SUPPLEMENTARY INFORMATION

A solid-phase affinity labeling method for target-selective isolation and modification of proteins

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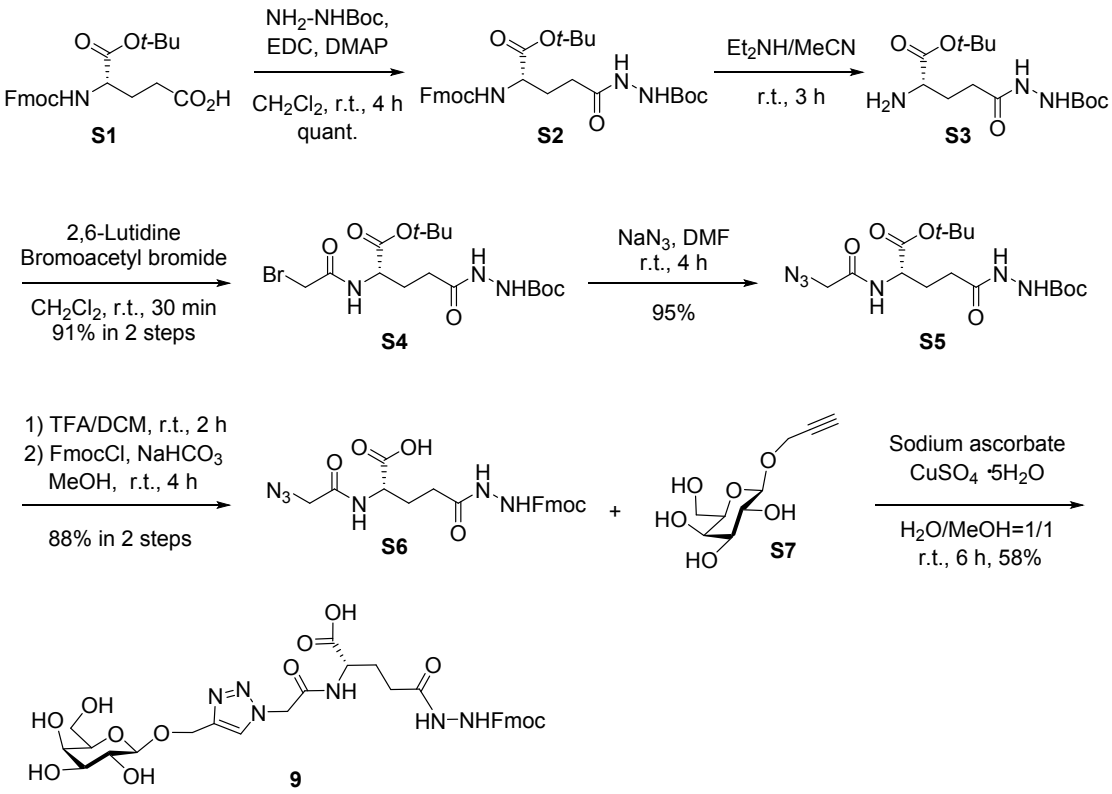
General methods for chemical synthesis.

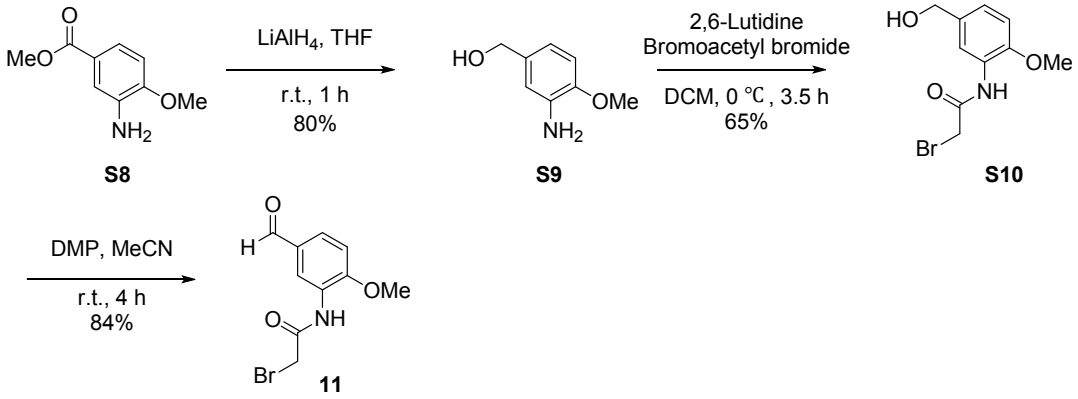
Melting points were determined on a micro hot-stage (Yanako MP-S3). Optical rotations were measured on a JASCO P-2200 plarimeter. UV/vis spectra were recorded on a JASCO V-550 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL ECA-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrometer in the indicated solvents. ^{1}H NMR data are reported as follows; chemical shift in parts par million (ppm) downfield or upfield from tetramethylsilane (δ 0.00) or CD_3OD (δ 3.31), integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet) and coupling constants (Hz). ^{13}C chemical shifts are reported in ppm downfield or upfield from $CDCl_3$ (δ 77.0), CD_3OD (δ 49.0) or acetone-d_6 (δ 29.8). ESI-TOF MS spectra were measured on a Waters LCT premier XE. The reactions were monitored by thin layer chromatography carried out on Merck TLC 60F-254 (0.25 mm) using UV light and *p*-anisaldehyde or 10% ethanolic phosphomonolybdic acid as developing agent. Column chromatography separations were performed using silica gel 60 N (spherical, neutral) (Kanto Chemical Co. Inc.). Reverse phase column chromatography separations were performed using Wakosil 40C18 (Wako Pure Chemical Industries, LTD.). Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon using oven-dried glassware. In general, organic solvents were purified and dried using an appropriate procedure, and evaporation and concentration were carried out under reduced pressure below 30 ºC, unless otherwise noted.

Synthetic scheme of the compound 9.



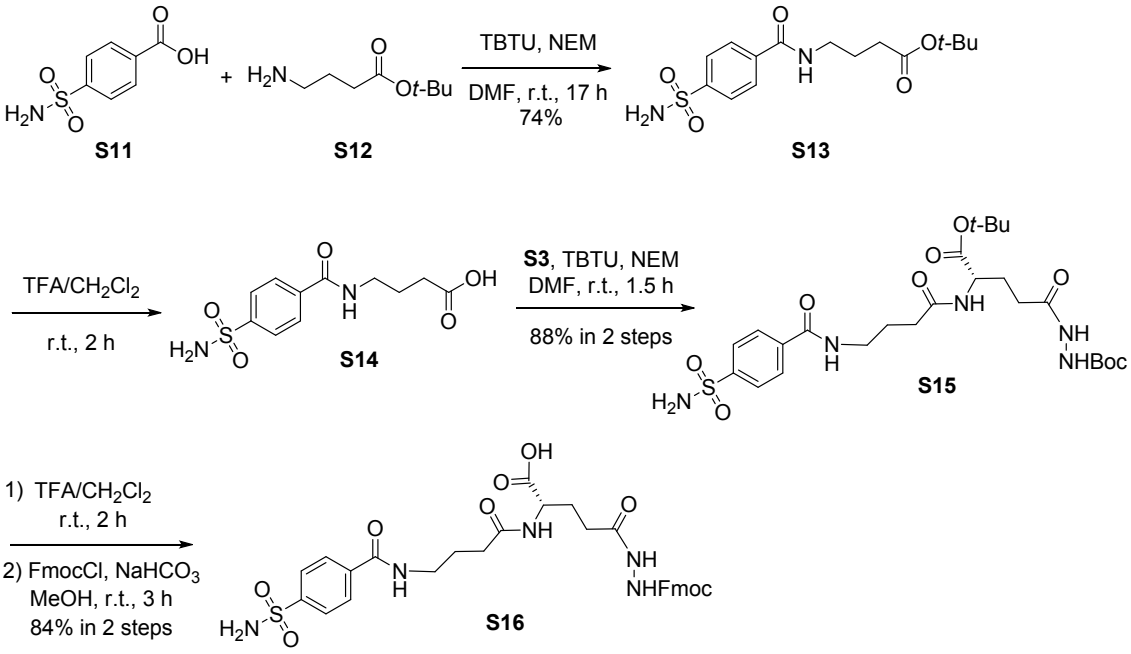
Scheme S1. Synthetic scheme of the compound **9**. Boc = *tert*-butyloxycarbonyl; EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DMAP = *N,N*-dimethyl-4-aminopyridine; DMF = *N,N*-dimethylformamide; FmocCl = 9-fluorenylmethyl chloroformate.

Synthetic scheme of the compound 11.



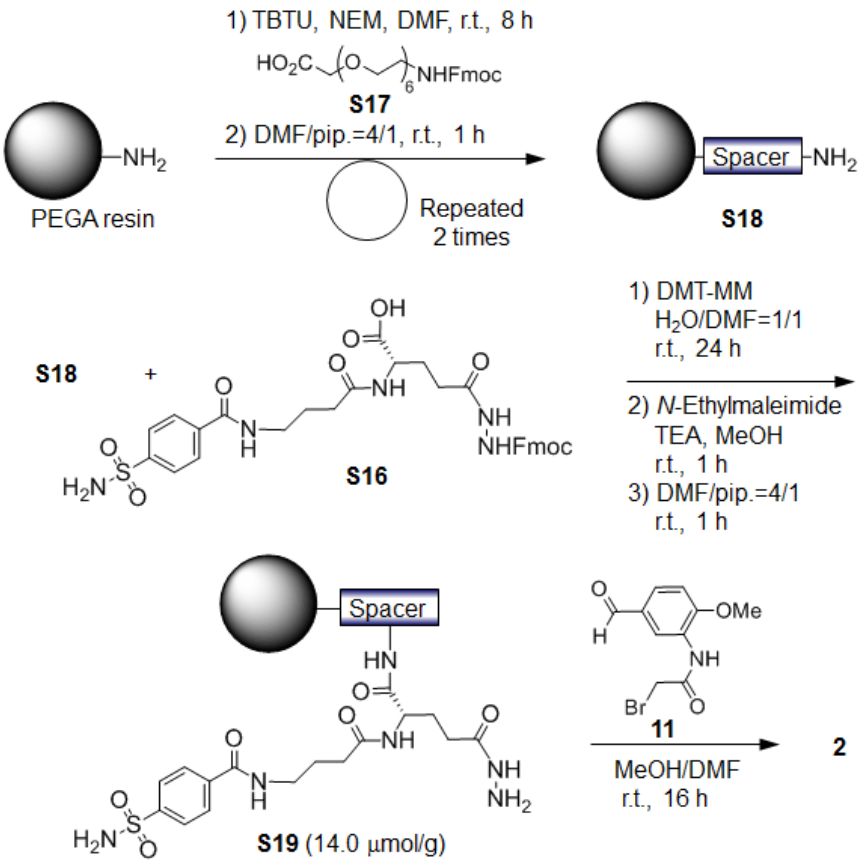
Scheme S2. Synthetic scheme of the compound **11**. DMP = Dess-Martin periodinane.

Synthetic scheme of the compound S16.



Scheme S3. Synthetic scheme of the compound **S16**. TBTU = *N*,*N*,*N'*,*N'*-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate; NEM = *N*-ethylmorphorine; DMF = *N*,*N*-dimethylformamide; TFA = trifluoroacetic acid; Boc = *tert*-butyloxycarbonyl; FmocCl = 9-fluorenylmethyl chloroformate.

Synthetic scheme of the solid-supported chemical tool 2.



Scheme S4. Synthetic scheme of the solid-supported chemical tool **2**. TBTU = *N*,*N*,*N'*,*N'*-tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate; NEM = *N*-ethylmorphorine; DMF = *N*,*N*-dimethylformamide; TFA = trifluoroacetic acid; Fmoc = 9-fluorenylmethyloxycarbonyl.

Synthesis of the compounds 9, 11, and S16.

Synthesis of the compound 9 in Scheme S1.

Compound S2: To a solution of Fmoc-Glu-*O*-*tert*-Bu (**S1**) (1.49 g, 3.49 mmol) in CH_2Cl_2 (30.0 mL) were added Boc-hydrazine (553 mg, 4.19 mmol), EDC (1.00 g, 5.24 mmol) and DMAP (42.6 mg, 0.349 mmol) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 4 h at room temperature, the reaction was quenched with sat. aq. NH_4Cl (100 mL). The resulting solution was extracted with EtOAc (100 mL×3). The extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by

silica-gel column chromatography (90 g, 7/3→1/1 toluene-acetone) gave **S2** (1.89 g, 3.49 mmol, quant.) as a pale yellow oil. R_f 0.39 (1/1 toluene-acetone); $[\alpha]^{25}_{D}$ +5.19 (*c* 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) (**Fig. S4**) δ 8.04 (1H, m), 7.76 (2H, d, *J*=7.5 Hz), 7.60 (2H, d, *J*=7.5 Hz), 7.39 (2H, t, *J*=7.5 Hz), 7.31 (2H, t, *J*=7.5 Hz), 6.55 (1H, br-s), 5.70 (1H, m), 4.40 (2H, d, *J*=6.5 Hz), 4.31 (1H, m), 4.21 (1H, t, *J*=6.5 Hz), 2.23-2.27 (2H, m), 1.92 (1H, br-s), 1.76 (1H, m), 1.46 (18 H, s×2); ^{13}C-NMR (125 MHz, $CDCl_3$) (**Fig. S5**) δ 171.7, 171.0, 156.6, 155.4, 143.8, 143.6, 141.3, 129.0, 128.2, 127.7, 127.1, 125.1×2, 120.0, 82.7, 81.7, 67.0, 53.7, 47.1, 30.2, 29.1, 28.1, 27.9; HRMS (ESI-TOF) *m/z* 540.2684 (540.2710 calcd. for $C_{29}H_{38}N_3O_7$, $[M+H]^+$).

Compound S4: To a solution of **S2** (1.89 g, 3.49 mmol) in acetonitrile (40.0 mL) was added diethylamine (8.00 mL) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 3 h at room temperature, the reaction mixture was concentrated in *vacuo*. The residue was co-evaporated with toluene (100 mL×3), and then the resulting residue was diluted with CH_2Cl_2 (24.0 mL). To the resulting solution were added 2,6-lutidine (1.30 mL, 10.5 mmol) and bromoacetylbromide (334 μL, 3.84 mmol) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 30 min at room temperature, the reaction was quenched with 1 N HCl (100 mL). The resulting solution was extracted with $CHCl_3$ (100 mL×3). The extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (90 g, 4/1→7/3 toluene-acetne) gave **S4** (1.34 g, 3.18 mmol, 91% yield in 2 steps) as a pale yellow oil. R_f 0.62 (1/1 toluene-acetone); $[\alpha]^{25}_{D}$ +52.3 (*c* 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) (**Fig. S6**) δ 8.37 (1H, br-s), 7.41 (1H, d, *J*=7.5 Hz), 6.79 (1H, br-s), 4.54 (1H, m), 3.94&3.88 (2H, ABq, *J*=13.0 Hz), 2.27-2.34 (3H, m), 1.99-2.08 (1H, m), 1.48 (9H, s), 1.47 (9H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) (**Fig. S7**) δ 171.7, 170.4, 166.5, 155.6, 83.0, 81.8, 52.9, 30.1, 28.6, 28.1, 27.9; HRMS (ESI-TOF) *m/z* 436.1083 (436.1085 calcd. for $C_{16}H_{27}N_3O_6Br$, $[M+H]^+$).

