

Syntheses of Siderophore-Drug Conjugates Using a Convergent Thiol-Maleimide System

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EXPERIMENTAL

Chemistry: General materials and methods. All solvents and reagents were obtained from commercial sources and used without further purification unless otherwise stated. Dichloromethane (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled from CaH_2 . Tetrahydrofuran (THF) was distilled from a mixture of sodium metal and benzophenone ketyl. Diisopropylethylamine (DIPEA), was used from Acros Seal® anhydrous bottle. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were obtained on a 500 MHz, or 600 MHz Varian DirectDrive spectrometer and FIDs were processed using ACD/SpecManager version 11. Chemical shifts (δ) are given in parts per million (ppm) and are referenced to residual solvent peaks as internal standards. Coupling constants (J) are reported in hertz (Hz). High resolution, accurate mass measurements were obtained with a Bruker micrOTOF II electrospray ionization time-of-flight mass spectrometer in positive ion mode. LCMS was performed on a Waters ZQ instrument consisting of a chromatography module Alliance HT, photodiode array detector 2996, and mass spectrometer Micromass ZQ with an MS electrospray source operate at capillary voltage 3.5 kV and a desolvation temperature of 300 °C. The LCMS instrument used a YMC Pro C18 reverse phase column (3.0 x 50 mm) fit with precolumn frit (0.5 μm) and YMC Pro C18 guard column (2.0 x 10 mm) for all analyses. Mobile phases used were 10 mM ammonium acetate in HPLC grade water (A) and HPLC grade acetonitrile (B). A gradient was formed from 5%-80% of B in 10 min, hold 80% of B for 2 min, then 80%-5% of B in 0.5 min at a flow rate of 0.7 mL/min (total run time of 12.5 min). Semi-preparative HPLC purifications were performed on a Waters semi-preparative binary pump system at a flow rate of 15 mL/min with detection by UV detection at 254 nm with separation facilitated by a YMC-Pack Pro C18 column, 150 x 20 mm (I.D.), particle size 5 μm fit with a guard column. All reactions were conducted under argon gas unless otherwise noted. Solvents were removed *in vacuo* on a rotary evaporator. All reactions were carried out at ambient temperature (~22 °C) unless stated otherwise. Reactions were monitored by thin layer chromatography (TLC) performed with aluminum-backed Merck 60-F₂₅₄ silica gel plates using a 254 nm lamp, ceric ammonium molybdate (CAM) stain or FeCl_3 stain for visualization. Silica gel column chromatography was performed using Sorbent Technologies silica gel 60 (32-63 μm). Melting points were determined in capillary tubes using a Thomas Hoover melting point apparatus and are uncorrected.

S-Triptyl protected acid 4.²³⁻²⁴ To a solution of 5.95 g (30.5 mmol) of commercially available 6-bromohexanoic acid and 8.56 g (30.9 mmol) of triphenyl methanethiol in 60 mL of freshly distilled CH_2Cl_2 under argon, 7 mL (46.9 mmol) of DBU (1,8-diazabicycloundec-7-ene) were added dropwise. After 3 h, the crude mixture was poured over a mixture of ice/1 M H_2SO_4 (60 mL), and the aqueous layer was further extracted with EtOAc (2 x 20 mL), the organic volumes were combined and sequentially washed with brine/ice (2 x 60 mL), and brine (60 mL); the EtOAc layer was separated, dried over Na_2SO_4 , filtered, concentrated under vacuum, purified by silica gel chromatography using CH_2Cl_2 as the eluent TLC R_f = 0.61 (2:8, EtOAc/ CH_2Cl_2 , CAM stain), and recrystallized from CH_2Cl_2 /hexanes to afford 4.84 g (40%) of **7**²³⁻²⁴ as white crystals: mp 91-92 °C (lit. 90-91 °C)²³; $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 7.44 - 7.40 (m, 6H), 7.31 - 7.26 (m, 6H), 7.24 - 7.19 (m, 3H), 2.27 (t, J = 7.5 Hz, 2H), 2.15 (t, J = 7.3 Hz, 2H), 1.51 (dt, J = 15.3, 7.4 Hz, 2H), 1.43 - 1.36 (m, 2H), 1.33 - 1.26 (m, 2H); $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 179.5, 145.2, 129.8, 128.0, 126.8, 66.7, 33.9, 31.9, 28.6, 28.5, 24.4; HRMS (ESI) m/z [$\text{M}+\text{Na}$]⁺: calcd for $\text{C}_{25}\text{H}_{26}\text{NaO}_2\text{S}^+$, 413.1546; found, 413.1535.

S-Triptyl protected NHS ester 5. To a solution of 2.40 g (6.15 mmol) of S-triptyl protected acid **4**, and 2.24 g (19.5 mmol) of *N*-hydroxysuccinimide in 40 mL of freshly distilled CH_2Cl_2 under argon, 3.13 g (16.3 mmol) of EDC•HCl were added in one portion. After 16 h, the crude mixture was poured over 0.001 M HCl (pH = 3, 70 mL), the layers were separated and the organic volume was sequentially washed with 0.001 M HCl (50 mL), brine (50 mL), 10% NaHCO_3 (50 mL), and brine (2 x 50 mL); the EtOAc layer was separated, dried over Na_2SO_4 , filtered, and concentrated under vacuum to afford 3.25 g of **5** as a colorless, viscous oil in quantitative yield. The desired product could be purified by silica gel chromatography using CH_2Cl_2 as the eluent if necessary, TLC R_f = 0.76 (1:9, EtOAc/ CH_2Cl_2 , CAM stain): $^1\text{H NMR}$ (600 MHz, CDCl_3) δ 7.45 - 7.40 (m, 6H), 7.31 - 7.27 (m, 6H), 7.24 - 7.20 (m, 3H), 2.83 (d, J = 7.3 Hz, 4H), 2.52 (t, J = 7.5 Hz, 2H), 2.17 (t, J = 7.2 Hz, 2H), 1.64 - 1.56 (m, 2H), 1.43 - 1.32 (m, 4H); $^{13}\text{C NMR}$ (150 MHz, CDCl_3) δ 169.3, 168.6, 145.1, 129.8, 128.0, 126.8, 66.7, 31.7, 30.9, 28.3, 28.3, 25.8, 24.2; HRMS (ESI) m/z [$\text{M}+\text{Na}$]⁺: calcd for $\text{C}_{29}\text{H}_{29}\text{NNaO}_4\text{S}^+$, 510.1710; found, 510.1693.

S-Triptyl protected ampicillin 6. A mixture of 0.105 g (0.26 mmol) of ampicillin trihydrate in 2 mL of THF/ H_2O (4:1) and 0.09 mL of Et_3N (0.64 mmol) was added slowly to a solution of 0.116 g (0.24 mmol) of S-triptyl protected NHS ester **5** in 3 mL of THF. After 7 h, the crude mixture was diluted with EtOAc (40 mL) and poured over ice/1 M H_2SO_4 (40 mL), the aqueous layer was further extracted with EtOAc (2 x 15 mL), the organics were combined and washed with ice/1 M H_2SO_4 (30 mL), and brine (2 x 30 mL). The EtOAc layer was separated, dried over Na_2SO_4 , filtered, concentrated under vacuum, purified by silica gel chromatography using a gradient of 1:99 to 4:96, isopropyl alcohol/ CH_2Cl_2 , TLC R_f = 0.36 (2:8, isopropyl alcohol/ CH_2Cl_2 , CAM stain),

and triturated with diethyl ether to afford 0.052 g (30%) of **6** as a white, powdery solid: mp 120-122 °C (sinters and decomp.); ¹H NMR (600 MHz, CD₃OD) δ 7.43 - 7.36 (m, 8H), 7.34 - 7.25 (m, 9H), 7.22 - 7.18 (m, 3H), 5.57 (s, 1H), 5.52 - 5.49 (m, 1H), 5.43 (d, *J* = 4.1 Hz, 1H), 4.31 (s, 1H), 2.20 (td, *J* = 7.5, 0.9 Hz, 2H), 2.12 (t, *J* = 7.3 Hz, 2H), 1.55 (s, 3H), 1.49 - 1.43 (m, 5H), 1.37 - 1.31 (m, 2H), 1.27 - 1.20 (m, 2H); ¹³C NMR (150 MHz, CD₃OD) δ 175.8, 174.6, 172.5, 171.2, 146.6, 138.6, 130.9, 129.9, 129.4, 129.0, 127.8, 72.1, 68.9, 65.3, 60.1, 58.3, 36.5, 33.0, 31.3, 29.7, 29.6, 27.5, 26.6; HRMS (ESI) *m/z* [M+Na]⁺: calcd for C₃₆H₄₃N₅NaO₇S₂⁺, 744.2496; found, 744.2516.

S-Trityl protected loracarbef 7. A suspension of 0.25 g (0.72 mmol) of loracarbef in 3 mL of DMF, 2 mL of pyridine and 1 mL of H₂O, was added to a solution of 0.23 g (0.47 mmol) of *S*-trityl protected NHS ester **5** in 2 mL of DMF, to improve solubility, 2 mL of a solution THF/H₂O (4:1) was added to the reaction mixture. After 3 h, 0.11 mL (0.78 mmol) of Et₃N were added and after 3 h the solvent was removed *in vacuo* to afford a yellow solid that was dissolved in EtOAc (50 mL), the solution was sequentially washed with 0.001 M HCl (pH = 3) until the pH of the aqueous layer was approximately 2.5-3 (by paper). The layers were separated, and the aqueous volume was extracted with EtOAc (2 × 30 mL), the organics were combined, washed with H₂O (pH = 3, 50 mL), brine (2 × 70 mL), the layers were then separated, dried over Na₂SO₄, concentrated and purified by silica gel chromatography using a gradient of 2:98 to 4:96 isopropyl alcohol/CH₂Cl₂ as the eluent, TLC R_f = 0.25 (plate developed twice, 2:8, isopropyl alcohol/CH₂Cl₂, CAM stain), to afford 0.19 g (56%) of **7** as an off-white, solidified foam: mp 118-120 °C (sinters); ¹H NMR (600 MHz, CDCl₃) δ 7.55 (br. s., 1H), 7.42 - 7.34 (m, 8H), 7.30 - 7.23 (m, 9H), 7.22 - 7.17 (m, 3H), 7.07 (br. s., 1H), 5.65 (d, *J* = 7.6 Hz, 1H), 5.24 (t, *J* = 5.0 Hz, 1H), 3.89 - 3.80 (m, 1H), 2.57 - 2.40 (m, 2H), 2.19 - 2.06 (m, 4H), 1.60 - 1.52 (m, 1H), 1.42 (quin, *J* = 7.6 Hz, 2H), 1.38 - 1.25 (m, 3H), 1.23 - 1.17 (m, 2H); ¹³C NMR (150 MHz, CDCl₃) δ 173.6, 171.2, 164.6, 145.2, 137.9, 129.8, 129.2, 128.7, 128.0, 127.3, 126.7, 66.6, 58.9, 56.5, 53.1, 36.5, 31.9, 31.6, 28.6, 28.5, 25.1, 21.1; HRMS (ESI) *m/z* [M+Na]⁺: calcd for C₄₁H₄₀ClN₃NaO₅S⁺, 744.2269; found, 744.2254.

S-Trityl protected ciprofloxacin 8. A mixture of 0.67 g (2.01 mmol) of ciprofloxacin in 12 mL of DMF/H₂O (1:1) and 0.6 mL of Et₃N (4.27 mmol) was added slowly to a solution of 0.49 g (1.01 mmol) of *S*-trityl protected NHS ester **5** in 12 mL of THF. After 4 h, the crude mixture was diluted with EtOAc (70 mL) and poured over 0.001 M HCl (pH = 3, 70 mL), then added 1M HCl until pH = 2 (by paper); the aqueous layer was further extracted with EtOAc (40 mL), the organics were combined and washed with 0.001 M HCl (3 × 70 mL), and brine (2 × 70 mL). The EtOAc layer was separated, dried over Na₂SO₄, filtered, concentrated under vacuum, purified by silica gel chromatography using a gradient of 2:98 to 3:97, isopropyl alcohol/CH₂Cl₂ as the eluent, TLC R_f = 0.32 (5:95, isopropyl alcohol/CH₂Cl₂, CAM stain), and recrystallized from CH₂Cl₂/hexanes to afford 0.36 g (51%) of **8** as a light-yellow colored powder: mp 165-166 °C; ¹H NMR (500 MHz, CDCl₃) δ 8.70 (s, 1H), 7.96 (d, *J* = 12.8 Hz, 1H), 7.44 - 7.37 (m, 6H), 7.34 (d, *J* = 7.0 Hz, 1H), 7.30 - 7.24 (m, 6H), 7.22 - 7.18 (m, 3H), 3.89 - 3.82 (m, 2H), 3.72 - 3.65 (m, 2H), 3.56 - 3.49 (m, 1H), 3.38 - 3.25 (m, 4H), 2.34 - 2.28 (m, 2H), 2.17 (t, *J* = 7.3 Hz, 2H), 1.56 (quin, *J* = 7.6 Hz, 2H), 1.48 - 1.29 (m, 6H), 1.23 - 1.17 (m, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 177.1, 177.1, 171.7, 166.9, 154.7, 152.7, 147.7, 145.6, 145.5, 145.1, 139.1, 129.7, 128.0, 126.7, 120.3, 120.2, 112.7, 112.5, 108.2, 105.3, 105.3, 66.6, 50.4, 50.3, 49.6, 49.6, 45.5, 41.3, 35.5, 33.1, 32.0, 29.0, 28.6, 25.0; HRMS (ESI) *m/z* [M+H]⁺: calcd for C₄₂H₄₃FN₃O₄S⁺, 704.2953; found, 704.2959.

S-Trityl protected nadifloxacin 9. To a solution of 98.8 mg (0.25 mmol) of *S*-trityl protected hexanoic acid **4**, and 81.4 mg (0.23 mmol) of commercially available nadifloxacin in 5 mL of anhydrous CH₂Cl₂, was added 0.12 g (0.62 mmol) of EDC•HCl and 0.04 g (0.03 mmol) of DMAP. After 13 h, the reaction mixture was diluted with CH₂Cl₂ (20 mL), and washed with H₂O (pH = 3, 25 mL), the aqueous layer was extracted with CH₂Cl₂ (25 mL) and the combined organics were washed with H₂O (pH = 3, 30 mL), brine (2 × 30 mL), dried over Na₂SO₄, concentrated *in vacuo*, and purified by silica gel chromatography using a gradient of 1:99 to 3:97 isopropyl alcohol/CH₂Cl₂ as the eluent, TLC R_f = 0.75 (2:8, isopropyl alcohol/CH₂Cl₂, CAM stain), to afford 39.6 mg (24%) of **9** as an off-white residue: ¹H NMR (600 MHz, CDCl₃) δ 8.70 (s, 1H), 8.03 (d, *J* = 12.3 Hz, 1H), 7.44 - 7.39 (m, 6H), 7.31 - 7.25 (m, 6H), 7.23 - 7.18 (m, 3H), 5.00 (br. s., 1H), 4.54 (qt, *J* = 6.7, 3.4 Hz, 1H), 3.37 - 3.00 (m, 4H), 2.87 (ddd, *J* = 17.8, 11.2, 6.9, 1H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.23 - 2.13 (m, 3H), 2.13 - 1.95 (m, 2H), 1.93 - 1.72 (m, 2H), 1.68 - 1.59 (m, 1H), 1.59 - 1.49 (m, 5H), 1.48 - 1.37 (m, 2H), 1.36 - 1.20 (m, 4H); ¹³C NMR (150 MHz, CDCl₃) δ 177.7, 177.7, 173.1, 167.5, 146.6, 145.2, 133.8, 133.8, 129.8, 128.0, 126.8, 111.1, 110.9, 108.0, 66.6, 58.1, 34.6, 32.0, 29.9, 28.6, 28.5, 26.2, 24.8, 20.4, 19.0; HRMS (ESI) *m/z* [M+H]⁺: calcd for C₄₄H₄₆FN₂O₅S⁺, 733.3106; found, 733.3128.

