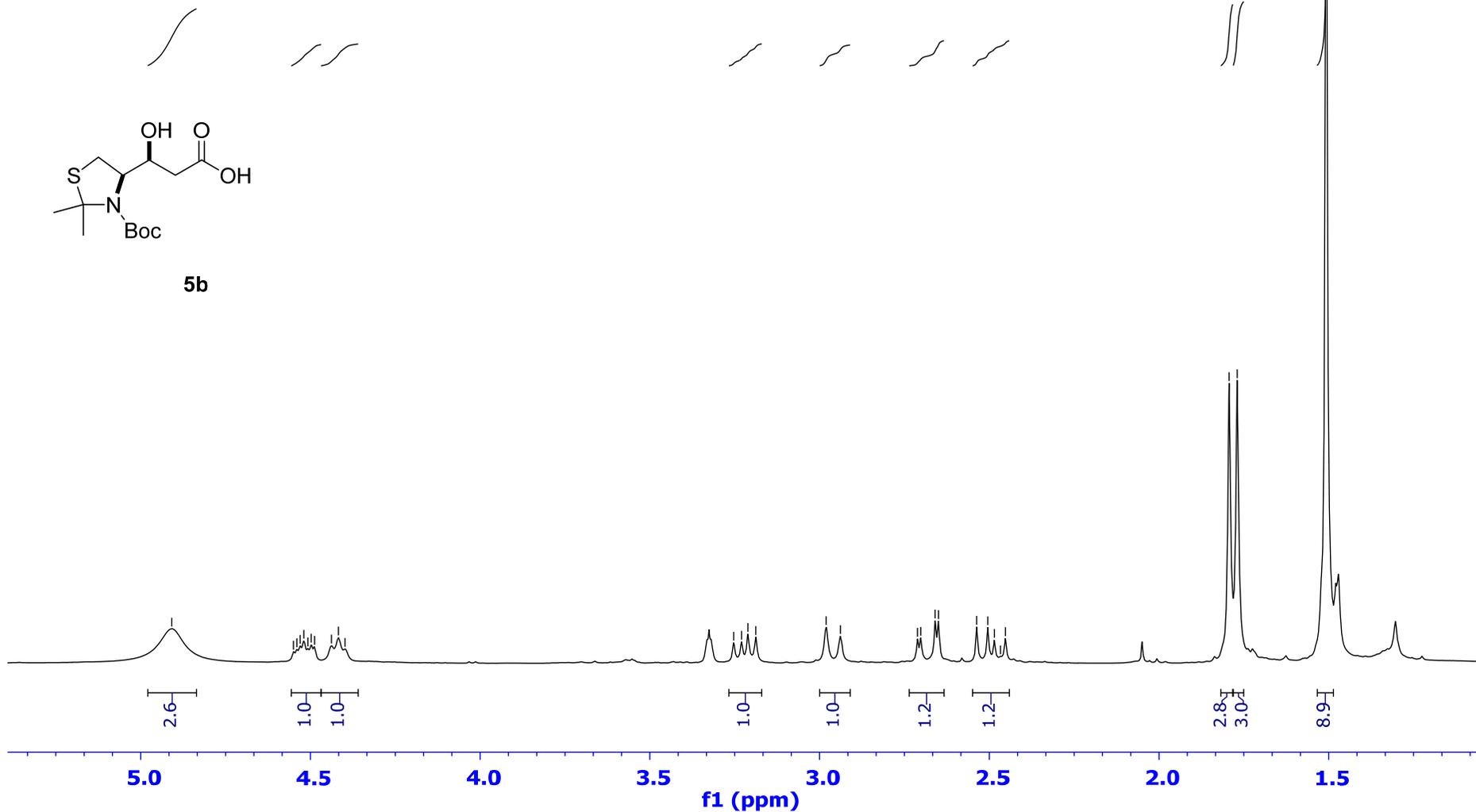


5b

4.9087
4.5502
4.5401
4.5304
4.5194
4.5076
4.4977
4.4877
4.4381
4.4174
4.3980

3.2531
3.2299
3.2112
3.1880
2.9807
2.9387
2.7116
2.7018
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2.5044
2.4850
2.4671
2.4524

1.7933
1.7696

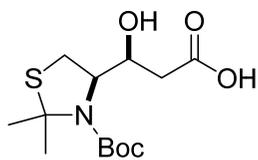


¹³C-NMR (125 MHz, CD₃OD) of 5b

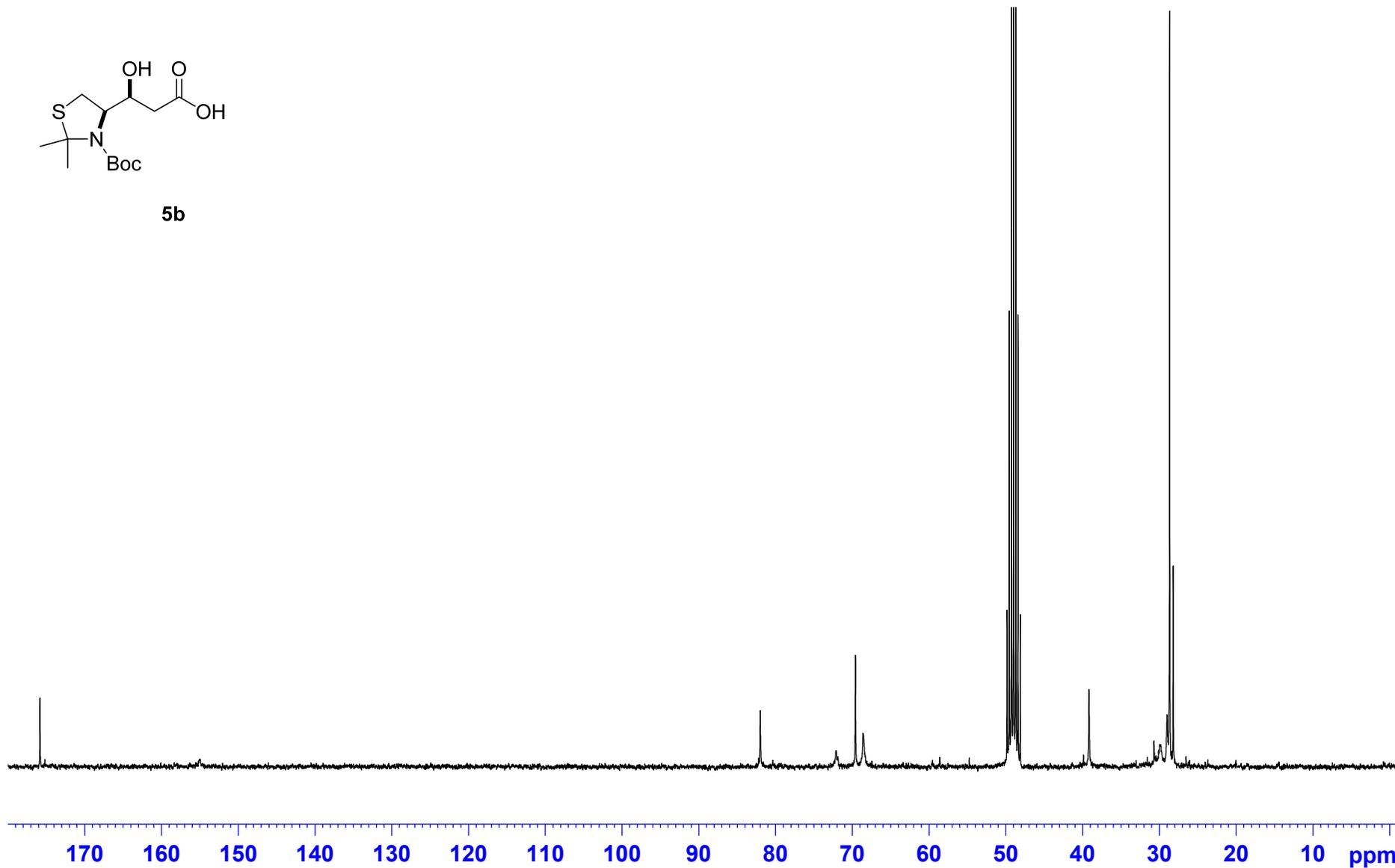
175.8
155.0

82.0
72.2
69.6
68.6
67.5

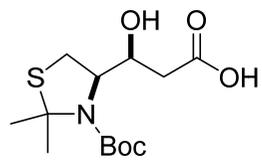
39.2
28.7
28.2



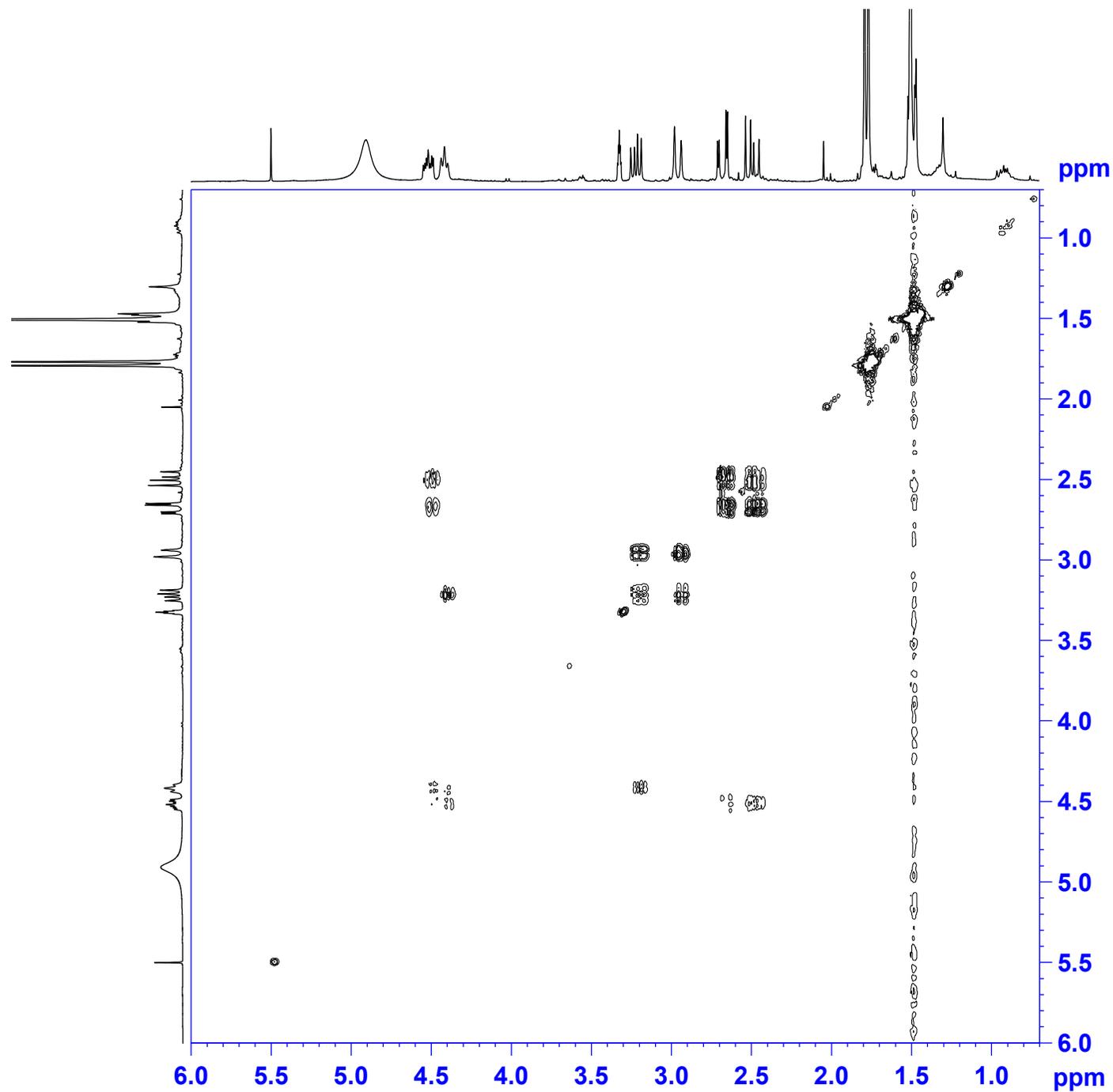
5b



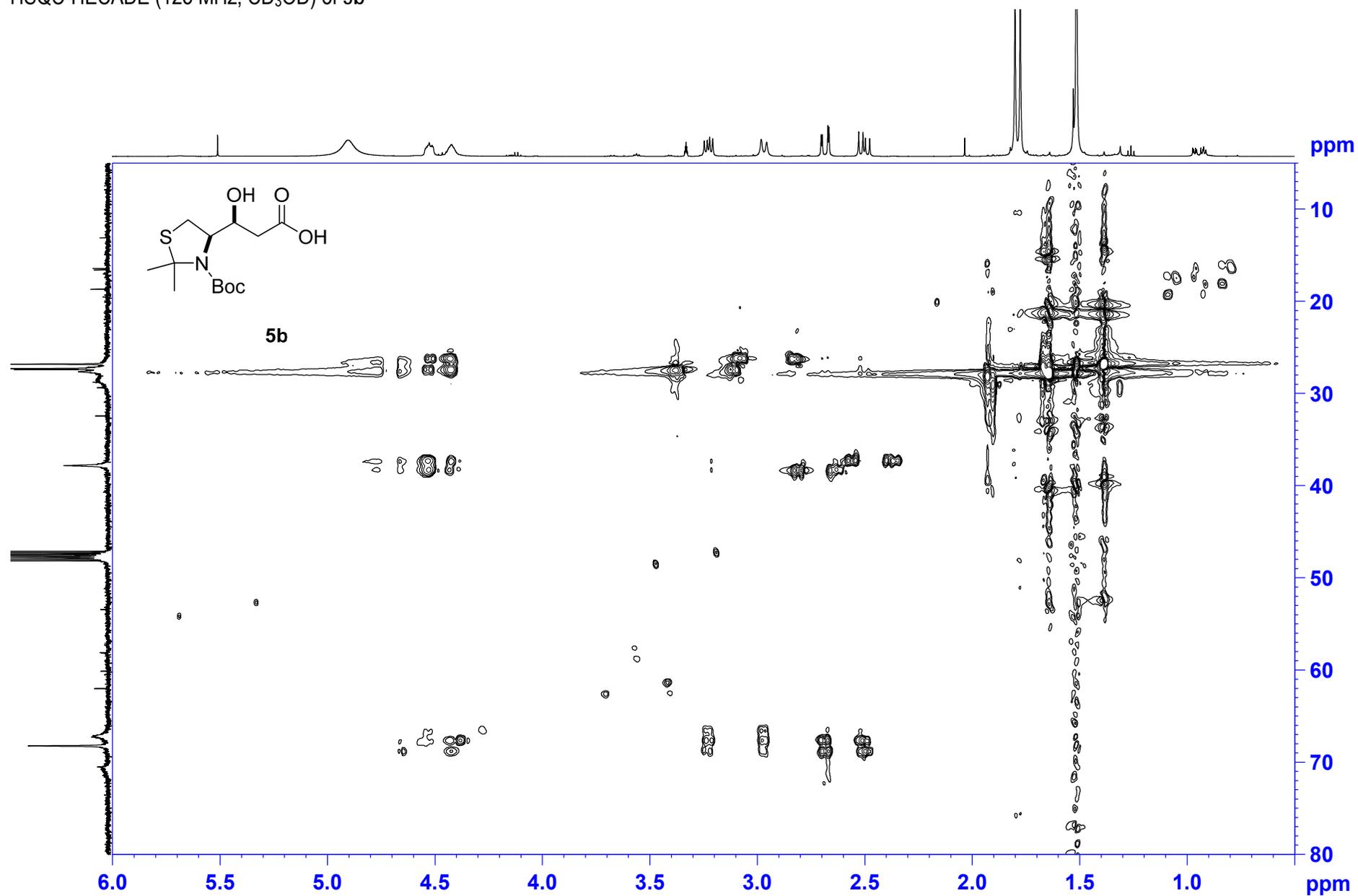
^1H - ^1H -COSY
(300 MHz, CDCl_3)



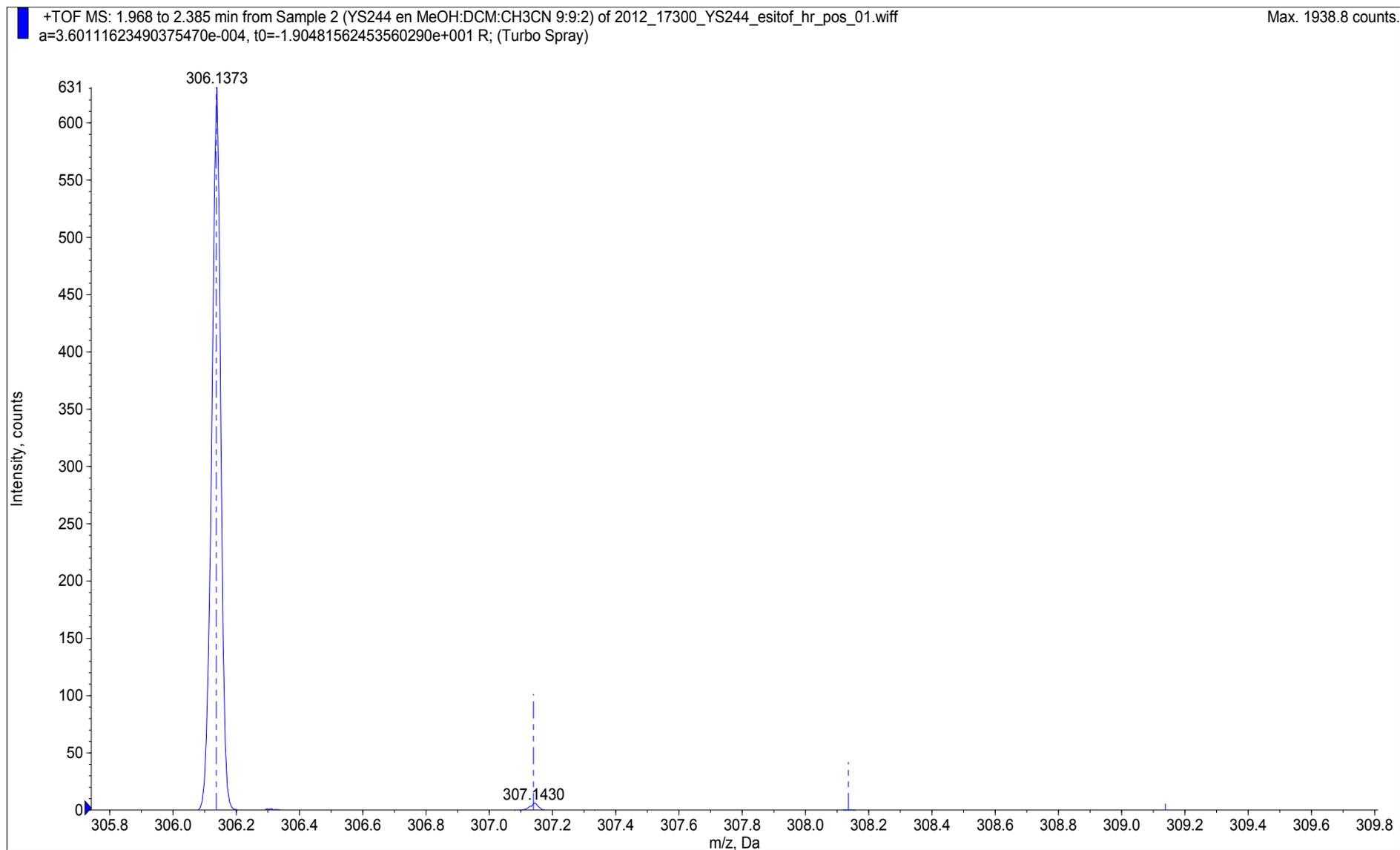
5b



HSQC-HECADE (125 MHz, CD₃OD) of 5b

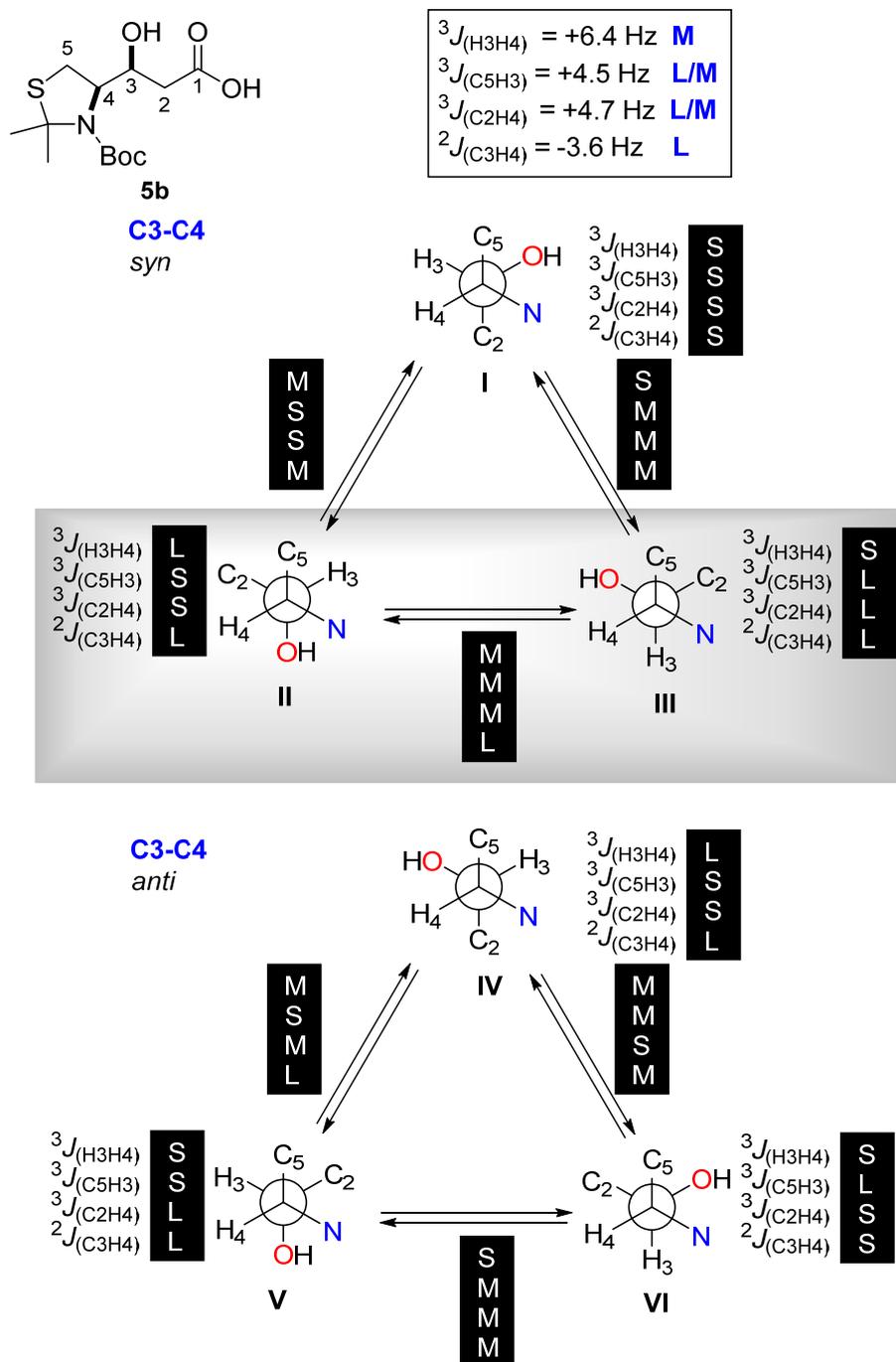


HRESIMS of 5b: Ion m/z 306.1373, $([M+H]^+)$



Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
<chem>C13H24NO5S</chem>	306.1369	0.3286	1.0734	2.5

J -Based configurational analysis of the C3-C4 bond of 5b



¹H-NMR (500 MHz, CDCl₃) of 6a

4.5760
4.4868
4.4800
4.4712
4.4635
4.4566
4.4432
4.4302
4.4184
4.4048
4.3155
4.2979
4.2810
4.2468
4.2413
4.2287
4.2231

3.2424
3.2296
3.2178
3.2048

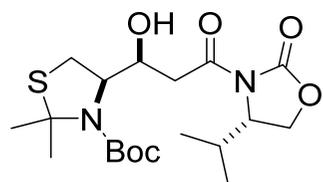
2.8679
2.8432

2.4551
2.4488
2.4411
2.4349
2.4272
2.4209
2.4133
2.4069

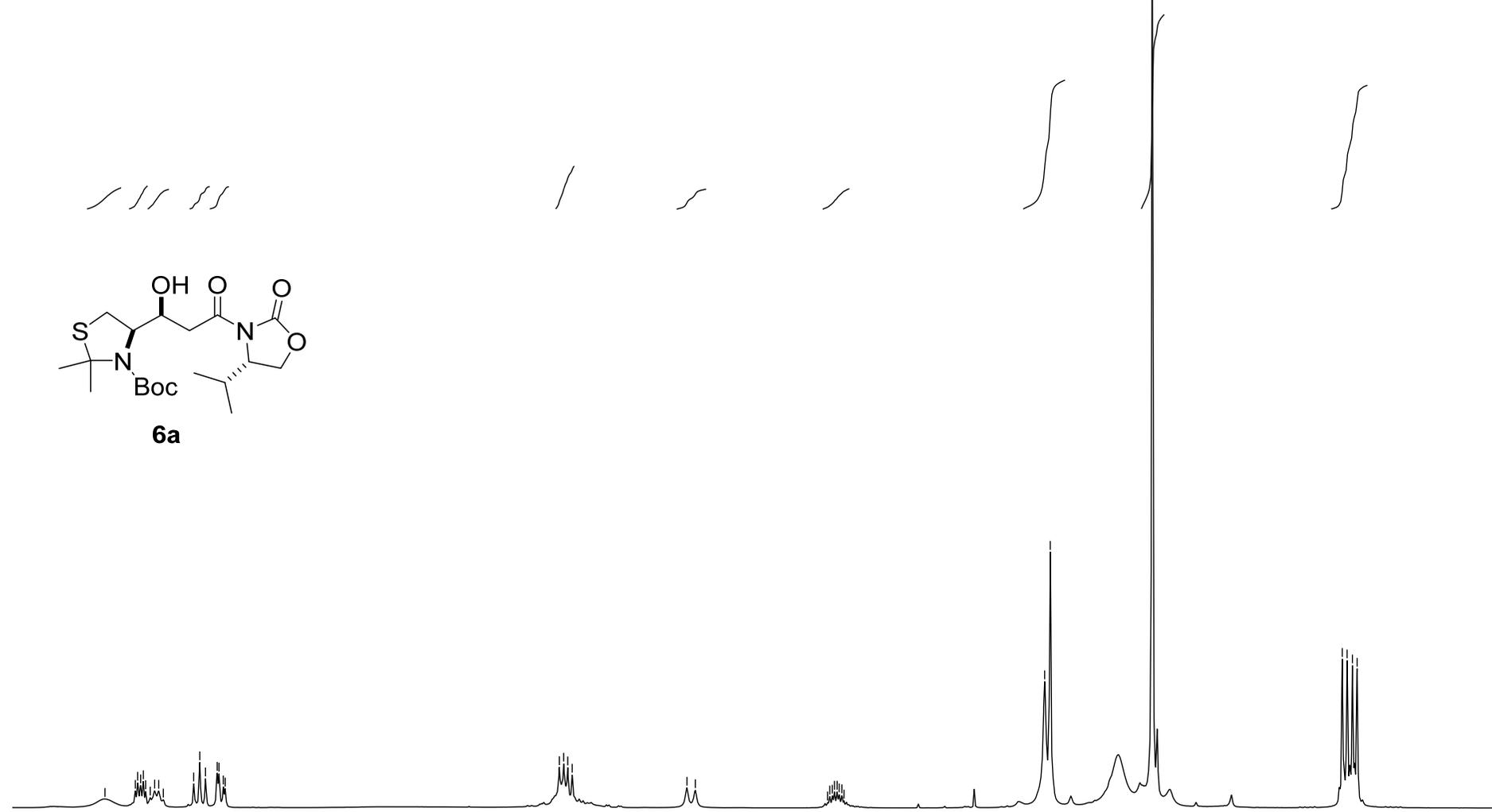
1.8179
1.8013

1.5022

0.9443
0.9303
0.9146
0.9008



6a



1.0
1.1
0.9
1.1
1.1

2.0

0.9

1.0

6.1

9.2

5.8

4.5

4.0

3.5

3.0

f1 (ppm)