Compound S5: To a solution of **S4** (1.34 g, 3.18 mmol) in DMF (40.0 mL) was added NaN_3 (414 mg, 6.36 mmol) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 4 h at room temperature, the reaction mixture was dilluted with water (100 mL). The resulting solution was extracted with $CHCl_3$ (100 mL×3). The extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (70 g, 7/3→6/4 toluene-acetone) gave **S5** (1.21 g, 3.02 mmol, 95% yield) as a pale yellow oil. R_f 0.62 (1/1 toluene-acetone); $[\alpha]^{25}_{D}$ +14.5 (*c* 1.00, $CHCl_3$);

^{1}H-NMR (500 MHz, $CDCl_3$, TMS) (**Fig. S8**) δ 8.31 (1H, br-s), 7.22 (1H, d, *J*=8.0 Hz), 6.69 (1H, br-s), 4.57 (1H, m), 4.04&3.97 (2H, ABq, *J*=16.5 Hz), 2.23-2.32 (3H, m), 1.90-2.03 (1H, m), 1.48 (9H, s), 1.47 (9H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) (**Fig. S9**) δ 171.7, 170.5, 167.5, 155.5, 83.0, 81.8, 52.3, 30.3, 28.1, 27.9; HRMS (ESI-TOF) *m/z* 399.1977 (399.1992 calcd. for $C_{16}H_{27}N_6O_6$, $[M-H]^-$).

Compound S6: To a solution of **S5** (1.16 g, 2.90 mmol) in CH_2Cl_2 (12.0 mL) was added TFA (12.0 mL) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 2 h at room temperature, the reaction mixture was concentrated in *vacuo*. The residue was co-evaporated with toluene (100 mL×3), and then the resulting residue was diluted with MeOH (24 mL). To the resulting solution were added $NaHCO_3$ (731 mg, 8.70 mmol) and FmocCl (750 mg, 2.90 mmol) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 4 h at room temperature, the reaction was quenched with 1N HCl (100 mL). The resulting solution was extracted with EtOAc (100 mL×3). The extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (70 g, 1/0→4/1 $CHCl_3$-MeOH containing 2% AcOH) gave **S6** (1.19 g, 2.55 mmol, 88% yield in 2 steps) as a white solid. R_f 0.36 (4/1 $CHCl_3$-MeOH containing 2% AcOH); mp 89-90 ºC; $[\alpha]^{25}_D$ +10.1 (*c* 1.00, CH_3OH); ^{1}H-NMR (500 MHz, CD_3OD, TMS) (**Fig. S10**) δ 7.79 (2H, m), 7.67 (2H, m), 7.38-7.40 (2H, m), 7.30-7.32 (2H, m), 4.40-4.49 (3H, m), 4.24 (1H, m), 3.93 (2H, s), 2.15-2.35 (3H, m), 1.99-2.04 (1H, m); ^{13}C-NMR (125 MHz, CD_3OD) (**Fig. S11**) δ 174.5, 174.3, 170.4, 158.5, 145.1, 142.6, 128.8, 128.2, 126.2, 121.0, 79.4, 68.6, 53.2, 52.7, 31.0, 30.7, 28.0, 24.2; HRMS (ESI-TOF) *m/z* 467.4538 (467.4541 calcd. for $C_{22}H_{25}N_6O_6$, $[M+H]^+$).

Compound 9: To a solution of **S6** (600 mg, 1.29 mmol) and **S7**[1] (299 mg, 1.54 mmol) in H_2O/MeOH (1/1) (18.0 mL) were added sodium ascorbate (76.6 mg, 0.387 mmol) and $CuSO_4$•$5H_2O$ (48.4 mg, 0.194 mmol) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 4 h at room temperature, the reaction mixture was concentrated in *vacuo*. Purification of the residue by reverse-phase column chromatography (1/0→0/1 H_2O-MeCN) gave (512 mg, 0.748 mmol, 58% yield) as a white solid. R_f 0.24 (10/10/1 $CHCl_3$-MeOH-water); mp 142-143 ºC; $[\alpha]^{25}_D$ −7.05 (*c* 1.00, CH_3OH); ^{1}H-NMR (500 MHz, CD_3OD, TMS) (**Fig. S12**) δ 8.05 (1H, s), 7.79 (2H, d, *J*=7.5 Hz), 7.66-7.67 (2H, m), 7.38 (2H, t, *J*=7.5 Hz), 7.31 (2H, t, *J*=7.5 Hz), 5.22 (2H, s), 4.97&4.79 (2H, ABq, *J*=12.6 Hz), 4.45-4.46 (1H, m), 4.40 (2H, d, *J*=6.9 Hz), 4.33 (1H, d, *J*=7.5 Hz), 4.25 (1H, m), 3.77-3.82 (2H, m), 3.70-3.73 (1H, m), 3.53-3.56 (2H, m), 2.15-2.41 (3H, m), 2.05 (1H, m); ^{13}C-NMR (125 MHz, CD_3OD) (**Fig. S13**) δ 174.5,

167.9, 158.6, 145.1, 142.6, 128.9, 128.2, 126.3, 121.0, 104.1, 76.8, 74.9, 72.5, 70.4, 68.6, 62.9, 62.6, 52.9, 39.7, 39.5, 39.4, 31.0, 28.0; HRMS (ESI-TOF) *m/z* 685.2478 (685.2469 calcd. for $C_{31}H_{37}N_6O_{12}$, $[M+H]^+$).

Synthesis of the compound 11 in Scheme S2.

Compound S9: To a solution of methyl 3-amino-4-methoxybenzoate (**S8**)[2] (1.58 g, 8.70 mmol) in THF (32.0 mL) was added $LiAlH_4$ (496 mg, 13.1 mmol) under Ar atmosphere in ice bath. After the reaction mixture was stirred for 1 h at room temperature, the reaction was quenched with MeOH (20 mL). The resulting solution was extracted with EtOAc (100 mL×3). The extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (70 g, 1/1 toluene-acetone) gave **S9** (1.07 g, 6.99 mmol, 80% yield) as a white solid. R_f 0.24 (1/1 toluene-acetone); mp 96-97 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) (**Fig. S14**) δ 6.76-6.70 (3H, m), 4.53 (2H, s), 3.85 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) (**Fig. S15**) δ 146.9, 136.2, 133.8, 117.2, 114.1, 110.2, 65.2, 55.5; HRMS (ESI-TOF) *m/z* 154.0875 (154.0868 calcd. for $C_8H_{12}NO_2$, $[M+H]^+$).

Compound S10: To a solution of (3-amino-4-methoxyphenyl)methanol (**S9**) (1.07 g, 6.99 mmol) in CH_2Cl_2 (54.0 mL) were added 2,6-lutidine (2.21 mL, 19.1 mmol) and bromoacetyl bromide (553 μL, 6.35 mmol) under Ar atmosphere in ice bath. After the reaction mixture was stirred for 3.5 h at 0 °C, the reaction was quenched with 1 N HCl (100 mL). The resulting solution was extracted with EtOAc (100 mL×3). The extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (170 g, 9/1→1/1 toluene-acetone) gave **S10** (1.24 g, 4.54 mmol, 65% yield) as a brown solid. R_f 0.39 (1/1 toluene-acetone); mp 101-102 ºC; ^{1}H-NMR (500 MHz, CD_3OD) (**Fig. S16**) δ 8.06 (1H, s), 7.13 (1H, d, *J*=8.3 Hz), 7.00 (1H, m), 4.52 (2H, s), 4.10 (2H, s), 3.89 (3H, s); ^{13}C-NMR (125 MHz, CD_3OD) (**Fig. S17**) δ 167.1, 150.3, 134.8, 127.6, 125.1, 121.6, 111.6, 64.9, 56.4, 29.9; HRMS (ESI-TOF) *m/z* 274.0071 (274.0073 calcd. for $C_{10}H_{13}BrNO_3$, $[M+H]^+$).

Compound 11: To a solution of 2-bromo-*N*-(5-(hydroxymethyl)-2-methoxyphenyl) acetamide (**S10**) (715 mg, 2.61 mmol) in acetonitrile (22.0 mL) was added Dess-Martin periodinane (1.22 g, 2.87 mmol) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 4 h at room temperature, the reaction was quenched with a solution of sat. aq. $Na_2S_2O_3$/sat. aq. $NaHCO_3$ (1/9, 50 mL). The resulting solution

was extracted with EtOAc (50 mL×3). The extracts were washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (71 g, 3/1→3/7 toluene-acetone) gave **11** (594 mg, 2.19 mmol, 84% yield) as a brown solid. R_f 0.72 (1/1 toluene-acetone); mp 141-142 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) (**Fig. S18**) δ 9.91 (1H, s), 8.85 (1H, d, *J*=2.0 Hz), 8.83 (1H, bs), 7.71 (1H, dd, *J*=8.3 Hz, *J*=2.0 Hz), 7.04 (1H, d, *J*=8.3 Hz), 4.06 (2H, s), 4.02 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) (**Fig. S19**) δ 190.9, 163.5, 152.8, 130.0, 127.3, 126.4, 121.3, 110.2, 56.4, 29.5; HRMS (ESI-TOF) *m/z* 271.9922 (271.9917 calcd. for $C_{10}H_{11}BrNO_3$, $[M+H]^+$).

Synthesis of the compound S16 in Scheme S3.

Compound S13: To a solution of 4-carboxybenzenesulfonamide (**S11**) (379 mg, 1.88 mmol) in DMF (19.0 mL) were added *tert*-butyl 4-aminobutanoate (**S12**) (200 mg, 1.26 mmol), TBTU (605 mg, 1.26 mmol) and NEM (239 μL, 1.26 mmol) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 17 h at room temperature, the reaction was quenched with sat. aq. NH_4Cl (50 mL). The resulting solution was extracted with EtOAc (50 mL×3). The extracts were washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (22 g, 1/1 hexane-ethyl acetate) gave **S13** (318 mg, 0.929 mmol, 74%) as a white solid. R_f 0.62 (1/1 hexane-ethyl acetate); mp 174-175 ºC; ^{1}H-NMR (500 MHz, acetone-d_6, TMS) (**Fig. S20**) δ 8.03 (2H, dd, *J*=6.6 Hz, *J*=1.9 Hz), 7.99 (1H, bs), 7.96 (2H, dd, *J*=6.6 Hz, *J*=1.9 Hz), 6.73 (1H, bs), 3.44 (2H, m), 2.32 (2H, t, *J*=2.4 Hz), 1.87 (2H, m), 1.43 (9H. s); ^{13}C-NMR (125 MHz, acetone-d_6) (**Fig. S21**) δ 172.9, 166.3, 147.2, 139.0, 128.6×2, 126.9×2, 80.3, 40.0, 33.4, 28.2×3, 25.7; HRMS (ESI-TOF) *m/z* 343.1340 (343.1328 calcd. for $C_{15}H_{23}N_2O_5S$, $[M+H]^+$).

Compound S15: To a solution of **S13** (306 mg, 0.895 mmol) in CH_2Cl_2 (7.70 mL) was added TFA (7.70 mL) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 2 h at room temperature, the reaction mixture was concentrated in *vacuo*. The residue was co-evaporated with toluene (20 mL×3), and then the resulting residue was diluted with DMF (15.4 mL). To the resulting solution were added **S6** (341 mg, 1.07 mmol), TBTU (431 mg, 1.34 mmol) and NEM (167 μL, 1.34 mmol) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 1.5 h at room temperature, the reaction was quenched with sat. aq. NH_4Cl (50 mL). The resulting solution was extracted with EtOAc (50 mL×3). The extracts were washed with

brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (50 g, 9/1→8/2 $CHCl_3$-MeOH) gave **S15** (463 mg, 0.791 mmol, 88% yield in 2 steps) as a white solid. R_f 0.67 (4/1 $CHCl_3$-MeOH); mp 115-116 ºC; $[\alpha]^{27}_D$ −11.5 (*c* 0.93, CH_3OH); ^{1}H-NMR (500 MHz, CD_3OD) (**Fig. S22**) δ 7.98 (4H, m), 4.29 (1H, m), 3.45 (2H, m), 2.37-2.30 (4H, m), 2.19-2.15 (1H, m), 1.97-1.91 (3H, m), 1.47 (9H, s), 1.46 (9H, s); ^{13}C-NMR (125 MHz, CD_3OD) (**Fig. S23**) δ 175.7, 174.5, 172.4, 168.8, 157.8, 147.7, 139.0, 129.0×2, 127.3×2, 83.0, 81.9, 54.1, 40.5, 34.0, 31.1, 28.5×3, 28.2×3, 28.0, 26.5; HRMS (ESI-TOF) *m/z* 586.2562 (586.2547 calcd. for $C_{25}H_{40}N_5O_9S$, $[M+H]^+$).

Compound S16: To a solution of **S15** (453 mg, 0.773 mmol) in CH_2Cl_2 (11.3 mL) was added TFA (11.3 mL) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 2 h at room temperature, the reaction mixture was concentrated in *vacuo*. The residue was co-evaporated with toluene (20 mL×3), and then the resulting residue was diluted with MeOH (23.2 mL). To the resulting solution were added $NaHCO_3$ (260 mg, 3.09 mmol) and FmocCl (240 mg, 0.928 mmol) under Ar atmosphere at room temperature. After the reaction mixture was stirred for 3 h at room temperature, the reaction mixture was concentrated in *vacuo*. Purification of the residue by reverse-phase column chromatography (1/0→0/1 H_2O-MeOH) gave **S16** (425 mg, 0.652 mmol, 84% yield in 2 steps) as a white solid. R_f 0.18 (1/1 $CHCl_3$-MeOH containing 2% AcOH); mp 147-148 ºC; $[\alpha]^{29}_D$ +12.4 (*c* 2.43, CH_3OH); ^{1}H-NMR (500 MHz, CD_3OD) (**Fig. S24**) δ 7.97 (4H, m), 7.78 (2H, m), 7.66 (2H, m), 7.38 (2H, m), 7.30 (2H, m), 4.37-4.30 (3H, m), 4.22 (1H, m), 3.48-3.34 (2H, m), 2.37-2.25 (4H, m), 2.18 (1H, m), 2.03-1.90 (3H, m); ^{13}C-NMR (125 MHz, CD_3OD) (**Fig. S25**) δ 178.2, 175.3, 174.8, 168.7, 158.5, 147.6, 145.1, 142.5, 139.0, 129.0×2, 128.8×2, 128.2×2, 127.3×2, 126.3×2, 120.9×2, 79.5, 68.6, 55.7, 49.8, 48.2, 40.6, 34.4, 31.6, 29.7, 26.2; HRMS (ESI-TOF) *m/z* 650.1912 (650.1921 calcd. for $C_{31}H_{32}N_5O_9S$, $[M-H]^-$).

Synthesis of the solid-supported chemical tools 1 and 2.

Synthesis of the solid-supported chemical tool 1 in Scheme 1.

