Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Selective lysine modification of native peptides via aza-Michael addition

Hongli Chen,* Rong Huang, Zhihong Li, Wei Zhu, Jiakang Chen, Yuexiong Zhan, Biao Jiang*

Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University, Shanghai, 201210, P.R.China

Chemical synthesis and characterization:

General information:

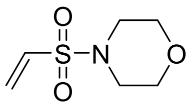
All chemical reagents were of analytical grade, used as supplied without further purification unless indicated. NMR spectra were recorded on a Bruker-500 instrument. Chemical shifts were given in ppm with respect to referenced solvent peaks. Spectra were analyzed with MestReNova. High-resolution mass spectra (HRESIMS) were obtained on an Agilent Technologies 6230 Accurate Mass TOF LC/MS instrument or Thermo fisher EASY1000-Fusion instrument and were reported as m/z (relative intensity). HPLC was performed using Waters 1525. MS^2 were recorded on Thermo fisher EASY1000-Fusion instrument.

Synthetic procedures

General procedure for the synthesis of vinylsulfonamides 1-10

To a stirred solution of amine (1 eq.) and trimethylamine (3 eq.) in DCM at 0℃, 2-chloroethanesulfonyl chloride (2.5 eq.) was added slowly. The resulting mixture was stirred at 0℃ to room temperature (r.t.) until the amine was consumed as determined by TLC. The reaction was quenched with water and the mixture was extracted with DCM. The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by column chromatography.

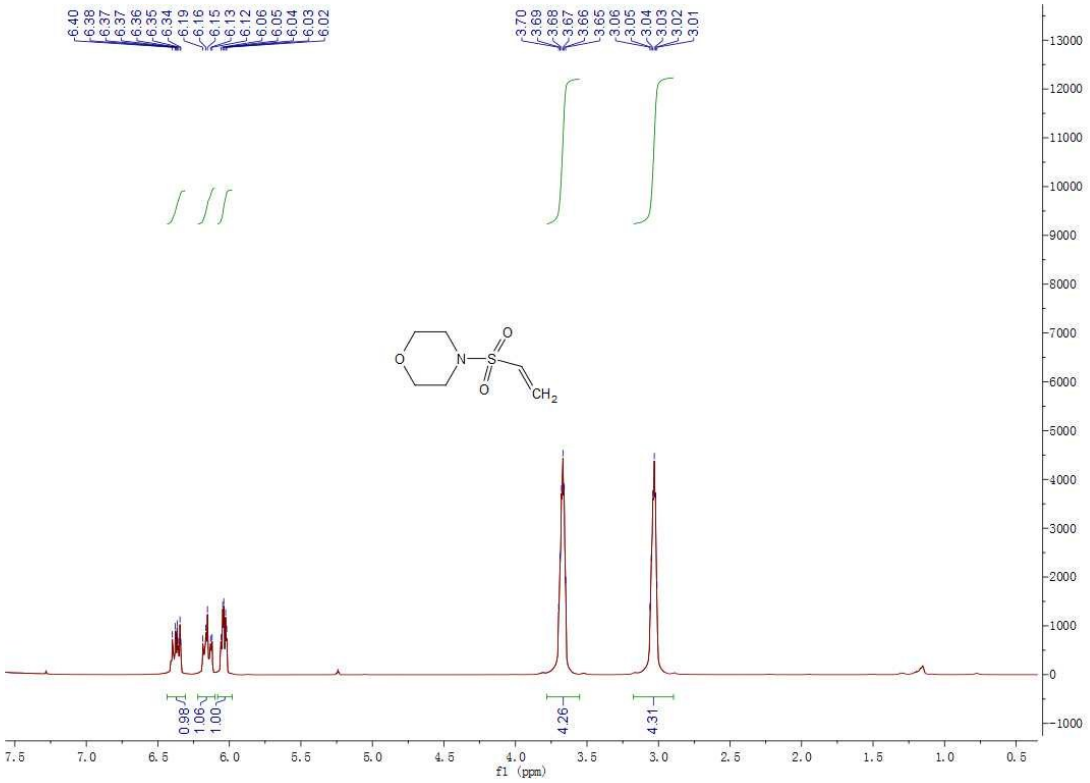
Characterization details for compound 1-10:



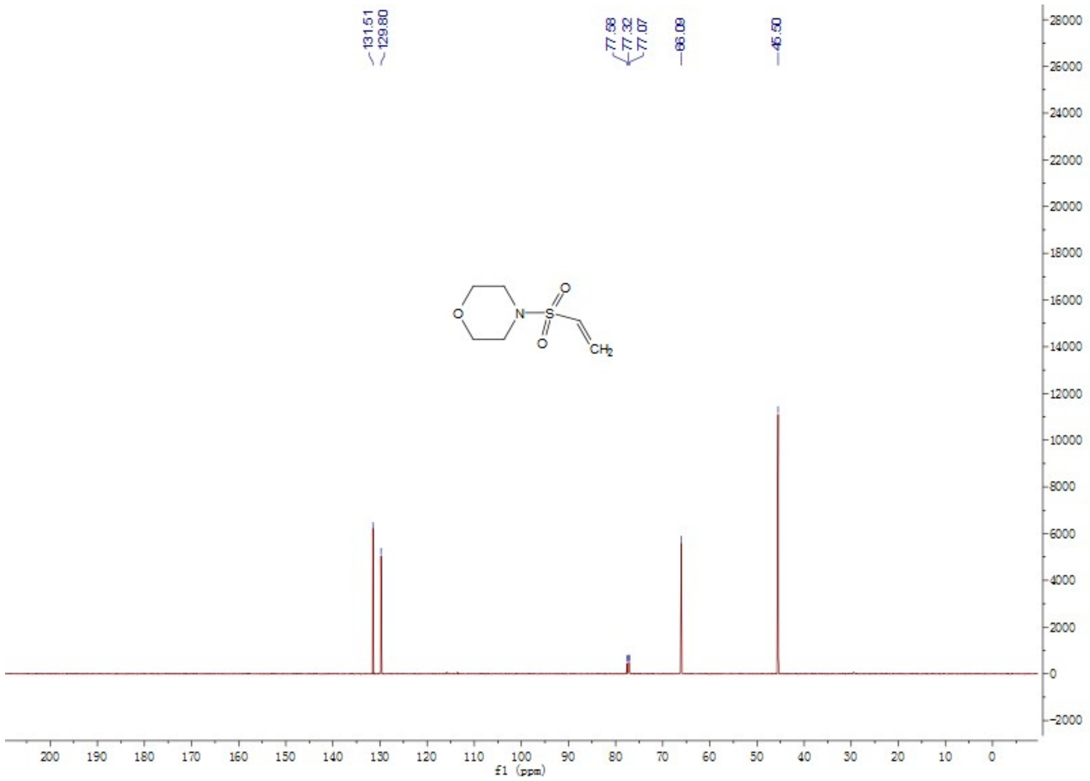
1

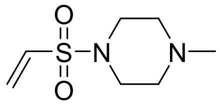
4-(vinylsulfonyl)morpholine (1): ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.44 – 6.31 (m, 1H), 6.22 – 6.10 (m, 1H), 6.04 (dt, *J* = 10.2, 5.1 Hz, 1H), 3.67 (dq, *J* = 9.4, 4.7 Hz, 4H), 3.03 (dq, *J* = 9.4, 4.7 Hz, 4H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 131.51, 129.80, 66.09, 45.50.HRMS (ESI) [M+H]$^+$ Calculated for $C_6H_{12}NO_3S$, 178.0538, Found 178.0540.

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



^{13}C NMR of compound **1**

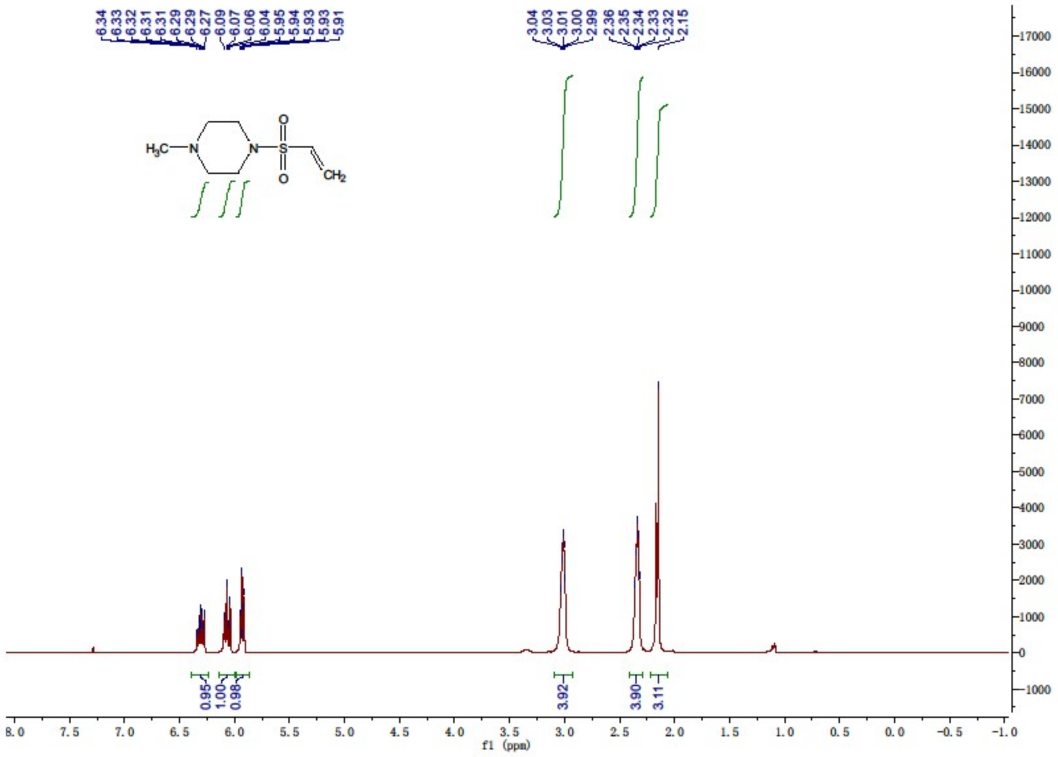




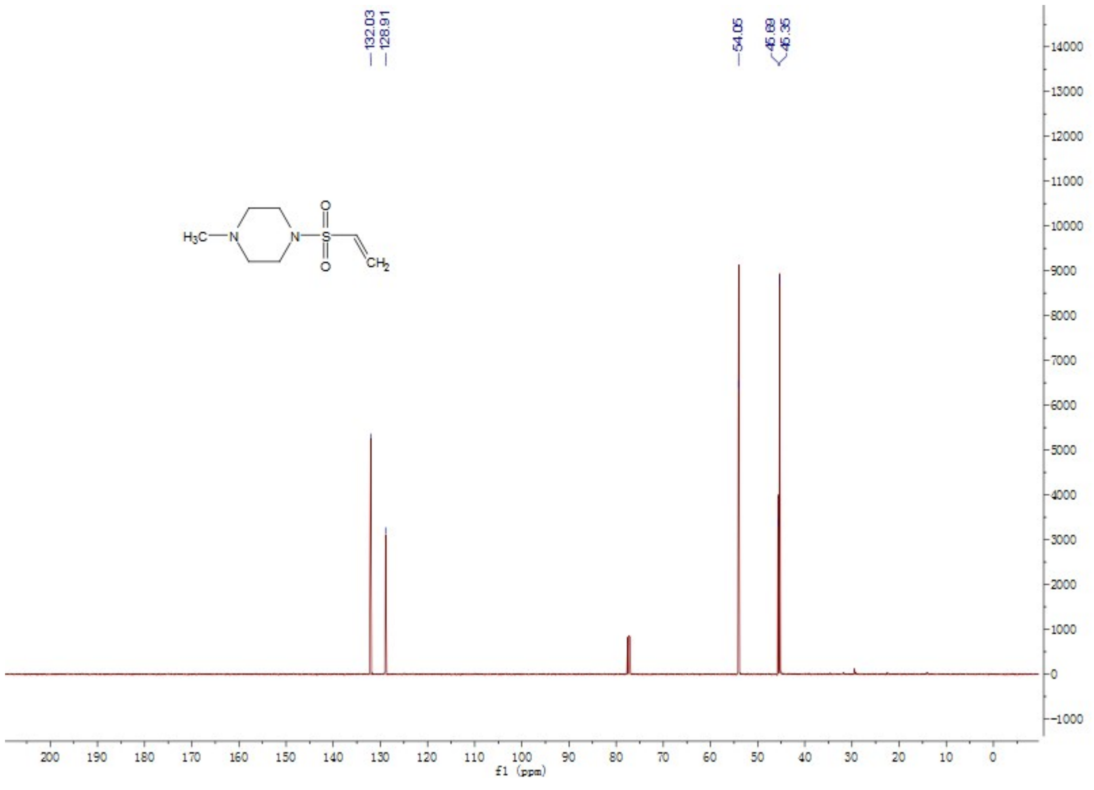
2

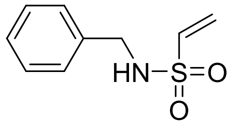
1-methyl-4-(vinylsulfonyl)piperazine (2): ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.22 (ddd, *J* = 16.7, 9.9, 7.6 Hz, 1H), 5.98 (dd, *J* = 16.6, 11.1 Hz, 1H), 5.84 (t, *J* = 9.2 Hz, 1H), 3.03 – 2.84 (m, 4H), 2.25 (m, *J* = 9.5, 4.7 Hz, 4H), 2.07 (s, 3H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 132.03, 128.91, 54.05, 45.69, 45.35. HRMS (ESI) [M+H]$^+$ Calculated for $C_7H_{15}N_2O_2S$, 191.0854, Found 191.0918.

^{1}H NMR of compound **2**



^{13}C NMR of compound **2**

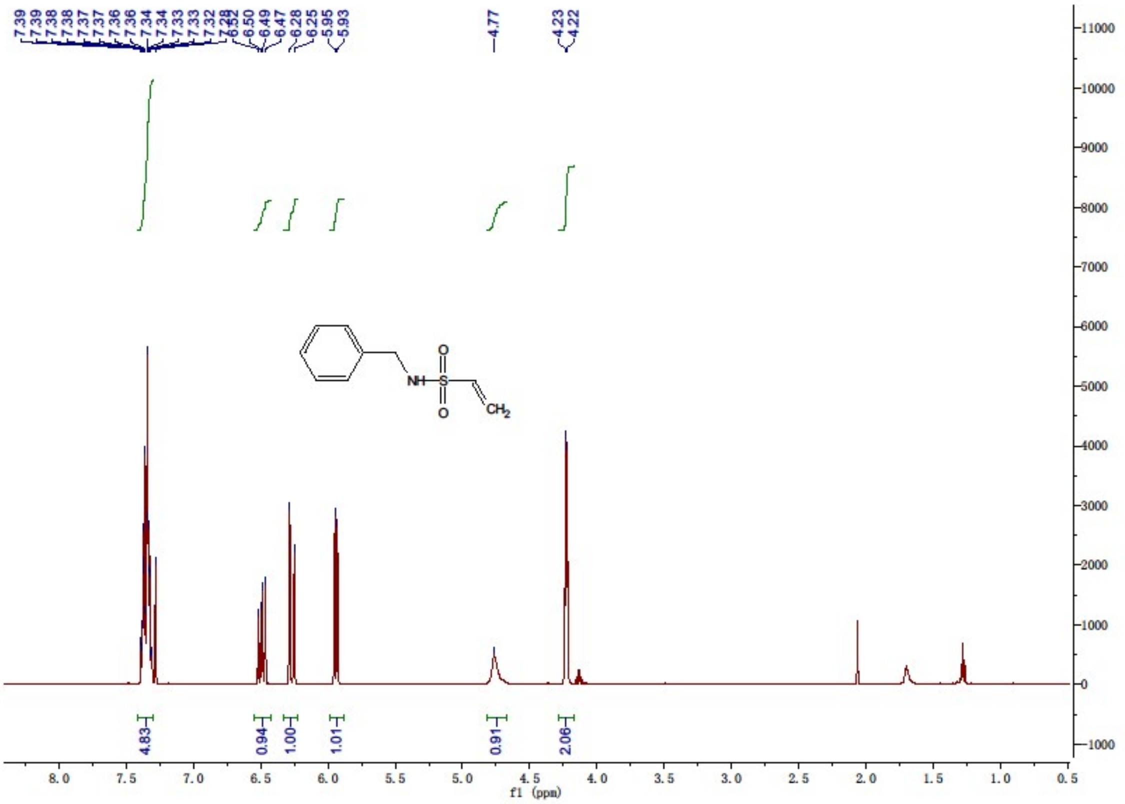


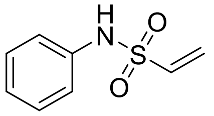


3

***N*-benzylethenesulfonamide (3)**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.42 – 7.30 (m, 5H), 6.49 (dd, *J* = 16.6, 9.9 Hz, 1H), 6.27 (d, *J* = 16.6 Hz, 1H), 5.94 (d, *J* = 9.9 Hz, 1H), 4.77 (s, 1H), 4.23 (d, *J* = 6.0 Hz, 2H). HRMS (ESI) $[M+H]^+$ Calculated for $C_9H_{12}NO_2S$, 198.0589, Found 198.0582.

