Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

# Design and Synthesis of a Calcium-Sensitive Photocage

*Laurel M. Heckman, Jonathan B. Grimm, Eric R. Schreiter, Charles Kim, Mark A. Verdecia, Brenda C. Shields, and Luke D. Lavis**

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**SUPPORTING INFORMATION**

# Design and synthesis of a calcium-sensitive photocage

Laurel M. Heckman, Jonathan B. Grimm, Eric R. Schreiter, Charles Kim, Mark A. Verdecia, Brenda C. Shields, and Luke D. Lavis *

*Janelia Research Campus, Howard Hughes Medical Institute,*
*Ashburn, VA 20147*

Email: lavisl@janelia.hhmi.org

## S1: Experimental Information

## GENERAL EXPERIMENTAL INFORMATION: SYNTHESIS

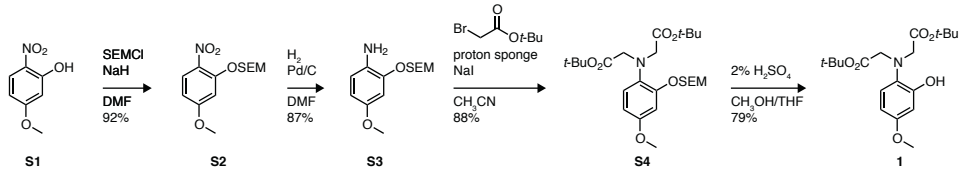
Commercial reagents were obtained from reputable suppliers and used as received. All solvents were purchased in septum-sealed bottles stored under an inert atmosphere. All reactions were sealed with septa through which a nitrogen atmosphere was introduced unless otherwise noted. Reactions were conducted in round-bottomed flasks or septum-capped crimp-top vials containing Teflon-coated magnetic stir bars. Heating of reactions was accomplished with a silicon oil bath or an aluminum reaction block on top of a stirring hotplate equipped with an electronic contact thermometer to maintain the indicated temperatures.

Reactions were monitored by thin layer chromatography (TLC) on precoated TLC glass plates (silica gel 60 $F_{254}$, 250 μm thickness) or by LC/MS (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 10–95% or 50–95% $CH_3CN/H_2O$, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm). TLC chromatograms were visualized by UV illumination or developed with *p*-anisaldehyde, ceric ammonium molybdate, or $KMnO_4$ stain. Flash chromatography was performed on an automated purification system using pre-packed silica gel columns. High-resolution mass spectrometry was performed by the Mass Spectrometry Center in the Department of Medicinal Chemistry at the University of Washington.

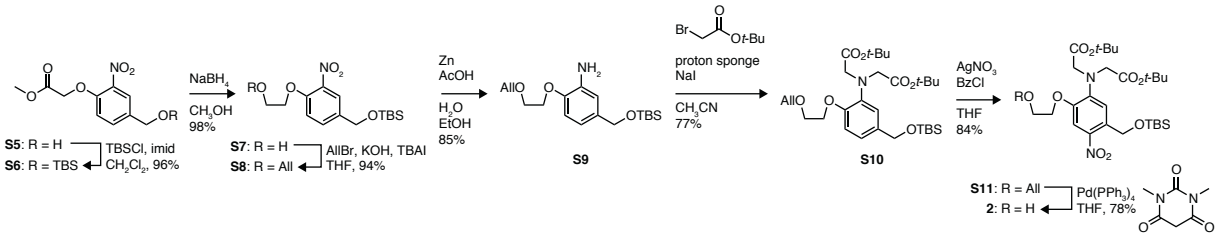
NMR spectra were recorded on a 400 MHz spectrometer. $^1H$ and $^{13}C$ chemical shifts (δ) were referenced to TMS or residual solvent peaks, and $^{19}F$ chemical shifts (δ) were referenced to $CFCl_3$. Data for $^1H$ NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constant (Hz), integration. Data for $^{13}C$ NMR spectra are reported by chemical shift (δ ppm) with hydrogen multiplicity (C, CH, $CH_2$, $CH_3$) information obtained from DEPT spectra.

**Scheme S1.** (**a**) Synthesis of phenol **1**. (**b**) Synthesis of alcohol **2**.

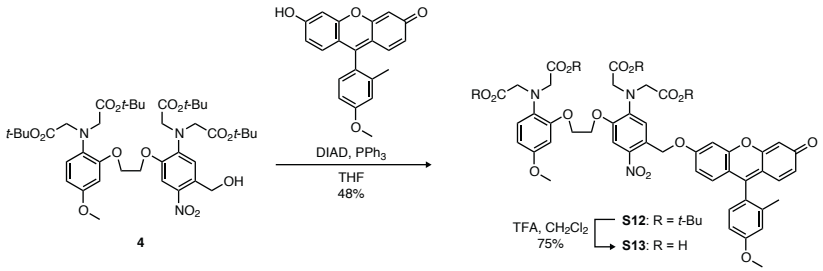
**a**



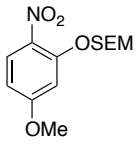

**b**



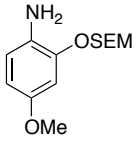

**Scheme S2.** Synthesis of caged Tokyo Green derivative **S13**.



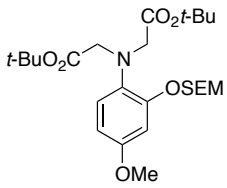
**EXPERIMENTALS AND CHARACTERIZATION DATA FOR ALL NEW COMPOUNDS**



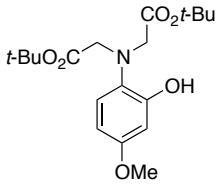

**(2-((5-Methoxy-2-nitrophenoxy)methoxy)ethyl)trimethylsilane (S2):** To a solution of 5-methoxy-2-nitrophenol (**S1**, 576 mg, 3.41 mmol) in DMF (60 mL) under nitrogen was added NaH (60%, 163 mg, 4.09 mmol, 1.2 eq). After stirring for 10 minutes, 2-(trimethylsilyl)ethoxymethyl chloride (723 μL, 4.09 mmol, 1.2 eq) was added. The reaction was stirred for 4 h at room temperature while shielded from light. The reaction was diluted with saturated $NH_4Cl$ and extracted with EtOAc (3×). The organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (0–20% EtOAc/hexanes, linear gradient; dry load with Celite) to afford **S2** as an off-white, waxy solid (935 mg, 92%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.95 (d, $J$ = 9.2 Hz, 1H), 6.82 (d, $J$ = 2.6 Hz, 1H), 6.56 (dd, $J$ = 9.2, 2.6 Hz, 1H), 5.34 (s, 2H), 3.87 (s, 3H), 3.85 – 3.79 (m, 2H), 1.01 – 0.92 (m, 2H), 0.01 (s, 9H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 164.49 (C), 153.6 (C), 134.0 (C), 128.0 (CH), 106.8 (CH), 102.8 (CH), 94.0 ($CH_2$), 67.4 ($CH_2$), 56.0 ($CH_3$), 18.2 ($CH_2$), -1.3 ($CH_3$); HRMS (ESI) calcd for $C_{13}H_{21}NO_5SiNa$ $[M+Na]^+$ 322.1081, found 322.1080.



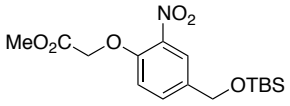

**4-Methoxy-2-((2-(trimethylsilyl)ethoxy)methoxy)aniline (S3):** Compound **S2** (900 mg, 3.01 mmol) was dissolved in DMF (12 mL). After adding Pd/C (10% Pd, 90 mg), the reaction was flushed with nitrogen for 10 minutes. An $H_2$ balloon was attached to the flask, and the reaction vessel was flushed with $H_2$. The reaction was then stirred at room temperature under $H_2$ for 24 h (shielded from light). The reaction was filtered through Celite with $CH_2Cl_2$ and concentrated *in vacuo* to give a brown residue. Flash chromatography (5–60% EtOAc/hexanes, linear gradient) provided 702 mg (87%) of **S3** as a yellow oil. $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.73 (d, $J$ = 2.7 Hz, 1H), 6.66 (d, $J$ = 8.5 Hz, 1H), 6.42 (dd, $J$ = 8.5, 2.7 Hz, 1H), 5.22 (s, 2H), 3.81 – 3.75 (m, 2H), 3.74 (s, 3H), 3.52 (s, 2H), 1.02 – 0.93 (m, 2H), 0.01 (s, 9H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 153.2 (C), 146.2 (C), 130.5 (C), 116.0 (CH), 106.8 (CH), 103.0 (CH), 93.9 ($CH_2$), 66.5 ($CH_2$), 56.0 ($CH_3$), 18.2 ($CH_2$), -1.2 ($CH_3$); HRMS (ESI) calcd for $C_{13}H_{24}NO_3Si$ $[M+H]^+$ 270.1520, found 270.1524.



**Di-*tert*-butyl 2,2'-((4-methoxy-2-((2-(trimethylsilyl)ethoxy)methoxy)phenyl)azanediyl)diacetate (S4):** Aniline **S3** (400 mg, 1.49 mmol) was dissolved in MeCN (30 mL); 1,8-bis(dimethylamino)naphthalene (1.274 g, 5.945 mmol, 4 eq), *tert*-butyl bromoacetate (1.739 g, 8.917 mmol, 6 eq) and NaI (67 mg, 0.446 mmol, 0.3 eq) were added sequentially. The resulting mixture was stirred at reflux for 18 h while being shielded from light. The reaction was cooled to room temperature, and the solvent was evaporated. The residue was diluted into $CH_2Cl_2$, washed with water (3×) and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by flash chromatography (0–20% EtOAc/hexanes, linear gradient) yielded **S4** as a pale yellow gum (650 mg, 88%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.83 (d, *J* = 8.8 Hz, 1H), 6.74 (d, *J* = 2.8 Hz, 1H), 6.45 (dd, *J* = 8.8, 2.8 Hz, 1H), 5.22 (s, 2H), 3.95 (s, 4H), 3.81 – 3.75 (m, 2H), 3.74 (s, 3H), 1.42 (s, 18H), 1.02 – 0.89 (m, 2H), 0.01 (s, 9H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 170.8 (C), 155.6 (C), 150.8 (C), 133.6 (C), 120.8 (CH), 106.3 (CH), 104.1 (CH), 93.8 ($CH_2$), 81.1 (C), 66.4 ($CH_2$), 55.7 ($CH_3$), 55.2 ($CH_2$), 28.3 ($CH_3$), 18.2 ($CH_2$), -1.2 ($CH_3$); HRMS (ESI) calcd for $C_{25}H_{44}NO_7Si$ $[M+H]^+$ 498.2882, found 498.2891.

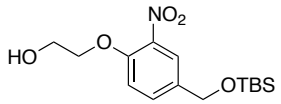



**Di-*tert*-butyl 2,2'-((2-hydroxy-4-methoxyphenyl)azanediyl)diacetate (1):** To a solution of **S4** (624 mg, 1.25 mmol) in 1:1 THF/MeOH (76 mL) was added $H_2SO_4$ (2.51 mL) in MeOH (38 mL). The reaction was stirred at room temperature for 2 h (shielded from light). The reaction was carefully quenched with 0.1 M $NaHCO_3$, diluted with additional saturated $NaHCO_3$, and extracted with EtOAc (3×). The organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The brown residue was purified by silica gel chromatography (2–10% *i*PrOH/hexanes, linear gradient) to afford 362 mg (79%) of **1** as a photosensitive, off-white solid. $^1$H NMR ($CDCl_3$, 400 MHz) δ 8.25 (s, 1H), 7.24 (d, *J* = 8.7 Hz, 1H), 6.51 (d, *J* = 2.9 Hz, 1H), 6.34 (dd, *J* = 8.7, 2.9 Hz, 1H), 3.75 (s, 3H), 3.70 (s, 4H), 1.45 (s, 18H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 171.6 (C), 159.2 (C), 155.1 (C), 130.6 (C), 127.4 (CH), 105.8 (CH), 100.8 (CH), 81.9 (C), 57.7 ($CH_2$), 55.5 ($CH_3$), 28.2 ($CH_3$); HRMS (ESI) calcd for $C_{19}H_{30}NO_6$ $[M+H]^+$ 368.2068, found 368.2062.

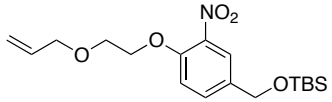



**Methyl 2-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-nitrophenoxy)acetate (S6):** Methyl 2-[4-(Hydroxymethyl)-2-nitrophenoxy]acetate (**S5**, 1.000 g, 4.146 mmol), TBSCl (750 mg, 4.98 mmol, 1.2 eq), and imidazole (423 mg, 6.22 mmol, 1.5 eq) were combined in $CH_2Cl_2$ (83 mL) and stirred at room temperature for 2 h while shielded from light. The reaction was diluted with brine and extracted with $CH_2Cl_2$ (3×). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Flash chromatography of the crude product (0–20% EtOAc/hexanes, linear gradient, with constant 20% v/v $CH_2Cl_2$ additive) provided **S6** (1.418 g, 96%) as a pale yellow solid. $^1$H NMR

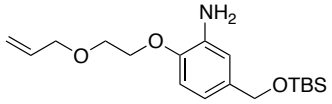
($CDCl_3$, 400 MHz) δ 7.82 (d, *J* = 2.2 Hz, 1H), 7.47 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.97 (d, *J* = 8.6 Hz, 1H), 4.77 (s, 2H), 4.70 (s, 2H), 3.80 (s, 3H), 0.94 (s, 9H), 0.10 (s, 6H); $^{13}C$ NMR ($CDCl_3$, 101 MHz) δ 168.5 (C), 150.2 (C), 140.4 (C), 136.0 (C), 131.5 (CH), 123.5 (CH), 115.5 (CH), 67.0 ($CH_2$), 63.6 ($CH_2$), 52.6 ($CH_3$), 26.0 ($CH_3$), 18.5 (C), -5.2 ($CH_3$); HRMS (ESI) calcd for $C_{16}H_{25}NO_6SiNa$ $[M+Na]^+$ 378.1343, found 378.1347.



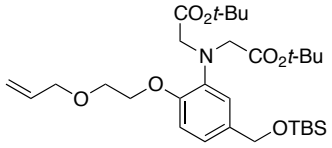

**2-(4-(((*tert*-Butyldimethylsilyl)oxy)methyl)-2-nitrophenoxy)ethanol (S7):** Ester **S6** (1.392 g, 3.916 mmol) was taken up in MeOH (20 mL) under nitrogen and cooled to 0°C. $NaBH_4$ (1.481 g, 39.16 mmol, 10 eq) was added portionwise. The reaction was covered in foil and stirred at 0°C for 4 h. It was subsequently quenched with $H_2O$ and extracted with EtOAc (3×). The combined organics were washed with brine, dried ($MgSO_4$), filtered, and evaporated. The resulting residue was purified by silica gel chromatography (0–30% EtOAc/hexanes, linear gradient, with constant 20% v/v $CH_2Cl_2$ additive) to give 1.260 g (98%) of **S7** as a yellow oil. $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.83 (d, *J* = 2.2 Hz, 1H), 7.50 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 4.71 (s, 2H), 4.27 – 4.20 (m, 2H), 3.98 (q, *J* = 5.0 Hz, 2H), 2.46 (t, *J* = 6.3 Hz, 1H), 0.94 (s, 9H), 0.11 (s, 6H); $^{13}C$ NMR ($CDCl_3$, 101 MHz) δ 151.3 (C), 139.9 (C), 135.0 (C), 132.0 (CH), 123.5 (CH), 115.4 (CH), 71.7 ($CH_2$), 63.7 ($CH_2$), 61.2 ($CH_2$), 26.0 ($CH_3$), 18.5 (C), -5.1 ($CH_3$); HRMS (ESI) calcd for $C_{15}H_{25}NO_5SiNa$ $[M+Na]^+$ 350.1394, found 350.1396.



