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Supporting Information

Multi-Color, One- and Two-Photon Imaging of Enzymatic Activities in Live Cells with Fluorescently Quenched Activity-Based Probes (qABPs)

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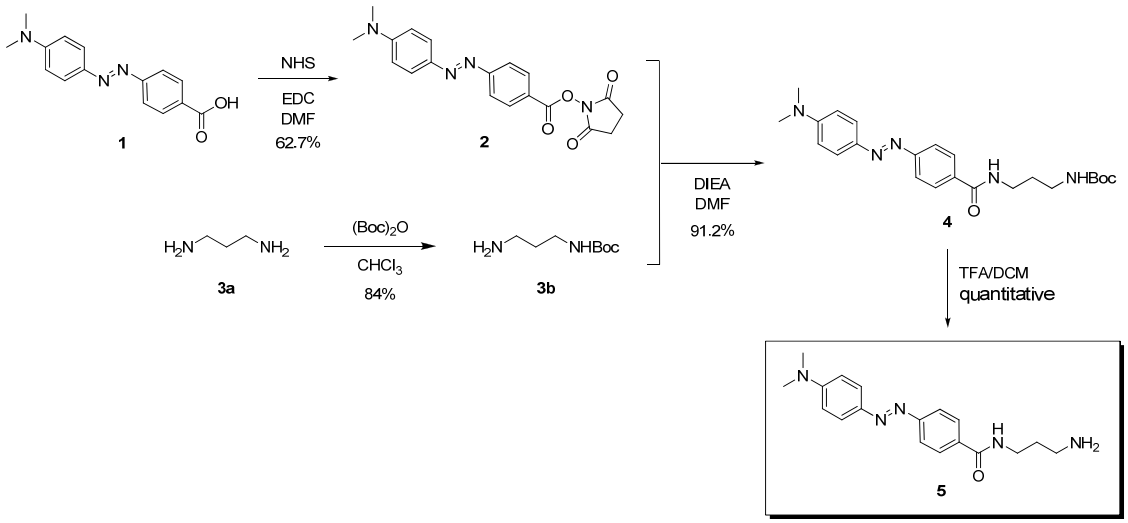
1. Chemical Synthesis



Scheme S1. Synthesis of Dabcyl-containing linker

Dabcyl-NHS (**2**)

N-hydroxysuccinimide (0.51 g, 4.5 mmol) and EDC (1.3 g, 6.8 mmol) were added to a solution of Dabcyl sodium salt (1.0 g, 3.4 mmol) in 60 mL DMF. The reaction mixture was stirred at room temperature overnight. The crude product was concentrated *in vacuo* and purified by flash chromatography (Ethyl Acetate : Hexane = 2:1) to obtain a purple solid compound **2** (0.78 g, 62.7%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.25 (d, J = 8.6 Hz, 2H), 7.92 (d, J = 8.9 Hz, 4H), 6.76 (d, J = 9.2 Hz, 2H), 3.49-3.46 (m, 2H), 3.19-3.13 (m, 2H), 2.95 (s, 6H). IT-TOF: *m/z* $[M+H]^+$ calcd: 367.13, found 367.12.

tert-Butyl 3-aminopropylcarbamate (**3b**)

Under a nitrogen atmosphere, to a solution of propane-1,3-diamine **3a** (41.7 mL, 500 mmol) in anhydrous $CHCl_3$ (100 mL) cooled to 0 °C was added dropwise di-*tert*-butyldicarbonate (10.9 g, 50 mmol) in 50 mL $CHCl_3$. After being stirred for 24 hrs at room temperature, the

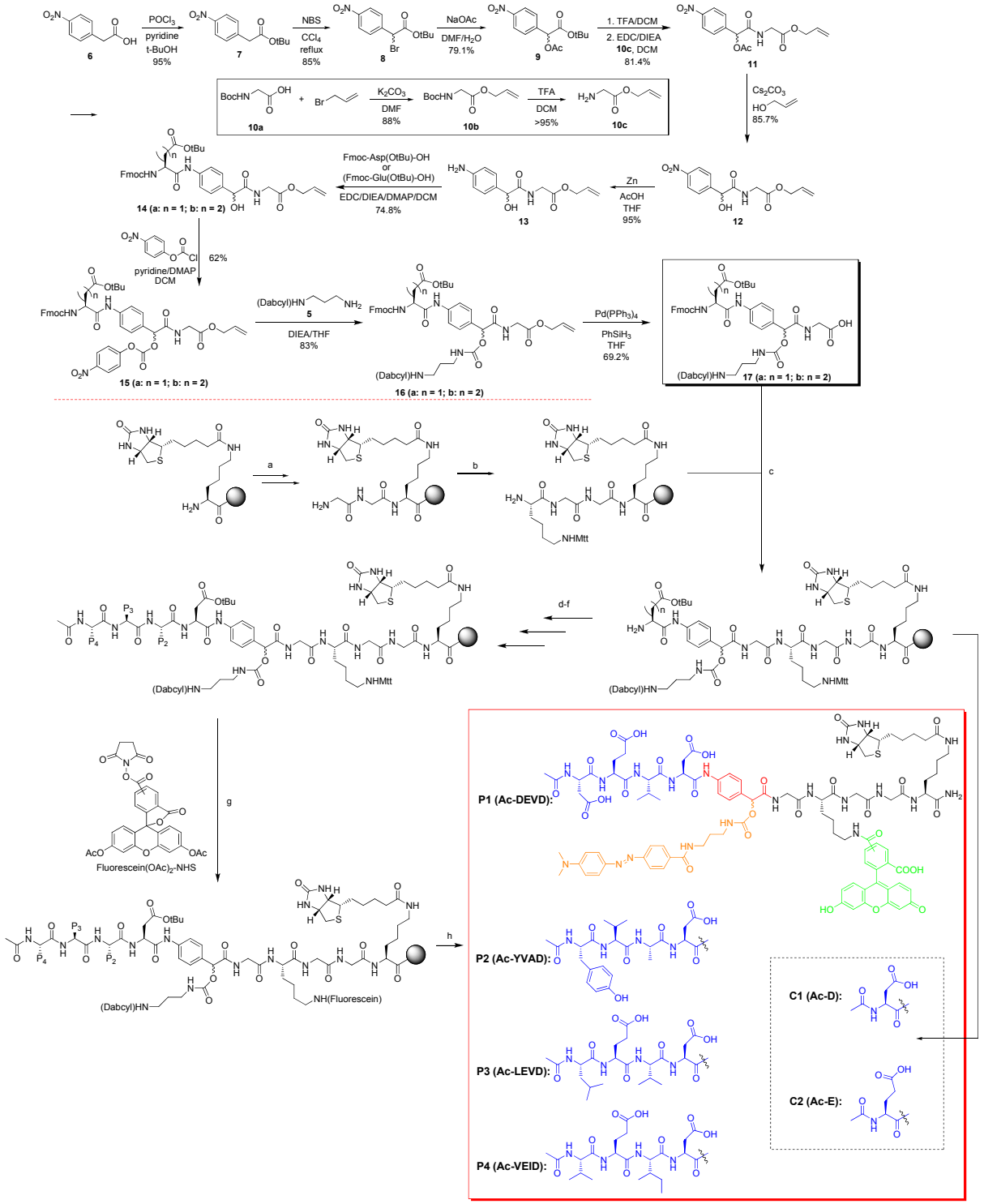
solvent was evaporated *in vacuo*. The thick oil obtained was taken up in CH_2Cl_2 (100 mL). The organic layer was successively washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to afford 7.3 g (84%) of crude compound **3b**. This material was used without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.13 (q, *J* = 6.2 Hz, 2H), 2.69 (t, *J* = 6.6 Hz, 2H), 1.55 (m, 2H), 1.37 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 156.08, 78.87, 39.40, 38.16, 33.23, 28.28.

Dabcyl derivative (**4**)

Dabcyl-NHS (**2**) (0.78 g, 2.1 mmol) dissolved in 50 mL DMF was added *tert*-butyl 3-aminopropylcarbamate (**3b**) (0.44 g, 2.5 mmol) followed by DIEA (0.4 mL, 2.5 mmol). The reaction mixture was stirred at room temperature overnight. The resulting solution was concentrated *in vacuo* and purified by flash chromatography (MeOH : DCM = 1:9) to afford Dabcyl derivative **4** (0.81 g, 91.2%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.97-7.87 (m, 6H), 6.76 (d, *J* = 9.2 Hz, 2H), 4.01 (m, 2H), 3.27 (m, 2H), 3.11 (s, 6H), 1.93 (m, 2H), 1.45 (s, 9H). IT-TOF: *m/z* $[M+H]^+$ calcd: 426.24, found 426.23.

Dabcyl derivative (**5**)

Compound **4** (0.81 g, 1.9 mmol) was vigorously stirred in 20% TFA/DCM solution for around 3 hrs. The reaction solution was concentrated and dried *in vacuo* to afford Dabcyl derivative **5.** The crude product was directly used without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.83-7.77 (t, *J* = 9.0 Hz, 4H), 7.73-7.70 (d, *J* = 9.0 Hz, 2H), 6.75-6.71 (d, *J* = 12.0 Hz, 2H), 3.40-3.36 (t, *J* = 6.0 Hz, 2H), 3.05 (s, 6H), 2.85-2.81 (t, *J* = 6.0 Hz, 2H), 1.86-1.82 (t, *J* = 6.0 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 168.52, 153.67, 142.40, 133.14, 128.08, 126.75, 121.14, 112.47, 40.38, 36.57, 35.92, 27.00. IT-TOF: *m/z* $[M+H]^+$ calcd: 326.19, found 326.19.



Scheme S2. Synthesis of **P1**-**P4**. The modularity of the probes were highlighted by showing the key components of each probe with a different color (Blue: enzymatic WH; Red: quinone methide; Orange: quencher; Green: fluorophore). Reagents and conditions: a) i. Fmoc-Gly-OH, HBTU, HOBt, DIEA, DMF; ii. 20% piperidine in DMF; b) i. Fmoc-Lys(Mtt)-OH, HBTU, HOBt, DIEA, DMF; ii. 20% piperidine in DMF; c) i. compound **17**, HATU, HOAt, DIEA, DMF; ii. 20% piperidine in DMF; d) i. Fmoc-Val-OH, HBTU, HOBt, DIEA, DMF; ii. 20% piperidine in DMF; e) i. Fmoc-Glu(OtBu)-OH, HBTU, HOBt, DIEA, DMF; ii. 20% piperidine in DMF; f) i. Fmoc-Asp(OtBu)-OH, HBTU, HOBt, DIEA, DMF; ii. 20% piperidine in DMF; iii. Acetic anhydride, DIEA; g) i. 1% TFA 5% TIS in DCM, 30 mins; ii. Fluorescein(OAc)$_2$-NHS, DIEA, DMF; iii. 20% piperidine in DMF; h). 95% TFA 2.5% TIS 2.5% H_2O, 2 hrs.

tert-Butyl 2-(4-nitrophenyl)acetate (**7**)

To a solution of 2-(4-nitrophenyl)acetic acid **6** (9.06 g, 50 mmol) in 100 mL $CHCl_3$, pyridine (20 mL, 250 mmol) and *t*-BuOH (47 mL, 500 mmol) were added followed by $POCl_3$ (6 mL, 65 mmol) dropwise over 2 mins. After 5 hrs, the reaction mixture was poured into a solution of ice containing 20 mL DCM and 10 mL 10% HCl. The organic layer was separated and washed with brine, dried over anhydrous Na_2SO_4. The pure product **7** was obtained by flash chromatography (Ethyl Acetate : Hexane = 1:9, 11.3 g, 95%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.19 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 3.64 (s, 2H), 1.44 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 173.11, 169.36, 142.08, 130.17, 123.63, 81.73, 42.35, 27.96. IT-TOF: *m/z* $[M+H]^+$ calcd: 238.10, found 238.09.

tert-Butyl 2-bromo-2-(4-nitrophenyl)acetate (**8**)[1]

NBS (9.3 g, 52 mmol) was added to a solution of compound **7** (11.3 g, 47.5 mmol) in 150 mL of CCl_4 in a reaction flask equipped with a condenser. The flask was irradiated with a 100W tungsten lamp for 5 hrs resulting in the formation of a white precipitate. Following filtration, the filtrate was concentrated, subjected to silica gel column chromatography (Ethyl Acetate : Hexane = 1:9) to give the product **8** (12.7 g, 85%) as a pale yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.22 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 8.8 Hz, 2H), 5.28 (s, 1H), 1.47 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 166.23, 148.01, 143.11, 129.71, 123.77, 83.95, 45.99, 27.62. IT-TOF: *m/z* $[M+H]^+$ calcd: 316.01, found 316.00.

tert-Butyl 2-acetoxy-2-(4-nitrophenyl)acetate (**9**)

Compound **8** (12.7 g, 40.4 mmol) was dissolved in a mixture of 100 mL DMF and 50 mL H_2O under nitrogen with constant stirring. Following addition of CH_3COONa (6.6 g, 48 mmol), the mixture was stirred at 100 °C. After 3 hrs, the solvent was removed by evaporation under reduced pressure, and the residue was partitioned between ethyl acetate (150 mL) and brine (150 mL). The organic layer was then separated, washed with 10% hydrochloric acid (100 mL) and brine (100 mL), dried with anhydrous Na_2SO_4 and then concentrated under reduced pressure to give a brown oil which was subsequently purified by flash chromatography (Ethyl Acetate : Hexane = 1:8)to afford the pale yellow solid **9** (9.4 g, 79.1%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.24 (d, J = 8.8 Hz, 2H), 7.66 (d, J = 8.8 Hz, 2H), 5.90 (s, 1H), 2.22 (s, 3H), 1.40 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.84, 166.57, 148.17, 141.19, 128.16, 123.79, 83.47, 73.84, 27.75, 20.62. IT-TOF: *m/z* $[M+H]^+$ calcd: 296.11, found 296.10.

2-Acetoxy-2-(4-nitrophenyl)acetic acid (**9b**)

Compound **9** (9.4 g, 32.0 mmol) was vigorously stirred in 20% TFA/DCM solution for around 8 hrs. The reaction solution was concentrated and dried *in vacuo* to afford compound **9b.** The crude product was directly used without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.26 (d, J = 8.9 Hz, 2H), 7.70 (d, J = 8.6 Hz, 2H), 6.05 (s, 1H), 2.24 (s, 3H). IT-TOF: *m/z* $[M+H]^+$ calcd: 240.04, found 240.03.

Allyl 2-(*tert*-butoxycarbonylamino)acetate (**10b**)

To a solution of compound **10a** (8.76 g, 50 mmol) in 100 mL DMF, K_2CO_3 was added at 0 °C. Then allyl bromide was added and the reaction mixture was stirred overnight. Most of DMF was removed *in vacuo*. The residue was redissolved in EtOAc and washed with water, brine, dried over anhydrous Na_2SO_4 to afford the crude product **10b** (9.5 g, 88%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.98-5.85 (m, 1H), 5.33 (d, J = 17.1 Hz, 1H), 5.25 (d, J = 10.4 Hz, 1H), 4.64 (d, J = 5.7 Hz, 2H), 3.93 (d, J = 5.6 Hz, 2H), 1.45 (s, 9H).

Allyl 2-aminoacetate (**10c**)

Compound **10b** (9.5 g, 44 mmol) was vigorously stirred in 20% TFA/DCM solution for around 8 hrs. The reaction solution was concentrated and dried *in vacuo* to afford compound **10c.** The crude product was directly used without further purification.

Allyl 2-(2-acetoxy-2-(4-nitrophenyl)acetamido)acetate (**11**)

To a solution of compound **9b** (5.98 g, 25 mmol) in 100 mL DCM, EDC (5.7 g, 30 mmol) and DIEA (5.1 mL, 30 mmol) were added under N_2 protection. Then a solution of allyl 2-aminoacetate (**10c**) (3.5 g, 30 mmol) was added slowly and the reaction mixture was stirred at room temperature for overnight. The mixture was diluted by DCM and washed with 1 M HCl, water and brine, dried over anhydrous Na_2SO_4. The pure compound **11** was obtained after flash chromatography (Ethyl Acetate : Hexane = 1:7, 6.8 g, 81.4%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.26 (d, *J* = 8.9 Hz, 2H), 7.66 (d, *J* = 8.7 Hz, 2H), 6.90 (s, 1H), 6.21 (s, 1H), 5.96-5.83 (m, 1H), 5.33 (d, *J* = 17.3 Hz, 1H), 5.27 (d, *J* = 11.5 Hz, 1H), 4.66 (d, *J* = 5.9 Hz, 2H), 4.10 (q, *J* = 4.1 Hz, 2H), 2.24 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.04, 168.63, 167.32, 142.11, 131.14, 128.25, 123.86, 119.31, 74.18, 66.35, 41.13, 20.85. IT-TOF: *m/z* $[M+H]^+$ calcd: 337.10, found 337.10.

Allyl 2-(2-hydroxy-2-(4-nitrophenyl)acetamido)acetate (**12**)

A solution of compound **11** (2.6 g, 7.7 mmol) in 50 mL allyl alcohol, was added Cesium carbonate (1.2 g, 3.8 mmol) slowly. The reaction was monitored closely by TLC and quenched by 1 M HCl once the starting material disappeared. The reaction mixture was concentrated *in vacuo* and redissolved in Ethyl Acetate, washed with water, brine and dried over anhydrous Na_2SO_4. A yellow solid product **12** was obtained after flash chromatography (Ethyl Acetate : Hexane = 1:4, 1.9 g, 85.7%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.7 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.16 (s, 1H), 5.94-5.81 (m, 1H), 5.34 (d, *J* = 1.2 Hz, 1H), 5.26 (d, *J* = 11.0 Hz, 2H), 4.63 (d, *J* = 5.8 Hz, 2H), 4.21 (s, 1H), 4.05 (dq, 2H), 1.92 (s, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.41, 169.31, 147.89, 145.81, 131.13, 127.41, 123.73, 119.27, 73.21, 66.33, 41.05. IT-TOF: *m/z* $[M+H]^+$ calcd: 295.09, found 295.08.

Allyl 2-(2-(4-aminophenyl)-2-hydroxyacetamido)acetate (**13**)

A mixture of **12** (1.9 g, 6.5 mmol), AcOH (1.9 ml, 32.5 mmol), and powdered Zn (4.3 g, 65 mmol) was stirred in THF (10 mL) at room temperature for 2 hrs. The mixture was diluted with EtOAc, filtered through Celite, washed with sat. $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The product was chromatographed on silica gel (Ethyl Acetate : Hexane = 3:1) to give compound **13** (1.6 g, 95%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.5 Hz, 2H), 6.67 (d, *J* = 8.5 Hz, 2H), 5.96-5.83 (m, 1H), 5.33 (d, *J* = 17.2 Hz, 1H), 5.27 (d, *J* = 10.5 Hz, 1H), 5.00 (s, 1H), 4.64 (d, *J* = 5.8 Hz, 2H), 4.17-3.99 (m, 2H). IT-TOF: *m/z* $[M+H]^+$ calcd: 287.11, found 287.09.

tert-Butyl 3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-(4-(2-(2-(allyloxy)-2-oxoethylamino)-1-hydroxy-2-oxoethyl)phenylamino)-4-oxobutanoate (**14a**)

To a solution of compound **13** (1.6 g, 6.1 mmol), Fmoc-Asp(OtBu)-OH (2.76 g, 6.7 mmol), EDC (1.4 g, 7.3 mmol), DMAP (0.074 g, 0.61 mmol) in 50 mL DCM, DIEA (1.24 mL, 7.3 mmol) was added under N_2 protection. The reaction mixture was stirred at room temperature overnight. The mixture was diluted by DCM and washed with 10% citric acid, water and brine, dried over anhydrous Na_2SO_4. The pure compound **14** was obtained after flash chromatography (Ethyl Acetate : Hexane = 1:2)3.0 g, 74.8%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 7.6 Hz, 2H), 7.56 (t, *J* = 5.0 Hz, 2H), 7.40-7.35 (m, 4H), 7.31-7.24 (m, 4H), 7.17 (d, *J* = 2.6 Hz, 1H), 6.20 (s, 1H), 5.89-5.81 (m, 1H), 5.31-5.21 (m, 2H), 4.99 (d, *J* = 3.7 Hz,

1H), 4.65 (s, 1H), 4.59 (d, *J* = 5.6 Hz, 2H), 4.41 (t, *J* = 6.9 Hz, 2H), 4.26 (d, *J* = 3.8 Hz, 1H), 4.19 (t, *J* = 6.9 Hz, 1H), 4.04-3.97 (m, 2H), 1.43 (s, 9H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.78, 172.76, 171.16, 170.89, 169.39, 168.89, 143.60, 143.56, 141.24, 137.53, 135.40, 131.35, 127.74, 127.55, 127.07, 124.95, 120.33, 119.97, 118.96, 82.08, 73.67, 67.30, 66.04, 60.36, 51.85, 47.03, 41.04, 38.54, 37.61, 27.97, 20.97, 14.13. IT-TOF: *m/z* $[M+H]^+$ calcd: 658.27, found 658.26.

(4*S*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((4-(2-((2-(allyloxy)-2-oxoethyl)amino)-1-hydroxy-2-oxoethyl)phenyl)amino)-5-oxopentanoic acid (**14b**)

Yield: 77.1%. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.73-7.71 (d, *J* = 6.0 Hz, 1H), 7.69-7.66 (t, *J* = 5.0 Hz, 1H), 7.57-7.53 (t, *J* = 5.7 Hz, 2H), 7.41-7.39 (t, *J* = 6.4 Hz, 1H), 7.37-7.33 (m, 3H), 7.31-7.30 (d, *J* = 4.2 Hz, 2H), 7.26-7.24 (t, *J* = 2.6 Hz, 2H), 7.22-7.20 (dd, J_1 = 4.5 Hz, J_2 = 9.5 Hz, 2H), 6.20-6.19 (d, *J* = 4.5 Hz, 1H), 5.88-5.80 (m, 1H), 5.29-5.20 (m, 2H), 5.02-5.01 (d, *J* = 5.0 Hz, 1H), 4.59-4.57 (d, *J* = 3.2 Hz, 2H), 4.38-4.31 (m, 3H), 4.17-3.97 (m, 4H), 1.42 (s, 9H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.20, 172.98, 170.21, 169.39, 169.34, 156.64, 143.58, 141.22, 137.59, 131.34, 128.42, 127.71, 127.57, 127.08, 126.40, 125.05, 120.38, 119.93, 118.98, 116.37, 111.58, 81.25, 73.73, 67.26, 66.09, 47.02, 41.09, 38.58, 31.69, 28.03. IT-TOF: *m/z* $[M+H]^+$ calcd: 672.28, found 672.27.

tert-Butyl 3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-(4-(2-(2-(allyloxy)-2-oxoethylamino)-1-((4-nitrophenoxy)carbonyloxy)-2-oxoethyl)phenylamino)-4-oxobutanoate (**15a**)

To a solution of compound **14** (3.0 g, 4.6 mmol), DMAP (0.056 g, 0.46 mmol) and pyridine (2.2 mL, 27.6 mmol) in 80 mL DCM, was added *p*-nitrophenyl chloroformate (1.9 g, 9.2 mmol) portion-wise at 0 °C. After 24 hrs, 10% citric acid was added to quench the reaction. The organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. The pure compound **15** was obtained by flash chromatography (Ethyl Acetate : Hexane = 2:1, 2.3 g, 62%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.27 (d, *J* = 8.8 Hz, 1H), 7.77 (d, *J* = 7.6 Hz, 2H), 7.59-7.37 (m, 10H), 7.31 (d, *J* = 7.4 Hz, 2H), 6.05 (s, 1H), 5.95-5.84 (m, 1H), 5.39-5.26 (m, 2H), 4.67 (d, *J* = 5.7 Hz, 2H), 4.48 (d, *J* = 6.7 Hz, 2H), 4.23 (t, *J* = 6.7 Hz, 2H), 4.16-4.09 (m, 2H), 2.95 (dd, 1H), 2.67 (dd, 1H), 1.46 (s, 9H). IT-TOF: *m/z* $[M+H]^+$ calcd: 823.27, found 823.26.

(4*S*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((4-(2-((2-(allyloxy)-2-oxoethyl)amino)-1-(((4-nitrophenoxy)carbonyl)oxy)-2-oxoethyl)phenyl)amino)-5-oxopentanoic acid (**15b**)

Yiled: 39.9%. The product is quite unstable, so we directly proceeded to the next step. IT-TOF: *m/z* $[M+H]^+$ calcd: 837.29, found 837.29.

(*E*)-allyl 9-(4-(2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-*tert*-butoxy-4-oxobutanamido)phenyl)-1-(4-((4-(dimethylamino)phenyl)diazenyl)phenyl)-1,7,10-trioxo-8-oxa-2,6,11-triazatridecan-13-oate (**16a**)

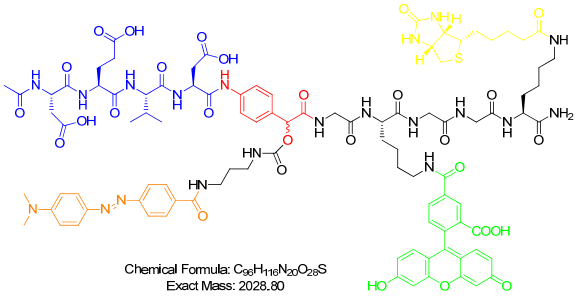
To a solution of compound **15** (2.3 g, 2.9 mmol) and DIEA (0.64 mL, 3.8 mmol) in 50 mL THF was added compound **5** (1.1 g, 3.5 mmol). The reaction was stirred overnight and concentrated *in vacuo*, redissolved in EtOAc. The solution was washed with 10% citric acid, water, brine and dried over anhydrous Na_2SO_4. An orange compound **16** was obtained after flash chromatography (Ethyl Acetate : Hexane = 1:1)2.4 g, 83%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.91-7.85 (m, 6H), 7.75 (d, *J* = 7.6 Hz, 2H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.44-7.37 (m, 4H), 7.30 (d, *J* = 7.4 Hz, 2H), 6.91 (s, 2H), 6.75 (d, *J* = 9.2 Hz, 2H), 6.07 (s, 1H), 5.92-5.81 (m, 2H), 5.33-5.22 (m, 2H), 4.62 (d, *J* = 5.9 Hz, 3H), 4.46 (d, *J* = 7.1 Hz, 2H), 4.22 (t, *J* = 6.3 Hz, 2H), 4.09 (t, *J* = 5.9 Hz, 2H), 3.51 (q, *J* = 5.9 Hz,

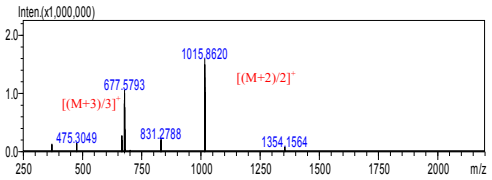
2H), 3.30 (s, 2H), 3.10 (s, 6H), 2.91 (dd, 1H), 2.69 (dd, 1H), 1.45 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.10, 169.65, 169.29, 168.82, 163.23, 156.34, 155.11, 152.86, 143.59, 143.55, 141.28, 140.69, 131.28, 128.35, 127.78, 127.09, 126.06, 125.46, 124.94, 122.25, 120.20, 120.01, 119.06, 115.68, 111.48, 82.26, 75.40, 66.15, 51.81, 49.37, 47.08, 41.14, 40.67, 40.24, 33.76, 29.65, 28.00. IT-TOF: *m/z* $[M+H]^+$ calcd: 1009.44, found 1009.43.

(4*S*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-((4-(1-(4-((*E*)-(4-(dimethylamino)phenyl)diazenyl)phenyl)-1,7,10,13-tetraoxo-8,14-dioxa-2,6,11-triazaheptadec-16-en-9-yl)phenyl)amino)-5-oxopentanoic acid (**16b**)

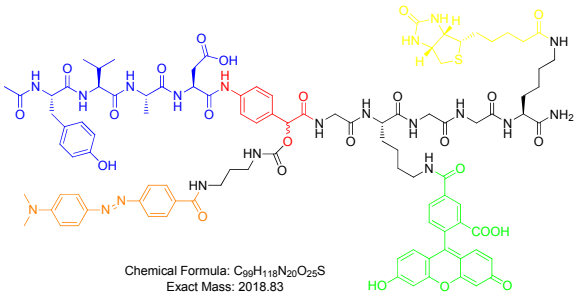
Yiled: 69.9%. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.89-7.85 (t, J = 6.2 Hz, 2H), 7.82-7.81 (d, J = 3.8 Hz, 2H), 7.73-7.70 (t, J = 6.2 Hz, 2H), 7.61-7.60 (d, J = 3.8 Hz, 1H), 7.55-7.53 (t, J = 4.2 Hz, 2H), 7.47-7.46 (d, J = 1.5 Hz, 2H), 7.43-7.33 (m, 6H), 7.26 (s, 2H), 6.72-6.70 (d, J = 5.3 Hz, 1H), 6.03 (s, 1H), 5.89-5.78 (m, 1H), 5.77 (s, 1H), 5.37-5.16 (m, 3H), 4.66-4.65 (d, J = 7.2 Hz, 1H), 4.58-4.54 (dd, J_1 = 3.4 Hz, J_2 = 7.6 Hz, 1H), 4.35-4.30 (t, J = 15.2 Hz, 2H), 4.18-4.14 (q, J = 4.5 Hz, J = 9.1 Hz, 2H), 4.09-3.97 (m, 2H), 3.42-3.41 (d, J = 3.4 Hz, 2H), 3.22-3.18 (m, 2H), 3.06 (s, 6H), 2.11 (s, 2H), 1.94- 1.93 (d, J = 3.8 Hz, 2H), 1.73-1.68 (m, 2H), 1.41 (s, 9H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 172.79, 170.62, 165.69, 1.56.54, 155.38, 154.92, 154.40, 152.72, 143.51, 143.50, 141.14, 141.11, 131.27, 130.80, 127.62, 127.60 , 126.97, 125.28, 124.91, 122.05, 119.84, 119.82, 111.35, 80.91, 67.05, 66.71, 65.92, 54,63, 46.96, 40.56, 40.08, 37.86, 31.37, 27.91. IT-TOF: *m/z* $[M+H]^+$ calcd: 1023.44, found 1023.43.

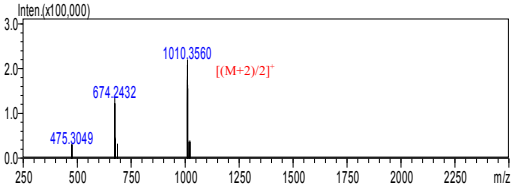
P1 (Ac-DEVD)



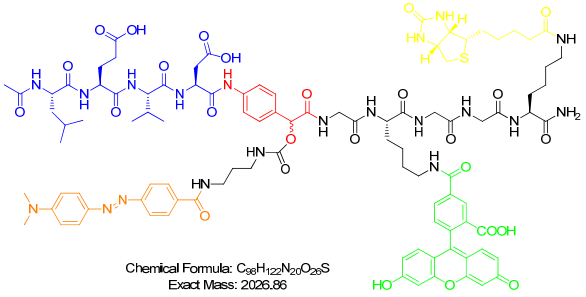


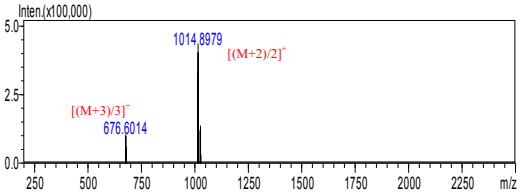
P2 (Ac-YVAD)



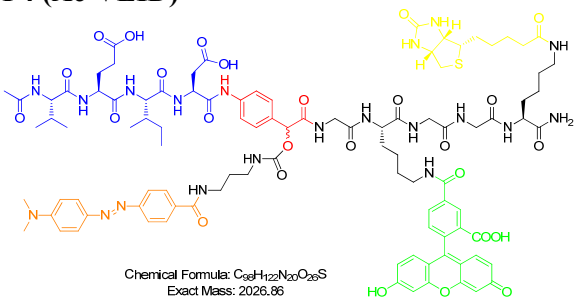


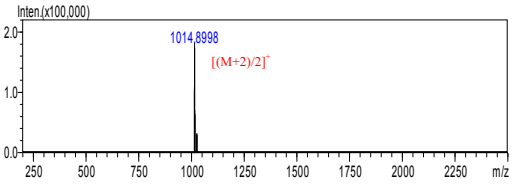
P3 (Ac-LEVD)



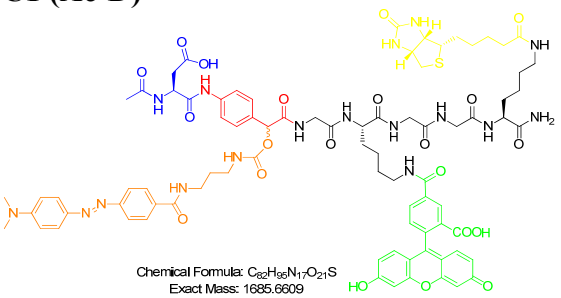


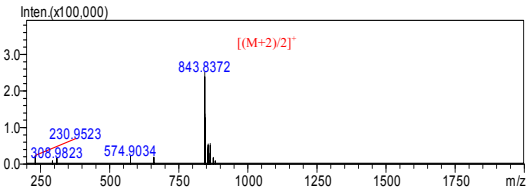
P4 (Ac-VEID)



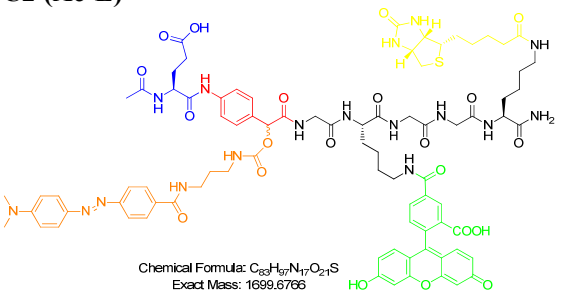


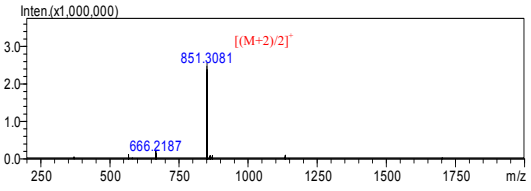
C1 (Ac-D)

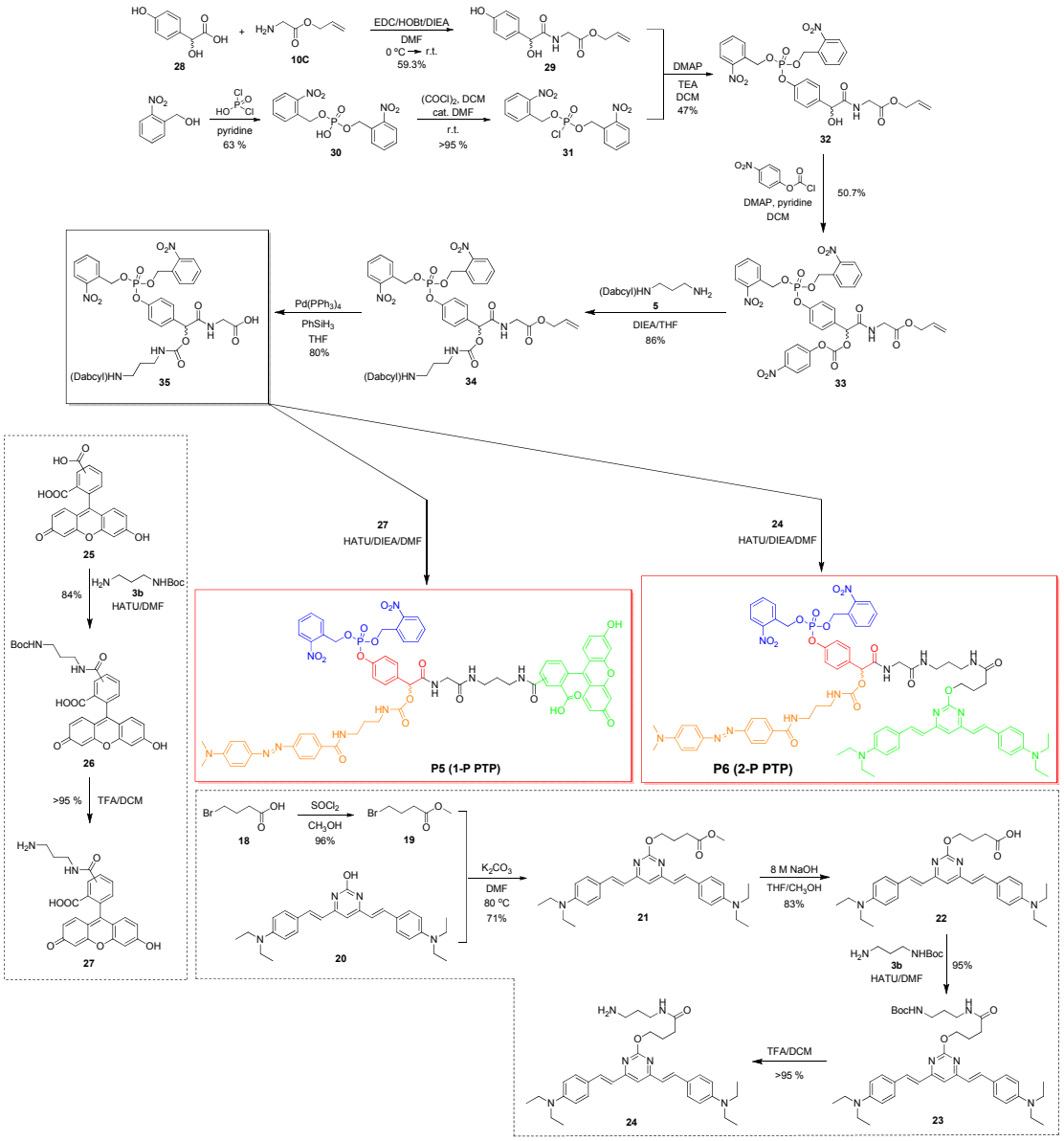




C2 (Ac-E)







Scheme S3. Synthesis of **P5** (1-P PTP) and **P6** (2-P PTP)

Methyl 4-bromobutanoate (**19**)

A mixture of 4-bromobutanoic acid (4.15 g, 25 mmol) and 25 mL $SOCl_2$ was stirred in 100 mL round-bottom flask in an ice bath for 30 mins. Then 25 mL MeOH was added slowly. After the completion of the reaction, the solvent was removed *in vacuo* to give the product as a colorless oil (4.30 g, 96%). ^{1}H-NMR: (300 MHz, $CDCl_3$), δ 3.69 (s, 3H), 3.47 (t, J = 6.6 Hz, 2H), 2.51 (t, J = 7.2 Hz, 2H), 2.17 (m, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.72, 51.51, 32.55, 32.03, 27.62.

Methyl 4-(4,6-bis(4-(diethylamino)styryl)pyrimidin-2-yloxy)butanoate (**21**)

4,6-bis(4-(diethylamino)styryl)pyrimidin-2-ol (**20**)[2] (2.16 g, 4.89 mmol) and K_2CO_3 (1.38 g, 100 mmol) were stirring in 70 mL DMF at 80 ºC for more than 1 hr. Then, methyl 4-bromobutanoate (1.76 g, 9.77 mmol) was added into the mixture slowly. After further stirring for more than 24 hrs, DMF was removed *in vacuo*. The residue was resuspended in 50 mL CH_2Cl_2 and washed with water twice. The organic layer was dried with anhydrous Na_2SO_4. After removing the CH_2Cl_2, the mixture was purified with silica-gel column (Ethyl Acetate :

Hexane = 1:2) to give the product as yellow solid (1.88 g, 71%). ^{1}H-NMR: (300 MHz, $CDCl_3$), δ 7.80 (d, *J* = 15.9 Hz, 2H), 7.45 (d, *J* = 9.0 Hz, 4H), 6.80 (s, 1H), 6.76 (d, *J* = 15.9 Hz, 2H), 6.65 (d, *J* = 9.0 Hz, 4H), 4.52 (t, *J* = 6.3 Hz, 2H), 3.69 (s, 3H), 3.39 (q, *J* = 6.9 Hz, 8H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.20 (m, 2H), 1.18 (t, *J* = 7.2 Hz, 12H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 173.89, 165.43, 165.19, 148.57, 136.92, 129.39, 123.39, 120.61, 111.44, 109.91, 65.84, 51.57, 44.43, 30.77, 24.54, 12.66. IT-TOF: *m/z* $[M+H]^+$ calcd: 543.33, found: 543.32.

4-(4,6-Bis(4-(diethylamino)styryl)pyrimidin-2-yloxy)butanoic acid (**22**)

Methyl 4-(4,6-bis(4-(diethylamino)styryl)pyrimidin-2-yloxy)butanoate (1.00 g, 1.84 mmol) and 5 mL NaOH (8 M) were stirring in 20 mL THF/CH_3OH (1:1) for 10 hrs. After reaction completed, the mixture was adjusted to *p*H = 5. The precipitate was collected and dried *in vacuo* to give the product as orange-yellow solid (810 mg, 83%). ^{1}H-NMR (300 MHz, $CDCl_3$), δ 7.80 (d, *J* = 15.6 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 4H), 6.81 (s, 1H), 6.76 (d, *J* = 15.9 Hz, 2H), 6.65 (d, *J* = 8.7 Hz, 4H), 4.52 (t, *J* = 6.0 Hz, 2H), 3.39 (q, *J* = 6.9 Hz, 8H), 2.64 (t, *J* = 7.2 Hz, 2H), 2.19 (m, 2H), 1.18 (t, *J* = 7.2 Hz, 12H). ^{13}C-NMR (75MHz, $CDCl_3$) δ 169.31, 165.43, 165.10, 148.61, 137.12, 129.44, 123.14, 120.52, 111.48, 109.72, 65.86, 44.45, 30.86, 24.46, 12.66. IT-TOF: *m/z* $[M+H]^+$ calcd: 529.31, found 529.32.

Two-Photon dye derivative (**24**)

To a solution of **22** (60 mg, 0.11 mmol) in DMF, HATU (53 mg, 0.14 mmol) and *tert*-butyl 3-aminopropylcarbamate (28 mg, 0.16 mmol) were added and the reaction mixture was stirred at room temperature for 8 hrs. Upon removal of DMF, the residue was purified by flash chromatography (Ethyl Acetate : Hexane = 2:1) to obtain compound **23** as a green-yellow oil (71 mg, 95%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.9 Hz, 2H), 7.45 (d, *J* = 9.1 Hz, 4H), 6,76 (t, *J* = 15.7 Hz, 3H), 6.64 (d, *J* = 2.7 Hz，4H), 6.42 (broad, NH), 5.08 (broad, NH), 4.50 (t, *J* = 5.7 Hz, 2H), 3.37 (dd, *J* = 6.9, 3.5 Hz, 8H), 3.30-3.26 (M, 2H), 3.11 (d, *J* = 2.8 Hz, 2H), 2.45 (t, *J* = 7.5 Hz, 2H), 2.19 (t, *J* = 6.3 Hz, 2H), 2.01 (s, 1H), 1.59 (t, *J* = 6.3 Hz, 3H), 1.41 (s, 9H), 1.17 (t, *J* = 6.9 Hz, 12H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 173.8, 166.0, 165.8, 157.1, 149.2, 137.6, 130.0, 123.7, 121.2, 112.3, 110.3, 66.6, 45.0, 37.3, 36.8, 33.8, 29.0, 25.9, 13.2. IT-TOF: *m/z* $[M+H]^+$ calcd: 685.44, found 685.44.

Compound **23** was further dissolved in DCM (0.4 mL) and TFA (80 μL, 1 mmol), and the reaction was stirred at room temperature for 2 hrs. Then the solvent was removed *in vacuo* to give compound **24** as a dark green oil. IT-TOF: *m/z* $[M+H]^+$ calcd: 585.28, found 585.27.

Fluorescein derivative (**27**)

Fluorescein **25** was synthesized according to reported procedures.[3] After dissolving **25** (0.11 g, 0.3 mmol) in DMF, HATU (0.14 g, 0.36 mmol) and *tert*-butyl 3-aminopropylcarbamate (0.8 g, 0.4 mmol) were added and the reaction mixture was stirred at room temperature for 8 hrs. Upon removal of DMF, the residue was purified by flash chromatography (Ethyl Acetate : Hexane = 3:1)to give compound **26** as a yellow oil (0.13 g, 84%). ^{1}H-NMR (500 MHz, MeOD) δ 8.21 (q, *J* = 4.1, 0.6 Hz, 1H), 7.94 (s, 1H), 7.27 (d, *J* = 3.8 Hz, 2H), 6.70 (s, 2H), 6.59-6.51 (m, 4H), 3.47 (t, *J* = 6.9 Hz, 2H), 3.16 (t, *J* = 6.6 Hz, 2H), 1.80 (t, *J* = 6.9 Hz, 2H), 1.41 (s, 9H). ^{13}C-NMR (125MHz, MeOD) δ 172.8, 170.4, 168.1, 164.7, 161.2, 158.4, 156.4, 153.9, 137.6, 135.4, 130.1, 130.0, 128.5, 125.6, 124.7, 113.6, 110.8, 103.6, 80.0, 61.4, 37.1, 38.8, 38.7, 38.5, 36.9, 31.7, 30.6, 28.8, 20.9, 14.4. IT-TOF: *m/z* $[M+H]^+$ calcd: 533.18, found 533.18.

Next, **26** was dissolved in DCM (2 mL) and TFA (0.2 mL, 2.5 mmol), and the reaction was stirred at room temperature for 2 hrs. Subsequently, the solvent was removed *in vacuo*, giving product **27** as a red-yellow solid (0.13 g, >95%). IT-TOF: *m/z* $[M+H]^+$ calcd: 451.18, found 451.17.

2-Hydroxy-2-(4-hydroxyphenyl) acetic acid (**28**)

2-hydroxy-2-(4-hydroxyphenyl)acetic acid was prepared following published procedures[4] in 63% yield. ^{1}H-NMR (300 MHz, MeOD) δ 7.28 (d, *J* = 4.3 Hz, 2H), 6.78 (d, *J* = 4.3 Hz, 2H), 5.06 (s, 1H). ^{13}C-NMR (75 MHz, MeOD) δ 176.54, 158.53, 131.61, 129.27, 116.16, 73.80.

Allyl 2-(2-hydroxy-2-(4-hydroxyphenyl)acetamido)acetate 2-hydroxy-2-(4-hydroxyphenyl)acetate (**29**)

To a solution of **28** (3.36 g, 20 mmol) in DMF (50 mL) in an ice bath, EDC (4.6 g, 24 mmol) and HOBt (3.24 g, 24 mmol) were added slowly. The reaction mixture was stirred for 10 mins. Then **10C** (5.5 g, 24 mmol) was added dropwise to the above solution, followed by DIEA (2.4 eq, 8.2 mL). The ice bath was removed after 1 hr and the reaction mixture was stirred at room temperature overnight.[6] Upon removal of DMF *in vacuo*, the residue was dissolved in ethyl acetate and washed with H_2O, brine and dried over anhydrous Na_2SO_4. Subsequent purification by flash chromatography (Ethyl Acetate : Hexane = 4:1) afforded compound **29** as a light yellow oil (3.1 g, 59.3%). ^{1}H-NMR (300 MHz, MeOD) δ 7.29 (d, *J* = 4.3 Hz, 2H), 6.76 (d, *J* = 4.3 Hz, 2H), 5.98-5.85 (m, 1H), 5.36-5.20 (m, 2H), 4.98 (s, 1H), 4.62 (dt, *J* = 2.9, 1.3 Hz, 2H), 4.02 (q, *J* = 11.8, 8.8 Hz, 2H). ^{13}C-NMR (75 MHz, MeOD) δ 176.43, 170.80, 158.49, 133.18, 129.68, 118.41, 116,10, 111.52, 75.15, 66.74, 41.71. IT-TOF: *m/z* $[M+Na]^+$ calcd: 288.08, found 288.08.

bis(2-nitrobenzyl) hydrogen phosphate (**30**)

The protected phosphate was synthesized according to reported procedures (in 63% yield).[5] ^{1}H-NMR (300 MHz, MeOD) δ 8.08 (d, 2H), 7.69-7.78 (m, 4H), 7.54 (t, *J* = 9.0 Hz, 2H), 5.43 (d, *J* = 3.8 Hz, 4H). ^{13}C-NMR (75 MHz, MeOD) δ 166.60, 148.50, 135.11, 130.13, 129.76, 125.87. ^{31}P-NMR (121 MHz, MeOD) δ -0.71. IT-TOF: *m/z* $[2M+H]^+$ calcd: 737.09, found 737.09.

Allyl 2-(2-(4-(bis(2-nitrobenzyloxy)phosphoryloxy)phenyl)-2-hydroxyacetamido)acetate (**32**)

30 (5.5g, 15mmol) was added to an oxalyl chloride solution (6.5ml, 75mmol; 85% in distilled DCM), followed by addition of a catalytic amount of DMF (2 drops) to initiate the reaction. After 2 hrs at room temperature, the reaction was concentrated *in vacuo* to give the desired product **31** as a light yellow oil.

29 (1.4 g, 5 mmol) was dissolved in distilled dichloromethane (30 mL), followed by addition of DMAP (0.06 g, 0.5 mmol). The mixture was stirred at room temperature for 10 mins before being cooled on an ice bath. Then a solution of **31** (15 mmol) in 10 mL distilled dichloromethane was added dropwise, followed by triethyl amine (1.4 mL, 10 mmol). The reaction was left on ice for 2 hrs before being warmed to room temperature for another 4 hrs. The reaction mixture was concentrated *in vacuo* and purified by flash chromatography (Ethyl Acetate : Hexane = 1:1) to afford **32** as a light yellow oil (1.4 g, 47%). ^{1}H-NMR (300 MHz, MeOD) δ 8.08 (d, *J* = 4.1 Hz, 2H), 7.71-7.61 (m, 4H), 7.58-7.49 (m, 4H), 7.19 (d, *J* = 3.9 Hz, 2H), 5.96-5.83 (m, 1H), 5.59 (d, *J* = 4.0 Hz, 4H), 5.33-5.17 (m, 2H), 5.07 (s, 1H), 4.59 (dt, *J* = 2.8, 1.3 Hz, 2H), 4.02(q, *J* = 10.8, 1.8 Hz, 2H). ^{13}C-NMR (75 MHz, MeOD) δ 175.35, 170.69, 151.12, 151.01, 148.12, 139.17, 135.22, 133.07, 132.24, 130.52, 129.95, 129.79, 125.98, 120.90, 118.70, 74.40, 68.12, 66.66, 41.66. ^{31}P-NMR (121 MHz, MeOD) δ -6.44. IT-TOF: *m/z* $[2M+H]^+$ calcd: 1231.26, found 1231.26.

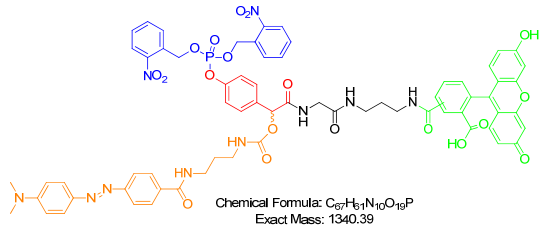
(E)-allyl9-(4-(bis(2-nitrobenzyloxy)phosphoryloxy)phenyl)-1-(4-((4-(dimethylamino)phenyl) diazenyl) phenyl)-1,7,10-trioxo-8-oxa-2,6,11-triazatridecan-13-oate (**34**)

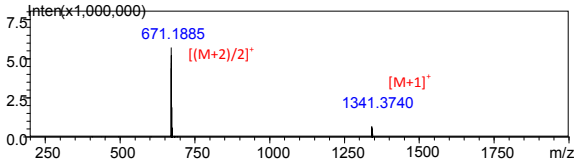
Compound **32** (1.3 g, 2.1 mmol) in distilled dichloromethane (20 mL) was added DMAP (0.03 g, 0.24 mmol) over 10 mins in an ice bath. Then anhydrous pyridine (0.96 mL, 12

mmol) was added slowly followed by addition of 4-nitrophenyl carbonochloridate (0.85 g, 4.2 mmol dissolved in 2 mL DCM) dropwise. The reaction mixture was kept in ice bath for 2 hrs before being warmed to room temperature for another 5 hrs. Upon solvent removal, the resulting residue was purified by flash chromatography (Ethyl Acetate : Hexane = 4:1), giving the desired compound **33** as a light yellow oil (0.79 g, 50.7%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.21 (d, J = 2.7 Hz, 2H), 8.10 (d, J = 3.9 Hz, 2H), 7.69-7.62 (m, 4H), 7.54-7.46 (m, 4H), 7.39-7.34 (m, 2H), 7.28-7.21 (m, 2H), 6.09 (s, 1H), 5.93-5.80 (m, 1H), 5.63 (d, J = 3.8 Hz, 4H), 5.32-5.20 (m, 3H), 4.61 (d, J = 2.9 Hz, 2H). ^{13}C-NMR (75MHz, $CDCl_3$) δ 169.5, 167.9, 155.7, 151.7, 151.6, 147.2, 146.1, 134.8, 132.1, 131.9, 130.3, 129.8, 129.1, 125.9, 125.7, 122.3, 121.1, 121.0, 119.6, 79.1, 67.5, 66.7, 41.7. ^{31}P-NMR (121 MHz, $CDCl_3$) δ -6.54.

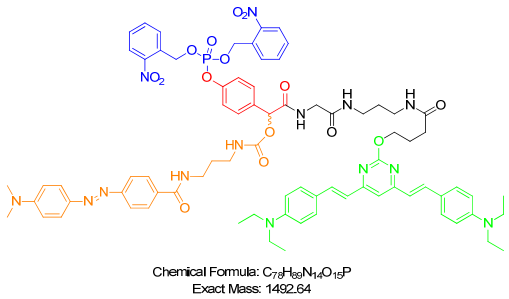
After dissolving **33** (0.79 g, 1.01 mmol) in distilled THF (20 mL), **5** (0.53 g, 1.2 mmol) was added, followed by addition of DIEA (0.22 mL, 1.2 mmol). The mixture was stirred at room temperature for 2 hrs before being concentrated *in vacuo* and purified by flash chromatography (Ethyl Acetate : Hexane = 2:1)to afford **34** as a red powder (0.83 g, 86%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.07-8.00 (m, 2H), 7.87-7.76 (m, 6H), 7.61-7.57 (m, 4H), 7.46-7.41 (m, 4H), 7.34 (t, J = 5.9 Hz, NH), 7.16 (d, J = 4.1 Hz, 2H), 6.82-6.67 (m, 2H), 6.26 (t, J = 6.1 Hz, NH), 5.87-5.74 (m, 1H), 5.60 (d, J = 8.3 Hz, 4H), 5.26-5.15 (m, 2H), 4.54 (d, J = 2.9 Hz, 2H), 4.02 (q, J = 5.6, 2.8 Hz, 2H), 3.44-3.41 (m, 2H), 3.22-3.14 (m, 2H), 3.04 (s, 1H), 1.69 (s, 2H). ^{13}C-NMR (75MHz, $CDCl_3$) δ 170.1, 169.8, 168.3, 155.7, 155.6, 153.4, 151.0, 150.9, 147.2, 144.1, 134.8, 134.7, 134.1, 129.9, 129.8, 129.1, 128.5, 126.7, 126.0, 125.7, 122.7, 120.8, 120.7, 119.5, 112.0, 75.4, 67.5, 67.4, 66.6, 41.6, 40.8, 38.6, 37.2, 30.17. ^{31}P-NMR (121 MHz, MeOD) δ -6.47. IT-TOF: m/z $[M+H]^+$ calcd: 967.29, found 967.30.

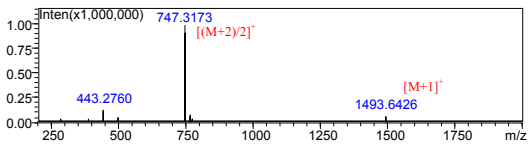
P5 (1-P PTP)

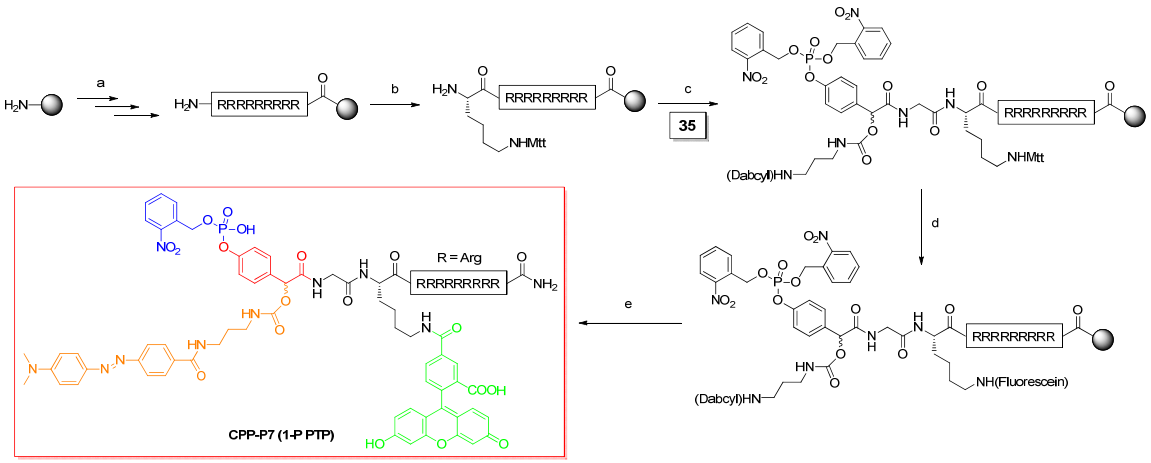




P6 (2-P PTP)

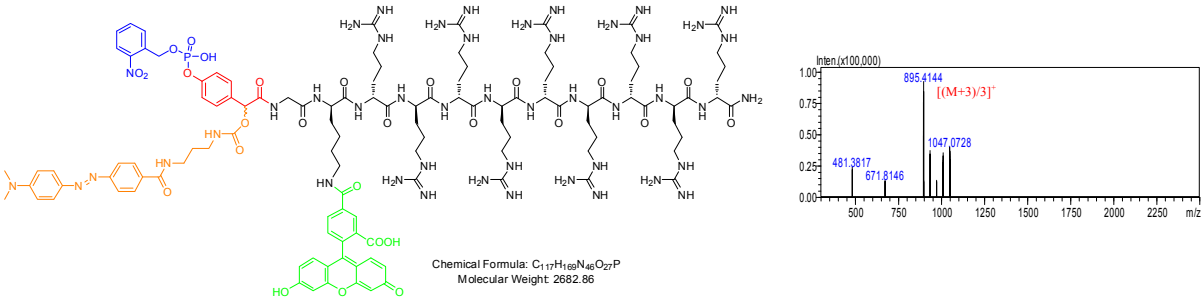






Scheme S4. Synthesis of CPP-containing **P7**. Reagents and conditions: a) i. Fmoc-Arg-OH, HBTU, HOBt, DIEA, DMF; ii. 20% piperidine in DMF; b) i. Fmoc-Lys(Mtt)-OH, HBTU, HOBt, DIEA, DMF; ii. 20% piperidine in DMF; c) i. compound **35**, HATU, HOAt, DIEA, DMF; ii. 20% piperidine in DMF; g) i. 1% TFA 5% TIS in DCM, 30 mins; ii. Fluorescein(OAc)$_2$-NHS, DIEA, DMF; iii. 20% piperidine in DMF; h). 95% TFA 2.5% TIS 2.5% H_2O, 2 hrs.

P7



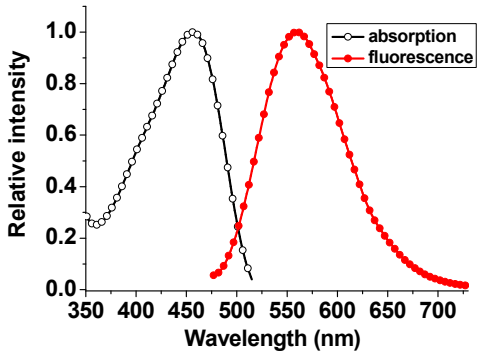
2. Optical Measurements

Table S1. Photophysical properties of Fluorescein, dye **22**, **P5** and **P6** before/after uncaging and treating with PTPB in Hepes buffer.

Sample name	$\lambda_{max}^{(ab)}$ [a]	ε	$\lambda_{max}^{(em)}$ [b]	Φ[c]	$\delta\Phi$/ GM[d]
Fluorescein	490	68000	511	0.49	30
P5 (before uncaging)	496	67200	523	0.01	1
P5 (after uncaging and treatment with PTPB)	492	79600	520	0.26	17
Dye **22**	455	32000	560	0.35	188
P6 (before uncaging)	480	35000	592	0.06	35
P6 (after uncaging and treatment with PTPB)	479	35000	569	0.26	140

[a] Peak position of the longest absorption band. [b] Peak position of emission, exited at the absorption maximum. [c] Quantum yields determined by using fluorescein aqueous NaOH (*p*H = 13) as standard, respectively. [d] The maxima two-photon action cross section values upon excitation from 750 to 860 nm in GM (1 GM = 10^{-50} cm^4 s photon^{-1}).

(a)



(b)

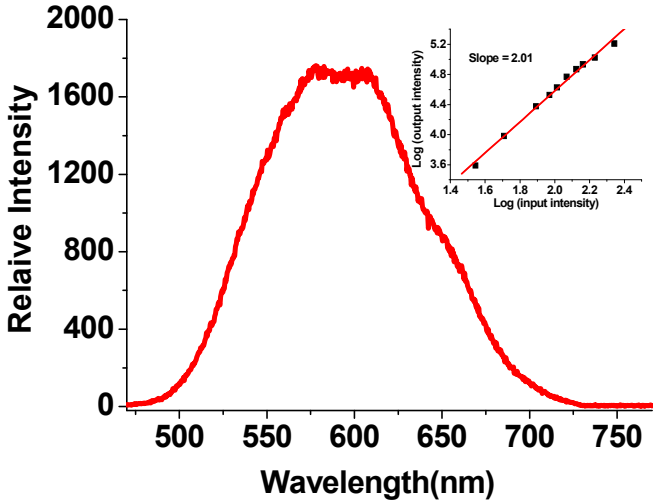


Figure S1. (a) One-photon absorption and excitation fluorescence spectra of the dye **22** in Hepes buffer; (b) Two-photon (76 MHz Ti:sapphire laser) excitation fluorescence spectra of the dye **22** at 820 nm in Hepes buffer ($C = 1.0 \times 10^{-6}$ mol L^{-1}). Inset is the power dependence of the two-photon excitation fluorescence intensity on the input intensity of dye **22** in Hepes buffer

3. Activity Assay against Different Enzymes

Enzymatic assay of pure Caspase-3 and -7

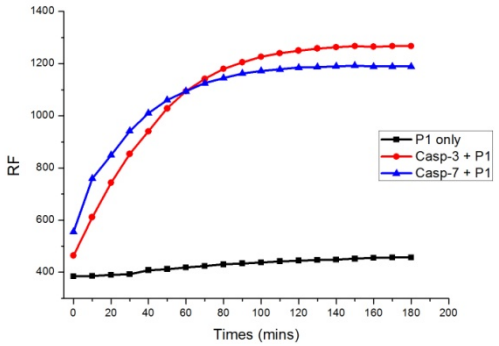


Figure S2. Enzymatic screening of **P1** against pure Caspase-3 and -7.

Table S2. Kinetic Data of **P1** with Caspase-3 and Caspase-7

	Caspase-3			Caspase-7		
	K_M (μM)	k_{cat}(s^{-1})	k_{cat}/K_M ($\mu M^{-1}\cdot s^{-1}$)	K_M (μM)	k_{cat}(s^{-1})	k_{cat}/K_M ($\mu M^{-1}\cdot s^{-1}$)
Ac-DEVD-AFC	9.09 ± 0.09	10.8 ± 0.1	1.19 ± 0.03	8.26 ± 0.08	6.8 ± 0.5	0.82 ± 0.05
P1	31.99 ± 0.13	7.1 ± 0.1	0.22 ± 0.05	69.83 ± 0.11	5.0 ± 0.3	0.07 ± 0.01

The assay conditions were as previously described.[6] Briefly, appropriate dilutions of substrate or Probe-1 were added to reaction mixtures containing enzyme and buffer in a total volume of 50 μL. Liberation of AFC or fluorescein was monitored continuously at room temperature using a BioTek Synergy 4 plate reader. Kinetic constants were computed by direct fits of the data to the Michaelis-Menton Equation using a non-linear regression via GraphPad Prism software. The value was taken in mean ± S.D. in two sets of data with duplicate.

Enzymatic assay of different PTPs

Uncaging by UV irradiation

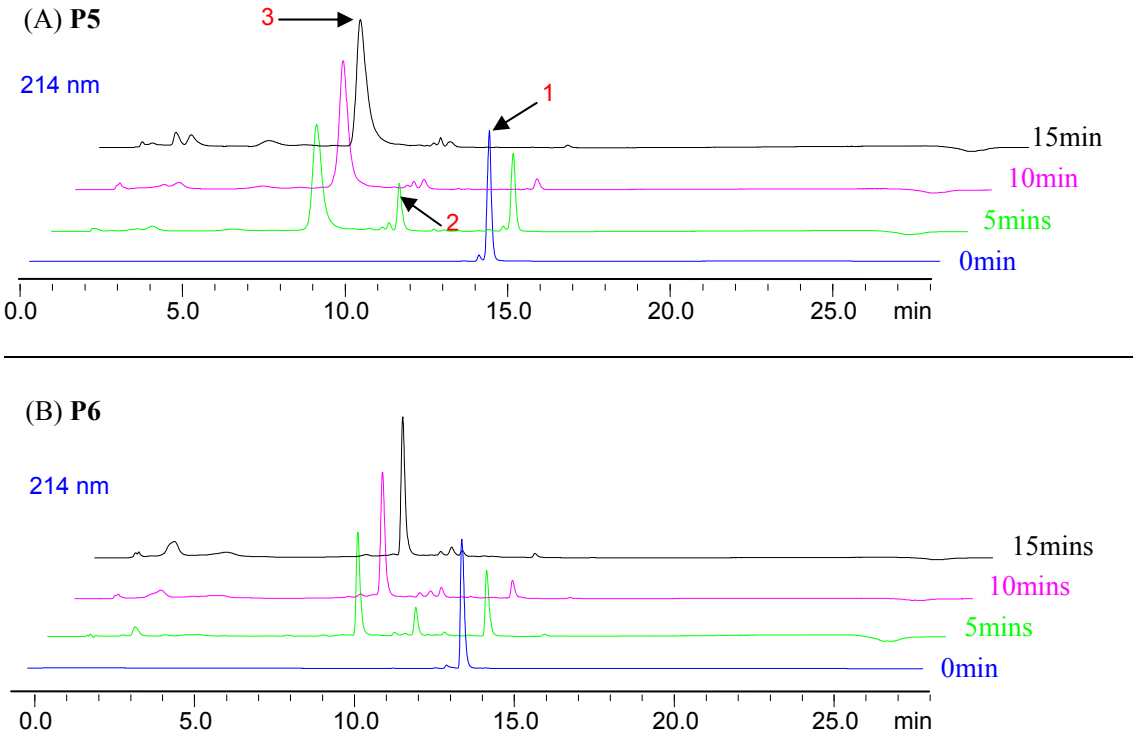


Figure S3. Uncaging experiment monitored by LCMS. (A): **P5**, (B): **P6**. The reactions were carried out in Hepes Buffer (1×) and the UV condition is 1000 μJ/cm^2. The results indicated that the reactions were completed within 15 min. Peak 1: Caged compound; Peak 2: mono-caged product; Peak 3: uncaged product.

PTP dephosphorylation

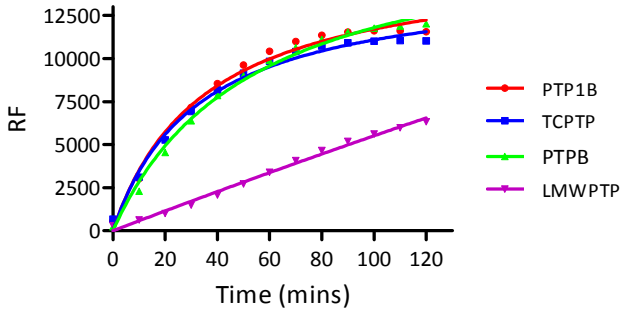


Figure S4. Enzymatic screening of uncaged **P5** against recombinant PTPs (PTP1B, TCPTP, PTPB, LMWPTP) monitored by microplate reader.

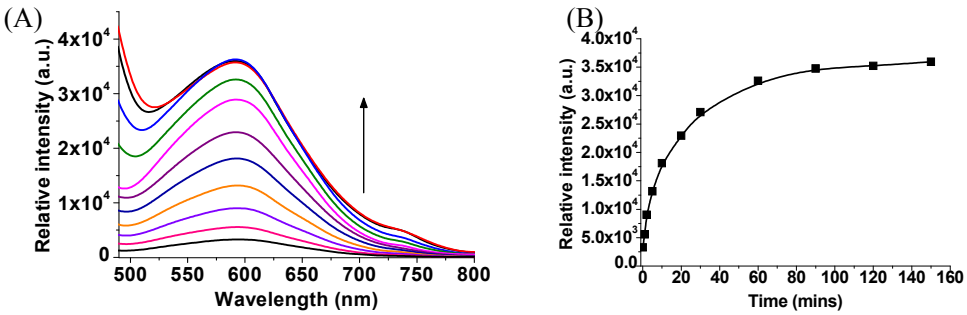


Figure S5. (A) Time-dependent emission spectra of uncaged **P6** after adding PTP1B (protein:probe = 1:40). (B) The time-dependent steady state fluorescence emission spectra of uncaged **P6** react with PTP1B from 0 min to 150 min.

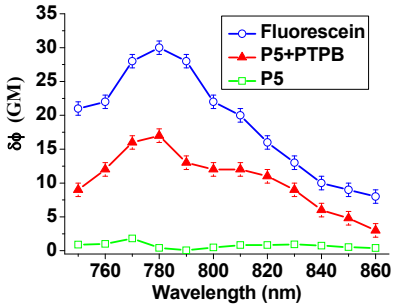


Figure S6. Two-photon action spectra of Fluorescein, uncaged **P5** + PTPB (protein:probe = 1:40) and uncaged **P5** only in Hepes buffer (1×).

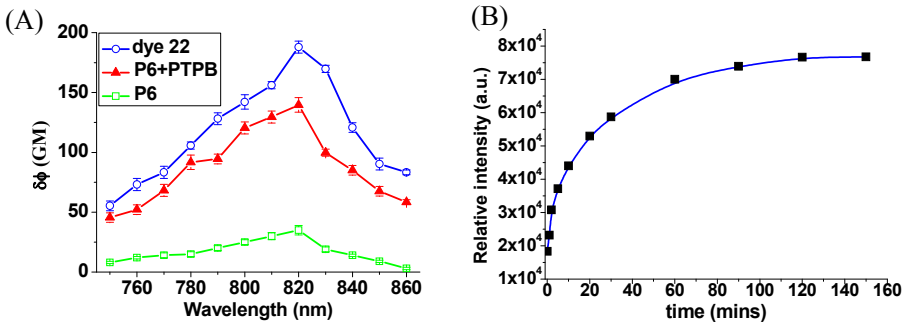


Figure S7. (A) Two-photon action spectra of dye **22**, uncaged **P6** + PTPB (protein:probe = 1:40) and uncaged **P6** only in Hepes buffer (1×). (B) The time dependent two-photon excited fluorescence emission spectra of uncaged **P6** react with PTPB from 0 to 150 min.

4. Labeling Experiments

Labeling Experiment with Pure Caspases and PTPs

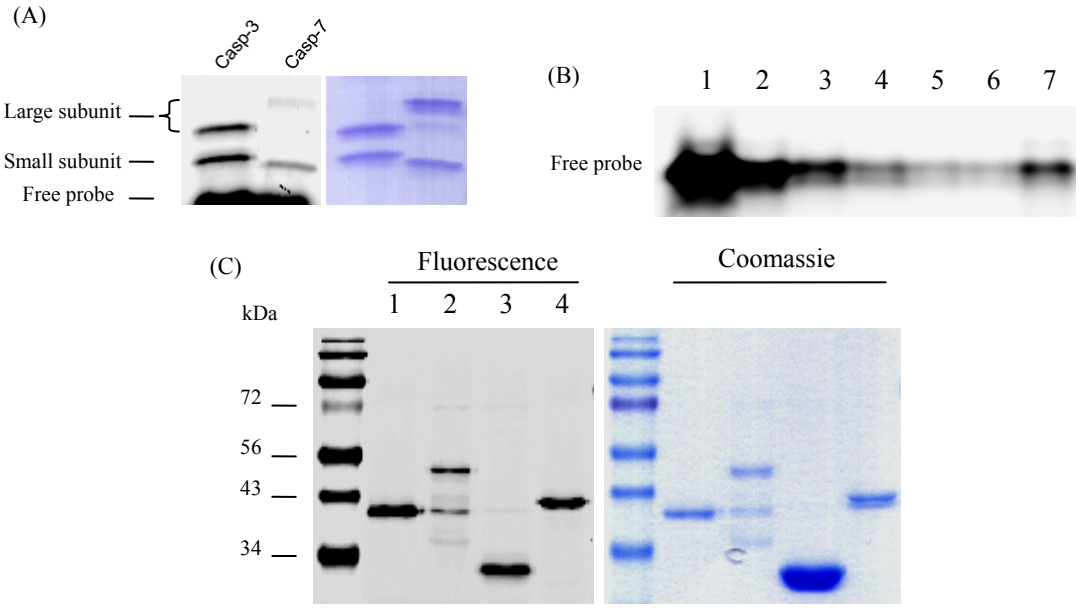


Figure S8. (A) Activity-based labeling of purified Caspase-3/7 with **P1**. The figure was reproduced in the maintext as Figure 4B. (B) Most of the fluorescence generated in (A) was from the free probe (Pathway A in Figure 2 in the maintext) and could be readily washed away from the solution. The experiment was carried out as previous described and the labeling reaction was subjected to extensive washings with PBS buffer using MW 3000 filter. Eluents as well as labeling reactions before and after the washes were loaded onto SDS-PAGE, giving the resulting fluorescent gel highlighting the band corresponding to the free probe after extensive washings. Lane 1: free probe before washing; Lane 2: 1stwash eluent; Lane 3: 2nd-wash eluent; Lane 4: 3rd-wash eluent; Lane 5: 4th-wash eluent; Lane 6: 5th-wash eluent; Lane 7: free probe after 5 washes. (C) Labeling of different PTPs with uncaged **P5**. Lane1: PTP1B, Lane2: TCPTP; lane3: PTPB; Lane4: LMWPTP.

Labeling Experiment with Heat- and Inhibitor- Inactivated Enzymes

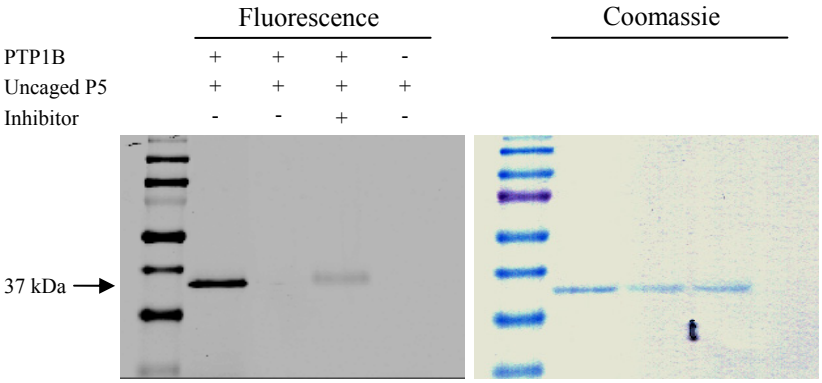


Figure S9. The labeling results with denatured or inhibited PTP1B. Lane 1: PTP1B with **P5**; Lane 2: 95 ^{o}C denatured PTP1B with **P5**; Lane 3: inhibited PTP1B (by inhibitor Na_3VO_4) with **P5**; Lane 4: **P5** only in buffer. Results indicated the labeling of PTPs with **P5** was activity-dependent. Similar experiments were done with Caspase-3/7 and results indicated that the labeling of caspases with **P1** was also activity-dependent (data not shown). Briefly, the enzyme was pre-treated with Capase-3/7 inhibitor (Calbiochem #218826, 25 μM) for 1 h or boiled at 95 ^{o}C for 15 min, respectively. Then 25 μM (final conc., caspase:probe = 1:20) of **P1** was added and the mixture was incubated at room temperature for another 3 h. These reactions were subsequently analyzed by SDS-PAGE and in-gel fluorescence scanning.

Comparison of Enzymes Activities Before and After Probe Labeling

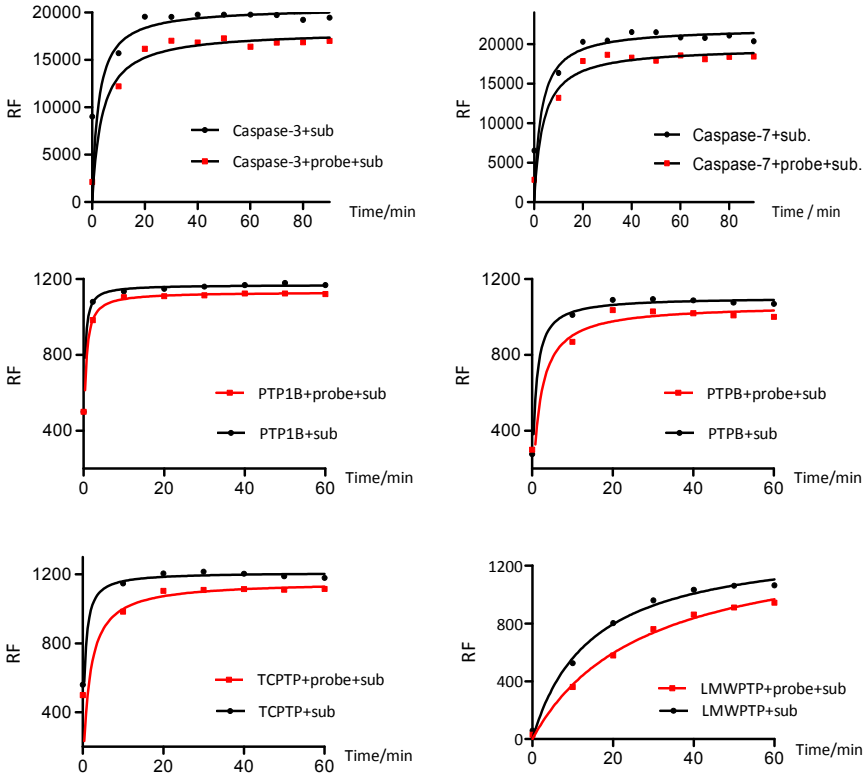


Figure S10. Comparison of enzyme's activity with and without probe labeling indicates that, no significant decrease of activity was observed.

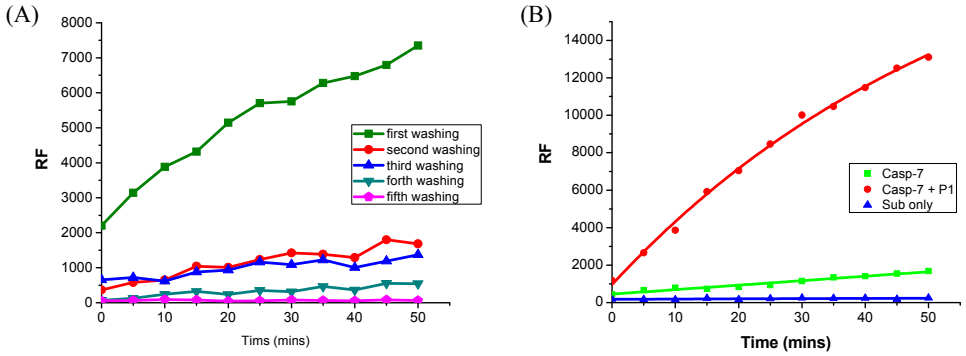


Figure S11. (A) The washing eluents were tested the caspase activity using substrate Ac-DEVD-AFC separately. The results showed that after 5 times of washings, there was almost no caspase activity remaining in the eluents, indicating all free (unlabeled) Caspase-7 has come off the beads. What remains on the beads can only be the probe-labeled Caspase-7. (B) Bead-bound (thus probe-labeled) Caspase-7 was tested using substrate Ac-DEVD-AFC; results (Red curve) indicated the probe-labeled Caspase-7 still retained most of the catalytic activity. The corresponding control beads (mixed with a control labeling reaction of Caspase-7 + DMSO) showed only background activity (Green curve). Above results were also summarized in Figure 4C in the maintext.

Lysate Labeling Experiments

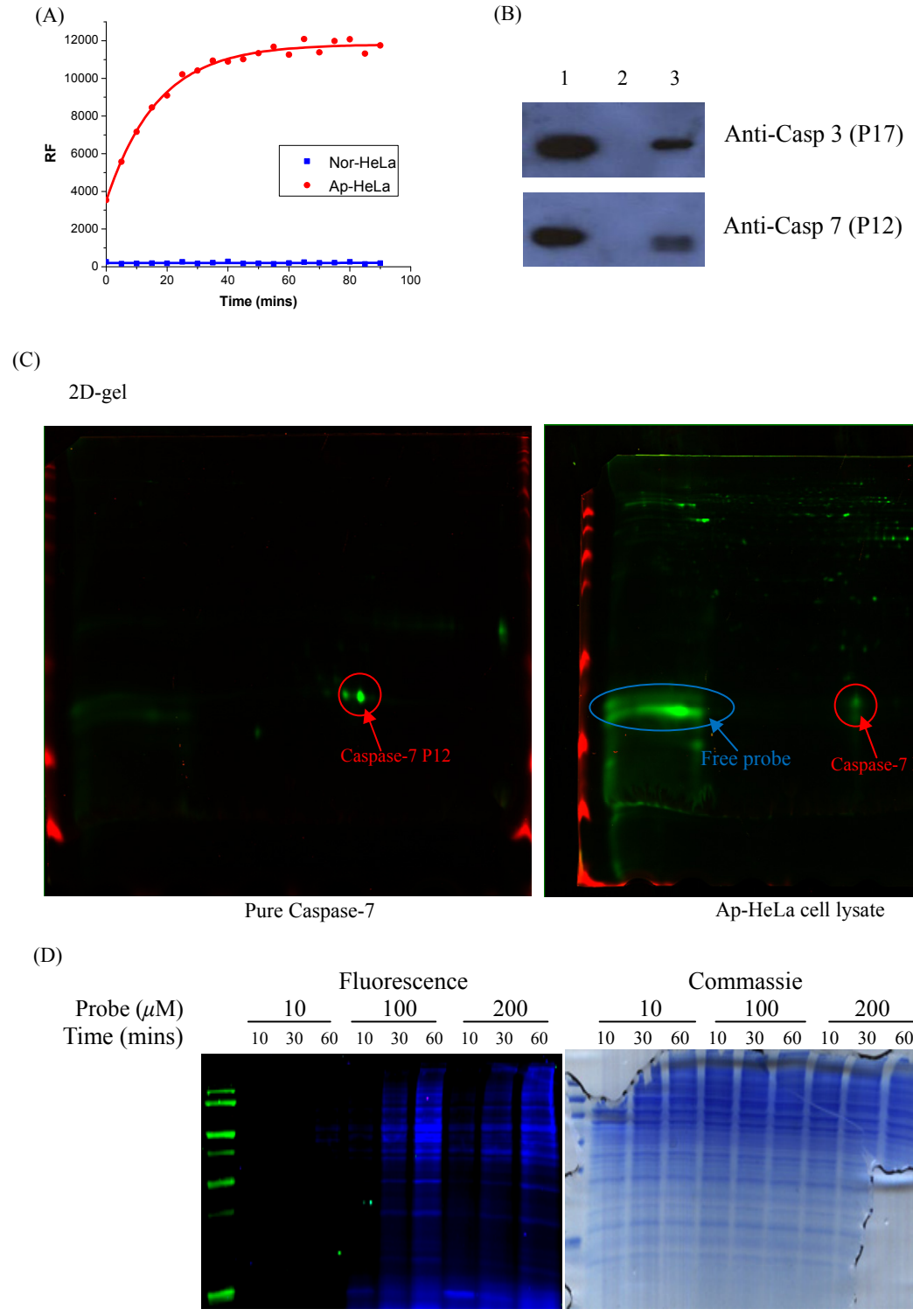


Figure S12. (A) Caspase-3/7 activity detected by fluorogenic substrate Ac-DEVD-AFC. Red: apoptotic Hela cell lysate (Ap-Hela); Blue: normal Hela cell lysate (Nor-Hela). (B) Immunoblotting results of the Hela cell lysates detected with anti Caspase-3 (P17) and anti Caspase-7 (P12) antibodies. Lane 1: pure Caspase-3 (top) or Caspase-7 (bottom); Lane 2: normal Hela cell lysate; Lane 3: apoptotic Hela cell lysate. (C) 2D-PAGE of **P1** labeled recombinant Caspase-7 (left) and apoptotic Hela cell lysates (right). Positions of the released Free Probe (Blue Oval) and labeled Caspase-7 (large subunit, Red Circle) were highlighted. Other non-specifically labeled protein bands were also clearly visible (above). (D) Labeling of Hela cell lysates with uncaged **P5** (10, 100 & 200 μM) for 10, 30 and 60 min, respectively.

5. Bioimaging Experiments

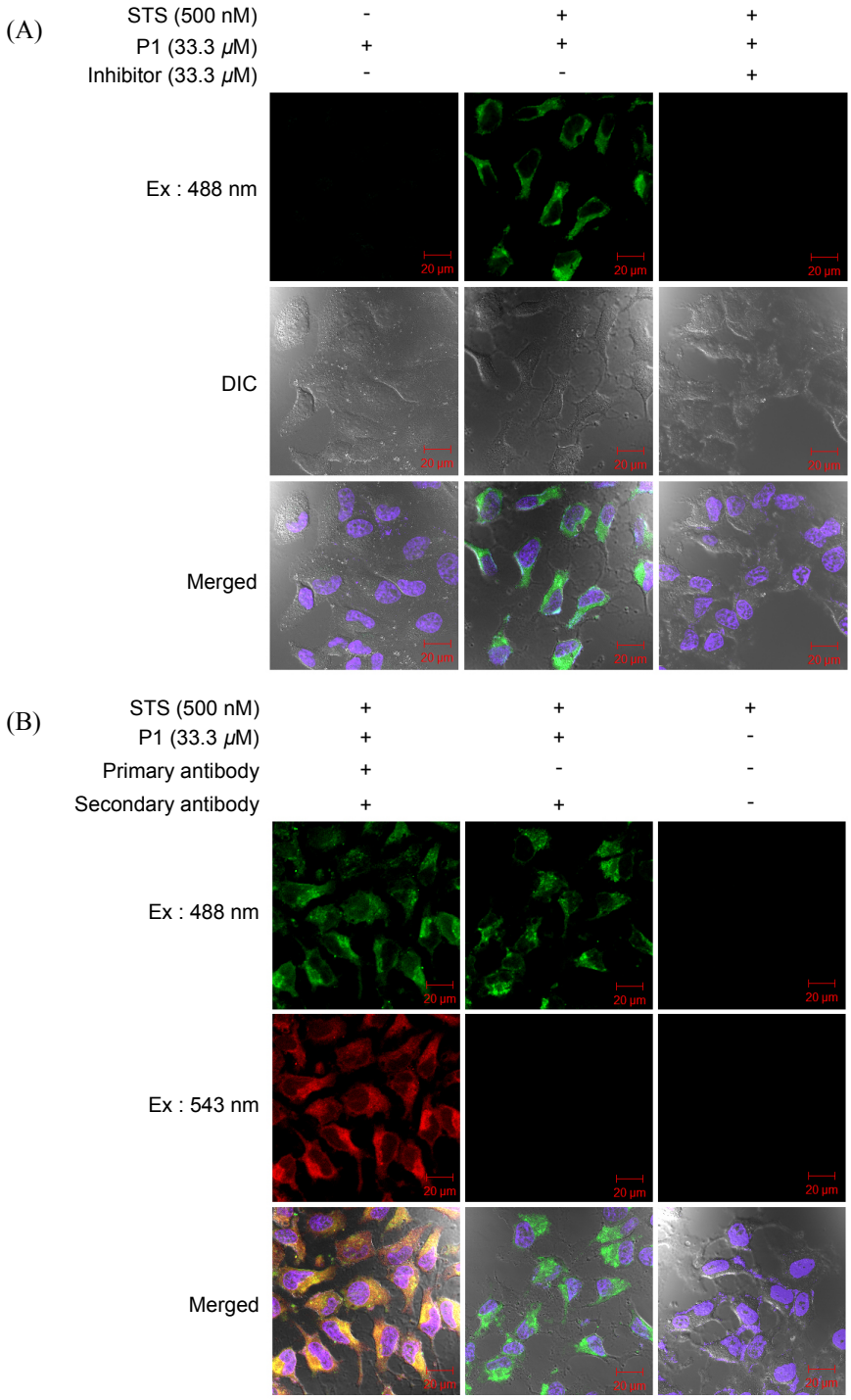


Figure S13. (A) Live cell imaging. (Left) normal Hela cells treated with **P1**; (Middle) apoptotic Hela cells treated with **P1** (33.3 μM, 0.3% DMSO); (Right) apoptotic Hela cells treated with **P1** (33.3 μM, 0.3% DMSO) and inhibitor (Calbiochem # **218826**, 33.3 μM) mixture. Scale Bar: 20 μm. All images were acquired the same way. (B) Immunofluorescence with anti-Caspase 3 antibody. (Left) Apoptotic Hela cells treated with **P1** and primary & secondary antibodies. Both 488 and 543 channels were showed strong fluorescent signals and could be merged well. (Middle): Apoptotic Hela cells treated with **P1** and secondary antibody only. The 488 channel showed the Caspase-3 activities in the cells. The 543 channel showed background level of secondary antibody. (Right) Apoptotic Hela cells only. Both 488 and 543 channels showed the background levels. Nucleus was stained with Hoechest (pseudo-colored in Blue). Parts of the images were reproduced as Figure 5B in the maintext.

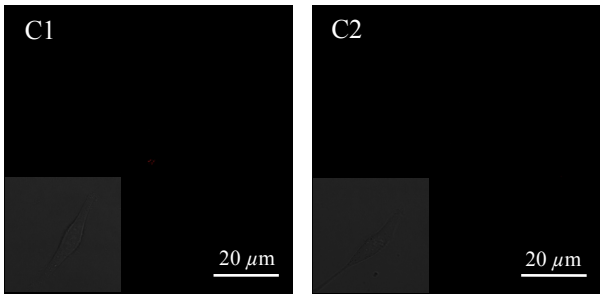


Figure S14. (A) Live cell imaging using **C1** and **C2**. The Apoptotic HeLa cells were treated with **C1** and **C2** respectively at 33.3 μM concentration. Both of them showed negative results in the 488/520 nm channel, which is consistent with our existing *in vitro* results.

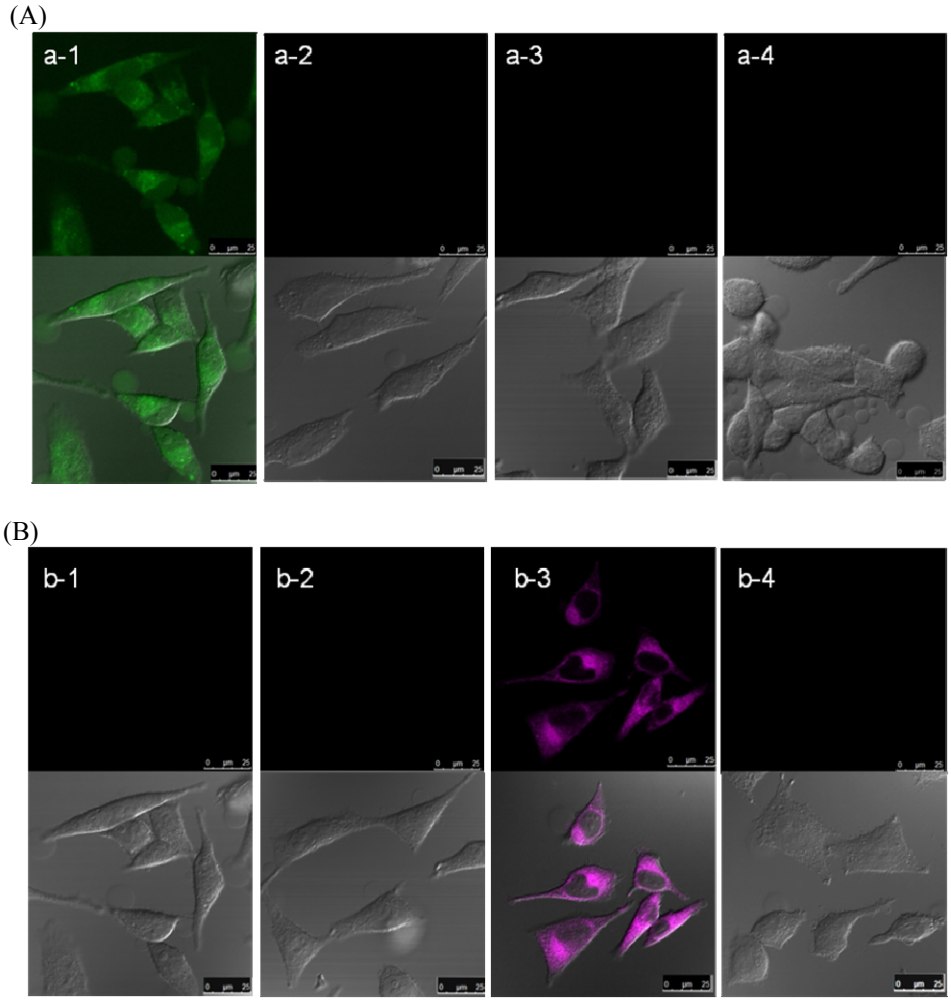


Figure S15. (A) Fluorescence microscopic images of Hela cells treated with **P5** (20 μM, 1 hr) and exposed under UV (1000 μJ/cm^2) for 10 min. A strong green fluorescence was observed when the cells were excited at 488 nm (a-1) comparing with the negative controls: **P5** without UV explosure (a-2), DMSO (a-3) and blank (a-4). All images were acquired the same way. (B) Fluorescence microscopic images of **P5** and **P6** excited at 800 nm. (b-1) **P5** with UV exposure; (b-2) **P5** without UV exposure; (b-3) **P6** with UV exposure; (b-4) **P6** without UV exposure. Scale Bar: 25 μm. All images were acquired the same way. Parts of the images were reproduced as Figure 6 in the maintext.

Endogenous PTP Activities in Hela Cells

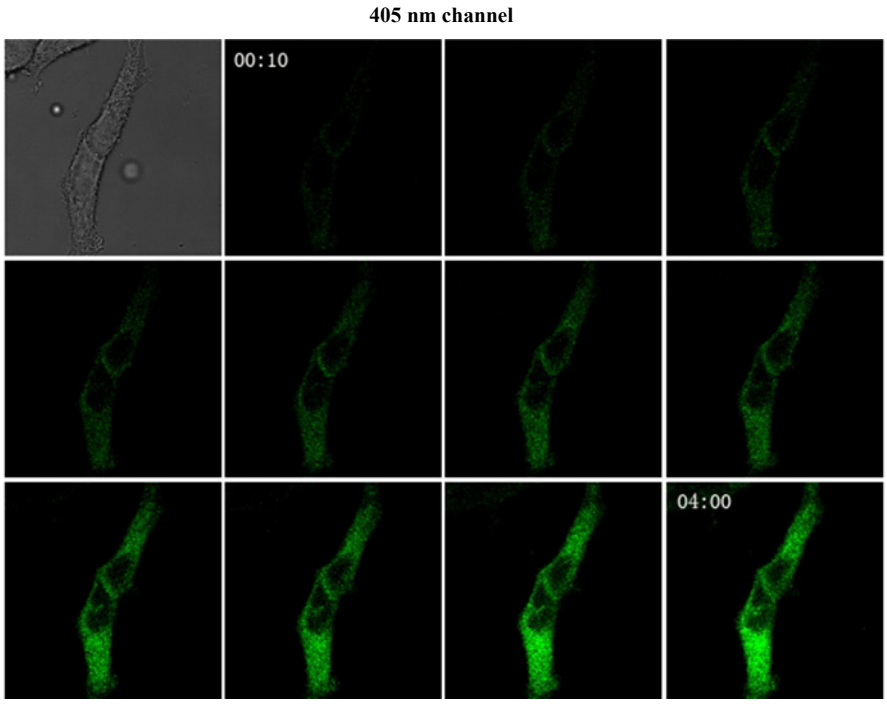


Figure S16. The ELF® 97 Endogenous Phosphatase Detection Kit (Invitrogen, E6601) was used to detect endogenous PTP activity *in vivo*. Without permeabilizing the cell, ELF® 97 was incubated with live HeLa cells. The fluorescence signals were monitored by every 20 seconds. Results indicated most endogenous PTP activities in live Hela cells were cytosolic, consistent with what was observed in Figure 6A (maintext).

Live Cell Imaging Experiments with **P7**

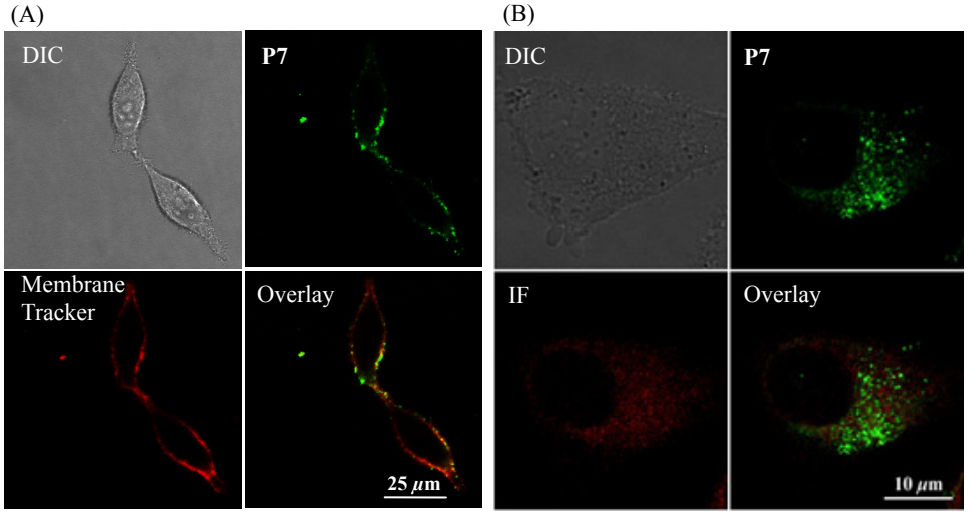
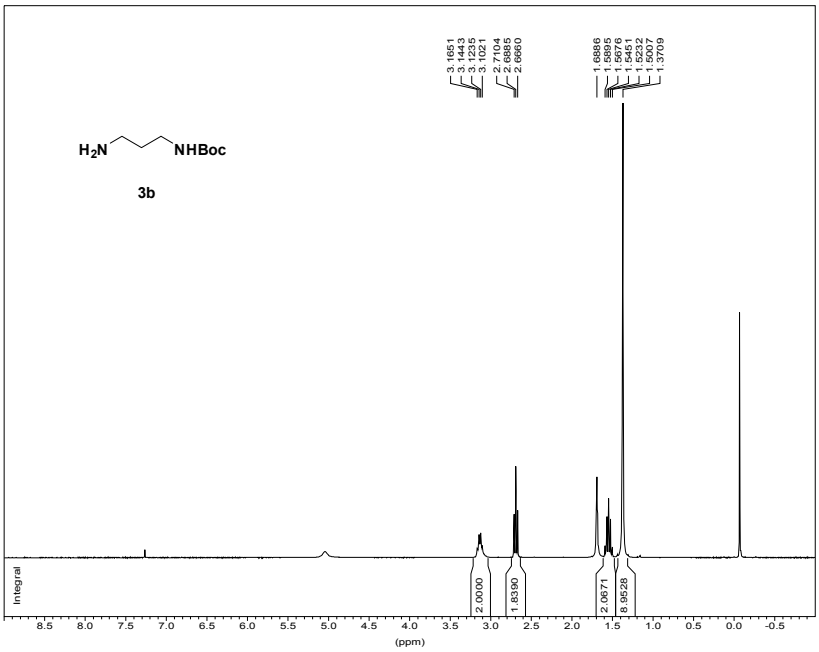


Figure S17. (A) Imaging of endogenous PTP activities with **P7** in live Hela cells. The probe was first uncaged by UV irradiation, then incubated with live Hela cells. After 1 h, the cells were imaged (green channel). Cells were further stained with membrane tracker and imaged (red channels). The merged image was reproduced in Figure 7B (Panel i). Scale Bar = 25 μm. (B) Imaging of PTP activities with **P7** in PTP1B over-expressed Hela cells (by transient transfection of pJ3H-PTP1B plasmid). **P7** was first incubated with live Hela cells for 1 h, followed by IF staining of PTP1B localization with mouse-anti-HA antibody (543 nm), then uncaged and followed by imaging, Scale bar = 10 μm. All images were acquired in the same way.

6. References

[1] R. Srinivasan, X. Huang, S. L. Ng, and S. Q. Yao, *ChemBioChem*. **2006**, *7*, 32-36.
[2] Z. J. Liu, P. Shao, Z. L. Huang, B. Liu, T. Chen, and J. G. Qin, *Chem. Commun.* **2008**, 2260.
[3] W. C. Sun, K. R. Gee, D. H. Klaubert, and R. P. Haugland, *J. Org. Chem.* **1997**, *62,* 6469-7475.
[4] C. Cativiela, *et al.*, *Appl. Cat. A: Gen.* **2004**, *274*, 9-14.
[5] M. Rubinstein, and A. Ptchornik, *Tetrhedron* **1975**, *31*, 2107-2110.
[6] S. L. Ng, P.-Y. Yang, K.Y.-T. Chen, R. Srinivasan, S.Q. Yao, *Org. Biomol. Chem.*, **2008**, *6*, 844-847.

1H normal range AC300 BocNH---NH2



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*** 1D NMR Plot Parameters ***
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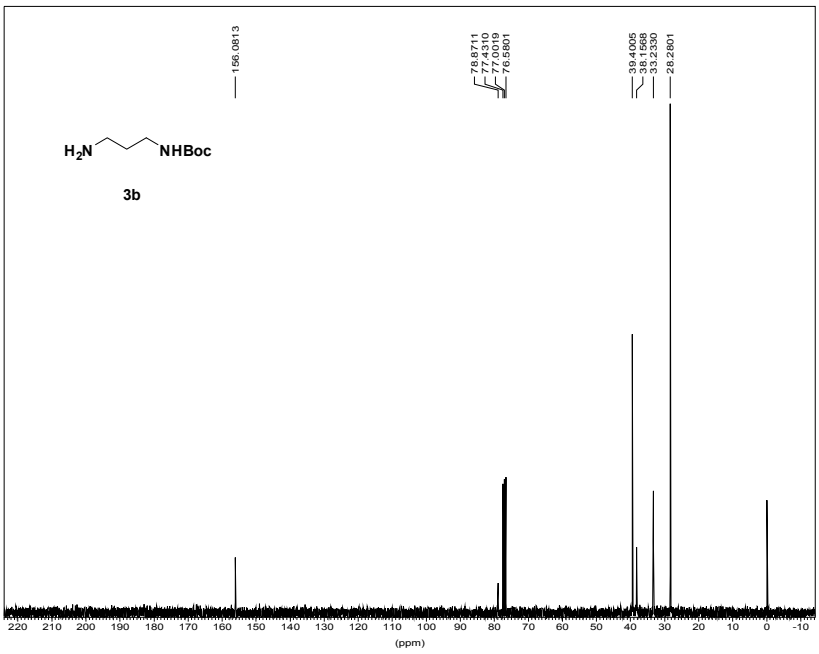
13C Standard AC300 BocNH---NH2



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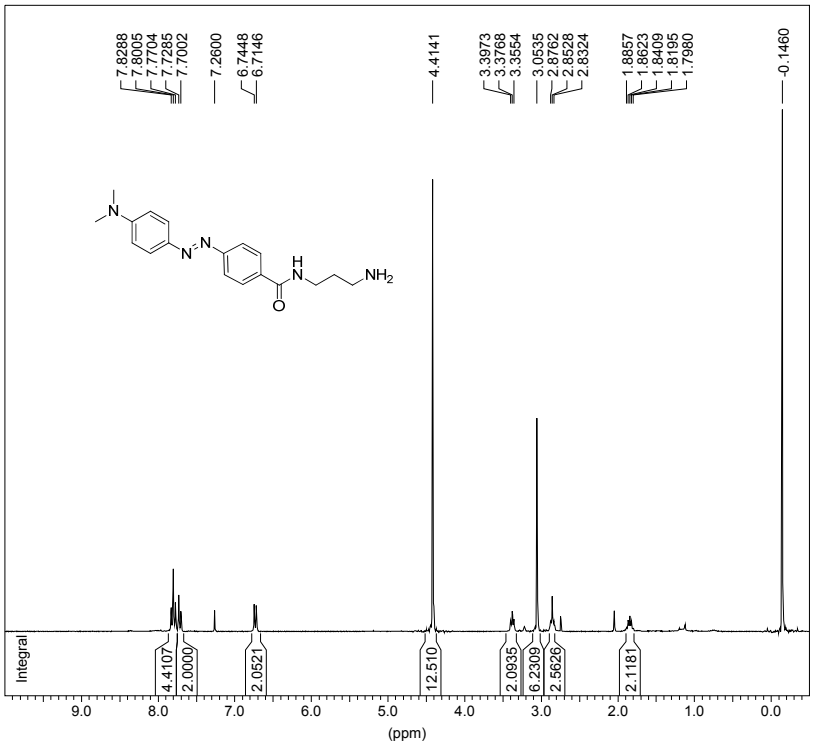

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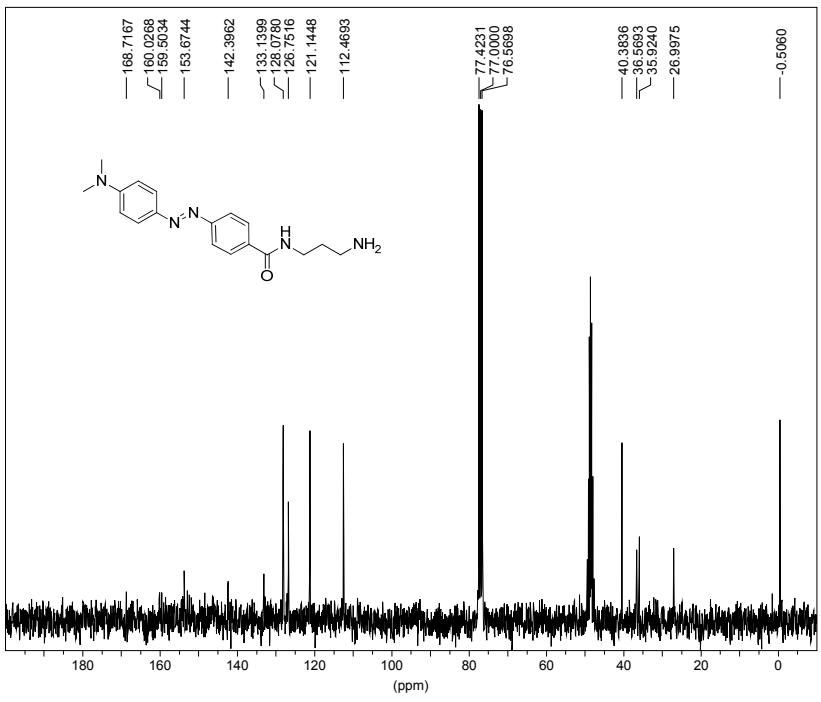
nv15suy
dabcyl-linkerNH2



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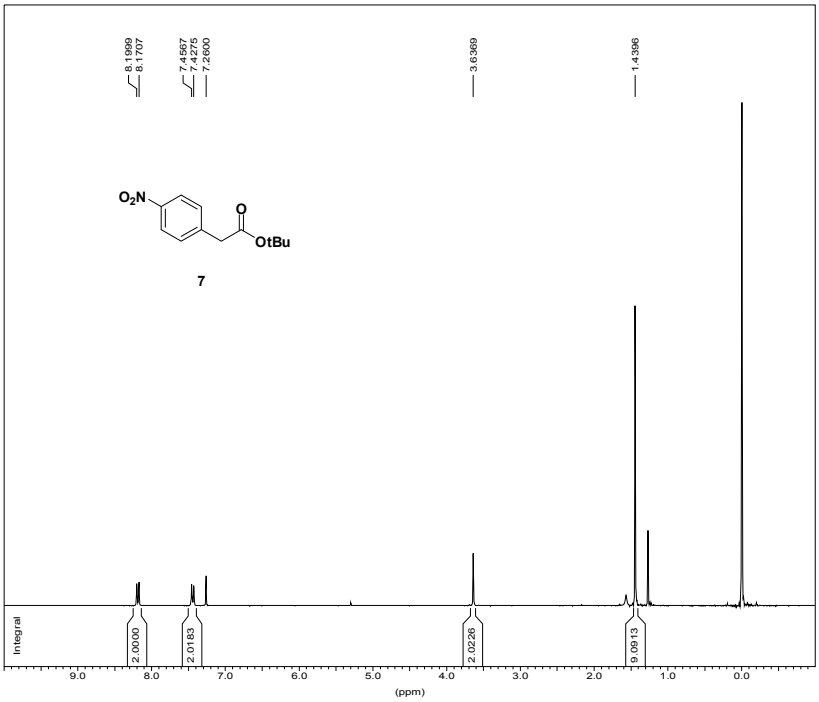
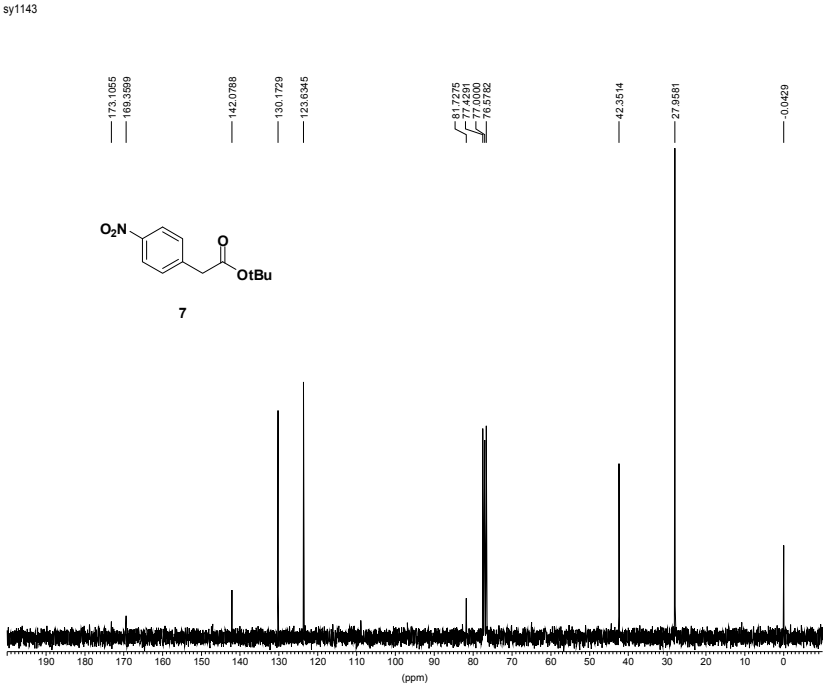
NS	:	106
NUCLEUS	:	off
O1	:	6792.09 Hz
PULPROG	:	zgdc
SFO1	:	75.4745111 MHz
SOLVENT	:	CDCl3
SW	:	234.9203 ppm
TD	:	32768
TE	:	299.2 K
*** Processing Parameters ***		
LB	:	3.00 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off



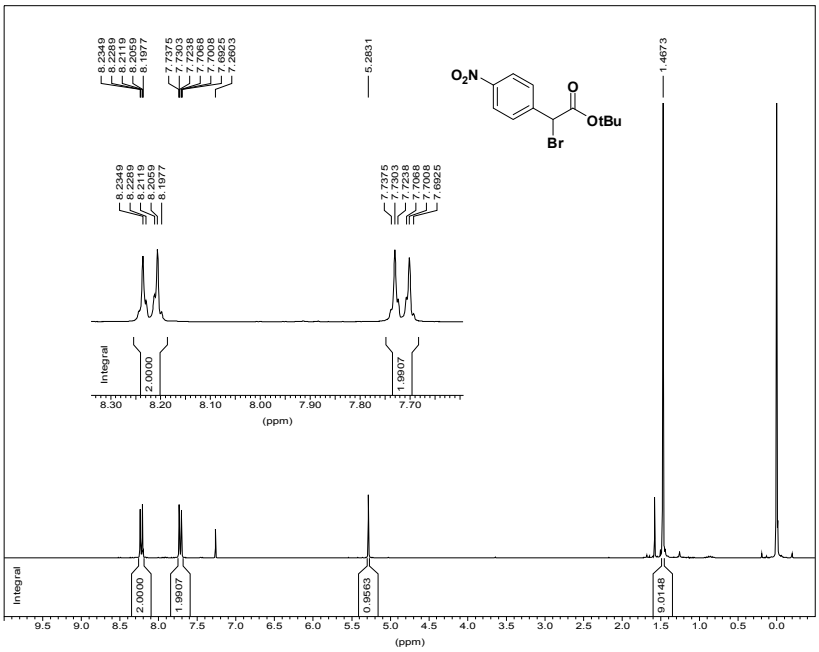
*** Current Data Parameters ***
NAME : fe09hmy
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 00:14:02
DATE_d : Feb 09 2009
NS : 8
SFO1 : 300.1312500 MHz
SOLVENT : DMSO
*** Processing Parameters ***
SF : 300.1298200 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 10.00 ppm
Stop : -1.00 ppm
YScale : 100.00 %
SR : -180.04 Hz
Hz_cm : 158.72

13C Standard AC300
de01suy
sy1143



*** Current Data Parameters ***
NAME : de01suy
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
AQ_mod : dqd
AUNM : au_zg
BF1 : 75.4677490 MHz
BF2 : 300.1300000 MHz
BF3 : 75.4677490 MHz
DATE_t : 19:47:46
DATE_d : Nov 30 2009
DBP07 : edit
INSTRUM : spect
NS : 204
O1 : 7924.11 Hz
O2 : 1200.52 Hz
PARMODE : 1D
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
SW : 238.2968 ppm
TE : 298.6 K
*** Processing Parameters ***
AZFW : 0.500 ppm
LB : 1.00 Hz
MAXI : 10000.00 cm
MI : 0.00 cm
PC : 1.40
WDW : EM
*** 1D NMR Plot Parameters ***
Start : 224.13 ppm
Stop : -14.20 ppm
Hz_cm : 848.38
AQ_time : 0.9109500 sec

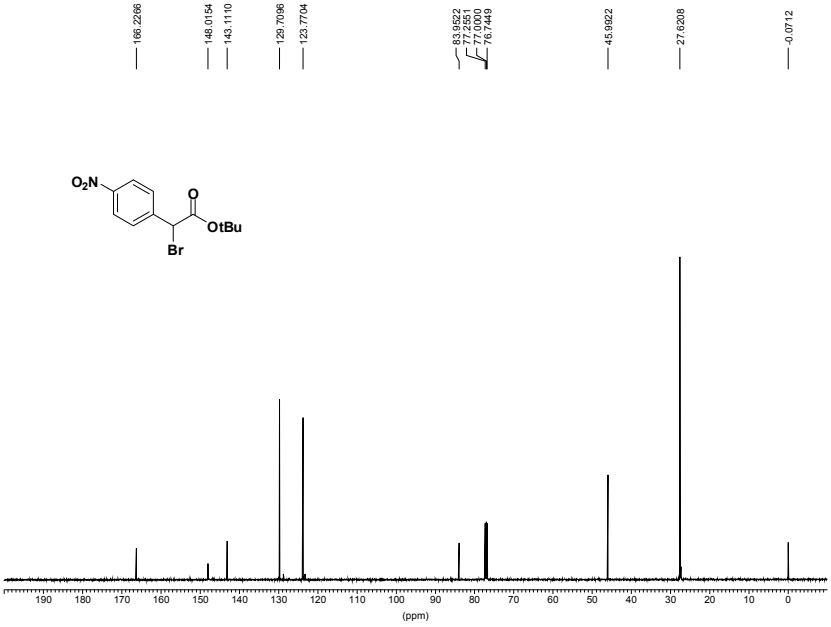
1H normal range AC300 Br-Tri(NO2)-OtBu



*** Current Data Parameters ***

NAME	:	ju09hmy
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
DATE_t	:	23:37:50
DATE_d	:	Jun 08 2009
NS	:	8
SFO1	:	300.1318534 MHz
SOLVENT	:	CDCl3
*** Processing Parameters ***		
SF	:	300.1300116 MHz
XDIM	:	8192
*** 1D NMR Plot Parameters ***		
Start	:	10.00 ppm
Stop	:	-0.50 ppm
YScale	:	100.00 %
SR	:	11.64 Hz
Hz_cm	:	151.50

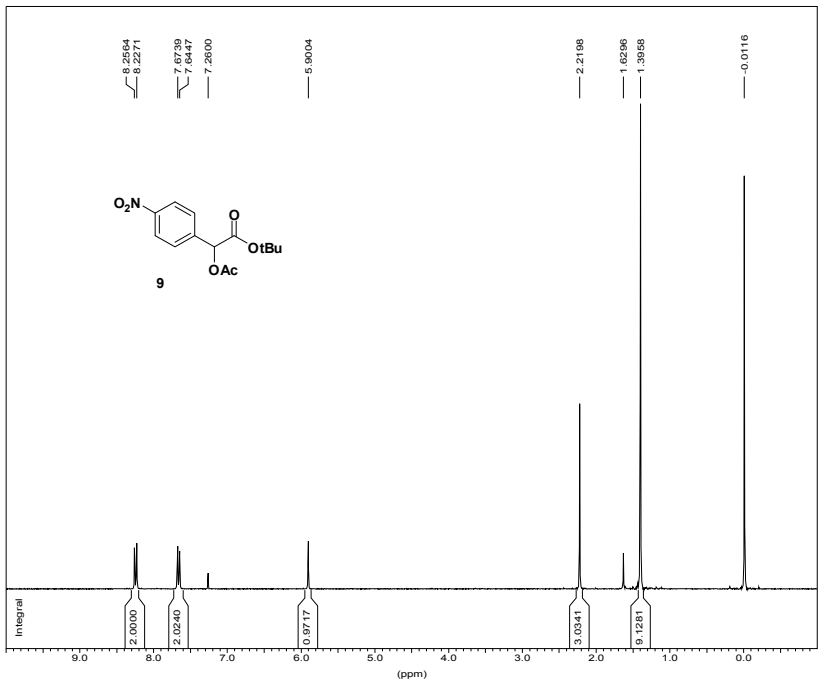
13C AMX500
suy1213 2
NO2-Br-OtBu



*** Current Data Parameters ***

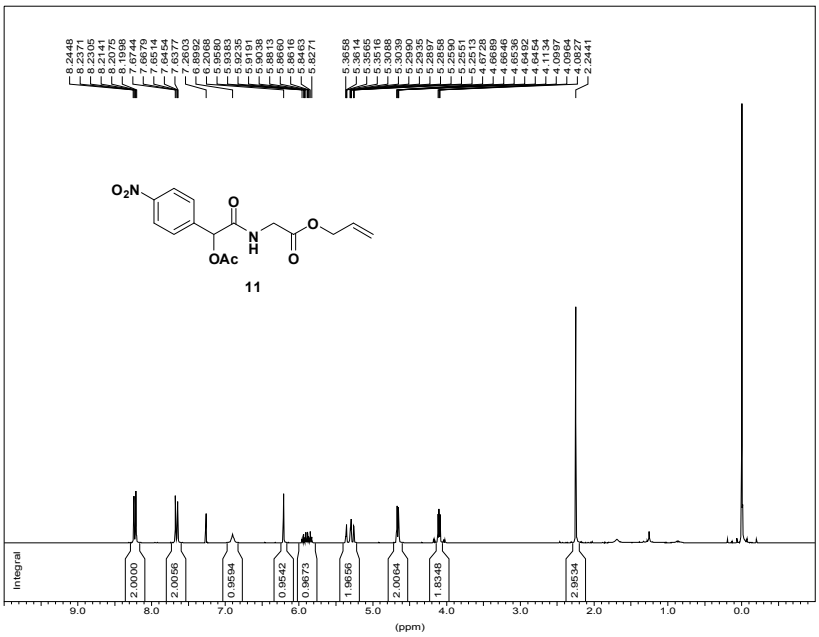
NAME	:	suy1213
EXPNO	:	2
PROCNO	:	1
*** Acquisition Parameters ***		
AQ_mod	:	qsim
AUNM	:	au_zg
BF1	:	125.7577890 MHz
BF2	:	500.1300000 MHz
BF3	:	125.7577890 MHz
DATE_t	:	23:24:22
DATE_d	:	Dec 12 2010
DBP07	:	edit
INSTRUM	:	av500
NS	:	404
O1	:	13204.57 Hz
O2	:	2000.52 Hz
PARMODE	:	1D
SFO1	:	125.7709936 MHz
SOLVENT	:	CDCl3
SW	:	238.7675 ppm
TE	:	300.0 K
*** Processing Parameters ***		
AZFW	:	0.500 ppm
LB	:	1.00 Hz
MAXI	:	10000.00 cm
MI	:	0.00 cm
PC	:	1.40
WDW	:	EM
*** 1D NMR Plot Parameters ***		
Start	:	223.98 ppm
Stop	:	-14.45 ppm
Hz_cm	:	1414.35
AQ_time	:	1.0911740 sec

AcO-Tri(NO2)-OtBu



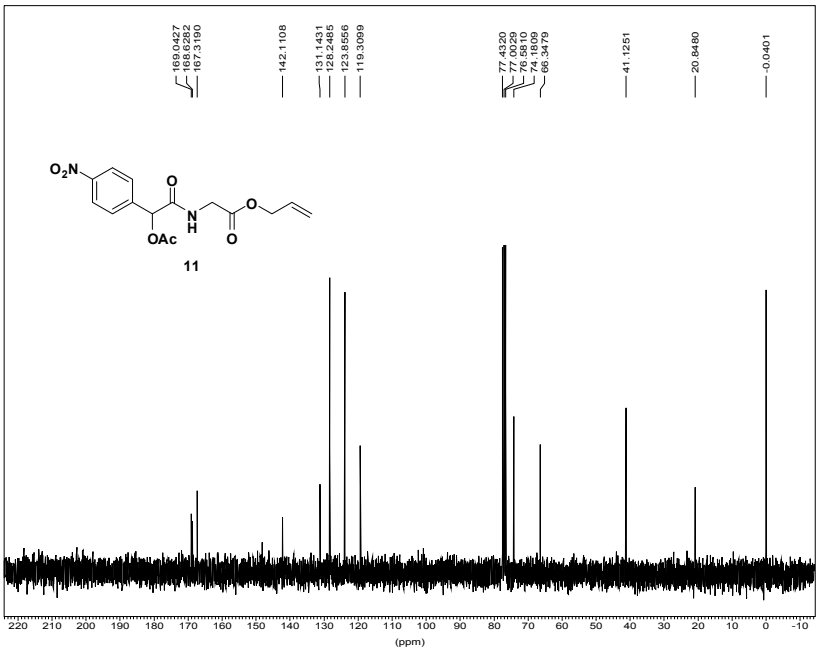
*** Current Data Parameters ***
NAME : ma24hmy
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 21:49:09
DATE_d : Mar 23 2009
NS : 8
SFO1 : 300.1312500 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 300.1298200 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 10.00 ppm
Stop : -1.00 ppm
YScale : 100.00 %
SR : -180.04 Hz
Hz_cm : 158.72

1H normal range AC300 AcO-Tri(NO2)-Gly-Allyl



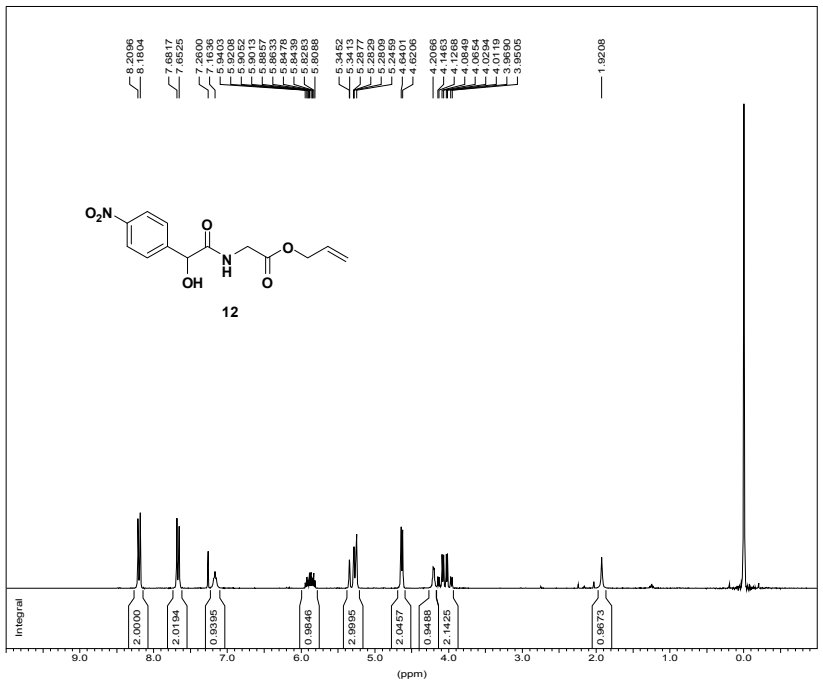
*** Current Data Parameters ***
NAME : fe18hmy
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 01:19:16
DATE_d : Feb 18 2009
NS : 8
SFO1 : 300.1318534 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 300.1300121 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 10.00 ppm
Stop : -1.00 ppm
YScale : 50.00 %
SR : 12.13 Hz
Hz_cm : 158.72

13C Standard AC300 AcO-Tri(NO2)-Gly-Allyl



*** Current Data Parameters ***
NAME : fe18hmy
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 01:22:26
DATE_d : Feb 18 2009
NS : 100
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 75.4677520 MHz
XDIM : 64
*** 1D NMR Plot Parameters ***
Start : 224.12 ppm
Stop : -14.46 ppm
YScale : 144.58 %
SR : 3.00 Hz
Hz_cm : 865.64

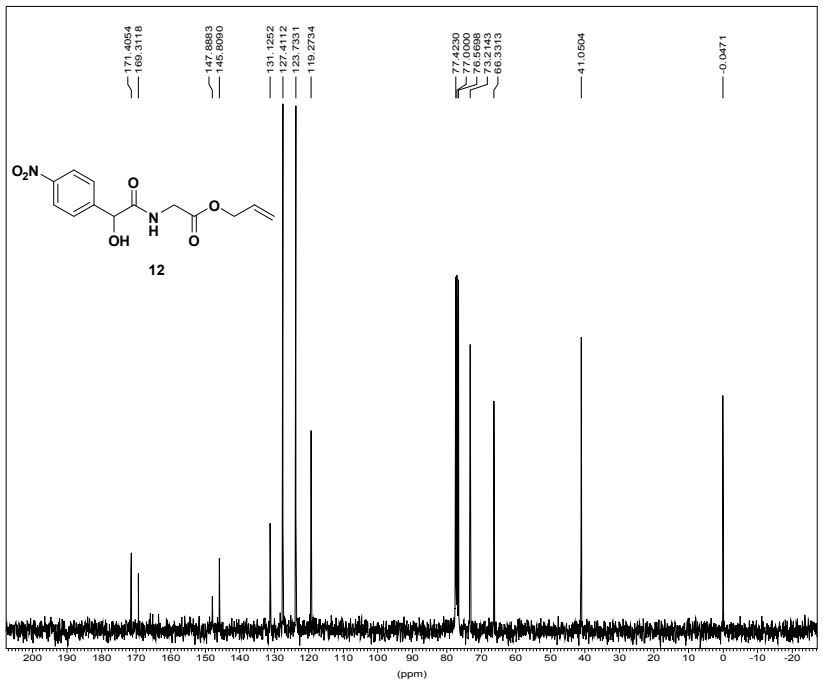
HO-Tri(NO2)-Gly(Allyl)



12

*** Current Data Parameters ***
NAME : ma24hmy
EXPNO : 3
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 22:00:30
DATE_d : Mar 23 2009
NS : 8
SFO1 : 300.1312500 MHz
SOLVENT : DMSO
*** Processing Parameters ***
SF : 300.1298200 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 10.00 ppm
Stop : -1.00 ppm
YScale : 50.00 %
SR : -180.04 Hz
Hz_cm : 158.72

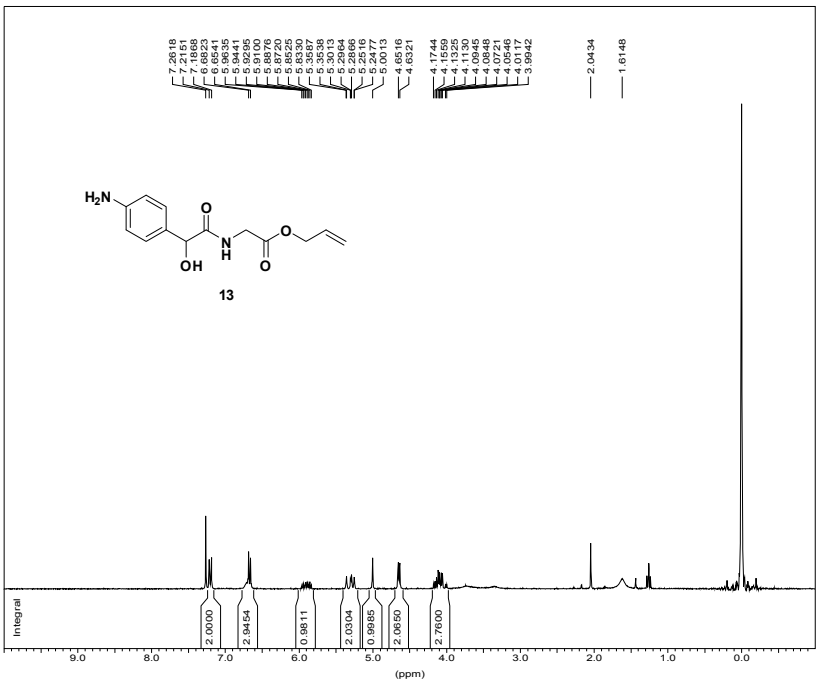
HO-Tri(NO2)-Gly(Allyl)



12

*** Current Data Parameters ***
NAME : ma24hmy
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 22:03:19
DATE_d : Mar 23 2009
NS : 200
SFO1 : 75.4745111 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 75.4677045 MHz
XDIM : 64
*** 1D NMR Plot Parameters ***
Start : 207.66 ppm
Stop : -27.28 ppm
YScale : 100.00 %
SR : -14.53 Hz
Hz_cm : 852.43

HO-Tri(NH2)-Gly-Allyl



*** Current Data Parameters ***

NAME	:	ma18hmy
EXPNO	:	1
PROCNO	:	1

*** Acquisition Parameters ***

DATE_t	:	00:11:30
DATE_d	:	Mar 18 2009
NS	:	8
SFO1	:	300.1312500 MHz
SOLVENT	:	CDCl3

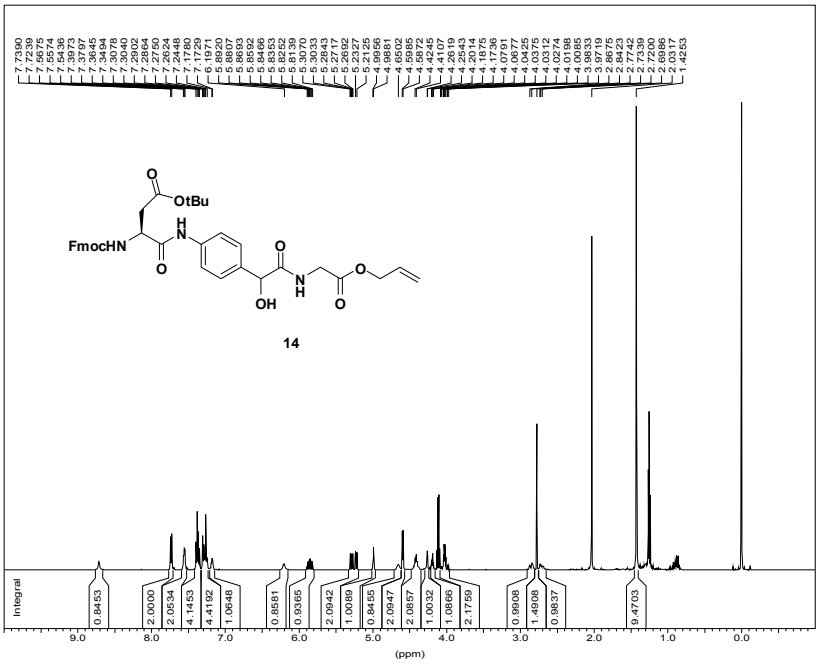
*** Processing Parameters ***

SF	:	300.1298194 MHz
XDIM	:	8192

*** 1D NMR Plot Parameters ***

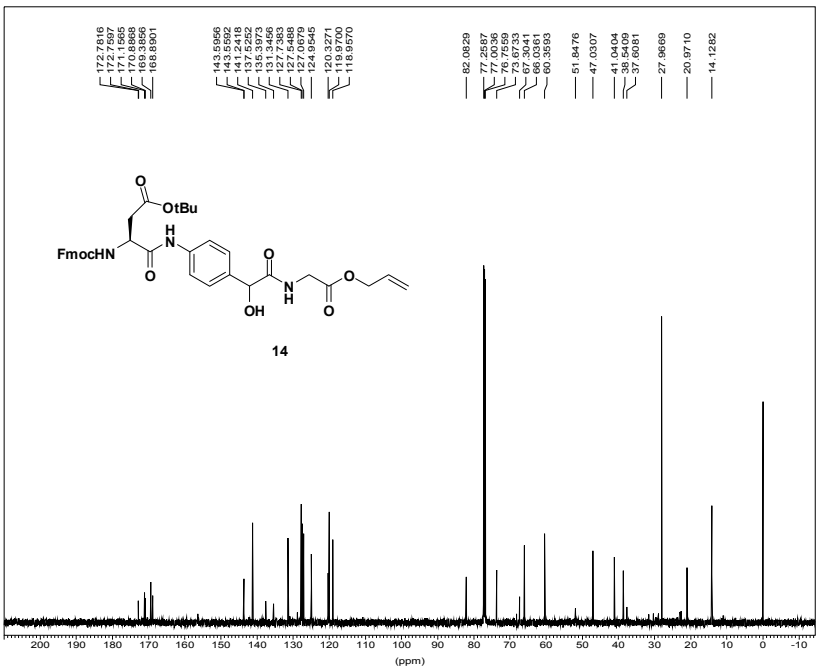
Start	:	10.00 ppm
Stop	:	-1.00 ppm
YScale	:	25.00 %
SR	:	-180.58 Hz
Hz_cm	:	158.72

1H AMX500
HO-Tri(D)-Gly(Allyl)



*** Current Data Parameters ***
NAME : my0309
EXPNO : 1
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 01:36:08
DATE_d : Mar 09 2009
NS : 8
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 500.1300125 MHz
XDIM : 8192
*** 1D NMR Plot Parameters ***
Start : 10.00 ppm
Stop : -1.00 ppm
YScale : 50.00 %
SR : 12.47 Hz
Hz_cm : 264.49

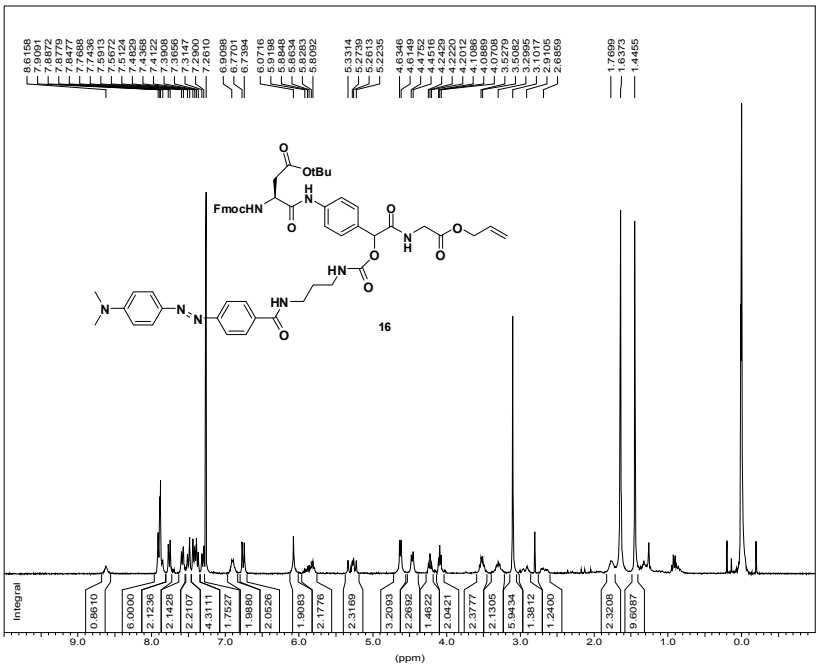
13C AMX500
HO-Tri(D)-Gly(Allyl)



*** Current Data Parameters ***
NAME : my0309
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 01:41:13
DATE_d : Mar 09 2009
NS : 214
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 125.7577961 MHz
XDIM : 64
*** 1D NMR Plot Parameters ***
Start : 210.01 ppm
Stop : -14.69 ppm
YScale : 146.30 %
SR : 7.12 Hz
Hz_cm : 1358.54

1H normal range AC300

Dabcyl-3C-Tri(D)-Gly(Allyl)



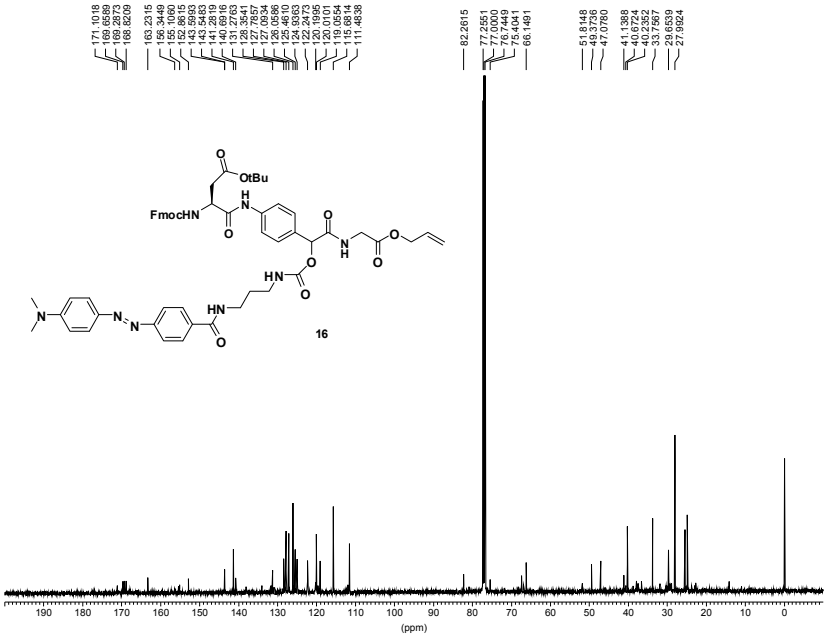
*** Current Data Parameters ***

NAME	:	ap22hmy
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
DATE_t	:	20:53:17
DATE_d	:	Apr 21 2009
DBPNAM0	:	
INSTRUM	:	spect
LOCNUC	:	2H
NS	:	16
NUCLEUS	:	off
O1	:	1853.43 Hz
SFO1	:	300.1318534 MHz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

13C AMX500

suy1213 6

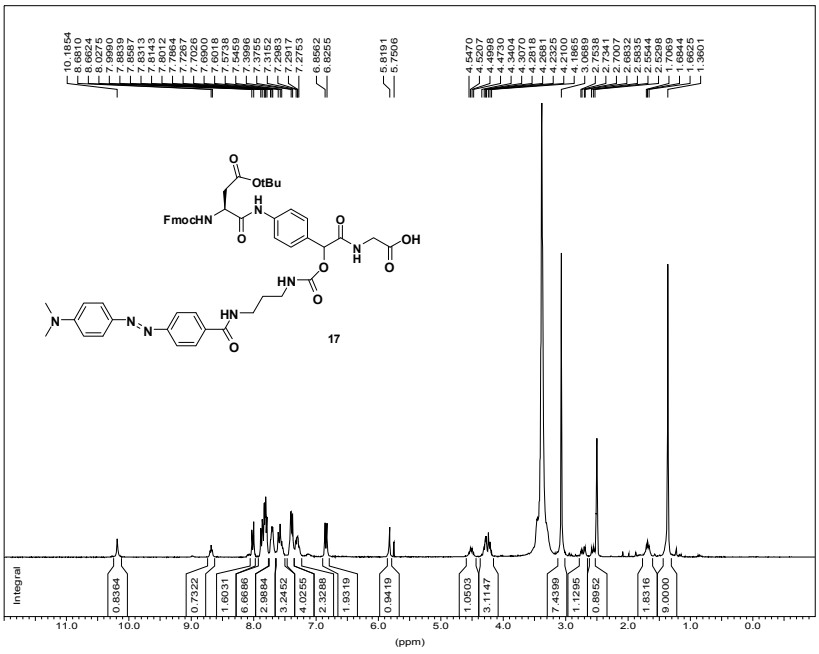
Dabcyl-Asp-Allyl(before deallylation)



*** Current Data Parameters ***

NAME	:	suy1213
EXPNO	:	6
PROCNO	:	1
*** Acquisition Parameters ***		
AQ_mod	:	qsim
AUNM	:	au_zg
BF1	:	125.7577890 MHz
BF2	:	500.1300000 MHz
BF3	:	125.7577890 MHz
DATE_t	:	00:32:49
DATE_d	:	Dec 13 2010
DBP07	:	edit
INSTRUM	:	av500
NS	:	2608
O1	:	13204.57 Hz
O2	:	2000.52 Hz
PARMODE	:	1D
SFO1	:	125.7709936 MHz
SOLVENT	:	CDCl3
SW	:	238.7675 ppm
TE	:	300.0 K
*** Processing Parameters ***		
AZFW	:	0.500 ppm
LB	:	1.00 Hz
MAXI	:	10000.00 cm
MI	:	0.00 cm
PC	:	1.40
WDW	:	EM
*** 1D NMR Plot Parameters ***		
Start	:	224.36 ppm
Stop	:	-14.43 ppm
Hz_cm	:	1416.51
AQ_time	:	1.0911740 sec

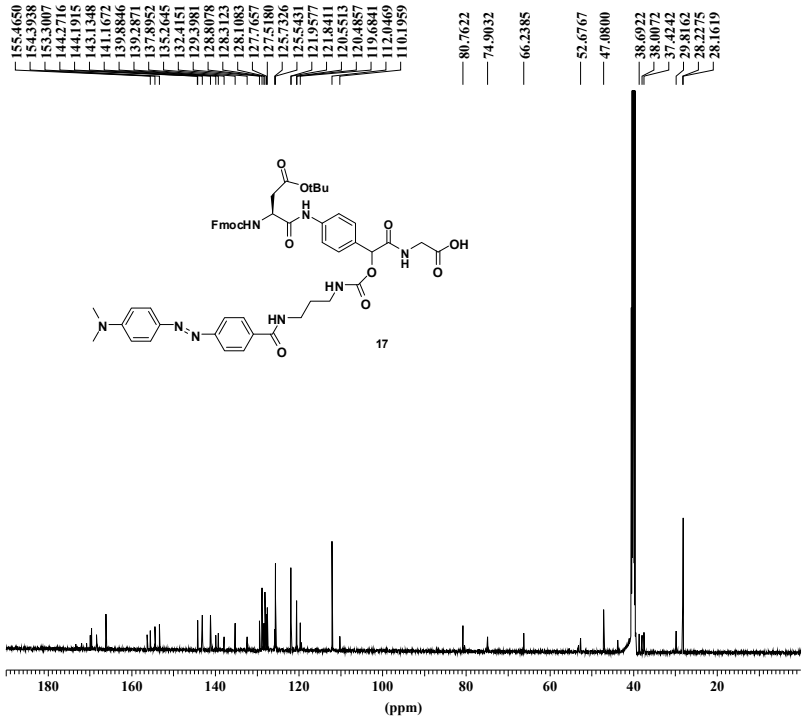
1H normal range AC300
Dabcyl-3C-Tri(D)-Gly-COOH



*** Current Data Parameters ***

NAME	:	ma20hmy
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
AQ_mod	:	dqd
AUNM	:	au_zg
BF1	:	300.1300000 MHz
BF2	:	300.1300000 MHz
BF3	:	300.1300000 MHz
BYTORDA	:	0
CPDPRG	:	
CPDPRGB	:	
CPDPRGT	:	
DATE_t	:	03:21:08
DATE_d	:	Mar 20 2009
DBP07	:	edit
DBPNAM0	:	
DBPNAM1	:	
DBPNAM2	:	
DBPNAM3	:	
DBPNAM4	:	
DBPNAM5	:	
DBPNAM6	:	
DBPNAM7	:	
DE	:	6.0 usec
DECBNUC	:	off
DECIM	:	32
DECNUC	:	off
DECSTAT	:	DO
DIGTYP	:	8
DP07	:	edit
DPNAME0	:	
DPNAME1	:	

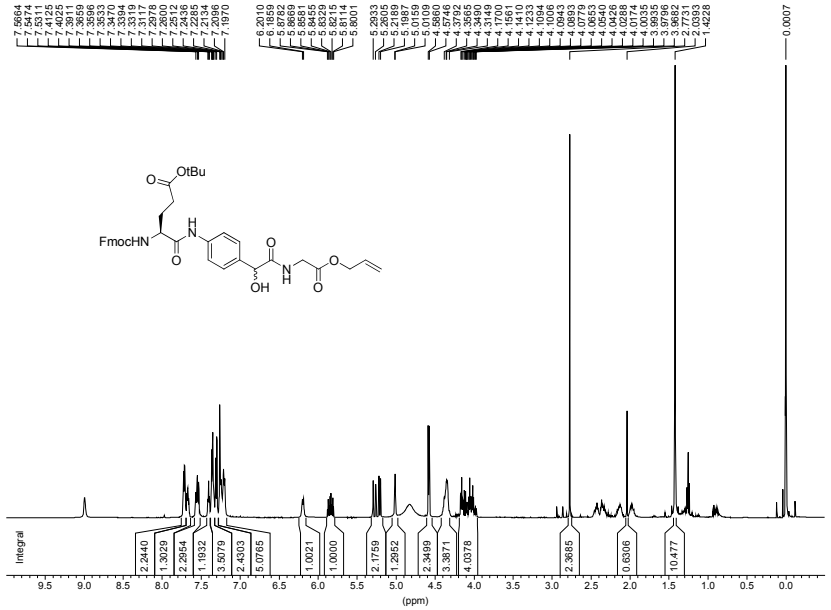
13C AMX500 Dabcyl-3C-Tri(D)-COOH



***** Current Data Parameters *****

NAME	**:**	**my1228**
EXPNO	**:**	**2**
PROCNO	**:**	**1**
***** Acquisition Parameters *****		
LOCNUC	**:**	**2H**
NS	**:**	**15901**
NUCLEUS	**:**	**off**
O1	**:**	**13204.57 Hz**
PULPROG	**:**	**zgpg30**
SFO1	**:**	**125.7709936 MHz**
SOLVENT	**:**	**DMSO**
SW	**:**	**238.7675 ppm**
TD	**:**	**65536**
TE	**:**	**300.0 K**
***** Processing Parameters *****		
LB	**:**	**1.00 Hz**
SF	**:**	**125.7577890 MHz**
***** 1D NMR Plot Parameters *****		
NUCLEUS	**:**	**off**

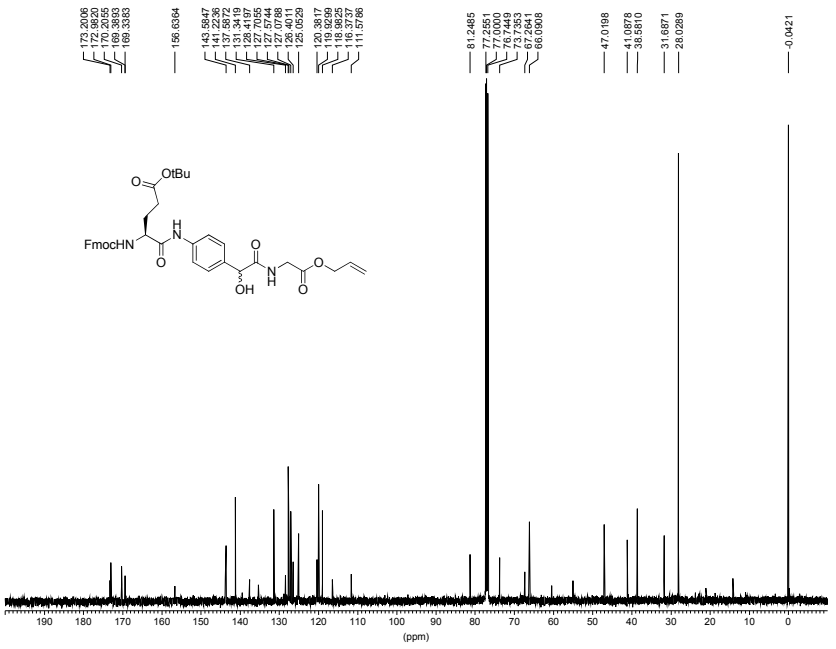
1H AMX500
suy1111 3
Glu-NH



*** Current Data Parameters ***

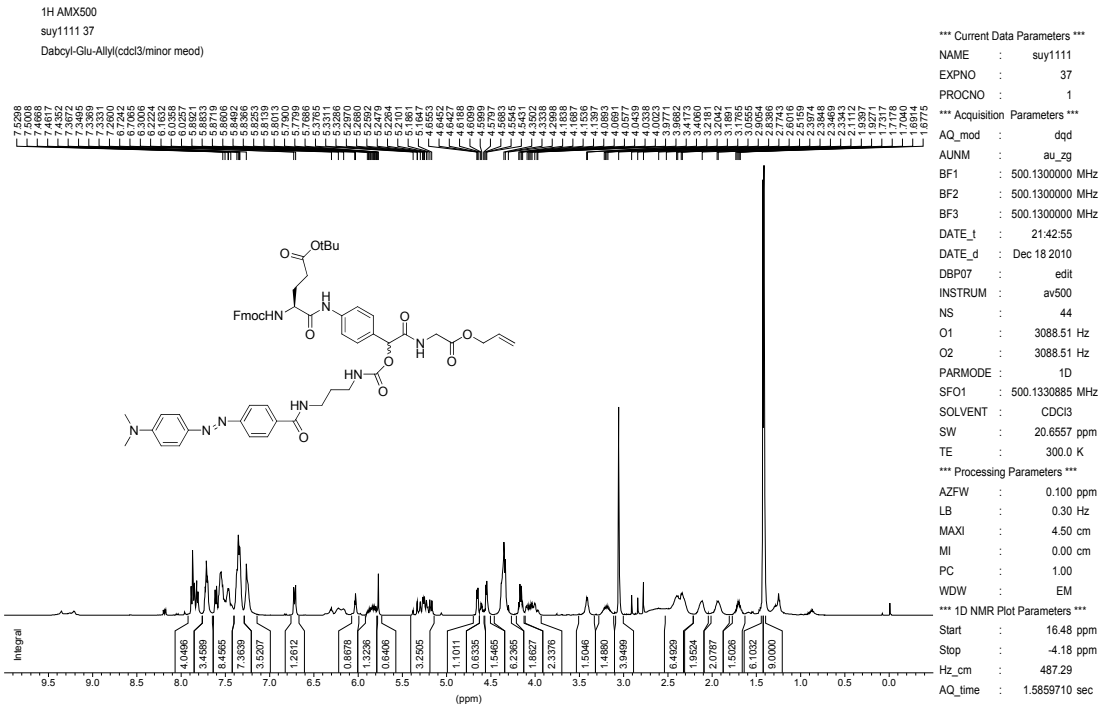
NAME	:	suy1111
EXPNO	:	3
PROCNO	:	1
*** Acquisition Parameters ***		
AQ_mod	:	dqd
AUNM	:	au_zg
BF1	:	500.1300000 MHz
BF2	:	500.1300000 MHz
BF3	:	500.1300000 MHz
DATE_t	:	05:17:25
DATE_d	:	Nov 11 2010
DBP07	:	edit
INSTRUM	:	av500
NS	:	175
O1	:	3088.51 Hz
O2	:	3088.51 Hz
PARMODE	:	1D
SFO1	:	500.1330885 MHz
SOLVENT	:	CDCl3
SW	:	20.6557 ppm
TE	:	296.2 K
*** Processing Parameters ***		
AZFW	:	0.100 ppm
LB	:	0.30 Hz
MAXI	:	10000.00 cm
MI	:	0.00 cm
PC	:	1.00
WDW	:	EM
*** 1D NMR Plot Parameters ***		
Start	:	8.73 ppm
Stop	:	3.57 ppm
Hz_cm	:	121.82
AQ_time	:	1.5859710 sec

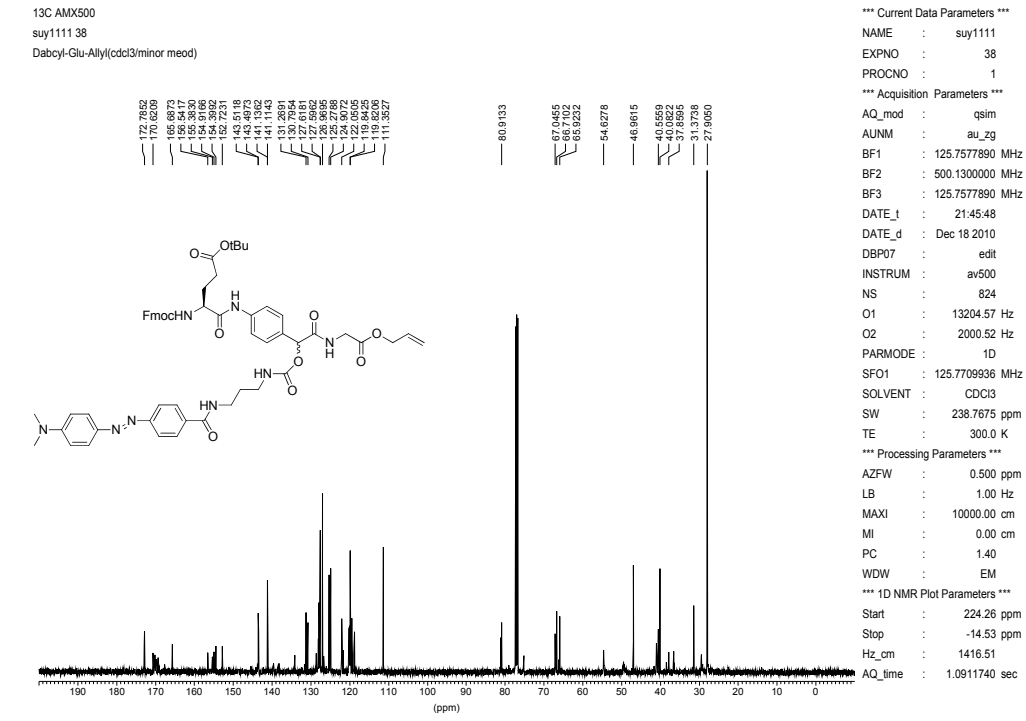
13C AMX500
suy1111 4
Glu-NH-Allyl



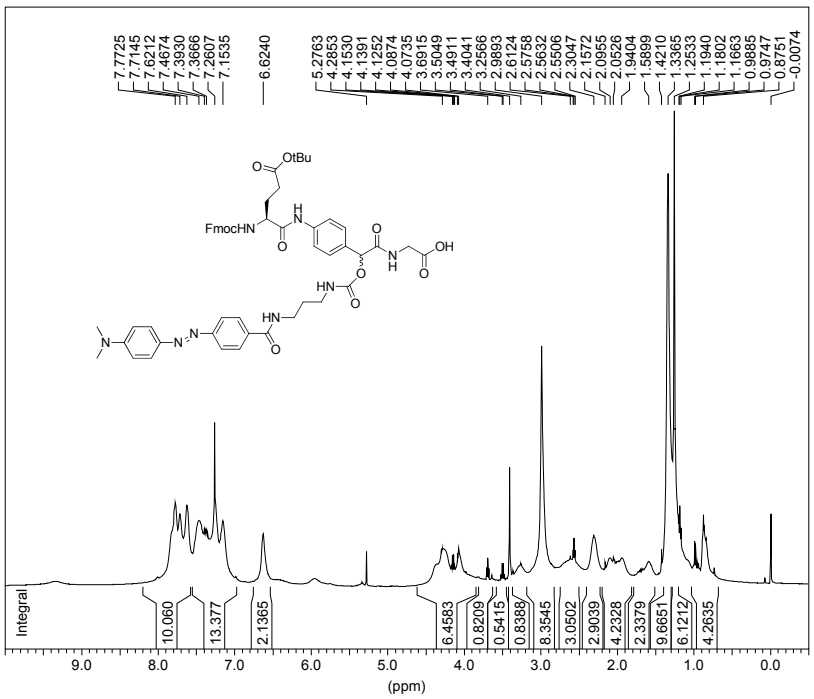
*** Current Data Parameters ***

NAME	:	suy1111
EXPNO	:	4
PROCNO	:	1
*** Acquisition Parameters ***		
AQ_mod	:	qsim
AUNM	:	au_zg
BF1	:	125.7577890 MHz
BF2	:	500.1300000 MHz
BF3	:	125.7577890 MHz
DATE_t	:	05:19:38
DATE_d	:	Nov 11 2010
DBP07	:	edit
INSTRUM	:	av500
NS	:	657
O1	:	13204.57 Hz
O2	:	2000.52 Hz
PARMODE	:	1D
SFO1	:	125.7709936 MHz
SOLVENT	:	CDCl3
SW	:	238.7675 ppm
TE	:	296.4 K
*** Processing Parameters ***		
AZFW	:	0.500 ppm
LB	:	1.00 Hz
MAXI	:	10000.00 cm
MI	:	0.00 cm
PC	:	1.40
WDW	:	EM
*** 1D NMR Plot Parameters ***		
Start	:	224.36 ppm
Stop	:	-14.43 ppm
Hz_cm	:	1416.51
AQ_time	:	1.0911740 sec


1H AMX500
suy1111 37
Dabcyl-Glu-Allyl(cdcl3/minor meod)
OtBu
FmocHN
HN
Integral
(ppm)
*** Current Data Parameters ***
NAME : suy1111
EXPNO : 37
PROCNO : 1
*** Acquisition Parameters ***
AQ_mod : dqd
AUNM : au_zg
BF1 : 500.1300000 MHz
BF2 : 500.1300000 MHz
BF3 : 500.1300000 MHz
DATE_t : 21:42:55
DATE_d : Dec 18 2010
DBP07 : edit
INSTRUM : av500
NS : 44
O1 : 3088.51 Hz
O2 : 3088.51 Hz
PARMODE : 1D
SFO1 : 500.1330885 MHz
SOLVENT : CDCl3
SW : 20.6557 ppm
TE : 300.0 K
*** Processing Parameters ***
AZFW : 0.100 ppm
LB : 0.30 Hz
MAXI : 4.50 cm
MI : 0.00 cm
PC : 1.00
WDW : EM
*** 1D NMR Plot Parameters ***
Start : 16.48 ppm
Stop : -4.18 ppm
Hz_cm : 487.29
AQ_time : 1.5859710 sec


13C AMX500
suy1111 38
Dabcyl-Glu-Allyl(cdcl3/minor meod)
OtBu
FmocHN
HN
(ppm)
*** Current Data Parameters ***
NAME : suy1111
EXPNO : 38
PROCNO : 1
*** Acquisition Parameters ***
AQ_mod : qsim
AUNM : au_zg
BF1 : 125.7577890 MHz
BF2 : 500.1300000 MHz
BF3 : 125.7577890 MHz
DATE_t : 21:45:48
DATE_d : Dec 18 2010
DBP07 : edit
INSTRUM : av500
NS : 824
O1 : 13204.57 Hz
O2 : 2000.52 Hz
PARMODE : 1D
SFO1 : 125.7709936 MHz
SOLVENT : CDCl3
SW : 238.7675 ppm
TE : 300.0 K
*** Processing Parameters ***
AZFW : 0.500 ppm
LB : 1.00 Hz
MAXI : 10000.00 cm
MI : 0.00 cm
PC : 1.40
WDW : EM
*** 1D NMR Plot Parameters ***
Start : 224.26 ppm
Stop : -14.53 ppm
Hz_cm : 1416.51
AQ_time : 1.0911740 sec

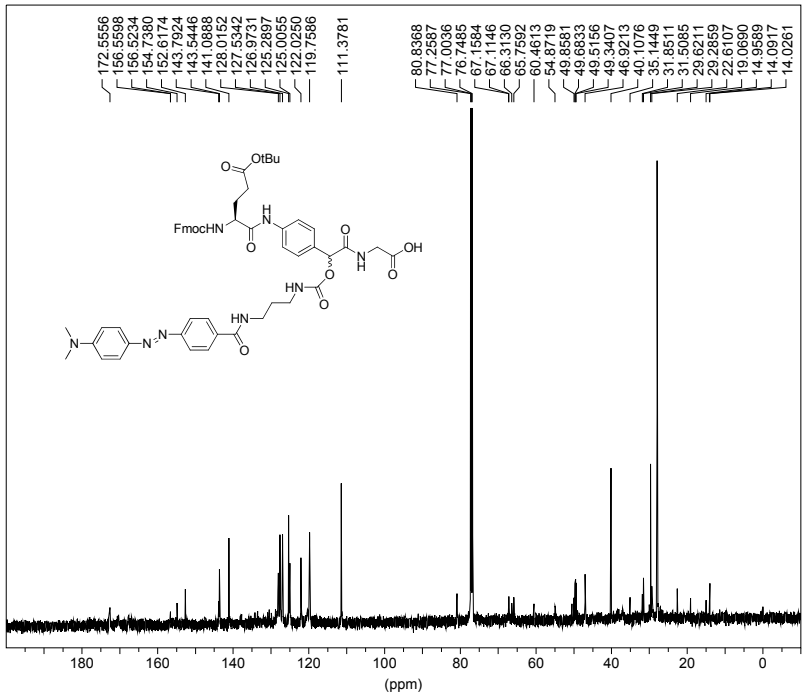
1H AMX500
suy1111 49
Dabcyl-Glu-COOH(repurifiedCDCl3/minor MeOD)



*** Current Data Parameters ***

NAME	:	suy1111
EXPNO	:	49
PROCNO	:	1
*** Acquisition Parameters ***		
INSTRUM	:	av500
LOCNUC	:	2H
NS	:	201
NUCLEUS	:	off
O1	:	3088.51 Hz
PULPROG	:	zg30
SFO1	:	500.1330885 MHz
SOLVENT	:	CDCl3
SW	:	20.6557 ppm
TD	:	32768
*** Processing Parameters ***		
LB	:	0.30 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

13C AMX500
suy1111 50
Dabcyl-Glu-COOH(repurifiedCDCl3/minor MeOD)



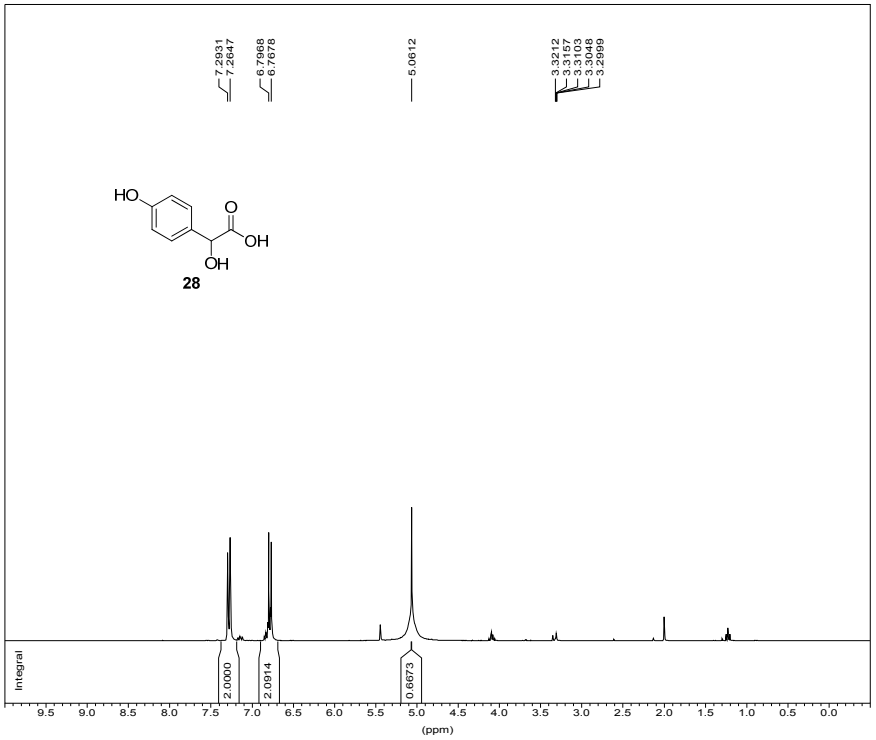
*** Current Data Parameters ***

NAME	:	suy1111
EXPNO	:	50
PROCNO	:	1
*** Acquisition Parameters ***		
INSTRUM	:	av500
LOCNUC	:	2H
NS	:	1824
NUCLEUS	:	off
O1	:	13204.57 Hz
PULPROG	:	zgpg30
SFO1	:	125.7709936 MHz
SOLVENT	:	CDCl3
SW	:	238.7675 ppm
TD	:	65536
*** Processing Parameters ***		
LB	:	1.00 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

1H normal range AC300

ma06suy-8

suy first acid



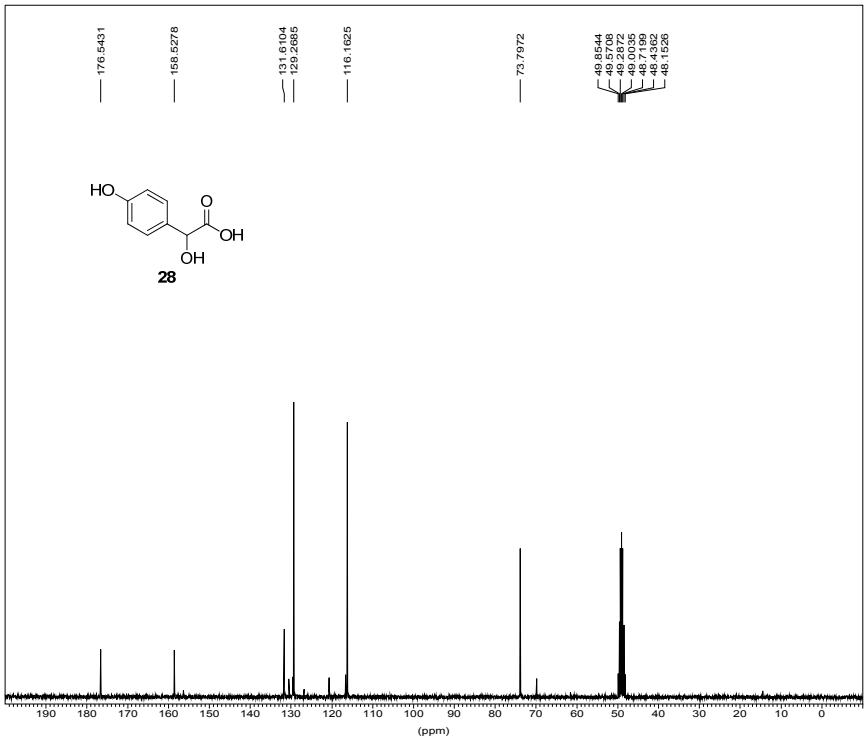
*** Current Data Parameters ***

NAME	:	ma06suy
EXPNO	:	8
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	300.1300000 MHz
LOCNUC	:	2H
NS	:	16
O1	:	1853.43 Hz
PULPROG	:	zg30
SFO1	:	300.1318534 MHz
SOLVENT	:	MeOD
SW	:	17.9519 ppm
*** Processing Parameters ***		
LB	:	0.30 Hz
PHC0	:	56.246 degree
PHC1	:	7.907 degree

13C Standard AC300

ma06suy-9

suy first acid



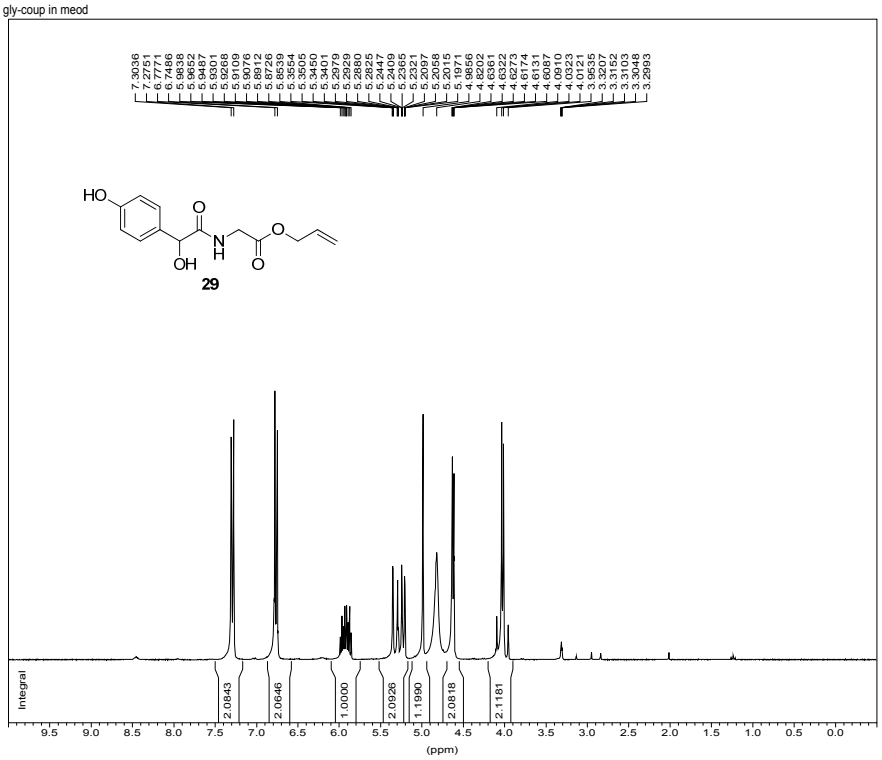
*** Current Data Parameters ***

NAME	:	ma06suy
EXPNO	:	9
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	75.4677490 MHz
LOCNUC	:	2H
NS	:	256
O1	:	7924.11 Hz
PULPROG	:	zgpg30
SFO1	:	75.4756731 MHz
SOLVENT	:	MeOD
SW	:	238.2968 ppm
*** Processing Parameters ***		
LB	:	1.00 Hz
PHC0	:	-12.847 degree
PHC1	:	8.024 degree

1H normal range AC300

ag04suy 1

gly-coup in meod



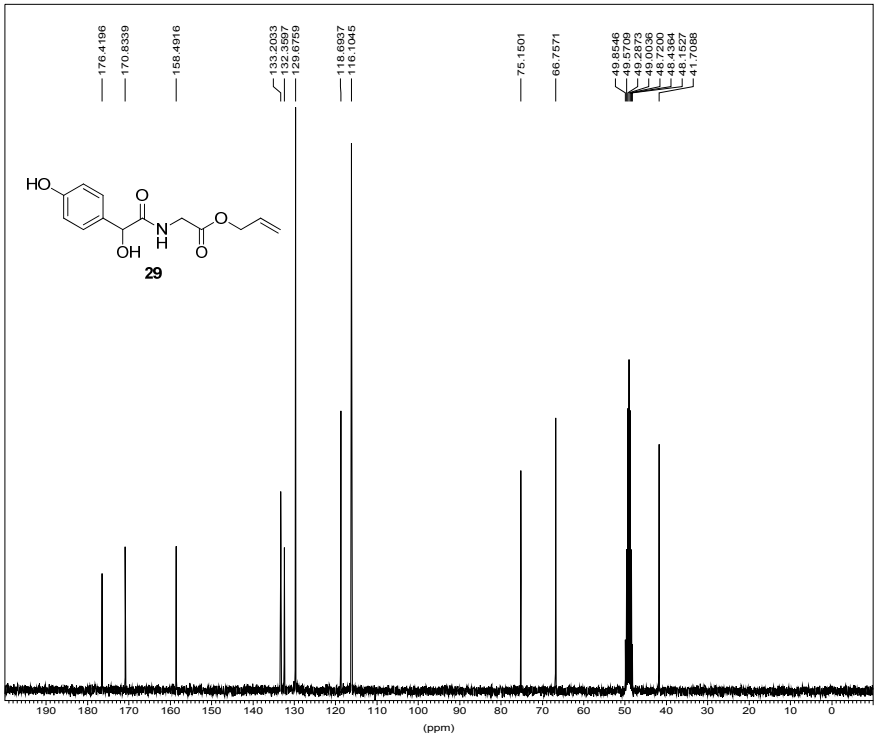
*** Current Data Parameters ***

NAME	:	ag04suy
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	300.1300000 MHz
LOCNUC	:	2H
NS	:	33
O1	:	1853.43 Hz
PULPROG	:	zg30
SFO1	:	300.1318534 MHz
SOLVENT	:	MeOD
SW	:	17.9519 ppm
*** Processing Parameters ***		
LB	:	0.30 Hz
PHC0	:	322.536 degree
PHC1	:	-14.204 degree

13C Standard AC300

ag04suy 2

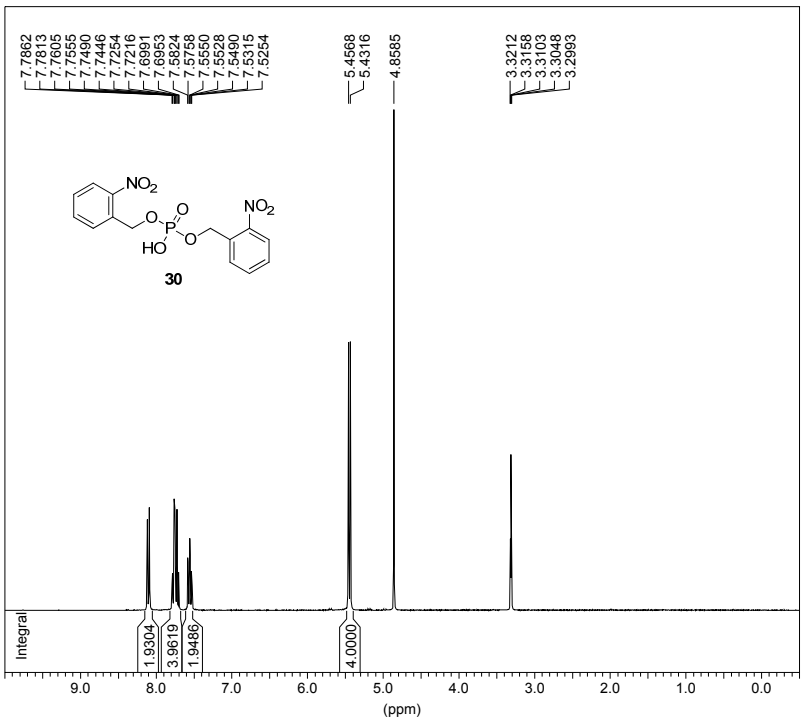
gly-coup in meod



*** Current Data Parameters ***

NAME	:	ag04suy
EXPNO	:	2
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	75.4677490 MHz
LOCNUC	:	2H
NS	:	241
O1	:	7924.11 Hz
PULPROG	:	zgpg30
SFO1	:	75.4756731 MHz
SOLVENT	:	MeOD
SW	:	238.2968 ppm
*** Processing Parameters ***		
LB	:	1.00 Hz
PHC0	:	37.651 degree
PHC1	:	-2.381 degree

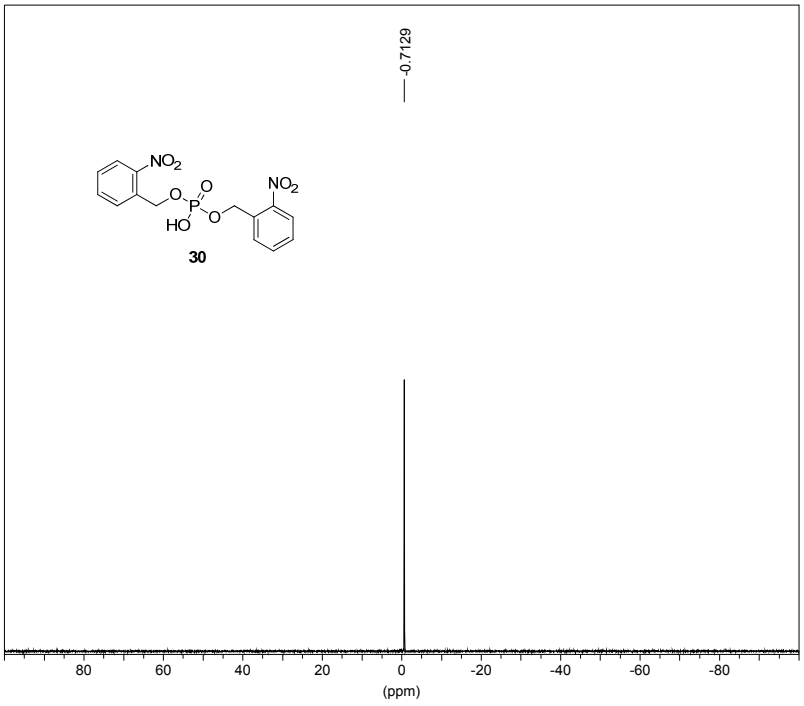
1H normal range AC300
jl28suy(ACX) 1
P-OH



*** Current Data Parameters ***

NAME	:	jl28suy
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
INSTRUM	:	spect
LOCNUC	:	2H
NS	:	100
NUCLEUS	:	off
O1	:	1853.43 Hz
PULPROG	:	zg30
SFO1	:	300.1318534 MHz
SOLVENT	:	MeOD
SW	:	17.9519 ppm
TD	:	32768
TE	:	300.0 K
*** Processing Parameters ***		
LB	:	0.30 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

31P AC300
jl28suy(ACX) 2
P-OH



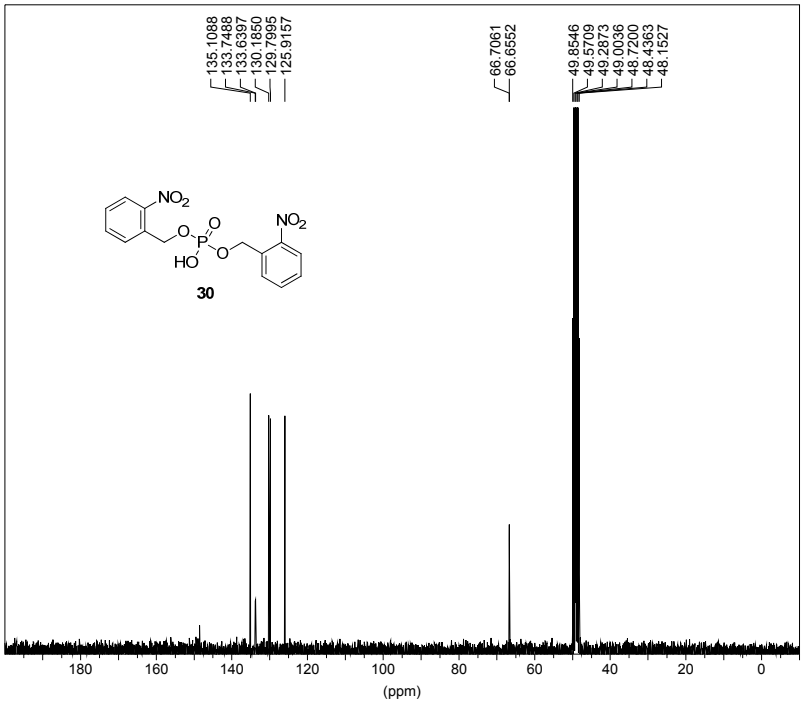
*** Current Data Parameters ***

NAME	:	jl28suy
EXPNO	:	2
PROCNO	:	1
*** Acquisition Parameters ***		
INSTRUM	:	spect
LOCNUC	:	2H
NS	:	121
NUCLEUS	:	off
O1	:	-6074.78 Hz
PULPROG	:	zgpg30
SFO1	:	121.4887762 MHz
SOLVENT	:	MeOD
SW	:	399.5734 ppm
TD	:	65536
TE	:	300.0 K
*** Processing Parameters ***		
LB	:	1.00 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

13C Standard AC300

jl28suy(ACX) 3

P-OH



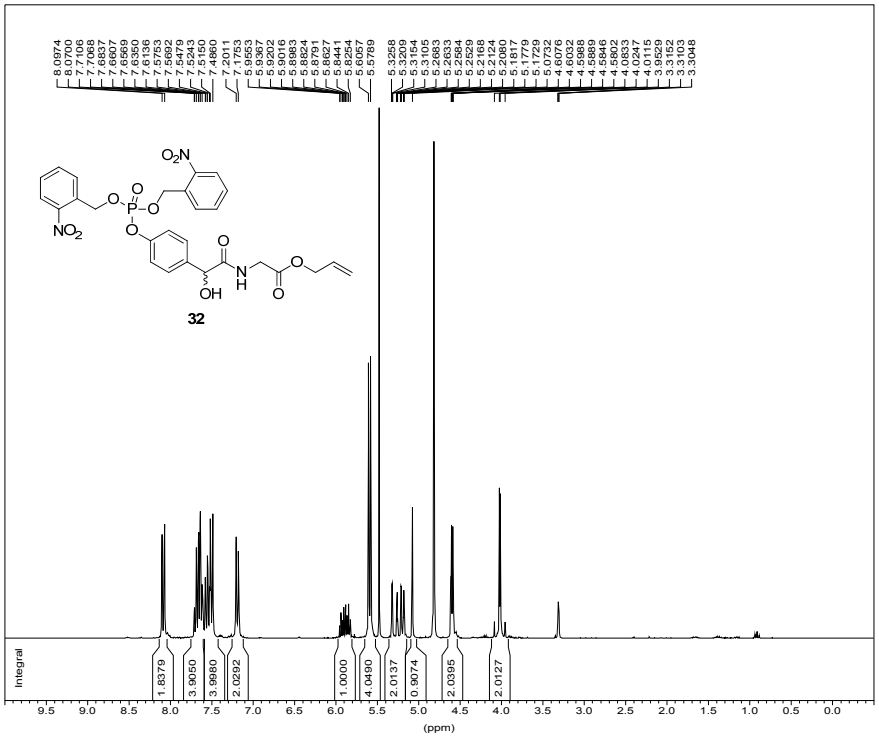
*** Current Data Parameters ***

NAME	:	jl28suy
EXPNO	:	3
PROCNO	:	1
*** Acquisition Parameters ***		
INSTRUM	:	spect
LOCNUC	:	2H
NS	:	1000
NUCLEUS	:	off
O1	:	7924.11 Hz
PULPROG	:	zgpg30
SFO1	:	75.4756731 MHz
SOLVENT	:	MeOD
SW	:	238.2968 ppm
TD	:	32768
TE	:	300.0 K
*** Processing Parameters ***		
LB	:	1.00 Hz
*** 1D NMR Plot Parameters ***		
NUCLEUS	:	off

1H normal range AC300

ag03suy 1

P-Cl coupling



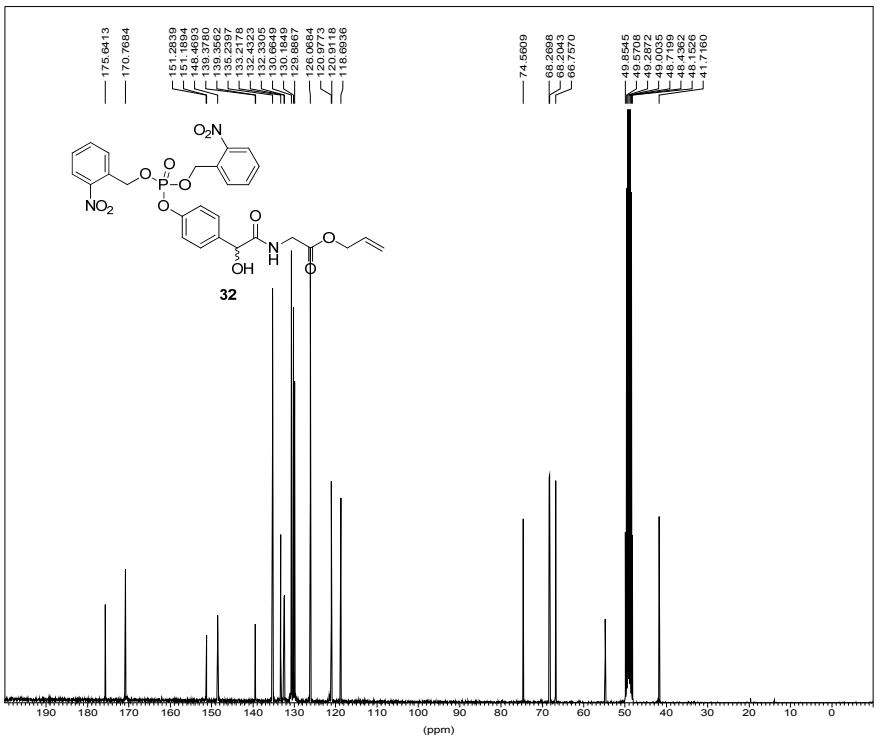
*** Current Data Parameters ***

NAME	:	ag03suy
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	300.1300000 MHz
LOCNUC	:	2H
NS	:	33
O1	:	1853.43 Hz
PULPROG	:	zg30
SFO1	:	300.1318534 MHz
SOLVENT	:	MeOD
SW	:	17.9519 ppm
*** Processing Parameters ***		
LB	:	0.30 Hz
PHC0	:	91.634 degree
PHC1	:	-1.046 degree

13C Standard AC300

ag03suy 3

P-Cl coupling



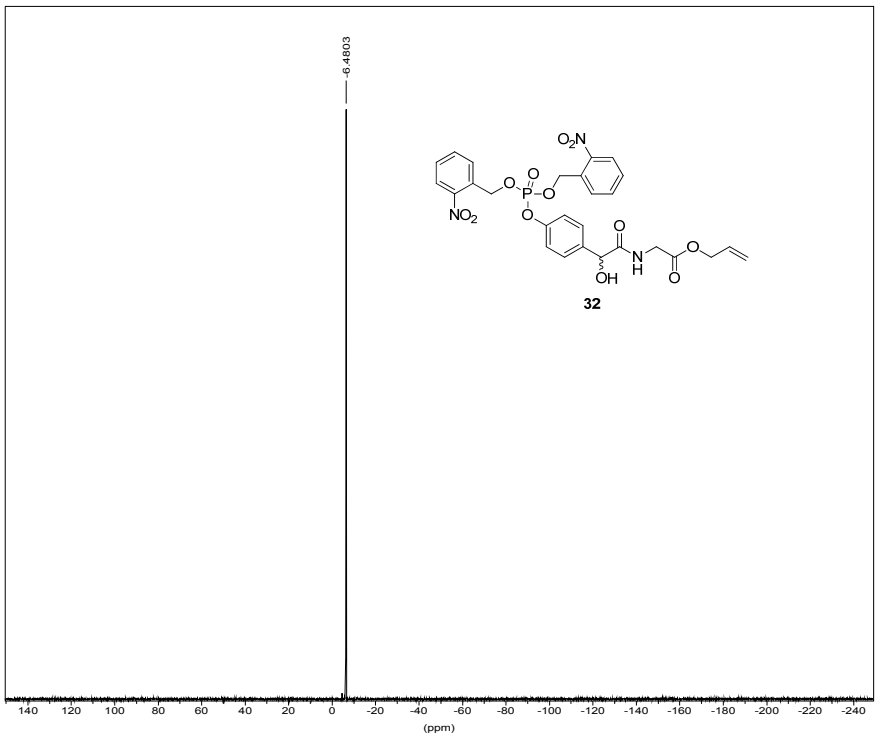
*** Current Data Parameters ***

NAME	:	ag03suy
EXPNO	:	3
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	75.4677490 MHz
LOCNUC	:	2H
NS	:	18013
O1	:	7924.11 Hz
PULPROG	:	zgpg30
SFO1	:	75.4756731 MHz
SOLVENT	:	MeOD
SW	:	238.2968 ppm
*** Processing Parameters ***		
LB	:	1.00 Hz
PHC0	:	30.978 degree
PHC1	:	2.752 degree

31P AC300

ag03suy 2

P-Cl coupling



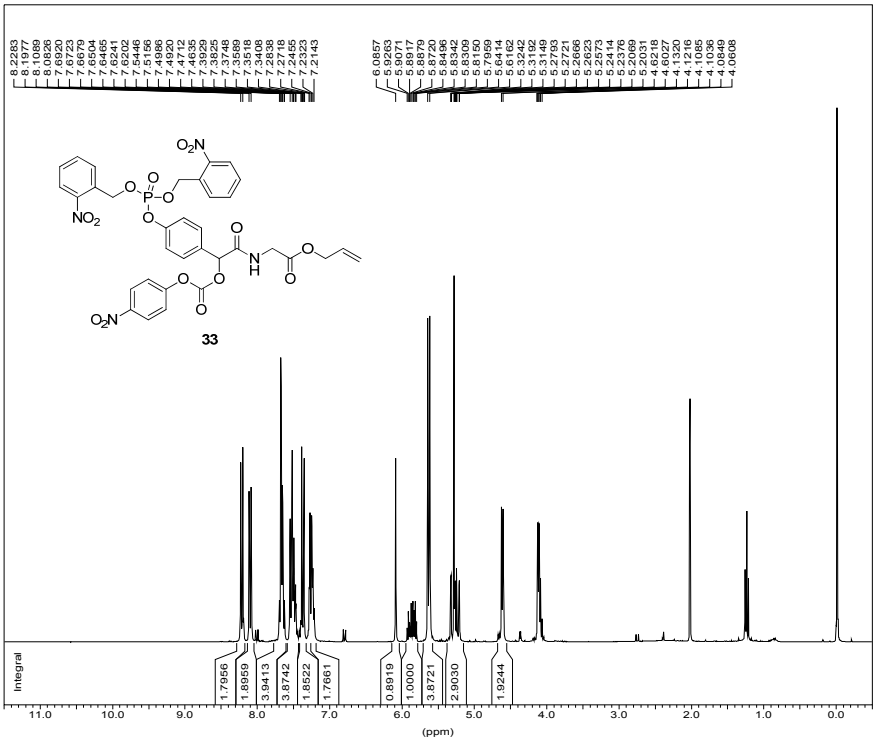
*** Current Data Parameters ***

NAME	:	ag03suy
EXPNO	:	2
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	121.4948510 MHz
LOCNUC	:	2H
NS	:	25
O1	:	-6074.78 Hz
PULPROG	:	zgpg30
SFO1	:	121.4887762 MHz
SOLVENT	:	MeOD
SW	:	399.5734 ppm
*** Processing Parameters ***		
LB	:	1.00 Hz
PHC0	:	182.515 degree
PHC1	:	-20.719 degree

1H normal range AC300

ag04suy 4

chloroformate coup in cdcl3



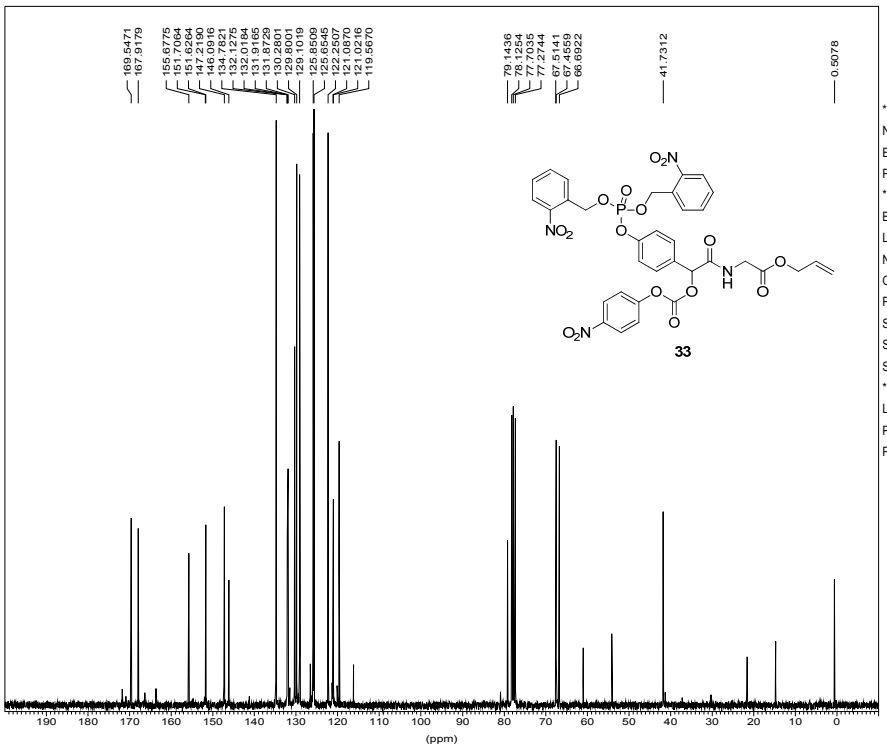
*** Current Data Parameters ***

NAME	:	ag04suy
EXPNO	:	4
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	300.1300000 MHz
LOCNUC	:	2H
NS	:	84
O1	:	1853.43 Hz
PULPROG	:	zg30
SFO1	:	300.1318534 MHz
SOLVENT	:	CDCl3
SW	:	17.9519 ppm
*** Processing Parameters ***		
LB	:	0.30 Hz
PHC0	:	323.248 degree
PHC1	:	2.337 degree

13C Standard AC300

ag04suy 5

chloroformate coup in cdcl3



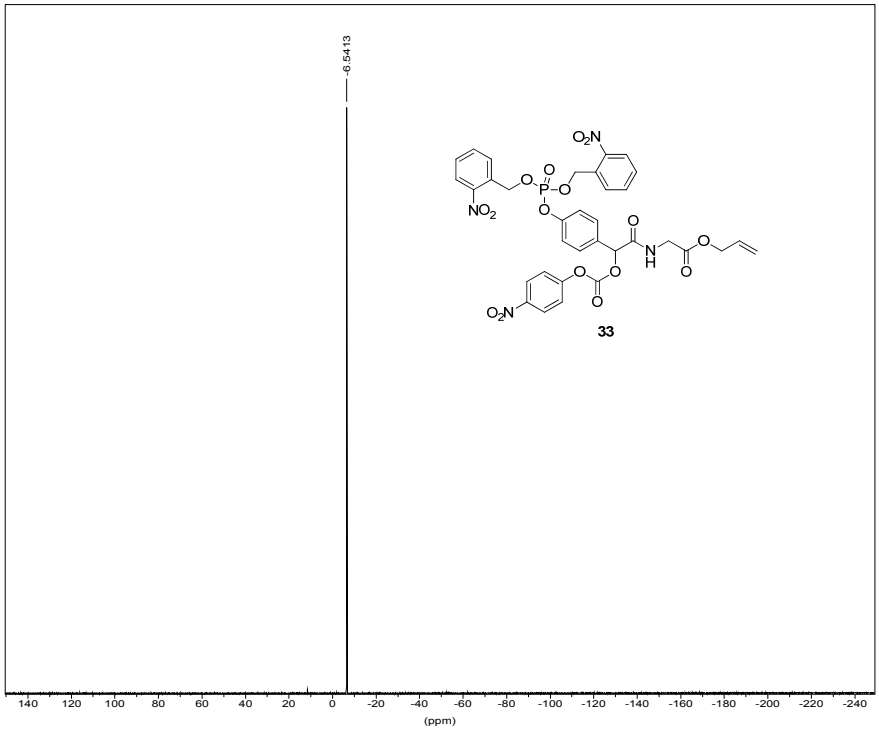
*** Current Data Parameters ***

NAME	:	ag04suy
EXPNO	:	5
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	75.4677490 MHz
LOCNUC	:	2H
NS	:	1279
O1	:	7924.11 Hz
PULPROG	:	zgpg30
SFO1	:	75.4756731 MHz
SOLVENT	:	CDCl3
SW	:	238.2968 ppm
*** Processing Parameters ***		
LB	:	1.00 Hz
PHC0	:	35.873 degree
PHC1	:	10.557 degree

31P AC300

ag04suy 3

chloroformate coup in cdcl3



33

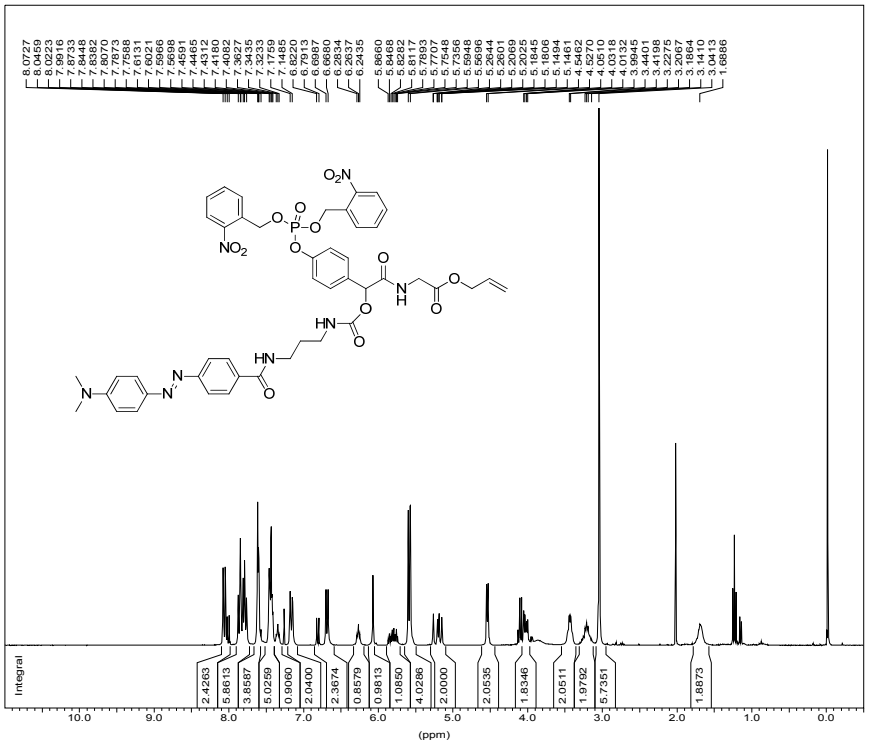
*** Current Data Parameters ***

NAME	:	ag04suy
EXPNO	:	3
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	121.4948510 MHz
LOCNUC	:	2H
NS	:	15
O1	:	-6074.78 Hz
PULPROG	:	zgpg30
SFO1	:	121.4887762 MHz
SOLVENT	:	CDCl3
SW	:	399.5734 ppm
*** Processing Parameters ***		
LB	:	1.00 Hz
PHC0	:	-69.162 degree
PHC1	:	11.896 degree

1H normal range AC300

ag04suy 7

before de-allyl in cdcl3



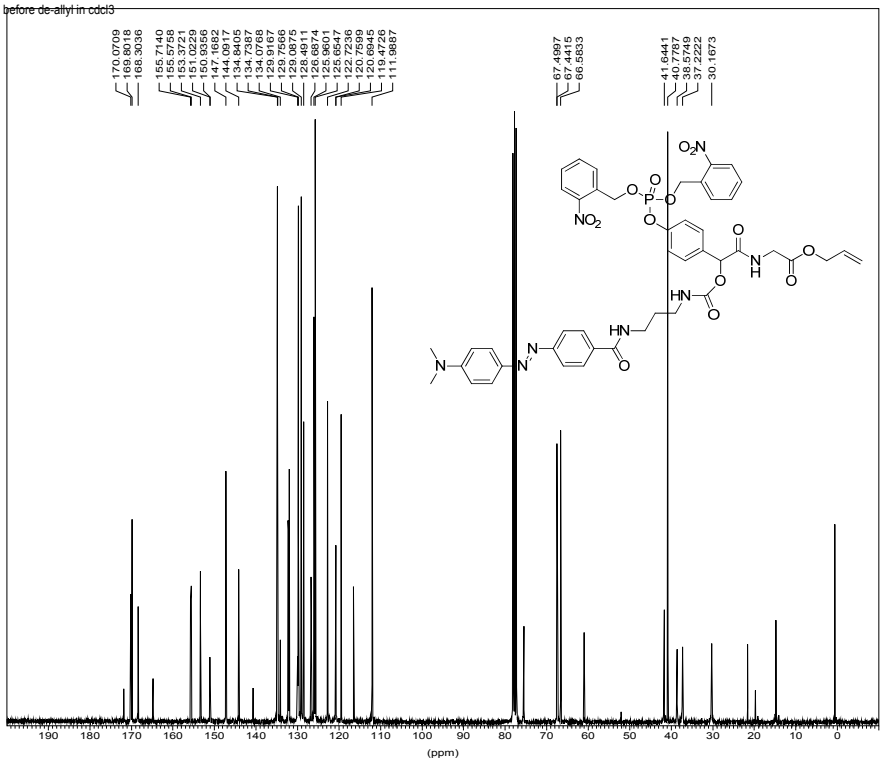
*** Current Data Parameters ***

NAME	:	ag04suy
EXPNO	:	7
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	300.1300000 MHz
LOCNUC	:	2H
NS	:	32
O1	:	1853.43 Hz
PULPROG	:	zg30
SFO1	:	300.1318534 MHz
SOLVENT	:	CDCl3
SW	:	17.9519 ppm
*** Processing Parameters ***		
LB	:	0.30 Hz
PHC0	:	320.653 degree
PHC1	:	2.436 degree

13C Standard AC300

ag04suy 8

before de-allyl in cdcl3



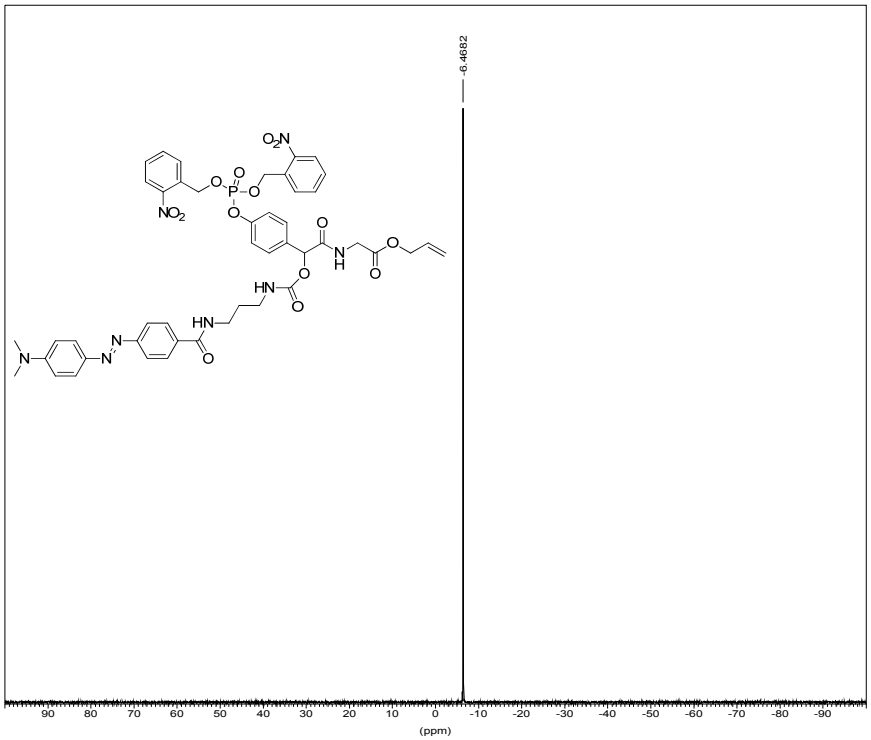
*** Current Data Parameters ***

NAME	:	ag04suy
EXPNO	:	8
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	75.4677490 MHz
LOCNUC	:	2H
NS	:	13545
O1	:	7924.11 Hz
PULPROG	:	zgpg30
SFO1	:	75.4756731 MHz
SOLVENT	:	CDCl3
SW	:	238.2968 ppm
*** Processing Parameters ***		
LB	:	1.00 Hz
PHC0	:	40.610 degree
PHC1	:	1.021 degree

31P AC300

ag04suy 6

before de-allyl in cdcl3



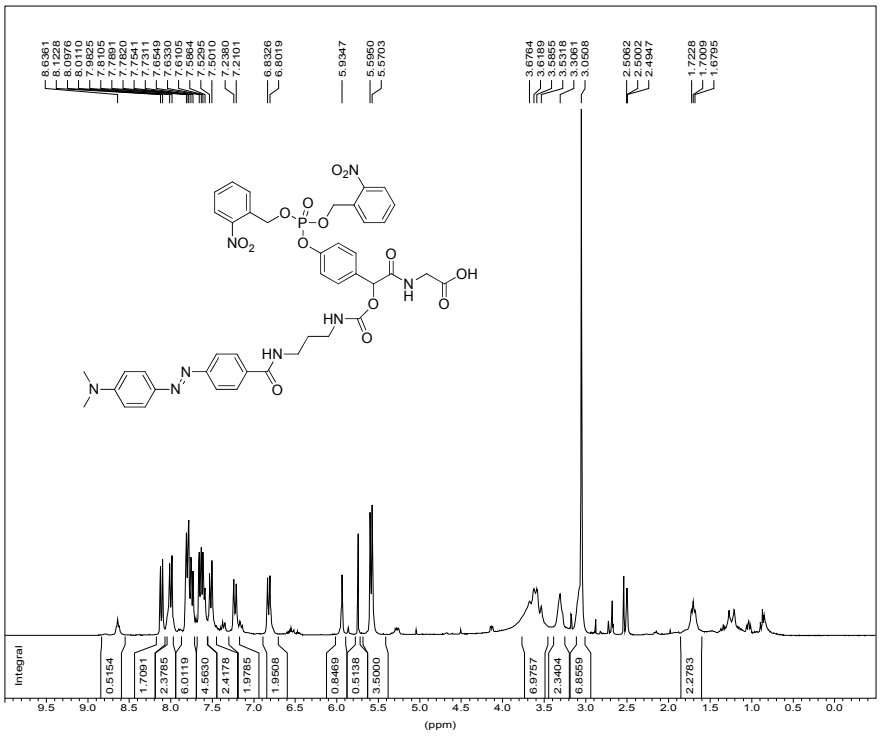
*** Current Data Parameters ***

NAME	:	ag04suy
EXPNO	:	6
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	121.4948510 MHz
LOCNUC	:	2H
NS	:	16
O1	:	-6074.78 Hz
PULPROG	:	zgpg30
SFO1	:	121.4887762 MHz
SOLVENT	:	CDCl3
SW	:	399.5734 ppm
*** Processing Parameters ***		
LB	:	1.00 Hz
PHC0	:	-78.535 degree
PHC1	:	34.595 degree

1H normal range AC300

ma15suy 1

after deallyl-63mg



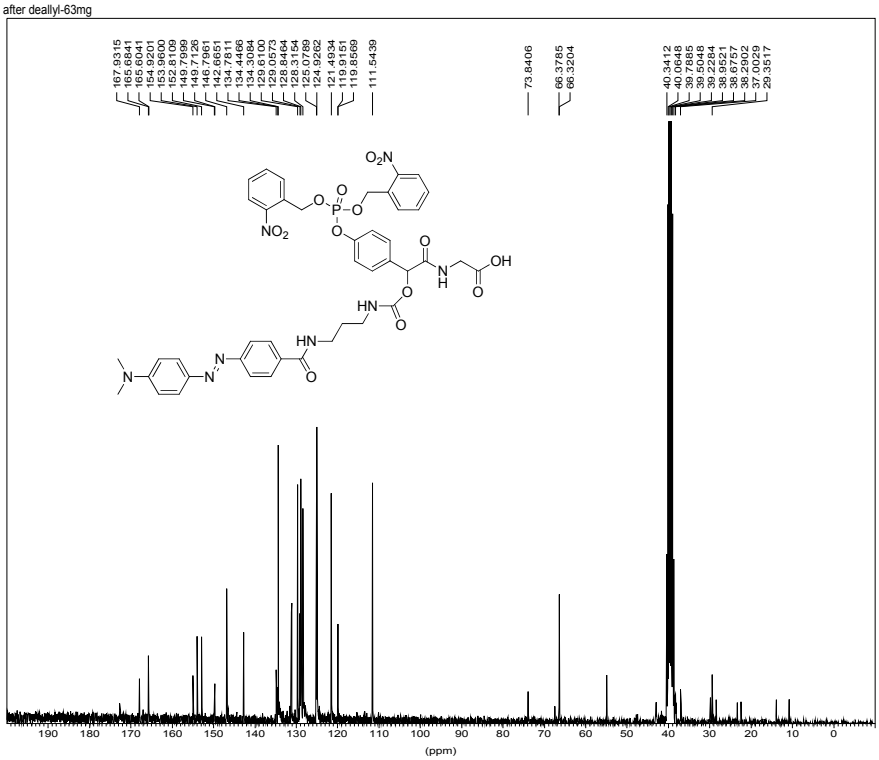
*** Current Data Parameters ***

NAME	:	ma15suy
EXPNO	:	1
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	300.1300000 MHz
LOCNUC	:	2H
NS	:	32
O1	:	1853.43 Hz
PULPROG	:	zg30
SFO1	:	300.1318534 MHz
SOLVENT	:	DMSO
SW	:	17.9519 ppm
*** Processing Parameters ***		
LB	:	0.30 Hz
PHC0	:	58.372 degree
PHC1	:	-10.187 degree

13C Standard AC300

ma15suy 3

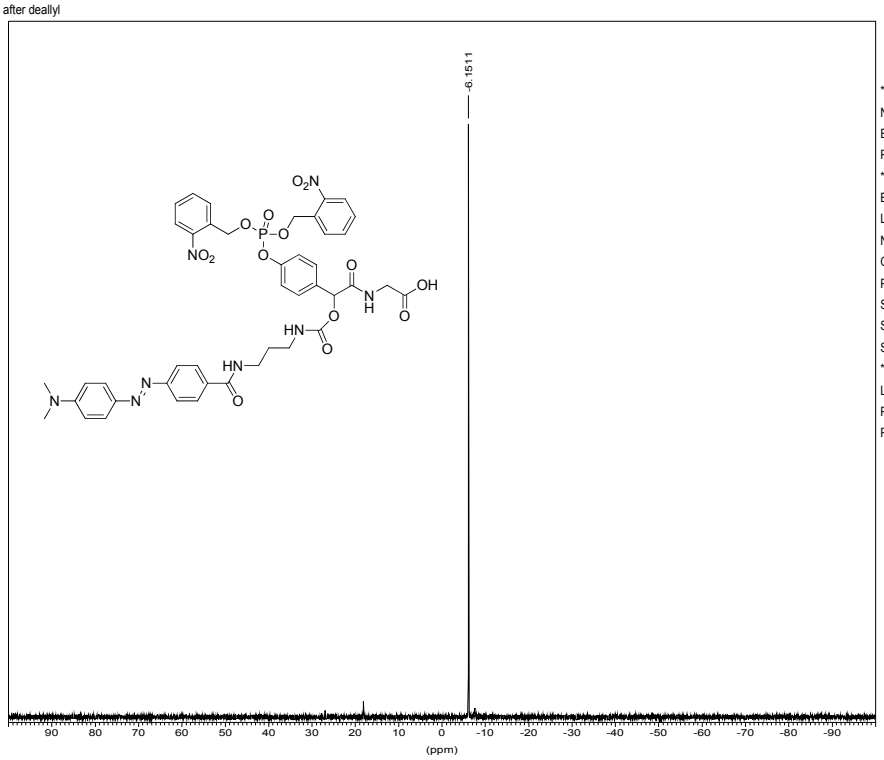
after deallyl-63mg



*** Current Data Parameters ***

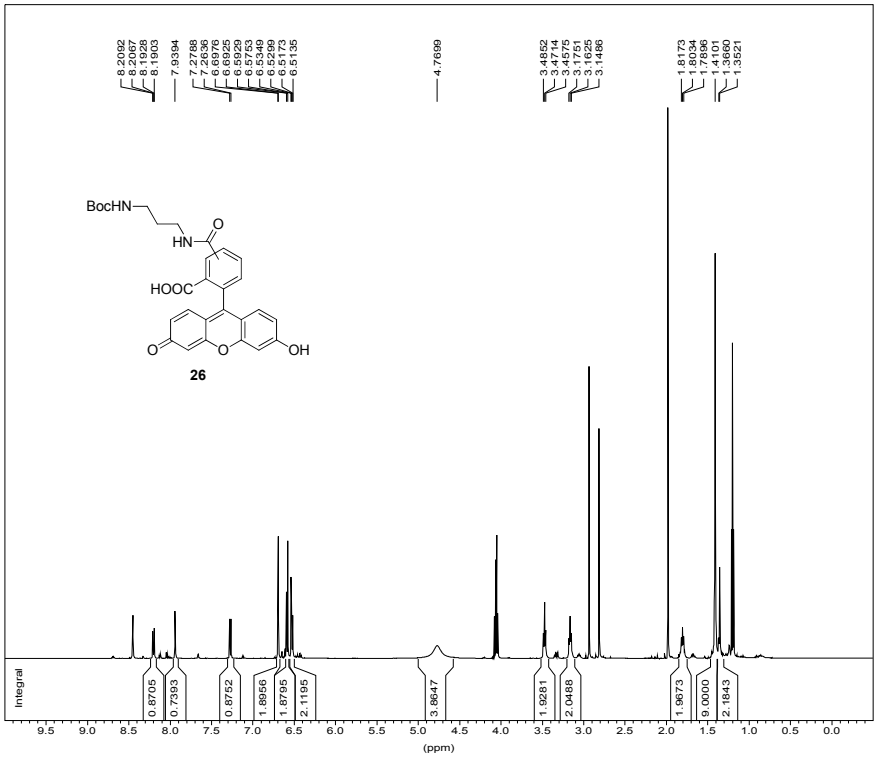
NAME	:	ma15suy
EXPNO	:	3
PROCNO	:	1
*** Acquisition Parameters ***		
BF1	:	75.4677490 MHz
LOCNUC	:	2H
NS	:	2609
O1	:	7924.11 Hz
PULPROG	:	zgpg30
SFO1	:	75.4756731 MHz
SOLVENT	:	DMSO
SW	:	238.2968 ppm
*** Processing Parameters ***		
LB	:	1.00 Hz
PHC0	:	8.017 degree
PHC1	:	-24.869 degree

31P AC300
ma15suy 2
after deallyl



*** Current Data Parameters ***
NAME : ma15suy
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 121.4948510 MHz
LOCNUC : 2H
NS : 8
O1 : -6074.78 Hz
PULPROG : zgpg30
SFO1 : 121.4887762 MHz
SOLVENT : DMSO
SW : 399.5734 ppm
*** Processing Parameters ***
LB : 1.00 Hz
PHC0 : -62.449 degree
PHC1 : 23.903 degree

suying 1-P linker 1H 500MHz

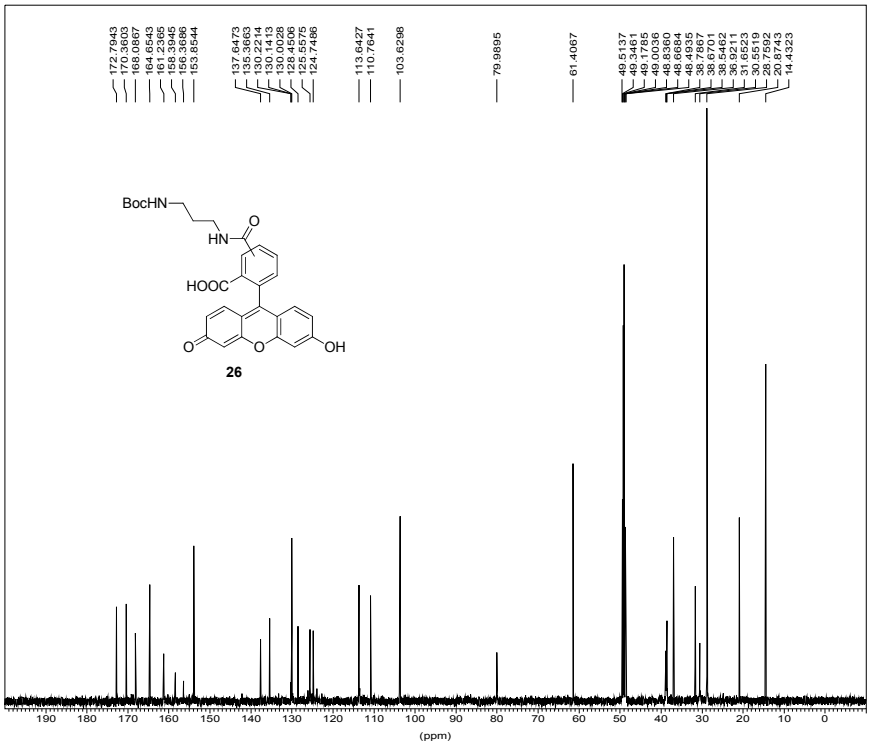


26

*** Current Data Parameters ***
NAME : gjy0807
EXPNO : 4
PROCNO : 1
*** Acquisition Parameters ***
BF1 : 500.1300000 MHz
LOCNUC : 2H
NS : 29
O1 : 3088.51 Hz
PULPROG : zg30
SFO1 : 500.1330885 MHz
SOLVENT : MeOD
SW : 20.6557 ppm
*** Processing Parameters ***
LB : 0.30 Hz
PHC0 : 146.720 degree
PHC1 : -0.006 degree

suying

1-p LINKER



*** Current Data Parameters ***

NAME	:	gjy0808
EXPNO	:	1
PROCNO	:	1

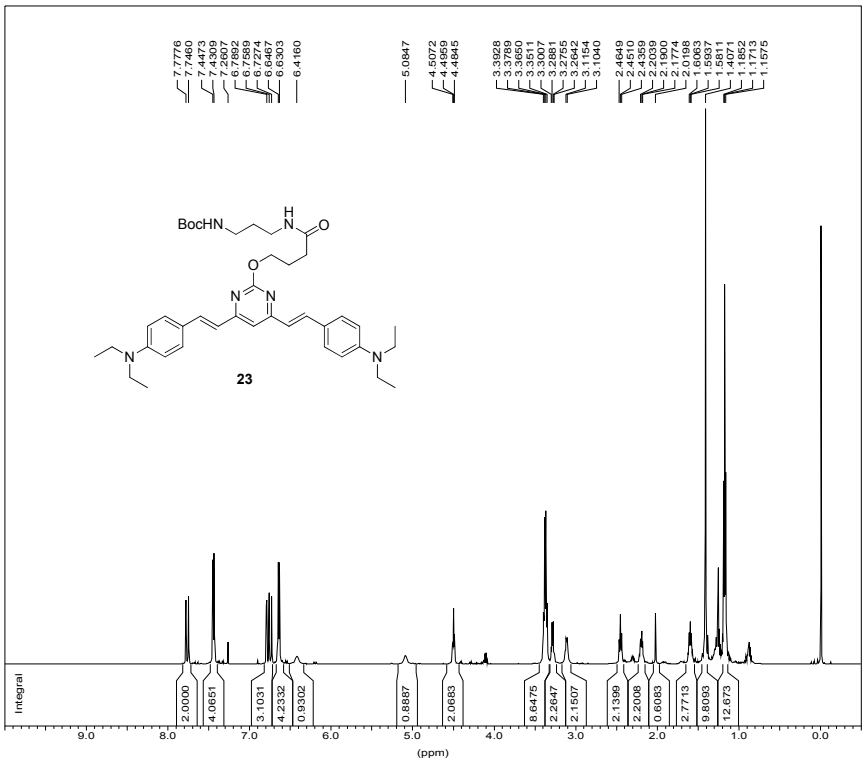
*** Acquisition Parameters ***

BF1	:	125.7577890 MHz
LOCNUC	:	2H
NS	:	71
O1	:	13204.57 Hz
PULPROG	:	zgpg30
SFO1	:	125.7709936 MHz
SOLVENT	:	MeOD
SW	:	238.7675 ppm

*** Processing Parameters ***

LB	:	1.00 Hz
PHC0	:	185.355 degree
PHC1	:	18.272 degree

suying 2-P linker 1H 500MHz



*** Current Data Parameters ***

NAME	:	gjy0807
EXPNO	:	2
PROCNO	:	1

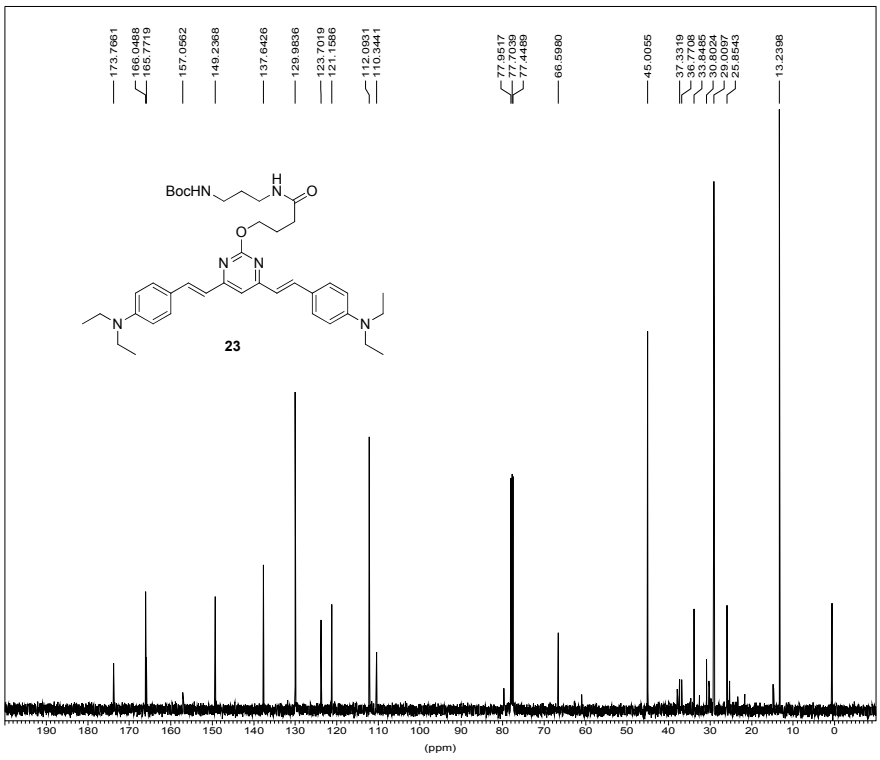
*** Acquisition Parameters ***

BF1	:	500.1300000 MHz
LOCNUC	:	2H
NS	:	21
O1	:	3088.51 Hz
PULPROG	:	zg30
SFO1	:	500.1330885 MHz
SOLVENT	:	CDCl3
SW	:	20.6557 ppm

*** Processing Parameters ***

LB	:	0.30 Hz
PHC0	:	133.966 degree
PHC1	:	-1.018 degree

suying 2-P linker



*** Current Data Parameters ***

NAME	:	gjy0807
EXPNO	:	3
PROCNO	:	1

*** Acquisition Parameters ***

BF1	:	125.7577890 MHz
LOCNUC	:	2H
NS	:	105
O1	:	13204.57 Hz
PULPROG	:	zgpg30
SFO1	:	125.7709936 MHz
SOLVENT	:	CDCl3
SW	:	238.7675 ppm

*** Processing Parameters ***

LB	:	1.00 Hz
PHC0	:	169.946 degree
PHC1	:	16.046 degree