^{1}H NMR of compound **3**

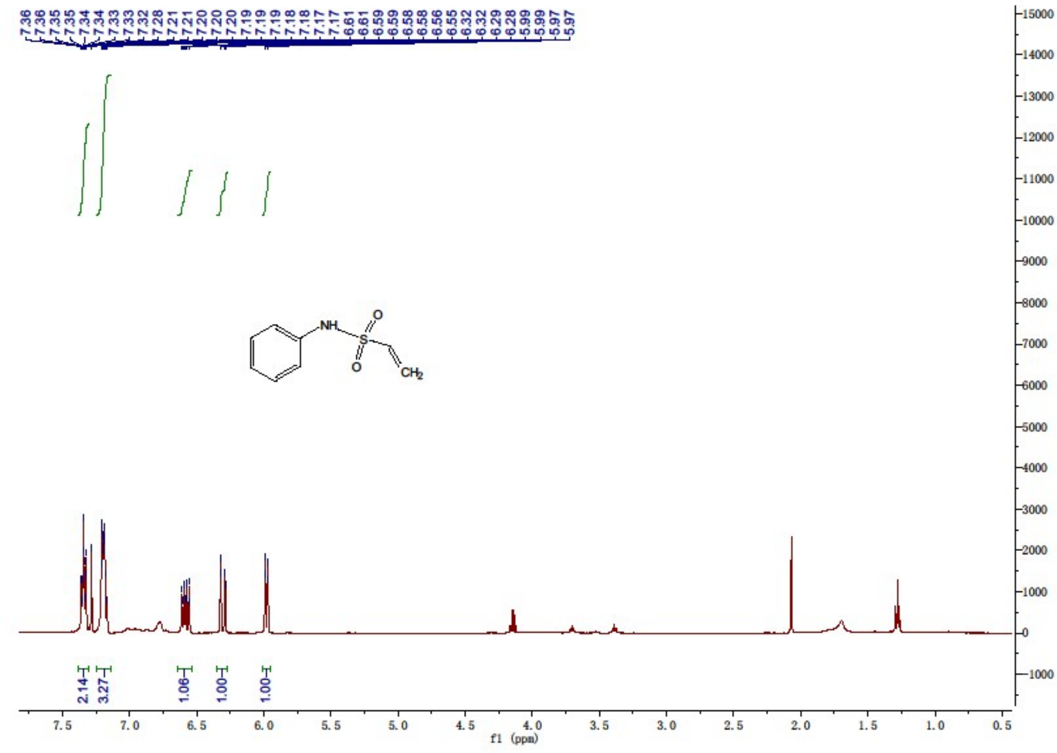




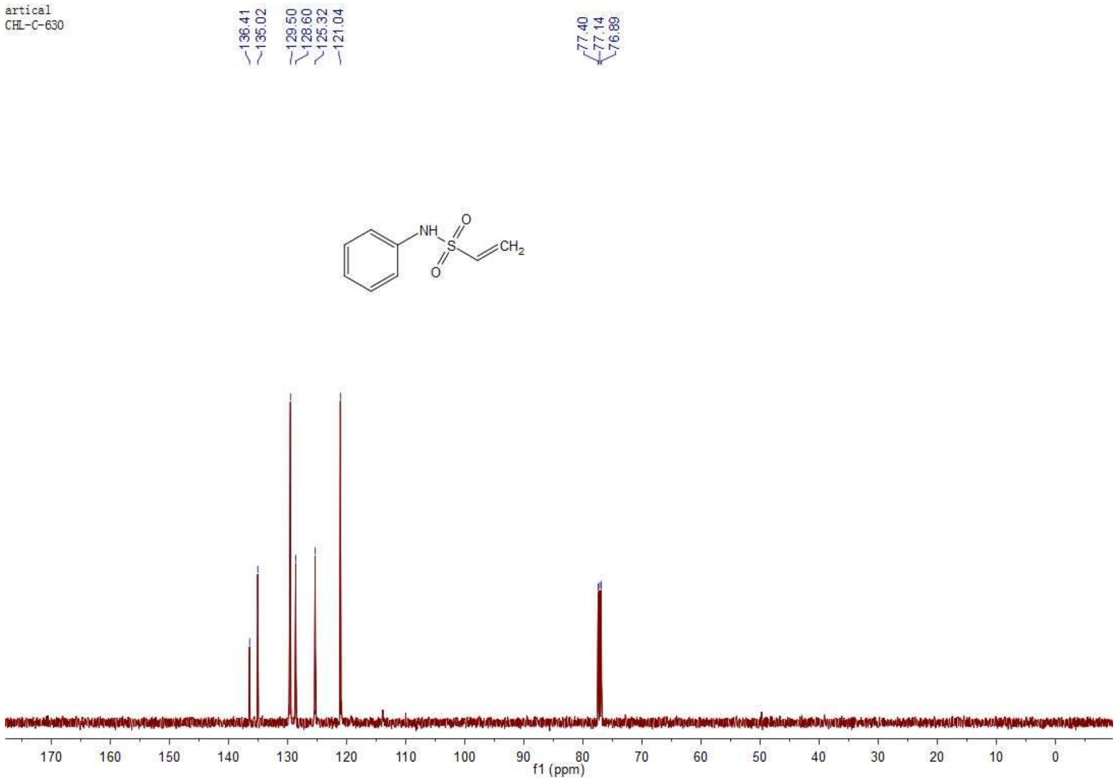
4

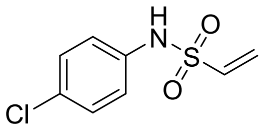
***N*-phenylethenesulfonamide (4)**: ^1H NMR (500 MHz, Chloroform-*d*) δ 7.34 (tt, *J* = 7.2, 2.0 Hz, 2H), 7.24 – 7.14 (m, 3H), 6.58 (ddd, *J* = 16.6, 10.0, 1.4 Hz, 1H), 6.30 (dd, *J* = 16.5, 2.6 Hz, 1H), 5.98 (dd, *J* = 9.8, 2.3 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.41, 135.02, 129.50, 128.60, 125.32, 121.04, 77.40, 77.14, 76.89. HRMS (ESI) $[M+H]^+$ Calculated for $C_8H_{10}NO_2S$, 184.0432, Found 184.1090 $[M+H]^+$, 206.0950 $[M+Na]^+$.

^1H NMR of compound **4**



^{13}C NMR of compound **4**

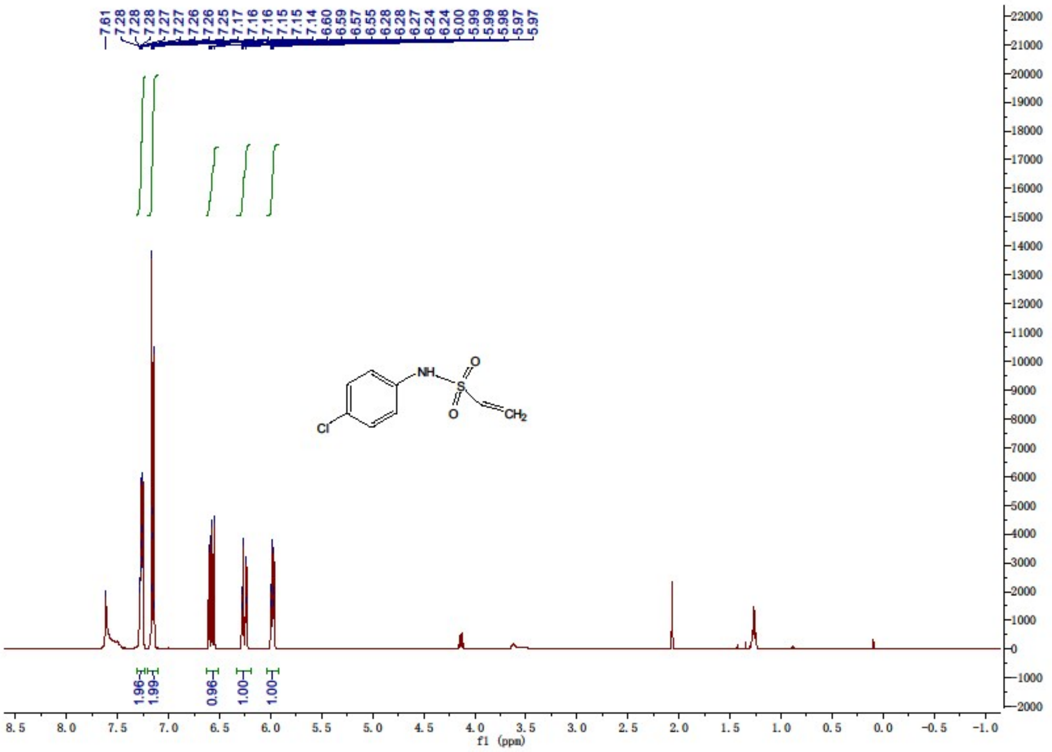




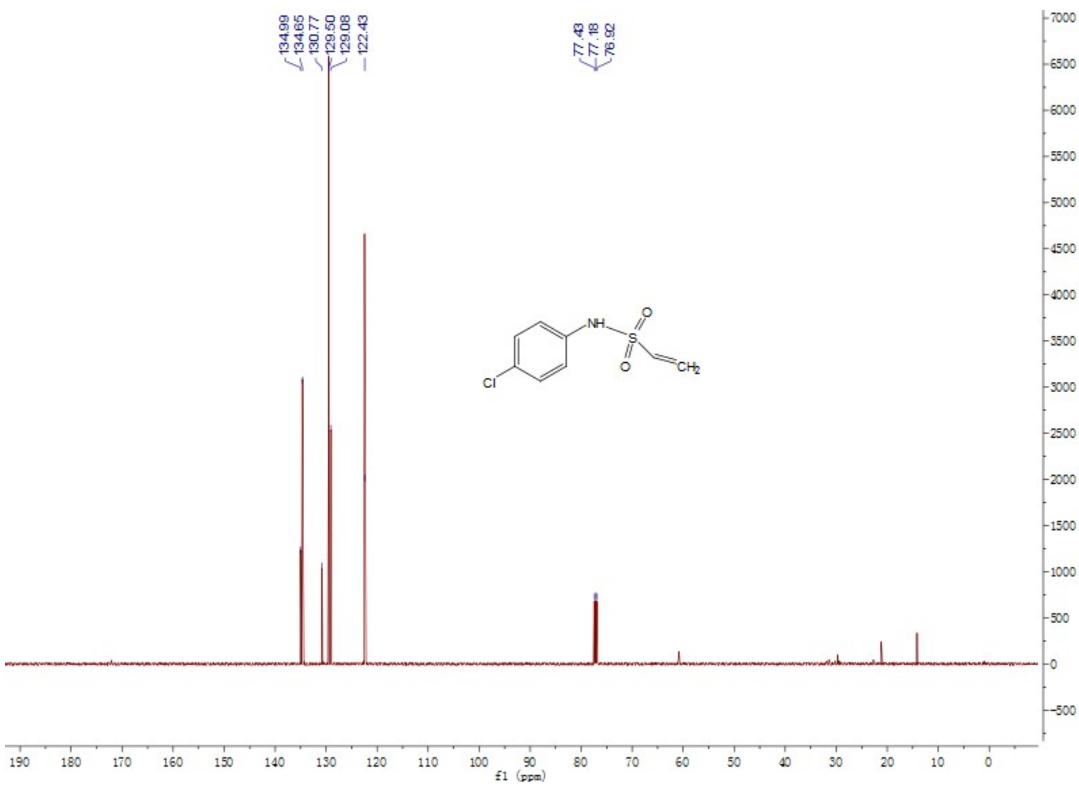
5

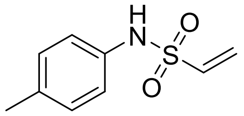
***N*-(4-chlorophenyl)ethenesulfonamide (5)**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.26 (d, *J* = 8.6 Hz, 2H), 7.15 (d, *J* = 8.8 Hz, 2H), 6.58 (dd, *J* = 16.5, 9.9 Hz, 1H), 6.25 (d, *J* = 16.5 Hz, 1H), 5.98 (d, *J* = 9.9 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.99, 134.65, 130.77, 129.50, 129.08, 122.43. HRMS (ESI) $[M+K]^+$ Calculated for $C_8H_8ClNO_2SK$ 255.9601, Found 255.9520.

^{1}H NMR of compound **5**



^{13}C NMR of compound **5**

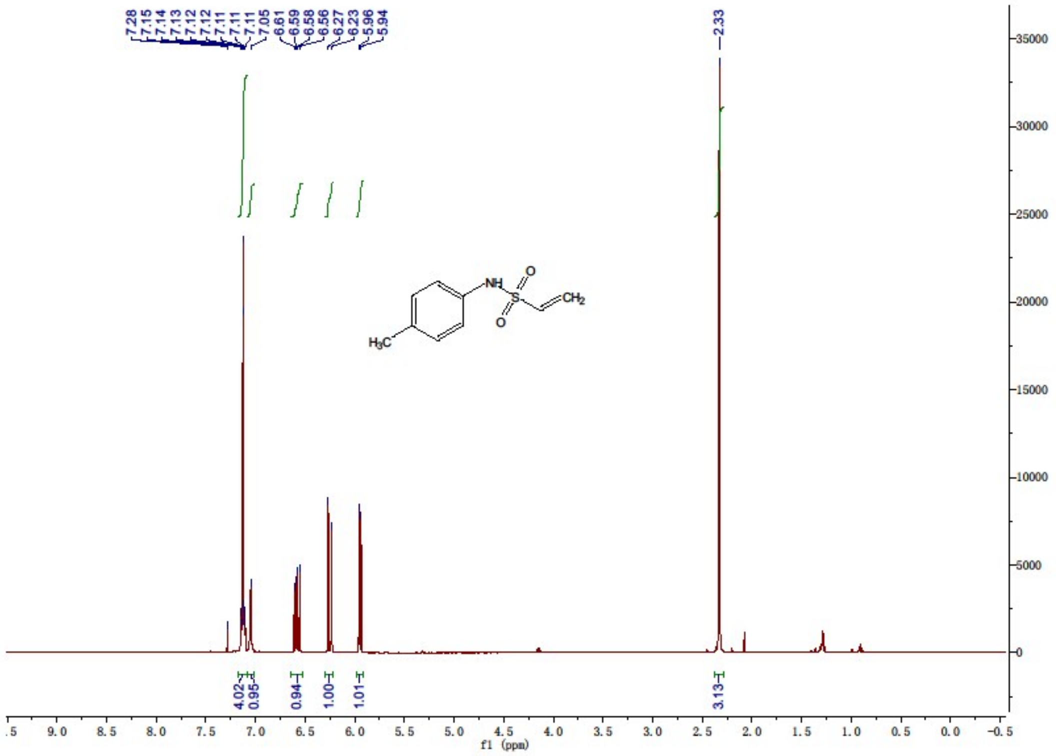




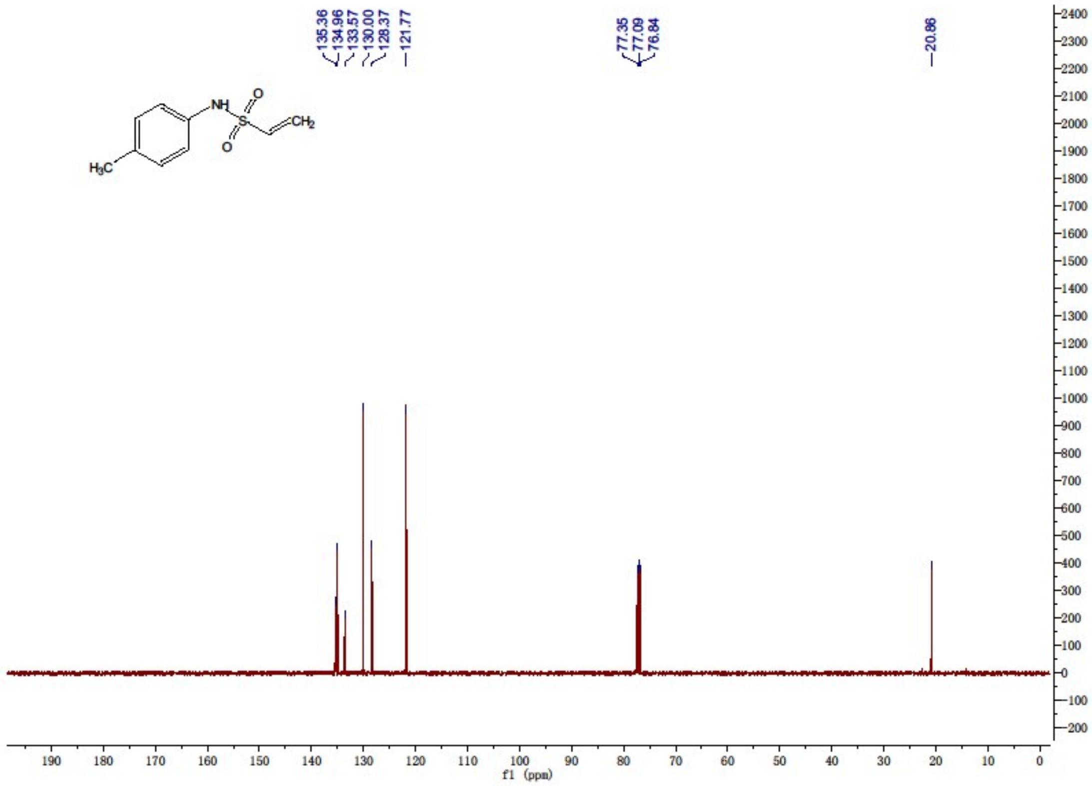
6

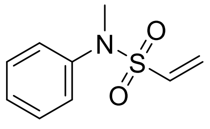
***N*-(*p*-tolyl)ethenesulfonamide (6)**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.17 – 7.08 (m, 5H), 7.05 (s, 1H), 6.58 (dd, *J* = 16.5, 9.9 Hz, 1H), 6.25 (d, *J* = 16.5 Hz, 1H), 5.95 (d, *J* = 9.9 Hz, 1H), 2.33 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 135.36, 134.96, 133.57, 130.00, 128.37, 121.77, 20.86. HRMS (ESI) $[M+H]^+$ Calculated for $C_9H_{12}NO_2S$, 198.0589, Found 198.0401 $[M+H]^+$, 220.0218 $[M+Na]^+$.

^{1}H NMR of compound **6**



^{13}C NMR of compound **6**

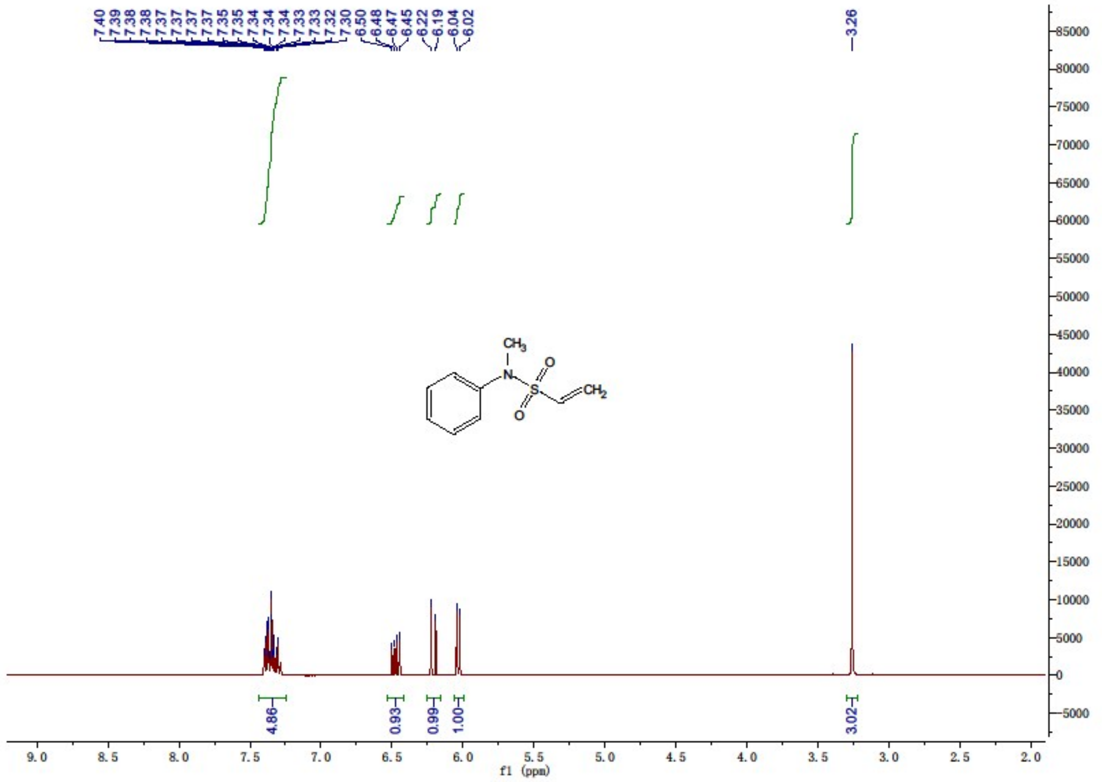




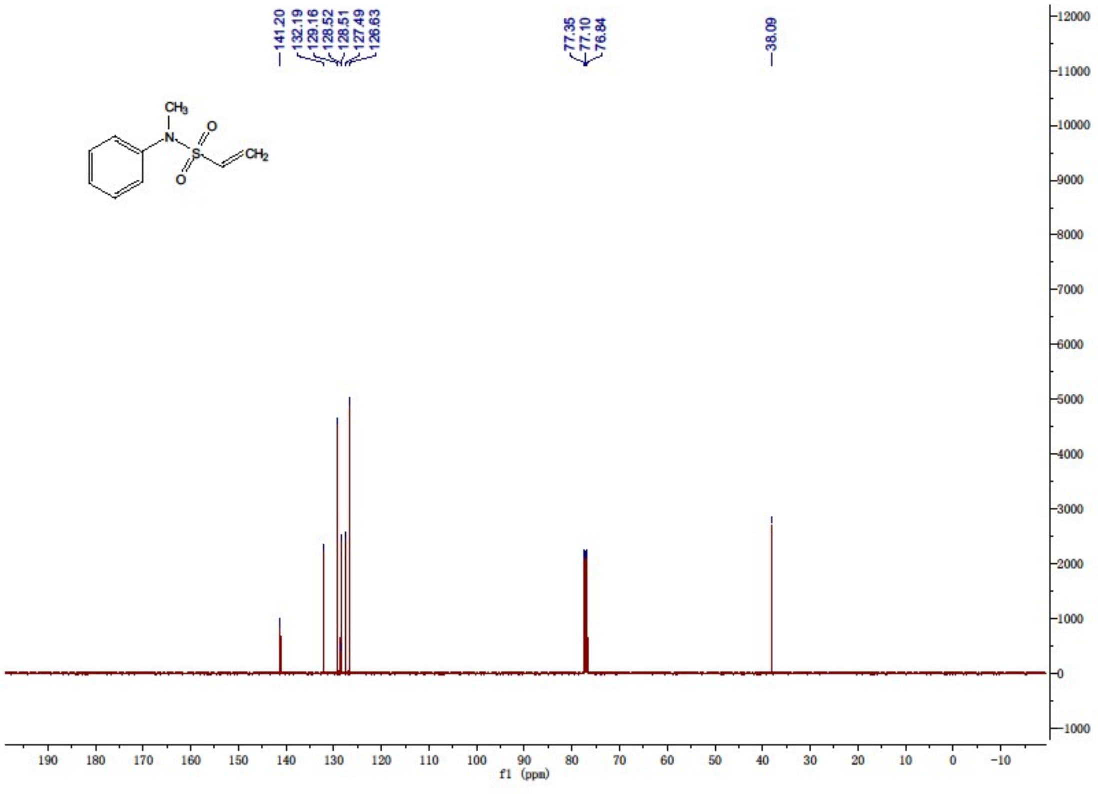
7

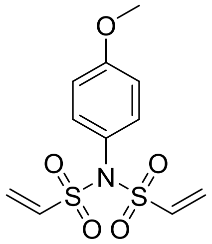
***N*-methyl-*N*-phenylethenesulfonamide (7)**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.43 – 7.25 (m, 5H), 6.47 (dd, *J* = 16.6, 9.9 Hz, 1H), 6.21 (d, *J* = 16.6 Hz, 1H), 6.03 (d, *J* = 10.0 Hz, 1H), 3.26 (s, 3H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 141.20, 132.19, 129.16, 128.52, 128.51, 127.49, 126.63, 38.09. HRMS (ESI) [M+H]$^+$ Calculated for C$_9$H$_{12}$NO$_2$S, 198.0589, Found 198.0249 [M+H]$^+$, 220.0061 [M+Na]$^+$.