2.5

2.0

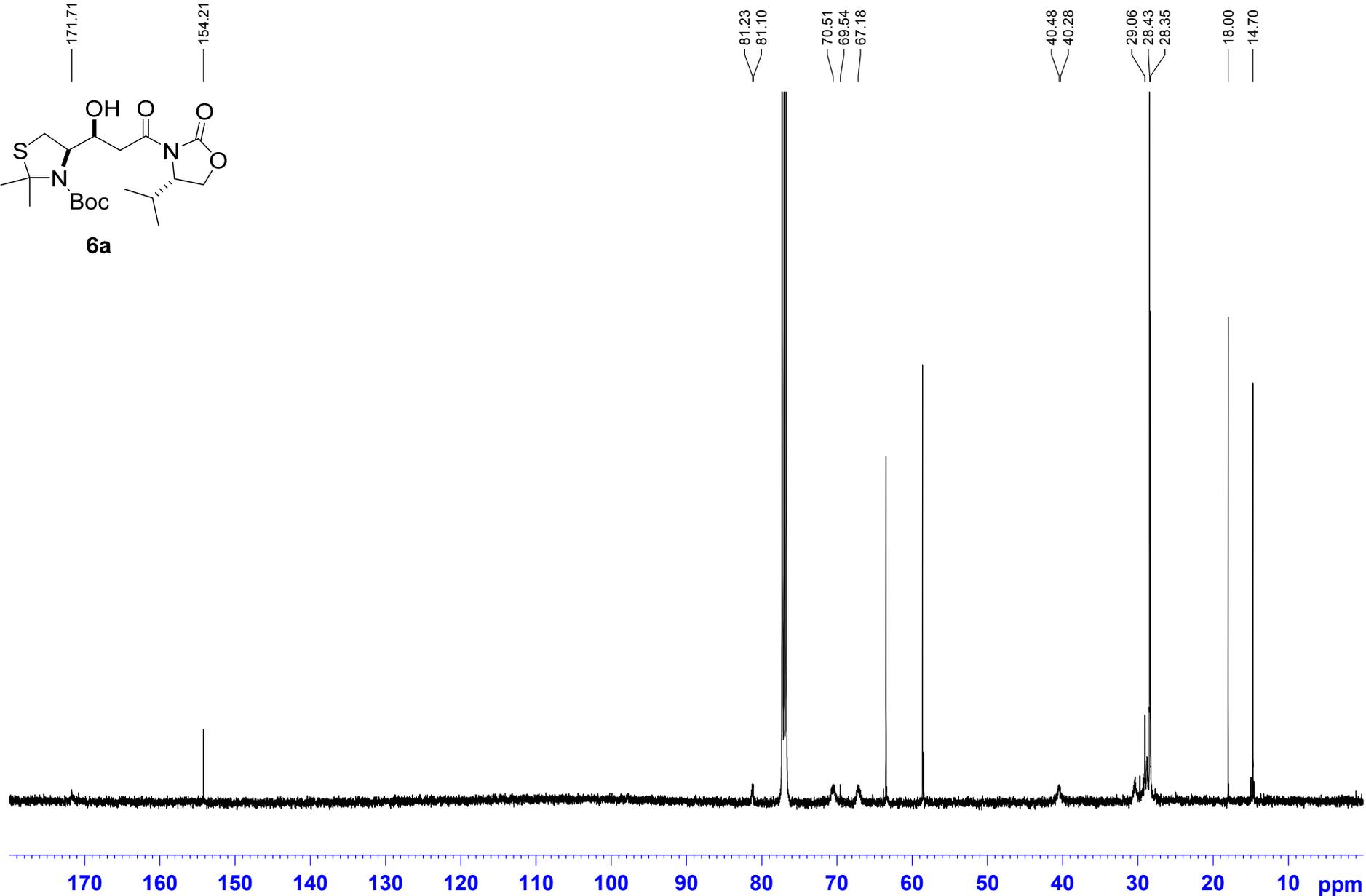
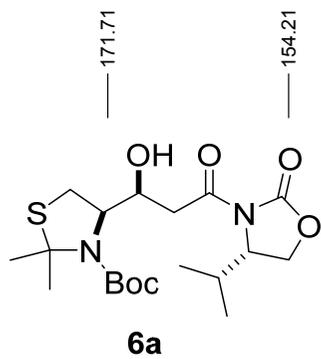
1.5

1.0

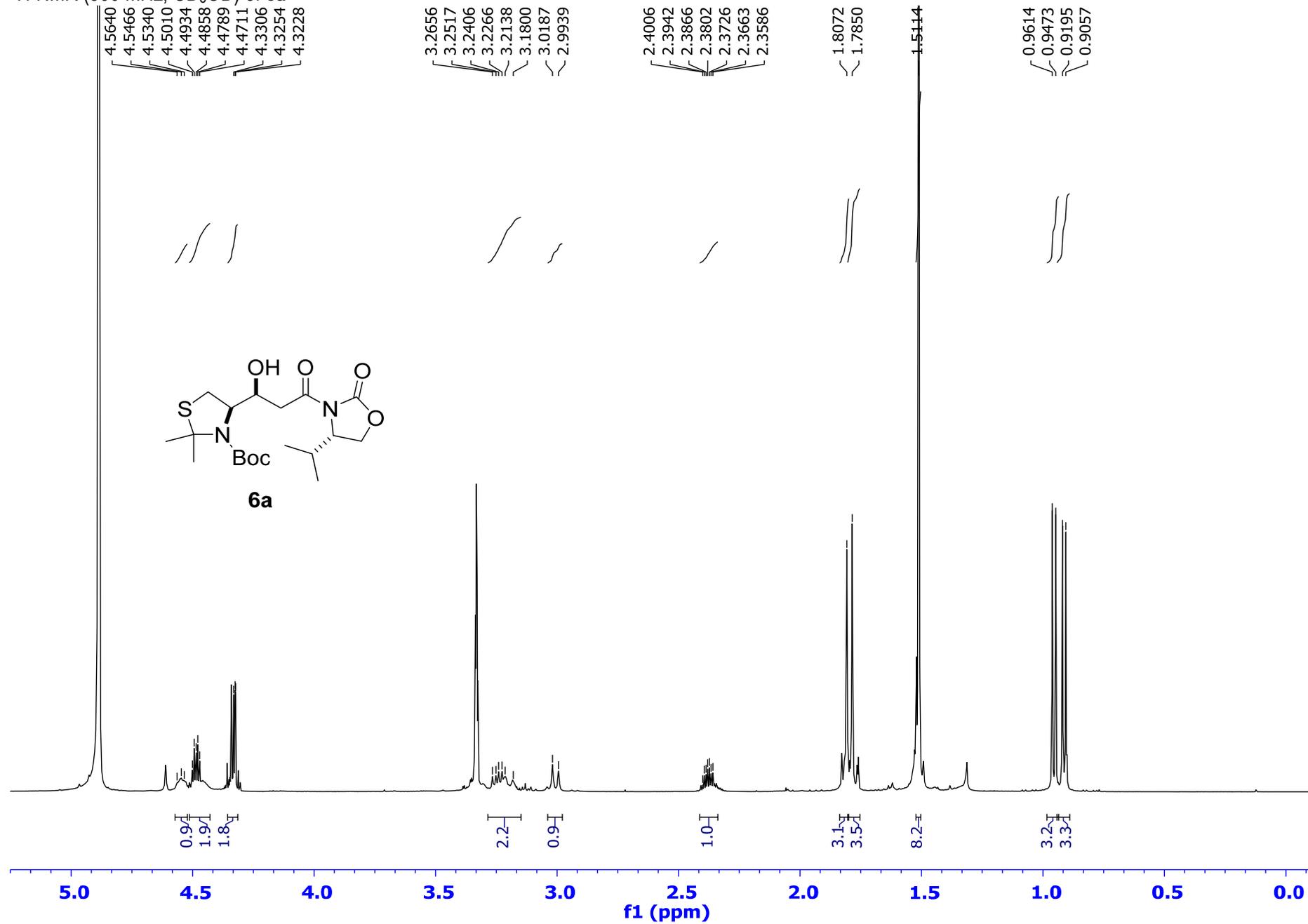
0

SI30

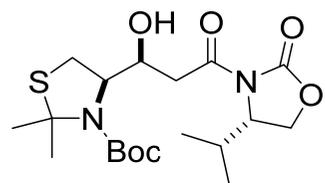
$^{13}\text{C-NMR}$ (125 MHz, CDCl_3)



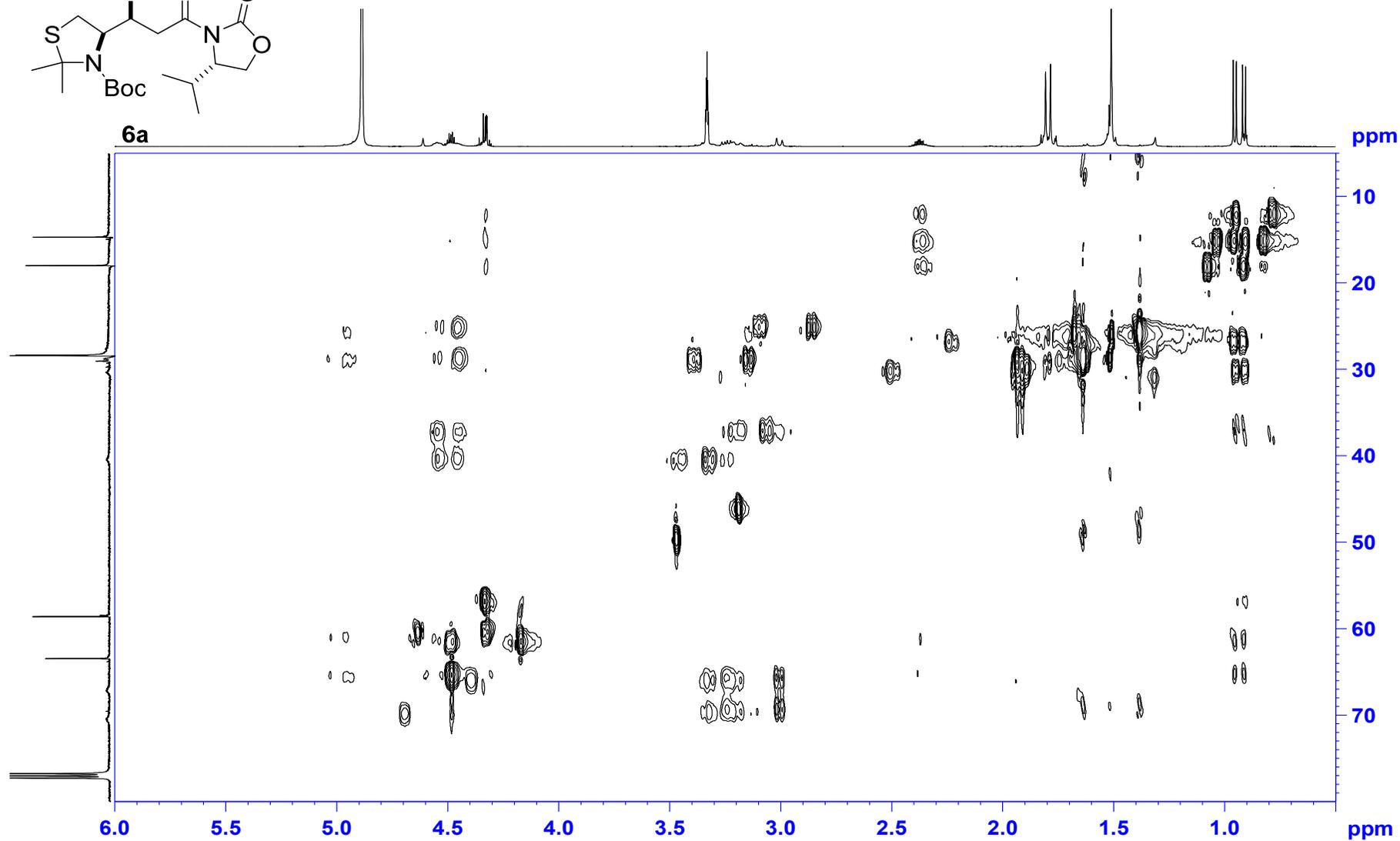
¹H-NMR (500 MHz, CD₃OD) of 6a



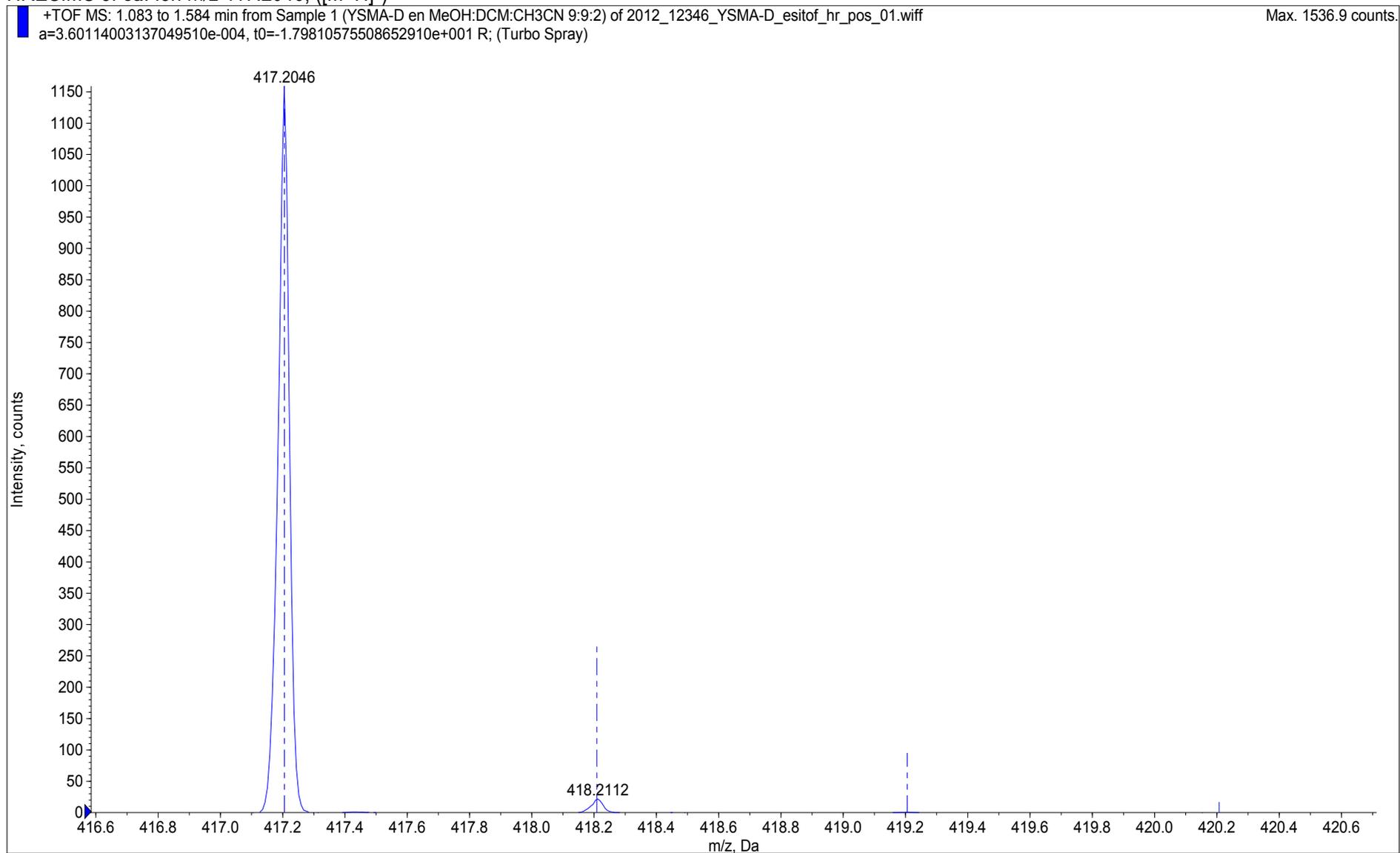
HSQC-HECADE (500 MHz, CD₃OD) of 6a



6a



HRESIMS of 6a: Ion m/z 417.2046, $([M+H]^+)$



Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
$C_{19}H_{33}N_2O_6S$	417.2053	-0.7853	-1.8825	4.5

¹H-NMR (500 MHz, CDCl₃) of 6b

4.4838
4.4772
4.4695
4.4675
4.4605
4.4534
4.4315
4.4130
4.4072
4.3941
4.2281
4.2159
4.2099

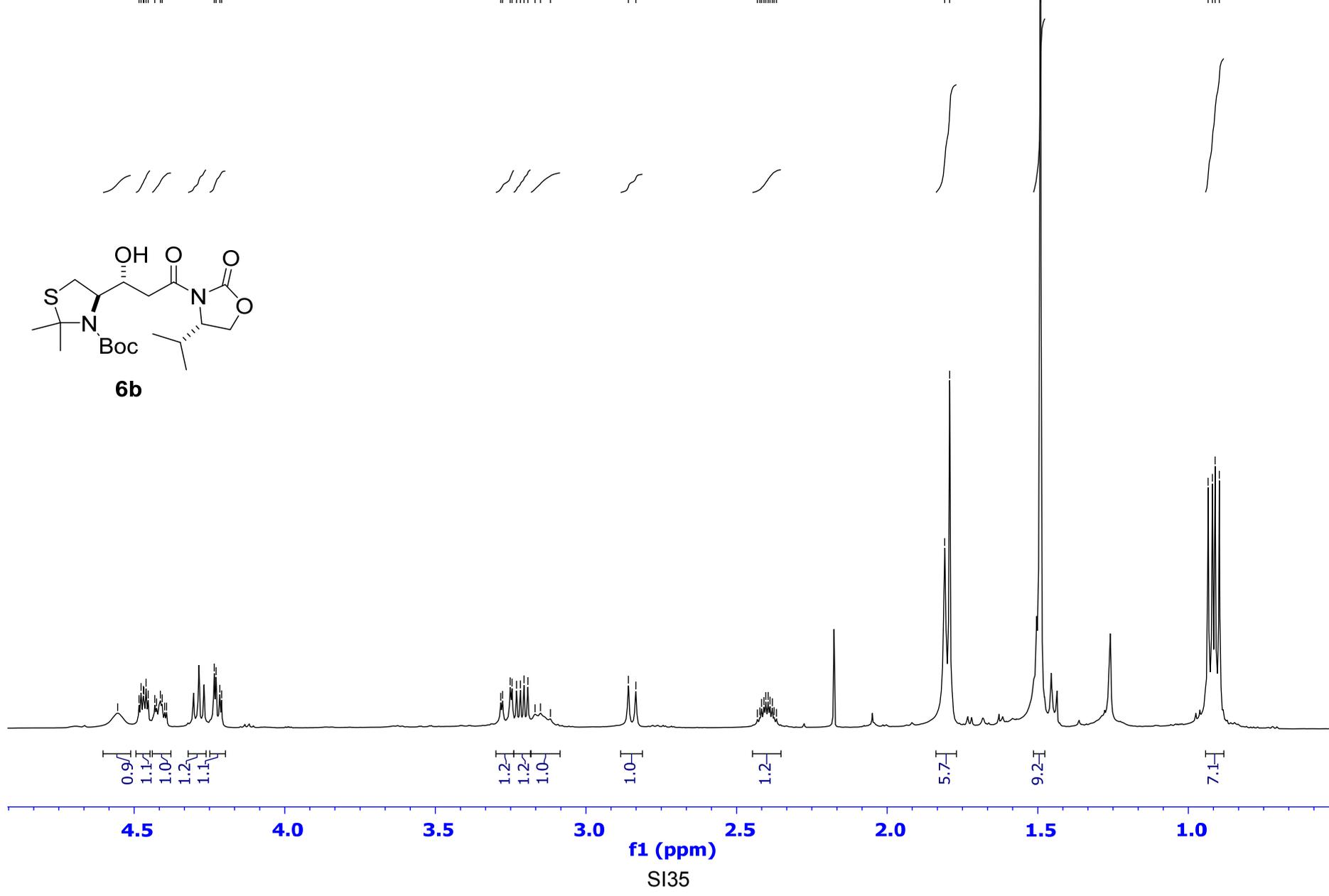
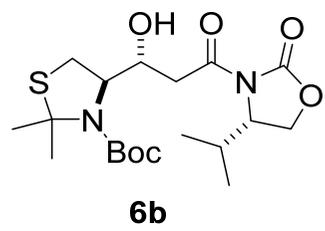
3.2830
3.2769
3.2514
3.2454
3.2304
3.2176
3.2057
3.1929
3.1689
3.1509
3.1180
2.8591
2.8344

2.4308
2.4227
2.4170
2.4090
2.4031
2.3952
2.3892
2.3813
2.3755
2.3675

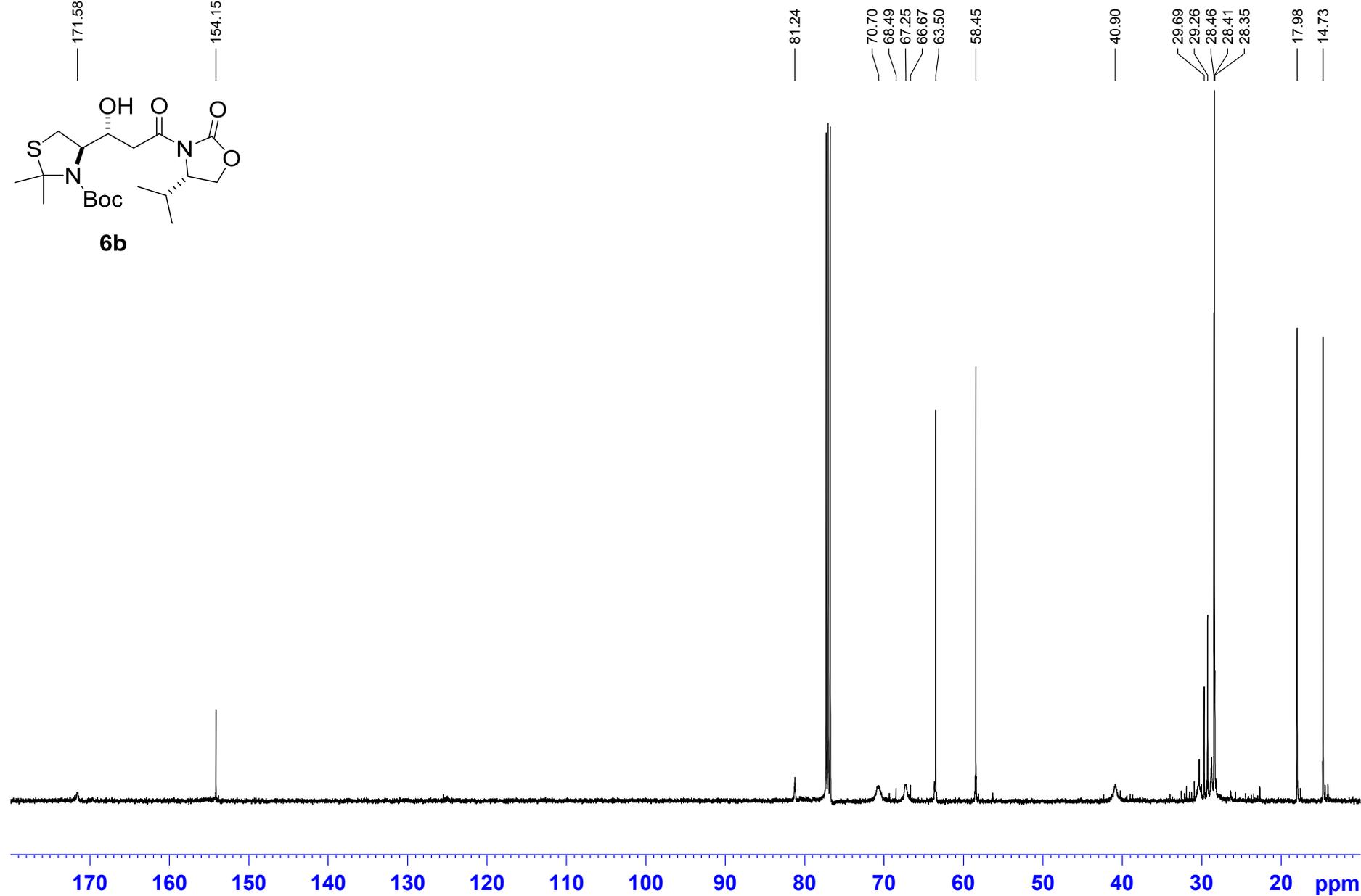
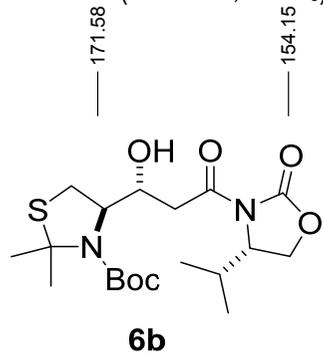
1.8089
1.7925

