Supporting Information

Divinylsulfonamides as specific linkers for stapling disulfide bonds in peptides

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1. General information

Oxytocin was purchased from GL Biochem Ltd without further purification. Sequence – [Cys^1-Tyr^2-Ile^3-Gln^4-Asn^5-Cys^6-Pro^7-Leu^8-Gly^9-$COONH_2$]. Disulfide bridge [Cys^1-Cys^6]. Salmon calcitonin was purchased from Meilunbio without further purification. Sequence – [Cys^1-Ser^2-Asn^3-Leu^4-Ser^5-Thr^6-Cys^7-Val^8-Leu^9-Gly^{10}-Lys^{11}-Leu^{12}-Ser^{13}-Gln^{14}-Gln^{15}-Leu^{16}-His^{17}-Lys^{18}-Leu^{19}-Gln^{20}-Thr^{21}-Tyr^{22}-Pro^{23}-Arg^{24}-Thr^{25}-Asn^{26}-Thr^{27}-Gly^{28}-Ser^{29}-Gly^{30}-Thr^{31}-Pro^{32}-NH_2]. Disulfide bridge [Cys^1-Cys^7]. The other commercial available chemicals were used as received.

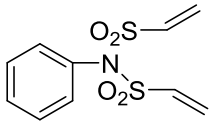
NMR spectra were recorded on a Bruker-500 instrument. The deuterated solvents employed were purchased from Energy Chemical. Chemical shifts are given in ppm with respect to referenced solvent peaks.

High-resolution mass spectra (HRMS-ESI) were obtained on an Agilent Technologies 6230 Accurate Mass TOF LC/MS instrument and are reported as m/z (relative intensity). GCMS-EI were obtained on an Thermo Scienticific ISQ QD instrument and are reported as m/z (relative intensity).

HPLC was performed using an Waters 1525. Mobile pHases are: 0.5% CF_3COOH on water (solvent A) and 0.5% CF_3COOH on acetonitrile (solvent B).
Method A: LC conditions: SunFire C18 column: 4.6X150mm, 5μm, column temperature: 30°C, λ=254nm,
gradient: 0-10 minutes 10-100%B, 10-12 minutes 100%B, flow rate: 1ml/min.
Method B: LC conditions: SunFire C18 column: 4.6X150mm, 5μm, column temperature: 30°C, λ=280nm,
gradient: 1-18 minutes 10-43%B, 18-19 minutes 43-100%B, 19-20 minutes 100%B, flow rate: 1ml/min.

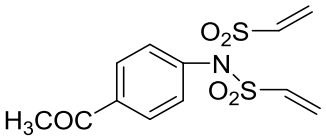
2. Synthetic procedures

N-phenyl-N-(vinylsμlfonyl)ethenesμlfonamide (1a)



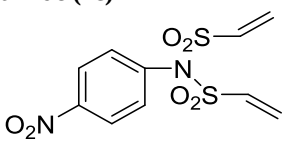
A stirred solution of aniline (0.365 ml, 4.0 mmol) and triethylamine (4.26 ml, 32.0 mmol) in DCM (30 ml) was cooled to 0 °C with ice-bath and 2-chloroethanesμlfonyl chloride (1.25 ml, 12.0 mmol) was then injected slowly. The reaction mixture was stirred at 0 °C for 30 minutes and then quenched with water (10 ml). The product was extracted with DCM (3 X 30 ml). The combined organic extracts were washed with brine (10 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and residue was purified by column chromatography on a gradient form petroleum ether to 10% ethyl acetate in petroleum ether to afford 339 mg (1.24 mmol, 32%) yield of **1a** as a white solid. R_f=0.36 (petroleum ether/ethyl acetate=5/1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.48 – 7.39 (m, 3H), 7.27 – 7.23 (m, 2H), 7.04 (dd, *J* = 16.6, 9.9 Hz, 2H), 6.27 (d, *J* = 16.6 Hz, 2H), 6.13 (d, *J* = 9.8 Hz, 2H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 136.22, 133.79, 131.02, 130.69, 129.84, 129.72 ppm. **ESI-HRMS** calcd for $C_{10}H_{12}NO_4S_2$ [$(M+H)^+$]: 274.0208, found: 274.0208

N-(4-acetylpHenyl)-N-(vinylsμlfonyl)ethenesμlfonamide (1b)



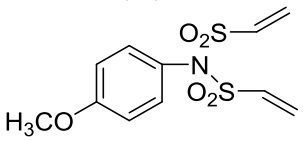
A stirred solution of 4-aminoacetopHenone (675.8 mg, 5.0 mmol) and triethylamine (5.6 ml, 40.0 mmol) in DCM (30 ml) was cooled to 0 °C with ice-bath and 2-chloroethanesμlfonyl chloride (1.3 ml, 12.5 mmol) was then injected slowly. The reaction mixture was stirred at 0 °C for 10 minutes and then quenched with water (10 ml). The product was extracted with DCM (3 X 30 ml). The combined organic extracts were washed with brine (10 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and residue was purified by column chromatography on a gradient form petroleum ether to 20% ethyl acetate in petroleum ether to afford 770 mg (2.45 mmol, 49%) yield of **1b** as a white solid. R_f=0.80 (petroleum ether/ethyl acetate=2/1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.01 (d, *J* = 8.5 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.07 (dd, *J* = 16.5, 9.9 Hz, 2H), 6.30 (d, *J* = 16.6 Hz, 2H), 6.18 (d, *J* = 9.8 Hz, 2H), 2.62 (s, 3H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 196.95, 138.48, 137.71, 136.07, 131.33, 130.30, 129.62, 26.94 ppm. **ESI-HRMS** calcd for $C_{12}H_{14}NO_5S_2$ [$(M+H)^+$]: 316.0313, found: 316.0296

N-(4-nitropHenyl)-N-(vinylsμlfonyl)ethenesμlfonamide (1c)



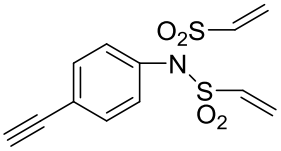
A stirred solution of 4-nitroaniline (552.5 mg, 4.0 mmol) and triethylamine (1.66 ml, 12.0 mmol) in DCM (30 ml) was cooled to 0 °C with ice-bath and 2-chloroethanesµlfonyl chloride (1.05 ml, 10.0 mmol) was then injected slowly. The reaction mixture was stirred at 0 °C for 1 hour and then quenched with water (10 ml). The product was extracted with DCM (3 X 30 ml). The combined organic extracts were washed with brine (10 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and residue was purified by column chromatography on a gradient form petroleum ether to 10% ethyl acetate in petroleum ether to afford 100 mg (0.32 mmol, 8%) yield of **1c** as a slightly yellow solid. R_f=0.52 (petroleum ether/ethyl acetate=2/1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.33 – 8.24 (m, 2H), 7.49 – 7.42 (m, 2H), 7.07 (dd, *J* = 16.5, 9.8 Hz, 2H), 6.31 (dd, *J* = 16.5, 0.9 Hz, 2H), 6.22 (dd, *J* = 9.9, 0.9 Hz, 2H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 148.85, 139.24, 135.86, 132.19, 130.80, 124.90 ppm. **EI-GCMS** calcd for $C_{10}H_{11}N_2O_6S_2$ (M): 317.9980, found: 318.20

N-(4-methoxypHenyl)-N-(vinylsµlfonyl)ethenesµlfonamide (1d)

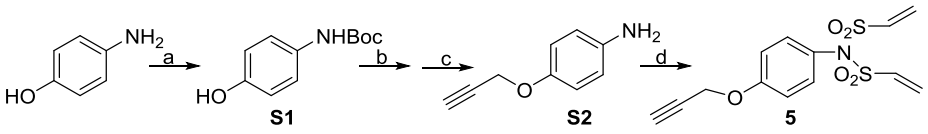


A stirred solution of 4-methoxyaniline (1.23 g, 10.0 mmol) and triethylamine (11 ml, 80.0 mmol) in THF (30 ml) was cooled to 0 °C with ice-bath and 2-chloroethanesµlfonyl chloride (2.6 ml, 25 mmol) was then injected slowly. The reaction mixture was stirred at 0 °C for 10 minutes and then quenched with water (10 ml) after THF was removed under vacuum. The product was extracted with DCM (3 X 30 ml). The combined organic extracts were washed with brine (10 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and residue was purified by column chromatography on a gradient form petroleum ether to 20% ethyl acetate in petroleum ether to afford 1.98 g (6.4 mmol, 64%) yield of **1d** as a white solid. R_f=0.62 (petroleum ether/ethyl acetate=2/1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.22 – 7.14 (m, 2H), 7.04 (dd, *J* = 16.6, 9.9 Hz, 2H), 6.94 – 6.88 (m, 2H), 6.28 (d, *J* = 16.6 Hz, 2H), 6.13 (d, *J* = 9.8 Hz, 2H), 3.82 (s, 3H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 161.17, 136.21, 132.19, 129.70, 125.97, 114.91, 55.67 ppm. **ESI-HRMS** calcd for $C_{11}H_{14}NO_5S_2[(M+H)^+]$: 304.0313, found:304.0298

N-(4-ethynylpHenyl)-N-(vinylsµlfonyl)ethenesµlfonamide (1e)



A stirred solution of 4-ethynylaniline (234.3 mg, 2.0 mmol) and triethylamine (2.2 ml, 16.0 mmol) in DCM (20 ml) was cooled to 0 °C with ice-bath and 2-chloroethanesµlfonyl chloride (0.62 ml, 5.0 mmol) was then injected slowly. The reaction mixture refluxed at 45 °C for 1 hour, after cooling to room temperature, water (5 ml) was added. The product was extracted with DCM (3 X 20 ml). The combined organic extracts were washed with brine (10 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and residue was purified by column chromatography on a gradient form petroleum ether to 20% ethyl acetate in petroleum ether to afford 251 mg (0.85 mmol, 43%) yield of **1e** as a white solid. R_f=0.76 (petroleum ether/ethyl acetate=2/1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 7.07 (dd, *J* = 16.5, 9.9 Hz, 2H), 6.32 (d, *J* = 16.6 Hz, 2H), 6.18 (d, *J* = 9.8 Hz, 2H), 3.20 (s, 1H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 136.11, 133.83, 133.40, 131.05, 130.13, 124.89, 82.34, 79.89 ppm. **ESI-HRMS** calcd for $C_{12}H_{12}NO_4S_2$ $[(M+H)^+]$: 298.0208, found: 298.0239.

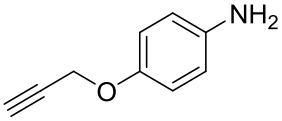


a) di-tert-butyl dicarbonate, Et_3N, THF, rt, overnight. b) 3-bromopropyne, K_2CO_3, DMF, rt, overnight.
c) CF_3COOH,0 °C, 1 h. d) Et_3N , 0 °C, DCM, 20 minutes.

tert-butyl (4-hydroxypHenyl)carbamate (S1)

A stirred solution of 4-aminopHenol (2.0 g, 18.3 mmol), di-tert-butyl dicarbonate (5.0 ml, 22 mmol) and triethylamine (5.0 ml, 36.6 mmol) in THF (50 ml) was stirred for overnight at room temperature. The mixture was concentrated under vacuum and residue was purified by column chromatography to afford 3.27 g (15.6 mmol, 96%) yield of **S1** as a white solid. **^{1}H NMR** (500 MHz, DMSO) δ 9.04 (s, 1H), 8.99 (br, 1H), 7.22 (d, J = 7.1 Hz, 2H), 6.71 – 6.58 (m, 2H), 1.45 (s, 9H) ppm. **^{13}C NMR** (126 MHz, DMSO) δ 153.06 , 152.56 , 131.08 , 119.99 , 115.07 , 78.45 , 28.24 ppm.

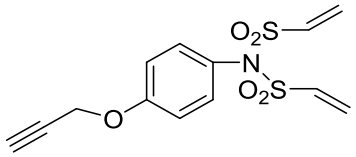
4-(prop-2-yn-1-yloxy)aniline (S2)



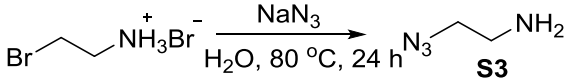
Tert-butyl (4-hydroxypHenyl)carbamate (**S1**) (1.7 g, 8.1 mmol), 3-bromopropyne (0.84 ml, 9.7 mmol) and potassium carbonate (3.3 g, 24.3 mmol) were dissolved in DMF (30 ml). The reaction mixture was stirred at room temperature for overnight followed that water (600 ml) and ethyl acetate(3 X 50 ml) were added to extract the product. The combined organic extracts were washed sequentially with saturated $NaHCO_3$ (20 ml), NH_4Cl (20 ml) and brine (20 ml) and dried over anhydrous Na_2SO_4. The solvent was

removed under vacuum and residue was dissolved in DCM (30 ml). The solution was cooled to 0°C with ice-bath followed trifluoroacetic acid (12 ml) added, and then the reaction mixture was stirred for 1 hour under ice-bath. The mixture was concentrated under vacuum and residue was purified by column chromatography on a gradient form petroleum ether to 10% ethyl acetate/0.5% triethylamine in petroleum ether to afford 1.0 g (6.8 mmol, 84%) yield of **S2** as red oil. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.80 (dd, *J* = 8.7, 1.6 Hz, 2H), 6.60 (dd, *J* = 8.8, 3.1 Hz, 2H), 4.58 (t, *J* = 2.4 Hz, 2H), 3.49 (s, 2H), 2.51 (d, *J* = 2.3 Hz, 1H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 150.38, 140.99, 116.23, 116.09, 79.15, 75.27, 56.57 ppm. **ESI-HRMS** calcd for $C_9H_{10}NO$ [$(M+H)^+$]: 148.0762, found:148.0815

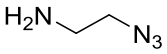
alkyne-divinylsulfonamide (5)



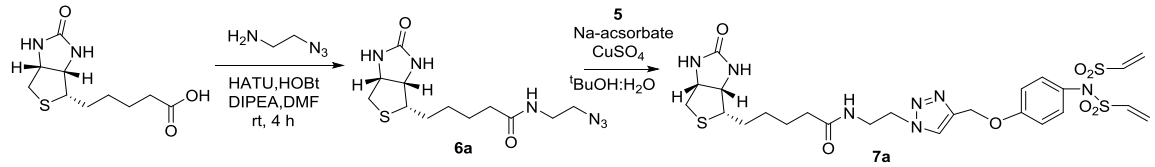
A stirred solution of 4-(prop-2-yn-1-yloxy)aniline (**S2**) (180 mg, 1.22 mmol) and triethylamine (1.0 ml, 7.32 mmol) in DCM (15 ml) was cooled to 0 °C with ice-bath and 2-chloroethanesµlfonyl chloride (288µl, 2.69 mmol) was then injected slowly. The reaction mixture was stirred at 0 °C for 20 minutes, and water (5 ml) was added. The product was extracted with DCM (3 X 15 ml). The combined organic extracts were washed with brine (10 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and residue was purified by column chromatography on a gradient form petroleum ether to 20% ethyl acetate in petroleum ether to afford 252 mg (0.77 mmol, 64%) yield of **5** as a slightly yellow solid. R_f=0.74 (petroleum ether/ethyl acetate=2/1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.23 – 7.17 (m, 2H), 7.09 – 6.96 (m, 4H), 6.28 (d, *J* = 16.6 Hz, 2H), 6.14 (d, *J* = 9.9 Hz, 2H), 4.70 (d, *J* = 2.4 Hz, 2H), 2.56 (t, *J* = 2.4 Hz, 1H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 159.16, 136.18, 132.24, 129.79, 126.81, 115.76, 77.97, 76.37, 56.17 ppm. **ESI-HRMS** calcd for $C_{13}H_{14}NO_5S_2$ [$(M+H)^+$]: 328.0313, found:328.1552



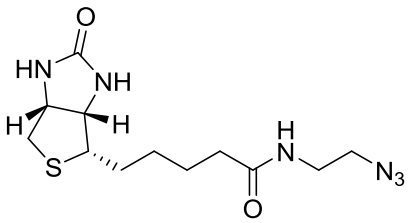
2-azidoethanamine (S3)



Sodium azide (1.9 g, 29.3 mmol) was dissolved in water(10 ml) and 2-bromoethylamine hydrobromide (2.0 g, 9.76 mmol) was added to the solution. The mixture was stirred at 45 °C for 24 hours. After cooling to room temperature, potassium hydroxide (3.0 g, 53.3 mmol) was added at 0 °C with ice-bath. The product was extracted with diethyl ether (3 X 10 ml). The combined organic extracts were washed with brine (10 ml) and dried over anhydrous Na_2SO_4. The solvent was removed by water pump to afford to afford 626 mg (7.28 mmol, 75%) yield of **S3** as a colorless oil. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.30 – 3.24 (m, 2H), 2.79 – 2.74 (t, *J* = 5.9 Hz, 2H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 54.50, 41.20 ppm. **ESI-HRMS** calcd for $C_4H_{12}N_8Na$ [$(2M+Na)^+$]: 195.1083, found:195.1340

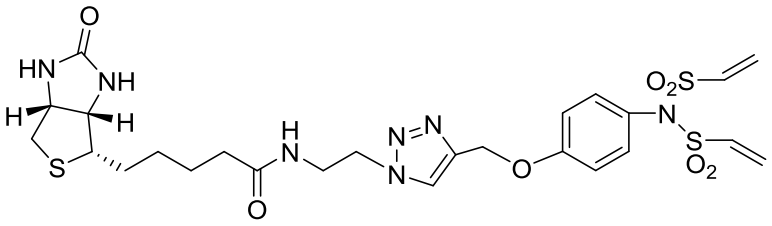


Biotin-N_3 (6a)

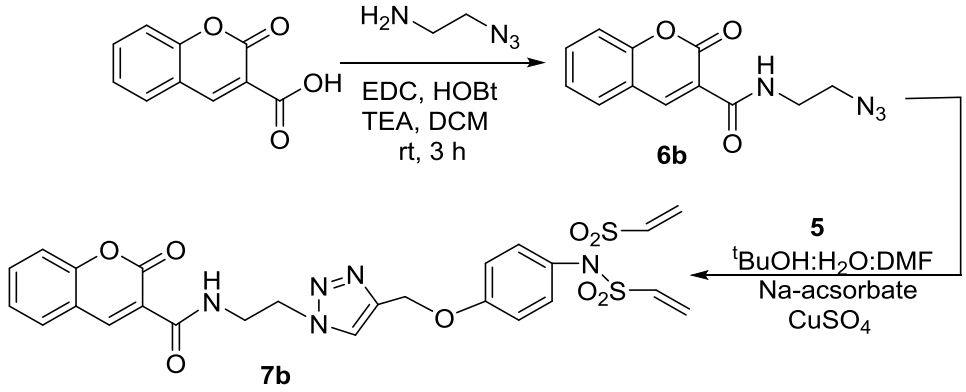


Prepared following a modified literature procedure.[1] Biotin (100 mg, 0.41 mmol), 2-azidoethanamine (**S3**) (70 mg, 0.82 mmol), HATU (171 mg, 0.45 mmol) and HOBt (69 mg, 0.45 mmol) were dissolved in DMF (2 ml), after which DIPEA (0.35 ml) was added. The reaction mixture was stirred at room temperature for 4 hours and then the mixture purified directly by column chromatography on a gradient form DCM to 10% methanol/0.5% triethylamine in DCM to afford 46 mg (0.15 mmol, 36%) yield of **6a** as a white solid. R_f=0.53 (DCM / methanol within a drop of triethylamine =2/1). **^{1}H NMR** (500 MHz, DMSO) δ 8.07 (t, *J* = 5.4 Hz, 1H), 6.44 (s, 1H), 6.38 (s, 1H), 4.30 (dd, *J* = 7.5, 5.3 Hz, 1H), 4.15 – 4.08 (m, 1H), 3.33 (t, *J* = 5.8 Hz, 2H), 3.22 (dd, *J* = 11.5, 5.7 Hz, 2H), 3.11 – 3.05 (m, 1H), 2.81 (dd, *J* = 12.5, 5.1 Hz, 1H), 2.57 (d, *J* = 12.4 Hz, 1H), 2.07 (t, *J* = 7.4 Hz, 2H), 1.30 – 1.20 (m, 6H) ppm. **^{13}C NMR** (126 MHz, DMSO) δ 172.42, 162.73, 61.02, 59.19, 55.43, 53.42, 49.99, 38.15, 35.14, 28.21, 28.05, 25.17 ppm. **ESI-HRMS** calcd for $C_{12}H_{21}N_6O_2S$ [$(M+H)^+$]: 313.1447, found:313.1473

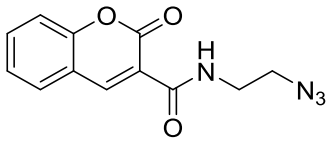
Biotin-divinylsulfonamide (7a)



Prepared following a modified literature procedure.[2] Biotin-N_3 (**6a**) (46 mg, 0.147 mmol), **5** (58 mg, 0.177 mmol), Na ascorbate (29.2 mg, 0.147 mmol) and $CuSO_4$ (23.5 mg, 0.1474 mmol) were dissolved in $^tBuOH/H_2O$ (1/1)(2 ml). The reaction mixture was stirred at room temperature overnight. The resulting crud mixture was purified by HPLC to afford 31 mg (0.05 mmol, 33%) yield of **7a** as a slightly yellow solid. **^{1}H NMR** (500 MHz, MeOD) δ 8.07 (s, 1H), 7.22 (d, *J* = 8.8 Hz, 2H), 7.14 – 7.03 (m, 4H), 6.23 (d, *J* = 4.1 Hz, 2H), 6.21 (d, *J* = 1.9 Hz, 2H), 5.18 (s, 2H), 4.53 (t, *J* = 5.6 Hz, 2H), 4.42 (dd, *J* = 7.7, 4.8 Hz, 1H), 4.25 (dd, *J* = 7.8, 4.4 Hz, 1H), 3.65 (d, *J* = 3.2 Hz, 2H), 3.34 (s, 1H), 3.19 – 3.11 (m, 1H), 2.86 (dd, *J* = 12.7, 4.9 Hz, 1H), 2.64 (d, *J* = 12.7 Hz, 1H), 2.14 (td, *J* = 7.1, 2.0 Hz, 2H), 1.68 (dt, *J* = 13.4, 7.2 Hz, 1H), 1.62 – 1.47 (m, 4H), 1.34 (dt, *J* = 15.0, 7.5 Hz, 3H) ppm. **^{13}C NMR** (126 MHz, MeOD) δ 176.46, 166.05, 161.11, 144.49, 137.52, 133.61, 130.76, 128.13, 125.88, 116.42, 63.25, 62.68, 61.56, 56.89, 50.61, 41.07, 40.36, 36.57, 29.60, 29.42, 26.69 ppm. **ESI-HRMS** calcd for $C_{25}H_{34}N_7O_7S_3$ [$(M+H)^+$]: 640.1682, found:640.1714

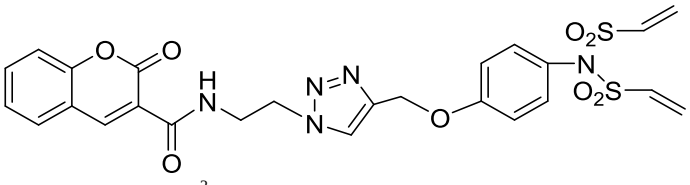


Coumarin-N_3 (6b)

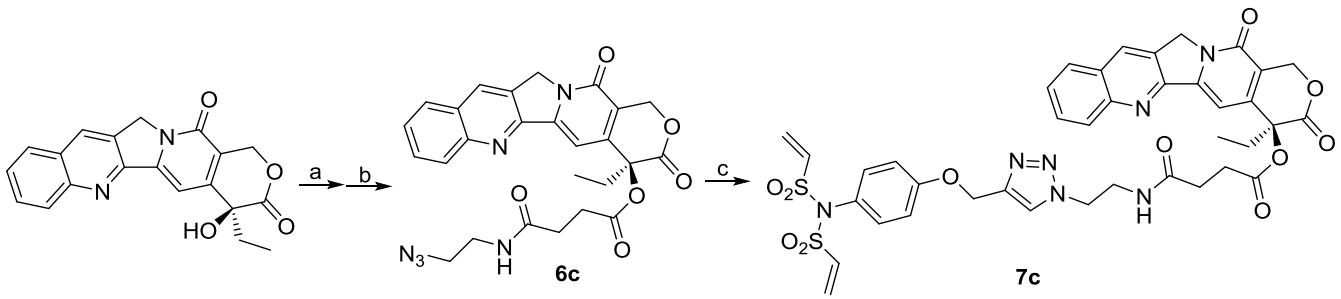


Coumarin-3-carboxylic acid (380.3 mg, 2.0 mmol), 2-azidoethanamine (**S3**) (206 mg, 2.4 mmol), HOBt (810.7 mg, 6.0 mmol), EDC (1.15 g, 6.0 mmol) and triethylamine (0.83 ml, 6.0 mmol) were dissolved in DCM (30 ml). The reaction mixture was stirred at room temperature for 3 hours and water (10 ml) was added. The product was extracted with DCM (3 X 30 ml). The combined organic extracts were washed sequentially with saturated $NaHCO_3$ (20 ml), NH_4Cl (20 ml) and brine (20 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and residue was purified by column chromatography on a gradient form petroleum ether to 50% ethyl acetate/0.5% triethylamine in petroleum ether to afford 336 mg (1.3 mmol, 65%) yield of **6b** as a white solid. R_f=0.44 (petroleum ether/ethyl acetate=2/1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.91 (s, 1H), 7.69 (ddd, *J* = 15.8, 8.2, 1.3 Hz, 2H), 7.40 (dd, *J* = 16.4, 8.0 Hz, 2H), 3.66 (dd, *J* = 11.7, 5.8 Hz, 2H), 3.55 (t, *J* = 5.8 Hz, 2H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 162.15, 161.57, 154.65, 148.86, 134.45, 130.05, 125.55, 118.71, 118.22, 116.88, 50.68, 39.38 ppm. **ESI-HRMS** calcd for $C_{12}H_{11}N_4O_3$ [$(M+H)^+$]: 259.0831, found:259.0451

Coumarin-divinylsulfonamide (7b)

