Supplementary Information

Specific Detection of IMP-1 metallo β-lactamase with a *trans*-Substituted Cephalosporin-based Fluorogenic Probe

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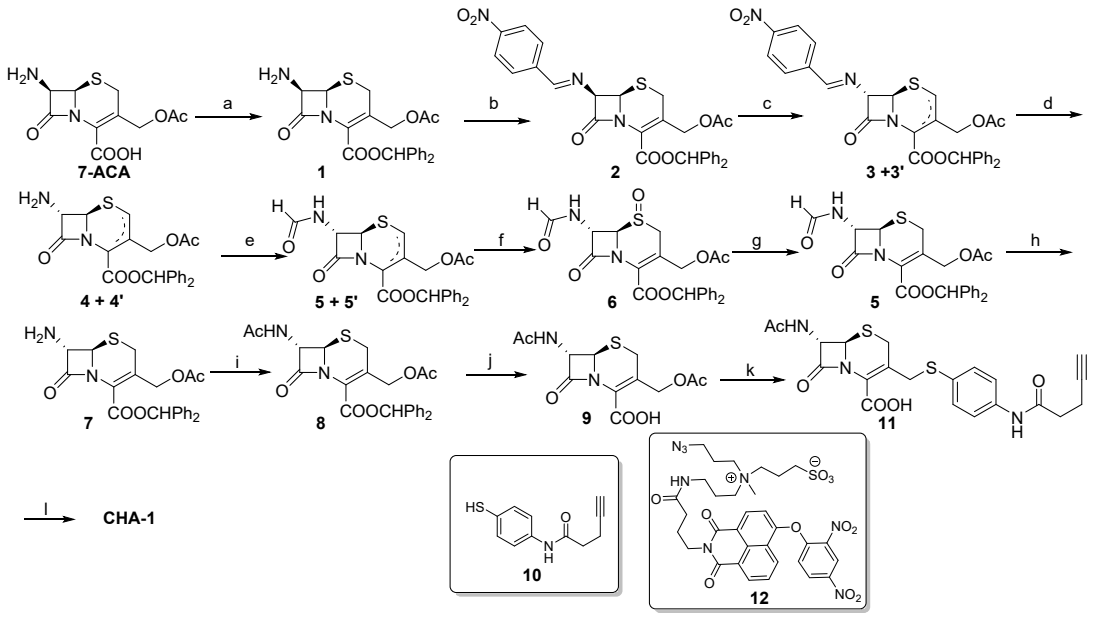
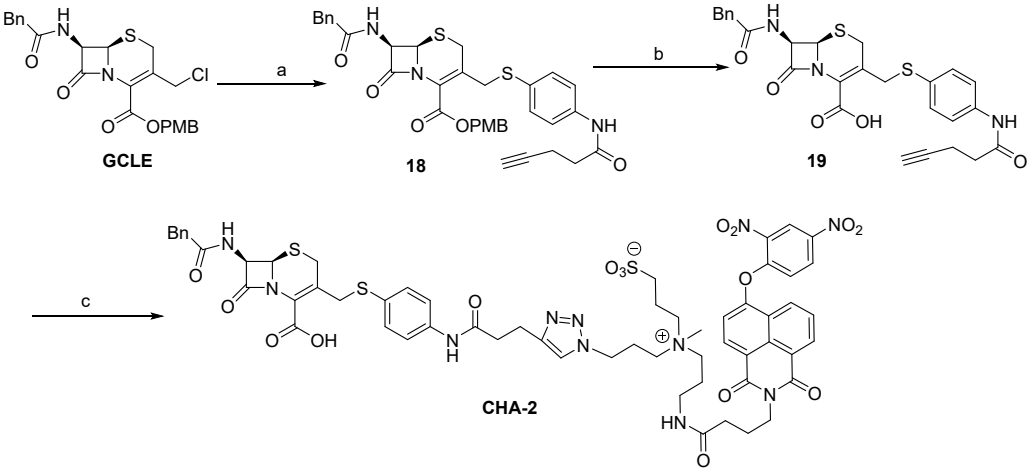


Scheme S1. Preparation of fluorogenic probe **CHA-1**. a) Ph_2CN_2, MeOH/DCM, 56%; b) *p*-nitrobenzaldehyde, $MgSO_4$, DCM, quant.; c) Et_3N, $CHCl_3$; d) Girard's reagent T, MeOH; e) acetic formic anhydride, pyridine, DCM; f) *m*CPBA, DCM; g) NaI, TFAA, acetone, 35% from **2**; h) concd. HCl, MeOH; i) AcCl, pyridine, MeCN, 48% from **5**; j) DCM/TFA/TIPS = 85/10/5; k) **10**, $NaHCO_3$, phosphate buffer (pH 6.4), 21% from **8**; l) **12**, L-(+)-ascorbic acid, THPTA, $CuSO_4$, DMSO, 40%.



Scheme S2 Synthesis of control probe **CHA-2**. a) **10**, NaI, NaHCO3, DMF; b) DCM/TFA/TIPS; c) **12**, L-(+)-ascorbic acid, THPTA, $CuSO_4$, DMSO

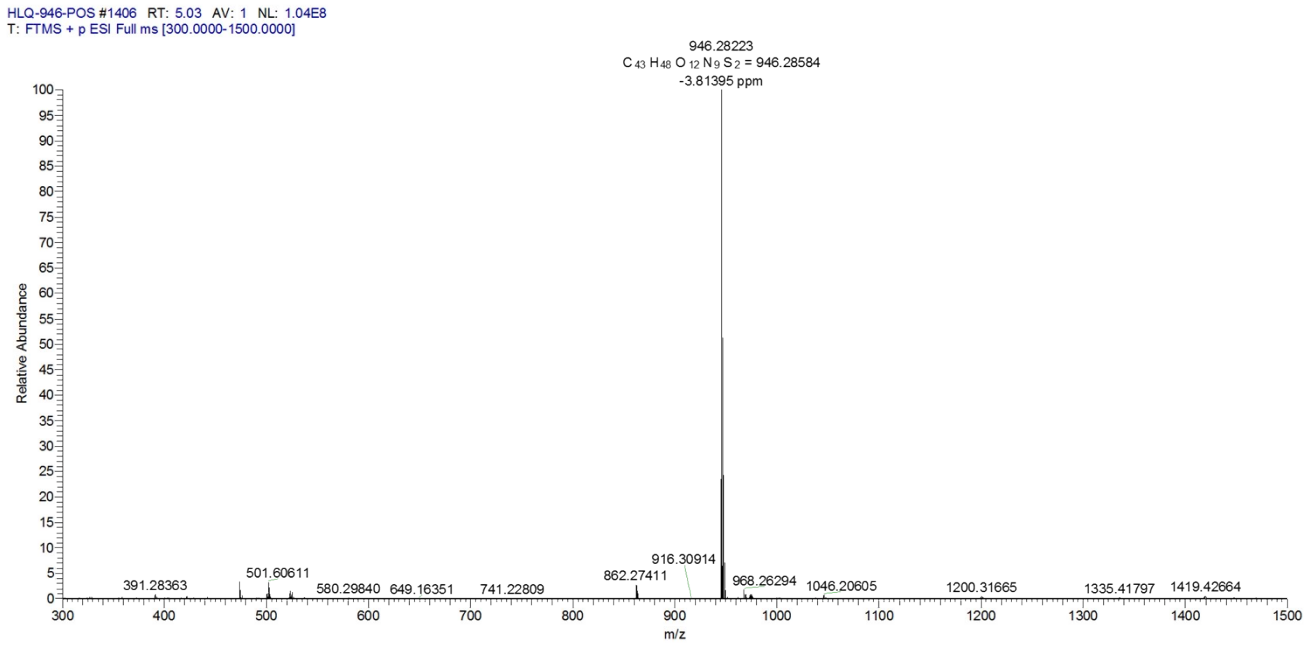


Fig. S1 HR-MS analysis of the major hydrolyzed product in Fig. 2c.

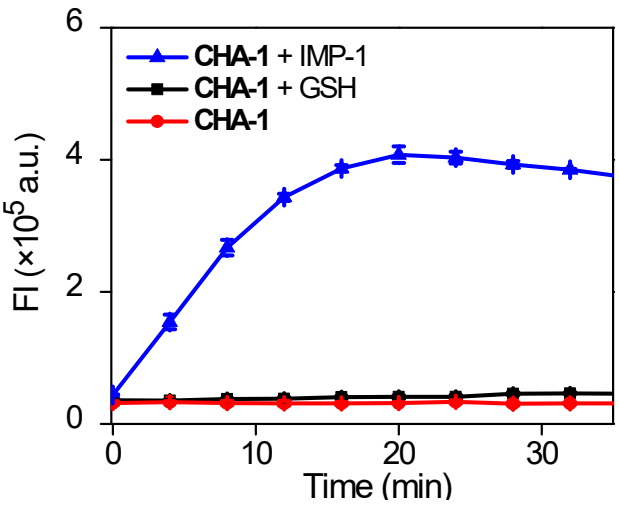
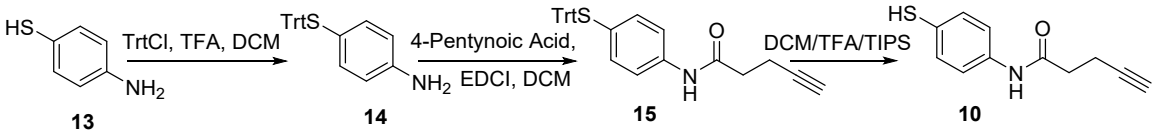


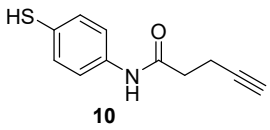
Fig. S2 Time-course fluorescence intensity of **CHA-1** (10 μM) in the presence of GSH (100 μM) in PBS at rt. Data are average of three experiments. Error bars are standard deviation.

General Information

Unless otherwise noted, all chemicals were purchased from commercial sources (e.g. Adamas-Beta, Energy Chemical and TCI China) and used without further purification. Phosphate-buffered saline (PBS, pH 7.4) were obtained from Invitrogen Corporation. Analytical thin layer chromatography was performed with 0.20 mm silica gel 60F plates with fluorescent indicator (254 nm), and visualized by ultraviolet light. HPLC was performed on a Shimadzu HPLC System equipped with a LC-20AT gradient pump and an inline diode array UV-Vis detector. An analytic or semi-preparative reversed-phase C18 column (Phenomenex, 5 μm) was used with a MeCN/H_2O gradient mobile phase containing 0.1% or 0.01% or no trifluoroacetic acid at a flow of 1 or 3 mL/min for the analysis or purification. ^{1}H and ^{13}C NMR spectra were taken on Bruker nuclear magnetic resonance spectrometer (400 MHz for ^{1}H NMR; 150 MHz for ^{13}C NMR). Data for ^{1}H NMR spectra are reported as follows: chemical shifts are reported as δ in units of parts per million (ppm) relative to tetramethylsilane (δ = 0, s); multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), or br (broadened); coupling constants are reported as a *J* value in Hertz (Hz); the number of protons (n) for a given resonance is indicated nH, and based on the spectral integration values. High-resolution mass spectra (HRMS) were recorded on a Bruker micro-TOF-QII time of flight mass spectrometer with electrospray ionization. The PBS used in this study contains 0.1% 3-[(3-cholamidopropyl)dimethylammonium]-1-propane sulfonate (CHAPS) as surfactant. Fluorescence spectra were obtained on a wavelength-calibrated FluoroMax-3 fluorometer (Horiba Jobin Yvon). Kinetic experiments were conducted in a microplate reader (Molecular Devices, SpectraMax *i*3). TEM-1, CTX-M-9, KPC-2, NDM-1, VIM-27, IMP-1, and AmpC β-lactamases were expressed and purified as previously described.[1]

