Electronic Supplementary Information (ESI)

A two photon-activatable amino acid linker for the induction of fluorescence

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General Information

All starting materials were obtained from commercially available sources and used without further purification. Activated zinc was bought from *Rieke Metals Inc*. ATTO565-NHS ester was obtained from *ATTO-TEC GmbH*. The hydrogel kit 3D Life PVA-PEG was obtained from *Cellendes*.

NMR spectra were recorded on Bruker AM250, AV400 and AV500 MHz spectrometers. To assign the ^{1}H and ^{13}C NMR additional spectra like $^{1}H^{1}H$-COSY, $^{1}H^{13}C$-HSQC and $^{1}H^{13}C$-HMBC were measured. The chemical shifts are reported in parts per million (ppm). The spectra were referenced to the chemical shift of the solvent according to G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176 ($CDCl_3$ ^{1}H = 7.26, ^{13}C = 77.16; DMSO-d_6 ^{1}H = 2.50, ^{13}C = 39.52). Multiplicity was indicated as follows: s (singlet), d (doublet), dd (doublet of doublet), ddd (doublet of doublet of doublet), t (triplet), dt (doublet of triplet), m (multiplet), br s (broad singlet). Coupling constants *J* were reported in Hz.

Flash chromatography was performed with silica gel 60 from *Machery Nagel* with a particle size of 40 – 63 µm. The typical solvents were cyclohexane (CH) and ethyl acetate (EE). For TLC analysis aluminum plates coated with silica gel 60 F_{254} from *Merck* were used. Compounds were visualized with 254 nm.

HPLC purification was performed with MultoKrom RP-C18 columns (either 250 * 4.6 mm, flow 1 mL/min or 250 * 8 mm, flow 4 mL/min). Three different gradients with solvents A: acetonitrile and B: 0.1% aqueous trifluoroacetic acid were used.

1.			**2.**			**3.**		
Time [min]	A	B	Time [min]	A	B	Time [min]	A	B
0	5	95	0	5	95	0	35	65
30	60	40	30	60	40	60	35	65
32	100	0	40	60	40	64	100	0
36	100	0	42	100	0	68	100	0
39	5	95	46	100	0	72	35	65
43	5	95	50	5	95	76	35	65
			55	5	95			

ESI mass spectra were recorded on a VG Plattform II spectrometer from *Fisons*. High resolution MS was measured with a MALDI Orbitrap XL from *Thermo*. For low resolution MALDI experiments a Voyager DE-PRO from *Applied Biosystems* was used.

Elemental analysis was conducted with a Vario Micro Cube from *Elementar Analysesysteme GmbH*. The elements Carbon, Hydrogen and Nitrogen were analyzed.

One-photon excitation was performed with a custom built setup containing LEDs from *Roithner Lasertechnik*. 365 nm with 250 mW power was used. The one-photon actinometry was conducted with a cuvette holder, a 365 nm LED, and a LED controller from *Thorlabs*. Irradiation time was controlled by an external trigger.

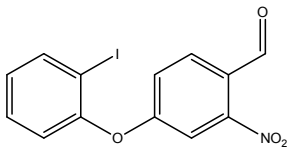
For fluorescence measurements a *TECAN* plate reader infinite M200 PRO in fluorescence top reading mode was used. Excitation wavelength was 530 nm (bandwidth 9 nm) and the emission was recorded from 560 – 850 nm (bandwidth 20 nm).

For the two-photon setup see section “Two-photon excitation”.

For activation with 405 nm and 568 nm and imaging see section “One-photon widefield and super-resolution imaging”.

Chemical synthesis

4-(2-Iodophenoxy)-2-nitrobenzaldehyde



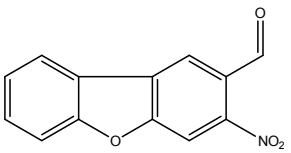
This synthesis was reproduced from H. Lusic, R. Uprety and A. Deiters, *Org. Lett.*, 2010, **12**, 916.

6.8 g (40.4 mmol) of 4-Fluoro-2-nitro-benzaldehyde were dissolved in 100 mL of dry pyridine. 8.9 g (40.4 mmol) of 2-Iodophenol, 5.8 g (40.4 mmol) of copper(I)bromide and 11.2 g (80.2 mmol) of K_2CO_3 were sequentially added. The reaction was purged with Ar and stirred at 60 °C. The reaction progress was followed by TLC. After 16 h the reaction was cooled to room temperature and the pyridine was removed on a rotary evaporator. The residue was mixed with 200 mL of Et_2O and filtered. The Et_2O phase was washed with 200 mL of 1 M NaOH, 200 mL of H_2O, and 200 mL of brine. The organic phase was dried with anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (CH:EE 20:1). 12.1 g (81.2%) of product were isolated.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 10.33 (s, 1H, COH), 7.98 (d, $^3J_{HH}$ = 8.8, 1H, H_{ar}), 7.95 (dd, $^3J_{HH}$ = 8.0, $^4J_{HH}$ = 1.5, 1H, H_{ar}), 7.50 – 7.42 (m, 2 H, H_{ar}), 7,22 (dd, $^3J_{HH}$ = 8.8, $^4J_{HH}$ = 2.5, 1H, H_{ar}), 7.15 – 7.08 (m, 2H, H_{ar}).

ESI-MS: *m/*z found $[M-H]^-$ = 368.

3-Nitrodibenzofuran-2-carbaldehyde



12.0 g (32.5 mmol) of 4-(2-Iodophenoxy)-2-nitrobenzaldehyde were dissolved in 150 mL of toluene. 10.9 mL (195 mmol) of Ethylene glycol and 1.0 g of *p*-toluene sulfonic acid monohydrate (5.3 mmol) were added. The reaction was purged with argon and stirred at 120 °C. Reaction progress was monitored by TLC. After 20 h, the reaction was cooled to room temperature, diluted with 100 mL of CH_2Cl_2 and extracted with 50 mL of $NaHCO_3$. The aqueous phase was washed three times with 50 mL of CH_2Cl_2. The combined organic extracts were dried with anhydrous $MgSO_4$, evaporated to dryness and purified by column chromatography (CH:EE 5:1). 8.2 g (61.0%) of the intermediate product were isolated.

^{1}H-NMR (250 MHz, $CDCl_3$): δ = 7.91 (dd, $^3J_{HH}$ = 7.8, $^4J_{HH}$ = 1.5, 1H, H_{ar}), 7.75 (d, $^3J_{HH}$ = 8.6, 1H, H_{ar}), 7.45 – 7.35 (m, 2H, H_{ar}), 7.15 (dd, $^3J_{HH}$ = 8.6, $^4J_{HH}$ = 2.5, 1H, H_{ar}), 7.08 – 6.94 (m, 2H, H_{ar}), 6.40 (s, 4H, CH_2).

8.1 g (19.6 mmol) of the intermediate product were dissolved in 40 mL of dimethylacetamide. 12.8 g (39.2 mmol) of Cs_2CO_3, 1.0 g (4.5 mmol) of $Pd(OAc)_2$ and 1 mL of H_2O were added. The reaction was stirred at 80 °C. The progress of the reaction was followed by NMR. The reaction was complete after one week. After filtration over a Celite® pad, the filtrate was diluted with 80 mL of EE, three times washed with 80 mL of H_2O and once with 80 mL of brine. The organic extract was dried with anhydrous $MgSO_4$ and the solvents evaporated to yield 5.4 g (96.6%) of the product 2-(1,3-dioxolan-2-yl)-3-nitrodibenzofuran, which was used in the next step without further purification.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.38 (s, 1H, H_{ar}), 8.18 (s, 1H, H_{ar}), 8.06 – 8.02 (m, 1H, H_{ar}), 7.68 – 7.54 (m, 2H, H_{ar}), 7.48 – 7.41 (m, 1H, H_{ar}), 6.63 (s, 1H, CH), 4.12 (s, 4H, CH_2).

5.3 g (18.6 mmol) of 2-(1,3-dioxolan-2-yl)-3-nitrodibenzofuran were dissolved in 50 mL of THF. After addition of 40 mL of 1M aqueous HCl, the reaction was stirred at room temperature. Reaction progress was monitored by TLC. After 48 h the reaction was extracted with 100 mL of ethyl acetate three times. The combined organic extracts were dried with anhydrous $MgSO_4$ and evaporated to dryness. The crude product was crystallized from $CHCl_3$ by adding n-hexane to yield 2.8 g (63%) of the pure product.

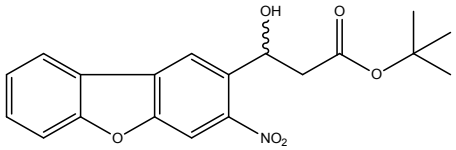
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 10.50 (s, 1H, COH), 8.56 (s, 1H, H_{ar}), 8.32 (s, 1H, H_{ar}), 8.08 (d, $^3J_{HH}$ = 7.8, 1H, H_{ar}), 7.71 – 7.63 (m, 2H, H_{ar}), 7.52 – 7.48 (m, 1H, H_{ar}).

^{13}C-NMR (62.5 MHz, $CDCl_3$): δ = 187.8, 158.8, 157.2, 131.5, 130.4, 129.5, 127.4, 124.7, 122.4, 122.3, 122.2, 112.7, 109.0.

ESI-MS: *m/*z found $[M-H]^-$ = 239.5, $[M+H]^+$ = 242.6, $[M+Na]^+$ = 264.2.

MALDI-HRMS: *m/*z calcd for $C_{13}H_9N_1O_3$ $[M-O+2H]^+$ = 227.05769, found 227.05790 (Δm = 0.00021, error 0.9 ppm) (reduction of NO_2 during MALDI-HRMS analysis is well-known, it is induced by the laser and results in a nitroso group).

tert-Butyl 3-hydroxy-3-(3-nitrodibenzofuran-2-yl)propanoate



3.2 mL (22.4 mmol) of *tert*-butyl chloroacetate were placed in a dried flask and dissolved in 40 mL of Et_2O. The flask was purged with argon and cooled to 0 °C. 58.4 mL of a solution of 2.5 g of activated zinc in 100 mL of THF (22.4 mmol) were added dropwise. The reaction was allowed to warm in 1 h to room temperature to form the Reformatzki reagent. In a second dried flask 2.7 g (11.2 mmol) of 3-nitrodibenzofuran-2-carbaldehyde were dissolved in 50 mL of dry THF. This solution was also purged with argon and cooled to 0 °C. The Reformatzki reagent was slowly added to this solution via syringe. The reaction was allowed to stir for 1 h at 0 °C. TLC control showed complete conversion of the starting material. 100 mL of 1M HCl were added and the mixture was extracted three times with 100 mL of ethyl acetate. The combined organic extracts were dried with anhydrous $MgSO_4$ and purified

by column chromatography (gradient from CH:EE 10:1 to CH:EE 1:1) to yield 3.9 g (98%) of the product.

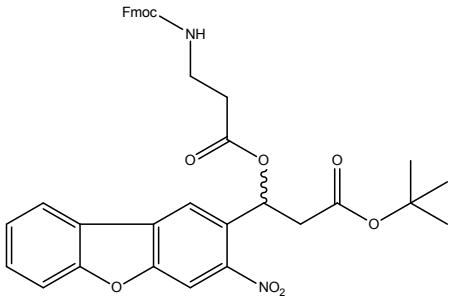
^{1}H-NMR (250 MHz, $CDCl_3$): δ = 8.45 (s, 1H, H_{ar}), 8.20 (s, 1H, H_{ar}), 8.02 (dd, $^{3}J_{HH}$ = 7.7, $^{4}J_{HH}$ = 1.1, 1H, H_{ar}), 7.65 – 7.54 (m, 2H, H_{ar}), 7.45 – 7.39 (m, 1H, H_{ar}), 5.77 (dd, $^{3}J_{HH}$ = 9.2, $^{3}J_{HH}$ = 2.7, 1H, CH), 3.86 (br s, 1H, OH), 2.97 (dd, $^{2}J_{HH}$ = 16.6, $^{3}J_{HH}$ = 2.7, 1H, CHH), 2.64 (dd, $^{2}J_{HH}$ = 16.7, $^{3}J_{HH}$ = 9.3, 1H, CHH), 1.48 (s, 9H, ^{t}Bu-Me).

^{13}C-NMR (62.5 MHz, $CDCl_3$): δ = 172.1, 158.6, 154.2, 146.0, 133.8, 129.6, 129.5, 124.0, 122.8, 122.0, 120.1, 112.4, 108.7, 82.1, 66.7, 43.9, 28.3.

ESI-MS: *m/*z found $[M-H]^-$ = 357.3, $[M+Na]^+$ = 380.6.

EA: calcd C: 63.86%, H: 5.36%, N: 3.92%; found C: 63.83% (Δ = 0.03%), H: 4.94% (Δ = 0.42%), N: 3.91% (Δ = 0.01%).

tert-Butyl 3-(3-(Fmoc)amino)propanoyloxy)-3-(3-nitrodibenzofuran-2-yl)propanoate



3.8 g (10.6 mmol) of *tert*-Butyl 3-hydroxy-3-(3-nitrodibenzofuran-2-yl)propanoate were placed together with 4.9 g (15.9 mmol) of Fmoc-β-alanine, 12.2 g (63.8 mmol) of EDC and 390 mg (3.2 mmol) of DMAP in a 250 mL flask. The flask was purged with Ar. The reagents were dissolved in 100 mL of CH_2Cl_2 and stirred under protection of light. TLC showed complete conversion of starting material after 18 h, so the reaction was poured into ice water. The organic phase was separated and the aqueous phase extracted with EE twice. The combined organic extracts were washed with brine, dried with anhydrous $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography (gradient from CH:EE 10:1 to 1:1) to yield 4.2 g (61%) of the desired product.

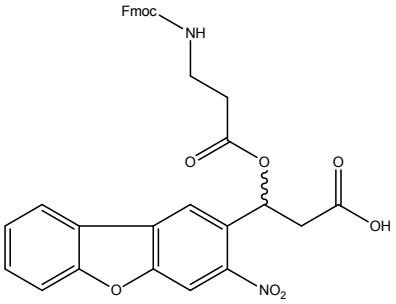
^{1}H-NMR (500 MHz, DMSO-d_6): δ = 8.60 (s, 1H, H_{ar}), 8.44 (s, 1H, H_{ar}), 8.33 (d, $^{3}J_{HH}$ = 7.9, 1H, H_{ar}), 7.85 (d, $^{3}J_{HH}$ = 7.6, 2H, 2x Fmoc-H), 7.78 (d, $^{3}J_{HH}$ = 8.3, 1H, H_{ar}), 7.63 (t, $^{3}J_{HH}$ = 7.6, 1H, H_{ar}), 7.58 (d, $^{3}J_{HH}$ = 7.4, 1H, Fmoc-H), 7.55 (d, $^{3}J_{HH}$ = 7.4, 1H, Fmoc-H), 7.45 (t, $^{3}J_{HH}$ = 7.6, 1H, H_{ar}), 7.41 – 7.34 (m, 3H, 2x Fmoc-H, NH), 7.28 – 7.22 (m, 2H, 2x Fmoc-H), 6.52 (dd, $^{3}J_{HH}$ = 5.4, $^{3}J_{HH}$ = 2.8, 1H, CH), 4.22 – 4.14 (m, 2H, Fmoc-CH_2), 4.07 (t, $^{3}J_{HH}$ = 6.8, 1H, Fmoc-CH), 3.27 – 3.14 (m, 2H,CH_2), 3.06 – 2.99 (m, 2H, CH_2), 2.59 – 2.51 (m, 2H, CH_2), 1.40 (s, 9H , ^{t}Bu-H).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 172.3, 168.4, 157.7, 155.8, 153.5, 146.0, 143.7, 143.6, 140.7, 140.6, 130.0, 129.7, 128.6, 127.6, 127.5, 127.0, 126.9, 125.1, 125.0, 124.1, 122.6, 121.9, 120.7, 120.1, 120.0, 112.2, 108.7, 80.8, 68.9, 65.6, 49.3, 46.5, 41.4, 27.7, 16.7.

ESI-MS: *m/*z found $[M+Na]^+$ = 673.3, $[M+K]^+$ = 689.3.

MALDI-HRMS: *m/*z calcd for $C_{37}H_{34}N_2O_9Na$ $[M+Na]^+$ = 673.21565, for $C_{37}H_{34}N_2O_9K$ $[M+K]^+$ = 689.18959, found 673.21488 (Δm = 0.00077, error 1.14 ppm) and 689.18978 (Δm = 0.00019, error 0.27 ppm).

3-(3-(Fmoc)amino)propanoyloxy)-3-(3-nitrodibenzofuran-2-yl)propanoate



4.1 g (6.3 mmol) of *tert*-butyl 3-(3-(Fmoc)amino)propanoyloxy)-3-(3-nitrodibenzofuran-2-yl)propanoate were dissolved in a mixture of 30 mL of CH_2Cl_2 and 5 mL of trifluoroacetic acid. The reaction progress was monitored by TLC. The reaction was complete after 3 h at room temperature. The solvents were evaporated and the residue coevaporated with 10 mL of toluene three times to yield the final product in 3.5 g (93%) yield.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 12.64 (br s, 1H, COOH), 8.57 (s, 1H, H_{ar}), 8.43 (s, 1H, H_{ar}), 8.34 (d, ^3J = 7.7, 1H, H_{ar}), 7.85 (d, ^3J = 7.6, 2H, 2x Fmoc-H), 7.78 (d, ^3J = 8.3, 1H, H_{ar}), 7.63 (t, ^3J = 7.8, 1H, H_{ar}), 7.59 (d, ^3J = 7.4, 1H, Fmoc-H), 7.54 (d, ^3J = 7.4, 1H, Fmoc-H), 7.45 (t, ^3J = 7.7, 1H, H_{ar}), 7.41 - 7.33 (m, 3H, NH, 2x Fmoc-H), 7.29 – 7.22 (m, 2H, 2x Fmoc-H), 6.53 (dd, ^3J = 8.8, ^3J = 4.2, 1H, CH), 4.24 – 4.12 (m, 2H, Fmoc-CH_2), 4.10 – 4.00 (m, 1H, Fmoc-CH), 3.29 – 3.13 (m, 2H, CH_2), 3.12 – 2.97 (m, 2H, CH_2), 2.58 – 2.52 (m, 2H, CH_2).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 171.3, 170.9, 158.1, 156.4, 153.9, 146.9, 144.3, 144.2, 141.1, 141.1, 130.5, 130.4, 129.0, 128.0, 127.5, 127.4, 125.5, 124.5, 123.3, 122.4, 121.2, 120.5, 112.6, 108.9, 68.5, 65.8, 47.1, 40.8, 40.4, 36.6, 34.4.

ESI-MS: *m/*z found $[M+NH_4]^+$ = 613.1, $[M+H]^+$ = 596.1, $[M-H]^-$ = 593.1.

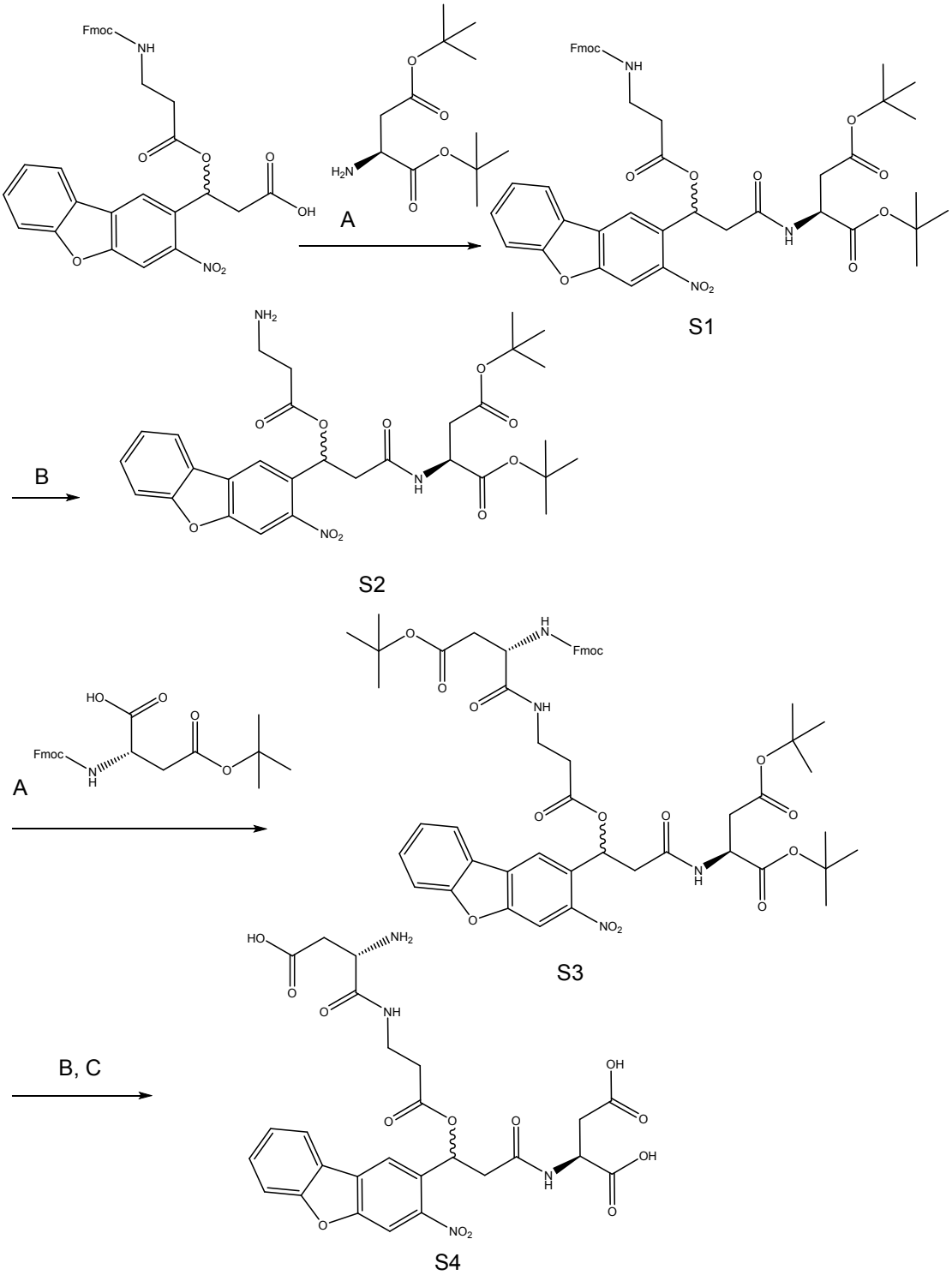
MALDI-HRMS: *m/*z calcd for $C_{33}H_{26}N_2O_9Na$ $[M+Na]^+$ = 617.15305, found = 617.15298 (Δm = 0.00007, error 0.11 ppm).

Peptide synthesis

Procedure A: 1 eq. of carboxylic acid was dissolved in dry THF (for 0.5 mmol 40 mL). 1.1 eq. HOBt, 1.1 eq. EDC, 2 eq. of DIPEA and 1.1 eq. of the amine were added. The reaction was stirred at room temperature for 16 h. The solvent was evaporated and the residue dissolved in a mixture of 100 mL EE and 100 mL H_2O. The organic phase was separated and washed with 100 mL H_2O twice, three times with 60 mL 10% aqueous Na_2CO_3 and 50 mL brine once. The organic phase was dried with $MgSO_4$ and evaporated to dryness.

Procedure B: The Fmoc protected starting material was dissolved in a mixture of 4 mL DMF with 1 mL piperidine. The reaction was stirred for 30 minutes and evaporated to dryness.

Procedure C: Per mg starting material 100 µl CH_2Cl_2, 80 µl trifluoroacetic acid and 20 µl H_2O were added. The reaction was stirred at room temperature for 16 h. The solvents were evaporated and the residue coevaporated with 200 µl toluene three times.



S1:

Yield: Starting with 715 mg of the new linker molecule, 940 mg (95%) of product were isolated.

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 8.49 (d, 1H, ^3J = 6.1, H_{ar}), 8.45 – 8.38 (m, 2H, NH, H_{ar}), 8.34 (d, 1H, ^3J = 7.7, H_{ar}), 7.85 (d, 2H, ^3J = 8.1, 2x Fmoc-H), 7.78 (d, 1H, ^3J = 8.3, H_{ar}), 7.63 (t, 1H, ^3J = 7.7, H_{ar}), 7.58 (d, 1H, ^3J = 7.6, Fmoc-H), 7.56 (dd, 1H, ^3J = 7.7, ^4J = 2.4, Fmoc-H), 7.45 (m, 1H, H_{ar}), 7.38 (t, 2H, ^3J = 7.5, 2x Fmoc-H), 7.36 – 7.30 (m, 1H, NH), 7.29 – 7.23 (m, 2H, 2x Fmoc-H), 6.55 (dd, 1H, ^3J = 9.1, ^3J = 4.0,

CH), 4.56 – 4.49 (m, 1H, CH), 4.21 – 4.12 (m, 2H, Fmoc-CH_2), 4.10 – 4.05 (m, 1H, Fmoc-CH), 3.30 – 3.14 (m, 2H, CH_2), 3.02 (ddd, 1H, ^2J = 15.9, ^3J = 9.2, ^4J = 1.8, CHH), 2.85 (dd, 1H, ^2J = 15.6, ^4J = 3.9, CHH), 2.67 – 2.60 (m, 1H, CHH), 2.57 – 2.51 (m, 3H, CHH, CH_2), 1.37 (m, 9H, ^{t}Bu-H), 1.35 (m, 9H, ^{t}Bu-H).

