Supplementary Information

Enzyme-Responsive Reporter Molecules for Selective Localization and Fluorescent Imaging of Pathogenic Biofilms

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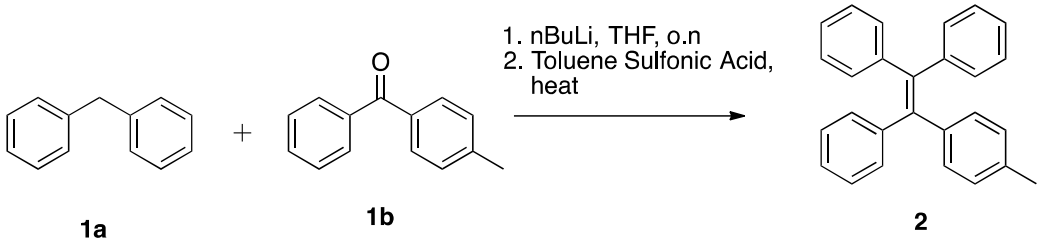
Experimental Section

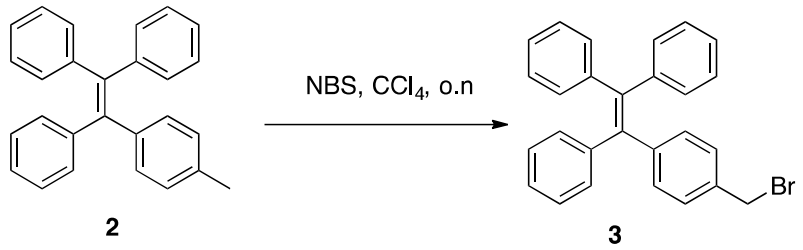
General Information: All the chemicals were purchased from Sigma Aldrich. Commercially available reagents were used without further purification. Three bacterial strains *Escherichia coli* (*E. coli*) DH5*α* (ATCC 53868), *Escherichia coli (E. coli)* Bl-21 (ATCC 13032) and *Enterobacter cloacae (E. cloacae)* (ATCC 13047) were purchased from American Type Culture Collection (ATCC), USA. Respective biofilms were constructed on glass slides surface in 50 mL centrifuge tube under constant shaking in laboratory shaker overnight. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance 300 spectrometer or Bruker Avance 400 spectrometer. Mass spectra (MS) were measured with Thermo LCQ Deca XP Max or Thermo Finnigan MAT 95 XP mass spectrometer for electrospray ionization mass spectra (ESI). Flash column chromatography was performed using Merck silica gel 60 with distilled solvents. Reverse-phase HPLC analysis was performed on a Shimadzu HPLC system using an Alltima C-18 (250 × 10 mm) column at a flow rate of 3.0 mL/min for preparation and a C-18 (250×4.6 mm) at a flow rate of 1.0 mL/min for analysis. Fluorescence emission spectra were performed on a Varian Cary eclipse Fluorescence Spectrophotometer. UV absorption spectra were recorded in a 10 mm path quartz cell on a Beckman coulter DU800 spectrometer. Dynamic light scattering (DLS) measurements were performed using Brookhaven 90 Plus Nanoparticle Size Analyzer. Fluorescence microscopic imaging and confocal laser scanning microscopic imaging were conducted with Zeiss LSM 800 Confocal Microscope.

Synthesis of ERM-1 and ERM-2



Synthesis of 2:

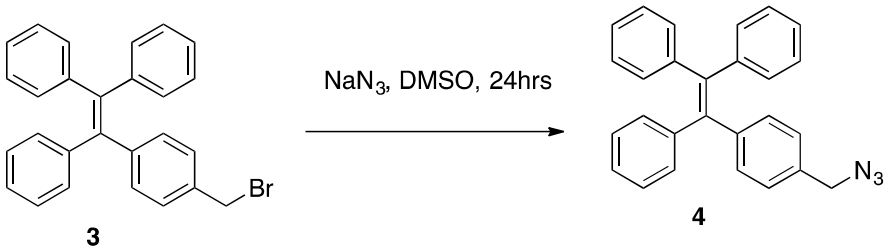
Diphenylmethane (2.03 g, 15 mmol) was dissolved into anhydrous THF (50 mL) and was subsequently cooled to 0°C. Next, n-butyllithium (7.50 mL, 2.5 M) was added drop wise into the reaction mixture. After stirring for 1 hr, 4-methylbenzophenone (2.45 g, 12.50 mmol) was added and allowed to slowly warm to room temperature while being stirred overnight. The reaction mixture was then cooled to room temperature and quenched with aqueous ammonium chloride. The organic materials were extracted thrice with ethyl acetate. The combined extracts were washed thrice with water and once with brine, and dried over $MgSO_4$. The solvents were removed under reduced pressure and the crude was purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate = 99:1) and is followed by purification with flash chromatography to afford **2** as a white solid (1.69 g, 4.88 mmol, yield 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.08-7.21 (m, 15H), 7.04 (m, 4H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 144.08, 141.07, 140.90, 140.62, 136.15, 131.48, 131.44, 131.36, 128.51, 127.81, 127.75, 126.46, 126.41, 21.33. MS (ESI) m/z: 347.24, calculated for $[M+H]^+$: 347.47.



Synthesis of 3:

Compound **2** (1.04 g, 3 mmol) was dissolved in CCl_4 (20 mL), followed by addition of *N*-bromosuccinimide (0.55 g, 3.2 mmol) and a catalytic amount of benzoyl peroxide. Reaction mixture was stirred under reflux condition for 12 h. The organic materials were extracted thrice with CH_2Cl_2. The combined extracts were washed thrice with

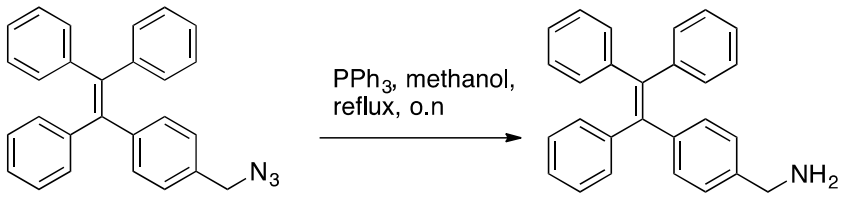
water and once with brine, and dried over $MgSO_4$. The solvents were removed under reduced pressure and the crude was purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate = 99:1) to afford **3** as a white solid (0.71 g, 1.67 mmol, yield 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.16−7.00 (m, 19H), 4.43 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 145.01, 144.11, 143.74, 143.44, 141.49, 140.14, 139.09, 135.79, 131.66, 131.28, 128.51, 127.68, 126.56, 33.64. MS (ESI) m/z: 425.54, calculated for $[M]^+$: 425.37



3 **4**

Synthesis of 4:

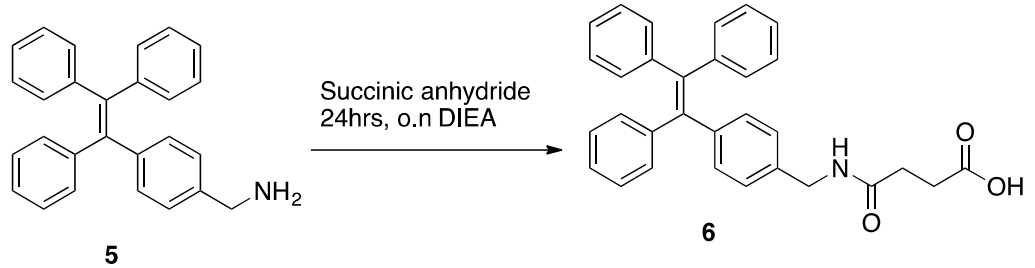
Compound **3** (0.85 g, 1.67 mmol) was dissolved in dimethyl sulfoxide (10 mL), followed by addition of sodium azide (0.20 g, 3 mmol). The reaction mixture was stirred under a nitrogen atmosphere at room temperature overnight. Subsequently, mixture was quenched with water (50 mL). The organic materials were extracted thrice with diethyl ether. The combined extracts were washed thrice with water and once with brine, and dried over $MgSO_4$. The solvents were removed under reduced pressure and the crude was purified by flash column chromatography (silica gel, *n*-hexane/Chloroform = 80:20) to afford **4** as a white solid (0.73 g, 1.88 mmol, yield 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.08-7.17 (m, 21H), 4.30 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 144.01, 143.61, 141.59, 140.39, 133.40, 131.84, 131.41, 127.87, 127.76, 127.67, 126.63, 54.59. MS (ESI) m/z: 409.64, calculated for $[M+Na]^+$: 409.49.



4 **5**

Synthesis of 5:

Compound **4** (0.73 g, 1.88 mmol) was dissolved in anhydrous methanol (10 mL), followed by addition of triphenylphosphine (1.00 g). The reaction mixture was stirred under reflux condition overnight. After confirmation with TLC, crude mixture was concentrated under reduced pressure and purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate = 20:80) to afford **5** as a white solid (0.55 g, 1.52 mmol, yield 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.12−7.01 (m, 19H), 3.84 (s, 2H), 1.75 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 143.70, 143.54, 143.51, 141.40, 140.23, 134.61, 131.76, 131.26, 127.78, 127.72, 127.66, 127.45, 126.57, 126.53, 43.88, 23.22. MS (ESI) m/z: 361.18, calculated for $[M]^+$: 361.49



5 **6**

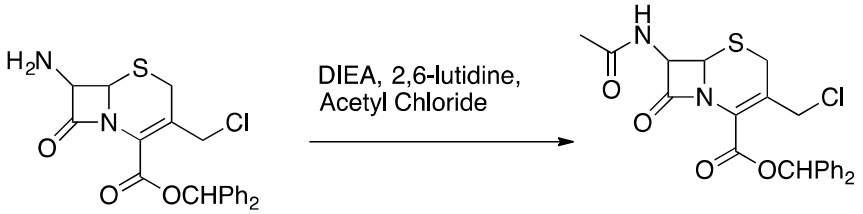
Synthesis of 6:

Compound **5** (0.55 g, 1.52 mmol) was dissolved in CH_2Cl_2 (10 mL), followed by addition of succinic anhydride (0.15 g, 15 mmol) and *N*,*N*-diisopropylethylamine (261 μ L). The reaction mixture was stirred at room temperature overnight. After confirmation with TLC, crude mixture was concentrated under reduced pressure and purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate = 50:50), to afford **6** as a white solid (0.31 g, 0.67 mmol, yield 45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.02-7.14 (m, 20H), 4.38 (d, J = 6.00 Hz, 2H), 2.74 (t, J = 6.00Hz, 2H), 2.54 (t, J = 6.00 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 143.60, 143.26, 141.28, 140.32, 135.54, 131.70, 131.28, 127.75, 127.70, 127.65, 127.03, 126.50, 43.61, 30.73, 29.69. MS (ESI) m/z: 462.09, calculated for $[M+H]^+$: 462.56

7

Synthesis of 7:

2-(2-amino-1,3-thiazol-4-yl)- 2-(methoxyimino) acetic acid (500 mg, 4.97 mmol) was dissolved in $CHCl_3$/DMF 2:1 (20 mL) and TEA (1.5 mL, 11 mmol). The reaction mixture was stirred at 0℃. Subsequently, 4-methyl trityl chloride (800 mg, 5.47 mmol) was added to the solution. The reaction mixture was slowly warmed to room temperature and stirred overnight. After confirmation with TLC, chemical mixture was quenched with aqueous hydrogen chloride (1M). The organic materials were extracted thrice with $CHCl_3$. The combined extracts were washed thrice with water and once with brine, and dried over $MgSO_4$. The solvents were removed under reduced pressure and the crude was purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate = 80:20) to afford **7** as a white solid (413 mg, 1.00 mmol, yield 45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.12-7.31 (m, 15H), 6.58 (s, 2H), 3.94 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 169.38, 141.91, 136.83, 129.38, 128.39, 127.92, 108.25, 72.94, 63.08, 29.71, 14.14. MS (ESI) m/z: 479.99, calculated for $[M+Na]^+$: 480.55

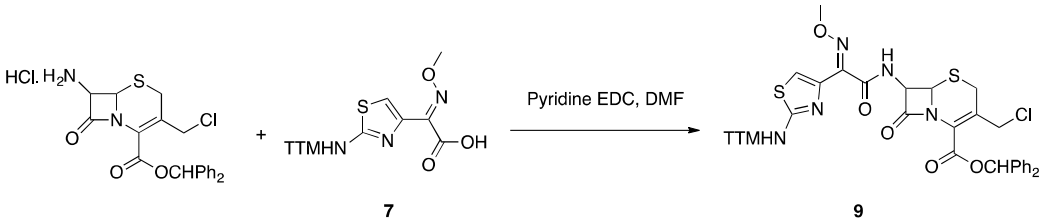


8

Synthesis of 8:

7-Amino-3-chloromethyl-3-cephem-4-carboxylic acid diphenylmethyl ester hydrochloride (451 mg, 1.00 mmol) was dissolved in CH_2Cl_2 (10 mL), followed by addition of N,N-diisopropylethylamine (174 μL), acetyl chloride (71 μL) and 2,6-lutidine (116 μL). The reaction mixture was stirred under room temperature overnight. After confirmation with TLC, organic materials were extracted thrice with CH_2Cl_2. The combined extracts were washed thrice with water and once with brine, and dried over $MgSO_4$. The solvents were removed under reduced pressure and the crude was purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate = 50:50) to afford **8** as a white solid (390 mg, 0.78 mmol, yield 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.25-7.44 (m, 10H), 6.75 (d, J = 8.91 Hz, 1H), 5.90 (dd,J = 4.92 Hz, 8.91 Hz,

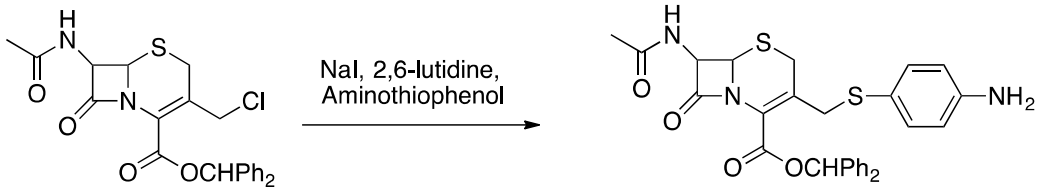
1H), 4.99 (d, *J* = 4.98 Hz, 1H), 4.40 (s, 2H), 3.53 (q, *J* = 17.97 Hz, 2H), 2.03 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.64, 165.44, 160.54, 139.11, 138.96, 128.66, 128.54, 128.34, 128.22, 127.71, 127.39, 126.95, 125.57, 79.89, 77.53, 77.11, 76.69, 59.20, 57.75, 43.18, 27.24, 22.74. MS (ESI) m/z: 456.87, calculated for $[M]^+$: 456.94



7 **9**

Synthesis of 9*:*

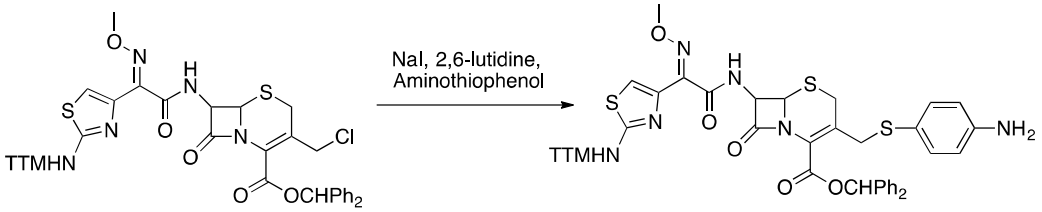
7-Amino-3-chloromethyl-3-cephem-4-carboxylic acid diphenylmethyl ester hydrochloride (451 mg, 1 mmol) was dissolved in dimethylformamide (10 mL), followed by addition of pyridine (79.1mg, 1 mmol), EDC hydrochloride (191.7 mg, 1 mmol) and compound **7** (447 mg, 1 mmol). The mixture was stirred under room temperature overnight. After confirmation with TLC, organic materials were extracted thrice with ethyl acetate. The combined extracts were washed thrice with water and once with brine, and dried over $MgSO_4$. The solvents were removed under reduced pressure and the crude was purified by flash column chromatography (silica gel, *n*-hexane/ethyl acetate = 50:50) to afford a yellow solid **9** (469.70 mg, 0.87 mmol, 55%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.26-7.37 (m, 19H), 7.11-7.19 (m, 7H), 6.87 (d, *J* = 8.94 Hz, 1H), 6.73 (s, 1H), 5.96 (dd, *J* = 4.92 Hz, 8.91Hz, 1H), 5.08 (d, *J* = 4.95 Hz, 1H), 4.40 (d, *J* = 2.97 Hz, 2H), 4.04 (s, 3H), 3.56 (q, *J* = 18.00 Hz, 2H), 2.33 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 168.62, 163.97, 162.31, 160.42, 147.19, 143.40, 143.24, 140.80, 140.05, 139.05, 138.92, 137.43, 129.27, 129.21, 129.17, 128.97, 128.65, 128.57, 128.36, 128.24, 127.75, 127.55, 126.99, 125.75, 113.03, 71.58, 63.32, 58.94, 57.70, 43.10, 27.36, 21.04MS (ESI) m/z: 854.65, calculated for $[M+H]^+$: 854.44



8 **10**

Synthesis of 10:

Compound **8** (250 mg, 0.50 mmol) was dissolved in dimethyl formamide (2 mL), followed by addition of 4-aminothiophenol (63 mg, 0.50 mmol). The reaction mixture was stirred under room temperature for 10 minutes. Subsequently, sodium iodide (150 mg, 1.00 mmol) and 2,6-lutidine (58 μL) was added. The chemical mixture was stirred overnight. After confirmation with TLC, organic materials were extracted twice with ethyl acetate, water and brine. The combined extracts were then dried over reduced pressure and purified using flash chromatography to afford **10** (201 mg, 0.37 mmol, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.27-7.34 (m, 11H), 7.12 (d, J = 6.00 Hz, 2H), 6.89 (d, J = 8.76 Hz, 1H), 6.45 (d,J = 6.00 Hz, 2H), 5.80 (dd,J = 4.71 Hz, 8.85 Hz, 1H), 4.88 (d, J = 4.71 Hz, 1H), 4.20 (d,J = 8.79 Hz, 1H), 3.59 (d,J = 13.08 Hz, 1H), 3.36 (q, J = 17.76 Hz, 2H), 2.04 (s, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.55, 165.32, 160.66, 147.11, 139.64, 139.42, 135.70, 133.71, 128.75, 128.12, 127.97, 127.18, 123.97, 120.57, 79.26, 59.08, 58.01, 38.91, 28.75, 22.80. MS (ESI) m/z: 545.98, calculated for $[M]^+$: 545.67

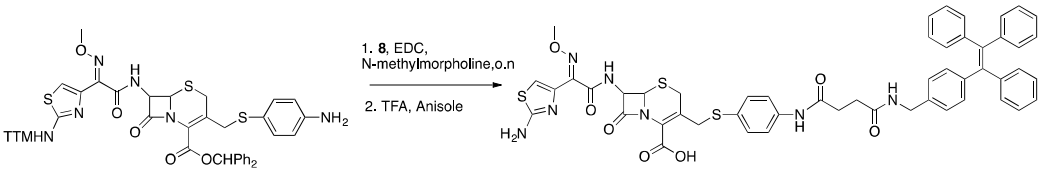


9 **11**

Synthesis of 11:

Compound **9** (427 mg, 0.50 mmol) was dissolved in dimethyl formamide (2 mL), followed by addition of 4-aminothiophenol (63 mg, 0.50 mmol). The reaction mixture was stirred under room temperature for 10 minutes. Subsequently, sodium iodide (150 mg, 1.00 mmol) and 2,6-lutidine (58 μL) was added. The chemical mixture was stirred overnight. After confirmation with TLC, organic materials were extracted

twice with ethyl acetate, water and brine. The combined extracts were then dried over reduced pressure and purified using flash chromatography to afford **11** (349 mg, 0.37 mmol, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.02-7.40 (m, 27H), 6.89 (s, 1H), 6.86 (d, J = 6.00 Hz, 2H), 6.76 (d, J = 6.00 Hz, 2H), 6.46 (d, J = 6.00 Hz, 2H), 5.89 (dd, J = 4.68 Hz, 8.76 Hz, 1H), 4.98 (d, J = 4.74 Hz, 1H), 4.13 (s, 3H), 3.67 (d, J = 13.29 Hz, 2H), 3.61 (s, 2H), 3.36 (d, J = 17.80 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 168.62, 163.68, 162.24, 160.54, 147.22, 147.03, 143.33, 143.27, 140.03, 139.58, 139.38, 137.45, 135.66, 132.82, 129.20, 128.96, 128.54, 128.44, 128.23, 128.10, 127.96, 127.62, 127.57, 127.02, 124.16, 120.66, 115.47, 113.22, 79.22, 71.58, 63.32, 58.76, 57.80, 38.91, 28.86, 21.02. MS (ESI) m/z: 944.77, calculated for $[M]^+$: 944.17

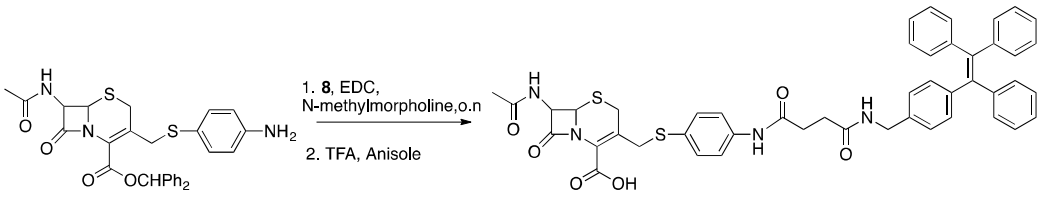


11 ERM-1

Synthesis of ERM-1:

Compound **11** (170 mg, 0.18 mmol) was dissolved in dimethyl formamide (10 mL), followed by addition of compound **8** (81.80 mg, 0.18 mmol), ethylcarbodiimide hydrochloride (70.30 mg, 0.37 mmol) and *N*-methylmorpholine N-oxide (20 μL). The reaction mixture was stirred overnight under room temperature. After confirmation by TCL, organic materials were extracted thrice with ethyl acetate. The combined extracts were washed thrice with water and once with brine, and dried over $MgSO_4$. The crude mixture was subsequently added with CH_2Cl_2 (1 mL), trifluoroacetic acid (300 μL), and anisole (20 μL) was added into the reaction mixture, which was stirred under room temperature for 1 h. The solvents were removed under reduced pressure and subsequently crude mixture was purified by reverse phase HPLC to afford white solid **ERM-1** (38.3 mg, 22%). ^{1}H NMR (300 MHz, Acetone-D_6) δ (ppm): 6.91-7.31 (m, 21H), 6.75 (d, J = 8.28 Hz, 1H), 6.64 (d, J =8.49 Hz, 2H), 5.71 (t, J = 4.92 Hz, 1H), 5.15 (d, J = 4.74, 1H), 4.35 (s, 2H), 3.88 (s, 3H), 3.94 (d, J = 17.76 Hz, 1H), 3.47 (d, 18.69 H), 3.47 (d, J = 18.21 Hz, 1H), 2.63 (t, J = 6.30 Hz, 2H), 2.58 (t, J = 5.61 Hz, 2H). ^{13}C NMR (75 MHz, Acetone-D_6) δ (ppm):δ 174.45, 171.38, 148.16, 144.07, 141.0, 131.01, 130.98, 127.98,127.72, 127.64, 127.44, 126.69, 126.65, 118.46, 115.05, 60.09,

58.38, 54.28, 42.33, 35.85, 20.14, 16.72. MS (ESI) m/z: 964.08, calculated for $[M]^+$: 964.14



Synthesis of ERM-2:

Compound **10** (100 mg, 0.18 mmol) was dissolved in dimethyl formamide (10 mL), followed by addition of compound **8** (81.80 mg, 0.18 mmol), ethylcarbodiimide hydrochloride (70.30 mg, 0.37 mmol) and *N*-methylmorpholine N-oxide (20 μL). The reaction mixture was stirred overnight under room temperature. After confirmation by TCL, organic materials were extracted thrice with ethyl acetate. The combined extracts were washed thrice with water and once with brine, and dried over $MgSO_4$. The crude mixture was then added with 1 ml of dichloromethane, 300μL trifluoroacetic acid (300 μL), and anisole (20 μL) was added into the reaction mixture, which was stirred under room temperature for 1 h. The solvents were removed under reduced pressure and subsequently crude mixture was purified by reverse phase HPLC to afford white solid **ERM-2** (21.10 mg, 14%). ^{1}H NMR (300 MHz, Acetone-D_6) δ (ppm): 7.12 (d, J = 3.00 Hz, 2H), 6.96-7.11 (m, 20H), 6.99 (d, J = 8.37, 2H), 6.64 (d, J = 9.00 Hz, 2H), 5.73 (t, J = 4.83 Hz, 1H), 5.05 (dd, J = 3.00 Hz, 6.00 Hz, 1H), 4.36 (s, 2H), 4.34 (d, J = 7.95, 1H), 4.13 (d, J = 13.38 Hz, 1H), 3.92 (d, J = 13.38 Hz, 1H), 3.88 (d, J = 9.33 Hz, 1H), 3.70-3.79 (m, 2H), 3.63 (q, J = 16.38, 2H), 2.63 (t, J = 6.00 Hz, 2H), 2.52 (t, J = 6.00 Hz, 2H), 2.01 (s, 3H). ^{13}C NMR (75 MHz, Acetone-D_6,) δ (ppm): 173.22, 171.04, 169.58, 143.86, 142.32, 140.78, 137.71, 135.47, 133.94, 130.99, 127.72, 127.64, 127.62, 126.58, 126.44, 126.40, 126.37, 114.87, 59.52, 57.87, 53.98, 42.21, 38.81, 37.64, 34.23, 21.25. MS (ESI) m/z: 823.02, calculated for $[M]^+$: 823.00

Enzyme Hydrolysis Assay:

Reaction mixtures (100 µL each) containing ERM-1 and ERM-2 (10 µM) were incubated separately with 50 nM of AmpC and TEM-1 respectively in 0.1 M PBS buffer (pH 7.4) at 37 ℃ for 1 hr and subjected to fluorescent measurement. In control experiments, ERM-1 and ERM-2 was incubated with PBS buffer at 37 ℃ for 1 hr. To study the enzyme specificity, the enzyme, TEM-1 and AmpC is pretreated with inhibitors CA and AZT (100 µM) respectively and with ERM-1 and ERM-2 for fluorescent studies.

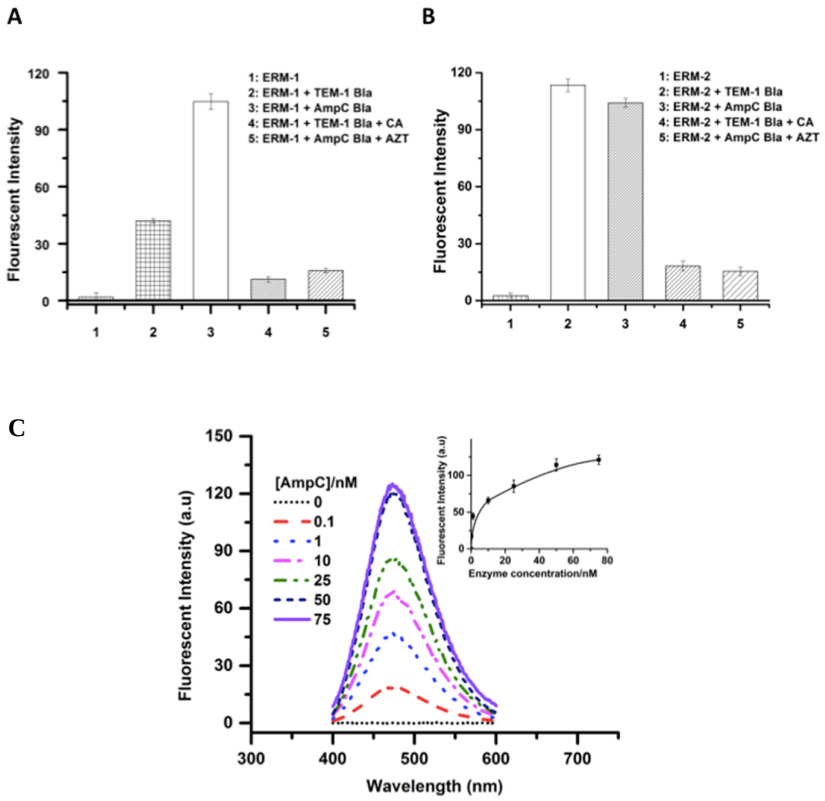


Fig. S1. Fluorescence enhancement of (A) ERM-1 and (B) ERM-2 (10 µM) in 0.1 M of PBS buffer, pH 7.4 incubated with TEM-1, AmpC (50 nM) and inhibitors Calvanic Acid (CA) and Aztreonam AZT (100 mM). (C) ERM-1 (10 µM) reaction in a range of different concentration of AmpC.

Enzyme Kinetics

The kinetic experiment were caried out at 37 ℃ in 0.1 M PBS buffer with pH 7.4. The fluorescence was measured using a fluorescence spectrometer. To a series of difference concentration of ERM-1 and ERM-2 (10 µM to 50 µM) were incubated separately with

TEM-1 and AmpC (50 nM) and the fluorescence was monitored over a period of 60 minutes with excitation wavelength at 312 nm and emission wavelength at 478 nm. The values of the kinetic parameters (K_M and K_{cat}) were determined by least-squares fit of a double-reciprocal plot of the hydrolysis rate versus probe concentrations.

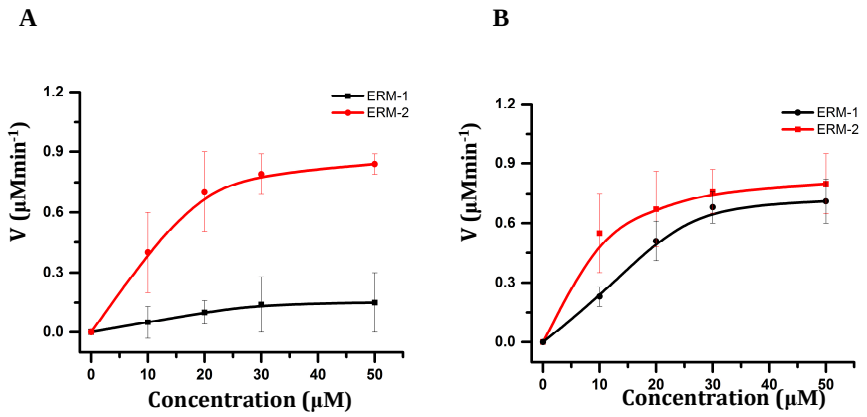


Fig. S2. Enzyme kinetics of ERM-1 and ERM-2 with TEM-1 and AmpC (50 nM). (A) ERM-1 and ERM-2 with TEM-1 enzyme. (B) ERM-1 and ERM-2 with AmpC enzyme.

HPLC Analysis

ERM-1 and ERM-2 were incubated separately with TEM-1 and AmpC in PBS (pH 7.4, 0.1 M) at 37℃ for 1 hr and subjected to HPLC analysis. The Bla (TEM-1 and AmpC 50 nM) hydrolysis of BSLP and NSPC were confirmed with analytical reverse-phase high performance liquid chromatography. RP-HPLC was performed on Alltima C-18 column (250 × 3.0 mm) at a flow rate of 1.0 mL/min. An eluting system consisting of A (water with 0.1% TFA) and B (acetonitrile with 0.1% TFA) was used under a linear gradient to elute the products, which was monitored by UV-Visible absorbance at 312 nm. The linear gradient started from 80% solution A and 40 % solution B, changed to 20 % solution A and 80 % solution B in 40 minute and to 0 % solution A and 100% solution B in the following 5 minutes, and then back to 80 % solution A and 40 % solution B in the next 5 minutes.

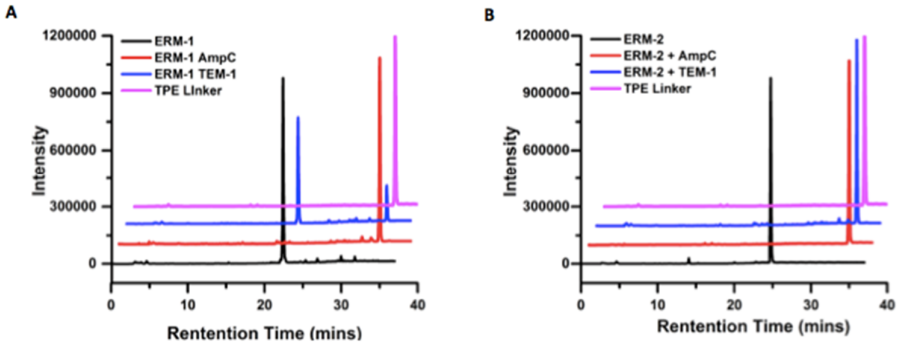


Fig. S3. HPLC analysis of ERM-1 and ERM-2 (100 μM in 0.1M PBS) with AmpC and TEM-1 (50 nM) at absorbance 312 nm.

Dynamic Light Scattering for size Distribution

The sizes and size population distributions of ERM-1 and ERM-2 after treatment with TEM-1 and AmpC (50 nM) respectively were determined on a Brookhaven 90 Plus Nanoparticle Size Analyzer. Dust-free solution vials were used for the aqueous solutions, and measurements were performed at an angle of 90˚ in room temperature.

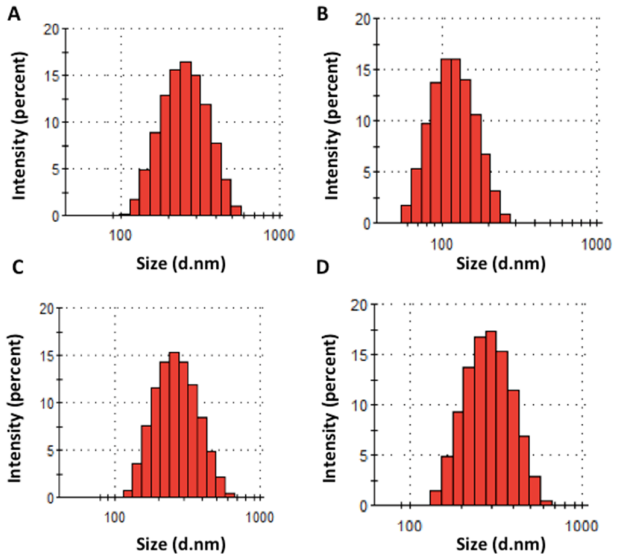


Fig. S4. Hydrodynamic diameter of the residue of ERM-1 and ERM-2 (10 μM in 0.1 M PBS) after interaction with AmpC and TEM-1 (50 nM) in DMSO/PBS (v/v=1/199).

(A) ERM-1 with AmpC. (B) ERM-1 with TEM-1. (C) ERM-2 with AmpC. (D) ERM-2 with TEM-1.

Confocal Microscopic Bacteria Imaging

The overnight culture of bacteria suspension were diluted to 10^8 cells/ml and incubated with ERM-1 and ERM-2 (20 µM). After washing the PBS, the bacteria cells were spotted on poly-L-lysine pretreated glass slides and immobilized with coverslips. Bacteria imaging tests were conducted with Zeiss LSM 800 confocal microscope. To study the enzyme specificity, the bacteria were pretreated with enzyme inhibitor CA and AZT and labeled with ERM-1 and ERM-2 for imaging studies.[1]

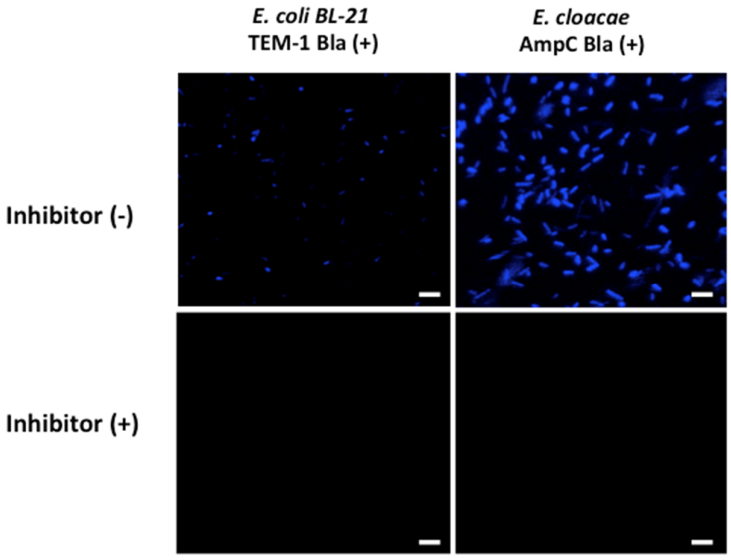


Fig. S5. Confocal imaging of penicillin resistant bacteria *E. cloacae and E. coli* BL21 pretreated with inhibitors (100 µM) with 20 µM of ERM-1 in 0.1 M PBS, pH 7.4. Ex = 350/50 nm; Em = 450/50 nm. Scale bar: 5 µm.

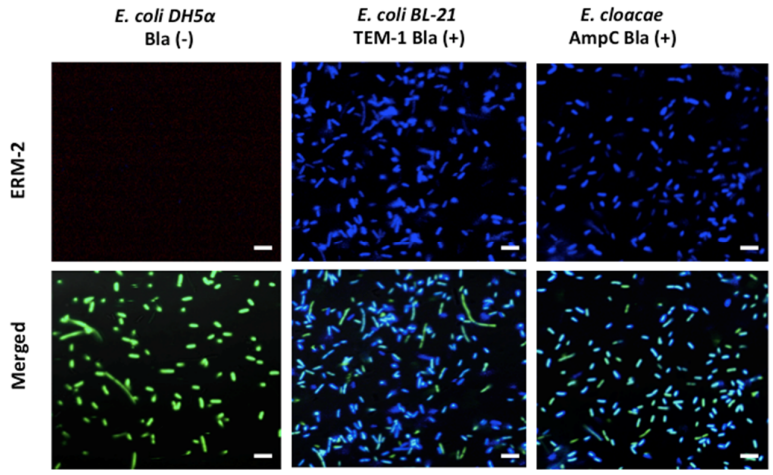


Fig. S6. Confocal imaging of penicillin resistant bacteria *E. cloacae and E. coli* BL21, and antibiotic susceptible *E. coli* DH5 α bacteria with 20 M of ERM-2 in 0.1 M PBS, pH 7.4. Ex = 350/50 nm; Em = 450/50 nm. Scale bar: 5 µm.