1.4912

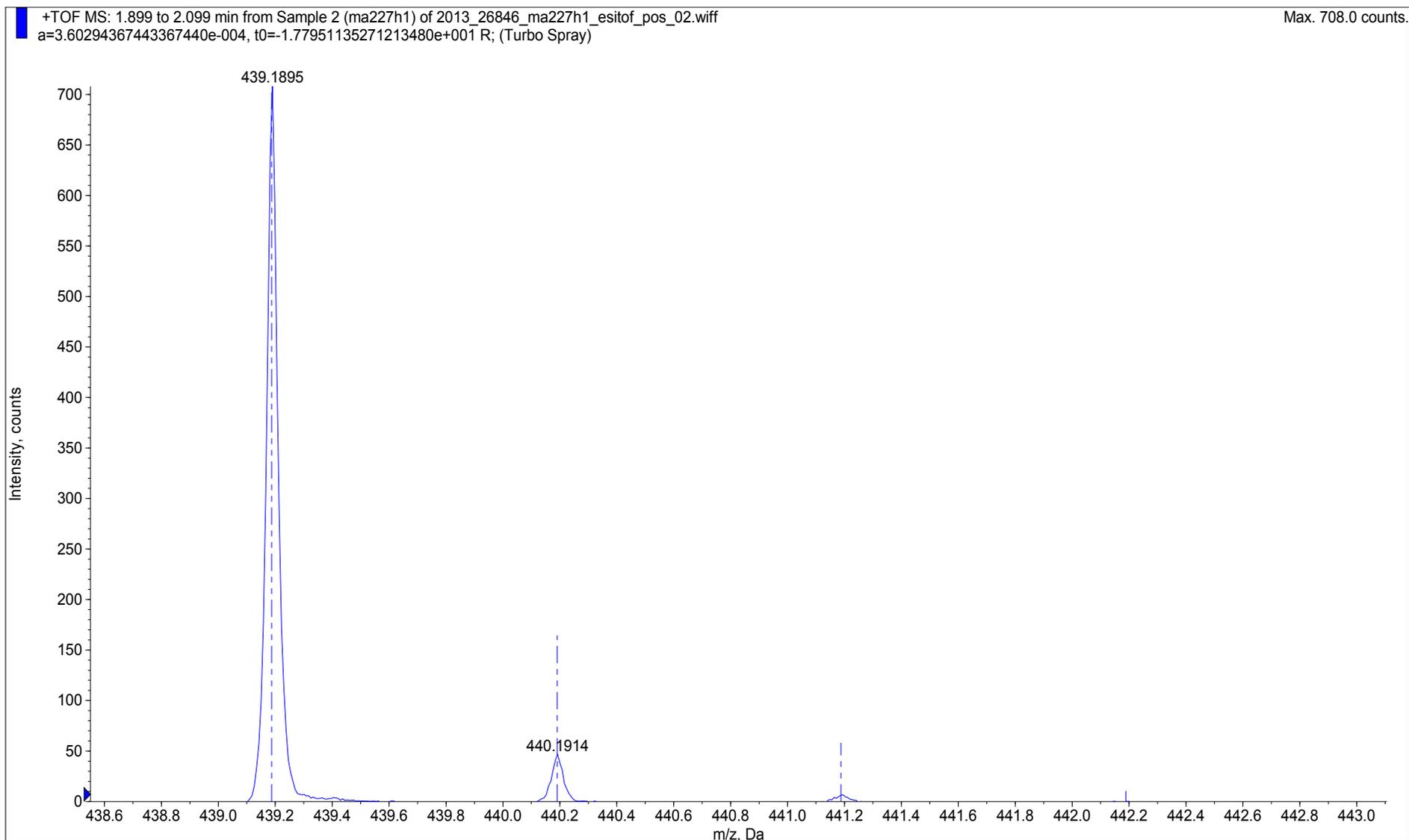
0.9344
0.9203
0.9111
0.8973



$^{13}\text{C-NMR}$ (125 MHz, CDCl_3) of **6b**

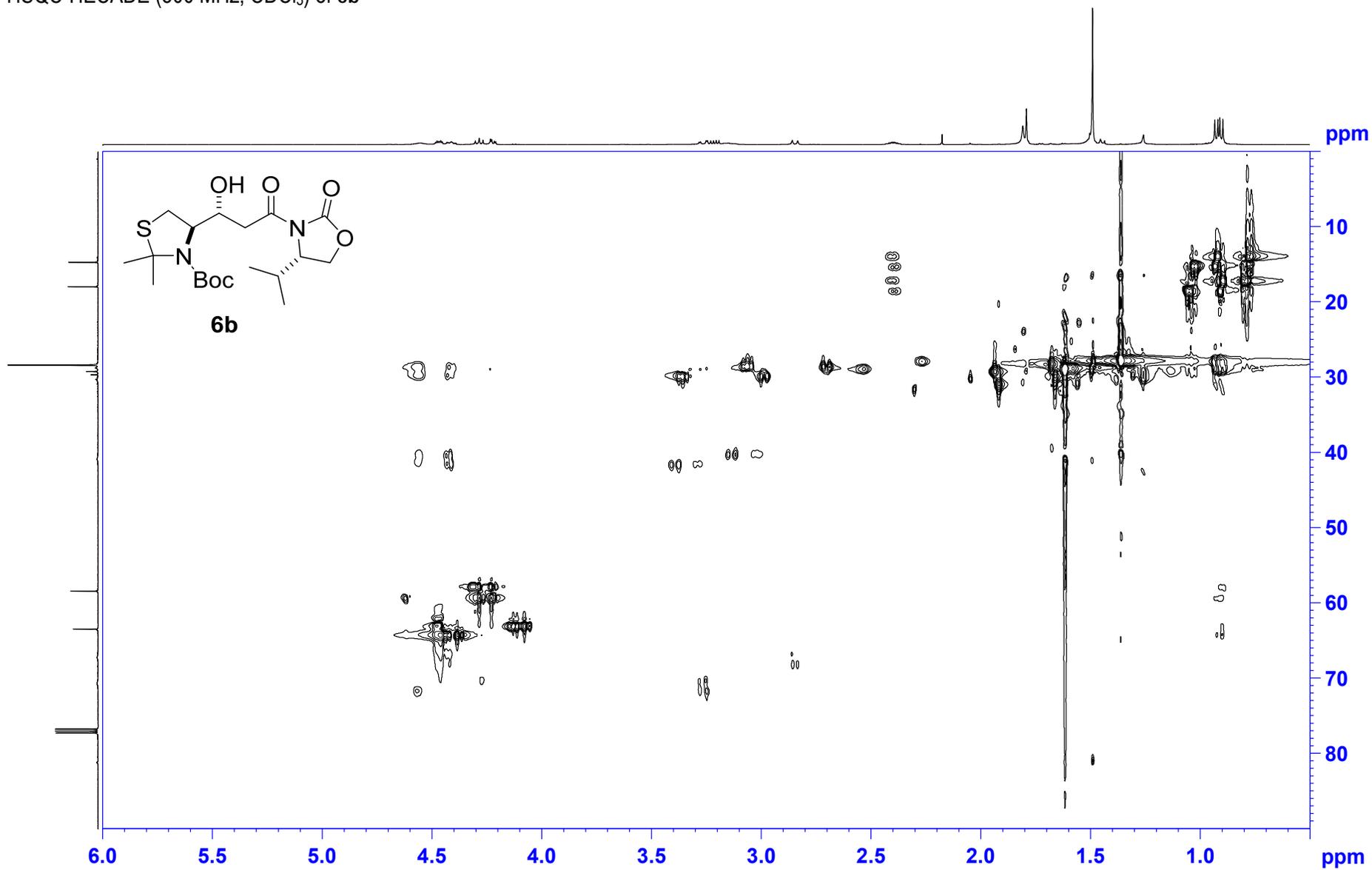


HRESIMS of 6b: Ion m/z 439.1895, $([M+Na]^+)$

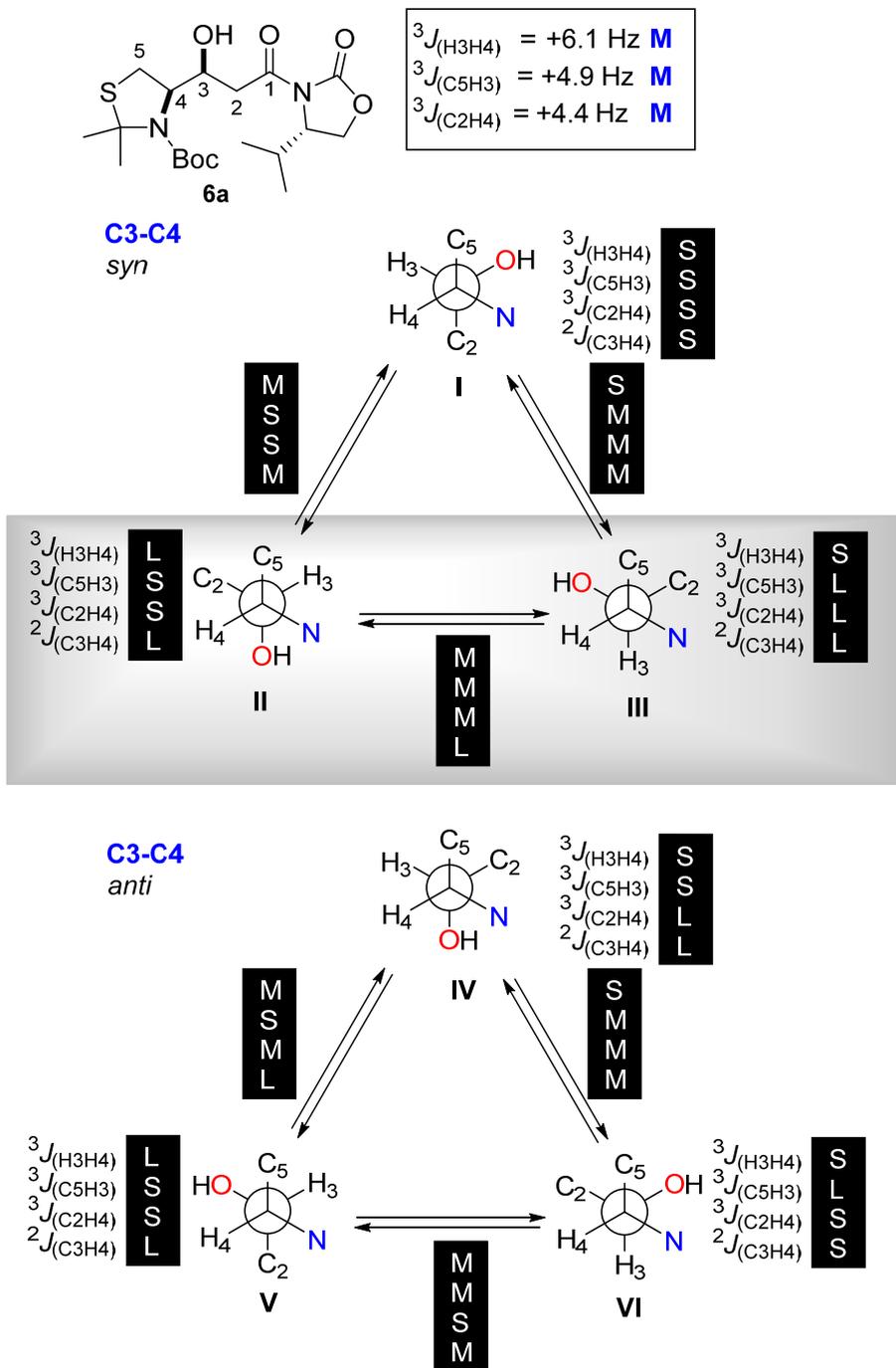


Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
$C_{19}H_{32}N_2O_6NaS$	439.1873	2.1699	4.9407	4.5

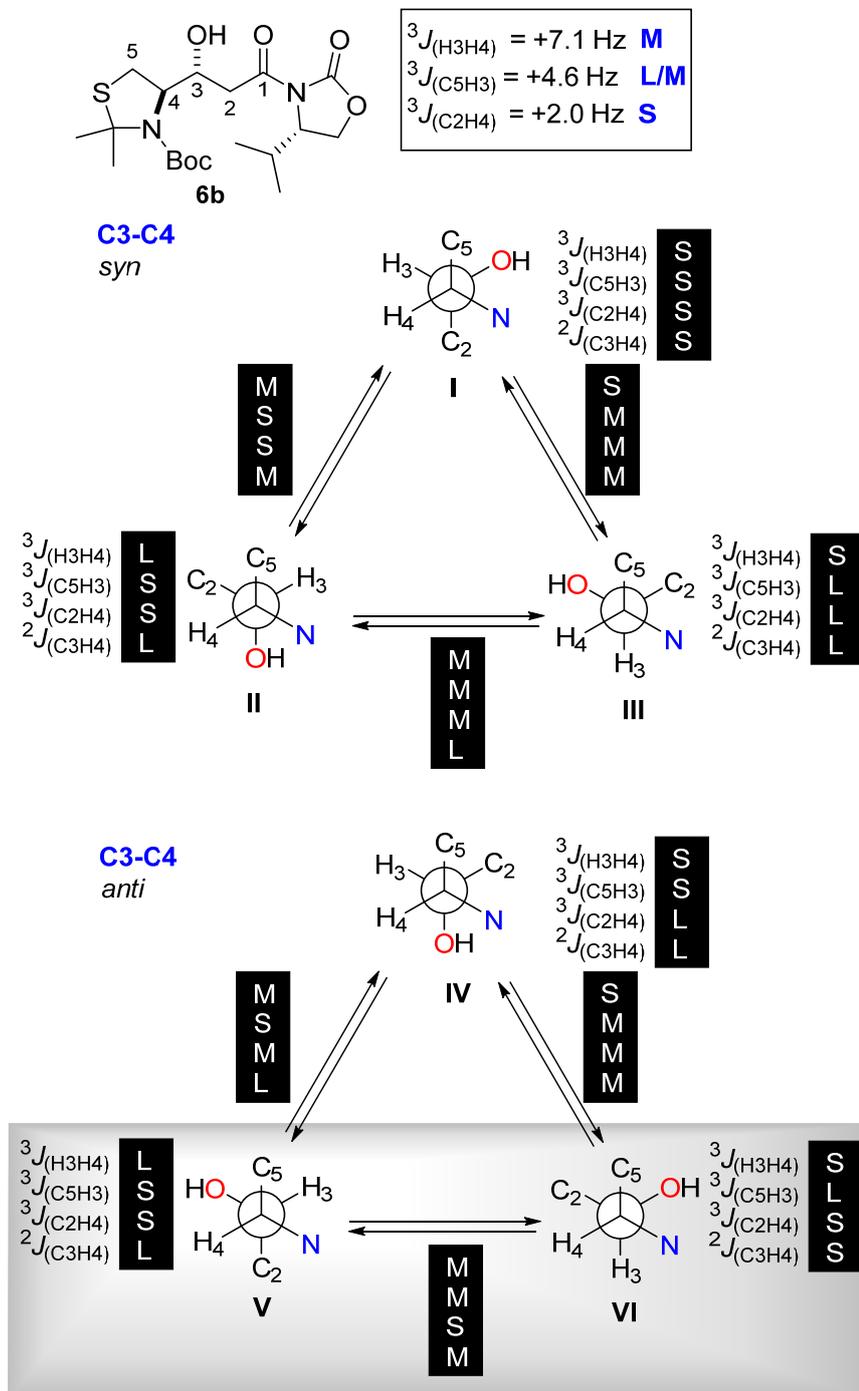
HSQC-HECADE (500 MHz, CDCl₃) of 6b



J -Based configurational analysis of the C3-C4 bond of 6a



J -Based configurational analysis of the C3-C4 bond of 6b

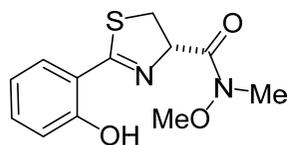


¹H-NMR (300 MHz, CDCl₃) of 7a

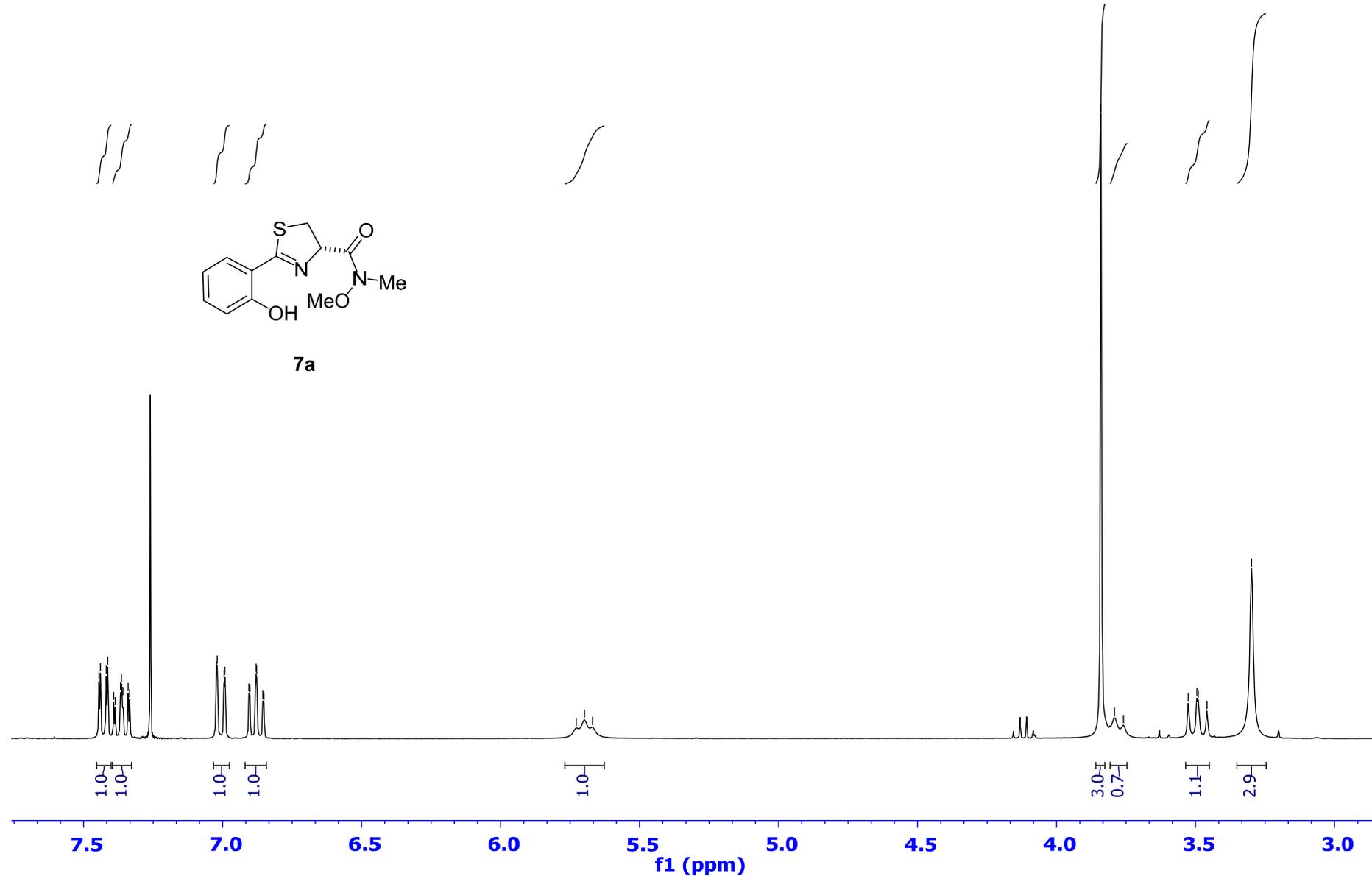
7.4447
7.4395
7.4185
7.4134
7.3919
7.3864
7.3675
7.3639
7.3587
7.3398
7.3343
7.0226
7.0197
6.9948
6.9920
6.9058
6.9021
6.8798
6.8778
6.8554
6.8516

5.7278
5.6985
5.6693

3.8401
3.7917
3.7593
3.5262
3.4952
3.4903
3.4589
3.2985



7a



¹³C-NMR (75 MHz, CDCl₃) of 7a

174.1
169.7
158.9

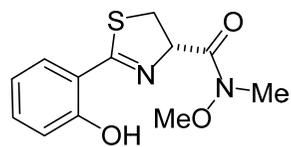
133.3
130.8

118.9
117.0
116.1

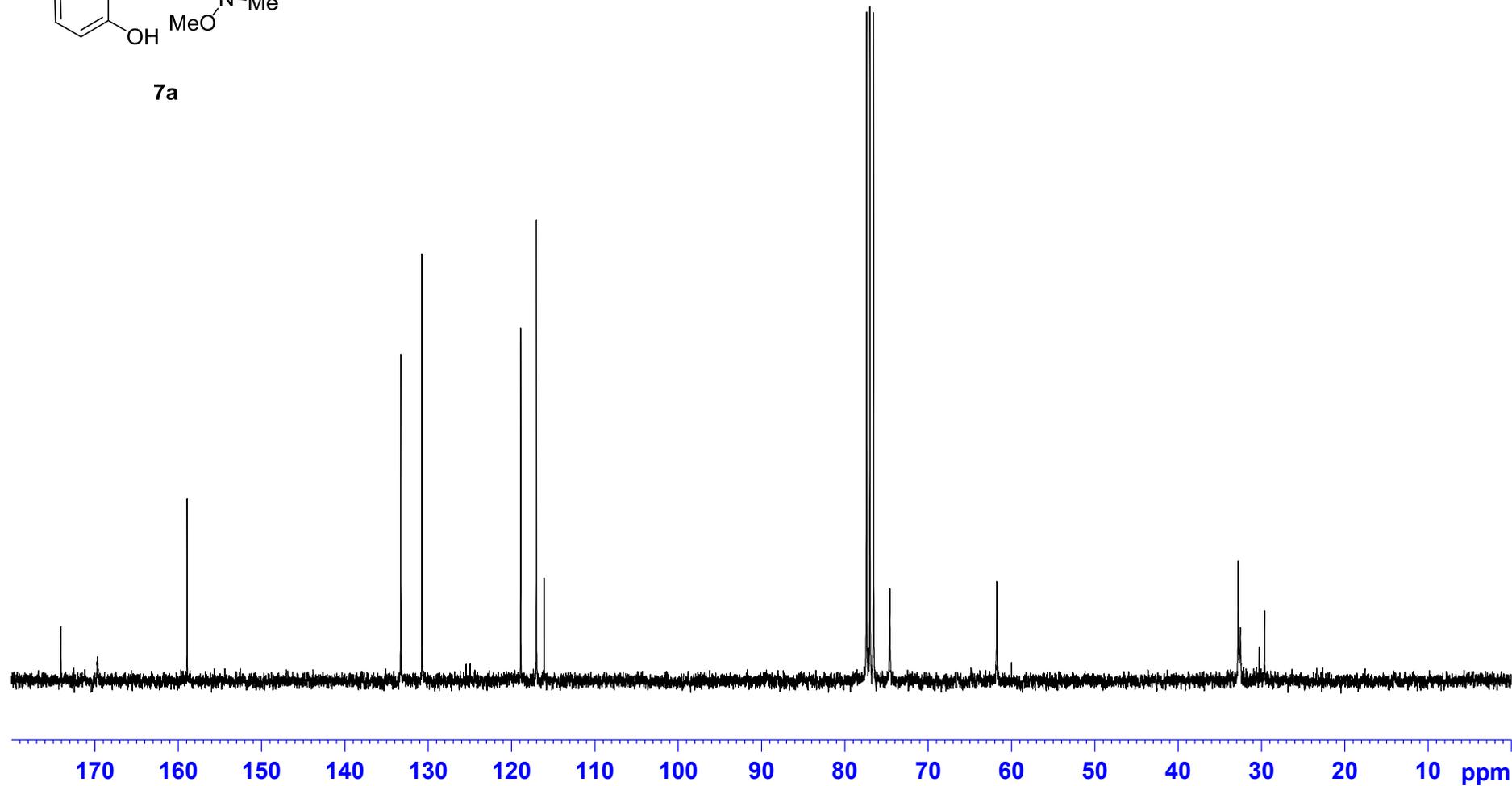
74.6

61.8

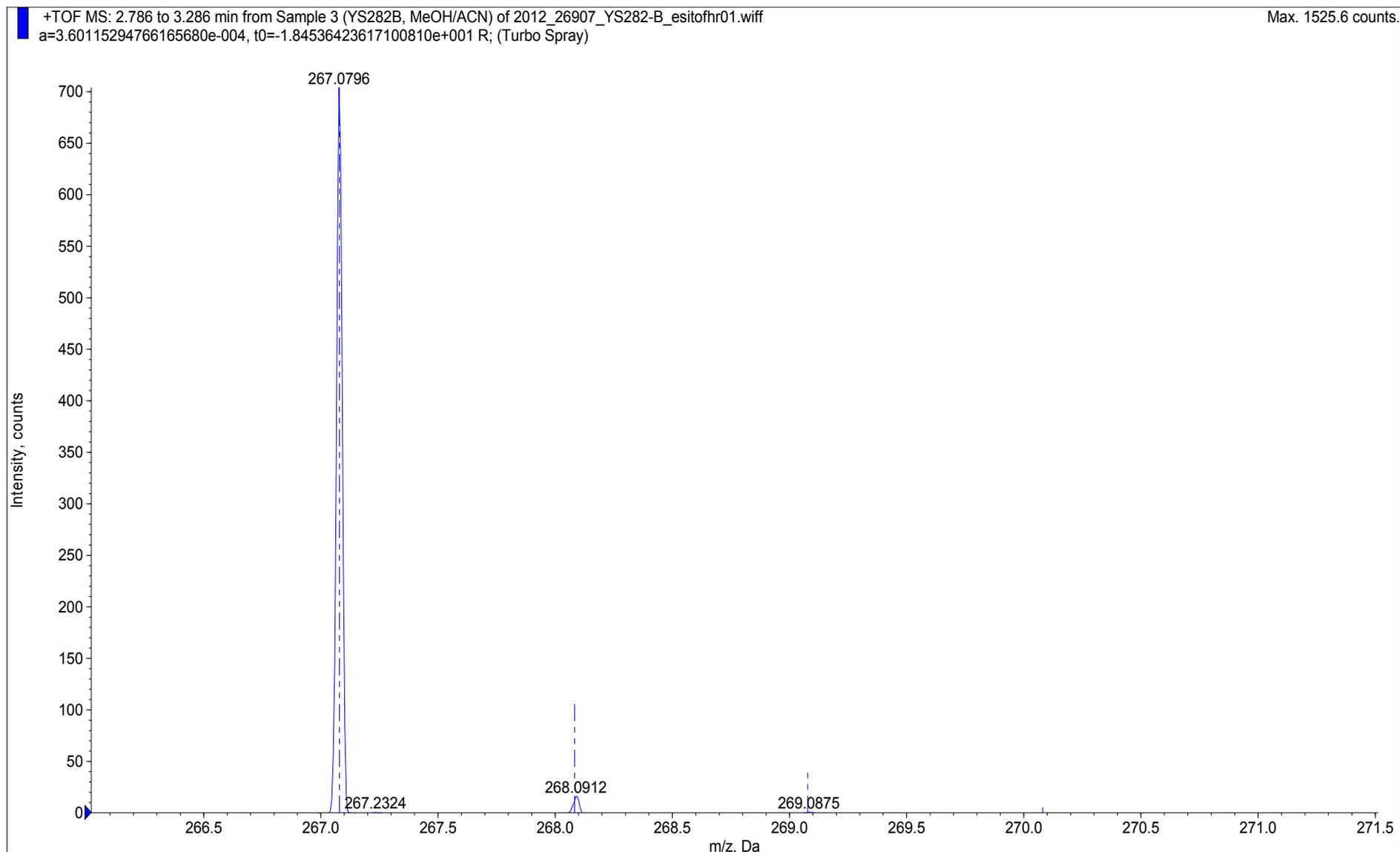
32.8
32.5
29.6



7a



HRESIMS of 7a: Ion m/z 267.0796, ([M+H]⁺)

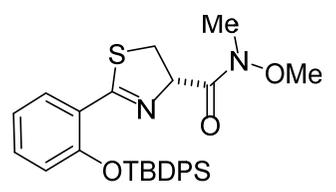
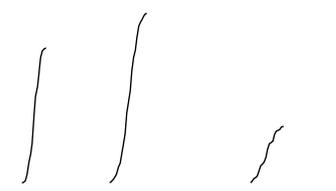


Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
C ₁₂ H ₁₅ N ₂ O ₃ S	267.0797	-0.1907	-0.7142	6.5

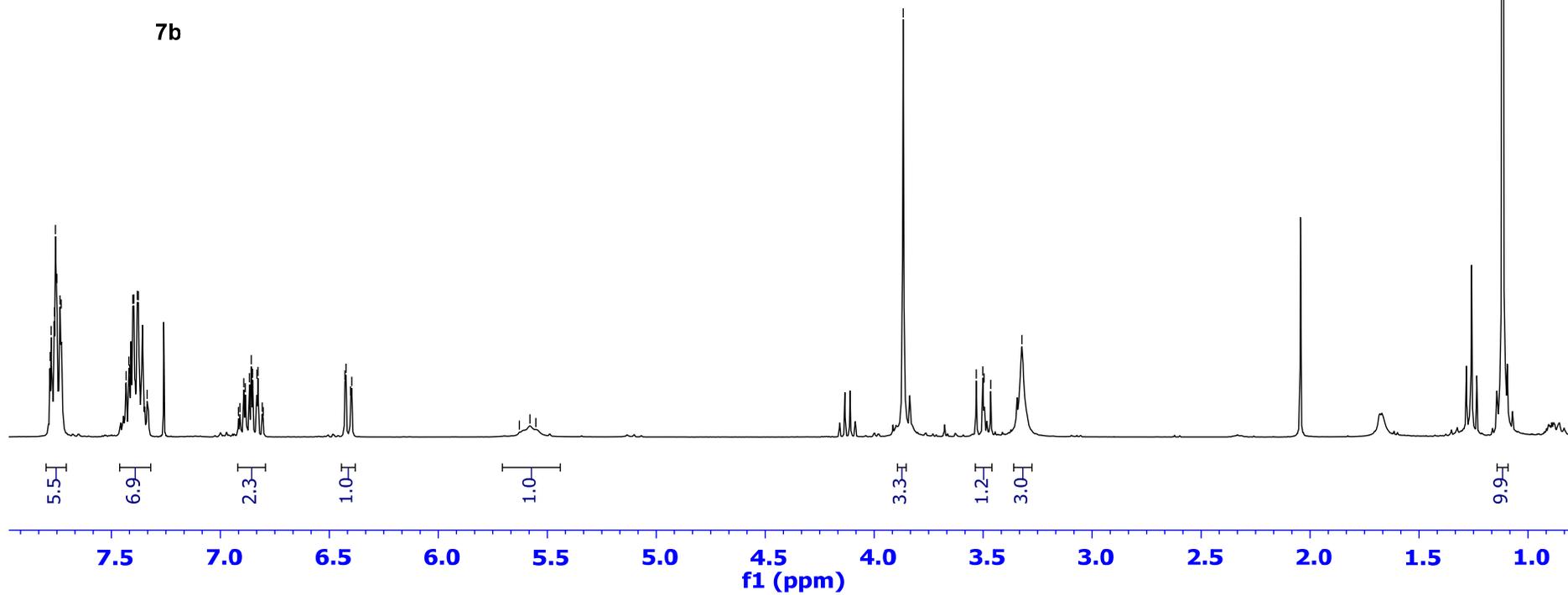
¹H-NMR (300 MHz, CDCl₃) of 7b

7.7812
7.7761
7.7617
7.7563
7.7500
7.7356
7.7301
7.4322
7.4196
7.4157
7.4016
7.3968
7.3814
7.3760
7.3558
7.3360
6.9169
6.9101
6.8924
6.8856
6.8665
6.8578
6.8519
6.8322
6.8276
6.8076
6.8032
6.4280
6.4233
6.4023
6.3969
5.6280
5.5797
5.5523

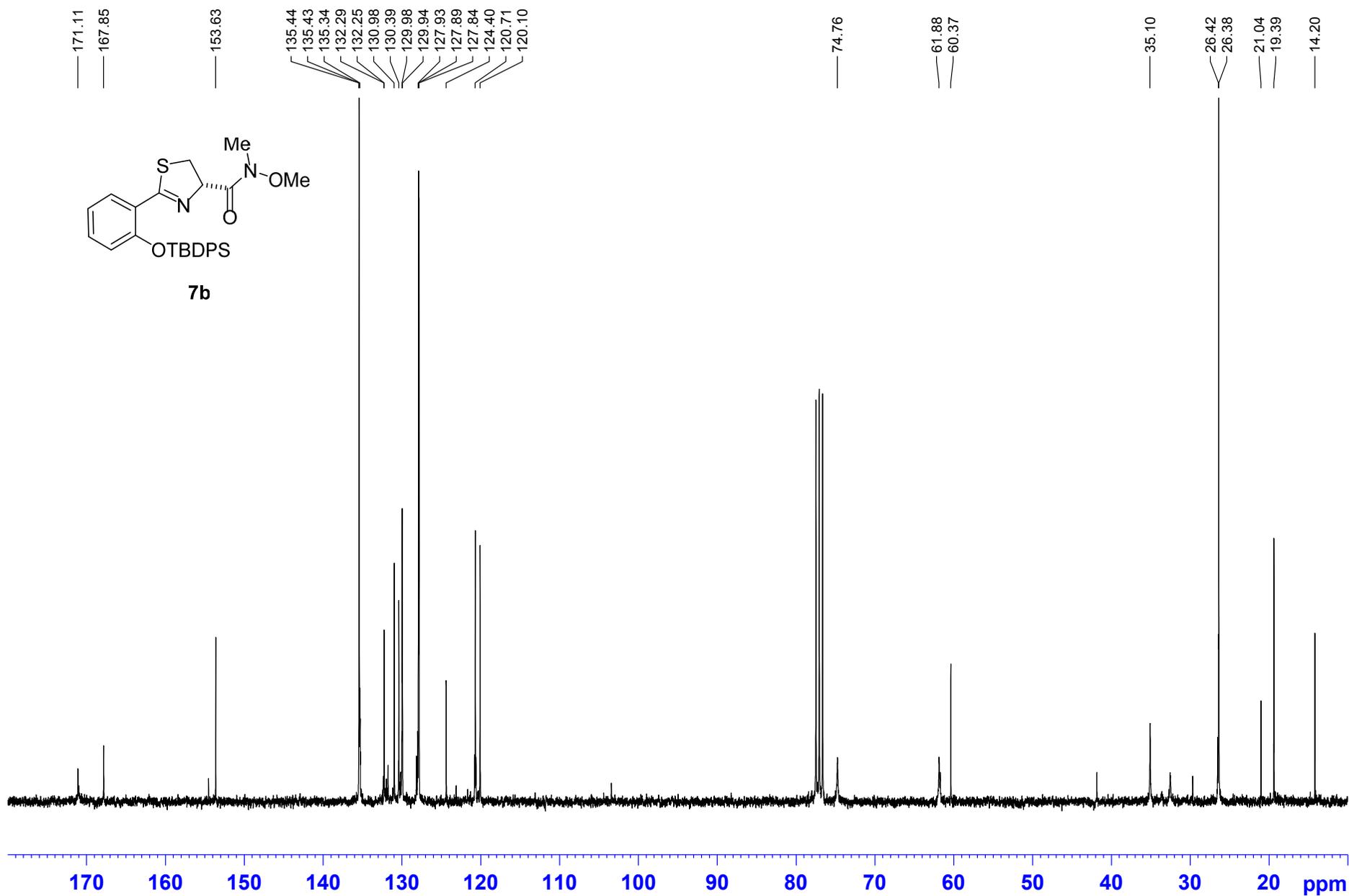
3.8668
3.5317
3.5014
3.4956
3.4653
3.3220



