Electronic Supplementary Material (ESI) for Chemical Communications

FRET-Based probe with a chemically deactivatable quencher

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Supporting Information

General Methods

General experimental procedures: Unless otherwise indicated, reactions were carried out under an atmosphere of argon in flame-dried glassware with magnetic stirring. Air and/or moisture-sensitive liquids were transferred *via* syringe. When required, solutions were degassed by bubbling of argon through a needle. Organic solutions were concentrated by rotary evaporation at 25-60 °C at 15-30 torr. Analytical thin layer chromatography (TLC) was performed using plates cut from glass sheets (silica gel 60F-254 from Merck). Visualization was achieved under a 254 or 365 nm UV light and by immersion in an ethanolic solution of cerium sulfate, followed by treatment with a heat gun. Column chromatography was carried out as "Flash Chromatography" using silica gel G-25 (40-63 μM) from Macherey-Nagel.

Materials: All reagents were obtained from commercial sources and used without further purifications. Dry MeOH and DMF were obtained from Aldrich. Dichloromethane was passed through a column of activated alumina under nitrogen. For enzymatic activity study, Human recombinant caspase-3, buffer (20 mM HEPES, pH 7.4 with 2 mM EDTA, 0.1% CHAPS, and 5 mM DTT), fluorescent probe Ac-DEVD-AMC (**4**) and capase-3 inhibitor Ac-DEVD-CHO (**5**) were purchased from Aldrich in a Caspase-3 Assay Kit, Fluorimetric (Catalog Number CASP3F).

Instrumentation: UV-Vis spectra and kinetic were recorded on Shimadzu UV-1800 spectrophotometer. Melting points were taken on a Stuart Scientific SMP3 apparatus from Bibby and are uncorrected. IR spectra were recorded on a Nicolet 380 FT-IR spectrometer from Thermo Electron Corporation as a CH_2Cl_2 solution or solid on a diamond plate. ^{1}H and ^{13}C NMR spectra were recorded at 23°C on Bruker 400 and 500 spectrometers. Recorded shifts are reported in parts per million (δ) and calibrated using residual undeuterated solvent. Data are represented as follows: Chemical shift, mutiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad), coupling constant (*J*, Hz) and integration. High resolution mass spectra (HRMS) were obtained using a Agilent Q-TOF (time of flight) 6520 and low resolution mass spectra using a Agilent MSD 1200 SL (ESI/APCI) with a Agilent HPLC1200 SL. GC-MS analyses were performed by means of Agilent 7890A Gas Chromatograph equipped with DB-5MS 30 m x 0.25 mm column and JEOL AccuTOF-GCv. The semi-preparative HPLC system consisted of a Waters 600 pump, a 2487 detector (Waters), a 5 ml sample loop and a Sunfire C18 column (150 mm × 19 mm i.d., 5 μm, Waters) with a 40 minutes gradient from 5% to 95% acetonitrile.

Dithionite cleavage procedure

Dithionite cleavage procedure for CDQ and Dabcyl: A 10 mM azo solution was prepared in DMSO and diluted in phosphate buffer (100 mM, pH 7.4) to obtain 50 μM of azo compound solution. Then, 950 μL of this solution was added to 50 μL of freshly prepared solution of sodium dithionite at 20.2 mM. Color disappearance was followed by UV/VIS spectrophotometry at 438 nm for **CDQ** and 460 nm for **Dabcyl**.

Dithionite cleavage procedure for 2 and 3: A 1 mM probe solution was prepared in DMSO and diluted in phosphate buffer (100 mM, pH 7.4) to obtain 2 µM of probe solution. Then, 950 µL of this solution was added to 50 µL of freshly prepared solution of sodium dithionite at 20.2 mM. Increase in fluorescence intensity was followed by fluorescence spectrophotometry. Excitation and emission wavelengths were 430 and 476 nm, respectively.

Enzymatic assay

Procedure for fluorimetric assay of caspase-3 activity using **3** and **4**: A 1 mM probe solution was prepared in DMSO and diluted in buffer (20 mM HEPES, pH 7.4 with 2 mM EDTA, 0.1% CHAPS, and 5 mM DTT). 965 µL of this solution was added into a cuvette and 10 µL of buffer or caspase-3 inhibitor was added. Then 25 µL of buffer or 1 µg/mL Human recombinant caspase-3 was added to the reaction mixture and kinetic was monitored at room temperature. (excitation - 360 nm for **4** and 430 for **3**) (emission - 430 nm for **4** and 476 for **3**). When saturation curves were observed, 50 µL of freshly prepared solution of sodium dithionite (21 mM) were added in the reaction including **3**. After 1 min of reaction at room temperature, fluorescence emission was recorded.

Cell Culture

HeLa cells were cultured in Dulbecco's modified Eagle medium (D-MEM, high glucose, Gibco-invitrogen) supplemented with 10% (v/v) fetal bovine serum (FBS, Lonza), 1% antibiotic solution (penicillin-streptomycin, Gibco-invitrogen) in a humidified incubator with 5% CO_2 /95% air atmosphere at 37°C. Cells plated on a 75 cm^2 flask at a density of 10^6 cells/flask were harvested at 80% confluence with trypsin–EDTA (Sigma) and seeded onto a chambered coverglass (IBiDi) at a density of 5×10^4 cells/IBiDi 24h before the microscopy measurements. To prepare apoptotic cells, the cells were treated with actinomycin D (0.5 µg/ml) for 18h prior the experiments.
Finally, cells in iBiDi dishes were washed with HBSS Hank's Buffered Salt Solution (SIGMA). Then, a solution of peptide in Opti-MEM was added. Microscopy images were taken after 1h of incubation at 37°C with the peptide.

Fluorescence spectroscopy and microscopy

Fluorescence spectra were recorded on a Fluorolog (Jobin Yvon, Horiba) spectrofluorometer at room temperature. Fluorescence images were taken on a Leica DMIRE2 inverted microscope equipped with a Leica DC350FX CCD camera and a thermostated chamber (Life Imaging Services, Basel Switzerland) for maintaining the temperature at 37°C. A 40× HCX PL APO (1.25 NA) objective and a CFP Filter (excitation 436/20nm, dichroic mirror 455nm, emission 480/40nm) were used.

ATP/Luciferase assay

In vitro cytotoxicity was measured using an Adenosine triphosphate monitoring luminometric assay (ATPlite ™, Perkin Elmer). The experiments were performed in 96-well plates containing MCF-7 cells grown to confluence in culture media (DEMEM media at 1 g/L of Gluc., supplemented with 10% fetal calf serum and non essential aminoacids (NEAA, 0.1mM) 100 µl per well). Cells were incubated with sodium dithionite at indicated concentrations (100 µM to 20 mM) at 37°C for 1 hour and the content in ATP was measured according to the supplier's instructions. Briefly, after the one hour dithionite treatment, the supernatant was discarded and replaced by PBS (100 µl). 50µl of a mammalian cell lysis solution were added to each well. After a five min shaking step, 50µl of a substrate solution were added to each well. After a further five min shaking step, the plate was allowed to stand in the dark for ten min before the luminescence was measured using a microplate luminometric reader (Synergy HT, Biotek). The cell viabilities were expressed as percent of untreated control cells.

Cleavage kinetics of CDQ and Dabcyl

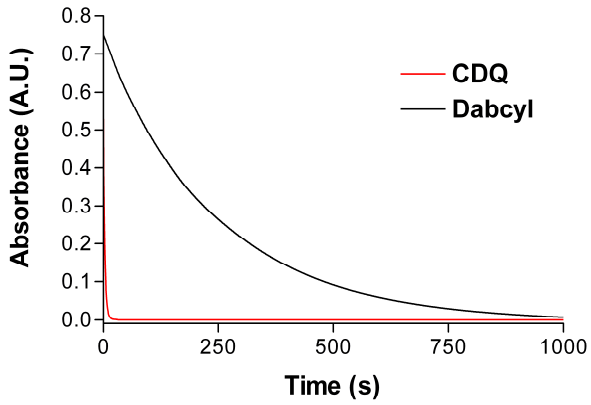


Fig. S1: Cleavage kinetics of **CDQ** and **Dabcyl** (50µM) with 1 mM of sodium dithionite in phosphate buffer (100 mM, pH 7.4), monitored by the absorbance at 449 and 463 nm, respectively.

Dithionite effect on absorption spectra of CDQ

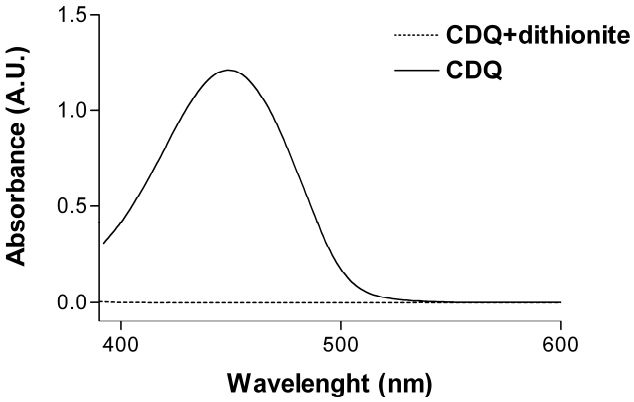


Fig. S2: Absorbance spectra of compound **CDQ** (50µM) with (dashed line) and without (solid line) 1 mM of dithionite solution.

Absorption spectra of CDQ, Dabcyl and DEAC

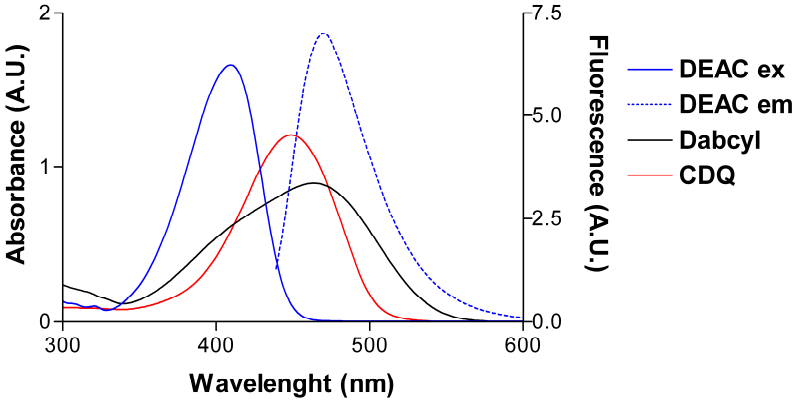


Fig. S3: Absorption spectra of compound **CDQ** (red line), **Dabcyl** (black line) and **DEAC** (blue line) at 50 µM in phosphate buffer (pH 7.4, 100 mM). Fluorescence spectrum of **DEAC** (dashed blue line) at 2 µM in phosphate buffer (pH 7.4, 100 mM).

Absorption spectra of 1 and 2 with and without dithionite

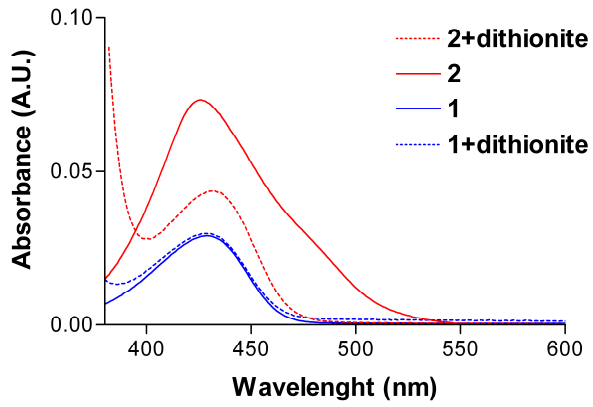


Fig. S4 : Absorption spectra of compounds **1** (blue lines) and **2** (red lines), at 50 µM in phosphate buffer (pH 7.4, 100 mM), before (solid lines) and after deactivation with 1 mM of dithionite solution (dashed lines).

Fluorescence spectra of 3 with and without dithionite

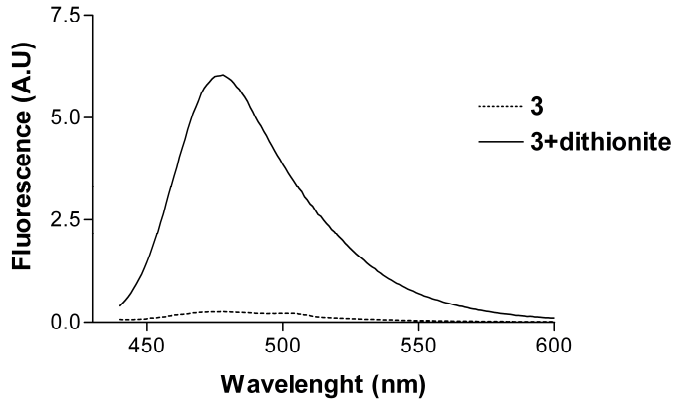


Fig. S5: Fluorescence spectra of compound **3** at 2 µM in phosphate buffer (pH 7.4, 100 mM) with (solid line) and without (dashed line) 1 mM of dithionite solution.

Cleavage kinetics of 4 by Human recombinant caspase-3

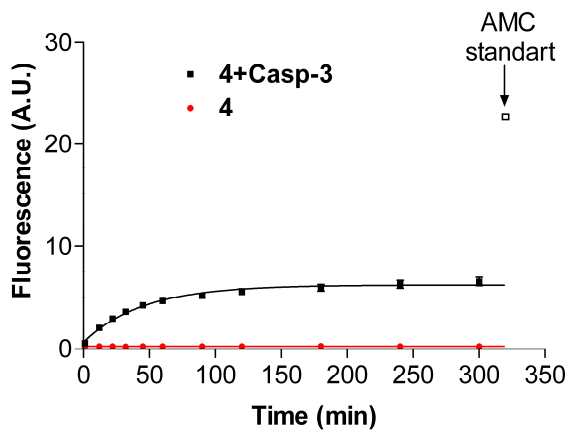


Fig. S6: Cleavage kinetics of **4** at 2 µM by Human recombinant caspase-3 at 25 or 0 ng/mL in buffer, (black and red lines respectively) monitored by fluorescence intensity at 430 nm. To calculate the rate of conversion, fluorescence intensity of a standard AMC solution at the same concentration (2 µM) was also measured.

Competitive inhibition study of caspase-3

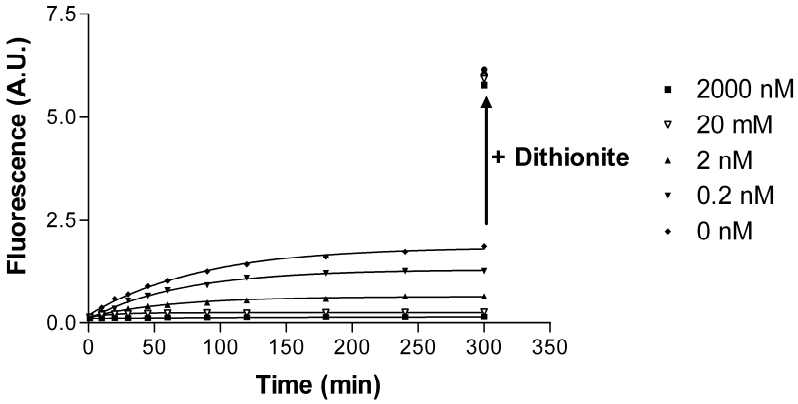


Fig. S7: Cleavage kinetics of **3** at 2 µM by Human recombinant caspase-3 at 25 ng/mL in buffer with different concentration of caspase-3 inhibitor **5**, monitored by fluorescence intensity at 476 nm vs time. After 300 minutes, dithionite solution (1 mM, 1 min) was added to the reaction mixture to reveal unreacted probe

Cell internalization of 3

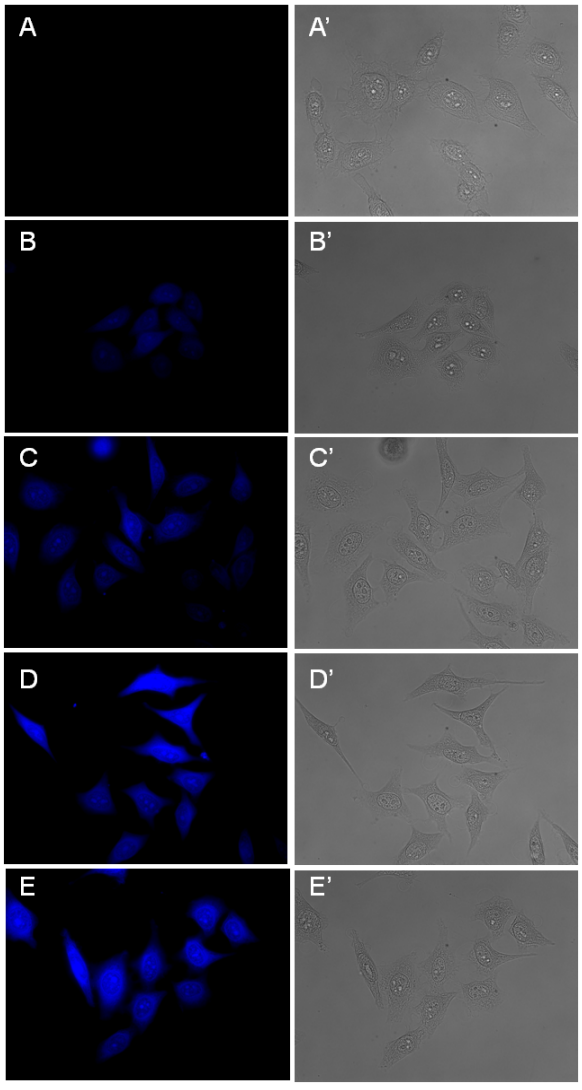


Fig. S8: Cell internalization of probe **3** as a function of incubation time. Cells were treated with 40 µM of **3** for different times (15, 30, 60 and 120 min), washed and incubated with 10 mM of dithionite for 45 min. The fluorescence images (B, C, D and E 15, 30, 60 and 120 min respectively) were acquired and presented with the same instrumental settings and the fluorescence scale ranging from 7880 to 36010. B', C', D', and E' correspond to brighfield images of 15, 30, 60 and 120 min, respectively. A control experiment consisted of incubating HeLa cells with 10 mM of dithionite for 45 min (pictures A and A'). Image size was 219×163 µm.