^{1}H NMR of compound **7**



^{13}C NMR of compound **7**

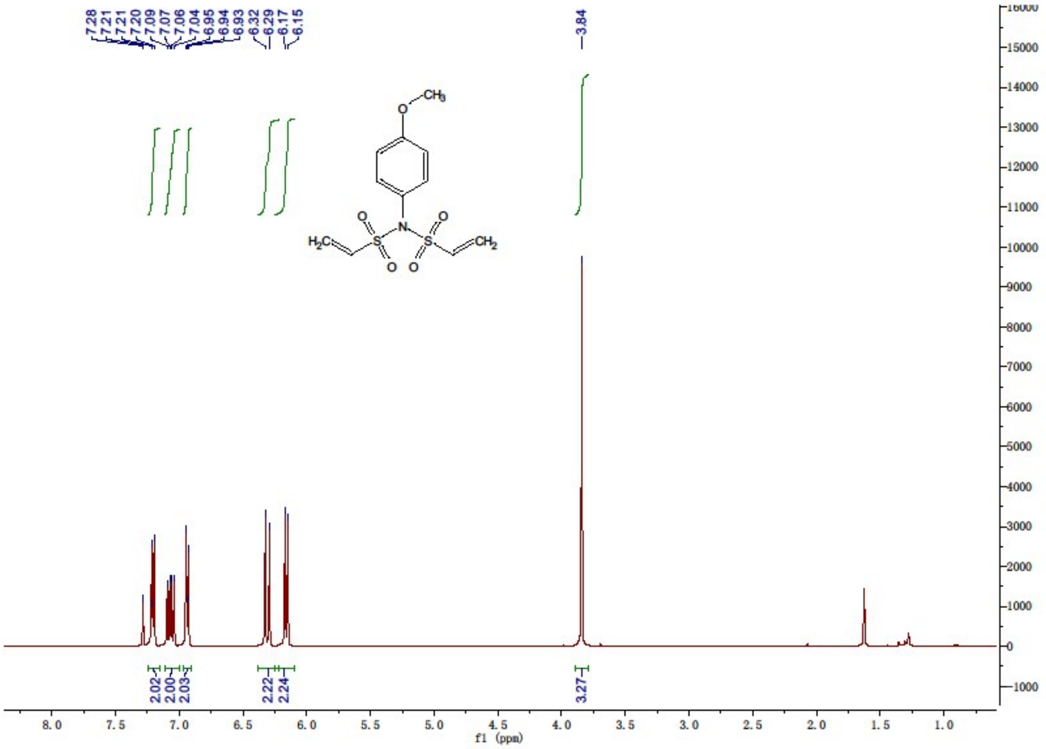




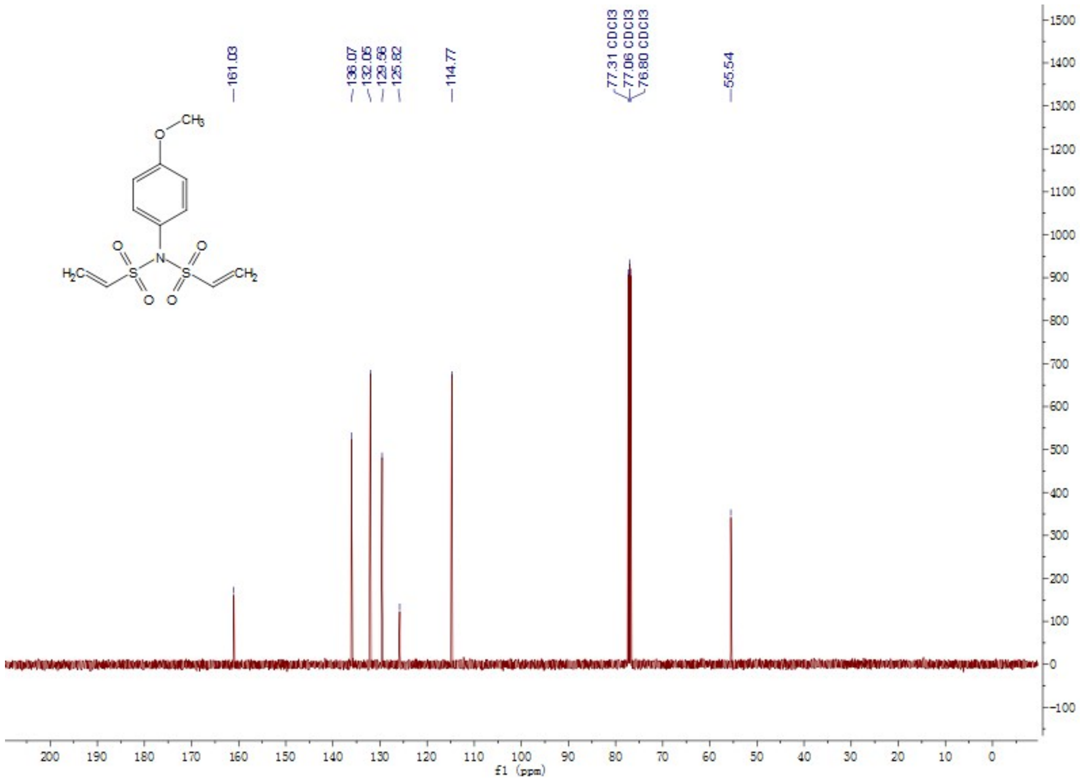
8

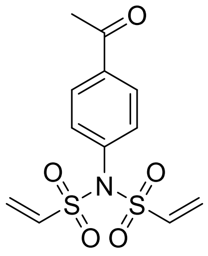
***N*-(4-methoxyphenyl)-*N*-(vinylsulfonyl)ethenesulfonamide (8)**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.21 (d, J = 8.7 Hz, 2H), 7.07 (dd, J = 16.5, 9.9 Hz, 2H), 6.94 (d, J = 8.7 Hz, 2H), 6.31 (d, J = 16.5 Hz, 2H), 6.16 (d, J = 9.8 Hz, 2H), 3.84 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.03, 136.07, 132.05, 129.56, 125.82, 114.77, 55.54. HRMS (ESI) $[M+H]^+$ Calculated for $C_{11}H_{14}NO_5S_2$, 304.0313, Found 304.0298.

^{1}H NMR of compound **8**



^{13}C NMR of compound **8**

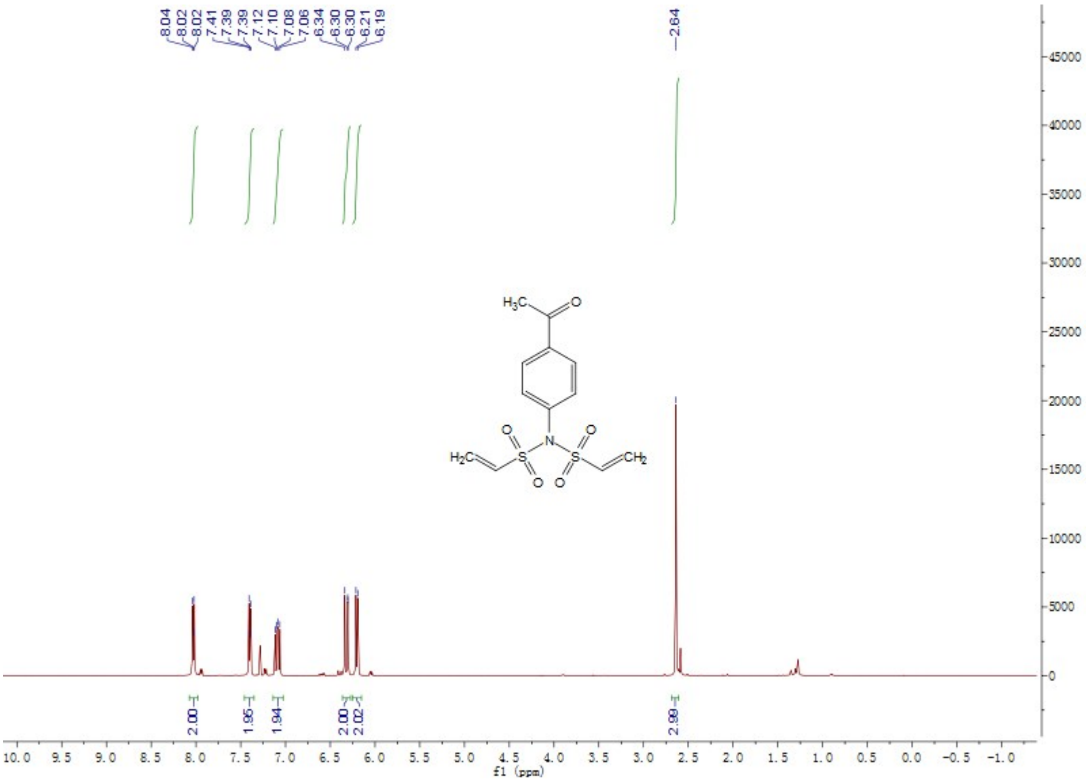




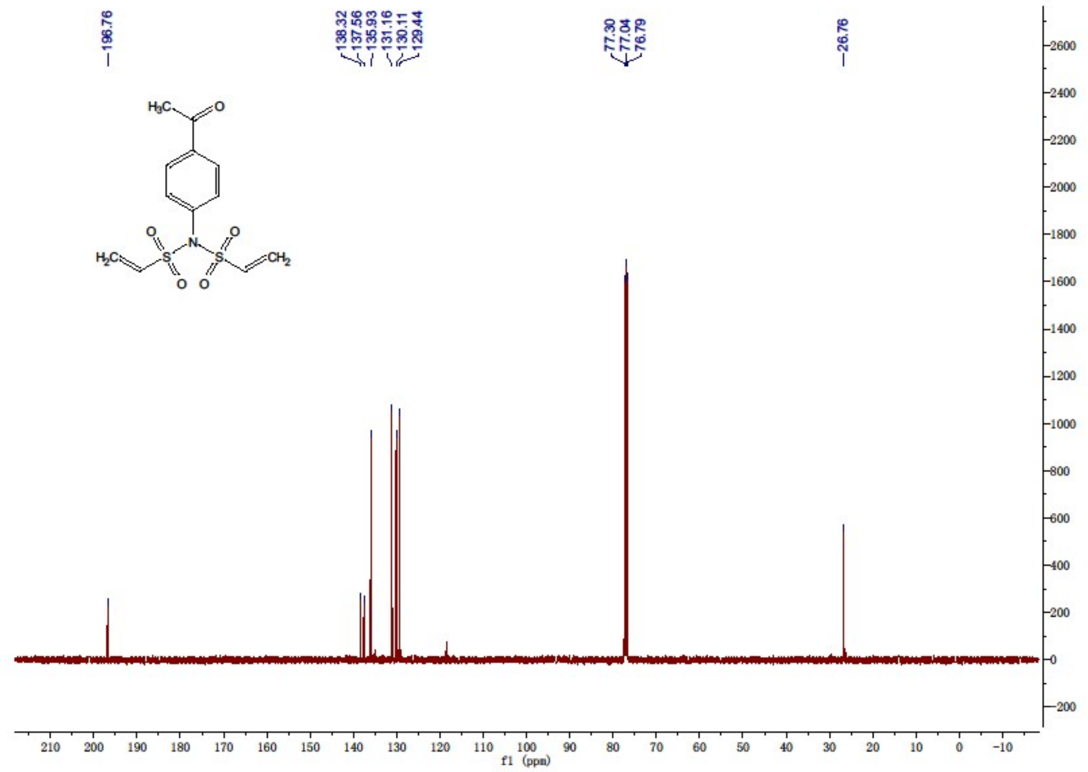
9

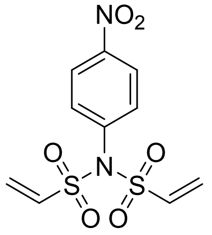
***N*-(4-acetylphenyl)-*N*-(vinylsulfonyl)ethenesulfonamide (9)**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.03 (d, *J* = 8.5 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 7.09 (dd, *J* = 16.5, 9.8 Hz, 2H), 6.32 (d, *J* = 16.4 Hz, 2H), 6.20 (d, *J* = 9.9 Hz, 2H), 2.64 (s, 3H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 196.76, 138.32, 137.56, 135.93, 131.16, 130.11, 129.44, 26.76. HRMS (ESI) [M+H]$^+$ Calculated for $C_{12}H_{14}NO_5S_2$, 316.0313, Found 316.0313.

^{1}H NMR of compound **9**



^{13}C NMR of compound **9**

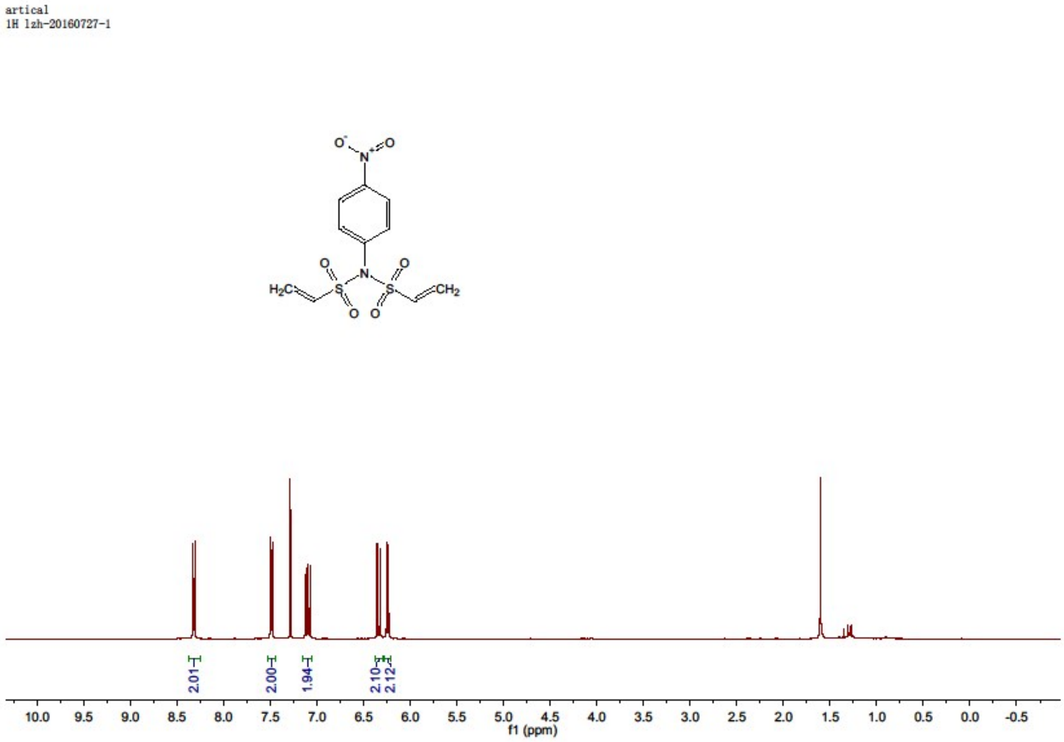




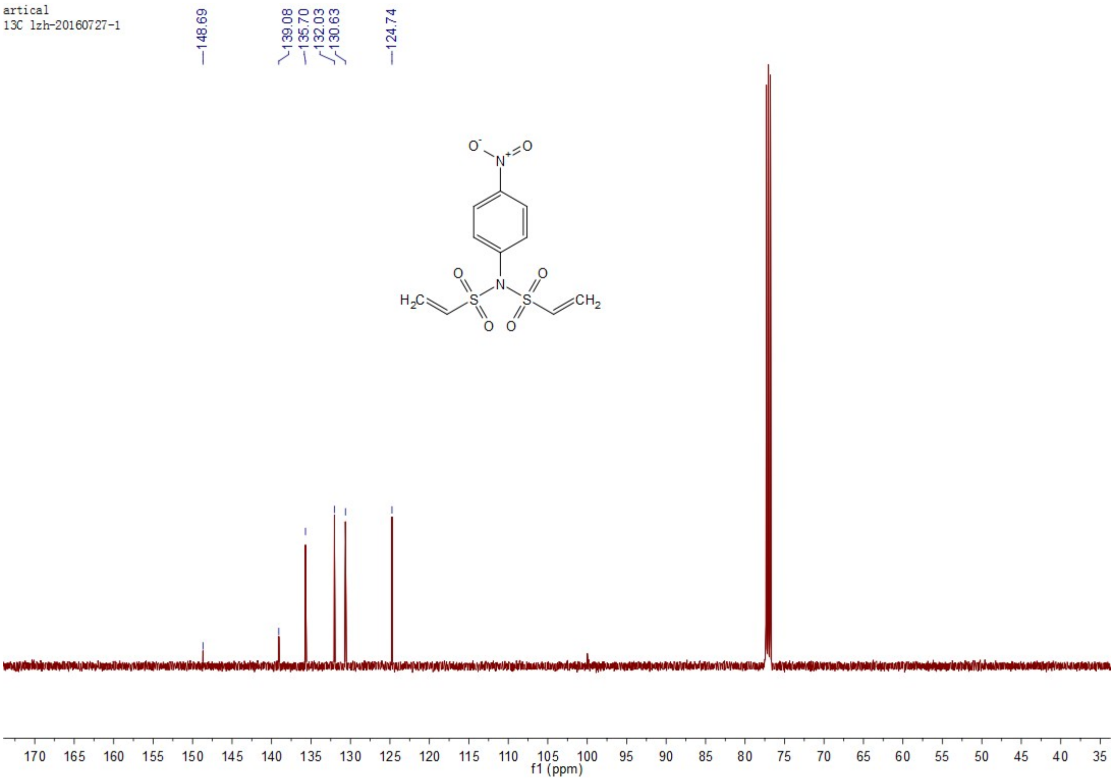
10

***N*-(4-nitrophenyl)-*N*-(vinylsulfonyl)ethenesulfonamide (10)**: ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.32 (d, *J* = 8.9 Hz, 2H), 7.49 (d, *J* = 8.9 Hz, 2H), 7.10 (dd, *J* = 16.5, 9.8 Hz, 2H), 6.34 (dd, *J* = 16.5, 1.0 Hz, 2H), 6.24 (dd, *J* = 9.8, 1.0 Hz, 2H); ^{13}C NMR (126 MHz, CDCl$_3$) δ 148.69, 139.08, 135.70, 132.03, 130.63, 124.74. HRMS (ESI) [M+H]$^+$ Calculated for $C_{10}H_{11}N_2O_6S_2$, 319.0059, Found 318.9974.

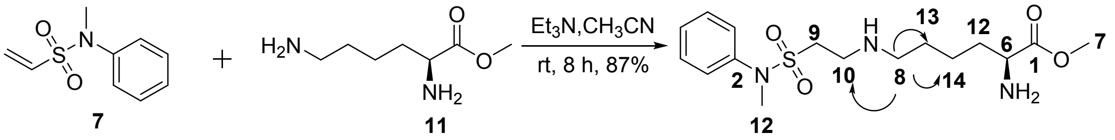
^{1}H NMR of compound **10**



^{13}C NMR of compound **10**



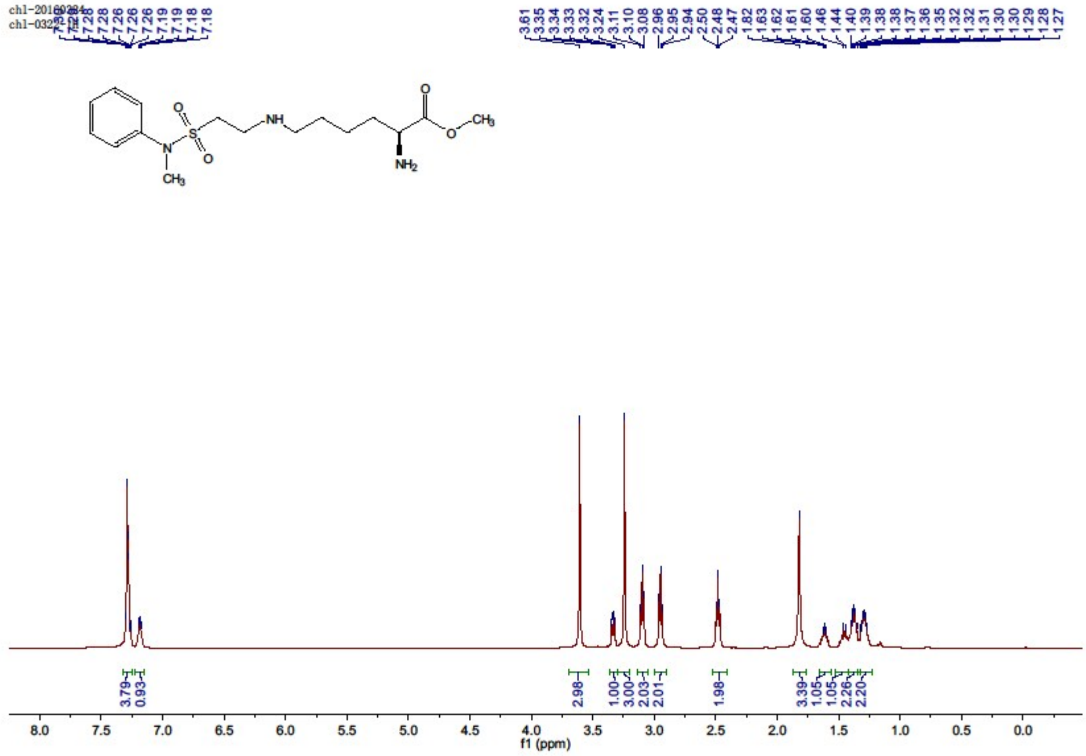
General procedure for the synthesis of compound 12



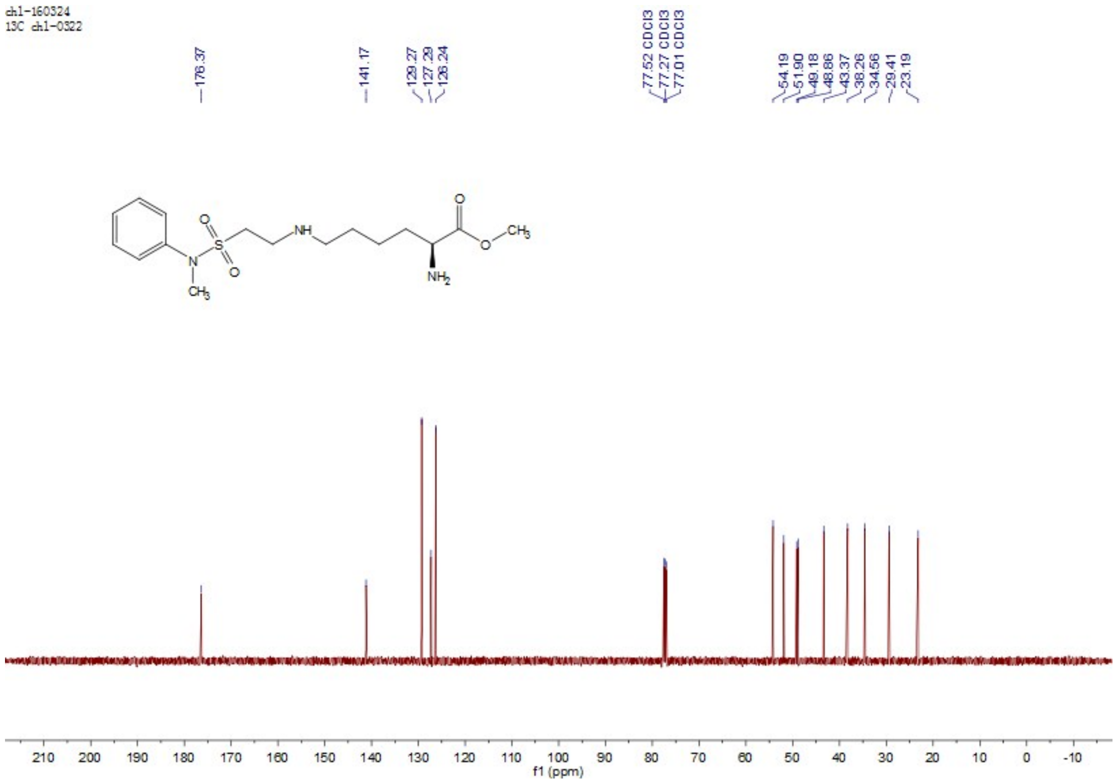
To a solution of compound **7** (100 mg, 0.51 mmol) and L-Lysine methyl ester dihydrochloride (355 mg, 1.52 mmol) in CH_3CN, Et_3N (307 mg, 3.05 mmol) was added. The resulting solution was stirred at rt overnight. The solvent was removed under vacuum, the residue was dissolved in DCM, and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The product was purified by column chromatography using CH_3OH:DCM=1:10 as eluent (158 mg, 87% yield).

Methyl *N*^{6}-(2-(*N*-methyl-*N*-phenylsulfamoyl)ethyl)-*L*-lysinate (12): ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.29 (m, 4H), 7.18 (m, 1H), 3.61 (s, 3H), 3.33 (dd, *J* = 7.6, 5.3 Hz, 1H), 3.24 (s, 3H), 3.10 (t, *J* = 6.5 Hz, 2H), 2.95 (t, *J* = 6.5 Hz, 2H), 2.48 (t, *J* = 7.0 Hz, 2H), 1.82 (s, 3H), 1.61 (m, 1H), 1.45 (m, 1H), 1.38 (m, 2H), 1.34 – 1.23 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.37, 141.17, 129.27, 127.29, 126.24, 54.19, 51.90, 49.18, 48.86, 43.37, 38.26, 34.56, 29.41, 23.19. HRMS (ESI) $[M+H]^+$ Calculated for $C_{16}H_{28}N_3O_4S$, 358.1801, Found 358.1917.

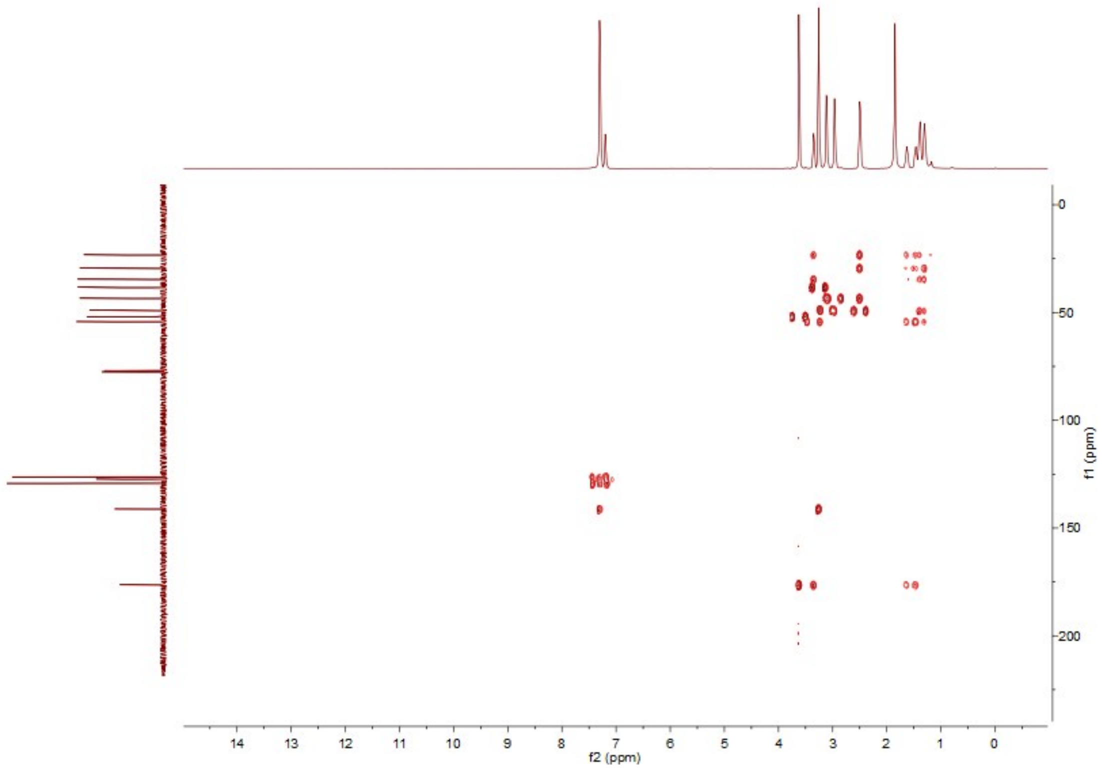
^{1}H NMR of compound **12**



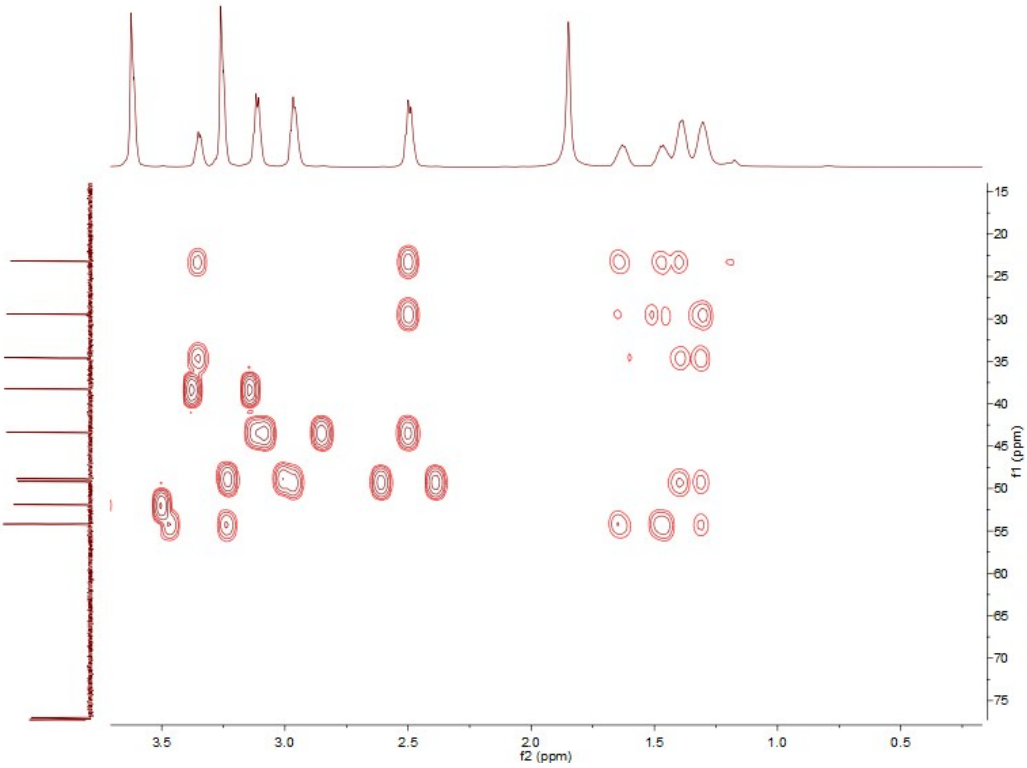
^{13}C NMR of compound **12**



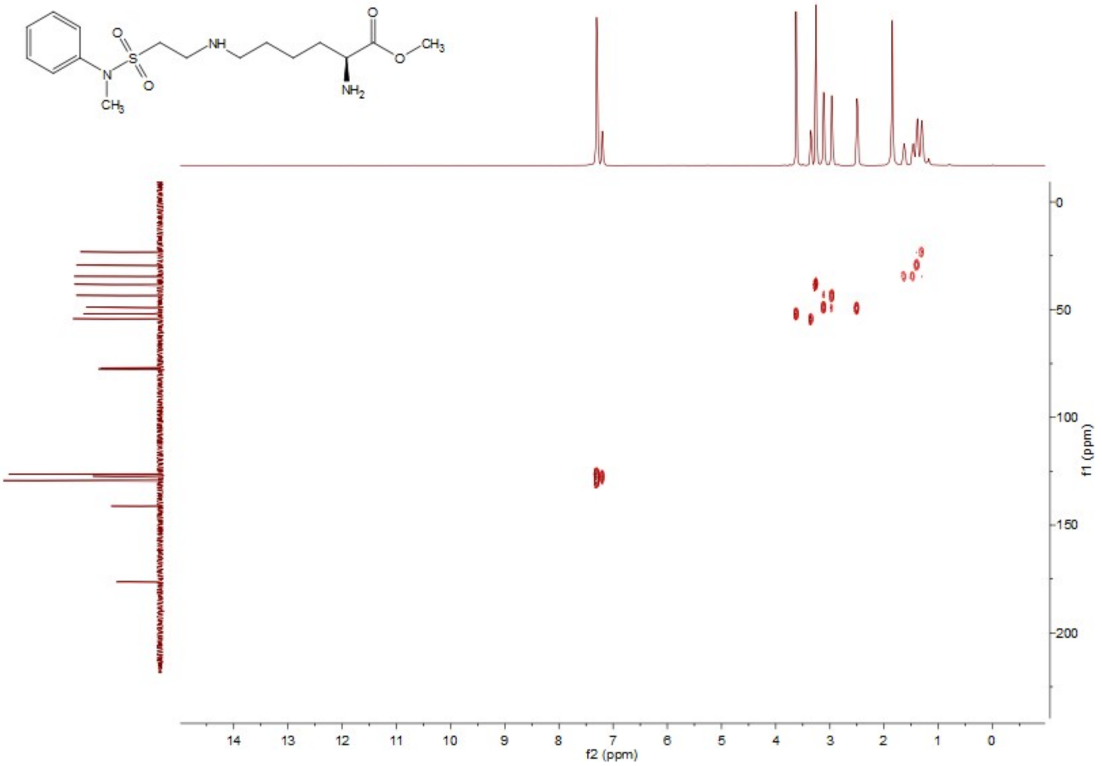
HMBC of compound **12**



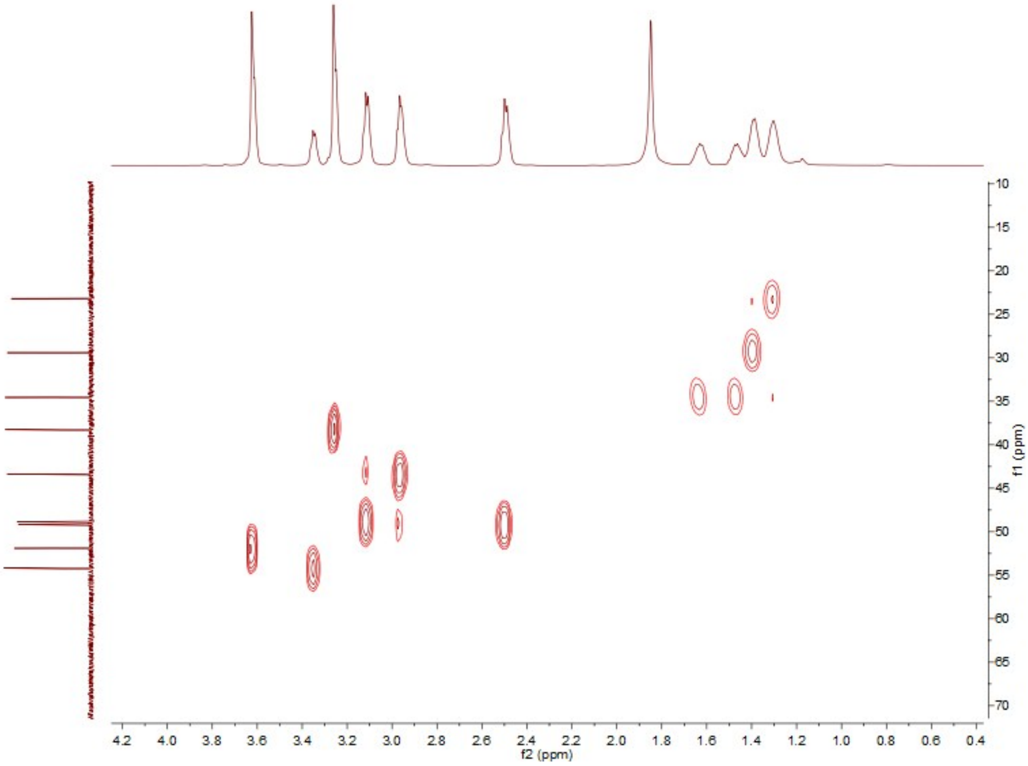
HMBC of compound **12**-enlarged view



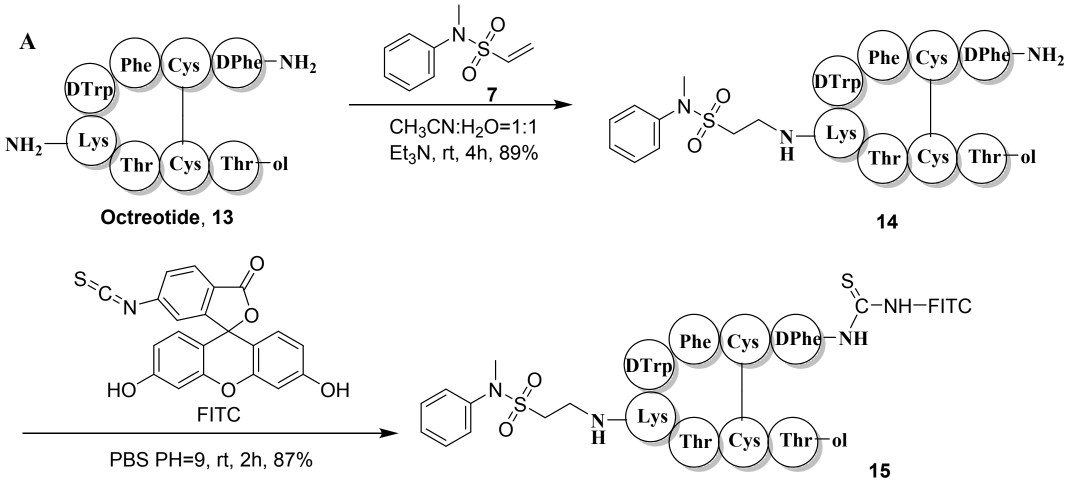
HSQC of compound **12**



HSQC of compound **12**-enlarged view



General procedure for the synthesis of compounds 14 and 15



To a solution of octreotide (5 mg, 0.005 mmol) and compound **7** (2.9 mg, 0.015 mmol) in CH_3CN and H_2O (1 ml : 1 ml), Et_3N (3 mg, 0.030 mmol) was added. The resulting solution was stirred at rt. The reaction was analyzed by RP-HPLC and LC-MS. The purified product **14** was obtained using semi-preparative RP-HPLC (89% yield) and determined by HRESIMS, MS^2 and ^1H NMR.

