Electronic Supplementary Material (ESI) for Chemical Communications

A Self-Immobilizing and Fluorogenic Unnatural Amino Acid that Mimics Phosphotyrosine

Supporting Information

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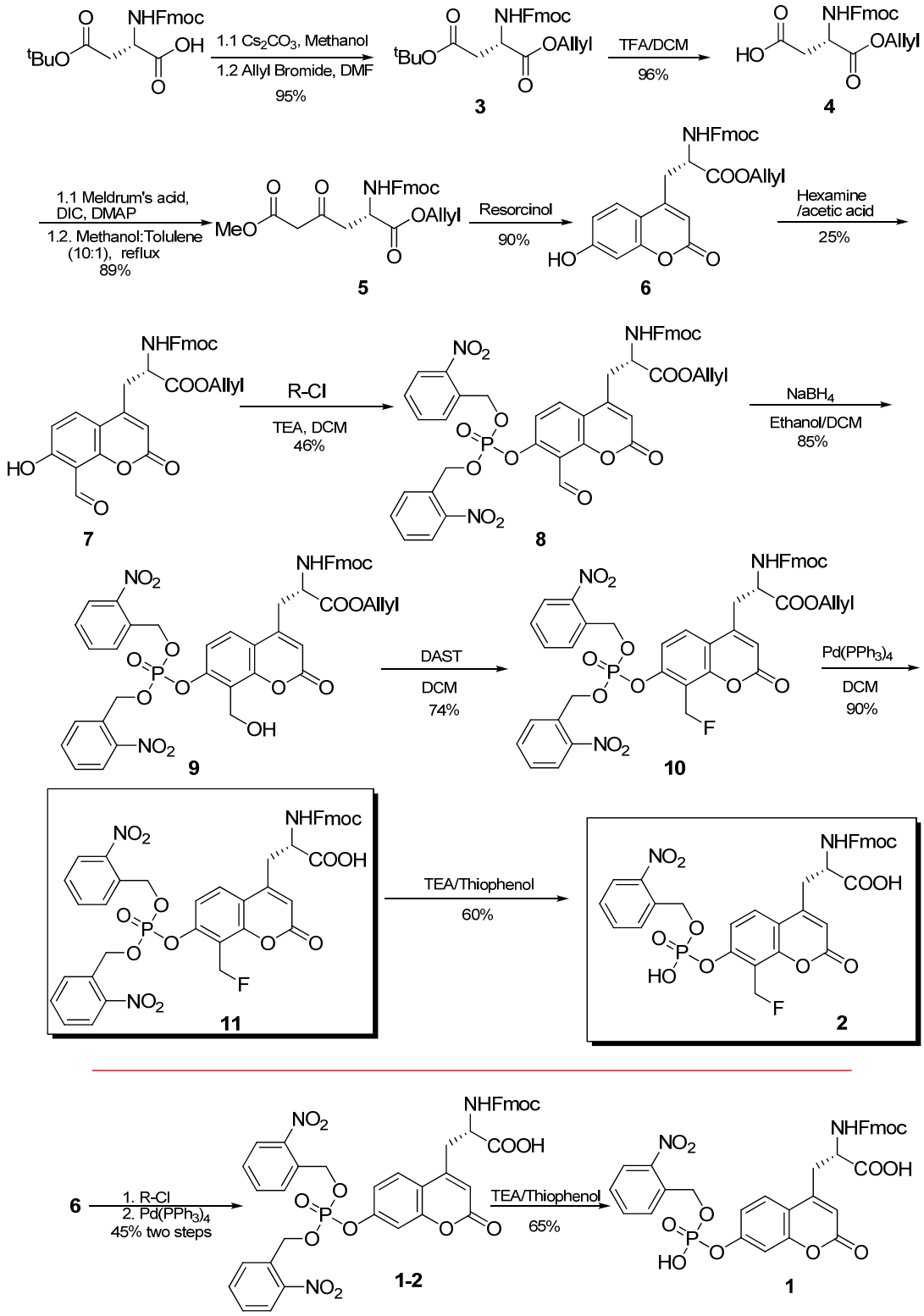
Contents

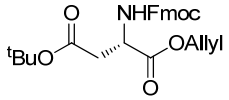
1. General Information

All chemicals were purchased from commercial vendors and used without further purifications, unless otherwise noted. ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR were recorded on Bruker 300 MHz, 500 MHz or DPX-300 NMR spectrometers. Chemical shifts are reported in parts per million (ppm) referenced with respect to residual solvent ($CDCl_3$ = 7.26 ppm, 77.00 ppm and d_6-DMSO = 2.5 ppm, 39.50 ppm). The following abbreviation was used in reporting spectra: s = singlet, d = doublet, t = triplet, q = quarter, m = multiplet, dd = doublet of doublets. All solvents used were of HPLC grade, unless otherwise indicated. All reactions requiring anhydrous conditions were conducted under a nitrogen or argon atmosphere in flame-dried glasswares. Dichloromethane (DCM) was dried over calcium hydride and distilled under room temperature. All LC profiles and mass spectra were recorded on Shimadzu LC-IT-TOF systems equipped with an autosampler, using reverse-phase Phenomenex Luna 5 C_{18} (2) 100 Å 50 × 3.0 mm column.

2. Synthetic procedure of unnatural amino acids

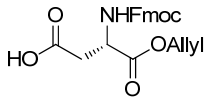


Scheme S1. Synthesis of unnatural amino acids



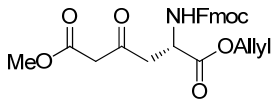
3

(*S*)-1-allyl 4-tert-butyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)succinate (**3**). The compound FmocNH-Asp(O^tBu)-COOH (20.0 g, 48.6 mmol) was dissolved in MeOH (150 mL). Cs_2CO_3 (7.9 g, 24.3 mmol) was added into the solution and the reaction was stirred for 30 min. Subsequently, MeOH was removed *in vacuo*. The residue was redissolved in DMF (200 mL). Allyl bromide (8.4 mL, 72.9 mmol) was added dropwise and the reaction mixture was stirred overnight. DMF was removed under reduced pressure. Water was added and the solution was extracted with EtOAc. The combined organic phase was washed with water, 10% $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The product **3** was obtained as a white solid (16.6 g, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.62 (t, *J* = 7.1 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.4 Hz, 2H), 5.97 (d, *J* = 8.5 Hz, 1H), 5.87 - 5.96 (m, 1H), 5.34 (d, *J* = 17.2 Hz, 1H), 5.24 (d, *J* = 10.4 Hz, 1H), 4.63 - 4.73 (m, 3H), 4.33 - 4.47 (m, 2H), 4.25 (t, *J* = 7.2 Hz, 1H), 2.90 (dd, *J* = 16.8, 4.5 Hz, 2H), 1.47 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.39, 169.71, 155.77, 143.70, 141.06, 131.35, 127.48, 126.84, 124.95, 119.75, 118.46, 81.52, 67.02, 65.99, 50.45, 46.88, 37.56, 27.79. IT-TOF: m/z $[M+H]^+$ calcd: 452.207, found: 452.216.



4

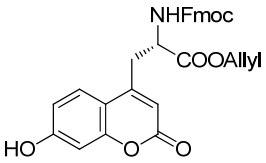
(*S*)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-(allyloxy)-4-oxobutanoic acid (**4**). The compound **3** (10 g, 22.2 mmol) was dissolved in DCM:TFA (1:1, 80 mL) and the mixture was stirred at room temperature for 5 h. The solvent was then evaporated under reduced pressure and the residue was precipitated by addition of cold water (400 mL). The solution was stirring overnight. The resulting precipitate was filtered, dried to give the desired product as a white solid (8.4 g, 96%). ^{1}H NMR (500 MHz, d_6-DMSO) δ 7.87 (t, *J* = 7.8 Hz, 2H), 7.71 (d, *J* = 7.4 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 2H), 5.84 - 5.92 (m, 1H), 5.30 (d, *J* = 17.3 Hz, 1H), 5.19 (d, *J* = 10.5 Hz, 1H), 4.59 (d, *J* = 5.1 Hz, 2H), 4.49 (dd, *J* = 13.7, 7.9 Hz, 1H), 4.29 - 4.36 (m, 2H), 4.23 (t, *J* = 6.9 Hz, 1H), 2.73 (dd, *J* = 16.6, 6.8 Hz, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 171.44, 170.85, 155.88, 143.78, 140.75, 132.24, 127.65, 127.09, 125.20, 120.11, 117.56, 65.80, 65.07, 50.60, 46.63, 35.90. IT-TOF: m/z $[M+H]^+$ calcd: 396.145, found: 396.121. m/z $[M+Na]^+$ calcd: 418.127, found: 418.109.



5

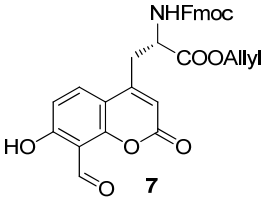
(*S*)-1-allyl 6-methyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-oxohexanedioate (**5**). Compound **4** (10 g, 25.3 mmol), Meldrum's acid (4.7 g, 32.6 mmol) and DMAP (3.1 g, 25.3 mmol) in 100 mL DCM was cooled in an ice bath. To this reaction mixture, *N*,*N*'-diisopropylcarbodiimide (DIC) was added very slowly. The reaction mixture was allowed to stir around 5 h, or till the disappearance of the starting material was detected by TLC. The resulting solution was washed with 10% aqueous solution of $KHSO_4$ (75 mL, 3×) and brine. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in methanol/toluene (10:1). The solution was refluxed overnight. After evaporation of the solvent, the desired product was obtained by recrystallization in hexane/EtOAc (10.1 g, 89%).[1] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, *J* = 7.5 Hz, 2H), 7.55 - 7.62 (m, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 2H), 5.96 (d, *J* = 7.1 Hz, 1H), 5.82 - 5.90 (m, 1H), 5.30 (d, *J* = 17.2 Hz, 1H), 5.23 (d, *J* = 10.4 Hz, 1H), 4.61 - 4.67 (m, 3H), 4.29 - 4.42 (m, 2H), 4.21 (t, *J* = 7.0 Hz, 1H), 3.68 (s, 3H), 3.46 (s, 2H), 3.22 (dd, *J* = 18.4, 4.4 Hz, 2H). ^{13}C

NMR (125 MHz, $CDCl_3$) δ 200.48, 170.19, 166.74, 155.80, 143.61, 141.02, 131.27, 127.47, 126.83, 124.92, 119.73, 118.48, 66.96, 66.08, 52.14, 49.68, 48.52, 46.83, 44.26. IT-TOF: m/z $[M+H]^+$ calcd: 452.171, found: 452.143.



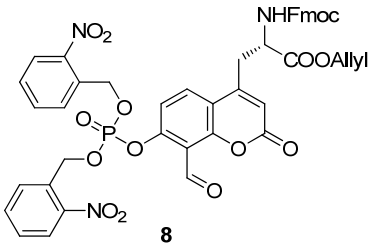
6

(*S*)-allyl-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(7-hydroxy-2-oxo-2H-chromen-4-yl)propanoate (**6**). Compound **5** (8 g, 17.7 mmol) was dissolved in methanesulfonic acid (100 mL). The solution was cooled down in an ice bath. Resorcinol (1.95 g, 17.7 mmol) was added into the solution. The mixture was stirred in 4 °C for 4 h, when TLC showed the starting material was completely consumed. The reaction mixture was then slowly poured into ice water, and the resulting white precipitate was collected by vacuum filtration. Upon recrystallization in ethanol/water, the desired product was obtained (8.1 g, 90%).[1] ^{1}H NMR (500 MHz, d_6-DMSO) δ 10.58 (s, 1H), 8.01 (d, J = 8.2 Hz, 1H), 7.87 (d, J = 7.5 Hz, 2H), 7.60 - 7.65 (m, 3H), 7.40 (t, J = 7.3 Hz, 2H), 7.29 (q, J = 7.3 Hz, 2H), 6.83 (d, J = 8.7 Hz, 1H), 6.74 (d, J = 2.0 Hz, 1H), 6.17 (s, 1H), 5.83 - 5.91 (m, 1H), 5.29 (d, J = 17.2 Hz, 1H), 5.20 (d, J = 10.5 Hz, 1H), 4.60 (d, J = 4.0 Hz, 2H), 4.42 - 4.45 (m, 1H), 4.17 - 4.20 (m, 2H), 4.19 (t, J = 6.9 Hz, 1H), 3.06 - 3.30 (m, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 170.71, 161.22, 159.98, 155.88, 155.09, 152.16, 143.64, 140.66, 132.08, 127.59, 127.01, 125.92, 125.07, 120.06, 117.83, 113.11, 111.23, 110.88, 102.54, 65.78, 65.25, 52.80, 46.50, 32.36. IT-TOF: m/z $[M+H]^+$ calcd: 512.171, found: 512.135.



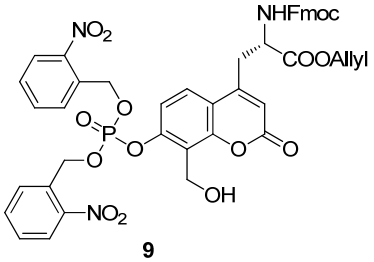
7

(*S*)-allyl-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(8-formyl-7-hydroxy-2-oxo-2H-chromen-4-yl)propanoate (**7**). Compound **6** (5 g, 9.78 mmol) was added into acetic acid (50 mL). The mixture was heated to around 100 °C, until compound **6** was dissolved. Hexamine (4.1 g, 29.4 mmol) was added slowly. Then the reaction was heated to 150 °C and stirred for 3 h. The solvent was removed *in vacuo*. 1 M HCl was added to the residue and the mixture was further stirred for 1 h. The solution was extracted with EtOAc (3 ×), washed with water and brine (2 ×). The organic layer was dried over Na_2SO_4 and concentrated. The product was obtained by purification with flash column chromatography (1.32 g, 25%).[2] ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.21 (s, 1H), 10.56 (s, 1H), 7.90 (d, J = 9.0 Hz, 1H), 7.74 (d, J = 7.5 Hz, 2H), 7.54 (d, J = 7.3 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.29 (t, J = 7.4 Hz, 2H), 6.88 (d, J = 9.0 Hz, 1H), 6.13 (s, 1H), 5.80 - 5.91 (m, 1H), 5.66 (d, J = 7.6 Hz, 1H), 5.27 - 5.38 (m, 2H), 4.69 (dd, J = 13.9, 6.9 Hz, 1H), 4.63 (d, J = 5.8 Hz, 2H), 4.41 (d, J = 6.8 Hz, 2H), 4.17 (t, J = 6.7 Hz, 1H), 3.19 (d, J = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.10, 170.00, 165.22, 158.53, 156.29, 155.63, 150.85, 143.41, 141.13, 132.58, 130.64, 127.69, 126.98, 124.80, 119.92, 114.51, 113.11, 110.73, 108.69, 67.17, 66.67, 53.11, 46.85, 34.99. IT-TOF: m/z $[M+H]^+$ calcd: 540.167, found: 540.136.



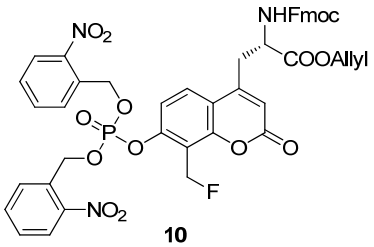
8

(*S*)-allyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(7-(bis(2-nitrobenzyloxy)- phosphoryloxy)-8-formyl-2-oxo-2H-chromen-4-yl)propanoate (**8**). To a suspension of di(2-nitrobenzyl) phosphate (1.22 g, 3.3 mmol) in distilled DCM (20 mL) was added two drops of DMF as a catalyst. Oxalyl chloride (2.12 g, 16.5 mmol) was then added dropwise at room temperature to the mixture. The reaction was stirred for 1 h. Upon solvent removal *in vacuo*, the product obtained was directly used in the next step without further purification. Compound **7** (900 mg, 1.67 mmol; dissolved in 20 mL DCM) was added the above product (dissolved in DCM with ~1 mg of DMAP) in an ice bath. Subsequently, triethylamine (0.86 g, 4.18 mmol) was added dropwise. The reaction mixture was stirred for 5 h until the starting material was completely consumed. Upon solvent removal *in vacuo*, the resulting residue was purified by flash column chromatography (1:1 hexane/EtOAc) to give **8** as a slightly yellow solid (1.34 g, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.61 (s, 1H), 8.11 (d, *J* = 8.2 Hz, 2H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.73 - 7.77 (m, 4H), 7.63 - 7.69 (m, 2H), 7.55 (d, *J* = 7.4 Hz, 2H), 7.47 - 7.50 (m, 3H), 7.36 - 7.39 (m, 2H), 7.30 (td, *J* = 7.3, 3.1 Hz, 2H), 6.27 (s, 1H), 5.80 - 5.90 (m, 1H), 5.74 (t, *J* = 7.1 Hz, 4H), 5.62 (d, *J* = 6.8 Hz, 1H), 5.25 - 5.35 (m, 2H), 4.63 - 4.66 (m, 3H), 4.42 (d, *J* = 5.8 Hz, 2H), 4.18 (t, *J* = 6.6 Hz, 1H), 3.21 - 3.22 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.48, 170.62, 158.61, 156.74, 156.42, 152.06, 150.62, 147.55, 144.21, 142.00, 134.82, 132.13, 131.32, 131.07, 129.90, 129.51, 128.50, 127.80, 125.76, 125.57, 121.05, 120.72, 118.14, 117.40, 116.79, 116.62, 68.11, 68.01, 67.57, 53.78, 47.76, 36.21. ^{31}P NMR (121 MHz, $CDCl_3$) δ -7.84. IT-TOF: m/z $[M+CF_3COO]^-$ calcd: 1002.173, found: 1002.143.



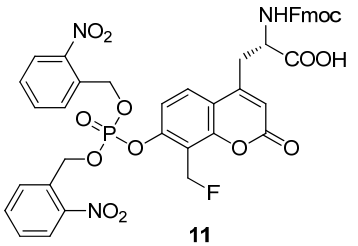
9

(*S*)-allyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(7-(bis(2-nitrobenzyloxy) phosphoryloxy)-8-(hydroxymethyl)-2-oxo-2H-chromen-4-yl)propanoate (**9**). Compound **8** (0.93 g, 1.0 mmol) was dissolved in DCM/Ethanol (1:1, 30 mL) and cooled to 0 °C. $NaBH_4$ (9.8 mg, 0.25 mmol) in 1 mL water was then added dropwise into the reaction. The reaction mixture was stirred at 0 °C for 5 min. The reaction was quenched with 0.25 M HCl. The organic solvents were removed *in vacuo*. The residue was purified by flash column chromatography (1:1 hexane/EtOAc) to obtain **9** as a white solid (757 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.2 Hz, 2H), 7.68 - 7.80 (m, 4H), 7.65 (t, *J* = 7.5 Hz, 2H), 7.55 (d, *J* = 7.0 Hz, 2H), 7.49 (t, *J* = 7.7 Hz, 2H), 7.36 - 7.39(m, 2H), 7.27 - 7.34 (m, 3H), 6.23 (s, 1H), 5.78 - 5.89 (m, 1H), 5.63 - 5.66 (m, 5H), 5.23 - 5.34 (m, 2H), 4.89 (s, 2H), 4.65 - 4.47 (m, 1H), 4.61 (d, *J* = 5.7 Hz, 2H), 4.39 (d, *J* = 6.7 Hz, 2H), 4.17 (t, *J* = 6.7 Hz, 1H), 3.22 (d, J = 6.4 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.15, 159.28, 155.71, 153.26, 150.93, 150.23, 146.89, 143.53, 141.28, 134.17, 131.20, 131.14, 130.73, 129.40, 128.86, 127.79, 127.11, 125.15, 124.92, 121.49, 120.12, 120.01, 116.83, 115.85, 67.46, 67.32, 66.76, 53.18, 47.07, 35.24. ^{31}P NMR (121 MHz, $CDCl_3$) δ -6.11. IT-TOF: m/z $[M+H]^+$ calcd: 892.212, found: 892.171. m/z $[M+CF_3COO]^-$ calcd: 1004.189, found: 1004.135.