ATP/luciferase assay for measurement of dithionite cytotoxicity

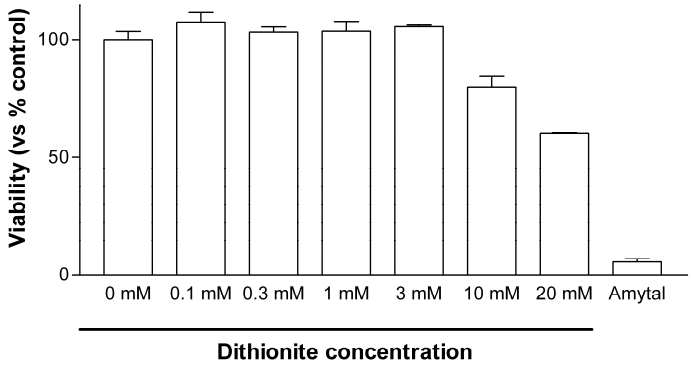
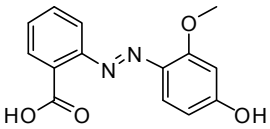


Fig. S9: ATP/luciferase assay on MCF-7 cells with different concentration of dithionite. Controls consist of cells without dithionite and cells+amytal.

Synthesis of CDQ

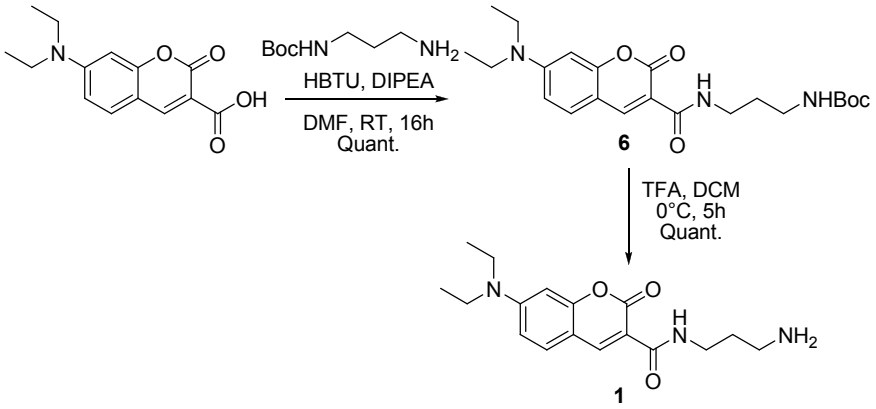
2-(4-hydroxy-2-methoxyphenylazo)benzoic acid ***CDQ***



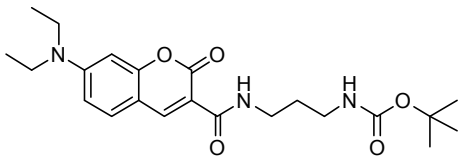
Compound **CDQ** was synthesized in two steps according to a previous described method.[1]

Rf: 0.05 (dichloromethane/MeOH 95:5); decomposition at 218 °C; ^{1}H NMR (400 MHz, dmso-d_6) δ 7.84 (dd, J = 1.6, 7.7 Hz, 1H), 7.66-7.68 (m, 2H), 7.52 (d, J = 8.9 Hz, 1H), 7.49-7.45 (m, 1H), 6.55 (d, J = 2.2 Hz, 1H), 6.49 (dd, J = 2.2, 8.9 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (101 MHz, dmso-d_6) δ 168.1, 158.6, 150.2, 134.3, 132.3, 129.9, 128.6, 127.5, 123.8, 116.6, 110.6, 100.6, 55.9; IR (neat) : 3445, 3155, 1738, 1631, 1584, 1489, 1440, 1325, 995 cm^{-1}; ESI-MS 273.0 $[M+H]^+$. HRMS $[M+H]^+$ *m/z calcd* 287.0954 for $C_{15}H_{15}N_2O_4$, found 287.1034.

Synthesis of 1



Tert-butyl 3-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido)propylcarbamate **6**



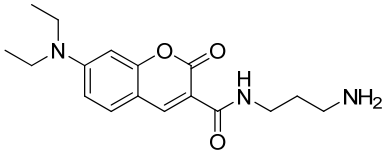
To a stirred solution of 7-(diethylamino)coumarin-3-carboxylic acid (100 mg, 0.38 mmol) in DMF (3.8 mL), were added successively, DIPEA (200 µL, 1.15 mmol) and tert-butyl 3-aminopropylcarbamate (73 mg, 0.42 mmol). The reaction mixture was stirred and cooled to 0°C using an ice bath then HBTU (160 mg, 0.42 mmol) was added. Stirring was continued for 16 hours while the mixture was warmed up to room temperature. The reaction mixture was poured into a solution of 1M HCl solution (40 mL) and extracted two times by EtOAc (2*50 mL). The organics layers were washed successively with two times water (100 mL) and brine (100 mL) then dried over Na_2SO_4 and concentrated under reduce pressure. Silica gel chromatography (DCM/MeOH 100% to 95/5) gave the compound **6** (159 mg, quant.) as a yellow powder.

Rf : 0.87 (DCM/MeOH 9:1); m.p. 118°C; ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 8.82 (t, J = 5.6 Hz, 1H), 8.62 (s, 1H), 7.35 (d, J = 9.0 Hz, 1H), 6.60 (dd, J = 2.4, 9.0 Hz, 1H), 6.42 (d, J = 2.4

[1] Leriche, G.; Budin, G.; Brino, L.; Wagner, A. *Eur. J. Org. Chem.* **2010**, *2010*, 4360-4364.

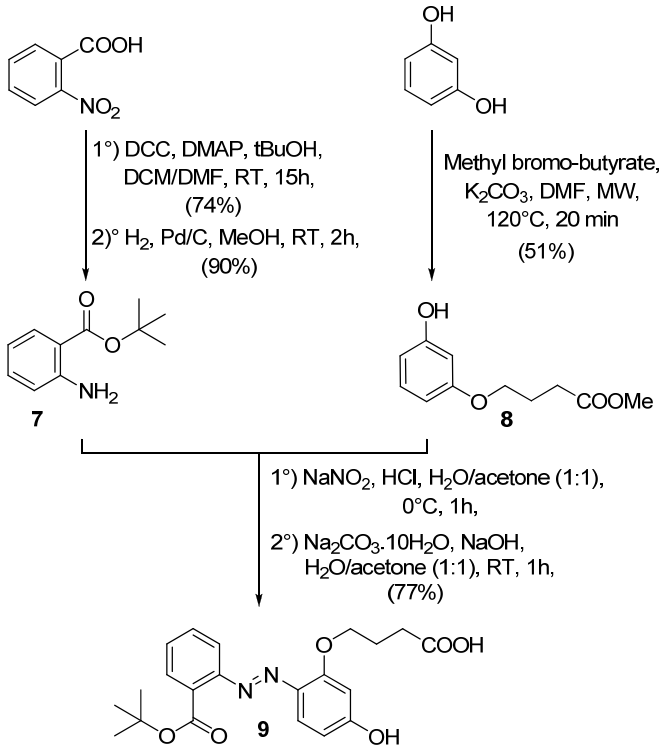
Hz, 1H), 5.30 (m, 1H), 3.48-3.44 (m, 2H), 3.43-3.36 (m, 4H), 3.12 (m, 2H), 1.71 (m, 2H), 1.38 (s, 9H), 1.17 (d, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ 163.7, 162.7, 157.6, 156.1, 152.6, 148.1, 131.1, 110.0, 108.3, 96.5, 78.8, 45.1, 37.4, 36.5, 30.2, 28.4, 12.4; IR (neat) : 3334, 1694, 1615, 1507, 1229, 1132 cm^{-1}; ESI-MS: 418 $[M+H]^+$ and 440 $[M+Na]^+$. HRMS calcd 417.2263 for $C_{22}H_{31}N_3O_5$, found 417.2268.

N-(3-aminopropyl)-7-(diethylamino)-2-oxo-2H-chromene-3-carboxamide ***1***

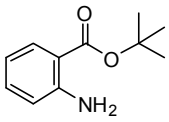


Compound **6** (125 mg, 0.30 mmol) was dissolved in DCM (3.6 ml). TFA (1.2 mL) was added at 0°C and the solution was stirred at room temperature for 5 hours then concentrated. The residue was diluted with a saturated solution of $NaHCO_3$ (3 mL) and extracted three times with EtOAc (2*6 mL). Organics layers were washed with brine (20 mL), dried over Na_2SO_4 and concentrated under reduce pressure giving product **1** (131 mg, quant.) as a yellow powder.
Rf : 0.21 (DCM/MeOH 9:1); m.p. 170-173°C; ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.61 (s, 1H), 7.53 (d, J = 9.1 Hz, 1H), 6.81 (dd, J = 2.5, 9.1 Hz, 1H), 6.55 (d, J = 2.5 Hz, 1H), 3.55-3.50 (m, 6H), 3.00 (t, J = 7.1 Hz, 2H), 1.99-1.92 (m, 2H), 1.23 (t, J = 7.1 Hz, 6H); ^{13}C NMR (100 MHz, MeOD-d_4) δ 166.4, 164.0, 159.3, 154.8, 149.5, 132.7, 111.7, 109.7, 109.5, 97.3, 46.0, 38.3, 37.1, 29.0, 12.7; IR (neat) : 3081, 1677, 1616, 1537, 1508, 1351, 1132 cm^{-1}; ESI-MS 318 $[M+H]^+$. HRMS calcd 317.1739 for $C_{17}H_{23}N_3O_3$, found 317.1745.

Synthesis of 2



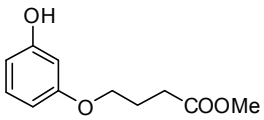
Tert-butyl 2-nitrobenzoate **7**



This compound was synthesized in two steps according to the described procedure.[2] The literature NMR data for this compound also matched those obtained for compound **7**.

^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 7.84 (dd, J = 8.0 Hz, 1.7 Hz, 1H), 7.29-7.25 (m, 1H), 6.69-6.45 (m, 2H), 5.62 (br, 2H), 1.62 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$-d_1) δ 167.6, 150.0, 133.5, 131.4, 116.8, 116.3, 112.8, 80.7, 28.3.

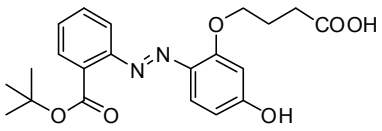
Methyl 4-(3-hydroxyphenoxy)butanoate **8**



Resorcinol (2.03 g, 14.80 mmol) was dissolved in acetonitrile (18 mL). K_2CO_3 (2.04 g, 14.80 mmol) and methyl 4-bromobutanoate (0.54 g, 27.61 mmol) were added. The mixture was heated under micro-waves at 120 °C for 20 minutes. After cooling, 1M HCl solution (200 mL) was added to the reaction mixture and extracted wit EtOAc (3*100 mL). Combined organic layers were then washed with water (200 mL), brine (200 mL) and dried over Na_2SO_4. Silica gel chromatography (cyclohexane/EtOAc 1:0 to 7:3) gave the compound **8** (0.31 g, 51%) as colorless oil.

Rf: 0.55 (cyclohexane/EtOAc 5:5); ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 7.09 (t, J = 8.1 Hz, 1H), 6.46-6.36 (m, 3H), 3.95 (t, J = 6.1 Hz, 2H), 3.67 (s, 3H), 2.50 (t, J = 7.3 Hz, 2H), 2.11-2.03 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$-d_1) δ 173.1, 160.8, 156.5, 130.7, 107.5, 107.1, 102.1, 68.1, 51.9, 29.9, 24.3; IR (neat) : 3391, 1710, 1593, 1144 cm^{-1}; GC-MS : 210.

4-(2-(2-(tert-butoxycarbonyl)phenylazo)-5-hydroxyphenoxy)butanoic acid **9**

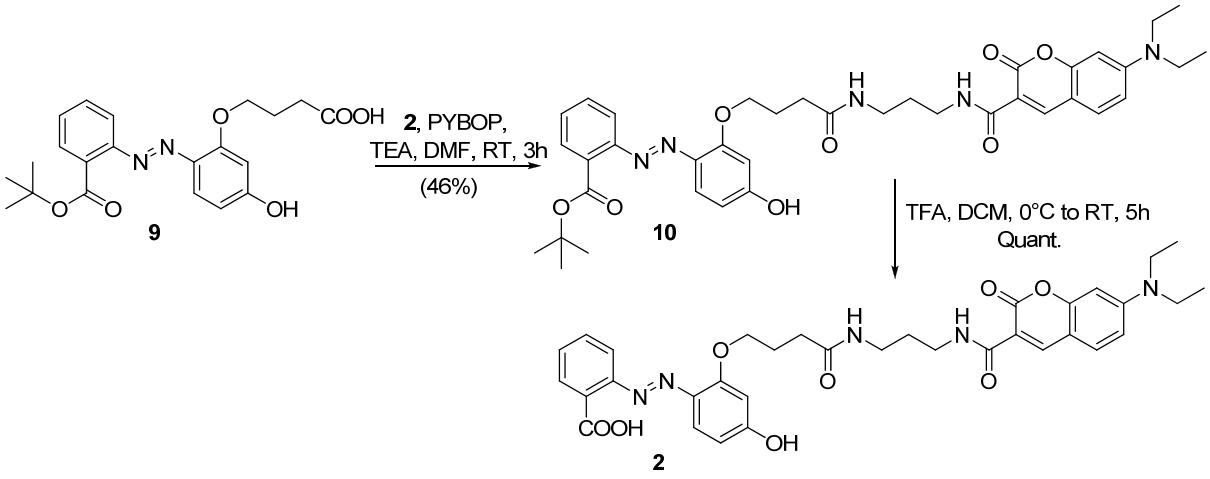


Compound **7** (1.34 g, 6.9 mmol) was dissolved in a solution of acetone/water (1:1) (31.0 mL). The mixture was cooled to 0° and concentrated HCl (3.5 mL) was added. After a few minutes sodium nitrite (0.56 g, 8.1 mmol) dissolved in water (7.0 mL) was added dropwise and the mixture was stirred for 1 hour at 0°C. In the same time, were solubilized compound **8** (1.25 g, 5.95 mmol), $Na_2CO_3.10H_2O$ (4.00 g, 13.9 mmol) and NaOH (0.95 g, 23.8 mmol) in a solution of acetone/water (1:1) (31 mL). The first solution was added dropwise at the second at 0°C. After the addition was completed, the mixture was allowed to reach room temperature and stirred for an additional hour. Reaction mixture was acidified with 1M HCl (50 mL) and then extracted with DCM (3*50 mL). Organic phases was dried over Na_2SO_4, concentrated and purified by silica gel chromatography (DCM/MeOH 1:0 to 9:1). Compound **9** was obtained as an orange/red solid (2.16 g, 77%).

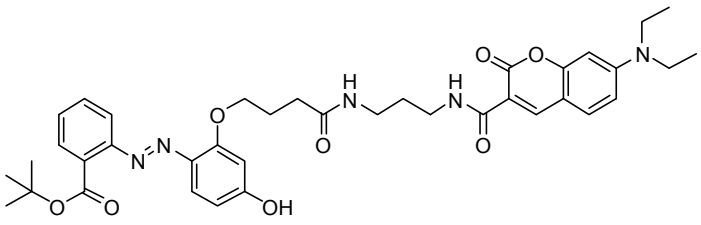
Rf: 0.28 (DCM/MeOH 95:5); m.p. 181°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65-7.58 (m, 2H), 7.52-7.46 (m, 3H), 6.59 (d, J = 2.3 Hz, 1H), 6.46 (dd, J = 9.0 Hz, 2.3 Hz, 1H), 4.13 (t, J = 6.4 Hz, 2H), 2.43 (t, J = 7.3 Hz, 2H), 1.99 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (101 MHz,

[2] Drewe, W. C.; Nanjunda, R.; Gunaratnam, M.; Beltran, M.; Parkinson, G. N.; Reszka, A. P.; Wilson, W. D.; Neidle, S. *J. Med. Chem.* **2008**, *51*, 7751-7767.

DMSO-d_6) δ 178.4, 165.9, 161.3, 153.7, 151.4, 133.3, 127.8, 126.8, 125.0, 122.9, 116.9, 116.2, 107.8, 102.4, 81.7, 68.1. IR (neat) : 3225, 1713, 1684, 1619, 1488, 1367, 1321, 1276, 1237 cm^{-1}; ESI-MS: 401 $[M+H]^+$. HRMS 400.1634 calcd for $C_{21}H_{24}N_2O_6$, found 400.1646.



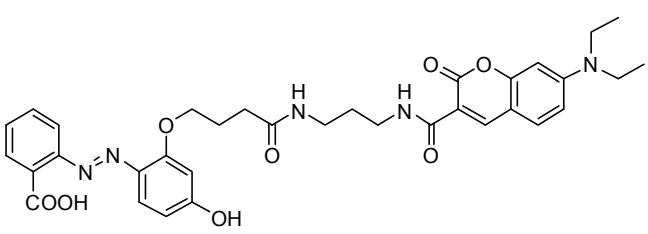
tert-butyl-2-(2-(4-(3-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido)propyl amino)-4-oxobutoxy)-4-hydroxyphenylazo)benzoate ***10***



Compound **1** (247 mg, 0.59 mmol) was dissolved in DMF (6.0 mL) and TEA (205 µL, 1.47 mmol), compound **9** (196 mg, 0.49 mmol) were added. The mixture was cooled to 0°C and PyBOP (307 mg, 0.59 mmol) was added. The solution was allowed to reach room temperature and stirred for 3 hours. 1M HCl (50 mL) was added to the reaction mixture and was extracted with EtOAc (3*50 mL). The organic layers were washed successively with 1M HCl (50 mL), water (50 mL), brine (50 mL) and dried over Na_2SO_4. The crude product was purified by column chromatography on silica gel (DCM/MeOH 100:0 to 95:5) to yield **10** as an orange solid (157 mg, 46%).