Biofilm Confocal Microscopic Imaging

The bacteria cells were grown overnight in LB medium at 37 °C and subsequently diluted with LB in 1:100. Sterile glass cover slides were covered with 10 ml of bacteria suspension and incubated in 37 °C 24h. Bacteria suspension was discarded and slides were washed gently with 1 ml LB. ERM-1 and ERM-2 probes (10 µM) were dissolved in LB medium and added to the slides for incubation for 1 hr 37°C. Slides were washed gently with sterile PBS (pH = 7.4, 0.1M) two times and observed by LSM 800 Zeiss Confocal Laser Microscope.[2-3]

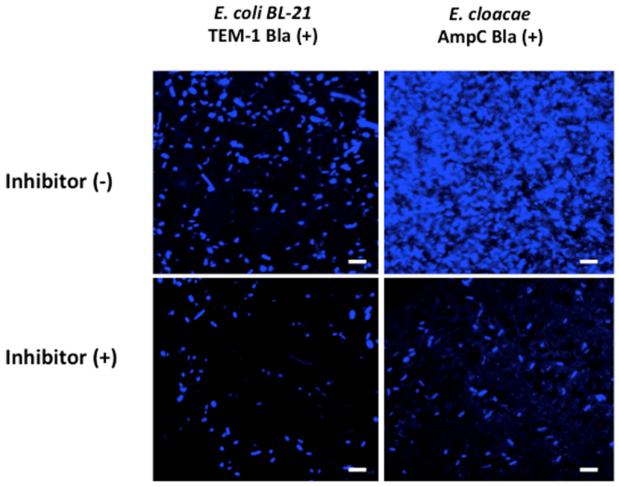


Fig. S7. Confocal imaging of penicillin resistant bacteria *E. cloacae and E. coli* BL21 biofilms pretreated with inhibitors (100 µM) and with 20 µM of ERM-1 in 0.1 M PBS, pH 7.4. Ex = 350/50 nm; Em = 450/50 nm. Scale bar: 5 µm.

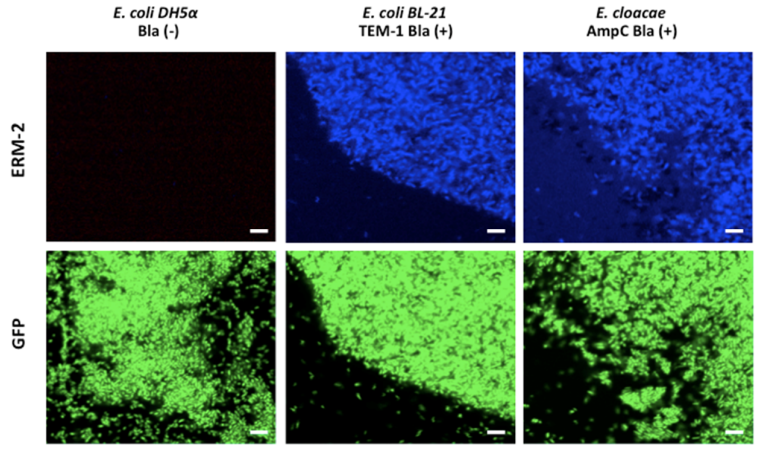


Fig. S8. Confocal imaging of penicillin resistant bacteria *E. cloacae and E. coli* BL21, and antibiotic susceptible *E. coli* DH5 α bacteria biofilms with 20 µM of ERM-2 in 0.1 M PBS, pH 7.4. Ex = 350/50 nm; Em = 450/50 nm. Scale bar: 5 µm.

Flow-Cytometric Analysis

The overnight cultures of *E. coli* DH5 α, BL-21 and *E. cloacae* cells (10^8 CFU/ml) were first treated with 10 µM of ERM-1 and ERM-2 for 1 hr and washed with PBS. The fluorescence of the cells was detected by a high sensitivity flow cytometer with a solid-state 355 nm continuous wave laser as excitation source. The emitted light was collected and split into two light paths for side scatter and blue fluorescence detection respectively.

Stability Test

Typically, the prepared enzyme responsive molecule, e.g. ERM-1 (100 μM), was incubated in several biological samples: 0.10 mL of PBS buffer (pH = 6.0, 7.4 and 8.0), LB (Lysogeny Broth) and human serum (10%) for different time intervals at room temperature. At different time point, the mixture was re-suspended in MeOH (0.3 mL) and then centrifuged at 16,000 *g* for 5 min. The supernatant was analyzed by HPLC to evaluate the stability. There was no obvious ERM-1 degradation observed in PBS (in different pH), LB and human serum (10%) (for 24 hours) indicated the sufficient stability of ERM-1 *in vitro* and living bacteria experiments.

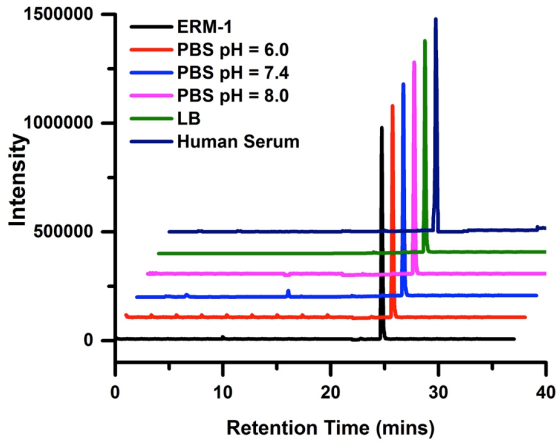


Figure S9. HPLC analysis of ERM-1 (100 μM) with PBS (pH = 6.0, 7.4 and 8.0), LB and human serum (10%) at absorbance 312 nm for 24 hrs.

References:

1. Q. Shao, Y. Zheng, X. Dong, K. Tang, X. Yan, B. Xing, *Chem. Eur. J*, 2013, 19, 10903

2. J. Kim., S. Sahu., Y. Yau., X. Wang., S.G. Shochat., P.H. Nielsen., M.S. Dueholm., D.E. Otzen., J.Lee., M.M.S.D. Santos., J.K.H. Yam., N. Kang., S. Park., H. Kwon., T. Seviour., L. Yang., M. Givskov., Y. Chang. *J. Am. Chem. Soc.* 2016, 138, 402

3. S.L. Chua., J.K.H. Yam., P. Hao., S.S. Adav, M.M. Salido., Y. Liu., M. Givskov., S.K. Sze., T. Tolker-Nielsen., and L. Yang. *Nat. Comm.* 2016, 7, 1-6

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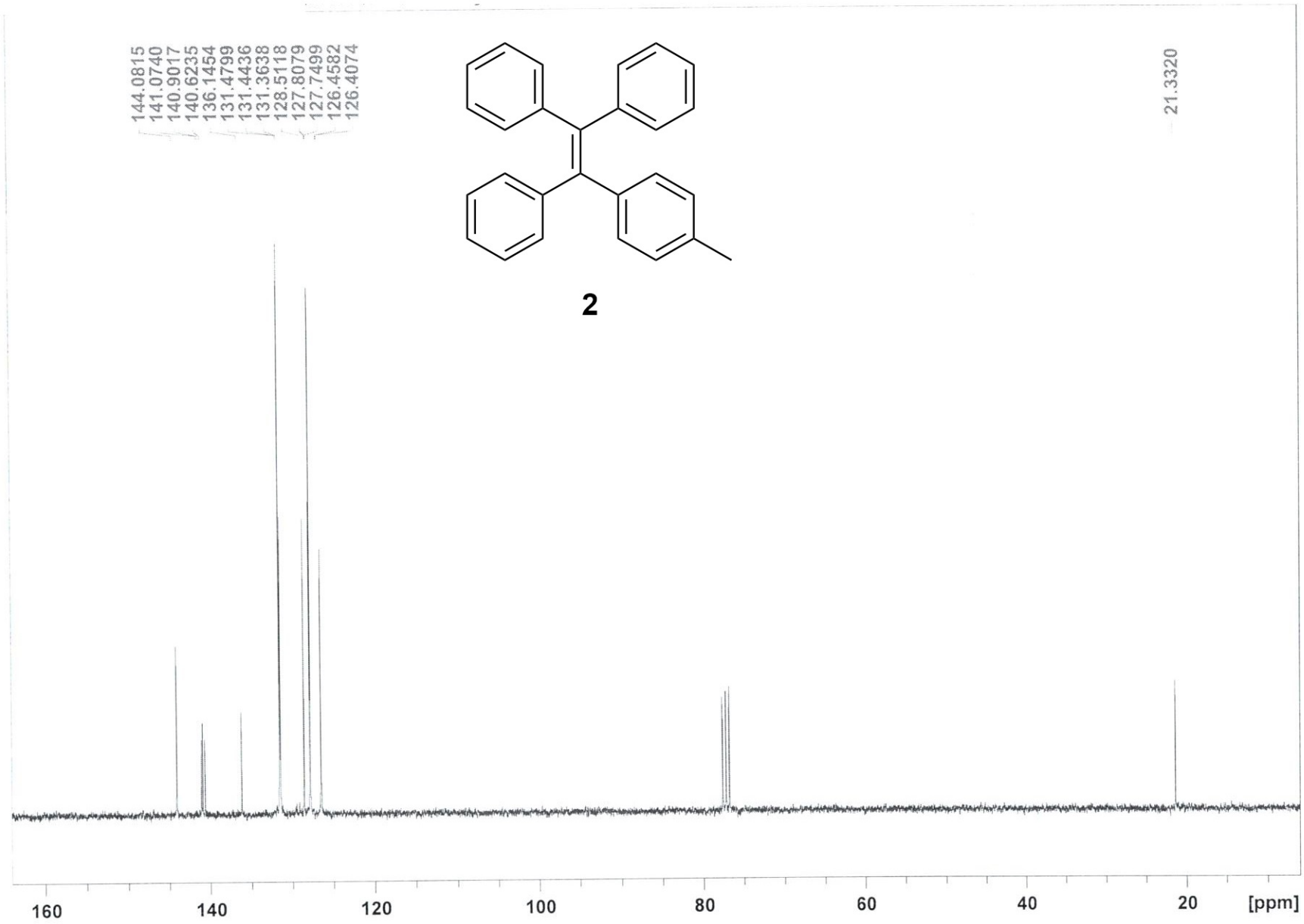
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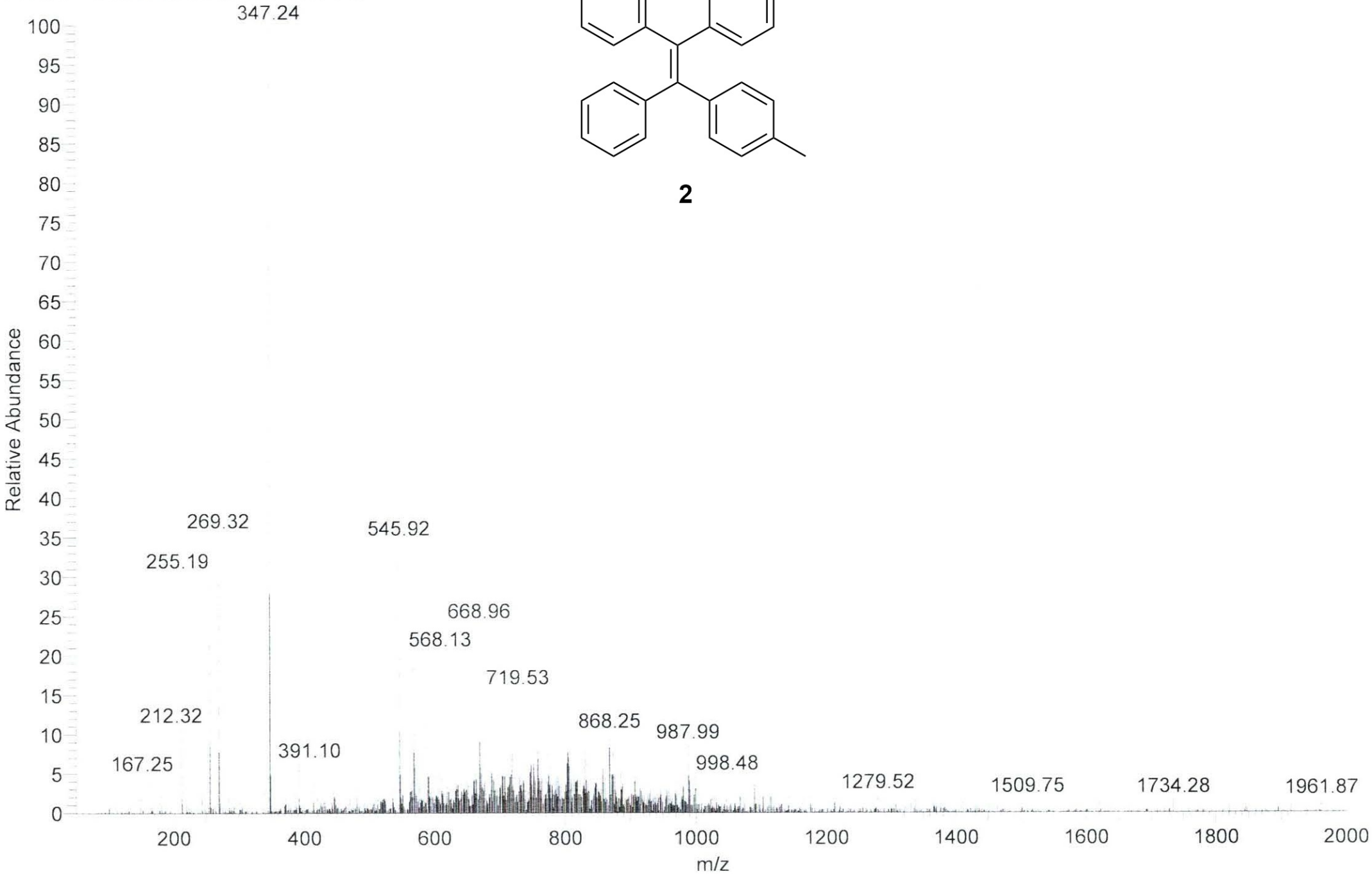

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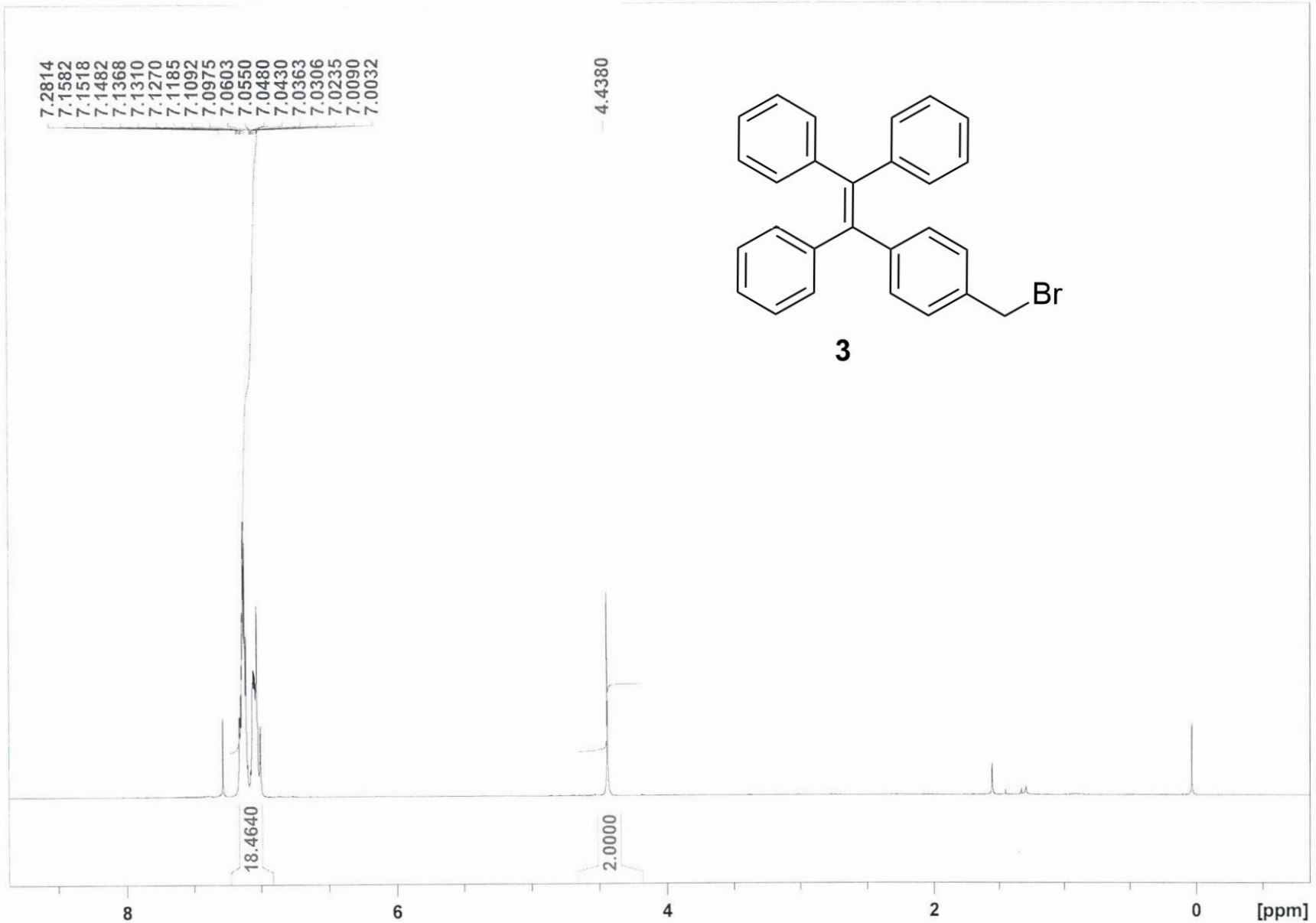
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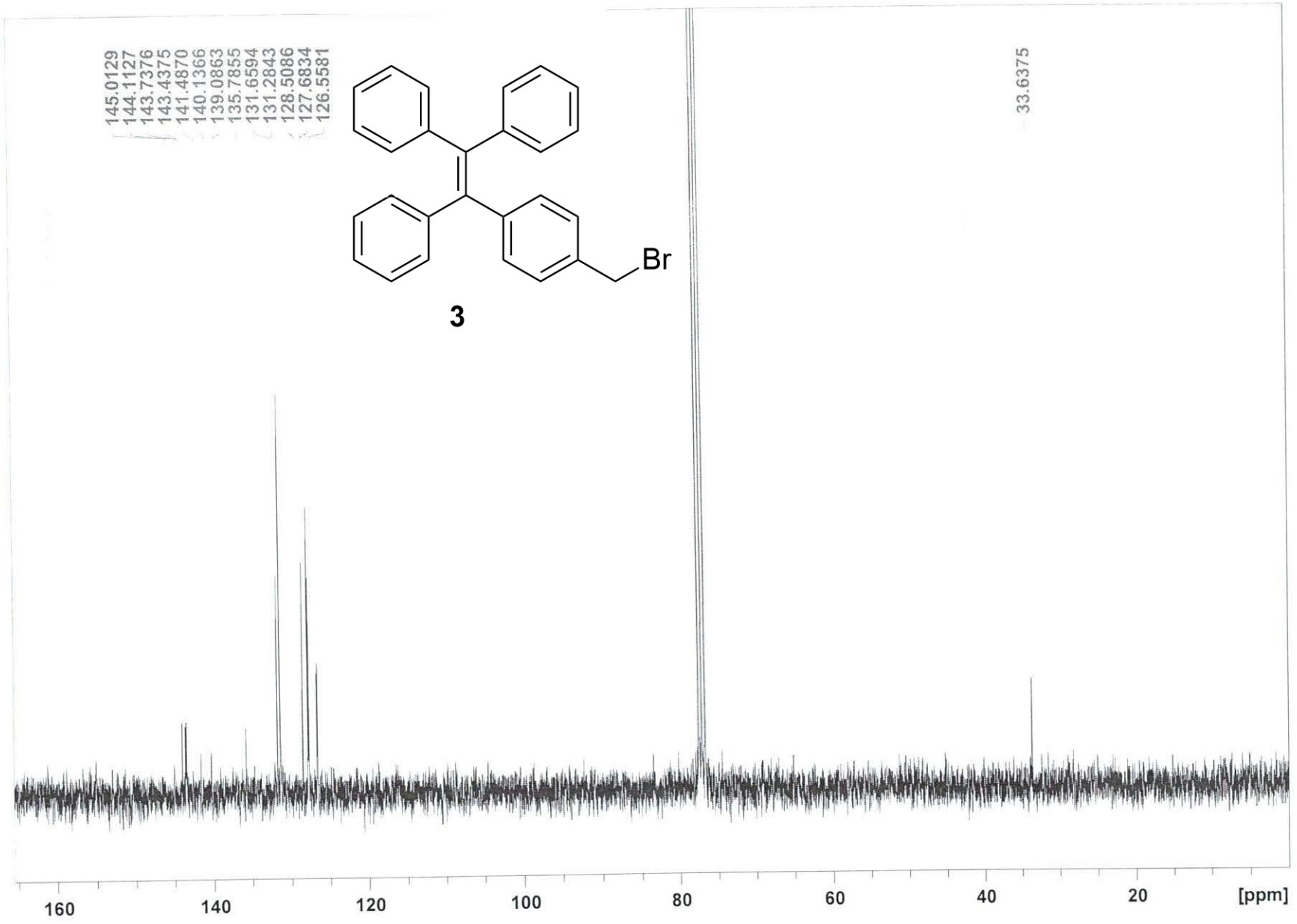
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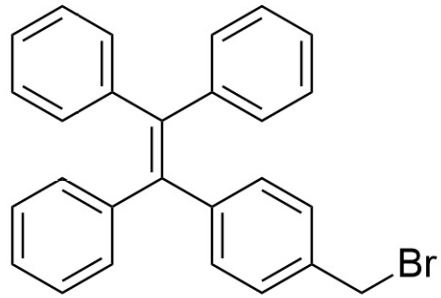

