Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

for

Biotin-guided anticancer drug delivery with acidity-triggered drug release

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Experimental Section

Synthetic Materials, methods and instrumentations.

2-chloroethanol (Fluka), sodium azide (Aldrich), biotin (Aldrich), EDC.HCl (GLS), 4-dimethylaminopyridine (Aldrich), 4-nitrosalicyclic acid (Aldrich), 1-butanol (Aldrich), N,N'-dicyclohexylcarbodiimide (Fluka), propargyl bromide (TCI), potassium carbonate (Aldrich), trifluoroacetic acid (Acros), *tert*-butyl carbazate (Alfa aesor), HATU (Iris Biotech), *N,N*-diisopropylethylamine (Aldrich), L-ascorbic acid sodium salt (TCI), copper sulphate pentahydrate (Duksan), doxorubicin·HCl (Aldrich) were received and were used without further purification. Most of the reactions were carried out under nitrogen atmosphere. Only compound **7** was synthesized under argon atmosphere. Compounds **1**, **3** and prodrug **9** containing the hydrazone bond were synthesized according to previously reported procedures. Silica gel 60 (Merck, 0.063~0.2 mm) was used for column chromatography as a stationary phase. Analytical thin layer chromatography was performed using Merck 60 F254 silica gel (precoated sheets, 0.25 mm thick). The ESI mass spectra were obtained using a LC/MS-2020 Series (Shimadzu). ^{1}H and ^{13}C NMR spectra were collected in $CDCl_3$ or DMSO-d6 in a Varian 300 and 400 MHz NMR spectrometer. All chemical shifts are reported in ppm value using the peak of TMS as an internal reference.

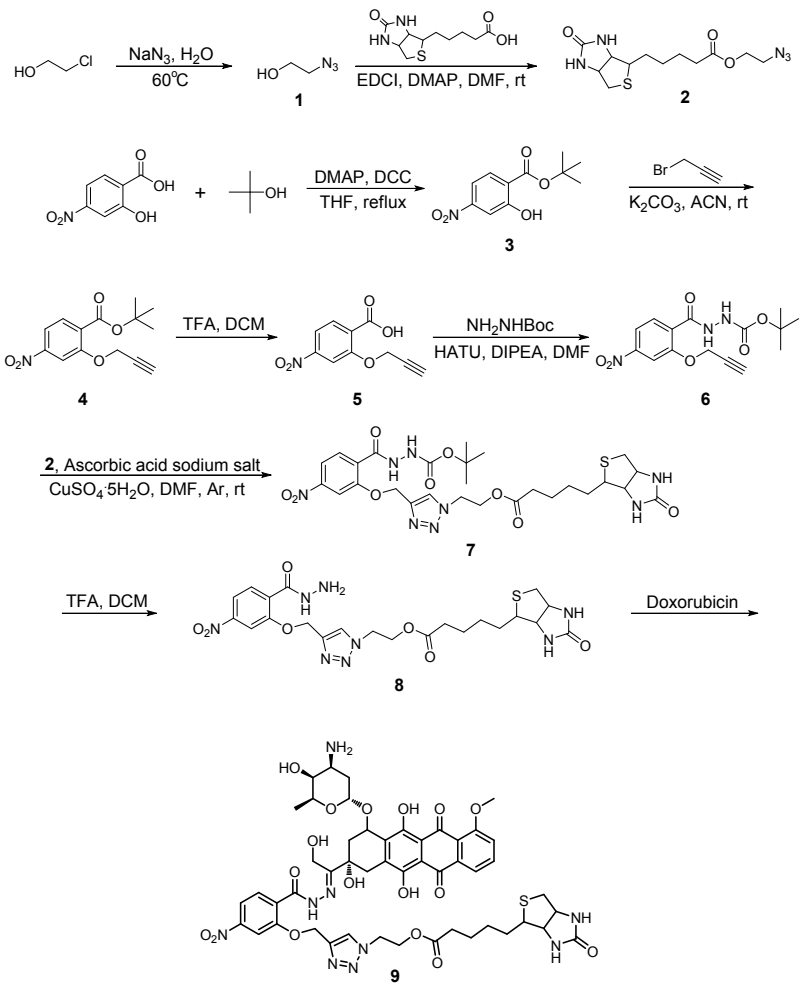
Spectroscopic measurements.

Stock solutions of prodrug **9** and free doxorubicin were prepared in DMSO. Excitation was carried out at 501 nm with all excitation and emission slit widths at 5 nm. The concentrations of each of the samples were fixed 5 μM at a total volume of 3 mL, while 20 μM samples were used in the UV/Vis spectra.

Cell line and cell culture.

In this study, HepG2 (human hepatocellular carcinoma) and, WI 38 (human lung fibroblast) were used. HepG2 and WI-38 cell lines were cultured in RPMI 1640 containing 10% FBS and 1% penicillin-streptomycin. Cells were grown at 37°C under a humidified atmosphere containing 5% CO_2.

Synthesis



Prodrug **9** was synthesized according to this scheme.

Preparation and Characterization of 1

A solution of 2-chloroethanol (20.0 g, 248 mmol) and NaN_3 (48.3 g, 744 mmol) in water (200 mL) was stirred at 60 °C for 48 h. After the solution was allowed to cool to room temperature, the crude product was extracted with diethyl ether (3 × 300 mL) to afford, after evaporation of the solvent under reduced pressure and at room temperature, 2-azidoethanol as a colorless liquid (15.5 g, 71.9%). CAUTION: Handling this low molar mass azide is hazardous as [nC+nO]/nN = 1.42. Therefore, it should be manipulated with extreme caution, and the crude product was thus directly used in the next step without further purification, handling, or storage.

Preparation and Characterization of 2

Biotin (2.24 g, 9.19 mmol) was dissolved in 20 mL DMF and a solution of compound **1** (1.00 g, 11.5 mmol) in DMF (3 mL) was added followed by EDC·HCl (5.35 g, 34.4 mmol) and, DMAP (9.12 g, 74.6 mmol). After being stirred for 16 h at room temperature, the reaction mixture was concentrated in vacuo and diluted with 30 mL of ethyl acetate and the organic layer was washed by water (3 × 10 mL), dried over $MgSO_4$ and then concentrated in vacuo. The crude product was purified by column chromatography DCM/methanol (9:1) to obtain the product 1.91 g (66.3%) ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.29 (s, 1H), 5.98 (s, 1H), 4.47–4.43 (m, 1H), 4.27–4.23 (m, 1H), 4.19 (t, J = 5.01 Hz, 2H), 3.44 (t, J = 5.22 Hz, 2H), 3.13–3.07 (m, 1H), 2.88–2.82 (m, 1H), 2.71–2.67 (m, 2H), 2.34 (t, J = 7.44 Hz, 2H), 1.59–1.69 (m, 4H), 1.37–1.45 (m, 2H). ^{13}C ($CDCl_3$, 100 MHz): 173.6, 163.8, 63.1, 62.1, 55.6, 50.0, 40.8, 33.9, 28.4, 24.9 ppm. ESI-MS: m/z calcd for $C_{12}H_{19}N_5O_3S$ [M+Na+], 336.11; found 336.

Preparation and Characterization of 3

DCC (1.10 equiv, 1.86 g, 9.00 mmol) dissolved in dry THF (12.0 mL) was added dropwise over 7 min to a stirred solution of 4-nitrosalicyclic acid (1.00 equiv, 1.50 g, 8.20 mmol) and DMAP (1.00 eqiuv, 990 mg, 8.20 mmol) in dry tert-butyl alcohol (30.0 mL). The mixture was stirred and heated to reflux overnight. The reaction was monitored by TLC. After the reaction was complete, the solvents were removed under reduced pressure. Then 15 mL of ethyl acetate was added, and the solution was filtered to remove the 1,3-dicyclohexylurea (DCU) formed. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography using ether/ethyl acetate (30:1) as the eluent. Pale yellow crystals, 1.62 g (81.7% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.27 (s, 1H), 7.94 (d, J = 8.61 Hz, 1H), 7.78 (s, 1H), 7.67 (d, J = 8.64 Hz, 1H), 1.64 (s, 9H).

Preparation and Characterization of 4

To a solution of Compound **3** (1.00 equiv, 1.50 g, 6.27 mmol) in CH_3CN (15.0 mL), K_2CO_3 (2.00 equiv, 1.73 g, 12.5 mmol) and propargyl bromide (1.20 equiv, 0.600 mL, 7.54 mmol) were added. The mixture was stirred for 15 hours at room temperature. The color changed to pale yellow. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography using ethyl acetate/hexane (1:9) as the eluent. Light yellow solid, 2.00 g (97.1% yield) ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.94 (d, J = 2.0 Hz, 1H), 7.88 (dd, J = 8.57, 1.97 Hz, 1H), 7.81 (d, J = 8.40 Hz, 1H), 4.87 (d, J = 2.44 Hz, 2H), 2.60 (t,

J = 2.39 Hz, 1H), 1.60 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): 164.1, 156.9, 150.2, 131.8, 129.3, 116.2, 109.2, 83.1, 77.3, 77.1, 57.4, 28.4 ppm.

Preparation and Characterization of 5

Compound **4** (850 mg, 3.07mmol) was dissolved in 8.00 mL of CH_2Cl_2 where trifluoroacetic acid (2.00 mL) was added. The reaction mixture was stirred for 4 hours at room temperature and subsequently the solvent was co-evaporated with toluene and the crude product was purified by column chromatography using DCM/methanol (9:1) as an eluent. White solid, 650 mg (95.8% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, J = 1.74 Hz, 1H), 7.93 (s, 1H), 7.91 (d, J = 1.74 Hz, 1H), 4.90 (d, J = 2.40 Hz, 2H), 2.63 (t, J = 2.45 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 169.8, 154.5, 147.4, 139.2, 129.6, 116.8, 109.1, 79.7, 79.4, 57.1 ppm. ESI-MS: m/z calcd for $C_{10}H_7NO_5$ [M-H+], 220.03; found 219.9.