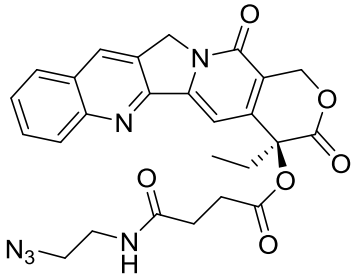


Prepared following a modified literature procedure.[2] Coumarin-N_3 (**6b**) (45 mg, 0.174 mmol), **5** (68.5 mg, 0.2 mmol), Na ascorbate (34.6 mg, 0.174 mmol) and $CuSO_4$ (27.8 mg, 0.174 mmol) were dissolved in $^tBuOH/H_2O$/DMF (1/1/1)(6 ml). The reaction mixture was stirred at room temperature for 3 hours. The resulting crud mixture was purified by HPLC to afford 29 mg (0.05 mmol, 29%) yield of **7b** as a white solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.89 (s, 1H), 7.73 – 7.69 (m, 2H), 7.68 (s, 1H), 7.40 (t, *J* = 8.6 Hz, 2H), 7.17 (d, *J* = 8.8 Hz, 2H), 7.08 – 6.94 (m, 4H), 6.27 (d, *J* = 16.6 Hz, 2H), 6.13 (d, *J* = 9.8 Hz, 2H), 5.21 (s, 2H), 4.65 (t, *J* = 6.0 Hz, 2H), 3.97 (dd, *J* = 11.9, 5.9 Hz, 2H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 162.48, 161.48, 159.84, 154.67, 149.07, 143.86, 136.19, 134.68, 132.28, 130.12, 129.78, 126.49, 125.64, 123.60, 118.62, 117.92, 116.92, 115.73, 62.36, 49.55, 40.13 ppm. **ESI-HRMS** calcd for $C_{25}H_{24}N_5O_8S_2$ [$(M+H)^+$]: 586.1066, found:586.0504



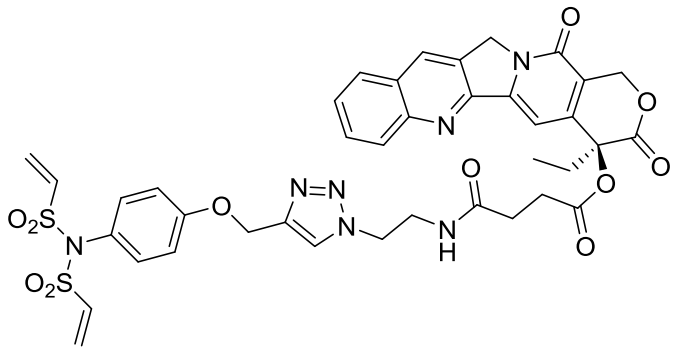
a) succinic anhydride, DBU, 1%HCl, DCM, rt, 4 h. b) 2-azidoethanamin, HOBt, EDC, Et_3N, DCM, rt, 5 h.
c) **5**, Na-ascorbate, $CuSO_4$, $^tBuOH/H_2O/DMF(1/1/1)$

Camptothecin-N_3 (6c)



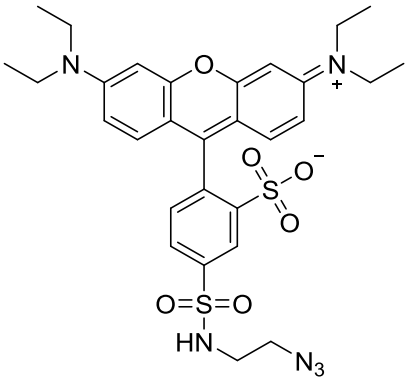
Prepared following a modified literature procedure.[3] A stirred solution of camptothecin (174.2 mg, 0.5 mmol) and succinic anhydride (150 mg, 1.5 mmol) in DCM (15 ml) was cooled to 0 °C with ice-bath and DBU (0.23 ml, 1.5 mmol) was then added dropwise. The mixture was stirred at room temperature overnight and water (10 ml) was added. After 1%HCl was used to acidize the reaction mixture, the product was extracted with DCM (3 X 20 ml). The combined organic extracts were washed with brine (10 ml) and dried over anhydrous Na_2SO_4. The solution was concentrated under vacuum to next step without further purification. 2-azidoethanamine (**S3**) (51.6 mg, 0.6 mmol), HOBt (202.7 mg, 1.5 mmol), EDC (287.6 mg, 1.5 mmol) and triethylamine (0.21 ml, 1.5 mmol) were added to former solution. The mixture was stirred for 5 hours at room temperature followed that water (10 ml) and DCM (3 X 15 ml) were added to extract the product. The combined organic extracts were washed sequentially with saturated $NaHCO_3$ (10 ml), NH_4Cl (10 ml) and brine (10 ml) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and residue was purified by column chromatography on a gradient form DCM to 5% methanol/0.5% triethylamine in DCM to afford 208 mg (0.4mmol, 81%) yield of **6c** as a slightly yellow solid. R_f=0.68 (DCM / methanol within a drop of triethylamine =15/1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.26 (d, *J* = 8.3 Hz, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 7.85 (t, *J* = 7.5 Hz, 1H), 7.68 (t, *J* = 7.4 Hz, 1H), 7.35 (s, 1H), 6.38 (s, 1H), 5.66 (d, *J* = 17.0 Hz, 1H), 5.36 (d, *J* = 17.0 Hz, 1H), 5.33 – 5.19 (m, 2H), 3.31 (s, 4H), 2.98 – 2.88 (m, 1H), 2.87 – 2.76 (m, 1H), 2.61 – 2.46 (m, 2H), 2.22 (td, *J* = 14.4, 7.2 Hz, 1H), 2.11 (dq, *J* = 14.4, 7.1 Hz, 1H), 0.99 (t, *J* = 7.3 Hz, 3H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.09, 167.75, 157.52, 151.88, 148.25, 146.72, 145.87, 132.16, 131.34, 129.05, 128.71, 128.50, 128.44, 128.38, 119.98, 97.43, 77.40, 76.50, 66.97, 50.62, 50.29, 39.20, 31.66, 30.85, 29.53, 7.74 ppm. **ESI-HRMS** calcd for $C_{26}H_{25}N_6O_6$ [$(M+H)^+$]: 517.1836, found:517.1914

Camptothecin-divinylsulfonamide (7c)



Prepared following a modified literature procedure.[2] Camptothecin-N_3 (**6c**) (176 mg, 0.34 mmol), **5** (133.8 mg, 0.41 mmol), Na ascorbate (67.4 mg, 0.34 mmol) and $CuSO_4$ (54.3 mg, 0.34 mmol) were dissolved in $^tBuOH/H_2O/DMF$ (1/1/1)(9 ml). The reaction mixture was stirred at room temperature for 2 hours. The resulting crud mixture was purified by HPLC to afford 138 mg (0.164 mmol, 49%) yield of **7c** as a slightly yellow solid. R_f=0.37 (DCM / methanol =20/1). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 7.9 Hz, 1H), 7.82 (t, *J* = 7.1 Hz, 1H), 7.65 (t, *J* = 7.2 Hz, 1H), 7.53 (s, 1H), 7.30 (s, 1H), 7.11 (d, *J* = 8.1 Hz, 2H), 6.98 (dd, *J* = 16.2, 9.9 Hz, 2H), 6.91 (d, *J* = 7.9 Hz, 2H), 6.64 (s, 1H), 6.23 (d, *J* = 16.5 Hz, 2H), 6.10 (d, *J* = 9.7 Hz, 2H), 5.58 (d, *J* = 16.9 Hz, 1H), 5.31 (d, *J* = 16.9 Hz, 1H), 5.17 (dd, *J* = 37.5, 18.8 Hz, 2H), 5.04 (s, 2H), 4.27 (s, 2H), 3.56 (d, *J* = 30.6 Hz, 2H), 2.95 – 2.70 (m, 2H), 2.43 (s, 2H), 2.14 (ddd, *J* = 49.5, 13.7, 7.1 Hz, 2H), 0.97 (t, *J* = 6.9 Hz, 3H) ppm. **^{13}C NMR** (126 MHz, $CDCl_3$) δ 172.20, 172.14, 167.67, 159.49, 157.27, 151.91, 148.35, 146.47, 146.00, 135.95, 132.10, 131.83, 131.03, 129.81, 129.73, 129.13, 128.61, 128.37, 128.26, 128.22, 126.39, 124.29, 119.61, 115.64, 96.70, 76.47, 66.80, 61.70, 50.07, 49.22, 39.76, 31.42, 30.43, 29.24, 7.66 ppm. **ESI-HRMS** calcd for $C_{39}H_{38}N_7O_{11}S_2$ [$(M+H)^+$]: 844.2071, found:844.2049

Rhodamine-N_3 (6d)



Prepared following a modified literature procedure.[4] Lissamine rhodamine (144.3 mg, 0.25 mmol), 2-azidoethanamine (**S3**) (32.25 mg, 0.38 mmol) and triethylamine (0.83 ml, 6.0 mmol) were dissolved in DMF (10 ml). The reaction mixture was stirred overnight at room temperature under dark condition. The resulting crud mixture was purified by HPLC to afford 119 mg (0.19 mmol, 76%) yield of **6d** as a red solid. **^{1}H NMR** (500 MHz, DMSO) δ 8.42 (d, *J* = 1.6 Hz, 1H), 8.29 (t, *J* = 5.9 Hz, 1H), 7.95 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.04 (dd, *J* = 9.6, 2.0 Hz, 2H), 6.96 (dd, *J* = 14.8, 5.7 Hz, 4H), 3.70 – 3.58 (m, 2H), 3.55 – 3.29 (m, 2H), 3.09 (m, 8H), 1.17 (t, *J* = 7.3 Hz, 12H). **ESI-HRMS** calcd for $C_{29}H_{35}N_6O_6S_2$ [$(M+H)^+$]: 627.2059, found:627.2108

3. Condition optimization for the reaction of thoil-divinylsulfonamides

3.1 Screening of divinylsulfonamides

*HPLC analytical data of stapling crude reaction with **1a-e***

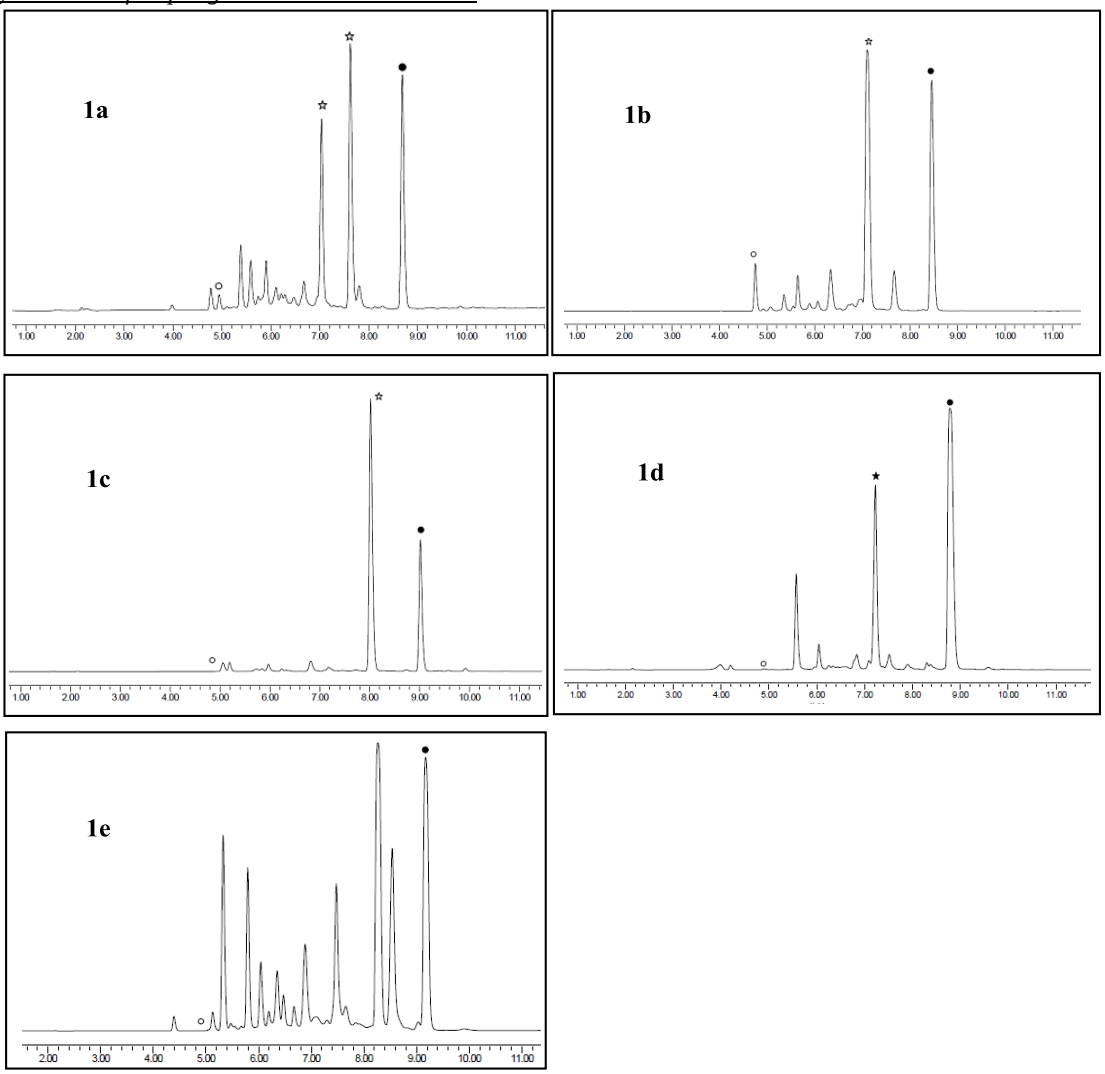


Figure S1 A 330 µl LABTIDE 96 Round Well contained 6 µl of oxytocin **2** (25 mM stock solution in H_2O), 10 µl of TCEP (25 mM stock solution in H_2O, pH 7.07 was adjusted by NaOH and H_3PO_4), 60 µl of PBS buffer (Cat. NO. SH30256.01, pH 7.07) and 40 µl of CH_3CN. The mixture was reacted at Micro plate fast oscillator for 1.5 hours to attain reduced oxytocin followed 18 µl of **1a-e** (25 mM stock solution in CH_3CN) added. The mixture was reacted on Micro plate fast oscillator at room temperature for 2 hours and the resulting mixture was subjected to HPLC *Method A* analysis. Signal of starting material **2** and **1a-e** marked with an empty circle (○) and a full black circle (●). Signal of the conjugation byproduct **2b** (yield = 47%) is marked with a full black star (★) and signal of undesired products marked with an empty star (☆)

3.2 The effects of PBS (pH) to the reaction

HPLC analytical data of stapling crude reaction under different pH value of PBS

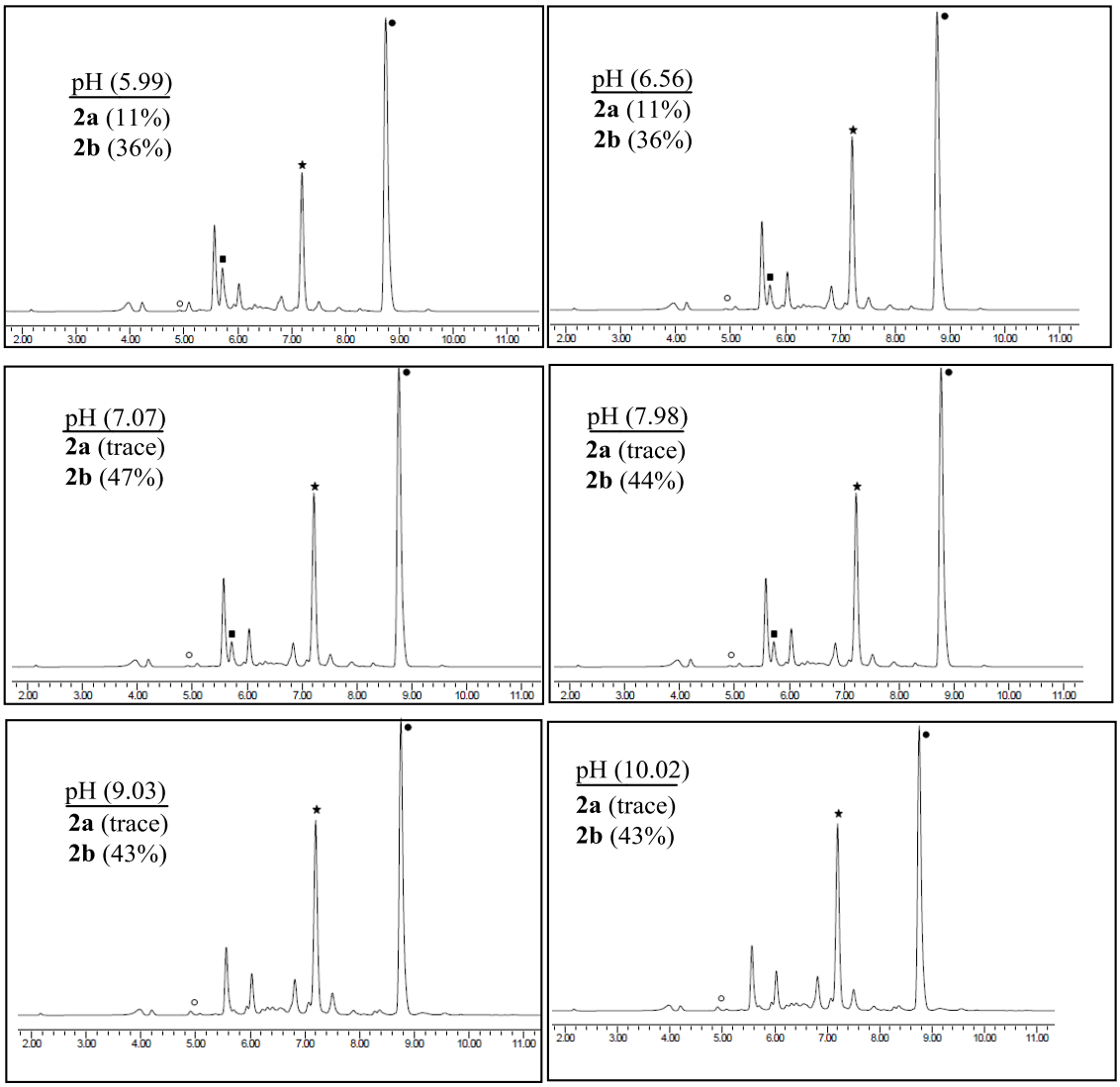


Figure S2 A 330 µl LABTIDE 96 Round Well contained 12 µl of oxytocin **2** (25 mM stock solution in H_2O), 20 µl of TCEP (25 mM stock solution in H_2O, pH 7.07 was adjusted by NaOH and H_3PO_4), 120 µl of PBS buffer (Cat. NO. SH30256.01, different pH was adjusted by NaOH and H_3PO_4) and 80 µl of CH_3CN. The mixture was reacted on Micro plate fast oscillator at room temperature for 1.5 hours to attain reduced oxytocin followed 36 µl of **1d** (25 mM stock solution in CH_3CN) added. The mixture was reacted on Micro plate fast oscillator at room temperature for 2 hours and the resulting mixture was subjected to HPLC *Method A* analysis. Signal of starting material **2** and **1d** marked with an empty circle (○) and a full black circle (●). Signal of the byproduct **2b** is marked with a full black star (★). Signal of the desired product **2a** is marked with a full black square (■).

3.3 The effects of equivalent (1d) to the reaction

*HPLC analytical data of stapling crude reaction with different equivalent **1d***

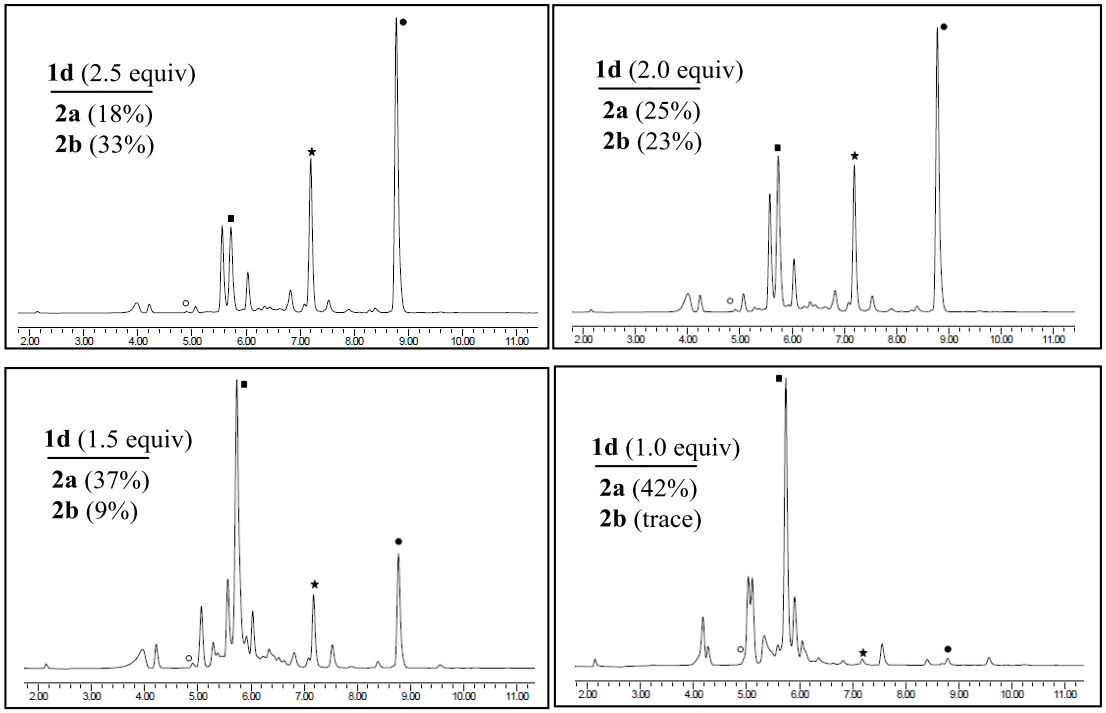


Figure S3 A 330 µl LABTIDE 96 Round Well contained 12 µl of oxytocin **2** (25 mM stock solution in H_2O), 20 µl of TCEP (25 mM stock solution in H_2O, pH 7.07 was adjusted by NaOH and H_3PO_4), 120 µl of PBS buffer (Cat. NO. SH30256.01, pH 7.07) and 80 µl of CH_3CN. The mixture was reacted on Micro plate fast oscillator at room temperature for 1.5 hours to attain reduced oxytocin followed 12-36 µl of **1d** (25 mM stock solution in CH_3CN) added. The mixture was reacted on Micro plate fast oscillator at room temperature for 2 hours and the resulting mixture was subjected to HPLC *Method A* analysis. Signal of starting material **2** and **1d** marked with an empty circle (○) and a full black circle (●). Signal of the byproduct **2b** is marked with a full black star (★). Signal of the desired product **2a** is marked with a full black square (■).

3.4 The effects of concentration (2) and temperature to the reaction

*HPLC analytical data of stapling crude reaction under different final concentration of oxytocin **2***

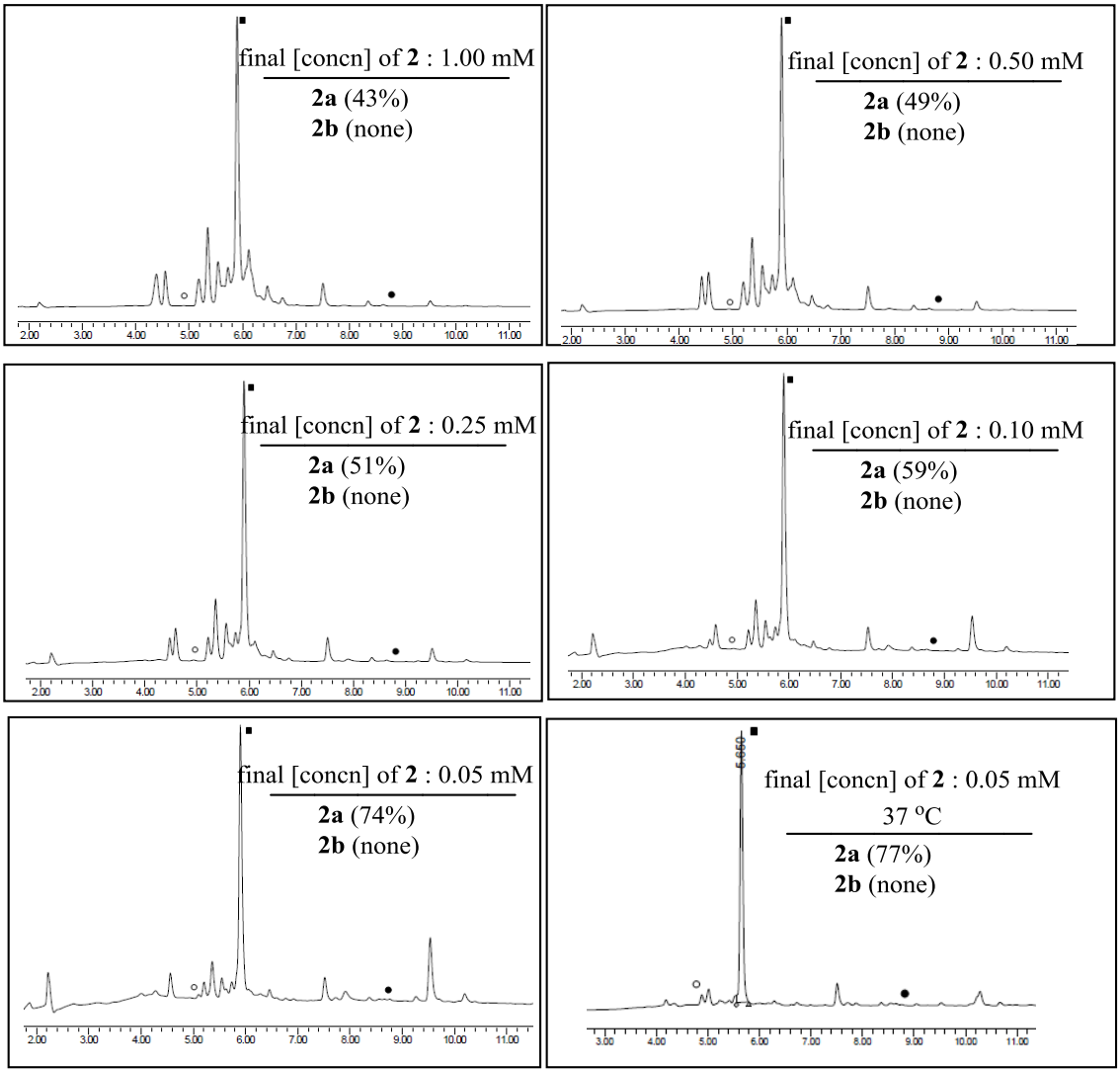


Figure S4 A 330 µl LABTIDE 96 Round Well contained 12 µl of oxytocin **2** (1-25 mM stock solution in H_2O), 20 µl of TCEP (1-25 mM stock solution in H_2O, pH 7.07 was adjusted by NaOH and H_3PO_4), 120 µl of PBS buffer (Cat. NO. SH30256.01, pH 7.07) and 80 µl of CH_3CN. The mixture was reacted on Micro plate fast oscillator at room temperature for 1.5 hours to attain reduced oxytocin followed 36 µl of **1d** (1-25 mM stock solution in CH_3CN) added. The mixture was reacted on Micro plate fast oscillator at room temperature for 2 hours and the resulting mixture was subjected to HPLC *Method A* analysis. However, the last was performed at 37 °C. Signal of starting material **2** and **1d** marked with an empty circle (○) and a full black circle (●). Signal of the byproduct **2b** is marked with a full black star (★). Signal of the desired product **2a** is marked with a full black square (■).