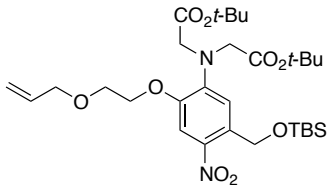

**((4-(2-(Allyloxy)ethoxy)-3-nitrobenzyl)oxy)(*tert*-butyl)dimethylsilane (S8):** A vial was charged with alcohol **S7** (1.250 g, 3.817 mmol), allyl bromide (1.778 g, 14.70 mmol, 3.85 eq), KOH (1.008 g, 7.291 mmol, 1.91 eq), and tetrabutylammonium iodide (71 mg, 0.191 mmol, 0.05 eq). The resulting mixture was stirred at room temperature for 1 h while being shielded from light. The reaction was then diluted with $CH_2Cl_2$, combined with Celite, and evaporated to dryness. Flash chromatography (0–20% EtOAc/hexanes, linear gradient; dry load with Celite) afforded **S8** as a yellow solid (1.316 g, 94%). $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, *J* = 2.2 Hz, 1H), 7.47 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.08 (d, *J* = 8.6 Hz, 1H), 5.92 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H), 5.31 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.20 (dq, *J* = 10.4, 1.4 Hz, 1H), 4.70 (s, 2H), 4.29 – 4.22 (m, 2H), 4.11 (dt, *J* = 5.6, 1.4 Hz, 2H), 3.88 – 3.81 (m, 2H), 0.93 (s, 9H), 0.10 (s, 6H); $^{13}C$ NMR ($CDCl_3$, 101 MHz) δ 151.4 (C), 140.1 (C), 134.61 (C), 134.60 (CH), 131.7 (CH), 123.3 (CH), 117.5 ($CH_2$), 115.3 (CH), 72.7 ($CH_2$), 70.0 ($CH_2$), 68.3 ($CH_2$), 63.7 ($CH_2$), 26.0 ($CH_3$), 18.5 (C), -5.1 ($CH_3$); HRMS (ESI) calcd for $C_{18}H_{29}NO_5SiNa$ $[M+Na]^+$ 390.1707, found 390.1712.



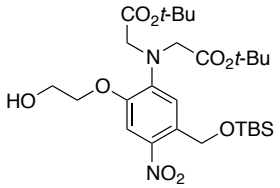
**2-(2-(Allyloxy)ethoxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)aniline (S9):** A flask was charged with **S8** (1.300 g, 3.537 mmol); EtOH (35.4 mL), $H_2O$ (6.8 mL), and AcOH (3.8 mL) were added sequentially. Zinc powder (7.170 g, 109.7 mmol, 31 eq) was then added portionwise. The reaction was stirred at room temperature for 30 min (shielded from light). The mixture was filtered, and the filtrand was rinsed with warm EtOH. The combined filtrate was diluted with brine and extracted with $CH_2Cl_2$ (3×). The combined organics were washed with water (3×) and saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The brown residue was purified by flash chromatography (0–40% EtOAc/hexanes, linear gradient) to yield 1.014 g (85%) of **S9** as a yellow oil. $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.76 (d, *J* = 8.2 Hz, 1H), 6.70 (d, *J* = 2.0 Hz, 1H), 6.63 (dd, *J* = 8.1, 2.0 Hz, 1H), 5.94 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H), 5.31 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.20 (dq, *J* = 10.4, 1.3 Hz, 1H), 4.60 (s, 2H), 4.17 – 4.11 (m, 2H), 4.08 (dt, *J* = 5.6, 1.4 Hz, 2H), 3.85 (s, 2H), 3.82 – 3.76 (m, 2H), 0.93 (s, 9H), 0.08 (s, 6H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 145.6 (C), 137.0 (C), 135.1 (C), 134.8 (CH), 117.4 ($CH_2$), 116.3 (CH), 113.7 (CH), 112.9 (CH), 72.4 ($CH_2$), 68.9 ($CH_2$), 68.7 ($CH_2$), 65.1 ($CH_2$), 26.2 ($CH_3$), 18.6 (C), -5.0 ($CH_3$); HRMS (ESI) calcd for $C_{18}H_{32}NO_3Si$ $[M+H]^+$ 338.2146, found 338.2145.



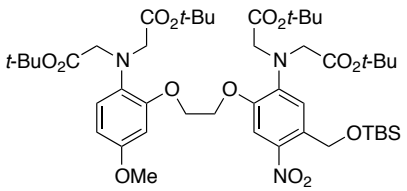

**Di-*tert*-butyl 2,2'-((2-(2-(allyloxy)ethoxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)phenyl)azanediyl)diacetate (S10):** Aniline **S9** (1.000 g, 2.963 mmol) was dissolved in MeCN (30 mL); 1,8-bis(dimethylamino)naphthalene (2.540 g, 11.85 mmol, 4 eq), *tert*-butyl bromoacetate (3.467 g, 17.78 mmol, 6 eq) and NaI (133 mg, 0.889 mmol, 0.3 eq) were added sequentially. The resulting mixture was stirred at reflux for 18 h while being shielded from light. The reaction was cooled to room temperature, and the solvent was evaporated. The residue was diluted into $CH_2Cl_2$, washed with water (3×) and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Purification by flash chromatography (0–20% EtOAc/hexanes, linear gradient) yielded **S10** as a pale yellow oil (1.295 g, 77%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 6.86 – 6.76 (m, 3H), 5.93 (ddt, *J* = 17.2, 10.5, 5.6 Hz, 1H), 5.29 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.18 (dq, *J* = 10.4, 1.3 Hz, 1H), 4.61 (s, 2H), 4.16 (dd, *J* = 5.7, 4.8 Hz, 2H), 4.06 (dt, *J* = 5.7, 1.5 Hz, 2H), 4.05 (s, 4H), 3.78 (dd, *J* = 5.7, 4.8 Hz, 2H), 1.42 (s, 18H), 0.92 (s, 9H), 0.07 (s, 6H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 170.7 (C), 149.6 (C), 139.6 (C), 134.9 (CH), 134.7 (C), 119.9 (CH), 117.8 (CH), 117.2 ($CH_2$), 114.9 (CH), 81.1 (C), 72.3 ($CH_2$), 68.8 ($CH_2$), 68.6 ($CH_2$), 65.1 ($CH_2$), 54.6 ($CH_2$), 28.3 ($CH_3$), 26.2 ($CH_3$), 18.6 (C), -5.0 ($CH_3$); HRMS (ESI) calcd for $C_{30}H_{52}NO_7Si$ $[M+H]^+$ 566.3508, found 566.3508.



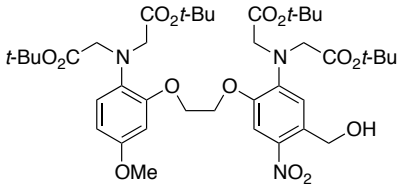
**Di-*tert*-butyl 2,2'-((2-(2-(allyloxy)ethoxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-4-nitrophenyl)azanediyl) diacetate (S11):** Compound **S10** (1.280 g, 2.262 mmol) was taken up in MeCN (22.6 mL) under nitrogen. $AgNO_3$ (422 mg, 2.49 mmol, 1.1 eq) and benzoyl chloride (384 mg, 2.49 mmol, 1.1 eq) were added sequentially while maintaining the temperature at or below 25 °C. The reaction was then shielded from light and stirred at ≤25 °C for 30 min. The reaction mixture was filtered to remove AgCl, and the filtrand was washed with saturated $NaHCO_3$. The combined filtrate was extracted with EtOAc (3×). The organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The yellow residue was purified by silica gel chromatography (0–30% EtOAc/hexanes, linear gradient; dry load with Celite) to afford 1.155 g (84%) of **S11** as a yellow solid. $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.76 (s, 1H), 7.17 (s, 1H), 5.93 (ddt, *J* = 17.2, 10.5, 5.6 Hz, 1H), 5.30 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.20 (dq, *J* = 10.4, 1.3 Hz, 1H), 5.06 (d, *J* = 0.8 Hz, 2H), 4.24 – 4.19 (m, 2H), 4.17 (s, 4H), 4.06 (dt, *J* = 5.6, 1.4 Hz, 2H), 3.82 – 3.76 (m, 2H), 1.45 (s, 18H), 0.97 (s, 9H), 0.13 (s, 6H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 169.4 (C), 146.9 (C), 145.0 (C), 137.6 (C), 134.6 (CH), 134.4 (C), 117.4 ($CH_2$), 114.6 (CH), 111.1 (CH), 81.9 (C), 72.3 ($CH_2$), 69.0 ($CH_2$), 68.2 ($CH_2$), 62.8 ($CH_2$), 55.1 ($CH_2$), 28.3 ($CH_3$), 26.2 ($CH_3$), 18.6 (C), -5.1 ($CH_3$); HRMS (ESI) calcd for $C_{30}H_{50}N_2O_9SiNa$ $[M+Na]^+$ 633.3178, found 633.3182.



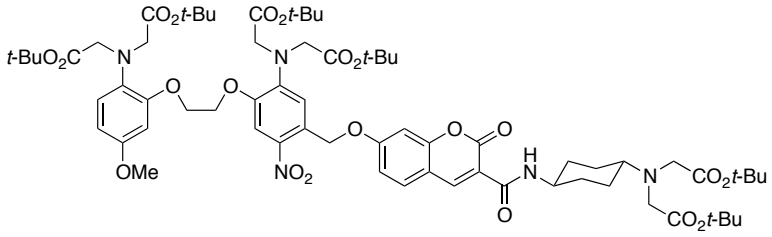

**Di-*tert*-butyl 2,2'-((5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(2-hydroxyethoxy)-4-nitrophenyl)azanediyl) diacetate (2):** A round-bottomed flask equipped with a condenser was charged with **S11** (100 mg, 0.164 mmol), 1,3-dimethylbarbituric acid (51 mg, 0.327 mmol, 2 eq), and $Pd(PPh_3)_4$ (95 mg, 0.0819 mmol, 0.5 eq). After thoroughly flushing the reaction with nitrogen, THF (1.64 mL) was added. The reaction was shielded from light and stirred at reflux for 8 h. It was subsequently diluted with saturated $NaHCO_3$ and extracted with EtOAc (3×). The combined organics were washed with brine, dried ($MgSO_4$), filtered, and evaporated. The resulting orange residue was purified by flash chromatography (2–10% *i*PrOH/hexanes, linear gradient) to provide **2** (73 mg, 78%) as a yellow solid. $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.72 (s, 1H), 7.15 (s, 1H), 5.06 (d, *J* = 0.7 Hz, 2H), 4.16 – 4.12 (m, 2H), 4.11 (s, 4H), 3.92 – 3.85 (m, 2H), 3.41 (t, *J* = 7.1 Hz, 1H), 1.49 (s, 18H), 0.96 (s, 9H), 0.13 (s, 6H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 170.3 (C), 147.0 (C), 145.0 (C), 137.8 (C), 134.4 (C), 114.2 (CH), 110.5 (CH), 82.7 (C), 72.2 ($CH_2$), 62.7 ($CH_2$), 60.8 ($CH_2$), 55.5 ($CH_2$), 28.2 ($CH_3$), 26.2 ($CH_3$), 18.6 (C), -5.1 ($CH_3$); HRMS (ESI) calcd for $C_{27}H_{47}N_2O_9Si$ $[M+H]^+$ 571.3045, found 571.3059.



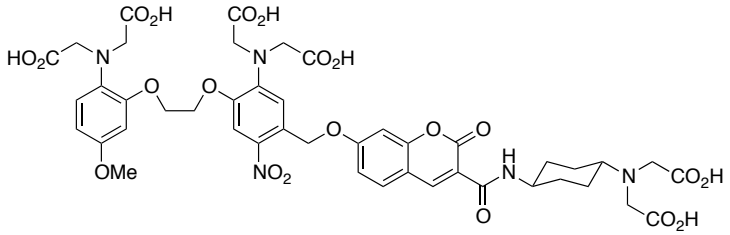
**Di-*tert*-butyl 2,2'-((2-(2-(2-(bis(2-(*tert*-butoxy)-2-oxoethyl)amino)-4-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-nitrophenoxy)ethoxy)-4-methoxyphenyl)azanediyl)diacetate (3):** A vial was charged with phenol **1** (77 mg, 0.210 mmol, 1.2 eq), alcohol **2** (100 mg, 0.175 mmol), and $PPh_3$ (51 mg, 0.193 mmol, 1.1 eq). The vial was sealed and evacuated/backfilled with nitrogen (3×). THF (160 µL) was added, and the resulting mixture was sonicated for 5 min to give a yellow slurry. DIAD (38 µL, 0.193 mmol, 1.1 eq) was added over 5 min (5 portions of 7-8 µL each, 1 min apart) while sonicating. The reaction was sonicated for an additional 20 min. It was then directly purified by silica gel chromatography (0–30% EtOAc/hexanes, linear gradient) to afford **3** as a yellow gum (129 mg, 80%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.78 (s, 1H), 7.21 (s, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.53 (d, *J* = 2.8 Hz, 1H), 6.46 (dd, *J* = 8.7, 2.8 Hz, 1H), 5.07 (s, 2H), 4.38 (s, 4H), 4.18 (s, 4H), 3.97 (s, 4H), 3.74 (s, 3H), 1.42 (s, 18H), 1.39 (s, 18H), 0.97 (s, 9H), 0.14 (s, 6H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 170.5 (C), 169.4 (C), 155.8 (C), 151.9 (C), 146.9 (C), 145.1 (C), 137.7 (C), 134.5 (C), 133.8 (C), 121.7 (CH), 114.9 (CH), 111.2 (CH), 106.1 (CH), 103.6 (CH), 82.0 (C), 81.1 (C), 68.4 ($CH_2$), 67.6 ($CH_2$), 62.7 ($CH_2$), 55.7 ($CH_3$), 55.1 ($CH_2$), 54.9 ($CH_2$), 28.27 ($CH_3$), 28.26 ($CH_3$), 26.2 ($CH_3$), 18.6 (C), -5.1 ($CH_3$); HRMS (ESI) calcd for $C_{46}H_{73}N_3O_{14}SiNa$ $[M+Na]^+$ 942.4754, found 942.4756.