Synthesis and Characterization



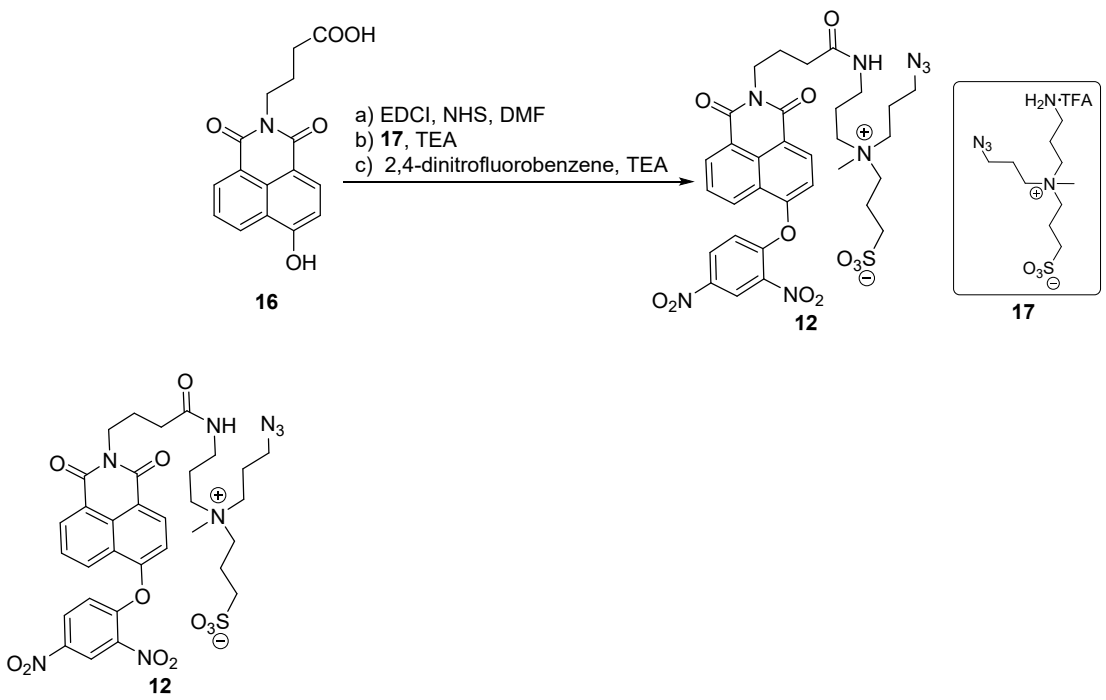


***N*-(4-mercaptophenyl)pent-4-ynamide (10).**

To a solution of trityl chloride (1.23 g, 4.40 mmol) and 4-aminothiophenol **13** (0.50 g, 4.00 mmol) in DCM (4 mL) at 0 °C was added dropwise trifluoroacetic acid (0.68 mL) and the resulting solution was stirred for 10 h, allowing reaction temperature raise to room temperature. The reaction was then quenched by the addition of aqueous solution of sodium hydroxide (1 M, 4.0 mL). The organic phase was separated and the aqueous phase was extracted with ethyl acetate (10 mL x 3). The combined organic phases were washed with brine (10 mL x 1) and dried over Na_2SO_4. Filtration followed by evaporation under vacuum to obtain compound **14** as a crude product, which was used in the next step without further purification.

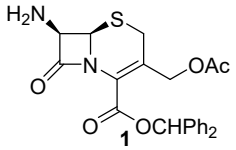
Compound **14** prepared above was treated with a solution of 4-pentynoic acid (0.52 g, 4.80 mmol), and EDCI (0.92 g, 4.80 mmol) in DCM (40 mL) at room temperature for 3 h. The reaction was then diluted with DCM (20 mL) and washed with HCl (aq. 1 M, 20 mL x 1) and brine (20 mL x 1), subsequently. Filtration followed by evaporation under vacuum to obtain compound **15** as a crude product.

The compound **15** prepared above was treated with a solution of DCM/TFA/TIPS = 9/1/0.5 (5.0 mL) at room temperature for 30 min. The reaction was then diluted with DCM and washed with water (10 mL x 3) and bine (10 mL x 1), subsequently. Purification by chromatography on a silica gel column gave compound **10** (0.71 g, 87% from **13**). 1H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 8.4 Hz, 2H), 7.25 (d, J = 9.2 Hz, 2H), 3.43 (s, 1H), 2.87 – 2.42 (m, 4H), 2.06 (s, 1H), 1.60 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.12, 135.85, 130.67, 125.52, 120.64, 82.73, 69.82, 36.27, 14.78. HRMS (ESI): Calculated for $C_{11}H_{10}NOS$ $(M-H)^-$ 204.0483, found 204.0482.



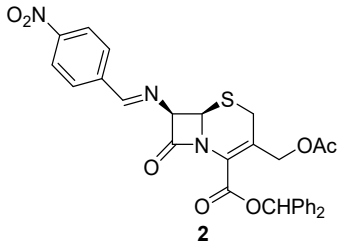
3-((3-azidopropyl)(3-(4-(6-(2,4-dinitrophenoxy)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)butanamido)propyl)(methyl)ammonio)propane-1-sulfonate (12).

A solution of **16**[2] (50.0 mg, 0.17 mmol), *N*-hydroxysuccinimide (23.5 mg, 0.20 mmol) and EDCI (49.8 mg, 0.26 mmol) in DMF (1.0 mL) was stirred at room temperature for 5 h (monitored by HPLC) before TEA (70.8 μL, 0.51 mmol) and a solution of **17**[3] (62.7 mg, 0.19 mmol) in DMF (1.0 mL) were added and stirred for 5 h at the same temperature. 2,4-Dinitrofluorobenzene (47.5 mg, 0.26 mmol) and TEA (70.8 μL, 0.51 mmol) were then added and stirred for another 2 h. Diethyl ether (20 mL) was added and the resulting solution was vortexed for a few minutes. Centrifugation to remove the diethyl ether layer and the residue was purified by preparative HPLC on a C18 column to give compound **12** (66.7 mg, 53%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.55 (d, J = 2.8 Hz, 1H), 8.16 – 8.08 (m, 3H), 8.04 (d, J = 8.2 Hz, 1H), 7.55 – 7.48 (m, 2H), 7.11 (d, J = 9.2 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 3.62 (t, J = 7.0 Hz, 3H), 3.06 – 2.95 (m, 4H), 2.84 (dd, J = 10.1, 5.3 Hz, 4H), 2.65 (dd, J = 11.2, 5.5 Hz, 2H), 2.06 (dd, J = 6.7, 4.8 Hz, 4H), 1.74 (t, J = 7.4 Hz, 2H), 1.57 – 1.33 (m, 9H). ^{13}C NMR (150 MHz, d_6-DMSO) δ 172.34, 163.85, 163.23, 155.88, 153.47, 143.62, 140.76, 132.63, 132.24, 130.60, 129.52, 128.55, 128.19, 123.98, 122.94, 122.78, 122.72, 119.58, 115.19, 60.46, 59.63, 58.78, 48.28, 48.18, 47.88, 33.44, 24.22, 22.55, 21.91, 18.92. MS (ESI): Calculated for $C_{32}H_{37}N_8O_{11}S$ $(M+H)^+$ 741.2, found 741.2.



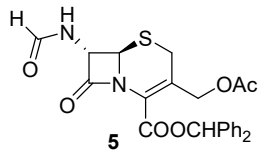
1

(6*R*,7*R*)-benzhydryl 3-(acetoxymethyl)-7-amino-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (1). To a mixture of 7-ACA (5.0 g, 22.03 mmol) in CH_2Cl_2/MeOH = 3/2 (70 mL) at 0 °C was added a solution of diphenyldiazomethane in CH_2Cl_2 (see Supplementary Information for detail) and the resulting mixture were stirred at room temperature for 20 h. The reaction solution were washed with water (20 mL x 3) and brine (20 mL x 1), subsequently, and dried over $MgSO_4$. Upon filtration to remove drying agent, the filtrate was concentrated under reduced pressure and the residual was crystallized with EA/PE = 1/20 to afford compound **1** (4.55 g, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.24 (m, 10H), 6.95 (s, 1H), 4.99 (d, *J* = 13.4 Hz, 1H), 4.93 (d, *J* = 5.1 Hz, 1H), 4.79 – 4.72 (m, 2H), 3.55 (d, *J* = 18.5 Hz, 1H), 3.37 (d, *J* = 18.5 Hz, 1H), 2.01 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.54, 168.95, 161.05, 139.25, 139.07, 128.52, 128.46, 128.19, 128.05, 127.76, 127.53, 127.09, 126.53, 126.00, 125.59, 79.65, 63.71, 63.18, 58.73, 26.20, 20.68. HRMS (ESI): calculated for $C_{23}H_{22}N_2NaO_5S$ $(M+Na)^+$ 461.1147, found 461.1146.



2

(6*R*,7*R*)-benzhydryl 3-(acetoxymethyl)-7-((E)-(4-nitrobenzylidene)amino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (2). Compound **2** was prepared according to reported procedure.[4] In brief, a mixture of **1** (4.5 g, 10.27 mmol), *p*-nitro-benzaldehyde (1.6 g, 11.29 mmol), and $MgSO_4$ (8.8 g, 72.63 mmol) in CH_2Cl_2 (anhydrous, 60 mL) were stirred at room temperature for 24 h. The drying agent was then removed by filtration and the filtrate was concentrated in *vacuo* to and then filtered, and the solution was removed in *vacuo* to give title compound (5.93 g, quant.). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.71 (d, *J* = 1.7 Hz, 1H), 8.27 (d, *J* = 8.7 Hz, 2H), 7.94 (d, *J* = 8.7 Hz, 2H), 7.46 (d, *J* = 7.4 Hz, 2H), 7.40 – 7.27 (m, 8H), 6.98 (s, 1H), 5.50 (dd, *J* = 5.0, 1.6 Hz, 1H), 5.20 (d, *J* = 5.0 Hz, 1H), 5.02 (d, *J* = 13.5 Hz, 1H), 4.77 (d, *J* = 13.5 Hz, 1H), 3.59 (d, *J* = 18.5 Hz, 1H), 3.40 (d, *J* = 18.5 Hz, 1H), 2.02 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.50, 163.78, 162.95, 160.80, 149.53, 140.58, 139.20, 139.07, 130.47, 129.28, 128.55, 128.50, 128.23, 128.09, 127.68, 127.07, 126.61, 125.65, 124.29, 123.91, 79.82, 74.14, 63.18, 57.74, 26.62, 20.66. HRMS (ESI): Calculated for $C_{30}H_{25}N_3NaO_7S$ $(M+Na)^+$ 594.1311, found 594.1312.



5

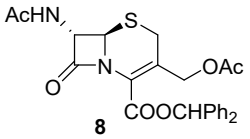
(6*R*,7*S*)-benzhydryl 3-(acetoxymethyl)-7-formamido-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (5). A solution of **2** (5.9 g, 10.27 mmol) and Et_3N (5.8 mL, 41.52 mmol) in $CHCl_3$ (210 mL) were stirred at room temperature for 1 h, and the reaction solution was washed with aqueous HCl (1 M) several times until the pH of solution became about 6.5. The organic layer was washed with brine (30 mL x 1) and dried with $MgSO_4$. Filtration to remove drying agent and the filtrate was concentrated to give a mixture of **3** and **3'**, which were dissolved in MeOH (100 mL) and treated with Girard's reagent T (5.2 g, 31.13 mmol) at room temperature for 100 minutes being stirred vigorously. The reaction solution was then poured into a mixture of EtOAc (200 mL) and water (200 mL). The aqueous layer was separated and the organic layer was washed with water (30 mL x 3), brine (30 mL x 1), subsequently. The resulting solution was dried over $MgSO_4$, and filtrated. The filtrate was concentrated under reducing pressure to give a mixture of **4** and **4'** as crude products, which were used in the next step without further purification.