Preparation and Characterization of 6

Compound **5** (1.00 equiv, 1.15 g, 4.35 mmol) was dissolved in 15.0 mL DMF. *tert*-Butyl carbazate (1.20 equiv, 688 mg, 5.21 mmol), HATU (1.20 equiv, 1.98 g, 5.21 mmol) and DIPEA (0.940 ml) were added. The reaction mixture was stirred for 5 hours at room temperature. After removal of the solvent under reduced pressure, the crude product was diluted by 30.0 mL of ethyl acetate and the organic layer was washed by water (3 × 10.0 mL), dried over $MgSO_4$ and then concentrated in vacuum. The crude product was purified by column chromatography ethyl acetate/hexane (1:3) to obtain the product 1.34 g (92.0%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.36 (s, 1H), 8.39 (d, J = 8.52 Hz, 1H), 8.00 (dd, J = 4.93, 2.02 Hz, 1H), 7.97 (d, J = 2.02 Hz, 1H), 6.98 (s, 1H), 5.01 (d, J = 2.40 Hz, 2H), 2.70 (t, J = 2.40 Hz, 1H), 1.51 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.1, 155.7, 155.1, 150.8, 134.1, 125.6, 117.1, 108.6, 82.4, 78.8, 76.0, 57.8, 28.4 ppm. ESI-MS: m/z calcd for $C_{15}H_{17}N_3O_6$ [M-H+], 334.11; found 333.95.

Preparation and Characterization of 7

To a solution of compound **6** (1.00 equiv, 264 mg, 0.790 mmol) in DMF (5.00 mL), **2** (1.10 eqiuv, 270 mg, 0.860 mmol) and sodium ascorbate (1.00 equiv, 156 mg, 0.790 mmol) were added. The reaction mixture was degassed for 30 min by purging argon gas. Then $CuSO_4.\cdot 5H_2O$ (1.00 equiv, 197 mg, 0.790 mmol) in DMF (3.00 mL) was added to the reaction mixture and the mixture was stirred for 2 hours. After removal of solvent under

reduced pressure, the crude product was diluted by 30.0 mL of ethyl acetate and the organic layer was washed by water (3 × 10.0 mL), dried over $MgSO_4$ and then concentrated in vacuum. The crude product was purified by column chromatography DCM/methanol (9:1) to obtain the product 380 mg (74.4%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.22 (d, J = 8.63 Hz, 1H), 8.06 (s, 1H), 7.92 (dd, J = 8.63, 1.92 Hz, 1H), 6.38 (s, 1H), 5.57 (s, 2H), 4.70-4.59 (m, 2H), 4.55-4.44 (m, 3H), 4.37-4.28 (m, 1H), 3.14 (dd, J = 7.20, 4.56 Hz, 1H), 2. 92 (dd, J = 13.3, 4.79 Hz, 1H), 2.71 (d, J = 12.7 Hz, 1H), 2.27 (t, J = 7.08 Hz, 2H), 1.62-1.56 (m, 4H), 1.5 (s, 9H), 0.92-0.80 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 173.3, 164.4, 163.5, 156.5, 155.8, 150.7, 142.4, 133.3, 126.6, 124.8, 116.7, 108.9, 81.8, 64.3, 62.3, 62.1, 60.4, 55.9, 49.7, 40.8, 33.6, 29.9, 28.4, 28.3, 24.7 ppm. ESI-MS: m/z calcd for $C_{27}H_{36}N_8O_9S$ [M+Na+], 671.23; found 671.30.

Preparation and Characterization of 8

Compound **7** (550 mg, 0.850 mmol) was dissolved in a mixture of 8.00 mL CH_2Cl_2. And trifluoroacetic acid (2.00 mL). The reaction mixture was stirred for 4 hours at room temperature. The solvent was co-evaporated with toluene and the crude product was purified by column chromatography DCM/methanol (9:1) as the eluent. The product was obtained 330 mg (70.9% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.22 (d, J = 8.68 Hz, 1H), 8.04 (d, J = 1.77 Hz, 1H), 7.98 (s, 1H), 7.91 (dd, J = 8.68, 1.77 Hz, 1H), 5.49 (s, 2H), 4.70 (t, J = 4.61 Hz, 2H), 4.56-4.49 (m, 3H), 4.35-4.29(m, 1H), 3.21-3.11 (m, 1H), 2.92 (dd, J = 12.7, 4.96 Hz, 1H), 2.72 (d, J = 12.7 Hz, 1H), 2.30 (t, J = 6.91 Hz, 2H), 1.69-1.50 (m, 4H), 1.45-1.32 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 173.3, 164.7, 164.4, 156.3, 150.4, 142.1, 133.1, 126.5, 124.5, 116.6, 108.4, 77.8, 63.1, 62.2, 60.3, 55.8, 49.7, 40.5, 33.5, 29.8, 28.4, 24.7 ppm. ESI-MS: m/z calcd for $C_{22}H_{28}N_8O_7S$ [M+Na+], 571.18; found 571.25.

Preparation and Characterization of 9

Doxorubicin·HCl (1.00 equiv, 60.0 mg, 0.110 mmol) and **8** (1.30 equiv, 75.0 mg, 0.140 mmol) were dissolved in anhydrous methanol (15.0 mL) and the mixture was treated with TFA (7.80 µl). After stirring overnight at room temperature in the dark all volatiles were removed under reduced pressure, the residue was redissolved in methanol (5.00 mL) and diethyl ether (35.0 mL) was added to precipitate the product. The red solid was collected by centrifugation and dried in vacuum. 91.0 mg (60.5% yield). ^{1}H NMR (300 MHz, DMSO-d6): δ 8.28-8.20 (m, 1H), 7.94-7.89 (m, 2H), 7.88-7.85 (m, 1H), 7.83-7.77 (m, 2H), 7.61-7.53 (m,

1H), 5.55 (s, 1H), 5.43 (d, J = 9.16 Hz, 2H), 5.25 (t, J = 13.9 Hz, 2H), 4.58 (br, 2H), 4.49-4.43 (br, 1H), 4.39-4.31 (m, 1H), 4.28-4.22 (m, 2H), 4.07 (br, 1H), 3.93 (s, 3H), 3.08-2.93 (m, 4H), 2.76 (br, 2H), 2.23-2.10 (m, 4H), 1.91-1.80 (m, 2H), 1.75-1.65 (m, 2H), 1.57-1.49 (m, 2H), 1.43-1.31 (m, 4H), 1.25-1.13 (m, 4H), 1.04 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d6): 187.0, 186.9, 173.2, 163.4, 161.4, 159.9, 158.5, 157.0, 156.4, 155.1, 150.4, 142.1, 136.9, 136.5, 135.9, 135.8, 135.3, 133.4, 133.0, 128.1, 126.3, 120.5, 119.6, 116.6, 111.3, 111.1, 109.8, 100.0, 72.5, 71.9, 66.9, 66.8, 65.6, 62.7, 61.7, 59.8, 57.2, 56.0, 49.3, 47.3, 47.2, 33.7, 28.5, 24.9, 15.8 ppm. ESI-MS: m/z calcd for $C_{49}H_{55}N_9O_{17}S$ [M+H+] 1074.34, [M+Na+] 1096. 34; found 1074. 40, 1096.40.

H-NMR, ^{13}C-NMR and MS Analyses:

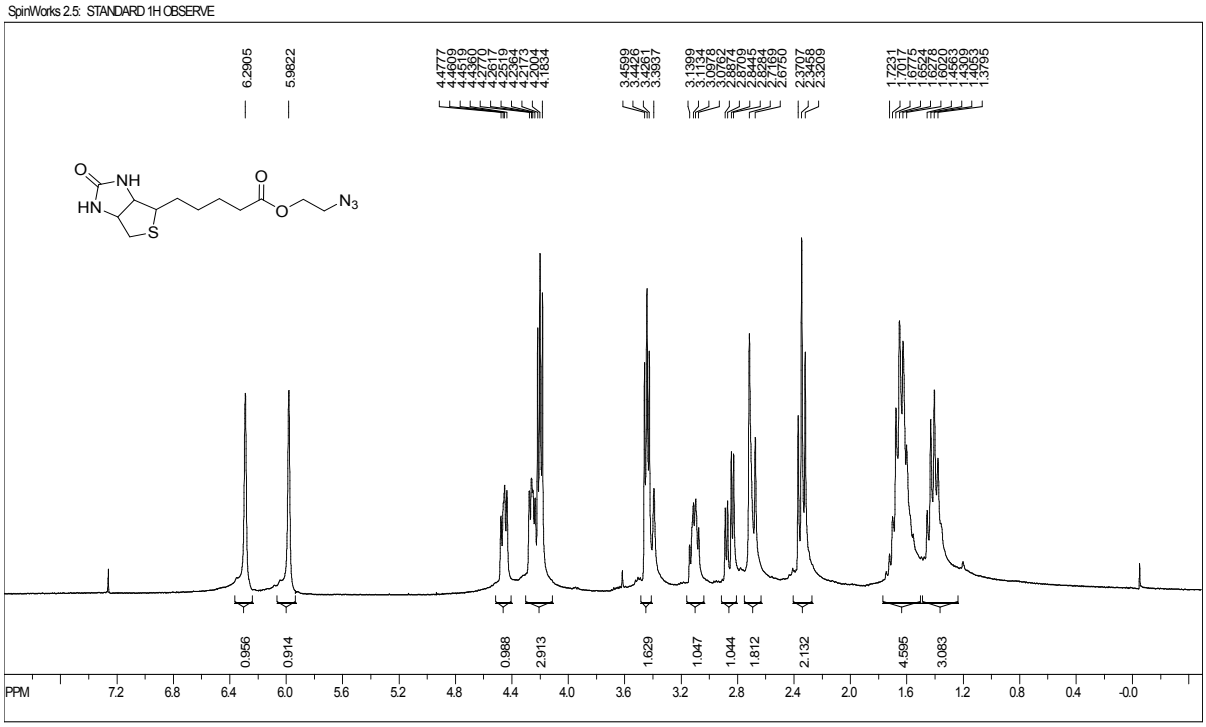


Fig. S1 ^{1}H NMR spectrum of compound **2** in $CDCl_3$.