Solid-supported chemical tool 1: To a NH_2-PEGA resin (NH_2-PEGA resin was purchased from WATANABE CHEMICAL INDUSTRIES, LTD.) (150 mg, 50.0 μmol/g as a swollen form in MeOH) was added a pre-mixed solution of TBTU (38.5 mg, 120 μmol, 80.0 mM), *N*-ethylmorpholine (22.8 μL, 180 μmol, 120 mM), and **7** (23.1 mg, 60.0 μmol, 40.0 mM) in DMF (1.50 mL). After the suspension was shaken at room temperature for 8 h, the resulting resin was washed with DMF (1.50 mL×5), CH_2Cl_2 (1.50 mL×5), and MeOH (1.50 mL×5). To the above resin was added a solution of 20% piperidine in DMF (1.50 mL) at room temperature. After the suspension was shaken for 1 h at room temperature, the resulting resin was washed with DMF (1.50 mL×5), CH_2Cl_2 (1.50 mL×5), and MeOH (1.50 mL×5). These reaction sequences were repeated further four times to give the amino resin **8**. Completion of the each coupling reaction was monitored by the Kaiser test.[3]

To the above resin **8** was added a pre-mixed solution of **9** (51.3 mg, 75.0 μmol, 50.0 mM) and DMT-MM (166 mg, 600 μmol, 400 mM) in H_2O/DMF (1/1) (1.50 mL) at room temperature. After the suspension was shaken at room temperature for 24 h, the resulting resin was washed with DMF (1.50 mL×5), CH_2Cl_2 (1.50 mL×5), and MeOH (1.50 mL×5). The remaining free amino groups were capped with a solution of *N*-ethylmaleimide (56.3 mg, 450 μmol, 300 mM) and TEA (10.5 μL, 75.0 μmol, 50.0 mM) in MeOH (1.50 mL) at room temperature for 1 h. Completion of the capping reaction was confirmed by the Kaiser test.

To the above resin (30.0 mg as a swollen form in MeOH) was added a solution of 20% piperidine in DMF (300 μL) at room temperature. After the suspension was shaken for 1 h at room temperature, the resulting resin was washed with DMF (300 μL×5), CH_2Cl_2 (300 μL×5), and MeOH (300 μL×5) to give **10**. The loading value of **10** was determined by measuring the UV absorbance of the piperidine-dibenzofulvene adduct in the filtrates at 301 nm to be 30.4 μmol/g (as a dry form).

To the above resin **10** (906 μM) was added a solution of aldehyde **11** (8.16 mg, 30 μmol, 100 mM) in DMF/MeOH (1/1) (300 μL) at room temperature. After the suspension was shaken at room temperature for 16 h, the resulting resin was washed with DMF (300 μL×5), CH_2Cl_2 (300 μL×5), and MeOH (300 μL×5) to give the solid-supported chemical tool **1**.

Synthesis of the solid-supported chemical tool 2 in Scheme S4.

Solid-supported chemical tool 2: To a NH_2-PEGA resin (150 mg, 50.0 μmol/g as a swollen form in MeOH) was added a pre-mixed solution of TBTU (38.5 mg, 120 μmol, 80.0 mM), *N*-ethylmorpholine (22.8 μL, 180 μmol, 120 mM), and **S17**[4] (33.7 mg, 60.0 μmol, 40.0 mM) in DMF (1.50 mL). After the suspension was shaken at room temperature for 8 h, the resulting resin was washed with DMF (1.50 mL×5), CH_2Cl_2 (1.50 mL×5), and MeOH (1.50 mL×5). To the above resin was added a solution of 20% piperidine in DMF (1.50 mL) at room temperature. After the suspension was shaken for 1 h at room temperature, the resulting resin was washed with DMF (1.50 mL×5), CH_2Cl_2 (1.50 mL×5), and MeOH (1.50 mL×5). These reaction sequences were repeated once more to give the amino resin **S18**. Completion of the each coupling reaction was monitored by the Kaiser test.

To the above resing **S18** was added a pre-mixed solution of **S16** (48.9 mg, 75.0 μmol, 50.0 mM) and DMT-MM (166 mg, 600 μmol, 400 mM) in H_2O/DMF (1/1) (1.50 mL) at room temperature. After the suspension was shaken at room temperature for 24 h, the resulting resin was washed with DMF (1.50 mL×5), CH_2Cl_2 (1.50 mL×5), and MeOH (1.50 mL×5). The remaining free amino groups were capped with a solution of *N*-ethylmaleimide (56.3 mg, 450 μmol, 300 mM) and TEA (10.5 μL, 75.0 μmol, 50.0 mM) in MeOH (1.50 mL) at room temperature for 1 h. Completion of the capping reaction was confirmed by the Kaiser test.

To the above resin (30.0 mg as a swollen form in MeOH) was added a solution of 20% piperidine in DMF (300 μL) at room temperature. After the suspension was shaken for 1 h at room temperature, the resulting resin was washed with DMF (300 μL×5), CH_2Cl_2 (300 μL×5), and MeOH (300 μL×5) to give **S19**. The loading value of **S19** was determined by measuring the UV absorbance of the piperidine-dibenzofulvene adduct in the filtrates at 301 nm to be 14.0 μmol/g (as a dry form).

To the above resin **S19** (329 μM) was added a solution of aldehyde **11** (8.16 mg, 30.0 μmol, 100 mM) in DMF/MeOH (1/1) (300 μL) at room temperature. After the suspension was shaken at room temperature for 16 h, the resulting resin was washed with DMF (300 μL×5), CH_2Cl_2 (300 μL×5), and MeOH (300 μL×5) to give the solid-supported chemical tool **2**.

Materials and experimental methods for solid-phase affinity labeling.

Materials: Peanut agglutinin (PNA) was purchased from Wako Pure Chemical Industries, LTD. Human carbonic anhydrase II (hCAII), bovine serum albumin (BSA), fetuin, papain, and ribonuclease A (RNase A) were purchased from Sigma-Aldrich Co. LLC. Red blood cells (RBCs, human) was purchased from Lee Biosolutions. The bradford assay was conducted using Bradford assay reagent (Thermo Scientific), plastic 96-well microplate (Corning), and Safire microplate reader (TECAN). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was conducted using a Bruker Ultraflex mass spectrometer with detection in the reflector mode. α-Cyano-4-hydroxycinnamic acid (CHCA) and sinapinic acid (SA) were used as the matrix, with positive ionization mode.

Solid-phase affinity labeling reaction of PNA using 1: To the resin **1** (1.0 eq., 50.0 μM) was added a solution of PNA (4.0 eq., 200 μM) in 50 mM HEPES buffer (pH 8.0, 1.00 mL) at room temperature. After the suspension was gently shaken at room temperature for 24 h, the resulting resin was washed twice with the reaction buffer to give the resin-bound PNA **12**. The chemical yield of the affinity labeling reaction was calculated based on the Bradford method.[5]

Ligand exchange reaction of 12 with 13: To the resin-bound PNA **12** (1.0 eq., 200 μM) was added a solution of *O*-alkoxyamine **13** (20 mM, 100 eq.) in 50 mM acetate buffer (pH 5.5, 250 μL) at room temperature. After the suspension was gently shaken at 37 °C for 72 h, the resulting resin was washed twice with the reaction buffer to give labeled PNA **14**. The chemical yield of the ligand exchange reaction was calculated based on the Bradford method.

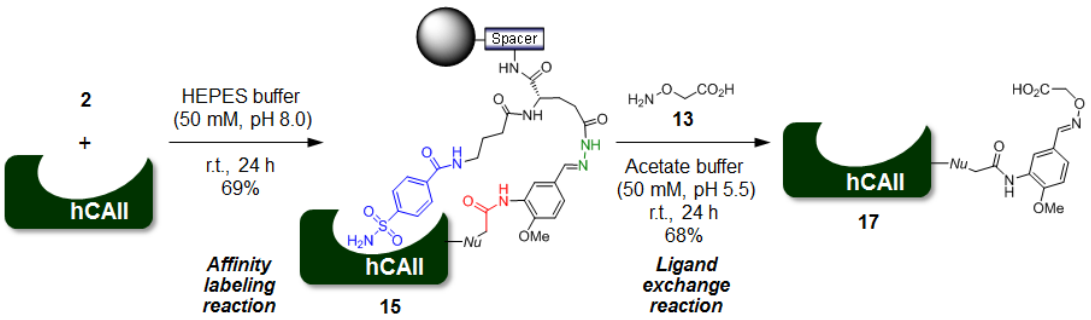
Solid-phase affinity labeling reaction of PNA using 1 in the protein mixtures, and ligand exchange reaction with 13: To the resin **1** (1.0 eq., 6.25 μM) was added a solution of PNA (4.0 eq., 25.0 μM), RNase A (4.0 eq., 25.0 μM), fetuin (4.0 eq., 25.0 μM), and BSA (4.0 eq., 25.0 μM) in 50 mM HEPES buffer (pH 8.0, 4.75 mL) at room temperature. After the suspension was gently shaken at room temperature for 24 h, the resulting resin was washed twice with the reaction buffer to remove the unreacted proteins. To the resulting resin-bound PNA **12** (1.0 eq., 200 μM) was added *O*-alkoxyamine **13** (100 eq., 20 mM) in 50 mM acetate buffer (pH 5.5, 150 μL) at room

temperature. After the suspension was gently shaken at room temperature for 37 °C for 48 h, the resin was washed twice with the reaction buffer to give labeled PNA **14**. The progress of the each reaction step was monitored by SDS-PAGE analysis (Fig. 3b).

Solid-phase affinity labeling reaction of papain using 1: To the resin **1** (1.0 eq., 50.0 μM) was added a solution of papain (1.0 eq., 200 μM) in 50 mM HEPES buffer (pH 8.0, 1.00 mL) at room temperature. After the suspension was gently shaken at room temperature for 24 h, the resulting resin was washed twice with the reaction buffer. The chemical yield of the recovered papain was calculated based on the Bradford method to be 99% yield.

Protocol for electrophoresis: SDS/polyacrylamide gel electrophoresis (SDS-PAGE) experiments were performed as reported previously.[6] Electrophoresis buffer consisted of SDS (5%, wt/vol), glycerol (27%, vol/vol), DTT (0.5%, wt/vol) and bromophenol blue (0.007%, wt/vol); 4.8 μL of buffer was added to the samples. 8% Polyacrylamide gels were run by applying 30 mA for 1.5 h. The gels were stained with Coomassie Brilliant Blue Stain (Wako Pure Chemical Industries, LTD.) or SYPRO Ruby Protein Gel Stain (Bio-Rad Laboratories Inc.) for 3 h, destained in acetic acid (7%, vol/vol) and methanol (10%, vol/vol) for 0.5 h, and then washed with water. The gels were scanned with a Molecular Imager FX (Bio-Rad Laboratories Inc.) and the images were processed using Adobe Photoshop software. Molecular weight markers were used in each gel for calibration purposes. The in-gel fluorescence analysis was performed with a Molecular Imager FX.

Solid-phase affinity labeling reaction of purified hCAII using 2, and ligand exchange reaction of 15 with 13: To the resin **2** (493 μM) was added a solution of purified hCAII (5 μM) in 50 mM HEPES buffer (pH 8.0, 200 μL) at room temperature. After the suspension was gently shaken at room temperature for 24 h, the resulting resin was washed twice with the reaction buffer to give the resin-bound hCAII **15**. To the resin-bound hCAII **15** was added a solution of *O*-alkoxyamine **13** (20 mM) in 50 mM acetate buffer (pH 5.5, 300 μL) at room temperature. After the suspension was gently shaken at room temperature for 24 h, the resulting resin was washed twice with the reaction buffer to give labeled hCAII **17**. The chemical yields of the affinity labeling reaction and the ligand exchange reaction were calculated based on the Bradford method to be 69% and 68% yield, respectively .



Scheme S5. Solid phase affinity labeling reaction of hCAII using **2**, and ligand exchange reaction of **15** with **13**.

Solid-phase affinity labeling reaction of hCAII using 2 in the hRBC lysate, and ligand exchange reaction: hRBCs were lysed[7] with the same volum of ice-cold water. The lysate was centrifuged at 20,000 rpm for 30 min at 4 ºC to remove cell membranes and non-lysed cells. The protein concentration was determined by the Bradford method. To the resin **2** (329 μM) was added the above solution of hRBC lysate (10 mg/mL) in 50 mM HEPES buffer (pH 8.0, 300 μL) at room temperature. After the suspension was gently shaken at room temperature for 24 h, the resulting resin was washed twice with the reaction buffer to remove the unreacted proteins. To the resulting resin-bound hCAII **15** was added *O*-alkoxyamine **13** or **16** (20 mM) in 50 mM acetate buffer (pH 5.5, 300 μL) at room temperature. After the suspension was gently shaken at room temperature for 24 h, the resin was washed twice with the reaction buffer to give the labeled hCAII **17** or **18**. The process of the each reaction step was monitored by SDS-PAGE analysis (Fig. 4b).

Huisgen reaction of N_3-hCAII 18: To a solution of N_3-hCAII **18** in 25 mM HEPES buffer (pH 8.0, 60.0 μL) was added 40.0 μL of a pre-mixed solution of **19**[8] (100 μM), $[Cu(MeCN)_4]PF_6$ (1.00 mM), and triazole ligand **20**[9] (1.00 mM) in 25 mM HEPES buffer (pH 8.0) containing 2.5% DMSO at room temperature. After the reaction mixture was stirred for 2 h at room temperature, the solution was dialyzed against H_2O with Specra/Pro® Dialysis Membrane (13-14000 MWCO, Spectrum Laboratories, Inc., USA), to remove the excess amount of Huisgen reagents, and the resulting solution was lyophilized to afford the functionalized hCAII **21**.

The determination of PNA concentration: PNA was dissolved in HEPES buffer (50 mM, pH 8.0). A volume of 300 μL Bradford assay reagent was added to 10.0 μL protein samples in test tube, and the resulting mixture was blended by gentle vortex mixing. After 5 min, absorbance at 595 nm was measured in plastic 96-well microplate against a reagent blank using Safire microplate reader. The calibration curve was built using PNA samples (12.5−75.0 μM).

The determination of hCAII concentration: hCAII was dissolved in distilled water. A volume of 300 μL Bradford assay reagent was added to 10.0 μL protein samples in test tube, and the resulting mixture was blended by gentle vortex mixing. After 5 min, absorbance at 595 nm was measured in plastic 96-well microplate against a reagent blank using Safire microplate reader. The calibration curve was built using hCAII samples (5.0−25.0 μM).

The determination of protein concentration in hRBC lysate: hRBC lysate was diluted with distilled water. A volume of 500 μL bradford assay reagent was added to 10.0 μL protein samples in test tube, and the resulting mixture was blended by gentle vortex mixing. After 5 min, absorbance at 595 nm was measured in plastic 96-well microplate against a reagent blank using Safire microplate reader. The calibration curve was built using BSA samples as a standard protein (0.2−1.0 mg/mL).

The estimation of hCAII concentration in hRBC lysate: hCAII concentration in hRBC lysate (10 mg/mL), which was used in the solid-phase affinity labeling reaction, was estimated to be 4.6 μM based on comparison to a density of SDS-PAGE band of the standard hCAII using the Image J program (NIH, Bethesda, MD).

