

SUPPORTING INFORMATION

H-Bond Surrogate-Stabilized Shortest Single-turn α -Helices: sp^2 Constraints and Residue Preferences for the Highest α - Helicities

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List of Abbreviations

1. Structure

| | | |
|--------|---|-------------------------|
| Ala, A | : | alanine |
| Gly, G | : | glycine |
| Moc | : | methyloxycarbonyl |
| Phe, F | : | phenylalanine |
| Prp | : | propyl linker |
| HBS | : | hydrogen bond surrogate |

2. Synthesis

| | | |
|---------------------------------|---|--|
| ACN | : | acetonitrile |
| anhyd. | : | anhydrous |
| aq. | : | aqueous |
| Bn | : | benzyl |
| Boc | : | <i>tert</i> -butyloxycarbonyl |
| Boc ₂ O | : | Di- <i>tert</i> -butyldicarbonate |
| Cbz, Z | : | benzyloxycarbonyl |
| CH ₃ CN | : | acetonitrile |
| DCM | : | dichloromethane |
| DIAD | : | diisopropyl azodicarboxylate |
| DMF | : | dimethyl formamide |
| DIPEA | : | <i>N,N</i> -Diisopropylethylamine |
| ECF | : | ethylchloroformate |
| EDC | : | 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride |
| eq. or equiv. | : | equivalent |
| EtOAc | : | ethyl acetate |
| H ₂ | : | hydrogen gas |
| HOBt | : | 1-hydroxybenzotriazole |
| K ₂ CO ₃ | : | potassium carbonate |
| LiOH | : | lithium hydroxide |
| Me | : | methyl |
| MeOH | : | methyl alcohol |
| mg | : | milligram(s) |
| ml | : | milliliter |
| mM | : | millimole(s) |
| Moc | : | methyloxycarbonyl |
| μl | : | microliter |
| Na ₂ SO ₄ | : | sodium sulphate |
| NaHCO ₃ | : | sodium bicarbonate |
| NMM | : | <i>N</i> -methylmorpholine |

| | | |
|----------------------|---|----------------------------------|
| Ns | : | 2-Nitrobenzenesulphonyl chloride |
| Pd-C | : | palladium on carbon |
| Ph | : | phenyl |
| PhSH | : | thiophenol |
| PPh ₃ | : | triphenylphosphine |
| Qu. | : | quantitative |
| <i>R_f</i> | : | retardation factor (in TLC) |
| TEA | : | triethylamine |
| THF | : | tetrahydrofuran |
| TFA | : | trifluoroacetic acid |
| TLC | : | thin layer chromatography |

3. Characterization

| | | |
|---------------------|---|---|
| Å | : | angstrom |
| Calcd. | : | calculated |
| CDCl ₃ | : | deuterated chloroform |
| CD ₃ CN | : | deuterated acetonitrile |
| cm | : | centimeter |
| nm | : | nanometer |
| d | : | doublet |
| dm | : | decimolar |
| DMSO-d ₆ | : | hexadeuterated dimethyl sulfoxide |
| dd | : | doublet of a doublet |
| dt | : | doublet of a triplet |
| g | : | gram(s) |
| h | : | hour(s) |
| HRMS | : | High resolution mass spectrum |
| HSQC | : | Heteronuclear Single Quantum Coherence |
| Hz | : | Hertz |
| K | : | Kelvin (temperature) |
| m | : | multiplet |
| MHz | : | mega hertz |
| μM | : | micromolar |
| mM | : | millimolar |
| ¹³ J | : | through bond scalar coupling constant |
| MHz | : | mega hertz |
| min | : | minute(s) |
| nm | : | nanometers (wavelength) |
| NMR | : | nuclear magnetic resonance |
| pH | : | -log ₁₀ c (c is proton concentration in molar units) |
| ppm | : | parts per million |
| quin | : | quintet |
| q | : | quartet |

| | | |
|-----------------------|---|--|
| RT or rt | : | room temperature |
| ROESY | : | Rotating-frame nuclear overhauser effect correlation spectroscopy |
| s | : | singlet |
| sec | : | secondary |
| t | : | triplet |
| td | : | triplet of doublet |
| TOCSY | : | Total Correlation Spectroscopy |
| θ | : | (Theta) ellipticity ($\text{deg}\cdot\text{cm}^2\cdot\text{dmol}^{-1}$) |
| φ | : | (phi) $\text{C}'_{i-1}\text{-N}_i\text{-C}^\alpha\text{-C}'_i$ dihedral angle of an amino acid residue |
| ψ | : | (psi) $\text{N}_i\text{-C}^\alpha\text{-C}'_i\text{-N}_{i+1}$ dihedral angle of an amino acid residue |
| $\pi\rightarrow\pi^*$ | : | (pi to pi*) electronic transition from bonding π to anti-bonding π^* orbital in the peptide motif |
| $n\rightarrow\pi^*$ | : | (non-bonding to pi*) electronic transition from non-bonding n to anti-bonding π^* orbital in the peptide motif |
| λ (nm) | : | (lambda) wavelength in nanometers (nm) |

1. Materials

All reactions were performed in oven-dried apparatus and were stirred using magnetic stir-bars. Column chromatography was performed on silica gel (100-200 mesh) (Acme's) purchased from S D Fine chem Ltd, India. Thin Layer Chromatography (TLC) was carried out on Merck DC Kieselgel 60 F254 aluminum sheets. Compounds were visualized by one (or all of the) following methods: (1) fluorescence quenching, (2) spraying with a 0.2% (w/v) ninhydrin solution in absolute ethanol, (3) spraying with 1% H₂SO₄ solution in EtOH/H₂O (1:5 v/v), (4) charring on hot plate. Ethyl acetate and hexanes (or low boiling fractions of petroleum ether) were obtained from SD-fine chemicals, India and were fractionally distilled at their respective boiling points, before use. Dichloromethane (DCM) was dried by distillation over phosphorus pentoxide (P₂O₅). N-methyl morpholine (NMM) was distilled over calcium hydride (CaH₂). Nuclear Magnetic Resonance (NMR) spectra of compounds were recorded on BRUKER-AV400 spectrometer (Bruker Co., Faellanden, Switzerland). Chemical shifts are expressed as δ values in parts per million (ppm) from the residual non-deuterated chloroform in CDCl₃ ($\delta_{\text{H}} = 7.26$ ppm, $\delta_{\text{C}} = 77.00$ ppm). ^{1,3}J_{H,H} coupling constant values are expressed in hertz (Hz). Multiplicities are indicated using the following abbreviations: s (singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplet), t (triplet), q (quartet), quin (quintet), sext (sextet), hept (heptet), m (multiplet), bs (broad singlet). Mass spectra were obtained with Micromass Q-ToF (ESI-HRMS). Melting points (m.p.) analyses were performed in VEEGO melting point apparatus (VEEGO Inst. Co., Mumbai, India). Far-UV CD spectra were recorded using a JASCO CD spectrometer (model No - J-815) equipped with a peltier temperature-controlled cell holder using a 0.1 cm path length Suprasil quartz cell (Hellma, Forest Hills, NY, USA).

2. Methods:

Nuclear Magnetic Resonance (NMR) spectroscopy Experiments:

¹H and 2D NMR spectra were recorded on a Bruker Avance (Bruker Co., Faellanden, Switzerland) 400 MHz spectrometer. 2D NMR spectra were recorded in phase sensitive mode using time-proportional phase incrementation for quadrature detection in the *t*₁ dimension. ¹H, ¹³C NMR experiments of molecules (except macrocyclic peptides) were performed typically at 10 mM sample and 60 mM concentrations respectively. ¹H, ¹³C and

2D NMR experiments of molecules were performed (for macrocyclic 1373 and 1363 peptides) in 3.8-5.0 mM solutions. The solvents used were CDCl₃, 40% CDCl₃ in CD₃CN or 10% D₂O in H₂O.

Heteronuclear Single Quantum Correlation (HSQC) Experiment:

The HSQC spectra were recorded at 298 K with a mixing time of 200 ms using the hsqctgpsi2 pulse sequence. An HSQC continuous wave spin-lock of 1.5 KHz was used to collect 2K points in the *f*₂ domain and 512 points in the *f*₁ domain. The data were processed using Bruker TOPSPIN 3.6 version software.

Total Correlation Spectroscopy (TOCSY) Experiment:

The TOCSY spectra were recorded at 298 K with mixing time of 200 ms using the MLEVPH pulse sequence. A TOCSY continuous wave spin-lock of 1.5 KHz was used to collect 2K points in the *f*₂ domain and 512 points in the *f*₁ domain. The data were processed using Bruker TOPSPIN 3.6 version software. A 90° sine-squared window function was applied in both directions.

Rotating Frame Nuclear Overhauser Effect Correlation Spectroscopy (ROESY) Experiment:

The ROESY spectra were recorded at 298 K with mixing time of 300 ms using ROESYPH pulse sequence. A ROESY continuous wave spin-lock of 1.5 KHz was used to collect 2K points in the *f*₂ domain and 512 points in the *f*₁ domain. The data were processed using Bruker TOPSPIN 3.6 version software. A 90° sine-squared window function was applied in both directions.

CD spectroscopic analyses:

CD spectra were recorded in the far-UV range, from 185 nm to 270 nm at 295 K with scan speed of 50 nm/min and with each reading averaged over 3 scans. Spectral baselines were obtained under conditions analogous as those for the samples. The blank solvent spectra for each solution were recorded under the same conditions. Solutions were prepared by weighing out the peptide in a volumetric flask and adding the solvent for dilution up to the marks, ensuring the dissolving of the peptide, followed by filtering of the solution through a 0.2 micron polyvinylidene Difluoride (PVDF) membrane filter (Pall India

Pvt. Ltd. Mumbai). All the spectra are baseline corrected and θ values were recorded in mdeg units. Each data point was then converted to uniform scale molar ellipticity θ (deg cm² dmol⁻¹) values. The corresponding mean molar residue ellipticity (θ_{MRE}) values were then calculated by the equation θ/n (where n = the number of peptide bonds in the peptide) and the θ_{MRE} (deg cm² dmol⁻¹) values were plotted as a function of corresponding λ_{nm} . Temperature-dependent CD experiments were performed by varying the temperature of the samples using JASCO peltier instrument, allowing 15 min equilibration time at each temperature before recording each data point.

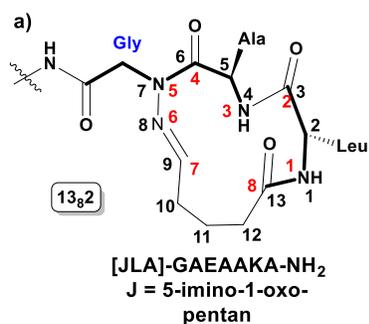
FTIR spectroscopic analyses:

IR spectra were recorded by using a Bruker spectrophotometer. Cell with path length 0.1 cm (with KCl window) was used for the solution measurements. All the spectra were baseline corrected with respect to the blank solvent (Spectrograde CH₃CN purchased from Merck) with minimum of 16 scans were signal-averaged. For acquiring the FTIR for the deuterated analogues of **1a,b** the peptides were dissolved (5 mM concentration) in 100% CD₃OD (99.8 % deuterated) and kept standing for 1 h and ¹H NMR were taken to ensure the complete exchange of NHs to NDs. Then CD₃OD was removed under reduced pressure until complete dryness. Further the peptides were redissolved in CH₃CN (2 mM concentration) and recorded the spectra.

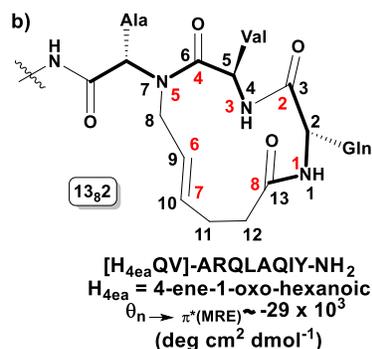
Procedure for Energy minimization.

Energy minimization was carried out on all the three 1373 analogues **1a-c**, starting from the structure which is built based on the 2D ROESY constraints. The interproton distances are taken care of based on the ROE intensities. The molecule was then uploaded into ATB topology builder to generate the initial optimized geometry using semi empirical QM theory (SCF level of theory) and MOPAC charges. Bonded and van der Waals parameters were taken from the GROMOS 54A7 parameter set. All the three molecules were then placed in a suitable cubic box with the box edge adjusted to 1 Å from the peptide's periphery. The box was filled with suitable number of pre-equilibrated SPC water molecules. Then the energy minimization was done using steep (steepest descent minimization) algorithm for all the three molecules, were restrained to its initial coordinates with a force constant of 100 kJ mol⁻¹.

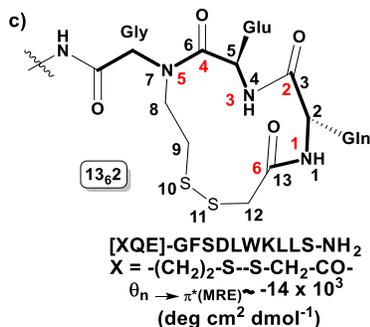
3. Nomenclature of HBS-constrained cyclic peptides:



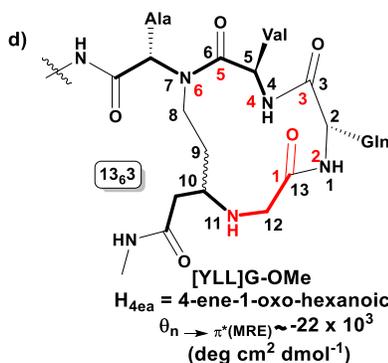
i+1st residue absent
Satterthwait et al.
J. Am. Chem. Soc., **1999**, 121, 3862



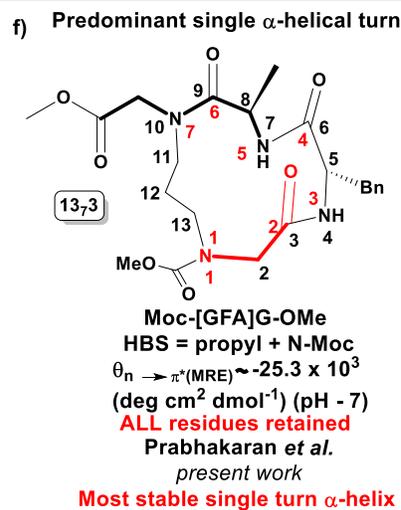
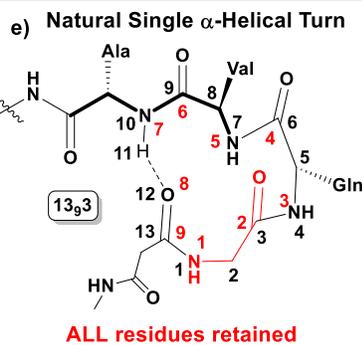
i+1st residue absent
Arora et al.
J. Am. Chem. Soc., **2008**, 130, 14343
Acc. Chem. Res., **2008**, 41, 1289



i+1st residue absent
Arora et al.
Tetrahedron, **2012**, 68, 4434



ALL residues retained
Alewood et al.
Angew. Chem. Int. Ed., **2009**, 48, 5675
Helix propagation at both N- and C-termini



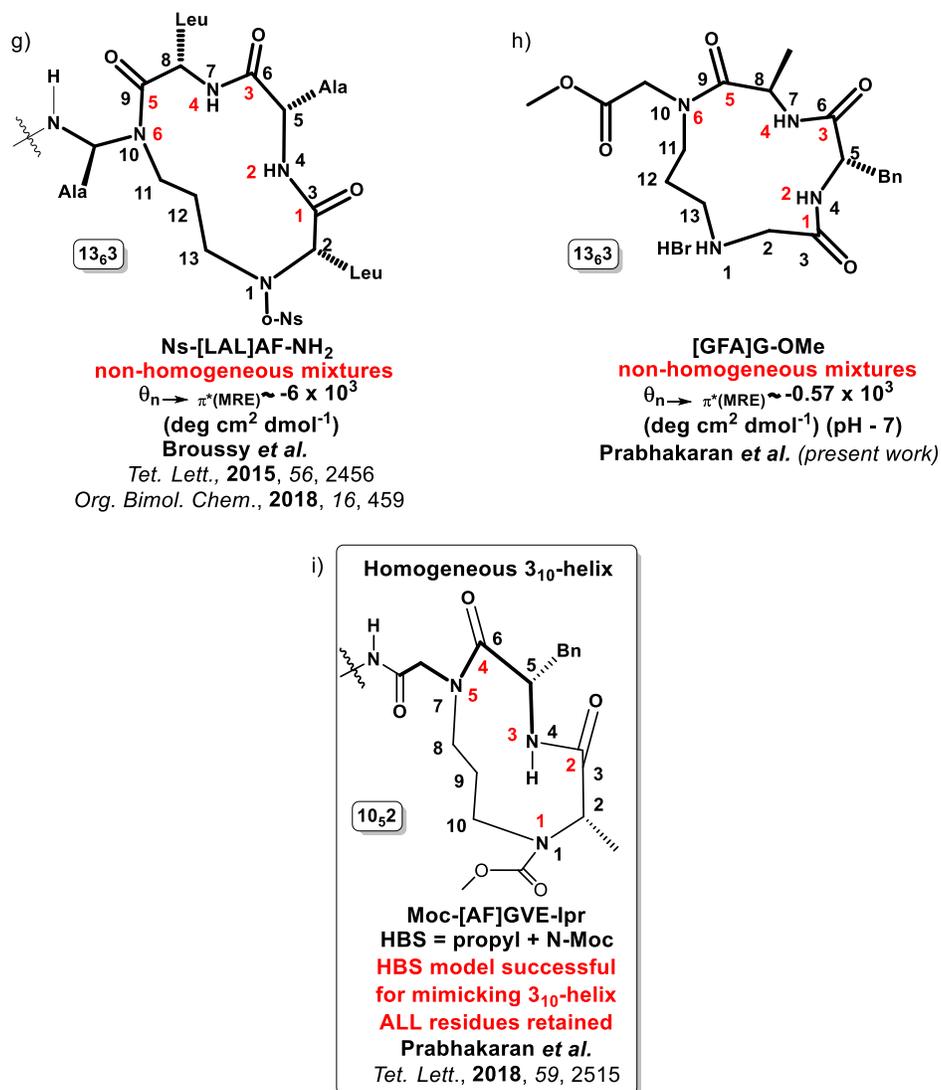
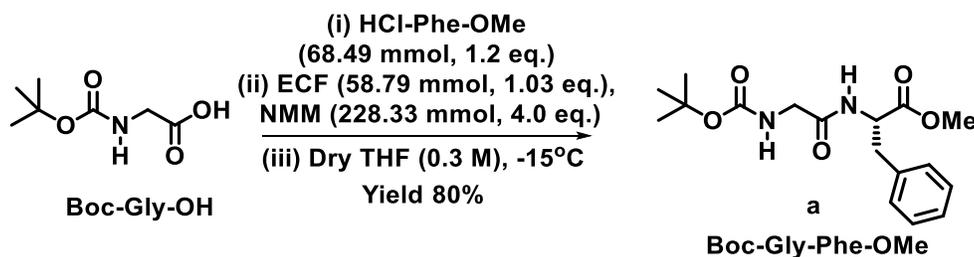


Figure S1. The nomenclature presented in current work for hydrogen bonded e) or HBS-constrained cyclic peptides – a) Satterthwait *et al.*, b) Arora *et al.*, c) Arora *et al.*, d) Alewood *et al.*, f) Prabhakaran *et al.* g) Broussy *et al.*, h) Prabhakaran *et al.*, i) Prabhakaran *et al.*. Numbers written along the outside of the backbone, in black color, indicate the total number of atoms present in the macrocyclic ring. Numbers written along the inside of the backbone in red color represent the number of sp² atoms in the macrocyclic ring. The backbone bonds of residues that are directly bonded to the HBS group and hence are in an unnatural environment are shown in bold bonds. The i+1st residues are shown in red in order to highlight the HBS models that retain this residue. The application of current HBS model in constraining short peptides in 3₁₀-helical conformation is also shown.

4. Experimental

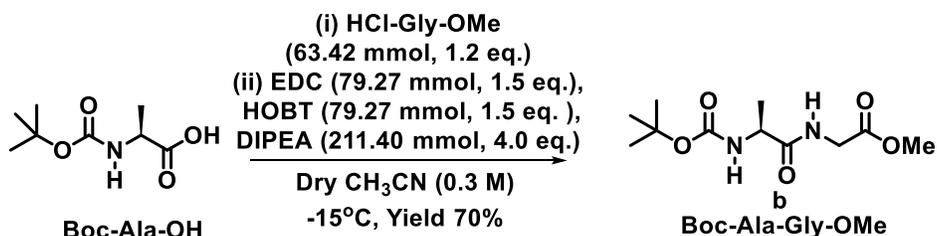
4.1. Synthesis

Scheme S1. Synthesis of Boc-Gly-Phe-OMe ((S)-methyl 2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-phenylpropanoate) (**a**):



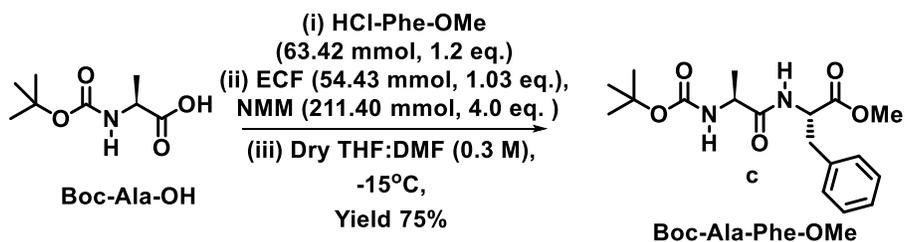
To a cold (-15°C) stirring solution of Boc-Gly-OH (10.0 g, 57.08 mmol) in dry tetrahydrofuran (THF) (190.0 ml) was added N-methyl morpholine (NMM) (9.39 ml, 85.62 mmol) and ethyl chloroformate (ECF) (5.62 ml, 58.79 mmol) and stirred until TLC indicated that all the acid had been consumed to form corresponding mixed anhydride (4 min). To this was added a solution of HCl-Phe-OMe (14.77 g, 68.49 mmol) in dry dimethylformamide (DMF) (25.0 ml) and NMM (15.66 ml, 142.70 mmol). After 2 h the ice-salt bath was removed and the mixture was stirred at room temperature until TLC indicated the complete consumption of the mixed anhydride. Removal of solvent under vacuum resulted in a residue which was dissolved in ethyl acetate (EtOAc) (60.0 ml), washed with water (2 X 25.0 ml), 1N HCl (2 X 25.0 ml) and saturated NaHCO₃ (2 X 25.0 ml). The organic layer was dried over anhydrous sodium sulphate (anhyd. Na₂SO₄) and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 1:3) to yield the desired product as a viscous liquid (15.36 g, 45.66, mmol, 80% yield); (TLC- EtOAc:Hexane (1:1) – *R_f* = 0.2); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 7.32 – 7.22 (m, 3H, H^{Aro}Phe), 7.09 (d, *J* = 6.98, 2H, H^{Aro}Phe), 6.52 (d, *J* = 7.19 Hz, 1H, H^NPhe), 5.08 (s, 1H, H^NGly), 4.89 (q, *J* = 5.76 Hz, 1H, H^αPhe), 3.84 (dd, *J* = 16.64, 4.62 Hz, 1H, H^αGly), 3.76 (d, *J* = 5.85 Hz, 1H, H^αGly), 3.72 (s, H^{Me}OMe), 3.17 – 3.08 (m, 2H, H^βPhe), 1.44 (s, 9H, H^{Me}Boc); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: 171.7, 169.1, 155.9, 135.6, 129.2, 128.7, 127.2, 80.4, 60.4, 53.1, 52.4, 44.3, 37.9, 14.2; **HRMS** *m/z* Calculated for C₁₇H₂₄N₂O₅Na 359.1583, Found 359.1584.

Scheme S2. Synthesis of Boc-Ala-Gly-OMe ((S)-methyl 2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-phenylpropanoate) (**b**):



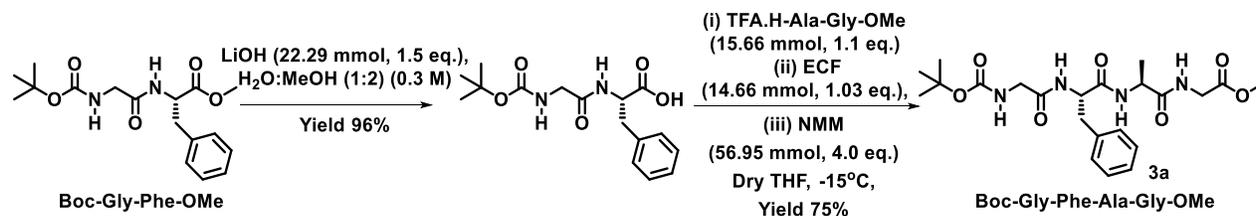
To a cold (0°C) stirring solution of Boc-Ala-OH (10.0 g, 52.85 mmol) and HCl-Gly-OMe (7.96 g, 63.42 mmol), in dry CH₃CN (ACN) (176.5 ml) was added ethylcarbodiimide hydrochloride (EDC) (15.19 g, 79.27 mmol), 1-hydroxybenzotriazole (HOBT) (1.4 g, 79.27 mmol) and N,N-diisopropylethylamine (DIPEA) (36.82 ml, 211.40 mmol). After 30 mins the ice-salt bath was removed, and the mixture was stirred at ambient conditions for further 10 hrs, when TLC indicated complete consumption of the acid. Removal of solvent resulted in a residue which was dissolved in EtOAc (70.0 ml), washed with water (2 X 25.0 ml), 1N HCl (2 X 25.0 ml) and saturated NaHCO₃ (2 X 25.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc : Hexane – 1:3) to yield the desired product as a viscous liquid (9.62 g, 36.99 mmol, 70% yield); (TLC- EtOAc:Hexane (1:1) – *R_f* = 0.2); **¹H NMR** (400 MHz, CDCl₃) δ ppm: major: 6.70 (s, 0.9 H, H^NGly), 5.02 (bs, 0.9H, H^αAla), 4.05 (t, *J* = 4.78 Hz, 2H, H^αGly), 3.76 (s, 3H, H^{Me}OMe), 1.45 (s, 9H, H^{Me}Boc), 1.38 (d, *J* = 7.08 Hz, 3H, H^βAla); minor: 6.89 (s, *J* = 0.37 Hz, H^NGly), 5.14 (bs, 0.25 H, H^αAla); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: 173.3, 170.2, 155.6, 80.3, 52.4, 50.0, 41.2, 28.3, 18.2; **FT-IR** (ν̄ cm⁻¹): 3581, 3371, 3303, 2980, 2936, 1755, 1710, 1660, 1528, 1449, 1368, 1298, 1252, 1214, 1168, 1069, 1027; **HRMS** *m/z* Calculated for C₁₁H₂₀N₂O₅Na 283.1271, Found 283.1271.