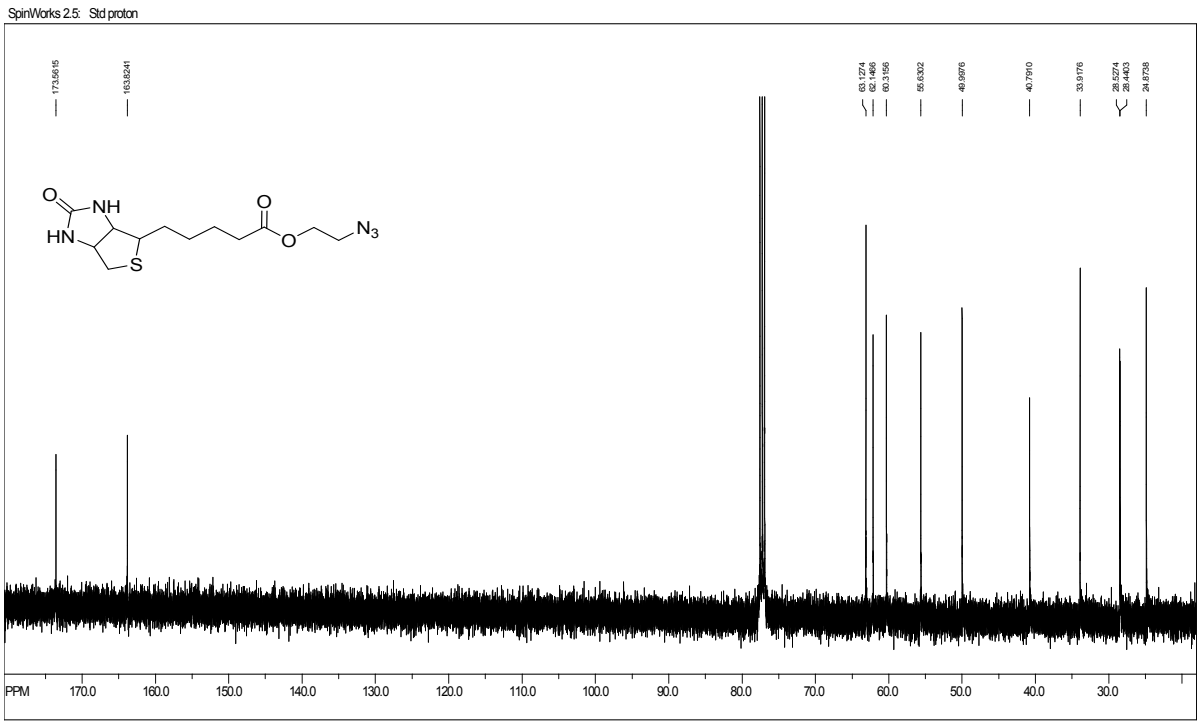


Fig. S2 ^{13}C NMR spectrum of compound **2** in $CDCl_3$.

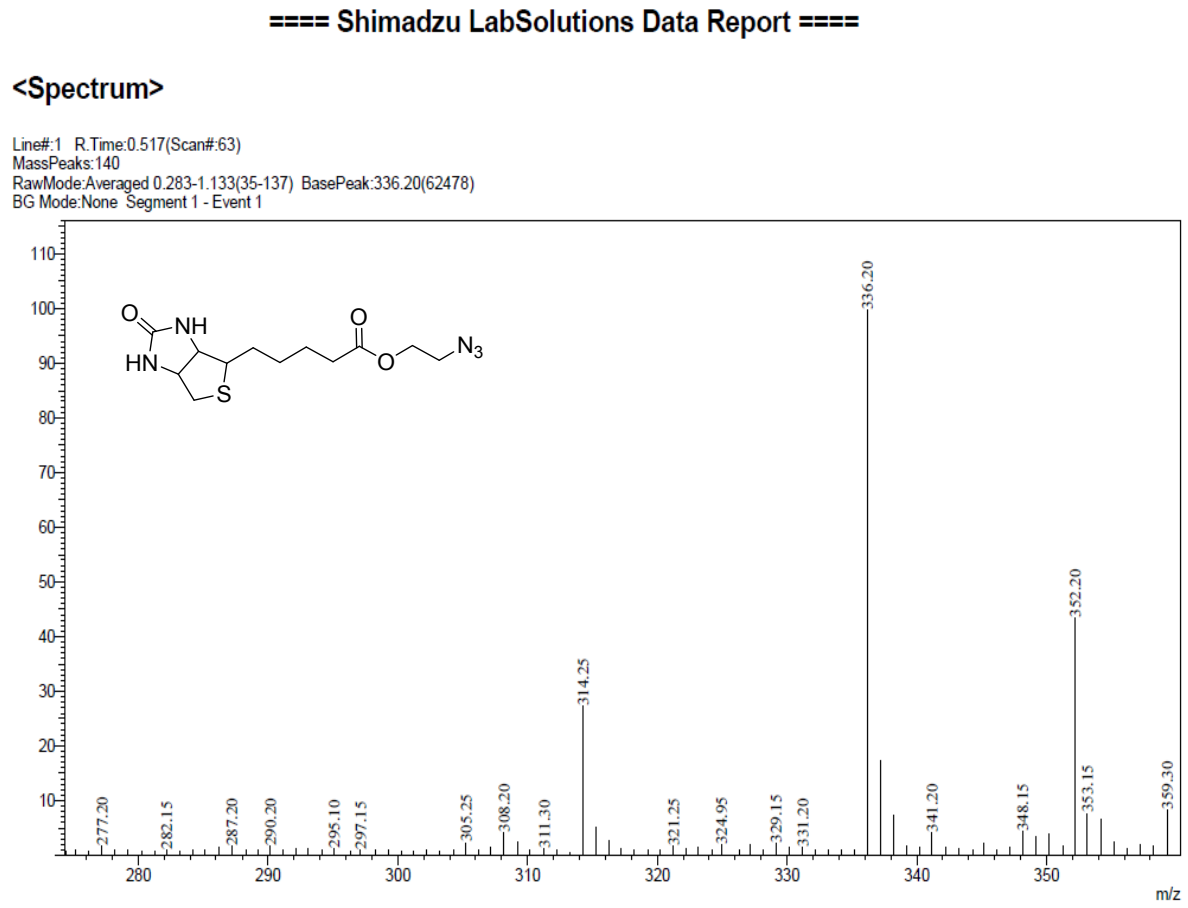


Fig. S3 Mass spectrum of compound **2**.

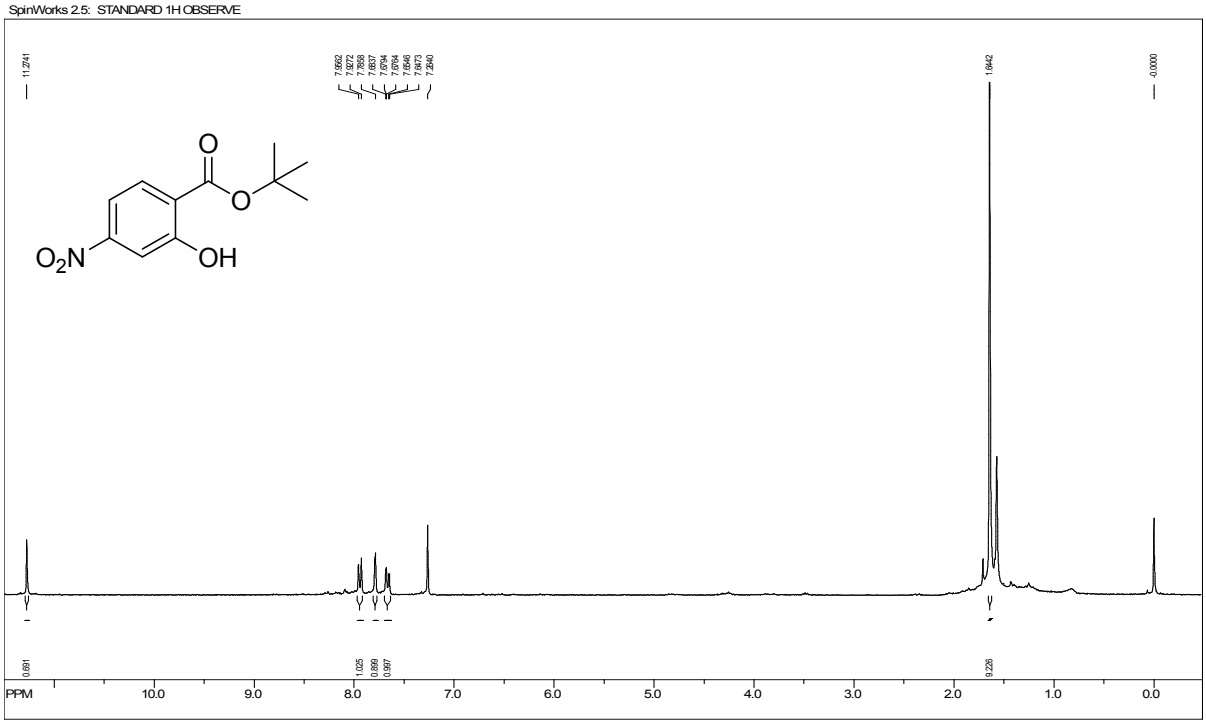


Fig. S4 ^{1}H NMR spectrum of compound **3** in $CDCl_3$.

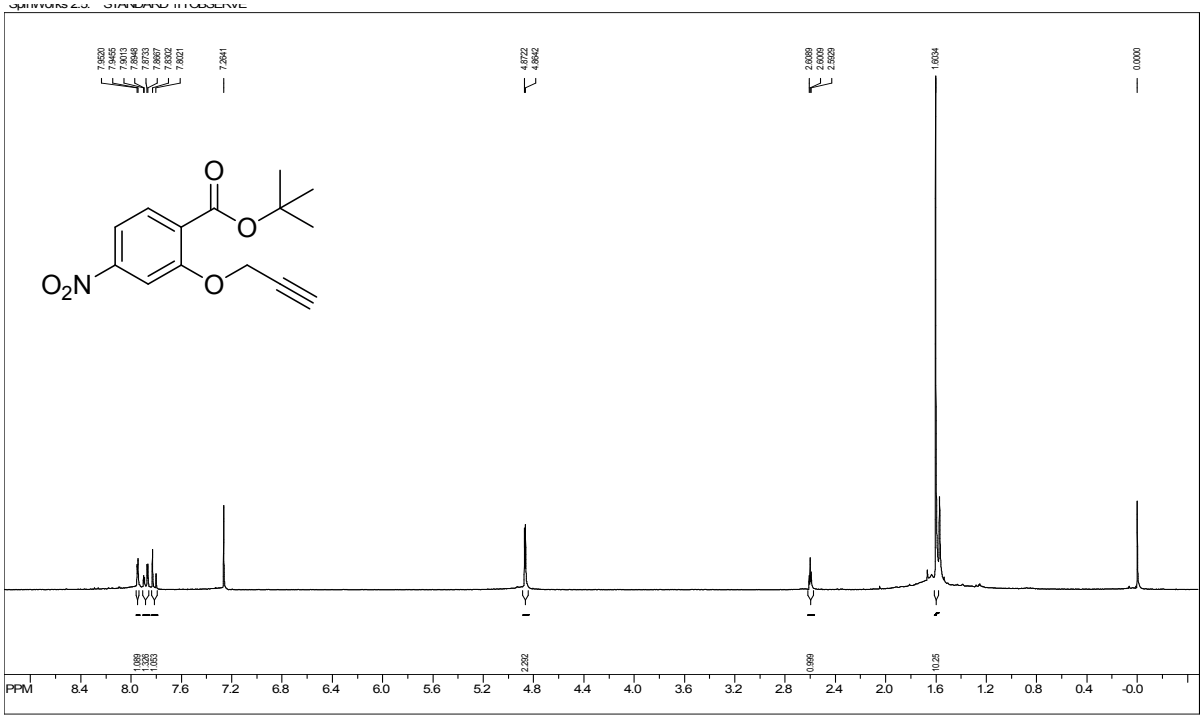


Fig. S5 ^{1}H NMR spectrum of compound **4** in $CDCl_3$.