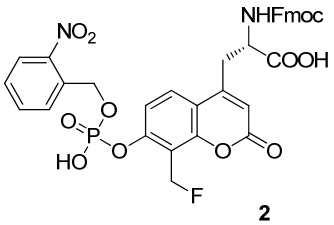
10

(*S*)-allyl 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(7-(bis(2-nitrobenzyloxy) phosphoryloxy)-8-(fluoromethyl)-2-oxo-2H-chromen-4-yl)propanoate (**10**). Compound **9** (602 mg, 0.67 mmol) was dissolved into distilled DCM (15 mL) at 0 °C. Diethylaminosulfur trifluoride (DAST, 218 mg, 1.35 mmol) was added slowly under an Ar atmosphere. The reaction was stirred for 10 min. The solvent was evaporated and the residue was purified by flash column chromatography to obtain compound **10** as a white foam solid (436 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.2 Hz, 2H), 7.86 (d, *J* = 8.5 Hz, 1H), 7.69 - 7.75 (m, 4H), 7.65 (td, *J* = 7.8, 3.5 Hz, 2H), 7.55 (d, *J* = 7.0 Hz, 2H), 7.46 - 7.52 (m, 3H), 7.36 - 7.41 (m, 2H), 7.29 - 7,32 (m, 2H), 6.24 (s, 1H), 5.78 - 5.89 (m, 1H), 5.71/5.61(d, *J* = 47.4 Hz, 2H, CH_2F), 5.67 (dd, *J* = 7.7, 2.5 Hz, 4H), 5.58 (d, *J* = 7.4 Hz, 1H), 5.26 – 5.35 (m, 2H), 4.68 (d, *J* = 6.9 Hz, 1H), 4.61 (d, *J* = 5.2 Hz, 2H), 4.41 (s, 2H), 4.18 (t, *J* = 6.7 Hz, 1H), 3.22 (d, *J* = 6.2 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.07, 158.85, 155.68, 153.35, 152.23, 150.08, 146.82, 143.51, 141.30, 134.16, 131.29, 131.23, 130.65, 129.33, 128.69, 127.80, 127.12, 126.91, 125.14, 124.98, 120.20, 120.02, 116.45, 116.08, 115.86, 115.74, 72.48 (d, *J* = 166.3 Hz, 1C, C-F), 67.32, 66.79, 53.15, 47.08, 35.33. ^{19}F NMR (282 MHz, $CDCl_3$) δ -135.37 (t, *J* = 47.6 Hz, 1F). ^{31}P NMR (121 MHz, $CDCl_3$) δ -7.03. IT-TOF: m/z $[M+H]^+$ calcd: 894.207, found: 894.131. m/z $[M+CF_3COO]^-$ calcd: 1006.185, found: 1006.100.



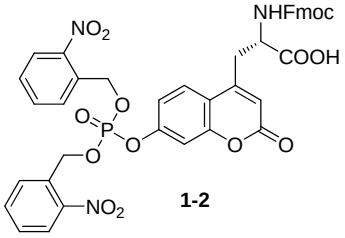
11

(*S*)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(7-(bis(2-nitrobenzyloxy)phosphoryloxy)-8-(fluoromethyl)-2-oxo-2H-chromen-4-yl)propanoic acid (**11**). Compound **10** (220 mg, 0.25 mmol) was dissolved in DCM under an Ar atmosphere. Phenylsilane (107 mg, 1 mmol) and $Pd(PPh_3)_4$ (14 mg, 0.01 mmol) were added. After 5 h, the solution became brown dark, with TLC showing the completion of the reaction. The solvent was removed *in vacuo*, and the resulting residue was purified by flash chromatography to give the desired product **11** (190 mg, 90%). ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.11 (dd, *J* = 8.1, 2.2 Hz, 2H), 7.96 (d, *J* = 7.2 Hz, 1H), 7.86 (dd, *J* = 8.0, 3.3 Hz, 2H), 7.74 - 7.78 (m, 2H), 7.68 - 7.70 (m, 2H), 7.58 - 7.64 (m, 4H), 7.43 (d, *J* = 8.9 Hz, 1H), 7.38 (t, *J* = 6.5 Hz, 2H), 7.27 (q, *J* = 7.3 Hz, 2H), 6.45 (s, 1H), 5.65 (d, *J* = 7.6 Hz, 4H), 5.57 (d, *J* = 47.8 Hz, 2H), 4.31 - 4.37 (m, 1H), 4.25 (d, *J* = 7.1 Hz, 2H), 4.14 - 4.21 (m, 2H), 3.24 (ddd, *J* = 25.4, 14.7, 7.2 Hz, 2H). ^{13}C NMR (75 MHz, d_6-DMSO) δ 173.41, 159.69, 157.02, 153.89, 153.17, 152.27, 147.93, 144.74, 141.72, 135.33, 131.90, 131.80, 130.75, 130.08, 128.65, 128.05, 126.18, 125.97, 121.12, 117.34, 116.83, 115.83, 115.70, 73.62 (d, *J* = 162.2 Hz, 1C, C-F), 67.92, 66.80, 53.55, 47.61, 33.61. ^{19}F NMR (282 MHz, d_6-DMSO) δ -133.34 (t, *J* = 47.9 Hz, 1F). ^{31}P NMR (121 MHz, d_6-DMSO) δ -6.93. IT-TOF: m/z $[M-H]^-$ calcd: 852.161, found: 852.165.



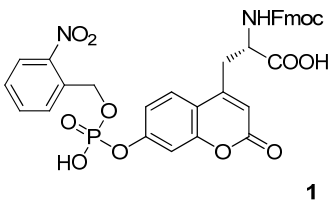
2

(2*S*)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(8-(fluoromethyl)-7-(hydroxy(2-nitrobenzyloxy)phosphoryloxy)-2-oxo-2H-chromen-4-yl)propanoic acid (**2**). Compound **11** (20 mg, 23 µmol) was dissolved in 1 mL DCM. 500 µL of 1 M thiophenol and triethylamine were subsequently added. After 1 h, the solvent was removed under reduced pressure. The residue was purified by preparative HPLC, giving the desired product **2** (10 mg, 60%). ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.09 (d, J = 8.1 Hz, 1H), 7.92 (d, J = 8.9 Hz, 1H), 7.86 (d, J = 7.8 Hz, 2H), 7.70 - 7.78 (m, 2H), 7.62 (dd, J = 12.1, 7.6 Hz, 2H), 7.55 (t, J = 8.3 Hz, 1H), 7.47 (d, J = 8.9 Hz, 1H), 7.39 (dd, J = 7.3, 5.2 Hz, 2H), 7.28 (q, J = 7.7 Hz, 2H), 6.38 (s, 1H), 5.55 (d, J = 47.9 Hz, 2H), 5.42 (d, J = 7.9 Hz, 2H), 4.32 - 4.39 (m, 1H), 4.25 (d, J = 7.2 Hz, 2H), 4.18 (t, J = 7.0 Hz, 1H), 3.23 (ddd, J = 25.2, 14.6, 7.3 Hz, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 172.35, 158.89, 158.06, 155.90, 153.20, 152.93, 152.28, 146.69, 143.65, 140.61, 134.06, 132.57, 132.51, 128.94, 128.59, 127.54, 127.10, 126.96, 125.10, 124.64, 120.00, 116.00, 114.88, 114.31, 113.72, 73.27, 71.98, 65.69, 64.94, 52.47, 46.51, 32.53. ^{19}F NMR (282 MHz, d_6-DMSO) δ -132.67 (t, J = 47.9 Hz, 1F). ^{31}P NMR (121 MHz, d_6-DMSO) δ -6.67. IT-TOF: m/z [M+H] $^{+}$ calcd: 719.144, found: 719.118. m/z [M-H] $^{-}$ calcd: 717.129, found: 717.092.



1-2

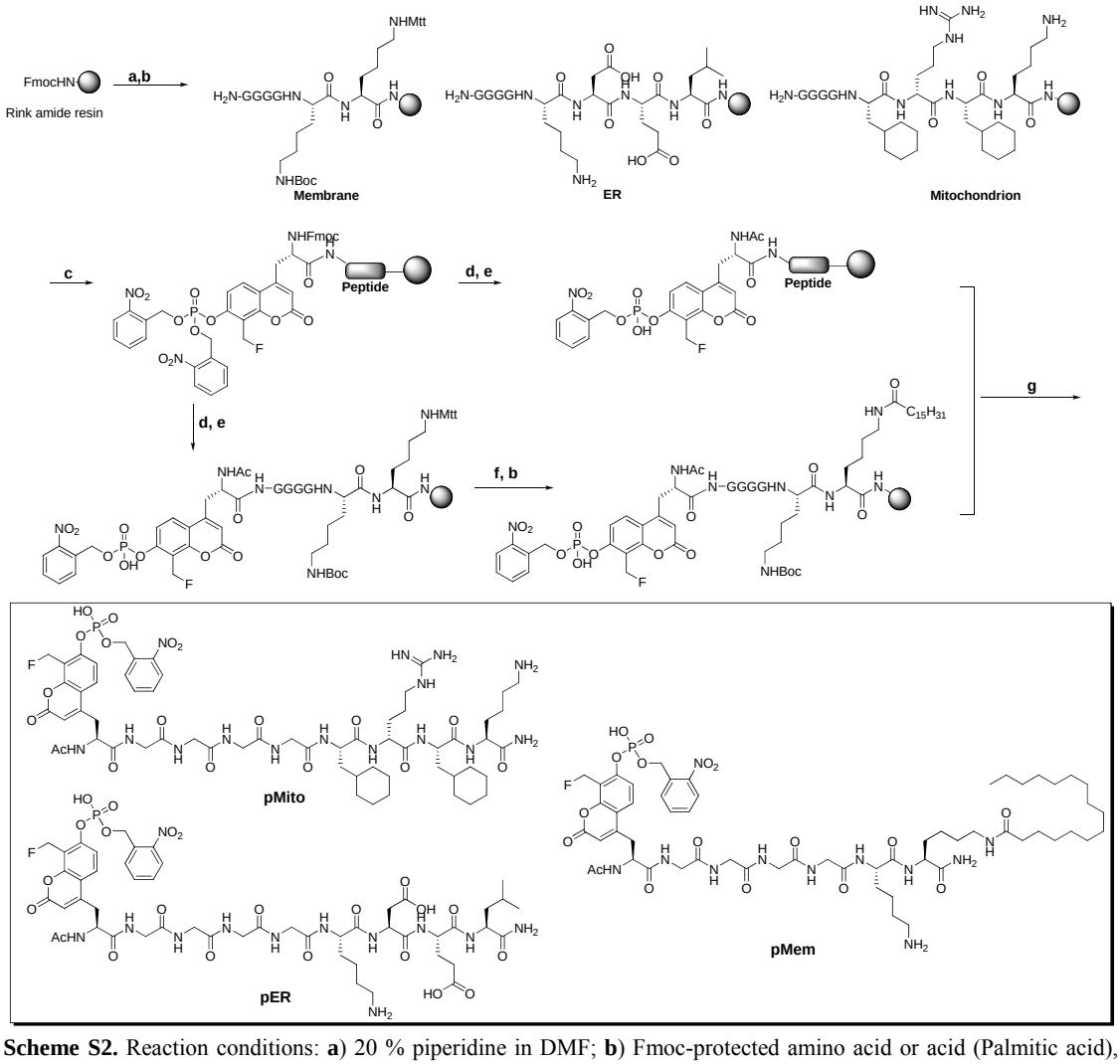
(*S*)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(7-(bis(2-nitrobenzyloxy) phosphoryloxy)-2-oxo-2H-chromen-4-yl)propanoic acid (**1-2**).Prepared according to procedures for preparation of compound **8** using di(2-nitrobenzyl) chlorophosphate [prepared from di(2-nitrobenzyl) phosphate (0.55 g, 1.5 mmol)], compound **6** (0.38 g, 0.75 mmol), triethylamine (0.38 g, 1.88 mmol). The allyl group of the phosphorylated product was deprotected using $Pd(PPh_3)_4$ and $PhSiH_3$, and purified by flash chromatography to give the desired product **1-2** (276 mg, two steps in 45% yield). ^{1}H NMR (500 MHz, d_6-DMSO) δ 8.11 (d, J = 8.1 Hz, 2H), 7.84 - 7.89 (m, 3H), 7.76 (t, J = 7.4 Hz, 2H), 7.69 (d, J = 7.6 Hz, 2H), 7.57 - 7.65 (m, 4H), 7.39 (t, J = 7.3 Hz, 2H), 7.26 - 7.30 (m, 4H), 6.39 (s, 1H), 5.63 (d, J = 7.7 Hz, 4H), 4.31 - 4.37 (m, 1H), 4.25 (d, J = 7.2 Hz, 2H), 4.17 (t, J = 7.1 Hz, 1H), 3.22 (ddd, J = 25.3, 14.6, 7.3 Hz, 2H). ^{13}C NMR (125 MHz, d_6-DMSO) δ 172.88, 159.70, 156.45, 154.42, 152.39, 147.40, 144.18, 141.16, 134.72, 131.38, 131.32, 130.15, 129.58, 128.08, 127.48, 126.94, 125.62, 125.38, 120.55, 117.05, 116.74, 115.09, 108.94, 67.14, 66.23, 53.00, 47.04, 33.01. ^{31}P NMR (121 MHz, d_6-DMSO) δ -6.70. IT-TOF: m/z [M-H] $^{-}$ calcd: 820.154, found: 820.055.



1

(2*S*)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(7-(hydroxy(2-nitrobenzyloxy)phosphoryloxy)-2-oxo-2H-chromen-4-yl)propanoic acid (**1**). Prepared according to procedures for the preparation of comopound **2** using compound **1-2** (18 mg, 22 µmol), triethylamine and thiophenol (500 µmol each). The product was purified by preparative HPLC (9.8 mg, 65%).^{1}H NMR (500 MHz, d_6-DMSO) δ 8.08 (d, J = 8.2 Hz, 1H), 7.86 (t, J = 6.8 Hz, 2H), 7.79 - 7.81 (m, 1H), 7.70 - 7.77 (m, 2H), 7.60 - 7.64 (m, 2H), 7.52 - 7.59 (m, 1H), 7.39 (t, J = 6.9 Hz, 2H), 7.28 (dd, J = 15.0, 7.5 Hz, 2H), 7.20 - 7.21 (m, 2H), 6.33 (s, 1H), 5.41 (d, J = 7.8 Hz, 2H), 4.30 - 4.36 (m, 1H), 4.23 - 4.26 (m, 2H), 4.15 - 4.20 (m, 1H), 3.21 (ddd, J = 25.3, 14.6, 7.3 Hz, 2H). ^{13}C NMR (75 MHz, d_6-DMSO) δ 172.45, 159.47, 155.93, 153.98, 152.08, 146.73, 143.61, 140.63, 134.09, 132.63, 132.52, 128.97, 128.65, 127.58, 126.99, 125.94, 125.06, 124.68, 120.05, 116.62, 114.90, 113.75, 107.91, 65.71, 64.87, 52.51, 46.51, 32.52. ^{31}P NMR (121 MHz, d_6-DMSO) δ -6.59. IT-TOF: m/z $[M+H]^+$ calcd: 687.138, found: 687.106. m/z $[M-H]^-$ calcd: 685.122, found: 685.088.

3. Procedure and LC-MS profiles of functionalized peptides



Scheme S2. Reaction conditions: **a**) 20 % piperidine in DMF; **b**) Fmoc-protected amino acid or acid (Palmitic acid), HOBT, HBTU, DIEA, DMF; **c**) compound **11**, HOAT, HATU, DIEA, DMF; **d**) 2% DBU in DMF; **e**) acetic anhydride, DIEA, DCM; **f**) 1% TFA in DCM; **g**) TFA/TIS/H_2O (95:2.5:2.5).

Procedures for condition **a**

The Fmoc-protected amino-functionalized resin was treated with 20% piperidine in DMF for 20 min at room temperature. After that, the resin was washed with DMF (3 ×), DCM (3 ×), DMF (3 ×) and DCM (3 ×).

Procedures for condition **b**

The Fmoc-protected amino acid or palmitic acid (4 equiv) was pre-activated using HOBT (4 equiv), HBTU (4 equiv), DIEA (8 equiv) in DMF for 10 min. Then the resin was put into the solution and shaken for 4 h or overnight. Next, the resin was washed with DMF (3 ×), DCM (3 ×) and DMF (3 ×).

Procedures for condition **c**

The Fmoc-protected unnatural amino acid, compound **11** (4 equiv) was pre-activated using HOAT (4 equiv), HATU (4 equiv), DIEA (8 equiv) in DMF for 10 min. Then the resin was put into the solution and shaken for 4 h or overnight. Next, the resin was washed with DMF (3 ×), DCM (3 ×) and DMF (3 ×).

Procedures for condition **d**

The Fmoc-protected amino-functionalized resin was treated with 2% DBU in DMF for 20 min at room temperature. After that, the resin was washed with DMF (3 ×), DCM (3 ×) and DMF (3 ×). If next step is using DCM as solvent, then the resin was washed with DCM (3 ×) again.

Procedures for condition **e**

The resin was swelled in DCM and washed with DCM. Then DIEA (10 equiv) was added to the reactor, followed by addition of acetic anhydride (5 equiv). The reaction was shaken for 2 h. Then the resin was washed with DMF (3 ×), DCM (3 ×), DMF (3 ×), MeOH (3 ×), and dried *in vacuo*.

Procedures for condition **f**

The resin was swelled in DCM and washed with DCM. Then 1% TFA in DCM was added to the reactor. The reaction was shaken for 30 min, then washed with DMF (3 ×), DCM (3 ×) and DMF (3 ×).

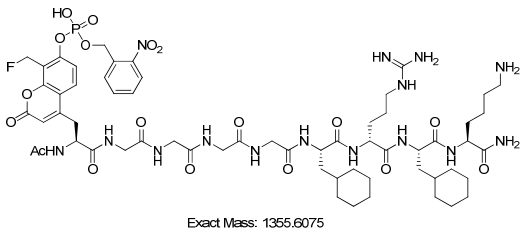
Procedures for condition **g**

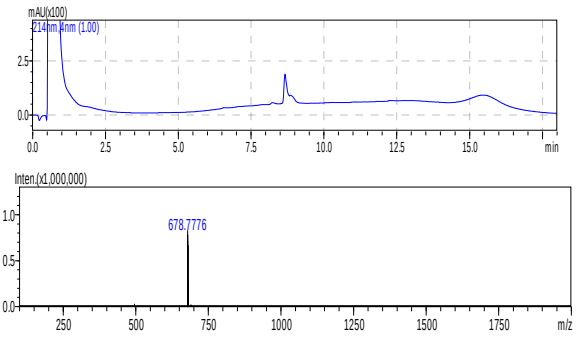
A cleavage cocktail TFA/TIS/DCM (95:2.5:2.5) was added to the resin and the reaction was stirred for 2 h. Subsequently, the resin was filtered off, followed by removal of the solvent *in vacuo*. The resulting residue was precipitated with cold diethyl ether and purified by preparative HPLC.