Scheme S3. Synthesis of Boc-Ala-Phe-OMe ((S)-methyl 2-((S)-2-((tert-butoxycarbonyl)amino)propanamido)-3-phenylpropanoate) (**c**):



To a cold (-15°C) stirring solution of Boc-Ala-OH (10.0 g, 52.85 mmol) in dry THF (176.0 ml) was added NMM (8.70 ml, 79.27 mmol) and ECF (5.20 ml, 54.43 mmol) and stirred until TLC indicated that all the acid had been consumed to form corresponding mixed anhydride (4 min). To this was added a solution of HCl-Phe-OMe (13.67 g, 63.42 mmol) in dry dimethylformamide (DMF) (10.0 ml) and NMM (14.50 ml, 132.12 mmol). After 2 h the ice-salt bath was removed and the mixture was stirred at room temperature until TLC indicated the complete consumption of the mixed anhydride. Removal of solvent under vacuum resulted in a residue which was dissolved in EtOAc (65.0 ml), washed with water (2 X 25.0 ml), 1N HCl (2 X 25.0 ml) and saturated NaHCO₃ (2 X 25.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc : Hexane – 1:4) to yield the desired product as a white solid (13.88 g, 39.63 mmol, 75% yield); (TLC-EtOAc:Hexane (1:1) – *R_f* = 0.6); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 7.30 – 7.22 (m, 3H), 7.10 (d, *J* = 6.9 Hz, 2H), 6.51 (d, *J* = 7.3 Hz, 1H), 4.92 (bs, 1H), 4.85 (q, *J* = 6.0, 13.3 Hz, 1H), 4.13 (s, 1H), 3.71 (s, 3H), 3.17 (dd, *J* = 13.86, 5.8 Hz, 1H), 3.08 (dd, *J* = 13.86, 5.8 Hz, 1H), 1.44 (s, 9H), 1.31 (d, *J* = 6.9 Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: 172.2, 171.7, 155.3, 135.7, 129.3, 128.6, 127.2, 80.2, 53.2, 52.3, 50.1, 37.9, 28.3, 18.3; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3407, 3338, 3306, 3066, 3030, 2979, 2933, 1727, 1662, 1632, 1531, 1500, 1450, 1398, 1368, 1332, 1286, 1250, 1164, 1112, 1051, 1027; **HRMS** *m/z* Calculated for C₁₈H₂₆N₂O₅Na 373.1739, Found 373.1739.

Scheme S4. Synthesis of Boc-Gly-Phe-Ala-Gly-OMe ((9*S*,12*S*)-methyl 9-benzyl-2,2,12-trimethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate) (**3a**):

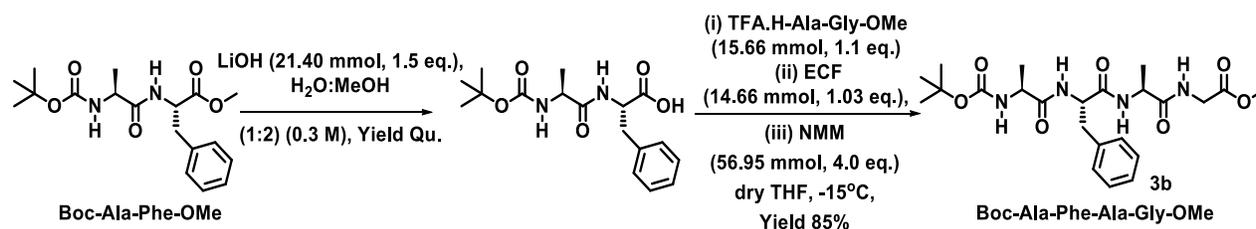


To a solution of Boc-Gly-Phe-OMe (5.0 g, 14.86 mmol) in methanol (33.04ml) was added a solution of LiOH (0.92 g, 22.29 mmol) in H₂O (16.50 ml) and stirred for 30 mins, at which point TLC indicated the complete consumption of the methyl ester. Methanol was evaporated and the resulting aqueous solution was acidified with 1N HCl (20.0 ml) to pH

1, followed by the extraction with dichloromethane (DCM) (2 X 25.0 ml) to get Boc-Gly-Phe-OH (4.59 g, 14.26 mmol, 96% yield) which was used without further purification.

To a cold (-15°C) stirring solution of Boc-Gly-Phe-OH (4.59 g, 14.23 mmol) in dry THF (47.0 ml) was added NMM (2.34 ml, 21.35 mmol) and ECF (1.40 ml, 14.66 mmol) and stirred until TLC indicated that all the acid had been consumed to form corresponding mixed anhydride (4 min). To this was added a solution of TFA.H-Ala-Gly-OMe (4.29 g, 15.66 mmol) in dry DMF (12.0 ml) and NMM (3.90 ml, 35.59 mmol). After 2 h the ice-salt bath was removed, and the mixture was stirred at room temperature until TLC indicated the complete consumption of the mixed anhydride. Removal of solvent resulted in a residue which was dissolved in EtOAc (70.0 ml), washed with water (2 X 20.0 ml), 1N HCl (2 X 20.0 ml) and saturated NaHCO₃ (2 X 20.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Methanol – 9:1) to yield the desired product as a white solid (4.96 g, 10.67 mmol, 75% yield); (TLC- EtOAc:Hexane (1:0) – *R_f* = 0.1); **¹H NMR** (400 MHz, DMSO-d₆, 10 mM) δ ppm: 8.28 (d, *J* = 7.25 Hz, 1H), 8.21 (t, *J* = 5.25 Hz, 1H), 7.93 (d, *J* = 8.25 Hz, 1H), 7.26 – 7.17 (m, 5H), 6.91 (t, *J* = 5.91 Hz, 1H), 4.54 (td, *J* = 9.17, 3.27 Hz, 1H), 4.32 (quin, *J* = 7.25 Hz, 1H), 3.90 – 3.78 (m, 2H), 3.63 (s, 3H), 3.54 (dd, *J* = 16.99, 5.91 Hz, 1H), 3.40 (dd, *J* = 16.62, 5.66 Hz, 1H), 3.00 (dd, *J* = 13.83, 4.10 Hz, 1H), 2.76 (dd, *J* = 13.83, 9.44 Hz, 1H), 1.36 (s, 9H), 1.24 (d, *J* = 7.04 Hz, 3H); **¹³C NMR** (100 MHz, DMSO-d₆, 60 mM) δ ppm: 172.6, 170.6, 170.1, 169.1, 155.7, 137.7, 129.2, 128.0, 126.2, 78.0, 53.5, 51.7, 47.9, 43.0, 40.5, 37.6, 28.1, 18.2; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3418, 3279, 3248, 3089, 3061, 2973, 2924, 2364, 2330, 1751, 1719, 1662, 1510, 1450, 1400, 1366, 1297, 1248, 1209, 1163, 1029; **HRMS** *m/z* Calculated for C₂₂H₃₂N₄O₇Na 487.2169, Found 487.2168.

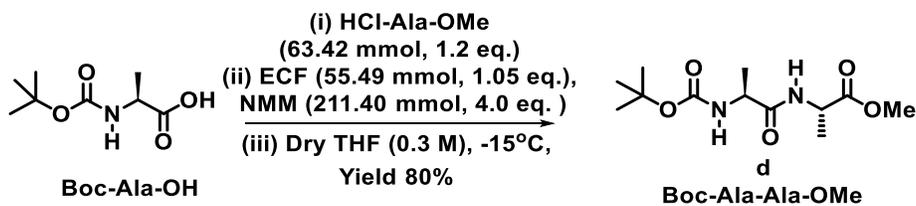
Scheme S5. Synthesis of Boc-Ala-Phe-Ala-Gly-OMe ((6*S*,9*S*,12*S*)-methyl 9-benzyl-2,2,6,12-tetramethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate) (**3b**):



To a solution of Boc-Ala-Phe-OMe (5.0 g, 14.26 mmol) in methanol (32.0 ml) was added a solution of LiOH (0.88 g, 21.40 mmol) in H₂O (16.0 ml) and stirred for 30 mins. Most of the methanol was evaporated and the resulting aqueous solution was acidified with 1N HCl (20 ml) (pH 1) followed by the extraction with dichloromethane (DCM) (2 X 25.0 ml) to get Boc-Ala-Phe-OH (4.79 g, 14.26 mmol, quantitative yield) which was used without further purification.

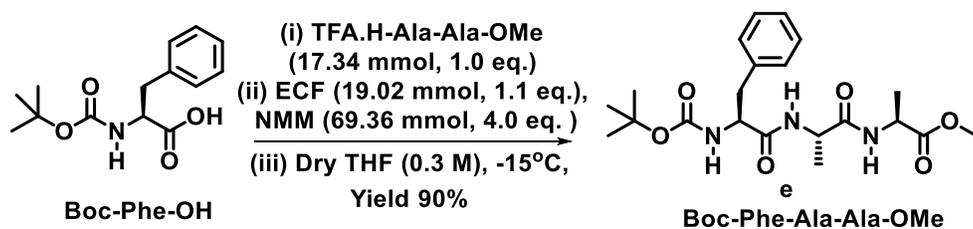
To a cold (-15°C) stirring solution of Boc-Ala-Phe-OH (4.79g, 14.23 mmol) in dry THF (47.0 ml) was added NMM (2.34 ml, 21.35 mmol) and ECF (1.40 ml, 14.66 mmol) and stirred until TLC indicated that all the acid had been consumed to form corresponding mixed anhydride (4 min). To this was added a solution of TFA.H-Ala-Gly-OMe (4.29 g, 15.66 mmol) in dry DMF (12.0 ml) and NMM (3.90 ml, 35.59 mmol). After 2 h the ice-salt bath was removed and the mixture was stirred at room temperature until TLC indicated the complete consumption of the mixed anhydride. Removal of solvent resulted in a residue which was dissolved in EtOAc (60.0 ml), washed with water (2 X 25.0 ml), 1N HCl (2 X 25.0 ml) and saturated NaHCO₃ (2 X 25.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc : Methanol – 4:1) to yield the desired product as a white solid (5.79 g, 12.09 mmol, 85% yield); (TLC- EtOAc:Hexane (1:0) – *R_f* = 0.1); **¹H NMR** (400 MHz, DMSO-d₆, 10 mM) δ ppm: 8.35 (d, J = 5.19 Hz, 1H), 7.86 (d, J = 7.99 Hz, 1H), 7.25 – 7.13 (m, 5H), 6.93 (d, J = 7.59 Hz, 1H), 4.51 (td, J = 9.06, 3.76 Hz, 1H), 4.30 (quin, J = 7.25 Hz, 1H), 3.90 – 3.78 (m, 3H), 3.62 (s, 3H), 3.02 (dd, J = 13.98, 4.37 Hz, 1H), 2.79 (dd, J = 13.57, 9.15 Hz, 1H), 1.36 (s, 9H), 1.22 (d, J = 7.03 Hz, 3H), 1.07 (d, J = 7.18 Hz, 3H); **¹³C NMR** (100 MHz, DMSO-d₆, 60 mM) δ ppm: 172.6, 172.4, 170.4, 170.1, 154.9, 137.6, 129.3, 127.9, 126.1, 78.1, 53.4, 51.6, 48.0, 40.5, 37.4, 28.1, 24.5, 18.2, 18.1; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3625, 3584, 3506, 3452, 3420, 3313, 3263, 3084, 2979, 2931, 2365, 1755, 1674, 1636, 1556, 1529, 1449, 1400, 1365, 1269, 1216, 1170, 1110, 1073, 1051, 1020; **HRMS** *m/z* Calculated for C₂₃H₃₄N₄O₇Na 501.2325, Found 501.2321.

Scheme S6. Synthesis of Boc-Ala-Ala-OMe ((S)-methyl 2-((S)-2-((tert butoxy carbonyl) amino) propanamido) propanoate) (**d**):



To a cold (-15°C) stirring solution of Boc-Ala-OH (10.0 g, 52.85 mmol) in dry THF (176.0 ml) was added NMM (8.70 ml, 79.27 mmol) and ECF (5.30 ml, 55.49 mmol) and stirred until TLC indicated that all the acid had been consumed to form corresponding mixed anhydride (4 min). To this was added a solution of HCl-Ala-OMe (8.85 g, 63.42 mmol) in dry DMF (15.0 ml) and NMM (14.50 ml, 132.12 mmol). After 2 h the ice-salt bath was removed and the mixture was stirred at room temperature until TLC indicated the complete consumption of the mixed anhydride. Removal of solvent resulted in a residue which was dissolved in ethyl acetate (EtOAc) (60.0 ml), washed with water (2 X 25.0 ml), 1N HCl (2 X 25.0 ml) and saturated NaHCO₃ (2 X 25.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc : Hexane – 1:2) to yield the desired product as a white solid (11.59 g, 42.25 mmol, 80% yield); (TLC- EtOAc:Hexane (1:1) – *R_f* = 0.5); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 6.62 (s, 1H), 4.99 (s, 1H), 4.57 (quin, J = 7.28 Hz, 1H), 4.17 (bs, 1H), 3.75 (s, 3H), 1.45 (s, 9H), 1.41 (d, J = 6.9 Hz, 3H), 1.36 (d, J = 6.69 Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: 173.2, 172.2, 155.4, 80.2, 52.5, 50.1, 48.0, 28.3, 18.4, 18.3; **FT-IR** (ν̄ cm⁻¹): 3583, 3305, 3080, 2981, 2936, 2878, 2365, 1749, 1711, 1662, 1530, 1502, 1452, 1393, 1367, 1317, 1249, 1213, 1166, 1105, 1061, 1020; **HRMS** *m/z* Calculated for C₁₂H₂₂N₂O₅Na 274.1529, Found 274.1531.

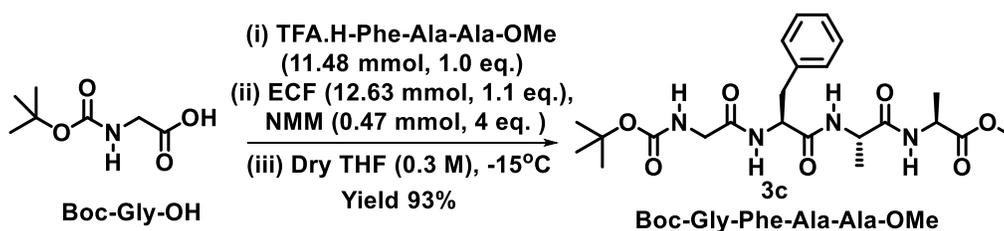
Scheme S7. Synthesis of Boc-Phe-Ala-Ala-OMe ((6*S*,9*S*)-methyl 6-benzyl-2,2,9,12-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oate) (**e**):



To a cold (-15°C) stirring solution of Boc-Phe-OH (5.52 g, 20.81 mmol) in dry THF (58.0 ml) was added NMM (2.85 ml, 26.02 mmol) and ECF (1.82 ml, 19.02 mmol) and stirred

until TLC indicated that all the acid had been consumed to form corresponding mixed anhydride (4 min). To this was added a solution of TFA.H-Ala-Ala-OMe (5.0 g, 17.34 mmol) (TFA is trifluoroacetic acid) in dry THF (15.0 ml) and NMM (4.75 ml, 43.36 mmol). After 2 h the ice-salt bath was removed and the mixture was stirred at room temperature until TLC indicated the complete consumption of the mixed anhydride. Removal of solvent resulted in a residue which was dissolved in EtOAc (60.0 ml), washed with water (2 X 25.0 ml), 1N HCl (2 X 25.0 ml) and saturated NaHCO₃ (2 X 25 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 1:1) to yield the desired product as a white solid (6.58 g, 15.61 mmol, 90% yield); (TLC- EtOAc:Hexane (3:1) – *R_f* = 0.5); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 7.34 – 7.16 (m, 5H), 6.64 (d, *J* = 6.24 Hz, 1H), 6.52 (d, *J* = 7.40 Hz, 1H), 4.95 (bs, 1H), 4.51 (quin, *J* = 7.35 Hz, 1H), 4.44 (quin, *J* = 7.06 Hz, 1H), 4.36 (d, *J* = 4.47 Hz, 1H), 3.75 (s, 3H), 3.13 – 3.01 (m, 2H), 1.40 (s, 9H), 1.38 (3H, merged), 1.32 (d, *J* = 6.99 Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: 173.1, 171.4, 171.2, 155.5, 136.4, 129.3, 128.7, 127.1, 80.4, 55.7, 52.5, 48.9, 48.2, 38.2, 28.3, 18.2, 18.1; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3300, 3277, 3066, 2980, 2932, 2365, 1748, 1694, 1646, 1549, 1530, 1498, 1451, 1392, 1367, 1249, 1214, 1167, 1052, 1019; **HRMS** *m/z* Calculated for C₂₁H₃₁N₃O₆Na 471.2213, Found 471.2210.

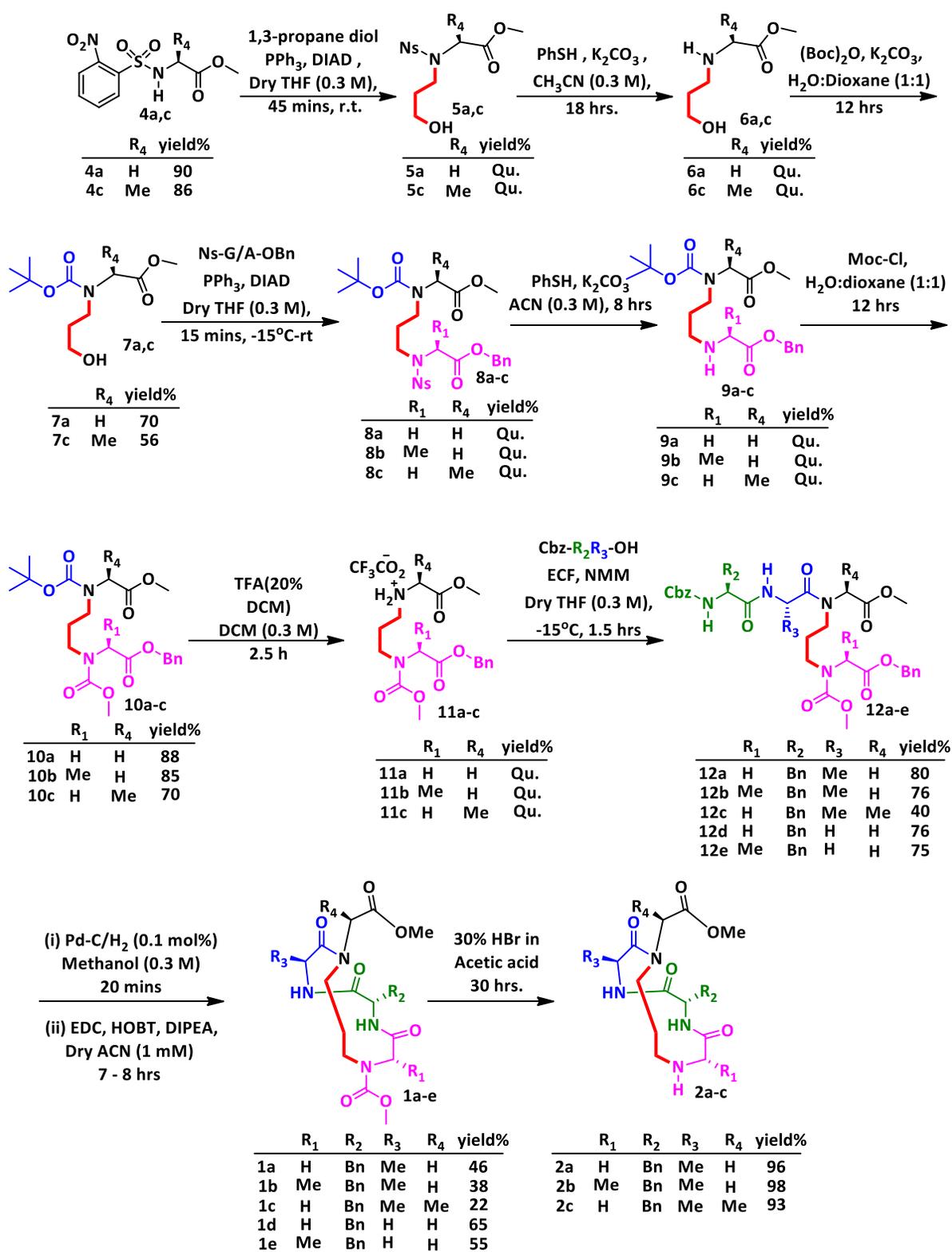
Scheme S8. Synthesis of Boc-Gly-Phe-Ala-Ala-OMe ((9*S*,12*S*,15*S*)-methyl 9-benzyl-2,2,12,15-tetramethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oate) (**3c**):



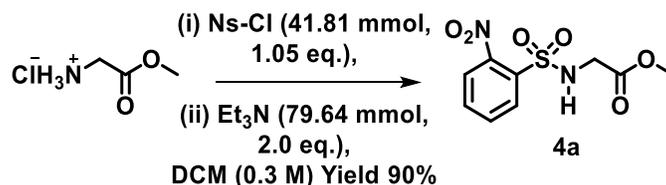
To a cold (-15°C) stirring solution of Boc-Gly-OH (2.1 g, 13.78 mmol) in dry THF (30.0 ml) was added NMM (1.89 ml, 17.22 mmol) and ECF (1.20 ml, 12.63 mmol) and stirred until TLC indicated that all the acid had been consumed to form corresponding mixed anhydride (4 min). To this was added a solution of TFA.H-Phe-Ala-Ala-OMe (5.0 g, 11.48 mmol) in dry THF (8.0 ml) and NMM (3.15 ml, 28.70 mmol). After 2 h the ice-salt bath was removed and the mixture was stirred at room temperature until TLC indicated the

complete consumption of the mixed anhydride. Removal of solvent resulted in a residue which was dissolved in EtOAc (70.0 ml), washed with water (2 X 20.0 ml), 1N HCl (2 X 20.0 ml) and saturated NaHCO₃ (2 X 20.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 3:1) to yield the desired product as a white solid (5.11 g, 10.67 mmol, 93% yield); (TLC- EtOAc:Hexane (3:1) – R_f = 0.4); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 7.33 – 7.21 (m, 2H), 7.2 – 7.14 (d, J = 7.12 Hz, 3H), 6.92 (d, J = 5.78 Hz, 1H), 6.82 (d, J = 7.16 Hz, 1H), 5.21 (s, 1H), 4.70 (q, J = 6.53 Hz, 1H), 4.51 (quin, J = 7.40 Hz, 1H), 3.77 – 3.72 (m, 5H), 3.16 (dd, J = 14.19, 6.54 Hz, 1H), 3.05 (dd, J = 13.82, 6.29 Hz, 1H), 1.42 – 1.38 (m, 12H), 1.32 (d, J = 7.12 Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: 173.2, 171.8, 170.5, 169.4, 156.2, 136.2, 129.3, 128.6, 127.1, 80.2, 54.2, 52.4, 48.8, 48.1, 42.2, 38.8, 28.3, 18.7, 18.0; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3294, 3078, 2979, 2930, 2365, 1748, 1639, 1545, 1500, 1451, 1394, 1367, 1283, 1247, 1215, 1166, 1053; **HRMS** m/z Calculated for C₂₃H₃₄N₄O₇Na 501.2325, Found 501.2326.

Scheme S9. Synthesis of Hydrogen Bond Surrogate (HBS) constrained α -turns (**1a-e**):

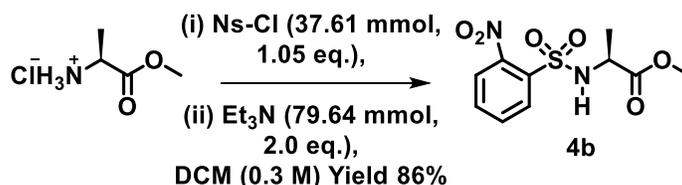


Scheme S10. Synthesis of Ns-Gly-OMe (methyl 2-(2-nitrophenylsulfonamido)acetate) (**4a**):



To a cold (0°C) stirring mixture of methylglycinate hydrochloride (5.0 g, 39.82 mmol) and o-nosyl chloride (NsCl) (9.26 g, 41.81 mmol) in DCM (133.0 ml) was added triethylamine (TEA) (11.12 ml, 79.64 mmol) drop-wise and the mixture was stirred at room temperature until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (70.0 ml), washed with water (2 x 20.0 ml) and 1N HCl solution (2 X 20.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane -2:3) to yield the desired product (Ns-Gly-OMe, **4a**) as a white solid (9.82 g, 35.84 mmol, 90% yield); m.p. 110°C; (TLC- EtOAc:Hexane (1:1) – *R_f* = 0.5); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 8.11-8.08 (m, 1H, H^{ArO}Ns), 7.95-7.93 (m, 1H, H^{ArO}Ns), 7.76-7.73 (m, 2H, H^{ArO}Ns), 6.03 (s, 1H, H^NGly), 4.02 (d, J = 5.86 Hz, 2H, H^αGly), 3.61 (s, 3H, H^{Me}OMe); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: 169.2, 148.0, 134.2, 133.9, 133.1, 130.8, 125.8, 52.7, 45.0; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3324, 3099, 1754, 1639, 1593, 1537, 1465, 1417, 1358, 1335, 1257, 1239, 1160, 1126, 1056, 1008; **HRMS** *m/z* Calculated for C₉H₁₀N₂O₆SNa 297.0157, Found 297.0161.

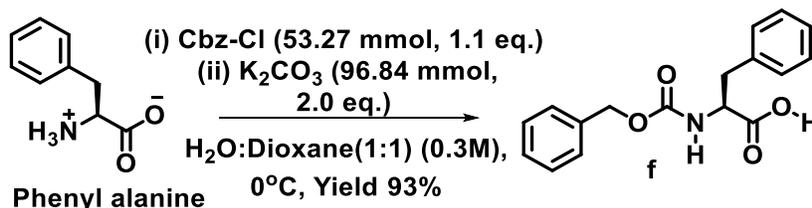
Scheme S11. Synthesis of Ns-Ala-OMe ((S)-methyl 2-(2-nitrophenylsulfonamido)propanoate) (**4b**):



To a cold (0°C) stirring mixture of methyl alaninate hydrochloride (5.0 g, 35.82 mmol) and NsCl (8.33 g, 37.61 mmol) in DCM (120.0 ml) was added TEA (10.00 ml, 71.64 mmol) dropwise and the mixture was stirred at room temperature until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue

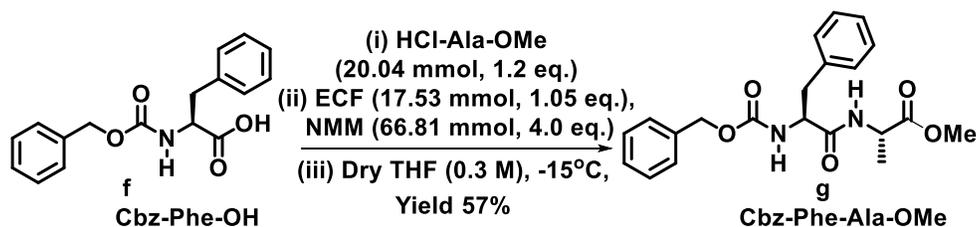
which was dissolved in EtOAc (70.0 ml), washed with water (2 x 25.0 ml) and 1N HCl solution (2 X 25.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane -2:3) to yield the desired product (Ns-Ala-OMe, **4b**) as a white solid (8.88 g, 30.80 mmol, 86% yield); m.p. 110°C; (TLC- EtOAc:Hexane (1:1) – *R_f* = 0.6); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 8.10-8.08 (m, 1H, H^{Aro}Ns), 7.94-7.92 (m, 1H, H^{Aro}Ns), 7.75-7.72 (m, 2H, H^{Aro}Ns), 6.10 (d, *J* = 8.25, 1H, H^NAla), 4.26 (d, *J* = 7.59 Hz, 2H, H^αAla), 3.53 (s, 3H, H^{Me}OMe); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: 171.9, 147.7, 134.3, 133.6, 132.9, 130.5, 125.6, 52.5, 52.47, 19.7; **FT-IR** (ν̄ cm⁻¹): 3461, 3302, 3099, 3015, 2995, 2956, 2887, 1738, 1594, 1543, 1443, 1419, 1382, 1357, 1333, 1299, 1217, 1171, 1055; HRMS *m/z* Calculated for C₁₀H₁₂N₂O₆SNa 311.0314, Found 311.0313.

