Supporting Information

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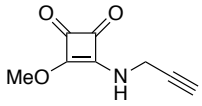
1. General information

Unless otherwise specified, starting materials were purchased from commercial suppliers and used as received. The precursor of modified carbapenems, ß-methyl-vinyl-phosphate, was purchased from AK scientific and magnetic beads dynabeads™ M-280 streptavidin were purchased from Invitrogen (Thermofischer), other molecules were purchased from Merck, TCI, Alfa Aesar, Fluorochem. All air and water sensitive reactions were performed under argon atmosphere. Solvents were purchased from commercial sources. If stated as dry, the solvents were distilled using standard methods or received already dried from suppliers. Microwave-assisted reactions were performed in a microwave reactor (Monowave 450, Anton Paar, Les Ulis, France). For the organic syntheses, the reactions were monitored by thin layer chromatography (TLC): precoated silica gel thin layer sheets 60 F254 (Merck, Darmstadt, Germany). The detection was performed by using a UV lamp (254 nm) or by staining with phosphomolybdic acid (PMA), $KMnO_4$ or ninhydrin when appropriate. Small scale reactions were performed using a Grant-Bio thermoshaker plate (Dutscher, Brumath, France). Purifications were carried out using silica gel (60 Å, 180-240 mesh from Merck, Darmstadt, Germany). Final compounds were purified using a HPLC system with a reverse phase C18 column (Agilent, 250 mm x 21.2 mm, 5 µm) using a solvent system consisting of solvent A (H_2O) and solvent B (acetonitrile) (linear gradient, from 0 to 100% B over 45 minutes) at a flow rate of 12 $mL.min^{-1}$ and UV detection at 299 nm. The purity of final compounds was established by analytical HPLC, performed on a Vydac C_{18} (250 mm x 4.6 mm, 5 µm) at a flow rate of 1.3 $mL.min^{-1}$ with UV detection at 299 nm. NMR spectra were recorded using Bruker spectrometers Bruker Advance II 500, and Bruker Advance III HD 4000 (Bruker Biospin, Fällanden, Switzerland). Chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual proton or carbon resonance of the solvents: $CDCl_3$ (δ 7.26), CD_3OD (δ 3.31), $(CD_3)_2SO$ (δ 2.50) or D_2O (δ 4.79) for ^1H and $CDCl_3$ (δ 77.16), CD_3OD (δ 49.00), $(CD_3)_2SO$ (δ 39.52) for ^{13}C. Coupling constants (J) are reported in Hz and splitting patterns are indicated as follows: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, bs = broad signal. High-resolution mass spectroscopy (HRMS) was recorded using LTQ Orbitrap XL (Thermo Scientific, Illkirch, France) or Bruker Daltonics maXis, Type: ESI Q TOF, acquisition mode "profile" with m/z scan range of 500 to 2200. Low-resolution mass spectroscopy was recorded on a LCQ Deca XP-Max (Thermo Scientific, Illkirch, France). Fluorescence experiments were performed on a spectrofluorometer (Cary Eclipse Varian, Mulgrave, Victoria, Australia) and the appropriate parameters were reported in the corresponding sections.

2. Synthesis of alkyne-linkers

2-1. Synthesis of alkyne-linker 1

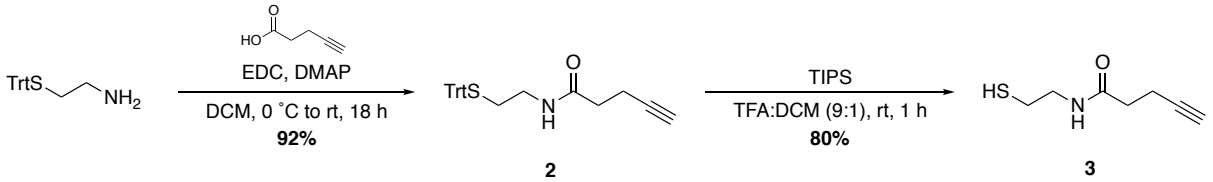
3-Methoxy-4-(prop-2-yn-1-ylamino)cyclobut-3-ene-1,2-dione (1)



1

Propargylamine (40 μL, 0.6 mmol) was added to a solution of 3,4-dimethoxycyclobut-3-ene-1,2-dione (100 mg, 0.7 mmol) in H_2O (5 mL) at 0 ˚C. A precipitate was formed within the first minutes. The reaction mixture was stirred for 5 minutes while maintaining the temperature between 0 and 5 ˚C. The precipitate was filtered and washed with cold water. The obtained crude solid was dissolved in a mixture of H_2O:CH_3CN (7:3) and purified by HPLC (H_2O:CH_3CN, linear gradient from 0 to 50% of CH_3CN over 30 minutes). The appropriate fractions were lyophilized to afford compound **1** as a white powder (50 mg, 50%). **^{1}H NMR (500 MHz, MeOD):** δ 4.43 – 4.21 (m, 5H), 2.83 (s, 1H). **HRMS (ESI):** *m/z* Calcd for $C_8H_8NO_3$ $[M + H]^+$ 166.0498; found: 166.0496.

2-2. Synthesis of thiol-alkyne-linker 3

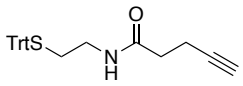


2 **3**

Scheme S1. Synthesis of alkyl-linker **3**.

General condition for removal of the trityl group. Protected thiol (1 equiv.) was dissolved in a mixture of TFA:DCM (9:1) and TIPS (1.2 equiv.) was added. The reaction mixture was stirred for 30 min at room temperature. The solvents were removed by using a stream of compressed air. The crude solid was purified by silica gel chromatography (DCM:MeOH eluent system) to afford the thiol.

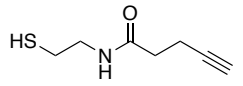
***N*-(2-(Tritylthio)ethyl)pent-4-ynamide (2)**



2

To a solution of protected cysteamine[1] (775 mg, 2.4 mmol) and 4-pentynoic acid (200 mg, 2.1 mmol) in dry DCM (15 mL) were added DMAP (25 mg, 0.2 mmol) and 3 successive portions of EDC (195 mg (x3), 3.1 mmol) at 0 ˚C. The mixture was warmed up to room temperature and stirred for 18 hours. The crude mixture was dissolved in EtOAc (100 mL) and washed with a solution of 1 M HCl (2 x 50 mL), H_2O (2 x 50 mL), brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford compound **2** as a white foam (772 mg, 92%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.48 (d, J = 7.9 Hz, 6H), 7.31 (t, J = 7.7 Hz, 6H), 7.24 (t, J = 7.3 Hz, 3H), 6.26 (t, J = 5.8 Hz, 1H), 3.14 (q, J = 6.3 Hz, 2H), 2.52 – 2.43 (m, 4H), 2.32 (t, J = 7.4 Hz, 2H), 2.00 (t, J = 5.0 Hz, 1H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 170.8, 144.5 (3C), 129.4 (6C), 127.8 (6C), 126.6 (3C), 82.8, 69.3, 66.6, 38.2, 34.9, 31.7, 14.7. **HRMS (ESI):** *m/z* calcd for $C_{26}H_{26}NNaOS$ $[M + Na]^+$ 422.1549; found: 422.1542.

***N*-(2-Mercaptoethyl)pent-4-ynamide (3)**

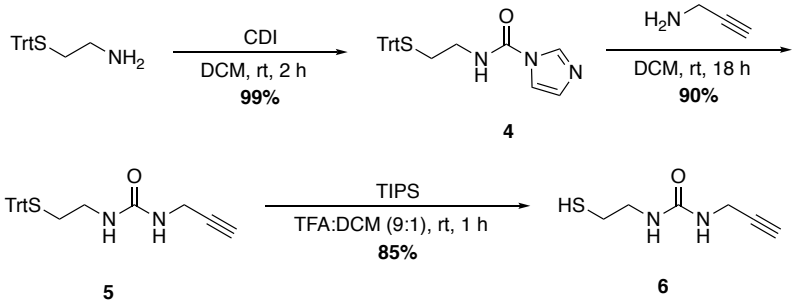


3

Following the general condition for thiol deprotection (p. 4), compound **2** (500 mg, 1.3 mmol) in presence of TIPS (330 µL, 1.6 mmol) afforded compound **3** after silica gel chromatography purification (DCM:MeOH 99:1) as a whitish foam (164 mg, 80%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 6.10 (s, 1H), 3.46 (q, J = 6.2 Hz, 2H), 2.68 (dt, J = 8.5, 6.3 Hz, 2H), 2.54 (td, J = 7.1, 2.8 Hz, 2H), 2.42 (t, J = 7.1 Hz, 2H), 2.02 (t, J = 2.7 Hz, 1H), 1.38 (t, J = 8.5 Hz, 1H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 171.2, 83.1, 69.7, 42.6, 35.5, 24.8, 15.1. **HRMS (ESI):** *m/z* calcd for $C_7H_{12}NOS$ $[M + H]^+$ 158.0634; found: 158.0632.

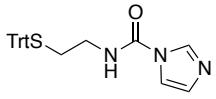
[1] Vishwanatha, T. M.; Bergamaschi, E.; Dömling, A. *Org. Lett.* **2017**, *19*, 3195–3198.

2-3. Synthesis of thiol-alkyne-linker 6



Scheme S2. Synthesis of alkyl-linker **6**.

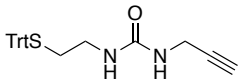
N-(2-(Tritylthio)ethyl)-1*H*-imidazole-1-carboxamide (4)



4

Carbonyl diimidazole (610 mg, 3.8 mmol) was added to a solution of protected cysteamine[1] (1.0 g, 3.1 mmol) in dry DCM (20 mL) and the reaction mixture was stirred for 2 hours at room temperature. H_2O (100 mL) was added, and the crude product was extracted with DCM (3 x 100 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford compound **4** as white foam (1.3 g, 99%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.95 (s, 1H), 7.34 – 7.33 (m, 6H), 7.20 – 7.12 (m, 10H), 6.99 (s, 1H), 6.11 (t, J = 5.2 Hz, 1H), 3.15 (dd, J = 12.3 Hz, 6.0 Hz, 2H), 2.50 (t, J = 6.3 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 148.7, 144.6 (3C), 136.0, 130.7, 129.6 (6C), 128.2 (6C), 127.1 (3C), 116.0, 67.3, 39.7, 31.8. **HRMS (ESI):** *m/z* calcd for $C_{25}H_{24}N_3OS$ $[M + H]^+$ 414.1635; found: 414.1629.

1-(Prop-2-yn-1-yl)-3-(2-(tritylthio)ethyl)urea (5)

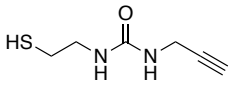


5

To a solution of *N*-(2-(tritylthio)ethyl)-1*H*-imidazole-1-carboxamide **4** (500 mg, 1.2 mmol) in dry DCM (10 mL) was added propargylamine (93 µL, 1.5 mmol). The mixture was stirred at room temperature for 18 hours. The crude mixture was then dissolved in EtOAc (50 mL),

washed with 1 M HCl (50 mL), H_2O (50 mL), brine (50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (cyclohexane:EtOAc 4:6) afforded compound **5** as a white foam (433 mg, 90%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.32 (d, J = 7.7 Hz, 6H), 7.20 (t, J = 7.3 Hz, 6H), 7.13 (t, J = 7.2 Hz, 3H), 4.73 (t, J = 5.6 Hz, 1H), 4.69 (t, J = 5.0 Hz, 1H), 3.80 (dd, J = 5.4 Hz, 2.5 Hz, 2H), 2.92 (dd, J = 12.4 Hz, J = 6.2 Hz, 2H), 2.31 (t, J = 6.35 Hz, 2H), 2.07 (t, J = 2.45 Hz, 1H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 157.6, 145.1 (3C), 130.0 (6C), 128.3 (6C), 127.2 (3C), 81.0, 71.6, 67.2, 39.5, 33.1, 30.5. **HRMS (ESI):** *m/z* calcd for $C_{25}H_{24}N_2NaOS$ $[M + Na]^+$ 423.1501; found: 423.1498.

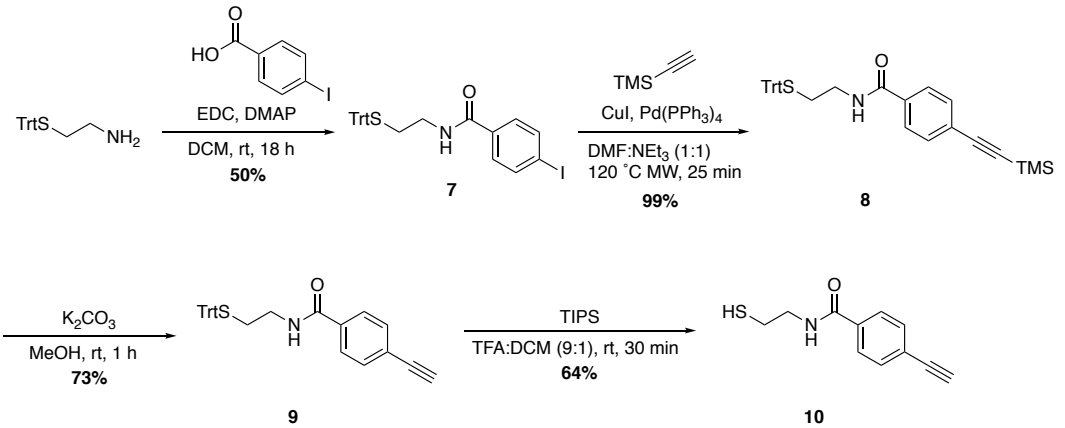
1-(2-Mercaptoethyl)-3-(prop-2-yn-1-yl)urea (6)



6

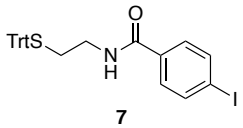
Following the general condition for thiol deprotection (p. 4), deprotection of compound **5** (400 mg, 1.0 mmol) in presence of TIPS (247 µL, 1.2 mmol) afforded compound **6** after silica gel chromatography purification (DCM:MeOH 99:1) as yellowish foam (135 mg, 85%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 4.80 (bs, 1H), 4.47 (bs, 1H), 4.00 (dd, J = 5.5, 2.5 Hz, 2H), 3.40 (q, J = 6.1 Hz, 2H), 2.68 (dt, J = 8.5, 6.3 Hz, 2H), 2.24 (t, J = 2.5 Hz, 1H), 1.35 (t, J = 8.5 Hz, 1H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 157.2, 80.5, 71.7, 43.5, 30.5, 25.5. **HRMS (ESI):** *m/z* calcd for $C_6H_{11}N_2OS$ $[M + H]^+$ 159.0586; found: 159.0586.

2-4. Synthesis of thiol-alkyne-linker 10



Scheme S3. Synthesis of acetylene-linker **10**.

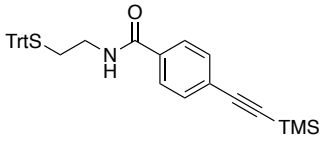
4-Iodo-*N*-(2-(tritylthio)ethyl)benzamide (7)



7

To a solution of protected cysteamine[1] (1.5 g, 4.7 mmol) and 4-iodobenzoic acid (1.1 g, 4.2 mmol) in dry DCM (30 mL) were added EDC (1.3 g, 6.7 mmol) and DMAP (104 mg, 0.9 mmol) at 0 ˚C. The reaction mixture was warmed up to room temperature and stirred for 18 hours. A white precipitate was formed. The mixture was dissolved in EtOAc (100 mL) and washed with 1 M HCl (100 mL), H_2O (100 mL), brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (cyclohexane:EtOAc 9:1) afforded the compound **7** as a white foam (1.3 g, 50%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.68 (d, J = 8.4 Hz, 2H), 7.34 – 7.32 (m, 8H), 7.20 – 7.17 (m, 6H), 7.15 – 7.10 (m, 3H), 6.16 (t, J = 5.7 Hz, 1H), 3.19 (q, J = 6.1 Hz, 2H), 2.45 (t, J = 6.2 Hz, 2H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 166.6, 144.7 (3C), 137.9 (2C), 134.0, 129.7 (6C), 128.7 (2C), 128.2 (6C), 127.0 (3C), 98.5, 67.1, 38.7, 32.2. **HRMS (ESI):** *m/z* calcd for $C_{28}H_{24}INNaOS$ $[M + Na]^+$ 572.0515; found: 572.0509.

4-((Trimethylsilyl)ethynyl)-*N*-(2-(tritylthio)ethyl)benzamide (8)

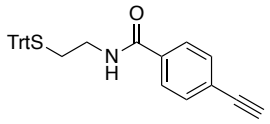


8

To a solution of compound **7** (1.0 g, 1.8 mmol) and ethynyl-trimethylsilane (300 µL, 2.0 mmol) in a mixture of DMF and triethylamine (1:1, 6 mL) were added $Pd(PPh_3)_4$ (210 mg, 0.2 mmol) and CuI (20 mg, 0.1 mmol). The reaction was stirred for 25 minutes at 120 ˚C in a microwave. After cooling down to room temperature, the mixture was acidified to pH = 5.0 using 1 M HCl. The product was extracted with Et_2O (3 x 50 mL). The combined organic layers were washed with H_2O (150 mL) and brine (150 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude solid was purified by silica gel chromatography (cyclohexane:EtOAc 99:1) to afford compound **8** as a yellow pale foam (0.93 g, 99%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.47 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 7.24 (d, J = 7.9 Hz, 6H), 7.07 (t, J = 7.6 Hz, 6H), 7.01 (t, J = 7.2 Hz, 3H), 6.52 (t, J = 5.9 Hz, 1H), 3.10 (q, J = 6.3 Hz, 2H), 2.33 (t, J = 6.4 Hz, 2H), 0.12 (s, 9H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 166.5, 144.6

(3C), 133.9, 132.0 (2C), 129.5 (6C), 128.0 (2C), 126.9 (6C), 126.8 (3C), 126.3, 104.2, 96.9, 66.9, 38.7, 32.0, -0.1 (3C). **HRMS (ESI):** *m/z* calcd for $C_{33}H_{33}NNaOSSi$ $[M + Na]^+$ 542.1944; found: 542.1941.

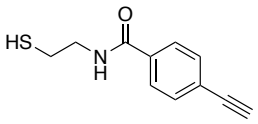
4-Ethynyl-*N*-(2-(tritylthio)ethyl)benzamide (9)



9

Compound **8** (750 mg, 1.4 mmol) was treated with K_2CO_3 (400 mg, 2.9 mmol) in MeOH (20 mL). The reaction mixture was stirred for 1 hour at room temperature and then filtered. MeOH was removed under reduced pressure and the crude solid was dissolved in EtOAc (50 mL). The organic phase was washed with sat. solution of $NaHCO_3$ (50 mL), H_2O (50 mL), brine (50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained crude solid was purified by silica gel chromatography (hexane:EtOAc 7:3) to afford compound **9** as a white foam (457 mg, 73%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.71 – 7.65 (m, 2H), 7.59 – 7.53 (m, 2H), 7.47 – 7.41 (m, 6H), 7.29 (d, J = 6.9 Hz, 6H), 7.26 – 7.21 (m, 3H), 6.26 (bs, 1H), 3.32 (q, J = 5.7 Hz, 2H), 3.25 – 3.12 (m, 1H), 2.58 – 2.56 (m, 2H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 166.5, 144.6 (3C), 134.3, 132.2 (2C), 129.5 (6C), 128.0 (2C), 127.0 (6C), 126.8 (3C), 125.3, 82.8, 79.7, 66.9, 38.7, 32.0. **HRMS (ESI):** *m/z* calcd for $C_{30}H_{25}NNaOS$ $[M + Na]^+$ 470.1549; found: 470.1545.

4-Ethynyl-*N*-(2-mercaptoethyl)benzamide (10)



10

Following the general condition for thiol deprotection (p. 4), compound **9** (450 mg, 1.0 mmol) in presence of TIPS (224 µL, 1.2 mmol) afforded compound **10** after silica gel chromatography purification (DCM:MeOH 99:1) as yellowish foam (131 mg, 64%). NMR analyses.[2] **HRMS (ESI):** *m/z* calcd for $C_{11}H_{12}NOS$ $[M + H]^+$ 206.0634; found: 206.0633.

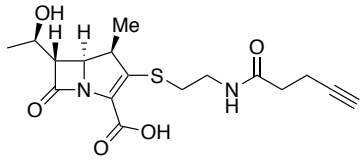
[2] Tsumoto, H.; Ogasawara, D.; Hashii, N.; Suzuki, T.; Akimoto, Y.; Endo, T.; Miura, Y. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 2645–2649.

3. Synthesis of alkyne-carbapenems

Carbapenem-alkynes (**CBA**) were synthesized following the 2 synthetic routes A and B (Scheme 1).

3-1. Synthesis of CBA-1, CBA-2 and CBA-3 following route A

(4*R*,5*S*,6*S*)-6-((*R*)-1-Hydroxyethyl)-4-methyl-7-oxo-3-((2-(pent-4-ynamido)ethyl)thio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (CBA-1)

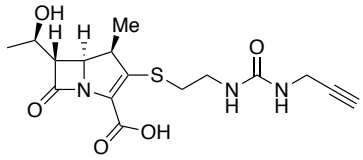


CBA-1

To a solution of ß-methyl-thienamycin[3] (8.5 mg, 0.03 mmol) in a mixture of DMF:H_2O (9:1), sodium bicarbonate (3.7 mg, 0.04 mmol) and NHS activated pentynoic acid[4] (5.8 mg, 0.03 mmol) were added. The mixture was stirred for 1 hour at 25 ˚C. The reaction mixture was dissolved in water and filtered through a PTFE 0.2 µm syringe filter. Purification by HPLC (H_2O:CH_3CN; linear gradient 50% CH_3CN over 30 minutes) afforded the corresponding carbapenem **CBA-1** as a white foam (5.4 mg, 49%). **^{1}H NMR (500 MHz, D_2O):** δ 4.26 (p, J = 6.3 Hz, 1H), 4.20 (dd, J = 9.0, 2.5 Hz, 1H), 3.55 – 3.36 (m, 4H), 3.07 (dt, J = 13.0, 6.2 Hz, 1H), 2.87 (dt, J = 13.8, 6.9 Hz, 1H), 2.54 – 2.44 (m, 4H), 2.40 (bs, 1H), 1.31 (d, J = 6.3 Hz, 3H), 1.20 (d, J = 7.2 Hz, 3H). **HRMS (ESI):** *m/z* calcd for $C_{17}H_{23}N_2O_5S$ $[M + H]^+$ 367.1322; found: 367.1331. **HPLC purity:** 90%. **HPLC t_R:** 15.0 min.

[3] Iannazzo, L.; Soroka, D.; Triboulet, S.; Fonvielle, M.; Compain, F.; Dubée, V.; Mainardi, J.-L.; Hugonnet, J.-E.; Braud, E.; Arthur, M.; Etheve-Quelquejeu, M. *J. Med. Chem.* **2016**, *59*, 3427–3438.
[4] Hacker, S. M.; Backus, K. M.; Lazear, M. R.; Forli, S.; Correia, B. E.; Cravatt, B. F. *Nat. Chem.* **2017**, *9*, 1181–1190.

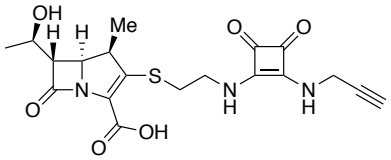
(4*R*,5*S*,6*S*)-6-((*R*)-1-Hydroxyethyl)-4-methyl-7-oxo-3-((2-(3-(prop-2-yn-1-yl)ureido)ethyl) thio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (CBA-2)



CBA-2

To a solution of ß-methyl-thienamycin[3] (9.4 mg, 0.03 mmol) in a mixture of DMF:H_2O (9:1), sodium bicarbonate (3.3 mg, 0.04 mmol) and *N*-(prop-2-yn-1-yl)-1*H*-imidazole-1-carboxamide[5] (5.8 mg, 0.04 mmol) were added. The mixture was stirred for 1 hour at 25 ˚C. The reaction mixture was dissolved in water and filtered through a PTFE 0.2 µm syringe filter. Purification by HPLC (H_2O:CH_3CN; linear gradient 50% CH_3CN over 30 minutes) afforded the corresponding carbapenem **CBA-2** as a white foam (7.4 mg, 67%). **^{1}H NMR (500 MHz, D_2O):** δ 4.21 – 4.16 (m, 1H), 4.13 (dd, J = 10.0 Hz, J = 5.0 Hz, 1H), 3.83 (s, 2H), 3.40 – 3.35 (m, 3H), 3.33 – 3.27 (m, 1H), 3.02 – 2.98 (m, 1H), 2.75 – 2.70 (m, 1H), 2.54 (s, 1H), 1.25 (d, J = 5.0 Hz, 3H), 1.12 (d, J = 7.5 Hz, 3H). **HRMS (ESI):** *m/z* calcd for $C_{16}H_{21}N_3NaO_5S$ $[M + Na]^+$ 390.1094; found: 390.1090. **HPLC purity:** 95%. **HPLC t_R:** 14.4 min.

(4*R*,5*S*,6*S*)-3-((2-((3,4-Dioxo-2-(prop-2-yn-1-ylamino)cyclobut-1-en-1-yl)amino)ethyl) thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (CBA-3)



CBA-3

To a solution of ß-methyl-thienamycin[3] (6.5 mg, 0.02 mmol) in a mixture of DMF:H_2O (9:1), sodium bicarbonate (4 mg, 0.05 mmol) and compound **1** (6 mg, 0.03 mmol) were added. The mixture was stirred for 1 hour at 40 ˚C. The reaction mixture was dissolved in water and filtered through a PTFE 0.2 µm syringe filter. Purification by HPLC (H_2O:CH_3CN; linear gradient 50% CH_3CN over 30 minutes) afforded the corresponding **CBA-3** as a white foam (1.2 mg, 14%).

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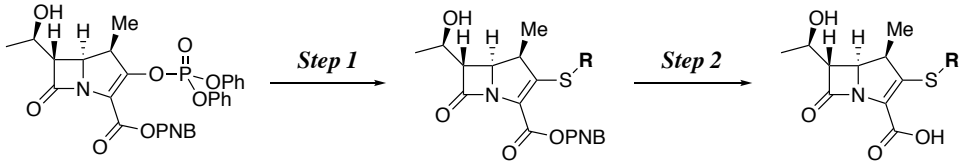
[5] Rillahan, C. D.; Schwartz, E.; Rademacher, C.; McBride, R.; Rangarajan, J.; Fokin, V. V.; Paulson, J. C. *ACS Chem. Biol.* **2013**, *8*, 1417–1422.

^{1}H NMR (500 MHz, D_2O): δ 4.33 (m, 3H), 4.19 (t, J = 5.0 Hz, 1H), 4.12 (bs, 1H), 3.85 (bs, 1H), 3.50 – 3,47 (m, 1H), 3.41 (dd, J = 4.2, 1.95 Hz, 1H), 3.26 – 3.21 (m, 1H), 2.99 – 2.94 (m, 1H), 2.92 – 2.77 (m, 2H), 2.74 (s, 1H), 1.25 (d, J = 6.25 Hz, 3H), 1.16 (d, J = 7.15 Hz, 3H). **HRMS (ESI):** *m/z* calcd for $C_{19}H_{21}N_3NaO_6S$ $[M + Na]^+$ 442.1043; found: 442.1037. **HPLC purity:** 88%. **HPLC t_R:** 14.9 min.

3-2. Synthesis of CBA-1, CBA-2, CBA-4 and CBA-5 following route B



ß-Methyl-vinyl-phosphate **CBA**

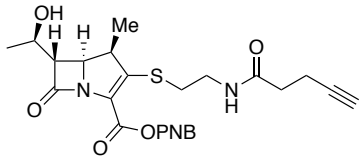
Scheme S4. Synthesis of carbapenems **CBA** using route B; *Step 1*: RSH, DIPEA, DMF, 0 ˚C to 4 ˚C, 24 h; *Step 2*: Zn dust, THF:phosphate buffer (1:2) (0.35 M, pH = 6.0), rt, 1 h.

General condition for *Step 1* (Scheme S4): To a solution of ß-methyl-vinyl-phosphate (1 equiv.) and thiol-alkyne linker (2 equiv., up to 4 equiv.) in dry DMF was added dropwise DIPEA (2 equiv.) at 0 ˚C. The reaction mixture was stirred for 24 hours at 4 ˚C. The reaction mixture was dissolved in EtOAc and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude mixture was purified by silica gel chromatography (DCM:MeOH eluent system) to afford the protected carbapenem.

General condition for *Step 2* (Scheme S4): Following the procedure described in the literature,[6] zinc dust (10 equiv. mass) was activated in 1 M HCl for 15 minutes at room temperature. Zinc dust was then filtrated and washed with H_2O, and phosphate buffer (0.35 M, pH = 6.0). To a solution of protected carbapenem (1 equiv.) in THF:phosphate buffer (1:2) (0.35 M, pH = 6.0) was added activated zinc dust. The reaction mixture was stirred for 1 hour at room temperature. THF was removed under reduced pressure. The aqueous phase was filtered through a 0.2 μm PTFE syringe filter to obtain a homogenous transparent solution. This solution was purified by HPLC (H_2O:CH_3CN; linear gradient of CH_3CN over 30 minutes) and the appropriate fractions were collected and lyophilized to afford the **CBA**.

[6] Kumagai, T.; Abe, T.; Fujimoto, Y.; Hayashi, T.; Inoue, Y.; Nagao, Y. *Heterocycles* **1993**, *36*, 1729–1734.

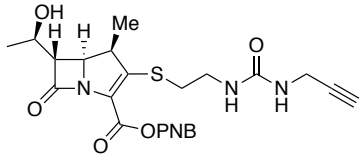
4-Nitrobenzyl (4*R*,5*S*,6*S*)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-3-((2-(pent-4-ynamido)ethyl)thio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (11a)



11a

Following the general condition for step 1 (p. 12), mixing ß-methyl-vinyl-phosphate (192 mg, 0.3 mmol) and compound **3** (100 mg, 0.6 mmol) in presence of DIPEA (105 µL, 0.6 mmol) afforded compound **11a** after silica gel chromatography purification (cyclohexane:EtOAc 2:8) as a yellowish foam (146 mg, 97%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 8.21 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 8.6 Hz, 2H), 6.18 (t, J = 6.0 Hz, 1H), 5.49 (d, J = 13.7 Hz, 1H), 5.21 (d, J = 13.8 Hz, 1H), 4.30 – 4.20 (m, 2H), 3.65 – 3.54 (m, 2H), 3.38 – 3.31 (m, 1H), 3.28 (dd, J = 6.7, 2.6 Hz, 1H), 3.14 – 3.09 (m, 1H), 2.88 – 2.82 (m, 1H), 2.51 (td, J = 7.0, 2.9 Hz, 2H), 2.40 (t, J = 7.0 Hz, 2H), 2.02 (t, J = 2.6 Hz, 1H), 1.35 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 7.2 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 172.9, 171.7, 160.6, 151.5, 147.8, 143.1, 128.3 (2C), 124.7 (2C), 123.9, 82.8, 69.8, 66.0, 65.4, 60.0, 56.3, 43.1, 40.2, 35.3, 31.1, 22.0, 17.0, 14.9. **HRMS (ESI):** *m/z* calcd for $C_{24}H_{26}N_3NaO_8S$ $[M + Na]^+$ 524.1461; found: 524.1456.

4-Nitrobenzyl (4*R*,5*S*,6*S*)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-3-((2-(3-(prop-2-yn-1-yl)ureido)ethyl)thio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (11b)

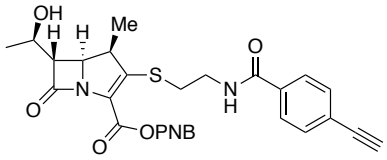


11b

Following the general condition for step 1 (p. 12), mixing ß-methyl-vinyl-phosphate (150 mg, 0.3 mmol) and compound **6** (80 mg, 0.5 mmol) in presence of DIPEA (105 µL, 0.6 mmol) afforded compound **11b** after silica gel chromatography purification (DCM:MeOH 95:5) as yellowish foam (116 mg, 77%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 8.16 (d, J = 8.8 Hz, 2H), 7.61 (d, J = 8.7 Hz, 2H), 5.54 (bs, 1H), 5.44 (d, J = 13.8 Hz, 1H), 5.29 (bs, 1H), 5.17 (d, J = 13.9 Hz, 1H), 4.30 (dd, J = 9.2, 2.6 Hz, 1H), 4.25 (q, J = 6.3 Hz, 1H), 4.03 (dd, J = 17.7, 2.5 Hz, 1H), 3.90 (dd, J = 17.7, 2.6 Hz, 1H), 3.59 – 3.54 (m, 1H), 3.52 – 3.44 (m, 1H), 3.41 – 3.35 (m, 1H), 3.27 (dd, J = 6.3, 2.6 Hz, 1H), 3.17 – 3.12 (m, 1H), 2.85 – 2.80 (m, 1H), 2.25 (t, J = 2.5

Hz, 1H), 1.34 (d, *J* = 6.3 Hz, 3H), 1.22 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$):** *δ* 173.2, 160.9, 157.9, 152.9, 147.7, 143.0, 128.3 (2C), 124.0 (2C), 123.9, 80.8, 71.6, 65.8, 65.6, 60.0, 56.1, 43.2, 41.3, 32.1, 30.5, 21.8, 17.0. **HRMS (ESI):** *m/z* calcd for $C_{23}H_{27}N_4O_7S$ $[M + H]^+$ 503.1595; found: 503.1590.