Mitochondrion probe (**pMito**)

Ac-X-GGGGF$_x$rF$_x$K-NH$_2$

IT-TOF-MS: Calcd: [M+2H]/2 = 678.804, found: 678.778

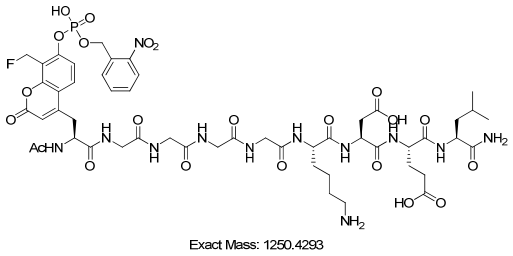


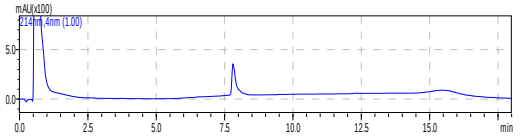


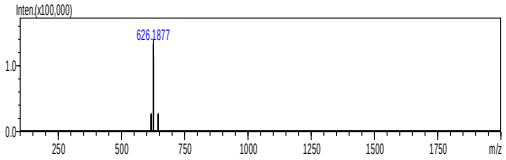
Endoplasmic Reticulum (**pER**)

Ac-X-GGGGKDEL-NH_2

IT-TOF-MS: Calcd: [M+2H]/2 = 626.215, found: 626.187



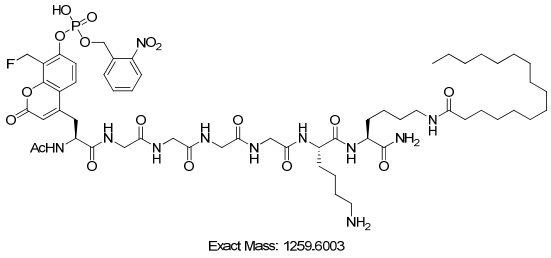


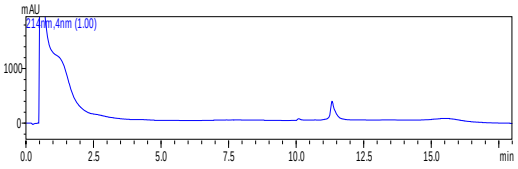


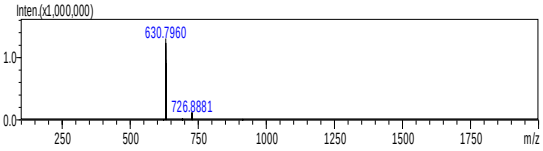
Membrane (**pMem**)

Ac-X-GGGGKK(palmitoyl)-NH_2

IT-TOF-MS: Calcd: [M+2H]/2 = 630.800, found: 630.796





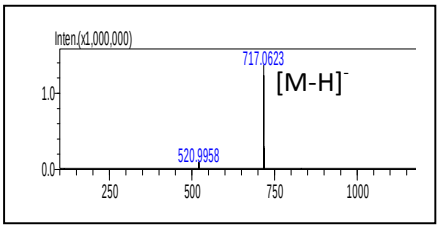


4. UV-initiated uncaging experiments

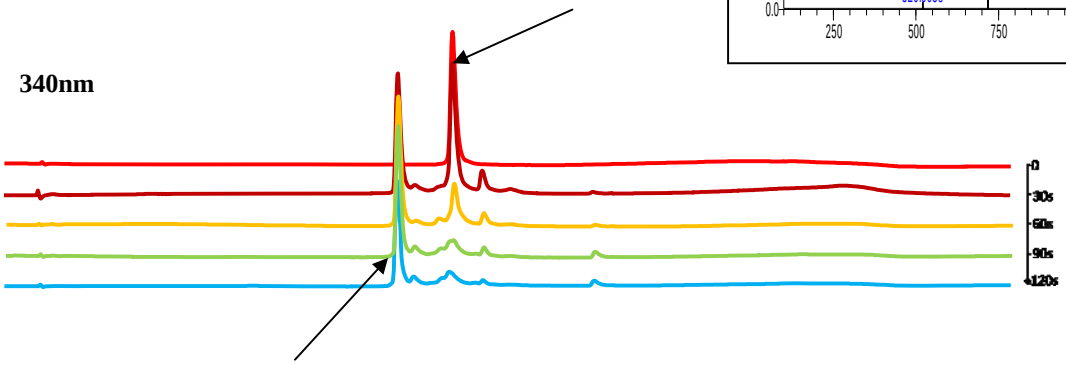
Compound **2** (50 μM) and **pER** peptide (50 μM), prepared in 25 mM Tris buffer with 50 mM NaCl at *p*H = 7.2, were photolyzed using a UVP CL-1000 Ultraviolet Crosslinker (500 μJ/cm^2 at 340 nm). The reaction was monitored by LCMS.

(A) Compound **2**

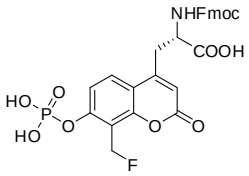
Before UV- irradiation



340nm

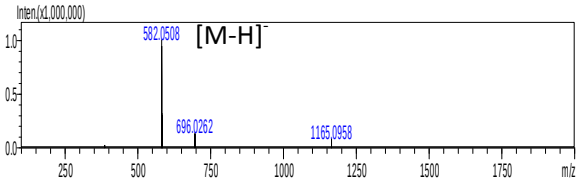


After UV-irradiation



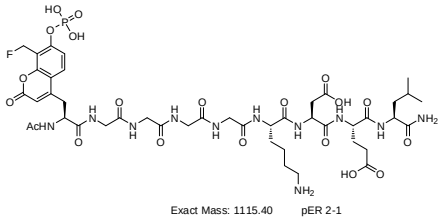
Exact Mass: 583.10

2'



(B) **pER** peptide

After UV- irradiation



Exact Mass: 1115.40 pER 2-1

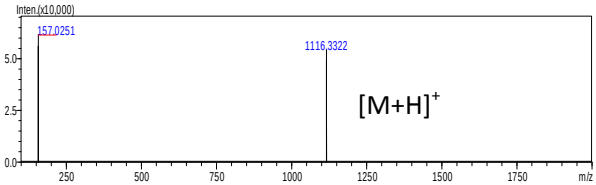


Fig. S1. (A) The LC profiles of **2** (50 μM) upon UV-irradiation for different lengths of time (0, 30 s, 60 s, 90 s and 120 s). All peaks were identified and confirmed based on LCMS results. (B) MS result of **pER** peptide (50 μM) upon UV-irradiation for 120 s, showing the complete removal of the "caged" group. The HPLC profiles are shown in Fig. 3a in the maintext.

5. Microplate experiments

a) Enzyme specificity. The microplate assays were performed in 384 wells. Compound **2** (20 μM final conc.) was first uncaged by UV-irradiation before being dispensed into 5 different wells. Subsequently, to these wells were added individually each of the following five enzymes (final 0.02 mg/mL) - including

recombinant PTP1B, a recombinant glycosidase, a recombinant Src (expressed and purified using the same protocol as reference 4), an esterase (Fluka, No. 46069) and trypsin (Fluka, No. 93610). The reactions were monitored continuously over 30 min using a BioTek multi-mode fluorescence microplate reader (λ_{ex} = 360 ± 10 nm; λ_{em} = 460 ± 10 nm). The result is shown in Figure S2. Only PTP1B produced significance fluorescence.

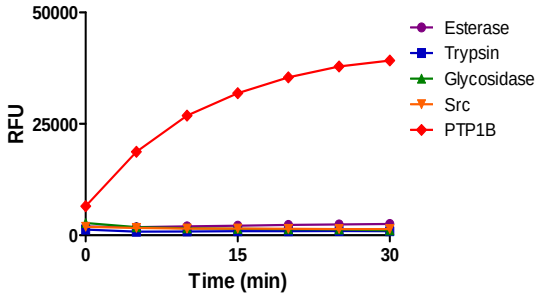


Fig. S2. Microplate-based enzymatic assay of PTP1B and four other enzymes with uncaged **2** as the enzyme substrate.

b) Dephophorylation reaction with uncaged compound 2. Compound **2** (4 µM in 25 mM Tris buffer, 100 mM NaCl , *p*H = 7.26; final volume is 25 µL), which were first UV-irradiated, or "uncaged", for different lengths of time. Subsequently, recombinant PTP1B (80 nM final conc.)[3] was added and the dephosphorylation reaction was monitored continuously over 2 h using a BioTek multi-mode fluorescence microplate reader (λ_{ex} = 360 ± 10 nm; λ_{em} = 460 ± 10 nm) (Fig. S3). Microplate experiments were also performed to compare the dephosphorylation of caged and uncaged **pER** peptide (Fig. 2a in the maintext). Briefly, HeLa cells were lysed using a buffer containing 50 mM Tris, 150 mM NaCl 0.02 % NP-40, at *p*H = 8.0. The total lysate was collected and its protein concentration was determined using Bradford assay. In each reaction, 15 µg (protein content) of the lysates was used, together with the **pER** peptide (4 µM final conc.), either non-irradiated or UV-irradiated (120 s), in an 25 µL final reaction volume. The reaction was then continuously monitored over 2 h in the BioTek microplate reader as above described. Results are shown in Fig. 3a in the maintext.

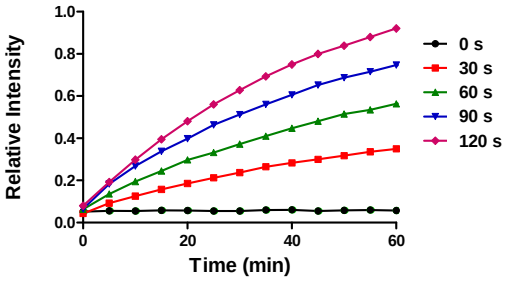


Fig. S3. Fluorescence measurements of **2**, upon UV-irradiation (for 0, 30, 60, 90, 120s), then treated with PTP1B. The measurements were taken under coumarin channels (λ_{ex} = 360 ± 10 nm; λ_{em} = 460 ± 10 nm).

c) Kinetic data of 1 and 2 with PTP1B. The assays were performed in Hepes buffer (25 mM Hepes, 0.05 M NaCl, 2.5 mM EDTA and 0.02% Triton X-100, *p*H 7.5). PTP1B was incubated with different dilutions of compounds **1** and **2** (final concentration: 250, 125, 62.5, 50, 25, 12.5, 6.25, 1.25 µM). The dephosphorylation reaction was monitored continuously over 2 h using a BioTek multi-mode fluorescence microplate reader (λ_{ex} = 360 ± 10 nm; λ_{em} = 460 ± 10 nm). Kinetic constants were computed by fitting the

data to the Michaelis-Menton equation using a non-linear regression via GraphPad Prism software. The value was taken in mean ± S.D. from duplicated experiments.

Table S1. Kinetic Data of **1** and **2** with PTP1B.

	K_M (mM)	k_{cat} (s^{-1})	K_{cat}/K_M ($mM^{-1}\cdot s^{-1}$)
Compound **1**	0.77 ± 0.20	11.8 ± 2.4	15.2 ± 0.2
Compound **2**	1.1 ± 0.4	8.8 ± 2.6	7.9 ± 0.1

d) Comparison of enzyme activities before and after probe reaction. The assays were performed in Hepes buffer (25 mM Hepes, 0.05 M NaCl, 2.5 mM EDTA and 0.02% Triton X-100, *p*H 7.5). PTP1B (2 μg/mL final conc.) was incubated with compound **1** or **2** (0.2 μM final conc.; 5 x of PTP1B), or H_2O (as a negative control). After 1 h incubation, DiFMUP (4 μM final conc., Invitrogen, No. D6567; 100 x of PTP1B) was then added. An identical set of experiments with PTP1B + compound **1/2** ONLY were carried out simultaneously as controls to account for any fluorescence produced by compound **1/2**. The dephosphorylation reaction was monitored continuously over 10 min using a BioTek multi-mode fluorescence microplate reader (λ_{ex} = 360 ± 10 nm; λ_{em} = 460 ± 10 nm). The kinetic curve was given after subtracting the background fluorescence from the dephosphorylation reaction of PTP1B with compound **1**/**2** ONLY. Results indicated that pre-incubation of PTP1B with either compound **1** or **2** didn't cause any significant inhibition of PTP1B activity.

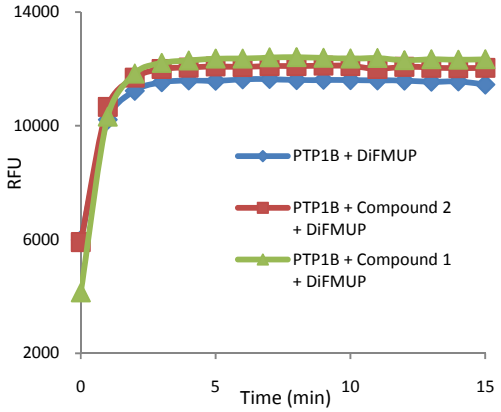


Fig. S4. Comparison of PTP1B's activity, with and without compound **1** and **2** labeling, indicates that no significant decrease of activity was observed.

6. Gel-based labeling experiments

a) Competitive gel analysis. Since the coumarin fluorescence in the probes could not be detected with our fluorescence gel scanner, we first used an indirect competition assay by labelling pre-treated PTP1B (with either different amounts of uncaged **2**, or the same amount of uncaged **2** incubated for different lengths of time) with a previously reported PTP1B peptide probe **P3** (Rhodamine-GGKAVDGXVKPQ-NH_2; X = 2-**FMPT**[4]). We also used uncaged **1** as a control for comparison (Fig. S5); both dose- and time-dependent inhibitions of labeled PTP1B-**P3** band were observed, but only at a very high concentration of the compound. In addition, similar inhibitory effect was observed for both compounds **1** and **2**. These results indicated that although some amount of uncaged **2** might have competed for covalent labelling of PTP1B with **P3** to a certain extent, the majority of it failed to do so (likely as a result of self-quenching).

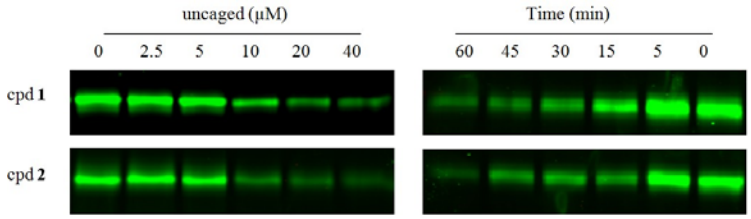


Fig. S5. Covalent labeling of PTP1B with **P3**, after PTP1B was pre-incubated with uncaged **1** or **2**. (Left) Dose-dependent experiments: different concentrations of compound **1** and **2** (upon 300s UV-irradiation) were incubated with PTP1B (1 μg) for 1.5 h in 25 mM Tris buffer (containing 50 mM NaCl at *p*H = 7.26). Subsequently, **P3** (final conc.: 20 μM) was added to the mixture and the reaction was further incubated for 1 h. Finally, the reaction was quenched by addition of 6 × SDS loading dye followed by heating at 95 °C for 10 min, then resolved on a 12% SDS-PAGE gel and visualized by in-gel fluorescence scanning with Typhoon 9200 fluorescence gel scanner. (Right). Time-dependent experiments: 80 μM of compound **1** and **2** (upon 300s UV-irradiation) were incubated with PTP1B (1 μg) for different lengths of time (0. 5, 15, 30, 45, 60 min) in a 25 mM Tris buffer (containing 50 mM NaCl at *p*H = 7.26). Subsequently, **P3** (final conc.: 20 μM) was added to the mixture and the reaction was further incubated for 15 min. Finally, the reaction was quenched by addition of 6 × SDS loading dye followed by heating at 95°C for 10 min, then resolved on a 12% SDS-PAGE gel and visualized by in-gel fluorescence scanning.

b) Direct labeling experiments with bacterial lysates over-expressing PTP1B. *Escherichia coli* BL21 DE3 cell lysates over-expressed with PTP1B were prepared in 50 mM Tris buffer, 150 mM NaCl , 1% Triton X-100 *p*H = 7.26.[3,4] Uncaged compound **1** (500 μM) and compound **2** (1 mM) were each incubated with 30 μg of the cell lysate (protein content) for 2 h. Then the reaction was quenched by addition of SDS loading dye followed by heating at 95 °C for 10 min, then resolved on a 10% SDS-PAGE gel and photographed under a UV transilluminator using CHEMI GENIUS Bio imaging system (Syngene). Results are shown in Fig. 3b.

7. Mass spectrometry

25 μg of recombinant PTP1B was labeled by uncaged compound **2** (1:10 PTP1B/**2** molar ratio) overnight at room temperature. This sample, together with unlabelled PTP1B as a control, was analyzed with a micromass Q-TOF 2 tandem mass spectrometer (Fig. S6); results indicated most of protein still retained its original molecular (MW = 39344), therefore was mostly not covalently modified.

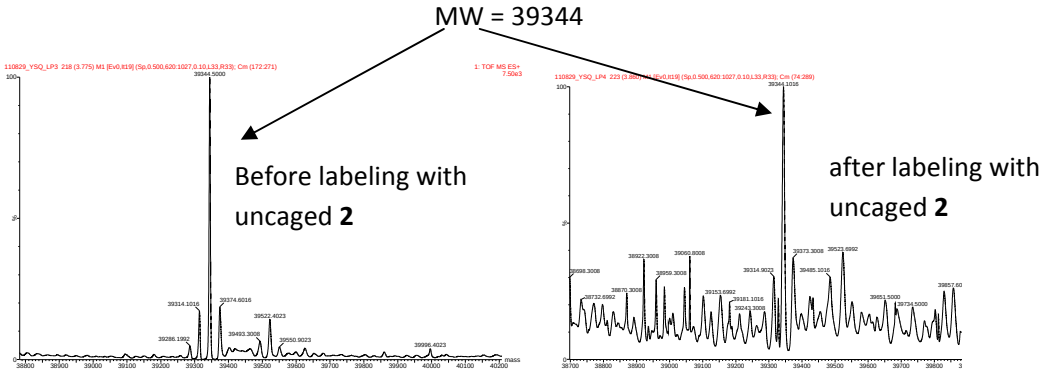


Fig. S6. MS profiling of PTP1B before and after incubation with compound **2**.