Scheme S12. Synthesis of Cbz-Phe-OH (2-(((benzyloxy)carbonyl)amino)-3-phenylpropionic acid) (**f**):



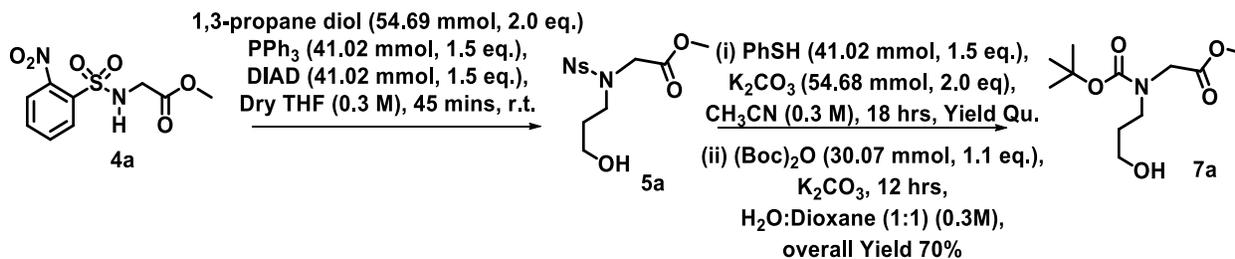
To a cold (0°C) stirring mixture of phenylalanine (8.0g, 48.42 mmol) and K₂CO₃ (13.36 g, 96.84 mmol) in water (80.0 ml) was added drop wise as solution of benzyl chloroformate (7.60 ml, 53.27 mmol) in dioxane (80.0 ml). The mixture was stirred at 0°C for 1 h and warmed to stir at ambient conditions until TLC indicated the complete consumption of starting material (12 h). The dioxane was removed under vacuum to get an aqueous solution which was extracted with EtOAc (2 X 30.0 ml) and the organic layer was acidified with 1N HCl solution and extracted with EtOAc (3 X 20.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and the solvent was removed under vacuum to get a hygroscopic white solid (13.47 g, 93 %), which was used for further reaction without any purification.

Scheme S13. Synthesis of Cbz-Phe-Ala-OMe ((S)-methyl 2-((S)-2-(((benzyloxy) carbonyl)amino)-3-phenylpropanamido)propanoate) (**g**):



To a cold (-15°C) stirring solution of Cbz-Phe-OH (**f**) (5.0 g, 16.70 mmol) in dry THF (40 ml) was added NMM (2.74 ml, 25.05 mmol) and ECF (1.67 ml, 17.53 mol) and stirred until TLC indicated that all the acid had been consumed to form corresponding mixed anhydride (4 min). To this was added a solution of HCl-Ala-OMe (2.79 g, 20.04 mmol) in dry DMF (15.0 ml) and NMM (4.58 ml, 41.75 mmol). After 2 h the ice-salt bath was removed and the mixture was stirred at ambient conditions for further 5 h. Removal of solvent resulted in a residue which was dissolved in ethyl acetate (EtOAc) (60.0 ml), washed with water (2 X 25.0 ml), 1N HCl (2 X 25.0 ml) and saturated NaHCO₃ (2 X 25.0ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 2:3) to yield the desired product as a white solid (3.66 g, 9.52 mmol, 57% yield); m.p. – 134°C; (TLC- EtOAc:Hexane (1:1) – *R_f* = 0.6); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 7.39 – 7.15 (m, 10H, H^{Aro}Cbz & H^{Aro}Phe), 6.28 (d, *J* = 5.85 Hz, 1H, H^NAla), 5.29 (d, *J* = 6.67 Hz, 1H, H^NPhe), 4.50 (quin, *J* = 7.16 Hz, 1H, H^αAla), 4.42 (q, *J* = 7.57 Hz, 1H, H^αPhe), 3.71 (s, 3H, H^{Me}OMe), 3.13 (dd, *J* = 13.76 Hz, 6.43 Hz, 1H, H^βPhe), 3.04 (dd, *J* = 13.83 Hz, 6.92 Hz, 1H, H^βPhe), 1.33 (d, *J* = 7.06 Hz, 3H, H^βAla); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: 172.9, 170.4, 156.0, 136.3, 129.5, 128.9, 128.7, 128.4, 128.2, 127.3, 67.3, 56.3, 52.7, 48.4, 38.7, 18.5; **FT-IR** ($\tilde{\nu}$ cm⁻¹): - 3778, 3778, 3582, 3298, 3065, 3031, 2950, 2364, 2331, 1746, 1706, 1659, 1544, 1497, 1451, 1382, 1264, 1217, 1148, 1051; **HRMS** *m/z* Calculated for C₂₁H₂₄N₂O₅Na 407.1583, Found 407.1582.

Scheme S14. Synthesis of methyl 2-((tert-butyloxycarbonyl)(3-hydroxypropyl)amino)acetate (**7a**):

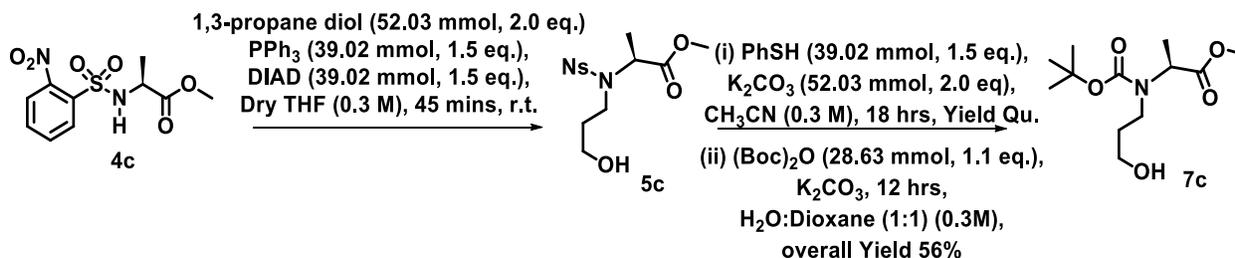


To a cold (0°C) stirring mixture of Ns-Gly-OMe (**4a**) (7.5 g, 27.34 mmol) and triphenylphosphine (PPh₃) (10.74 g, 41.02 mmol) in dry THF (91.0 ml) under N₂ atmosphere was added 1,3-propane diol (3.95 ml, 54.69 mmol) drop-wise followed by diisopropyl azodicarboxylate (DIAD) (8.12 ml, 41.02 mmol). After 15 mins of stirring the ice bath was removed and the reaction was continued at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue which was dissolved in (EtOAc (50.0 ml) and washed with water (2 X 25.0 ml). The organic layer was concentrated to get a residue which was immediately subjected to denosylation reaction conditions by the addition of thiophenol (4.2 ml, 41.02 mmol) and K₂CO₃ (7.54 g, 54.68 mmol) in CH₃CN (91.0 ml). The reaction was continued at ambient conditions until TLC indicated the complete consumption of the starting material. After that the solvent was removed and the resulting residue was acidified using 1N HCl (50.0 ml). The aqueous mixture was thoroughly washed with diethyl ether (3 x 20.0 ml) to remove all the water-insoluble organic impurities.

The resulting aqueous solution was then treated with K₂CO₃ until it reaches a pH of 10. and di-tert-butylidicarbonate (6.90 ml, 30.07 mmol) was added and the reaction mixture was stirred at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of the solvent resulted in a residue which was dissolved in EtOAc (70.0 ml) and the organic layer was washed with water (3 x 20 ml) and dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane -1:10) to yield the desired product as a viscous oil (4.73g, 19.12 mmol, 70% yield); (TLC- EtOAc:Hexane (1:0) – R_f = 0.5); ¹H NMR (400 MHz, CDCl₃, 10 mM) δ ppm: (mixture of two rotamers) 3.86 (s) & 3.96 (s) (2H, H^αGly), 3.74 (s, 3H, H^{Me}OMe), 3.65, (t, J = 5.3 Hz), & 3.71-3.68 (m), (2H, HPrp^β);

3.45 (t, $J = 6.0$ Hz) & 3.41-3.38 (m), (2H, HPrp^a); 1.66-1.64 (m) & 1.77-1.74 (m) (2H, HPrp^b); 1.48 (s) & 1.42 (s), (9H, H^{Me}Boc); ¹³C NMR (100 MHz, CDCl₃, 60 mM) δ ppm: (mixture of two rotamers) 170.8, 170.6, 156.7, 155.9, 81.2, 80.9, 60.0, 58.6, 52.2, 49.8, 49.2, 45.5, 44.8, 31.4, 30.8, 28.5, 28.3; FT-IR ($\tilde{\nu}$ cm⁻¹): 3413, 2974, 2956, 2875, 1753, 1682, 1479, 1437, 1406, 1368, 1313, 1250, 1210, 1061; HRMS m/z Calcd for C₁₁H₂₁NO₅Na 270.1317, Found 270.1320.

Scheme S15. Synthesis of methyl 2-((tert-butyloxycarbonyl)(3-hydroxypropyl) amino) acetate (**7c**):

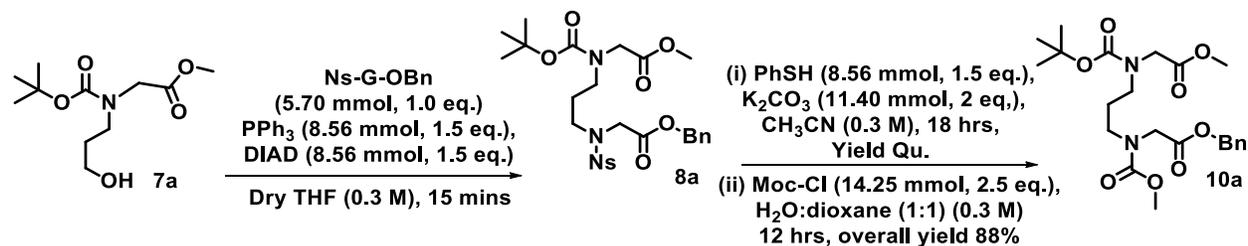


To a cold (0°C) stirring mixture of Ns-Ala-OMe (**4c**) (7.5 g, 26.03 mmol) and PPh₃ (10.22 g, 39.02 mmol) in dry THF (87.0 ml) under N₂ atmosphere was added 1,3-propane diol (3.76 ml, 52.03 mmol) drop-wise followed by DIAD (7.72 ml, 39.02 mmol). After 15 mins of stirring the ice bath was removed and the reaction was continued at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (40.0 ml) and washed with water (2 X 20.0 ml). The organic layer was concentrated to get a residue which was immediately subjected to denosylation reaction conditions by the addition of thiophenol (4.00 ml, 39.02 mmol) and K₂CO₃ (7.18 g, 52.03 mmol) in CH₃CN (87.0 ml). The reaction was continued at ambient conditions until TLC indicated the complete consumption of the starting material. After that the solvent was removed and the resulting residue was acidified using 1N HCl (50.0 ml). The aqueous mixture was thoroughly washed with diethyl ether (3 x 20.0 ml) to remove all the water-insoluble organic impurities.

The resulting aqueous solution was then treated with K₂CO₃ until it reaches a pH of 10, and di-tert-butylidicarbonate (6.11 ml, 26.63 mmol) was added and the reaction mixture was stirred at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of the solvent resulted in a residue which was dissolved in

EtOAc (70.0 ml) and washed with water (3 x 20 ml) and the organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane -1:4) to yield the desired product as a viscous oil (3.80g, 14.56 mmol, 56% yield); (TLC- EtOAc:Hexane (1:0) – *R_f* = 0.4); ¹H NMR (400 MHz, CDCl₃, 10 mM) δ ppm: (mixture of two rotamers) 4.45 (m) & 3.93 (q, *J* = 7.09) (2H, H^αAla), 3.72 (s, 3H, H^{Me}OMe), 3.70 – 3.59, (m), & 3.32-3.20 (m), (4H, HPrp^c & HPrp^a), 3.45 (t, *J* = 6.0 Hz), 1.75-1.66 (m) (2H, HPrp^b), 1.50 – 1.44 (m, H^βAla), 1.43 (s), (9H, H^{Me}Boc); ¹³C NMR (100 MHz, CDCl₃, 60 mM) δ ppm: (only one rotamer found) 172.4, 156.1, 81.1, 58.3, 56.1, 52.0, 43.2, 31.4, 28.2, 15.7; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3451, 2978, 2949, 2882, 1747, 1689, 1478, 1456, 1433, 1368, 1297, 1255, 1222, 1164, 1103, 1056, 1013; **HRMS** *m/z* Calcd for C₁₂H₂₃NO₅Na 284.1474, Found 284.1471.

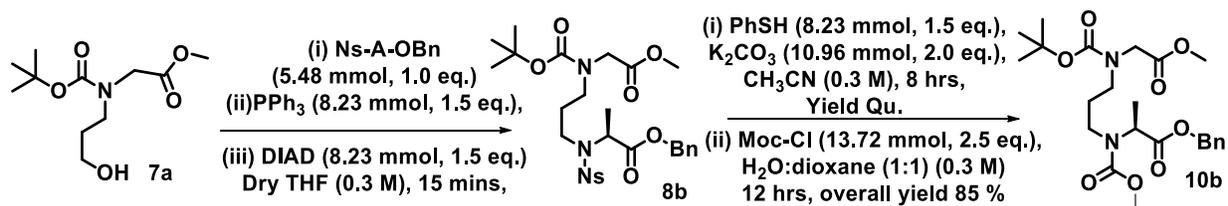
Scheme S16. Synthesis of (S)-benzyl 2-((3-((tert-butyloxycarbonyl)(2-methoxy-2-oxoethyl) amino) propyl) (methoxycarbonyl)amino)propanoate (**10a**):



To a cold (0°C) stirring mixture of Ns-Gly-OBn (2.0 g, 5.70 mmol) and PPh₃ (2.24 g, 8.56 mmol) in dry THF (19.0 ml) under N₂ atmosphere was added N-Boc-(3-hydroxypropyl) benzylglycinate (**7a**) (2.11 gm, 8.56 mmol) dissolved in THF followed by DIAD (1.69 ml, 8.56 mmol). After 15 minutes the ice bath was removed, and the mixture was continued to stir at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (30.0 ml) and washed with water (2 X 15.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to denosylation reaction by the addition of thiophenol (0.87 ml, 8.56 mmol) and K₂CO₃ (1.57g, 11.40 mmol) in CH₃CN (19.0 ml). The mixture was continued to stir at ambient conditions until TLC indicated the complete consumption of the starting material. The solvent was removed followed by acidification using 1N HCl (20.0 ml). The aqueous mixture was washed with diethyl ether to remove all the water-insoluble organic impurities.

The aqueous solution was then treated with K_2CO_3 until the pH became 10.0. This was followed by the addition of methylchloroformate (MCF) (1.10 ml, 14.25 mmol) to the reaction mixture. The mixture was continued to stir at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (30.0 ml), washed with water (2 X 10.0 ml) and the organic layer was dried over anhyd. Na_2SO_4 and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane -1:5) to yield the desired product as a viscous oil (2.26g, 4.99 mmol, 88% yield); (TLC- EtOAc:Hexane (1:1) – R_f = 0.4); 1H NMR (400 MHz, $CDCl_3$, 10 mM) δ ppm: (mixture of four rotamers) 7.40-7.30 (m, 5H, H^{AroBn}), 5.16 (s, 2H, H^{CH_2Bn}), 4.055 (d, J = 4.08 Hz, 1H, $H^\alpha Gly$), 3.99 (d, J = 11.65 Hz, 1H, $H^\alpha Gly$), 3.93 (s, 1H, $H^\alpha Gly$), 3.84 (d, J = 13.15, 1H, $H^\alpha Gly$), 3.75 – 3.70 (m), 3.64 – 3.59 (m) (H^{MeOMe} & H^{MeMoc}), 3.36 – 3.25 (m, 4H, $HPrp^c$ & $HPrp^a$), 1.77 – 1.71 (m, 2H, $HPrp^b$), 1.45 (s) & 1.40 (s) (9H, H^{MeBoc}); ^{13}C NMR (100 MHz, $CDCl_3$, 60 mM) δ ppm: (mixture of four conformers) 170.8, 170.7, 170.6, 169.9, 169.7, 157.1, 156.6, 155.7, 155.2, 135.5, 135.4, 128.6, 128.5, 80.52, 80.46, 80.37, 80.26, 66.94, 66.86, 66.81, 53.0, 52.8, 52.0, 49.4, 49.3, 49.2, 49.0, 48.9, 46.8, 46.7, 46.4, 46.3, 45.9, 28.34, 28.20, 27.5, 27.2, 27.1, 26.7, ; FT-IR ($\tilde{\nu}$ cm^{-1}): 3583, 2975, 2954, 1752, 1703, 1476, 1403, 1367, 1245, 1209, 1175, 1143, 1070, 1012; HRMS m/z Calculated for $C_{23}H_{34}N_2O_8K$ 491.1796, Found 491.1796.

Scheme S17. Synthesis of (S)-benzyl 2-((3-((tert-butyloxycarbonyl)(2-methoxy-2-oxoethyl) amino) propyl) (methoxycarbonyl)amino)propanoate (**10b**):

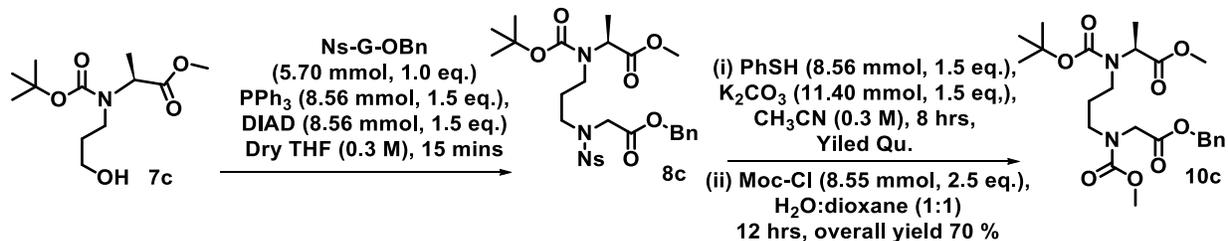


To a cold ($0^\circ C$) stirring mixture of Ns-Ala-OBn (**3**) (2.0 g, 5.48 mmol) and PPh_3 (2.15 g, 8.23 mmol) in dry THF (18.0 ml) under N_2 atmosphere was added N-Boc-(3-hydroxypropyl) benzylglycinate (**7a**) (2.03 g, 8.23 mmol) dissolved in THF followed by DIAD (1.62 ml, 8.23 mmol). After 15 minutes the ice bath was removed, and the mixture was continued to stir at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue which was dissolved in

EtOAc (30.0 ml) and washed with water (2 X 15.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to denosylation reaction by the addition of thiophenol (0.842 ml, 8.23 mmol) and K₂CO₃ (1.51g, 10.96 mmol) in CH₃CN (18.0 ml). The mixture was continued to stir at ambient conditions until TLC indicated the complete consumption of the starting material. The solvent was removed followed by acidification using 1N HCl (20.0 ml). The aqueous mixture was washed with diethyl ether to remove all the water-insoluble organic impurities.

The aqueous solution was then treated with K₂CO₃ until the medium became basic (pH – 10.0). This was followed by the addition of MCF (1.06 ml, 13.72 mmol) to the reaction mixture. The mixture was continued to stir at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (30.0 ml), washed with water (2 X 20.0 ml) and the organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane -1:4) to yield the desired product as a viscous oil (2.17 g, 4.66 mmol, 85% yield); (TLC- EtOAc:Hexane (1:1) – *R_f* = 0.5); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: (mixture of four cis/trans rotamers) 7.38-7.33 (m, 5H, H^{Ar}Bn), 5.19-5.11 (m, 2H, H^{CH₂}Bn), 4.51 – 4.23 (m, 1H, H^αAla), 3.96 – 3.80 (m, 2H, H^αGly), 3.71 (m, 3H, H^{Me}OMe), 3.71 (m, 3H, H^{Me}Moc), 3.53 – 3.42 (m, 2H, HPrp^c), 3.30 – 3.23 (m, 2H, HPrp^a), 3.30 – 3.23 (m, 2H, H^βPhe), 1.80 (m, 2H, HPrp^b), 1.48 – 1.45 (m, 9H, H^{Me}Boc), 1.41 (s, 3H, H^βAla); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: (mixtures of four rotamers) 171.8, 170.6, 156.3, 155.7, 155.2, 135.8, 128.6, 128.2, 128.1, 80.4, 55.7, 55.5, 52.8, 52.6, 52.0, 51.9, 49.3, 48.8, 46.4, 46.0, 44.0, 28.4, 15.9, 15.3; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 2977, 2955, 2874, 1748, 1703, 1547, 1478, 1459, 1405, 1301, 1251, 1211, 1153, 1084, 1062, 1028; **HRMS** *m/z* Calculated for C₂₃H₃₄N₂O₈K 505.1952, Found 505.1951.

Scheme S18. Synthesis of (S)-benzyl 2-((3-((tert-butyloxycarbonyl)(2-methoxy-2-oxoethyl) amino) propyl) (methoxycarbonyl)amino)propanoate (**10c**):

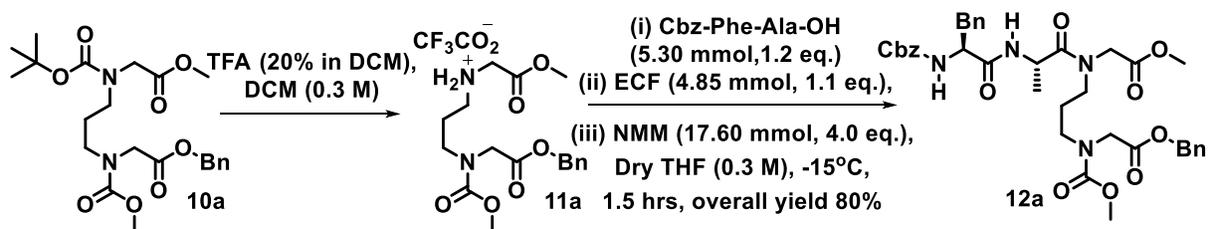


To a cold (0°C) stirring mixture of Ns-Gly-OBn (2.0 g, 5.70 mmol) and PPh₃ (2.24 g, 8.56 mmol) in dry THF (19.0 ml) under N₂ atmosphere was added N-Boc-(3-hydroxypropyl) benzylalaninate (**7c**) (2.23 g, 8.56 mmol) dissolved in THF followed by DIAD (1.69 ml, 8.56 mmol). After 15 minutes the ice bath was removed, and the mixture was continued to stir at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue which was dissolved in ethyl acetate (EtOAc) (30.0 ml) and washed with water (2 X 15.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to denosylation reaction by the addition of thiophenol (0.876 ml, 8.56 mmol) and K₂CO₃ (1.57 g, 11.40 mmol) in CH₃CN (19.0 ml). The mixture was continued to stir at ambient conditions until TLC indicated the complete consumption of the starting material. The solvent was removed followed by acidification using 1N HCl (20.0 ml). The aqueous mixture was washed with diethyl ether to remove all the water-insoluble organic impurities.

The aqueous solution was then treated with K₂CO₃ until the medium became basic (pH – 10.0). This was followed by the addition of MCF (1.10 ml, 14.25 mmol) to the reaction mixture. The mixture was continued to stir at ambient conditions until TLC indicated the complete consumption of the starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (30.0 ml), washed with water (2 X 15.0 ml) and the organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane - 1:3) to yield the desired product as a viscous oil (1.86 g, 3.98 mmol, 70% yield); (TLC - EtOAc:Hexane (1:1) – R_f = 0.4); ¹H NMR (400 MHz, CDCl₃, 10 mM) δ ppm: (mixture of four rotmers) 7.45-7.33 (m, 5H, H^{ArO}Bn), 5.20 (s, 2H, H^{CH₂}Bn), 4.47 – 4.33 (m) & 3.99 – 3.88 (m) (1H, H^αAla) 4.07 (d, J = 19.79 Hz, 2H, H^αGly), 3.76 (s) & 3.66 (s) (3H, H^{Me}Moc), 3.71 (m, 3H, H^{Me}OMe), 3.51 – 3.31 (m) & 3.27 – 3.15 (m) (4H, HPrp^a & HPrp^c), 1.92 – 1.76 (m, 2H, HPrp^b), 1.55 – 1.37 (m) (12H, H^{Me}Boc & H^βAla); ¹³C NMR (100 MHz, CDCl₃,

60 mM) δ ppm: 172.7, 169.7, 157.1, 156.5, 135.5, 128.6, 128.4, 128.2, 80.3, 66.9, 55.9, 52.9, 52.8, 49.2, 46.7, 46.2, 45.0, 28.3; **FT-IR** ($\tilde{\nu}$ cm^{-1}): 2975, 2952, 2360, 1748, 1700, 1473, 1410, 1367, 1306, 1248, 1168, 1087, 1010; **HRMS** m/z Calculated for $\text{C}_{23}\text{H}_{34}\text{N}_2\text{O}_8\text{K}$ 505.1952, Found 505.1952.

Scheme S19. Synthesis of precursor of cyclization (**12a**):

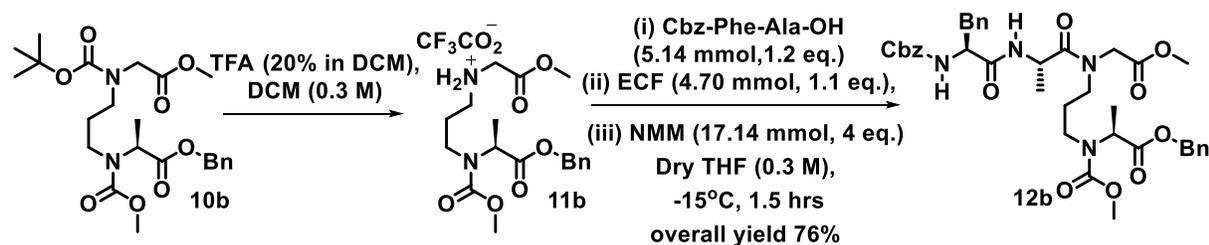


To a cold (0°C) solution of **10a** (2.0 g, 4.41 mmol) in DCM (11.76 ml) was added trifluoroacetic acid (TFA) (2.94 ml) dropwise and the mixture was allowed to stir for 2 hrs by which time TLC indicated the complete consumption of the starting material. Removal of solvent resulted in the Boc-deprotected product **11a** as a liquid (2.06 g, 4.41 mmol, quantitative yield) which was used for further reaction as it is.