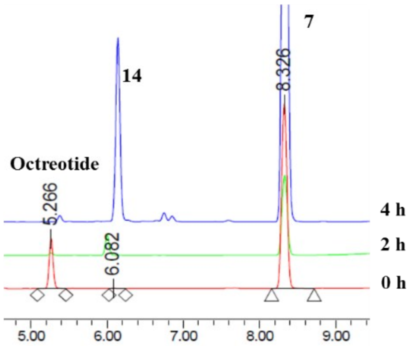


Fig. 1SA HPLC of the reaction that compound **7** conjugated with octreotide to produce **14**

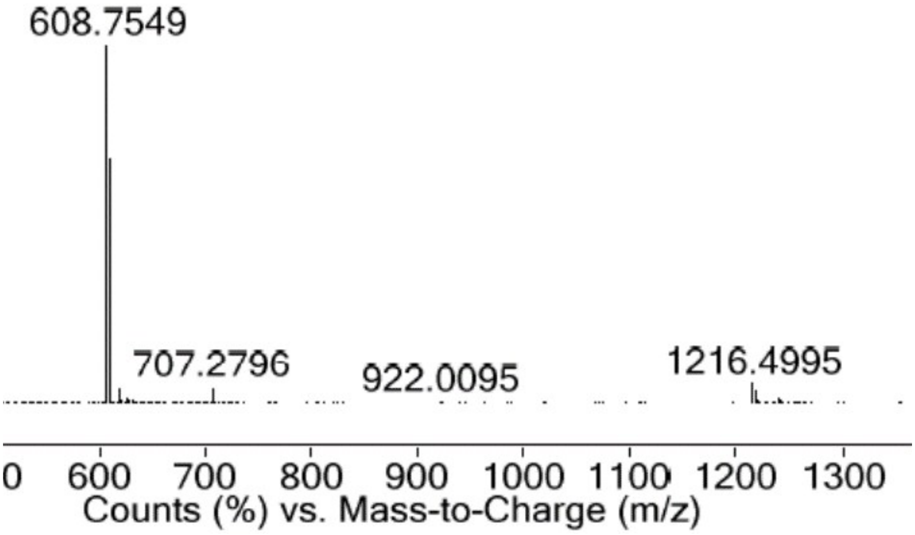


Fig. 1SB The HRESIMS of compound **14**

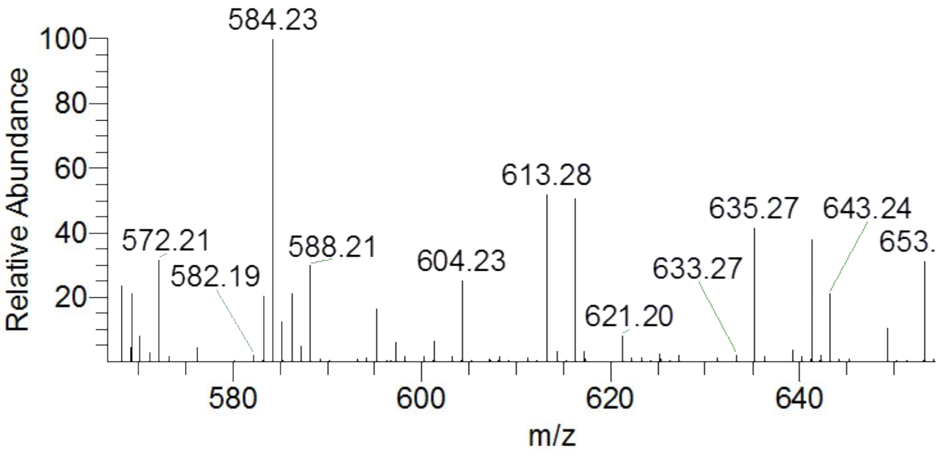


Fig. 1SC The MS^2 of compound **14**

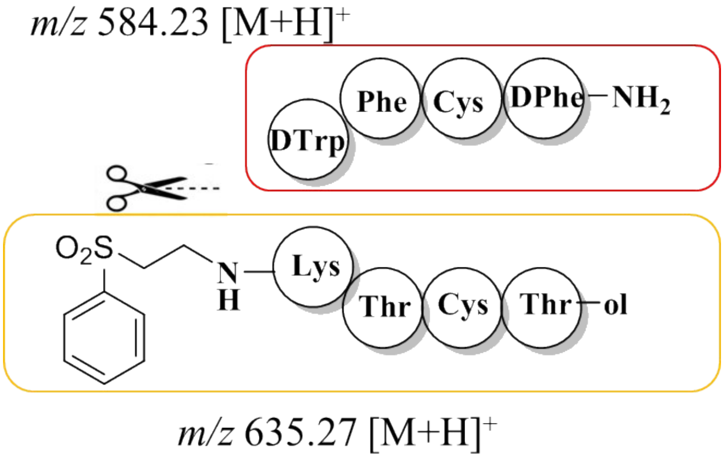
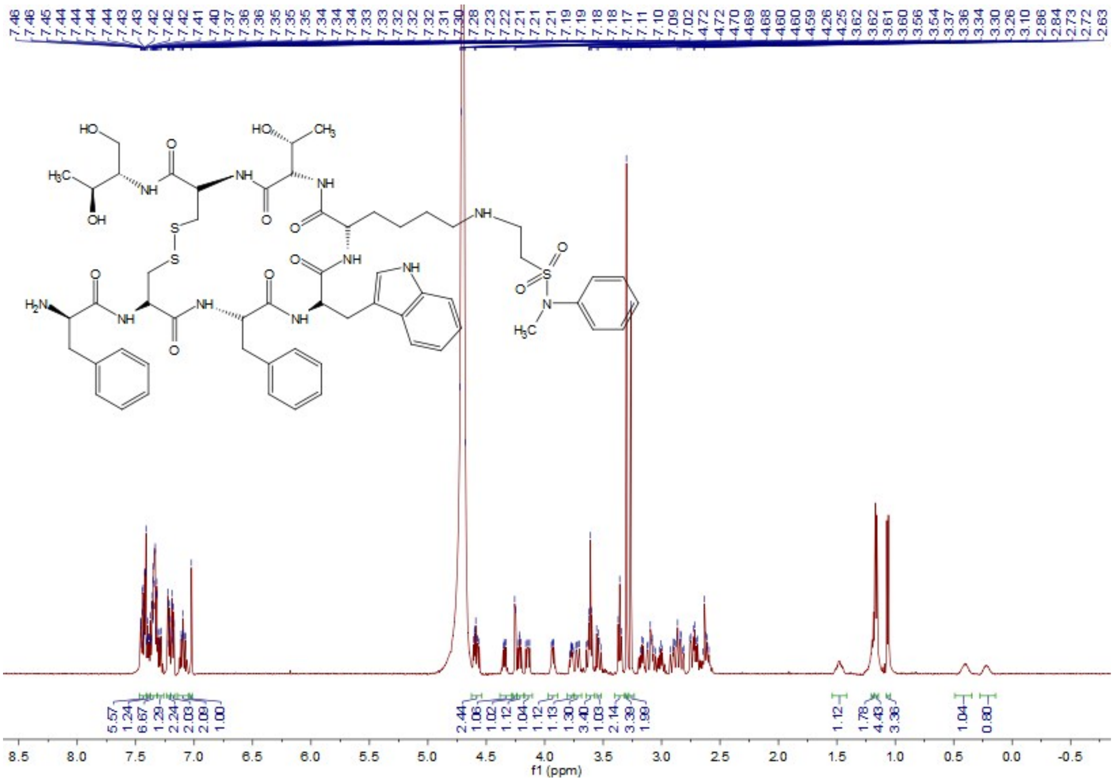


Fig. 1SD Peptide fragments

^{1}H NMR of compound **14**



To a solution of compound **14** (2 mg, 0.0016 mmol) in 1.6 ml PBS buffer (50 mmol, PH=9), Fluorescein isothiocyanate (FITC) (0.7 mg, 0.0018 mmol) was added. The resulting solution was stirred at rt for 2 h. The reaction was analyzed by RP-HPLC and LC-MS. The product **15** was determined by HRESIMS and MS^2.

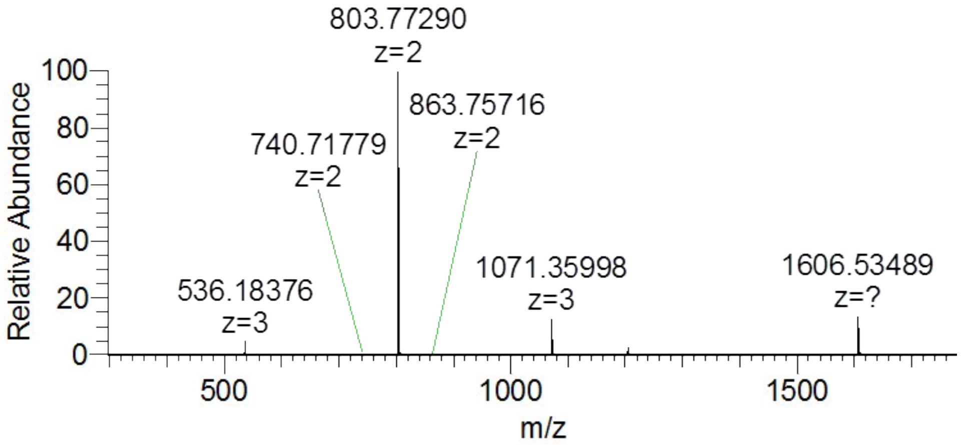


Fig. 1SE HRESIMS of compound **15**

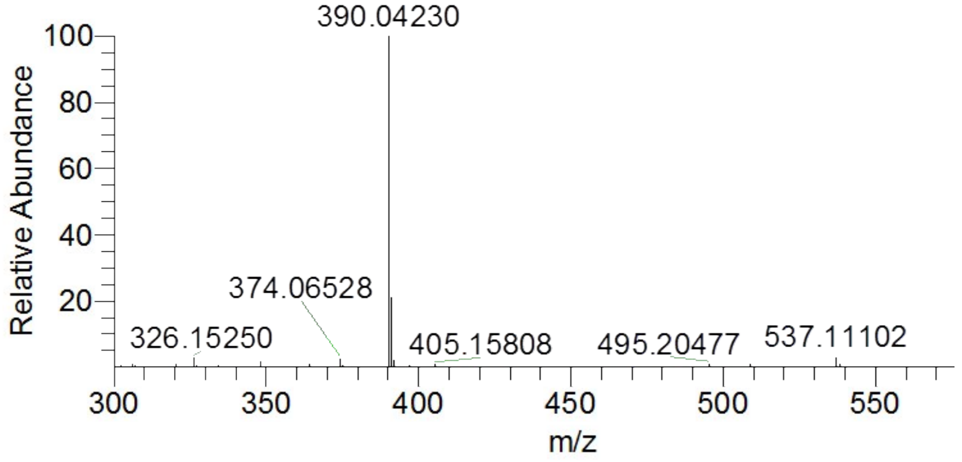


Fig. 1SF MS^2 of compound **15**

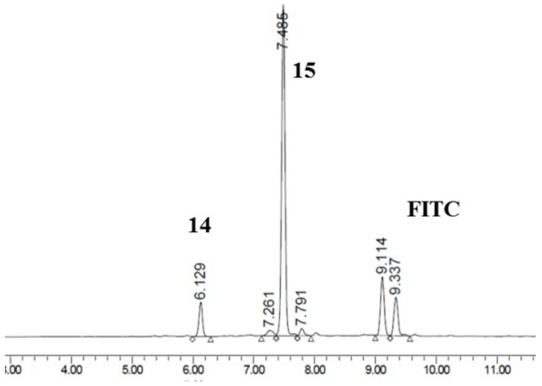
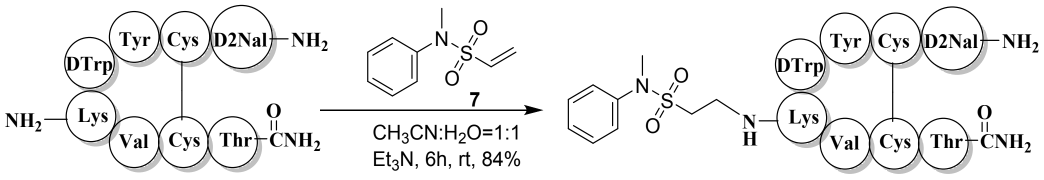


Fig. 1SG HPLC of the reaction that compound **14** reacted with FITC to produce **15**

General procedure for the synthesis of compound 18



Lanreotide (16)　　**18**

The procedure was same to the synthesis of compound **14** (84% yield).

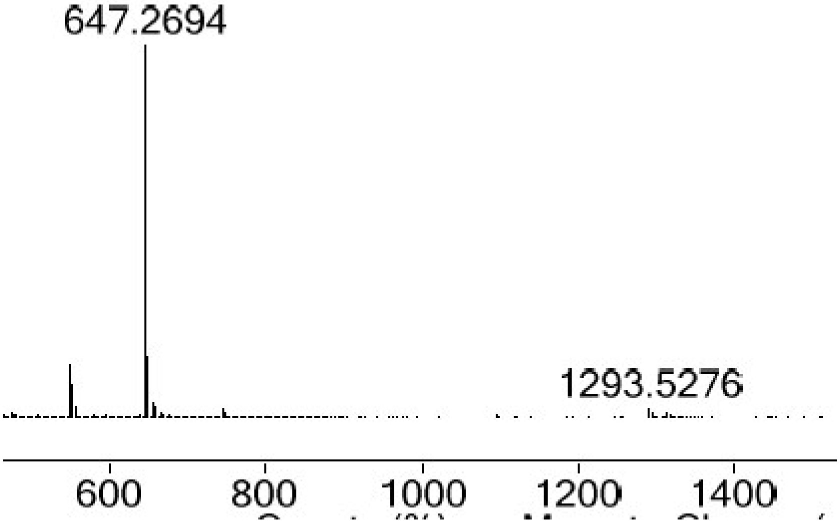


Fig. 2SA The HRESIMS of compound **18**

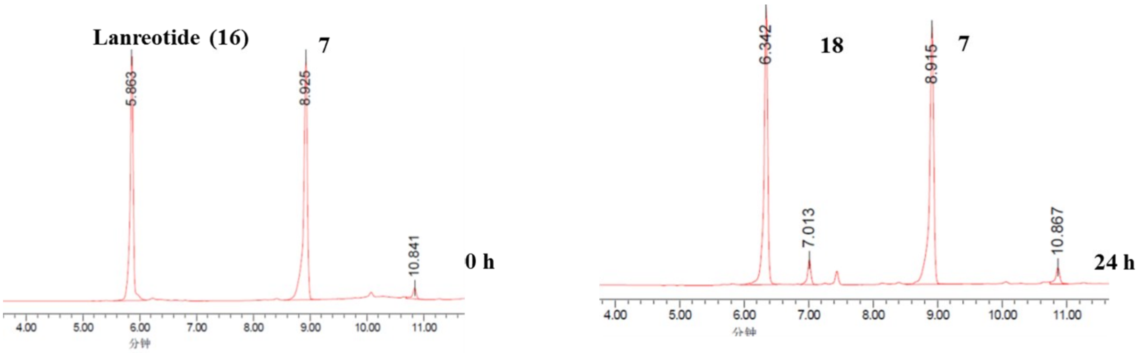
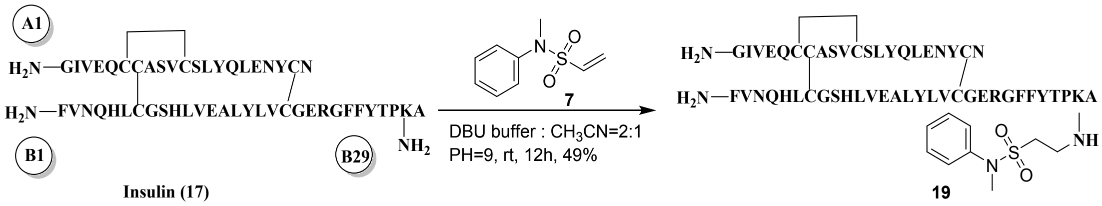


Fig. 2SB HPLC of the reaction that lanreotide **16** reacted with **7** to produce **18**

General procedure for the synthesis of compound 19



To a solution of insulin (1.57 mg, 0.3 μmol) in the mixture of DBU buffer (10 mmol, PH=9, 320 c) and CH_3CN (160 μl), compound **7** (1M/L in CH_3CN, 6 μl) was added. The resulting solution was shaking at rt (49% yield and 90% yield based on recovered insulin).