**Di-*tert*-butyl 2,2'-((2-(2-(2-(bis(2-(*tert*-butoxy)-2-oxoethyl)amino)-4-(hydroxymethyl)-5-nitrophenoxy)ethoxy)-4-methoxyphenyl)azanediyl)diacetate (4):** To a solution of silyl ether **3** (170 mg, 0.185 mmol) in THF (5 mL) was added TBAF (1 M in THF, 222 µL, 0.222 mmol, 1.2 eq). After stirring for 10 min at room temperature, the reaction was diluted with saturated $NH_4Cl$ and extracted with EtOAc (2×). The organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Flash chromatography on silica gel (0–50% EtOAc/hexanes, linear gradient) provided 141 mg (95%) of **4** as a yellow solid. $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.76 (s, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.86 (s, 1H), 6.52 (d, *J* = 2.8 Hz, 1H), 6.46 (dd, *J* = 8.7, 2.8 Hz, 1H), 4.87 (d, *J* = 6.7 Hz, 2H), 4.39 (s, 4H), 4.18 (s, 4H), 3.96 (s, 4H), 3.74 (s, 3H), 2.64 (t, *J* = 6.9 Hz, 1H), 1.45 (s, 18H), 1.39 (s, 18H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 170.5 (C), 169.5 (C), 155.9 (C), 151.9 (C), 147.5 (C), 145.2 (C), 138.8 (C), 133.8 (C), 132.9 (C), 121.8 (CH), 116.9 (CH), 111.2 (CH), 106.1 (CH), 103.7 (CH), 82.2 (C), 81.1 (C), 68.4 ($CH_2$), 67.5 ($CH_2$), 63.4 ($CH_2$), 55.7 ($CH_3$), 55.1 ($CH_2$), 54.9 ($CH_2$), 28.3 ($CH_3$), 28.2 ($CH_3$); HRMS (ESI) calcd for $C_{40}H_{60}N_3O_{14}$ $[M+H]^+$ 806.4070, found 806.4074.

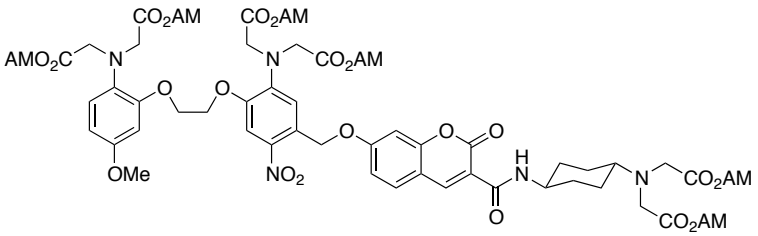


**Di-*tert*-butyl 2,2'-((2-(2-(2-(bis(2-(*tert*-butoxy)-2-oxoethyl)amino)-4-(((3-(((1*r*,4*r*)-4-(bis(2-(*tert*-butoxy)-2-oxoethyl)amino)cyclohexyl)carbamoyl)-2-oxo-2H-chromen-7-yl)oxy)methyl)-5-nitrophenoxy)ethoxy)-4-methoxyphenyl)azanediyl)diacetate (6):** A vial was charged with alcohol **4** (30 mg, 37.2 μmol), 7-hydroxycoumarin-3-carboxamide **5**[1] (23.7 mg, 44.7 μmol, 1.2 eq), and $PPh_3$ (11.7 mg, 44.7 μmol, 1.2 eq). The vial was sealed and evacuated/backfilled with nitrogen (3×). THF (75 μL) was added, and the resulting mixture was sonicated for 5 min to give a yellow solution. DIAD (8.8 μL, 44.7 μmol, 1.2 eq) was added over 15 min (3 portions of ~3 μL each, 5 min apart) while sonicating. The reaction was sonicated for an additional 30 min. It was then directly purified by silica gel chromatography (10–50% EtOAc/hexanes, linear gradient) to afford **6** as a yellow gum (35 mg, 71%). $^1$H NMR ($CDCl_3$, 400 MHz) δ 8.83 (s, 1H), 8.61 (d, *J* = 7.9 Hz, 1H), 7.85 (s, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.03 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.01 (s, 1H), 6.97 (d, *J* = 2.3 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 1H), 6.53 (d, *J* = 2.8 Hz, 1H), 6.47 (dd, *J* = 8.7, 2.8 Hz, 1H), 5.55 (s, 2H), 4.41 (s, 4H), 4.15 (s, 4H), 3.97 (s, 4H), 3.92 – 3.84 (m, 1H), 3.74 (s, 3H), 3.47 (s, 4H), 2.77 – 2.67 (m, 1H), 2.16 – 2.08 (m, 2H), 2.00 – 1.92 (m, 2H), 1.46 (s, 18H), 1.42 (s, 18H), 1.40 (s, 18H), 1.38 – 1.23 (m, 4H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 171.8 (C), 170.5 (C), 169.3 (C), 163.3 (C), 161.8 (C), 161.2 (C), 156.6 (C), 155.9 (C), 151.8 (C), 148.1 (CH), 147.7 (C), 145.2 (C), 138.0 (C), 133.8 (C), 131.2 (CH), 127.8 (C), 121.8 (CH), 115.6 (C), 114.6 (CH), 114.2 (CH), 113.2 (C), 111.1 (CH), 106.2 (CH), 103.8 (CH), 102.1 (CH), 82.2 (C), 81.1 (C), 80.8 (C), 68.4 ($CH_2$), 68.3 ($CH_2$), 67.5 ($CH_2$), 60.4 (CH), 55.7 ($CH_3$), 55.3 ($CH_2$), 54.9 ($CH_2$), 53.9 ($CH_2$), 48.6 (CH), 32.0 ($CH_2$), 29.4 ($CH_2$), 28.28 ($CH_3$), 28.26 ($CH_3$), 28.22 ($CH_3$); HRMS (ESI) calcd for $C_{68}H_{95}N_5O_{21}Na$ $[M+Na]^+$ 1340.6412, found 1340.6461; HRMS (ESI) calcd for $C_{68}H_{95}N_5O_{21}Na_2$ $[M+2Na]^{2+}$ 681.8152, found 681.8155.

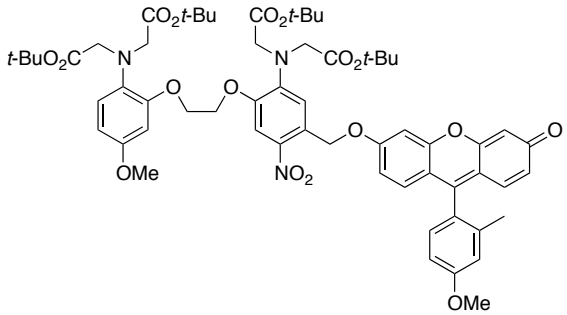



**2,2'-((2-(2-(2-(Bis(carboxymethyl)amino)-4-(((3-(((1*r*,4*r*)-4-(bis(carboxymethyl)amino)cyclohexyl)carbamoyl)-2-oxo-2*H*-chromen-7-yl)oxy)methyl)-5-nitrophenoxy)ethoxy)-4-methoxyphenyl)azanediyl)diacetic acid (7):** To a solution of ester **6** (32 mg, 24.3 μmol) in $CH_2Cl_2$ (2.5 mL) was added TFA (0.5 mL). The reaction was stirred at room temperature for 24 h while shielded from light. Toluene (2 mL) was added, and the resulting mixture was concentrated to dryness. The residue was purified by reverse phase HPLC (10–95% MeCN/$H_2O$, linear gradient, with constant 0.1% v/v TFA additive) to afford **7** as a yellow solid (TFA salt, 22.3 mg, 84%). $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 12.50 (s, 6H), 8.82 (s, 1H), 8.47 (d, *J* = 7.7 Hz, 1H), 7.94 (d, *J* = 8.9 Hz, 1H), 7.76 (s, 1H), 7.17 (d, *J* = 2.1 Hz, 1H), 7.09 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.85 (s, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 6.56 (d, *J* = 2.7 Hz, 1H), 6.45 (dd, *J* = 8.8, 2.7 Hz, 1H), 5.56 (s, 2H), 4.41 – 4.23 (m, 4H), 4.17 (s, 4H), 3.94 (s, 4H), 3.82 – 3.58 (m, 5H), 3.69 (s, 3H), 2.91 – 2.81 (m, 1H), 2.06 – 1.77 (m, 4H), 1.50 – 1.20 (m, 4H); Analytical HPLC: 98.2% purity (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 10–95% $CH_3CN/H_2O$, linear gradient, with constant 0.1% v/v TFA additive; 20

min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{44}H_{47}N_5O_{21}Na$ $[M+Na]^+$ 1004.2656, found 1004.2654.

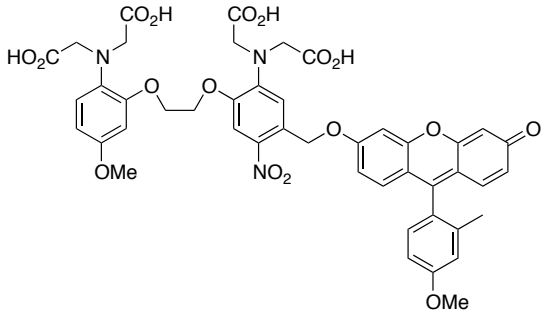



**Bis(acetoxymethyl) 2,2'-((2-(2-(2-(bis(2-(acetoxymethoxy)-2-oxoethyl)amino)-4-(((3-(((1*r*,4*r*)-4-(bis(2-(acetoxymethoxy)-2-oxoethyl)amino)cyclohexyl)carbamoyl)-2-oxo-2*H*-chromen-7-yl)oxy)methyl)-5-nitrophenoxy)ethoxy)-4-methoxyphenyl)azanediyl)diacetate (8):** Acid **7** (15 mg, 13.7 µmol) was dissolved in DMF (2 mL); bromomethyl acetate (34 µL, 342 µmol, 25 eq) and DIEA (60 µL, 342 µmol, 25 eq) were added, and the reaction was stirred at room temperature for 24 h while shielded from light. The crude reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by reverse phase HPLC (30–95% MeCN/$H_2O$, linear gradient, with constant 0.1% v/v TFA additive). Product fractions were combined, concentrated to remove MeCN, diluted with saturated $NaHCO_3$, and extracted with $CH_2Cl_2$ (2×). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated. Flash chromatography on silica gel (50–100% EtOAc/hexanes, linear gradient) afforded 9.8 mg (51%) of **8** as a yellow foam. $^1$H NMR ($CDCl_3$, 400 MHz) δ 8.83 (s, 1H), 8.60 (d, $J$ = 7.9 Hz, 1H), 7.84 (s, 1H), 7.63 (d, $J$ = 8.8 Hz, 1H), 7.04 (dd, $J$ = 8.7, 2.4 Hz, 1H), 7.01 – 6.93 (m, 3H), 6.49 – 6.43 (m, 2H), 5.76 (s, 4H), 5.66 (s, 4H), 5.59 (s, 4H), 5.56 (s, 2H), 4.42 – 4.38 (m, 2H), 4.34 – 4.29 (m, 2H), 4.27 (s, 4H), 4.11 (s, 4H), 3.91 – 3.82 (m, 1H), 3.76 (s, 3H), 3.64 (s, 4H), 2.78 – 2.69 (m, 1H), 2.15 – 2.10 (m, 2H), 2.13 (s, 6H), 2.072 (s, 6H), 2.066 (s, 6H), 1.99 – 1.90 (m, 2H), 1.44 – 1.24 (m, 4H); Analytical HPLC: 98.9% purity (4.6 mm x 150 mm 5 µm C18 column; 5 µL injection; 30–95% $CH_3CN/H_2O$, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{62}H_{71}N_5O_{33}Na$ $[M+Na]^+$ 1436.3924, found 1436.3960; HRMS (ESI) calcd for $C_{62}H_{71}N_5O_{33}Na_2$ $[M+2Na]^{2+}$ 729.6908, found 729.6903.




**Di-*tert*-butyl 2,2'-((2-(2-(2-(bis(2-(*tert*-butoxy)-2-oxoethyl)amino)-4-(((9-(4-methoxy-2-methylphenyl)-3-oxo-3*H*-xanthen-6-yl)oxy)methyl)-5-nitrophenoxy)ethoxy)-4-methoxyphenyl)azanediyl)diacetate (S12):** This compound (48%, orange solid) was prepared from **4** and TokyoGreen[2] according to the procedure described for **6**.

$^1$H NMR ($CDCl_3$, 400 MHz) δ 7.85 (s, 1H), 7.11 – 7.04 (m, 3H), 7.01 (s, 1H), 6.99 (d, *J* = 9.7 Hz, 1H), 6.95 – 6.89 (m, 3H), 6.88 – 6.83 (m, 1H), 6.57 (dd, *J* = 9.7, 1.9 Hz, 1H), 6.52 (d, *J* = 2.8 Hz, 1H), 6.47 (dd, *J* = 8.7, 2.8 Hz, 1H), 6.45 (d, *J* = 1.9 Hz, 1H), 5.57 (s, 2H), 4.40 (s, 4H), 4.16 (s, 4H), 3.97 (s, 4H), 3.90 (s, 3H), 3.74 (s, 3H), 2.06 (s, 3H), 1.42 (s, 18H), 1.40 (s, 18H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 186.0 (C), 170.5 (C), 169.4 (C), 162.8 (C), 160.5 (C), 159.0 (C), 155.9 (C), 154.6 (C), 151.8 (C), 149.4 (C), 147.6 (C), 145.2 (C), 138.0 (C), 137.8 (C), 133.8 (C), 130.8 (CH), 130.6 (CH), 130.3 (CH), 129.9 (CH), 128.1 (C), 124.7 (C), 121.8 (CH), 119.2 (C), 116.2 (CH), 115.5 (C), 114.6 (CH), 113.4 (CH), 111.7 (CH), 111.1 (CH), 106.2 (CH), 106.0 (CH), 103.9 (CH), 102.2 (CH), 82.2 (C), 81.1 (C), 68.4 ($CH_2$), 68.2 ($CH_2$), 67.5 ($CH_2$), 55.7 ($CH_3$), 55.5 ($CH_3$), 55.3 ($CH_2$), 54.9 ($CH_2$), 28.3 ($CH_3$), 28.2 ($CH_3$), 20.1 ($CH_3$); HRMS (ESI) calcd for $C_{61}H_{73}N_3O_{17}Na$ $[M+Na]^+$ 1142.4832, found 1142.4816.




**2,2'-((2-(2-(2-(Bis(carboxymethyl)amino)-4-(((9-(4-methoxy-2-methylphenyl)-3-oxo-3*H*-xanthen-6-yl)oxy)methyl)-5-nitrophenoxy)ethoxy)-4-methoxyphenyl)azanediyl)diacetic acid (S13):** This compound (TFA salt, 75%, yellow-orange solid) was prepared from **S12** according to the procedure described for **7**. $^1$H NMR (DMSO-$d_6$, 400 MHz) δ 12.47 (s, 4H), 7.78 (s, 1H), 7.45 (d, *J* = 2.3 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.17 (d, *J* = 9.0 Hz, 1H), 7.14 – 7.08 (m, 3H), 7.03 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.86 (s, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 6.68 (dd, *J* = 9.6, 1.9 Hz, 1H), 6.58 – 6.53 (m, 2H), 6.45 (dd, *J* = 8.8, 2.7 Hz, 1H), 5.64 (s, 2H), 4.39 – 4.32 (m, 2H), 4.31 – 4.25 (m, 2H), 4.18 (s, 4H), 3.94 (s, 4H), 3.87 (s, 3H), 3.69 (s, 3H), 2.01 (s, 3H); Analytical HPLC: 97.9% purity (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 10–95% $CH_3CN/H_2O$, linear gradient, with constant 0.1% v/v TFA additive; 20 min run; 1 mL/min flow; ESI; positive ion mode; UV detection at 254 nm); HRMS (ESI) calcd for $C_{45}H_{42}N_3O_{17}$ $[M+H]^+$ 896.2509, found 896.2505.

## OPTICAL SPECTROSCOPY AND MICROSCOPY METHODS

**General.** All measurements were taken at ambient temperature (23 ± 2 °C). Fluorogenic molecules were prepared as stock solutions in DMSO and diluted such that the DMSO concentration did not exceed 1% v/v. Spectroscopy was performed using 1-cm path length quartz or polymethacrylate cuvettes (Starna or Fisher). Absorption measurements were recorded on a Cary Model 100 spectrometer (Varian). Fluorescence measurements spectra were recorded on a Cary Eclipse fluorometer (Varian). Buffer pH was adjusted with KOH. Curve fitting was accomplished with GraphPad Prism 5.