8. Microscopy experiments

HeLa cells were seeded in glass-bottom dishes (CELLview™, Cat. No. 627861) and grown till ~70% confluence. The cells were incubated with 20 μM of the peptide (either **pMito**, **pMem** or **pER**) in fresh Dulbecco's Modified Eagle Medium (DMEM). The cells were further incubated (1.5 h for **pER** and **pMito**, 30 min for **pMem**). Then the medium was refreshed. Upon UV-irradiation for 1.2 min, the cells were

further incubated for 1.5 h. Next, 0.25 μg/mL of a corresponding organelle tracker was added (ER-Tracker™: Red glibenclamide BODIPY® TR; CellMask™ Plasma Membrane Stains C10045; MitoTracker®: Red CMXRos dye; all from Invitrogen). Upon further incubation according to the vendor's protocols, the cells were washed with PBS (3 ×), then fixed with 3.7 % formaldehyde for 15 min at 37 °C. The stained cells were finally imaged with a Leica TCS SP5X confocal microscope system and processed with the Leica application suite advanced fluorescence (LAS AF). Excitation channel: peptide channel (405 nm Diode laser); tracker channel (white laser, 590 nm for ER-Tracker, 543 nm for CellMask, 554 nm for Mito-Tracker, respectively). Fig. S7 is the fluorescence images HeLa cells treated with different non-irradiated peptide probes (negative controls). Fig. 4 in the maintext shows the fluorescence images of HeLa cells treated with the different uncaged peptide probes.

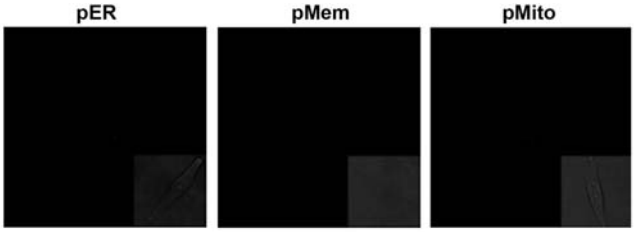


Fig. S7. Fluorescence images of HeLa cells treated with different non-irradiated peptide probes (20 μM, 30-90 min incubation). Without UV-irradiation, the cells were directly imaged. No fluorescence was observed in any of the images, indicating the temporal control of our "caging" strategy.

9. FACS experiments

Flow Cytometry analysis was performed on a BD LSR II Bioanalyzer equipped with a 355 nm UV air-cooled laser. The data was analyzed using BD FACSDiva software. Cells were seeded in Nunc™ (Cat. No. 153066) 3.5 cm dishes and grown till ~90% confluence. Two sets of cells were prepared. One set was UV-irradiated for 1 min. After collecting the cells by centrifugation, fresh PBS buffer was added. Propidium iodide (PI, 2 μM final conc.) was added and the cells were stained for 10 min at room temperature. Then the cells were rinsed with 1 × PBS and directly analyzed by flow Cytometry (Fig. S8); results indicated that UV-treated cells and untreated cells didn't appear to show any difference. Manual inspection of the cells also showed 1-min UV irradiation on cells didn't appear to have any noticeable effect (data not shown).

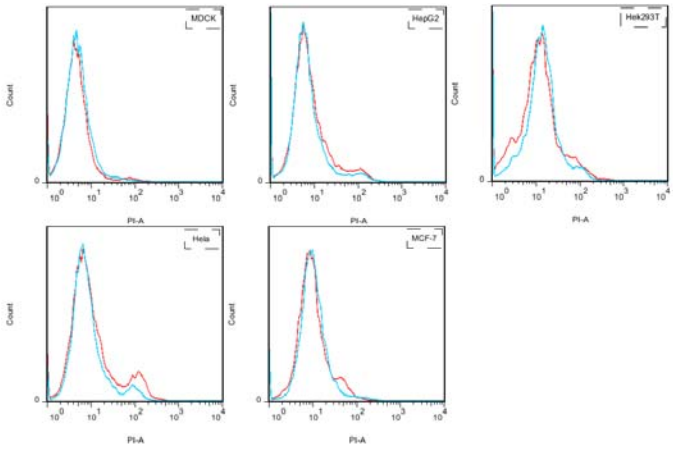
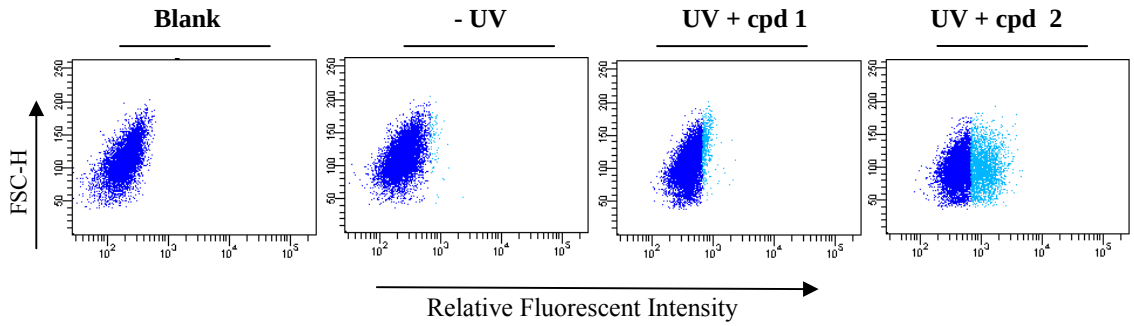


Fig. S8. Histogram of FACS analysis of cells upon 1-min UV irradiation. All five cell line (MDCK, HEK293T, HepG2, Hela, MCF-7) were tested. Red line: untreated cells stained by PI; Blue line: UV-irradiated cells stained by PI.

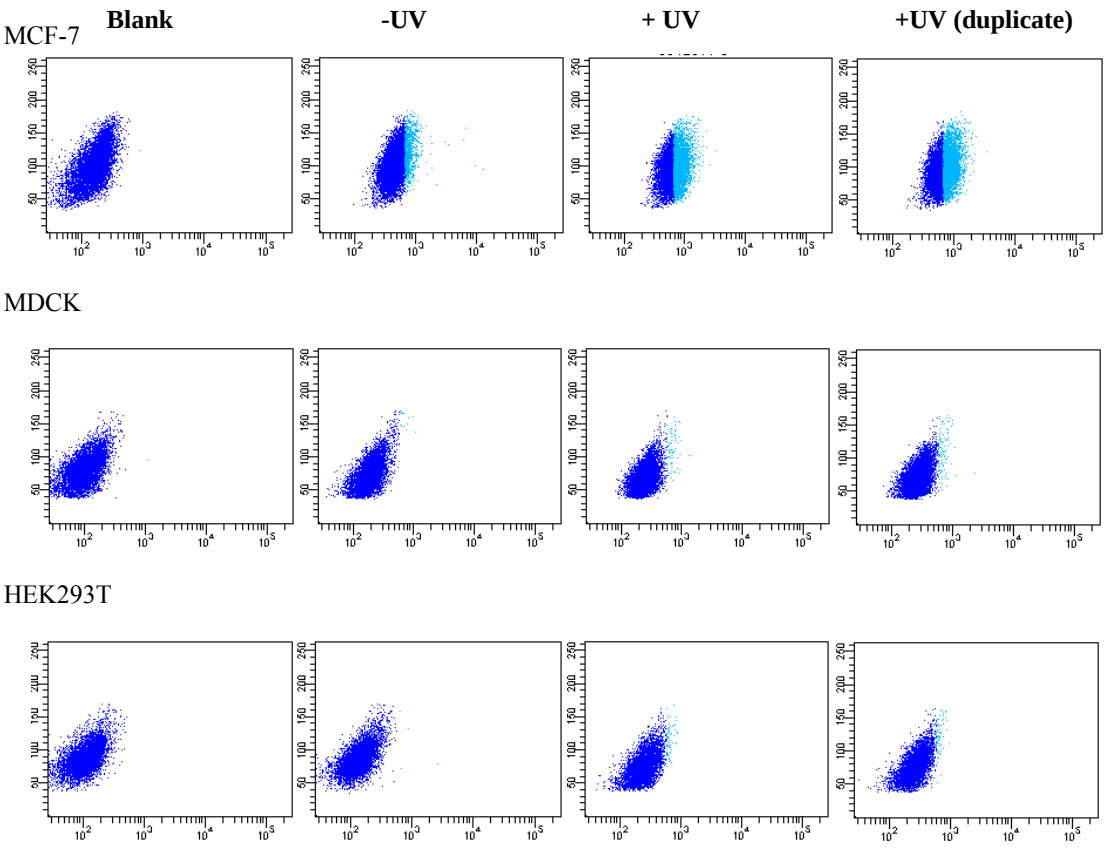
Cells were added different amounts of compound **2** (or compound **1** – as a control). Incubation was continued further for 30 min. Cells were subsequently UV-irradiated for 1 min then further incubated for 2 h. Upon washing with PBS (2 ×), the cells were detached and finally fixed with 1% paraformaldehyde in

PBS before being subjected to FACS analysis (Fig. S9a); results clearly indicated that **2** was able to strongly and permanently "stain" the cells thru its self-immobilizing property. After initial washing, no further leakage of **2** from the cells was observed even with repeated washing of cells. On the other hand, cells stained with **1** (the non self-immobilizing analog of **2**) showed much weaker fluorescence, which rapidly disappeared upon repeated washing (not shown). For FACS experiments with normal and cancer cells (Fig. 4b), the cells were seeded in Nunc™ (Cat. No. 153066) 3.5 cm dishes and grown till ~90% confluence. Next, compound **2** (80 μM) was added and the cells were incubated further for 30 min. Upon UV-irradiation for 1 min, cells were further incubated for 2 h, then washed with PBS (2 ×), detached from dishes, washed (with PBS), fixed with 1% paraformaldehyde in PBS for 10 min, then analyzed by FACS (Fig. S9b).

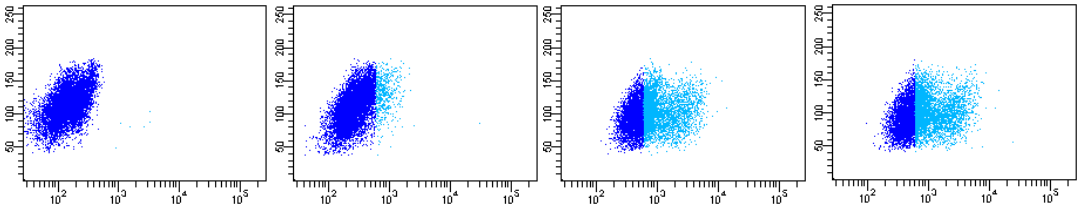
(A)



(B)



HeLa



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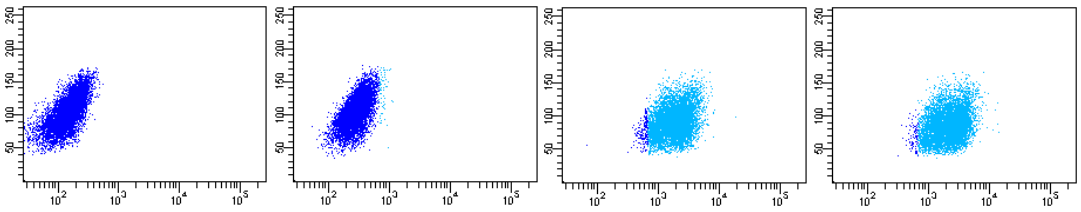
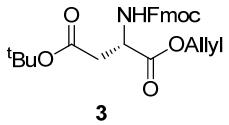


Fig. S9. (A) Density-plot of FACS analysis of HeLa cells treated with 80 μM of **1** and **2**. Blank: only cells (without any added compounds). "– UV": cells treated with compound **2** but without UV-irradiation. UV + cpd **1/2**: cells treated with compound **1**/**2** followed by UV-uirradiation. The boundary between two colors corresponds to the maximum fluorescence in the blank sample. (B) Density-plot of FACS analysis of five cell lines treated with 80 μM of compound **2**. Blank: only cells (without any added compounds). The UV-irradiated samples were performed in duplicate (two right panels). The boundary between two colors corresponds to the maximum fluorescence in the blank sample. The data were extrapolated and summarized in Fig. 3d in the maintext.

10. References

1. S. Mitra, A. M. Barrio, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 5142-5145
2. R. M. Naik, V. M. Thakor, *J. Org. Chem.*, 1957, **22**, 1626-1629
3. H. Sun, L. P. Tan, L. Gao, S. Q. Yao, *Chem. Commun.*, 2009, 677-679
4. K. A. Kalesh, L. P. Tan, K. Liu, L. Gao, J. Wang, S. Q. Yao, *Chem. Commun.*, 2010, **46**, 589-591

11. ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra



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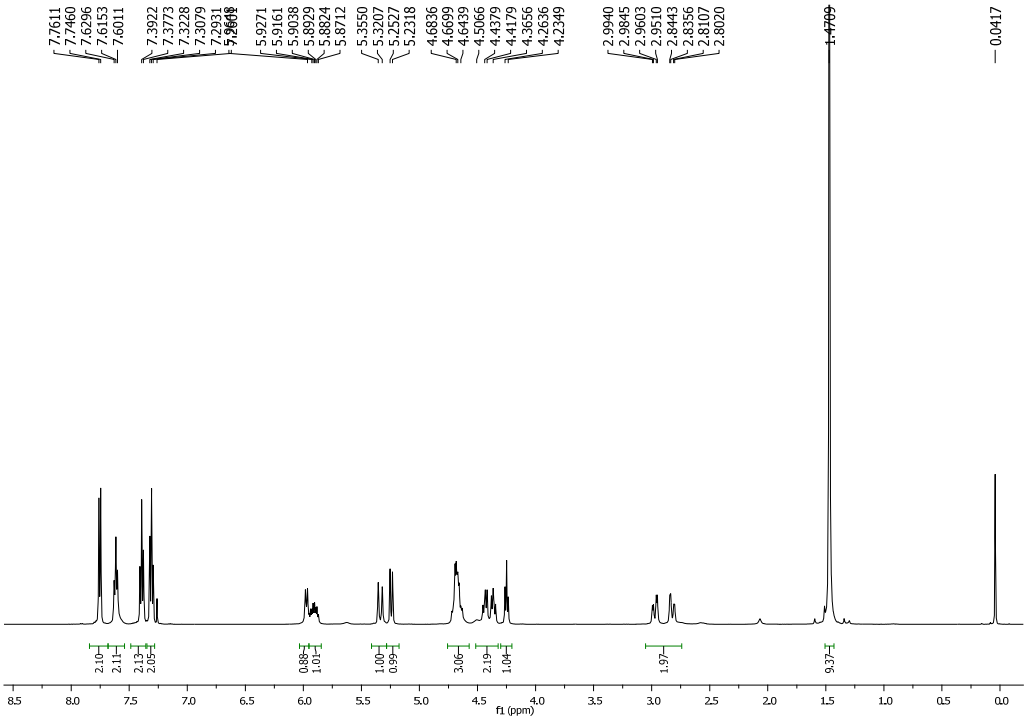

