

Supporting Information

Unprecedented biological cyclopropanation in the biosynthesis of FR-900848

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General

Unless otherwise noted, nonaqueous reactions were carried out under an argon atmosphere. Dichloromethane (CH₂Cl₂), *N,N*-dimethylformamide (DMF) and tetrahydrofuran (THF) were dried over 4A molecular sieves. [²H₄]methanol (99.8 atom% ²H) and ²H₂O (99.9 atom% ²H) were purchased from the Cambridge Isotope Lab. All other commercially supplied reagents were used as received. Optical rotations were recorded on a JASCO DIP-360 digital polarimeter. NMR spectra were obtained on a JEOL ECP-300, ECP-400, Alpha-400 and a Bruker AM-500 spectrometer for solutions of CDCl₃. ¹H and ¹³C chemical shifts were referenced to the solvent signals (7.26 and 77.0 ppm). Infrared (IR) spectra were obtained on a Hitachi 270-30 infrared spectrometer. High-resolution mass spectra (HR-MS) were obtained on a JEOL JMS-T100LP mass spectrometer (ESI).

[4,4,4-²H₃]-1-(*tert*-butyldiphenylsilyloxy)-2-butyne (**10**).

To a solution of 1-(*tert*-Butyldiphenylsilyloxy)-2-propyne (**9**, 4.02 g, 13.7 mmol) in THF (22 ml) was added dropwise a solution of BuLi (9.6 ml of a 1.56M solution in hexane, 15.0 mmol) at -78 °C. After stirring at 0 °C for 1 h, a solution of C²H₃OTs (prepared from [²H₄]methanol and TsCl, 3.36 g, 17.8 mmol) in THF (5.0 ml) was added. The resulting solution was stirred at 25 °C for 10 h. Then satd. NH₄Cl (40 ml) was added, and the mixture was extracted with Et₂O. The organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (SiO₂, hexane/EtOAc=100:1–50:1) to give **10** (4.06 g, 95%). **10**: a colorless oil; ¹H-NMR (300 MHz, CDCl₃) δ 7.74-7.71 (4H, m), 7.44-7.26 (6H, m), 4.28 (2H, s), 1.07 (9H, s); ¹³C-NMR (75 MHz, CDCl₃) δ 135.6, 133.3, 129.7, 127.6, 81.1, 77.5, 52.9, 26.7, 19.1; IR (film) 3072, 2932, 2856, 1474, 1430, 1376, 1178, 1112, 998, 824, 738, 702, 612 cm⁻¹; EI-HR-MS (positive) calcd for C₁₆H₁₂D₃OSi [M-*t*Bu] 254.1077, found *m/z* 254.1078.

Thioester **14**.

To a solution of **10** (2.56 g, 8.20 mmol) in THF (40 ml) was added HF-pyridine (64 μl, 3.5 mmol). After stirring at 25 °C for 3 h, satd. NaHCO₃ (70 ml) was added. The resulting mixture was extracted with Et₂O. The organic layers were dried over anhydrous Na₂SO₄, and concentrated *in vacuo*. The residue was purified by silica gel column

chromatography (pentane/Et₂O =100/1–1/2) to give [4,4,4-²H₃]-2-butyne-1-ol containing organic solvents. To a solution of [4,4,4-²H₃]-2-butyne-1-ol and NaOMe (650 mg, 12.1 mmol) in THF (30 ml) at -10 °C was added and LiAlH₄ (844 mg, 22.3 mmol) in portions. The resulting mixture was heated under reflux for 8 h. The reaction mixture was cooled to -10 °C, and quenched with water (5 ml). After 2M NaOH (2.5 ml) and water (2.5 ml) were added, the resulting mixture was stirred at 25 °C for 1 h. The mixture was filtered through celite, dried over anhydrous Na₂SO₄, and concentrated *in vacuo* to give **11** which was used in the next reaction without further purification. **11**: a yellow oil; ¹H-NMR (300 MHz, CDCl₃) δ 5.64 (2H, m), 4.08 (2H, br).

CH₂I₂ (1.34 ml, 16.3 mmol) was added slowly to a stirred solution of Et₂Zn (1.0M in hexane, 16.3 ml, 16.3 mmol) in CH₂Cl₂ (23 ml) at -78 °C. After a white slurry was formed, a solution of **11** and **12** (998 mg, 3.68 mmol) in CH₂Cl₂ (9 ml) was added dropwise. The reaction mixture was stirred at 0 °C for 2 h, and quenched with satd. NH₄Cl (15 ml). The resulting mixture was extracted with Et₂O (40 ml x 3). To the organic layers was added 5M KOH (70 ml), and the mixture was stirred for 10 h. The organic layer was washed with 5% HCl, satd. NaHCO₃, and brine, dried over anhydrous Na₂SO₄, and concentrated *in vacuo* to give **13** which contained organic solvents and was used in the next reaction without further purification. **13**: a colorless oil; ¹H-NMR (300 MHz, CDCl₃) δ 3.50-3.36 (2H, m), 0.88-0.78 (1H, m), 0.65-0.59 (1H, m), 0.39-0.33 (1H, m), 0.30-0.24 (1H, m).