N-Maleimide NHS ester 10. To a solution of 0.043 g (0.22 mmol) of 5-maleimidopentanoic acid³³ and 0.076 g (0.66 mmol) of *N*-hydroxysuccinimide in 5 mL of anhydrous CH₂Cl₂, were added 0.202 g of EDC•HCl (1.05 mmol) in one portion, the reaction mixture stirred under argon for 3 h before it was judged complete by TLC R_f = 0.25 (5:95, isopropyl alcohol/CH₂Cl₂, CAM stain). The organic solution was sequentially washed with 10% citric acid (10 mL), brine (10 mL), 10% NaHCO₃ (10 mL), and brine (10 mL), then dried over Na₂SO₄, filtered,

concentrated under vacuum and purified by silica gel chromatography using 3:97, isopropyl alcohol/CH₂Cl₂ as the eluent to afford 0.058 g (90%) of **10** as an off-white solid: mp 135-137 °C; ¹H NMR (600 MHz, CDCl₃) δ 6.69 (s, 2H), 3.59 - 3.53 (m, 2H), 2.83 (d, *J* = 4.1 Hz, 4H), 2.68 - 2.62 (m, 2H), 1.77 - 1.67 (m, 4H); ¹³C NMR (150 MHz, CDCl₃) δ 170.9, 169.3, 168.3, 134.3, 37.2, 30.5, 27.7, 25.8, 21.9; HRMS (ESI) *m/z* [M+Na]⁺: calcd for C₁₃H₁₄N₂NaO₆⁺, 317.0744; found, 317.0741.

N-Maleimide containing DfoB 11. To remove traces of iron, all the glassware involved was washed with 6 M HCl, rinsed thoroughly with deionized H₂O, until pH > 6 (pH paper), washed with acetone and oven-dried. To a solution of 63.1 mg (0.10 mmol) of commercially available DfoB mesylate salt in 2 mL of phosphate buffer (pH = 8)²¹, were added 57.6 mg (0.20 mmol) of *N*-maleimide containing NHS ester **10** in 2 mL of THF, and the solution was allowed to stir for 47 h before the solvent was removed under vacuum, reverse phase TLC R_f = 0.36 (1:2, CH₃CN/H₂O, FeCl₃ stain). The crude material was suspended in H₂O (20 mL), cooled down to 0 °C, and filtered, the obtained precipitate was sequentially washed with cold H₂O (50 mL), cold sat. NaHCO₃ (50 mL), cold H₂O (50 mL), cold acetone (20 mL), and cold CH₂Cl₂ (50 mL), to afford 35.4 mg (50%) of **11** as an off-white solid: mp 163-166 °C (decomp.); ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.67 (br. s., 2H), 7.84 - 7.68 (m, 3H), 7.00 (s, 2H), 3.56 (br. s., 1H), 3.49 - 3.39 (m, 5H), 3.37 (t, *J* = 6.6 Hz, 2H), 3.04 - 2.92 (m, 6H), 2.62 - 2.52 (m, 4H), 2.32 - 2.22 (m, 4H), 2.04 (t, *J* = 6.9 Hz, 2H), 1.96 (s, 3H), 1.61 - 1.30 (m, 16H), 1.27 - 1.14 (m, 6H); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 171.9, 171.5, 171.3, 171.3, 171.1, 170.1, 134.5, 47.1, 46.8, 38.4, 38.3, 36.9, 36.6, 34.8, 29.9, 28.8, 27.6, 27.3, 26.0, 25.2, 23.5, 22.5, 20.4; HRMS (ESI) *m/z* [M+H]⁺: calcd for C₃₄H₅₈N₇O₁₁⁺, 740.4189; found, 740.4214.

N-Maleimide containing Ga-DfoB 12. To a suspension of 0.051 g (0.069 mmol) of maleimide-containing DfoB **11** in 18 mL of anhydrous methanol under argon, 0.028 g (0.077 mmol) of Ga(acac)₃ were added in one portion. The mixture was stirred at 40 °C for 6 h, and at room temperature for 16 h, before the volatiles were removed by evaporation. The resulting crude was purified by iron-free³² silica gel chromatography using 9:91, methanol/CH₂Cl₂ as the eluent, TLC R_f = 0.15 (plate developed twice using 1:9, methanol/CH₂Cl₂, CAM stain), and precipitated from chloroform/diethyl ether to afford 0.047 g (84%) of **12** as a white solid: ¹H NMR (600 MHz, CDCl₃) δ 6.69 (s, 2H), 3.94 - 3.62 (m, 8H), 3.53 (t, *J* = 6.6 Hz, 4H), 3.39 - 3.27 (m, 2H), 3.21 - 2.75 (m, 8H), 2.52 (br. s., 3H), 2.36 (br. s., 3H), 2.30 - 2.19 (m, 3H), 2.16 (s, 3H), 2.06 (br. s., 2H), 1.86 - 1.15 (m, 25H); HRMS (ESI) *m/z* [M+H]⁺: calcd for C₃₄H₅₅GaN₇O₁₁⁺, 806.3210; found, 806.3206; HPLC-LCMS retention time 3.65 min.

Ga-DfoB-loracarbef conjugate 16. *Sulfur deprotection:* To a solution of 28.9 mg (0.04 mmol) of *S*-trityl protected loracarbef **7** in 4.8 mL of freshly distilled and degassed CH₂Cl₂ (argon bubbled for 30 minutes), were added 0.12 mL (2% v/v) of triisopropylsilane (*i*Pr₃SiH) and 1 mL (20% v/v) of TFA, the solution was stirred under argon for 5 min before being concentrated under vacuum, and co-evaporated with CH₂Cl₂ (2 × 2 mL) to afford thiol-containing loracarbef **13** as a white solid that was used without further characterization. *Siderophore conjugation:* To a suspension of 0.04 mmol of **13** and 13.2 mg (0.016 mmol) of Ga-DfoB **12** in 2 mL of anhydrous and degassed THF, were added 1 mL of CH₃CN and 9 μL (0.052 mmol) of anhydrous DIPEA and the mixture was stirred under argon for 70 h, until judged complete by complete consumption of **12** under LCMS conditions. The crude mixture was concentrated under vacuum, the resulting solid was triturated with hexanes, and precipitated from CHCl₃/ether: the material was dissolved in 0.5 mL of CHCl₃ and slowly added to cold ether (0 °C, 19 mL), after removal of the mother liquor, an off-white solid was obtained and purified by preparative HPLC. *Purification:* The crude material was dissolved in 0.7 mL of DMSO and purified in 7 injections (7 × 0.1 mL) using 10 mM ammonium acetate in HPLC grade water (A) and HPLC grade acetonitrile (B) as the eluent, using a gradient of 5-80% B in 7 min, then increase to 90% of B for 2 min, hold 90% of B for 2 min, then decrease to 5% B in 1 min (total run time = 12 min). Pure fractions of **16** (HPLC-LCMS retention time 4.55 min) were combined and concentrated under vacuum to afford 11.8 mg (56%) of a light tan residue: ¹H NMR (600 MHz, CDCl₃) δ 7.66 - 7.41 (m, 2H), 7.39 - 7.23 (m, 3H), 3.94 - 3.58 (m, 6H), 3.49 (br. s., 3H), 3.38 - 2.67 (m, 8H), 2.57 - 2.18 (m, 7H), 2.14 (br. s., 2H), 2.07 (s, 3H), 1.78 - 1.13 (m, 19H); HRMS (ESI) *m/z* [M+H]⁺: calcd for C₅₆H₈₁ClGa₁₀O₁₆S⁺, 1285.4492; found, 1285.4536.

Ga-DfoB-ciprofloxacin conjugate 17. *Sulfur deprotection:* To a solution of 30.4 mg (0.04 mmol) of *S*-trityl protected ciprofloxacin **8** in 4.8 mL of freshly distilled and degassed CH₂Cl₂ (argon bubbled for 30 minutes), were added 0.12 mL (2% v/v) of triisopropylsilane (*i*Pr₃SiH) and 1 mL (20% v/v) of TFA, the solution was stirred under argon for 5 min before being concentrated under vacuum, and co-evaporated with CH₂Cl₂ (2 × 2 mL) to afford thiol-containing ciprofloxacin **14** as a white solid that was used without further characterization. *Siderophore conjugation:* To a suspension of 0.04 mmol of **14** and 12.3 mg (0.015 mmol) of Ga-DfoB **12** in 2 mL of anhydrous and degassed THF, were added 1 mL of CH₃CN and 9 μL (0.052 mmol) of anhydrous DIPEA and the mixture was stirred under argon for 69 h, until judged complete by complete consumption of **14** under LCMS conditions. The crude mixture was concentrated under vacuum, the resulting solid was triturated with

hexanes, and precipitated from CHCl₃/ether: the material was dissolved in 0.5 mL of CHCl₃ and slowly added to cold ether (0 °C, 19 mL), after removal of the mother liquor, an off-white solid was obtained and purified by preparative HPLC. *Purification*: The crude material was dissolved in 0.7 mL of DMSO and purified in 7 injections (7 × 0.1 mL) using 10 mM ammonium acetate in HPLC grade water (A) and HPLC grade acetonitrile (B) as the eluent, using a gradient of 5-80% B in 7 min, then increase to 90% of B for 2 min, hold 90% of B for 2 min, then decrease to 5% B in 1 min (total run time = 12 min). Pure fractions of **17** (HPLC-LCMS retention time 5.85 min) were combined and concentrated under vacuum to afford 4.6 mg (24%) of a light yellow residue: ¹H NMR (600 MHz, CDCl₃) δ 8.81 (s, 1H), 8.08 (d, *J* = 12.6 Hz, 1H), 7.39 (d, *J* = 7.0 Hz, 1H), 3.92 - 3.83 (m, 2H), 3.77 - 3.61 (m, 8H), 3.59 - 3.44 (m, 5H), 3.39 - 3.26 (m, 5H), 3.19 - 3.10 (m, 2H), 3.08 - 2.73 (m, 8H), 2.54 - 2.46 (m, 3H), 2.45 - 2.31 (m, 5H), 2.26 - 2.18 (m, 3H), 2.16 (s, 3H), 2.09 (br. s., 2H), 1.77 - 1.14 (m, 38H); HRMS (ESI) *m/z* [M+H]⁺: calcd for C₅₇H₈₃FGaN₁₀O₁₅S⁺, 1267.4994; found, 1267.5016.

Ga-DfoB-nadifloxacin conjugate 18. *Sulfur deprotection*: To a solution of 31.8 mg (0.04 mmol) of *S*-trityl protected nadifloxacin **9** in 4.8 mL of freshly distilled and degassed CH₂Cl₂ (argon bubbled for 30 minutes), were added 0.12 mL (2% v/v) of triisopropylsilane (*t*Pr₃SiH) and 1 mL (20% v/v) of TFA, the solution was stirred under argon for 5 min before being concentrated under vacuum, and co-evaporated with CH₂Cl₂ (2 × 2 mL) to afford thiol-containing nadifloxacin **15** as a tan residue that was used without further characterization. *Siderophore conjugation*: To a suspension of 0.04 mmol of **15** and 18.5 mg (0.023 mmol) of Ga-DfoB **12** in 3 mL of anhydrous and degassed THF, were added and 10 μL (0.057 mmol) of anhydrous DIPEA and the mixture was stirred under argon for 89 h. The crude mixture was concentrated under vacuum, co-evaporated with CHCl₃ (3 × 4 mL), triturated with hexanes, and precipitated from CHCl₃/ether: the material was dissolved in 0.5 mL of CHCl₃ and slowly added to cold ether (0 °C, 19 mL), after removal of the mother liquor, an off-white solid was obtained and purified by preparative HPLC. *Purification*: The crude material was dissolved in 0.7 mL of DMSO and purified in 7 injections (7 × 0.1 mL) using 10 mM ammonium acetate in HPLC grade water (A) and HPLC grade acetonitrile (B) as the eluent, using a gradient of 5-90% B in 7 min, then hold 90% of B for 7 min, then decrease to 5% B in 1 min (total run time = 15 min). Pure fractions of **18** (HPLC-LCMS retention time 5.07 min: 5-90% B in 7 min, hold 90% B for 5 min, 90-5% B in 0.5 min. Total run time of 12.5 min) were combined and concentrated under vacuum to afford 6.2 mg (21%) of a yellow residue: ¹H NMR (600 MHz, CDCl₃) δ 8.71 (s, 1H), 8.04 (d, *J* = 12.3 Hz, 1H), 4.60 - 4.51 (m, 1H), 3.72 (d, *J* = 3.8 Hz, 4H), 3.60 - 3.46 (m, 3H), 3.38 - 3.27 (m, 2H), 3.20 - 3.10 (m, 2H), 2.95 - 2.73 (m, 5H), 2.56 - 2.45 (m, 2H), 2.37 (br. s., 3H), 2.29 - 1.16 (m, 39H); HRMS (ESI) *m/z* [M+H]⁺: calcd for C₅₉H₈₆FGaN₉O₁₆S⁺, 1296.5148; found, 1296.5151.

Microbiology: Antibiotic Susceptibility Testing by the Agar Diffusion Method: General Materials and Methods. All liquids and media were sterilized by autoclaving (121 °C, 15 min) before use. All aqueous solutions and media were prepared using distilled, deionized, and filtered water (Millipore Milli-Q Advantage A10 Water Purification System). Luria broth (LB) was purchased from VWR. Mueller-Hinton no. 2 broth (MHII broth; cation adjusted) was purchased from Sigma-Aldrich (St. Louis, MO). Mueller-Hinton no. 2 agar (MHII agar; HiMedia Laboratories) was purchased from VWR. McFarland BaSO₄ turbidity standards were purchased from bioMérieux, Inc. Sterile plastic Petri dishes (145 mm × 20 mm; Greiner Bio-One) were purchased from VWR. Ciprofloxacin was purchased from Sigma-Aldrich (St. Louis, MO).