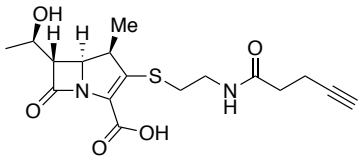
4-Nitrobenzyl (4*R*,5*S*,6*S*)-3-((2-(4-ethynylbenzamido)ethyl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (11d)



11d

Following the general condition for step 1 (p. 12), mixing ß-methyl-vinyl-phosphate (350 mg, 0.6 mmol) and compound **10** (250 mg, 1.2 mmol) in presence of DIPEA (210 µL, 0.12 mmol) afforded compound **11d** after silica gel chromatography purification (DCM:MeOH 95:5) as yellowish foam (178 mg, 54%). **^{1}H NMR (500 MHz, $CDCl_3$):** *δ* 8.15 (d, *J* = 8.9 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.6 Hz, 2H), 7.50 (d, *J* = 8.2 Hz, 2H), 7.00 (t, *J* = 6.0 Hz, 1H), 5.43 (d, *J* = 13.8 Hz, 1H), 5.15 (d, *J* = 13.8 Hz, 1H), 4.25 – 4.14 (m, 2H), 3.76 – 3.68 (m, 1H), 3.60 – 3.50 (m, 2H), 3.26 (dd, *J* = 6.5, 2.5 Hz, 1H), 3.23 – 3.16 (m, 2H), 2.96 – 2.90 (m, 1H), 1.31 (d, *J* = 6.3 Hz, 3H), 1.21 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$):** *δ* 173.0, 167.3, 160.6, 151.5, 147.6, 143.0, 133.8, 132.5 (2C), 128.2 (2C), 127.1 (2C), 125.9, 124.8, 123.9 (2C), 82.7, 80.1, 65.8, 65.4, 59.9, 56.2, 43.1, 40.6, 31.0, 21.8, 17.0. **HRMS (ESI):** *m/z* calcd for $C_{28}H_{28}N_3O_7S$ $[M + H]^+$ 550.1642; found: 550.1638.

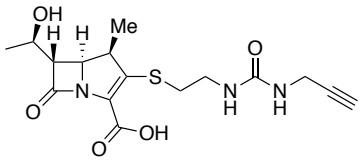
(4*R*,5*S*,6*S*)-6-((*R*)-1-Hydroxyethyl)-4-methyl-7-oxo-3-((2-(pent-4-ynamido)ethyl)thio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid(CBA-1)



CBA-1

Following the general condition for step 2 (p. 12), compound **11a** (70 mg, 0.14 mmol) was deprotected in presence of Zn dust (1.4 g, 20 equiv. mass) to afford **CBA-1** as a white foam (5.8 mg, 11%). Analytical data were identical to those obtained with route A. **HPLC purity:** 99%.

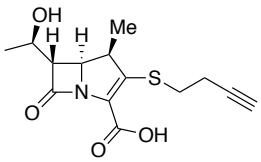
(4*R*,5*S*,6*S*)-6-((*R*)-1-Hydroxyethyl)-4-methyl-7-oxo-3-((2-(3-(prop-2-yn-1-yl)ureido) ethyl)thio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (CBA-2)



CBA-2

Following the general condition for step 2 (p. 12), compound **11b** (50 mg, 0.01 mmol) was deprotected in presence of Zn dust (1.0 g, 20 equiv. mass) to afford **CBA-2** as a white foam (28 mg, 77%). Analytical data were identical to those obtained with route A. **HPLC purity:** 99%.

(4*R*,5*S*,6*S*)-3-(But-3-yn-1-ylthio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid (CBA-4)

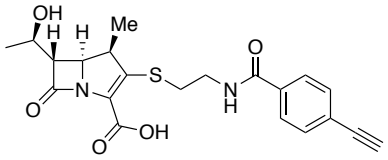


CBA-4

Following the general condition for step 2 (p. 12), compound **11c**[3] (50 mg, 0.12 mmol) was deprotected in presence of Zn dust (1.0 g, 20 equiv. mass) to afford **CBA-4** as a white foam (14.2 mg, 40%). **^{1}H NMR (500 MHz, D_2O):** δ 4.27 (p, J = 6.3 Hz, 1H), 4.21 (dd, J = 9.1, 2.6 Hz, 1H), 3.53 (p, J = 7.2 Hz, 1H), 3.44 (dd, J = 6.2, 2.6 Hz, 1H), 3.15 – 3.09 (m, 1H), 2.94 –

2.88 (m, 1H), 2.62 – 2.55 (m, 2H), 2.49 – 2.46 (m, 1H), 1.32 (d, J = 6.3 Hz, 3H), 1.22 (d, J = 7.2 Hz, 3H). **HRMS (ESI):** *m/z* calcd for $C_{14}H_{18}NO_4S$ $[M + H]^+$ 296.0951; found: 296.0946. **HPLC purity:** 88%. **HPLC t_R:** 16.2 min.

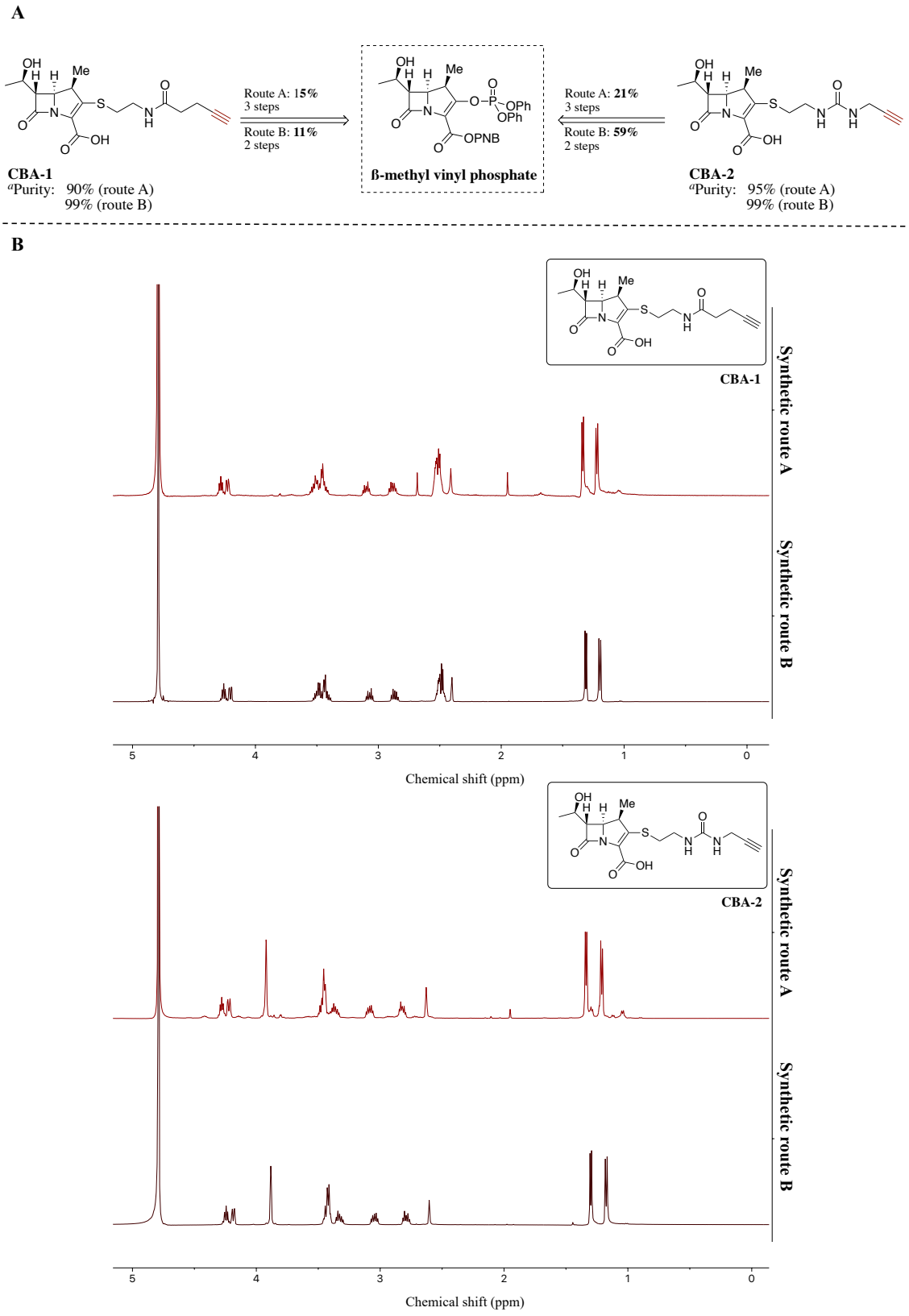
(4*R*,5*S*,6*S*)-3-((2-(4-Ethynylbenzamido)ethyl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (CBA-5)



CBA-5

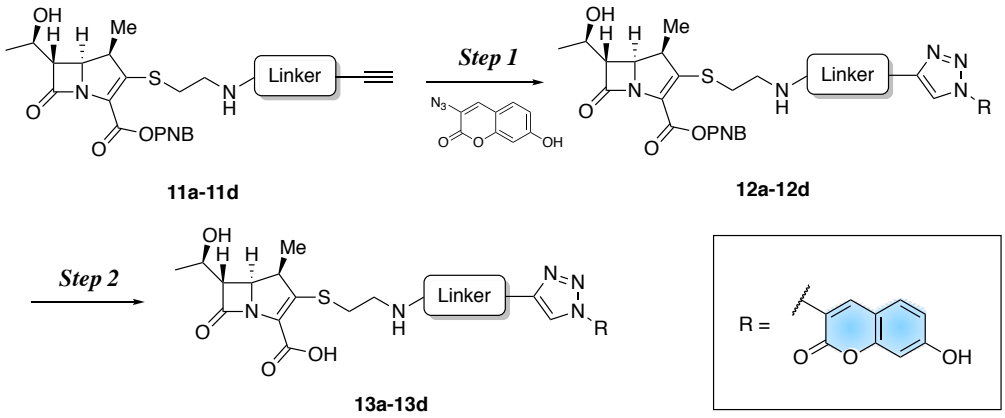
Following the general condition for step 2 (p. 12), compound **11d** (50 mg, 0.091 mmol) was deprotected using Zn dust (1.0 g, 20 equiv. mass) to afford **CBA-5** as a white foam (16 mg, 43%). **^{1}H NMR (500 MHz, D_2O):** δ 7.78 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 6.9 Hz, 2H), 4.20 – 4.12 (m, 1H), 3.80 – 3.72 (m, 2H), 3.70 (d, J = 1.5 Hz, 1H), 3.65 – 3.59 (m, 1H), 3.44 – 3.34 (m, 2H), 3.26 – 3.20 (m, 1H), 3.01 – 2.92 (m, 1H), 1.26 (d, J = 4.9 Hz, 3H), 1.18 (d, J = 6.2 Hz, 3H). **HRMS (ESI):** *m/z* calcd for $C_{21}H_{23}N_2O_5S$ $[M + H]^+$ 415.1322; found: 415.1317. **HPLC purity:** 96%. **HPLC t_R :** 18.1 min.

3-3. Comparison of route A and route B



Scheme S5. A brief comparative of synthetic routes A and B. A: retrosynthesis of **CBA-1** and **CBA-2** from ß-methyl-vinyl-phosphate using route A and B and their overall yield and purity. B: ^{1}H NMR spectra of **CBA-1** and **CBA-2** obtained *via* route A (up) and route B (down). Frequency 500 MHz at 298 K. [a]Purity calculated by analytical HPLC.

4. Synthesis of fluorescent carbapenems 13a-13d



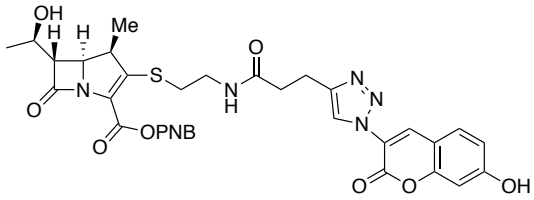
Scheme S6. Synthesis of fluorescent carbapenems **13a-13d**; *Step 1*: 3-azido-7-hydroxycoumarin, $CuSO_4$, sodium ascorbate, THF:H_2O (1:1), rt, 2 h; *Step 2*: Pt/C 10% wt., H_2 3.5 bars, THF:triethylammonium bicarbonate buffer (2:1) (1 M, pH = 8.5), rt, 2 h.

General condition for *Step 1* (Scheme S6): To a solution of alkynylated carbapenem **11a-11d** (1 equiv.) in a mixture of THF:H_2O (1:1) was added 3-azido-7-hydroxycoumarin[7] (1.5 equiv.), $CuSO_4.5H_2O$ (30 mol%) and sodium ascorbate (60 mol%). The reaction mixture was stirred at room temperature until the starting material was fully consumed (monitored by TLC, ≈ 2 hours). If not the case, additional amount of sodium ascorbate was introduced (60 mol%). The crude mixture was dissolved in EtOAc, washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude solid was purified by silica gel chromatography (DCM:MeOH eluent system) to afford the corresponding protected fluorescent carbapenems **12a-12d**.

General condition for *Step 2* (Scheme S6): The solution of protected fluorescent carbapenem **12a-12d** in a mixture of THF:triethylammonium bicarbonate (1 M, pH = 8.5) (2:1) was treated with 10 wt. % Pt/C (1 equiv. mass.). The reaction mixture was hydrogenated at 3.5 bars using a PARR apparatus for 2 hours at room temperature. THF was removed under reduced pressure. The aqueous phase was filtered through a PTFE 0.2 µm syringe filter and purified by HPLC (using H_2O:CH_3CN; linear gradient of CH_3CN over 30 minutes) to afford the deprotected fluorescent carbapenems **13a-13d**.

[7] Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603–4606.

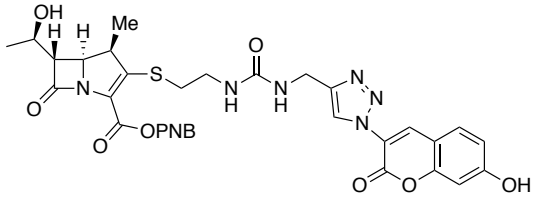
4-Nitrobenzyl (4*R*,5*S*,6*S*)-3-((2-(3-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)propanamido)ethyl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (12a)



12a

Following the general condition for step 1 (p. 18), mixing compound **11a** (50 mg, 0.1 mmol) and 3-azido-7-hydroxycoumarin (26 mg, 0.13 mmol) afforded compound **12a** after silica gel chromatography purification (DCM:MeOH 9:1) as yellowish foam (45 mg, 64%). **^{1}H NMR (500 MHz, $(CD_3)_2CO$):** δ 8.46 (s, 1H), 8.32 (s, 1H), 8.22 (d, J = 8.8 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 8.6 Hz, 1H), 6.95 (dd, J = 8.5, 2.3 Hz, 1H), 6.88 (d, J = 2.2 Hz, 1H), 5.48 (d, J = 14.1 Hz, 1H), 5.24 (d, J = 14.2 Hz, 1H), 4.30 (dd, J = 9.3, 2.6 Hz, 1H), 4.13 (p, J = 6.4 Hz, 1H), 3.77 – 3.71 (m, 1H), 3.51 – 3.46 (m, 1H), 3.43 – 3.35 (m, 1H), 3.31 (dd, J = 6.9, 2.6 Hz, 1H), 3.17 – 3.11 (m, 2H), 3.08 (t, J = 7.3 Hz, 3H), 2.94 – 2.89 (m, 2H), 2.62 (t, J = 7.3 Hz, 2H), 1.28 (d, J = 6.2 Hz, 3H), 1.24 (d, J = 7.3 Hz, 3H). **^{13}C NMR (126 MHz, $(CD_3)_2CO$):** δ 174.3, 172.8, 163.1, 161.1, 157.1, 155.8, 152.9, 148.5, 147.4, 145.0, 135.4, 131.6, 129.1 (2C), 124.9, 124.3 (2C), 123.4, 121.0, 115.1, 111.9, 103.3, 66.2, 65.5, 61.0, 57.0, 43.7, 40.6, 40.4, 35.9, 31.4, 22.2, 17.4. **HRMS (ESI):** *m/z* calcd for $C_{33}H_{33}N_6O_{10}S$ $[M + H]^+$ 705.1973; found: 705.1962.

4-Nitrobenzyl (4*R*,5*S*,6*S*)-3-((2-(3-((1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)methyl)ureido)ethyl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (12b)

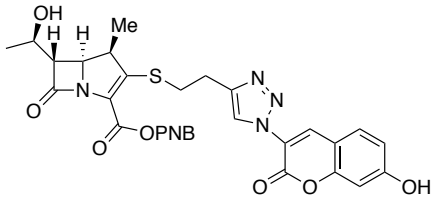


12b

Following the general condition for step 1 (p. 18), mixing compound **11b** (40 mg, 0.08 mmol) and 3-azido-7-hydroxycoumarin (21 mg, 0.1 mmol) afforded compound **12b** after silica gel chromatography purification (DCM:MeOH 9:1) as yellowish foam (31 mg, 55%). **^{1}H NMR**

(500 MHz, $(CD_3)_2CO$): δ 8.43 (s, 1H), 8.41 (s, 1H), 8.20 (d, J = 8.8 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H), 7.69 (d, J = 8.1 Hz, 1H), 6.93 (dd, J = 8.5, 2.4 Hz, 1H), 6.84 (s, 1H), 5.49 (d, J = 14.2 Hz, 1H), 5.23 (d, J = 14.1 Hz, 1H), 4.50 (t, J = 4.2 Hz, 2H), 4.28 (dd, J = 9.2, 2.6 Hz, 1H), 4.12 (p, J = 6.4 Hz, 1H), 3.77 – 3.71 (m, 1H), 3.45 – 3.39 (m, 2H), 3.29 (dd, J = 7.0, 2.7 Hz, 1H), 3.19 – 3.14 (m, 2H), 2.93 – 2.87 (m, 2H), 1.27 (d, J = 6.1 Hz, 3H), 1.22 (d, J = 7.3 Hz, 3H). **^{13}C NMR (126 MHz, $(CD_3)_2CO$):** δ 174.3, 163.3, 161.2, 159.2, 157.1, 155.9, 153.5, 148.4, 147.3, 145.0, 135.7, 131.6, 129.1 (2C), 124.6 (2C), 124.3, 124.0, 120.9, 115.2, 111.8, 103.3, 66.3, 65.5, 60.9, 57.1, 43.8, 41.7, 36.4, 32.4, 22.2, 17.4. **HRMS (ESI):** *m/z* calcd for $C_{32}H_{32}N_7O_{10}S$ $[M + H]^+$ 706.1925; found: 706.1914.

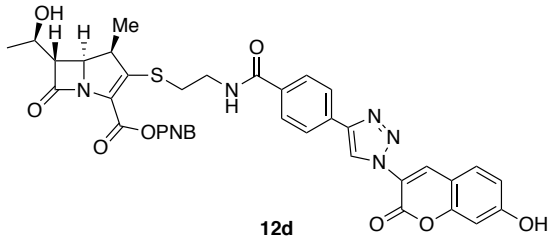
4-Nitrobenzyl (4*R*,5*S*,6*S*)-3-((2-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)ethyl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (12c)



12c

Following the general condition for step 1 (p. 18), mixing compound **11c** (50 mg, 0.12 mmol) and 3-azido-7-hydroxycoumarin (33 mg, 0.16 mmol) afforded compound **12c** after silica gel chromatography purification (DCM:MeOH 95:5) as yellowish foam (48 mg, 63%). **^{1}H NMR (500 MHz, $(CD_3)_2CO$):** δ 8.50 (s, 1H), 8.37 (s, 1H), 8.20 (d, J = 8.6 Hz, 2H), 7.75 (d, J = 8.6 Hz, 2H), 7.72 (d, J = 8.5 Hz, 1H), 6.96 (dd, J = 8.5, 2.4 Hz, 1H), 6.84 (d, J = 2.4 Hz, 1H), 5.49 (d, J = 14.2 Hz, 1H), 5.24 (d, J = 14.1 Hz, 1H), 4.25 (dd, J = 9.4, 2.8 Hz, 1H), 4.15 (p, J = 6.3 Hz, 1H), 3.62 – 3.56 (m, 1H), 3.42 – 3.36 (m, 1H), 3.34 – 3.29 (m, 1H), 3.28 – 3.21 (m, 1H), 3.20 – 3.11 (m, 2H), 1.28 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 7.3 Hz, 3H). **^{13}C NMR (126 MHz, $(CD_3)_2CO$):** δ 174.4, 163.0, 161.0, 157.1, 155.9, 152.3, 148.4, 145.9, 144.9, 135.8, 131.7, 129.1 (2C), 125.2, 124.4, 124.2 (2C), 120.9, 115.0, 112.0, 103.2, 65.9, 65.5, 61.0, 56.6, 43.8, 31.3, 27.4, 22.2, 17.2. **HRMS (ESI):** *m/z* calcd for $C_{30}H_{26}N_5O_9S$ $[M - H]^-$ 632.1456; found: 632.1467.

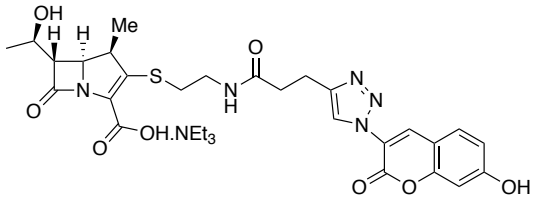
4-Nitrobenzyl (4*S*,5*R*,6*R*)-3-((2-(4-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)benzamido)ethyl)thio)-6-((*S*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylate (12d)



12d

Following the general condition for step 1 (p. 18), mixing compound **11d** (40 mg, 0.07 mmol) and 3-azido-7-hydroxycoumarin (18 mg, 0.1 mmol) afforded compound **12d** after silica gel chromatography purification (DCM:MeOH 95:5) as yellowish foam (29 mg, 55%). **^{1}H NMR (500 MHz, $(CD_3)_2CO$):** δ 9.00 (s, 1H), 8.61 (s, 1H), 8.23 (d, J = 8.5 Hz, 2H), 8.10 (d, J = 8.0 Hz, 2H), 8.01 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 8.4 Hz, 3H), 7.02 (d, J = 8.6 Hz, 1H), 6.93 (s, 1H), 5.53 (d, J = 14.2 Hz, 1H), 5.29 (d, J = 14.1 Hz, 1H), 4.28 (dd, J = 9.3, 2.6 Hz, 1H), 4.13 (p, J = 6.5 Hz, 1H), 3.83 (p, J = 7.3 Hz, 1H), 3.73 – 36.8 (m, 1H), 3.66 – 3.59 (m, 1H), 3.37 – 3.26 (m, 2H), 3.09 – 3.03 (m, 1H), 1.28 (d, J = 6.4 Hz, 6H). **^{13}C NMR (126 MHz, $(CD_3)_2CO$):** δ 174.3, 167.3, 163.3, 161.1, 157.1, 156.1, 152.6, 148.5, 147.2, 145.0, 136.1, 134.9, 134.5, 131.9, 129.2 (2C), 128.8 (2C), 126.4, 125.2, 124.3 (2C), 122.8 (2C), 120.9, 115.2, 111.9, 103.3, 66.2, 65.5, 61.0, 57.1, 43.7, 41.3, 31.2, 22.2, 17.4. **HRMS (ESI):** *m/z* calcd for $C_{37}H_{31}N_6O_{10}S$ $[M - H]^-$ 751.1827; found: 751.1839.

(4*R*,5*S*,6*S*)-3-((2-(3-(1-(7-Hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl) propanamido)ethyl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid (13a)

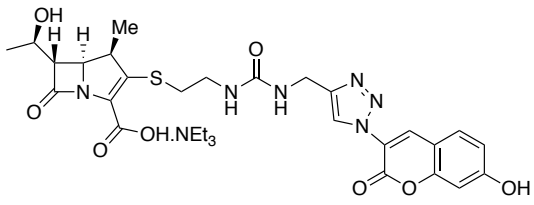


13a

Following the general condition for step 2 (p. 18), compound **12a** (44 mg, 0.06 mmol) was deprotected using Pt/C (44 mg, 1 equiv. mass.) to afford compound **13a** as a yellow foam (6.8 mg, 17%). **^{1}H NMR (500 MHz, D_2O):** δ 8.30 (s, 1H), 8.26 (s, 1H), 7.59 (d, J = 8.5 Hz, 1H), 6.92 (dd, J = 8.5, 2.4 Hz, 1H), 6.83 (s, 1H), 4.18 (p, J = 6.3 Hz, 1H), 4.09 (dd, J = 9.0, 2.4 Hz,

1H), 3.51 – 3.46 (m, 1H), 3.41 – 3.31 (m, 3H), 3.27 – 3.22 (m, 6H, $3CH_2$ of NEt_3), 3.11 (t, *J* = 7.1 Hz, 2H), 2.95 – 2.90 (m, 1H), 2.84 – 2.79 (m, 1H), 2.69 (t, *J* = 7.1 Hz, 2H), 1.32 (t, *J* = 7.3 Hz, 9H, $3CH_3$ of NEt_3), 1.28 (d, *J* = 6.4 Hz, 3H), 1.09 (d, *J* = 7.3 Hz, 3H). **HRMS (ESI):** *m/z* calcd for $C_{26}H_{28}N_5O_8S$ $[M + H]^+$ 570.1653; found: 570.1642. **HPLC purity:** 89%. **HPLC t_R:** 17.0 min.

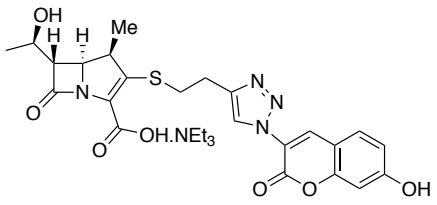
(4*R*,5*S*,6*S*)-3-((2-(3-((1-(7-Hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl) methyl)ureido)ethyl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid (13b)



13b

Following the general condition for step 2 (p. 18), compound **12b** (31 mg, 0.044 mmol) was deprotected using Pt/C (31 mg, 1 equiv. mass.) to afford compound **13b** as a yellow foam (13.9 mg, 47%). Triethylammonium salts are slightly in excess as observed by ^1H NMR, consequently the precise quantity of **12b** was calculated by NMR integration. **^1H NMR (500 MHz, D_2O):** δ 8.30 (s, 1H), 8.24 (d, *J* = 1.3 Hz, 1H), 7.52 (dd, *J* = 8.7, 1.5 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 6.73 (s, 1H), 4.47 (s, 2H), 4.21 (p, *J* = 6.3 Hz, 1H), 4.11 (dd, *J* = 9.2, 2.4 Hz, 1H), 3.42 (q, *J* = 7.2, 6.6 Hz, 3H), 3.37 (dd, *J* = 6.2, 2.5 Hz, 1H), 3.24 (q, *J* = 7.1 Hz, 9H, $3CH_2$ of NEt_3), 3.07 – 3.02 (m, 1H), 2.86 – 2.81 (m, 1H), 1.32 (t, *J* = 7.3 Hz, 13H, $3CH_3$ of NEt_3), 1.28 (d, *J* = 6.3 Hz, 3H), 1.15 (d, *J* = 7.2 Hz, 3H). **HRMS (ESI):** *m/z* calcd for $C_{25}H_{27}N_6O_8S$ $[M + H]^+$ 571.1605; found: 571.1577. **HPLC purity:** 89%. **HPLC t_R:** 16.7 min.

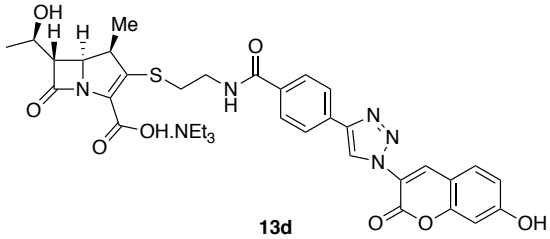
(4*R*,5*S*,6*S*)-3-((2-(1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl)ethyl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (13c)



13c

Following the general condition for step 2 (p. 18), compound **12c** (42 mg, 0.07 mmol) was deprotected using Pt/C (42 mg, 1 equiv. mass.) to afford compound **13c** as a yellow foam (19.7 mg, 47%). Triethylammonium salts are slightly in excess as observed by ^{1}H NMR, consequently the precise quantity of **13c** was calculated by NMR integration. **^{1}H NMR (500 MHz, D_2O):** δ 8.20 (s, 1H), 8.13 (s, 1H), 7.45 (d, J = 9.0 Hz, 1H), 6.84 – 6.75 (m, 1H), 6.66 (d, J = 2.5 Hz, 1H), 4.26 – 4.21 (m, 1H), 4.05 (dd, J = 8.5 Hz, 1H), 3.39 (dd, J = 6.2, 2.6 Hz, 1H), 3.34 – 3.30 (m, 1H), 3.26 – 3.17 (m, 8H, NEt_3), 3.18 – 3.11 (m, 1H), 3.10 – 2.97 (m, 3H), 1.35 – 1.24 (m, 15H, 12H of NEt_3), 1.16 (d, J = 7.4 Hz, 3H). **HRMS (ESI):** *m/z* calcd for $C_{23}H_{23}N_4O_7S$ $[M + H]^+$ 499.1282; found: 499.1269. **HPLC purity:** 95%. **HPLC t_R:** 18.0 min.

(4*R*,5*S*,6*S*)-3-((2-(4-(1-(7-Hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl) benzamido)ethyl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (13d)

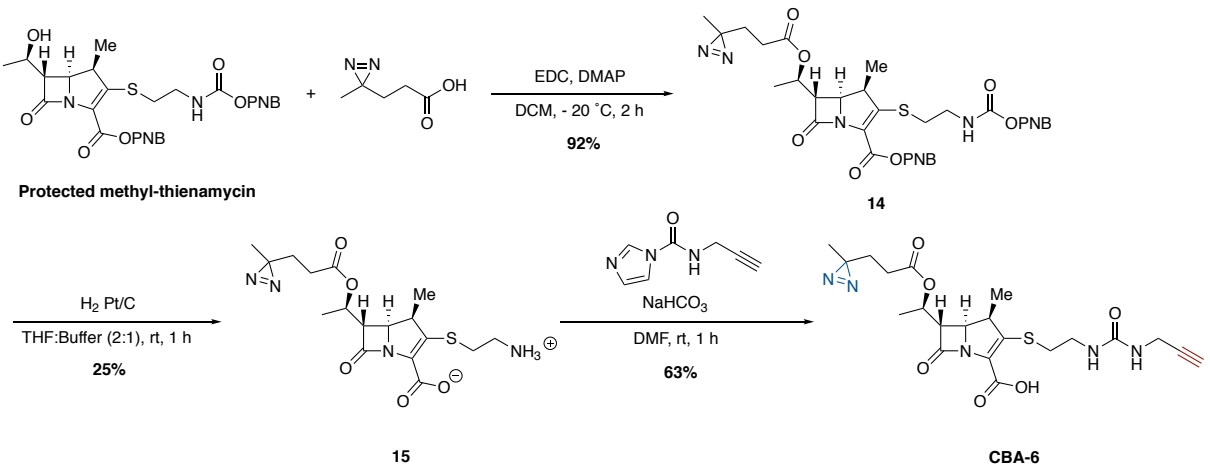


13d

Following the general condition for step 2 (p. 18), compound **12d** (20 mg, 0.02 mmol) was deprotected using Pt/C (20 mg, 1 equiv. mass.) to afford compound **13d** as a yellow foam (4.2 mg, 29%). Triethylammonium salts are in excess as observed by ^{1}H NMR, consequently the precise quantity of **13d** was calculated by NMR integration. **^{1}H NMR (500 MHz, $(CD_3)_2CO$):** δ 9.01 (s, 1H), 8.61 (s, 1H), 8.11 (d, J = 8.1 Hz, 2H), 8.02 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.5 Hz, 1H), 7.03 (dd, J = 8.6, 2.4 Hz, 1H), 6.95 (d, J = 2.1 Hz, 1H), 4.22 (dd, J = 9.4, 2.5 Hz, 1H),

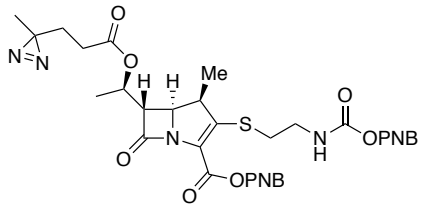
4.10 (p, *J* = 6.7 Hz, 1H), 3.72 – 3.67 (m, 1H), 3.60 (q, *J* = 6.5 Hz, 1H), 3.27 (dd, *J* = 7.3, 2.8 Hz, 1H), 3.26 – 3.24 (m, 1H), 3.18 – 3.16 (m, 12H, 2 x 3CH_2 of NEt_3), 3.00 (dt, *J* = 14.3, 7.6 Hz, 2H), 1.36 (t, *J* = 7.2 Hz, 18H, 2 x 3CH_3 of NEt_3), 1.26 (d, *J* = 6.9 Hz, 6H). **HRMS (ESI):** *m/z* calcd for $C_{30}H_{26}N_5O_8S$ $[M - H]^-$ 616.1507; found: 616.1505. **HPLC purity:** 90%. **HPLC t_R:** 19.6 min.