**Determination of Photochemical Quantum Yield.** Photochemistry was performed in 1-cm path length/3.5 mL quartz cuvettes (Starna) in a Luzchem LZC 4V photoreactor equipped with 365 nm UV lamps, a carousel, and a timer. The irradiation was determined by potassium ferrioxalate actinometry.[3] A solution of 60 mM $K_3Fe(C_2O_4)_3$ was irradiated using the photoreactor setup and released $Fe^{2+}$ was determined by complexometry with 1,10 phenanthroline. Using the known photochemical quantum yield of this process, we determined the photon flux ($I$) = $3.57 \times 10^{-7}$ ein/min·cm$^2$. Samples (10 μM, 3.5 mL) were irradiated in either zero calcium buffer (30 mM MOPS pH 7.2, 10 mM EGTA, 100 mM KCl) or calcium-containing buffer (30 mM MOPS pH 7.2, 10 mM $CaCl_2$, 100 mM KCl) and a small aliquot (30 μL) was removed and diluted 100-fold in matching buffer. The fluorescence of these samples was then measured (coumarin **7**: $\lambda_{ex}/\lambda_{em}$ = 402/447 nm; Tokyo Green compound **S13**: $\lambda_{ex}/\lambda_{em}$ = 496/520 nm). The photochemical quantum yield ($\Phi$, mol/ein) was determined by fitting a plot of fluorescence vs. irradiation time to a one-phase association described by equation 1:

$$F_t = F_{max} - F_{max}(e^{-I\sigma\Phi t}) \quad (1)$$

where $F_{max}$ = maximal fluorescence, $t$ = time (min), $F_t$ = fluorescence at time $t$, $I$ = irradiation (ein/min·cm$^2$), and $\sigma$ = decadic extinction coefficient (in units of cm$^2$/mol; 1000-fold higher than the $\varepsilon$ value with units of $M^{-1}cm^{-1}$ based on cuvette geometry). For compound **7** we determined $\Phi$ = 4.8 ± 0.2% (mean ± SE) in the presence of 10 mM $Ca^{2+}$ and $\Phi$ = 0.0079 ± 0.0003% in the presence of 10 mM EGTA (Fig. 2A). For compound **S13** we found $\Phi$ = 1.63 ± 0.05% in the presence of 10 mM $Ca^{2+}$ and $\Phi$ = 0.0110 ± 0.0004% in the presence of 10 mM EGTA (Fig. S1A).

**Determination of $K_d$.** For [$Ca^{2+}$] < 40 μM, the free calcium concentration was controlled by a commercial EGTA buffer system (Invitrogen).[4] Briefly, different proportions of EGTA buffer (30 mM MOPS pH 7.2, 10 mM EGTA, 100 mM KCl) or Ca·EGTA buffer (30 mM MOPS pH 7.2, 10 mM Ca·EGTA, 100 mM KCl) were mixed to give solutions with different free [$Ca^{2+}$] based on a $K_d$ of Ca·EGTA = 141.5 nM (value from maxchelator.stanford.edu at 25 °C. 0.1 M ionic strength, pH 7.2). For [$Ca^{2+}$] > 40 μM, buffers were prepared with 1 mM EGTA and excess $CaCl_2$ in 30 mM MOPS pH 7.2 and 100 mM KCl. To determine $K_d$ from the initial rate of photochemical conversion, different buffer solutions containing 100 nM of **7** or **S13** were irradiated in clean glass vials for 1 min using the photoreactor setup described above; this brief irradiation keeps the photoreaction below saturation (Fig. 1b). The samples were then transferred to a black, clear bottom, 96-well polystyrene microplate with a nonbinding surface coating (Corning). Fluorescence was read from the bottom on a FlexStation3 fluorescence microplate reader

(Molecular Devices; for coumarin **7**: $\lambda_{ex}/\lambda_{em}$ = 402/447 nm; for Tokyo Green compound **S13**: $\lambda_{ex}/\lambda_{em}$ = 496/520 nm). The plot of fluorescence *vs*. [$Ca^{2+}$] was fit to one site-specific binding curve to determine the $K_d$. Found: 34.7 ± 2.0 μM (±SE) for **7** and 28.0 ± 0.8 μM for **S13**. To determine $K_d$ from the change in absorption at 430 nm, the absorbance spectra of different buffer solutions containing 5 μM of **7** were measured (Figure 1a). The plot of change in absorbance at 430 nm ($\Delta A$) *vs*. [$Ca^{2+}$] was fit to one site-specific binding curve to determine the $K_d$. Found: 31.0 ± 2.1 μM (±SE) for **7**.

**Cell Culture.** HEK293T/17 cells (ATCC) were cultured according to instructions in poly-D-lysine-coated 24-well CellBind plates (Corning) in Dulbecco's modified eagle medium (DMEM; Invitrogen) supplemented with 10% v/v fetal bovine serum (FBS; Invitrogen) and maintained at 37 °C in a humidified 5% $CO_2$ v/v environment. Primary rat hippocampal neurons were prepared and cultured as described previously.[5] Imaging buffer for HEK293T/17 cells consisted of Dulbecco's phosphate buffered saline (DPBS; Invitrogen). Imaging buffer for cultured neurons consisted of: 145 mM NaCl, 2.5 KCl, 10 mM glucose, 10 mM HEPES pH 7.4, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. To load cells with the dosimeter, a stock solution of **8** (1 mM) in DMSO containing 10% w/v Pluronic F-127 (Sigma) was added to a final concentration of 10 μM in imaging buffer. Cells were incubated in this solution for 1 h at 37 °C, after which the cells were washed with imaging buffer (3×) and imaged. The final imaging buffer wash for cultured neurons additionally contained pharmacological agents (10 μM CNQX, 10 μM (R)-CPP, 10 μM gabazine, and 1 mM (S)-MCPG; Tocris) to block spontaneous cellular activity.

**Live Cell Wide-Field Fluorescence Microscopy.** Cells were imaged on a Olympus IX-81 microscope using a 10× or 20× objective and a DAPI filter set (Semrock DAPI-5060C-000; excitation: 352–402 nm, dichroic cutoff: 409 nm, emission: 417–477 nm) filtering a mercury arc lamp. Excitation/photoactivation light was attenuated using a neutral density filter giving a light intensity of ~60 mW/$cm^2$. To raise intracellular calcium ion levels (Figs. 3B and S2B), cells were incubated with 10 μM ionomycin (Sigma) and 4 mM $CaCl_2$ for 2 min prior to imaging.

**Light-Gated Integration of $Ca^{2+}$ Evoked by Electrical Stimulation.** Cultured neurons loaded with compound **8** were stimulated electrically to induce action potential firing at 80 Hz for one second prior to and during illumination with excitation/photoactivation light. A Grass S48 Stimulator (Grass Technologies) was used with a custom-built platinum wire field stimulation electrode as described.[5]

**Modeling of the rate of uncaging in stimulated and unstimulated cells.** The expected release rate of compound **7** under different $Ca^{2+}$ concentrations can be calculated using equation 1:

$$k = I(\sigma_f C_f \Phi_f + \sigma_b C_b \Phi_b) \quad (1)$$

where $k$ = the rate of photochemical reaction, $I$ = irradiation intensity (ein/min·$cm^2$), $\sigma$ = the decadic extinction coefficient (in units of $cm^2$/mol; 1000-fold higher than the $\varepsilon$ value with units of $M^{-1}cm^{-1}$ based on cuvette geometry), $C$ = the concentration of indicator, $\Phi$ = the photochemical quantum yield of the cage, and subscripts $b$ and $f$ indicate $Ca^{2+}$-bound and $Ca^{2+}$-free, respectively. To find $C_b$ and $C_f$ we use the following Equations S1–S3 where the $[Ca^{2+}]_t$ is the total calcium ion concentration and $[Ca^{2+}]_b$ and $[Ca^{2+}]_f$ are the concentrations of bound and free calcium ion, respectively:

$$K_d = \frac{C_f \times [Ca^{2+}]_f}{C_b} \quad \text{(S1)}$$

$$C_f = C_t - C_b \quad \text{(S2)}$$

$$[Ca^{2+}]_f = [Ca^{2+}]_t - C_b \quad \text{(S3)}$$

Putting everything in terms of $C_b$ gives Equation S4

$$K_d = \frac{(C_t - C_b) \times ([Ca^{2+}]_t - C_b)}{C_b} \quad \text{(S4)}$$

Rearrange and simplify to yield Equation S5:

$$C_b^2 - C_b([Ca^{2+}]_t + C_t + K_d) + (C_t \times [Ca^{2+}]_t) = 0 \quad \text{(S5)}$$

Solving the quadratic equation gives Equation 2:

$$C_b = \frac{(C_t + [Ca^{2+}]_t + K_d) - \sqrt{(C_t + [Ca^{2+}]_t + K_d)^2 - 4(C_t \times [Ca^{2+}]_t)}}{2} \quad \text{(2)}$$

where $C_t$ = total concentration of indicator, $[Ca^{2+}]$ is the calcium ion concentration in the cells, and $K_d$ is the dissociation constant of the $Ca^{2+}$-sensitive cage indicator. The concentration of free indicator ($C_f$) can be calculated from $C_t$ and $C_b$. We first modeled the rate of uncaging in stimulated cells relative to the rate in resting cells (Fig. 3a; $k_{stim}/k_{rest}$, the uncaging contrast) as a function of $K_d$, assuming average $[Ca^{2+}]$ of 245 nM and 62 nM in stimulated and resting cells, respectively,[14] an indicator concentration ($C_t$) of 10 μM (Figure 3a) and an irradiation time of 5 s. To model the increase in fluorescence over time in simulated and resting cells we assumed the same parameters as above and a light intensity ($I$) of $1.67 \times 10^{-8}$ ein/s·cm$^2$, correcting for the depletion of indicator concentration ($C$) during the course of the reaction.

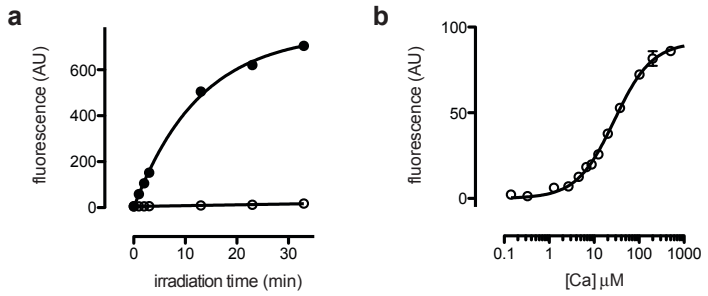



**Figure S1.** (**A**) Fluorescence of released Tokyo Green *vs.* irradiation (365 nm) of **S13** in the presence (●; 10 mM $CaCl_2$) or absence (O; 10 mM EGTA) of $Ca^{2+}$. (**B**) Determination of $K_d$ of compound **S13**.

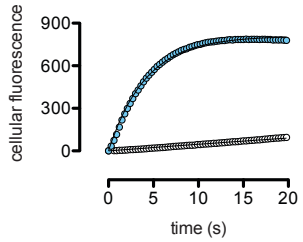



**Figure S2.** Quantification of cellular fluorescence *vs.* irradiation time of untreated cultured neuron (black, Figure 2b, $k$ = 0.0060 $s^{-1}$) and ionomycin-treated cultured neuron (cyan, Figure 2c, $k$ = 0.240 $s^{-1}$).

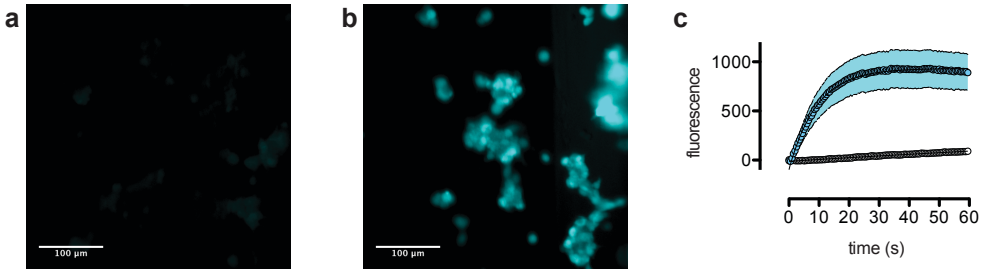



**Figure S3.** **Function of light-gated chemdosimeter in HEK293 cells.** (**a**–**b**) Representative images of live cultured HEK 293T/17 cells incubated with AM ester **8** (10 μM) for 1 h and then illuminated with 365 nm light for 20 s. (**a**) Untreated cells. (**b**) Cells treated with ionomycin. (**c**) Quantification of cellular fluorescence *vs.* irradiation time of untreated cells in **a** (black) and ionomycin-treated cells in **b** (cyan). Shading shows ± standard error (n = 11).

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(1) Guo, Y. M.; Chen, S.; Shetty, P.; Zheng, G.; Lin, R.; Li, W. *Nat. Methods* **2008**, *5*, 835-841.

(2) Urano, Y.; Kamiya, M.; Kanda, K.; Ueno, T.; Hirose, K.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 4888-4894.

(3) Hatchard, C.; Parker, C. *Proc. R. Soc. A* **1956**, *235*, 518-536.

(4) Tsien, R. Y. *Biochemistry* **1980**, *19*, 2396-2404.

(5) Akerboom, J. *et al. J. Neurosci.* **2012**, *32*, 13819-13840.