To a cold (-15°C) solution of Cbz-Phe-Ala-OH (1.96 g, 5.30 mmol) in dry THF (12.7 ml) and NMM (0.726 ml, 6.61 mmol) was added ECF (0.463 ml, 4.85 mmol). The mixture was stirred for (4 min) when TLC indicated that all the acid was consumed to form the corresponding mixed anhydride. To this mixture was added a solution of **11a** (2.06 g, 4.41 mmol) in dry THF (2.0 ml) followed by NMM (1.21 ml, 11.02 mmol). After 2 h of vigorous stirring, the mixture was warmed and stirred until TLC indicated the complete consumption of starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (40.0 ml), washed with water (2 X 20.0 ml) and 1N HCl (2 X 20.0 ml) and saturated NaHCO_3 (2 X 20.0 ml). The organic layer was dried over anhyd. Na_2SO_4 and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 1:1) to yield the desired product as a liquid (2.48 g, 3.51 mmol, 80% yield); (TLC- EtOAc:Hexane (1:0) – R_f = 0.6); **$^1\text{H NMR}$** (400 MHz, CDCl_3 , 10 mM) δ ppm: (mixture of four rotamers) 7.40 – 7.09 (m, 15H, H^{AroPhe} , H^{AroBn} , H^{AroCbz}), 6.89 (bs, 1H, H^{NAla}), 5.34 (d, J = 6.83 Hz, 1H, H^{NPhe}), 5.16 (d, J = 6.83 Hz, 2H) & 5.06 (t, J = 13.65 Hz, 2H) ($\text{H}^{\text{CH}_2\text{Bn}}$, $\text{H}^{\text{CH}_2\text{Cbz}}$, can't distinguish between benzyl

protons of Cbz and Bn groups), 4.86 (quin, $J = 7.06$ Hz) & 4.59 (quin, $J = 6.85$ Hz) (1H, H^{α} Ala), 4.50 – 4.41 (1H, H^{α} Phe); 4.40 – 4.32 (m) & 3.99 (d, $J = 17.77$ Hz) (2H, H^{α} Gly), 4.22 (d, $J = 16.88$ Hz) & 3.85 (d, $J = 16.88$) (2H, H^{α} Gly), 3.76 (s) & 3.62 (d, $J = 10.66$) (3H, $H^{\text{Me}}\text{Moc}$), 3.71 (s, $H^{\text{Me}}\text{OMe}$), 3.42– 3.28 (m) (4H, HPrp^{c} & HPrp^{a}), 3.08 – 3.01 (m, 2H, H^{β} Phe), 1.91 – 1.79 (m) & 1.78 – 1.66 (m) (2H, HPrp^{b}). 1.31 (d, $J = 6.51$ Hz) & 1.24 (d, $J = 6.51$ Hz) (3H, H^{β} Ala); ^{13}C NMR (100 MHz, CDCl_3 , 60 mM) δ ppm: (mixtures of four conformers) 174.6, 173.1, 172.9, 170.3, 170, 169.8, 169.5, 157.0, 156.9, 156.0, 136.4, 136.3, 135.6, 135.55, 129.5, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 127.2, 67.2, 56.1, 53.3, 53.2, 53.1, 52.8, 52.3, 49.9, 49.6, 49.2, 48.1, 47.9, 46.9, 46.8, 46.6, 46.3, 45.9, 45.7, 45.5, 45.3, 38.7, 28.0, 27.8, 25.9, 20.7, 19.0, 18.7; FT-IR ($\tilde{\nu}$ cm^{-1}): 3397, 2951, 2929, 2361, 2341, 1748, 1706, 1642, 1539, 1475, 1455, 1368, 1255, 1218, 1132, 1066; HRMS m/z Calculated for $\text{C}_{37}\text{H}_{44}\text{N}_4\text{O}_{10}\text{Na}$ 727.2955, Found 727.2958.

Scheme S20. Synthesis of precursor of cyclization (**12b**):

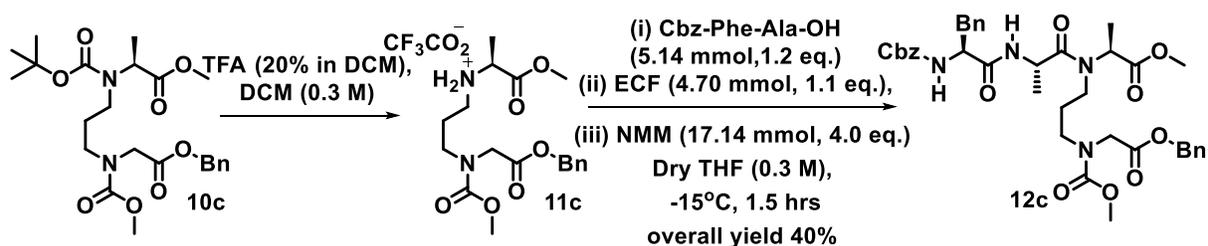


To a cold (0°C) solution of **10b** (2.0 g, 4.28 mmol) in DCM (11.43 ml) was added TFA (2.85 ml) dropwise and the mixture was allowed to stir for 2 hrs by which time TLC indicated the complete consumption of the starting material. Removal of solvent resulted in the Boc-deprotected product **11b** as a liquid (2.05 g, 4.28 mmol) which was used for further reaction as it is.

To a cold (-15°C) solution of Cbz-Phe-Ala-OH (1.90 g, 5.14 mmol) in dry THF (10.0 ml) and NMM (0.704 ml, 6.42 mmol) was added ECF (0.450 ml, 4.70 mmol). The mixture was stirred for (4 min) when TLC indicated that all the acid was consumed to form the corresponding mixed anhydride. To this mixture was added a solution of **11b** (2.05 g, 4.28 mmol) in dry THF (2.0 ml) followed by NMM (1.17 ml, 10.71 mmol). After 2 h of vigorous stirring, the mixture was warmed and stirred until TLC indicated the complete consumption of starting material. Removal of solvent resulted in a residue which was

dissolved in EtOAc (40.0 ml), washed with water (2 X 20.0 ml) and 1N HCl (2 X 20.0 ml) and saturated NaHCO₃ (2 X 20.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 1:1) to yield the desired product as a liquid (2.33 g, 3.24 mmol, 76% yield); (TLC- EtOAc:Hexane (1:0) – *R_f* = 0.6); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 7.39 – 7.10 (m, 15H, H^{Aro}Phe, H^{Aro}Bn, H^{Aro}Cbz), 6.76 (d, J = 6.16, 1H, H^NAla), 5.24 (d, J = 6.81 Hz, 1H, H^NPhe), 5.16 (q, J = 12.33 Hz, 2H) & 5.07 (s, 2H) (H^{CH2}Bn, H^{CH2}Cbz, can't distinguish between the benzyl protons of Cbz and Bn groups), 4.83 (quin, J = 7.01 Hz) & 4.58 (quin, J = 7.01 Hz) (1H, H^αAla), 4.50 (quin, J = 6.70, 1H, H^αPhe); 4.30 (d, J = 18.97 Hz) & 3.96 (d, J = 16.0 Hz) (1H, H^αGly), 4.17 (d, J = 20.15 Hz) & 3.87 – 3.78 (m) (1H, H^αGly), 3.76 (s) & 3.60 – 3.49 (m) (3H, H^{Me}Moc), 3.71 (s, H^{Me}OMe), 3.47– 3.43 (m) & 3.20 – 3.10 (m) (2H, H^{Prp^c}), 3.37 – 3.26 (m, 2H, H^{Prp^a}) 3.10 – 3.00 (m, 2H, H^βPhe), 1.95 – 1.80 (m) & 1.79 – 1.73 (m) (2H, H^{Prp^b}), 1.48 (t, J = 6.87, H^βAla), 1.31 (d, J = 6.43 Hz) & 1.24 (d, J = 6.58 Hz) (3H, H^βAla); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: (mixtures of four conformers) 172.5, 171.7, 169.9, 169.6, 169.3, 156.4, 155.8, 136.2, 136.1, 135.6, 129.4, 128.7, 128.6, 128.5, 128.2, 128.1, 127.1, 67.0, 66.9, 55.9, 55.6, 52.6, 52.2, 49.2, 47.6, 46.6, 45.3, 38.5, 18.9, 18.6; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3781, 3720, 3606, 3536, 3298, 3065, 3033, 2948, 2856, 2380, 2305, 1957, 1744, 1705, 1642, 1537, 1450, 1408, 1375, 1297, 1210, 1170, 1096, 1044; **HRMS** *m/z* Calculated for C₃₈H₄₆N₄O₁₀Na 741.3112, Found 741.3116.

Scheme S21. Synthesis of precursor of cyclisation (**12c**):

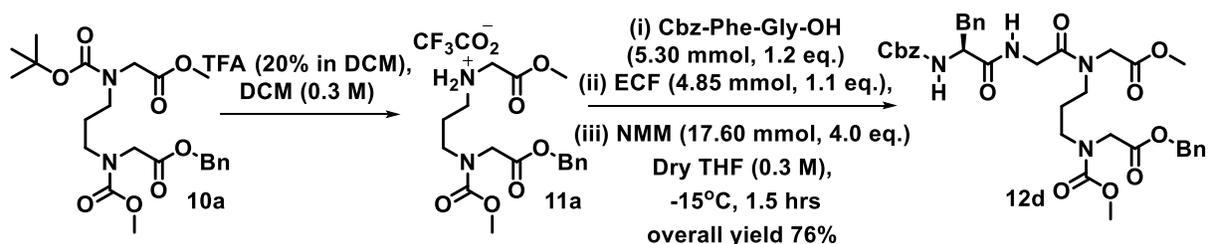


To a cold (0°C) solution of **10c** (2.0 g, 4.28 mmol) in DCM (11.43 ml) was added TFA (2.85 ml) dropwise and the mixture was allowed to stir for 2 hrs by which time TLC indicated the complete consumption of the starting material. Removal of solvent resulted in the Boc-deprotected product **11c** as a liquid (2.06 g, 4.28 mmol) which was used for further reaction as it is.

To a cold (-15°C) solution of Cbz-Phe-Ala-OH (1.90 g, 5.14 mmol) in dry THF (12.26 ml) and NMM (0.704 ml, 6.42 mmol) was added ECF (0.450 ml, 4.70 mmol). The mixture was stirred for (4 min) when TLC indicated that all the acid was consumed to form the corresponding mixed anhydride. To this mixture was added a solution of **11c** (2.06 g, 4.28 mmol) in dry THF (2.0 ml) followed by NMM (1.17l, 10.71 mmol). After 2 h of vigorous stirring, the mixture was warmed and stirred until TLC indicated the complete consumption of starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (40.0 ml), washed with water (2 X 20.0 ml) and 1N HCl (2 X 20.0 ml) and saturated NaHCO₃ (2 X 20.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 3:2) to yield the desired product as a liquid (1.23g, 1.71 mmol, 40% yield); (TLC- EtOAc:Hexane (1:0) – *R_f* = 0.6); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: (mixture of four rotamers) 7.44 – 7.09 (m, 15H, H^{Aro}Phe, H^{Aro}Bn, H^{Aro}Cbz), 6.93 (bs), 6.81 (d, *J* = 5.08 Hz) & 6.64 – 6.56 (m) (1H, H^NAla), 5.34 – 5.23 (m, 1H, H^NPhe), 5.18 (s, 2H) & 5.06 (s, 2H) (H^{CH2}Bn, H^{CH2}Cbz, can't distinguish between Cbz and Bn groups), 4.85 – 4.70 (m, 1H, H^αAla), 4.54 – 4.39 (m, 1H, H^αPhe), 4.13 – 3.93 (m, 2H, H^αGly), 3.74 (d, *J* = 7.90 Hz) & 3.64 – 3.59 (m, merged with the H^{Me}OMe) (3H, H^{Me}Moc), 3.66 (s, H^{Me}OMe), 3.45– 3.27 (m, 4H, HPrp^c & HPrp^a), 3.06 (bs, 2H, H^βPhe), 1.90 - 1.69 (m) (2H, HPrp^b merged with water signal), 1.43 (d, *J* = 5.38, 3H, H^βAla), 1.27 (d, *J* = 8.60 Hz, 3H, H^βAla); **¹³C NMR** (100 MHz, CDCl₃) δ ppm: (mixtures of four

conformers) 172.1, 171.6, 169.8, 156.9, 155.9, 136.2, 136.1, 135.4, 129.4, 128.6, 128.5, 128.3, 128.1, 128.05, 127.1, 67.03, 54.9, 53.2, 53.04, 52.3, 49.2, 45.5, 38.4, 29.7, 28.9, 19.2, 18.9, 14.5; **HRMS** m/z Calcd for $C_{38}H_{46}N_4O_{10}Na$ 741.3112, Found 741.3115.

Scheme S22. Synthesis of precursor of cyclization (**12d**):

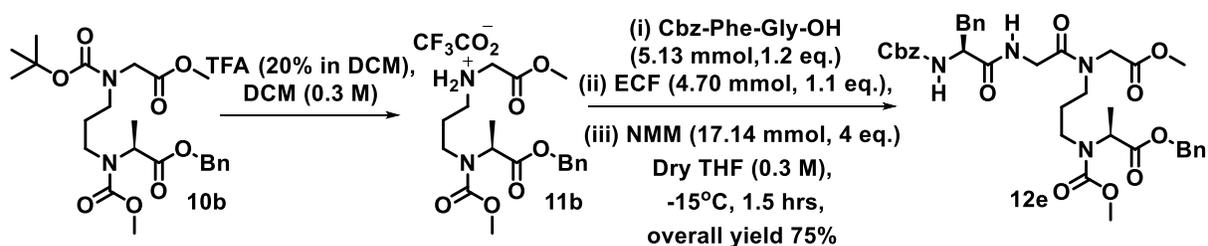


To a cold (0°C) solution of **10a** (2.0 g, 4.41 mmol) in DCM (11.76 ml) was added TFA (2.94 ml) dropwise and the mixture was allowed to stir for 2 hrs by which time TLC indicated the complete consumption of the starting material. Removal of solvent resulted in the Boc-deprotected product **11a** as a liquid (2.06 g, 4.41 mmol, quantitative yield) which was used for further reaction as it is.

To a cold (-15°C) solution of Cbz-Phe-Gly-OH (1.88 g, 5.30 mmol) in dry THF (12.70 ml) and NMM (0.726 ml, 6.61 mmol) was added ECF (0.463 ml, 4.85 mmol). The mixture was stirred for (4 min) when TLC indicated that all the acid was consumed to form the corresponding mixed anhydride. To this mixture was added a solution of **11a** (2.06 g, 4.41 mmol) in dry THF (2.0 ml) followed by NMM (1.21 ml, 11.02 mmol). After 2 h of vigorous stirring, the mixture was warmed and stirred until TLC indicated the complete consumption of starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (40.0 ml), washed with water (2 X 20.0 ml) and 1N HCl (2 X 20.0 ml) and saturated $NaHCO_3$ (2 X 20.0 ml). The organic layer was dried over anhyd. Na_2SO_4 and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 4:1) to yield the desired product as a liquid (2.35 g, 3.34 mmol, 76% yield); (TLC- EtOAc:Hexane (1:0) – R_f = 0.2); **¹H NMR** (400 MHz, $CDCl_3$, 10 mM) δ ppm: 7.47 – 7.11 (m, 15H, $H^{Aro}Phe$, $H^{Aro}Bn$, $H^{Aro}Cbz$), 6.82 (bs, 1H, $H^N Gly$), 5.25 (d, J = 8.62 Hz, 1H, $H^N Phe$), 5.21 – 5.15 (m, 2H) & 5.07 (dd, J = 21.20, 12.09 Hz, 2H) ($H^{CH_2}Bn$, $H^{CH_2}Cbz$, can't distinguish between benzyl protons of Cbz and Bn groups), 4.86 (dd, J = 15.12, 9.48 Hz) (1H, $H^{\alpha}Phe$), 4.09 – 4.03 (m, 3H), 3.98 (s, 2H),

3.76 (s, 3H), 3.72 (s, 3H), 3.63 (d, $J = 10.29$ Hz, 2H), 3.44 – 3.33 (m, 4H), 3.19 – 3.01 (m, 2H), 1.86 – 1.77 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3 , 60 mM) δ ppm: (mixtures of four rotamers) 171.0, 169.8, 169.4, 168.4, 156.7, 155.9, 136.3, 136.2, 135.4, 135.3, 129.3, 128.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.0, 67.1, 67.0, 56.1, 53.1, 52.7, 52.3, 49.5, 49.2, 48.0, 46.7, 46.1, 45.8, 41.2, 41.1, 38.6, 27.5, 27.3; **FT-IR** ($\tilde{\nu}$ cm^{-1}): 3302, 3064, 3032, 2958, 2924, 2848, 1751, 1708, 1646, 1529, 1476, 1443, 1405, 1375, 1323, 1254, 1212, 1181, 1135, 1084, 1051, 1014; **HRMS** m/z Calculated for $\text{C}_{37}\text{H}_{44}\text{N}_4\text{O}_{10}\text{Na}$ 713.2799, Found 713.2795.

Scheme S23. Synthesis of precursor of cyclization (**12e**):

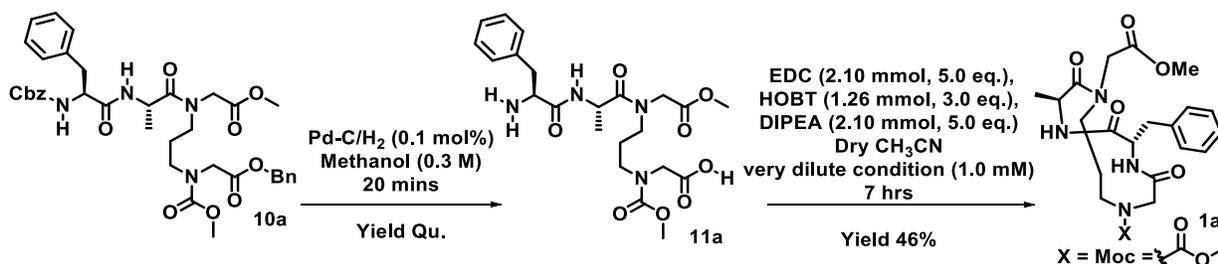


To a cold (0°C) solution of **10b** (2.0 gm, 4.28 mmol) in DCM (11.43 ml) was added TFA (2.85 ml) dropwise and the mixture was allowed to stir for 2 hrs by which time TLC indicated the complete consumption of the starting material. Removal of solvent resulted in the Boc-deprotected product **11b** as a liquid (2.06 g, 4.28 mmol, 100 yield) which was used for further reaction as it is.

To a cold (-15°C) solution of Cbz-Phe-Gly-OH (1.83 g, 5.13 mmol) in dry THF (12.26 ml) and NMM (0.704 ml, 6.42 mmol) was added ECF (0.450 ml, 4.70 mmol). The mixture was stirred for (4 min) when TLC indicated that all the acid was consumed to form the corresponding mixed anhydride. To this mixture was added a solution of **11b** (2.06 g, 4.28 mmol) in dry THF (2.0 ml) followed by NMM (1.17 ml, 10.71 mmol). After 2 h of vigorous stirring, the mixture was warmed and stirred until TLC indicated the complete consumption of starting material. Removal of solvent resulted in a residue which was dissolved in EtOAc (40.0 ml), washed with water (2 X 20.0 ml) and 1N HCl (2 X 20.0 ml) and saturated NaHCO_3 (2 X 20.0 ml). The organic layer was dried over anhyd. Na_2SO_4 and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 4 :1) to yield the desired product as a liquid

(2.26g, 3.20 mmol, 75% yield); (TLC- EtOAc:Hexane (4:1) – R_f = 0.5); **¹H NMR** (400 MHz, CDCl₃, 10 mM) δ ppm: 7.40 – 7.11 (m, 15H, H^{Aro}Phe, H^{Aro}Bn, H^{Aro}Cbz), 6.85 (bs, 1H, H^NGly), 5.26 (d, J = 6.78 Hz, 1H, H^NPhe), 5.22 – 5.12 (m, 2H) & 5.07 (dd, J = 21.43, 12.22 Hz, 2H) (H^{CH₂}Bn, H^{CH₂}Cbz, can't distinguish between benzyl protons of Cbz and Bn groups), 4.51 (d, J = 5.91 Hz, 1H), 4.14 (d, J = 17.75, 1H), 4.09 – 3.90 (m, 4H), 3.76 (s, 2H) & 3.72 (s, 3H), 3.53 (d, J = 15.39, 2H), 3.47 – 3.26 (m, 4H), 3.24 – 2.99 (m, 4H), 1.48 (m, 3H); **¹³C NMR** (100 MHz, CDCl₃, 60 mM) δ ppm: (mixtures of four rotamers) 171.7, 171.0, 169.4, 168.3, 168.2, 156.4, 156.0, 136.3, 136.2, 135.6, 129.2, 128.7, 128.62, 128.57, 128.5, 128.3, 128.1, 128.0, 127.0, 67.1, 66.9, 56.1, 55.3, 52.3, 52.28, 48.6, 47.7, 46.0, 45.7, 41.2, 41.1, 38.5, 15.8, 15.3; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3302, 3064, 3032, 2958, 2924, 2847, 1748, 1704, 1646, 1528, 1478, 1445, 1408, 1375, 1308, 1258, 1212, 1148, 1094, 1028; **HRMS** m/z Calculated for C₃₇H₄₄N₄O₁₀Na 727.2955, Found 727.2957.

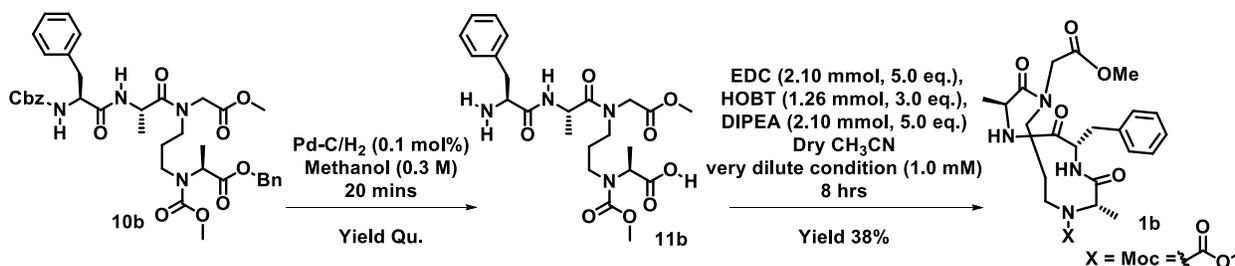
Scheme S24. Synthesis of HBS-constrained single α -helical turn 13₇3 analogue **1a**:



MeOH (3.0 ml) was added to a mixture of **10a** (300 mg, 0.43 mmol) and Pd-C (0.1 mol%) in a sealed round-bottom flask kept under H₂-atmosphere and stirred for 20 mins by which time TLC indicated the complete consumption of starting material. Filtering the mixture through Whatman-40 filter paper and concentration of the organic filtrate gave the N- and C-terminal double de-protected derivative of **10a** as a viscous liquid **11a** (208.8 mg, 0.43 mmol). Next **11a** was dissolved in dry CH₃CN (1.0 mM) (420.0 ml) followed by addition of EDC (410.6 mg, 2.15 mmol), HOBT (174.1 mg, 1.29 mmol) and DIPEA (375.0 μ l, 2.15 mmol) under N₂ atmosphere. The mixture was stirred for further 7 hrs. Removal of solvent resulted in a residue which was dissolved in DCM (30.0 ml) and washed with water (2 X 15.0 ml), 1N HCl (2 X 15.0 ml) and saturated NaHCO₃ (2 X 15.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 9:1) to yield the

desired product as a white solid (89.35 mg, 0.19 mmol, 46% yield); (TLC-EtOAc:Methanol (9:1) – R_f = 0.7); **¹H NMR** (400 MHz, 40% CDCl₃ in CD₃CN, 5 mM) δ ppm: major conformation: 7.33 – 7.21 (m, 5H, H^{Ar}O₂Phe), 6.61 (d, J = 9.29 Hz, 2H, H^NPhe+Ala), 4.75 (dt, J = 16.57, 10.07 Hz, 1H, H ^{α} Ala₃), 4.50 (d, J = 15.11 Hz, 1H, H ^{α} Gly₁), 4.44 (d, J = 19.05 Hz, 1H, H ^{α} Gly₄), 4.31 (q, J = 6.12 Hz, 1H, H ^{α} Phe₂), 4.11 (t, J = 13.52 Hz, 1H, H^{prp}^c), 3.77 (d, J = 19.12 Hz, 1H, H ^{α} Gly₄), 3.71 (s, 3H, H^{Me}OMe), 3.66 (d, J = 9.27 Hz, 1H, H^{Me}Moc), 3.54 (d, J = 16.17 Hz, 1H, H ^{α} Gly₁), 3.45 – 3.32 (m, 1H, H^{prp}^a), 3.30 – 3.16 (m, 1H, H^{prp}^a), 3.04 – 2.96 (m, 2H, H ^{β} Phe₂), 2.52 (d, J = 14.53 Hz, 1H, H^{prp}^c), 2.29 – 2.17 (m, 1H, H^{prp}^b), 1.87 – 1.74 (m, 1H, H^{prp}^b), 1.33 (d, J = 6.11 Hz, 1H, H ^{β} Ala₃), 1.21 (d, J = 6.37 Hz, 3H, H ^{β} Ala₃); minor conformation: 7.17 – 7.09 (m, 0.11H, H^NPhe₂), 7.07 – 6.98 (m, 0.19H, H^NPhe₂), 6.91 – 6.83 (m, 0.11H, H^NAla₃), 6.76 – 6.67 (m, 0.19H, H^NAla₃); **¹³C NMR** (100 MHz, 40% CDCl₃ in CD₃CN, 5 mM) δ ppm: (mixtures of major and minor conformers) 173.2, 171.5, 170.9, 170.1, 136.7, 129.7, 129.1, 127.6, 58.1 (C ^{α} Phe₂), 52.9 (C^{Me}Moc), 52.6 (C^{Me}OMe), 52.5 (C ^{α} Gly₁), 48.2 (C ^{α} Gly₂), 46.0 (C^{prp}^a), 44.4 (C ^{α} Ala₃), 42.9 (C^{prp}^c), 36.8 (C ^{β} Phe₂), 16.9 (C ^{β} Ala₃); **FT-IR** ($\tilde{\nu}$ cm⁻¹) (**CH₃CN, 2mM**): 2978, 2868, 1749, 1700, 1657, 1528, 1472, 1358, 1315, 1290, 1220; **HRMS** m/z Calcd for C₂₂H₃₀N₄O₇Na 481.2012, Found 481.2014.