*ESI-HRMS analytical data of **2**, **2a** **2b** and **2c***

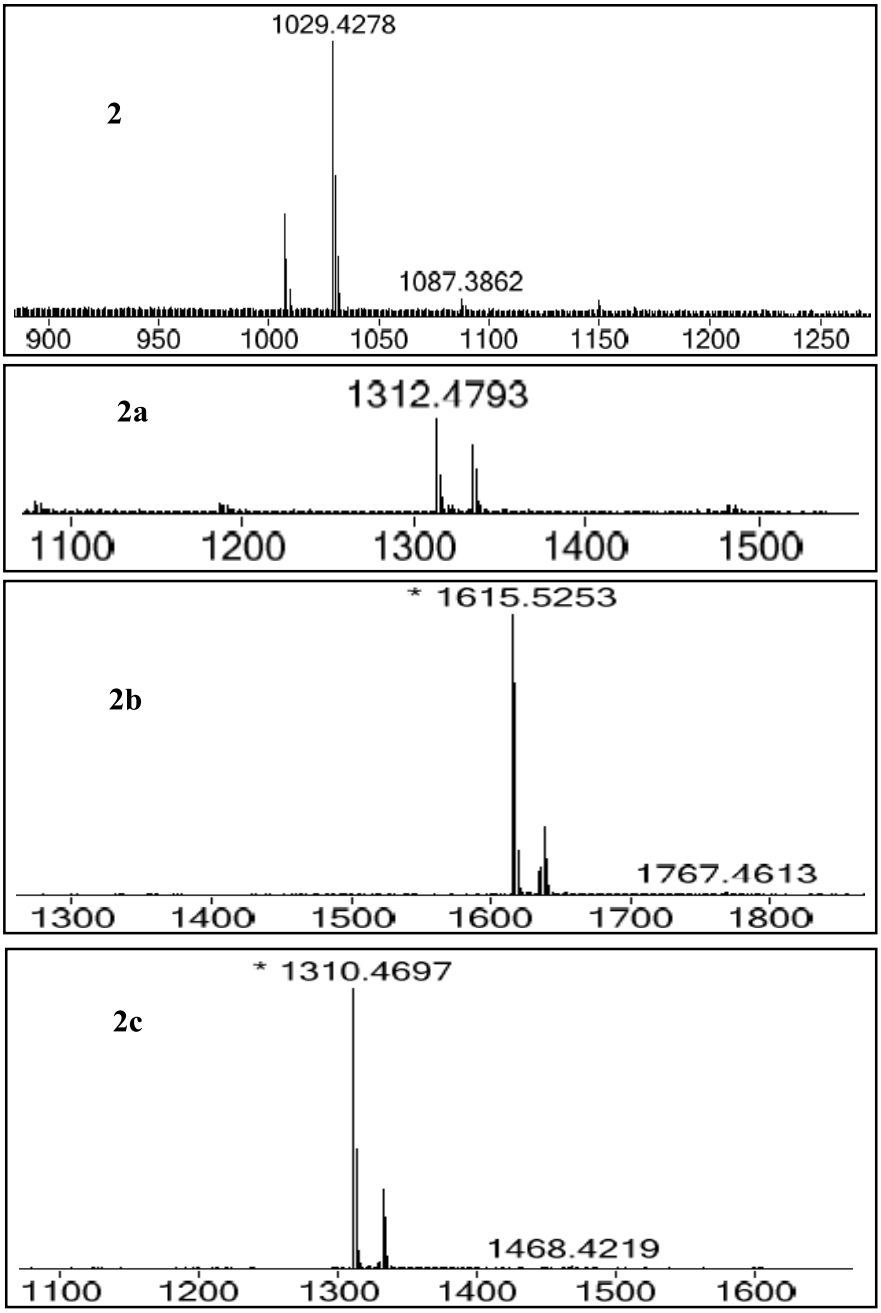
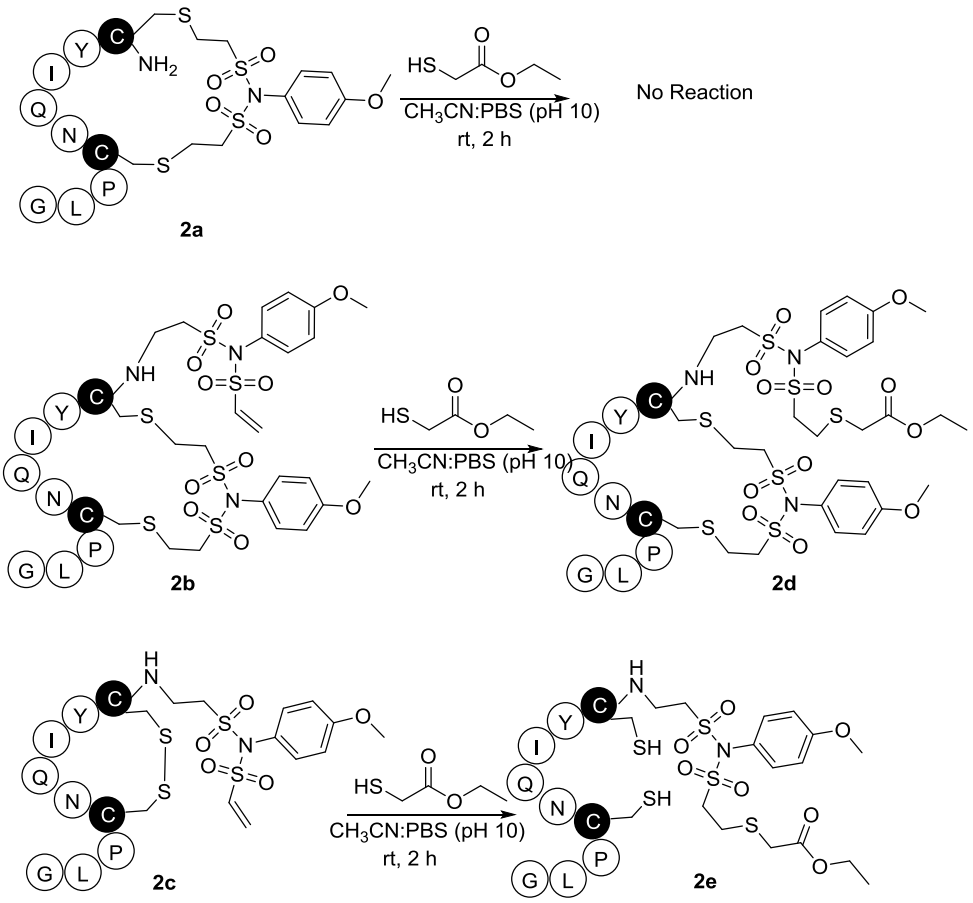


Figure S5 Analytical data for oxytocin **2**: m/z calcd for $C_{43}H_{66}N_{12}O_{12}S_2Na$ [$(M+Na)^+$]: 1029.4262, found:1029.4278; **2a**: m/z calcd for $C_{54}H_{82}N_{13}O_{17}S_4$ [$(M+H)^+$]: 1312.4834, found:1312.4793; **2b** : m/z calcd for $C_{65}H_{95}N_{14}O_{22}S_6$ [$(M+H)^+$]: 1615.5070, found:1615.5253; **2c** : m/z calcd for $C_{54}H_{80}N_{13}O_{17}S_4$ [$(M+H)^+$]: 1310.4687, found:1310.4697

4. Confirmatory experiments of product 2a, 2b and 2c



Scheme S1. Quenching reaction for remaining vinylsulfonamide using thioglycolate

Prepared using 100 µl of **2a**, **2b** or **2c** solution (received from analytical HPLC), 20 µl of ethyl thioglycolate (25 mM in CH_3CN) and 20 µl of PBS (pH 10.02) at room temperature for 2 hours. The resulting mixture was analyzed by HRMS.

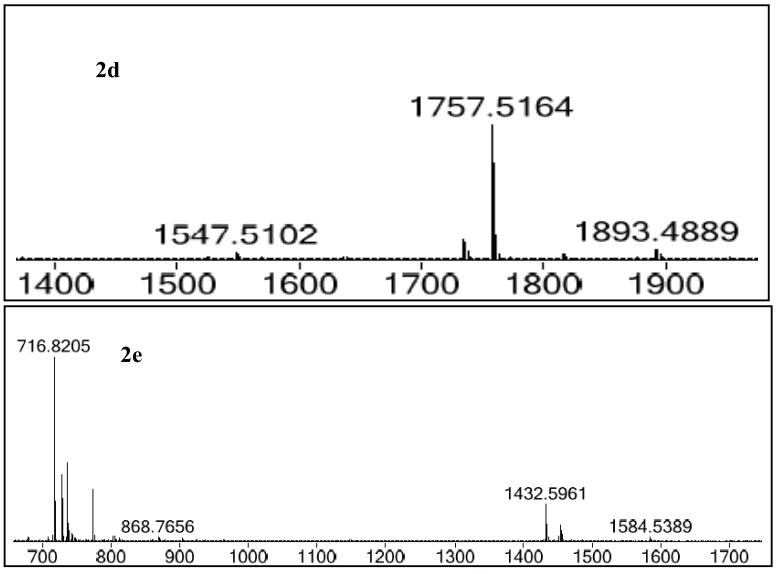
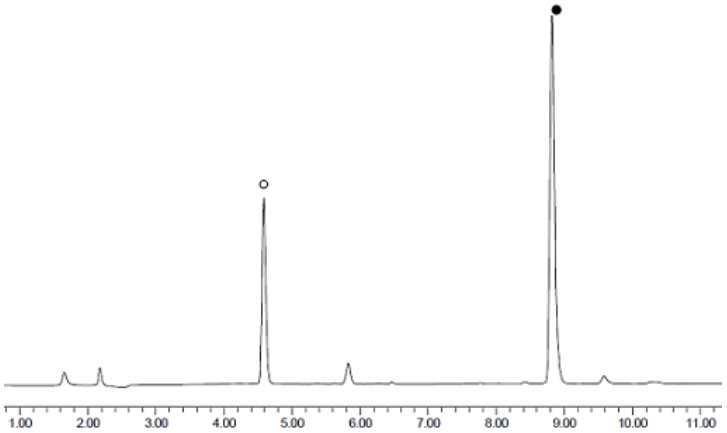


Figure S6 Analytical data for **2d** : m/z calcd for $C_{69}H_{102}N_{14}O_{24}S_7Na$ [$(M+Na)^+$]: 1757.5134, found:1757.5164. Analytical data for **2e**: m/z calcd for $C_{58}H_{90}N_{13}O_{19}S_5$ [$(M+H)^+$]: 1432.5079, found:1432.5960. The disulfide bridge was reduced by excessive ethyl thioglycolate.

5. The investigation of site-selective modification of 1d at low concentration

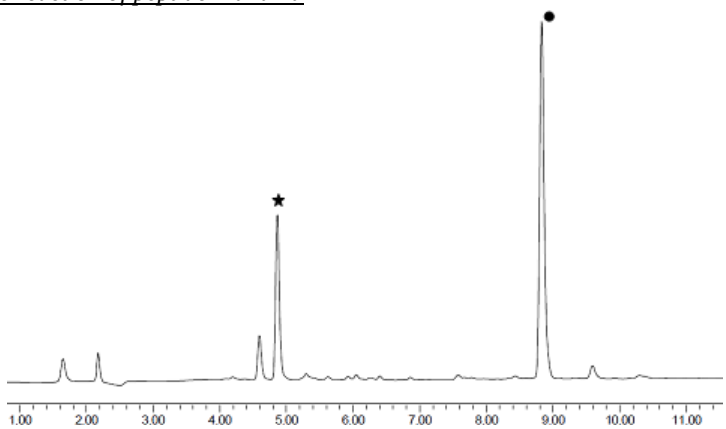
Protocol: A 330 µl LABTIDE 96 Round Well contained 12 µl of peptide **3** or **4** (1 mM stock solution in H_2O), 120 µl of PBS buffer (Cat. NO. SH30256.01, pH 7.07) and 80 µl of CH_3CN followed 12 µl of **1d** (1 mM stock solution in CH_3CN) added. The mixture was reacted on Micro plate fast oscillator at room temperature for 2 hours and the resulting mixture was subjected to HPLC analysis via *Method A.*.

*HPLC analytical data of crude reaction of peptide **3** and **1d***



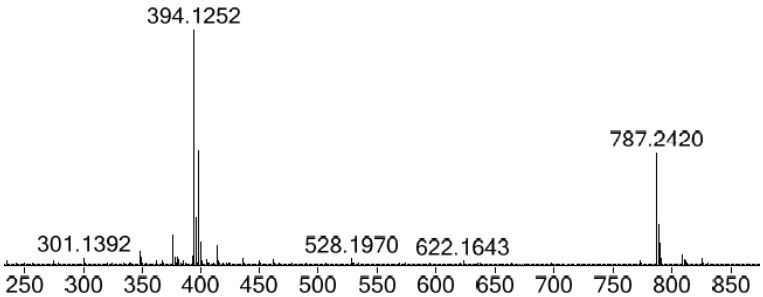
Signal of starting material peptide **3** and **1d** marked with an empty circle (○) and a full black circle (●).

*HPLC analytical data of crude reaction of peptide **4** and **1d***



Signal of starting material **1d** and conjugate product **4a** marked with a full black circle (●) and .a full black star (★). The starting material peptide **4** had not obvious peak using *Method A*.

*ESI-HRMS analytical data of product **4a***



Analytical data for **4a**: m/z calcd for $C_{32}H_{47}N_6O_{11}S_3$ $[(M+H)^+]$: 787.2465, found:787.2420.

6. The conjugation between oxytocin or modified oxytocin with functional materials

6.1 The conjugation of oxytocin and functional divinylsulfonamide 5, 7a, 7b or 7c

*HPLC analytical data of conjugation crude reaction using **5, 7a, 7b or 7c***

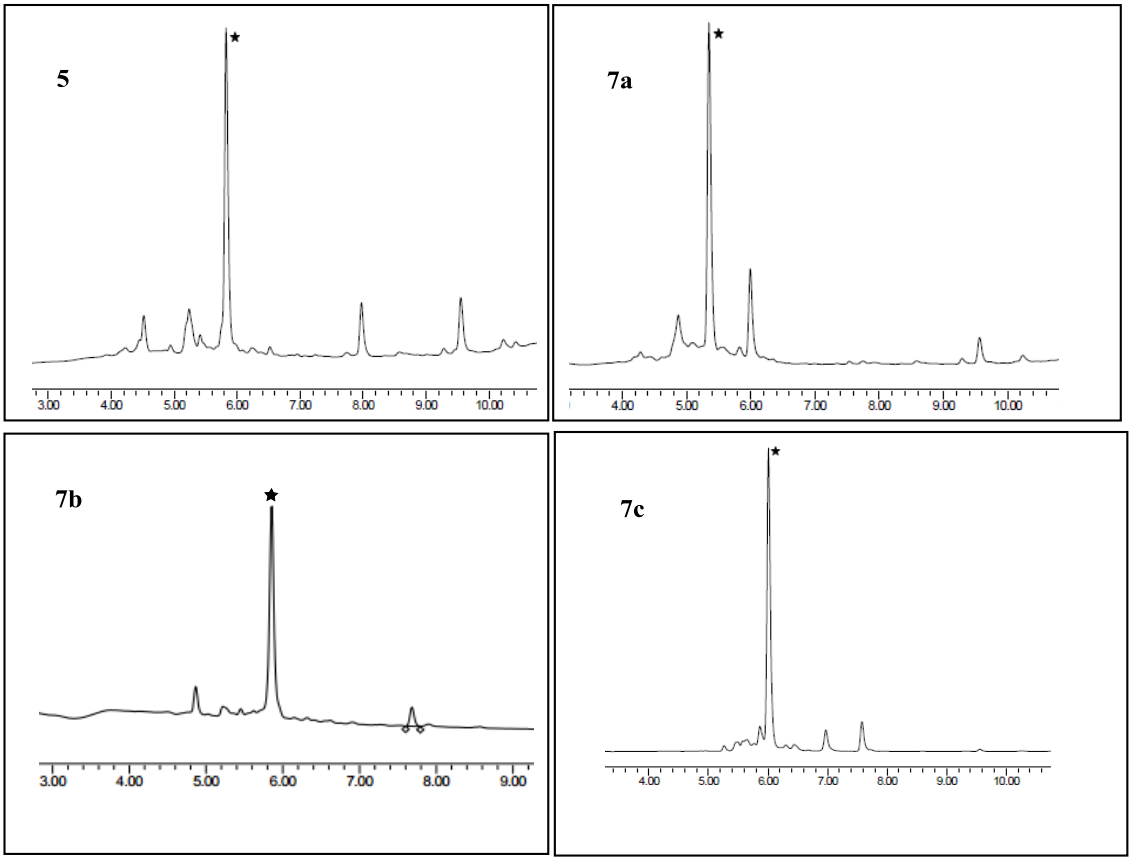


Figure S7 A 330 µl LABTIDE 96 Round Well contained 12 µl of oxytocin **2** (1 mM stock solution in H_2O), 20 µl of TCEP (1 mM stock solution in H_2O, pH 7.07 was adjusted by NaOH and H_3PO_4), 120 µl of PBS buffer (Cat. NO. SH30256.01, pH 7.07) and 80 µl of CH_3CN. The mixture was reacted on Micro plate fast oscillator at room temperature for 1.5 hours to attain reduced oxytocin followed 12 µl of **5** (1 mM stock solution in CH_3CN), **7a**, **7b** or **7c** (1 mM stock solution in DMF) added. The mixture was reacted on Micro plate fast oscillator at room temperature for 2 hours and the resulting mixture was subjected to HPLC *Method A* analysis. Signal of the relevant conjugation product **8** (yield=76%), **9** (yield= 79%), **10** (yield= 81%) or **11** (yield= 70%) are marked with a full black star (★).

*ESI-HRMS analytical data of product **8**, **9**, **10** and **11***

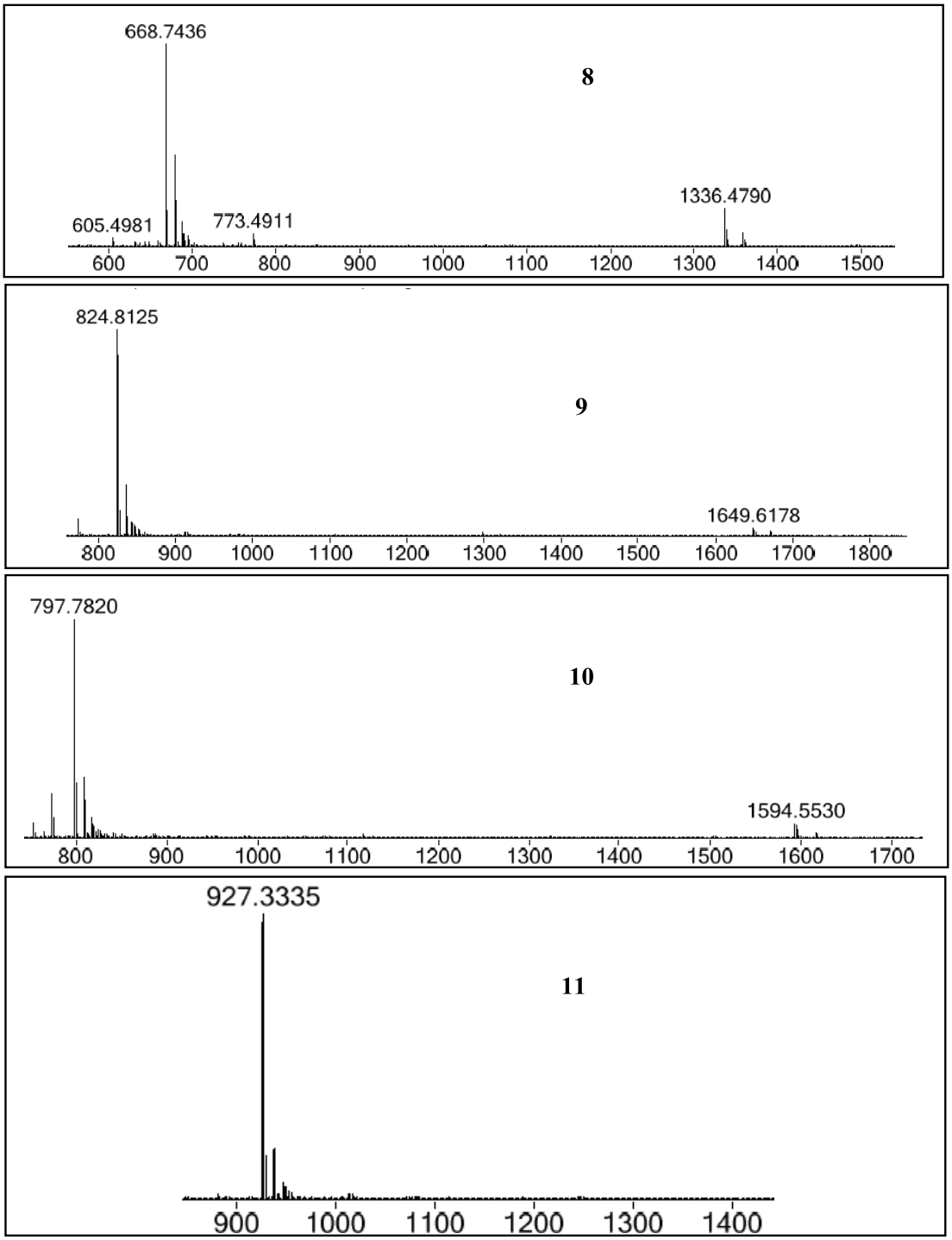


Figure S8 Analytical data for **8**: m/z calcd for $C_{56}H_{82}N_{13}O_{17}S_4$ [$(M+H)^+$]: 1336.4834, found:1336.4791; **9**: m/z calcd for $(C_{68}H_{103}N_{19}O_{19}S_5)/2$ [$(M/2+H)^+$]: 824.8140, found:824.8125; **10**: m/z calcd for $(C_{68}H_{93}N_{17}O_{20}S_4)/2$ [$(M/2+H)^+$]: 797.7833, found:797.7820; **11**: m/z calcd for $(C_{82}H_{107}N_{19}O_{23}S_4)/2$ [$(M/2+H)^+$]: 926.8335, found:927.3335.

6.2 The conjugation of modified oxytocin 8 and 6b, 6c or 6d via click chemistry

*HPLC analytical data of click chemistry crude reaction using **6b**, **6c** or **6d***

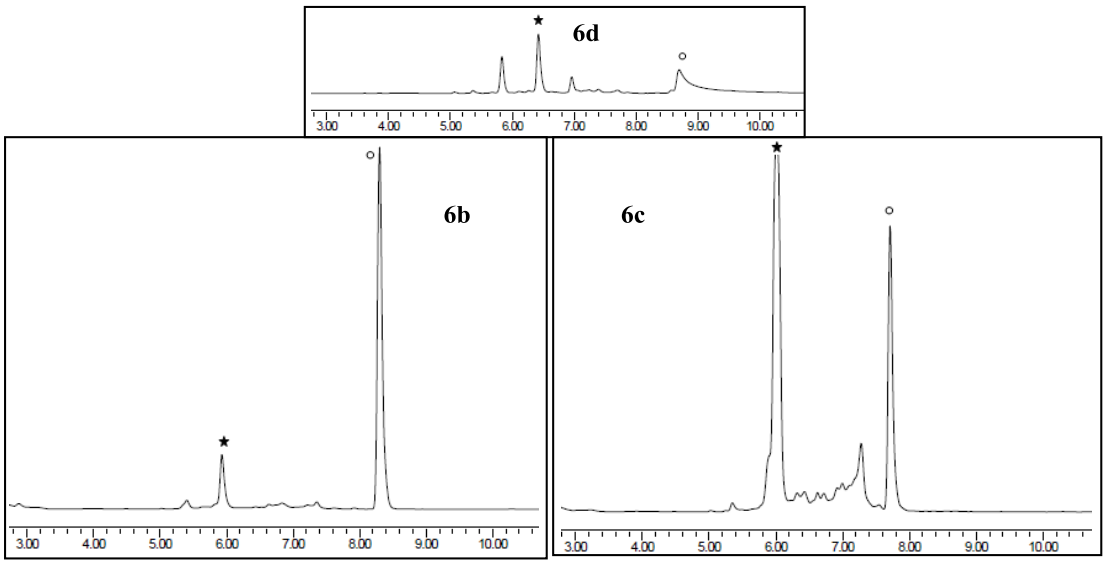


Figure S9 A 330 µl LABTIDE 96 Round Well contained modified oxytocin **8** (1 mg), Na ascorbate (0.15 mg), ^{t}BuOH/H_2O/DMF (1/1/1) (300 µl), $CuSO_4$ (0.12 mg) and **6b** (2.8 mg), **6c** (2.8 mg) or **6d** (0.7 mg). The mixture was reacted on Micro plate fast oscillator at room temperature for 2 hours to attain product **10**, **11** or **12** and the resulting mixture was subjected to HPLC *Method A* analysis. Signal of starting material **6b**, **6c** or **6d** marked with an empty circle (○). Signal of the relevant conjugation product **10** (yield = 56%), **11** (yield = 62%) or **12** (yield = 42%) are marked with a full black star (★).