^{13}C-NMR (125 MHz, DMSO-d_6): δ = 170.40, 169.62, 169.18, 169.13, 168.07, 167.98, 157.59, 155.95, 153.44, 146.46, 146.43, 143.78, 143.72, 140.66, 130.32, 130.29, 129.90, 128.34, 127.57, 126.99, 125.07, 123.98, 122.72, 121.90, 120.60, 120.54, 120.08, 112.15, 108.44, 80.97, 80.41, 68.15, 67.02, 65.37, 49.29, 49.22, 46.57, 37.17, 34.09, 34.01, 27.66, 27.61, 27.49, 27.48, 25.13.

ESI-MS: *m/*z found $[M+H]^+$ = 822.5, $[M+Na]^+$ = 844.6.

MALDI-HRMS: *m/*z calcd for $C_{45}H_{47}N_3O_{12}Na$ $[M+Na]^+$ = 844.30519, found = 844.30718 (Δm = 0.00199, error 2.36 ppm).

S2:

Yield: Starting with 390 mg of S1, the product was isolated in 285 mg (99%) yield.

This product is not stable, so it was directly used in the next step.

ESI-MS: *m/*z found $[M+H]^+$ = 600.2.

MALDI-HRMS: *m/*z calcd for $C_{30}H_{38}N_3O_{10}$ $[M+H]^+$ = 600.25517, found = 600.25407 (Δm = 0.0011, error 1.8 ppm).

S3

Yield: Starting with 110 mg of S2, 90 mg (50%) of product were isolated.

ESI-MS: *m/*z found $[M+H]^+$ = 993.5.

MALDI-HRMS: *m/*z calcd for $C_{53}H_{60}N_4O_{15}Na$ $[M+Na]^+$ = 1015.39474, found = 1015.37496 (Δm = 0.01978, error 19.5 ppm).

S4

Yield: Starting with 60 mg of S3, 26 mg (52%) of product were isolated.

The product was purified by HPLC method 1.

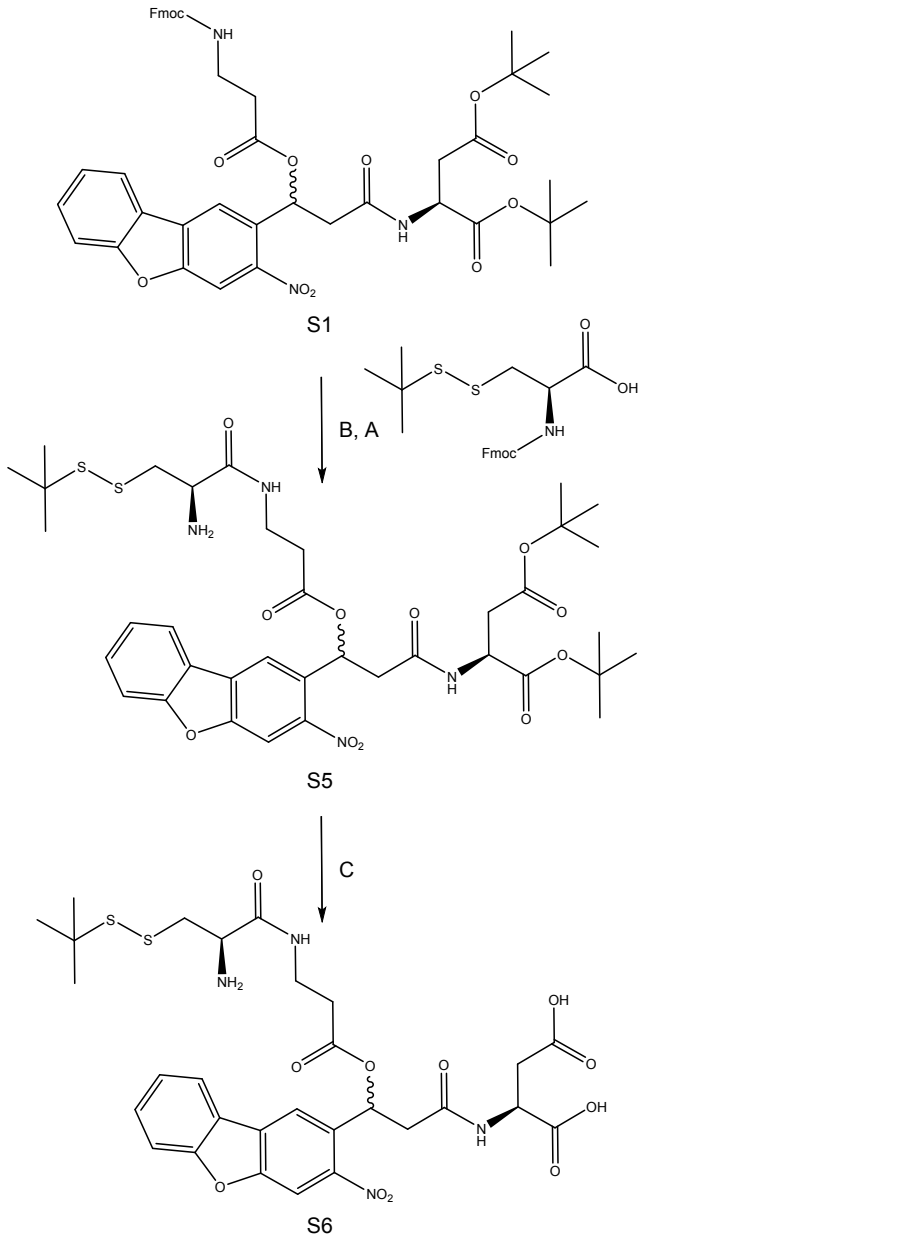
HPLC: two diastereomers eluted at 17.5 and 17.9 min in quantitative HPLC (big column with flow of 4 mL/min). In analytical HPLC the retention time shifted to 20.4 and 20.7 min.

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 12.74 (br s, 3H, 3x COOH), 8.50 (d, 1H, ^{4}J = 2.25, H_{ar}), 8.49 – 8.40 (m, 3H, H_{ar}, 2x NH), 8.34 (d, 1H, ^{3}J = 7.8, H_{ar}), 8.12 (br s, 2H, NH_2), 7.82 (dd, 1H, ^{3}J = 7.9, ^{4}J = 0.9, H_{ar}), 7.68 (dt, 1H, ^{3}J = 7.2, ^{4}J = 1.2, H_{ar}), 7.52 (dt, 1H, ^{3}J = 7.9, ^{4}J = 0.8, H_{ar}), 6.56 – 6.51 (m, 1H, CH), 4.62 – 4.53 (m, 1H, CH), 4.00 – 3.94 (m, 1H, CH), 3.37 – 3.23 (m, 2H, CH_2), 3.13 – 3.07 (m, 1H, CHH), 3.06 – 2.99 (m, 1H, CHH), 2.90 – 2.83 (m, 1H, CHH), 2.78 – 2.51 (m, 5H, CHH, 2x CH_2).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 172.73, 172.16, 172.12, 171.32, 171.28, 170.97, 170.95, 168.52, 168.50, 168.10, 168.08, 158.84, 158.59, 158.33, 158.10, 158.09, 153.93, 146.99, 146.92, 130.72, 130.68, 130.43, 128.84, 124.60, 123.12, 122.38, 121.08, 120.90, 118.53, 116.16, 112.69, 108,94, 108.92, 68.90, 68.84, 49.37, 49.32, 49.14, 48.99, 41.53, 41.41, 36.53, 36.39, 35.79, 35.07, 33.64, 33.60.

ESI-MS: *m/*z found $[M-H]^-$ = 601.1.

MALDI-HRMS: *m/*z calcd for $C_{26}H_{27}N_4O_{13}$ $[M+H]^+$ = 603.15691, found = 603.15752 (Δm = 0.00061, error 1.0 ppm).



S5

The crude product was purified by column chromatography with a gradient of methanol in CH_2Cl_2 from 1% to 5%. The isolated product did no longer possess an Fmoc group.

Yield: Starting with 400 mg of S1, the product yield over the two steps was 140 mg (21%).

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 8.48 (d, 1H, ^4J = 2.3, H_{ar}), 8.42 (d, 1H, ^3J = 8.2, NH), 8.39 (d, 1H, ^4J = 1.5, H_{ar}), 8.33 (d, 1H, ^3J = 8.2, H_{ar}), 8.05 (t, 1H,^3J = 6.1, NH), 7.78 (d, 1H, ^3J = 8.3, H_{ar}), 7.65 (t, 1H, ^3J = 7.6, H_{ar}), 7.48 (t, 1H, ^3J = 7.3, H_{ar}), 6.56 (dd, 1H, ^3J = 9.4, ^3J = 4.2, CH), 4.59 – 4.50 (m, 1H, CH), 3.40 – 3.22 (m, 3H, CH, CH_2), 3.08 – 2.99 (m, 1H, CHH), 2.93 (m, 1H, CHH), 2.89 – 2.83 (m, 1H, CHH), 2.74 – 2.62 (m, 2H, CHH, CHH), 2.59 – 2.52 (m, 3H, CHH, CH_2), 1.88 (br s, 2H, NH_2), 1.40 – 1.38 (m, 9H, ^{t}Bu-H), 1.36 (m, 9H, ^{t}Bu-H), 1.22 (m, 9H, ^{t}Bu-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 173.09, 173.08, 170.62., 170.59, 169.62, 169.17, 169.14, 168.05, 167.99, 162.26, 157.60, 153.42, 146.45, 146.39, 130.30, 129.89, 128.38, 128.36, 123.99, 122.68, 121.90, 120.57, 120.52, 112.10, 108.38, 80.96, 80,94, 80.40, 80.38, 68.17, 54.44, 54.38, 49.29, 49.22, 48.60, 47.52, 47.51, 46.32, 46.27, 41.08, 40.89, 37.18, 35.76, 34.33, 33.79, 33.75, 30.74.

ESI-MS: *m/*z found $[M+H]^+$ = 790.9.

MALDI-HRMS: *m/*z calcd for $C_{37}H_{51}N_4O_{11}S_2$ $[M+H]^+$ = 791.29903, found = 791.29864 (Δm = 0.00039, error 0.5 ppm).

S6

Yield: Starting with 4 mg of S5, 3 mg (87%) of product were isolated.

HPLC (method 1): two diastereomers eluted at 23.6 and 24.2 min.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 12.65 (br s, 2H, 2x COOH), 8.61 (m, 1H, NH), 8.50 (d, 1H, ^3J = 4.5, H_{ar}), 8.46 – 8.40 (m, 2H, H_{ar}, NH), 8.34 (d, 1H, ^3J = 7.7, H_{ar}), 8.32 – 8.21 (m, 2H, NH_2), 7.83 (dd, 1H, ^3J = 8.4, ^4J = 0.5, H_{ar}), 7.69 (t, 1H, ^3J = 7.4, H_{ar}), 7.52 (t, 1H, ^3J = 7.2, H_{ar}), 6.57 – 6.50 (m, 1H, CH), 4.64 – 4.52 (m, 1H, CH), 3.96 – 3.79 (m, 1H, CH), 3.44 – 3.19(m, 2H, CH_2), 3.11 – 2.98 (m, 2H, CH_2), 2.93 (d, 1H, ^3J = 6.2, CHH), 2,90 – 2.82 (m, 1H, CHH), 2.74 – 2.54 (m, 4H, 2x CH_2), 1.20 (m, 9H, ^{t}Bu-H).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 172.27, 171.66, 170.54, 170.46, 168.08, 167.99, 166.77, 166.71, 158.60, 158.32, 158.04, 157.76, 157.67, 157.64, 153.48, 146.56, 146.44, 137.37, 130.32, 130.22, 129.99, 128.41, 128.36, 125.34, 124.15, 122.68, 121.95, 120.66, 120.61, 117.16, 114.83, 112.25, 108.47, 68.38, 51.65, 51.39, 48.70, 48.54, 48.05, 47.99, 41.16, 41.00, 40.81, 40.53, 36.11, 35.94, 34.64, 34.59, 33.14, 33.08, 31.32, 29.35, 29.26.

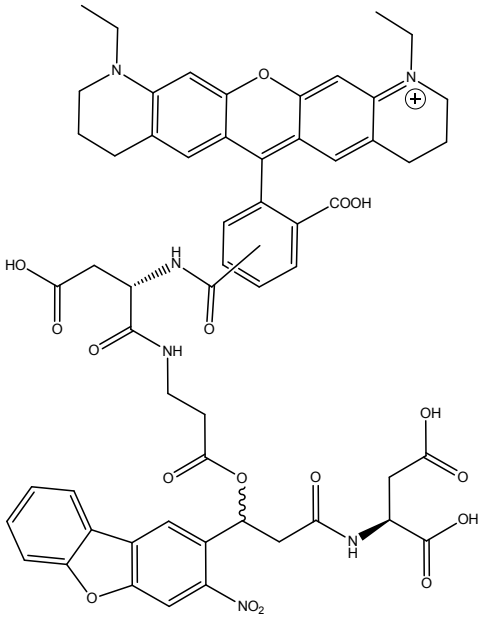
ESI-MS: *m/*z found $[M+H]^+$ = 679.4.

MALDI-HRMS: *m/*z calcd for $C_{29}H_{35}N_4O_{14}S_2$ $[M+H]^+$ = 679.17383, found = 679.17414 (Δm = 0.00031, error 0.5 ppm).

ATTO565-labeling

1.0 mg (1.4 µmol) of ATTO565 was dissolved in 50 µL of dry DMSO. 1.4 µmol of peptide were dissolved in the recommended coupling buffer (mixture of phosphate and sodium bicarbonate buffer, pH = 8.3, see *ATTO TEC* homepage for further information). After one hour at room temperature, the reaction was purified by HPLC.

Peptide **1** (reaction of ATTO565 with S4)



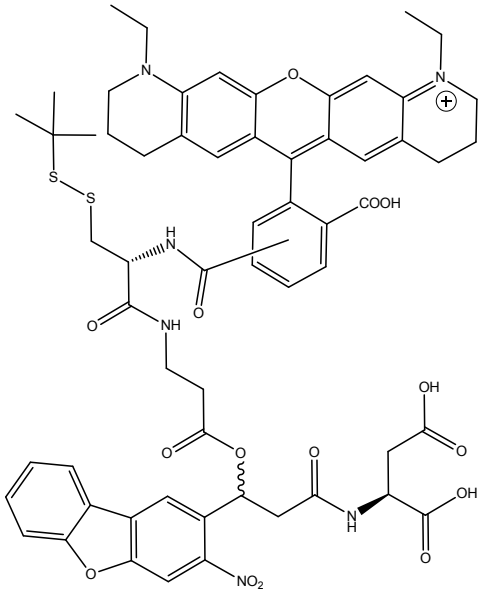
1

HPLC (method 3): product eluted at 45.4 min. Isocratic conditions were necessary to separate the product from an impurity that elutes in method 1 at the same retention time. Attached is an analytical spectrum of the pure product after purification with isocratic conditions with method 1, retention time 24.4 min.

ESI-MS: *m/*z found $[M-2H]^-$ = 1093.5, radical cation $[M]^{+\bullet}$ = 1095.5.

MALDI-HRMS: *m/*z calcd for $C_{57}H_{55}N_6O_{17}$ radical cation$[M]^{+\bullet}$ = 1095.36182, found = 1095.36264 (Δm = 0.00082, error 0.7 ppm).

S7 (from reaction of S6 with ATTO565)



S7

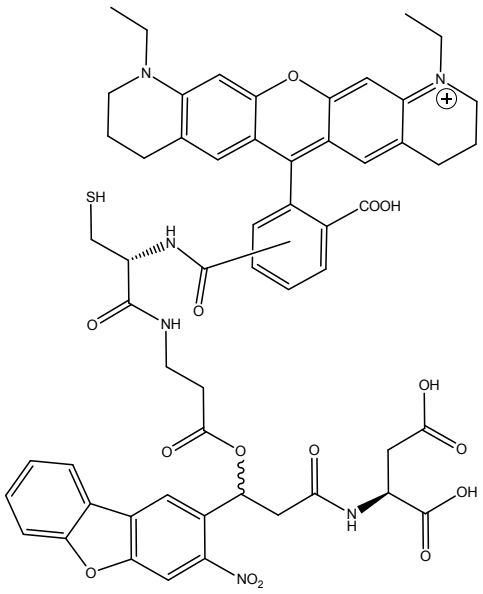
HPLC (method 2): product eluted at 33.6 min.

ESI-MS: *m/*z found radical cation $[M]^{+\bullet}$ = 1171.8.

MALDI-HRMS: *m/*z calcd for $C_{60}H_{63}N_6O_{15}S_2$ radical cation $[M]^{+\bullet}$ = 1171.37873, found = 1171.38168 (Δm = 0.00295, error 2.5 ppm).

Disulfide cleavage

Peptide **2**



2

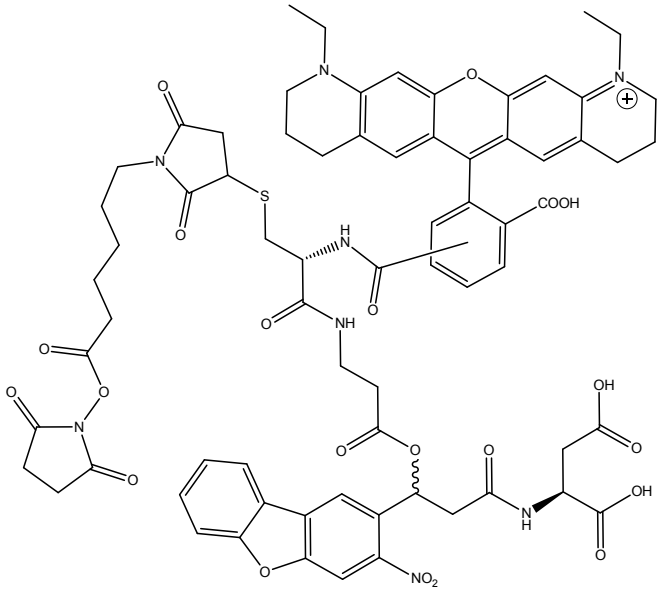
The disulfide containing peptide was dissolved in 50 mM TCEP, 100 mM Tris buffer pH 7.4. If solubility problems were observed small amounts of acetonitrile were added. After deprotection for 16 h the reaction was purified by HPLC method 2.

HPLC: diastereomers eluted at 30.0 and 30.4 min.

MALDI-MS: *m/z* found radical cation $[M]^{+\bullet}$ = 1082.5.

MALDI-HRMS: *m/z* calcd for $C_{56}H_{55}N_6O_{15}S$ radical cation $[M]^{+\bullet}$ =1083.34406, found = 1083.34527 (Δm = 0.00121, error 1.1 ppm).

NHS ester



S8

0.5 mg (0.46 µmol) of Peptide **2** were reacted with 1.3 mg (4.3 µmol) 6-maleimidohexanoic acid *N*-hydroxysuccinimide ester in 100 µL DMF. 1 µL (57 µmol) DIPEA was added and the reaction was stirred for 16 h. The solvent was evaporated and the residue dissolved in H_2O:MeCN 1:1. The crude product was purified by HPLC method 2.

HPLC: diastereomers eluted between 31.3 and 31.7min.

MALDI-MS: *m/z* found $[M-2H]^+$ = 1390.8.

Actinometry

The procedure was conducted according to support protocol 4 from A. Rodrigues-Correia, P. Seyfried and A. Heckel, *Current Protocols in Nucleic Acid Chemistry*, eds. M. Egli, P. Herdewijn, A. Matusda and Y. S. Sanghvi, John Wiley & Sons, Inc., 2014, unit 6.11.1.

A calibrated ferrioxalate actinometer was used (Fig. S1, see reference for further information).

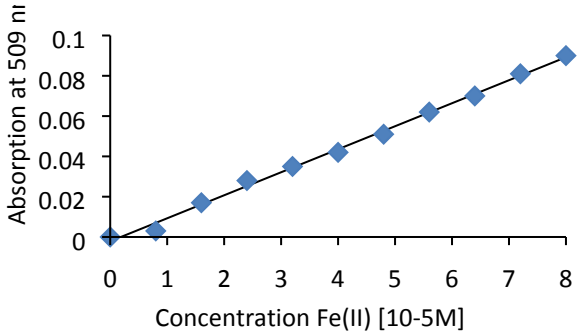


Fig. S1: Calibration curve for ferrioxalate actinometry. The absorption at 509 nm is recorded in dependence of an increasing concentration of Fe(II) ions in a solution with phenanthroline.

In the beginning five different solutions were prepared:

1. Iron(III) oxalate solution (130 mg $(NH_4)_3Fe(C_2O_4)_3$ * 3 H_2O solved in 45 mL MilliQ water and 5 mL 0.05 M H_2SO_4).
2. Phenanthroline solution (60 mg 1,10-Phenanthroline solved in 50 mL MilliQ water).
3. Sodium acetate buffer (4.08 g NaOAc solved in 32 mL MilliQ water and 18 mL 0.5 M H_2SO_4).
4. Buffer B (16 mL 0.05 M H_2SO_4 mixed with 10 mL sodium acetate buffer and 16 mL MilliQ water).
5. Internal standard (2 mg of uracil dissolved in 10 mL of MilliQ water).

13 µL of the iron(III) oxalate solution were irradiated with 365 nm (184 mW) in a 1 cm cuvette. Irradiation time increased from 1 s, 2 s, 3 s, 5 s to 7 s. All experiments were repeated three times. After irradiation, each sample was vortexed in a 2 mL Eppendorf tube. Afterwards 4 µL of the irradiated ferrioxalate solution were mixed with 4 µL phenanthroline solution and 42 µL of buffer B. After waiting for 30 minutes, the absorption at 509 nm was measured (Fig. S2).

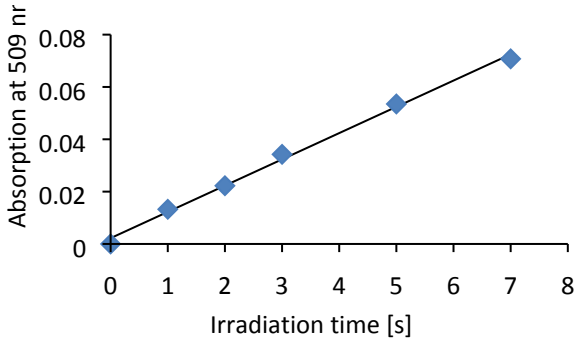


Fig. S2: The absorption of ferrioxalate actinometry at 509 nm with 184 mW showed linear behavior with increasing irradiation time.

As long as the results show a linear behavior (Fig. S2), the amount of generated Fe(II) can be determined from the increase in the absorption and the slope of the calibration curve (Fig. S1).

$$n_{Fe(II)} = \frac{A_t - A_0}{a_{calc} * d} * V = 7.7\ nmol$$

$n_{Fe(II)}$: Amount of generated iron(II) by irradiation for time t.
A_t: Absorption at 509 nm at time t ($A_{t\ =\ 5\ s} = 0.0865$).
A_0: Absorption at 509 nm without irradiation ($A_0 = 0.0325$).
a_{calc}: Increase of the absorption in dependence of the concentration of Fe(II), slope from calibration curve (Fig. S1, $\frac{0.00114}{\mu M}$).
d: Factor to correct the dilution of the sample (4 µL diluted to 50 µL; 0.08).
V: Measured volume (13 µL).

The numbers of photons emitted by the LED per second can be calculated from the amount of Fe(II) and the quantum yield of iron(III) oxalate.

$$\frac{n_{Photons}}{s} = \frac{n_{\ Fe(II)}}{\phi_{Ferrioxalate} * t} = 1.27\ \frac{nmol}{s}$$

$n_{Photons}$: Amount of photons emitted by the LED at 365 nm.
$\phi_{Ferrioxalate}$: Quantum yield of ferrioxalate, 1.21.
t: Time of the irradiation (5 s).

A solution of 492 µL 45 µM peptide **1** in HBS buffer pH 7.5 was mixed with 23 µL of internal standard. 13 µL of this solution were irradiated for 0, 2, 5, 8, 10, 12, 15, 20, 25, 50 and 60 minutes using the same light source as in the previous experiments. After irradiation the samples were analyzed by HPLC (method 1, internal standard at 3.94 min, **1** at 27.52 min). The decrease of the peptide was measured by integration of the corresponding peak in the HPLC chromatogram. The values were referenced to the internal standard. The experiments were repeated thrice. The initial slope of the photoreaction was determined by exponential fit of the obtained data.