Antibacterial activity of the compounds was determined by a modified Kirby-Bauer agar diffusion assay.²⁷ Overnight cultures of test organisms were grown in LB broth for 18-24 h and standard suspensions of 1.5 × 10⁶ CFU/mL were prepared in saline solution (0.9% NaCl) according to a 0.5 BaSO₄ McFarland Standard.²⁸ Each standardized suspension (0.1 mL) was added to 34 mL of sterile, melted MHII agar tempered to 47-50 °C. After gentle mixing, the inoculated agar media was poured into a sterile plastic Petri dish (145 mm × 20 mm) and allowed to solidify near a flame with the lid cracked for ≈ 30 min. Wells of 9.0 mm diameter were cut from the Petri dish agar and filled with exactly 50 μL of the test sample solution. The Petri dish was incubated at 37 °C for 18-24 h, and the inhibition zone diameters were measured (mm) with an electronic caliper after 24-48 h.

Determination of MIC Values by the Broth Microdilution Assay: Antibacterial activity of the synthesized compounds was determined by measuring their minimum inhibitory concentrations (MICs) using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS) guidelines.²⁹ Each well of a 96-well microtiter plate was filled with 50 μL of sterile broth media (MHII or MHII + 100 μM of 2,2'-bipyridine). Each test compound was dissolved in DMSO making a 20 mM solution, then diluted with sterile broth media to the screened initial concentration (100-200 μM). In certain cases, the experiment was repeated at a different initial concentration to accurately determine the MIC values. Exactly 50 μL of the compound solution was added to the first well of the microtiter plate and 2-fold serial dilutions were made down each row of the plate. Exactly 50 μL of bacterial inoculum (5 × 10⁵ CFU/mL in broth media) was then added to each well giving a total volume of 100 μL/well. The plate was incubated at 37 °C for 18 h and then each well was examined for bacterial growth. The MIC was recorded as the lowest compound concentration

(μM) required to inhibit 90% of bacterial growth as judged by turbidity of the culture media relative to a row of wells filled with a DMSO standard. Ciprofloxacin was included in a control row at a concentration gradient of 32 μM –0.0156 μM .

Table S3. Antibacterial activity of compounds in the agar diffusion assay^{a-j}

Compound	<i>B. subtilis</i>	<i>S. aureus</i>	<i>M. luteus</i>	<i>M. vaccae</i>	<i>M. smegmatis</i>	<i>P. aeruginosa</i>		<i>E. coli</i>		
	ATCC 6633	SG511	ATCC 10240	IMET 10670	MC ² 155	K799/wt	K799/61	X580	DC0	DC2
6	21	27	20	13	0	20	24	23	NT	NT
7	15	23	23	17 P	17 V	0	16 U	20/29 U	0	14 V
8	0 P	0 P	0 P	H, P	0 P	14 U, P	16 U, P	22 P	0 P	0 P
9^e	21	16	0	0	0	0	0	26	0	0
Ga(acac)₃^e	0	0	0	0	0	0	0	0	0	0
Ga(NO₃)₃^e	0	0	0	0	0	0	0	0	0	0
11^e	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	14 U	13 U	0	0	H
16	17 U	22/29 U	30	16 V	0	15 U	25	25/38 U	0	14
17	14	22	18	17	0	14 U	14 V, P	24	17 V	H
18^e	36	17	0	24/30 U	0	0	0	26	0	0
Cipro	27/34 U ^c	26 ^b	0 ^b	19 ^b	17 ^b	24 ^c	28 ^c	29 ^d	21/26 V ^b	26 ^b

^aExactly 50 μL of each compound in solution (2 mM in 10:1, MeOH/DMSO) were added to 9 mm wells in agar media (MHII) inoculated with $\sim 5 \times 10^3$ CFU/mL. Diameters of growth inhibition zones were measured (mm) after incubation at 37 °C for 24 h, or 48 h for *M. vaccae* and *M. smegmatis*.²⁷⁻²⁸ Ciprofloxacin (Cipro) was used as a standard at ^b0.015 mM (5 $\mu\text{g}/\text{mL}$), ^c0.005 mM and ^d0.001mM in H₂O. ^eTested at 0.2 mM. ^fH, hint of inhibition. ^gNT, not tested. ^hP, observed precipitation. ⁱU, unclear inhibition zone. ^jV, very unclear inhibition zone.

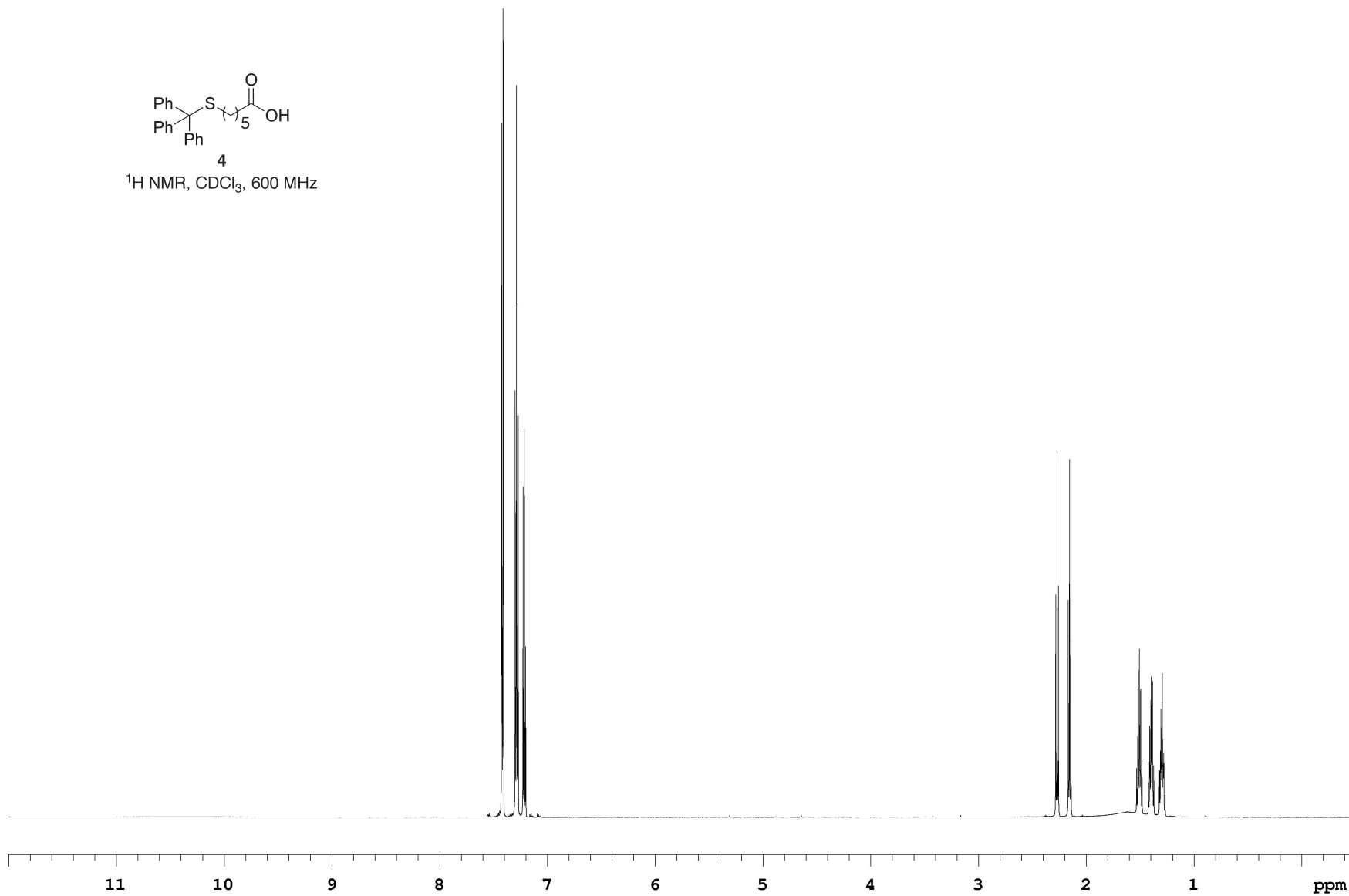
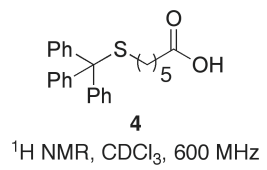
Table S4. Minimum Inhibitory Concentration of compounds (MIC μM) determined in MHII media + 100 μM 2,2'-bipyridine^{a-d}

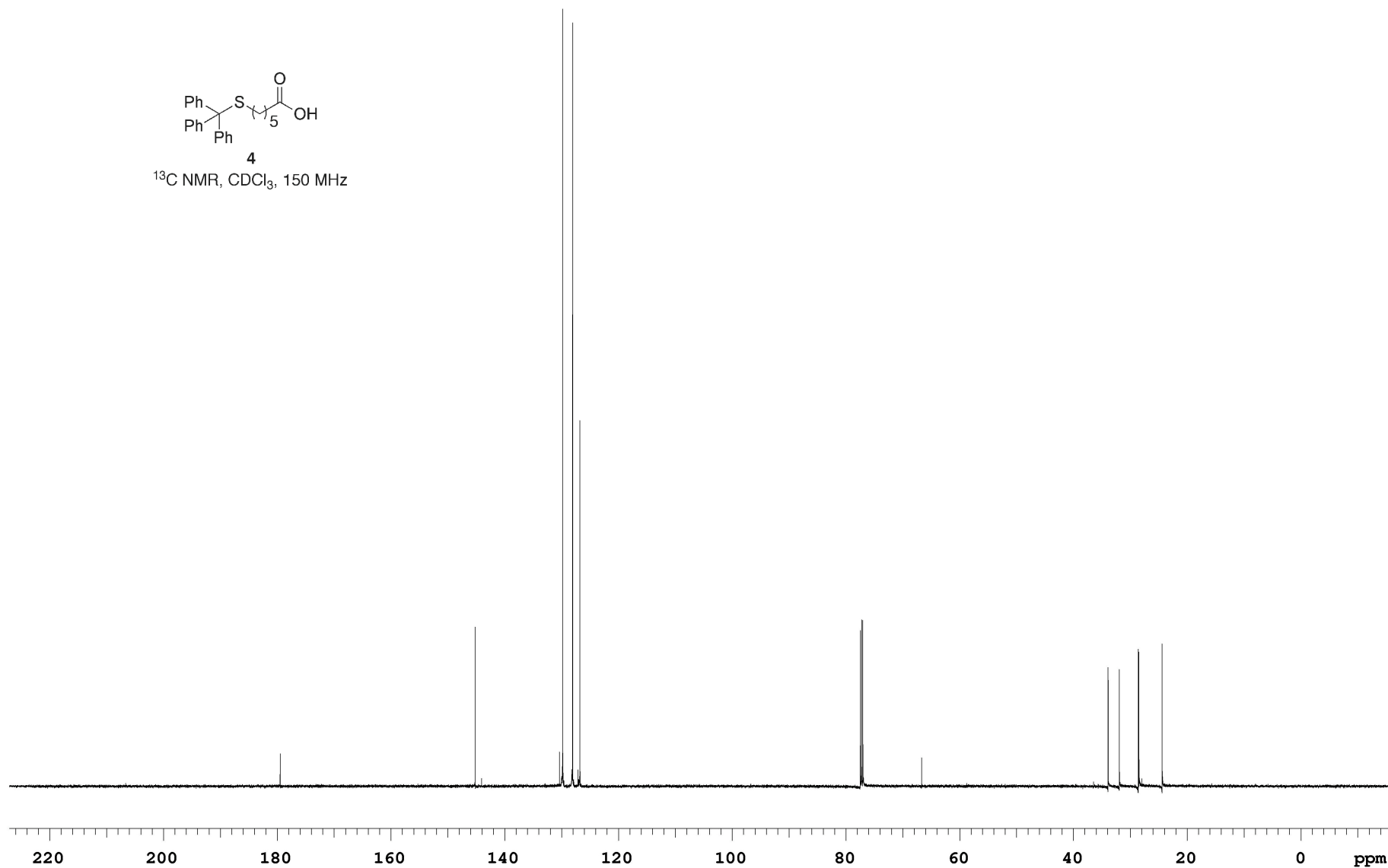
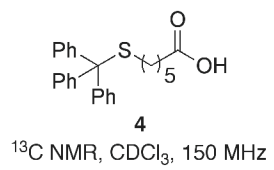
Compound	<i>B. subtilis</i>	<i>S. aureus</i>	<i>M. luteus</i>	<i>M. vaccae</i>	<i>P. aeruginosa</i>
	ATCC 6633	SG511	ATCC 10240	IMET 10670	K799/wt
6	0.78	0.39	>0.4	NG	>200
7	3.13	3.13	>0.4	-	>200
8	200	>200	25	-	>200
9	1.56	25	100	-	>200
12	>200	>200	>200	-	>200
16	7.8	3.9	0.003	-	>200
17	62.5	31.3	125	-	>200
18	<0.1	12.5	50	-	>50
Cipro	0.06	0.47	3.77	-	0.94

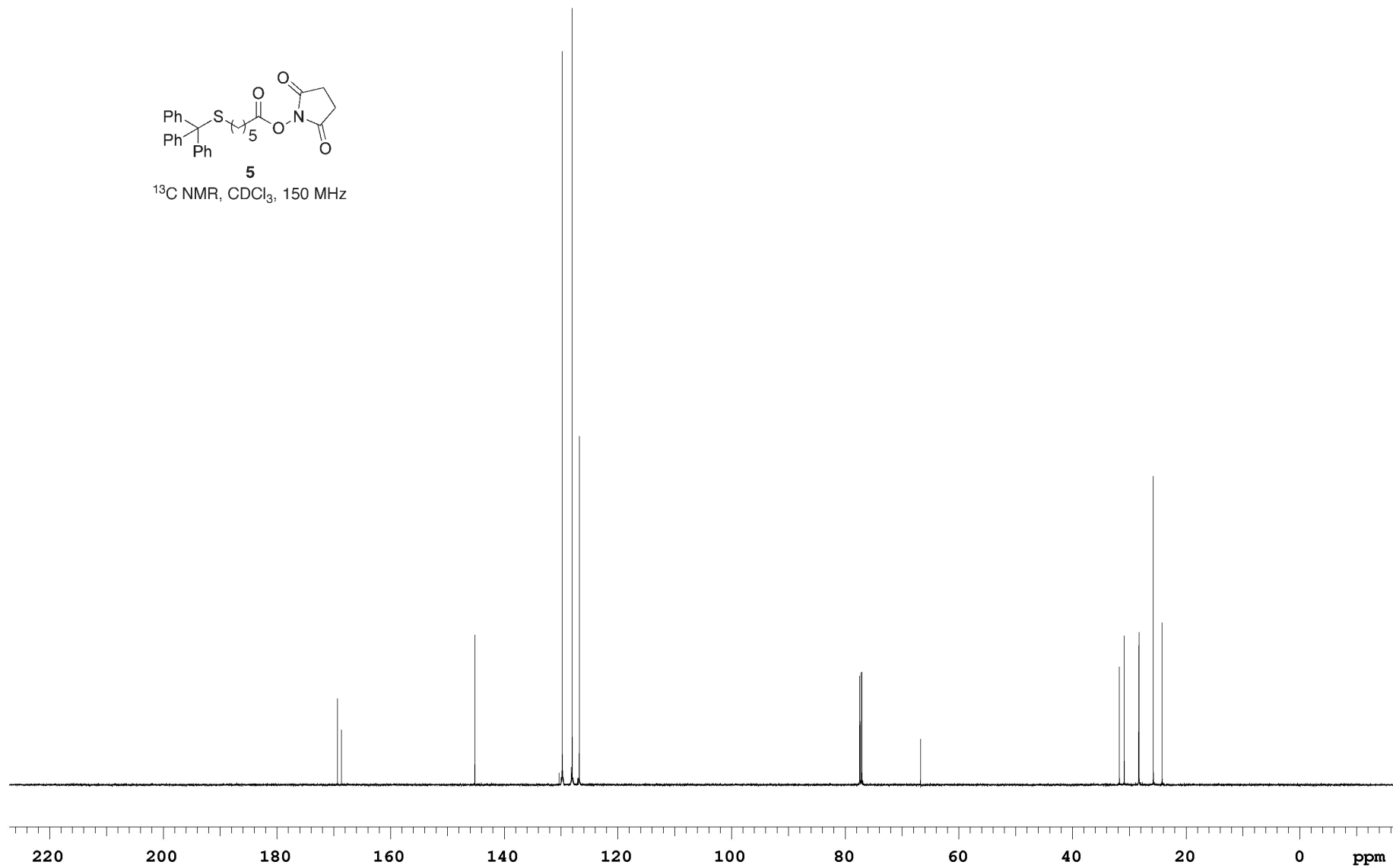
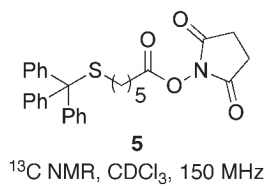
^aMICs were determined by the visual end point broth microdilution method following CLSI guidelines.²⁹ ^bReported values are the average of triplicates ($N = 3$). ^cNG, No growth. ^dP, observed precipitation.