*ESI-HRMS analytical data of product **12***

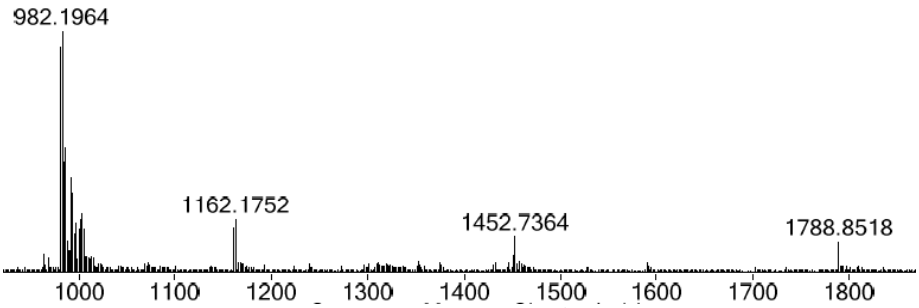


Figure S10 Analytical data for **12**: m/z calcd for $(C_{85}H_{117}N_{19}O_{23}S_6)/2$ $[(M/2+H)^+]$: 981.8447, found:982.1964.

7. The conjugation between salmon calcitonin with functional materials

*HPLC analytical data of sCT **13** and conjugation crude reaction using **1d**, **5** or **7a***

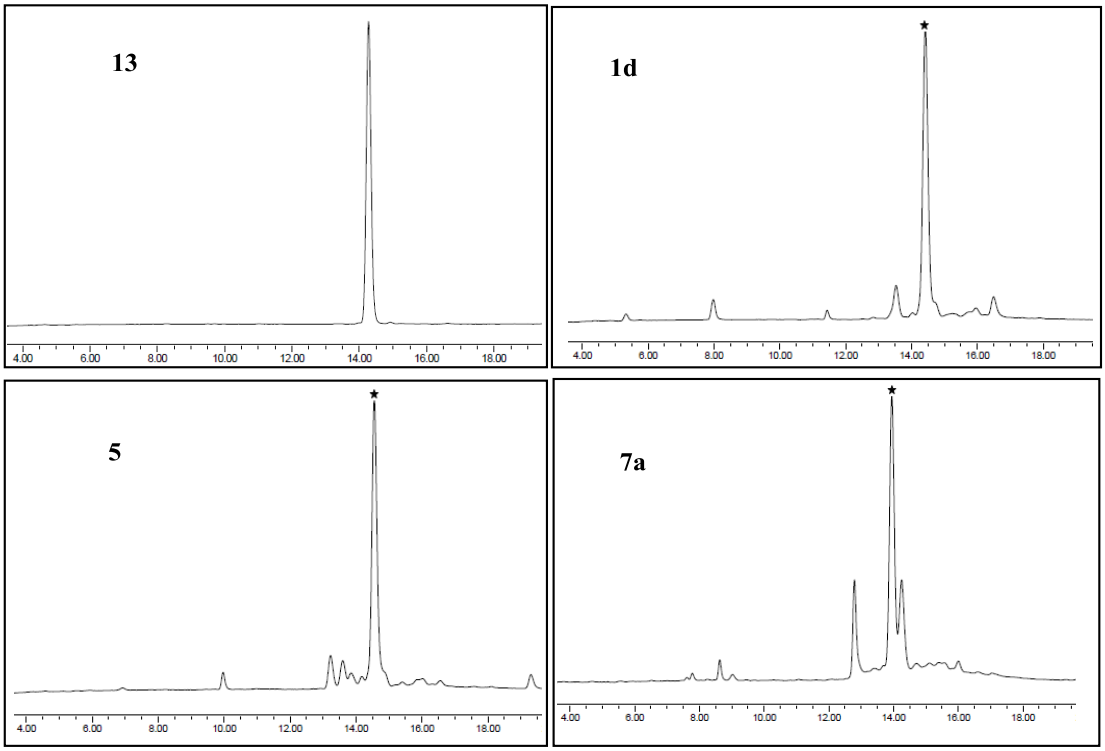


Figure S11 A 330 µl LABTIDE 96 Round Well contained 12 µl of sCT **13** (1 mM stock solution in H_2O), 20 µl of TCEP (1 mM stock solution in H_2O, pH 7.07 was adjusted by NaOH and H_3PO_4), 120 µl of PBS buffer (Cat. NO. SH30256.01, pH 7.07) and 80 µl of CH_3CN. The mixture was reacted on Micro plate fast oscillator at room temperature for 1.5 hours to attain reduced oxytocin followed 12 µl of **1d**, **5** or **7a** (1 mM stock solution in CH_3CN or DMF) added. The mixture was reacted on Micro plate fast oscillator at room temperature for 2 hours and the resulting mixture was subjected to HPLC *Method B* analysis. Signal of the relevant conjugation product **14** (yield = 81%), **15** (yield = 83%) or **16** (yield = 60%) are marked with a full black star (★).

*ESI-HRMS analytical data of sCT**13** and product **14**, **15** and **16***

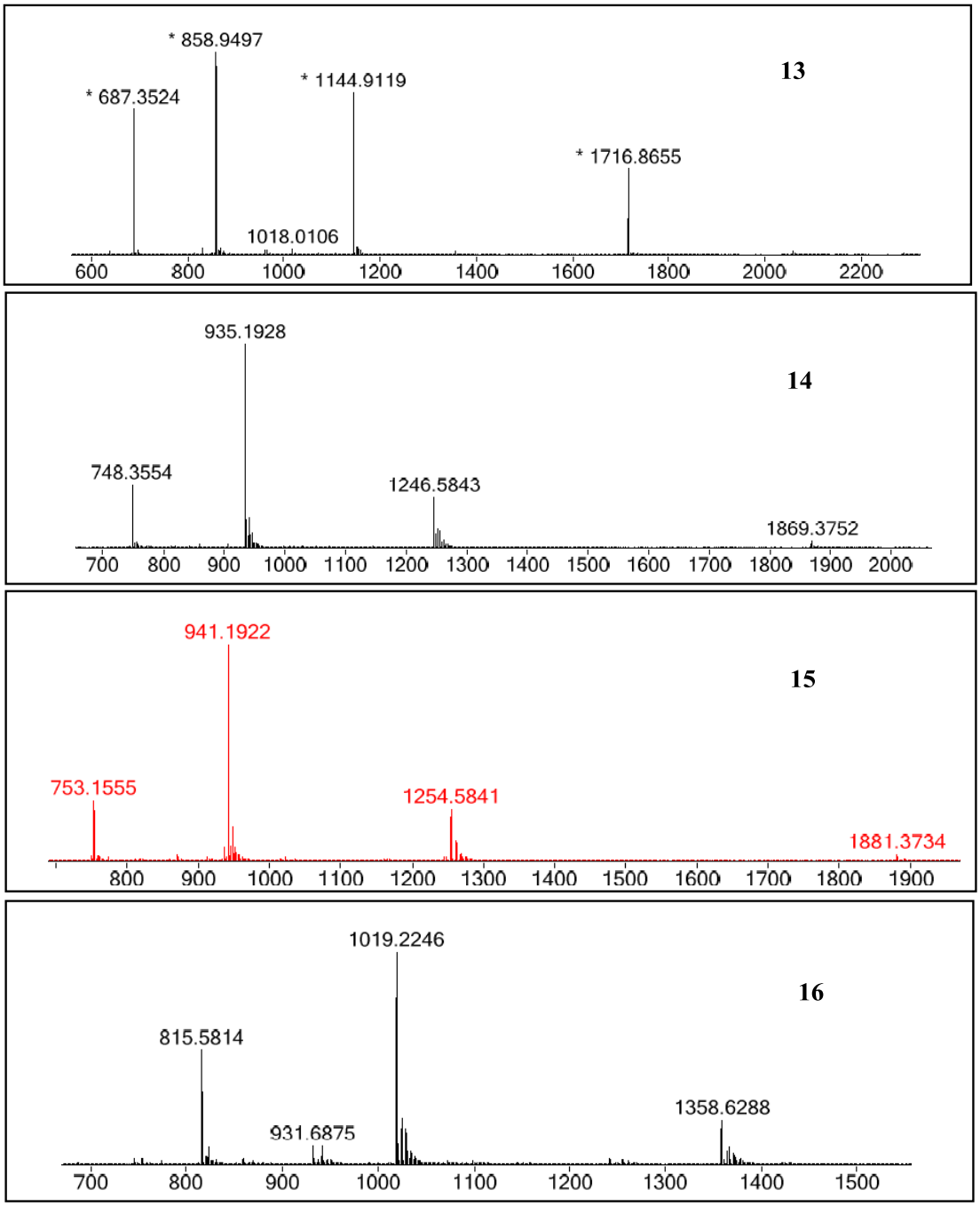


Figure S12 Analytical data for sCT **13**: m/z calcd for $(C_{145}H_{244}N_{44}O_{48}S_2)/4$ $[(M/4+H)^+]$: 858.4362, found:858.9497; **14**: m/z calcd for $(C_{156}H_{259}N_{45}O_{53}S_4)/4$ $[(M/4+H)^+]$: 934.6960, found:935.1928; **15**: m/z calcd for $(C_{158}H_{259}N_{45}O_{53}S_4)/4$ $[(M/4+H)^+]$: 940.6960, found:941.1922; **16**: m/z calcd for $(C_{170}H_{279}N_{51}O_{55}S_5)/4$ $[(M/4+H)^+]$: 1018.7302, found:1019.2246.

8. The Fluorescence measurements of 2, 10 and 7b.

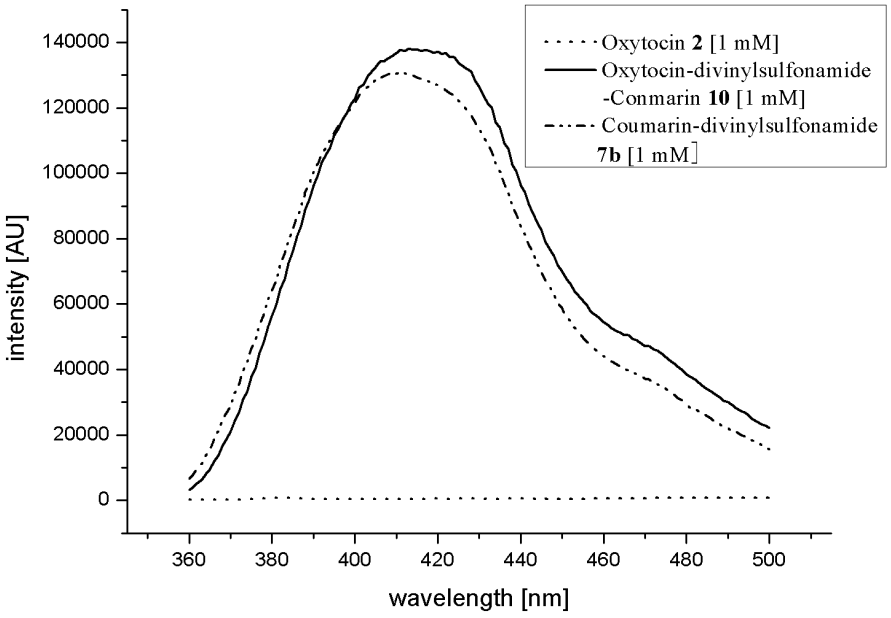
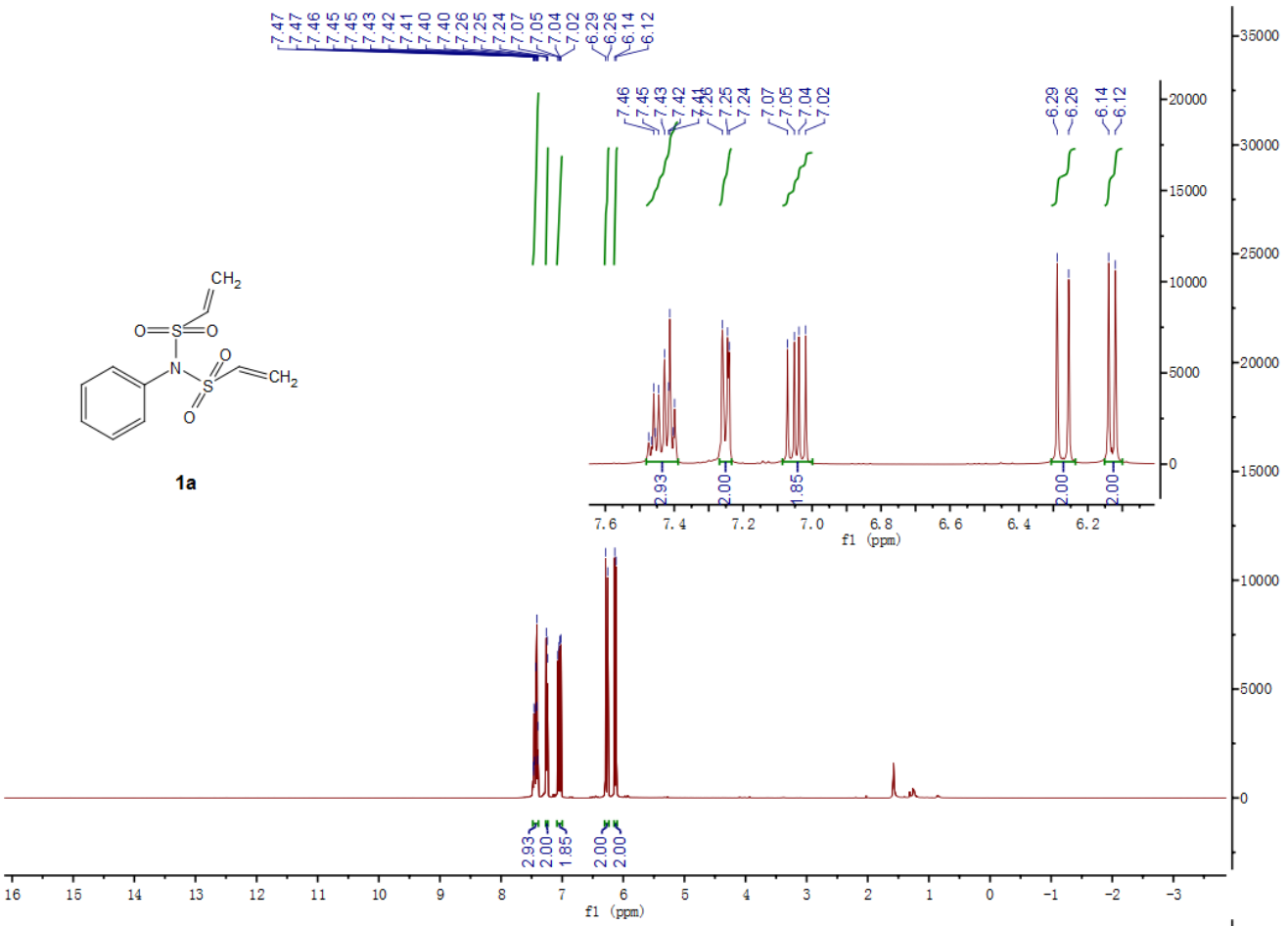
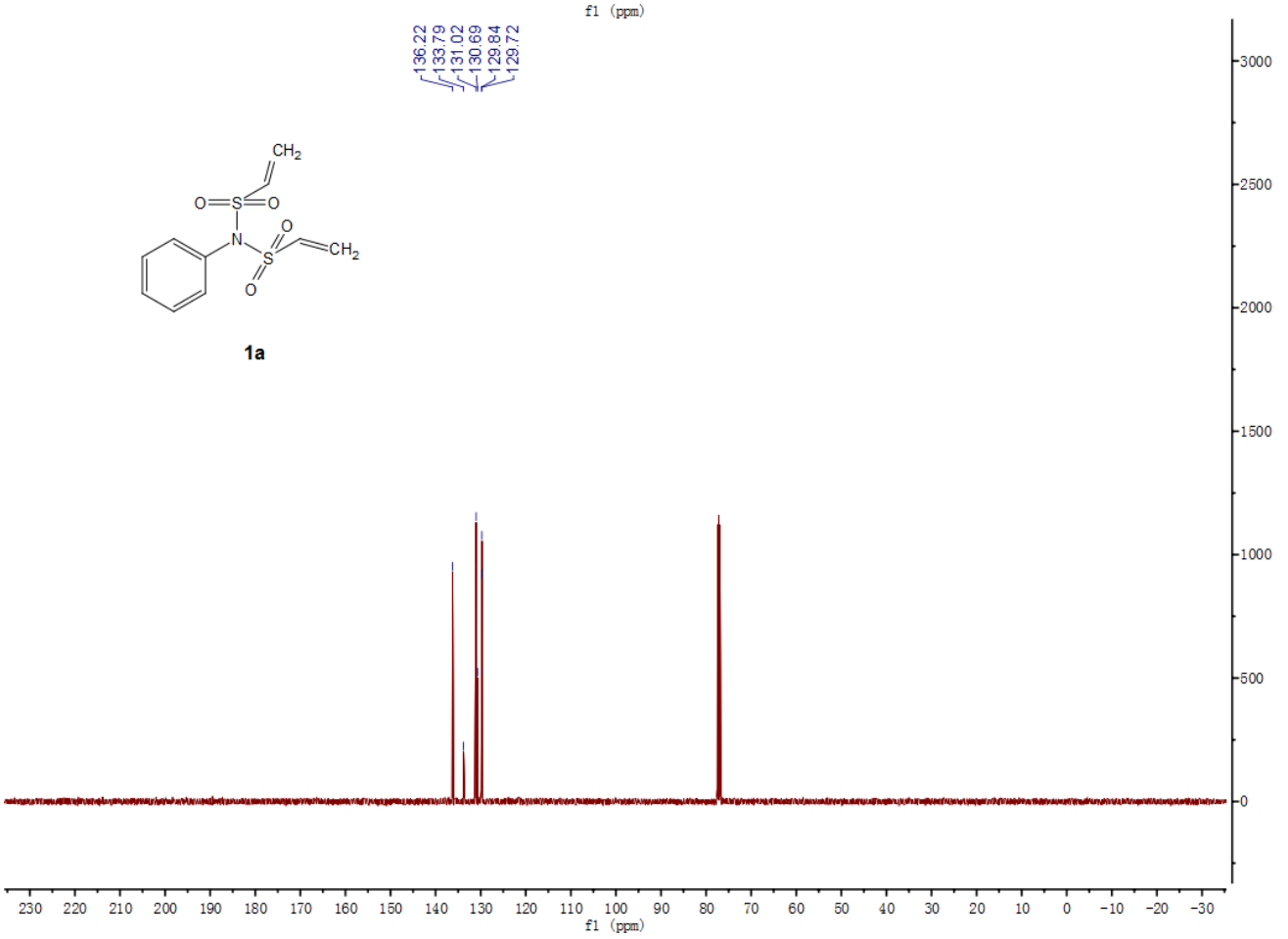
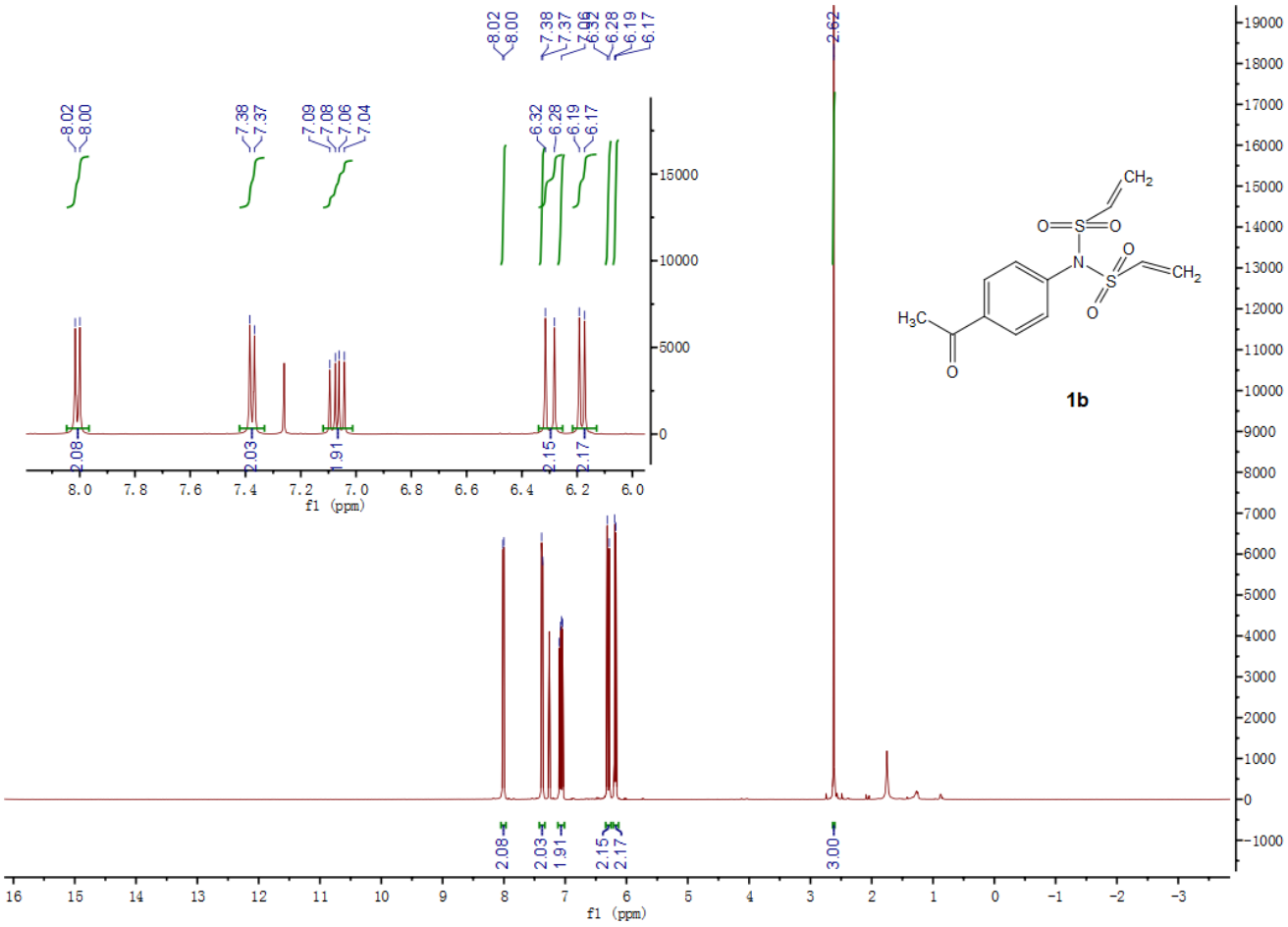


