

Absolute Configuration and Total Synthesis of a Novel Antimalarial Lipopeptide by the De Novo Preparation of Chiral Nonproteinogenic Amino Acids

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SUPPORTING INFORMATION

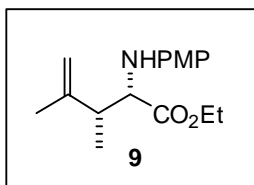
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1. General Procedures and Methods

Unless stated otherwise, all non-aqueous reactions were performed in flame-dried round bottom flasks under an inert argon atmosphere with freshly distilled dry solvents under anhydrous conditions. Tetrahydrofuran (THF) was distilled over sodium/benzophenone. Dichloromethane (CH_2Cl_2) was distilled over CaH_2 . Commercial reagents were purchased from Sigma Aldrich, Fluka, Merck, Alfa Aesar or Strem Chemicals, and used as received without further purification. 4Å molecular sieves were activated by heating in a furnace at 300 °C for 20 h before storing in a dry desiccator, which would be heated at 200 °C under high vacuum for 15-20 min immediately prior to use. Yields refer to chromatographically and spectroscopically homogeneous materials, unless otherwise stated. Reaction progress was monitored by analytical thin layer chromatography (TLC) with 0.25 mm E. Merck pre-coated silica gel plates (60F-254) using UV light (254 nm) as visualizing agent, and ceric ammonium molybdate, KMnO_4 or ninhydrin as developing stains. Flash chromatography was performed on silica gel 60 (0.040 – 0.063 mm) purchased from SiliCycle or ACME Research Support. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AMX500 (500 MHz) and Bruker ACF300 (300 MHz) NMR spectrometer at ambient atmosphere. 2D NMR was performed on Bruker AMX500 (500 MHz) NMR spectrometer. The deuterated solvents used were CDCl_3 and CD_3OD . Chemical shifts are reported in parts per million (ppm), and residual undeuterated solvent peaks were used as internal reference: proton (δ 7.26), carbon (δ 77.0) for CDCl_3 and proton (δ 3.31), carbon (δ 49.0) for CD_3OD . ^1H NMR coupling constants (J) are reported in Hertz (Hz), and multiplicities are presented as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublet), and br (broad). Low resolution mass spectra were obtained on a Finnigan/MAT LCQ spectrometer in ESI mode. High resolution ESI mass spectra were obtained on a Bruker micrOTOF-Q II. Shimadzu LCMS-IT-TOF spectrometer was used for comparing retention time. Mass samples were dissolved in CH_3OH (HPLC Grade), unless otherwise stated. Samples for infra-red measurements were prepared as thin films neat or in CH_2Cl_2 solution spread on NaCl cells, and spectra were recorded on a IRPrestige-21 Shimadzu FTIR spectrometer. Optical rotations were recorded on a Jasco DIP-1000 polarimeter with a sodium lamp of wavelength 589 nm. Enantiomeric excess was determined by chiral-phase HPLC analysis on Shimadzu LC-10AT using the indicated chiral column.

2. Experimental Procedures and Characterization Data

(2S,3R)-ethyl 2-(4-methoxyphenylamino)-3,4-dimethylpent-4-enoate (9): To a solution of

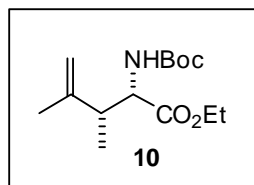


compound **8** (565 mg, 2.02 mmol) in dry THF (10 mL) was added Tebbe reagent (4.45 mL, 2.2 mmol) slowly at -78 °C and the reaction mixture was stirred at that temperature for 2 h and then slowly warmed up to room temperature and allowed to stirred for 12 h. THF (5 mL) was added to the reaction mixture and then 2.5 mL of 5% NaOH solution was added at -15 °C. After stirring for 10 min., it was warmed up to room temperature and filtered through short silica plug and washed with Et_2O . The collected

filtrate was extracted with Et_2O and the organic layer was washed with brine and dried over Na_2SO_4 . The solvent was removed under reduced pressure to get the crude material which was carefully purified over silica gel (7.5% EtOAc /hexane) to afford compound **9** (252 mg, 45%) as a yellow oil.

R_f = 0.5 (silica, 15% EtOAc /hexane); $[\alpha]_D^{25}$ = -90.2 (c = 2.0, CHCl_3); IR (thin film) ν_{max} = 3373, 2979, 2832, 1729, 1516, 1369, 1241, 1182, 1036, 895, 821 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ 6.77-6.74 (2H, d, J = 8.9 Hz), 6.61-6.59 (2H, d, J = 8.9 Hz), 4.82 (2H, s), 4.14-4.07 (2H, m), 3.92-3.90 (1H, d, J = 7.56 Hz), 3.77-3.73 (4H, m), 2.63-2.60 (1H, m), 1.78 (3H, s), 1.22-1.18 (6H, m); ^{13}C NMR (CDCl_3 , 125 MHz) δ 173.71, 152.78, 145.94, 141.41, 115.29, 114.83, 112.84, 61.69, 60.67, 55.70, 44.70, 19.52, 15.43, 14.18; HRMS (ESI): m/z calcd for $\text{C}_{16}\text{H}_{23}\text{O}_3\text{NNa}^+$ $[\text{M}+\text{Na}]^+$ 300.1570, found 300.1579.

(2S,3R)-ethyl 2-(tert-butoxycarbonylamino)-3,4-dimethylpent-4-enoate (10): To a solution of ceric

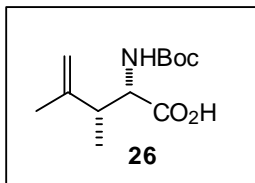


ammonium nitrate (CAN) (643.3 mg, 0.47 mmol) in H_2O (10 mL) was added compound **9** (130 mg, 1.2 mmol) in acetonitrile (5 mL) dropwise at 0 °C and the reaction mixture was stirred at that temperature for 45 min. The reaction mixture was diluted with Et_2O and 1M HCl (5 mL) was added and it was extracted. The aqueous layer was basified with sat. NaHCO_3 and extracted with EtOAc . The organic layer was washed with brine and dried over Na_2SO_4 . The solvent was removed under reduced pressure to get the crude material which was dissolved in Dioxane/ H_2O (3.0 mL/1.0 mL). To this soln. were added Et_3N

(68 μ L, 0.5 mmol) and (Boc)₂O (142 μ L, 0.62 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was extracted with EtOAc and washed with brine, dried over Na₂SO₄. The solvent was removed under reduced pressure to get the crude material which was purified over silica gel (7.5% EtOAc/hexane) to afford compound **10** (80 mg, 63%) as a colourless oil.

R_f = 0.6 (silica, 15% EtOAc/hexane); $[\alpha]_D^{25}$ = +16.2 (c = 1.25, CHCl₃); IR (thin film) ν_{\max} = 3360, 2979, 2935, 1720, 1504, 1367, 1344, 1249, 1165, 1027 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 4.98-4.89 (1H, br), 4.81-4.75 (2H), 4.40-4.37 (1H, m), 4.20-4.14 (2H, m), 2.58-2.55 (1H, m), 1.78 (3H, s), 1.43 (9H, s), 1.26 (3H, t, J = 7.6 Hz), 1.06-1.04 (3H, d, J = 6.95 Hz); ¹³C NMR (CDCl₃, 125 MHz) δ 172.29, 155.49, 145.56, 112.46, 79.75, 61.05, 56.18, 43.87, 28.29, 20.13, 14.41, 14.16; HRMS (ESI): m/z calcd for C₁₄H₂₅O₄NNa⁺ [M+Na]⁺ 294.1676, found 294.1672.

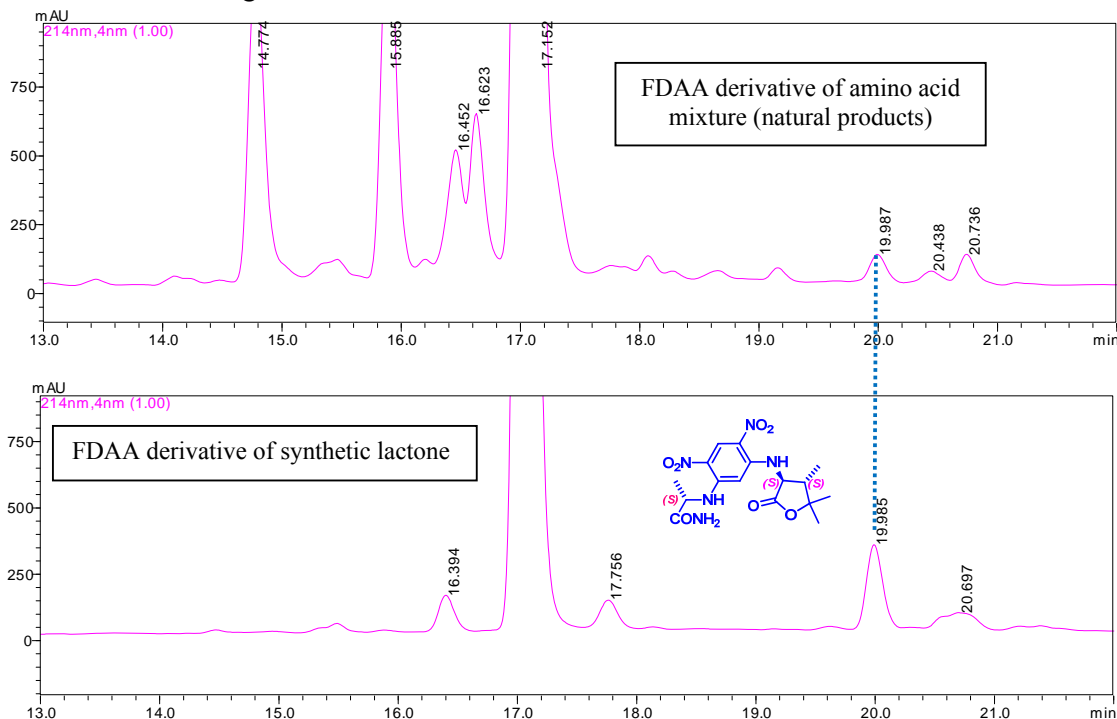
(2S,3R)-2-(tert-butoxycarbonylamino)-3,4-dimethylpent-4-enoic acid (26): To a solution of Compound **10** (110 mg, 0.41 mmol) in THF/H₂O/MeOH (2 mL/2 mL/0.4 mL) was added LiOH (11 mg, 0.45 mmol) at 0 °C and the reaction mixture was allowed to stir for 2 h at that temperature. After completion of the reaction (monitored by TLC), the reaction mixture was extracted with Et₂O. The aqueous layer was acidified with 10% NaHSO₄ to pH 2-3 and then extracted with EtOAc, washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure to afford compound **26** (102



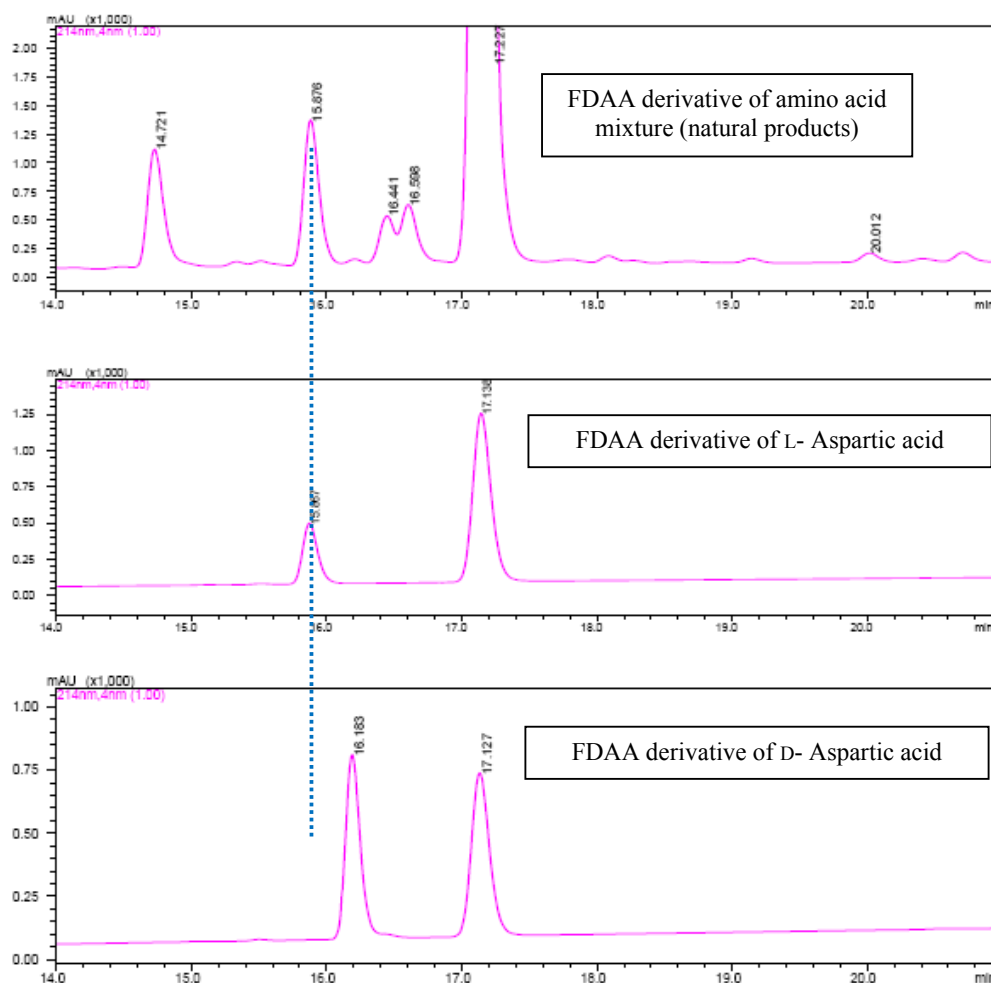
mg, 85%) as a colourless oil.

R_f = 0.2 (silica, 5% CH₃OH/CH₂Cl₂); $[\alpha]_D^{25}$ = +17.8 (c = 0.64, CHCl₃); IR (thin film) ν_{\max} = 3324, 2977, 2363, 1734, 1507, 1163 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 4.98-4.97 (1H, br), 4.85-4.78 (2H), 4.45 (1H, s), 2.65-2.64 (1H, br), 1.78 (3H, s), 1.42 (9H, s), 1.07-1.06 (3H, d, J = 6.95 Hz); ¹³C NMR (CDCl₃, 125 MHz) δ 176.91, 155.67, 145.31, 112.52, 80.07, 55.99, 42.94, 28.24, 20.57, 13.99; HRMS (ESI): m/z calcd for C₁₂H₂₀O₄N [M-H]⁻ 242.1398, found 242.1398.

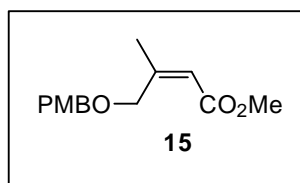
LCMS chromatogram:



LCMS chromatogram for determining aspartic acid:

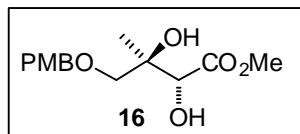


(Z)-methyl 4-(4-methoxybenzyloxy)-3-methylbut-2-enoate (15): To the suspension of CuI (640 mg, 3.36 mmol) in dry THF (10 mL) was added MeLi (3.35 mL, 6.70 mmol) at 0 °C and the reaction mixture was allowed to stir for 30 min. at that temperature. To this colourless soln. was added compound **14** (555 mg, 2.24 mmol) dropwise at -78 °C and stirred at that temperature for 4 h. To this reaction mixture was added 16 mL sat. NH₄Cl soln dropwise and after complete addition it was kept at -78 °C for 10 min. The reaction mixture was then warmed up slowly to room temperature. The precipitate was filtered and the filtrate was extracted with Et₂O. The organic layer was washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure to get the crude material which was purified over silica gel (1.5% EtOAc/hexane) to obtain compound **15** (400 mg, 69%, exclusive Z-isomer) as a colourless oil.



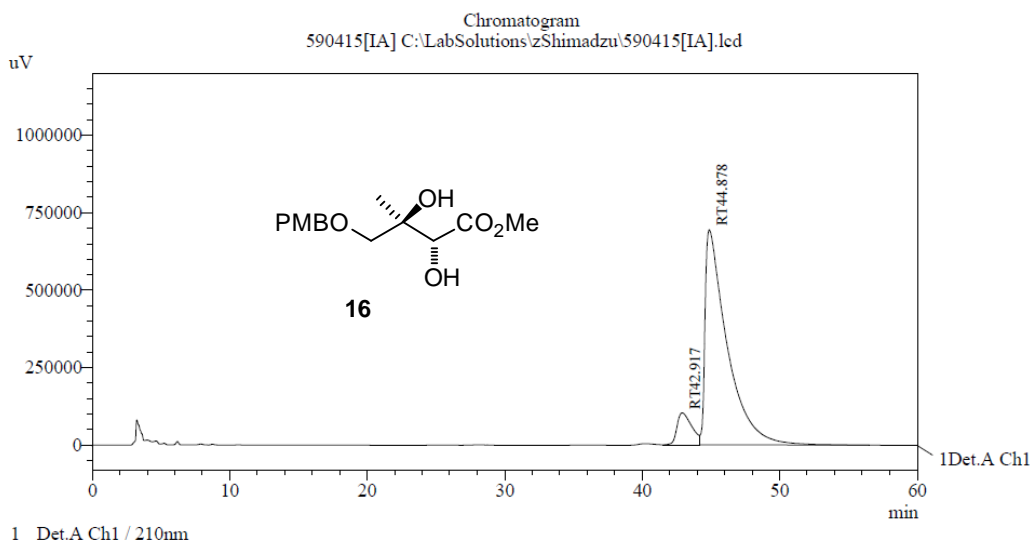
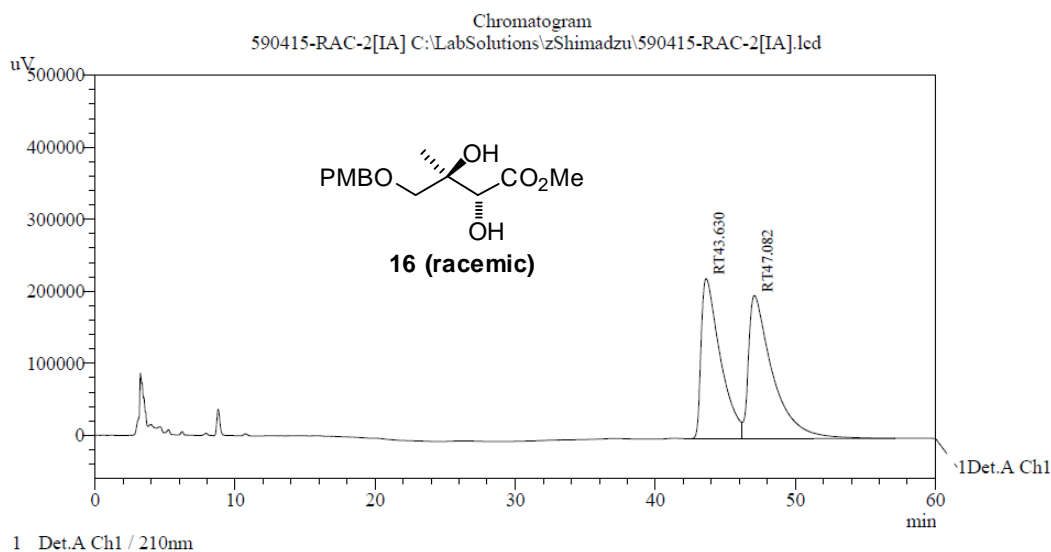
$R_f = 0.7$ (silica, 5% EtOAc/hexane); IR (thin film) $\nu_{\max} = 2952, 2838, 1716, 1613, 1515, 1444, 1363, 1248, 1153, 1035, 1032, 820 \text{ cm}^{-1}$; ¹H NMR (CDCl₃, 500 MHz) δ 7.26-7.24 (2H, d, $J = 8.2$ Hz), 6.87-6.85 (2H, d, $J = 8.85$ Hz), 5.74 (1H, d, $J = 1.9$ Hz), 4.61 (2H, s), 4.43 (4H, s), 3.78 (3H, s), 3.66 (3H, s), 1.98 (3H, s); ¹³C NMR (CDCl₃, 125 MHz) δ 166.29, 156.18, 157.47, 130.32, 129.23, 116.70, 113.74, 72.35, 68.99, 55.20, 50.97, 21.69; HRMS (ESI): m/z calcd for C₁₄H₁₈O₄Na⁺ [M+Na]⁺ 273.1097, found 273.1106.

(2R,3R)-methyl 2,3-dihydroxy-4-(4-methoxybenzyloxy)-3-methylbutanoate (16) : To a solution of compound **15** (80 mg, 0.30 mmol) in ¹BuOH/H₂O (1:1, 8 mL) were added AD-mix α (420 mg, 1.4 gm/mmol) and methanesulfonamide (30 mg, 100 mg/mmol) at -1 °C and the reaction mixture was allowed to stir



for 96 h at that temperature. The reaction was quenched by addition of Na₂SO₃ (444 mg, 1.48 mg/mmol) and stirred for 1 h at room temperature until it became colourless. CH₂Cl₂ was used for extraction and the organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product which was purified over silica gel (20% EtOAc/hexane) to obtain compound **16** (75 mg, 85%) as a colourless oil. The enantiomeric excess was determined through HPLC analysis with Chiralpak-IA column (0.46 cm □x25 cm) using hexanes/2-propanol (90:10) at a flow rate of 1.0 mL/min; detection UV 210 nm; 82 % *ee*.

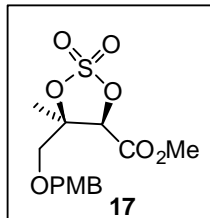
R_f = 0.3 (silica, 25% EtOAc/hexane); [α]_D²⁵ = -16.2 (*c* = 4.0, CHCl₃); IR (thin film) ν_{\max} = 3473, 2954, 2863, 1728, 1613, 1515, 1248, 1174, 1091, 1032 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 7.23 (2H, d, *J* = 8.85 Hz), 6.88 (2H, d, *J* = 8.85 Hz), 4.43 (2H, s), 4.1 (1H, s), 3.80 (3H, s), 3.7 (3H, s), 3.47 (1H, d, *J* = 9.45 Hz), 3.37 (1H, d, *J* = 8.85 Hz), 1.22 (3H, s); ¹³C NMR (CDCl₃, 125 MHz) δ 173.39, 159.35, 129.44, 113.8, 75.26, 74.31, 73.32, 73.18, 55.25, 52.39, 20.65; HRMS (ESI): *m/z* calcd for C₁₄H₂₀O₆Na⁺ [M+Na]⁺ 307.1152, found 307.1159.