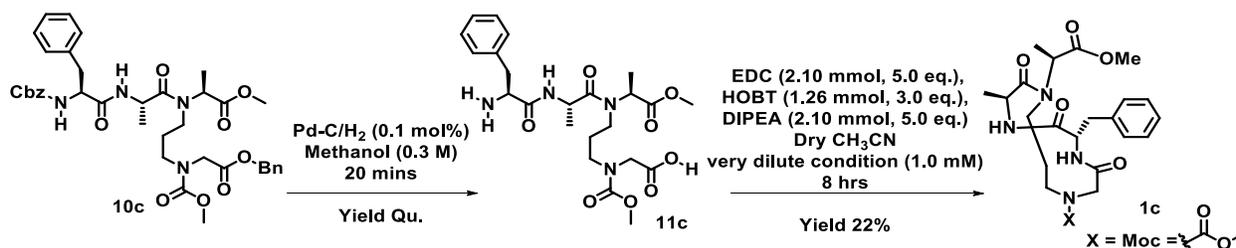
Scheme S25. Synthesis of HBS-constrained single α -helical turn 13₇₃ analogue **1b**:



MeOH (3.0 ml) was added to a mixture of **10b** (300.0 mg, 0.42 mmol) and Pd-C (0.1 mol%) in a sealed round-bottom flask kept under H₂-atmosphere and stirred for 20 mins by which time TLC indicated the complete consumption of starting material. Filtering the mixture through Whatman-40 filter paper and concentration of the organic filtrate gave the N- and C-terminal double de-protected derivative of **10b** as a viscous liquid **11b** (208.0 mg, 0.42 mmol). Next **11b** was dissolved in dry CH₃CN (1.0 mM) (420.0 ml) followed by addition of EDC (401.1 mg, 2.10 mmol), HOBT (170.1 mg, 1.26 mmol) and DIPEA (365.0

μl , 2.10 mmol) under N_2 atmosphere. The mixture was stirred for further 8 hrs. Removal of solvent resulted in a residue which was dissolved in DCM (10 ml) and washed with water (2 X 15.0 ml), 1N HCl (2 X 15.0 ml) and saturated NaHCO_3 (2 X 15.0 ml). The organic layer was dried over anhyd. Na_2SO_4 and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 2:3) to yield the desired product as a white solid (77.6 mg, 0.16 mmol, 38% yield); (TLC-EtOAc:Methanol (9:1) – $R_f = 0.8$); **$^1\text{H NMR}$** (400 MHz, 40% CDCl_3 in CD_3CN , 5 mM) δ ppm: major conformation: 7.36 – 7.20 (m, 5H, $\text{H}^{\text{ArO}}\text{Phe}_2$), 6.68 (d, $J = 9.86$ Hz, 1H, $\text{H}^{\text{N}}\text{Ala}_3$), 6.51 (s, 1H, $\text{H}^{\text{N}}\text{Phe}_2$), 4.78 (dt, $J = 17.02$, 6.70 Hz, 1H, $\text{H}^{\alpha}\text{Ala}_3$), 4.69 – 4.62 (m, 1H, $\text{H}^{\alpha}\text{Ala}_1$), 4.58 (d, $J = 19.13$ Hz, 1H, $\text{H}^{\alpha}\text{Gly}_4$), 4.25 – 4.17 (m, 1H, $\text{H}^{\alpha}\text{Phe}_2$), 4.1 (t, $J = 13.63$ Hz, 1H), 3.76 (d, $J = 18.99$ Hz, 1H, $\text{H}^{\alpha}\text{Gly}_4$), 3.71 (s, 3H, $\text{H}^{\text{Me}}\text{OMe}$), 3.29 – 3.19 (m, 1H, Hprp^{a}), 3.21 – 3.17 (m, 1H, Hprp^{a}), 3.07 – 2.97 (m, 2H, $\text{H}^{\beta}\text{Phe}_2$), 1.76 – 1.66 (m, 1H, Hprp^{b}), 1.28 (d, $J = 7.07$ Hz, 3H, $\text{H}^{\beta}\text{Ala}_1$), 1.24 (d, $J = 6.27$ Hz, 3H, $\text{H}^{\beta}\text{Ala}_3$); minor conformation: 7.17 – 7.12 (m, 0.36H, $\text{H}^{\text{N}}\text{Phe}_2$), 7.12 – 7.07 (m, 0.36H, $\text{H}^{\text{N}}\text{Ala}_3$); **$^{13}\text{C NMR}$** (100 MHz, 40% CDCl_3 in CD_3CN , 5 mM) δ ppm: (mixtures of major and minor conformers) 174.3, 172.0, 171.8, 171.7, 170.9, 138.3, 137.6, 130.6, 130.5, 130.3, 130.9, 129.8, 129.7, 128.4, 128.2, 59.2 ($\text{C}^{\alpha}\text{Phe}_2$), 58.3 ($\text{C}^{\alpha}\text{Ala}_1$), 53.5 ($\text{C}^{\text{Me}}\text{Moc}$), 53.4 ($\text{C}^{\text{Me}}\text{OMe}$), 48.9 ($\text{C}^{\alpha}\text{Gly}_4$), 45.0 ($\text{C}^{\alpha}\text{Ala}_3$), 43.5 (Cprp^{c}), 41.4 (Cprp^{a}), 37.6 ($\text{C}^{\beta}\text{Phe}_2$), 28.9 (Cprp^{b}), 17.4 ($\text{C}^{\beta}\text{Ala}_1$), 15.9 ($\text{C}^{\beta}\text{Ala}_3$); **FT-IR** ($\tilde{\nu}$ cm^{-1}) (**CH_3CN , 2 mM**): 2992, 2946, 1750, 1691, 1657, 1527, 1507, 1472, 1440, 1402, 1375, 1302, 1210, 1112, 1059, 1003; **HRMS** m/z Calcd for $\text{C}_{23}\text{H}_{32}\text{N}_4\text{O}_7\text{Na}$ 499.2169, Found 499.2166.

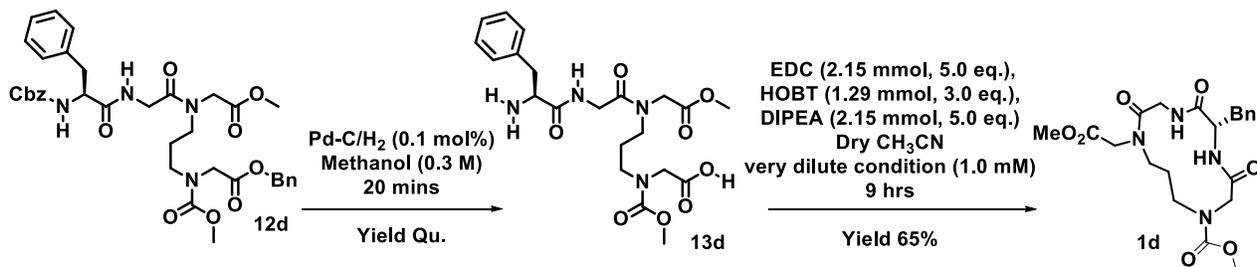
Scheme S26. Synthesis of HBS-constrained single α -helical turn 13₇₃ analogue **1c**:



MeOH (3.0 ml) was added to a mixture of **10c** (300.0 mg, 0.42 mmol) and Pd-C (0.1 mol%) in a sealed round-bottom flask kept under H₂-atmosphere and stirred for 20 mins by which time TLC indicated the complete consumption of starting material. Filtering the mixture through Whatman-40 filter paper and concentration of the organic filtrate gave the N- and C-terminal double de-protected derivative of **10c** as a viscous liquid **11c** (208.0 mg, 0.42 mmol). Next **11c** was dissolved in dry CH₃CN (1.0 mM) (420.0 ml) followed by addition of EDC (401.1 mg, 2.10 mmol), HOBT (170.1 mg, 1.26 mmol) and DIPEA (365.0 μ l, 2.10 mmol) under N₂ atmosphere. The mixture was stirred for further 8 hrs. Removal of solvent resulted in a residue which was dissolved in DCM (10 ml) and washed with water (2 X 15.0 ml), 1N HCl (2 X 15.0 ml) and saturated NaHCO₃ (2 X 15.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 2:3) to yield the desired product as a white solid (45 mg, 0.09 mmol, 22% yield); (TLC-EtOAc:Methanol (9:1) – *R_f* = 0.8); **¹H NMR** (400 MHz, 40% CDCl₃ in CD₃CN, 5 mM) δ ppm: major conformation: 7.14 (d, *J* = 8.49 Hz, 1H, H^NPhe₂), 7.35 – 7.23 (m, 5H, H^{ArO}Phe), 7.16 (d, *J* = 9.49 Hz, 1H, H^NAla₃), 4.76 (q, *J* = 6.72 Hz, 1H, H ^{α} Ala₃), 4.65 (q, *J* = 7.90 Hz, 1H, H ^{α} Phe₂), 3.99 (d, *J* = 13.44 Hz, 1H, H ^{α} Gly₁), 3.86 (q, *J* = 6.88 Hz, H ^{α} Ala₄), 3.74 (s, 3H, H^{Me}OMe), 3.70 – 3.67 (m, 1H, H ^{α} Gly₁), 3.62 (s, 3H, H^{Me}Moc), 3.38 – 3.28 (m, 1H, H_{prp}^c), 3.27 – 3.18 (m, 1H, H_{prp}^a), 3.13 – 3.04 (m, 1H, H ^{β} Phe₂), 3.03 – 2.97 (m, 1H, H_{prp}^c), 2.97 – 2.91 (m, 1H, H ^{β} Phe₂), 1.91 – 1.79 (m, 1H, H_{prp}^b), 1.72 – 1.60 (m, 1H, H_{prp}^b), 1.40 (d, *J* = 6.58 Hz, 3H, H ^{β} Ala₄), 0.94 (d, *J* = 6.49 Hz, 3H, H ^{β} Ala₃); minor conformation: 6.99 – 6.91 (m, H^NAla₃), 6.84 – 6.74 (m, H^NAla₃); **¹³C NMR** (100 MHz, 40% CDCl₃ in CD₃CN, 5 mM) δ ppm: (mixtures of major and minor conformers) 173.3, 172.0, 171.4, 171.3, 158.6, 138.3, 130.54, 130.48, 129.9, 129.7, 128.2, 128.0, 57.6 (C ^{α} Ala₄), 57.4, 56.0 (C ^{α} Phe₂), 54.3 (C ^{α} Gly₁ + C^{Me}OMe), 53.0 (C^{Me}Moc), 48.1 (C_{prp}^c), 47.5 (C_{prp}^a),

46.9 (C^αAla₃), 38.6 (C^βPhe₂), 29.5 (C^γPrp^b), 18.0 (C^βAla₃), 16.0 (C^βAla₄); **FT-IR** ($\tilde{\nu}$ cm⁻¹) (**CH₃CN**, 2 mM): 3092, 3037, 2603, 2265, 2117, 1880, 1741, 1701, 1639, 1534, 1477, 1245, 1193; **HRMS** *m/z* Calcd for C₂₃H₃₂N₄O₇Na 499.2169, Found 499.2168.

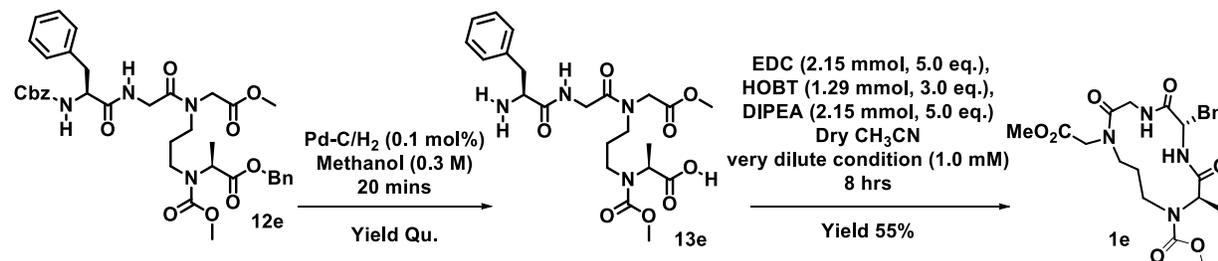
Scheme S27. Synthesis of HBS-constrained single α -helical turn 13₇₃ analogue **1d**:



MeOH (3.0 ml) was added to a mixture of **12d** (300.0 mg, 0.434 mmol) and Pd-C (0.1 mol%) in a sealed round-bottom flask kept under H₂-atmosphere and stirred for 20 mins by which time TLC indicated the complete consumption of starting material. Filtering the mixture through Whatman-40 filter paper and concentration of the organic filtrate gave the N- and C-terminal double de-protected derivative of **12d** as a white solid **13d** (200.5 mg, 0.43 mmol). Next **13d** was dissolved in dry CH₃CN (1.0 mM) (420.0 ml) followed by addition of EDC (410.6 mg, 2.15 mmol), HOBT (174.1 mg, 1.29 mmol) and DIPEA (374.0 μ l, 2.15 mmol) under N₂ atmosphere. The mixture was stirred for further 9 hrs. Removal of solvent resulted in a residue which was dissolved in DCM (30.0 ml) and washed with water (2 X 15.0 ml), 1N HCl (2 X 15.0 ml) and saturated NaHCO₃ (2 X 15.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Methanol – 9:1) to yield the desired product as a white solid (125.3 mg, 0.28 mmol, 65% yield); (TLC-EtOAc:Methanol (9:1) – *R_f* = 0.6); **¹H NMR** (400 MHz, DMSO-d₆, 5 mM) δ ppm: 8.82 (t, *J* = 9.29 Hz, 2H), 7.28 (t, *J* = 7.74 Hz, 2H), 7.23 – 7.17 (m, 3H), 4.70 (q, *J* = 8.91 Hz, 1H), 4.54 (quin, *J* = 7.41 Hz, 1H), 3.82 (dd, *J* = 16.92, 6.18 Hz, 1H), 3.61 (s) & 3.57 (s) (6H), 3.51 (t, *J* = 12.51 Hz, 1H), 3.20 (t, *J* = 12.88 Hz, 1H), 3.02 – 2.89 (m, 2H), 2.88 – 2.66 (m, 3H), 1.96 – 1.76 (m, 1H), 1.40 (quin, *J* = 11.90 Hz, 1H); **¹³C NMR** (100 MHz, DMSO-d₆) δ ppm: 170.3, 169.3, 169.0, 168.3, 168.1, 156.0, 155.3, 137.2, 128.8, 128.2, 126.3, 53.3, 52.6, 52.4, 51.6, 51.0, 47.3, 45.7, 44.4, 44.0, 42.9, 36.6, 27.3, 26.6; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3784, 3765, 3661, 3265, 3083, 2959, 2923, 2847, 1812, 1736, 1640, 1590, 1554, 1492, 1461,

1407, 1376, 1304, 1264, 1214, 1190, 1158, 1126, 1082, 1020; **HRMS** m/z Calcd for $C_{21}H_{28}N_4O_7H$ 449.2036, Found 449.2039.

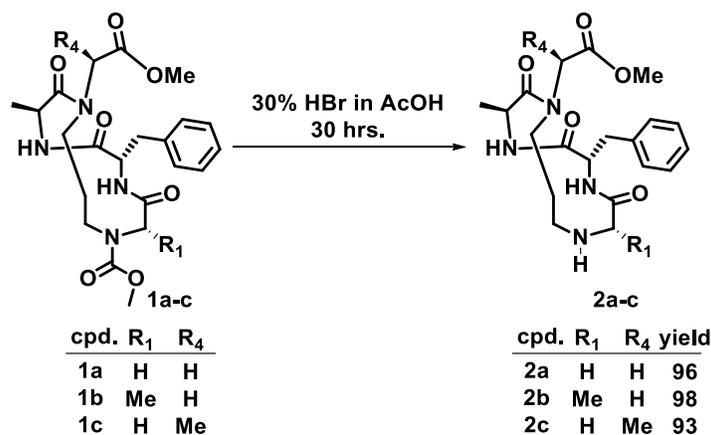
Scheme S28. Synthesis of HBS-constrained single α -helical turn 1373 analogue **1e**:



MeOH (3.0 ml) was added to a mixture of **12e** (300.0 mg, 0.43 mmol) and Pd-C (0.1 mol%) in a sealed round-bottom flask kept under H₂-atmosphere and stirred for 20 mins by which time TLC indicated the complete consumption of starting material. Filtering the mixture through Whatman-40 filter paper and concentration of the organic filtrate gave the N- and C-terminal double de-protected derivative of **1e** as a white solid **13e** (206.6 mg, 0.43 mmol). Next **13e** was dissolved in dry CH₃CN (1.0 mM) (420.0 ml) followed by addition of EDC (410.6 mg, 2.15 mmol), HOBT (174.1 mg, 1.29 mmol) and DIPEA (374.0 μ l, 2.15 mmol) under N₂ atmosphere. The mixture was stirred for further 8 hrs. Removal of solvent resulted in a residue which was dissolved in DCM (30.0 ml) and washed with water (2 X 15.0 ml), 1N HCl (2 X 15.0 ml) and saturated NaHCO₃ (2 X 15.0 ml). The organic layer was dried over anhyd. Na₂SO₄ and concentrated to get a residue which was subjected to purification by silica gel flash column chromatography (EtOAc:Hexane – 9:1) to yield the desired product as a white solid (111 mg, 0.24 mmol, 55% yield); (TLC-EtOAc:Methanol (9:1) – R_f = 0.5); **¹H NMR** (400 MHz, DMSO-d₆, 5 mM) δ ppm: 8.80 (d, J = 6.02 Hz, 1H), 8.16 (d, J = 8.65 Hz, 1H), 7.28 (t, J = 7.09, 2H), 7.21 (d, J = 7.09 Hz, 3H), 4.63 (q, J = 7.42 Hz, 1H), 4.57 – 4.44 (m, 1H), 4.03 (d, J = 17.10 Hz, 1H), 3.78 (d, J = 17.10 Hz, 1H), 3.61 (s, 3H), 3.59 (s, 3H), 3.23 (t, J = 12.18 Hz, 2H), 2.99 (d, J = 7.68 Hz, 2H), 2.89 (t, J = 10.07 Hz, 1H), 2.77 (t, J = 13.03 Hz, 1H), 2.05 – 1.83 (m, 1H), 1.20 (d, J = 7.07 Hz, 3H); **¹³C NMR** (100 MHz, 40% CDCl₃ in CD₃CN, 5 mM) δ ppm: 170.4, 169.4, 169.01, 137.5, 129.0, 128.2, 126.4, 55.2, 54.3, 52.5, 51.6, 47.2, 45.4, 43.1, 36.4, 31.2, 28.9, 22.0, 13.9; **FT-IR** ($\tilde{\nu}$ cm⁻¹): 3289s, 3252, 3085, 2958, 2923, 2847, 1734, 1703,

1640, 1534, 1468, 1442, 1404, 137, 1292, 1264, 1223, 1193, 1141, 1107, 1082, 1052, 1008; **HRMS** m/z Calcd for $C_{22}H_{30}N_4O_7Na$ 485.2012, Found 485.2007.

Scheme S29. General procedure for N-Moc deprotection to synthesize the 13₆₃ analogues **2a-c** from the 13₇₃ analogues **1a-c**:



To a cold (0 °C) solution of 30 mg of **1a-c** (~20 mg) in DCM (1 ml) was added 30% HBr in acetic acid (1 ml) dropwise and the mixture was allowed to stir for 30 hrs by which time TLC indicated the complete consumption of the starting material. Removal of solvent resulted in the corresponding Moc-deprotected product **2a-c** as an oil in high yields.

2a: ¹H NMR (400 MHz, 10% D₂O in H₂O, 5 mM) δ ppm (mixture of multiple conformation): 8.56 (t, J = 7.62 Hz, 0.36H), 8.39 (d, J = 7.39 Hz, 0.42H), 8.39 (d, J = 6.76 Hz, 0.36H), 8.27 (d, J = 6.56 Hz, 0.08H), 7.32 – 7.17 (m, 6H), 4.3 – 4.19 (m, 0.44H), 4.15 (t, J = 7.34 Hz, 0.31H), 4.01 (q, J = 6.59 Hz, 0.33H), 3.85 (d, J = 5.91 Hz, 3H), 3.83 – 3.77 (m, 1H), 3.77 – 3.69 (m, 1H), 3.18 – 3.12 (m, 1H), 3.12 – 3.03 (m, 3H), 3.01 – 2.89 (2H), 2.14 – 1.95 (m, 2H), 1.45 (d, J = 7.47 Hz, 1H), 1.28 (d, J = 7.47 Hz, 2H), 1.11 (d, J = 7.62 Hz, 0.4H); **HRMS** m/z Calcd for $C_{20}H_{28}N_4O_5H$ 406.2216, Found 406.2212.

2b: ¹H NMR (400 MHz, 10% D₂O in H₂O, 5 mM) δ ppm (mixture of multiple conformation): 8.81 (d, J = 8.50 Hz, 0.11H), 8.73 (d, J = 8.72 Hz, 0.63H), 8.44 (d, J = 6.66 Hz, 0.52H), 8.34 (d, J = 6.66 Hz), 7.44 – 7.29 (m, 5H), 3.98 (s, 5H), 3.23 (dd, J = 14.11, 5.83 Hz, 1H), 3.13 (quin, J = 8.47 Hz, 2H), 3.02 – 2.92 (m, 1H), 2.92 – 2.80 (m, 1H), 2.53 – 2.32 (m, 1H), 2.15 – 1.93 (m, 2H), 1.56 (d, J = 7.65 Hz, 0.38H), 1.49 (d, J = 7.29 Hz, 3H), 1.41 (d,

J = 7.29 Hz, 2H), 1.28 (d, J = 7.34 Hz, 0.54H); **HRMS** *m/z* Calcd for C₂₁H₃₀N₄O₅H 420.2373, Found 420.2370.

2c: ¹H NMR (400 MHz, CDCl₃, 5 mM) δ ppm (mixture of multiple conformation): 7.36 – 7.16 (m, 6H), 7.10 (d, J = 5.78 Hz, 0.27H), 6.72 (d, J = 6.90 Hz, 0.66H), 4.68 (q, J = 6.98 Hz, 1H), 4.49 (quin, J = 7.05 Hz, 1H), 3.72 (s, 3H), 3.70 – 3.64 (m, 3H), 3.17 – 3.06 (m, 4H), 1.90 – 1.72 (m, 2H), 1.39 – 1.35 (m, 2H), 1.33 – 1.24 (m, 5H); **HRMS** *m/z* Calcd for C₂₁H₃₀N₄O₅H 420.2373, Found 420.2369.

4.2 NMR analyses

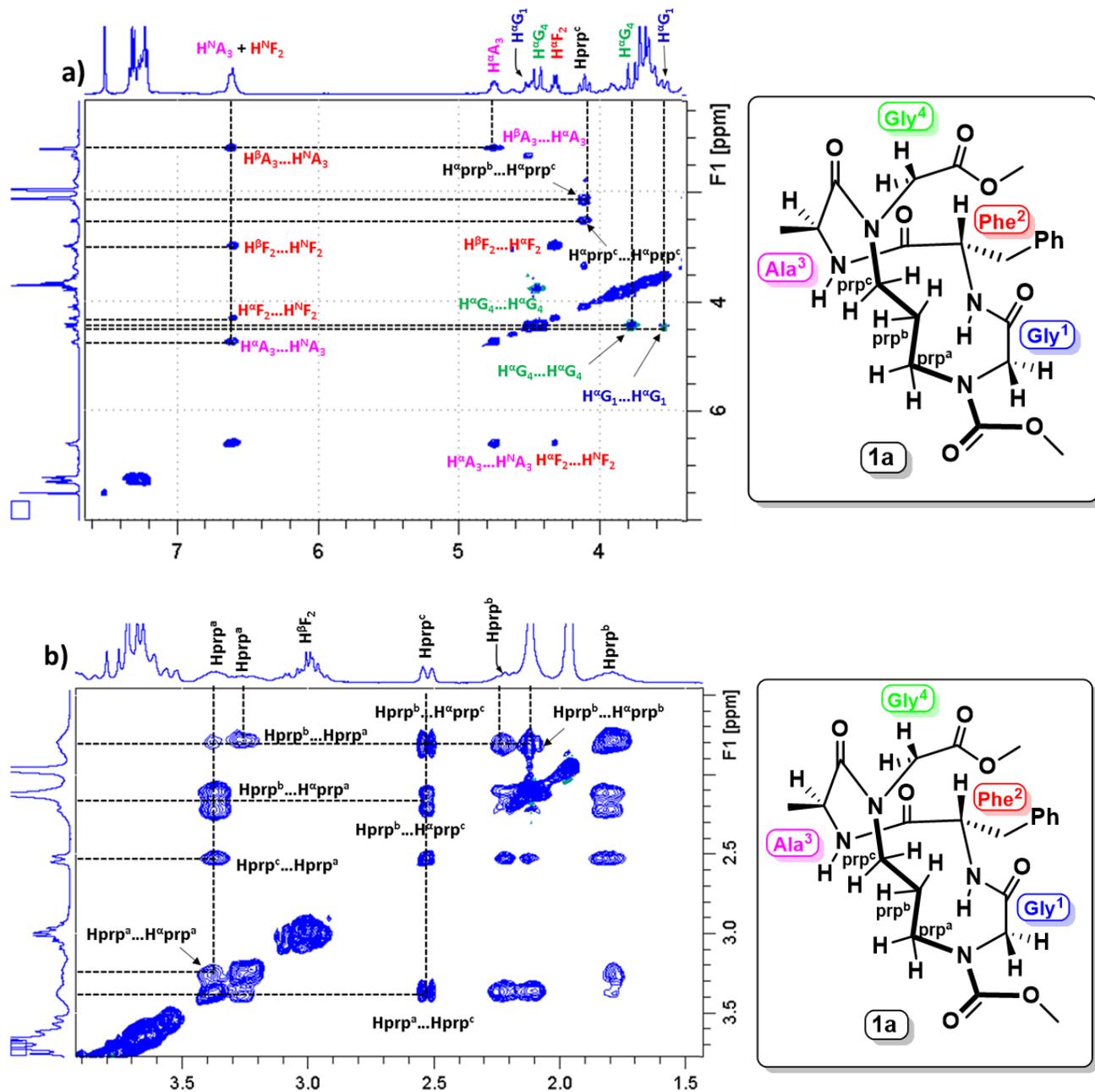


Figure S2. a), b) Portions of Total Correlation Spectroscopy (TOCSY) spectrum of major conformer of Moc[GFA]G-OMe (**1a**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C). a) Showing the through bond coupling of all the four amino acids, b) showing the through bond coupling between the prp protons of HBS part.