Figure S13. Fluorescence measurements of samples excited at 340 nm

9. ^{1}H NMR and ^{13}C NMR



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7.38
7.37
7.09
7.08
7.06
7.04
6.32
6.28
6.19
6.17
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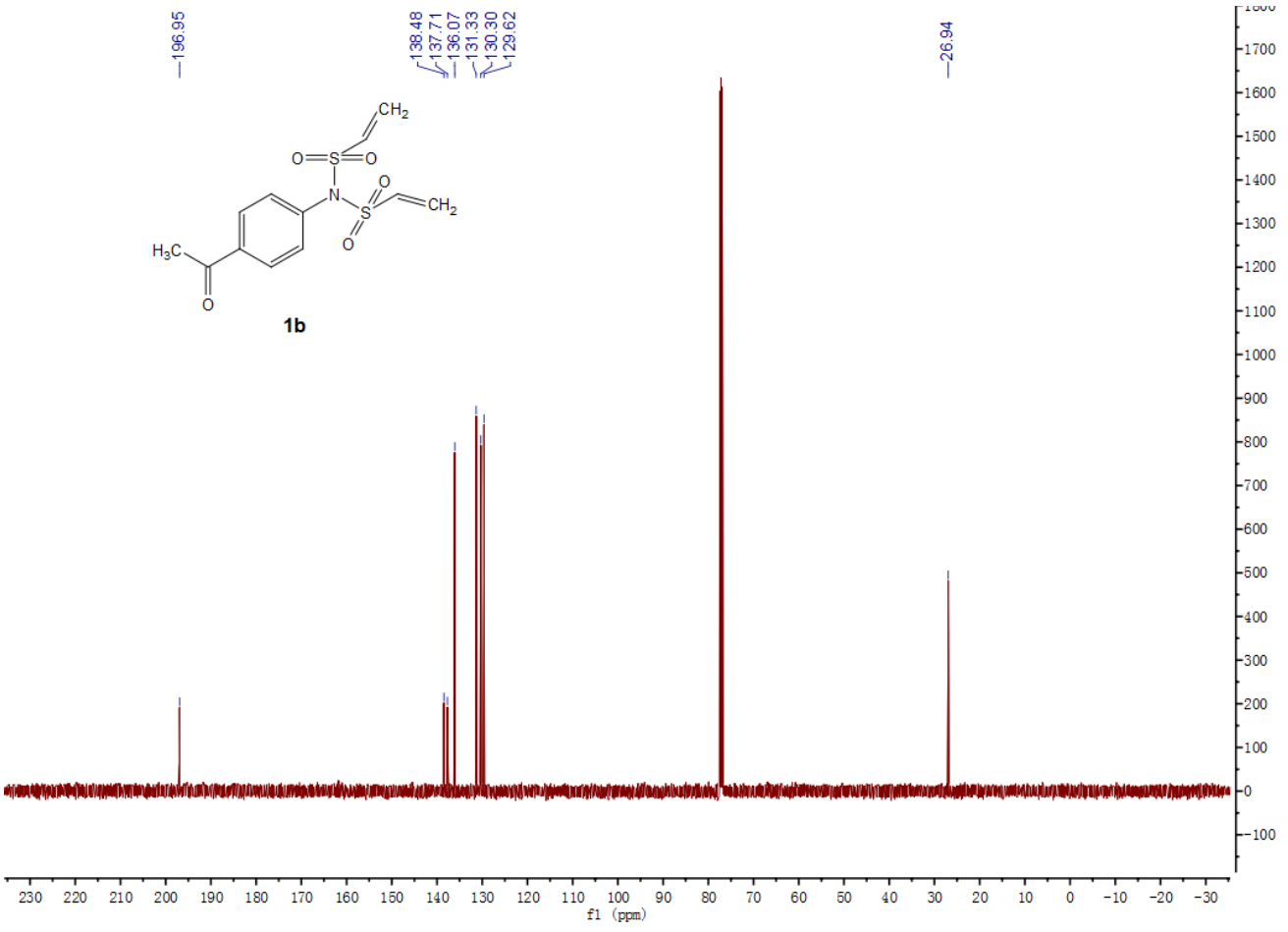
2.15
2.17
3.00
f1 (ppm)
1b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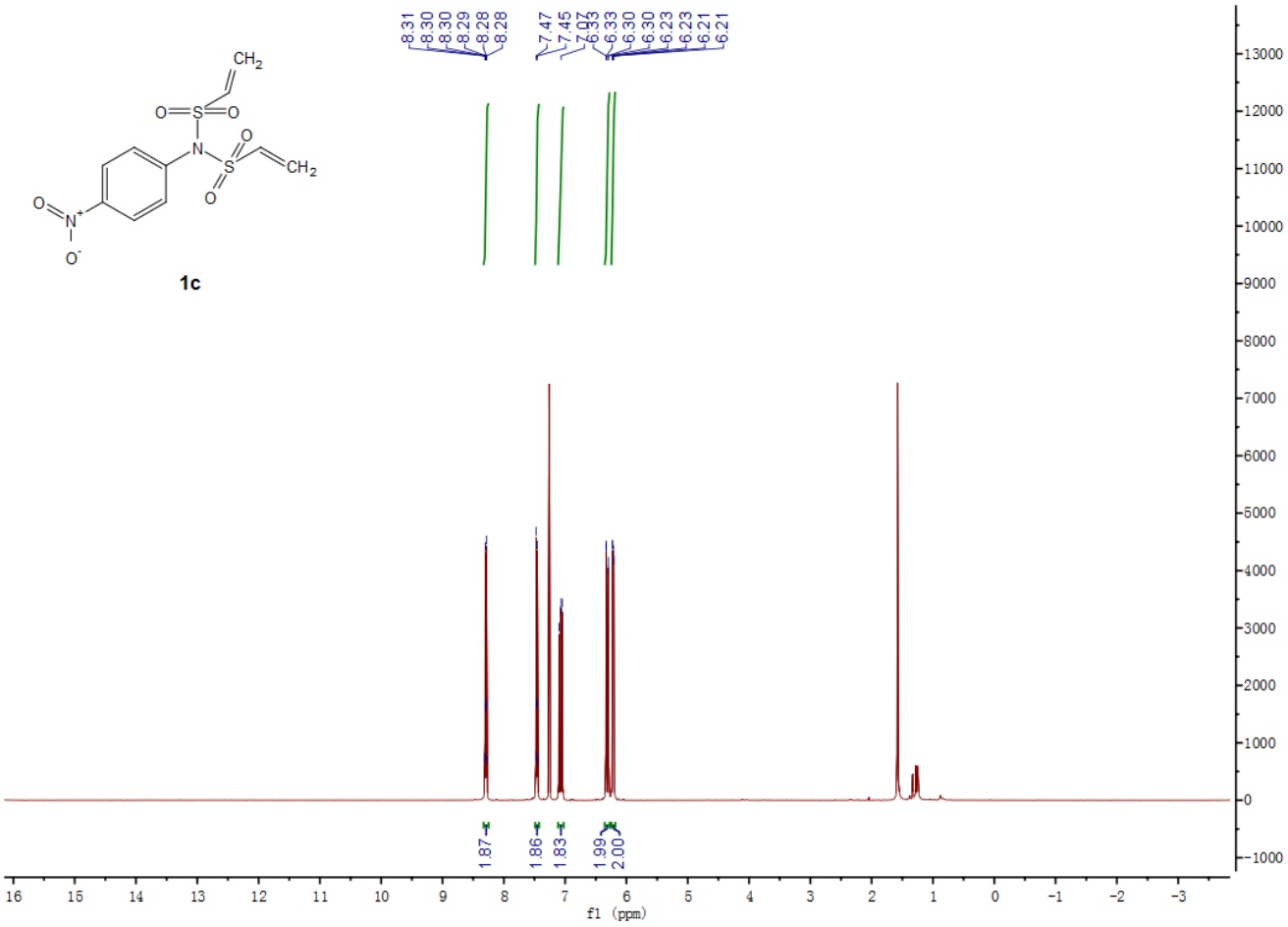
130.30
129.62
26.94
f1 (ppm)
1b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8.29
8.28
8.28
7.47
7.45
7.07
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6.33
6.30
6.30
6.23
6.23
6.21
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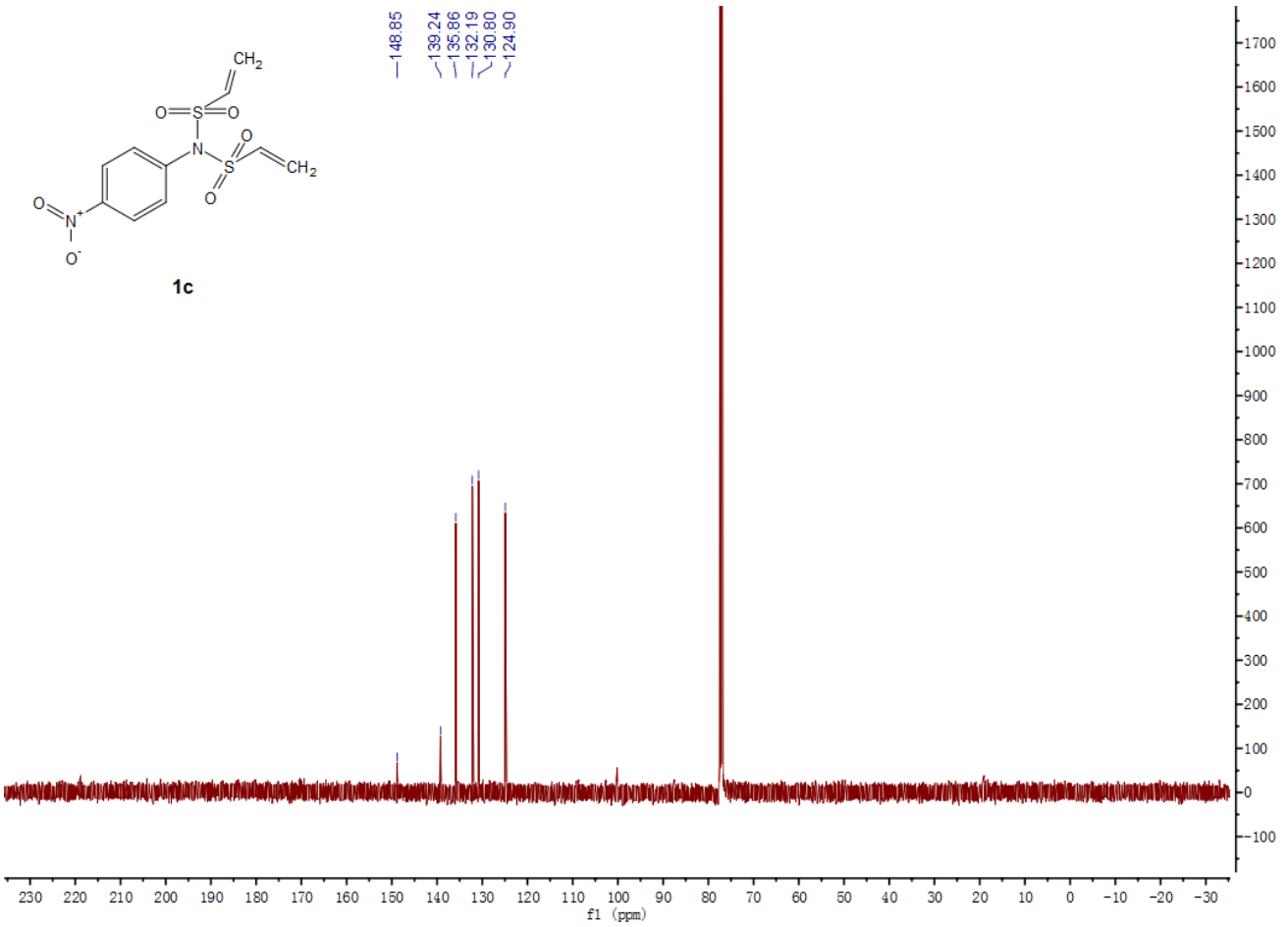
1.86
1.83
1.99
2.00
f1 (ppm)

1c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132.19
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124.90
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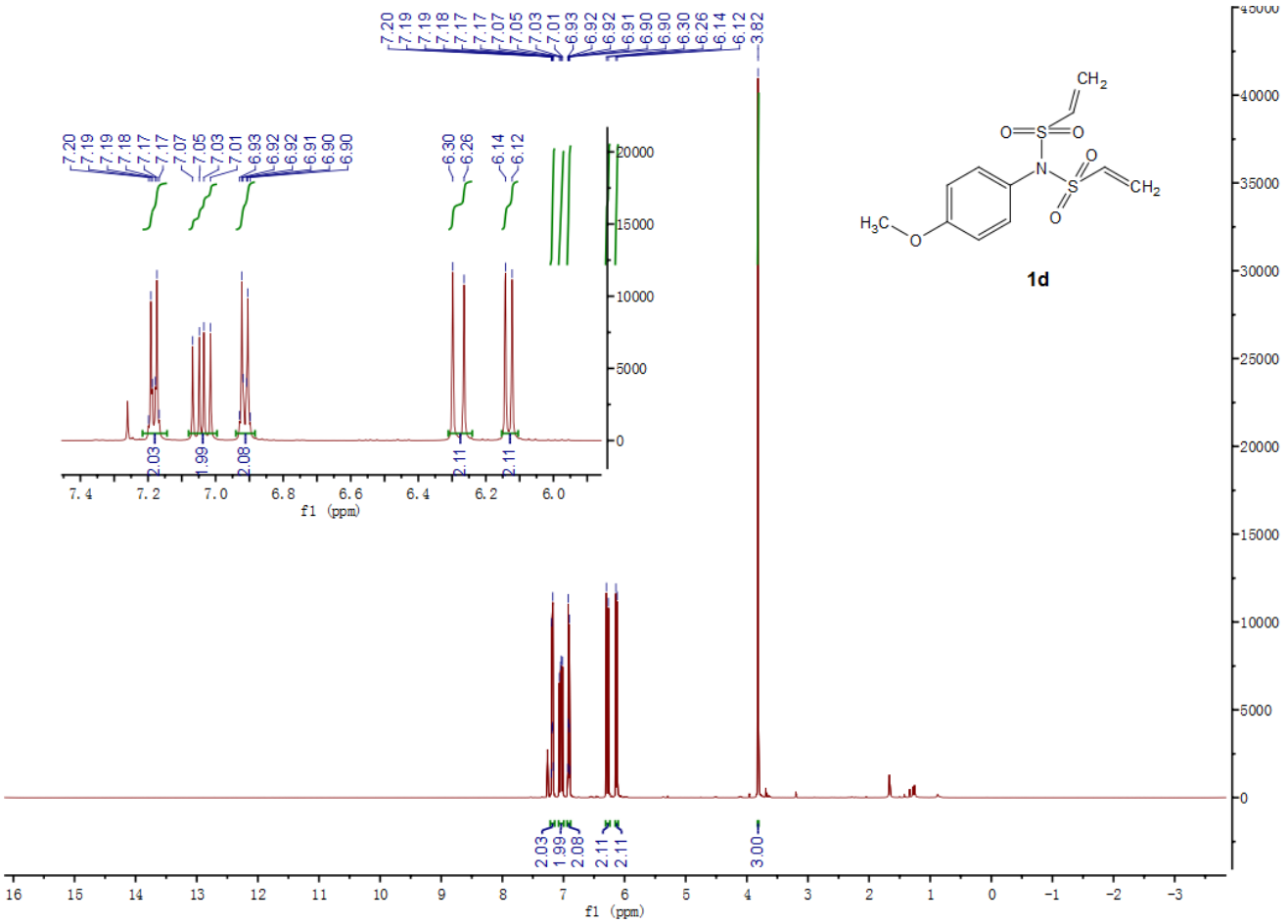
1c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7.18
7.17
7.17
7.07
7.05
7.03
7.01
6.93
6.92
6.92
6.91
6.90
6.90
6.30
6.26
6.14
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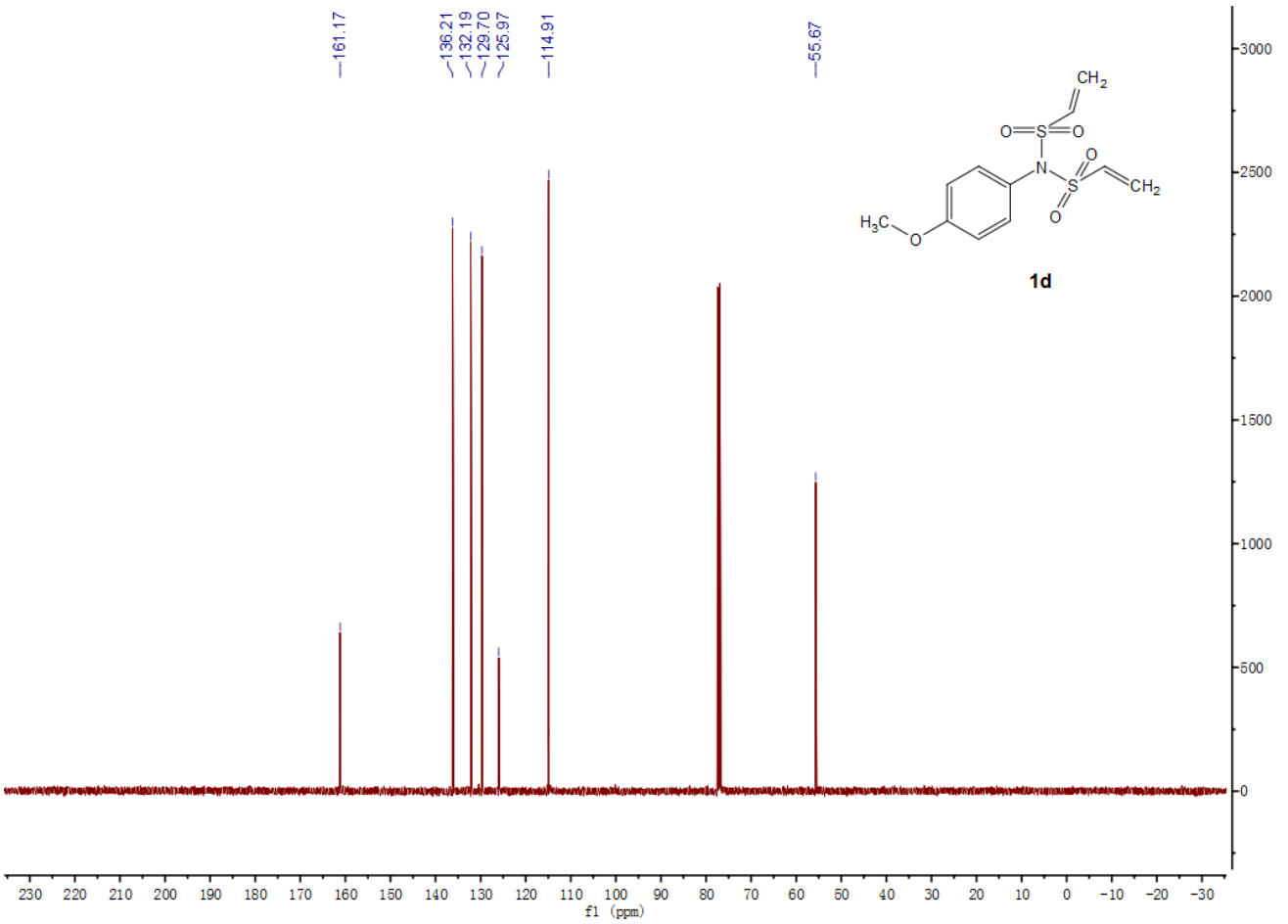
1.99
2.08
2.11
2.11
3.00
f1 (ppm)
1d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114.91
55.67
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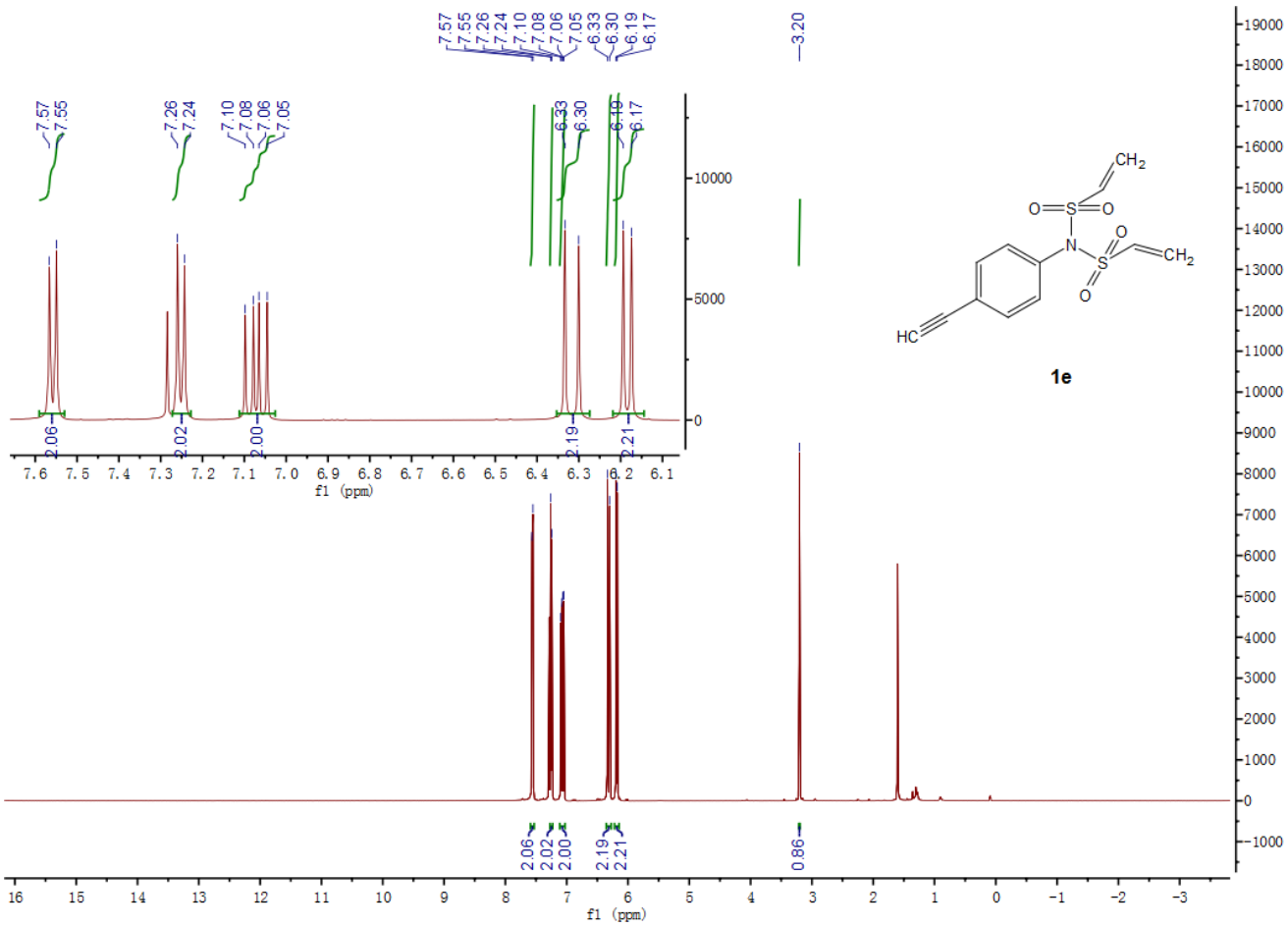
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7.05
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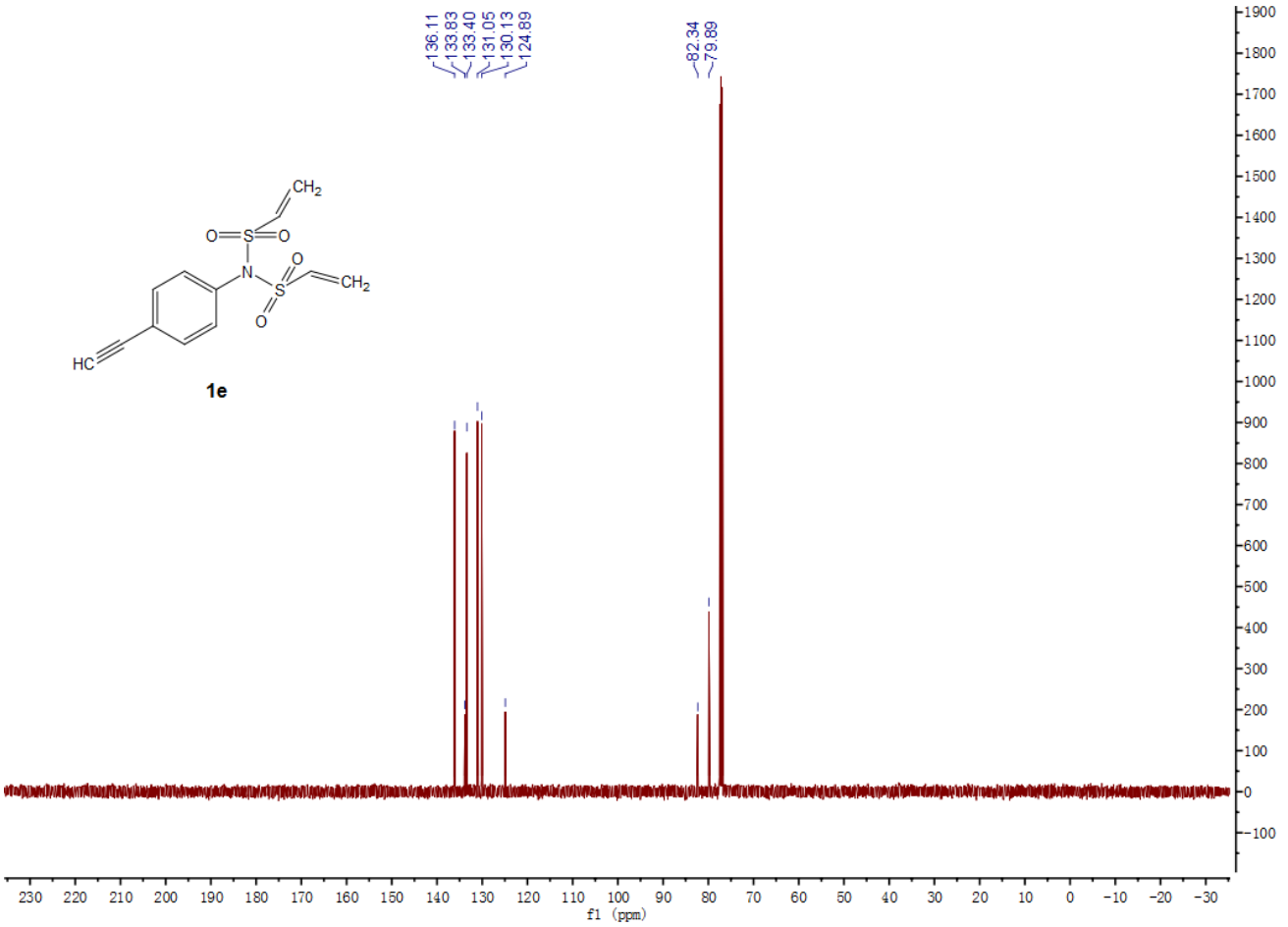
2.02
2.00
2.19
2.21
0.86
f1 (ppm)

1e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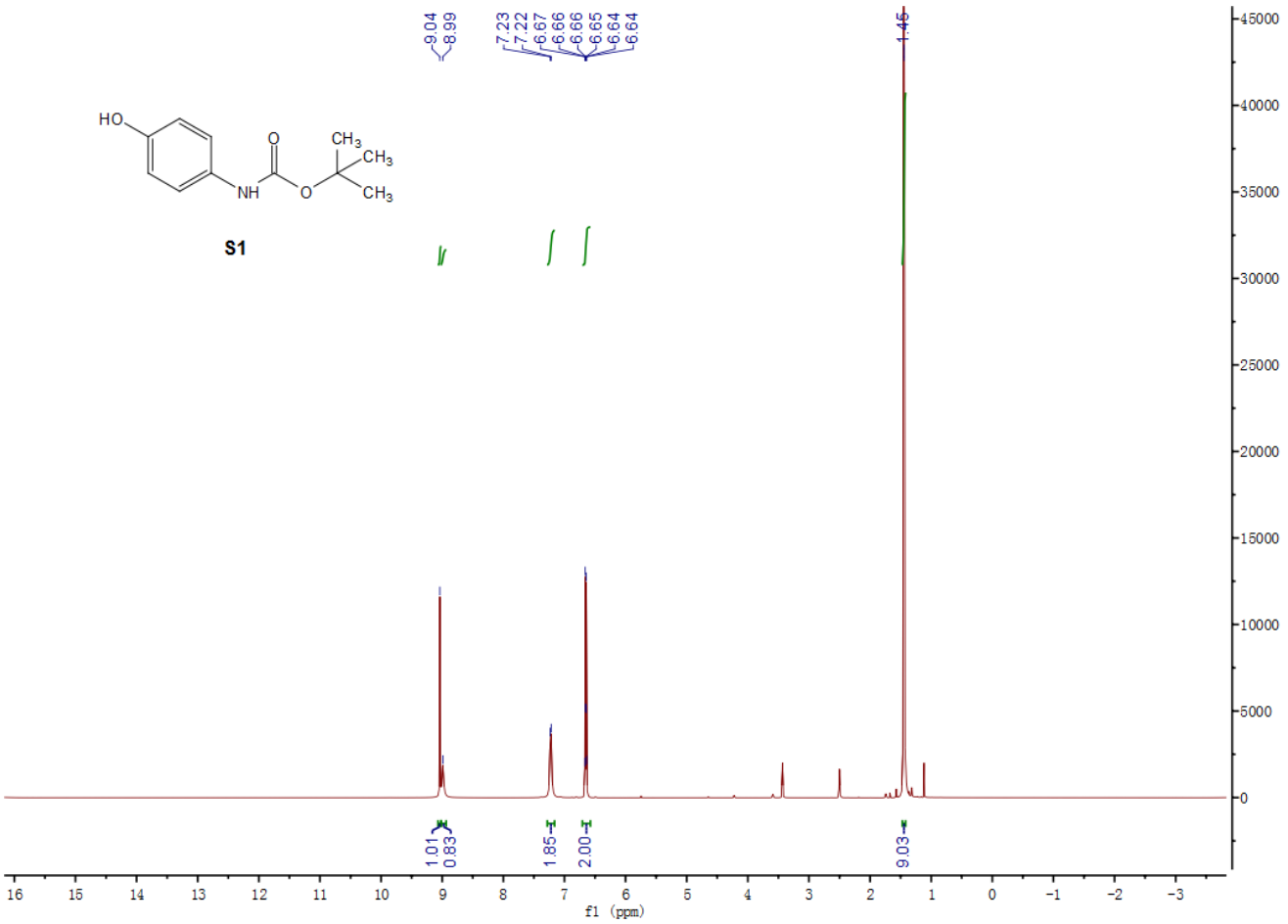
f1 (ppm)

1e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O
O
CH3
CH3
CH3
S1
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8.99
7.23
7.22
6.67
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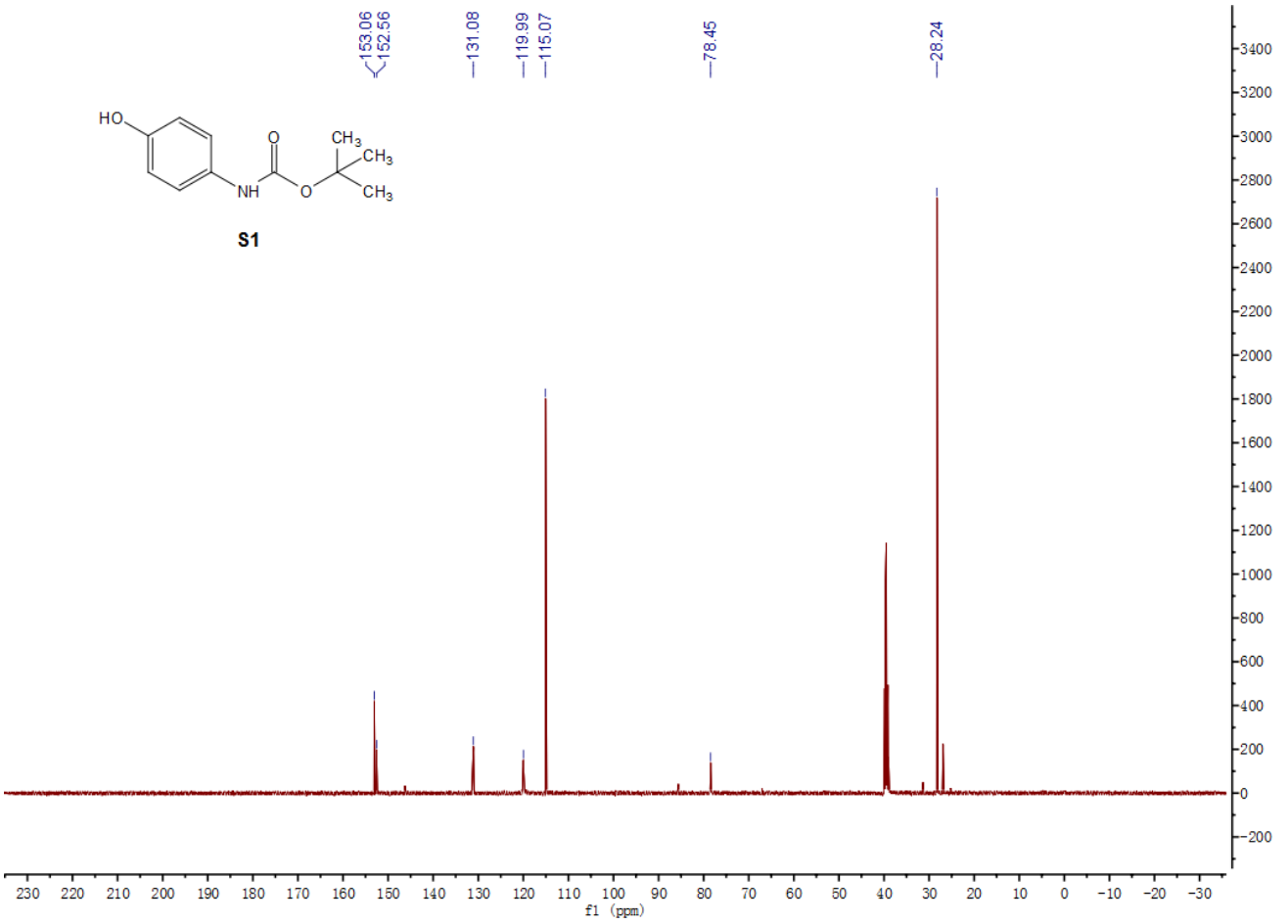
6.66
6.65
6.64
6.64
1.46
1.01
0.83
1.85
2.00
9.03
f1 (ppm)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CH3
CH3
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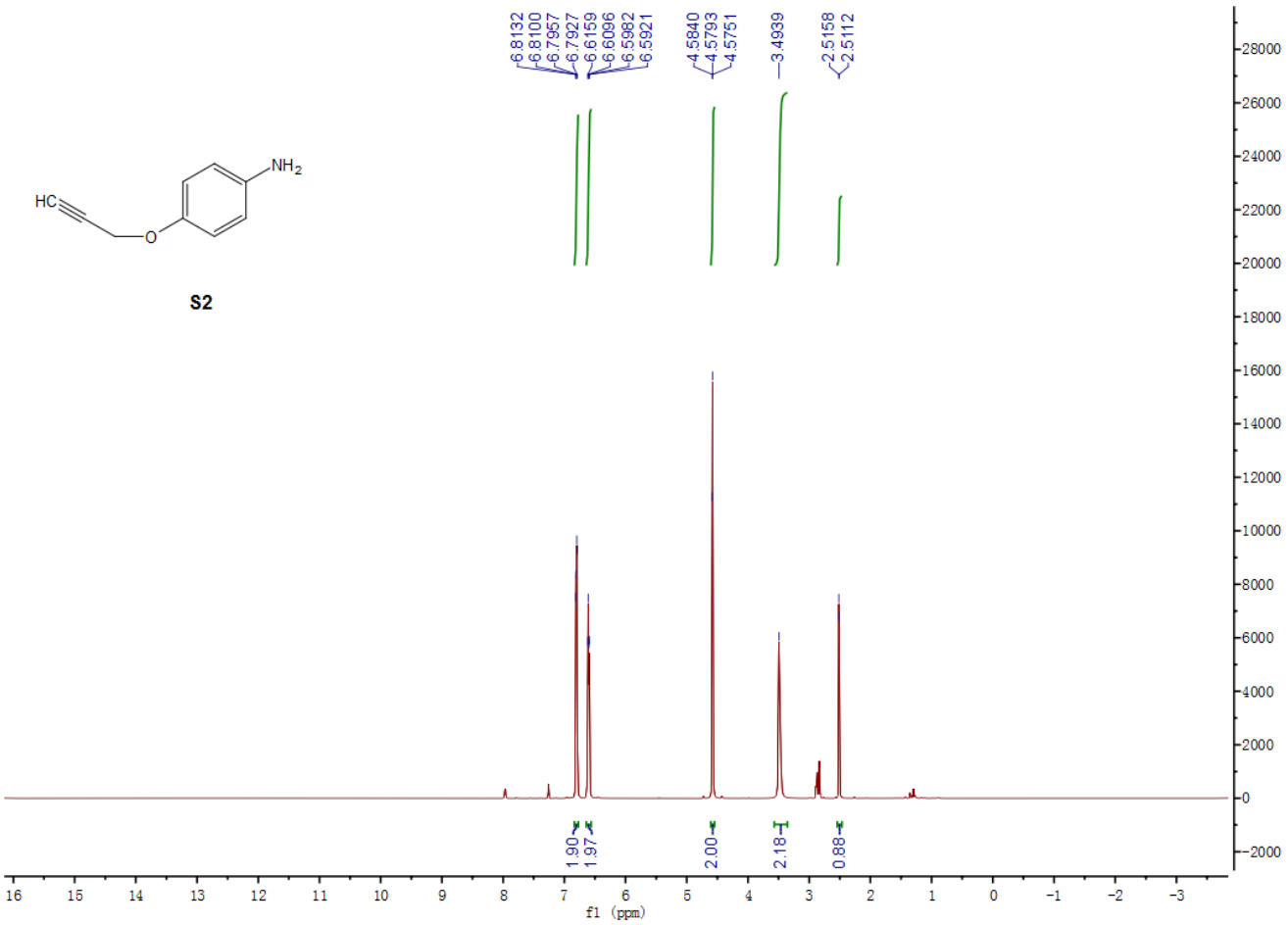
f1 (ppm)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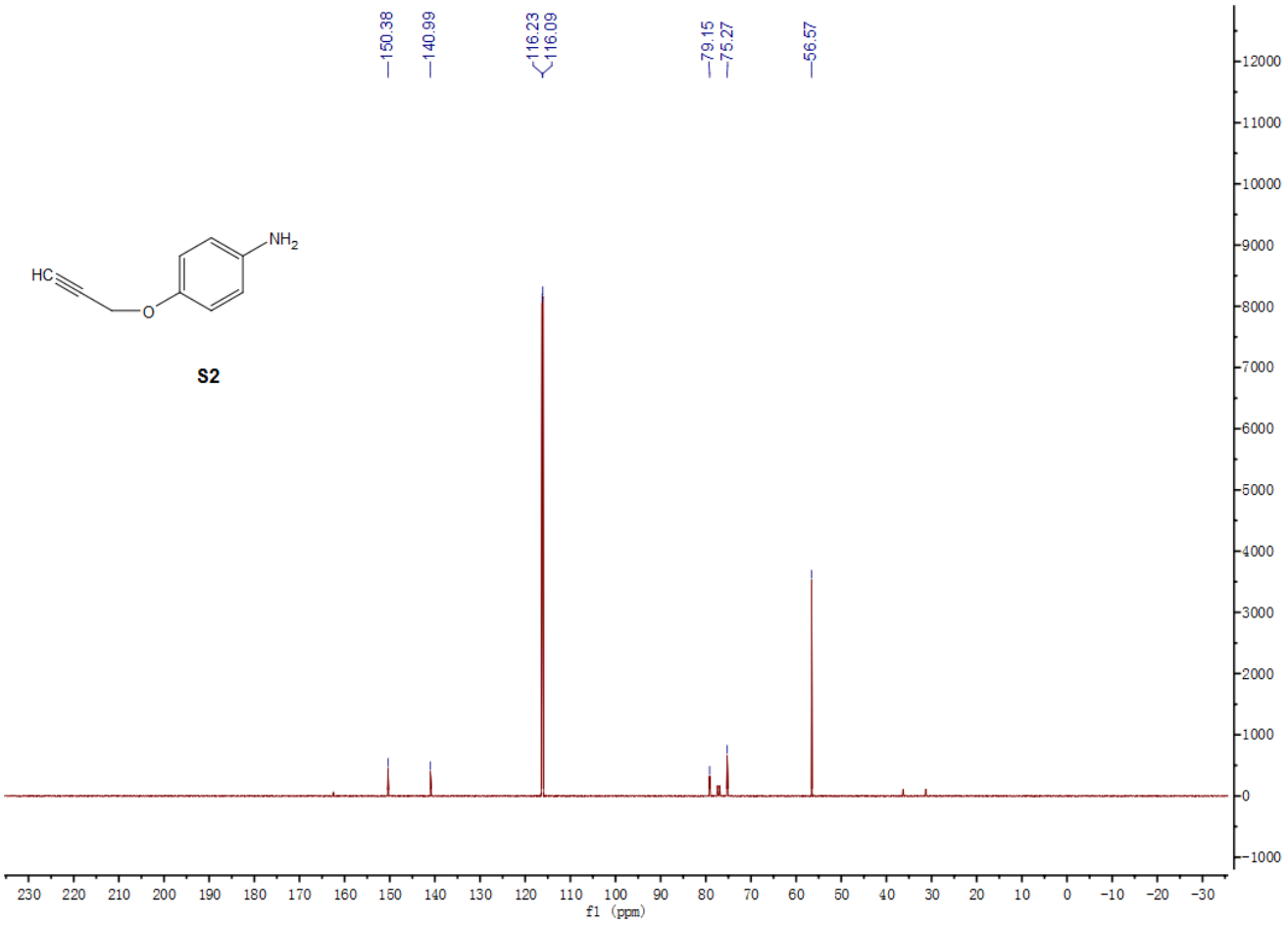
6.7957
6.7927
6.6159
6.6096
6.5982
6.5921
4.5840
4.5793
4.5751
3.4939
2.5158
2.5112
NH2
HC
O
S2
1.90
1.97
2.00
2.18
0.88
f1 (ppm)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75.27
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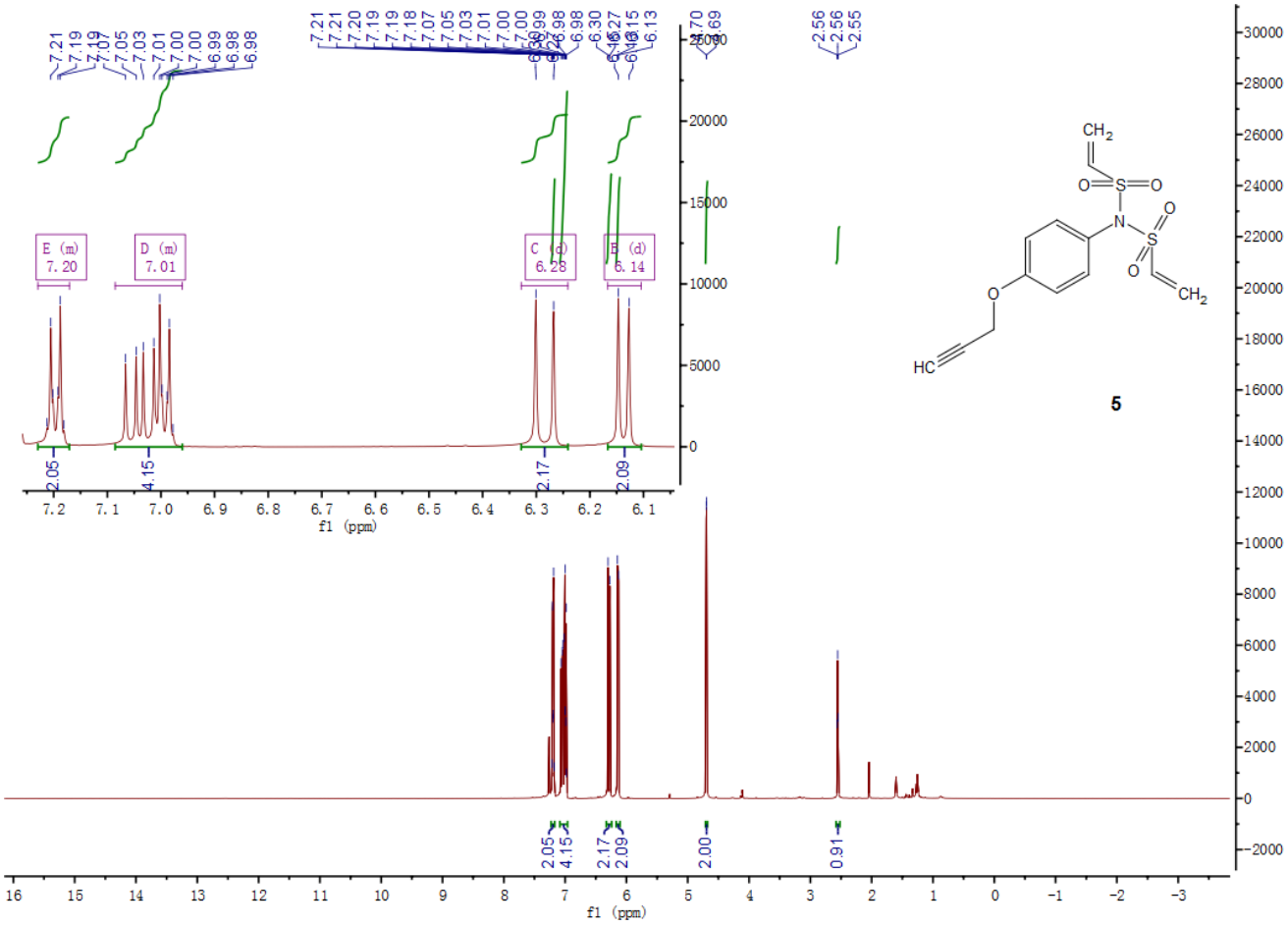
NH2
HC
O
S2
f1 (ppm)


E (m)
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D (m)
7.01
C (d)
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B (d)
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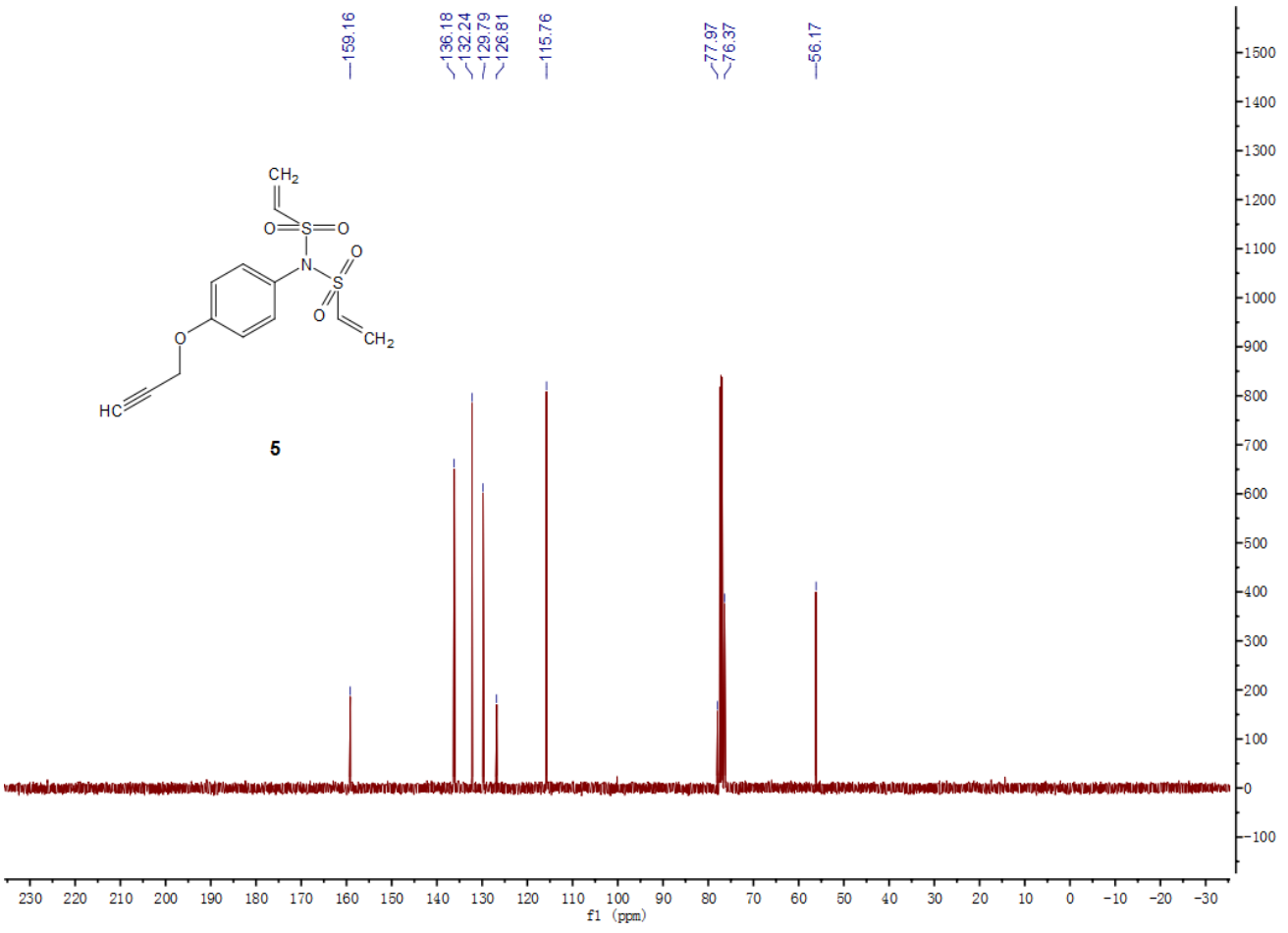
2.05
4.15
2.17
2.09
2.00
0.91
f1 (ppm)
5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76.37
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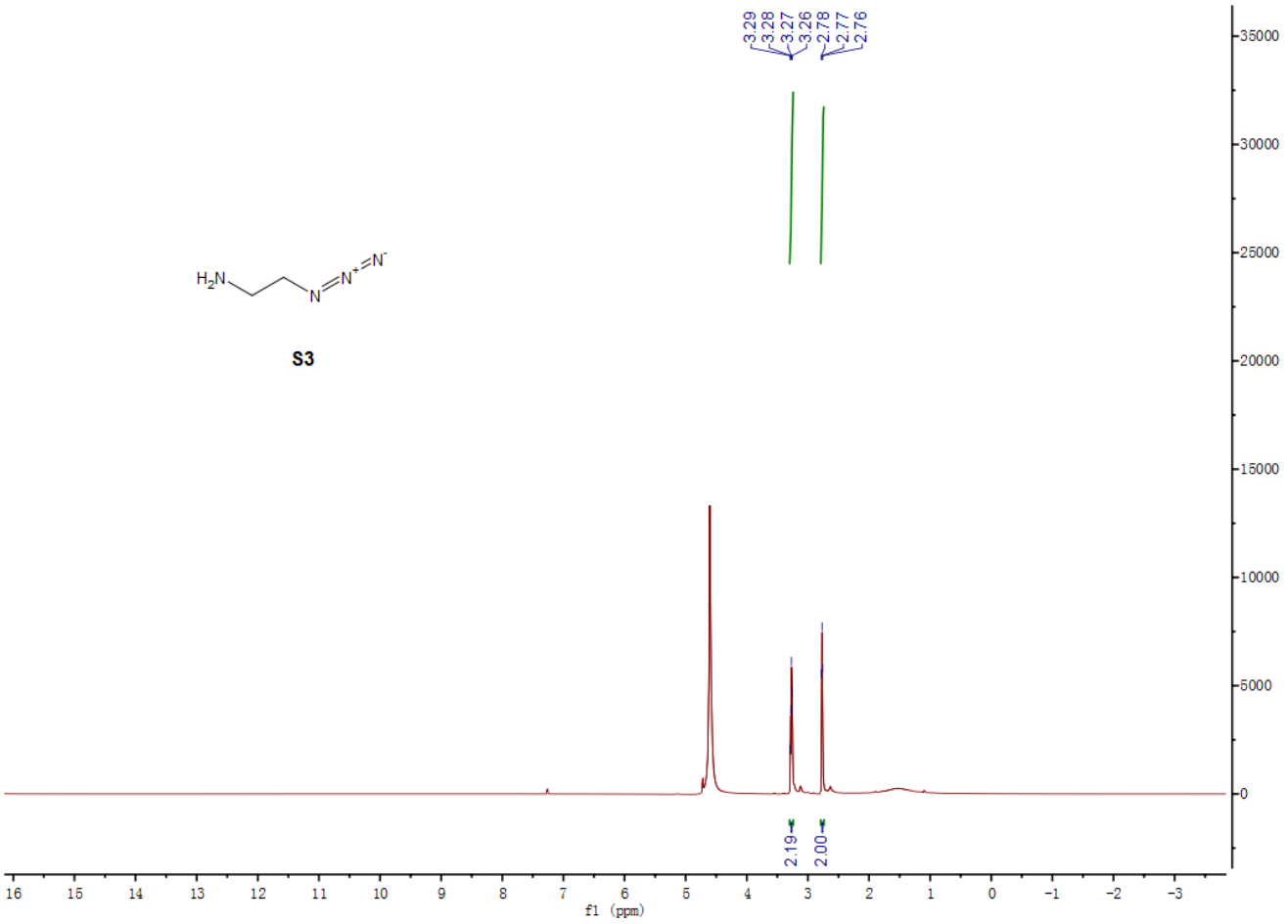
5



