A Photoswitchable Tryptophan Zipper – (Un)folding Fibrils in Seconds

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Supporting Information

Contents

1. Materials and Methods

1.1. Solvents and Reagents

All reagents and chemicals were used as supplied. Dry solvents were obtained from Acros Organics (now Thermo Fisher Scientific, Nidderau, Germany) and stored over molecular sieves (4 Å). Solvents for HPLC were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany) in HPLC grade (99.9%). DMF for peptide synthesis was purchased from Fisher Scientific GmbH (Schwerte, Germany) in peptide grade (99.8%). Other solvents were used from Carl Roth GmbH + Co. KG (Karlsruhe, Germany), VWR International GmbH (Darmstadt, Germany), and Sigma-Aldrich Chemie GmbH in technical grade. Ultra-pure, demineralized water ($^{demi}H_2O$) was obtained by purifying water using the arium® mini from Sartorius AG (Göttingen, Germany). Fmoc- or Boc-protected amino acids (AAs) were obtained from Carbolution Chemicals GmbH (St. Ingbert, Germany), BLD Pharmatech GmbH (Kaiserslautern, Germany), Bachem AG (Bubendorf, Switzerland), Sigma-Aldrich Chemie GmbH and GL Biochem (Shanghai, China). Resins and coupling reagents were purchased from Novabiochem, Merck KgaA (Darmstadt, Germany), Iris Biotech GmbH (Marktredwitz, Germany), Sigma-Aldrich Chemie GmbH, Carl Roth GmbH + Co. KG, Carbolution Chemicals GmbH and Fisher Scientific GmbH. Other chemicals were purchased from Acros Organics, BLD Pharmatech GmbH, Sigma-Aldrich Chemie GmbH, Fluorochem (Dublin, Ireland) and abcr GmbH (Karlsruhe, Germany) as well as TCI Deutschland GmbH (Eschborn, Germany), J&K Scientific LLC (San Jose, USA) and Merck KgaA. Liquid nitrogen for freezing samples and cooling reactions as well as Argon was provided by Air Liquide (Düsseldorf, Germany).

1.2. General Methods

Reactions

Reactions sensitive to water and air were executed under Schlenk conditions. Therefore, air was removed with a vacuum pump from the apparatus and the line was ventilated with argon gas (> 99.996%). In between, the glassware was heated with a heat gun under reduced pressure to remove any remaining humidity. The process was repeated three times.

Solid Phase Peptide Synthesis

For the synthesis of peptide strands, a Liberty Blue Peptide Synthesizer from CEM was used. Rink Amide with a 0.37 mmol/g loading was used as solid support and always swollen for 30 minutes in DMF prior to loading into the synthesizer. All amino acids were dissolved in DMF to a 0.2 M final concentration with the exception of the photoresponsive one, which was

dissolved into a 1:5 mixture of DMSO/NMP and filtered prior to loading. The solutions were prepared into 50 mL Falcon Tubes.
Activator, activator base and deprotection solutions were prepared in pressure-resistant glass bottles according to CEM recommendations.
All solutions were prepared fresh for every synthesis.

Lyophilization

To remove water from samples and gently dry peptides, an *Alpha 2-4 LD plus* lyophilizer with an attached centrifuge, model *AVC 2-18 CD plus* from *Christ* (Osterode am Harz, Germany) was used. Samples were frozen in liquid nitrogen and dried under reduced pressure.

Aliquoting of Peptides

To prepare 25 or 50 nmol aliquots of the peptides, the lyophilized pure compound obtained after HPLC was redissolved into a 50:50 mix of $^{demi}H_2O$ + 0.1% TFA and MeCN + 9.1% TFA. Precise mL amount of the solution were collected into Eppendorf tubes and lyophilized once more.

Storage

Crude and pure peptides were kept at room temperature for up to one week. For long-term storage, they were stored in a freezer at – 20 °C. Non-preloaded resins were stored in a fridge at 8 °C, preloaded resins were kept in a desiccator under vacuum at room temperature. Sensitive chemicals were layered with argon or nitrogen and stored at room temperature or, if not moisture sensitive, in the fridge. Buffers were stored in the fridge up to six weeks. All other chemicals were handled as recommended by the supplier.

Centrifugation

After peptide precipitation in diethyl ether, peptides were centrifuged in a *Thermo Scientific Haraeus Megafuge 8R* at 8000 rpm for 5 min or in a benchtop centrifuge at maximum speed for 5 min.

1.3. Chromatographic Methods

Flash Chromatography

The purification of small organic compounds was performed using a packed glass column with Geduran® silica gel 60, 230 – 450 mesh from *Merck KgaA* at an overpressure of 0.5 to 0.8 bar. The sample was loaded either after being absorbed onto silica gel, or in solution. Alternatively to manual column chromatography, an automated flash chromatography system, namely the Isolera One™ from *Biotage* (Uppsala, Sweden) was used. Hereby, the sample was loaded onto the commercially available prepacked *Sfär* silica columns either by

dry or liquid loading and collected with an autosampler after detection at 220 nm and 254 nm.

High-Performance Liquid Chromatography (HPLC).

Semi-preparative reverse-phase HPLC purifications were performed with a system from JASCO (Tokyo, Japan), consisting of two pumps PU-2020Plus, a 3-line degasser DG2080-53, and a diode array detector MD-2010Plus.
Crude compounds were eluted with a linear gradient of two phases: phase A ($^{demi}H_2O$ + 0.1 % TFA) and phase B (MeCN + 0.1 % TFA). The column was placed in an external oven model 2155 from *Pharmacia LKB*. An MN Nucleodor 100-5-C18, 250mm x 10mm, 5µm column from MACHEREY-NAGEL (Düren, Germany) was used for purification, with a flow rate of 3mL/min. UV detection was measured at 220 and 254 nm for unmodified peptides. For photoswitchable peptides, absorbance was also monitored at 365 nm.
Detailed information regarding the peptide purification can be found in the Standard Operating Procedures section.

Ultra High-Performance Liquid Chromatography (UHPLC)

To analyze the purity of peptides, samples were run on an UltiMate 3000 UHPLC system from *Thermo Fisher Scientific* and injected by an autosampler with an injection volume of 10–20 µL. Samples were filtered prior to use. As mobile phase, water with trifluoroacetic acid (TFA) and MeCN, *i.e.* $^{demi}H_2O$ +0.1% TFA (A) and MeCN+0.1% TFA (B), were used. The UHPLC was equipped with a C18 column (10 x 2.1 mm, 2 µm, 100 Å) from *Avantor* and peptides were detected with a diode array detector mainly at 215 and 245 nm. As gradient, 30–70% B in 15 min at 50 °C (UHPLC Method A) was used, with a total running time of 20 min and a flow of 0.3 mL/min. The analysis of the chromatograms was performed with the software Chromeleon 7.

Liquid Chromatography – Mass Spectrometry (LC-MS)

For simultaneous separation and identification of compounds, HPLC was combined with mass spectrometry. Chromatographic analysis was performed on an *Agilent* 1260 Infinity II LC system (*Agilent Technologies Deutschland GmbH*, Waldbronn, Germany) with an attached LC MSD iQ mass section. Samples were injected with a volume of 1 µL and separated over an *Agilent* Poroshell 120 ES C18 column (50 x 2.1 mm, 2.7 µm, 120 Å). As mobile phase, water with formic acid (FA) and MeCN, *i.e.* $^{demi}H_2O$ + 0.1% FA as A and MeCN+0.1% FA as B, were used. As gradient, 5-95% B in 10 min (LC-MS method A) with a flow of 0.5 mL/min at 40 °C was used.

1.4. Characterization Methods

1.4.1. Mass spectrometry (MS)

Electrospray ionization (ESI) mass spectra and high-resolution ESI (HR-MS) spectra were recorded at a maXis or MicroTOF spectrometer by BRUKER 42 DALTONIK GMBH (Bremen, Germany). The data were analyzed with Compass Data analysis software (version 4.0) by Bruker. The samples were dissolved in $^{demi}H_2O$, $^{demi}H_2O$ MeCN, or MeCN. The values are given in m/z ratio, along with the relative intensity of the peak.

1.4.2. Nuclear magnetic resonance spectroscopy (NMR)

NMR of unnatural amino acids and their precursors

^1H and ^{13}C NMR spectra for characterizing the unnatural amino acids and their precursors, were recorded on a Bruker Avance III HD 400 or Avance III HD 300 at 25 °C at frequencies of 300 or 400 MHz, respectively, (^1H-NMR) and 75 or 101 MHz, respectively, (^{13}C-NMR). Chemical shifts (δ) are given in parts per million (ppm) relative to the residual solvent signal (for ^1H detection, δ = 7.26 ppm ($CDCl_3$), 1.94 (CD_3CN), 3.31 (CD_3OD), 2.50 (DMSO-*d6*); for ^{13}C detection, δ = 77.16 ppm ($CDCl_3$), 1.32 ppm (CD_3CN), 49.00 (CD_3OD), 39.25 (DMSO-*d6*). The splitting pattern of peaks is designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), or combinations of these signals. ^{13}C APT NMR is indicated with a (+) for positive or upwards pointing signals with an odd number of attached protons and is indicated (-) for negative or downwards pointing signals. Coupling constants (*J*) are given in Hz.

For photostationary distribution (PSD) determination via *ex-situ* illumination, 5 mM samples of the individual photoswitches were prepared and first measured after being thermally equilibrated by keeping them in the dark, then irradiated with a 365 nm LED torch (Alonefire SV003 10W UV torch) at a distance of *ca*. 5 cm for 10 min and measured, and lastly irradiated with a 455 nm LED from ThorLabs at a distance of *ca*. 5 cm for 20 min and then measured.

The NMR-spectra were evaluated with MestReNova 14.2.0-26256 from Mestrelab Research S.L.

Characterization of A3Tz5 via NMR with in-situ irradiation

Sample preparation

NMR samples of the A3Tz5 peptide were prepared with 5 mM, 1 mM or 200µM A3Tz5 in PBS-buffer (1X physiological concentration), pH 7.4, 92% H_2O, 8% D_2O, with 0.094 mM (± 2%) TSP-d_4 as internal standard. Errors of nominal sample concentration, as reported in Table S4 were estimated assuming peptide purity, using the balance and pipetting errors specified on the instruments. NMR samples were prepared in the dark. First spectra were measured

without illumination and then illumination proceeded during the NMR measurements, using the in-situ illumination device described below.

NMR Instrumentation for Peptide Characterization

NMR data was collected on an Avance III HD spectrometer (Bruker Biospin GmbH) operating at 700.1 MHz ^{1}H frequency. The system was equipped with a cryogenically cooled 5 mm quadruple-resonance inverse probe (^{1}H/^{19}F-P/C/N-D) equipped with a z-gradient. Hard pulse lengths of 10.25 µs on ^{1}H, 12.0 µs on ^{13}C and 35.0 µs on ^{15}N were used throughout this work. Sample temperature was regulated between 278 and 308 K. The samples were equipped with a setup for *in-situ* illumination, as described below.

Setup for NMR with in-situ illumination

We used an NMR sample chamber equipped with glass-fibers for *in-situ* illumination, akin to the setup described by Feldmeier *et al.*[1]. In contrast to the system described in the literature, the system was equipped with two light fibers and two LED light sources, for irradiation with two different wavelengths. The light sources were switched manually during the experiments.

A Nichia NVSU 233B SMD LED, driven at 700 mA constant current was used as light source with emission at 370 nm (see Figure S8). For irradiation at 455 nm a Cree XP-E2 550 mW SMD LED, driven at 350 mA was used. Both LEDs were centered onto the flat-cut end of a multimode glass fiber (one glass fiber for each LED; FT100UMT, Thorlabs, Inc.). The glass-fibers were roughened at the opposing end with sand paper, and inserted into a 3.3 mm outer diameter (OD) coaxial insert (WGS-5BL-SP, Wilmad Labglass, Inc.). Samples of ca. 300 µL were transferred into a 4.2 mm inner diameter (ID) NMR tube (5.0 mm OD) and the insert containing the glass-fibers was immersed into the solution for measurements.

Before and after the NMR measurements, LED emission spectra at the outlet of the glass-fiber were measured using an integration sphere (Avantes AvaSphere-50) coupled to a spectrophotometer (Avantes AvaSpec-3648), to check for stability of the light source.

In-Situ Illumination in the NMR

^{1}H spectra (Fig. 5 a of the main article) were collected with excitation sculpting for water suppression using a water flip-back pulse[2]. Series of these spectra were collected with 45 s time spacing between individual spectra. Each spectrum was collected with eight scans, four dummy scans, an acquisition duration of 3.28 s and 3.75 s between two consecutive scans (recycle delay 0.47 s). Further settings are detailed in section “Settings used throughout NMR experiments with in-situ illumination”. During measurement, in-situ

illumination was applied, as detailed in section "Setup for NMR with in-situ illumination" and Figure 5 a – d of the main article.

To reduce bias from baseline distortions caused by water suppression during the analysis of ^{1}H spectra series, the first spectra acquired without sample illumination were averaged and were subtracted from the spectrum series before integration. This applies to the data shown in Fig. 5 b – d of the main article.

NMR Characterization of Photostationary State

The photostationary state generated by irradiation with 370 nm was characterized under continuous irradiation, collecting T_1- and T_2-data, NOESY, TOCSY, DQF-COSY, ROESY, HSQC and HMBC data. The 2D-data collected is summarized in Figures S11 – S14.

All NOESY spectra were collected with gradient-selected NOESY experiments[3] with excitation sculpting[2] for water suppression and with States-TPPI[4] for quadrature detection in the indirect dimension (*F*1). The NOESY shown in Fig. 5a of the main article was acquired with 32 dummy scans and 64 scans per increment 512 complex points in *F*1 and a 200 ms mixing time. For NOESY spectra shown in the SI the number of dummy scans, scans per increment points in *F*1 and mixing time are given in the figure legends. All other experimental parameters are given in "Settings used throughout NMR experiments with in-situ illumination".

T_1- and T_1-data was collected with inversion recovery and with CPMG[5] experiments with excitation sculpting[2] for water suppression. The T_1 experiment hereby used a water flip-back. Four dummy scans and 16 scans were collected for eight spectra with varying relaxation delay. A 5 s relaxation delay was used in-between scans. Signal integration ranges were chosen to extract averaged relaxation rates for regions containing overlapped signals (Table S5) or individual relaxation rates for selected signals with sufficient baseline separation (Table S6). The integrals obtained were fitted against $I(\tau) = I_0\left(1 - a\, e^{-\frac{t}{T_1}}\right)$ or with $I(\tau) = I_0 e^{-\frac{t}{T_2}}$ for extraction of T_1 and T_2, respectively.

TOCSY spectra were collected with DIPSI-2 mixing[6, 7], excitation sculpting[2] for water suppression and with States-TPPI [4] for quadrature detection in *F*1. Number of dummy scans, scans per increment points in *F*1 and mixing time are given in the figure legends. All other experimental parameters are given in "Settings used throughout NMR experiments with in-situ illumination".

DQF-COSY[8, 9] spectra were collected with excitation sculpting[2] for water suppression and with pulsed z-gradients for coherence selection.

ROESY spectra were collected using a variant of the EASY-ROESY[10] sequence using presaturation and excitation sculpting[2] for water suppression. Spin-locking was applied for 150 ms (2x 75 ms) at ± 6500 Hz offset from the transmitter with 6500 Hz spinlock strength

($\gamma_H B_1$). Adiabatic ramps of 1.9 ms length with quarter-sine profile were used to ramp the spinlock up and down. Pulsed z-gradients of 0.04 T/m and 0.075 T/m gradient strength and 1 ms duration were used for coherence selection before and after the spin-locking period, respectively.

[^{1}H,^{13}C]-HSQCs were collected with gradient-selected, sensitivity-improved HSQC experiments[11]. INEPT delays were optimized for $^1J_{CH}$ = 145 Hz. Adiabatic pulses were used for inversion and refocusing on ^{13}C, and adiabatic bilevel decoupling[12] was used during acquisition. 80 kHz Chirp pulses were used for inversion and refocusing, and 42 kHz Chirp pulses were used for decoupling. A spectral width of 165 ppm and an offset of 75 ppm were used on ^{13}C.

[^{1}H,^{15}N]-HSQCs were collected with gradient-selected HSQCs without sensitivity-improvement[13]. INEPT delays were optimized for $^1J_{CH}$ = 90 Hz. Hard pulses were used for inversion and refocusing on ^{15}C, GARP-4 decoupling[14] was used during acquisition. A spectral width of 100 ppm and an offset of 120 ppm were used on ^{15}N.

The [^{1}H,^{13}C]-HMBC in Figure S14e was collected with gradient-selected HMBC experiments with echo/antiecho gradient selection and with a three-fold low-pass filter for $^1J_{CH}$ suppression[15]. The long-range coupling evolution delay was optimized for $^1J_{CH}$ = 10 Hz. Adiabatic 80 kHz composite Chirp pulses were used for refocusing on ^{13}C. A spectral width of 227.2 ppm and an offset of 100 ppm were used on ^{13}C.

The CO-selective [^{1}H,^{13}C]-HMBC in Figure S14f was collected with a ^{13}C-selective HMBC experiment with phase-insensitive detection in the indirect dimension ("shmbcgpndqf" from TopSpin 3.5pl7 pulse sequence library), which was equipped with water presaturation for improved water suppression (34 Hz during 0.8 s long recycling delay). The long-range coupling evolution delay was optimized for $^1J_{CH}$ = 8 Hz. A 1.5 ms Gauss pulse was used for selective inversion of the CO-region, otherwise hard pulses were used. A spectral width of 9.0 ppm and an offset of 173.5 ppm were used on ^{13}C.

Settings used throughout NMR experiments with in-situ illumination

All ^{1}H data was collected with 10 kHz (14.28 ppm) spectral width and with the transmitter set on resonance with the solvent signal. All 1D spectra were acquired with 32768 complex data points (3.28 s acquisition), all homonuclear 2D correlation spectra were acquired with 8192 complex data points (819 ms acquisition) in the direct dimension (*F*2*)*. [^{1}H-^{13}C]-HSQCs and HMBCs were collected with 1024 complex data points (102 ms acquisition) in *F*2, and [^{1}H-^{15}N]-HSQCs with 768 complex data points (76.8 ms acquisition) in *F*2. 2D spectra were collected with 256 complex data points in the indirect dimension (*F*1) and 16 dummy scans, unless stated differently. 2D datasets were collected with 16 scans per increment for the 5 mM and 1 mM sample, and with 64 scans per increment for the 200 µM sample, unless stated differently. NOESY, TOCSY, DQF-COSY and ROESY spectra were collected with

States-TPPI[4] for quadrature detection in *F*1, whereas HSQC and HMBC experiments used echo-antiecho encoding[13].
For water suppression using excitation sculpting[2], a 2 ms pulse with Sinc-profile (normalized Sinc-function between -1 and +1) on resonance with the water was used in all cases. Pairs of pulsed z-gradients with 0.16 T/m and 0.055 T/m gradient strength and 1 ms duration were applied during excitation sculpting. Water flip-back pulses used a Sinc-profile of 4 ms length. All pulsed field-gradients used smoothed-square profiles with squared-sine ramps.
NMR data with collected with in-situ illumination was processed, analyzed and plotted in Topspin 4.3.0 (Bruker Biospin GmbH, Ettlingen, Germany). Photoswitching kinetics were plotted and fitted in IgorPro 6.3.7.2 (WaveMetrics, Inc., Lake Oswego, OR/USA).

1.4.3. CD – sample preparation - Irradiation

Circular Dichroism measurements were performed on a *Jasco* J-1500 CD device in a 1 mm quartz cell from *Hellma Optik GmbH* (Jena, Germany). The instrument was used with the following settings:

Start-end wavelength: 260-185 nm
Data pitch: 1 nm
Scanning speed: 50 nm/min
CD scale: 200 mdeg/1.0 dOD
Band width: 1 nm
Response: 1 s

The samples were prepared by dissolving 25 nmol aliquots of peptides into 250 µL of desired solvents and transferred into a HELLMA cuvette type 110-macro QS with PTFE stopper (1 mm optical path length, 350 µL volume, 200-2500 nm wavelength).
For irradiation, the samples were placed in front of LEDs from ThorLabs (365 and 455 nm) and irradiated at a distance of *ca*. 10 cm for 15 min at ambient conditions without additional temperature control.

1.4.4. UV-Vis Absorption Spectroscopy, Time-resolved Irradiation Experiments, and Half-Lives

UV/VIS-spectroscopy and irradiation experiments were conducted using a SPECORD S600 UV-Vis spectrometer. Quartz cuvettes from HELLMA with a layer thickness of 1 cm were used. Stock solutions of the compounds of interest were prepared in the range of 10 to 50 mM in either MeOH, MeOD-*d4*, or DMSO-*d6* to match the solubility of the individual compound. The stock solutions were then diluted into the final concentration and a spectral

range between 200 nm and 800 nm was recorded. For the determination of the molar absorption coefficient ε, spectra of at least three different concentrations were recorded and a linear fit was conducted at, typically, the ππ* transition maximum of the *trans* isomers of the compounds.
For irradiation, LEDs in a custom-built setup from Mountain Photonics (365 nm and 405 nm) or from ThorLabs (455 nm, 505 nm, 625 nm) were used. Samples were prepared at a concentration of 25–50 µM (as specified with the individual measurements) and spectra were recorded at defined time intervals, while stirring the samples and keeping the temperature constant at 20 °C.
The thermal half-lives were determined at a defined temperature using either a SPECORD S600 or a JASCO V-670 UV-Vis spectrometer and the irradiation setup described above.
The data evaluation was performed with Spectragryph v1.2.15, GraphPad Prism 8.0.2, and OriginPro 2020 softwares.

1.4.5. Optical Light Microscopy with Irradiation

Optical light microscopy was performed with a Digital Microscope UltraZoom Pro from Dnt Innovation GmbH. The samples were prepared in a HELLMA cuvette type 110-macro QS with PTFE stopper (1 mm optical path length, 350 µL volume, 200-2500 nm wavelength) and magnified up to 200x. For irradiation, a 365 nm LED torch (Alonefire SV003 10W UV torch) positioned at a distance of ca. 5 cm and a 455 nm LED from ThorLabs at a distance of ca. 10 cm were employed.

1.4.6. CryoSEM and CryoTEM

Sample plunge-freezing and Imaging

Samples were prepared by dissolving 25 nmol aliquots of peptides into either 500 or 125 µL of PBS pH 7.4 in a glass vial or an Eppendorf tube, in order to obtain a final concentration of 50 µM or 200 µM, respectively. The samples were either further processed directly (“dark”), or irradiated with 365 nm light using LEDs from ThorLabs, and further processed (“365 nm”), or subsequently irradiated with 455 nm light using LEDs from ThorLabs, and then further processed (“455 nm”).
The so-treated samples were used to obtain 3-µL samples, which were applied to freshly glow-discharged Quantifoil Cu R2/1, 200 mesh grids, which were plunge-frozen with a Vitrobot Mark IV (Thermo Fisher Scientific) at 10 °C with 100% humidity.
To look for larger objects, the grids were loaded to a cryo-dual beam microscope Aquilos2 (Thermo Fisher Scientific) FIB-SEM (focused ion beam - scanning electron microscope) for imaging.

To search for microscopic objects, the same grids were loaded into a Titan Krios G4 (Thermo Fisher Scientific) operating at 300 kV for imaging. Images were acquired at a magnification of 64 kX and 165 kX (1.89 and 0.72 Å/pixel respectively) using a Selectris energy filter with a Falcon4i detector (Thermo Fisher Scientific).

Cryo-EM SPA

Cryo-EM SPA (single particle analysis) was performed, and two datasets were collected using a Titan Krios G4 with F4i detector and Selectris energy filter, at a magnification of 165 kX (0.72 Å/pixel) with a total dose of 40 e/ $Å^2$ in a range of defocus from -1 µm to -2 µm.
The first dataset comprised 2,643 images. The images were first motion corrected in cryosparc v4.4.0, followed by patch Contrast Transfer Function (CTF) estimation. Filament coordinates were picked manually in Relion 4.0.
The second dataset comprised 1,410 images. The images were first motion corrected in Relion 4.0, followed by CTF estimation using CTFFIND4. Filaments were picked using a trained cryolo network. 2D classifications were performed using Relion 4.0.

1.5. Standard Operating Protocols (SOPs)

SOP1 – Ring Closing Reaction

Compound **2** (1.85 g, 4.75 mmol, 1 eq) was dissolved in EtOH (10 mL), and either $NH_2NH_2 \cdot H_2O$ (0.28 mL, 4.75 mmol, 1 eq), $CH_3(NH)NH_2$ (2.47 mL, 4.75 mmol, 1 eq), or $NH_2OH \cdot HCl$ (0.32 g, 4.75 mmol, 1 eq) was added to the solution. For the synthesis of the isoxazole ring, 1 eq of solid $NaHCO_3$ was added to neutralize the HCl released by the salt.
The reaction was left stirring under reflux for 6 h, then the mixture was dried under a N_2 stream.

SOP2 – Boc Deprotection of UAAs

Boc deprotection was carried out by dissolving the Boc-protected UAA in a 1:1 mixture of DCM/TFA (20 mL). The reaction was left stirring for 4 h at room temperature.
Afterwards, the reaction mixture was concentrated under a N_2 stream, and the product was precipitated in ice-cold Et_2O. The product was isolated after centrifugation and elimination of the supernatant.

SOP3 – Automated Microwave-Assisted Solid Phase Peptide Synthesis

The non-preloaded resin (1.0 eq, 0.44 mmol/g, 50 µmol, 114 mg) was placed in the reaction vessel of the peptide synthesizer and was swollen for 30 min in DMF. First, the *N*-terminal Fmoc protecting group was removed by addition of piperidine (20 % in DMF, *v/v*, 2.5 mL). To achieve a complete cleavage of the Fmoc-group, the deprotection step was repeated twice.

After washing of the resin with DMF (5 x 4.0 mL) the amino acid (5 eq, 0.2 M, 1.25 mL), *N*,*N*'-Diisopropylcarbodiimide (DIC, 0.5 M, 1.25 mL), and activator base (0.5 M, 1.25 mL) were added to the reaction vessel, and the coupling reaction was performed (see **table S1** for microwave conditions). For the coupling of Fmoc-Arg(Pbf)-OH, specific coupling cycles were used to suppress the possible γ-lactam formation, as reported in **table S1**.

Table S1 – Overview of coupling and deprotection microwave parameters on the Liberty Blue Peptide Synthesizer utilized for the syntheses.

Synthetic Step	Temperature [°C]	Power [W]	Time [s]	Δ Temperature [°C]
Coupling step 1	75	170	15	2
Coupling step 2	90	30	225	1
Arg Coupling step 1	25	0	1500	2
Arg Coupling step 2	70	30	300	1
Deprotection step 1	75	155	15	2
Deprotection step 2	90	30	50	1

After the peptide synthesis was completed, the resin was transferred into a BD Syringe with PE-frit and was washed with DMF (6 x 4.0 mL), DCM (6 x 4.0 mL), and Et_2O (3 x 4.0 mL). Following, the resin was dried under reduced pressure.

SOP4 – Cleavage from Resin

The cleavage of the peptides from the resin was performed in a BD syringe with a PE frit. The resin was shaken at room temperature in a TFA/Triisopropylsilane (TIPS)/$^{demi}H_2O$ solution (95:2.5:2.5 v/v/v).

For test cleavages, 10 mg of resin were treated with 1 mL of cleavage cocktail for 1.5 hrs. Afterwards, the solution was filtered into a 2 mL Eppendorf tube and reduced under nitrogen stream. The crude peptide was precipitated in Et_2O and centrifuged in a Carl Roth™ Mini-Centrifuge at 6000 rpm. The supernatant was discarded, and the peptide was redissolved into a 50:50 MeCN/$^{demi}H_2O$ (+0.1% TFA) solution for ESI-MS and UHPLC analysis.

For full cleavage, the whole resin was treated with 4 mL of cleavage cocktail for 3 hrs. Afterwards, the solution was filtered into a 15 mL falcon tube and reduced under nitrogen stream. The crude peptide was precipitated in ice-cold Et_2O. The precipitate was isolated by centrifugation (9000 rpm, -10 °C, 10 min), washed twice with ether, redissolved in a 20:80 MeCN/$^{demi}H_2O$ (+0.1% TFA) solution, and lyophilized.

SOP5 – HPLC Purification

For the preparation of the samples, they were dissolved in an 80:20 or a 70:30 solution of $^{demi}H_2O$/MeCN, according to their solubility and the elution gradient used for purification. Samples were then sonicated and filtered through a Chromafil filter from MACHEREY-NAGEL. A maximum of 1.8 mL of solution with a maximum concentration of 2 mg/mL was injected per every run with a semi-preparative scale.

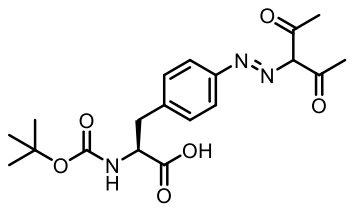
The flow of the HPLC was set to 3 mL/min, and the temperature for the column-hosting oven was set to 40 °C.

Gradient A – 20 to 50% of MeCN (+0.1% TFA) in $^{demi}H_2O$ (+0.1% TFA) in 20 min.

Before each run, the HPLC system was purged with the respective starting gradient for 30 minutes. After collecting, fractions containing the product were combined, frozen with liquid nitrogen and lyophilized to obtain a fluffy compound. Purity was always controlled by UHPLC.

2. Synthetic Procedures

(S,E)-2-((Tert-butoxycarbonyl)amino)-3-(4-((2,4-dioxopentan-3-yl)diazenyl)phenyl)propanoic acid

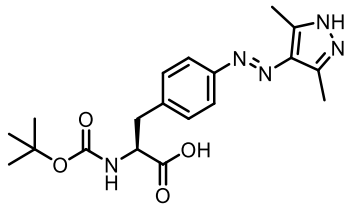


2

Boc-4-amino-phenylalanine (1.4 g, 5.0 mmol, 1 eq) was dissolved in a mixture of H_2O (10 mL) and aqueous HCl (1M, 1.1 mL), and the reaction was cooled to 0 °C. Then, solid $NaNO_2$ (0.38 g, 5.5 mmol, 1.1 eq) was added, and the reaction was left stirring for 2 h.
$NaOAc \cdot 3H_2O$ (2.4 g, 17.5 mmol, 3.5 eq) and 2,4-pentadione (0.52 mL, 5.0 mmol, 1 eq) were dissolved in a mixture of H_2O (25 mL) and EtOH (2.5 mL) and added to the reaction, which was then left stirring for 1 h at room temperature.
Afterwards, the solid residue was isolated by suction filtration, washed with H_2O, H_2O/EtOH 1:1, and *n*-hexane, and dried under vacuum to afford compound **2** as a yellow solid (1.9 g, 4.8 mmol, 95% yield).

^{1}H NMR (400 MHz, DMSO) δ 7.43 (d, *J* = 8.5 Hz, 2H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.42 (s, 0H), 3.94 (s, NH), 3.05 (dd, *J* = 13.6, 4.9 Hz, 1H), 2.89 – 2.81 (m, 1H), 2.46 (s, 3H), 2.39 (s, 3H), 1.33 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 198.0, 197.0, 156.3, 140.4, 135.2, 133.2, 130.7, 116.4, 80.2, 56.3, 37.7, 31.8, 28.4, 26.6, 21.5.
HR-MS (ESI+) m/z calculated for $C_{19}H_{26}N_3O_6$ ($[M+H]^+$): 392.1816; found: 392.1819; m/z calculated for $C_{19}H_{25}N_3O_6Na$ ($[M+Na]^+$): 414.1636; found: 414.1650.

(S,E)-2-((Tert-butoxycarbonyl)amino)-3-(4-((3,5-dimethyl-1H-pyrazol-4-yl)diazenyl)phenyl)propanoic acid

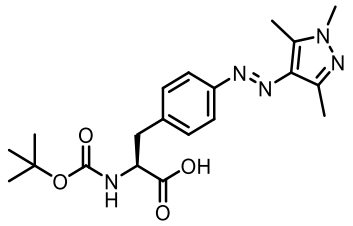


3

Compound **3** was synthesized according to **SOP1** and obtained in quantitative yield as a yellow solid, which was used in the next steps without further purification.

^{1}H NMR (400 MHz, MeOD) δ 6.97 (AA'BB', 2H), 6.64 (AA'BB', 2H), 4.16 – 4.08 (m, 1H), 3.06 – 2.99 (m, 1H), 2.80 (dd, *J* = 13.9, 7.2 Hz, 1H), 1.90 (s, 3H), 1.40–1.30 (m, 9H).
LCMS (ESI+)

(S,E)-2-((Tert-butoxycarbonyl)amino)-3-(4-((1,3,5-trimethyl-1H-pyrazol-4-yl)diazenyl)phenyl)propanoic acid

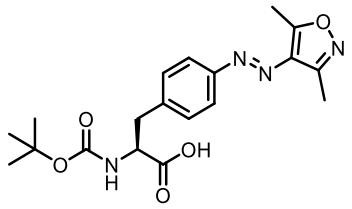


4

Compound **4** was synthesized according to **SOP1** and obtained in quantitative yield as a yellow solid, which was used in the next steps without further purification.

^{1}H NMR (300 MHz, MeOD) δ 7.69 (AA'BB', 2H), 7.35 (AA'BB', 2H), 4.44 – 4.34 (m, 1H), 3.77 (s, 3H), 3.23 (dd, *J* = 13.8, 5.3 Hz, 1H), 2.97 (dd, *J* = 13.8, 8.8 Hz, 1H), 2.59 (s, 3H), 2.44 (s, 3H), 1.38 (s, 9H).

(S,E)-2-((Tert-butoxycarbonyl)amino)-3-(4-((3,5-dimethylisoxazol-4-yl)diazenyl)phenyl)propanoic acid

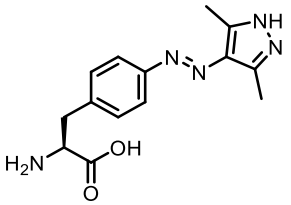


5

Compound **5** was synthesized according to **SOP1** and obtained in quantitative yield as a yellow solid, which was used in the next steps without further purification.

^{1}H NMR (300 MHz, MeOD) δ 7.76 (AA'BB', 2H), 7.39 (AA'BB', 2H), 4.38 (s, 1H), 3.27 – 3.21 (m, 1H), 2.99 (dd, J = 13.6, 8.9 Hz, 1H), 2.75 (s, 3H), 2.50 (s, 3H), 1.38 (s, 9H).

(S,E)-2-Amino-3-(4-((3,5-dimethyl-1H-pyrazol-4-yl)diazenyl)phenyl)propanoic acid

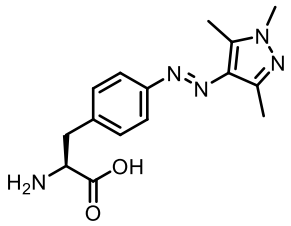


6

Boc deprotection of compound **3** was carried out following **SOP2**. Compound **6** was obtained as a yellow powder in quantitative yield and utilized in the next step without further purification.

MS (ESI+): m/z calculated for $C_{14}H_{18}N_5O_2$: 287.1; found: 288.1 ($[M+H]^+$).

5.2.9 (S,E)-2-Amino-3-(4-((1,3,5-trimethyl-1H-pyrazol-4yl)diazenyl)phenyl)propanoic acid

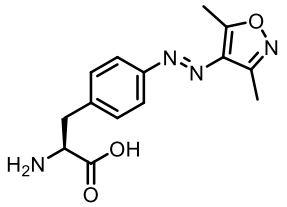


7

Boc deprotection of compound **4** was carried out following **SOP2**. Compound **7** was obtained as a yellow powder in quantitative yield and utilized in the next step without further purification.

^{1}H NMR (300 MHz, DMSO) δ 8.38 (s, 1H), 7.68 (AA'BB', 2H), 7.40 (AA'BB', 2H), 4.22 (br, 1H), 3.74 (s, 3H), 3.18 (d, *J* = 5.9 Hz, 2H), 2.54 (s, 3H), 2.36 (s, 3H).
^{13}C NMR (101 MHz, DMSO) δ 170.8, 158.8, 158.5, 152.7, 140.8, 136.8, 134.9, 130.8, 122.0, 119.1, 116.1, 65.4, 53.6, 36.4, 36.0, 15.6, 14.2, 9.9.
MS (ESI+): m/z calculated for $C_{15}H_{20}N_5O_2$: 301.2; found: 302.2 ($[M+H]^+$).

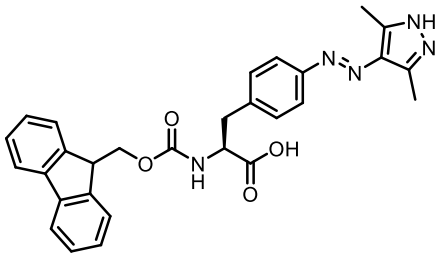
(S,E)-2-Amino-3-(4-((3,5-dimethylisoxazol-4-yl)diazenyl)phenyl)propanoic acid



8

Boc deprotection of compound **5** was carried out following **SOP2**. Compound **8** was obtained as a yellow powder in quantitative yield and utilized in the next step without further purification.
MS (ESI+): m/z calculated for $C_{14}H_{17}N_4O_3$: 288.1; found: 289.1 ($[M+H]^+$).

(S,E)-2-((2-(9H-Fluoren-9-yl)acetoxy)amino)-3-(4-((3,5-dimethyl-1H-pyrazol-4-yl)diazenyl)phenyl)propanoic acid



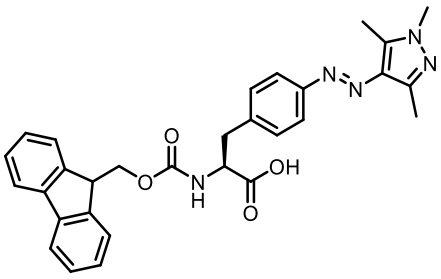
9

Compound **6** (0.22 g, 0.77 mmol, 1 eq) was suspended in MeCN (20 mL) together with Fmoc-Osu (0.73 g, 2.15 mmol, 1.2 eq). To this, a solution of $NaHCO_3$ (0.38 g, 4.48 mmol, 2.5 eq) in H_2O (10 mL) was added, and the mixture was left stirring overnight at room temperature. Afterwards, the reaction mixture was dried under vacuum to eliminate the MeCN. The aqueous suspension was acidified with HCl (37% v/v) until precipitation occurred. The mixture was filtered under vacuum and the precipitate was isolated and washed 3x30mL with a 7:3 mixture of H_2O/iPrOH acidified with HCl (37% v/v), and 2x20 mL of ice-cold Et_2O. Compound **9** was obtained as a bright yellow powder (0.14 g, 0.27 mmol, 35% yield).

