Supporting Information

Enzymatic Macrocyclization of 1,2,3-Triazole Peptide Mimetics

Emilia Oueis, Marcel Jaspars, Nicholas J. Westwood, and James H. Naismith*

anie_201601564_sm_miscellaneous_information.pdf
I. STRUCTURES OF PRECURSOR PePTIDES P AND CYCLIC PEPTIDES CP ............ S2

II. GENERAL INFORMATION AND MATERIALS .................................................. S4

III. GENERAL PROCEDURES ........................................................................... S6

IV. MS AND HPLC DATA OF STARTING AND FINAL CYCLIC PEPTIDES ..........S14

V. NMR DATA OF FINAL CYCLIC PEPTIDES ................................................ S16

VI. NMR SPECTRA OF FINAL CYCLIC PEPTIDES ..................................... S33

VII. EXSY NMR OF FINAL CYCLIC PEPTIDES ......................................... S42

VIII. MS-MS DATA OF FINAL CYCLIC PEPTIDES .................................... S47

IX. LC-MS TRACES OF CRUDE MIXTURES OF CP3, CP13, AND CP135 ..........S52

X. LC-MS TRACES OF FINAL CYCLIC PEPTIDES .................................... S55

XI. LC-MS TRACES OF STARTING PEPTIDES ........................................... S59

XII. REFERENCES ........................................................................................... S69
I. Structures of precursor peptides P and cyclic peptides CP

1. Precursor peptides P
2. Cyclic peptides CP
II. General information and materials

The HPLC grade acetonitrile (MeCN) was purchased from Fisher. Aqueous buffers and aqueous mobile-phases for HPLC were prepared using water purified with an Elga® Purelab® Milli-Q water purification system (purified to 18.2 MΩ.cm). DMSO, THF and dichloromethane (CH₂Cl₂) were purchased from Fisher. Amino acids and coupling reagents were purchased commercially from different sources. Other chemicals and reagents were purchased from Sigma and used without any further purification.

NMR spectra (¹H, 2D) were recorded on a Bruker Ascend 700 spectrometer {δH (700 MHz), δC (175 MHz)} at ambient temperature in the deuterated solvent stated. Chemical shifts are expressed in parts per million (ppm) from DMSO-d6 (δH = 2.50).[1] Multiplicities are described as s (singlet), d (doublet), q (quadruplet), qt (quintuplet), dd (doublet of doublets), t (triplet), m (multiplet), br (broad). Coupling constants J are quoted in Hertz (Hz) to the nearest 0.1 Hz. Signals of protons and carbons were assigned, as far as possible, by using the following two dimensional NMR spectroscopy techniques: [¹H, ¹H] COSY (Correlation Spectroscopy), [¹H, ¹H] TOCSY (Total Correlation Spectroscopy), [¹H, ¹H] ROESY (Rotating-frame NOE Spectroscopy), [¹H, ¹³C] HSQC (Heteronuclear Single Quantum Coherence) and long range [¹H, ¹³C] HMBC (Heteronuclear Multiple Bond Connectivity). EXSY (Exchange Spectroscopy) experiment was used to identify equilibrium chemical exchange either at rt or with heating.

Low-resolution mass spectra were obtained with an Agilent 6130 single quad apparatus equipped with an electrospray ionization source. High-resolution mass spectra (HRMS) were obtained with a Thermo Exactive Orbitrap mass spectrometer. Reactions performed in the enzymatic media were monitored using MALDI-MS acquired using a 4800 MALDI TOF/TOF Analyser (ABSciex, Foster City, CA) equipped with a Nd:YAG 355 nm laser and calibrated using a mixture of peptides. The spot was analysed in positive MS mode between 5000 and 4000 m/z, by averaging 1000 laser spots. The samples, diluted in water to reduce the buffer concentration, (0.5 µL) were applied to the MALDI target along with alpha-cyano-4-hydroxycinnamic acid matrix (0.5 µL, 10 mg/mL in 50:50 acetonitrile:0.1% TFA) and allowed to dry. MSMS data were acquired using a TripleTOF 5600+. The sample was
subjected to chromatography on an Acclaim PepMap 100 C18 trap and an Acclaim PepMap RSLC C18 column (ThermoFisher Scientific), using a nano-LC Ultra 2D plus loading pump and nano-LC as-2 autosampler (Eksigent). The sample was injected at neutral pH to avoid acid catalyzed ring opening. The trap was washed with 2% acetonitrile, 0.05% trifluoroacetic acid, and the patellamide was then eluted with a gradient of increasing acetonitrile, containing 0.1 % formic acid (15-40% acetonitrile in 5 min, 40-95% in a further 1 min, followed by 95% acetonitrile to clean the column, before re-equilibration to 15% acetonitrile). The eluent was sprayed into a TripleTOF 5600+ electrospray tandem mass spectrometer (Sciex) operating with standard nanospray conditions, and analyzed in Product Ion Scan mode isolating the m/z of interest. The collision energy was adjusted to give optimal fragmentation. The MSMS fragmentation pattern was interrogated for diagnostic peaks.

Automated solid-phase peptide synthesis (SPPS) was accomplished on a Biotage® Syro Wave™ system in polypropylene (PP) syringe with a PTFE frit. Manual SPPS was performed using a vacuum manifold and a shaker. Final cleavage and deprotection were completed manually.

Analytical RP-HPLC was performed on an Agilent infinity 1260 series equipped with either a VWD or an MWD detector and a single quadrupole MS using a Macherey-Nagel Nucleodur C18 column (10 µm x 4.6 x 250 mm) using the following chromatographic system: 1 mL/min flow rate with MeCN and 0.1 % aqueous TFA [95% TFA (5 min), linear gradient from 5 to 95% of MeCN (35 min), 95% MeCN (40 min)] and UV detection at 220 nm.

Semi-preparative RP-HPLC was performed on an Agilent infinity 1260 series equipped with a VWD or an MWD detector using a Macherey-Nagel Nucleodur C18 column (10 µm x 10 x 250 mm at 4 mL/min, a 10 µm x 16 x 250 mm at 10 mL/min, or 10 µm x 21 x 250 mm at 16 mL/min). Several chromatographic systems were used; System Pa: MeCN and 0.1 % aqueous TFA [95% TFA (5 min), linear gradient from 5 to 95% of MeCN (35 min), 95% MeCN (40 min)] and UV detection at 220 nm. System Pb: MeCN and 0.1 % aqueous TFA [95% TFA (5 min), linear gradient from 5 to x-3% of MeCN (1%/min), x+12% MeCN (0.1%/min)] and UV detection at 220 nm; where x is the % of MeCN at which the corresponding peak elutes on the analytical run of a similar column type. The runs were stopped as soon as the peptide eluted off the column.
III. General procedures

*PatG*<sub>mac</sub> cloning, expression and purification:

The *PatG*<sub>mac</sub> enzyme was cloned from genomic DNA (*Prochloron* sp.) into the pHISTEV vector, expressed in *Escherichia coli* BL21 (DE3) cells grown on autoinduction medium, and purified as previously described by Koehnke *et al.*[^2]^ However, subsequent to the Nickel column eluting with 250 mM imidazole, the remaining purification steps were replaced with dialysis in a bicine buffered solution [20 mM Bicine, 150 mM NaCl, pH = 8.1] to remove the imidazole and the reducing agent.

Solid-phase peptide synthesis

The different precursor peptides were synthesized on solid-phase (SPPS) on a Rink amide resin (RAR) using the Fmoc strategy and Fmoc-protected amino acids (aa). Both automatic (A) and manual (M) procedures were employed. The introduction of the triazole moiety was accomplished by first reacting azido acids to the growing peptide (A or M) and then by manually introducing the triazole through a copper-catalyzed azide alkyne cycloaddition (CuAAC) between the azide-terminal peptide and the Fmoc-protected alkyne amino acid analogue (Scheme S1).

1- Resin preparation

The rink amide resin (RAR) is weighed into the fritted PP-syringe then swelled in DMF for half an hour.

2- Automated SPPS

A double coupling strategy was used for all amino acids using the following conditions for each step:

5 eq. HBTU in DMF; 8 eq. DIEA in NMP; 5 eq. aa in DMF Reaction time of 30 min with mixing/shaking for 15 s every two minutes at rt followed by 4x DMF washing cycles.

Fmoc deprotection is done in 40% piperidine/DMF in two steps:
3 min reaction with mixing/shaking for 10 s every minute at rt then 12 min reaction with mixing/shaking for 10 s every two minutes at rt followed by 6x DMF washing cycles.

Scheme S1: General SPPS strategy for the synthesis of triazole-containing peptides.

3- Manual SPPS

A double coupling strategy was used for all amino acids using the following conditions for each step:

5 eq. HBTU in DMF; 10 eq. DIEA in NMP; 5 eq. aa in DMF Reaction time of 1h 30 min with shaking at rt followed by 4x DMF, 4x CH$_2$Cl$_2$, and 4x DMF washing cycles. Fmoc deprotection is done in 20% piperidine/DMF for 10 min followed by 4x DMF, 4x CH$_2$Cl$_2$, and 4x DMF washing cycles.

4- Manual CuAAC reaction; solid phase (SP)

After the coupling of an azido acid to the growing peptidic chain (A or M), the resin is washed with 5x THF. The corresponding amino alkyne (5 eq.) is diluted in THF and added to the resin. DIEA (10 eq.) is then added followed by CuI (5 eq.). The mixture is bubbled with dry nitrogen for 1 min then sealed, protected from light, and left overnight with shaking. After the reaction is complete (mini work up of a few
beads; repeat the procedure if reaction not complete), the resin is washed with 4x THF, 4x H₂O, 4x THF, and 4x DMSO. The resin is swelled in DMSO for 10 min before the Fmoc deprotection step (if needed) with 20% piperidine/DMF for 10 min followed by 4x DMF, 4x CH₂Cl₂, and 4x DMF washing cycles.