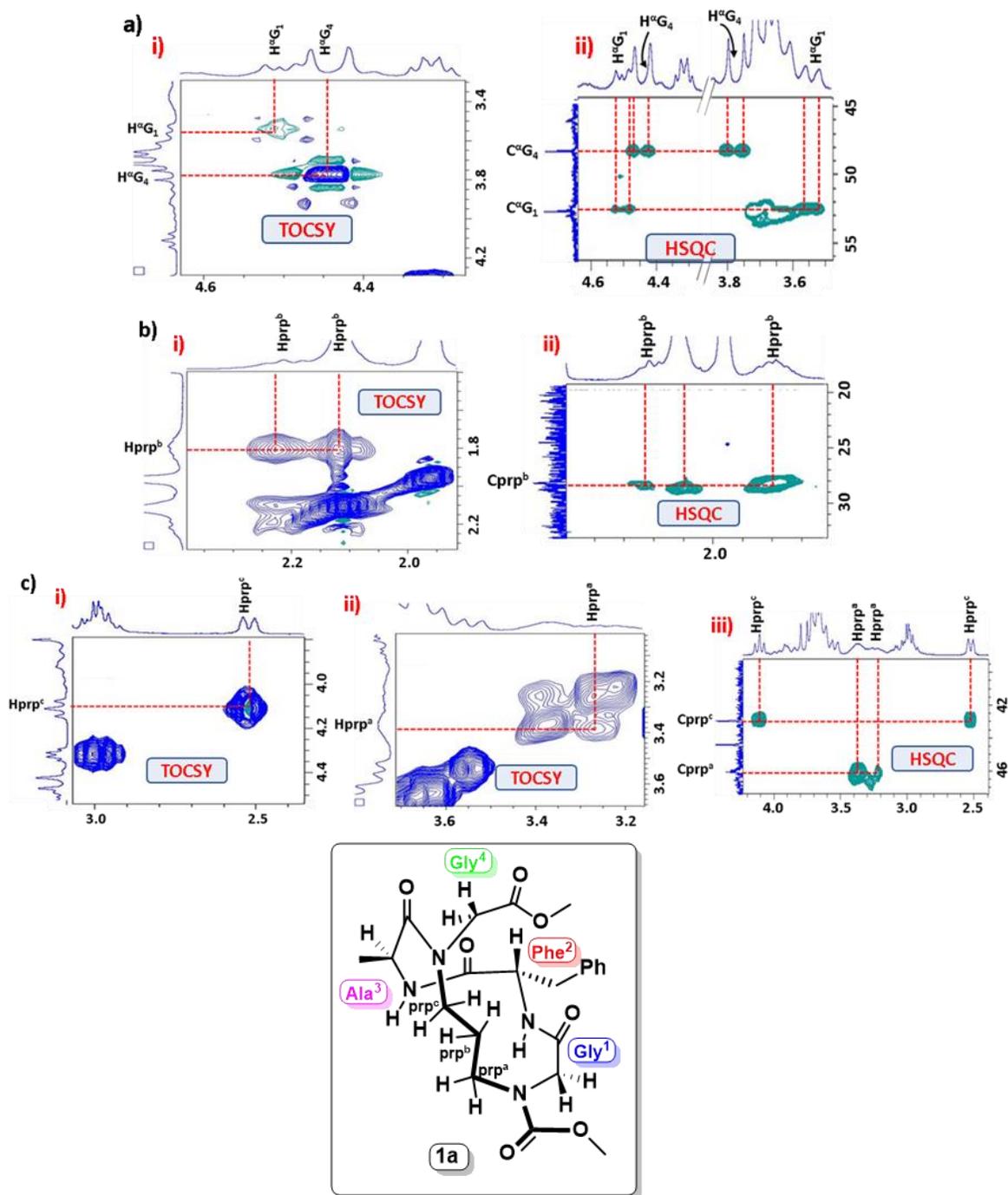


Figure S3. Relevant portions of the TOCSY and HSQC spectra for a) $H^{\alpha}G_1$ and $H^{\alpha}G_4$ b) $Hprp^b$ c) $Hprp^a$ and $Hprp^c$ spin systems of major conformer of Moc[GFA]G-OMe (**1a**) (400 MHz, 5 mM, 40% $CDCl_3$ in CD_3CN , 22 °C).

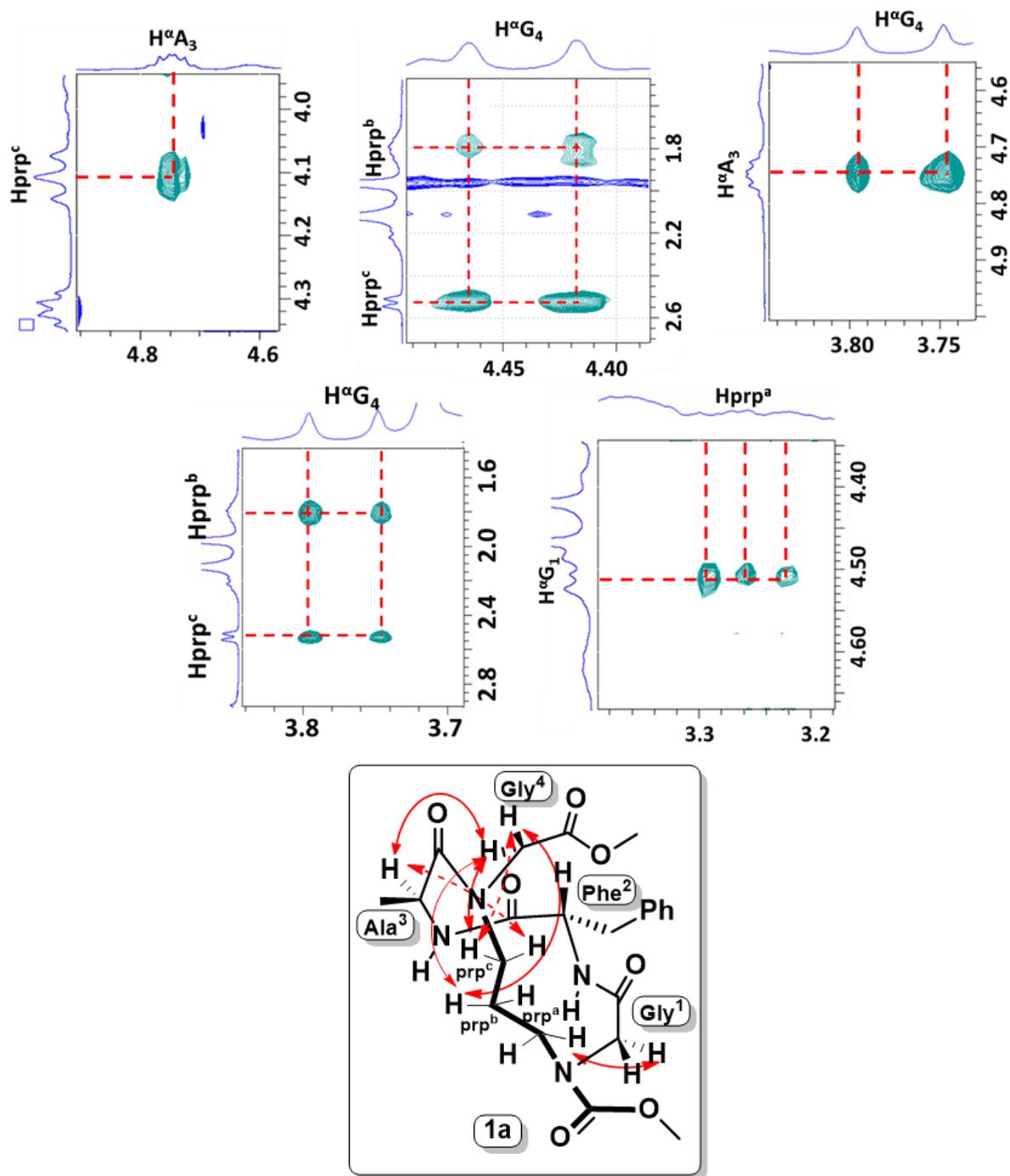


Figure S5. Rotating Frame Overhauser Effect Spectroscopy (ROESY) spectrum of major conformer of Moc[GFA]G-OMe (**1a**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C):

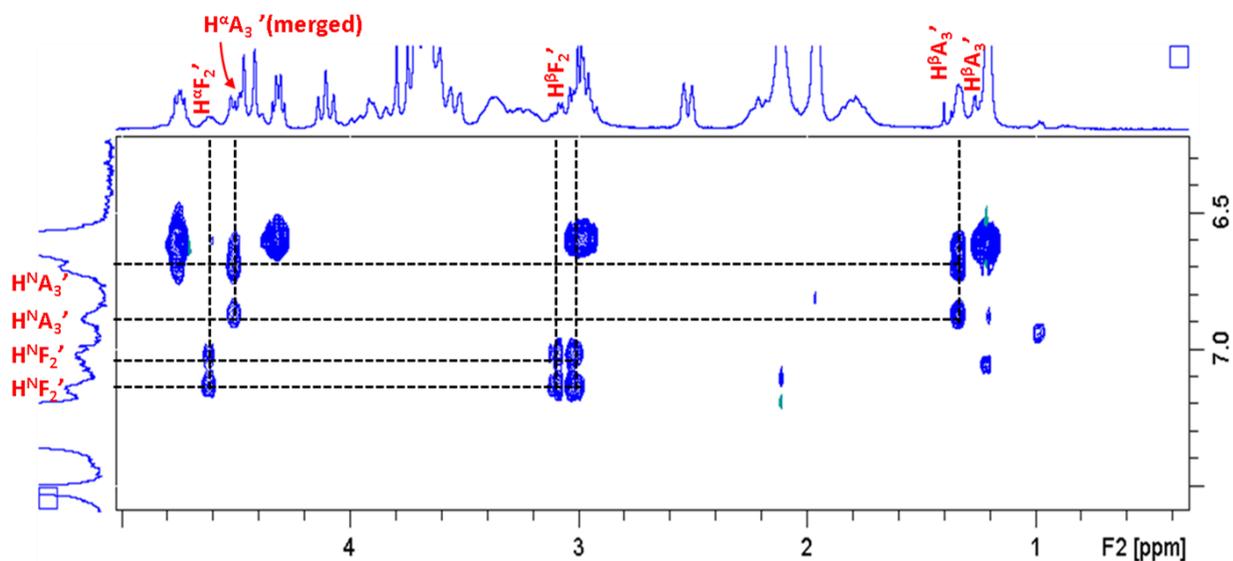


Figure S6. Total Correlation Spectroscopy (TOCSY) spectrum of minor conformer of Moc[GFA]G-OMe (**1a**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C).

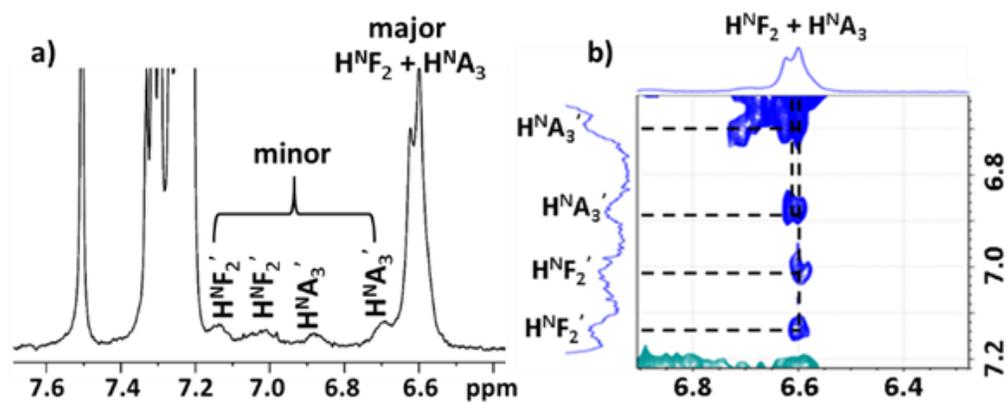


Figure S7. a) Expanded amide region of **1a** indicating major and minor sets of signals. b) Conformational exchange (EXSY) crosspeaks between major and minor conformers in Moc[GFA]G-OMe (**1a**).

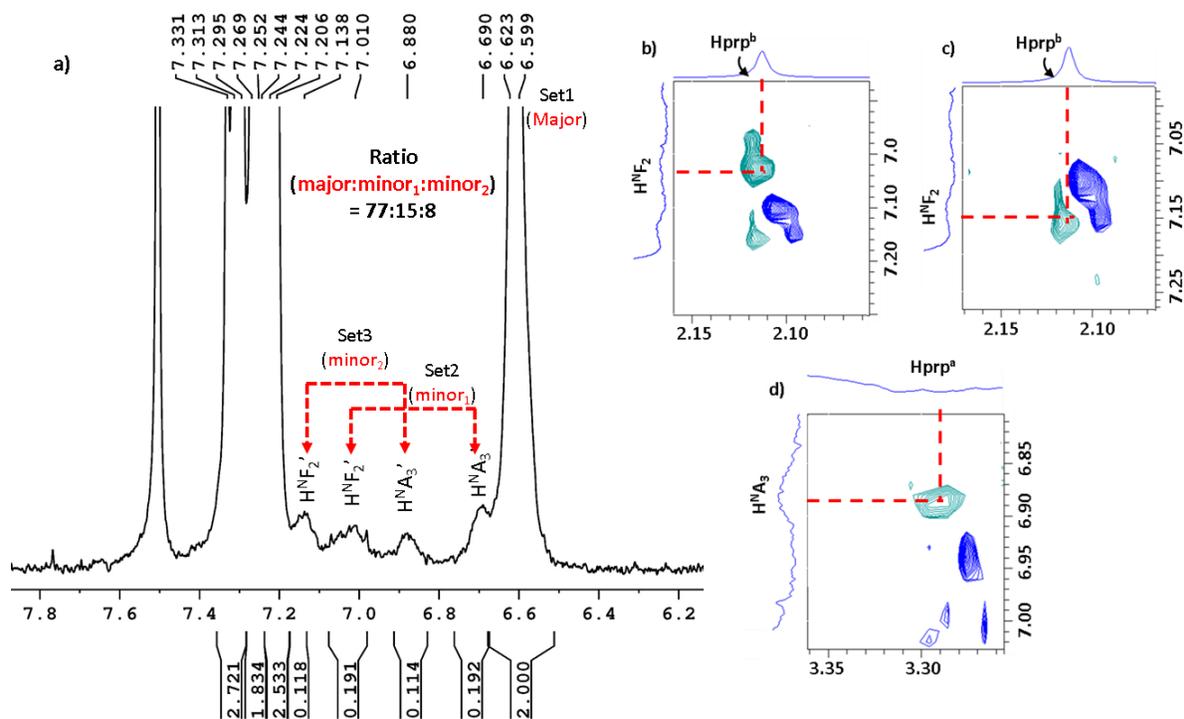


Figure S8. a) The NH region showing the major and minor peaks and their integrations of Moc[GFA]G-OMe (**1a**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C). The ROEs for the minor peaks are shown in **b**), **c**) and **d**).

Table S1. The list of chemical shifts for the major and the minor peaks for the NH regions and their corresponding ROE cross peaks of Moc[GFA]G-OMe (**1a**).

| | | | | | |
|---------------------------------|------------------------------------|------------------------------------|---------------------------------|------------------------|------------------------------------|
| 6.61 (F ₂) | 7.01 (F ₂) | 7.16 (F ₂) | 6.61 (A ₃) | 6.70 (A ₃) | 6.87 (A ₃) |
| (1) | (0.19) | (0.11) | (1) | (0.19) | (0.11) |
| Major | Minor ₁ | Minor ₂ | Major | Minor ₁ | Minor ₂ |
| (ROEs are discussed separately) | 2.11 (Hprp ^b) (ROE) | 2.11 (Hprp ^b) (ROE) | (ROEs are discussed separately) | - | 3.29 (Hprp ^a) (ROE) |

Ratio (major : minor₁ : minor₂) – (1.00 : 0.19 : 0.11) = (77 : 15 : 8) (Based on H^N proton)

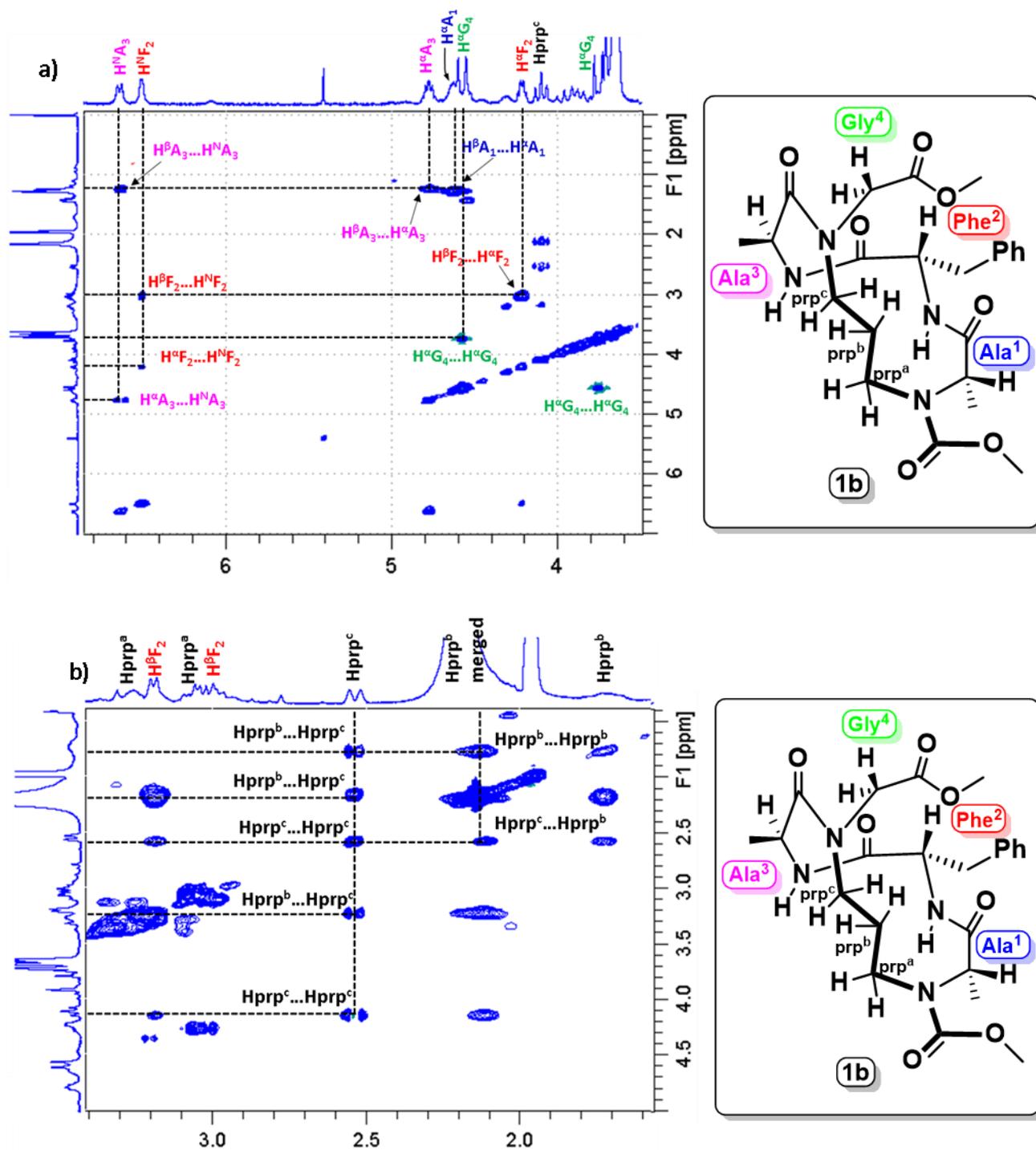
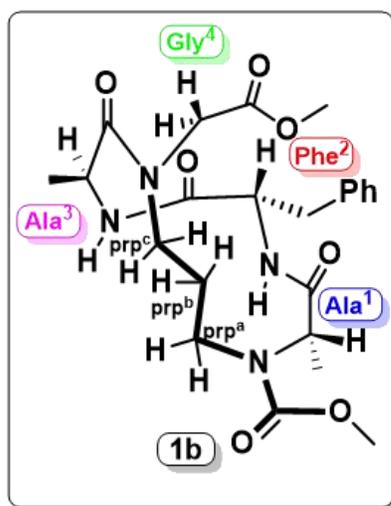
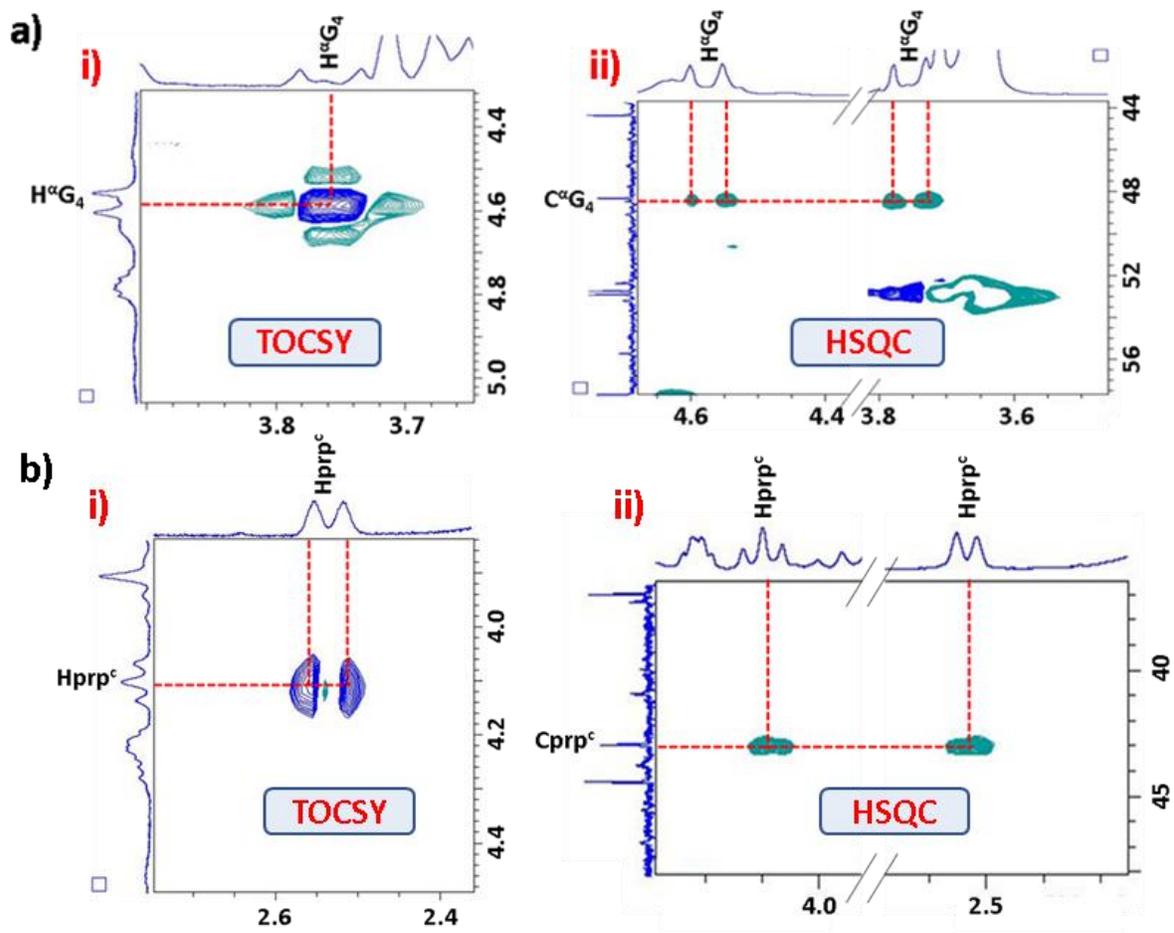


Figure S9. a), b) Total Correlation Spectroscopy (TOCSY) spectrum of major conformer of Moc[AFA]G-OMe (**1b**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C). a) Showing the through bond coupling of all the four amino acids, b) showing the through bond coupling between the prp protons of HBS part.



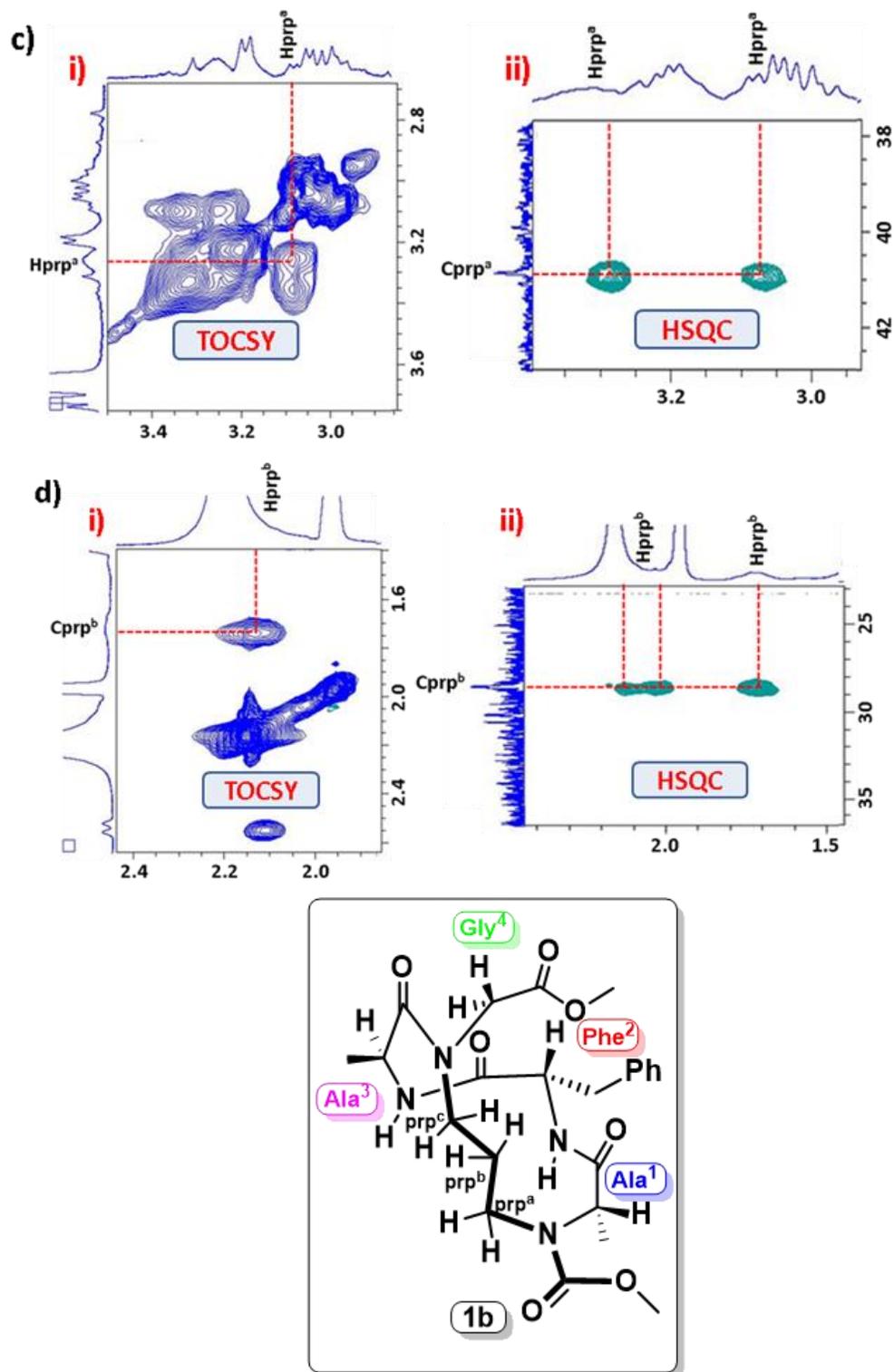


Figure S10. Relevant portions of TOCSY and HSQC spectra of a) H^aG₄ b)Hprp^c c) Hprp^a and d) Hprp^b spin systems of major conformer of Moc[AFA]G-OMe (1b) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C).

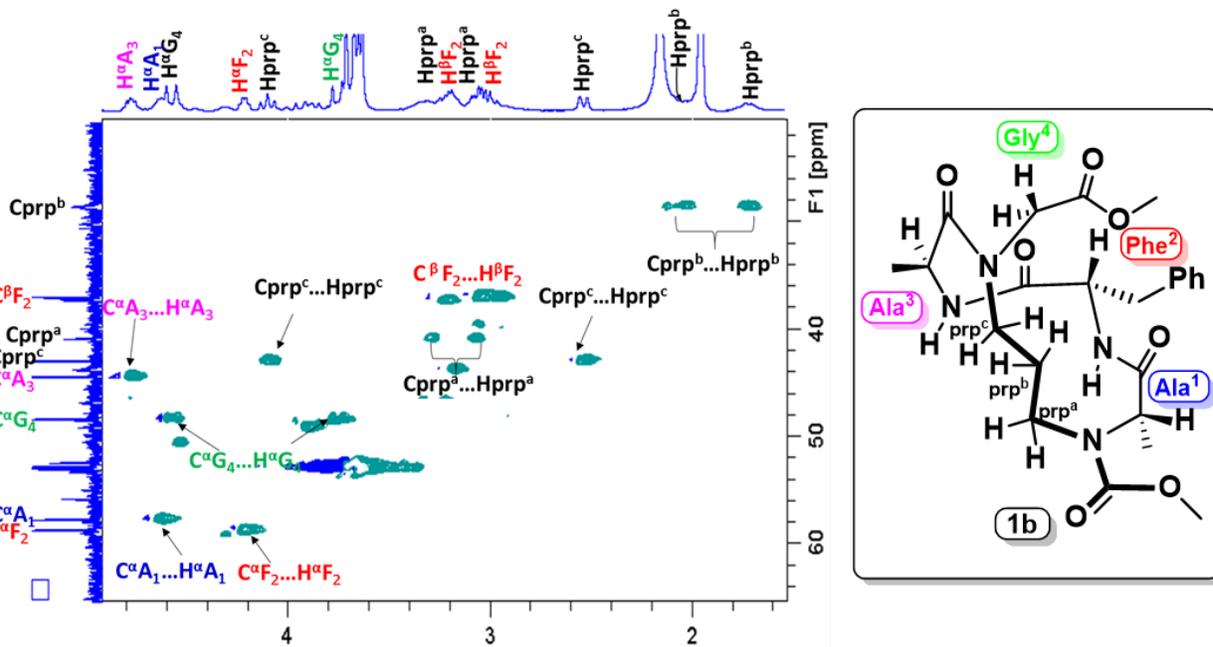


Figure S11. Heteronuclear Single Quantum Coherence (HSQC) spectrum of Moc[AFA]G-OMe (**1b**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C).

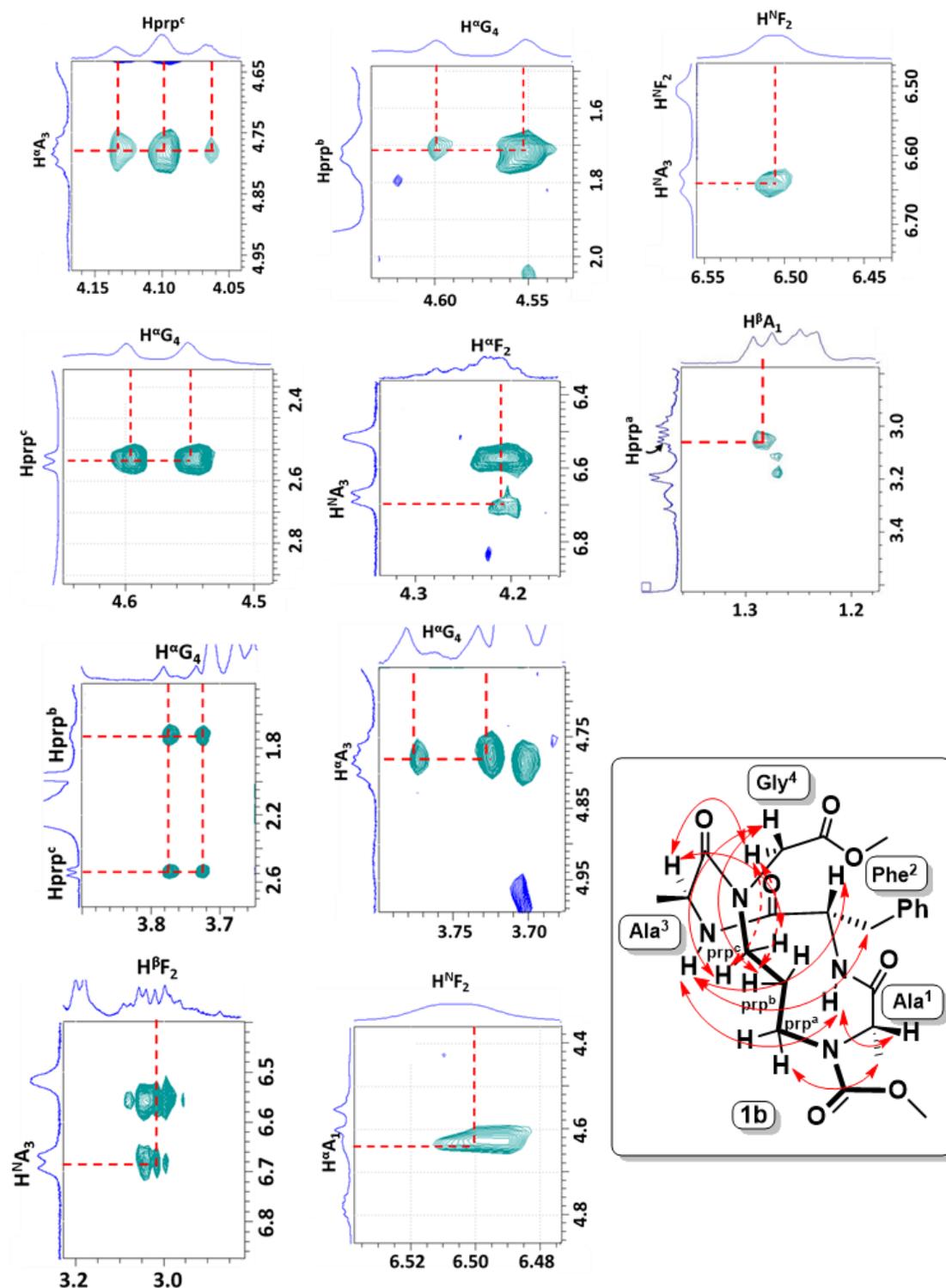


Figure S12. Rotating Frame Overhauser Effect Spectroscopy (ROESY) spectrum of major conformer of Moc[AFA]G-OMe (**1b**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C):

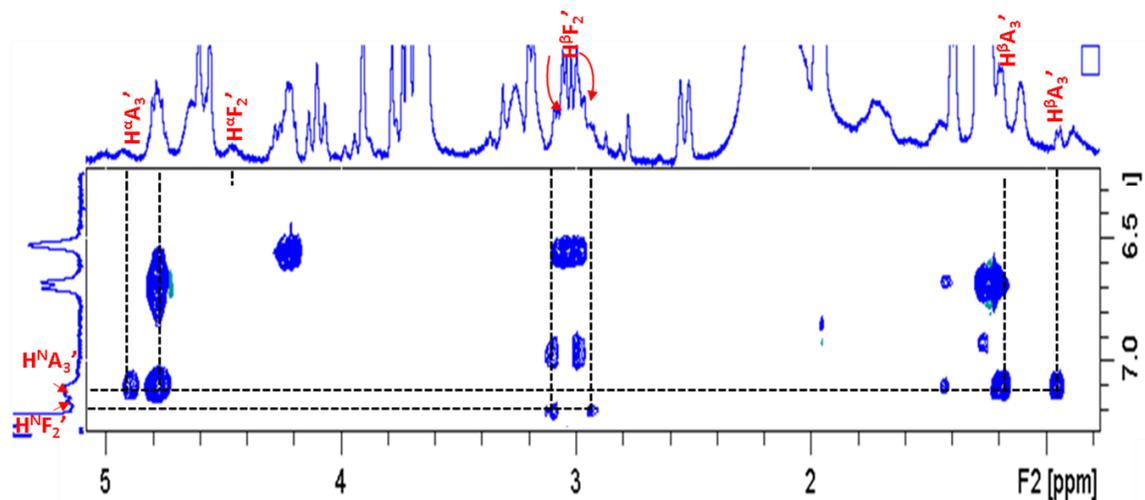


Figure S13. Total Correlation Spectroscopy (TOCSY) spectrum of minor conformer of Moc[AFA]G-OMe (**1b**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C).

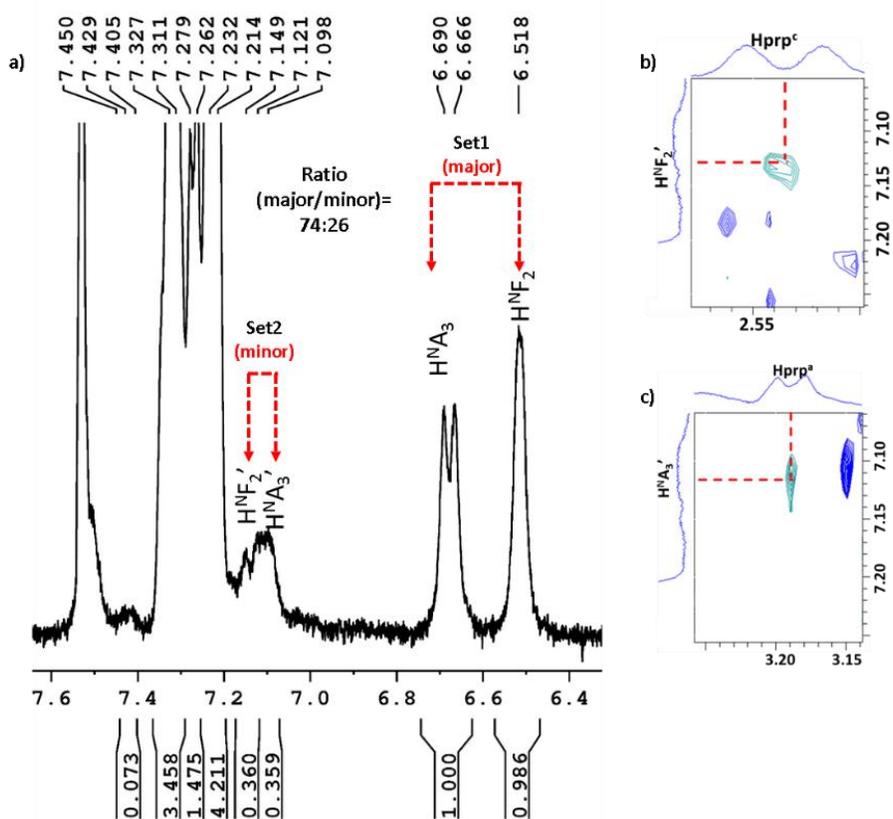


Figure S14. a) The NH region showing the major and minor peaks and their integrations of Moc[AFA]G-OMe (**1b**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C). The ROEs for the minor peaks are shown in **b**) and **c**).

Table S2. The list of chemical shifts for the major and the minor peaks for the NH regions and their corresponding ROE cross peaks of Moc[AFA]G-OMe (**1b**).

| | | | |
|---|--|---|--|
| 6.53 (F ₂) (1.0) Major | 7.14 (F ₂) (0.36) Minor | 6.66 (A ₃) (1.0) Major | 7.11 (A ₃) (0.36) Minor |
| (ROEs are discussed separately) | 2.53 (HPrp ^c) (ROE) | (ROEs are discussed separately) | 3.20 (HPrp ^a) (ROE) |

Ratio (major : minor) – (1 : 0.36) = 74 : 26 (Based on H^N proton relative integrals)

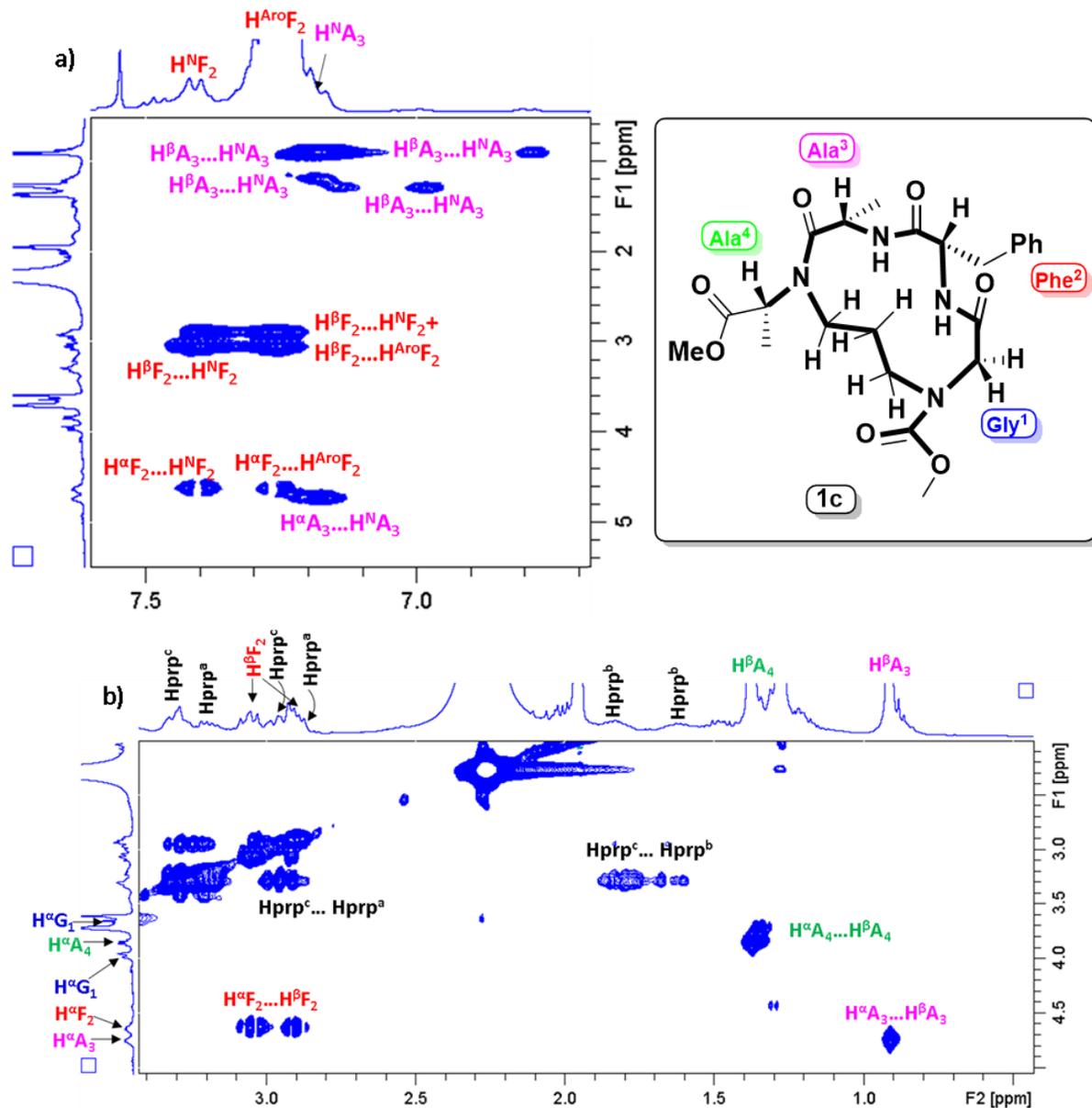


Figure S15. a), b) Total Correlated Spectroscopy (TOCSY) spectrum of major conformer of Moc[GFA]A-OMe (**1c**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C). a) Showing the through bond coupling of all the four amino acids, b) showing the through bond coupling between the prp protons of HBS part.

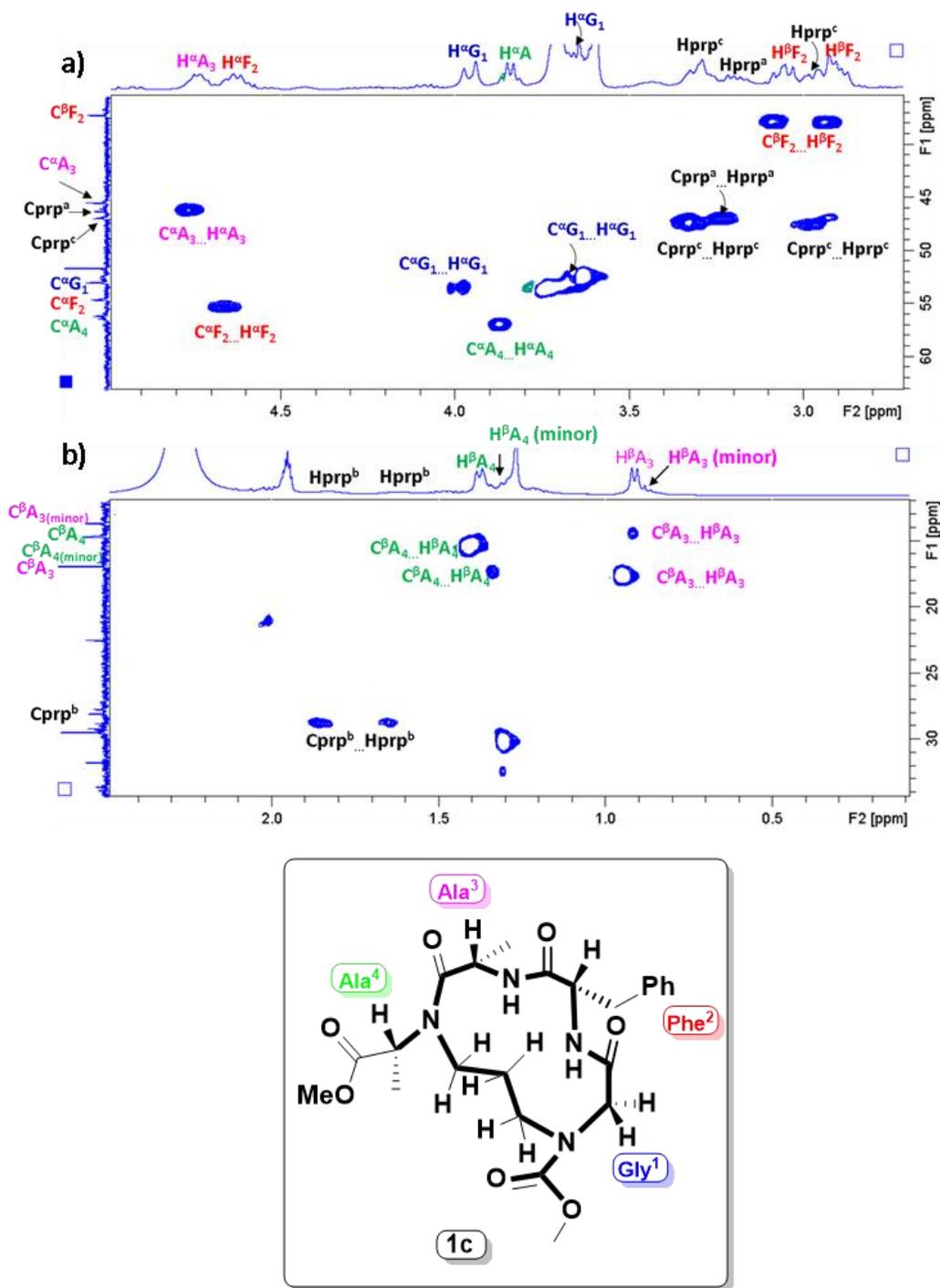


Figure S16. a), b) Heteronuclear Single Quantum Coherence (HSQC) spectrum of major conformer of Moc[GFA]A-OMe (1c) (400 MHz, 5 mM, 40% CDCl_3 in CD_3CN , 22 °C):

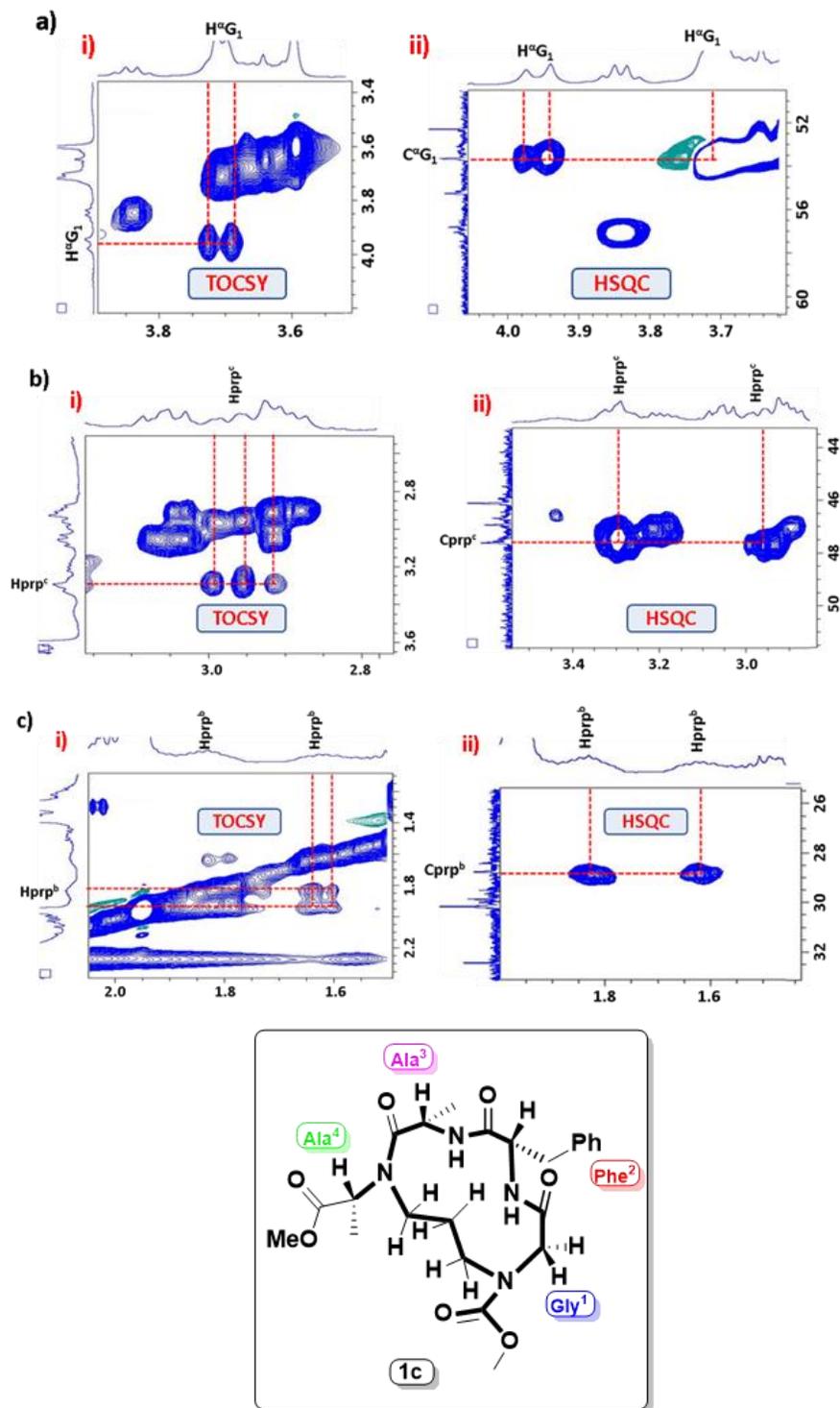


Figure S17. Relevant portions of TOCSY and HSQC spectra of a) $H^a G_1$ b) $Hprp^c$ c) $Hprp^b$ spin systems of major conformer of Moc[GFA]A-OMe (1c) (400 MHz, 5 mM, 40% $CDCl_3$ in CD_3CN , 22 °C).

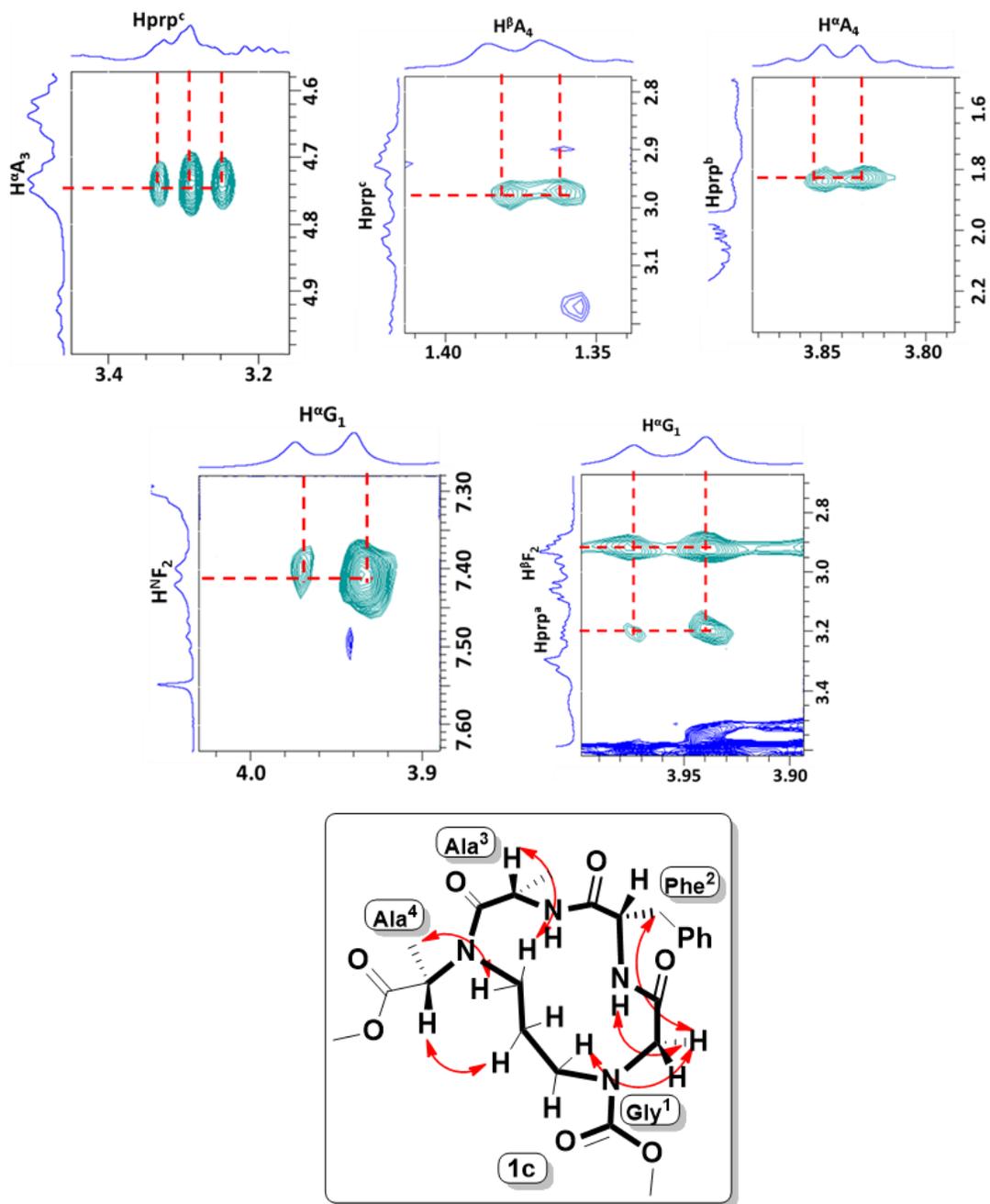


Figure S18. ROESY spectrum of major conformer of Moc[GFA]A-OMe (**1c**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C):

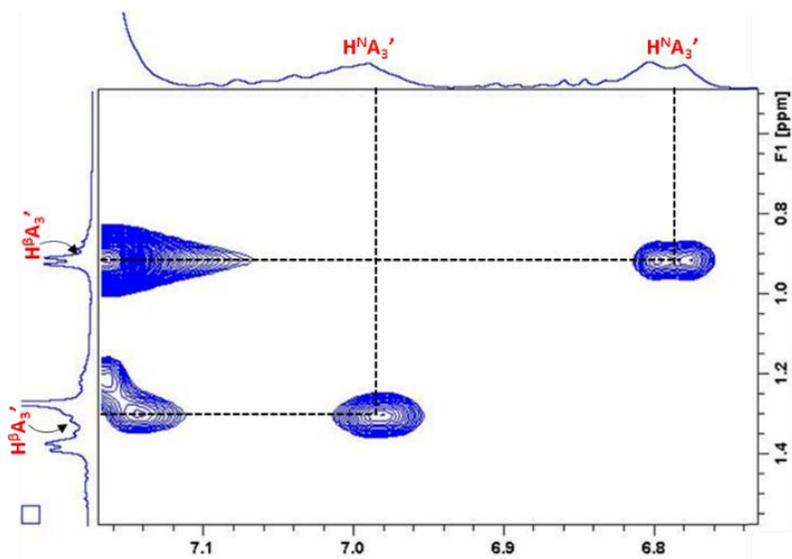


Figure S19. TOCSY spectrum of minor conformer of Moc[GFA]A-OMe (**1c**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C).