5. Synthesis of bi-functionalized carbapenem CBA-6



Scheme S7. Synthesis of bi-functionalized carbapenem **CBA-6**. Buffer: triethylammonium bicarbonate buffer (1 M, pH = 8.5).

4-Nitrobenzyl (4*R*,5*S*,6*S*)-4-methyl-6-((*R*)-1-((3-(3-methyl-3*H*-diazirin-3-yl)propanoyl) oxy)ethyl)-3-((2-((((4-nitrobenzyl)oxy)carbonyl)amino)ethyl)thio)-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylate (14)

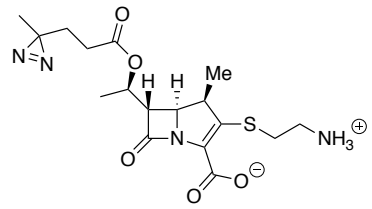


Applying procedure found in literature:[8] protected methyl-thienamycin[3] (100 mg, 0.17 mmol) and 3-(3-methyl-3*H*-diazirin-3-yl)propanoic acid[9] (90 mg, 0.67 mmol) were mixed in dry DCM (5 mL) in presence of DMAP (20 mg, 0.15 mmol) and EDC (260 mg, 1.34 mmol) at – 20 ˚C. The reaction mixture was stirred for 2 hours between – 10 to – 20 ˚C. After completion of the reaction (monitored by TLC), the crude mixture was dissolved in EtOAc (50 mL) and washed with a sat. solution of $NaHCO_3$ (50 mL), H_2O (50 mL), brine (50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude solid was purified by silica gel chromatography (cyclohexane:EtOAc 2:8) to afford compound **14** as a yellowish foam (111

[8] Singh, S. B.; Rindgen, D.; Bradley, P.; Suzuki, T.; Wang, N.; Wu, H.; Zhang, B.; Wang, L.; Ji, C.; Yu, H.; Soll, R. M.; Olsen, D. B.; Meinke, P. T.; Nicoll-Griffith, D. A. *J. Med. Chem.* **2014**, *57*, 8421–8444.
[9] Ahad, A. M.; Jensen, S. M.; Jewett, J. C. *Org. Lett.* **2013**, *15*, 5060–5063.

mg, 92%). **^{1}H NMR (500 MHz, $CDCl_3$):** δ 8.24 – 8.21 (m, 4H), 7.65 (d, J = 8.7 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 5.50 (d, J = 13.7 Hz, 1H), 5.34 – 5.27 (m, 1H), 5.25 (d, J = 13.7 Hz, 1H), 5.20 (s, 2H), 4.20 (dd, J = 9.2, 2.6 Hz, 1H), 3.54 – 3.45 (m, 2H), 3.45 – 3.33 (m, 2H), 3.13 – 3.06 (m, 1H), 2.94 – 2.89 (m, 1H), 2.18 – 2.15 (m, 2H), 1.75 (dt, J = 7.4, 1.6 Hz, 2H), 1.42 (d, J = 6.3 Hz, 3H), 1.25 (d, J = 7.1 Hz, 4H), 1.03 (s, 3H). **^{13}C NMR (126 MHz, $CDCl_3$):** δ 171.5, 170.9, 160.4, 155.9, 151.3, 147.7 (2C), 143.8, 143.0, 128.2 (4C), 124.5, 123.9 (4C), 68.9, 65.4, 65.4, 60.5, 56.9, 43.2, 41.3, 31.4, 29.5, 29.0, 25.3, 21.1, 16.8, 14.3. **HRMS (ESI):** *m/z* calcd for $C_{32}H_{34}N_6NaO_{11}S$ $[M + Na]^+$ 733.1898; found: 733.1886.

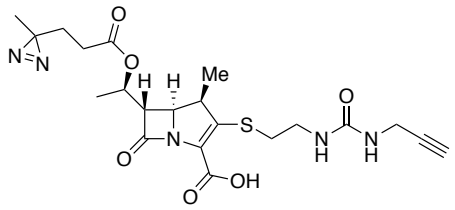
(4*R*,5*S*,6*S*)-3-((2-Ammonioethyl)thio)-4-methyl-6-((*R*)-1-((3-(3-methyl-3*H*-diazirin-3-yl)propanoyl)oxy)ethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (15)



15

Compound **14** (195 mg, 0.3 mmol) was dissolved in a mixture of THF:triethylammonium bicarbonate (1 M, pH = 8.4) (2:1) and treated with 10% wt. Pt/C (195 mg, 1 equiv. mass). The reaction mixture was stirred under 1 atm of H_2 at room temperature for 1 hour. THF was removed under reduced pressure and the crude mixture was dissolved in H_2O (2 mL), filtered through a 0.2 µm PTFE syringe filter and purified by HPLC (H_2O:CH_3CN, linear gradient, 50% of CH_3CN over 30 minutes). The appropriate fractions were collected and lyophilized to afford compound **15** as a yellowish foam (30 mg, 25%). **^{1}H NMR (500 MHz, D_2O):** δ 5.39 – 5.33 (m, 1H), 4.34 (dd, J = 9.4, 2.8 Hz, 1H), 3.74 (dd, J = 5.2, 2.8 Hz, 1H), 3.47 – 3.41 (m, 1H), 3.37 – 3.32 (m, 1H), 3.31 – 3.15 (m, 2H), 3.02 – 2.96 (m, 1H), 2.40 – 2.37 (m, 2H), 1.78 (t, J = 7.3 Hz, 2H), 1.41 (d, J = 6.4 Hz, 3H), 1.26 (d, J = 7.1 Hz, 3H), 1.07 (s, 3H). **HRMS (ESI):** *m/z* calcd for $C_{17}H_{25}N_4O_5S$ $[M + H]^+$ 397.1540; found: 397.1534. **HPLC purity:** 93%. **HPLC t_R:** 17.4 min.

(4*R*,5*S*,6*S*)-4-Methyl-6-((*R*)-1-((3-(3-methyl-3*H*-diazirin-3-yl)propanoyl)oxy)ethyl)-7-oxo-3-((2-(3-(prop-2-yn-1-yl)ureido)ethyl)thio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (CBA-6)

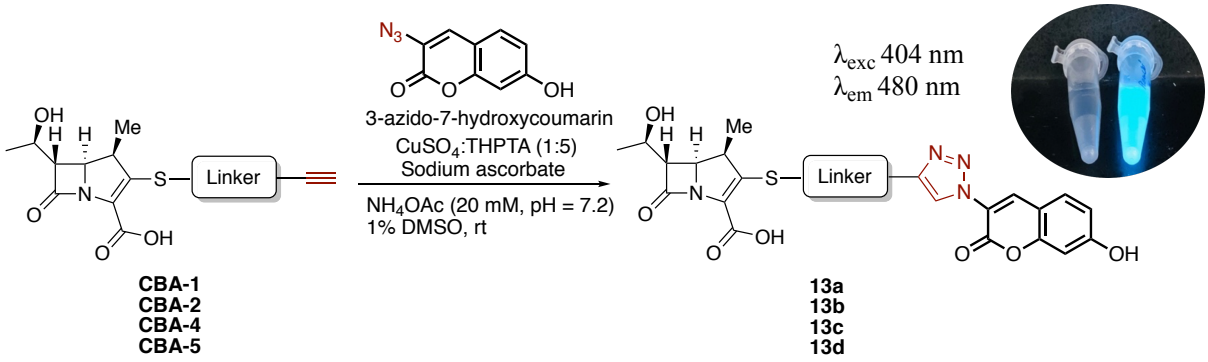


CBA-6

To a solution of compound **15** (2 mg, 0.005 mmol) in DMF (500 µL), sodium bicarbonate (5 mg, 0.006 mmol) and *N*-(prop-2-yn-1-yl)-1*H*-imidazole-1-carboxamide[3] (11 mg, 0.008 mmol) were added. The mixture was stirred for 30 minutes at 25 ˚C. The reaction mixture was dissolved in water (2 mL) and filtrated through a PTFE 0.2 µm syringe filter. Purification by HPLC (H_2O:CH_3CN; linear gradient, 50% of CH_3CN over 30 minutes) afforded the corresponding carbapenem **CBA-6** as a yellowish foam (1.5 mg, 63%). **^{1}H NMR (500 MHz, D_2O):** δ 5.38 – 5.34 (m, 1H), 4.29 (dd, J = 9.1, 2.7 Hz, 1H), 3.93 – 3.91 (m, 2H), 3.69 (dd, J = 5.2, 2.6 Hz, 1H), 3.54 – 3.43 (m, 2H), 3.36 – 3.30 (m, 1H), 3.13 – 3.07 (m, 1H), 2.85 – 2.80 (m, 1H), 2.64 (t, J = 2.5 Hz, 1H), 2.41 – 2.37 (m, 2H), 1.79 (t, J = 7.3 Hz, 2H), 1.42 (d, J = 6.4 Hz, 3H), 1.22 (d, J = 7.1 Hz, 3H), 1.08 (s, 3H). **HRMS (ESI):** *m/z* calcd for $C_{21}H_{28}N_5O_7S$ $[M + H]^+$ 478.1754; found: 478,1755. **HPLC purity:** 91%. **HPLC t_R:** 19.8 min.

6. Reactivity of CBAs in the CuAAC reaction

6-1. Kinetic studies based on a fluorescent assay



Scheme S8. The CuAAC reaction of **CBA-1**, **CBA-2**, **CBA-4**, and **CBA-5** with fluorogenic 3-azido-7-hydroxycoumarin[7] afforded fluorescent carbapenems **13a-13d**, respectively**.**

The CuAAC reaction was performed with each of the **CBA** carbapenem (100 µM), 3-azido-7-hydroxycoumarin (120 µM), $CuSO_4$:THPTA (25 µM:125 µM), and 1% DMSO in ammonium acetate (20 mM, pH 7.2) (final volume of 150 µl). Sodium ascorbate was added (750 µM; final volume 150 µl) and fluorescence was measured (Carry Eclipse; λ excitation = 404 nm; λ emission = 480 nm; slit = 5 nm, photomultiplier tube voltage = 550 V) every 30 seconds for one hour. The rate of formation of fluorescent carbapenems **13a-13d** was determined using calibration curves obtained with compounds purified by HPLC.

6-2. HPLC analysis of the products of the CuAAC reaction

The reagents and products of the CuAAC reaction were separated by HPLC on a C_{18} column (Vydac 250 x 4.6 mm; 5 µm) at a flow rate of 1.3 $mL.min^{-1}$ (Solvent A: 0.1% TFA; Solvent B: CH_3CN, 0.1% TFA). The gradient (0 to 100%) was applied for 30 minutes. The relative area of the **CBA-1**, **CBA-2**, **CBA-4,** and **CBA-5** peaks (λ = 299 nm) at 0 and 30 min was used to estimate the percentage of conversion.

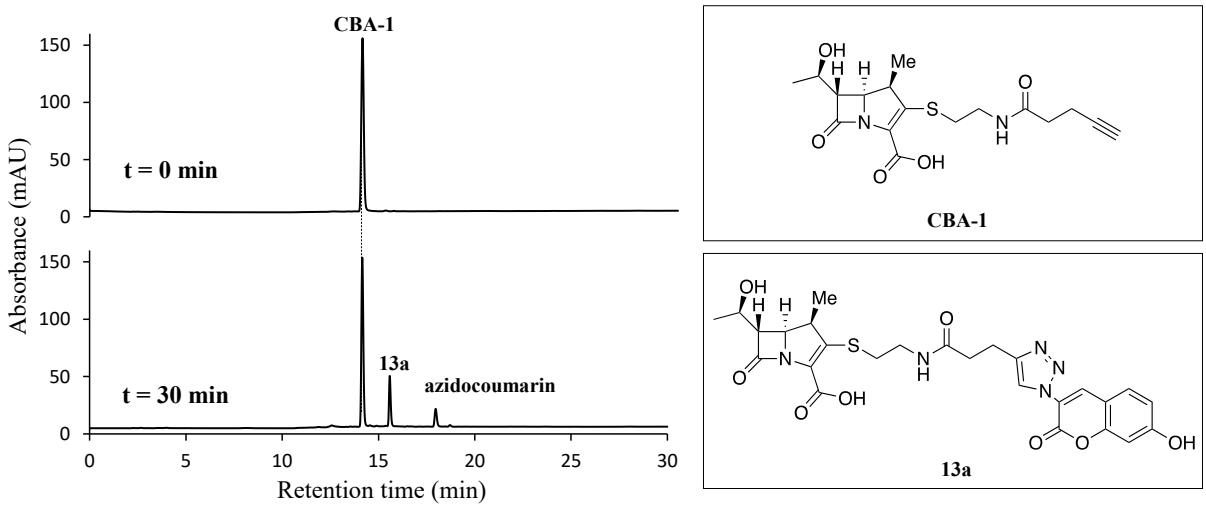


Figure S1. HPLC analysis of the reagents and products of the CuAAC reaction performed with **CBA-1** and 3-azido-7-hydroxycoumarin.

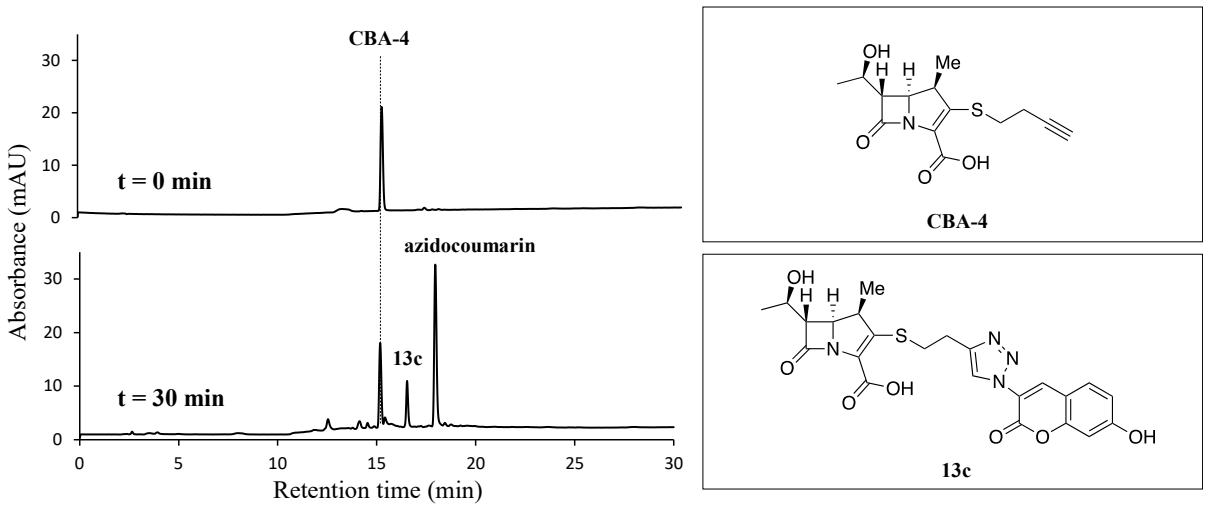


Figure S2. HPLC analysis of the reagents and products of the CuAAC reaction performed with **CBA-4** and 3-azido-7-hydroxycoumarin.

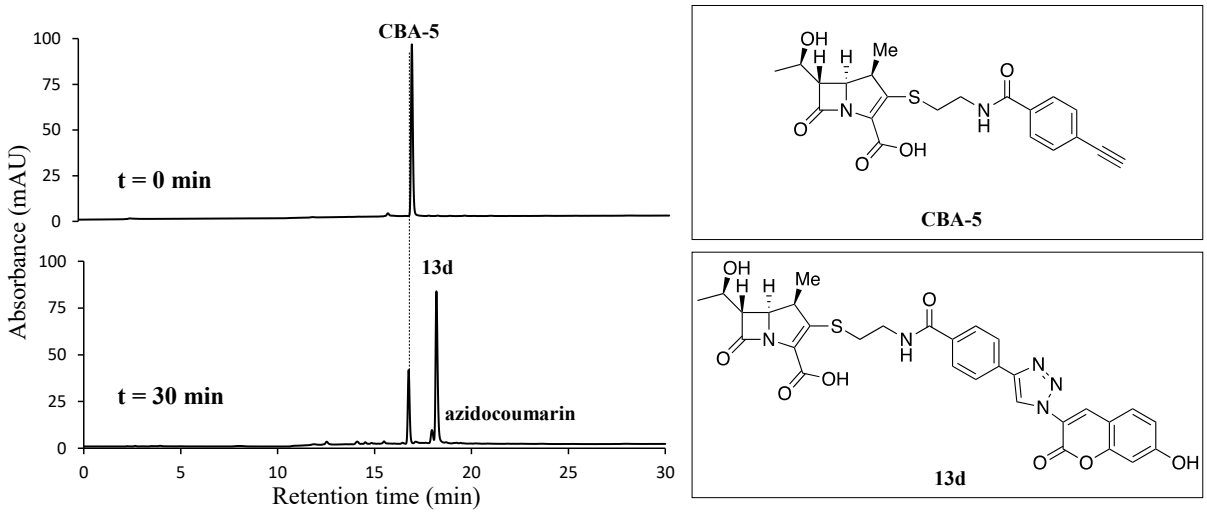


Figure S3. HPLC analysis of the reagents and products of the CuAAC reaction performed with **CBA-5** and 3-azido-7-hydroxycoumarin.

7. Purification of Ldt_{fm} and kinetic studies

7-1 Protein purification

A soluble fragment of the Ldt_{fm} L,D-transpeptidase (residues 341 to 466) was produced in *E. coli* BL21(DE3) (Invitrogen) harboring plasmid pET-TEVΩ*ldt*$_{fm}$[10]. Bacteria were grown in brain-heart infusion broth (BHI; Difco) containing kanamycin (50 mg.L^{-1}). Ldt_{fm} was purified from clarified lysates by metal-affinity chromatography in 100 mM sodium phosphate (pH 6.4) containing NaCl 300 mM followed by size-exclusion chromatography in 50 mM ammonium acetate (pH 6.4). The purified enzyme was concentrated by ultrafiltration (Amicon Ultra-4 centrifugal filter devices, Millipore) and stored at -65 °C in the same buffer. The Ldt_{fm} derivative harboring the $C^{442}A$ substitution was prepared by using the same procedures.

7-2. Acylation assays with CBAs monitored by mass spectrometry

Ldt_{fm} (6.5 µM) was incubated with carbapenems (39 µM) in ammonium acetate buffer (20 mM, pH = 7.2) for 15 min at 25 ˚C. Excess carbapenem was removed by gel filtration (G25 MicroSpin™ column; GE healthcare). The eluate was diluted 10 fold in the same buffer and 2.5 µL were injected in the mass spectrometer (LCQ DECA XP-Max, Thermo Scientific) at a flow rate of 10 µL.min^{-1} (50% ACN; 0.1% acetic acid). Mass spectra were acquired in the

[10] S. Triboulet, V. Dubee, L. Lecoq, C. Bougault, J. L. Mainardi, L. B. Rice, M. Etheve-Quelquejeu, L. Gutmann, A. Marie, L. Dubost, J. E. Hugonnet, J. P. Simorre, M. Arthur *PLoS One* **2013**, *8*, e67831.

positive mode. The observed mass was the mean ± standard deviation deduced from the *m*/*z* values obtained for nine charge states (*z* = 20 to 28). The Ldt_{fm}-**CBA-6** adduct generated by photoactivation was further characterized by mass spectrometry on a Bruker Daltonics maXis high-resolution mass spectrometer (Bremen, Germany). The acquisition was performed in the profile mode over a 500-2200 *m*/*z* range. The data were converted to the standard mzML format[11] in order to be scrutinized using the mineXpert software.[12] The mass calculations were performed in massXpert.[13] Protein average masses were determined manually by looking at the isotopic clusters. The M_r of the Ldt_{fm}-**CBA-6** adduct was determined to be 29,458.3 Da using two consecutive charge state envelope peaks (*m*/*z* values of 819.297 and 842.679, with 36 and 35 charges, respectively). Over eight pairs of the best-shaped protein peaks, the measured mass of the adduct was found to be 29,458.3 ± 0.2 Da.

Table S1. Mass spectrometry analysis of Ldt_{fm}-carbapenem acyl-enzymes

Carbapenems[a]	**Calculated mass (Da)**	**Observed mass (Da)**[b]
None (NA)	29,008.9	29,011.5 ± 2.3
Meropenem (383.5 Da)	29,392.4	29,394.3 ± 1.6
CBA-1 (366.4 Da)	29,375.3	29,378.2 ± 2.5
CBA-2 (367.4 Da)	29,376.3	29,376.3 ± 0.2
CBA-3 (419.5 Da)	29,428.4	29,432.3 ± 3.6
CBA-4 (295.4 Da)	29,304.3	29,305.3 ± 1.0
CBA-5 (414.5 Da)	29,423.4	29,425.6 ± 1.8
CBA-6 (477.5 Da)	29,486.4	29,489.1 ± 2.3
CBA-6 + h*ν*[c] (449.5 Da)	29,458.5	29,458.3 ± 0.2

[a]The expected mass increment upon Ldt_{fm} acylation is indicated in parentheses. NA, not applicable.

[b]Determined by low-resolution mass spectrometry on an LCQ Deca XP-Max mass spectrometer except for **CBA-2** and the irradiated **CBA-6** adducts, which were analysed on a high-resolution Bruker Daltonics maXis mass spectrometer.

[c]Photoactivation of the diazirine of **CBA-6** led to the loss of 2 nitrogen atoms (-28.0 Da).

[11] Adusumilli, R.; Mallick, P. Data Conversion with ProteoWizard MsConvert. In *Proteomics: Methods and Protocols*; Comai, L., Katz, J. E., Mallick, P., Eds.; Methods in Molecular Biology; Springer: New York, NY, 2017; pp 339–368.
[12] Rusconi, F. *J. Proteome Res.* **2019**, *18*, 2254–2259.
[13] Rusconi, F. *Bioinformatics* **2009**, *25*, 2741–2742.

7-3. Stability of Ldt_{fm}-CBA adducts monitored by mass spectrometry

Ldt_{fm}-**CBA** adducts purified by gel filtration (above) were incubated at room temperature for 0, 1, and 3 h in the elution buffer, diluted 10 fold in the same buffer, and 2.5 µL of this solution were analyzed by mass spectrometry as described above. The relative abundance of the Ldt_{fm}-CBA adducts and of Ldt_{fm} was estimated from the intensity of peaks with nine charge states (*z* = 20 to 28). The error bars represent the standard deviation of these nine ratios.

7-4. Determination of kinetic constants for inactivation of Ldtfm

Acylation of Ldt_{fm} (10 µM) by meropenem, **CBA-2**, and **CBA-6** (three concentrations for each carbapenem, 50, 100, and 200 µM) was detected by stopped-flow spectrofluorometry (λ_{ex} = 224 nm, λ_{em} = 335 nm) at 20 °C in 20 mM ammonium acetate buffer (pH 7.2). The kinetic constants were calculated as previously described.[14]3/11/21 4:54:00 PM

7-5. Determination of the stability of Ldt_{fm}-CBA adducts by spectrophotometry

Meropenem, **CBA-2**, and **CBA-6** (200 µM) were incubated with Ldt_{fm} (0 and 10 µM) at 20 °C in 20 mM ammonium acetate buffer (pH 7.2) for 1,000 minutes. Hydrolysis of the carbapenems was detected by spectrophotometry at 299 nm.

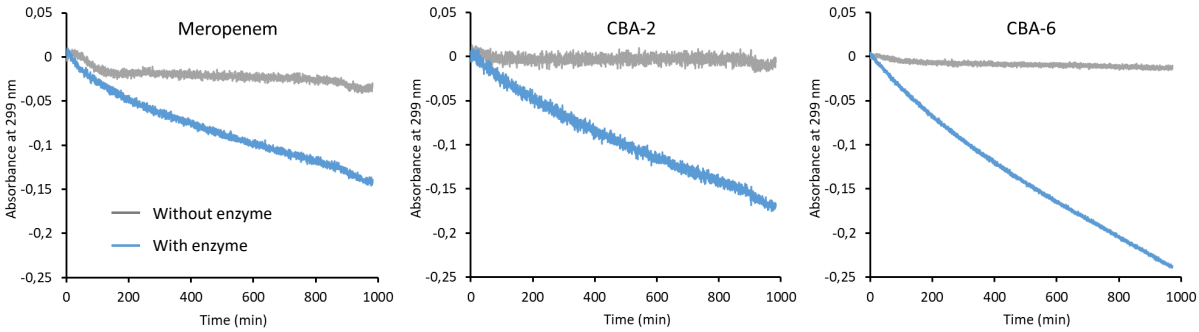


Figure S4. Kinetics of carbapenem hydrolysis in absence or presence of Ldt_{fm}.

8. Capture and release experiments

8-1. Conditions for the four steps of the capture and release procedure

***Step 1* – Acylation of Ldt_{fm} by CBA-2.** Ldt_{fm} (6.5 µM) was incubated with **CBA-2** (39 µM) in ammonium acetate buffer (20 mM, pH 7.2) for 15 min at 25 ˚C. Excess carbapenem was removed by gel filtration (G25 MicroSpin™ column; GE Healthcare). The appropriate fraction

[14] Triboulet, S.; Edoo, Z.; Compain, F.; Ourghanlian, C.; Dupuis, A.; Dubée, V.; Sutterlin, L.; Atze, H.; Etheve-Quelquejeu, M.; Hugonnet, J.-E.; Arthur, M. *ACS Infect. Dis.* **2019**, *5*, 1169–1176.

was collected (100 µL), analyzed by mass spectrometry, and protein concentration was determined by the absorbance at 280 nm.

***Step 2* – Copper-catalyzed alkyne-azide cycloaddition (CuAAC).** The eluate of step 1 (90 µL, 5 µM) was mixed with the trifunctional probe (54 µM, 10.8 equiv.), THPTA (6.75 mM), $CuSO_4$ (1.35 mM), and freshly prepared sodium ascorbate (10 mM) in ammonium acetate buffer (20 mM, pH 7.2) (V_t = 120 µL). The reaction mixture was gently shaken at 25 ˚C for 90 min. The excess of reagents was removed by gel filtration (G25 MicroSpin™ column; GE Healthcare).

***Step 3* – Capture of the Ldt_{fm}-platform adduct on streptavidin beads.** The eluate from step 2 was mixed with 1 mg of streptavidin beads (Dynabeads™ M-280; Invitrogene) equilibrated with ammonium acetate (20 mM, pH 7.2). The mixture was incubated at 25 ˚C for 15 minutes with gentle shaking (180 rpm). Beads were washed three times with 1 M NaCl (3 x 100 µL), three times with ammonium acetate (20 mM, pH 7.2) containing 0.1% Tween® 20 (3 x 100 µL), and three times with ammonium acetate (20 mM, pH 7.2) (3 x 100 µL).

***Step 4* – Elution by the click and release reaction.** Washed beads (from Step 3) were suspended in 39 µL of ammonium acetate buffer (20 mM, pH 7.2), 1 µL of a 20 mM solution of DBCO-TAMRA was added, and the suspension was incubated for 90 minutes at 25 ˚C with gentle shaking (180 rpm). The supernatant was collected (40 µl), the beads were washed with 10 µL of ammonium acetate buffer (20 mM, pH 7.2), and the two supernatants were combined (50 µL). The eluate was concentrated under reduced pressure (SpeedVac; Savant) to a final volume of 10 µL.

***Step 5* – SDS-PAGE analysis.** Two µL of Laemmli buffer (Bio-Rad) and 2 µL of glycerol were added to the eluate form step 4 (10 µl). The sample was loaded onto a 16% polyacrylamide gel and electrophoresis was performed at 200 V for 90 minutes. Protein bands were detected by fluorescence (Transilluinator; Herolab UV-B 8 watt) followed by Coomassie blue staining.

8-2. Capture and release of Ldt_{fm} added to a bacterial lysate

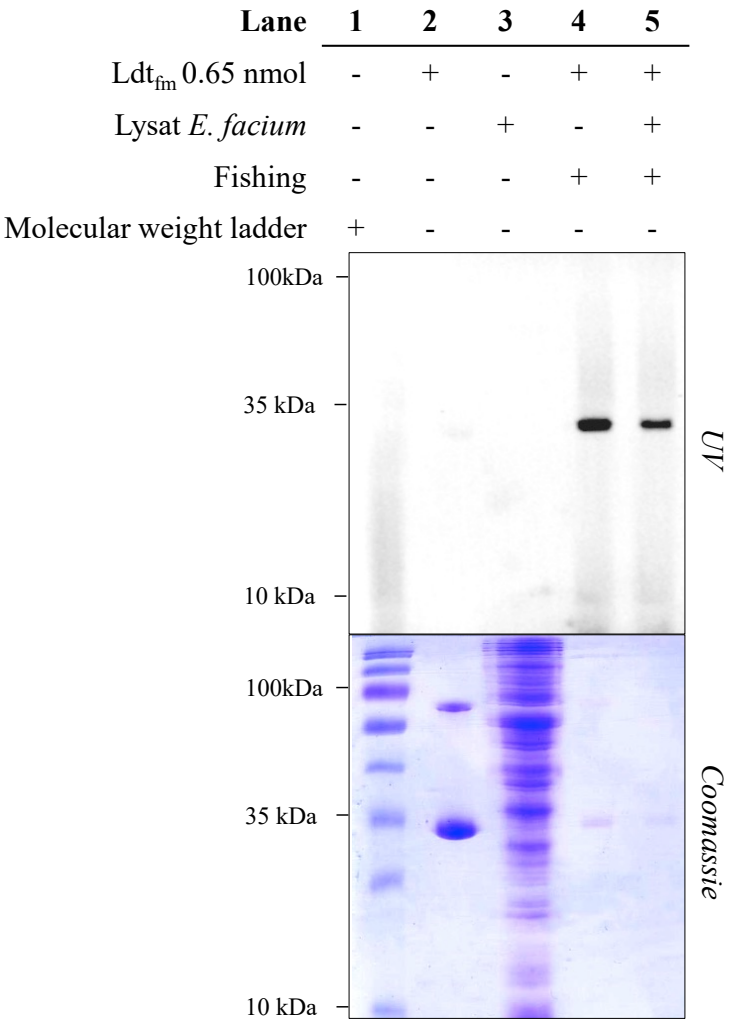


Figure S5. Assessing the specificity of the capture and release reactions in the presence of whole protein extract from *E. faecium.* Soluble proteins from *E. faecium* M512 were prepared by sonication of bacterial cells followed by centrifugation to remove cell debris. The protein concentration was determined by the Bio-Rad assay using bovine serum albumin as a standard.

8-3. Capture and release of Ldt_{fm} using photoactivable carbapenem CBA-6

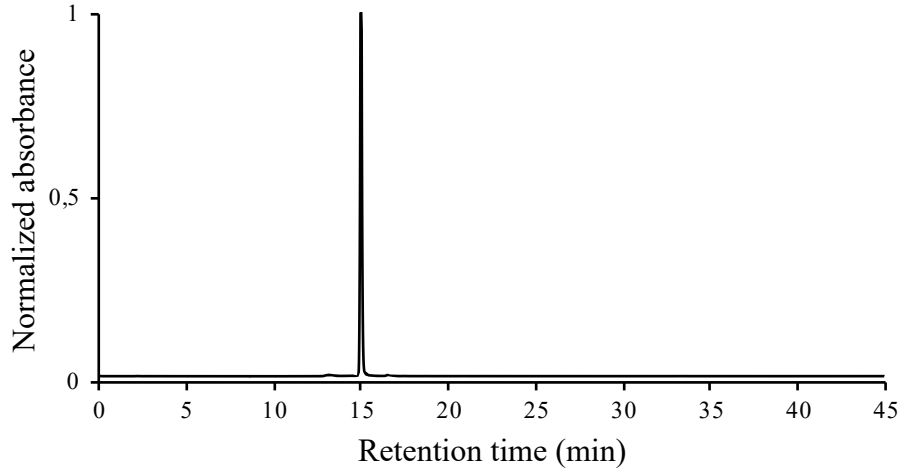
An additional irradiation step was performed between Step 1 and Step 2 of the general procedure described above and in Figure 2 of the main text. For this additional step, the Ldt_{fm}-CBA-6 adduct was placed at the surface of a parafilm deposited on ice. Irradiation was performed in the dark for 30 minutes with a UVGL-58 handheld UV lamp (6W; λ = 365 nm; UVP Cambridge UK) placed at a distance of 1 cm from the sample.