To a solution of **13** in CCl₄-H₂O-CH₃CN (2/2/3, 7 ml) were added RuCl₃ (4.3 mg, 0.021 mmol) and NaIO₄ (920 mg, 4.31 mmol). After stirring at 25 °C for 1.5 h, the reaction mixture was filtered through celite. The filtrate was extracted with CH₂Cl₂ (20 ml x 3), and the organic layers were extracted with satd. NaHCO₃ (60 ml x 4). The aqueous layers acidified with 2M HCl were extracted with CH₂Cl₂. The organic layer was dried over anhydrous Na₂SO₄, and concentrated *in vacuo* to give a carboxylic acid. To a solution of the carboxylic acid and *N*-acetyl cysteamine (86 mg, 0.72 mmol) in CH₂Cl₂ (1.5 ml) was added DCC (89 mg, 0.43 mmol), Ph₃P (38 mg, 0.14 mmol), and DMAP (1 crystal). The resulting mixture was stirred at 0 °C for 3 h, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO₂, CH₂Cl₂/MeOH=100:0–99:1) to give **14** (52.9 mg, 3.0% in 5 steps). **14**: a colorless oil; [α]_D²⁵ -54 (*c* 0.37, CHCl₃); ¹H-NMR (300 MHz, CDCl₃) δ 6.30-5.70 (1H, br), 3.42 (2H, q, *J* = 6.1 Hz), 3.02 (2H, t, *J* = 6.3 Hz), 1.96 (3H, s), 1.82-1.68 (1H, m), 1.59-1.47 (1H, br), 1.40-1.29 (1H, m), 0.87-0.77 (1H, m); ¹³C-NMR (75 MHz, CDCl₃) δ 199.0, 170.3, 39.7, 31.1, 28.4, 23.0, 20.0, 19.4; ESI-HR-MS (positive) calcd for C₉H₁₂D₃NO₂SNa (M+Na) 227.0906, found *m/z* 227.0930.

Thioester *ent*-14.

Following the procedure just described for **14**, *ent*-**14** was derived from **10** in a 2.8% yield (5 steps). *ent*-**14**: a colorless oil; ESI-HR-MS (positive) calcd for C₉H₁₂D₃NSO₂Na (M+Na) 227.0906, found *m/z* 227.0880.

Thioester 16.

To a solution of [4,4,4-²H₃]-2-butyne-1-ol prepared from **10** (3.50 g, 11.2 mmol) in THF (39 ml) at -10 °C were added NaOMe (1.06 g, 19.6 mmol) and LiAlH₄ (602 mg, 15.9 mmol). The resulting mixture was heated under reflux for

8 h. The reaction mixture was cooled to -10 °C, and quenched with ²H₂O (620 mg). After 2M NaOH (1.8 ml) and water (1.8 ml) were added, the resulting mixture was stirred at 25 °C for 1 h. The mixture was filtered through celite, dried over anhydrous Na₂SO₄, and concentrated *in vacuo* to give a mixture of **15** and **15'** which contained THF and was used in the next reaction without further purification. The mixture of **15** and **15'**: a yellow oil; ¹H-NMR (400 MHz, CDCl₃) δ 5.64 (1H, m), 4.08 (2H, br).

Using the procedure just described for **14**, a mixture of **16** and **16'** was obtained in a 5.5% yield (5 steps). The mixture of **16** and **16'**: a colorless oil; [α]_D²⁵ -53 (c 0.22, CHCl₃); ¹H-NMR (400 MHz, CDCl₃) δ 4.8 (1H, br, NH), 3.42 (2H, q, *J* = 6.2 Hz), 3.02 (2H, t, *J* = 6.2 Hz), 1.96 (3H, s), 1.82-1.68 (0.72H, m), 1.59-1.47 (0.42H, br), 1.40-1.29 (1H, m), 0.87-0.77 (1H, m); ¹³C-NMR (100 MHz, CDCl₃) δ 199.4, 170.2, 39.8, 31.1, 28.5, 23.1, 20.1, 19.5; ESI-HR-MS (positive) calcd for C₉H₁₂D₄NO₂SNa (M+Na) 228.0968, found *m/z* 228.0974.

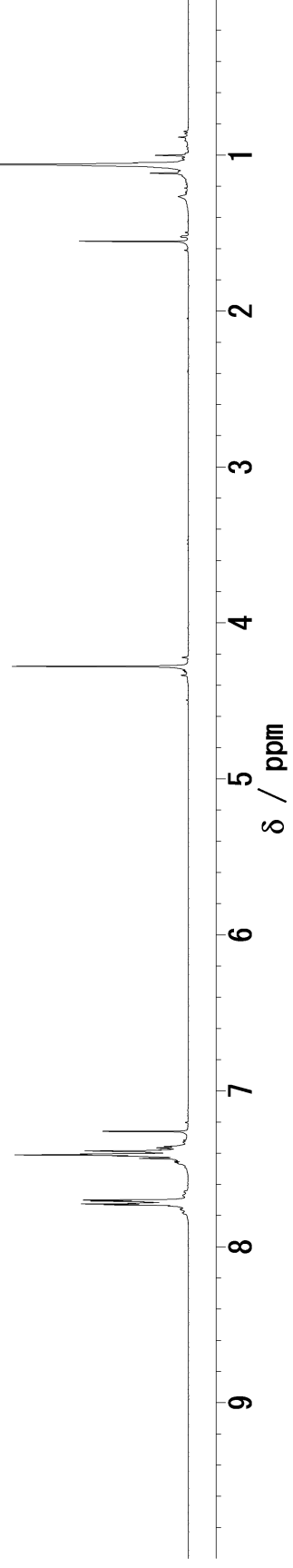
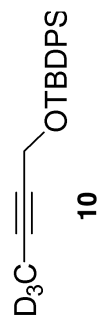
MTPA esters of **13**.