5- Final cleavage

The resin is washed with CH₂Cl₂ and dried for a minute on the vacuum manifold. The beads are then transferred into a flacon tube and the cleavage cocktail is added: 95% TFA, 3.5% H₂O, 1.5% TIS. The mixture is vortexed to mix everything and the flacon tube is left on the shaker for 2h. The resin is then filtered, washed with 3x CH₂Cl₂ and the filtrate is evaporated under vacuum until a few milliliters of TFA are left (< 2mL).

6- Peptide precipitation and HPLC purification

To a falcon tube containing cold diethyl ether, the remaining TFA solution is added dropwise. The peptide precipitates upon contact with the cold ether. The falcon tube is placed for 5 min in the cold and centrifuged at 4 °C and 4000 rpm for 10 min. The supernatant is decanted and the remaining solid left a few minutes under nitrogen flow to dry. The crude is then solubilized in a minimum volume of 20% acetic acid and MeCN (ultrasonic bath helps) and immediately purified by HPLC.

Peptides P0, P13, P14, P15, P24, P25, P26, P35, P36, P46, P135, and P246 were purified using system Pa and peptides P1, P2, P3, P4, P5, P6, and P16 were purified using system Pb.

Pat Gmac macrocyclisation reaction

The reactions are conducted in 20 mM bicine buffer, 500 mM NaCl, and 5% DMSO solution, pH = 8.1 and incubated at 37 °C (without shaking) until full consumption of the starting peptide (MALDI monitoring). The reaction set-ups are prepared in the following order; final concentrations:

1- A solution of the linear peptide in DMSO (between 10 and 50 mM); 100 µM
2- DMSO; 5%
3- 20 mM Bicine, 150 mM NaCl, pH=8.1 buffer
4- 5 mM NaCl; 500 mM
5- PatGmac enzyme; 60 µM

The large scale reaction mixture is then extracted 3 times with n-butanol (BuOH): H₂O (1/1, v/v). BuOH is added to the aqueous reaction, vigorously mixed, and then centrifuged for 10 min at high speed to help separate the two phases. The combined BuOH fractions are evaporated under reduced pressure to dryness. The crude is solubilized in a minimum volume of H₂O/MeCN and immediately purified by HPLC using system Pa.

**Synthesis of amino acids analogues**

1- **Synthesis of imidazole-1-sulfonyl azide hydrochloride**

\[
\text{NaN}_3 + \text{SO}_2\text{Cl}_2 \xrightarrow{\text{CH}_3\text{CN, rt, 18h}} \text{SO}_2\text{CIN}_3 \xrightarrow{1-\text{Imidazole, 3h}} \text{ImSO}_2\text{N}_3\text{HCl}
\]

Scheme S2: Synthesis of the diazotransfer reagent.

A suspension of NaN₃ (2g, 0.03 mol) in anhydrous MeCN (30 mL) is cooled to 0 °C. Sulfuryl chloride (2.5 mL, 0.03 mol) is then added drop-wise and the mixture stirred overnight at room temperature. Imidazole (3.98 g, 0.06 mmol) is added slowly to the reaction at 0 °C and the mixture is stirred for 3 h at room temperature. EtOAc (60 mL) is added and the reaction is washed with H₂O (2x 40 mL) and saturated aqueous NaHCO₃ (2x 40 mL). The organic fraction is then dried over MgSO₄ and filtered. In a separate flask, a solution of anhydrous HCl in EtOH is prepared at 0 °C by the dropwise addition of acetyl chloride (3.28 mL, 0.046 mol) to dry ethanol (7.5 mL). The EtOAc solution is cooled to 0 °C and while stirring, the HCl solution in EtOH is added dropwise to precipitate a white solid. The precipitate is then filtered, washed with EtOAc (3x 15 mL), and dried under pressure to give imidazole-1-sulfonyl azide hydrochloride as white solid (5g, 76%). The NMR spectroscopic data were in agreement with those described in the literature.[³] **Caution!** Imidazole-1-sulfonyl azide and its salts (including chloride salt) are known to be energetic materials that are sensitive to chock and friction. Care should be taken while handling this material.
and proper safety precautions should be used. Plastic container and materials are recommended for use.\[4\]

2- General procedure for the synthesis of azido acids 1a, 1b, and 1d

\[
\begin{align*}
\text{H}_2\text{N} & \quad \rightarrow \quad \text{ImSO}_2\text{N}_3\cdot\text{HCl} \\
\text{CuSO}_4\cdot\text{H}_2\text{O}, \text{K}_2\text{CO}_3 & \quad \text{MeOH/H}_2\text{O} \\
\text{rt, O/N} & \quad \rightarrow \\
\text{N}_3 & \quad \rightarrow \quad \text{OH} \\
\text{aa} & \quad \rightarrow \quad \text{1a} \\
\text{aa} = \text{Ala, 1a} & \quad 90\% \\
\text{aa} & \quad \rightarrow \quad \text{1b} \\
\text{aa} = \text{Ile, 1b} & \quad 90\% \\
\text{aa} & \quad \rightarrow \quad \text{1d} \\
\text{aa} = \text{Phe, 1d} & \quad 100\%
\end{align*}
\]

Scheme S3: Synthesis of azido acids 1.

To a solution of the amino acid (1 eq.), K$_2$CO$_3$ (2.7 eq.), and CuSO$_4$.5H$_2$O (0.01 eq.) in a MeOH/H$_2$O mixture (5/1, v/v), ImSO$_2$N$_3$.HCl is added and the mixture stirred vigorously for 18h at room temperature. The reaction is then concentrated under reduced pressure, diluted with H$_2$O, acidified with 1M HCl to pH = 1, and extracted with 3x CH$_2$Cl$_2$. The organic layer is dried over MgSO$_4$, filtered, and concentrated. The crude obtained is then diluted in saturated aqueous NaHCO$_3$ and washed with Et$_2$O. The aqueous phase is acidified to pH with conc. HCl solution and extracted with 3x CH$_2$Cl$_2$. The combined organic layers are dried over MgSO$_4$, filtered, and concentrated. The corresponding azido acids are then used without any further purification.\[3\]

1a: L-Ala (1g, 11.2 mmol) in MeOH/H$_2$O (50 mL/10 mL) was treated according to the abovementioned procedure. Compound 1a was obtained as pale yellow oil (755 mg, 59%). The NMR spectroscopic data were in agreement with those described in the literature.\[5\]

1b: L-Ile (1g, 7.6 mmol) in MeOH/H$_2$O (34 mL/7 mL) was treated according to the abovementioned procedure. Compound 1b was obtained as colourless oil (1.03 g, 86%). The NMR spectroscopic data were in agreement with those described in the literature.\[5\]

1d: L-Phe (1g, 6.05 mmol) in MeOH/H$_2$O (27 mL/5.5 mL) was treated according to the abovementioned procedure. Compound 1d was obtained as colourless oil (1.1 g, 100%). The NMR spectroscopic data were in agreement with those described in the literature.\[5\]
3- General procedure for the synthesis of amino alkynes 2a, 2b, and 2c[6]

Scheme S4: Synthesis of Fmoc protected amino alkynes 2.

3a-c: To a solution of BOC-protected amino acids (1 eq.) and DIEA (1.5 eq.) in DMF is added PyBop (1.1 eq.) and the reaction is stirred at rt for 0.5 h. Weinreb amine (1.1 eq.), DIEA (1.1 eq.) and DMF are then added to the reaction mixture and left at room temperature overnight. The reaction mixture is diluted with H2O and extracted with 3x EtOAc. The combined organic fractions are washed successively with saturated aqueous NaHCO3, KHSO4 1M, and brine. The organic fraction is dried over MgSO4, filtered, and evaporated under reduced pressure. Purification over silica gel afforded the corresponding weinreb amide 3a-c.

3a: Boc-L-Ala (1.5g, 7.9 mmol) in DMF (16 mL; 10 mL) was treated according to the abovementioned procedure. Purification over silica gel (30% EtOAc/70% CH2Cl2) afforded compound 3a as white solid (1.3 g, 72%). The NMR spectroscopic data were in agreement with those described in the literature.[7]

3b: Boc-L-Ile (1g, 4.3 mmol) in DMF (8.5 mL; 5.5 mL) was treated according to the above mentioned procedure. Purification over silica gel (15% EtOAc/85% CH2Cl2) afforded compound 3b as colourless oil (964 mg, 81%). The NMR spectroscopic data were in agreement with those described in the literature.[7]

3c: Boc-L-Val (1g, 4.6 mmol) in DMF (9 mL; 6 mL) was treated according to the abovementioned procedure. Purification over silica gel (20% EtOAc/80% CH2Cl2) afforded compound 3c as colourless oil (919 mg, 77%). The NMR spectroscopic data
were in agreement with those described in the literature.\textsuperscript{[7]}

4a-c: To a solution of the Weinreb amides 3a-c (1 eq.) in anhydrous THF at 0 °C, LiAlH\textsubscript{4} (1.2 eq.) is added slowly and the reaction is stirred at 0 °C for 1.5 h. The reaction mixture is quenched by the addition of a solution of HCl 0.1M and extracted with 3x EtOAc. The combined organic fractions are washed with brine, dried over MgSO\textsubscript{4}, filtered, and evaporated under reduced pressure. Purification over silica gel afforded the corresponding aldehydes 4a-c.

