Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information for:

Tailored photocleavable peptides: fragmentation and neutralization pathways in high vacuum

M. Debiossac,[a] J. Schätti,[b] M. Kriegleder,[a] P. Geyer,[a] A. Shayeghi,[a] M. Mayor,[c,d,e] M. Arndt,[a] and V. Köhler[b]

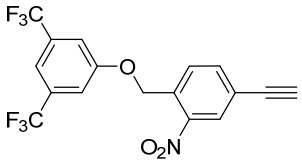
[a] Vienna Center for Quantum Science and Technology, Faculty of Physics, University of Vienna, Boltzmanngasse 5, A-1090 Vienna, Austria
[b] Department of Chemistry, University of Basel, Mattenstrasse 24a, BPR 1096, CH-4058 Basel
[c] Department of Chemistry, University of Basel, St. Johannsring 19, CH-4056 Basel, Switzerland
[d] Institute of Nanotechnology (INT), Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, D-76344 Eggenstein-Leopoldshafen, Germany
[e] Lehn Institute of Functional Materials (LIFM), Sun Yat-Sen University (SYSU), Xingang Rd. W., Guangzhou, China

Contents

Synthesis

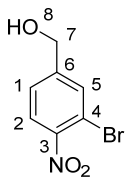
General

Chemicals were purchased from Sigma Aldrich, Fluorochem, Novabiochem or Bachem and used as received unless otherwise noted. UPLC experiments were performed with an Acquity UPLC-H Class Bio from Waters equipped with a PDA and a SQ detector 2 with the following column: ACQUITY UPLC, HSS T3 1.8 µm, 2.1 x 100 mm. Solvents were water and acetonitrile, respectively, each containing 0.1 % formic acid, later on referred to as (A) and (B). The flow rate was set to 0.61 ml/min and the temperature to 40 °C. Method 1: 0 min – 90 % A; 1 min – 90 % A; 6 min – 100 % B; 7.5 min – 100 % B. Method 2: 0 min – 100 % A; 1 min – 100 % A; 3 min – 80 % A; 13.5 min – 20 % A. Method 3: 0 min – 100 % A; 1 min – 100 % A; 7.5 min – 10 % A. Mass detection was performed in scan mode for positive ions (cone voltage 40 V, desolvation temperature: 600°C). A Water Prep LC 4000 System equipped with a Waters 2487: Dual λ Absorbance Detector was used for preparative separations with the following column: Agilent: XDB-C18, 21.2 x 150 mm, 5 µm. HRMS experiments were conducted with a Bruker maXis 4G. NMR experiments were performed at 25°C on Bruker Avance III NMR spectrometers operating at 250, 500 or 600 MHz proton frequency. The NMR spectrometers were equipped with inverse or direct observe, broadband probe heads or with a four-channel cryogenic QCI-F probe (600 MHz) all with self-shielded *z*-gradients. ^{13}C shifts were determined by 2D NMR experiments (HMBC and HMQC). ^{1}H and ^{13}C signals were assigned by 2D NMR experiments (COSY, HMBC, HMQC and ^{19}F-^{13}C HSQC for **1b**). Chemical shifts are reported in δ values (ppm) and are relative to the solvent residual signal (for samples in $CDCl_3$ ^{1}H = 7.26; ^{13}C = 77.0 ppm; for samples in DMSO-d_6 ^{1}H = 2.50 ppm; ^{13}C = 39.5 ppm). ^{19}F chemical shifts are referenced externally to CCl_3F in $CDCl_3$ (= 0 ppm).



S1

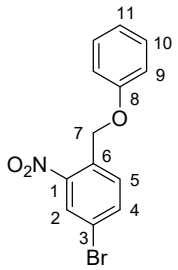
S1. The synthesis of the precursor alkyne for attachment of the photocleavable unit by click chemistry to the peptide followed a procedure published previously. [1]



S2

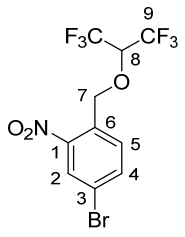
S2. 3-bromo-4-nitrobenzoic acid (800 mg, 3.25 mmol, 1.00 eq.) was dissolved in a solution of BH_3 in THF (1 M BH_3 in THF, 26.0 ml, 26.0 mmol, 8.00 eq.) and the resulting mixture heated to 50 °C for 4 h. The solvent was removed under reduced pressure and the residue subjected to column chromatography (cyclohexane /ethyl acetate 3:1). **S2** was isolated as a pale yellow solid (580 mg, 77 %). ^{1}H-NMR: (500 MHz, $CDCl_3$, 298 K) δ 7.85 (d, $^{3}J_{H\text{-}H}$ = 8.3 Hz, 1 H, H-2), 7.76 (m, 1 H, H-5), 7.43 (m, 1 H, H-1), 4.78 (d, $^{3}J_{H\text{-}H}$ = 5.5 Hz, 2 H, H-7), 2.03 (t, $^{3}J_{H\text{-}H}$ = 5.5 Hz, 1 H, H-8); δ ^{13}C (determined by HMQC and HMBC experiments) 148.5 (C-3), 146.9 (C-6), 132.6 (C-5), 125.8 (C-1 and C-2),

114.7 (C- 4), 63.2 (C-7); UPLC Method 1, T_R = 3.85 min; HRMS (ESI-) calcd. for $C_7H_5BrNO_3^-$ (M-H$^+$): 229.9458, found: 229.9461.



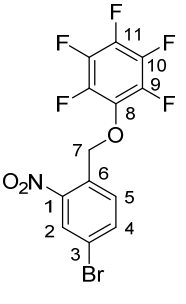
S3

S3. *General procedure 1.* The reaction was performed in analogy to the preparation of **S1** and under exclusion of light.[1] (3-Bromo-4-nitrophenyl)methanol (1.20 g, 5.17 mmol, 1.00 eq.), phenol (584 mg, 6.20 mmol, 1.20 eq.) and triphenylphosphine (1.63 g, 6.20 mmol, 1.20 eq.) were dissolved in dry THF and cooled to 0°C. Diisopropyl azodicarboxylate (1.52 ml, 7.76 mmol, 1.50 eq.) was added dropwise over 15 min. After completed addition the reaction was allowed to warm to room temperature and stirring continued overnight. For work up volatiles were removed under reduced pressure and the remaining residue subjected to column chromatography (cyclohexane /ethyl acetate 20:1). **S3** was isolated as a pale yellow solid (542 mg, 1.76 mmol, 34 %). ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K) δ 8.30 (d, $^4J_{H\text{-}H}$ = 2.1 Hz, 1 H, H-2), 7.99 (dd, $^3J_{H\text{-}H}$ = 8.3 Hz, $^4J_{H\text{-}H}$ = 2.1 Hz, 1 H, H-4), 7.72 (d, $^3J_{H\text{-}H}$ = 8.3 Hz, 1 H, H-5), 7.35-7.29 (m, 2 H, H-10), 7.02- 6.96 (m, 3 H, H-9 and H-11), 5.40 (s, 2 H, H-7); δ ^{13}C (determined by HMQC and HMBC experiments) 157.3 (C-8), 147.8 (C-1), 136.2 (C-4), 131.7 (C-6), 130.6 (C-5), 129.4 (C-10), 127.0 (C-2), 121.0 (C-9), 120.6 (C-3),114.4 (C-11), 65.5 (C-7); UPLC Method 1, T_R = 5.88 min; HRMS (ESI-) calcd. for $C_{13}H_9BrNO_3^-$ (M-H$^+$): 305.9771, found: 305.9775.



S4

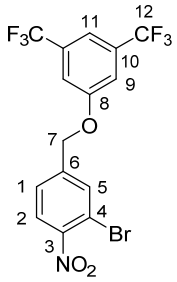
S4. The synthesis followed the protocol described for **S3** (*General procedure 1*) with 1,1,1,3,3,3-hexafluoropropan-2-ol (652 µl, 6.20 mmol, 1.20 eq.) instead of phenol. **S4** was obtained as a pale yellow solid and contained 21 wt% diisopropyl azodicarboxylate (DIAD) as judged by ^{1}H NMR (858 mg, 1.77 mmol, 34 % corrected yield). ^{1}H-NMR (500 MHz, $CDCl_3$, 298 K) δ 8.31 (d, $^4J_{H\text{-}H}$ = 2.0 Hz, 1 H, H-2), 7.84 (dd, $^3J_{H\text{-}H}$ = 8.4 Hz, $^4J_{H\text{-}H}$ = 2.0 Hz, 1 H, H-4), 7.71 (d, $^3J_{H\text{-}H}$ = 8.4 Hz, 1 H, H-5), 5.27 (s, 2 H, H-7), 4.34 (hept, $^3J_{H\text{-}F}$ = 5.8 Hz, 1 H, H-8); δ ^{13}C (determined by HMQC and HMBC experiments) 146.9 (C-1), 137.3 (C-4), 131.1 (C-6), 129.9 (C-5), 128.1 (C-2), 122.3 (C-3), 121.3 (q, C-9), 76.5 (C-8), 72.7 (C-7); UPLC Method 1, T_R = 5.79 min; HRMS (ESI-) calcd. for $C_{10}H_5BrF_6NO_3^-$ (M-H$^+$): 379.9362, found: 379.9368.



S5

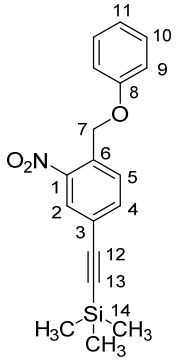
S5. The synthesis followed the protocol described for **S3** (*General procedure 1*) with pentafluorophenol (1.14 g, 6.20 mmol, 1.20 eq.) instead of phenol and has been previously reported.[2] **S5** was isolated as a slightly yellow solid (925 mg, 2.32 mmol, 45 %). ^{1}H-NMR (500 MHz, $CDCl_3$, 298 K) δ 8.32 (d, $^4J_{H\text{-}H}$ = 1.4 Hz, 1 H, H-2), 7.89-7.85 (m, 2 H, H-4 and H-5), 5.54 (s, 2 H, H-7); δ ^{13}C (determined by HMQC and HMBC experiments, C-9, C-10 and C-11 were assigned in analogy to compound **1b**) 147.0 (C-1), 143.8 (C-11), 140.0 (C-9 or C-10), 137.5 (C-4), 136.2 (C-9 or C-10), 133.0 (C-8), 131.4 (C-6), 130.1 (C-5),

128.1 (C-2), 122.3 (C-3), 73.0 (C-7); ^{19}F-NMR (235 MHz, $CDCl_3$, 298 K) δ -156.1 (m, 2 F, 9-F), -161.7 (m, 1 F, 11-F), -162.5 (m, 2 F, 10-F); UPLC Method 1, T_R = 6.09 min; HRMS (ESI-) calcd. for $C_{13}H_4BrF_5NO_3^-$ (M-H$^+$): 395.9300, found: 395.9304.



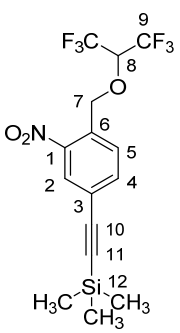
S6

S6. The synthesis followed the protocol described for **S3** (*General procedure 1*) starting with (3-bromo-4-nitrophenyl)methanol (541 mg, 2.33 mmol, 1.00 eq.) and 3,5-bis(trifluoromethyl)phenol (643 mg, 2.80 mmol, 1.20 eq). **S6** was isolated as a pale yellow solid (592 mg, 1.33 mmol, 57 %). ^{1}H-NMR (500 MHz, $CDCl_3$, 298 K) δ 7.91 (d, $^3J_{H\text{-}H}$ = 8.3 Hz, 1 H, H-2), 7.87-7.86 (m, 1 H, H-5), 7.55-7.52 (m, 2 H, H-1 and H-11), 7.41 (s, 2 H, H-9), 5.20 (s, $^3J_{H\text{-}H}$ = 5.5 Hz, 2 H, H-7); δ ^{13}C (determined by HMQC and HMBC experiments) 158.3 (C-8), 149.3 (C-3), 141.3 (C-6), 133.4 (C-5), 133.2 (C-10), 126.5 (C-1), 126.0 (C-2), 123.0 (q, C-12), 115.1 (C-4), 115.4 (C-11), 115.1 (C-9), 68.5 (C-7); UPLC-MS: Method 1, T_R = 6.24 min; m/z MS (ES-): 444.8, 442.7 [100%, M$^-$], 229.1 [40 %, $(CF_3)_2C_6H_3O^-$]; HRMS (ESI-) calcd. for $C_{15}H_7BrF_6NO_3^-$ (M-H$^+$): 441.9519, found: 441.9520.



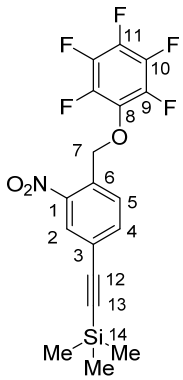
S7

S7. *General procedure 2*. The synthesis followed the procedure reported for the preparation of **S1**, but employed a twofold loading of catalyst.[1] The reaction flask was wrapped in aluminium foil to exclude light before 4-bromo-2-nitro-1-(phenoxymethyl)benzene (400 mg, 1.30 mmol, 1.00 eq.) was dissolved in triethylamine (15 ml). The solution was degassed by bubbling with nitrogen for 20 min before CuI (5.0 mg, 2 mol% and tetrakis-(triphenylphosphine)palladium (15 mg, 1 mol%) were added and the reaction mixture degassed for another 10 min. Trimethylsilylacetylene (277 µl, 2.00 mmol, 1.54 eq.) was added and the reaction stirred under reflux overnight. After filtration through Celite, *tert*-butyl methyl ether (100 ml) was added and the solution was washed with water (2 × 100 ml) and brine (100 ml). Volatiles were removed under reduced pressure and the residue purified by column chromatography (cyclohexane / ethyl acetate 40 : 1). **S7** was isolated as a slightly yellow solid which contained 32 wt% starting material **S3** (253 mg, 0.53 mmol, 41 % corrected yield). ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K) δ 8.13 (d, $^4J_{H\text{-}H}$ = 1.7 Hz, 1 H, H-2), 7.83 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^4J_{H\text{-}H}$ = 1.7 Hz, 1 H, H-4), 7.78 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-5), 7.33-7.28 (m, 2 H, H-10), 7.03-6.96 (m, 3 H, H-9 and H-11), 5.45 (s, 2 H, H-7), 0.26 (s, 9 H, H-14); δ ^{13}C (determined by HMQC and HMBC experiments) 157.4 (C-8), 147.0 (C-1), 136.1 (C-4), 133.0 (C-6), 129.4 (C-10), 129.2 (C-5), 127.2 (C-2), 122.3 (C-3), 121.0 (C-9), 114.5 (C-11), 101.9 (C-12), 96.9 (C-13), 65.7 (C-7), -0.7 (C-14); UPLC Method 1, T_R = 6.66 min; HRMS (ESI-) calcd. for $C_{18}H_{18}NO_3Si^-$ (M-H$^+$): 324.1061, found: 324.1065.



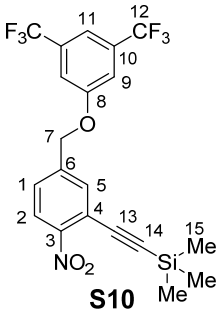
S8

S8. The synthesis followed the protocol described for the synthesis of **S7** (*General procedure 2*) employing **S4** (250 mg, 654 μmol, 1.00 eq., the DIAD impurity was not considered in the calculation). **S8** was isolated as a pale yellow solid 89 mg, 0.22 mmol, 34 %. ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K) δ 8.13 (d, $^{4}J_{H\text{-}H}$ = 1.7 Hz, 1 H, H-2), 7.89 (dd, $^{3}J_{H\text{-}H}$ = 8.1 Hz, $^{4}J_{H\text{-}H}$ = 1.7 Hz, 1 H, H-4), 7.72 (d, $^{3}J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-5), 5.77 (hept, $^{3}J_{H\text{-}F}$ = 6.4 Hz, 1 H, H-8), 5.30 (s, 2 H, H-7), 0.26 (s, 9 H, H-12); δ ^{13}C (determined by HMQC and HMBC) 146.9 (C-1), 136.5 (C-4), 132.0 (C-6), 129.0 (C-5), 127.3 (C-2), 122.8 (C-3), 121.6 (q, C-9), 102.0 (C-10), 97.3 (C-11), 74.0 (C-8), 71.7 (C-7), -0.7 (C-12); UPLC Method 1, T_R = 6.53 min; HRMS (ESI-) calcd. for $C_{15}H_{14}F_6NO_3Si^-$ (M-H$^+$): 398.0653, found: 398.0655.



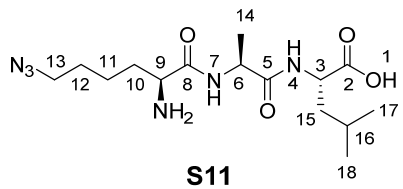
S9

S9. The synthesis followed the protocol for the synthesis of **S7** (*General procedure 2*) employing **S5** (250 mg, 628 μmol, 1.00 eq.). **S9** was isolated as a pale yellow solid (106 mg, 0.26 mmol, 39 %); ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K) δ 8.15 (d, $^{4}J_{H\text{-}H}$ = 1.7 Hz, 1 H, H-2), 7.91 (dd, $^{3}J_{H\text{-}H}$ = 8.0 Hz, $^{4}J_{H\text{-}H}$ = 1.7 Hz, 1 H, H-4), 7.86 (d, $^{3}J_{H\text{-}H}$ = 8.0 Hz, 1 H, H-5), 5.61 (s, 2 H, H-7), 0.26 (s, 9 H, H-14); ^{13}C NMR (126 MHz, DMSO-d_6, 298 K, C-9, C-10, C-11 were assigned in analogy to compound **1b**) 146.9 (C-1), 142.1 (C-9 or C-10), 140.1 (C-9 or C-10), 136.5 (C-4), 136.4 (C-11), 132.4 (C-8), 131.4 (C-6), 129.8 (C-5), 127.3 (C-2), 123.0 (C-3), 101.7 (C-12), 97.4 (C-13), 72.6 (C-7), -0.7 (C-14); UPLC Method 1, T_R = 6.78 min; HRMS (ESI-) calcd. for $C_{18}H_{13}F_5NO_3Si^-$ (M-H$^+$): 414.0590, found: 414.0592.



S10

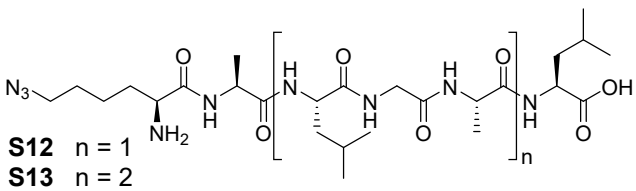
S10. The synthesis followed the protocol for the synthesis of **S7** (*General procedure 2*) employing **S6** (200 mg, 0.450 mmol, 1.00 eq.). **S10** was isolated as a pale yellow solid (126 mg, 0.27 mmol, 61 %). ^{1}H-NMR (500 MHz, $CDCl_3$, 298 K) δ 8.08 (d, $^{3}J_{H\text{-}H}$ = 8.5 Hz, 1 H, H-2), 7.75-7.73 (m, 1 H, H-5), 7.54 (s, 1 H, H-11), 7.53-7.50 (m, 1 H, H-1), 7.40 (s, 2 H, H-9), 5.18 (s, 2 H, H-7), 0.29 (s, 9 H, H-15); δ ^{13}C (determined by HMQC and HMBC experiments) 158.5 (C-8), 149.7 (C-3), 140.6 (C-6), 133.4 (C-5), 133,0 (C-10), 127.1 (C-1), 125.1 (C-2), 123.0 (q, C-12), 119.0 (C-4), 115.3 (C-11), 115.1 (C-9), 104.7 (C-14), 98.6 (C-13), 68.8 (C-7), -0.5 (C-15); UPLC-MS: Method 1, T_R = 6.78 min; m/z MS (ES-): 460.9 [100%, M - H$^+$], 229.5 [80%, $(CF_3)_2C_6H_3O^-$]; HRMS (ESI-) calcd. for $C_{20}H_{16}F_6NO_3Si^-$ (M-H$^+$):460.0809, found: 460.0817.



S11

S11. 2-Chlorotrityl chloride resin (1 mmol/g, 700 mg) was placed in a plastic syringe equipped with a filter frit. The resin was washed with CH_2Cl_2 (ca. 4 ml). Subsequently the syringe was filled with CH_2Cl_2 (ca. 4 ml) and placed on a shaker for 15 min. The CH_2Cl_2 was exchanged and DIPEA (150 µl, 0.908 mmol, 2.00 eq.), and Fmoc-Leu-OH (160 mg, 0.453 mmol, 1.00 eq.) were added.

After the sample was agitated on the shaker for 1.5 h the liquid phase was removed by filtration and the resin washed with CH_2Cl_2 (ca. 4 ml) and DMF (ca. 4 ml). The unreacted binding sites on the resin were capped by adding a basic MeOH-solution in dichloromethane (ca. 5 ml, 80 % CH_2Cl_2; 15 % MeOH, 5 % *N*,*N*-diisopropylethylamine) followed by 15 min agitation. Subsequently the sample was washed with CH_2Cl_2 (ca. 4 ml) and DMF (ca. 4 ml). For Fmoc deprotection a solution of piperidine in DMF (20 % piperidine, ca. 4 ml) was added and shaking continued for 5 mins. This step was repeated three times. Note: In the following, this procedure will be referred to as *Fmoc deprotection*. The sample was washed with DMF (ca. 4 ml) and CH_2Cl_2 (ca. 4 ml) and again with DMF (ca. 4 ml). Between the different washing steps the sample was agitated on a shaker for ca. 30 sec. Note: In the following this procedure will be referred to as *washing.* After the *washing*, DMF (ca. 4 ml), *N*,*N*-diisopropylethylamine (750 µl, 4.54 mmol, 10.0 eq.), Fmoc-Ala-OH (705 mg, 2.26 mmol, 5.00 eq.) and PyBOP (1.18 g, 2.27 mmol, 5.01 eq.) were added. The mixture was agitated on a shaker for 2 h. Note: In the following this procedure will be referred to as *peptide coupling*. This was followed by *washing*, *Fmoc deprotection* and another *washing*. For the next peptide coupling step DIPEA (300 µl, 1.81 mmol, 4.01 eq.), Fmoc-azidolysine (411 mg, 0.906 mmol, 2.00 eq.) and PyBOP (471 mg, 0.905 mmol, 2.00 eq.) were used. After *washing*, *Fmoc- deprotection* and another *washing* the resin was rinsed with CH_2Cl_2 (3 × ca. 4 ml) before a cleavage solution of trifluoro acetic acid, triisopropylsilane and water (5 ml, 92 % TFA, 5 % triisopropylsilane, 3 % H_2O) was added. For the cleavage procedure, the mixture was agitated on the shaker for 1 h. Subsequently the cleavage solution was removed and the resin washed with TFA (1 ml). The TFA containing filtrates were combined and concentrated under reduced pressure to ca. 0.5 ml. Ice cold Et_2O (50 ml) was added and the formed precipitate was collected by centrifugation (4400 rpm). The precipitated trifluoroacetate salt was washed with Et_2O (3 × 20 ml) and obtained after drying as a white powder (108 mg, 0.230 mmol, 51 %). ^{1}H-NMR: (500 MHz, DMSO-d_6, 298 K) δ 8.56 (d, $^3J_{H\text{-}H}$ = 7.7 Hz, 1 H, H-7), 8.16 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-4), 4.39 (quint, $^3J_{H\text{-}H}$ = 7.1 Hz, 1 H, H-6), 4.21 (q, $^3J_{H\text{-}H}$ = 7.9 Hz, 1 H, H-3), 3.71 (t, $^3J_{H\text{-}H}$ = 6.5 Hz, 1 H, H-9), 3.35-3.25 (m, 2 H, H-13), 1.72-1.58 (m, 3 H, H-10 and H-16), 1.54-1.45 (m, 4 H, H-12 and H-15), 1.35 (quint, $^3J_{H\text{-}H}$ = 7.2 Hz, 2 H, H-11), 1.24 (d, $^3J_{H\text{-}H}$ = 7.0 Hz, 3 H, H-14), 0.88 (d, $^3J_{H\text{-}H}$ = 6.5 Hz, 3 H, H-17 or H-18), 0.83 (d, $^3J_{H\text{-}H}$ = 6.5 Hz, 3 H, H-17 or H-18); ^{13}C NMR (126 MHz, DMSO-d_6, 298 K) 173.7 (C-2), 171.3 (C-5), 168.1 (C-8), 51.7 (C-9), 50.1 (C-13), 49.9 (C-3), 47.5 (C-6), 40.0 (C-15), 30.5 (C-10), 27.5 (C-12) 24.3 (C-16), 22.6 (C-17 or C-18), 21.2 (C-11), 21.1 (C-17 or C-18), 18.0 (C-14); UPLC-MS Method 2, T_R = 4.88 min; m/z MS (ESI+): 357.3 [100 %, M + H^+], 226.3 [65 %]; HRMS (ESI+) calcd. for $C_{15}H_{29}F_6O_4^+$ ($M+H^+$): 357.2245, found: 357.2249.