^{1}H NMR (300 MHz, DMSO) δ 7.86 (d, *J* = 7.6 Hz, 2H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.68 – 7.58 (m, 3H), 7.49 – 7.23 (m, 6H), 4.29 – 4.13 (m, 4H), 3.15 (dd, *J* = 13.5, 4.2 Hz, 1H), 3.00 – 2.86 (m, 1H), 2.44 (s, 3H).

HR-MS (ESI+) – m/z calculated for $C_{29}H_{28}N_5O_4$ ($[M+H]^+$): 510.2136.2; found: m/z 510.2139; m/z calculated for $C_{29}H_{27}N_5O_4Na$ ([M+Na]+): 532.1955; found: m/z 532.1964.

(S,E)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-((1,3,5-trimethyl-1H-pyrazol-4-yl)diazenyl)phenyl)propanoic acid



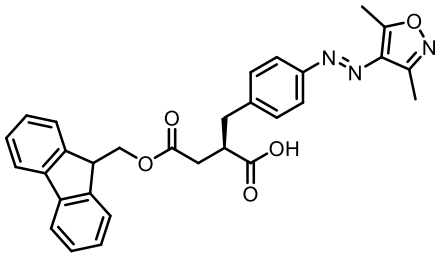
10

Compound **7** (0.54 g, 1.79 mmol, 1 eq) was suspended in MeCN (20 mL) together with Fmoc-Osu (0.73 g, 2.15 mmol, 1.2 eq). To this, a solution of $NaHCO_3$ (0.38 g, 4.48 mmol, 2.5 eq) in H_2O (10 mL) was added, and the mixture was left stirring overnight at room temperature. Afterwards, the reaction mixture was dried under vacuum to eliminate the MeCN. The aqueous suspension was acidified with HCl (37% v/v) until precipitation occurred. The

mixture was filtered under *vacuum* and the precipitate was isolated and washed 3x30mL with a 7:3 mixture of H_2O/iPrOH acidified with HCl (37% v/v), and 2x20 mL of ice-cold Et_2O. Compound **10** was obtained as a bright yellow powder (0.75 g, 1.43 mmol, 80% yield).

^{1}H NMR (300 MHz, DMSO) δ 7.84 (d, *J* = 7.5 Hz, 2H), 7.65 – 7.52 (m, 4H), 7.41 – 7.20 (m, 7H), 4.23 – 4.07 (m, 4H), 3.71 (s, 3H), 3.16 (dd, *J* = 13.2, 4.1 Hz, 1H), 2.92 (dd, *J* = 13.6, 9.5 Hz, 1H), 2.50 (s, 3H), 2.33 (s, 3H).
^{13}C NMR (101 MHz, DMSO) δ 173.3, 156.0, 151.7, 143.8, 140.7, 140.3, 139.7, 139.4, 134.4, 130.0, 127.7, 127.6, 127.1, 125.3, 121.3, 120.1, 65.7, 62.1, 55.5, 46.6, 36.4, 36.0, 25.5, 13.8, 9.5.
HR-MS (ESI+) – m/z calculated for [$C_{30}H_{29}N_5O_4$]: 523.2220; found: m/z 524.2296 [M+H]$^+$; m/z 546.2113 [M+Na]$^+$.

(S,E)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-((3,5-dimethylisoxazol-4-yl)diazenyl)phenyl)propanoic acid



11

Fmoc-protection of compound **8** (0.22 g, 0.76 mmol, 1 eq) was performed by suspending it and Fmoc-OSu (0.31 g, mmol, 0.91 eq) in MeCN (15 mL). A solution of $NaHCO_3$ (0.16 g, 1.90 mmol, 2.5 eq) in H_2O (7.5 mL) was added, and the mixture was left stirring overnight at room temperature.
Afterwards, the reaction mixture was dried under vacuum to eliminate the MeCN. The aqueous suspension was acidified with HCl (37% v/v) until precipitation occurred. The mixture was filtered under *vacuum* and the precipitate was isolated and washed 3x30mL with a 7:3 mixture of H_2O/iPrOH acidified with HCl (37% v/v), and 2x20 mL of ice-cold Et_2O.
To further purify the compound, reverse-phase flash column chromatography was carried out (H_2O/MeCN eluent, 5 to 95% gradient) and compound **11** was isolated as a bright yellow powder (22.0 mg, 0.043 mmol, 6% yield).

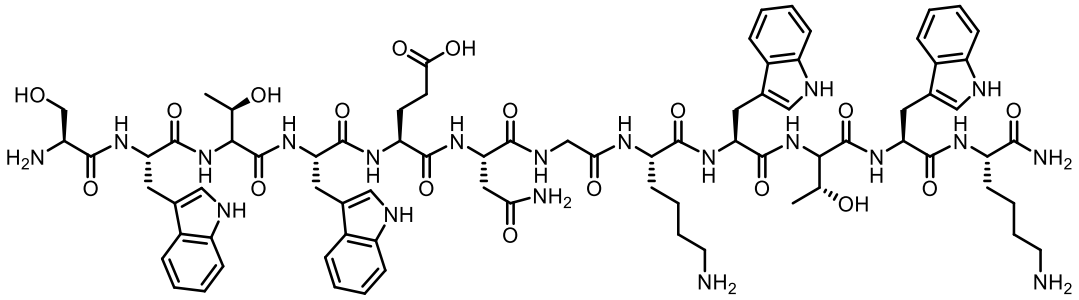
^{1}H NMR (400 MHz, DMSO) δ 7.85 (d, *J* = 7.6 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.60 (dt, *J* = 12.5, 6.2 Hz, 2H), 7.45–7.34 (m, 4H), 7.28 (dt, *J* = 10.7, 7.0 Hz, 2H), 4.24 – 4.09 (m, 4H), 3.19 (dd, *J* = 13.6, 4.5 Hz, 1H), 2.95 (dd, *J* = 13.6, 10.1 Hz, 1H), 2.71 (s, 3H), 2.42 (s, 3H).

^{13}C NMR (101 MHz, DMSO) δ 170.0, 156.2, 153.3, 151.2, 144.1, 144.0, 142.1, 141.0, 141.0, 132.1, 130.5, 127.9, 127.4, 125.6, 125.5, 122.1, 120.4, 65.9, 46.9, 36.9, 31.5, 28.8, 26.1, 22.4, 14.3, 12.0, 11.6.

HR-MS (ESI+) – m/z calculated for $C_{29}H_{27}N_4O_5$ ($[M+H]^+$): 511.1976; found: m/z 511.1981; m/z calculated for $C_{29}H_{26}N_4O_5Na$ ($[M+Na]^+$) 533.1795; found: m/z 533.1791.

HR-MS (ESI-) – m/z calculated for $C_{29}H_{25}N_4O_5$ ($[M-H]^-$): 509.1830; found: m/z 509.1839.

TrpZip2



1

TrpZip2 was synthesized *via* microwave-assisted solid phase peptide synthesis on a Liberty Blue Peptide Synthesizer (CEM) following **SOP3**.

The synthesis (on a 0.025 mmol scale) was carried out on a Rink Amide resin (0.67 mmol/g loading). DIC (0.25 M in DMF) and Oxyma (0.50 M in DMF + 0.10 mL DIPEA) were employed as activators, while deprotection was carried out with a 20% solution of piperidine in DMF.

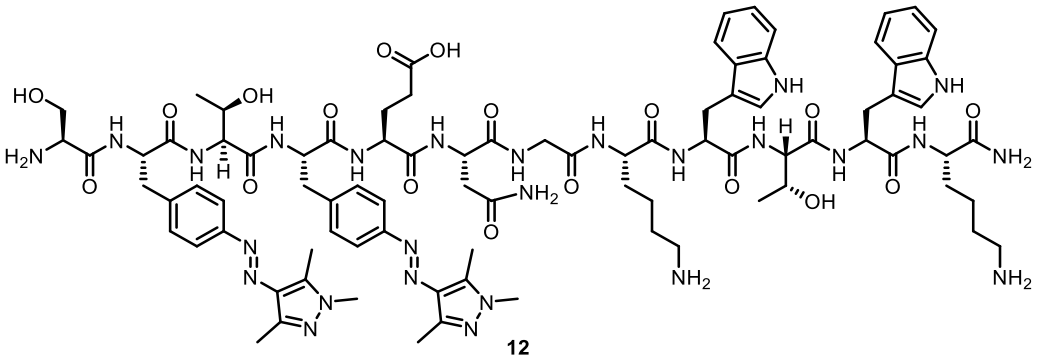
Table S2 – Overview of amounts of amino acids used.

Amino Acid	Molecular Weight [g/mol]	Concentration [M]	Mass [g]	DMF Volume [mL]
Fmoc-Asn(Trt)-OH	596.77	0.2	0.36	3
Fmoc-Gly-OH	297.31	0.2	0.26	3
Fmoc-Glu(OtBu)-OH	425.47	0.2	0.18	3
Fmoc-Lys-OH	468.50	0.2	0.57	6
Fmoc-Ser(OtBu)-OH	383.44	0.2	0.24	3
Fmoc-Thr(tBu)-OH	468.54	0.2	0.48	6
Fmoc-Trp(Boc)-OH	526.60	0.2	1.92	18

After the synthesis, the resin was collected in a BD-Syringe and cleavage was performed according to **SOP4**. The crude peptide was collected, redissolved in $^{demi}H_2O$, and lyophilized. RT-HPLC purification was carried out according to **SOP5**, and the pure peptide was obtained as a white solid (7.8 mg, 0.005 mmol, 20% yield).

UHPLC t_R (Gradient **A**) – 8.867 min.
Purity (UHPLC) – 94.8%.
HR-MS (ESI+) – m/z calculated for $C_{78}H_{102}N_{20}O_{18}$ ($[M+H]^+$): 1607.7754, found: m/z 1607.7742; m/z calculated for $C_{81}H_{108}N_{23}O_{18}$ ($[M+2H]^{2+}$): 804.3913, found: m/z 804.3920; m/z calculated for $C_{81}H_{109}N_{23}O_{18}$ ($[M+3H]^{3+}$): 536.60, found: m/z 536.60; m/z calculated for $C_{81}H_{107}N_{23}O_{18}K$ ($[M+Na]^+$): 1629.7573, found: m/z 1629.7540; m/z calculated for $C_{81}H_{107}N_{23}O_{18}Na$ ($[M+H+Na]^{2+}$): 815.3823, found: m/z 815.3806.

A3Tz5



A3Tz5 was synthesized *via* microwave-assisted solid phase peptide synthesis on a Liberty Blue Peptide Synthesizer (CEM) following **SOP3**.
The synthesis (on a 0.025 mmol scale) was carried out on a Rink Amide resin (0.67 mmol/g loading). DIC (0.25 M in DMF) and Oxyma (0.50 M in DMF + 0.10 M DIPEA) were employed as activators, while deprotection was carried out with a 20% solution of piperidine in DMF.

Table S3 – Overview of amounts of amino acids used.

Amino Acid	Molecular Weight [g/mol]	Concentration [M]	Mass [g]	DMF Volume [mL]
Fmoc-Asn(Trt)-OH	596.77	0.20	0.36	3
Fmoc-Gly-OH	297.31	0.20	0.26	3
Fmoc-Glu(OtBu)-OH	425.47	0.20	0.18	3
Fmoc-Lys-OH	468.50	0.20	0.57	6
Fmoc-Ser(OtBu)-OH	383.44	0.20	0.24	3
Fmoc-Thr(tBu)-OH	468.54	0.20	0.48	6
Fmoc-Trp(Boc)-OH	526.60	0.20	0.64	6
Fmoc-MePyrAzoF-OH	523.59	0.075	0.16	4

After the synthesis, the resin was collected in a BD-Syringe and cleavage was performed according to **SOP4**. The crude peptide was collected, redissolved in $^{demi}H_2O$, and lyophilized.

RT-HPLC purification was carried out according to **SOP5**, and the pure peptide was obtained as a yellow solid (5.5 mg, 0.003 mmol, 12% yield).

UHPLC t_R (Gradient **A**) – 9.913 min.
Purity (UHPLC) – 99.9%.
HR-MS (ESI+) – m/z calculated for $C_{86}H_{116}N_{25}O_{19}$ ($[M+H]^+$): 1801.8801, found: m/z 1801.9022; m/z calculated for $C_{86}H_{117}N_{25}O_{19}$ ($[M+2H]^{2+}$): 901.4553, found: m/z 901.4551; m/z calculated for $C_{86}H_{118}N_{25}O_{19}$ ($[M+3H]^{3+}$): 601.31, found: m/z 601.31.

3. Photophysical and Photochemical Analysis

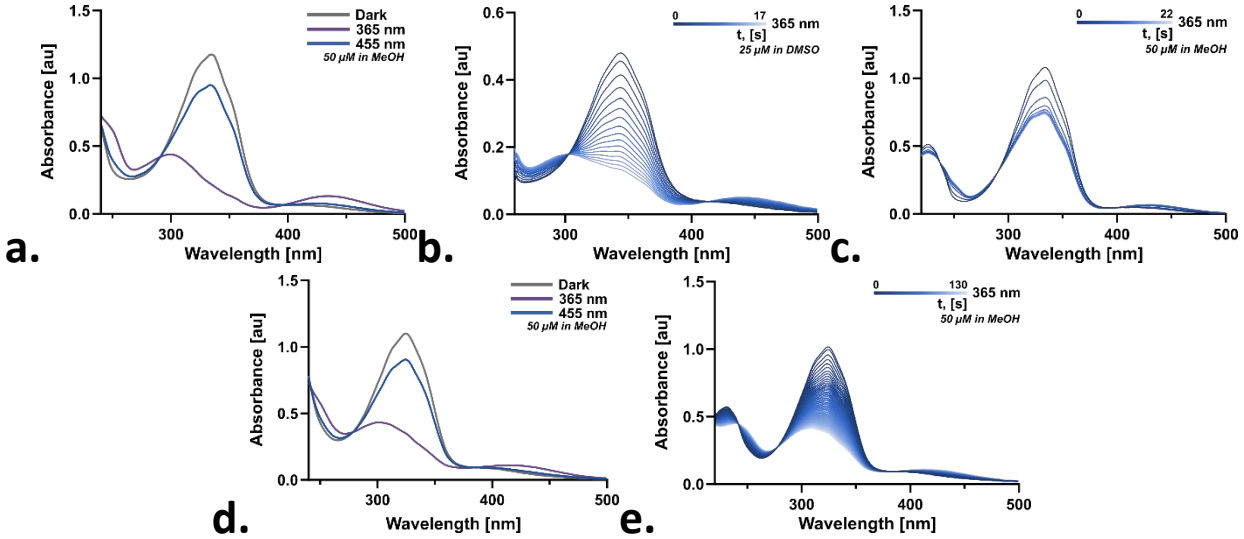


Figure S1 – a. UV-Vis absorption spectrum of Boc-PyrAzoF-OH dissolved to a 50 µM concentration in MeOH; **b.** photoswitching kinetics of Boc-PyrAzoF-OH dissolved in DMSO in a 25 µM concentration; **c.** time-resolved UV-Vis absorption spectra under irradiation with a 365 nm LED of Boc-PyrAzoF-OH dissolved in MeOH in a 50 µM concentration; **d.** UV-Vis absorption spectrum of Boc-OxAzoF-OH dissolved to a 50 µM concentration in MeOH; **e.** photoswitching kinetics of Boc-PyrAzoF-OH dissolved in MeOH in a 50 µM concentration. All spectra were recorded at 20 °C temperature.

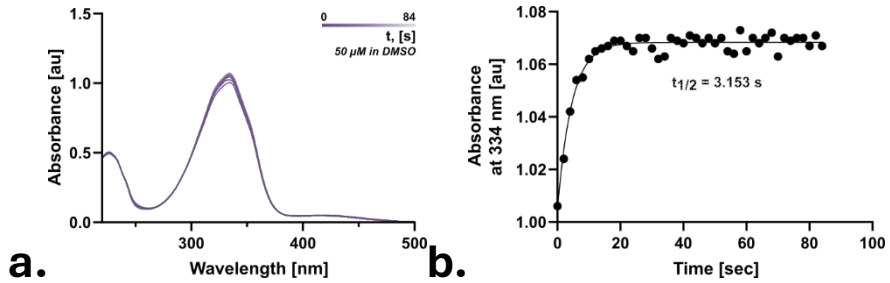
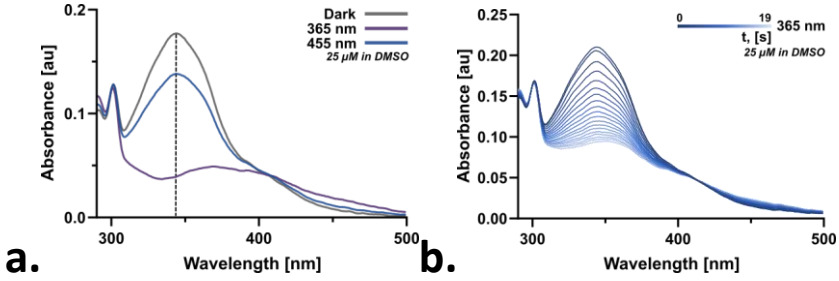


Figure S2 – a. Time-resolved UV-Vis absorption spectrum of Boc-PyrAzoF-OH (50 µM in DMSO) recorded at 20 °C in the dark after 365 nm irradiation; **b.** Change of the absorbance at 334 nm over time, extracted from (**a**), and fitted with a first order exponential fit to calculate the half-life at 20 °C for Boc-PyrAzoF-OH.



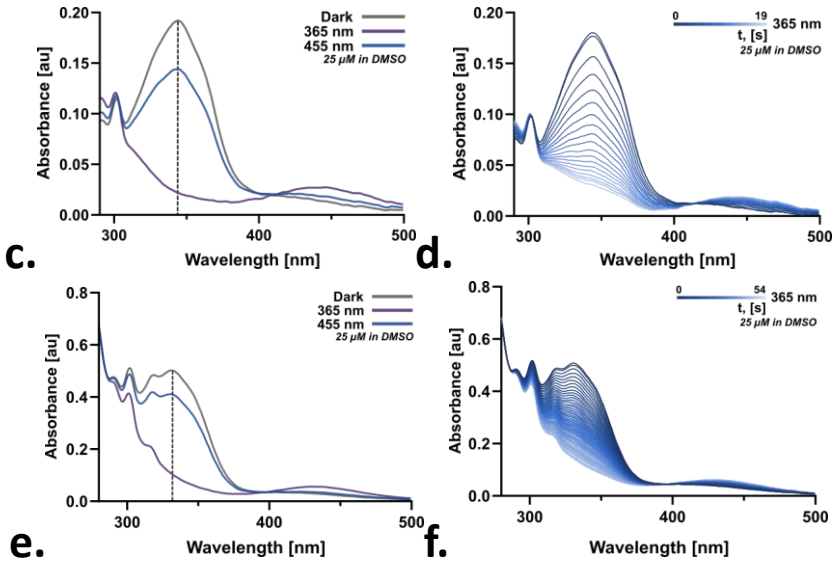


Figure S3 – a. UV-Vis absorption spectrum of Fmoc-PyrAzoF-OH dissolved to a 25 µM concentration in DMSO; **b.** Time-resolved UV-Vis absorption spectra under irradiation with a 365 nm LED of Fmoc-PyrAzoF-OH dissolved in DMSO in a 25 µM concentration; **c.** UV-Vis absorption spectrum of Fmoc-MePyrAzoF-OH dissolved to a 25 µM concentration in DMSO; **d.** Time-resolved UV-Vis absorption spectra under irradiation with a 365 nm LED of Fmoc-MePyrAzoF-OH dissolved in DMSO in a 25 µM concentration; **e.** UV-Vis absorption spectrum of Fmoc-OxAzoF-OH dissolved to a 25 µM concentration in DMSO; **f.** Time-resolved UV-Vis absorption spectra under irradiation with a 365 nm LED of Fmoc-OxAzoF-OH dissolved in DMSO in a 25 µM concentration. All spectra were recorded at 20 °C temperature.

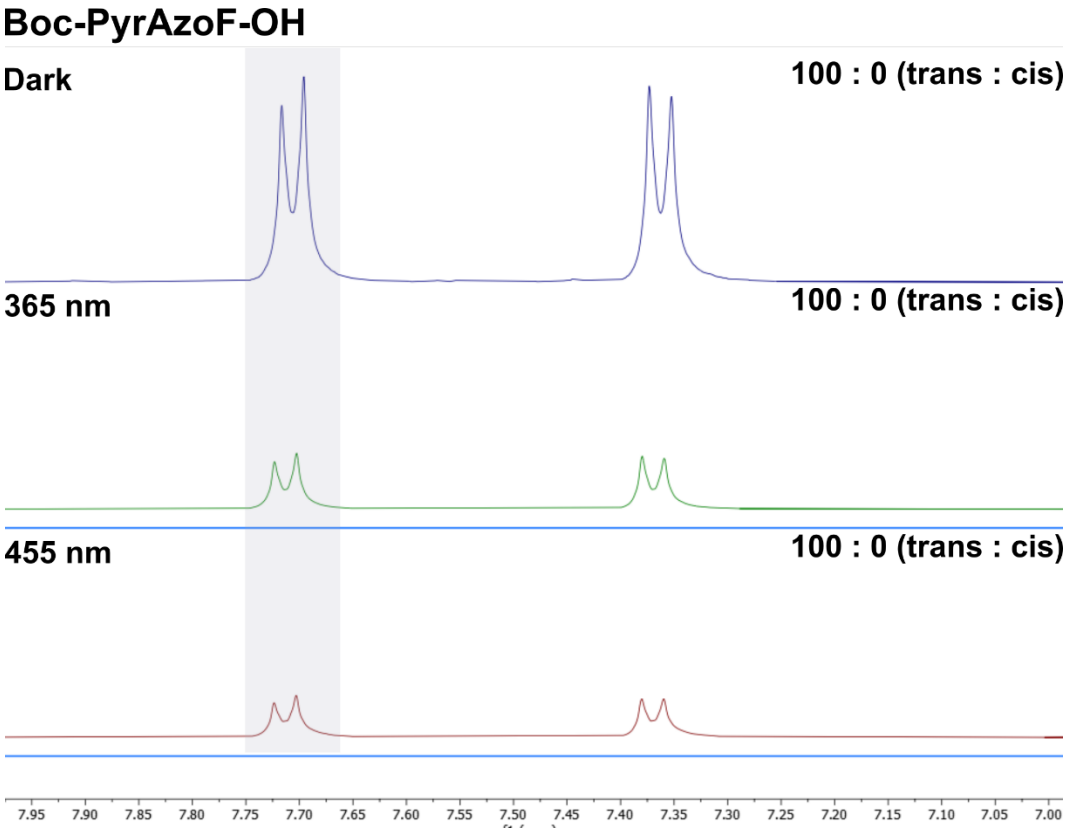


Figure S4 - NMR monitoring of photoswitching process for Boc-PyrAzoF-OH through ex-situ illumination. The NMR spectrum has been cut to the aromatic region, where signals of interest can be clearly observed, and their shifting upon light-irradiation can be measured. Highlighted in grey are the peaks that were used to quantify the photoswitching. From top to bottom, we report the NMR spectrum *previa* light irradiation (blue), after 365 nm light irradiation (green), and after 455 nm light irradiation (maroon).

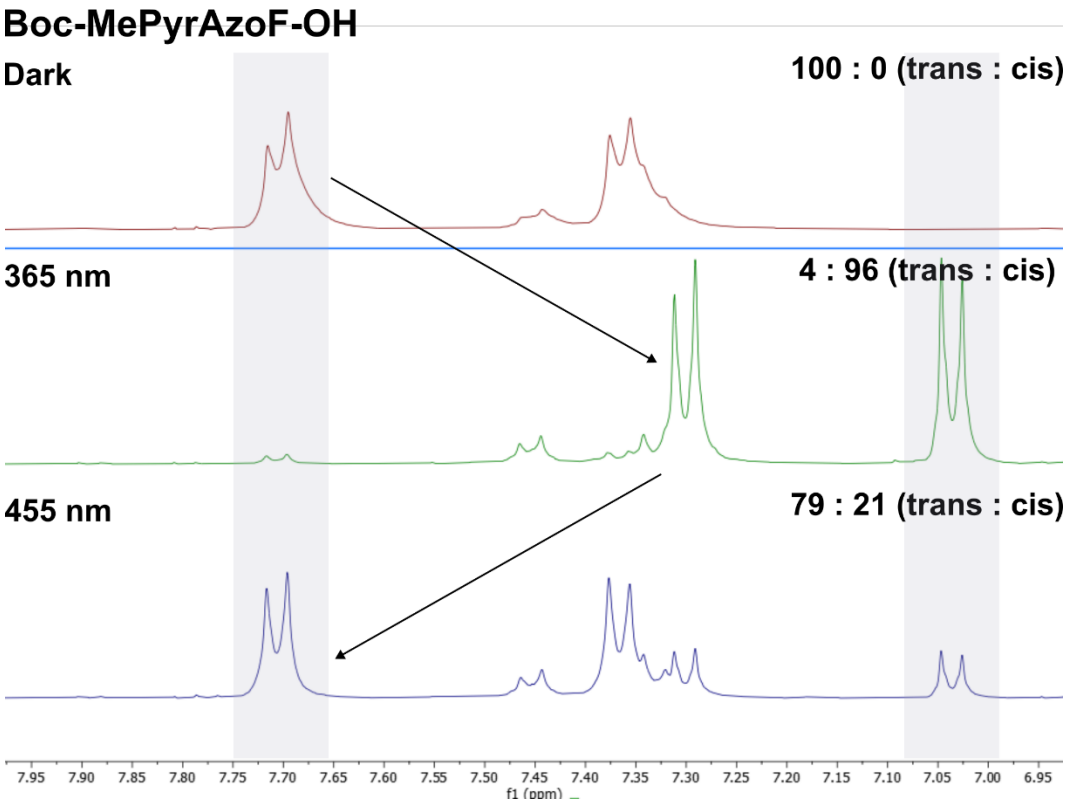


Figure S5 – NMR monitoring of photoswitching process for Boc-MePyrAzoF-OH through ex-situ illumination. The NMR spectrum has been cut to the aromatic region, where signals of interest can be clearly observed, and their shifting upon light-irradiation can be measured. Highlighted in grey are the peaks that were used to quantify the photoswitching. From top to bottom, we report the NMR spectrum *previa* light irradiation (maroon), after 365 nm light irradiation (green), and after 455 nm light irradiation (blue).

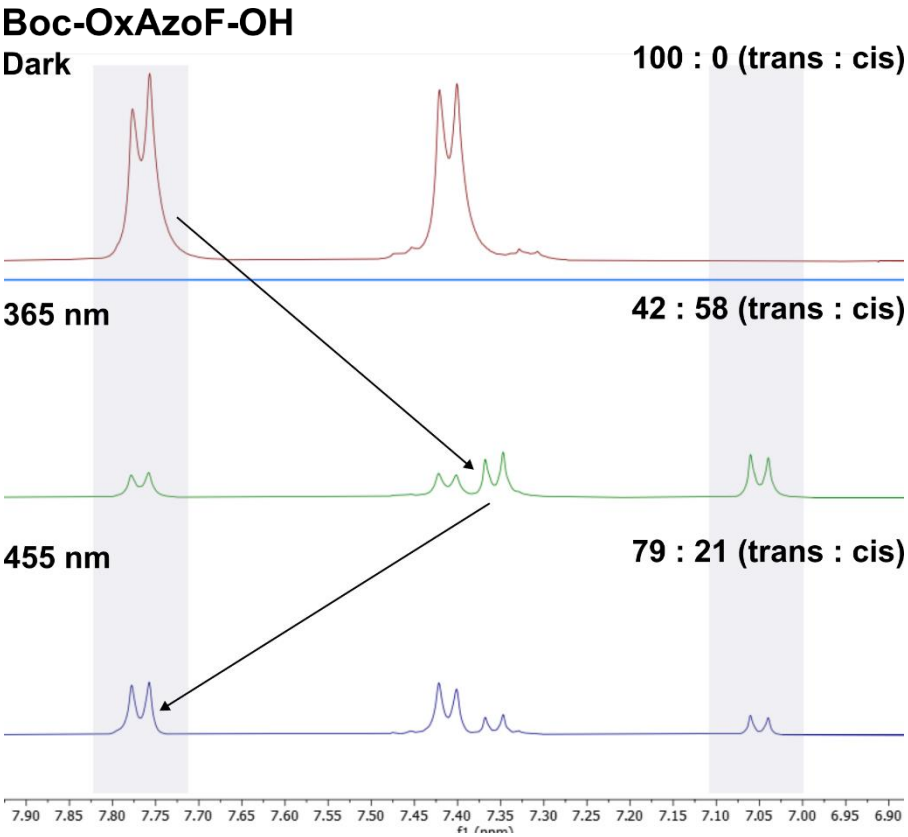


Figure S6 - NMR monitoring of photoswitching process for Boc-OxAzoF-OH through ex-situ illumination. The NMR spectrum has been cut to the aromatic region, where signals of interest can be clearly observed, and their shifting upon light-irradiation can be measured. Highlighted in grey are the peaks that were used to quantify the photoswitching. From top to bottom, we report the NMR spectrum *previa* light irradiation (maroon), after 365 nm light irradiation (green), and after 455 nm light irradiation (blue).

4. CD Measurements

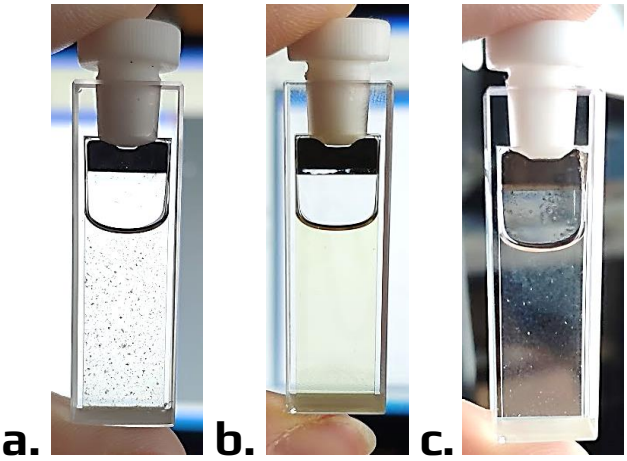


Figure S7 – a. Photo of the cuvette containing a 100 µM A3Tz5 sample dissolved in PBS buffer (pH 7.4) previa light irradiation; **b.** Photo of the cuvette containing a 100 µM A3Tz5 sample dissolved in PBS buffer (pH 7.4) after 15 minutes of irradiation with a 365 nm LED; **c.** Photo of the cuvette containing a 100 µM A3Tz5 sample dissolved in PBS buffer (pH 7.4) after 15 minutes of irradiation with a 455 nm LED.

5. Digital Microscope UltraZoom Pro Recording

A digital microscope was used to record the reversible formation of aggregates in real time at ambient conditions. A mp4 file is provided in a separate file as supporting information. The same sample preparation and cuvettes as for CD measurements (*vide supra*) was applied.

Pictures of the experimental setup are provided below.

Figure S8 – Photograph of the experimental setup for qualitative irradiation studies on a digital microscope. On the left side, a 455 nm LED from ThorLabs was placed and kept switched on during the whole course of the experiment (also visible in the video as constant blueish background). The focus was set of the peptide aggregates, visible in white on the screen. For *trans* to *cis* isomerization, a high power (10W) 365 nm LED torch, placed on the right side of the cuvette (*cf*. detail-view on the right photograph), was switched on for a period of 10 sec, which was the time in which the camera showed maximum disappearance of aggregates. Due to the higher power of the 365 nm light source, the cis to trans isomerization could be outcompeted, while the light was on. Switching off the 365 nm LED torch and illumination with blue light, led to re-appearance of the peptide aggregates on camera within 20 sec (*cf*. real-time video).

6. CryoTEM Imaging

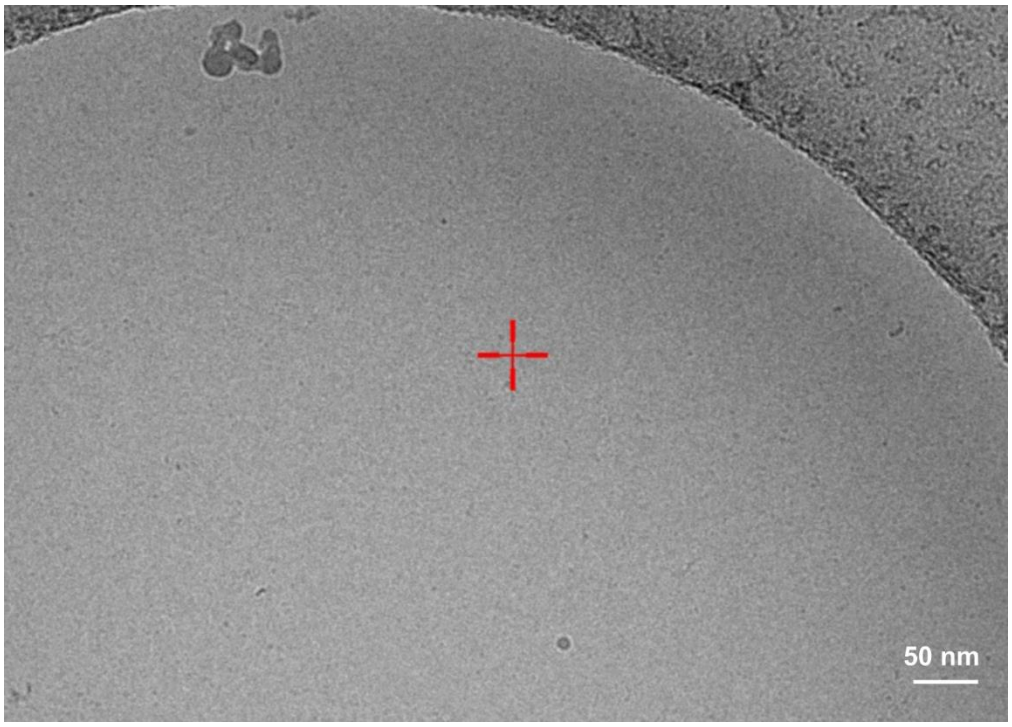
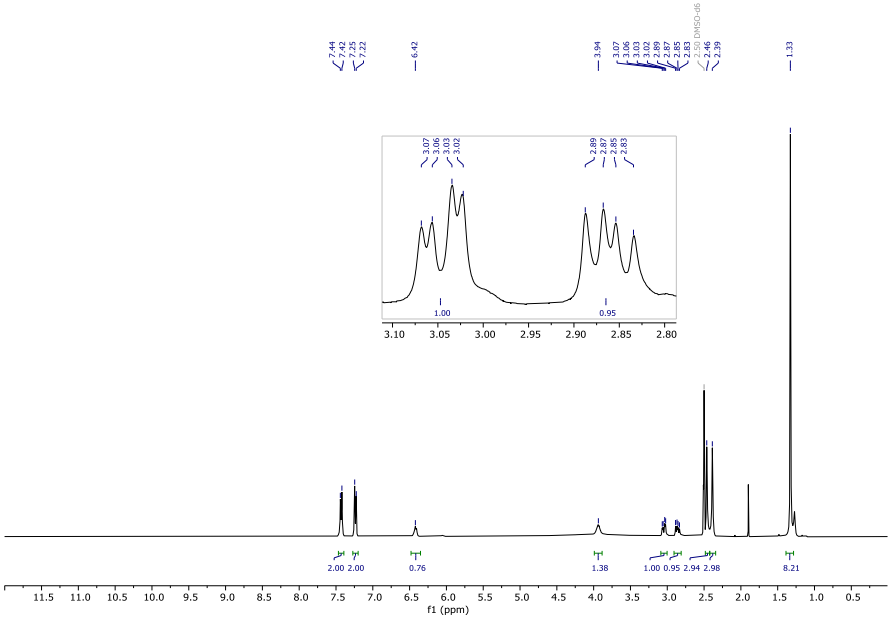


Figure S9 – CryoTEM imaging of a 50 μM A3Tz5 sample dissolved in PBS buffer (pH 7.4) kept in the dark (magnification 64kx).

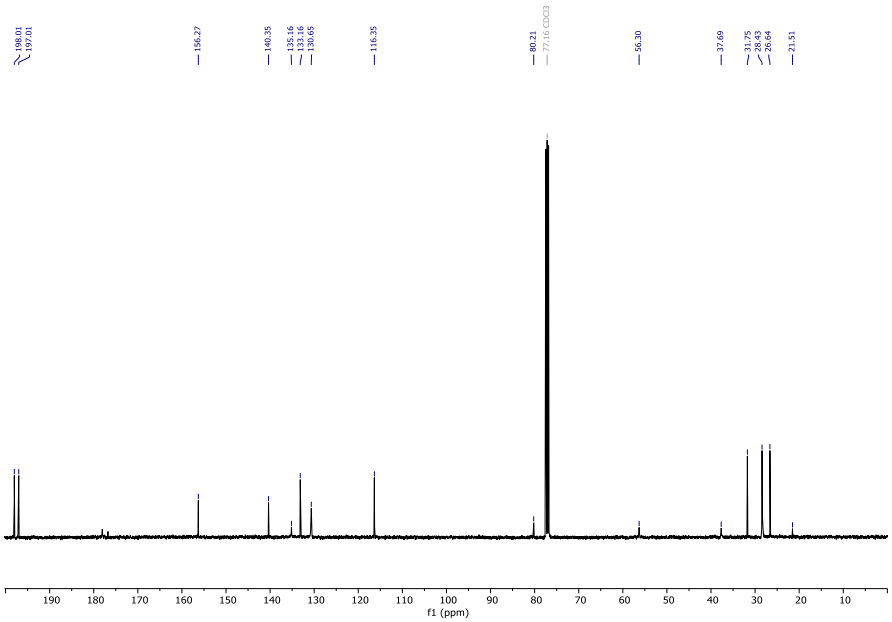
7. NMR Spectra of of Unnatural Amino Acids and their Precursors

Boc-diketone-OH

^{1}H-NMR

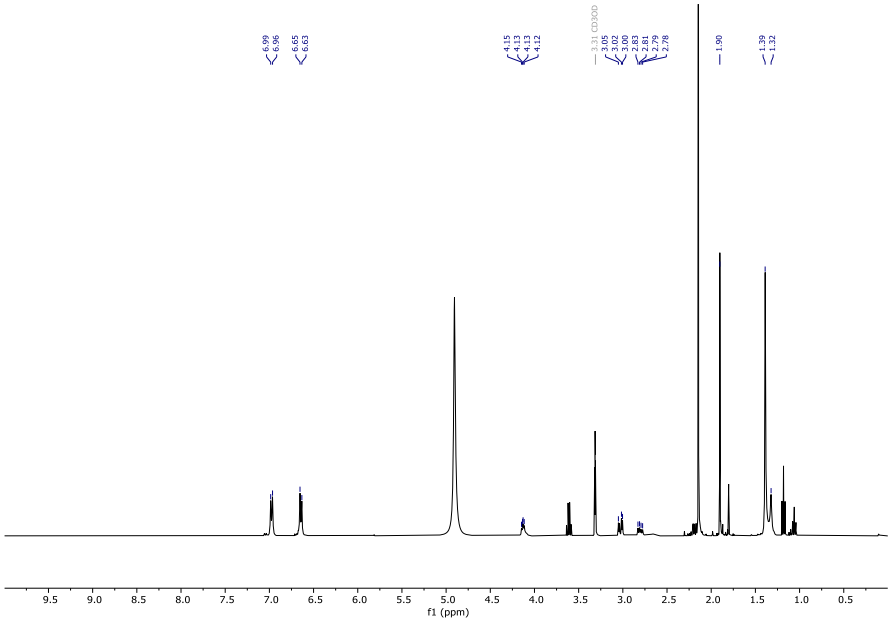


^{13}C-NMR



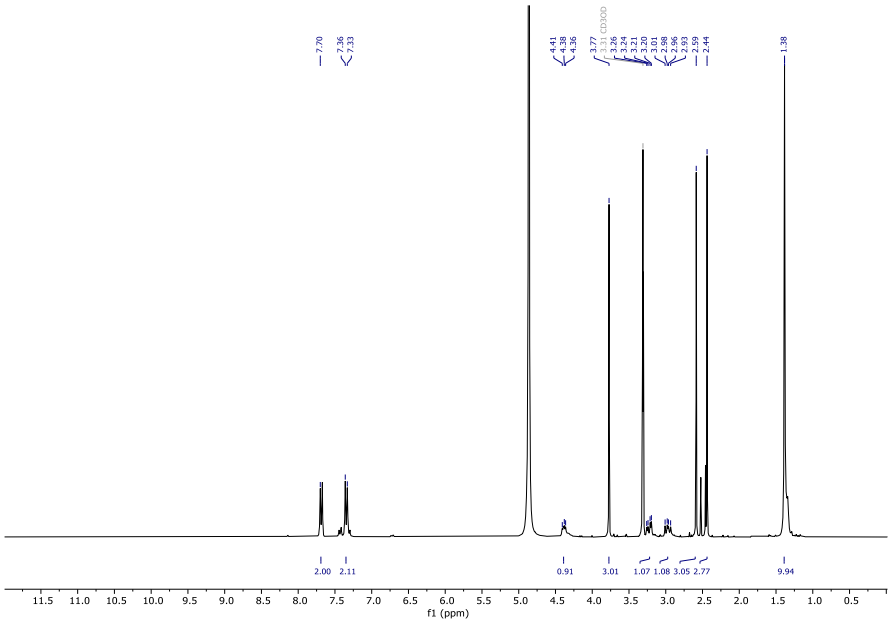
Boc-PyrAzoF-OH

^{1}H-NMR

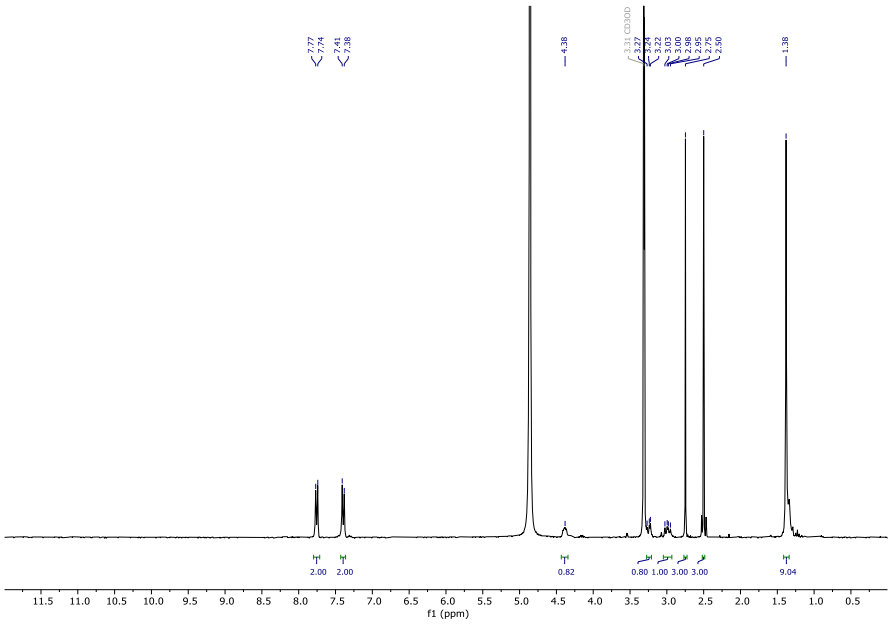


Boc-MePyrAzoF-OH

^{1}H-NMR

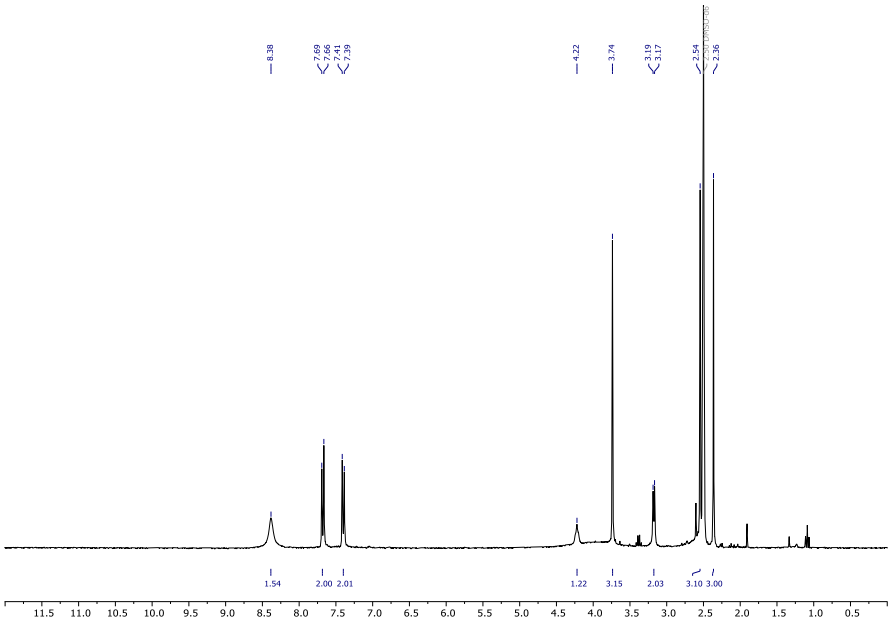


Boc-OxAzoF-OH

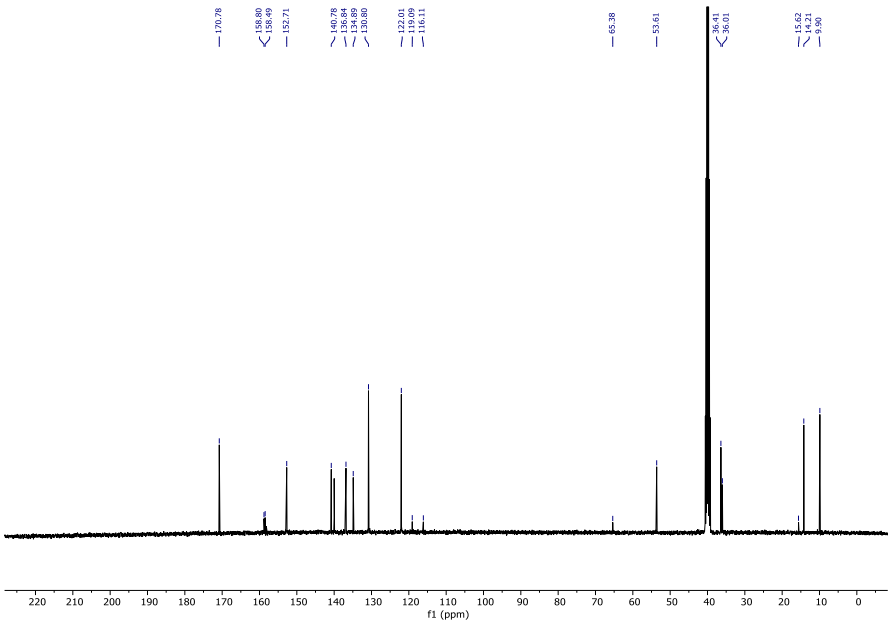


H-MePyrAzoF-OH

^{1}H-NMR

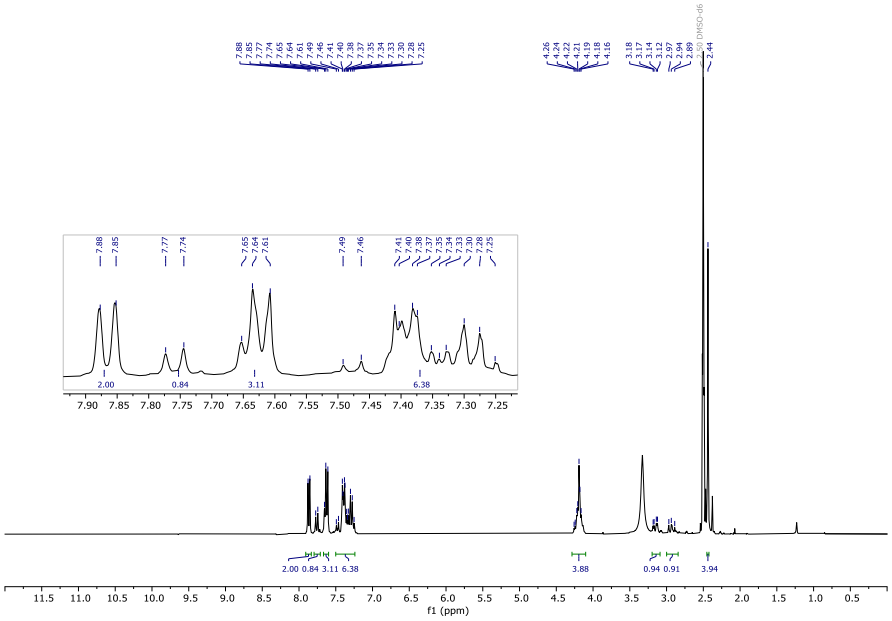


^{13}C-NMR



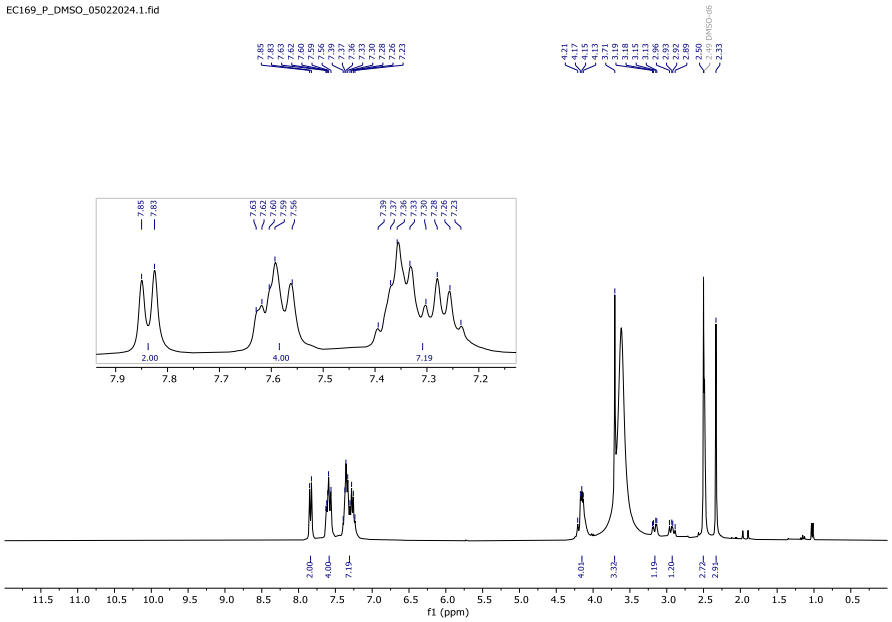
Fmoc-PyrAzoF-OH

^{1}H-NMR

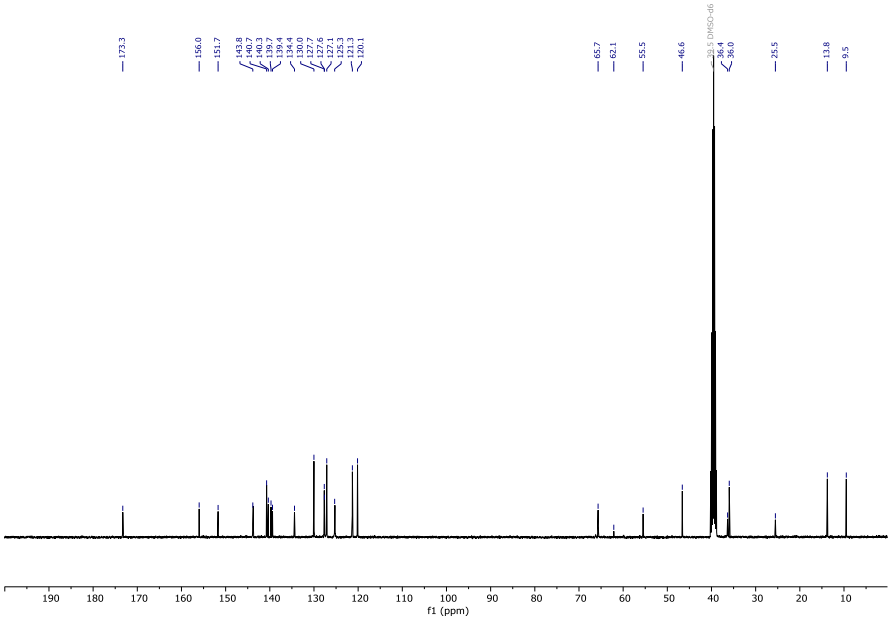


Fmoc-MePyrAzoF-OH

^{1}H-NMR

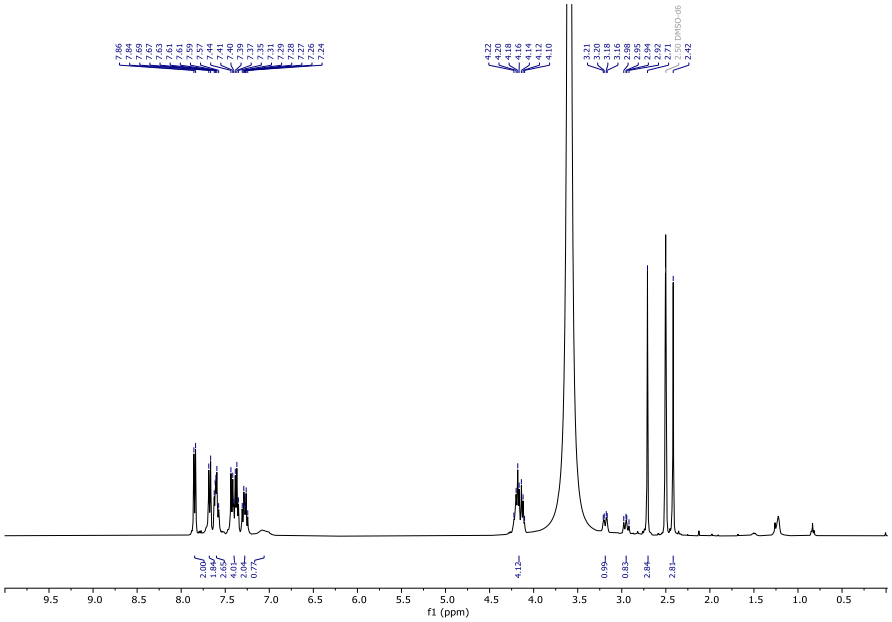


^{13}C-NMR

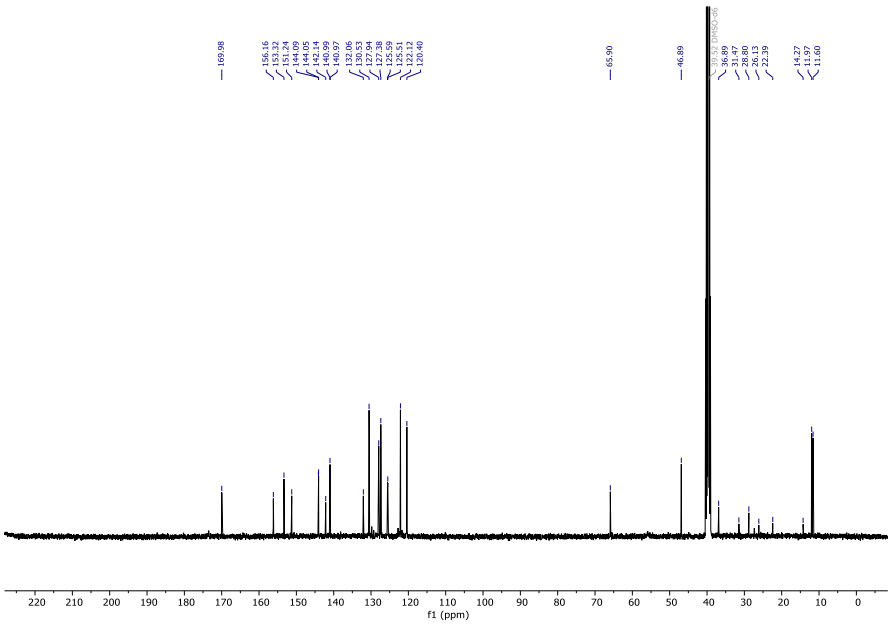


Fmoc-OxAzoF-OH

^{1}H-NMR



^{13}C-NMR



8. NMR of A3Tz5

Characterization of Photoswitching

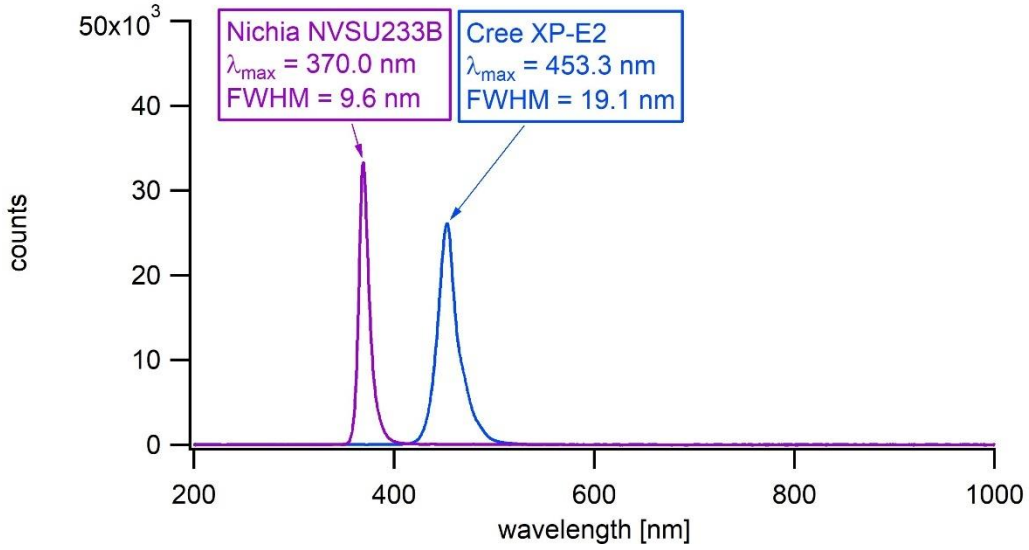


Figure S10: LED emission spectra measured after inserting the assembled NMR illumination device with the roughened end of the glass fibres into the integration sphere (Avantes AvaSphere-50), without adding a sample.

Table S4 – Summary of sample preparation and fitting results for photoswitching experiments performed at three different nominal A3Tz5 concentrations. Nominal concentrations refer to the concentrations determined by weighing the purified peptide, assuming chemical purity, and estimating the uncertainty based on the specified uncertainties of the balance and the pipets used. The estimated c_{max} is the estimated maximum concentration of dissolved peptide measured by ^{1}H-NMR (using TSP-d_4 as internal concentration reference standard). Pseudo-first-order rate constants for photoswitching with irradiation at 370 nm or at 455 nm are given as $\tau_{370\ nm}$ or $\tau_{455\ nm}$, respectively. Values and errors were determined by least-squares fitting monoexponential growth or decay functions to the concentration-time-profiles shown in Fig. 5 b. – d. of the main article. The number #N is the number of times irradiation was performed with the given wavelength. All data collected at 288 K.

nominal c(A3Tz5) [mM]	estimated c_{max}(A3Tz5) [mM]	$\tau_{370\ nm}$ [min]			$\tau_{455\ nm,\#1}$ [min]	
		illumination #1	illumination #2	illumination #3	illumination #1	illumination #2
5 ± 0.1	1.16 ± 0.04	2.72 ± 0.24	2.18 ± 0.14	2.13 ± 0.11	1.00 ± 0.13	0.84 ± 0.08
1 ± 0.05	0.15 ± 0.01	1.36 ± 0.08	0.46 ± 0.03	0.45 ± 0.02	0.95 ± 0.14	0.50 ± 0.01
0.2 ± 0.02	0.025 ± 0.010	0.89 ± 0.11	0.26 ± 0.11	0.25 ± 0.08	2.4 ± 0.6	1.30 ± 0.14

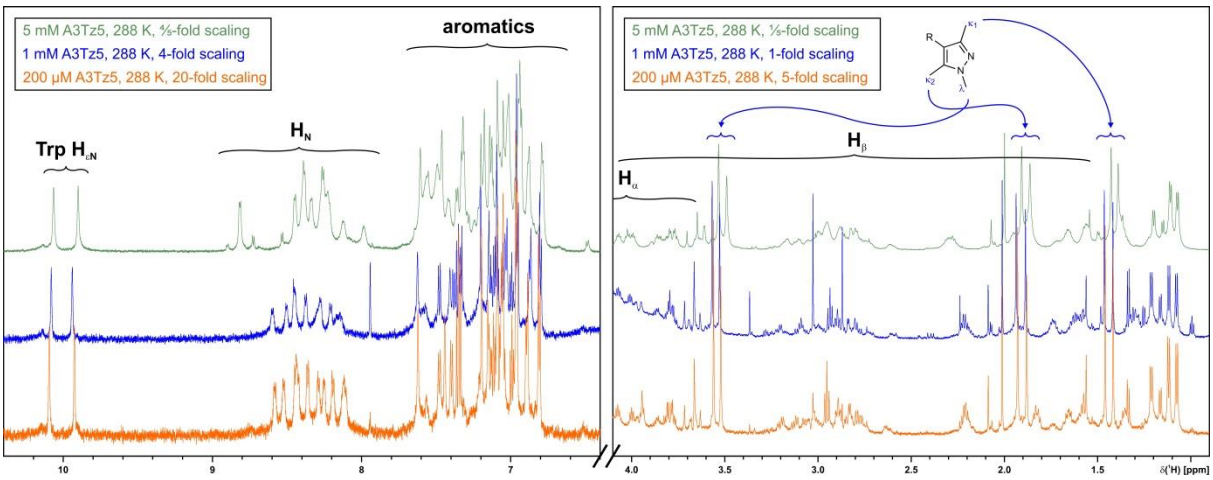


Figure S11: ^{1}H spectra of the A3Tz5 peptide collected at three different peptide concentrations (orange: 200 µM, blue: 1 mM, green: 5 mM) in the photostationary state obtained under continuous irradiation with 370 nm. Data was collected with water suppression, and the central part of the spectrum is omitted for simplicity. Selected signal regions are highlighted. For easier inspection, spectra are scaled with the peptide concentration and the number of scans used, and in addition intensities in the region 10.4 – 6.4 ppm are amplified 4x, with respect to the intensity scale used in the region 4.1 – 0.9 ppm .

^{1}H relaxation rates for A3Tz5

Table S5 — Relaxation time constants T_1 and T_2 measured for the A3Tz5 peptide at two different peptide concentrations. Different chemical shift ranges were integrated for data extraction and averaged relaxation rates are reported for these ranges. Data was collected with or without sample irradiation, labelled as "370 nm" or "off" respectively.

			1 mM				**5 mM**	
			T_1 [s]		T_2 [s]		T_1 [s]	T_2 [s]
range [ppm]		assignment	off	370 nm	off	370 nm	370 nm	370 nm
9.0	7.9	**H^N region**	0.27	0.27	0.086	0.087	0.50	0.063
7.7	6.7	**aromatics**	1.36	1.35	0.22	0.22	0.49	0.079
4.4	3.4	**H^α & Pyr-NMe**	1.12	1.13	0.27	0.28	0.56	0.12
3.3	2.5	**H^β & H^γ**	0.76	0.76	0.12	0.12	0.49	0.064
2.5	1.0	**sidechain**	0.80	0.80	0.24	0.25	0.50	0.11

Table S6 – Relaxation time constants T_1 and T_2 measured for selected residues of the A3Tz5 peptide and for the chemical shift standard TSP-d_4 at two different peptide concentrations. For the methyl peaks, intensity fitting was used rather than integral fitting, due to partial signal overlap. Data was collected with or without sample irradiation, labelled as "370 nm" or "off" respectively.

			1 mM				5 mM	
□(^{1}H) [ppm]			T_1 [s]		T_2 [s]		T_1 [s]	T_2 [s]
1 mM	**5 mM**	assignment	off	370 nm	off	370 nm	370 nm	370 nm
10.060	10.08	**11 Trp, $H_{\varepsilon N}$**	0.84	0.89	0.13	0.11	0.56	0.065
9.92	9.91	**9 Trp, $H_{\varepsilon N}$**	0.76	0.73	0.14	0.13	0.53	0.071
3.56	3.53	**4 MePyrAzoF $NCH_{3,\lambda}$**	1.53	1.56	0.38	0.40	0.69	0.11
3.52	3.49	**2 MePyrAzoF $NCH_{3,\lambda}$**	1.64	1.57	0.25	0.24	0.67	a)
1.93	1.92	**4 MePyrAzoF $CH_{3,\kappa1}$**	1.41	1.38	0.34	0.31	0.64	a)
1.88	1.86	**2 MePyrAzoF $CH_{3,\kappa1}$**	1.52	1.49	0.23	0.21	0.56	a)
1.46	1.43	**4 MePyrAzoF $CH_{3,\kappa2}$**	1.41	1.38	0.38	0.38	0.64	0.11
1.42	1.39	**2 MePyrAzoF $CH_{3,\kappa2}$**	1.50	1.44	0.28	0.27	0.62	a)
0.0	0.0	**TSP-d_4**	2.67	2.72	2.37	2.37	0.50	0.16

a) point eliminated due to poor fitting quality

^{1}H-NMR signal assignment for A3Tz5

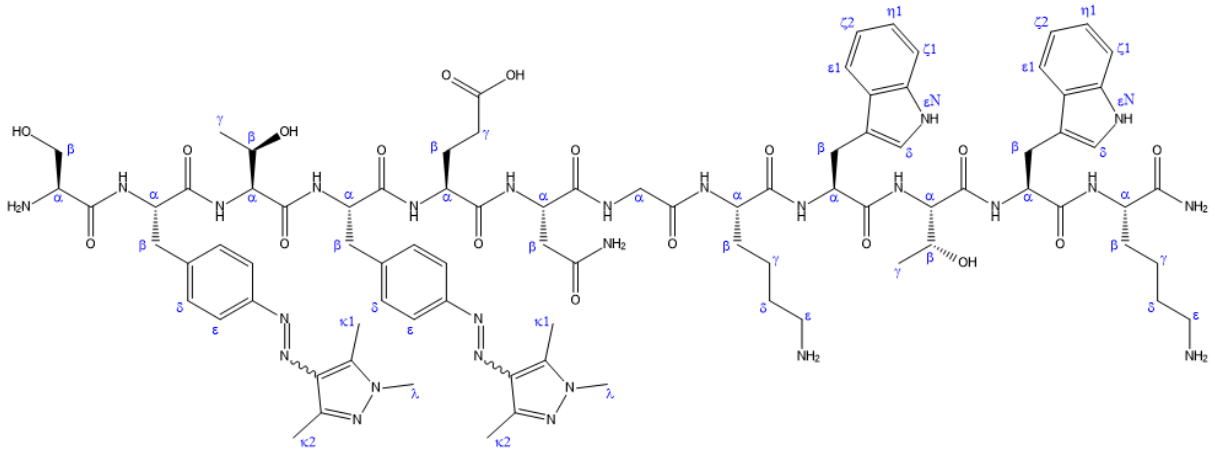


Figure S12: Chemical structure of A3Tz5 with ^{1}H position labels used throughout the signal assignment.

Table S7 – ^{1}H resonance assignments for the A3Tz5 peptide at 288 K at three different concentrations (200 µM, 1 mM, 5 mM). Samples were prepared in PBS-buffer (1x physiological concentration), pH 7.4, 92% H_2O, 8% D_2O, with 0.094 mM TSP-d_4. All chemical shifts are referenced against TSP-d_4. Data was collected in the photostationary state obtained under continuous irradiation with 370 nm. Selected inter-residue NOEs measured for the 5 mM sample are given in the rightmost column. For simpler identification of cross-peaks that may indicate folding into a tertiary structure, inter residue contacts between residues close to the N-terminus (2 & 4) and the C-terminus (9, 10 & 11) are highlighted in blue.

residue	position	δ(^{1}H, @288 K) [ppm]			selected Inter-residue NOEs
		200 µM	1 mM	5 mM	5 mM
1 Ser	H_N				
	H_α		4.35	4.36	
	$H_{\beta1}$		3.77	3.77	
	$H_{\beta2}$		3.81	3.79	
2 MePyrAzoF	H_N			8.26	1.11 (3 Thr $CH_{3,\gamma}$)
	H_α			4.62	
	$H_{\beta1}$	2.74	2.76	2.74	
	$H_{\beta2}$	3.02	3.04	3.02	
	H_δ	7.02	7.05	7.03	1.11 (3 Thr $CH_{3,\gamma}$) 1.39 (4 MePyrAzoF $CH_{3,\kappa2}$) 1.86 (4 MePyrAzoF $CH_{3,\kappa1}$) 3.49 (4 MePyrAzoF $NCH_{3,\kappa\lambda}$)
	H_ε	6.95	6.95	6.93	1.07 (10 Thr $CH_{3,\gamma}$) 1.11 (3 Thr $CH_{3,\gamma}$) 1.39 (4 MePyrAzoF $CH_{3,\kappa2}$) 1.86 (4 MePyrAzoF $CH_{3,\kappa1}$) 3.49 (4 MePyrAzoF $NCH_{3,\kappa\lambda}$)
	$CH_{3,\kappa1}$ [a]	1.94	1.93	1.91	6.79 (4 MePyrAzoF H_ε) 6.88 (4 MePyrAzoF H_δ) 7.09 (9 Trp H_δ or 11 Trp $H_{\eta1}$) 7.32[c] (9 Trp $H_{\zeta1}$ or 11 Trp $H_{\zeta1}$)
	$CH_{3,\kappa2}$ [b]	1.46	1.46	1.43	6.79 (4 MePyrAzoF H_ε) 6.88 (4 MePyrAzoF H_δ) 7.09 (9 Trp H_δ or 11 Trp $H_{\eta1}$) 7.18 (11 Trp H_δ) 7.32[c] (9 Trp $H_{\zeta1}$ or 11 Trp $H_{\zeta1}$)
	$NCH_{3,\lambda}$	3.57	3.56	3.53	6.79 (4 MePyrAzoF H_ε) 6.88 (4 MePyrAzoF H_δ) 7.09 (9 Trp H_δ or 11 Trp $H_{\eta1}$) 7.18 (11 Trp H_δ) 7.32[c] (9 Trp $H_{\zeta1}$ or 11 Trp $H_{\zeta1}$)
3 Thr	H_N			8.44	
	H_α	4.36	4.34	4.35	
	H_β	4.07	4.07	4.08	8.21 (4 MePyrAzoF H_N)
	$CH_{3,\gamma}$	1.12	1.12	1.11	6.88 (4 MePyrAzoF H_δ) 6.93 (2 MePyrAzoF H_ε) 7.03 (2 MePyrAzoF H_δ)

					7.09 (9 Trp H_{δ} or 11 Trp $H_{\eta 1}$) 7.18 (11 Trp H_{δ}) 8.21 (4 MePyrAzoF H_N) 8.26 (2 MePyrAzoF H_N)
4 MePyrAzoF	H_N			8.21	1.07 (10 Thr $CH_{3,\gamma}$) 1.11 (3 Thr $CH_{3,\gamma}$)
	H_{α}			4.65	8.34 (5 Glu H_N)
	$H_{\beta 1}$	2.81	2.83	2.82	8.34 (5 Glu H_N)
	$H_{\beta 2}$	2.61	2.63	2.61	8.34 (5 Glu H_N)
	H_{δ}	6.86	6.89	6.88	1.11 (3 Thr $CH_{3,\gamma}$) 1.43 (2 MePyrAzoF $CH_{3,\kappa 2}$) 1.91 (2 MePyrAzoF $CH_{3,\kappa 1}$) 3.53 (2 MePyrAzoF $NCH_{3,\kappa\lambda}$) 8.34 (5 Glu H_N)
	H_{ε}	6.80	6.80	6.79	1.43 (2 MePyrAzoF $CH_{3,\kappa 2}$) 1.91 (2 MePyrAzoF $CH_{3,\kappa 1}$) 3.53 (2 MePyrAzoF $NCH_{3,\kappa\lambda}$) 8.34 (5 Glu H_N)
	$CH_{3,\kappa 1}$ [a)]	1.89	1.88	1.86	6.93 (2 MePyrAzoF H_{ε}) 7.03 (2 MePyrAzoF H_{δ}) 7.09 (9 Trp H_{δ} or 11 Trp $H_{\eta 1}$) 7.18 (11 Trp H_{δ}) 7.32[c)] (9 Trp $H_{\zeta 1}$ or 11 Trp $H_{\zeta 1}$)
	$CH_{3,\kappa 2}$ [b)]	1.42	1.42	1.39	6.93 (2 MePyrAzoF H_{ε}) 7.03 (2 MePyrAzoF H_{δ}) 7.09 (9 Trp H_{δ} or 11 Trp $H_{\eta 1}$) 7.18 (11 Trp H_{δ}) 7.32[c)] (9 Trp $H_{\zeta 1}$ or 11 Trp $H_{\zeta 1}$)
	$NCH_{3,\lambda}$	3.53	3.52	3.49	6.93 (2 MePyrAzoF H_{ε}) 7.03 (2 MePyrAzoF H_{δ}) 7.09 (9 Trp H_{δ} or 11 Trp $H_{\eta 1}$) 7.18 (11 Trp H_{δ}) 7.32[c)] (9 Trp $H_{\zeta 1}$ or 11 Trp $H_{\zeta 1}$)
5 Glu	H_N			8.34	4.56 (4 MePyrAzoF H_{α}) 2.61 (4 MePyrAzoF $H_{\beta 1}$) 2.81 (4 MePyrAzoF $H_{\beta 2}$) 6.88 (4 MePyrAzoF H_{δ}) 6.79 (4 MePyrAzoF H_{ε})
	H_{α}		4.24	4.29	
	$H_{\beta 1}$	1.91	1.90	1.91	8.23 (6 Asn H_N)
	$H_{\beta 2}$	1.94	1.94	1.95	8.23 (6 Asn H_N)
	$H_{\gamma 1}$	2.21	2.20	2.28	
	$H_{\gamma 2}$	2.20	2.22	2.30	
6 Asn	H_N			8.23	1.91 (5 Glu $H_{\beta 1}$)

					1.95 (5 Glu $H_{\beta2}$)
	H_{α}			4.73	8.38 (7 Gly H_N)
	$H_{\beta1}$	2.84	2.84	2.82	
	$H_{\beta2}$	2.78	2.78	2.79	
7 Gly	H_N			8.38	8.23 (6 Asn H_{α})
	$H_{\alpha1}$	3.68	3.67	3.65	
	$H_{\alpha2}$	3.95	3.95	3.88	
8 Lys	H_N				
	H_{α}			4.03	
	$H_{\beta1\&2}$	1.73	1.82	1.86	8.82 (11 Trp H_N) 1.07 (10 Thr $CH_{3,\gamma}$)
	$H_{\gamma1\&2}$	1.31	1.35	1.37	1.07 (10 Thr $CH_{3,\gamma}$)
	$H_{\delta1\&2}$	1.63	1.65	1.66	1.07 (10 Thr $CH_{3,\gamma}$)
	$H_{\varepsilon1\&2}$	2.93	2.95	2.96	1.07 (10 Thr $CH_{3,\gamma}$)
9 Trp	H_N			8.81	4.03 (8 Lys H_N) 1.86 (8 Lys $H_{\beta1\&2}$) 1.37 (8 Lys $H_{\gamma1\&2}$)
	H_{α}			4.82	8.12 (10 Thr H_N)
	$H_{\beta1}$	2.99	3.06	2.99	4.39 (10 Thr H_{α})
	$H_{\beta2}$	3.11	3.13	3.12	4.39 (10 Thr H_{α})
	H_{δ}	7.09	7.09	7.09	
	$H_{\varepsilon N}$	9.94	9.92	9.90	
	$H_{\varepsilon1}$	7.38	7.40	7.36	
	$H_{\zeta1}$	7.35	7.35	7.33	
	$H_{\zeta2}$	6.99[d)]	6.99[d)]	6.96[d)]	
	$H_{\eta1}$	7.09[d)]	7.09[d)]	7.09[d)]	
10 Thr	H_N			8.12	
	H_{α}	4.38	4.38	4.39	2.99 (9 Trp $H_{\beta1}$) 3.12 (9 Trp $H_{\beta2}$)
	H_{β}	4.00	4.00	4.00	
	$CH_{3,\gamma}$	1.07	1.07	1.07	1.37 (8 Lys $H_{\gamma1\&2}$) 1.66 (8 Lys $H_{\delta1\&2}$) 1.86 (8 Lys $H_{\beta1\&2}$) 2.96 (8 Lys $H_{\varepsilon1\&2}$) 6.93 (2 MePyrAzoF H_{ε}) 7.09 (9 Trp H_{δ} or 11 Trp $H_{\eta1}$) 7.18 (11 Trp H_{δ}) 8.21 (4 MePyrAzoF H_N) 8.26 (2 MePyrAzoF H_N) 8.27 (11 Trp H_N)
11 Trp	H_N			8.27	1.07 (10 Thr H_{β}) 1.86 (8 Lys $H_{\beta1\&2}$)
	H_{α}			4.37	
	$H_{\beta1}$	3.08	3.07	3.05	

	$H_{\beta 2}$	3.22	3.21	3.18	
	H_{δ}	7.20	7.19	7.18	1.07 (10 Thr $CH_{3,\gamma}$) 1.11 (3 Thr $CH_{3,\gamma}$) 1.39 (4 MePyrAzoF $CH_{3,\kappa 2}$) 1.43 (2 MePyrAzoF $CH_{3,\kappa 2}$) 1.86 (4 MePyrAzoF $CH_{3,\kappa 1}$) 3.49 (4 MePyrAzoF $NCH_{3,\kappa\lambda}$) 3.53 (2 MePyrAzoF $NCH_{3,\kappa\lambda}$)
	$H_{\varepsilon N}$	10.08	10.09	10.06	
	$H_{\varepsilon 1}$	7.48	7.48	7.42	
	$H_{\zeta 1}$	7.33	7.34	7.32	
	$H_{\zeta 2}$	7.05[d)]	7.05[d)]	7.02[d)]	
	$H_{\eta 1}$	7.11[d)]	7.11[d)]	7.09[d)]	
12 Lys	H_N			8.41	
	H_{α}	4.23	4.23	4.23	8.27 (11 Trp H_N)
	$H_{\beta 1\&2}$	1.75	1.74	1.73	
	$H_{\gamma 1\&2}$	1.22	1.21	1.20	
	$H_{\delta 1\&2}$	1.59	1.58	1.57	
	$H_{\varepsilon 1\&2}$	2.89	2.89	2.89	
Ter $C(=O)NH_2$	H_N			7.99 & 8.39	
Fraction of ^1H assigned		66%	69%	85%	

a) Proximal to $NCH_{3,\lambda}$. b) Distal from $NCH_{3,\lambda}$. c) No discrimination between 9 Trp $H_{\zeta 1}$ and 11 Trp $H_{\zeta 1}$ possible based on apparent chemical shift. d) Positions $H_{\zeta 2}$ and $H_{\eta 1}$ may be interchanged in 9 Trp and 11 Trp.

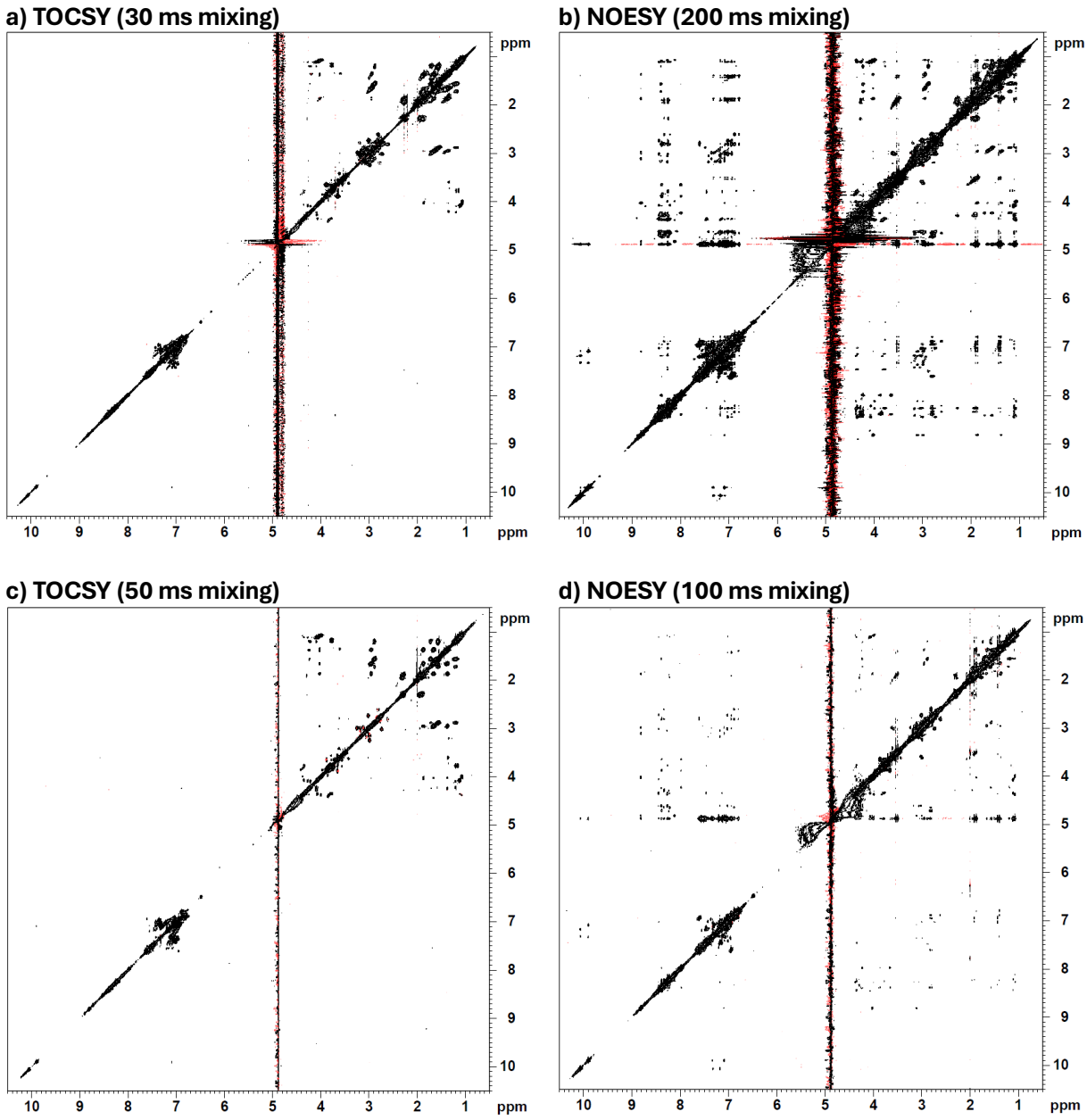


Figure S13: 2D-spectra utilized for ^{1}H signal assignment of the 5 mM A3Tz5 sample at 288 K. Contours are set just above the noise. Figure 5e of the main article shows selected regions of the spectrum shown in panel b). The NOESY spectra in panels b) and d) were collected with 32 or 16 dummy scans and 64 or 16 scans per increment 512 or 256 complex points in *F*1 and a 200 ms or 100 ms mixing time, respectively. The TOCSY spectra in panels a) and c) were collected with 32 or 16 dummy scans, 512 or 256 complex points in *F*1 and a 30 ms or 50 ms mixing time, respectively. 16 scans per increment were used for both TOCSY spectra.

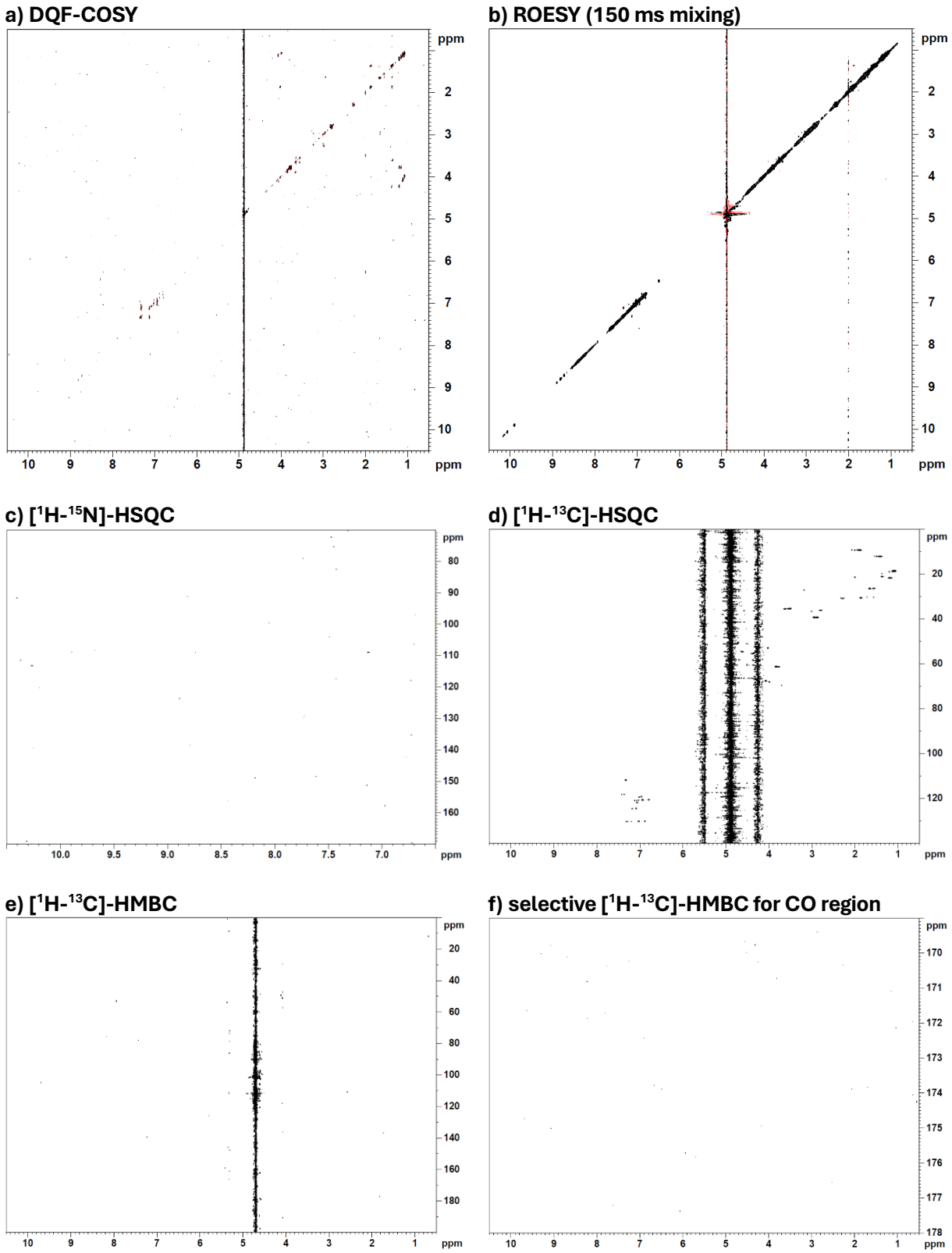
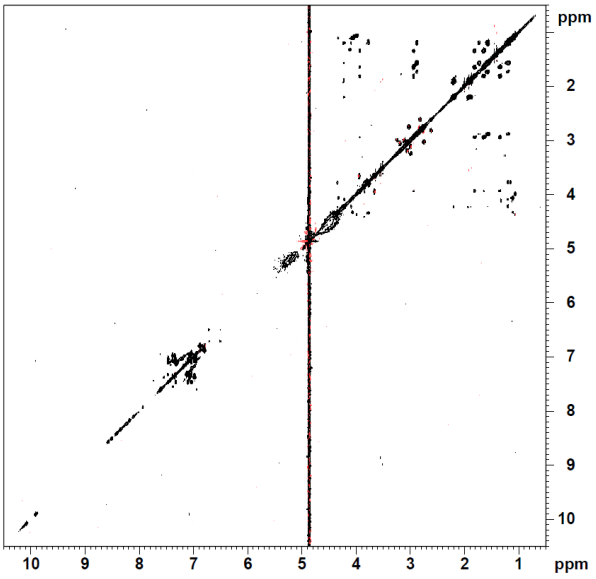


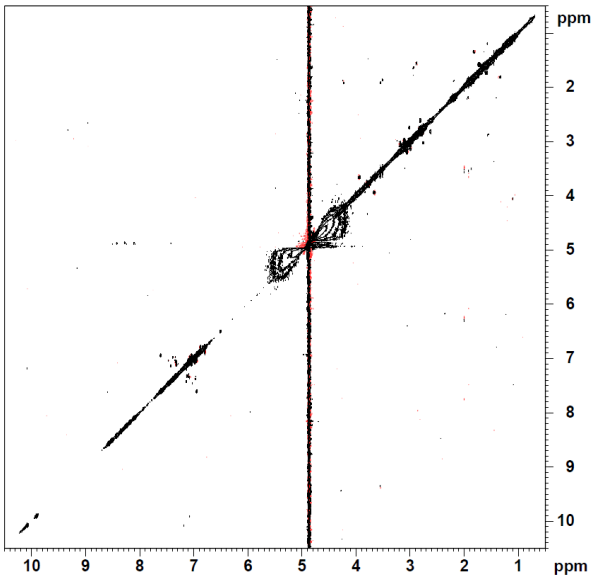
Figure S14: Exemplary 2D-spectra collected for 5 mM A3Tz5 sample at 288 K with experimental parameters comparable to those in Figure S & Figure S. Contours are set just above the noise. The DQF-COSY and the ROESY spectrum in panels a) and b) were collected with 16 dummy scans, 16 scans per increment in *F1* and 256 complex points in *F*1. The [^{1}H-^{15}N]-HSQC spectrum in panel c) was collected with 16 dummy scans, 64 scans per increment in *F1* and 256 complex points in *F*1. The [^{1}H-^{13}C]-HSQC and [^{1}H-

^{13}C]-HMBC spectra in panels d) and e) were collected with 32 dummy scans, 16 scans per increment in *F1* and 256 complex points in *F*1. The CO-selective [^{1}H-^{13}C]-HMBC in panel f) was collected with 32 dummy scans, 128 scans per increment in *F1* and 128 complex points in *F*1.

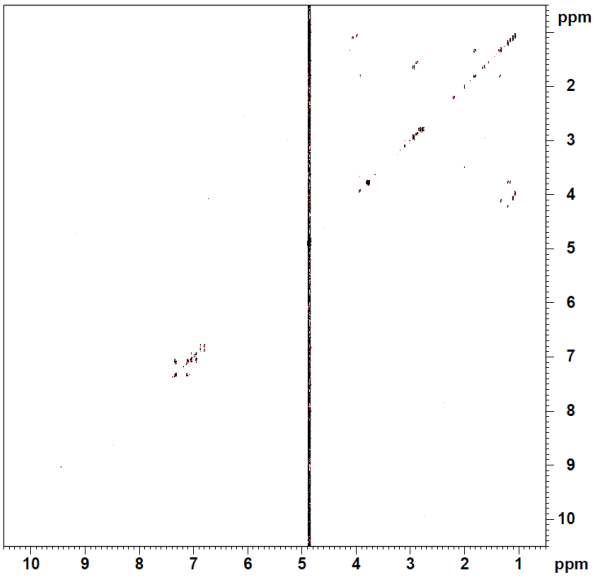
a) TOCSY (50 ms mixing)



b) NOESY (100 ms mixing)



c) DQF-COSY



d) ROESY (150 ms mixing)

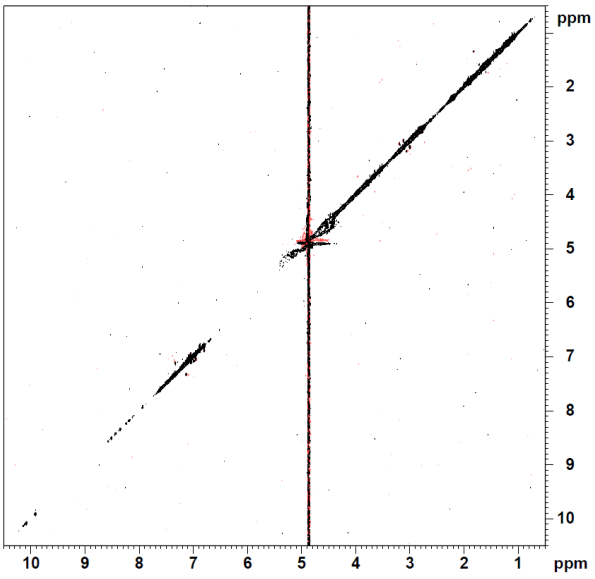


Figure S15: Exemplary 2D-spectra collected for 1 mM A3Tz5 sample at 288 K. Contours are set just above the noise. All spectra shown in this figure were collected with 16 dummy scans and 16 scans per increment in *F1*, 256 complex points in *F*1.