4a: Weinreb amide 3a (116 mg, 0.5 mmol) in THF (1.5 mL) was treated according to the abovementioned procedure. Purification over silica gel (10% EtOAc/90% CH\textsubscript{2}Cl\textsubscript{2}) afforded compound 4a as white solid (69 mg, 75%). The NMR spectroscopic data were in agreement with those described in the literature.\textsuperscript{[7]}

4b: Weinreb amide 3b (116 mg, 0.5 mmol) in THF (1.5 mL) was treated according to the abovementioned procedure. Purification over silica gel (5% EtOAc/95% CH\textsubscript{2}Cl\textsubscript{2}) afforded compound 4b as colourless oil (66 mg, 69%). The NMR spectroscopic data were in agreement with those described in the literature.\textsuperscript{[7]}

4c: Weinreb amide 3c (121 mg, 0.5 mmol) in THF (1.5 mL) was treated according to the abovementioned procedure. Purification over silica gel (5% EtOAc/95% CH\textsubscript{2}Cl\textsubscript{2}) afforded compound 4c as colourless oil (60 mg, 64%). The NMR spectroscopic data were in agreement with those described in the literature.\textsuperscript{[7]}

5a-c: To a solution of the aldehyde 4a-c (1 eq.) and K\textsubscript{2}CO\textsubscript{3} (2 eq.) in anhydrous MeOH, the Bestmann-Ohira reagent (dimethyl (1-diazo-2-oxopropyl)phosphonate; 1.2 eq.) is added and the reaction is stirred at rt overnight. The reaction mixture is diluted with Et\textsubscript{2}O and washed successively with 5% aqueous NaHCO\textsubscript{3} and H\textsubscript{2}O, dried over MgSO\textsubscript{4}, filtered, and evaporated under reduced pressure. Purification over silica gel afforded the corresponding alkynes 5a-c.

5a: Aldehyde 4a (40 mg, 0.23 mmol) in MeOH (3.5 mL) was treated according to the abovementioned procedure. Purification over silica gel (CH\textsubscript{2}Cl\textsubscript{2}) afforded compound 5a as white solid (24 mg, 61%). The NMR spectroscopic data were in agreement with those described in the literature.\textsuperscript{[8]}

5b: Aldehyde 4b (66 mg, 0.31 mmol) in MeOH (4.8 mL) was treated according to the abovementioned procedure. Purification over silica gel (CH\textsubscript{2}Cl\textsubscript{2}) afforded compound
5b as colourless oil. The NMR spectroscopic data were in agreement with those described in the literature.\[^8\]

5c: Aldehyde 4c (57 mg, 0.28 mmol) in MeOH (4.3 mL) was treated according to the abovementioned procedure. Purification over silica gel (10% pentane/90% CH\(_2\)Cl\(_2\)) afforded compound 5c as white solid. The NMR spectroscopic data were in agreement with those described in the literature.\[^8\]

6a-c: Alkyne 5a-c (1 eq.) is diluted in CH\(_2\)Cl\(_2\) (V) and TFA/H\(_2\)O (95:5; 2V) is added and stirred at rt until full deprotection of the compound. The solvent is evaporated and resuspended in toluene and evaporated 3 times until dryness. The crude is diluted in CH\(_2\)Cl\(_2\) (V) and DIEA (1.1 eq.) and FmocOSu (2 eq.) are added and the reaction stirred at room temperature overnight. The mixture is washed successively with 3x HCl 1M, 3x NaHCO\(_3\), and brine. The organic layer is dried over MgSO\(_4\), filtered, and evaporated under reduced pressure. Purification over silica gel afforded the Fmoc protected alkyne 2a-c.

2a: Boc-protected alkyne 5a (345 mg, 2.04 mmol) in CH\(_2\)Cl\(_2\) (11.9 mL) was treated according to the abovementioned procedure. Purification over silica gel (10% pentane/90% CH\(_2\)Cl\(_2\)) afforded compound 2a as white solid (419 mg, 71%). The NMR spectroscopic data were in agreement with those described in the literature.\[^9\]

2b: Boc-protected alkyne 5b (1.27 g, 6 mmol) in CH\(_2\)Cl\(_2\) (35 mL) was treated according to the abovementioned procedure. Purification over silica gel (10% pentane/90% CH\(_2\)Cl\(_2\)) afforded compound 2b as white solid (1.64 g, 82%). The NMR spectroscopic data were in agreement with those described in the literature.\[^10\]

2c: Boc-protected alkyne 5c (527 mg, 2.74 mmol) in CH\(_2\)Cl\(_2\) (16.1 mL) was treated according to the abovementioned procedure. Purification over silica gel (10% pentane/90% CH\(_2\)Cl\(_2\)) afforded compound 2c as white solid (599 mg, 69%). The NMR spectroscopic data were in agreement with those described in the literature.\[^11\]
### IV. MS and HPLC data of starting and final cyclic peptides

Table S1: MS data, HRMS, retention time, and HPLC purities of starting peptides $P$ (used for large scale synthesis of CP) and the MS data for the corresponding macrocyclic peptides $CP$

| Ref | Peptide                  | MS [M+H]$^+$ | HRMS* | rt    | Purity** | MS of CP [M+H]$^+$ |
|-----|--------------------------|---------------|-------|-------|----------|---------------------|
| P0  | VGAGIGFPAYD              | 1065.4        | 1065.5372 | 16.97 | 99%      | 699.4               |
| P1  | V$^{15}$GAGIGFPAYD       | 1089.4        | 1089.5473 | 17.32 | 99%      | 723.4               |
| P2  | V$^{15}$AGIGFPAYD        | 1089.4        | 1089.5468 | 17.26 | 99%      | 723.4               |
| P3  | V$^{15}$GIGFPAYD         | 1089.4        | 1089.5473 | 17.38 | 99%      | 723.4               |
| P4  | V$^{15}$GIGFPAYD         | 1089.4        | 1089.5477 | 17.61 | 93%      | 723.4               |
| P5  | V$^{15}$GIGFPAYD         | 1089.4        | 1089.5468 | 17.7  | 97%      | 723.4               |
| P6  | V$^{15}$GIGFPAYD         | 1089.4        | 1111.5291 | 17.51 | 98%      | 723.4               |
| P13 | V$^{15}$GAG$^T$GFPAYD    | 1113.4        | 1113.5600 | 17.64 | 96%      | 747.4               |
| P135| V$^{15}$GAG$^T$GFPAYD    | 1137.4        | 1137.5712 | 18.07 | 97%      | 771.4               |

* Calc. for $P0$ C$_{50}$H$_{72}$N$_{12}$O$_{14}$ [M+H]$^+$: 1065.5364; Calc. for $P1-6$ C$_{51}$H$_{72}$N$_{14}$O$_{13}$ [M+H]$^+$: 1089.5476; [M+Na]$^+$: 1111.5295; Calc. for $P13$ C$_{53}$H$_{72}$N$_{16}$O$_{12}$ [M+H]$^+$: 1113.5588; Calc. for $P135$ C$_{53}$H$_{72}$N$_{18}$O$_{13}$ [M+H]$^+$: 1137.5701.** Purity assessed by analytical HPLC at 220 nm UV absorption. LCMS traces of all precursor peptides $P$ can be found in section IX.

Table S2: MS data, retention time, and HPLC purities of the remaining starting peptides $P$ (used for small scale macrocyclisation reactions) and the MS data for the corresponding macrocyclic peptides.

| Ref | Peptide                  | MS [M+H]$^+$ | rt    | Purity** | MS of CP [M+H]$^+$ |
|-----|--------------------------|---------------|-------|----------|---------------------|
| P14 | V$^{15}$GAG$^T$GFPAYD    | 1113.4        | 17.86 | 98%      | 747.4               |
| P15 | V$^{15}$GAG$^T$GFPAYD    | 1113.4        | 17.61 | 99%      | 747.4               |
| P16 | V$^{15}$GAG$^T$GFPAYD    | 1113.4        | 17.59 | 99%      | 747.4               |
| P24 | V$^{15}$AG$^T$GFPAYD     | 1113.4        | 17.91 | 98%      | 747.4               |
| P25 | V$^{15}$AG$^T$GFPAYD     | 1113.4        | 17.69 | 98%      | 747.4               |
| P26 | V$^{15}$AG$^T$GFPAYD     | 1113.4        | 17.58 | 99%      | 747.4               |
| P35 | V$^{15}$G$^T$GFPAYD      | 1113.4        | 17.96 | 98%      | 747.4               |
| P36 | V$^{15}$G$^T$GFPAYD      | 1113.4        | 17.90 | 94%      | 747.4               |
| P46 | V$^{15}$AG$^T$GFPAYD     | 1113.4        | 18.00 | 97%      | 747.4               |
| P463| V$^{15}$AG$^T$GFPAYD     | 1137.4        | 18.28 | 99%      | 771.4               |

** Purity assessed by analytical HPLC at 220 nm UV absorption. LCMS traces of all precursor peptides $P$ can be found in section IX.
Table S3: HRMS, HPLC data, and yields of the final cyclic peptides CP

| Cyclic Peptide | Yield | Purity | rt | Found [M+Na]^+ | Expected [M+Na]^+ | Found [M+H]^+ | Expected [M+H]^+ | Molecular formula |
|----------------|-------|--------|----|----------------|------------------|--------------|------------------|------------------|
| CP0            | 32%   | 99%    | 20.27 | 721.3629 | 699.3816 | 34% | 97% | C_{35}H_{50}N_{10}O_{7} |
| CP1            | 43%   | 99%    | 20.45 | 745.3754 | 721.3644 | 34% | 99% | C_{35}H_{50}N_{10}O_{7} |
| CP2            | 48%   | 91%    | 21.08 | 745.3747 | 745.3756 | 34% | 99% | C_{35}H_{50}N_{10}O_{7} |
| CP3            | 55%   | 99%    | 18.37 | 745.3756 | 745.3756 | 34% | 99% | C_{35}H_{50}N_{10}O_{7} |
| CP4            | 58%   | 97%    | 20.63 | 745.3757 | 745.3757 | 34% | 99% | C_{35}H_{50}N_{10}O_{7} |
| CP5            | 40%   | 98%    | 20.54 | 745.3756 | 745.3756 | 34% | 99% | C_{35}H_{50}N_{10}O_{7} |
| CP6            | 54%   | 93%    | 20.63 | 745.3746 | 745.3746 | 34% | 99% | C_{35}H_{50}N_{10}O_{7} |
| CP13           | 39%   | 97%    | 21.32 | 769.3863 | 793.3980 | 34% | 99% | C_{36}H_{50}N_{12}O_{6} |
| CP135          | 39%   | 97%    | 21.54 | 793.3979 | 793.3979 | 34% | 99% | C_{37}H_{50}N_{14}O_{5} |

**Cyclic Peptide:**
- CP0: VGAGIGFP
- CP1: V^\beta\gamma GAGIGFP
- CP2: VGAG^\beta TzGIGFP
- CP3: VGAG^\beta TzAGIGFP
- CP4: VGAG^\beta TzAGIGFP
- CP5: VGAG^\beta TzGIGFP
- CP6: VGAG^\beta TzGIGFP
- CP13: V^\beta\gamma G^\beta\gamma A^\beta\gamma TzG^\beta\gamma G^\beta\gamma I^\beta\gamma G^\beta\gamma F^\beta\gamma P
- CP135: V^\beta\gamma G^\beta\gamma A^\beta\gamma TzG^\beta\gamma G^\beta\gamma I^\beta\gamma G^\beta\gamma F^\beta\gamma P
V. NMR data of final cyclic peptides

Copies of the proton NMR spectra for each compound depicting the different species by color code as well as copies of the HSQC spectra can be found in section V. Copies of the exchange spectroscopy (EXSY) spectra can be found in section VI.

CP0; Cyclo(-VGaAGbIGcFP-) 3:1 (conformers)

$^1$H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*

| Amino acid | Atom | $^1$H chemical shift          | $^{13}$C chemical shift |
|------------|------|------------------------------|------------------------|
| Val        | NH   | 8.10, d, $J = 8.5$          | -                      |
|            | $\alpha$ CH | 3.76, t, $J = 8.7$      | 61.4                   |
|            | $\beta$ CH | 2.02-1.96 m             | 29.3                   |
|            | $\gamma$ CH$_3$ | 0.82-0.78, m    | 15.5                   |
|            | CO   | -                           | 19.6                   |
| Gly (a)    | NH   | 7.25-7.21, m                | -                      |
|            | $\alpha$ CH$_2$ | 3.92-3.86, m;        | 41.7                   |
|            |      | 3.68-3.61, m               |                        |
|            | CO   | -                           | 169.9                  |
| Ala        | NH   | 8.56, d, $J = 6.1$          | -                      |
|            | $\alpha$ CH | 4.12-4.07, m             | 49.3                   |
|            | $\beta$ CH$_3$ | 1.27, d, $J = 7.2$     | 17.5                   |
|            | CO   | -                           | 173.3                  |
| Gly (b)    | NH   | 8.47, t, $J = 5.88$         | -                      |
|            | $\alpha$ CH$_2$ | 3.97-3.92, m;        | 43.2                   |
|            |      | 3.51-3.47, m               |                        |
|            | CO   | -                           | 170.4                  |
| Ile        | NH   | 7.85, d, $J = 7.7$          | -                      |
|            | $\alpha$ CH | 4.02, d, $J = 7.2$      | 58.8                   |
|            | $\beta$ CH | 1.81-1.75, m             | 36.2                   |
|            | $\gamma$ CH$_2$ | 1.42-1.35, m;           | 25.0                   |
|            |      | 1.20-1.15, m               |                        |
|            | $\gamma$ CH$_3$ | 0.85-0.83, m     | 15.7                   |
|            | $\delta$ CH$_3$ | 0.82-0.79, m    | 11.2                   |
|            | CO   | -                           | 170.8                  |
| Gly (c)    | NH   | 4.32, dd, $J = 4.9$, $J = 6.8$ | -                      |
|            | $\alpha$ CH$_2$ | 3.92-3.86, m;        | 41.7                   |
|            |      | 3.68-3.61, m               |                        |
|            | CO   | 170.7                      |                        |
**NMR data of the major conformer**

1H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*

| Amino acid* | Atom | 1H chemical shift | 13C chemical shift |
|-------------|------|-------------------|-------------------|
| Val         | NH   | 7.68, d, J = 7.5  | -                 |
|             | α CH | 4.12-4.07, m      | 58.3              |
|             | β CH | 2.09-2.03 m       | 30.7              |
|             | γ CH₃| 0.85-0.83, m;     | 19.6              |
|             | CO   | -                 | 169.2             |
| Gly (a)     | NH   | 8.24, t, J = 5.5  | -                 |
|             | α CH | 3.82, t, J = 5.3; 3.58-3.53, m | 43.0          |
|             | CO   | -                 | 169.2             |
| Ala         | NH   | 8.03, d, J = 7.5  | -                 |
|             | α CH | 4.18, qt, J = 7.2 | 48.9              |
|             | β CH₃| 1.22, d, J = 7.1  | 17.5              |
|             | CO   | -                 | 172.1             |
| Gly (b)     | NH   | 7.79, t, J = 5.6  | -                 |
|             | α CH | 3.80, t, J = 5.7; 3.71-3.68, m | 42.2          |
|             | CO   | -                 | 169.8             |
| Ile         | NH   | 8.18, d, J = 7.0  | -                 |
|             | α CH | 3.97-3.95, m      | 58.1              |
|             | β CH | 1.76-1.72, m      | 35.3              |
|             | γ CH₂| 1.53-1.48, m; 1.12-1.05, m | 24.9          |
|             | γ CH₃| 0.83-0.80, m;     | 15.5              |
|             | δ CH₃| 0.83-0.81, m      | 11.2              |
|             | CO   | -                 | ND                |
| Gly (c)     | NH   | 8.63, t, J = 5.6  | -                 |
|             | α CH | 3.97-3.92, m; 3.29-3.25, m | 42.8          |
|             | CO   | -                 | 172.3             |
| Phe         | NH   | 7.61, d, J = 8.4  | -                 |
|             | α CH | 4.71, dt, J = 4.4, J = 9.0 | 52.5          |
|             | β CH₂| 2.95, dd, J = 4.2, J = 14.2 | 37.6          |

*ND: Not determined*
| Amino acid* | Atome | $^1$H chemical shift | $^{13}$C chemical shift |
|------------|-------|----------------------|------------------------|
| Val        | NH    | 8.51, d, $J = 9.3$   | -                      |
|            | $\alpha$ CH | 4.63, t, $J = 9.6$   | 53.0                   |
|            | $\beta$ CH | 2.02-1.98 m         | 33.0                   |
|            | $\gamma$ CH$_3$ | 0.65, d, $J = 6.7$; | 20.3                   |
|            |        | 0.82, d, $J = 6.6$   | 19.8                   |
|            | Tz CH  | 7.75, s               | 124.4                  |
|            | Tz C   |                       | 148.9                  |
| Gly (a)    | $\alpha$ CH$_2$ | 5.16, s           | 51.6                   |
|            | CO     | -                    | 167.2                  |
| Ala        | NH    | 9.10, d, $J = 6.1$   | -                      |
|            | $\alpha$ CH | 3.93, q, $J = 6.9$   | 50.2                   |
|            | $\beta$ CH$_3$ | 1.29, d, $J = 7.0$  | 16.6                   |
|            | CO     | -                    | 171.9                  |
| Gly (b)    | NH    | 8.39, dd, $J = 5.8$, $J = 6.4$ | -                  |
|            | $\alpha$ CH$_2$ | 4.01-3.98, m; 3.40, dd, $J = 5.0$, $J = 16.3$ | 42.8 |
|            | CO     | -                    | 169.3                  |
| Ile        | NH    | 7.31                 | -                      |
|            | $\alpha$ CH | 4.01-3.98, m       | 57.7                   |
|            | $\beta$ CH | 1.58-1.54, m       | 35.8                   |
|            | $\gamma$ CH$_2$ | 1.35-1.31, m; 1.02-0.98, m | 25.2 |
|            | $\gamma$ CH$_3$ | 0.88, d, $J = 6.8$ | 15.9                   |
|            | $\delta$ CH$_3$ | 0.69, t, $J = 7.4$ | 10.8                   |
|            | CO     | -                    | 171.6                  |
| Gly (c)    | NH    | 7.95-7.92, m         | -                      |
|            | $\alpha$ CH$_2$ | 3.83, dd, $J = 7.4$, $J = 16.7$; 3.70, dd, $J = 4.2$, $J = 16.8$ | 41.9 |

*NMR data of the minor conformer

**CP1; Cyclo(-VTzGaAG$_6$IG$_6$FP-) 4:1**

$^1$H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*
| Amino acid* | Atome | $^1$H chemical shift | $^1$C chemical shift |
|------------|-------|----------------------|----------------------|
| Phe        | NH    | 8.49, d, $J = 3.6$   | -                    |
|            | $\alpha$ CH | 4.22-4.19, m;         | 54.0                 |
|            | $\beta$ CH$_2$ | 2.98, dd, $J = 6.1$, $J = 13.2$; 2.83, dd, $J = 10.0$, $J = 13.2$ | 37.5                |
|            | Ar CH  | 7.32-7.18, m         | 129.9, 129.5, 129.0  |
|            | Ar C   | -                    | 136.4                |
|            | CO     | -                    | ND                   |
| Pro        | $\alpha$ CH | 3.52-3.51, m         | 60.9                 |
|            | $\beta$ CH$_2$ | 2.02-1.97, m; 0.77-0.71, m | 29.8                |
|            | $\gamma$ CH$_3$ | 1.64-1.60, m; 1.32-1.27, m | 21.9                |
|            | $\delta$ CH$_2$ | 3.38-3.31, m; 3.17, t, $J = 10.0$ | 46.3                |
|            | CO     | -                    | 169.3                |

*NMR data of the major conformer

$^1$H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*
| Amino acid* | Atome | $^1$H chemical shift | $^{13}$C chemical shift |
|------------|-------|---------------------|------------------------|
| Val        | NH    | 7.75, d, $J = 8.6$  | -                      |
|            | $\alpha$ CH | 3.80-3.76, m   | 61.6                   |
|            | $\beta$ CH | 1.97-1.92 m     | 29.6                   |
|            | $\gamma$ CH$_3$ | 0.86-0.79, m | 19.8/18.7               |
|            | CO    |                   | 170.9                  |
| Gly (a)    | NH    | 7.43, d, $J = 6.7$  | -                      |
|            | $\alpha$ CH | 4.53, dd, $J = 7.7$, $J = 14.6$; | 34.2                   |
|            |       | 4.06-3.98, m       |                        |
|            | Tz CH | 8.12, s            | 122.3                  |
|            | Tz C  |                     | 144.1                  |
| Ala        | $\alpha$ CH | 5.63, q, $J = 6.5$ | 57.9                   |
|            | $\beta$ CH$_3$ | 1.67, d, $J = 6.8$ | 19.4                   |
|            | CO    | -                  | 170.9                  |
| Gly (b)    | NH    | 9.42-9.38, m       | -                      |
|            | $\alpha$ CH | 4.08-4.06, m;     | 43.7                   |
|            |       | 3.65, dd, $J = 6.1$, $J = 14.2$ |            |
|            | CO    | -                  | 171.9                  |
| Ile        | NH    | 8.61, d, $J = 5.9$  | -                      |
|            | $\alpha$ CH | 4.06-3.98, m     | 59.3                   |
|            | $\beta$ CH | 1.92-1.88, m    | 35.8                   |
|            | $\gamma$ CH$_3$ | 1.78-1.74, m; 1.35-1.28, m | 24.5                   |
|            | $\gamma$ CH$_3$ | 0.92, d, $J = 6.8$; | 15.9                   |
|            | $\delta$ CH$_3$ | 0.86-0.79, m | 12.2                   |
|            | CO    | -                  | 170.5                  |
| Gly (c)    | NH    | 7.87-7.85, m       | -                      |
|            | $\alpha$ CH | 3.76-3.71, m; 2.50 | 42.4                   |
|            | CO    |                   | 168.8                  |

Phe

NH

7.98-7.97, m

-
\[ \alpha \text{CH} \quad 4.68-4.63, \text{m}; \quad 52.6 \\
\beta \text{CH}_2 \quad 2.87-2.82, \text{m}; \quad 36.6 \\
\quad 2.70-2.65, \text{m} \\
\text{Ar CH} \quad 7.22-7.13, \text{m} \quad 129.7, 128.5, 126.3 \\
\text{Ar C} \quad - \quad \text{ND} \\
\text{CO} \quad - \quad \text{ND} \\
\text{Pro} \quad \alpha \text{CH} \quad 3.43, \text{d}, J = 7.6 \quad 60.5 \\
\beta \text{CH}_2 \quad 1.78-1.74, \text{m}; 0.72-0.64, \text{m} \quad 30.1 \\
\gamma \text{CH}_2 \quad 1.59-1.53, \text{m}; 1.35-1.28, \text{m} \quad 21.7 \\
\delta \text{CH}_2 \quad 3.30-3.25, \text{m}; 3.22-3.15, \text{m} \quad 46.1 \\
\text{CO} \quad - \quad 172.8 \\
\]

*NMR data of the first conformer

\[ ^1\text{H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*} \]

| Amino acid* | Atome | $^1\text{H}$ chemical shift | $^{13}\text{C}$ chemical shift |
|-------------|-------|-----------------------------|-----------------------------|
| Val         | NH    | 7.66, d, $J = 8.8$          | -                          |
|             | $\alpha$ CH | 4.06-3.98, m          | 59.3                       |
|             | $\beta$ CH | 2.08-2.03, m          | 30.1                       |
|             | $\gamma$ CH$_3$ | 0.86-0.79, m         | 19.8/14.4                  |
|             | CO    | -                          | 170.9                      |
| Gly (a)     | NH    | 8.19-8.15, m             | -                          |
|             | $\alpha$ CH$_2$ | 4.48, dd, $J = 6.3, J = 15.5$; 4.15, dd, $J = 4.5, J = 15.0$ | 34.9                       |
|             | Tz CH | 7.98, s                   | 122.7                      |
|             | Tz C  | 145.3                     |                            |
| Ala         | $\alpha$ CH | 5.43, q, $J = 6.8$     | 58.4                       |
|             | $\beta$ CH$_3$ | 1.62, d, $J = 6.8$    | 18.9                       |
|             | CO    | -                          | 169.0                      |
| Gly (b)     | NH    | 8.41-8.45, m             | -                          |
|             | $\alpha$ CH$_2$ | 4.11, dd, $J = 7.5, J = 16.9$; 3.58, d, $J = 15.6$ | 41.7                       |
|             | CO    | -                          | 169.3                      |
| Ile         | NH    | 8.39, d, $J = 7.1$       | -                          |
|             | $\alpha$ CH | 4.06-3.98, m          | 58.1                       |
|             | $\beta$ CH | 1.74-1.69, m          | 36.1                       |
|             | $\gamma$ CH$_3$ | 1.50-1.42, m; 1.13-1.09, m | 25.1                       |
|             | $\gamma$ CH$_3$ | 0.86-0.79, m         | 15.6                       |
|             | $\delta$ CH$_3$ | 0.86-0.79, m        | 11.2                       |
|             | CO    | -                          | 172.1                      |
| Gly (c)     | NH    | 8.63-8.66, m             | -                          |
|             | $\alpha$ CH$_2$ | 4.06-3.98, m; 3.25-3.22, m | 42.4                       |
|             | CO    | -                          | 170.7                      |
| Phe         | NH    | 7.38-7.35, m             | -                          |
|             | $\alpha$ CH | 4.08-4.06, m          | 53.5                       |
|             | $\beta$ CH$_2$ | 2.93, dd, $J = 3.8, J = 12.5$; 2.87-2.82, m | 37.9                       |
|             | Ar CH | 7.39-7.34, m; 7.33-7.29, m | 130.0, 129.2, 127.6       |
|             | Ar C  | -                          | 136.6                      |
**NMR data of the second conformer**

**CP3; Cyclo(-VGaATzGβIGcFP-) 1:0.9**

$^1$H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*

| Amino acid* | Atome     | $^1$H chemical shift | $^{13}$C chemical shift |
|-------------|-----------|----------------------|-------------------------|
| Val         | NH        | 7.89-7.83, m         | -                       |
|             | α CH      | 3.82-3.79, m         | 60.0                    |
|             | β CH      | 1.95-1.92 m          | 29.1/29.6               |
|             | γ CH$_3$  | 0.96, d, $J$ = 6.6;  | 19.6                    |
|             |           | 0.90-0.86, m         | 19.6                    |
|             | CO        | -                    | 171.9                   |
| Gly (a)     | NH        | 8.46-8.41, m         | -                       |
|             | α CH$_2$  | 4.09, dd, $J$ = 8.4, $J$ = 16.8; | 42.4                    |
|             |           | 3.44-3.38, m         |                         |
|             | CO        | -                    | 168.6                   |
| Ala         | NH        | 8.34, d, $J$ = 9.3   | -                       |
|             | α CH      | 5.12-5.07, m         | 41.4                    |
|             | β CH$_3$  | 1.37, d, $J$ = 6.9   | 21.5                    |
|             | Tz CH     | 7.69, s              | 123.2                   |
|             | Tz C      | -                    | 150.3                   |
| Gly (b)     | α CH$_2$  | 5.12-5.07, m         | 51.4                    |
|             | CO        | -                    | 166.9                   |
| Ile         | NH        | 8.79-8.76, m         | -                       |
|             | α CH      | 3.82-3.79, m         | 60.0                    |
|             | β CH      | 1.77-1.73, m         | 35.2                    |
|             | γ CH$_2$  | 1.65-1.58, m; 1.24-1.21, m | 25.4                    |
|             | γ CH$_3$  | 0.91-0.86, m         | 15.6                    |
|             | δ CH$_3$  | 0.90-0.84, m         | 11.0                    |
|             | CO        | -                    | 171.9                   |
| Gly (c)     | NH        | 8.79-8.76, m         | -                       |
|             | α CH$_2$  | 3.92-3.87, m; 3.21, dd, $J$ = 4.5, $J$ = 16.9 | 42.4                    |
|             | CO        | -                    | 168.4                   |
| Phe         | NH        | 7.65, d, $J$ = 9.2   | -                       |
**Amino acid** | Atome | $^1$H chemical shift | $^{13}$C chemical shift |
|---|---|---|---|
| Val | NH | 8.65-8.61, m | - |
| | α CH | 3.70, dd, $J = 6.3$, $J = 9.0$ | 61.6 |
| | β CH | 1.95-1.92 m | 29.1/29.6 |
| | γ CH$_3$ | 0.94, d, $J = 6.6$; 0.87-0.85, m | 20.0 |
| | CO | - | 172.3 |
| Gly (a) | NH | 8.13-8.07, m | - |
| | α CH$_2$ | 3.77, dd, $J = 6.0$, $J = 17.3$; 3.66-3.59, m | 42.0 |
| | CO | - | 168.7 |
| Ala | NH | 7.96, d, $J = 8.4$ | - |
| | α CH | 5.25-5.17, m | 42.6 |
| | β CH$_3$ | 1.26, d, $J = 7.0$ | 23.3 |
| | Tz CH | 8.01, s | 122.3 |
| | Tz C | - | 150.7 |
| Gly (b) | α CH$_2$ | 5.25-5.17, m; 5.15-5.12, m | 51.9 |
| | CO | - | 166.7 |
| Ile | NH | 8.79-8.76, m | - |
| | α CH | 3.94-3.90, m | 59.3 |
| | β CH | 1.81-1.77, m | 34.9 |
| | γ CH$_2$ | 1.57-1.52, m; 1.21-1.17, m | 25.0 |
| | s | 0.89-0.83, m | 15.6 |
| | δ CH$_3$ | 0.90-0.84, m | 11.0 |
| | CO | - | 171.6 |
| Gly (c) | NH | 8.65-8.61, m | - |
| | α CH$_2$ | 3.87-3.83, m; 3.54, dd, $J = 5.8$, $J = 16.9$ | 43.1 |
| | CO | - | 169.8 |
| Phe | NH | 7.45, d, $J = 5.8$ | - |
| | α CH | 4.55-4.51, m | 52.8 |
| | β CH$_2$ | 2.75, dd, $J = 8.3$, $J = 12.8$; 2.69-2.62, m | 38.2 |
| | Ar CH | 7.30-7.20, m; 7.10-7.06, m | 130.0, 128.8, 127.2 |

*NMR data of the major conformer*

$^1$H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*

α CH 4.55-4.51, m 52.8
β CH$_2$ 2.70-2.66, m; 2.13-2.07, m 36.6
Ar CH 7.22-7.13, m; 7.04-7.00, m 129.5, 128.3, 126.3
Ar C - ND
Pro α CH 4.30, t, $J = 7.4$ 60.2
β CH$_2$ 2.19-2.13, m; 1.75-1.68, m 29.4
γ CH$_2$ 1.92-1.89, m; 1.87-1.83, m 25.6
δ CH$_2$ 3.66-3.59, m; 3.50-3.46, m 47.7
CO - 168.6
\[ \text{Ar C} \quad \text{CO} \quad 136.40 \quad 169.8 \]

|         | $\alpha$ CH | $\beta$ CH | $\gamma$ CH | $\delta$ CH | CO |
|---------|-------------|-------------|-------------|-------------|----|
| Pro     | 3.97-3.94, m | 1.80-1.76, m; 1.53-1.48, m | 1.65-1.58, m; 1.51-1.46, m | 3.29-3.25, m; 3.17-3.12, m | - |
|         | 59.5        | 31.0        | 22.2        | 46.8        | 172.6 |

*NMR data of the minor conformer

**CP4:** Cyclo(-VGaAG$_b$TzIG$_c$FP-) 1:1

$^1$H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*

| Amino acid* | Atome | $^1$H chemical shift | $^{13}$C chemical shift |
|-------------|-------|----------------------|-------------------------|
| Val         | NH    | 7.88-7.85, m         | -                       |
|             | $\alpha$ CH | 4.08, dd, $J = 6.4$, $J = 8.3$ | 58.4 |
|             | $\beta$ CH | 2.04-1.95, m         | 30.3                    |
|             | $\gamma$ CH$_3$ | 0.82, d, $J = 6.7$; | 19.8                    |
|             |         | 0.81, d, $J = 6.7$   | 18.4                    |
|             | CO     | -                    | 171.4                   |
| Gly (a)     | NH    | 8.16-8.14, m         | -                       |
|             | $\alpha$ CH | 3.93, dd, $J = 6.4$, $J = 16.2$; | 42.6 |
|             |         | 3.40-3.38, m         | -                       |
|             | CO     | 168.4                | -                       |
| Ala         | NH    | 7.98-7.96, m         | -                       |
|             | $\alpha$ CH | 4.32, quintet, $J = 7.2$ | 48.4 |
|             | $\beta$ CH | 1.20, d, $J = 7.1$   | 19.1                    |
|             | CO     | -                    | 172.4                   |
| Gly (b)     | NH    | 8.53, dd, $J = 5.0$, $J = 7.0$ | -                       |
|             | $\alpha$ CH | 4.59-4.58, m; 4.05-4.00, m | 34.4 |
|             | Tz CH  | 8.02, s               | 121.4                   |
|             | Tz C   | -                    | 145.3                   |
| Ile         | $\alpha$ CH | 5.19, d, $J = 10.1$  | 66.8                    |
|             | $\beta$ CH | 2.16-2.09, m         | 37.7                    |
|             | $\gamma$ CH$_2$ | 1.01-0.95, m; 0.86-0.83, m | 24.7 |
|             | $\gamma$ CH$_3$ | 0.87, d, $J = 6.7$;  | 15.4                    |
|             | $\delta$ CH$_3$ | 0.78-0.73, m         | 10.5                    |
|             | CO     | -                    | 168.1                   |
| Gly (c)     | NH    | 8.99, dd, $J = 3.5$, $J = 7.9$ | -                       |
|             | $\alpha$ CH | 4.22, dd, $J = 7.9$, $J = 15.5$; | 42.6 |
|             |         | 3.25-3.22, m         | 168.8                   |
| Amino acid | Atome | $^1$H chemical shift | $^{13}$C chemical shift |
|------------|-------|----------------------|------------------------|
| Val        | NH    | 8.01, d, $J = 6.5$   | -                      |
|            | $\alpha$ CH | 3.87, t, $J = 6.5$ | 59.6                   |
|            | $\beta$ CH | 1.92-1.85, m         | 30.3                   |
|            | $\gamma$ CH$_3$ | 0.78-0.73, m; 0.72, d, $J = 6.8$ | 19.4 |
|            | CO    | 171.4                |                        |
| Gly (a)    | NH    | 8.00-7.98, m         | -                      |
|            | $\alpha$ CH$_2$ | 3.67, dddd, $J = 5.4$, $J = 16.4$, $J = 50.3$ | 42.8 |
|            | CO    | 169.3                |                        |
| Ala        | NH    | 7.87-7.85, m         | -                      |
|            | $\alpha$ CH | 4.17-4.13, m         | 48.9                   |
|            | $\beta$ CH$_3$ | 1.20, d, $J = 7.1$  | 17.7                   |
|            | CO    | 172.1                |                        |
| Gly (b)    | NH    | 8.20, dddd, $J = 5.4$, $J = 6.7$ | - |
|            | $\alpha$ CH$_2$ | 4.57-4.53, m; 4.01-3.99, m | 34.5 |
|            | Tz CH | 7.85, s              | 121.8                  |
|            | Tz C  | -                    | 145.6                  |
| Ile        | $\alpha$ CH | 5.07, d, $J = 10.1$ | 67.4                   |
|            | $\beta$ CH | 2.16-2.09, m         | 37.7                   |
|            | $\gamma$ CH$_2$ | 1.01-0.95, m; 0.86-0.83, m | 24.7 |
|            | $\gamma$ CH$_3$ | 0.87, d, $J = 6.7$  | 15.4                   |
|            | $\delta$ CH$_3$ | 0.78-0.73, m         | 10.5                   |
|            | CO    | 167.7                |                        |
| Gly (c)    | NH    | 8.62, dddd, $J = 4.1$, $J = 7.2$ | - |
|            | $\alpha$ CH$_2$ | 4.05-4.03, m; 3.40-3.37, m | 41.9 |
|            | CO    | 167.9                |                        |
| Phe        | NH    | 8.15-8.12, m         | -                      |
|            | $\alpha$ CH | 4.57-4.53, m         | 52.3                   |
|            | $\beta$ CH$_2$ | 2.85, dddd, $J = 7.3$, $J = 13.3$; 2.80-2.77, m | 39.1 |

*NMR data of the first conformer

$^1$H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*
| Amino acid* | Atome | \(^1\)H chemical shift | \(^{13}\)C chemical shift |
|------------|-------|------------------------|------------------------|
| Val        | NH    | 7.86, d, \(J = 8.8\) | -                      |
|            | \(\alpha\) CH | 4.13, dd, \(J = 5.5, J = 8.7\) | 58.1                  |
|            | \(\beta\) CH | 2.15-2.09, m | 29.7                  |
|            | \(\gamma\) CH\(_3\) | 0.67, t, \(J = 7.1\); | 19.5                  |
|            |       | 0.44, d, \(J = 6.9\) | 17.3                  |
|            | CO    | -                      | 17.4                  |
| Gly (a)    | NH    | 8.05, t, \(J = 4.7\) | -                      |
|            | \(\alpha\) CH\(_2\) | 3.79-3.74, m; | 44.4                  |
|            |       | 3.64, dd, \(J = 5.2, J = 14.8\) |                    |
|            | CO    | -                      | 171.5                 |
| Ala        | NH    | 8.76, d, \(J = 5.0\) | -                      |
|            | \(\alpha\) CH | 4.06-4.00, m | 50.6                  |
|            | \(\beta\) CH\(_3\) | 1.29, d, \(J = 7.4\) | 16.7                  |
|            | CO    | -                      | 172.9                 |
| Gly (b)    | NH    | 8.43, t, \(J = 6.3\) | -                      |
|            | \(\alpha\) CH\(_2\) | 3.82, dd, \(J = 6.8, J = 16.9\); | 43.1                  |
|            |       | 3.44-3.40, m |                    |
|            | CO    | -                      | 168.3                 |
| Ile        | NH    | 7.30-7.27, m | -                      |
|            | \(\alpha\) CH | 5.15-5.09, m | 49.2                  |
|            | \(\beta\) CH | 1.67-1.64, m | 40.4                  |
|            | \(\gamma\) CH\(_2\) | 1.35-2.30, m; 1.00-0.96, m | 25.6                  |
|            | \(\gamma\) CH\(_3\) | 0.77, d, \(J = 6.8\) | 14.5                  |
|            | \(\delta\) CH\(_3\) | 0.84, t, \(J = 7.4\) | 11.8                  |
|            | Tz CH | 8.18, s | 124.5                 |
|            | Tz C  | -                      | 147.8                 |

* NMR data of the second conformer

\(\text{CP5; Cyclo}(-\text{VGaAG}_9\text{TzG}_6\text{FP}-) 1:0.6\)

\(^1\)H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*
| Amino acid* | Atome | \(^1\)H chemical shift | \(^{13}\)C chemical shift |
|-------------|-------|------------------------|-------------------------|
| Val         | NH    | 7.94, d, \(J = 7.7\)   | -                       |
|             | \(\alpha\) CH | 3.79-3.74, m          | 60.1                    |
|             | \(\beta\) CH | 1.84-1.78, m          | 29.5                    |
|             | \(\gamma\) CH | 0.75, d, \(J = 6.8\); | 19.8                    |
|             |         | 0.67, t, \(J = 7.1\); | 19.5                    |
|             | CO    |                        | 171.5                   |
| Gly (a)     | NH    | 7.58, t, \(J = 5.7\)   | -                       |
|             | \(\alpha\) CH | 3.90-3.86, m;         | 42.6                    |
|             |         | 3.59, dd, \(J = 5.0\);| 170.3                   |
|             | CO    |                        | 170.3                   |
| Ala         | NH    | 8.50, d, \(J = 6.0\)   | -                       |
|             | \(\alpha\) CH | 3.95-3.90, m        | 49.8                    |
|             | \(\beta\) CH | 1.25, d, \(J = 7.0\) | 16.9                    |
|             | CO    | -                      | 172.4                   |
| Gly (b)     | NH    | 8.36, dd, \(J = 5.7\);| -                       |
|             | \(\alpha\) CH | 3.98-3.92, m;        | 43.0                    |
|             |         | 3.38-3.33, m           | 168.5                   |
|             | CO    | -                      | 168.5                   |
| Ile         | NH    | 7.63, d, \(J = 9.6\)   | -                       |
|             | \(\alpha\) CH | 4.88, dd, \(J = 7.7\);| 50.1                    |
|             | \(\beta\) CH | 1.74-1.70, m       | 39.3                    |
|             | \(\gamma\) CH | 1.44-1.41, m; 1.07-1.02, m | 24.9         |
|             | \(\gamma\) CH | 0.71, d, \(J = 6.7\) | 15.7                    |
|             | \(\delta\) CH | 0.80, t, \(J = 7.5\) | 11.6                    |
|             | Tz CH | 7.98, s                | 124.1                   |
|             | Tz C  | -                      | 148.6                   |
| Gly (c)     | \(\alpha\) CH | 5.28, d; 5.02, d, \(J = 16.8\) | 51.3                    |
|             | CO    |                        | 165.6                   |
| Phe         | NH    | 9.04-9.00, m           | -                       |
|             | \(\alpha\) CH | 4.54, q, \(J = 7.2\) | 53.3                    |
|             | \(\beta\) CH | 2.96-2.90, m       | 38.4                    |
|             | Ar CH | 7.36-7.21, m           | 128.9, 127.4, 129.8     |

*NMR data of the major species

\(^1\)H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*

S27
| Amino acid* | Atome | ¹H chemical shift | ¹³C chemical shift |
|------------|-------|------------------|-------------------|
| Val        | NH    | 7.80-7.78, m     | -                 |
|            | α CH  | 4.20-4.17, m     | 58.1              |
|            | β CH  | 2.06-2.00, m     | 31.4              |
|            | γ CH₃ | 0.88-0.85, m     | 19.8              |
|            |       | 0.87-0.84, m     | 18.6              |
|            | CO    | -                | 171.3             |
| Gly (a)    | NH    | 8.51, t, J = 5.7 | -                 |
|            | α CH₂ | 3.93, d, J = 15.5; 3.50-3.49, m | 43.3  |
|            | CO    | -                | 168.9             |
| Ala        | NH    | 8.07, d, J = 7.8 | -                 |
|            | α CH  | 4.32-4.25, m     | 48.6              |
|            | β CH₂ | 1.24, d, J = 7.1 | 18.4              |
|            | CO    | -                | 172.5             |
| Gly (b)    | NH    | 8.12-8.10, m     | -                 |
|            | α CH₂ | 3.87, dd, J = 6.7, J = 16.6; 3.63, dd, J = 4.8, J = 16.7 | 42.6  |
|            | CO    | -                | 168.7             |
| Ile        | NH    | 7.89, d, J = 8.5 | -                 |
|            | α CH  | 4.11-4.07, m     | 57.5              |
|            | β CH  | 1.80-1.76, m     | 36.3              |
|            | γ CH₂ | 1.50-1.43, m; 1.08-1.03, m | 24.9  |
|            | γ CH₃ | 0.87-0.83, m     | 15.8              |
|            | δ CH₃ | 0.84-0.80, m     | 11.4              |
|            | CO    | -                | 171.3             |
| Gly (c)    | NH    | 8.58, dd, J = 4.6, J = 7.3 | -                 |
|            | α CH₂ | 4.59, dd, J = 7.7, J = 15.3; | 34.7  |

*NMR data of the minor species

**CP6; Cyclo(-VGaAGbIGcTzFP-) 2:1**

¹H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*
**NMR data of the major conformer**

**1H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)**

| Amino acid* | Atome | ¹H chemical shift | ¹²C chemical shift |
|-------------|-------|-------------------|-------------------|
| Val         | NH    | 8.33, d, J = 5.1  | -                 |
|             | α CH  | 3.73, dd, J = 5.3, J = 7.5 | 61.0 |
|             | β CH  | 1.94-1.89, m      | 29.3              |
|             | γ CH₃ | 0.94, d, J = 6.7; | 19.8              |
|             |       | 0.88-0.85, m      | 19.8              |
|             | CO    |                   | 168.7             |
| gly (a)     | NH    | 8.27-8.22, m      | -                 |
|             | α CH  | 3.78-3.75, m; 3.53-3.50, m | 42.6 |
|             | CO    |                   | 168.7             |
| Ala         | NH    | 7.82, d, J = 7.6  | -                 |
|             | α CH  | 4.32-4.25, m      | 48.6              |
|             | β CH₃ | 1.11, d, J = 7.2  | 18.0              |
|             | CO    |                   | 173.2             |
| gly (b)     | NH    | 8.31, t, J = 5.4  | -                 |
|             | α CH₂ | 3.88, dd, J = 5.6, J = 15.7; | 43.3 |
|             |       | 3.45-3.42, m      | 169.4             |
|             | CO    |                   |                   |
| Ile         | NH    | 7.74, d, J = 8.4  | -                 |
|             | α CH  | 4.12-4.08, m      | 57.5              |
|             | β CH  | 1.89-1.82, m      | 36.5              |
|             | γ CH₂ | 1.36-1.31, m; 1.11-1.08, m | 24.5 |
|             | γ CH₃ | 0.83-0.80, m      | 15.8              |
|             | δ CH₃ | 0.81-0.78, m      | 11.4              |
|             | CO    |                   | 170.8             |
| gly (c)     | NH    | 8.27-8.22, m      | -                 |
|             | α CH₂ | 4.48-4.45, m; 4.17-4.13, m | 34.7 |
| Tz CH       |       | 7.79, s           | 123.0             |
| Tz C        |       |                   | 144.7             |
**Phe**
- α CH: 5.26, t, $J = 7.7$
- β CH: 3.49-3.47, m; 3.35, dd, $J = 7.9, J = 13.4$
- Ar CH: 7.29-7.26, m; 7.27-7.24, m; 7.18-7.16, m
- Ar C: -
- CO: -

**Pro**
- α CH: 4.17-4.13, m
- β CH: 1.95-1.91, m; 1.87-1.83, m
- γ CH: 1.63-1.55, m; 1.28-1.24, m
- δ CH: 3.34-3.30, m; 3.26-3.22, m
- CO: -

*NMR data of the minor conformer

---

**CP13; Cyclo(-VTz$_{a}$G$_{a}$ATz$_{b}$G$_{b}$IG$_{c}$FP-) 3:1**

$^1$H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*

| Amino acid* | Atome | $^1$H chemical shift | $^{13}$C chemical shift |
|-------------|-------|----------------------|-------------------------|
| Val         | NH    | 7.68, d, $J = 8.6$   | -                       |
|             | α CH  | 4.83, t, $J = 7.5$   | 51.4                    |
|             | β CH  | 2.13-2.07, m         | 32.6                    |
|             | γ CH  | 0.85-0.83, m         | 19.3                    |
|             |       | 0.83-0.80, m         | 18.9                    |
|             | Tz CH | 7.81, s              | 123.9                   |
|             | Tz C  | -                    | 147.4                   |
| Gly (a)     | α CH  | 5.16, d, $J = 16.0$  | 52.1                    |
|             |       | 5.01, d, $J = 16.4$  | 164.9                   |
|             | CO    | -                    | -                       |
| Ala         | NH    | 8.89, d, $J = 7.7$   | -                       |
|             | α CH  | 5.04, t, $J = 7.4$   | 41.7                    |
|             | β CH  | 1.44, d, $J = 6.9$   | 21.0                    |
|             | Tz CH | 7.71, s              | 123.2                   |
|             | Tz C  | -                    | 149.6                   |
| Gly (b)     | α CH  | 4.71, d, $J = 15.9$; | 51.2                    |
|             |       | 5.09, d, $J = 16.4$  | 165.9                   |
|             | CO    | -                    | -                       |
| Ile         | NH    | 8.65, d, $J = 7.0$   | -                       |
|             | α CH  | 4.08, t, $J = 7.6$   | 58.2                    |
|             | β CH  | 1.72-1.66, m         | 36.3                    |
|             | γ CH  | 1.49-1.45, m; 1.17-1.10, m | 29.3 |
| Amino Acid | Atome | $^1$H Chemical Shift | $^{13}$C Chemical Shift |
|------------|-------|----------------------|------------------------|
| Gly (c)    | NH    | 8.60-8.56, m         | -                      |
|            | $\alpha$ CH | 4.07-4.02, m; 3.30, dd, $J = 2.2, J = 16.5$ | 42.4 |
|            | CO    | -                   | 171.6                  |
| Phe        | NH    | 7.09, d, $J = 7.7$  | -                      |
|            | $\alpha$ CH | 4.67-4.62, m | 52.4 |
|            | $\beta$ CH | 2.99, dd, $J = 3.4, J = 14.2$; 2.78, dd, $J = 10.6, J = 14.2$ | 36.5 |
|            | Ar CH | 7.27-7.24, m; 7.21-7.18, m; 7.11-7.10, m | 128.8; 126.7; 129.8 |
|            | Ar C  | -                   | 138.5                  |
|            | CO    | -                   | ND                     |
| Pro        | $\alpha$ CH | 4.31, d, $J = 8.5$ | 61.0 |
|            | $\beta$ CH | 2.06-1.97, m; 1.90-1.85, m | 27.1 |
|            | $\gamma$ CH | 1.90-1.85, m; 1.84-1.80, m | 24.9 |
|            | $\delta$ CH | 3.60-3.56, m | 47.4 |
|            | CO    | -                   | 170.6                  |

*NMR data of the major conformer*

$^1$H NMR: (700 MHz, DMSO); HSQC and HMBC analysis (700 MHz, DMSO)*
| Gly (c) | NH     | 8.52, t, \( J = 5.7 \) |
|        | \( \alpha \text{ CH}_2 \) | 3.82-3.78, m; 3.69-3.65, m |
|        | CO     | - |

| Phe    | NH     | 7.69-7.67 |
|        | \( \alpha \text{ CH} \) | 4.13-4.09, m |
|        | \( \beta \text{ CH}_2 \) | 2.66-2.61, m; ND |
|        | Ar \text{ CH} | |
|        | Ar \text{ C} | |
|        | CO     | - |

| Pro    | \( \alpha \text{ CH} \) | 3.26-3.22, m |
|        | \( \beta \text{ CH}_2 \) | 1.59-1.54, m |
|        | \( \gamma \text{ CH}_2 \) | 1.31-1.27, m |
|        | \( \delta \text{ CH}_2 \) | 3.20-3.17, m; 3.14-3.13, m |
|        | CO     | - |

*NMR data of the minor conformer*
VI. NMR spectra of final cyclic peptides

**CP0;** Cyclo(-VG\textsubscript{a}AG\textsubscript{b}IG\textsubscript{c}FP-); $^1$H; DMSO-d6; 700 MHz; 3:1 ratio

![NMR spectra of CP0](image1)

**CP0;** Cyclo(-VG\textsubscript{a}AG\textsubscript{b}IG\textsubscript{c}FP-); HSQC; DMSO-d6; 700 MHz

![HSQC spectrum of CP0](image2)
**CP1**: Cyclo(-VTzGₐAGₐIGₐFP-); $^1$H; DMSO-d$_6$; 700 MHz; 4:1 ratio

**CP1**: Cyclo(-VTzGₐAGₐIGₐFP-); HSQC; DMSO-d$_6$; 700 MHz
**CP2**: Cyclo(-VG,TzAG,TzAG,Fp-); $^1$H; DMSO-d6; 700 MHz; 1:1 ratio

**CP2**: Cyclo(-VG,TzAG,TzAG,Fp-); HSQC; DMSO-d6; 700 MHz
**CP3**: Cyclo(-VG₉ATzG₉IG₃FP-); $^1$H; DMSO-d6; 700 MHz; 1:0.9 ratio

**CP3**: Cyclo(-VG₉ATzG₉IG₃FP-); HSQC; DMSO-d6; 700 MHz
**CP4:** Cyclo(-VG₄AG₆TzIG₃FP-); $^1$H; DMSO-d6; 700 MHz; 1:1 ratio

**CP4:** Cyclo(-VG₄AG₆TzIG₃FP-); HSQC; DMSO-d6; 700 MHz
**CP5**: Cyclo(-VG₉AG₉ITzG₉FP-); $^1$H; DMSO-d6; 700 MHz; 1:0.6 ratio

![NMR spectrum of CP5](image)

**CP5**: Cyclo(-VG₉AG₉ITzG₉FP-); HSQC; DMSO-d6; 700 MHz

![HSQC spectrum of CP5](image)
CP6; Cyclo(-VGaAGbIGcTzFP-); $^1$H; DMSO-d6; 700 MHz; 2:1 ratio

CP6; Cyclo(-VGaAGbIGcTzFP-); HSQC; DMSO-d6; 700 MHz
**CP13**: Cyclo(-VTzG₃ATzG₃IG₃FP-); $^1$H; DMSO-d$_6$; 700 MHz; 3:1 ratio

**CP13**: Cyclo(-VTzG₃ATzG₃IG₃FP-); HSQC; DMSO-d$_6$; 700 MHz
The full ratio of all four major species is not determined. The individual sequences of all four major species are not determined.

**CP135; Cyclo(-VTzG₄ATzG₃ITzG₄FP-); ¹H; DMSO-d₆; 700 MHz; 1:1.6:1.1:x ratio**
VII. EXSY NMR of final cyclic peptides

**CP0**: Cyclo(-VG\textsubscript{a}AG\textsubscript{b}IG\textsubscript{c}FP-); EXSY

![EXSY NMR of CP0](image1)

**CP1**: Cyclo(-VTzG\textsubscript{a}AG\textsubscript{b}IG\textsubscript{c}FP-); EXSY

![EXSY NMR of CP1](image2)

**CP2**: Cyclo(-VG\textsubscript{a}TzAG\textsubscript{b}IG\textsubscript{c}FP-); EXSY

![EXSY NMR of CP2](image3)
**CP3;** Cyclo(-VG\textsubscript{8}ATzG\textsubscript{8}IG\textsubscript{8}FP\textsubscript{8}); EXSY
CP4; Cyclo(-VG₃AG₅TzIG₃FP-); EXSY

EXSY NMR
1H NMR
CH₂-I

CP5; Cyclo(-VG₃AG₅ITzG₃FP-); EXSY

EXSY NMR
EXSY NMR
1H NMR
CH₃-F
**CP6; Cyclo(-VGAGIGcTzFP-); EXSY**

![EXSY NMR diagram of CP6]

**CP13; Cyclo(-VTzGATzGIGcFP-); EXSY**

![EXSY NMR diagram of CP13]
CP135; Cyclo(-VTzG\textsubscript{a}ATzG\textsubscript{b}ITzG\textsubscript{c}FP-); EXSY
VIII. MS-MS data of final cyclic peptides

**CP0; Cyclo(-VG\textsubscript{a}AG\textsubscript{b}IG\textsubscript{c}FP-)**

**CP1; Cyclo(-VTzG\textsubscript{a}AG\textsubscript{b}IG\textsubscript{c}FP-)**
The MS-MS fragmentation data of both cyclic compounds observed in the crude mixture were identical.
CP4; Cyclo(-VGₐAGₜTZGₜFP-)
The MS-MS fragmentation data of both cyclic compounds observed in the crude mixture were identical.
The MS-MS fragmentation data of both cyclic compounds observed in the crude mixture were identical.
IX. LC-MS traces of crude mixtures of CP3, CP13, and CP135

LC-MS trace of the crude reaction mixture of CP3 showing two peaks with the same desired mass. Both of these peaks were determined to be the cyclic peptide after analysis by HRMS (Table S2) and MS-MS fragmentation (section VIII).
LC-MS trace of the crude reaction mixture of **CP13** showing two peaks with the same desired mass. Both of these peaks were determined to be the cyclic peptide after analysis by HRMS (Table S2) and MS-MS fragmentation (section VIII).
LC-MS trace of the crude reaction mixture of \textbf{CP135} showing two peaks with the same desired mass. Both of these peaks were determined to be the cyclic peptide after analysis by HRMS (Table S2) and MS-MS fragmentation (section VIII).
X. LC-MS traces of final cyclic peptides

For each of the cyclic peptides CP, the UV trace at 220 nm obtained by HPLC is complemented by its corresponding LCMS trace at the desired molecular weight (Single Ion Monitoring SIM mode) The HPLC methods used are described in section I.
CP2, 220 nm, 91%

CP3, 220 nm, 99%
CP4, 220 nm, 98%

CP5, 220 nm, 93%
CP6, 220 nm, 98%

CP13, 220 nm, 93%
XI. LC-MS traces of starting peptides

For each of the precursor peptides \( \mathbf{P} \), the UV trace at 220 nm obtained by HPLC is complemented by its corresponding LCMS trace at the desired molecular weight (Single Ion Monitoring SIM mode). The HPLC methods used are described in section I.
P1, 220 nm, 99%

P2, 220 nm, 99%
P3, 220 nm, 99%

P4, 220 nm, 93%
P5, 220 nm, 97%

MWD1 A, Sig=220

MSD2 TIC, MS

ES-API, Pos, SIM, Frag: 125

P6, 220 nm, 98%

MWD1 A, Sig=220

MSD2 TIC, MS

ES-API, Pos, SIM, Frag: 125
P15, 220 nm, 99%

P16, 220 nm, 99%
P24, 220 nm, 98%

P25, 220 nm, 98%
XII. References

[1] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics* **2010**, *29*, 2176.

[2] J. Koehnke, A. Bent, W. E. Houssen, D. Zollman, F. Morawitz, S. Shirran, J. Vendome, A. F. Nneyoigbe, L. Trembleau, C. H. Botting, M. C. M. Smith, M. Jaspars, J. H. Naismith, *Nat Struct Mol Biol* **2012**, *19*, 767.

[3] E. D. Goddard-Borger, R. V. Stick, *Organic Letters* **2007**, *9*, 3797.

[4] N. Fischer, E. D. Goddard-Borger, R. Greiner, T. M. Klapötke, B. W. Skelton, J. Stierstorfer, *The Journal of Organic Chemistry* **2012**, *77*, 1760-1764.

[5] Lundquist, J. C. Pelletier, *Organic Letters* **2001**, *3*, 781.

[6] H. D. Dickson, S. C. Smith, K. W. Hinkle, *Tetrahedron Letters* **2004**, *45*, 5597-5599.

[7] J.-A. Fehrentz, B. Castro, *Synthesis* **1983**, *1983*, 676.

[8] G. Reginato, A. Mordini, F. Messina, A. Degl’Innocenti, G. Poli, *Tetrahedron* **1996**, *52*, 10985.

[9] I. E. Valverde, A. Bauman, C. A. Kluba, S. Vomstein, M. A. Walter, T. L. Mindt, *Angew. Chem. Int. Ed.* **2013**, *52*, 8957.

[10] M. Tischler, D. Nasu, M. Empting, S. Schmelz, D. W. Heinz, P. Rottmann, H. Kolmar, G. Bunktowsky, D. Tietze, O. Avrutina, *Angew. Chem. Int. Ed.* **2012**, *51*, 3708.

[11] A. Tam, U. Arnold, M. B. Soellner, R. T. Raines, *J. Am. Chem. Soc.* **2007**, *129*, 12670.