9. Analytical data

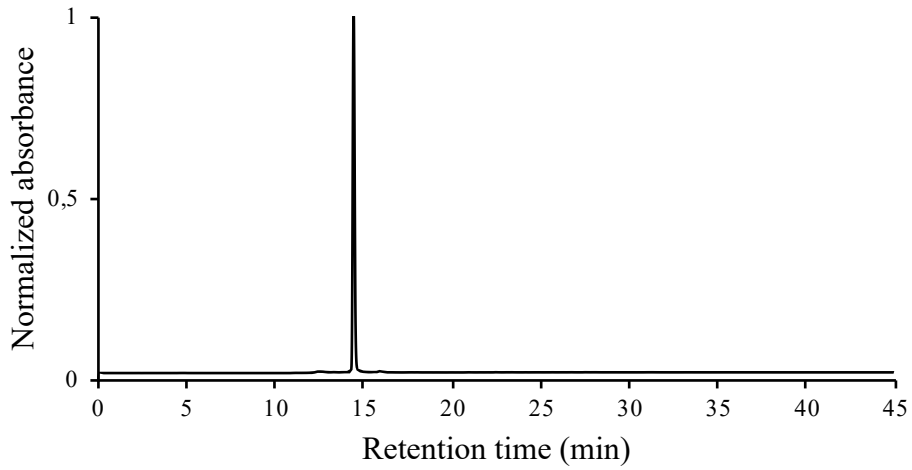
9-1. Analytical HPLC spectra

Analytical HPLC was performed on a Vydac C_{18} (250 mm x 4.6 mm, 5 µm). Detection at 299 nm, flow 1.3 mL.min^{-1}. **Solvent A:** H_2O + 0.1% TFA; **Solvent B:** ACN + 0.1% TFA. Gradient 0 to 100% of Solvent B over 45 minutes.

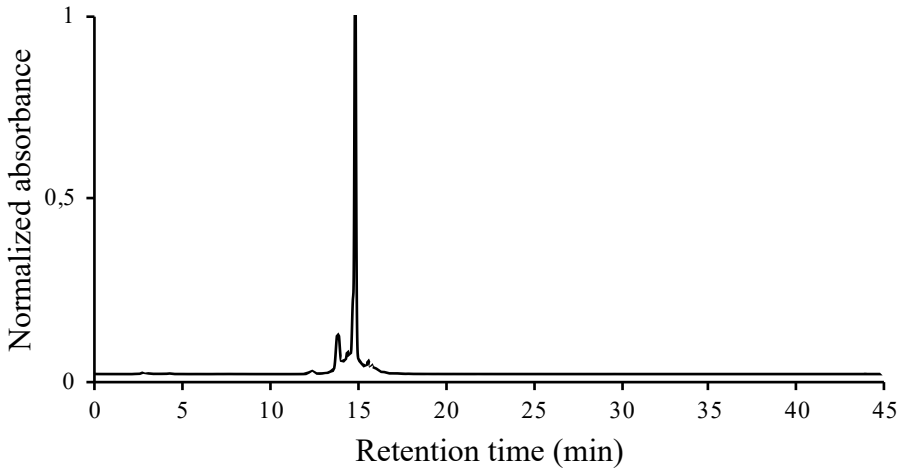
CBA-1



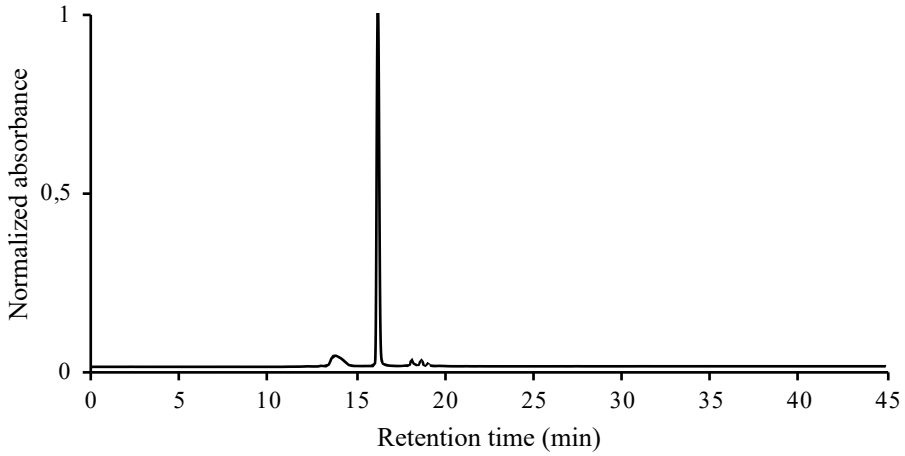
CBA-2



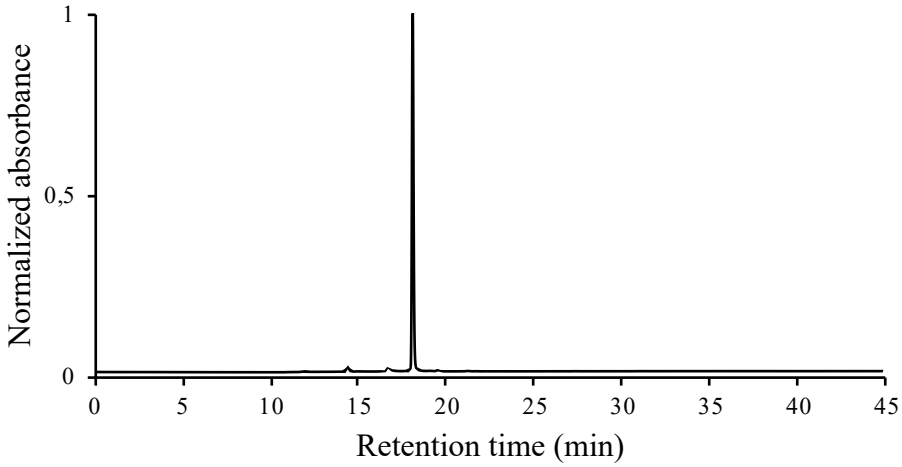
CBA-3



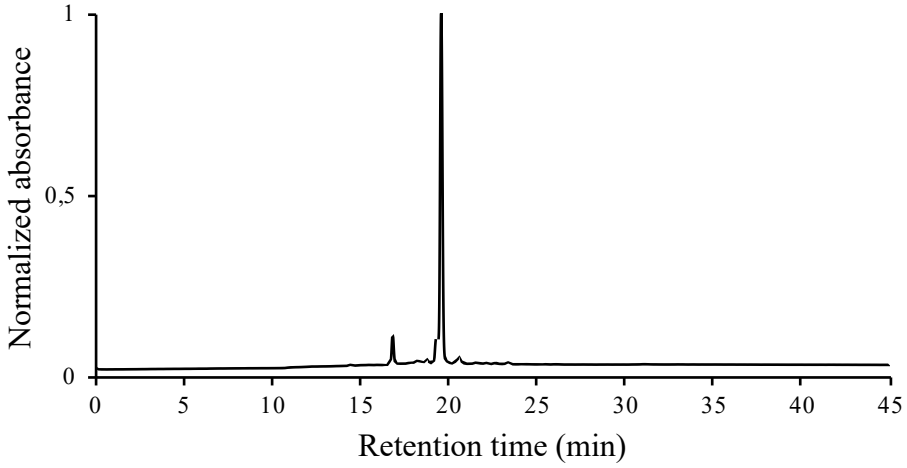
CBA-4



CBA-5



CBA-6


Normalized absorbance
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0,5
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5
10
15
20
25
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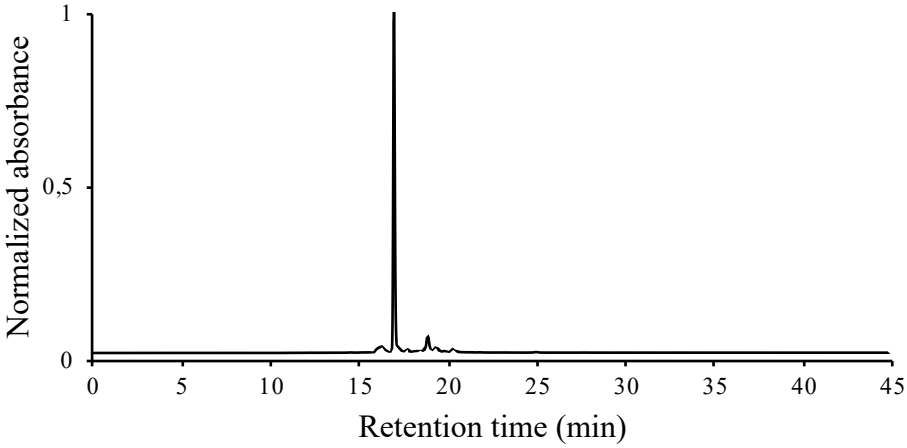
45
Retention time (min)

13a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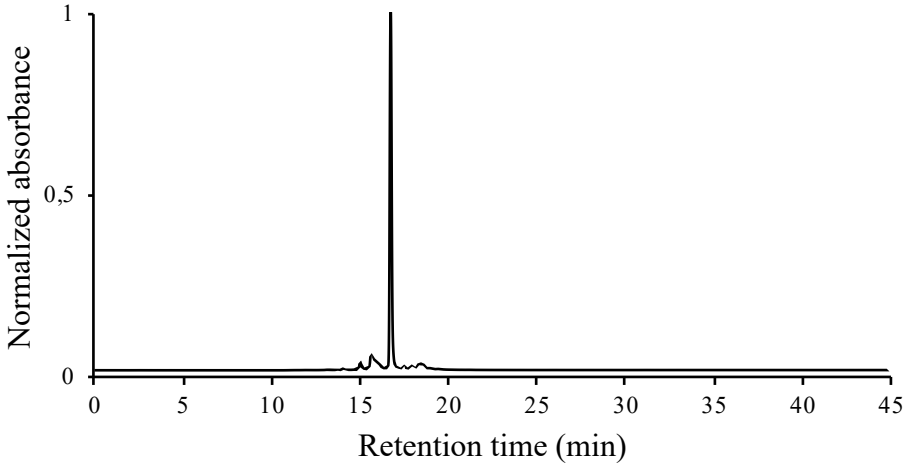
Retention time (min)

13b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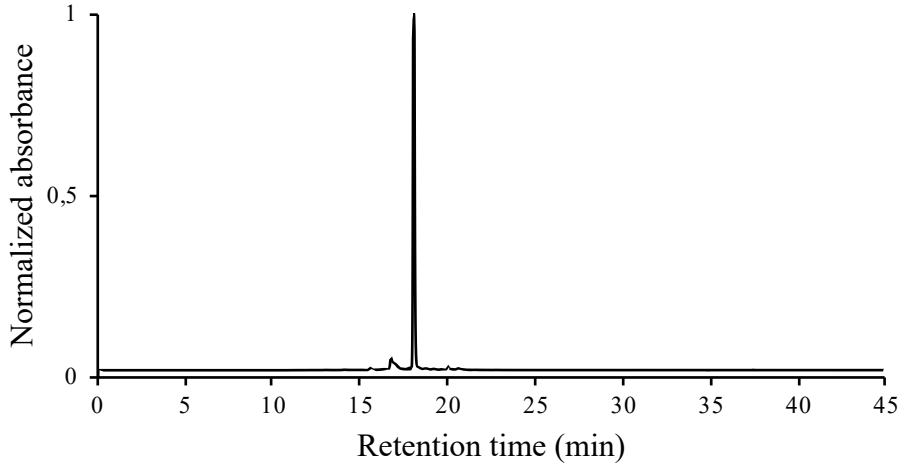
0
5
10
15
20
25
30
35
40
45
Retention time (min)

13c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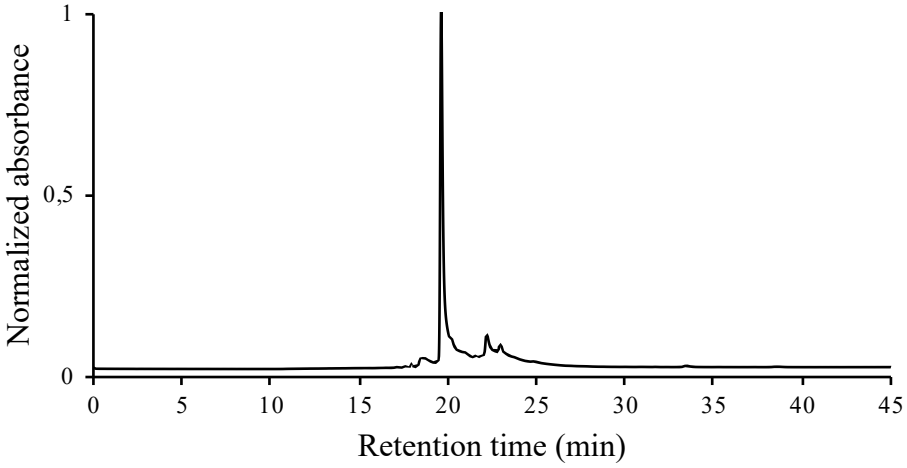
Retention time (min)

13d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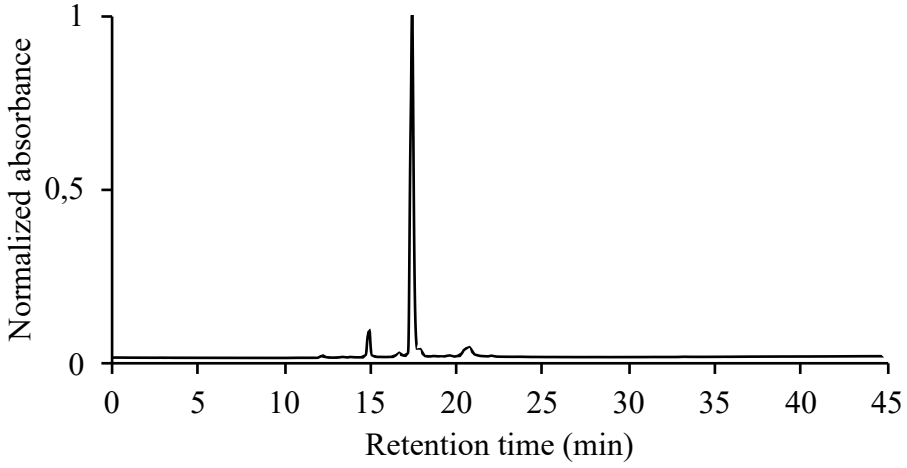
Retention time (min)

15


Normalized absorbance
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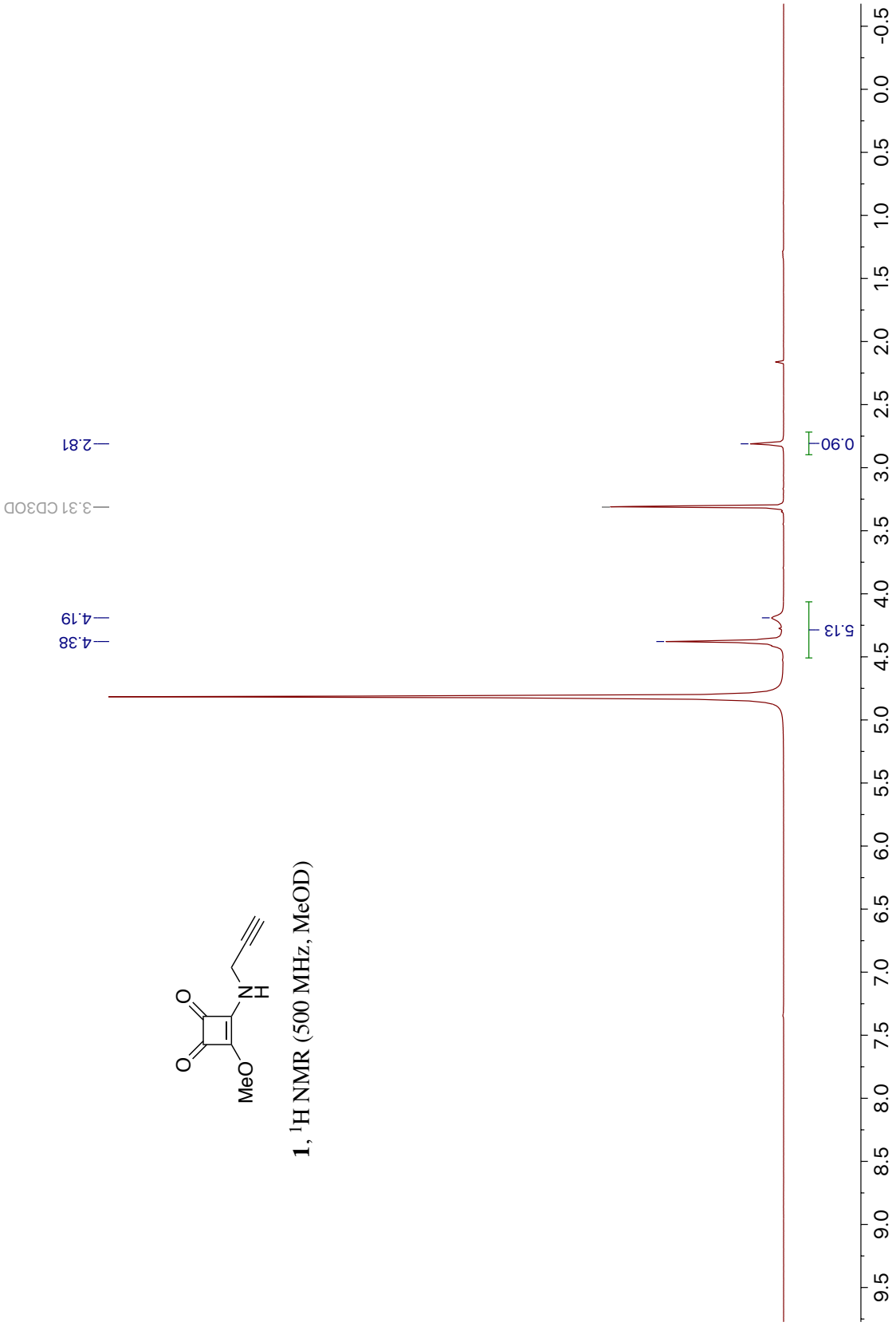
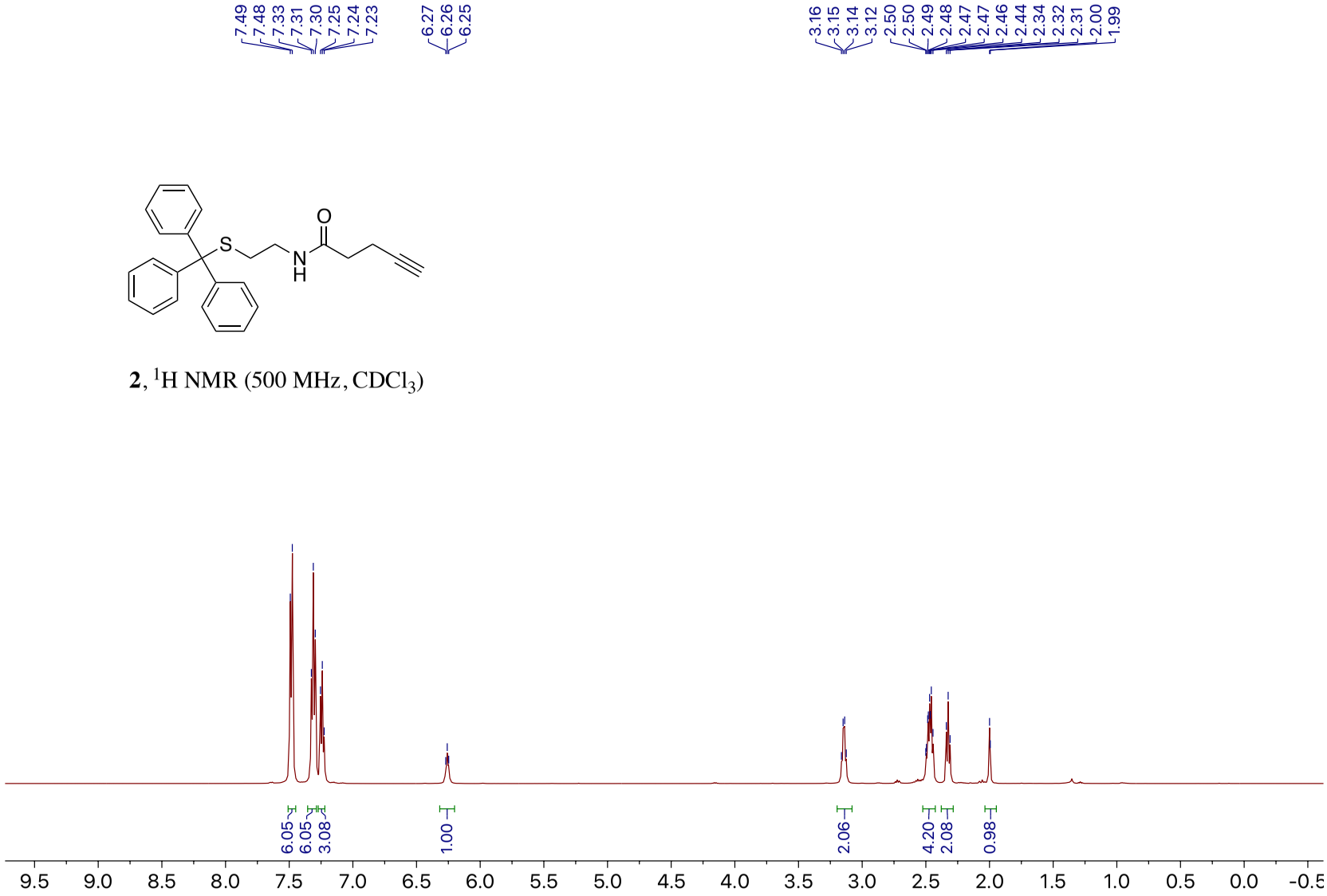
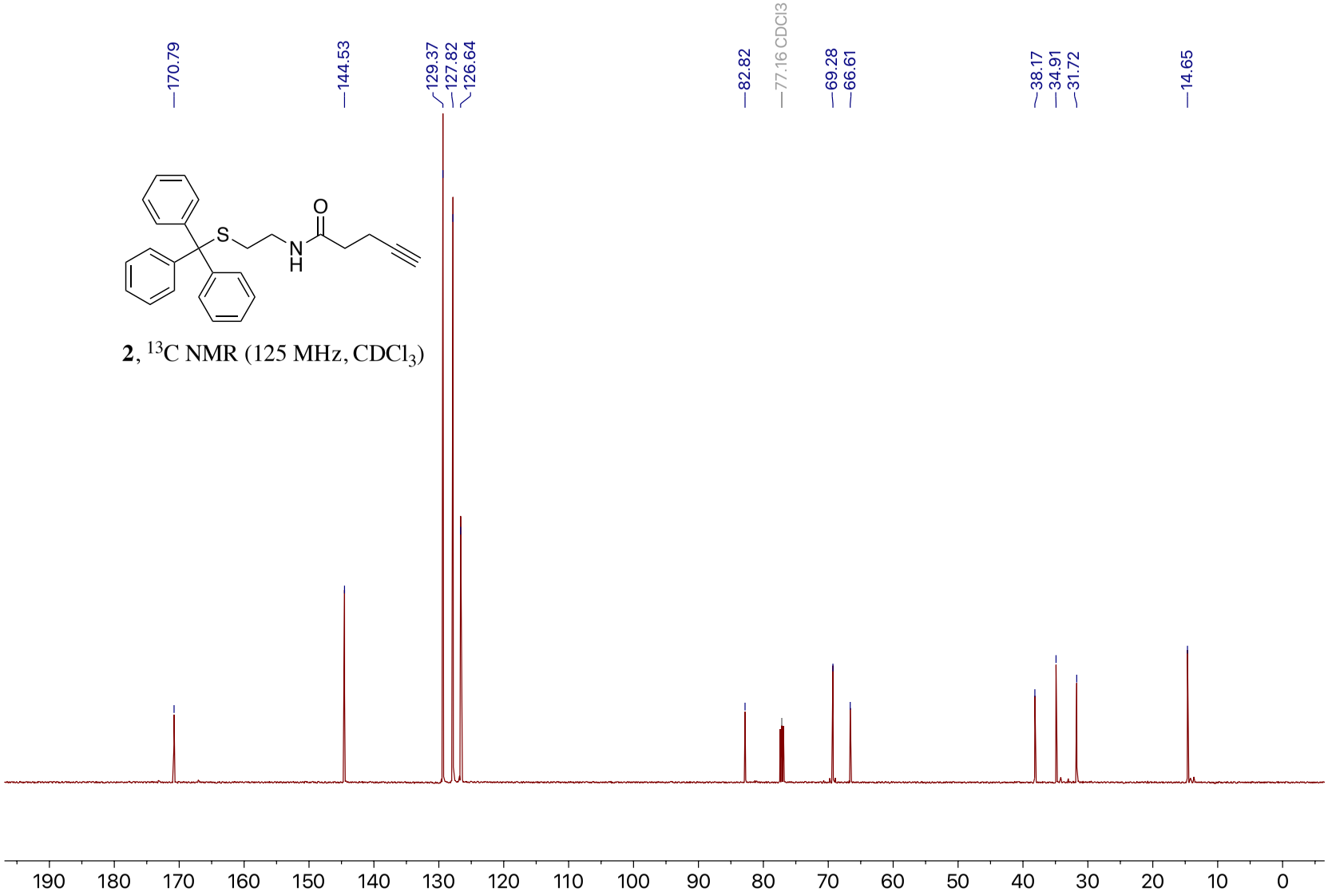
Retention time (min)

9-2. ^{1}H NMR & ^{13}C NMR spectra of new compounds

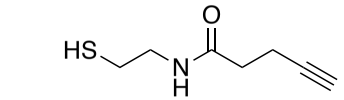




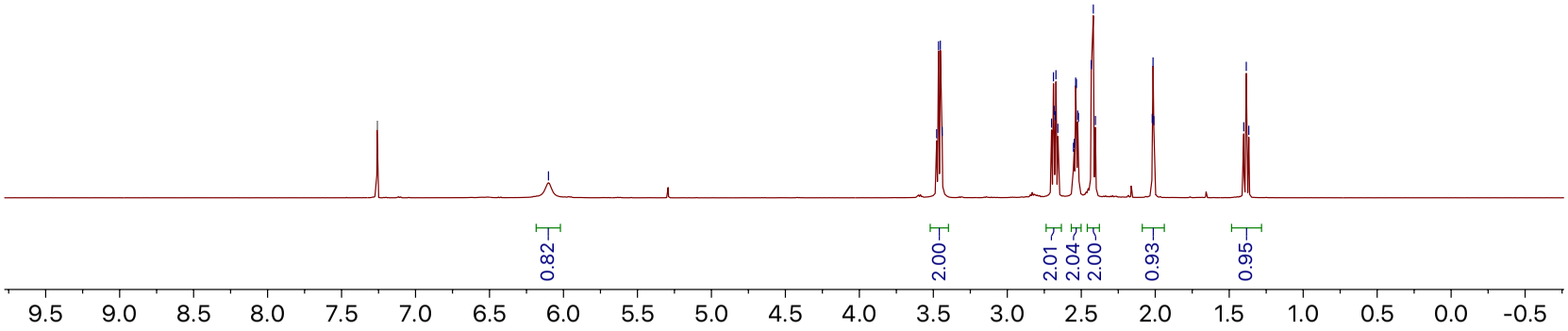
2, ^{1}H NMR (500 MHz, $CDCl_3$)

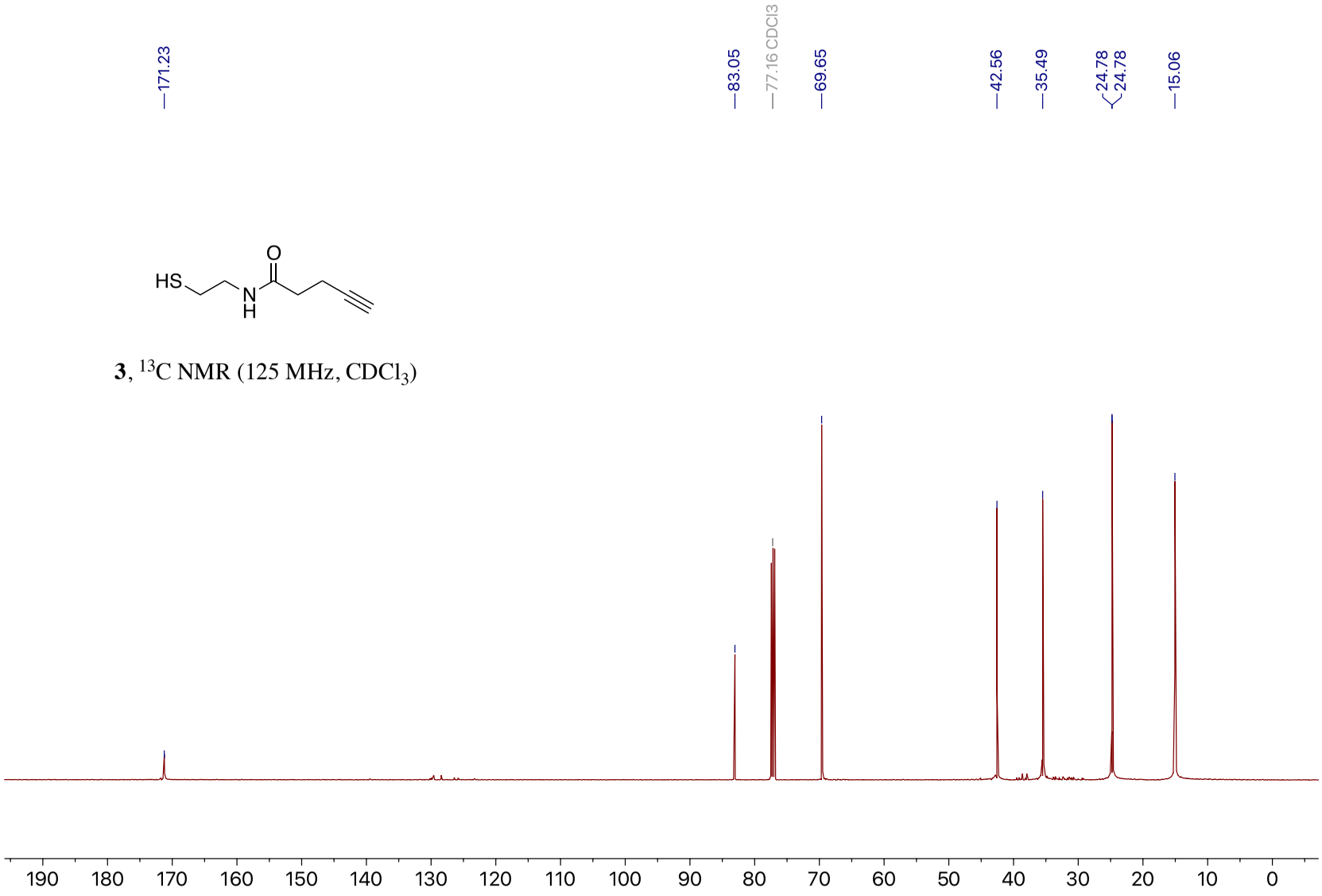


2, ^{13}C NMR (125 MHz, $CDCl_3$)



3, ^{1}H NMR (500 MHz, $CDCl_3$)