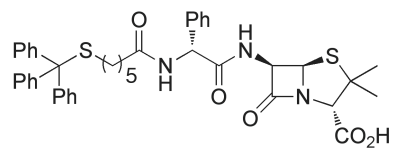
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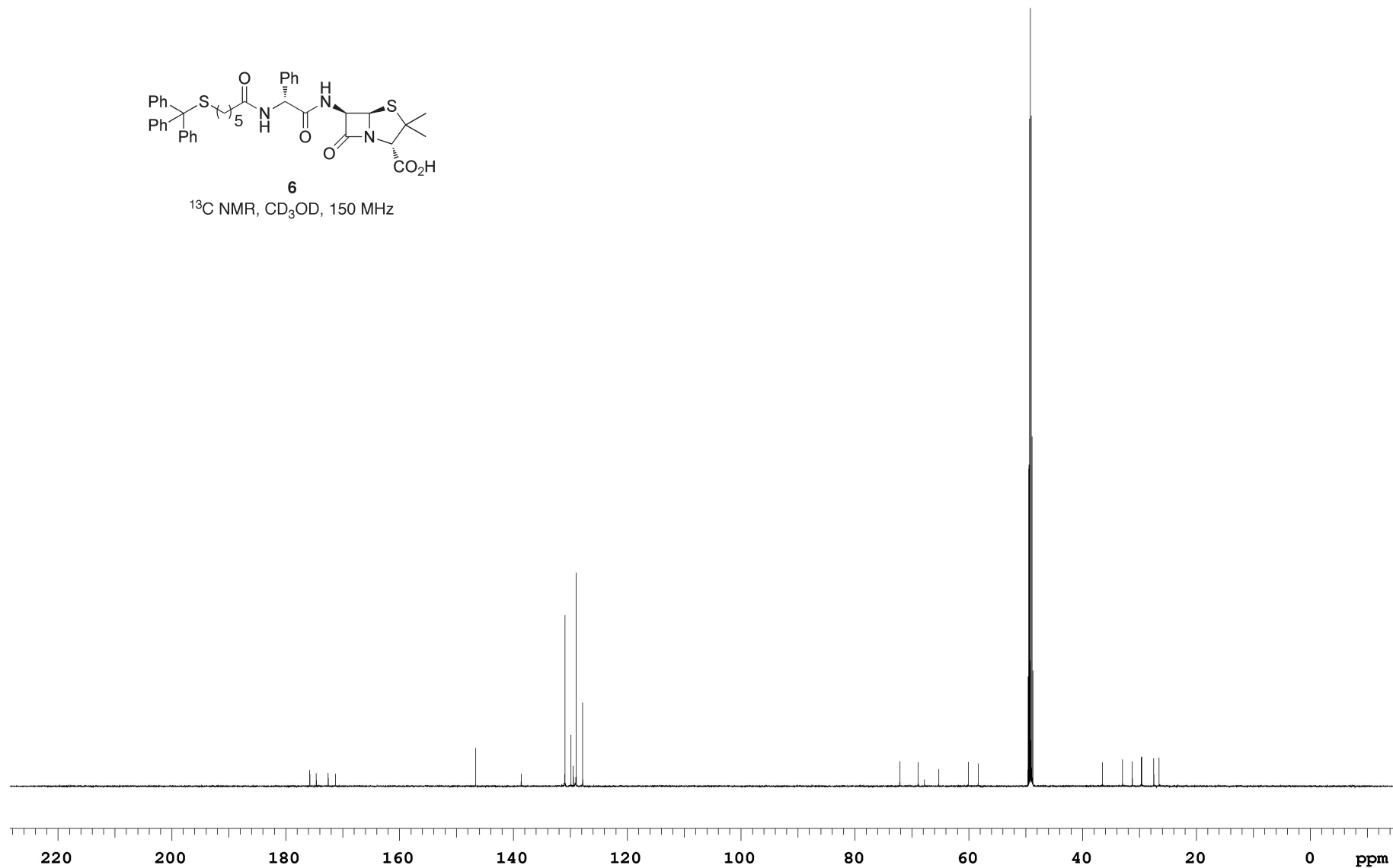


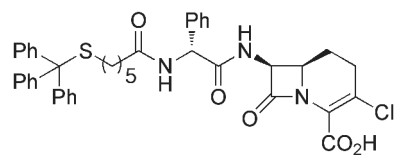




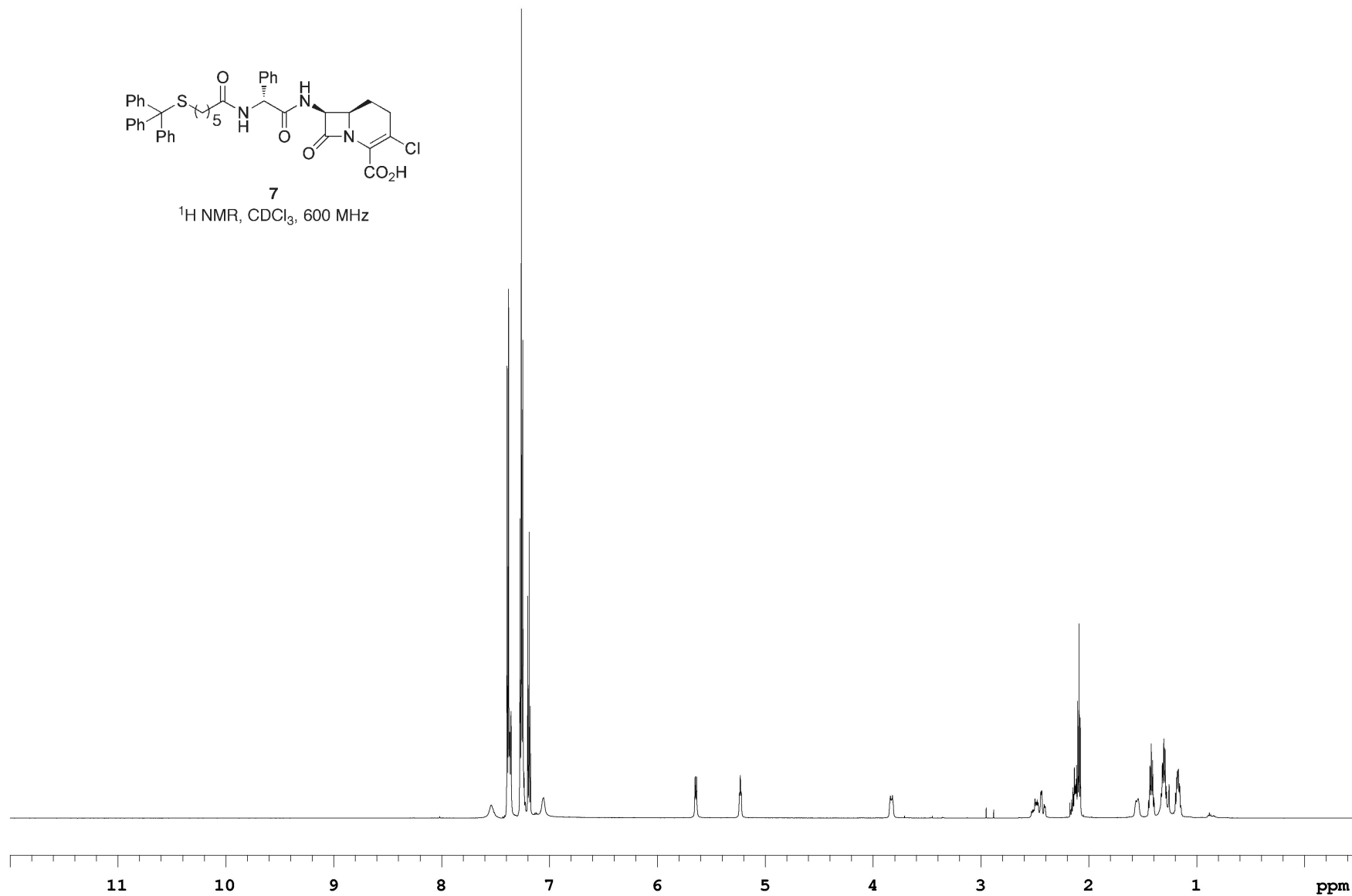


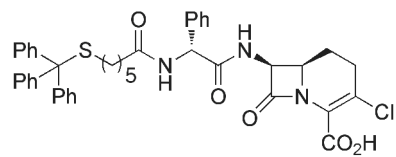
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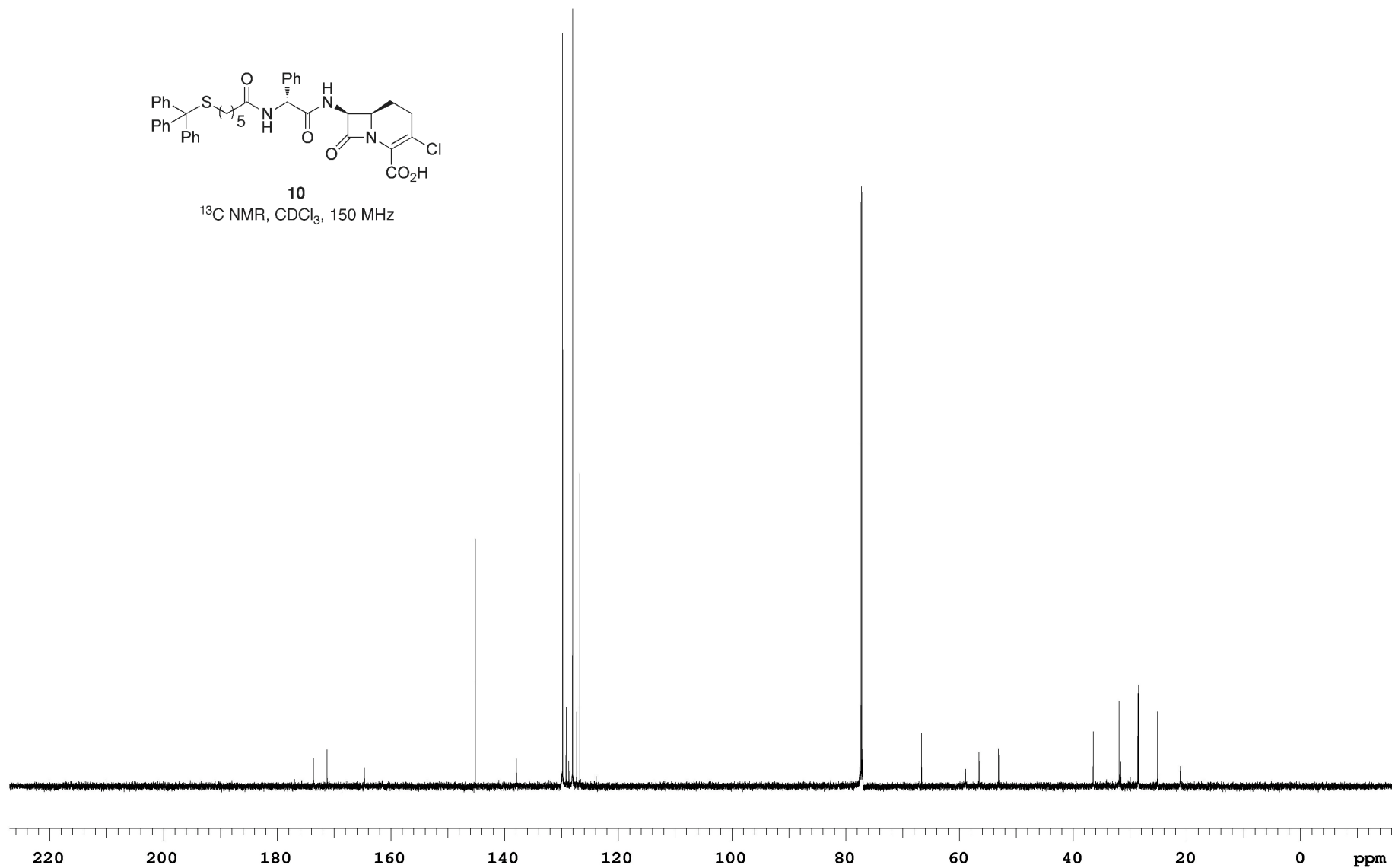
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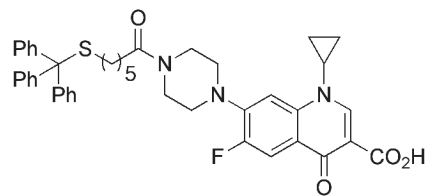