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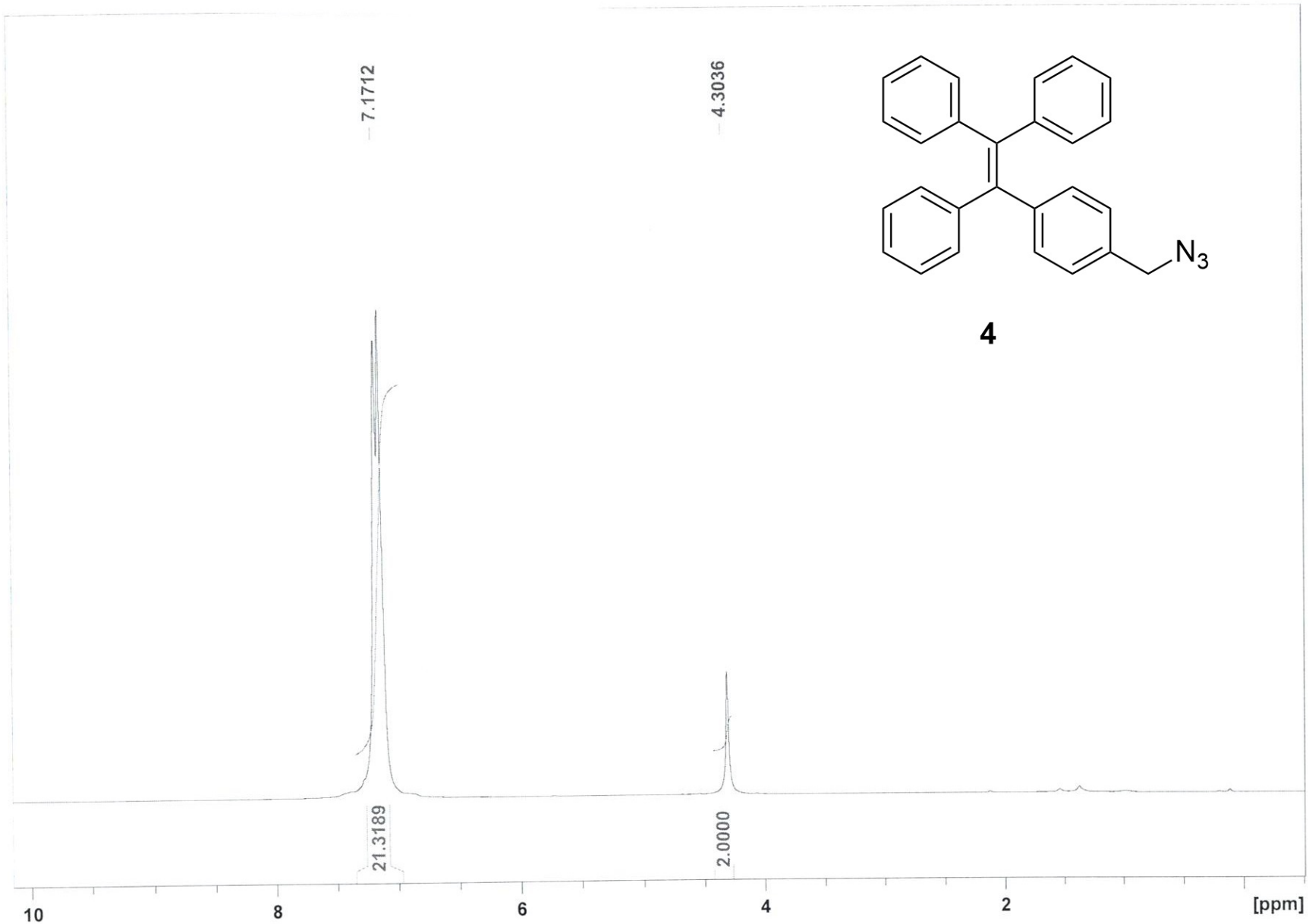

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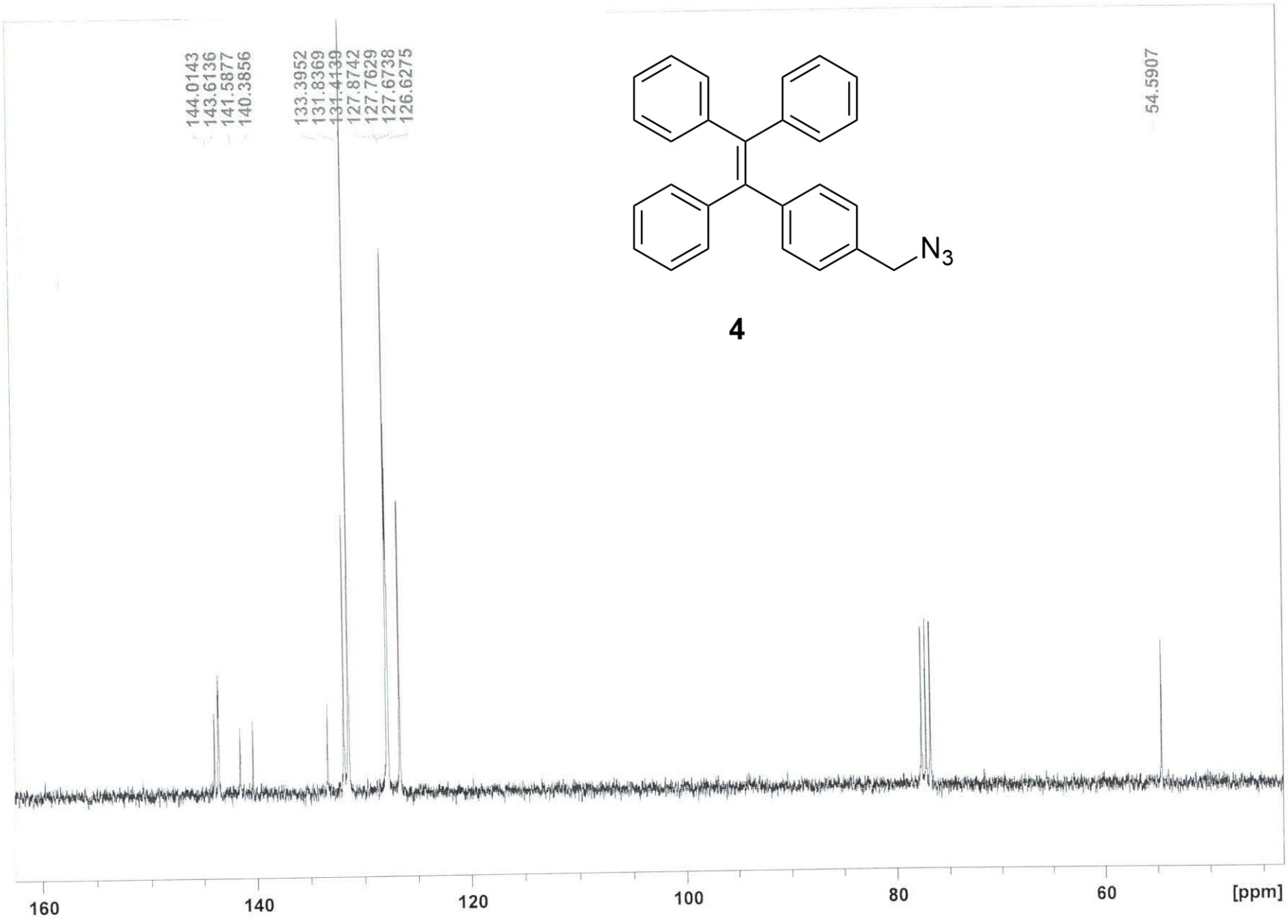
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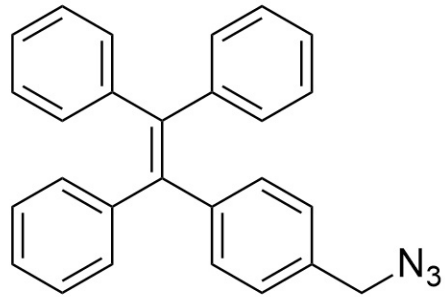

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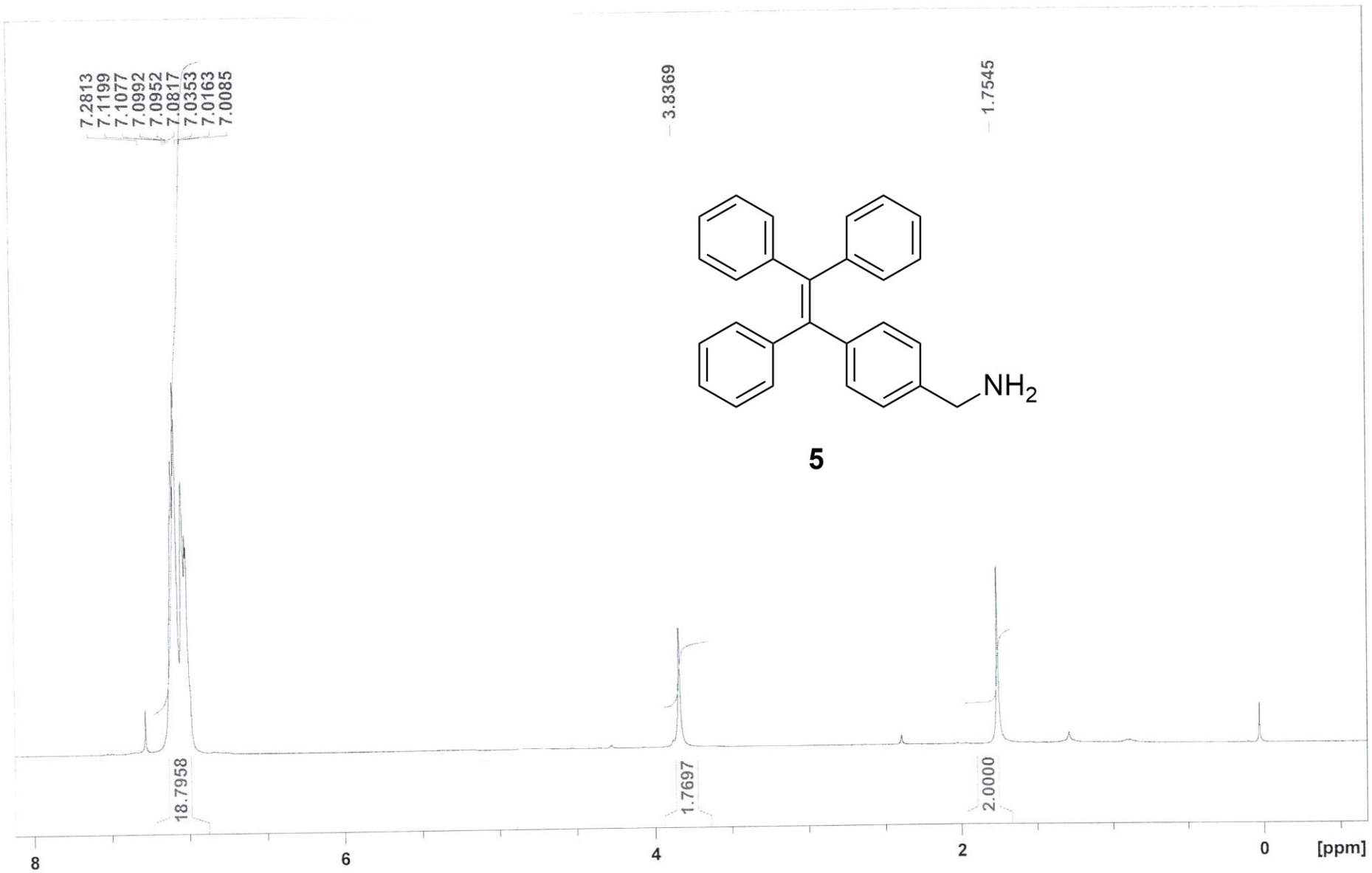
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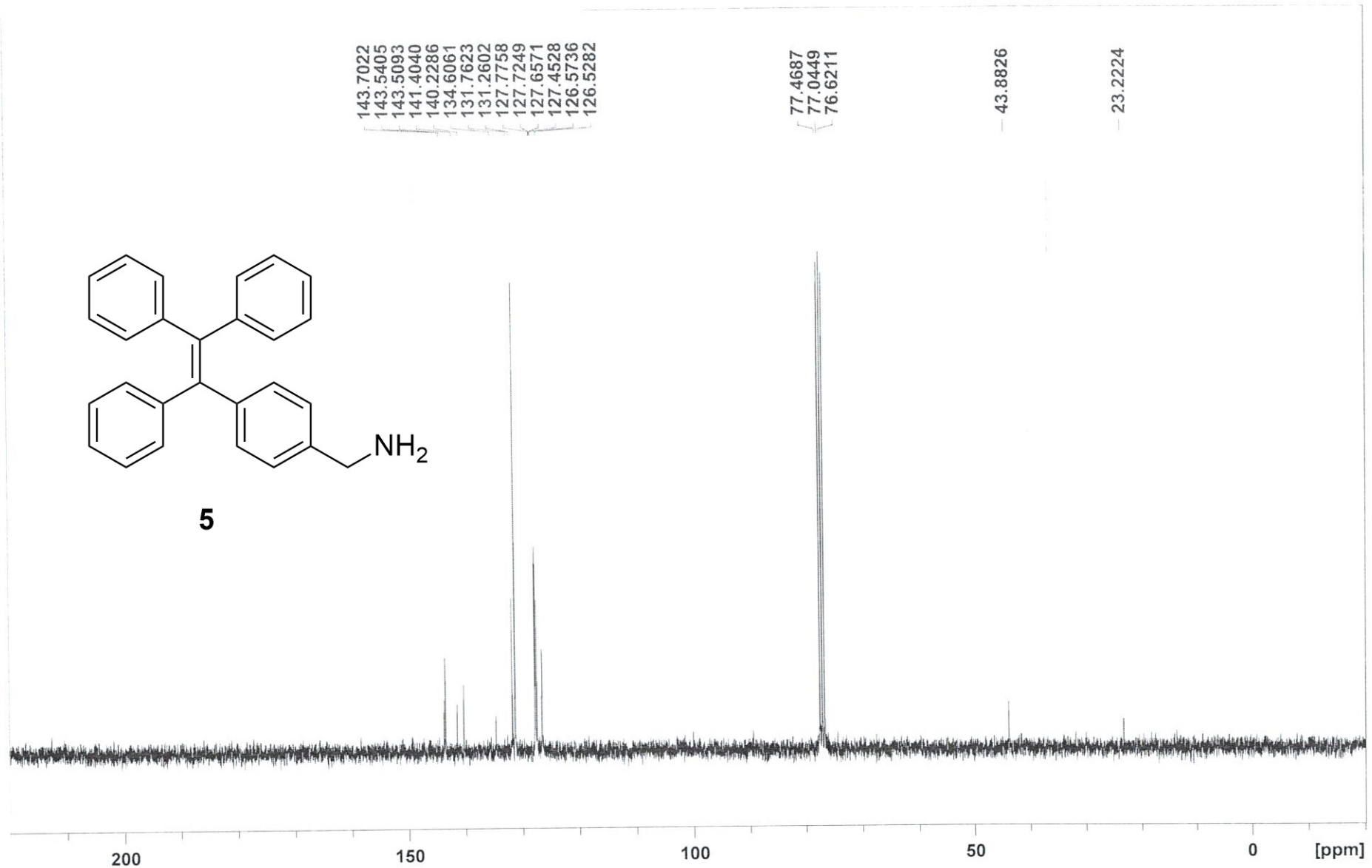
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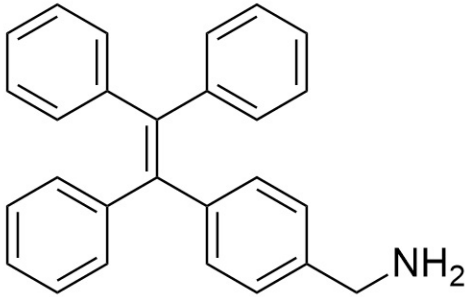

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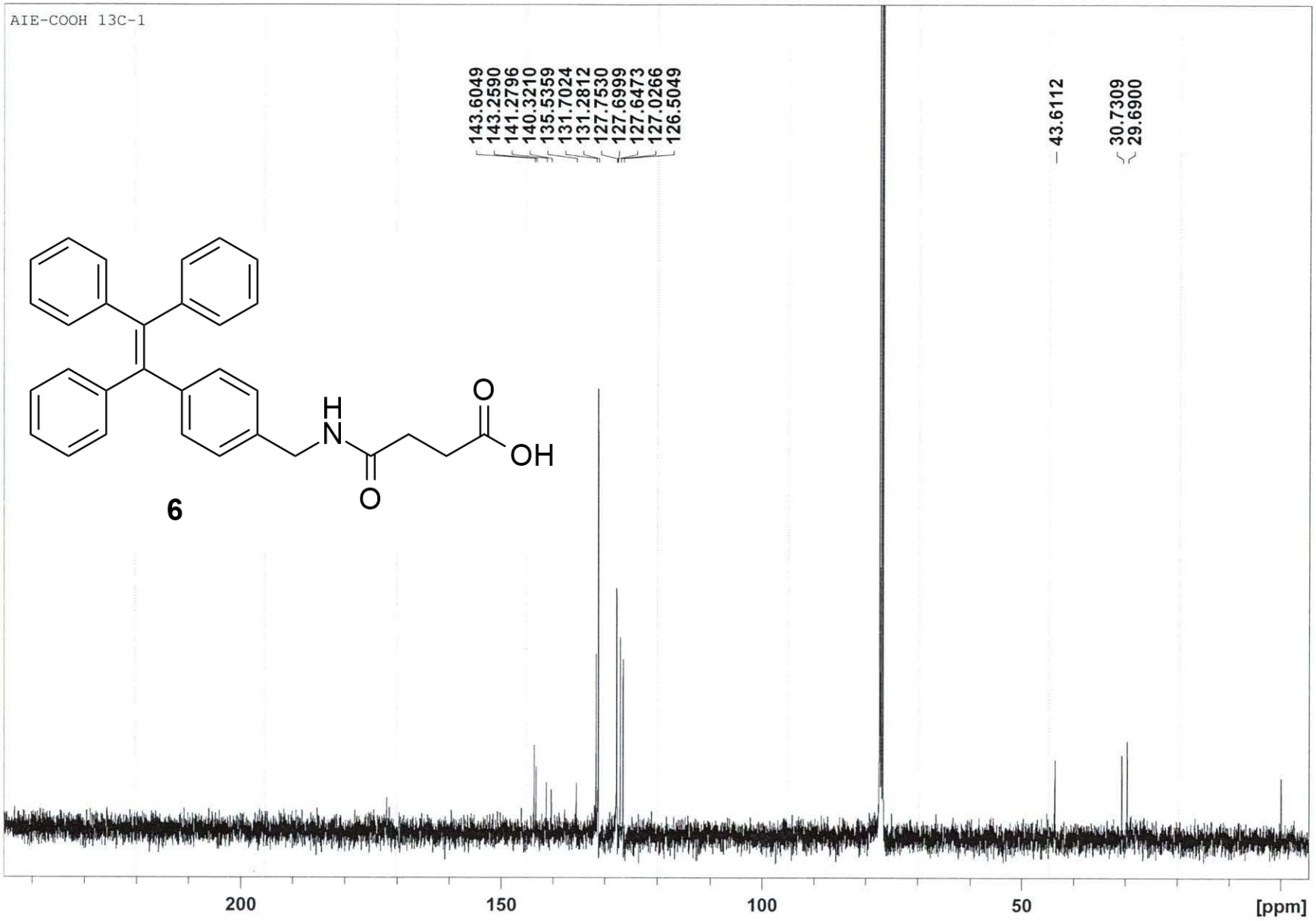
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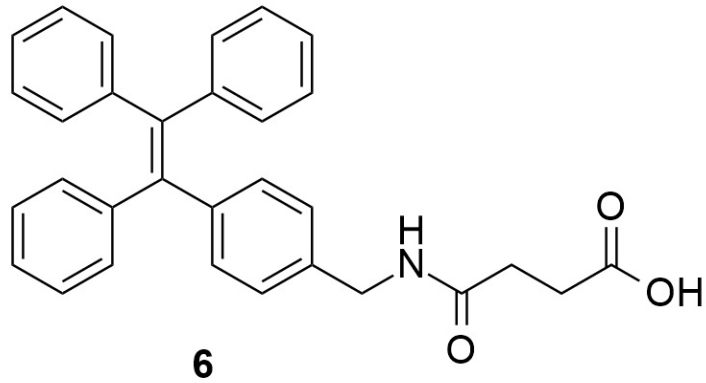

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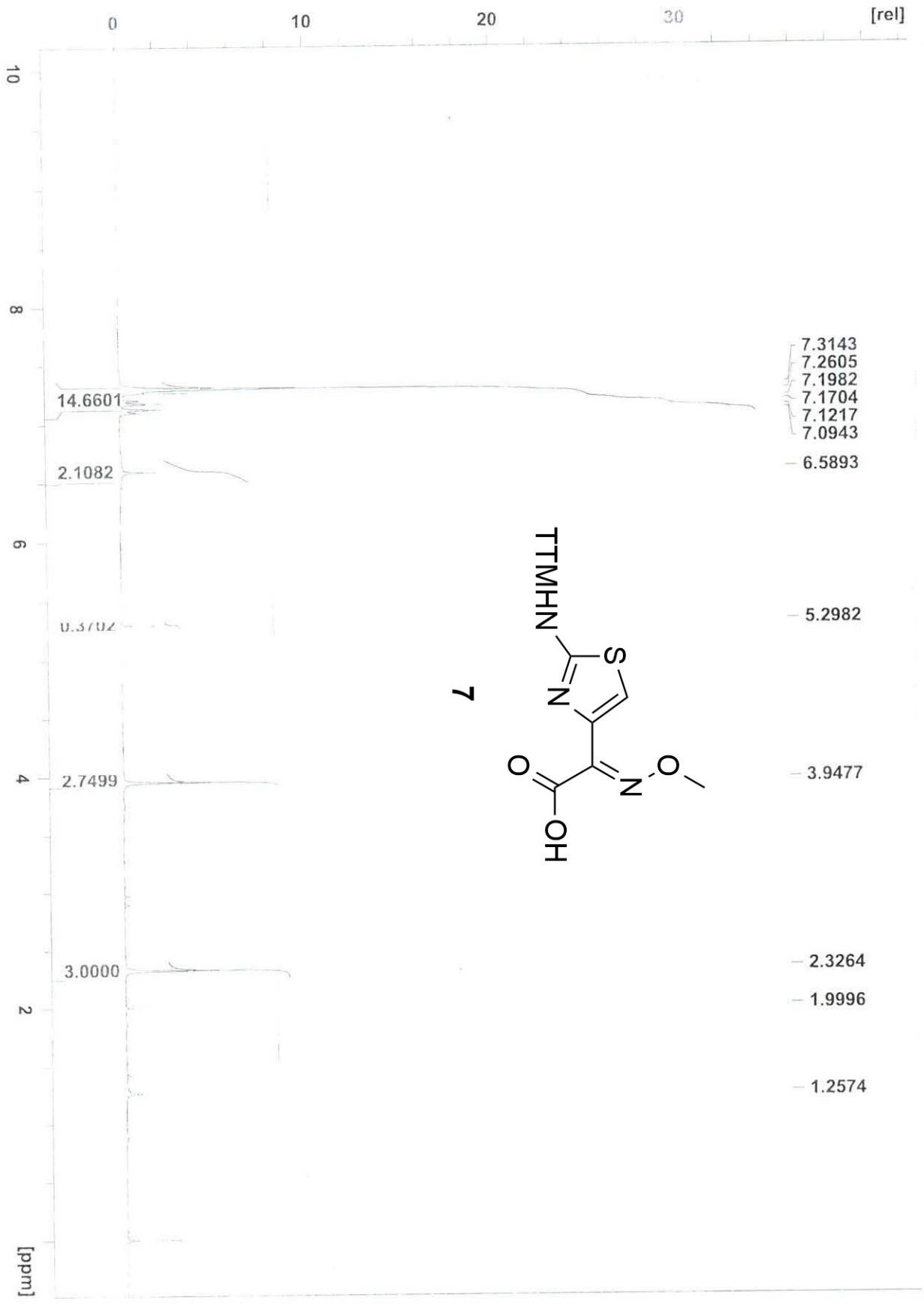
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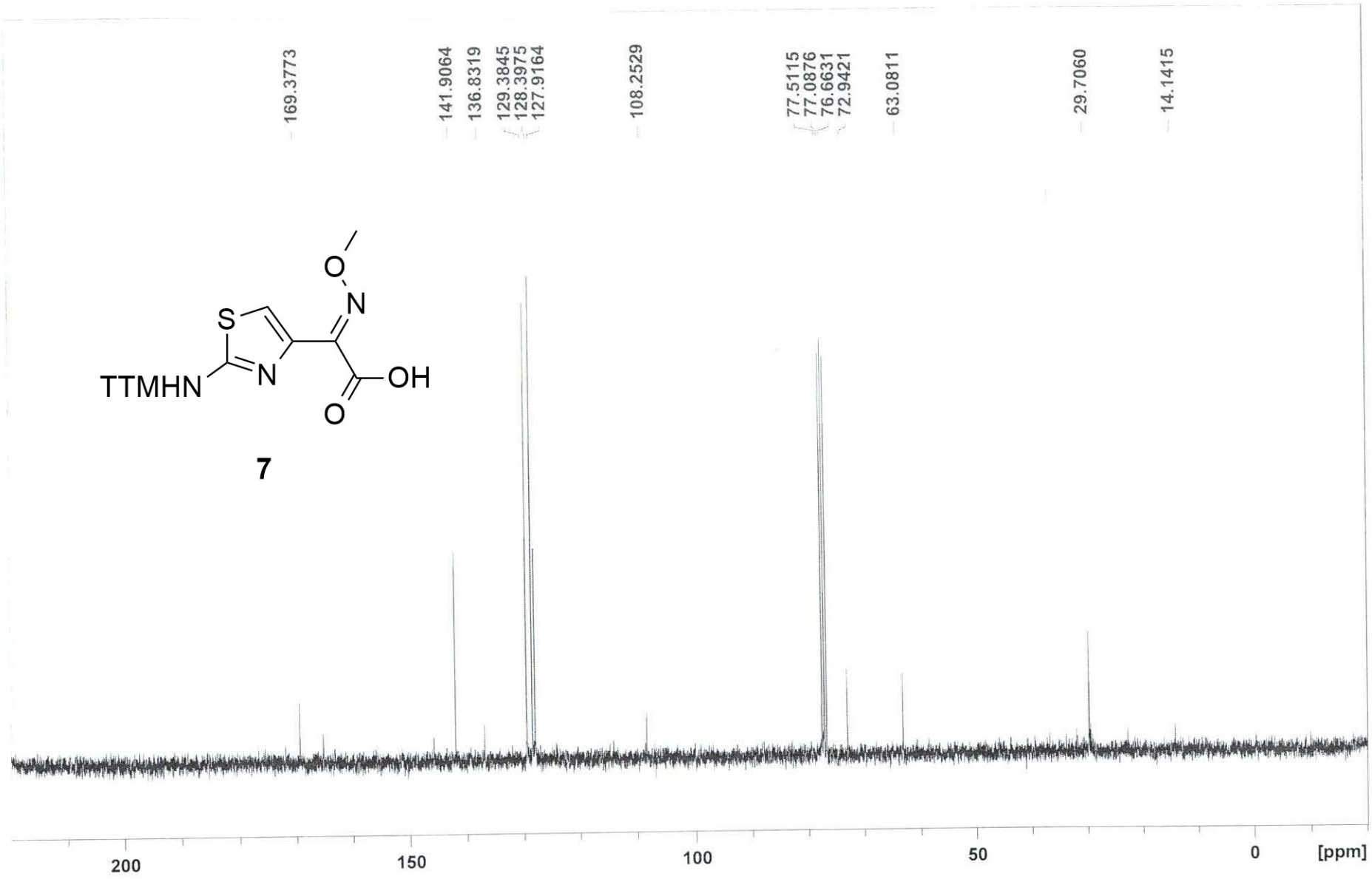
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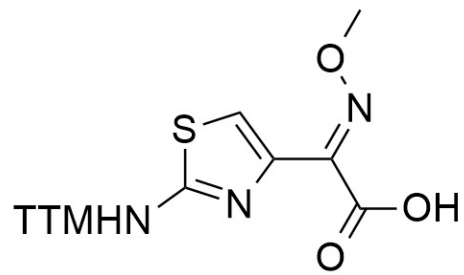

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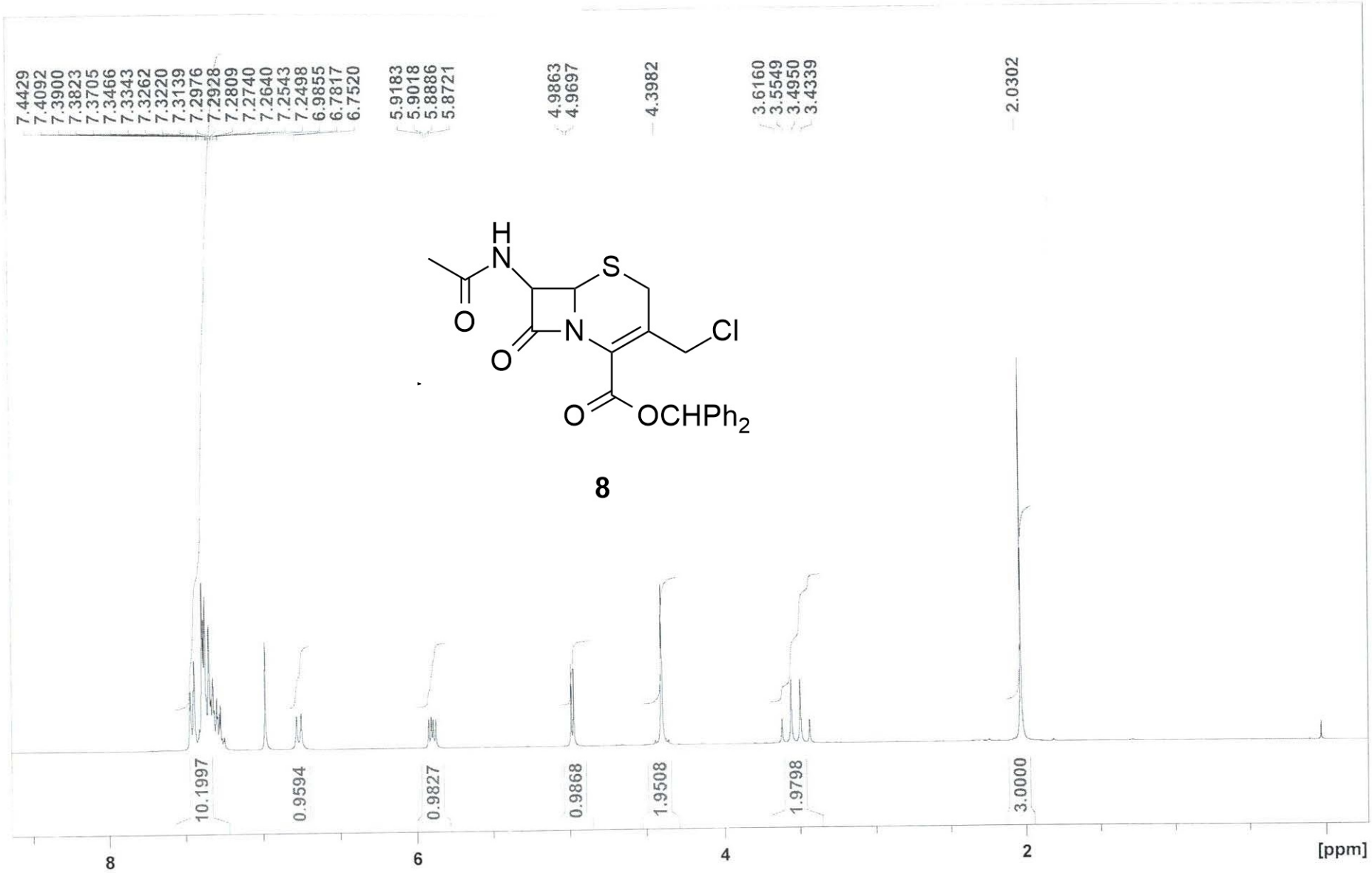
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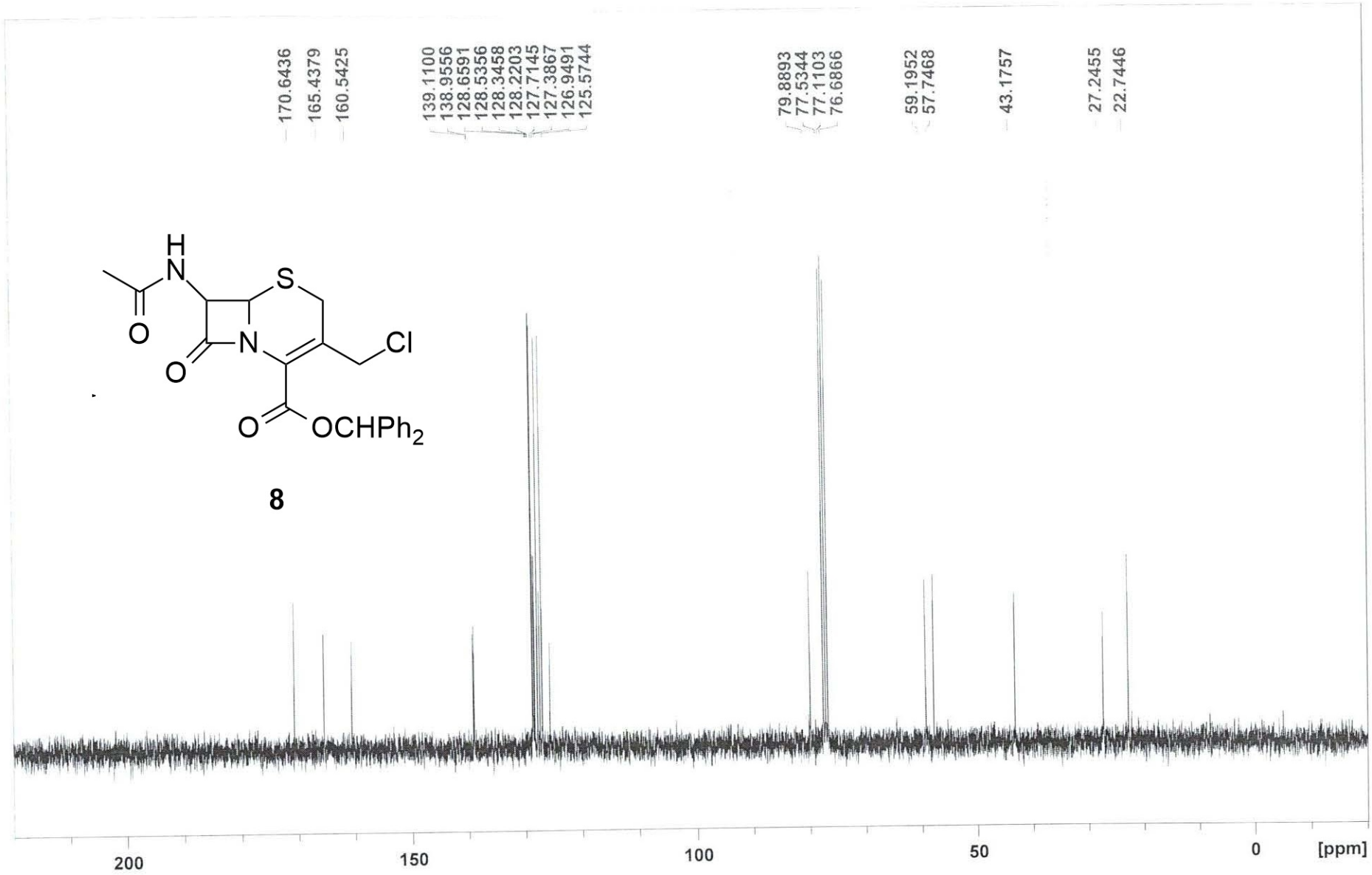
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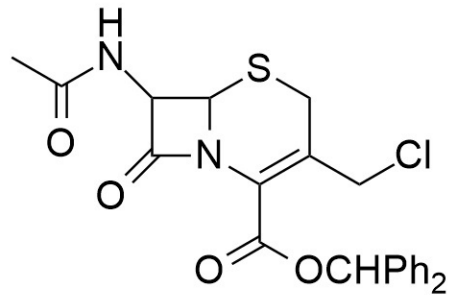