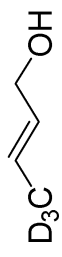
To a 0.3M solution of **13** in CH₂Cl₂ were added (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (2 eq), DCC (2 eq), and DMAP (1 crystal). After stirring at 25 °C for 8 h, the resulting mixture was filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (SiO₂, Hexane/EtOAc=5:1) to give the corresponding MTPA ester. Using this procedure, (*S*)-MTPA ester of *ent*-**13** and (*R*)-MTPA ester **17** were obtained. (*R*)-MTPA ester of **13**: colorless oil; ¹H-NMR (300 MHz, CDCl₃) δ 7.57-7.50 (2H, m), 7.44-7.38 (3H, m) 4.22 (1H, dd, *J* = 7.2, 11.3 Hz), 4.10 (1H, dd, *J* = 7.8, 11.3 Hz), 3.58 (3H, s), 0.98-0.87 (1H, m), 0.75 (1H, br), 0.47 (1H, dt, *J* = 4.7, 8.4 Hz), 0.42 (1H, dt, *J* = 5.1, 8.4 Hz).

Feeding experiments with cyclopropanated diketide precursors.

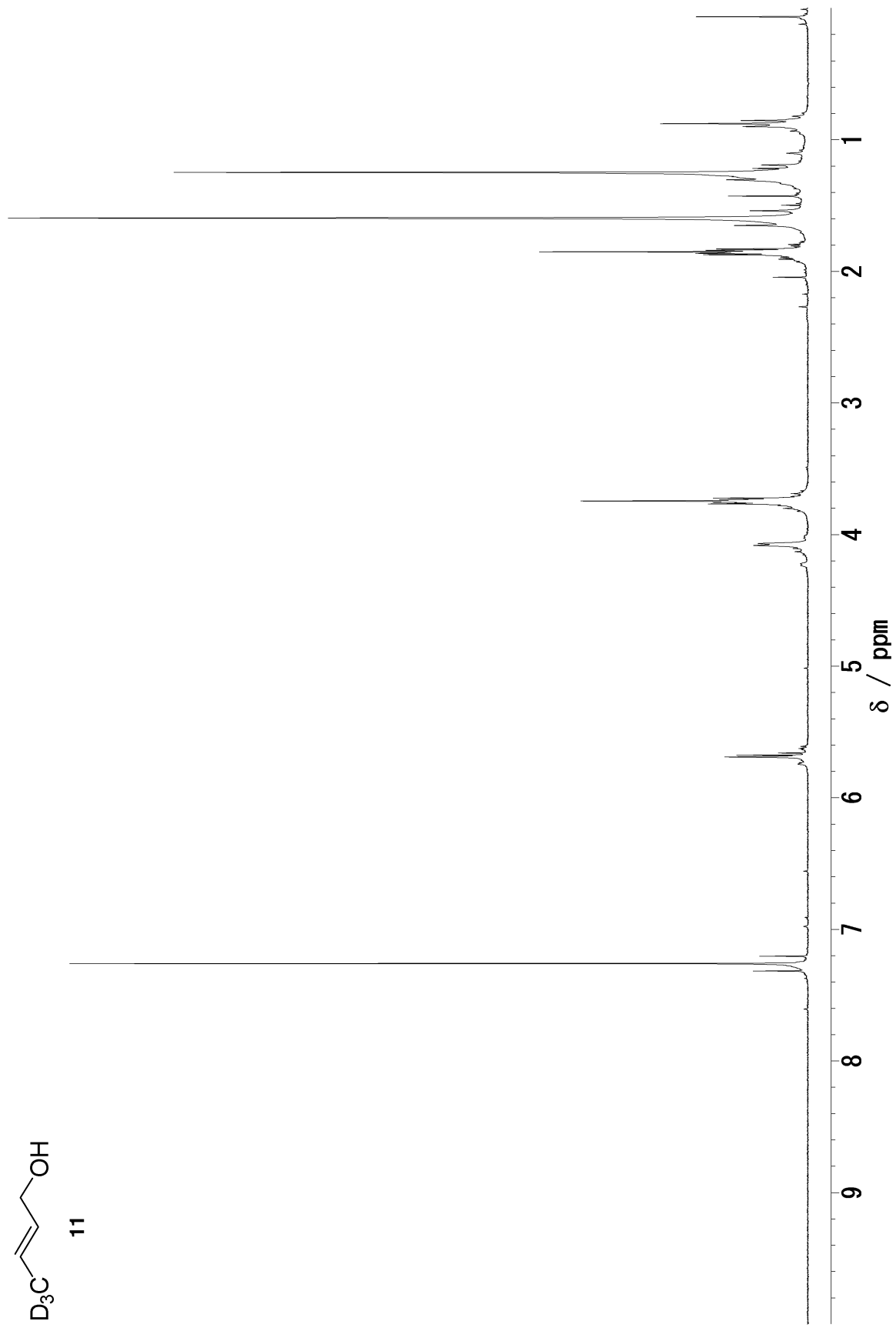
Culture medium and growth conditions for *Streptoverticillium fervens* HP-891 were as described by Yoshida *et al.*¹ and our previous reports⁵. On the fourth day after inoculation, the sterilized ethanol solution of diketide precursors **14**, *ent*-**14** or **16+16'** (5 mg per one flask containing 100 ml of a fermentation medium) was added to the cultures. After further incubation for 5 days, the mycelia were extracted with acetone. The acetone extracts were partitioned between acetonitrile and hexane, and the acetonitrile layer was concentrated *in vacuo*. The resulting residue was treated with acetic anhydride, pyridine, and 4-dimethylaminopyridine at 25 °C for 10 h. The product was purified by silica gel column chromatography twice (CHCl₃/MeOH=100/0-99/1 and toluene/MeOH=99/1-97/3) and reverse-phase HPLC (Inertsil ODS-2, φ10 x 250 mm, GL Science; eluent, CH₃CN/H₂O linear gradient from 5% to 100% CH₃CN; flow rate, 0.5 ml min⁻¹; UV 280 nm) affording FR-900848 diacetate **2** (1.5-3 mg/100 ml). The ¹³C NMR data and NMR spectra were previously reported.⁵ **2**: a yellow oil; ¹H-NMR (400 MHz, CDCl₃) δ 7.88 (1H, s, CO-NH-CO), 7.15 (1H, dd, *J* = 14.8, 11.1 Hz, H3), 6.25-6.21 (1H, m, CO-NH-CH₂), 6.14 (1H, dd, *J* = 14.9, 11.1 Hz, H4), 5.72 (1H, d, *J* = 14.8 Hz, H2), 5.66 (1H, d, *J* = 5.8 Hz, H1''), 5.58 (1H, dd, *J* = 14.9, 9.4 Hz, H5), 5.34 (1H, t, *J* = 5.8 Hz, H2''), 5.14 (1H, dd, *J* = 5.8, 5.4 Hz, H3''), 5.02-4.98 (2H, m, H14, 15), 4.12 (1H, dt, *J* = 5.4, 4.9 Hz, H4''), 3.72-3.62 (1H, m, H5''), 3.62-3.54 (1H, m, H5''), 3.51 (2H, t, *J* = 6.7 Hz, H6'), 2.70 (2H, t, *J* = 6.7 Hz, H5'), 2.10 (3H, s, Ac), 2.08 (3H, s, Ac), 1.26-1.16