Enzymatic digestion and peptide mapping experiments.

The labeled hCAII **17** was dialyzed aginast H_2O with Specra/Pro® Dialysis Membrane, and then the resulting solution was lyophilized. The residue was reconstituted at 0.47 μM in 50 mM Tris−HCl buffer (pH 9.0) containing 3 M urea. The solution was treated with Lysyl endopeptidase (LEP) (Wako Pure Chemical Industries, LTD.) at 37 °C overnight at the enzyme/substrate ratio of 1:50 (w/w). The digestion reaction was stopped by addition of TFA (final concentration: 0.1% (v/v)). The digested peptides

were analyzed by MALDI−TOF MS using CHCA as a matrix and the labeled fragment was further characterized by MALDI−TOF MS/MS analysis.

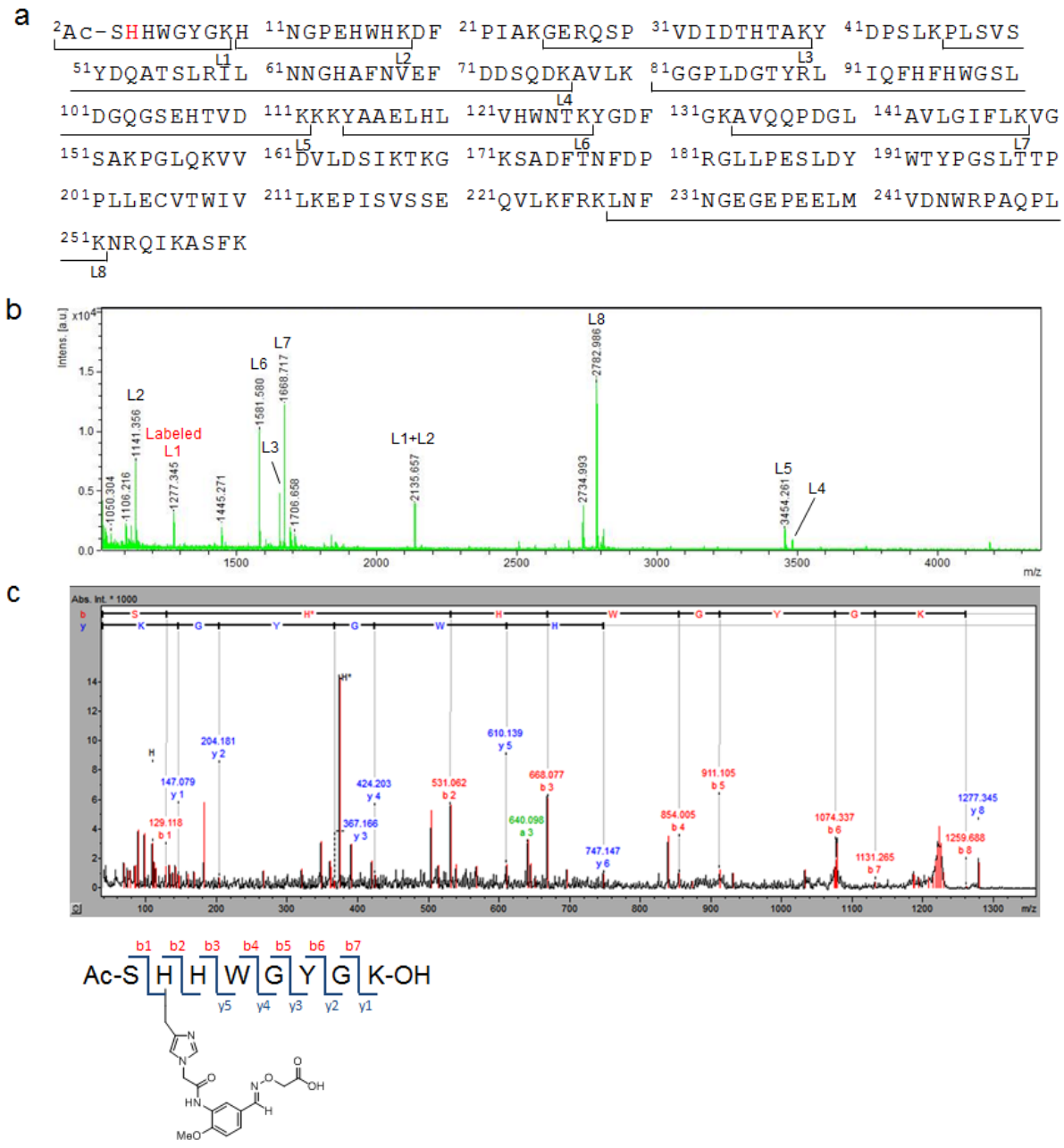


Fig. S1 Determination of the labeling site of the labeled hCAII **17**. (a) The primary sequence of hCAII and the assignment of each fragment generated by Lysyl endopeptidase (LEP) digestion. The N–terminal Ser-2 is acetylated. His-3 shown in red is the labeling site. (b) MALDI–TOF MS spectrum of peptide fragments generated by LEP digestion of the labeled hCAII **17**. The peak corresponding to the labeled L1 fragment was characterized by MALDI–TOF MS analysis. MALDI–TOF MS: calcd for

$[M+H]^+$ = 1277.534, obsd 1277.345. (c) MAIDI–TOF MS/MS analysis of the labeled L1 fragment.

Western blotting analysis of N_3-hCAII 18

The proteins were mixed with an one-fifth volume of 6 × SDS-PAGE loading buffer (Tris (0.35 M), SDS (10%, wt/vol), glycerol (36%, wt/vol), 2-mercaptoethanol (5%, wt/vol) and bromophenol blue (0.012%, wt/vol)). The samples were resolved by 8% SDS-PAGE and electrotransfered onto a nitrocellulose membrane Hybond™–ECL (GE Healthcare). The membrane was blocked with Tris-buffered saline-0.1% Tween 20 (TBST) containing 5% nonfat dry milk for 1 h at room temperature and was incubated with anti-hCAII antibody (Abcam) for 1 h at 4 °C. After washing five times with TBST, the membrane was incubated with anti-rabbit IgG antibody-HRP conjugate (GE Healthcare) for 1 h at 4 °C and then again washed five times. Then the complex was visualized in Medical Film Processer FPM100 (Fujifilm Co.) using the enhanced chemiluminescence reagents Immobilon™ Western (Milipore Co.).

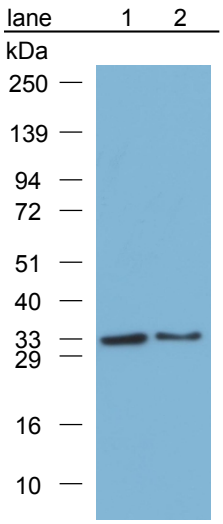


Fig. S2 Western blotting analysis of hCAII in hRBC lysate (lane 1) and N_3-hCAII **18** (lane 2).

MALDI-TOF MS analysis of N_3-hCAII 18 and fluorescein-labeled hCAII 21.

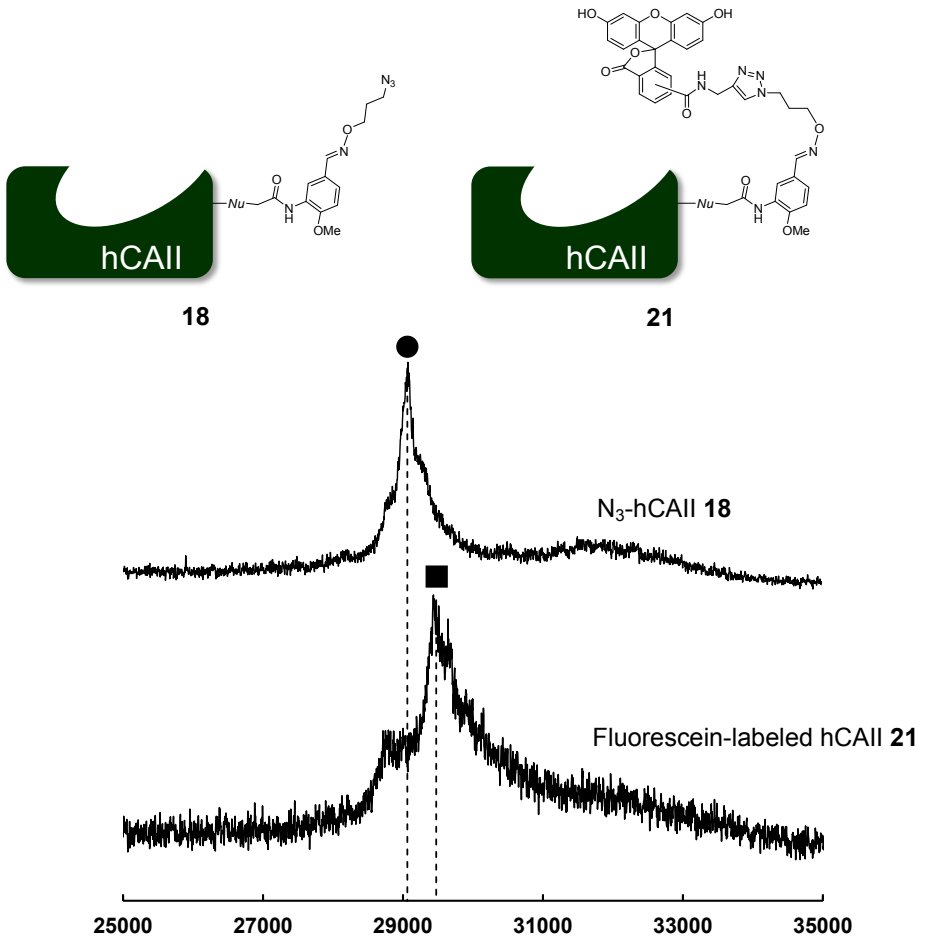


Fig. S3 MALDI-TOF MS analysis of N_3-hCAII **18** (●) (M_w 29069) and fluorescein-labeled-hCAII **21** (■) (M_w 29485).