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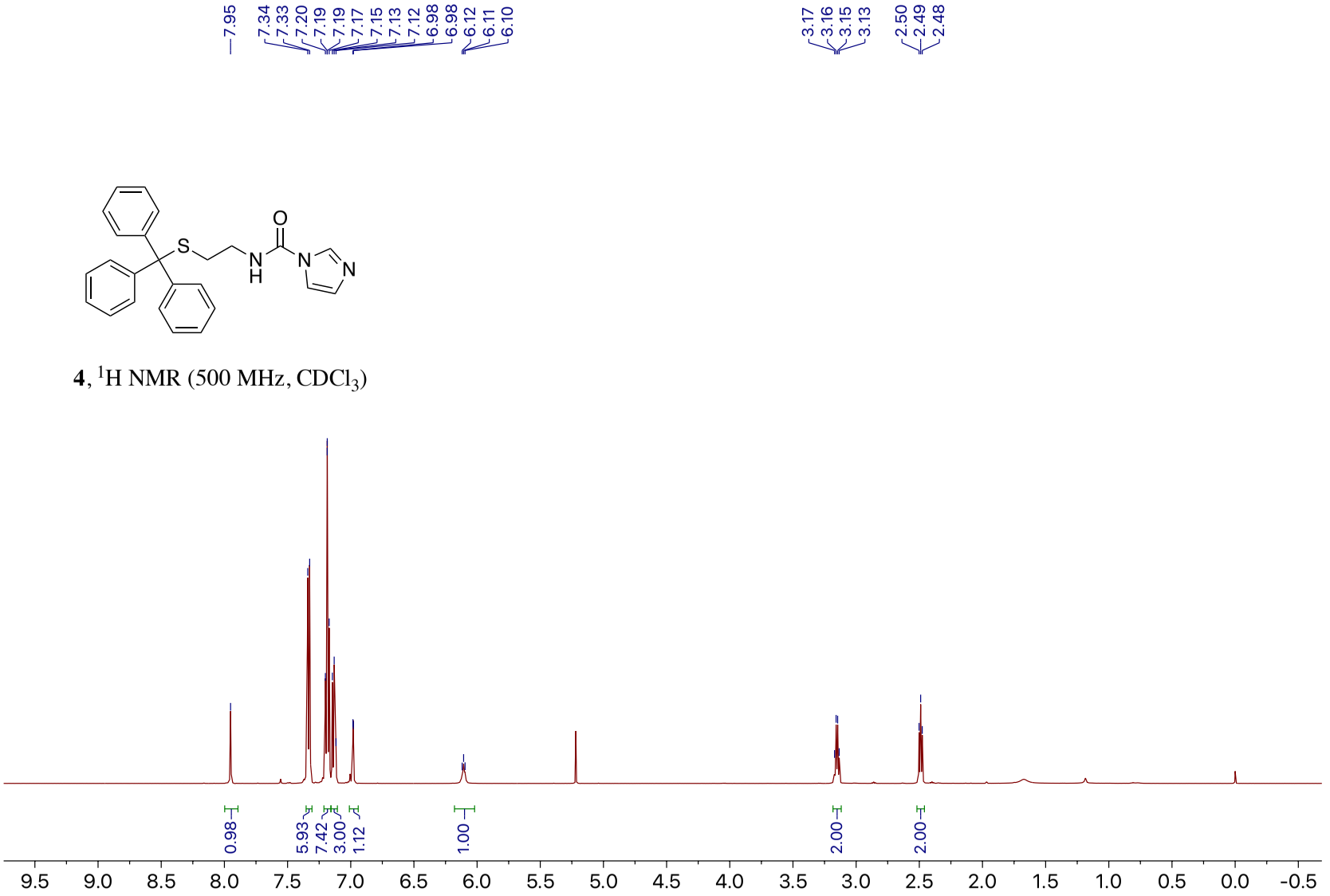
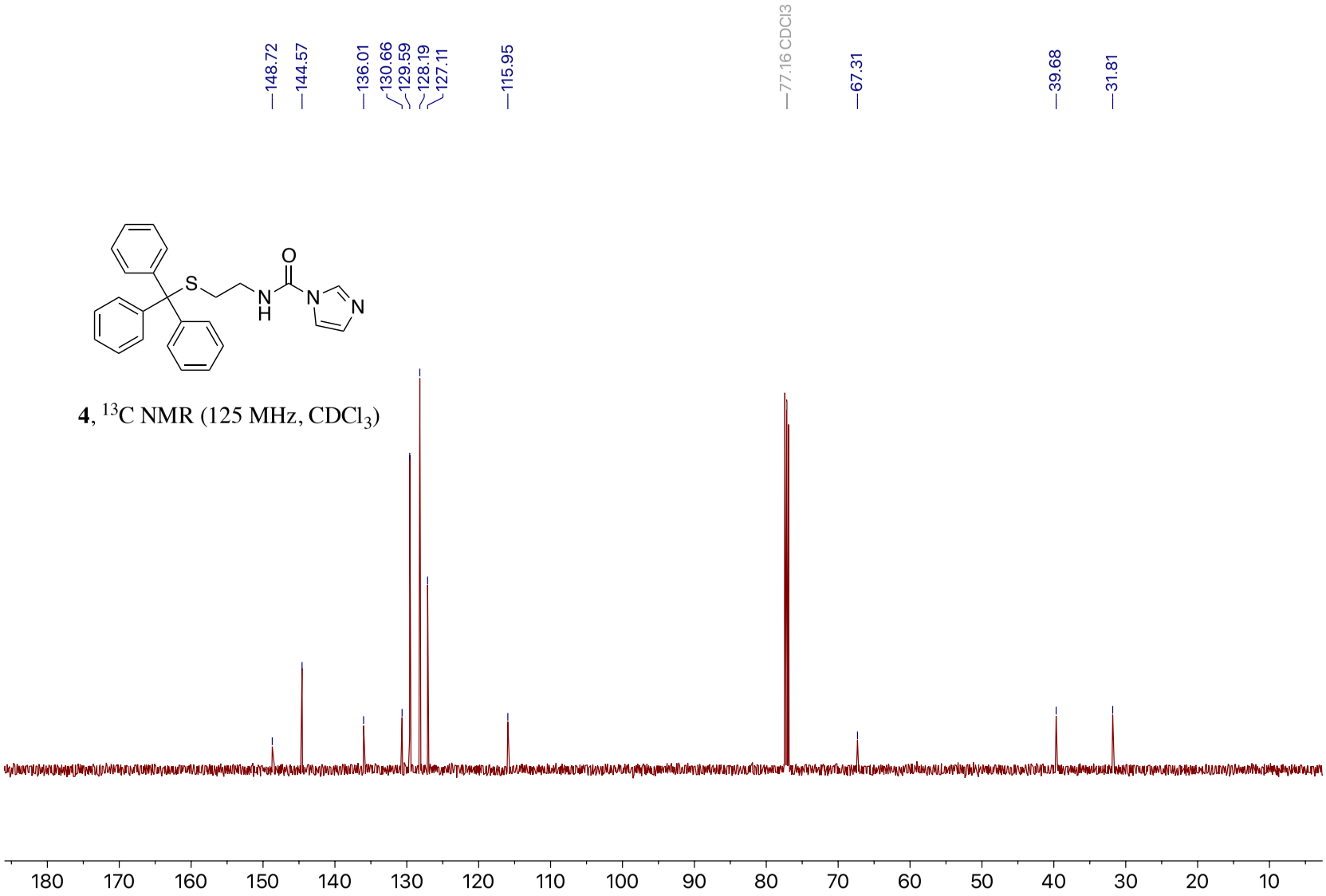
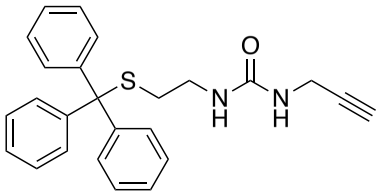


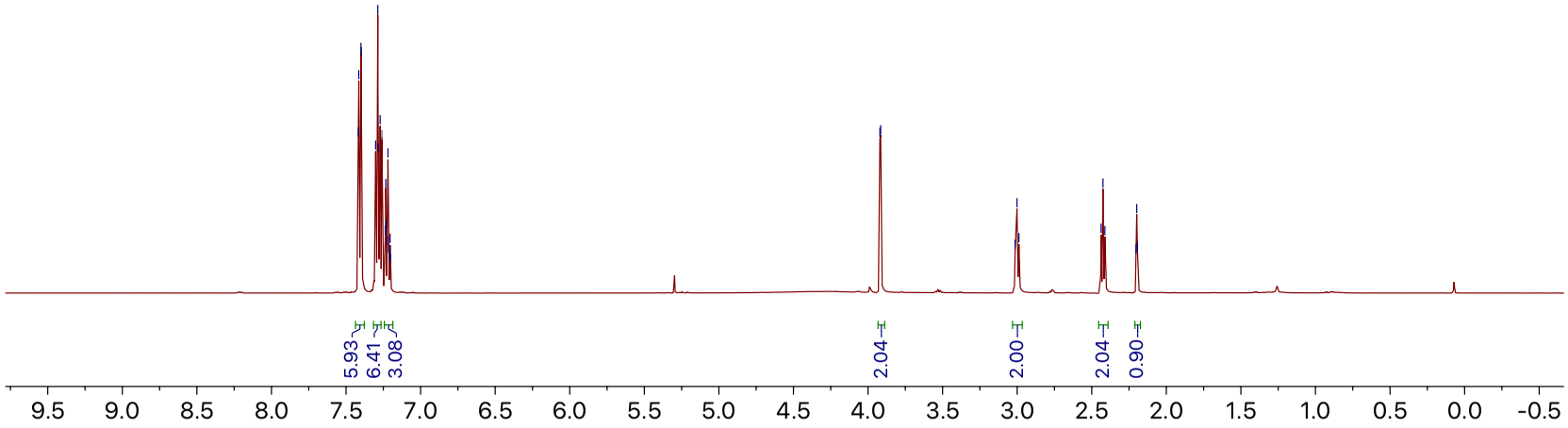
4, ^{1}H NMR (500 MHz, $CDCl_3$)

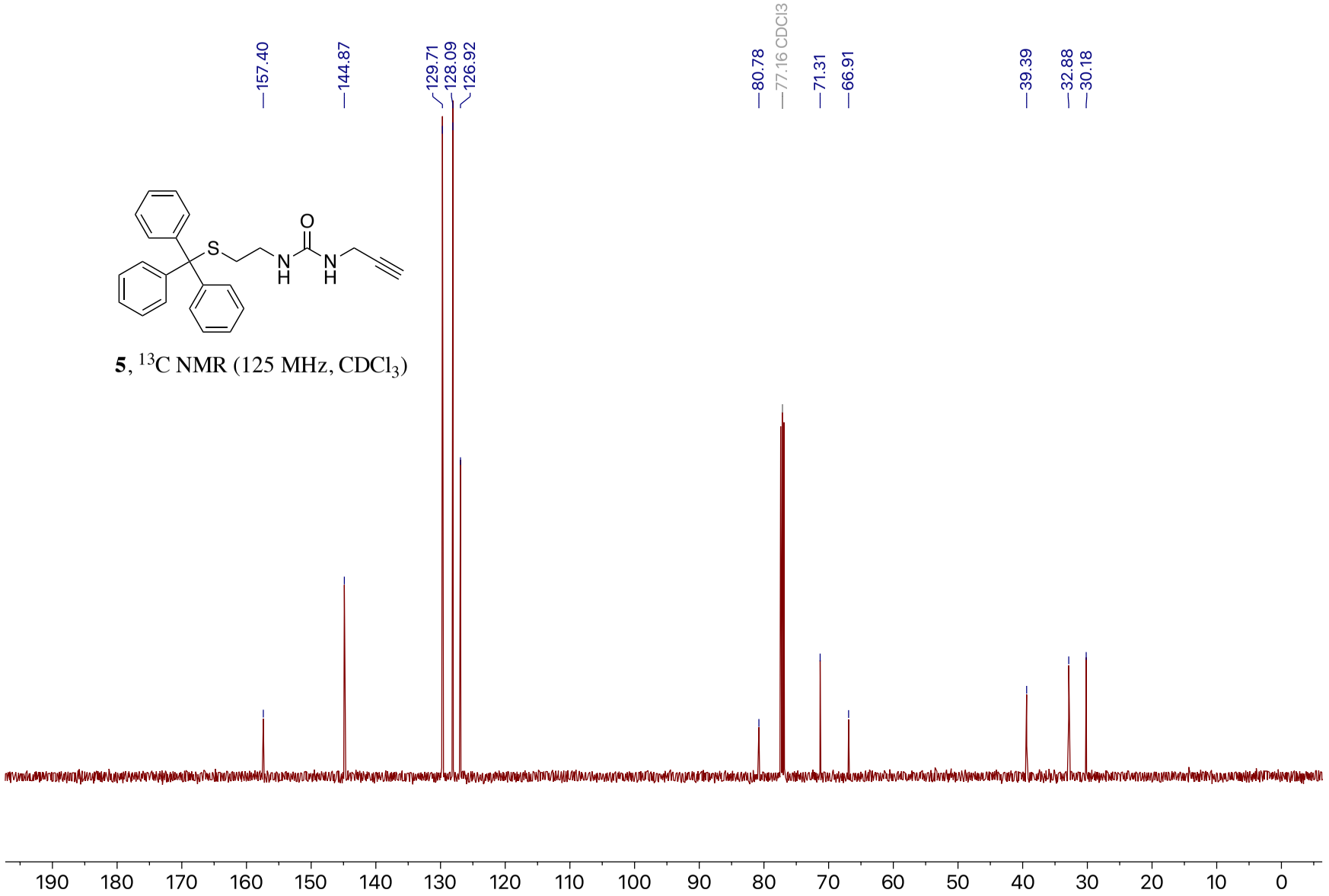


4, ^{13}C NMR (125 MHz, $CDCl_3$)

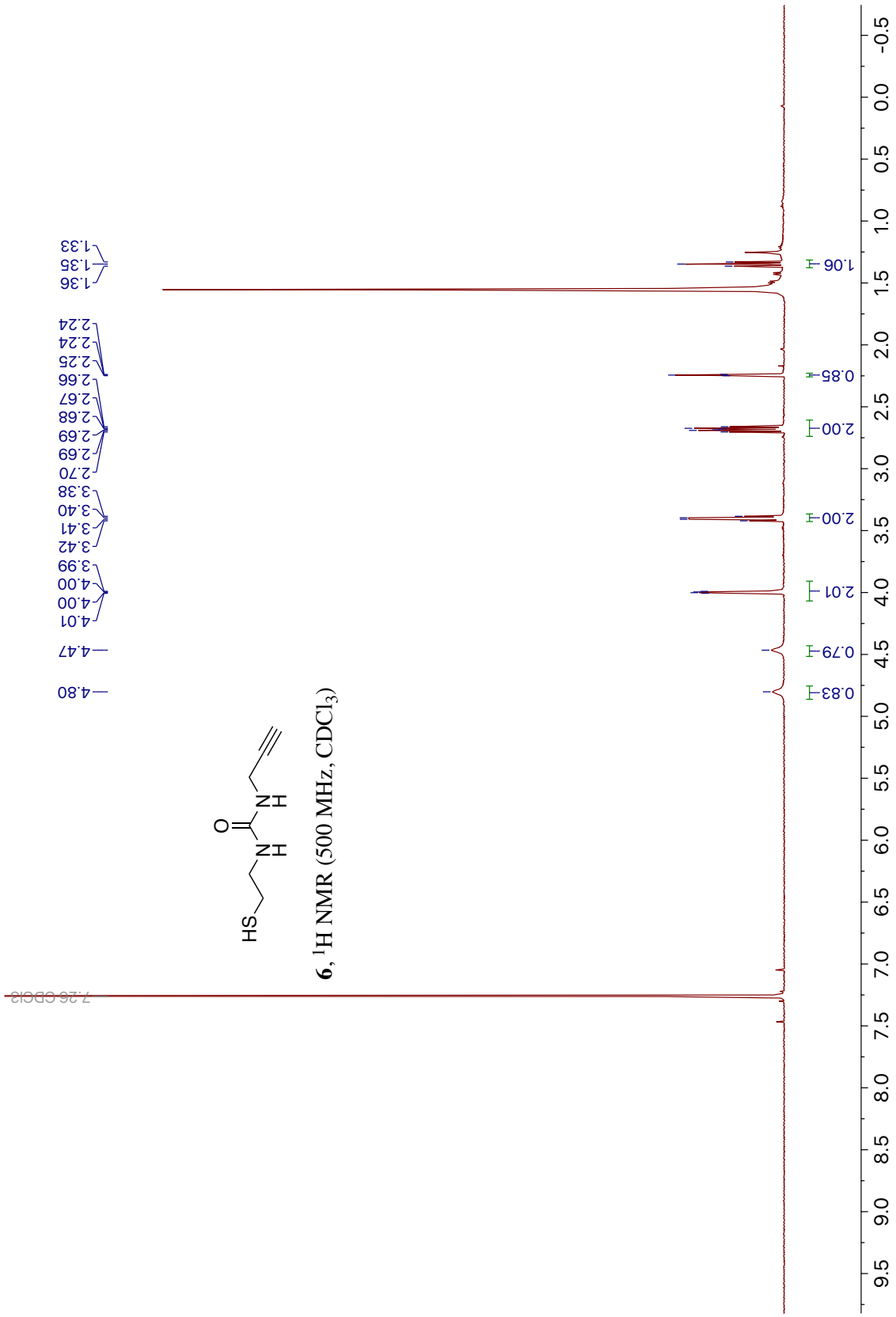


5, ^{1}H NMR (500 MHz, $CDCl_3$)

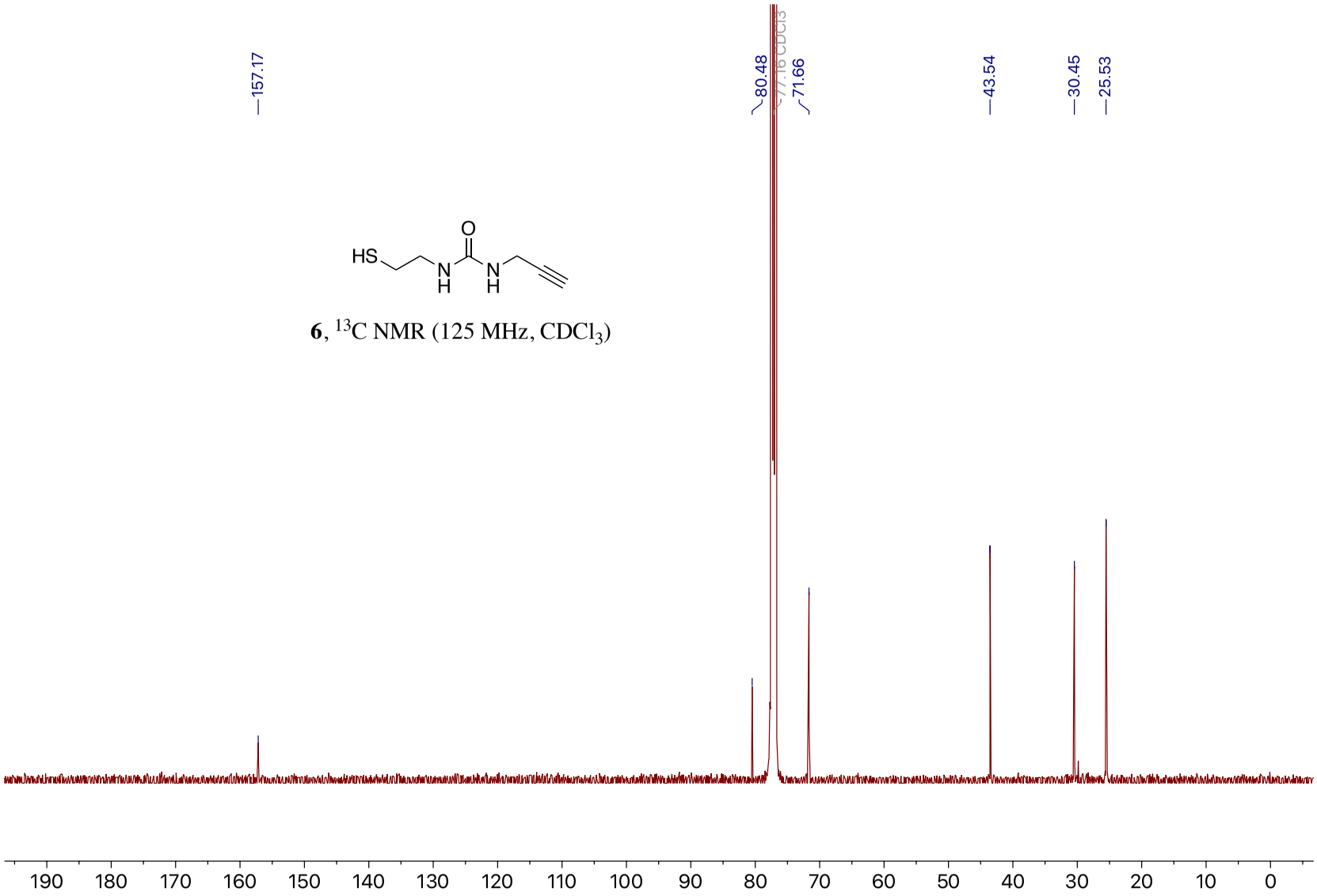




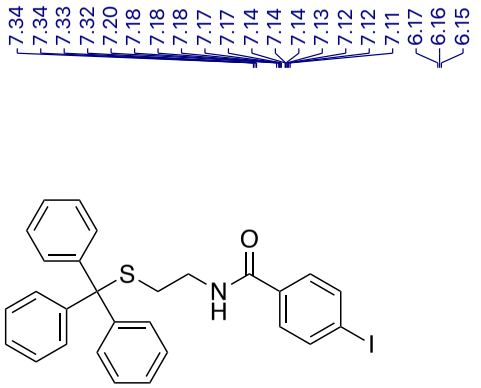
5, ^{13}C NMR (125 MHz, $CDCl_3$)



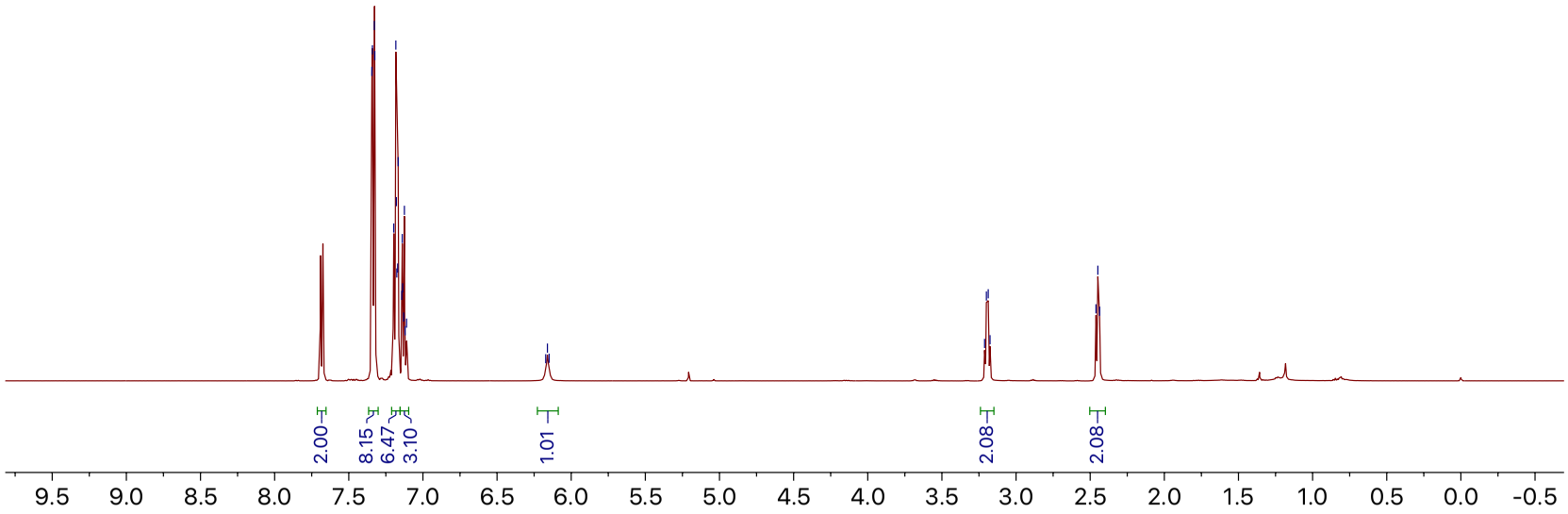
6, ^{1}H NMR (500 MHz, $CDCl_3$)

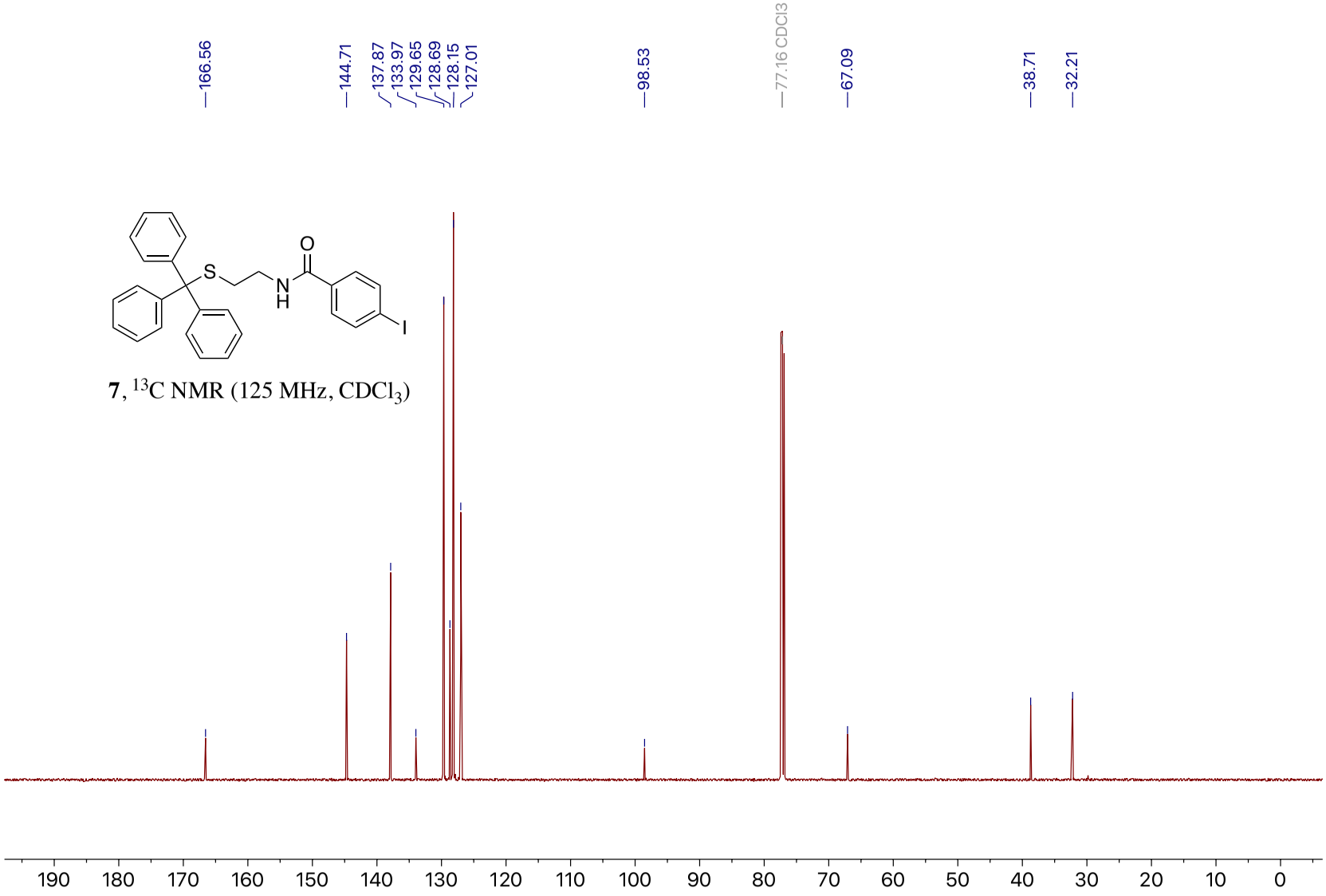


6, ^{13}C NMR (125 MHz, $CDCl_3$)

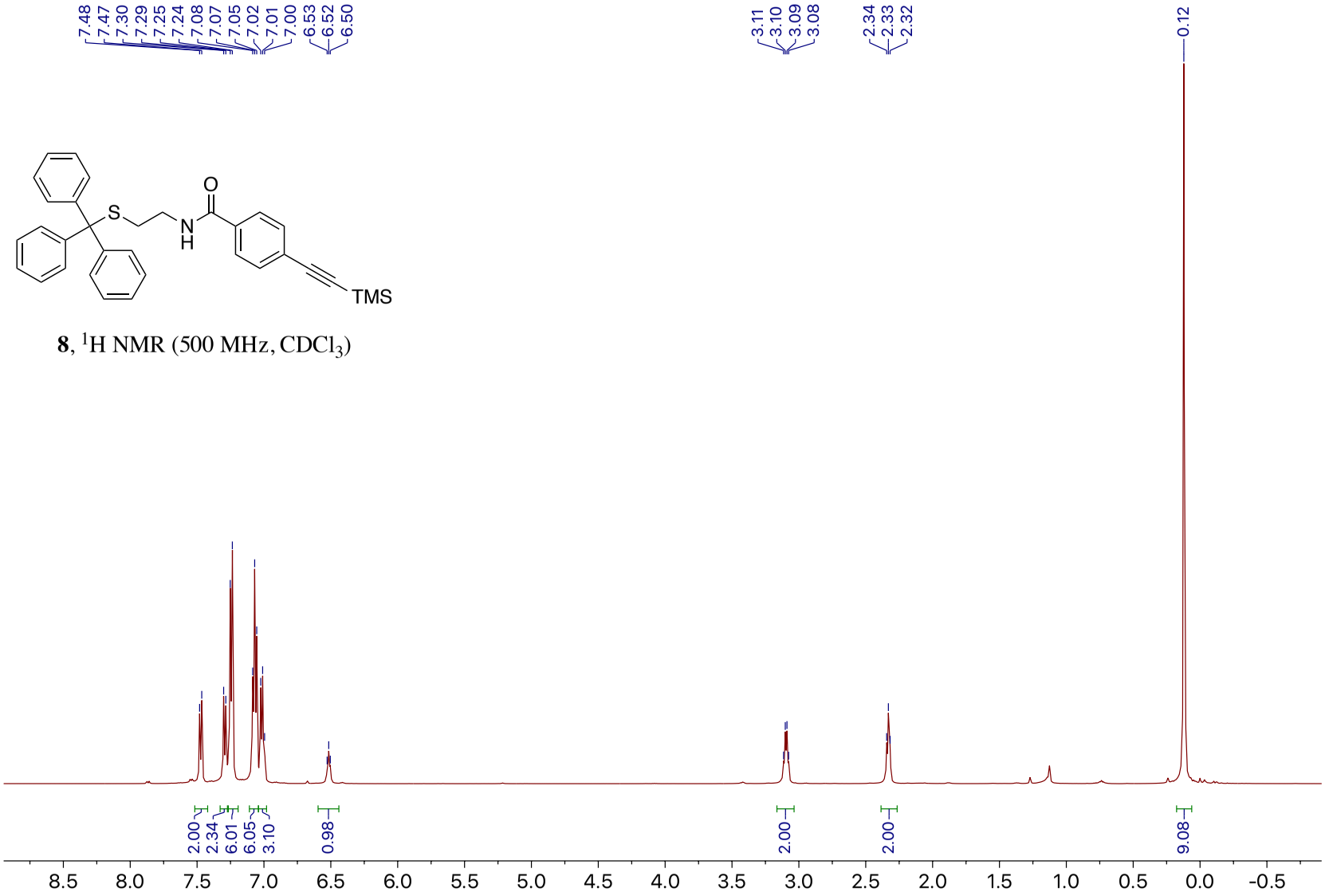


7, ^{1}H NMR (500 MHz, $CDCl_3$)