The mixture of **4** and **4'** were dissolved in CH_2Cl_2 (60 mL) and cooled to 0 °C. Pyridine (3.34 mL, 41.50 mmol) was then added, followed by the dropwise addition of acetic formic anhydride (2.71 g, 30.81 mmol). The reaction solution was stirred at room temperature for 2 h and then poured into water (50 mL). The organic layer was separated and washed with water (50 mL x 3), brine (50 mL x 1), subsequently. The resulting solution was dried over $MgSO_4$ and filtrated. Volatile reagents and solvent were removed under reduced pressure. The residue was purified by chromatography on a short silica gel column to afford a mixture of **5** and **5'** (4.03 g), which were used in the next step without further purification.

To a solution of **5** and **5'** (4.03 g, 9.01 mmol) in CH_2Cl_2 (100 mL) at 0 ºC, *m*-chloroperoxybenzoic acid (*m*CPBA, 68%, 2.28 g, 9.91 mmol) was added in several ports. The reaction mixture were stirred at 0 ºC for 30 min, all of the starting material disappeared (monitored by TLC). The reaction solution was diluted with CH_2Cl_2 and washed with $NaHSO_3$ (aq.) (20 mL x 1) and brine (20 mL x 1), subsequently. After drying over $MgSO_4$, purification by flash chromatography on a short silica gel column to afford compound **6** (2.01 g).

To a mixture of **6** (2.01 g, 4.17 mmol) and NaI (3.1 g, 20.83 mmol) in acetone (anhydrous, 90 mL) at 0 ºC was added dropwise trifluoroacetic anhydride (3.19 mL, 22.96 mmol). The reaction mixture were stirred at 0ºC for 1 h and then the reaction mixture were poured into a mixture EtOAc (150 mL) and saturated aqueous solution of Na_2SO_3 (20 mL) at 0 °C. The organic layer was separated and washed with water (20 mL x 3), brine (20 mL x 1), subsequently. Upon drying over $MgSO_4$ and purification by flash chromatography on a silica gel column, compound **5** was obtained as a solid (1.70 g, 35% from compound **2**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.44 – 7.22 (m, 10H), 7.03 (d, J = 8.4 Hz, 1H), 6.93 (s, 1H), 4.97 (d, J = 7.3 Hz, 1H), 4.80 (d, J = 13.1 Hz, 1H), 4.64 (d, J = 13.1 Hz, 1H),

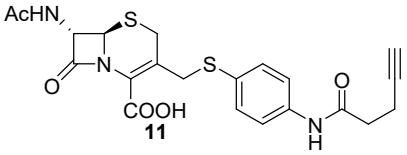
4.54 (s, 1H), 3.53 (d, J = 18.3 Hz, 1H), 3.30 (d, J = 18.3 Hz, 1H), 1.97 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.51, 161.52, 160.92, 138.92, 138.79, 128.64, 128.45, 128.22, 127.76, 127.00, 126.91, 122.63, 80.18, 62.81, 62.17, 56.81, 28.06, 20.65. HRMS: calculated for $C_{24}H_{22}N_2NaO_6S$ $(M+Na)^+$: 489.1096; Found: 489.1098.



8

(6R,7S)-benzhydryl 7-acetamido-3-(acetoxymethyl)-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylate (8). To a solution of **5** (120.0 mg, 0.26 mmol) in MeOH (2.7 mL) at 0 °C was added dropwise concd. HCl (216 μL) and the mixture were stirred at room temperature for 2 h. The mixture were then poured into a mixture of ethyl acetate (5 mL) and ice water (5 mL). $NaHCO_3$ was then added to adjust pH to 6.5. The organic layer was separated and washed with water (10 mL x 3), brine (10 mL x 1), subsequently. After drying over $MgSO_4$ and filtration, the filtrate was concentrated under reduced pressure to give compound **7** as a crude product, which was used in the next step without further purification.

A solution of **7**, acetyl chloride (40 μL, 0.56 mmol), and pyridine (66.0 μL, 0.82 mmol) in acetonitrile (2 mL) was stirred at room temperature for 2 h, and the reaction solution was then diluted with ethyl acetate (5 mL) and washed with water (10 mL x 3), brine (10 mL x 1), subsequently. The organic layer was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure to afford a residue, which was purified by flash chromatography on a silica gel column to give compound **8** as a solid (60.2 mg, 48% from compound **5**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.28 (m, 10H), 6.98 (d, J = 8.0 Hz, 1H), 6.94 (s, 1H), 4.96 (d, J = 7.5 Hz, 1H), 4.80 (d, J = 13.1 Hz, 1H), 4.65 (d, J = 13.1 Hz, 1H), 4.54 (s, 1H), 3.55 (d, J = 18.3 Hz, 1H), 3.30 (d, J = 18.3 Hz, 1H), 1.98 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.60, 170.53, 161.82, 160.87, 138.86, 138.69, 128.59, 128.56, 128.41, 128.17, 127.70, 126.96, 126.87, 122.79, 80.10, 63.58, 62.79, 56.96, 27.98, 22.53, 20.59. HRMS: calculated for $C_{25}H_{24}N_2NaO_6S$ $(M+Na)^+$ 503.1253, found 503.1252.

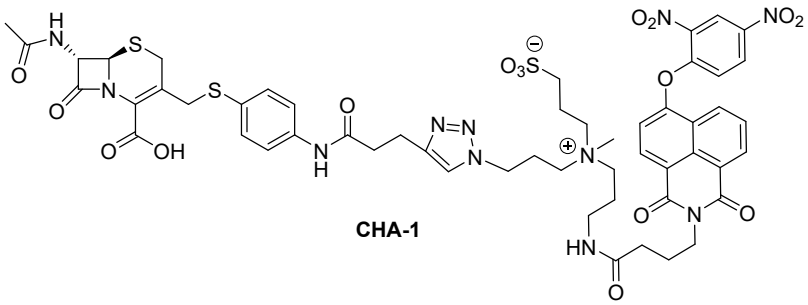


11

(6R,7S)-7-acetamido-8-oxo-3-(((4-(pent-4-ynamido)phenyl)thio)methyl)-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid (11). Compound **8** (53.2 mg, 0.11 mmol) was treated with a solution of DCM/TFA/TIPS = 85/10/5 (1 mL) at 0 °C for 30 min. Upon disappearance of starting material (monitored by HPLC), the reaction was diluted with acetonitrile (30 mL). The reaction solution was concentrated under reduced pressure, keeping the temperature of water bath below 20 °C,

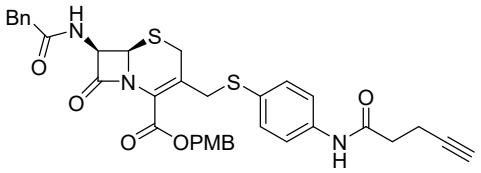
to give a residue, which was washed with PE/EA (30/1) to yield compound **9** as a crude product.

To a solution of **9** in phosphate buffer (pH 6.4, 1.4 mL), were added $NaHCO_3$ (18.5 mg, 0.22 mmol) and **10** (22.6 mg, 0.11 mmol) and the resulting mixture were heated at 60 °C for 8 h. After cooling to room temperature, ethyl acetate (5 mL) was added and the mixture was acidified with 1 M HCl to pH 2. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (15 mL x 3). The combined organic layers were dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. The residue was purified by preparative RP-HPLC on a C18 column to obtain **11** (10.6 mg, 21% from compound **8**). ^{1}H NMR (600 MHz, d_6-DMSO) δ 10.05 (s, 1H), 9.06 (dd, J = 71.2, 8.2 Hz, 1H), 7.55 (d, J = 8.6 Hz, 2H), 7.33 (d, J = 8.7 Hz, 2H), 4.80 (dd, J = 8.1, 2.2 Hz, 1H), 4.71 (d, J = 1.8 Hz, 1H), 4.06 (d, J = 13.2 Hz, 1H), 3.82 (d, J = 13.2 Hz, 1H), 3.68 (d, J = 17.4 Hz, 1H), 3.44 (d, J = 17.5 Hz, 1H), 2.80 (t, J = 2.6 Hz, 1H), 2.54 – 2.43 (m, 4H), 1.88 (s, 3H). ^{13}C NMR (150 MHz, d_6-DMSO) δ169.76, 189.45, 163.06, 161.57, 138.41, 121.97, 129.56, 128.14, 123.70, 119.49, 83.64, 71.55, 63.01, 57.04, 37.03, 35.23, 28.23, 22.25, 14.02. HRMS: Calculated for $C_{21}H_{21}N_3NaO_5S_2$ $(M+Na)^+$ 482.0820, found 482.0822.



3-((3-(4-(3-((4-((((6R,7S)-7-acetamido-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)thio)phenyl)amino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)propyl)(3-(4-(6-(2,4-dinitro phenoxy)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)butanamido)propyl)(methyl)ammonio) propane-1-sulfonate (CHA-1)

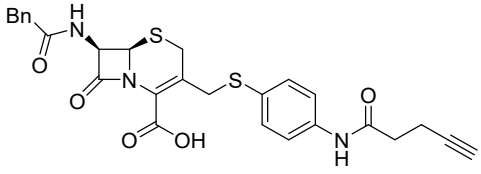
A mixture of **11** (5.0 mg, 0.011mmol), **12** (9.7 mg, 0.013 mmol), $CuSO_4$ (0.2 mg, 0.001 mmol), L-(+)-ascorbic acid (7.7 mg, 0.044 mmol), and THPTA (0.5 mg, 0.001 mmol) in DMSO: H_2O = 1:1 (200 μL) were stirred at room temperature for 0.5 h. Purification by preparative RP-HPLC on a C18 column afforded **CHA-1** (5.2 mg, 40%). The purity of product was confirmed by HPLC analysis. HRMS (ESI): calculated for $C_{53}H_{56}N_{11}O_{16}S_3$ $(M-H)^-$ 1198.3069, found 1198.3062.



18

(6R,7R)-4-methoxybenzyl 8-oxo-3-(((4-(pent-4-ynamido)phenyl)thio)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (18).

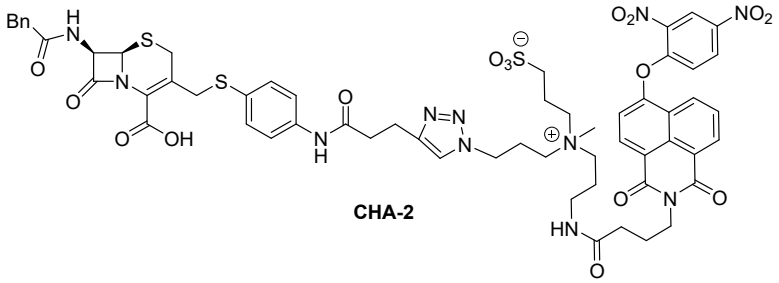
A mixture of **GCLE** (50.0 mg, 0.10 mmol) and NaI (15.0 mg, 0.1 mmol) in DMF (anhydrous, 1 mL) were stirred at rt for 30 min before **10** (30.8 mg, 0.15 mmol) and $NaHCO_3$ (8.4 mg, 0.10 mmol) were added and stirred for 1 h at room temperature. Upon completely consumption of **GCLE** (monitored by TLC), ethyl acetate (10 mL) was added. The reaction solution was washed with water (5 mL x 3) and dried over $MgSO_4$. Purification by chromatography on a silica gel column afforded compound **18** (59.2 mg, 90%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38 – 7.28 (m, 13H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.10 (d, *J* = 8.9 Hz, 1H), 5.72 (dd, *J* = 9.0, 4.8 Hz, 1H), 5.07 (d, *J* = 11.9 Hz, 1H), 5.02 (d, *J* = 11.9 Hz, 1H), 4.82 (d, *J* = 4.8 Hz, 1H), 4.13 (d, *J* = 13.3 Hz, 1H), 3.81 (s, 3H), 3.75 (d, *J* = 13.4 Hz, 1H), 3.68-3.63 (m, 2H), 3.59 (d, *J* = 18.4 Hz, 1H), 3.33 (d, *J* = 18.0 Hz, 1H), 2.63 – 2.50 (m, 4H), 2.07 (t, *J* = 2.3 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.12, 169.12, 164.29, 161.32, 159.83, 137.60, 134.00, 133.70, 130.61, 129.48, 129.20, 128.82, 128.60, 127.76, 124.42, 120.27, 113.94, 82.68, 69.90, 67.68, 59.07, 57.64, 55.33, 43.33, 38.02, 36.28, 28.23, 14.70. HRMS: Calculated for $C_{35}H_{33}N_3NaO_6S_2$ $(M+Na)^+$ 678.1708, found 678.1707.