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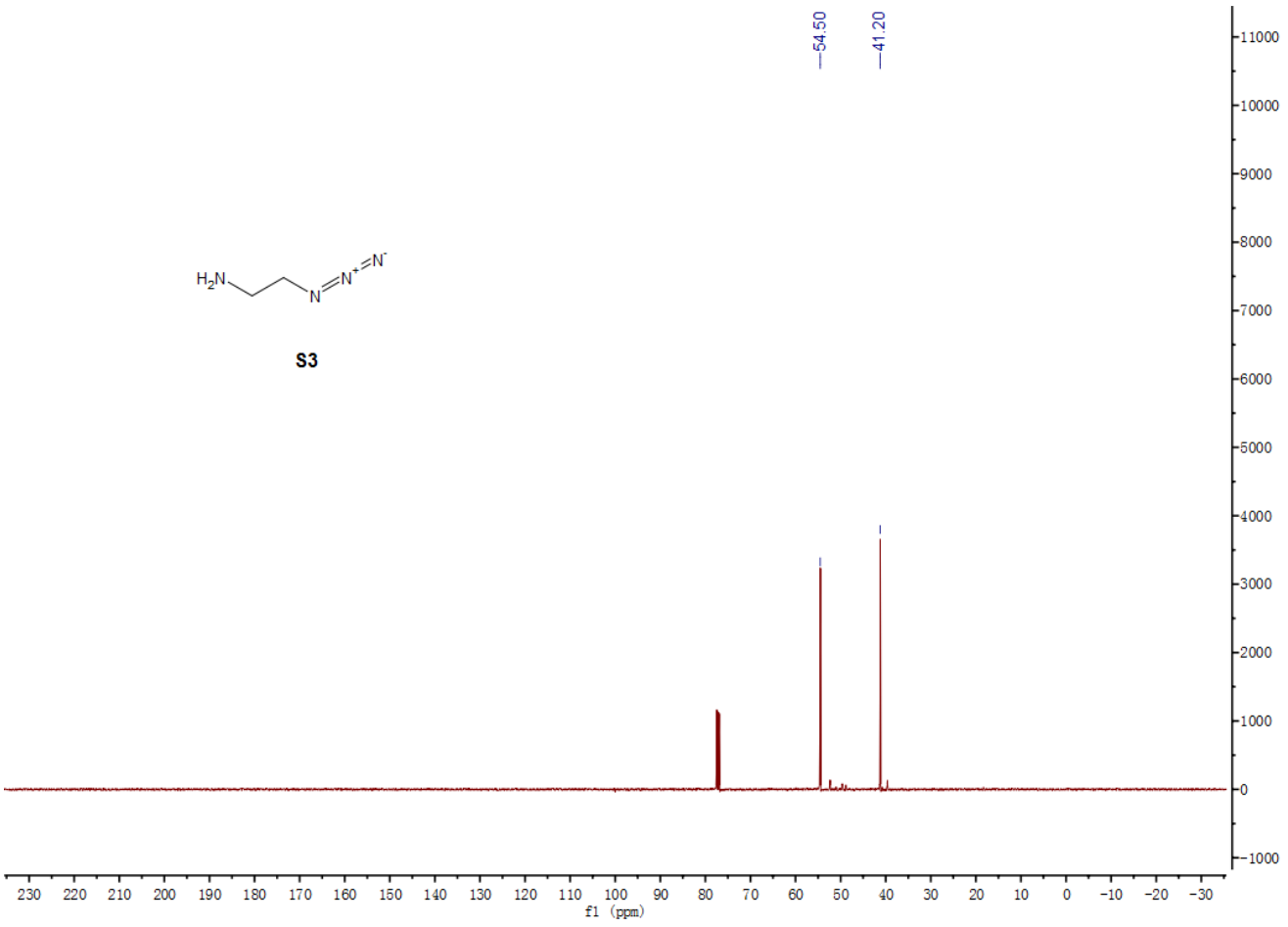
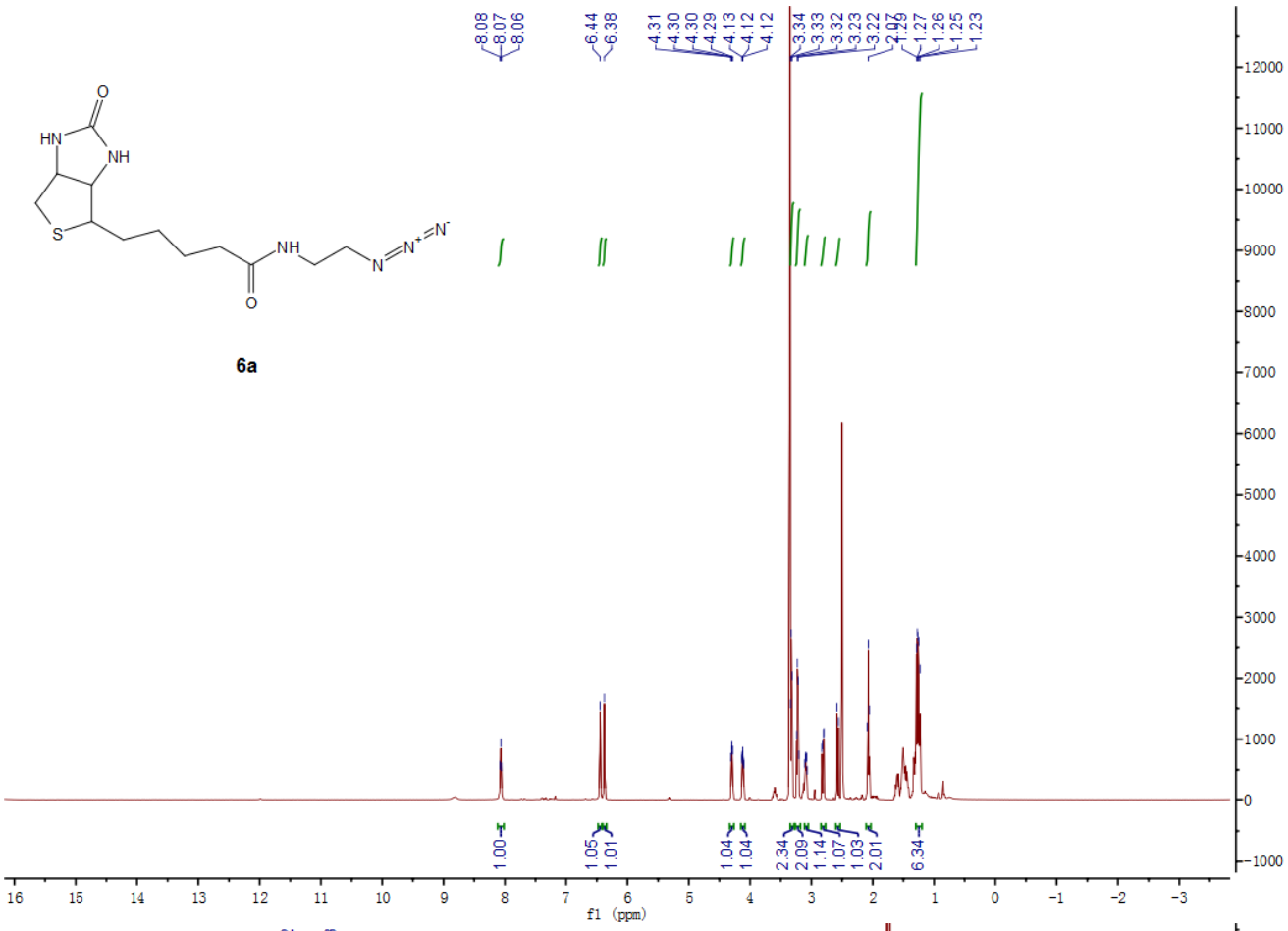


S3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4.30
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3.34
3.33
3.32
3.23
3.22
2.07
2.09
1.27
1.26
1.25
1.23
1.00
1.05
1.01
1.04
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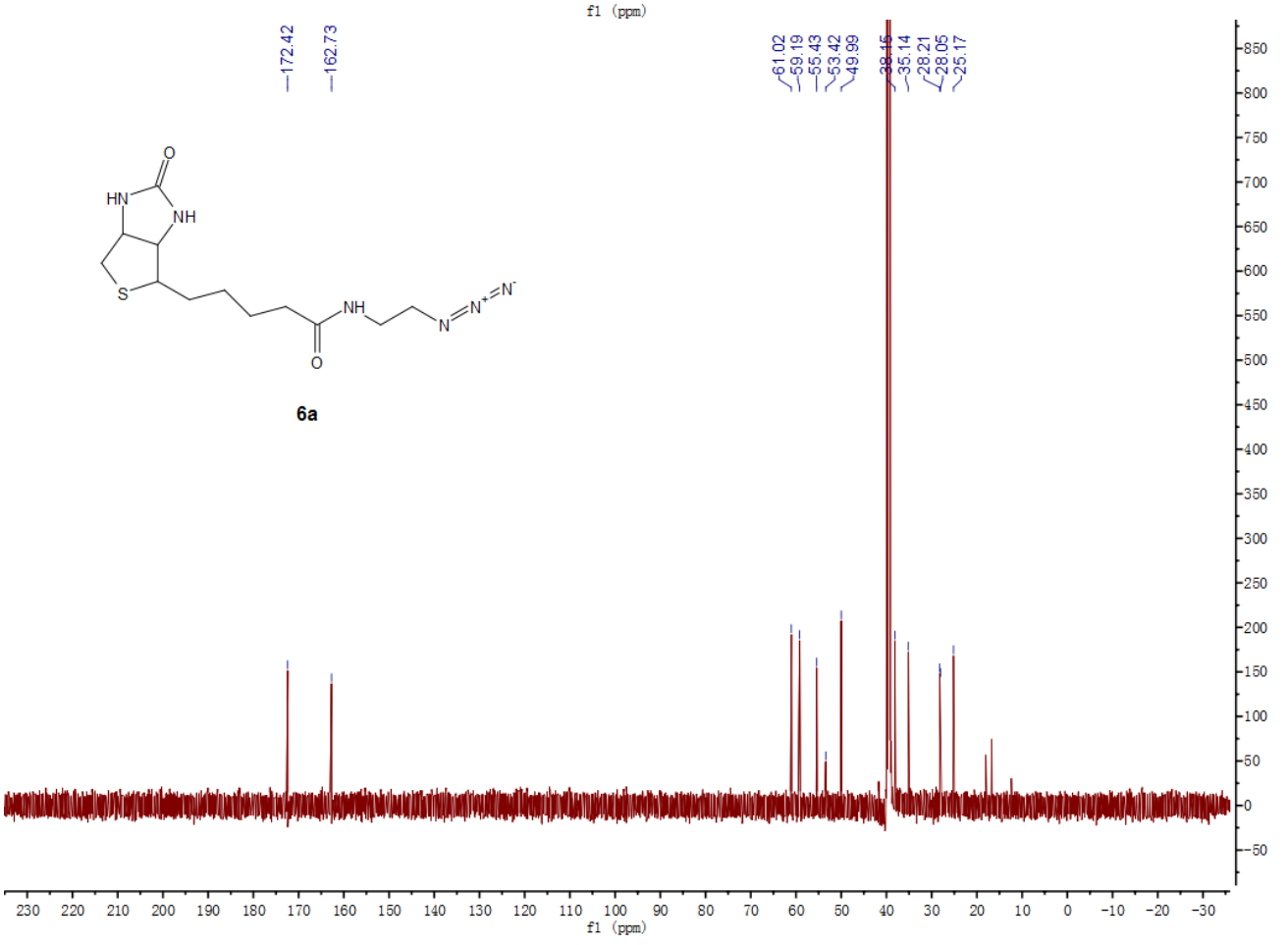
2.34
2.09
1.14
1.07
1.03
2.01
6.34
f1 (ppm)

6a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53.42
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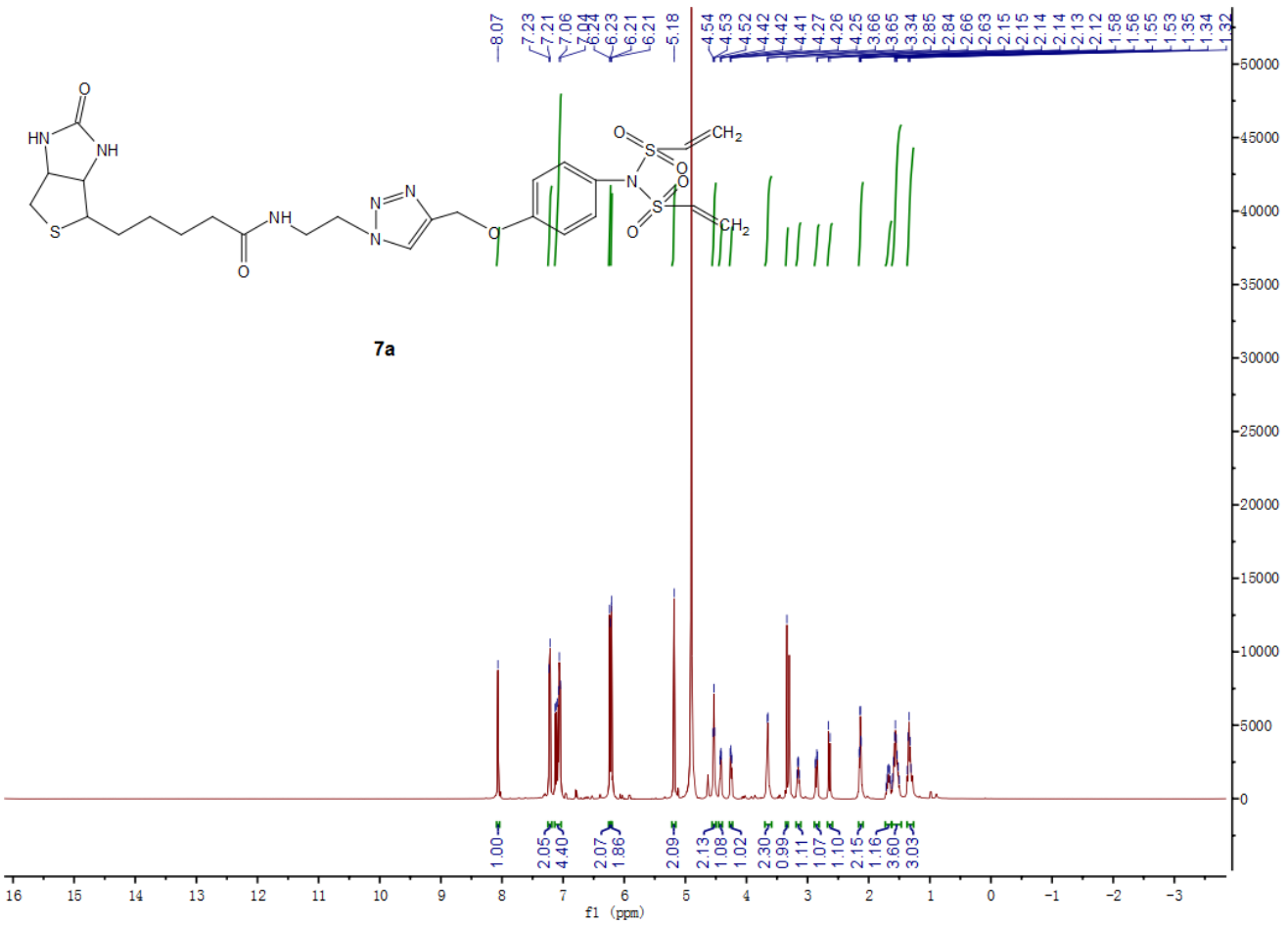
38.15
35.14
28.21
28.05
25.17
f1 (ppm)

6a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7.21
7.06
6.24
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6.21
5.18
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4.53
4.52
4.42
4.42
4.41
4.27
4.26
4.25
3.66
3.65
3.34
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2.84
2.66
2.63
2.15
2.15
2.14
2.14
2.13
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1.34
1.32
1.00
2.05
4.40
2.07
1.86
2.09
2.13
1.08
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2.15
1.16
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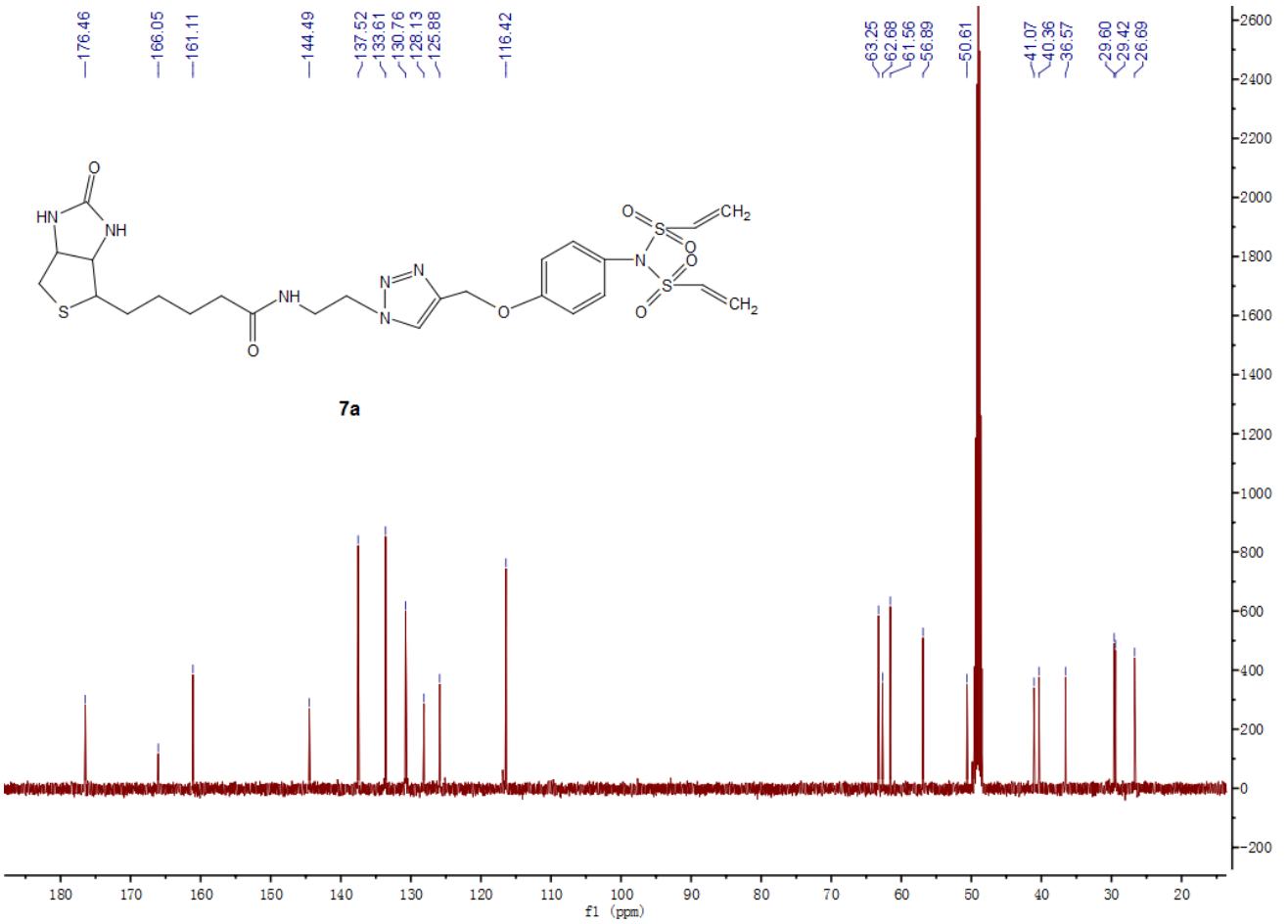
3.03
f1 (ppm)

7a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137.52
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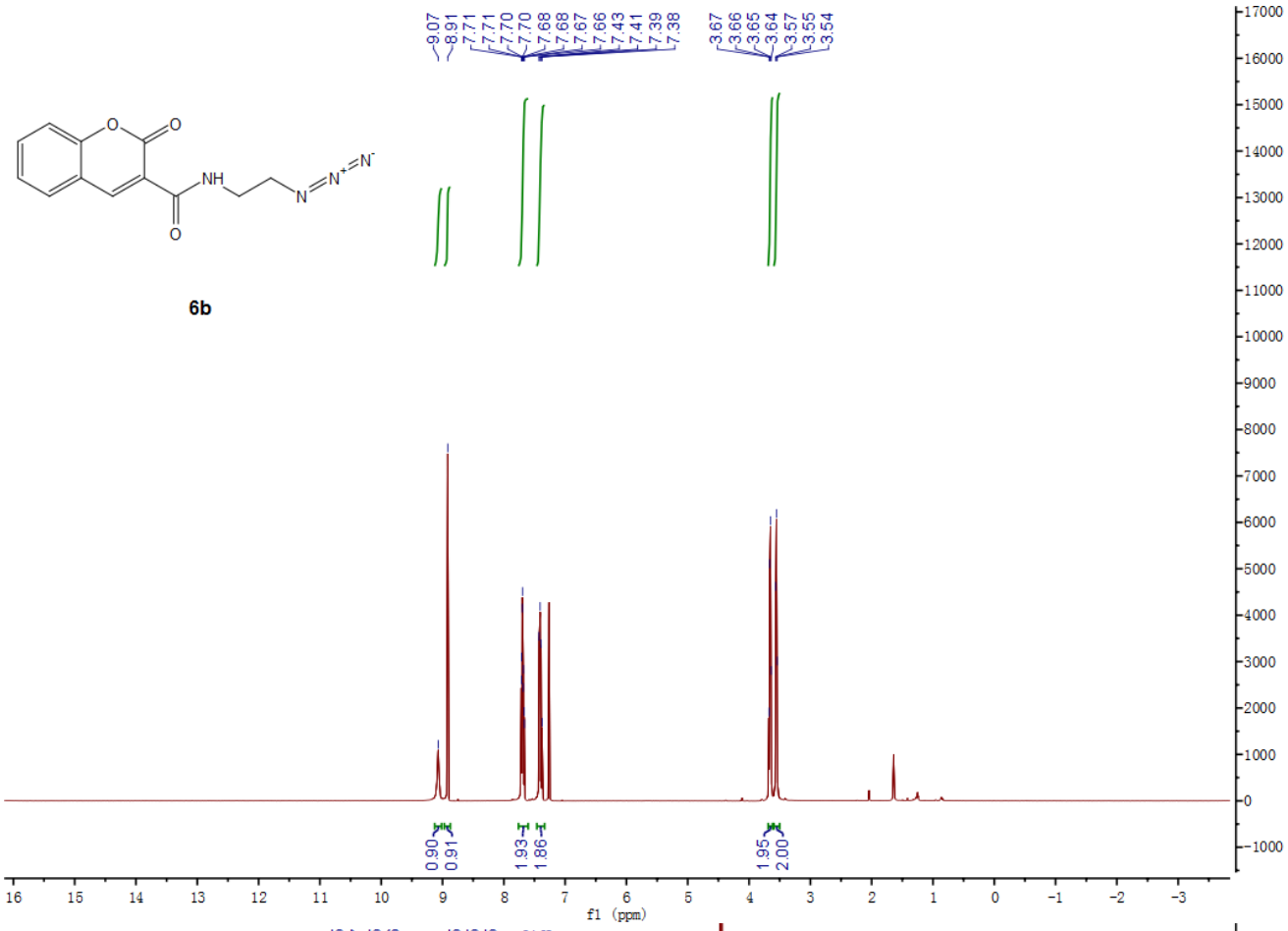
f1 (ppm)

7a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7.66
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7.38
3.67
3.66
3.65
3.64
3.57
3.55
3.54
NH
O
O
O
N
N+
N-
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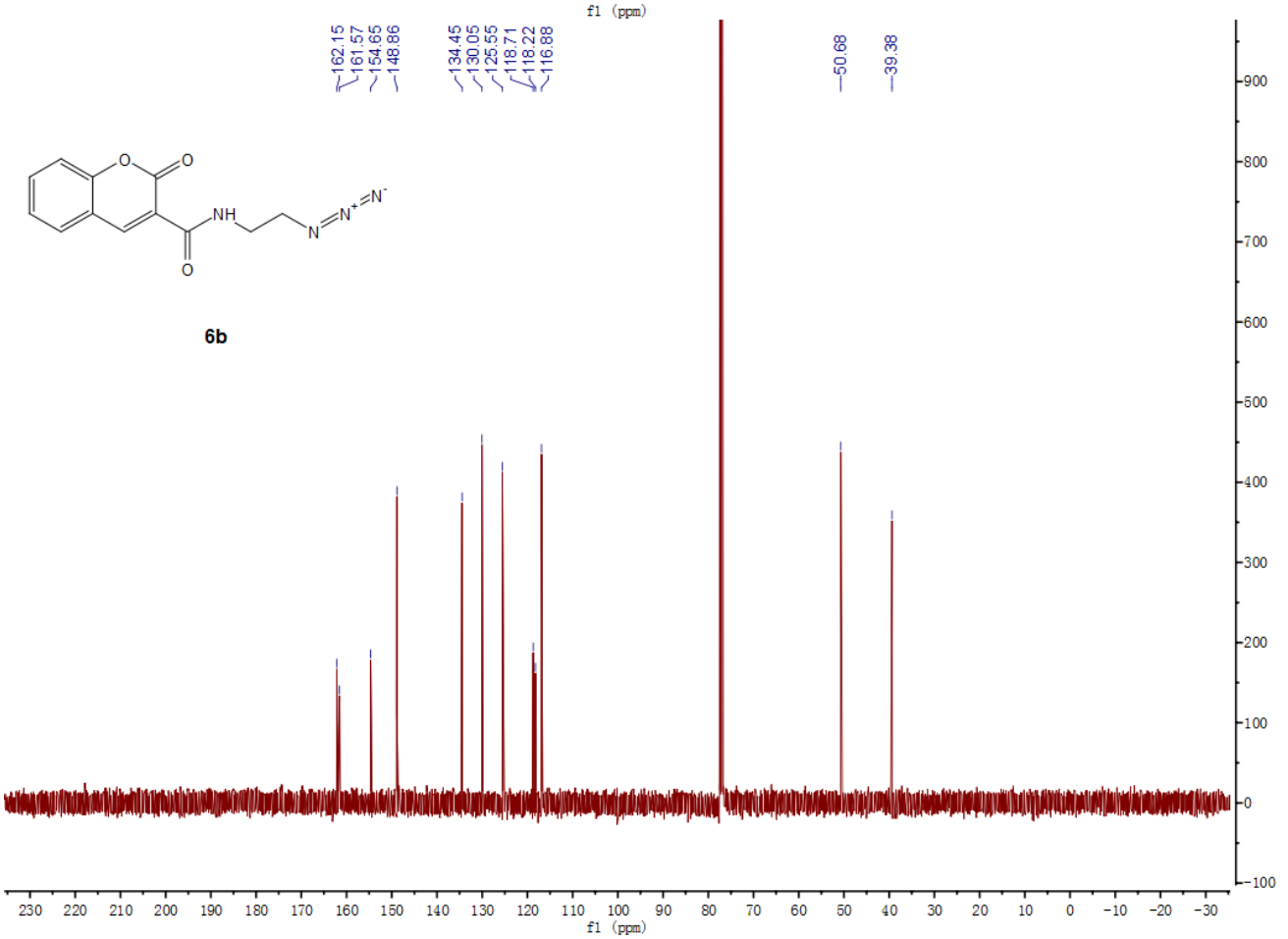
0.91
1.93
1.86
1.95
2.00
f1 (ppm)