(1H, m, H6), 1.05-1.00 (1H, m, H13), 1.02 (3H, d, $J = 6.1$ Hz, H18), 1.01-0.93 (2H, m, H7, 16), 0.80-0.72 (1H, m, H12), 0.71-0.63 (1H, m, H17), 0.63-0.51 (6H, m, H8, 9, 10, 11, 19), 0.49-0.42 (1H, m, H23), 0.41-0.36 (1H, m, H23), 0.36-0.31 (2H, m, H22), 0.12-0.01 (4H, m, H20, 21); IR (film) 3304 (br), 3064, 2998, 2926, 2374, 1749, 1707, 1557, 1443, 1377, 1242, 1218 cm^{-1} ; ESI-HR-MS (positive) calcd for $\text{C}_{36}\text{H}_{47}\text{N}_3\text{O}_8\text{Na}$ (M+Na) 672.3261, found m/z 672.3257.



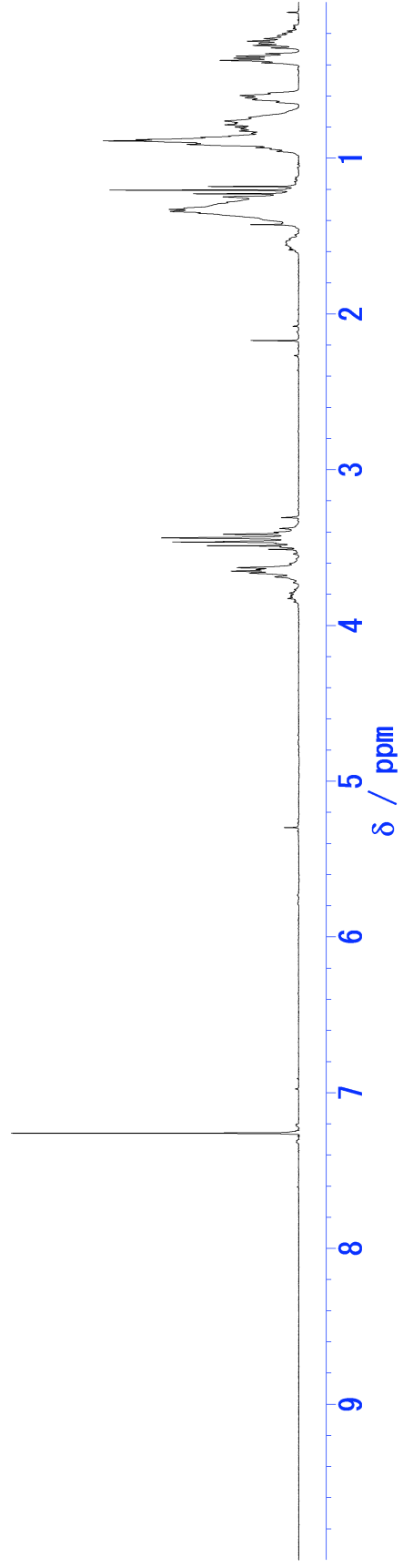


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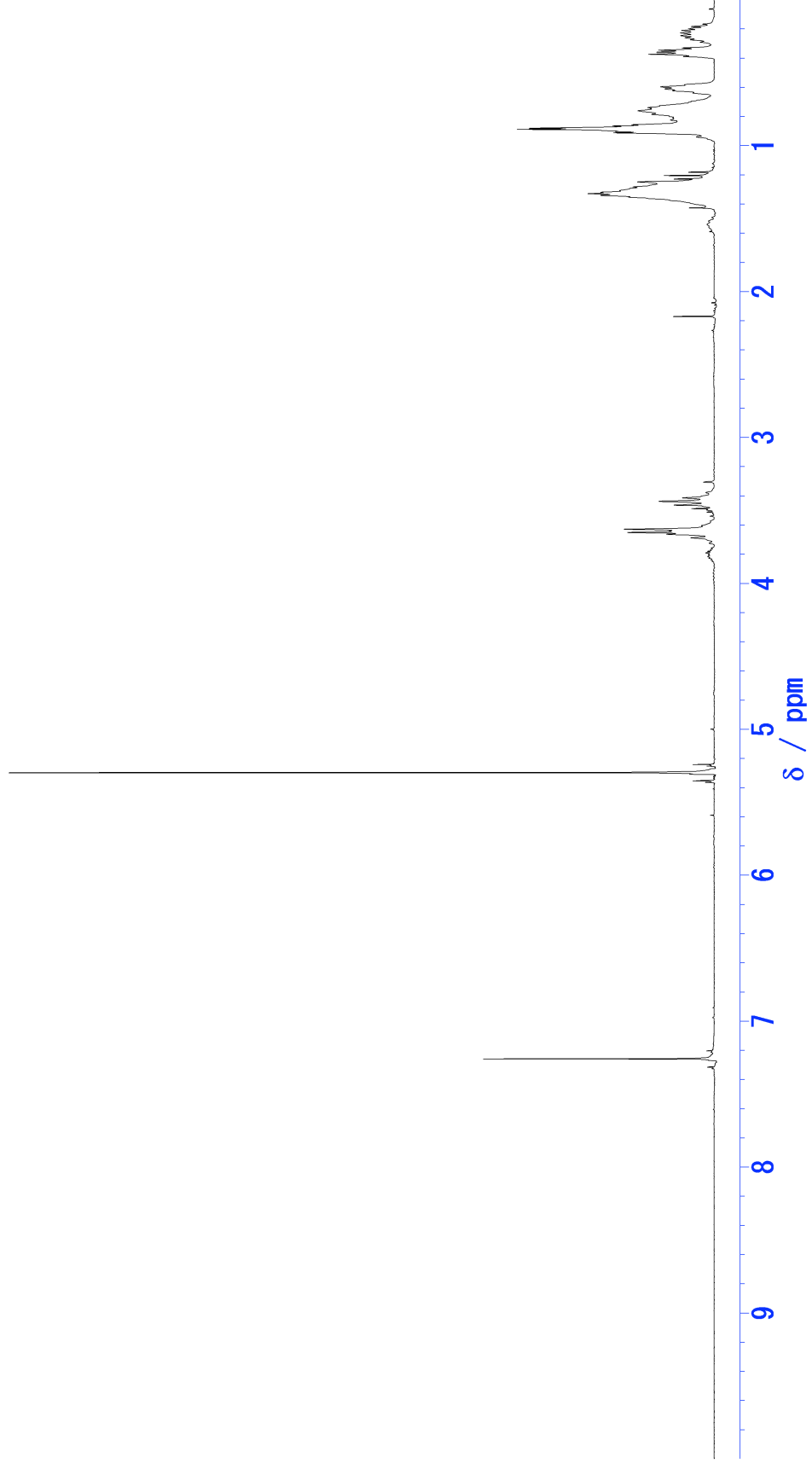