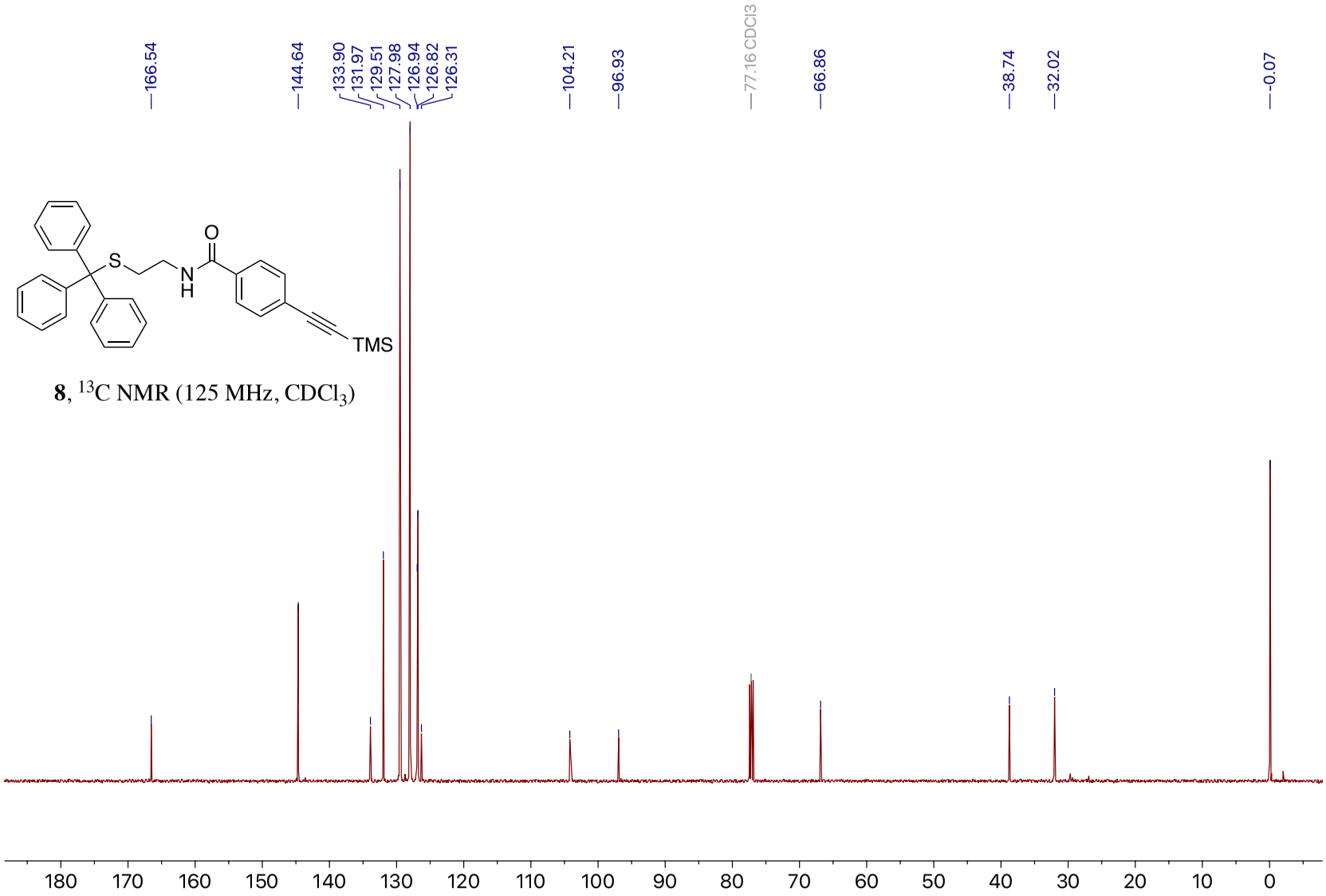




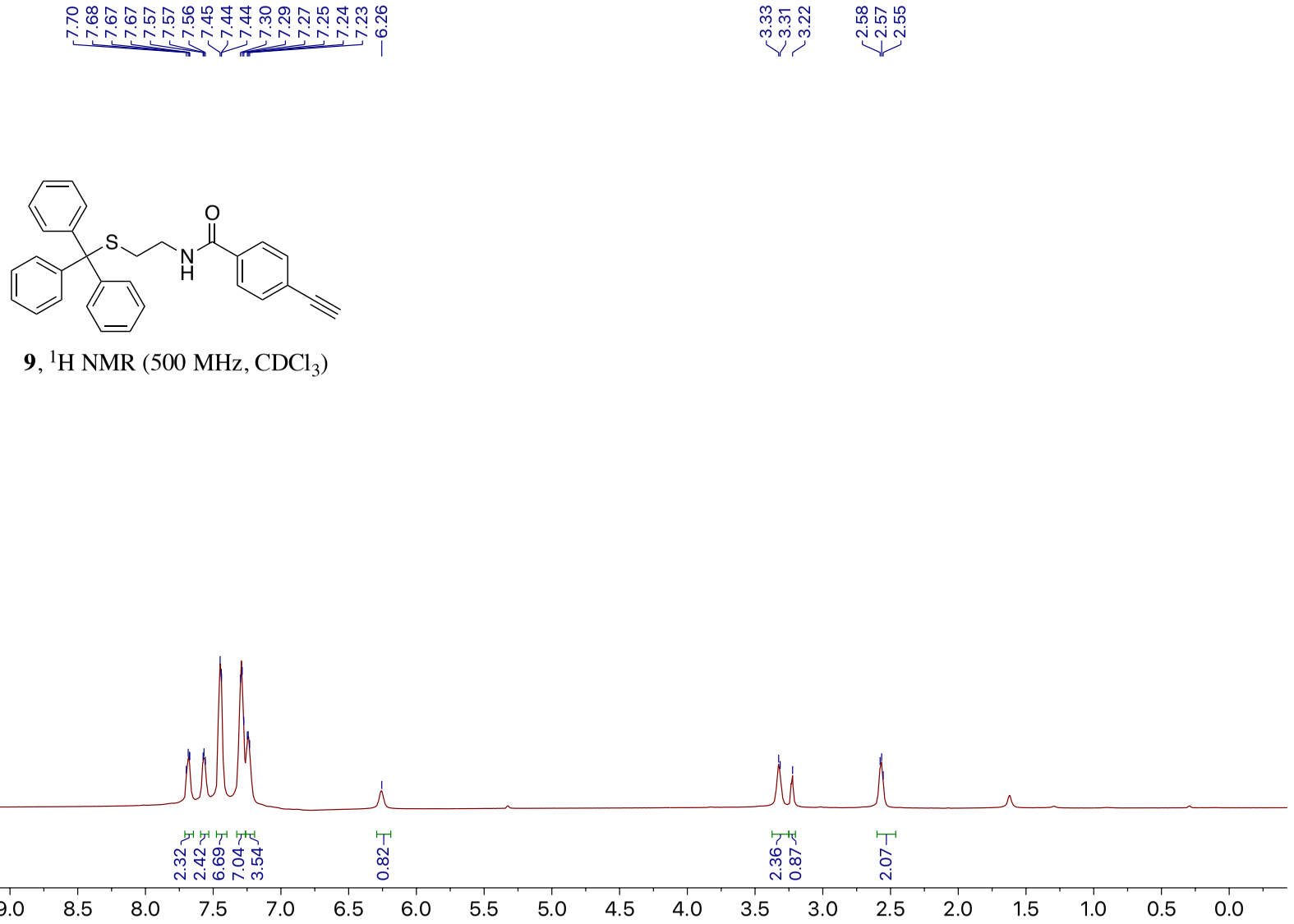
7, ^{13}C NMR (125 MHz, $CDCl_3$)



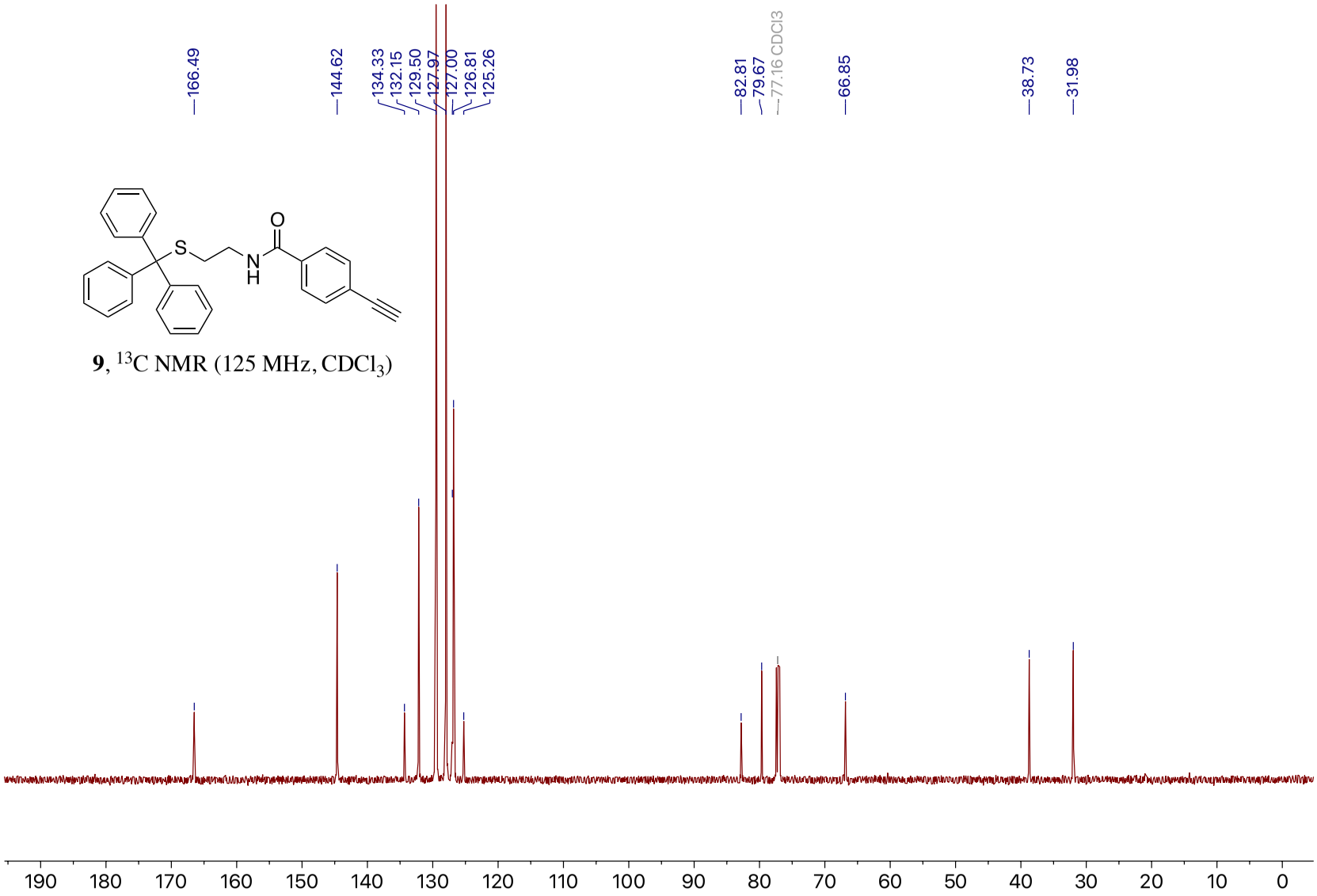
8, ^{1}H NMR (500 MHz, $CDCl_3$)



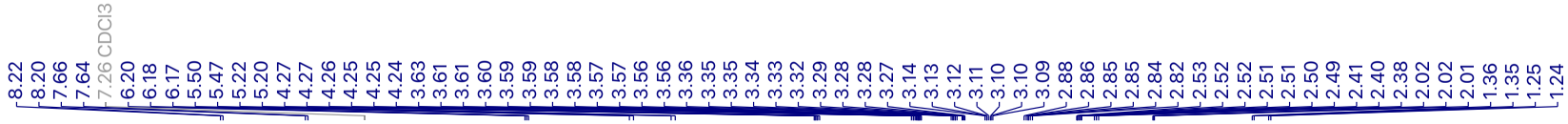
8, ^{13}C NMR (125 MHz, $CDCl_3$)

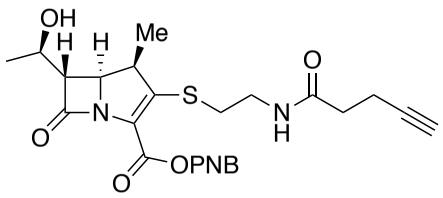


9, ^{1}H NMR (500 MHz, $CDCl_3$)

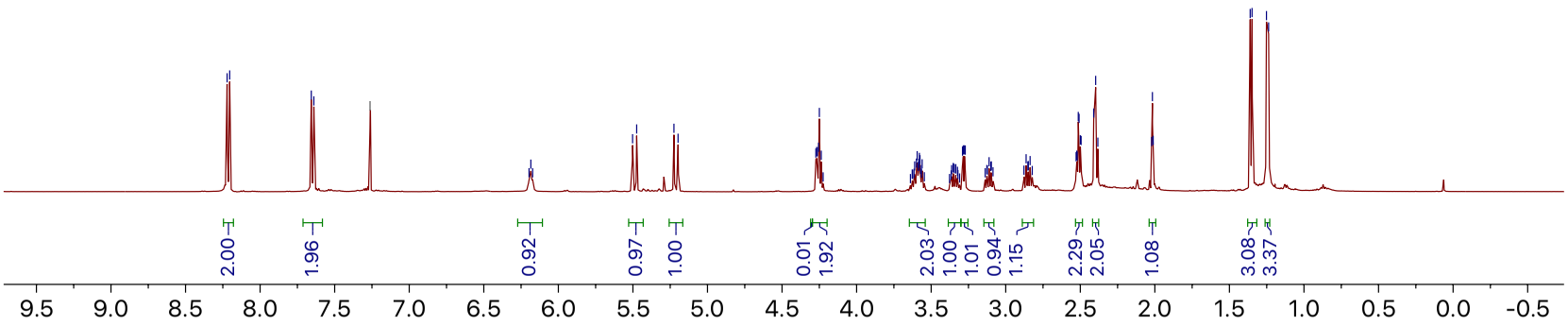


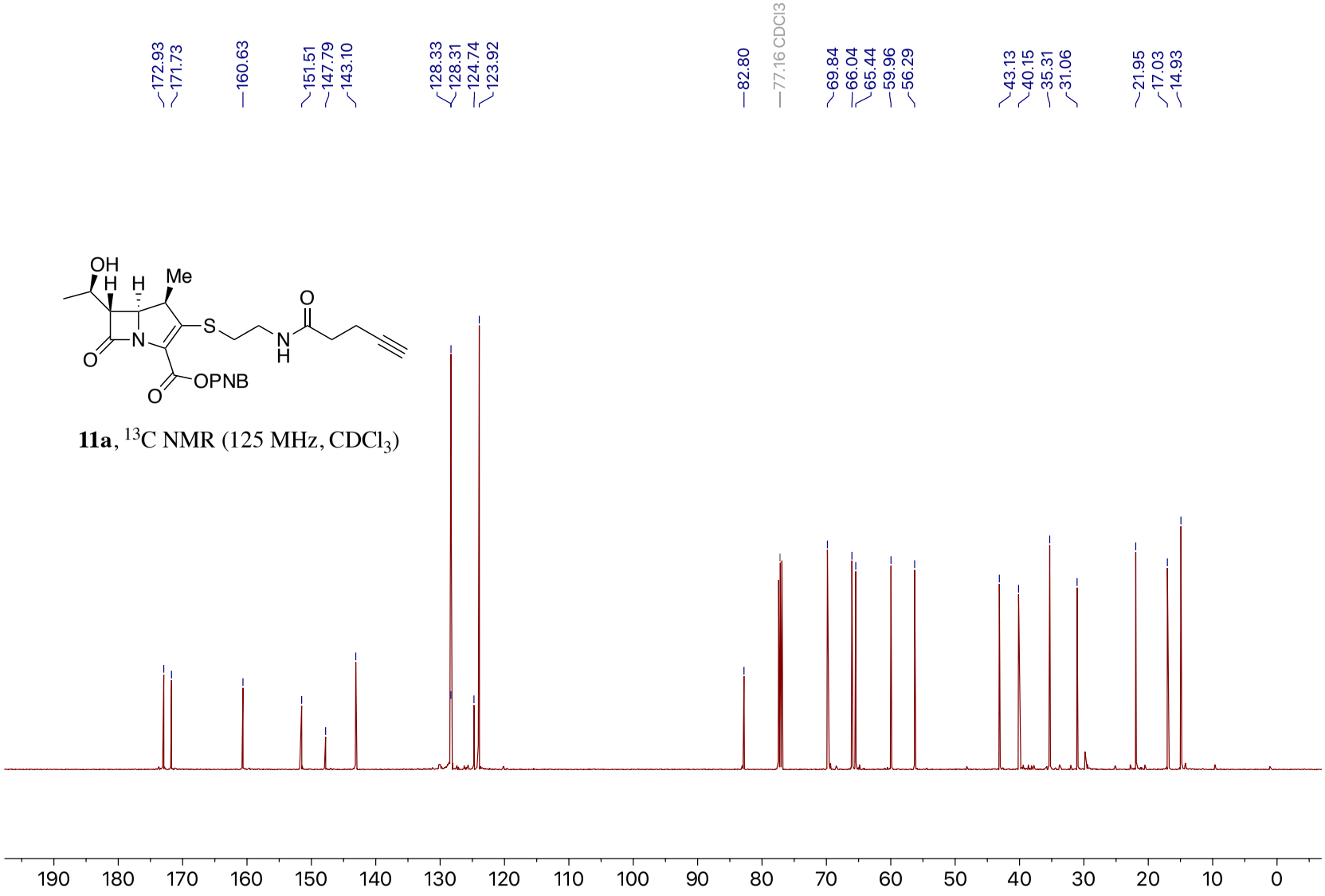
9, ^{13}C NMR (125 MHz, $CDCl_3$)



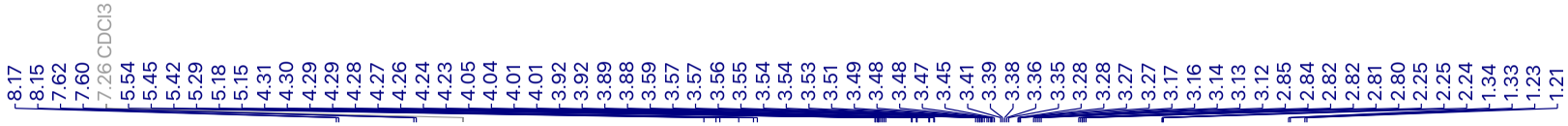


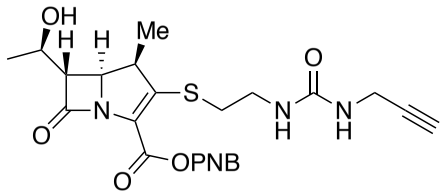
11a, ^{1}H NMR (500 MHz, $CDCl_3$)



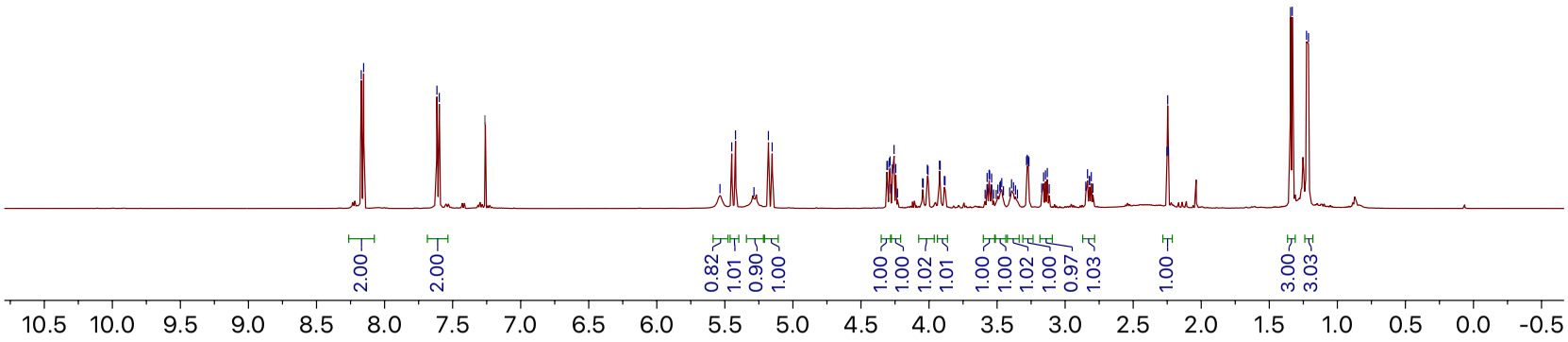


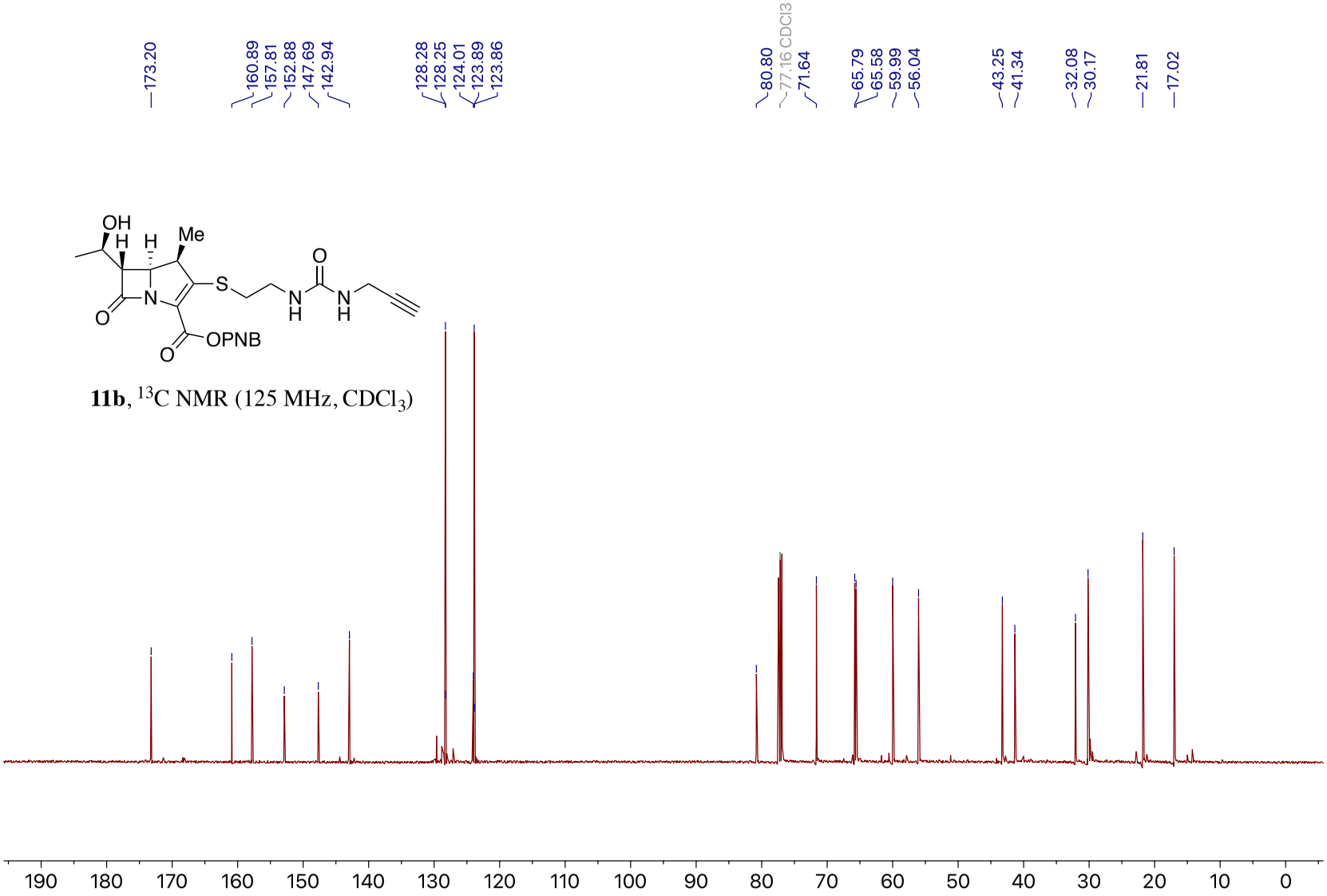
11a, ^{13}C NMR (125 MHz, $CDCl_3$)



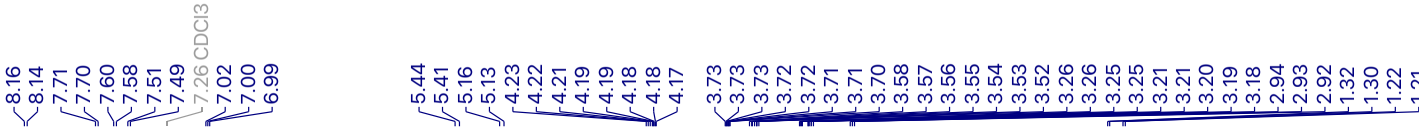


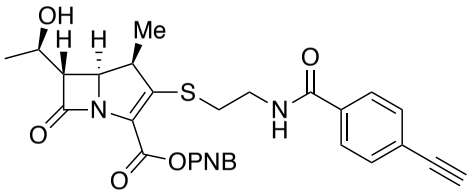
11b, ^{1}H NMR (500 MHz, $CDCl_3$)



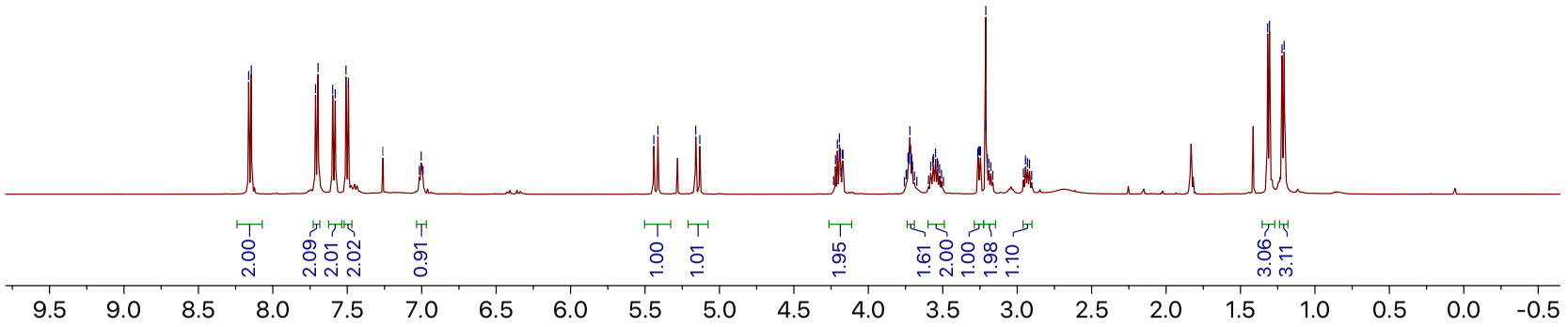


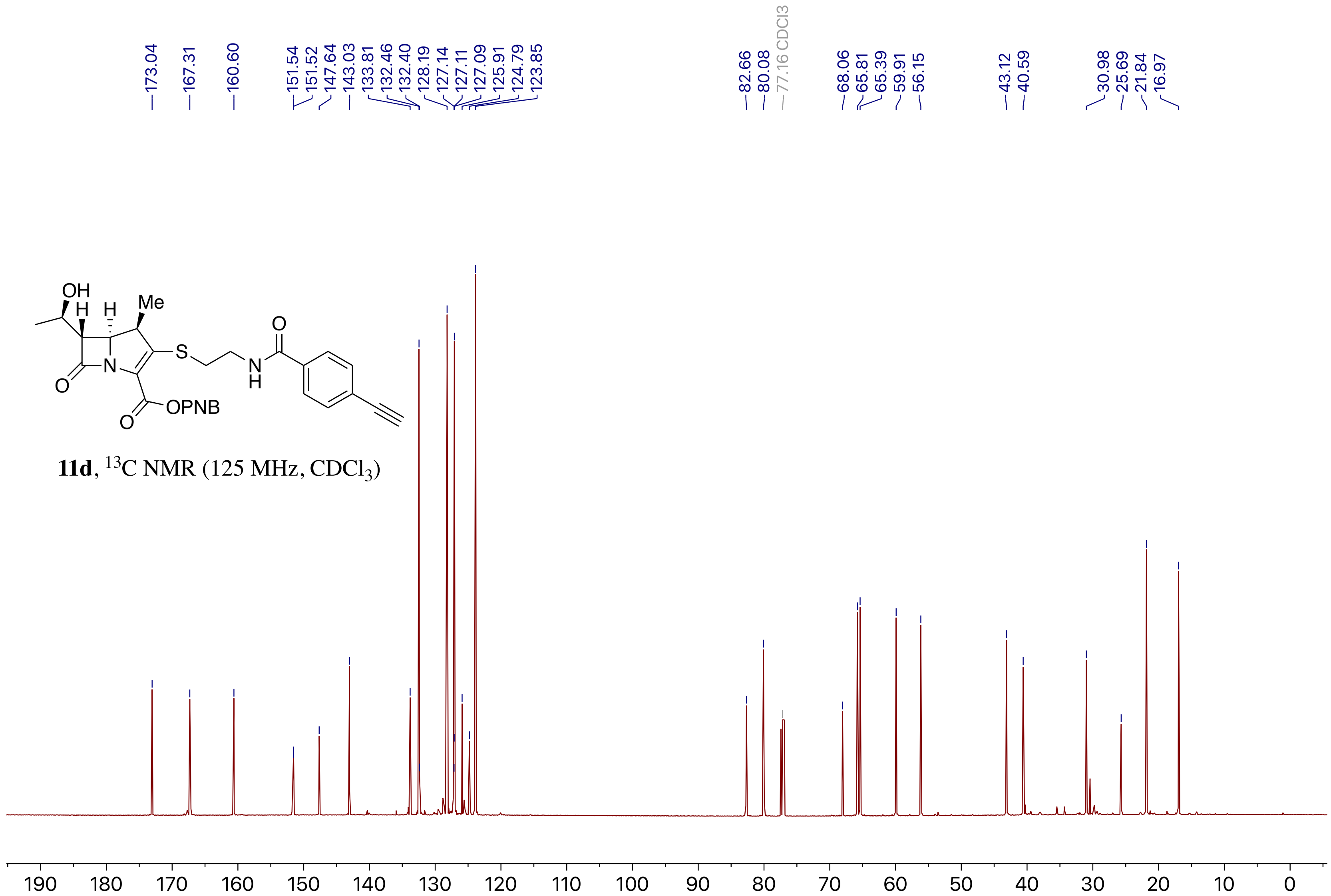
11b, ^{13}C NMR (125 MHz, $CDCl_3$)



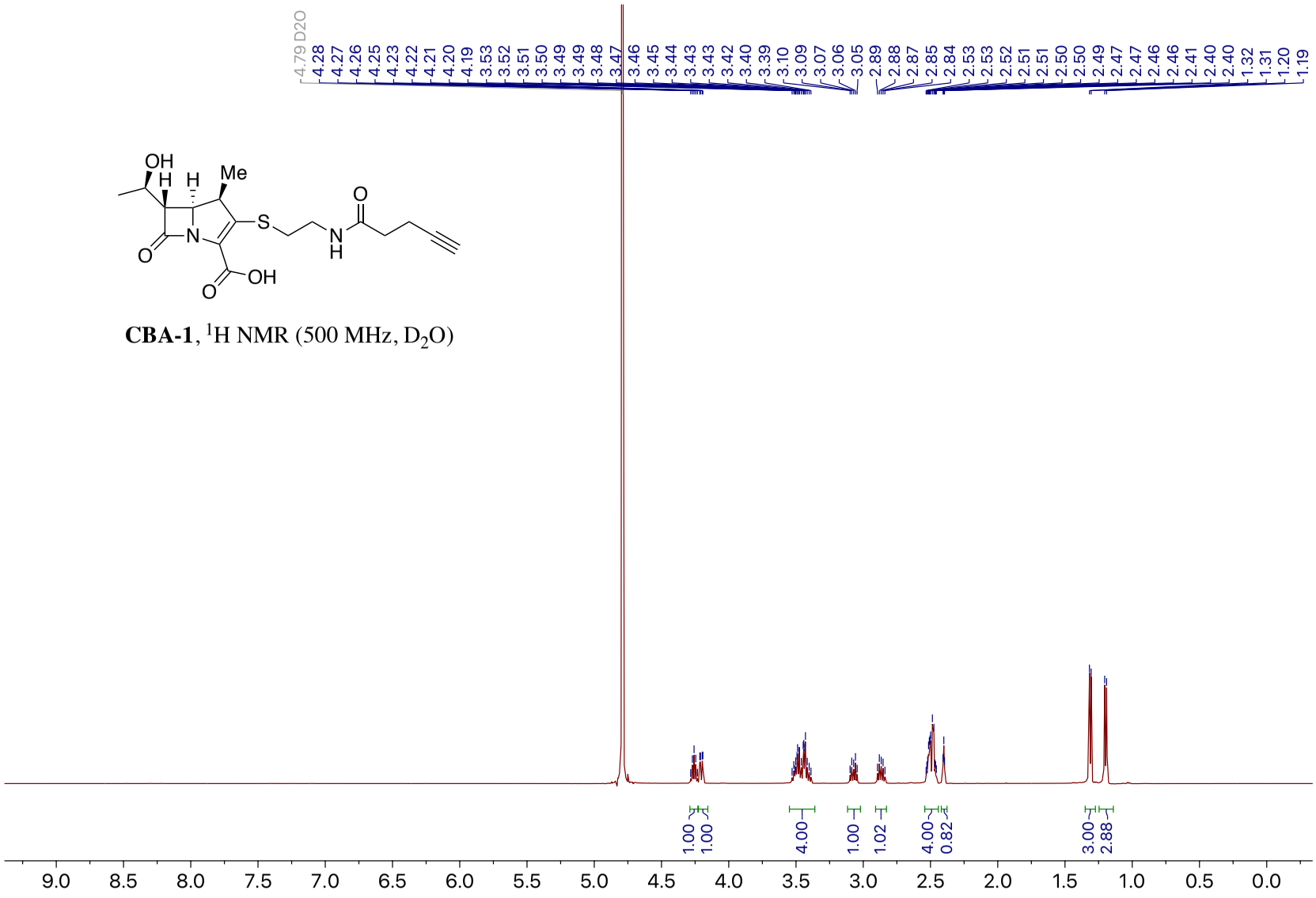


11d, ^{1}H NMR (500 MHz, $CDCl_3$)