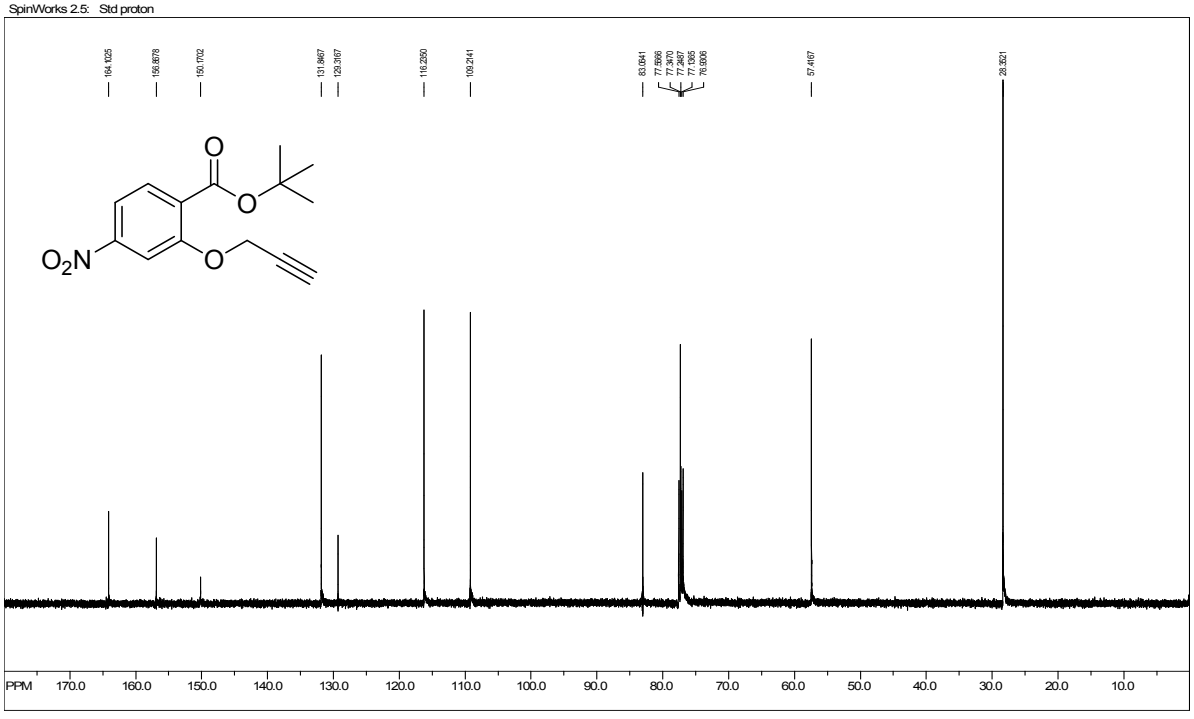


Fig. S6 ^{13}C NMR spectrum of compound **4** in $CDCl_3$.

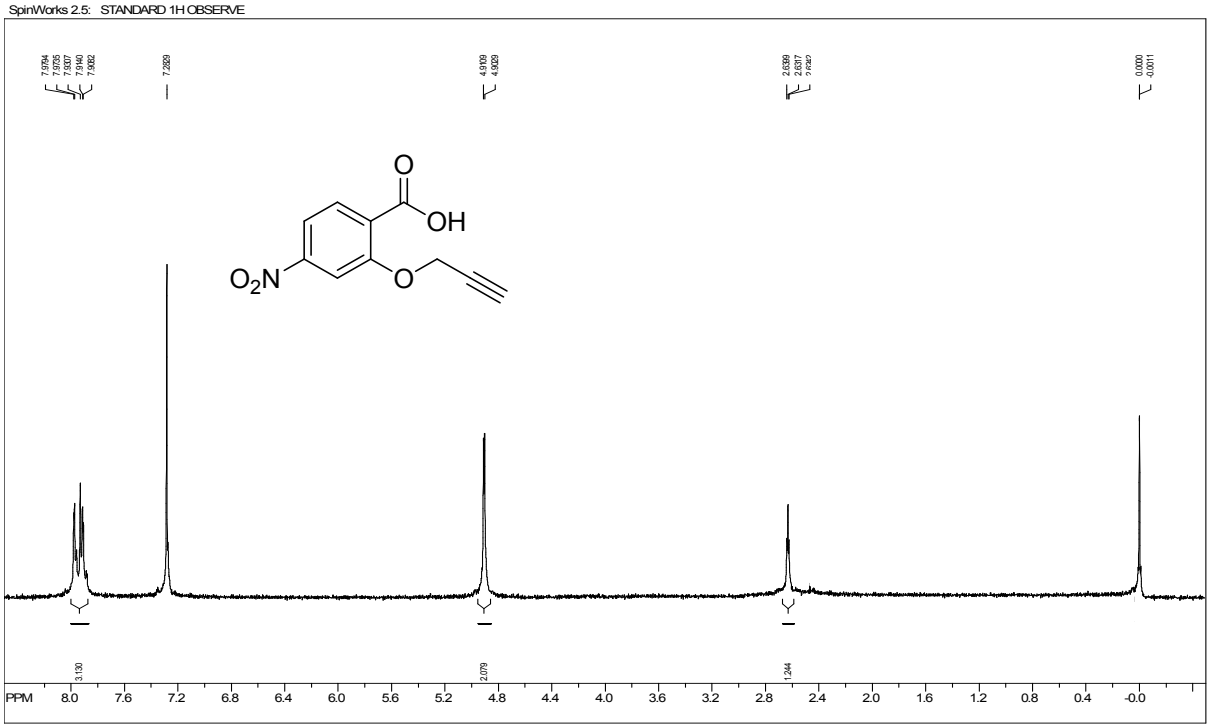


Fig. S7 ^{1}H NMR spectrum of compound **5** in $CDCl_3$.

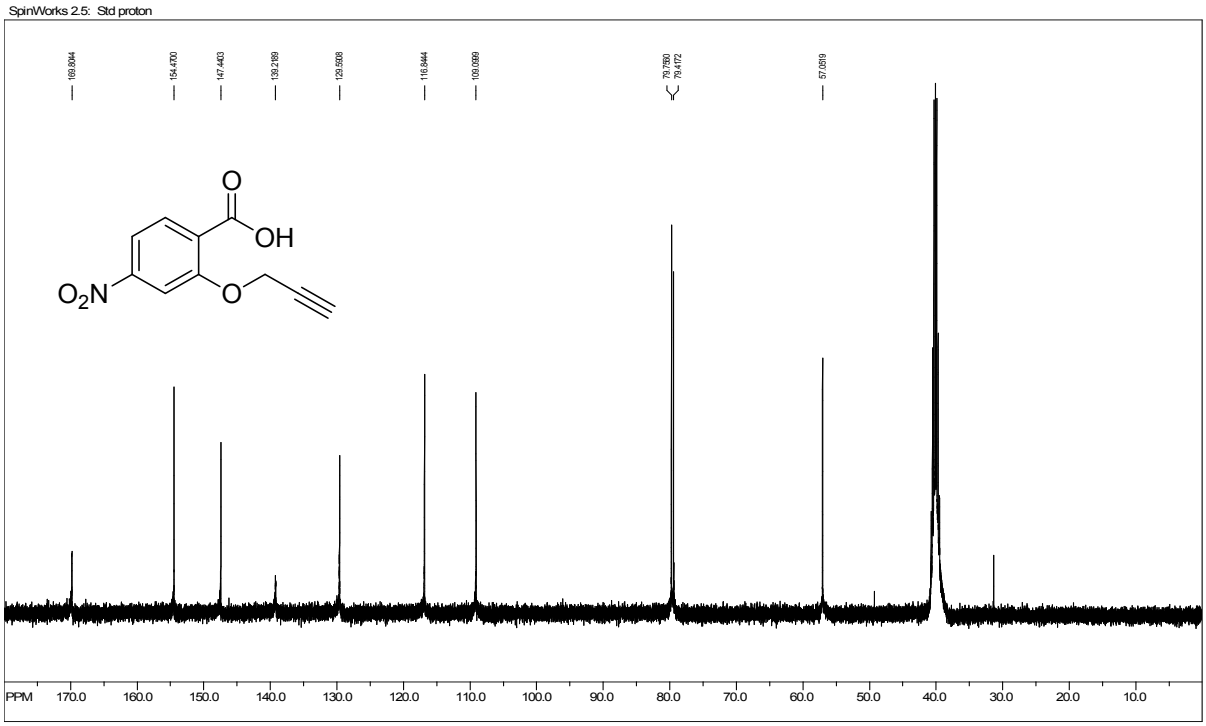


Fig. S8 ^{13}C NMR spectrum of compound **5** in $CDCl_3$.