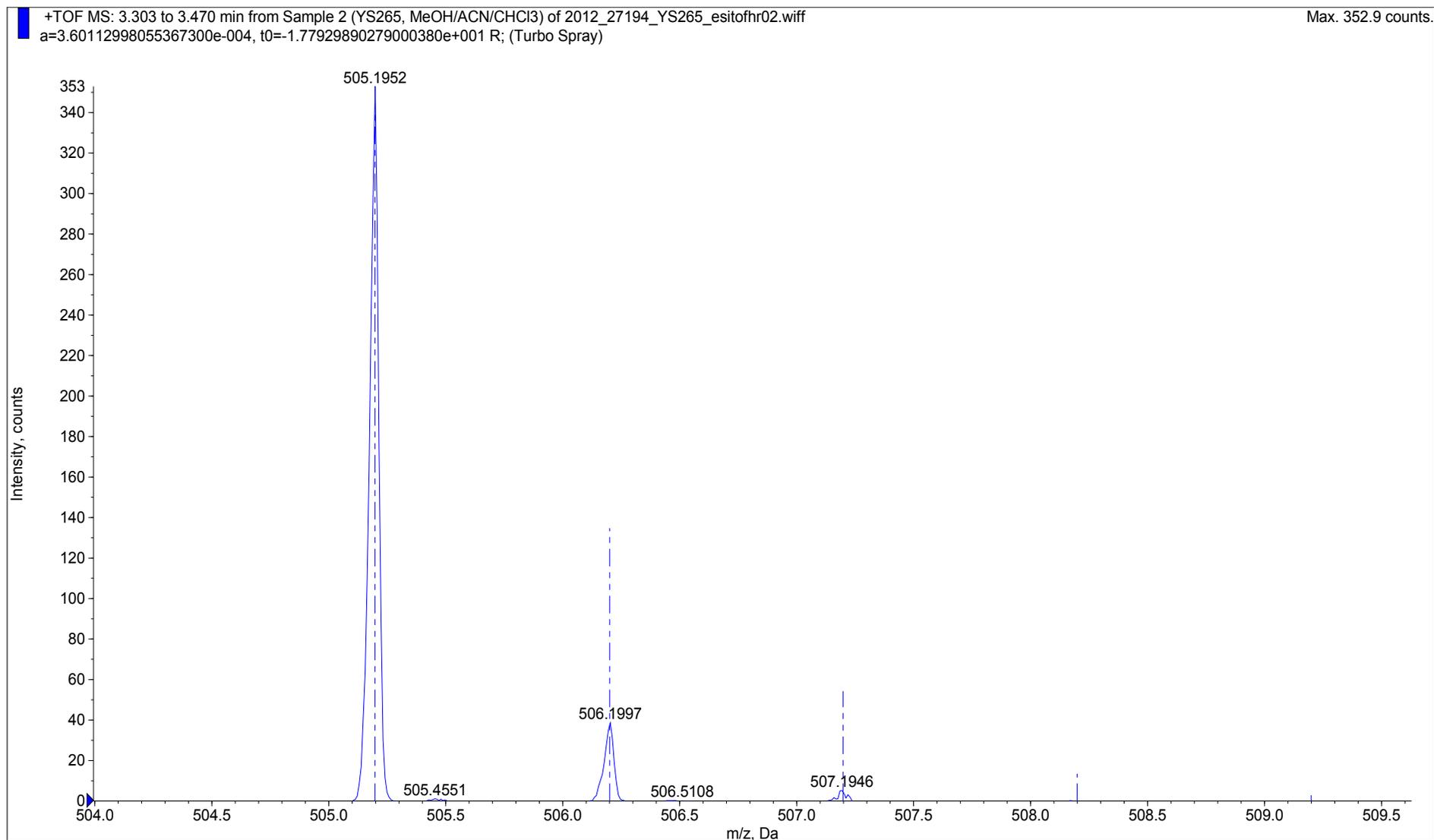
7b



¹³C-NMR (75 MHz, CDCl₃) of 7b

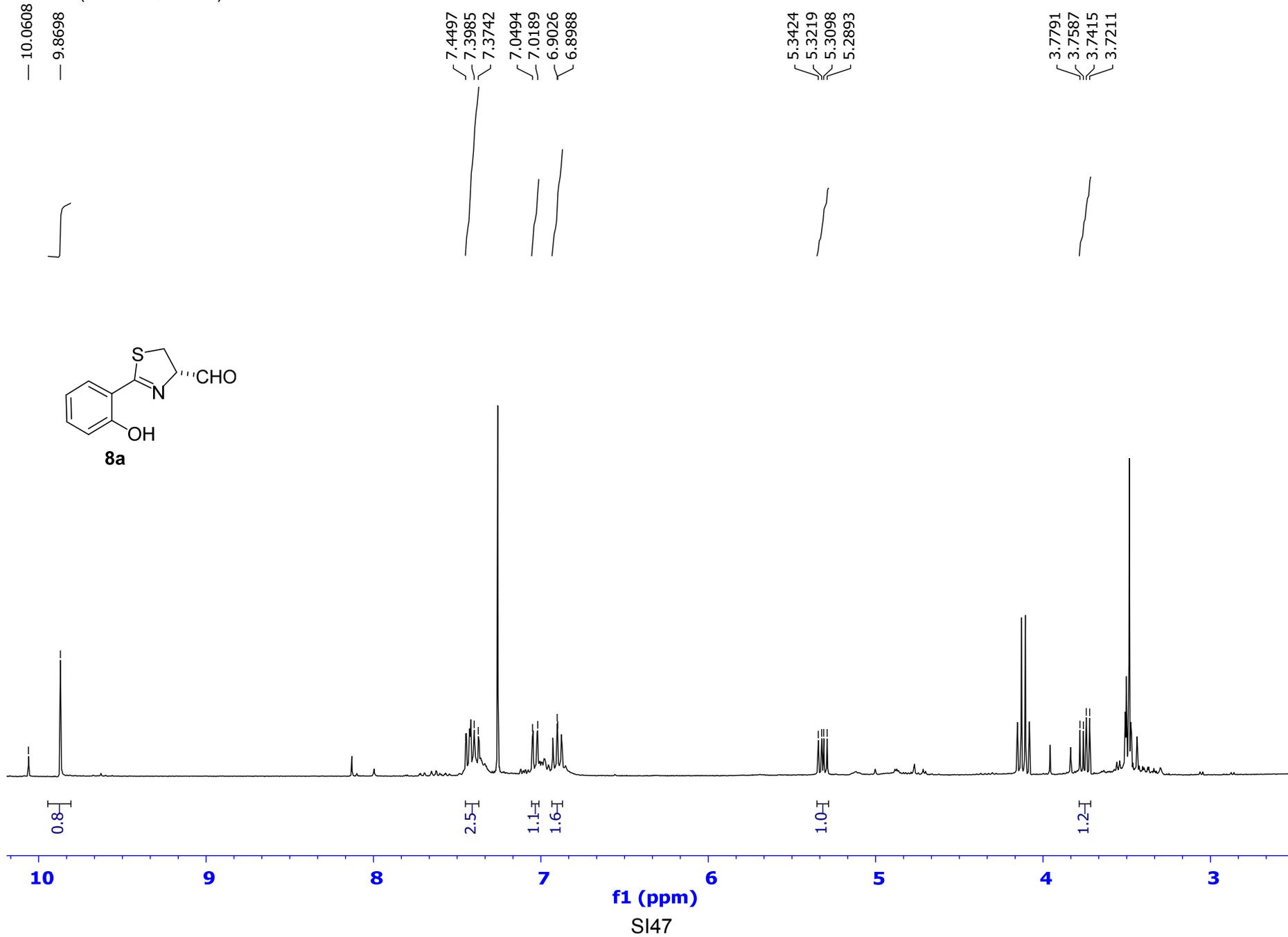


HRESIMS of 7b: Ion m/z 505.1952, $([M+H]^+)$

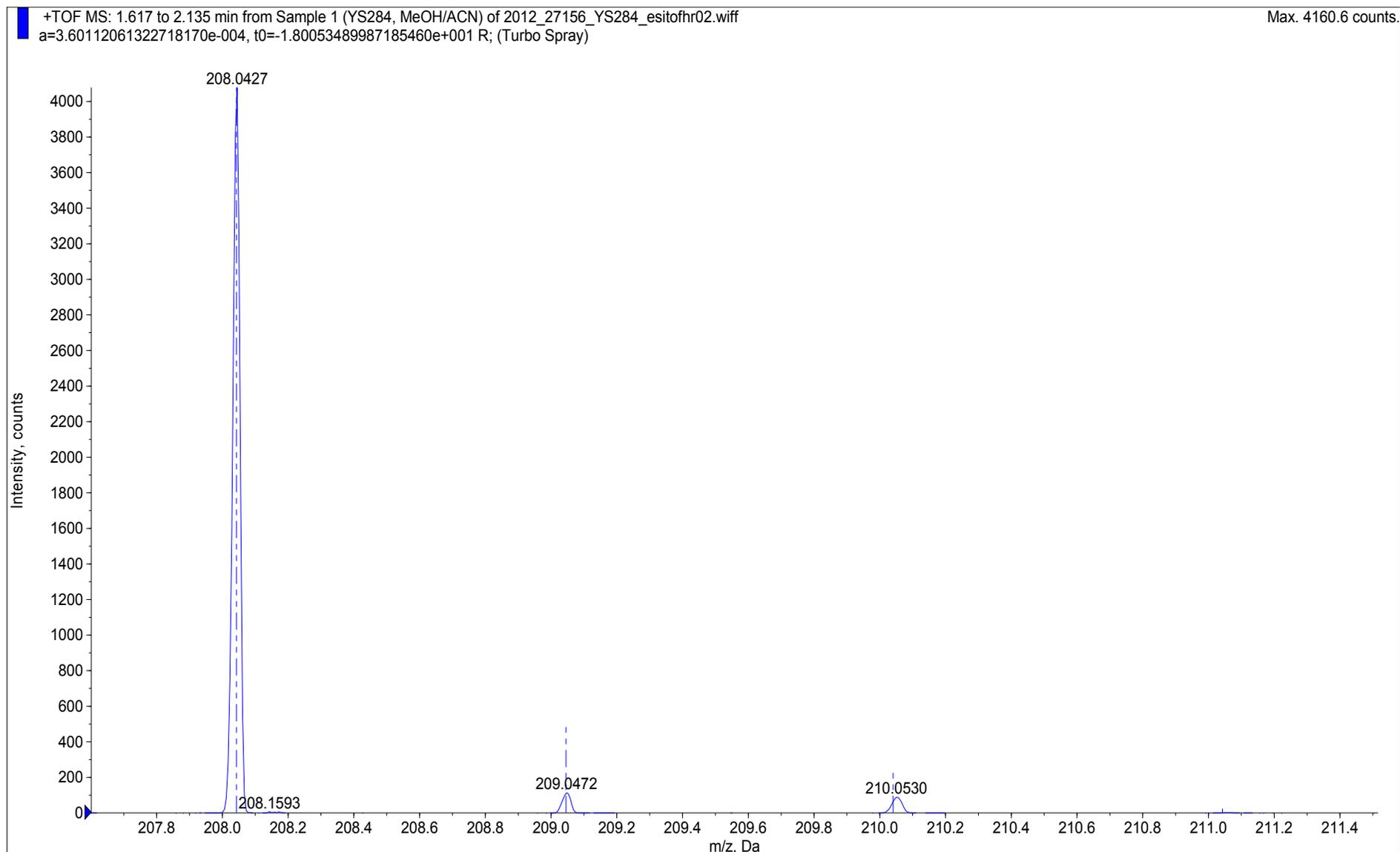


Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
C ₂₈ H ₃₃ N ₂ O ₃ SiS	505.1975	-2.3698	-4.691	14.5

¹H-NMR (300 MHz, CDCl₃) of 8a

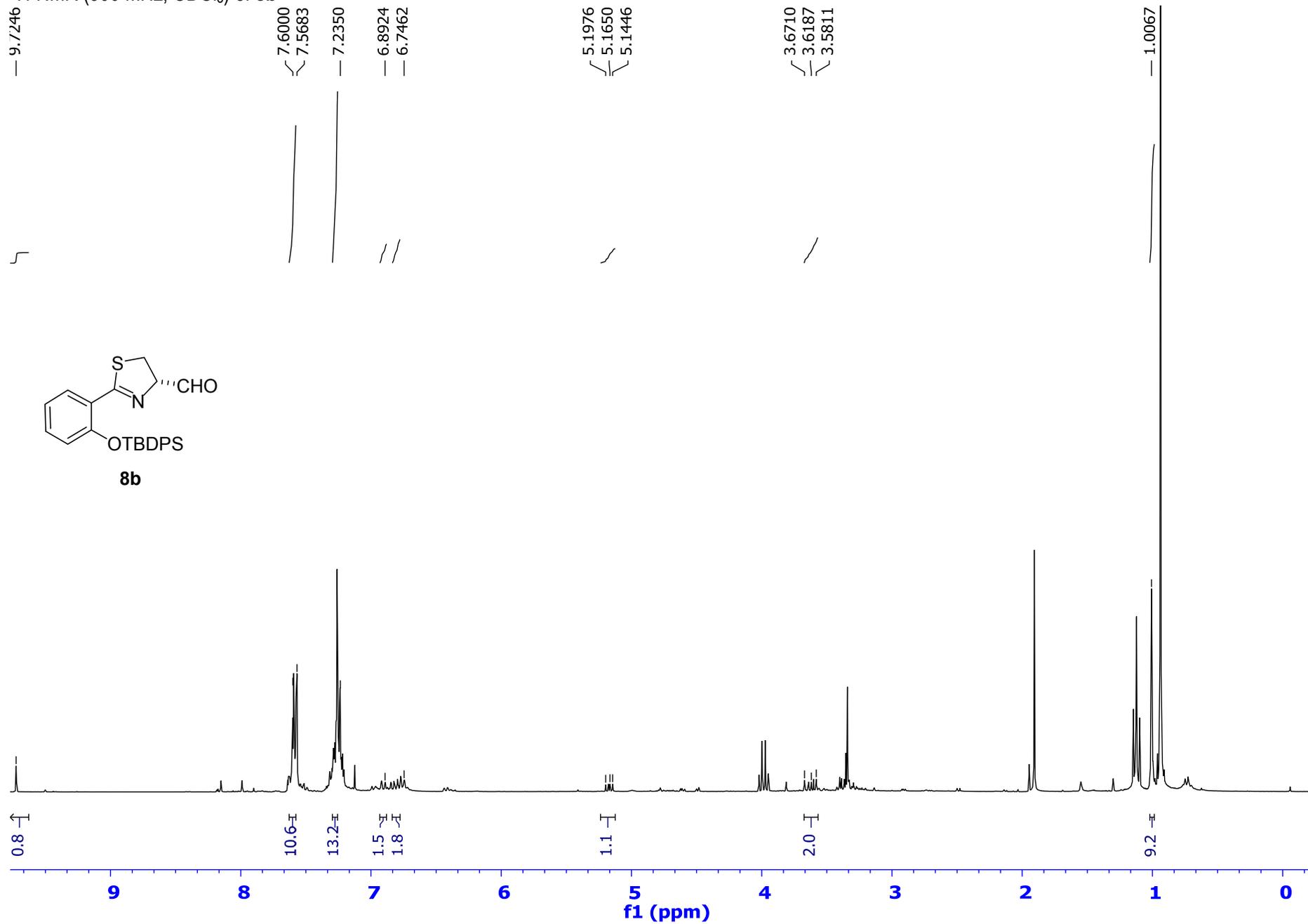


HRESIMS of 8a: Ion m/z 208.0427 ($[M+H]^+$)

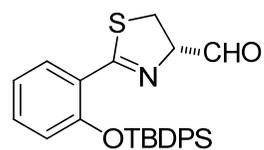


Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
C₁₀ H₁₀ N O₂ S	208.0426	0.023	0.1109	6.5

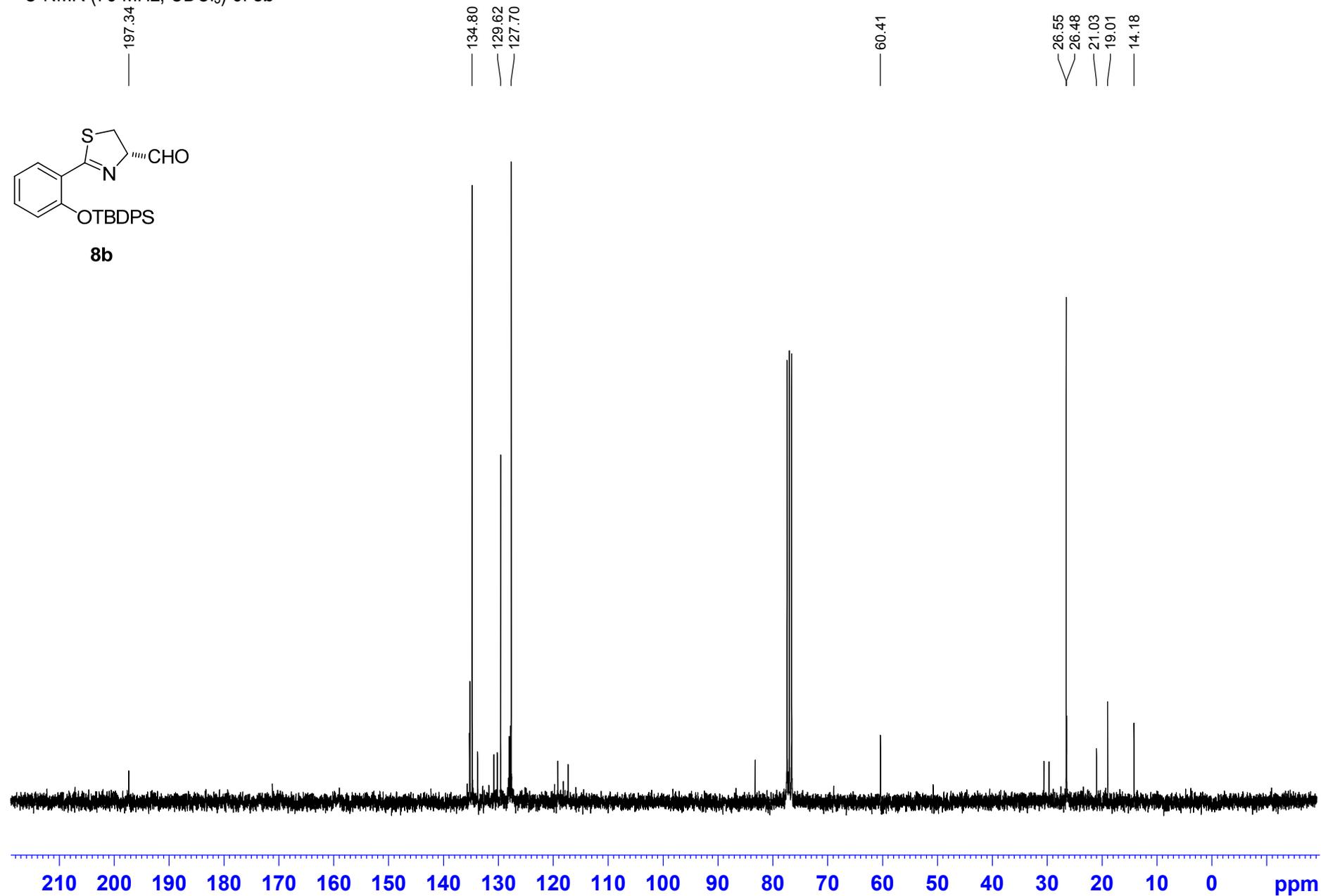
¹H-NMR (300 MHz, CDCl₃) of 8b



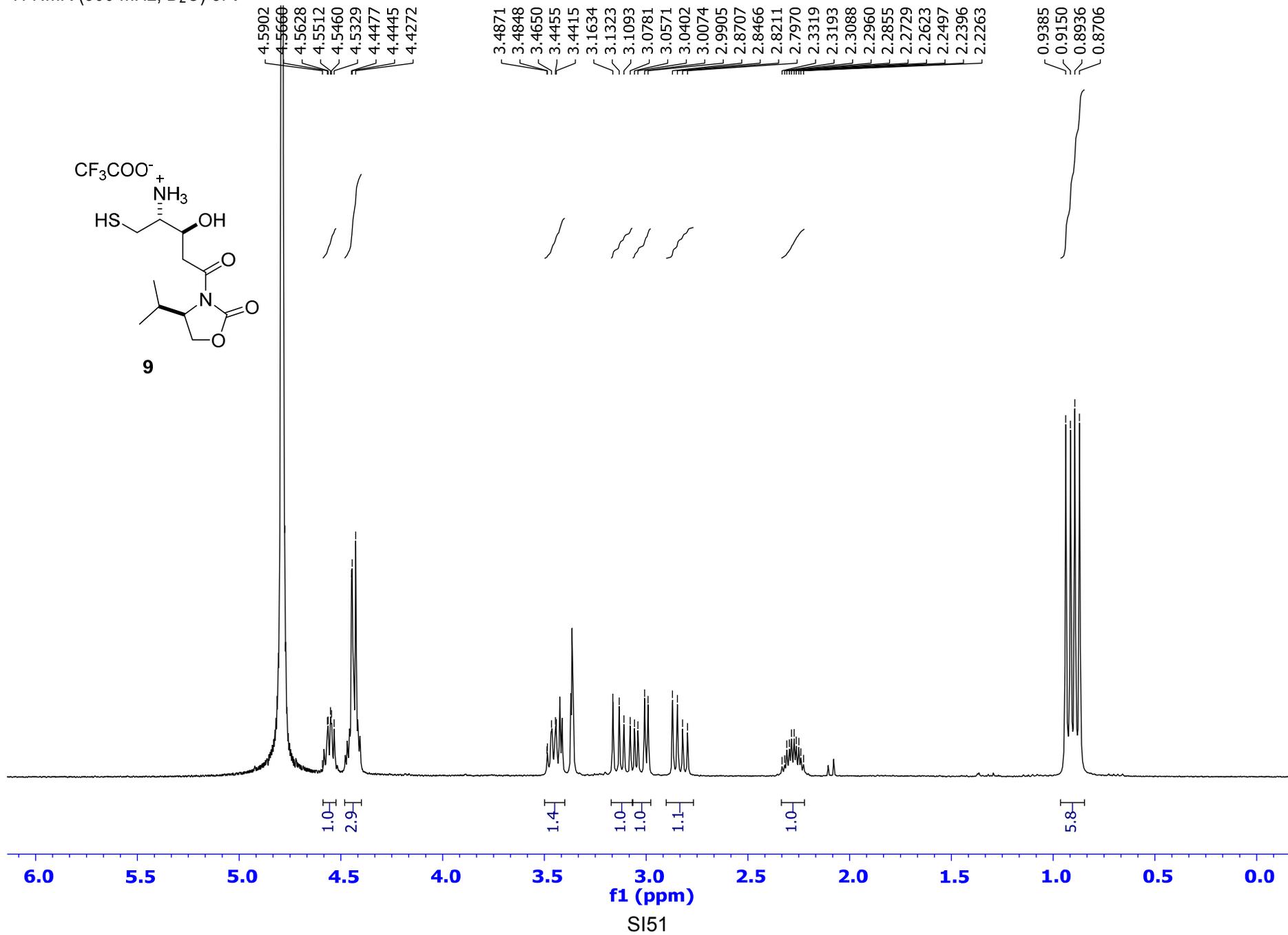
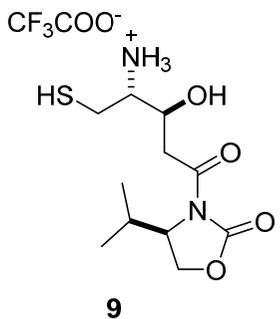
^{13}C -NMR (75 MHz, CDCl_3) of **8b**