PeakTable

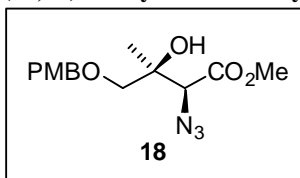
| Detector A Ch1 210nm | | | | | | |
|----------------------|----------|-----------|----------|--------|---------|-------|
| Peak# | Name | Ret. Time | Area | Height | Area % | Units |
| 1 | RT42.917 | 42.917 | 7642634 | 102964 | 8.989 | ppm |
| 2 | RT44.878 | 44.878 | 77378875 | 694312 | 91.011 | ppm |
| Total | | | 85021509 | 797275 | 100.000 | |

Cyclic sulfate (17): To a solution of compound **16** (835 mg, 2.94 mmol) in CH₂Cl₂ (20 mL) were added Et₃N (1.03 mL, 7.35 mmol) and SOCl₂ (540 μ L, 7.35 mmol) carefully at 0 °C and then the reaction mixture was allowed to stir for 30 min. at that temperature. After consumption of the starting material (checked by TLC), CH₂Cl₂ was evaporated in vacuo and the resulting cyclic sulphite was eluted with Et₂O through silica plug. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product which was dissolved in CH₃CN (14 mL). To this soln. were added RuCl₃ (2 mg), NaIO₄ (1.13 gm, 5.3 mmol), and H₂O (18 mL) at 0 °C and the reaction mixture was allowed to stir for 30 min. at that temperature. The reaction mixture was diluted with Et₂O and extracted. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product which was purified over silica gel (20% EtOAc/hexane) to afford compound **17** (800 mg, 79%) as a colourless oil.



R_f = 0.45 (silica, 20% EtOAc/hexane); $[\alpha]_D^{25}$ = +34.3 (c = 4.2, CHCl₃); IR (thin film) ν_{max} = 2957, 2875, 1766, 1742, 1612, 1516, 1440, 1418, 1302, 1249, 1215, 1176 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 7.21 (2H, d, J = 8.7 Hz), 6.88 (2H, d, J = 8.7 Hz), 4.99 (1H, s), 4.43 (2H, q), 3.8 (3H, s), 3.77 (1H, d, J = 10.35 Hz), 3.7 (3H, s), 3.59 (1H, d, J = 10.35 Hz), 1.75 (3H, s); ¹³C NMR (CDCl₃, 75 MHz) δ 163.65, 159.50, 129.38, 128.72, 113.85, 91.87, 81.56, 73.35, 69.89, 55.26, 53.02, 22.15; HRMS (ESI): m/z calcd for C₁₄H₁₈O₈Na⁺ [M+Na]⁺ 369.0615, found 369.0624.

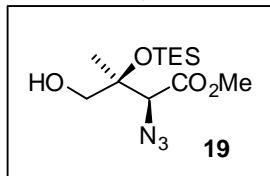
(2S,3S)-methyl 2-azido-3-hydroxy-4-(4-methoxybenzyloxy)-3-methylbutanoate (18): To a solution of compound **17** (800 mg, 2.31 mmol) in acetone/H₂O (56 mL/ 14 mL) was added NaN₃ (900 mg, 13.87 mmol) and the mixture was heated at 50 °C for 9 h. After consumption of the starting material (checked by TLC), acetone was evaporated in vacuo and the residue was dissolved in Et₂O. 20% H₂SO₄ (10 mL) was added carefully at 0 °C and the mixture was allowed to stir for 12 h at room temperature. Et₂O was used for extraction and the organic layer was washed with brine, dried over



Na₂SO₄, and concentrated under reduced pressure to get the crude product which was purified over silica gel (15% EtOAc/hexane) to obtain compound **18** (560 mg, 78%) as colourless oil.

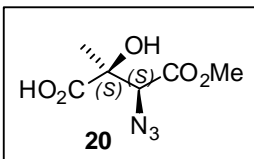
R_f = 0.48 (silica, 20% EtOAc/hexane); $[\alpha]_D^{25}$ = -34.4 (c = 4.5, CHCl₃); IR (thin film) ν_{max} = 3512, 2909, 2864, 2114, 1733, 1613, 1514, 1248, 1207, 1175, 1094, 1032, 821 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 7.23 (2H, d, J = 8.7 Hz), 6.88 (2H, d, J = 8.7 Hz), 4.43 (2H, s), 4.02 (1H, s), 3.80 (3H, s), 3.66 (3H, s), 3.47 (1H, d, J = 9.36 Hz), 3.37 (1H, d, J = 9.36 Hz), 1.26 (3H, s); ¹³C NMR (CDCl₃, 75 MHz) δ 169.43, 159.39, 129.62, 129.58, 113.77, 74.39, 73.7, 73.29, 66.7, 55.27, 52.52, 22.29; HRMS (ESI): m/z calcd for C₁₄H₁₉O₅N₃Na⁺ [M+Na]⁺ 332.1217, found 332.1229.

(2S,3S)-methyl 2-azido-3-(diethyl(propyl)silyloxy)-4-hydroxy-3-methylbutanoate (19): To a solution of compound **18** (560 mg, 1.81 mmol) in CH₂Cl₂ (20 mL) were added Et₃N (1.02 mL, 7.25 mmol) and TESOTf (1.02 mL, 4.53 mmol) slowly at 0 °C and it was allowed to stir for 45 min. at that temperature. CH₂Cl₂ was used for extraction and was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product which was purified over silica gel to get 680 mg (1.61 mmol) of pure protected compound. To a solution of the TES protected compound in CH₂Cl₂ (30 mL) were added 6.1 mL phosphate buffer (pH 7.5) and DDQ (912 mg, 4.02 mmol) at 0 °C and it was allowed to stir for 3 h at room temperature. After completion of the reaction, it was directly transferred into a silica gel column and purified (7.5% EtOAc/hexane) to obtain compound **19** (268 mg, 70% over two steps) as a colourless oil.



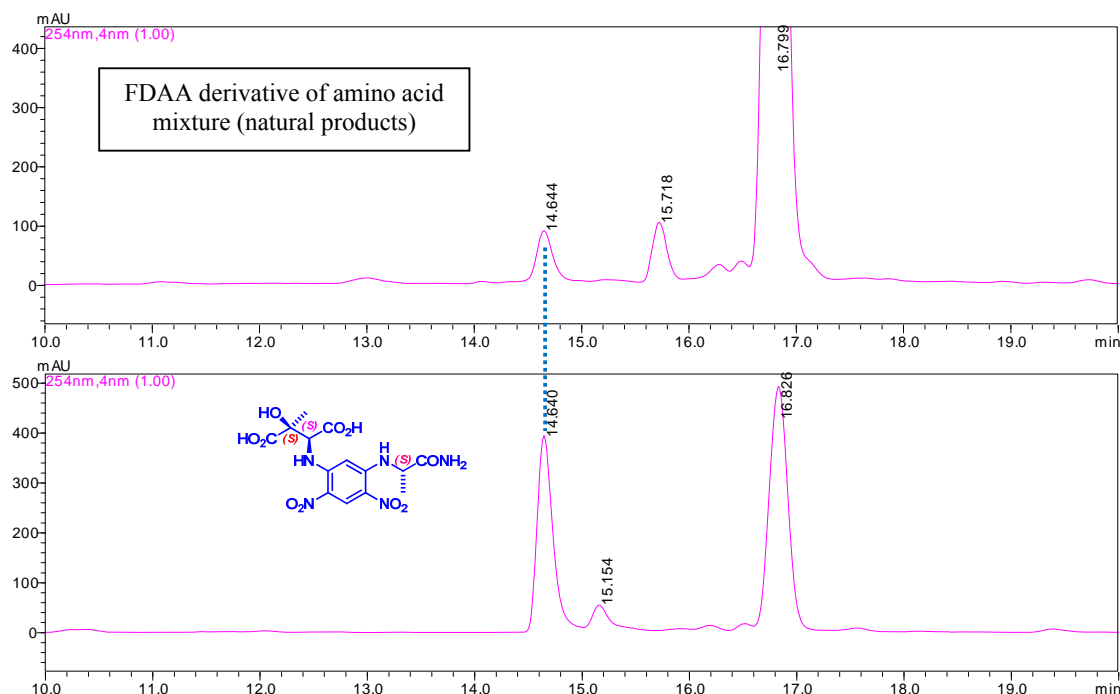
R_f = 0.5 (silica, 20% EtOAc/hexane); $[\alpha]_D^{25}$ = +15.6 (c = 1.0, CHCl₃); IR (thin film) ν_{max} = 3440, 2956, 2878, 2114, 1747, 1458, 1436, 1277, 1243, 1174, 1057, 1010, 746 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 3.94 (1H, s), 3.84 (3H, s), 3.65-3.57 (1H, dd, J = 11.35, 7.55 Hz), 3.56-3.53 (1H, dd, J = 11.35, 7.55 Hz), 2.0-1.98 (1H, m), 1.38 (3H, s), 0.97 (9H, t, J = 8.2), 0.64 (6H, q, J = 8.2); ¹³C NMR (CDCl₃, 125 MHz) δ 169.20, 78.31, 68.40, 67.38, 52.40, 21.93, 6.83, 6.58; HRMS (ESI): m/z calcd for C₁₂H₂₆O₄N₃Si [M+H]⁺ 304.1693, found 304.2841.

(2S,3S)-3-azido-2-hydroxy-4-methoxy-2-methyl-4-oxobutanoic acid (20): To a solution of compound **19** (240 mg, 0.8 mmol) in dry CH₂Cl₂ (25 mL) were added TEMPO (25 mg, 0.16 mmol) and diacetoxyiodobenzene (773 mg, 2.4 mmol) at 0 °C and it was allowed to stir for 10 h. at room temperature. CH₂Cl₂ was concentrated

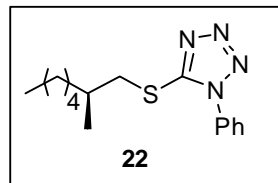


and directly transferred into a silica gel column and purified (3% EtOAc/hexane) to obtain 168 mg (70%) of aldehyde which was again dissolved in THF/H₂O/ⁱBuOH (12 mL/ 12 mL/ 2.4 mL) and to this soln. were added 4.8 mL 2-methyl-2-butene, NaClO₂ (160 mg, 1.8 mmol) and NaH₂PO₄ (400 mg, 3.34 mmol) in 6 mL H₂O at 0 °C and the reaction mixture was allowed to stir for 12 h at room temperature. THF was removed under reduced pressure and 50 mL 1M HCl was added and then extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the residue which was dissolved in 12 mL sat. NaHCO₃ and extracted with Et₂O. The aqueous layer was acidified with 10 mL 3M HCl and then extracted with EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to obtain compound **20** (101mg, 63% over two steps) as a colourless oil.

R_f = 0.2 (silica, 5% CH₃OH/CH₂Cl₂); $[\alpha]_D^{25}$ = -44.1 (c = 2.0, CHCl₃); IR (thin film) ν_{\max} = 3490, 2959, 2117, 1730, 1438, 1263, 1215, 1182, 1106, 1013, 847 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 4.16 (1H, s), 3.88 (3H, s), 1.57 (3H, s); ¹³C NMR (CDCl₃, 75 MHz) δ 176.61, 167.99, 66.37, 53.03, 23.05; HRMS (ESI): m/z calcd for C₆H₈O₃N₃ [M-H] 202.0469, found 202.0473.

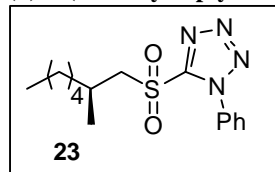


(S)-5-(2-methylheptylthio)-1-phenyl-1H-tetrazole (22): To a solution of compound **21a** (1.9 gm, 6.27 mmol) in dry Et₂O (25 mL) was added LiBH₄ (410 mg, 18.81 mmol) at 0 °C and it was allowed to stir for 2 h. at room temperature. MeOH was added slowly to quench and after getting the clear soln., the solvent was evaporated and the residue was extracted with CH₂Cl₂. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to obtain 600 mg of pure corresponding alcohol (74%). To the soln. of resulting alcohol in dry THF (50 mL) were added PPh₃ (1.7 gm, 6.46 mmol), 1-phenyl-1H-tetrazole-5-thiol (1.15 gm, 6.46 mmol) and DIAD (1.3 mL, 6.46 mmol) at 0 °C and it was allowed to stir for 8 h. at room temperature. THF was concentrated and directly transferred into silica gel column and purified (5% EtOAc/hexane) to obtain compound **22** (1.02 gm, 56% over two steps) as a colourless oil.



R_f = 0.6 (silica, 15% EtOAc/hexane); $[\alpha]_D^{25}$ = +1.4 (c = 1.84, CHCl₃); IR (thin film) ν_{\max} = 2957, 2927, 2856, 1600, 1500, 1386, 1240, 760 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 7.54-7.51 (4H, m), 5.24-5.21 (1H, m), 3.47-3.43 (1H, dd, J = 12.6, 5.7 Hz), 3.27-3.23 (1H, m), 1.93-1.90 (1H, m), 1.49-1.34 (2H, m), 1.31-1.22 (7H, m), 1.03-1.01 (3H, d, J = 6.3 Hz), 0.88-0.86 (3H, t, J = 6.95 Hz); ¹³C NMR (CDCl₃, 125 MHz) δ 154.72, 133.77, 130.00, 129.71, 123.85, 74.29, 40.53, 35.84, 32.91, 31.86, 26.42, 22.52, 21.54, 19.05, 13.98; HRMS (ESI): m/z calcd for C₁₅H₂₂N₄SN⁺ [M+Na]⁺ 313.1457, found 313.1455.

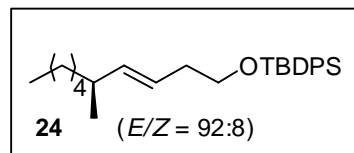
(S)-5-(2-methylheptylsulfonyl)-1-phenyl-1H-tetrazole (23): To a solution of compound **22** (1.02 gm, 3.52 mmol) in absolute EtOH (60 mL) was added (NH₄)₆Mo₇O₂₄·4H₂O (870 mg, 0.7 mmol) dissolved in 19 mL H₂O₂ at 0 °C and it was allowed to stir for 8 h. at room temperature. The solvent was evaporated and then the residue was extracted with EtOAc, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product which was purified over silica gel (7.5% EtOAc/hexane) to afford compound **23**



(1.1 gm, 98%) as a colourless oil.

R_f = 0.38 (silica, 15% EtOAc/hexane); $[\alpha]_D^{25}$ = -8.3 (c = 1.75, CHCl₃); IR (thin film) ν_{max} = 2957, 2930, 2859, 1497, 1339, 1153, 762 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 7.70-7.66 (2H, m), 7.62-7.56 (3H, m), 3.84-3.77 (1H, dd, J = 14.5, 4.9 Hz), 3.61-3.54 (1H, dd, J = 14.5, 8.07 Hz), 2.36-2.30 (1H, m), 1.57-1.50 (1H, m), 1.38-1.28 (7H, m), 1.16-1.14 (3H, d, J = 6.8 Hz), 0.90-0.85 (3H, t, J = 7.0 Hz); ¹³C NMR (CDCl₃, 75 MHz) δ 154.08, 133.07, 131.40, 129.63, 125.13, 61.84, 36.49, 31.57, 28.22, 25.93, 22.46, 19.68, 13.95; HRMS (ESI): m/z calcd for C₁₅H₂₂O₂N₄SN⁺ [M+Na]⁺ 345.1356, found 345.1348.

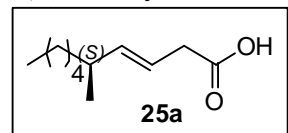
(S,E)-tert-butyl(5-methyldec-3-enyloxy)diphenylsilane (24): To a solution of compound **23** (200 mg, 0.62 mmol) in dry DME (4 mL) was added KHMDS (1.4 mL, 0.5M in toluene, 0.7 mmol) dropwise at -60 °C and it was stirred at that temperature for 1 h. Then 3-(tert-butyldiphenylsilyloxy)propanal (290 mg, 0.92 mmol) in DME (4 mL) was added slowly at -60 °C and it was allowed to stir for 12 h. To this reaction mixture 2 mL H₂O was added and it was vigorously stirred for 1 h. The reaction



mixture was extracted with Et₂O, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product which was purified over silica gel (1.5% EtOAc/hexane) to afford compound **24** (89 mg, 35%) as a colourless oil.

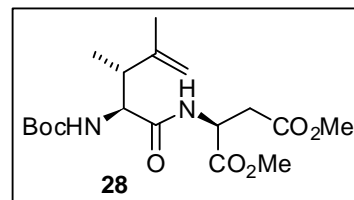
R_f = 0.8 (silica, 5% EtOAc/hexane); $[\alpha]_D^{25}$ = +13.5 (c = 0.6, CHCl₃); IR (thin film) ν_{max} = 2957, 2929, 2857, 1738, 1472, 1429, 1112, 969, 823, 736 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz) δ 7.69-7.67 (4H, d, J = 5.8 Hz), 7.42-7.35 (6H, m), 5.37-5.34 (2H, m), 3.70-3.66 (2H, t, J = 6.6 Hz), 2.29-2.23 (2H, m), 2.05 (1H, m), 1.44-1.25 (10H, m), 1.05 (9H, s), 1.01-0.93 (3H, d, J = 8.0 Hz), 0.90-0.85 (3H, t, J = 7.0 Hz); ¹³C NMR (CDCl₃, 75 MHz) δ 138.66, 135.60, 134.13, 129.48, 127.55, 124.51, 64.12, 37.07, 36.73, 36.08, 32.02, 26.96, 26.85, 22.63, 20.68, 19.23, 14.10; GCMS (M⁺Bu) 351.2.

(S,E)-5-methyldec-3-enoic acid (25a): To a solution of compound **24** (89 mg, 0.22 mmol) in dry THF (2 mL) was added TBAF (335 μ L, 0.33 mmol) at 0 °C and it was allowed to stir for 2 h. at room temperature. The reaction was quenched with sat. NH₄Cl and THF was evaporated and the residue was extracted with Et₂O, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product which was purified over silica gel (10% EtOAc/hexane) to get 26 mg (70%) of corresponding alcohol. To the soln. of CrO₃ (100 mg, 1.0 mmol) in 1 mL 3M H₂SO₄ was added the alcohol in 2 mL acetone at 0 °C and it was allowed to stir for 3 h. at room temperature. The reaction mixture was quenched with EtOH and filtered through filter paper. The filtrate was concentrated and the residue was extracted with EtOAc, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product which was purified over silica gel (25% EtOAc/hexane) to afford compound **25a** (20 mg, 72%) as a colourless oil.



R_f = 0.3 (silica, 50% EtOAc/hexane); $[\alpha]_D^{25}$ = +14.0 (c = 2.0, CHCl₃); IR (thin film) ν_{max} = 2957, 2927, 2855, 1713, 1703, 1462, 1248, 969, 805, 725 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 5.48-5.46 (2H, m), 3.07 (2H, d, J = 5.0 Hz), 1.29 (8H, br), 0.96 (3H, d, J = 8.0 Hz), 0.86 (3H, t, J = 7.0 Hz); ¹³C NMR (CDCl₃, 75 MHz) δ 176.66, 141.34, 118.95, 37.55, 36.77, 36.61, 31.93, 26.86, 22.61, 20.32, 14.05; HRMS (ESI): m/z calcd for C₁₁H₁₉O₂ [M-H]⁻ 183.1391, found 183.1389.

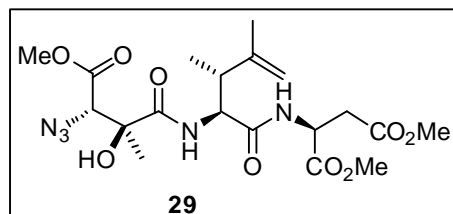
(S)-dimethyl-2-((2S,3R)-2-(tert-butoxycarbonylamino)-3,4-dimethylpent-4-enamido) succinate (28):



To a soln. of **26** (60 mg, 0.25 mmol) in dry CH₂Cl₂ (2mL) were added DIPEA (87 μ L, 0.5 mmol), dimethylaspartate hydrochloride salt (73 mg, 0.37 mmol), HATU (113 mg, 0.3 mmol) and HOAt (3.5 mg, 0.03 mmol) at -10 °C and it was allowed to stir for 12 h. at room temperature. The reaction was extracted with CH₂Cl₂, washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure to get the crude product which was purified over silica gel (35% EtOAc/hexane) to afford compound **28** (76 mg, 80%) as a colourless oil.

$R_f = 0.3$ (silica, 40% EtOAc/hexane); $[\alpha]_D^{25} = +22.4$ ($c = 0.8$, CHCl_3); IR (thin film) $\nu_{\max} = 3317, 3256, 3077, 2979, 1747, 1682, 1655, 1557, 1519, 1434, 1367, 1303, 1168, 1013 \text{ cm}^{-1}$; ^1H NMR (CDCl_3 , 500 MHz) δ 6.98 (1H, br), 4.87 (2H, s), 4.77 (2H, s), 4.2 (1H, br), 3.74 (3H, s), 3.67 (3H, s), 3.0 (1H, dd, $J = 17.6, 4.4 \text{ Hz}$), 2.86 (1H, dd, $J = 17.6, 4.4 \text{ Hz}$), 2.68 (1H, t, $J = 6.9$), 1.77 (3H, s), 1.42 (9H, s), 0.99 (3H, d, $J = 6.9$); ^{13}C NMR (CDCl_3 , 125 MHz) δ 171.21, 170.72, 155.63, 145.96, 112.34, 80.13, 57.04, 52.66, 51.91, 48.68, 42.39, 35.98, 28.19, 21.08, 13.84; HRMS (ESI): m/z calcd for $\text{C}_{18}\text{H}_{30}\text{O}_7\text{N}_2\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 409.1945, found 409.1953.