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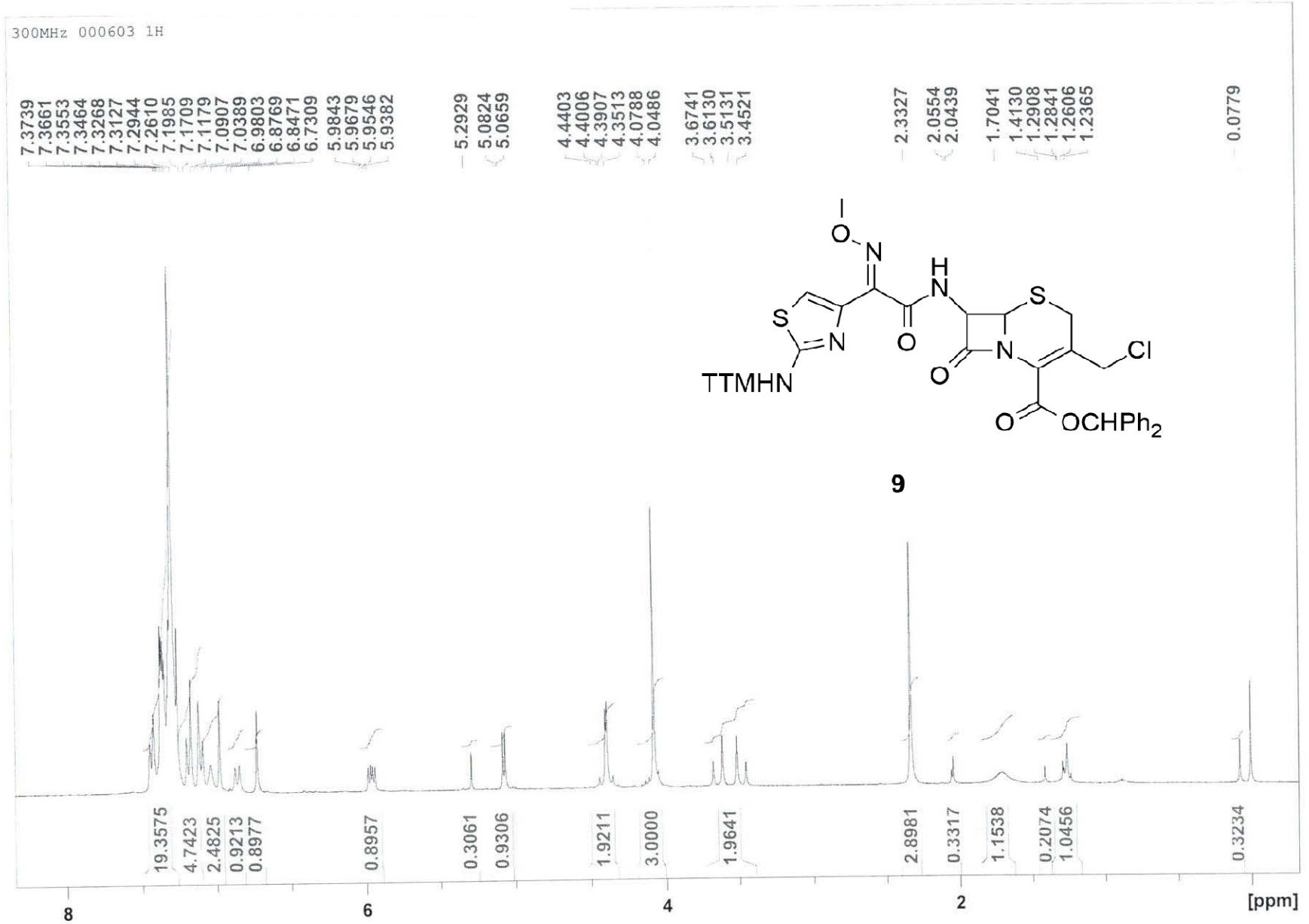
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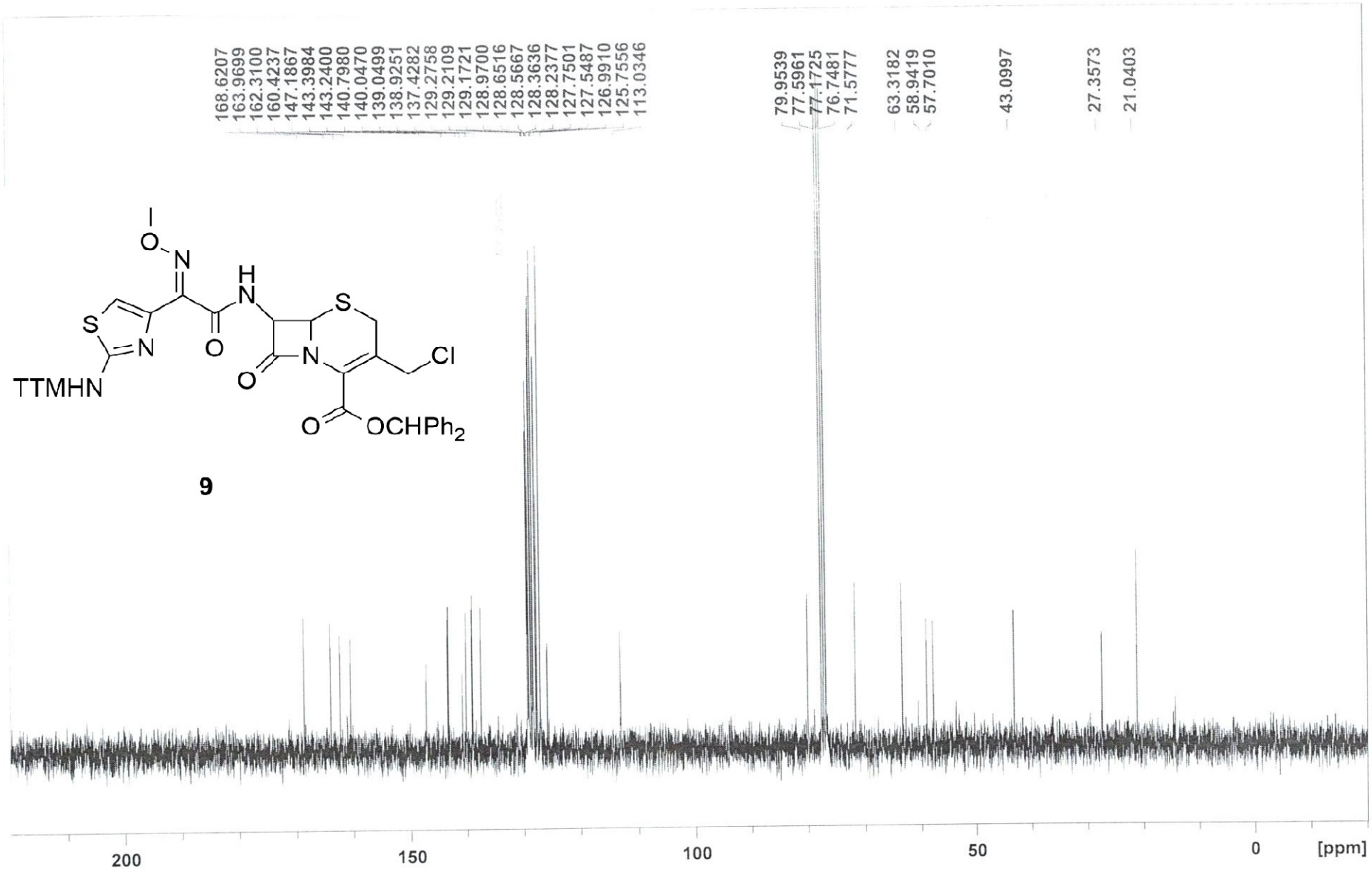
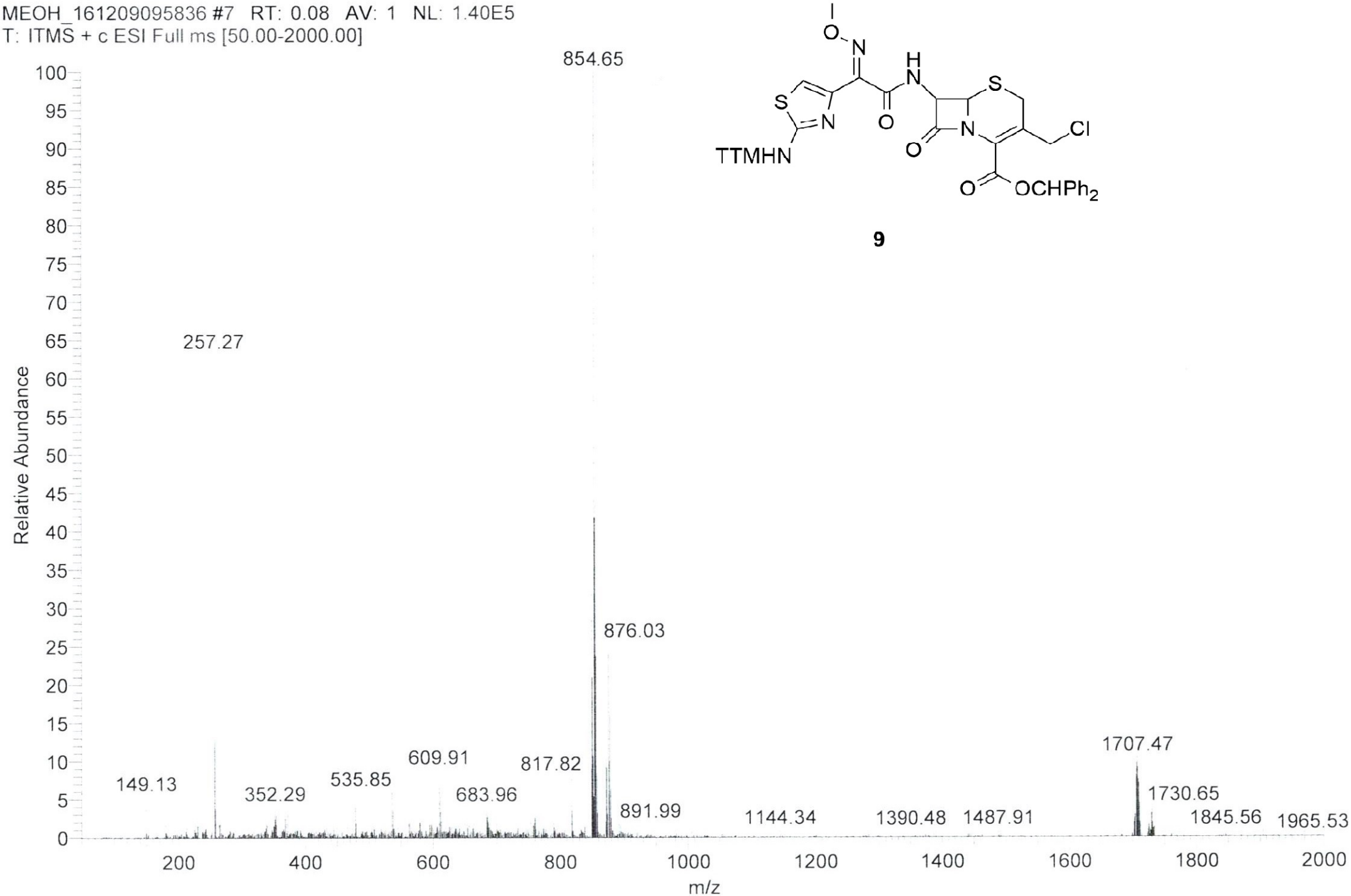
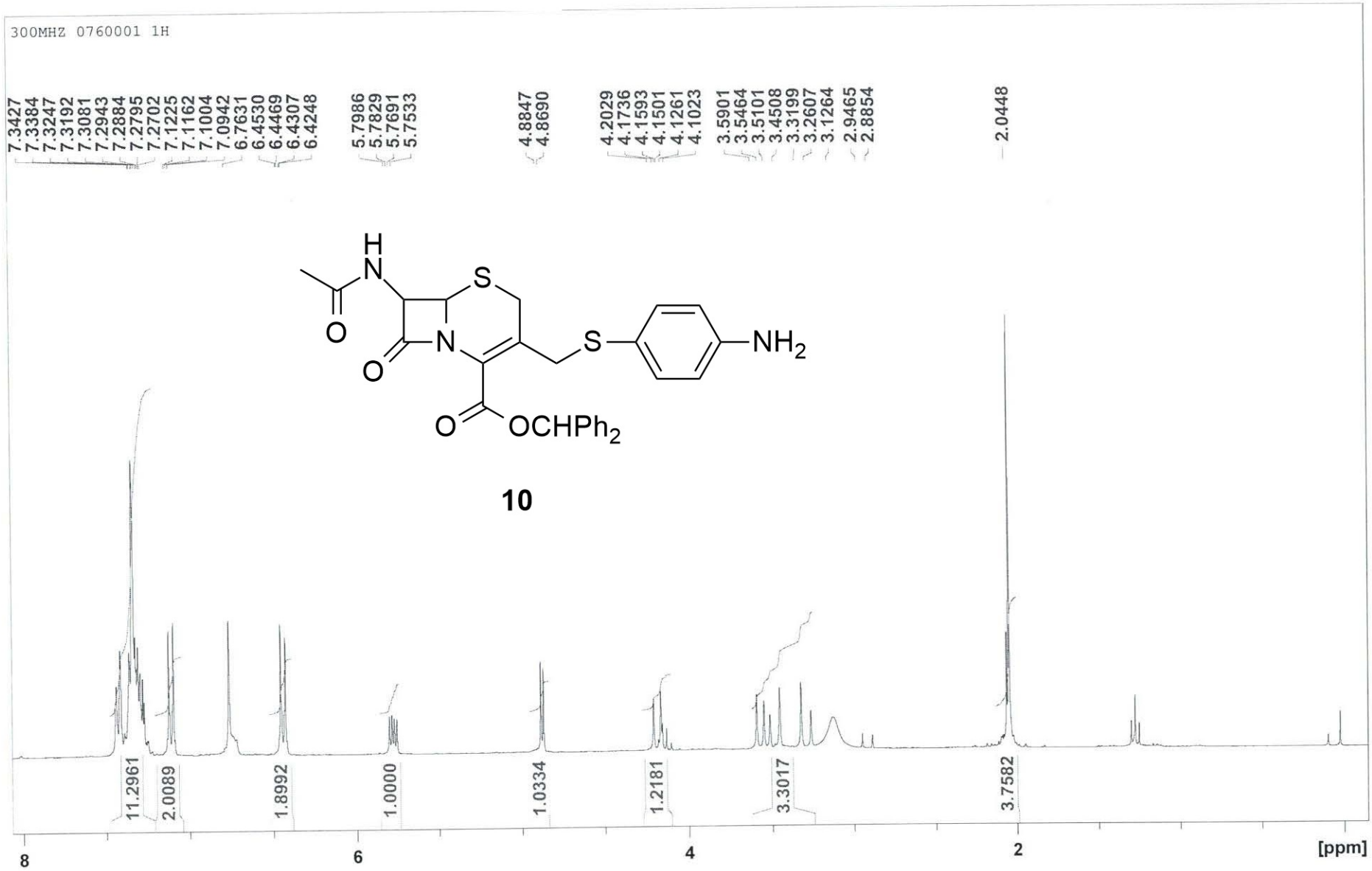
7.1709
7.1179
7.0907
7.0389
6.9803
6.8769
6.8471
6.7309
5.9843
5.9679
5.9546
5.9382
5.2929
5.0824
5.0659
4.4403
4.4006
4.3907
4.3513
4.0788
4.0486
3.6741
3.6130
3.5131
3.4521
2.3327
2.0554
2.0439
1.7041
1.4130
1.2908
1.2841
1.2606
1.2365
0.0779
TTMHN
Cl
OCHPh2
9
19.3575
4.7423
2.4825
0.9213
0.8977
0.8957
0.3061
0.9306
1.9211
3.0000
1.9641
2.8981
0.3317
1.1538
0.2074
1.0456
0.3234
8
6
4
2
[ppm]


168.6207
163.9699
162.3100
160.4237
147.1867
143.3984
143.2400
140.7980
140.0470
139.0499
138.9251
137.4282
129.2758
129.2109
129.1721
128.9700
128.6516
128.5667
128.3636
128.2377
127.7501
127.5487
126.9910
125.7556
113.0346
79.9539
77.5961
77.1725
76.7481
71.5777
63.3182
58.9419
57.7010
43.0997
27.3573
21.0403
TTMHN
Cl
OCHPh2
9
200
150
100
50
0
[ppm]

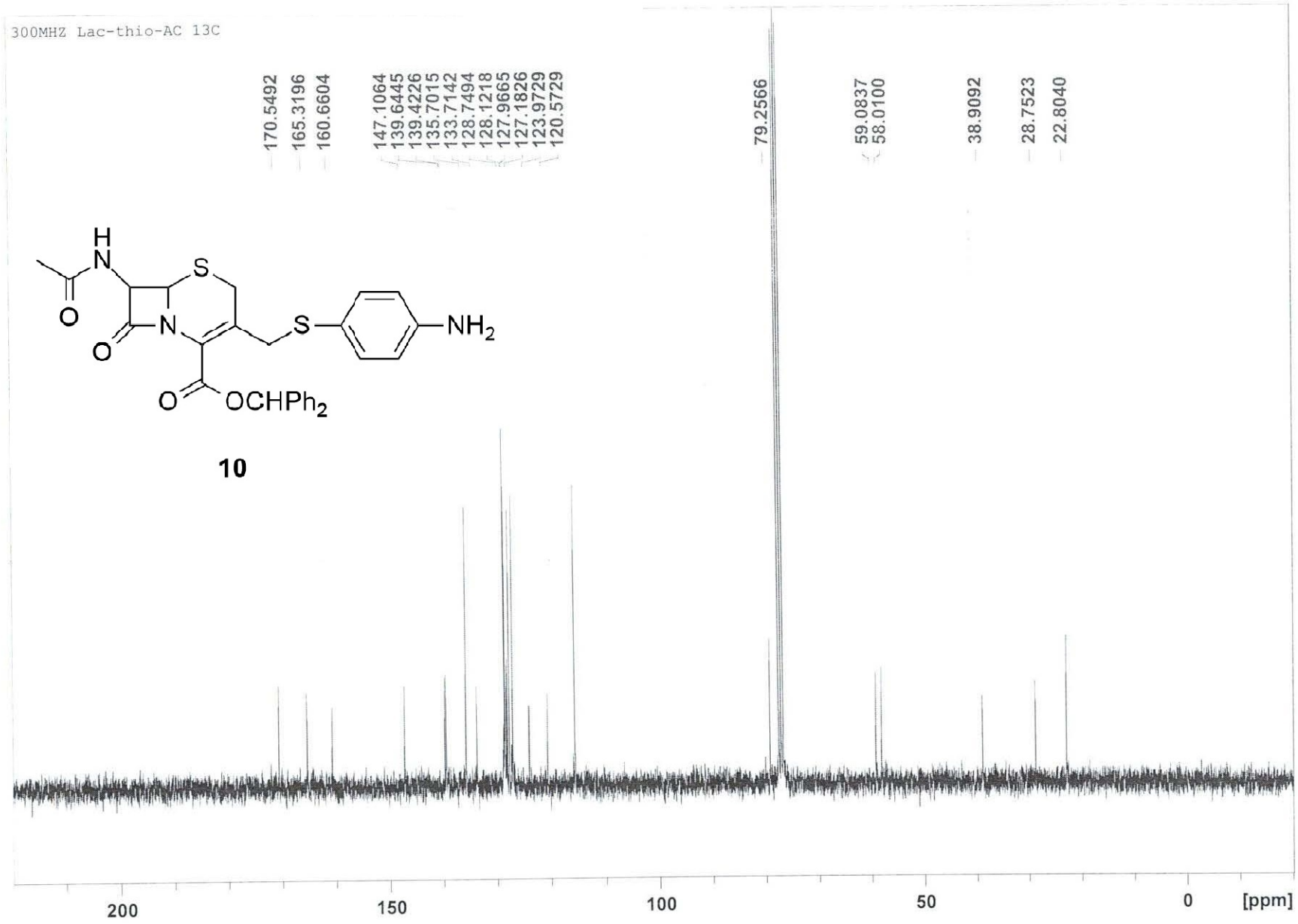
C:\Xcalibur\...\Junxin\MEOH_161209095836 M71-A7M4 4H c
MEOH
MEOH_161209095836 #7 RT: 0.08 AV: 1 NL: 1.40E5
T: ITMS + c ESI Full ms [50.00-2000.00]