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

spectrum charts

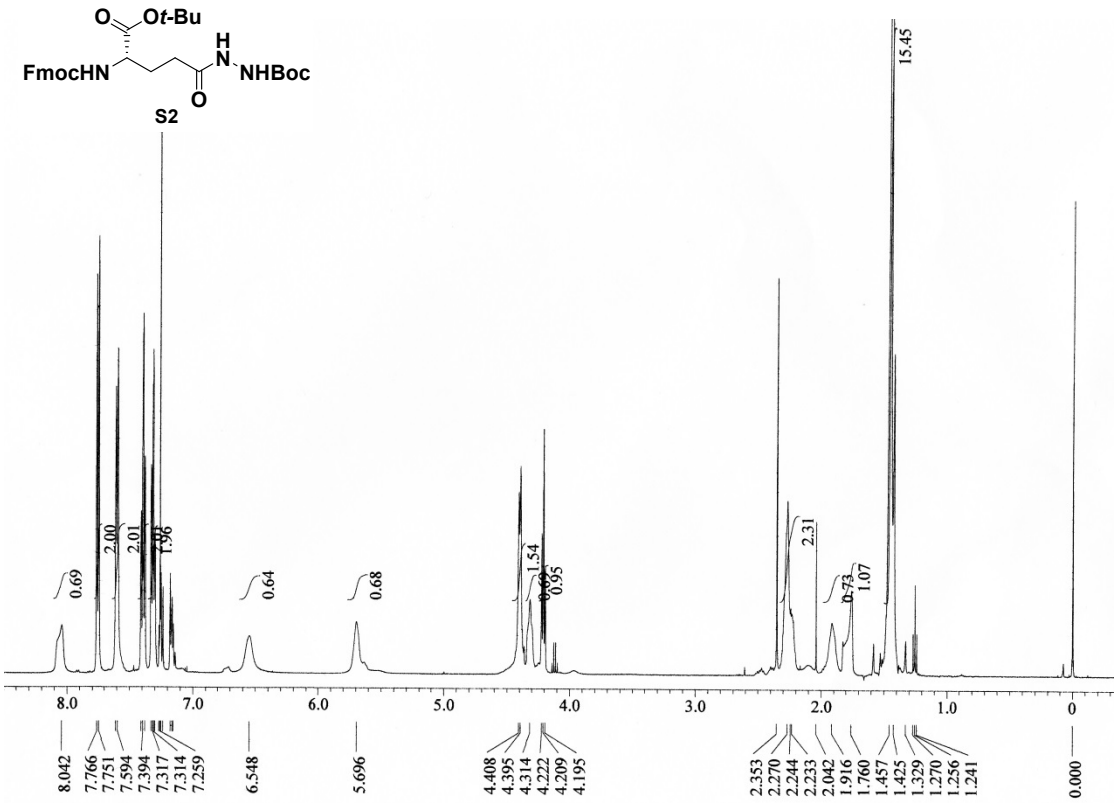


Fig. S4 ^{1}H-NMR spectrum of **S2**

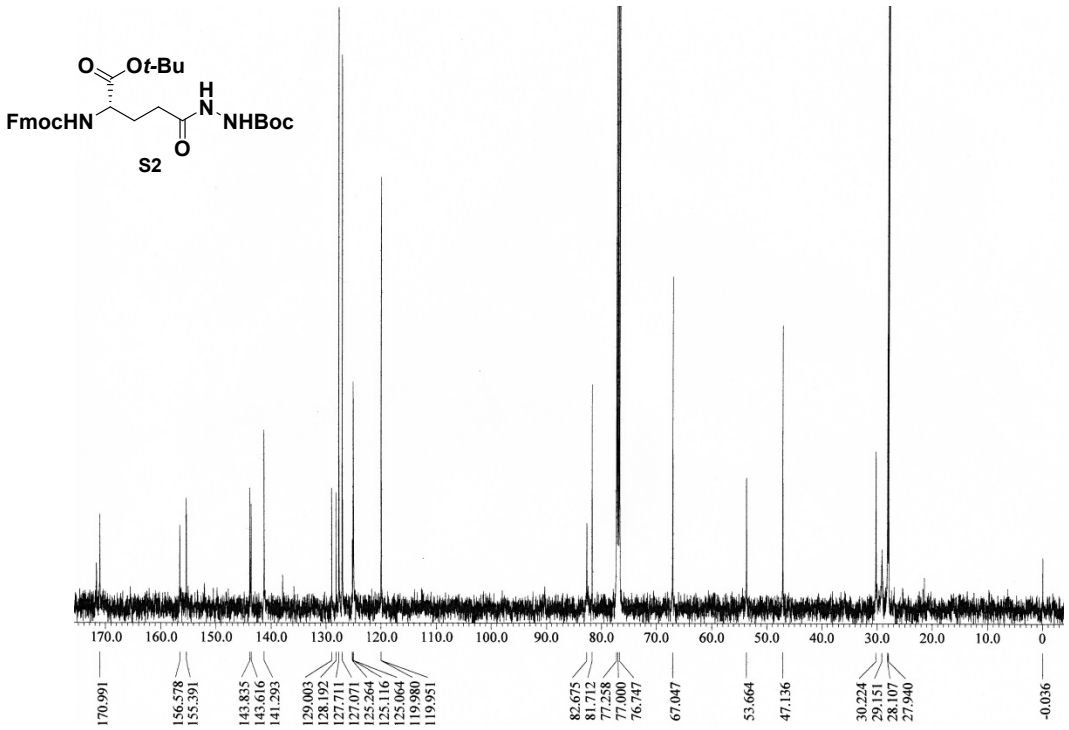


Fig. S5 ^{13}C-NMR spectrum of **S2**

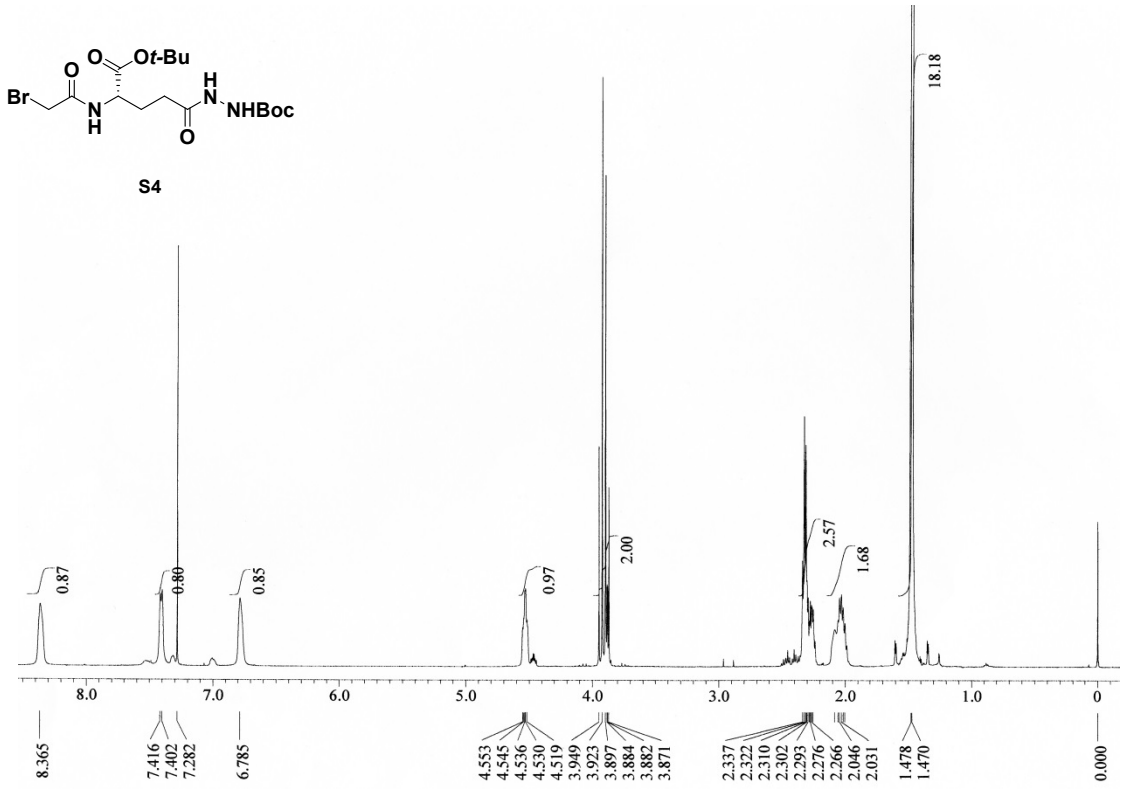


Fig. S6 ^{1}H-NMR spectrum of **S4**

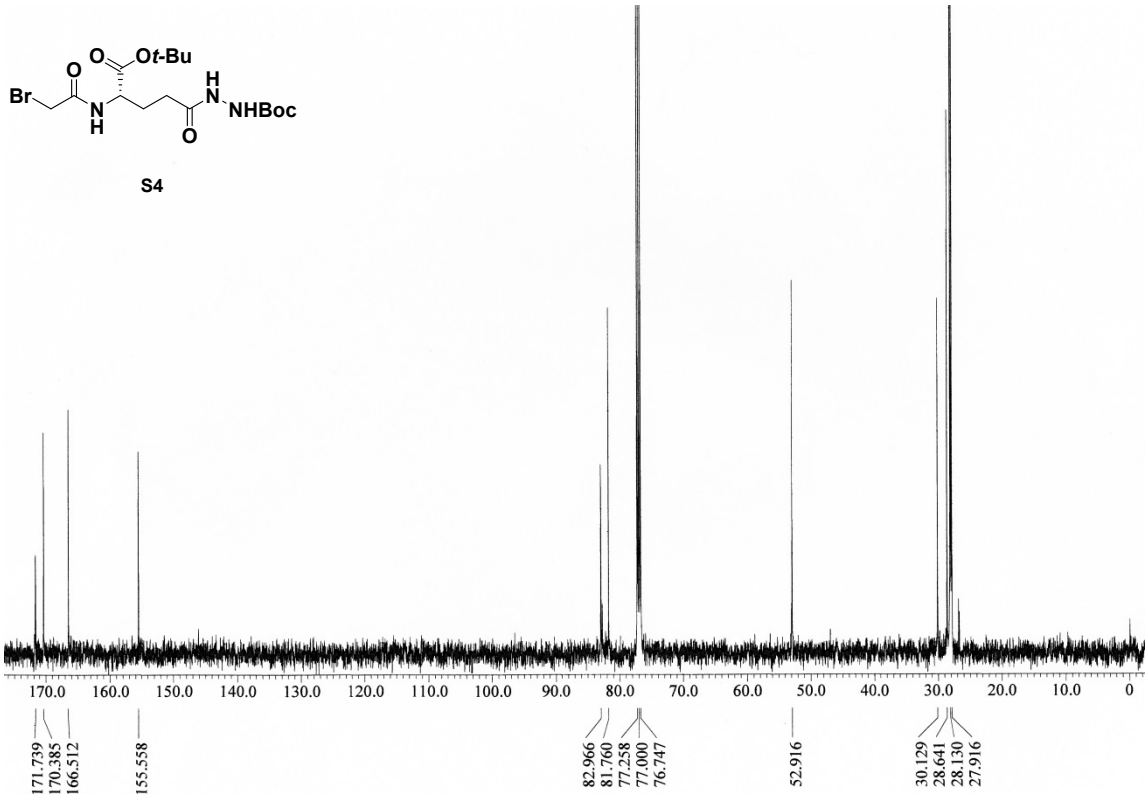


Fig. S7 ^{13}C-NMR spectrum of **S4**

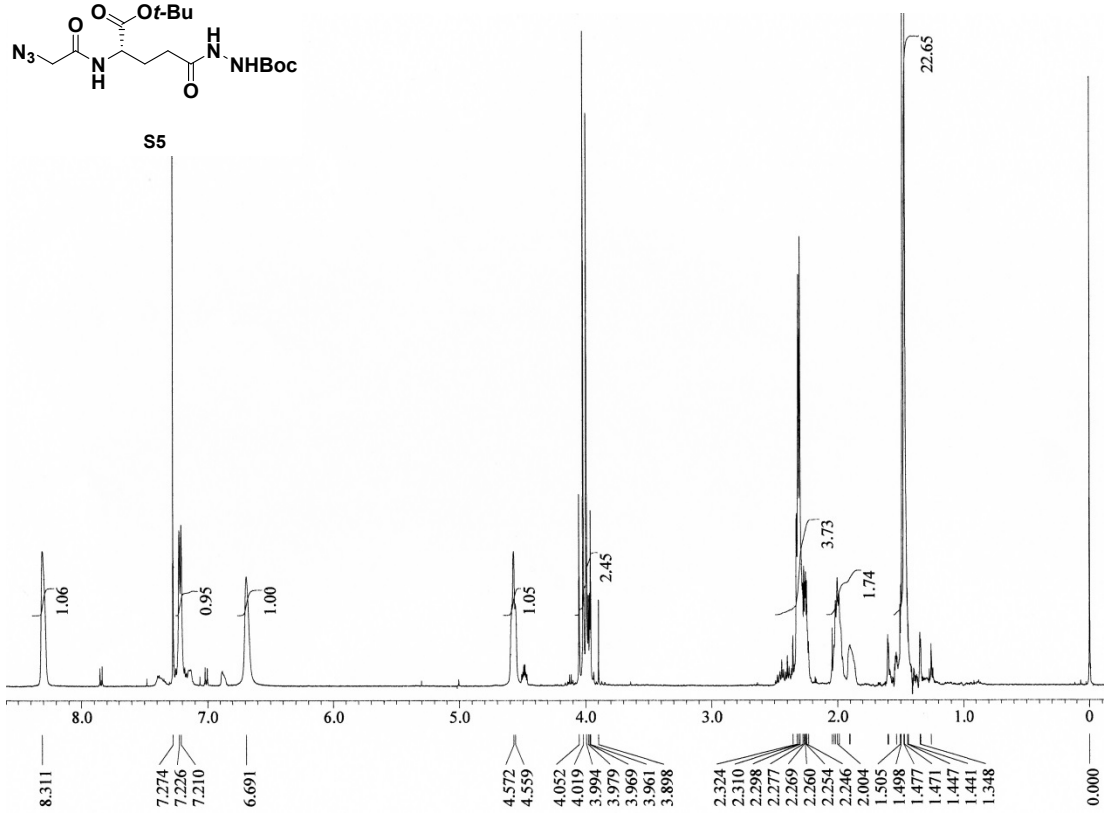


Fig. S8 ^{1}H-NMR spectrum of **S5**