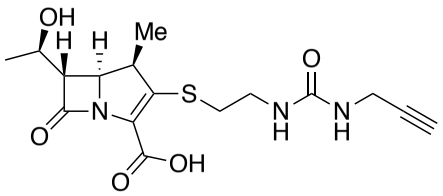




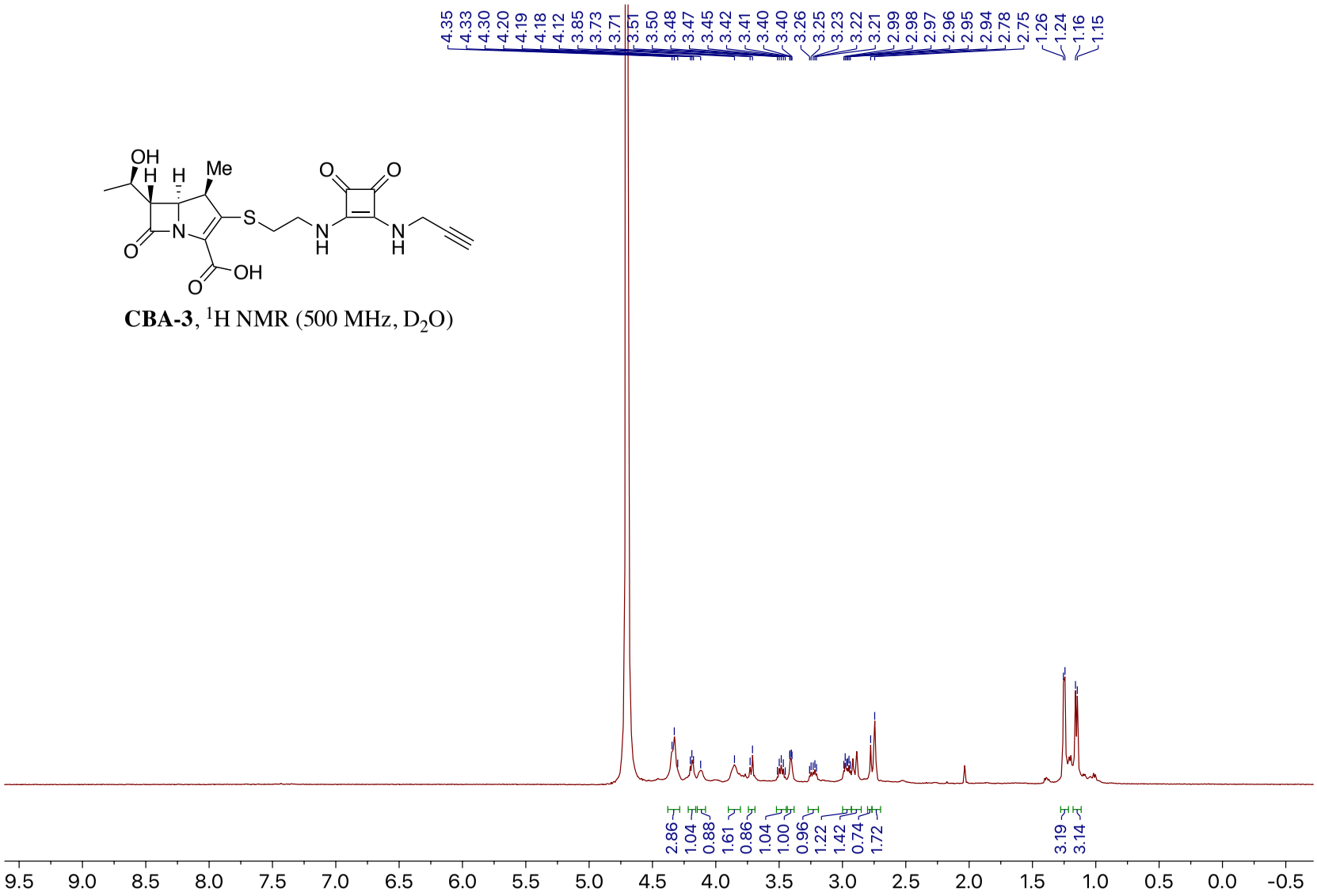
11d, ^{13}C NMR (125 MHz, $CDCl_3$)



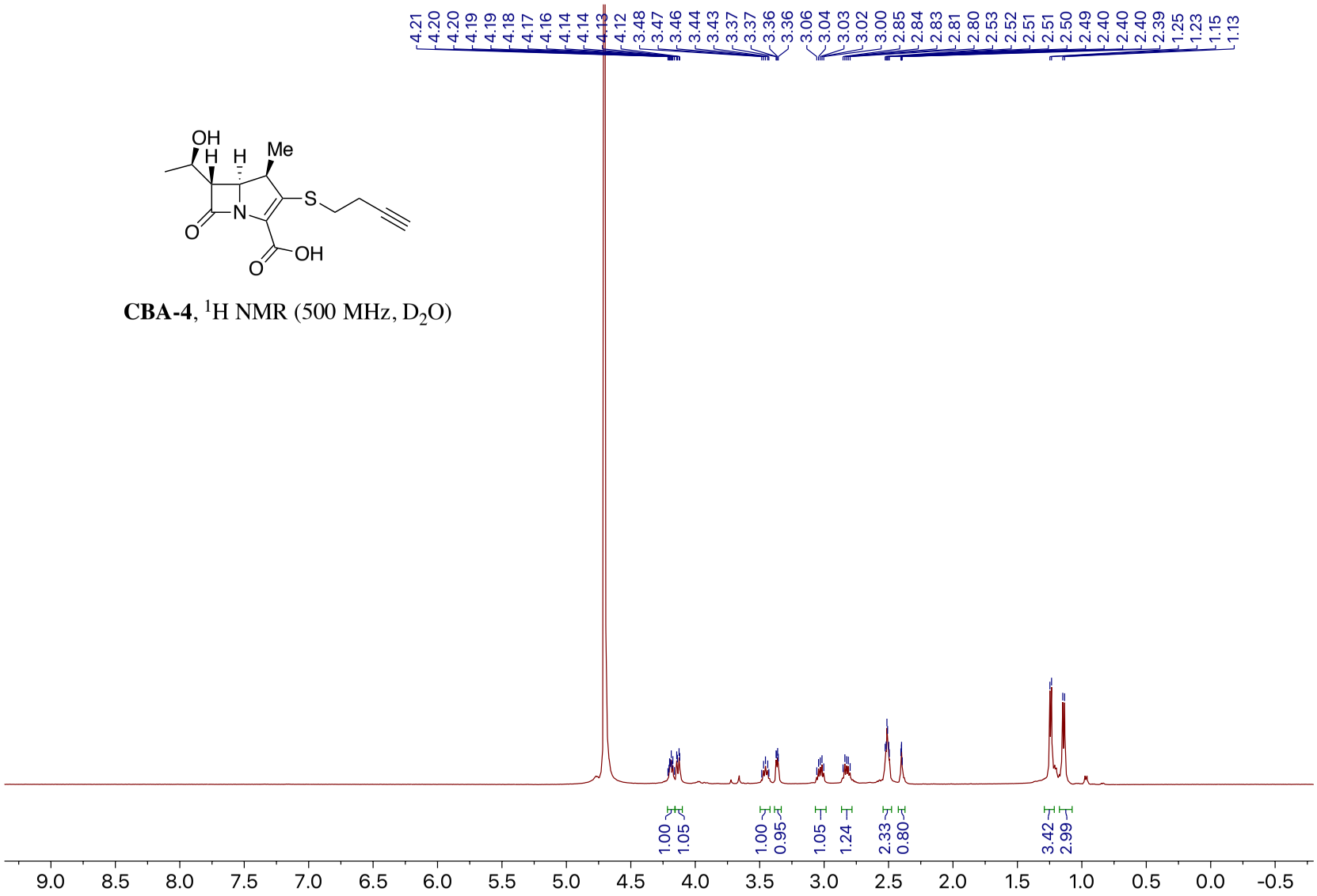
CBA-1, ^{1}H NMR (500 MHz, D_2O)



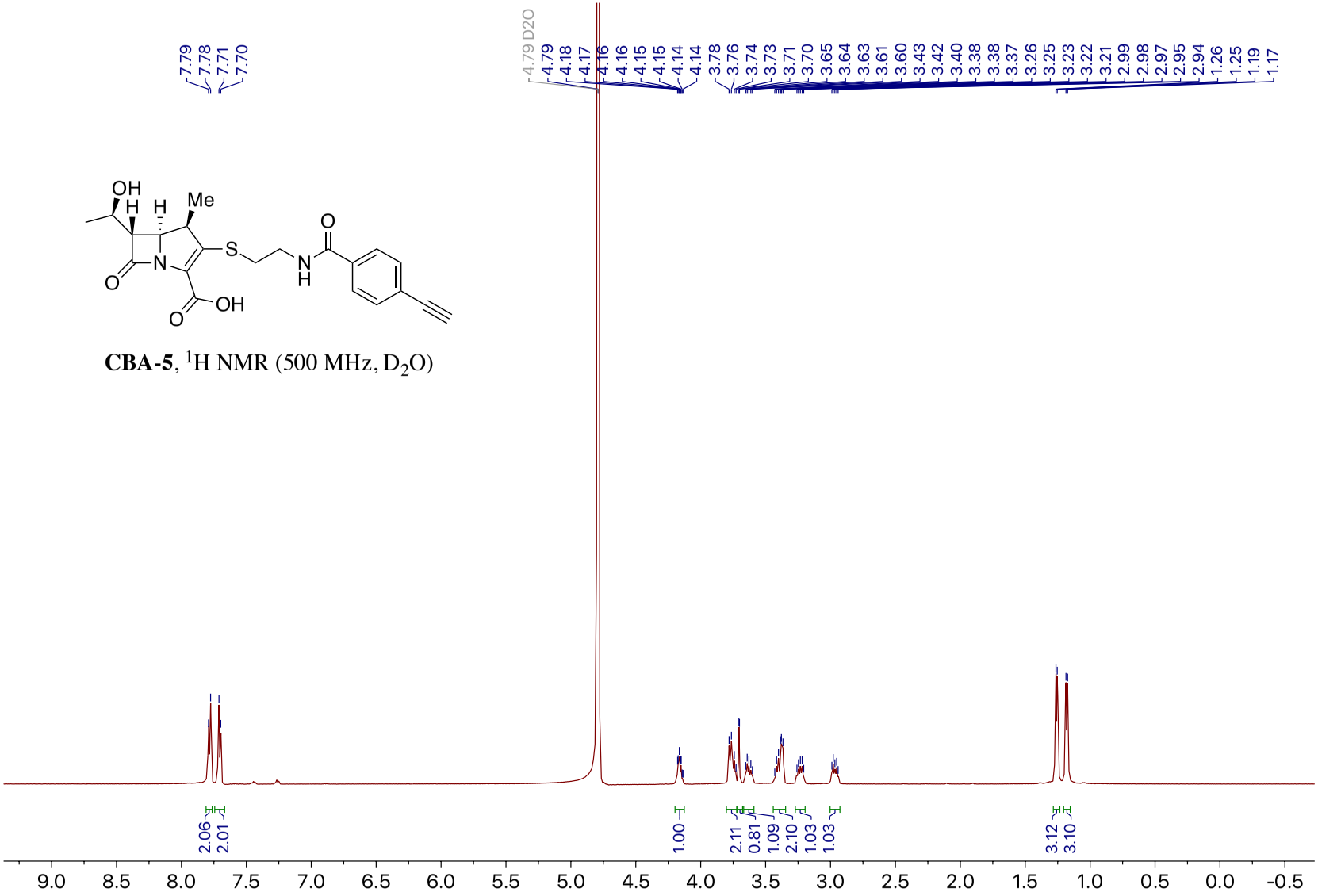
CBA-2, ^{1}H NMR (500 MHz, D_2O)



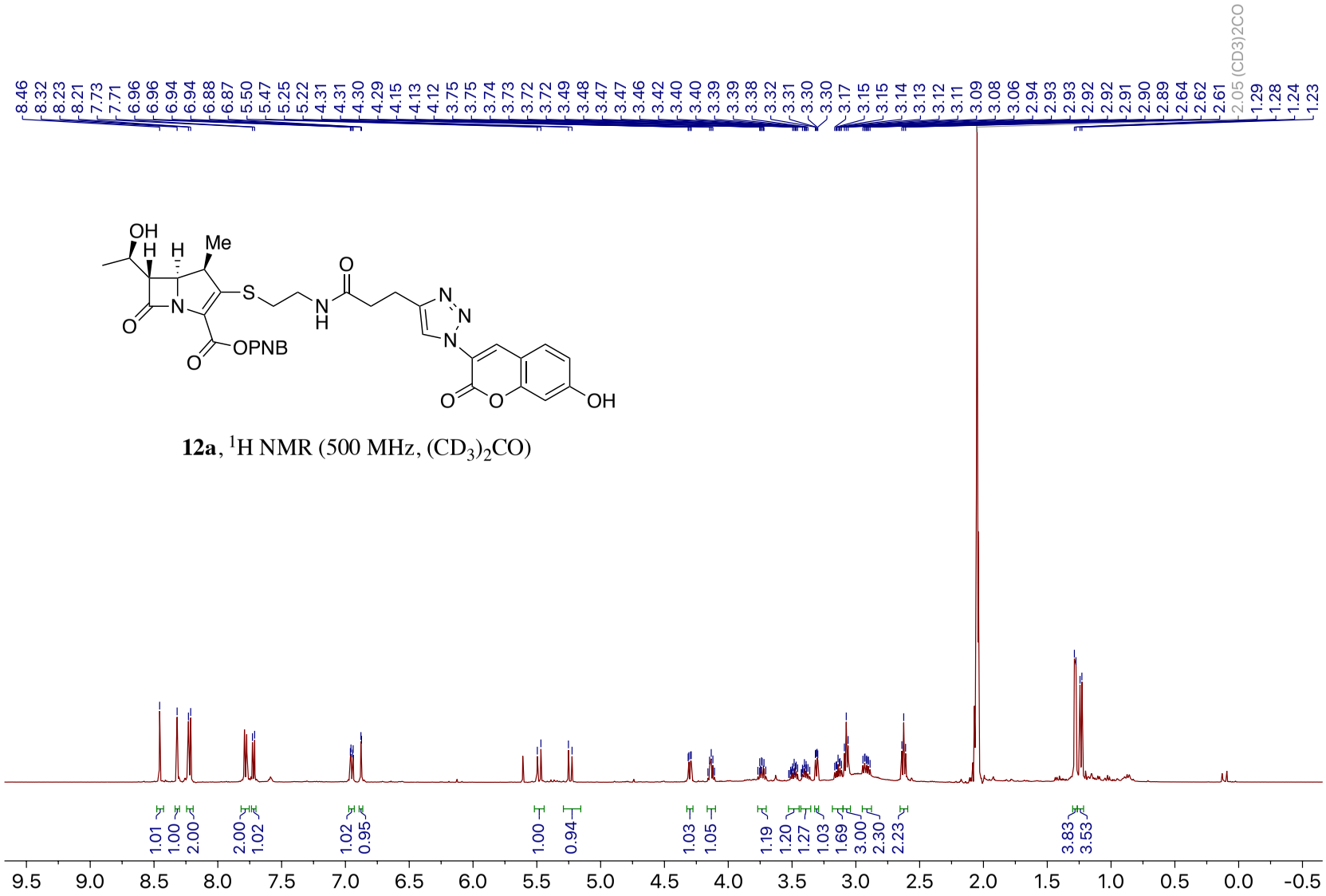
CBA-3, ^{1}H NMR (500 MHz, D_2O)



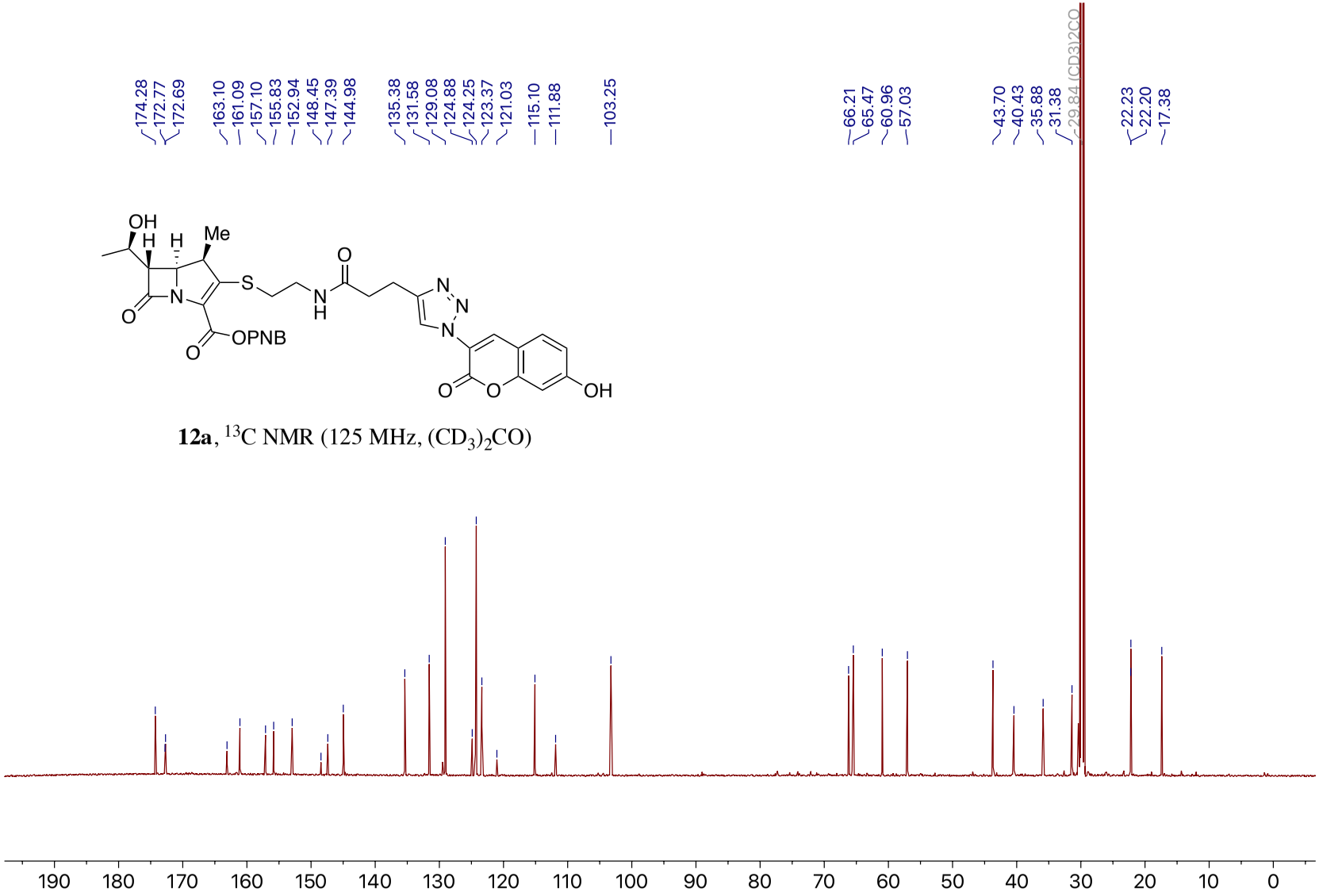
CBA-4, ^{1}H NMR (500 MHz, D_2O)



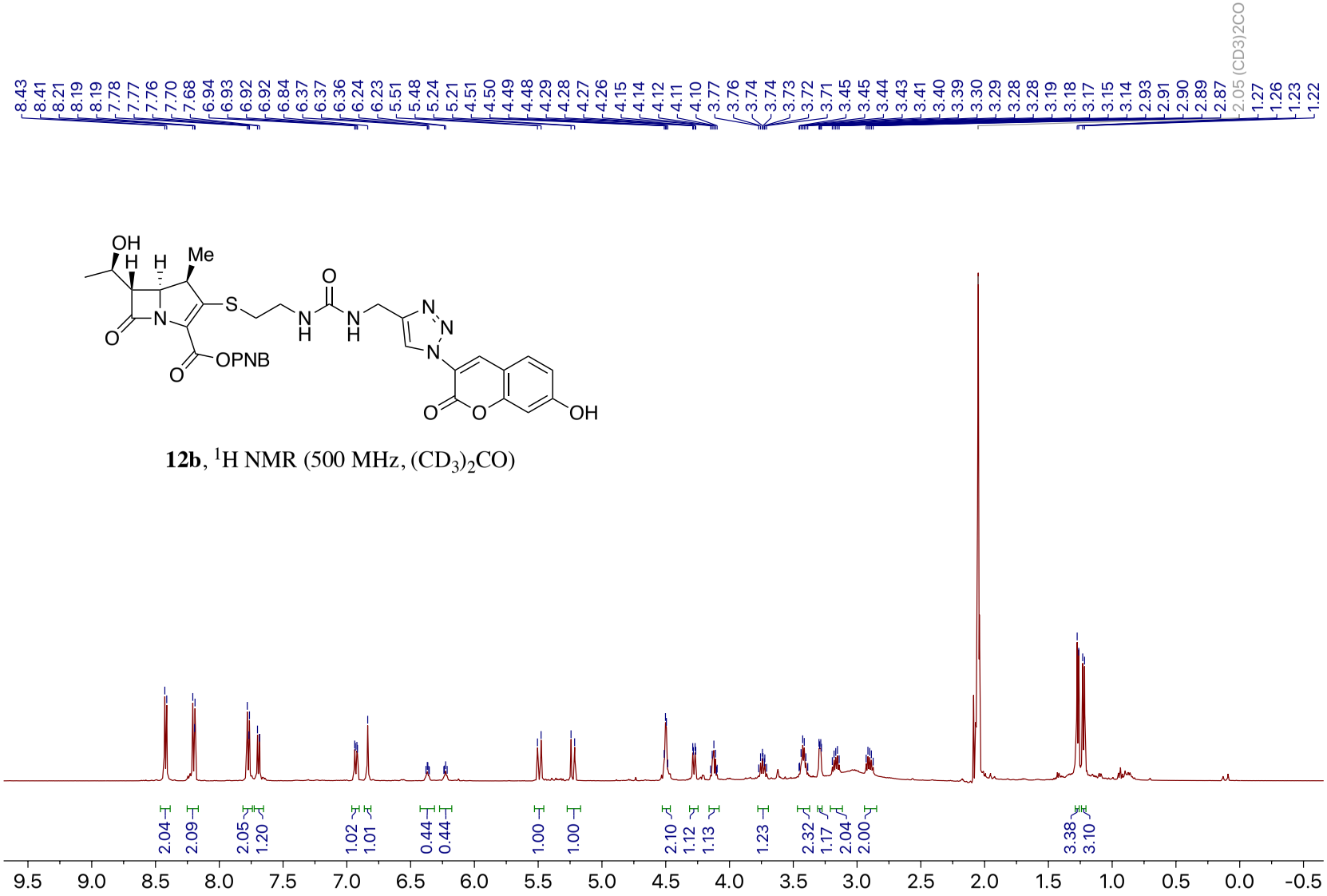
CBA-5, ^{1}H NMR (500 MHz, D_2O)



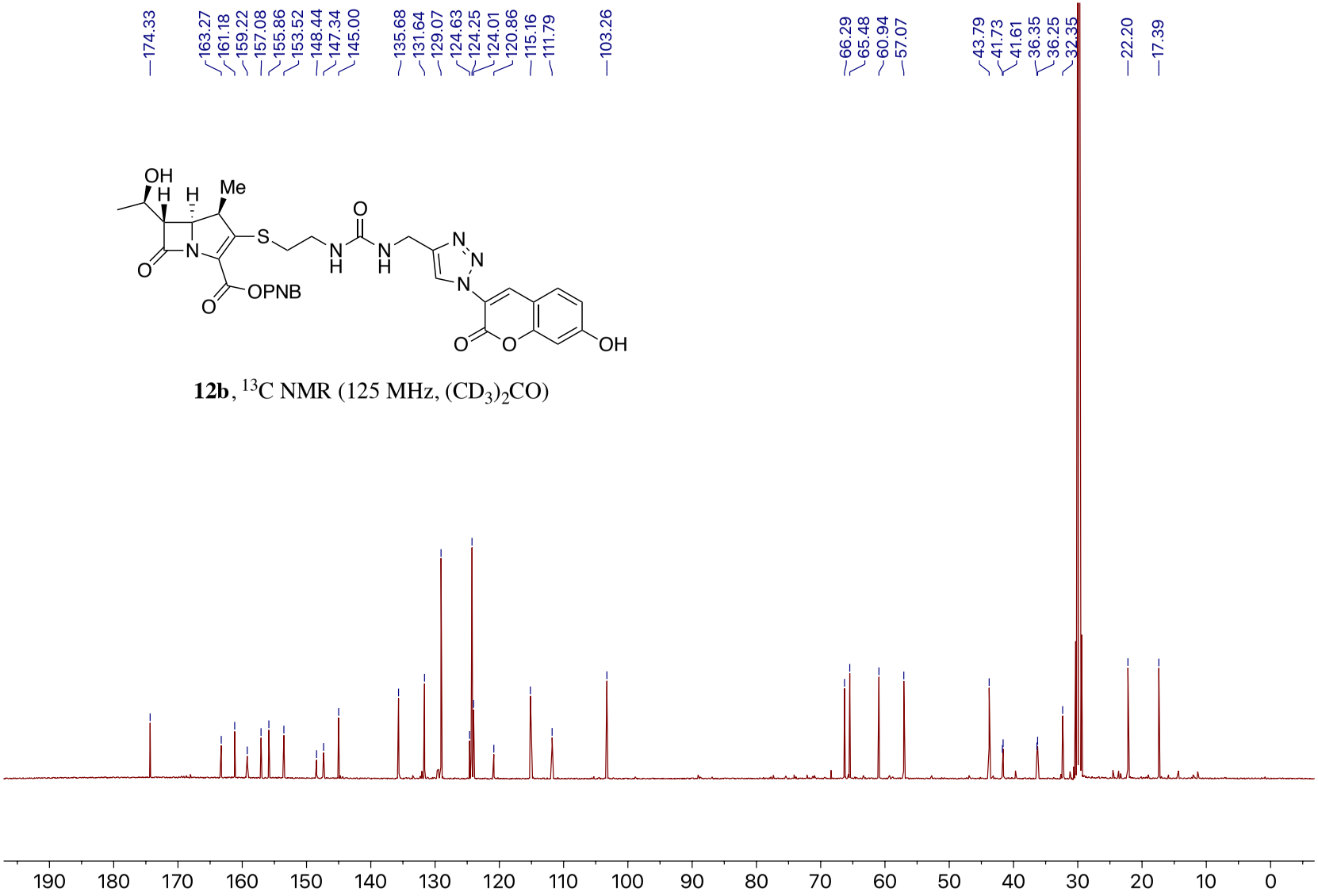
12a, ^{1}H NMR (500 MHz, $(CD_3)_2CO$)



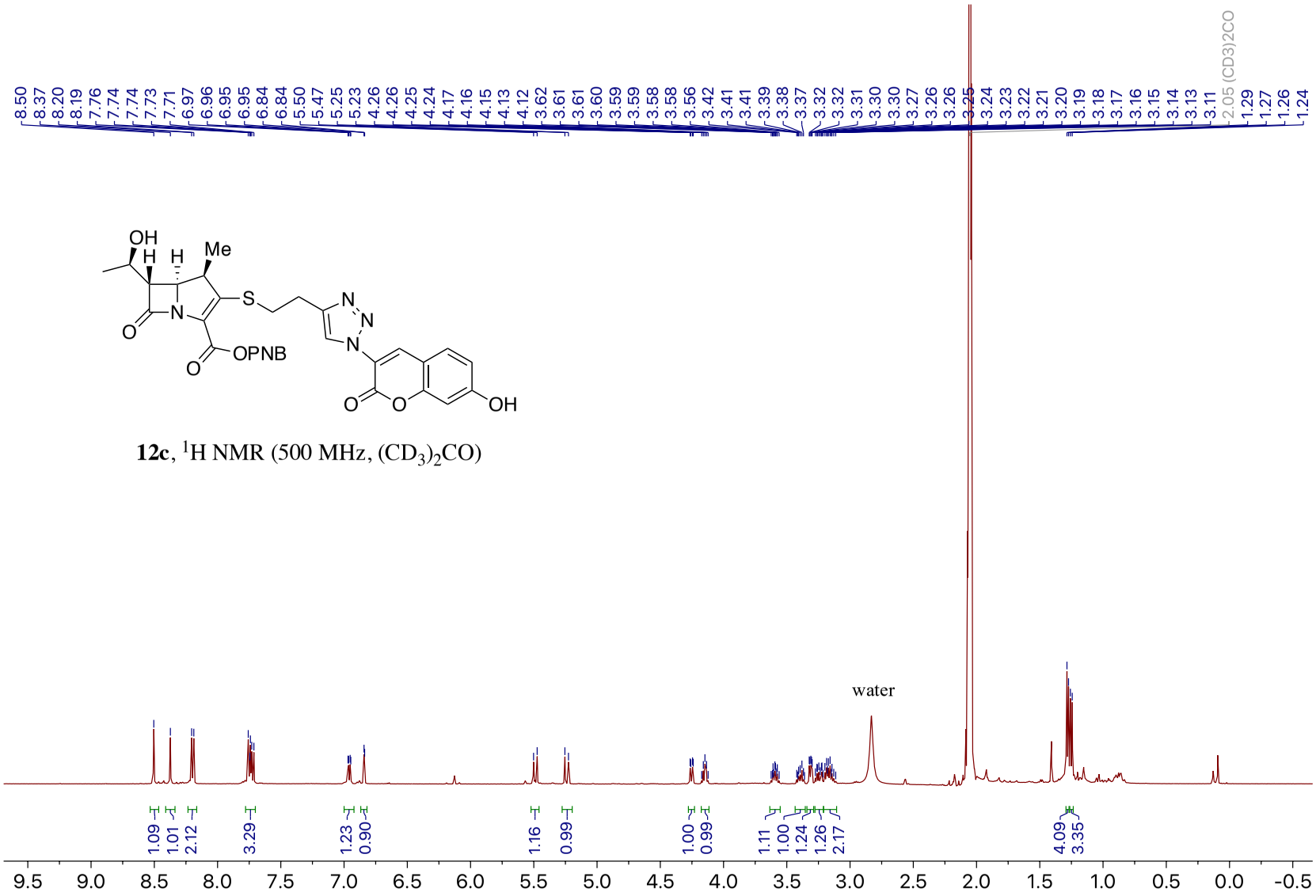
12a, ^{13}C NMR (125 MHz, $(CD_3)_2CO$)