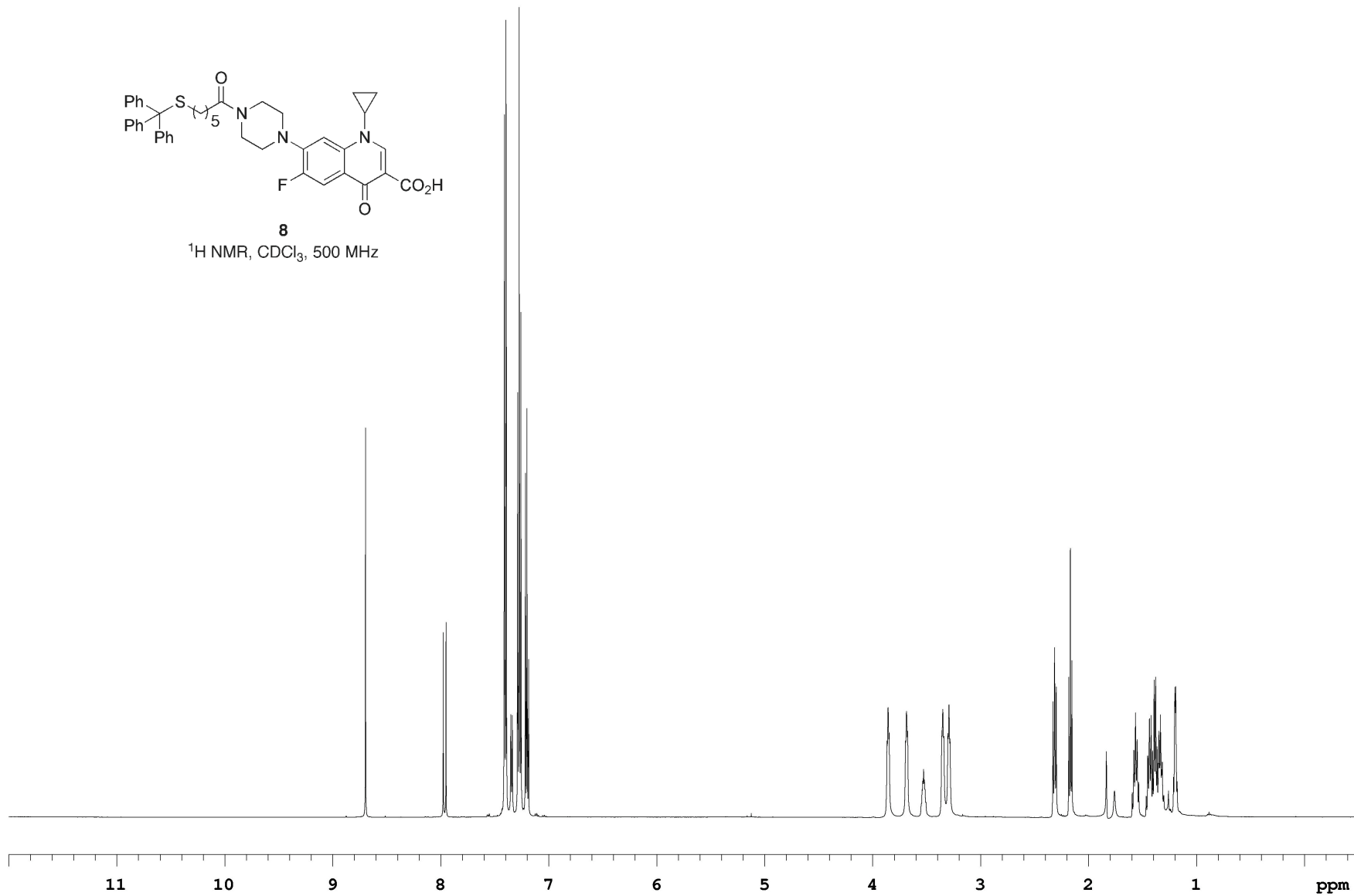
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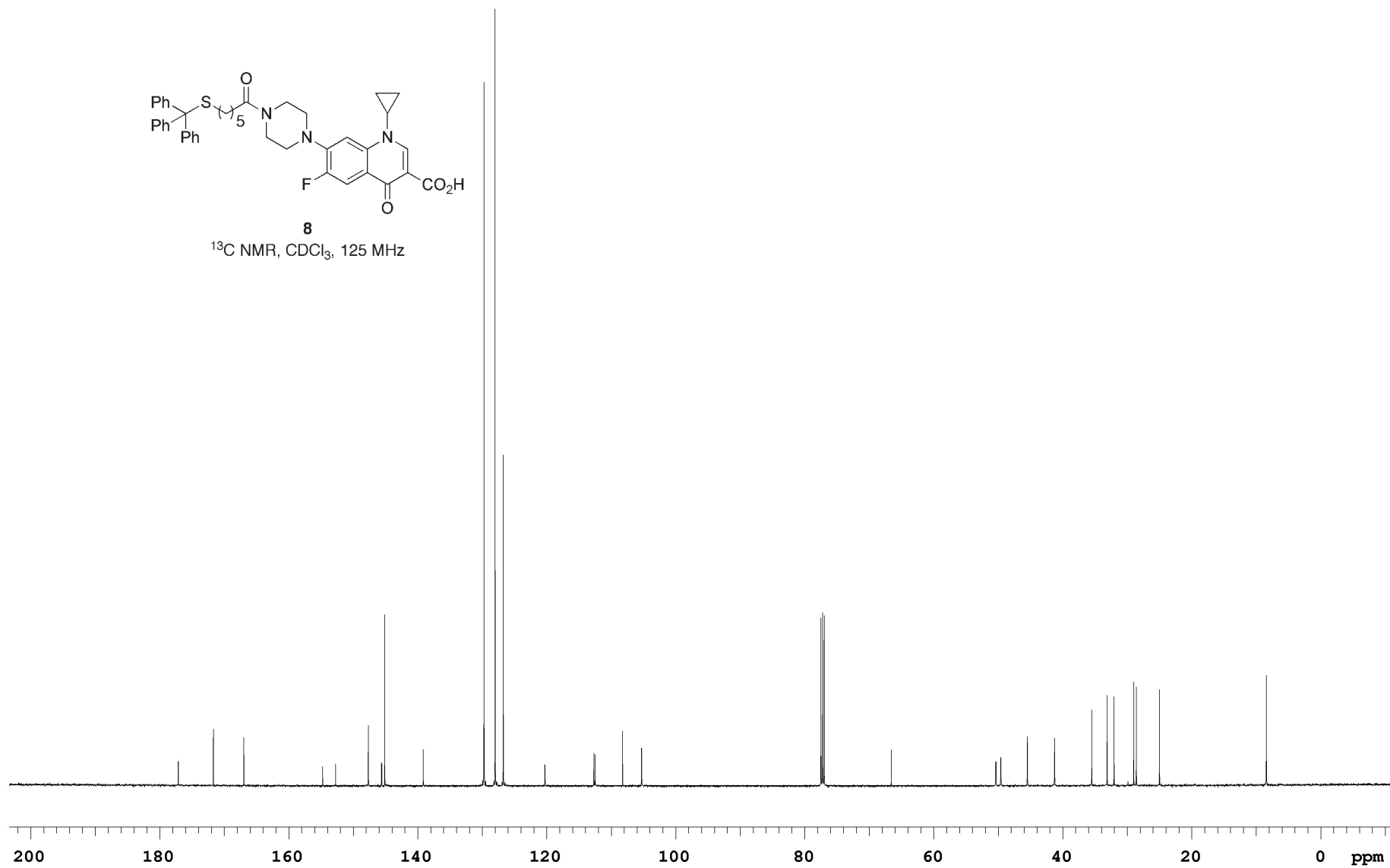
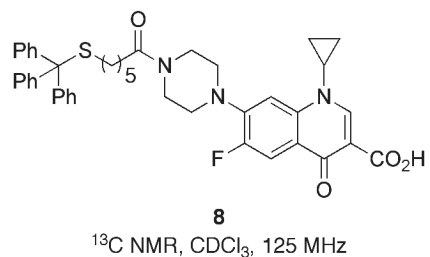
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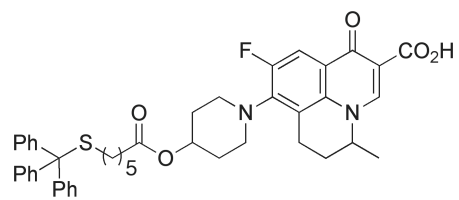




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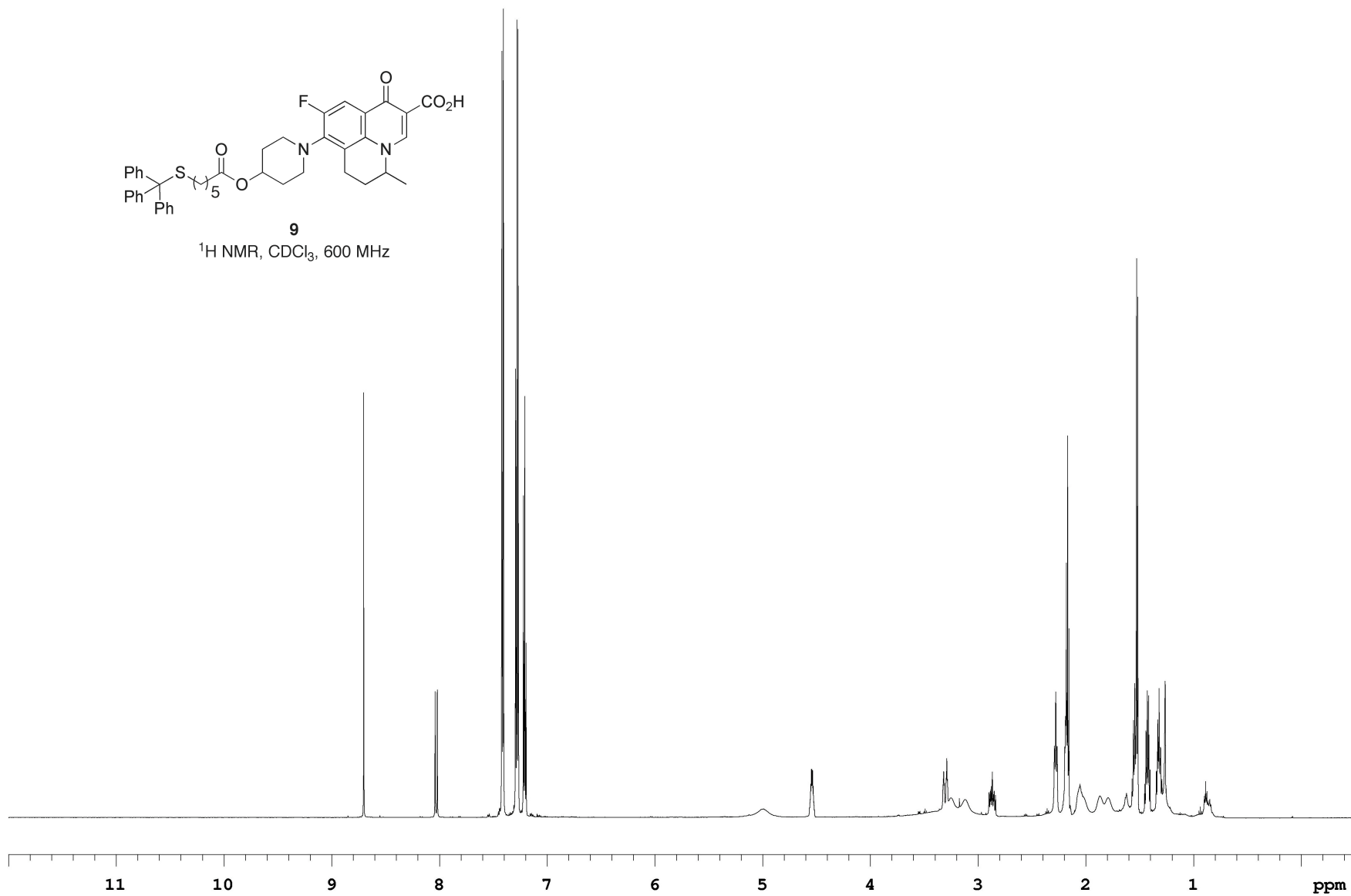