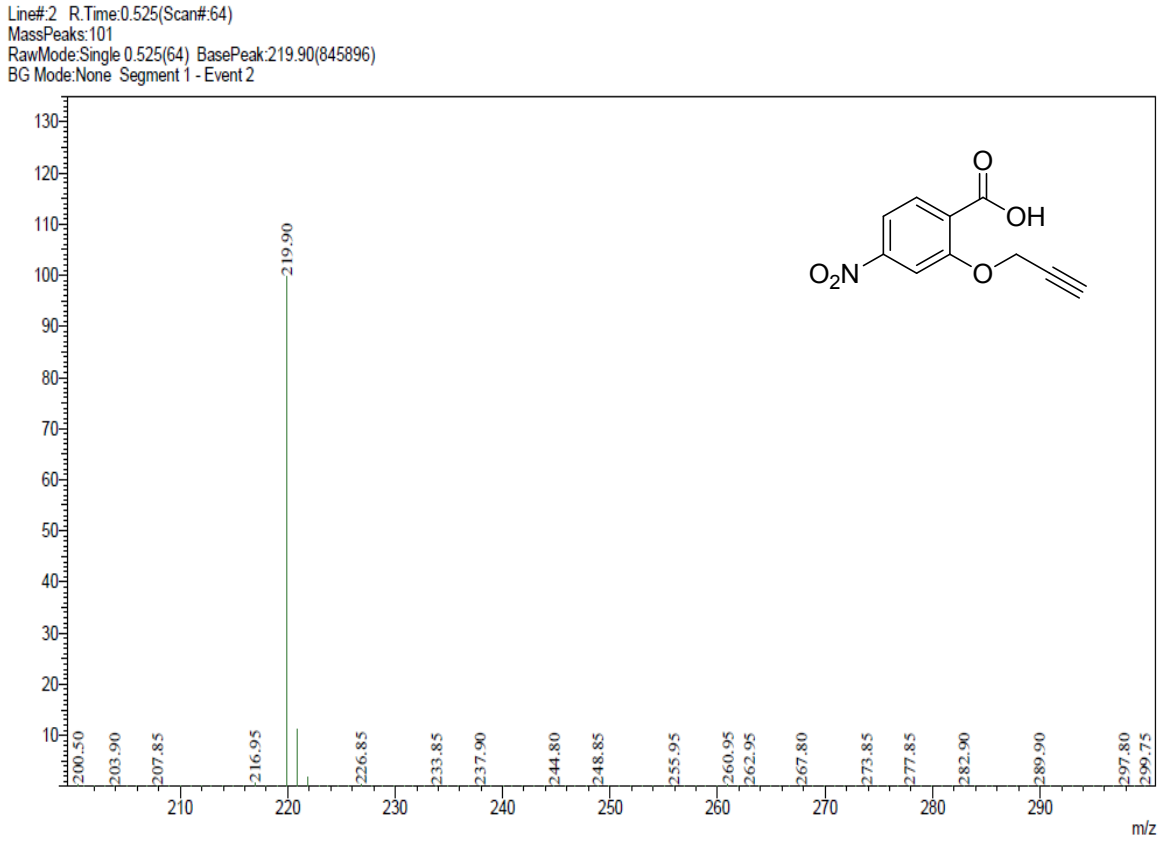


Fig. S9 Mass spectrum of compound **5**.

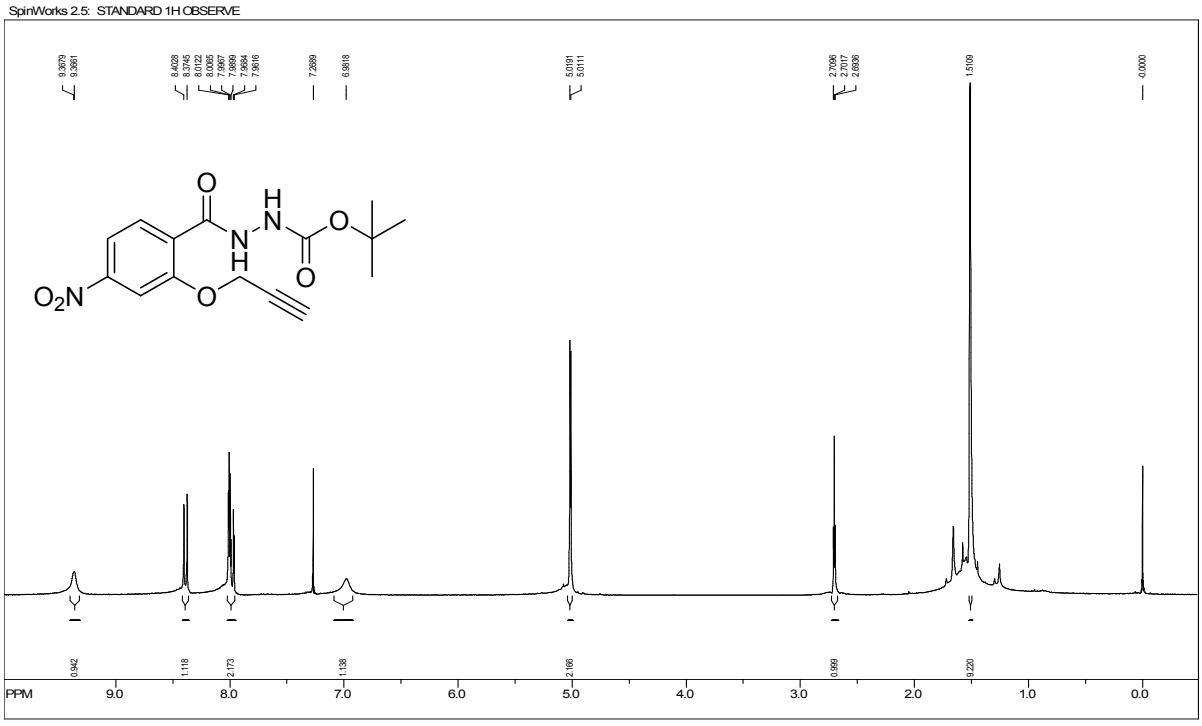


Fig. S10 ^{1}H NMR spectrum of compound **6** in $CDCl_3$.

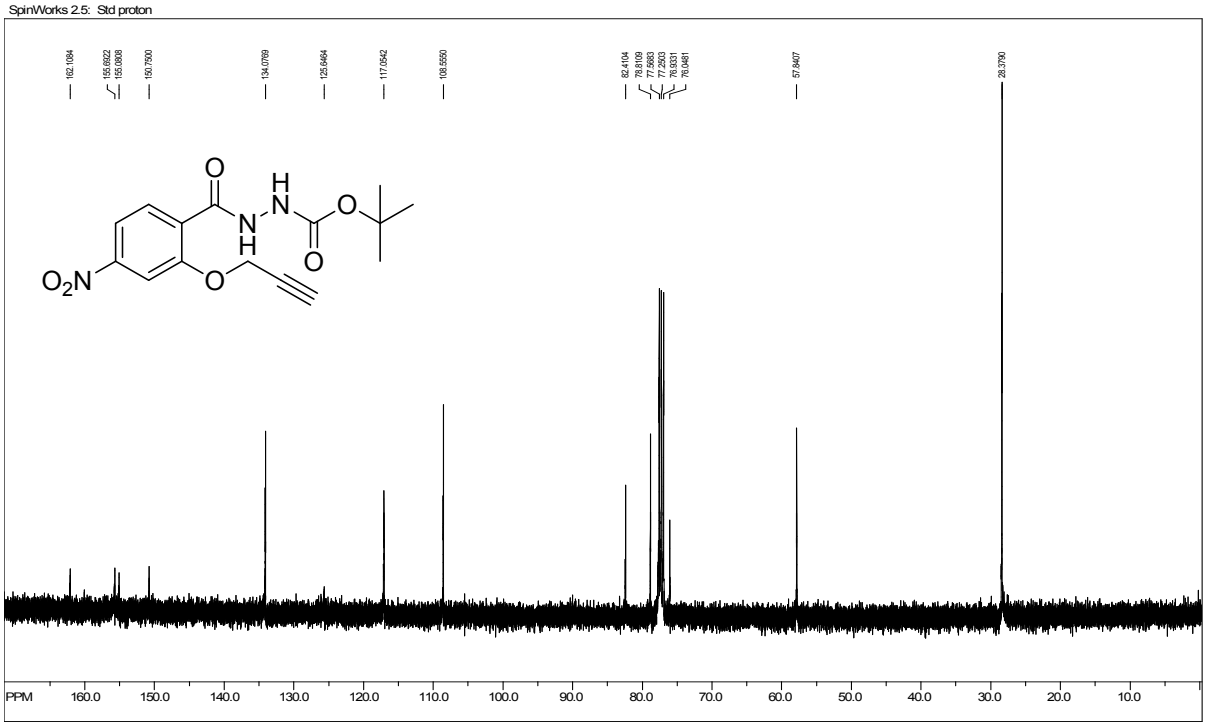


Fig. S11 ^{13}C NMR spectrum of compound **6** in $CDCl_3$.

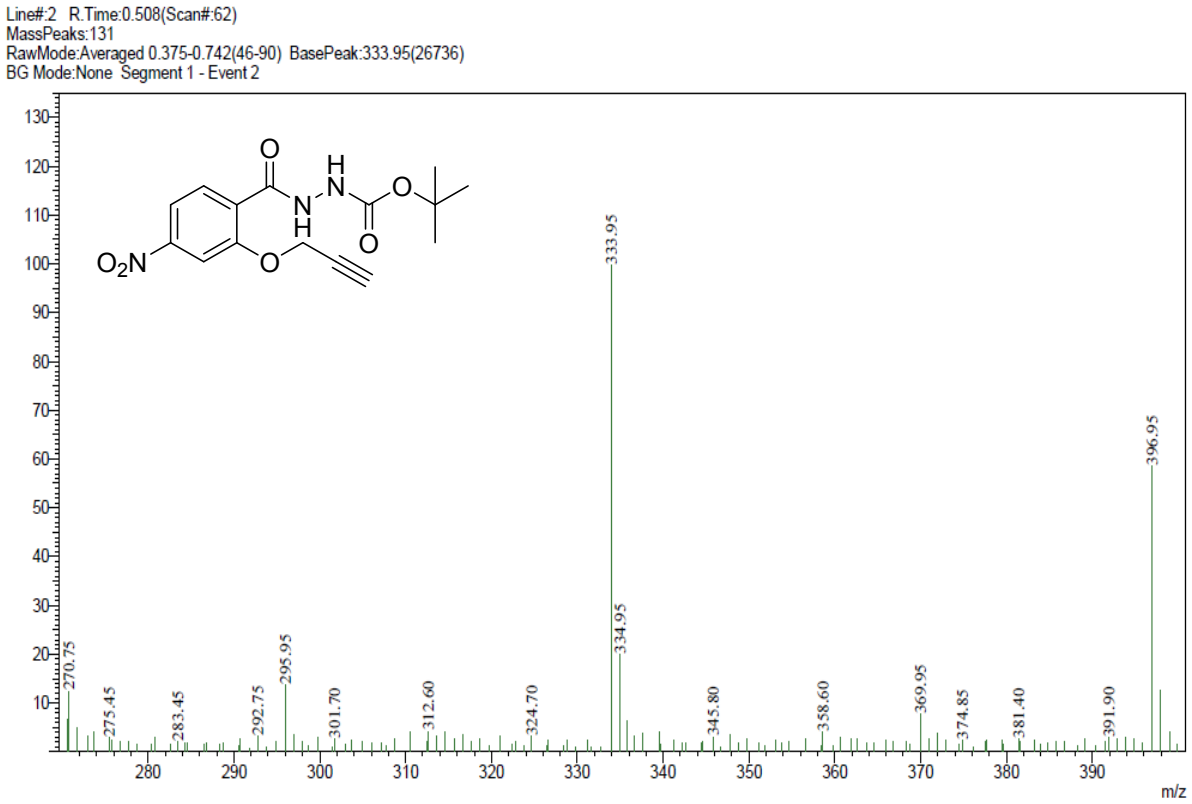


Fig. S12 Mass spectrum of compound **6**.