9

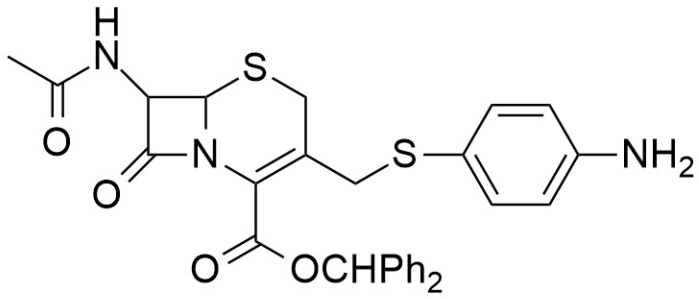

300MHZ 0760001 1H
7.3427
7.3384
7.3247
7.3192
7.3081
7.2943
7.2884
7.2795
7.2702
7.1225
7.1162
7.1004
7.0942
6.7631
6.4530
6.4469
6.4307
6.4248
5.7986
5.7829
5.7691
5.7533
4.8847
4.8690
4.2029
4.1736
4.1593
4.1501
4.1261
4.1023
3.5901
3.5464
3.5101
3.4508
3.3199
3.2607
3.1264
2.9465
2.8854
2.0448
H
N
S
O
N
O
S
NH2
O
OCHPh2
11.2961
2.0089
1.8992
1.0000
1.0334
1.2181
3.3017
3.7582
8
6
4
2
[ppm]

10

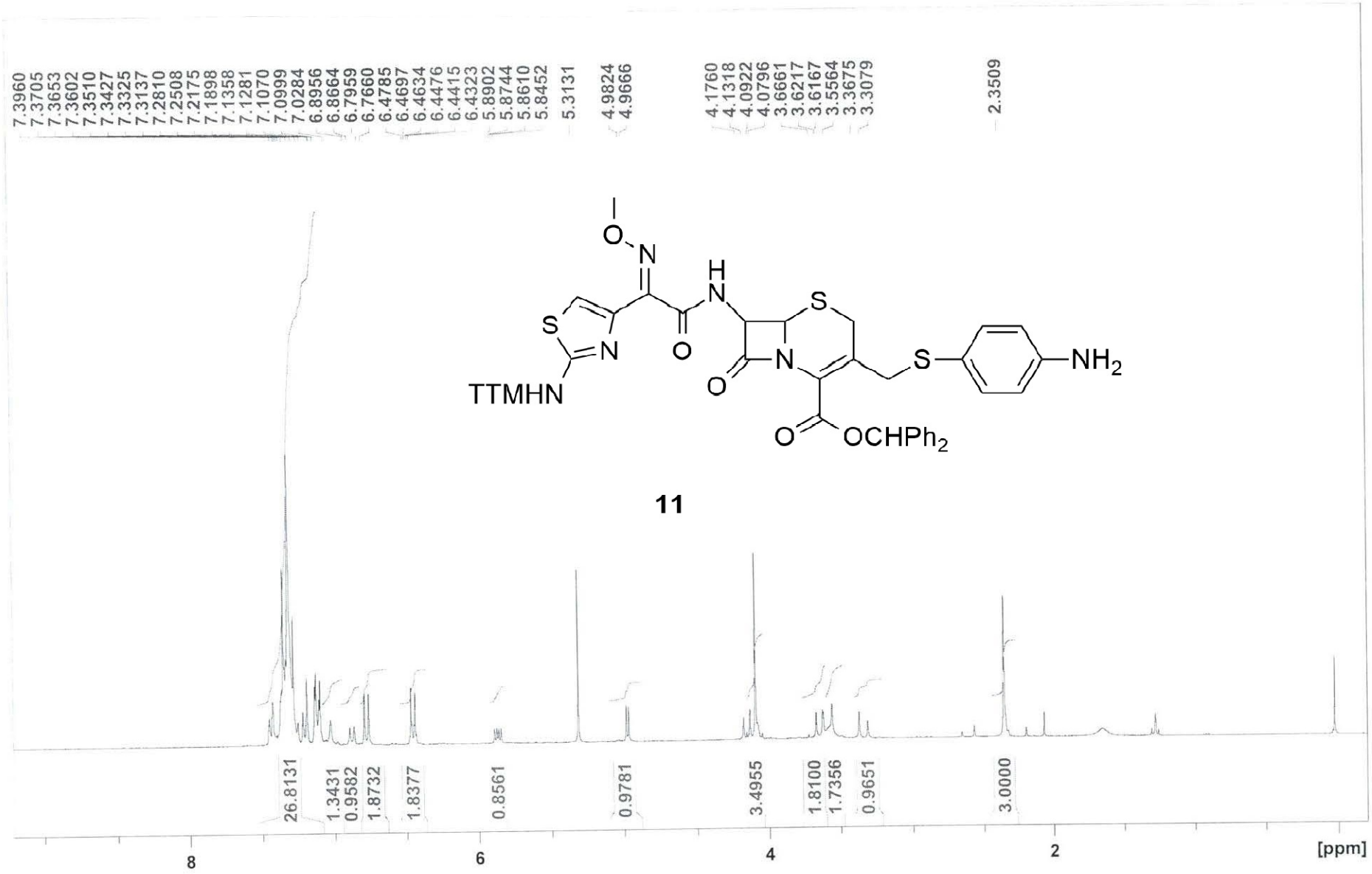

300MHZ Lac-thio-AC 13C
170.5492
165.3196
160.6604
147.1064
139.6445
139.4226
135.7015
133.7142
128.7494
128.1218
127.9665
127.1826
123.9729
120.5729
79.2566
59.0837
58.0100
38.9092
28.7523
22.8040
H
N
S
O
N
O
S
NH2
O
OCHPh2
200
150
100
50
0
[ppm]

10

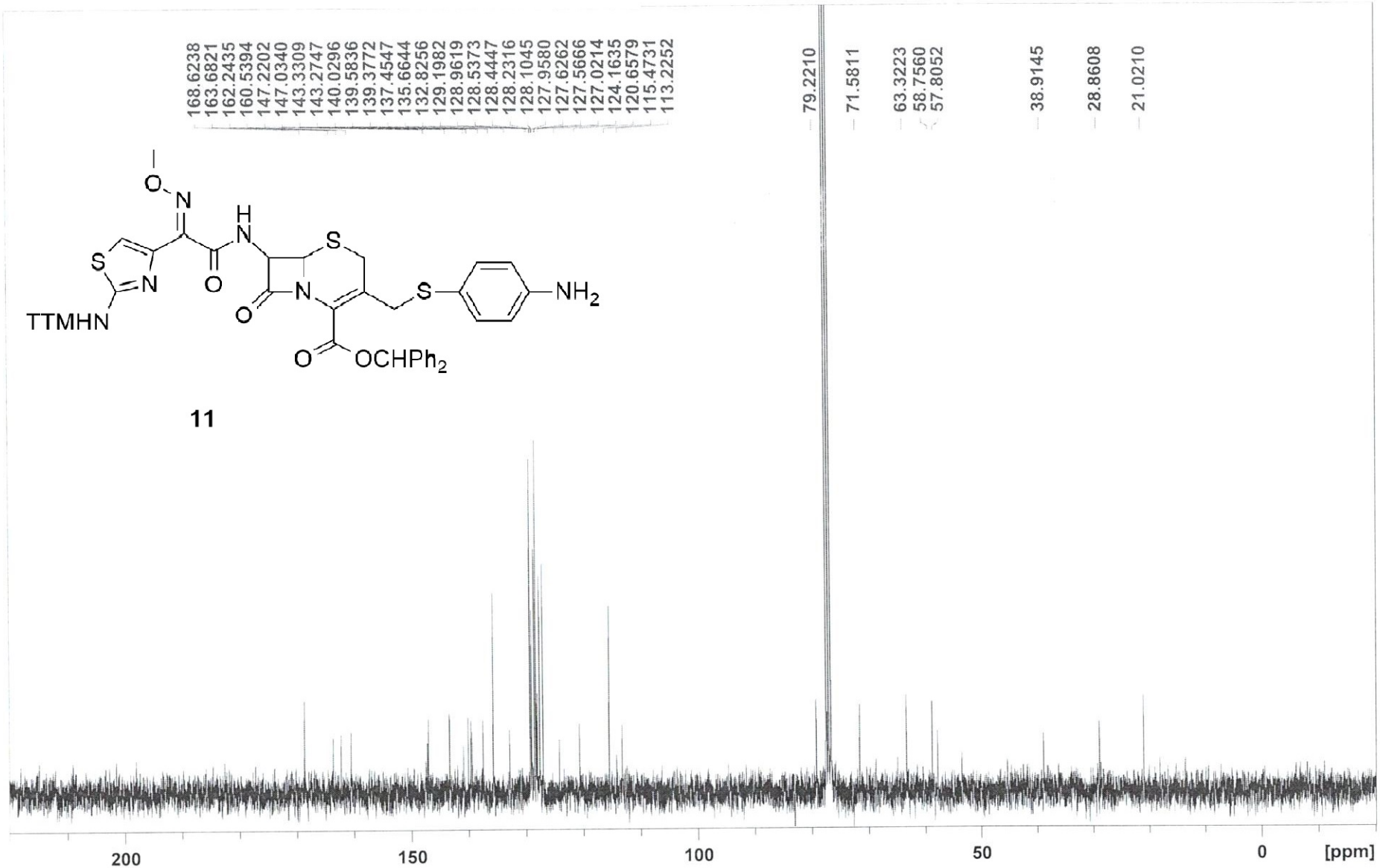
C:\Xcalibur\...\Junxin\ac-thio-phenol
MEOH
ac-thio-phenol #34 RT: 0.40 AV: 1 NL: 4.95E5
T: ITMS + c ESI Full ms [50.00-2000.00]



10

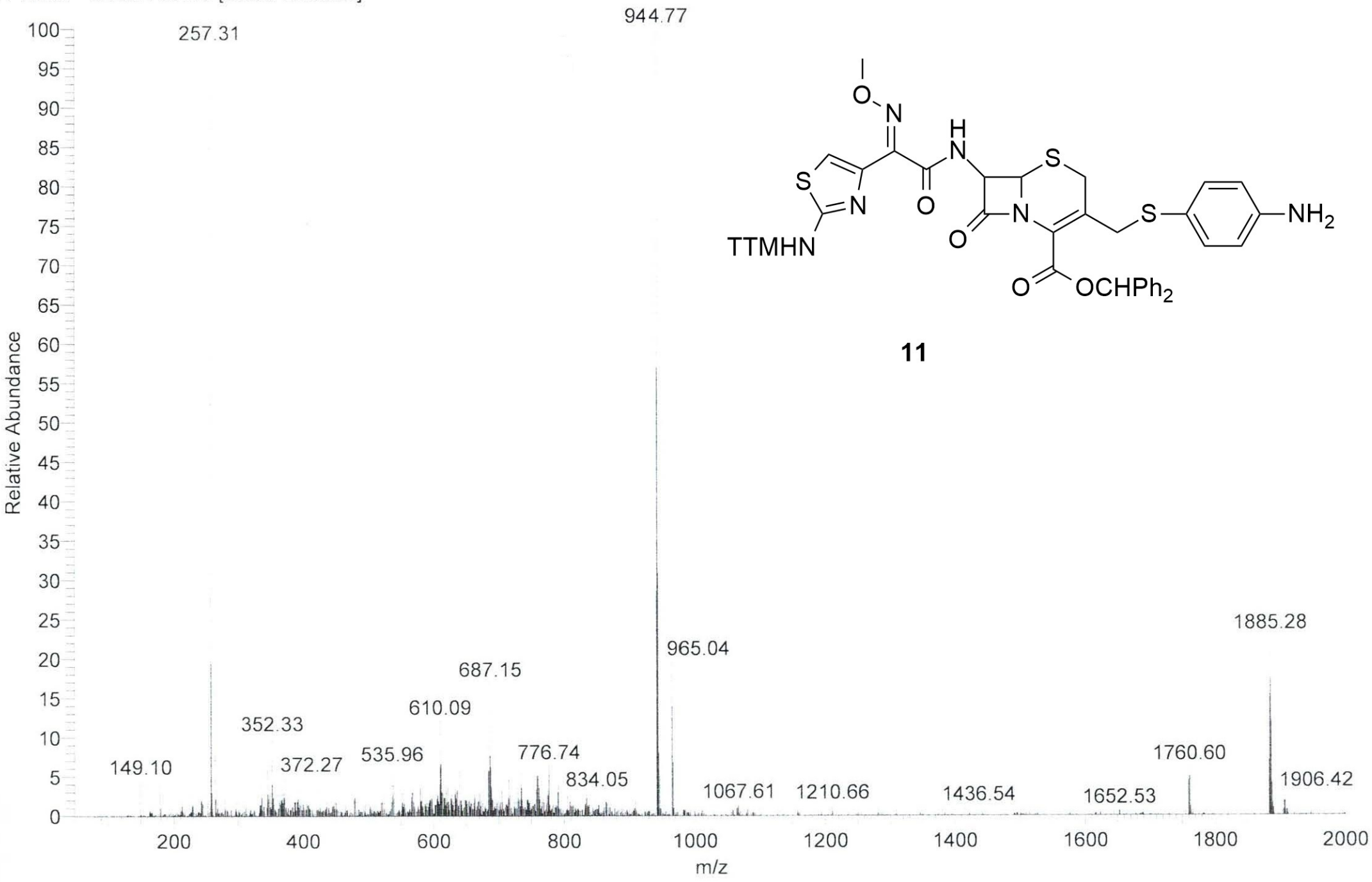

7.3960
7.3705
7.3653
7.3602
7.3510
7.3427
7.3325
7.3137
7.2810
7.2508
7.2175
7.1898
7.1358
7.1281
7.1070
7.0999
7.0284
6.8956
6.8664
6.7959
6.7660
6.4785
6.4697
6.4634
6.4476
6.4415
6.4323
5.8902
5.8744
5.8610
5.8452
5.3131
4.9824
4.9666
4.1760
4.1318
4.0922
4.0796
3.6661
3.6217
3.6167
3.5564
3.3675
3.3079
2.3509
TTMHN
NH2
OCHPh2
26.8131
1.3431
0.9582
1.8732
1.8377
0.8561
0.9781
3.4955
1.8100
1.7356
0.9651
3.0000
8
6
4
2
[ppm]

11

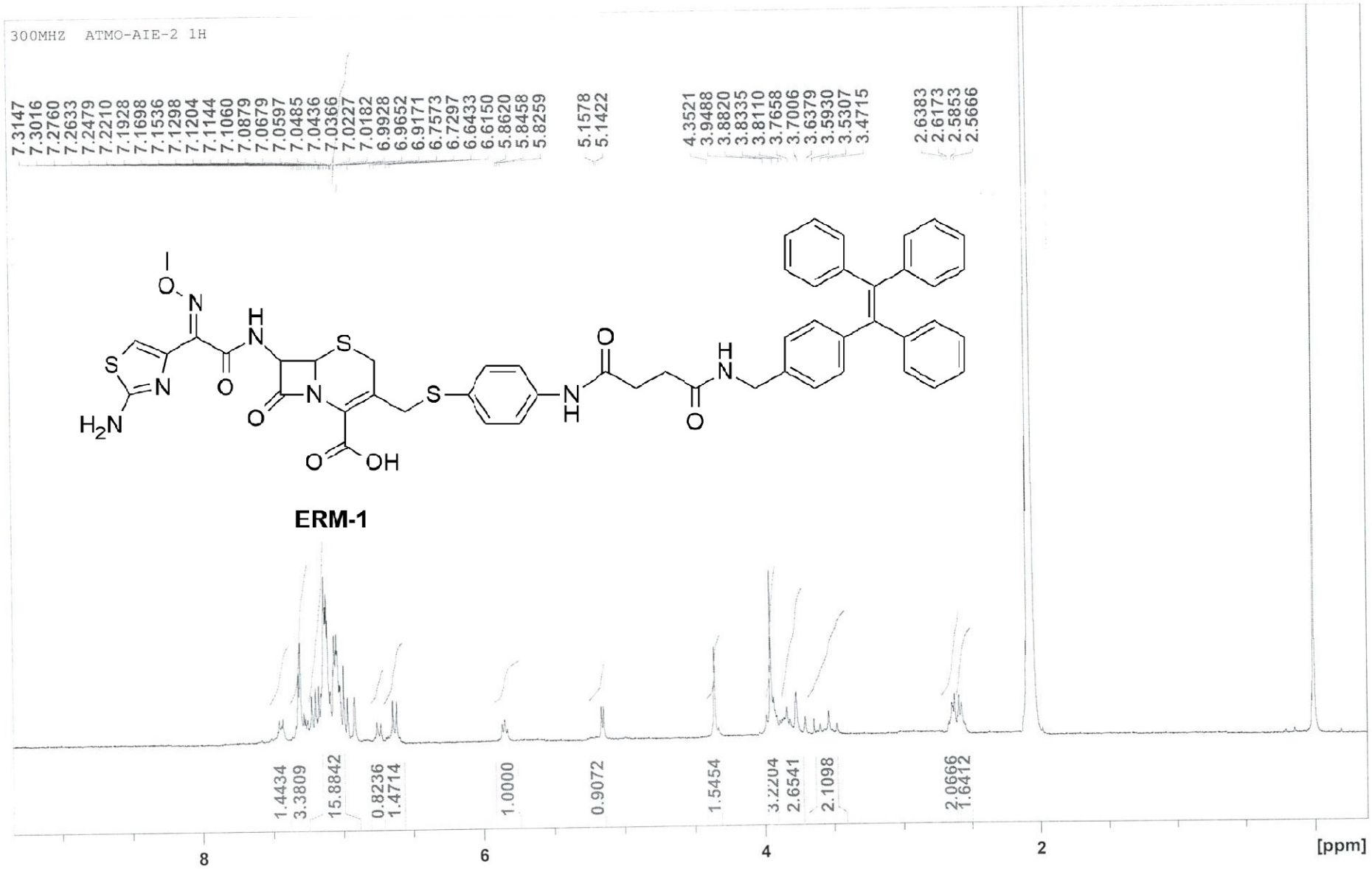

168.6238
163.6821
162.2435
160.5394
147.2202
147.0340
143.3309
143.2747
140.0296
139.5836
139.3772
137.4547
135.6644
132.8256
129.1982
128.9619
128.5373
128.4447
128.2316
128.1045
127.9580
127.6262
127.5666
127.0214
124.1635
120.6579
115.4731
113.2252
79.2210
71.5811
63.3223
58.7560
57.8052
38.9145
28.8608
21.0210
TTMHN
NH2
OCHPh2
200
150
100
50
0
[ppm]

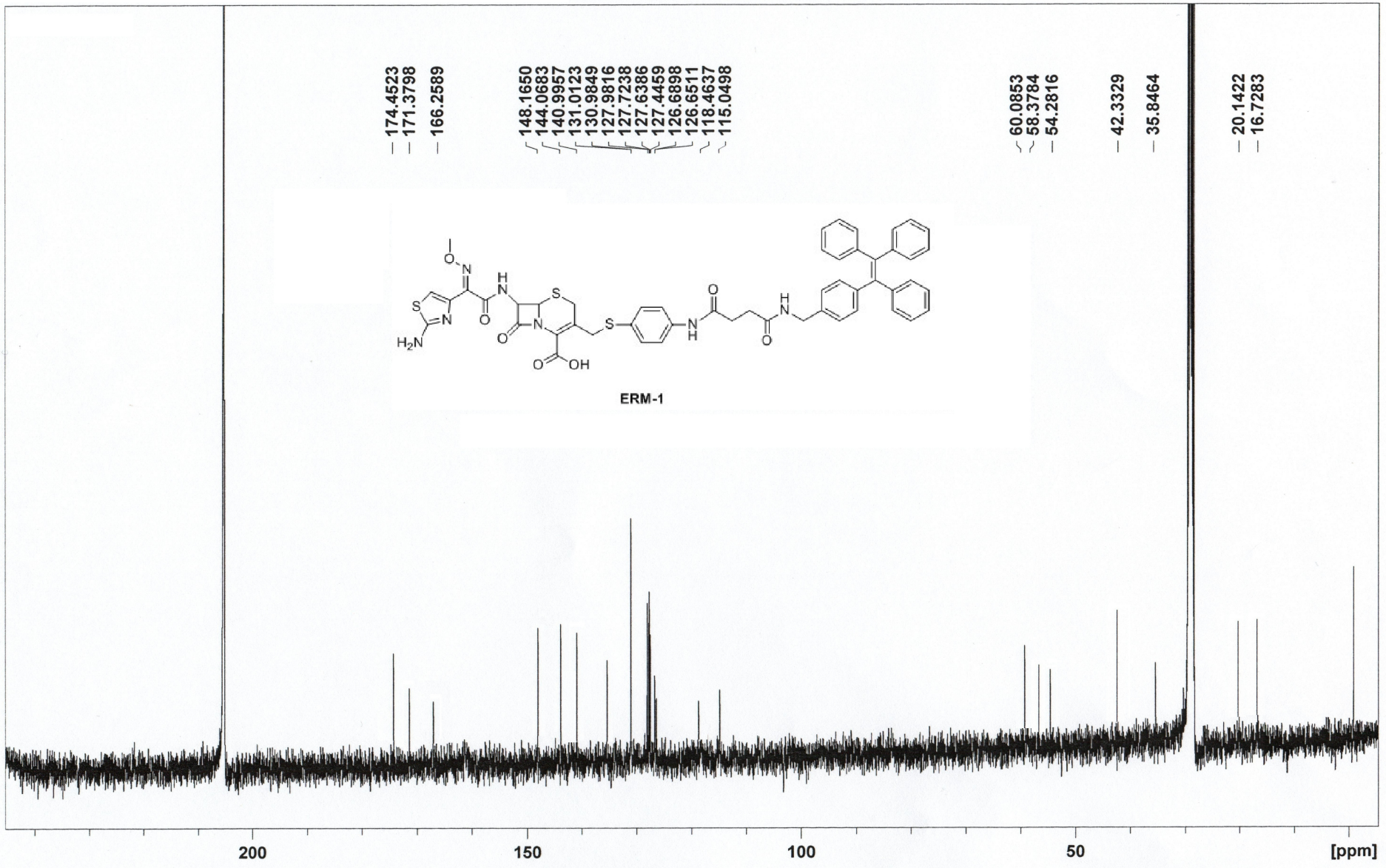
11

C:\Xcalibur\...\Junxin\MEOH_161209144745 ATMO -196-NH2
MEOH
MEOH_161209144745 #4 RT: 0.04 AV: 1 NL: 1.37E5
T: ITMS + c ESI Full ms [50.00-2000.00]

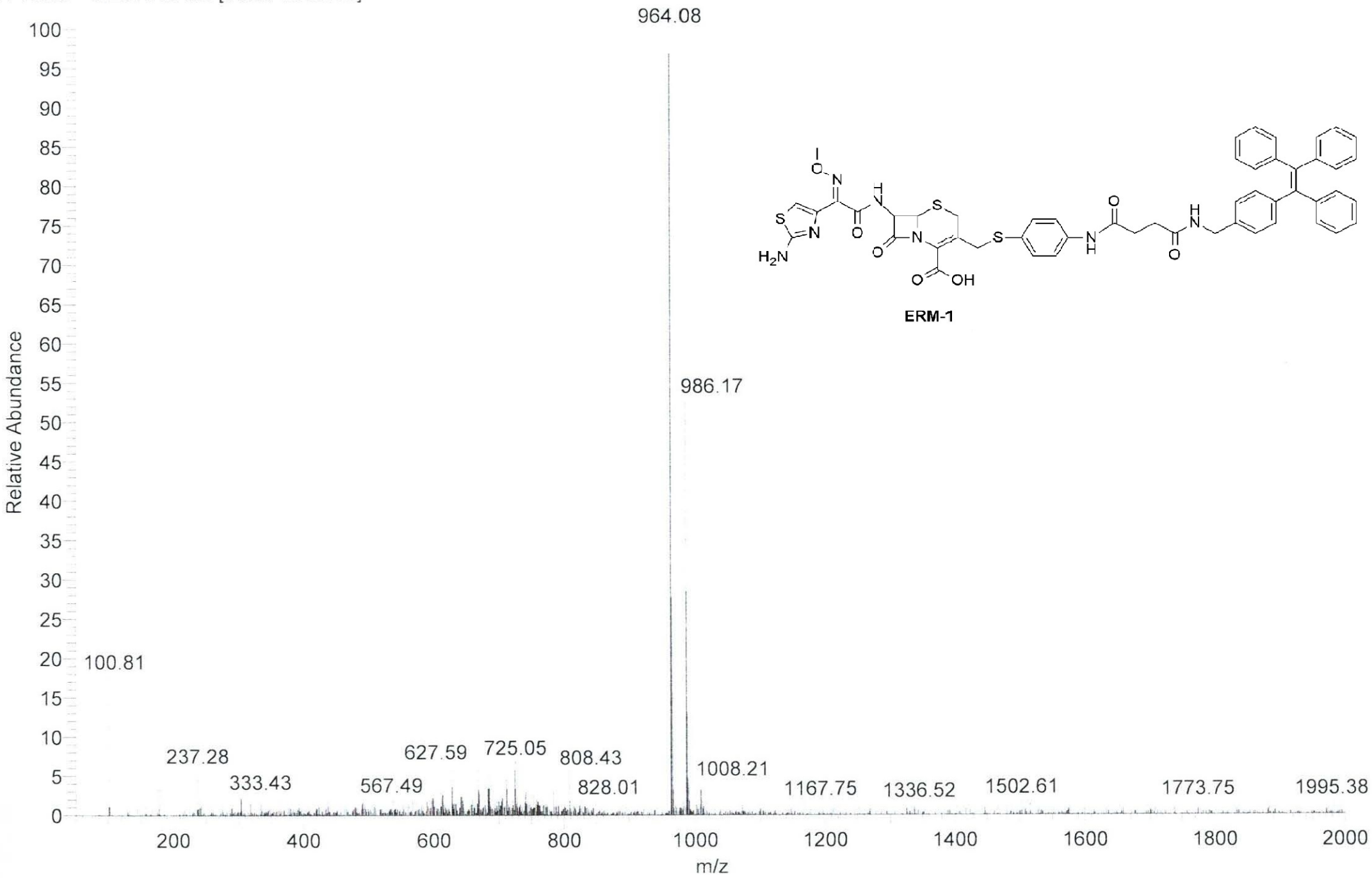


11

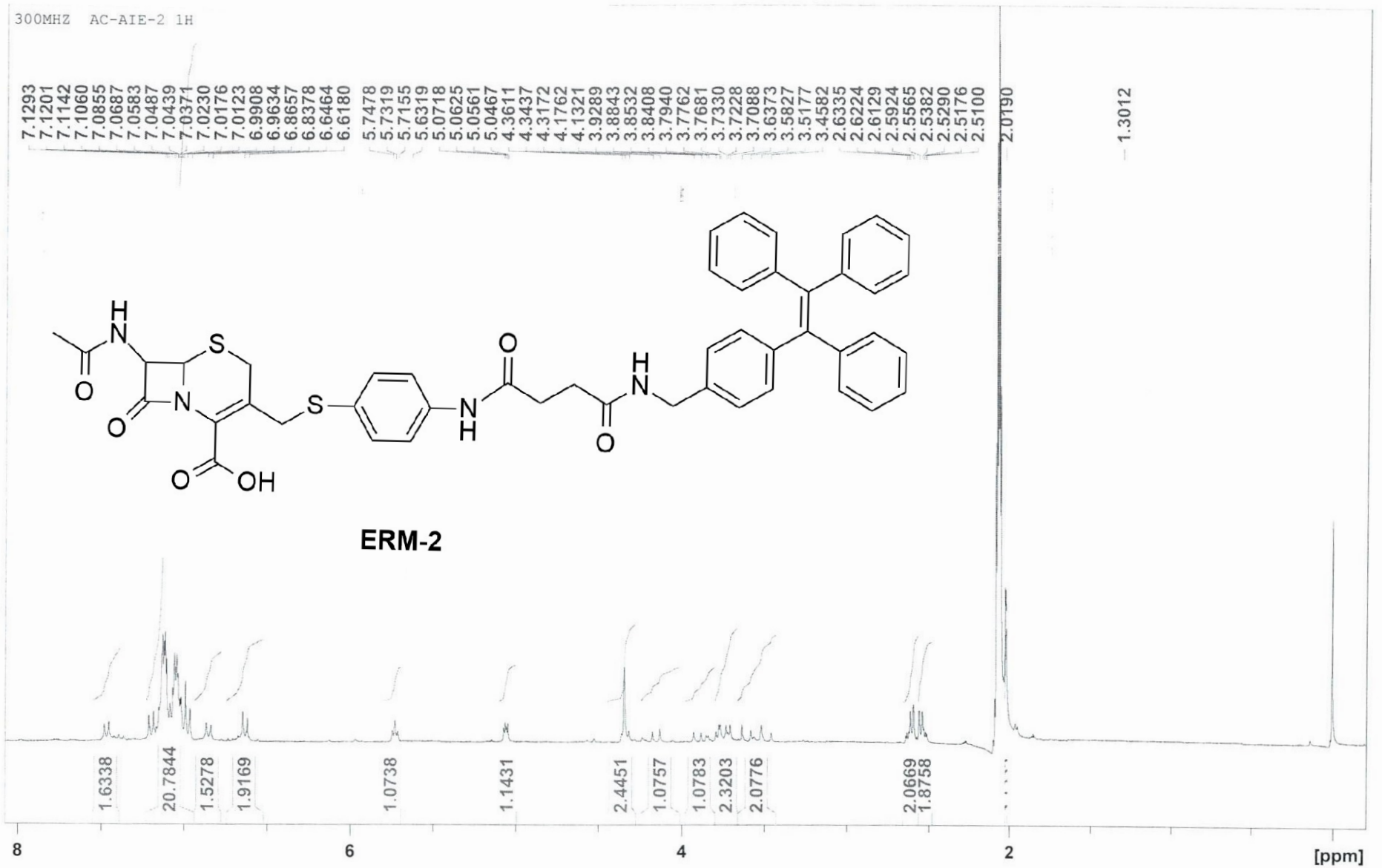

300MHZ ATMO-AIE-2 1H
7.3147
7.3016
7.2760
7.2633
7.2479
7.2210
7.1928
7.1698
7.1536
7.1298
7.1204
7.1144
7.1060
7.0879
7.0679
7.0597
7.0485
7.0436
7.0366
7.0227
7.0182
6.9928
6.9652
6.9171
6.7573
6.7297
6.6433
6.6150
5.8620
5.8458
5.8259
5.1578
5.1422
4.3521
3.9488
3.8820
3.8335
3.8110
3.7658
3.7006
3.6379
3.5930
3.5307
3.4715
2.6383
2.6173
2.5853
2.5666
ERM-1
1.4434
3.3809
15.8842
0.8236
1.4714
1.0000
0.9072
1.5454
3.2204
2.6541
2.1098
2.0666
1.6412
8
6
4
2
[ppm]

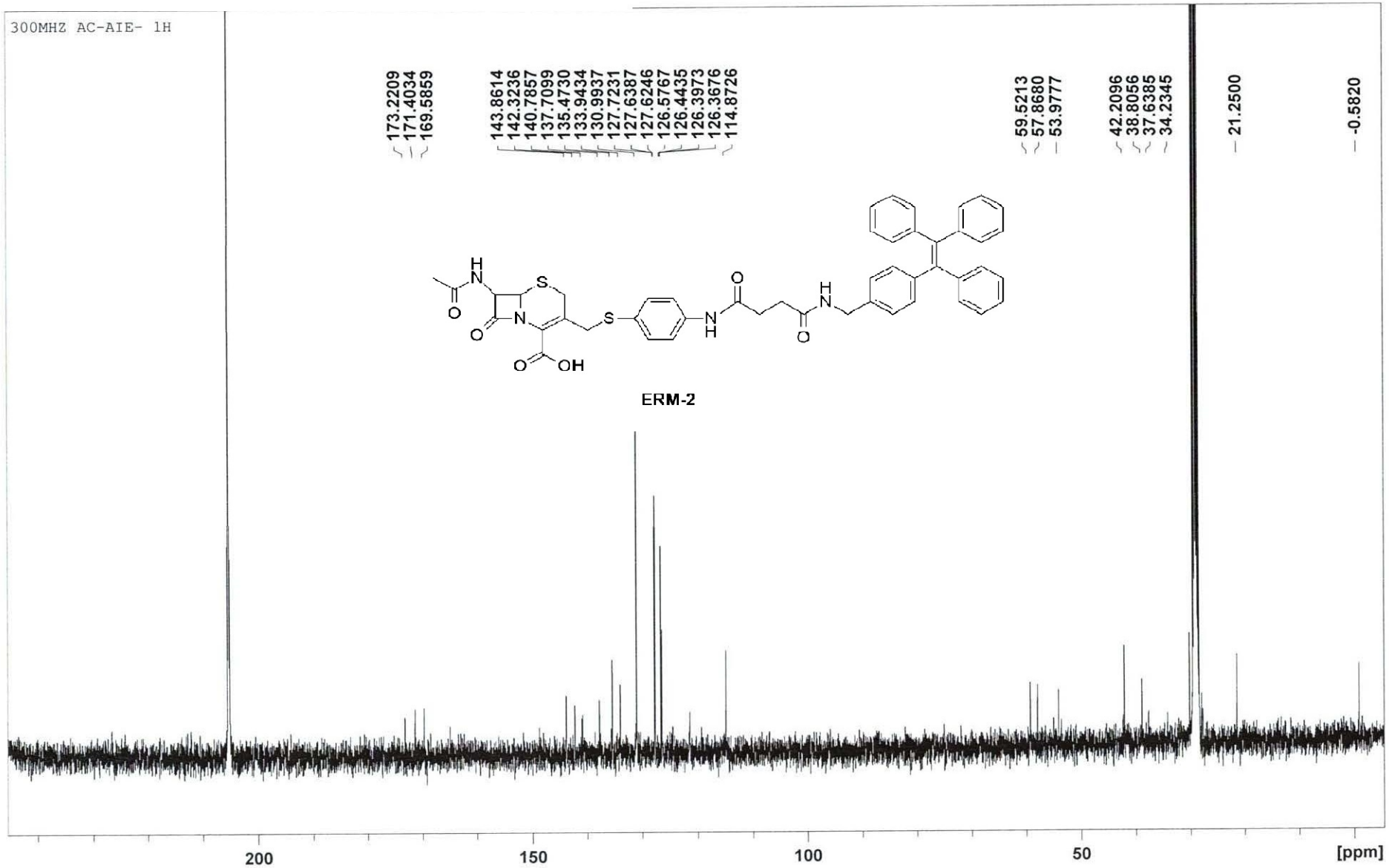

174.4523
171.3798
166.2589
148.1650
144.0683
140.9957
131.0123
130.9849
127.9816
127.7238
127.6386
127.4459
126.6898
126.6511
118.4637
115.0498
60.0853
58.3784
54.2816
42.3329
35.8464
20.1422
16.7283
ERM-1
200
150
100
50
[ppm]

C:\Xcalibur\...\Junxin\atmo-aie
MEOH
atmo-aie #7 RT: 0.08 AV: 1 NL: 5.22E4
T: ITMS + c ESI Full ms [50.00-2000.00]

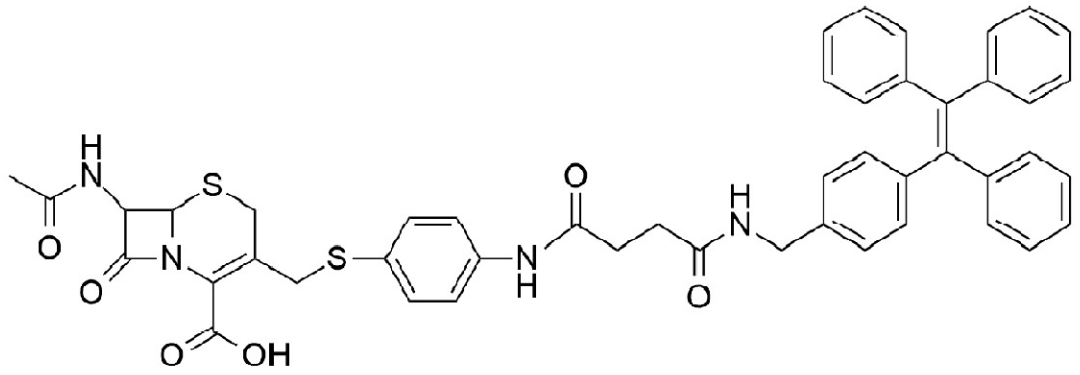


ERM-1


300MHZ AC-AIE-2 1H
7.1293
7.1201
7.1142
7.1060
7.0855
7.0687
7.0583
7.0487
7.0439
7.0371
7.0230
7.0176
7.0123
6.9908
6.9634
6.8657
6.8378
6.6464
6.6180
5.7478
5.7319
5.7155
5.6319
5.0718
5.0625
5.0561
5.0467
4.3611
4.3437
4.3172
4.1762
4.1321
3.9289
3.8843
3.8532
3.8408
3.7940
3.7762
3.7681
3.7330
3.7228
3.7088
3.6373
3.5827
3.5177
3.4582
2.6335
2.6224
2.6129
2.5924
2.5565
2.5382
2.5290
2.5176
2.5100
2.0190
1.3012
ERM-2
1.6338
20.7844
1.5278
1.9169
1.0738
1.1431
2.4451
1.0757
1.0783
2.3203
2.0776
2.0669
1.8758
8
6
4
2
[ppm]


300MHZ AC-AIE- 1H
173.2209
171.4034
169.5859
143.8614
142.3236
140.7857
137.7099
135.4730
133.9434
130.9937
127.7231
127.6387
127.6246
126.5767
126.4435
126.3973
126.3676
114.8726
59.5213
57.8680
53.9777
42.2096
38.8056
37.6385
34.2345
21.2500
-0.5820
ERM-2
200
150
100
50
[ppm]

C:\Xcalibur\...\Junxin\acetyl-aie
MEOH
acetyl-aie #4 RT: 0.04 AV: 1 NL: 1.94E5
T: ITMS + c ESI Full ms [50.00-2000.00]



ERM-2