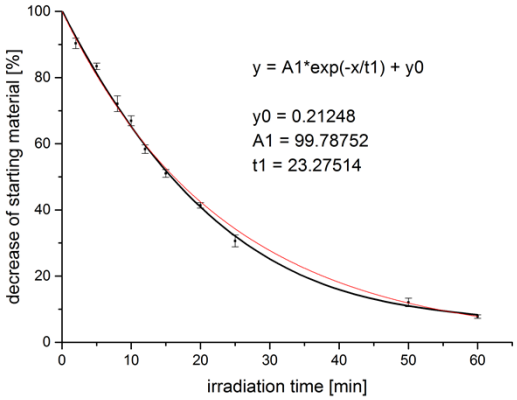


Fig. S3: Photolysis of peptide **1**. The initial slope of the decay was calculated by an exponential fit.

$$y' = -\frac{A_1}{t_1} * e^{-\frac{x}{t_1}}$$

$$y'(x=0) = -\frac{A_1}{t_1} = 4.3\,\frac{\%}{s} = 25.7\,\frac{pmol}{s}$$

The amount of photons absorbed by the new compound is calculated from Lambert Beer's law.

$$E_{365} = \varepsilon_{365} * c * d = -\log T$$

$$A = 1 - T = 1 - 10^{-E_{365}} = 0.158$$

E_{365}: Absorbance at 365 nm.
ε_{365}: Coefficient of the photolabile group at 365 nm ($1660\,\frac{L}{mol\ cm}$).
c: Concentration of the sample (45 µM).
d: Path length (1 cm).
T: Transmission.
A: Relative absorbance.

The quantum yield of the new linker is then calculated from the amount of photons absorbed by the sample and the calculated slope of the photoreaction.

$$\varphi = \frac{25.7\,\frac{pmol}{s}}{A * \frac{n_{Photons}}{s}} = 0.13$$

φ: quantum yield of the new molecule.

Determination of fluorescence quantum yield

For the determination of fluorescence quantum yields, peptide **1** was diluted directly in HBS buffer (20 mM HEPES, 150 mM NaCl, pH 7.5) and ATTO565 NHS ester (ATTO-TEC) was diluted in HBS from a 1 mM stock solution in absolute DMSO (≥ 99.5% (GC)). As reference dye we used Rhodamine 101 (Radiant Dyes) in absolute ethanol. The concentrations of the dye solutions were adjusted not to exceed an absorption of 0.1 in the S_0-S_1 maximum and to matching absorbances at 540 nm, which was chosen as excitation wavelength for all dye constructs. Absorption and fluorescence spectra were recorded on a V-650 UV/Vis photometer (*Jasco*) and on a FP-8500 fluorimeter (*Jasco*) by keeping the spectrometer settings constant. The measurements were carried out at 25 °C by using a 3x3 mm quartz cuvette (*Hellma Analytics*). We calculated the fluorescence quantum yields according to C. Würth, M. Grabolle, J. Pauli, M. Spieles and U. Resch-Genger, *Nat. Prot.* 2013, **8**, 1535 with the following equations:

$$\Phi_{f,x} = \Phi_{f,st} \cdot \frac{F_x}{F_{st}} \cdot \frac{f_{st}}{f_x} \cdot \frac{n_x^2(\lambda_{em})}{n_{st}^2(\lambda_{em})}$$

$$f = 1 - 10^{-A(\lambda_{ex})}$$

$$F = \int_{\lambda_{em,1}}^{\lambda_{em,2}} I_C(\lambda_{em}) \cdot \lambda_{em} d\lambda_{em}$$

The index x represents the respective sample and the index st the standard.
Φ_f : fluorescence quantum yield
f : absorption factor
$A(\lambda_{ex})$: absorbance at the excitation wavelength λ_{ex}
F : integral emitted photon flux
I_C : background-subtracted and corrected fluorescence spectrum
λ_{em}: emission wavelength
n : refractive index of the solvent

For the reference dye Rhodamine 101 we assumed a fluorescence quantum yield of 91.5% in ethanol (C. Würth, M. Grabolle, J. Pauli, M. Spieles and U. Resch-Genger, *Nat. Prot.* 2013, **8**, 1535). We obtained quantum yields of 2.8% for caged peptide **1,** 61.8% for uncaged peptide **1** and 78.6% for free ATTO565 NHS ester in HBS buffer. Note that the absorption spectrum of peptide **1** is blue-shifted of about 10 nm upon photoactivation, which results in an increased absorbance at the excitation wavelength. This yields a much higher fluorescence increase (81 fold) compared to the quantum yield increase (22 fold), for which the concentration of the uncaged compound was decreased to obtain a similar absorbance at the excitation wavelength compared to the caged sample.

Two-photon excitation

For two-photon irradiation, peptide **2** was immobilized in a commercially available maleimide containing hydrogel (*Cellendes*, see section "General Information"). 1.5 µL water, 0.63 µL CB buffer pH 5.5, 1.25 µL Mal PVA, 1 µL peptide (118 pmol in HBS buffer pH 7.5), and 1.87 µL PEG-Linker were mixed in a chambered cover glass according to manufactures instruction. The hydrogel was covered with 200 µL of HBS buffer pH 7.5. Irradiation was performed with an LSM 780 Axio Observer NLO with a C-Apochromat 63x/1.20 W Korr M27 objective from *Zeiss*. Two-photon excitation was performed with a Coherent Ultra II laser at 780 nm during simultaneous fluorescence excitation with a second laser at 561 nm.

The increase in the fluorescence was measured with 100 scans at 6 mW (Fig. S4).

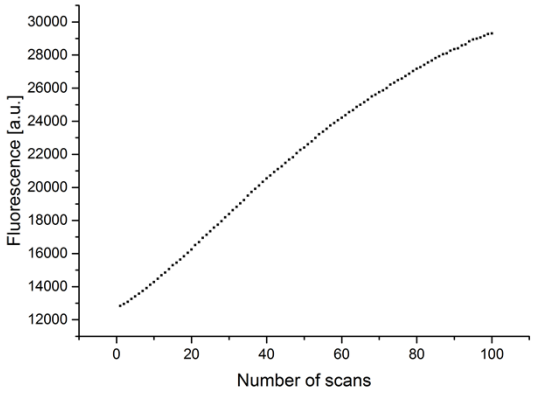


Fig S4: Fluorescence increase during two-photon irradiation with 780 nm. Saturation indicates that all molecules in the area of the hydrogel were activated.

One-photon widefield and super-resolution imaging

All following experiments were performed in aqueous media if not indicated elsewise. Phosphate buffered saline (PBS, 0.2 g/L KCl, 0.2 g/L KH_2PO_4 (anhydrous), 8.0 g/L NaCl, 1.15 g/L Na_2HPO_4 (anhydrous)) was diluted from a 10x concentrated stock (*Sigma*).

Antibody labeling

The unlabeled F(ab')$_2$ fragment of an IgG (H + L) goat anti-mouse antibody (A10534, *Life Technologies*) was diluted to a concentration of 1 mg/mL in 50 µL $NaHCO_3$ buffer (0.1 M) at pH 8.3 and vortexed, while adding 0.1 µL of a freshly prepared solution of the dye NHS ester in DMF (2 mM). The reaction mixture was slightly stirred for 30 min. The labeled protein was purified by size exclusion chromatography using an Illustra NAP-5 column (Sephadex G-25, DNA-free, *GE Healthcare*) according to the protocol of the supplier. PBS was used as column equilibration buffer and elusion buffer, respectively. The average number of fluorophores per antibody was determined to 0.5 by absorption spectroscopy on a Cary 100 UV-Vis spectrometer (*Agilent*).

Cell culture and staining

U2OS cells were cultured in Dulbecco's modified Eagle medium/nutrient mixture F-12 (DMEM/F-12) (3.15 g/L (+)D-glucose, Gibco, *Life Technologies*) supplemented with 1% Gluta MAX (Gibco, *Life Technologies*) and 10% fetal bovine serum (South America, Gibco, *Life Technologies*) at 37 °C in 5% CO_2 atmosphere. Cells were grown on 8-well chambered coverslips (170 µm thickness, *Sarstedt*; 1.5 x 10^4 cells per well) the day before fixation. Cell fixation and staining of microtubules was performed referring to a protocol from A. Desai (2012): Fluorescence procedures for the actin and tubulin cytoskeleton in fixed cells.
http://mitchison.med.harvard.edu/protocols/general/Fluorescence%20Procedures%20for%20the%20Actin%20and%20Tubulin%20Cytoskeleton%20in%20Fixed%20Cells.pdf

Cells were washed shortly with PBS and extracted for 1 min with a buffer containing 80 mM PIPES, 1 mM $MgCl_2$, 5 mM EGTA and 0.5% (v/v) Triton X-100 (pH adjusted to 6.8 with 6 M KOH). Glutaraldehyde was added from a 25% (v/v) stock solution (Grade I, *Sigma*) to final concentration of 0.5% (v/v) and incubated for 10 min. The solution was removed and cells were quenched with 0.1% (w/v) $NaBH_4$ in PBS for 7 min, followed by thorough washing with PBS. The cells were treated with a blocking buffer consisting of 2% (w/v) bovine serum albumin and 0.1% (v/v) Triton X-100 in PBS for 10 min, followed by incubation for 40 min with the primary mouse anti-β-tubulin antibody (*Invitrogen*) diluted to 1 µg/mL in the same buffer. After washing several times with a solution of PBS containing 0.1% (v/v) Triton X-100, the self-labeled secondary goat anti-mouse F(ab')$_2$ fragment diluted to 1 µg/mL in blocking buffer was added for 30 min, followed by another washing step with 0.1% (v/v) Triton X-100 in PBS and pure PBS. After post-fixation with 3.7% (v/v) formaldehyde in PBS, cells were rinsed with PBS and stored in PBS supplemented with 0.1% (w/v) NaN_3 at 4 °C.

Super-resolution imaging

One-photon activation and super-resolution imaging were performed on a custom-built widefield microscope setup suitable for single-molecule detection. A detailed description of the setup was published in F. Fricke et al., *Histochem. Cell. Biol.*, 2014, **142**, 91. Briefly, a 405 nm diode laser (CUBE 405-100C, *Coherent*) and a 568 nm laser (Saphire 568 LP, *Coherent*) were coupled into an IX-71 inverted microscope (*Olympus*) equipped with a 100x oil-immersion objective lens (PLAPO 100x

TIRFM, NA ≥ 1.45, *Olympus*). An additional mirror was inserted in the excitation path for illumination of the sample in total internal reflection mode. The respective laser intensities were adjusted via an acousto-optic tunable filter (*AA Optics*). The sample was placed on a nosepiece stage (IX2-NPS, *Olympus*) to reduce thermal drift. Excitation and emission beam were separated with a quad-band dichroic mirror (HC Quad 410/504/582/669, *AHF Analysentechnik*) and a bandpass filter (ET 605/52, *AHF Analysentechnik*) in the detection channel. The fluorescence signal was collected on a 512 x 512 pixel chip of an electron multiplying charge-coupled device camera (iXon3, *Andor*). The image pixel size was adjusted to 160 nm using appropriate lenses.

One-photon activation was performed with a short 405 nm laser pulse (100 W/cm^2), while the sample was continuously illuminated with 568 nm (80 W/cm^2). 500 frames were recorded with a frame rate of 33 Hz and an EM gain of 100.

For super-resolution imaging, the UV laser was switched off and the intensity of the 568 nm laser line was increased continuously to a value of 230 W/cm^2 during the measurement. Image sequences of 10000 frames were recorded in PBS buffer with a frame rate of 33 Hz and an EM gain of 200. For localization of single emitters and reconstruction of the super-resolution image, the data was processed with the open-source software rapidSTORM V2.21, described in detail in S. Wolter, A. Löschberger, T. Holm, S. Aufmkolk, M.-C. Dabauvalle, S. van de Linde and M. Sauer, *Nat. Meth.*, 2012, **9**, 1040.

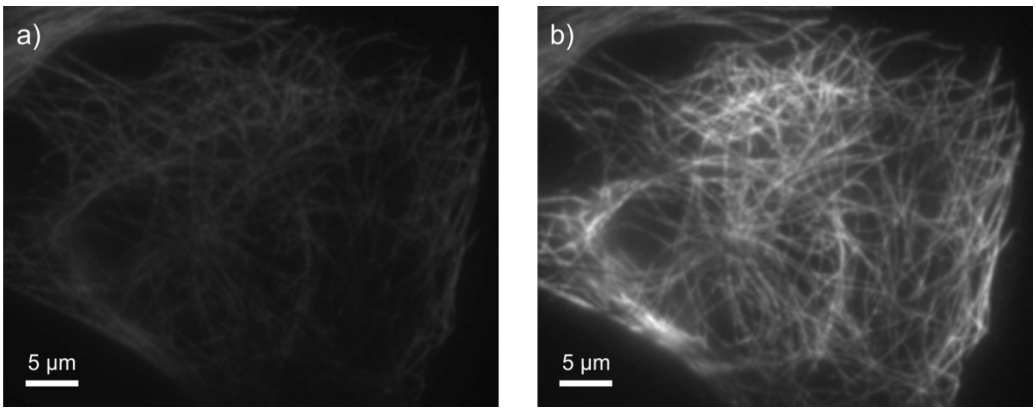


Fig S5: Widefield images of the microtubule network of an U2OS cell stained via immunofluorescence with the photoactivatable peptide **2** antibody conjugate: a) before activation with 405 nm, b) after activation with a short 405 nm puls. Images were averaged out of 50 frames without further image processing. Note that imaging in the microscope at a wavelength of 568 nm does lead to some photoactivation of the fluorophore ATTO565.

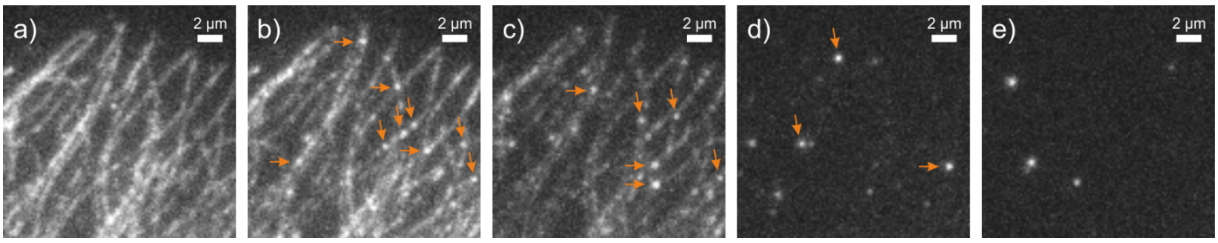
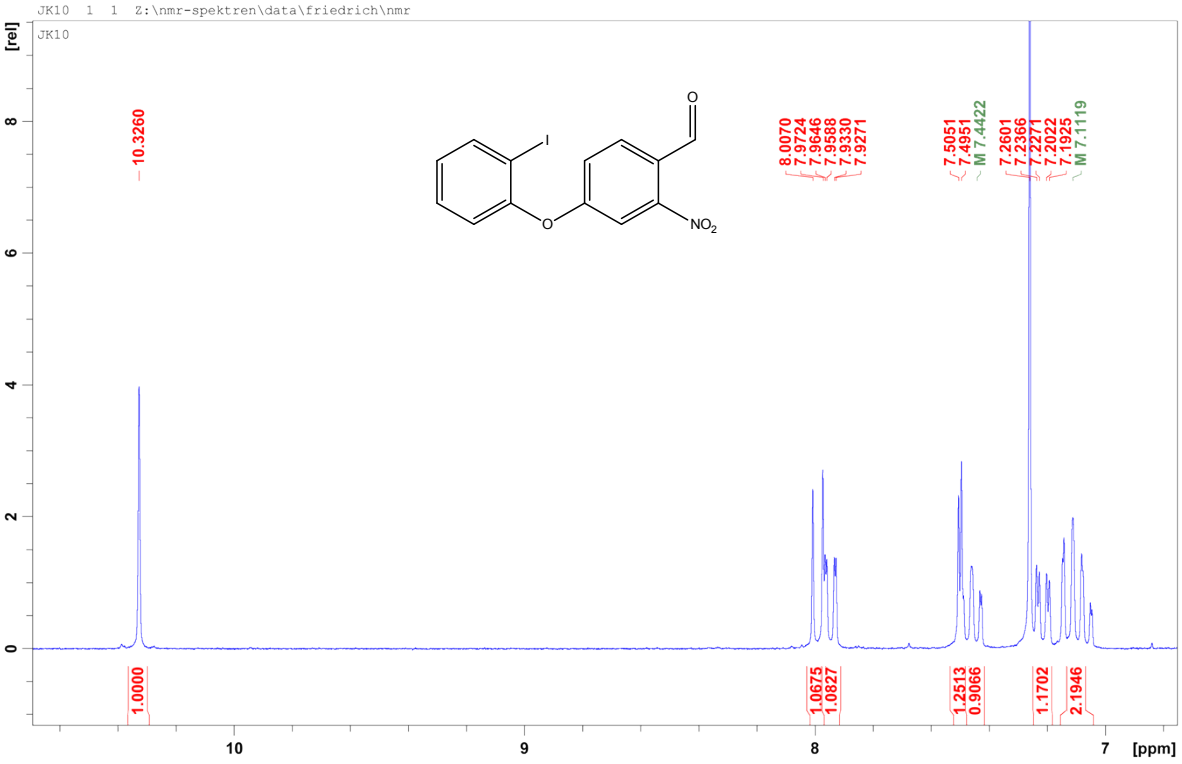


Fig S6: Widefield images of an U2OS cell stained via immunofluorescence against β-tubulin using the fluorogenic peptide **2** antibody conjugate. The sample was continuously illuminated with 568 nm. Images are selected from an image sequence at different time points: a) before activation, showing background fluorescence because of the non-zero quantum yield of the caged fluorophore; b) – d) consecutive activation and bleaching of single molecules, showing bright fluorescent spots (highlighted with orange arrows) that are successfully detected with rapidSTORM; e) blinking of residual ATTO565 conjugates. No further image processing was applied.