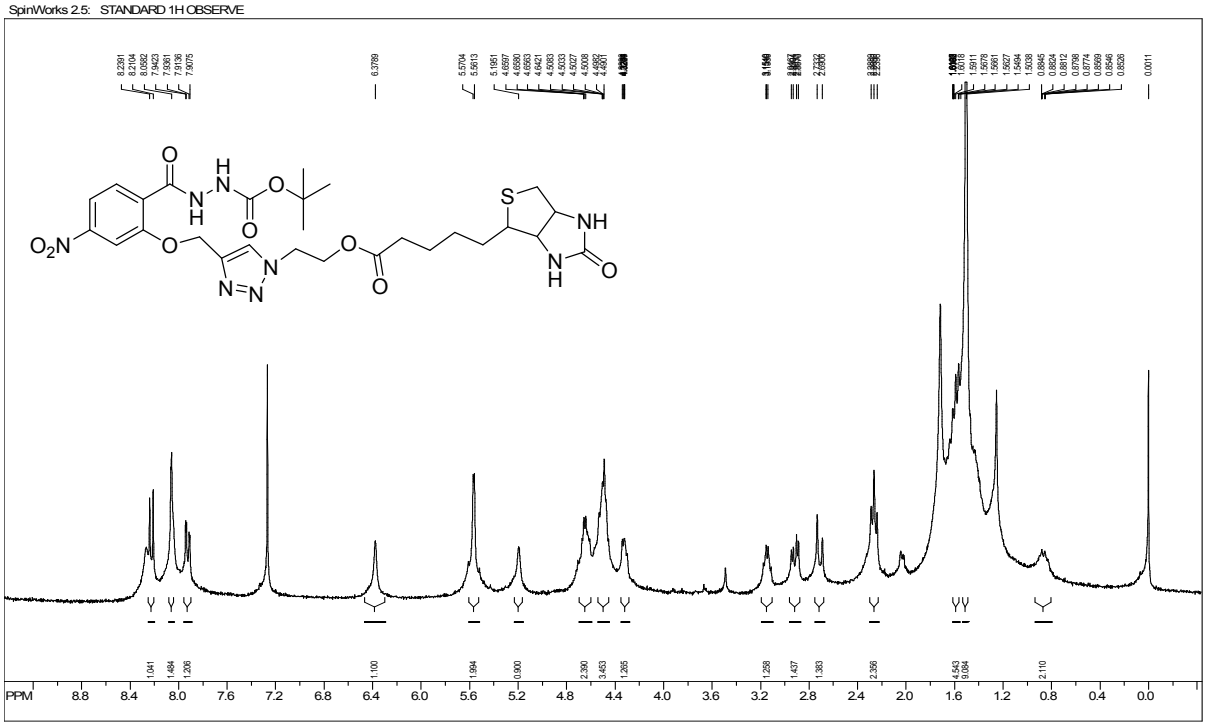


Fig. S13 ^{1}H NMR spectrum of compound **7** in $CDCl_3$.

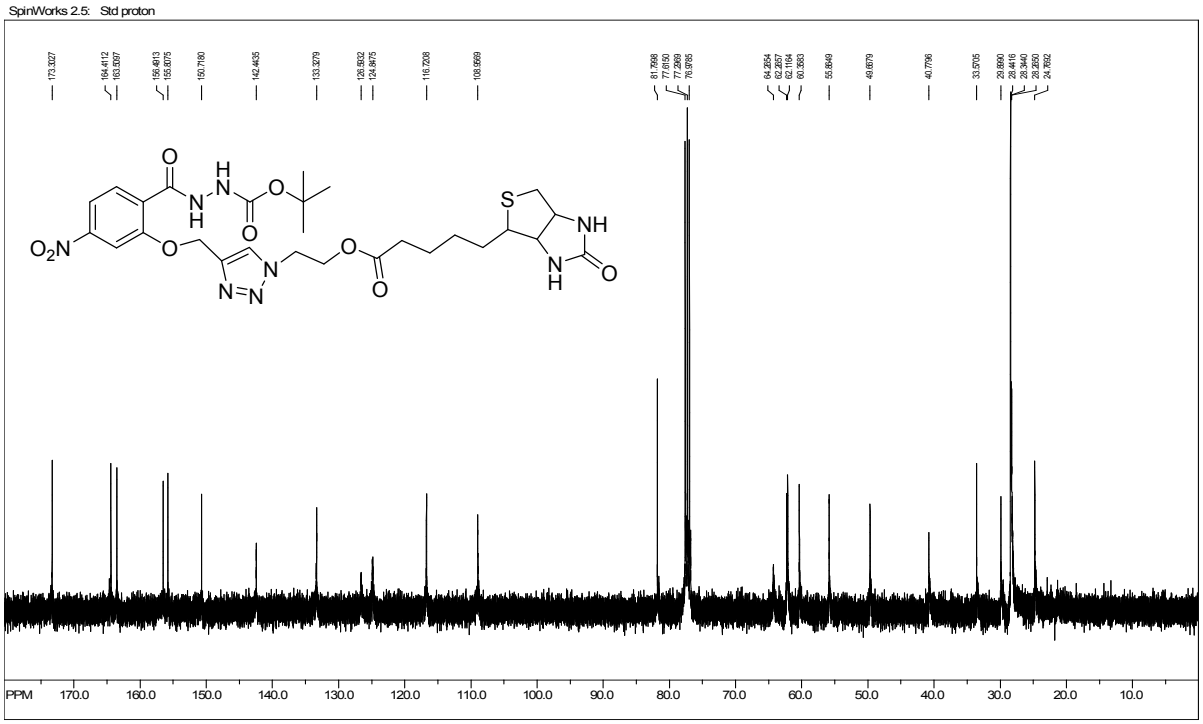


Fig. S14 ^{13}C NMR spectrum of compound **7** in $CDCl_3$.

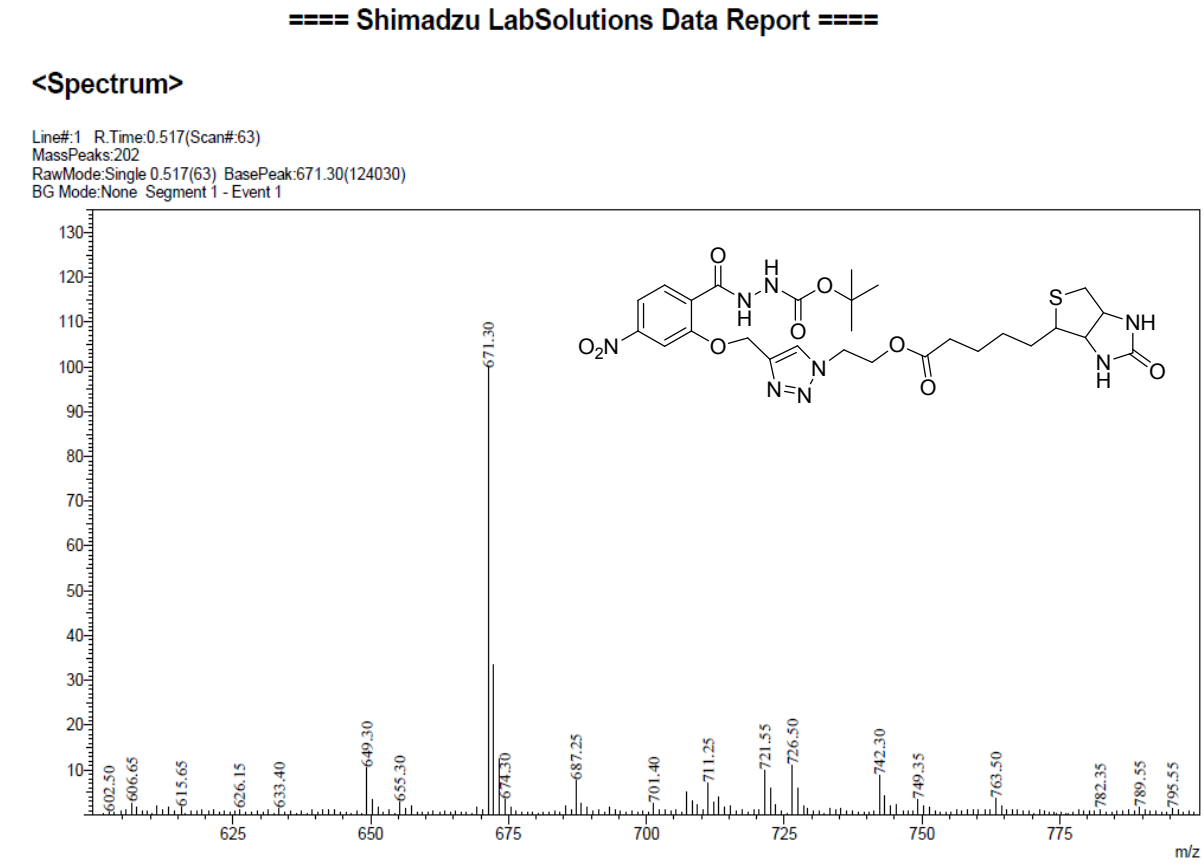


Fig. S15 Mass spectrum of compound **7**.