**SUPPORTING INFORMATION**

# S2: Characterization Spectra (NMR, HPLC)

| | |
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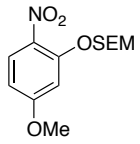
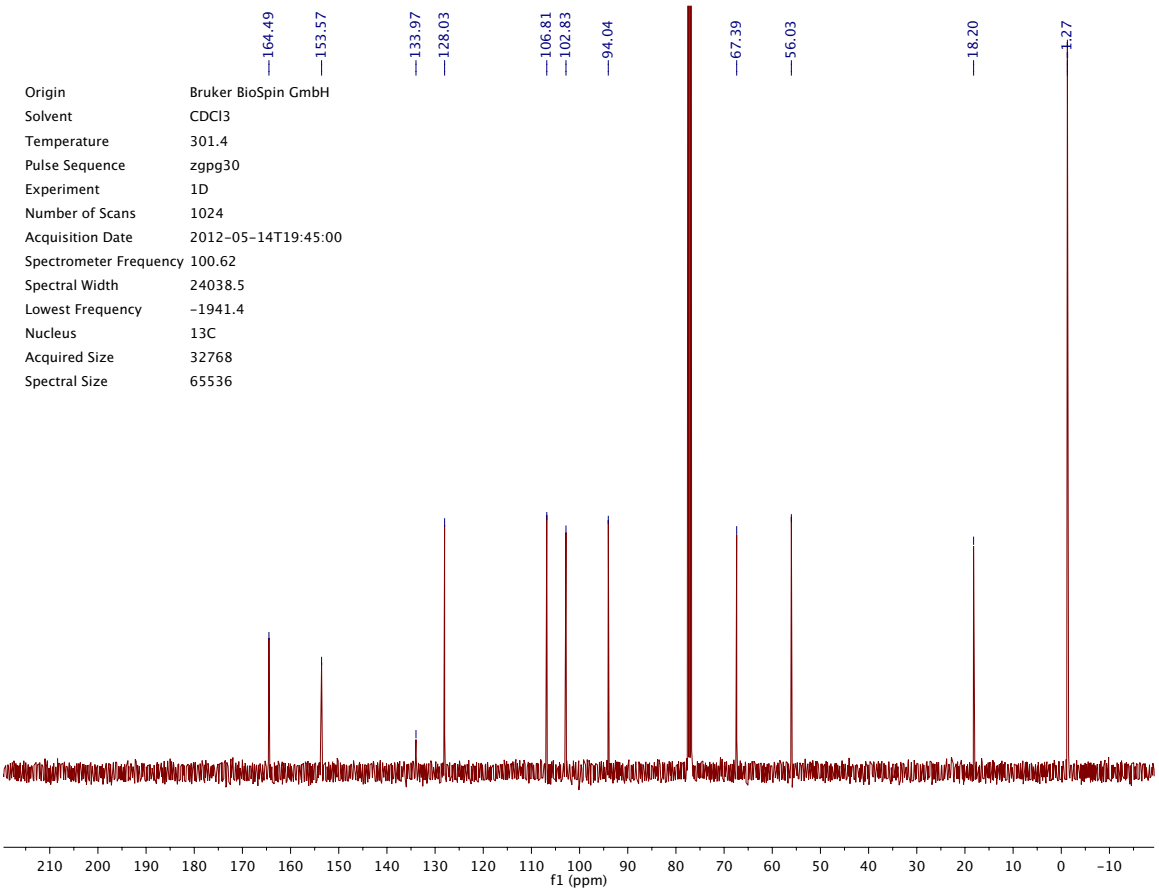
| Lowest Frequency | -1646.3 |
| Nucleus | 1H |
| Acquired Size | 32768 |
| Spectral Size | 65536 |




**S2**




| | |
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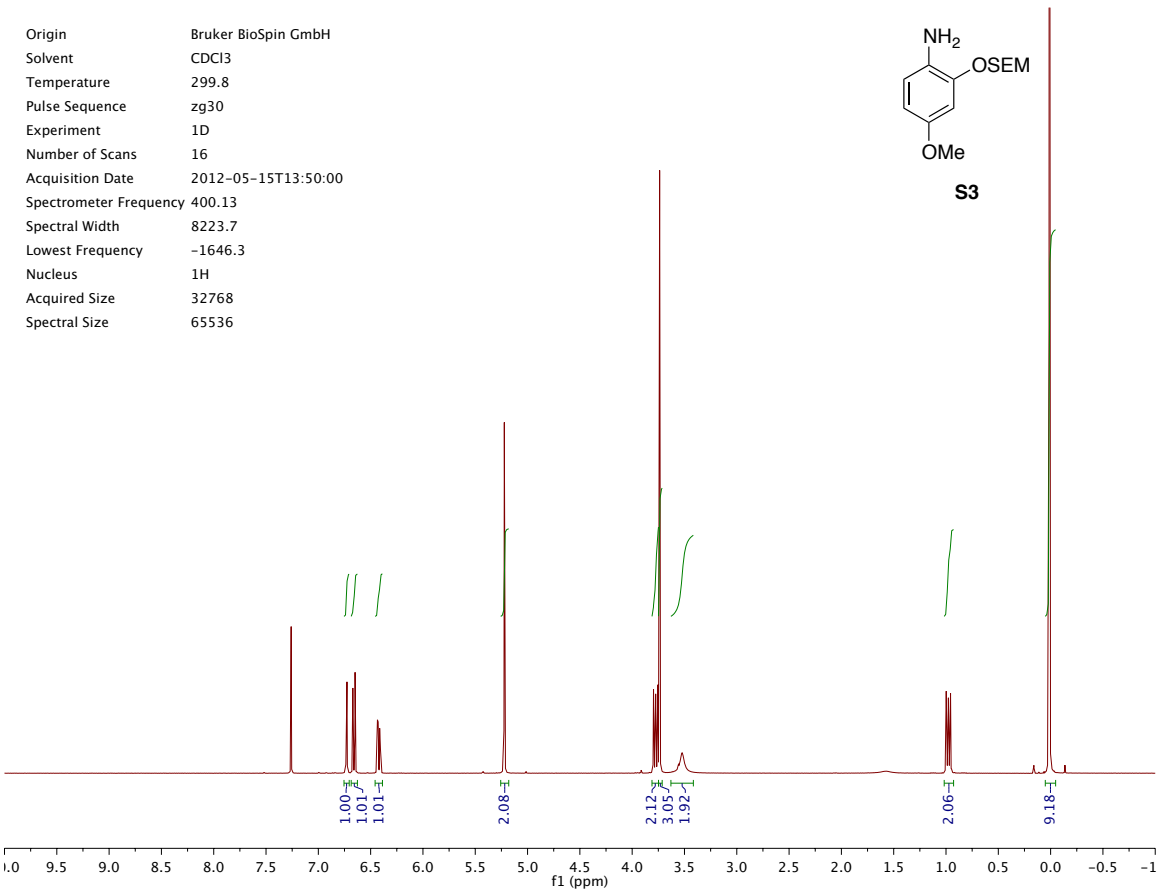
| Lowest Frequency | -1941.4 |
| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |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Nucleus 1H
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Spectral Size 65536
NH2
OSEM
OMe
S3
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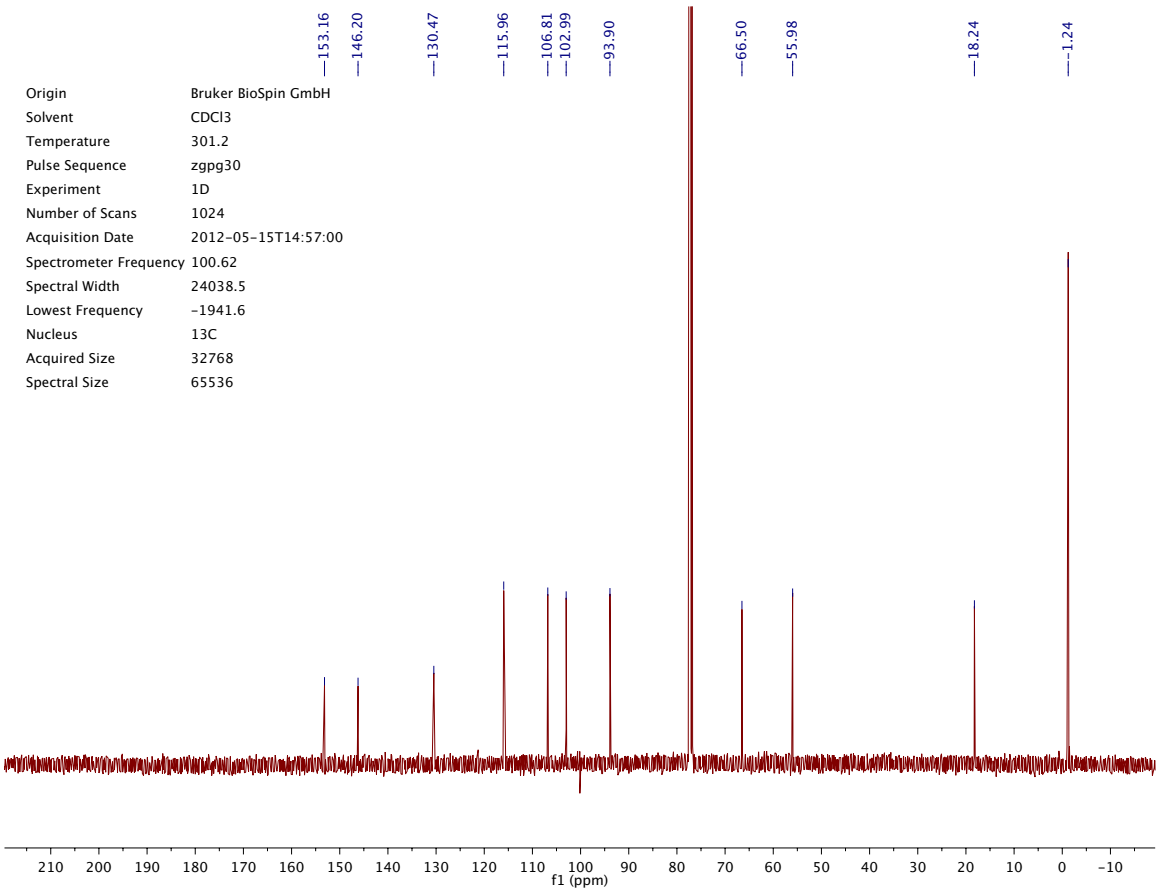
1.01
1.01
2.08
2.12
3.05
1.92
2.06
9.18
f1 (ppm)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Nucleus 13C
Acquired Size 32768
Spectral Size 65536
f1 (ppm)

| | |
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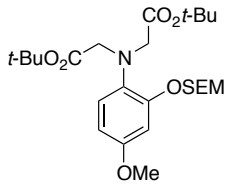


**S4**





| | |
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| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

| | |
|---|---|
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| Pulse Sequence | zg30 |
| Experiment | 1D |
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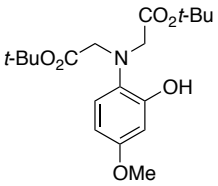


**1**





| | |
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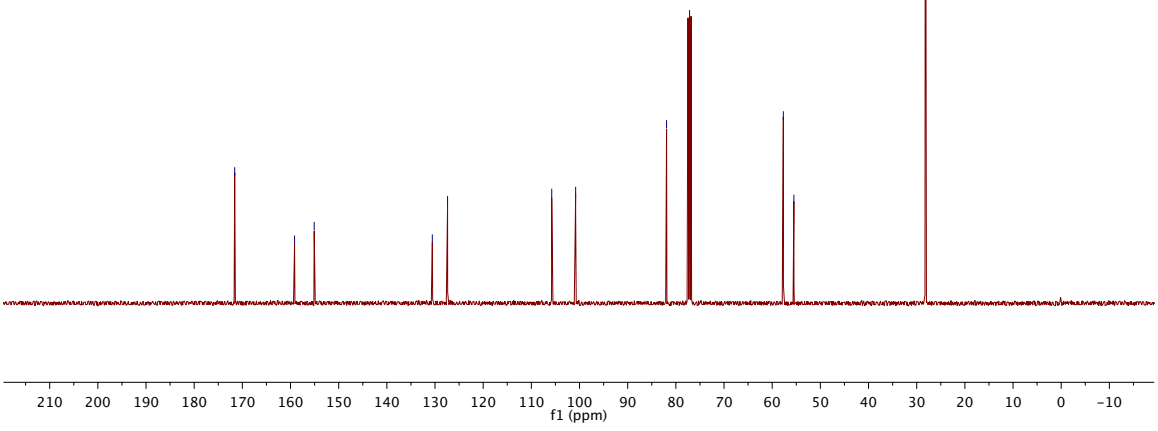

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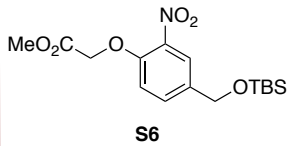
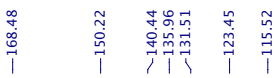



**S6**




| | |
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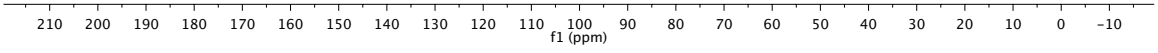

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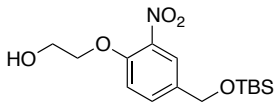


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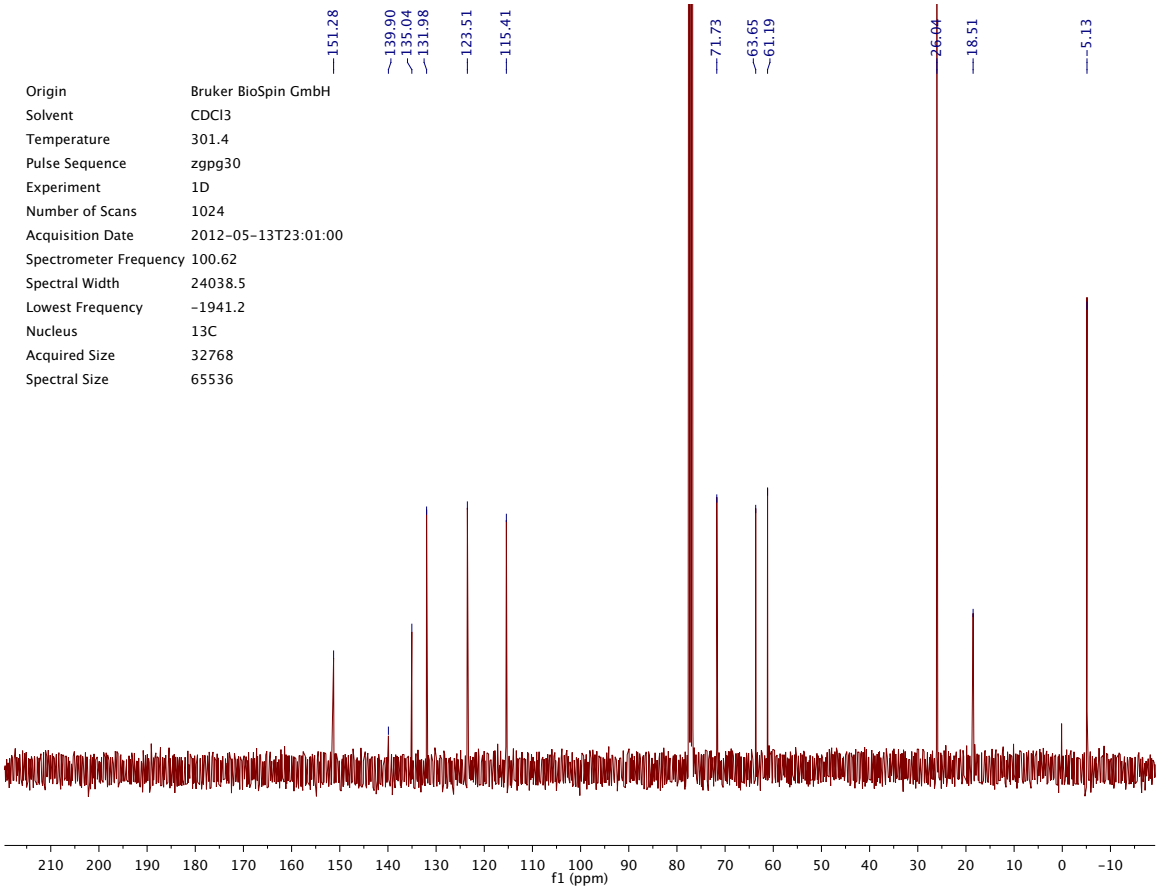


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| Lowest Frequency | -1941.2 |
| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

| | |
|---|---|
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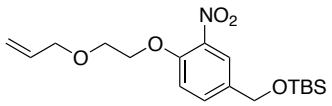