NMR spectra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7.9646
7.9588
7.9330
7.9271
7.5051
7.4951
M 7.4422
7.2601
7.2366
7.2271
7.2022
7.1925
M 7.1119
O
I
O
NO2
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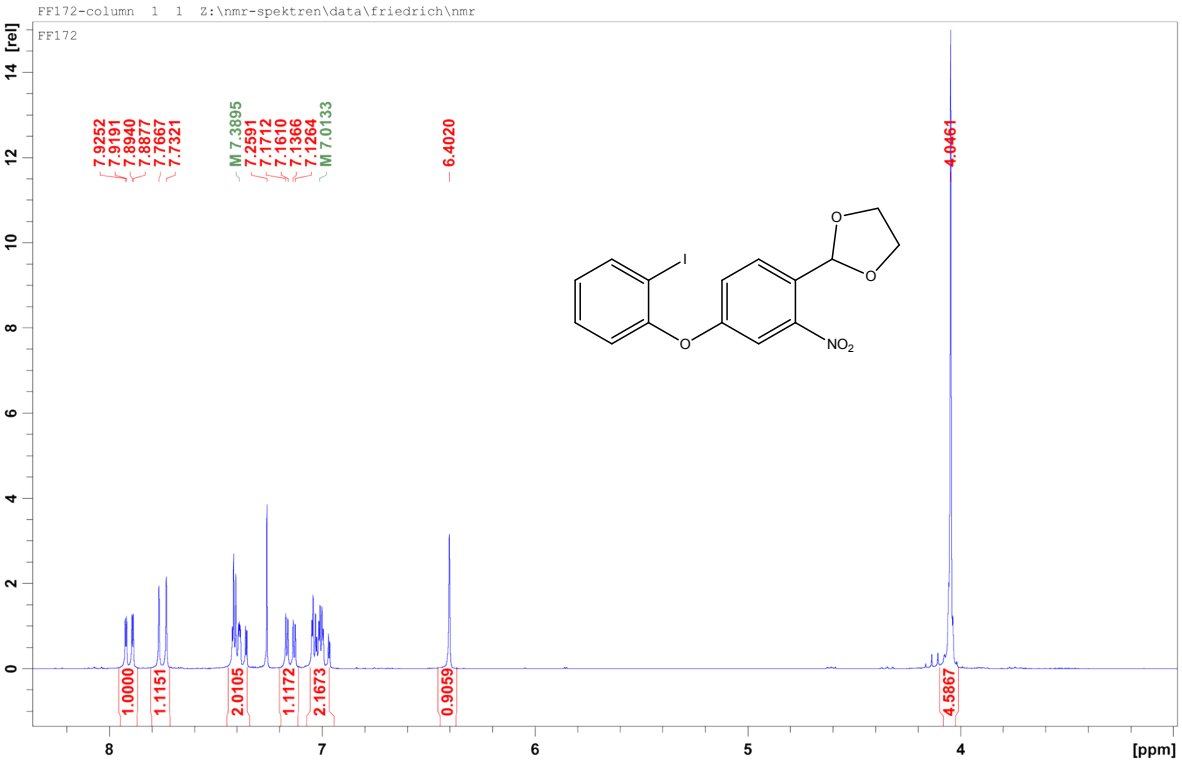
1.0827
1.2513
0.9066
1.1702
2.1946
10
9
8
7
[ppm]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7.1712
7.1610
7.1366
7.1264
M 7.0133
6.4020
4.8461
O
O
I
O
NO2
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6
5
4
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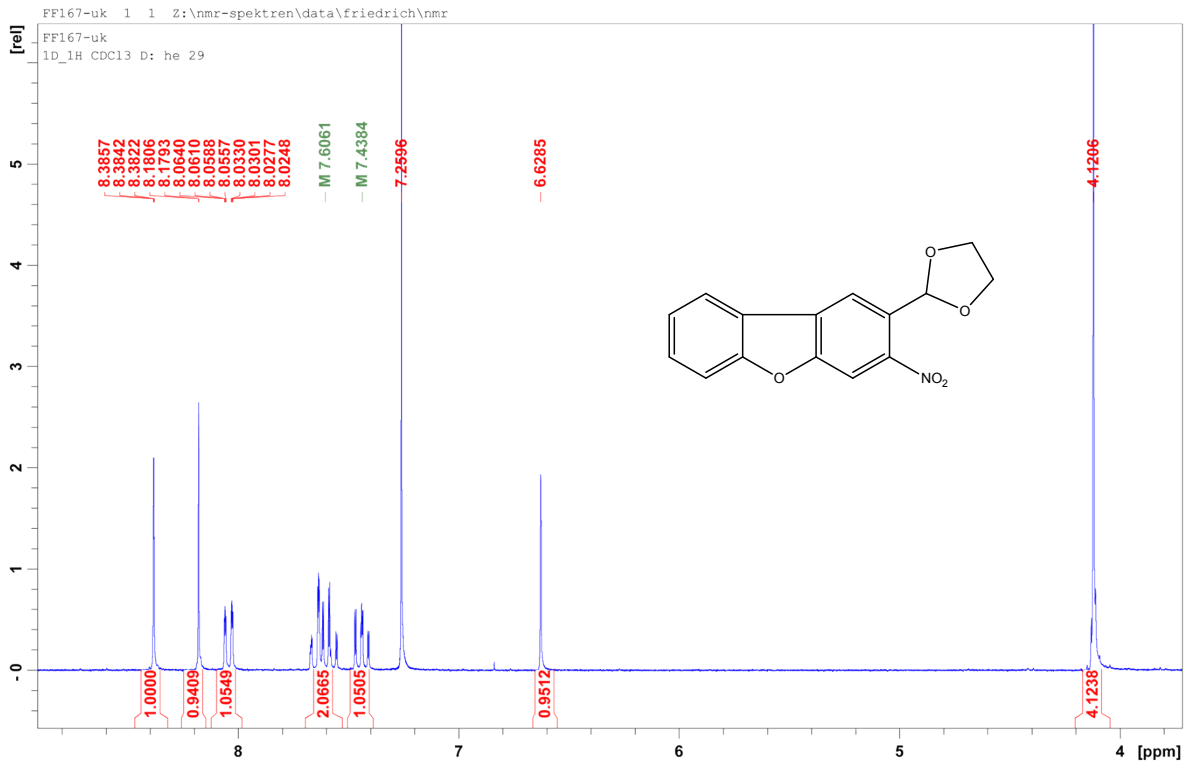
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8.0301
8.0277
8.0248
M 7.6061
M 7.4384
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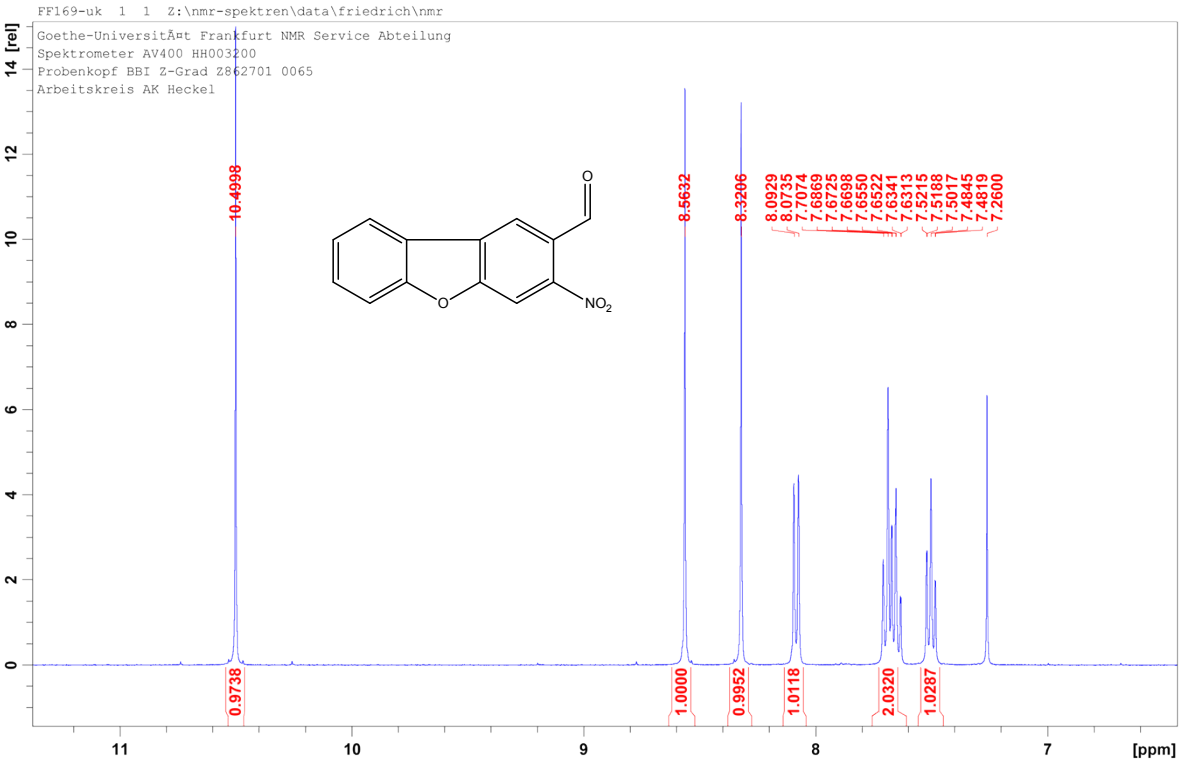
NO2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Goethe-UniversitÃ¤t Frankfurt NMR Service Abteilung
Spektrometer AV400 HH003200
Probenkopf BBI Z-Grad Z862701 0065
Arbeitskreis AK Heckel
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8.0735
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7.6725
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7.6522
7.6341
7.6313
7.5215
7.5188
7.5017
7.4845
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O
NO2
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1.0287
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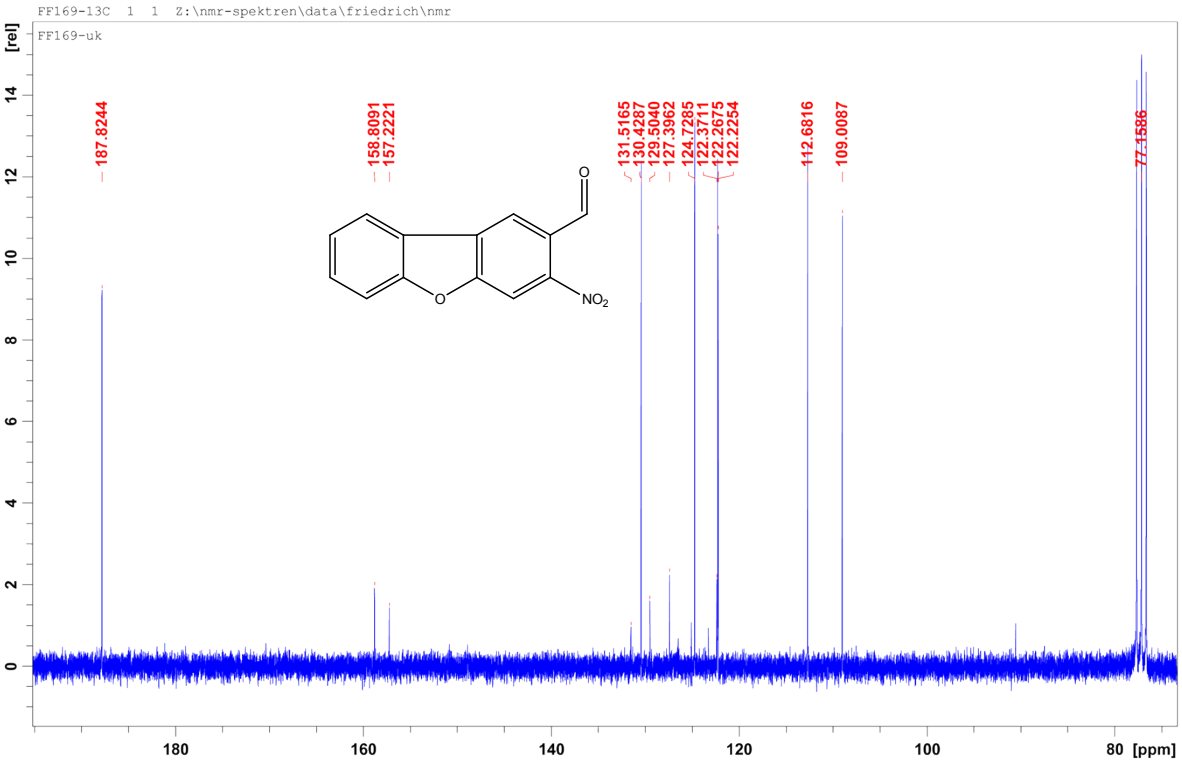
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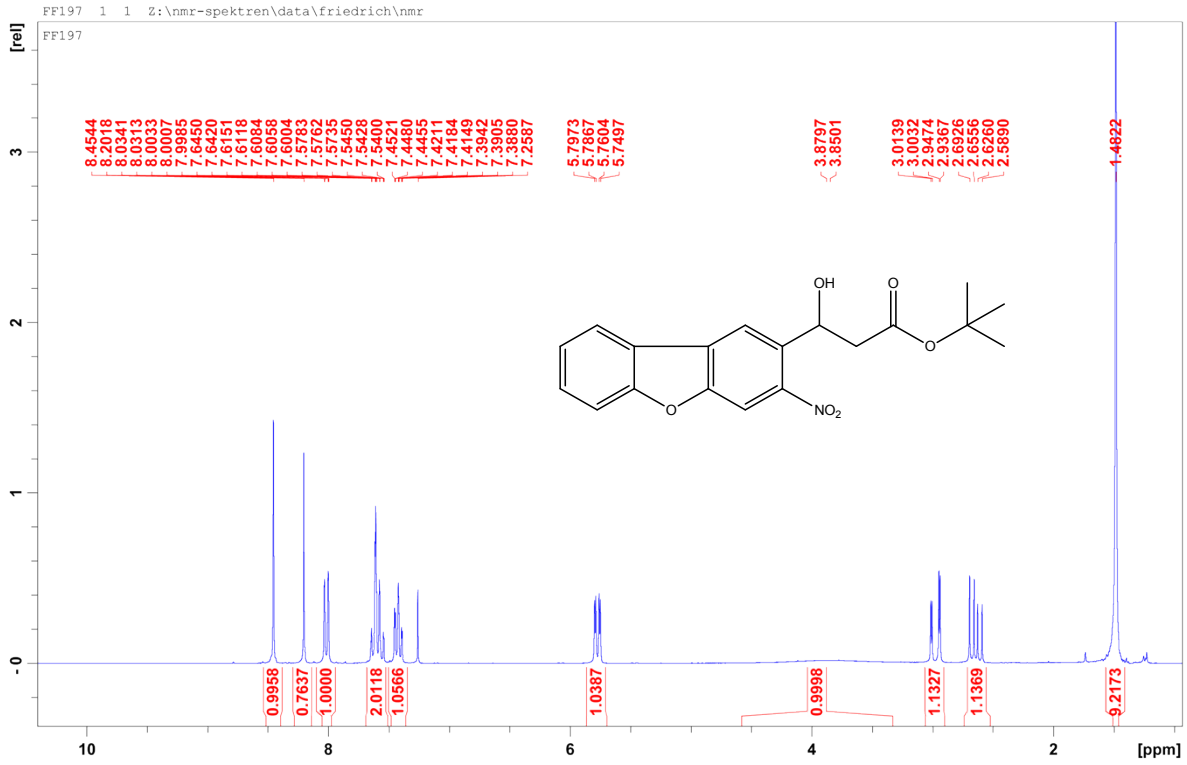
O
O
NO2
[ppm]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7.6058
7.6004
7.5783
7.5762
7.5735
7.5450
7.5428
7.5400
7.4521
7.4480
7.4455
7.4211
7.4184
7.4149
7.3942
7.3905
7.3880
7.2587
5.7973
5.7867
5.7604
5.7497
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3.8501
3.0139
3.0032
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O
O
NO2
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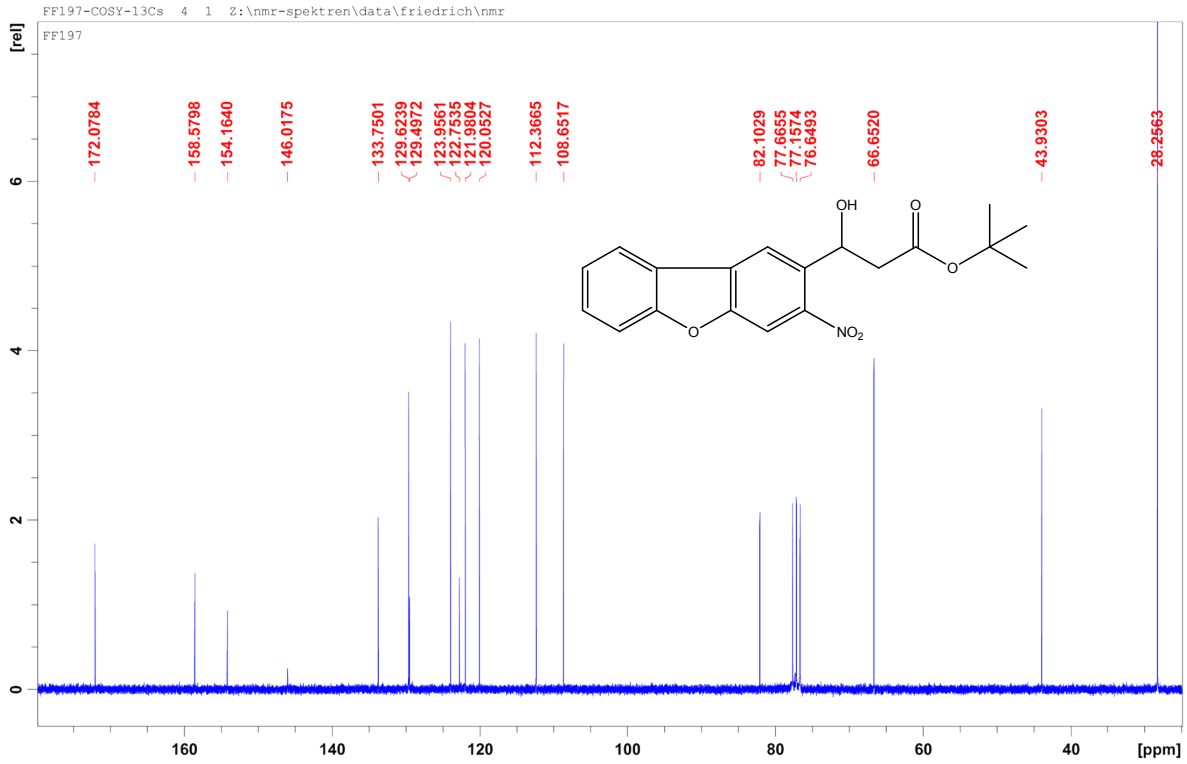
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O
NO2
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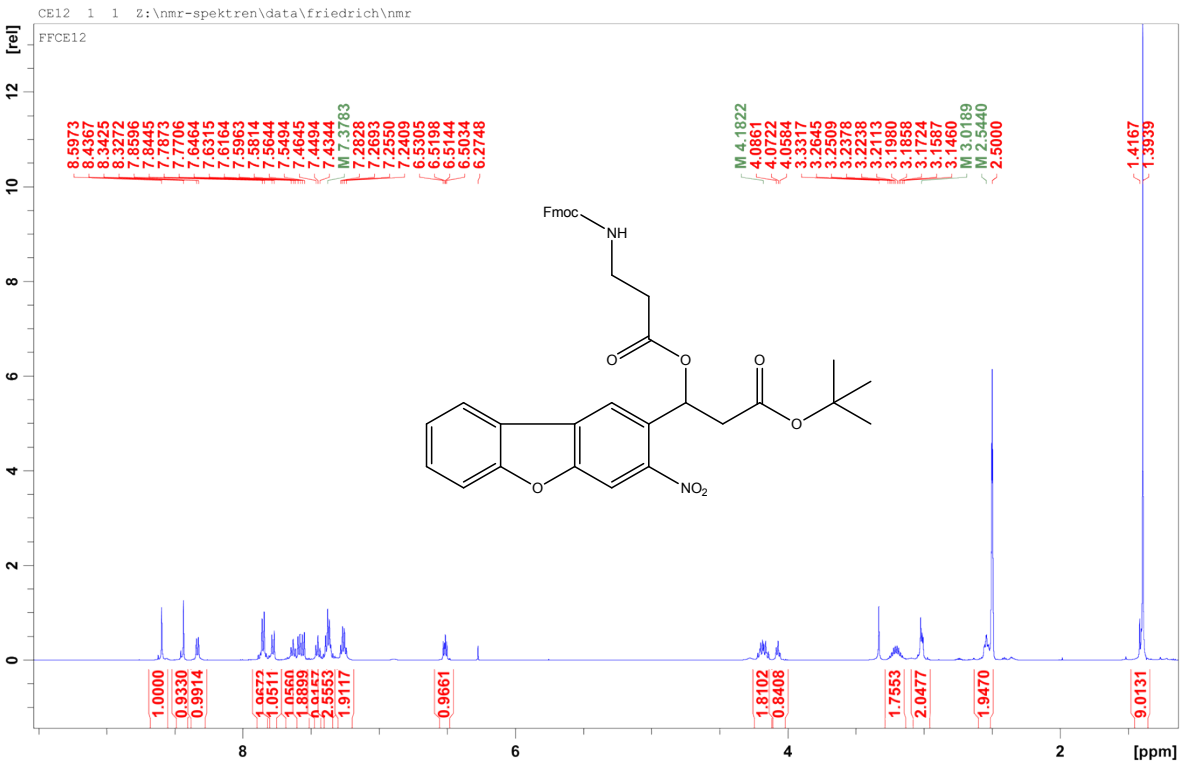
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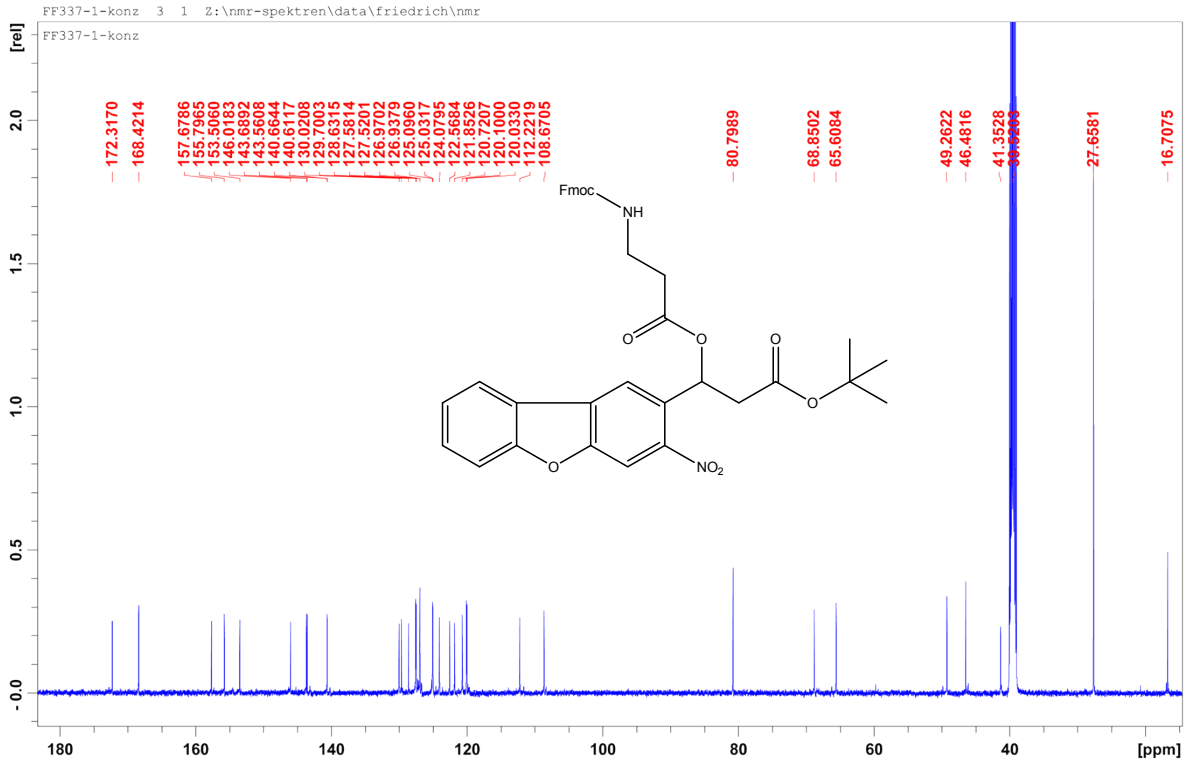
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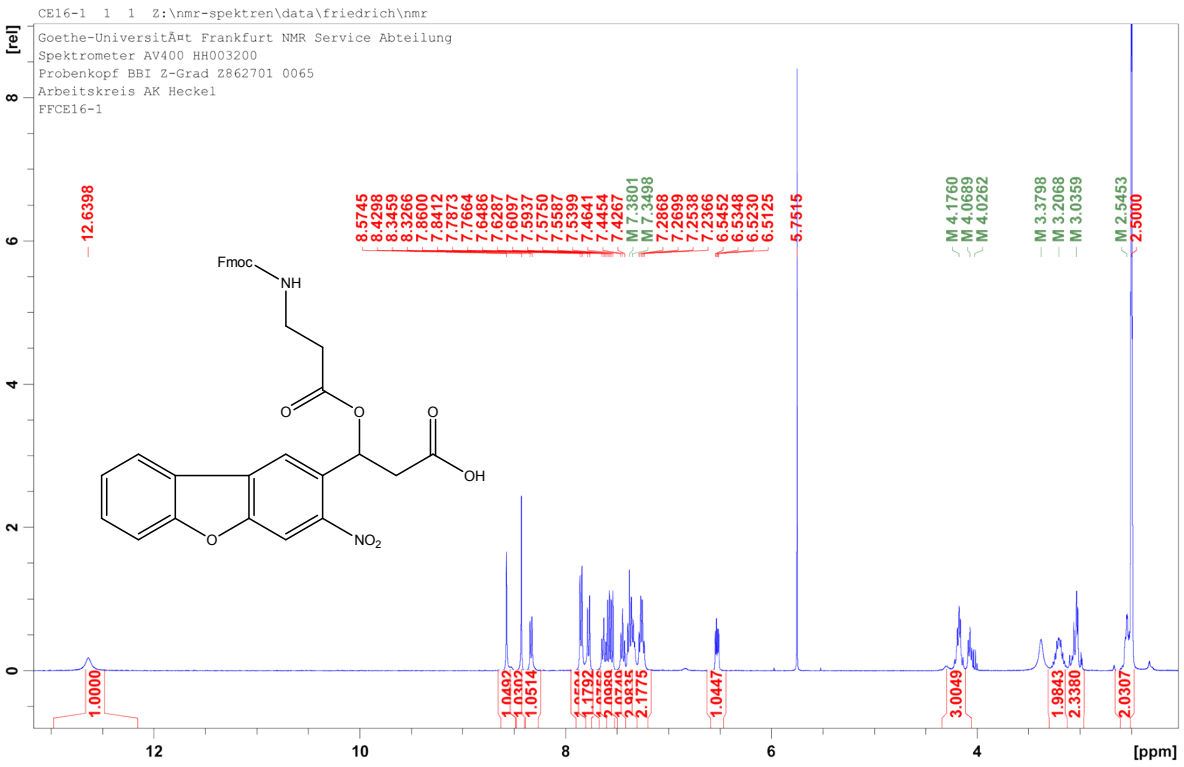
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Goethe-UniversitÃ¤t Frankfurt NMR Service Abteilung
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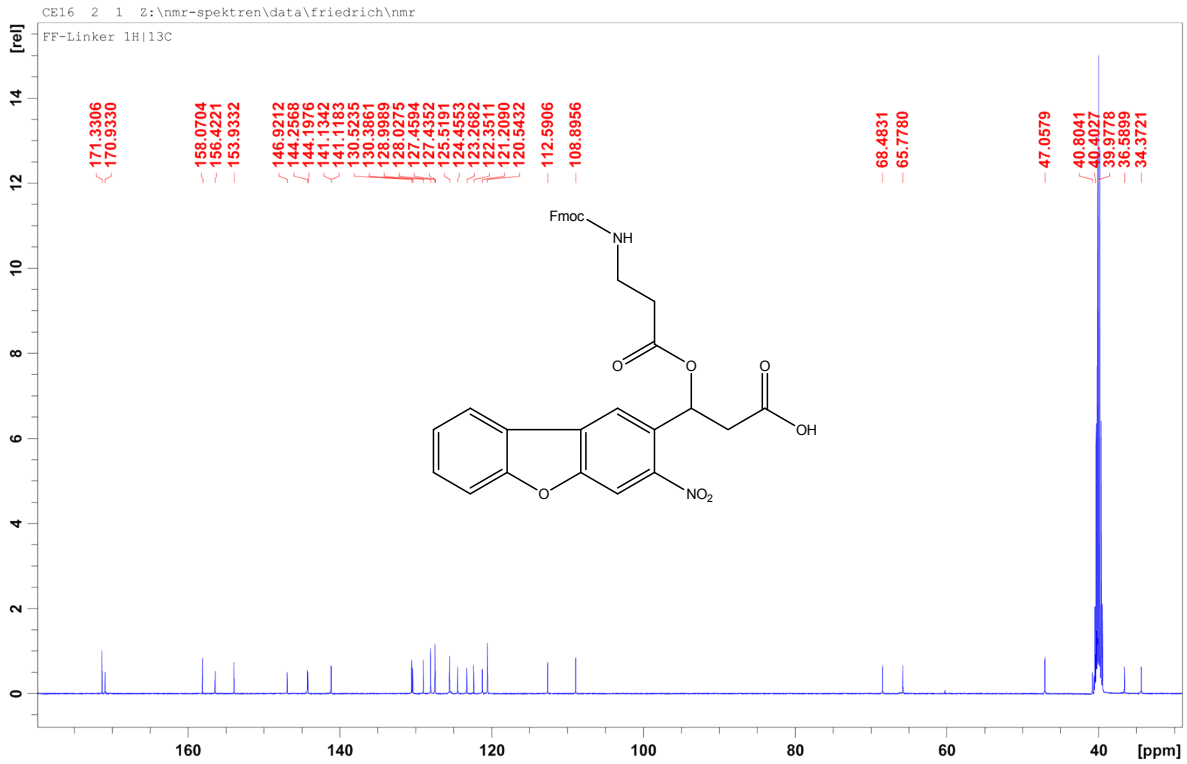
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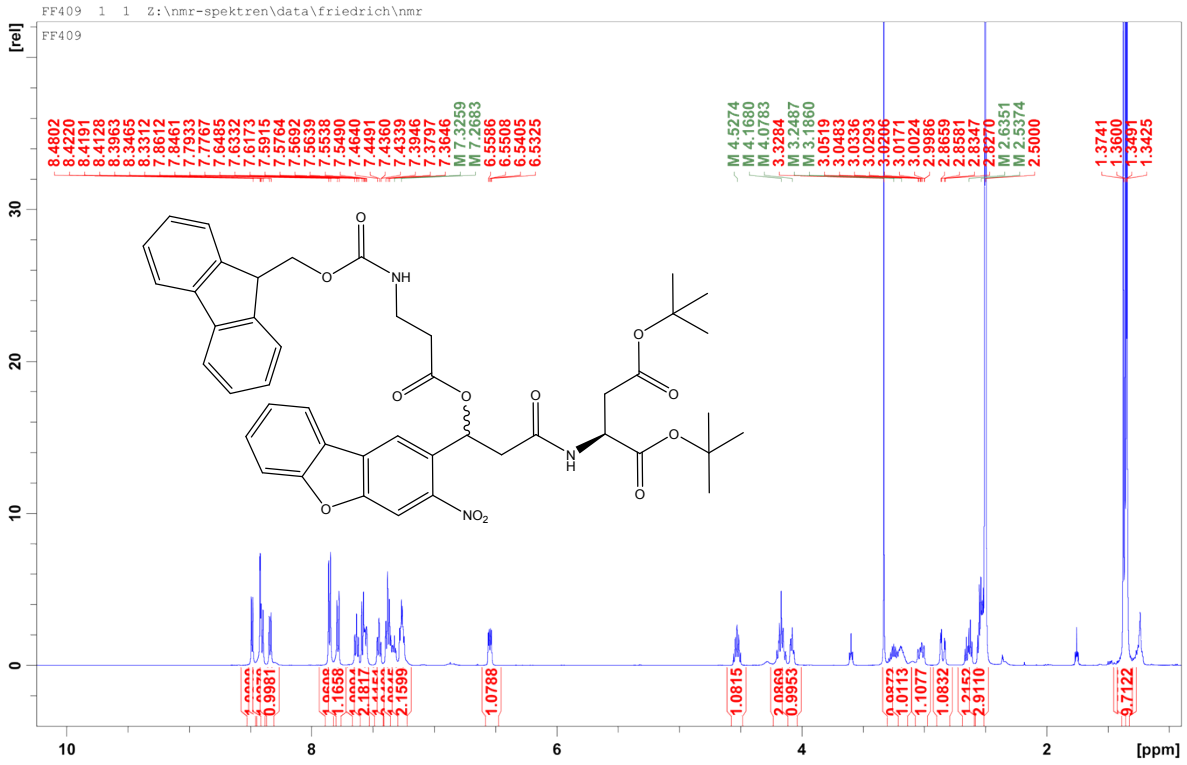
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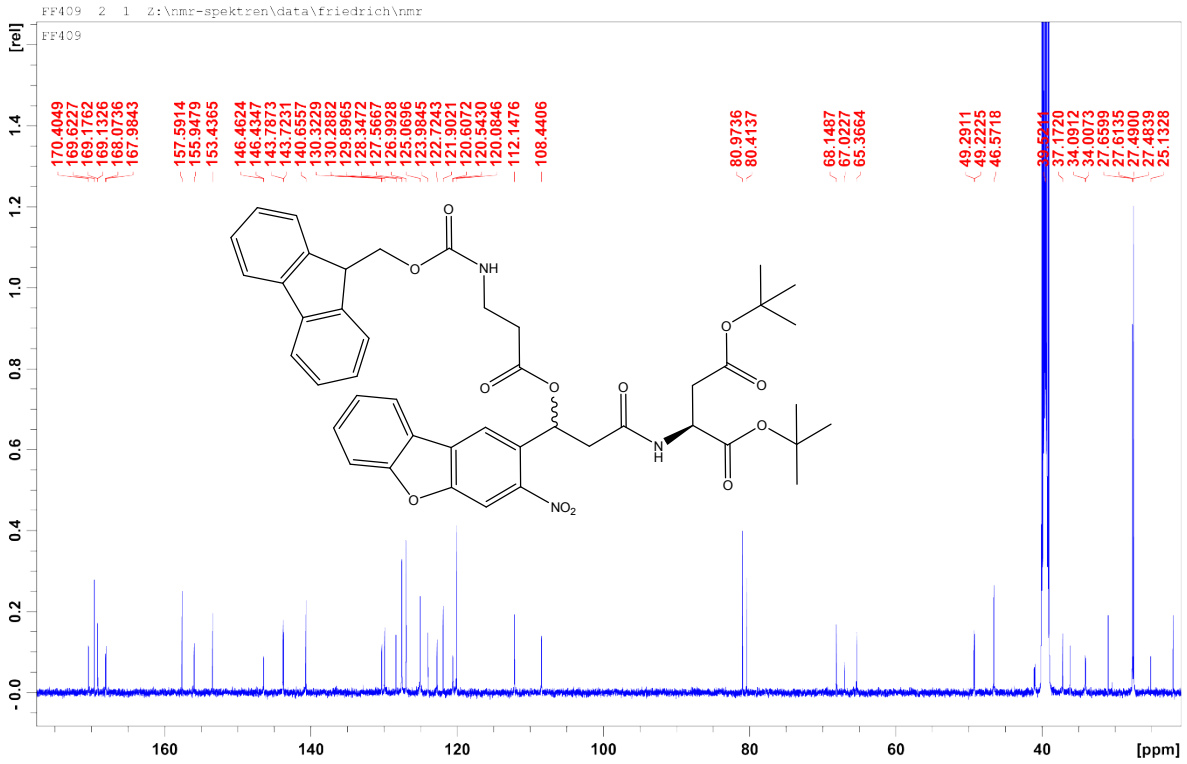
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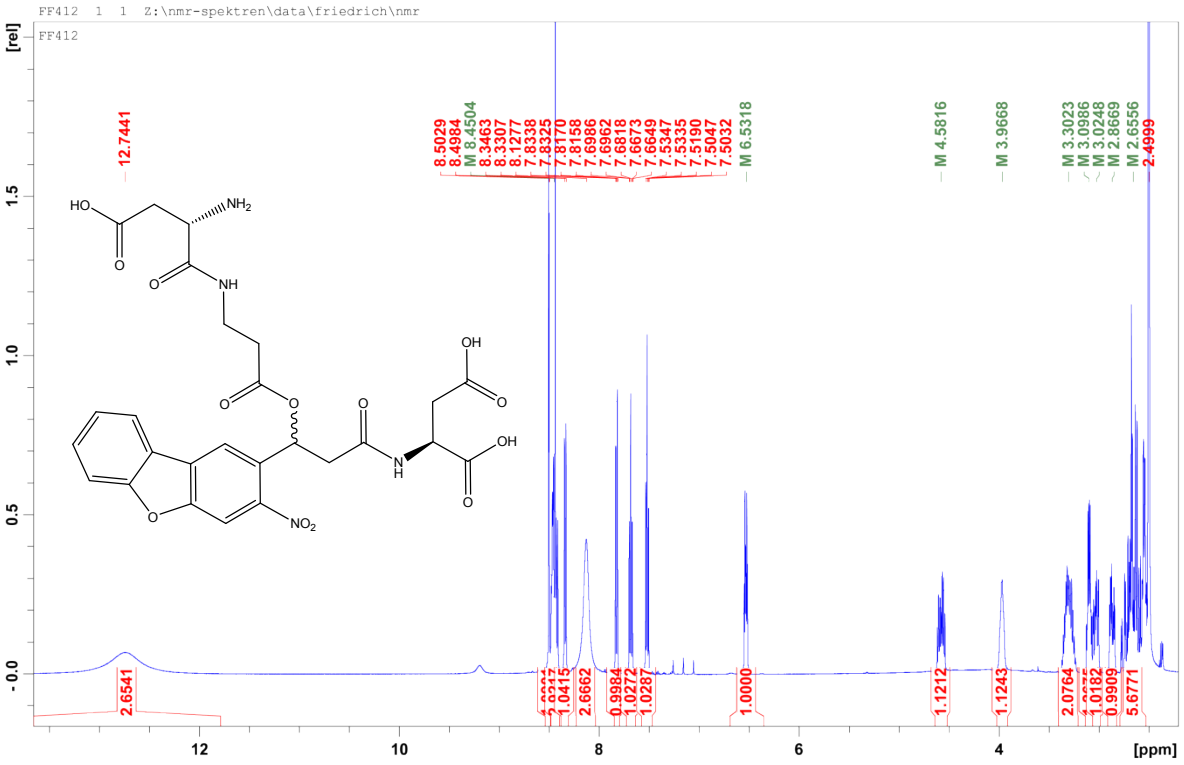
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7.6962
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7.5047
7.5032
M 6.5318
M 4.5816
M 3.9668
M 3.3023
M 3.0986
M 3.0248
M 2.8669
M 2.6556
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1.0415
2.6662
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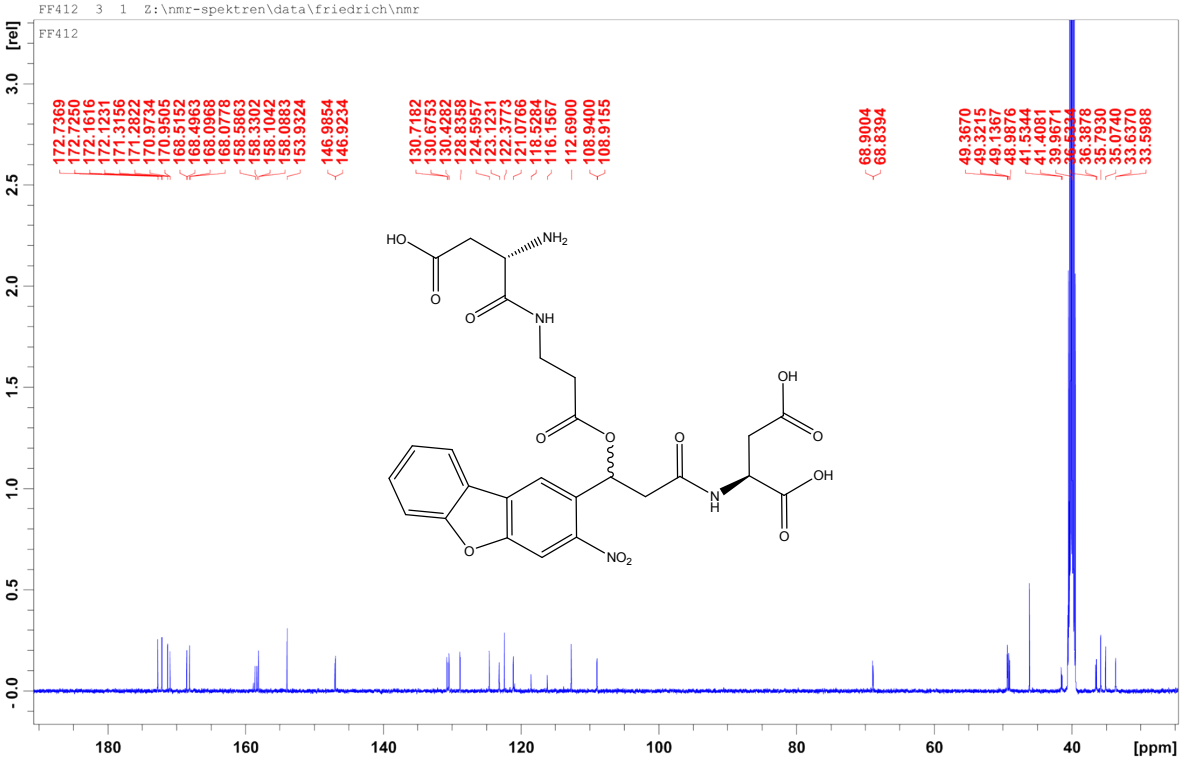
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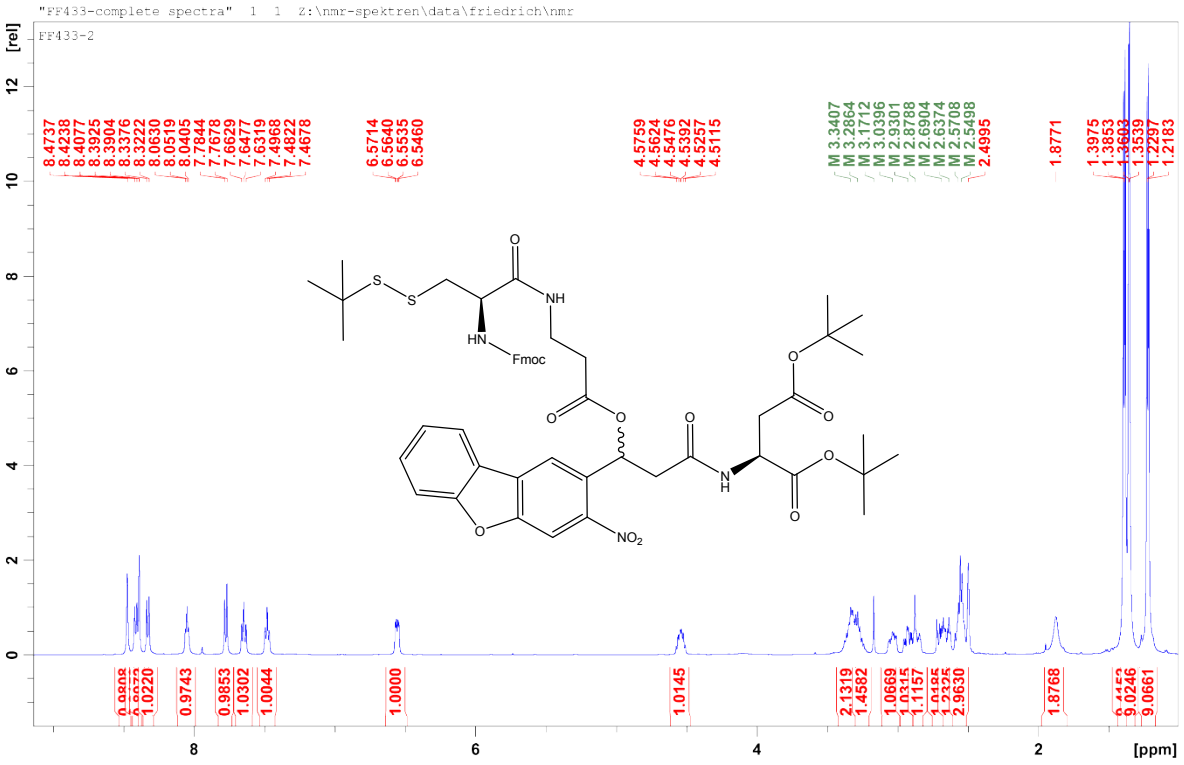
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M 3.2864
M 3.1712
M 3.0396
M 2.9301
M 2.8788
M 2.6904
M 2.6374
M 2.5708
M 2.5498
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1.3539
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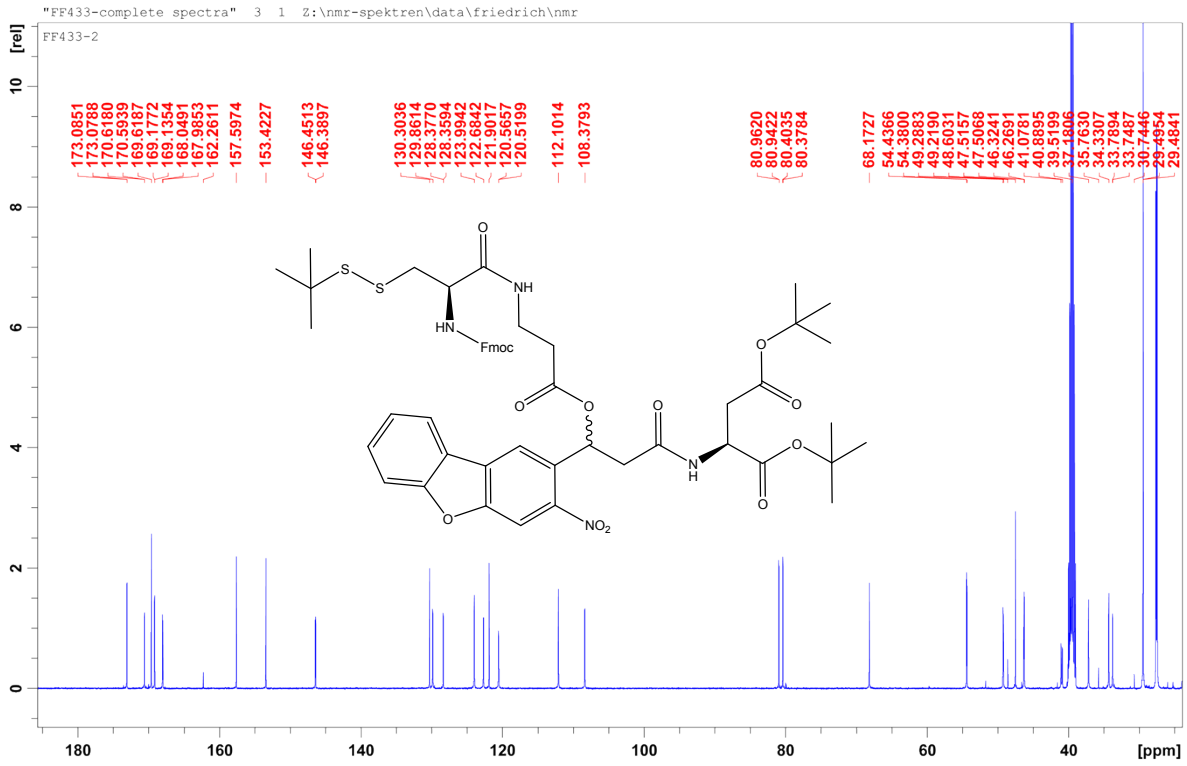
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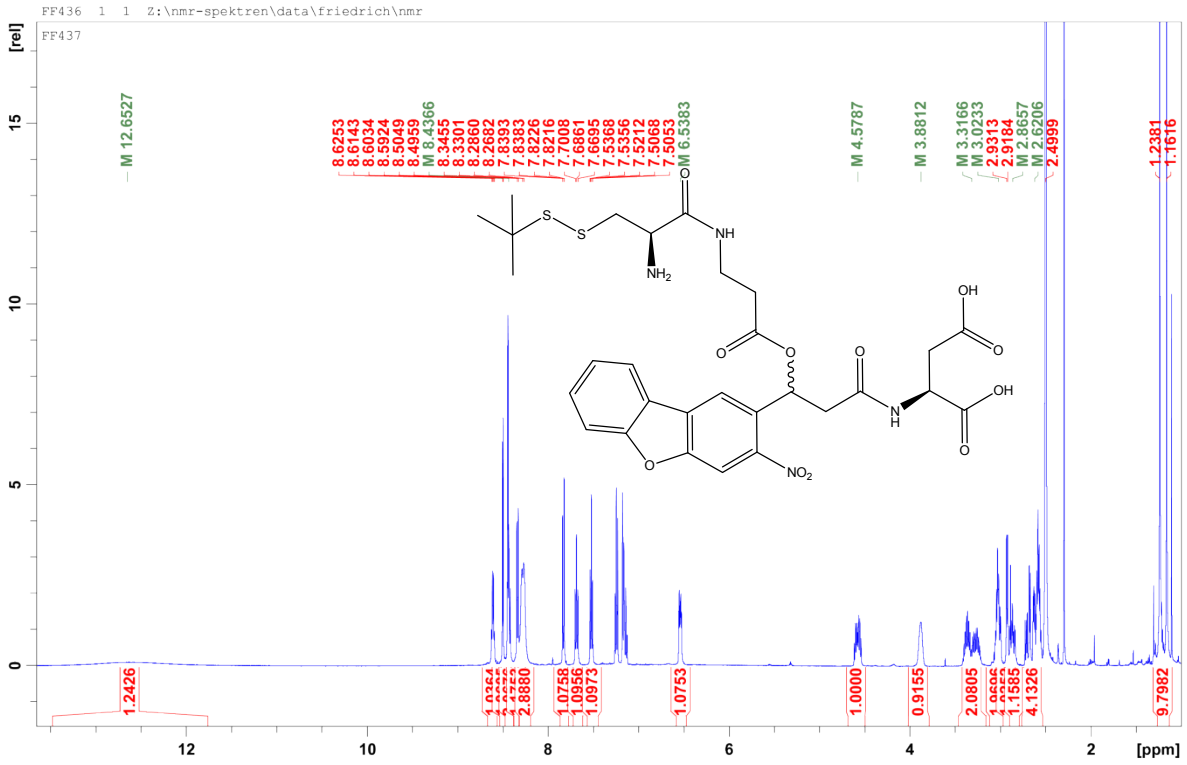
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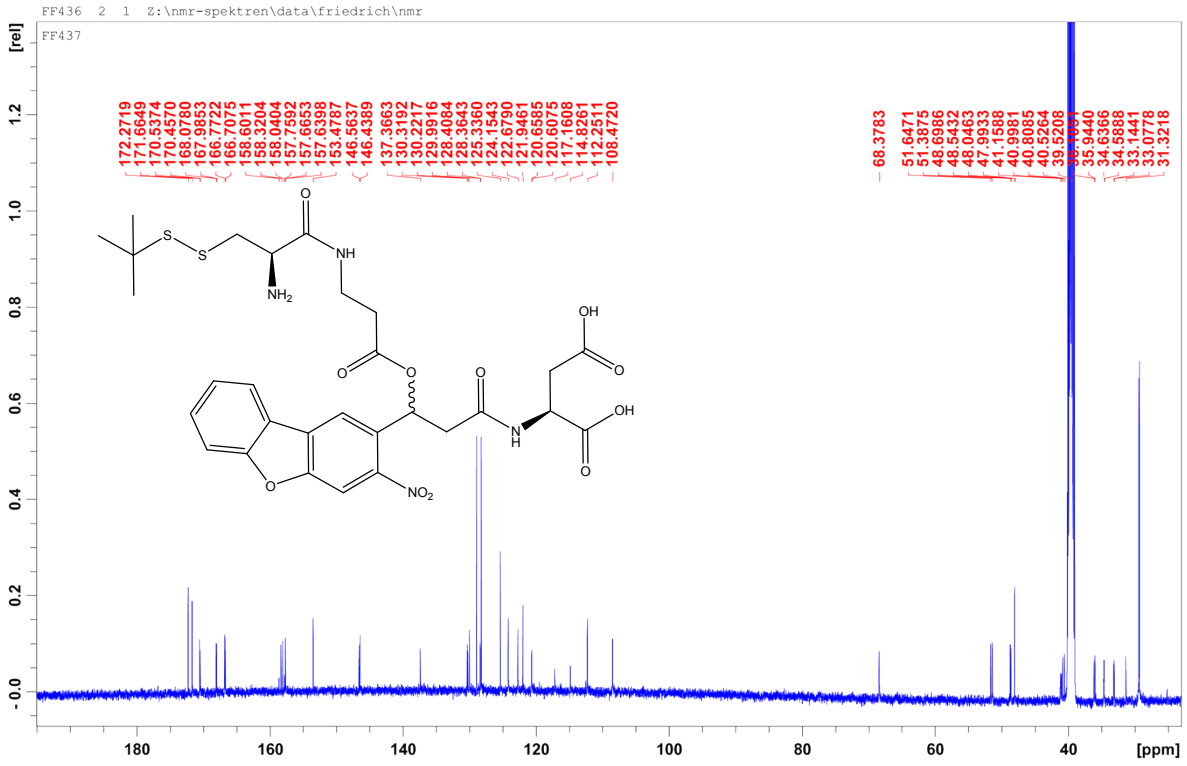
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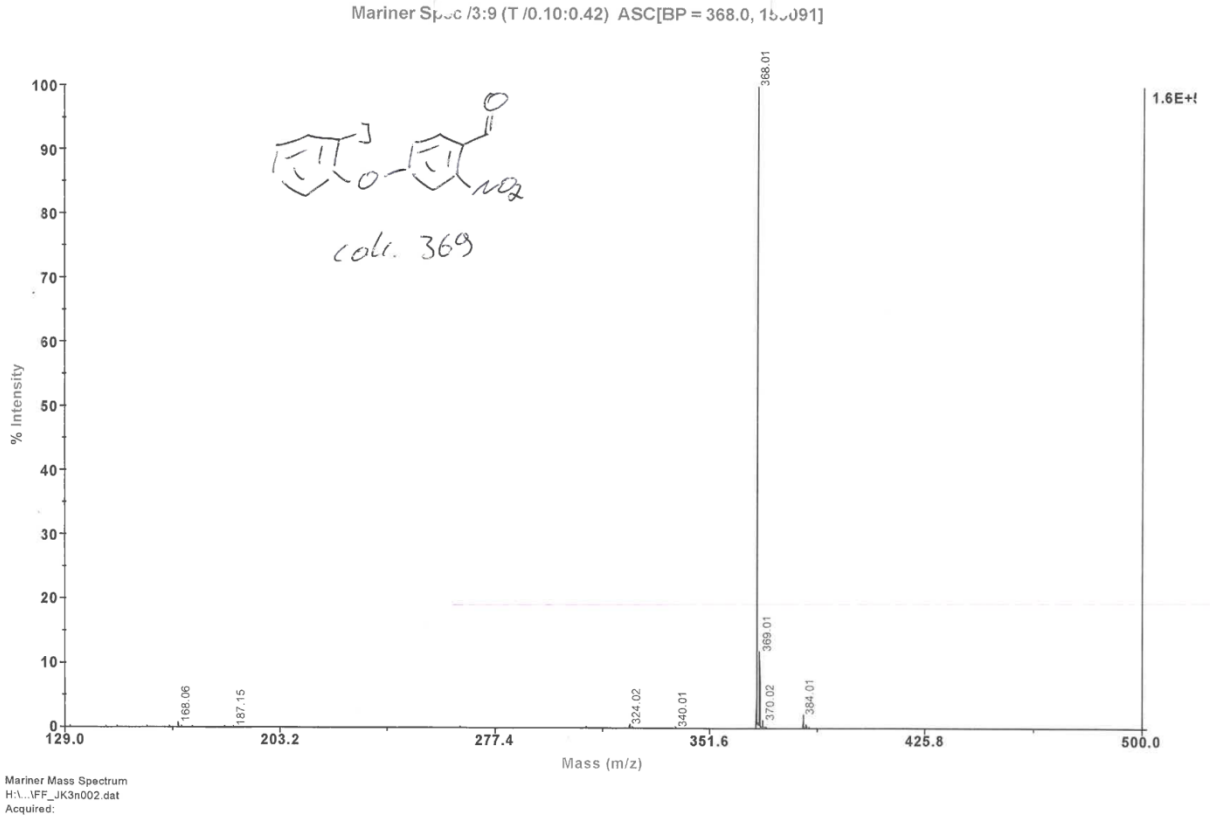
MS spectra


Mariner Spec /3:9 (T /0.10:0.42) ASC[BP = 368.0, 1
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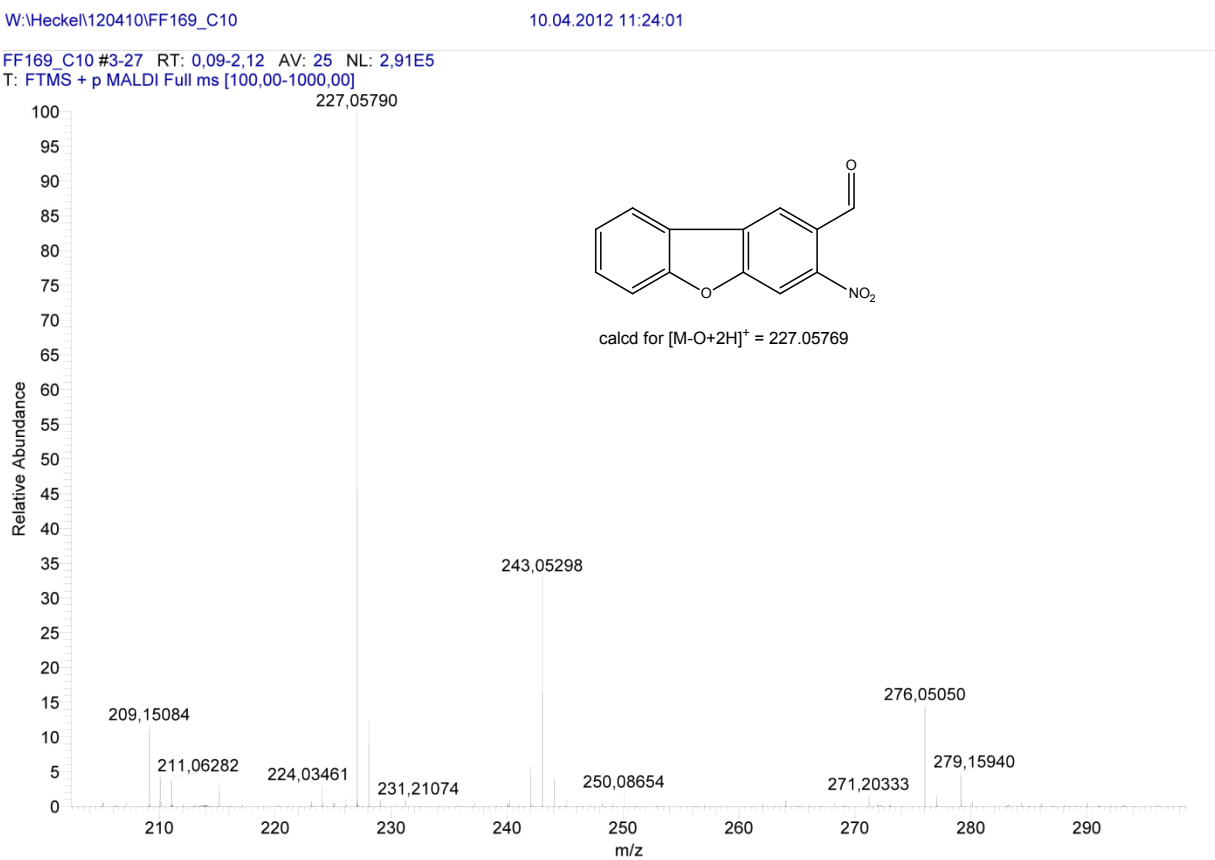
1.6E+
100
90
80
70
60
50
40
30
20
10
0
% Intensity
168.06
187.15
324.02
340.01
369.01
370.02
384.01
129.0
203.2
277.4
351.6
425.8
500.0
Mass (m/z)
Mariner Mass Spectrum
H:\...\FF_JK3n002.dat
Acquired: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10.04.2012 11:24:01
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T: FTMS + p MALDI Full ms [100,00-1000,00]
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243,05298
276,05050
209,15084
211,06282
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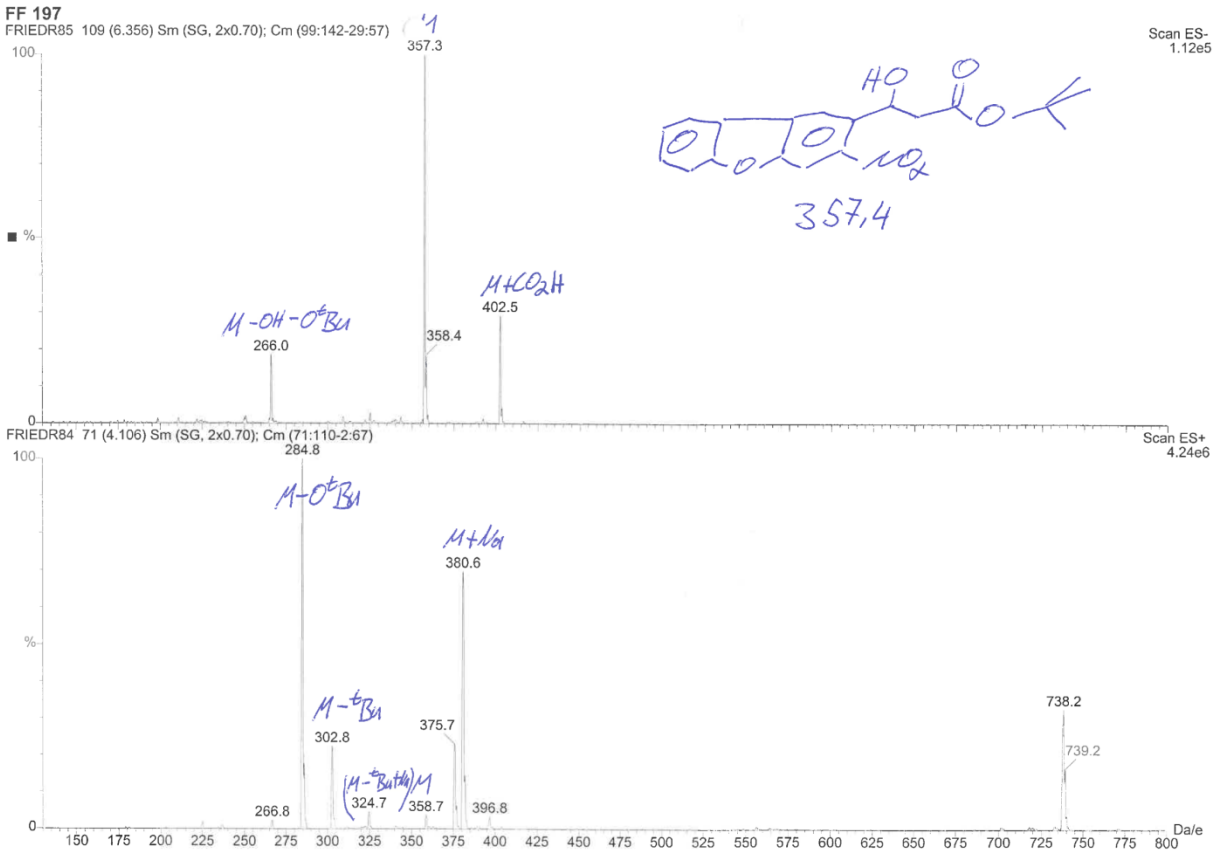
Relative Abundance
m/z
O
O
NO2
calcd for [M-O+2H]+ = 227.05769


FF 197
FRIEDR85 109 (6.356) Sm (SG, 2x0.70); Cm (99:142-29:57)
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1.12e5
357.3
358.4
402.5
266.0
M-OH-OtBu
M+CO2H
357,4
FRIEDR84 71 (4.106) Sm (SG, 2x0.70); Cm (71:110-2:67)
Scan ES+
4.24e6
284.8
M-OtBu
M+Na
380.6
M-tBu
302.8
375.7
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266.8
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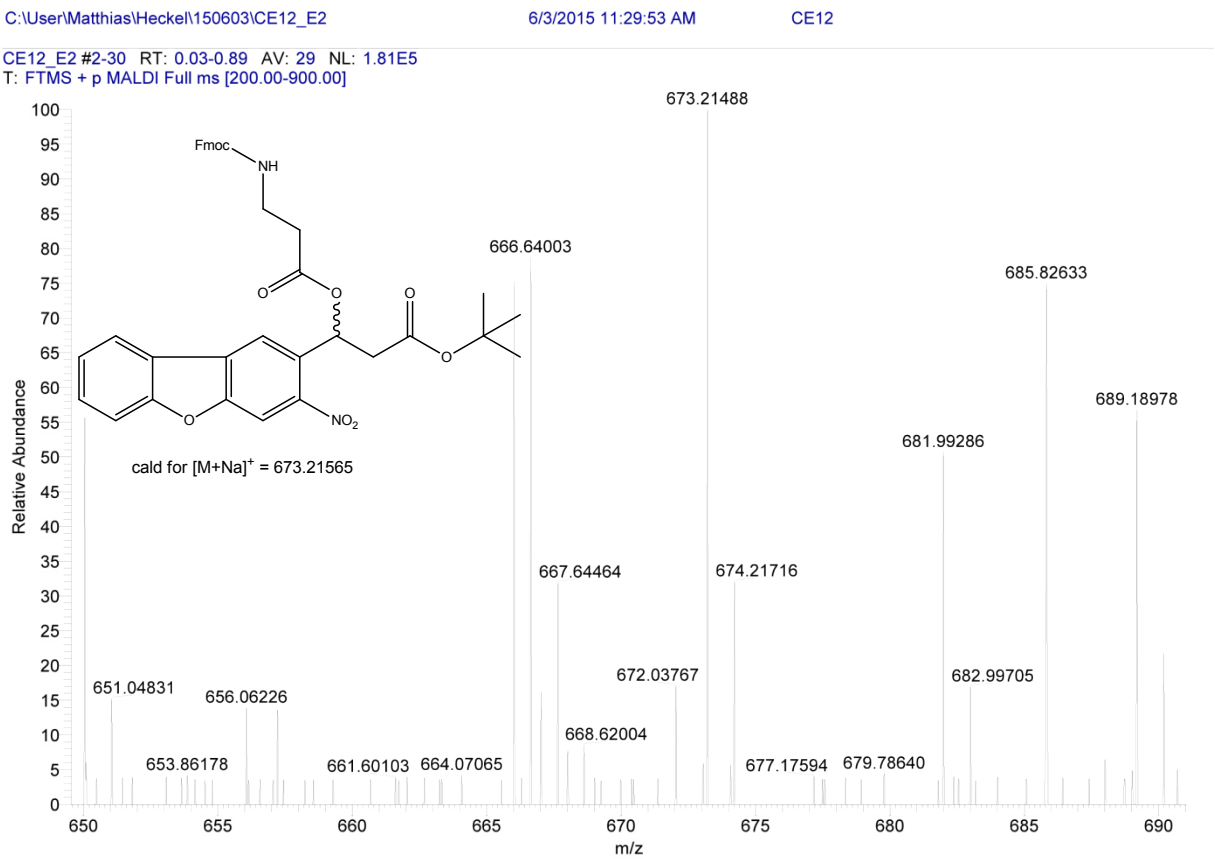
396.8
Da/e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6/3/2015 11:29:53 AM
CE12
CE12_E2 #2-30 RT: 0.03-0.89 AV: 29 NL: 1.81E5
T: FTMS + p MALDI Full ms [200.00-900.00]
Fmoc
NH
NO2
cald for [M+Na]+ = 673.21565
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666.64003
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672.03767
682.99705
651.04831
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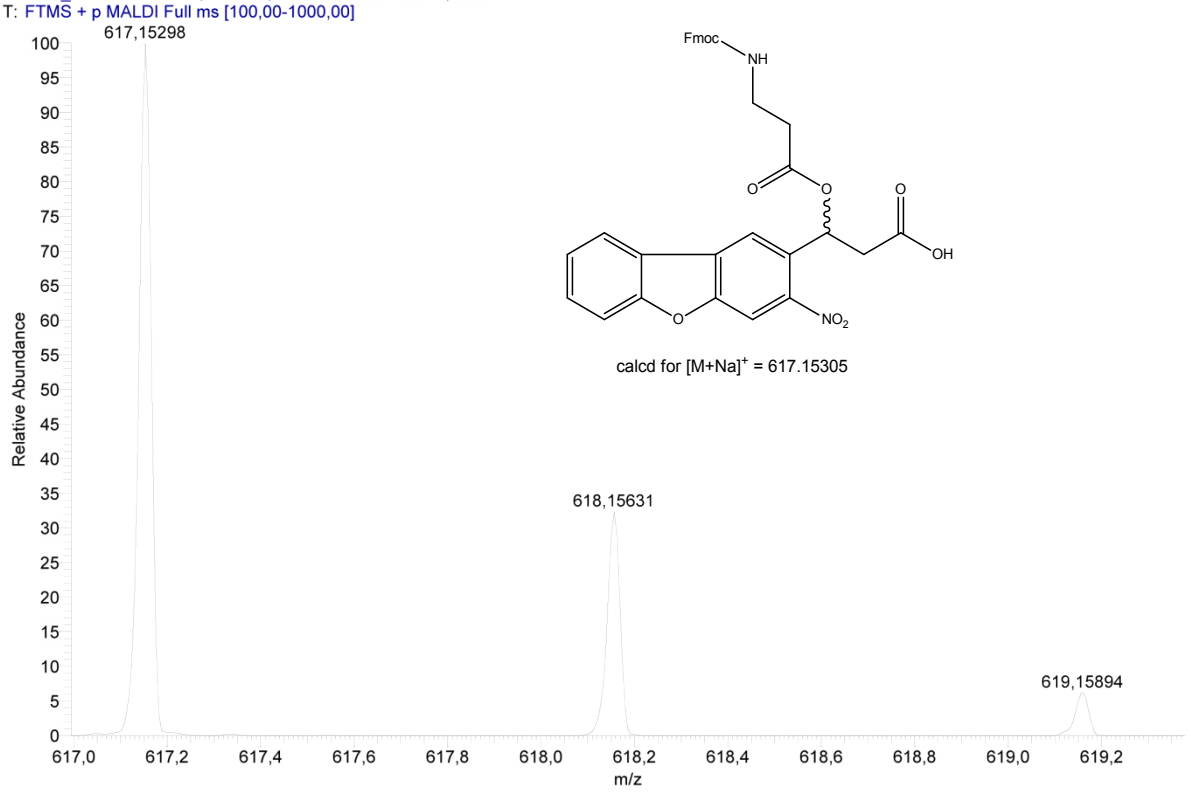
653.86178
661.60103
664.07065
677.17594
679.78640
Relative Abundance
m/z

X:\Andreas\Heckel\140507\CE16-1_E8 08.05.2014 09:30:32

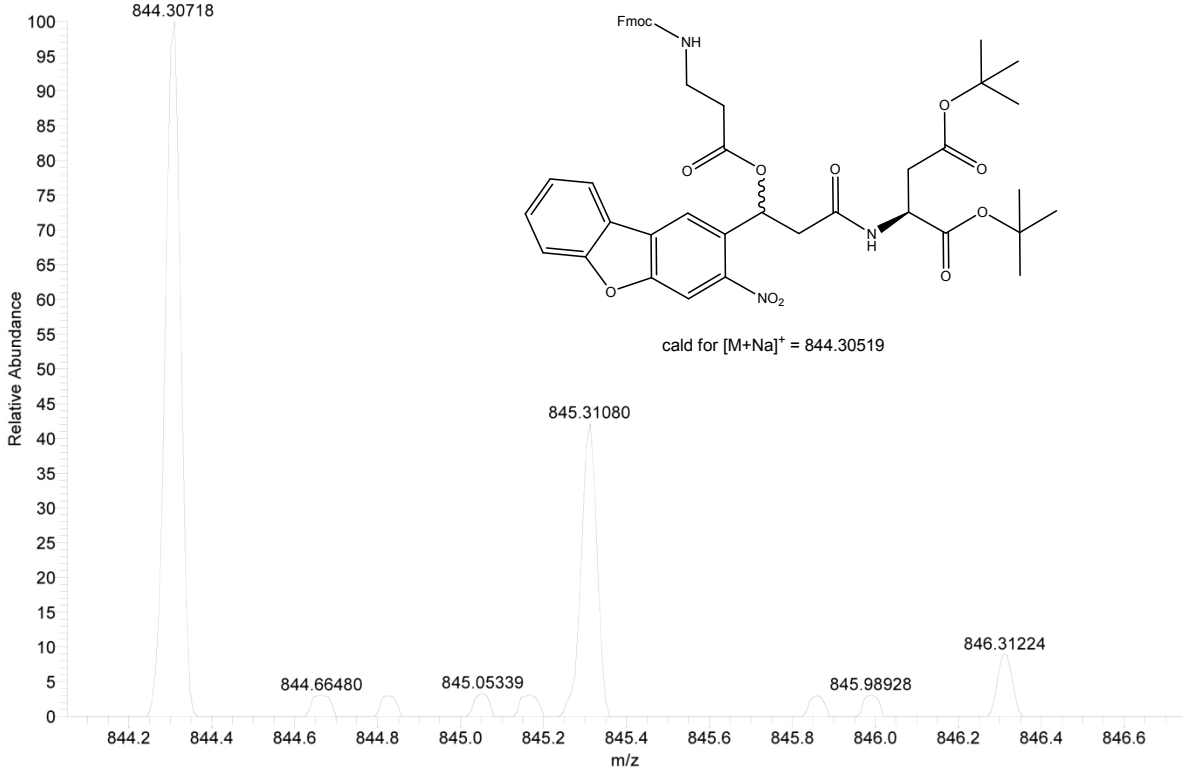
CE16-1_E8 #6-15 RT: 0,56548-1,37695 AV: 10 NL: 3,40E5
T: FTMS + p MALDI Full ms [100,00-1000,00]



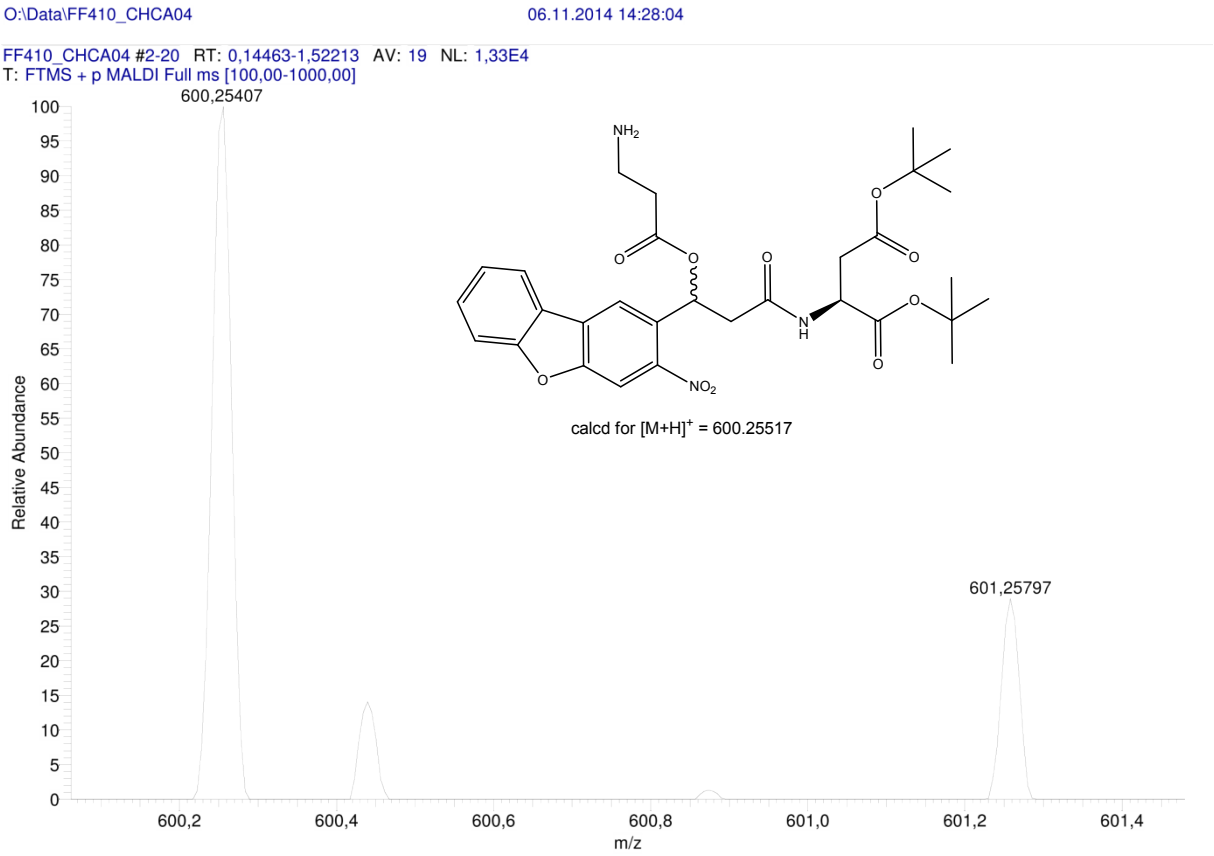
calcd for $[M+Na]^+$ = 617.15305

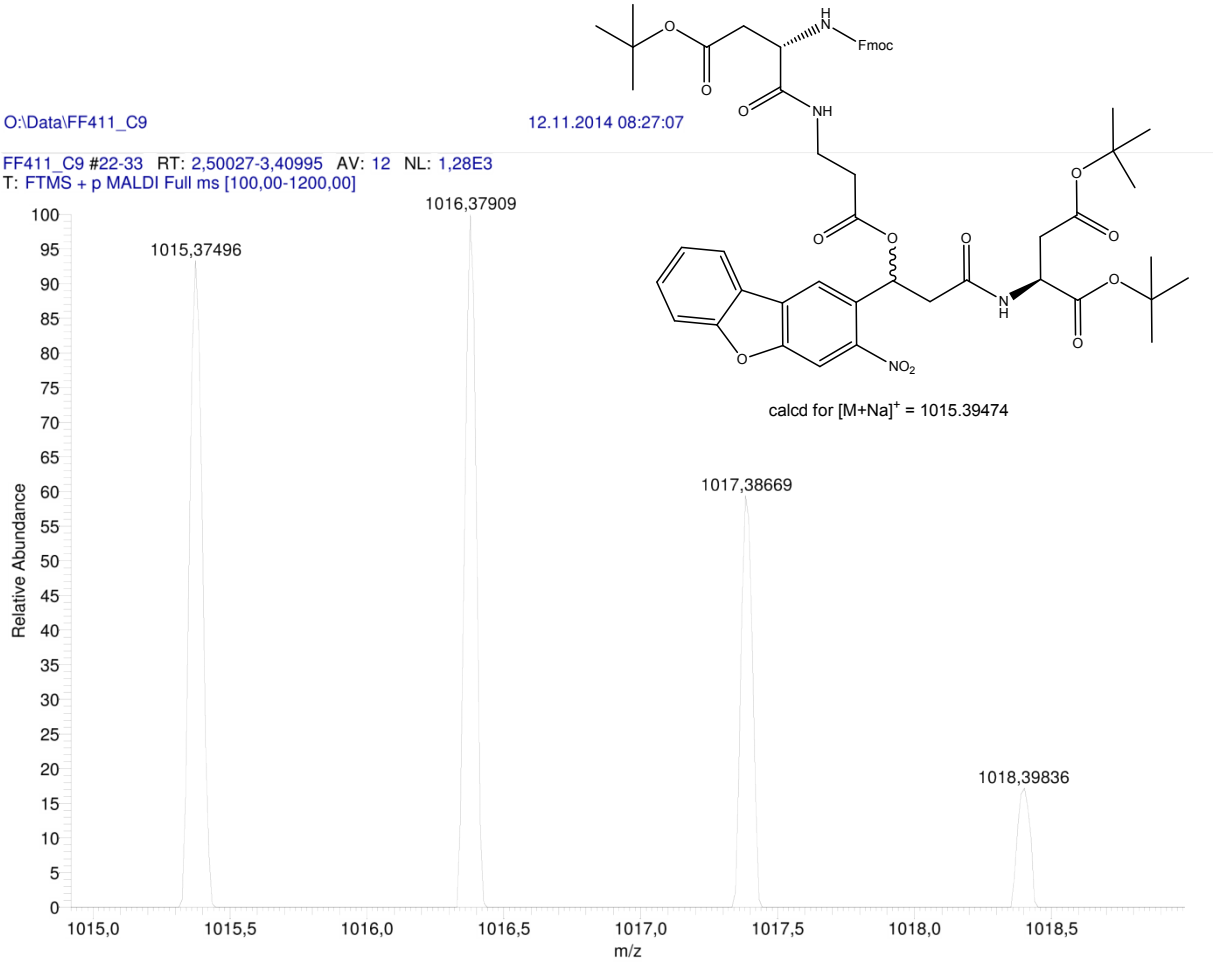
C:\User\Matthias\Heckel\150603\FF409_E1 6/3/2015 11:23:47 AM FF409 mit HCCA

FF409_E1 #1-30 RT: 0.00-0.89 AV: 30 NL: 2.04E5
T: FTMS + p MALDI Full ms [200.00-900.00]