Rf: 0.42 (DCM/MeOH 95:5); m.p. 83-87°C; ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 13.75 (s, 1H), 8.87 (t, J = 6.5 Hz, 1H), 8.58 (s, 1H), 7.92 (dd, J = 5.2, 8.3 Hz, 2H), 7.50 (t, J = 7.8 Hz, 1H), 7.38 (d, J = 9.0 Hz, 1H), 7.15 (d, J = 9.5 Hz, 1H), 7.03 (t, J = 7.8 Hz, 1H), 6.86 (t, J = 5.7 Hz, 1H), 6.61 (dd, J = 2.5, 9.0 Hz, 1H), 6.45 (d, J = 2.5 Hz, 1H), 6.32 (d, J = 9.5 Hz, 1H), 5.97 (s, 1H), 4.26 (t, J = 5.5 Hz, 2H), 3.44-3.39 (m, 6H), 3.23 (dt, J = 5.5, 6.1 Hz, 2H), 2.41-2.39 (m, 4H), 1.71-1.65 (m, 2H), 1.58 (s, 9H), 1.21 (t, J = 7.00 Hz, 6H);^{13}C NMR (101 MHz, $CDCl_3$-d_1) δ 187.1, 171.8, 166.9, 164.4, 162.9, 159.5, 157.9, 152.9, 148.3, 145.2, 139.8, 134.2, 131.4, 131.3, 130.7, 125.5, 122.7, 115.9, 115.5, 110.3, 109.9, 108.5, 106.1, 96.7, 82.5, 69.5, 45.3, 36.5, 36.1, 33.1, 29.9, 28.5, 24.2, 12.6; IR (neat) : 2975, 1698, 1618, 1582, 1533, 1509, 1437, 1417, 1135 cm^{-1}; ESI-MS: 700 $[M+H]^+$ and 722 $[M+Na]^+$. HRMS 699.3268 calcd for $C_{38}H_{45}N_5O_8$, found 699.3289.

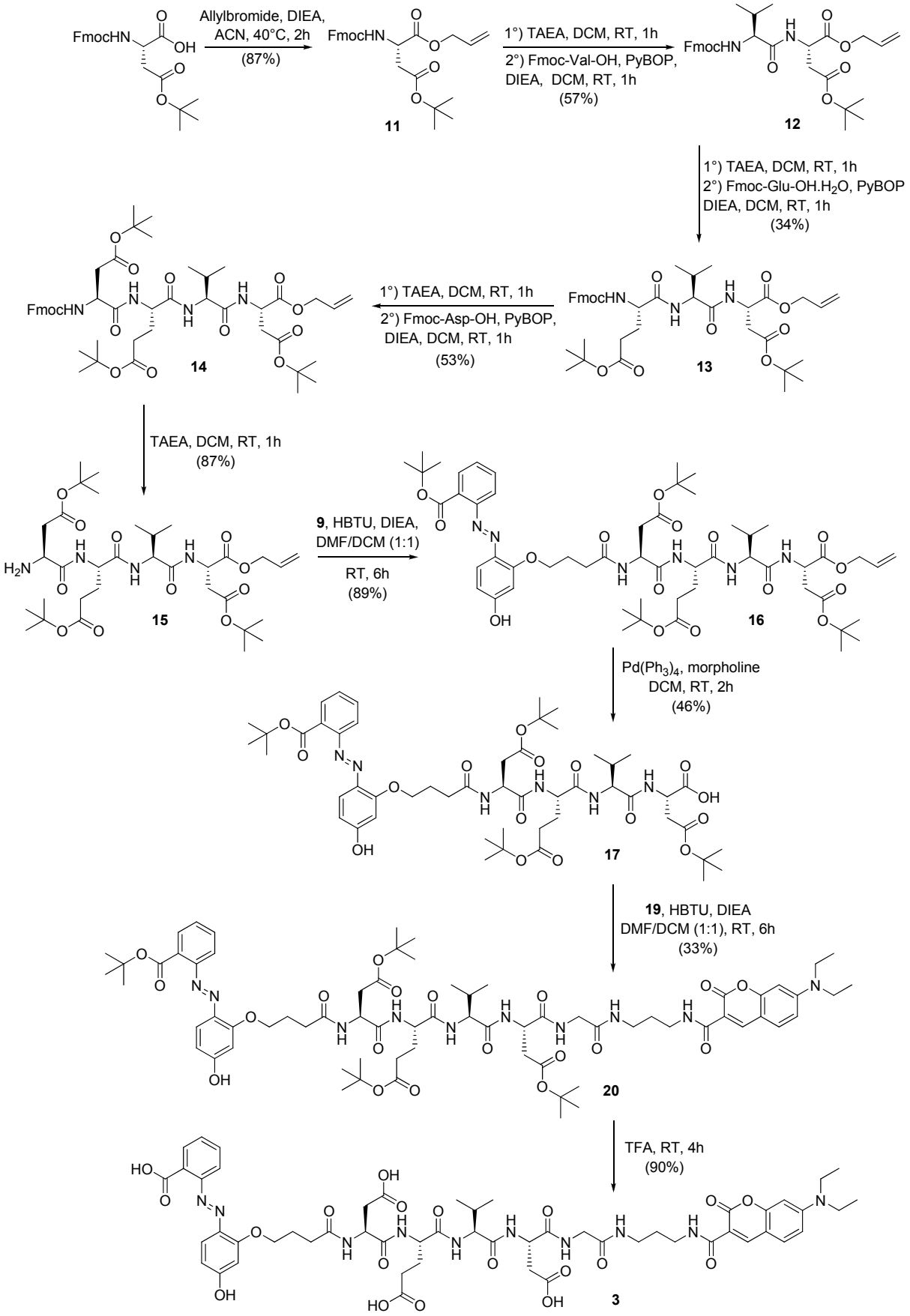
2-((2-(4-(3-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido)propylamino)-4-oxobutoxy)-4-hydroxyphenyl)diazenyl)benzoic acid ***2***



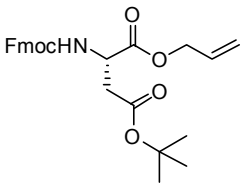
10 (66 mg, 0.1 mmol) was dissolved in DCM (1.2 mL), cooled to 0°C in an ice bath and TFA (0.6 mL) was added. The mixture was allowed to reach room temperature and stirred for 16 hours. The reaction was monitored by TLC. The TFA was removed under reduce pressure and coevaporated with toluene. The crude product was purified by preparative HPLC to yield compound **2** as a red solid (59 mg, quant.).

m.p. 126°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (t, d, J = 5.9 Hz, 1H), 8.64 (s, 1H), 7.89 (t, d, J = 5.9 Hz, 1H), 7.79 (d, J = 7.3 Hz, 1H), 7.67-7.57 (m, 3H), 7.52-7.46 (m, 3H), 6.79 (dd, J = 2.4, 9.0 Hz, 1H), 6.6 (d, J = 2.4 Hz, 1H), 6.56 (br, 1H), 6.46 (d, J = 9.0 Hz, 1H), 4.14 (t, J = 6.7 Hz, 2H), 3.47 (q, J = 7.2 Hz, 4H), 3.29 (q, J = 6.7 Hz, 2H), 3.09 (dt, J = 5.5, 6.7 Hz, 2H), 2.29 (t, J = 7.3 Hz, 2H), 2.07-1.99 (m, 2H), 1.65-1.68 (m, 2H), 1.13 (t, J = 7.03 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.6, 168.2, 162.2, 161.6, 158.3, 157.2, 152.4, 147.6, 131.9, 131.5, 129.5, 117.3, 110.1, 109.5, 107.6, 95.9, 68.3, 44.3, 36.7, 36.2, 31.6, 29.4, 24.7, 12.3; IR (neat) : 3324, 2973, 1698, 1618, 1509, 1233 cm^{-1}; ESI-MS: 644 $[M+H]^+$ and 667 $[M+Na]^+$. HRMS 643.2642 calcd for $C_{34}H_{37}N_5O_8$, found 643.2654.

Synthesis of 3



Fmoc-Asp(OtBu)-OAll ***11***



This compound was synthesized according to the described procedure.[3] Fmoc-Asp-OH (3.0 g, 7.4 mmol) was added to a mixture of acetonitrile (15.0 mL) and allyl bromide (17.6 mL). DIEA (2.7 mL, 15.5 mmol) was added and the reaction mixture was stirred for 4 hours at 40 °C. Ethyl acetate (200 mL) was added, and organic layer was washed with aq half-saturated $KHSO_4$ (100 mL), half-saturated $NaHCO_3$ (100 mL), brine (100 mL) and dried over Na_2SO_4. Silica gel chromatography (Cyclohexane/EtOAc 9:1 to 1:1) gave the compound **11** (3.0 g, 91%) as yellow oil.

Rf : 0.62 (Cyclohexane/EtOAc 7:3); ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 7.75 (d, J = 7.5 Hz, 2H), 7.60-7.57 (m, 2H), 7.38 (dd, J = 6.9, 7.5 Hz, 2H), 7.31-7.27 (m, 2H), 5.94-5.80 (m, 2H), 5.32 (dd, J = 1.2, 17.1 Hz, 1H), 5.23 (dd, J = 1.2, 10.6 Hz, 1H), 4.70-4.60 (m, 3H), 4.41 (dd, J = 7.4, 10.0 Hz, 1H), 4.33 (dd, J = 7.4, 10.0 Hz, 1H), 4.23 (t, J = 7.1 Hz, 1H), 2.96 (dd, J = 4.7, 17.0 Hz, 1H), 2.77 (dd, J = 4.7, 17.0 Hz, 1H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ 170.6, 170.0, 156.0, 143.9, 143.8, 141.3, 131.5, 127.7, 127.1, 125.2, 125.1, 120.0, 118.8, 81.9, 67.3, 66.3, 50.7, 47.2, 37.8, 28.1.

General procedure for peptide synthesis in solution

The deprotection and coupling conditions for peptide synthesis were used as previously described with slight modifications.[4] Fmoc-AA_1-R (1.0 mmol) was dissolved in dichloromethane (4.5 mL), TAEA (4.5 mL) was added and the solution was stirred at room temperature for 1 hour. After conversion has been verified by TLC, EtOAc (15 mL) was added and organic layer was washed with brine (10 mL) and extracted three times with phosphate buffer (Na_2HPO_4 NaH_2PO_4 pH 5.5) (10 mL). The aqueous fractions were discarded and the organic layer was dried over Na_2SO_4 and concentrate to dryness. Resulting reaction crude was directly engaged in peptide coupling without further purification. H_2N-AA_1-R was dissolved in DCM (3.6 mL) and Fmoc-AA_2-OH (1.1 mmol) was added to the solution. After DIEA addition (1.5 mmol), PyBOP (1.1 mmol) was directly added to the reaction mixture. After the solution was stirred for 1 hour at room temperature, 1M HCl solution (5 mL) was added and reaction mixture was extracted with DCM (2*10 mL). Organic layers were combined and washed with sat $NaHCO_3$ solution (20 mL), brine (20 mL) and dried over Na_2SO_4. Crude was purified by silica gel chromatography to give pure Fmoc-AA_2-AA_1-R.

Fmoc-Val-Asp(OtBu)-OAll ***12***

This compound was synthesized using **11** (3.01 mmol) and Fmoc-Val-OH (3.31 mmol), following the general procedure for peptide synthesis. The crude was purified by chromatography on silica gel using cyclohexane/EtOAc (1:0 to 1:1) to give **12** (2.1 g, 57%) as a white powder.

[3] Hamzavi, R.; Dolle, F.; Tavitian, B.; Otto Dahl O.; Nielsen, P. E. *Bioconjugate Chem* **2003**, *5*, 941-954.
[4] Peterson, Q. P.; Goode, D. R.; West, D. C.; Botham, R. C.; Hergenrother, P. J. *Nat. Protoc.* **2010**, *5*, 294-302.

Rf : 0.37 (Cyclohexane/EtOAc 7:3); m.p. 134-136°C; ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 7.74 (d, J = 7.6 Hz, 2H), 7.58 (d, J = 7.6 Hz, 2H), 7.40-7.36 (m, 2H), 7.31-7.27 (m, 2H), 6.80 (d, J = 8.5 Hz, 1H), 5.91-5.81 (m, 1H), 5.46 (d, J = 8.5 Hz, 1H), 5.31-5.20 (m, 2H), 4.87-4.83 (m, 1H), 4.67-4.56 (m, 2H), 4.42-4.30 (m, 2H), 4.21 (t, J = 6.9 Hz, 1H), 4.07 (dd, J = 5.5, 8.5 Hz, 1H), 2.97 (dd, J = 4.1, 17.0 Hz, 1H), 2.70 (dd, J = 4.1, 17.0 Hz, 1H), 2.18-2.10 (m, 1H), 1.40 (s, 9H), 0.98 (dd, J = 7.0, 15.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ 171.0, 170.4, 156.5, 144.1, 144.0, 141.5, 131.6, 127.9, 127.3, 125.3, 120.2, 119.1, 82.2, 67.3, 66.5, 60.3, 48.8, 47.4, 37.4, 31.8, 28.2, 19.2, 17.9; IR (neat) : 3293, 2964, 1724, 1649, 1535 cm^{-1}; ESI-MS 573 $[M+Na]^+$. HRMS 550.2679 calcd for $C_{31}H_{38}N_2O_7$, found 550.2696.

Fmoc-Glu(OtBu)-Val-Asp(OtBu)-OAll ***13***

This compound was synthesized using **12** (3.83 mmol) and Fmoc-Glu(OtBu)-OH.H_2O (4.21 mmol), following the general procedure for peptide synthesis. The crude was purified by chromatography on silica gel using DCM/EtOAc (1:0 to 8:2) to give **13** (0.95 g, 34%) as a white powder.
Rf : 0.42 (DCM/EtOAc 8:2); m.p. 182°C; ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 7.74 (d, J = 7.6 Hz, 2H), 7.57 (d, J = 7.6 Hz, 2H), 7.39-7.36 (m, 2H), 7.30-7.27 (m, 2H), 6.99 (d, J = 8.5 Hz, 1H), 6.85 (d, J = 8.5 Hz, 1H), 5.90-5.76 (m, 2H), 5.31-5.26 (m, 1H), 5.22-5.20 (m, 1H), 4.84-4.82 (m, 1H), 4.66-4.55 (m, 2H), 4.40-4.18 (m, 5H), 2.95 (dd, J = 4.3, 17.0 Hz, 1H), 2.70 (dd, J = 4.1, 17.0 Hz, 1H), 2.48-2.32 (m, 2H), 2.22-2.05 (m, 2H), 1.98-1.89 (m, 2H), 1.44 (s, 9H), 1.41 (s, 9H), 0.95 (dd, J = 6.4, 9.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ 173.3, 171.5 170.6, 170.4, 156.5, 144.1, 144.0, 141.5, 131.7, 127.9, 127.3, 125.3, 120.2, 119.1, 82.2, 81.4, 67.4, 66.5, 58.7, 54.7, 48.8, 47.4, 37.4, 32.1, 31.4, 28.5, 28.3, 28.2, 19.3, 17.9; IR (neat) : 3291, 1729, 1641, 1536, 1150 cm^{-1}; ESI-MS 758 $[M+Na]^+$. HRMS 735.3731 calcd for $C_{40}H_{53}N_3O_{10}$, found 735.3751.

Fmoc-Asp(OtBu)-Glu(OtBu)-Val-Asp(OtBu)-OAll ***14***

This compound was synthesized using **13** (1.29 mmol) and Fmoc-Asp(OtBu)-OH (1.42 mmol), following the general procedure for peptide synthesis. The crude was purified by chromatography on silica gel using cyclohexane/EtOAc (1:0 to 7:3) to give **14** (0.62 g, 53%) as a white powder.
Rf : 0.20 (DCM/EtOAc 8:2); m.p. 166°C; ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 7.73 (d, J = 7.6 Hz, 2H), 7.59-7.6 (m, 2H), 7.37 (t, J = 7.6 Hz, 2H), 7.28 (t, J = 7.6 Hz, 2H), 7.11 (d, J = 8.9 Hz, 1H), 6.95 (d, J = 8.5 Hz, 1H), 6.00 (d, J = 8.7 Hz, 1H), 5.89-5.80 (m, 1H), 5.29-5.25 (m, 1H), 5.21-5.18 (m, 1H), 4.84-4.80 (m, 1H), 4.65-4.19 (m, 8H), 2.94-2.88 (m, 2H), 2.74-2.62 (m, 2H), 2.48-2.31 (m, 2H), 2.23-1.94 (m, 3H), 1.43-1.40 (m, 27H), 0.93 (dd, J = 6.4, 8.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$-d_1) δ 173.7, 171.1, 170.8, 170.5, 170.3, 156.3, 144.0, 143.9, 141.5, 131.7, 127.9, 127.3, 125.3, 120.2, 119.0, 82.1, 82.0, 81.3, 67.6, 66.4, 58.8, 51.7, 48.9, 47.3, 37.7, 37.4, 32.0, 30.9, 28.3, 28.2, 27.4, 19.4, 18.0; IR (neat) : 3274, 1727, 1633, 1533, 1366, 1147 cm^{-1}; ESI-MS 929 $[M+Na]^+$. HRMS calcd 906.4626 for $C_{48}H_{66}N_4O_{13}$, found 906.4646.