S12 n = 1
S13 n = 2
S14 n = 3

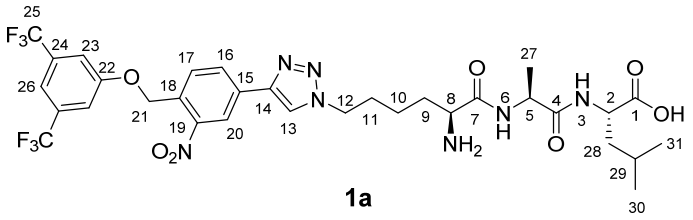
General procedure 3. 2-chlorotrityl chloride resin (1 mmol/g, 200 mg) was loaded with Fmoc-Leu-OH (18 mg, 51 μmol, 1.0 eq.) and unreacted binding sites capped as described above. Peptide coupling steps were performed by means of a peptide synthesizer (Intavis multi pep RSI) with Fmoc-amino acids (5.00 eq.), PyBOP (5.00 eq.), and DIPEA (5.00 eq.) in DMF (1.6 ml) at room temperature, with a reaction time of 45 min followed by a 2nd coupling of 90 min duration and identical concentrations. Fmoc-deprotection was performed with piperidine (20 % in DMF, 0.8 ml , 4 × 5 min) after the syringes were washed with DMF (5 × 1 ml). The coupling of Fmoc-azidolysine was performed with 1.50 eq. of Fmoc-amino acids, PyBOP and *N*,*N*-diisopropylethylamine in DMF (1.6 ml) for 2 h followed by another 8 h after exchange of the coupling reagents. In the end the resin was washed CH_2Cl_2 (5 × 1.6 ml) before cleavage. Cleavage, precipitation and washing in Et_2O were performed as described for **S11**. The trifluoroacetate salts of the products were isolated as white solids.

S12. 25 mg (contains ca. 5 wt% Et_2O, 65 % corrected yield). ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K) δ 8.60 (d, $^3J_{H\text{-}H}$ = 7.4 Hz, 1 H, NH), 8.16- 8.06 (m, 5 H, NH and NH_2), 7.88 (d, $^3J_{H\text{-}H}$ = 7.6 Hz, 1 H, NH), 4.40 (quint, $^3J_{H\text{-}H}$ = 7.1 Hz, 1 H, H-C_α), 4.35-4.26 (m, 2 H, H-C_α), 4.19 (m, 1 H, H-C_α), 3.82-3.62 (m, 3 H, H-C_α), 3.33-3.28 (m, 2 H, H-$C_{\varepsilon(Lys)}$), 1.71-1.45 (m, 10 H, H-$C_{\beta(Lys)}$, H-$C_{\delta\,(lys)}$, H-$C_{\beta(leu)}$, H-$C_{\gamma(leu)}$), 1.39-1.31 (m, 2 H, H-$C_{\gamma(lys)}$), 1.24 (d, $^3J_{H\text{-}H}$ = 7.0 Hz, 3 H, H-$C_{\beta(ala)}$), 1.20 (d, $^3J_{H\text{-}H}$ = 7.0 Hz, 3 H, H-$C_{\beta(ala)}$), 0.89 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3 H, H-$C_{\delta(leu)}$), 0.87 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3 H, 0.83 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3 H, H-$C_{\delta(leu)}$), 0.83 (d, $^3J_{H\text{-}H}$ = 6.4 Hz, 3 H, H-$C_{\delta(leu)}$). UPLC-MS Method 3, T_R = 4.18 min; m/z MS (ES+): 598.2 [100 %, M + H^+], 299.7 [60 %, M + 2 H^+]; HRMS (ESI+) calcd. for $C_{26}H_{48}N_9O_7^+$ (M+H^+): 598.3671, found: 598.3681.

S13. 30 mg (62 %). ^{1}H-NMR: (500 MHz, DMSO-d_6, 298 K): 8.60 (d, $^3J_{H\text{-}H}$ = 7.4 Hz, 1 H, amide NH), 8.16- 8.03 (m, 6 H, 4 amide NH and amine NH_2), 7.99 (d, $^3J_{H\text{-}H}$ = 7.9 Hz, 1 H, amide NH), 7.95 (d, $^3J_{H\text{-}H}$ = 7.2 Hz, 1 H, amide NH), 7.84 (d, $^3J_{H\text{-}H}$ = 7.5 Hz, 1 H, amide NH), 4.40 (quint., $^3J_{H\text{-}H}$ = 7.1 Hz, 1 H, H-C_α), 4.35-4.16 (m, 5 H, H-C_α), 3.81-3.62 (m, 5 H, H-C_α), 3.34-3.25 (m, 2 H, H-$C_{\varepsilon(Lys)}$), 1.71-1.43 (m, 13 H, H-$C_{\beta(lys)}$, H-$C_{\delta\,(lys)}$, H-$C_{\beta(leu)}$, H-$C_{\gamma(leu)}$), 1.39-1.31 (m, 2 H, H-$C_{\gamma(lys)}$), 1.24 (d, $^3J_{H\text{-}H}$ = 7.0 Hz, 3 H, H-$C_{\beta(ala)}$), 1.21 (d, $^3J_{H\text{-}H}$ = 7.0 Hz, 3 H, H-$C_{\beta(ala)}$), 1.20 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 3 H, H-$C_{\beta(ala)}$), 0.90-0.86 (m, 9 H, H-$C_{\delta(leu)}$), 0.85-0.83 (m, 9 H, H-$C_{\delta(leu)}$); UPLC-MS Method 3, T_R = 4.51 min; m/z MS (ES+): 839.2 [100 %, M + H^+], 420.4 [60 %, M + 2 H^+]; HRMS (ESI+) calcd. for $C_{37}H_{67}N_{12}O_{10}^+$ (M+H^+): 839.5098, found: 839.5096.

S14. 36 mg (contains ca. 4 wt% Et_2O, 57 % corrected yield). ^{1}H-NMR: (500 MHz, DMSO-d_6, 298 K): 8.59 (d, $^3J_{H\text{-}H}$ = 7.5 Hz, 1 H, amide NH), 8.14- 7.82 (m, 12 H, 10 amide NH and amine NH_2), 4.41 (quint, $^3J_{H\text{-}H}$ = 7.1

Hz, 1 H, H-C_α), 4.35-4.16 (m, 7 H, H-C_α), 3.80-3.62 (m, 7 H, H-C_α), 3.34-3.25 (m, 2 H, H-$C_{\varepsilon(Lys)}$), 1.71-1.43 (m, 16 H, H-$C_{\beta(lys)}$, H-$C_{\delta\ (lys)}$, H-$C_{\beta(leu)}$, H-$C_{\gamma(leu)}$), 1.39-1.31 (m, 2 H, H-$C_{\gamma(lys)}$), 1.24 (d, $^3J_{H\text{-}H}$ = 7.0 Hz, 3 H, H-$C_{\beta(ala)}$), 1.22-1.18 (m, 9 H, H-$C_{\beta(ala)}$), 0.90-0.86 (m, 12 H, H-$C_{\delta(leu)}$), 0.85-0.83 (m, 12 H, H-$C_{\delta(leu)}$); UPLC-MS Method 3, T_R = 4.78 min; m/z MS (ES+): 1080.2 [100 %, M + H^+], 540.9 [60 %, M + 2 H^+]; HRMS (ESI+) calcd. for $C_{48}H_{86}N_{15}O_{13}^+$ (M+H^+): 1080.6524, found: 1080.6516.

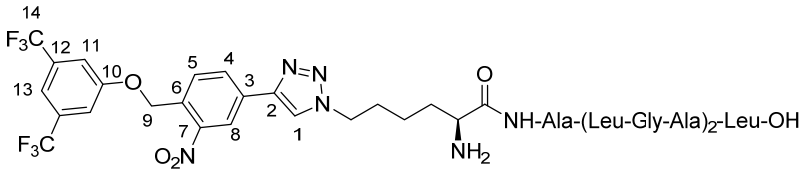


1a

1a. *General procedure 4*. To a suspension of **S1** (43.6 mg, 112 μmol, 1.00 eq.) and the trifluoroacetate salt of **S11** (40.0 mg, 85.1 μmol, 0.760 eq.) in water / *tert*-butanol (1 : 1, 10 ml) was added aq. sodium ascorbate (1 M, 400 μl, 3.57 eq.) and aq. $CuSO_4$ (1 M, 40 μl, 0.36 eq.) and the resulting mixture was stirred for 4 h at 55 °C. Subsequently volatiles were removed under reduced pressure and the resulting residue was purified by reversed phase HPLC. The trifluoroacetate salt of the **1a** was obtained as a white powder after lyophilisation (22 mg, 23 %). ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K) δ 8.91 (s, 1 H, H-13), 8.59 (d, $^4J_{H\text{-}H}$ = 1.7 Hz, 1 H, H-20), 8.25 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^4J_{H\text{-}H}$ = 1.7 Hz, 1 H, H-16), 8.24 (d, $^3J_{H\text{-}H}$ = 7.0, 1 H, H-6), 7.94 (d, $^3J_{H\text{-}H}$ = 8.3 Hz, 1 H, H-3), 7.92 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-17), 7.76 (s, 2 H, H-23), 7.70 (s, 1 H, H-26), 5.69 (s, 2 H, H-21), 4.42 (t, $^3J_{H\text{-}H}$ = 7.0 Hz, 2 H, H-12), 4.30 (quint, $^3J_{H\text{-}H}$ = 7.0 Hz, 1 H, H-5), 4.10 (td, $^3J_{H\text{-}H}$ = 8.2 Hz, $^3J_{H\text{-}H}$ = 5.7 Hz, 1 H, H-2), 3.29 (t, $^3J_{H\text{-}H}$ = 6.2 Hz, 1 H, H-8), 1.88 (quint, $^3J_{H\text{-}H}$ = 7.1 Hz, 2 H, H-11), 1.71-1.62 (m, 1 H, H-9), 1.65-1.57 (m, 1 H, H-29), 1.52-1.43 (m, 3 H, H-9, H-28), 1.37-1.29 (m, 2 H, H-10), 1.19 (d, $^3J_{H\text{-}H}$ = 7.0 Hz, 3 H, H-27), 0.85 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3 H, H-30 or H-31), 0.82 (d, $^3J_{H\text{-}H}$ = 6.5 Hz, 3 H, H-30 or H-31); δ ^{13}C (determined by HMQC and HMBC experiments) 173.6 (C-1), 171.4 (C-4), 158.6 (C-22), 147.8 (C-19), 143.6 (C-14), 132.6 (C-24), 132.0 (C-15), 130.2 (C-18), 130.1 (C-17), 129.8 (C-16), 122.9 (q, C-25), 122.6 (C-13), 120.7 (C-20), 115.8 (C-23), 114.3 (C-26), 67.1 (C-21), 53.5 (C-8), 50.8 (C-2), 49.3 (C-12), 47.6 (C-5), 40.4 (C-28), 33.1 (C-9), 29.1 (C-11), 24.2 (C-29), 22.8 (C-30 or C-31), 21.6 (C-10), 21.5 (C-30 or C-31), 18.2 (C-27), (C-7 was not detected); UPLC-MS Method 3, T_R = 6.10 min; m/z MS (ES+): 746.1 [100 %, M + H^+], 394.5 [40 %]; HRMS (ESI+) calcd. for $C_{32}H_{38}F_6N_7O_7^+$ (M+H^+): 746.2731, found: 746.2737.

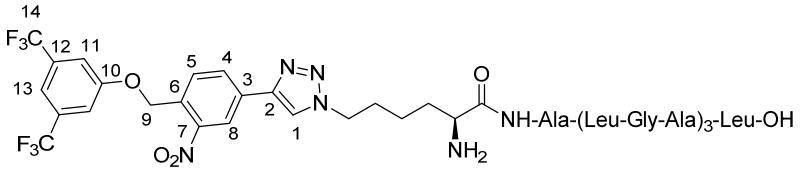
2a. The synthesis followed the protocol for the synthesis of **1a** (*General procedure 4*) starting with **S1** (23 mg, 50 μmol, 1.0 eq.) and the trifluoroacetate salt of **S12** (30 mg, 40 μmol, 0.80 eq.). The modified peptide was isolated as a white solid (8 mg, 7 μmol, 18 %). ^{1}H-NMR (600 MHz, DMSO-d_6, 298 K) δ 12.51 (br, 1 H, COOH), 8.83 (s, 1 H, H-1), 8.60-8.57 (m, 2 H, H-8 and amide NH), 8.24 (dd, $^3J_{H\text{-}H}$ = 8.0 Hz, $^4J_{H\text{-}H}$ = 1.8 Hz, 1 H, H-4), 8.16- 8.04 (m, 5 H, 3 amide NH, amine NH_2), 7.93 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-5), 7.89 (d, $^3J_{H\text{-}H}$ = 7.5 Hz, 1 H, amide NH), 7.76 (s, 2 H, H-11), 7.71 (s, 1 H, H-13), 5.70 (s, 2 H, H-9), 4.44-4.35 (m, 3 H, 2 H-$C_{\varepsilon(Lys)}$ and H-C_α), 4.35-4.26 (m, 2 H, H-C_α), 4.19 (m, 1 H, H-C_α), 3.77-3.62 (m, 3 H, H-C_α), 1.89 (quint, $^3J_{H\text{-}H}$ = 7.3 Hz, 2 H,

H-$C_{\delta\,(lys)}$), 1.75-1.69 (m, 2 H, H-$C_{\beta(lys)}$), 1.66-1.57 (m, 2 H, H-$C_{\gamma(leu)}$), 1.56-1.43 (m, 4 H, H-$C_{\beta(leu)}$), 1.38-1.31 (m, 2 H, H-$C_{\gamma(lys)}$), 1.24 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 3 H, H-$C_{\beta(ala)}$), 1.19 (d, $^3J_{H\text{-}H}$ = 7.2 Hz, 3 H, H-$C_{\beta(ala)}$), 0.89-0.85 (m, 6 H, H-$C_{\delta(leu)}$), 0.83-0.81 (m, 6 H, H-$C_{\delta(leu)}$).UPLC-MS: Method 3, T_R = 6.12 min; m/z MS (ES+): 987.0 [100 %, M + H^+], 856.4 [40 %], 494.1 [40 %, M + 2 H^+]; HRMS (ESI+) calcd. for $C_{43}H_{57}F_6N_{10}O_{10}^+$ (M+H^+): 987.4158, found: 987.4172.



3a

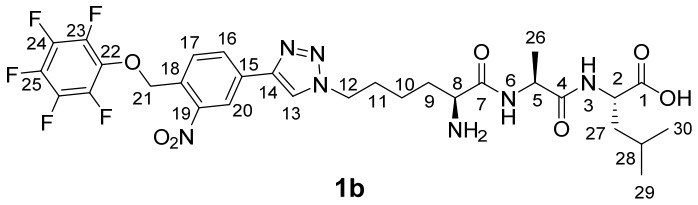
3a. The synthesis followed the protocol for the synthesis of **1a** (*General procedure 4*) starting with **S1** (23 mg, 50 μmol, 1 eq.) and the trifluoroacetate salt of **S13** (42 mg, 44 μmol, 0.88 eq.). The trifluoroacetate salt of the modified peptide was isolated as a white solid (7 mg, 5 μmol, 12 %). ^{1}H-NMR (600 MHz, DMSO-d_6, 298 K) δ 12.44 (br, 1 H, COOH), 8.83 (s, 1 H, H-1), 8.60-8.57 (m, 2 H, H-8 and amide NH), 8.24 (dd, $^3J_{H\text{-}H}$ = 8.0 Hz, $^4J_{H\text{-}H}$ = 1.8 Hz, 1 H, H-4), 8.11- 8.03 (m, 6 H, 4 amide NH, amine NH_2), 8.00 (d, $^3J_{H\text{-}H}$ = 7.9 Hz, 1 H, amide NH), 7.96 (d, 1 H, $^3J_{H\text{-}H}$ = 7.2 Hz, amide NH), 7.93 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-5), 7.85 (d, $^3J_{H\text{-}H}$ = 7.5 Hz, 1 H, amide NH), 7.76 (s, 2 H, H-11), 7.71 (s, 1 H, H-13), 5.70 (s, 2 H, H-9), 4.44-4.36 (m, 3 H, 2 H-$C_{\varepsilon(Lys)}$ and H-C_α), 4.35-4.14 (m, 5 H, H-C_α), 3.77-3.62 (m, 5 H, H-C_α), 1.89 (quint, $^3J_{H\text{-}H}$ = 7.1 Hz, 2 H, H-$C_{\delta\,(lys)}$), 1.76-1.60 (m, 2 H, H-$C_{\beta(lys)}$), 1.66-1.57 (m, 3 H, H-$C_{\gamma(leu)}$), 1.54-1.42 (m, 6 H, H-$C_{\beta(leu)}$), 1.40-1.32 (m, 2 H, H-$C_{\gamma(lys)}$), 1.24 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 3 H, H-$C_{\beta(ala)}$), 1.20 (d, $^3J_{H\text{-}H}$ = 7.2 Hz, 6 H, H-$C_{\beta(ala)}$), 0.89-0.85 (m, 9 H, H-$C_{\delta(leu)}$), 0.83-0.81 (m, 9 H, H-$C_{\delta(leu)}$); UPLC-MS Method 3, T_R = 6.47 min; m/z MS (ES+): 1250.1 [40 %, M + Na^+], 1228.2 [100 %, M + H^+], 614.9 [40 %, M + 2 H^+]; HRMS (ESI+) calcd. for $C_{54}H_{76}F_6N_{13}O_{13}^+$ (M+H^+): 1228.5584, found: 1228.5582.



4a

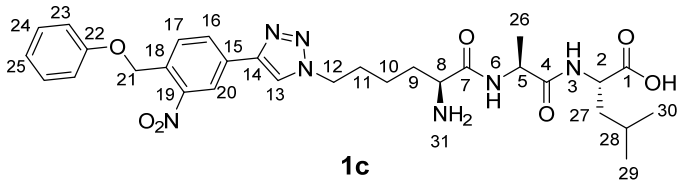
4a. The synthesis followed the protocol for the synthesis of **1a** (*General procedure 4*) starting with **S1** (14 mg, 30 μmol, 1.0 eq.) and the trifluoroacetate salt of **S14** (32 mg, 26 μmol, 0.86 eq.). The trifluoroacetate salt of the modified peptide was isolated as a white solid (6.9 mg, 17 %). The modified peptide was isolated as a white solid (6.9 mg, 9.0 %). ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K) δ 12.51 (br, 1 H, COOH), 8.83 (s, 1 H, H-1), 8.59 (m, 2 H, H-8 and amid), 8.24 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^4J_{H\text{-}H}$ = 1.7 Hz, 1 H, H-4), 8.14- 8.02 (m, 8 H, 6 amide, amine NH_2), 8.02-7.88 (m, 5 H, H-5 and amide NH), 7.84 (d, $^3J_{H\text{-}H}$ = 7.5 Hz, 1 H, amide NH), 7.76 (s, 2 H, H-11), 7.71 (s, 1 H, H-13), 5.70 (s, 2 H, H-9), 4.44-4.36 (m, 3 H, 2 H-$C_{\varepsilon(Lys)}$ and H-C_α), 4.35-4.14 (m, 7 H, H-C_α), 3.78-3.62 (m, 7 H, H-C_α), 1.89 (quint, $^3J_{H\text{-}H}$ = 7.1 Hz, 2 H, H-$C_{\delta\,(lys)}$), 1.76-1.69 (m, 2 H, H-$C_{\beta(lys)}$), 1.66-1.57 (m, 4 H, H-$C_{\gamma(leu)}$), 1.54-1.42 (m, 8 H, H-$C_{\beta(leu)}$), 1.40-1.32 (m, 2 H, H-$C_{\gamma(lys)}$), 1.24 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 3 H, H-$C_{\beta(ala)}$), 1.20 (d, $^3J_{H\text{-}H}$ = 7.2

Hz, 9 H, H-$C_{\beta(ala)}$), 0.90-0.80 (m, 24 H, H-$C_{\delta(leu)}$); UPLC-MS: Method 4, T_R = 7.02 min; m/z MS (ES+): 1467.2 [100 %, M + H^+], 733.4 [40 %, M + 2 H^+]; HRMS (ESI+) calcd. for $C_{65}H_{95}F_6N_{16}O_{16}^+$ (M+H^+): 1469.7011, found: 1469.7005.



1b

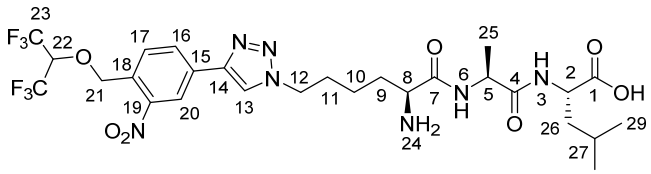
1b. *General procedure 5*. The trimethylsilyl-protected alkyne **S9** was deprotected *in situ*. **S9** (51 mg, 0.12 mmol, 1.0 eq.) and the trifluoroacetate salt of **S11** (44 mg, 94 μmol, 0.78 eq.) were suspended in water/*tert*-butanol (1:1, 10 ml). Aq. sodium ascorbate (1.00 M, 200 μl, 1.63 eq.), aq. $CuSO_4$ (1.00 M, 20 μl, 0.16 eq.) and TBAF (1 M in THF, 369 μl, 0.369 mmol, 3.00 eq.) were added and the mixture was stirred for 4 h at 55 °C. Subsequently volatiles were removed under reduced pressure and the residue purified by reversed phase preparative HPLC. The trifluoroacetate salt of the product was obtained as a white powder after lyophilisation. (8.0 mg, 11 %). ^{1}H-NMR (600 MHz, DMSO-d_6, 298 K) δ 8.85 (s, 1 H, H-13), 8.59 (d, $^3J_{H\text{-}H}$ = 7.5 Hz, 1 H, H-6), 8.57 (d, $^4J_{H\text{-}H}$ = 1.8 Hz, 1 H, H-20), 8.28 (dd, $^3J_{H\text{-}H}$ = 8.1 Hz, $^4J_{H\text{-}H}$ = 1.8 Hz, 1 H, H-16), 8.22 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-3), 7.94 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-17), 5.63 (s, 2 H, H-21), 4.47-4.35 (m, 3 H, H-12 and H-5), 4.22 (ddd, $^3J_{H\text{-}H}$ = 9.2 Hz, $^3J_{H\text{-}H}$ = 8.0 Hz, $^3J_{H\text{-}H}$ = 5.4 Hz, 1 H, H-2), 3.77 (t, $^3J_{H\text{-}H}$ = 6.5 Hz, 1 H, H-8), 1.89 (quint, $^3J_{H\text{-}H}$ = 7.2 Hz, 2 H, H-11), 1.76-1.69 (m, 2 H, H-9), 1.67-1.58 (m, 1 H, H-28), 1.56-1.47 (m, 2 H, H-27), 1.40-1.32 (m, 2 H, H-10), 1.25 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 3 H, H-26), 0.88 (d, $^3J_{H\text{-}H}$ = 6.6 Hz, 3 H, H-29 or H-30), 0.82 (d, $^3J_{H\text{-}H}$ = 6.5 Hz, 3 H, H-29 or H-30); ^{13}C NMR (150 MHz, DMSO-d_6, 298 K) δ 173.9 (C-1), 171.7 (C-4), 168.1 (C-7), 147.9 (C-19), 144.0 (C-14), 141.4 (C-23), 137.5 (C-24), 137.0 (C-25), 132.5 (C-15), 130.9 (C-17), 130.1 (C-18), 130.0 (C-16), 122.8 (C-13), 120.9 (C-20), 73.1 (C-21), 51.9 (C-8), 50.1 (C-2), 49.5 (C-12), 48.0 (C-5), 39.9 (C-27), 30.6 (C-9), 29.0 (C-11), 24.3 (C-28), 22.9 (C-29 or C-30), 21.3 (C-29 or C-30), 21.2 (C-10), 18.2 (C-26), C-22 was not detected; ^{19}F-NMR (565 MHz, DMSO-d_6, 298 K) δ -73.5 (s, trifluoroacetate), -156.1 (m, 2 F, 9-F), -161.7 (m, 1 F, 11-F), -162.5 (m, 2 F, 10-F); UPLC-MS Method 3, T_R = 5.59 min; m/z MS (ES+): 700.4 [100 %, M + H^+]; HRMS (ESI+) calcd. for $C_{30}H_{35}F_5N_7O_7^+$ (M+H^+): 700.2513, found: 700.2519.