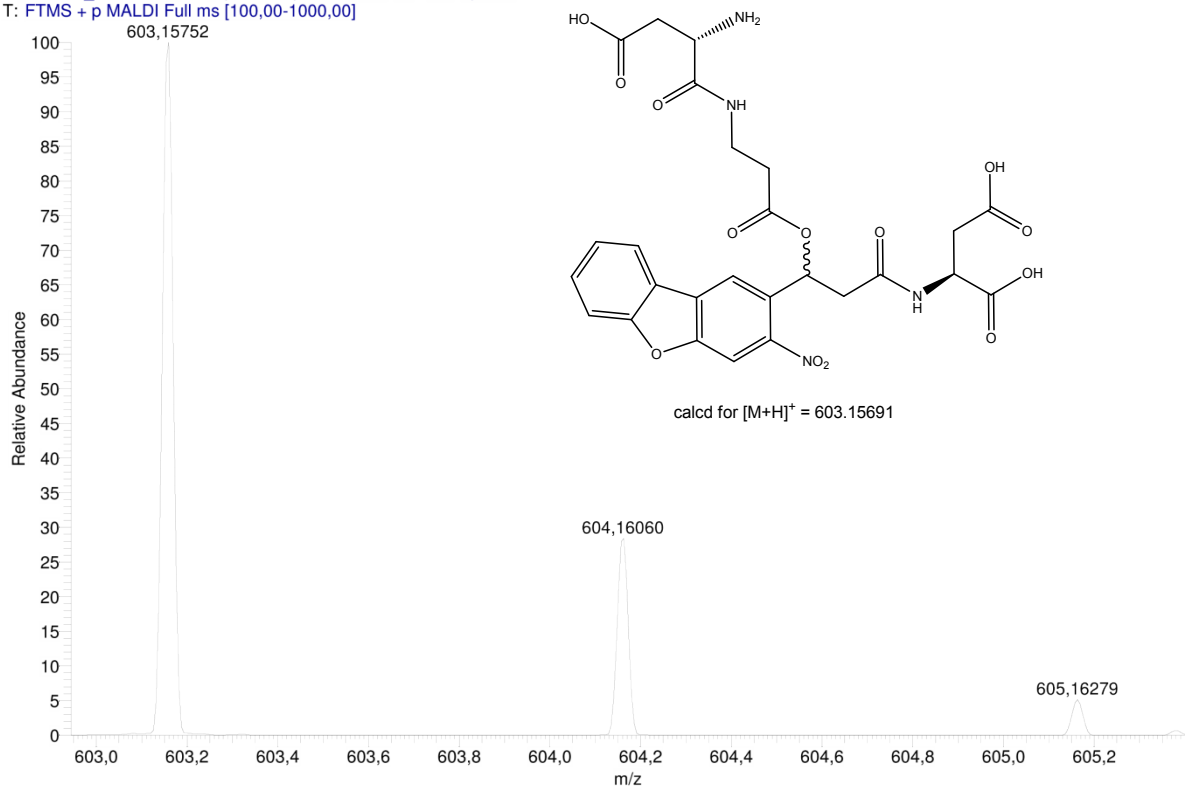
cald for $[M+Na]^+$ = 844.30519


O:\Data\FF410_CHCA04
06.11.2014 14:28:04
FF410_CHCA04 #2-20 RT: 0,14463-1,52213 AV: 19 NL: 1,33E4
T: FTMS + p MALDI Full ms [100,00-1000,00]
600,25407
601,25797
Relative Abundance
m/z
calcd for [M+H]+ = 600.25517


O:\Data\FF411_C9
12.11.2014 08:27:07
FF411_C9 #22-33 RT: 2,50027-3,40995 AV: 12 NL: 1,28E3
T: FTMS + p MALDI Full ms [100,00-1200,00]
1016,37909
1015,37496
1017,38669
1018,39836
Relative Abundance
m/z
calcd for [M+Na]+ = 1015.39474

O:\Data\FF412-MS_G2 01.12.2014 10:21:38

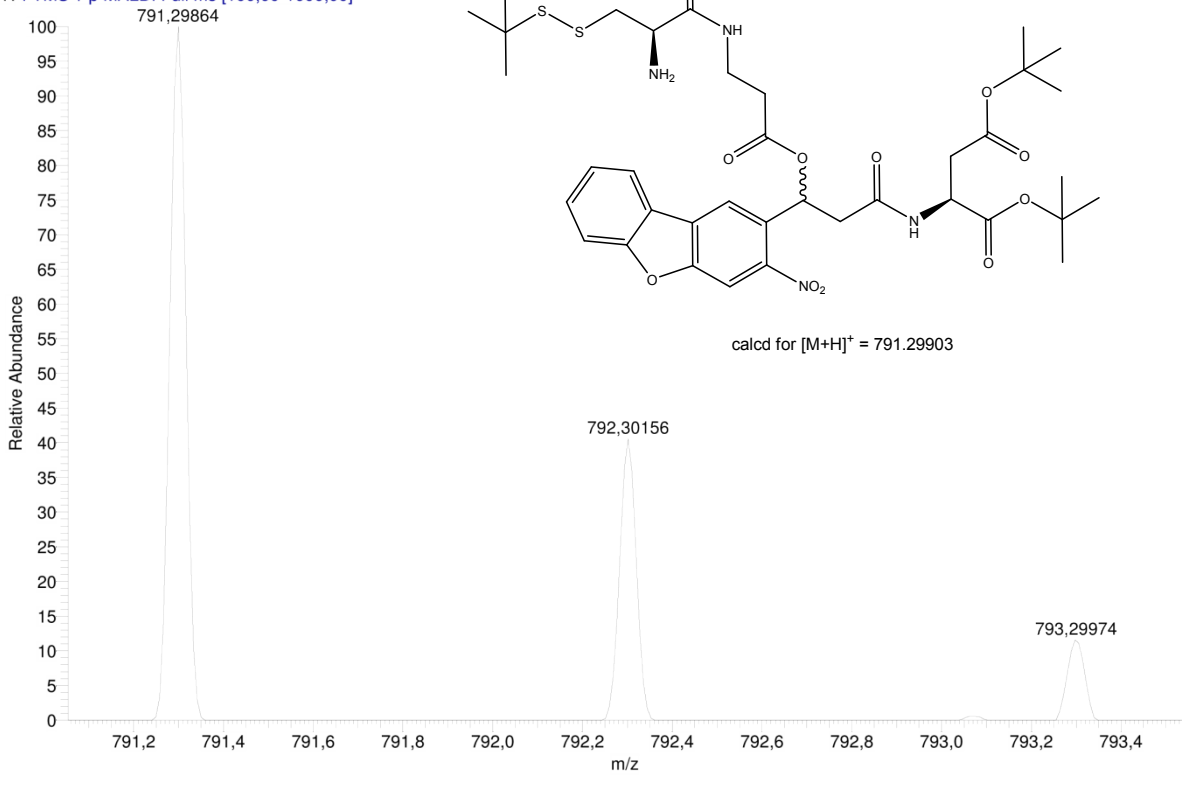
FF412-MS_G2 #1-13 RT: 0,00364-0,90119 AV: 13 NL: 1,87E6
T: FTMS + p MALDI Full ms [100,00-1000,00]



calcd for $[M+H]^+$ = 603.15691

O:\Data\FF433-2_F1 16.03.2015 09:43:03

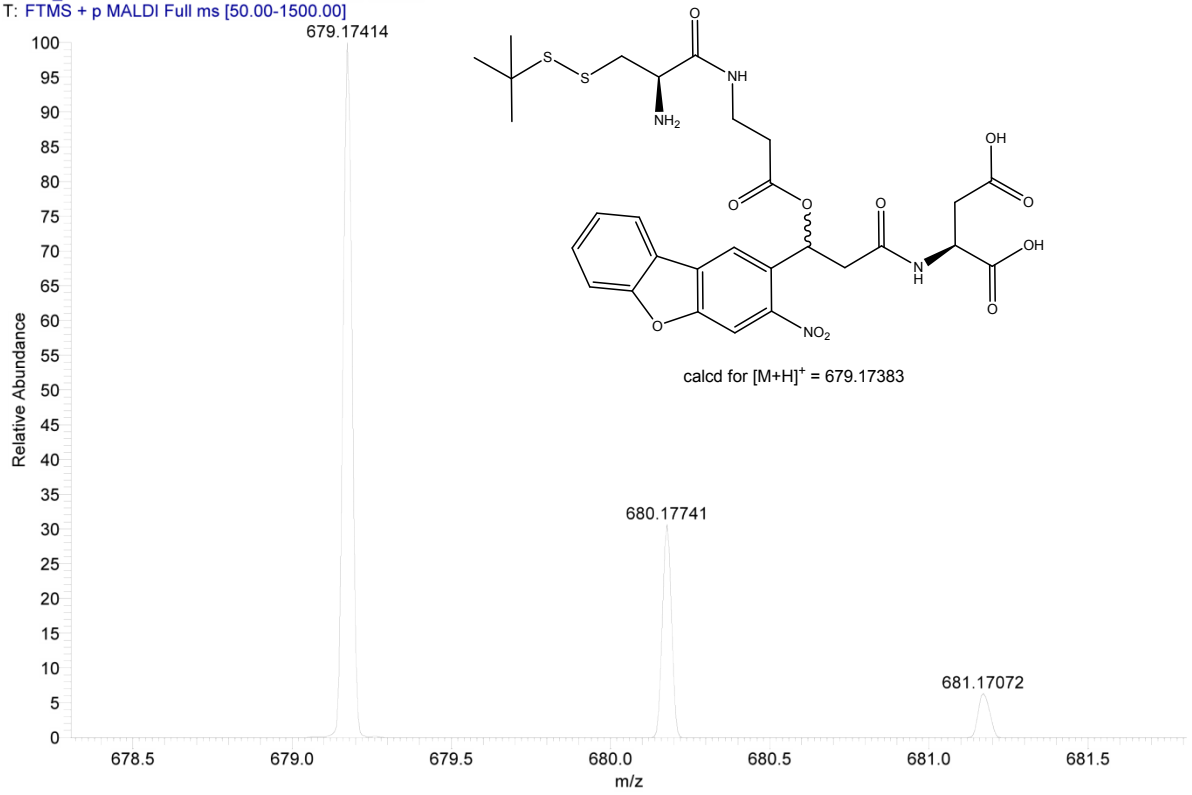
FF433-2_F1 #3-19 RT: 0,19954-1,45445 AV: 17 NL: 3,43E4
T: FTMS + p MALDI Full ms [100,00-1000,00]



calcd for $[M+H]^+$ = 791.29903

C:\User\Matthias\Heckel\150608\FF436_A2 6/8/2015 11:07:05 AM FF436

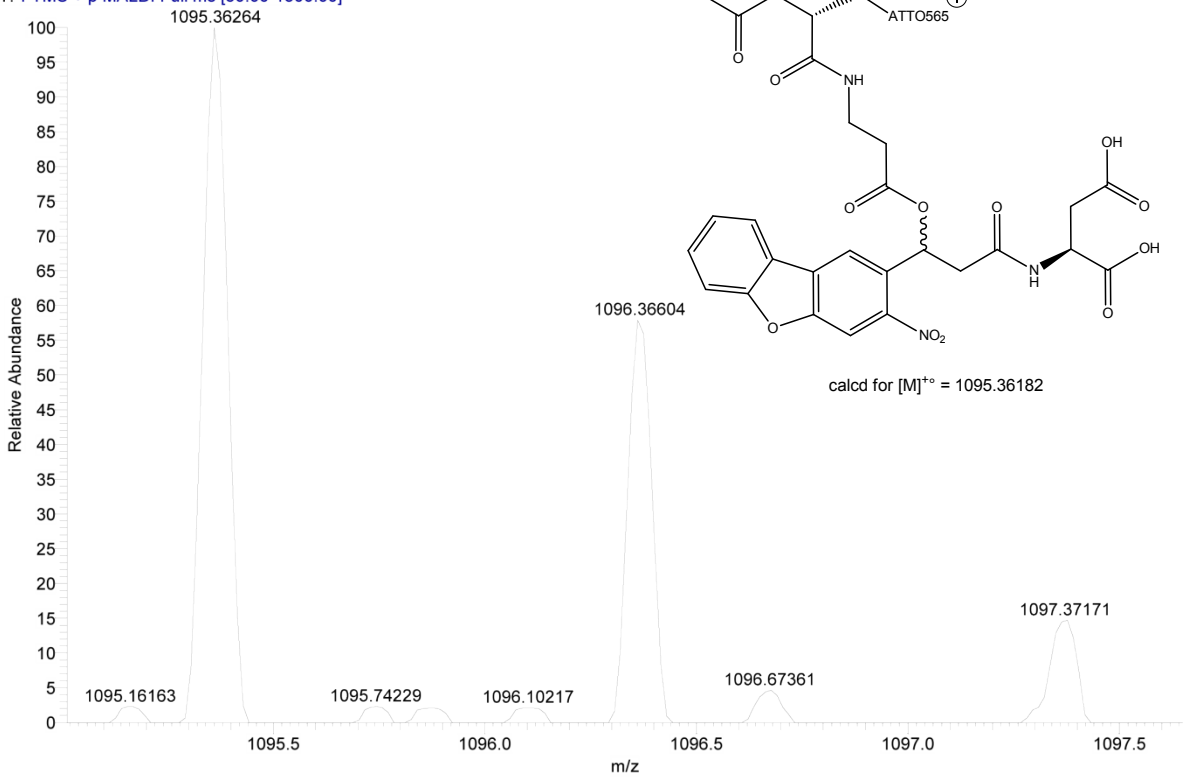
FF436_A2 #1-19 RT: 0.00-0.55 AV: 19 NL: 1.07E7
T: FTMS + p MALDI Full ms [50.00-1500.00]



calcd for $[M+H]^+$ = 679.17383

C:\User\Matthias\Heckel\150608\FF417_A6 6/8/2015 11:28:00 AM FF417

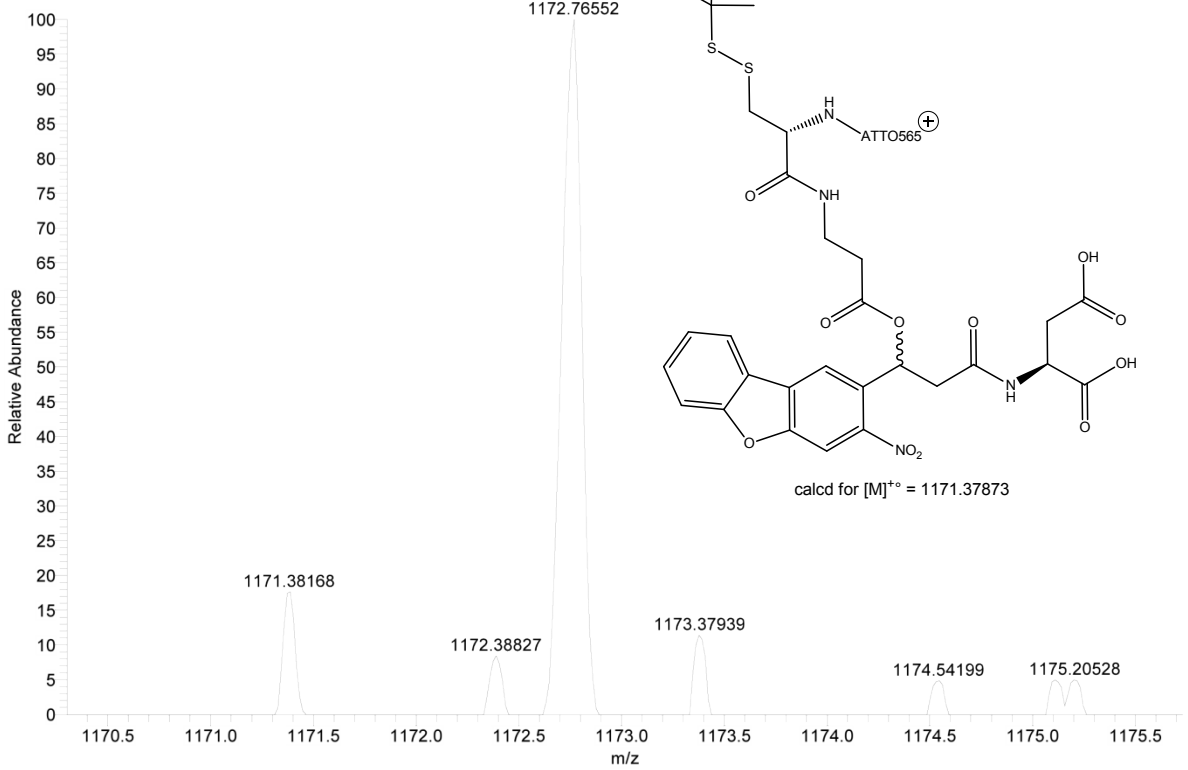
FF417_A6 #1-46 RT: 0.00-1.38 AV: 46 NL: 1.73E5
T: FTMS + p MALDI Full ms [50.00-1500.00]



calcd for $[M]^{+\circ}$ = 1095.36182

C:\User\Matthias\Heckel\150608\FF437_A3 6/8/2015 11:11:50 AM FF437

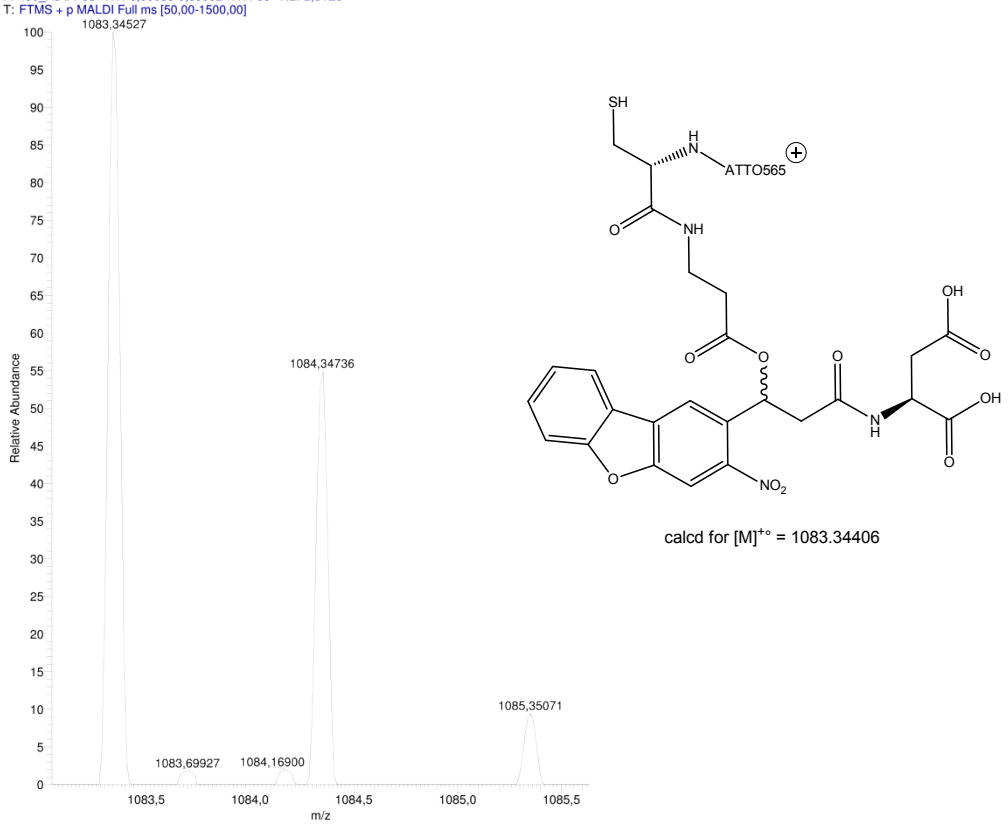
FF437_A3 #1-32 RT: 0.01-0.95 AV: 32 NL: 1.10E5
T: FTMS + p MALDI Full ms [50.00-1500.00]



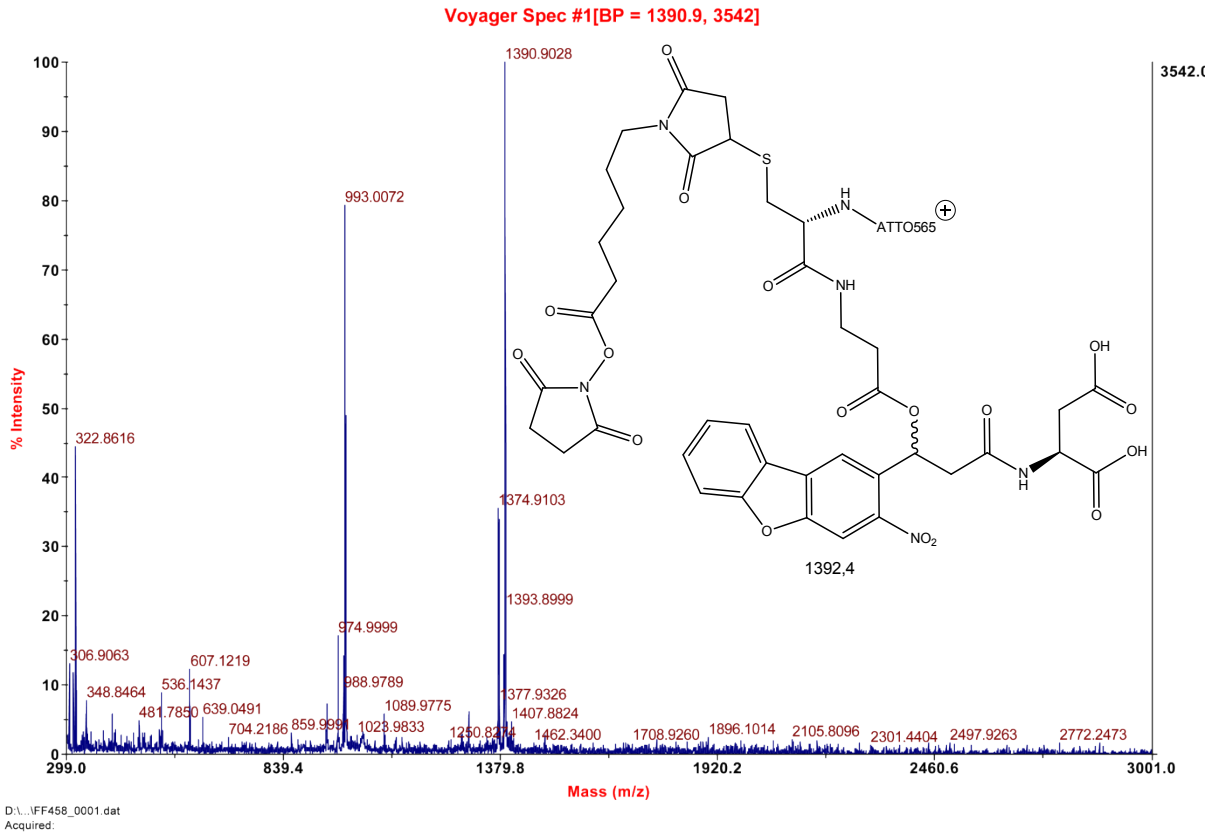
calcd for $[M]^{+\circ}$ = 1171.37873

Z:\Heckel\150608\FF439_A5 08.06.2015 11:22:33 FF439

FF439_A5 #1-33 RT: 0,00038-0,89952 AV: 33 NL: 2,81E5
T: FTMS + p MALDI Full ms [50,00-1500,00]

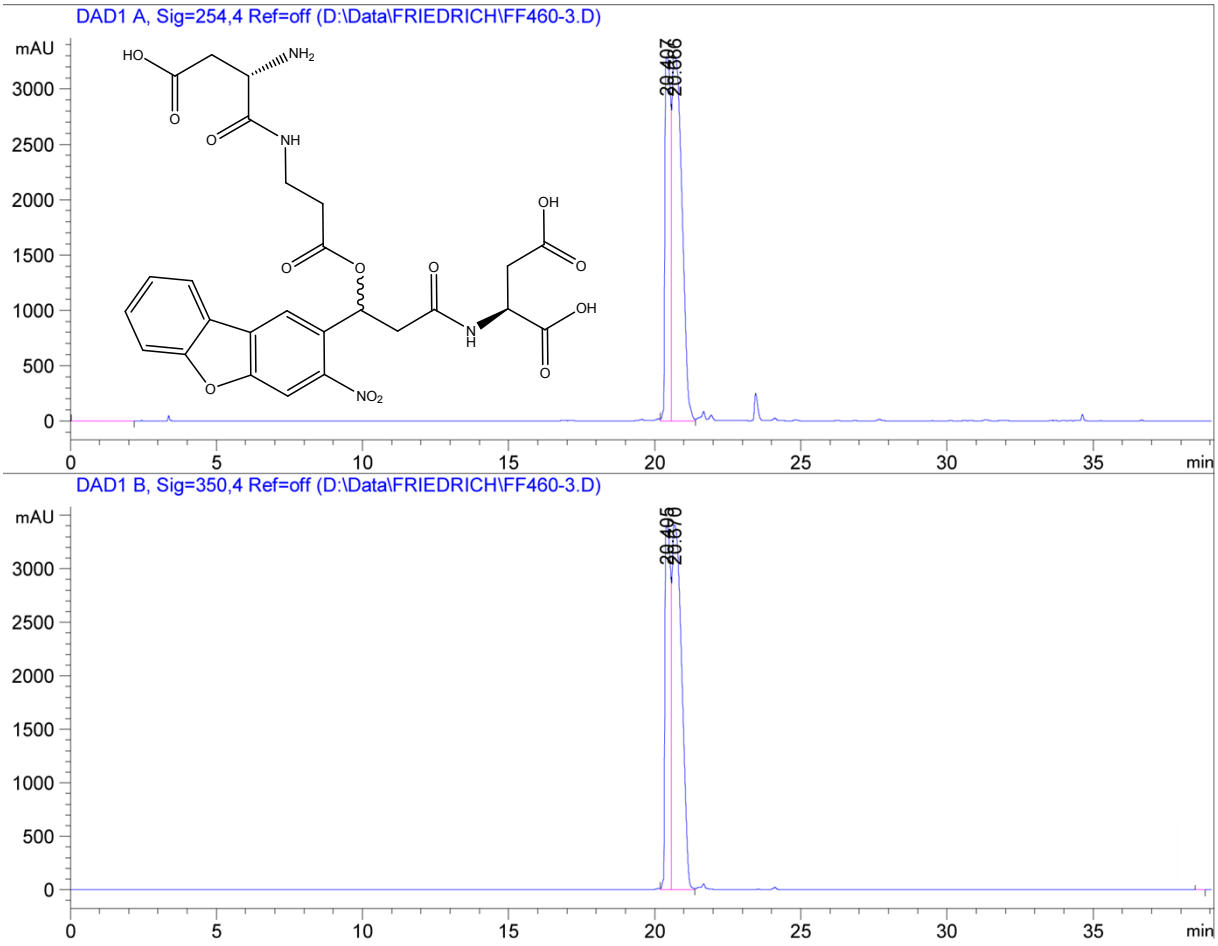


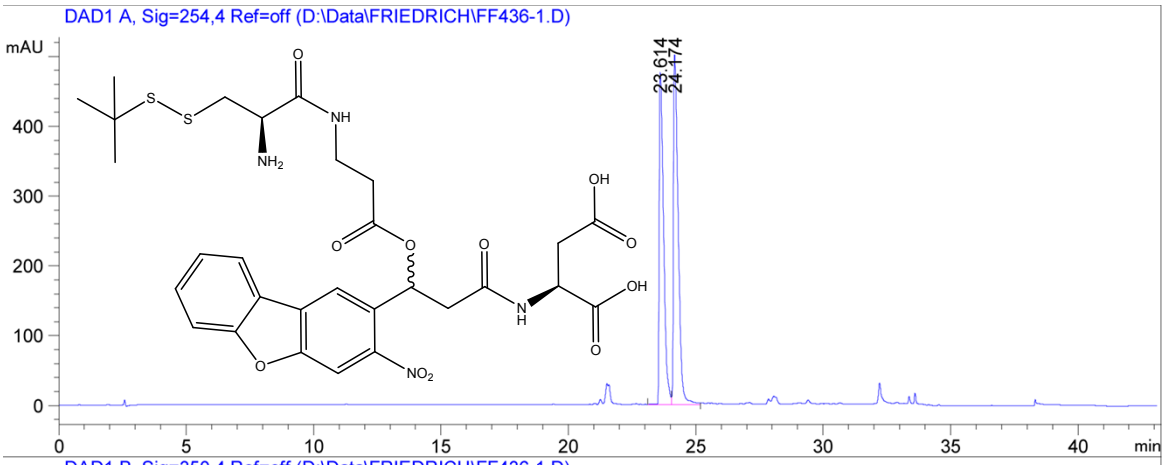
calcd for $[M]^{+\circ}$ = 1083.34406

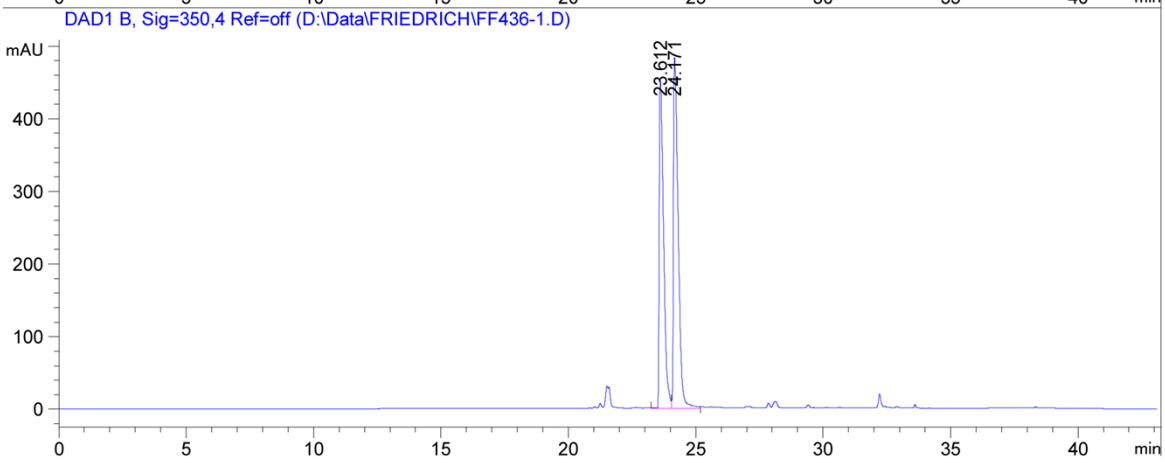

Voyager Spec #1[BP = 1390.9, 3542]
% Intensity
Mass (m/z)
1390.9028
993.0072
322.8616
1374.9103
1393.8999
974.9999
306.9063
607.1219
988.9789
348.8464
536.1437
1089.9775
1377.9326
481.7850
639.0491
1407.8824
704.2186
859.9991
1023.9833
1462.3400
1708.9260
1896.1014
2105.8096
2301.4404
2497.9263
2772.2473
299.0
839.4
1379.8
1920.2
2460.6
3001.0
ATTO565
1392,4
D:\...\FF458_0001.dat
Acquired:

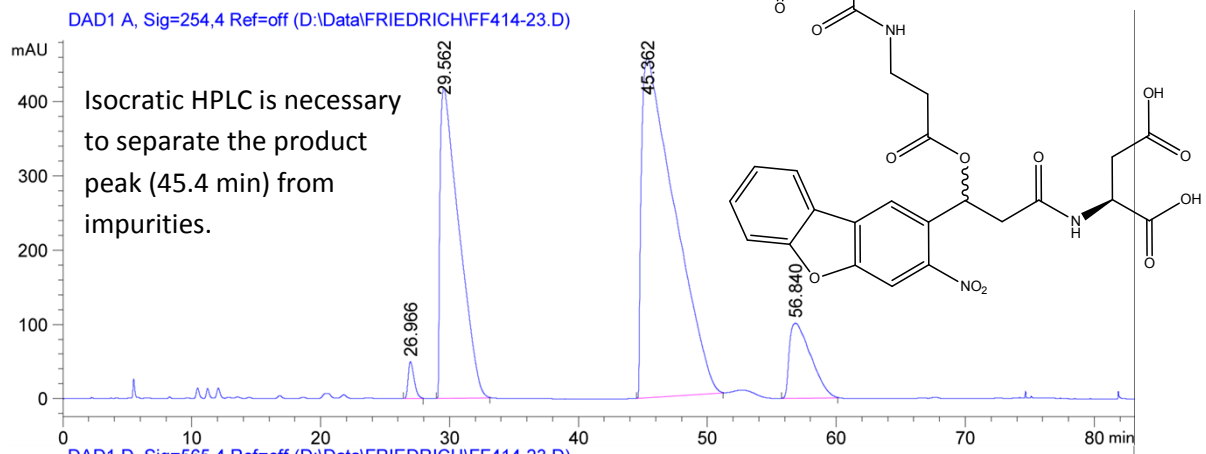
HPLC spectra

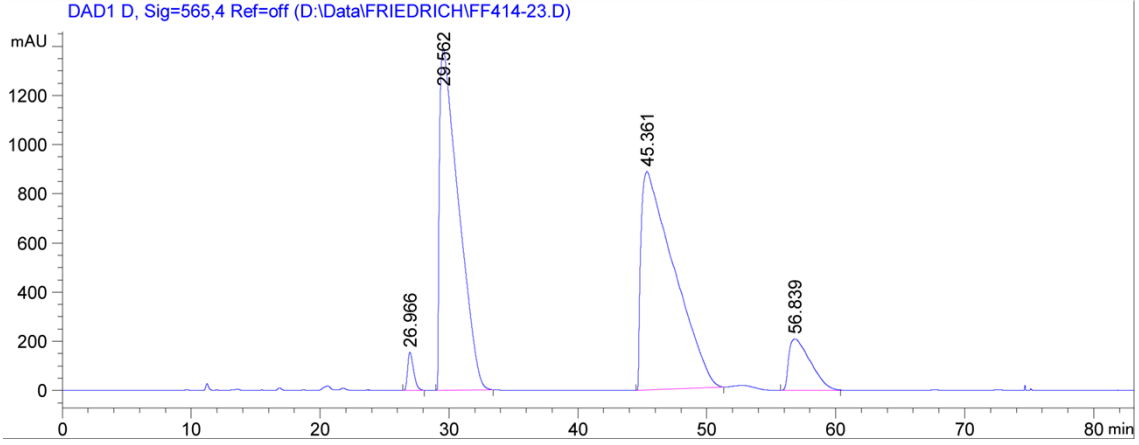
All attached spectra were recorded on 250 * 4.6 mm MultoKrom RP-C18 columns with a flow of 1 mL/min. The methods for each run are described in the experimental section.

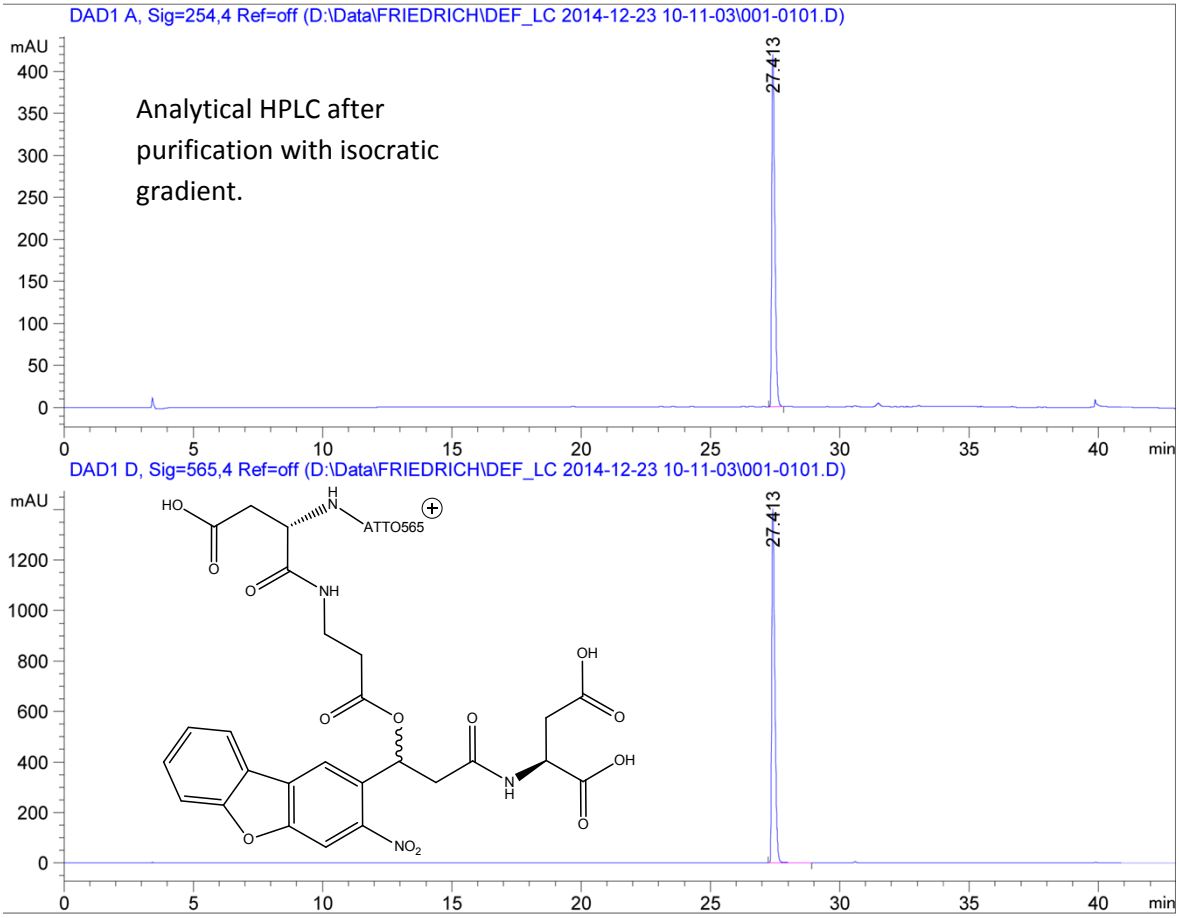



DAD1 A, Sig=254,4 Ref=off (D:\Data\FRIEDRICH\FF436-1.D)
mAU
23.614
24.174
min

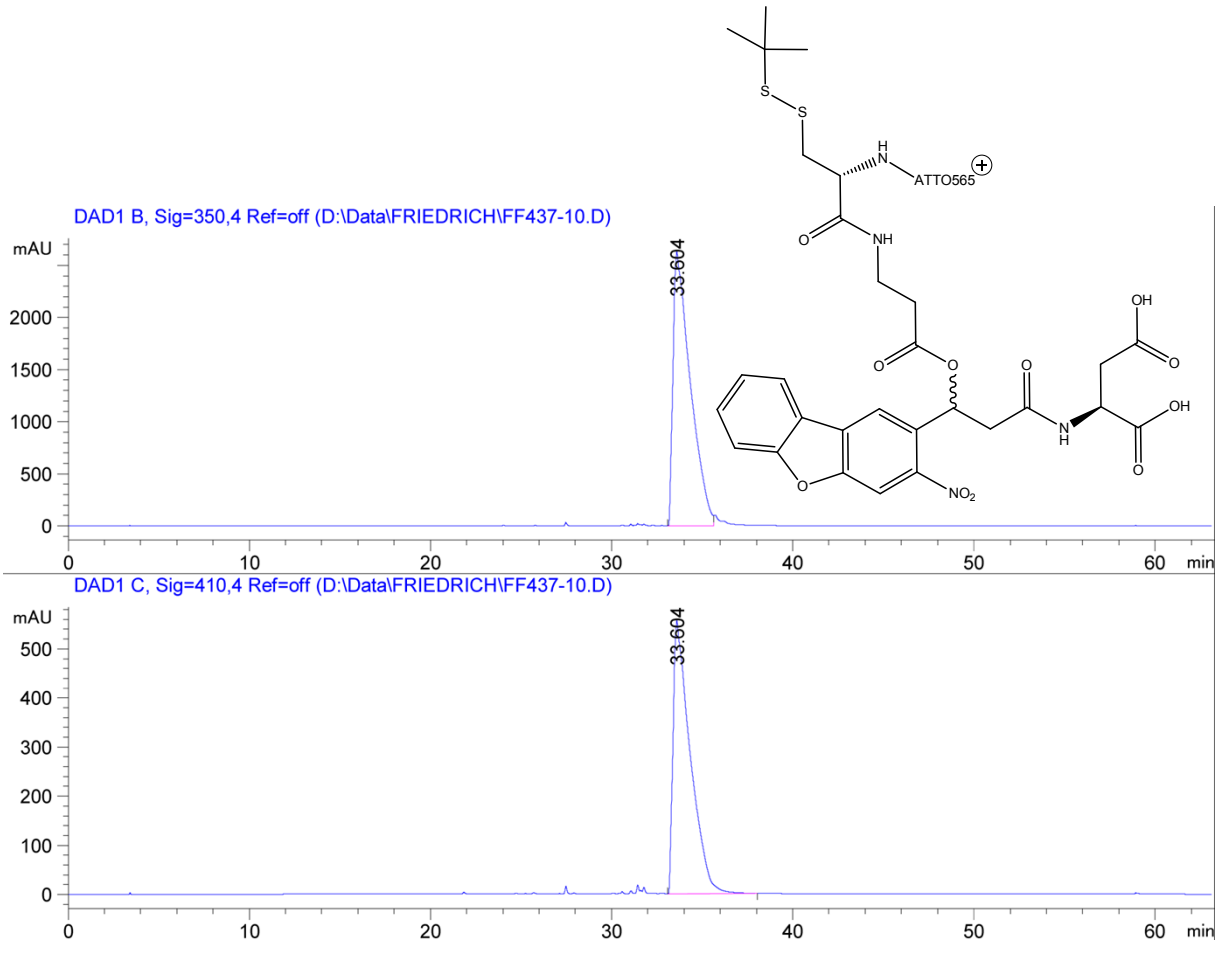

DAD1 B, Sig=350,4 Ref=off (D:\Data\FRIEDRICH\FF436-1.D)
mAU
23.612
24.171
min

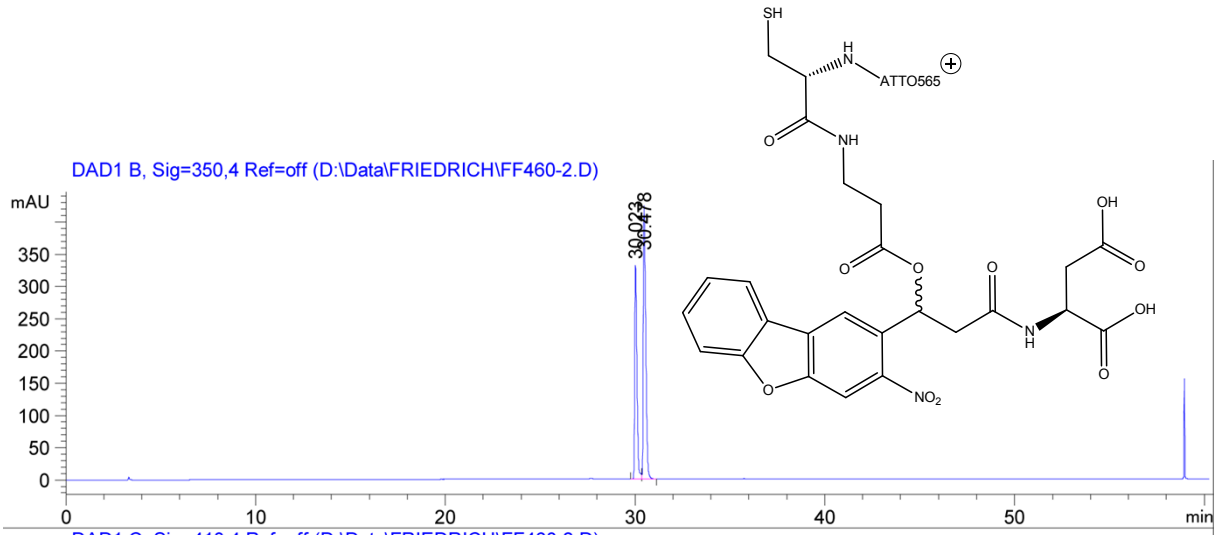

DAD1 A, Sig=254,4 Ref=off (D:\Data\FRIEDRICH\FF414-23.D)
mAU
Isocratic HPLC is necessary to separate the product peak (45.4 min) from impurities.
29.562
45.362
26.966
56.840
ATTO565
min

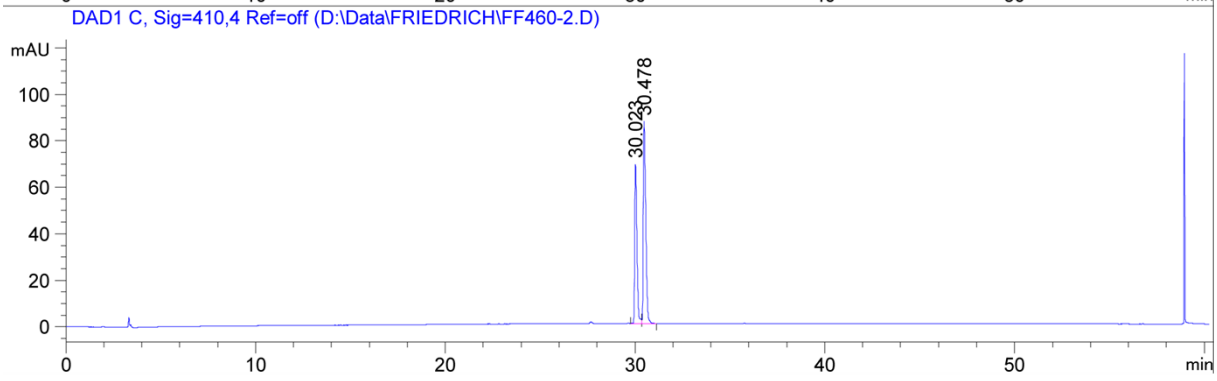

DAD1 D, Sig=565,4 Ref=off (D:\Data\FRIEDRICH\FF414-23.D)
mAU
29.562
45.361
26.966
56.839
min

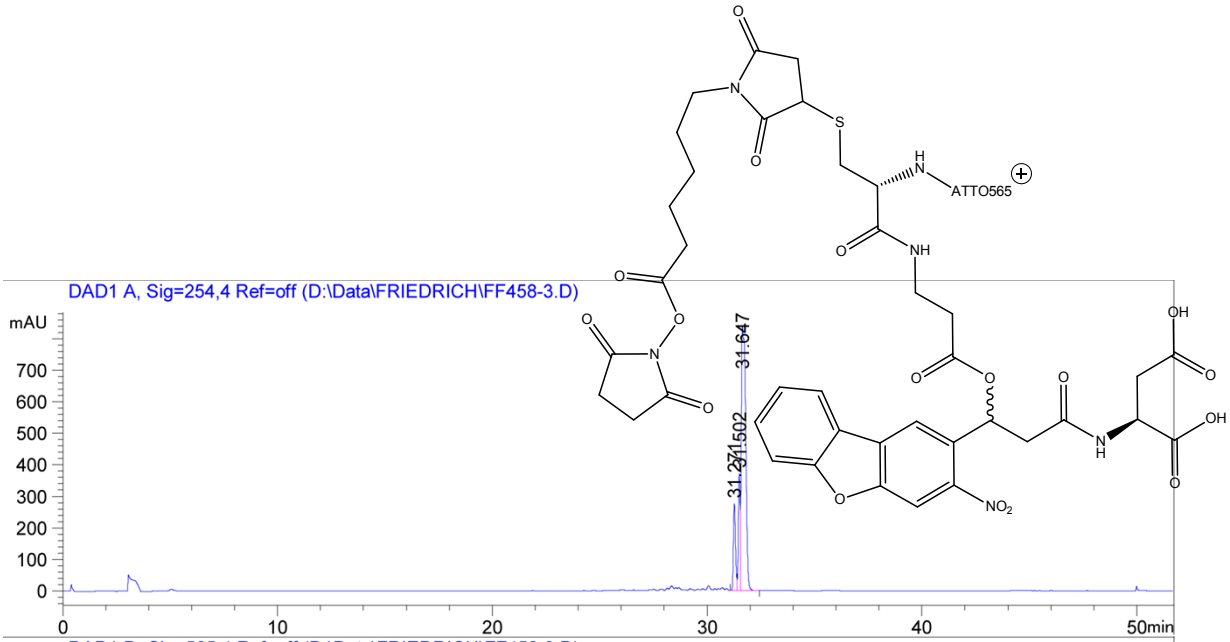


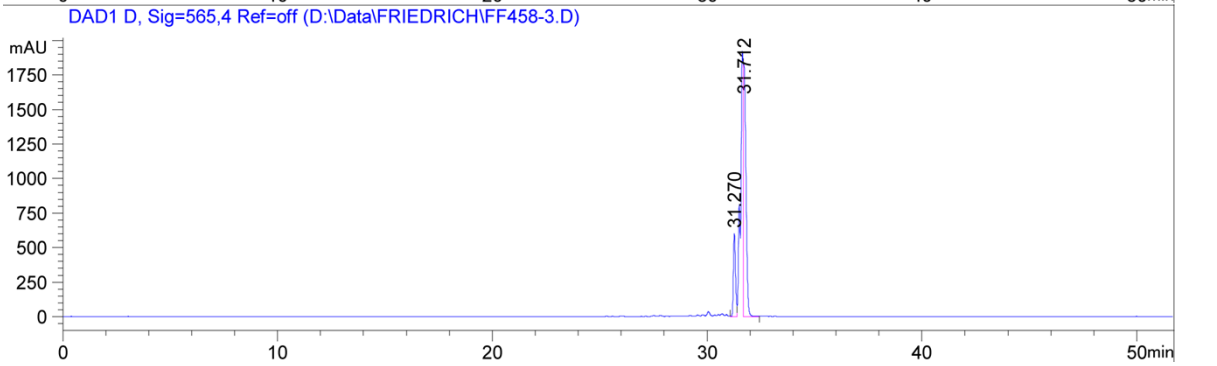
Analytical HPLC after purification with isocratic gradient.




DAD1 B, Sig=350,4 Ref=off (D:\Data\FRIEDRICH\FF460-2.D)
mAU
30.023
30.478
ATTO565
NO2
min


DAD1 C, Sig=410,4 Ref=off (D:\Data\FRIEDRICH\FF460-2.D)
mAU
30.023
30.478
min


DAD1 A, Sig=254,4 Ref=off (D:\Data\FRIEDRICH\FF458-3.D)
mAU
31.647
31.502
31.271
ATTO565
NO2
50min


DAD1 D, Sig=565,4 Ref=off (D:\Data\FRIEDRICH\FF458-3.D)
mAU
31.712
31.270
50min