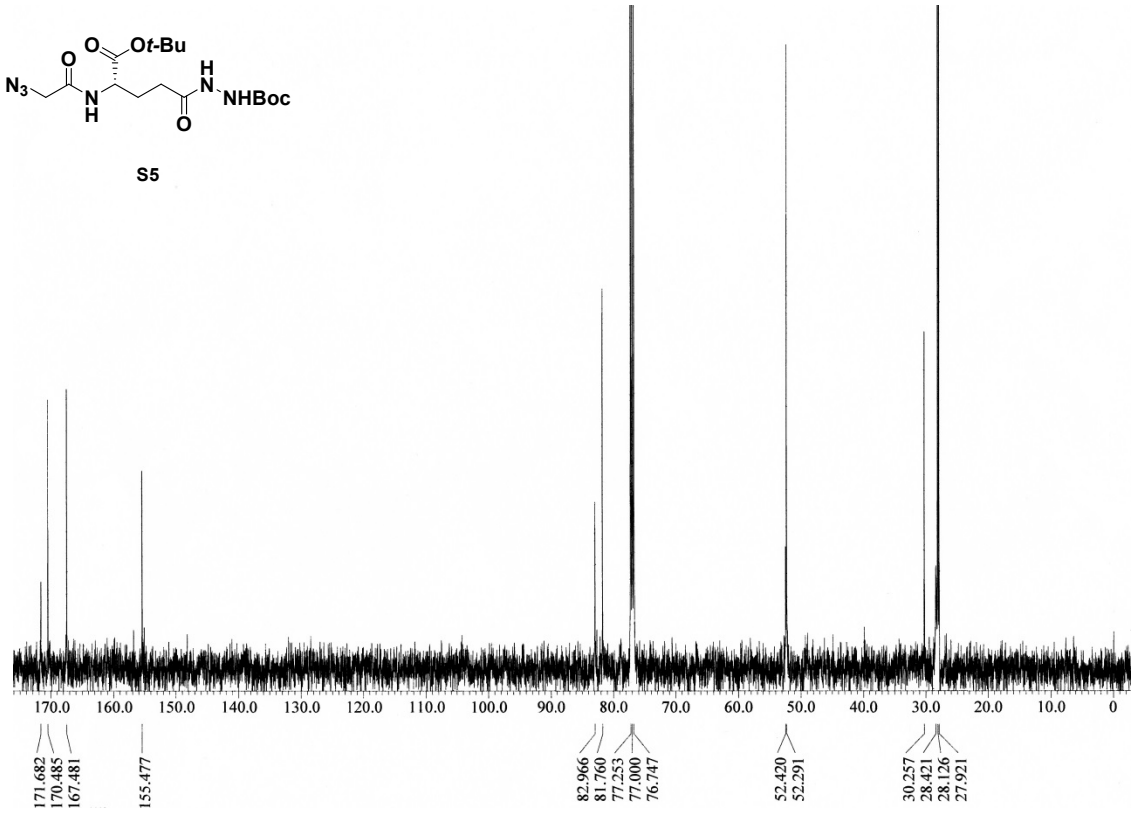


Fig. S9 ^{13}C-NMR spectrum of **S5**

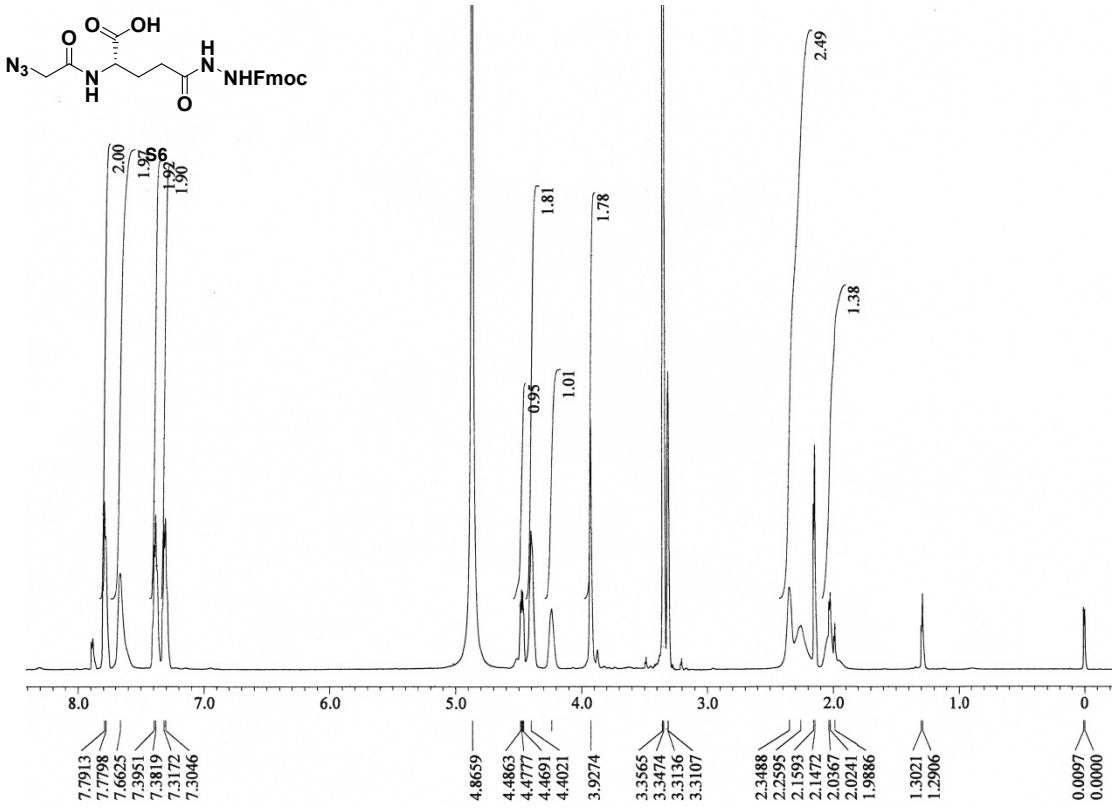


Fig. S10 ^{1}H-NMR spectrum of **S6**

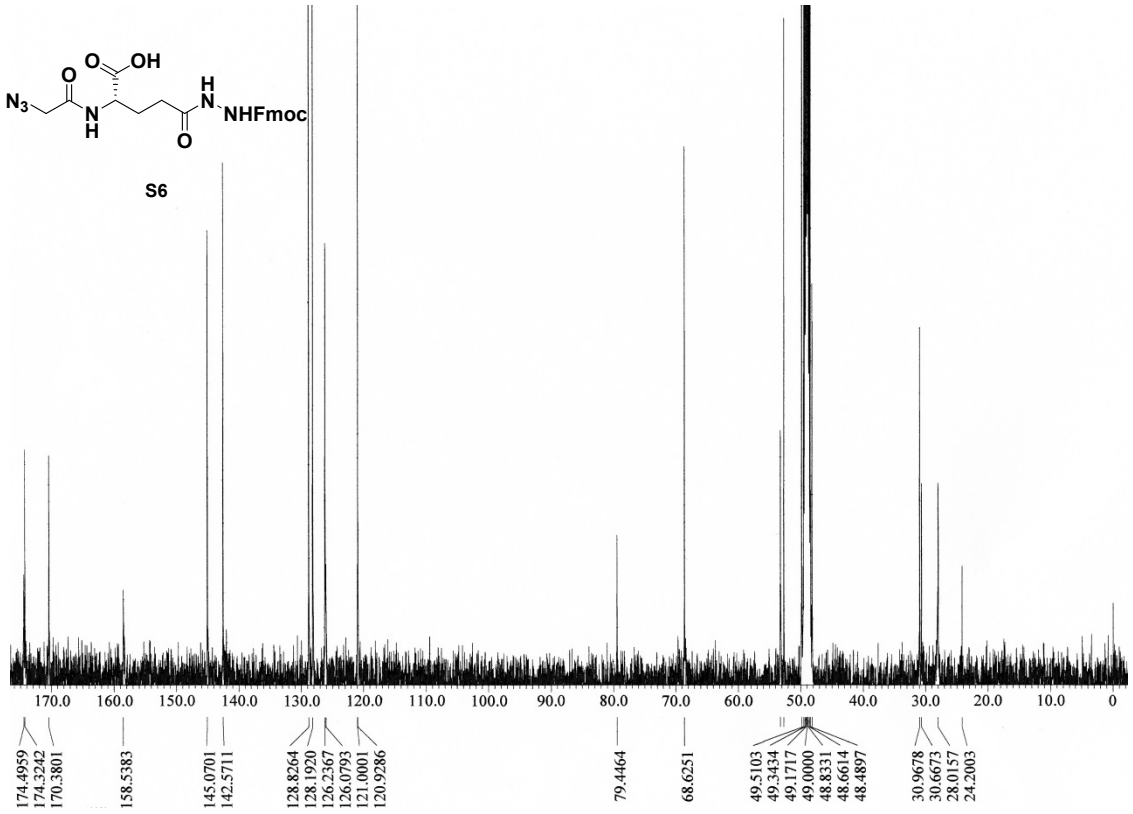


Fig. S11 ^{13}C-NMR spectrum of **S6**

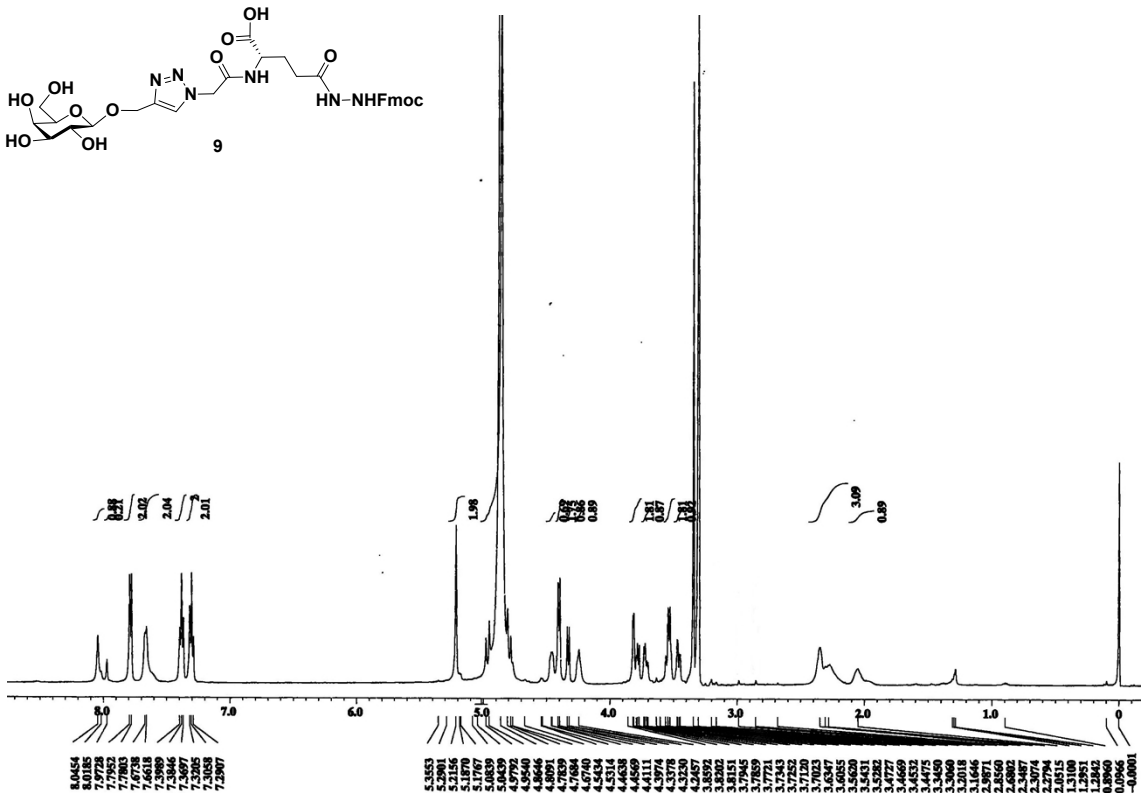


Fig. S12 ^{1}H-NMR spectrum of **9**

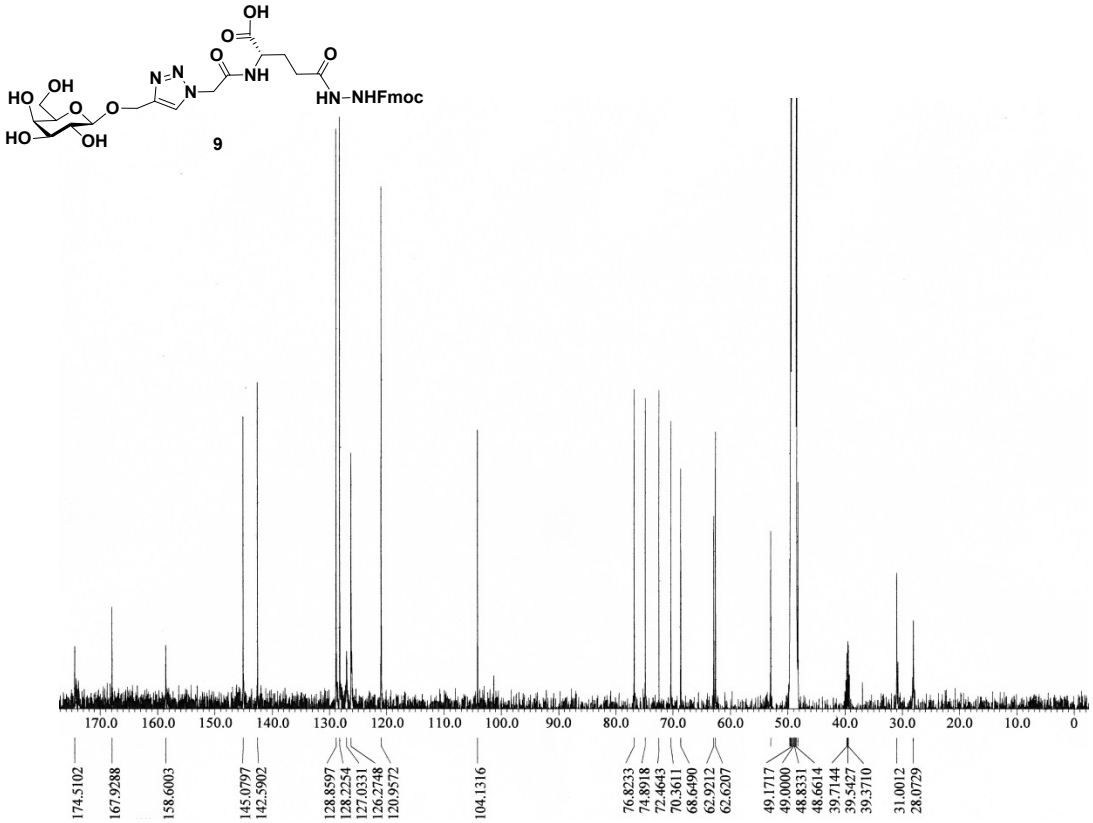


Fig. S13 ^{13}C-NMR spectrum of **9**

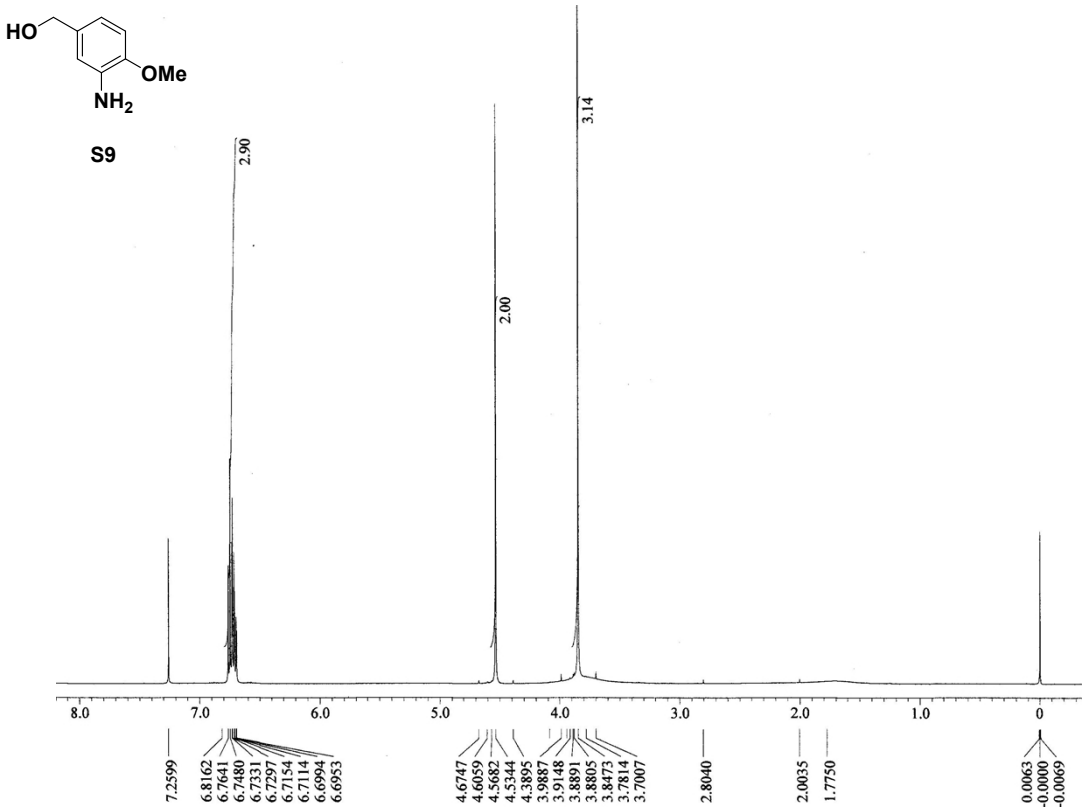


Fig. S14 ^{1}H-NMR spectrum of **S9**