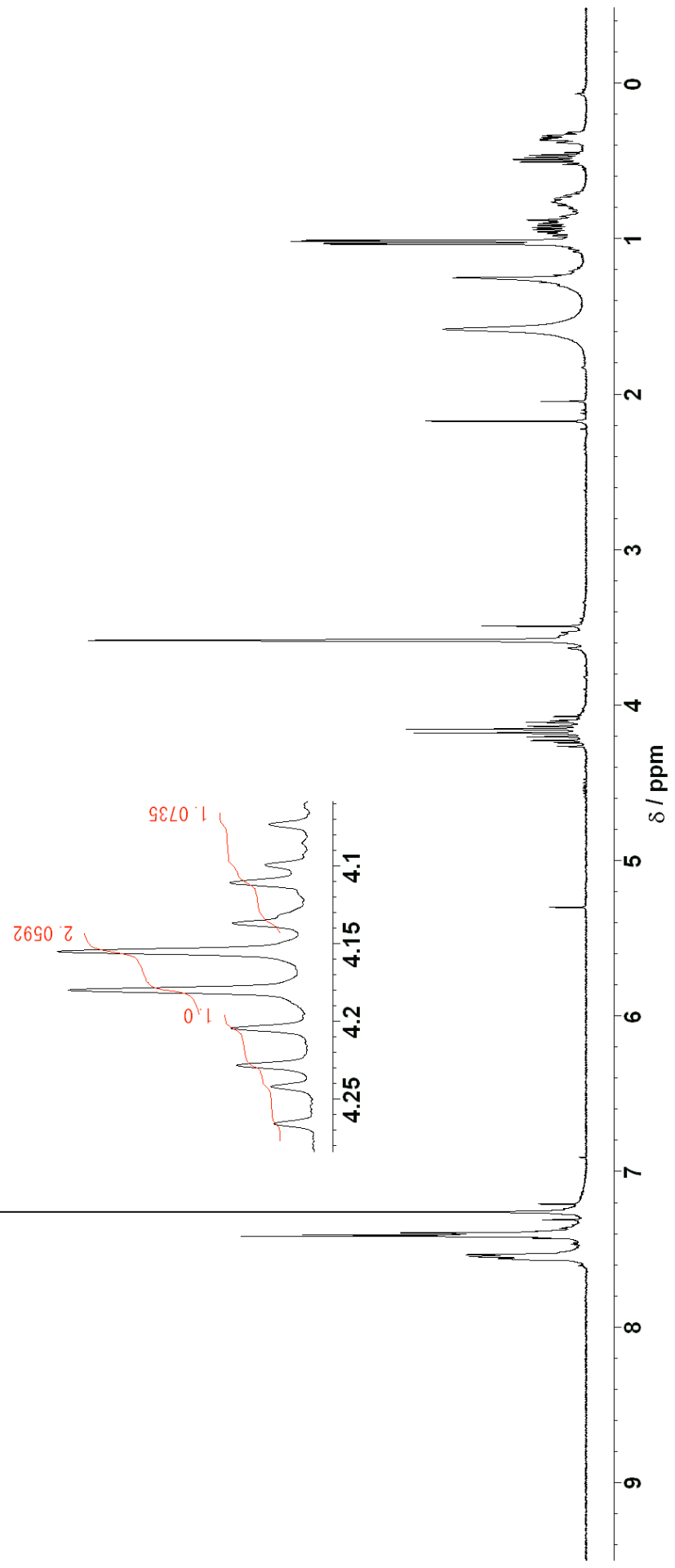
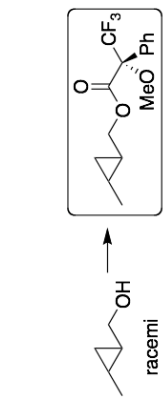
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