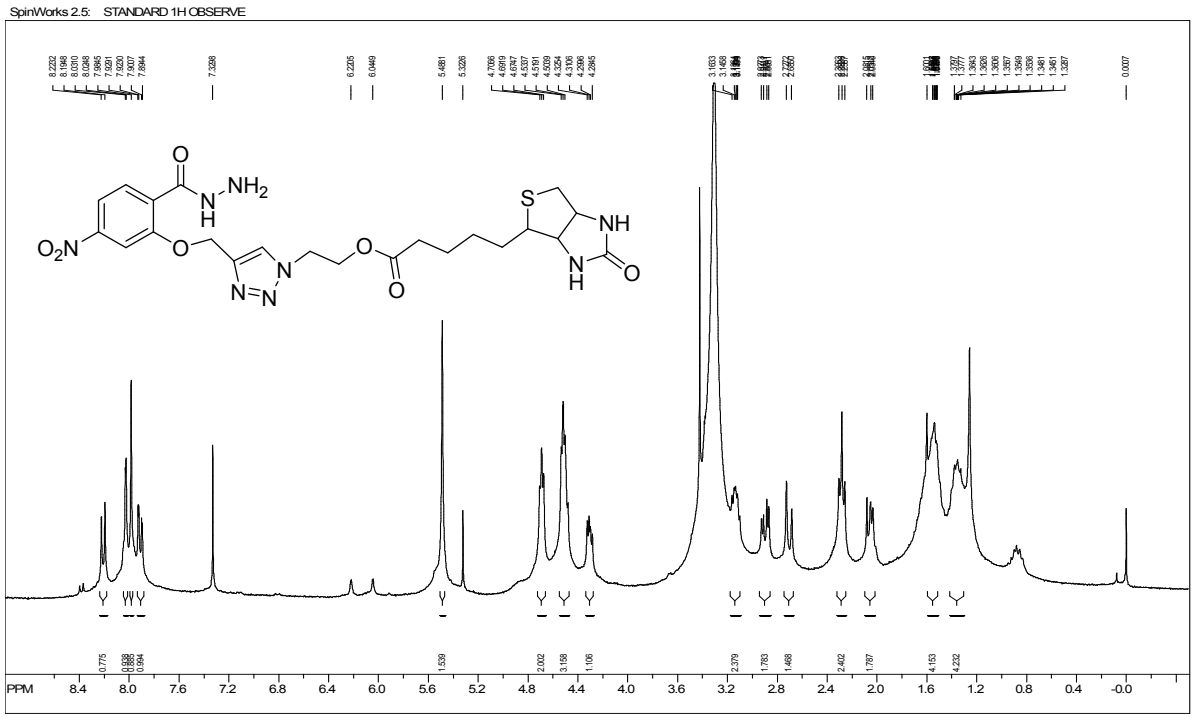


Fig. S16 ^{1}H NMR spectrum of compound **8** in $CDCl_3$.

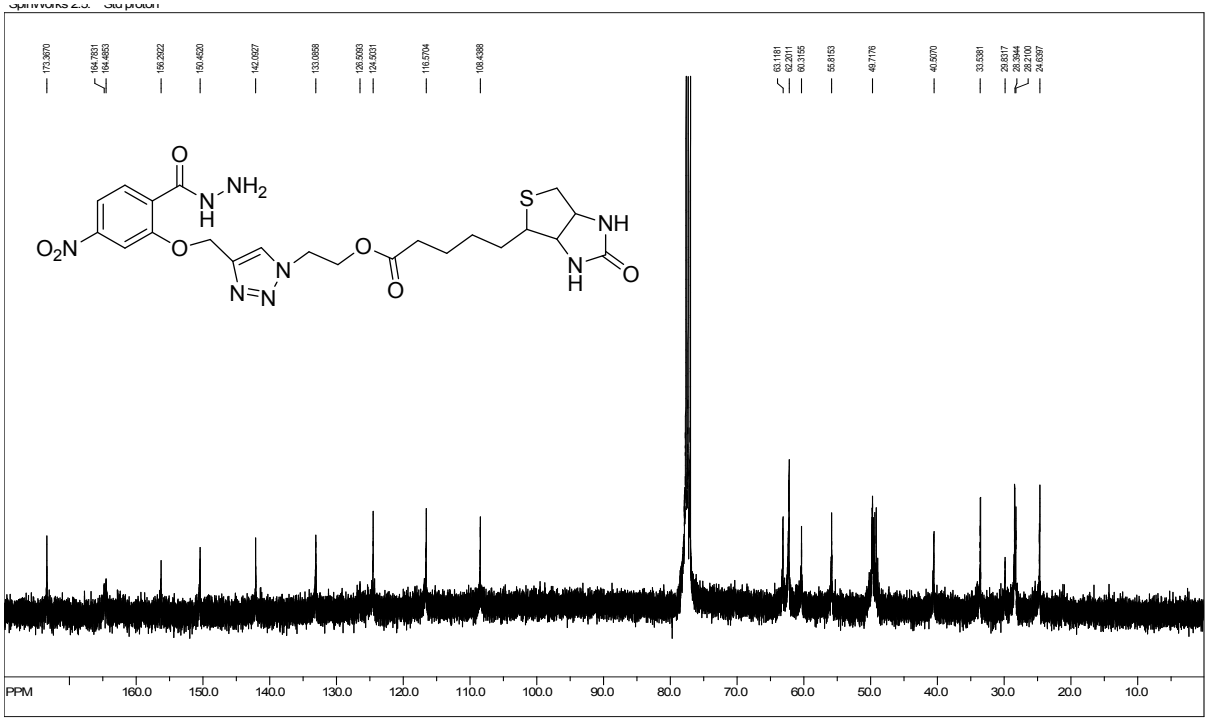


Fig. S17 ^{13}C NMR spectrum of compound **8** in $CDCl_3$.

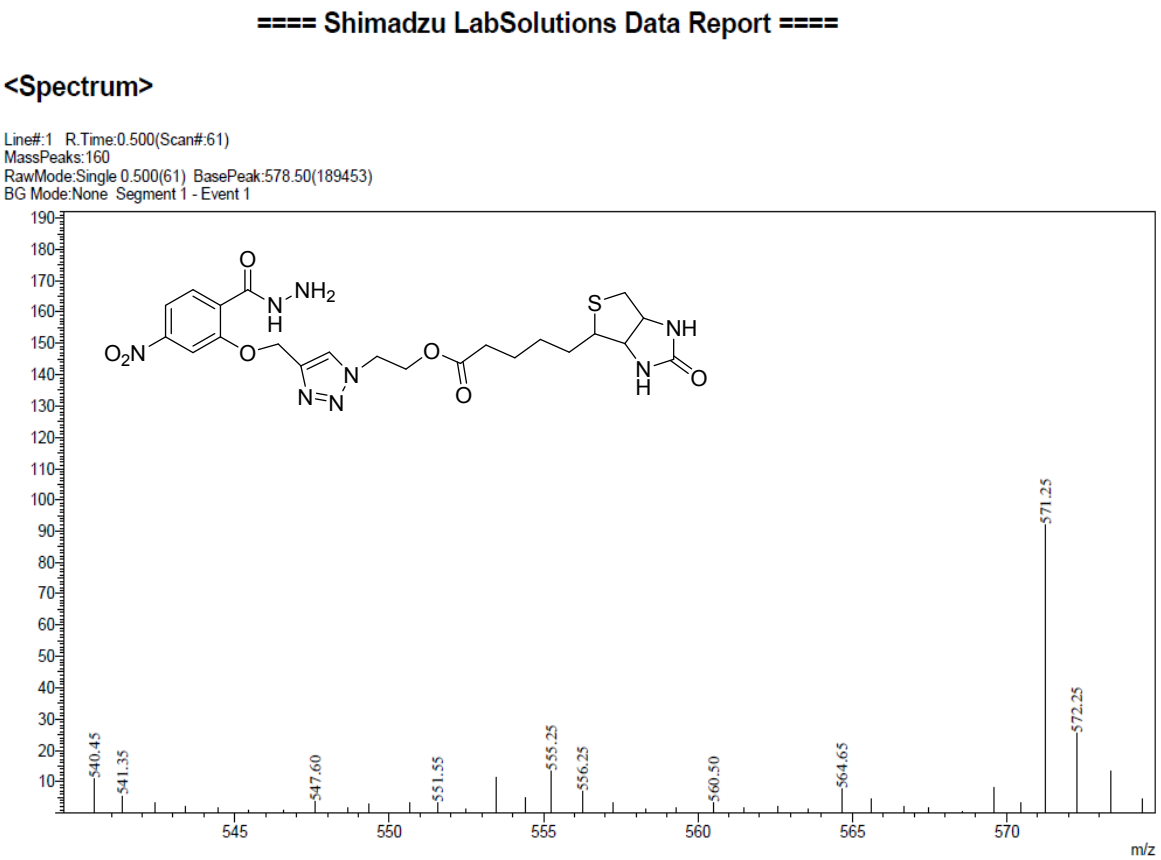


Fig. S18 Mass spectrum of compound **8**.

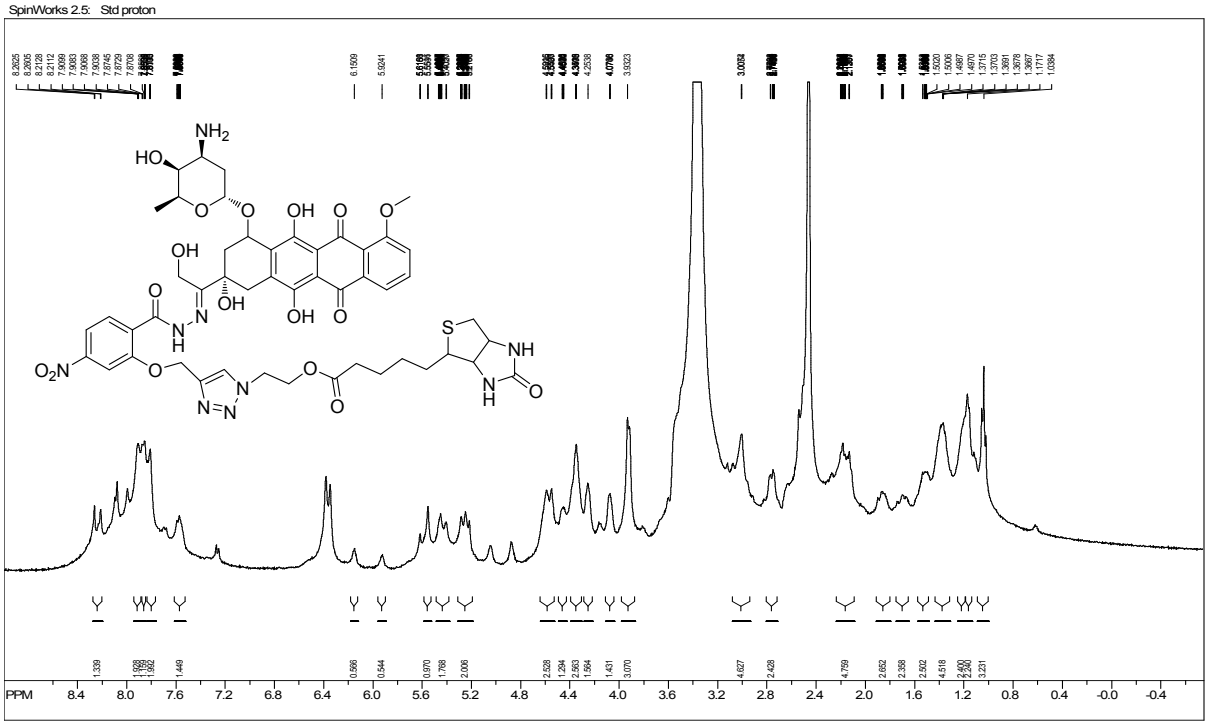


Fig. S19 ^{1}H NMR spectrum of prodrug **9** in DMSO-d6.

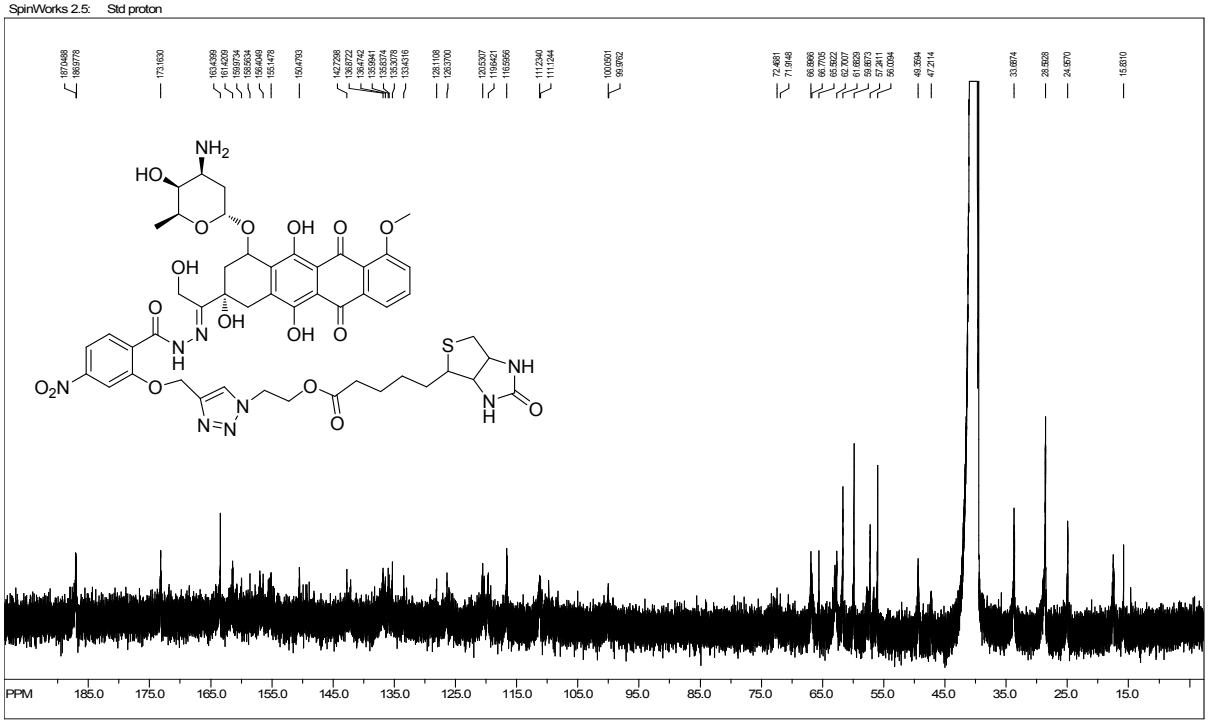


Fig. S20 ^{13}C NMR spectrum of prodrug **9** in DMSO-d6.

==== Shimadzu LabSolutions Data Report ====

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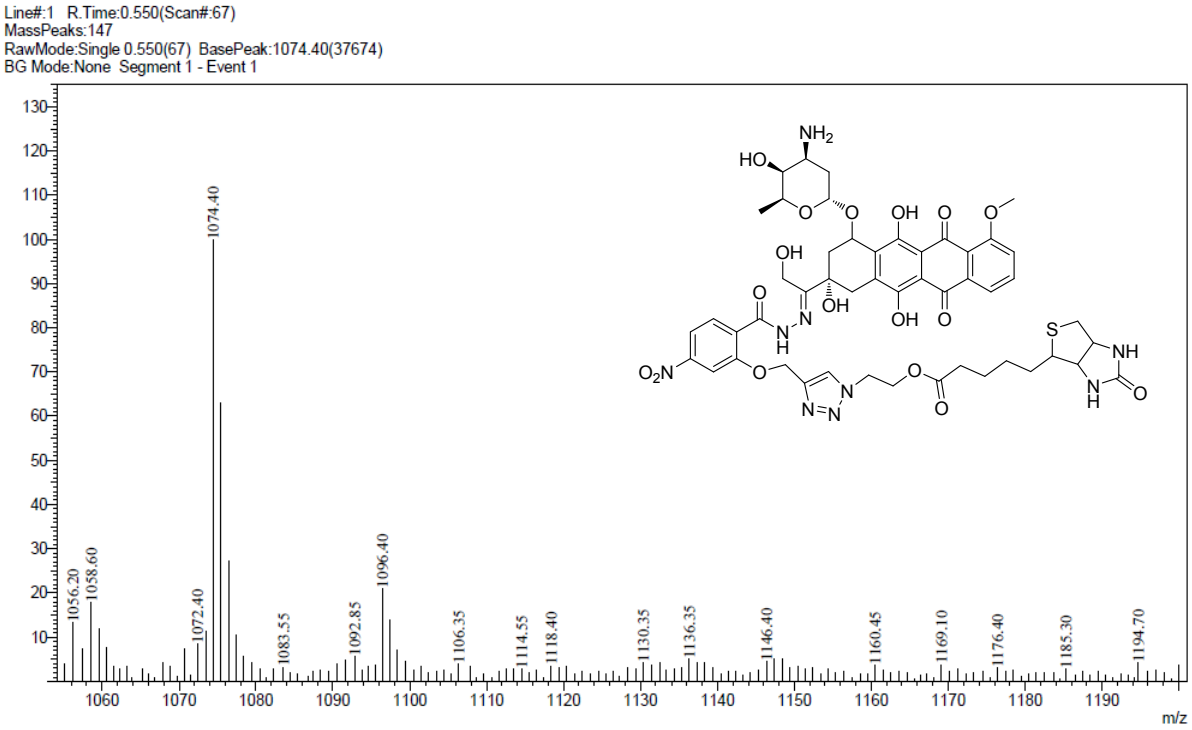

Fig. S21 Mass spectrum of prodrug **9**.

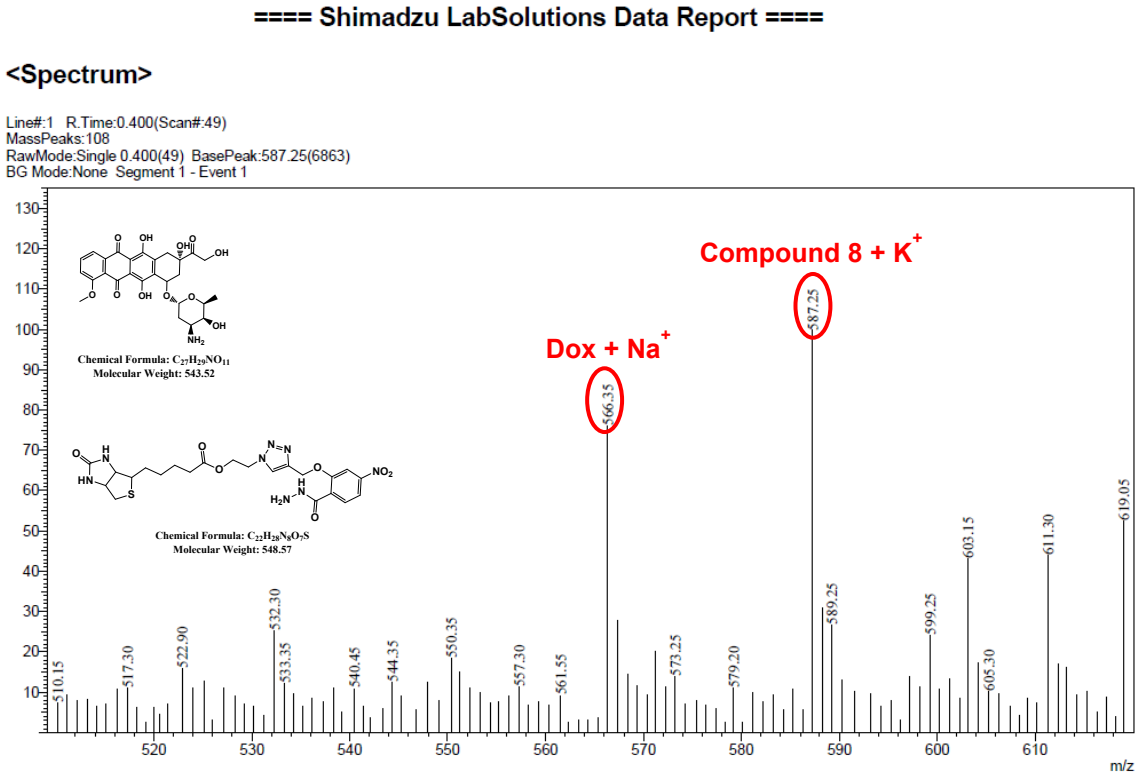

Fig. S22 Mass spectra of prodrug **9** (5 μM) at pH 5.0 after 3 days at 37℃. Mass peak of compound **8** shown at [M+K$^+$] and mass peak of Doxorubicin shown at [M+Na$^+$] (graph = cationic measurement).