H_2N-Asp(OtBu)-Glu(OtBu)-Val-Asp(OtBu)-OAll ***15***

14 (543 mg, 0.6 mmol) was dissolved in dichloromethane (15 mL), piperidine (15 mL) was added and the solution was stirred at room temperature for 1 hour. After conversion has been verified by TLC, EtOAc (50 mL) was added and organic layer was washed with 1M HCl solution (2*50 mL), sat. $NaHCO_3$ solution (50 mL), brine (50 mL) and dried over Na_2SO_4. The crude was purified by chromatography on silica gel using DCM/EtOAc (1:1) and then DCM/MeOH (95:5) to give **15** (358 mg, 87%) as a white powder.
Rf : 0.44 (DCM/MeOH 95:5); m.p. 117°C; ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 7.99 (d, J = 7.8 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.87 (d, J = 8.6 Hz, 1H), 5.90-5.80 (m, 1H), 5.30-5.26 (m, 1H), 5.22-5.19 (m, 1H), 4.81 (td, J = 4.5, 8.4 Hz, 1H), 4.65-4.55 (m, 2H), 4.42-4.37 (m, 1H), 4.27-4.24 (m, 1H), 3.64 (dd, J = 3.9, 8.0 Hz, 1H), 2.93 (dd, J = 4.4, 16.7 Hz, 1H), 2.80-2.67 (m, 2H), 2.56 (dd, J = 7.9, 16.7 Hz, 1H), 2.43-2.29 (m, 2H), 2.20-2.07 (m, 2H), 1.97-1.88 (m, 1H), 1.42-1.39 (m, 27H), 0.93 (dd, J = 7.0, 7.5 Hz, 6H); (100 MHz, $CDCl_3$-d_1) δ 173.9, 172.8, 171.2, 171.0, 170.5, 170.3, 170.2, 131.52, 118.9, 81.9, 81.3, 80.9, 66.2, 58.5, 52.8, 52.1, 48.6, 40.3, 37.2, 31.9, 31.0, 27.6, 19.1, 17.7; IR (neat) : 3272, 1725, 1627, 1540, 1366, 1147 cm^{-1}; ESI-MS 685 $[M+H]^+$ 707 $[M+Na]^+$. HRMS calcd 684.3945 for $C_{33}H_{56}N_4O_{11}$, found 684.3949.

CDQ(OtBu)-Asp(OtBu)-Glu(OtBu)-Val-Asp(OtBu)-OAll ***16***

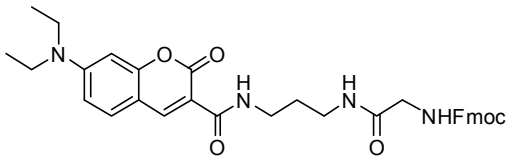
15 (325 mg, 0.48 mmol) was dissolved in a mixture of DCM/DMF (1:1) (4.0 mL) and DIEA (124 µL, 0.71 mmol), compound **9** (209 mg, 0.52 mmol) were added. The mixture was cooled to 0°C and HBTU (195 mg, 0.52 mmol) was added. The solution was allowed to reach room temperature and stirred for 6 hours. At the mixture was added 1N HCl (50 mL) and it was extracted with EtOAc (3*50 mL). The organic layers were washed successively with 1M HCl solution (2*50 mL), sat. $NaHCO_3$ solution (50 mL), brine (50 mL) and dried over Na_2SO_4. The crude product was purified by column chromatography on silica gel (DCM/EtOAc 1:1 to 0:1) to yield **16** as an orange solid (450 mg, 89%).
Rf : 0.27 (DCM/EtOAc 3:7); m.p. 124-128°C; ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 13.76 (s, 1H), 7.93 (t, J = 9.4, 16.7 Hz, 2H), 7.56-7.50 (m, 2H), 7.31 (d, J = 8.0 Hz, 1H), 7.17 (d, J = 9.7 Hz, 1H), 7.10-7.04 (m, 2H), 6.87 (d, J = 8.0 Hz, 1H), 6.32 (d, J = 9.8 Hz, 1H), 6.07 (s, 1H), 5.88-5.81 (m, 1H), 5.29-5.26 (m, 1H), 5.20-5.18 (m, 1H), 4.83-4.79 (m, 1H), 4.70-4.67 (m, 1H), 4.63-4.55 (m, 2H), 4.33-4.24 (m, 4H), 2.87 (dd, J = 5.0, 16.8 Hz, 2H), 2.72 (dd, J = 5.2, 16.8 Hz, 1H), 2.72 (dd, J = 6.0, 16.8 Hz, 1H), 2.46-2.33 (m, 4H), 2.17-2.03 (m, 2H), 1.94-1.87 (m, 1H), 1.58 (s, 9H), 1.39 (s, 9H), 1.38 (s, 18H), 0.91 (dd, J = 6.8, 9.7 Hz, 6H); (100 MHz, $CDCl_3$-d_1) δ 187.1, 173.7, 172.0, 171.4, 171.1, 171.0, 170.6, 170.2, 166.9, 159.6, 145.1, 140.0, 134.3, 131.8, 131.4, 130.6, 125.4, 122.9, 118.9, 115.9, 115.6, 106.2, 82.4, 82.2, 82.0, 81.3, 69.3, 66.4, 58.8, 53.6, 50.0, 49.0, 37.5, 37.1, 32.4, 32.0, 30.9, 28.4, 28.3, 28.2, 27.7, 23.8, 19.4, 18.2; IR (neat) : 3275, 1728, 1630, 1493, 1366, 1149 cm^{-1}; ESI-MS 1067 $[M+H]^+$. HRMS 1066.5474 calcd for $C_{54}H_{78}N_6O_{16}$, found 1066.5498.

CDQ(OtBu)-Asp(OtBu)-Glu(OtBu)-Val-Asp(OtBu)-OH ***17***

To a stirred solution of **16** (95 mg, 0.09 mmol) in degassed DCM (9 mL) were added morpholine (24 µL, 0.27 mmol) and tetrakis(triphenylphosphine)palladium (11 mg, 0.01

mmol). The reaction mixture was stirred at room temperature for 2 hours. After completion of the reaction, the mixture was diluted with water (10 mL), extracted with DCM and dried over Na_2SO_4. Product presence was confirmed by LC-MS and crude reaction was directly engaged in the next step.

Fmoc-Gly-DEAC ***18***



To a stirred solution of **1** (231 mg, 0.55 mmol) in DMF (3.7 mL), were added successively, DIEA (144 µL, 0.83 mmol) and Fmoc-Gly-OH (148 mg, 0.5 mmol). The reaction mixture was stirred and cooled to 0°C using an ice bath then HBTU (210 mg, 0.55 mmol) was added. Stirring was continued for 16 hours while the mixture was warmed up to room temperature. The reaction mixture was poured into a solution of 1M HCl solution (20 mL) and extracted two times by EtOAc (2*50 mL). The organics layers were washed successively with two times water (2*50 mL) and brine (50 mL) then dried over Na_2SO_4 and concentrated under reduce pressure. Silica gel chromatography (DCM/MeOH 1:0 to 97:3) gave the compound **18** (200 mg, 61%) as a yellow powder.
Rf : 0.27 (DCM/MeOH 95:5); m.p. 150°C; ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 8.89 (t, J = 6.2 Hz, 1H), 8.55 (s, 1H), 7.67 (d, J = 7.6 Hz, 2H), 7.59 (d, J = 7.6 Hz, 2H), 7.34-7.21 (m, 6H), 6.53 (dd, J = 2.5, 9.0 Hz, 1H), 6.4 (d, J = 2.5 Hz, 1H), 5.95 (t, J = 4.7 Hz, 1H), 4.35 (d, J = 7.2 Hz, 2H), 4.19 (t, J = 7.2, 1H), 3.93 (d, J = 5.5, 1H), 3.49-3.45 (m, 2H), 3.39-3.30 (6H), 1.77-1.71 (m, 2H), 1.16 (t, J = 7.1 Hz, 6H); (100 MHz, $CDCl_3$-d_1) δ 169.2, 164.1, 157.7, 156.7, 152.7, 148.3, 144.0, 141.3, 131.3, 127.7, 127.1, 125.3, 120.0, 110.1, 109.8, 108.4, 96.6, 67.2, 47.3, 45.2, 44.7, 38.7, 36.7, 12.5; IR (neat) : 3324, 2973, 2931, 1698, 1645, 1580, 1502, 1416, 1349, 1230 cm^{-1}; ESI-MS 597 $[M+H]^+$ and 619 $[M+Na]^+$. HRMS 596.2635 calcd for $C_{34}H_{36}N_4O_6$, found 596.2632.

H_2N-Gly-DEAC ***19***

To a stirred solution of **18** (121 mg, 0.20 mmol) in DCM (5 mL) was added piperidine (0.5 mL) and reaction mixture was stirred at room temperature for 2 hours. After completion of the reaction, the mixture was diluted with water (10 mL), extracted with DCM (20 mL) and dried over Na_2SO_4. Product presence was confirmed by LC-MS and crude reaction was directly used for peptide coupling.

CDQ(OtBu)-Asp(OtBu)-Glu(OtBu)-Val-Asp(OtBu)-Gly-DEAC ***20***

Crude reaction mixture of **17** (94 mg, 0.09 mmol) was dissolved in a mixture of DCM/DMF (1:1) (4.0 mL) and DIEA (41 µL, 0.23 mmol), compound **19** (76 mg, 0.20 mmol) were added. The mixture was cooled to 0°C and HBTU (38 mg, 0.1 mmol) was added. The solution was allowed to reach room temperature and stirred for 16 hours. 1M HCl (50 mL) was added to reaction mixture and it was extracted with EtOAc (3*50 mL). The organic layers were washed

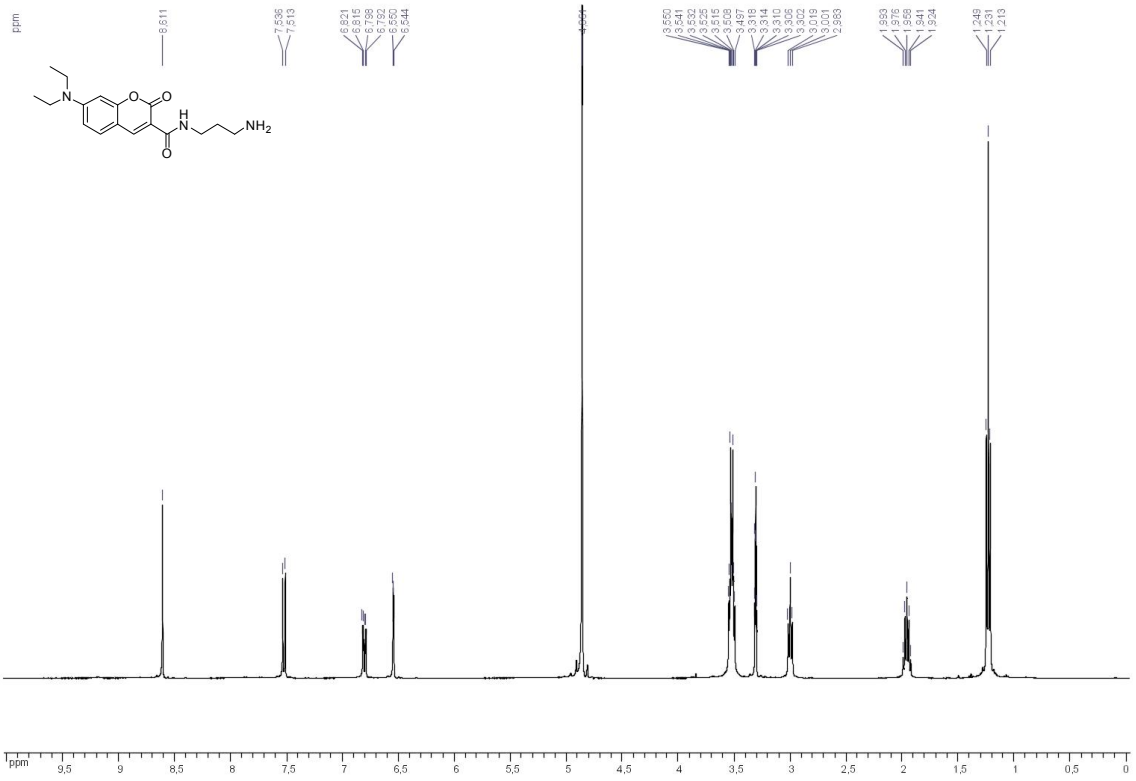
successively with 1M HCl solution (2*50 mL), brine (50 mL) and dried over Na_2SO_4. The crude was purified by column chromatography on silica gel (DCM/MeOH 97:3 to 95:5) to yield **20** as an orange solid (43 mg, 33% yield for two steps).
Rf : 0.39 (DCM/MeOH 95:5); m.p. 178°C; ^{1}H NMR (400 MHz, $CDCl_3$-d_1) δ 13.72 (s, 1H), 8.84 (t, J = 6.1 Hz, 1H), 8.59 (s, 1H), 7.93-7.88 (m, 3H), 7.85 (d, J = 7.8 Hz, 1H), 7.55-7.41 (m, 5H), 7.23-7.22 (m, 1H), 7.12 (d, J = 9.7 Hz, 1H), 7.05 (t, J = 7.7 Hz, 1H), 6.61 (dd, J = 2.5, 9.0 Hz, 1H), 6.47 (d, J = 2.5 Hz, 1H), 6.28 (dd, J = 1.8, 9.6 Hz, 1H), 6.09 (d, J = 1.8 Hz, 1H), 4.72-4.65 (m, 2H), 4.27-4.18 (m, 3H), 4.10-4.00 (m, 2H), 3.78 (dd, J = 5.8, 16.8 Hz, 1H), 3.44-3.38 (m, 5H), 3.35-3.27 (m, 2H), 3.19-3.13 (m, 1H), 2.85-2.74 (m, 4H), 2.56-2.31 (m, 6H), 2.16-1.91 (m, 3H), 1.79-1.70 (m, 2H), 1.57 (s, 9H), 1.39 (s, 9H), 1.36 (s, 9H), 1.33 (s, 9H), 1.21 (t, J = 7.11 Hz, 6H), 0.95 (d, J = 6.8 Hz, 6H); (100 MHz, $CDCl_3$-d_1) δ 187.1, 174.1, 172.7, 172.5, 172.4, 171.7, 171.5, 171.1, 170.9, 169.6, 166.9, 163.6, 162.8, 159.7, 157.8, 152.8, 148.3, 145.0, 140.0, 134.4, 131.5, 131.3, 130.5, 125.3, 123.0, 115.8, 115.6, 110.2, 110.1, 108.6, 106.0, 96.7, 82.4, 82.2, 81.7, 81.4, 69.3, 60.9, 54.9, 51.0, 50.2, 45.3, 43.7, 37.3, 37.1, 36.9, 36.7, 32.3, 32.1, 29.7, 29.5, 28.5, 28.3, 28.2, 27.0, 23.9, 19.5, 19.2, 12.7; IR (neat) : 3290, 2974, 1731, 1697, 1630, 1510, 1152, 1136 cm^{-1}; ESI-MS 1381 [M-H]$^-$. HRMS calcd 1382.7009 for $C_{70}H_{98}N_{10}O_{19}$, found 1382.7015.