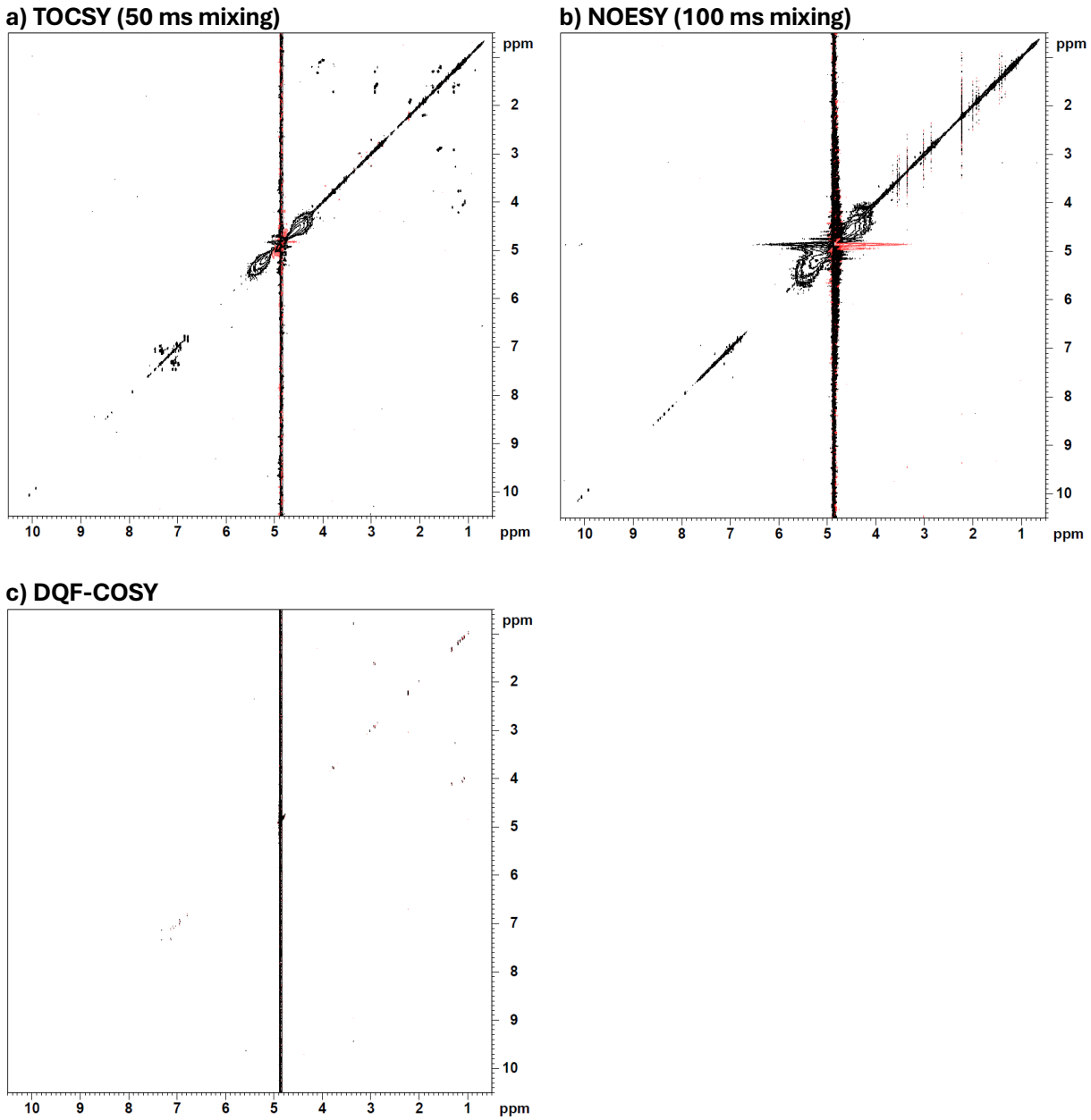
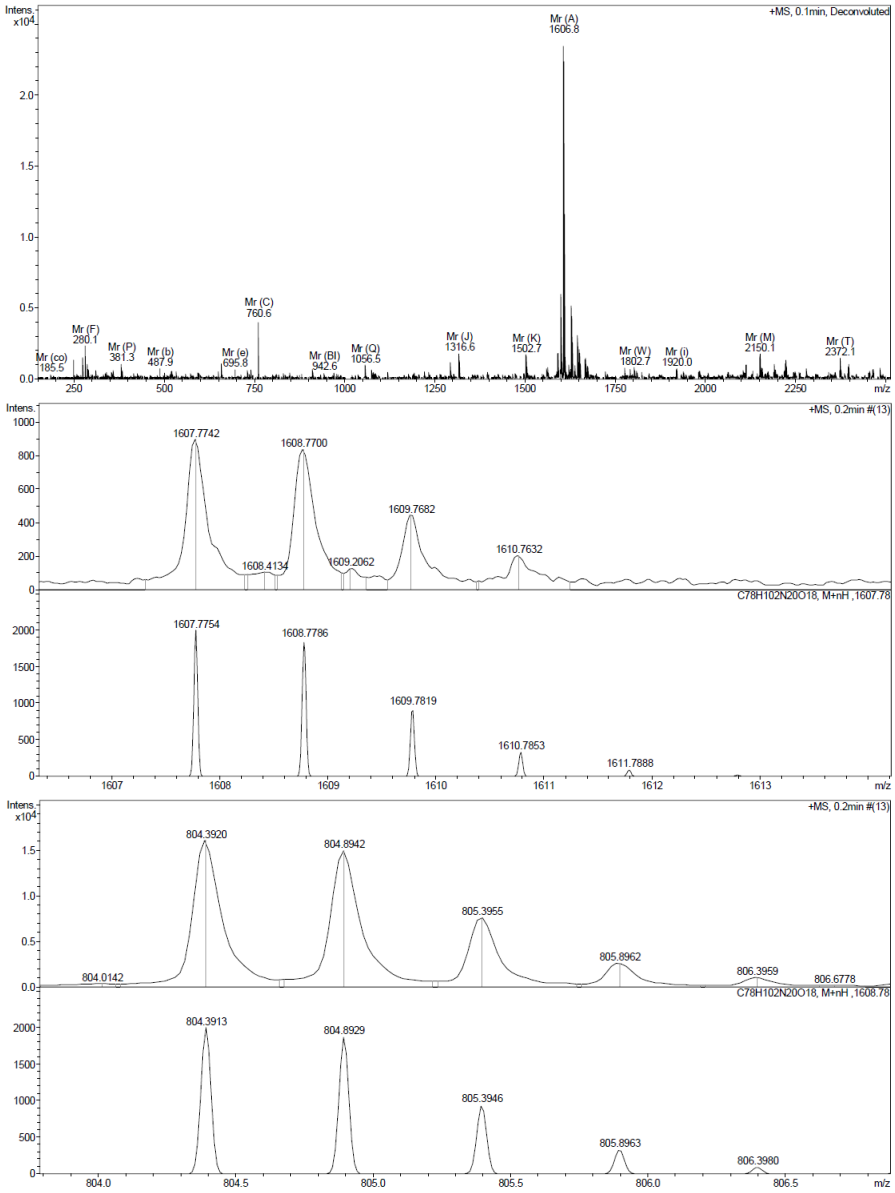
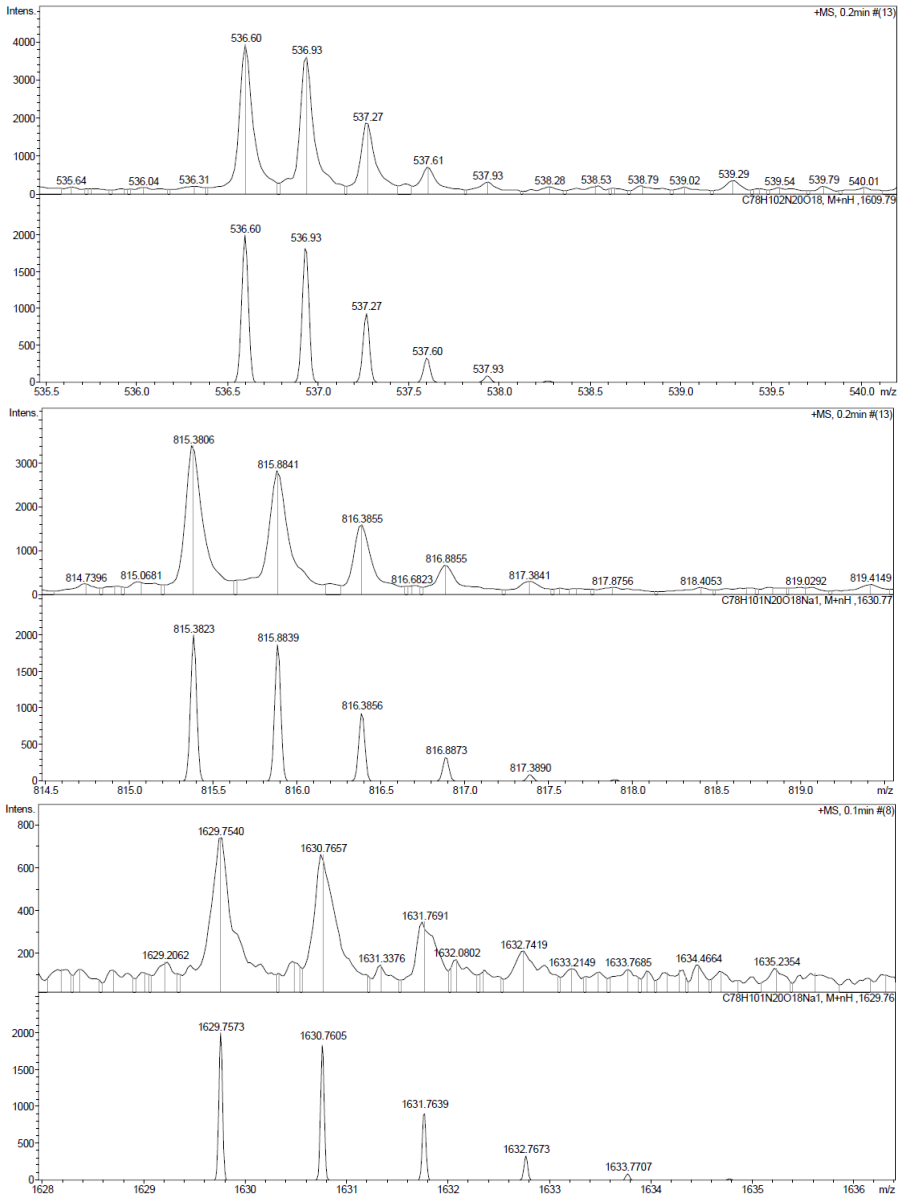


Figure S16: 2D-spectra collected for 200 µM A3Tz5 sample at 288 . Contours are set just above the noise. The TOCSY and NOESY spectra in panels a) and b) were collected with 16 dummy scans and 64 scans per increment in *F1*, 256 complex points in *F*1. The DQF-COSY spectrum in panels c) was collected with 16 dummy scans and 64 scans per increment in *F1*, 384 complex points in *F*1.

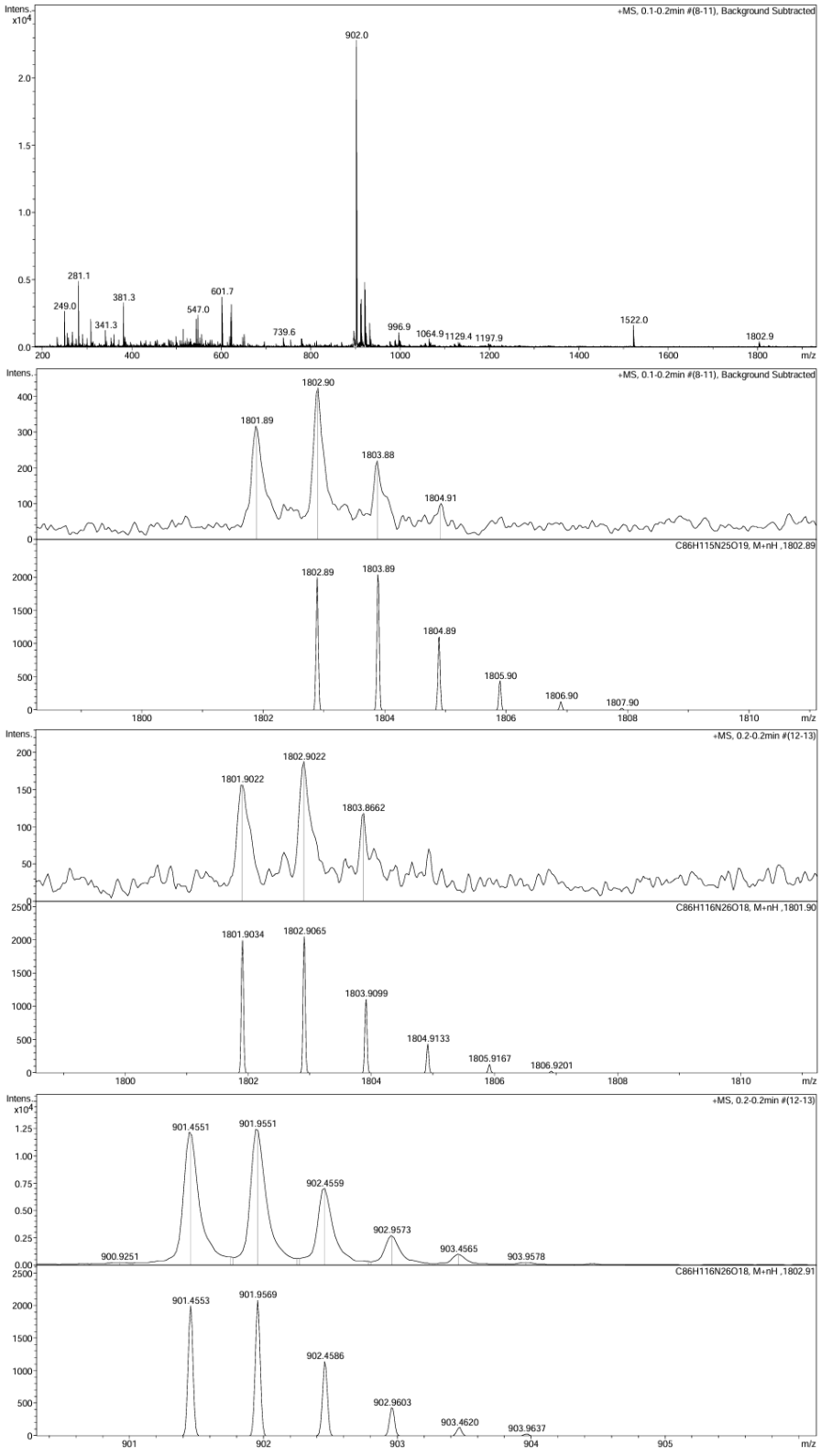
9. Mass Spectra

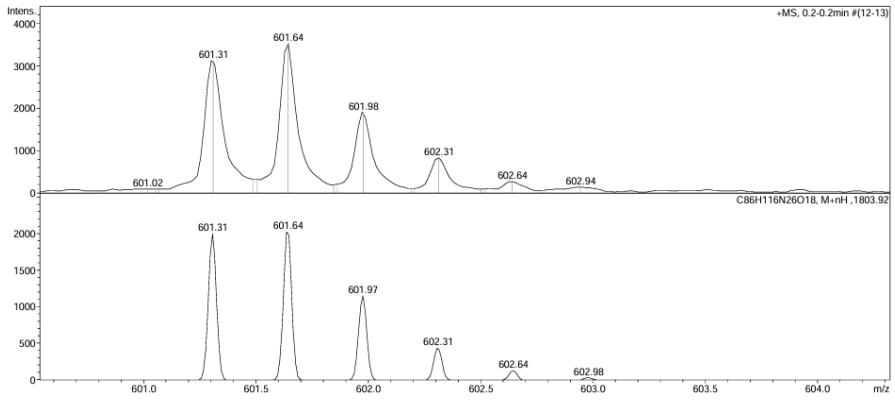
TrpZip2




Intens.
+MS, 0.2min #(13)
536.60
536.93
537.27
537.61
537.93
538.28
538.53
538.79
539.02
539.29
539.54
539.79
540.01
535.64
536.04
536.31
C78H102N20O18, M+nH, 1609.79
536.60
536.93
537.27
537.60
537.93
m/z
Intens.
+MS, 0.2min #(13)
815.3806
815.8841
816.3855
816.8855
814.7396
815.0681
816.6823
817.3841
817.8756
818.4053
819.0292
819.4149
C78H101N20O18Na1, M+nH, 1630.77
815.3823
815.8839
816.3856
816.8873
817.3890
m/z
Intens.
+MS, 0.1min #(8)
1629.7540
1630.7657
1631.7691
1629.2062
1631.3376
1632.0802
1632.7419
1633.2149
1633.7685
1634.4664
1635.2354
C78H101N20O18Na1, M+nH, 1629.76
1629.7573
1630.7605
1631.7639
1632.7673
1633.7707
m/z

A3Tz5

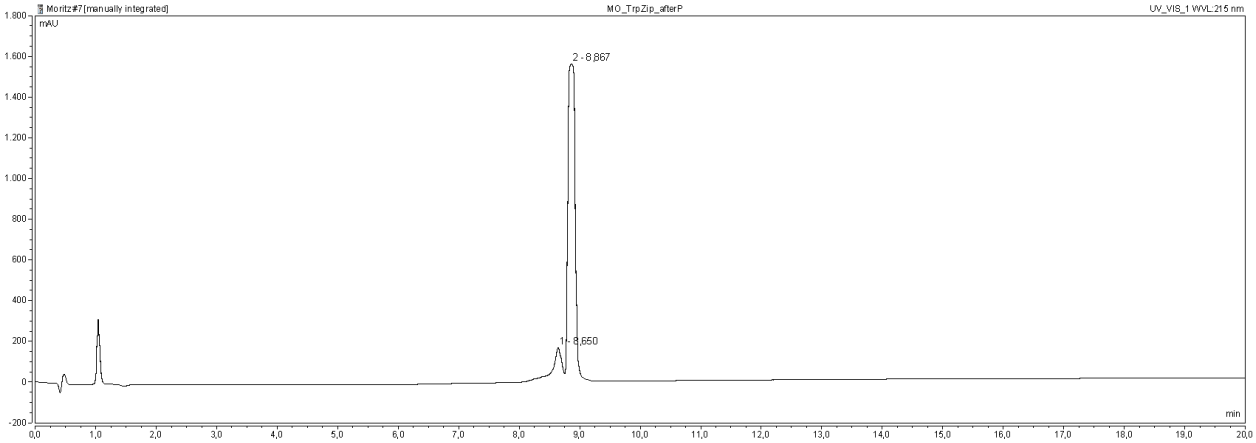

+MS, 0.1-0.2min #(8-11), Background Subtracted
902.0
281.1
249.0
381.3
341.3
547.0
601.7
739.6
996.9
1064.9
1129.4
1197.9
1522.0
1802.9
m/z
+MS, 0.1-0.2min #(8-11), Background Subtracted
1802.90
1801.89
1803.88
1804.91
C86H115N25O19, M+nH ,1802.89
1802.89
1803.89
1804.89
1805.90
1806.90
1807.90
+MS, 0.2-0.2min #(12-13)
1802.9022
1801.9022
1803.8662
C86H116N26O18, M+nH ,1801.90
1801.9034
1802.9065
1803.9099
1804.9133
1805.9167
1806.9201
+MS, 0.2-0.2min #(12-13)
901.4551
901.9551
902.4559
902.9573
903.4565
900.9251
903.9578
C86H116N26O18, M+nH ,1802.91
901.4553
901.9569
902.4586
902.9603
903.4620
903.9637


Intens.
+MS, 0.2-0.2min #(12-13)
601.31
601.64
601.98
602.31
602.64
602.94
601.02
C86H116N26O18, M+nH ,1803.92
601.31
601.64
601.97
602.31
602.64
602.98
601.0
601.5
602.0
602.5
603.0
603.5
604.0
m/z

10. UHPLC Chromatograms (Purity Grades)

TrpZip2

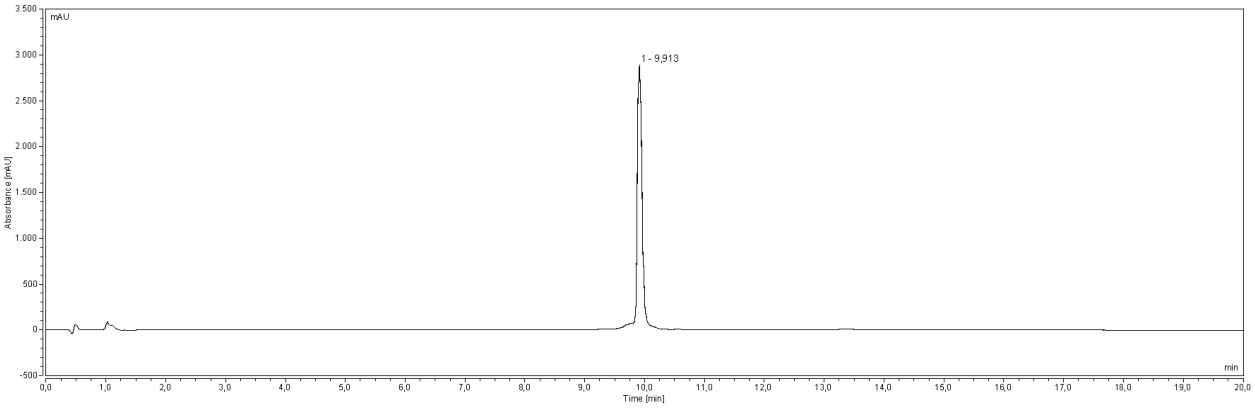
220 nm detector



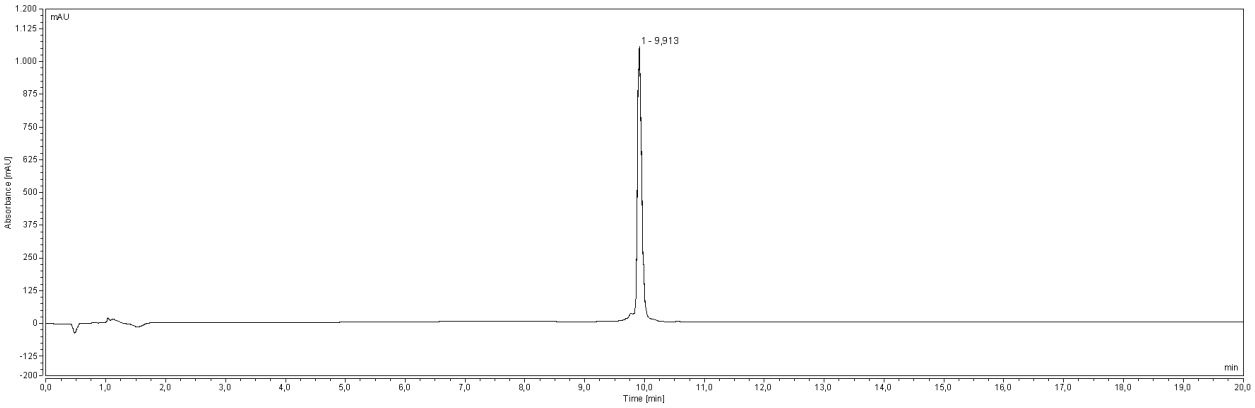
94.8% purity (220 nm detector)

A3Tz5

220 nm detector



365 nm detector



99.9% purity (220 nm detector)

11. Literature

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