19

(6R,7R)-8-oxo-3-(((4-(pent-4-ynamido)phenyl)thio)methyl)-7-(2-phenylacetamido) -5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (19).

A solution of **18** (33 mg, 0.0503 mmol) in DCM/TFA/TIPS = 50/40/10 (0.5 mL) was stirred at 0 °C for 30 min. Solvent and excess reagent were removed by Rota-vap, maintaining the temperature of water bath below 10 °C) and the resulting residue was purified by preparative HPLC on a C18 column to give compound **19** (19.3 mg, 71%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.06 (s, 1H), 9.09 (d, *J* = 8.3 Hz, 1H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.37 – 7.17 (m, 7H), 5.60 (dd, *J* = 8.3, 4.7 Hz, 1H), 5.04 (d, *J* = 4.7 Hz, 1H), 4.13 (d, *J* = 13.1 Hz, 1H), 3.90 (d, *J* = 13.1 Hz, 1H), 3.68 (d, *J* = 17.7 Hz, 1H), 3.56 (d, *J* = 13.9 Hz, 1H), 3.52 – 3.46 (m, 3H), 2.80 (t, *J* = 2.5 Hz, 1H), 2.53 – 2.43 (m, 4H). ^{13}C NMR (150 MHz, d_6-DMSO) δ 171.40, 169.91, 165.05, 163.31, 139.06, 136.30, 132.85, 129.49, 128.70, 128.30, 126.96, 125.36, 119.95, 84.08, 72.00, 59.41, 58.29, 42.04, 37.60, 35.68, 27.83, 14.46. HRMS: Calculated for $C_{27}H_{25}N_3NaO_5S_2$ $(M+Na)^+$ 558.1133, found 558.1134.



3-((3-(4-(3-((4-((((6R,7S)-2-carboxy-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)thio)phenyl)amino)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)propyl)(3-(4-(6-(2,4-dinitrophenoxy)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)butanamido)propyl)(methyl) ammonio)propane-1-sulfonate (CHA-2)

The title compound (6.1 mg, 44%) was prepared from **19** and **12** following the synthesis of **CHA-1**. The purity of **CHA-2** was confirmed by HPLC analysis. HRMS (ESI): Calculated for $C_{59}H_{60}N_{11}O_{16}S_3$ $(M-H)^-$ 1274.3382, found 1274.3383.

Enzymatic kinetics.

To a series of concentrations of **CHA-1** (2, 3, 4, 6, 8, 10 μM) in PBS (pH 7.4) in a 96-well plate (black and flat bottom) was added IMP-1 (50 pM). PBS was added to adjust the total volume to 100 μL/well. The fluorescence intensity at 550 nm was immediately measured at room temperature with a microplate reader (excitation at 450 nm) over 30 minutes. A double-reciprocal plot of the hydrolysis rate versus substrate concentration (Lineweaver-Burk plot) was used to determine the kinetic parameters (K_m and k_{cat}). All experiments were triplicated.

Determined of self-hydrolysis rate.

To a 96-well plate (black and flat bottom) was added **CHA-1** (8 μM) in PBS (pH 7.4) and the total volume was set to 100 μL/well. The fluorescence intensity of **CHA-1** at 550 nm (excitation at 450 nm) was monitored over a 2-day period. The self-hydrolysis rate (K_{obs}) was calculated from the plot of $Ln([S]_0/([S]_0-[P]))$ versus time. All experiments were triplicated.

Construction of the β-lactamase-expressing plasmids and *E. coli*.

TEM-1-expressing *E. coli* was obtained by transforming pBAD/myc-HisA plasmid into competent DH5α *E. coli.* The KPC-2-expression plasmid, KPC-2 pBAD, was constructed by simply replacing the ampicillin resistance gene *(bla_{TEM})* of pBAD/myc-HisA plasmid with KPC-2 gene, and the KPC-2-expressing *E. coli* was obtained by transforming this plasmid into competent *E. coli* DH5α*E.*as previously reported.[1c] The NDM-1, IMP-1-expressing *E. coli* were obtained in a similar manner. In brief, with pBAD/myc-HisA vector as template DNA and the following primers, PCRs were conducted to knock out of the ampicillin resistance gene.

NDM-1:

F-primer: 5'-TGGCCGACAAGCTGCGCTGACTGTCAGACCAAGTTTACTCATATATACTT-3'

R-primer: 5'-ATAATATTGGGCAATTCCATACTCTTCCTTTTTCAATATTATTGAAGCAT-3'

IMP-1:

F-primer: 5'-CATCAAAACCAAGCAACTAACTGTCAGACCAAGTTTACTCATATATACT-3'

R-primer: 5'-AATACAGATAACTTGCTCATACTCTTCCTTTTTCAATATTATTGAAGCAT-3'

The resulting PRC products were then treated with DpnI restriction enzyme to digest the template following manufacturer's protocol and the *bla_{TEM}*-free pBAD/myc-HisA vector was obtained upon purification.

PCRs were conducted with *Klebsiella pneumoniae* (ATCC-BAA-2146) as template DNA for NDM-1 or the pBAD-IMP-1 plasmid as template for IMP-1 and the following primers.

NDM-1:

F-primer: 5'-AATATTGAAAAAGGAAGAGTATGGAATTGCCCAATATTAT-3'

R-primer: 5'-GAGTAAACTTGGTCTGACAGTCAGCGCAGCTTGTCGGCCA-3'

IMP-1:

F-primer: 5'- CATCAAAACCAAGCAACTAACTGTCAGACCAAGTTTACTCATATATACT-3'

R-primer: 5'- AATACAGATAACTTGCTCATACTCTTCCTTTTTCAATATTATTGAAGCAT-3'

Homologous recombination of the purified PCR and the *bla_{TEM}*-free pBAD/myc-HisA vector were conducted with Ezmax One-step Cloning kit (Tolo Biotech). The resulting NDM-1 pBAD plasmid or IMP-1 pBAD plasmid was transformed into competent *E. coli* DH5α to obtain the desired NDM-1 or IMP-1 expressing *E. coli.*

Detection of resistant bacteria with CHA-1.

E. coli transformed by pBAD/myc-HisA plasmid, KPC pBAD, NDM-1 pBAD, and IMP-1 pBAD, as well as *E. coli* DH5α, were cultured in Luria-Broth (LB) at 37 ^{o}C overnight. Bacteria were harvested by centrifugation and resuspended in PBS (pH 7.4). The number of bacteria was determined by OD600 (OD600 of 1.0 = 8 x 10^8 cells/mL). To a 96-well plate (black and flat bottom) were added **CHA-1** (10 μM) and 1 x 10^6 CFU of *E. coli*. PBS was added to adjust the total volume to 100 μL/well. Upon

incubation at room temperature for 30 minutes, the fluorescence intensity at 560 nm (excitation at 450 nm) was measured with a microplate reader. All experiments were triplicated.

References

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3. S. C. Lange, E. van Andel, M. M. Smulders and H. Zuilhof, *Langmuir*, 2016, **32**, 10199-10205.

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^{1}H and ^{13}C NMR spectra

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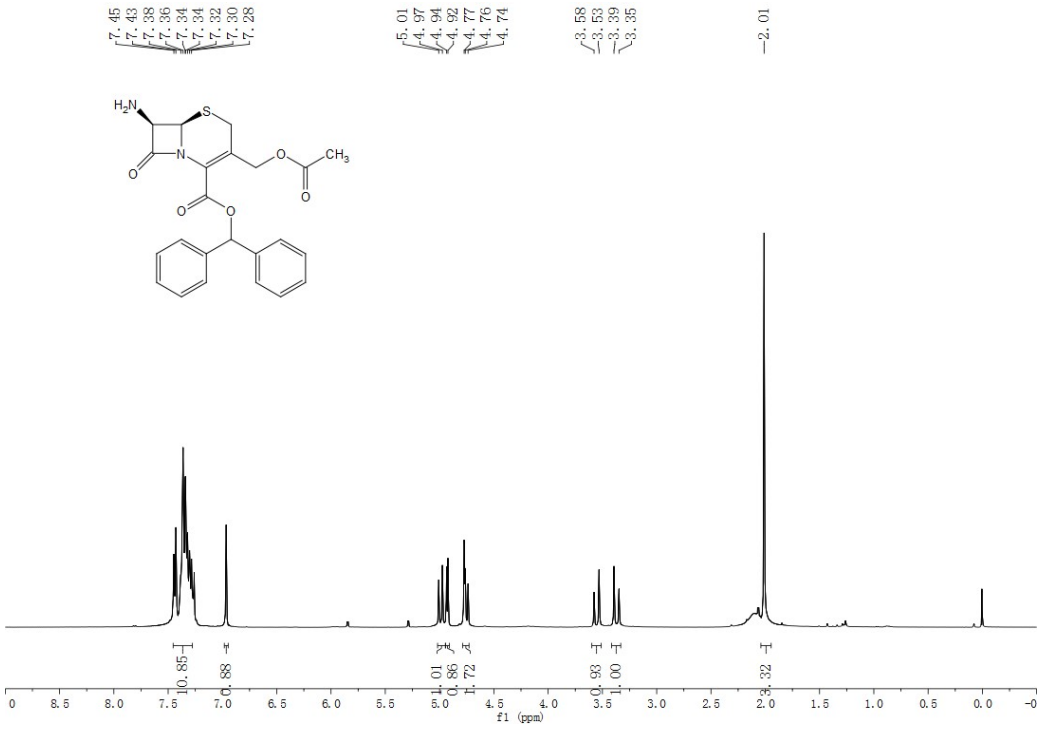

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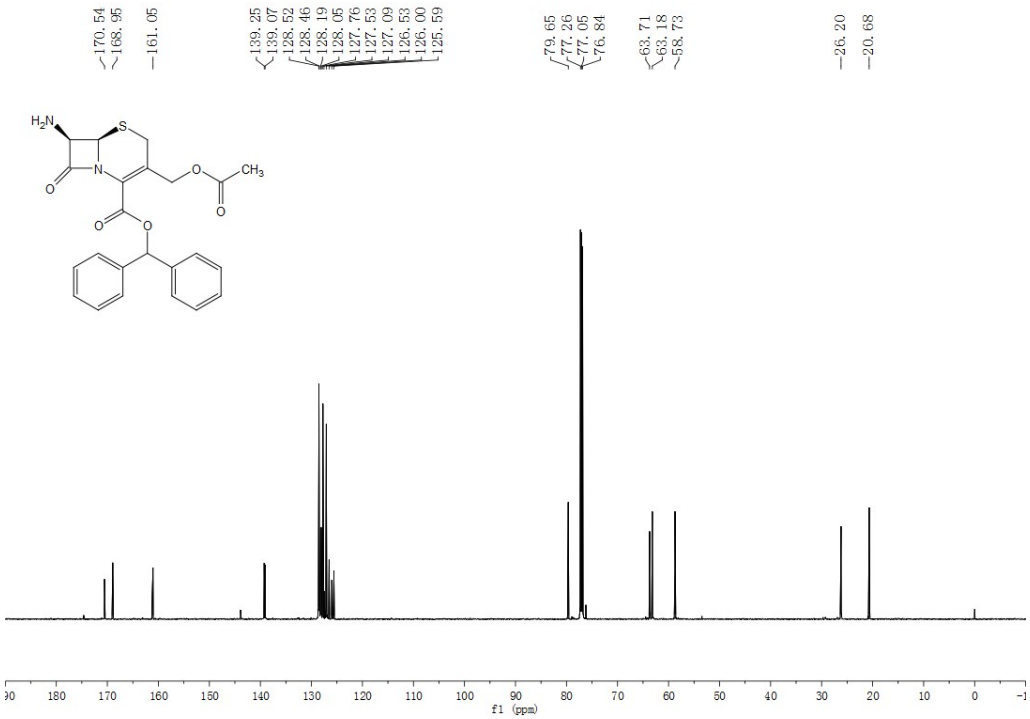