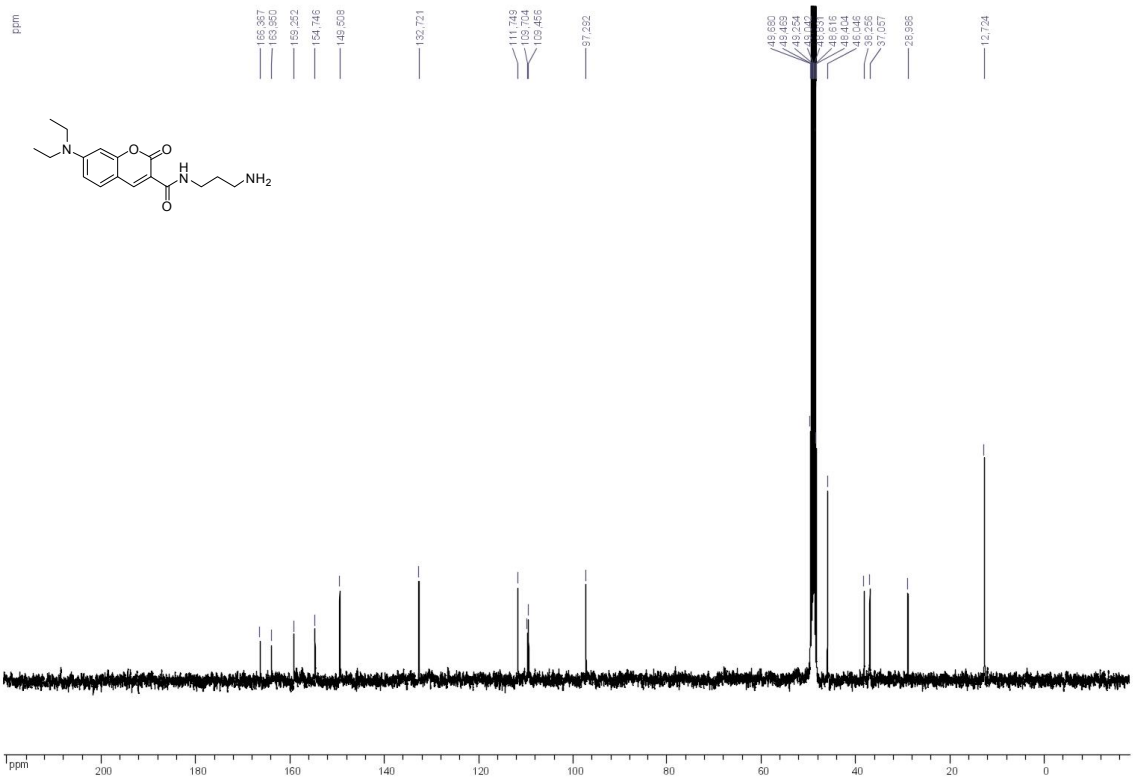
CDQ-Asp(OH)-Glu(OH)-Val-Asp(OH)-Gly-DEAC ***3***

20 (20 mg, 0.014 mmol) was dissolved in TFA (0.4 mL) and reaction mixture was stirred at room temperature for 4 hours. After completion of the reaction, crude was purified by preparative HPLC without further work-up and gave **3** (14 mg, 90%) as an orange solid.
^{1}H NMR (500 MHz, pyridine-d_5) δ 9.90 (d, J = 6.7 Hz, 1H), 9.80 (d, J = 6.7 Hz, 1H), 9.55 (d, J = 7.5 Hz, 1H), 9.34-9.29 (m, 2H), 9.05 (s, 1H), 8.88 (d, J = 7.6 Hz, 1H), 8.45-8.42 (m, 2H), 8.01 (d, J = 8.3 Hz, 1H), 7.61-7.59 (m, 3H), 7.54-7.51 (m, 2H), 7.24 (t, J = 7.5 Hz, 1H), 6.64 (dd, J = 2.1, 9.1 Hz, 1H), 6.60 (dd, J = 2.1, 9.1 Hz, 1H), 6.55 (s, 1H), 6.51 (d, J = 2.41 Hz, 1H), 5.58-5.45 (m, 1H), 5.17-5.13 (m, 2H), 4.82 (t, J = 7.9 Hz, 1H), 4.43 (dd, J = 5.8, 16.8 Hz, 1H), 4.33-4.26 (m, 3H), 3.7 (td, J = 6.1, 7.4 Hz, 2H), 3.63-3.56 (m, 4H), 3.42 (dd, J = 7.3, 16.5 Hz, 1H), 3.30 (dd, J = 7.3, 16.5 Hz, 1H), 3.23 (q, J = 7.0 Hz, 4H), 3.00-2.86 (m, 2H), 2.80-2.66 (m, 3H), 2.58-2.43 (m, 4H), 1.95 (quint , J = 7.1 Hz, 2H), 1.08 (dd, J = 6.8, 8.9 Hz, 6H), 1.01 (t, J = 7.0 Hz, 6H); (125 MHz, pyridine-d_5) δ 175.20, 173.7, 173.6, 172.9, 172.8, 172.6, 172.3, 171.6, 170.2, 169.3, 162.9, 162.1, 158.6, 157.3, 152.1, 147.7, 132.0, 131.1, 130.9, 115.4, 110.1, 109.6, 108.0, 103.9, 95.9, 68.4, 59.9, 54.0, 51.0, 50.9, 44.2, 43.3, 36.7, 36.5, 36.4, 36.3, 32.0, 30.8, 30.1, 29.5, 27.2, 24.4, 18.9, 18.5, 11.7; ESI-MS 1157 [M-H]$^-$. HRMS 1158.4505 calcd for $C_{54}H_{66}N_{10}O_{19}$, found 1158.4485.

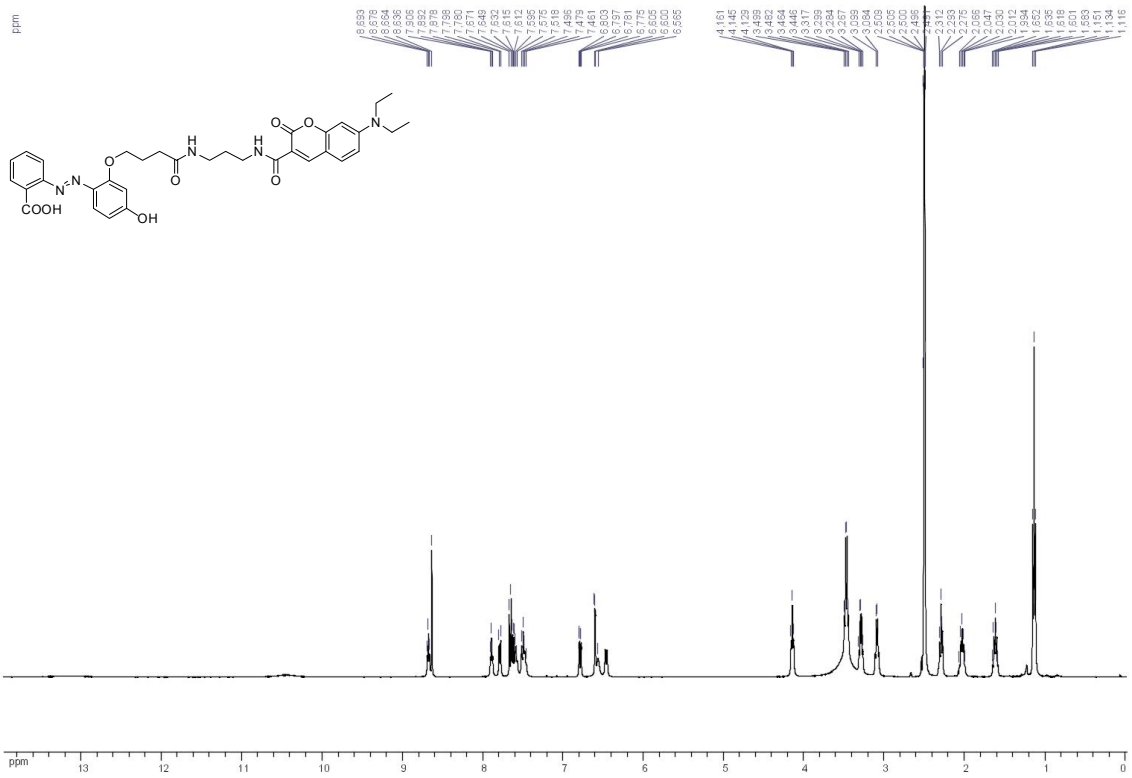
NMR spectra

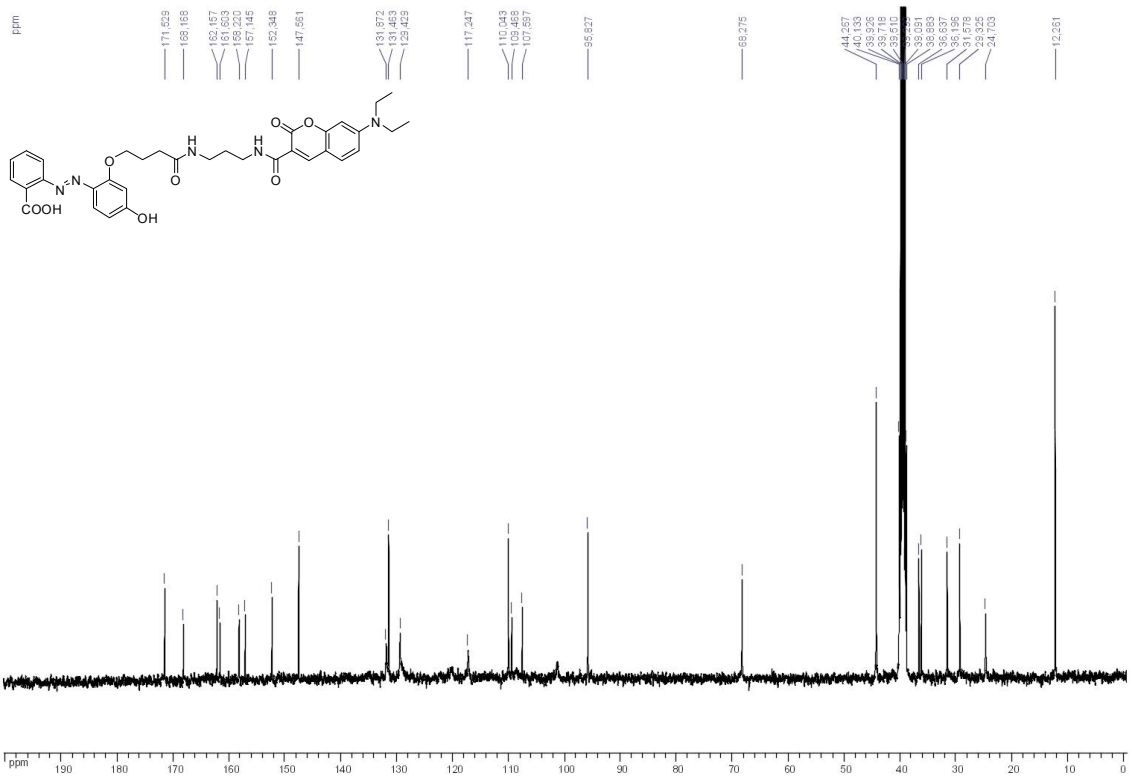
Spectra of compound **1**:



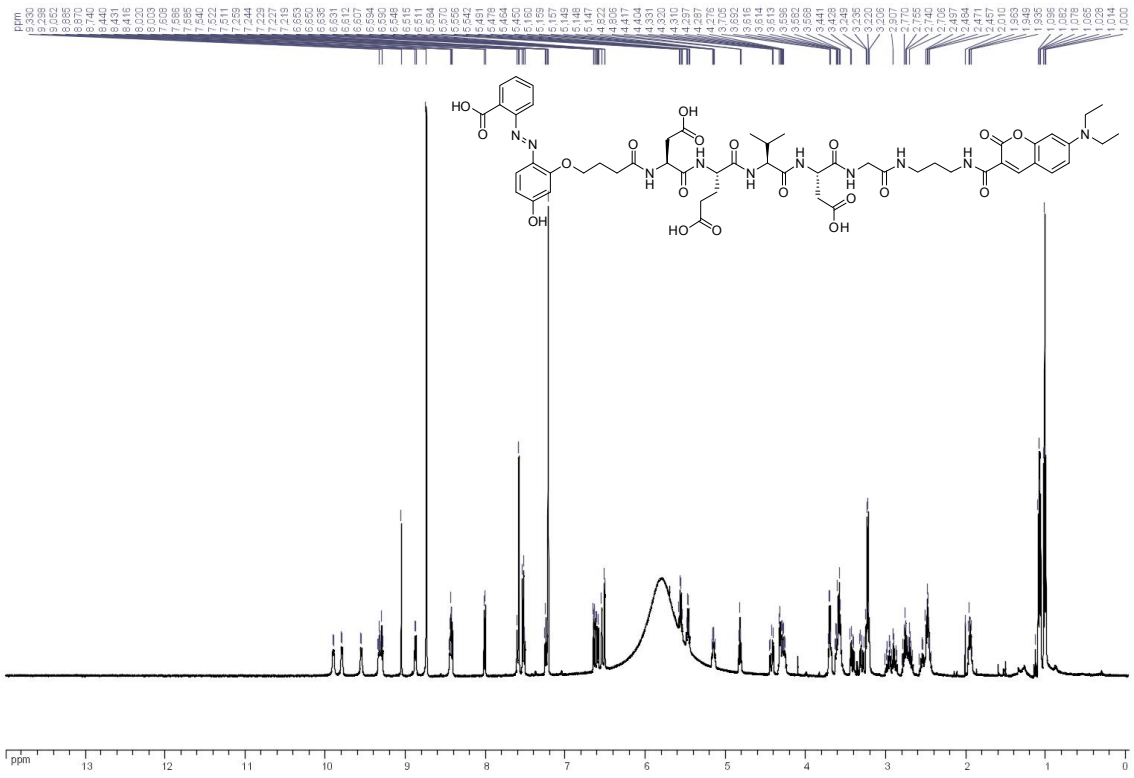


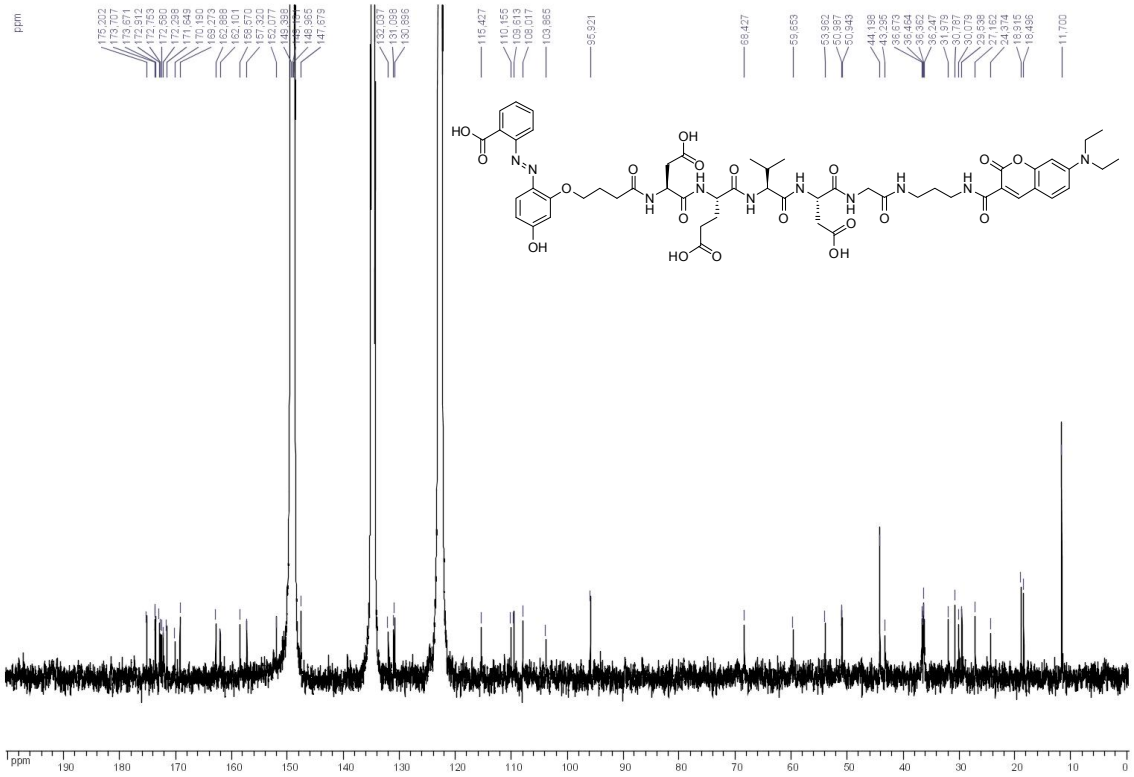
Spectra of compound **2**:



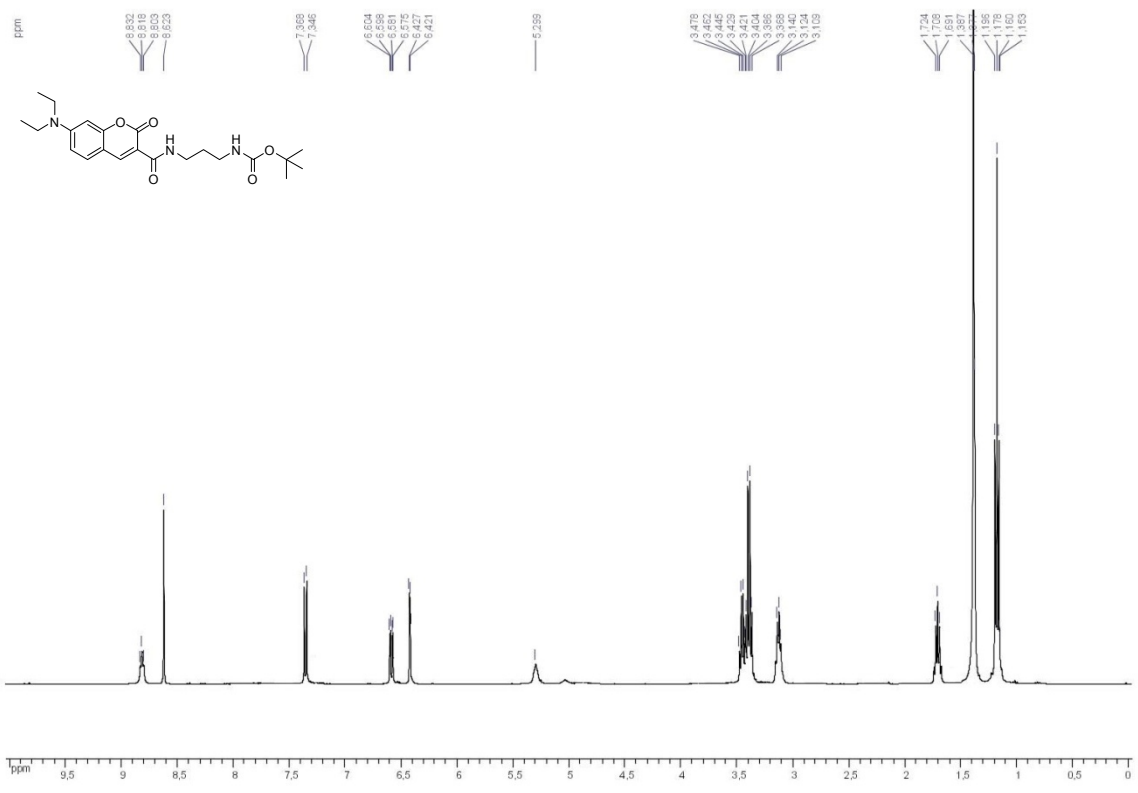


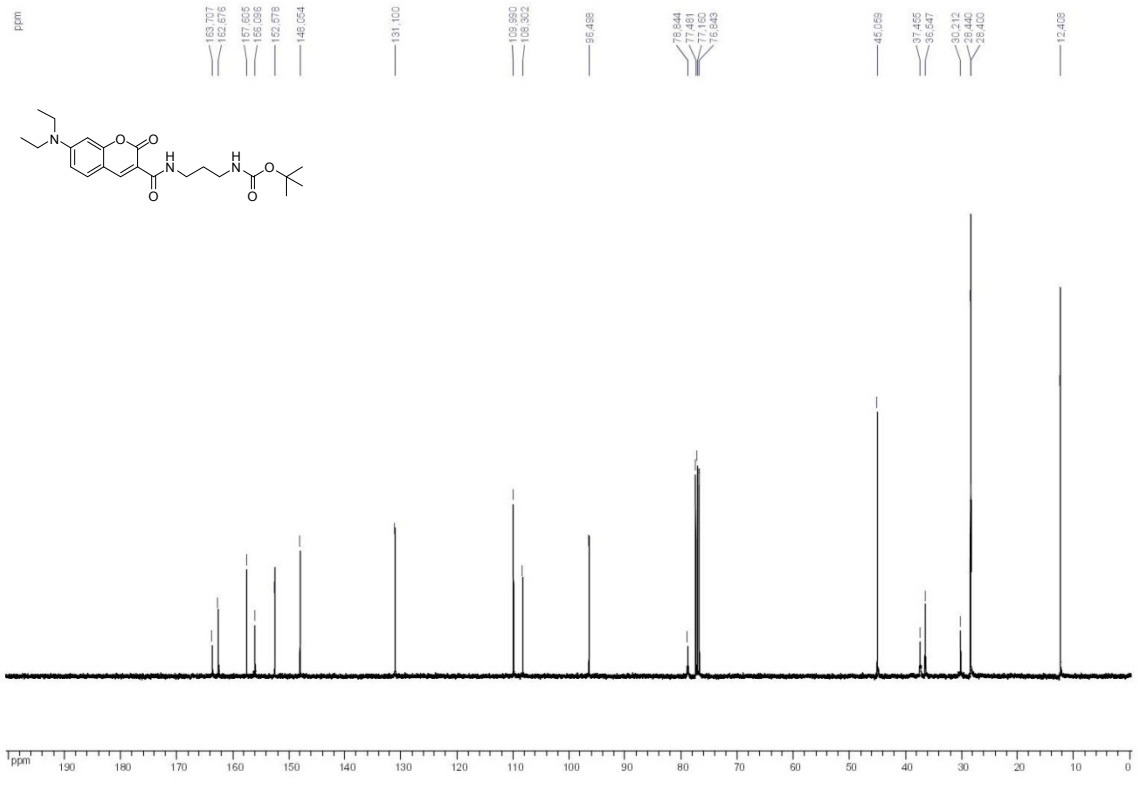
Spectra of compound **3**:



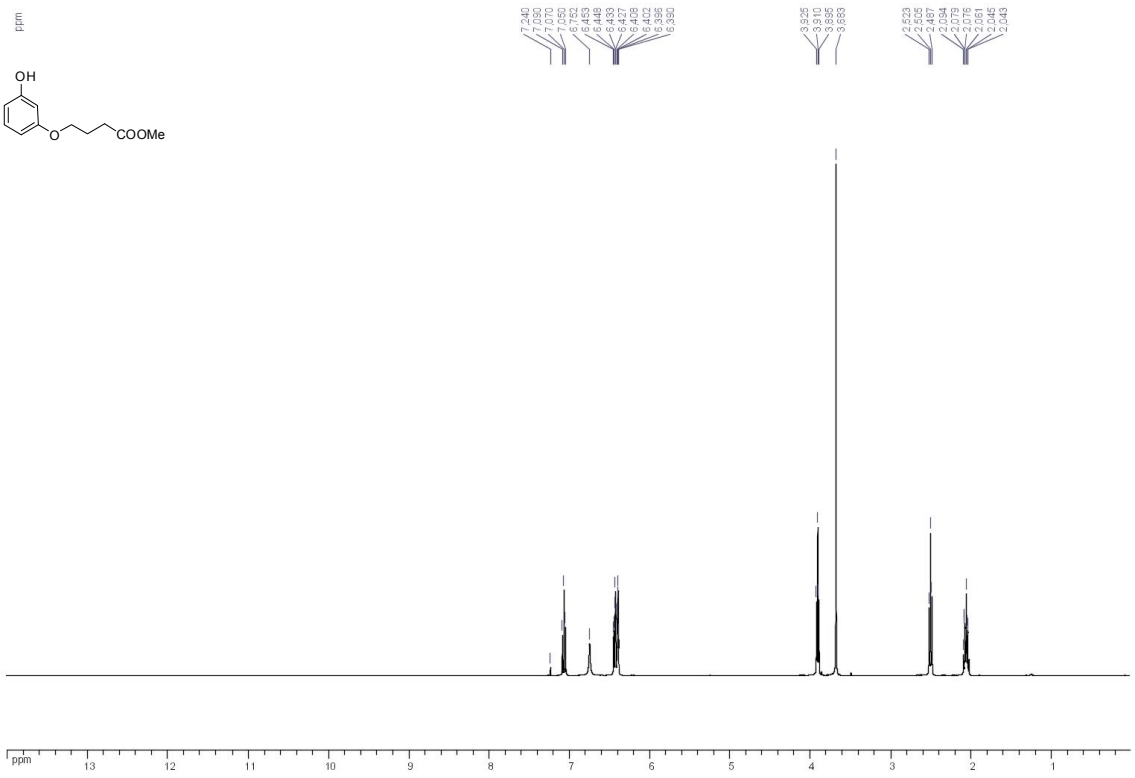


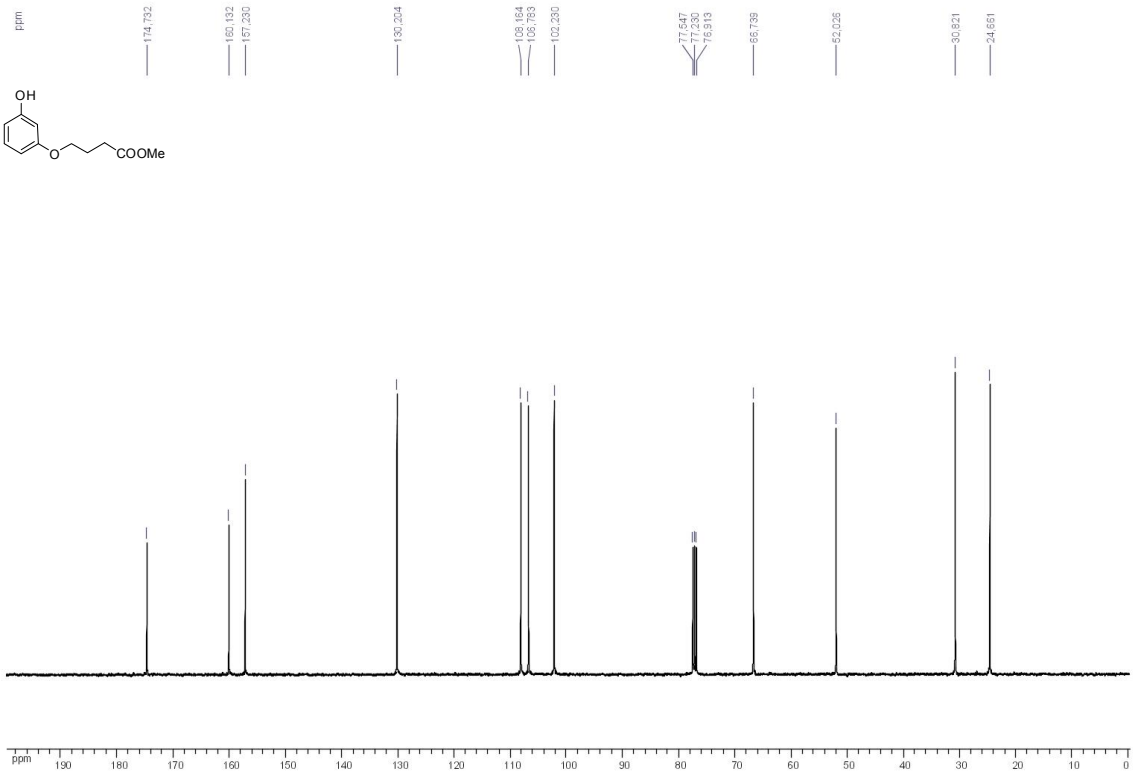
Spectra of compound **6**:



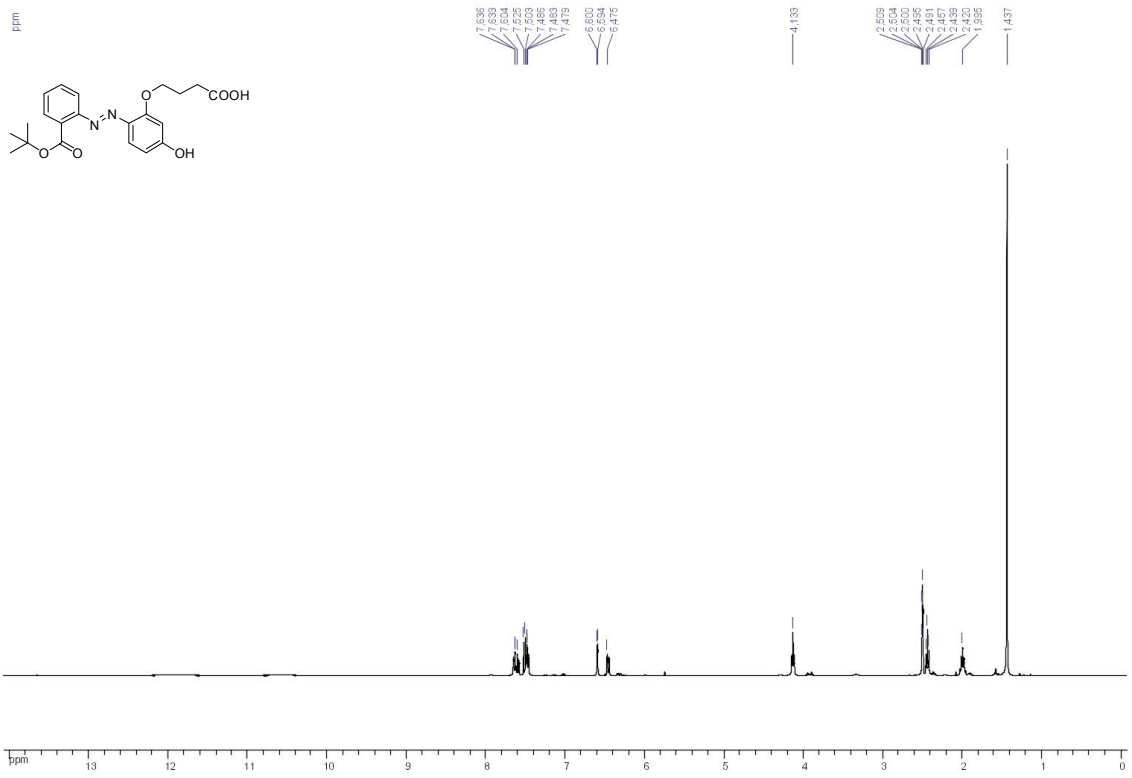


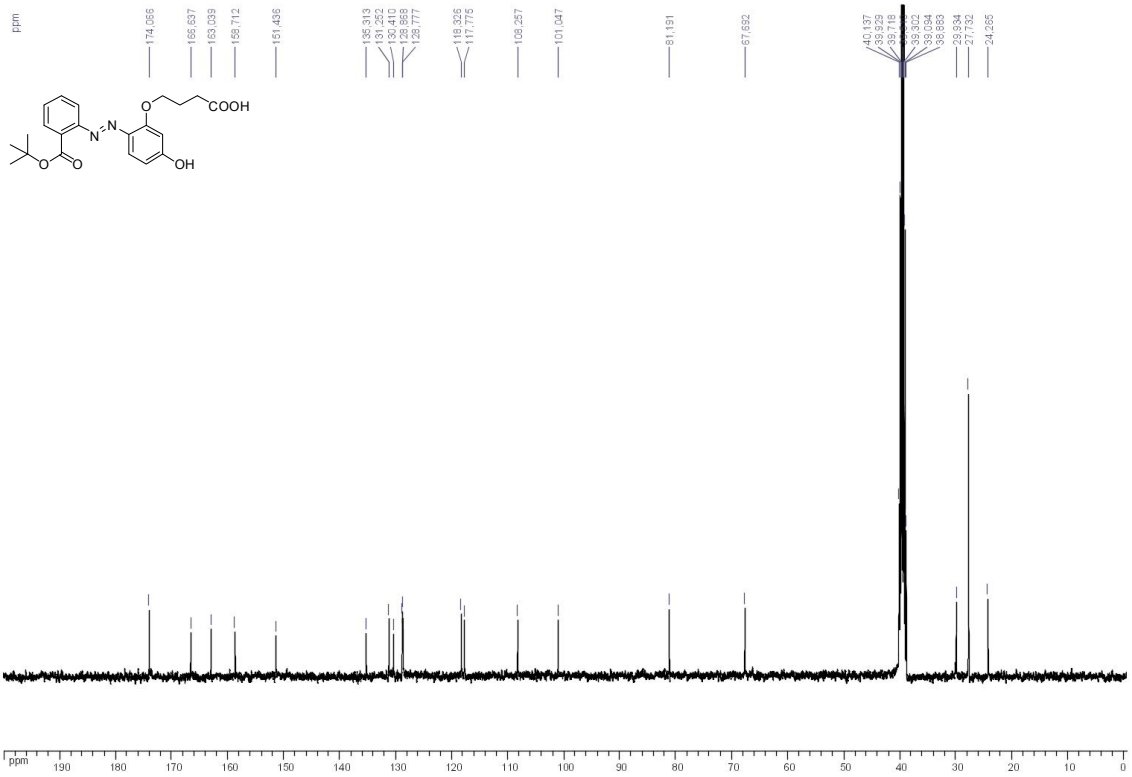
Spectra of compound **8**:



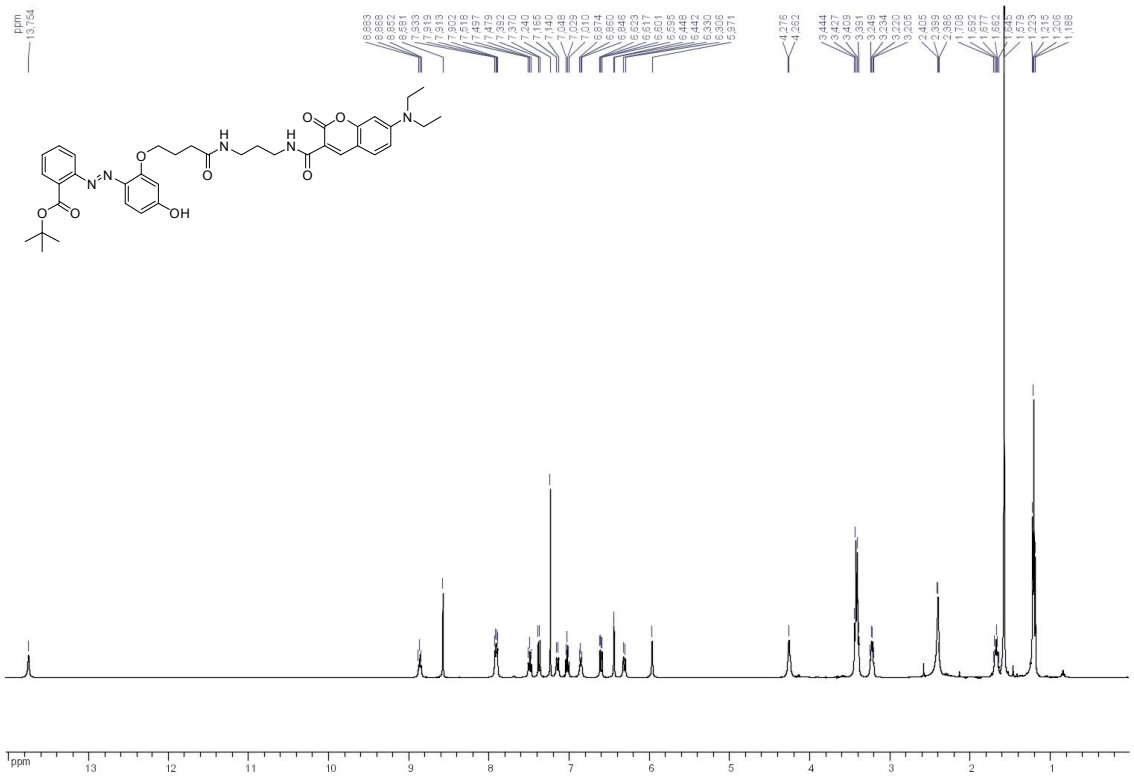


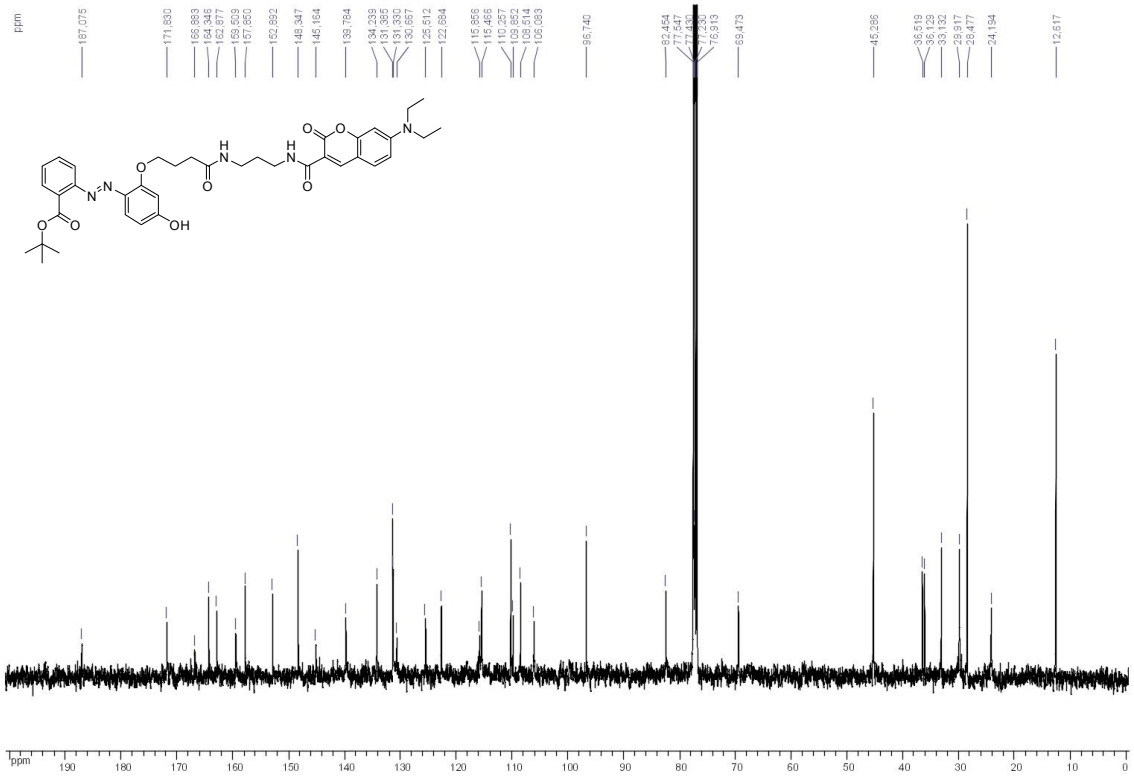
Spectra of compound **9**:



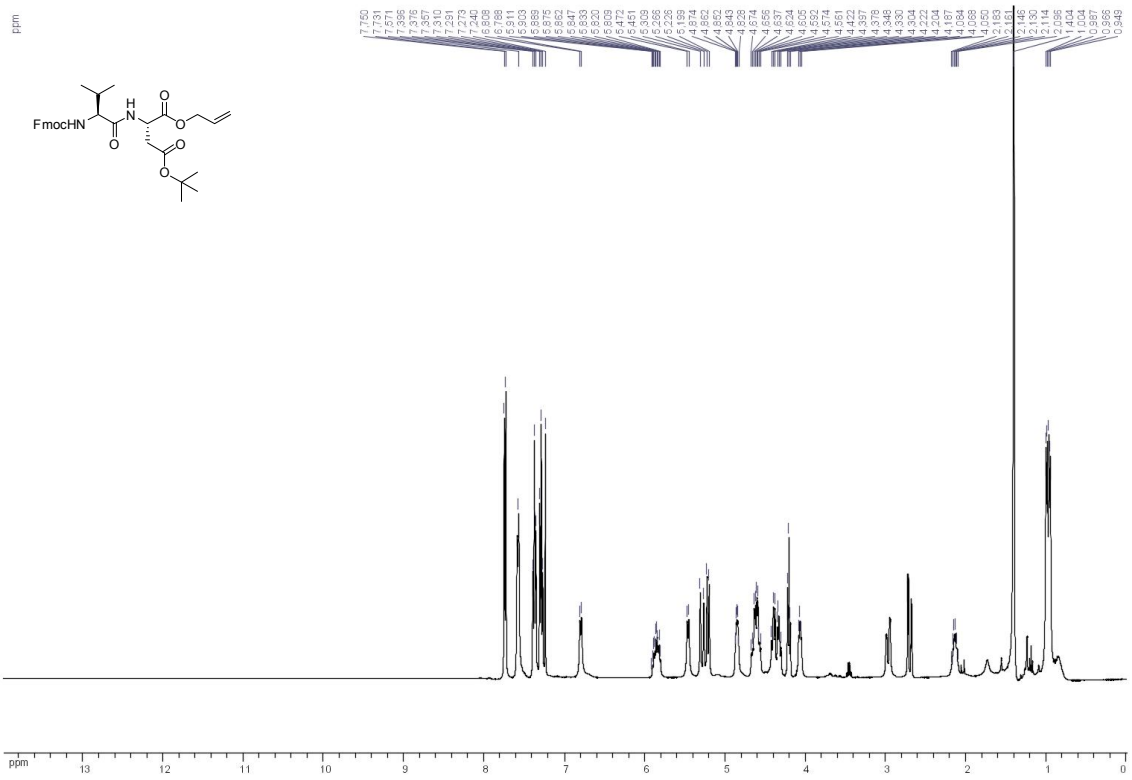


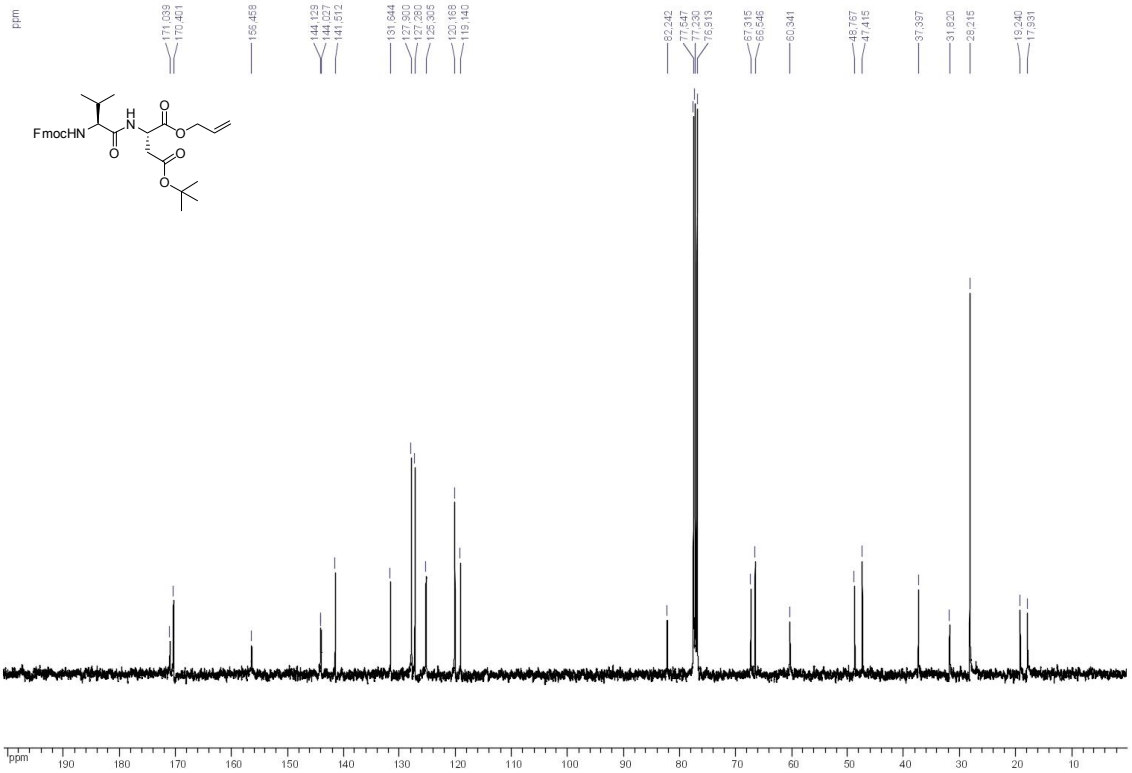
Spectra of compound **10**:



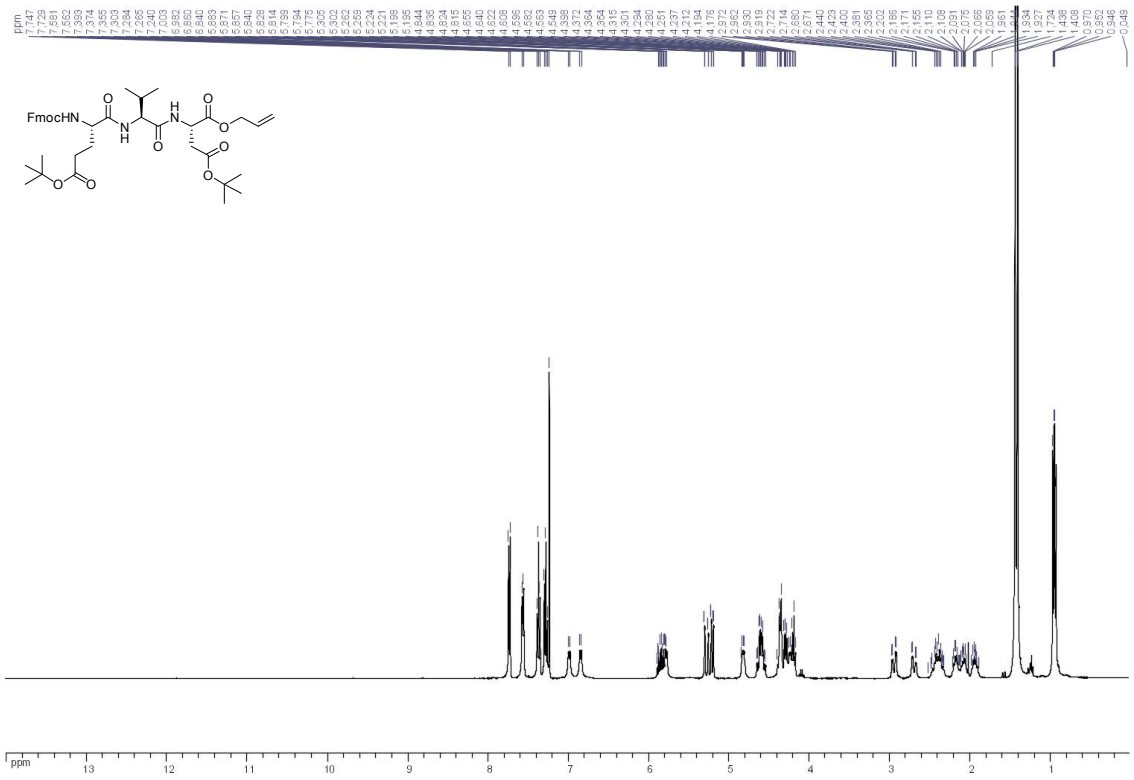


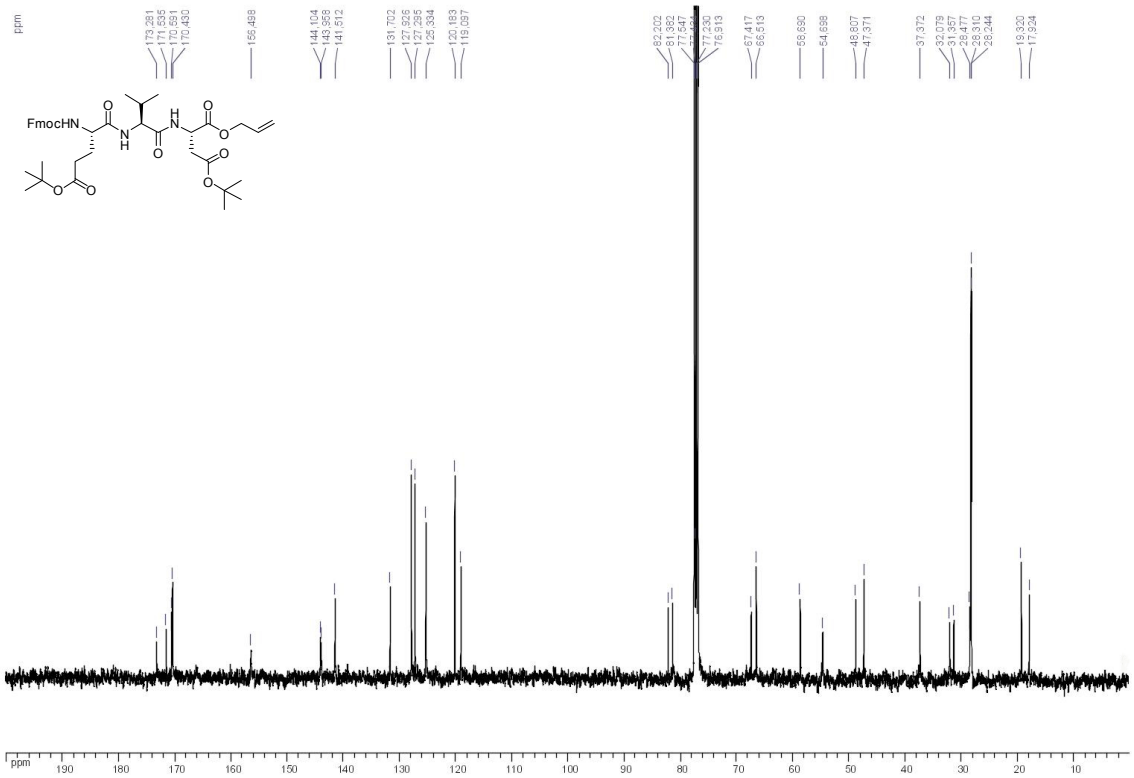
Spectra of compound **12**:



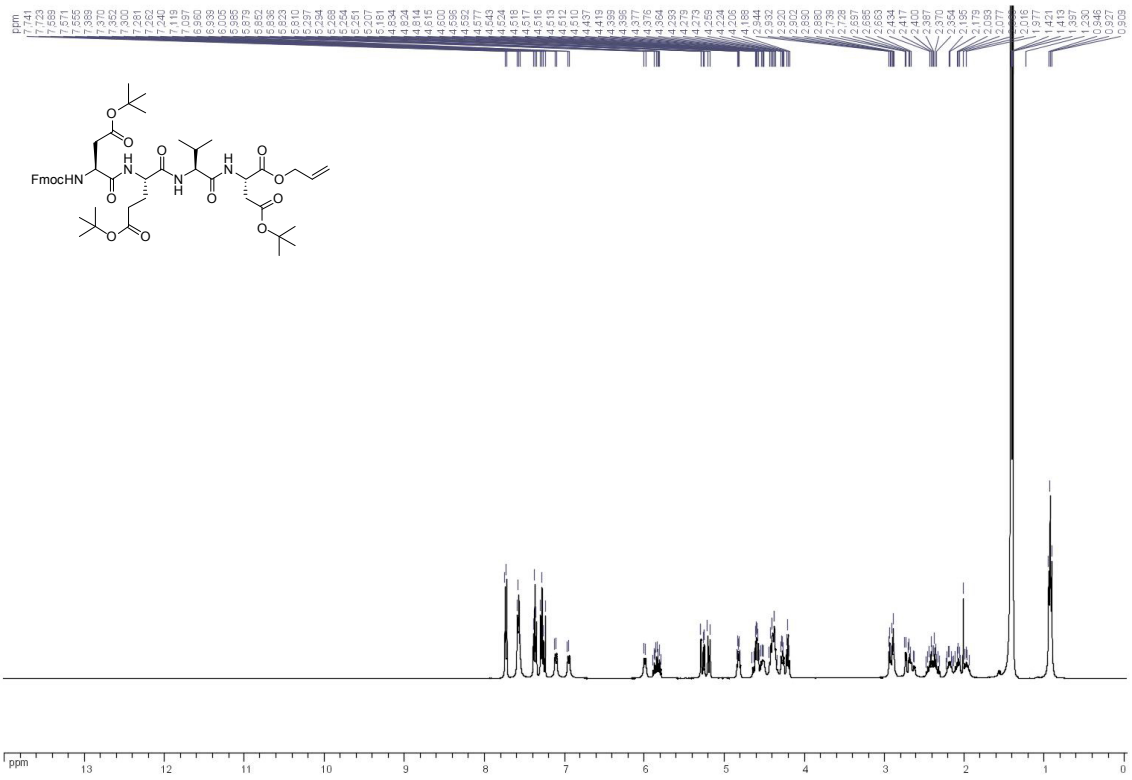


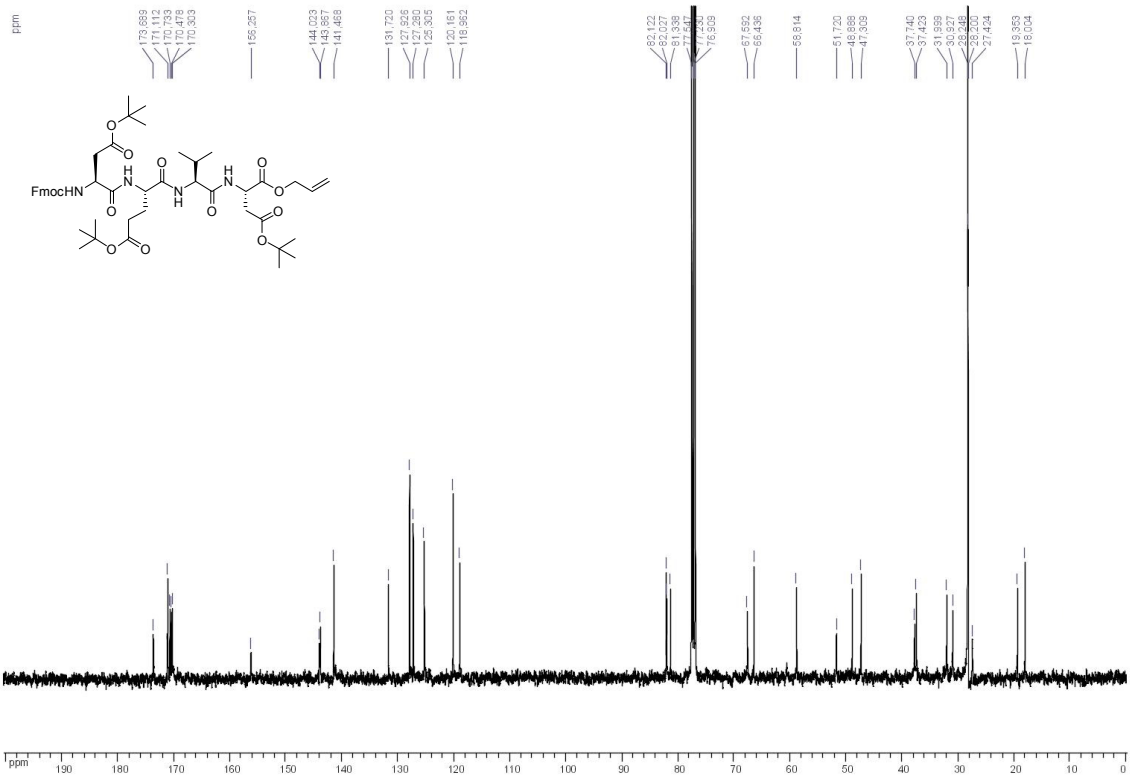
Spectra of compound **13**:



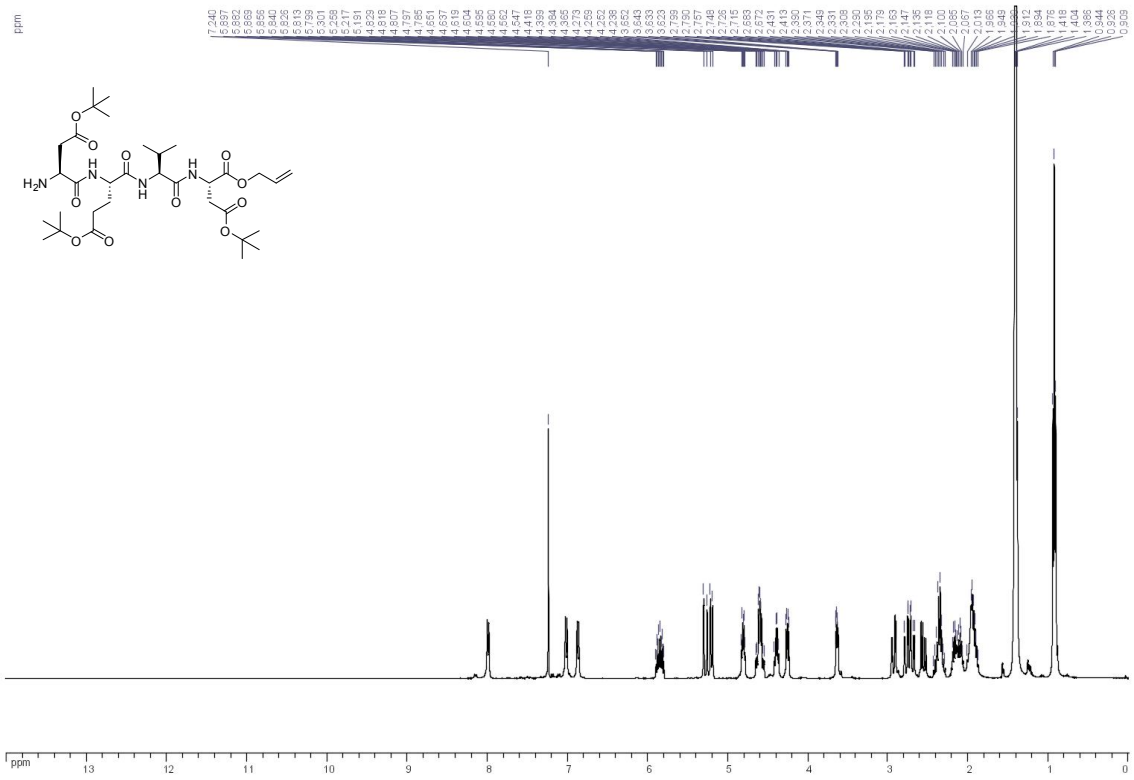


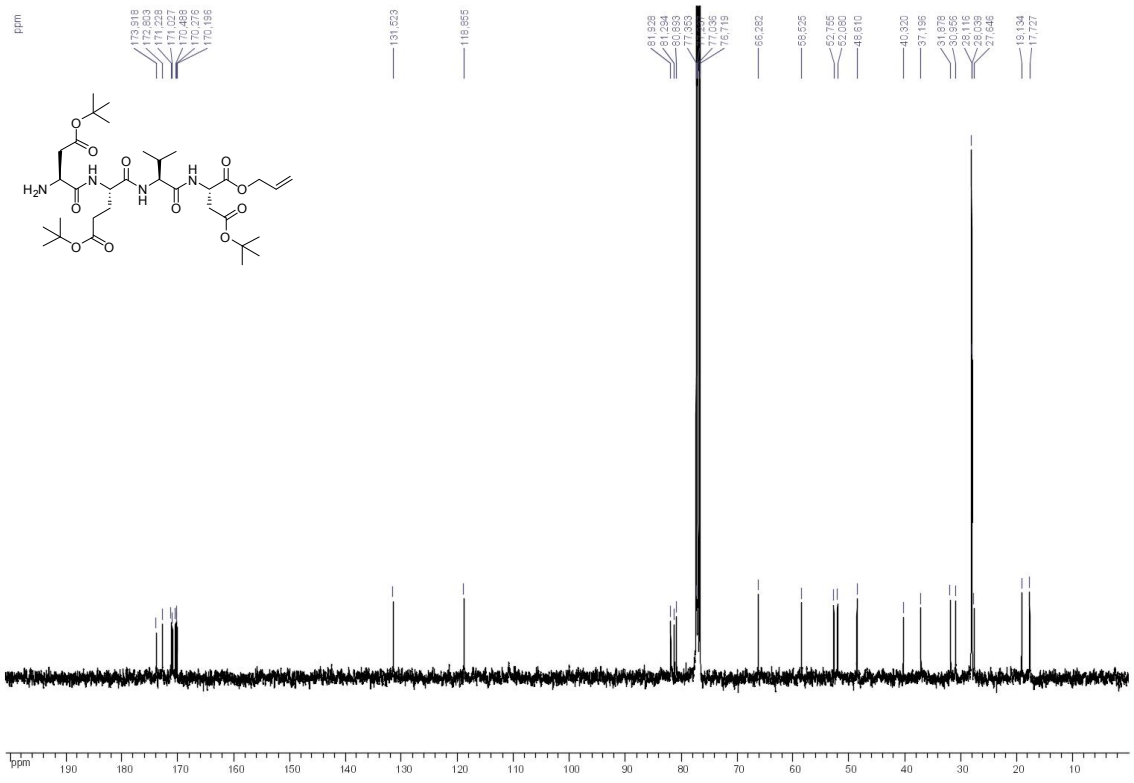
Spectra of compound **14**:



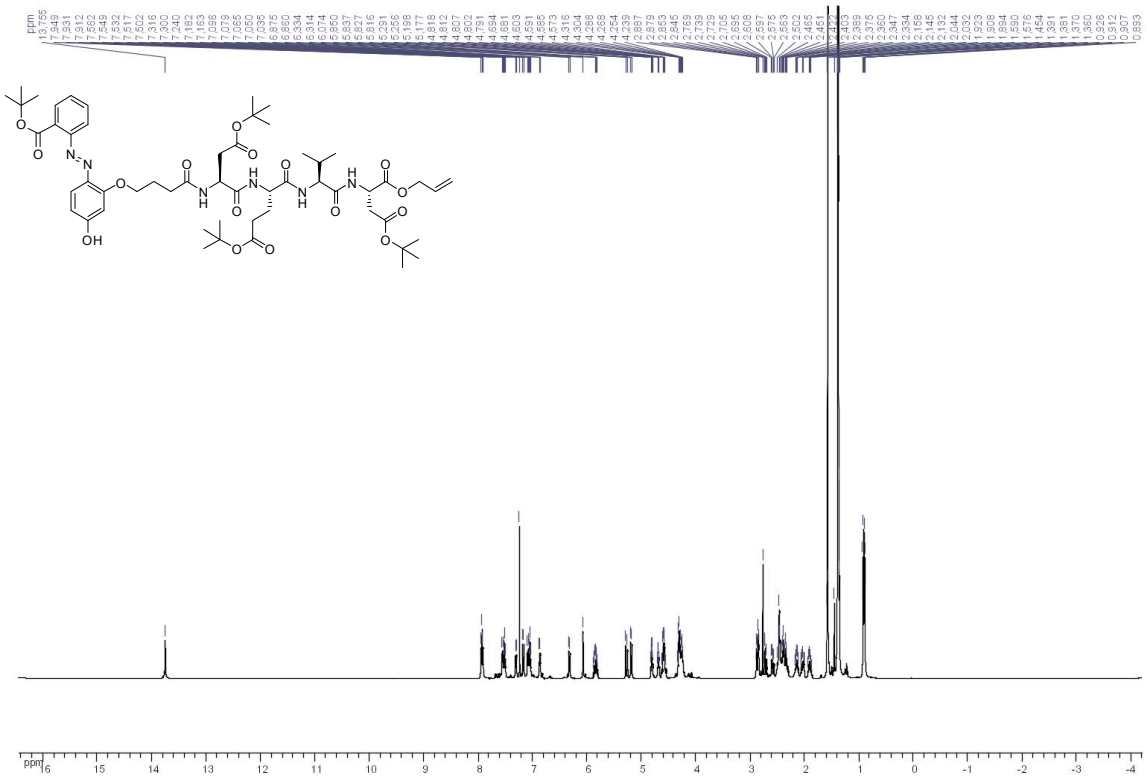


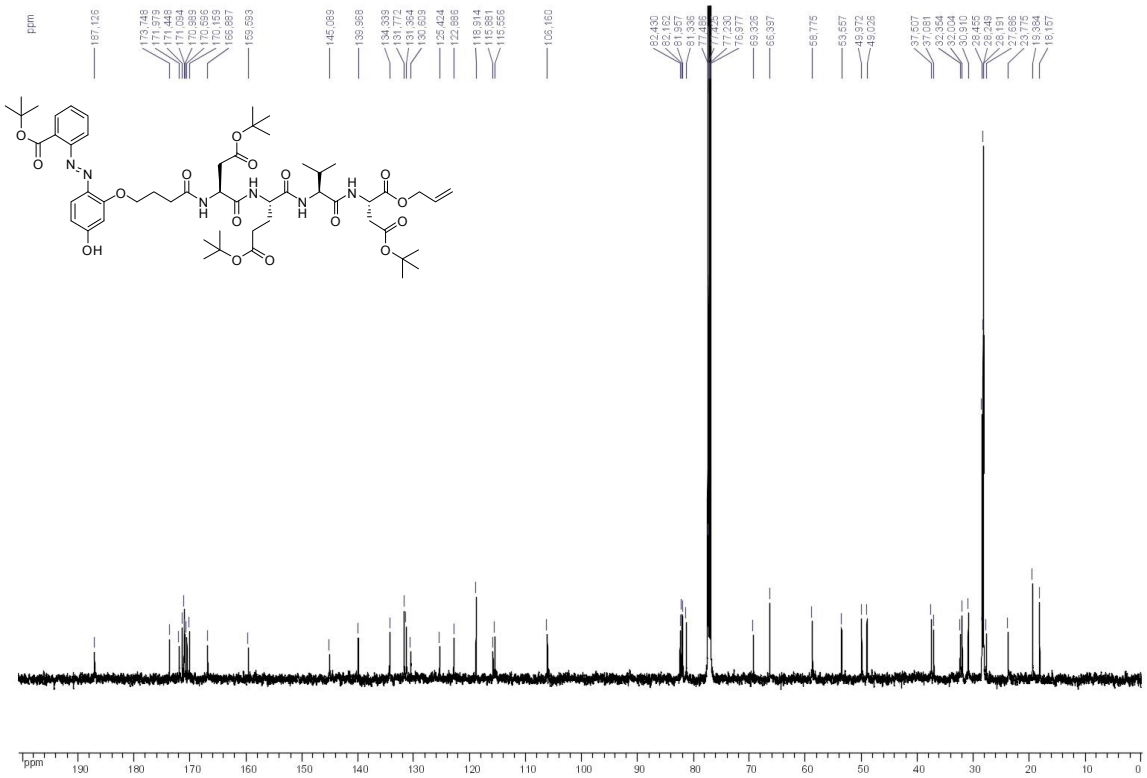
Spectra of compound **15**:



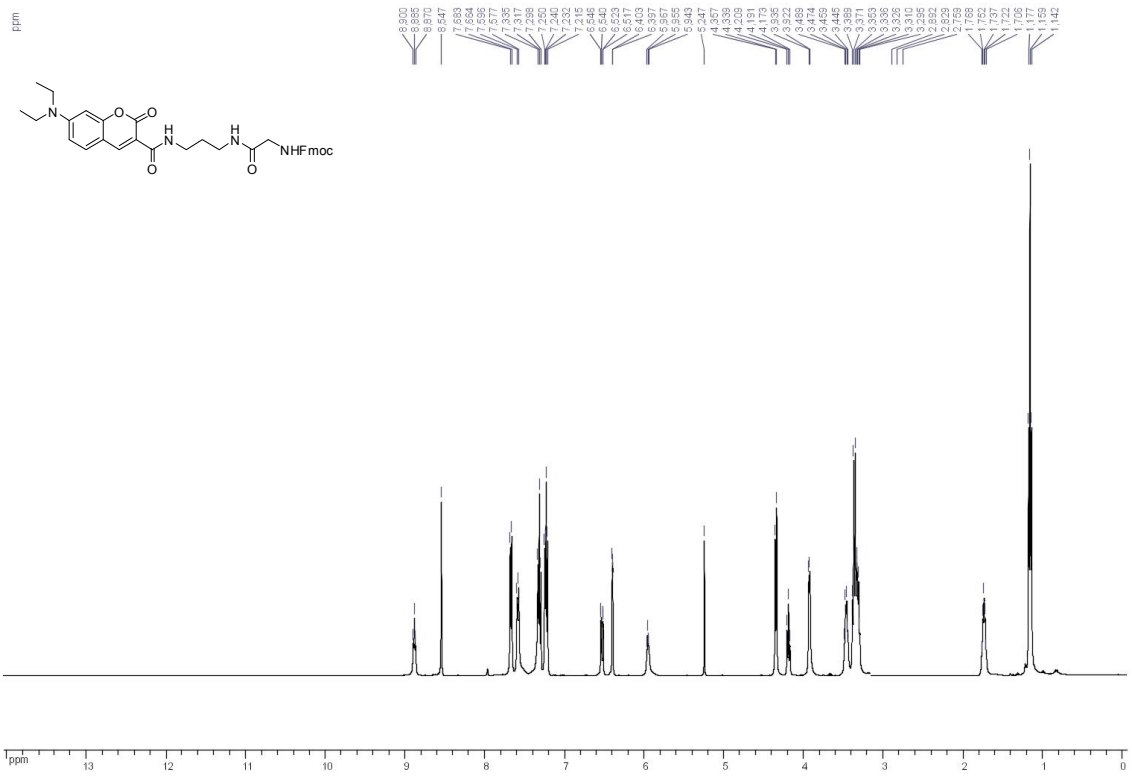


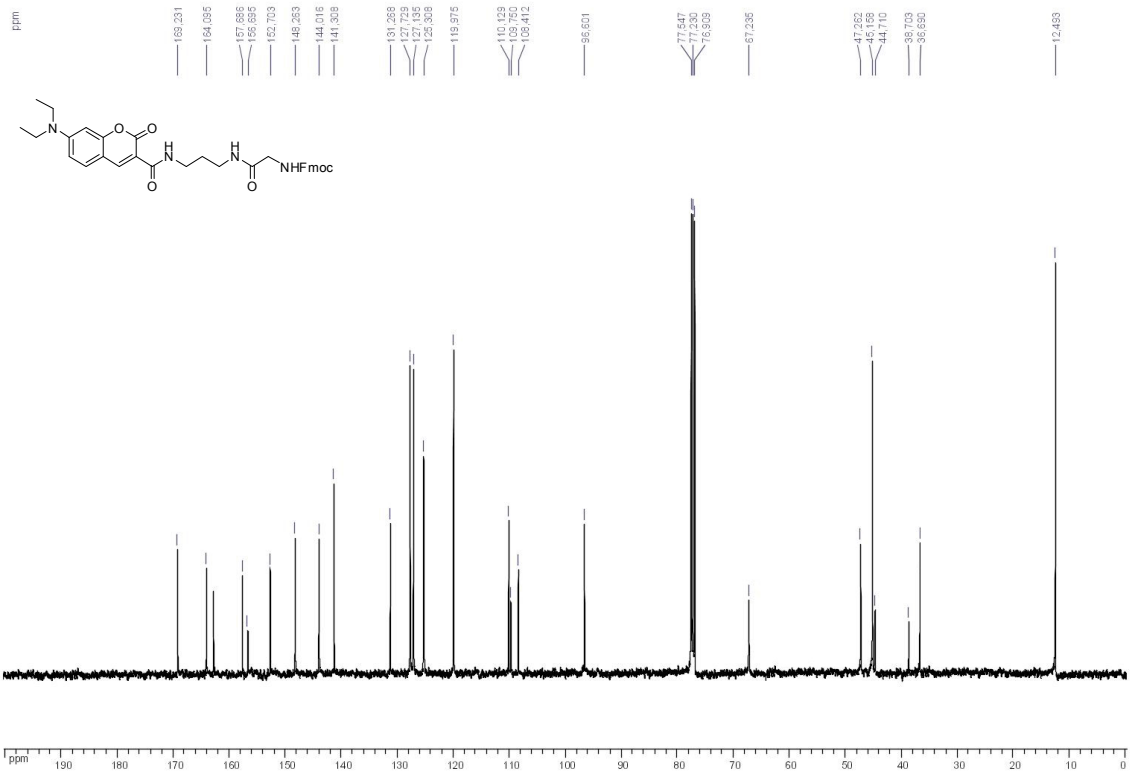
Spectra of compound **16**:





Spectra of compound **18**:





Spectra of compound **20**: