Supporting Information

Development of an Anilide-Type Scaffold for the Thioester Precursor *N*-Sulfanylethylcoumarinyl Amide

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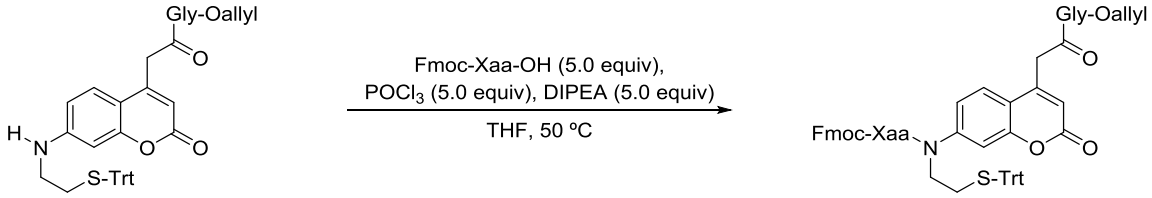
Contents

Table SI-1. Coupling of *N*-Fmoc-protected amino acids with *N*-sulfanylethylcoumarin linker **3**



3 **8**

Fmoc-Xaa-OH	reaction time (h)	product	isolated yield (%)
Gly	6	**8a**	95
Ala	18	**8b**	93
Asp(O*t*-Bu)	24	**8c**	60*
Glu(O*t*-Bu)	6	**8d**	78
Asn(Trt)	6	**8e**	87
Gln(Trt)	6	**8f**	83
Ser(*t*-Bu)	6	**8g**	87
Thr(*t*-Bu)	6	**8h**	84
Cys(Trt)	6	**8i**	79
Pro	24	**8j**	48**
Val	6	**8k**	86
Met	6	**8l**	83
Leu	6	**8m**	91
Ile	6	**8n**	85
Tyr(*t*-Bu)	6	**8o**	73
Phe	6	**8p**	81
His(MBom[#])	6	**8q**	70
Lys(Boc)	6	**8r**	70
Arg(Pbf)	12	**8s**	74
Trp	6	**8t**	87

[#]4-methoxybenzyloxymethyl.[S1]

*Recovery of substrate: 24%. **Recovery of substrate: 43%.

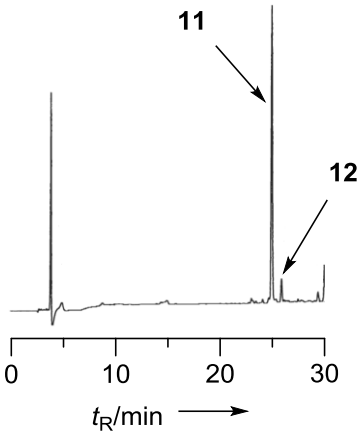


Figure SI-1. HPLC chart of crude model SECmide peptide. Analytical HPLC conditions: Cosmosil $5C_{18}$ AR-II column (4.6 × 250 mm) with a linear gradient of 0.1% (v/v) TFA–MeCN in 0.1% (v/v) TFA aq. (1:99–30:70 over 30 min) at a flow rate 1.0 mL/min, detection at 220 nm.

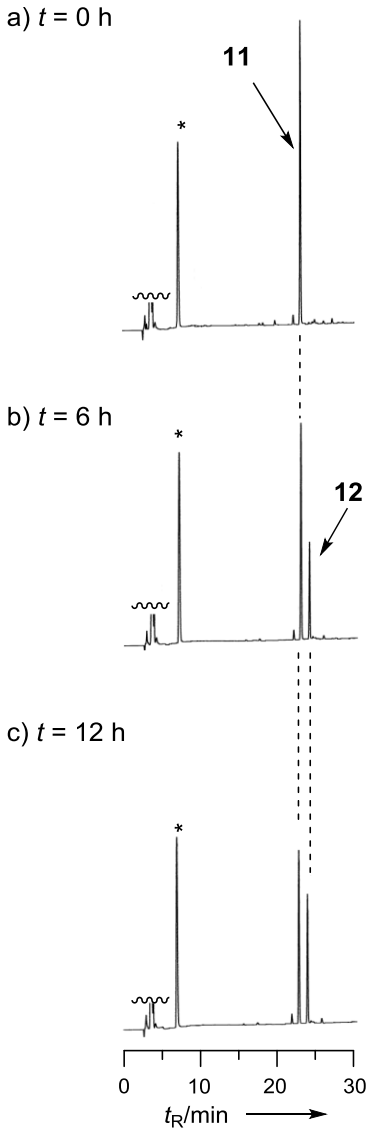


Figure SI-2. HPLC monitoring of N–S acyl transfer of SECmide peptide in 0.1% (v/v) TFA–MeCN: 0.1% (v/v) TFA aq. (1:4, (v/v)). Analytical HPLC conditions: Cosmosil $5C_{18}$ AR-II column (4.6 × 250 mm) with a linear gradient of 0.1% (v/v) TFA–MeCN in 0.1% (v/v) TFA aq. (1:99–30:70 over 30 min) at a flow rate 1.0 mL/min, detection at 220 nm. *Internal standard (benzenesulfonic acid).

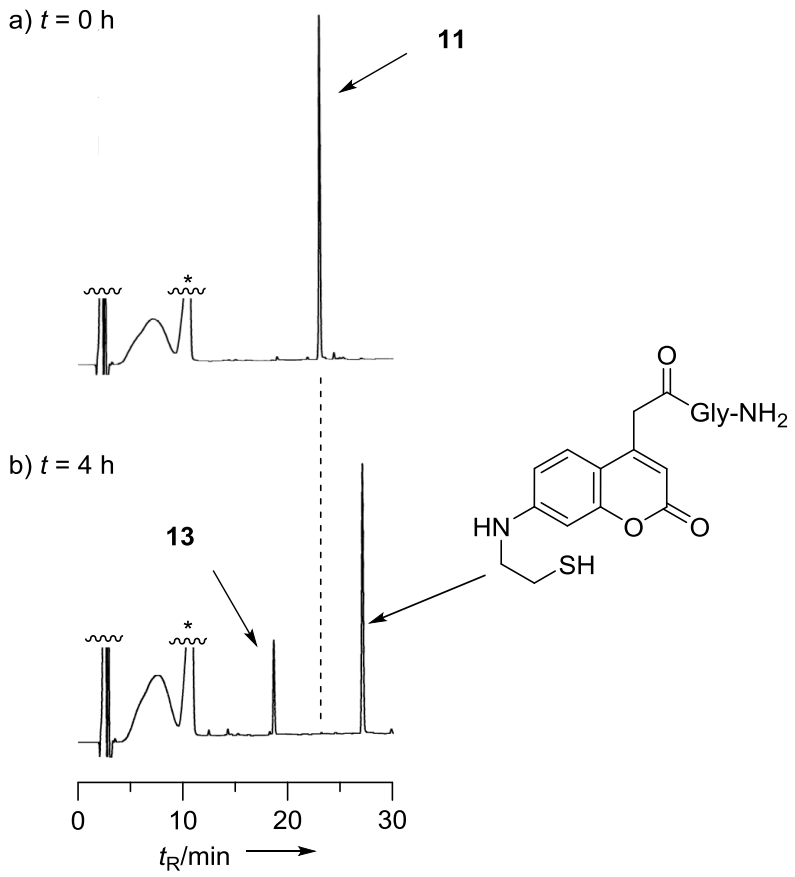


Figure SI-3. HPLC monitoring of preparation of peptide thioester **13**. Analytical HPLC conditions: Cosmosil $5C_{18}$ AR-II column (4.6 × 250 mm) with a linear gradient of 0.1% (v/v) TFA–MeCN in 0.1% (v/v) TFA aq. (1:99–30:70 over 30 min) at a flow rate 1.0 mL/min, detection at 220 nm.
*MPA.

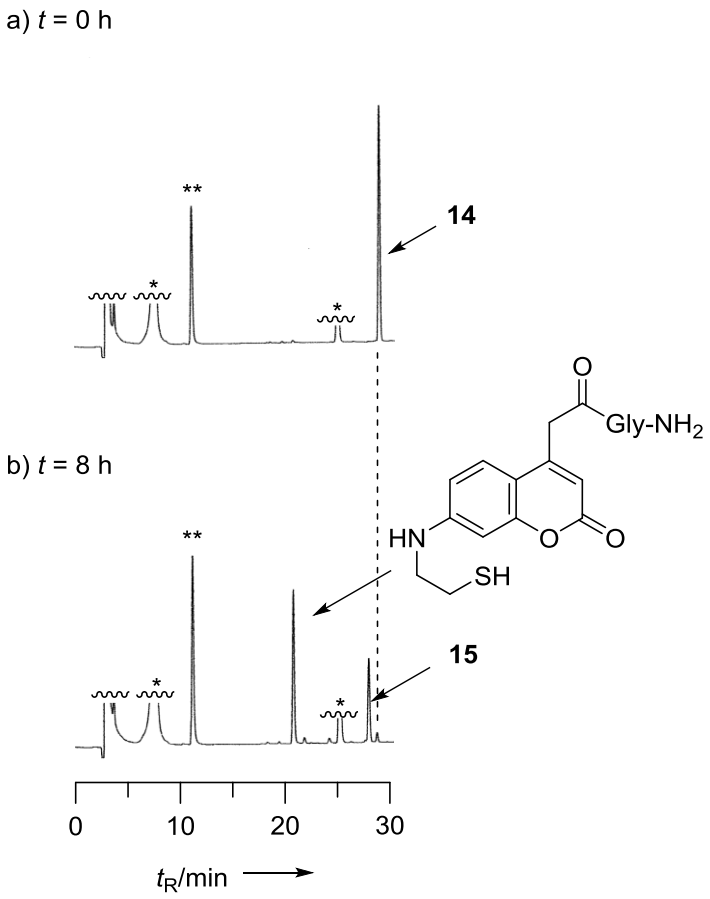


Figure SI-4. HPLC monitoring of preparation of peptide thioester **15**. Analytical HPLC conditions: Cosmosil $5C_{18}$ AR-II column (4.6 × 250 mm) with a linear gradient of 0.1% (v/v) TFA–MeCN in 0.1% (v/v) TFA aq. (10:90–30:70 over 30 min) at a flow rate 1.0 mL/min, detection at 220 nm.
*Nonpeptidic compounds. **Internal standard (benzenesulfonic acid).

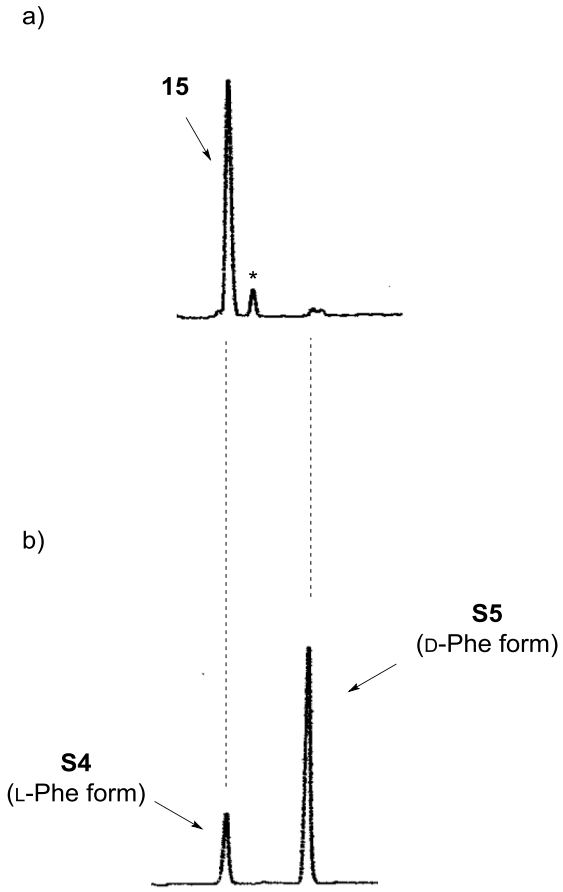
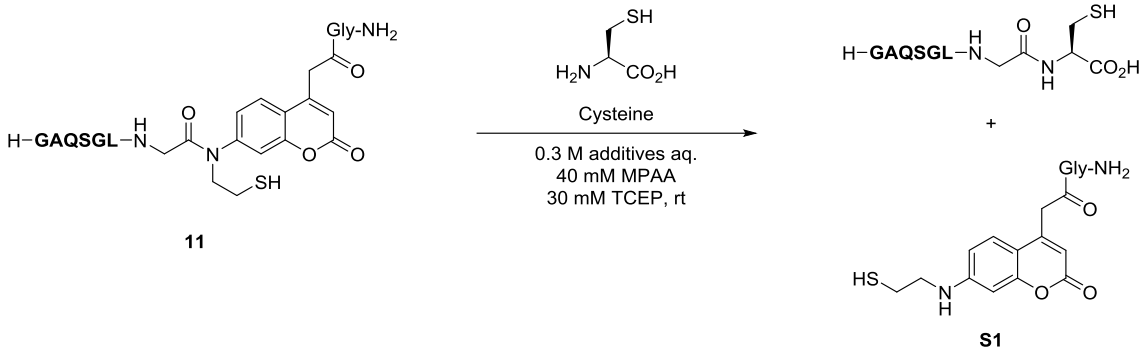


Figure SI-5. Verification of epimerization of C-terminal chiral amino acids during N–S acyl transfer mediated thioesterification. a) Peptide thioester **15** obtained via N–S acyl transfer of SECmide peptide **14**. b) Reference peptide thioesters **S4** and **S5** prepared using Boc SPPS. Analytical HPLC conditions: Cosmosil 5C_{18} AR-II column (4.6 × 250 mm) with a linear gradient of 0.1% (v/v) TFA–MeCN in 0.1% (v/v) TFA aq. (10:90–30:70 over 30 min) at a flow rate 1.0 mL/min, detection at 220 nm. Only a critical retention time region of the HPLC charts was enlarged. *Substrate **14**.



entry	additive	pH	half-life of **11** (h)
1	4-mercaptobenzyl phosphonic acid	6.0	1.38
2	〃	7.0	1.42
3	diphosphoric acid	6.0	0.80
4	〃	7.0	1.01
5	sodium phosphate	6.0	1.03
6	〃	7.0	0.94
7	sodium phosphite	6.0	1.30
8	〃	7.0	2.38
9	methylphosphonate	6.0	1.63
10	〃	7.0	1.62
11	sodium hypophosphite	6.0	11.42
12	〃	7.0	*
13	potassium carbonate	6.0	5.18
14	〃	7.0	7.36
15	imidazole	6.0	4.53
16	〃	7.0	2.08
17	citric acid	6.0	10.82
18	〃	7.0	*
19	ethylenediaminetetraacetic acid	6.0	7.91
20	〃	7.0	*
21	glycine	6.0	26.67
22	〃	7.0	*

entry	additive	pH	half-life of **11** (h)
23	none	6.0	39.95
24	〃	7.0	*
25	ammonium sulfate	6.0	19.58
26	〃	7.0	*
27	boronic acid	6.0	*
28	〃	7.0	*
29	mannose	6.0	*
30	〃	7.0	*
31	sodium nitrate	6.0	*
32	〃	7.0	*
33	hexamethylphosphoric triamide	6.0	*
34	〃	7.0	*
35	sodium sulfate	6.0	*
36	〃	7.0	*
37	tartaric acid	6.0	*
38	〃	7.0	*
39	oxalic acid	6.0	*
40	〃	7.0	*
41	tricine	6.0	*
42	〃	7.0	*

*: Half-life of **11** was over 50 h

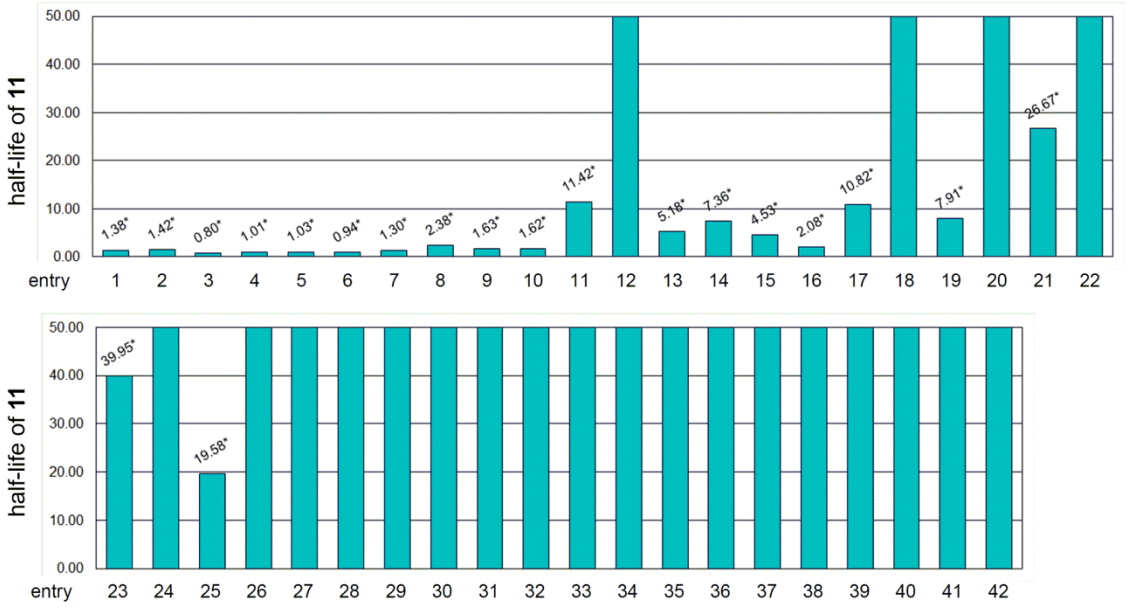


Figure SI-6. Exploration of N–S acyl transfer promoters of SECmide peptide **11**.

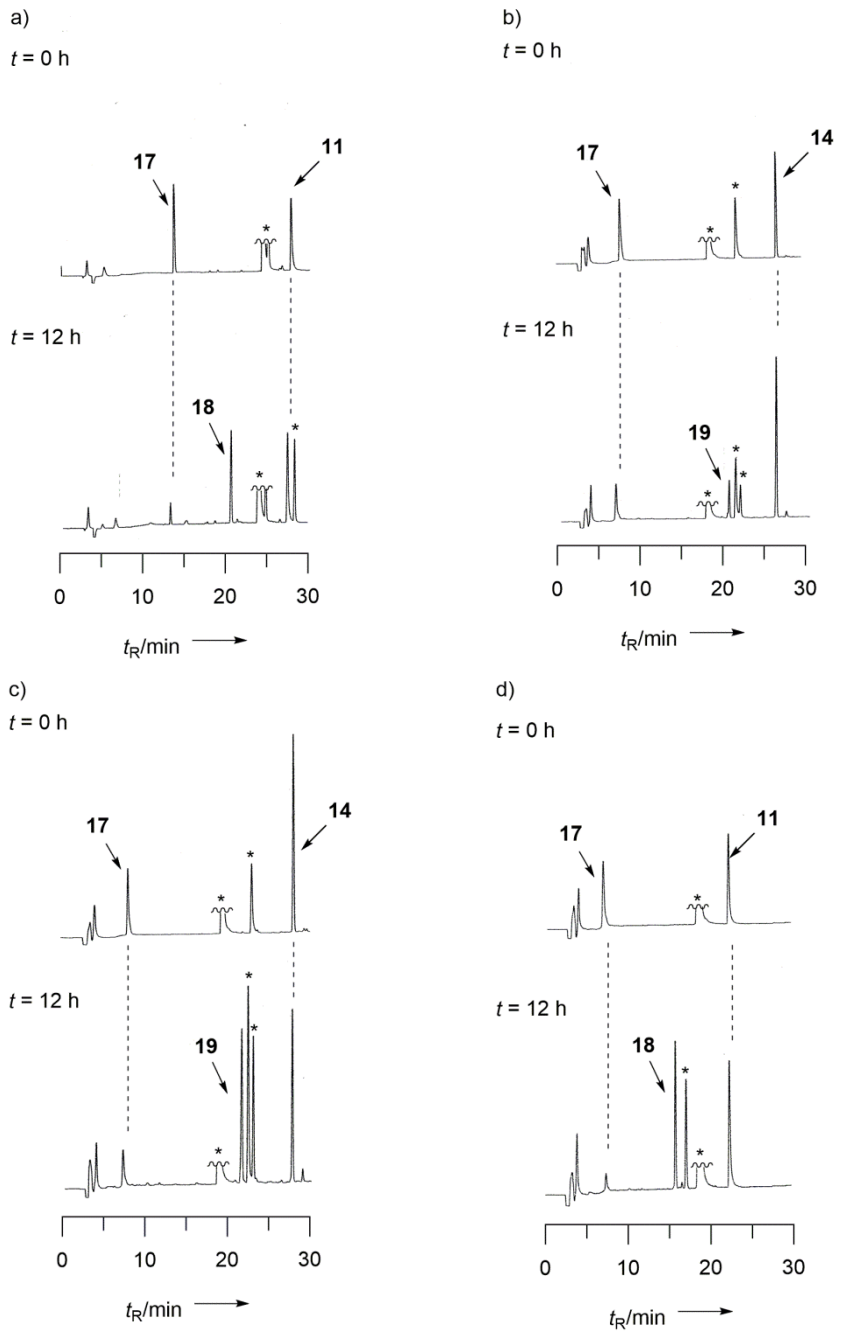


Figure SI-7-1. HPLC monitoring of NCL of SECmide or SEAlide peptide with N-terminal cysteinyl peptide. a) NCL condition: Table 2 entry 2, b) NCL condition: Table 2 entry 5, c) NCL condition: Table 2 entry 8, d) NCL condition: Table 2 entry 11. Analytical HPLC condition for a): Cosmosil 5C_{18}-AR-II analytical column (4.6 × 250 mm) with a linear gradient of solvent B in solvent A, 1% to 30% over 30 min. Analytical HPLC conditions for b), c) or d): Cosmosil 5C_{18}-AR-II analytical column (4.6 × 250 mm) with a linear gradient of solvent B in solvent A, 5% to 35% over 30 min. *Nonpeptidic compounds.

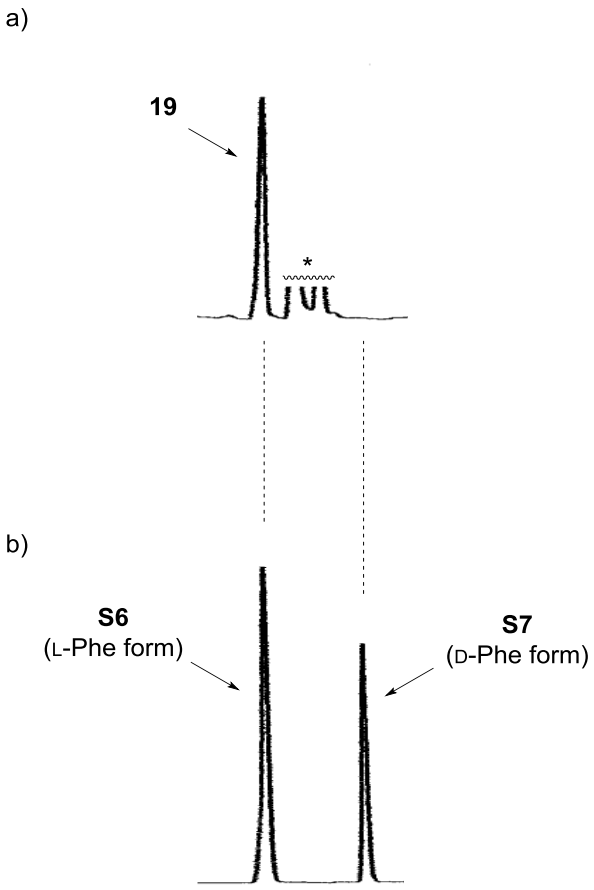


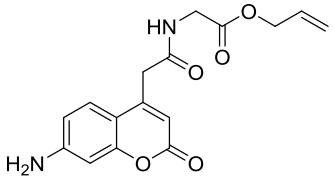
Figure SI-7-2. Verification of Epimerization of C-terminal chiral amino acids during NCL. a) Ligation product **19** obtained NCL between SECmide peptide **14** and N-terminal cysteinyl peptide **17**. b) Reference peptide **S6** or **S7** prepared NCL between peptide thioester **S2** or **S3** and N-terminal cysteinyl peptide **17**. Analytical HPLC conditions: Cosmosil 5C_{18}-AR-II analytical column (4.6 × 250 mm) with a linear gradient of solvent B in solvent A, 5% to 35% over 30 min. Only a critical retention time region of the HPLC charts was enlarged. *Nonpeptidic compounds.

General Methods

Reactions except for peptide synthesis were carried out under a positive pressure of argon. Mass spectra were recorded on a Waters MICROMASS® LCT PREMIER™ (ESI-TOF). For HPLC separation, a Cosmosil 5C_{18}-AR-II analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL/min), a Cosmosil 5C_{18}-AR-II semi-preparative column (Nacalai Tesque, 10 × 250 mm, flow rate 3.0 mL/min), or a Cosmosil 5C_{18}-AR-II preparative column (Nacalai Tesque, 20 × 250 mm, flow rate 10 mL/min) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% TFA aqueous solution (v/v, solvent A), 0.1% TFA in MeCN (v/v, solvent B), 10 mM aqueous ammonium acetate (pH 6.7) (solvent C) and MeCN (solvent D) was used for HPLC elution. For column chromatography, silica gel (KANTO KAGAKU N-60) was employed. Thin layer chromatography was performed on precoated plates (0.25 nm, silica gel Merck KGaA 60 F_{245}). NMR spectra were recorded using Bruker AV400N at 400 MHz frequency for ^{1}H, and JEOL JNM-AL300 at 75 MHz frequency for ^{13}C. The fluorescence intensity (FI) was measured on a Perkin Elmer Enspire® Multimode Plate Reader with an excitation wavelength of 373 nm and an emission wavelength of 465 nm (microplate: FlUOTRAC™ 600 (Greiner Bio-One)).

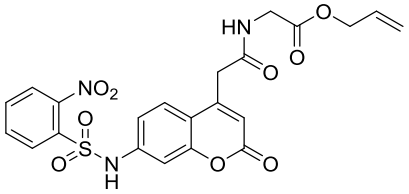
Preparation of *N*-sulfanylethylcoumarin linker **3**

[2-(7-aminocoumarin-4-acetylamino)]-acetic acid allyl ester (5)



To a solution of H-Gly-OAllyl·HCl (10.3 g, 68.2 mmol) in DMF (150 mL) was slowly added DIPEA (24.3 mL, 141 mmol) at an ice-salt bath temperature. The resulting solution was stirred for 15 min. Then, **4** (9.96 g, 45.4 mmol), DMAP (5.55 g, 45.4 mmol) and EDC·HCl (13.1 g, 68.2 mmol) were added and the mixture was warmed up to room temperature and stirred for another 16 h. After removal of the solvent in vacuo, water was added to the residual mixture. The yellow precipitate was collected by filtration and washed with water to afford **5** (12.0 g, 37.9 mmol, 83%) as a yellow powder: ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 3.63 (2H, s), 3.91 (2H, d, J = 5.9 Hz), 4.57 (2H, ddd, J = 5.2, 1.5 and 1.5 Hz), 5.20 (1H, ddt, J = 10.6, 1.5 and 1.5 Hz), 5.30 (1H, ddt, J = 17.2, 1.5 and 1.5 Hz), 5.88 (1H, ddt, J = 17.2, 10.6 and 5.2 Hz), 5.99 (1H, s), 6.13 (2H, s), 6.41 (1H, d, J = 2.2 Hz), 6.54 (1H, dd, J = 8.7 and 2.2 Hz), 7.42 (1H, d, J = 8.7 Hz), 8.67 (1H, t, J = 5.9 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 38.4, 40.9, 64.8, 98.5, 108.2, 108.7, 111.1, 117.9, 126.3, 132.3, 151.1, 153.0, 155.6, 160.7, 168.6, 169.4; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{16}N_2NaO_5$ ([M + Na]$^+$) 339.0957, found 339.0935.

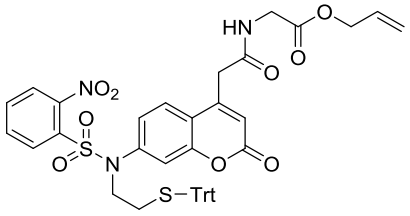
{2-[7-*N*-(2-nitrobenzenesulfonylamino)coumarin-4-acetylamino]}-acetic acid allyl ester (6)[S2]



To a solution of compound **5** (4.00 g, 12.6 mmol) in pyridine (63 mL) was added 2-Nitrobenzenesulfonyl chloride (5.61 g, 25.3 mmol). The reaction mixture was stirred at room temperature for 5 h. After, removal of the solvent *in vacuo* followed by addition of 5% (w/v) $KHSO_4$ aq, the resulting mixture was extracted with EtOAc. The organic phase was washed with 5% (w/v) $KHSO_4$ aq. followed by brine, filtered and concentrated in vacuo. The residue was purified by column chromatography ($CHCl_3$/MeOH = 100/0 to 100/7 then 0/100 (v/v)) to yield **6** (4.84g, 9.65 mmol, 77%) as a pale orange powder: ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 3.72 (2H, s), 3.89 (2H, d, J = 5.9 Hz), 4.54 (2H, ddd, J = 5.3, 1.5 and 1.5 Hz), 5.17 (1H, ddt, J = 10.6, 1.5 and 1.5 Hz), 5.27 (1H, ddt, J = 17.2, 1.5 and 1.5 Hz), 5.85 (1H, ddt, J = 17.2, 10.6 and 5.3 Hz), 6.36 (1H, s), 7.05-7.12 (2H, m), 7.68

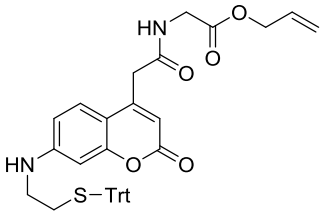
(1H, d, J = 8.4 Hz), 7.82-7.92 (2H, m), 8.01-8.04 (1H, m), 8.07-8.11 (1H, m), 8.68 (1H, t, J = 5.9 Hz), 11.44 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 38.2, 40.9, 64.8, 105.6, 114.4, 114.7, 115.1, 117.8, 124.9, 126.8, 129.9, 130.9, 132.2, 132.9, 135.1, 140.3, 147.9, 150.3, 153.7, 159.5, 168.2, 169.3; HRMS (ESI-TOF) m/z calcd for $C_{22}H_{19}KN_3O_9S$ ([M + K]$^+$) 540.0479, found 540.0473.

(2-{7-[*N*-(2-nitrobenzenesulfonyl)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (7)



To a stirred suspension of compound **6** (663 mg, 1.32 mmol), triphenylmethyl-sulfanylethyl alcohol (846 mg, 2.64 mmol) and Ph_3P (692 mg, 2.64 mmol) in THF (25 mL) was added 40% (v/v) DEAD/toluene (1.20 mL, 2.64 mmol) at 0 °C. After being stirred at room temperature for 17 h, the reaction mixture was diluted with EtOAc, sat. NH_4Cl aq. The solution was extracted with EtOAc, washed with sat. NH_4Cl aq., brine, dried over $NaSO_4$, filtered and concentrated. The residue was purified by column chromatography (hexane/EtOAc = 1/2 to 1/4 (v/v)) then (CH_2Cl_2/ EtOAc = 5/2 (v/v)) to yield **7** (614 mg, 0.764 mmol, 58%) as orange amorphous solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 2.18 (2H, t, J = 6.9 Hz), 3.68 (2H, t, J = 6.9 Hz), 3.84 (2H, s), 3.96 (2H, d, J = 5.9 Hz), 4.54 (2H, ddd, J = 5.3, 1.5 and 1.5 Hz), 5.17 (1H, ddt, J = 10.6, 1.5 and 1.5 Hz), 5.28 (1H, ddt, J = 17.2, 1.5 and 1.5 Hz), 5.86 (1H, ddt, J = 17.2, 10.6 and 5.3 Hz), 6.56 (1H, s), 7.09 (1H, dd, J = 8.5 and 2.2 Hz), 7.11-7.26 (16H, m), 7.70 (1H, dd, J = 8.0 and 1.3 Hz), 7.73-7.81 (2H, m), 7.88 (1H, td, J = 8.0 and 1.3 Hz), 9.96 (1H, dd, J = 8.0 and 1.3 Hz), 8.81 (1H, t, J = 5.9 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 29.8, 38.2, 40.9, 49.5, 64.8, 66.2, 116.0, 116.4, 117.8, 118.6, 124.3, 124.5, 126.0, 126.6, 127.9, 128.9, 129.4, 130.4, 132.2, 132.3, 135.2, 139.6, 144.0, 147.6, 150.1, 153.0, 159.2, 168.1, 169.3; HRMS (ESI-TOF) m/z calcd for $C_{43}H_{37}KN_3O_9S_2$ ([M + K]$^+$) 842.1608, found 842.1626.

(2-{7-[*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (3)



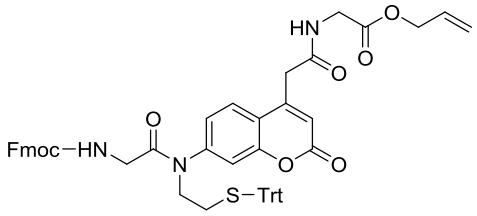
To a solution of compound **7** (4.90 g, 6.10 mmol) in MeCN (50 mL) were added K_2CO_3 (1.68 g, 12.2 mmol) and thiophenol (3.11 ml, 30.5 mmol) at 0 °C. The resulting solution was stirred for 15 min. Then the mixture was warmed up to room temperature and stirred for another 12 h. After evaporation, hexane was added to the residual mixture. The yellow precipitate was collected by filtration and washed with hexanes. The residue was dissolved in $CHCl_3$ and THF, and concentrated in vacuo. The residue was purified by column chromatography (hexane/EtOAc = 1/1 to 1/2 then 0/100 (v/v), and THF) to yield **3** (3.15 g, 5.09 mmol, 84%) as a pale yellow powder: ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 2.38 (2H, t, *J* = 6.7 Hz), 3.04 (2H, dt, *J* = 6.7 and 6.7 Hz), 3.65 (2H, s), 3.92 (2H, d, *J* = 5.9 Hz), 4.57 (2H, ddd, *J* = 5.3, 1.5 and 1.5 Hz), 5.20 (1H, ddt, *J* = 10.6, 1.5 and 1.5 Hz), 5.30 (1H, ddt, *J* = 17.2, 1.5 and 1.5 Hz), 5.88 (1H, ddt, *J* = 17.2, 10.6 and 5.3 Hz), 6.02 (1H, s), 6.24 (1H, d, *J* = 2.1 Hz), 6.43 (1H, dd, *J* = 8.8 and 2.1 Hz), 6.74 (1H, t, *J* = 6.7 Hz), 7.22-7.36 (15H, m), 7.43 (1H, d, *J* = 8.8 Hz), 8.68 (1H, t, *J* = 5.9 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) = 30.7, 38.4, 40.9, 41.2, 64.8, 66.3, 96.5, 108.4, 108.9, 110.0, 117.9, 126.1, 126.8, 128.0, 129.1, 132.3, 144.4, 151.0, 151.6, 155.7, 160.6, 168.5, 169.4; HRMS (ESI-TOF) *m*/*z* calcd for $C_{37}H_{34}KN_2O_5S$ ($[M + K]^+$) 657.1826, found 657.1829.

Preparation of Fmoc-Xaa-*N*-sulfanylethylcoumarin **8**

Typical procedure of coupling of Fmoc-Xaa-OH with 3

To a stirred solution of compound **3** (300 mg, 0.480 mmol) in THF (15 mL) were added Fmoc-Gly-OH (721 mg, 2.42 mmol), DIPEA (422 μL, 2.42 mmol) and $POCl_3$ (226 μL, 2.42 mmol) at 0 °C. After being stirred at 50 °C for 6 h, the reaction was quenched by the addition of sat. $NaHCO_3$ aq. After extraction with EtOAc, the obtained organic layer was washed with sat. $NaHCO_3$ aq., water, sat. NH_4Cl aq., and brine. The obtained solution was dried over Na_2SO_4 and evaporated in vacuo. The product was purified by column chromatography (CH_2Cl_2/ EtOAc = 5/1 to 5/2 (v/v)) to yield **8a** (414 mg, 0.461 mmol, 95%) as white amorphous solid.

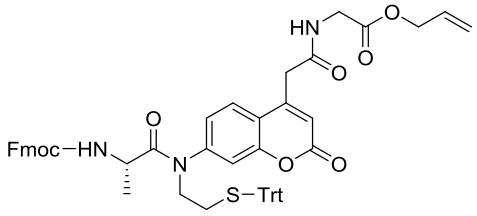
(2-{7-[*N*-(Fmoc-Gly)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (8a)



White amorphous solid; yield: 95% (414 mg); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.41 (2H, t, *J* = 7.3 Hz), 3.51 (2H, br t, *J* = 7.3 Hz), 3.61 (2H, br s), 3.75 (2H, s), 4.08 (2H, d, *J* = 5.2 Hz), 4.18 (1H, t, *J* =

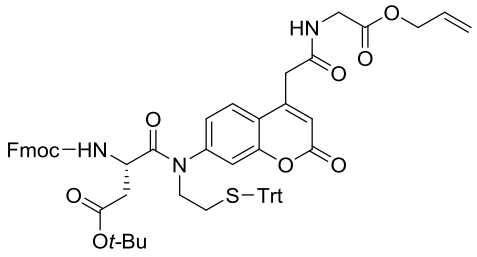
7.1 Hz), 4.31 (2H, d, *J* = 7.1 Hz), 4.63 (2H, d, *J* = 5.9 Hz), 5.25 (1H, ddt, *J* = 10.6, 1.2 and 1.1 Hz), 5.31 (1H, br ddt, *J* = 17.2, 1.2 and 1.2 Hz), 5.67 (1H, br t, *J* = 4.3 Hz), 5.88 (1H, ddt, *J* = 17.1, 10.6 and 5.9 Hz), 6.44-6.54 (2H, m), 6.89-7.00 (2H, m), 7.11-7.41 (19H, m), 7.57 (2H, d, *J* = 7.4 Hz), 7.67 (1H, d, *J* = 8.4 Hz), 7.75 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 29.6, 40.0, 41.7, 43.7, 47.2, 49.0, 66.4, 67.3, 116.9, 117.9, 119.2, 119.4, 120.1, 124.3, 125.2, 126.9, 127.2, 127.8, 128.0, 129.6, 131.4, 141.4, 143.2, 143.9, 144.5, 148.3, 154.4, 156.2, 159.6, 167.4, 167.8, 169.3; HRMS (ESI-TOF) *m*/*z* calcd for $C_{54}H_{47}KN_3O_8S$ ([M + K]$^+$) 936.2721, found 936.2723.

(2-{7-[*N*-(Fmoc-L-Ala)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (8b)



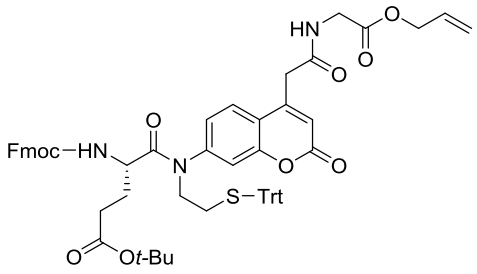
White amorphous solid; yield: 93% (206 mg); $[\alpha]^{26}_D$ 89.6 (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.12 (3H, br d, *J* = 6.0 Hz), 2.25-2.38 (1H, m), 2.45-2.59 (1H, m), 3.32-3.43 (1H, m), 3.47-3.59 (1H, m), 3.72 (1H, d, *J* = 15.7 Hz), 3.78 (1H, d, *J* = 15.7 Hz), 3.74 (2H, m), 4.09 (2H, d, *J* = 5.3 Hz), 4.18 (2H, m), 4.31 (2H, d, *J* = 7.1 Hz), 4.63 (2H, d, *J* = 6.0 Hz), 5.26 (1H, ddt, *J* = 10.7, 1.2 and 1.2 Hz), 5.32 (1H, ddt, *J* = 17.0, 1.2 and 1.2 Hz), 5.49 (1H, br d, *J* = 7.8 Hz), 5.88 (1H, ddt, *J* = 17.0, 10.7 and 6.0 Hz), 6.35 (1H, br t, *J* = 5.3 Hz), 6.48 (1H, s), 6.97-7.05 (2H, m), 7.08-7.42 (19H, m), 7.57 (1H, d, *J* = 7.0 Hz), 7.59 (1H, d, *J* = 7.0 Hz), 7.65 (1H, d, *J* = 8.4 Hz), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 19.1, 29.4, 39.9, 41.7, 47.2, 47.8, 49.4, 66.4, 67.1, 67.2, 116.9, 117.7, 118.9, 119.4, 120.1, 124.6, 125.3, 126.5, 126.8, 127.2, 127.8, 128.0, 129.6, 131.3, 141.4, 141.4, 143.9, 144.0, 144.1, 144.6, 148.3, 154.3, 155.6, 159.7, 167.4, 169.3, 172.6; HRMS (ESI-TOF) *m*/*z* calcd for $C_{55}H_{49}N_3NaO_8S$ ([M + Na]$^+$) 934.3138, found 934.3163.

[2-(7-{*N*-[Fmoc-L-Asp(O*t*-Bu)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8c)



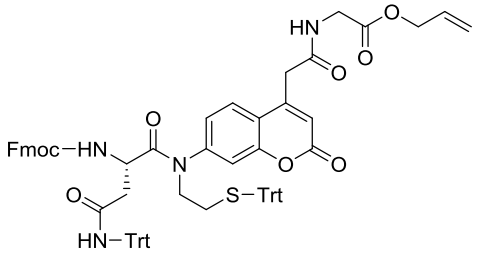
White amorphous solid; yield: 60% (146 mg); $[\alpha]^{22}_{D}$ 46.5 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.38 (9H, s), 2.26-2.60 (4H, m), 3.45 (2H, br t, *J* = 7.0 Hz), 3.65 (1H, d, *J* = 16.8 Hz), 3.69 (1H, d, *J* = 16.8 Hz), 4.06 (2H, d, *J* = 5.2 Hz), 4.13 (1H, t, *J* = 6.7 Hz), 4.17-4.30 (2H, br m), 4.47 (1H, br s), 4.62 (2H, d, *J* = 5.9 Hz), 5.26 (1H, ddt, *J* = 10.4, 1.3 and 1.3 Hz), 5.32 (1H, ddt, *J* = 17.1, 1.3 and 1.3 Hz), 5.53 (1H, br d, *J* = 8.4 Hz), 5.88 (1H, ddt, *J* = 17.1, 10.4 and 5.9 Hz), 6.24 (1H, br t, *J* = 5.2 Hz), 6.44 (1H, s), 6.95-7.06 (1H, m), 7.00 (1H, S), 7.08-7.36 (17H, m), 7.39 (1H, t, *J* = 7.2 Hz), 7.40 (1H, t, *J* = 7.5 Hz), 7.51-7.61 (1H, m), 7.55 (2H, d, *J* = 8.0 Hz), 7.76 (2H, d, *J* = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 28.1, 29.3, 38.4, 39.9, 41.7, 47.2, 49.1, 49.7, 66.4, 67.2, 81.6, 116.9, 117.6, 118.7, 119.4, 120.1, 124.6, 125.2, 126.3, 126.8, 127.2, 127.3, 127.9, 128.0, 129.7, 131.4, 141.4, 143.8, 143.9, 144.1, 144.6, 148.2, 154.2, 155.3, 159.7, 167.4, 169.3, 169.3, 170.1; HRMS (ESI-TOF) *m*/*z* calcd for $C_{60}H_{57}N_3NaO_{10}S$ ($[M + Na]^+$) 1034.3662, found 1034.3634.

[2-(7-{*N*-[Fmoc-L-Glu(O*t*-Bu)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8d)



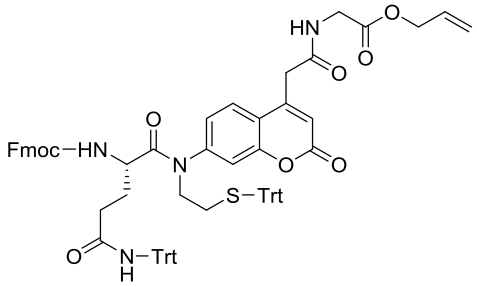
White amorphous solid; yield: 78% (194 mg); $[\alpha]^{22}_{D}$ 89.4 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.36 (9H, s), 1.67-1.80 (2H, br m), 2.06-2.17 (2H, br m), 2.25-2.36 (1H, br m), 2.45-2.59 (1H, br m), 3.29-3.42 (1H, br m), 3.50-3.61 (1H, m), 3.72 (1H, d, *J* = 15.8 Hz), 3.77 (1H, d, *J* = 15.8 Hz), 4.09 (2H, d, *J* = 5.2 Hz), 4.13-4.22 (2H, m), 4.31 (2H, d, *J* = 7.1 Hz), 4.64 (2H, d, *J* = 5.9 Hz), 5.26 (1H, ddt, *J* = 10.6, 1.3 and 1.3 Hz), 5.32 (1H, ddt, *J* = 17.0, 1.3 and 1.3 Hz), 5.55 (1H, br d, *J* = 8.3 Hz), 5.88 (1H, ddt, *J* = 17.0, 10.6 and 5.9 Hz), 6.30 (1H, br t, *J* = 5.2 Hz), 6.48 (1H, s), 6.99-7.08 (2H, m), 7.09-7.36 (17H, m), 7.39 (2H, t, *J* = 7.5 Hz), 7.57 (1H, d, *J* = 6.6 Hz), 7.59 (1H, d, *J* = 6.6 Hz), 7.63 (1H, d, *J* = 8.2 Hz), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 27.6, 28.2, 29.3, 30.8, 39.9, 41.8, 47.3, 49.4, 51.3, 66.4, 67.1, 67.2, 80.8, 117.1, 117.7, 118.9, 119.4, 120.1, 124.7, 125.3, 126.4, 126.8, 127.2, 127.8, 128.0, 129.6, 131.4, 141.4, 143.9, 144.0, 144.6, 148.2, 154.3, 155.9, 159.7, 167.3, 169.3, 171.2, 172.0; HRMS (ESI-TOF) *m*/*z* calcd for $C_{61}H_{59}N_3NaO_{10}S$ ($[M + Na]^+$) 1048.3819, found 1048.3817.

[2-(7-{*N*-[Fmoc-L-Asn(Trt)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8e)



White amorphous solid; yield: 87% (387 mg); $[\alpha]^{26}_{D}$ 5.5 (*c* 1.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.37 (2H, br t, *J* = 7.1 Hz), 2.44-2.66 (2H, br m), 3.26-3.56 (4H, m), 3.92 (2H, d, *J* = 4.9 Hz), 3.97-4.06 (1H, br m), 4.06-4.21 (2H, br m), 4.49 (1H, br s), 4.57 (2H, d, *J* = 5.9 Hz), 5.23 (1H, ddt, *J* = 10.7, 1.3 and 1.3 Hz), 5.29 (1H, ddt, *J* = 17.0, 1.3 and 1.3 Hz), 5.62 (1H, br s), 5.85 (1H, ddt, *J* = 17.0, 10.7 and 5.9 Hz), 6.23-6.42 (2H, br m), 6.69-6.89 (3H, br m), 7.06-7.42 (35H, m), 7.49 (2H, br d, *J* = 6.9 Hz), 7.74 (2H, br d, *J* = 5.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 29.2, 39.5, 39.8, 41.6, 47.1, 49.8, 66.2, 67.2, 67.3, 70.8, 116.5, 117.3, 118.5, 119.2, 120.1, 124.4, 125.2, 125.3, 126.1, 126.8, 127.2, 127.3, 127.9, 128.0, 128.1, 128.8, 129.6, 131.5, 141.3, 141.4, 143.7, 143.8, 144.5, 144.6, 148.3, 154.1, 155.2, 159.7, 167.6, 168.6, 169.3, 170.2; HRMS (ESI-TOF) *m*/*z* calcd for $C_{75}H_{64}N_4NaO_9S$ ($[M + Na]^+$) 1219.4292, found 1219.4301.

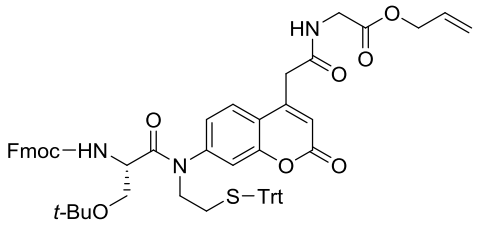
[2-(7-{*N*-[Fmoc-L-Gln(Trt)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8f)



White amorphous solid; yield: 83% (244 mg); $[\alpha]^{21}_{D}$ 72.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.68 (1H, br dt, *J* = 14.1 and 7.0 Hz), 1.74-1.86 (1H, br m), 2.15 (2H, br t, *J* = 7.0 Hz), 2.23-2.36 (1H, br m), 2.42-2.56 (1H, br m), 3.24-3.39 (1H, br m), 3.46-3.63 (1H, br m), 3.58 (2H, br s), 3.95 (1H, dd, *J* = 17.7 and 4.9 Hz), 4.01 (1H, dd, *J* = 17.7 and 4.9 Hz), 4.12-4.23 (2H, br m), 4.32 (2H, br d, *J* = 6.8 Hz), 4.61 (2H, d, *J* = 5.9 Hz), 5.25 (1H, ddt, *J* = 10.7, 1.3 and 1.3 Hz), 5.31 (1H, ddt, *J* = 17.0, 1.3 and 1.3 Hz), 5.66 (1H, br d, *J* = 6.9 Hz), 5.87 (1H, ddt, *J* = 17.0, 10.7 and 5.9 Hz), 6.29 (1H, br t, *J* = 4.9 Hz), 6.41 (1H, s), 6.50 (1H, br s), 6.92-7.02 (2H, m), 7.02-7.41 (34H, m), 7.49 (1H, d, *J* = 8.2 Hz), 7.56 (1H, d, *J* = 6.5 Hz), 7.57 (1H, d, *J* = 6.5 Hz), 7.75 (2H, d, *J* = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 27.8, 29.4, 32.6, 39.7, 41.7, 47.3, 49.4, 51.5, 66.3, 67.1, 67.2, 70.7, 116.8, 117.5, 118.9, 119.4, 120.1, 124.6, 125.3, 126.5, 126.8, 127.2, 127.8, 128.0, 128.1, 128.8, 129.6, 131.4, 141.4,

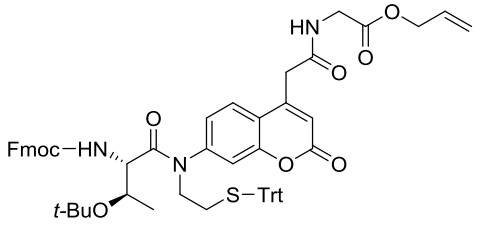
141.5, 143.8, 144.0, 144.6, 144.7, 148.2, 154.3, 156.1, 159.7, 167.4, 169.3, 170.5, 171.1; HRMS (ESI-TOF) *m*/*z* calcd for $C_{76}H_{66}N_4NaO_9S$ ($[M + Na]^+$) 1233.4448, found 1233.4451.

[2-(7-{*N*-[Fmoc-L-Ser(*t*-Bu)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8g)



White amorphous solid; yield: 87% (207 mg); $[\alpha]^{21}_D$ 36.6 (*c* 1.02, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ = 1.09 (9H, s), 2.45 (2H, br m), 3.24-3.42 (3H, m), 3.63 (1H, dt, *J* = 14.3 and 7.2 Hz), 3.72 (1H, d, *J* = 16.0 Hz), 3.76 (1H, d, *J* = 16.0 Hz), 4.09 (2H, d, *J* = 5.1 Hz), 4.17 (1H, t, *J* = 7.0 Hz), 4.25-4.39 (3H, m), 4.63 (2H, d, *J* = 5.9 Hz), 5.26 (1H, ddt, *J* = 10.7, 1.2 and 1.2 Hz), 5.32 (1H, ddt, *J* = 17.0, 1.2 and 1.2 Hz), 5.38 (1H, d, *J* = 7.0 Hz), 5.88 (1H, ddt, *J* = 17.0, 10.7 and 5.9 Hz), 6.28 (1H, br t, *J* = 5.1 Hz), 6.47 (1H, s), 7.00-7.23 (11H, m), 7.27-7.35 (8H, m), 7.39 (2H, t, *J* = 7.5 Hz), 7.56 (1H, d, *J* = 6.9 Hz), 7.57 (1H, d, *J* = 6.9 Hz), 7.61 (1H, d, *J* = 8.4 Hz), 7.75 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 27.4, 29.3, 29.8, 39.9, 41.7, 47.2, 49.5, 51.6, 62.8, 66.4, 67.2, 73.8, 117.4, 118.6, 119.3, 120.1, 124.8, 125.2, 126.0, 126.8, 127.2, 127.8, 128.0, 129.6, 131.3, 141.4, 143.8, 143.9, 144.4, 144.6, 148.4, 154.0, 155.6, 159.9, 167.5, 169.3, 170.5; HRMS (ESI-TOF) *m*/*z* calcd for $C_{59}H_{57}N_3NaO_9S$ ($[M + Na]^+$) 1006.3713, found 1006.3732.

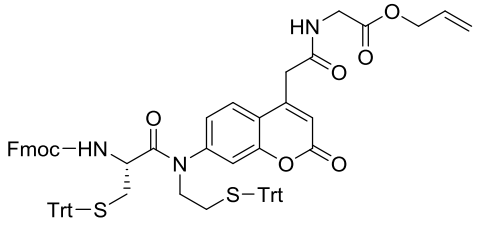
[2-(7-{*N*-[Fmoc-L-Thr(*t*-Bu)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8h)



White amorphous solid; yield: 84% (202 mg); $[\alpha]^{26}_D$ 110.1 (*c* 1.04, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ = 0.92 (3H, br d, *J* = 4.4 Hz), 1.03 (9H, br s), 2.19-2.36 (1H, br m), 2.50-2.65 (1H, br m), 3.17-3.33 (1H, br m), 3.56-3.71 (2H, br m), 3.72 (1H, d, *J* = 15.7 Hz), 3.77 (1H, d, *J* = 15.7 Hz), 4.08 (2H, d, *J* = 5.2 Hz), 4.13-4.25 (1H, m), 4.21 (1H, t, *J* = 7.1 Hz), 4.35 (2H, d, *J* = 7.1 Hz), 4.63 (2H, d, *J* = 6.0 Hz), 5.26 (1H, ddt, *J* = 10.6, 1.2 and 1.2 Hz), 5.32 (1H, ddt, *J* = 17.0, 1.2 and 1.2 Hz), 5.50

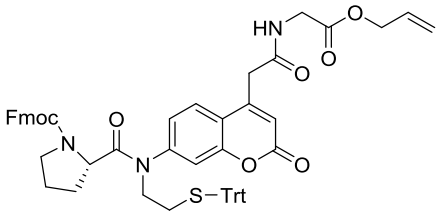
(1H, br d, *J* = 7.5 Hz), 5.88 (1H, ddt, *J* = 17.0, 10.6 and 6.0 Hz), 6.28 (1H, t, *J* = 5.2 Hz), 6.48 (1H, s), 6.94-7.05 (2H, m), 7.08-7.23 (9H, m), 7.28-7.36 (8H, m), 7.40 (2H, t, *J* = 7.5 Hz), 7.57-7.66 (3H, m), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 20.3, 28.4, 29.3, 39.9, 41.8, 47.3, 49.8, 56.8, 66.4, 67.2, 67.2, 68.1, 74.5, 116.8, 117.5, 118.5, 119.4, 120.1, 124.9, 125.3, 125.3, 126.2, 126.8, 127.2, 127.8, 128.0, 129.7, 131.4, 141.4, 143.9, 144.1, 144.6, 144.7, 148.3, 154.3, 156.2, 159.7, 167.3, 169.3, 170.2; HRMS (ESI-TOF) *m*/*z* calcd for $C_{60}H_{59}N_3NaO_9S$ ([M + Na]$^+$) 1020.3870, found 1020.3878.

[2-(7-{*N*-[Fmoc-L-Cys(Trt)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8i)



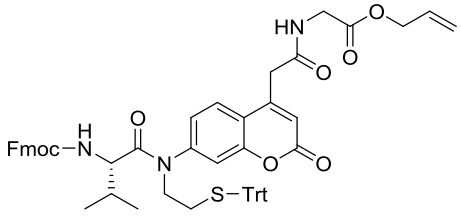
White amorphous solid; yield: 79% (227 mg); $[\alpha]^{22}_D$ 25.2 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.13 (1H, dd, *J* = 11.6 and 7.3 Hz), 2.24-2.37 (2H, m), 2.46 (1H, dt, *J* = 13.6 and 6.7 Hz), 3.39 (2H, br t, *J* = 6.7 Hz), 3.70 (1H, d, *J* = 15.8 Hz), 3.75 (1H, d, *J* = 15.8 Hz), 4.08 (2H, d, *J* = 5.1 Hz), 4.16-4.36 (4H, m), 4.62 (2H, d, *J* = 5.9 Hz), 5.25 (1H, ddt, *J* = 10.7, 1.3 and 1.3 Hz), 5.31 (1H, ddt, *J* = 17.0, 1.3 and 1.3 Hz), 5.41 (1H, d, *J* = 8.6 Hz), 5.87 (1H, ddt, *J* = 17.0, 10.7 and 5.9 Hz), 6.24 (1H, br t, *J* = 5.1 Hz), 6.48 (1H, s), 6.77 (1H, br s), 6.85 (1H, br d, *J* = 8.4 Hz), 7.08-7.42 (34H, m), 7.54 (1H, d, *J* = 8.4 Hz), 7.59 (1H, d, *J* = 6.9 Hz), 7.60 (1H, d, *J* = 6.9 Hz), 7.77 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 29.3, 34.4, 40.0, 41.8, 47.2, 49.7, 50.8, 66.5, 66.9, 67.2, 67.3, 116.8, 117.7, 118.8, 119.4, 120.1, 124.6, 125.3, 126.3, 126.8, 127.0, 127.2, 127.3, 127.9, 128.0, 128.1, 129.5, 129.6, 131.3, 141.4, 143.8, 143.9, 144.0, 144.3, 144.6, 148.1, 154.2, 155.5, 159.6, 167.3, 169.3, 169.9; HRMS (ESI-TOF) *m*/*z* calcd for $C_{74}H_{63}N_3NaO_8S_2$ ([M + Na]$^+$) 1208.3954, found 1208.3955.

(2-{7-[*N*-(Fmoc-L-Pro)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (8j)



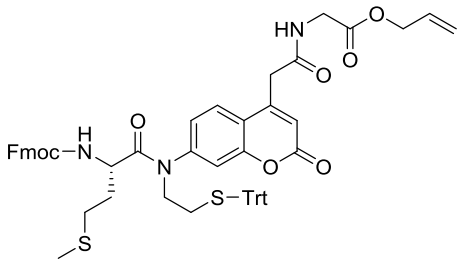
White amorphous solid; yield: 48% (109 mg); $[\alpha]^{23}_{D}$ 87.9 (*c* 1.01, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ = 1.67-2.16 (4H, m), 2.25-2.41 (1.3H, m), 2.50-2.59 (0.7H, m), 3.20-3.39 (1H, m), 3.45-3.53 (1H, m), 3.54-3.76 (4H, m), 4.06-4.15 (3H, m), 4.20-4.43 (3H, m), 4.59-4.67 (2H, m), 5.26 (1H, ddt, *J* = 10.4, 1.3 and 1.3 Hz), 5.32 (1H, ddt, *J* = 17.2, 1.3 and 1.3 Hz), 5.82-5.94 (1H, m), 6.31 (1H, br s), 6.46 (1H, s), 6.59 (0.3H, d, *J* = 7.2 Hz), 6.74 (0.3H, s), 7.04-7.50 (21.5H, m), 7.56-7.64 (2H, m), 7.75 (1.4H, d, *J* = 7.5 Hz), 7.80 (0.6H, d, *J* = 7.5 Hz),; ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 23.5, 24.7, 29.6, 30.5, 31.7, 39.9, 41.8, 47.1, 47.3, 47.5, 47.7, 49.3, 57.7, 66.5, 67.1, 67.6, 117.1, 117.5, 118.5, 119.5, 120.1, 120.2, 125.0, 125.3, 125.4, 126.2, 126.8, 127.1, 127.2, 127.8, 128.0, 129.6, 129.7, 131.3, 141.4, 144.0, 144.2, 144.3, 144.6, 144.7, 145.1, 148.3, 154.2, 154.9, 159.8, 167.4, 169.3, 172.1; HRMS (ESI-TOF) *m*/*z* calcd for $C_{57}H_{51}N_3NaO_8S$ ($[M + Na]^+$) 960.3295, found 960.3321.

(2-{7-[*N*-(Fmoc-L-Val)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (8k)



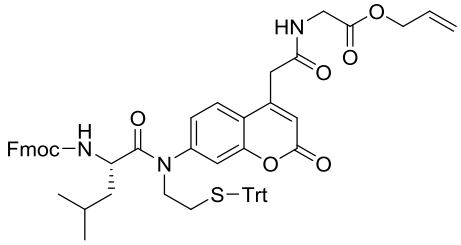
White amorphous solid; yield: 86% (260 mg); $[\alpha]^{23}_{D}$ 117.3 (*c* 1.01, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ = 0.72 (3H, d, *J* = 6.3 Hz), 0.77 (3H, d, *J* = 6.5 Hz), 1.73-1.88 (1H, m), 2.23-2.35 (1H, m), 2.49-2.62 (1H, m), 3.34-3.46 (1H, br m), 3.54 (1H, ddd, *J* = 13.4, 8.7 and 6.1 Hz), 3.72 (1H, d, *J* = 15.5 Hz), 3.78 (1H, d, *J* = 15.5 Hz), 4.02 (1H, dd, *J* = 9.3 and 6.5 Hz), 4.09 (2H, d, *J* = 5.2 Hz), 4.19 (1H, t, *J* = 7.1 Hz), 4.30 (1H, dd, *J* = 10.4 and 7.1 Hz), 4.38 (1H, dd, *J* = 10.4 and 7.1 Hz), 4.63 (2H, d, *J* = 5.9 Hz), 5.25 (1H, ddt, *J* = 10.6, 1.2 and 1.2 Hz), 5.28-5.38 (1H, m), 5.32 (1H, ddt, *J* = 17.0, 1.2 and 1.2 Hz), 5.88 (1H, ddt, *J* = 17.0, 10.6 and 5.9 Hz), 6.32 (1H, br s), 6.48 (1H, s), 6.91-7.04 (2H, m), 7.09-7.22 (9H, m), 7.27-7.36 (8H, m), 7.39 (2H, t, *J* = 7.5 Hz), 7.55-7.67 (3H, m), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 17.5, 19.6, 29.4, 31.9, 39.9, 41.7, 47.3, 49.5, 56.8, 66.4, 67.1, 67.2, 117.0, 117.6, 118.7, 119.4, 120.1, 124.9, 125.2, 126.3, 126.8, 127.2, 127.8, 128.0, 129.6, 131.4, 141.4, 143.9, 144.0, 144.4, 144.6, 148.3, 154.2, 156.1, 159.7, 167.4, 169.3, 171.7; HRMS (ESI-TOF) *m*/*z* calcd for $C_{57}H_{53}N_3NaO_8S$ ($[M + Na]^+$) 962.3451, found 962.3468.

(2-{7-[*N*-(Fmoc-L-Met)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (8l)



White amorphous solid; yield: 83% (196 mg); $[\alpha]^{21}_{D}$ 76.7 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.63-1.83 (2H, m), 1.89 (3H, s), 2.22-2.39 (3H, m), 2.47-2.60 (1H, br m), 3.37-3.58 (2H, m), 3.72 (1H, d, *J* = 15.6 Hz), 3.77 (1H, d, *J* = 15.6 Hz), 4.09 (2H, d, *J* = 5.2 Hz), 4.18 (1H, t, *J* = 7.0 Hz), 4.27-4.40 (3H, m), 4.64 (2H, d, *J* = 5.9 Hz), 5.26 (1H, ddt, *J* = 10.7, 1.3 and 1.3 Hz), 5.32 (1H, ddt, *J* = 17.0, 1.3 and 1.3 Hz), 5.44 (1H, br d, *J* = 8.1 Hz), 5.88 (1H, ddt, *J* = 17.0, 10.7 and 5.9 Hz), 6.26 (1H, br t, *J* = 5.2 Hz), 6.48 (1H, s), 6.95-7.05 (2H, m), 7.09-7.24 (9H, m), 7.28-7.37 (8H, m), 7.40 (2H, t, *J* = 7.5 Hz), 7.57 (1H, d, *J* = 6.8 Hz), 7.58 (1H, d, *J* = 6.9 Hz), 7.64 (1H, d, *J* = 8.2 Hz), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 15.6, 29.4, 29.9, 32.7, 40.0, 41.8, 47.3, 49.4, 51.1, 66.5, 67.1, 67.2, 116.9, 117.8, 118.9, 119.4, 120.1, 124.7, 125.2, 126.5, 126.9, 127.2, 127.9, 128.0, 129.6, 131.4, 141.4, 143.8, 144.0, 144.6, 148.2, 154.3, 155.9, 159.6, 167.3, 169.3, 171.4; HRMS (ESI-TOF) *m*/*z* calcd for $C_{57}H_{53}N_3NaO_8S_2$ ($[M + Na]^+$) 994.3172, found 994.3176.

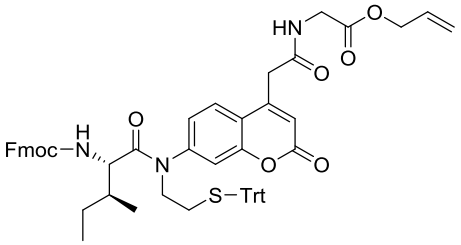
(2-{7-[*N*-(Fmoc-L-Leu)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (8m)



White amorphous solid; yield: 91% (563 mg); $[\alpha]^{27}_{D}$ 94.4 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 0.41 (3H, br d, *J* = 5.1 Hz), 0.74 (3H, br d, *J* = 5.9 Hz), 1.15-1.28 (1H, br m), 1.35-1.52 (2H, br m), 2.24-2.37 (1H, m), 2.48-2.60 (1H, br m), 3.27-3.40 (1H, br m), 3.48-3.60 (1H, m), 3.72 (1H, d, *J* = 15.6 Hz), 3.78 (1H, d, *J* = 15.6 Hz), 4.08 (2H, d, *J* = 5.2 Hz), 4.15-4.26 (2H, m), 4.27-4.37 (2H, m), 4.63 (2H, d, *J* = 5.9 Hz), 5.26 (1H, ddt, *J* = 10.6, 1.1 and 1.1 Hz), 5.28-5.38 (2H, m), 5.88 (1H, ddt, *J* = 17.0, 10.6 and 5.9 Hz), 6.43 (1H, br s), 6.49 (1H, s), 7.00 (1H, br s), 7.04 (1H, br d, *J* = 8.4 Hz), 7.09-7.21 (9H, m), 7.27-7.36 (8H, m), 7.39 (2H, t, *J* = 7.5 Hz), 7.57 (1H, d, *J* = 6.5 Hz), 7.59 (1H, d, *J* = 6.5 Hz), 7.65 (1H, d, *J* = 8.4 Hz), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 21.1, 23.3, 24.6, 29.4, 39.9, 41.7, 42.4, 47.3, 49.3, 50.5, 66.4, 67.1, 67.2, 116.9, 117.6, 118.7, 119.4, 120.1,

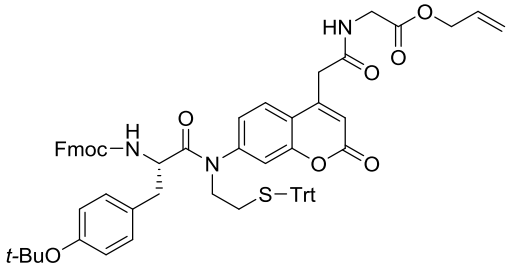
124.9, 125.3, 126.3, 126.8, 127.2, 127.8, 128.0, 129.6, 131.3, 141.4, 143.9, 144.0, 144.2, 144.6, 148.3, 154.2, 156.1, 159.7, 167.4, 169.3, 172.6; HRMS (ESI-TOF) *m*/*z* calcd for $C_{58}H_{55}KN_3O_8S$ ($[M + K]^+$) 992.3347, found 992.3329.

(2-{7-[*N*-(Fmoc-L-Ile)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (8n)



White amorphous solid; yield: 85% (195 mg); $[\alpha]^{22}_D$ 110.1 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 0.67-0.80 (6H, m), 0.81-0.95 (1H, m), 1.32-1.46 (1H, br m), 1.50-1.62 (1H, br m), 2.22-2.35 (1H, br m), 2.50-2.62 (1H, br m), 3.37-3.47 (1H, m), 3.49-3.59 (1H, m), 3.71 (1H, d, *J* = 15.6 Hz), 3.78 (1H, d, *J* = 15.6 Hz), 4.03 (1H, dd, *J* = 8.9 and 7.8 Hz), 4.09 (2H, d, *J* = 5.2 Hz), 4.19 (1H, t, *J* = 7.2 Hz), 4.31 (1H, dd, *J* = 10.4 and 7.2 Hz), 4.37 (1H, dd, *J* = 10.4 and 7.2 Hz), 4.63 (2H, d, *J* = 6.0 Hz), 5.22-5.36 (1H, m), 5.26 (1H, ddt, *J* = 10.6, 1.2 and 1.2 Hz), 5.32 (1H, ddt, *J* = 16.9, 1.2 and 1.2 Hz), 5.88 (1H, ddt, *J* = 16.9, 10.6 and 6.0 Hz), 6.23-6.35 (1H, br m), 6.48 (1H, s), 6.92-7.02 (2H, m), 7.09-7.24 (9H, m), 7.28-7.43 (10H, m), 7.54-7.66 (3H, m), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 11.4, 15.8, 24.2, 29.4, 38.5, 39.9, 41.8, 47.4, 49.4, 56.2, 66.4, 67.0, 67.2, 117.1, 117.6, 118.7, 119.4, 120.1, 124.9, 125.2, 126.2, 126.8, 127.2, 127.8, 128.0, 129.6, 131.3, 141.4, 143.9, 144.0, 144.4, 144.6, 148.3, 154.2, 156.0, 159.8, 167.4, 169.3, 171.8; HRMS (ESI-TOF) *m*/*z* calcd for $C_{58}H_{55}N_3NaO_8S$ ($[M + Na]^+$) 976.3608, found 976.3602.

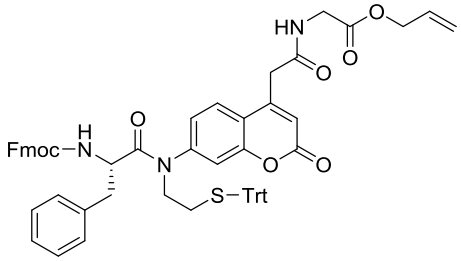
[2-(7-{*N*-[Fmoc-L-Tyr(*t*-Bu)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8o)



White amorphous solid; yield: 73% (188 mg); $[\alpha]^{26}_D$ 17.9 (*c* 1.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.32 (9H, s), 2.25-2.42 (2H, m), 2.69 (1H, dd, *J* = 12.9 and 5.8 Hz), 2.84 (1H, dd, *J* = 12.9 and 9.0 Hz), 3.24-3.42 (2H, m), 3.70 (1H, d, *J* = 15.7 Hz), 3.75 (1H, d, *J* = 15.7 Hz), 4.09 (2H, d, *J* = 5.1 Hz), 4.15 (1H, t, *J* = 6.9 Hz), 4.22-4.36 (3H, m), 4.64 (2H, d, *J* = 5.9 Hz), 5.26 (1H, ddt, *J* = 10.7, 1.2 and 1.2 Hz), 5.32 (1H, ddt, *J* = 17.0, 1.2 and

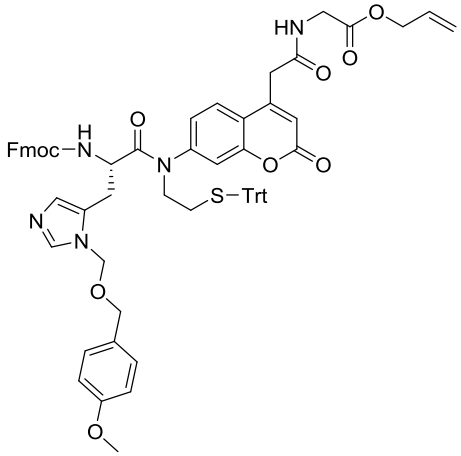
1.2 Hz), 5.39 (1H, br d, *J* = 8.1 Hz), 5.88 (1H, ddt, *J* = 17.0, 10.7 and 5.9 Hz), 6.27 (1H, br s), 6.46 (1H, s), 6.76-6.85 (4H, br s), 7.08-7.23 (10H, m), 7.28-7.36 (9H, m), 7.40 (2H, t, *J* = 7.5 Hz), 7.47 (1H, br d, *J* = 8.0 Hz), 7.55 (1H, d, *J* = 6.2 Hz), 7.57 (1H, d, *J* = 6.4 Hz), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 29.0, 29.1, 39.3, 39.9, 41.8, 47.3, 49.3, 53.1, 66.4, 67.1, 67.3, 78.6, 116.9, 117.7, 118.7, 119.4, 120.1, 124.2, 124.5, 125.2, 125.9, 126.8, 127.2, 127.9, 128.0, 129.6, 130.1, 130.4, 131.4, 141.4, 143.9, 144.6, 148.1, 154.0, 154.8, 155.4, 159.6, 167.3, 169.3, 171.1; HRMS (ESI-TOF) *m*/*z* calcd for $C_{65}H_{61}N_3NaO_9S$ ([M + Na]$^+$) 1082.4026, found 1082.4020.

(2-{7-[*N*-(Fmoc-L-Phe)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (8p)



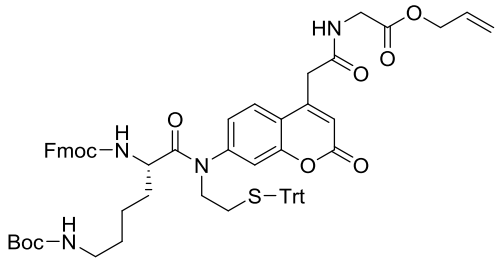
White amorphous solid; yield: 81% (194 mg); $[\alpha]^{20}_D$ 58.0 (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.24-2.40 (2H, m), 2.75 (1H, dd, *J* = 12.6 and 5.3 Hz), 2.87 (1H, dd, *J* = 12.6 and 9.4 Hz), 3.24-3.43 (2H, br m), 3.70 (1H, d, *J* = 16.0 Hz), 3.76 (1H, d, *J* = 16.0 Hz), 4.09 (2H, d, *J* = 5.0 Hz), 4.17 (1H, t, *J* = 7.0 Hz), 4.23-4.40 (3H, m), 4.63 (2H, d, *J* = 5.9 Hz), 5.26 (1H, ddt, *J* = 10.6, 1.2 and 1.2 Hz), 5.32 (1H, ddt, *J* = 17.0, 1.2 and 1.2 Hz), 5.41 (1H, br d, *J* = 8.7 Hz), 5.88 (1H, ddt, *J* = 17.0, 10.6 and 5.9 Hz), 6.25 (1H, br t, *J* = 5.0 Hz), 6.46 (1H, s), 6.89 (2H, d, *J* = 7.3 Hz), 7.08-7.23 (11H, m), 7.23-7.36 (11H, m), 7.40 (2H, t, *J* = 7.5 Hz), 7.48 (1H, d, *J* = 8.2 Hz), 7.56 (1H, d, *J* = 6.4 Hz), 7.57 (1H, d, *J* = 6.6 Hz), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 29.2, 39.9, 40.2, 41.8, 47.3, 49.3, 53.2, 66.5, 67.1, 67.2, 116.7, 117.6, 118.6, 119.5, 120.1, 124.5, 125.2, 125.3, 125.8, 126.8, 127.2, 127.5, 127.9, 128.0, 128.7, 129.6, 131.3, 135.7, 141.4, 143.8, 143.9, 144.6, 148.2, 153.9, 155.4, 159.7, 167.3, 169.3, 171.0; HRMS (ESI-TOF) *m*/*z* calcd for $C_{61}H_{53}KN_3O_8S$ ([M + K]$^+$) 1026.3190, found 1026.3214.

[2-(7-{*N*-[Fmoc-L-His(MBom)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8q)



White amorphous solid; yield: 70% (193 mg); $[\alpha]^{23}_{D}$ 58.0 (*c* 1.01, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ = 2.21-2.32 (1H, br m), 2.40-2.52 (1H, br m), 2.79 (1H, br dd, *J* = 14.9 and 6.5 Hz), 2.95 (1H, dd, *J* = 14.9 and 7.6 Hz), 3.20-3.33 (1H, m), 3.52-3.70 (3H, m), 3.77 (3H, s), 4.04 (2H, d, *J* = 5.2 Hz), 4.08-4.20 (3H, m), 4.27-4.39 (3H, m), 4.62 (2H, d, *J* = 5.9 Hz), 4.83 (1H, d, *J* = 10.9 Hz), 4.90 (1H, br d, *J* = 10.9 Hz), 5.24 (1H, ddt, *J* = 10.6, 1.2 and 1.2 Hz), 5.30 (1H, ddt, *J* = 16.9, 1.2 and 1.2 Hz), 5.57 (1H, br d, *J* = 7.7 Hz), 5.87 (1H, ddt, *J* = 16.9, 10.6 and 5.9 Hz), 6.42 (1H, s), 6.60-6.67 (3H, br m), 6.83 (2H, d, *J* = 8.6 Hz), 7.04-7.22 (11H, m), 7.24-7.42 (12H, m), 7.50 (1H, d, *J* = 8.4 Hz), 7.54 (1H, d, *J* = 6.5 Hz), 7.56 (1H, d, *J* = 6.5 Hz), 7.74 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 27.7, 29.2, 39.8, 41.7, 47.2, 49.2, 51.5, 55.5, 66.4, 67.1, 67.2, 69.4, 72.8, 114.2, 116.8, 117.7, 118.8, 119.4, 120.1, 124.4, 125.2, 126.3, 126.8, 127.2, 127.9, 128.0, 128.2, 129.6, 129.7, 131.4, 138.5, 141.4, 143.6, 143.8, 144.5, 148.2, 154.1, 155.4, 159.6, 159.7, 167.4, 169.3, 170.8; HRMS (ESI-TOF) *m*/*z* calcd for $C_{67}H_{62}N_5O_{10}S$ ($[M + H]^+$) 1128.4217, found 1128.4210.

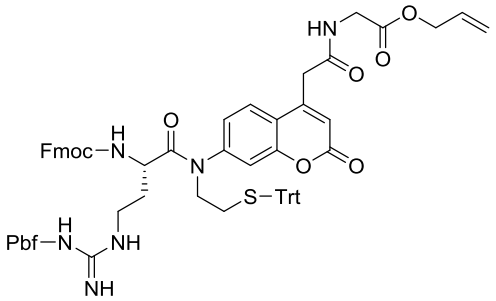
[2-(7-{*N*-[Fmoc-L-Lys(Boc)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8r)



White amorphous solid; yield: 70% (177 mg); $[\alpha]^{22}_{D}$ 90.0 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ = 1.02-1.24 (4H, br m), 1.36-1.47 (2H, m), 1.40 (9H, s), 2.23-2.37 (1H, br m), 2.47-2.61 (1H, br m), 2.77-2.97 (2H, br s), 3.28-3.40 (1H, br m), 3.53-3.66 (1H, br m), 3.76 (2H, s), 4.09 (2H, d, *J* = 5.3 Hz), 4.14-4.24 (1H, m), 4.18 (1H, t, *J* = 7.1 Hz), 4.32 (2H, d, *J* = 7.1 Hz), 4.49-4.59 (1H, br s),

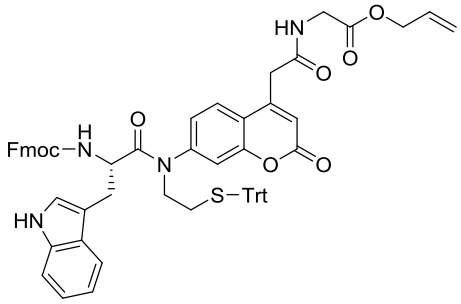
4.63 (2H, d, *J* = 5.9 Hz), 5.25 (1H, d, *J* = 10.7 Hz), 5.32 (1H, dd, *J* = 17.0 and 1.2 Hz), 5.36-5.48 (1H, br m), 5.88 (1H, ddt, *J* = 17.0, 10.7 and 5.9 Hz), 6.37-6.62 (2H, br s), 6.96-7.06 (2H, m), 7.09-7.21 (9H, m), 7.24-7.35 (8H, m), 7.40 (2H, t, *J* = 7.5 Hz), 7.58 (1H, d, *J* = 6.6 Hz), 7.59 (1H, d, *J* = 6.9 Hz), 7.65 (1H, d, *J* = 8.2 Hz), 7.76 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 22.3, 28.5, 29.2, 29.4, 29.8, 32.5, 39.8, 40.1, 41.7, 47.3, 49.3, 51.5, 66.4, 67.1, 67.2, 76.7, 77.2, 77.4, 77.6, 79.3, 116.9, 117.7, 118.9, 119.3, 120.1, 124.7, 125.3, 126.4, 126.8, 127.2, 127.8, 128.0, 129.6, 131.4, 141.4, 143.9, 144.0, 144.6, 148.3, 154.3, 156.1, 159.6, 167.6, 169.3, 171.9; HRMS (ESI-TOF) *m*/*z* calcd for $C_{63}H_{64}N_4NaO_{10}S$ ([M + Na]$^+$) 1091.4241, found 1091.4253.

[2-(7-{*N*-[Fmoc-L-Arg(Pbf)]-*N*-(2-tritylsulfanylethyl)amino}coumarin-4-acetylamino)]-acetic acid allyl ester (8s)



White amorphous solid; yield: 74% (222 mg); $[\alpha]^{22}_D$ 38.5 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.07-1.28 (1H, br m), 1.33-1.57 (9H, m), 2.05 (3H, s), 2.20-2.27 (1H, m), 2.44 (3H, s), 2.50 (3H, s), 2.39-2.57 (1H, m), 2.59-2.81 (2H, br m), 2.91 (2H, s), 3.24-3.38 (1H, m), 3.61-3.73 (1H, m), 3.87-4.10 (3H, m), 4.11-4.21 (2H, m), 4.23-4.42 (3H, m), 4.61 (2H, d, *J* = 5.7 Hz), 5.22 (1H, ddt, *J* = 10.7, 1.3 and 1.3 Hz), 5.31 (1H, ddt, *J* = 17.0, 1.3 and 1.3 Hz), 5.42-5.80 (2H, br m), 5.67 (1H, d, *J* = 9.0 Hz), 5.89 (1H, ddt, *J* = 17.0, 10.7 and 5.7 Hz), 6.04 (1H, br s), 6.67 (1H, s), 6.94 (1H, s), 6.97 (1H, dd, *J* = 8.4 and 1.6 Hz), 7.08-7.41 (19H, m), 7.53 (1H, d, *J* = 7.5 Hz), 7.56 (1H, d, *J* = 7.5 Hz), 7.74 (2H, dd, *J* = 7.5 and 3.0 Hz), 7.83 (1H, br d, *J* = 8.4 Hz), 7.99 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 12.6, 18.0, 19.4, 23.1, 28.7, 29.3, 39.4, 41.5, 43.3, 47.3, 49.0, 50.8, 66.1, 67.2, 67.3, 86.6, 116.6, 116.9, 117.7, 118.9, 119.6, 120.2, 120.2, 123.9, 124.8, 125.1, 126.9, 127.2, 127.2, 127.9, 128.0, 129.6, 131.7, 132.2, 132.9, 138.4, 141.4, 141.5, 143.0, 143.4, 143.8, 144.5, 149.5, 154.0, 155.7, 157.0, 158.9, 160.0, 168.8, 169.6, 171.3; HRMS (ESI-TOF) *m*/*z* calcd for $C_{71}H_{72}N_6NaO_{11}S_2$ ([M + Na]$^+$) 1271.4598, found 1271.4609.

(2-{7-[*N*-(Fmoc-L-Trp)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid allyl ester (8t)

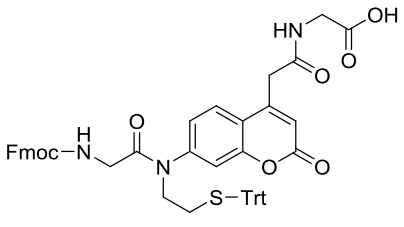


White amorphous solid; yield: 87% (217 mg); $[\alpha]^{21}_{D}$ 108.1 (*c* 1.01, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ = 2.16 (1H, ddd, *J* = 12.3, 9.3 and 6.2 Hz), 2.26 (1H, ddd, *J* = 12.3, 9.0 and 5.6 Hz), 2.94 (1H, dd, *J* = 13.6 and 4.2 Hz), 3.04 (1H, dd, *J* = 13.6 and 10.3 Hz), 3.17 (1H, ddd, *J* = 13.3, 9.0 and 6.2 Hz), 3.43 (1H, ddd, *J* = 13.3, 9.3 and 5.6 Hz), 3.59 (1H, d, *J* = 15.7 Hz), 3.67 (1H, d, *J* = 15.7 Hz), 4.08 (2H, d, *J* = 5.2 Hz), 4.22 (1H, t, *J* = 7.1 Hz), 4.36 (1H, dd, *J* = 10.4 and 7.1 Hz), 4.39 (1H, dd, *J* = 10.4 and 7.1 Hz), 4.53-4.69 (1H, m), 4.64 (2H, d, *J* = 5.9 Hz), 5.25 (1H, ddt, *J* = 10.7, 1.2 and 1.2 Hz), 5.31 (1H, ddt, *J* = 17.0, 1.2 and 1.2 Hz), 5.59 (1H, br d, *J* = 8.7 Hz), 5.88 (1H, ddt, *J* = 17.0, 10.7 and 5.9 Hz), 6.26 (1H, br t, *J* = 5.2 Hz), 6.38 (1H, s), 6.72 (1H, s), 6.75-6.84 (1H, br m), 7.01-7.24 (12H, m), 7.25-7.37 (11H, m), 7.41 (2H, t, *J* = 7.5 Hz), 7.60 (1H, d, *J* = 6.7 Hz), 7.61 (1H, d, *J* = 6.8 Hz), 7.78 (2H, d, *J* = 7.5 Hz), 8.03 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 29.2, 29.8, 30.5, 39.6, 41.7, 47.3, 49.0, 52.3, 66.4, 67.0, 67.1, 110.0, 111.4, 116.0, 117.1, 117.9, 118.4, 119.4, 120.1, 122.1, 123.0, 124.1, 125.3, 126.8, 127.2, 127.4, 127.9, 127.9, 129.6, 131.3, 136.0, 141.4, 143.6, 143.9, 143.9, 144.6, 148.3, 153.4, 155.7, 160.0, 167.5, 169.4, 171.9; HRMS (ESI-TOF) *m*/*z* calcd for $C_{63}H_{54}N_4NaO_8S$ ($[M + Na]^+$) 1049.3560, found 1049.3582.

Typical procedure of removal of an allyl group of 8

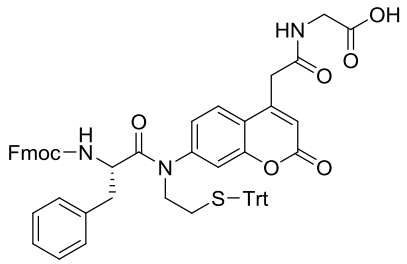
To a stirred mixture of compound **8a** (77.0 mg, 85.7 μmol) in THF (2mL), *N*-methylaniline (93.0 μL, 857 μmol) and $Pd(Ph_3P)_4$ (9.91 mg, 8.57 μmol) were added and the reaction mixture was stirred at room temperature for 1h. After removal of the solvent in vacuo, the product was purified by column chromatography ($CHCl_3$/MeOH = 100/0 to 100/7 then 3/2 (v/v)) to yield **9a** (53.3 mg, 62.1 μmol, 72 %) as a yellow amorphous solid.

(2-{7-[*N*-(Fmoc-Gly)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid (9a)



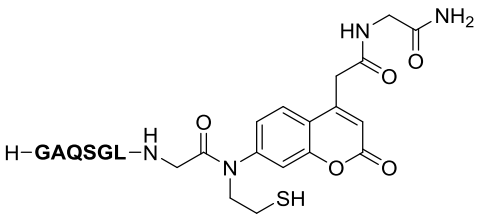
Yellow amorphous solid; yield 72% (53.3 mg); ^{1}H NMR ($CDCl_3$, 400 MHz) = 2.41 (2H, t, *J* = 7.1 Hz), 3.41-3.63 (4H, m), 3.76 (2H, s), 3.92 (2H, br d, *J* = 4.2 Hz), 4.15 (1H, t, *J* = 7.0 Hz), 4.29 (2H, d, *J* = 7.0 Hz), 5.87 (1H, br t, *J* = 3.9 Hz), 6.51 (1H, s), 6.57 (1H, br s), 6.89 (1H, dd, *J* = 8.3 and 1.4 Hz), 6.94 (1H, d, *J* = 1.4 Hz), 7.09-7.40 (19H, m), 7.54 (2H, d, *J* = 7.4 Hz), 7.63 (1H, d, *J* = 8.3 Hz), 7.73 (2H, d, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 29.6, 40.6, 41.3, 43.7, 47.1, 48.8, 67.3, 67.6, 116.7, 117.8, 119.2, 120.2, 124.5, 125.2, 126.9, 127.2, 127.9, 128.1, 129.6, 141.4, 143.0, 143.7, 144.5, 148.8, 154.3, 156.6, 160.0, 167.7, 168.3, 171.3; HRMS (ESI-TOF) *m/z* calcd for $C_{51}H_{43}N_3NaO_8S$ ([M + Na]$^+$) 880.2669, found 880.2656.

(2-{7-[*N*-(Fmoc-L-Phe)-*N*-(2-tritylsulfanylethyl)amino]coumarin-4-acetylamino})-acetic acid (9p)



Pale yellow amorphous solid; yield: 57% (217 mg); $[\alpha]^{28}_D$ 46.3 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.30 (2H, t, *J* = 7.6 Hz), 2.72 (1H, dd, *J* = 13.5 and 5.3 Hz), 2.83 (1H, dd, *J* = 13.5 and 9.3 Hz), 3.28-3.38 (2H,m), 3.72 (1H, d, *J* = 15.5 Hz), 3.79 (1H, d, *J* = 15.5 Hz), 4.07-4.19 (2H, m), 4.23 (1H, dd, *J* = 9.3 and 5.3 Hz), 4.28-4.43 (1H, m), 4.31 (1H, dd, *J* = 7.2 and 10.5 Hz), 4.39 (1H, dd, *J* = 7.2 and 10.5 Hz), 7.10-7.21 (12H, m), 7.28-7.35 (10H, m), 7.40 (2H, dd, J = 7.4 and 8.4 Hz), 7.50-7.60 (1H, m), 7.55 (2H, t, *J* = 7.2 Hz), 7.75 (2H, d, *J* = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 29.1, 39.7, 40.0, 41.9, 47.1, 49.2, 53.2, 67.2, 67.3, 76.7, 77.2, 77.4, 77.6, 116.5, 117.3, 118.7, 120.1, 124.4, 125.2, 126.2, 126.8, 127.2, 127.4, 127.9, 127.9, 128.7, 129.6, 135.5, 141.3, 143.5, 143.6, 144.5, 149.0, 153.6, 155.7, 160.5, 168.5, 171.0, 172.3; HRMS (ESI-TOF) *m/z* calcd for $C_{58}H_{49}N_3NaO_8S$ ([M+Na]$^+$) 970.3138, found 970.3142.

Preparation of SECmide peptide **11**

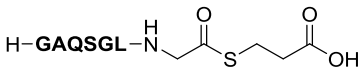


The protected peptide resin was constructed on NovaSyn® TGR resin (loading: 0.22 mmol/g) using

Fmoc SPPS (Acylation: Fmoc amino acid (5.0 equiv), DIC (5.0 equiv) and HOBt·H_2O (5.0 equiv) in DMF or **9a** (2.0 equiv), HATU (1.9 equiv) and DIPEA (1.9 equiv) in DMF for 2 h; Fmoc removal: 20% (v/v) piperidine DMF for 10 min). The completed resin (150 mg) was treated with TFA–TES–H_2O (95:2.5:2.5, (v/v), 7.5 mL) at room temperature for 2 h. The resin was filtered off and the filtrate was directly added to cold Et_2O to generate precipitate. The precipitate collected by centrifugation was washed with cold Et_2O and purified by preparative HPLC to give SECmide peptide **11** (6.2 mg, 25%).

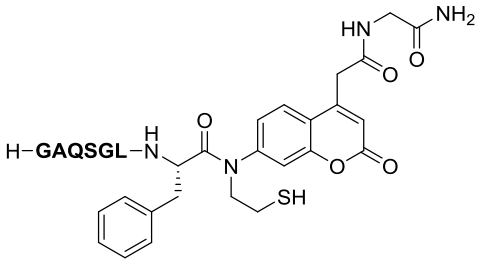
SECmide peptide **11**: Analytical HPLC conditions, linear gradient of solvent B in solvent A, 15 to 25% over 30 min, retention time = 24.6 min. Preparative HPLC condition: linear gradient of solvent D in solvent C, 15 to 25% over 30 min. LRMS (ESI-TOF) *m/z* calcd for ($[M + H]^+$) 906.4, found 906.1.

Preparation of peptide thioester **13**



SECmide peptide **11** (1.5 mg, 1.7 μmol) was dissolved in 0.5 M Na phosphate buffer containing 5% (v/v) 3-mercaptopropionic acid (MPA), 20 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl) and 50 mM Na ascorbate (pH 5.0, 1.7 mL). The reaction mixture was incubated at 50 °C for 4 h and reaction progress was monitored by analytical HPLC. After completion of the reaction, the crude material was purified by preparative HPLC to give **13** (0.89 mg, 1.1 μmol, 68% isolated yield). Analytical HPLC conditions, linear gradient of solvent B in solvent A, 5 to 30% over 30 min, retention time = 18.6 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 8 to 18% over 30 min. LRMS (ESI-TOF) *m/z* calcd for ($[M + H]^+$) 677.3, found 677.2.

Preparation of SECmide peptide **14**



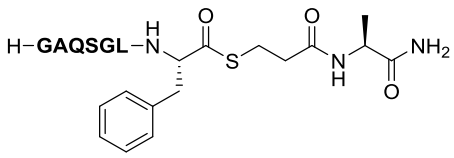
The protected peptide resin was constructed on NovaSyn® TGR resin (loading: 0.22 mmol/g) using Fmoc SPPS (Acylation: Fmoc amino acid (3.0 equiv), DIC (3.0 equiv) and HOBt·H_2O (3.0 equiv) in DMF or **9p** (2.0 equiv), HATU (1.9 equiv) and DIPEA (1.9 equiv) in DMF for 2 h; Fmoc removal: 20% (v/v) piperidine in DMF for 10 min). The completed resin (120 mg) was treated with TFA–TES–H_2O (95:2.5:2.5, (v/v), 6.0 mL) at room temperature for 2 h. The resin was filtered off and the filtrate

was directly added to cold Et_2O to generate precipitate. The precipitate collected by centrifugation was washed with cold Et_2O and purified by preparative HPLC to give SECmide peptide **14** (4.53 mg, 16.3%). Analytical HPLC conditions, linear gradient of solvent B in solvent A, 5 to 35% over 30 min, retention time = 27.8 min. Preparative HPLC conditions: linear gradient of solvent D in solvent C, 19 to 28% over 30 min. LRMS (ESI-TOF) *m/z* calcd for ($[M + H]^+$) 996.4, found 996.1.

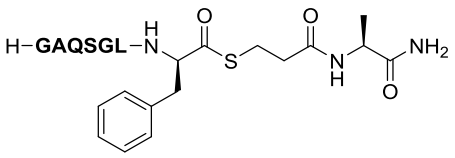
Examination of epimerization during N–S acyl transfer mediated thioesterification of SECmide peptide **14**

SECmide peptide **14** (0.12 mg, 0.10 μmol) was dissolved in 0.5 M Na phosphate buffer containing 5% (v/v) MPA, 20 mM TCEP·HCl and 50 mM Na ascorbate (pH 5.0, 0.10 mL). The reaction mixture was incubated at 50 °C for 8 h and reaction progress was monitored by analytical HPLC. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 10 to 30% over 30 min.

Preparation of peptide thioesters S2 and S3



S2



S3

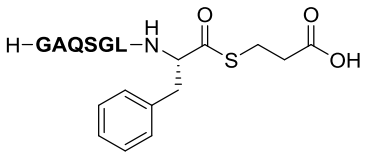
Typical procedure: On 4-methylbenzhydrylamine (MBHA) resin (0.70 mmol amine/g, 0.36 g, 0.25 mmol), introduction of Boc-Ala-OH (4.0 equiv) in the presence of DIC (4.0 equiv), $HOBt·H_2O$ (4.0 equiv) and DIPEA (2.0 equiv) in DMF at room temperature for 2 h followed by Boc removal by TFA–anisole–toluene (50:2:48 (v/v), 30 min) afforded the Boc-Ala-incorporated resin. Next, treatment of the resulting resin with *S*-Tr mercaptopropionic acid (4.0 equiv), DIC (4.0 equiv), $HOBt·H_2O$ (4.0 equiv) and DIPEA (2.0 equiv) in DMF at room temperature for 2 h followed by Trt removal by TFA–TES (95:5, 10 min) gave $HSCH_2CH_2CO$-Ala-MBHA resin. Activated Boc-$_L$-Phe-OH (4.0 equiv) with DIC (4.0 equiv), $HOBt·H_2O$ (4.0 equiv) and DIPEA (2.0 equiv) in DMF was coupled with $HSCH_2CH_2CO$-Ala-MBHA resin for 2 h, and the resin was subsequently subjected to Boc removal by TFA–anisole–toluene (50:2:48 (v/v), 30 min). On the resulting resin, standard *in situ* neutralization Boc SPPS (Acylation: Boc amino acid (4.0 equiv), DIC (4.0 equiv), $HOBt·H_2O$ (4.0 equiv) and DIPEA (2.0 equiv) in DMF at room temperature for 2 h; Boc removal: TFA–anisole–toluene (50:2:48 (v/v), 30 min)) was performed for chain elongation to give a protected peptide resin. The resulting completed resin (50 mg) was treated with 1 M TMSOTf–thioanisole in TFA and *m*-cresol (100/5 (v/v)) at 4 °C for 2 h. After filtration of the resin, cooled Et_2O was added to the filtrate to give precipitate. The

formed precipitate was collected by centrifugation and thoroughly washed with Et_2O to afford crude peptide thioester. The crude peptide thioester was purified by preparative HPLC to give the purified peptide **S2** (2.0 mg, 9.4%).

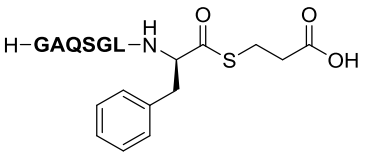
Peptide thioester **S2**: Analytical HPLC conditions, linear gradient of solvent B in solvent A, 10 to 30% over 30 min, retention time = 24.1 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 10 to 30% over 30 min. LRMS (ESI-TOF) *m/z* calcd for ($[M + H]^+$) 837.4, found 837.1.

Peptide thioester **S3** (2.0 mg, 9.2%): Analytical HPLC conditions, linear gradient of solvent B in solvent A, 10 to 30% over 30 min, retention time = 27.1 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 10 to 30% over 30 min. LRMS (ESI-TOF) *m/z* calcd for ($[M + H]^+$) 837.4, found 837.1.

Preparation of peptide thioesters S4 and S5



S4



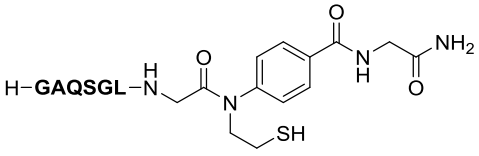
S5

Typical procedure: Peptide thioester **S2** (0.94 mg, 1.0 μmol) was dissolved in 6 M guanidine·HCl–0.1 M Na phosphate buffer containing 2% (v/v) MPA (pH 7.3, 1.0 mL). The reaction mixture was incubated at 37 °C for 1 h and reaction progress was monitored by analytical HPLC. After 1 h of the reaction, the crude material was purified by preparative HPLC to give **S4** (0.10 mg, 0.13 μmol, 13% isolated yield).

Peptide thioester **S4**: Analytical HPLC conditions, linear gradient of solvent B in solvent A, 10 to 30% over 30 min, retention time = 27.2 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 15 to 35% over 30 min. LRMS (ESI-TOF) *m/z* calcd for ($[M + H]^+$) 767.3, found 767.1.

Peptide thioester **S5** (0.25 mg, 0.33 μmol, 33% isolated yield): Analytical HPLC conditions, linear gradient of solvent B in solvent A, 10 to 30% over 30 min, retention time = 29.8 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 15 to 35% over 30 min. LRMS (ESI-TOF) *m/z* calcd for ($[M + H]^+$) 767.3, found 767.2.

Preparation of SEAlide peptide **16**



The protected peptide resin was constructed on NovaSyn® TGR resin (loading: 0.22 mmol/g) using Fmoc SPPS (Acylation: Fmoc amino acid (4.0 equiv), DIC (4.0 equiv) and HOBt·H_2O (4.0 equiv) in DMF or 4-[(Fmoc-Gly -2-tritylsulfanylethyl)amino]benzoic acid (2.0 equiv), HATU (1.9 equiv) and DIPEA (1.9 equiv) in DMF for 2 h; Fmoc removal: 20% (v/v) piperidine in DMF for 10 min). The completed resin (100 mg) was treated with TFA–TES–H_2O (95:2.5:2.5, (v/v), 5.0 mL) at room temperature for 2 h. The resin was filtered off and the filtrate was directly added to cold Et_2O to generate precipitate. The precipitate collected by centrifugation was washed with cold Et_2O and purified by preparative HPLC to give SEAlide peptide **16** (3.1 mg, 11%). Analytical HPLC conditions, linear gradient of solvent B in solvent A, 10 to 60% over 30 min, retention time = 11.2 min. Preparative HPLC conditions: linear gradient of solvent D in solvent C, 15 to 25% over 30 min. LRMS (ESI-TOF) *m/z* calcd for ($[M + H]^+$) 824.4, found 824.2.

Preparation of N-terminal cysteinyl peptide **17**

H–**CYRANK**–NH_2

The protected peptide resin was constructed on NovaSyn® TGR resin (loading: 0.22 mmol/g) using Fmoc SPPS (Acylation: Fmoc amino acid (3.0 equiv), DIC (3.0 equiv) and HOBt·H_2O (3.0 equiv) in DMF) in DMF for 2 h; Fmoc removal: 20% (v/v) piperidine in DMF for 10 min). The completed resin (200 mg) was treated with TFA–*m*-cresol–thioanisole–H_2O–1,2-ethanedithiol (80:5:5:5:5, (v/v), 10 mL) at room temperature for 2 h. The resin was filtered off and the filtrate was directly added to cold Et_2O to generate precipitate. The precipitate collected by centrifugation was washed with cold Et_2O and purified by preparative HPLC to give N-terminal cysteinyl peptide **17** (14 mg, 37%). Analytical HPLC conditions, linear gradient of solvent B in solvent A, 1 to 30% over 30 min, retention time = 11.0 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 1 to 13% over 30 min. LRMS (ESI-TOF) m/z calcd for ($[M + H]^+$) 753.4, found 753.2.

NCL between SECmide peptide **11, 14** or SEAlide peptide **16** and N-terminal cysteinyl peptide **17**

NCL between SECmide peptide 11 or SEAlide peptide 16 and N-terminal cysteinyl peptide 17:

NCL between SECmide peptide **11** or SEAlide peptide **16** and N-terminal cysteinyl peptide **17** was performed in 0.1 M HEPPS buffer containing 40 mM additive and 30 mM TCEP·HCl (pH 7, 100 μL,1 mM each peptide) at 37 °C.

Ligation product **18**: Analytical HPLC conditions, linear gradient of solvent B in solvent A, 5 to 35% over 30 min, retention time = 18.6 min. LRMS (ESI-TOF) m/z calcd for ($[M + 2H]^{2+}$) 662.3, found 662.4.

NCL between SECmide peptide 14 and N-terminal cysteinyl peptide 17:

NCL between SECmide peptide **14** and N-terminal cysteinyl peptide **17** was performed in 0.1 M HEPPS buffer containing 40 mM additive and 30 mM TCEP·HCl (pH 7, 100 μL,1 mM each peptide) at 50 °C.

Ligation product **19**: Analytical HPLC conditions, linear gradient of solvent B in solvent A, 5 to 35% over 30 min, retention time = 21.7 min. LRMS (ESI-TOF) m/z calcd for ($[M + 2H]^{2+}$) 707.3, found 707.3.

Preparation of S6 and S7

H−**GAQSGLX**−**CYRANK**−NH_2

Typical procedure: Peptide thioester **S2** (1.1 mg, 1.0 μmol) and N-terminal cysteinyl peptide **17** (1.2 mg, 1.0 μmol) was dissolved in 0.1 M HEPPS buffer containing 40 mM MPAA and 30 mM TCEP·HCl (pH 7, 100 μL,1 mM each peptide) at 37 °C. The reaction mixture was incubated for 1.5 h and reaction progress was monitored by analytical HPLC. After 1.5 h of the reaction, the crude material was purified by preparative HPLC to give **S6** (0.34 mg, 0.24 μmol, 24% isolated yield).

S6 (**X** = L-Phe): Analytical HPLC conditions, linear gradient of solvent B in solvent A, 5 to 35% over 30 min, retention time = 21.1 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 5 to 35% over 30 min. LRMS (ESI-TOF) *m/z* calcd for ($[M + 2H]^{2+}$) 707.8, found 707.4.

S7 (**X** = D-Phe, 0.25 mg, 0.078 μmol, 7.8% isolated yield): Analytical HPLC conditions, linear gradient of solvent B in solvent A, 5 to 35% over 30 min, retention time = 23.7 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 5 to 35% over 30 min. LRMS (ESI-TOF) m/z calcd for ($[M + 2H]^{2+}$) 707.8, found 707.5.

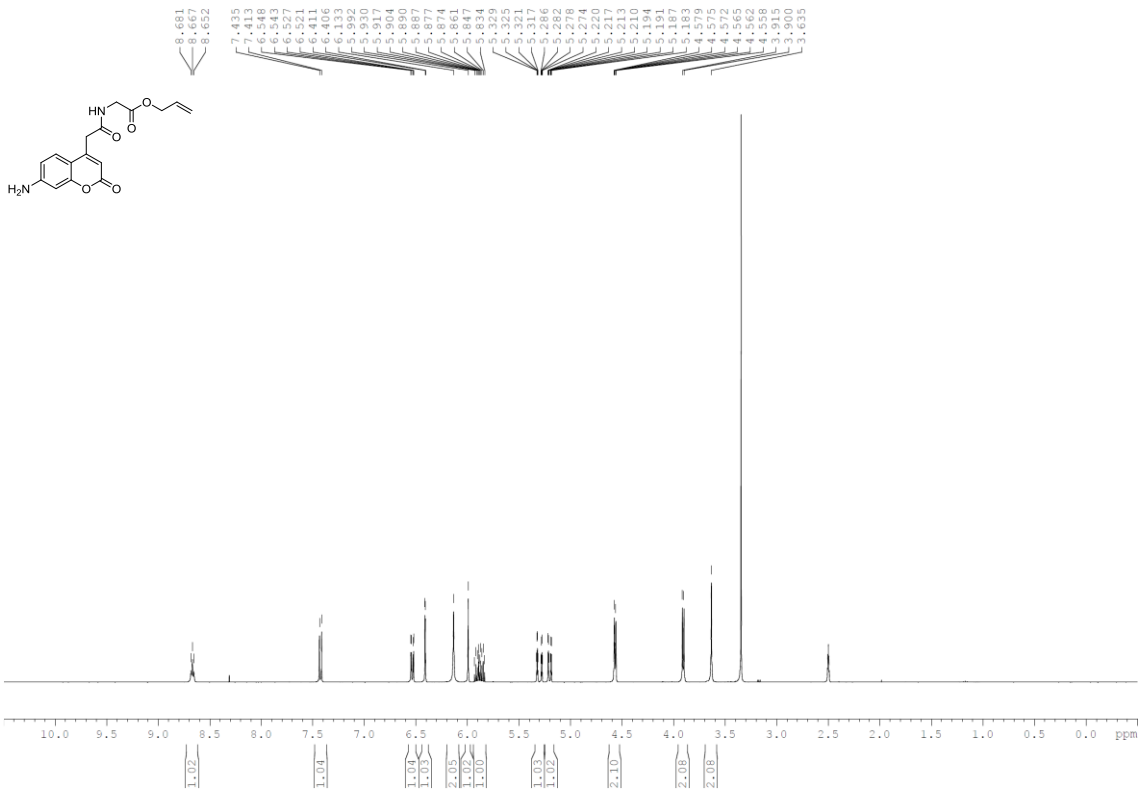
Kinetics measurement

Ligation of SECmide peptide **11** (0.020 μmol) and cysteine·HCl (4.0 μmol) were performed in 0.3 M additive aq. containing 40 mM TCEP·HCl and 30 mM MPAA (pH 7.0 or pH 6.0, 200 μL, SECmide

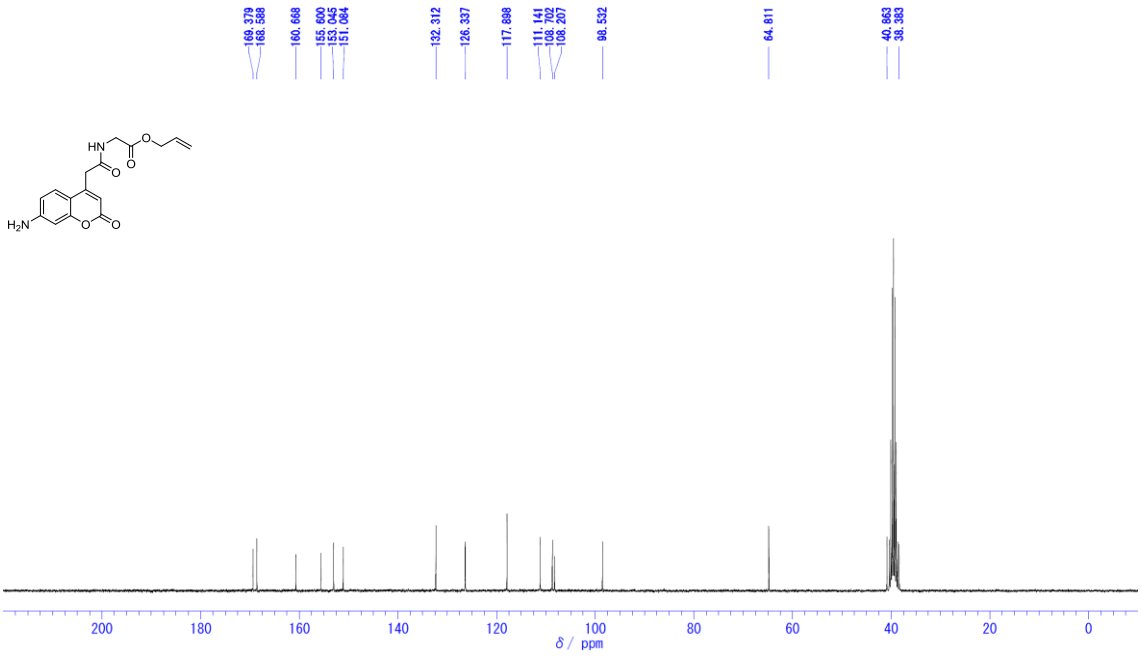
peptide: 0.10 mM, cysteine: 20 mM) at 37 °C. Fluorescence intensity of **S1** was measured (λex: 373 nm; λem: 465 nm) and it was defined as time = 0 min. Then, the fluorescence of the reaction mixture was recorded at 1, 2, 3, 6 and 12 h. Half-life of **11** was estimated based on GraphPad Prism 5 software.