1c

1c. The synthesis followed the protocol for the synthesis of **1b** (*General procedure 5*) employing **S7** (27 mg, 57 μmol, 1.0 eq.) and the trifluoroacetate salt of **S11** (30 mg, 64 μmol, 1.1 eq.). The trifluoroacetate salt of the product was isolated as a white solid (3 mg, 7 %); ^{1}H-NMR (500 MHz, DMSO-d_6, 298 K) δ 8.81 (s, 1 H, H-13), 8.59 (d, $^3J_{H\text{-}H}$ = 8.0 Hz, 1 H, H-6), 8.55(d, $^4J_{H\text{-}H}$ = 1.5 Hz, 1 H, H-20), 8.25-8.20 (m, 2 H, H-16 and H-3), 8.05 (br s, 2 H, H-31), 7.88 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-17), 7.34-7.30 (m, 2 H, H-24), 7.05-7.01 (m, 2 H, H-23), 7.00-6.95 (m, 1 H, H-25), 5.48 (s, 2 H, H-21), 4.45-4.35 (m, 3

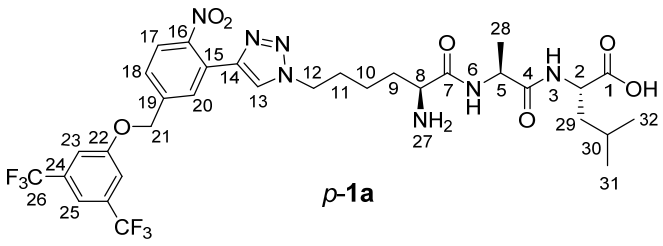
H, H-12 and H-5), 4.25-4.18 (m, 1 H, H-2), 3.78-3.73 (m, 1 H, H-8), 1.93-1.84 (m, 2 H, H-11), 1.76-1.69 (m, 2 H, H-9), 1.67-1.60 (m, 1 H, H-28), 1.57-1.46 (m, 2 H, H-27), 1.39-1.30 (m, 2 H, H-10), 1.25 (d, $^{3}J_{H\text{-}H}$ = 6.9 Hz, 3 H, H-26), 0.88 (d, $^{3}J_{H\text{-}H}$ = 6.1 Hz, 3 H, H-29 or H-30), 0.82 (d, $^{3}J_{H\text{-}H}$ = 6.1 Hz, 3 H, H-29 or H-30). δ ^{13}C (determined by HMQC and HMBC experiments) 171.3 (C-4), 167.7 (C-7), 157.5 (C-22), 147.7 (C-19), 143.6 (C-14), 131.3 (C-15 and C-18), 129.7 (C-17), 129.4 (C-16), 129.2 (C-24), 122.2 (C-13), 120.9 (C-25), 120.5 (C-20), 114.4 (C-23), 65.8 (C-21), 51.5 (C-8), 49.9 (C-2), 49.0 (C-12), 47.7 (C-26), 39.5 (C-27), 30.1 (C-9), 28.7 (C-11), 23.9 (C-28), 22.4 (C-29 or C-30), 20.9 (C-29 or C-30), 20.8 (C-10), 17.7 (C-26), C-1 was not detected; UPLC-MS Method 3, T_R = 5.25 min; m/z MS (ES+): 610.1 [100 %, M + H$^+$], 326.3 [40 %], 305.8 [40 %, M + 2 H$^+$]; HRMS (ESI+) calcd. for $C_{30}H_{40}N_7O_7^+$ (M+H$^+$): 610.2984, found: 610.2989.



1d

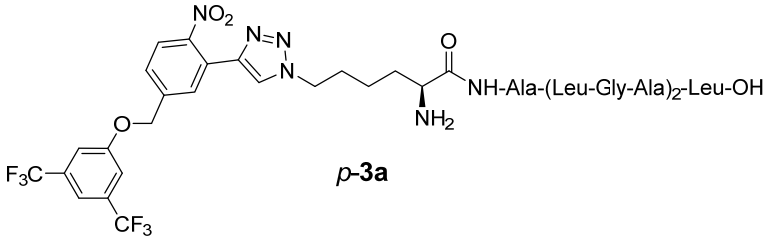
1d. The synthesis followed the protocol for the synthesis of **1b** (*General procedure 5*) starting from **S8** (30 mg, 75 μmol, 1.0 eq.) and the trifluoroacetate salt of **S11** (27 mg, 57 μmol, 0.76 eq.) The trifluoroacetate of the product was isolated as a white solid (5 mg, contains 23 wt% of a tetrabutylammonium salt, which was considered as the trifluoroacetate, corrected yield 8 %). ^{1}H-NMR (600 MHz, DMSO-d$_6$, 298 K) δ 12.57 (br, 1 H), 8.83 (s, 1 H, H-13), 8.61 (d, $^{3}J_{H\text{-}H}$ = 7.5 Hz, 1 H, H-6), 8.54 (d, $^{4}J_{H\text{-}H}$ = 1.8 Hz, 1 H, H-20), 8.28 (dd, $^{3}J_{H\text{-}H}$ = 8.1 Hz, $^{4}J_{H\text{-}H}$ = 1.8 Hz, 1 H, H-16), 8.22 (d, $^{3}J_{H\text{-}H}$ = 8.0 Hz 1 H, H-3), 8.09 (s, 2 H, H-24), 7.82 (d, $^{3}J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-17), 5.78 (hept, $^{3}J_{H\text{-}F}$ = 6.4 Hz, 1 H, H-22), 5.32 (s, 2 H, H-21), 4.46-4.36 (m, 3 H, H-12 and H-5), 4.22 (ddd, $^{3}J_{H\text{-}H}$ = 9.7 Hz, $^{3}J_{H\text{-}H}$ = 8.0 Hz, $^{3}J_{H\text{-}H}$ = 5.4 Hz, 1 H, H-2), 3.77 (t, $^{3}J_{H\text{-}H}$ = 6.4 Hz, 1 H, H-8), 1.89 (quint, $^{3}J_{H\text{-}H}$ = 7.2 Hz, 2 H, H-11), 1.75-1.70 (m, 2 H, H-9), 1.67-1.58 (m, 1 H, H-27), 1.56-1.47 (m, 2 H, H-26), 1.40-1.32 (m, 2 H, H-10), 1.25 (d, $^{3}J_{H\text{-}H}$ = 7.1 Hz, 3 H, H-25), 0.88 (d, $^{3}J_{H\text{-}H}$ = 6.6 Hz, 3 H, H-28 or H-29), 0.82 (d, $^{3}J_{H\text{-}H}$ = 6.6 Hz, 3 H, H-28 or H-29); ^{13}C NMR (150 MHz, DMSO-d$_6$, 298 K) δ 174.0 (C-1), 171.7 (C-4), 168.1 (C-8), 147.7 (C-19), 144.0 (C-14), 132.2 (C-15), 130.5 (C-18), 130.1 (C-17), 130.0 (C-16), 122.8 (C-13), 121.7 (q, $^{1}J_{C\text{-}F}$ ≈ 284 Hz, C-23), 120.9 (C-20), 74.2 (sept, $^{3}J_{H\text{-}F}$ = 32 Hz, C-22), 72.2 (C-21), 51.9 (C-8), 50.2 (C-2), 49.5 (C-12), 48.1 (C-5), 39.9 (C-26), 30.6 (C-9), 29.0 (C-11), 24.3 (C-27), 22.9 (C-28 or C-29), 21.3 (C-28 or C-29), 21.2 (C-10), 18.2 (C-25); UPLC-MS Method 3, T_R = 5.32 min; m/z MS (ES+): 683.9 [100 %, M + H$^+$], 363.9 [40 %]; HRMS (ESI+) calcd. for $C_{27}H_{36}F_6N_7O_7^+$ (M+H$^+$): 684.2575, found: 684.2576.

p-**1a**. The synthesis followed the protocol described for **1b** (*General procedure 5*) starting from **S10** (30 mg, 65 μmol, 1.0 eq.) and the trifluoroacetate salt of **S11** (23 mg, 49 μmol, 0.76 eq.). The trilfuoroacetate salt of the product was isolated as a white solid (5 mg, 9 %). ^{1}H-NMR (500 MHz, DMSO-d$_6$, 298 K) δ 12.57 (br, 1H), 8.61 (d, $^{3}J_{H\text{-}H}$ = 7.5 Hz, 1 H, H-6), 8.51 (s, 1 H, H-13), 8.22 (d, $^{3}J_{H\text{-}H}$ = 8.1 Hz, 1 H, H-3), 8.09 (d, $^{3}J_{H\text{-}H}$ =



p-**1a**

5.4 Hz, 2 H, H-27), 7.99 (d, $^{3}J_{H\text{-}H}$ = 8.3, 1 H, H-17), 7.97 (d, $^{4}J_{H\text{-}H}$ = 1.8 Hz, 1 H, H-20), 7.76 (s, 2 H, H-23), 7.72 (dd, $^{3}J_{H\text{-}H}$ = 8.3 Hz, $^{4}J_{H\text{-}H}$ = 1.8 Hz, 1 H, H-18), 7.70 (s, 1 H, H-25), 5.46 (s, 2 H, H-21), 4.43-4.35 (m, 3 H, H-5 and H-12), 4.23 (ddd, $^{3}J_{H\text{-}H}$ = 9.4 Hz, $^{3}J_{H\text{-}H}$ = 8.1 Hz, $^{3}J_{H\text{-}H}$ = 5.8 Hz, 1 H, H-2), 3.29 (m, 1 H, H-8), 1.88 (quint, $^{3}J_{H\text{-}H}$ = 7.3 Hz, 2 H, H-11), 1.76-1.70 (m, 2 H, H-9), 1.65-1.57 (m, 1 H, H-30), 1.52-1.46 (m, 2 H, H-29), 1.37-1.29 (m, 2 H, H-10), 1.25 (d, $^{3}J_{H\text{-}H}$ = 7.0 Hz, 3 H, H-28), 0.87 (d, $^{3}J_{H\text{-}H}$ = 6.6 Hz, 3 H, H-31 or H-32), 0.82 (d, $^{3}J_{H\text{-}H}$ = 6.5 Hz, 3 H, H-31 or H-32); δ ^{13}C (determined by HMQC and HMBC experiments) 173.6 (C-1), 171.3 (C-4), 167.8 (C-7), 158.7 (C-22), 147.2 (C-16), 141.0 (C-14), 140.5 (C-19), 131.4 (C-24), 128.6 (C-20), 127.8 (C-18), 124.1 (C-17), 123.7 (C-15), 123.2 (C-13), 122.7 (q, $^{3}J_{C\text{-}F}$ = 273 Hz, C-26), 115.8 (C-23), 114.0 (C-25), 68.7 (C-21), 51.5 (C-8), 49.8 (C-2), 49.2 (C-12), 47.7 (C-5), 39.6 (C-29), 30.3 (C-9), 29.0 (C-11), 24.1 (C-30), 22.8 (C-31 or C-32), 20.9 (C-31 or C-32), 20.8 (C-10), 17.8 (C-28); UPLC-MS: Method 3, T_R = 5.93 min; m/z MS (ES+): 746.5 [100 %, M + H$^+$], 394.4 [40 %], 373.9 [40 %]; HRMS (ESI+) calcd. for $C_{32}H_{38}F_6N_7O_7^+$ (M+H$^+$): 746.2731, found: 746.2738.



p-**3a**

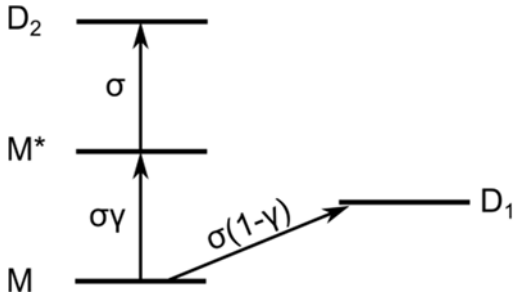
p-**3a**. The synthesis followed the protocol described for the synthesis of **1b** (*General procedure 5*) starting from **S10** (20 mg, 50 μmol, 1.0 eq.) and the trifluoroacetate salt of **S13** (42 mg, 44 μmol, 0.88 eq.). The trifluoroacetate salt of the modified peptide was isolated as a white solid (4 mg, 7%). ^{1}H-NMR (500 MHz, DMSO-d$_6$, 298 K) δ 12.49 (br, 1 H), 8.61 (d, $^{3}J_{H\text{-}H}$ = 7.2 Hz, 1 H, amid), 8.51 (s, 1 H, H-1), 8.13-8.03 (m, 6 H, 4 amide NH and amine NH$_2$), 8.02- 7.91 (m, 4 H, H-5, H-8 and 2 amid), 7.84 (d, $^{3}J_{H\text{-}H}$ = 7.5 Hz, 1 H, amide NH), 7.76 (s, 2 H, H-11), 7.72 (dd, $^{3}J_{H\text{-}H}$ = 8.5 Hz, $^{4}J_{H\text{-}H}$ = 2.0 Hz, 1 H, H-6), 7.70 (s, 1 H, H-13), 5.47 (s, 2 H, H-9), 4.44-4.36 (m, 3 H, 2 H-C$_{\varepsilon(Lys)}$ and H-C$_\alpha$), 4.35-4.14 (m, 5 H, H-C$_\alpha$), 3.77-3.62 (m, 5 H, H-C$_\alpha$), 1.87 (quint, $^{3}J_{H\text{-}H}$ = 7.1 Hz, 2 H, H-C$_{\delta\ (lys)}$), 1.76-1.70 (m, 2 H, H-C$_{\beta(lys)}$), 1.66-1.57 (m, 3 H, H-C$_{\gamma(leu)}$), 1.54-1.42 (m, 6 H, H-C$_{\beta(leu)}$), 1.40-1.32 (m, 2 H, H-C$_{\gamma(lys)}$), 1.24 (d, $^{3}J_{H\text{-}H}$ = 7.1 Hz, 3 H, H-C$_{\beta(ala)}$), 1.21 (d, $^{3}J_{H\text{-}H}$ = 7.2 Hz, 6 H, H-C$_{\beta(ala)}$), 0.89-0.85 (m, 9 H, H-C$_{\delta(leu)}$), 0.83-0.81 (m, 9 H, H-C$_{\delta(leu)}$); UPLC-MS Method 1, T_R = 4.57 min; m/z MS (ES+): 1250.1 [10 %, M + Na$^+$], 1228.2 [80 %, M + H$^+$], 614.9 [100 %, M + 2 H$^+$]. HRMS (ESI+) calcd. for $C_{54}H_{76}F_6N_{13}O_{13}^+$ (M+H$^+$): 1228.5584, found: 1228.5574.

Sample preparation for MS experiments

For the electrospray and depletion experiments 1 mg of peptide was added in dry form to 2 ml of a mixture of deionized water, methanol and ammonia (25 %) in a ratio of 1:1:0.001, resulting in a pH value of approximately 8.3. The electrospray was operated at a flow rate of 5 μl/min.

Kinetic rate equations

Figure S1: *idealized absorption scheme in the peptide photo-depletion experiments. The letter M designates a molecular electronic ground state, M* an excited state, while D_1 and D_2 refer to depletion states. The absorption cross section σ is weighted by the probability γ to require more than a single-photon process for successful beam depletion. We assume the dominant beam depletion process to be cleavage rather than electron detachment.*



Our derivation of the UV photo depletion and fragment yield, equation (1) in the main text, follows [3]. We assume a sequential 2-photon absorption, as illustrated in Figure S1, which can be generalized to the case of N photons: The populations of all levels change in dependence of the laser fluence F.

$$\frac{dM}{dF} = -\sigma M \qquad Eq.(S2)$$

$$\frac{dM^*}{dF} = \sigma\gamma M - \sigma M^*$$

$$\frac{dD_1}{dF} = \sigma(1-\gamma)M$$

$$\frac{dD_2}{dF} = \sigma M^*$$

With the initial condition $M(F=0)=1$, we obtain

$$M = e^{-\sigma F} \qquad Eq.(S3)$$

$$M^* = \gamma\sigma F e^{-\sigma F}$$

$$D_1 = (1-\gamma)(1-e^{-\sigma F})$$

$$D_2 = \gamma(1-(1+\sigma F)e^{-\sigma F})$$

The depletion of the intact molecular mass peak M is given by the *ultraviolet photo-depletion probability*

$$UVPD = 1 - D_1 - D_2 = 1 - \alpha + \alpha(1+\gamma\sigma F)e^{-\sigma F} \quad (Eq.S3),$$

where we have introduced the *beam overlap* α. It measures the fraction of the molecular beam overlapping with the depleting laser beam. The fragment yield is the sum of all fragment channels and given by

$$FY = D_1 + D_2 = \eta_{det}\alpha(1-(1+\gamma\sigma F)e^{-\sigma F}) \qquad (Eq.S4)$$

where η_{det} includes losses due to the limited detection efficiency for fragment ions as well as electron photodetachment as a depletion channel that does not generate new mass peaks.

UV photodepletion of nonapeptide 3a and dodecapeptide 4a

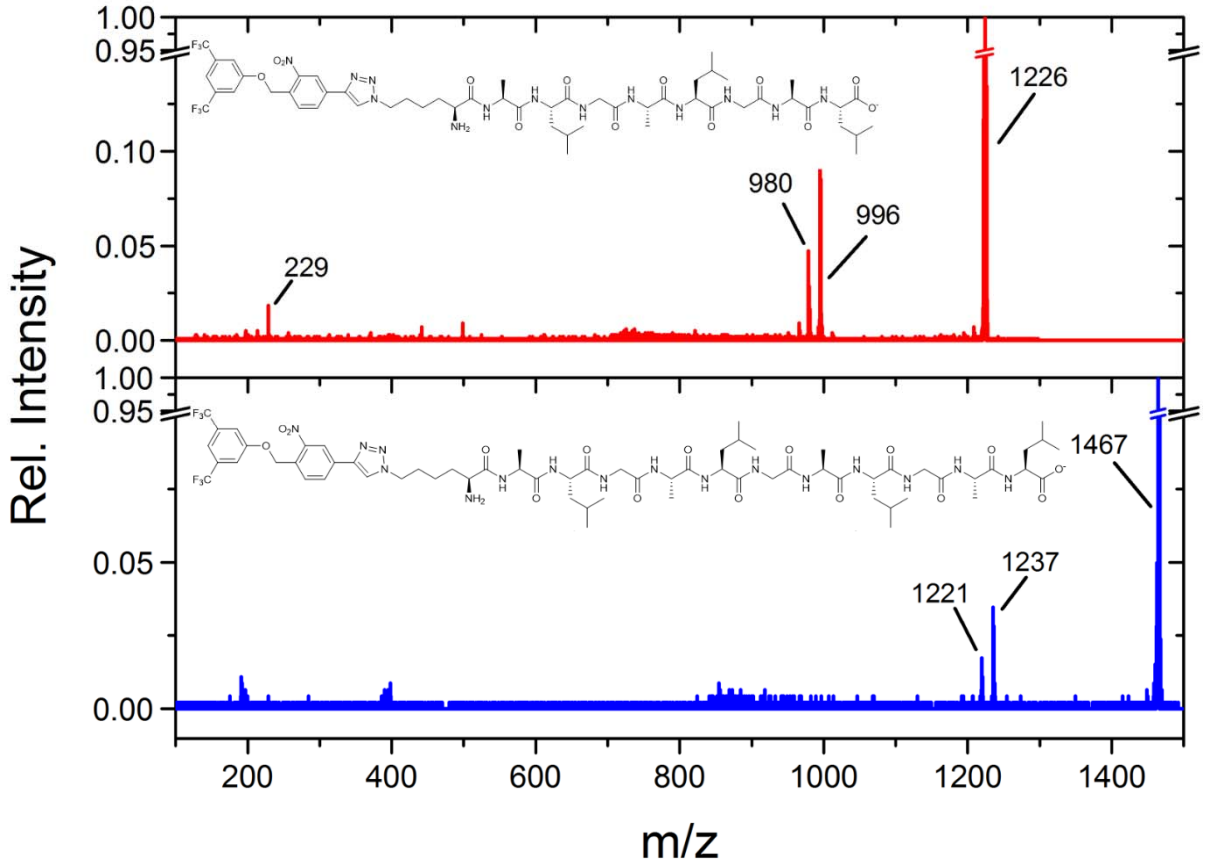


Figure S2: *UVPD mass spectra for the tagged nonapeptide **3a** (LG-PCG-Lys-Ala-Leu-Gly-Ala-Leu-Gly-Ala-Leu, upper panel) and the tagged dodecapeptide **4a** (LG-PCG-Lys-Ala-Leu-Gly-Ala-Leu-Gly-Ala-Leu-Gly-Ala-Leu, lower panel). Again, bond-selective heterolytic photocleavage can be observed, but the probability for this process shrinks with peptide length, while homolytic cleavage emerges.*

At an average laser power of 200 mW in a circular beam of 2 mm diameter, delivered in 10 ns pulses at a repetition rate of 250 Hz, we see again bond-selective photocleavage. The delay between the laser pulse and the mass spectrometer extraction voltage (ion pusher) was set to maximize the detection of the high mass fragment to assess the total depletion rate. In both cases, the dominant fragments appear at m/z=(M-230) u/e and (M-246) u/e, with M the mass of the parent peak. The fragment at (M-230) u/e can be assigned to homolytic cleavage and proton transfer to the LG. The UVPD mass spectrum of peptide **3a** still shows a small signal for the leaving group **a**, which is absent in the spectrum of the longer peptide **4a**.

Temperature dependence of the UVPD curves of hexapeptide 2a

We have studied the UVPD curves for the hexapeptide **2a** at 300 K and 60 K (Figure S3) to explore the influence of the molecular heat capacity. The depletion curves exhibit a clear multiphoton behaviour at both temperatures and the best fit is obtained for a sequential absorption of 2 photons (with $\gamma = 1$). In this model we use the same absorption cross section as for the tripeptide **1a**.

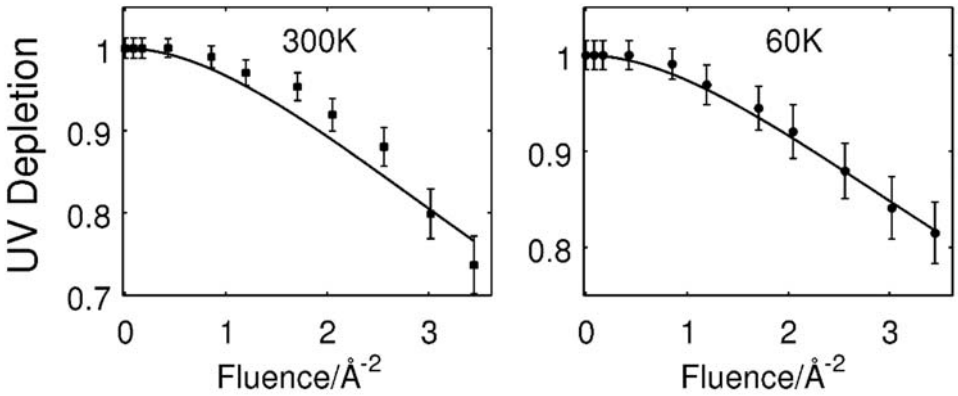


Figure S3: *Temperature dependence of the UV photodepletion curves for the hexapeptide* ***2a*** *at 300 K* ***(left)*** *and 60 K* ***(right)****. Points and error bars represent experimental values. Lines are fits assuming a multiphoton process and the same absorption cross section as for the tripeptide* ***1a****. The best fit parameters are found for a pure 2 sequential photons absorption (γ = 1).*

UVPD mass spectra of tripeptides 1b, 1c, 1d

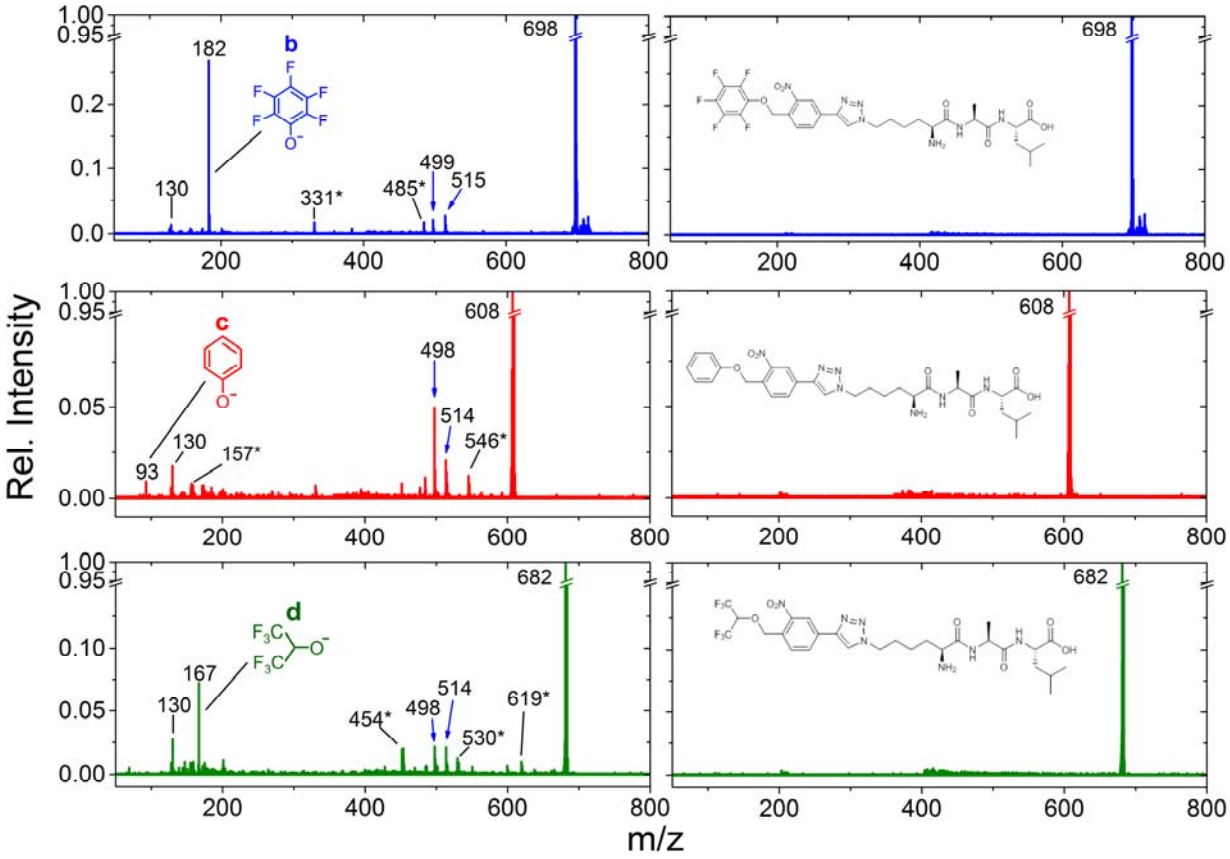


Figure S4: *Comparison of the UVPD mass spectra of tripeptide* ***1b, 1c, 1d*** *with* ***(left)*** *and without* ***(right)*** *laser radiation. This shows that signal-to-noise and mass-selectivity is good enough for the smallest peaks to be unambiguously assigned to the photo-cleavage process, even with peak amplitudes below 1% of the parent peak.*

To unambiguously establish that all observed fragments are caused by the incident laser radiation, we have recorded the UVPD mass spectra of three differently tagged tripeptides **1b, 1c, 1d** (see Figure **3b**) and compared them with their mass spectra in the absence of any laser light (see Figure S4, right column).

For tripeptide **1b**, the leaving group is expected at m/z = 182 u/e, for tripeptide **1c** at 93 u/e and for tripeptide **1d** at 167 u/e. The blue arrows indicate the proton transfer fragments (as depicted in Scheme 1 of the main text), which can be observed in all three cleavage processes. The fragment with m/z = 130 u/e is present in all spectra and arises from dissociation of the peptide backbone, that is common to all tested molecules. Note, that the dominant fragment of tripeptide **1d** is the proton transfer fragment, rather than the leaving group **d**. The fragments marked with an asterix * are assigned to backbone fragments.

Collison-induced dissociation mass spectra of peptides 1a-4a

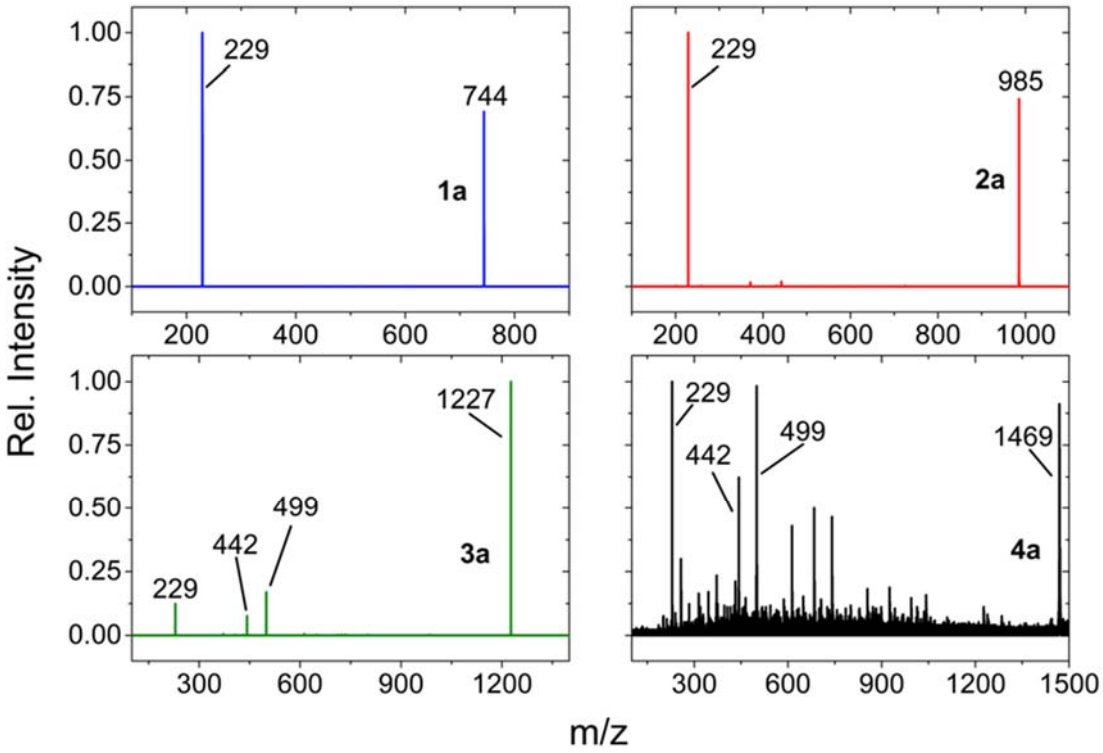


Figure S5: *Collision-induced dissociation mass spectra of peptides* ***1a*** *-* ***4a****.* ***Top left****: tagged tripeptide* ***1a****,* ***top right****: tagged hexapeptide* ***2a, bottom left****: tagged nonapeptide* ***3a, bottom right:*** *tagged dodecapeptide* ***4a****. The designed leaving group is always observed. At a given collision energy, the fragment yield decreases with increasing peptide length (33-73 eV, see Figure S6).*

The thermal nature of the selective bond cleavage can be explored by studying the collision-induced dissociation (CID) mass spectra of the peptides **1a**, **2a**, **3a** and **4a.** They were recorded at room temperature (300 K), with neutral argon atoms as the collision gas. The gas pressure is estimated to be 10^{-4} mbar. The spectra were recorded at kinetic energies between 33 and 73 eV (see Figure 2 of the main text), to achieve that each peptide parent peak is depleted by 90%. We find identical fragments, namely only the leaving group **a** in the CID and UVPD spectra of peptides **1a** and **2a.** Additional fragments arise in the CID spectra of the peptides **3a** and **4a**. They are attributed to backbone fragments whose probability increases with peptide length. Peptide **4a** has a smaller fragment yield. The scaled signal thus shows increased background noise. Figure S6 shows that the depletion of the parent peptide by CID requires a kinetic energy which increases linearly with peptide length.

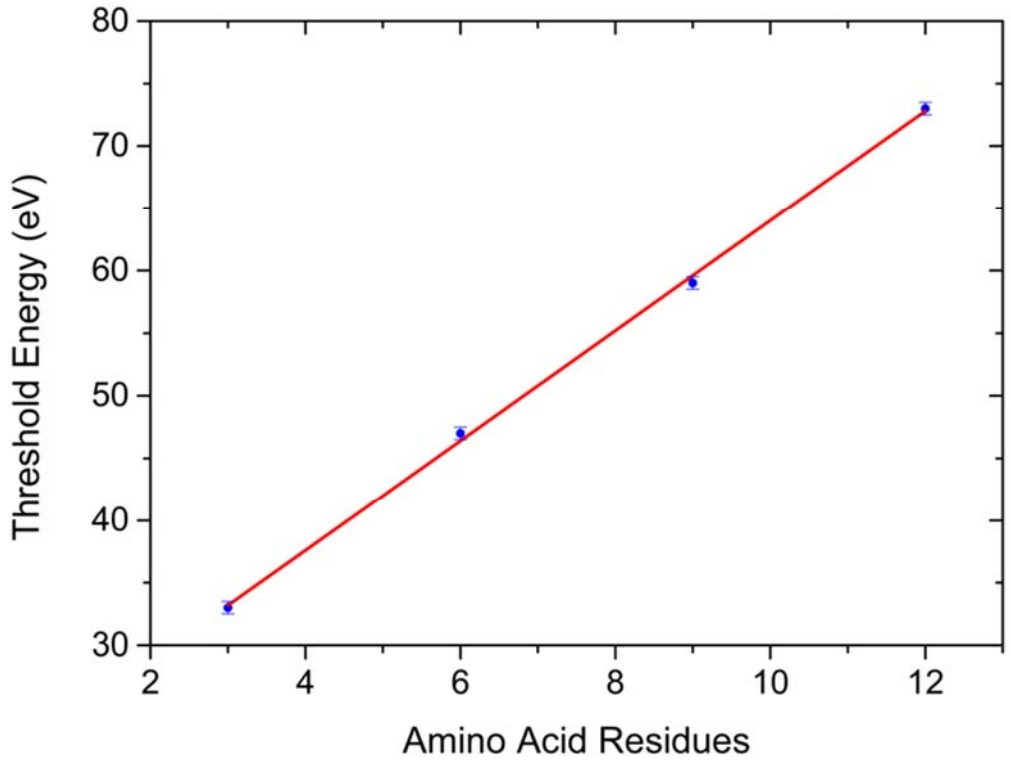


Figure S6: *CID threshold energy as function of the number of amino acid residues per tagged peptide (from tripeptide **1a** to dodecapeptide **4a**). The number shown on the y-axis is the threshold kinetic energy of the peptide ions in collision with the thermal (300 K) argon gas to achieve 90% depletion of the molecular parent peak. The heat capacity increases linearly with peptide length, and so does the CID threshold energy. A linear fit to the data finds a slope of 4.4 ± 0.1 eV/residue and a linear regression coefficient of R^2=0.998. The error bars represent the uncertainty in the energy setting.*

Computational Information

AIMD: Short ab initio molecular dynamics (AIMD) simulations are performed to additionally scan the conformational space of the peptides for structural candidates that are used in the following computations. Using the AIMD module of NWChem v6.6 [4] the nuclear motion of the peptides is integrated with the velocity-Verlet algorithm, while the electronic potential is provided by DFT at the PBE0/3-21G [5, 6] level of theory. Due to the high computational costs of AIMD simulations, we have only calculated several tens of picoseconds using a small basis set for a scan of the potential energy surface (PES). Starting peptide conformations are manually generated and dynamics are run at 300 K in 1 fs time steps using a stochastic velocity rescaling thermostat [7] to control the temperature with a relaxation time of 0.1 ps. The small basis set size can be justified since binding- and torsion angles, which are the relevant

geometric parameters for our scan, are generally less sensitive to basis set sizes than bond lengths, and because the peptides are further locally optimized at a higher level of theory.

DFT: Structural candidates extracted from the AIMD trajectories are further geometry optimized using the Gaussian09 program package [8] at the PBE0/Def2TZVP level of theory [5, 9]. Harmonic frequencies are calculated for the lowest energy conformations to ensure true minima on the PES. Conformations that converge to transition states are distorted along their imaginary modes and re-optimized. In case they do not converge to minima, they are excluded from further investigations. The computed harmonic frequencies are further used to estimate mean thermal energies derived by the frequency model [10] using known beam temperatures of 60 K and 300 K in the buffer gas from the knowledge of all 3*N*-6 harmonic modes:

$$\langle E \rangle_T = \sum_{i=1}^{3N-6} \frac{\hbar\omega_i}{\exp\left(\frac{\hbar\omega_i}{k_B T}\right) - 1}$$

BDE: The tripeptide anions, here named as complex AB^-, are locally relaxed (AB^-_{eq}) and then split heterolytically to evaluate the energies of the fragments A (a neutral zwitterion) and B^- (the negatively charged leaving group) from single-point calculations using the geometries of the fragments within the complex: BDE = E(A) + E(B^-) – E(AB^-_{eq}). Heterolytic bond dissociation energies are generally higher than typical homolytic values due to the additional coulomb attraction between fragments of opposite charge [11]. Adiabatic bond dissociation energies: Here, the fragments A and B^- were further relaxed to the next local minimum found in geometry optimization using tight convergence criteria in Gaussian09: BDE = (A_{eq}) + E(B^-_{eq})– E(AB^-_{eq}). The difference between BDE and adiabatic BDE may be considered as a maximum reorganization energy while the adiabatic values are representative for the strength of the interaction of the monomers A and B^- forming the complex AB^-.

VDE: Vertical electron detachment energies are calculated from the energies of the optimized tripeptide anions AB^-_{eq} and single-point energies after detachment of one electron within the geometries of the anions: VDE = E(AB) – E(AB^-_{eq}). Adiabatic detachment energies (ADE) for the tripeptide anions can be calculated by further relaxing the neutralized complexes: ADE = E(AB_{eq}) – E(AB^-_{eq}). However, any attempt to calculate the ADE leads to decarboxylation of the neutralized tripeptides and therefore E(AB_{eq}) could not be evaluated. In order to study the electron detachment process in more detail, Mulliken orbital population analysis was performed to estimate partial charges. Here, the smaller Def2SVP basis set was used to counter a common problem of predicting unphysical charges when using diffuse basis functions. Single-point calculations using the Def2SVP basis set for geometries optimized with the larger basis set Def2TZVP were performed for the deprotonated tripeptides AB^- and their respective neutral products AB within the geometry of the anion AB^-. Then partial charges were evaluated and the change in partial

charge between these two geometries was studied showing that the biggest change in partial charge by vertical electron detachment is found for the COO^- group in the complex AB^-.

Additionally, basis set superposition errors (BSSE) were estimated by computing counterpoise corrections [12], which tend to slightly reduce the calculated BDE (-0.1 eV). The influence of dispersion effects has also been tested using Grimmes D3 correction [13] leading to an increase in BDE (+0.1 eV) thereby counteracting the effect of BSSE corrections. Additional calculations using the CAM-B3LYP functional [14] provided slightly higher VDE (+0.2 eV) and significantly higher BDE (+1.0 eV). Binding energies for the homolytic dissociation case i.e. the proton transfer pathway were not calculated although they would be very interesting values to know for comparison purposes. However, due to the yet unknown mechanism of the proton transfer route BDE could not be evaluated.

Table S1: *Comparison of pK_a values [15] and experimental fragment yields for the differently tagged tripeptides* ***1a-1d****, with calculated VDE and BDE values and thermal energies at 300 K. All energies are given in eV. For all species the thermal energy at 60 K is below 0.1 eV and therefore negligible compared to the photon energy at 266 nm. All energies are obtained by DFT at the PBE0/Def2TZVP level of theory. BDE values calculated after geometry relaxation of the fragments are shown in parentheses.*

PCT	pK_a	Yield	VDE	BDE	$\langle E \rangle_{300K}$
1a	8.0	0.12	4.6	6.9 (3.7)	1.4
1b	5.5	0.10	4.6	6.7 (3.5)	1.2
1c	9.8	0.01	4.6	7.8 (4.5)	1.2
1d	9.9	0.02	4.6	7.5 (4.4)	1.4

TDDFT: For every tripeptide, TDDFT calculations have been performed at the PBE0/Def2TZVP level of theory using the Gaussian09 package. For every tripeptide 100 excited states have been considered. The calculated line spectra are presented as Gaussian convolutions with Gaussian functions with a FWHM=0.33 eV. Natural transition orbitals [16] of the main optical transitions are analyzed. UV transitions including the weak transitions around 350 nm show mainly contributions of the PCT and the transitions mainly consist of one or two pairs of NTOs with significant contributions. Figure S7 shows the NTOs with largest contribution for the transitions closest to 266 nm for tripeptides **1a**-**1d**.

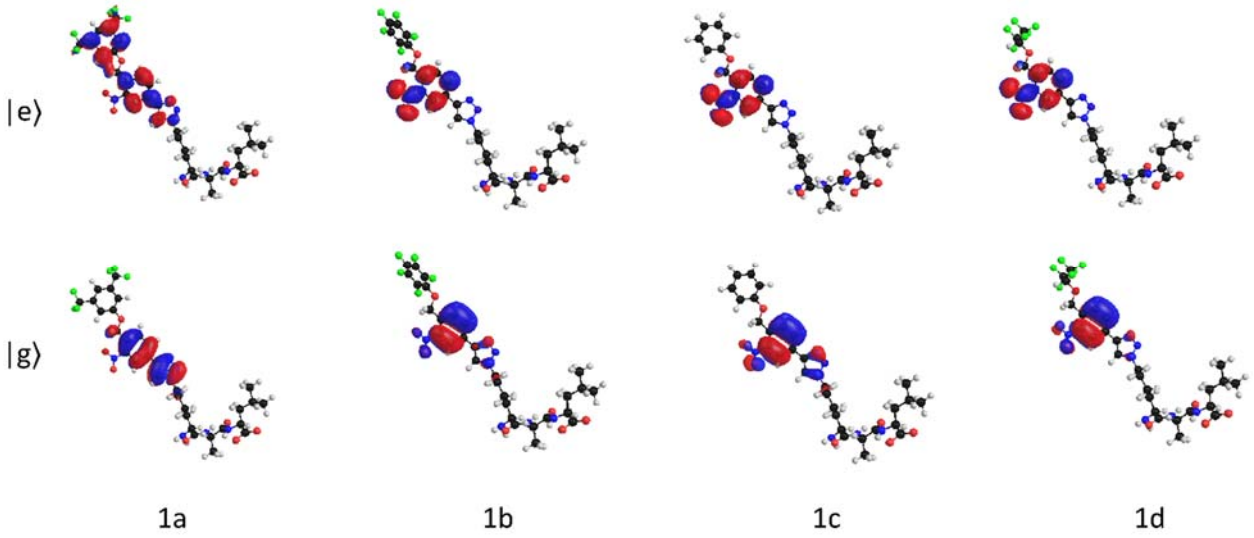


Figure S7: *NTO obtained by TDDFT at the PBE0/Def2TZVP level of theory for the tripeptides* ***1a***-***1d****. Only NTOs with the largest contributions to the calculated transitions closest to the experimental wavelength of 266 nm are shown. While only the NTO of* ***1a*** *shows a charge transfer towards the LG, all NTOs show that close to 266 nm only the PCT is the dominant absorber.*

Solution phase cleavage of 1a and *p*-1a

1a (3 mg, 4 μmol) or *p*-**1a**, respectively were dissolved in DMSO-d_6 (0.5 mL), placed in a standard 3.5 mL quartz cuvette and irradiated overnight in a TLC viewing chamber at 254 nm by positioning the cuvette directly in front of the lamp unit. NMR spectra of the samples were recorded before and after irradiation (Figure S8).

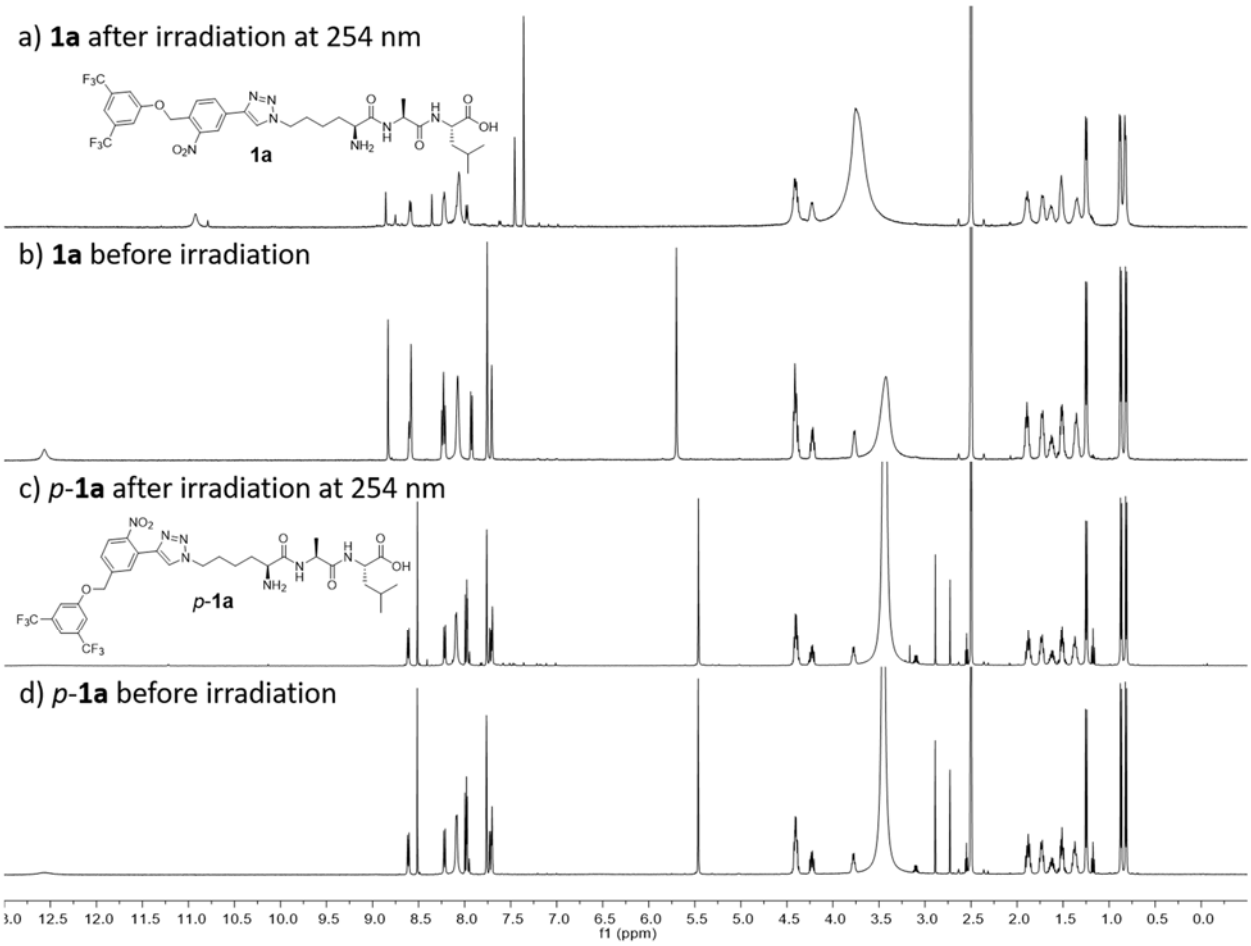
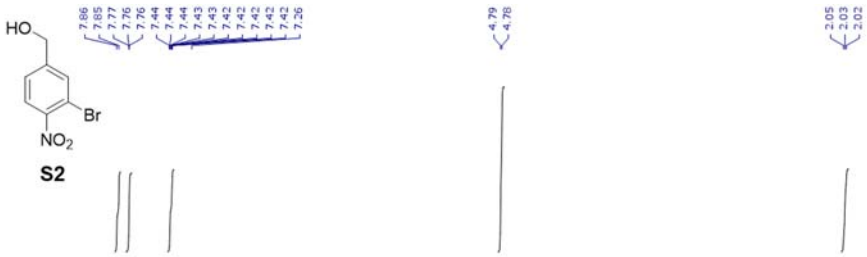
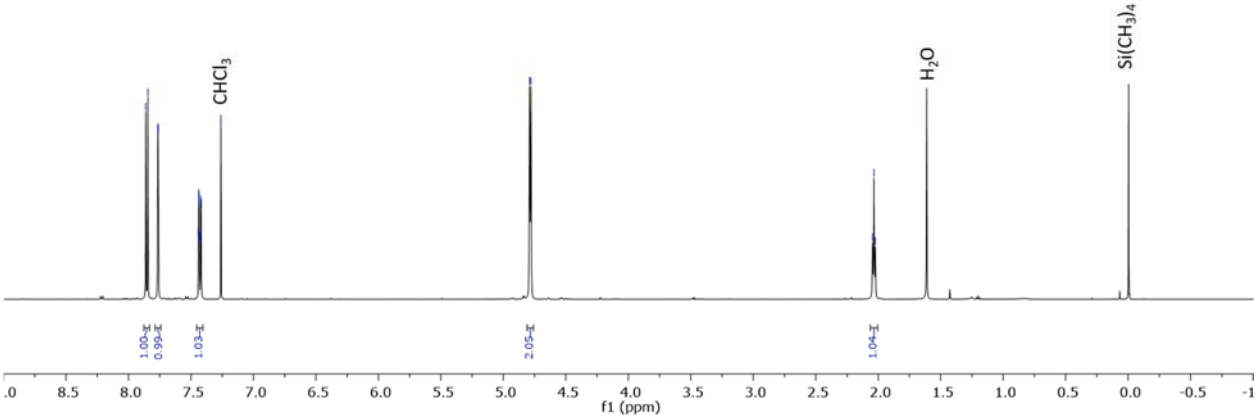


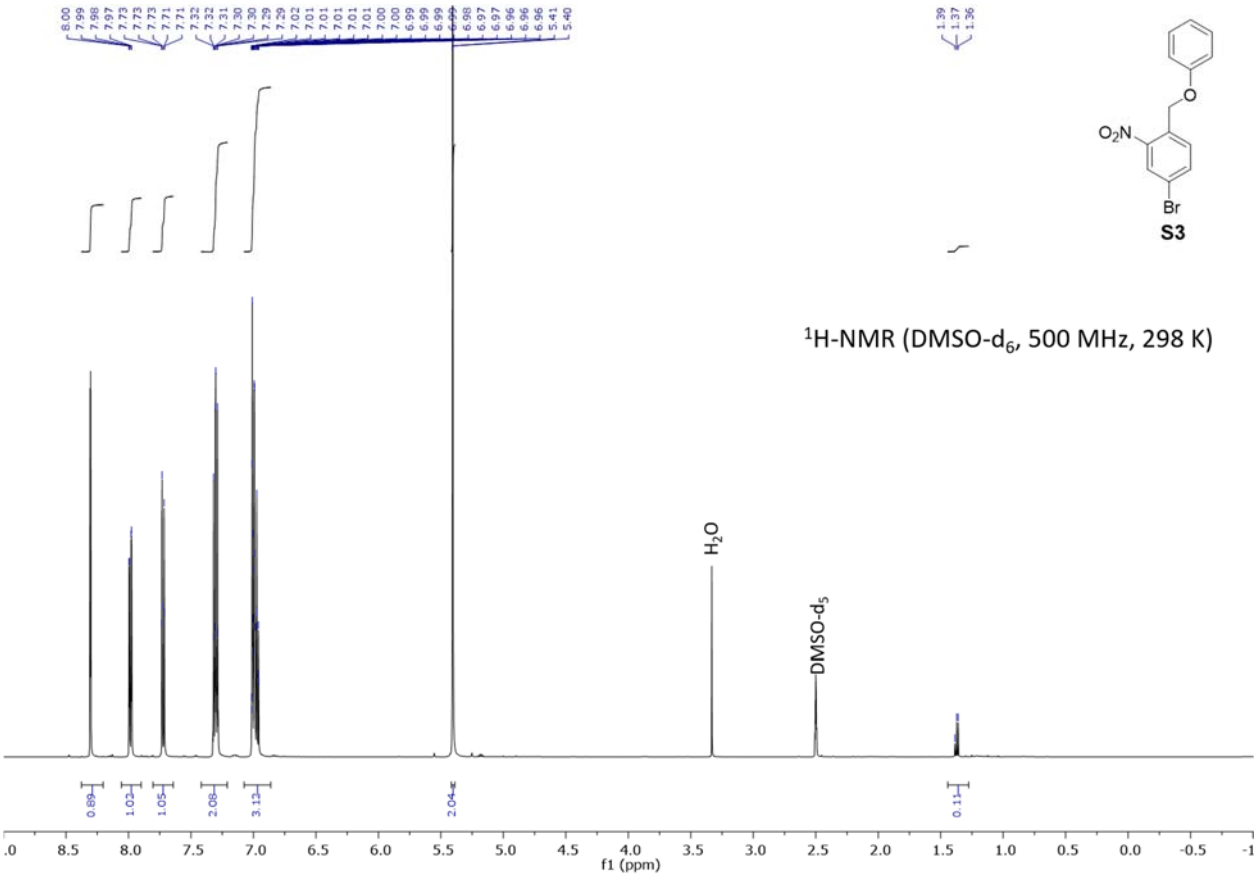
Figure S8. ^{1}H NMR spectra (500 MHz, DMSO-d_6, 298K) of solution phase irradiation experiments and references. a) **1a** after irradiation overnight; b) sample of **1a** before irradiation; c) *p*-**1a** after irradiation overnight d) sample of *p*-**1a** before irradiation. Only **1a,** which has the nitro-group in *ortho*-position to the aryl ether function, cleaves upon irradiation, whereas *p*-**1a** with the nitro-group in *para*-position to the aryl ether function does not cleave.

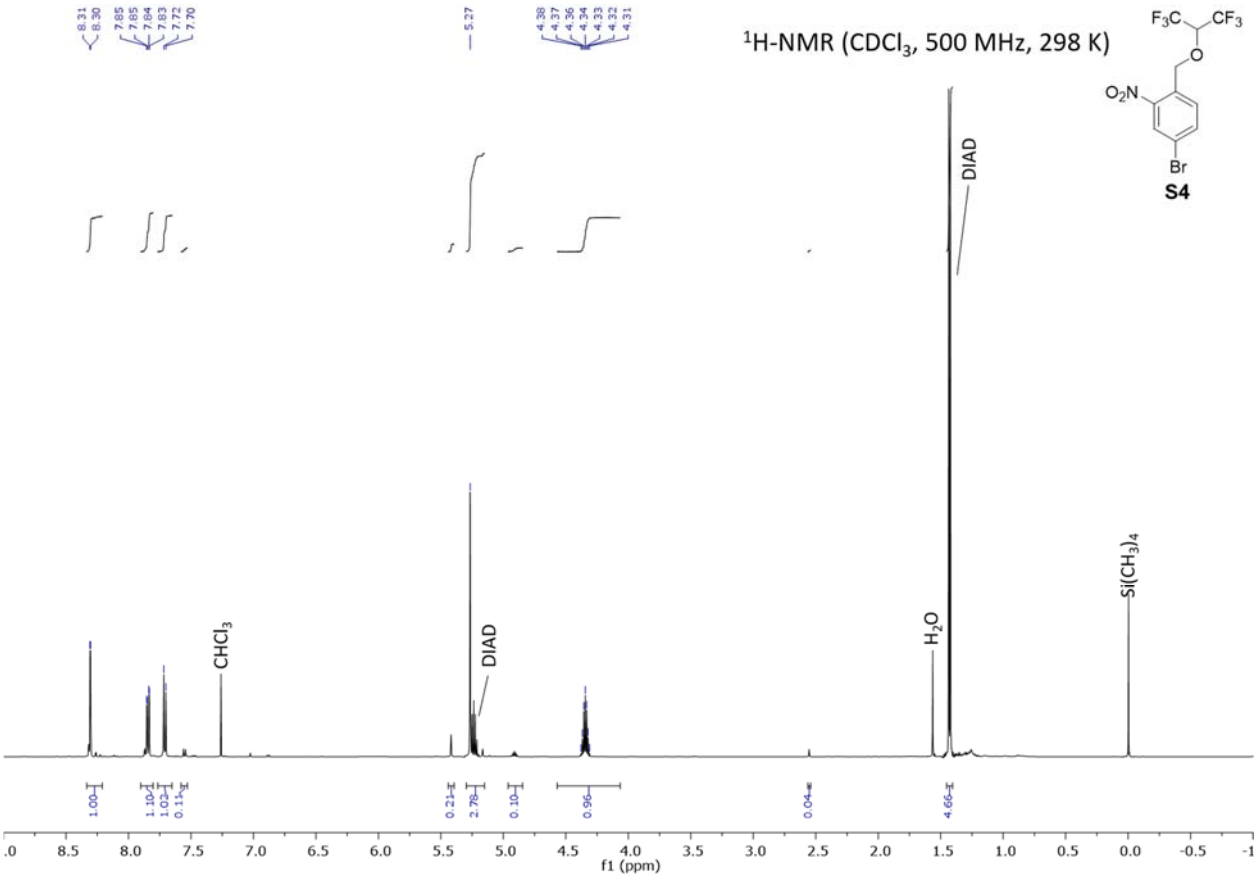
NMR-spectra and LC-traces of compounds synthesized



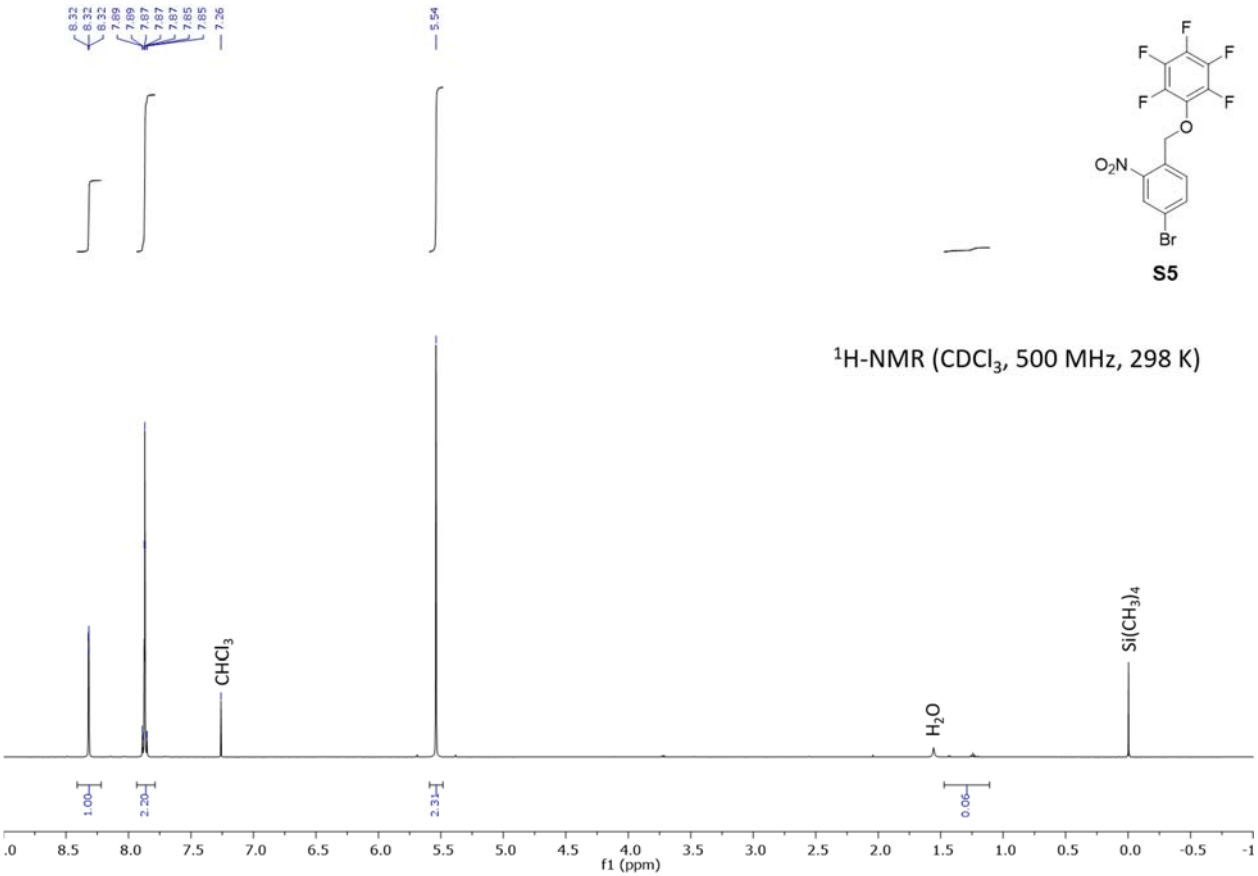
^{1}H-NMR ($CDCl_3$, 500 MHz, 298 K)

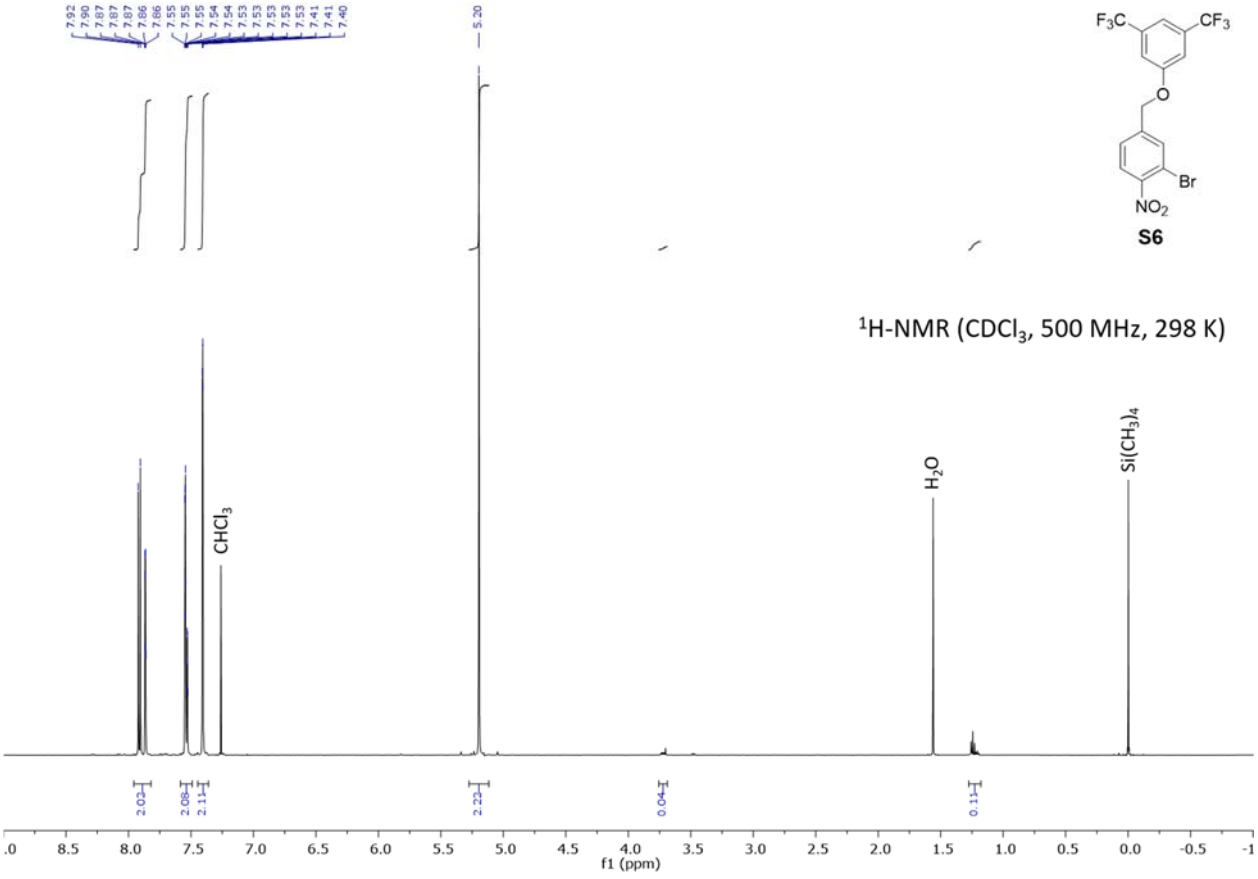


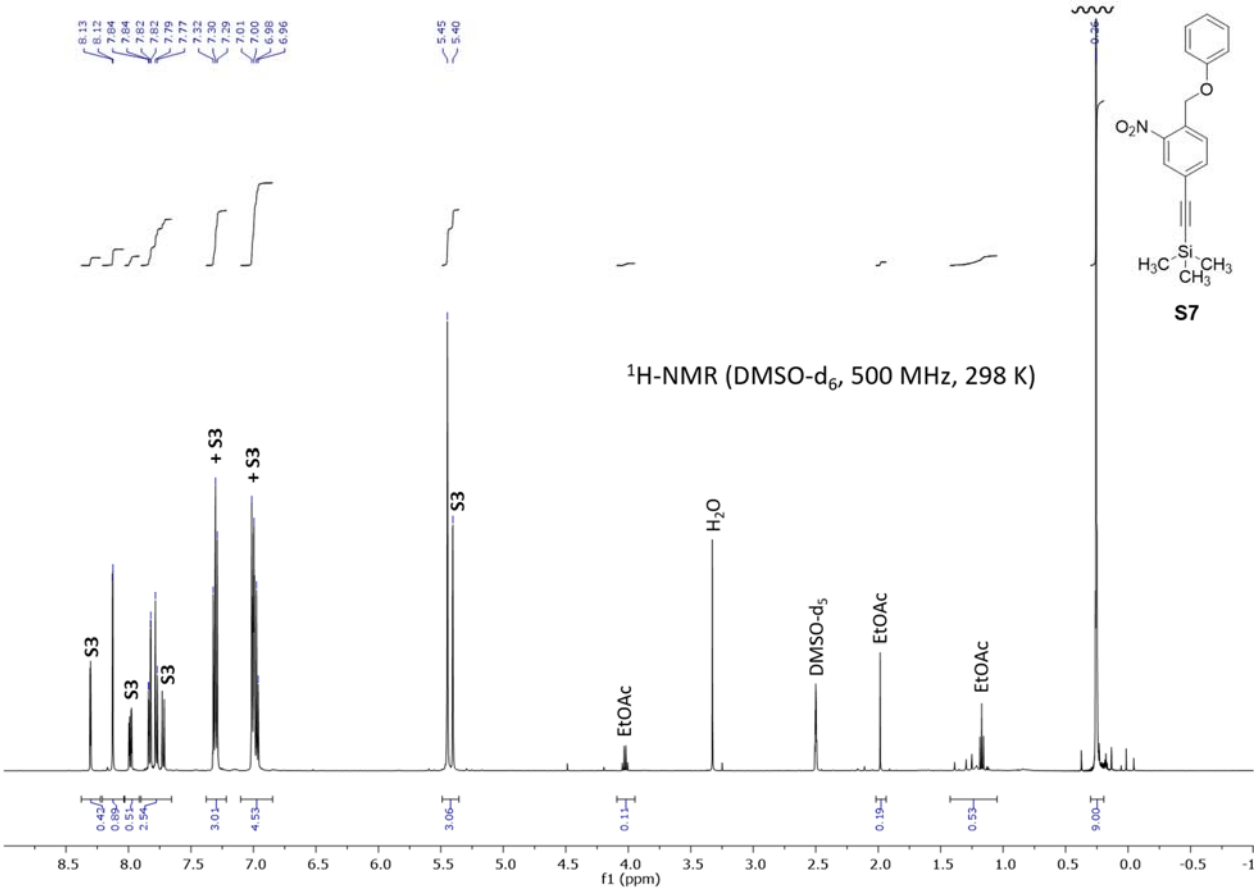




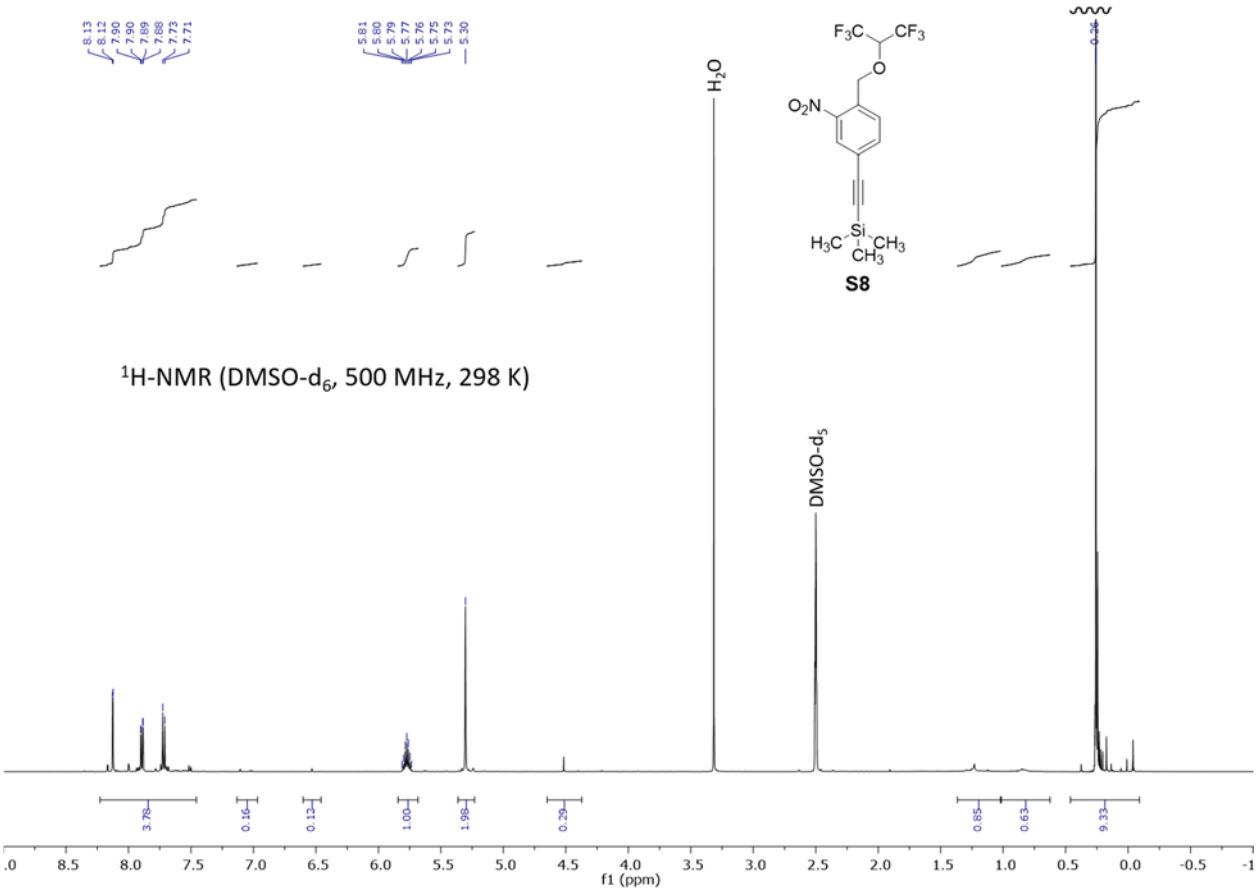
DIAD = diisopropyl azodicarboxylate


8.32
8.32
8.32
7.89
7.89
7.87
7.87
7.87
7.85
7.85
7.26
5.54
F
F
F
F
F
O
O2N
Br
S5
1H-NMR (CDCl3, 500 MHz, 298 K)
CHCl3
H2O
Si(CH3)4
1.00
2.20
2.31
0.06
f1 (ppm)

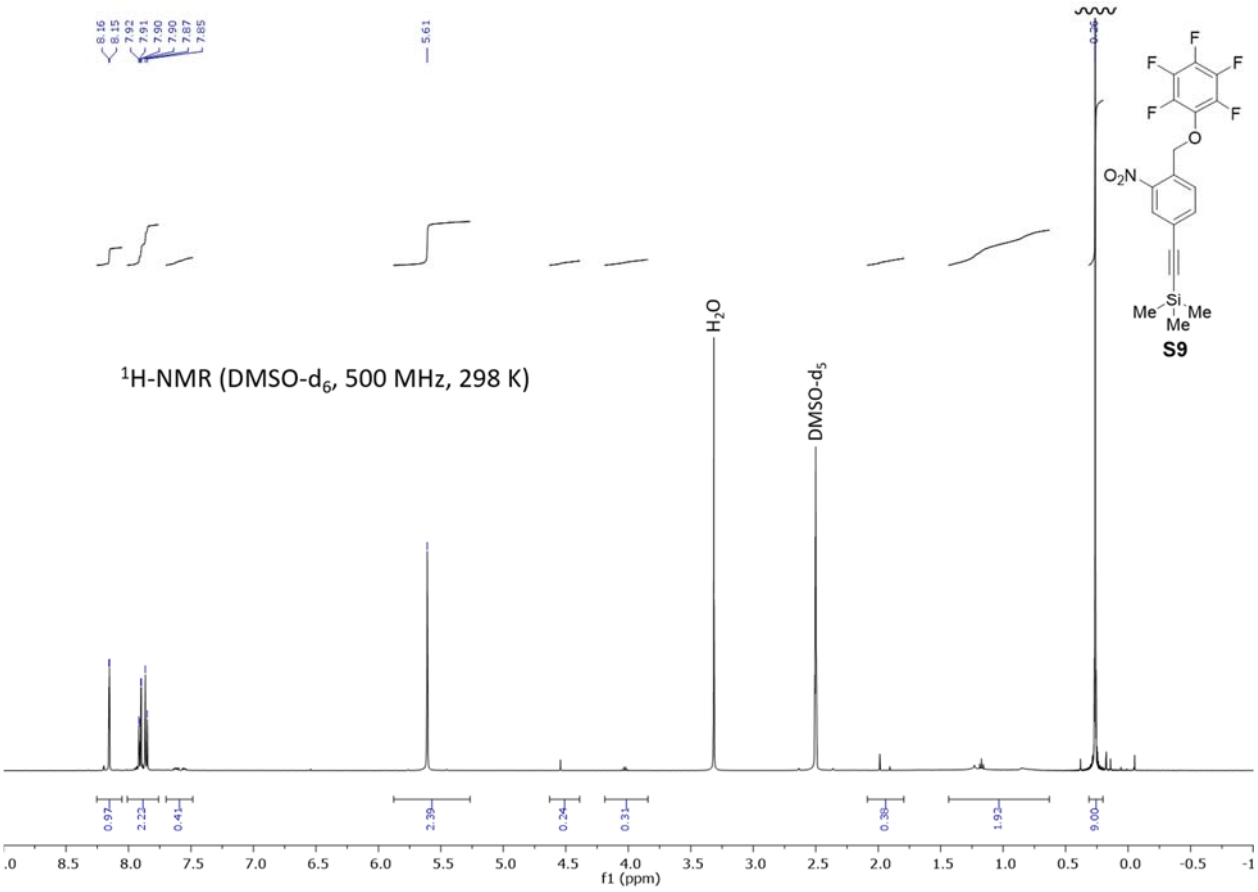

7.92
7.90
7.87
7.87
7.87
7.86
7.86
7.55
7.55
7.55
7.54
7.54
7.53
7.53
7.53
7.53
7.53
7.41
7.41
7.40
5.20
F3C
CF3
O
Br
NO2
S6
1H-NMR (CDCl3, 500 MHz, 298 K)
CHCl3
H2O
Si(CH3)4
2.02
2.08
2.11
2.22
0.04
0.11
f1 (ppm)


^{1}H-NMR (DMSO-d$_6$, 500 MHz, 298 K)
S3
+ S3
+ S3
S3
S3
S3
EtOAc
H$_2$O
DMSO-d$_5$
EtOAc
EtOAc
f1 (ppm)

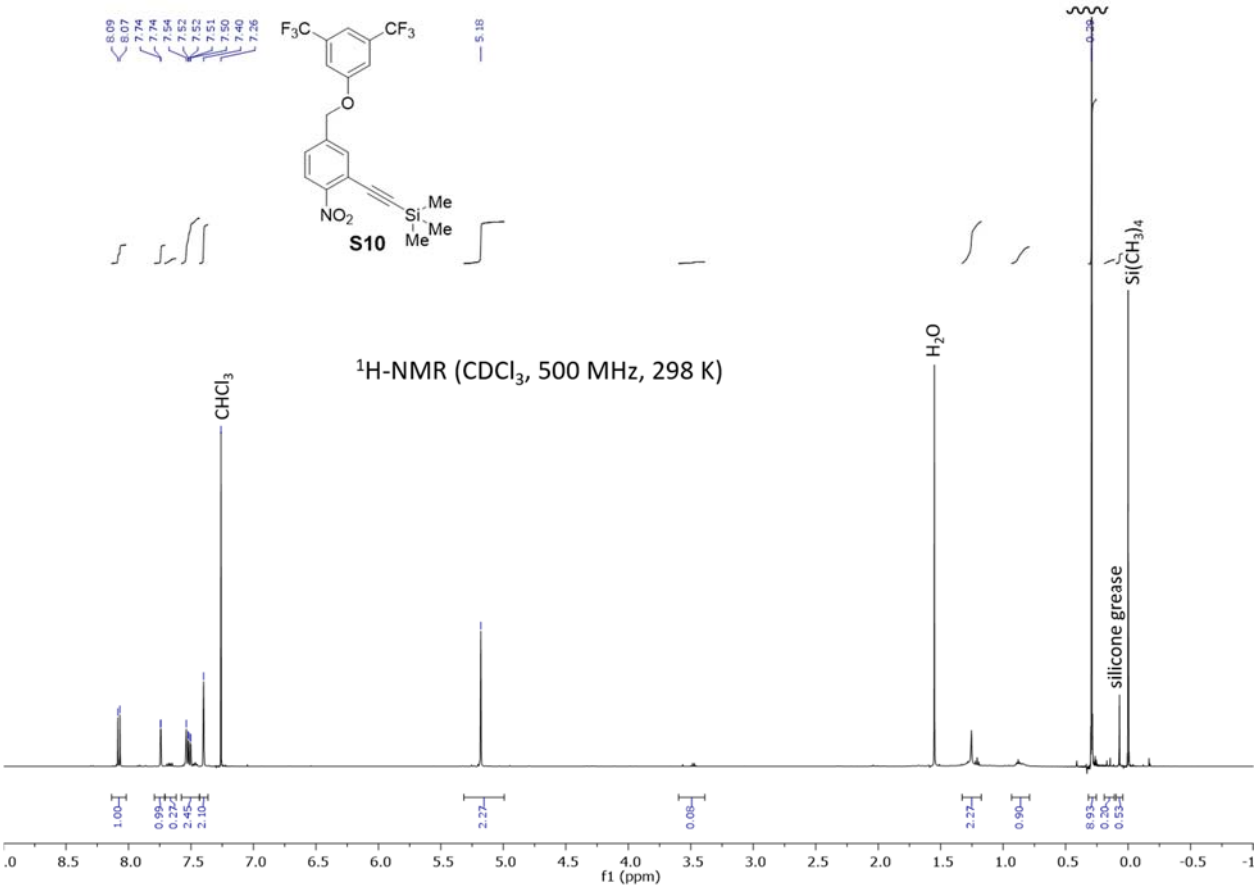
S7

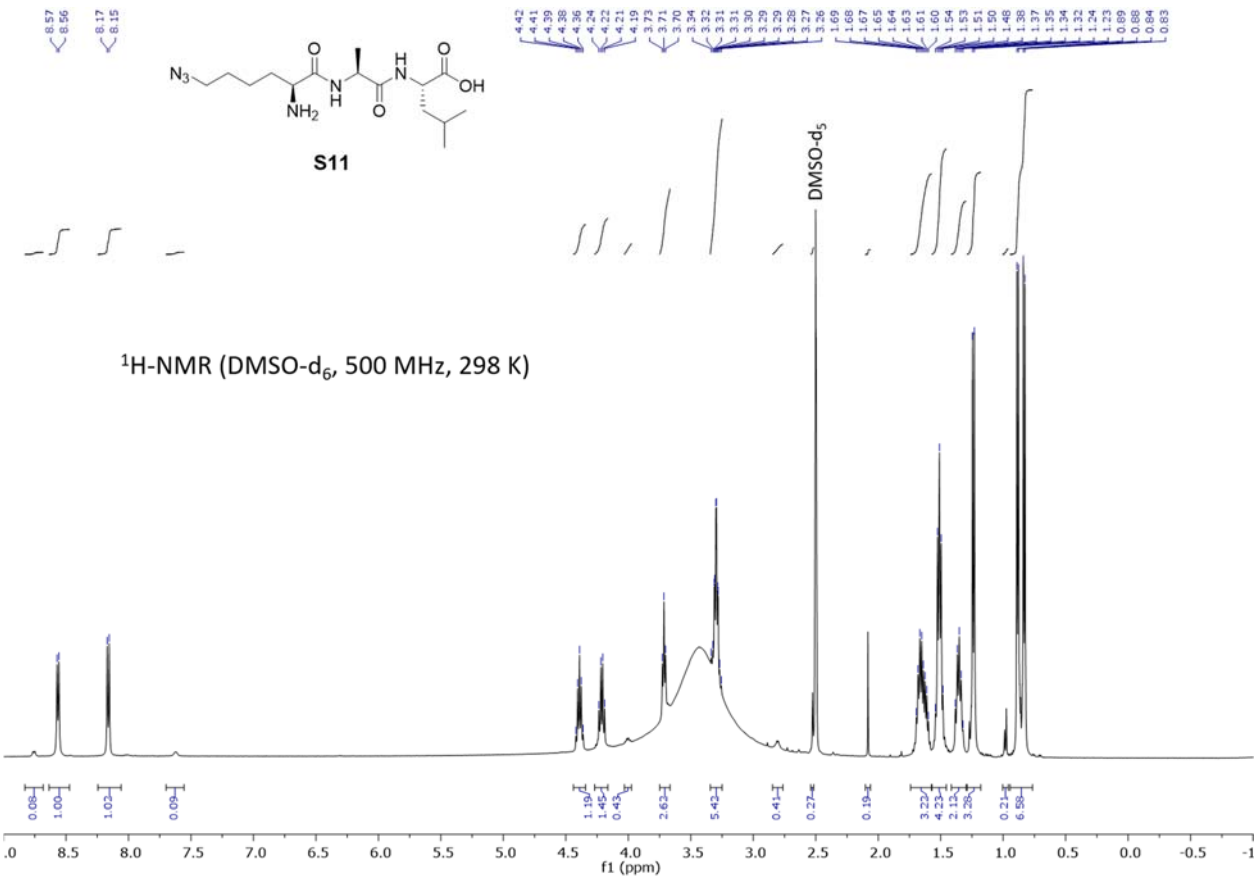

^{1}H-NMR (DMSO-d$_6$, 500 MHz, 298 K)
H$_2$O
DMSO-d$_5$
f1 (ppm)

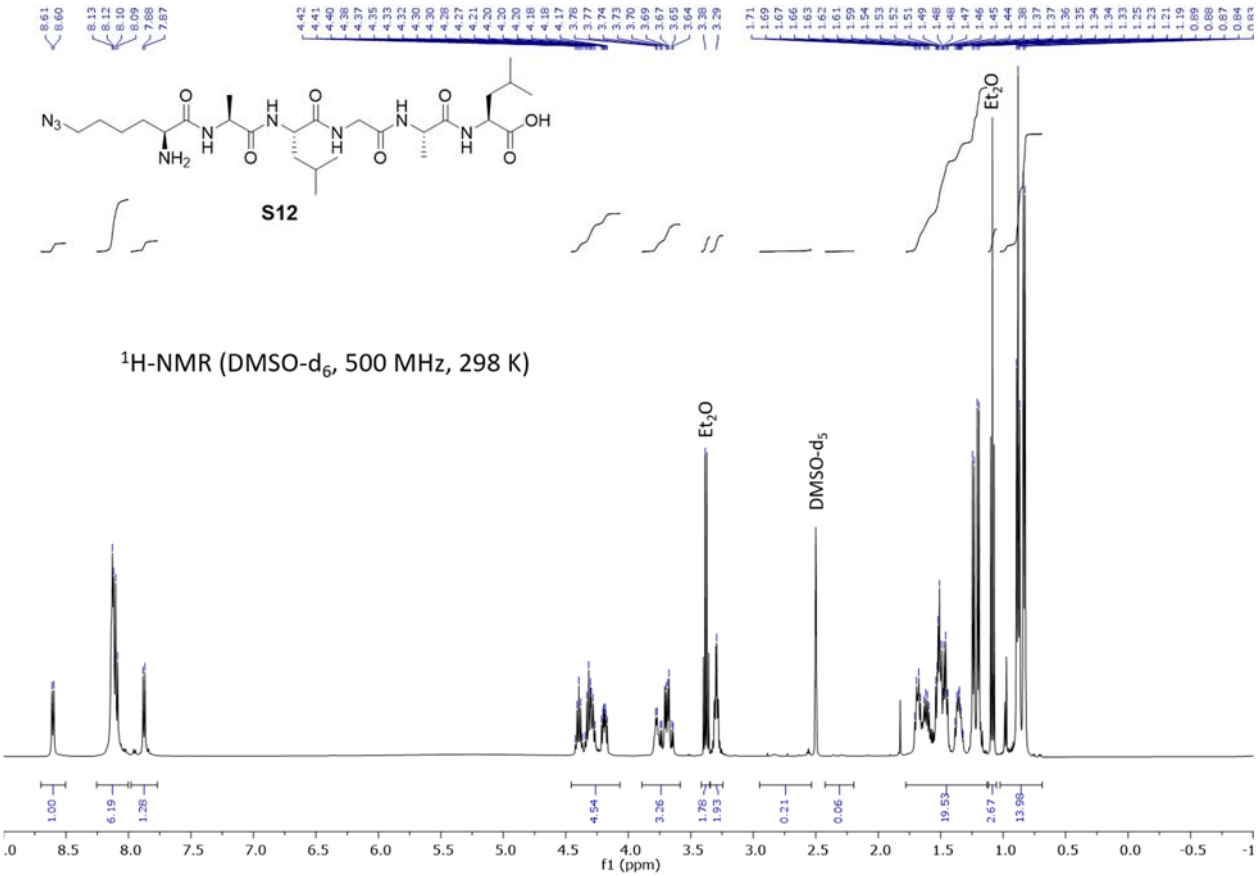
S8

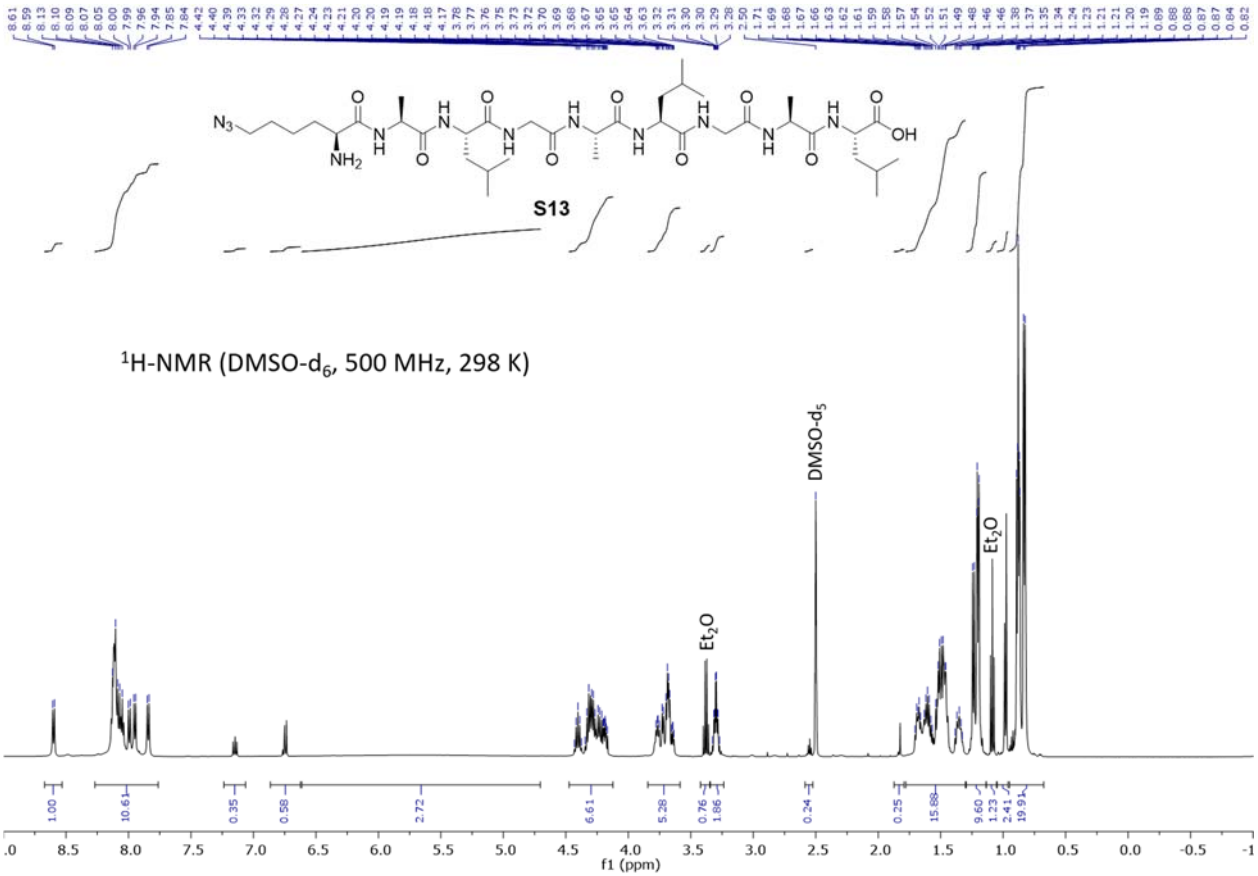


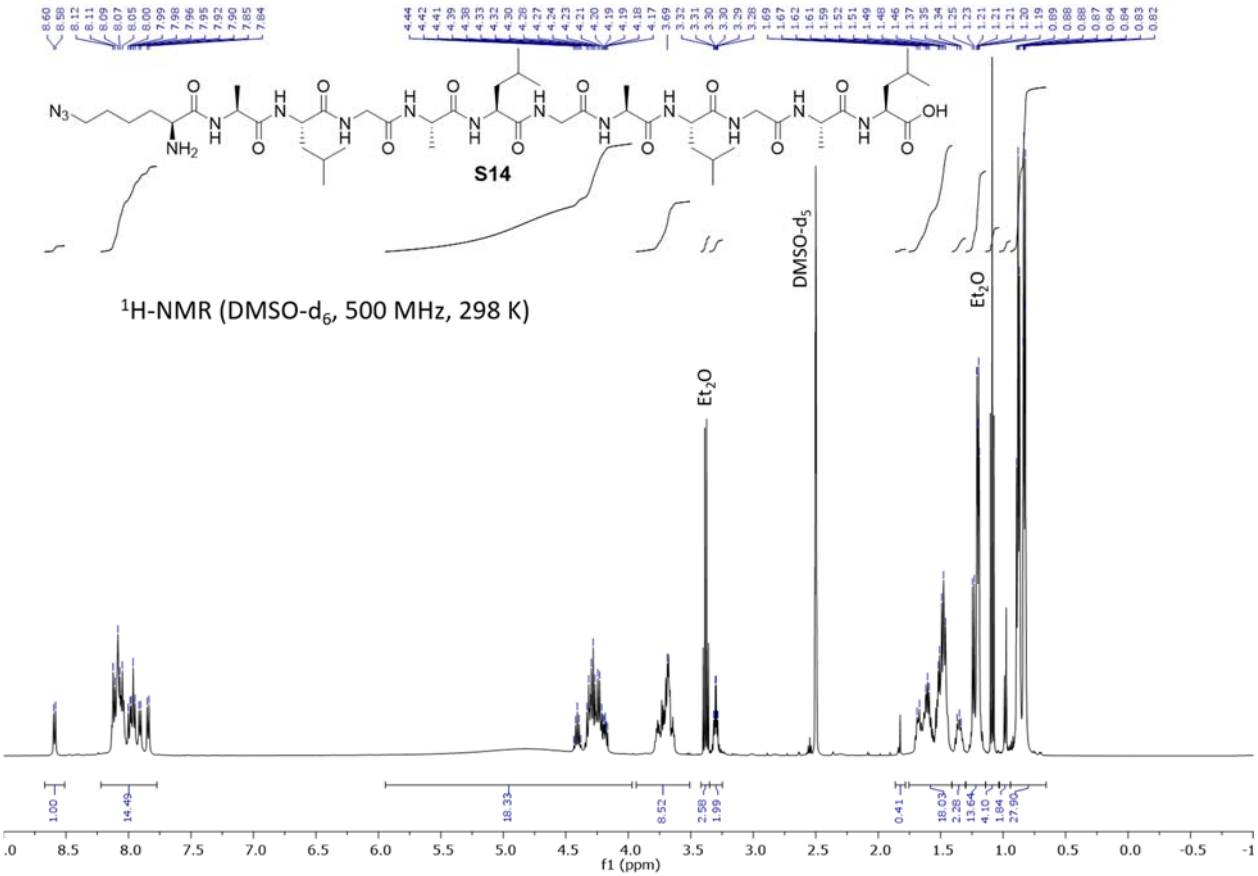
^{1}H-NMR (DMSO-d$_6$, 500 MHz, 298 K)

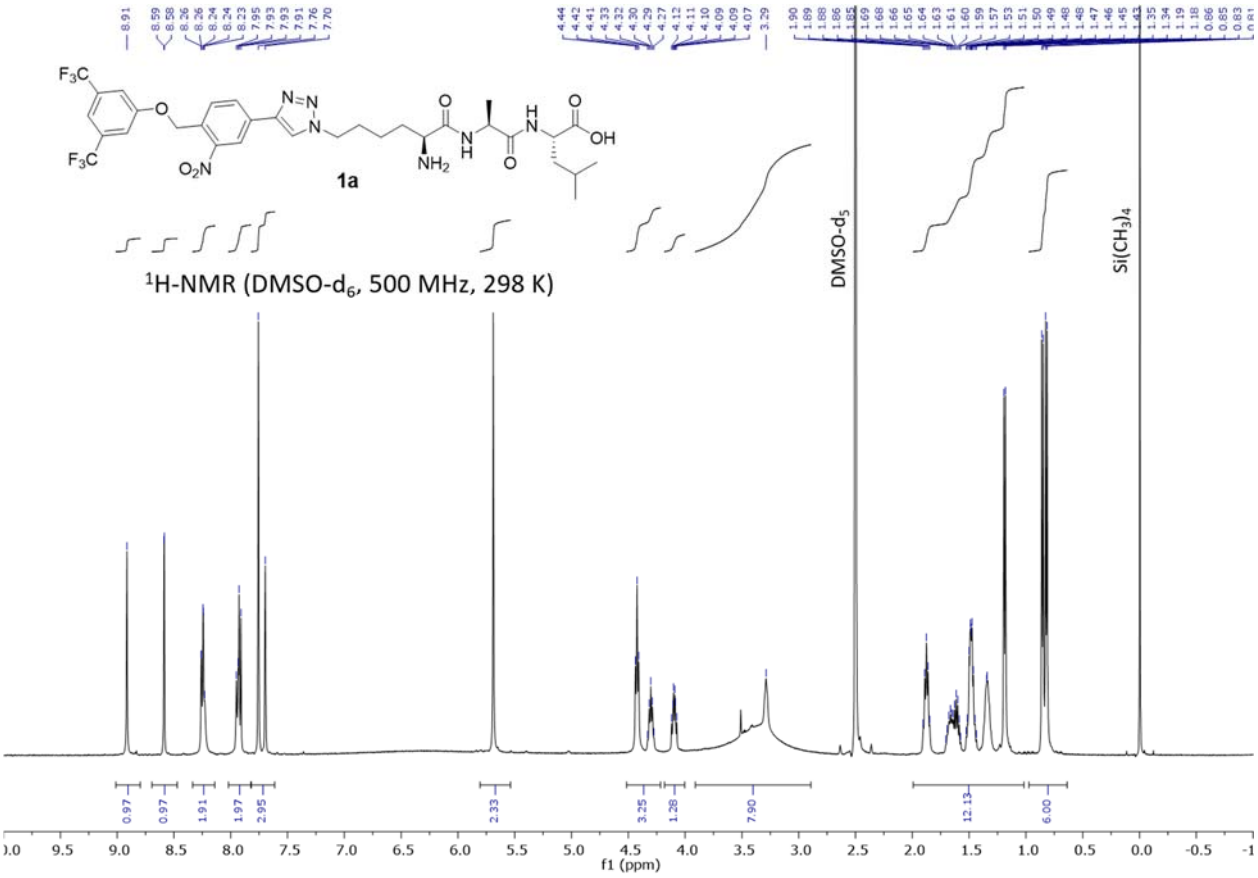

S10
^{1}H-NMR ($CDCl_3$, 500 MHz, 298 K)
$CHCl_3$
H_2O
$Si(CH_3)_4$
silicone grease
f1 (ppm)

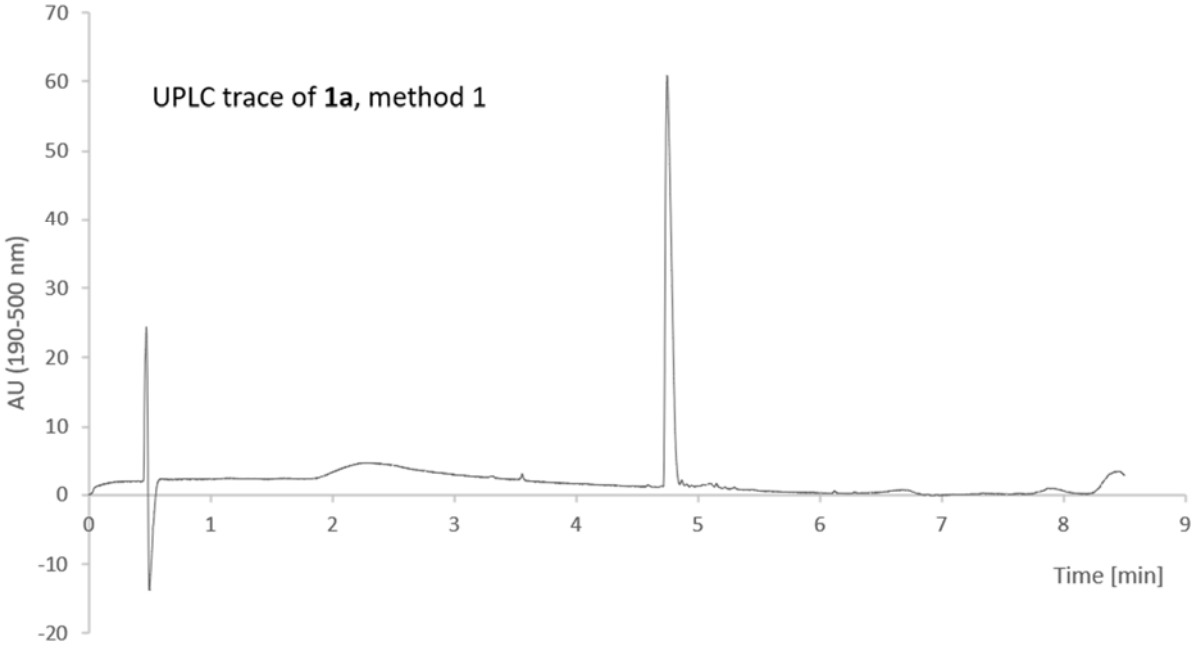

S11
^{1}H-NMR (DMSO-d_6, 500 MHz, 298 K)
DMSO-d_5
f1 (ppm)