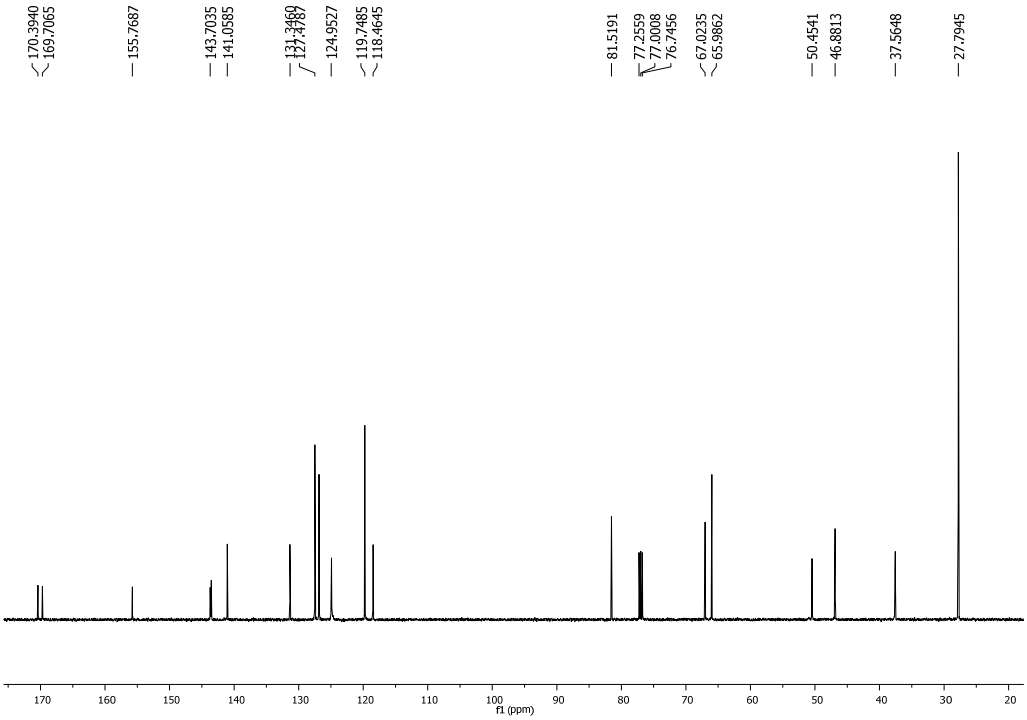

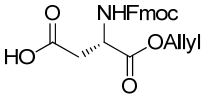
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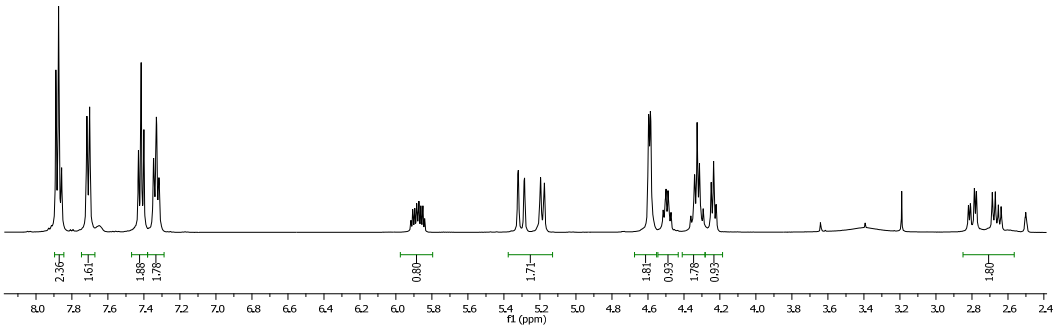
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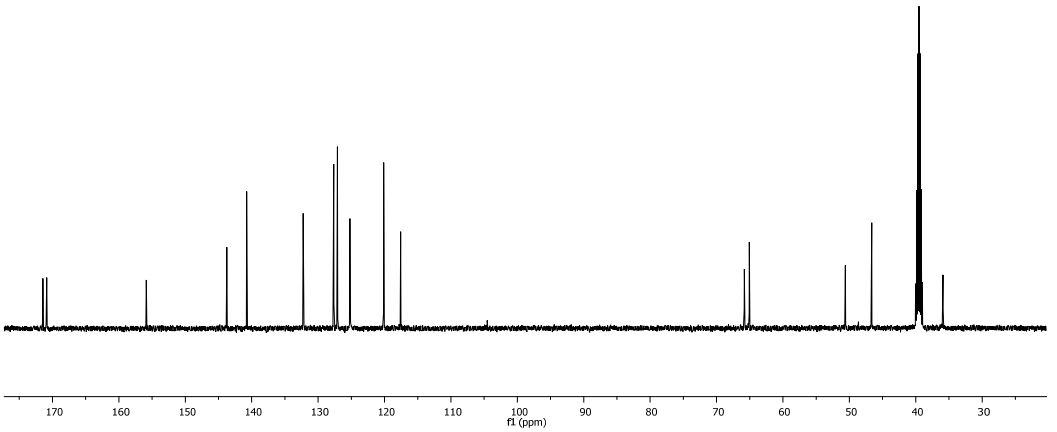
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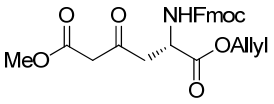
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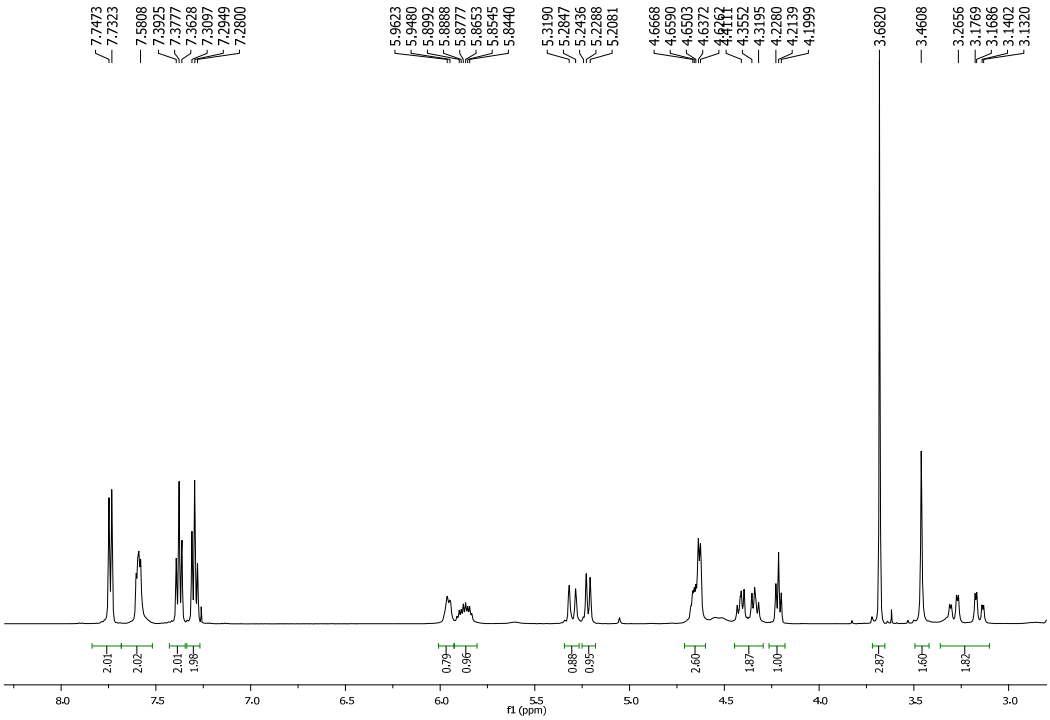
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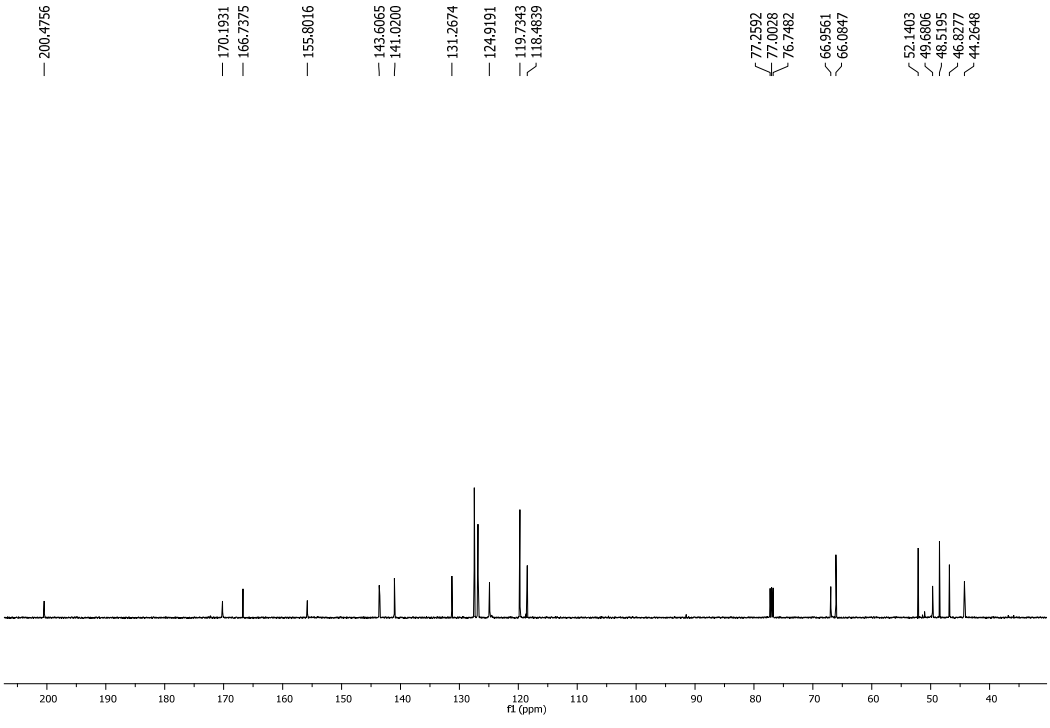
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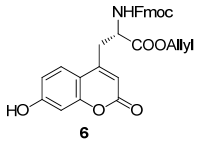
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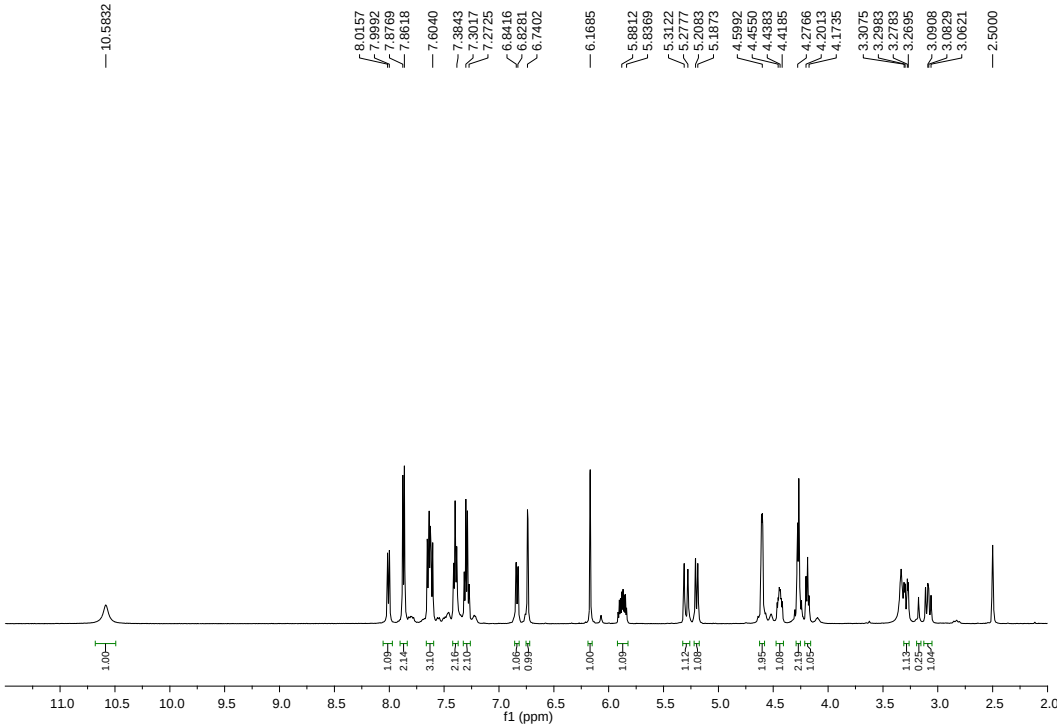
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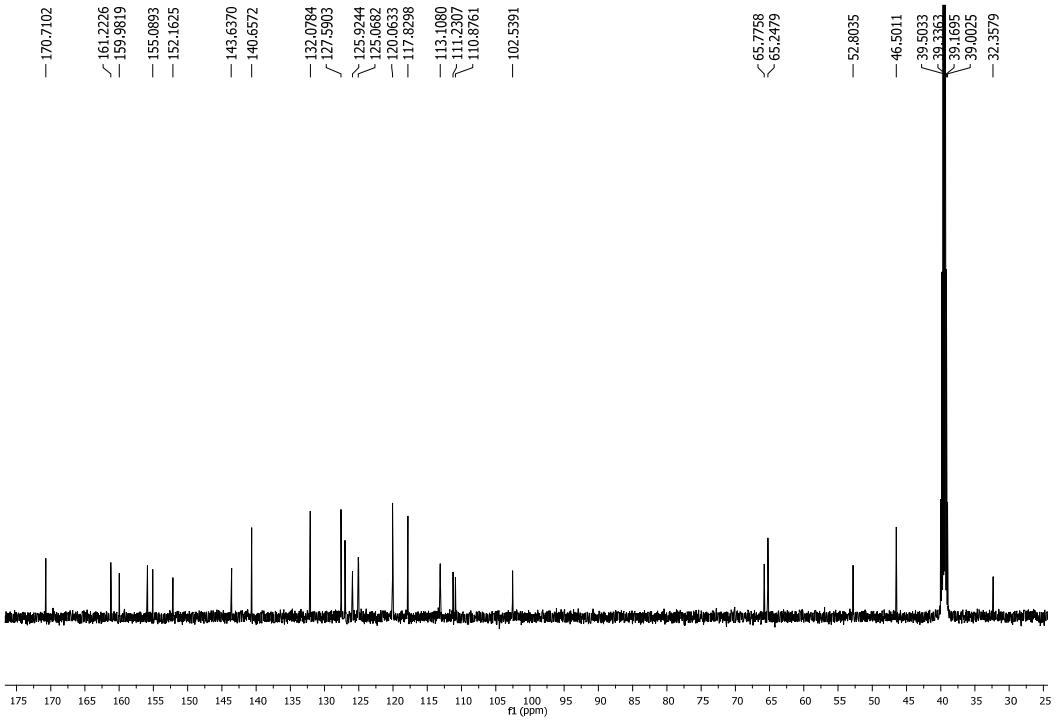
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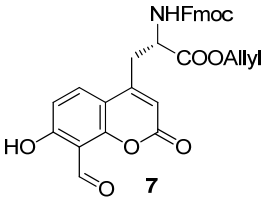
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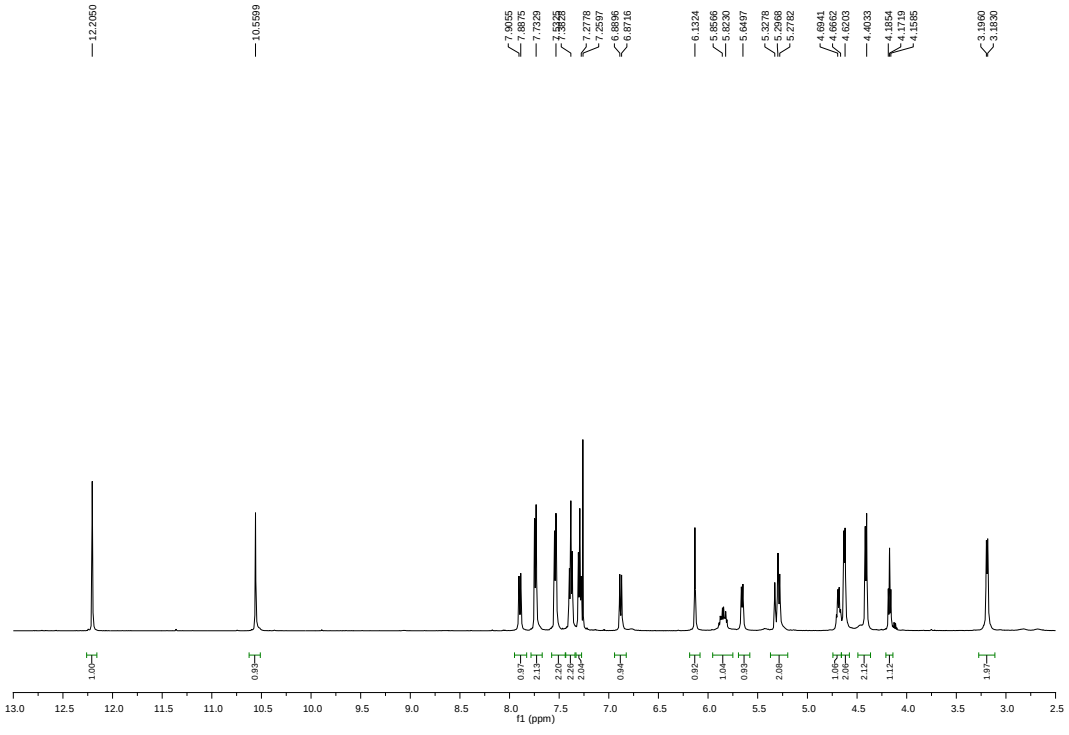
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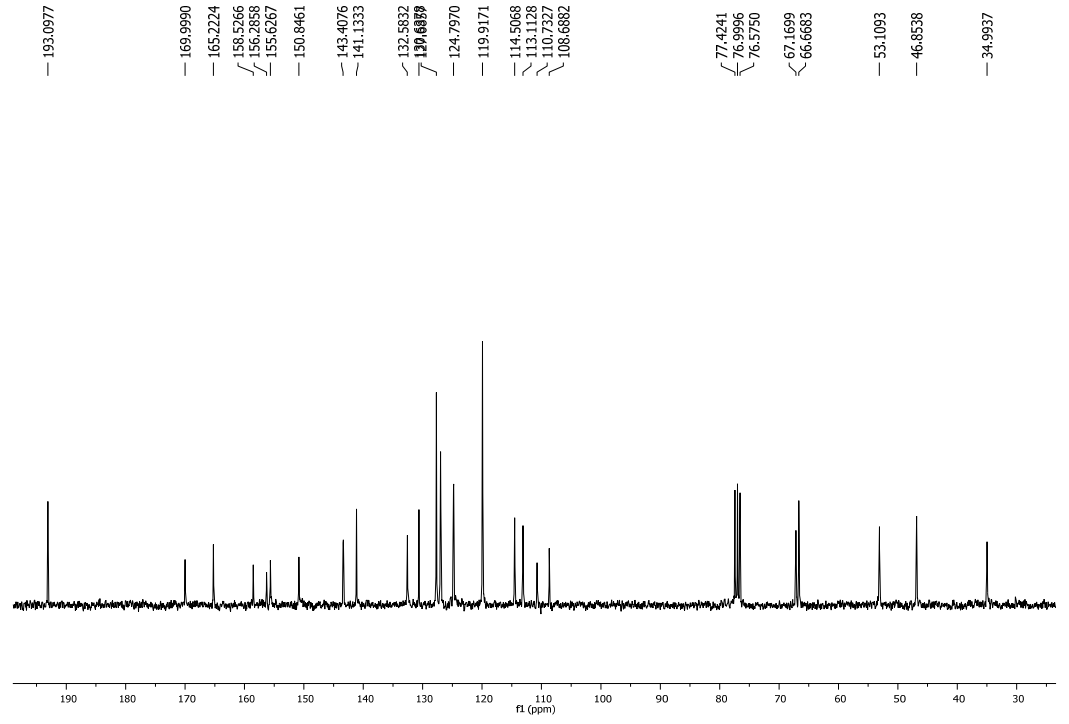
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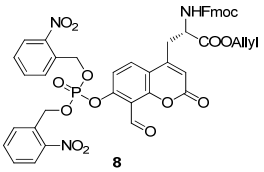
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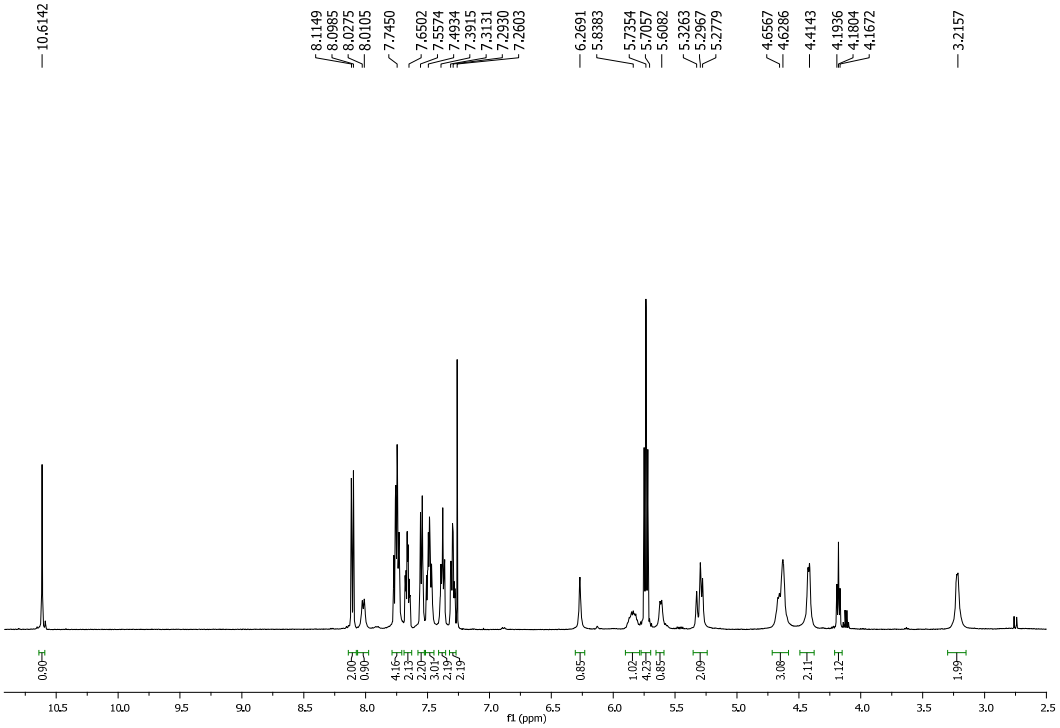
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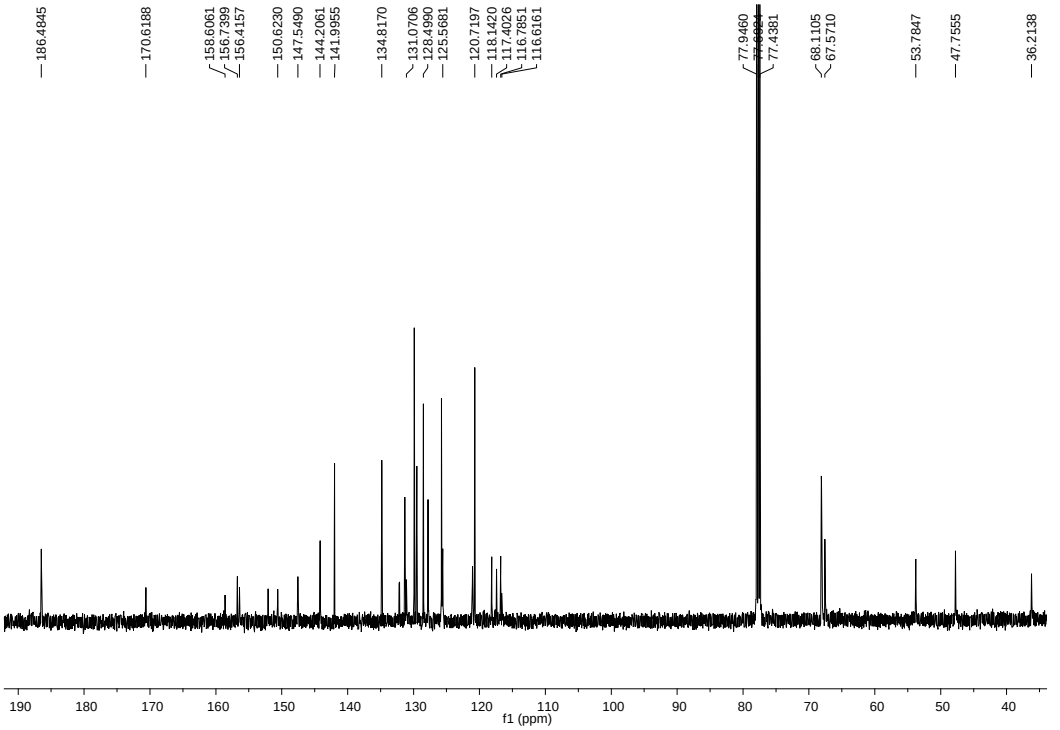
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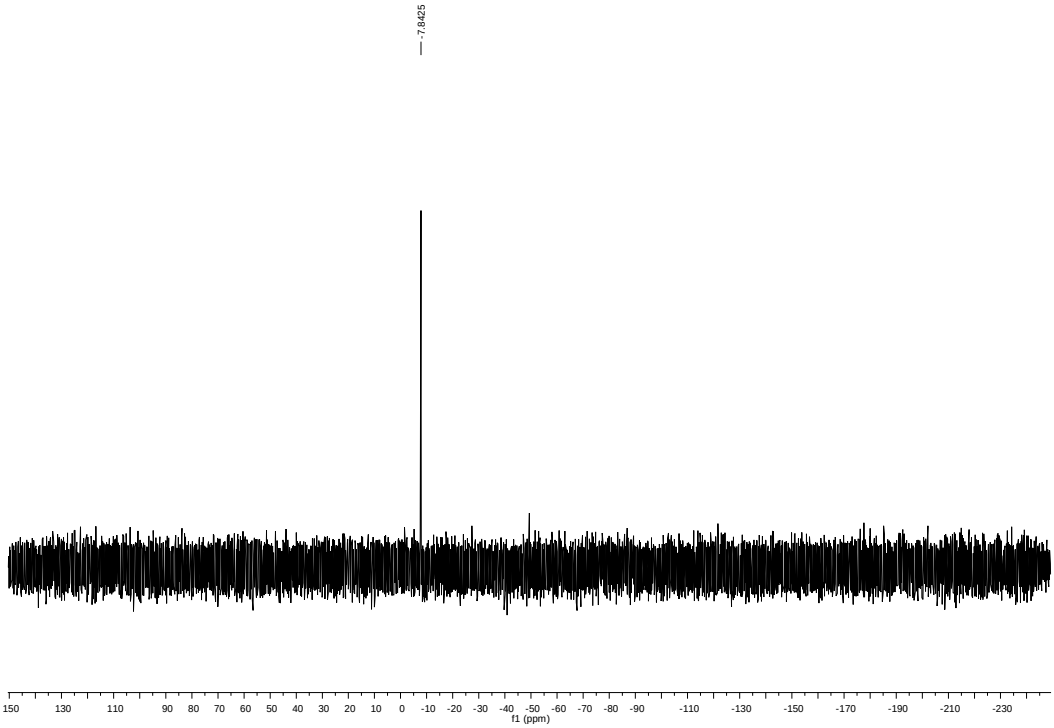

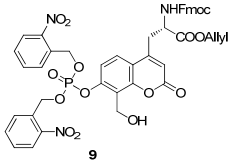

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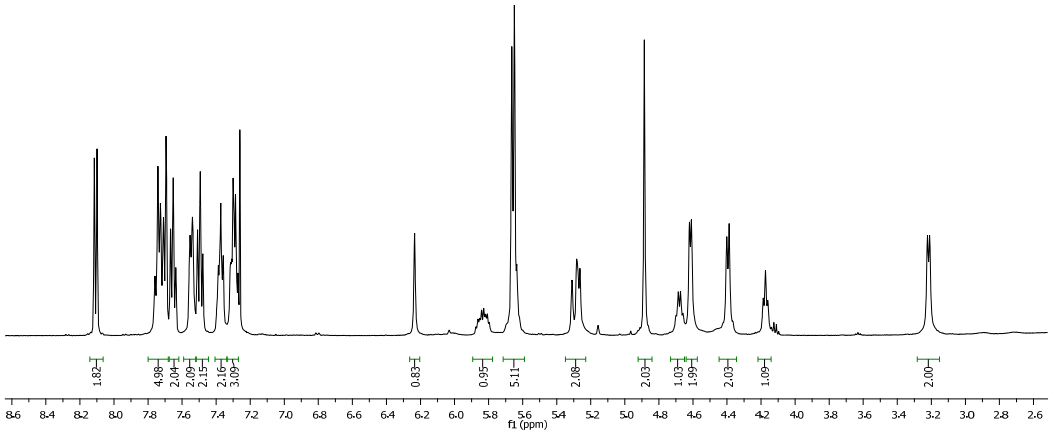

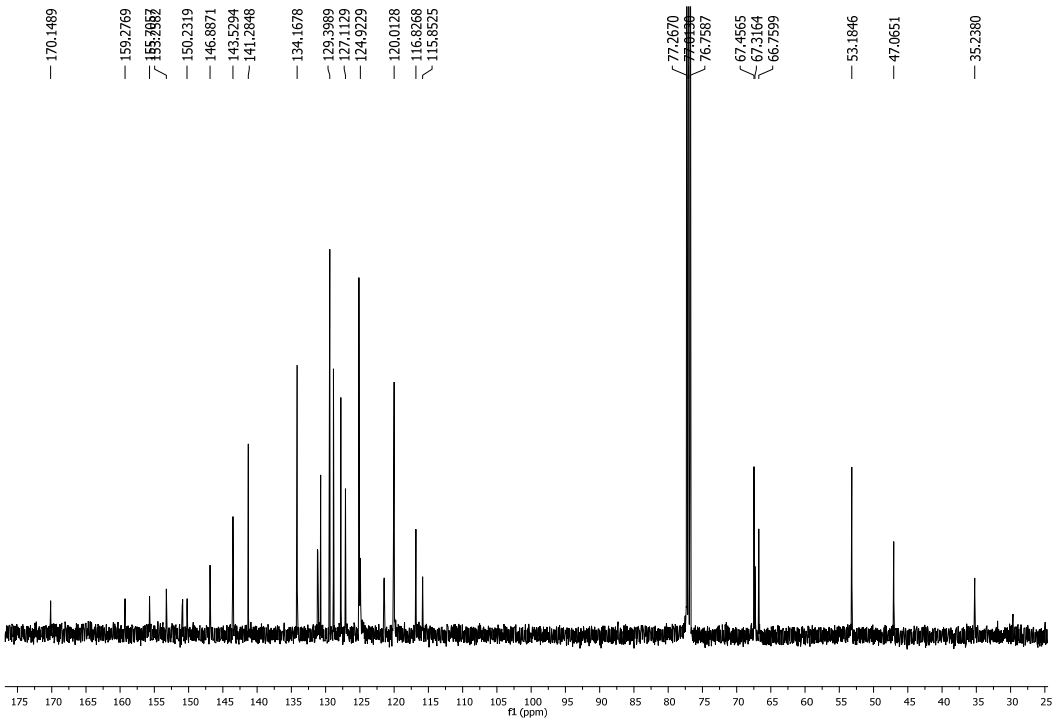
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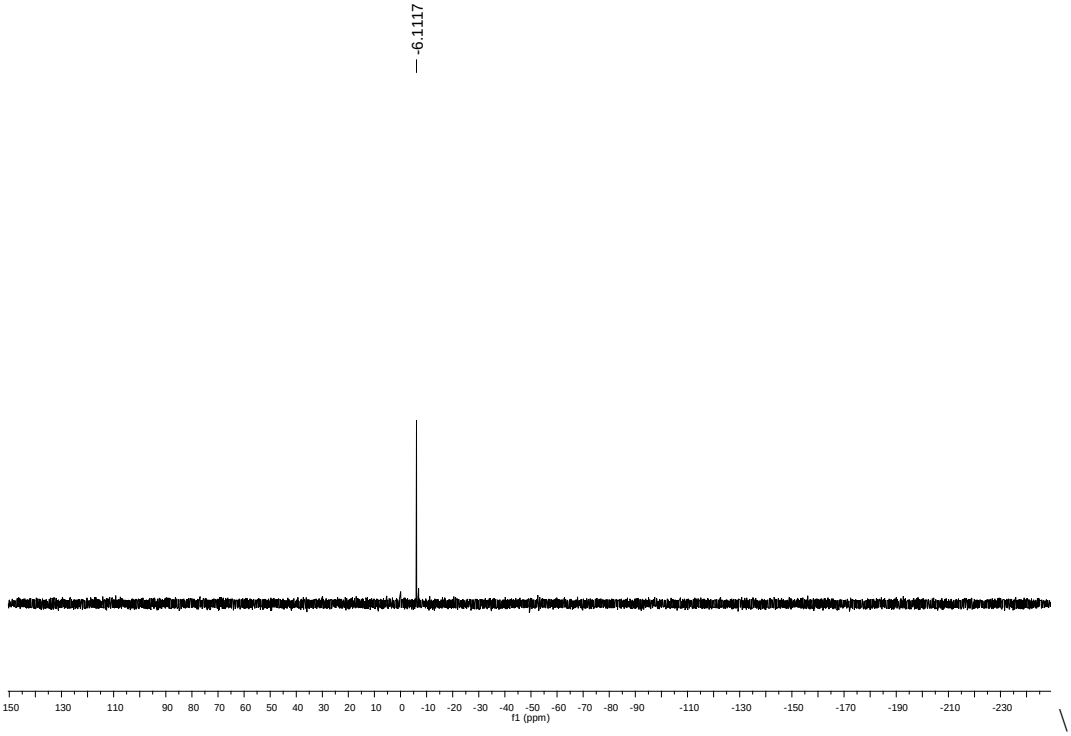
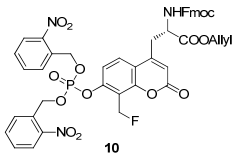
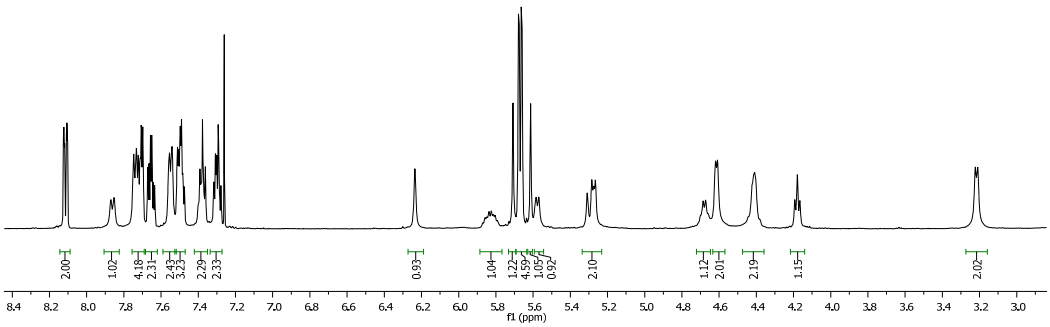
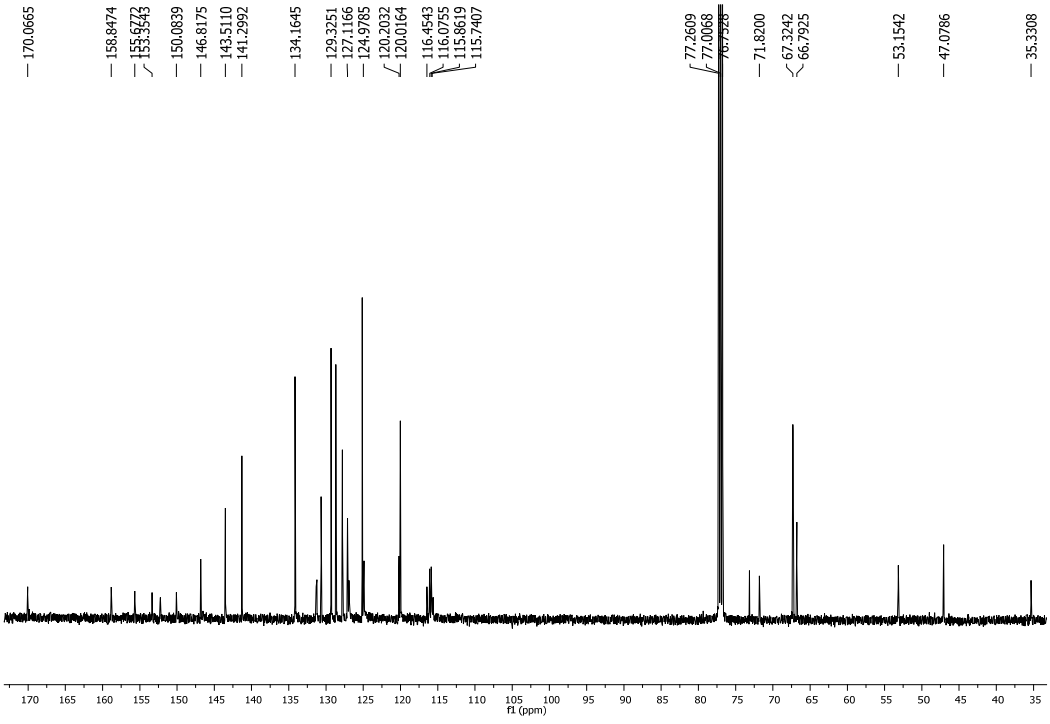

-6.1117
f1 (ppm)

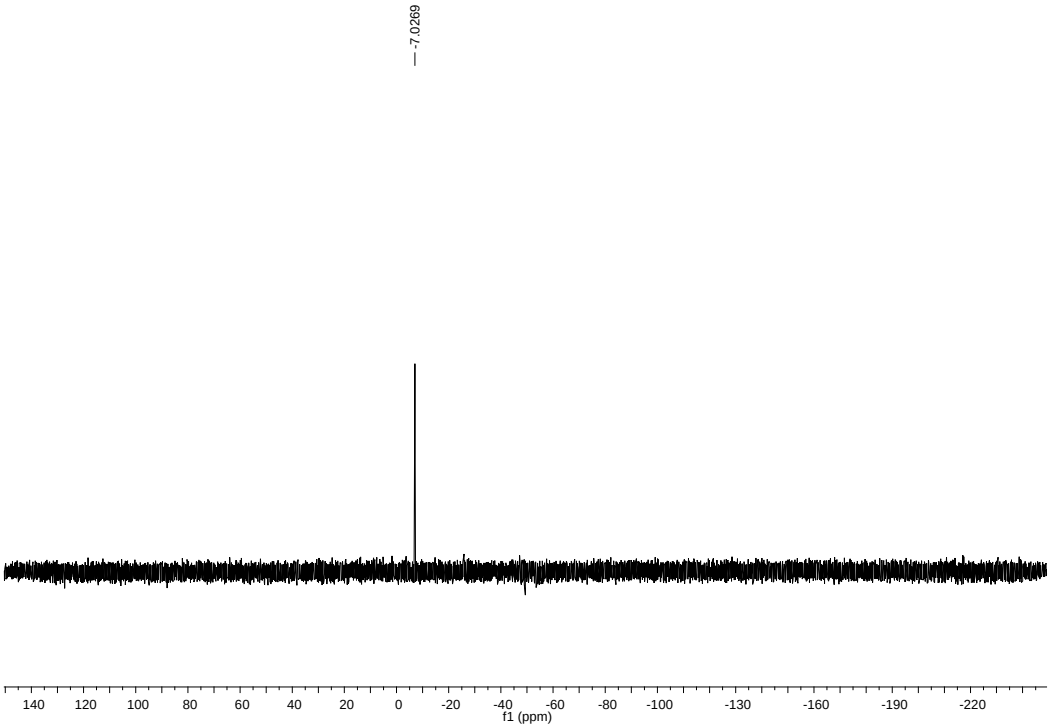

NO2
NHFmoc
COOAllyl
O
P
O
O
O
O
O
O
F
NO2

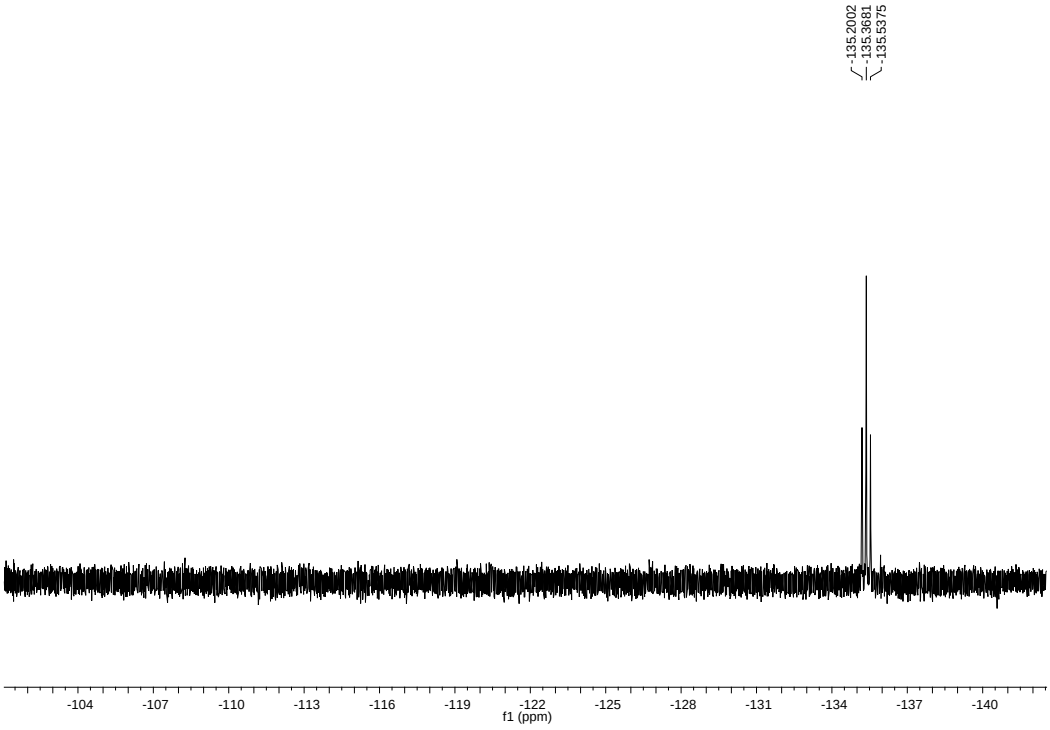
10

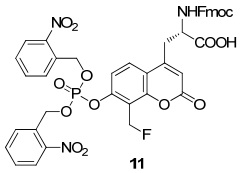

8.1227
8.1063
7.8699
7.8529
7.7067
7.6348
7.5408
7.5065
7.4970
7.4836
7.3993
7.3849
7.3621
7.3080
7.2931
7.2603
6.2353
5.8248
5.7079
5.6740
5.6587
5.6131
5.5695
5.3086
5.2846
5.2743
5.2651
4.6863
4.6725
4.6193
4.6088
4.4093
4.1928
4.1794
4.1660
3.2226
3.2103


2.00
1.02
4.18
2.31
2.43
3.25
2.29
2.33
0.93
1.04
1.22
4.59
1.05
0.92
2.10
1.12
2.01
2.19
1.15
2.02
f1 (ppm)

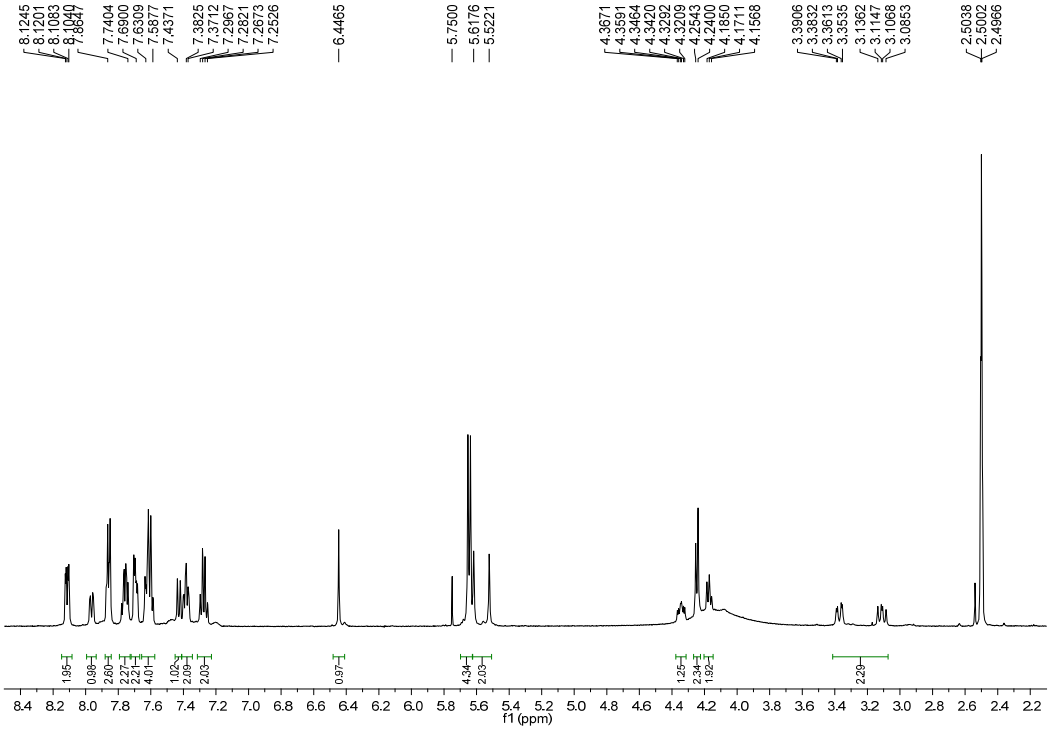

170.0665
158.8474
155.6772
153.3543
150.0839
146.8175
143.5110
141.2992
134.1645
129.3251
127.1166
124.9785
120.2032
120.0164
116.4543
116.0755
115.8619
115.7407
77.2609
77.0068
76.7528
71.8200
67.3242
66.7925
53.1542
47.0786
35.3308
f1 (ppm)

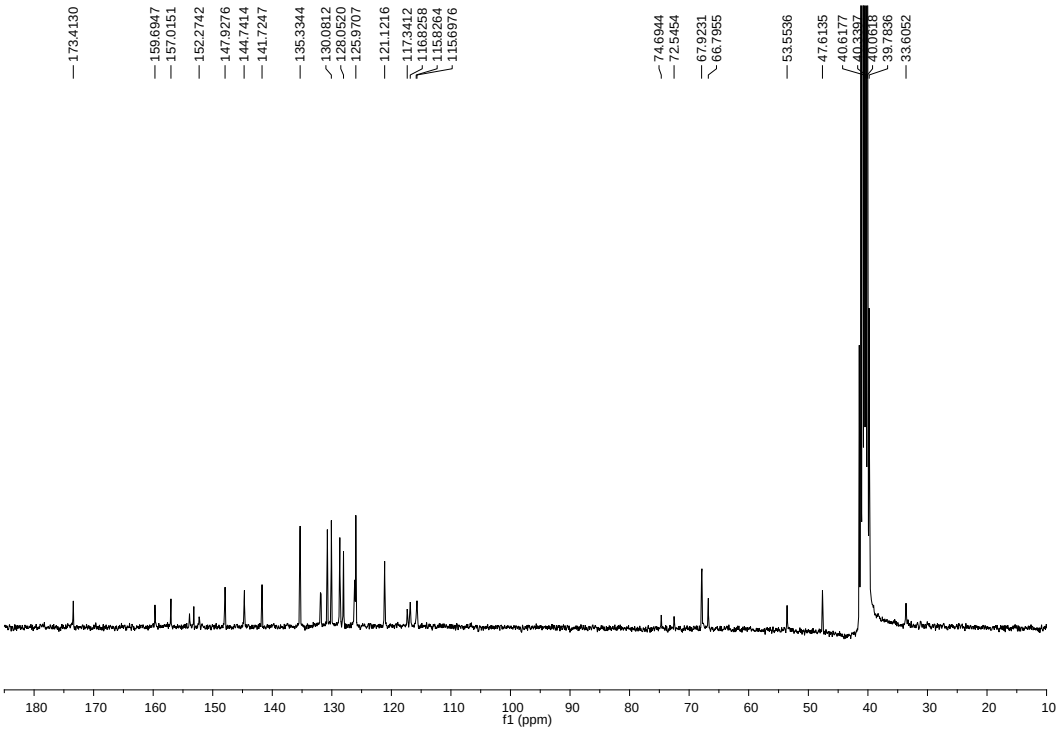

-7.0269
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-130
-160
-190
-220
f1 (ppm)

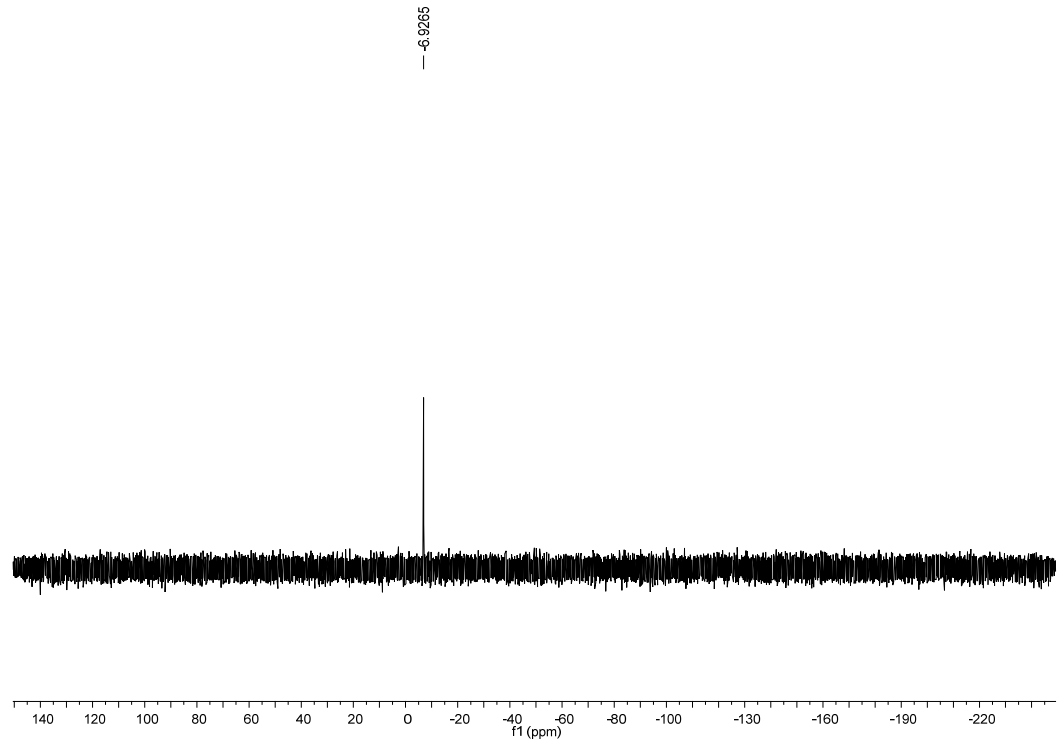

-135.2002
-135.3681
-135.5375
-104
-107
-110
-113
-116
-119
-122
-125
-128
-131
-134
-137
-140
f1 (ppm)

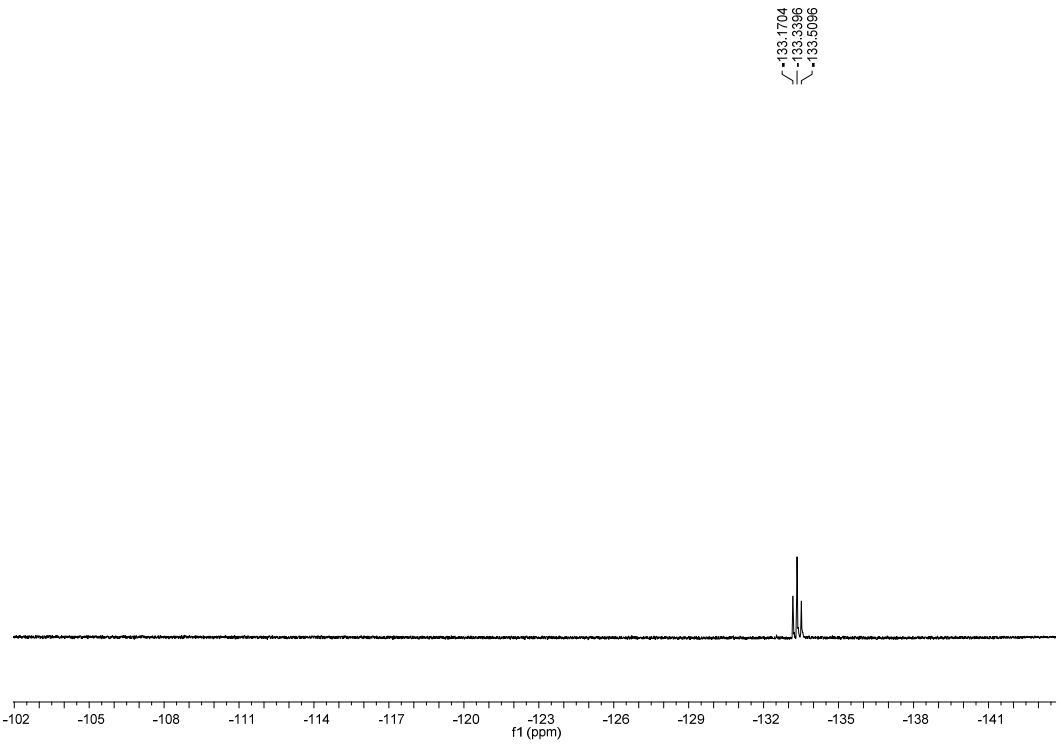

NO2
NHFmoc
COOH
O
O
P
O
O
O
O
F
NO2

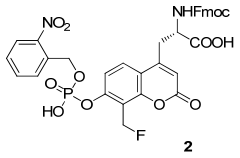
11


8.1245
8.1201
8.1083
8.1040
8.0847
7.7404
7.6900
7.6309
7.5877
7.4371
7.3825
7.3712
7.2967
7.2821
7.2673
7.2526
6.4465
5.7500
5.6176
5.5221
4.3671
4.3591
4.3464
4.3420
4.3292
4.3209
4.2543
4.2400
4.1850
4.1711
4.1568
3.3906
3.3832
3.3613
3.3535
3.1362
3.1147
3.1068
3.0853
2.5038
2.5002
2.4966
1.95
0.98
2.60
2.27
2.21
4.01
1.02
2.09
2.03
0.97
4.34
2.03
1.25
2.34
1.92
2.29
f1 (ppm)

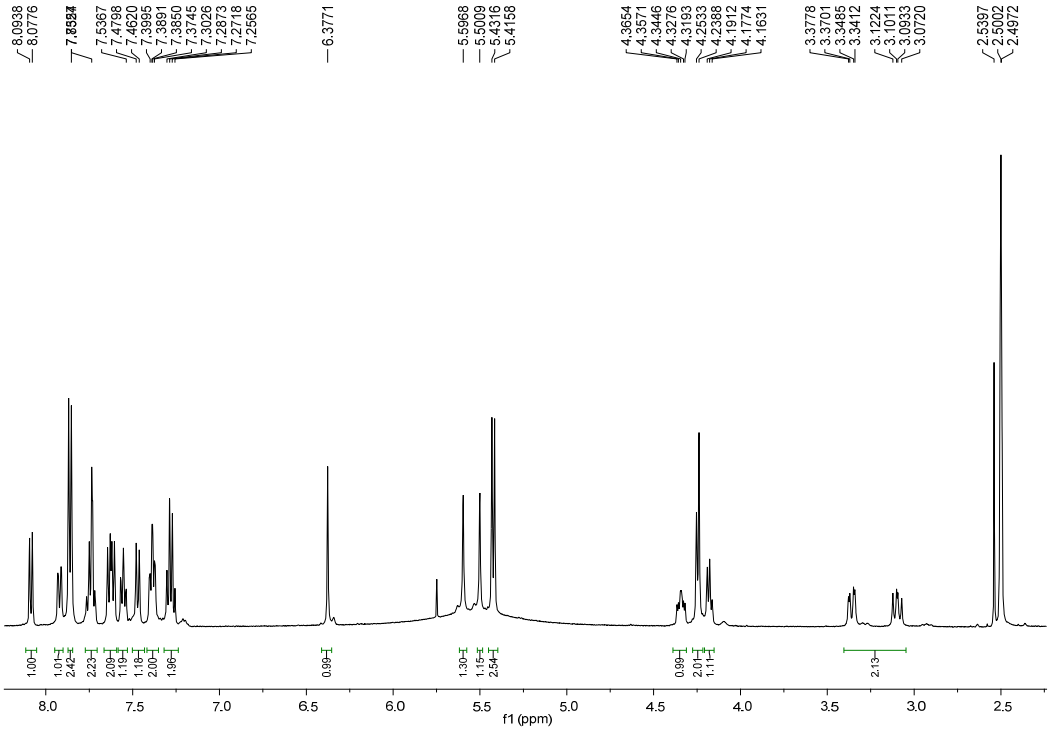

173.4130
159.6947
157.0151
152.2742
147.9276
144.7414
141.7247
135.3344
130.0812
128.0520
125.9707
121.1216
117.3412
116.8258
115.8264
115.6976
74.6944
72.5454
67.9231
66.7955
53.5536
47.6135
40.6177
40.3397
40.0618
39.7836
33.6052
f1 (ppm)

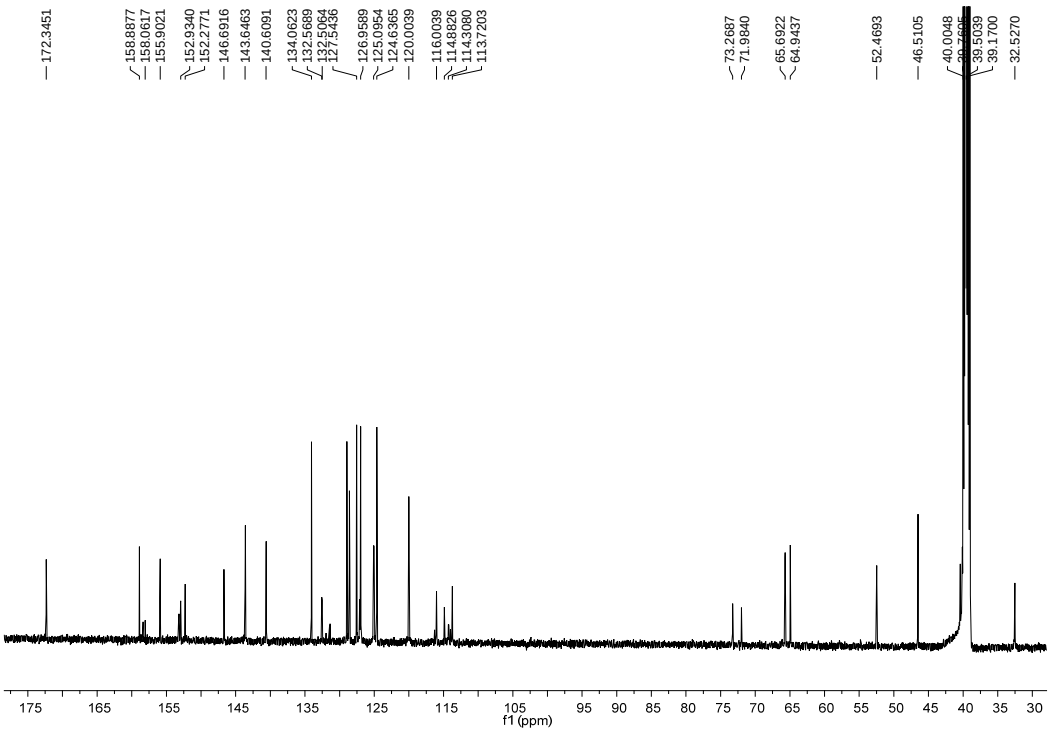

-6.9265
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-130
-160
-190
-220
f1 (ppm)

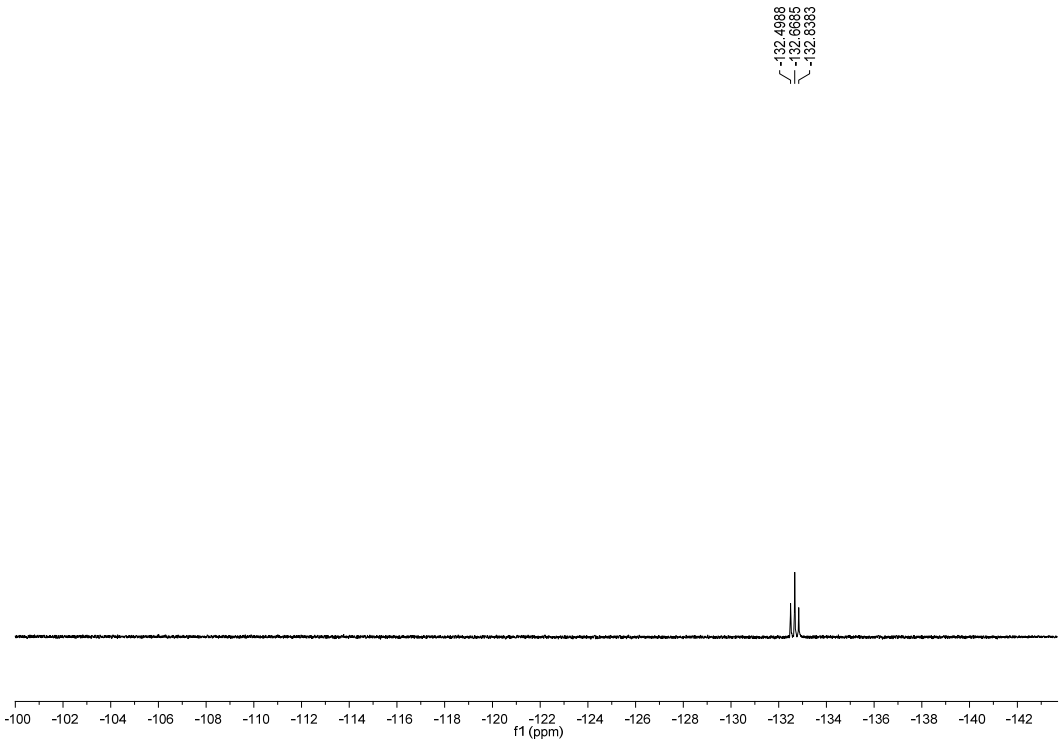

-133.1704
-133.3396
-133.5096
-102
-105
-108
-111
-114
-117
-120
-123
-126
-129
-132
-135
-138
-141
f1 (ppm)