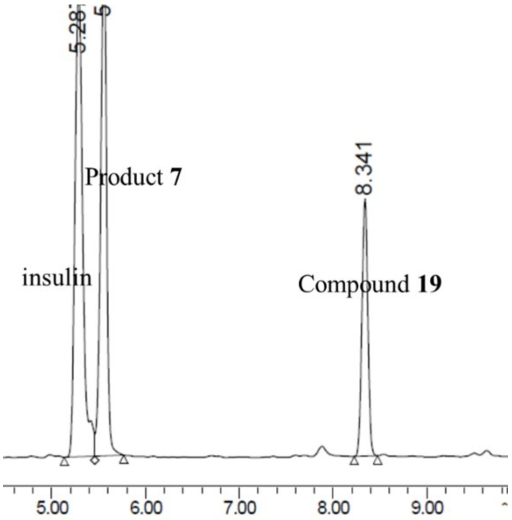


Fig. 3SA HPLC of the reaction that compound **7** conjugated with insulin to produce **19**

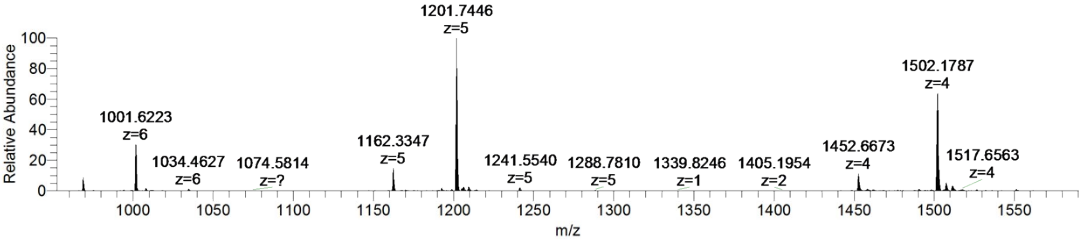


Fig. 3SB The HRESIMS of compound **19**

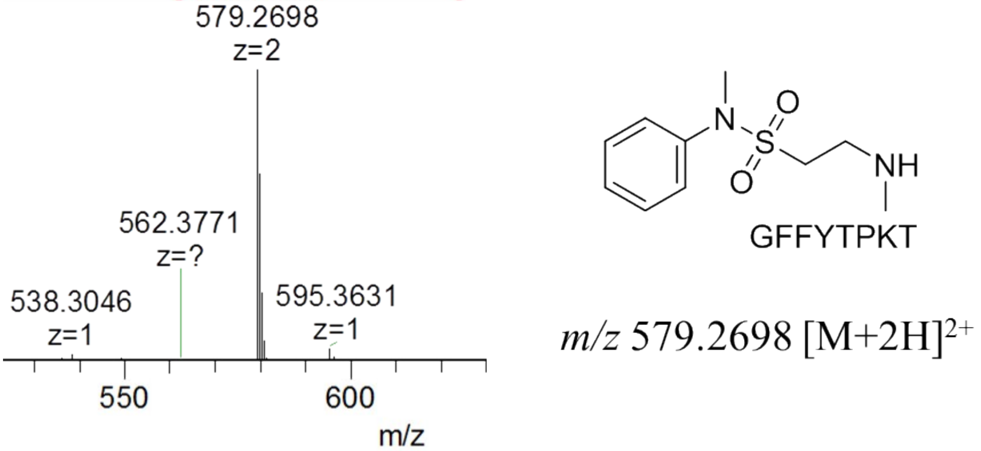
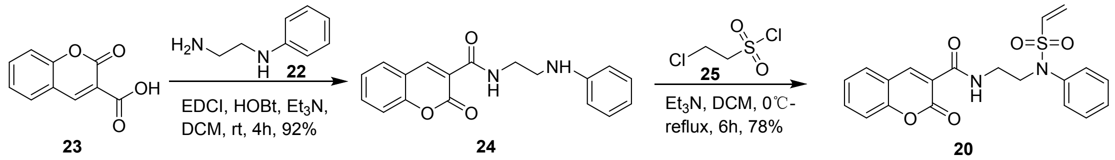


Fig. 3SC The MS^2 of compound **19**

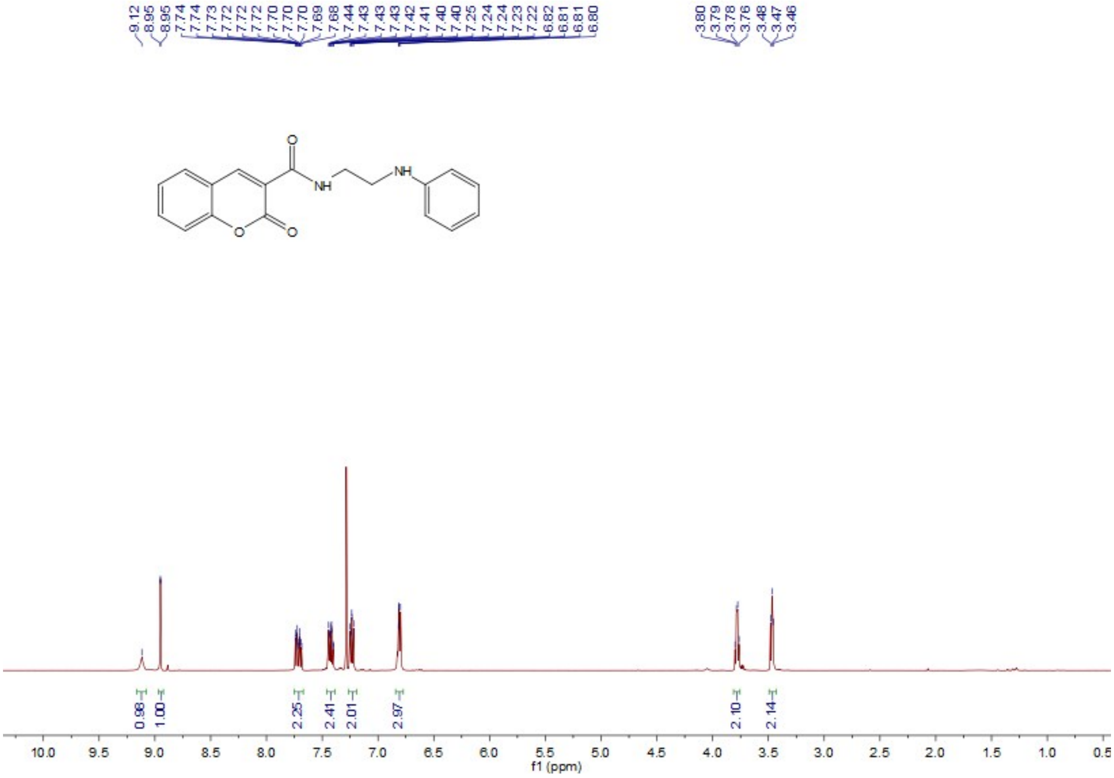
General procedure for the synthesis of compound 20



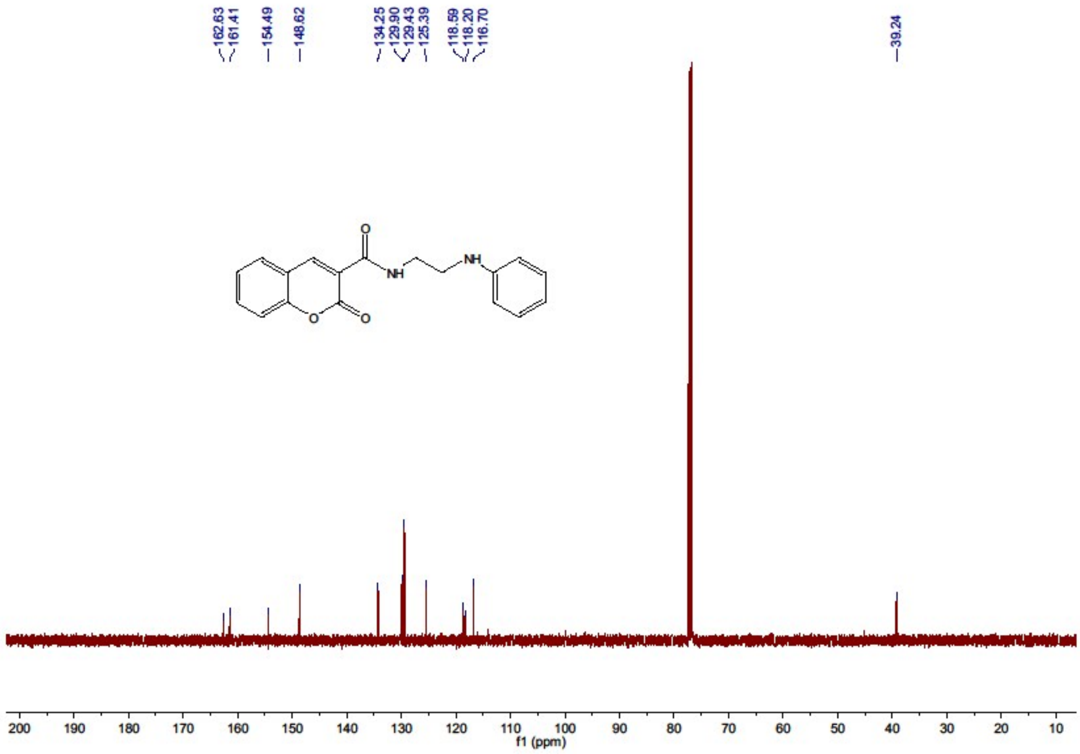
To a solution of compound coumarin-3-carboxylic acid (300 mg, 1.58 mmol), Hydroxybenzotriazole (HOBt, 426 mg, 3.16 mmol) and 3-(ethyliminomethyleneamino)-N,N-dimethyl-propan-1-amine (EDCI, 600 mg, 3.16 mmol) in DCM under Ar_2 and darkness, Et_3N (335 mg, 3.31 mmol) was added. The resulting solution was stirred at rt for 10 min. Then N-(2-aminoethyl)benzeneamine (**22**, 236 mg, 1.74 mmol) was added to the reaction mixture. The solution was stirred at rt for another 4h. The reaction was quenched with H_2O (50 mL) and extracted with DCM (3 × 50 mL). The organic layer was combined, washed with H_2O (1 × 50 mL) and brine (1 × 50 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by column chromatography (EA:PE=1:2) to give the target compound **24** (447 mg, 92% yield).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.12 (s, 1H), 8.99 – 8.89 (m, 1H), 7.79 – 7.60 (m, 2H), 7.50 – 7.32 (m, 2H), 7.27 – 7.15 (m, 2H), 6.85 – 6.70 (m, 3H), 3.78 (t, *J* = 6.0 Hz, 2H), 3.47 (t, *J* = 6.0 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.63, 161.41, 154.49, 148.62, 134.25, 129.90, 129.43, 125.39, 118.59, 118.20, 116.70, 39.24. HRMS (ESI) $[M+H]^+$ Calculated for $C_{18}H_{17}N_2O_3$, 309.1239, Found 309.1241, 331.1042 $[M+Na]^+$.

^{1}H NMR of compound **24**



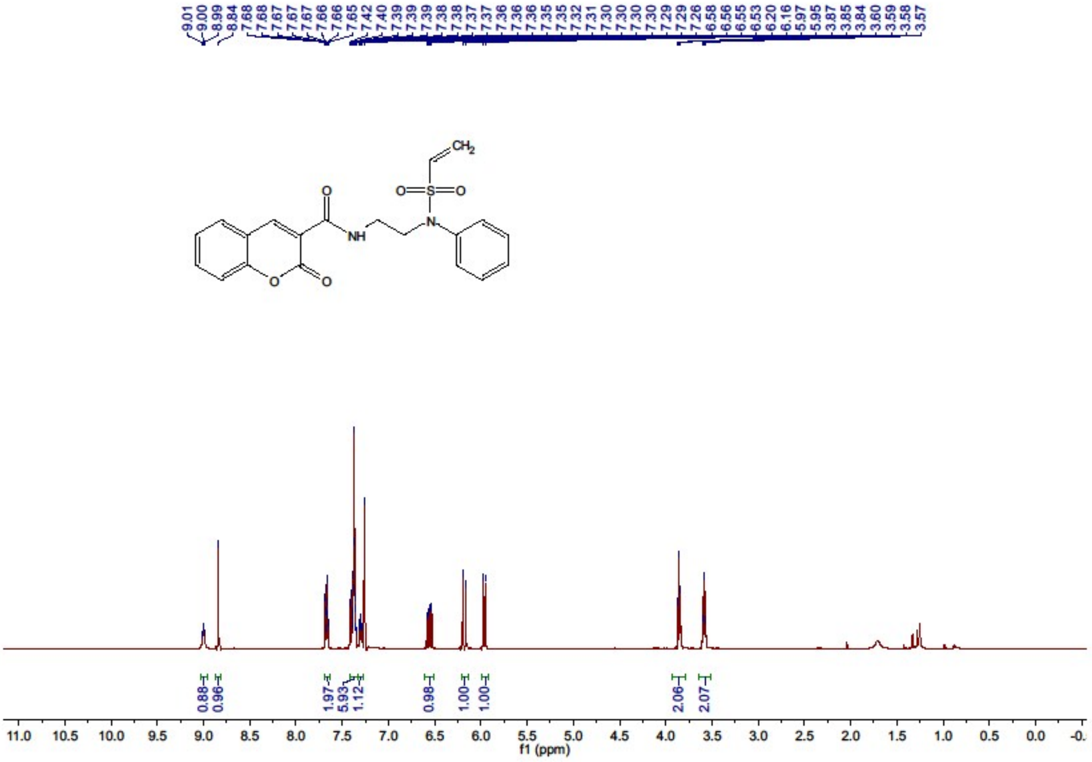
^{13}C NMR of compound **24**



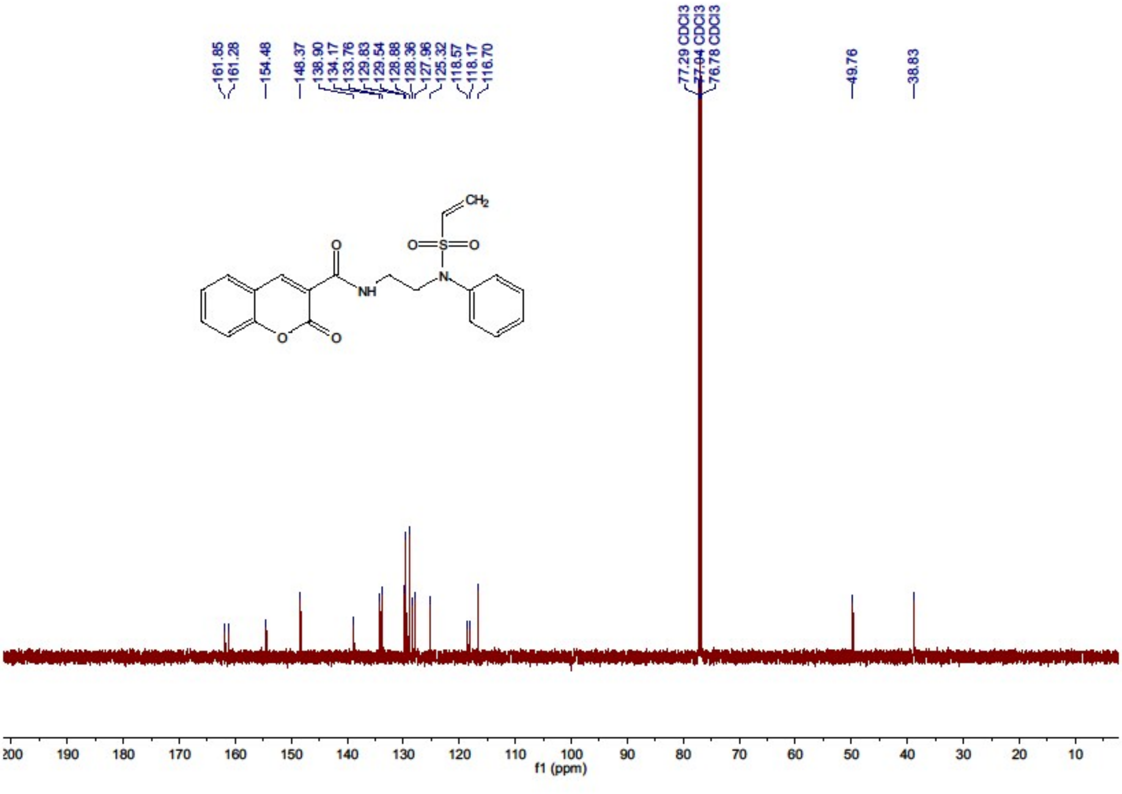
To a stirred solution of compound **24** (233 mg, 0.76 mmol) and trimethylamine (230 mg, 2.28 mmol) in DCM at 0℃, 2-chloroethanesulfonyl chloride (310 mg,1.9 mmol) was added slowly. The resulting mixture was stirred under reflux until compound **24** was consumed The reaction was quenched with water and the mixture was extracted with DCM. The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by column chromatography(EA:PE=1:2) to give the desired product **20** (234 mg, 78% yield).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.00 (t, *J* = 6.1 Hz, 1H), 8.84 (s, 1H), 7.67 (ddd, *J* = 7.5, 6.3, 1.8 Hz, 2H), 7.42 – 7.33 (m, 5H), 7.30 (ddd, *J* = 6.4, 5.2, 2.7 Hz, 1H), 6.56 (dd, *J* = 16.5, 9.9 Hz, 1H), 6.18 (d, *J* = 16.6 Hz, 1H), 5.96 (d, *J* = 9.9 Hz, 1H), 3.85 (t, *J* = 6.1 Hz, 2H), 3.59 (q, *J* = 6.0 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.85, 161.28, 154.48, 148.37, 138.90, 134.17, 133.76, 129.83, 129.54, 128.88, 128.36, 127.96, 125.32, 118.57, 118.17, 116.70, 49.76, 38.83. HRMS (ESI) $[M+H]^+$ Calculated for $C_{20}H_{19}N_2O_5S$, 399.1015, Found 399.1038.

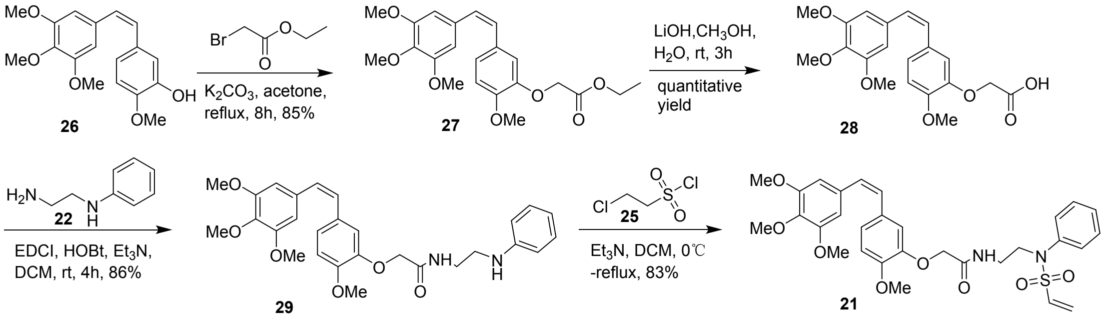
^{1}H NMR of compound **20**



^{13}C NMR of compound **20**

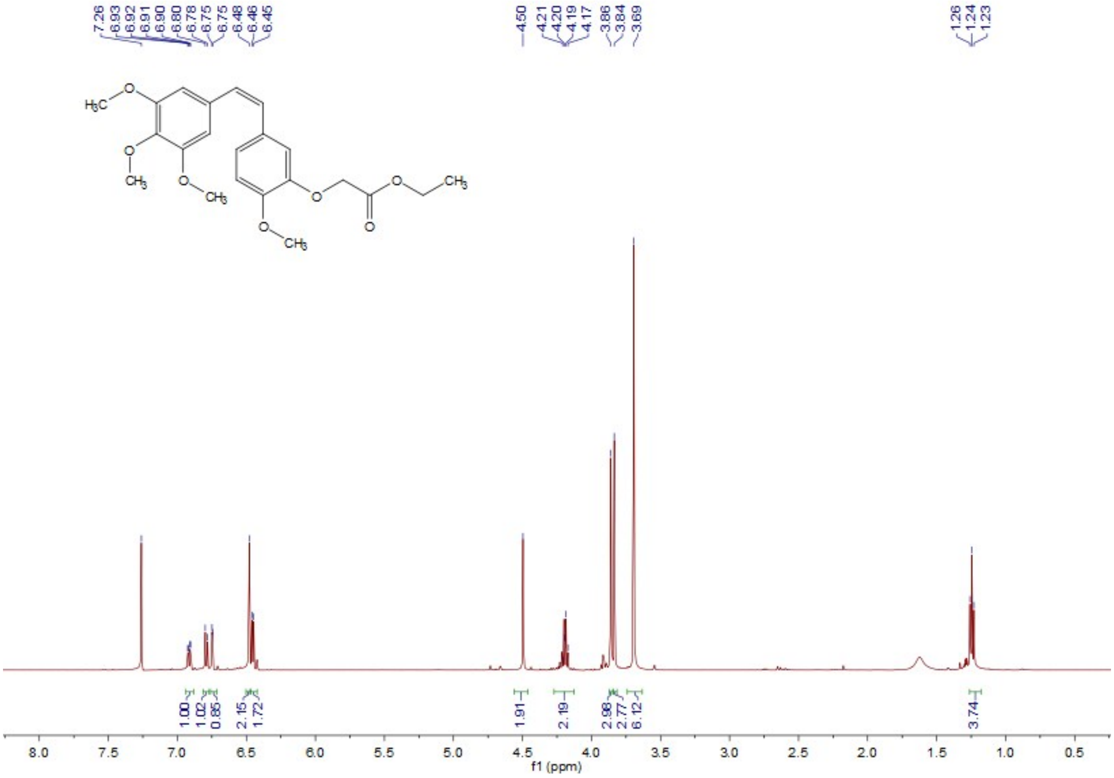


General procedure for the synthesis of compound 21



To a solution of combretastatin A-4 (CA4, 152 mg, 0.48 mmol) and K_2CO_3 (199 mg, 1.44 mmol) in acetone, α-bromoethyl acetate (120 mg, 0.72 mmol) was added. The mixture was stirred under reflux. The reaction was monitored by TLC. After the reaction was completed, the solid was filtered and washed with DCM. The filtrate was concentrated under reduced pressure. The residue was purified column chromatography (EA:PE=1:3) to give the desired product (165 mg, 85% yield).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.91 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.79 (d, *J* = 8.3 Hz, 1H), 6.75 (d, *J* = 1.9 Hz, 1H), 6.48 (s, 2H), 6.45 (d, *J* = 6.4 Hz, 2H), 4.50 (s, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 3.69 (s, 6H), 1.24 (t, *J* = 7.1 Hz, 3H).