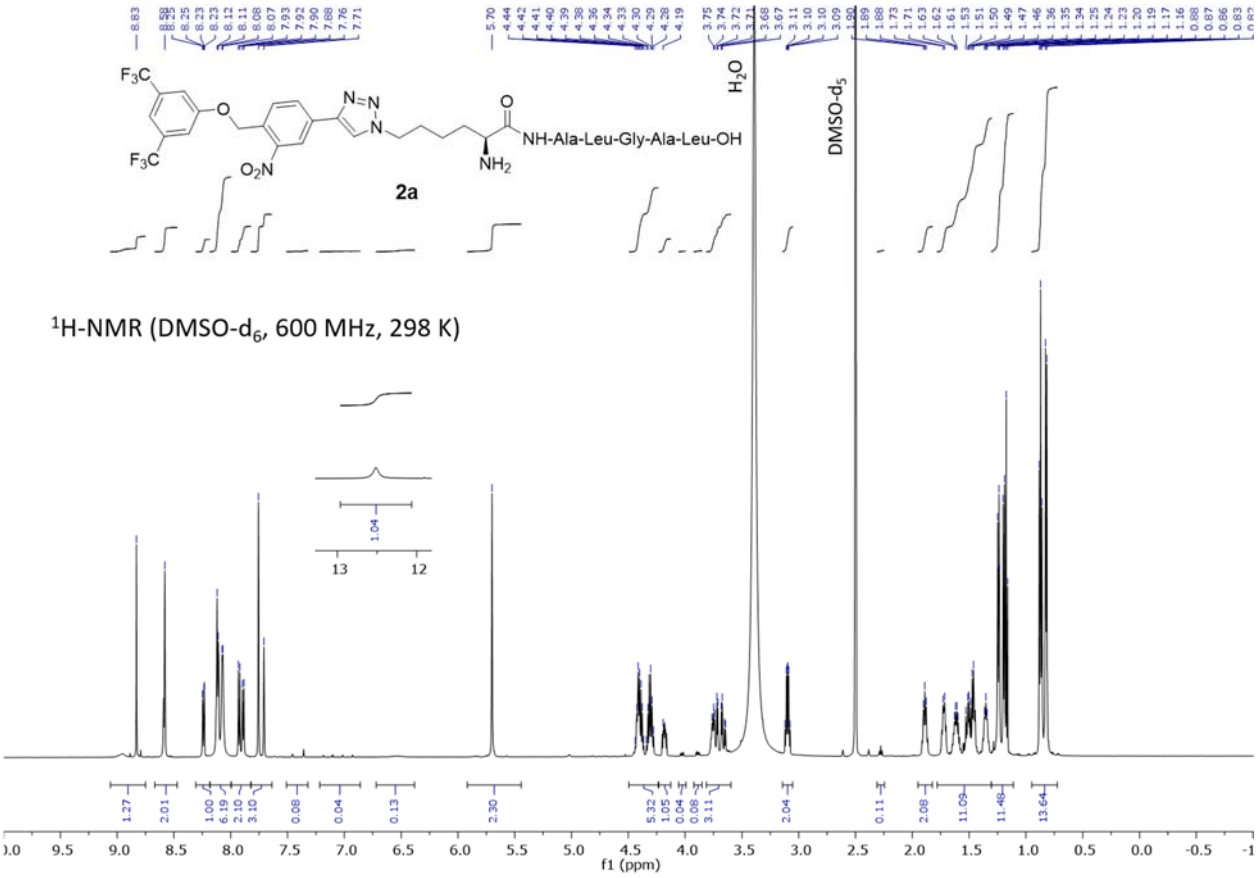

S12
^{1}H-NMR (DMSO-d$_6$, 500 MHz, 298 K)
Et$_2$O
DMSO-d$_5$
f1 (ppm)

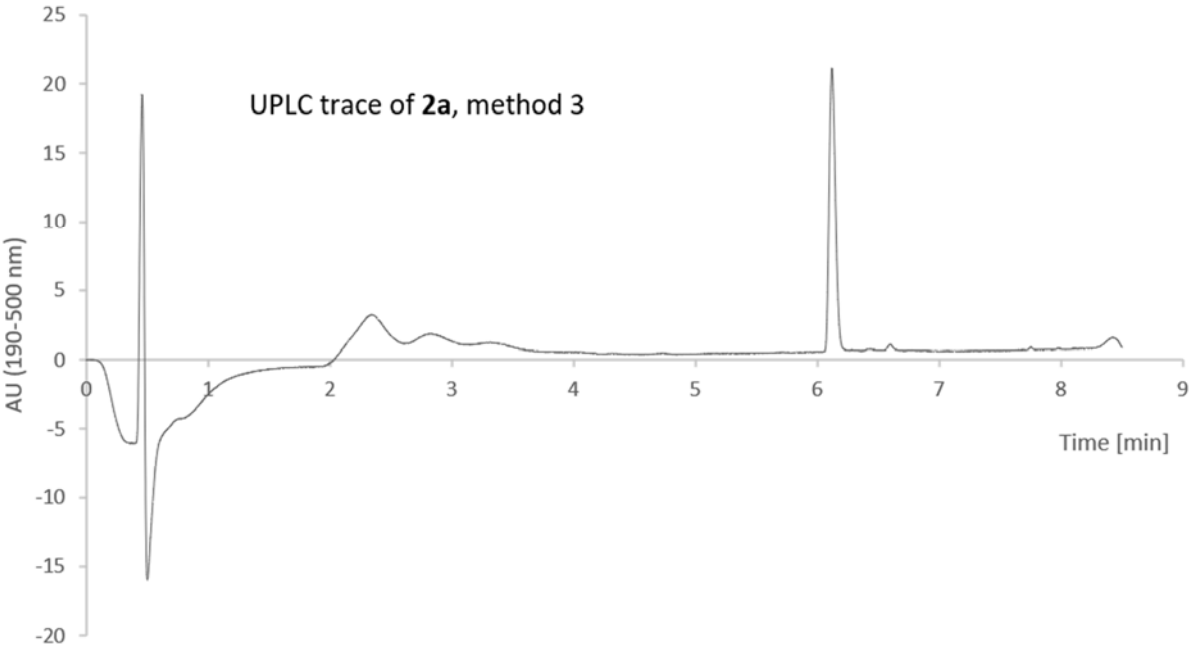

S13
^{1}H-NMR (DMSO-d$_6$, 500 MHz, 298 K)
DMSO-d$_5$
Et$_2$O
Et$_2$O
f1 (ppm)

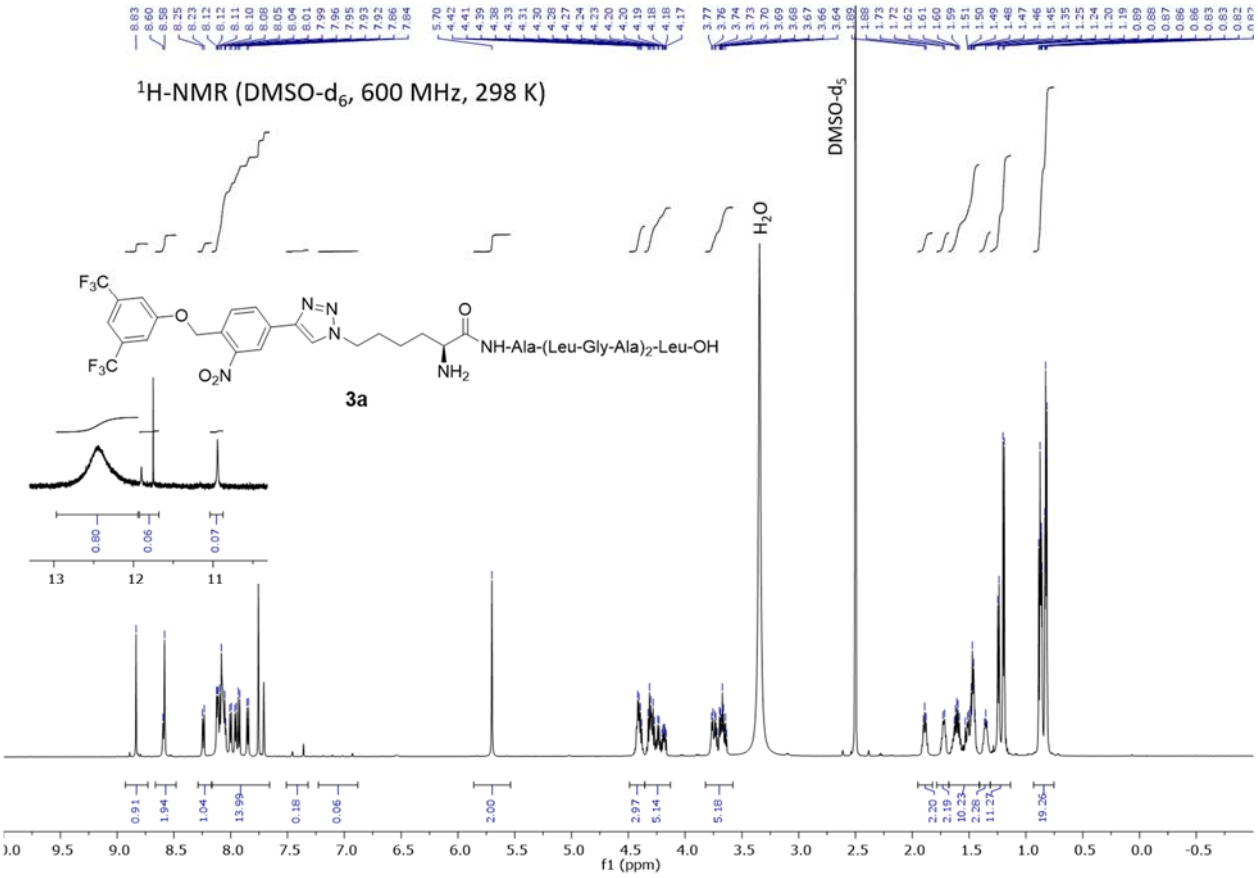

S14
^{1}H-NMR (DMSO-d$_6$, 500 MHz, 298 K)
DMSO-d$_5$
Et$_2$O
Et$_2$O
f1 (ppm)

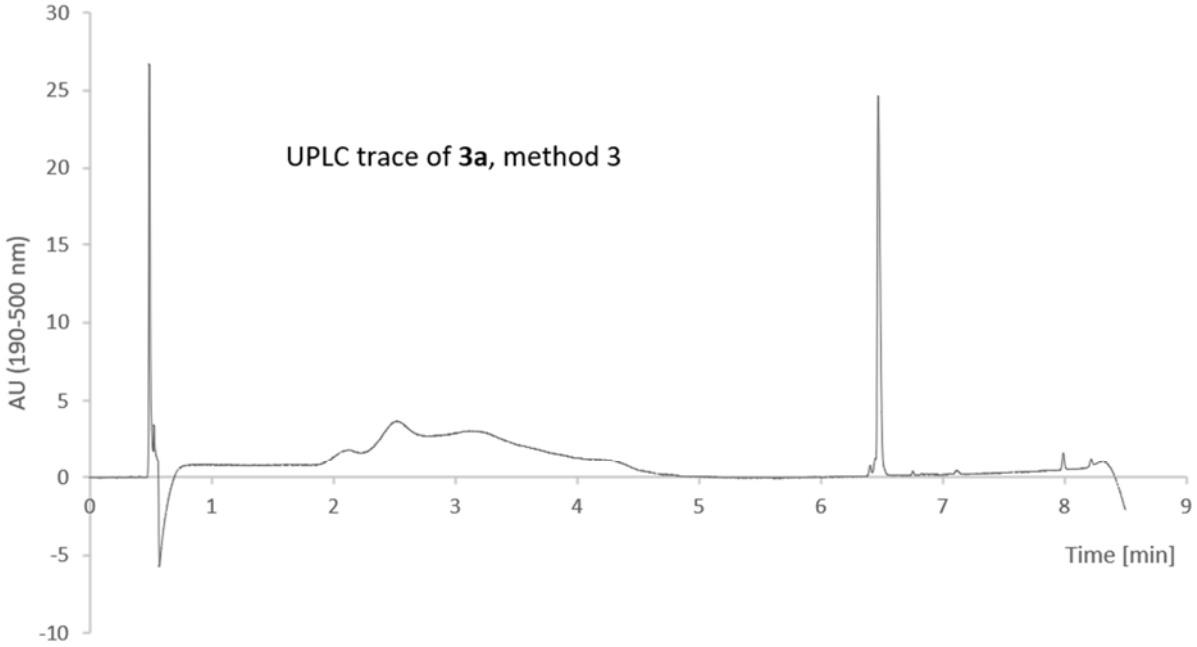

1a
DMSO-d5
Si(CH3)4
1H-NMR (DMSO-d6, 500 MHz, 298 K)
f1 (ppm)

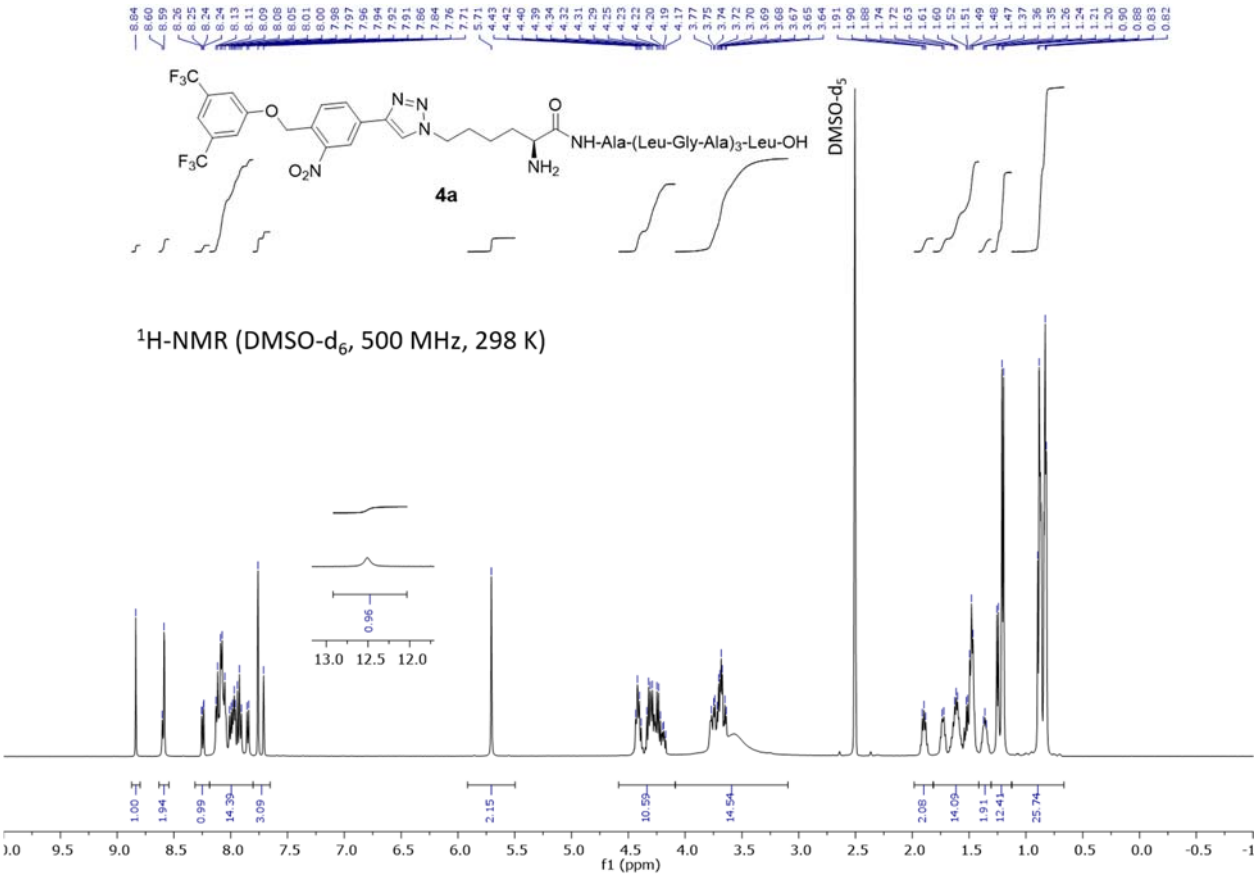

UPLC trace of 1a, method 1
AU (190-500 nm)
Time [min]

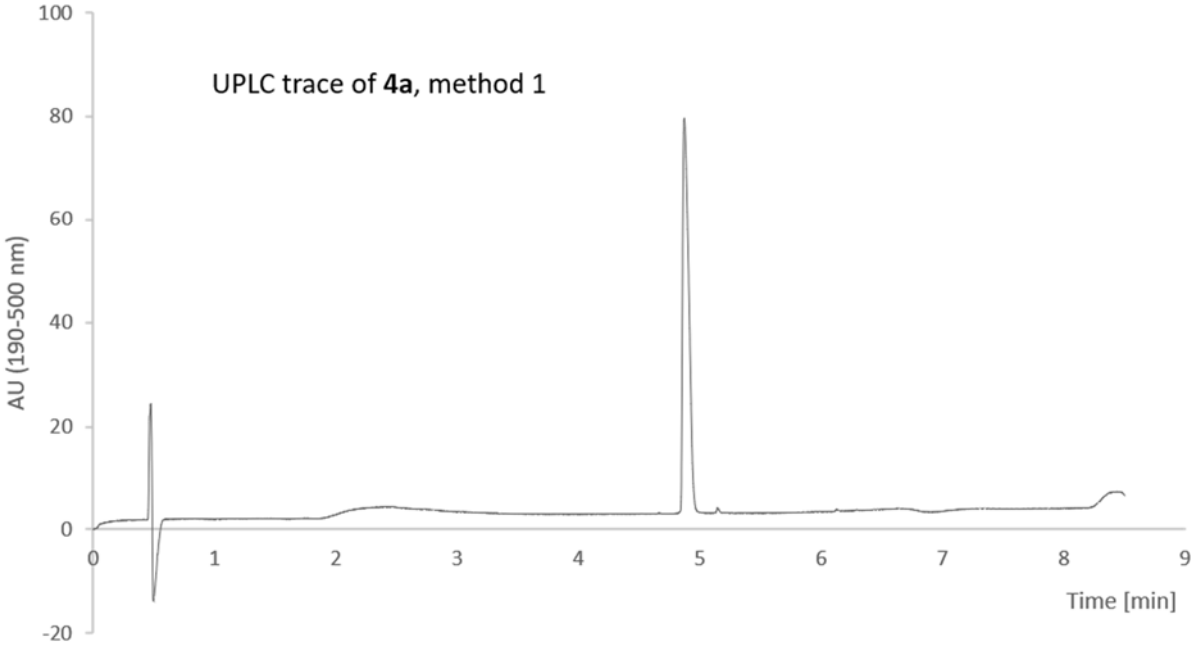

2a
NH-Ala-Leu-Gly-Ala-Leu-OH
H2O
DMSO-d5
1H-NMR (DMSO-d6, 600 MHz, 298 K)
f1 (ppm)


UPLC trace of 2a, method 3
AU (190-500 nm)
Time [min]


^{1}H-NMR (DMSO-d$_6$, 600 MHz, 298 K)
DMSO-d$_5$
H$_2$O
NH-Ala-(Leu-Gly-Ala)$_2$-Leu-OH
3a
f1 (ppm)


UPLC trace of **3a**, method 3
AU (190-500 nm)
Time [min]