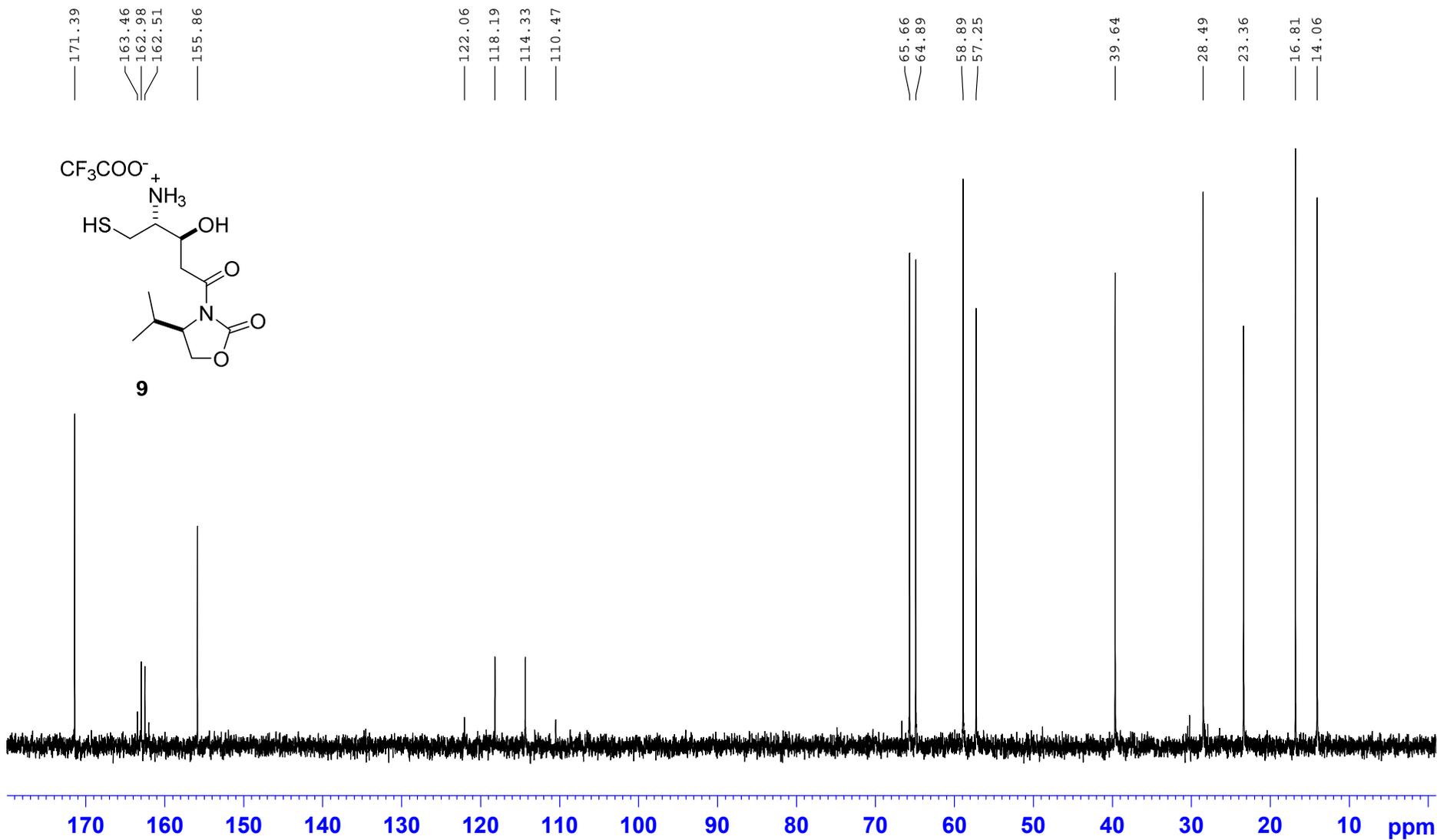
8b



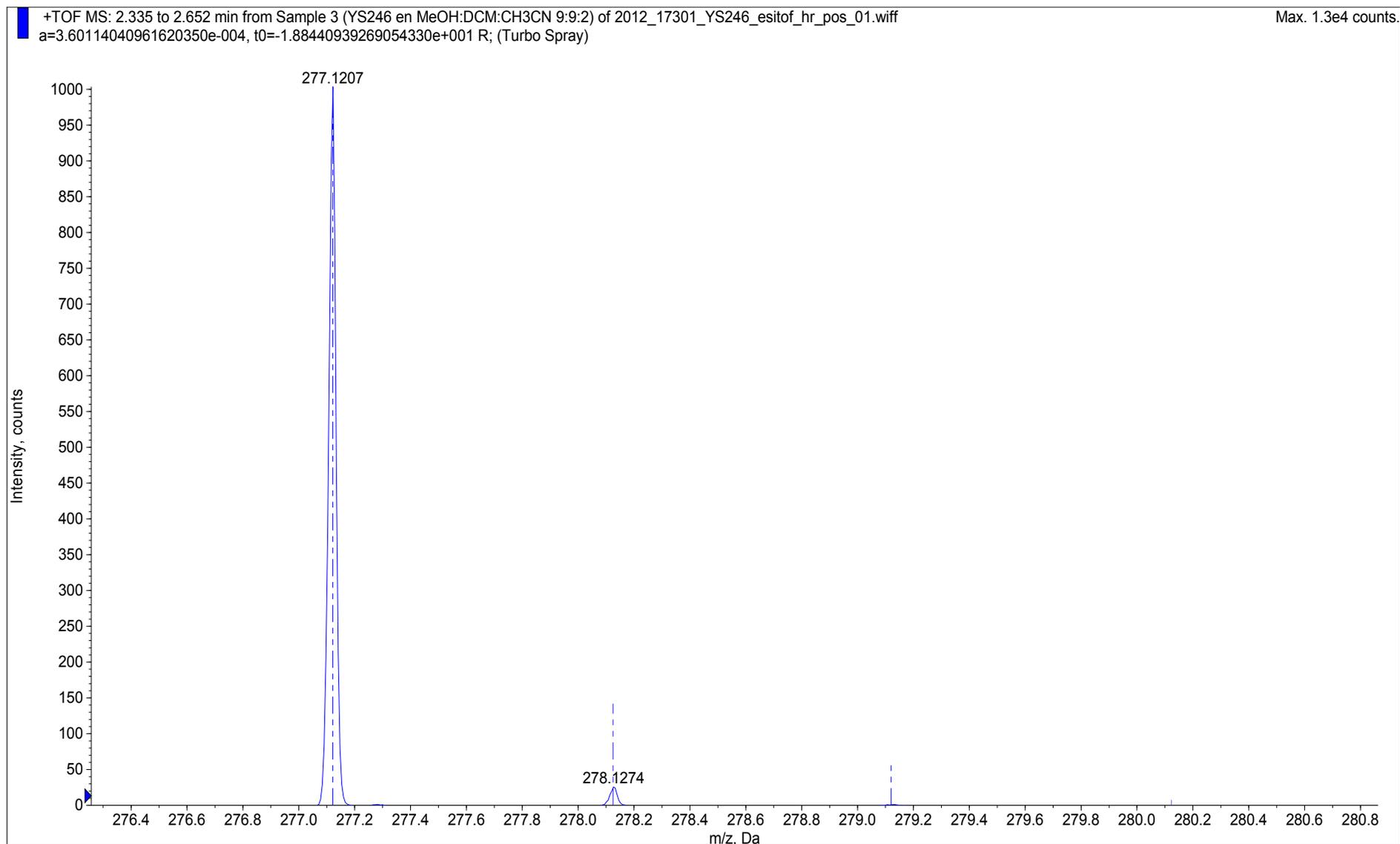
¹H-NMR (300 MHz, D₂O) of 9



¹³C-NMR (75 MHz, D₂O) of 9

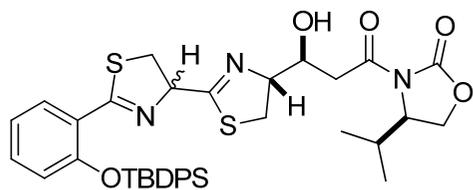


HRESIMS of 9: Ion m/z 277.1207, $([M+H]^+)$

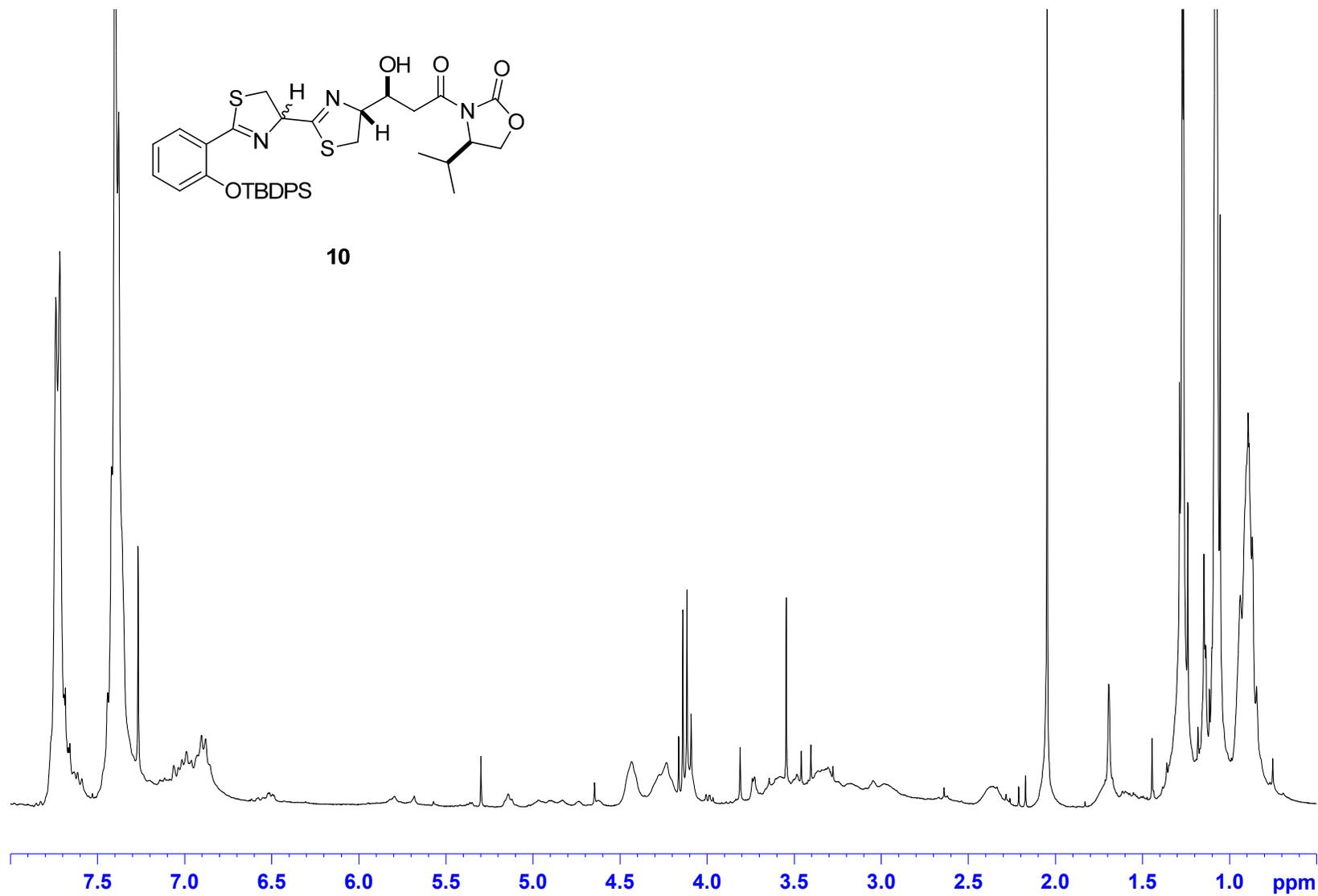


Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
<chem>C11H21N2O4S</chem>	277.1216	-0.9556	-3.4484	2.5

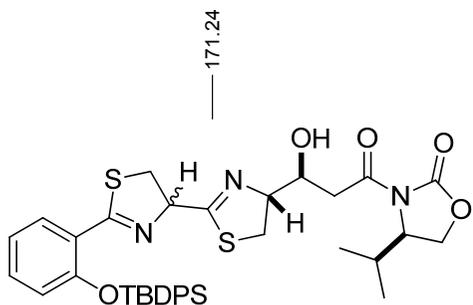
$^1\text{H-NMR}$ (300 MHz, CDCl_3) of 10



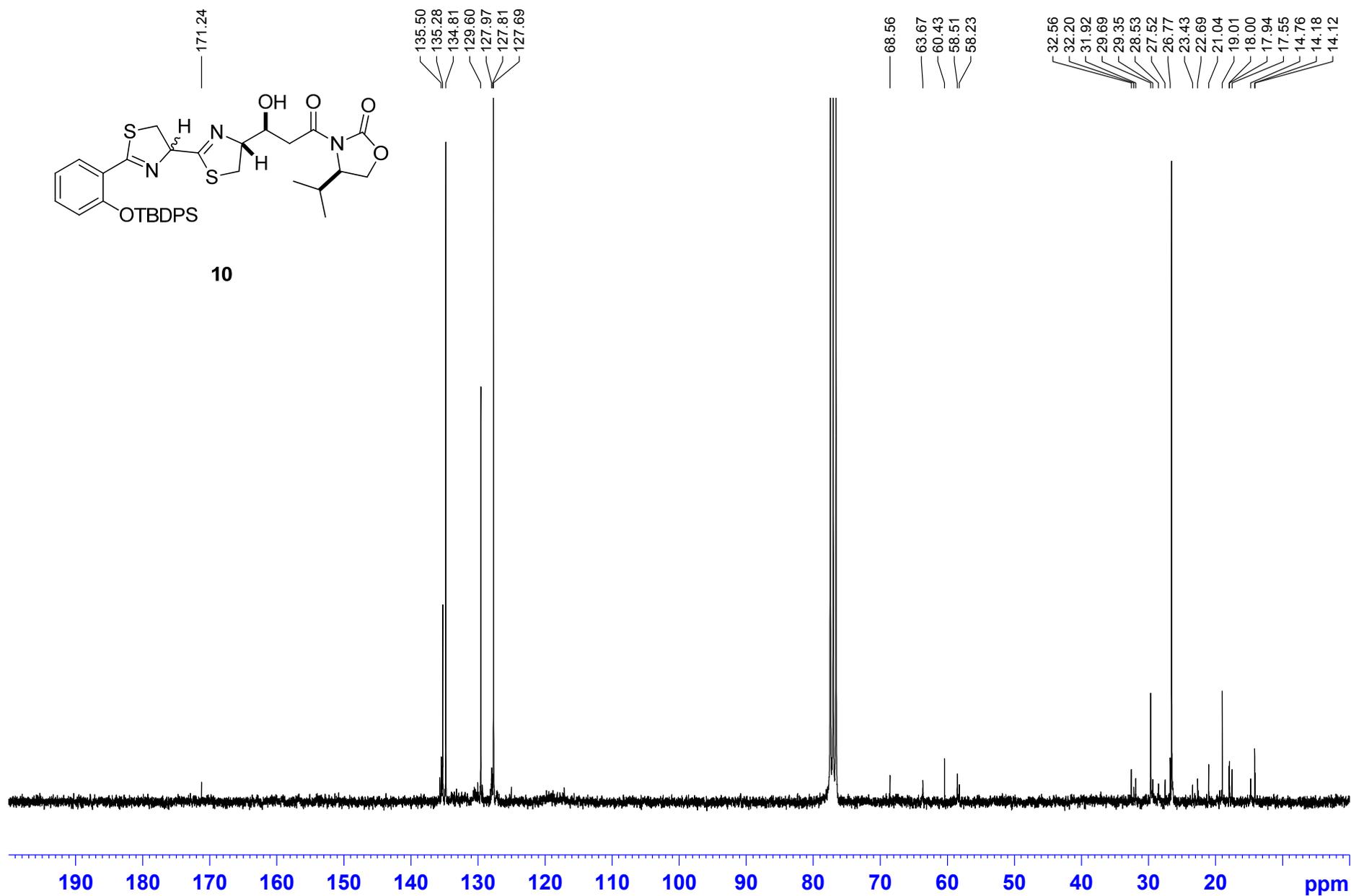
10



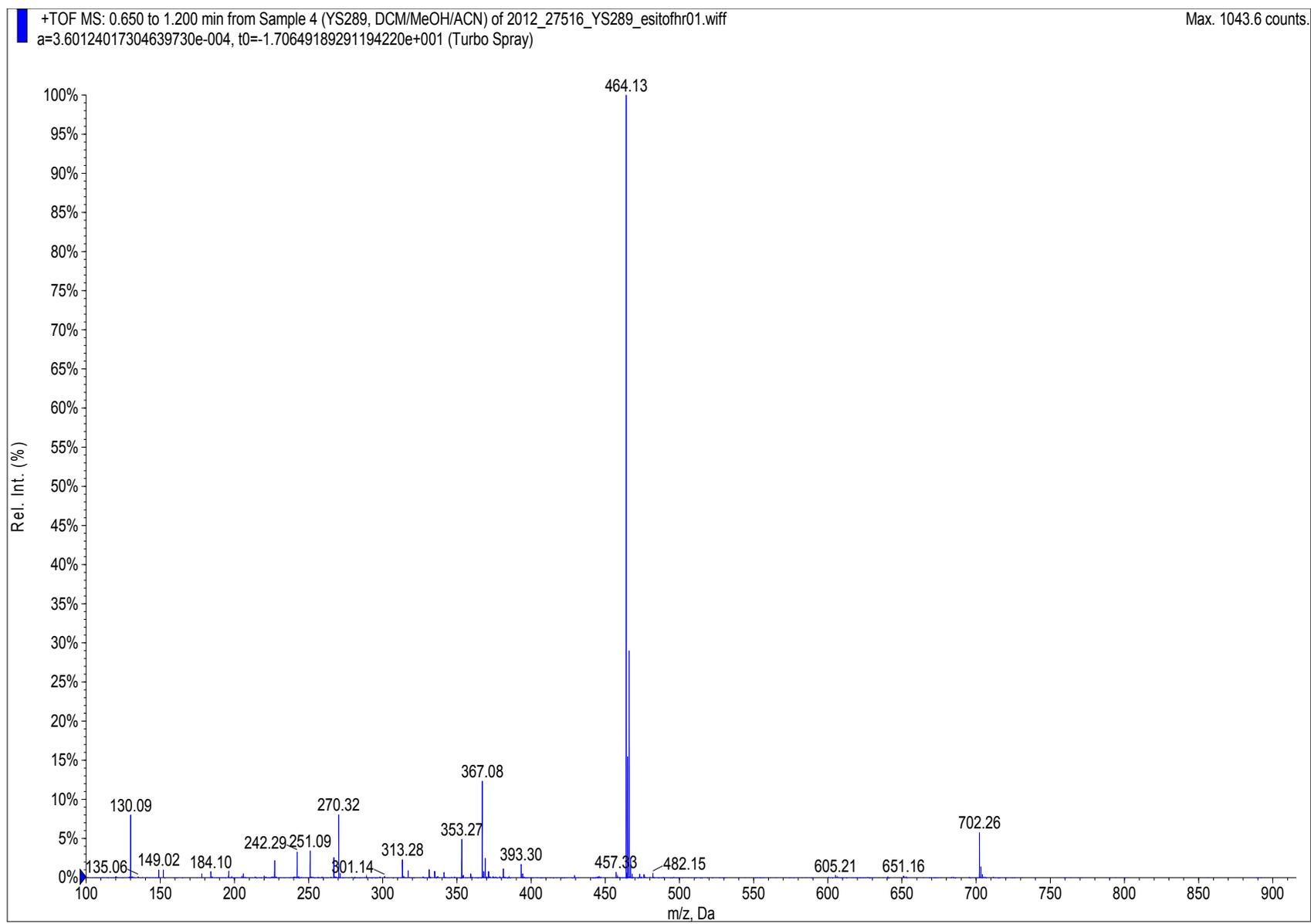
¹³C-NMR (75 MHz, CDCl₃) of 10



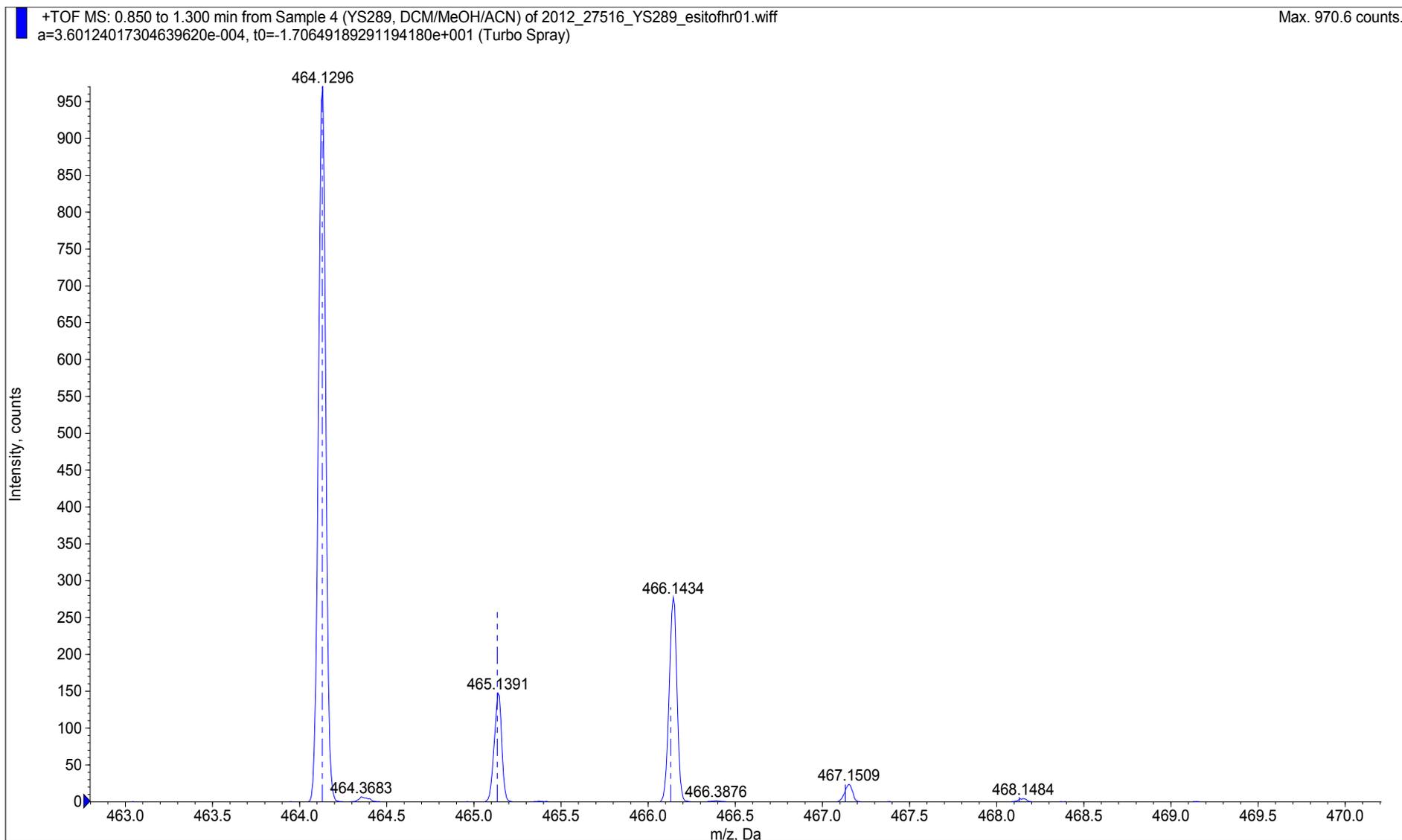
10



HRESIMS of 10: m/z 702 ($[M+H]^+$, 7), 464 ($[M-C_{16}H_{19}Si]^+$, 100)

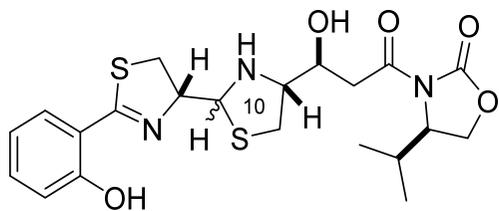


HRESIMS of 10: Ion m/z 464.1296, $([M-C_{16}H_{19}Si]^+)$



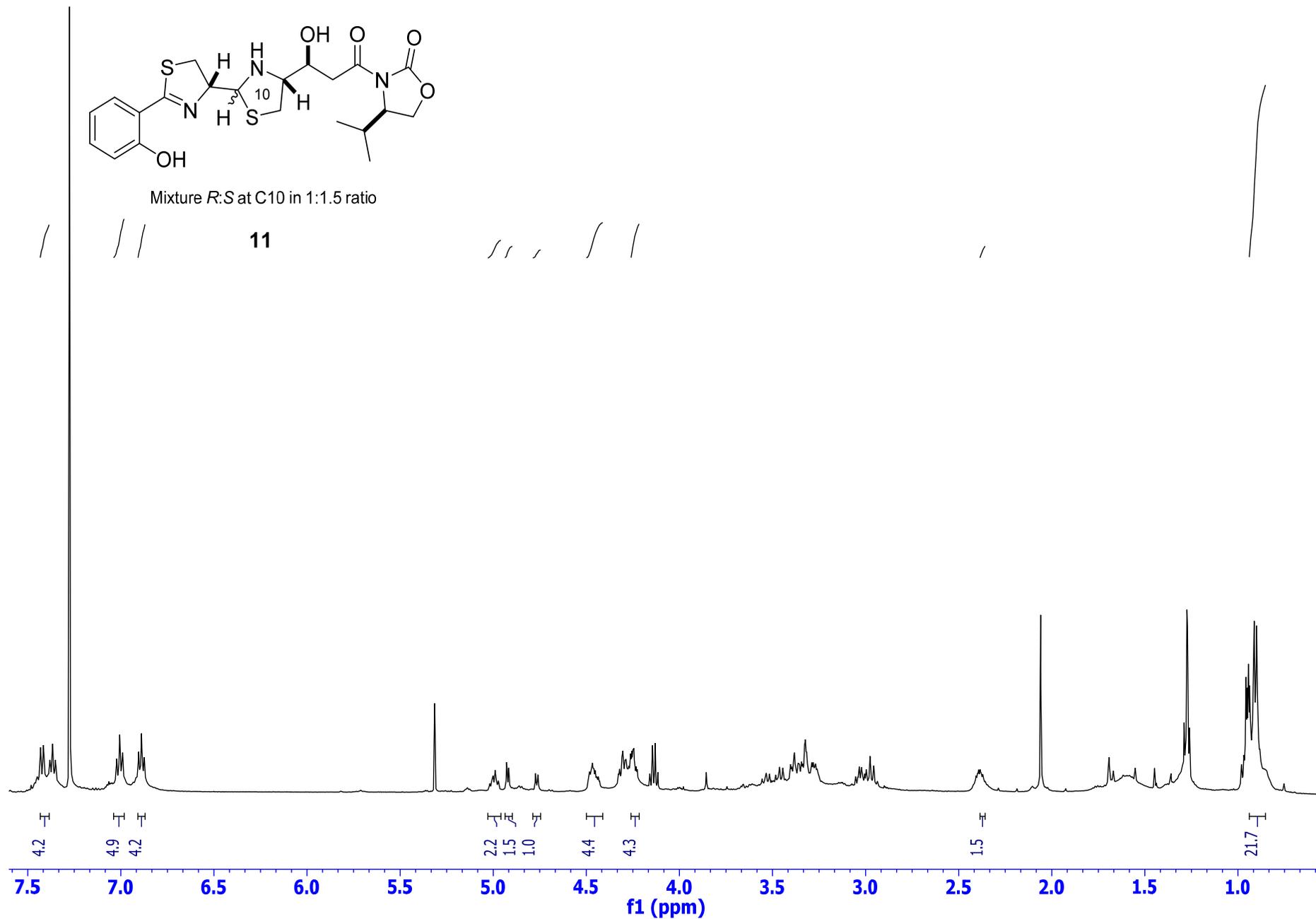
Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
C₂₁H₂₆N₃O₅S₂	464.1308	-1.2412	-2.6744	10.5