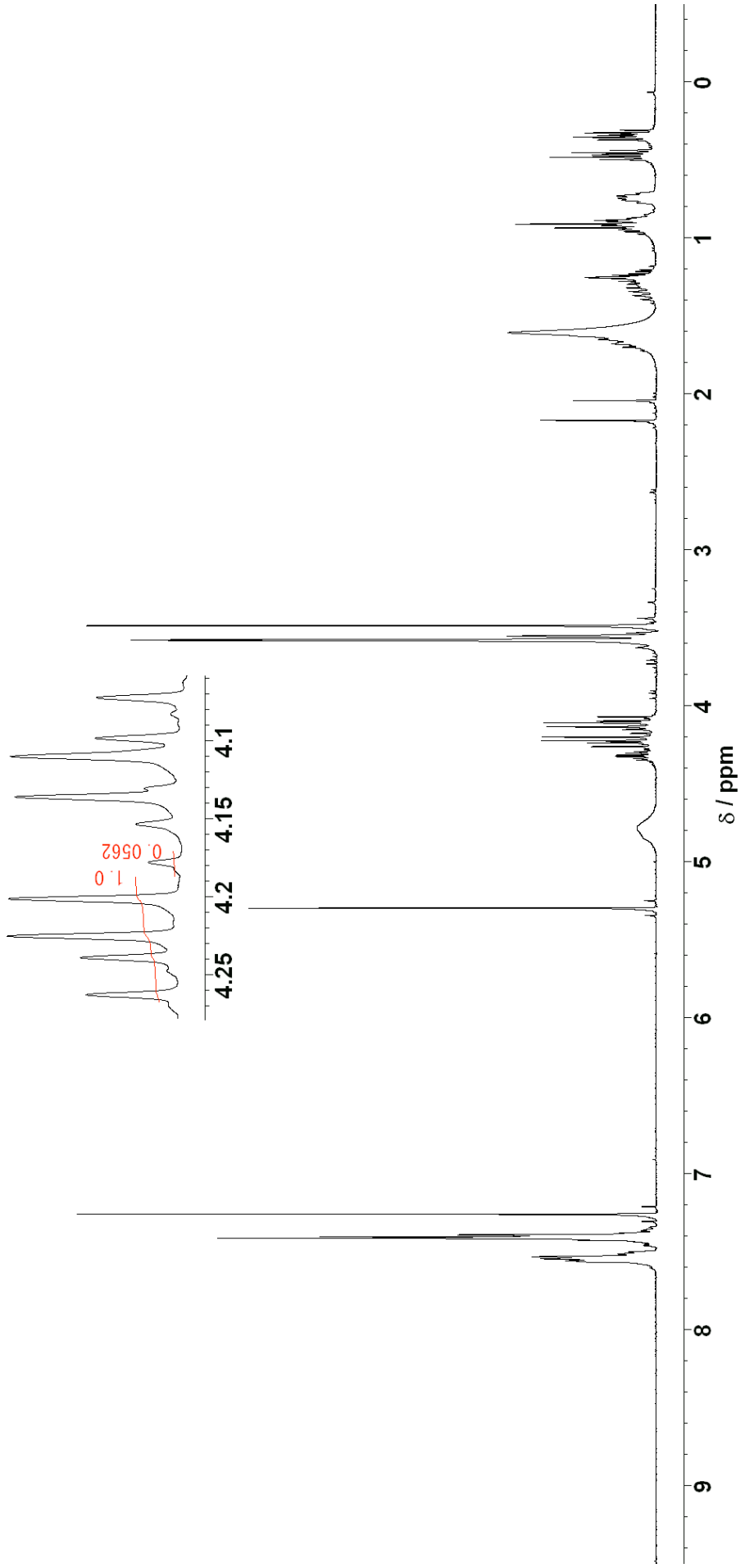
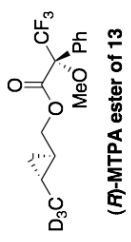