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

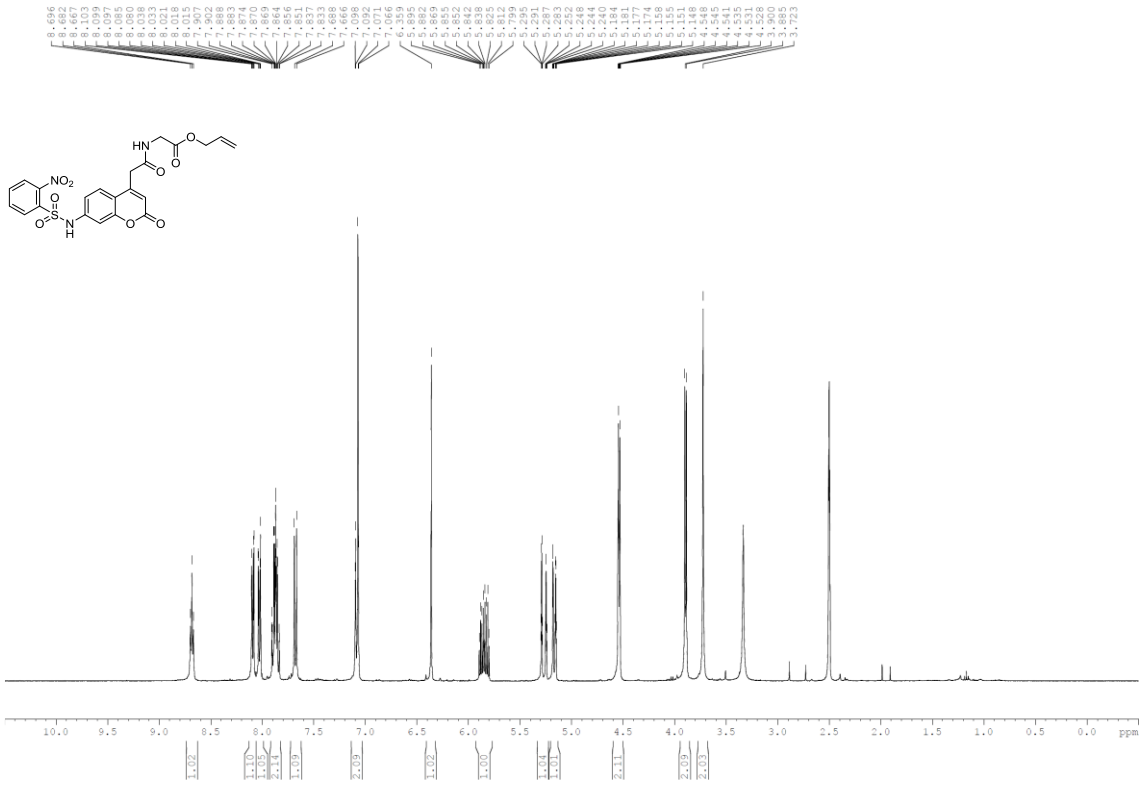
^{1}H NMR spectrum of **5**



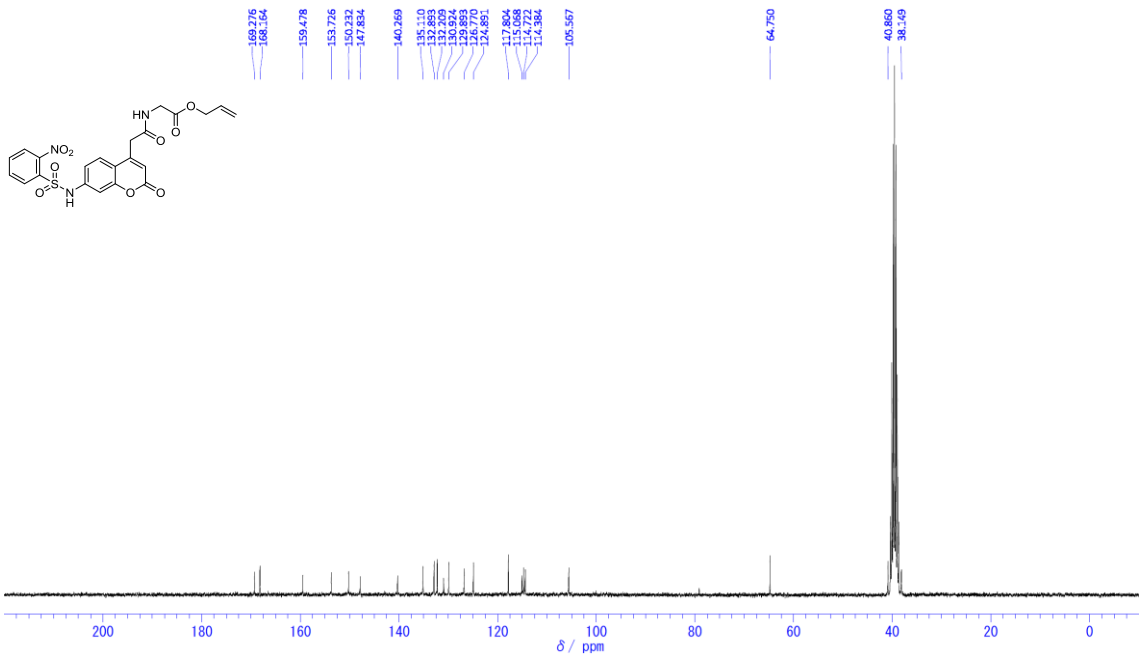
^{13}C NMR spectrum of **5**



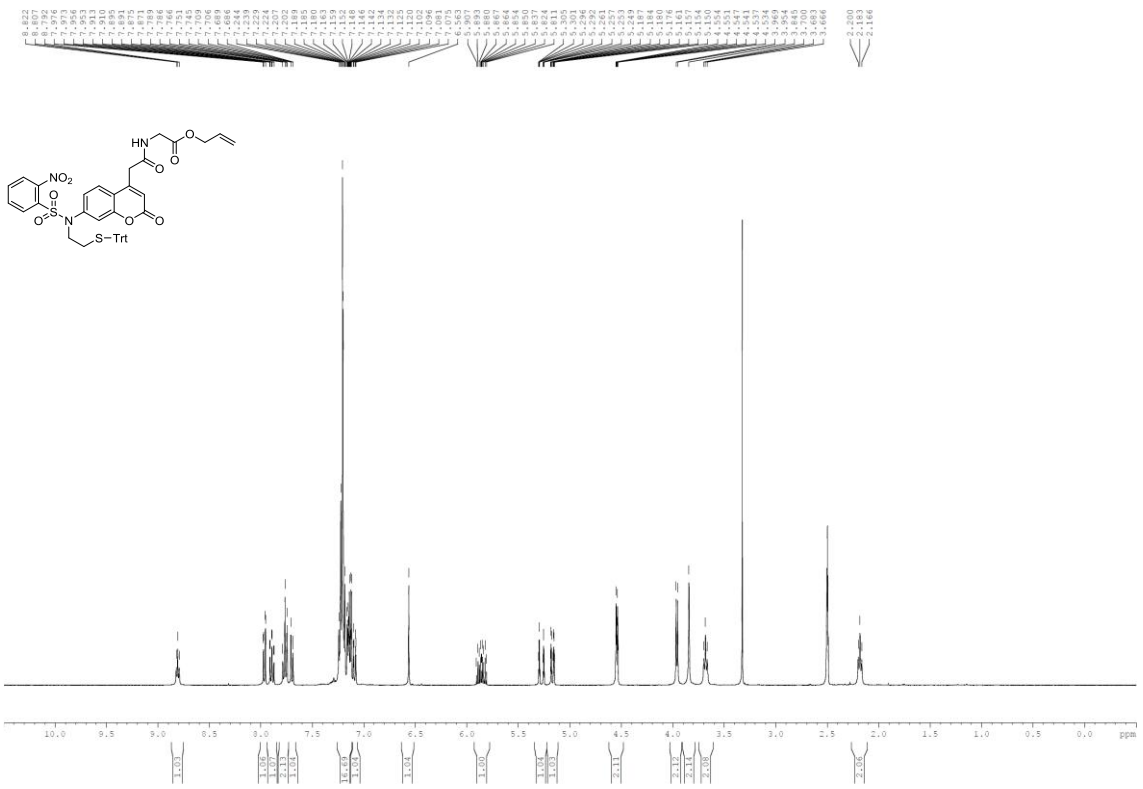
^{1}H NMR spectrum of **6**



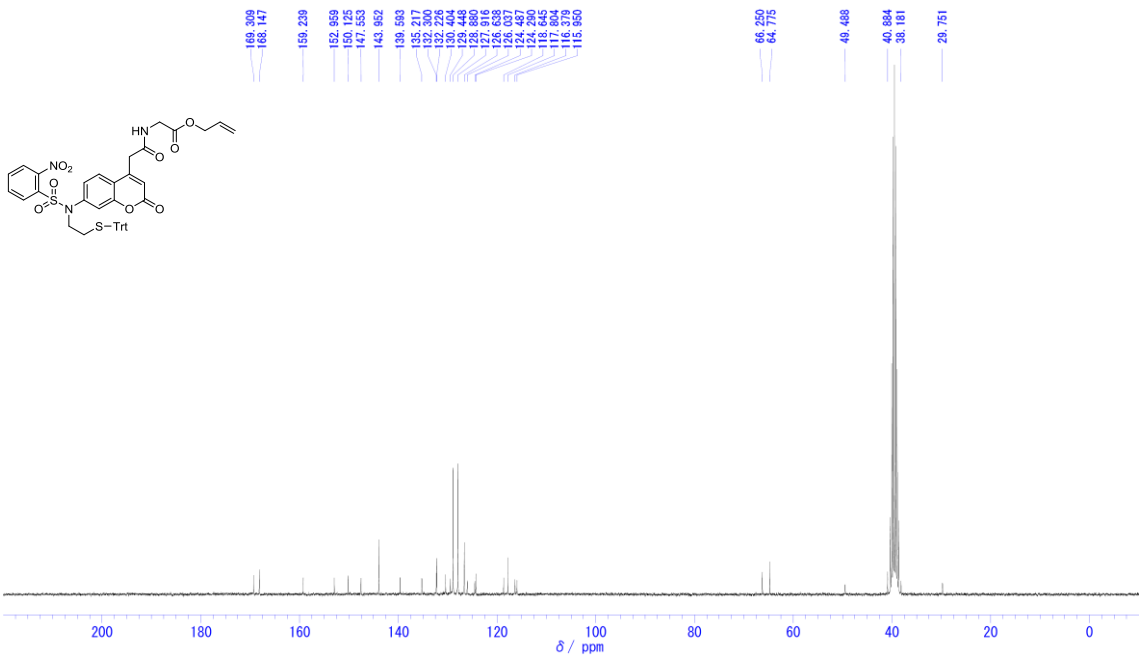
^{13}C NMR spectrum of **6**



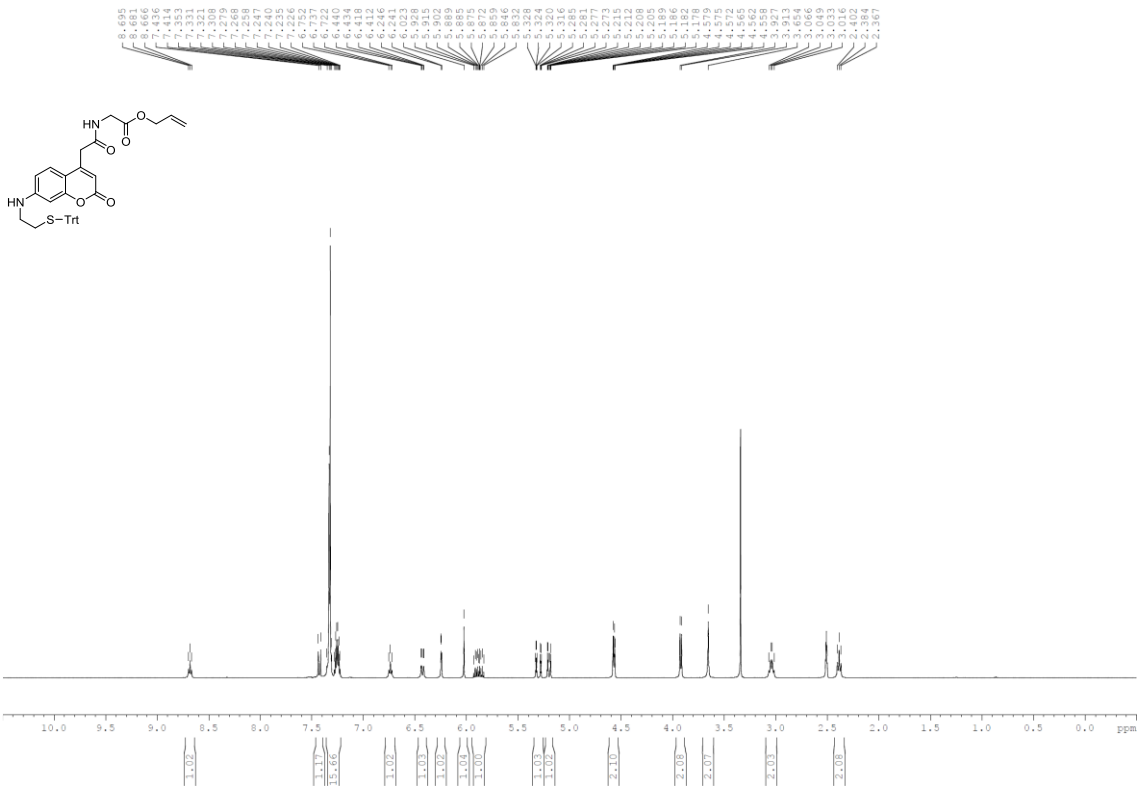
^{1}H NMR spectrum of **7**



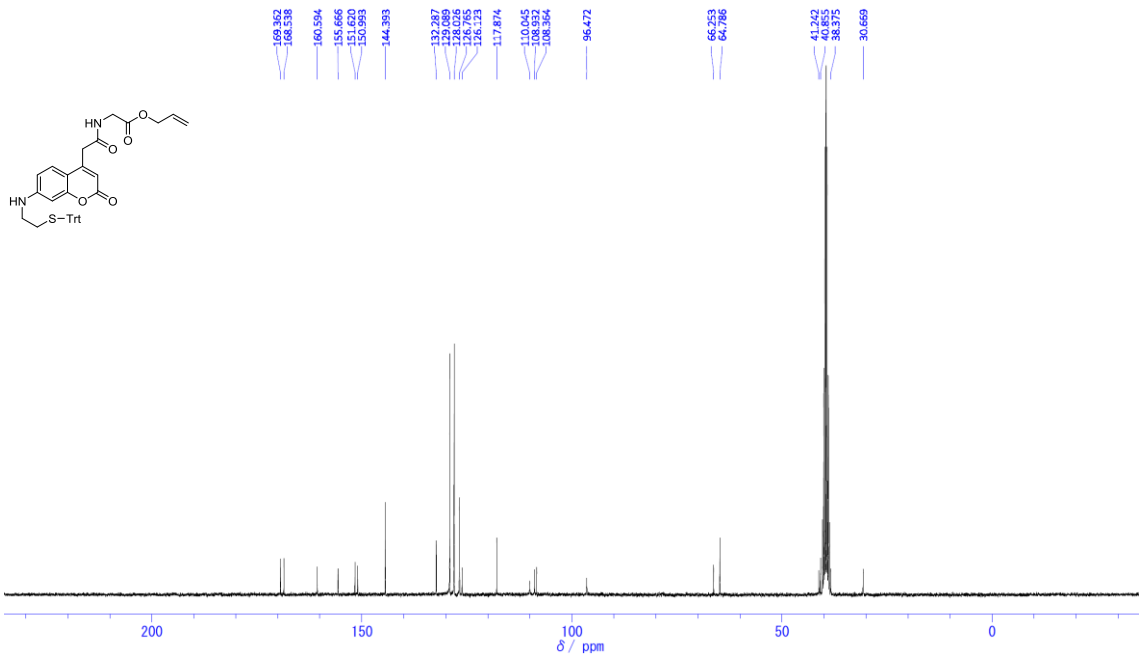
^{13}C NMR spectrum of **7**



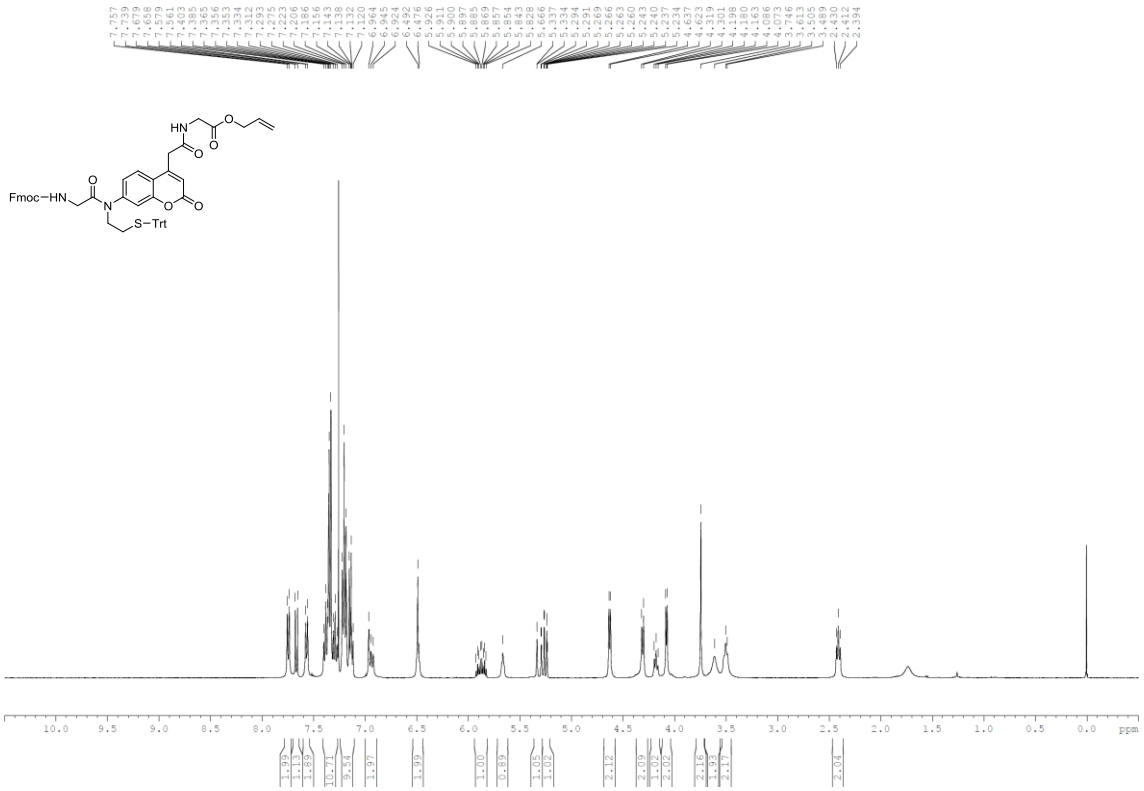
^{1}H NMR spectrum of **3**



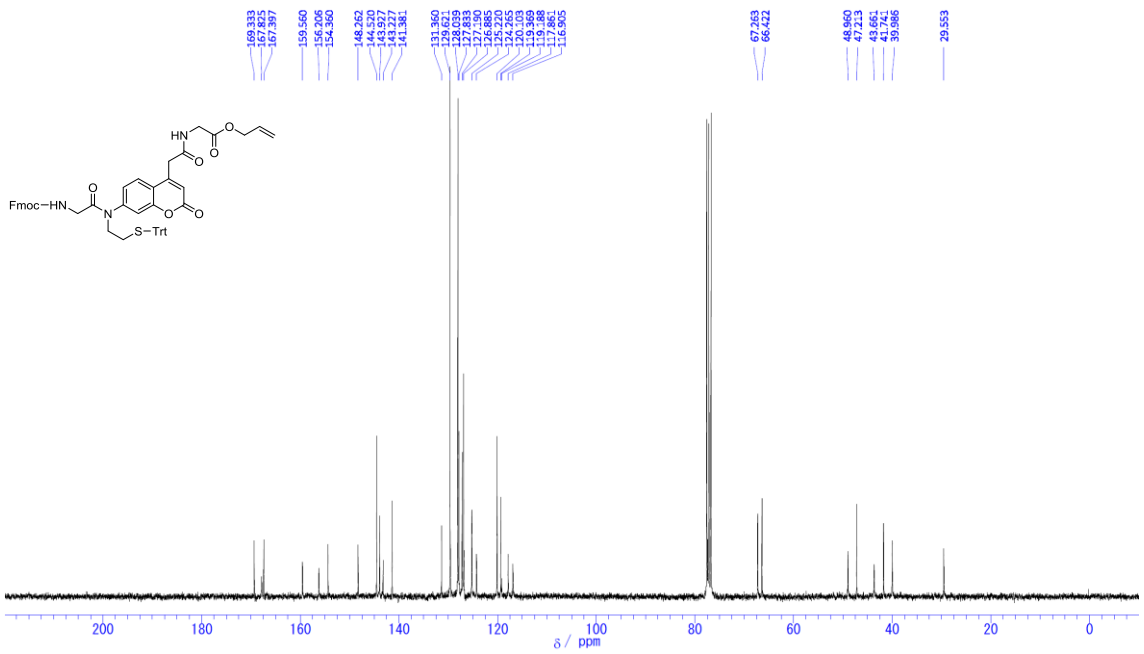
^{13}C NMR spectrum of **3**



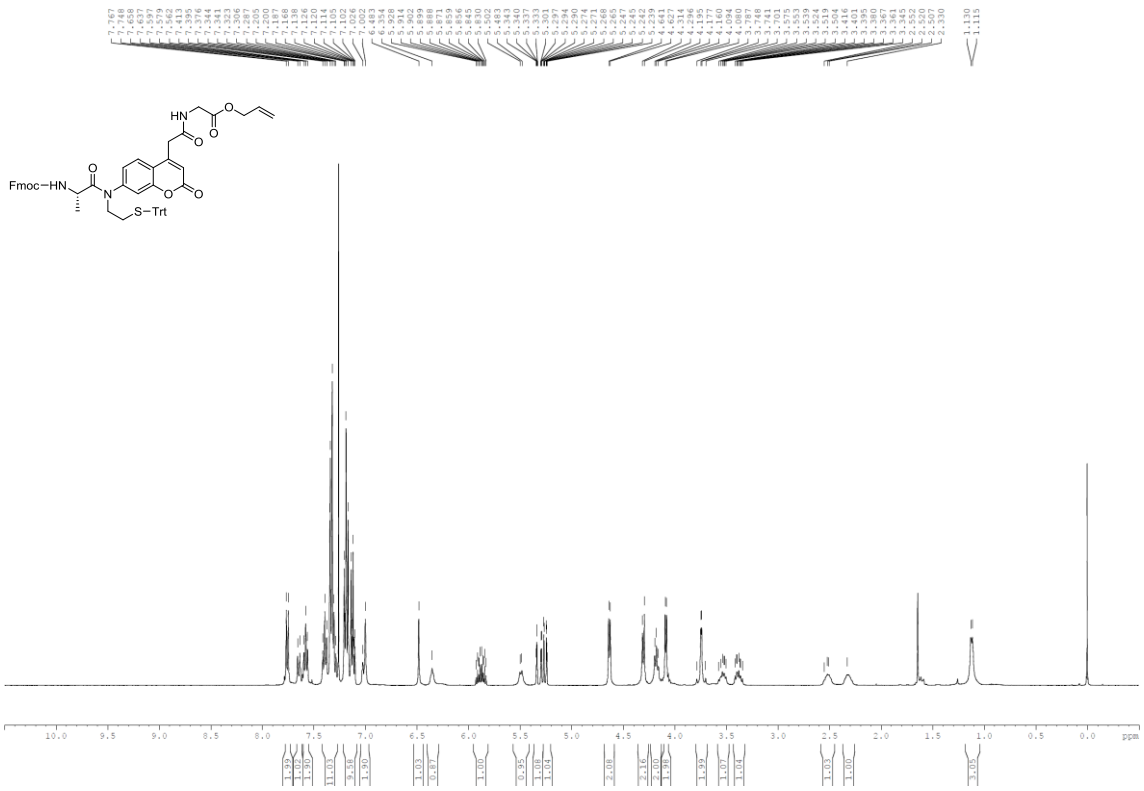
^{1}H NMR spectrum of **8a**



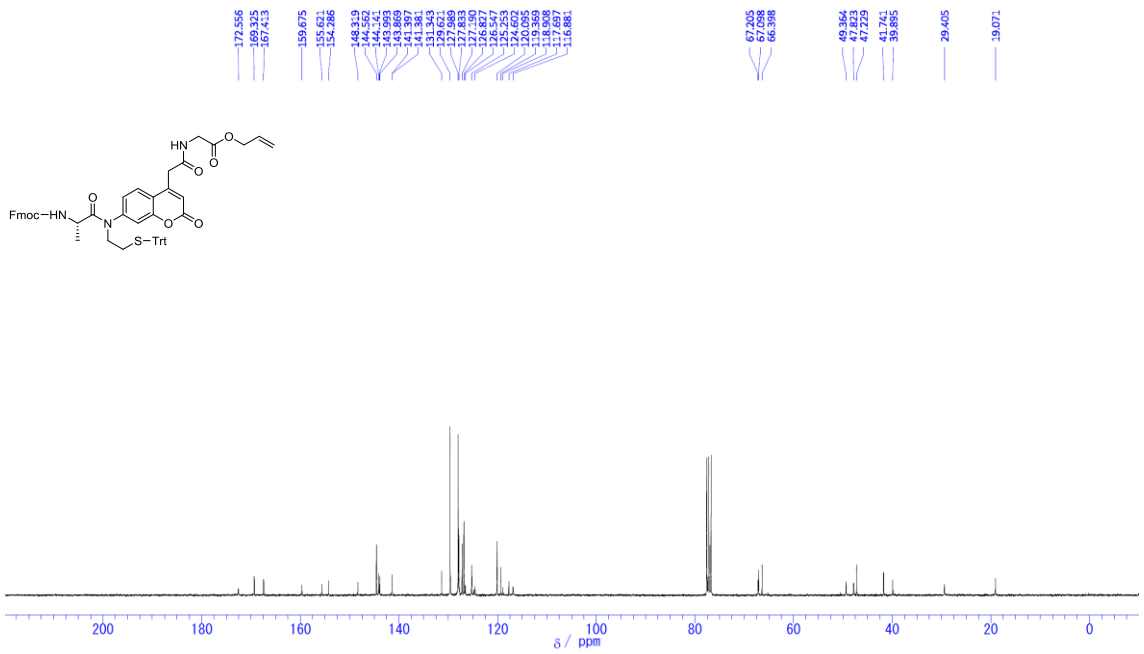
^{13}C NMR spectrum of **8a**



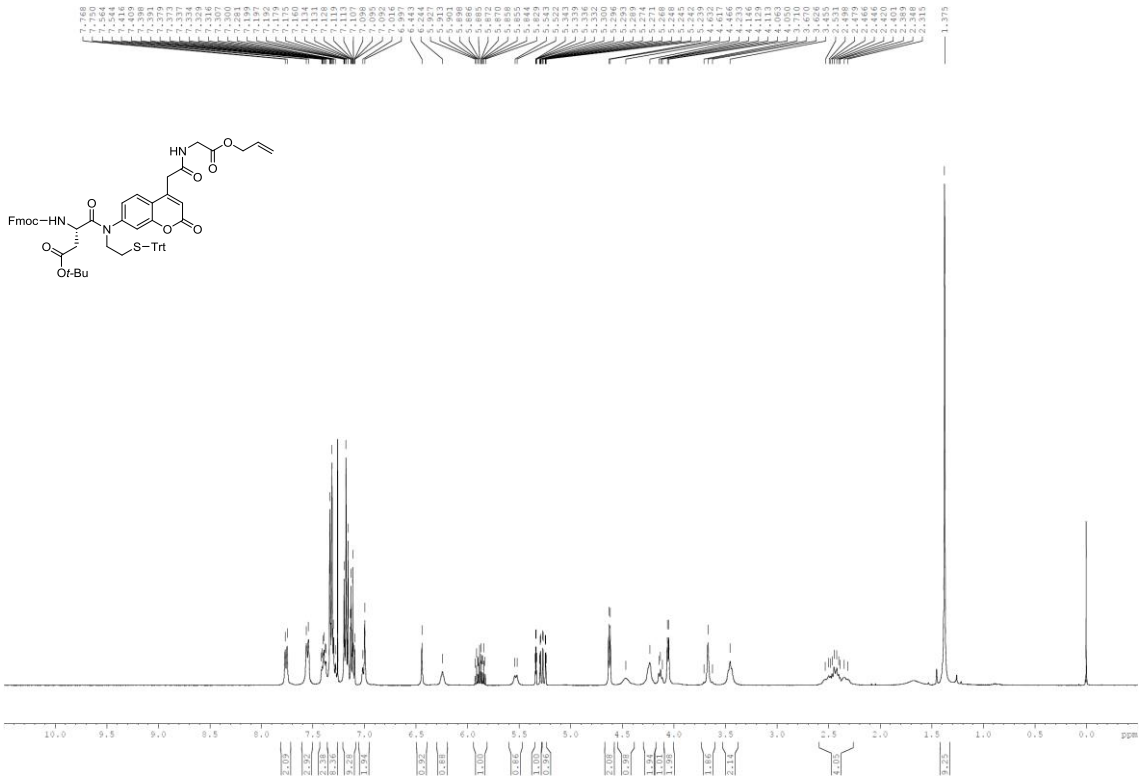
^{1}H NMR spectrum of **8b**



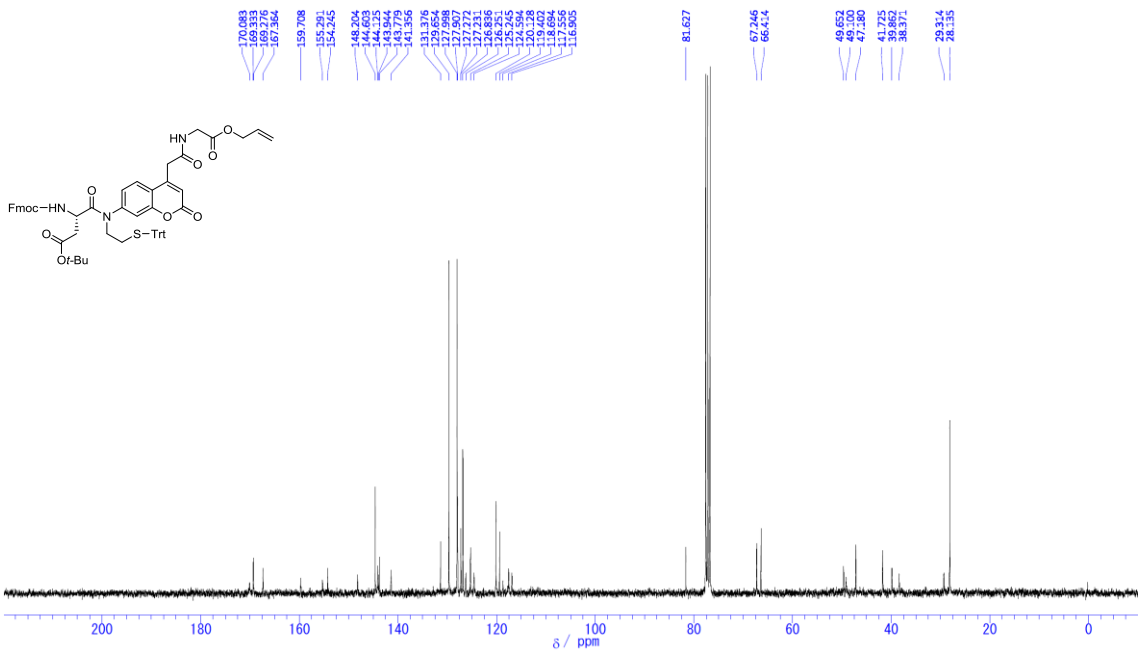
^{13}C NMR spectrum of **8b**



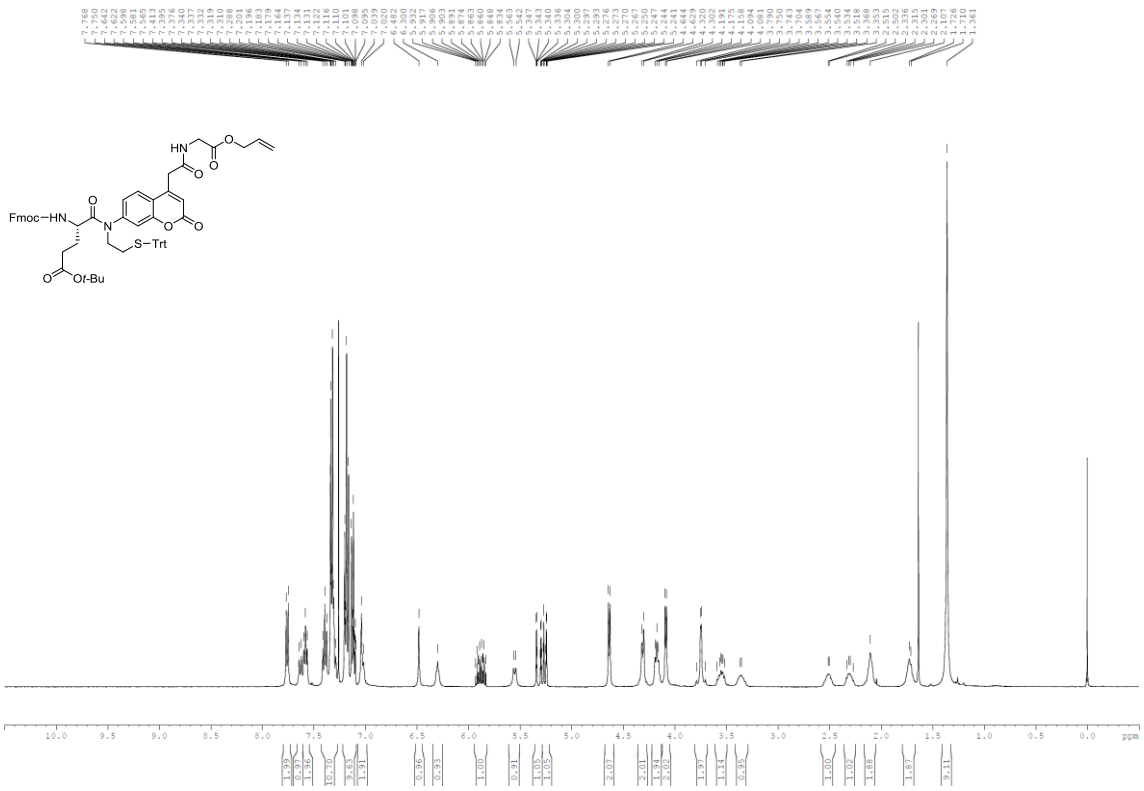
^{1}H NMR spectrum of **8c**



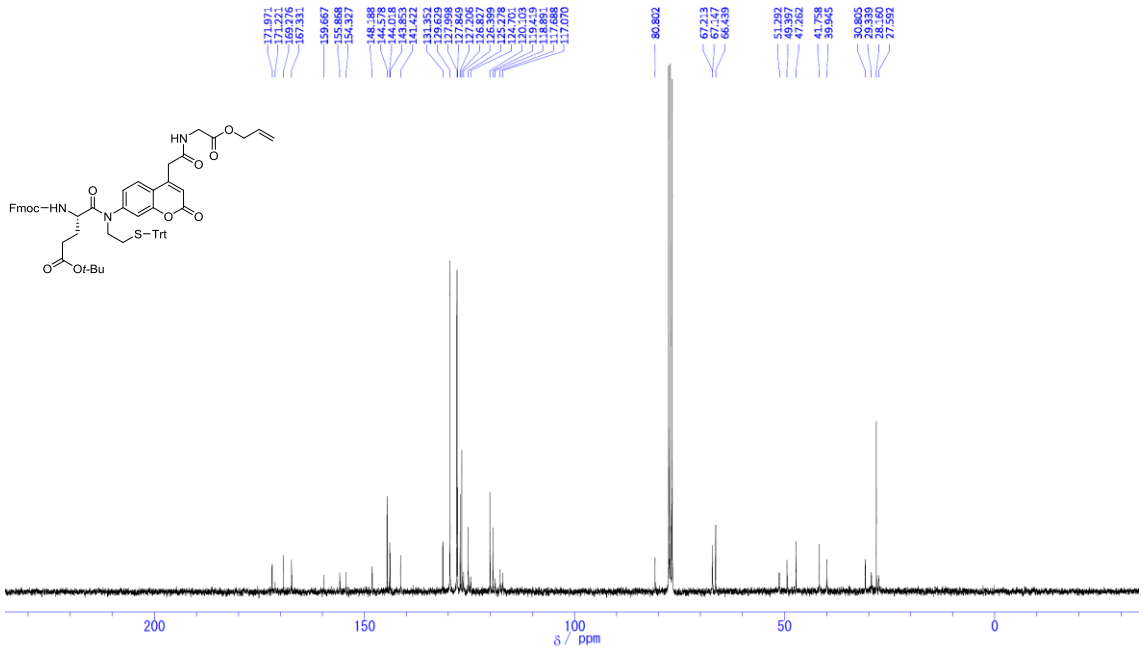
^{13}C NMR spectrum of **8c**



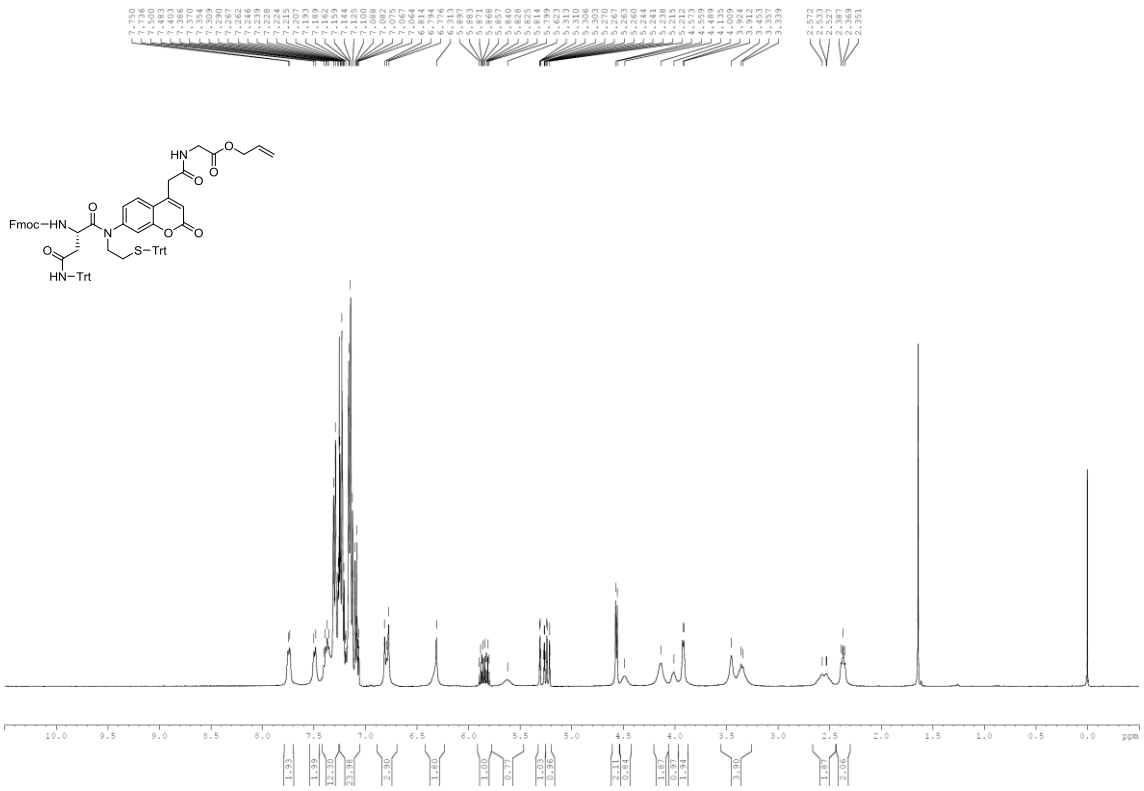
^{1}H NMR spectrum of **8d**



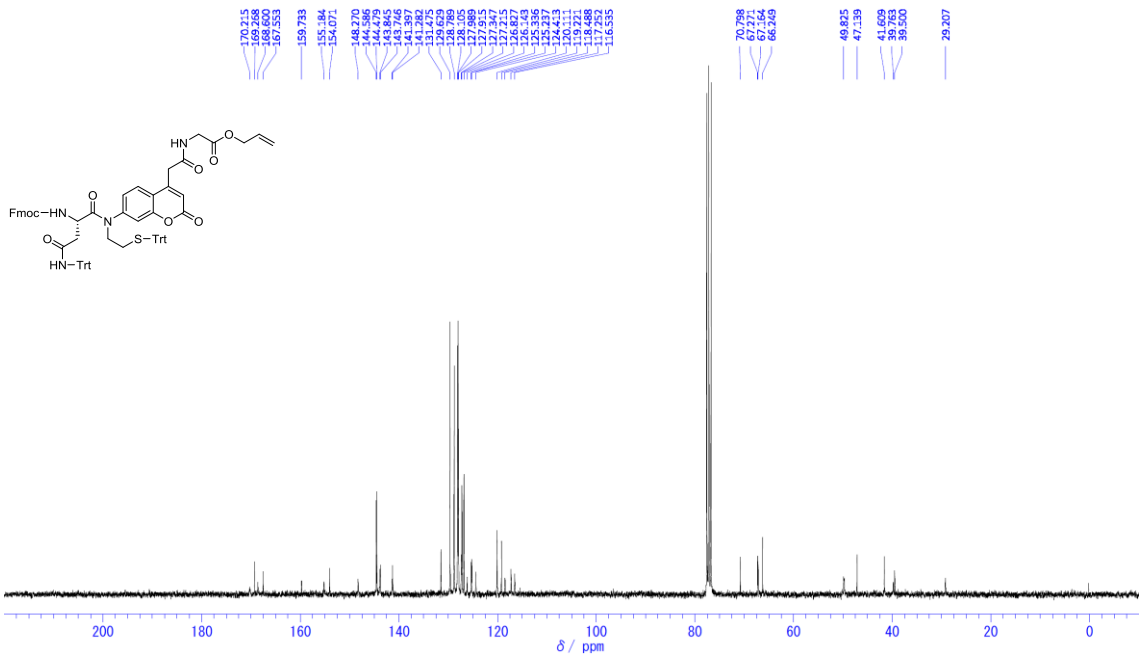
^{13}C NMR spectrum of **8d**



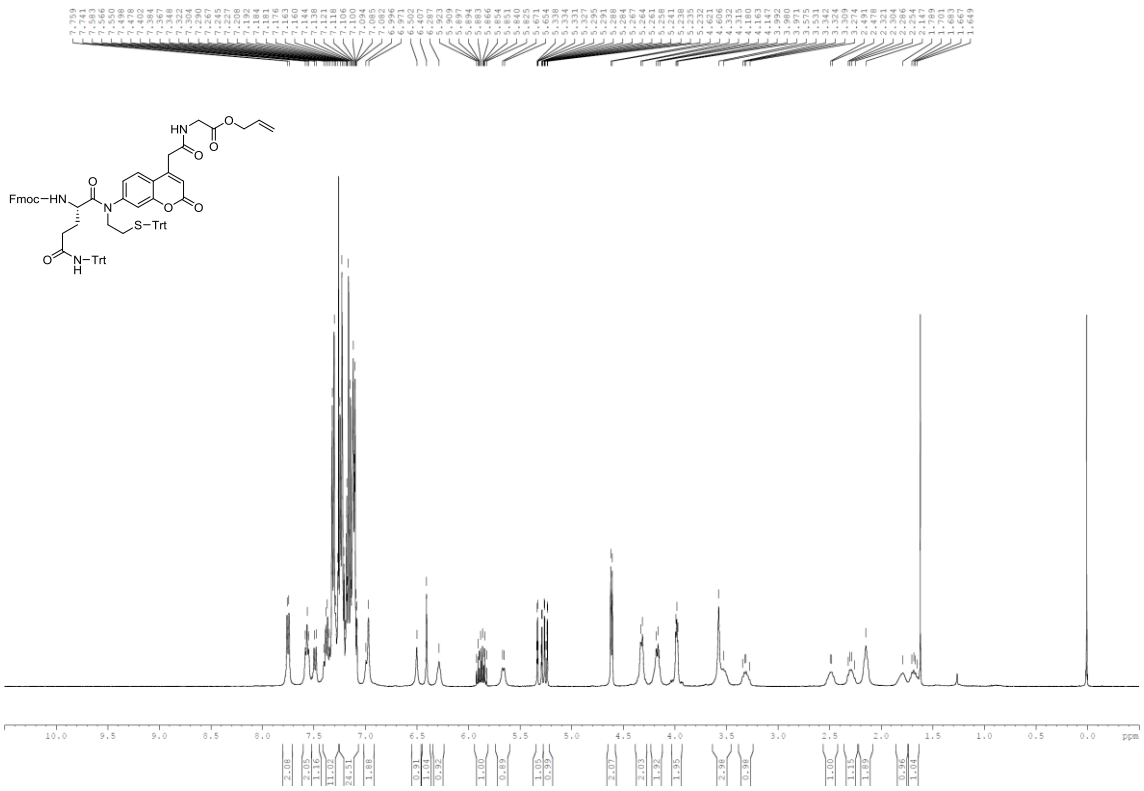
^{1}H NMR spectrum of **8e**



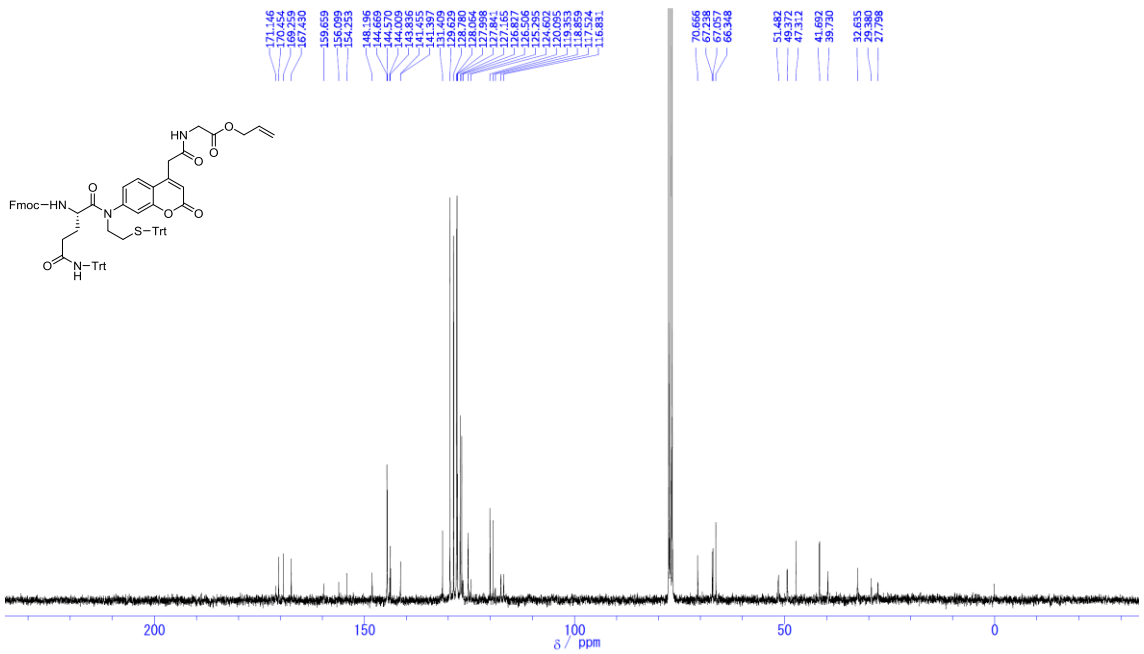
^{13}C NMR spectrum of 8**e**



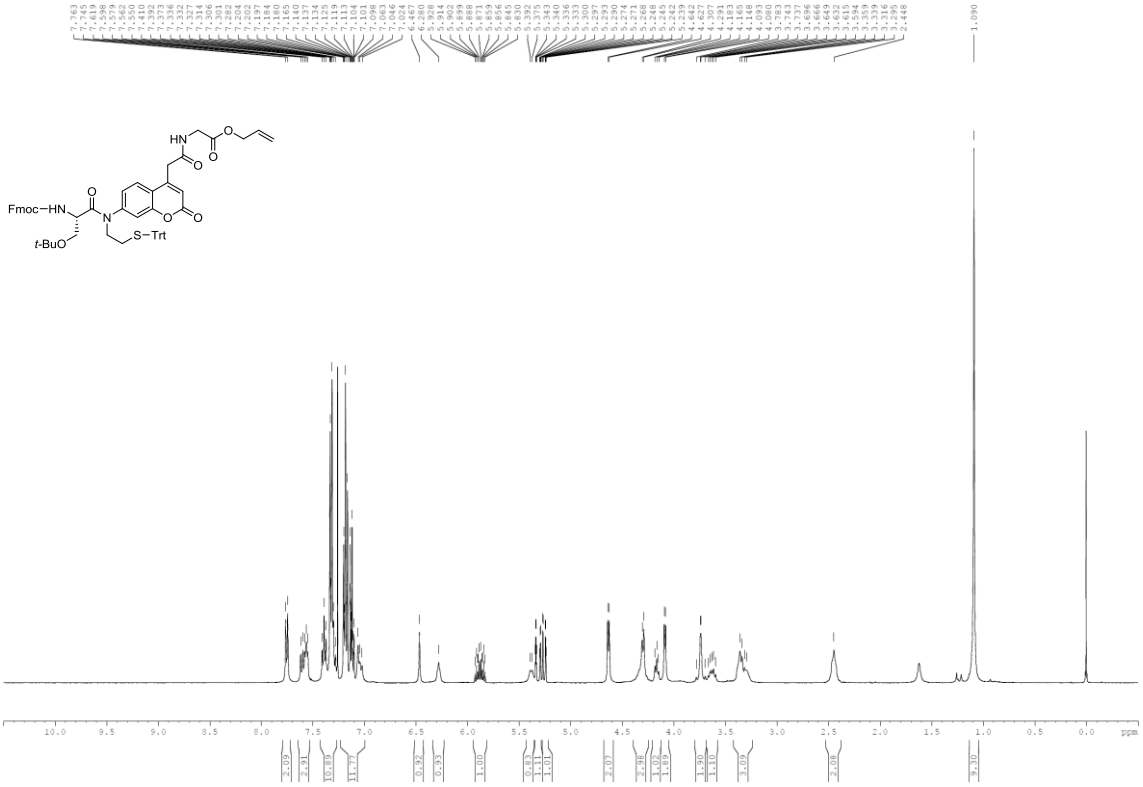
^{1}H NMR spectrum of **8f**



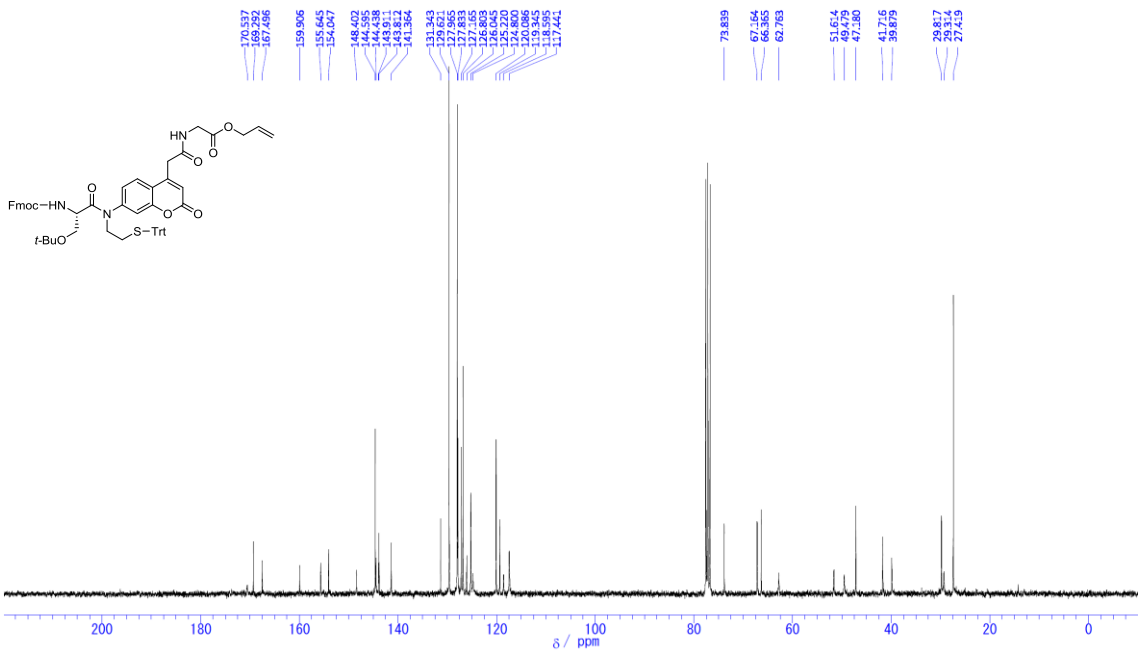
^{13}C NMR spectrum of **8f**



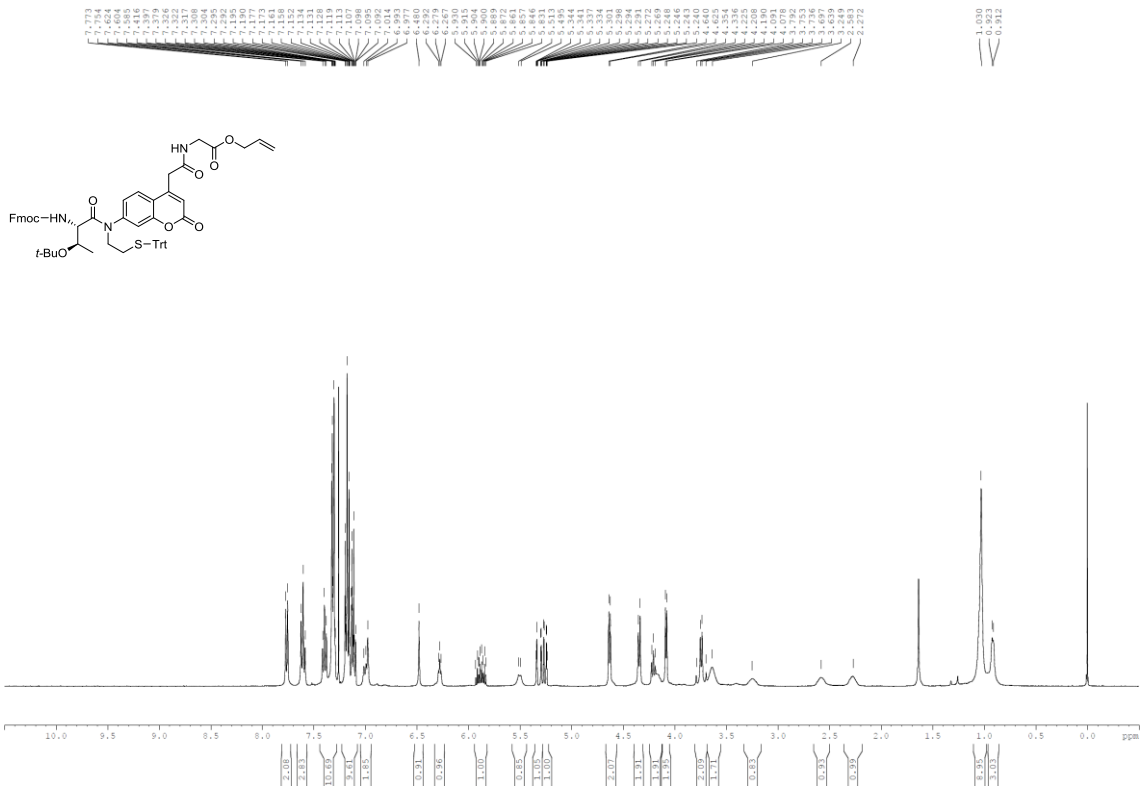
^{1}H NMR spectrum of **8g**



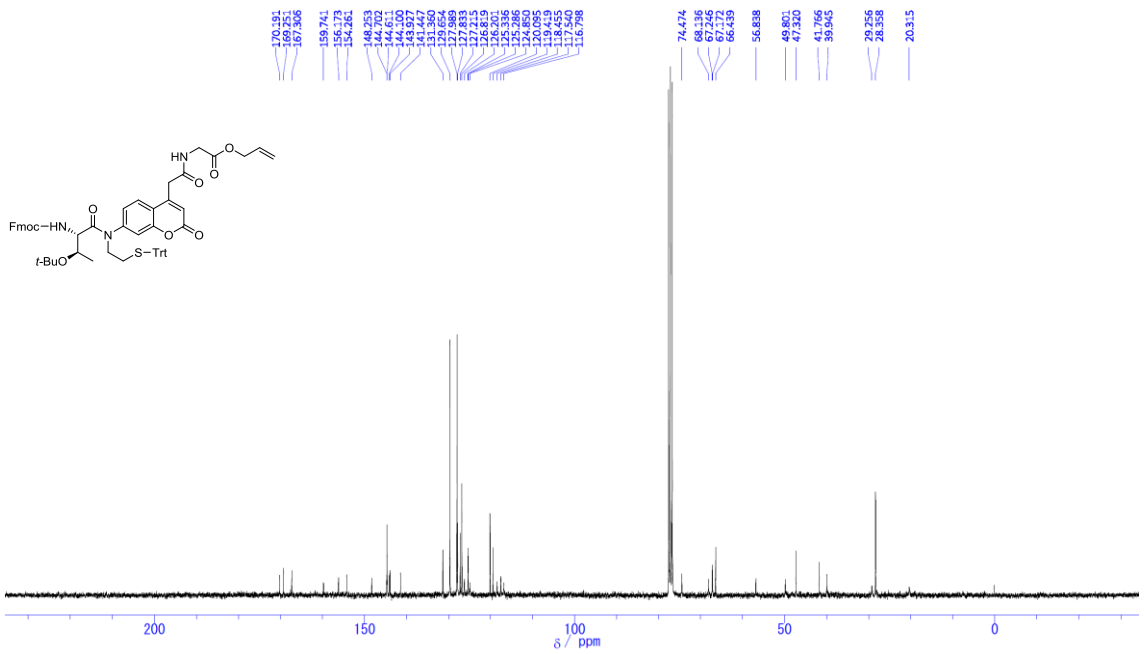
^{13}C NMR spectrum of **8g**



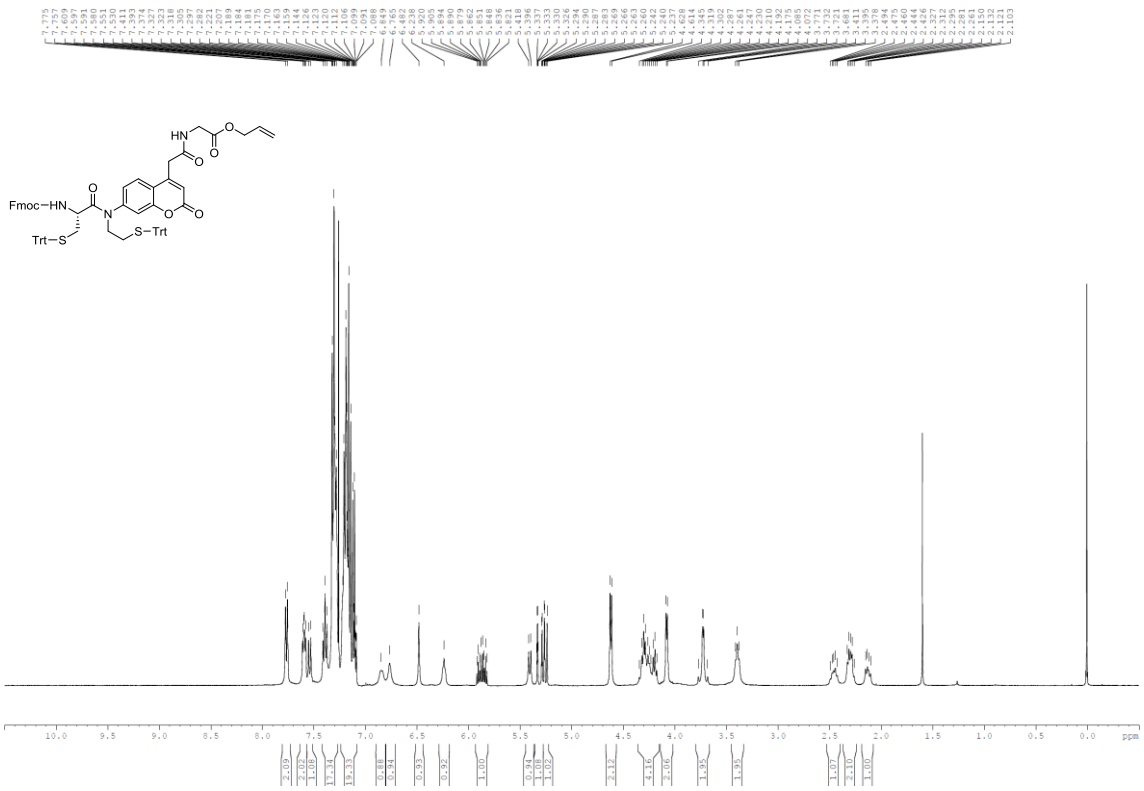
^{1}H NMR spectrum of **8h**



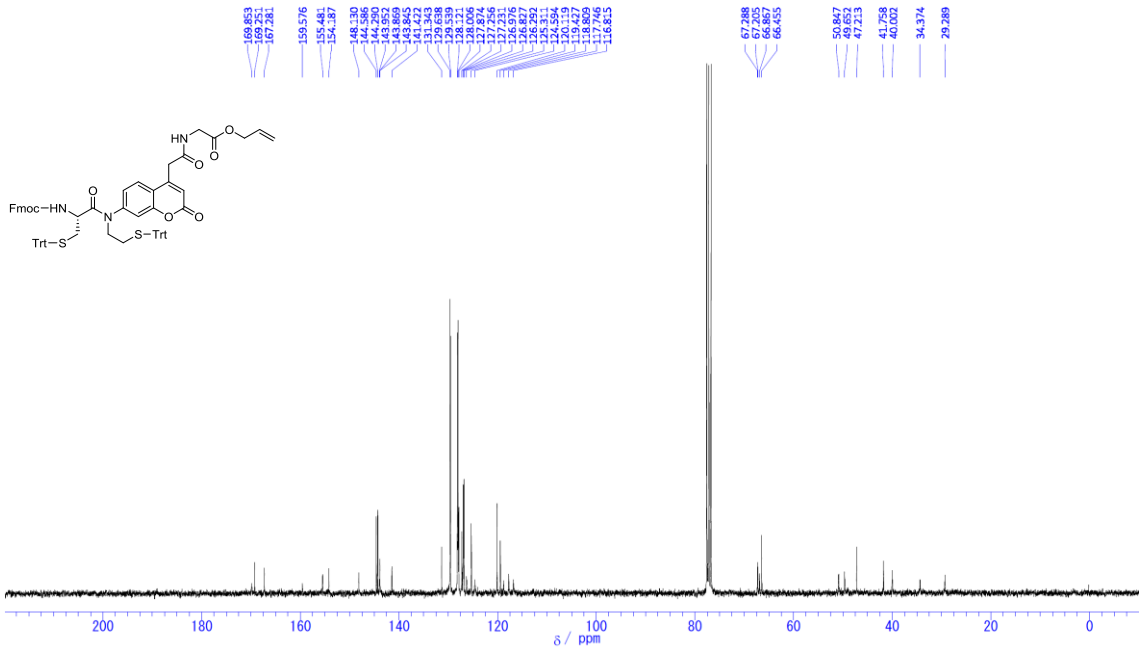
^{13}C NMR spectrum of **8h**



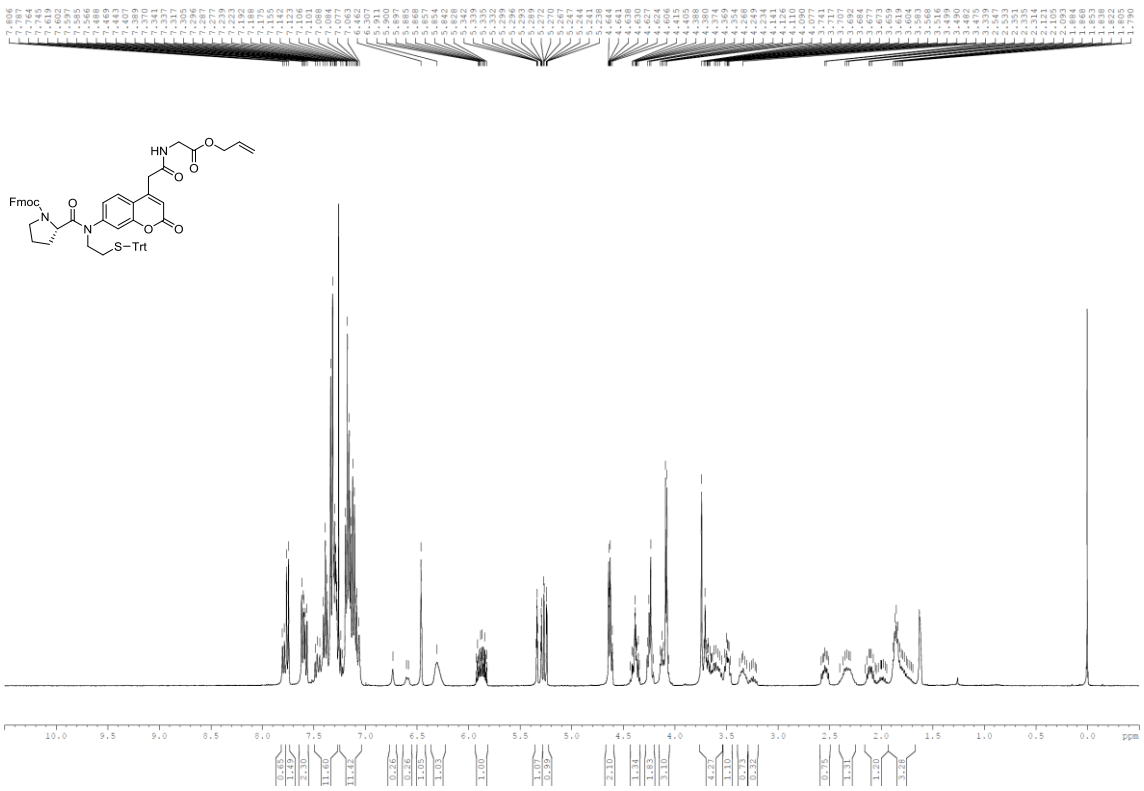
^{1}H NMR spectrum of **8i**



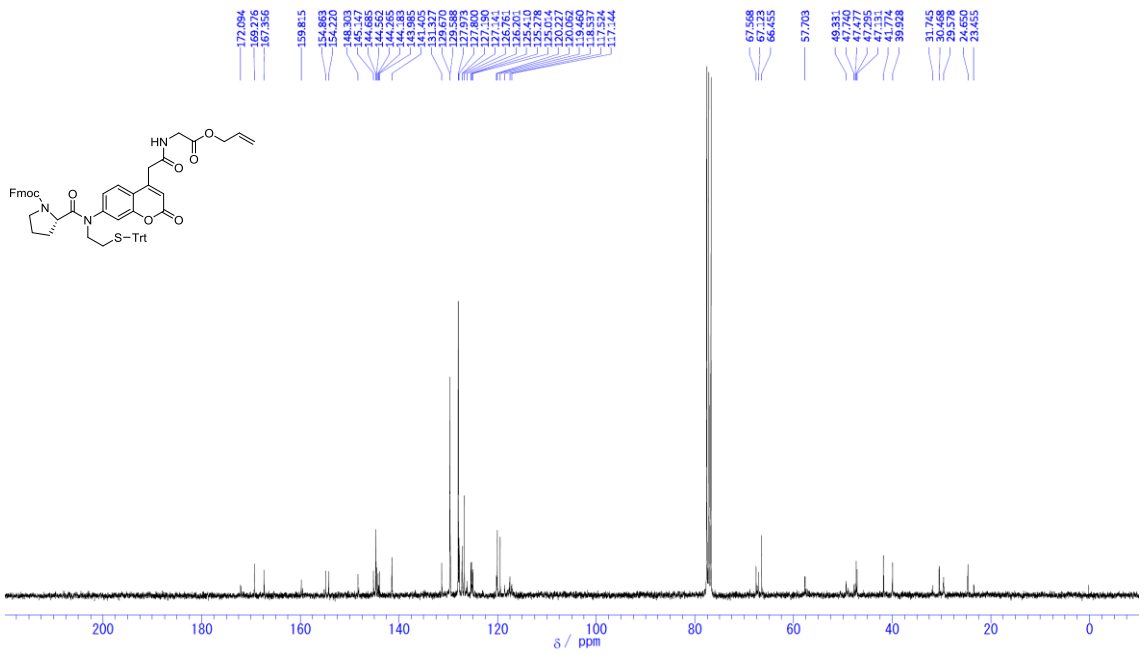
^{13}C NMR spectrum of **8i**



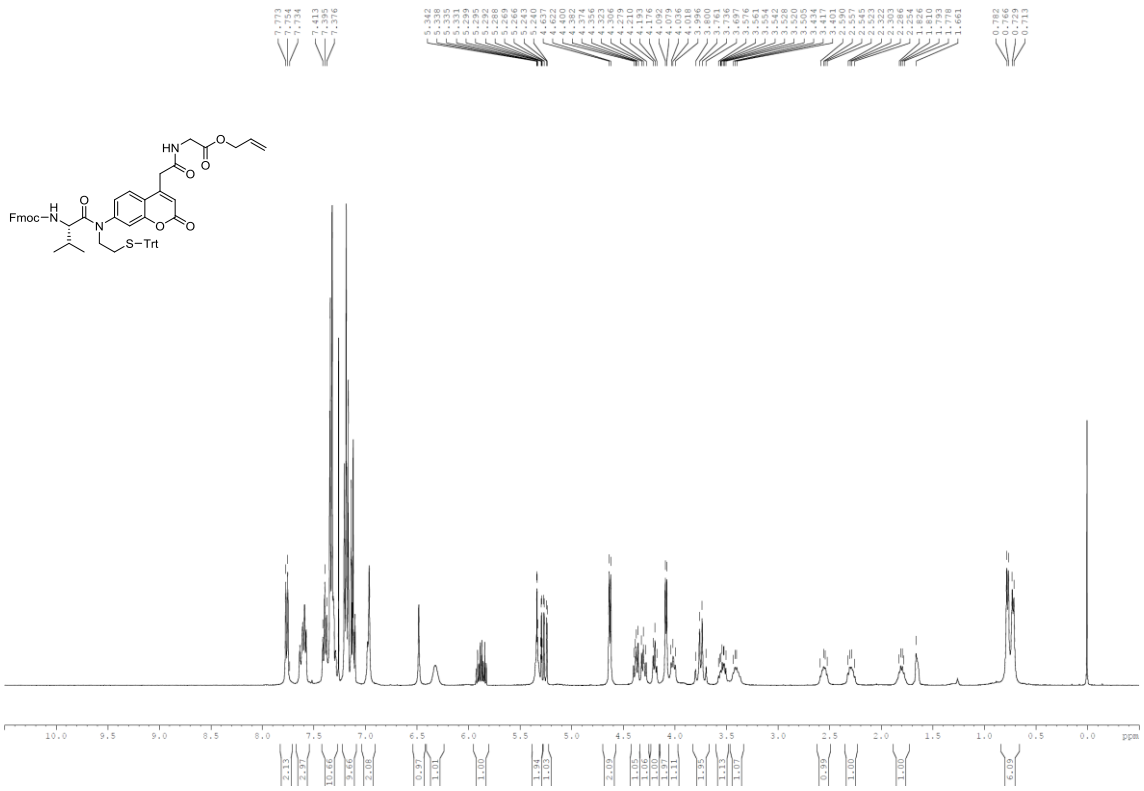
^{1}H NMR spectrum of **8j**



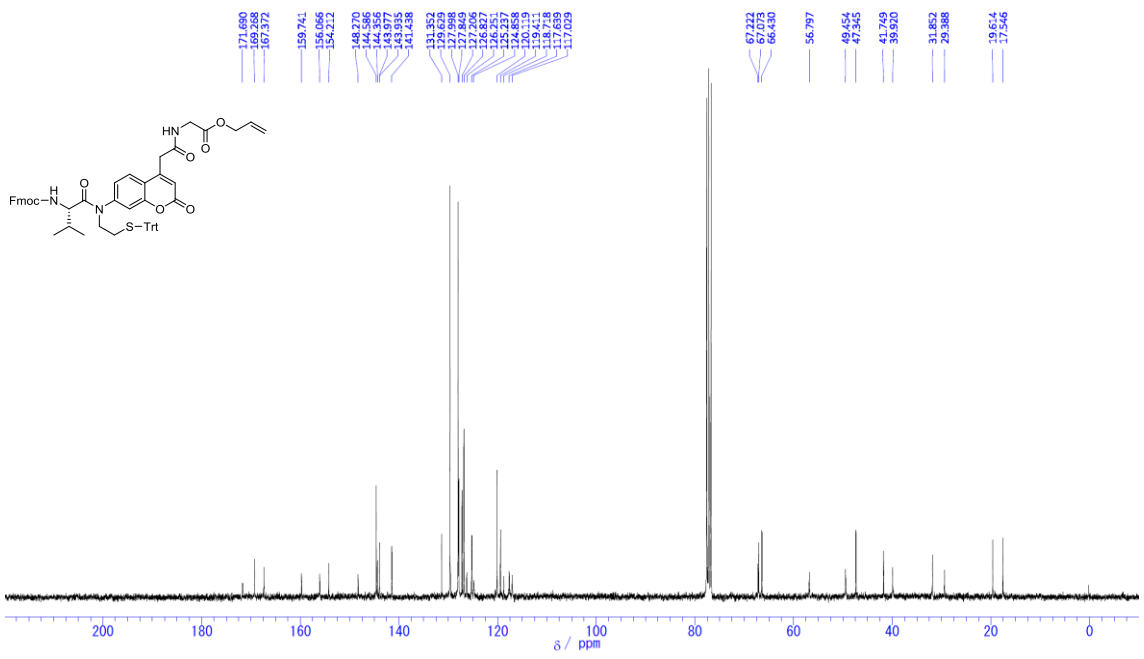
^{13}C NMR spectrum of **8j**



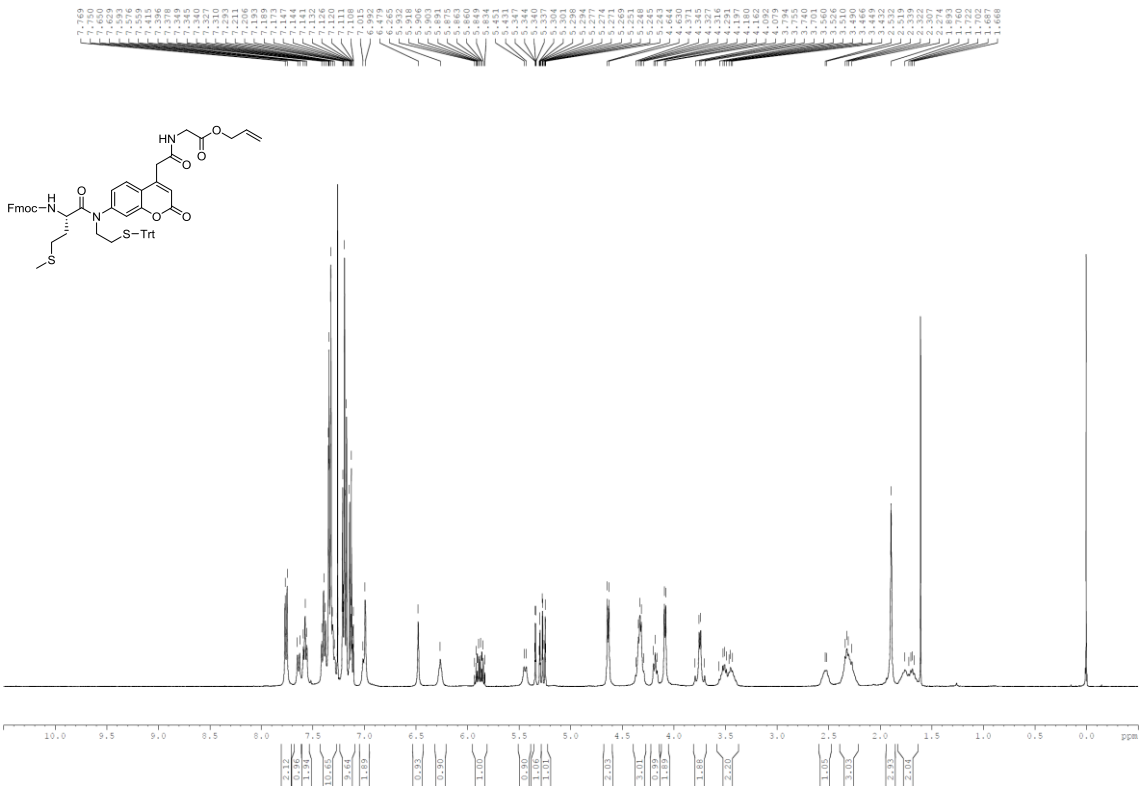
^{1}H NMR spectrum of **8k**



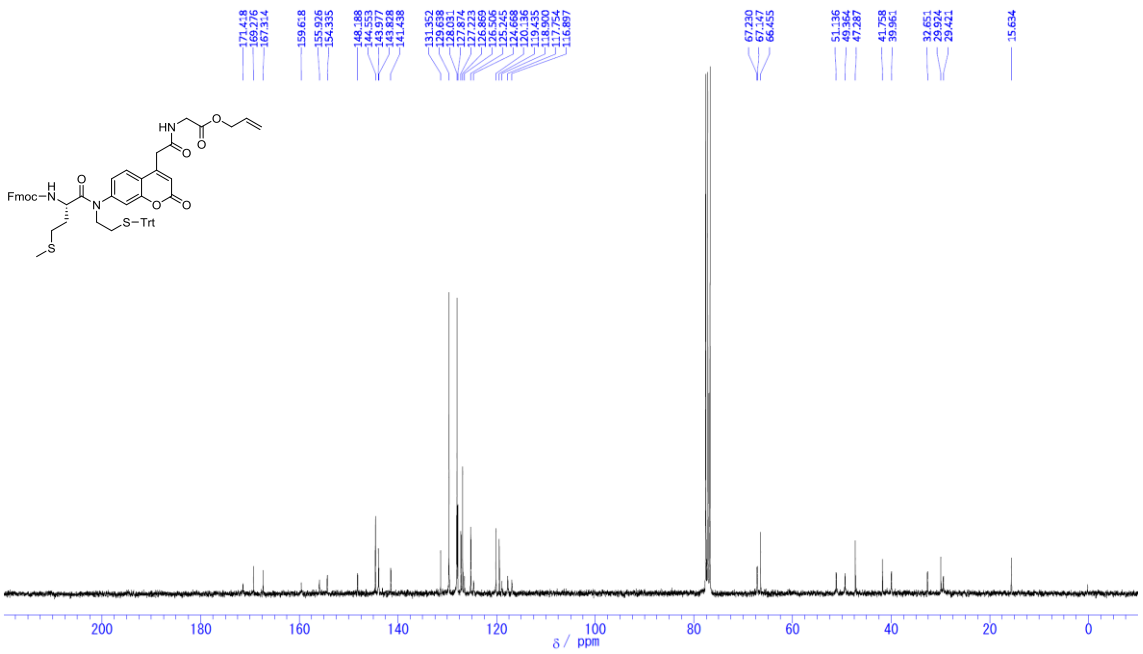
^{13}C NMR spectrum of **8k**



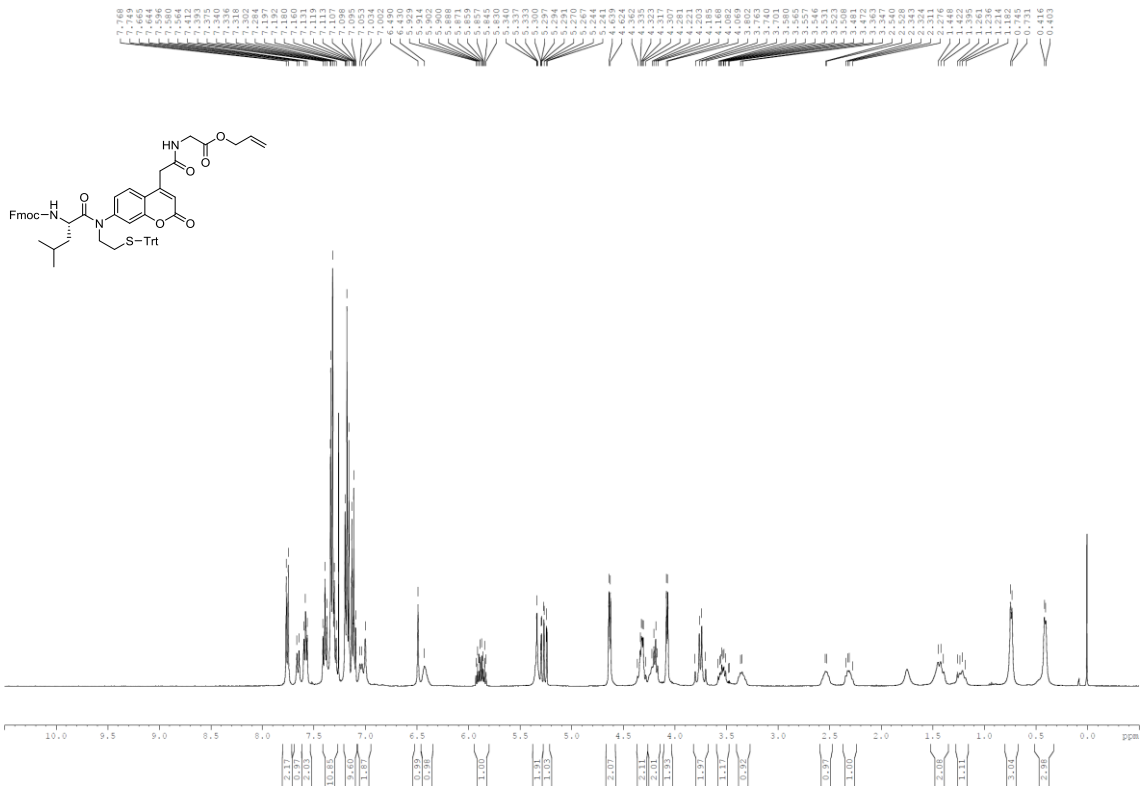
^{1}H NMR spectrum of **8l**



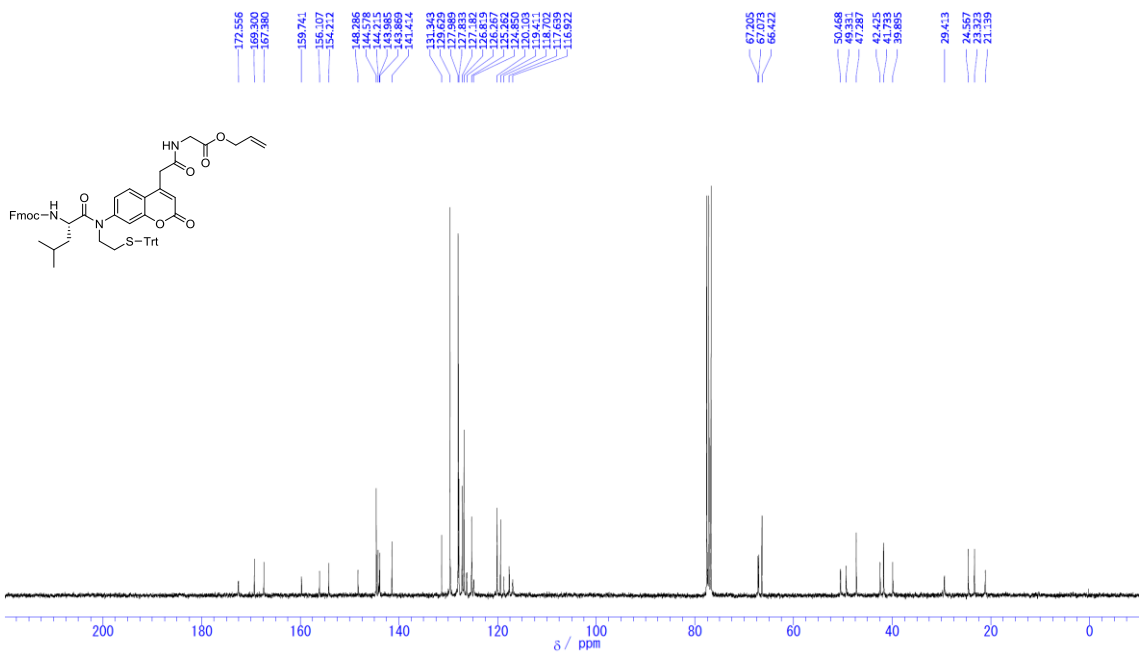
^{13}C NMR spectrum of **8l**



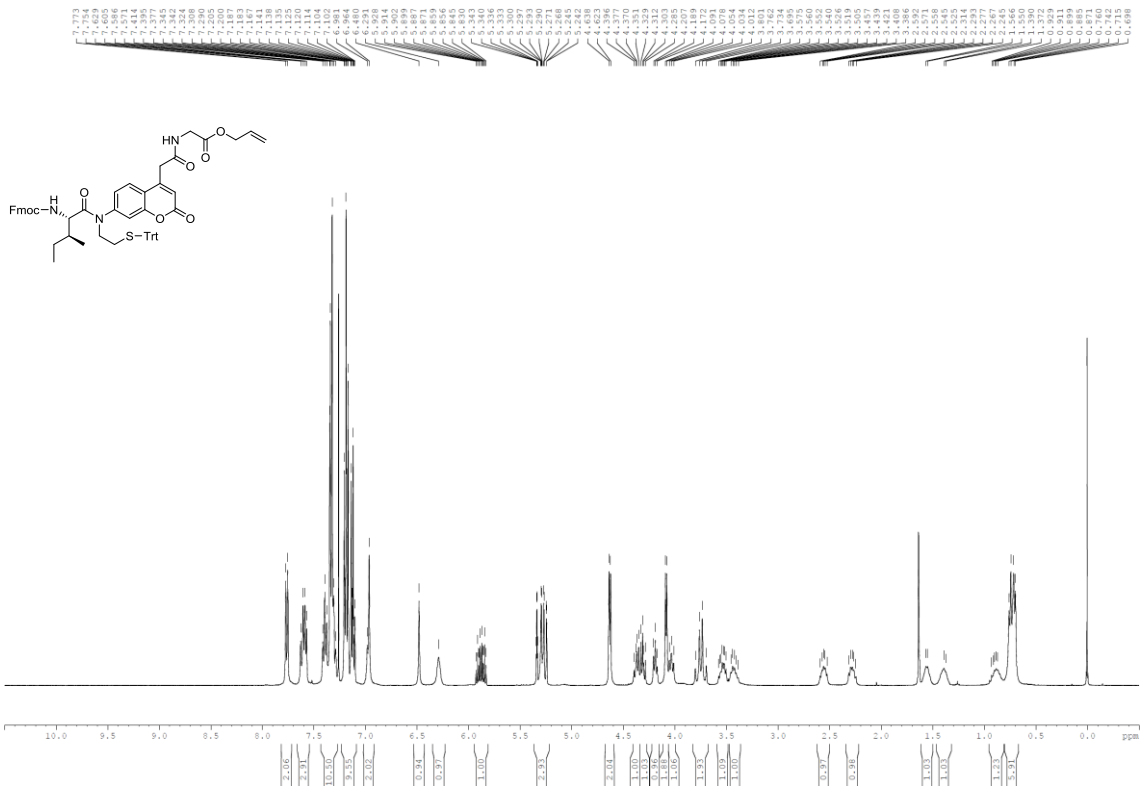
^{1}H NMR spectrum of **8m**



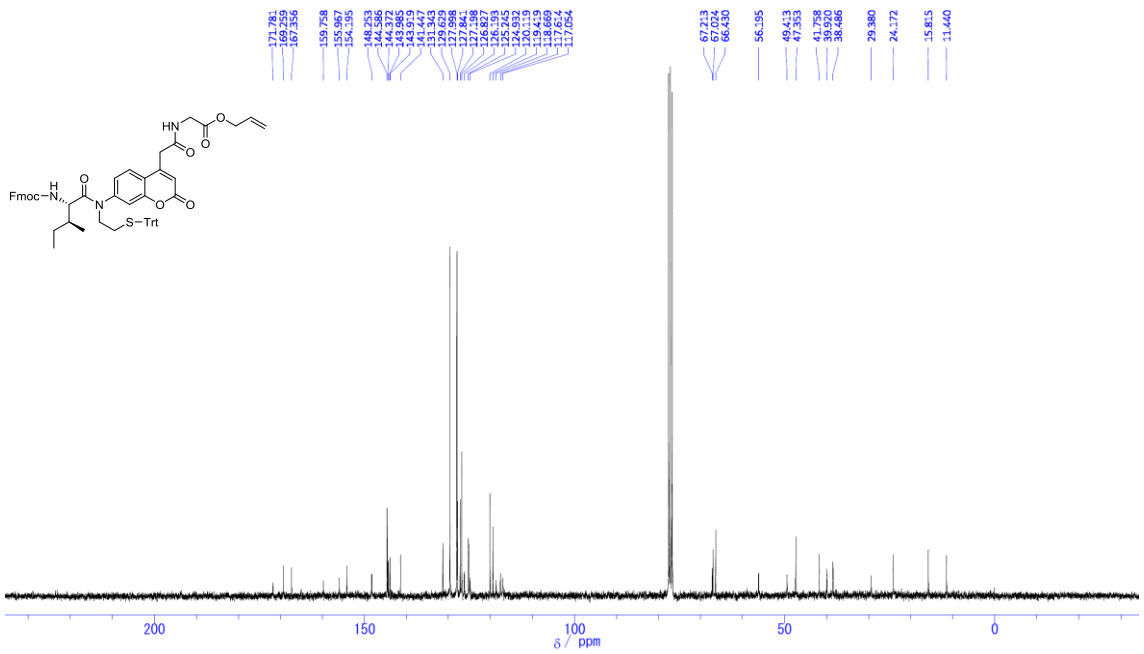
^{13}C NMR spectrum of **8m**



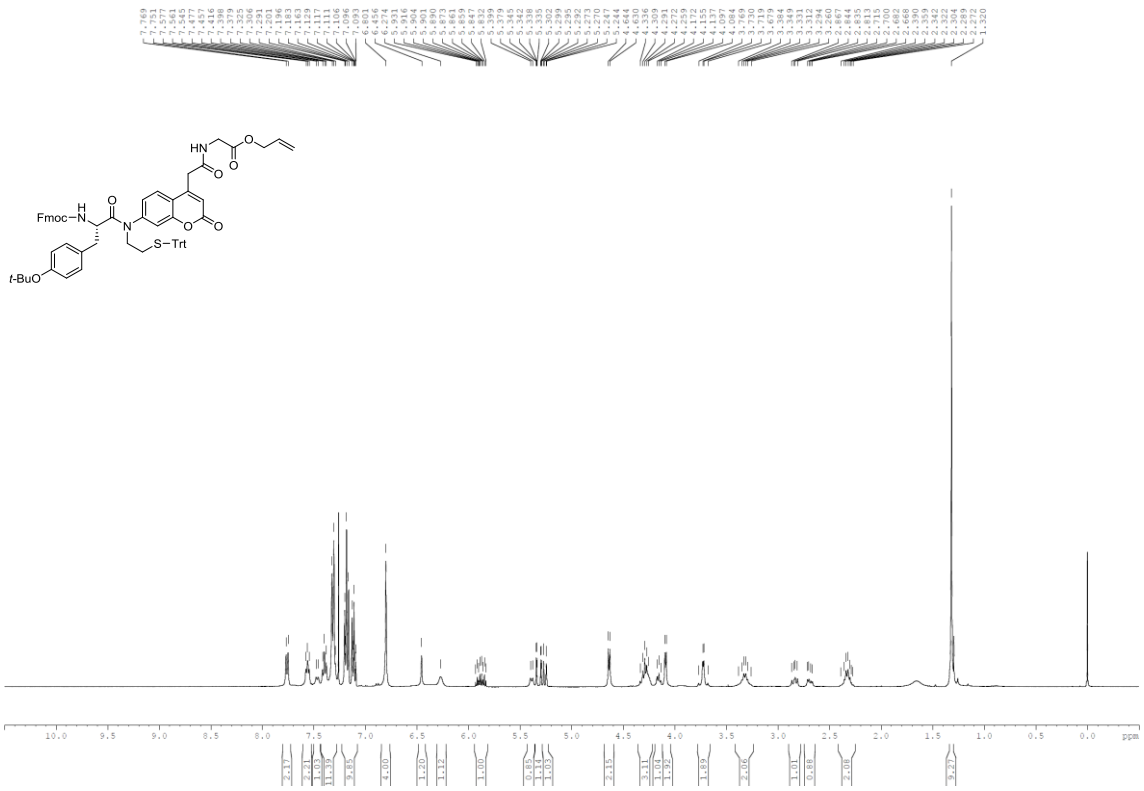
^{1}H NMR spectrum of **8n**



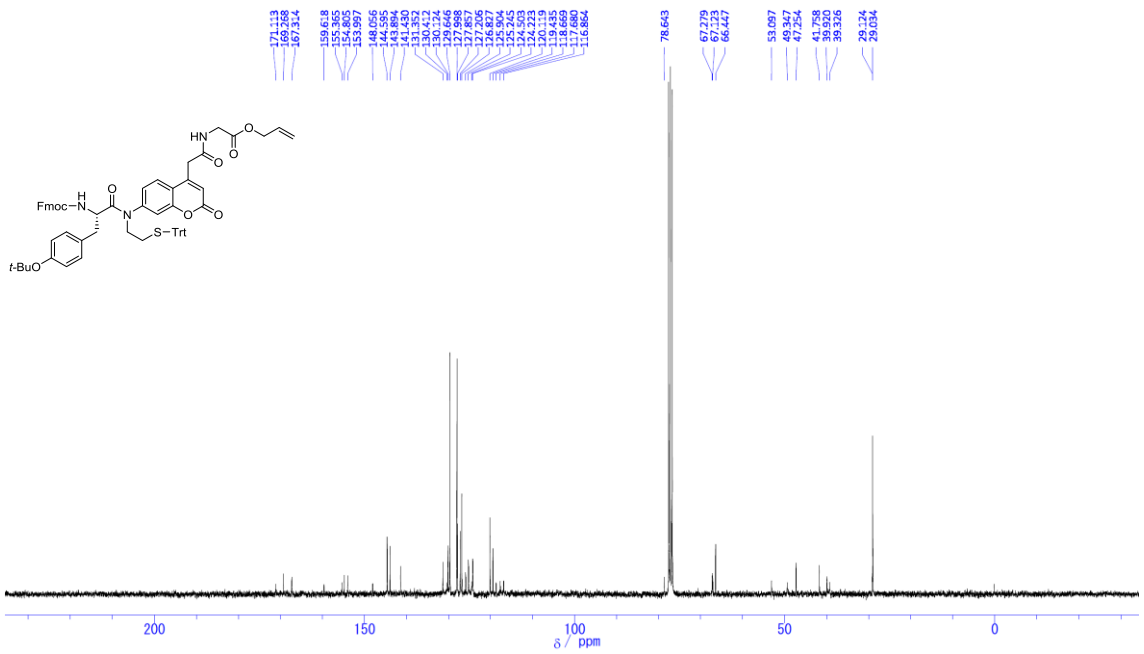
^{13}C NMR spectrum of **8n**



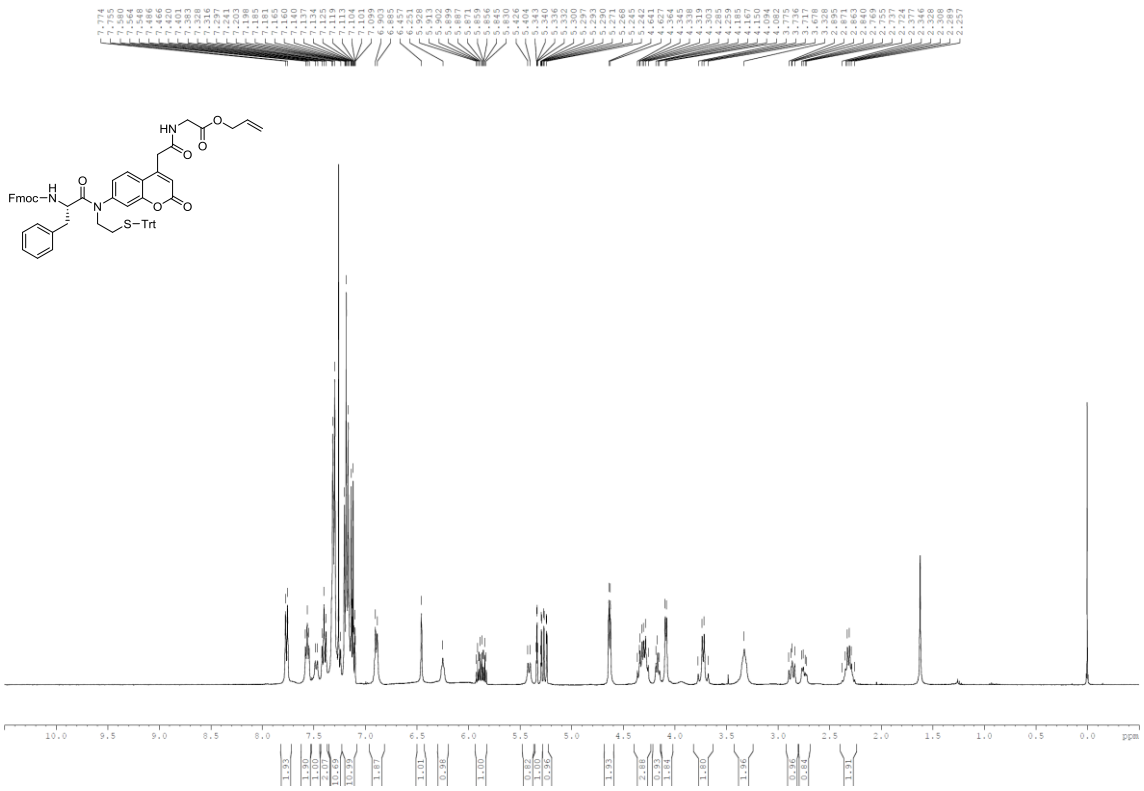
^{1}H NMR spectrum of **8o**



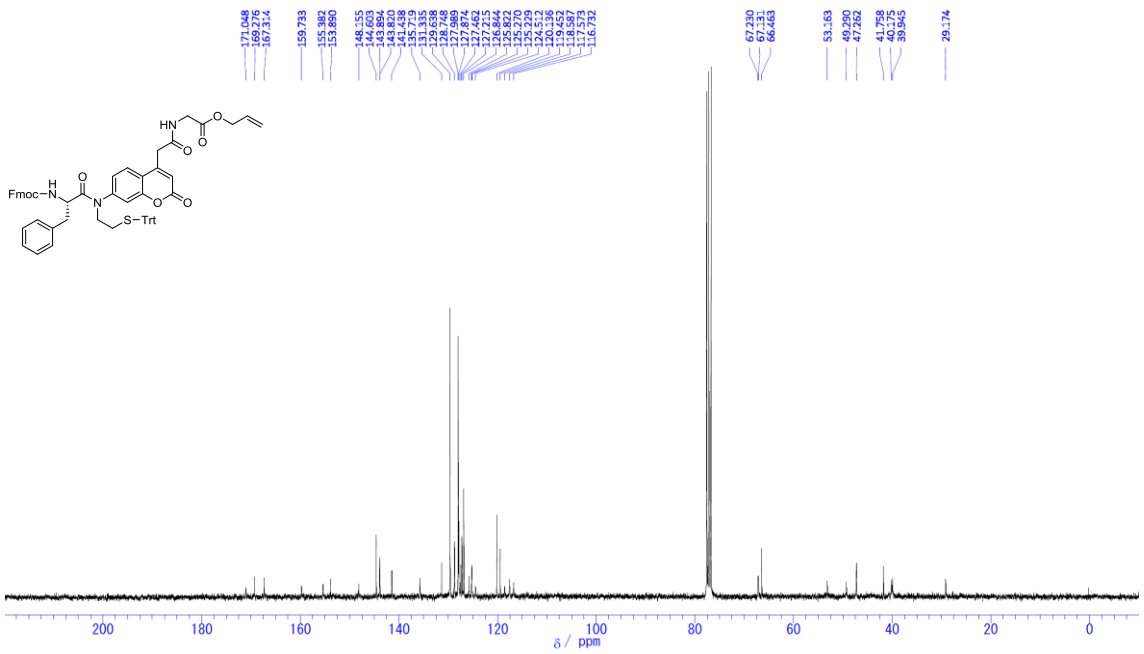
^{13}C NMR spectrum of **8o**



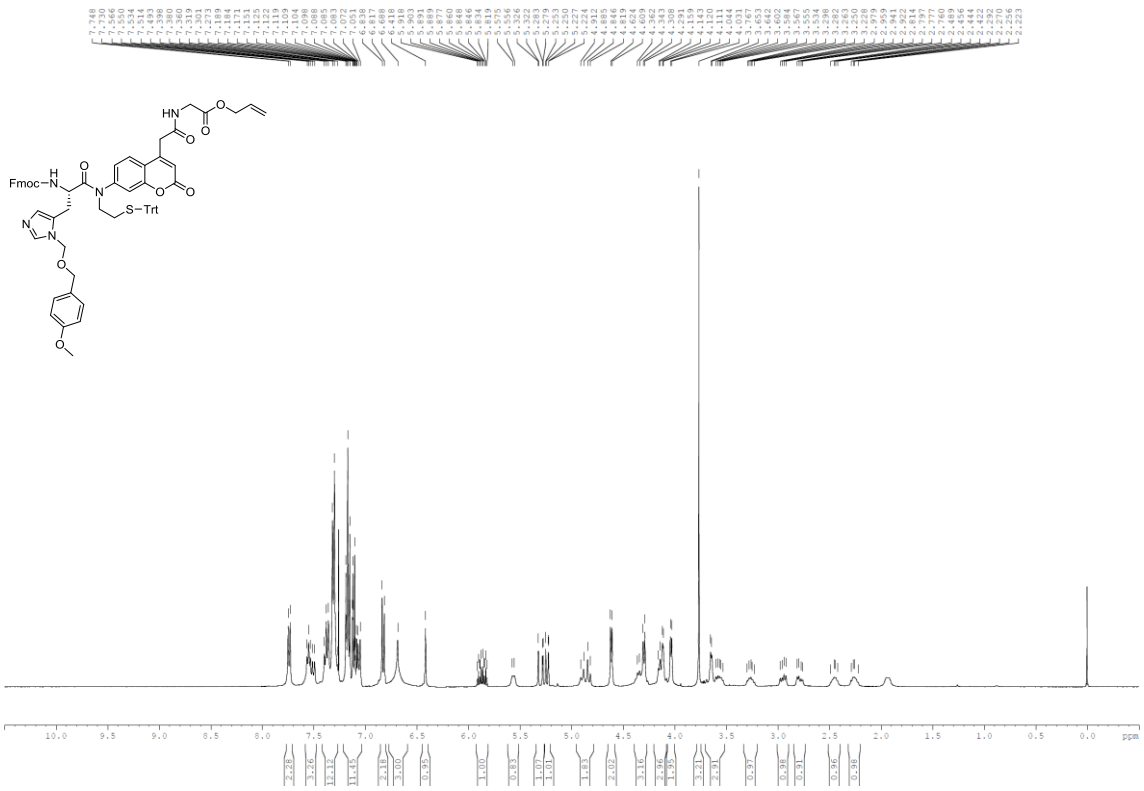
^{1}H NMR spectrum of **8p**



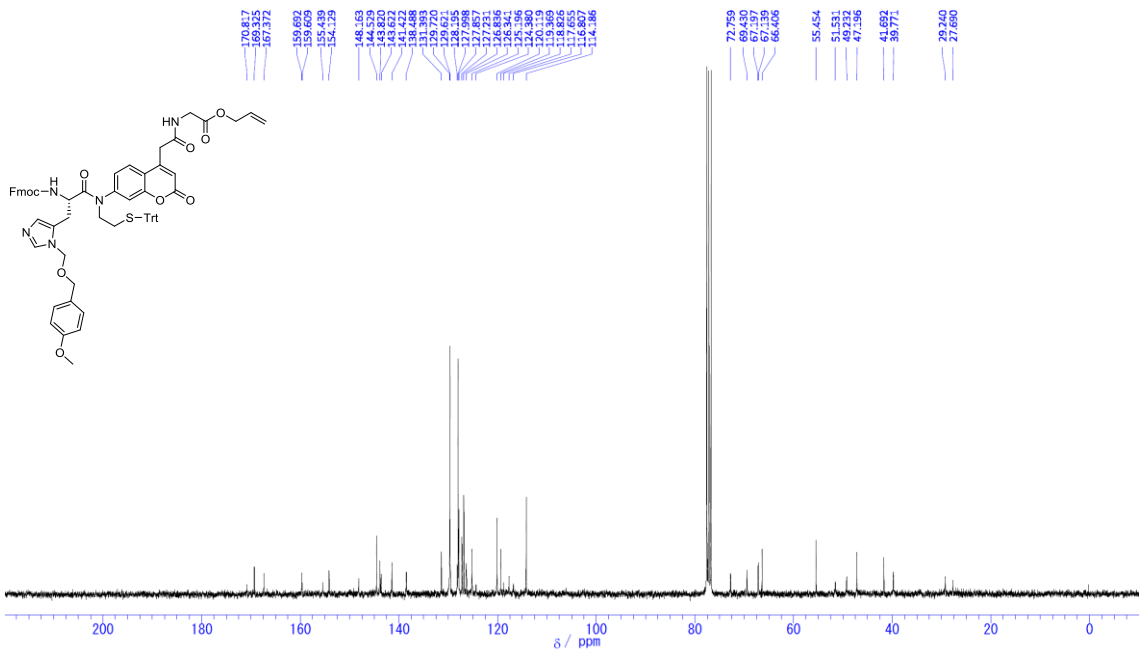
^{13}C NMR spectrum of **8p**



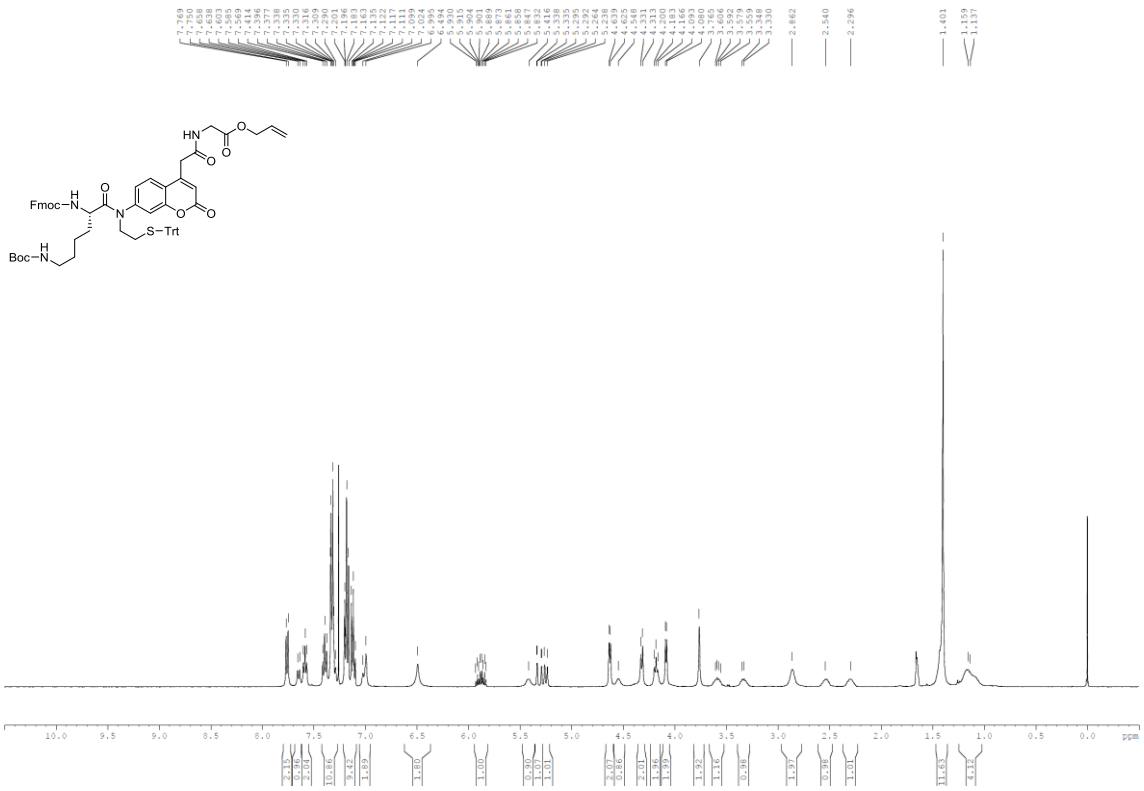
^{1}H NMR spectrum of **8q**



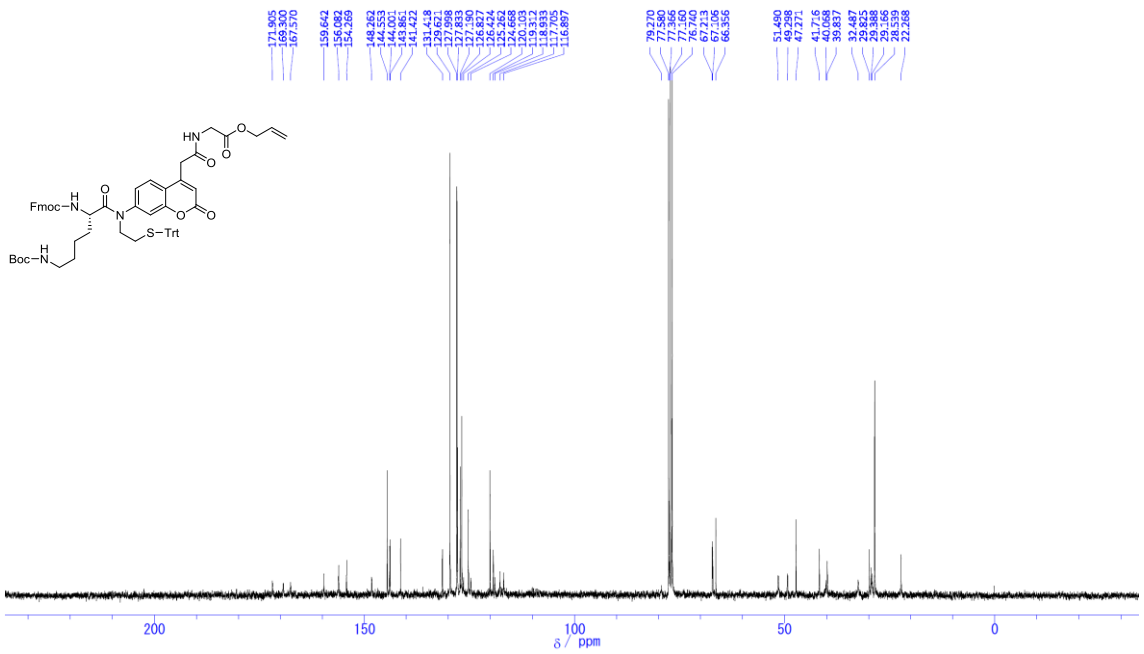
^{13}C NMR spectrum of **8q**



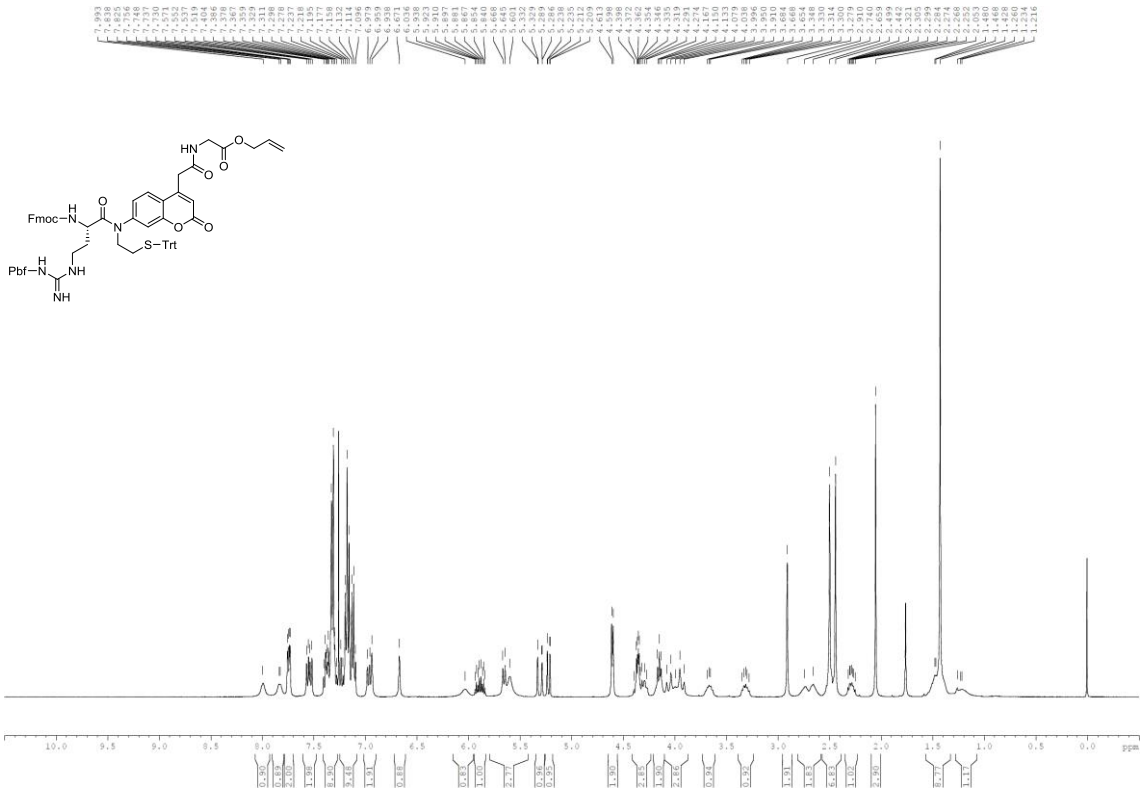
^{1}H NMR spectrum of **8r**



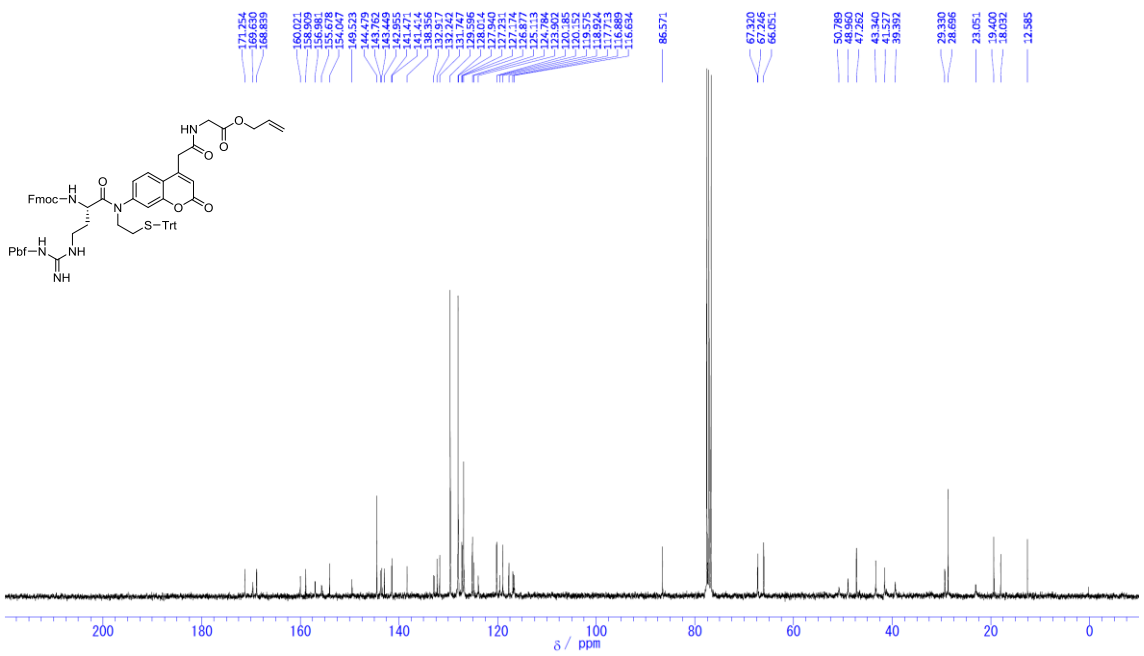
^{13}C NMR spectrum of **8r**



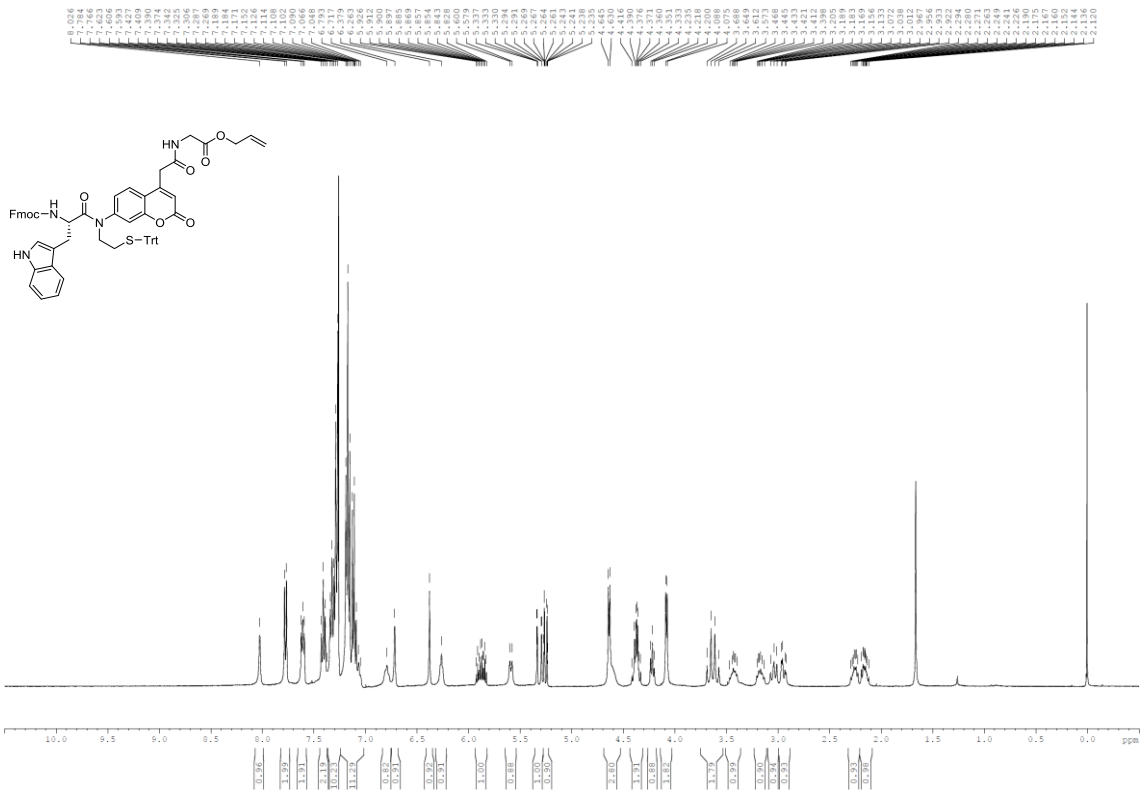
^{1}H NMR spectrum of **8s**



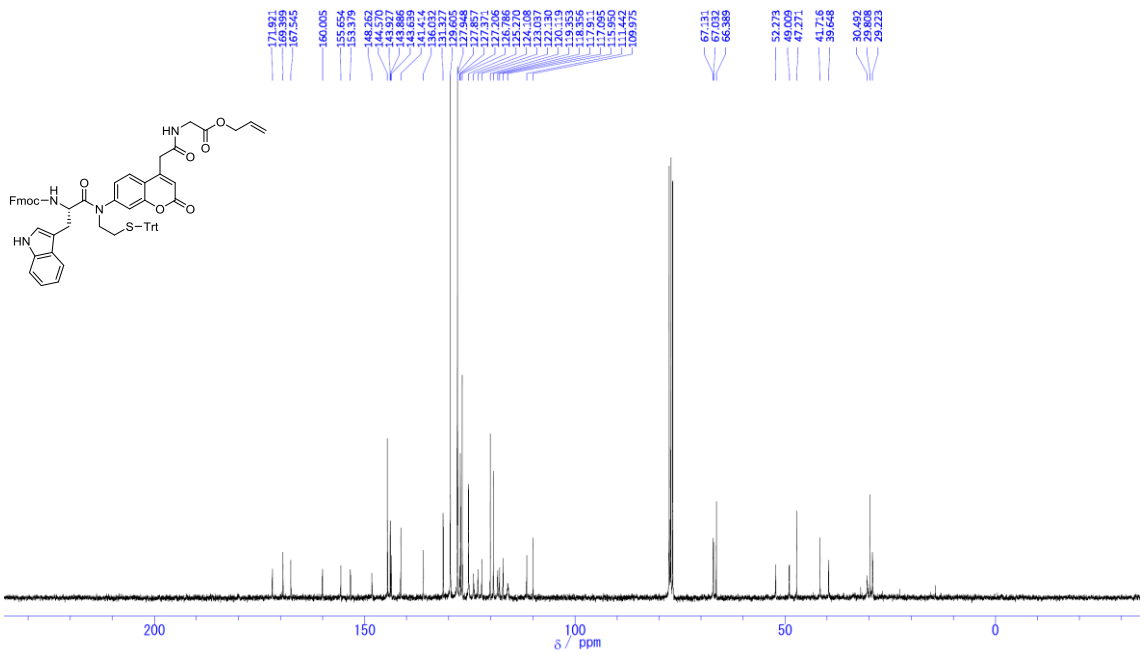
^{13}C NMR spectrum of **8s**



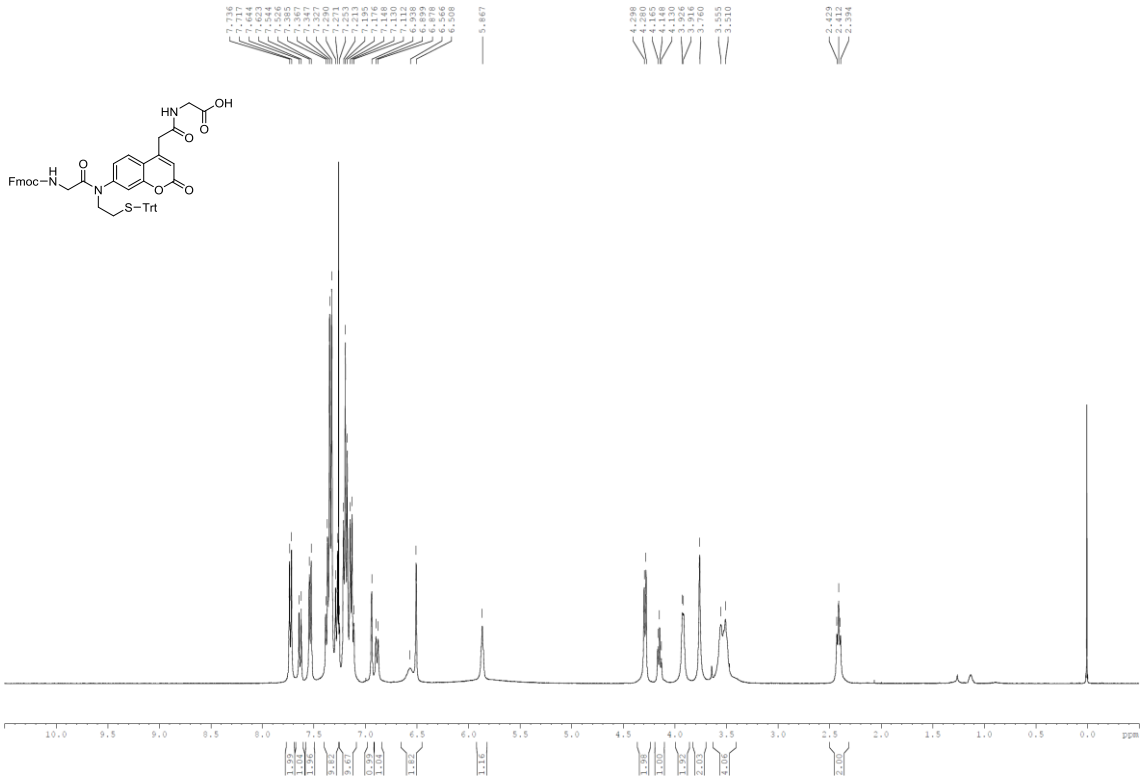
^{1}H NMR spectrum of **8t**



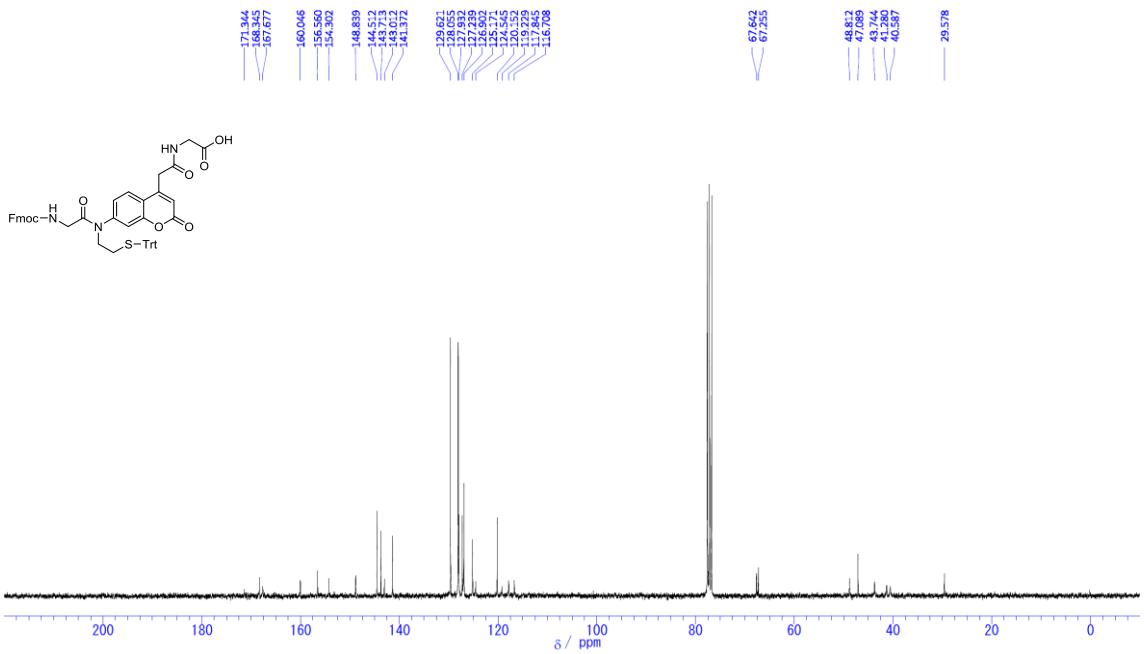
^{13}C NMR spectrum of **8t**



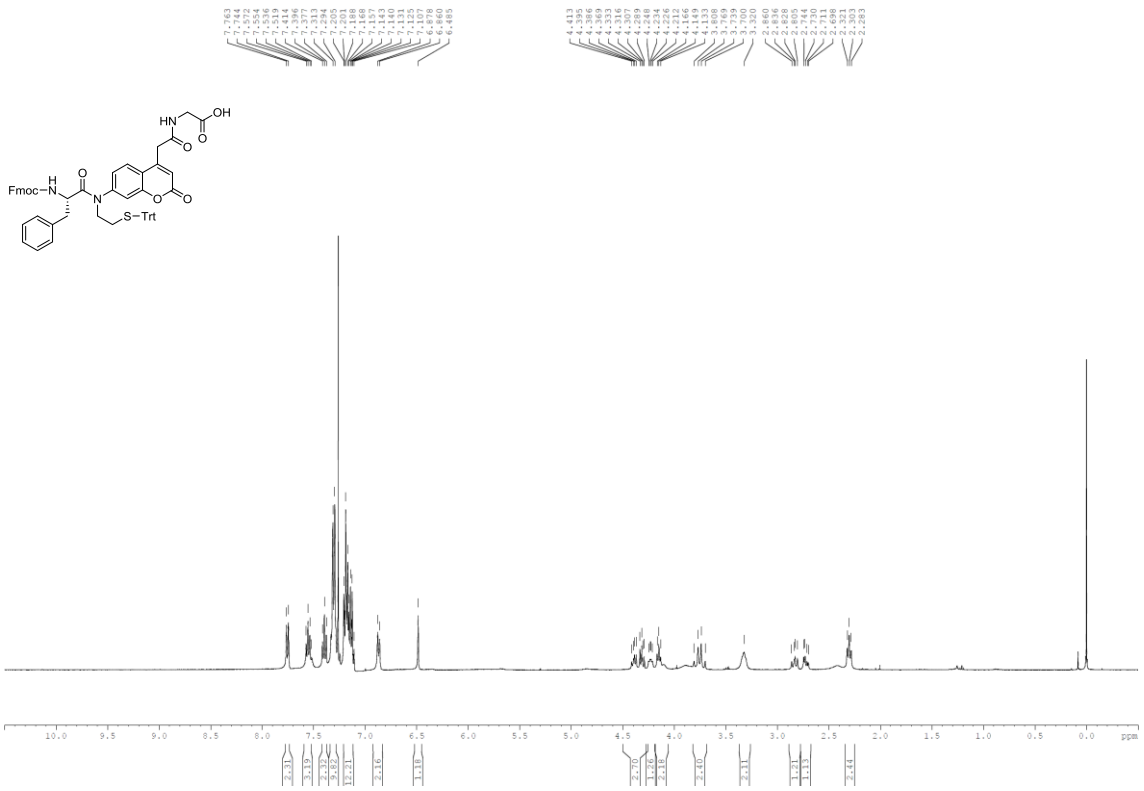
^{1}H NMR spectrum of **9a**



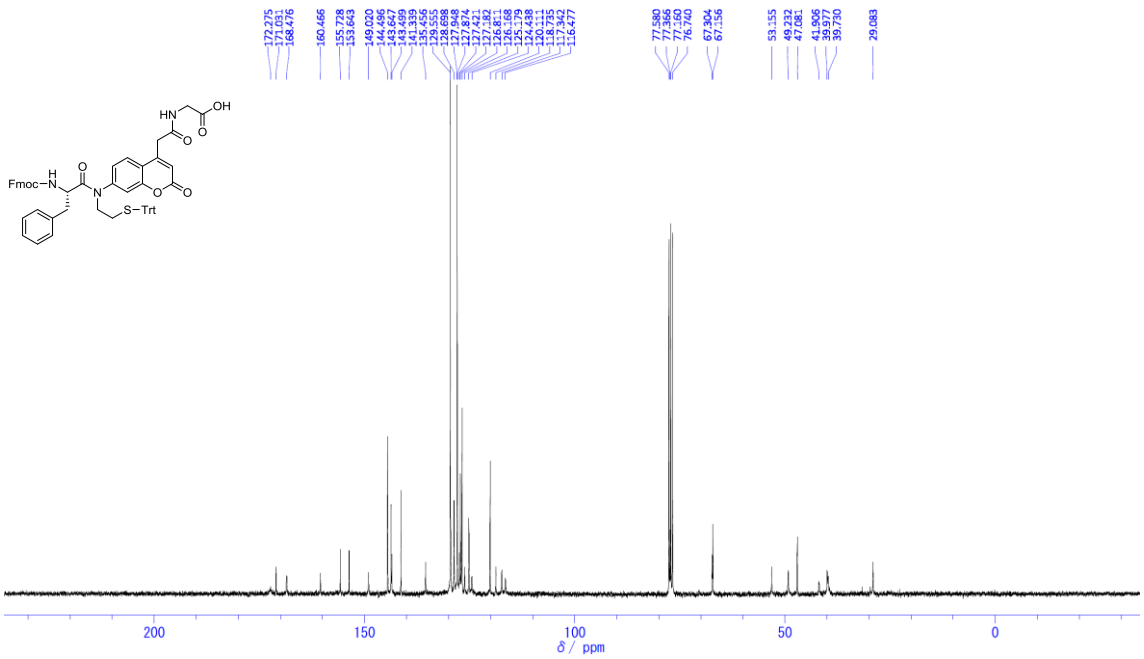
^{13}C NMR spectrum of **9a**



^{1}H NMR spectrum of **9p**



^{13}C NMR spectrum of **9p**



Reference

S1. Hibino, H.; Nishiuchi, Y. *Tetrahedron Lett.* **2011**, *52*, 4947-4949.

S2. Fukuyama, T.; Jow, C. K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373-6374.