(S)-dimethyl-2-((2S,3R)-2-((2S,3S)-3-azido-2-hydroxy-4-methoxy-2-methyl-4-oxobutanamido)-3,4-dimethylpent-4-enamido)succinate (29): To a soln. of **28** (30 mg, 0.08 mmol) in dry CH_2Cl_2 (1 mL) was added TFA (500 μL) at 0°C and it was allowed to stir for 2 h. at room temperature. CH_2Cl_2 was concentrated under reduced pressure to get the TFA salt which was dissolved in dry CH_2Cl_2 (1 mL) and to this soln. were added DIPEA (34 μL , 0.2 mmol), compound

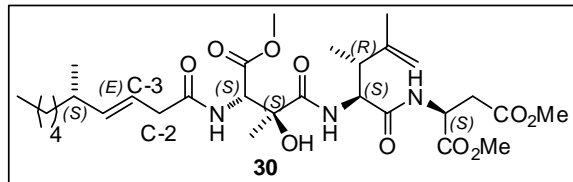


20 (16 mg, 0.08 mmol) in CH_2Cl_2 (1 mL), HATU (60 mg, 0.16 mmol) and HOAt (5 mg, 0.04 mmol) at -10°C and it was allowed to stir for 12 h. at room temperature.

The reaction was extracted with CH_2Cl_2 , washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (65% EtOAc/hexane) to obtain compound **29** (18 mg, 50% over two steps).

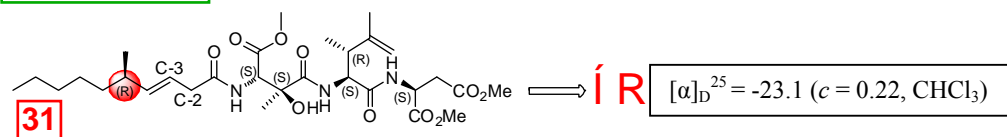
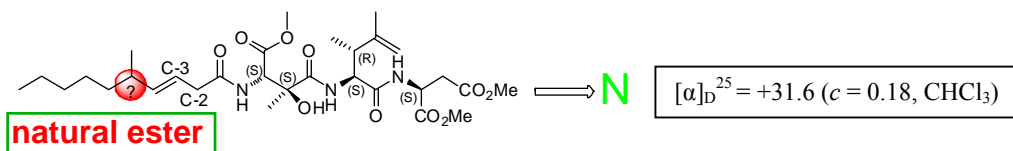
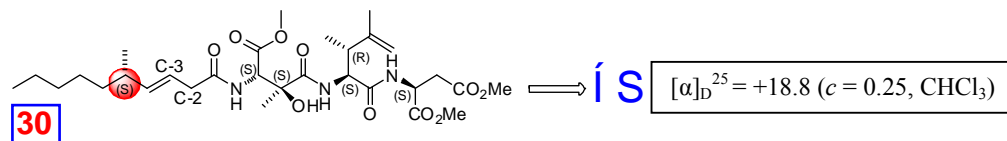
$R_f = 0.3$ (silica, 50% EtOAc/hexane); $[\alpha]_D^{25} = -34.7$ ($c = 1.0$, CHCl_3); IR (thin film) $\nu_{\max} = 3305, 2106, 1744, 1738, 1652, 1544, 1438, 1299, 1133, 1002, 912, 856, 732 \text{ cm}^{-1}$; ^1H NMR (CDCl_3 , 500 MHz) δ 7.19 (1H, d, $J = 8.8 \text{ Hz}$), 6.99 (1H, d, $J = 7.6 \text{ Hz}$), 4.91 (1H, s), 4.84 (1H, s), 4.80-4.78 (1H, m), 4.57-4.54 (1H, m), 4.23 (1H, s), 3.90 (1H, s), 3.85 (3H, s), 3.75 (3H, s), 3.69 (3H, s), 2.98 (1H, dd, $J = 17.3, 4.4 \text{ Hz}$), 2.88 (1H, dd, $J = 17.3, 4.4 \text{ Hz}$), 2.80-2.77 (1H, m), 1.8 (3H, s), 1.48 (3H, s), 1.08 (3H, d, $J = 6.9 \text{ Hz}$); ^{13}C NMR (CDCl_3 , 125 MHz) δ 172.62, 171.24, 170.67, 170.46, 169.28, 145.60, 112.59, 65.84, 55.53, 52.90, 52.76, 52.03, 48.71, 42.14, 38.58, 35.85, 29.66, 23.92, 20.86, 14.11; HRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{29}\text{O}_9\text{N}_5\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 494.1857, found 494.1851.

(S)-dimethyl-2-((2S,3R)-2-((2S,3S)-2-hydroxy-4-methoxy-2-methyl-3-((S,E)-5-methyldec-enamido)-4-oxobutanamido)-3,4-dimethylpent-4-enamido)succinate (30): To a soln. of **29** (20 mg, 0.04 mmol) in THF/ H_2O (3 mL/300 μL) was added Me_3P (300 μL , 1.0M in THF) at 0°C and it was allowed to stir for 1 h. After complete consumption of **29** THF was removed in



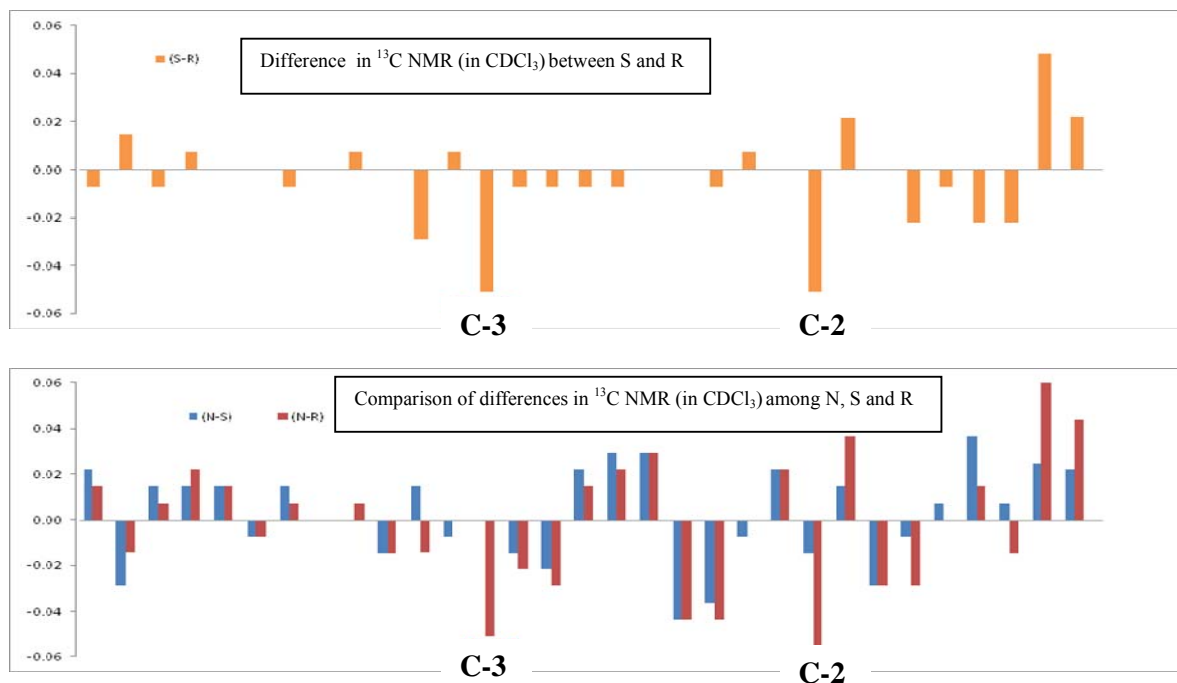
vacuo and extracted with EtOAc, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (5% MeOH/ CH_2Cl_2) to get 13 mg (0.03 mmol) of corresponding amine which was dissolved in dry CH_2Cl_2 (3 mL). To this soln. were added DIPEA (15 μL , 0.09 mmol), compound **25a** (8 mg, 0.04 mmol) in CH_2Cl_2 (2 mL), HATU (35 mg, 0.09 mmol) and HOAt (2 mg, 0.02 mmol) at -10°C and it was allowed to stir for 12 h. at room temperature. The reaction was extracted with CH_2Cl_2 , washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (50% EtOAc/hexane) to afford compound **30** (10 mg, 40% over two steps) as a colourless oil.

$R_f = 0.38$ (silica, 40% EtOAc/hexane); $[\alpha]_D^{25} = +18.8$ ($c = 0.25$, CHCl_3); IR (thin film) $\nu_{\max} = 3289, 2957, 2921, 2850, 1748, 1729, 1654, 1647, 1527, 1287, 1210, 1107 \text{ cm}^{-1}$; ^1H NMR (CDCl_3 , 500 MHz) δ 7.13 (1H, d, $J = 8.8 \text{ Hz}$), 7.08 (1H, d, $J = 8.2 \text{ Hz}$), 6.88 (1H, d, $J = 8.2 \text{ Hz}$), 5.55 (2H, m), 4.9 (1H, s), 4.84 (3H, m), 4.77 (1H, m), 4.43 (1H, m), 3.75 (3H, s), 3.72 (3H, s), 3.68 (3H, s), 3.0 (3H, m), 2.86 (1H, dd, $J = 17.3, 4.4 \text{ Hz}$), 2.75 (1H, t, $J = 7 \text{ Hz}$), 2.16-2.13 (1H, m), 1.76 (3H, s), 1.46 (3H, s), 1.29 (8H, br), 1.04 (3H, d, $J = 6.95 \text{ Hz}$), 0.97 (3H, d, $J = 7.0 \text{ Hz}$), 0.87 (3H, t, $J = 7.0 \text{ Hz}$); ^{13}C NMR (CDCl_3 , 125 MHz) δ 175.86, 172.74, 171.75, 171.29, 170.65, 170.03, 145.59, 142.48, 119.93, 112.68, 76.44, 57.56, 55.19, 52.92, 52.80, 52.04, 48.66, 42.16, 40.19, 36.77, 36.73, 35.88, 31.97, 29.69, 26.92, 23.97, 22.60, 20.93, 20.40, 14.15, 14.08; HRMS (ESI): m/z calcd for $\text{C}_{30}\text{H}_{49}\text{O}_{10}\text{N}_3\text{Na}^+$ $[\text{M}+\text{Na}]^+$ 634.3310, found 634.3291.

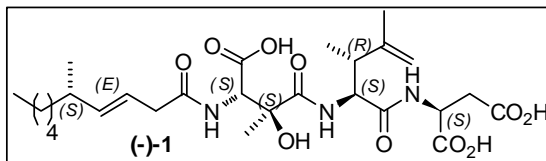


^{13}C NMR in CDCl_3 in 125 MHz :

| (7S)/52 | (N)/pentacyclic | (7R)/53 |
|---------|-----------------|---------|
| 14.08 | 14.11 | 14.09 |
| 14.15 | 14.12 | 14.14 |
| 20.40 | 20.42 | 20.41 |
| 20.93 | 20.95 | 20.93 |
| 22.60 | 22.61 | 22.60 |
| 23.97 | 23.96 | 23.97 |
| 26.92 | 26.94 | 26.93 |
| 29.69 | 29.69 | 29.69 |
| 31.97 | 31.97 | 31.97 |
| 35.88 | 35.87 | 35.88 |
| 36.73 | 36.74 | 36.76 |
| 36.77 | 36.76 | 36.76 |
| 40.19 | 40.19 | 40.24 |
| 42.16 | 42.15 | 42.17 |
| 48.66 | 48.63 | 48.66 |
| 52.04 | 52.07 | 52.05 |
| 52.80 | 52.83 | 52.81 |
| 52.92 | 52.95 | 52.92 |
| 55.19 | 55.14 | 55.19 |
| 57.56 | 57.52 | 57.57 |
| 76.44 | 76.44 | 76.44 |
| 112.68 | 112.70 | 112.68 |
| 119.93 | 119.91 | 119.98 |
| 142.48 | 142.50 | 142.46 |
| 145.59 | 145.56 | 145.59 |
| 170.03 | 170.03 | 170.06 |
| 170.65 | 170.66 | 170.66 |
| 171.29 | 171.32 | 171.31 |
| 171.75 | 171.76 | 171.78 |
| 172.74 | 172.76 | 172.69 |
| 175.86 | 175.88 | 175.83 |



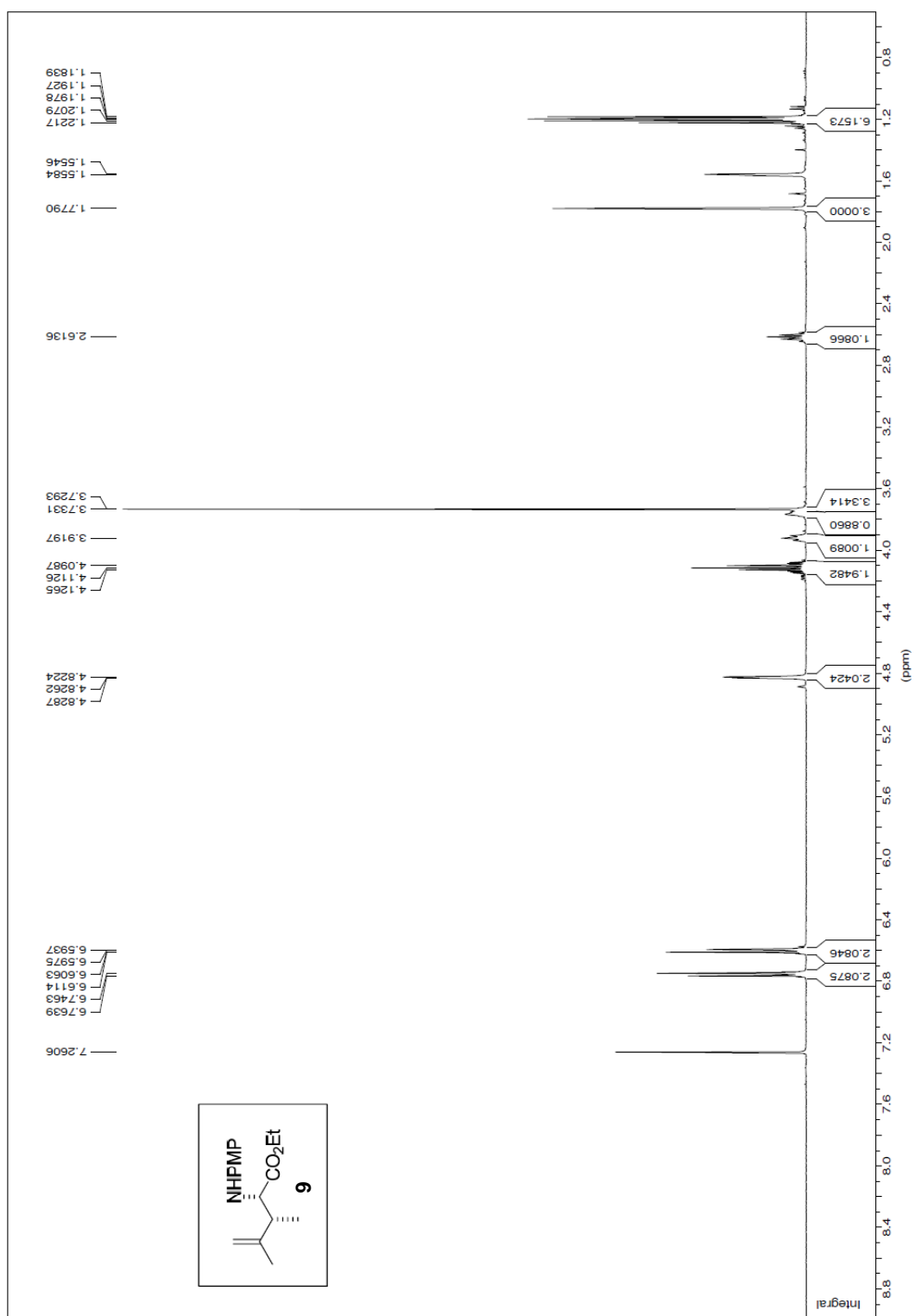
(S)-2-((2S,3R)-2-((2S,3S)-3-carboxy-2-hydroxy-2-methyl-3-((S,E)-5-methyldec-3-

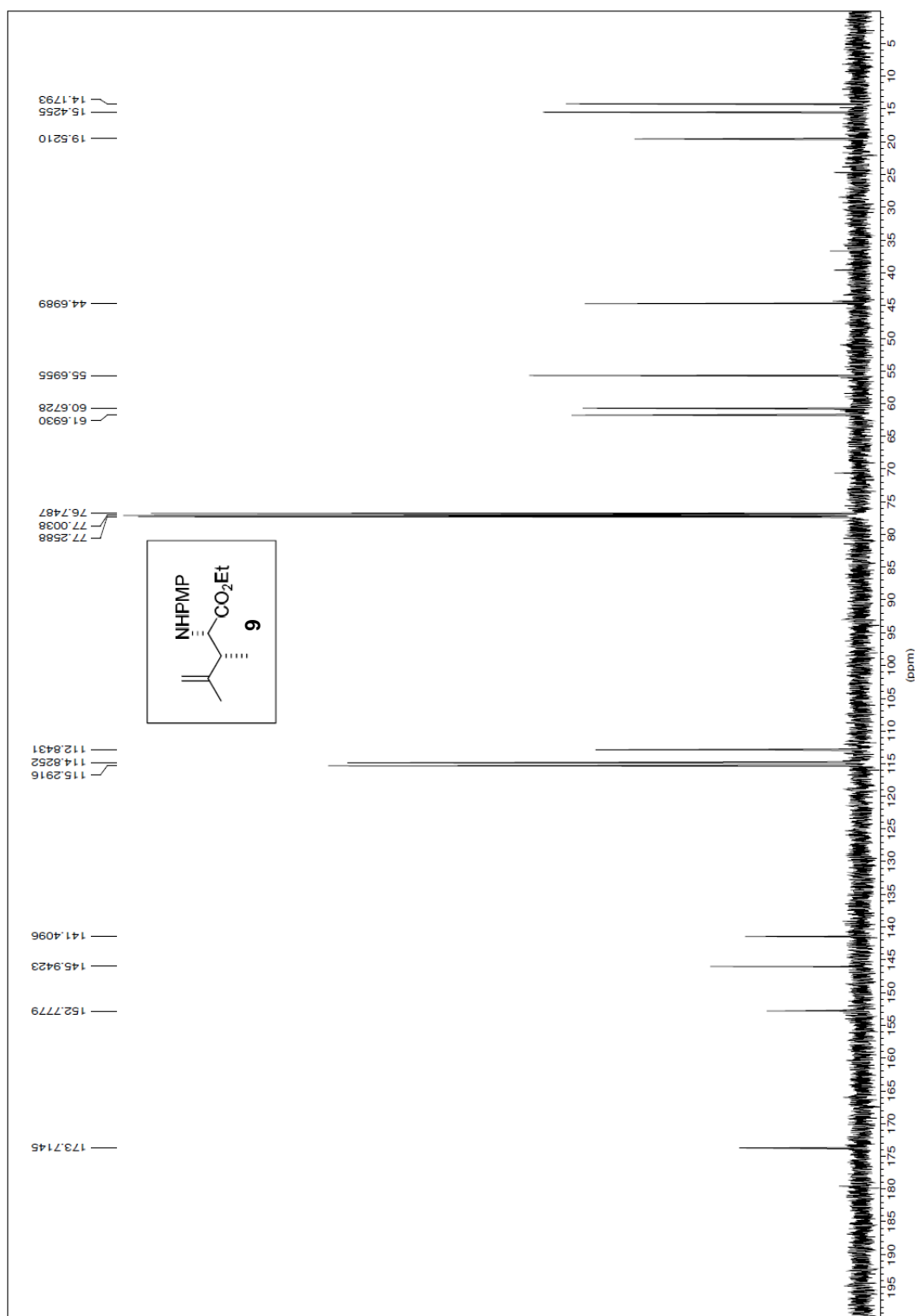


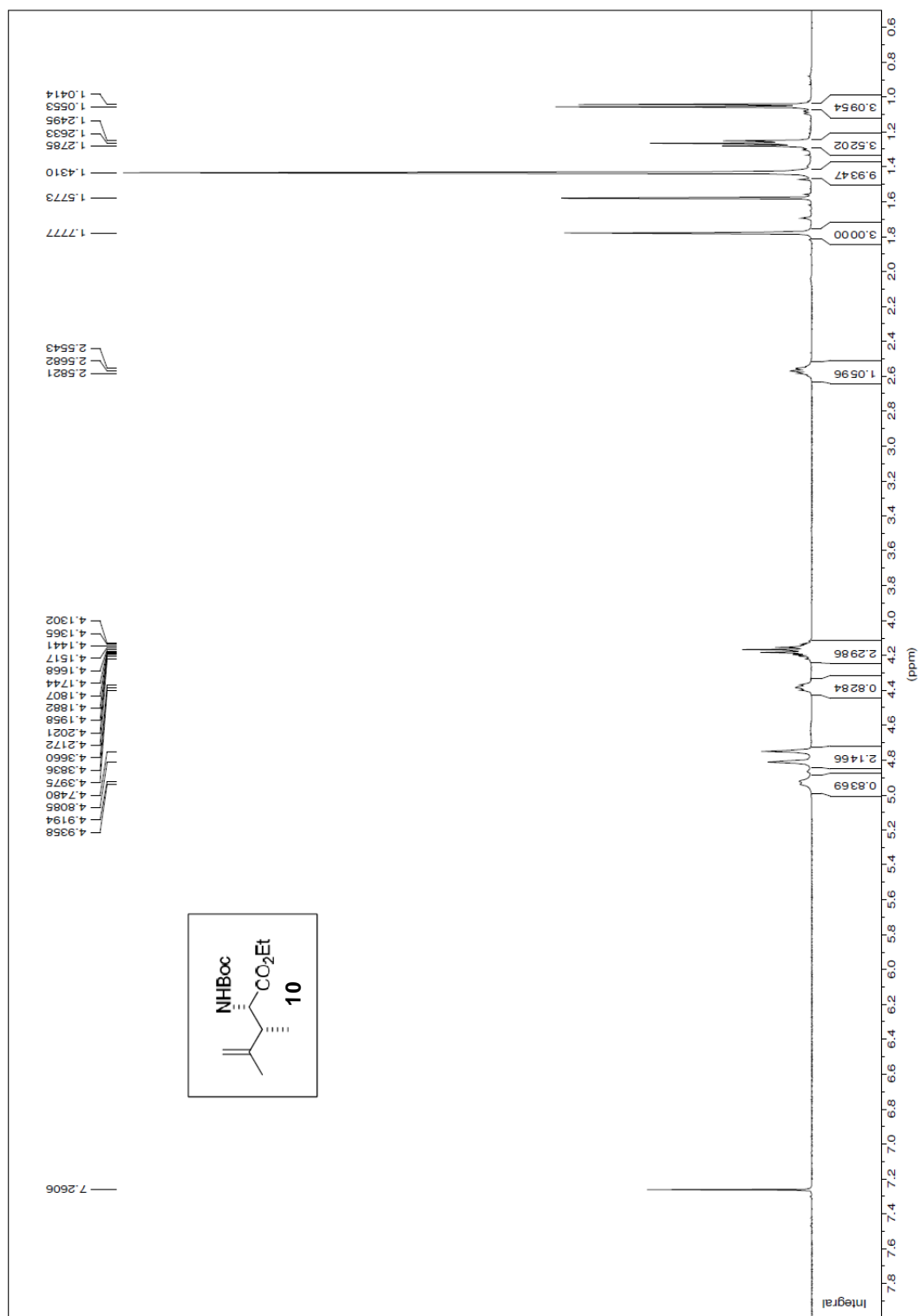
enamido)propanamido)-3,4-dimethylpent-4-enamido)succinic acid (1) : To a soln. of **30** (3 mg, 0.005 mmol) in $\text{ClCH}_2\text{CH}_2\text{Cl}$ (2 mL) was added Me_3SnOH (27 mg, 0.15 mmol) and the reaction mixture was heated at 80°C for 48 h. ESI mass was checked for crude reaction