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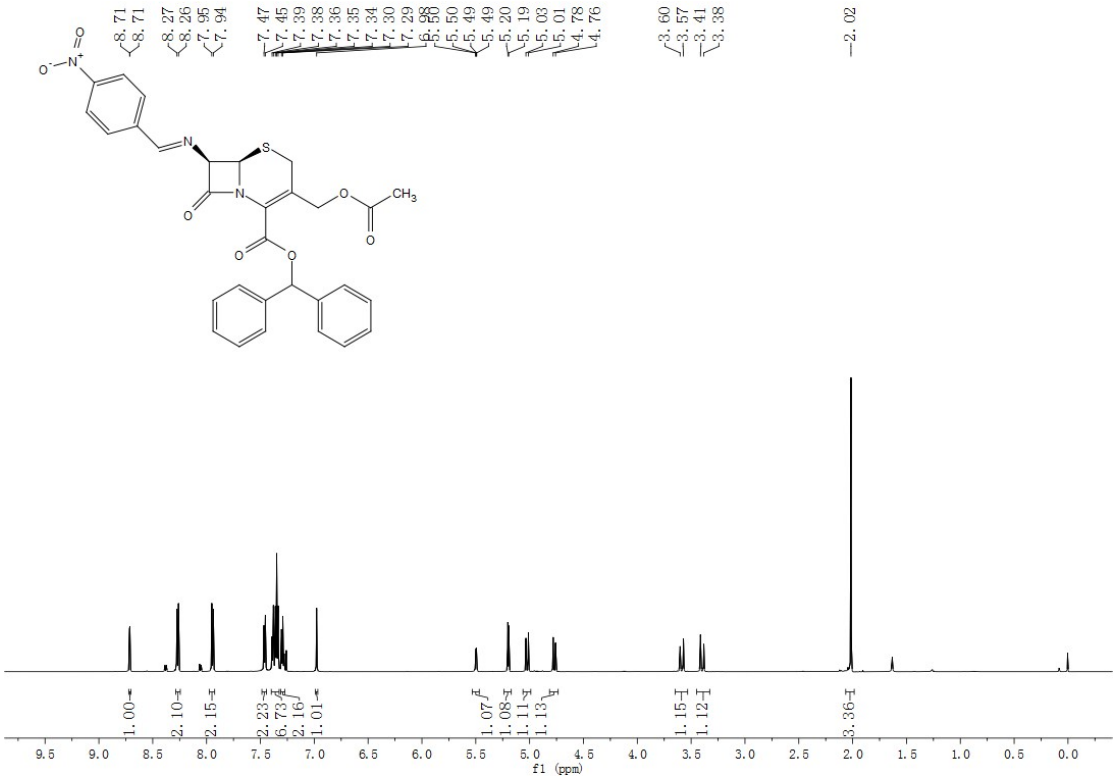

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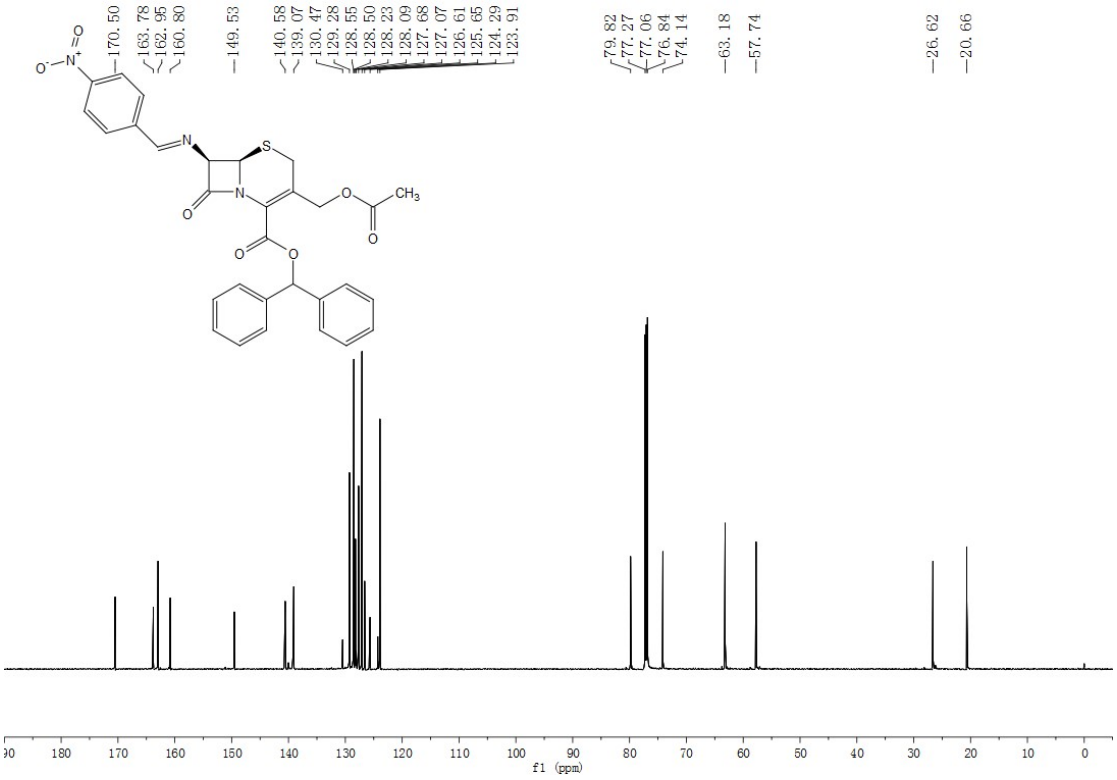

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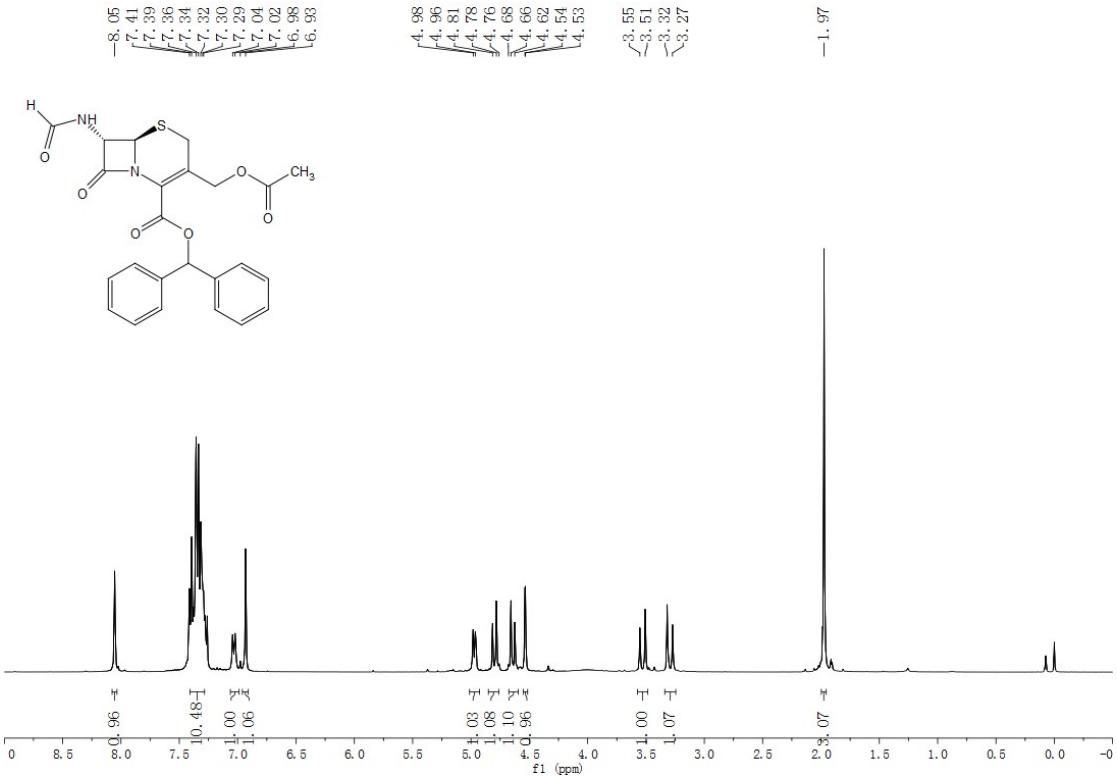

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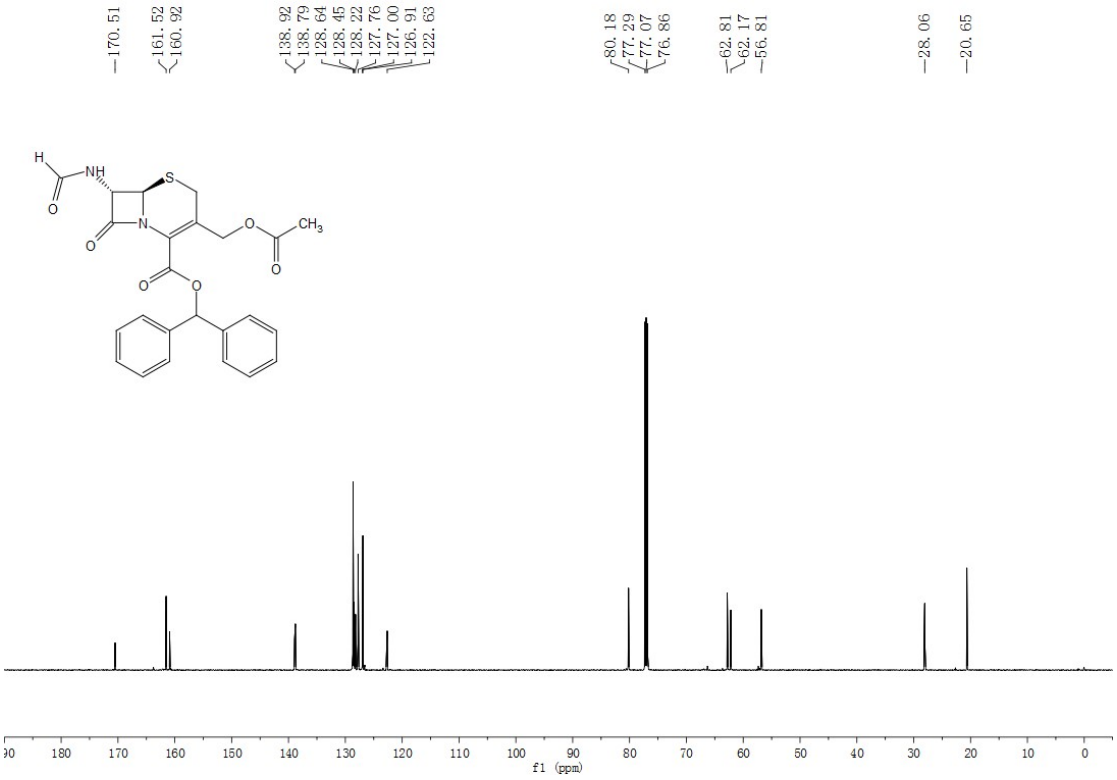