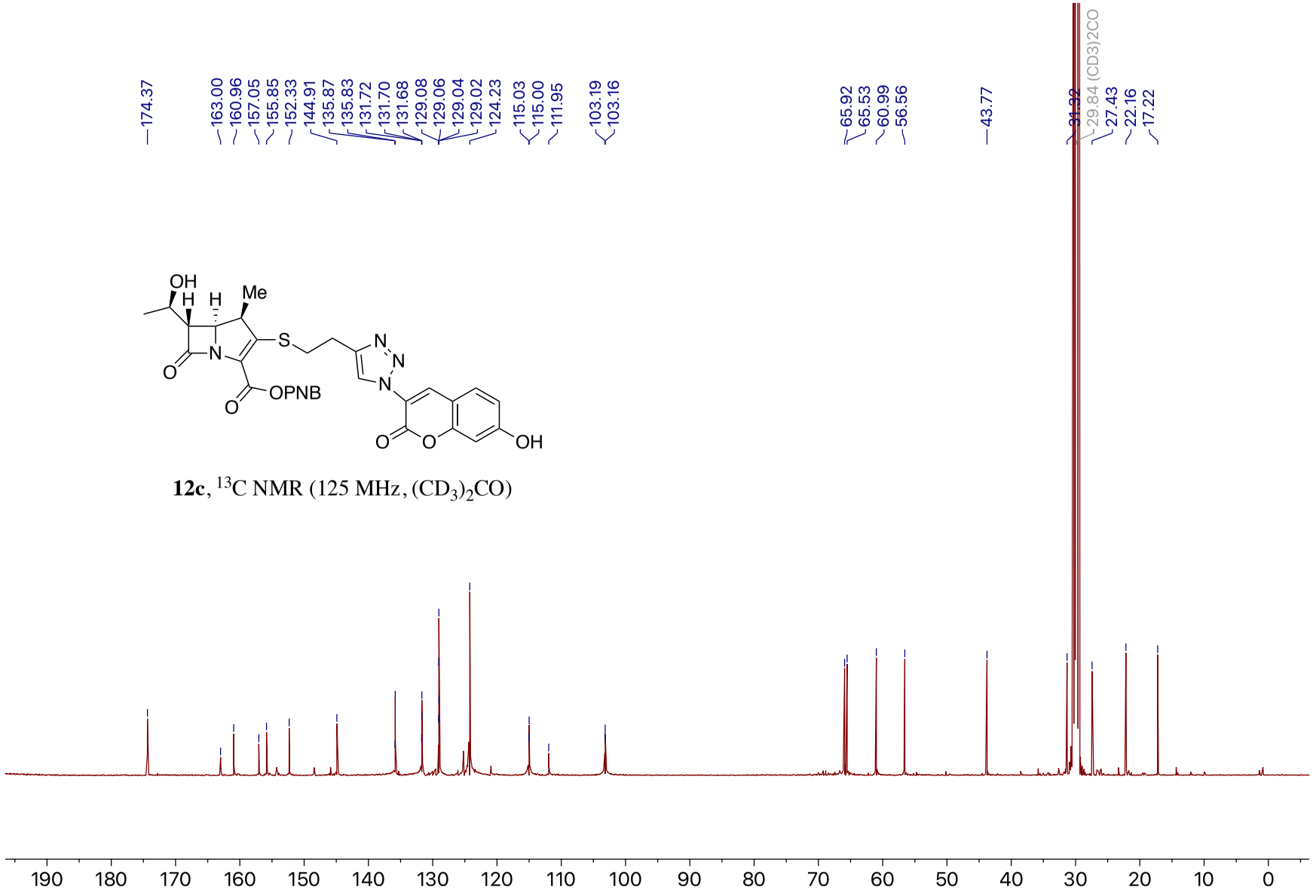
12b, ^{1}H NMR (500 MHz, $(CD_3)_2CO$)



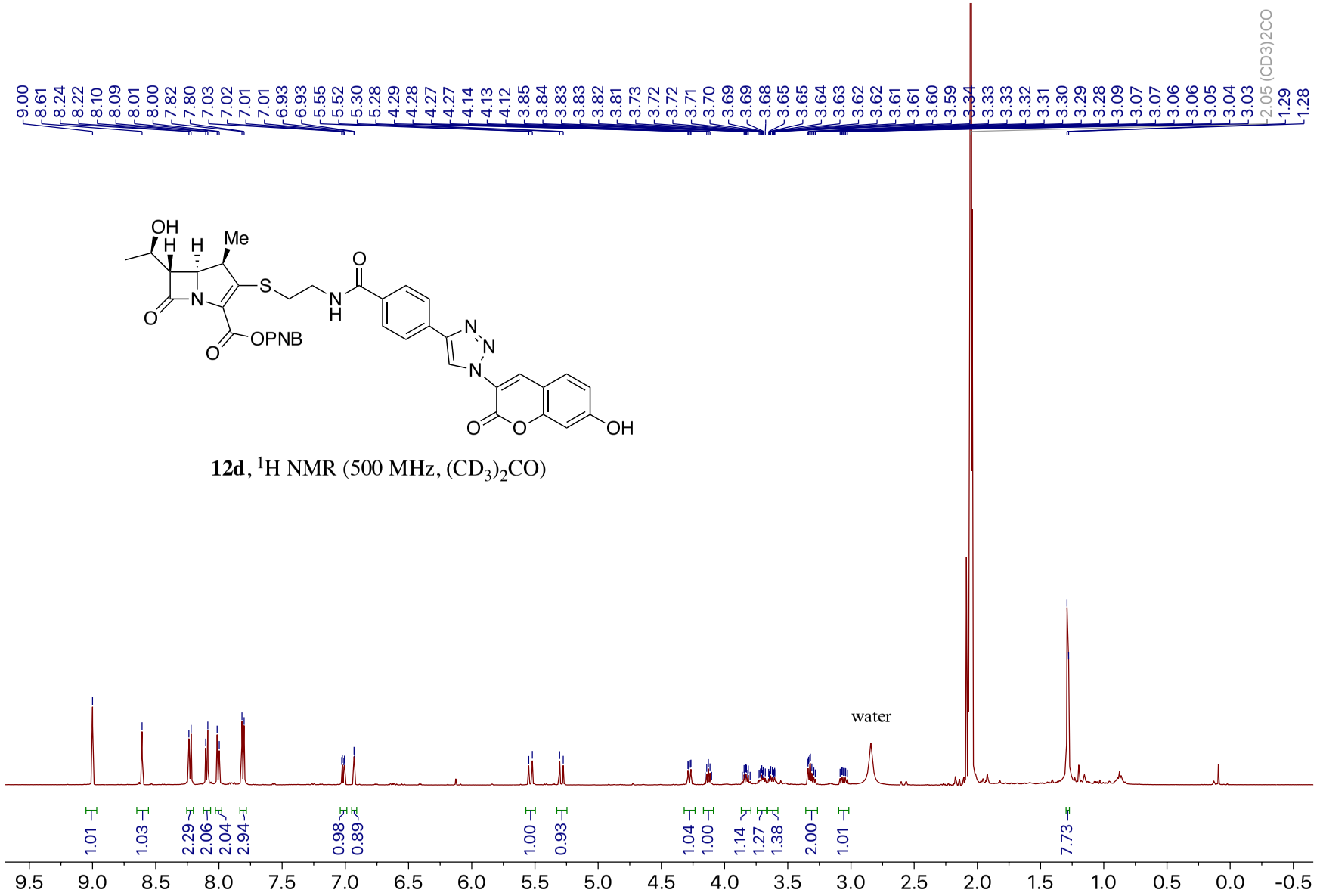
12b, ^{13}C NMR (125 MHz, $(CD_3)_2CO$)



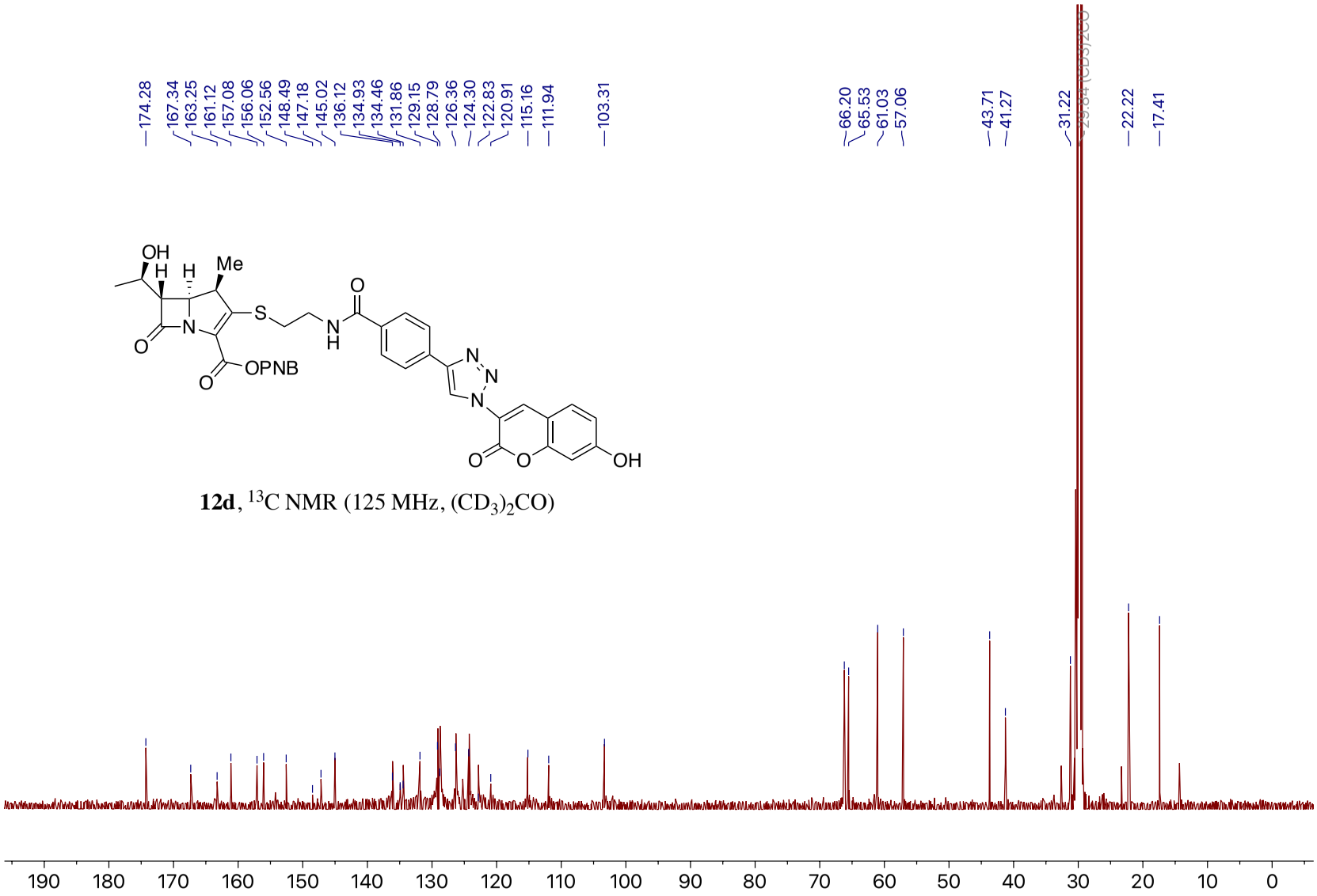
12c, ^{1}H NMR (500 MHz, $(CD_3)_2CO$)



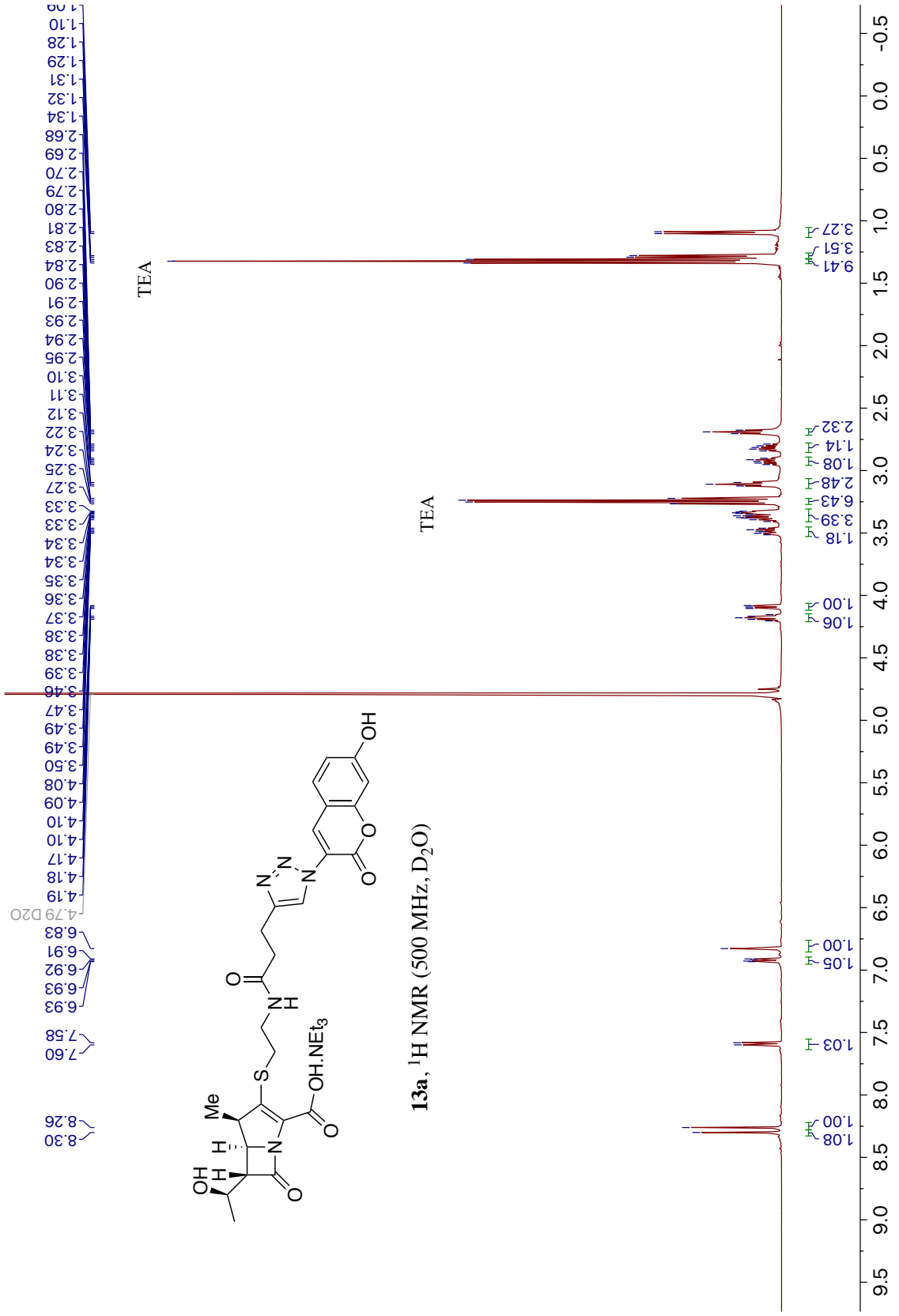
12c, ^{13}C NMR (125 MHz, $(CD_3)_2CO$)



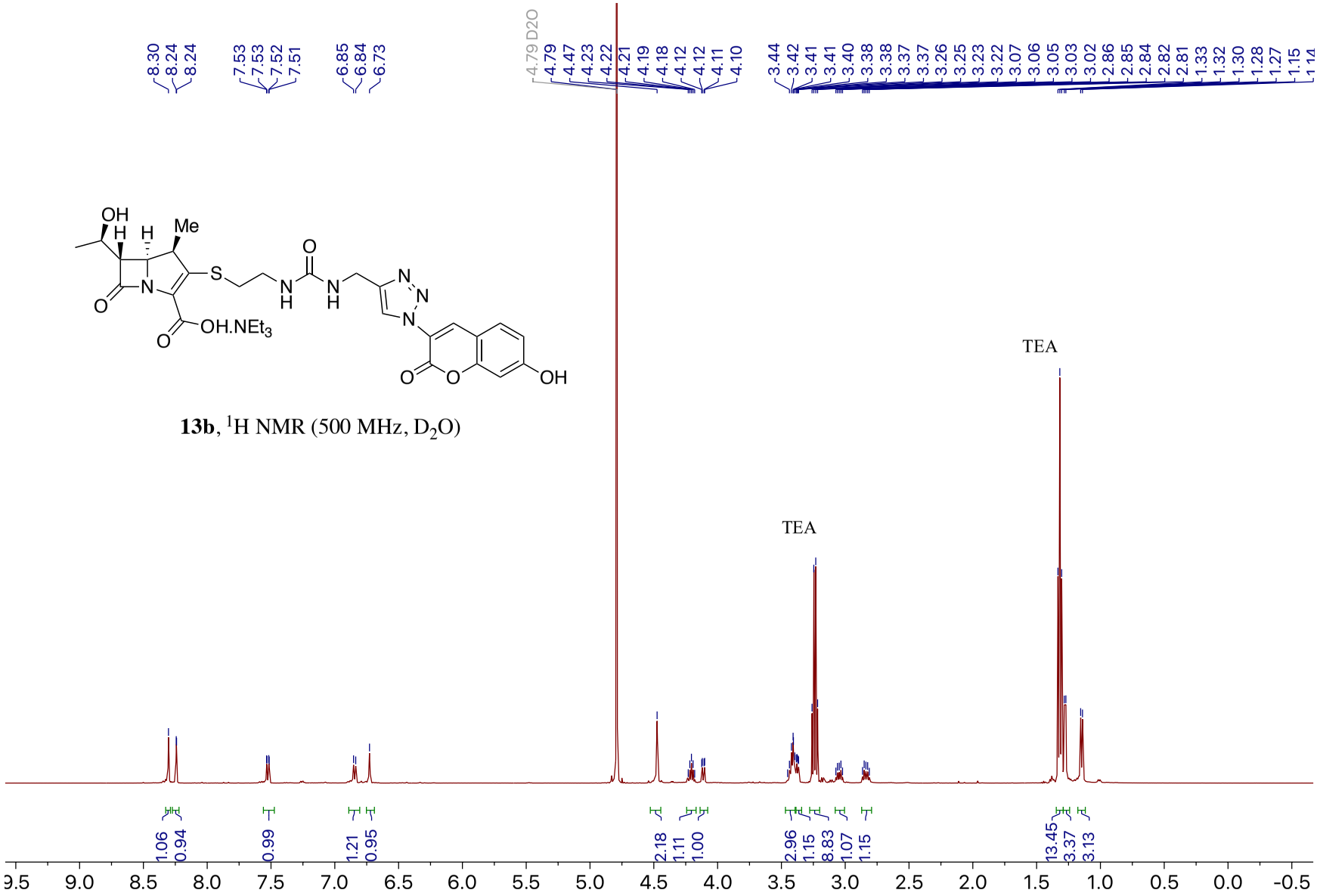
12d, ^{1}H NMR (500 MHz, $(CD_3)_2CO$)



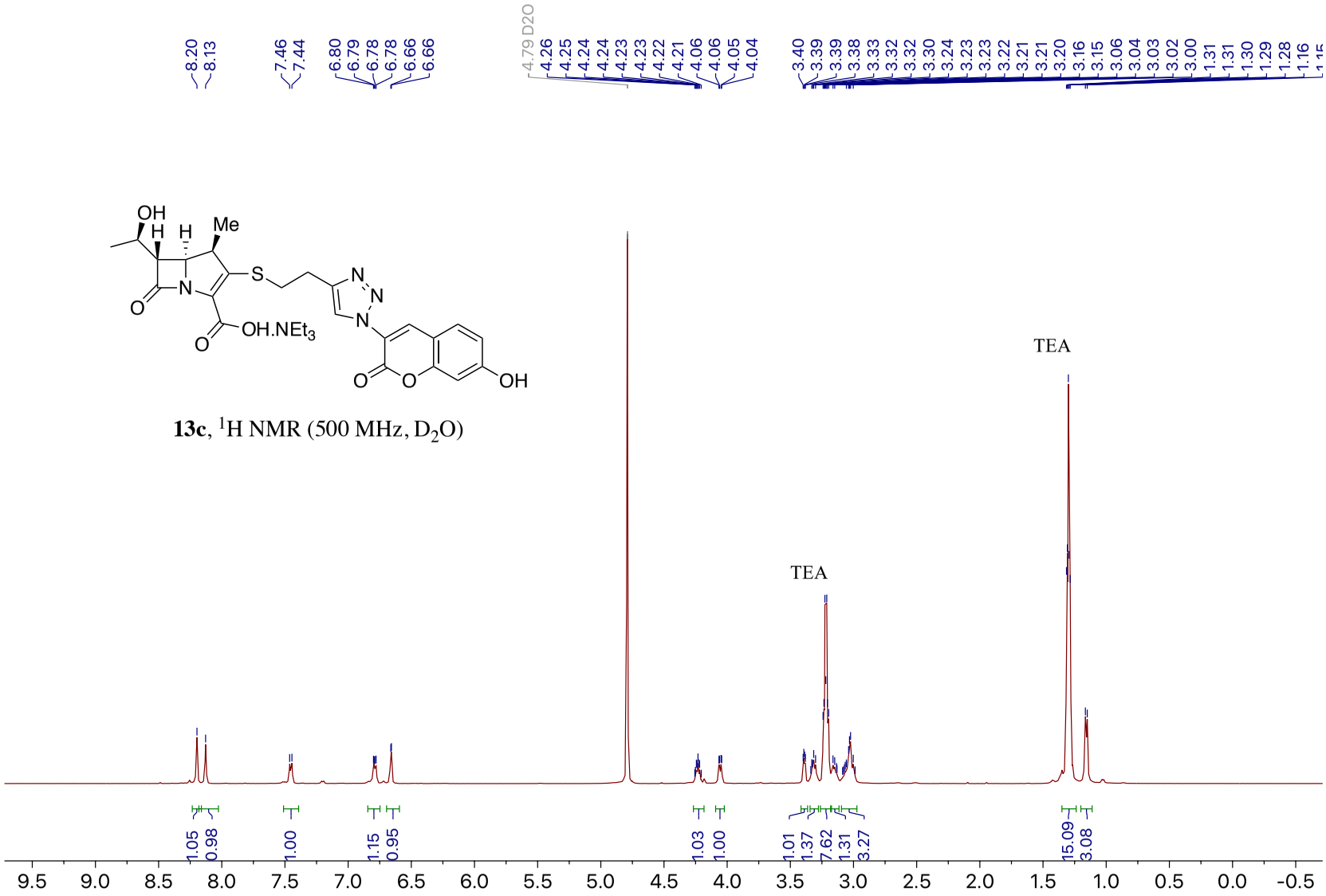
12d, ^{13}C NMR (125 MHz, $(CD_3)_2CO$)



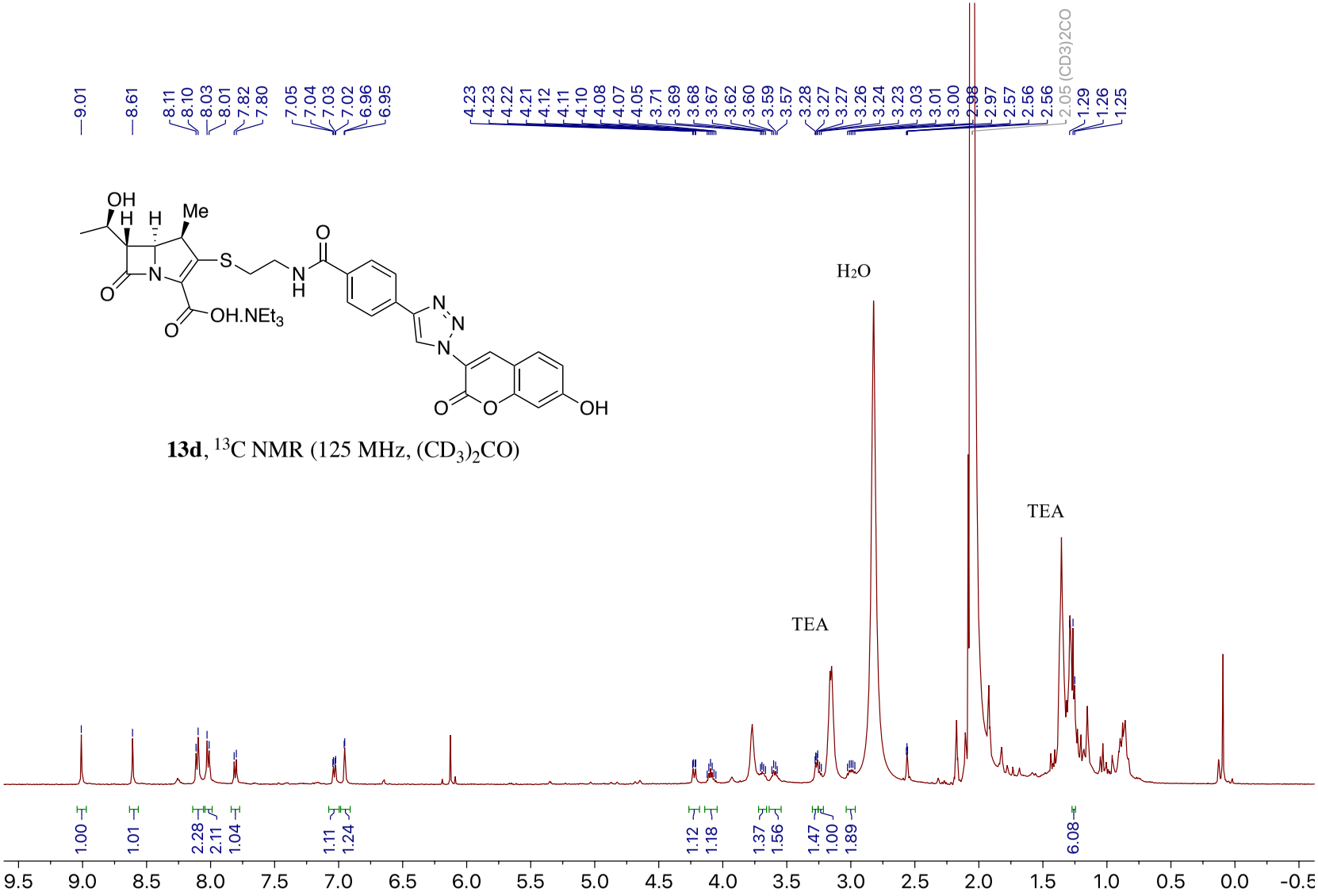
13a, ^{1}H NMR (500 MHz, D_2O)



13b, ^{1}H NMR (500 MHz, D_2O)



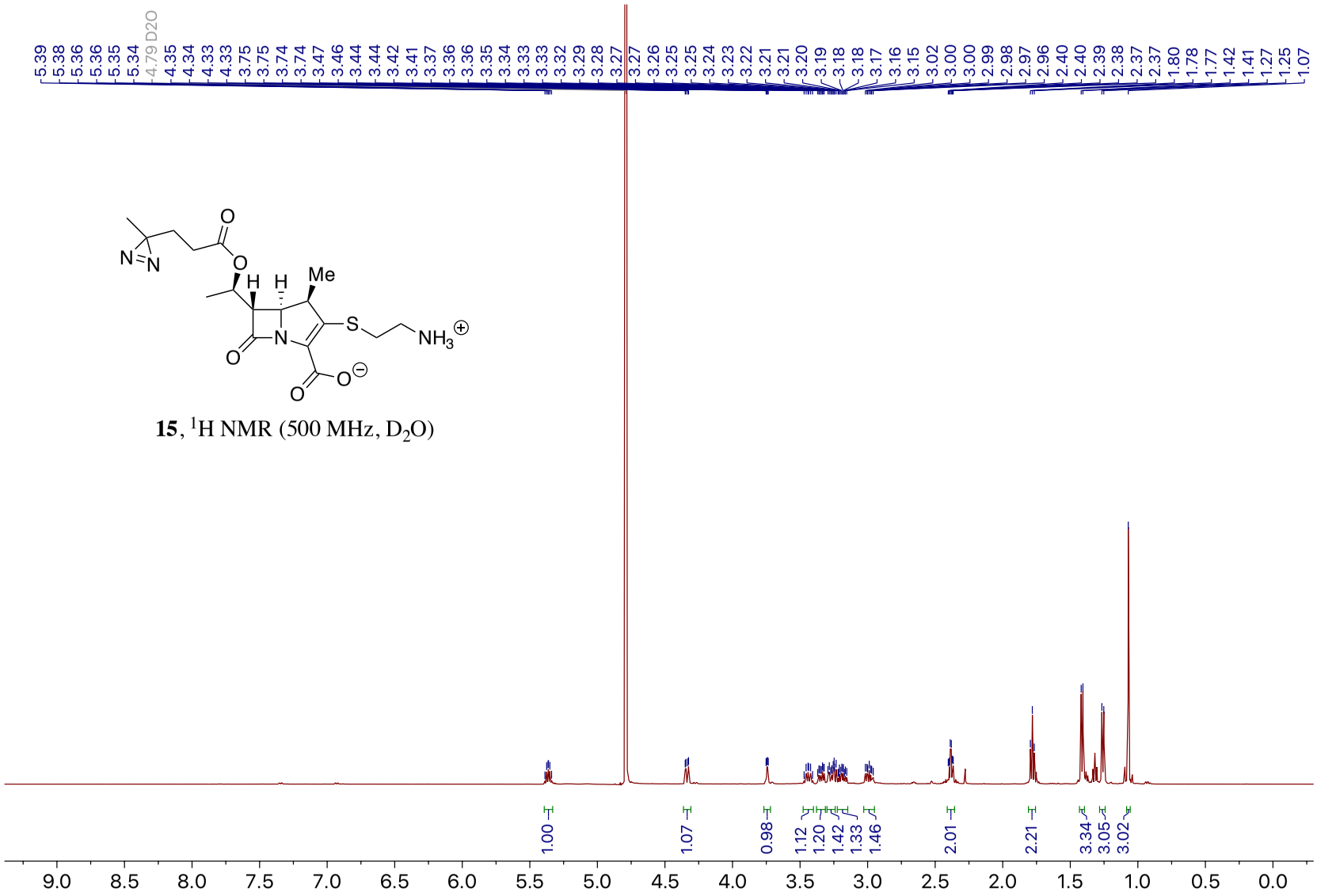
13c, ^{1}H NMR (500 MHz, D_2O)



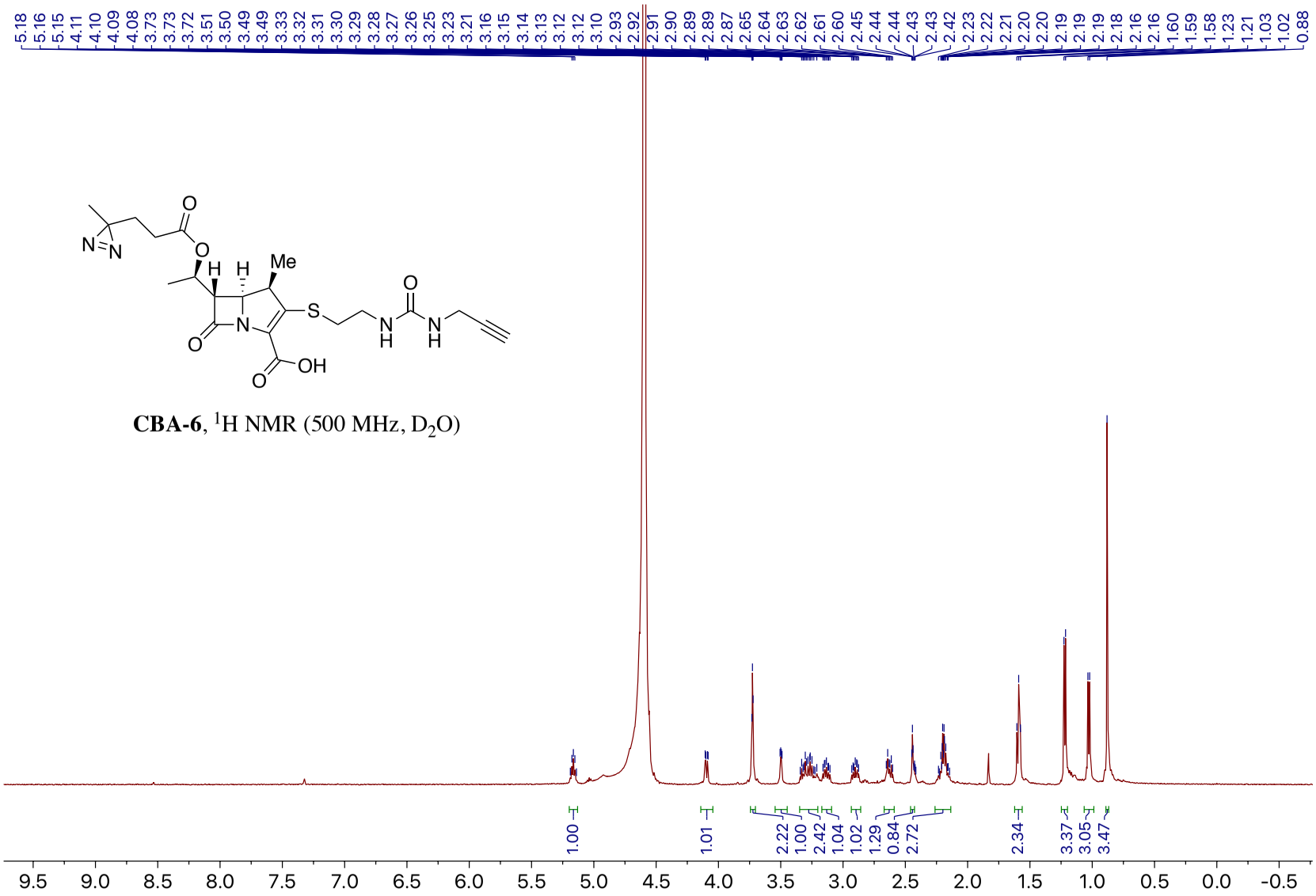
13d, ^{13}C NMR (125 MHz, $(CD_3)_2CO$)

14, ^{1}H NMR (500 MHz, $CDCl_3$)

14, ^{13}C NMR (125 MHz, $CDCl_3$)



15, ^{1}H NMR (500 MHz, D_2O)



CBA-6, ^{1}H NMR (500 MHz, D_2O)