¹H-NMR (300 MHz, CDCl₃) of 11



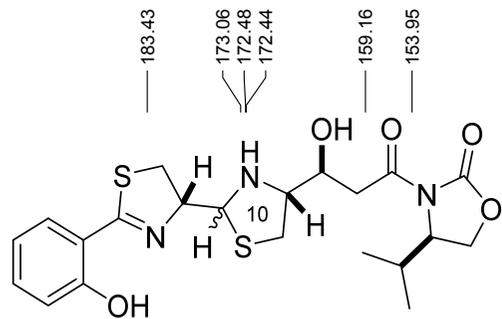
Mixture R:S at C10 in 1:1.5 ratio

11



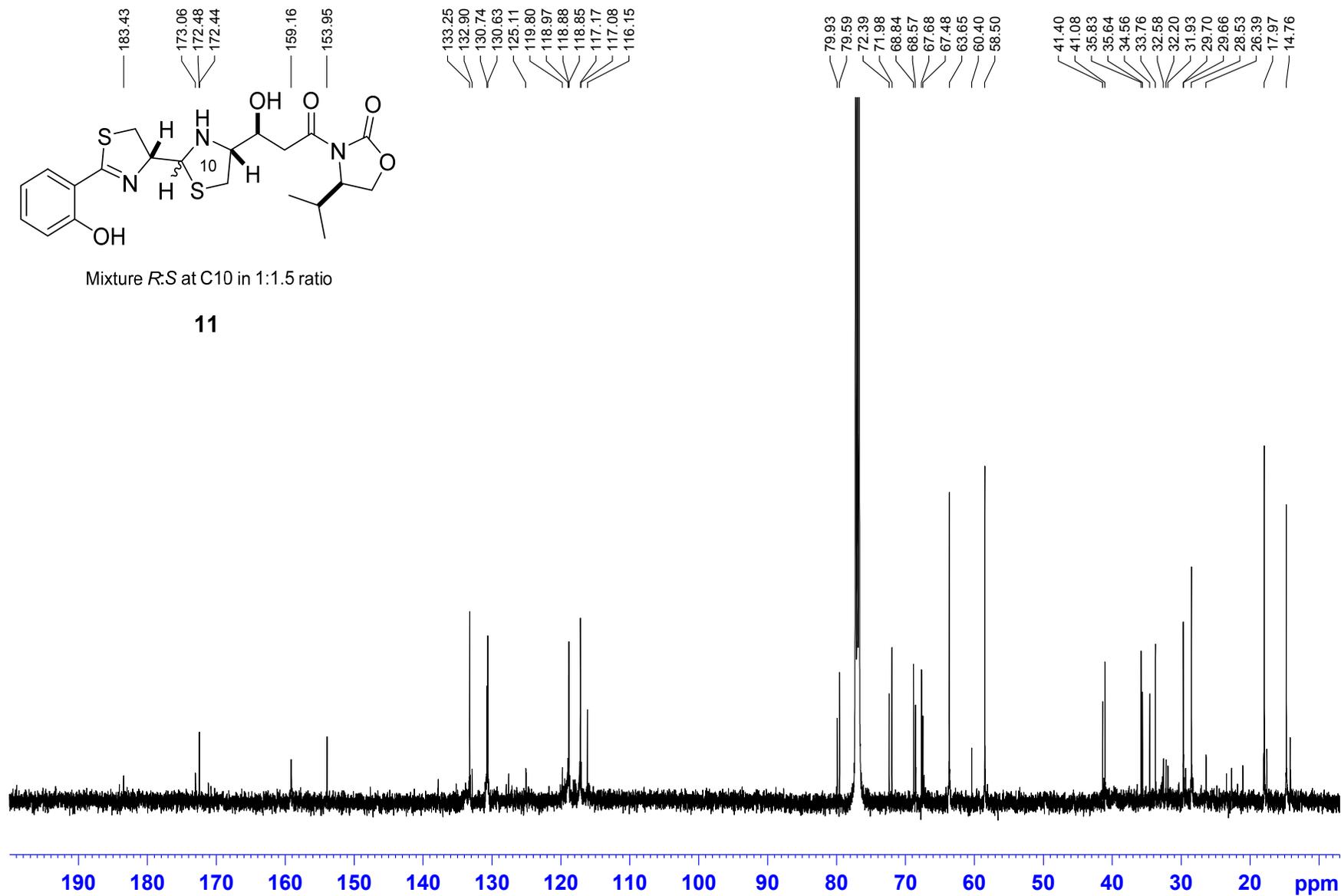
SI58

¹³C-NMR (75 MHz, CDCl₃) of 11

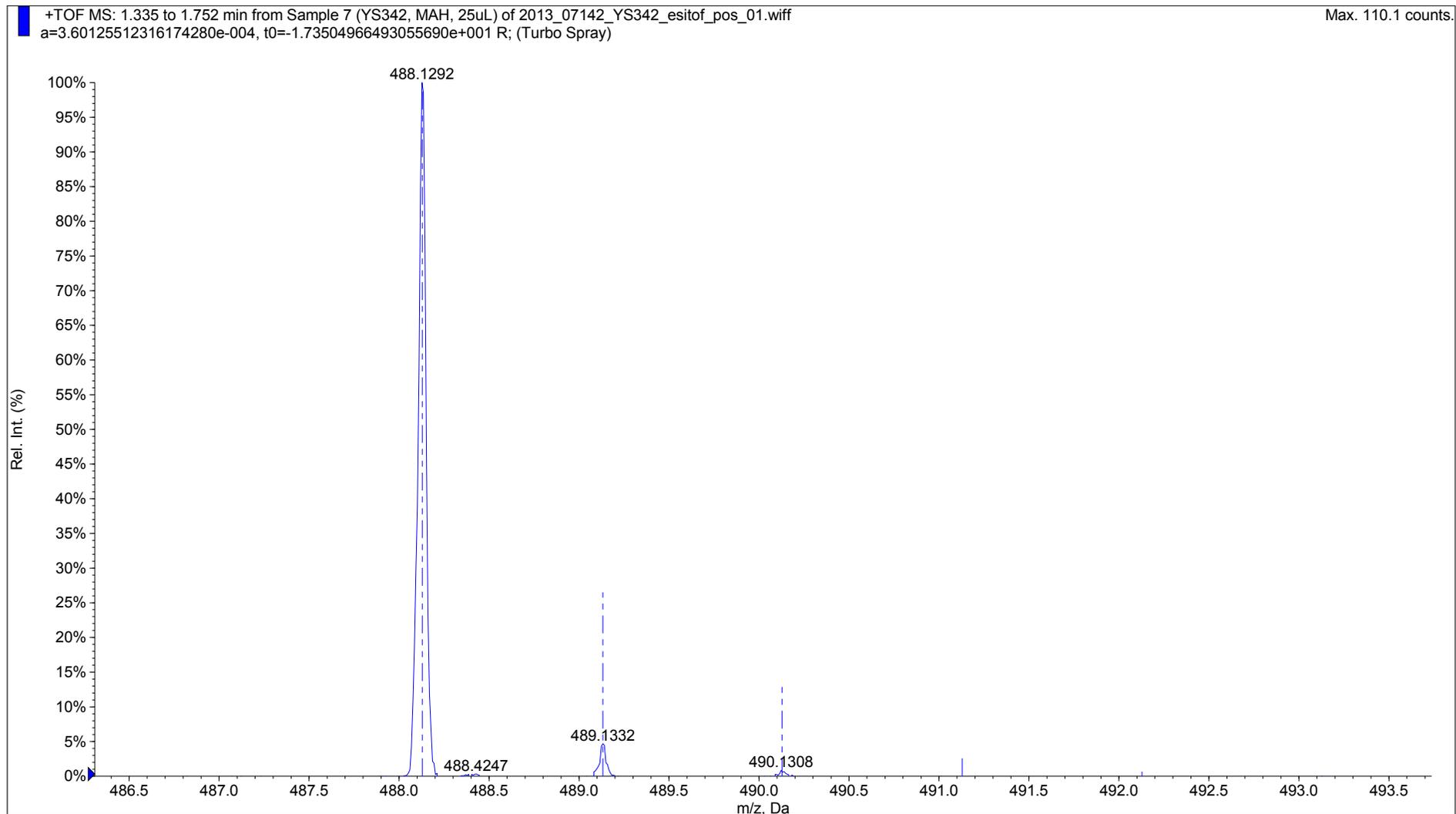


Mixture *R:S* at C10 in 1:1.5 ratio

11

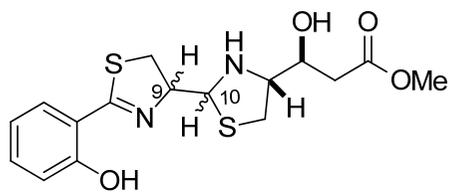


HRESIMS of 11: Ion m/z 488.1292, $([M+Na]^+)$

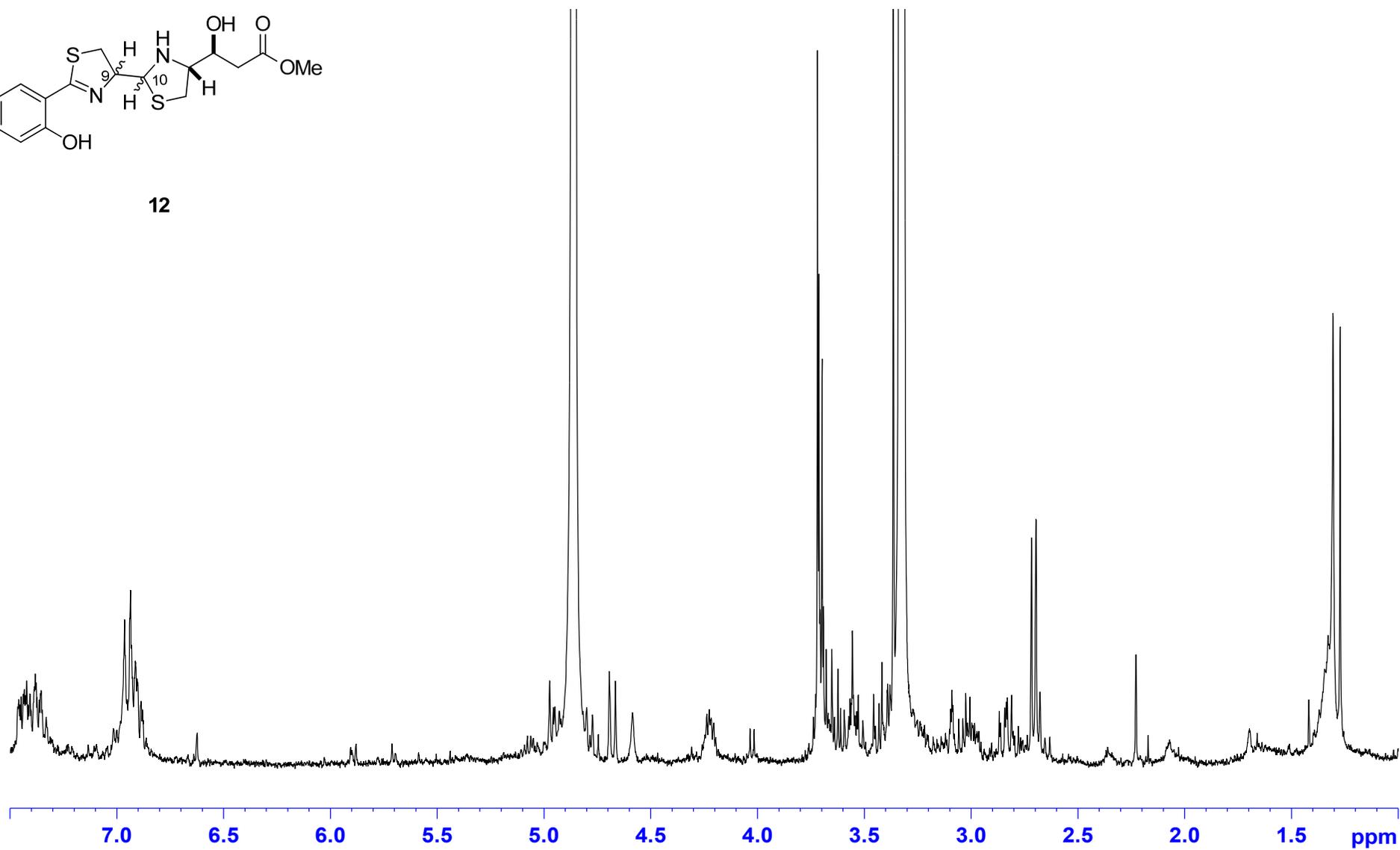


Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
$C_{21}H_{27}N_3O_5NaS_2$	488.1289	0.2153	0.4412	9.5

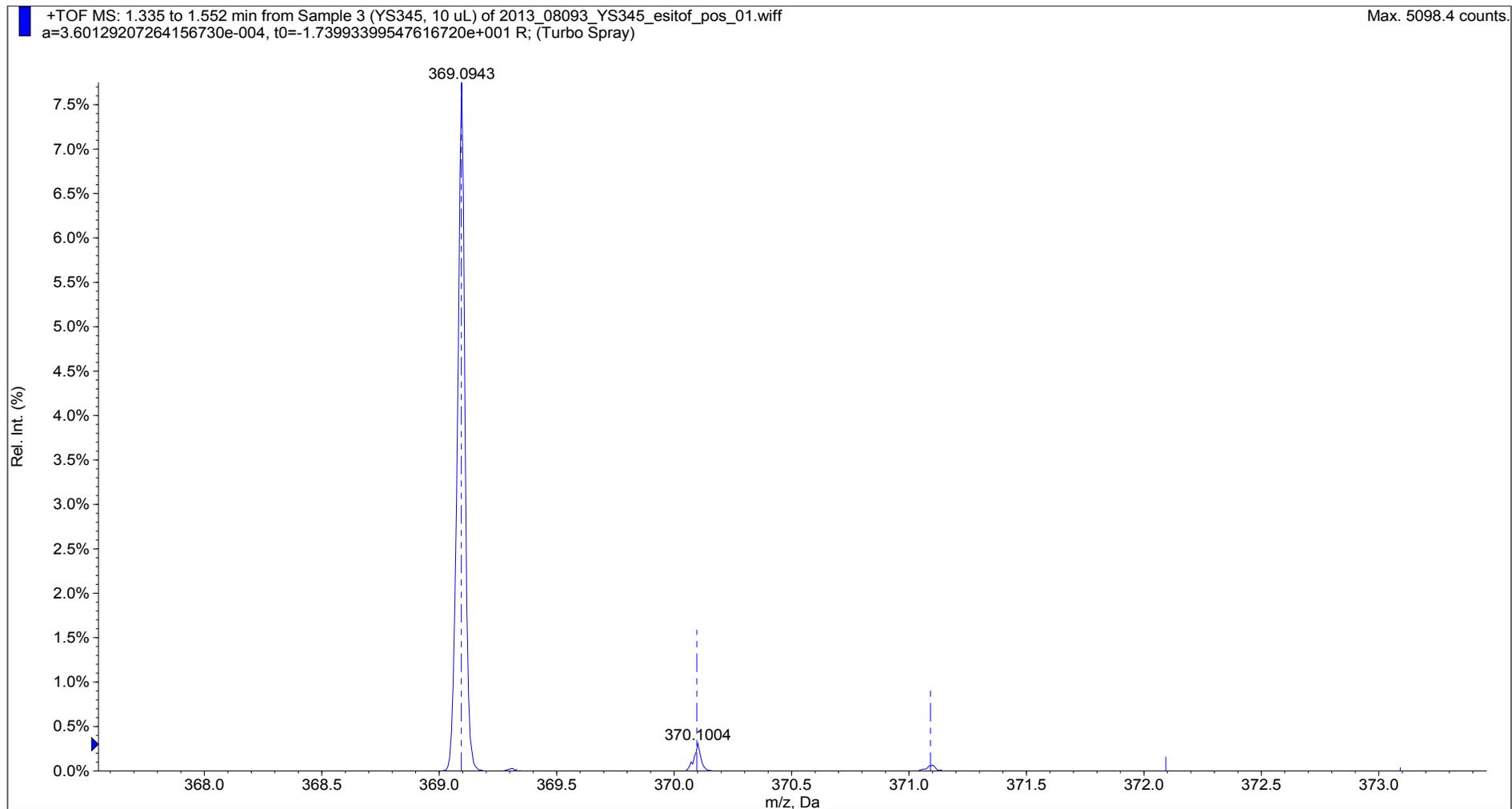
¹H-NMR (300 MHz, CD₃OD) of 12



12



HRESIMS of 12: Ion m/z 369.0943, $([M+H]^+)$



Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
$C_{16}H_{21}N_2O_4S_2$	369.0937	0.5725	1.5512	7.5

¹H-NMR (300 MHz, CDCl₃) of 13

4.5168
4.4052
4.3926
4.3817
4.3771
4.3692
4.3645
4.3537
4.2118
4.1916
4.1880
4.1677
4.1643
4.1438
4.1407

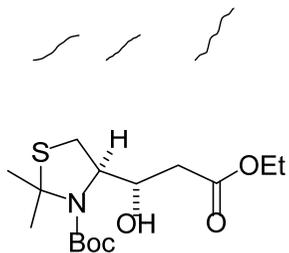
3.2275
3.2058
3.1859
3.1642

2.7857
2.7442
2.6525
2.6401
2.5999
2.5874
2.5765
2.5483
2.5239
2.4957

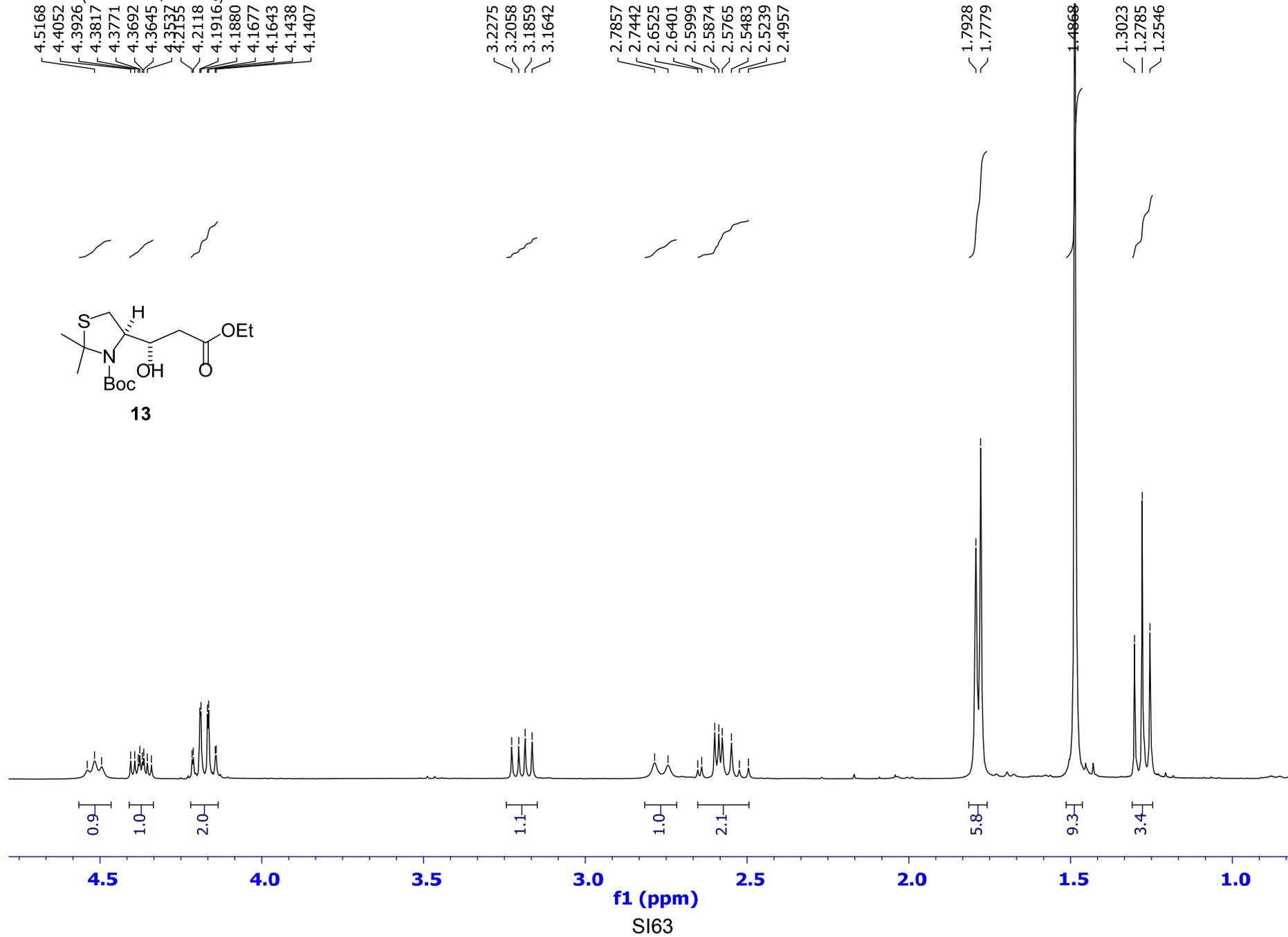
1.7928
1.7779

1.4868

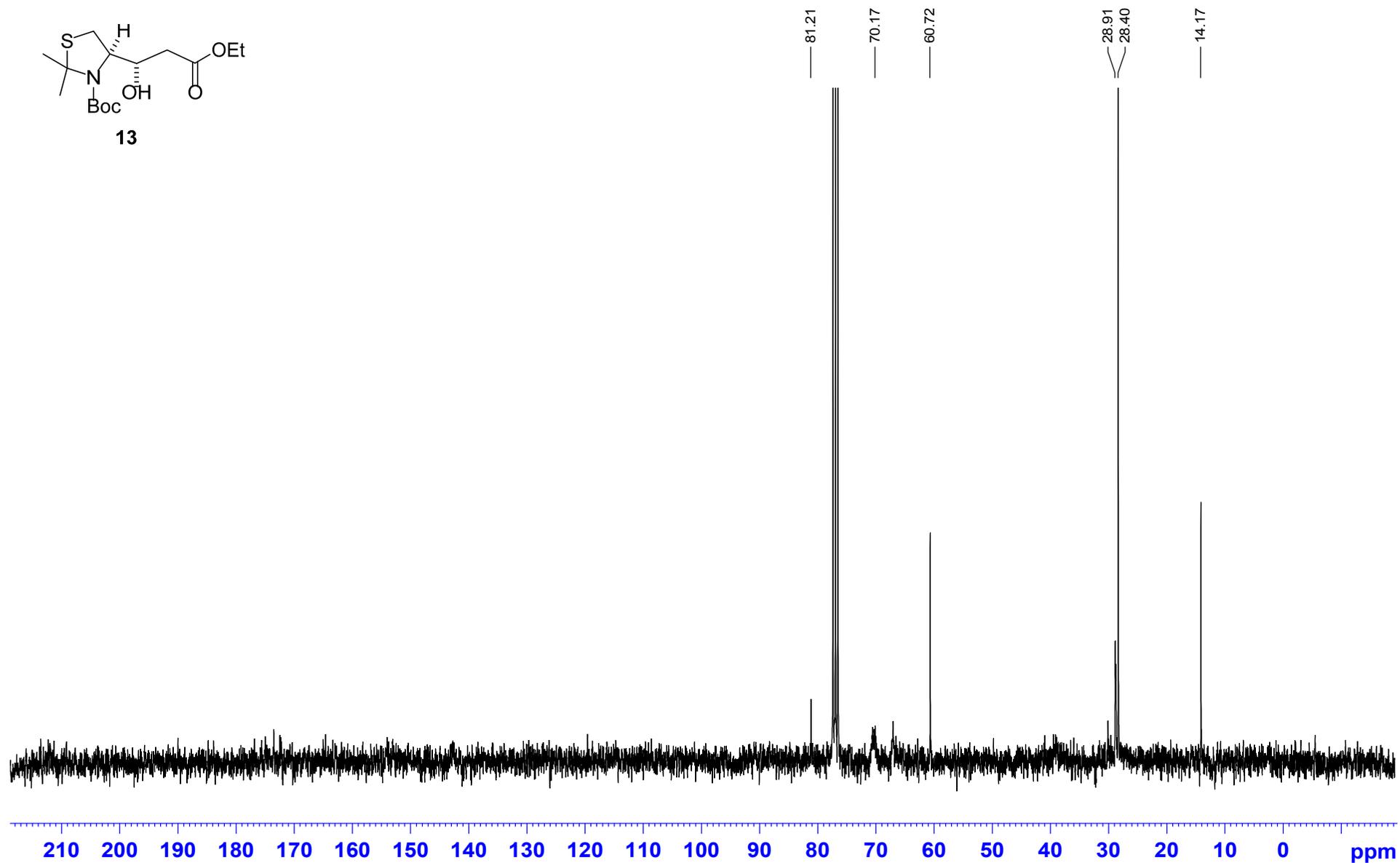
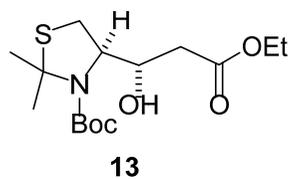
1.3023
1.2785
1.2546



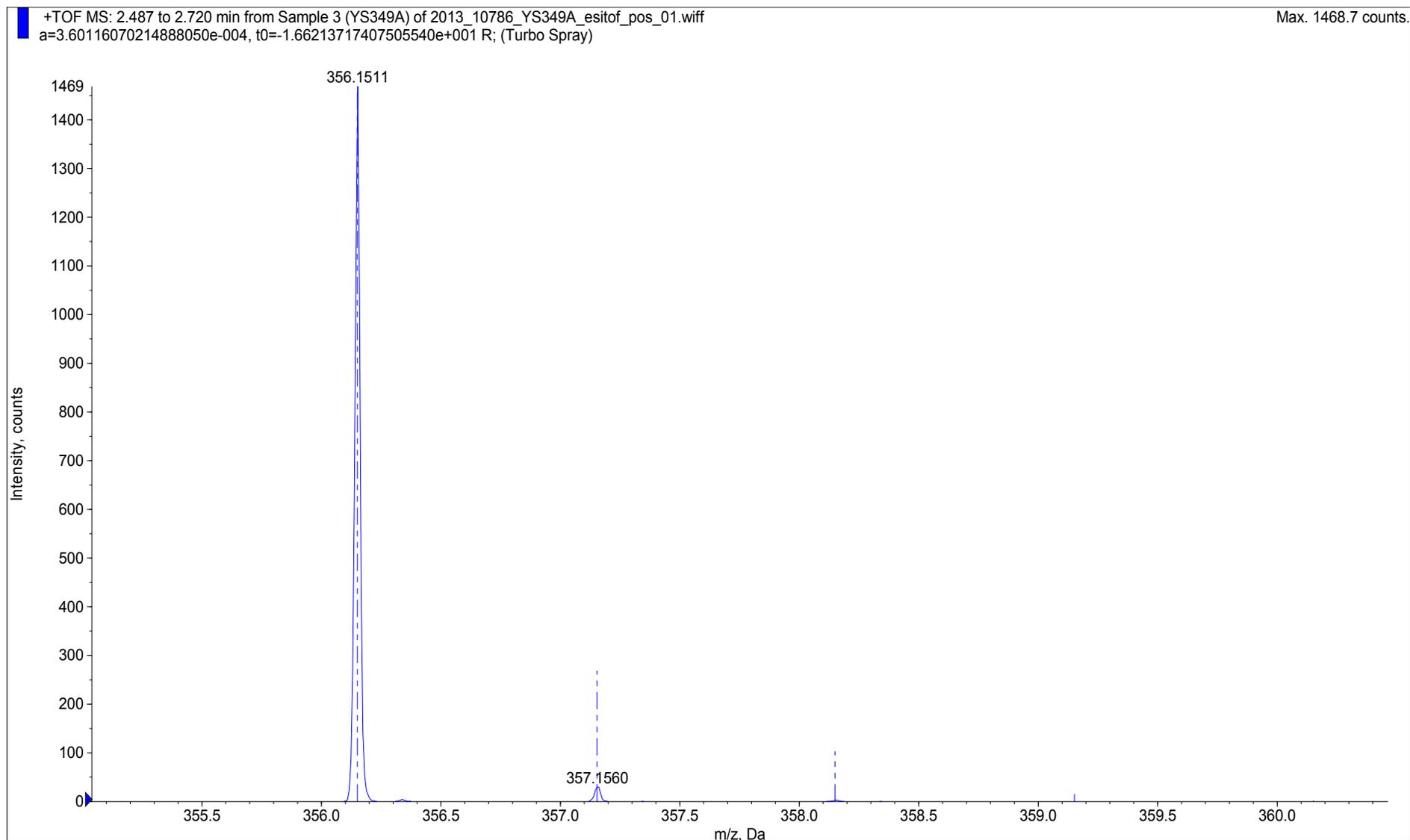
13



¹³C-NMR (75 MHz, CDCl₃) of 13

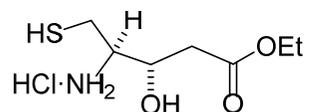


HRESIMS of 13: Ion m/z 356.1511, ([M+Na]⁺)



Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
<chem>C15H27NO5NaS</chem>	356.1502	0.8837	2.4815	2.5

¹H-NMR (300 MHz, CD₃OD) of 14

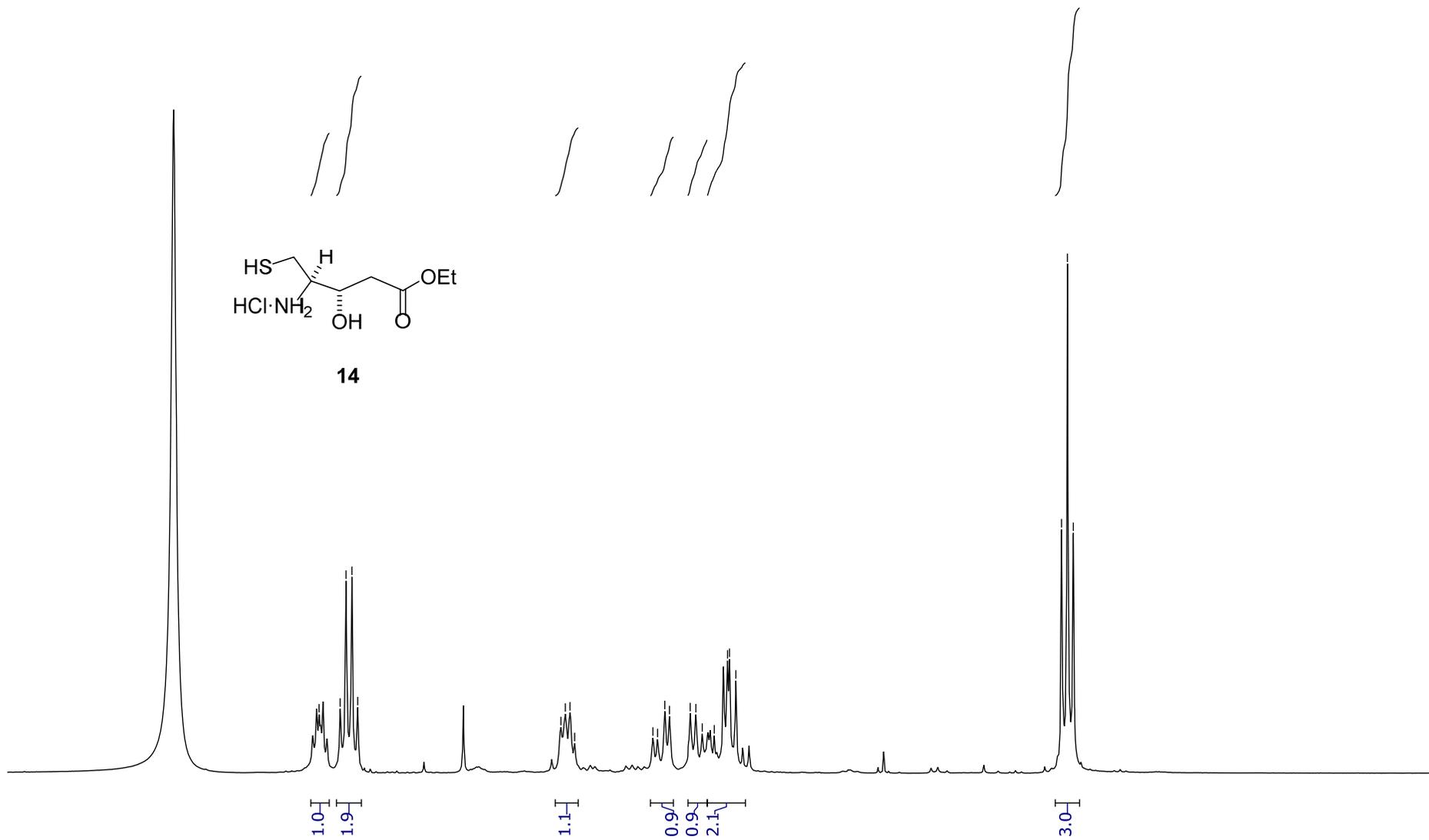


14

4.3036
4.2182
4.1946
4.1709
4.1473

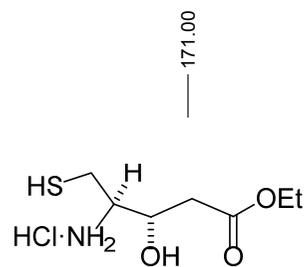
3.3258
3.3083
3.2890
3.2702
2.9539
2.9350
2.9056
2.8867
2.8029
2.7802
2.7547
2.7058
2.6525
2.6445
2.6186