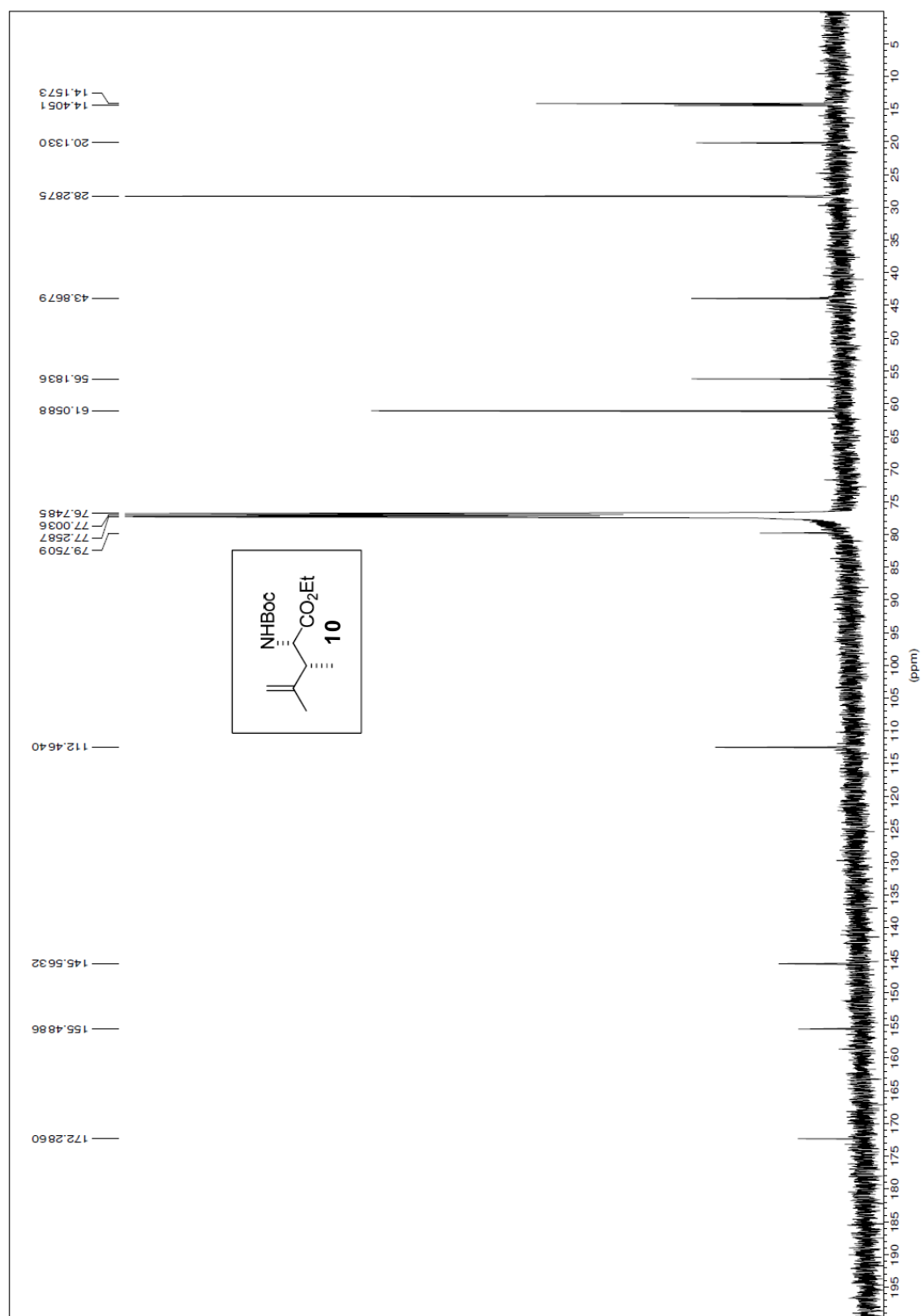
mixture and found di-acid as major product. The solvent was evaporated and the residue was extracted with EtOAc, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was dissolved in THF/ H_2O /MeOH (1 mL/1 mL/ 0.2 mL). To this soln. was added LiOH (10 mg, 0.42 mmol) at 0°C and it was allowed to stir for 12 h. at room temperature. The reaction mixture was acidified with 3M HCl and extracted with EtOAc, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified by prep. HPLC to afford compound **1** (1.3 mg, 46%) as a colourless oil.

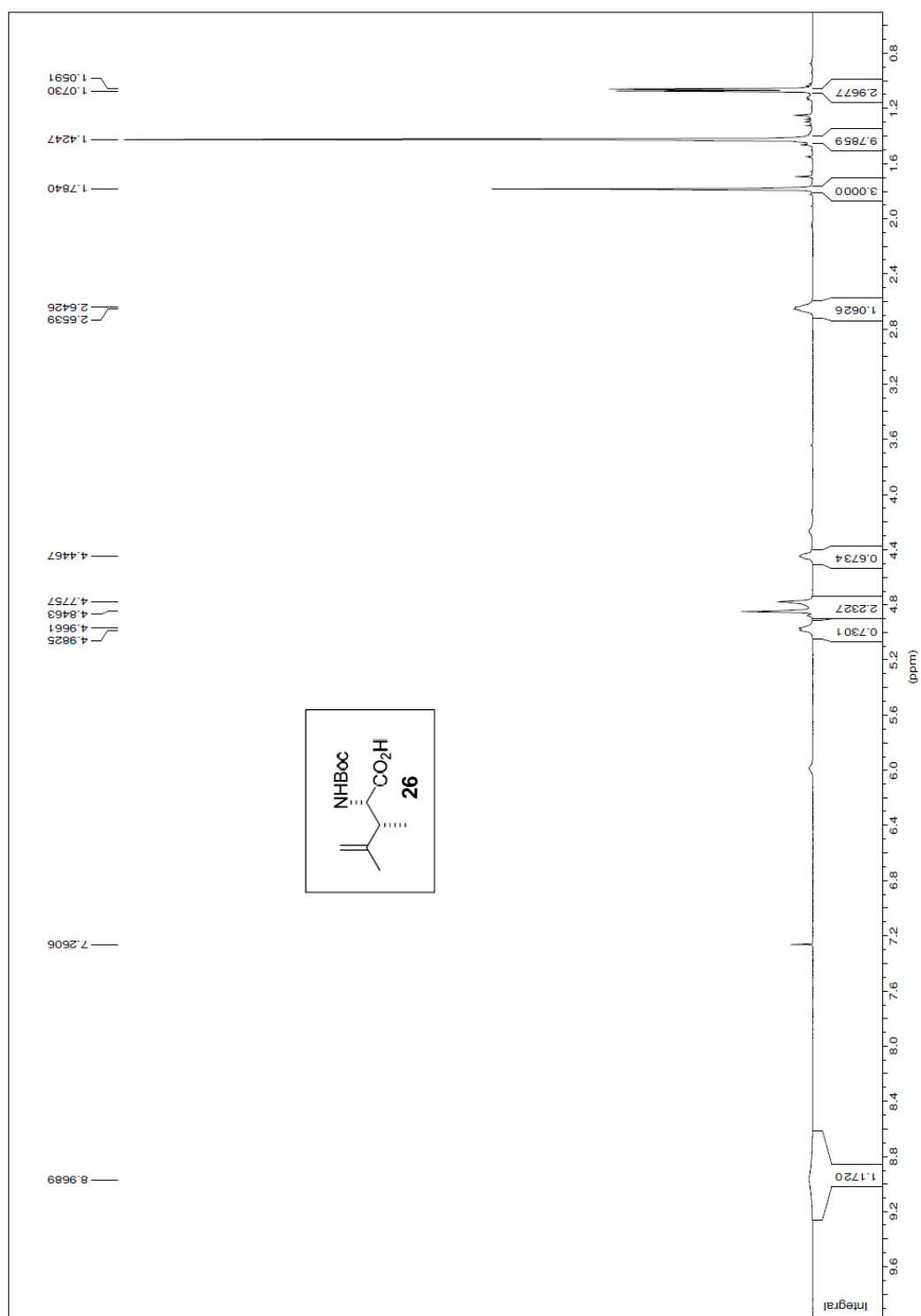
$R_f = 0.1$ (silica, 20% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$); $[\alpha]_D^{25} = -20.0$ ($c = 0.1$, CH_3OH); IR (thin film) $\nu_{\text{max}} = 2927, 2855, 1733, 1656, 1521, 1195 \text{ cm}^{-1}$; ^1H NMR ($\text{MeOD}-d_4$, 500 MHz) δ 5.52-5.0 (2H, m), 4.61-4.6 (2H, m), 4.43 (1H, d, $J = 6.9 \text{ Hz}$), 2.97 (2H, d, $J = 6.9 \text{ Hz}$), 2.81 (2H, t, $J = 7.0 \text{ Hz}$), 2.67-2.65 (2H, m), 2.13 (1H, m), 1.76 (3H, s), 1.44 (3H, s), 1.29 (8H, br), 1.07 (3H, d, $J = 6.9 \text{ Hz}$), 0.97 (3H, d, $J = 6.9 \text{ Hz}$), 0.89 (3H, t, $J = 7.0 \text{ Hz}$); ^{13}C NMR (CDCl_3 , 125 MHz) δ 176.35, 174.13, 173.95, 173.59, 173.28, 172.66, 147.01, 142.79, 121.74, 113.38, 76.51, 57.12, 50.33, 44.33, 40.63, 38.09, 38.00, 36.83, 33.15, 28.10, 24.45, 23.67, 20.95, 20.45, 15.55, 14.43; HRMS (ESI): m/z calcd for $\text{C}_{27}\text{H}_{42}\text{O}_{10}\text{N}_3[\text{M}-\text{H}]$ 568.2876, found 568.2868.