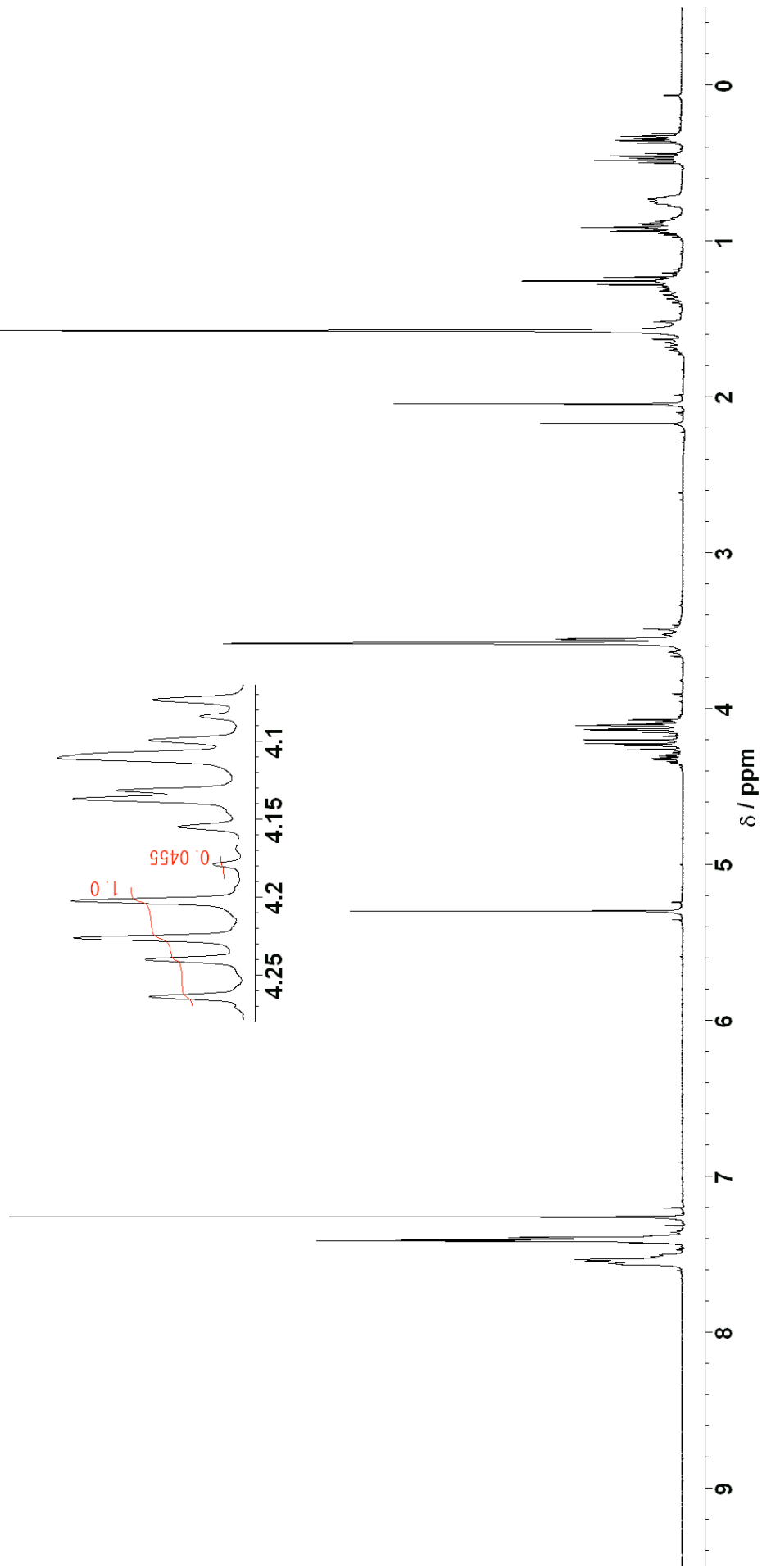
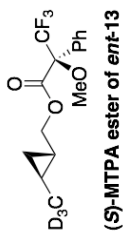


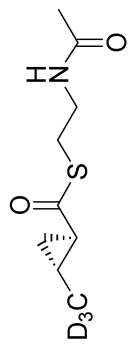
ent-13



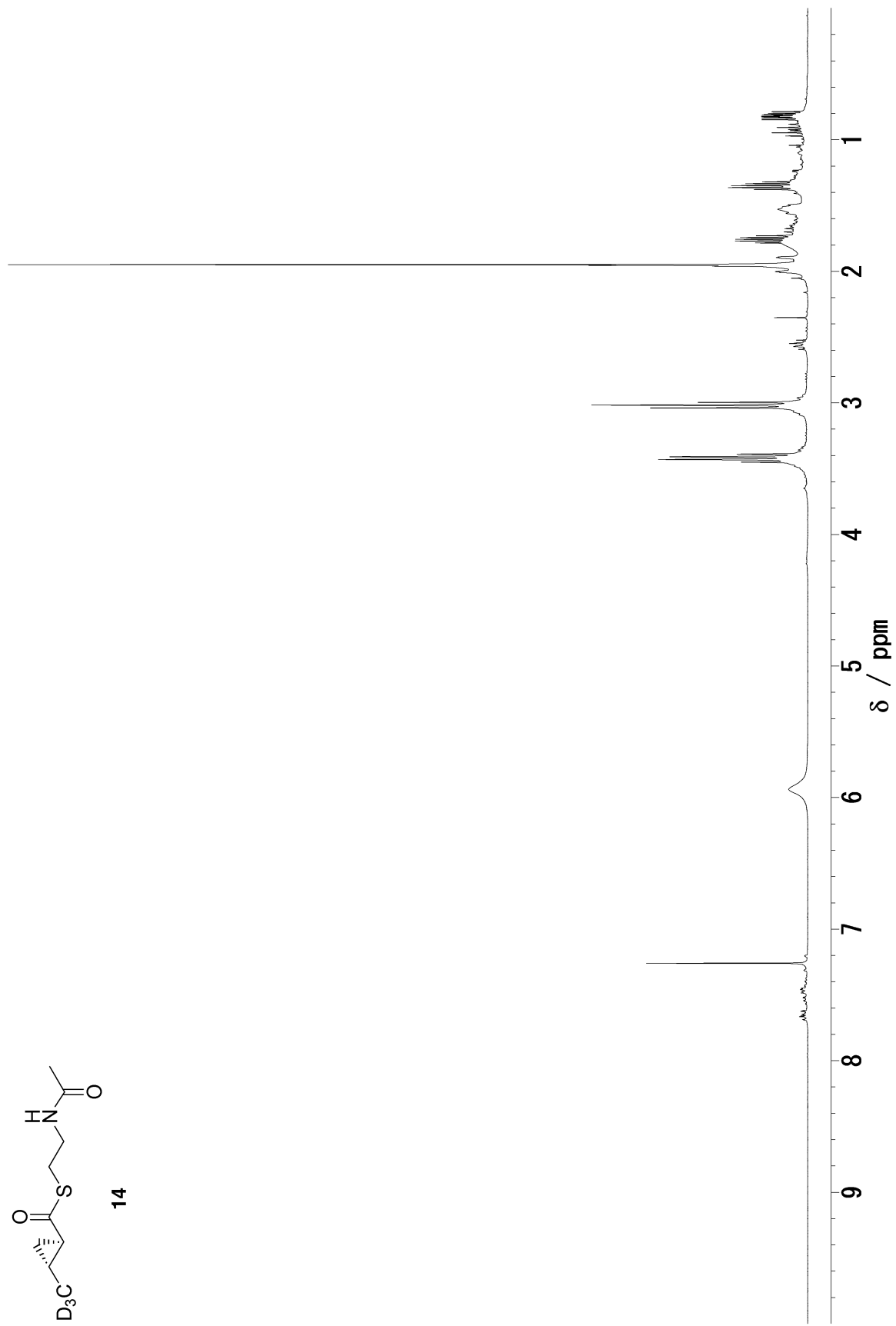


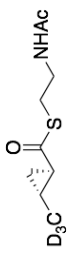




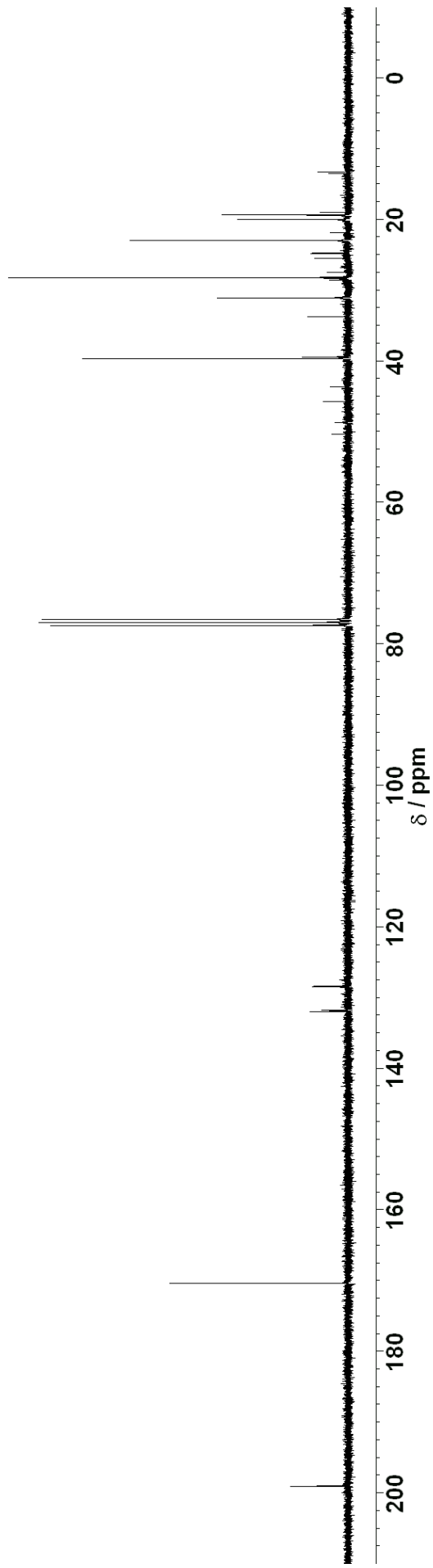


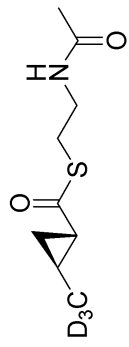
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ent-14