1.3019
1.2782
1.2545

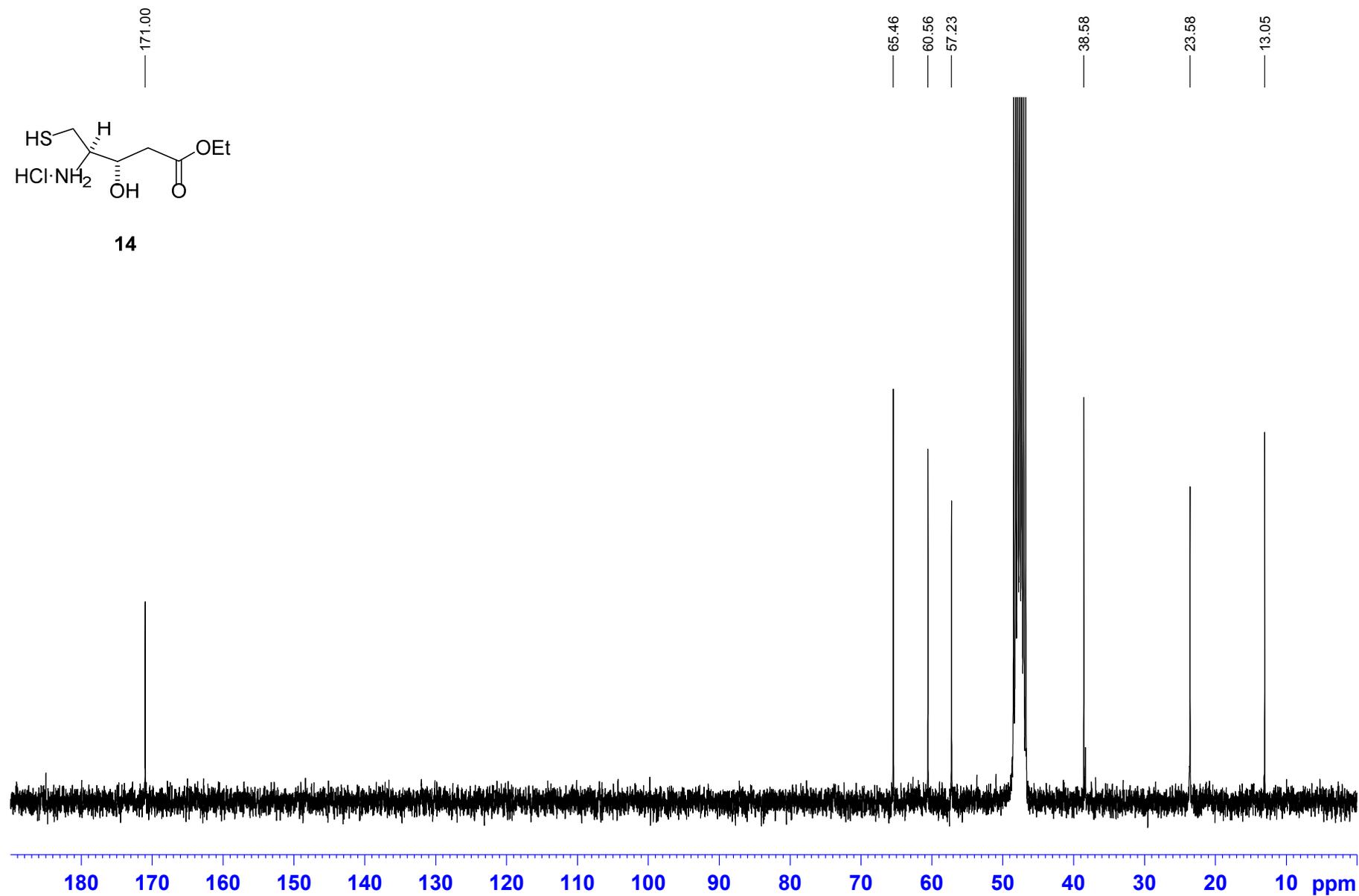


5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
SI66

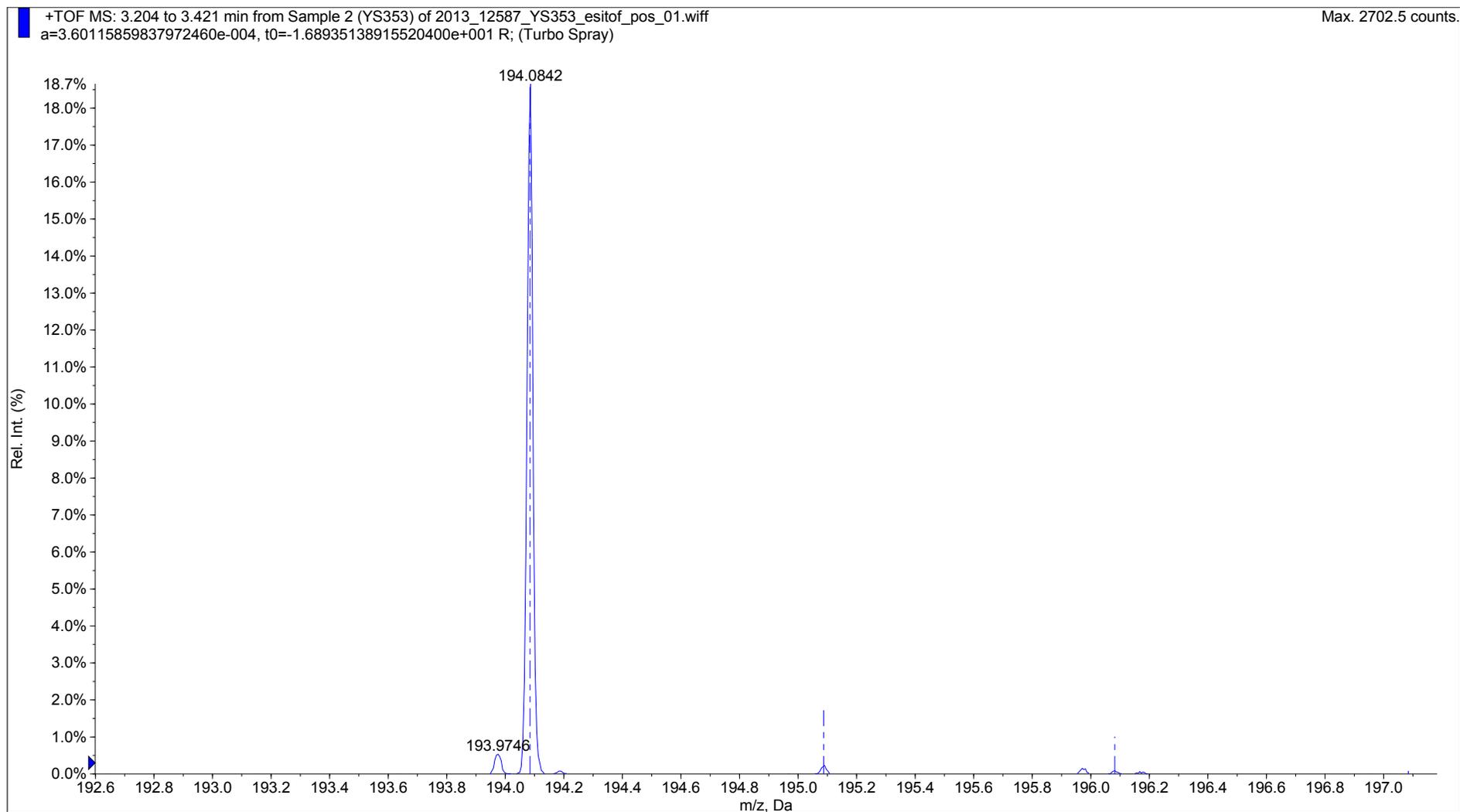
¹³C-NMR (75 MHz, CD₃OD) of 14



14



HRESIMS of 14: Ion m/z 194.0842, ([M+H]⁺)

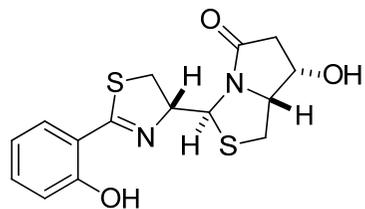
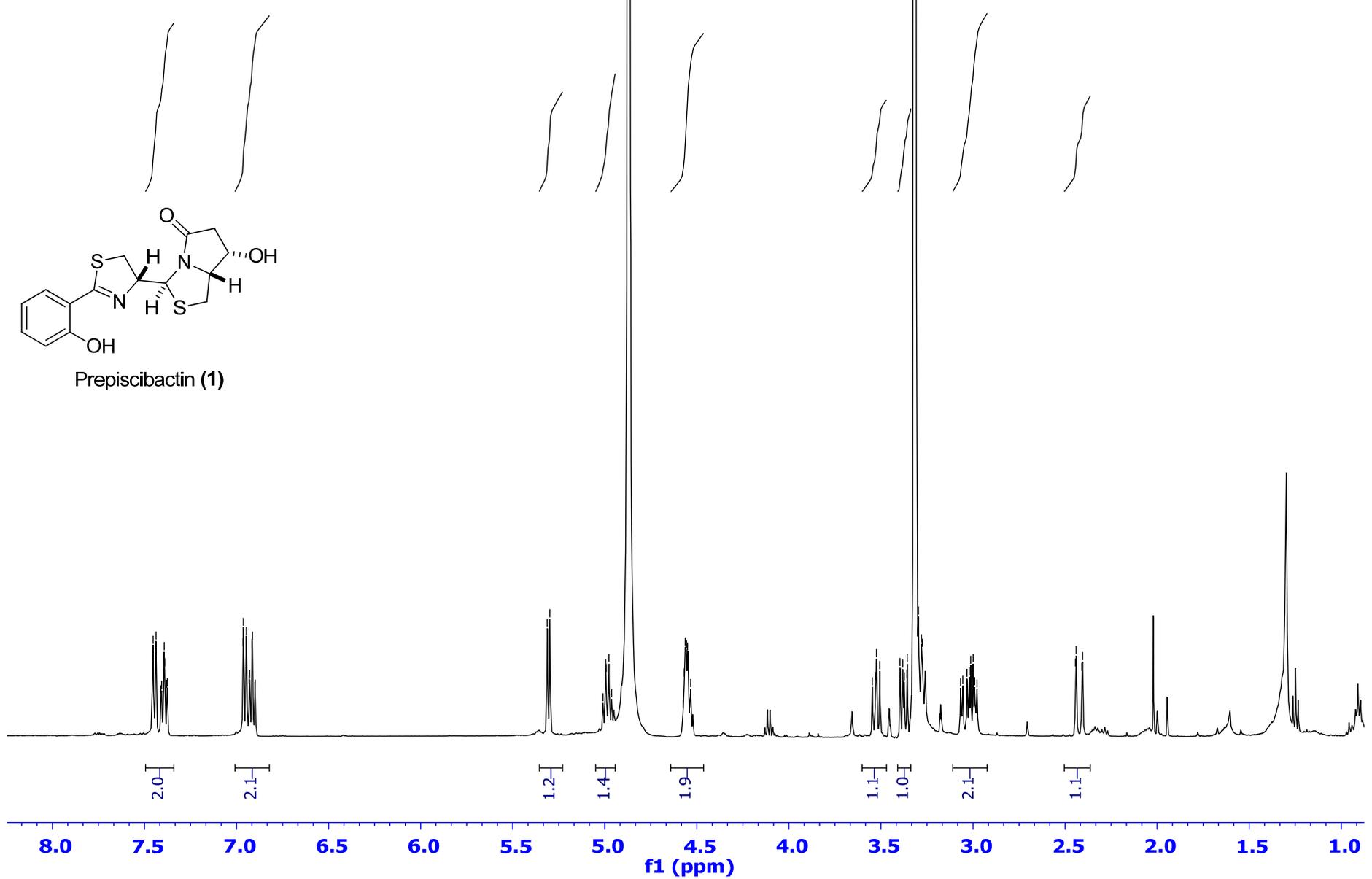


Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
C ₇ H ₁₆ NO ₃ S	194.0845	-0.3417	-1.761	0.5

¹H-NMR (500 MHz, CD₃OD) of 1

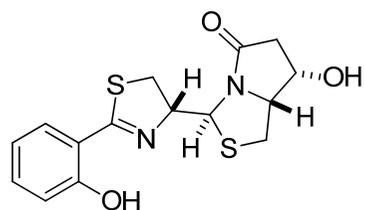
7.4562
7.4529
7.4406
7.4373
7.4070
7.3957
7.3931
7.3903
7.3790
6.9652
6.9629
6.9485
6.9461
6.9312
6.9288
6.9160
6.9137
6.9009
6.8985

5.3124
5.2984
5.0095
4.9950
4.9921
4.9806
4.9776
4.9629
4.5742
4.5680
4.5632
4.5593
4.5562
4.5502
4.5462
4.5386
4.5335
3.5468
3.5296
3.5241
3.5064
3.3961
3.3810
3.3733
3.3586
3.3570
3.2966
3.2802
3.2754
3.0674
3.0555
3.0330
3.0211
3.0126
2.9996
2.9914
2.9787
2.4437
2.4395
2.4095
2.4049

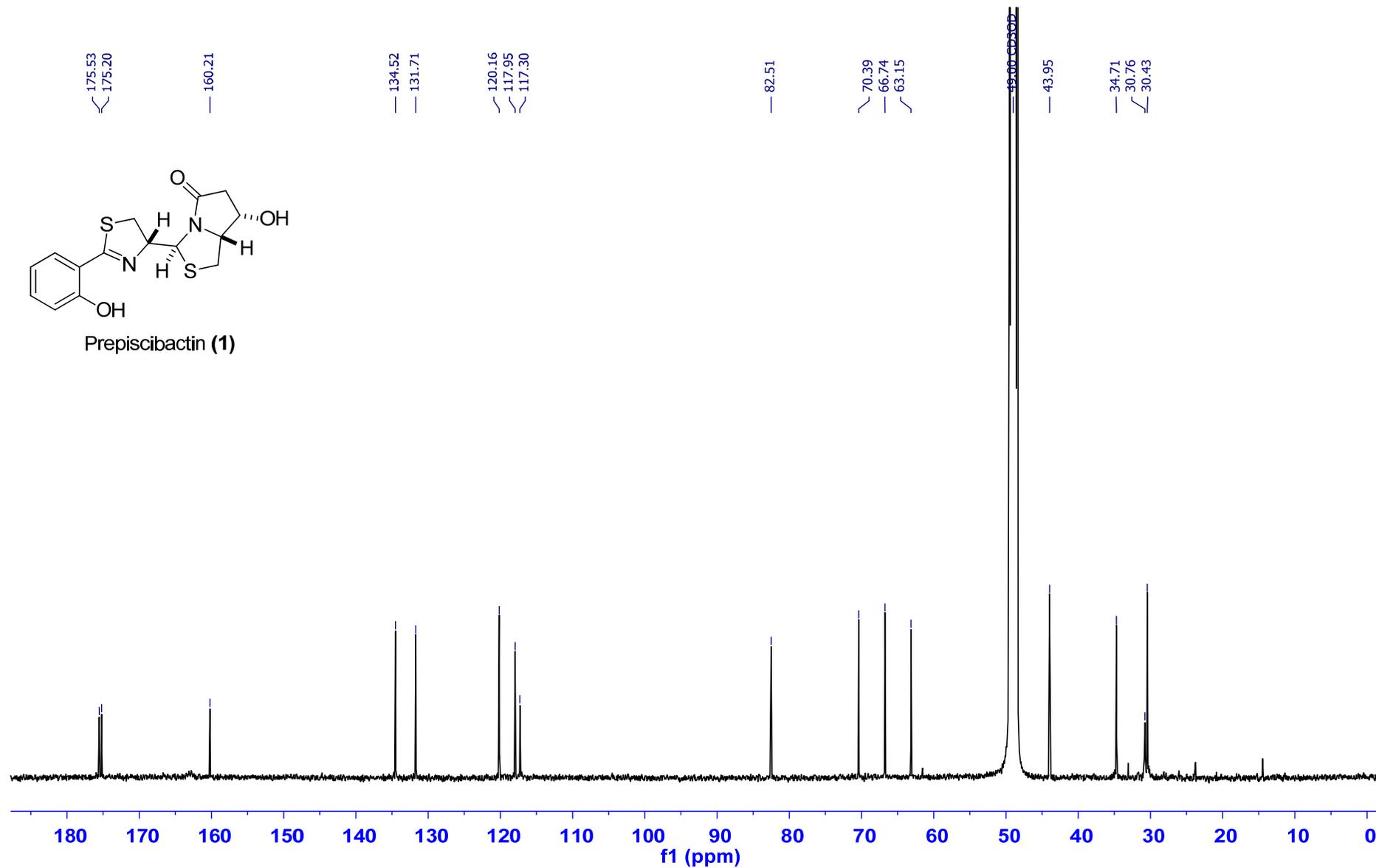


Prepiscibactin (1)

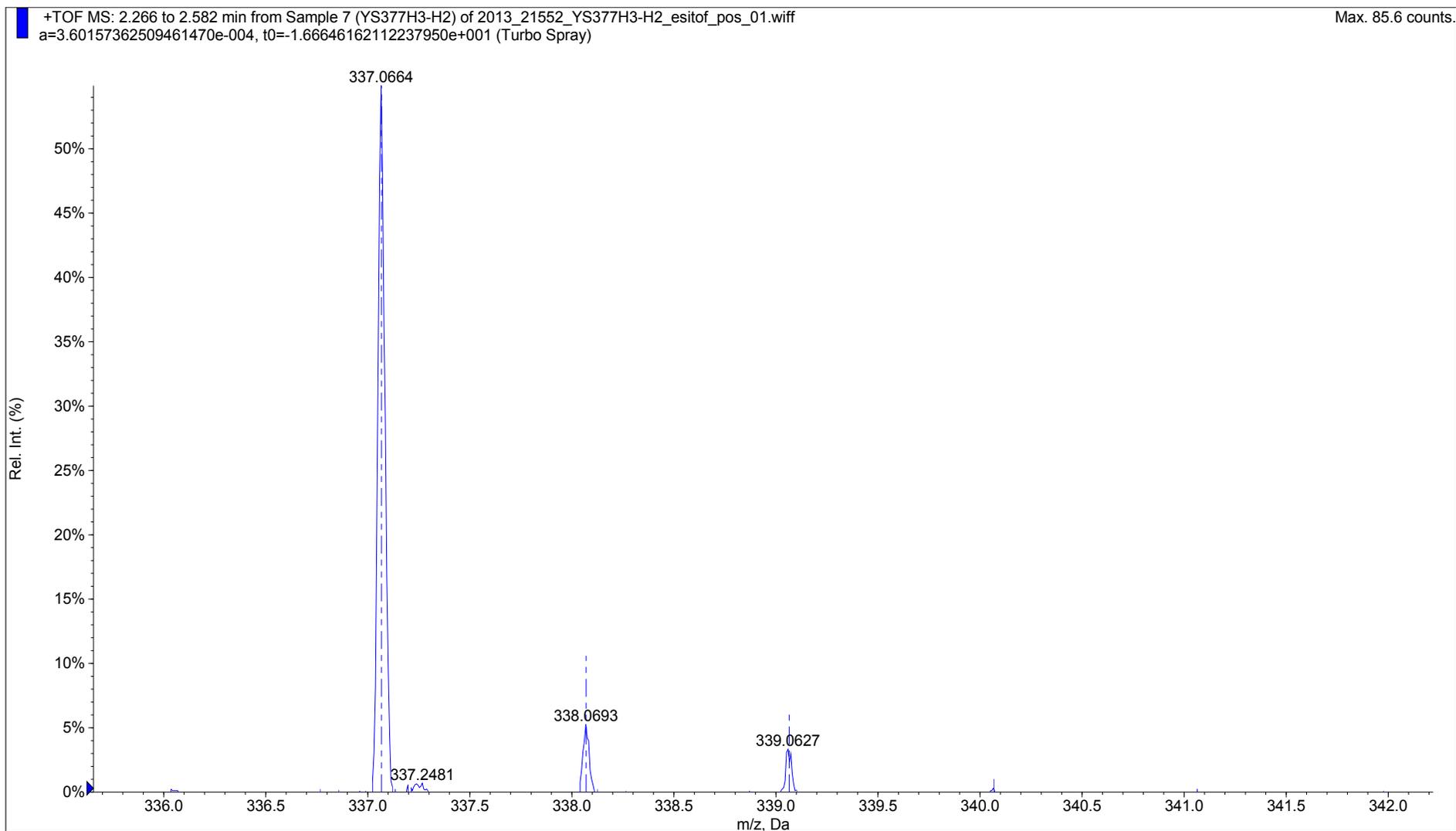
^{13}C -NMR (125 MHz, CD_3OD) of 1



Prepiscibactin (1)

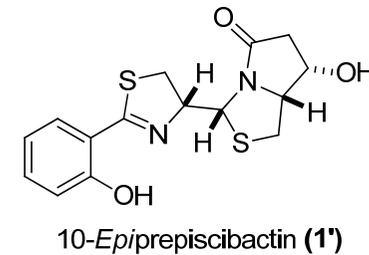
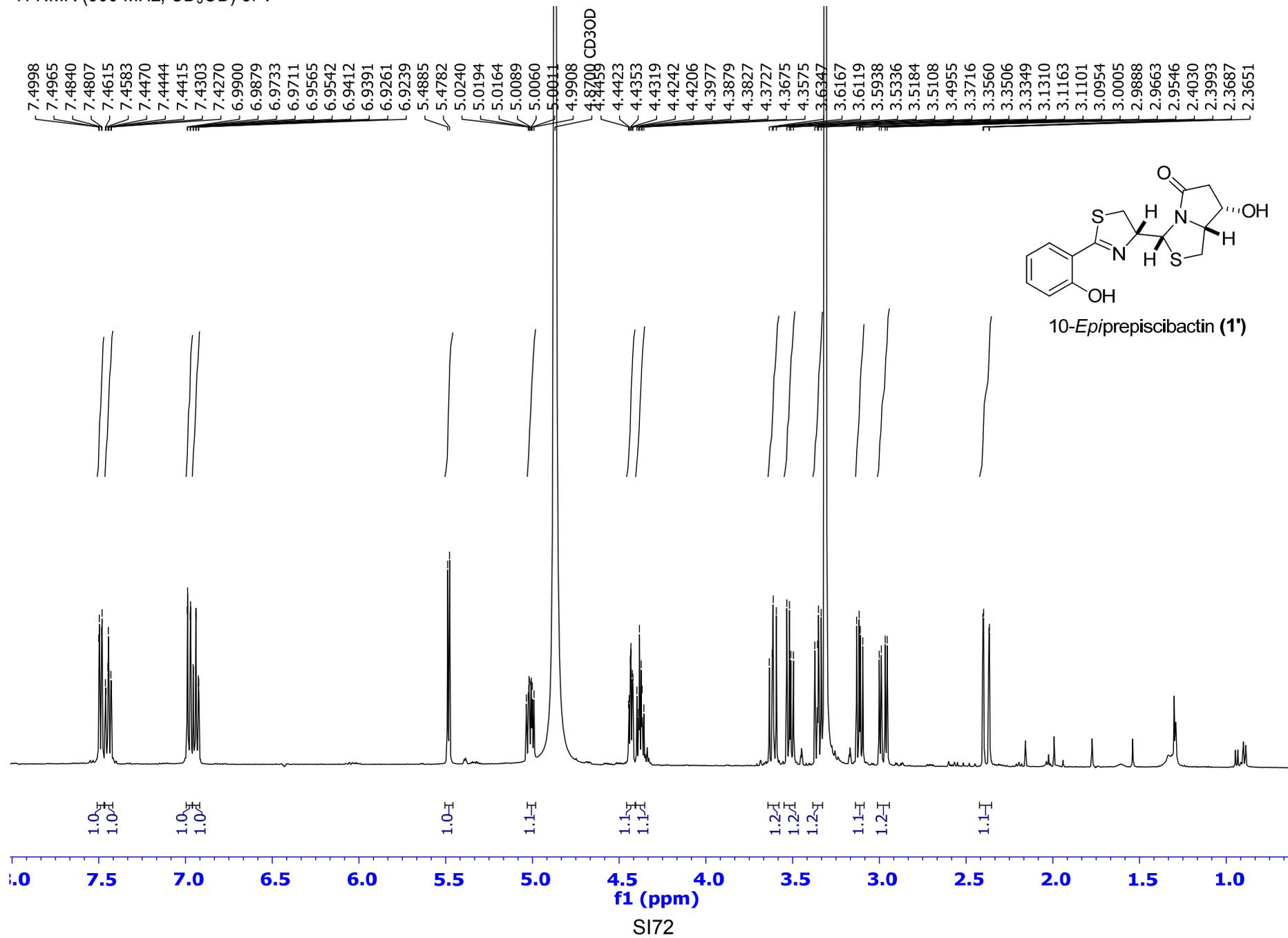


HRESIMS of 1: Ion m/z 337.0664, $([M+H]^+)$



Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
<chem>C15H17N2O3S2</chem>	337.0675	-1.1126	-3.3009	8.5

¹H-NMR (500 MHz, CD₃OD) of 1'



¹³C-NMR (125 MHz, CD₃OD) of 1'