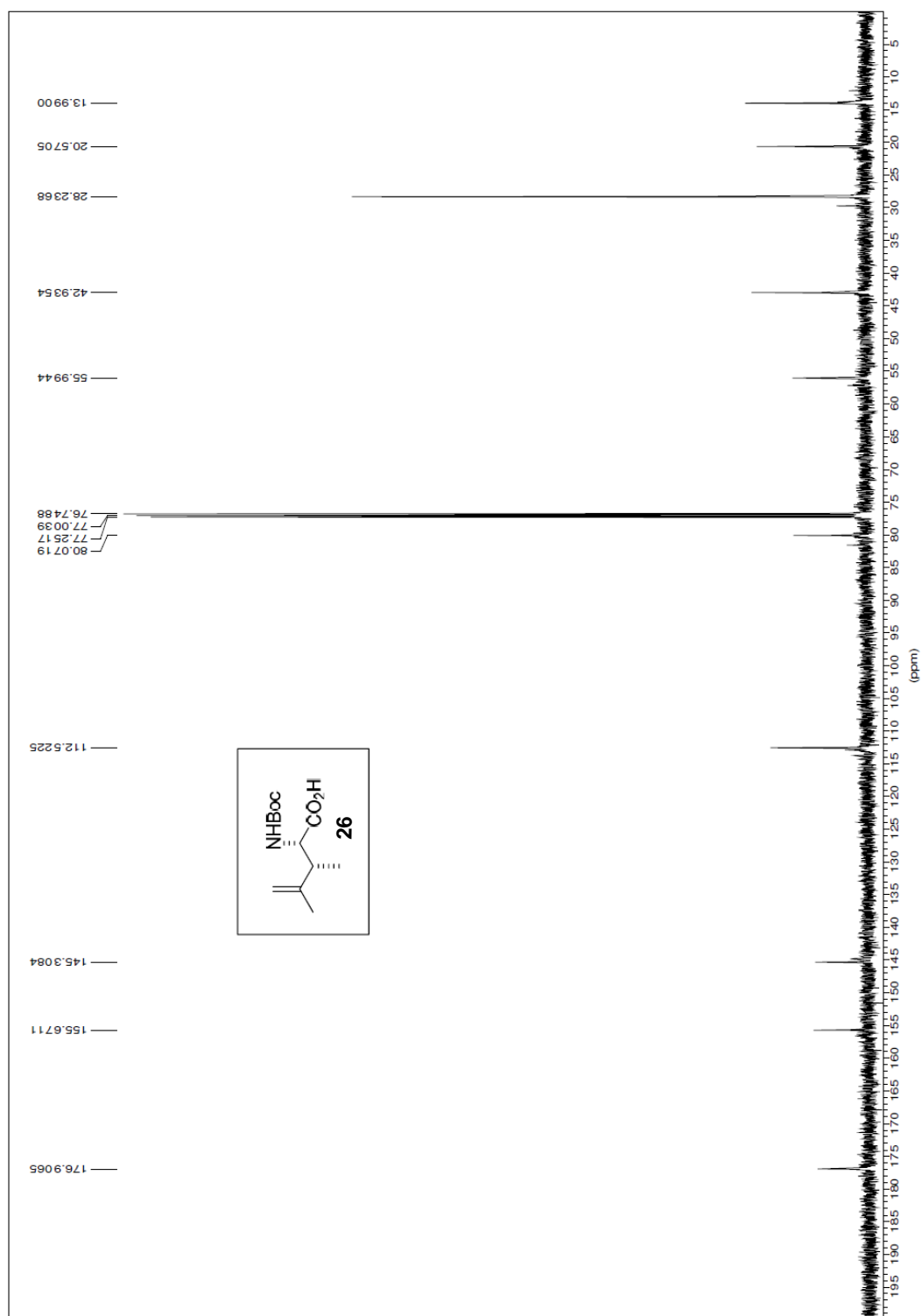


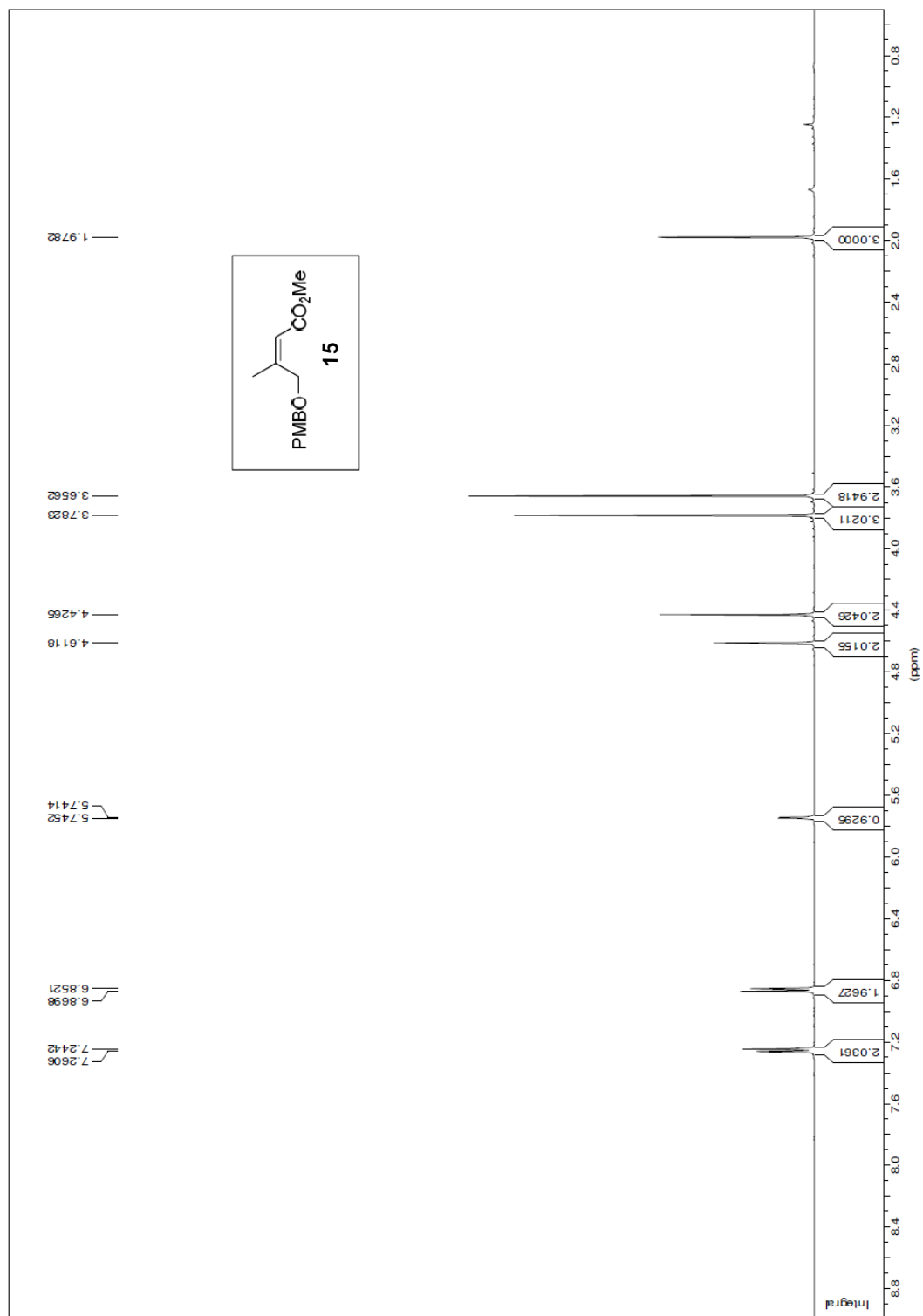


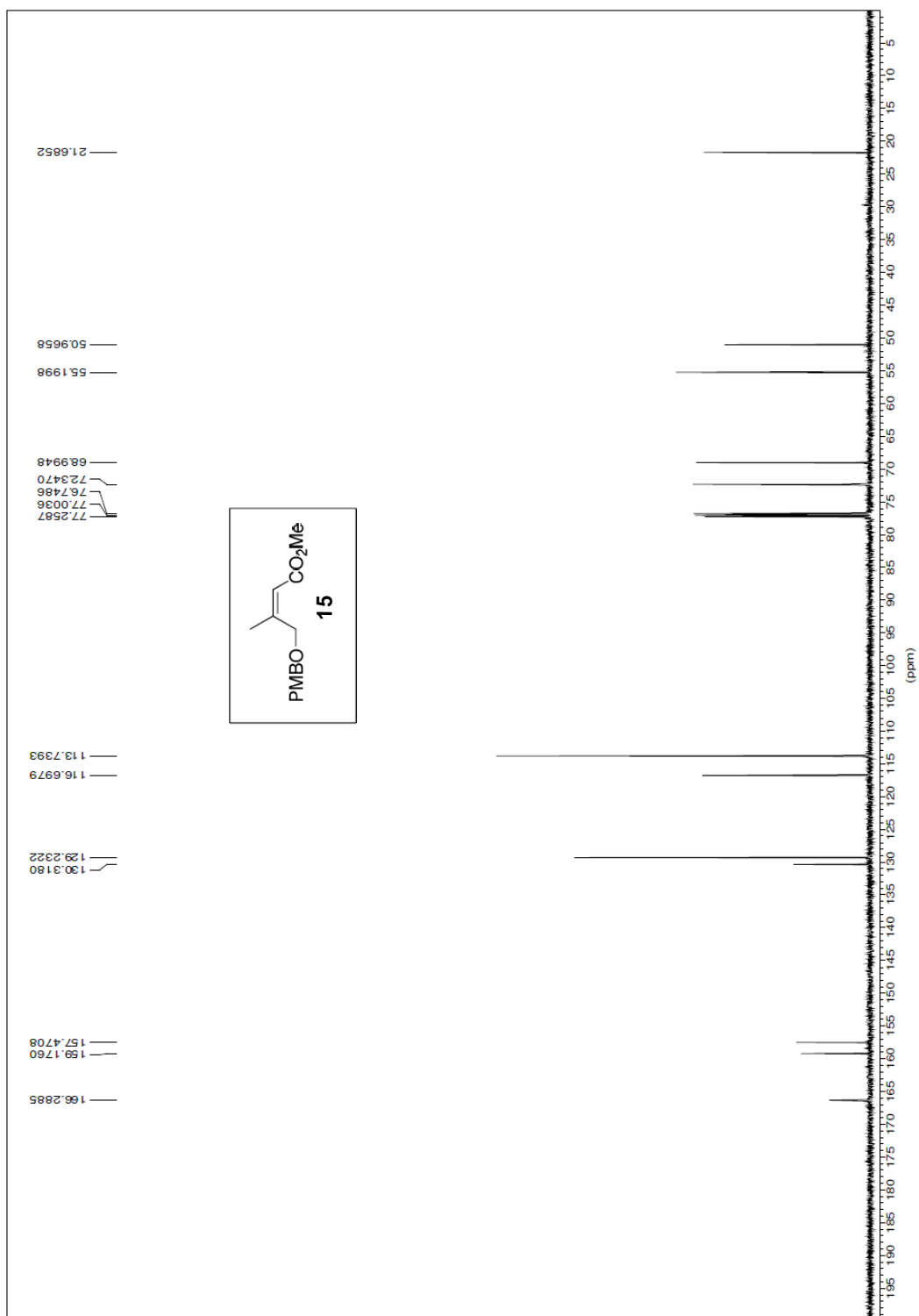


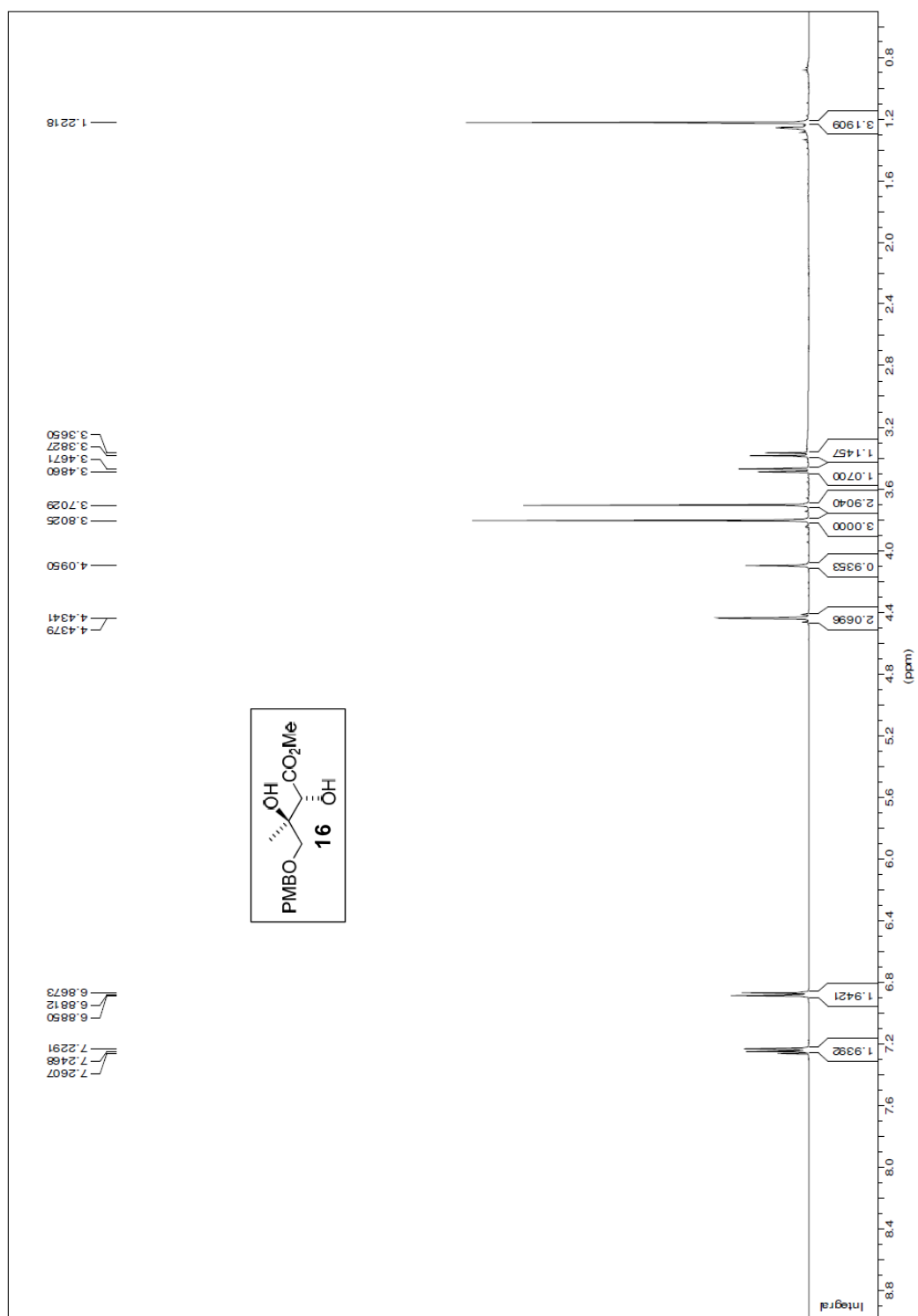


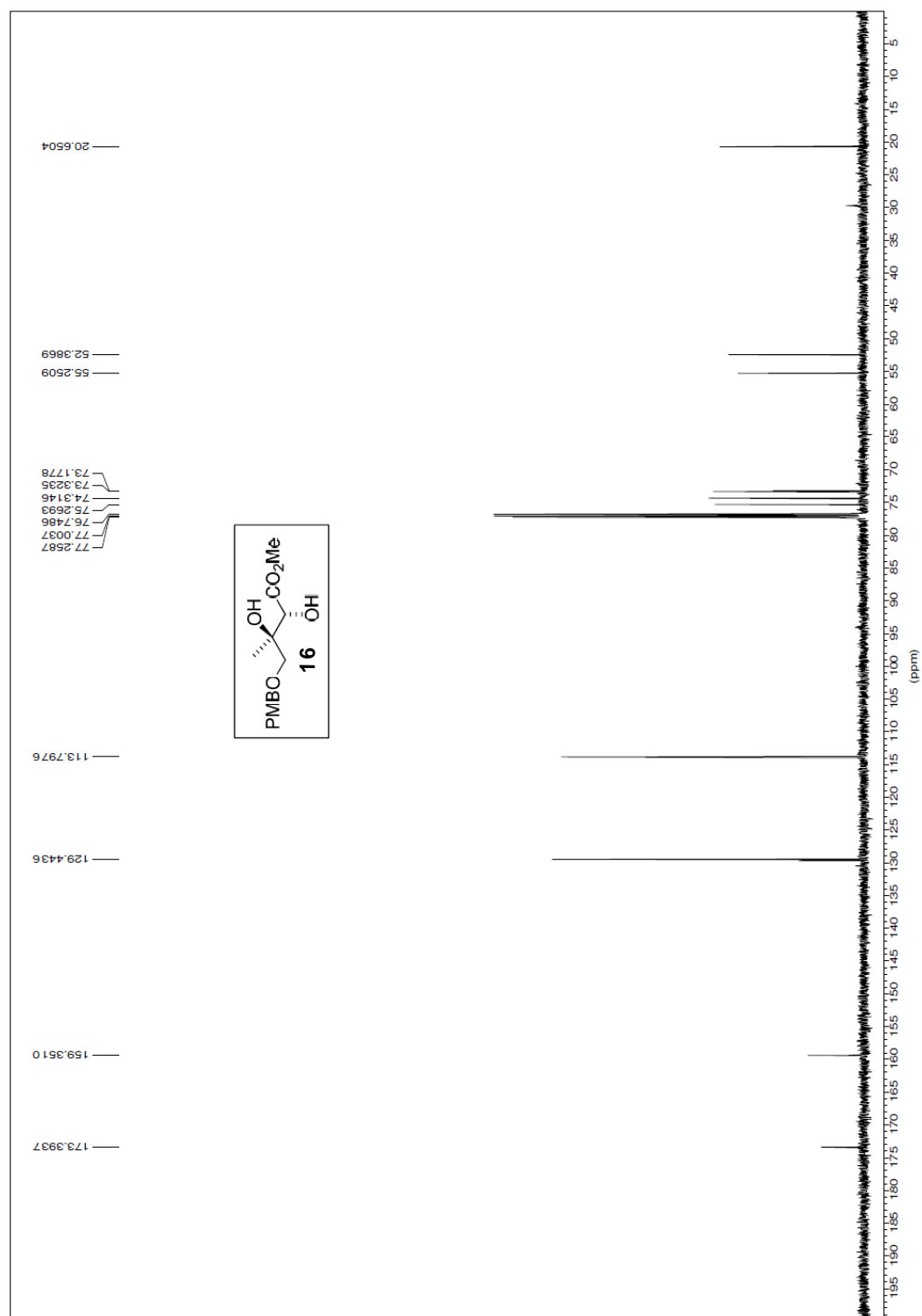


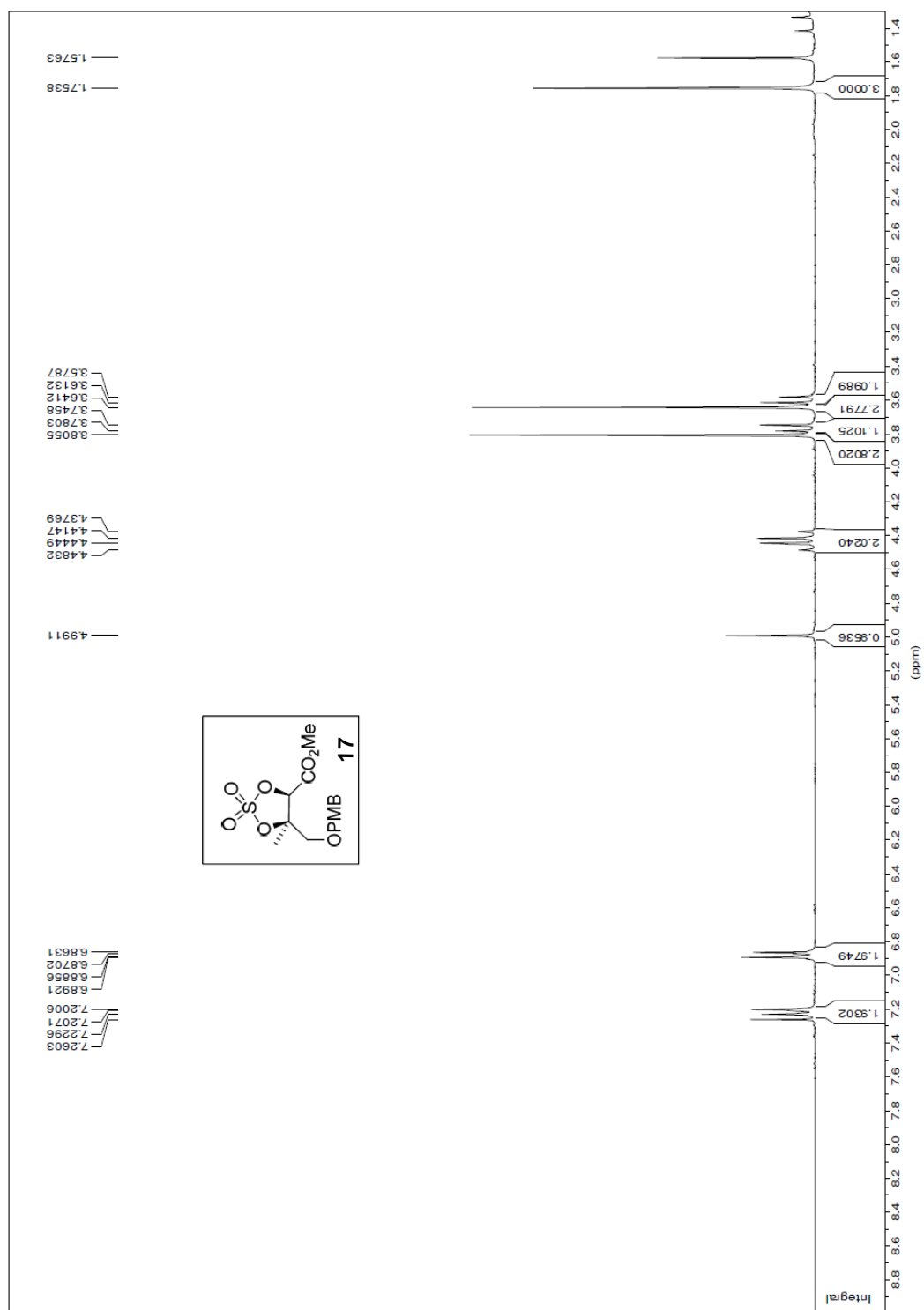


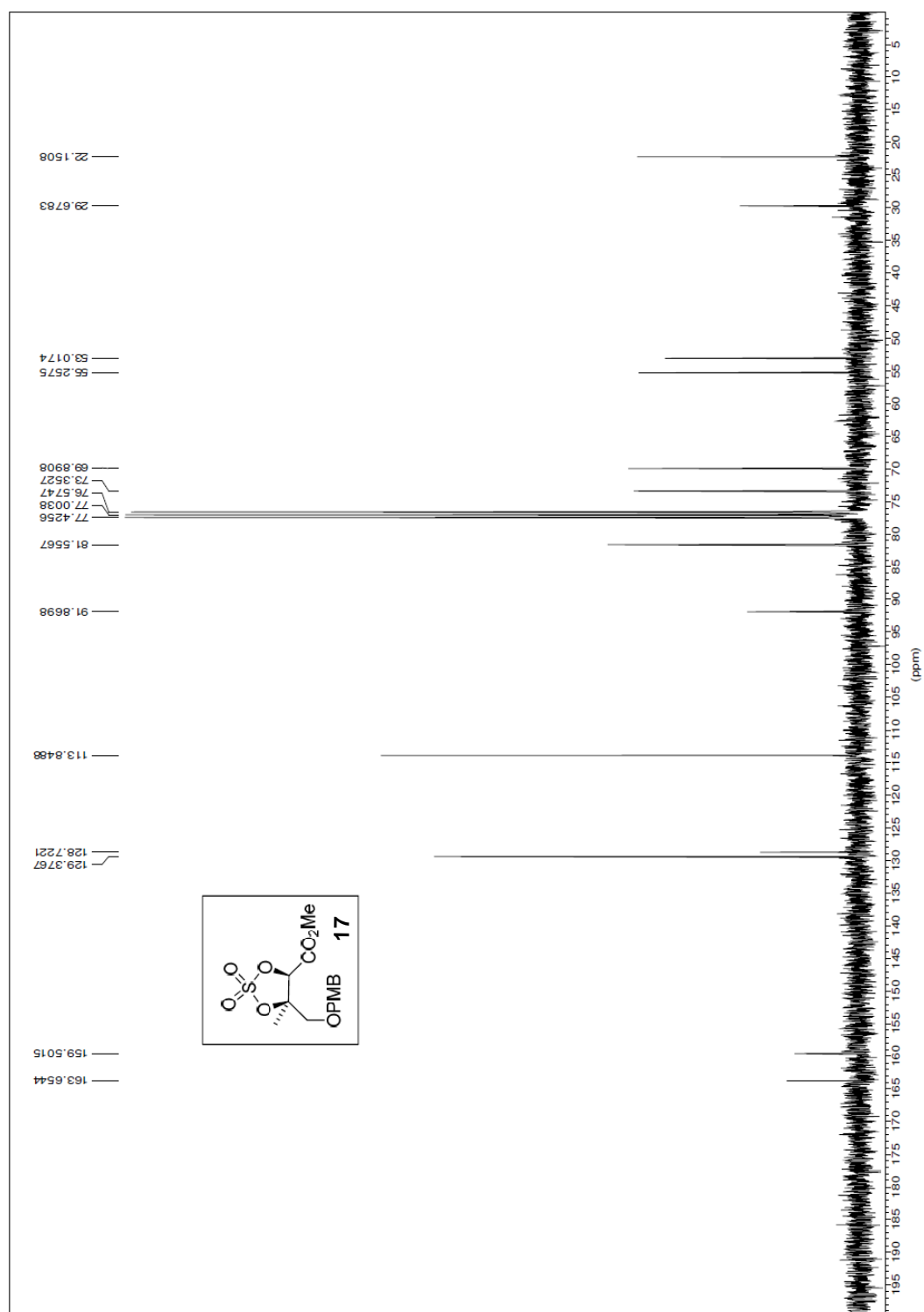




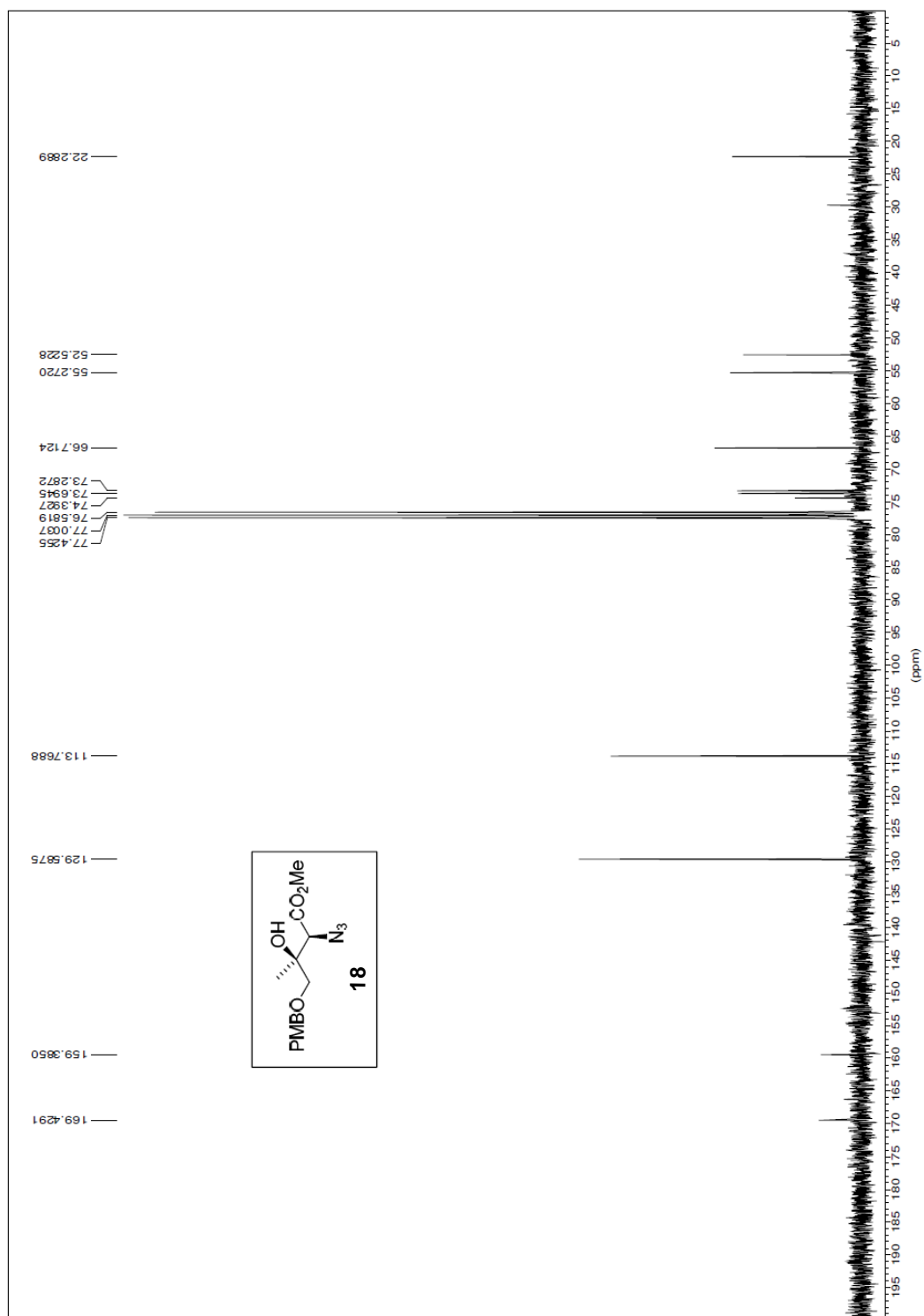


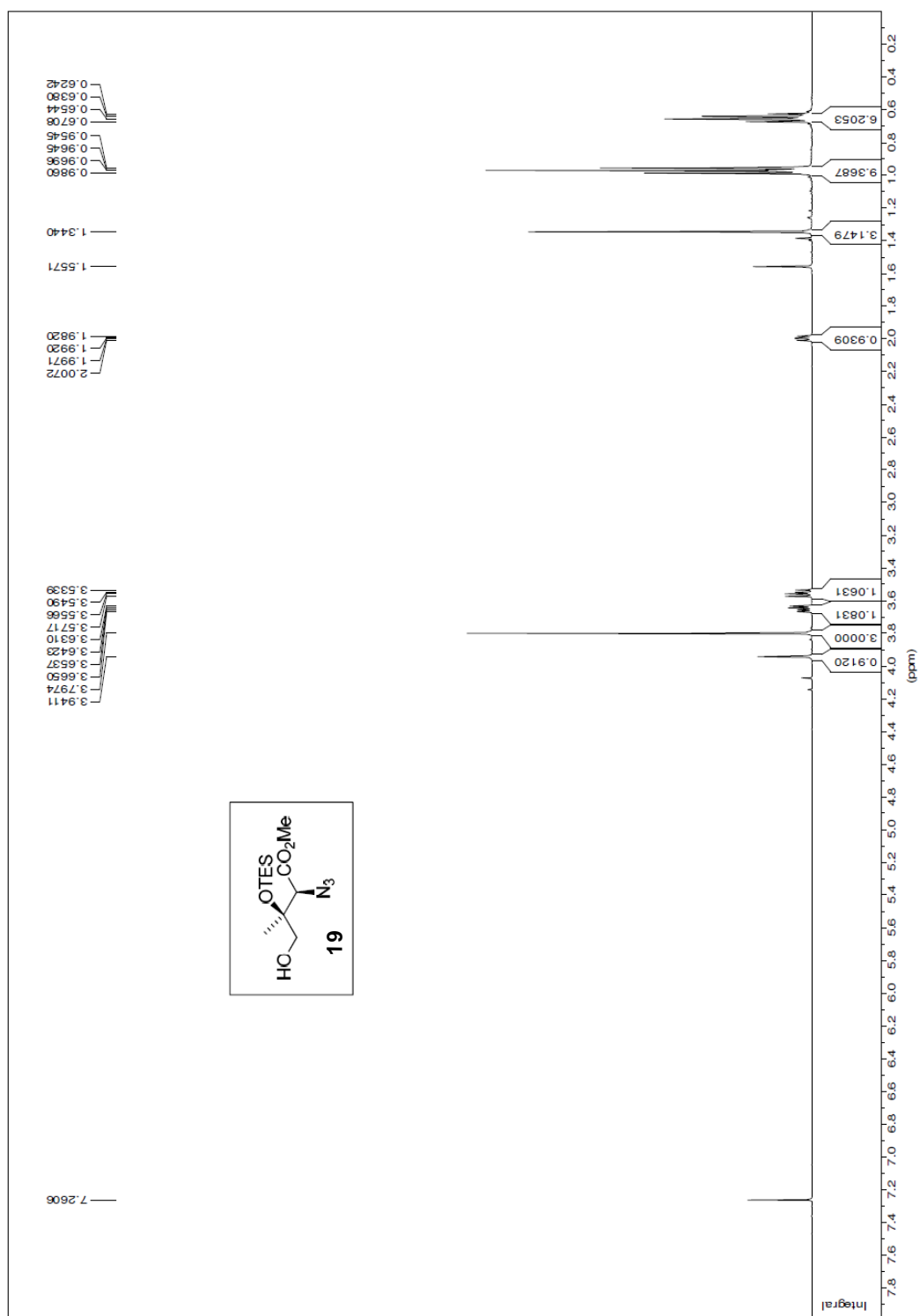


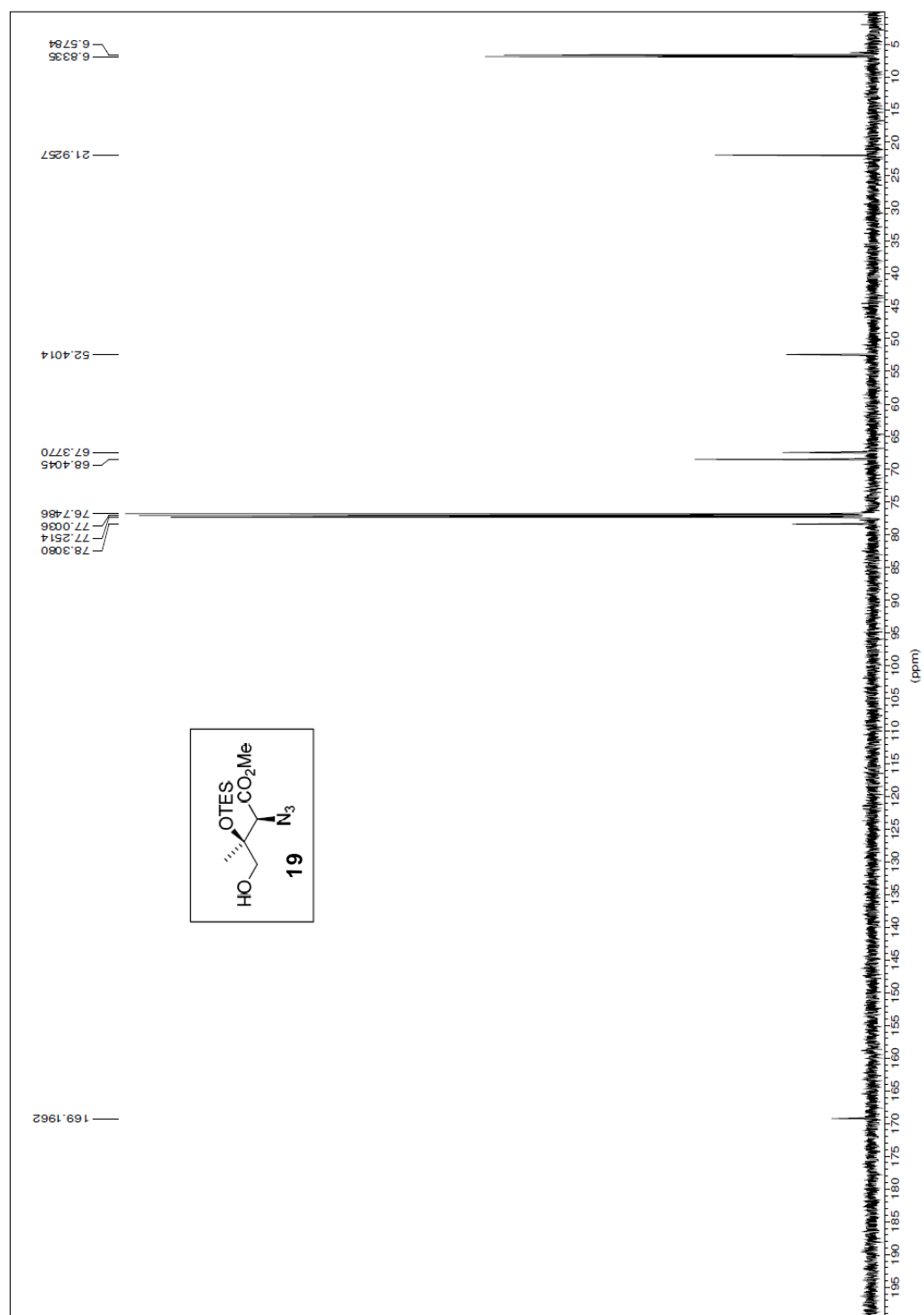


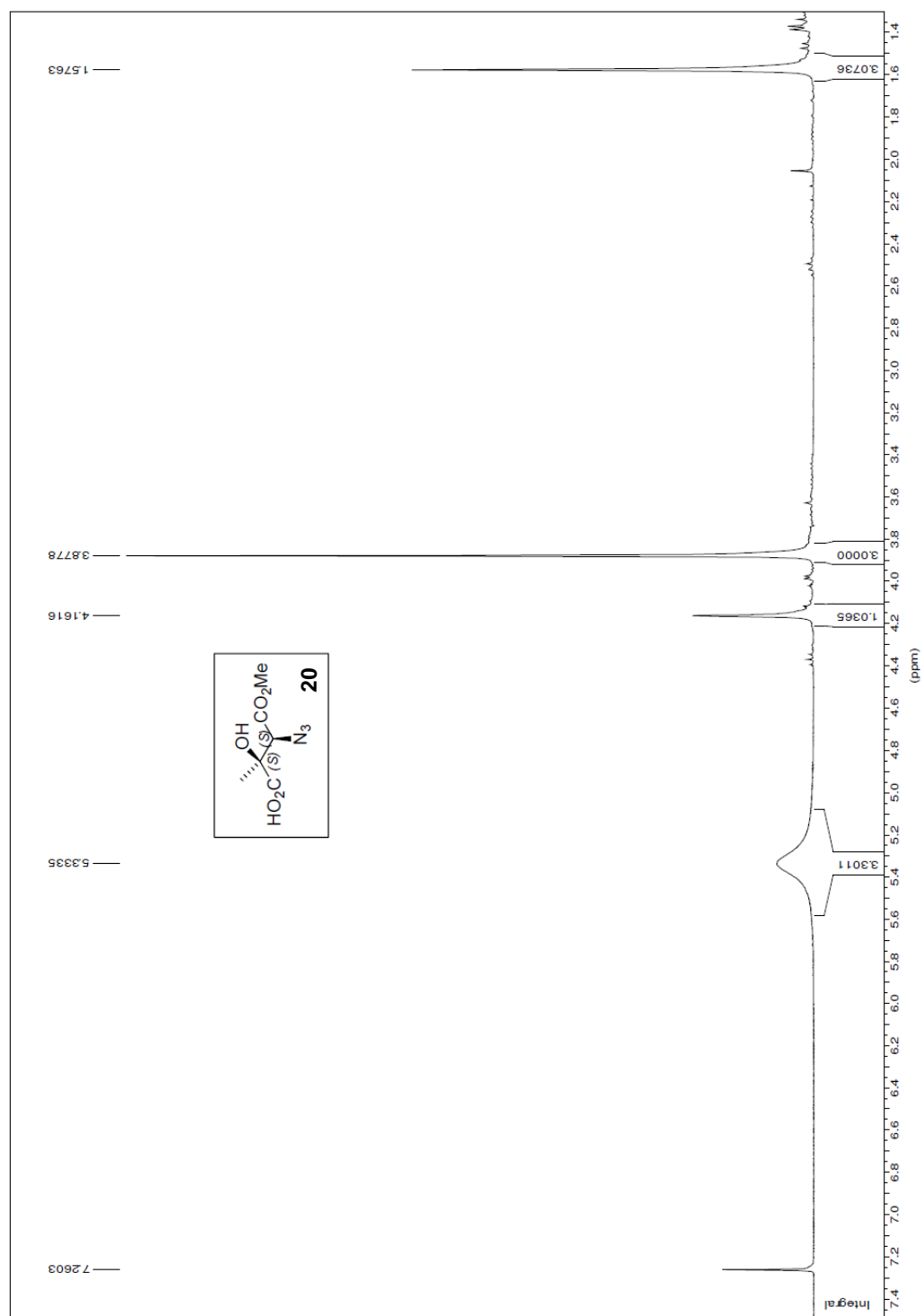


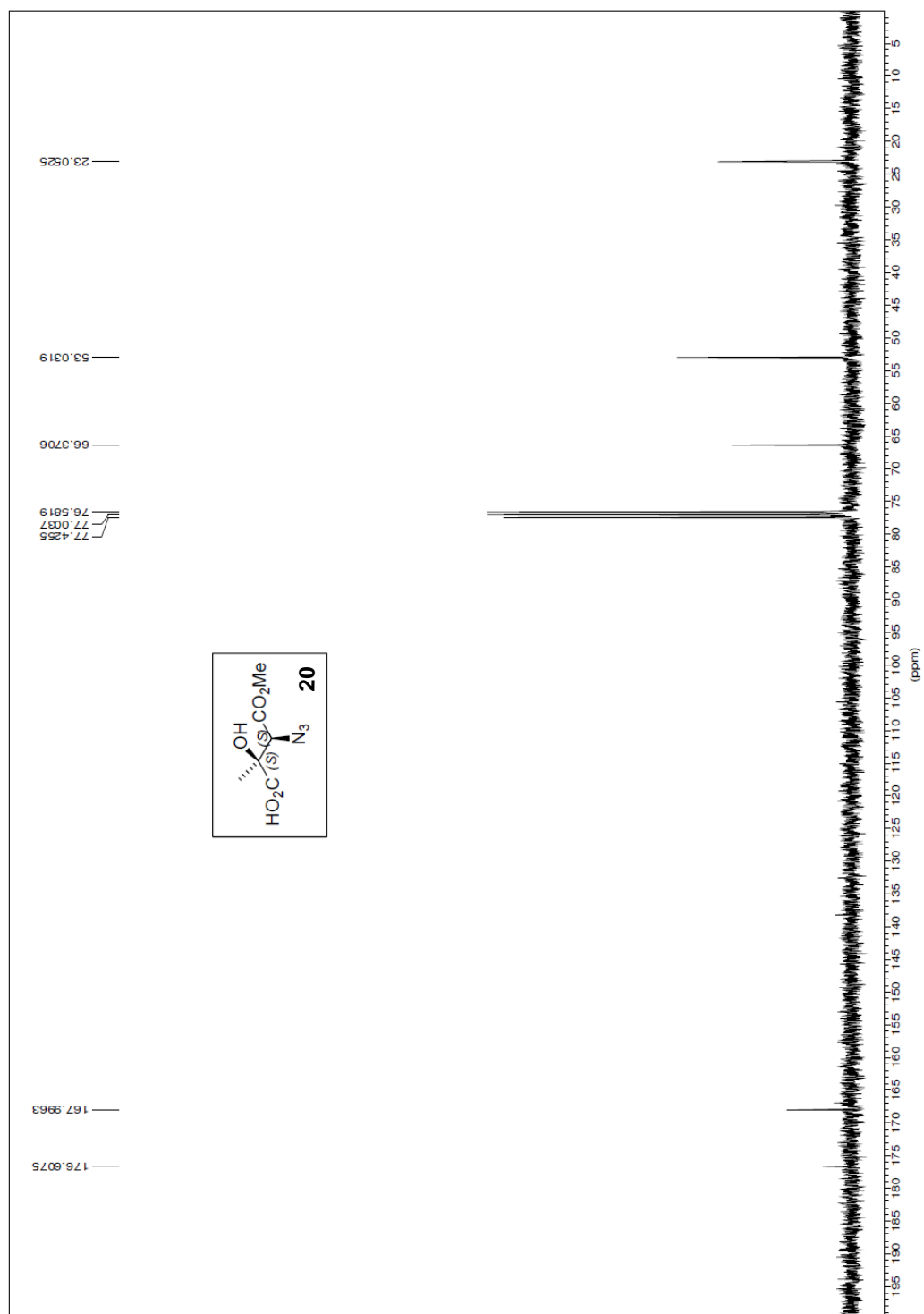