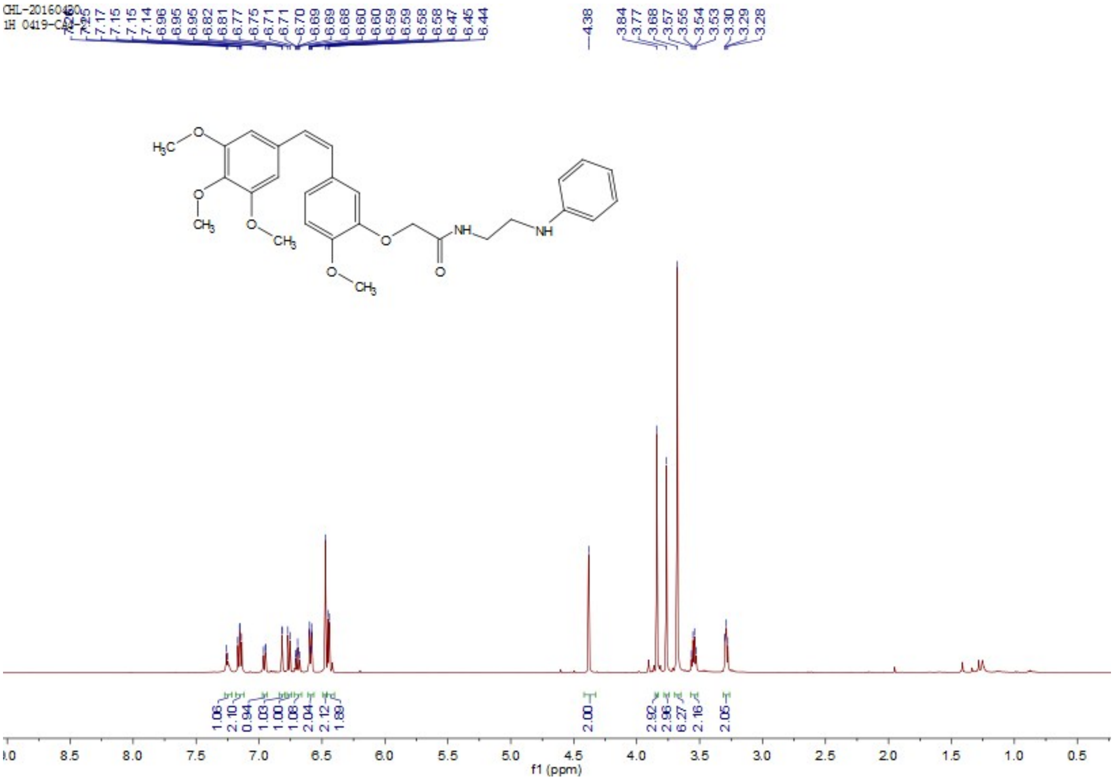
To a solution of the ester obtained above **27** (165 mg, 0.41 mmol) in CH_3OH and H_2O (10:1), LiOH·H_2O (21 mg, 0.49 mmol) was added. The resulting solution was stirred at rt for 3 h. The PH of the reaction solution was adjusted to 4 with diluted HCl. The mixture was diluted with water and extracted with DCM. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the desired product **28** (quantitative yield), which was used directly in the next step.

To a solution of compound **28** (122 mg, 0.33 mmol), Hydroxybenzotriazole (HOBt, 88 mg, 0.65 mmol) and 3-(ethyliminomethyleneamino)-N,N-dimethyl-propan-1-amine (EDCI, 125 mg, 0.65

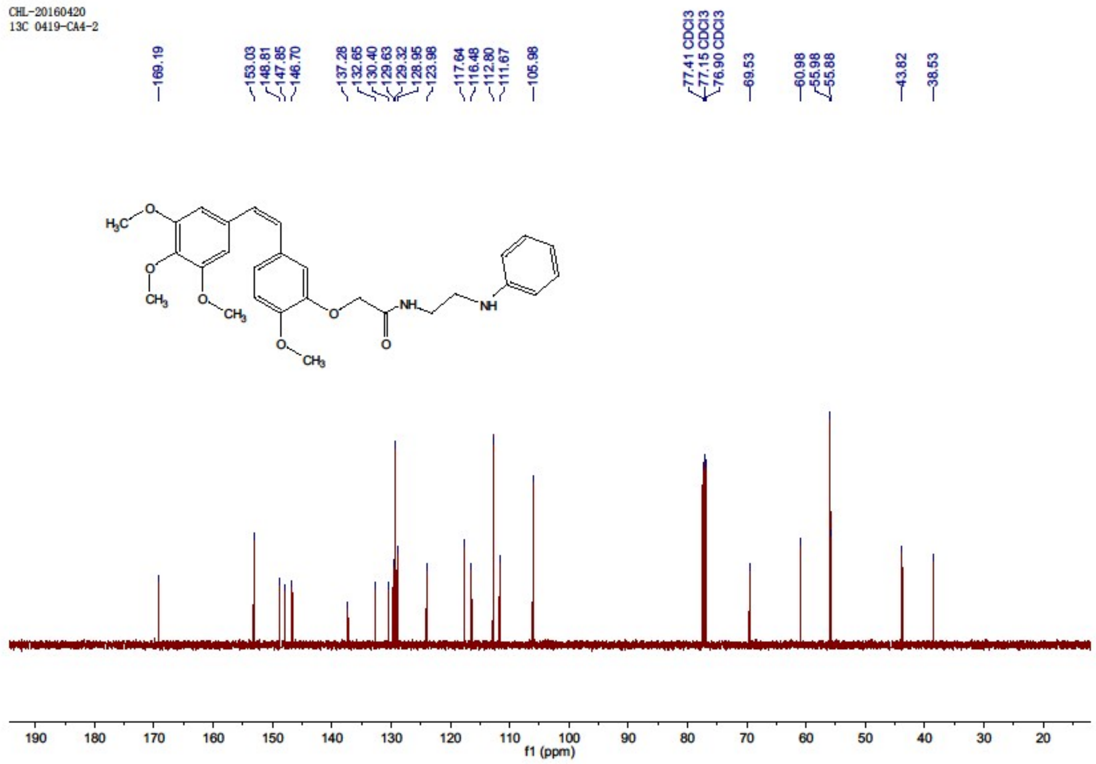
mmol) in DCM,, Et_3N (71mg, 0.7 mmol) was added. The resulting solution was stirred at rt for 10 min. Then N-(2-aminoethyl)benzeneamine (**22**, 49 mg, 0.36 mmol) was added to the reaction mixture. The solution was stirred at rt for another 4h. The reaction was quenched with H_2O (50 mL) and extracted with DCM (3 × 50 mL). The organic layer was combined, washed with H_2O (1 × 50 mL) and brine (1 × 50 mL), and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by column chromatography (EA:PE=1:1) to give the target compound **29** (138 mg, 86% yield).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.25 (t, *J* = 6.3 Hz, 1H), 7.18 – 7.12 (m, 2H), 6.96 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.82 (d, *J* = 2.0 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.73 – 6.65 (m, 1H), 6.62 – 6.56 (m, 2H), 6.47 (s, 2H), 6.45 (d, *J* = 5.3 Hz, 2H), 4.38 (s, 2H), 3.84 (s, 3H), 3.77 (s, 3H), 3.68 (s, 6H), 3.55 (d, *J* = 6.0 Hz, 2H), 3.29 (t, *J* = 5.9 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.19, 153.03, 148.81, 147.85, 146.70, 137.28, 132.65, 130.40, 129.63, 129.32, 128.95, 123.98, 117.64, 116.48, 112.80, 111.67, 105.98, 69.53, 60.98, 55.98, 55.88, 43.82, 38.53. HRMS (ESI) $[M+H]^+$ Calculated for $C_{28}H_{32}N_2O_6$, 493.2339, Found 493.2418, 515.2171 $[M+Na]^+$.

^{1}H NMR of compound **29**



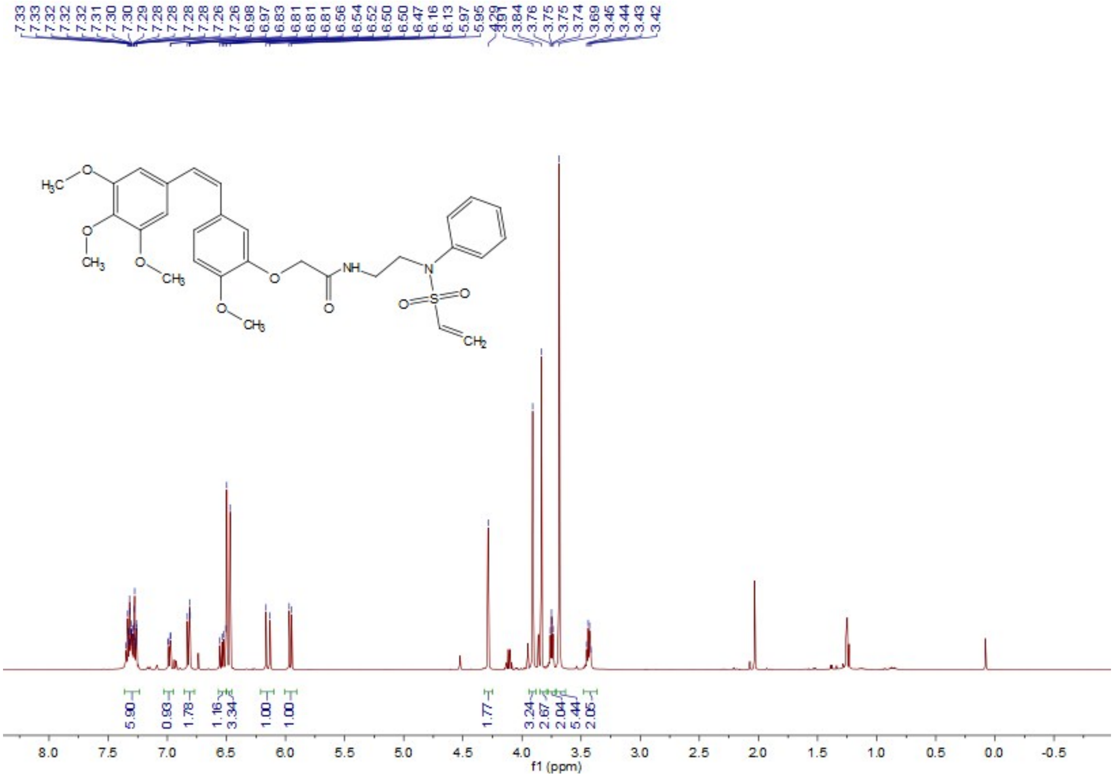
^{13}C NMR of compound **29**



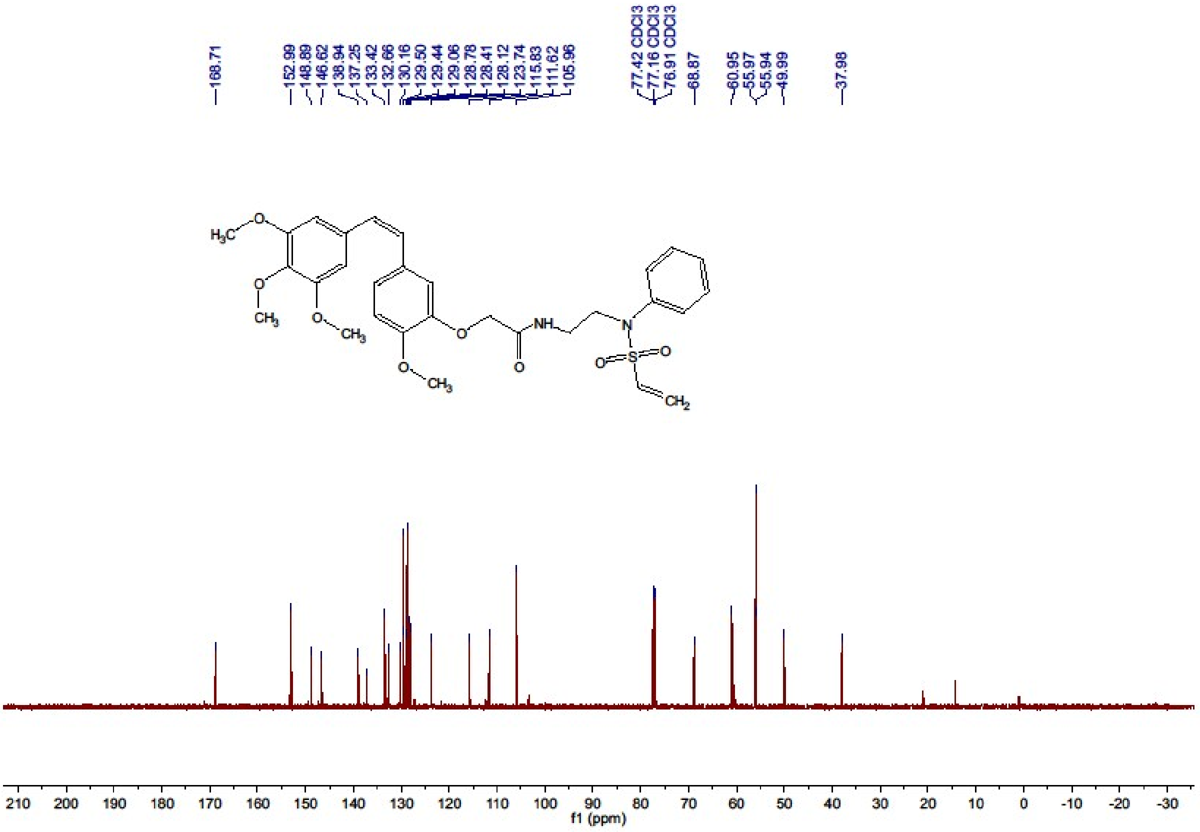
To a stirred solution of compound **22** (90 mg, 0.18 mmol) and trimethylamine (55 mg, 0.55mmol) in DCM at 0℃, 2-chloroethanesulfonyl chloride (59 mg, 0.36 mmol) was added slowly. The resulting mixture was stirred under reflux overnight. The reaction was quenched with water and the mixture was extracted with DCM. The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by column chromatography(EA:PE=1:1) to give the desired product **17** (88 mg, 83% yield).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.36 – 7.23 (m, 6H), 6.98 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.85 – 6.77 (m, 2H), 6.53 (dd, *J* = 16.5, 9.9 Hz, 1H), 6.48 (d, *J* = 13.8 Hz, 3H), 6.15 (d, *J* = 16.6 Hz, 1H), 5.96 (d, *J* = 9.9 Hz, 1H), 4.29 (s, 2H), 3.91 (s, 3H), 3.84 (s, 3H), 3.75 (dd, *J* = 6.5, 5.3 Hz, 2H), 3.69 (s, 6H), 3.43 (q, *J* = 5.9 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.71, 152.99, 148.89, 146.62, 138.94, 137.25, 133.42, 132.66, 130.16, 129.50, 129.44, 129.06, 128.78, 128.41, 128.12, 123.74, 115.83, 111.62, 105.96, 68.87, 60.95, 55.97, 55.94, 49.99, 37.98. HRMS (ESI) $[M+H]^+$ Calculated for $C_{30}H_{35}N_2O_8S$, 583.2114, Found 583.2025, 605.1844 $[M+Na]^+$

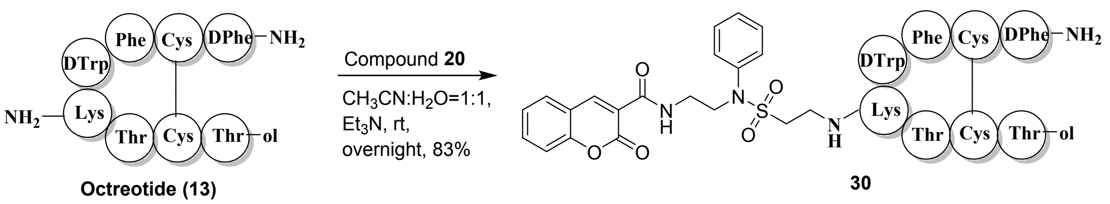
^{1}H NMR of compound **21**

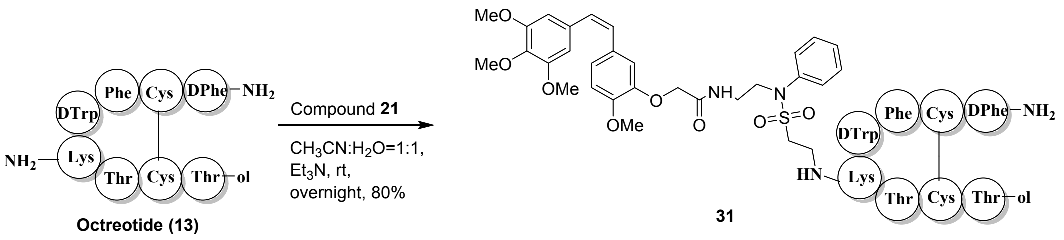


^{13}C NMR of compound **21**



General procedure for the synthesis of compounds 30 and 31





The procedure was same to the synthesis of compound **14**. The yields of compound **30** and **31** were determined by HPLC (For compound **30**: 83% yield, 94% yield based on recovered octreotide; For compound **31**: 80% yield, 95% yield based on recovered octreotide).

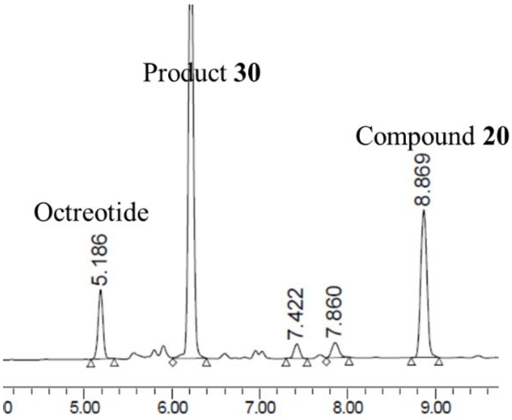


Fig. 4SA HPLC of the reaction that compound **20** conjugated with octreotide to produce **30**

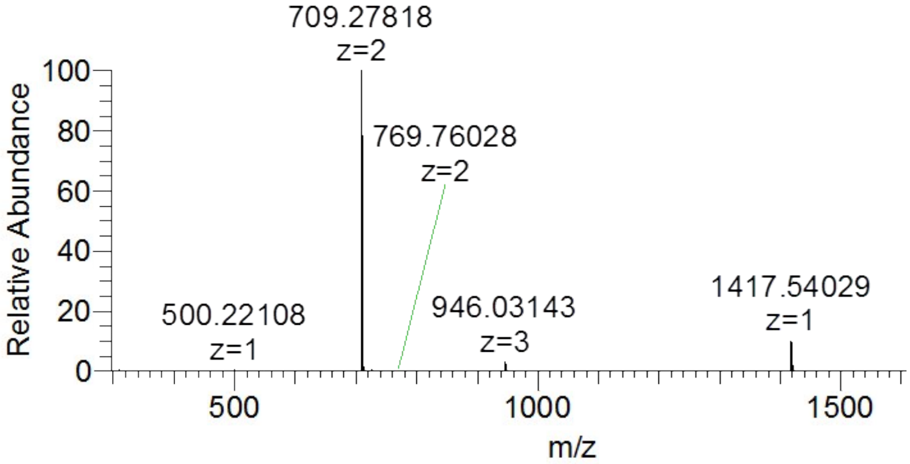


Fig. 4SB The HRESIMS of compound **30**

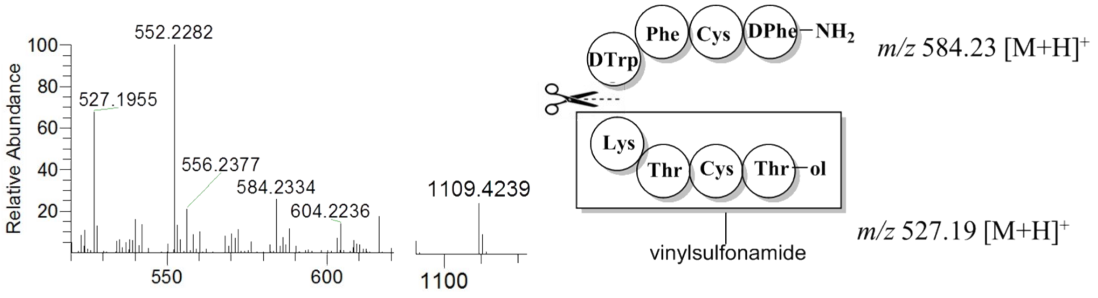
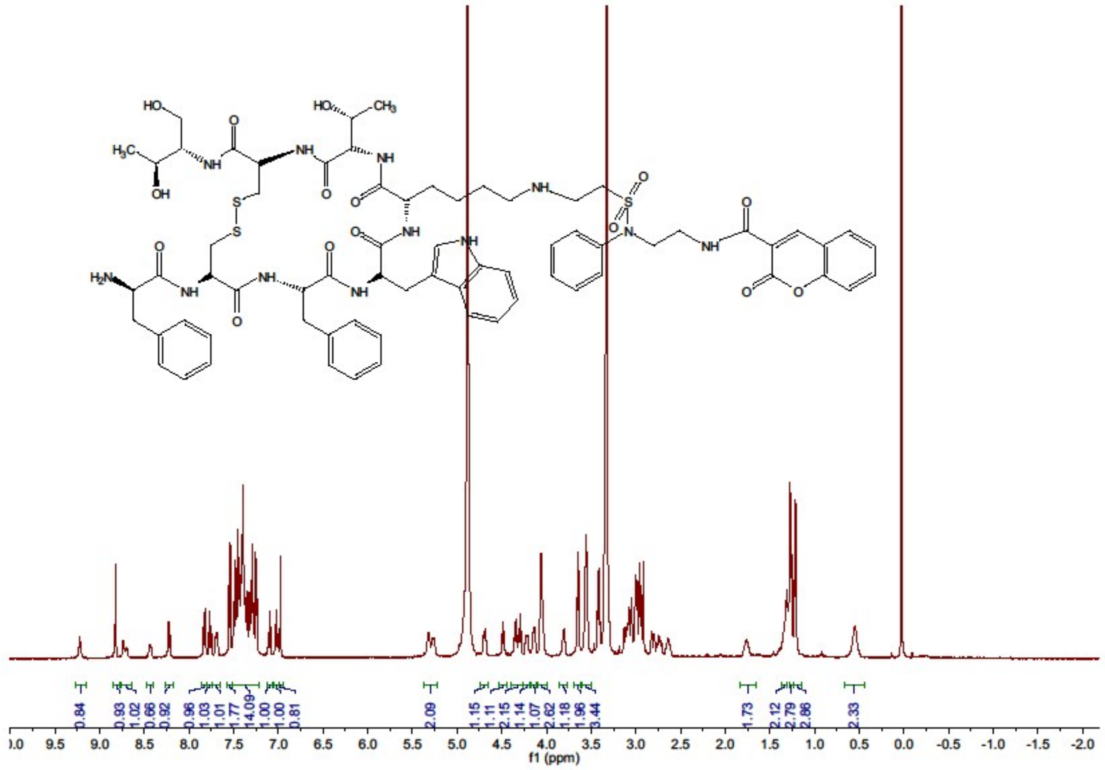


Fig. 4SC The MS^2 of compound **30**

^{1}H NMR of compound **30**



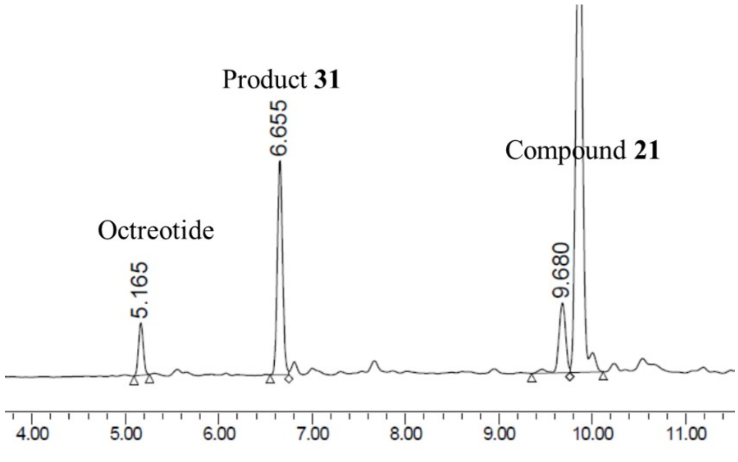


Fig. 4SD HPLC of the reaction that compound **21** conjugated with octreotide to produce **31**

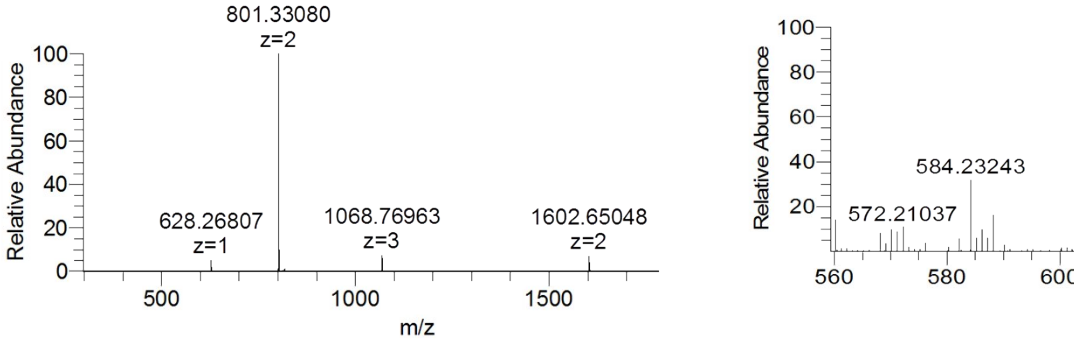
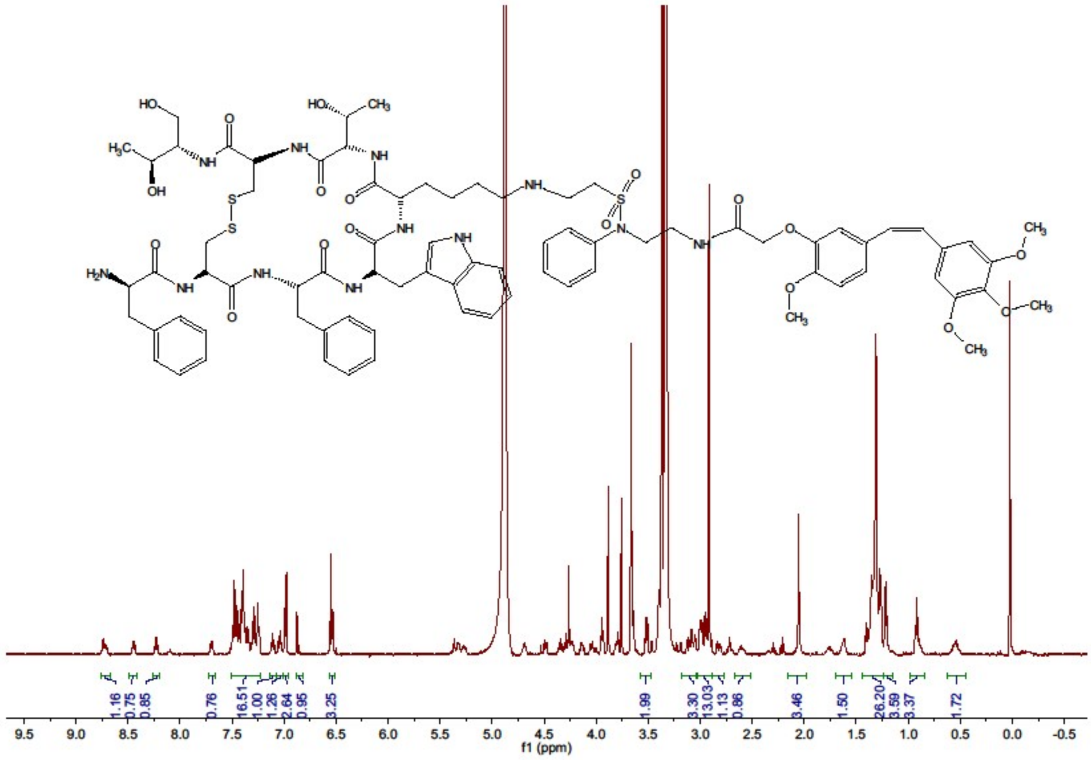


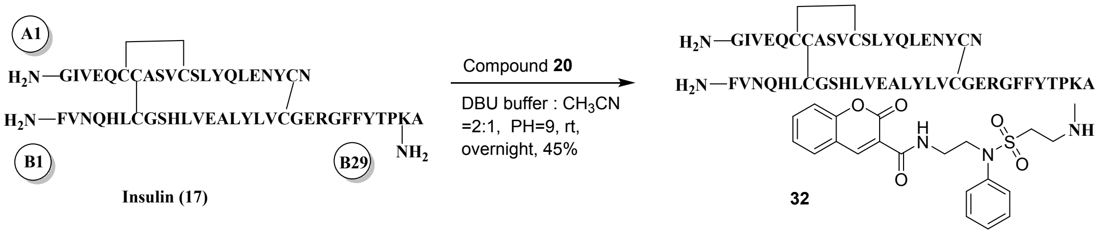
Fig. 4SE The HRESIMS and MS^2 of compound **31**

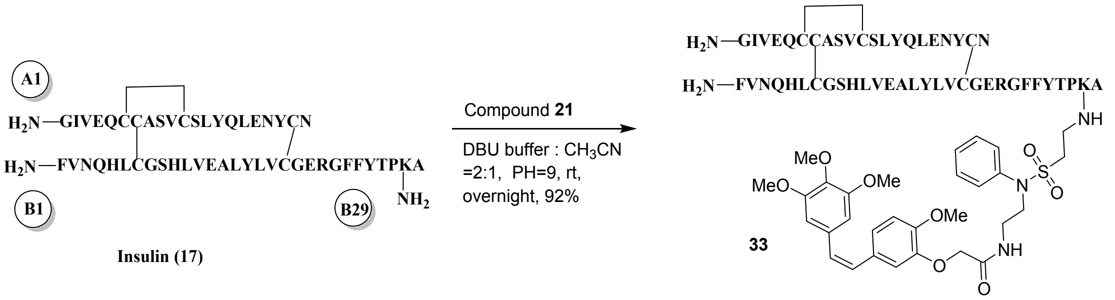
^{1}H NMR of compound **31**



General procedure for the synthesis of compounds 32 and 33

The procedure was same to the synthesis of compound **19**. The yields of compound **32** and **33** were determined by HPLC (For compound **32**: 45% yield, 79% yield based on recovered insulin; For compound **33**: 92% yield, 96% yield based on recovered insulin;).





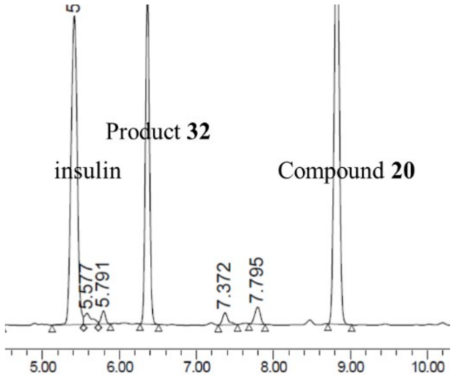
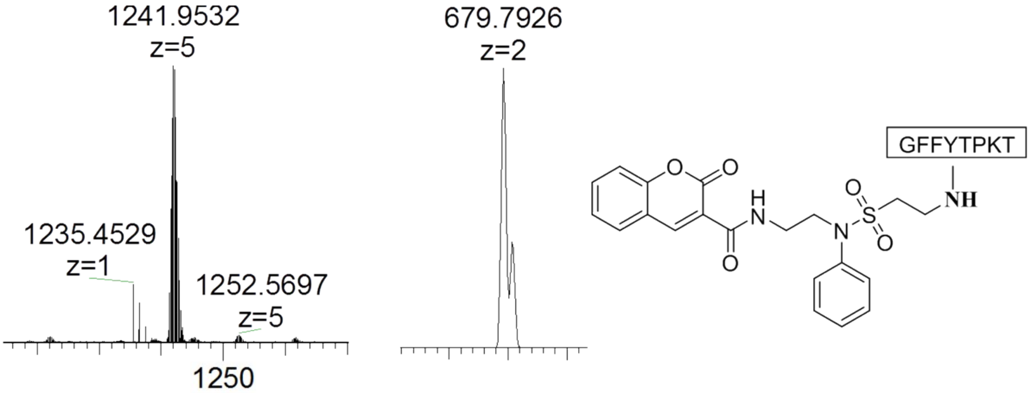
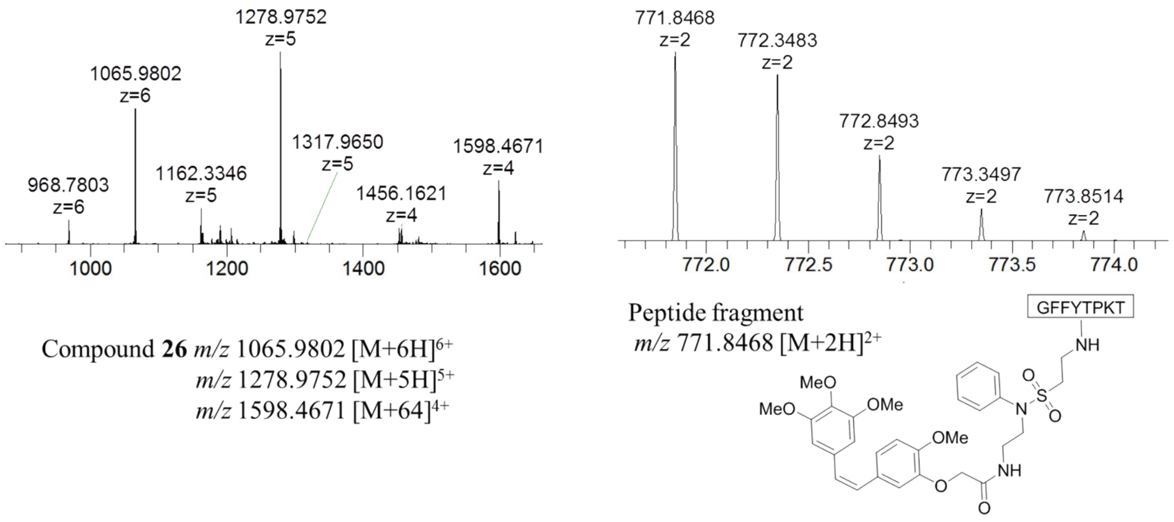


Fig. 5SA HPLC of the reaction that compound **20** conjugated with insulin to produce **32**



Compound **25** *m/z* 1241.9532 [M+5H]$^{5+}$ Peptide fragment *m/z* 679.7926 [M+2H]$^{2+}$

Fig. 5SB HRESIMS and MS^2 of compound **32**



Compound **26** *m/z* 1065.9802 $[M+6H]^{6+}$
m/z 1278.9752 $[M+5H]^{5+}$
m/z 1598.4671 $[M+64]^{4+}$

Peptide fragment
m/z 771.8468 $[M+2H]^{2+}$

Fig. 5SC The HRESIMS and MS^2 of compound **33**

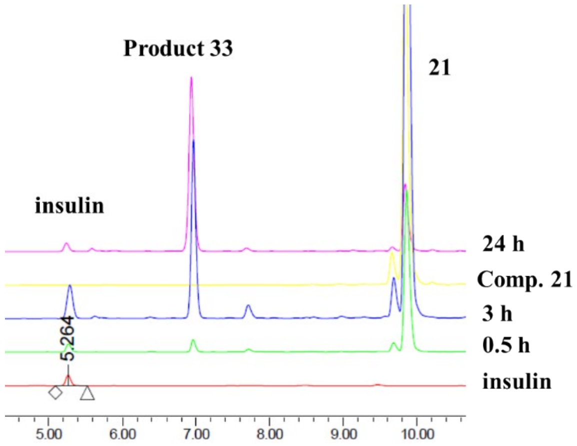


Fig. 5SD HPLC of the reaction that compound **21** conjugated with insulin to produce **33**

Table S1 Conditions tested during optimization

Entry	Compound	PH	Buffer	Yield	Selectivity
1	**1**	7.01	DABCO+H_3PO_4	\	\
2	**2**	7.01	DABCO+H_3PO_4	\	\
3	**3**	7.01	DABCO+H_3PO_4	10.2	3:1
4	**4**	7.01	DABCO+H_3PO_4	7.9	2:1
5	**5**	7.01	DABCO+H_3PO_4	16.6	>50:1
6	**6**	7.01	DABCO+H_3PO_4	13.4	>50:1
7	**7**	**7.01**	**DABCO+H_3PO_4**	**30.2**	**>50:1**
8	**7**	7.00	Et_3N+ H_3PO_4	0.7	\
9	**7**	7.01	TBD+H_3PO_4	1.9	\
10	**7**	7.09	MTBD+H_3PO_4	2.1	\
11	**7**	7.02	DBU+H_3PO_4	1.1	\
12	**7**	6.99	PBS	8.2	2:1
13	**7**	8.23	NH_4HCO_3	0.8	\
14	**7**	8.45	$NaHCO_3$	1.7	\
15	**7**	8.04	DABCO+H_3PO_4	31.4	20:1

16	**7**	9.09	DABCO+H_3PO_4	26.6	5:1
17	**7**	10.37	DABCO	20.5	4:1