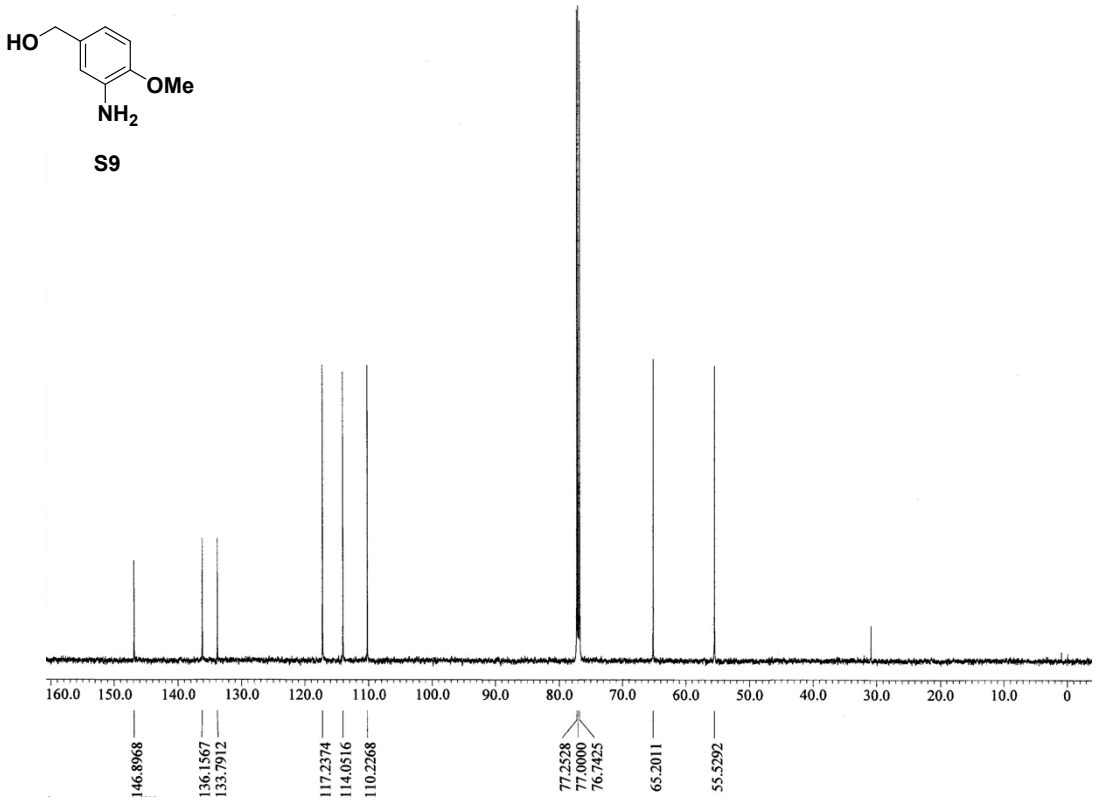


Fig. S15 ^{13}C-NMR spectrum of **S9**

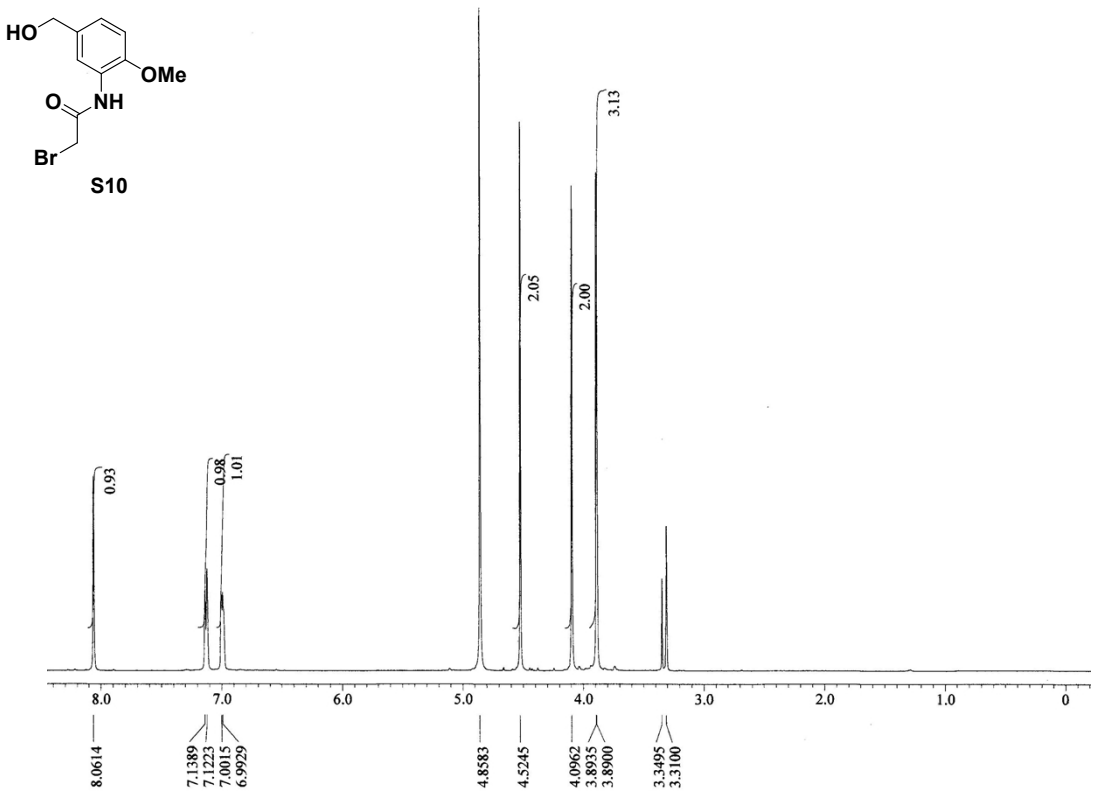


Fig. S16 ^{1}H-NMR spectrum of **S10**

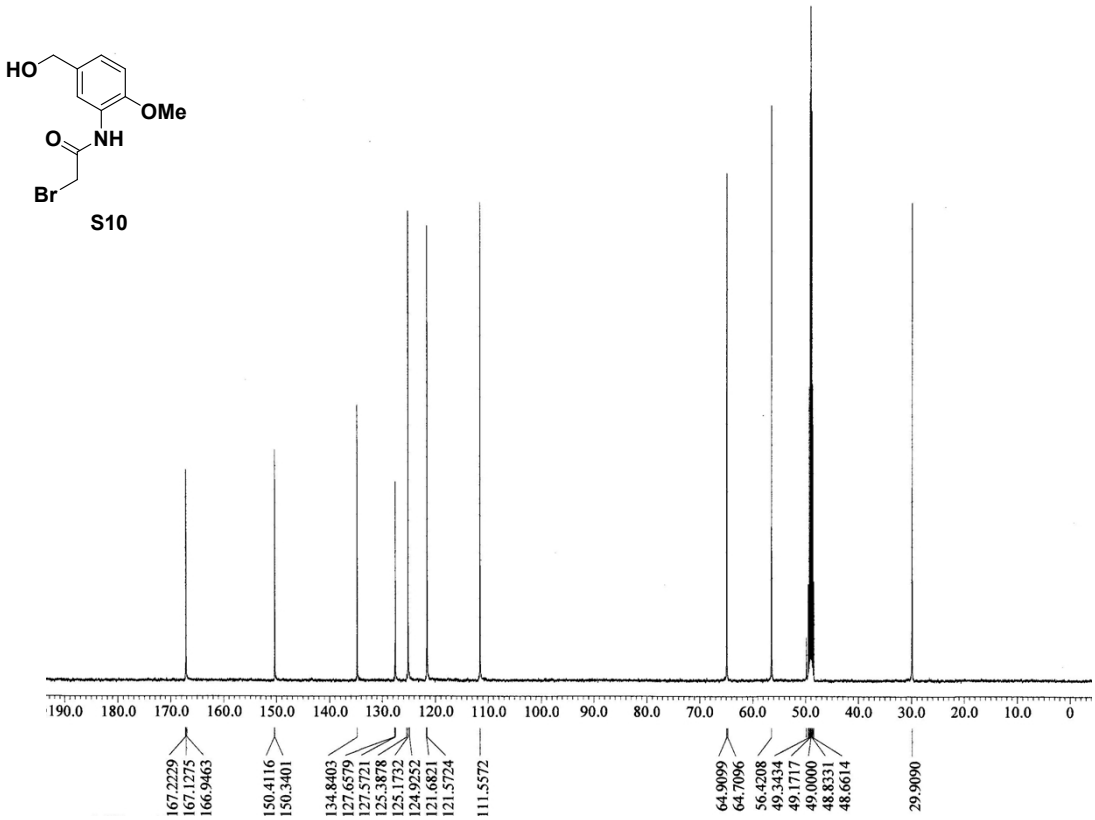


Fig. S17 ^{13}C-NMR spectrum of **S10**

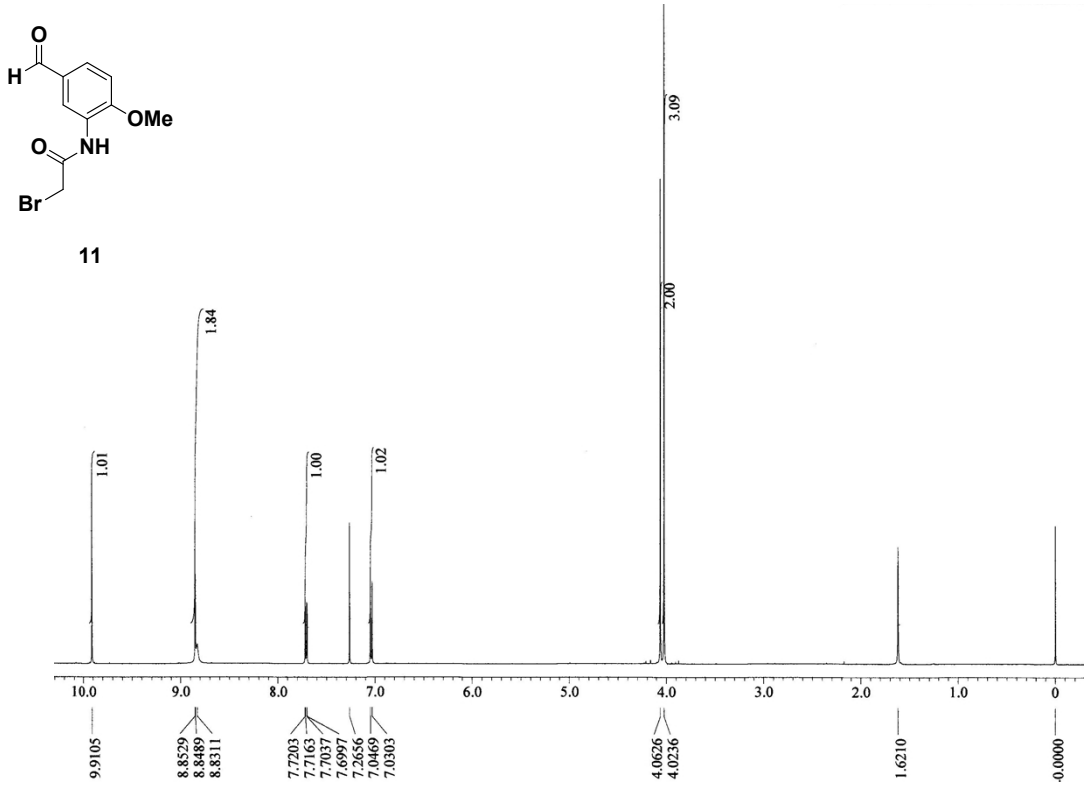


Fig. S18 ^{1}H-NMR spectrum of **11**

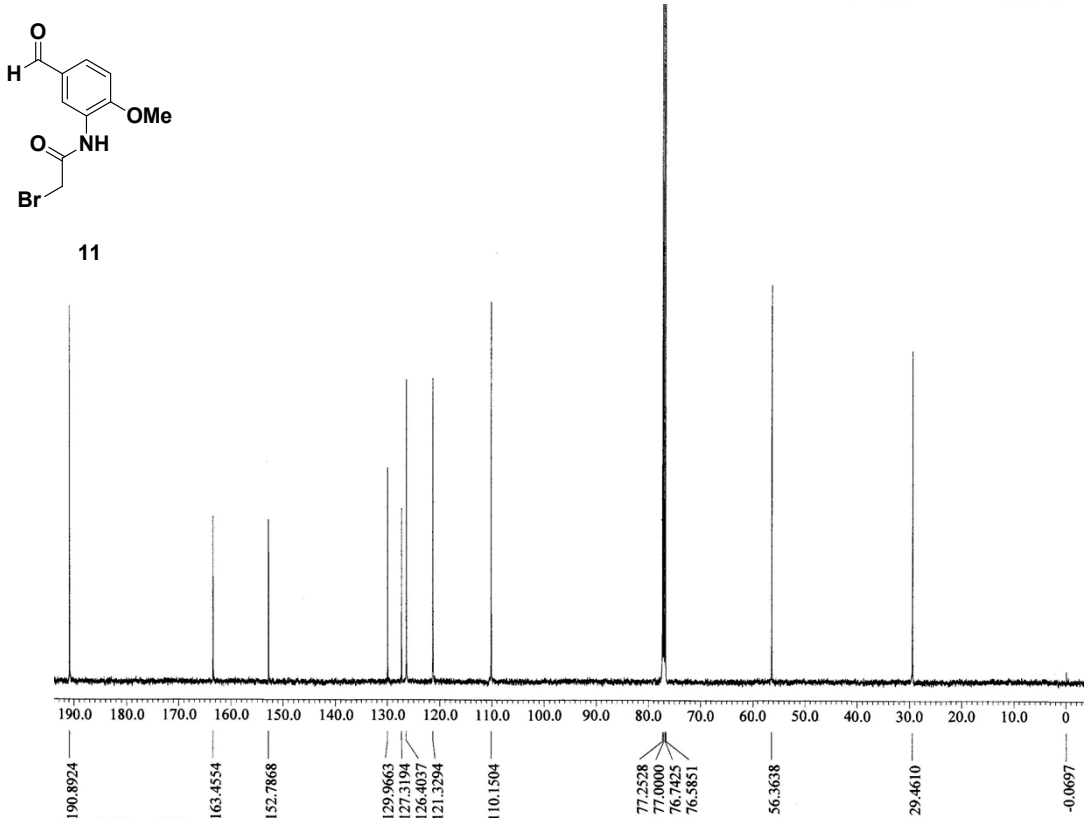


Fig. S19 ^{13}C-NMR spectrum of **11**

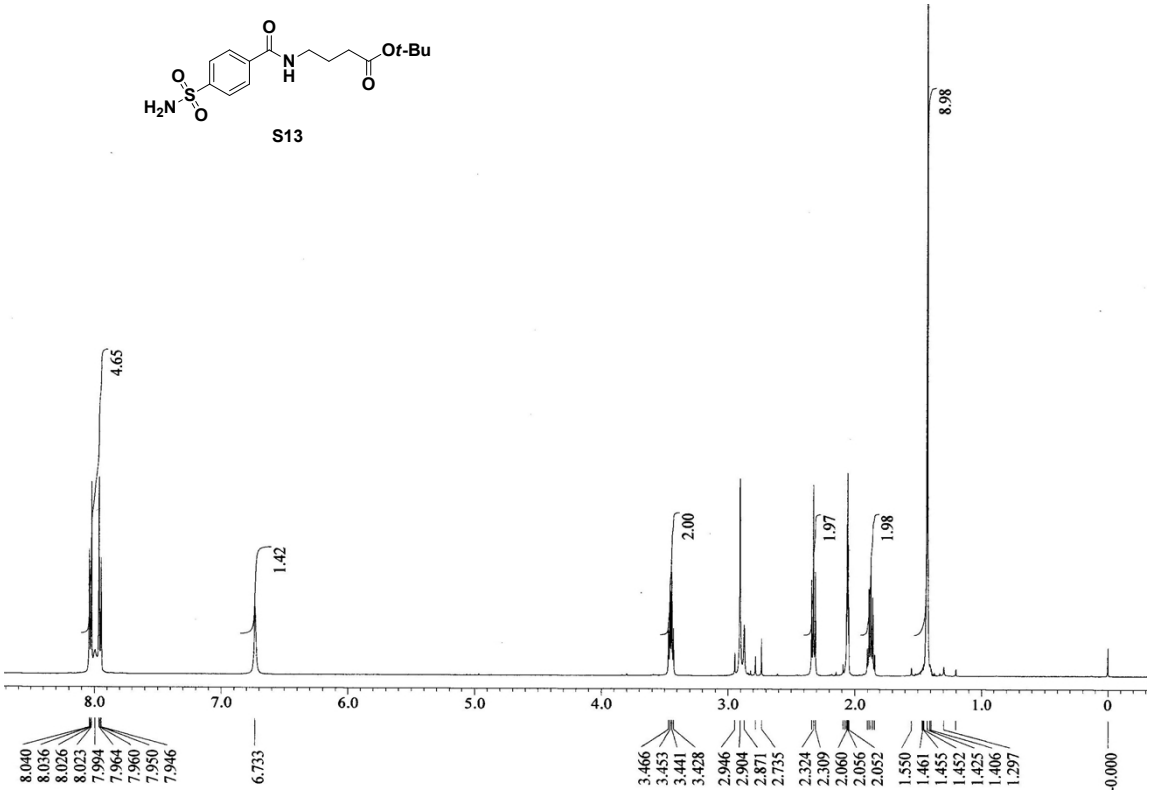


Fig. S20 ^{1}H-NMR spectrum of **S13**