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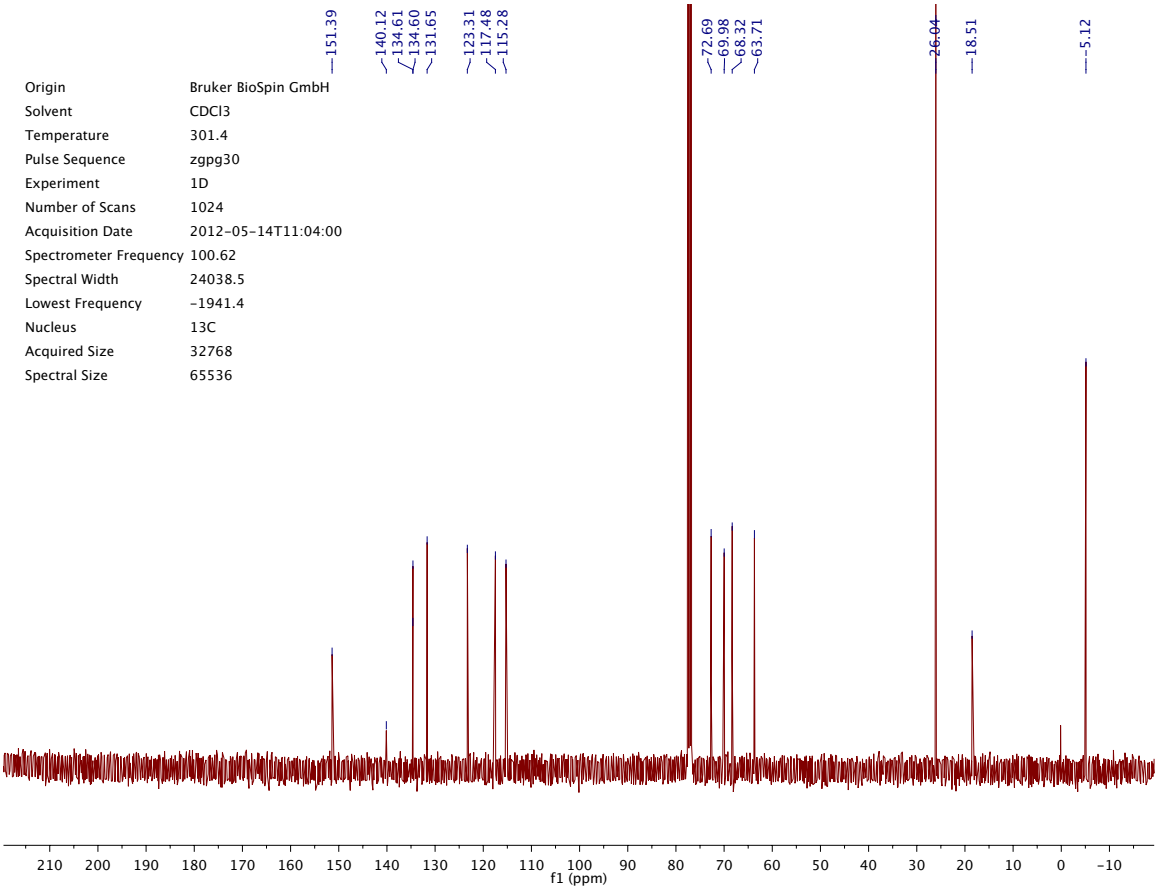


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| Lowest Frequency | -1941.4 |
| Nucleus | 13C |
| Acquired Size | 32768 |
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| | |
|---|---|
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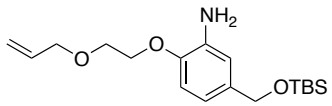


**S9**




| | |
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| Experiment | 1D |
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| Spectrometer Frequency | 100.62 |
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| Lowest Frequency | -1941.2 |
| Nucleus | 13C |
| Acquired Size | 32768 |
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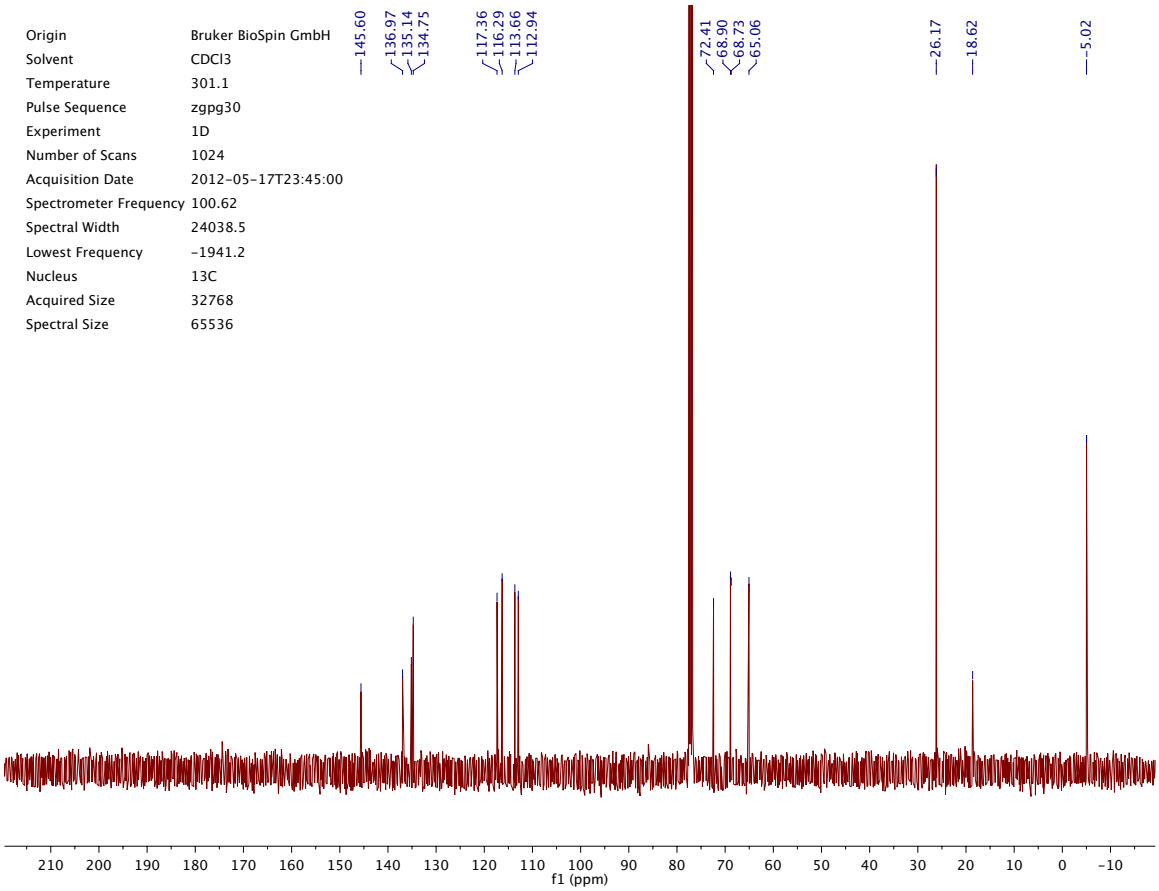

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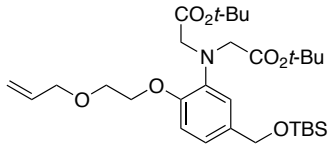


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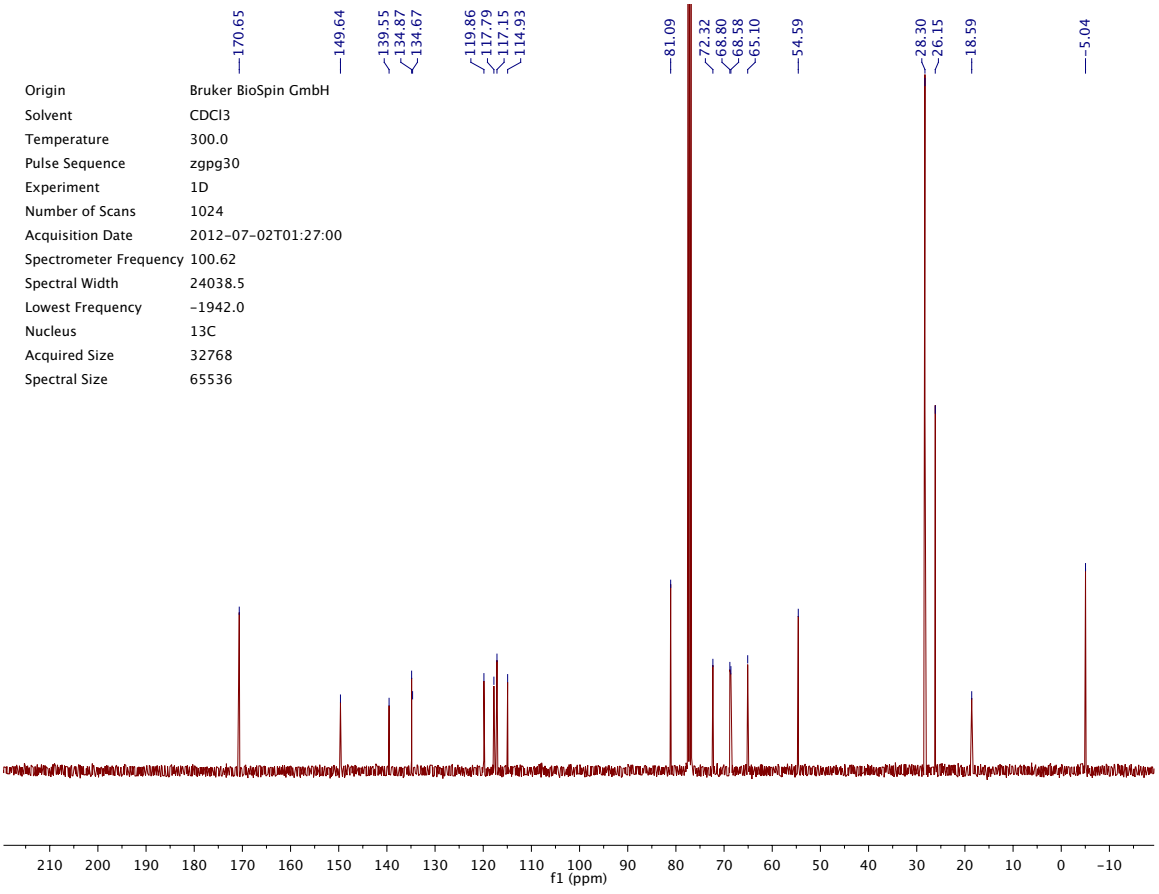


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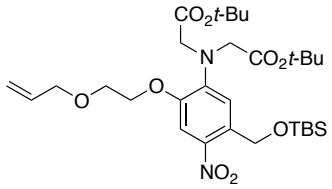
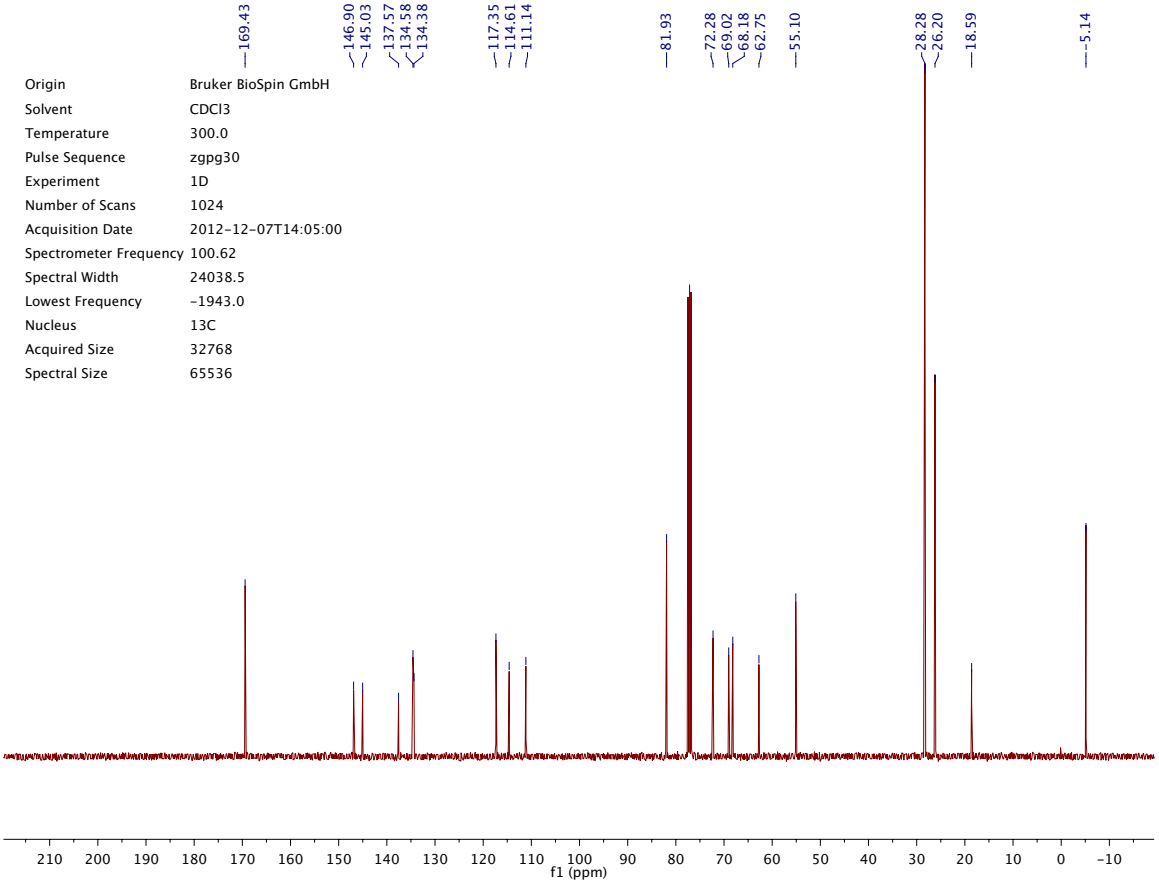
| Pulse Sequence | zgpg30 |
| Experiment | 1D |
| Number of Scans | 1024 |
| Acquisition Date | 2012-07-02T01:27:00 |
| Spectrometer Frequency | 100.62 |
| Spectral Width | 24038.5 |
| Lowest Frequency | -1942.0 |
| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.0 |
| Pulse Sequence | zg30 |
| Experiment | 1D |
| Number of Scans | 16 |
| Acquisition Date | 2012-12-07T13:05:00 |
| Spectrometer Frequency | 400.13 |
| Spectral Width | 8223.7 |
| Lowest Frequency | -1644.0 |
| Nucleus | 1H |
| Acquired Size | 32768 |
| Spectral Size | 65536 |