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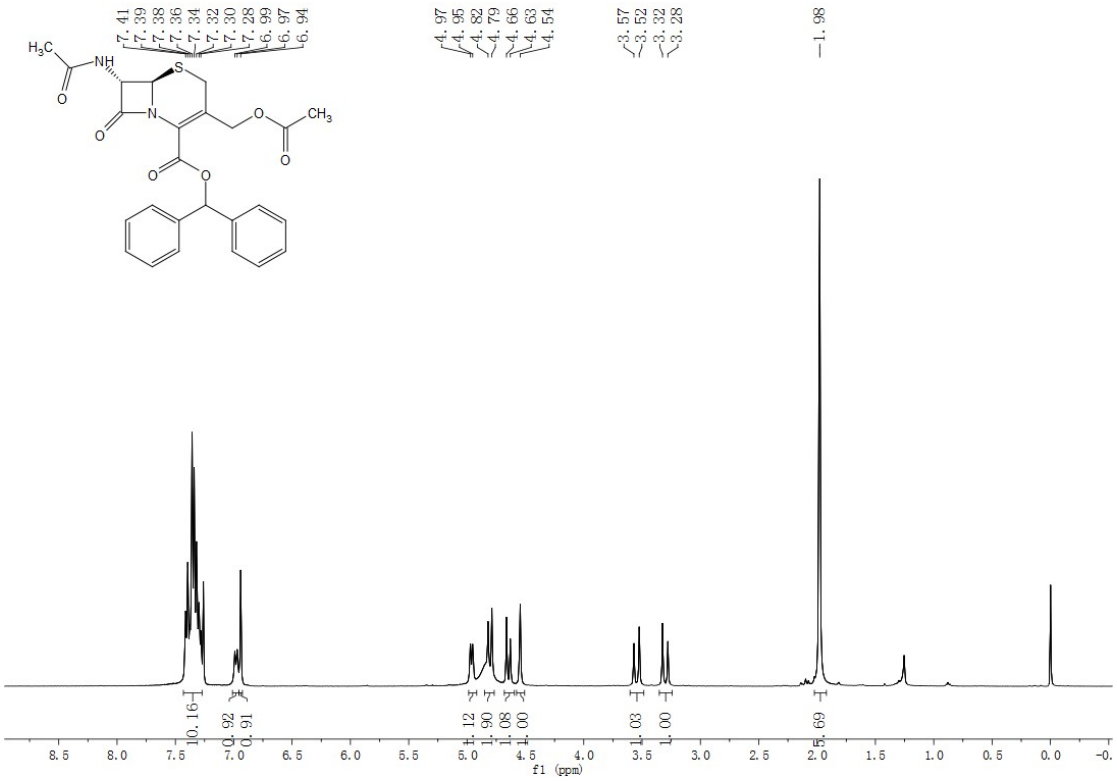

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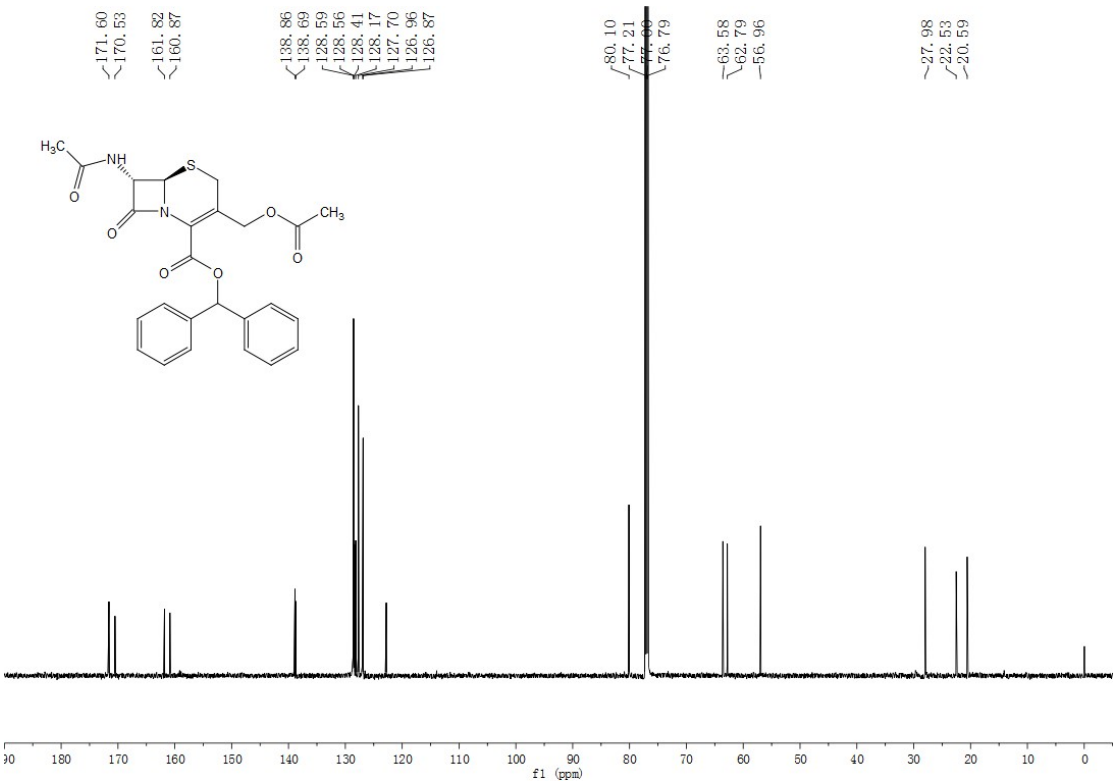

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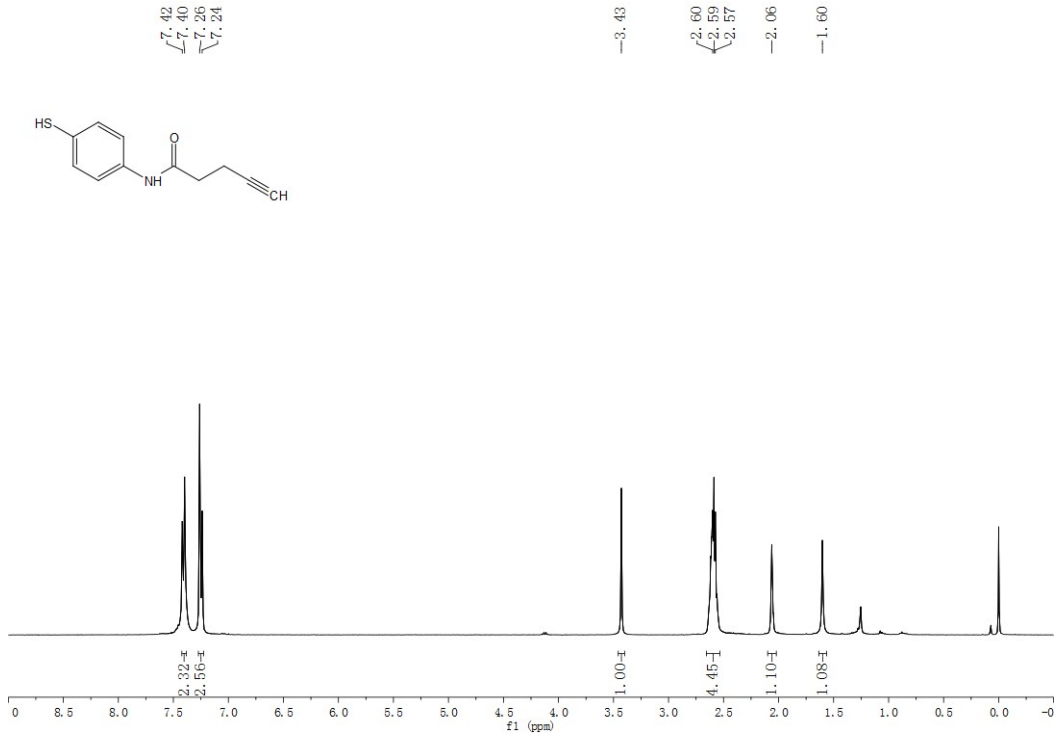

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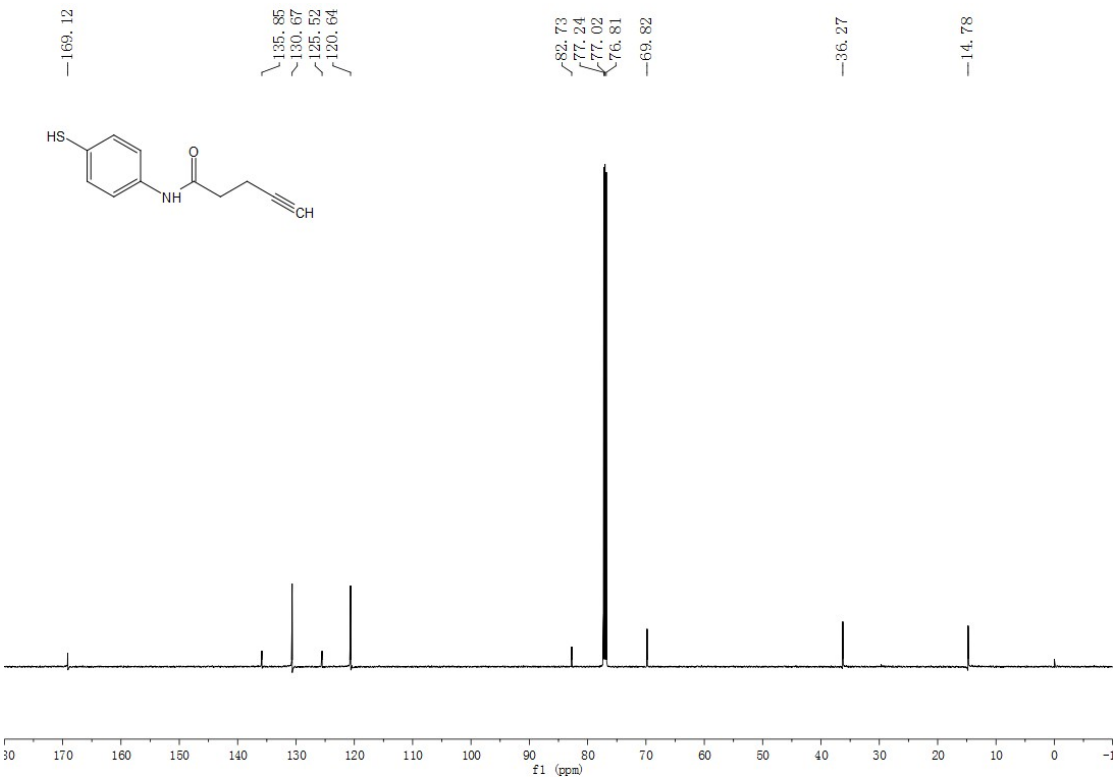

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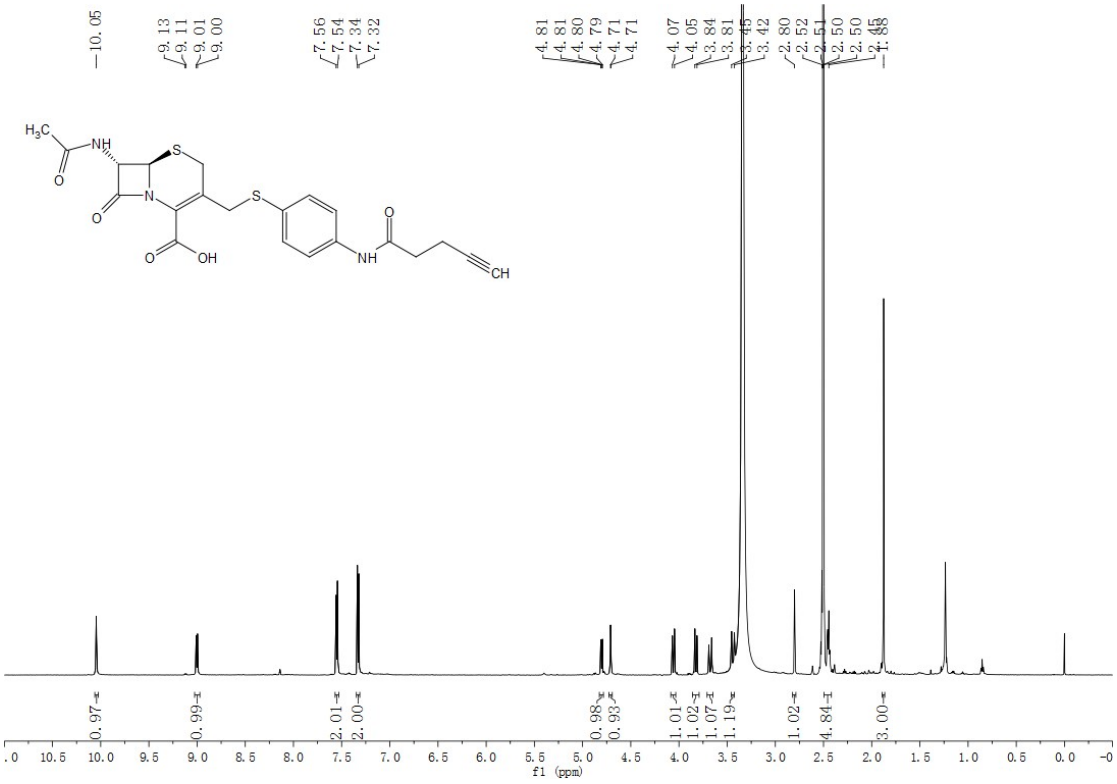

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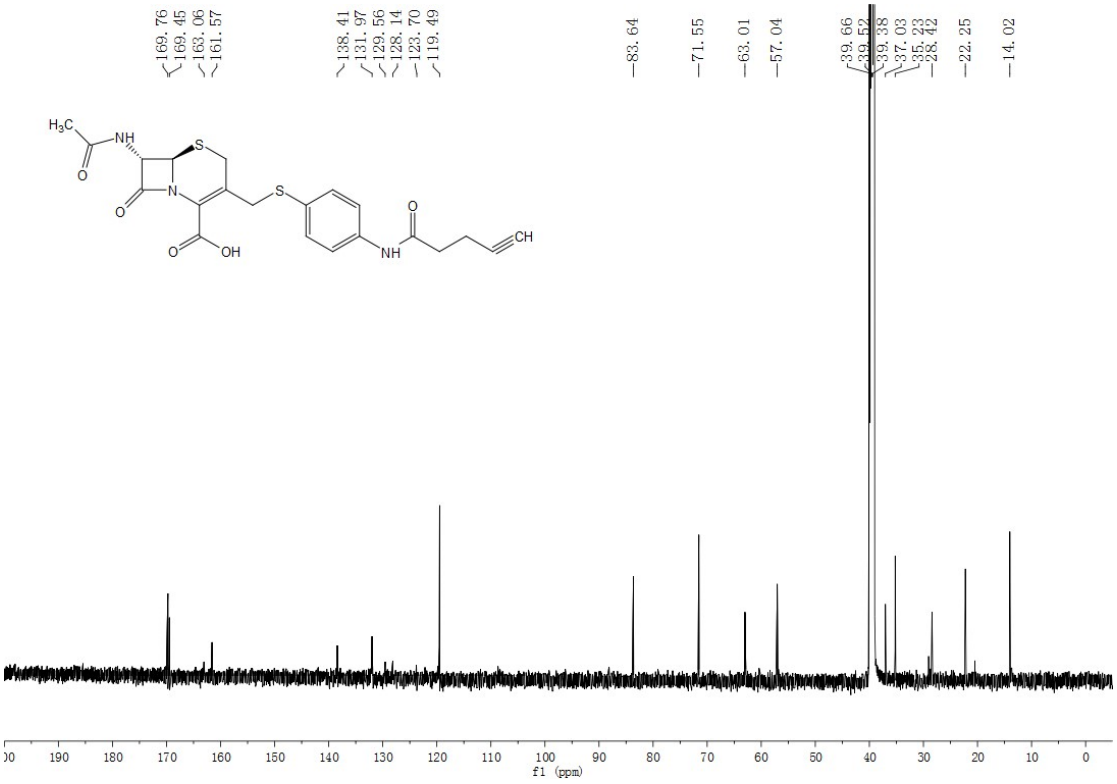

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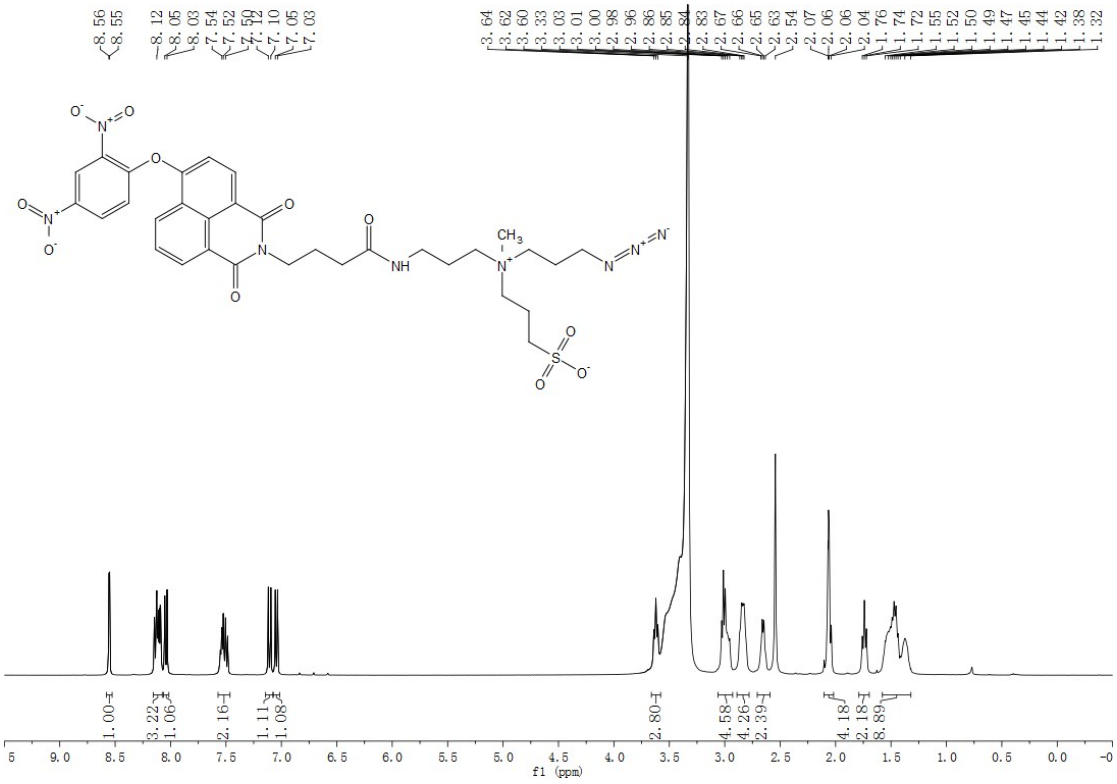

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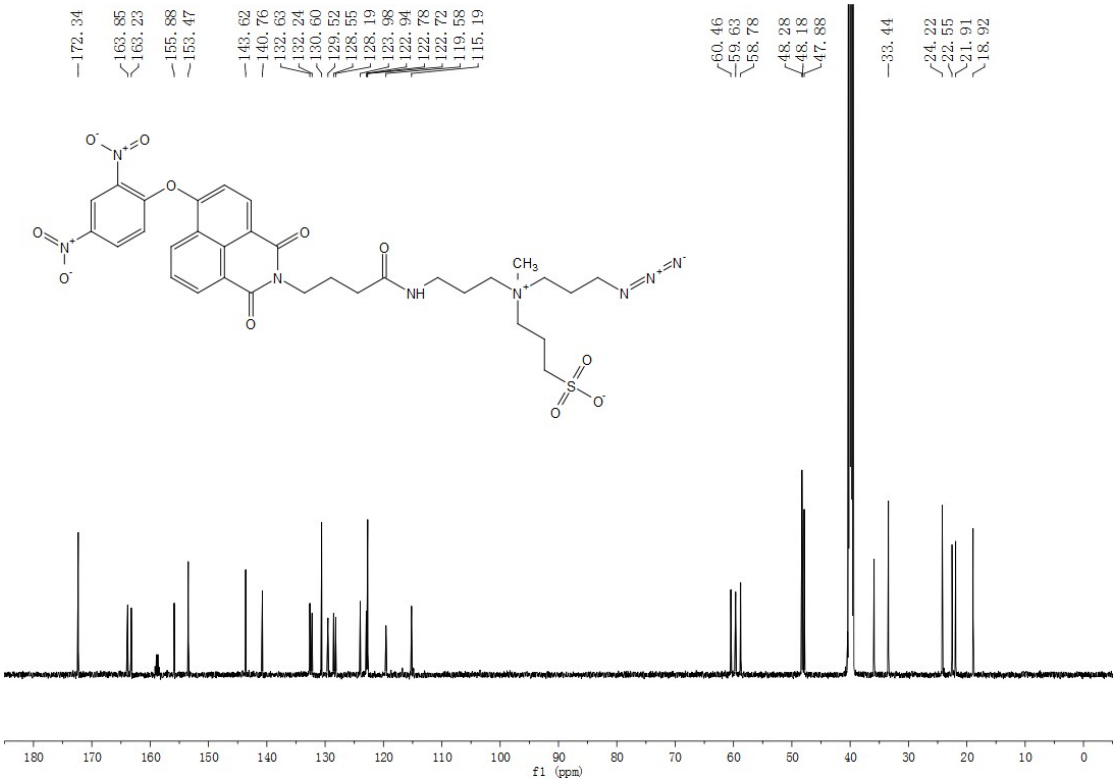
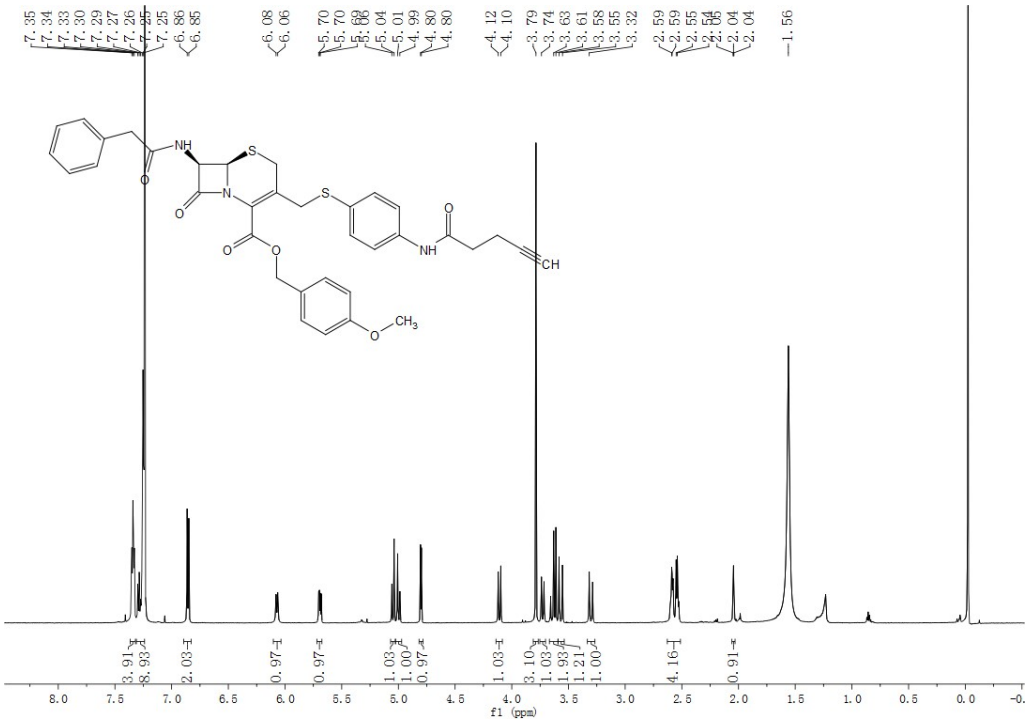


^{1}H NMR of **18**



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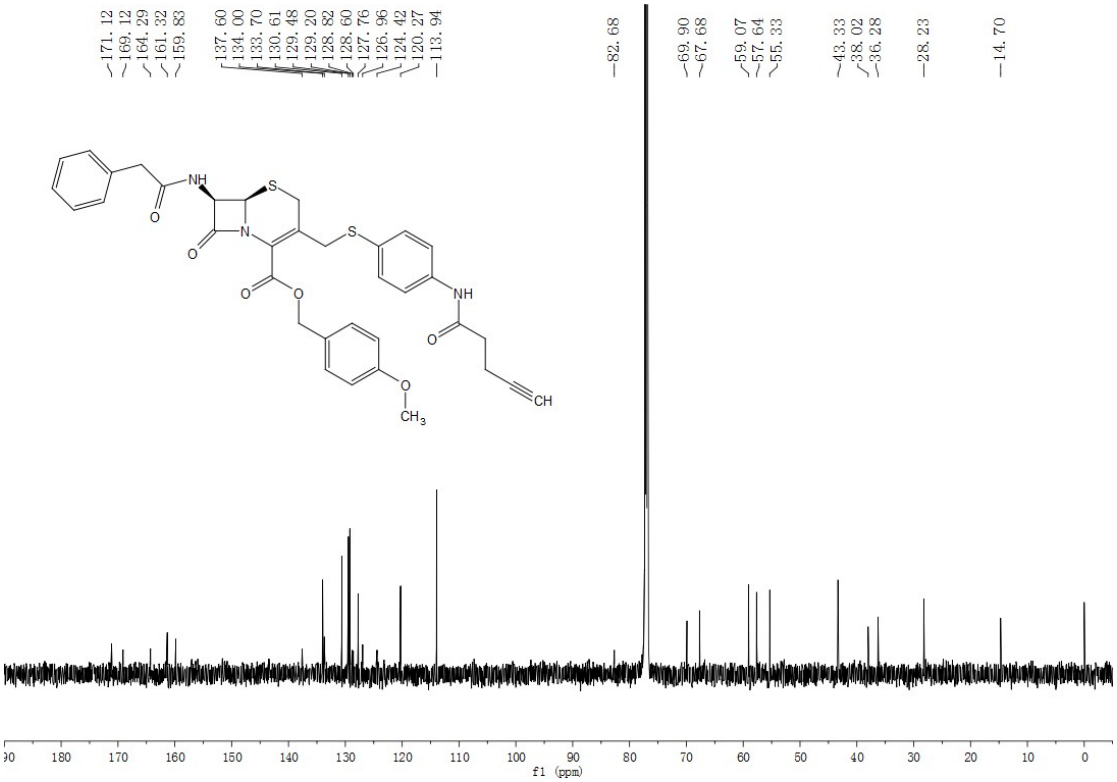

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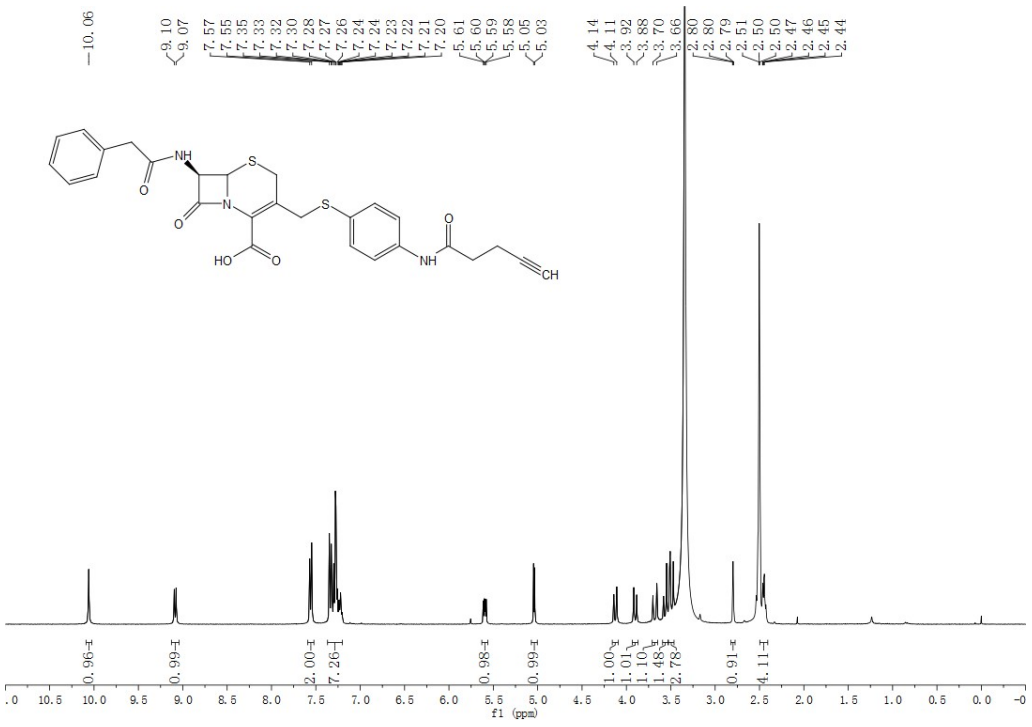

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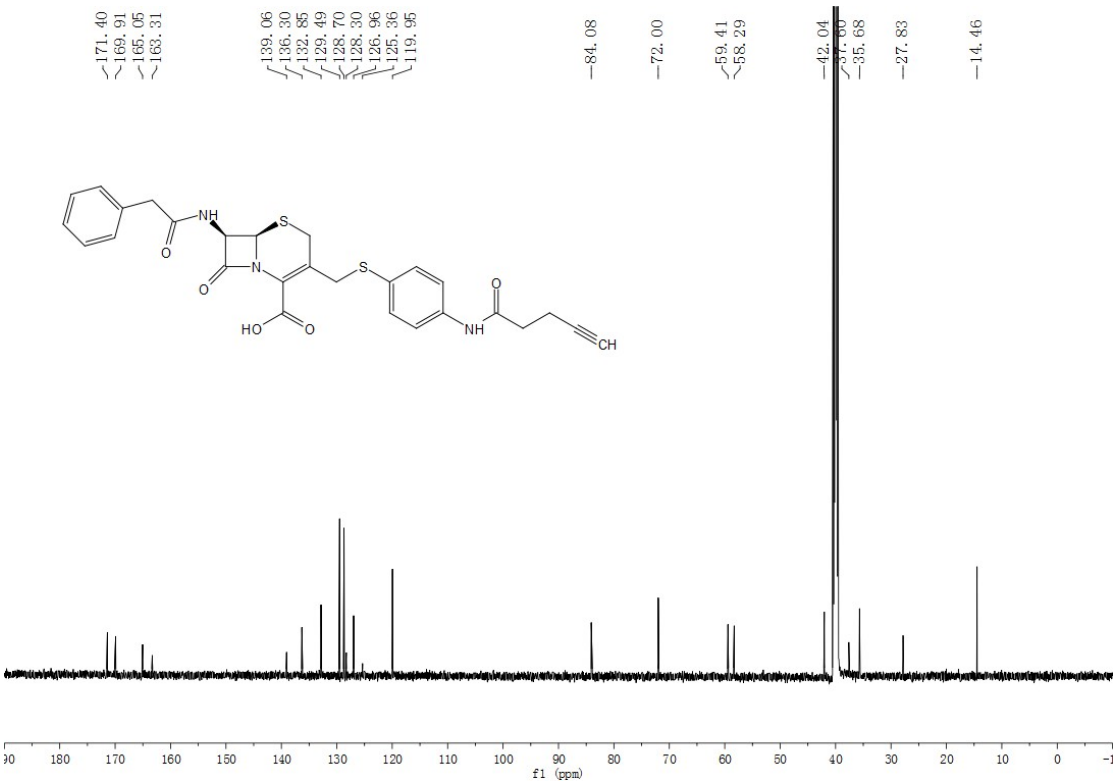
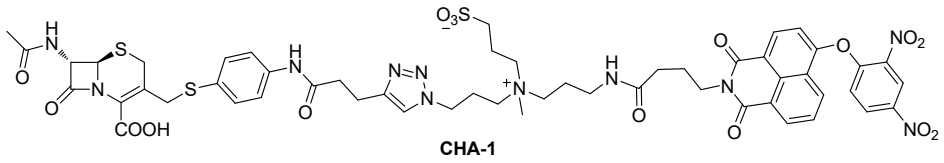
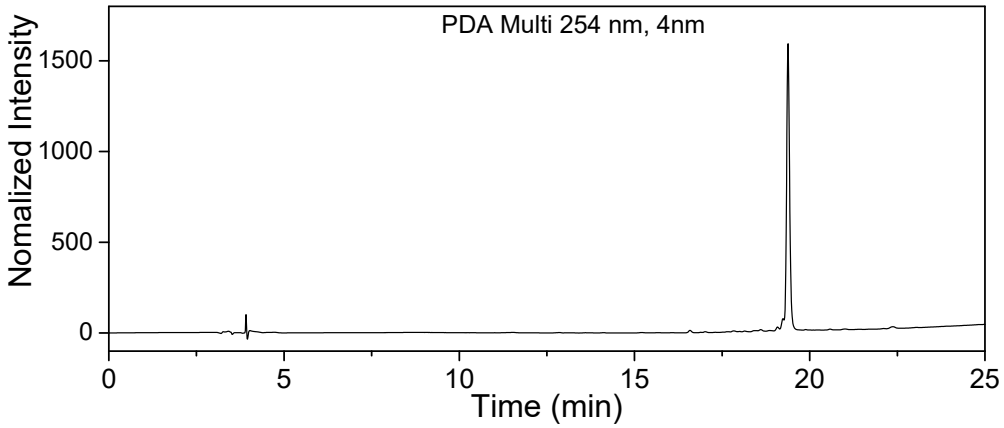
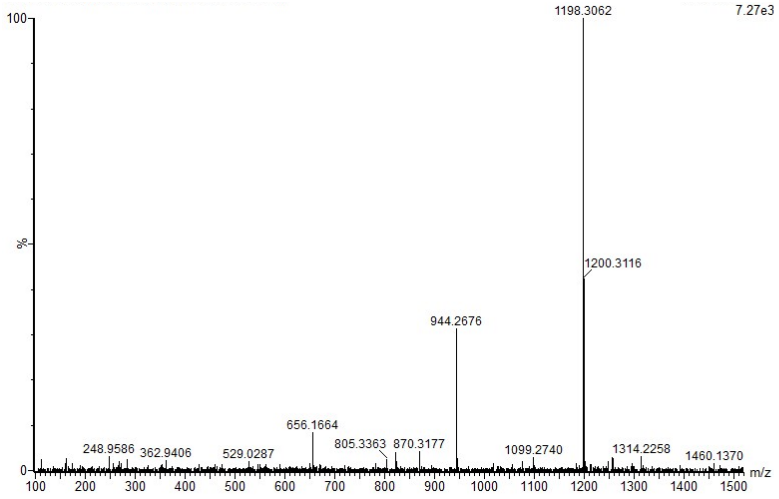


HPLC trace and HRMS (ESI) of CHA-1



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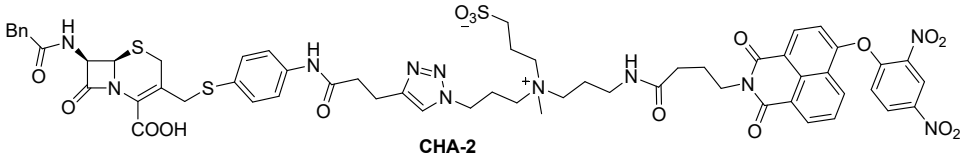
HPLC trace and HRMS (ESI) of CHA-2



CHA-2