F3C
F3C
O2N
NH-Ala-(Leu-Gly-Ala)3-Leu-OH
NH2
4a
DMSO-d5
1H-NMR (DMSO-d6, 500 MHz, 298 K)
f1 (ppm)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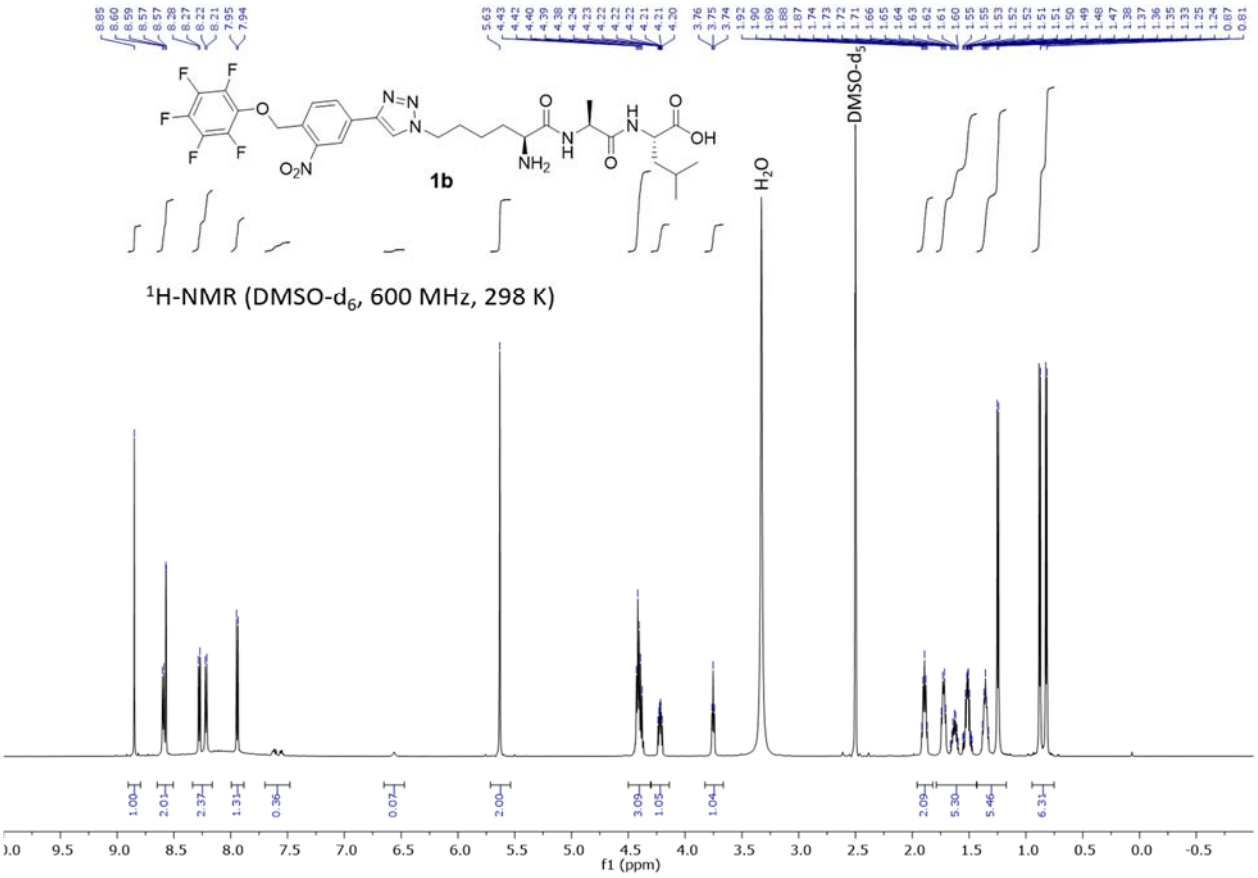
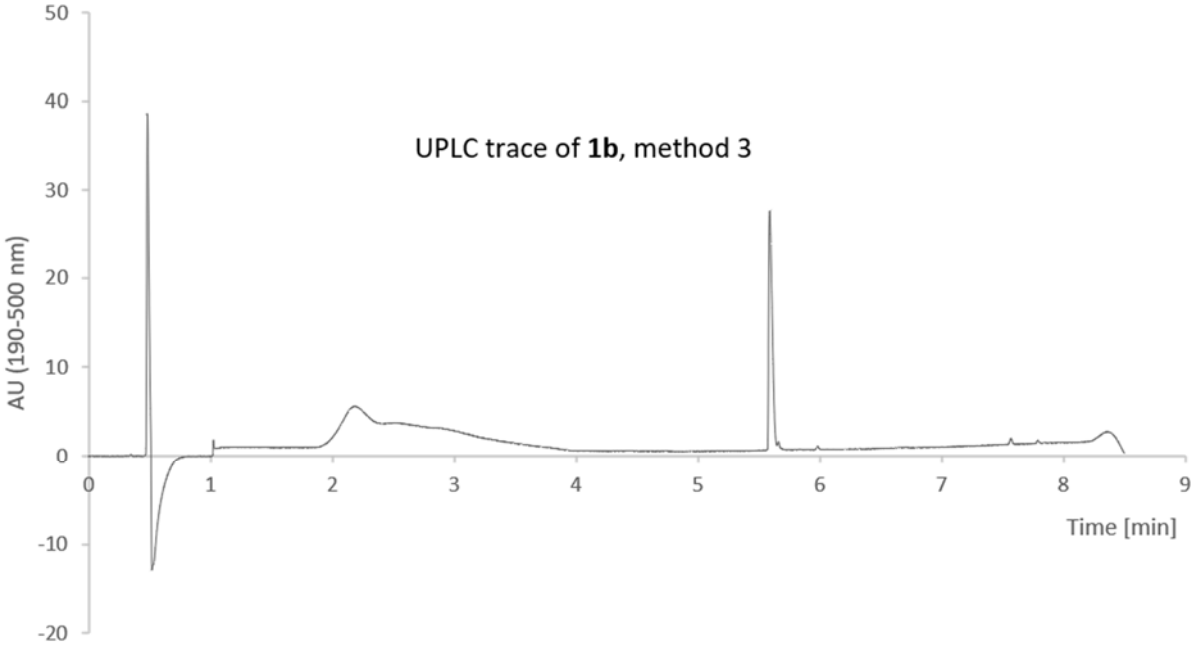
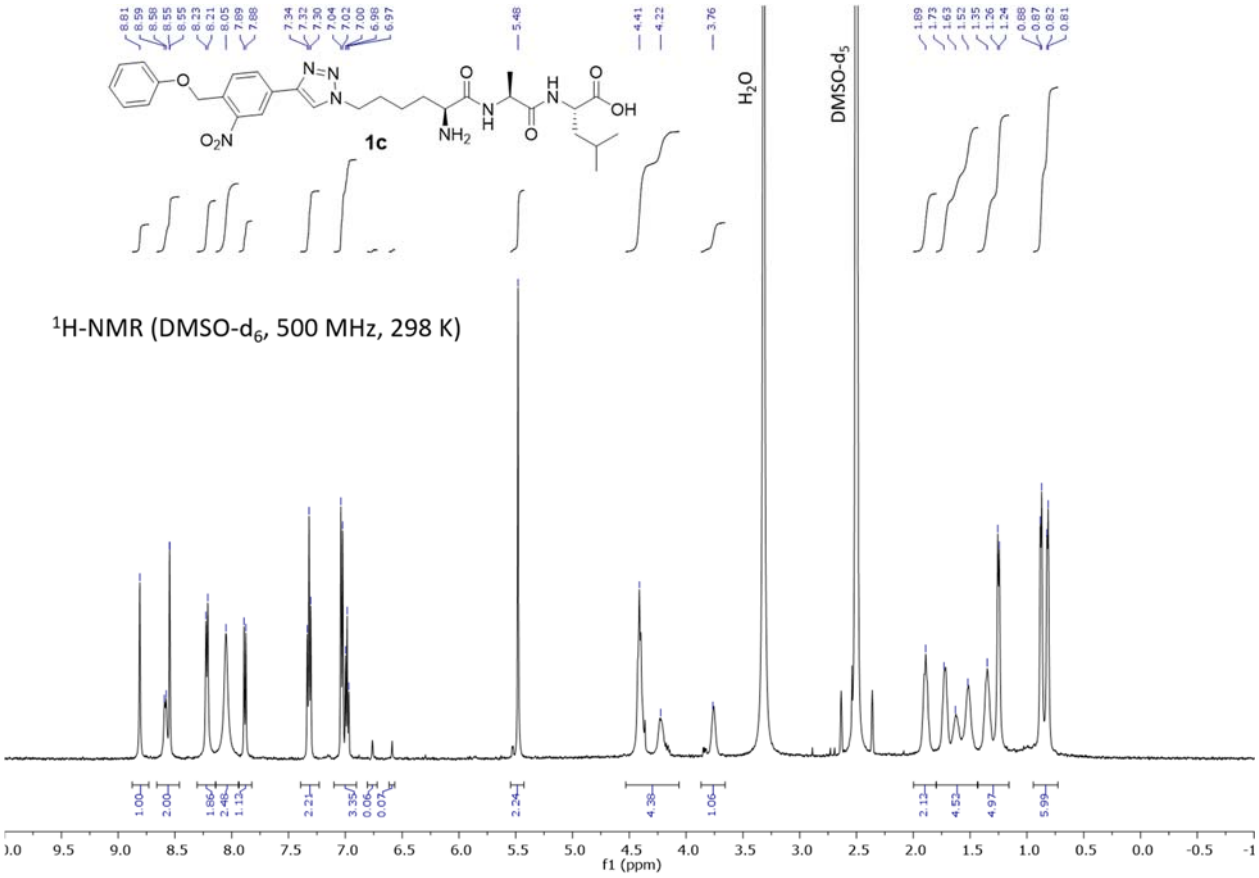
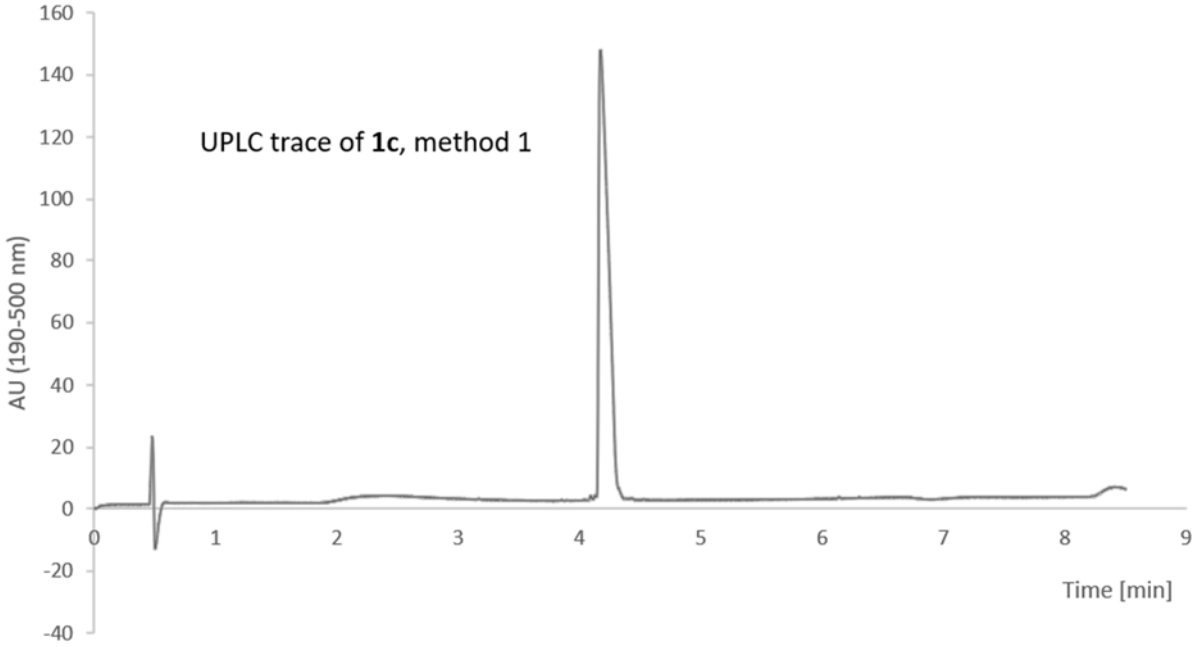
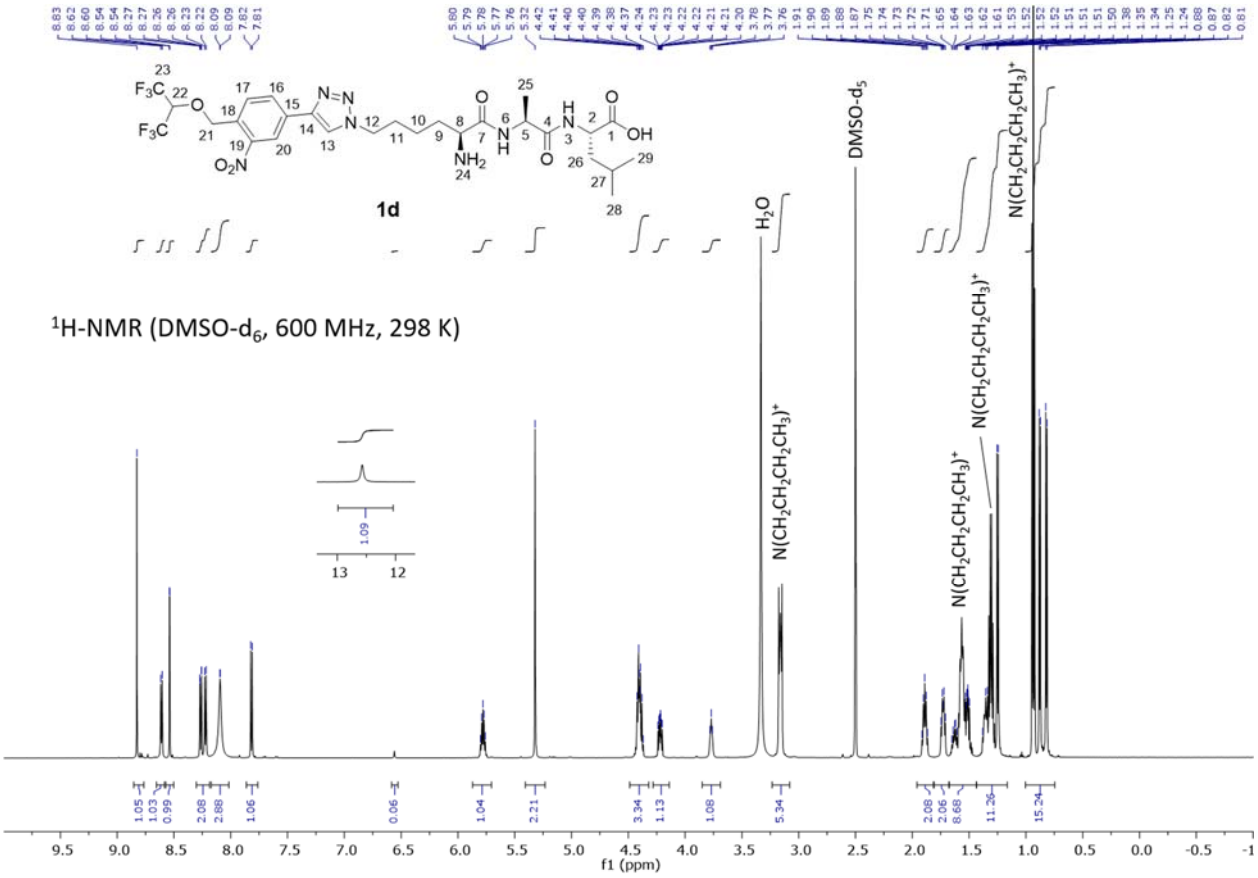
AU (190-500 nm)
Time [min]

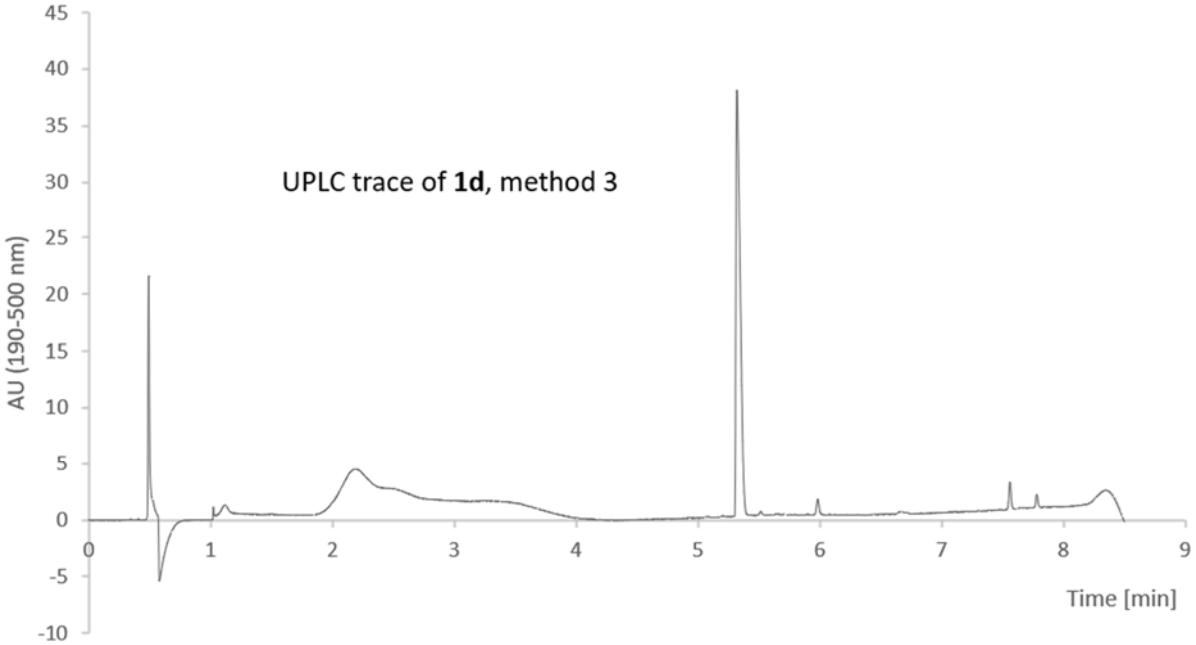

1b
DMSO-d5
H2O
1H-NMR (DMSO-d6, 600 MHz, 298 K)
f1 (ppm)

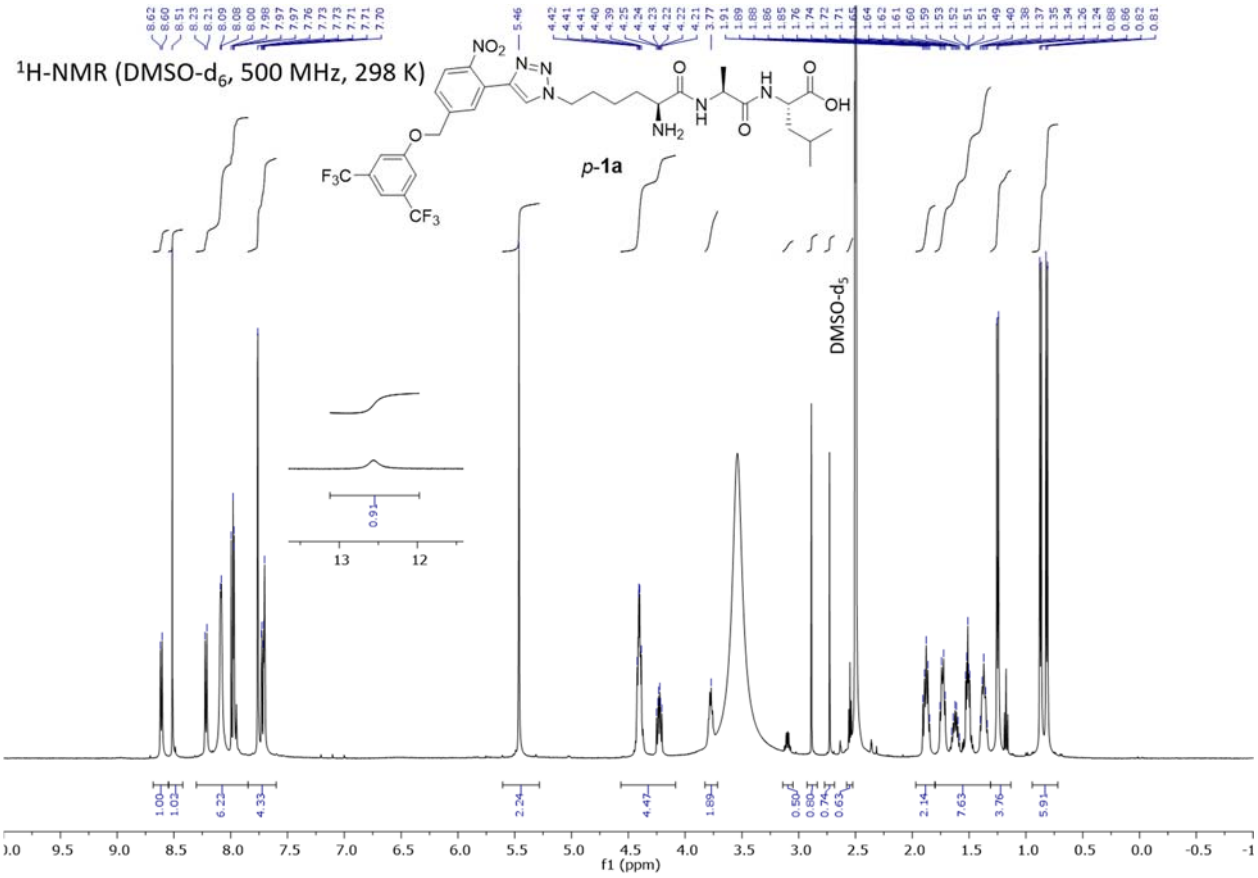

UPLC trace of 1b, method 3
AU (190-500 nm)
Time [min]

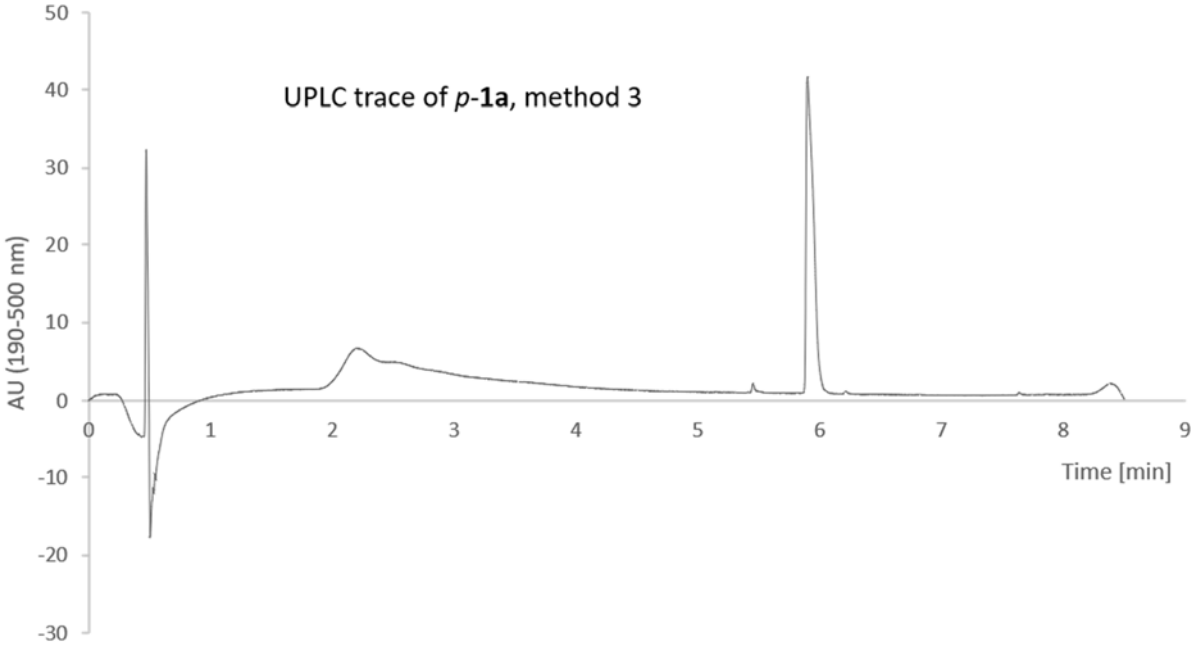

1c
H2O
DMSO-d5
1H-NMR (DMSO-d6, 500 MHz, 298 K)
f1 (ppm)

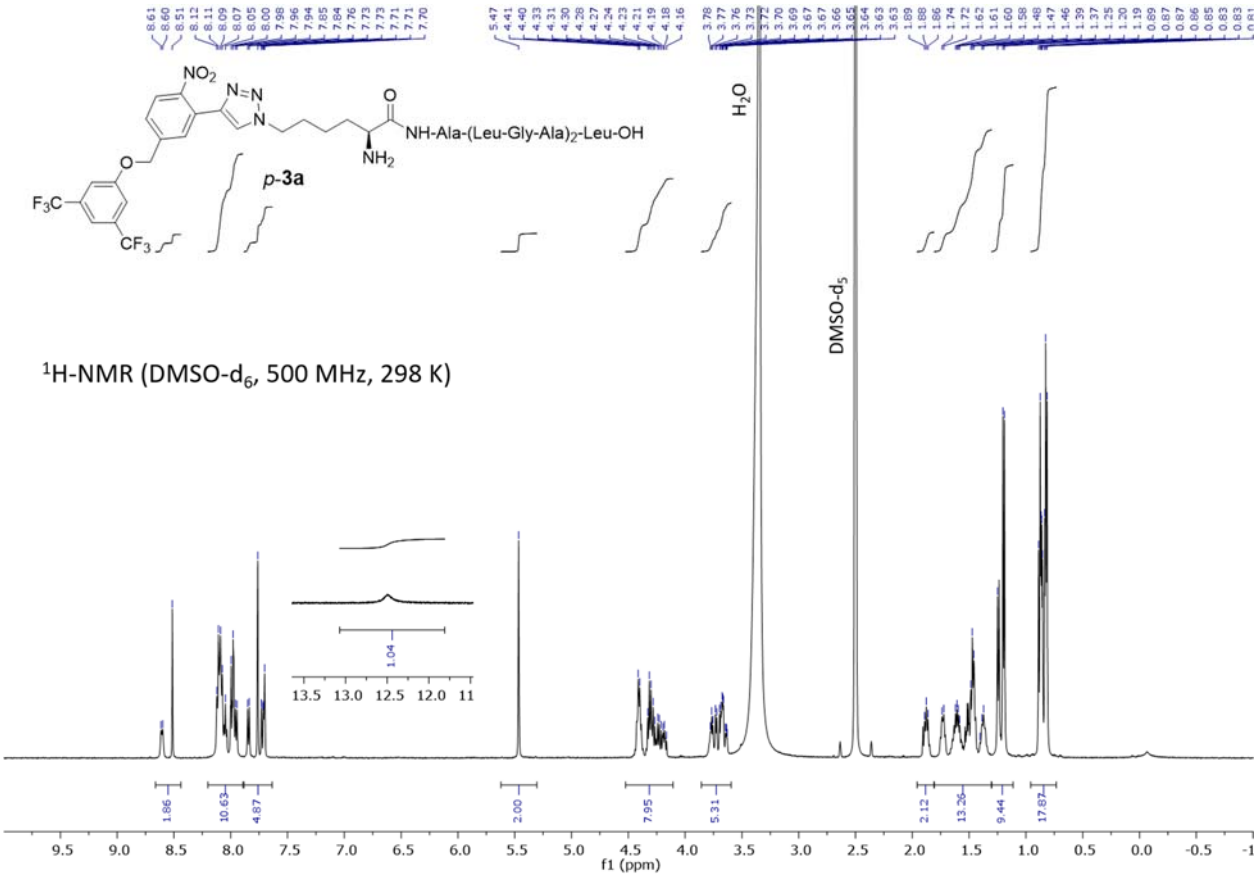

UPLC trace of 1c, method 1
AU (190-500 nm)
Time [min]

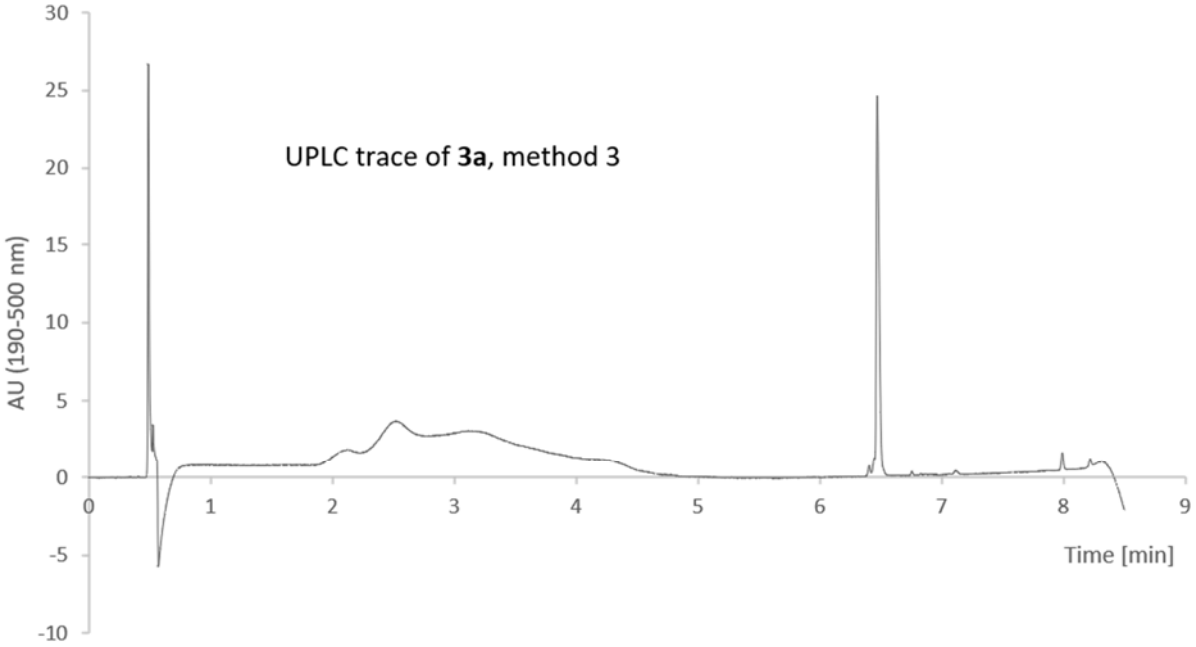

1d
^{1}H-NMR (DMSO-d$_6$, 600 MHz, 298 K)
H$_2$O
DMSO-d$_5$
N(CH$_2$CH$_2$CH$_2$CH$_3$)$^+$
f1 (ppm)


UPLC trace of **1d**, method 3
AU (190-500 nm)
Time [min]


¹H-NMR (DMSO-d6, 500 MHz, 298 K)
p-1a
DMSO-d5
f1 (ppm)


UPLC trace of p-1a, method 3
AU (190-500 nm)
Time [min]


NO2
N=N
N
O
NH-Ala-(Leu-Gly-Ala)2-Leu-OH
NH2
O
F3C
CF3
p-3a
H2O
DMSO-d5
1H-NMR (DMSO-d6, 500 MHz, 298 K)
1.04
13.5 13.0 12.5 12.0 11
1.86
10.63
4.87
2.00
7.95
5.31
2.12
13.26
9.44
17.87
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1
f1 (ppm)


UPLC trace of 3a, method 3
AU (190-500 nm)
30
25
20
15
10
5
0
-5
-10
0 1 2 3 4 5 6 7 8 9
Time [min]

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