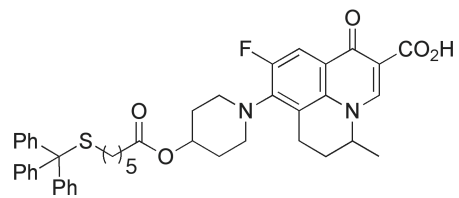




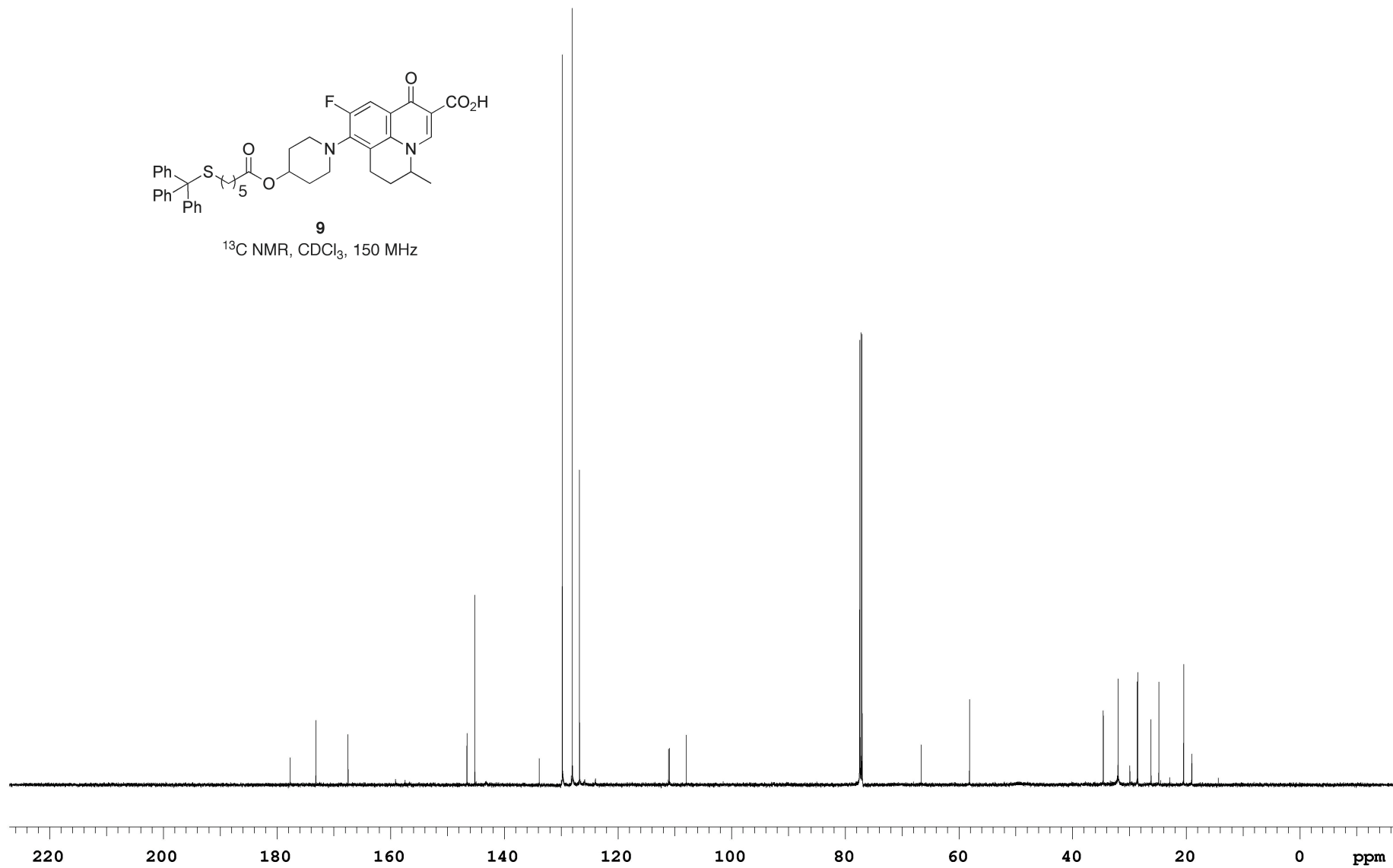
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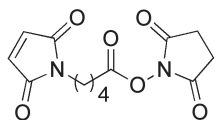
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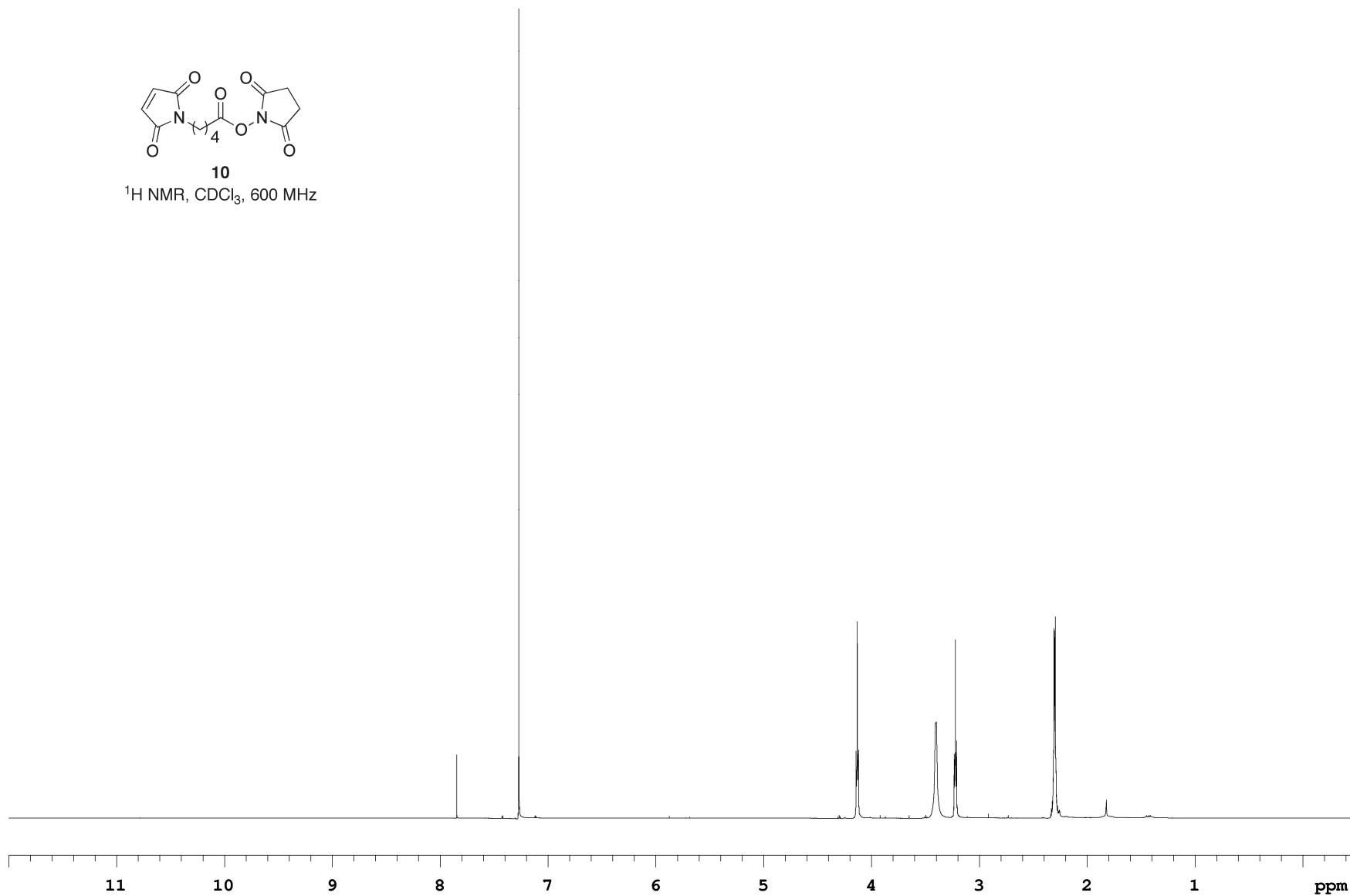
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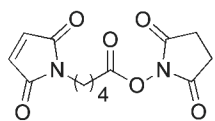




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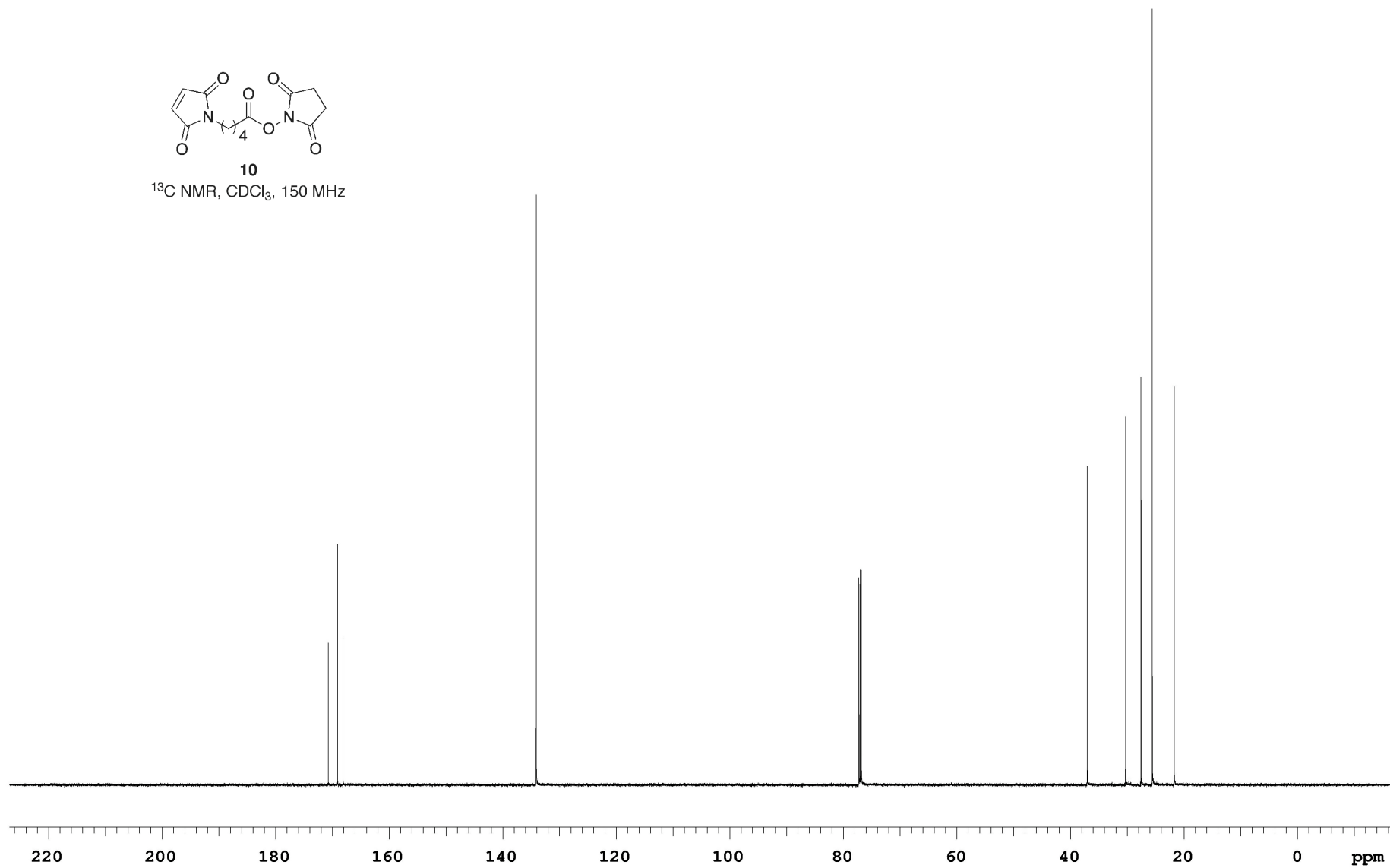
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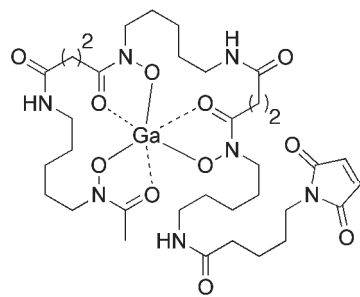




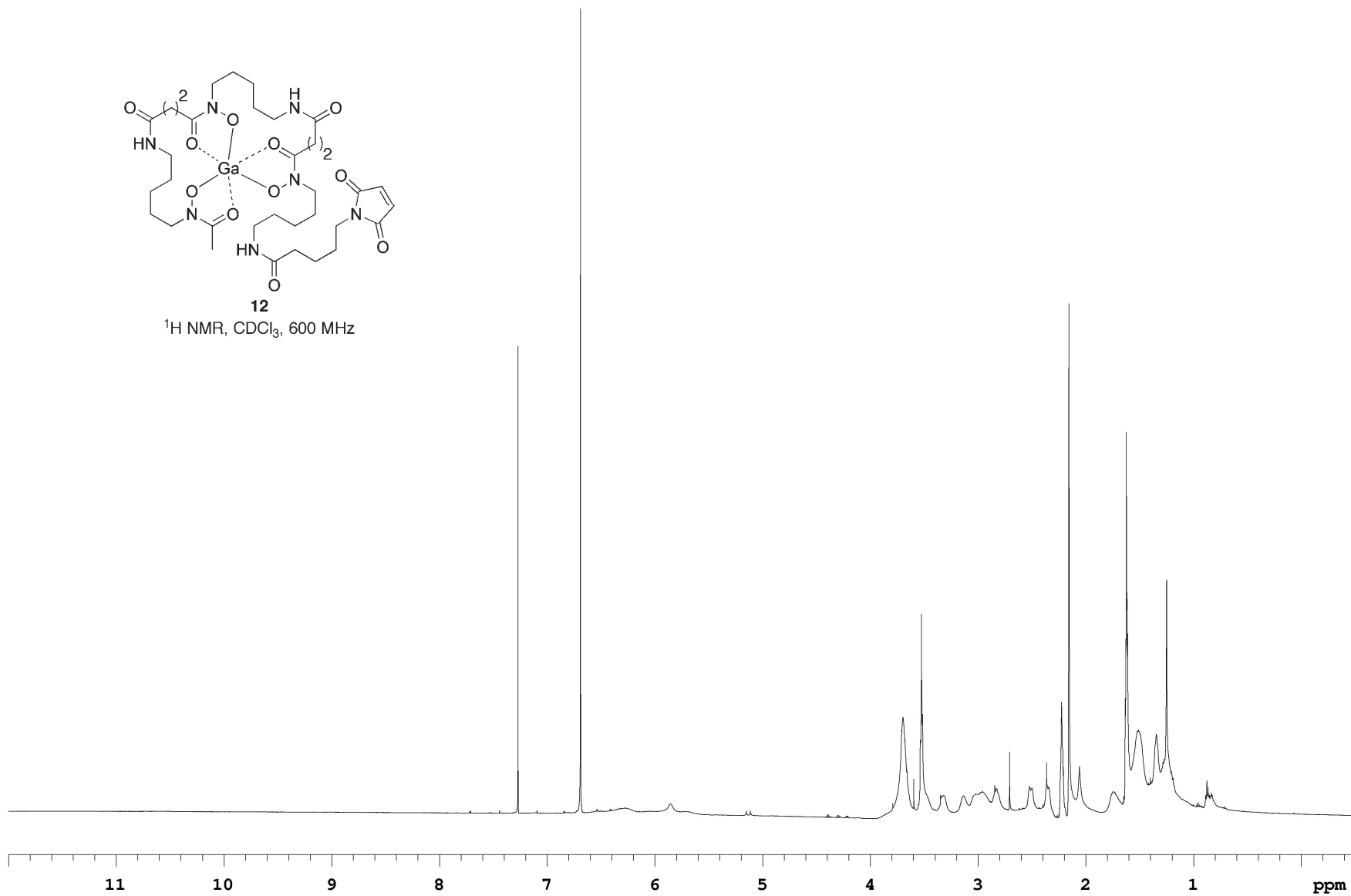
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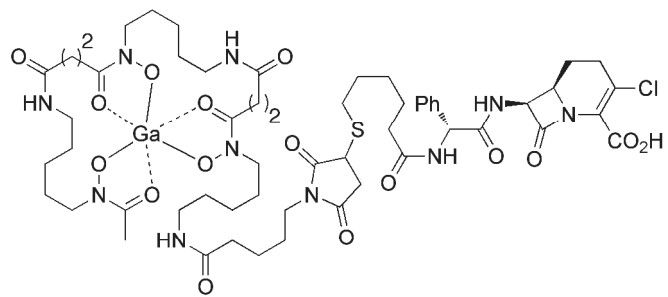
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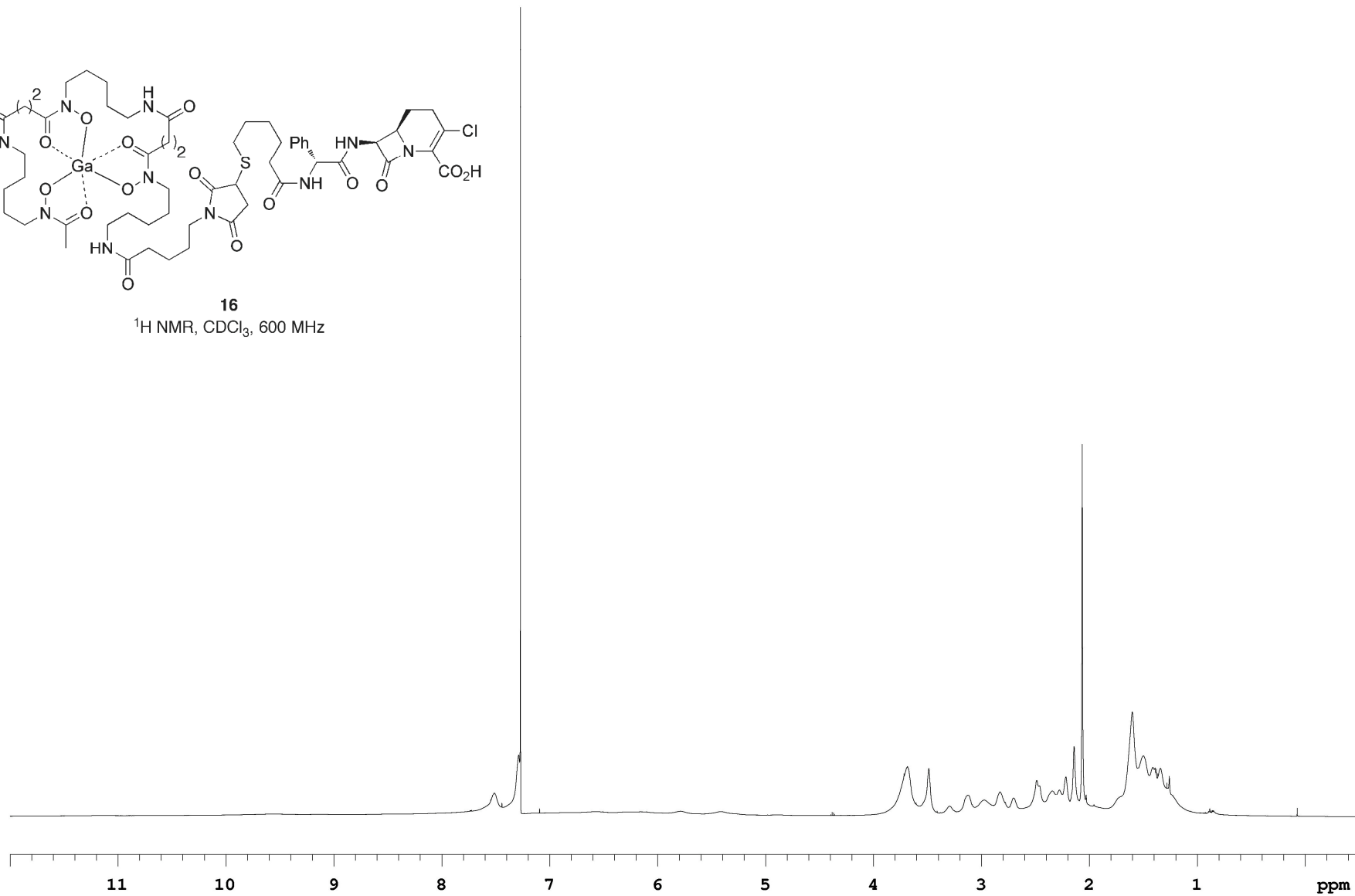
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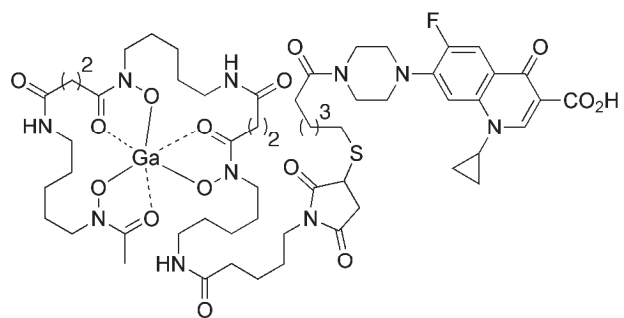