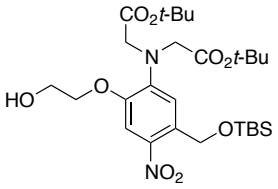

**S11**



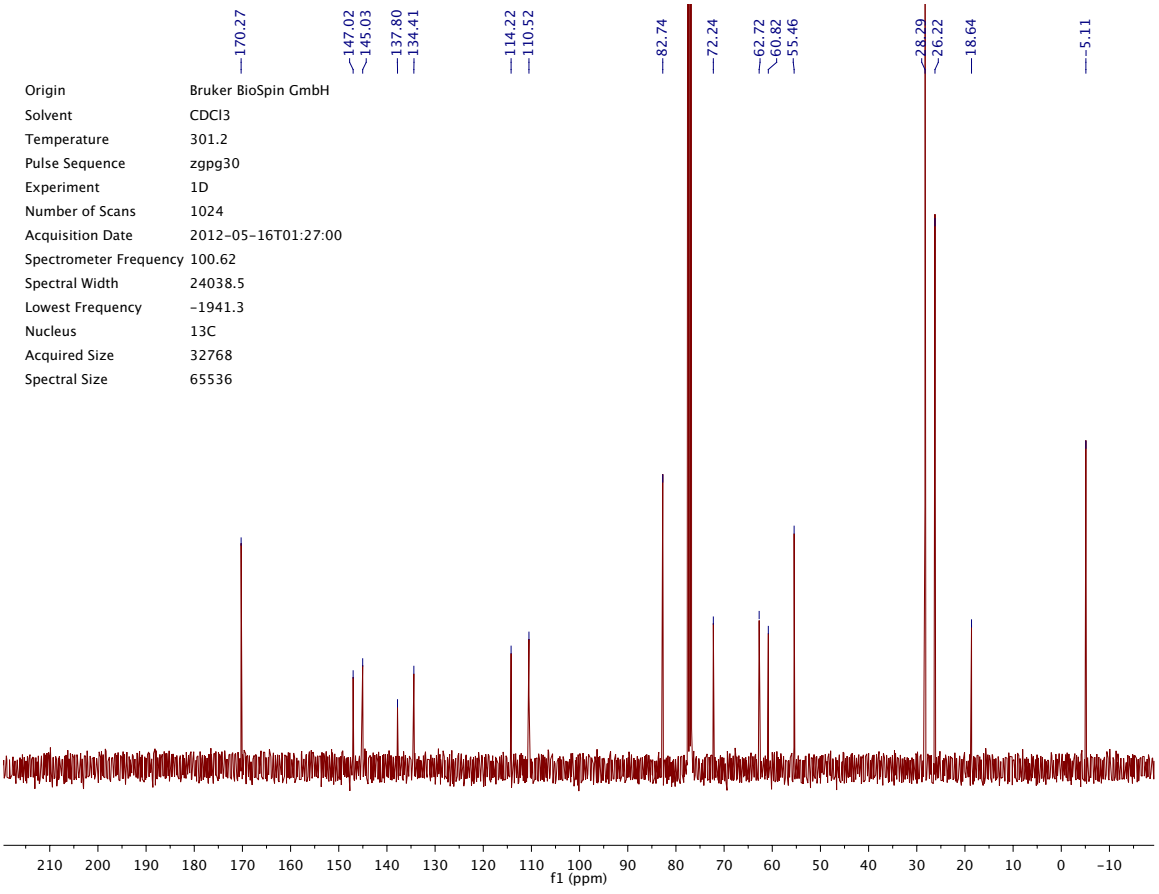

| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.0 |
| Pulse Sequence | zgpg30 |
| Experiment | 1D |
| Number of Scans | 1024 |
| Acquisition Date | 2012-12-07T14:05:00 |
| Spectrometer Frequency | 100.62 |
| Spectral Width | 24038.5 |
| Lowest Frequency | -1943.0 |
| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 299.9 |
| Pulse Sequence | zg30 |
| Experiment | 1D |
| Number of Scans | 16 |
| Acquisition Date | 2012-05-16T00:26:00 |
| Spectrometer Frequency | 400.13 |
| Spectral Width | 8223.7 |
| Lowest Frequency | -1646.1 |
| Nucleus | 1H |
| Acquired Size | 32768 |
| Spectral Size | 65536 |



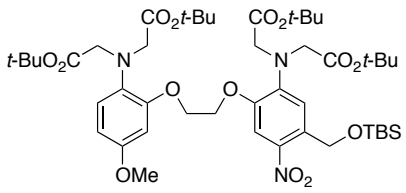

**2**




| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 301.2 |
| Pulse Sequence | zgpg30 |
| Experiment | 1D |
| Number of Scans | 1024 |
| Acquisition Date | 2012-05-16T01:27:00 |
| Spectrometer Frequency | 100.62 |
| Spectral Width | 24038.5 |
| Lowest Frequency | -1941.3 |
| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.0 |
| Pulse Sequence | zg30 |
| Experiment | 1D |
| Number of Scans | 16 |
| Acquisition Date | 2012-10-25T10:48:00 |
| Spectrometer Frequency | 400.13 |
| Spectral Width | 8223.7 |
| Lowest Frequency | -1646.3 |
| Nucleus | 1H |
| Acquired Size | 32768 |
| Spectral Size | 65536 |



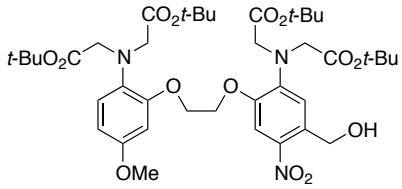

**3**





| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.1 |
| Pulse Sequence | zgpg30 |
| Experiment | 1D |
| Number of Scans | 2048 |
| Acquisition Date | 2012-11-07T13:50:00 |
| Spectrometer Frequency | 100.62 |
| Spectral Width | 24038.5 |
| Lowest Frequency | -1942.7 |
| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.0 |
| Pulse Sequence | zg30 |
| Experiment | 1D |
| Number of Scans | 16 |
| Acquisition Date | 2012-11-27T09:33:00 |
| Spectrometer Frequency | 400.13 |
| Spectral Width | 8223.7 |
| Lowest Frequency | -1646.3 |
| Nucleus | 1H |
| Acquired Size | 32768 |
| Spectral Size | 65536 |



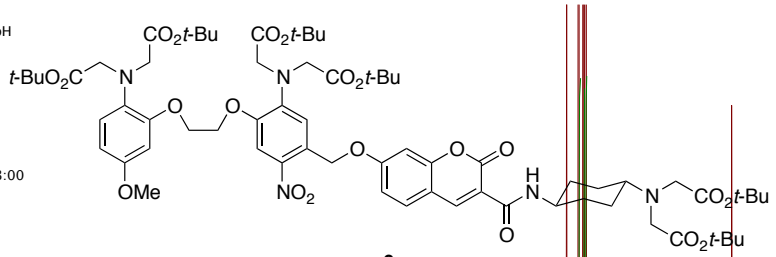

**4**





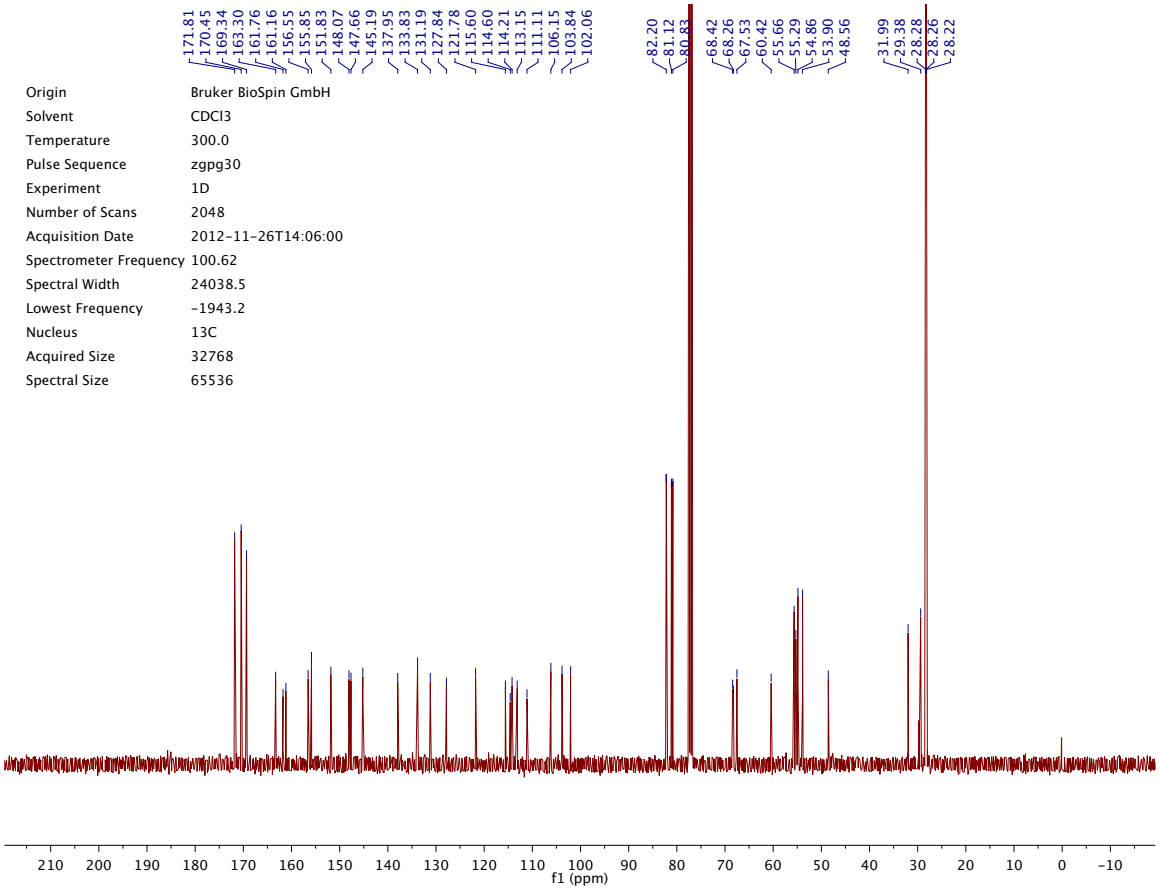
| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.0 |
| Pulse Sequence | zgpg30 |
| Experiment | 1D |
| Number of Scans | 2048 |
| Acquisition Date | 2012-11-28T12:53:00 |
| Spectrometer Frequency | 100.62 |
| Spectral Width | 24038.5 |
| Lowest Frequency | -1943.2 |
| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.0 |
| Pulse Sequence | zg30 |
| Experiment | 1D |
| Number of Scans | 16 |
| Acquisition Date | 2012-11-21T10:23:00 |
| Spectrometer Frequency | 400.13 |
| Spectral Width | 8223.7 |
| Lowest Frequency | -1645.5 |
| Nucleus | 1H |
| Acquired Size | 32768 |
| Spectral Size | 65536 |



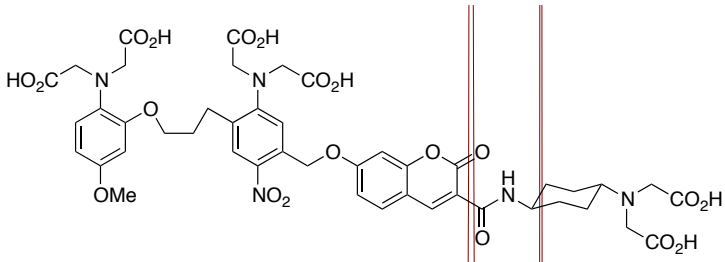

**6**



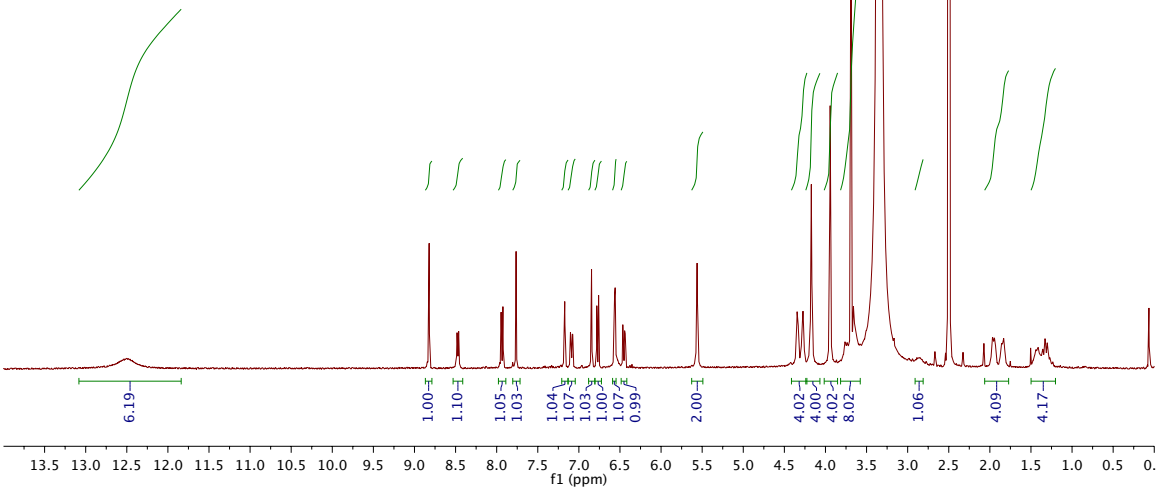



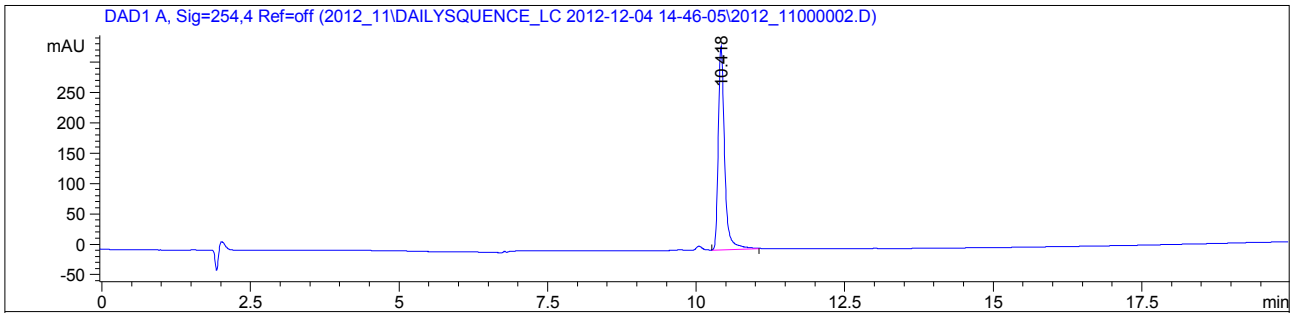

| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.0 |
| Pulse Sequence | zgpg30 |
| Experiment | 1D |
| Number of Scans | 2048 |
| Acquisition Date | 2012-11-26T14:06:00 |
| Spectrometer Frequency | 100.62 |
| Spectral Width | 24038.5 |
| Lowest Frequency | -1943.2 |
| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

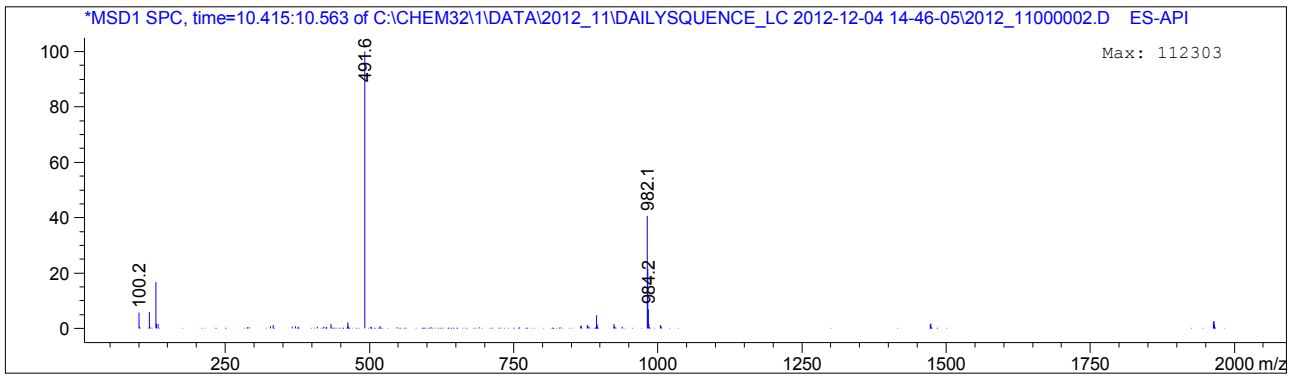
| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | DMSO |
| Temperature | 295.2 |
| Pulse Sequence | zg30 |
| Experiment | 1D |
| Number of Scans | 128 |
| Acquisition Date | 2013-03-29T12:18:00 |
| Spectrometer Frequency | 400.13 |
| Spectral Width | 8223.7 |
| Lowest Frequency | -1638.6 |
| Nucleus | 1H |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