~ 177.1197
~ 175.4460

— 160.2876

— 135.5589
— 131.9521

~ 120.4851
~ 118.0889
~ 116.5988

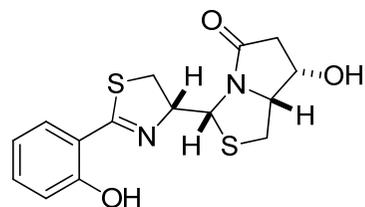
— 80.8469

~ 70.6362
~ 67.1919
~ 62.7843

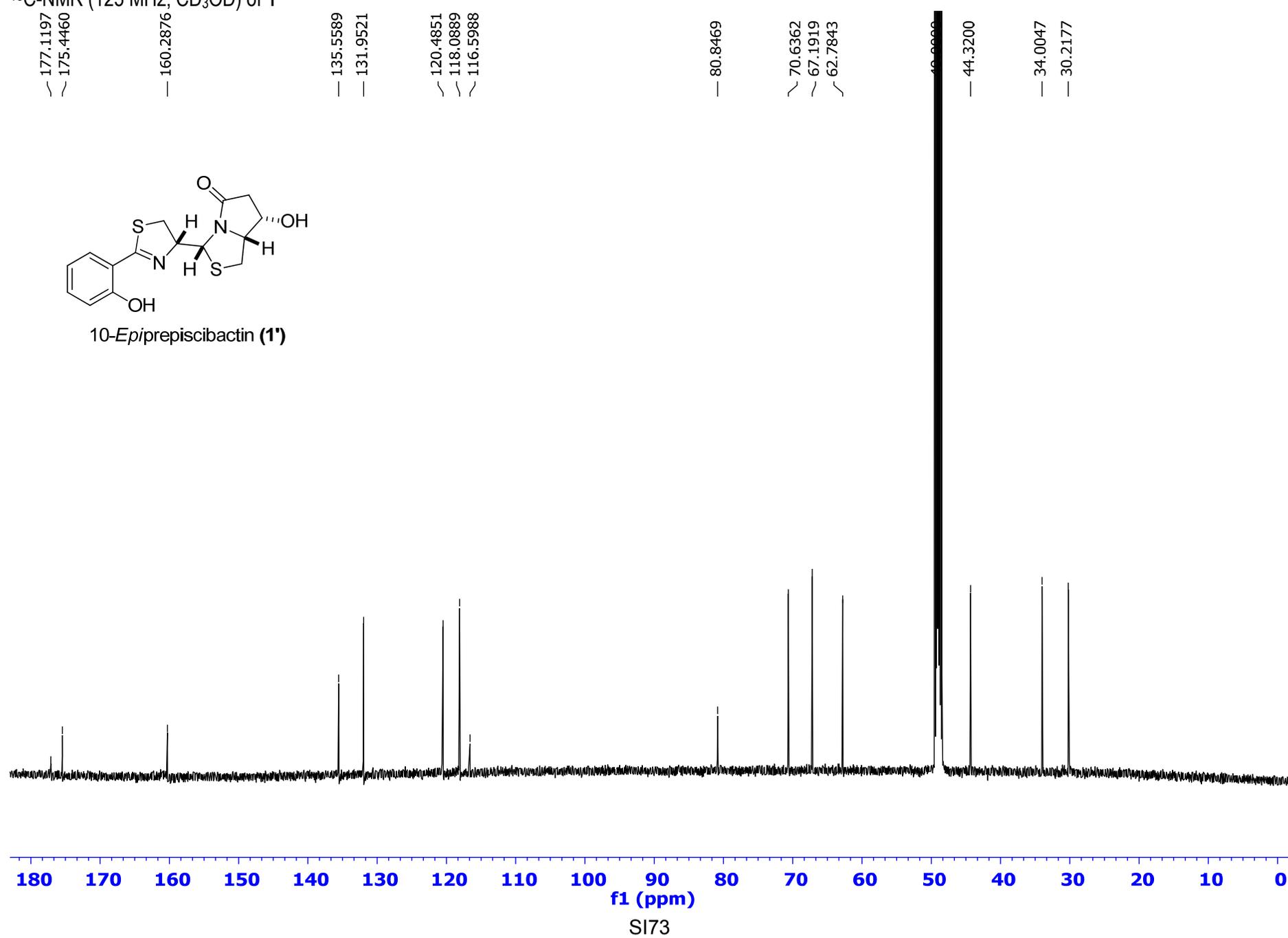
46.6666

— 44.3200

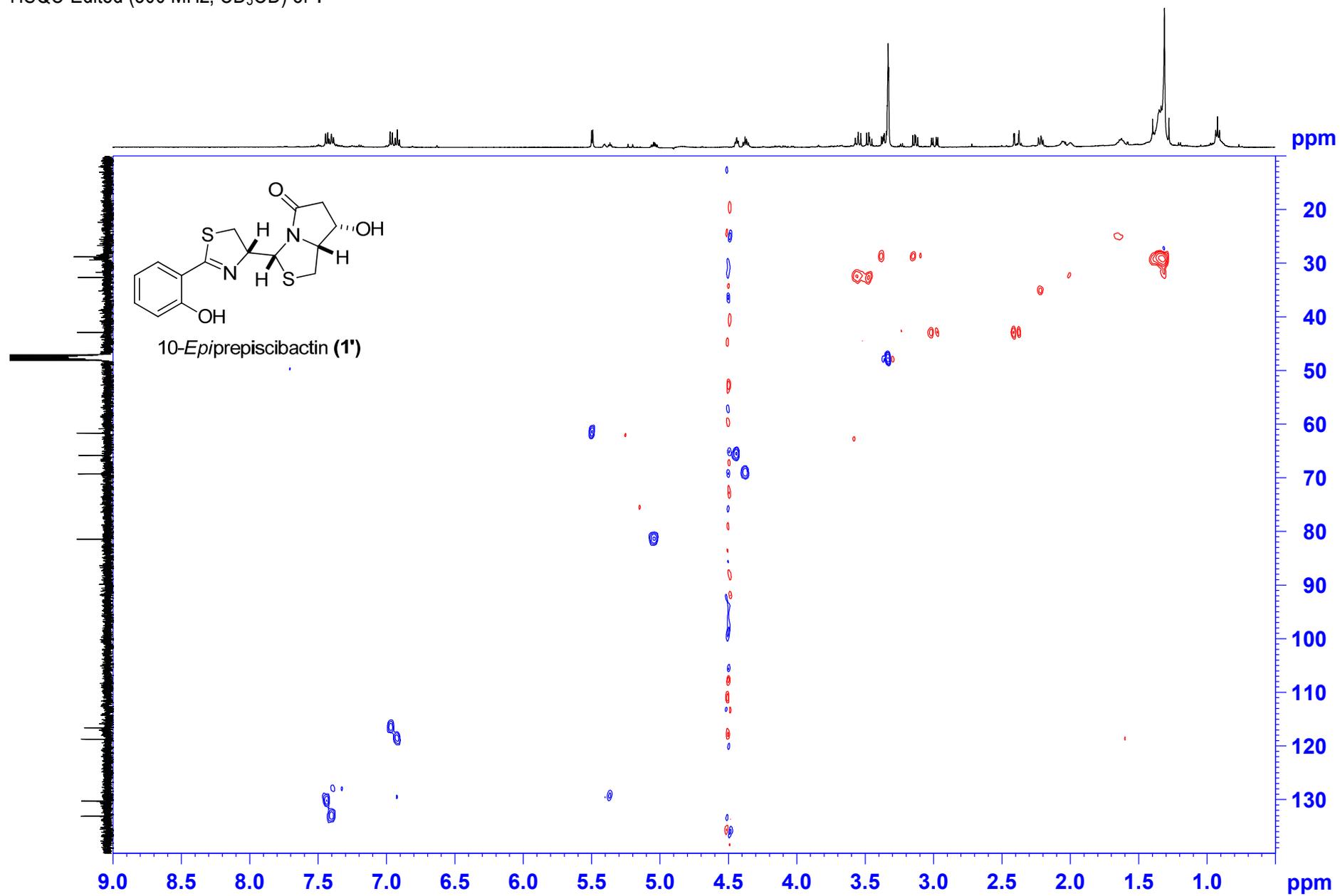
— 34.0047
— 30.2177



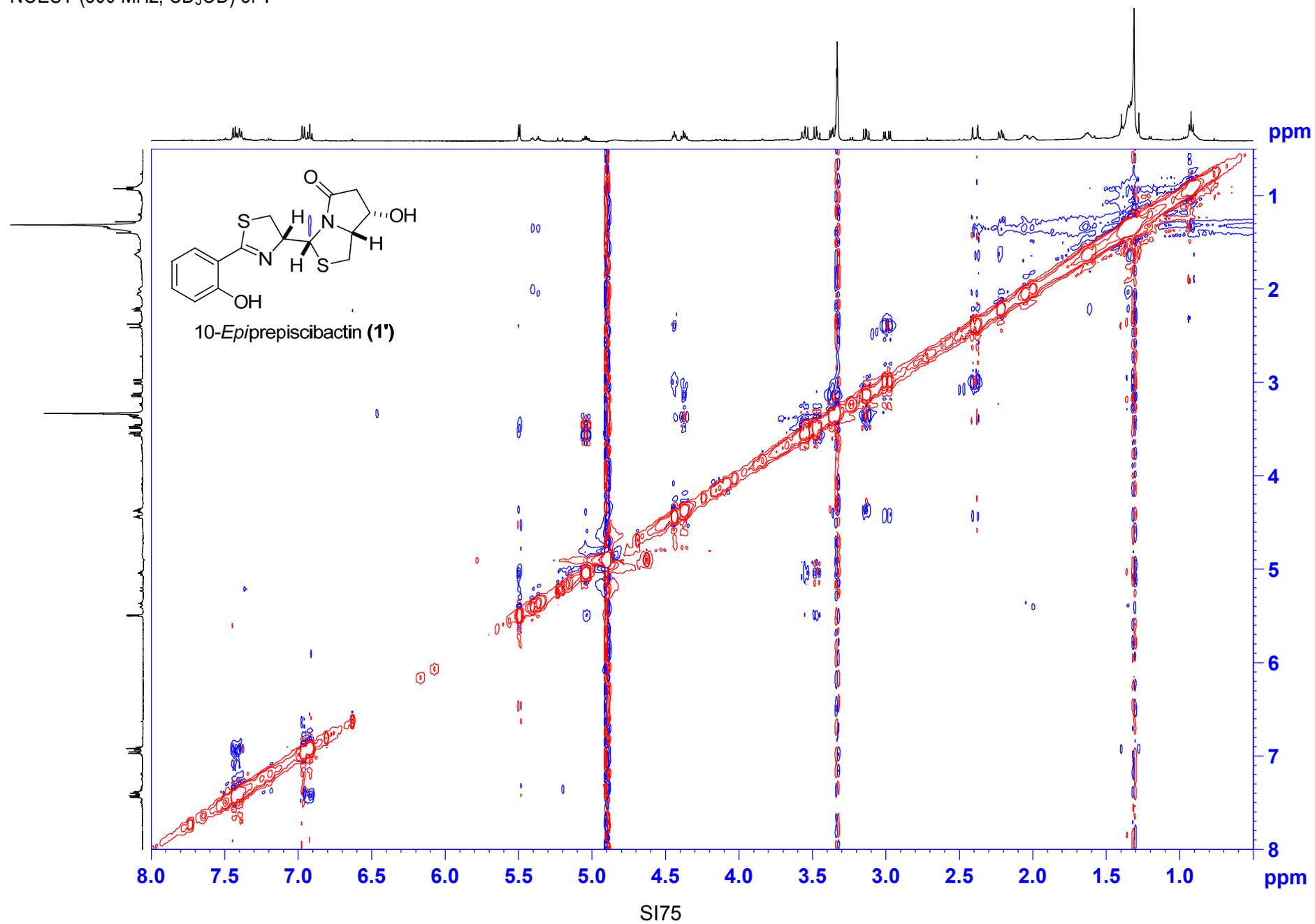
10-*Epiprepiscibactin* (**1'**)



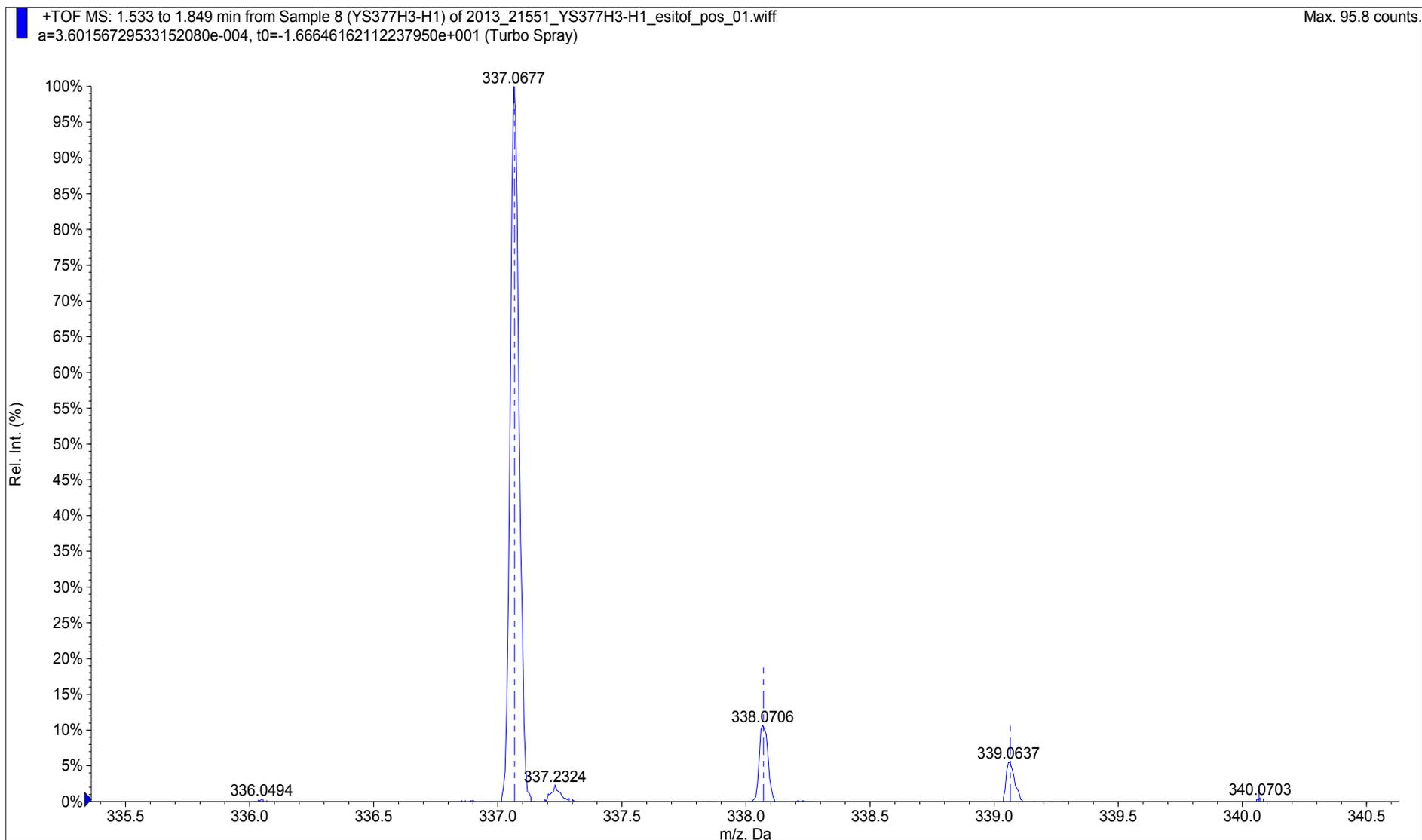
HSQC-Edited (500 MHz, CD₃OD) of 1'



NOESY (500 MHz, CD₃OD) of 1'



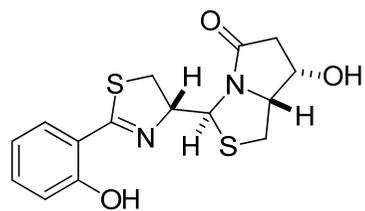
HRESIMS of 1': Ion m/z 337.0677, ($[M+H]^+$)



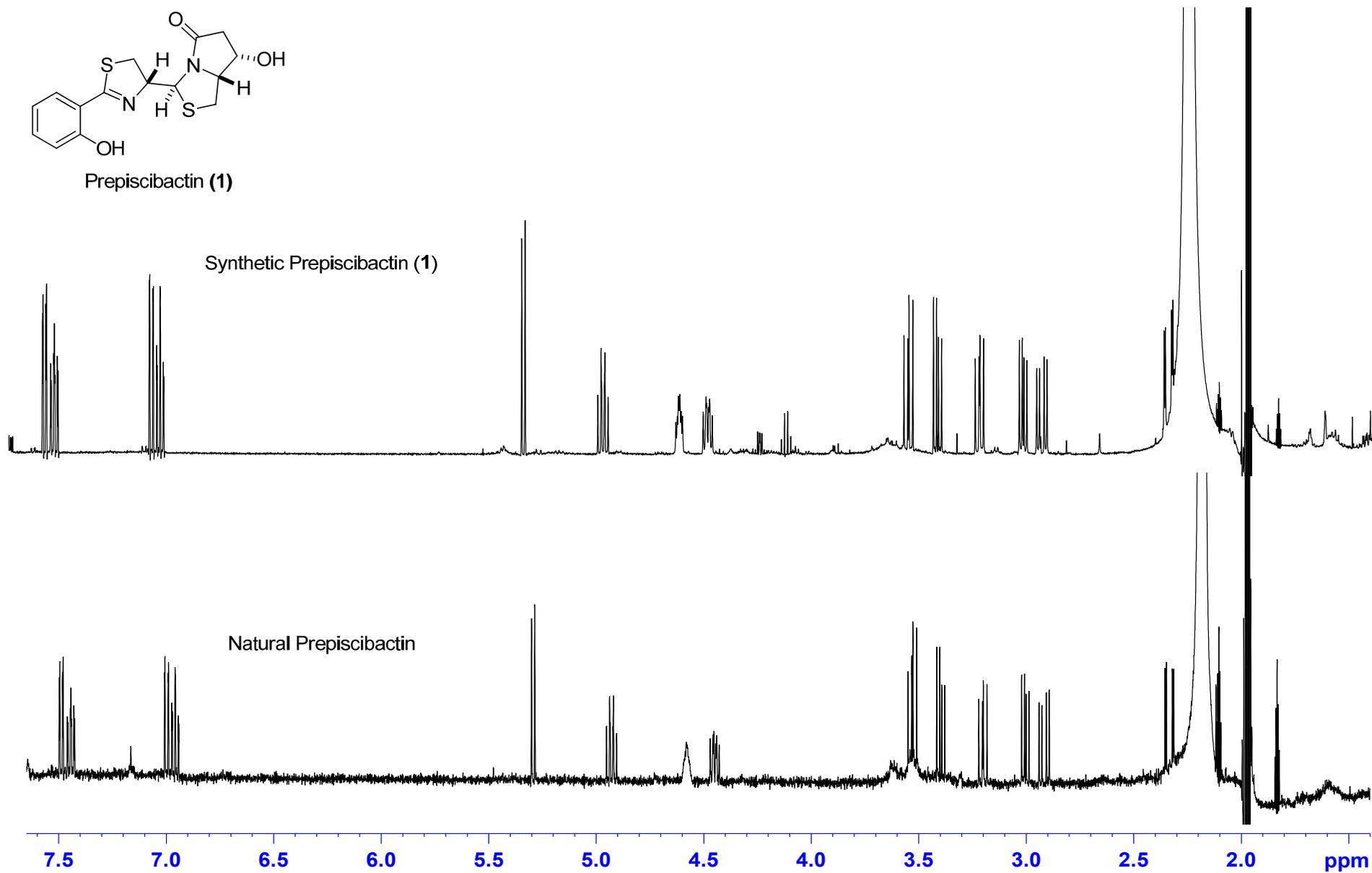
Formula	Calc. m/z	Δ , mDa	Δ , ppm	DBE
$C_{15}H_{17}N_2O_3S_2$	337.0675	0.1873	0.5558	8.5

IV. Comparison between spectra of natural and synthetic prepiscibactin (1)

Comparison between $^1\text{H-NMR}$ (500 MHz, CD_3CN) spectra of synthetic prepiscibactin (1) and natural prepiscibactin.

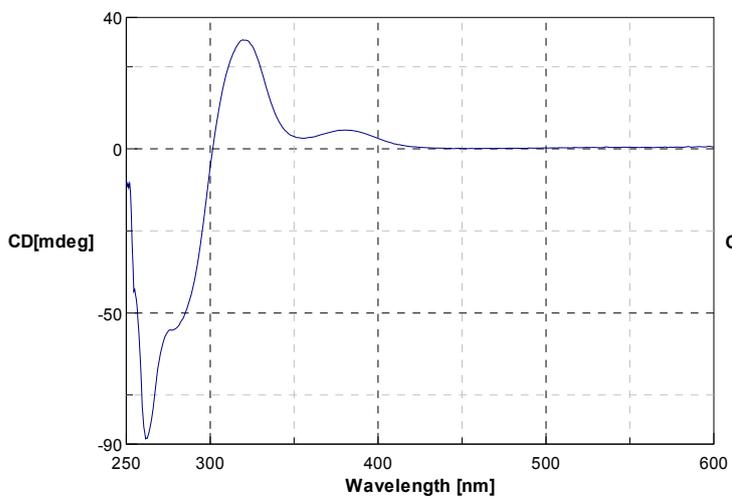


Prepiscibactin (1)

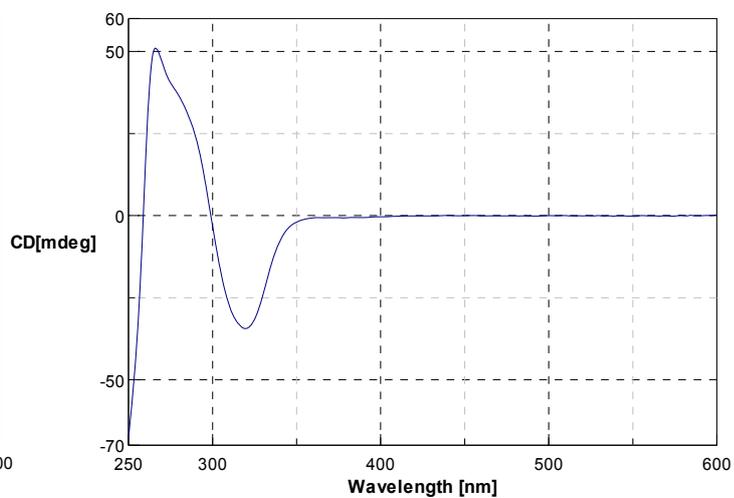


Comparison between CD-ORD spectrum of natural prepiscibactin, synthetic prepiscibactin (1) and 10-*ep*prepiscibactin (1').

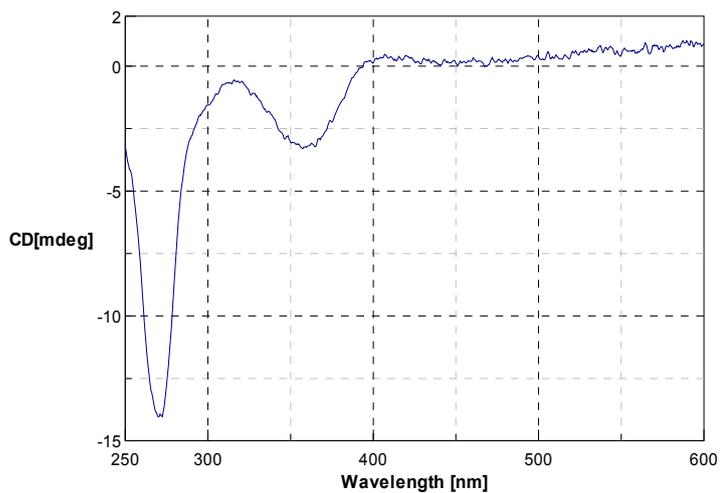
CD-ORD spectrum in MeOH of synthetic prepiscibactin (1)



CD-ORD spectrum in MeOH of 10-*ep*prepiscibactin (1')

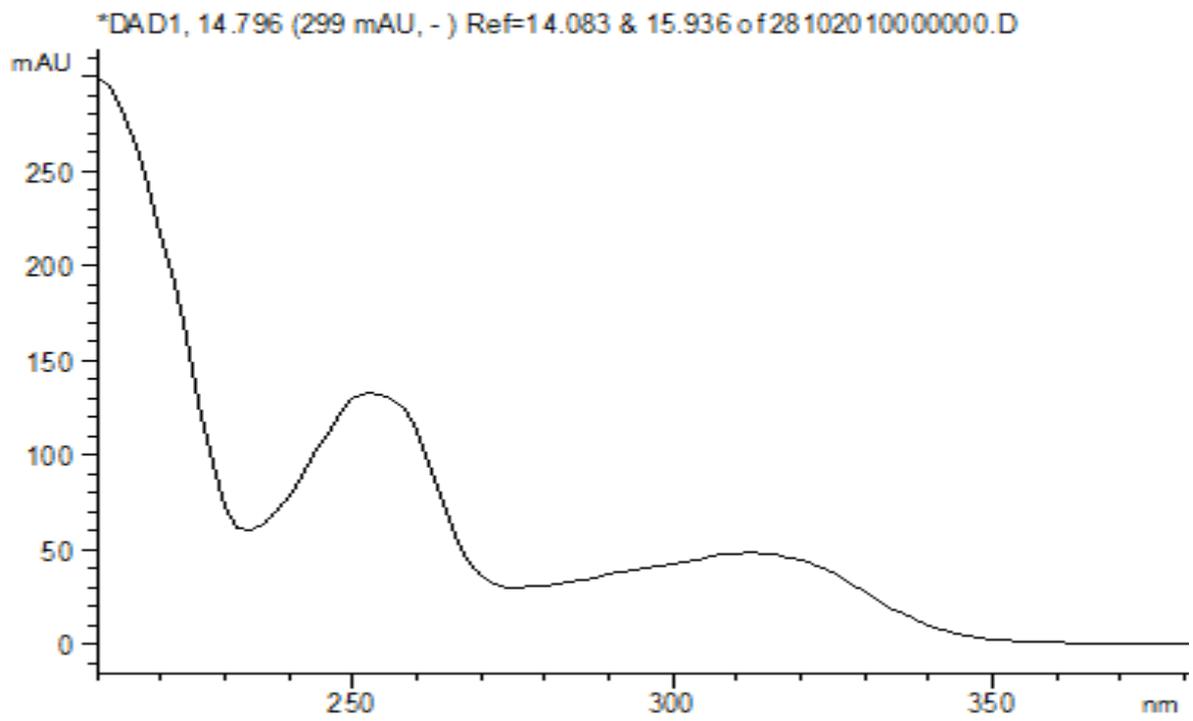


CD-ORD spectrum in MeOH of natural prepiscibactin

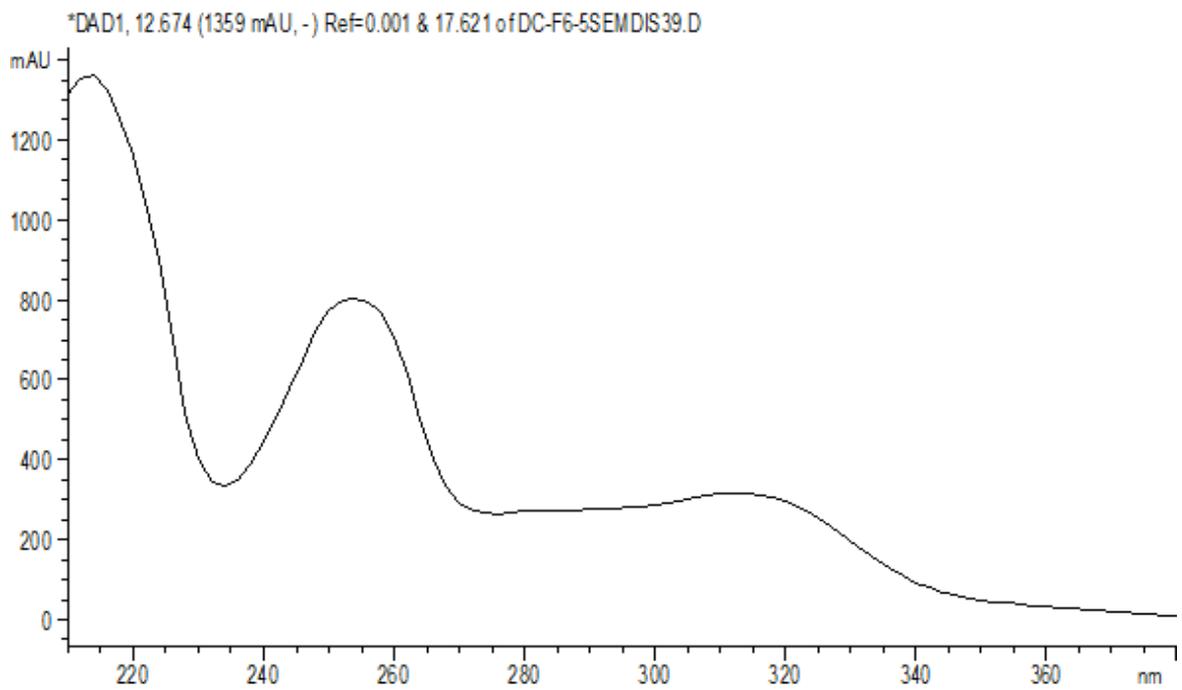


Comparison between UV spectra of natural prepiscibactin and synthetic prepiscibactin (1).

UV spectrum of natural prepiscibactin



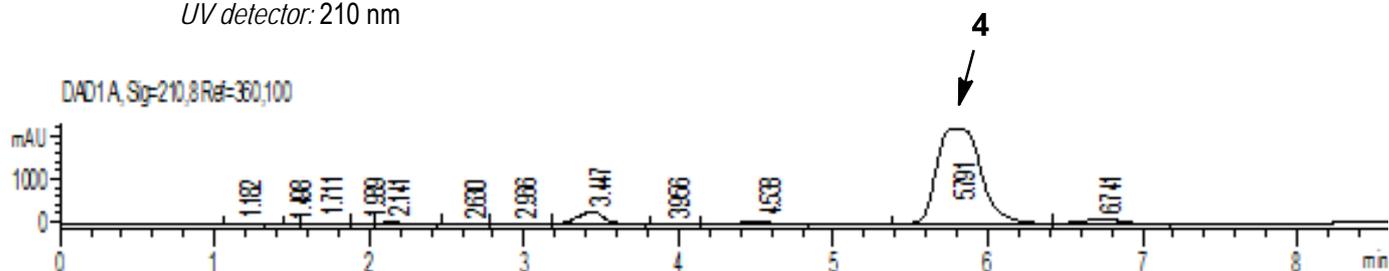
UV spectrum of synthetic prepiscibactin (1)



V. HPLC chromatograms of crude reactions

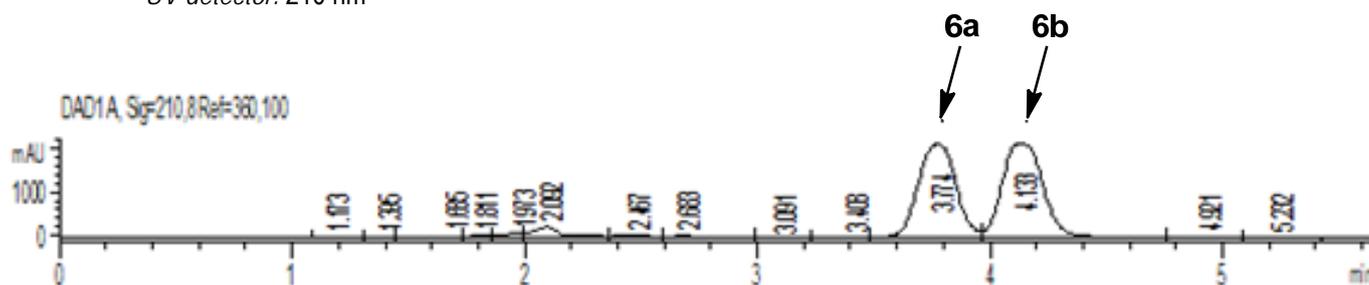
HPLC chromatogram of the crude of reaction from 2 to 4.

Conditions: *Column:* Eclipse® XDB-C18 (150x4.6 mm, 5 µm)
Mobile phase: isocratic 60% CH₃CN and 40% H₂O (v/v)
Flow rate: 1.0 mL/min.
UV detector: 210 nm



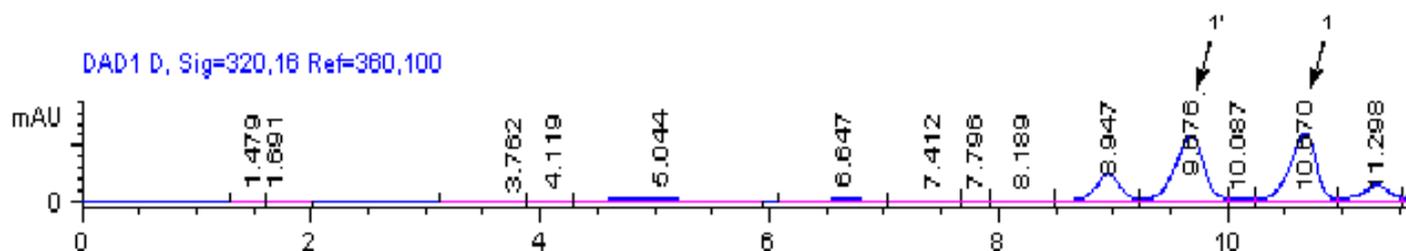
HPLC chromatogram of the crude of reaction from 2 to 6a and 6b.

Conditions: *Column:* Eclipse® XDB-C18 (150x4.6 mm, 5 µm)
Mobile phase: isocratic 60% CH₃CN and 40% H₂O (v/v)
Flow rate: 1.0 mL/min.
UV detector: 210 nm



HPLC chromatogram of formation's reaction of diastereoisomers 1 and 1' (crude) without ZnCl₂.

Conditions: *Column:* Discovery® HS F5 (100x4.6 mm, 5 µm)
Mobile phase: from 0 to 10 min., gradient from 10% to 30% CH₃CN in H₂O; from 10 to 13 min. isocratic 30% CH₃CN and 70% H₂O; from 13 to 15 min. gradient from 30% to 100% CH₃CN in H₂O (percentages are v/v, each containing 0.1% TFA).
Flow rate: 1.0 mL/min.
UV detector: 320 nm.



HPLC chromatogram of formation's reaction of diastereoisomers 1 and 1' (crude) with ZnCl₂..

Conditions: *Column:* Discovery® HS F5 (100x4.6 mm, 5 µm)

Mobile phase: from 0 to 10 min., gradient from 10% to 30% CH₃CN in H₂O; from 10 to 13 min. isocratic 30% CH₃CN and 70% H₂O; from 13 to 15 min. gradient from 30% to 100% CH₃CN in H₂O (percentages are v/v, each containing 0.1% TFA).

Flow rate: 1.0 mL/min.

UV detector: 320 nm.