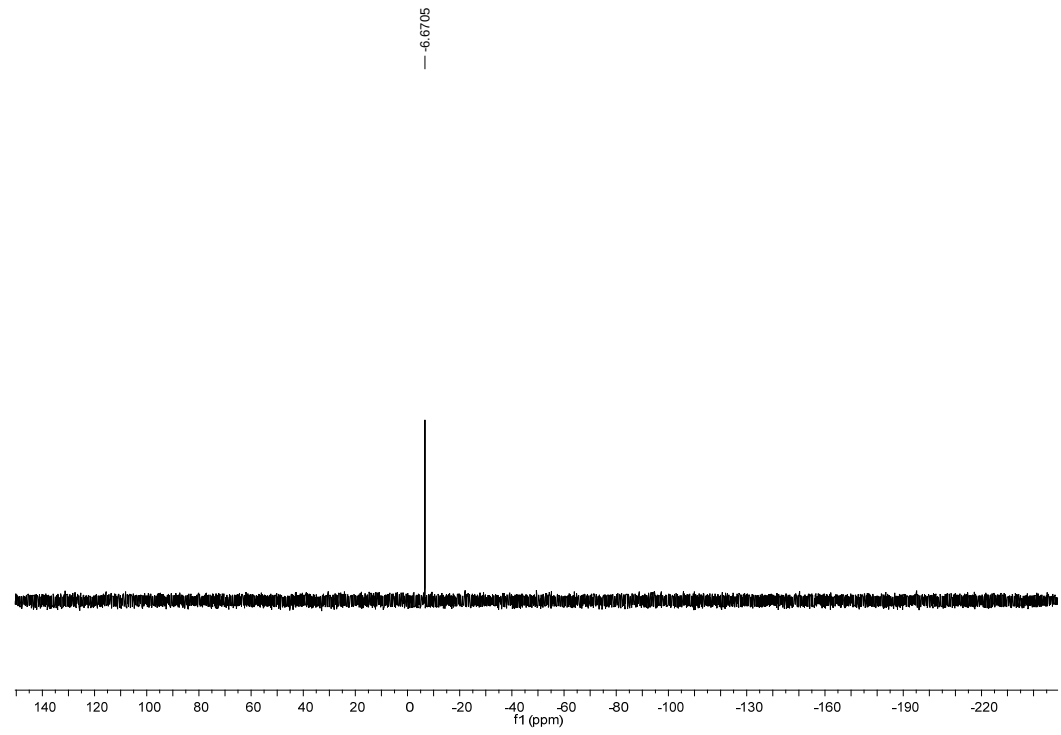

NO2
NHFmoc
COOH
O
O
P
HO
O
O
O
O
F

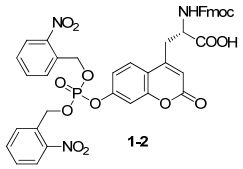
2


8.0938
8.0776
7.5367
7.4798
7.4620
7.3995
7.3891
7.3850
7.3745
7.3026
7.2873
7.2718
7.2565
6.3771
5.5968
5.5009
5.4316
5.4158
4.3654
4.3571
4.3446
4.3276
4.3193
4.2533
4.2388
4.1912
4.1774
4.1631
3.3778
3.3701
3.3465
3.3412
3.1224
3.1011
3.0933
3.0720
2.5397
2.5002
2.4972
1.00
1.01
2.42
2.23
2.09
1.19
1.18
2.00
1.98
0.99
1.30
1.15
2.54
0.99
2.01
1.11
2.13
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)


172.3451
158.8877
158.0617
155.9021
152.9340
152.2771
146.6916
143.6463
140.6091
134.0623
132.5689
132.5064
127.5438
126.9589
125.0954
124.6365
120.0039
116.0039
114.8826
114.3080
113.7203
73.2687
71.9840
65.6922
64.9437
52.4693
46.5105
40.0048
39.5039
39.1700
32.5270
175
165
155
145
135
125
115
105
95
90
85
80
75
70
65
60
55
50
45
40
35
30
f1 (ppm)


-132.4988
-132.6685
-132.8383
-100 -102 -104 -106 -108 -110 -112 -114 -116 -118 -120 -122 -124 -126 -128 -130 -132 -134 -136 -138 -140 -142
f1 (ppm)


-6.6705
140 120 100 80 60 40 20 0 -20 -40 -60 -80 -100 -130 -160 -190 -220
f1 (ppm)


NHFmoc
COOH
NO2
NO2
O
O
P
O
O
O
O

1-2


8.1163
8.1000
8.0853
7.7413
7.6826
7.6186
7.5876
7.4007
7.3861
7.3715
7.2987
7.2855
7.2712
7.2578
6.3949
5.6380
5.6227
4.3647
4.3567
4.3438
4.3268
4.3186
4.2526
4.2382
4.1868
4.1732
4.1586
3.3760
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3.0651
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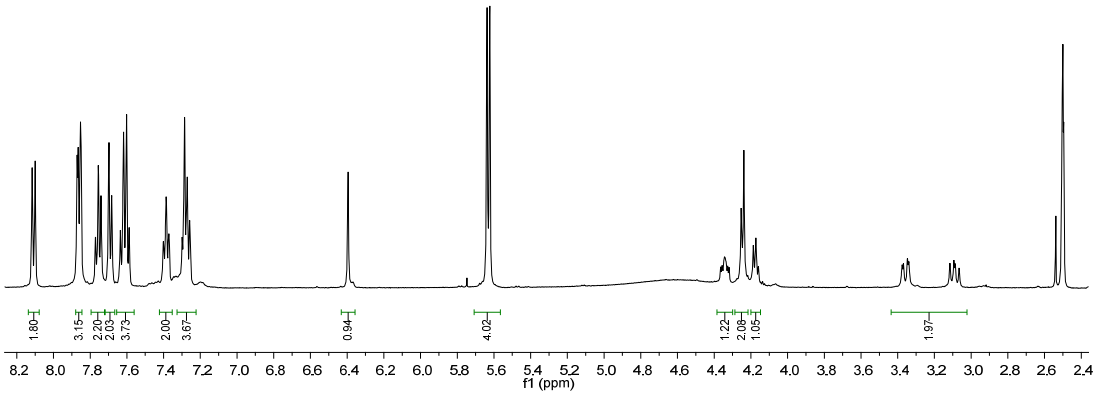
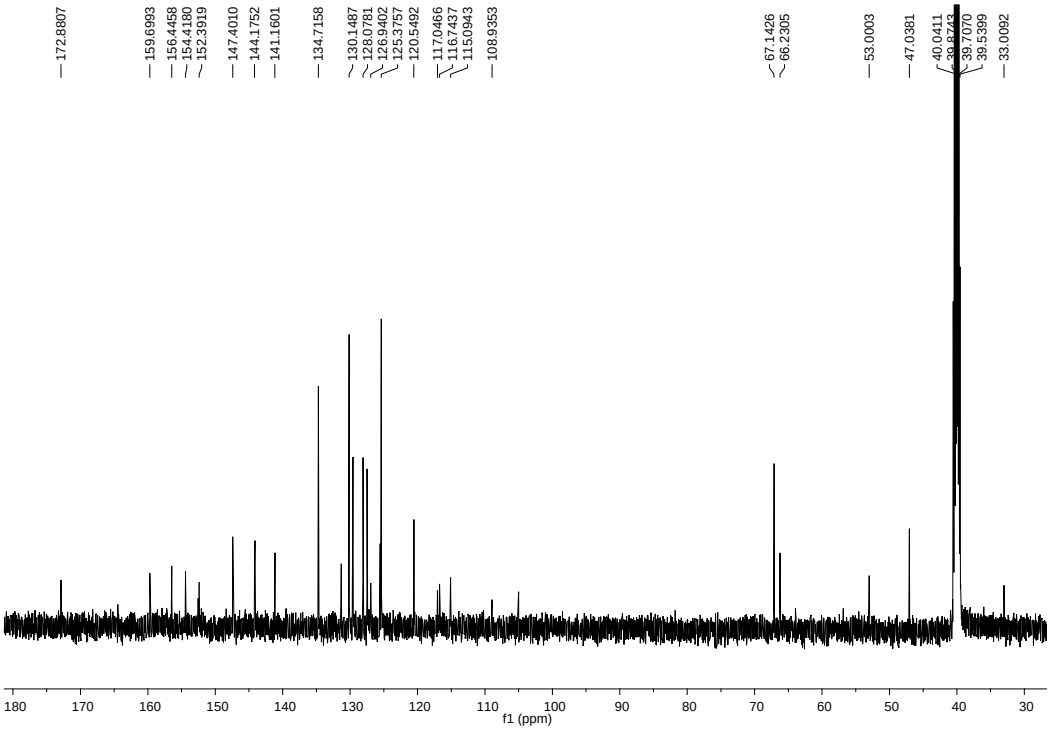
2.5000
2.4966


1.80
3.15
2.20
2.03
3.73
2.00
3.67
0.94
4.02
1.22
2.05
1.05
1.97
8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4
f1 (ppm)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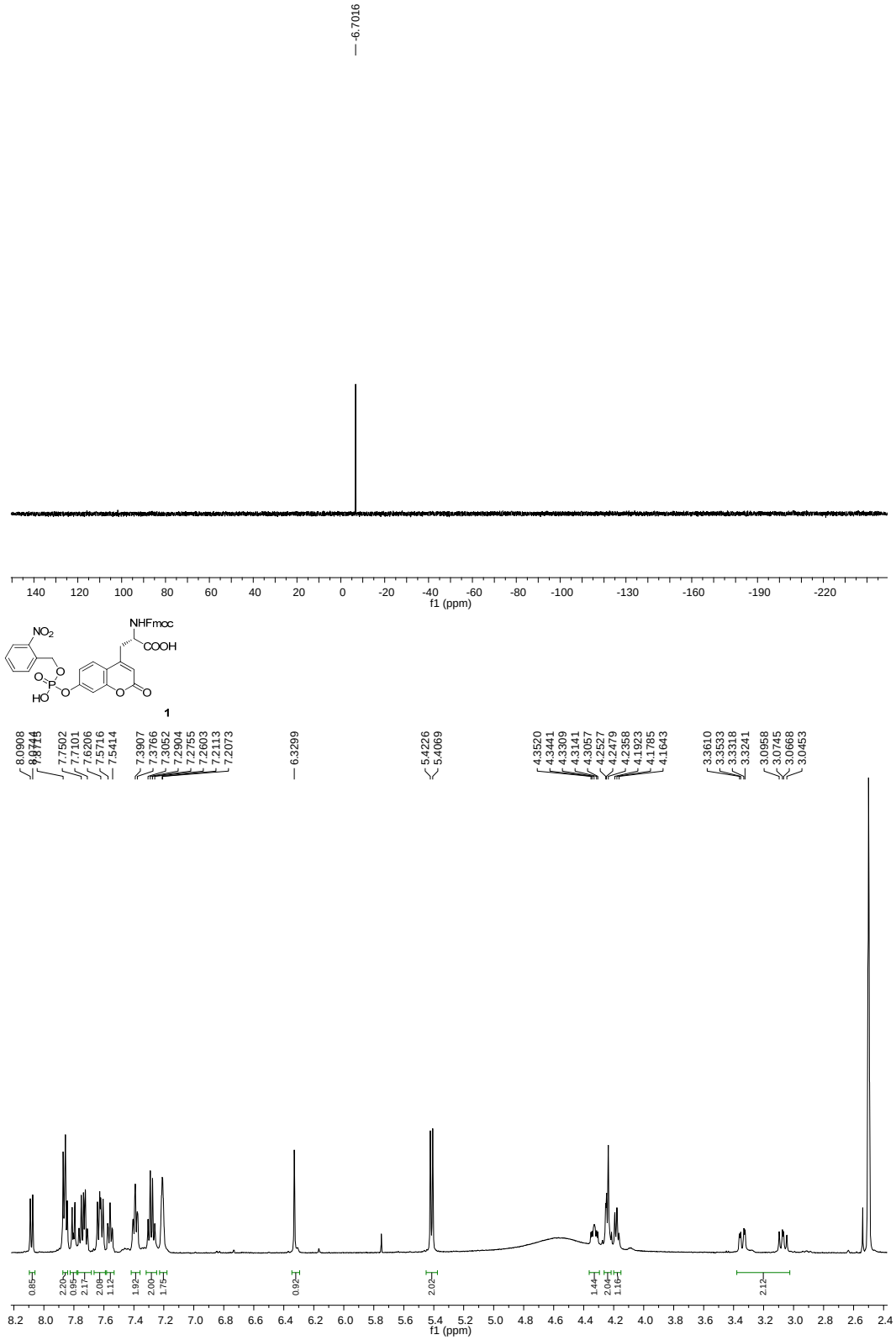
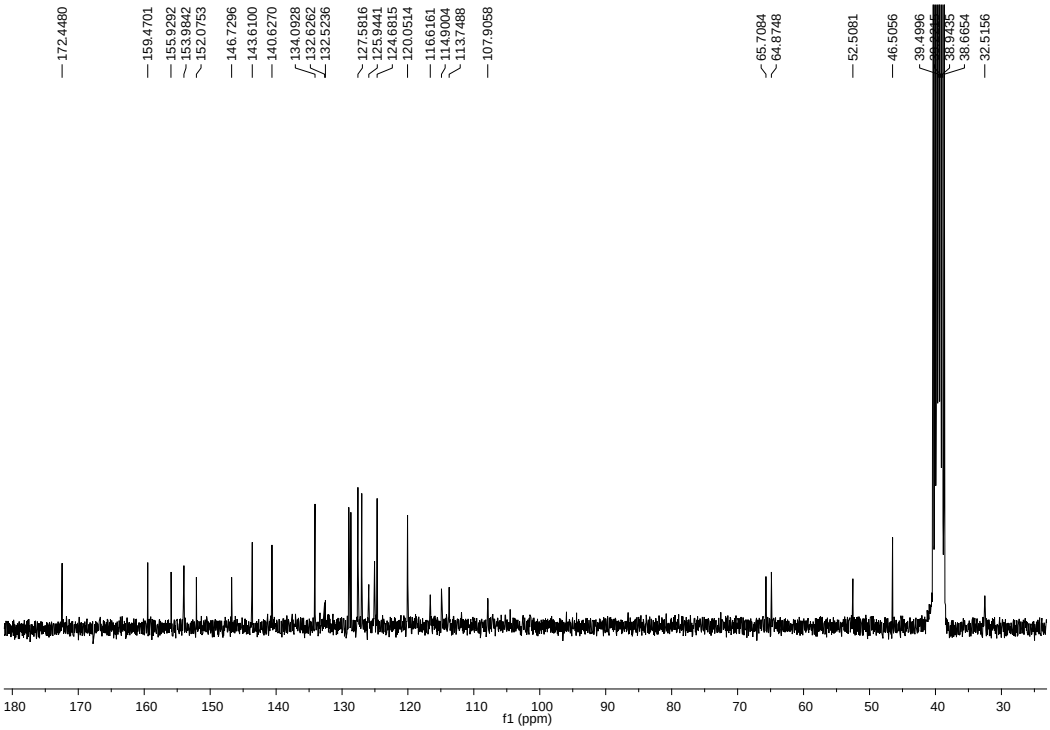
159.6993
156.4458
154.4180
152.3919
147.4010
144.1752
141.1601
134.7158
130.1487
128.0781
126.9402
125.3757
120.5492
117.0466
116.7437
115.0943
108.9353
67.1426
66.2305
53.0003
47.0381
40.0411
39.7070
39.5399
33.0092
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30
f1 (ppm)


-6.7016
f1 (ppm)
NHFmoc
COOH
NO2
HO
1
8.0908
8.0744
7.7502
7.7101
7.6206
7.5716
7.5414
7.3907
7.3766
7.3052
7.2904
7.2755
7.2603
7.2113
7.2073
6.3299
5.4226
5.4069
4.3520
4.3441
4.3309
4.3141
4.3057
4.2527
4.2479
4.2358
4.1923
4.1785
4.1643
3.3610
3.3533
3.3318
3.3241
3.0958
3.0745
3.0668
3.0453
0.85
2.20
0.95
2.17
2.09
1.12
1.92
2.00
1.75
0.92
2.02
1.44
2.04
1.16
2.12
f1 (ppm)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152.0753
146.7296
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140.6270
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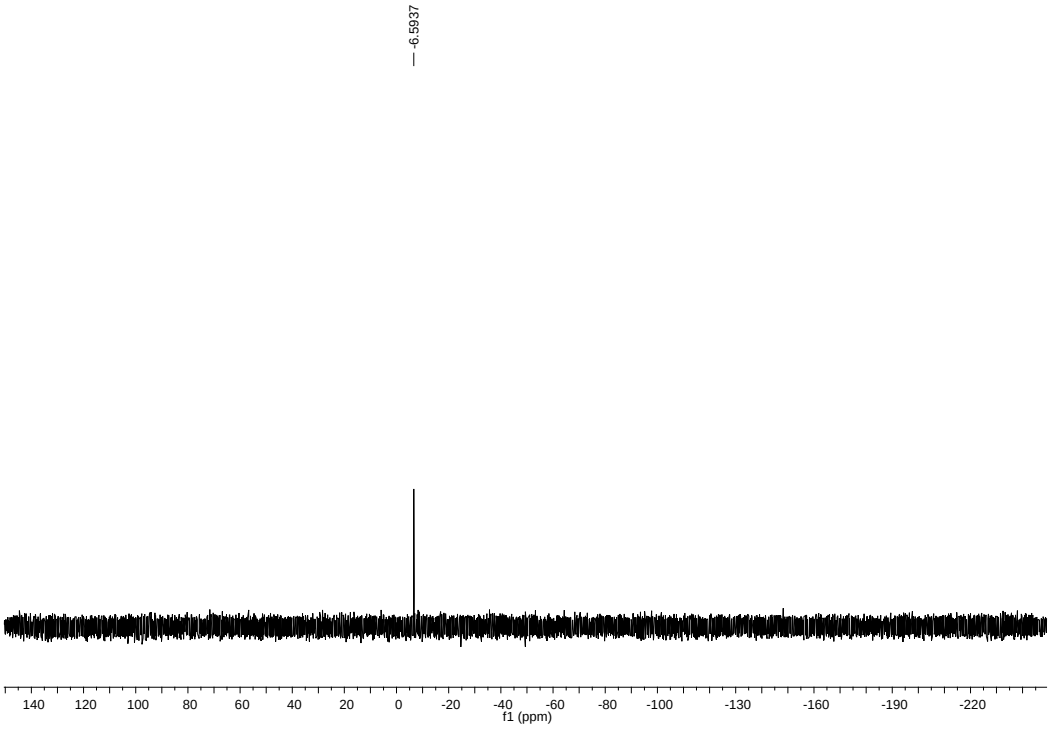
132.6262
132.5236
127.5816
125.9441
124.6815
120.0514
116.6161
114.9004
113.7488
107.9058
65.7084
64.8748
52.5081
46.5056
39.4996
38.9435
38.6654
32.5156
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)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140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
-130
-160
-190
-220
f1 (ppm)