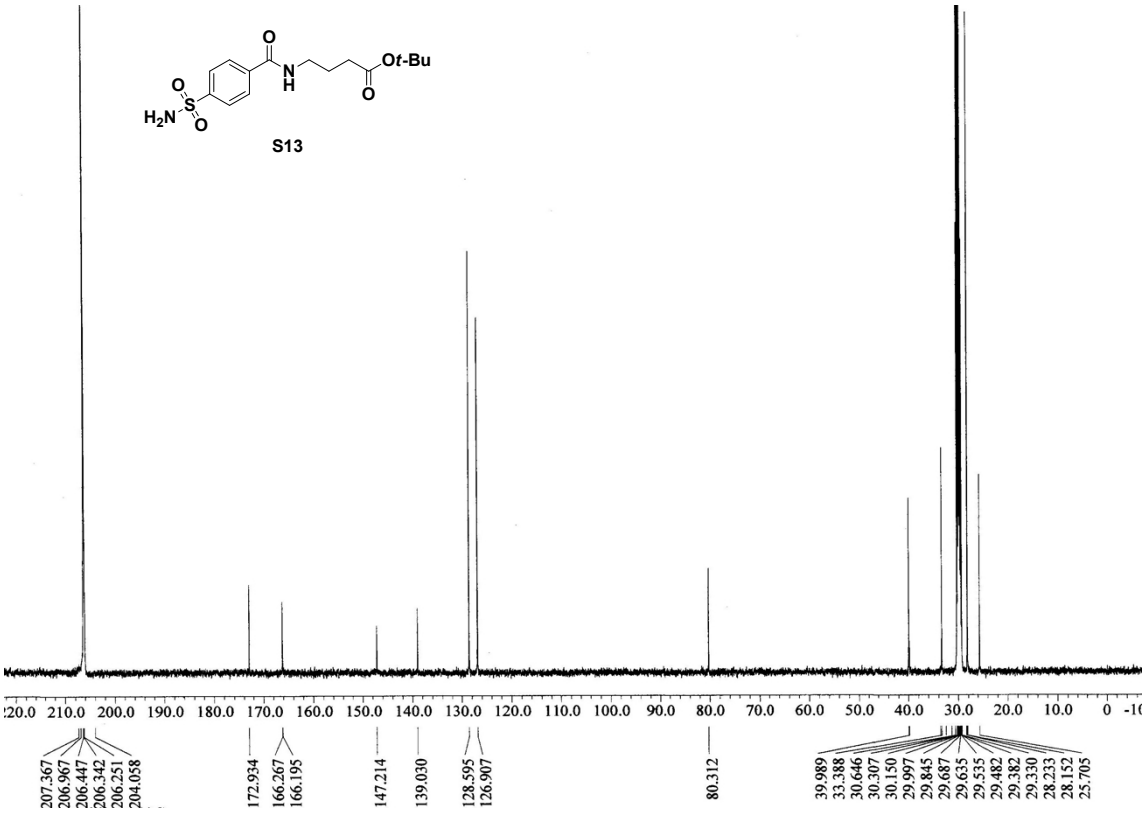


Fig. S21 ^{13}C-NMR spectrum of **S13**

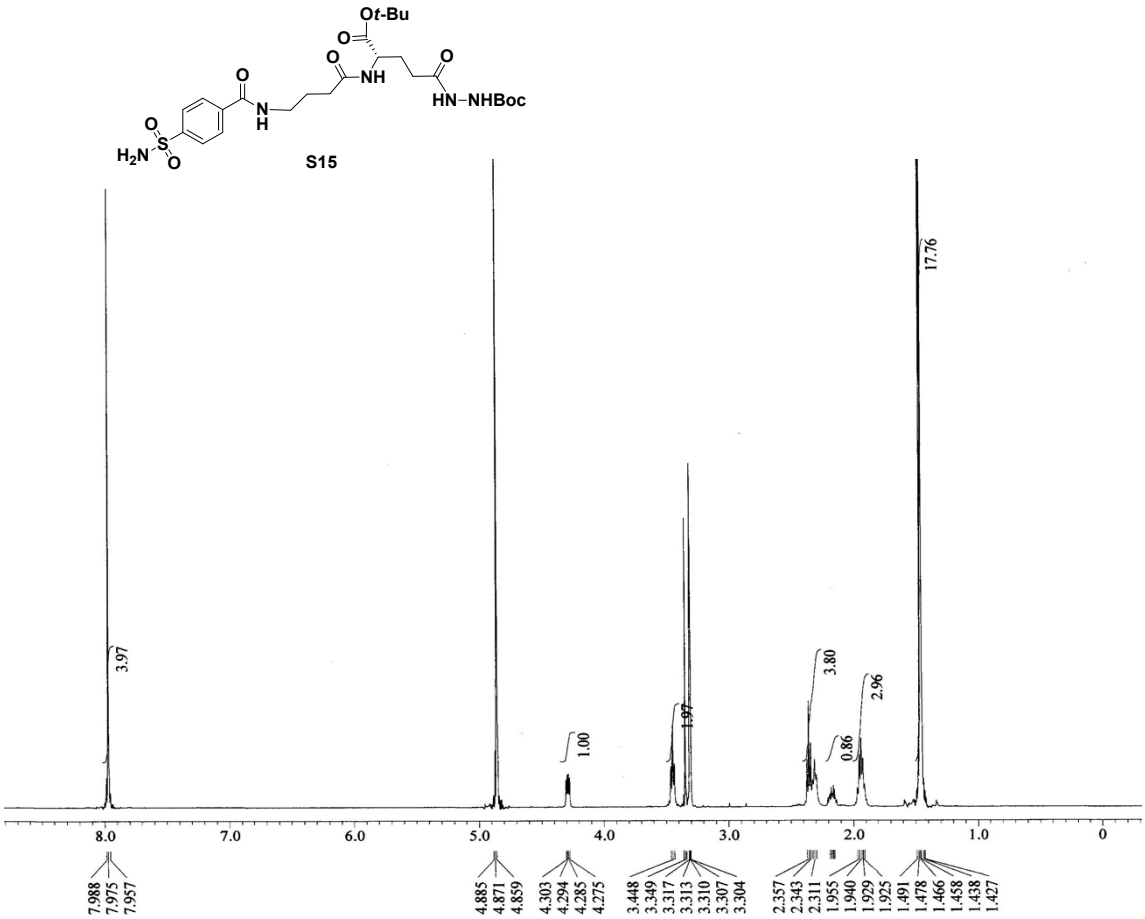


Fig. S22 ^{1}H-NMR spectrum of **S15**

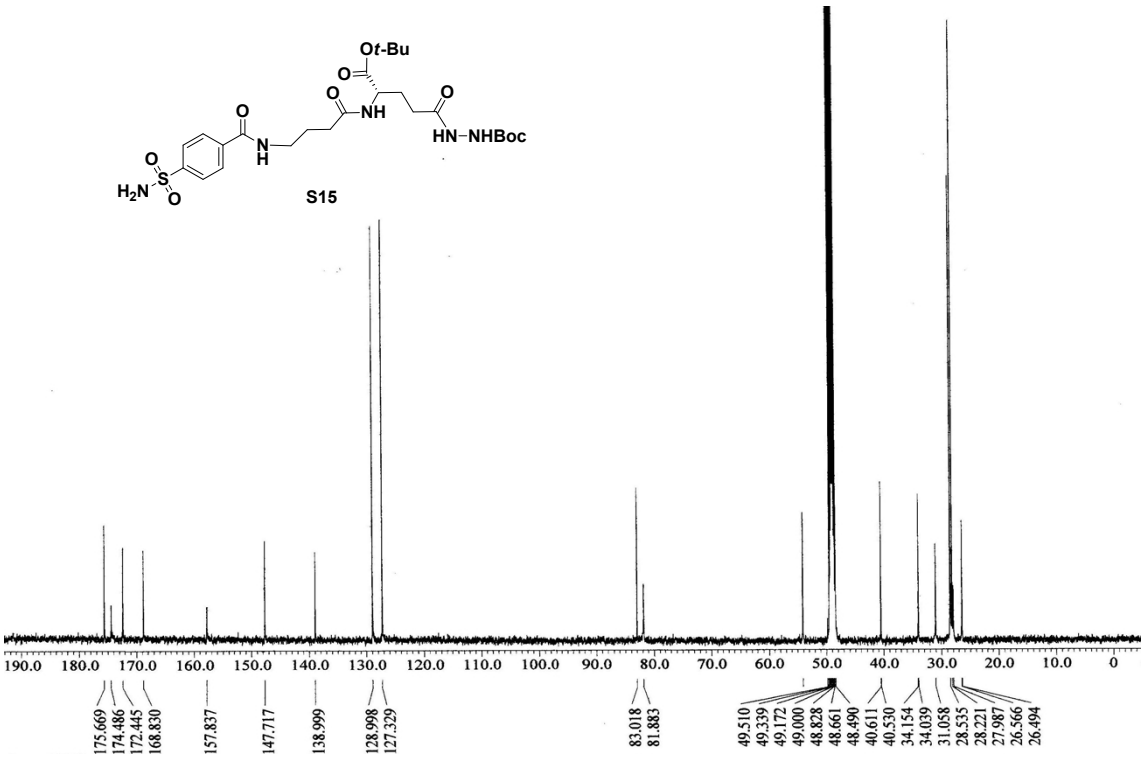


Fig. S23 ^{13}C-NMR spectrum of **S15**

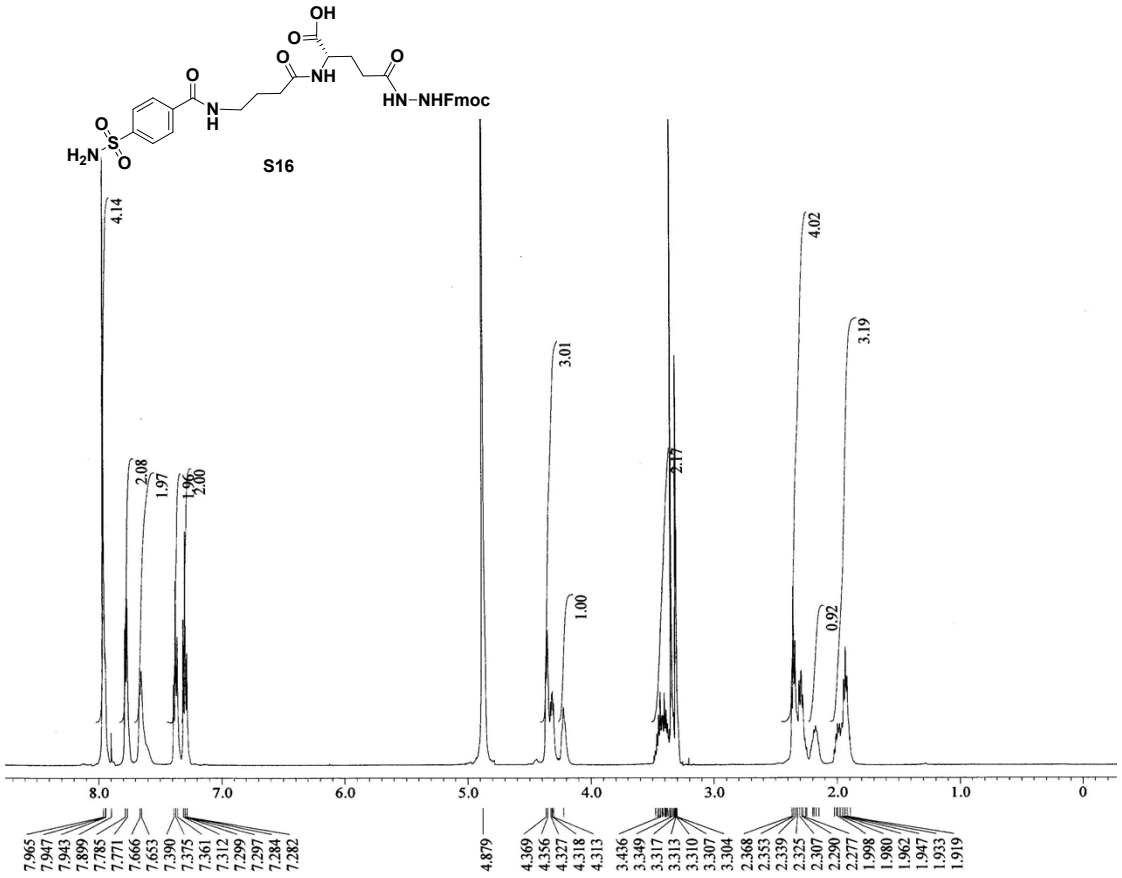


Fig. S24 ^{1}H-NMR spectrum of **S16**

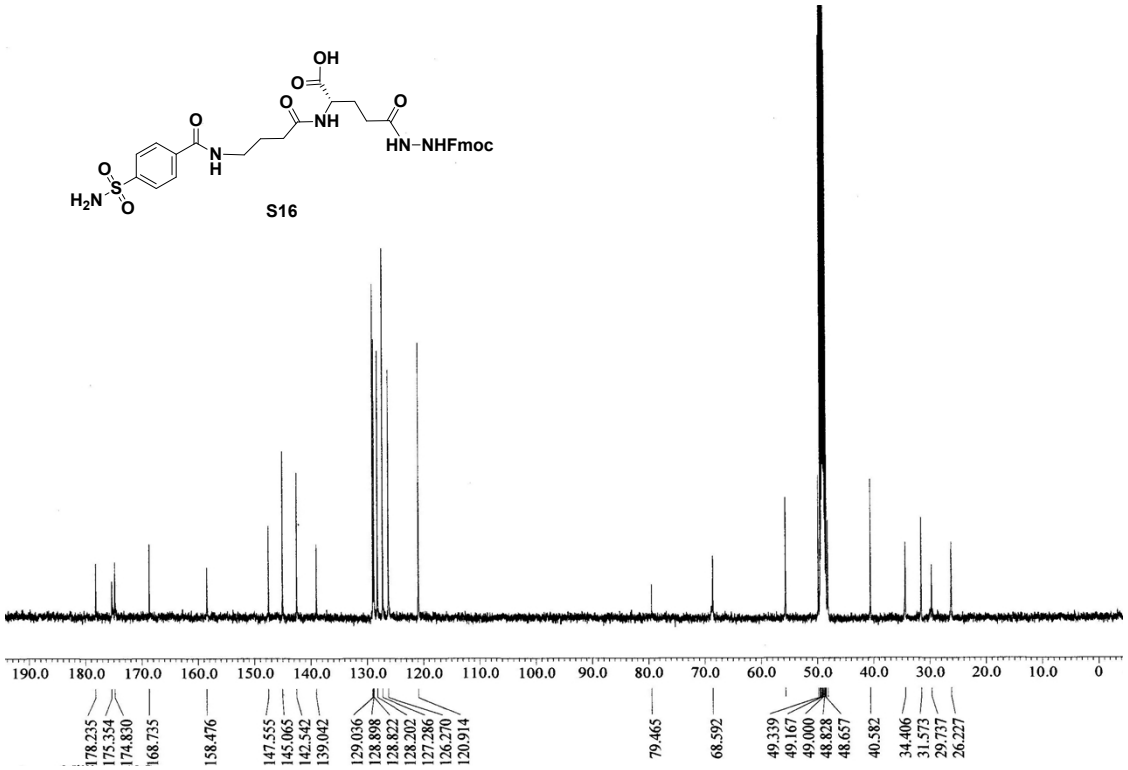


Fig. S25 ^{13}C-NMR spectrum of **S16**

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