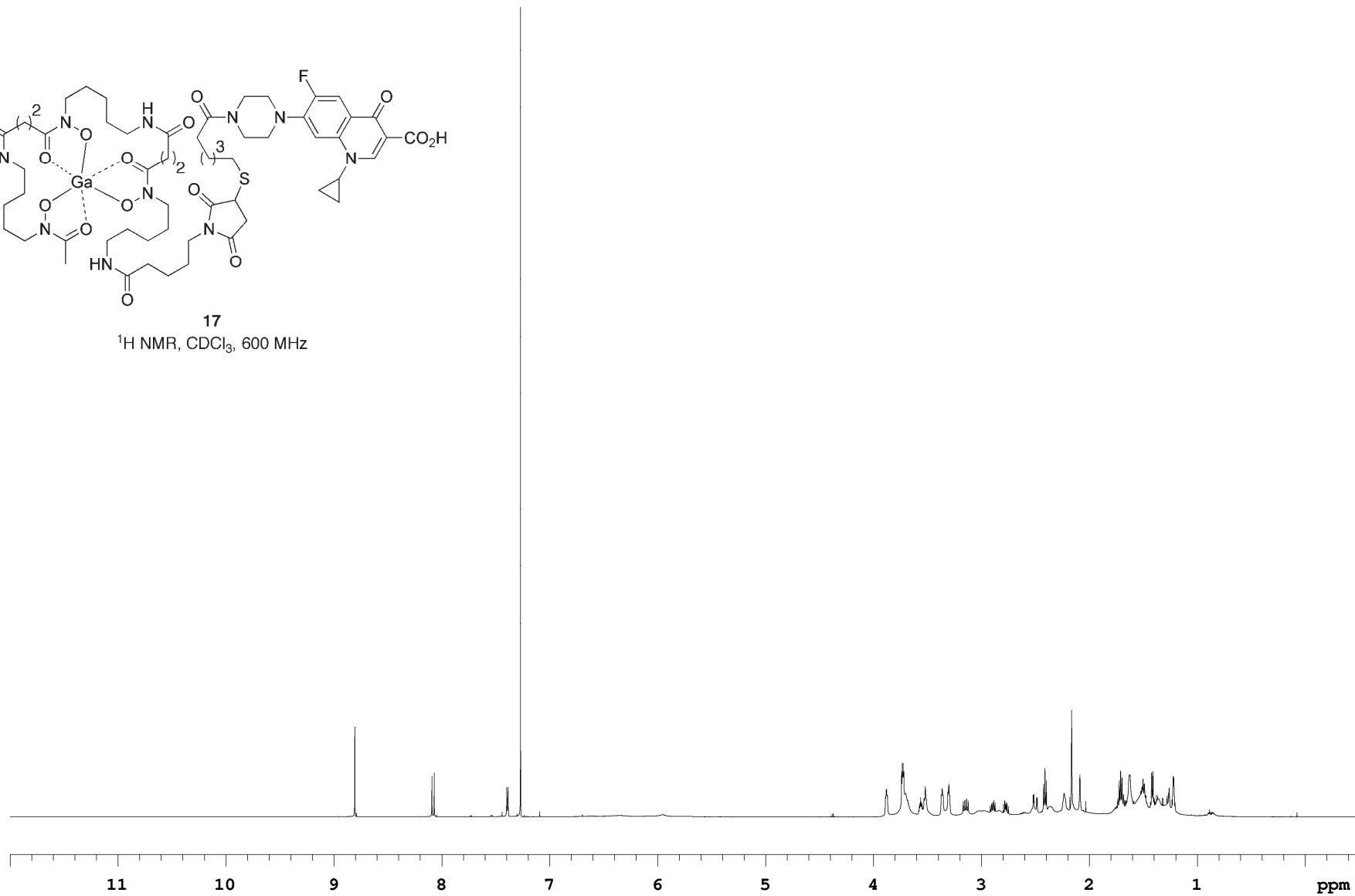
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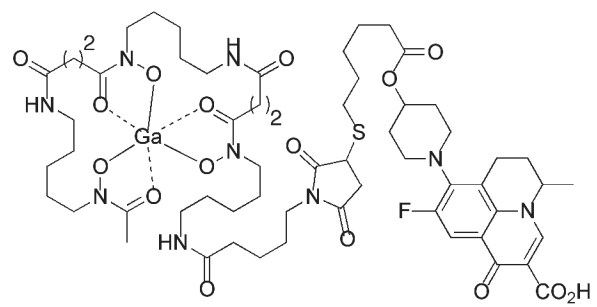
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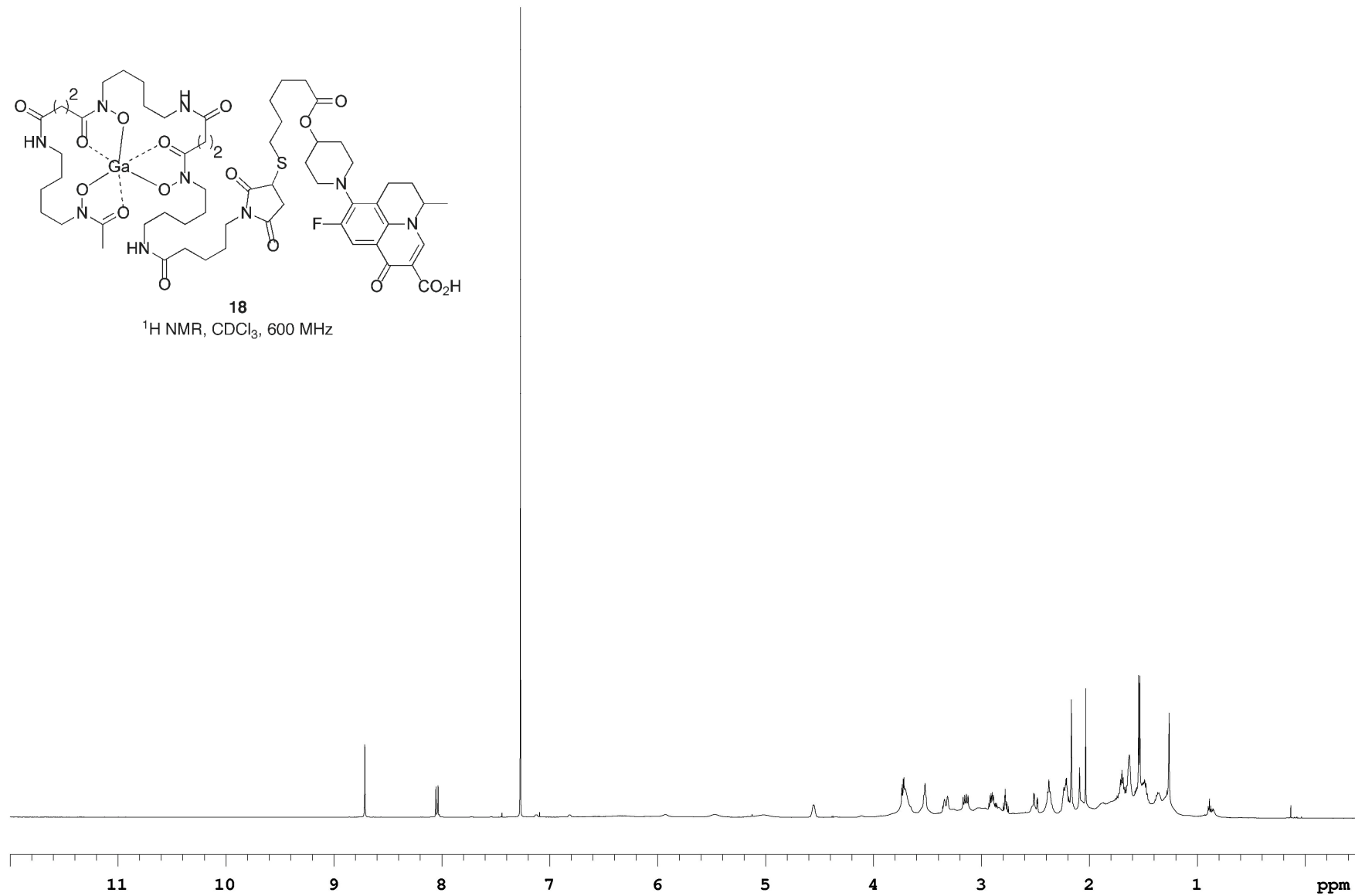
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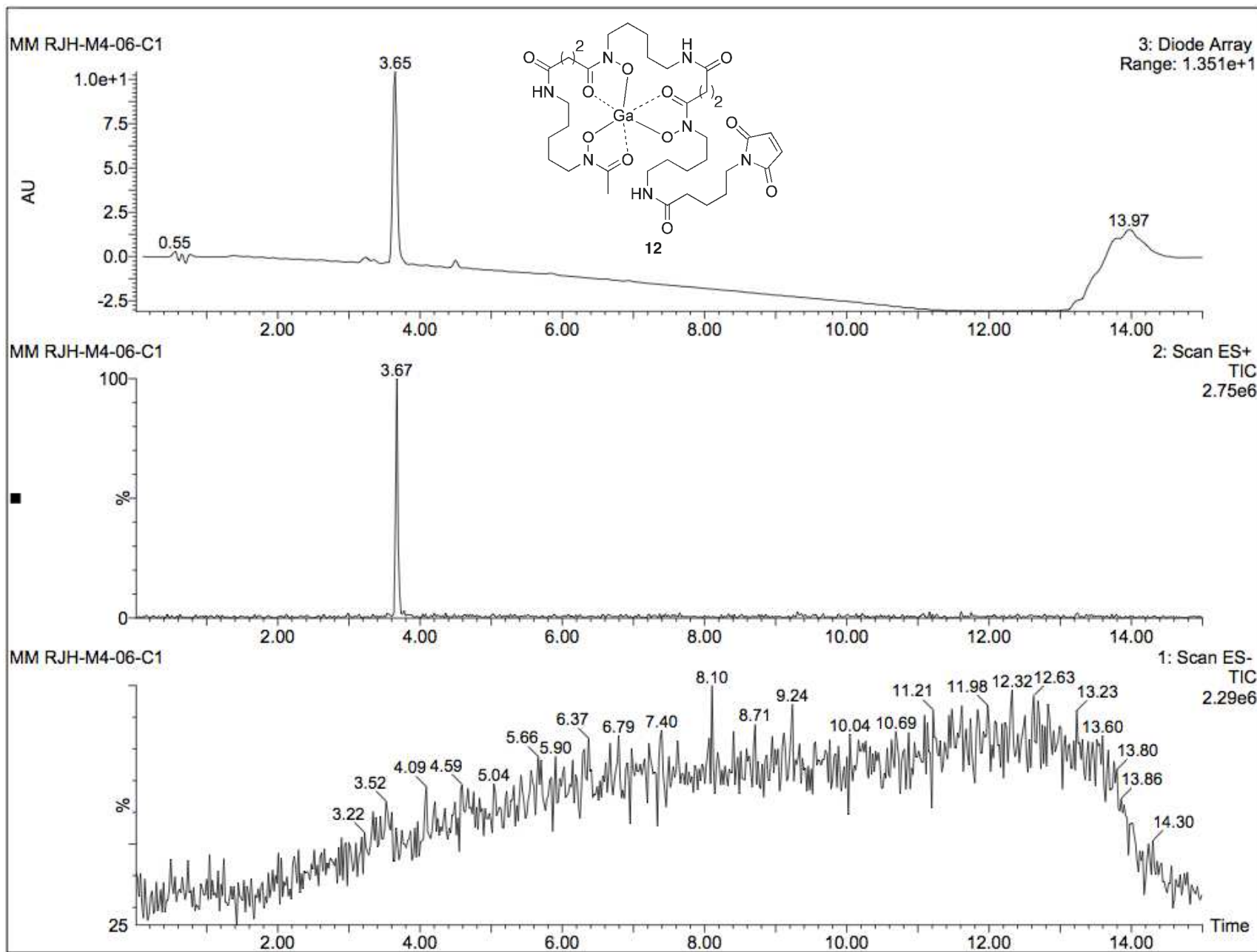


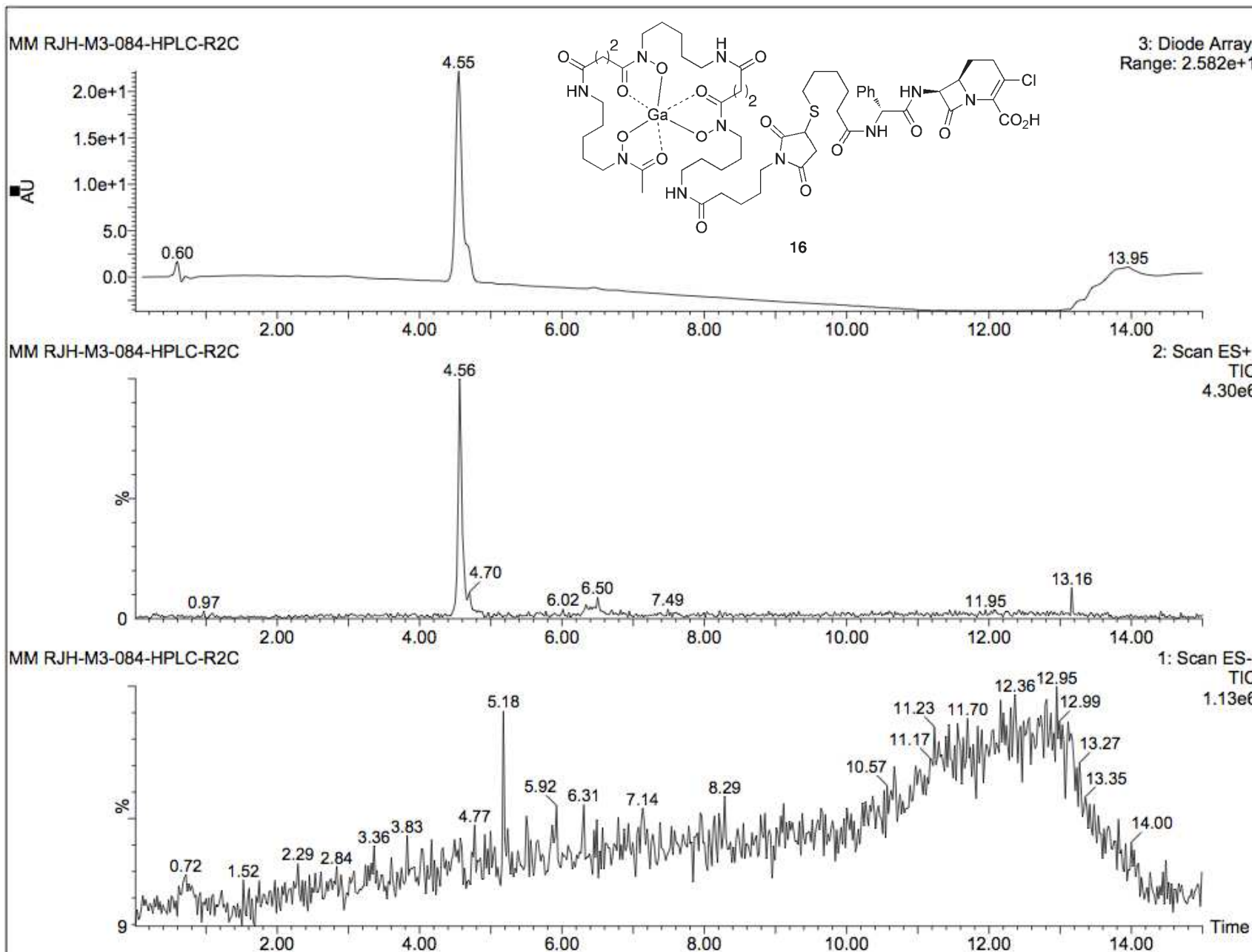


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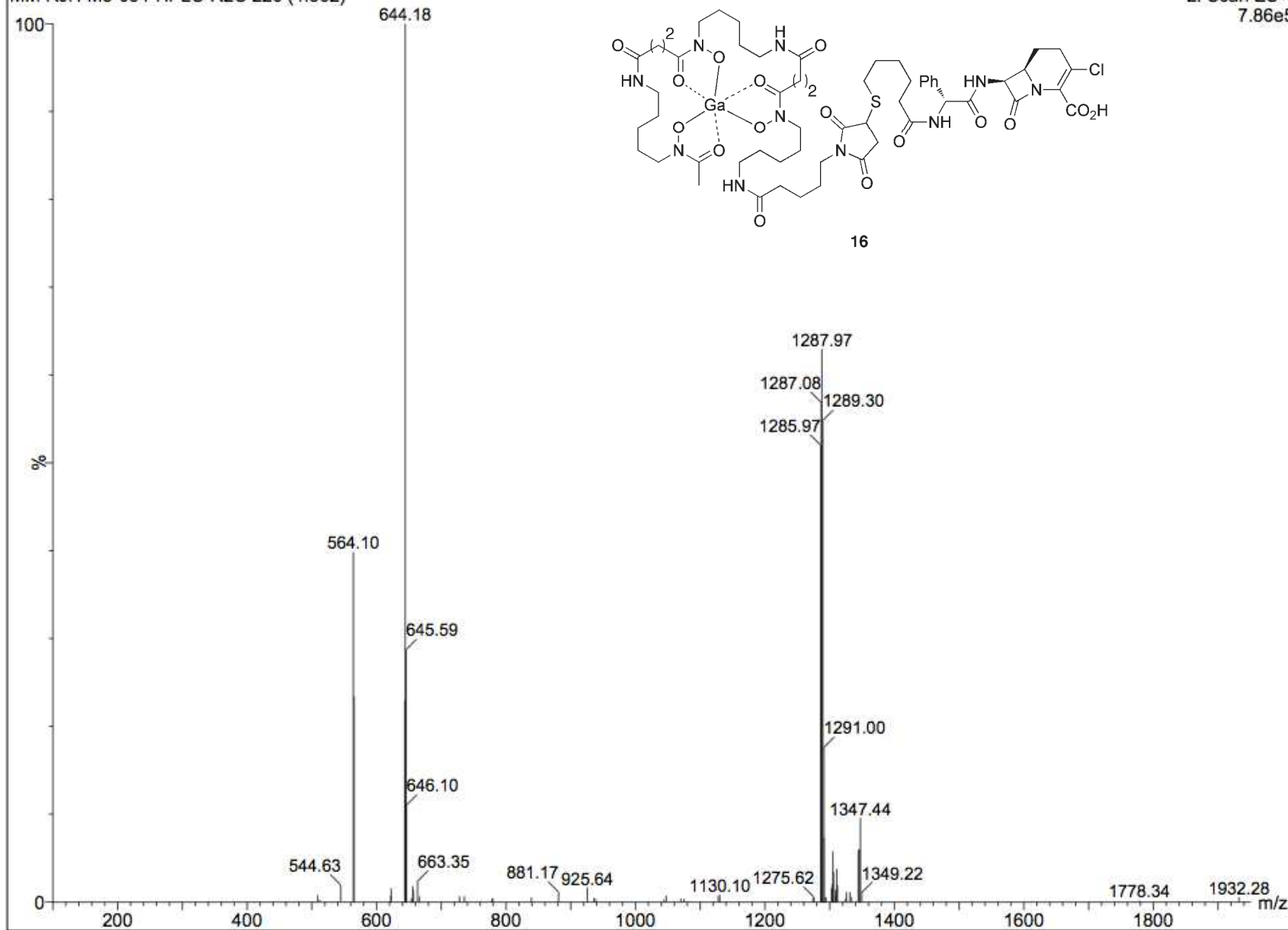


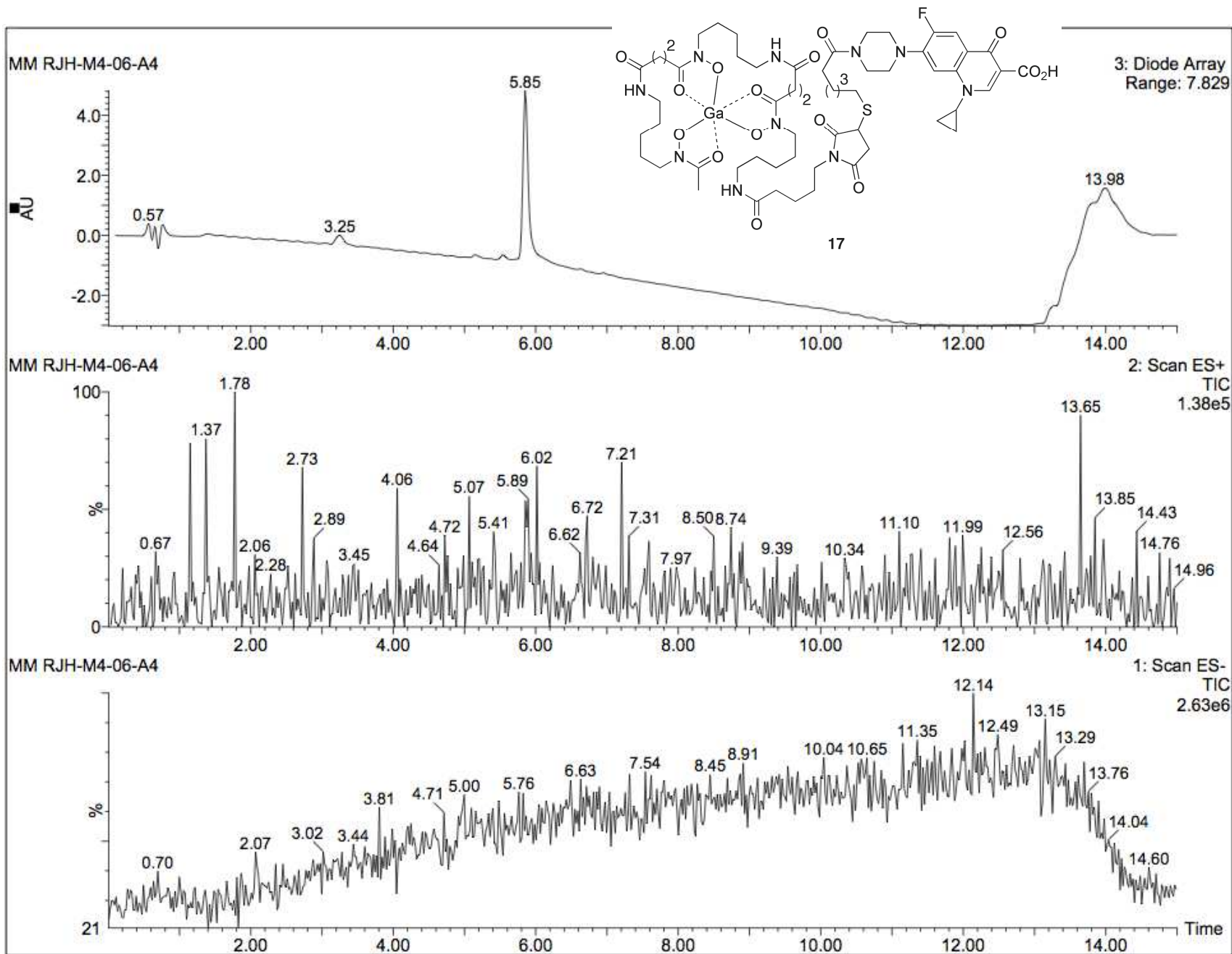




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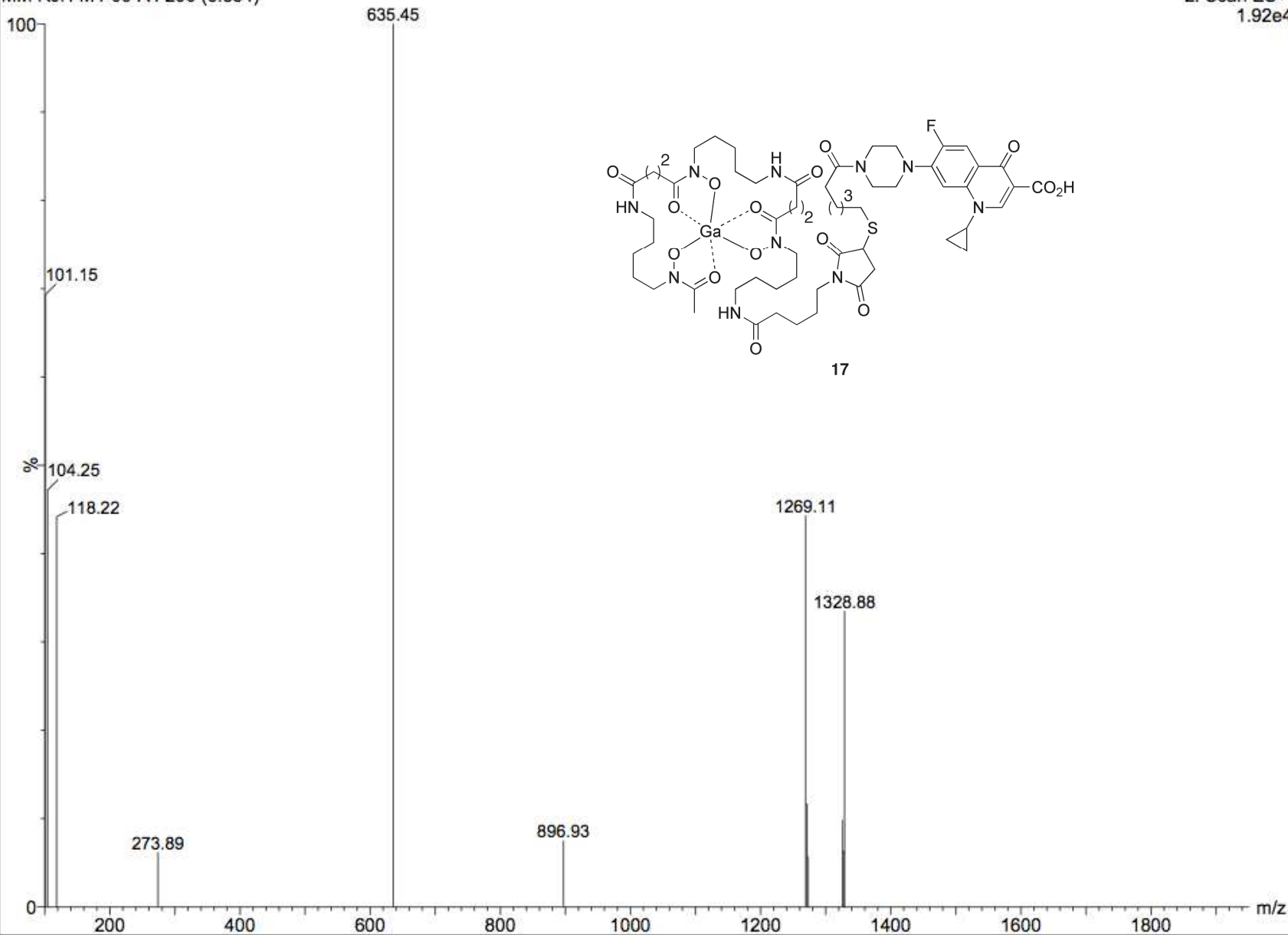
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