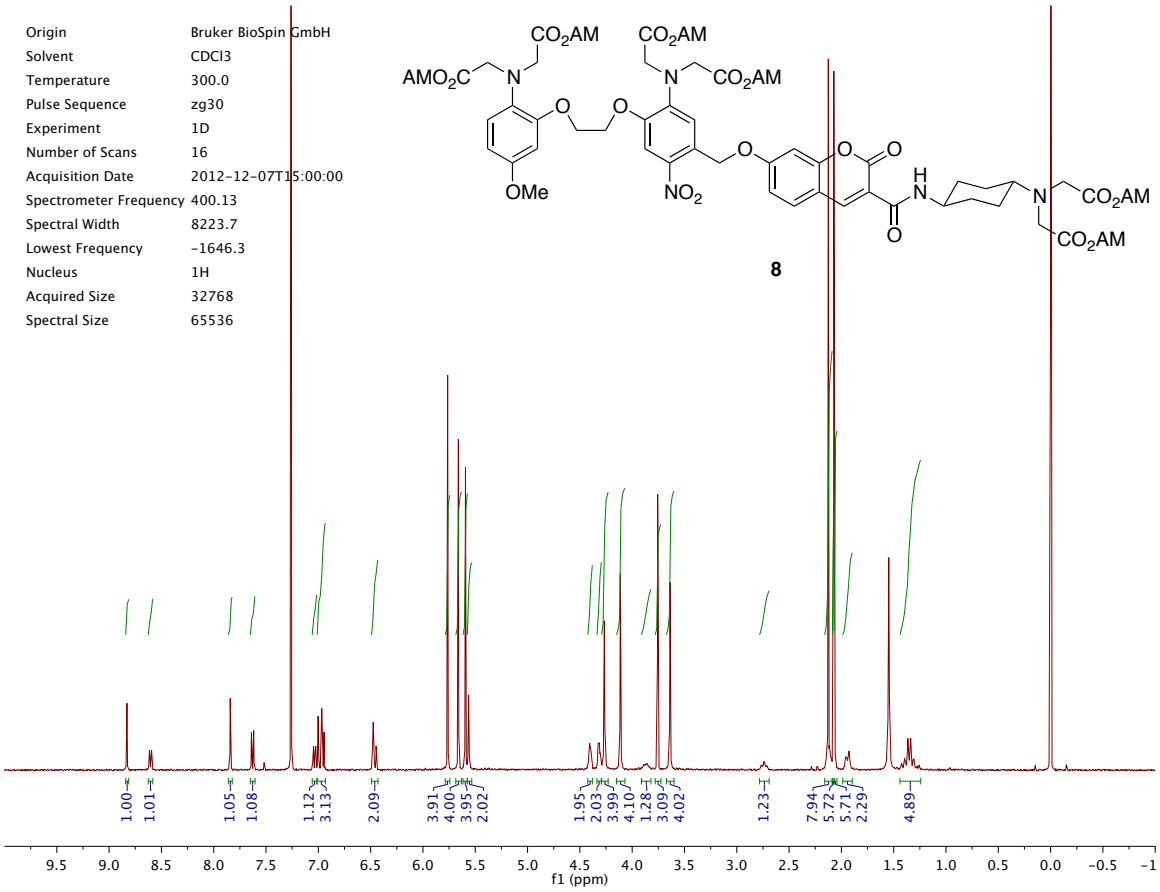


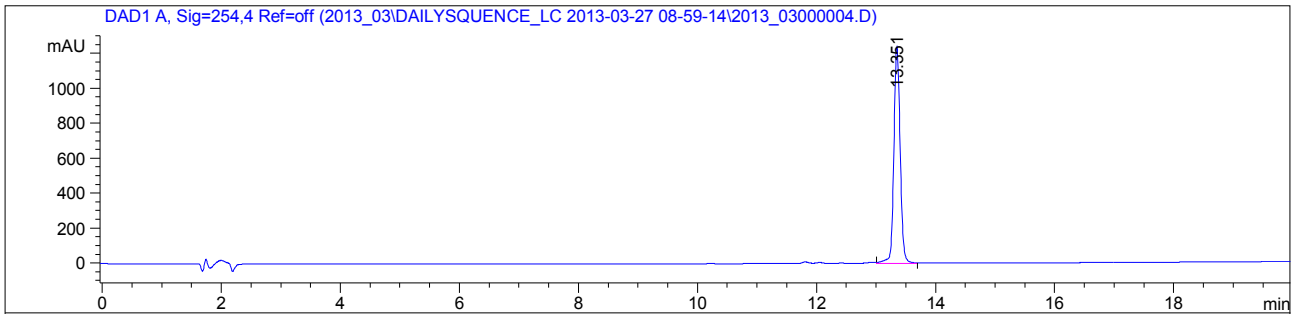

**7**

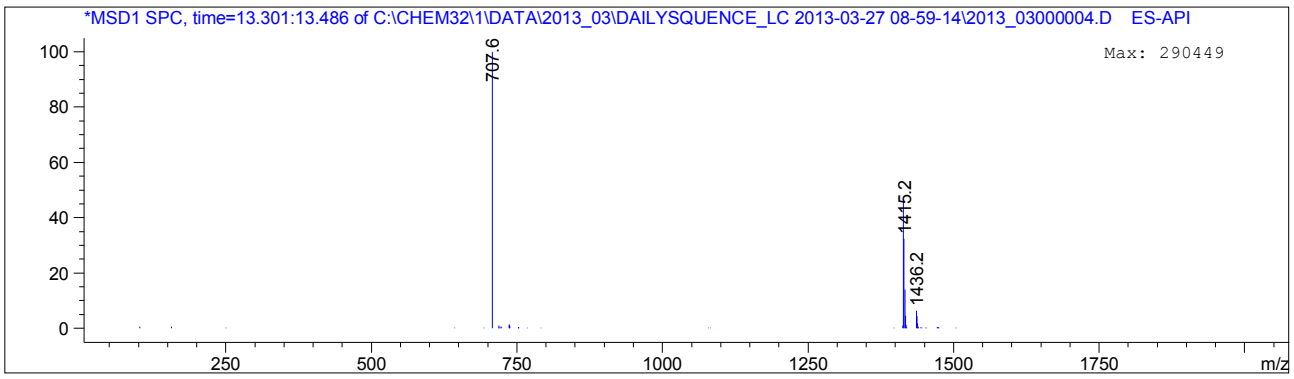


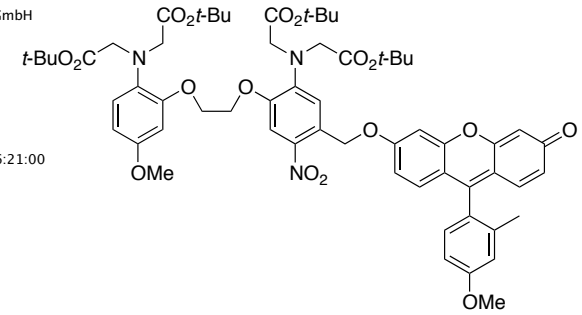




Origin Bruker BioSpin GmbH
Solvent CDCl3
Temperature 300.0
Pulse Sequence zg30
Experiment 1D
Number of Scans 16
Acquisition Date 2012-12-07T15:00:00
Spectrometer Frequency 400.13
Spectral Width 8223.7
Lowest Frequency -1646.3
Nucleus 1H
Acquired Size 32768
Spectral Size 65536
CO2AM
AMO2C
OMe
NO2
8
1.00 1.01 1.05 1.08 1.12 3.13 2.09 3.91 4.00 3.95 2.02 1.95 2.03 3.99 4.10 1.28 3.09 4.02 1.23 7.94 5.72 5.71 2.29 4.89
f1 (ppm)

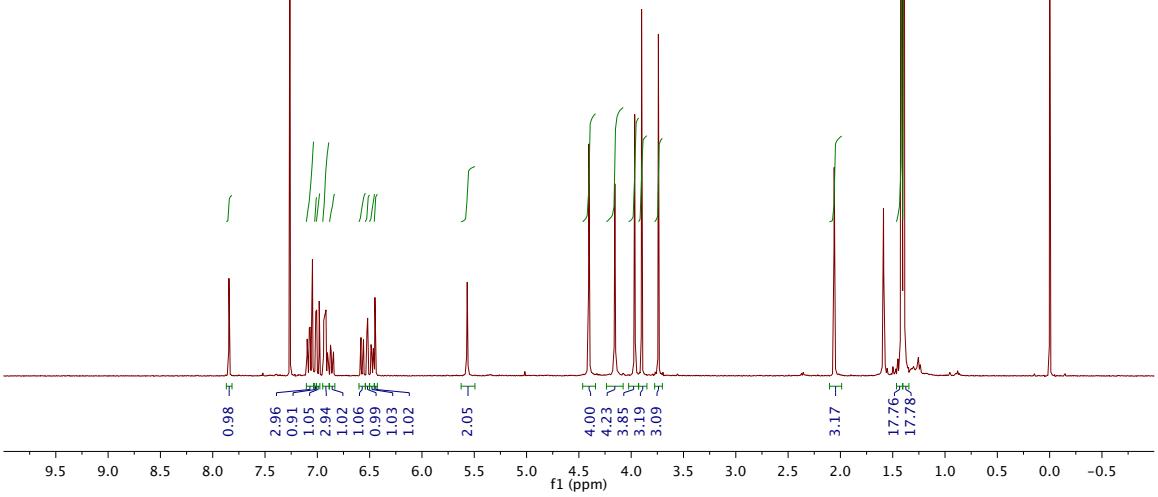

DAD1 A, Sig=254,4 Ref=off (2013_03\DAILYSQUENCE_LC 2013-03-27 08-59-14\2013_03000004.D)
mAU
13.351
min


*MSD1 SPC, time=13.301:13.486 of C:\CHEM32\1\DATA\2013_03\DAILYSQUENCE_LC 2013-03-27 08-59-14\2013_03000004.D ES-API
Max: 290449
707.6
1415.2
1436.2
m/z

| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.0 |
| Pulse Sequence | zg30 |
| Experiment | 1D |
| Number of Scans | 16 |
| Acquisition Date | 2013-01-10T16:21:00 |
| Spectrometer Frequency | 400.13 |
| Spectral Width | 8223.7 |
| Lowest Frequency | -1645.5 |
| Nucleus | 1H |
| Acquired Size | 32768 |
| Spectral Size | 65536 |

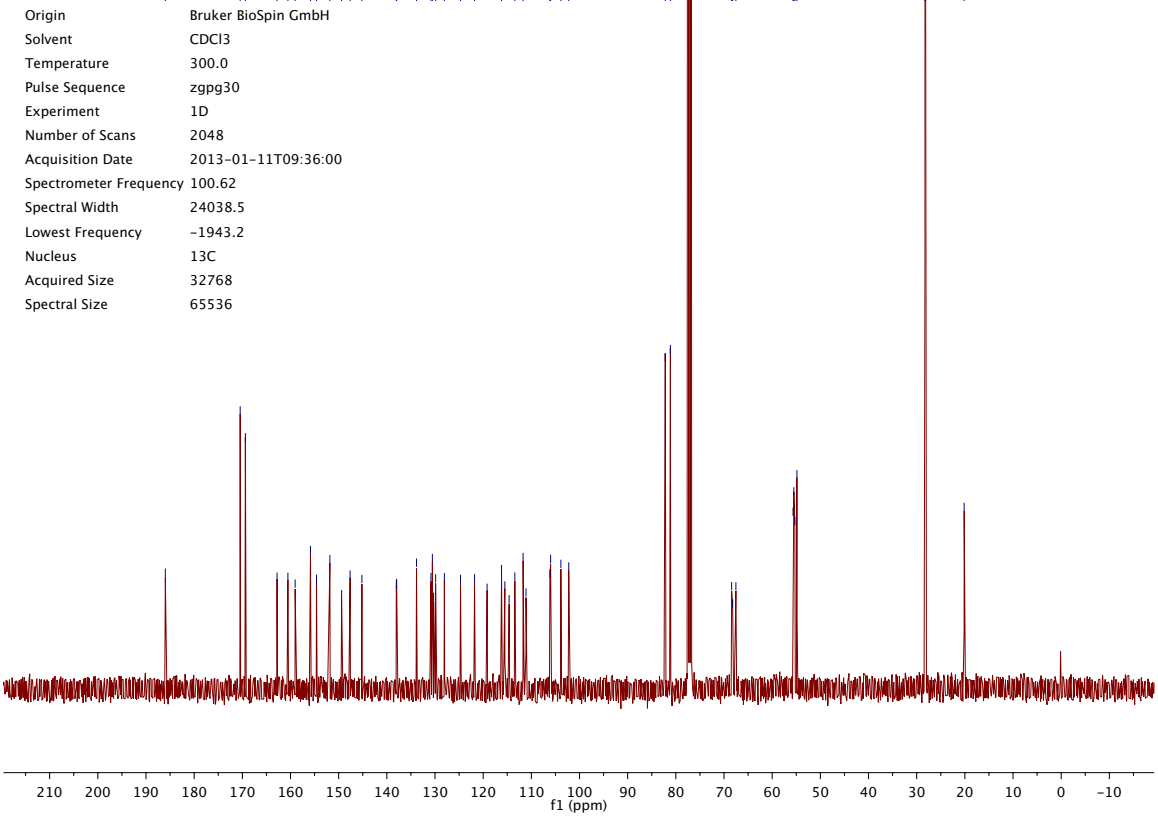



**S12**

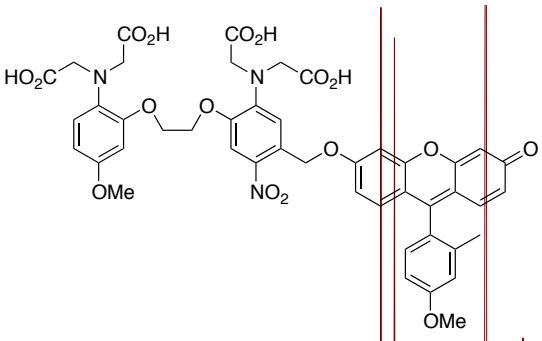




| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | CDCl3 |
| Temperature | 300.0 |
| Pulse Sequence | zgpg30 |
| Experiment | 1D |
| Number of Scans | 2048 |
| Acquisition Date | 2013-01-11T09:36:00 |
| Spectrometer Frequency | 100.62 |
| Spectral Width | 24038.5 |
| Lowest Frequency | -1943.2 |
| Nucleus | 13C |
| Acquired Size | 32768 |
| Spectral Size | 65536 |



| | |
|---|---|
| Origin | Bruker BioSpin GmbH |
| Solvent | DMSO |
| Temperature | 295.2 |
| Pulse Sequence | zg30 |
| Experiment | 1D |
| Number of Scans | 128 |
| Acquisition Date | 2013-04-03T16:55:00 |
| Spectrometer Frequency | 400.13 |
| Spectral Width | 8223.7 |
| Lowest Frequency | -1638.6 |
| Nucleus | 1H |
| Acquired Size | 32768 |
| Spectral Size | 65536 |




**S13**