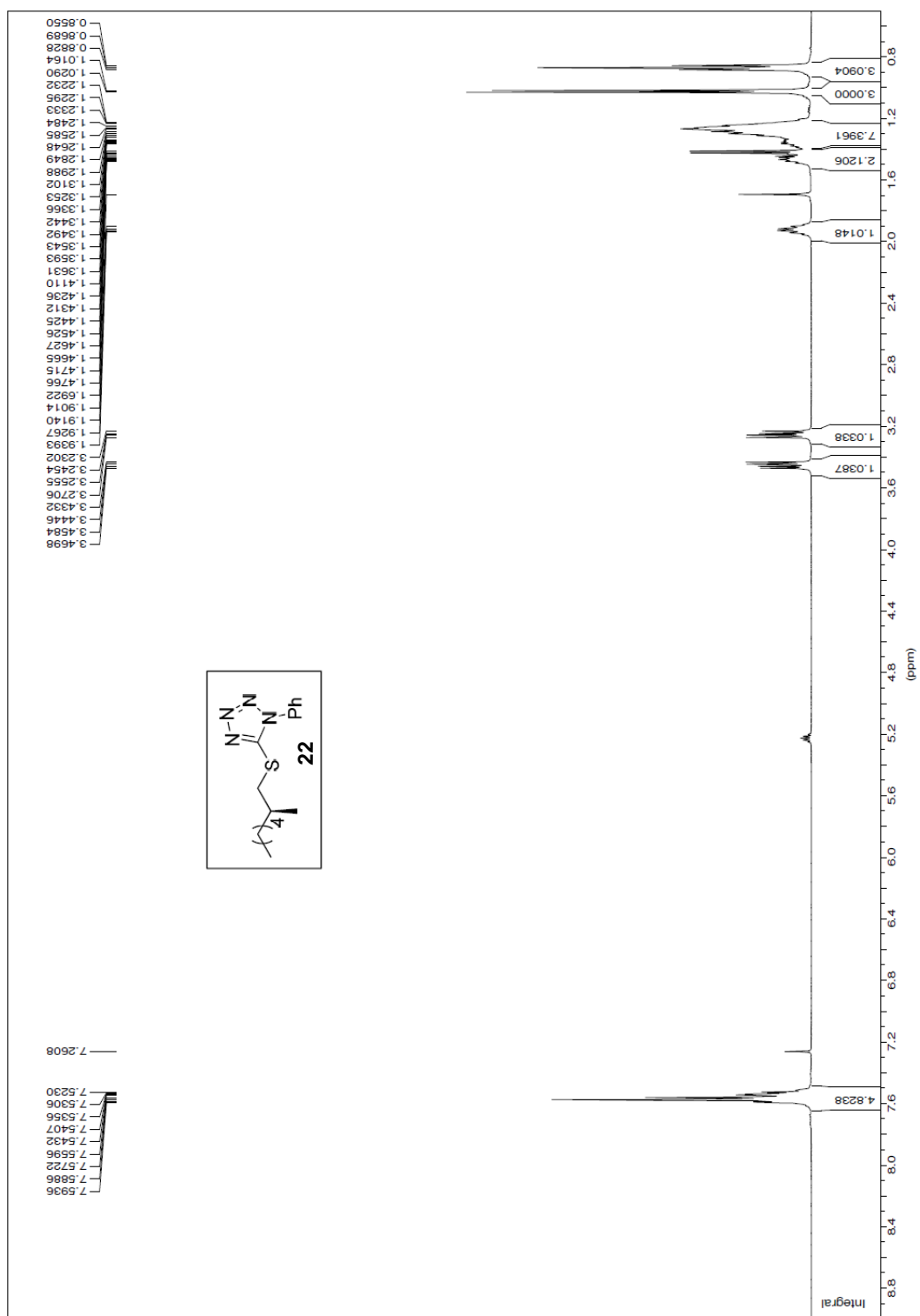


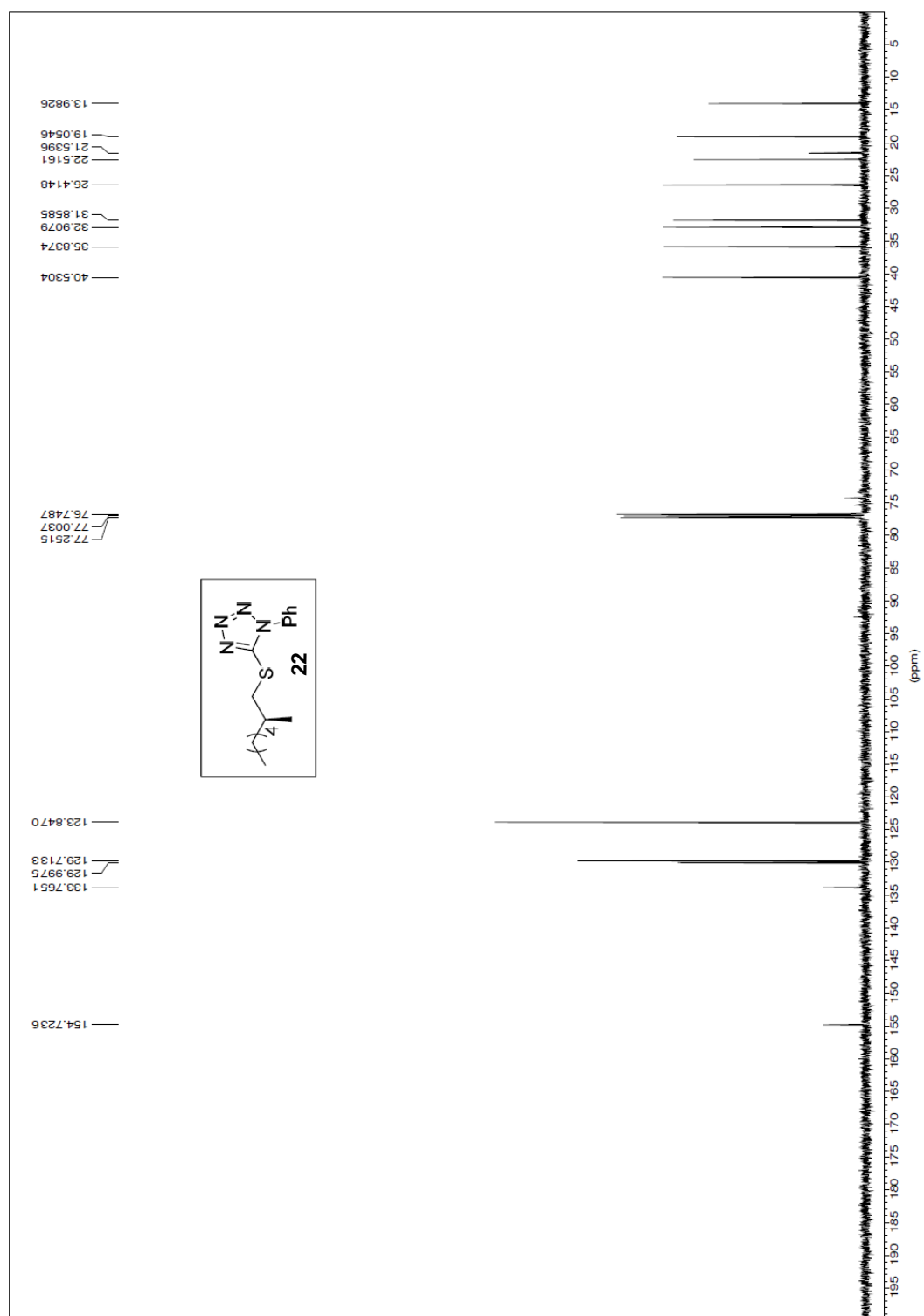


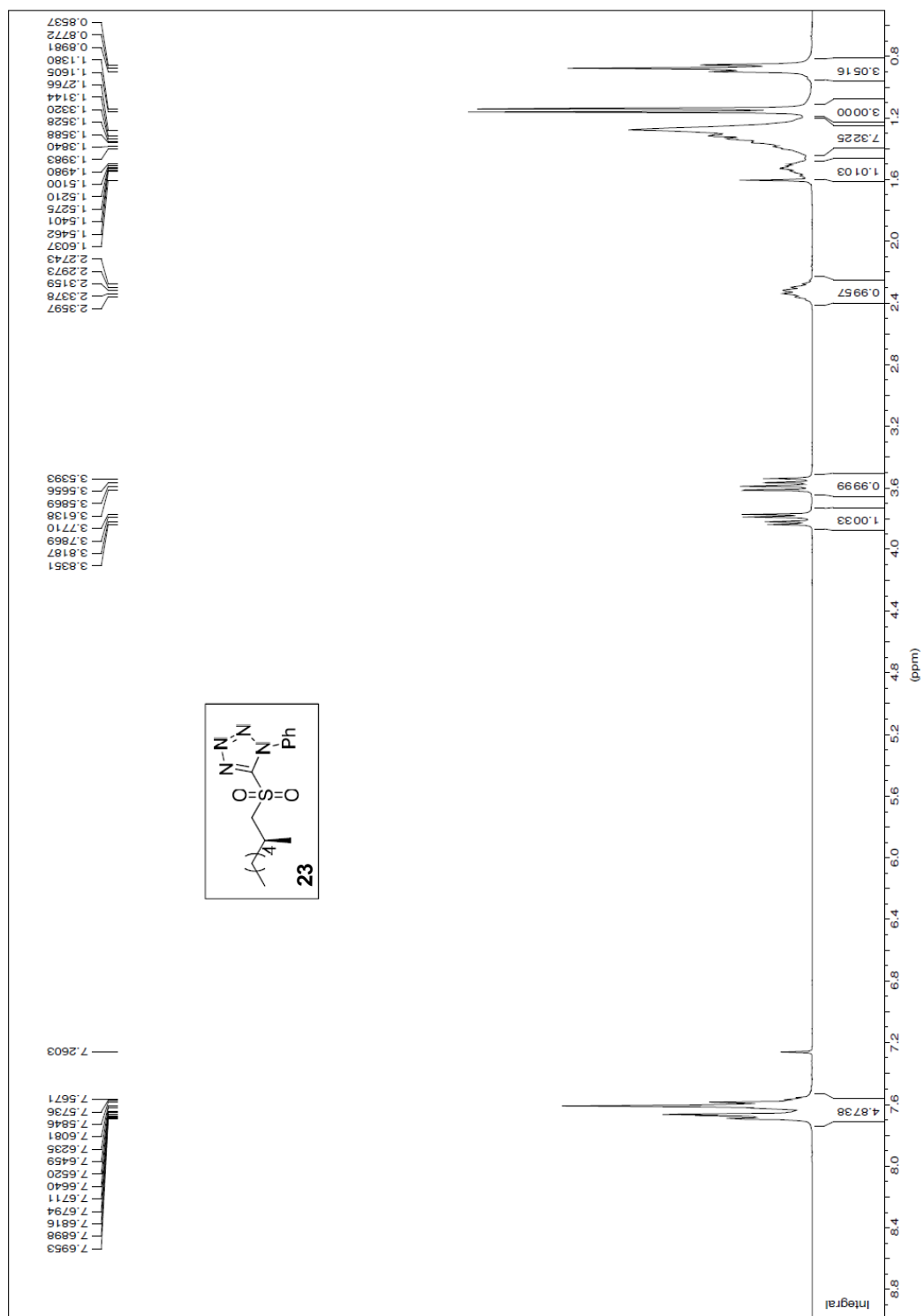


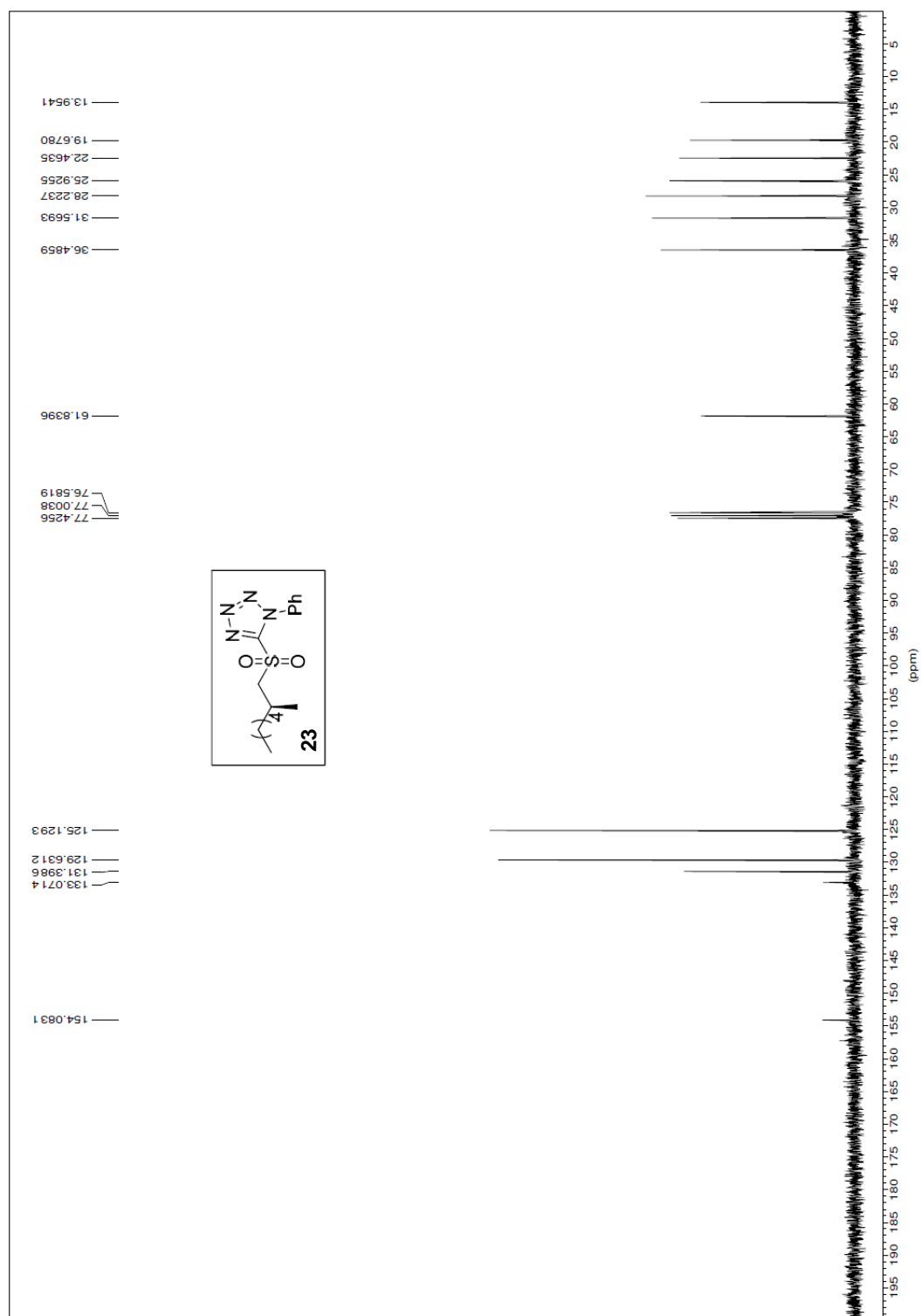


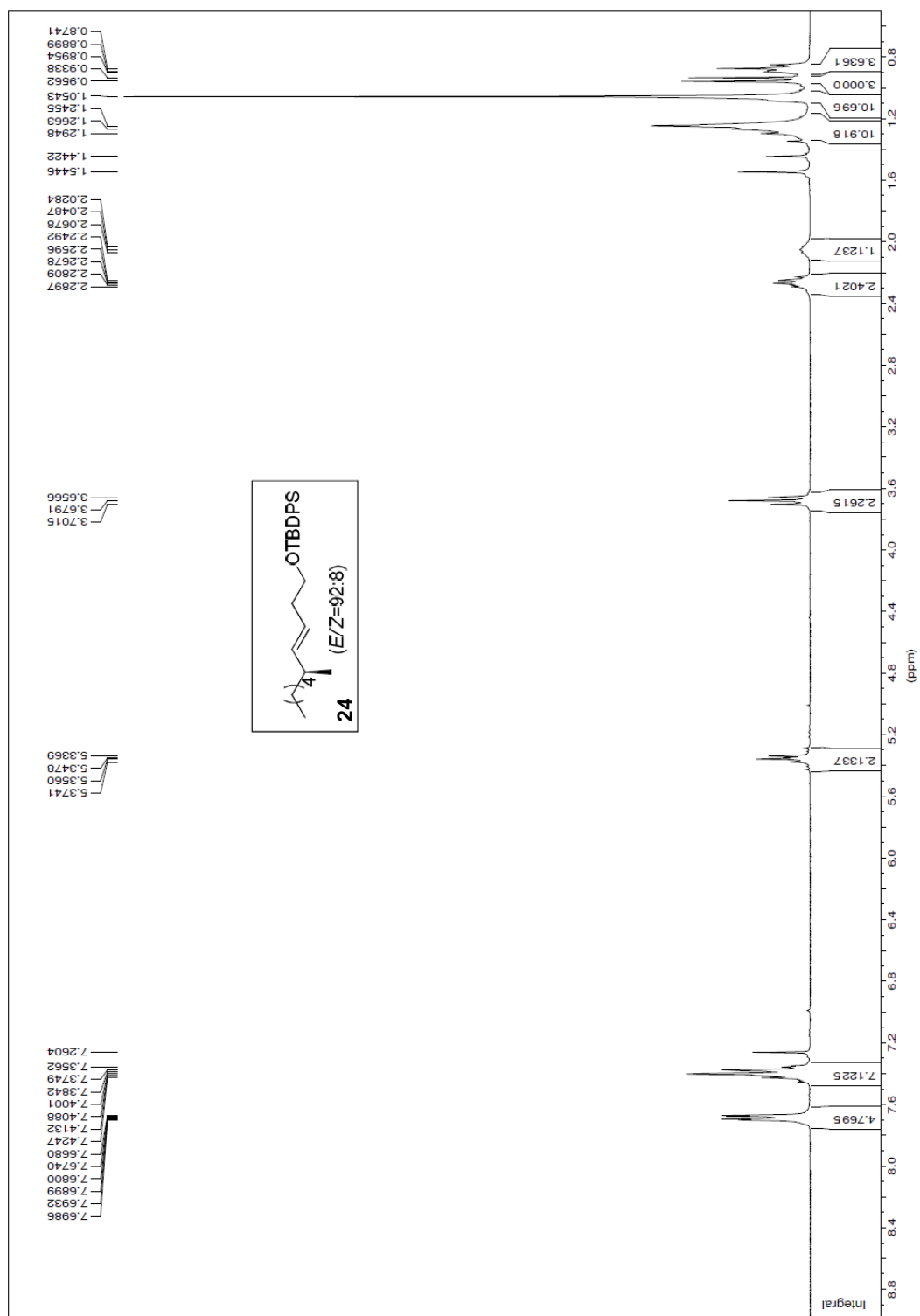


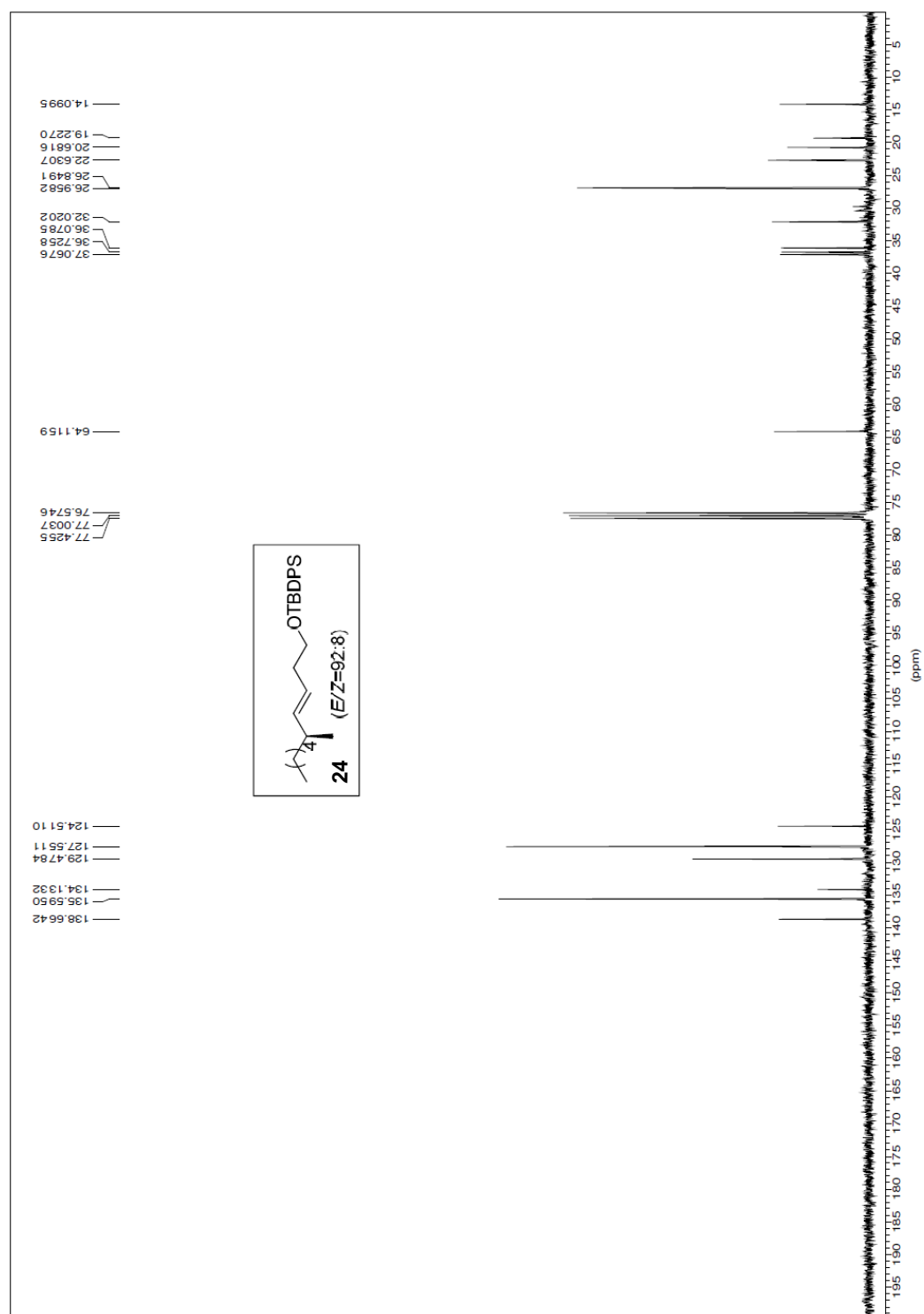


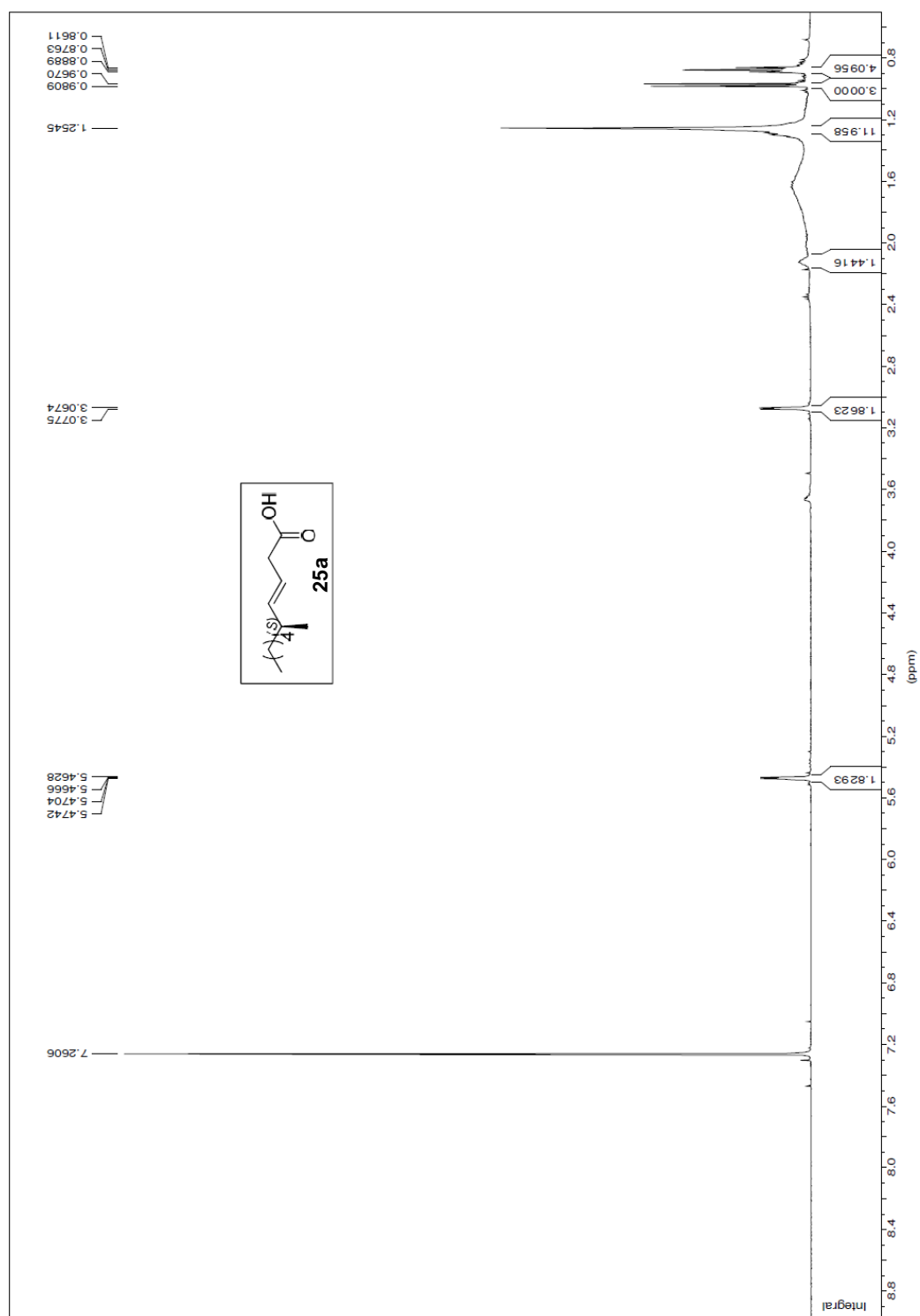


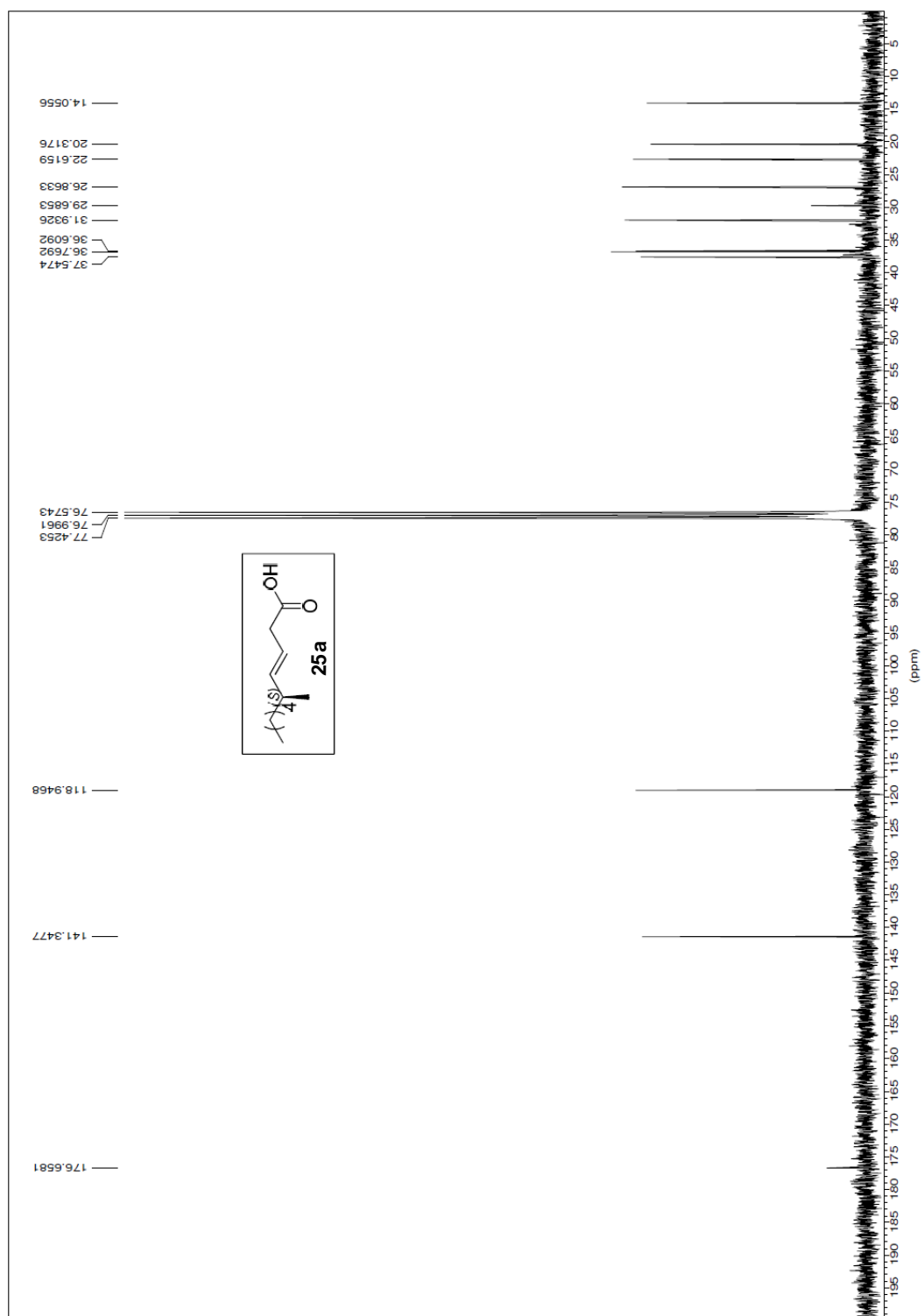


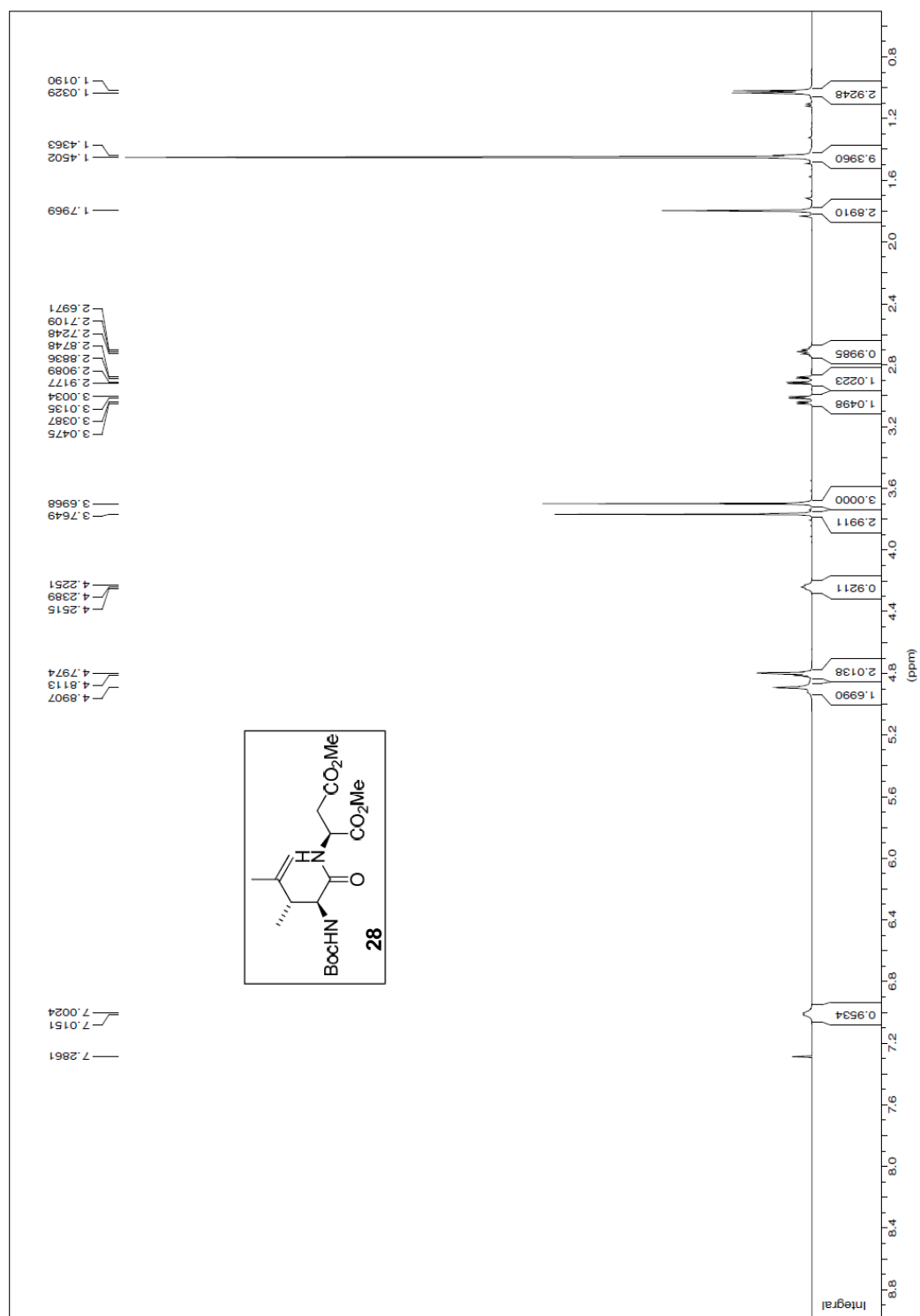


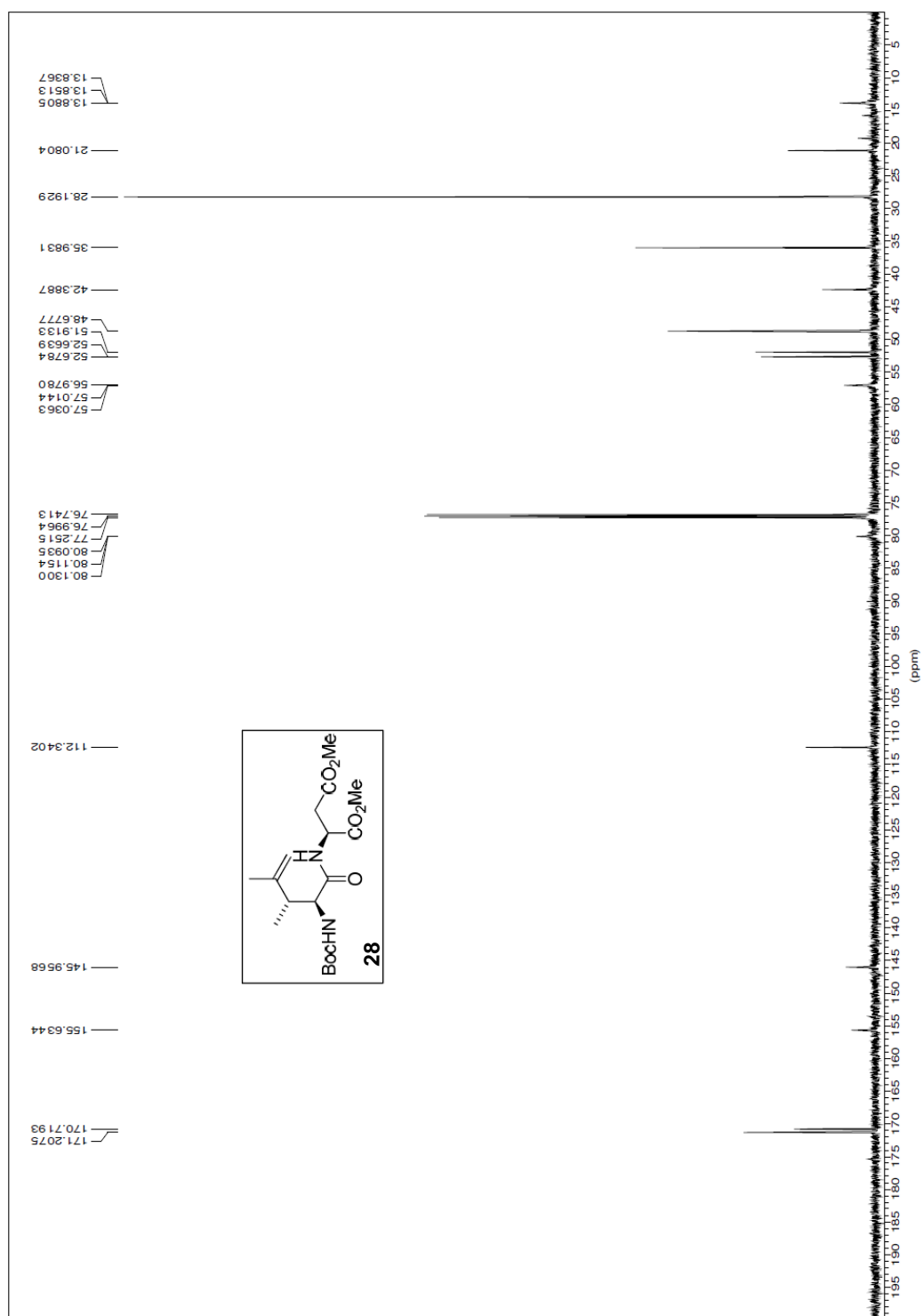


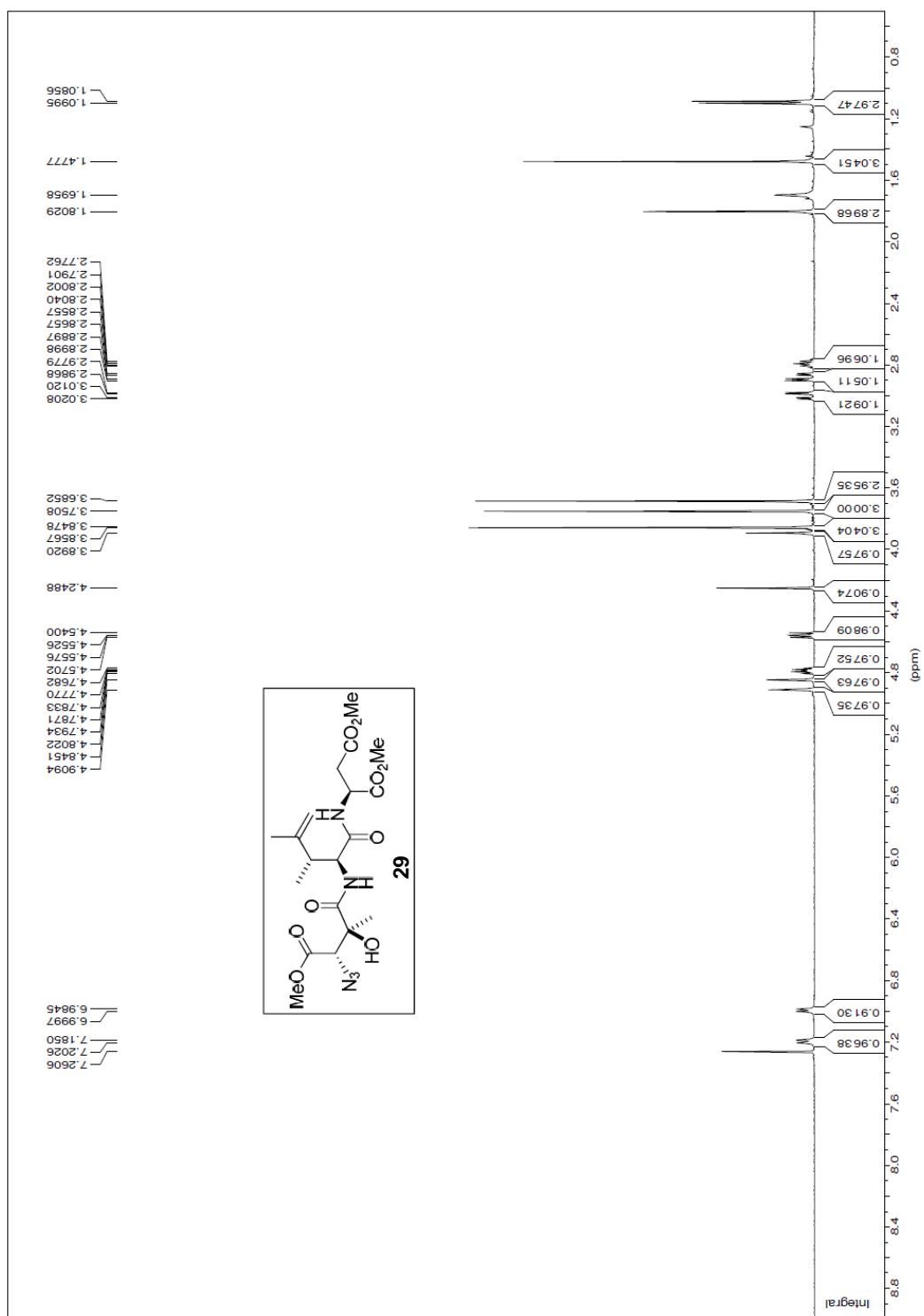


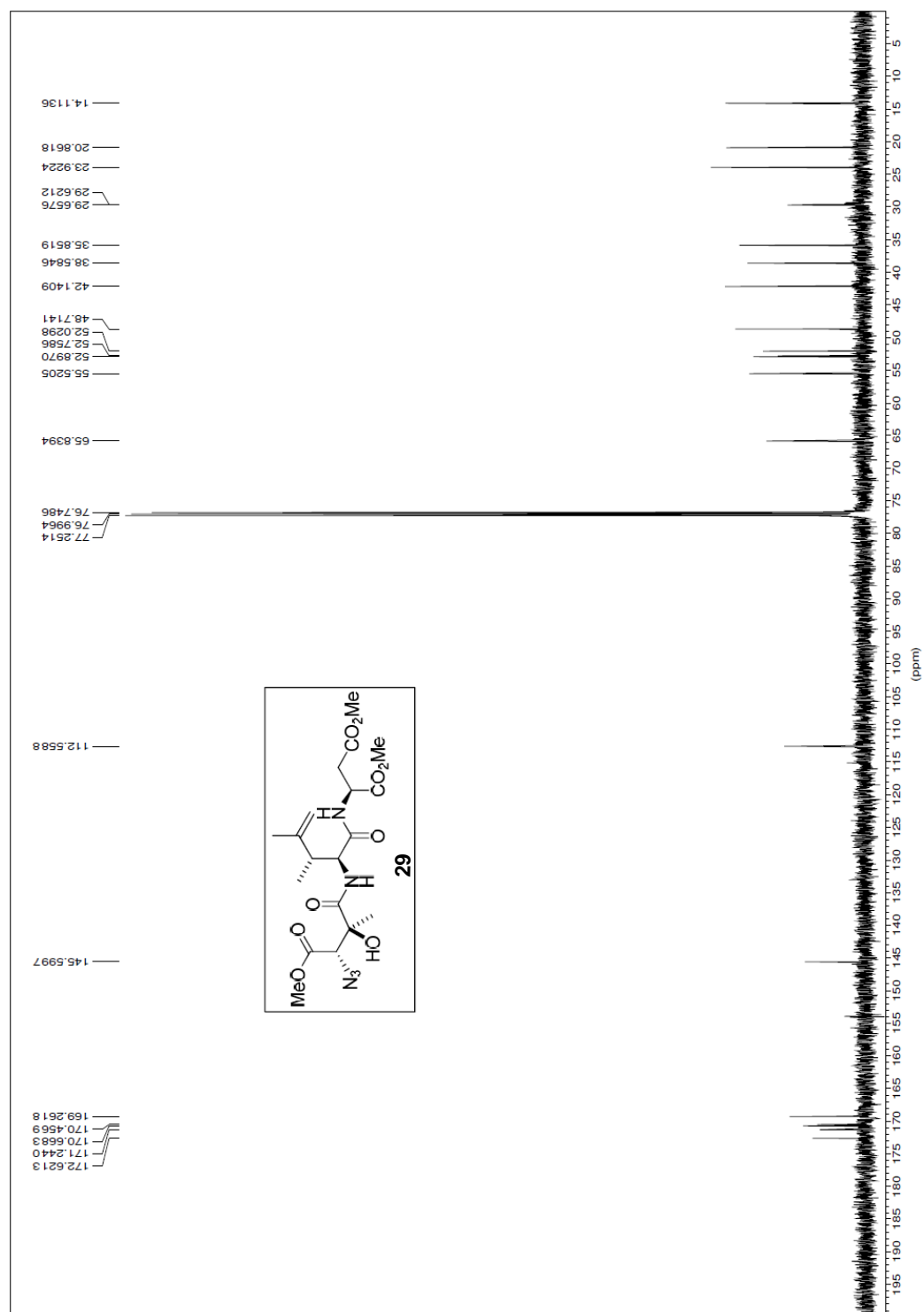


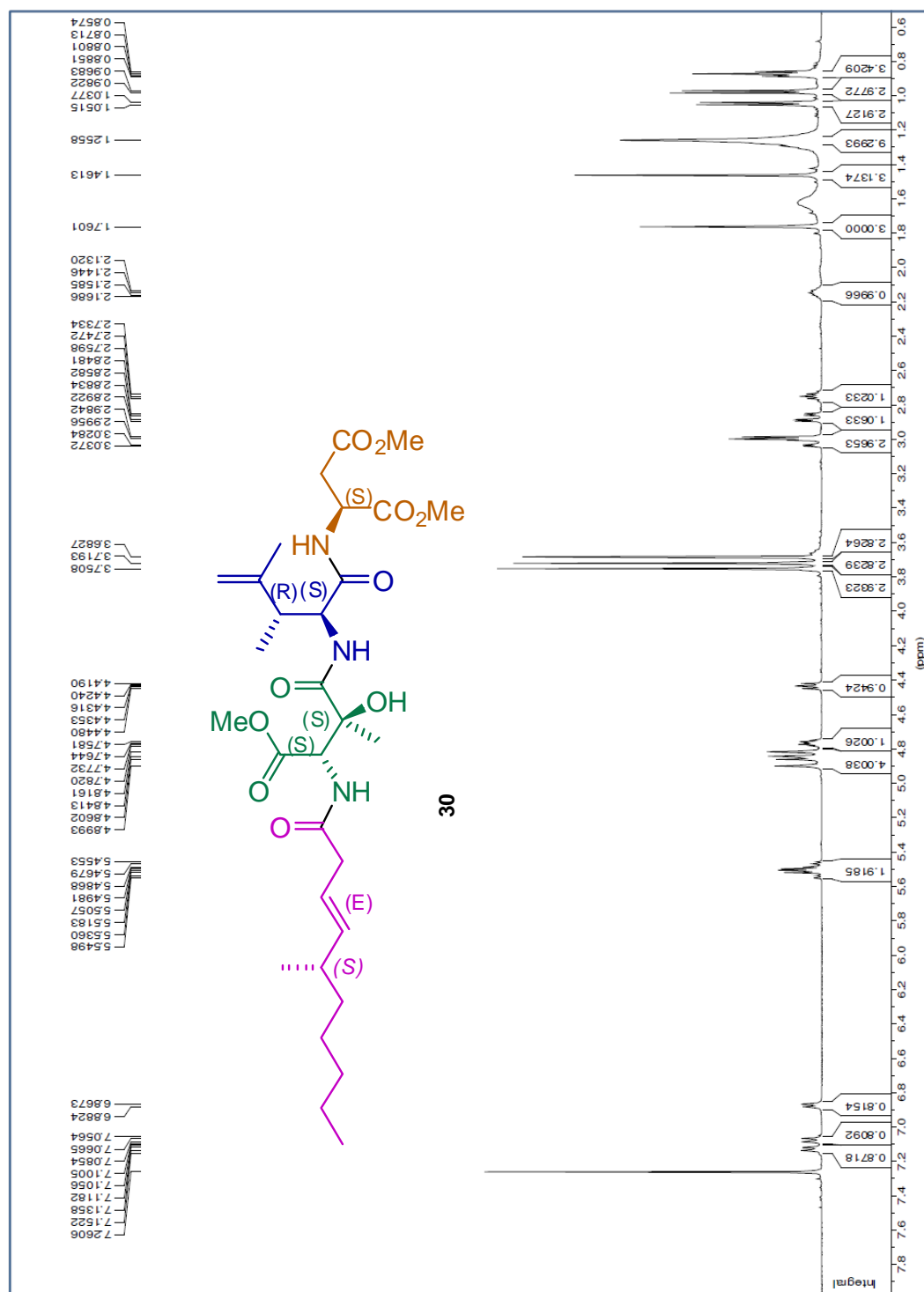


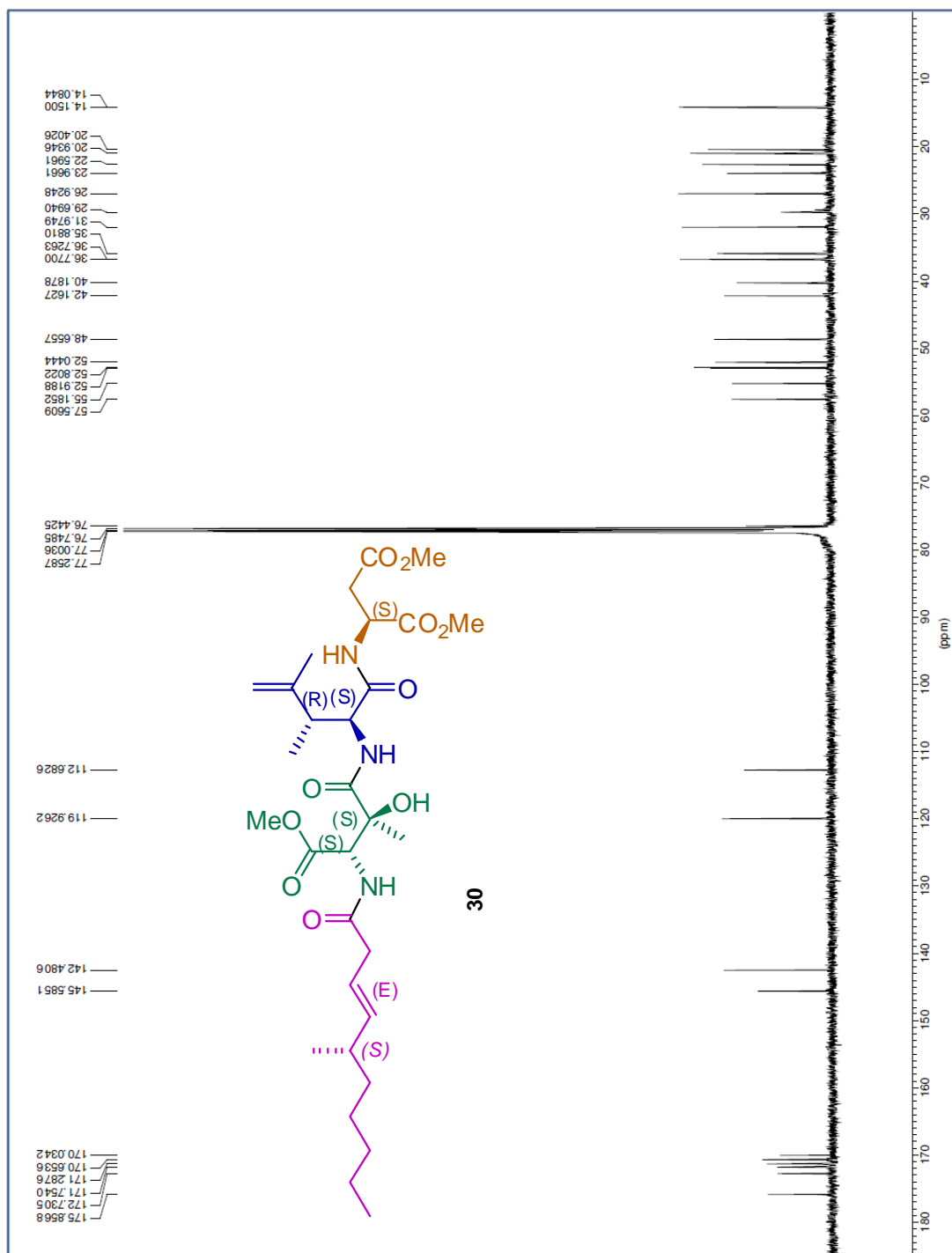














Current Data Parameters
 NAME: ak91022
 EXPNO: 2
 PROCNO: 1

F2 - Acquisition Parameters
 Date_: 20101022
 Time: 13.36

INSTRUM: spect
 PULPROG: 5 mm TXI 1H-13
 TD: 2048
 SOLVENT: C6D6
 NS: 16
 DS: 8
 SWH: 3822.630 Hz
 FIDRES: 1.860311 Hz
 AQ: 0.268092 sec
 RG: 181
 DW: 130.800 usec
 DE: 6.00 usec
 TE: 298.0 K

D0: 0.0000300 sec
 D1: 1.0000000 sec
 D13: 0.0000000 sec
 D16: 0.0002000 sec
 INO: 0.00026160 sec

===== CHANNEL f1 =====
 NUC1: 1H
 P0: 8.20 usec
 F1: 9.20 usec
 F11: 4.00 usec
 SFO1: 500.2320434 MHz

===== GRADIENT CHANNEL =====
 P16: 1500.00 usec

F1 - Acquisition parameters
 ND0: 1
 FIDRES: 500.232 MHz
 FIDRES: 7.466074 Hz
 SW: 7.642 ppm
 FMODE: QF

F2 - Processing parameters
 SI: 32
 SF: 500.2300003 MHz
 NPM: 0
 SSB: 0
 LB: 0.00 Hz
 GB: 0
 PC: 1.40

F1 - Processing parameters
 SI: 32
 SF: 500.2300003 MHz
 NPM: 0
 SSB: 0
 LB: 0.00 Hz
 GB: 0