6b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50.68
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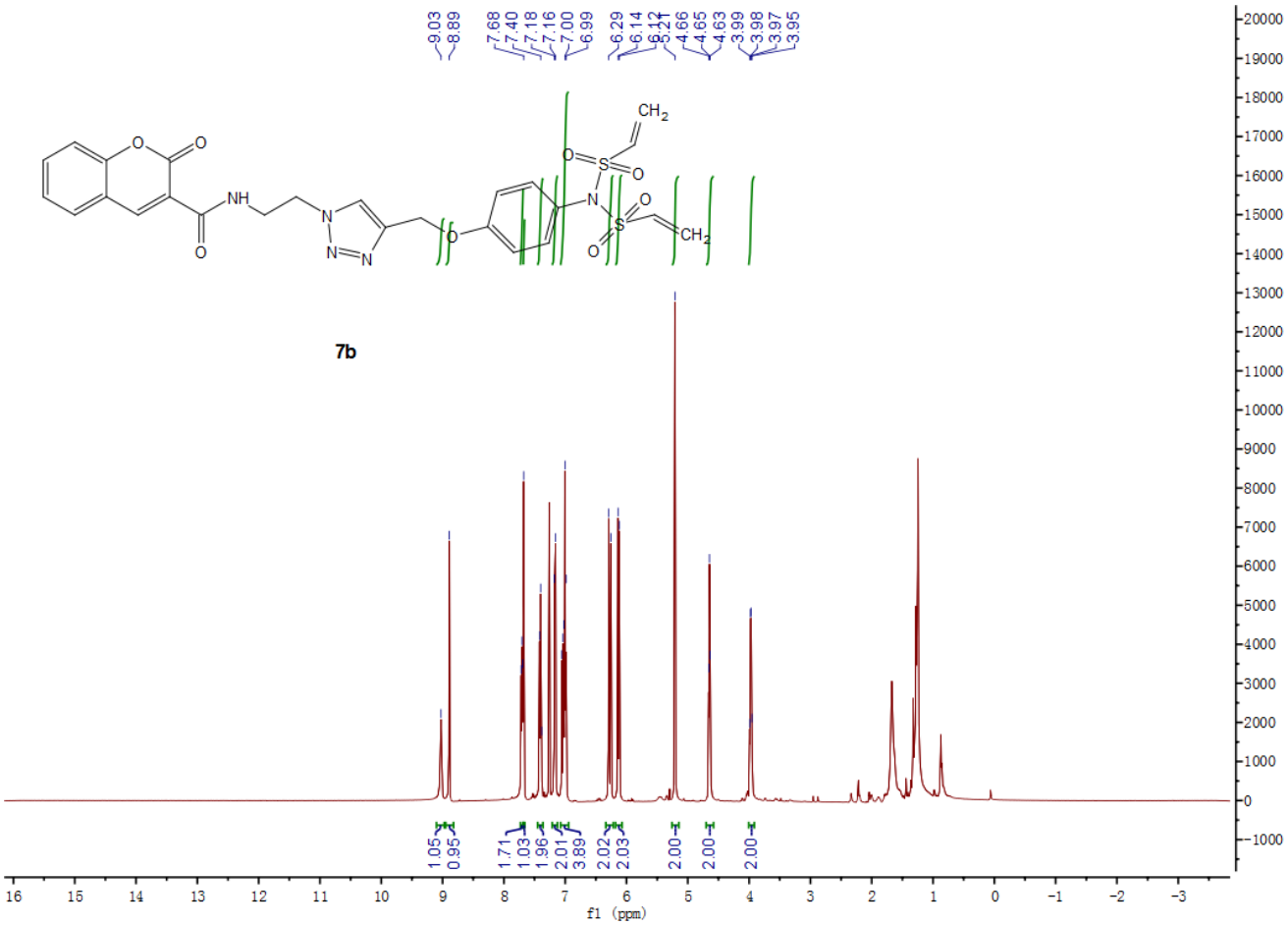
O
O
O
N
N+
N-
f1 (ppm)

6b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6.12
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4.65
4.63
3.99
3.98
3.97
3.95
7b
1.05
0.95
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3.89
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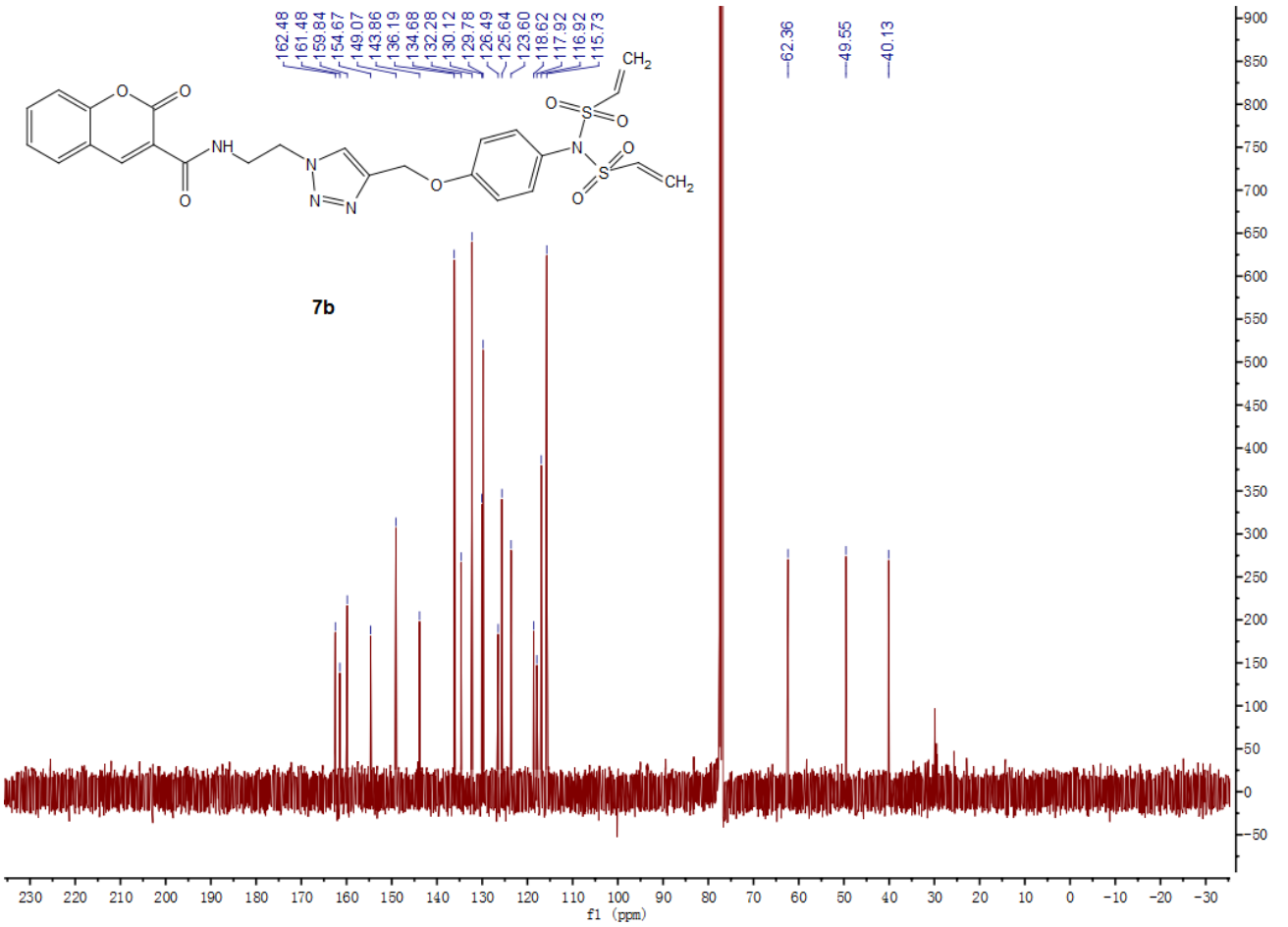
2.00
2.00
2.00
f1 (ppm)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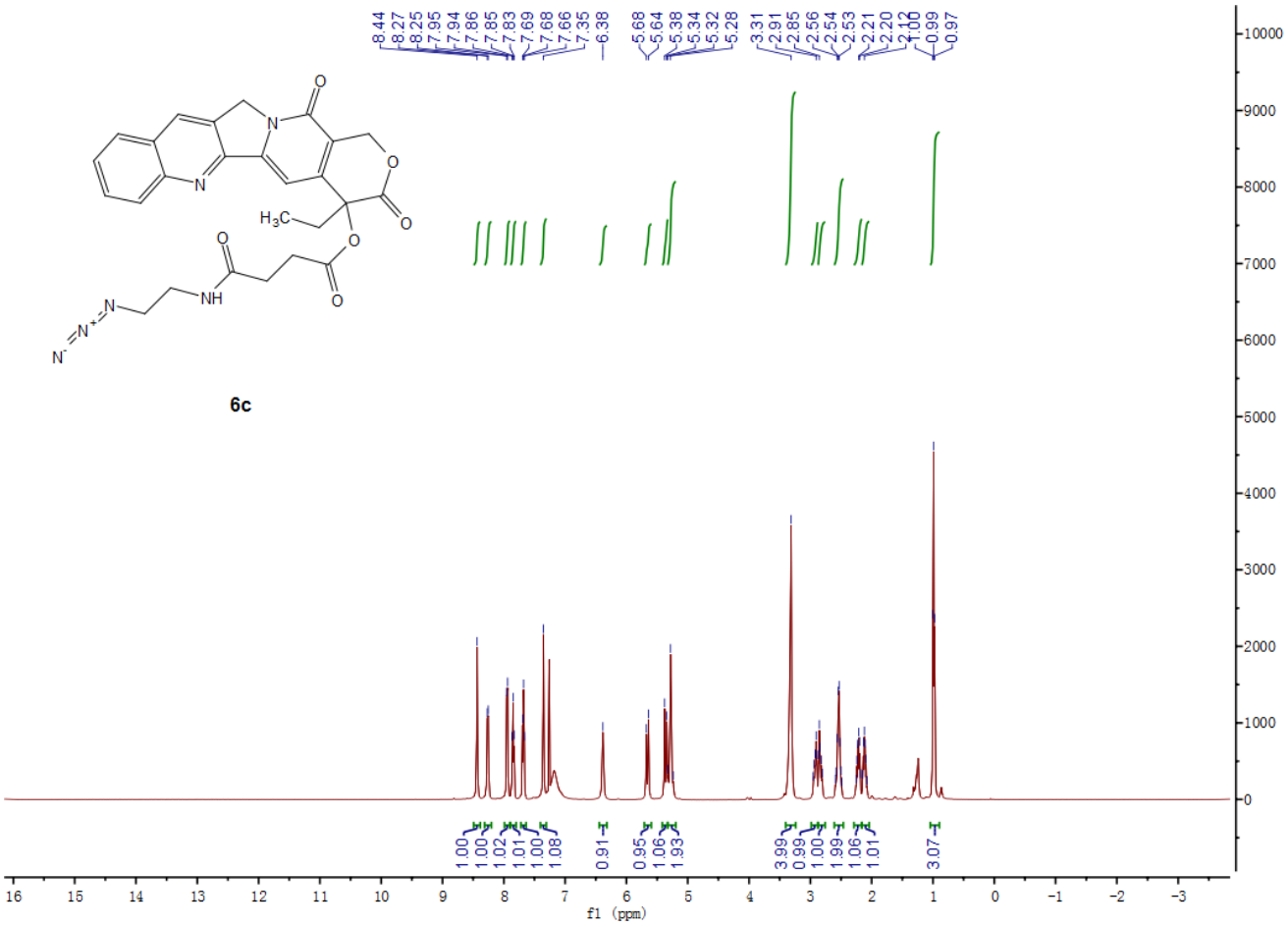
130.12
129.78
126.49
125.64
123.60
118.62
117.92
116.92
115.73
62.36
49.55
40.13
7b
f1 (ppm)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8.27
8.25
7.95
7.94
7.86
7.85
7.83
7.69
7.68
7.66
7.35
6.38
5.68
5.64
5.38
5.34
5.32
5.28
3.31
2.91
2.85
2.56
2.54
2.53
2.21
2.20
1.00
0.99
0.97
1.00
1.00
1.02
1.01
1.00
1.08
0.91
0.95
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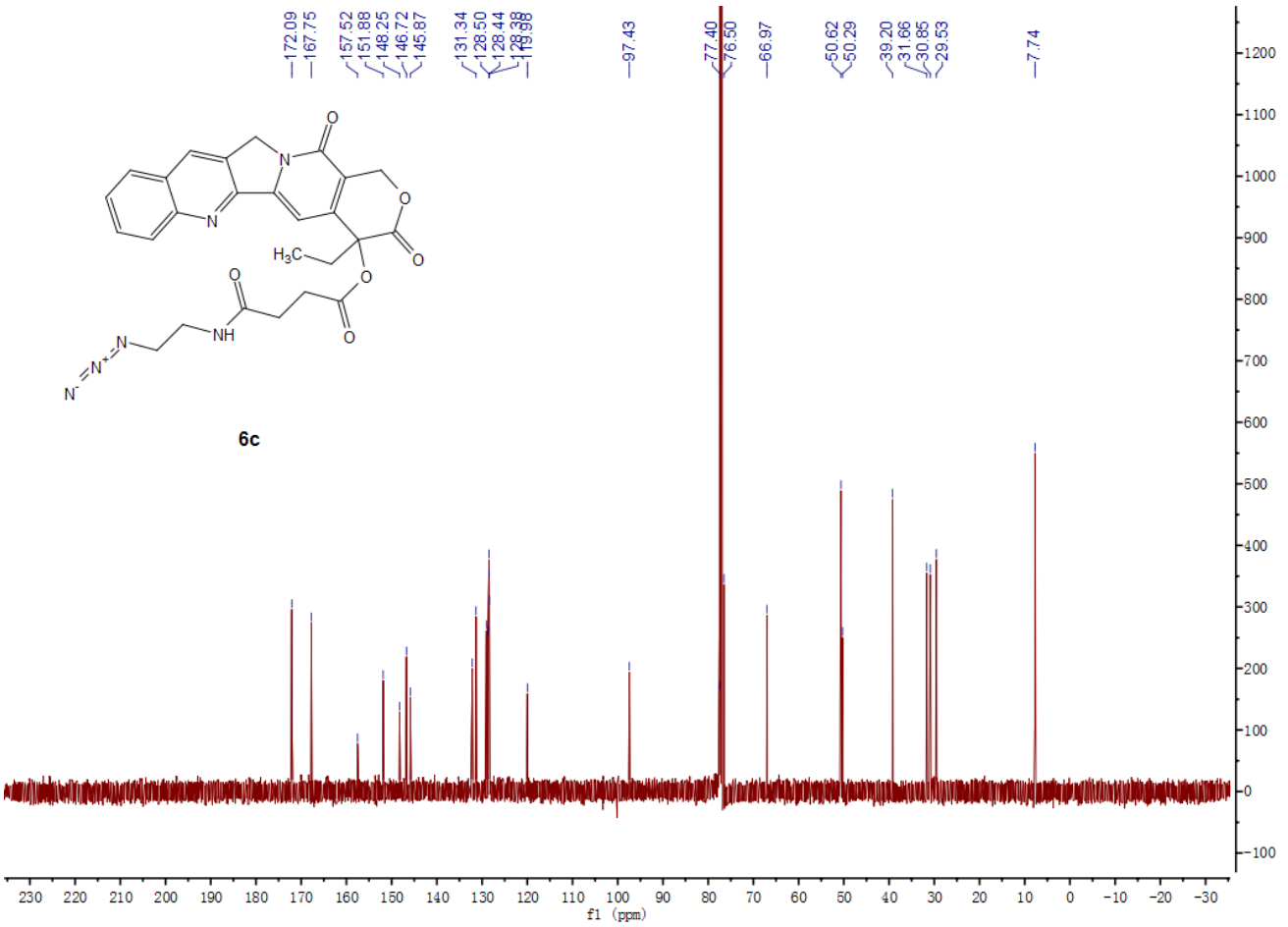
3.99
0.99
1.00
1.99
1.06
1.01
3.07
f1 (ppm)

6c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167.75
157.52
151.88
148.25
146.72
145.87
131.34
128.50
128.44
97.43
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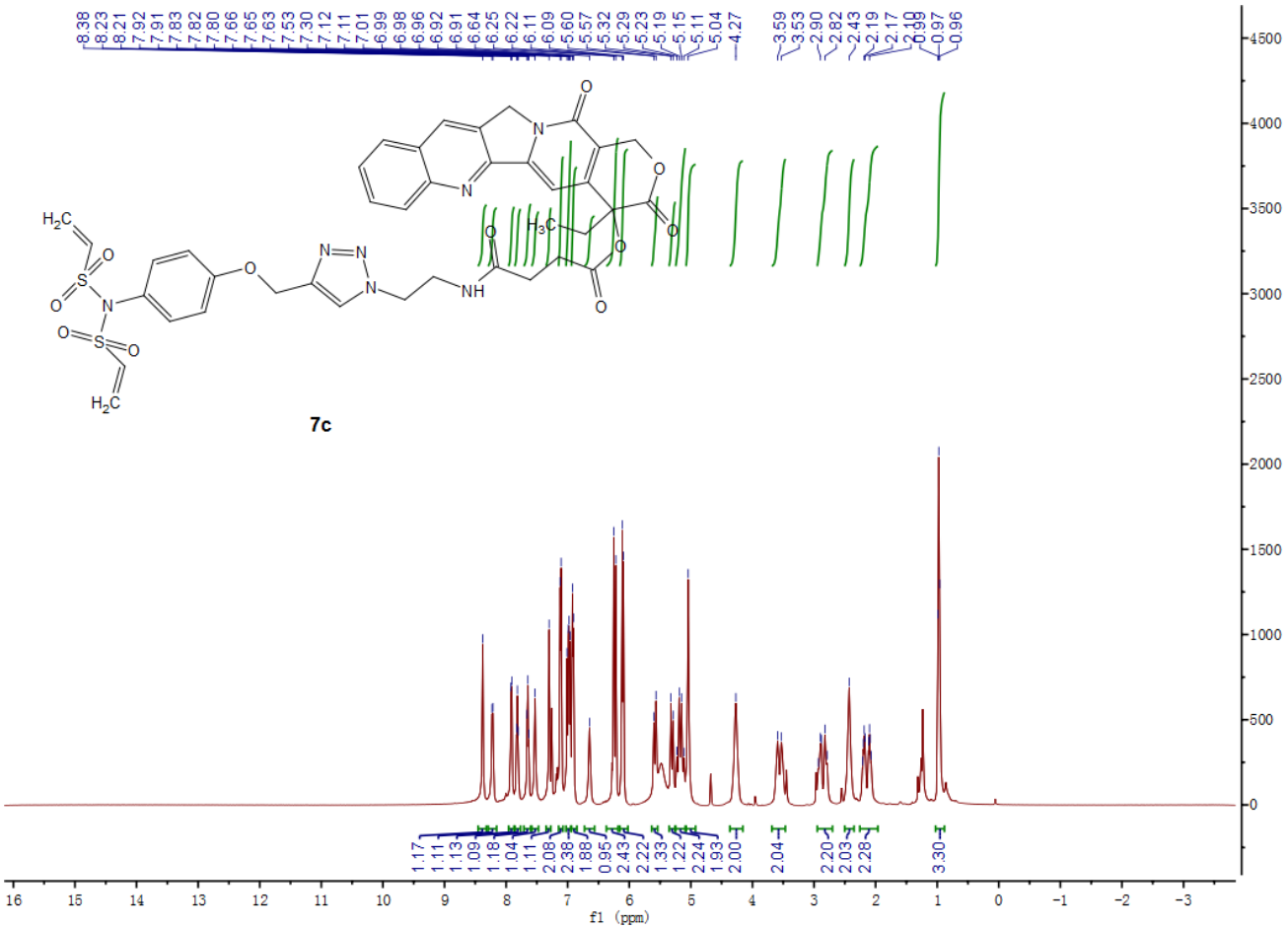
66.97
50.62
50.29
39.20
31.66
30.85
29.53
7.74
f1 (ppm)

6c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8.21
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7.91
7.83
7.82
7.80
7.66
7.65
7.63
7.53
7.30
7.12
7.11
7.01
6.99
6.98
6.96
6.92
6.91
6.64
6.25
6.22
6.11
6.09
5.60
5.57
5.32
5.29
5.23
5.19
5.15
5.11
5.04
4.27
3.59
3.53
2.90
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2.43
2.19
2.17
2.10
0.99
0.97
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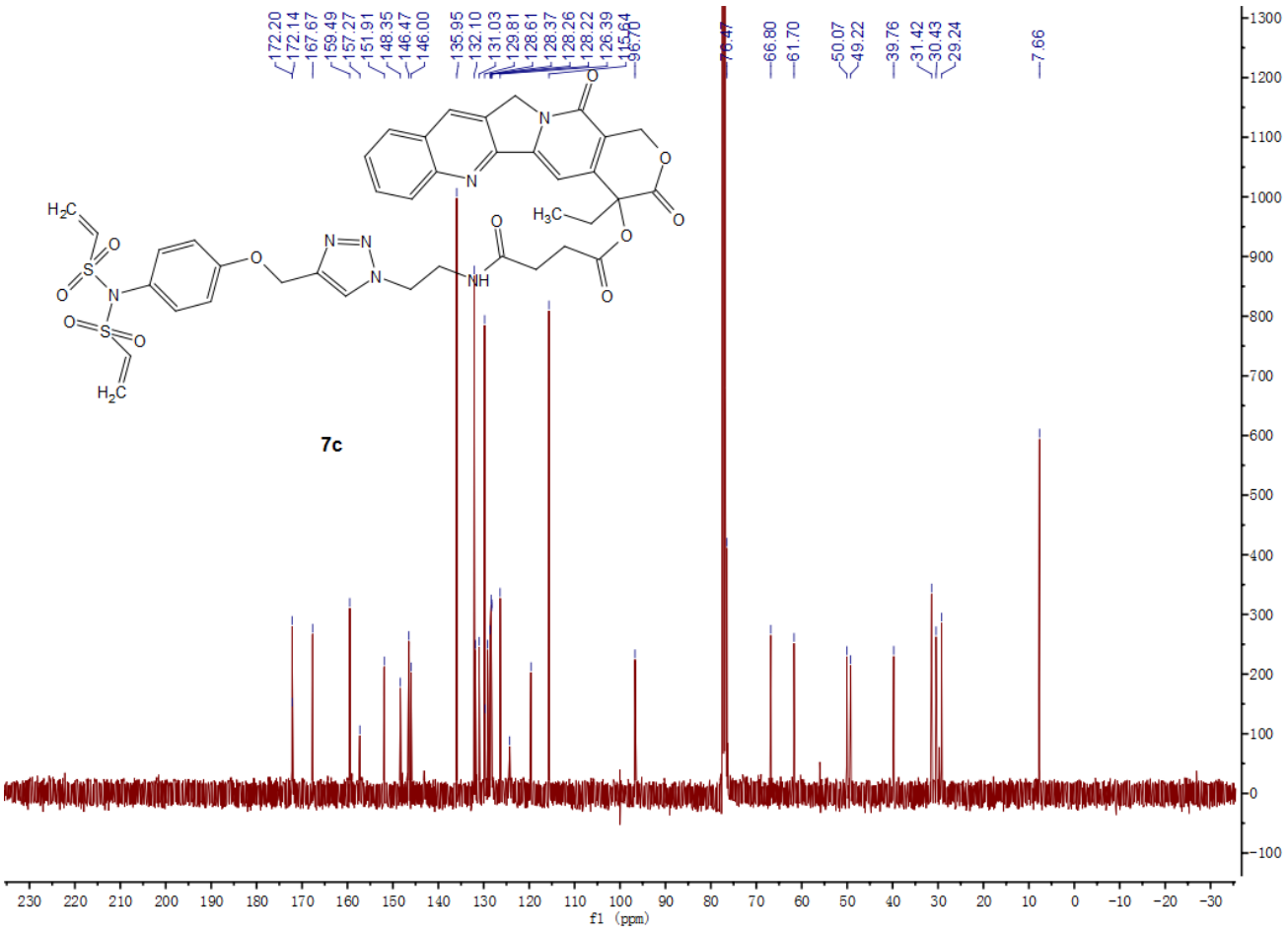
7c
f1 (ppm)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157.27
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148.35
146.47
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128.37
128.26
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126.39
115.64
96.70
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29.24
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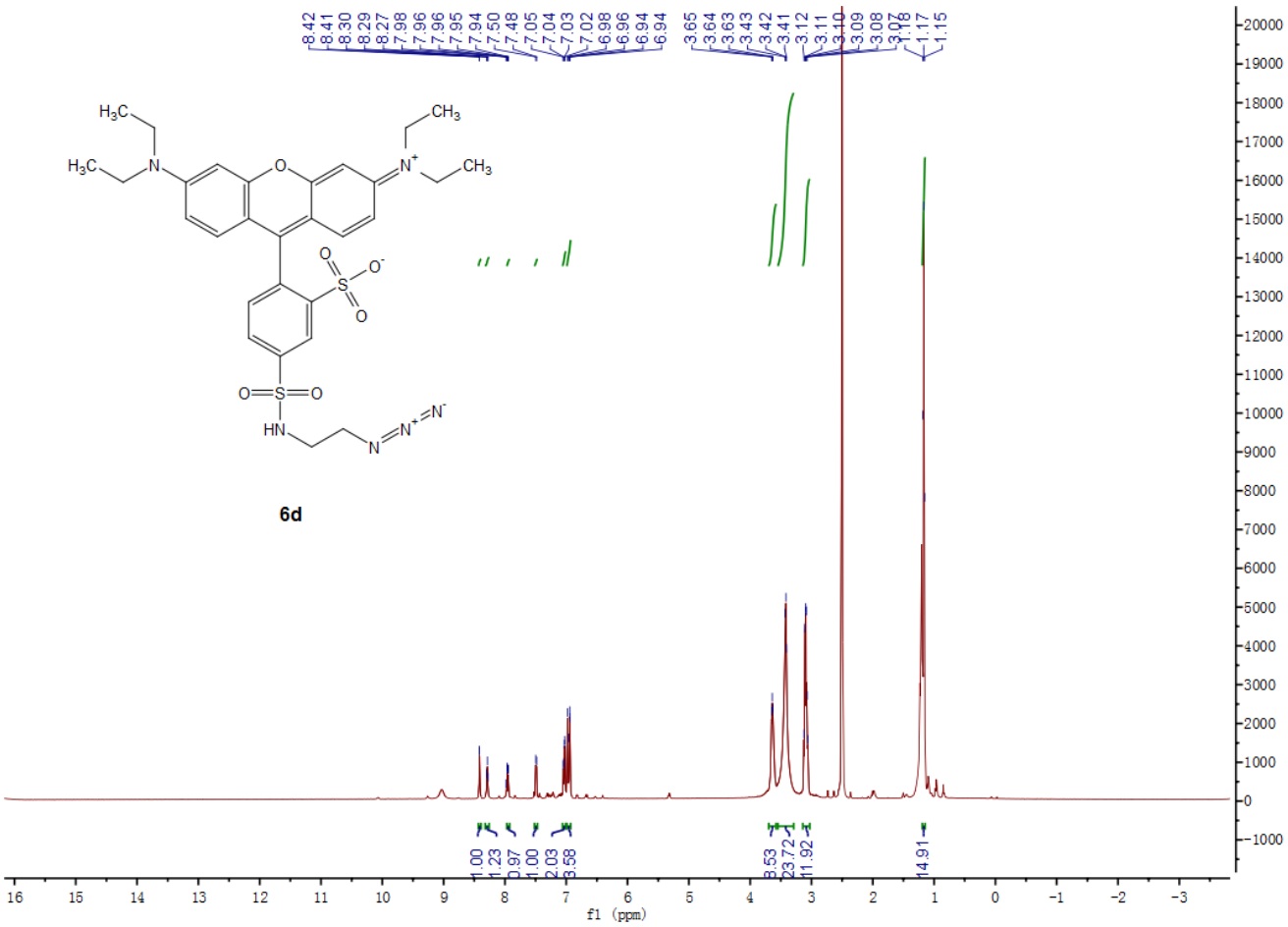
7c
f1 (ppm)



10. References

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