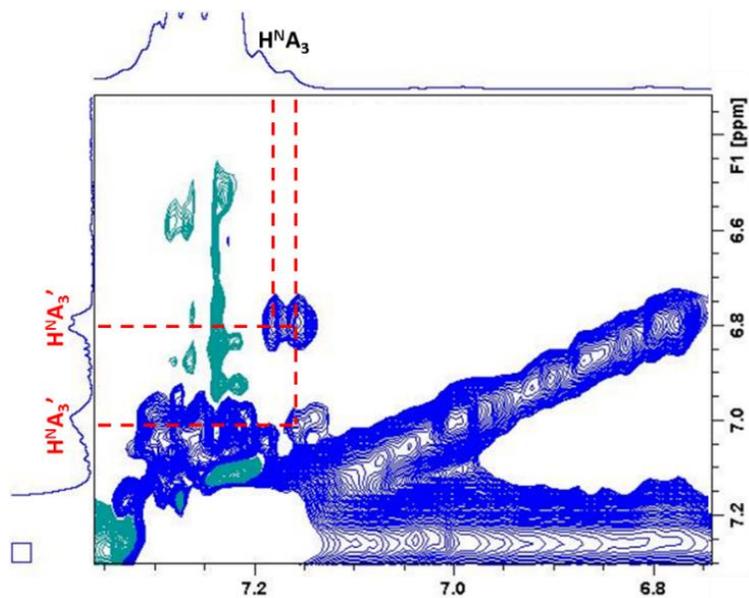
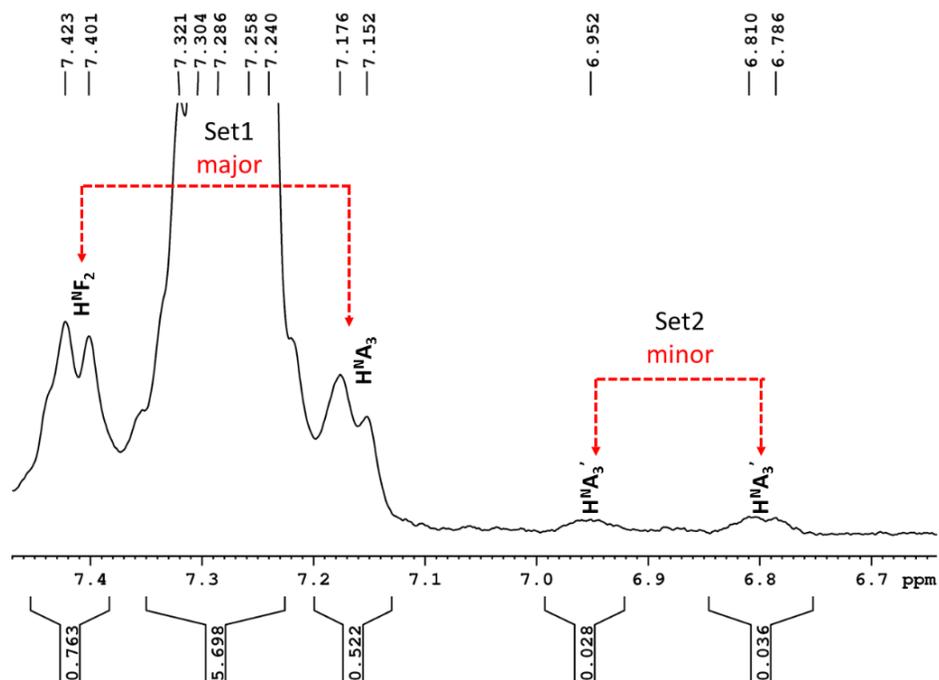


Figure S20. Conformational exchange (EXSY) crosspeaks between major and minor conformers in Moc[GFA]A-OMe (**1c**).



Ratio (major : minor) – $0.522 : (0.028+0.036) = 0.522 : 0.064 = 89 : 11$ (only the Ala NH shows the minor conformer, there is no trace of minor conformation for Phe)

Figure S21. The NH region showing the major and minor peaks and their integrations of Moc[GFA]A-OMe (**1c**) (400 MHz, 5 mM, 40% CDCl₃ in CD₃CN, 22 °C).

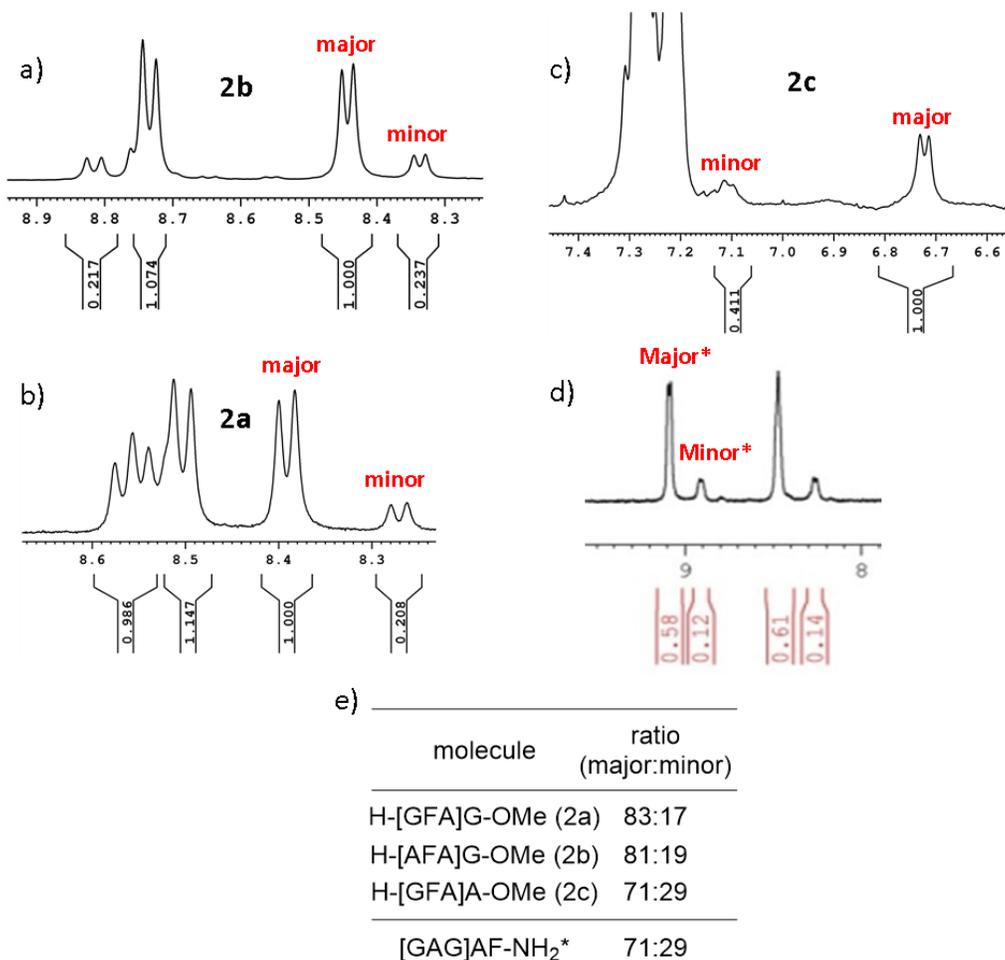


Figure S22. a) to c) shows the ¹H NMR amide NH chemical shift regions of the 13₆3 analogues **2a-c**, showing their major and minor peaks. In d) the similar analysis from Broussy et al. (*Org. Biomol. Chem.*, **2018**, *16*, 459) is reproduced. In the e) the relative peak integrals are tabulated.

* the integral ratio is reproduced from *Org. Biomol. Chem.*, **2018**, *16*, 459

Table S3. Tabulation of the (major:minor) ratios of **1a-c** and **2a-c** analogues.

| analogue | molecule | ratio (major:minor) |
|------------------------|------------------------------|---------------------|
| 13₇3 | Moc-[GFA]G-OMe (1a) | 77:23 |
| | Moc-[AFA]G-OMe (1b) | 74:26 |
| | Moc-[GFA]A-OMe (1c) | 89:11 |
| 13₆3 | H-[GFA]G-OMe (2a) | 83:17 |
| | H-[AFA]G-OMe (2b) | 81:19 |
| | H-[GFA]A-OMe (2c) | 71:29 |

Table S4. Comparative a) ^1H NMR and b) ^{13}C chemical shift values of **1a**, **1b** and **1c**.

| a) | | 1a | | A1 1b | | A4 1c | |
|-------|----------------------------------|------------------------------------|---------|---------------|-------|---------------|-------|
| | | $(\delta \text{ ppm}) \rightarrow$ | | | | | |
| Table | Ratio | major | minor | major | minor | major | minor |
| | | 77 | 23 | 70 | 30 | 88 | 12 |
| 1 | $\text{CH}_3(\text{Moc})$ | 3.66 | 3.66 | 3.65 | 3.65 | 3.62 | 3.62 |
| 2 | $\text{H}^\alpha(i+1)(\text{A})$ | 4.50 | - | 4.66 | - | 3.99 | - |
| 3 | $\text{H}^\alpha(i+1)(\text{B})$ | 3.54 | - | NA | NA | 3.69 | - |
| 4 | $\text{H}^\beta(i+1)$ | NA | NA | 1.28 | 1.44 | NA | NA |
| 5 | $\text{H}^N(i+2)$ | 6.61 | 7.13 bs | 6.51 | 7.42 | 7.41 | - |
| 6 | | 7.03 | 6.02 | 6.10 | 7.10 | | |
| 7 | $\text{H}^\alpha(i+2)$ | 4.31 | - | 4.21 | 4.31 | 4.65 | - |
| 8 | $\text{H}^\beta(i+2)$ | 3.04- 2.96 | - | 3.07- 2.97 | - | 3.13- 3.04 | - |
| 9 | $\text{H}^N(i+3)$ | 6.61 | 6.87 | 6.68 | 7.91 | 7.16 | 6.95 |
| 10 | | 6.72 | 5.97 | 6.68 | 7.15 | | 6.79 |
| 11 | $\text{H}^\alpha(i+3)$ | 4.75 | 4.61 | 4.78 | 4.92 | 4.76 | - |
| 12 | $\text{H}^\beta(i+3)$ | 1.21 | 1.33 | 1.24 | 1.17 | 0.94 | 0.92 |
| 13 | $\text{H}^\alpha(i+4)(\text{A})$ | 4.44 | - | 4.58 | - | 3.86 | - |
| 14 | $\text{H}^\alpha(i+4)(\text{B})$ | 3.77 | - | 3.76 | - | NA | NA |
| 15 | $\text{H}^\beta(i+4)$ | NA | NA | NA | NA | 1.40 | 1.34 |
| 16 | $\text{CH}_3(\text{MeO})$ | 3.71 | 3.71 | 3.71 | 3.71 | 3.74 | 3.74 |

1a. Moc-[GFA]-G-OMe; **1b.** Moc-[AFA]-G-OMe; **1c.** Moc-[GFA]-A-OMe

| b) | | 1a | | A1 1b | | A4 1c | |
|-------|---------------------------|---|-------|-------|-------|-------|-------|
| | | $(^{13}\text{C}, \delta \text{ ppm}) \rightarrow$ | | | | | |
| Table | Ratio | major | minor | major | minor | major | minor |
| | | 77 | 23 | 70 | 23 | 88 | 12 |
| 1 | $\text{CH}_3(\text{Moc})$ | 52.9 | 52.9 | 53.5 | 53.5 | 53.0 | 53.0 |
| 2 | $\text{C}^\alpha(i+1)$ | 52.5 | - | 58.3 | - | 46.9 | - |
| 3 | $\text{C}^\beta(i+1)$ | NA | NA | 17.4 | 18.1 | NA | NA |
| 4 | $\text{C}^\alpha(i+2)$ | 58.1 | - | 59.2 | 59.5 | 56.1 | - |
| 5 | $\text{C}^\beta(i+2)$ | 36.8 | - | 37.6 | - | 38.6 | - |
| 6 | $\text{C}^\alpha(i+3)$ | 44.4 | 56.5 | 45.0 | 50.4 | 46.9 | - |
| 7 | $\text{C}^\beta(i+3)$ | 16.9 | 17.5 | 15.9 | 18.3 | 18.3 | 18.0 |
| 8 | $\text{C}^\alpha(i+4)$ | 48.2 | - | 48.9 | - | 57.6 | - |
| 10 | $\text{C}^\beta(i+4)$ | NA | NA | NA | NA | 16.0 | 15.2 |
| 11 | Me(Est) | 52.6 | 52.6 | 53.4 | 53.4 | 54.3 | 54.3 |

1a. Moc-[GFA]-G-OMe; **1b.** Moc-[AFA]-G-OMe; **1c.** Moc-[GFA]-A-OMe

4.3 Circular Dichroism (CD) spectral analyses

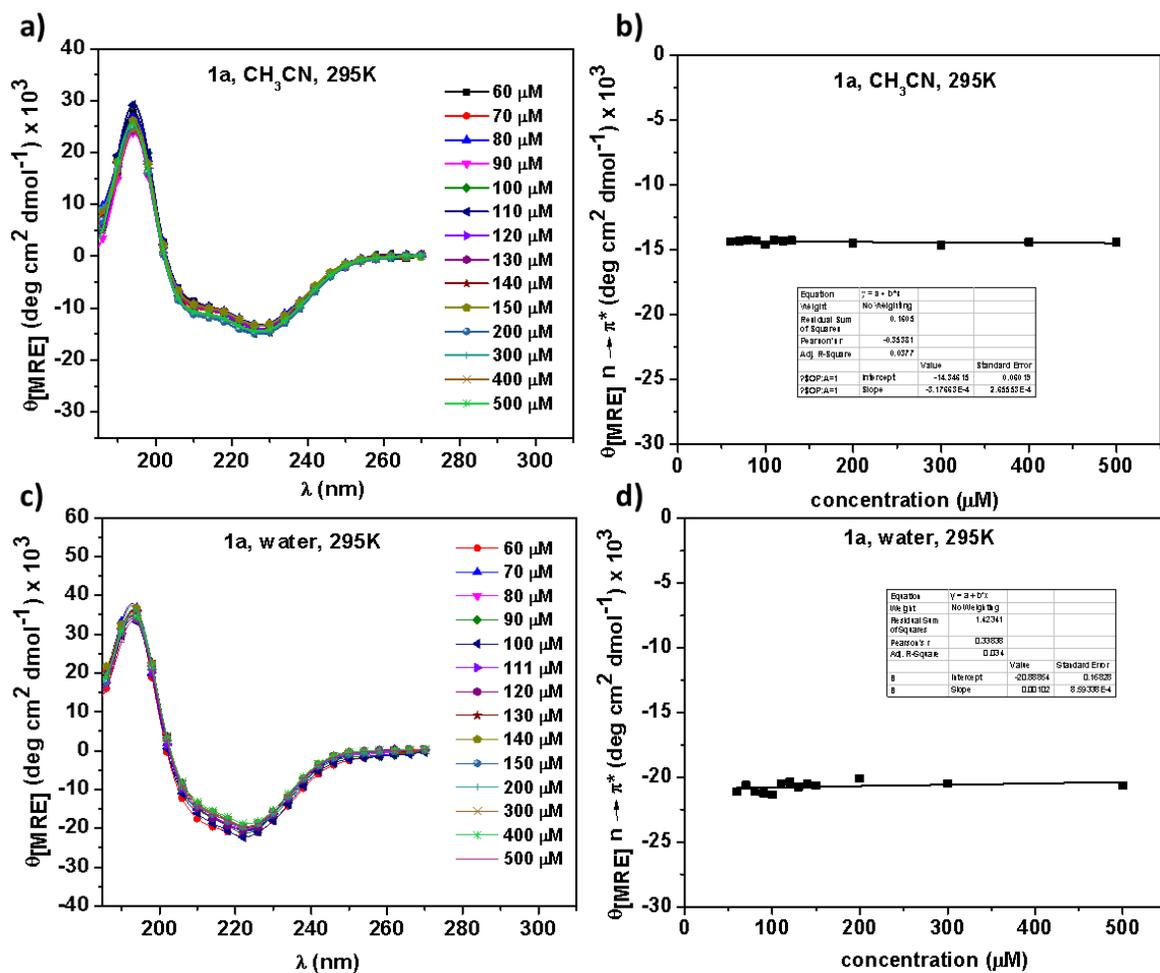


Figure S24. CD spectral plots of a), c) θ_{MRE} (mean molar residue ellipticity) vs λ_{nm} and b), d) $\theta_{\text{n} \rightarrow \pi^* \text{MRE}}$ vs concentration of (Moc[GFA]G-OMe, 1a) in CH₃CN (295 K) and water (295 K) respectively.

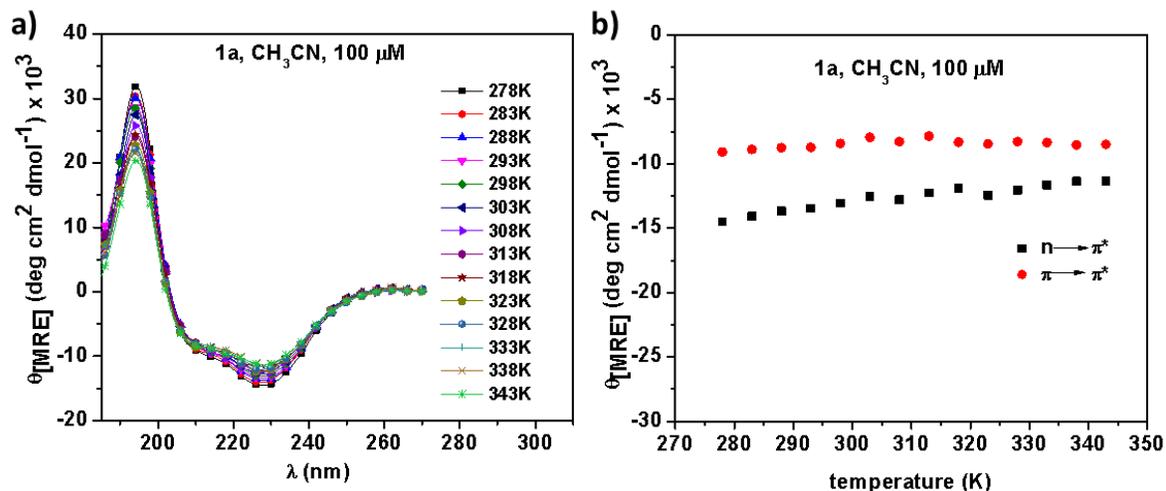


Figure S25. Temperature-dependent (278 – 343 K, CH₃CN, 100 μM) CD spectra of Moc[GFA]G-OMe, **1a**: Plot of: a) θ_{MRE} vs λ ; b) $\theta_{n \rightarrow \pi^* \text{MRE}}$ and $\theta_{\pi \rightarrow \pi^* \text{MRE}}$ vs temperature.

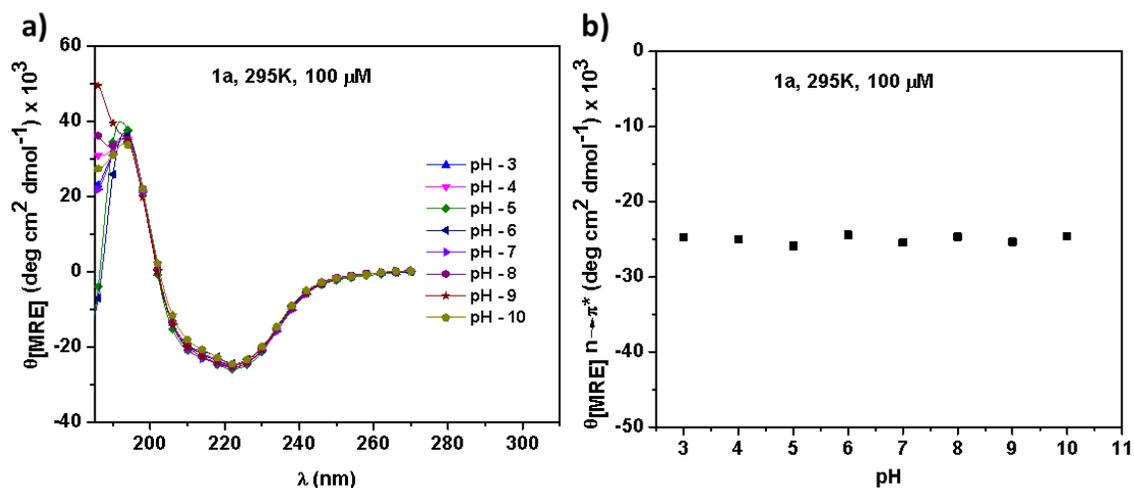


Figure S26. a) pH-dependent CD spectra of Moc[GFA]G-OMe, **1a**. pH – 3 – 4 (30 mM acetate buffer, 295 K, 100 μM), pH – 5 - 7 (30 mM phosphate buffer, 295 K, 100 μM), pH – 8 - 10 (30 mM carbonate buffer, 295 K, 100 μM). b) Plot of $\theta_{n \rightarrow \pi^* \text{MRE}}$ vs pH.

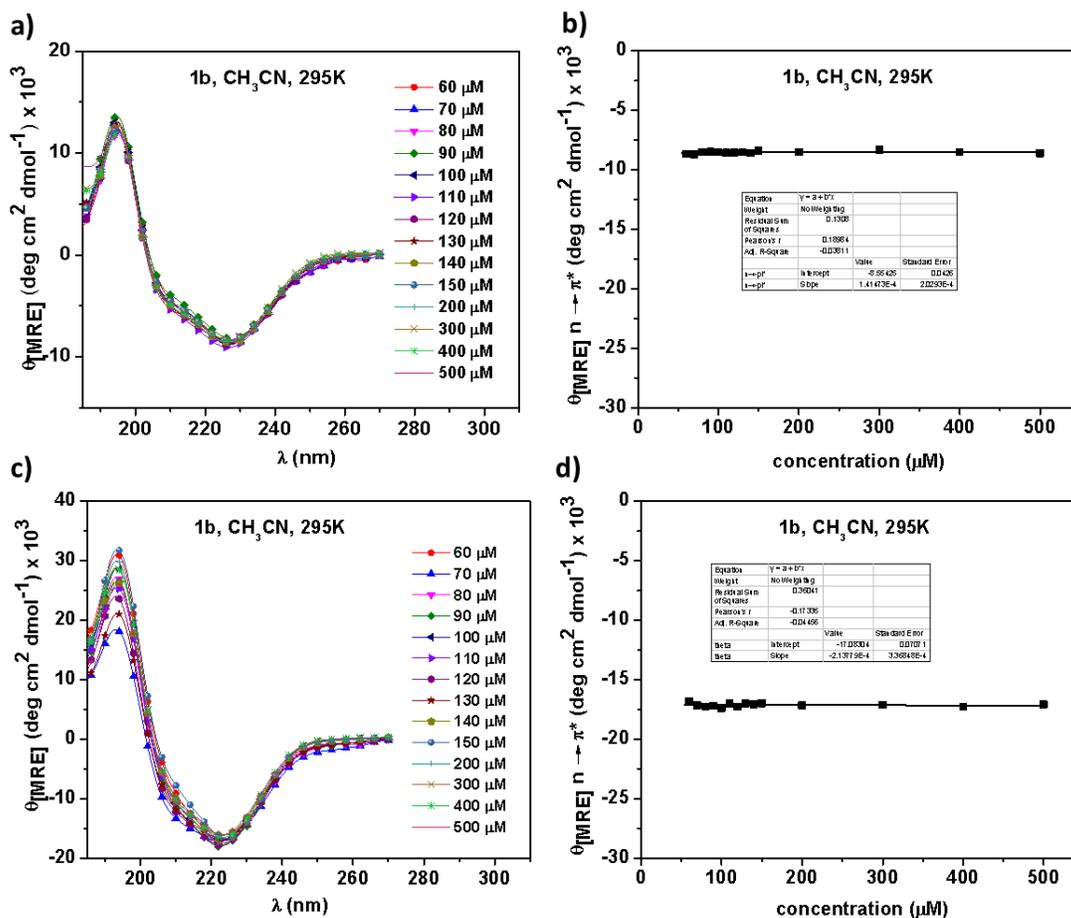


Figure S27. CD spectral plots of a), c) θ_{MRE} (mean molar residue ellipticity) vs λ_{nm} and b), d) $\theta_{n \rightarrow \pi^* \text{MRE}}$ vs concentration of Moc[AFA]G-OMe, **1b** in CH₃CN (295 K) and water (295 K) respectively.

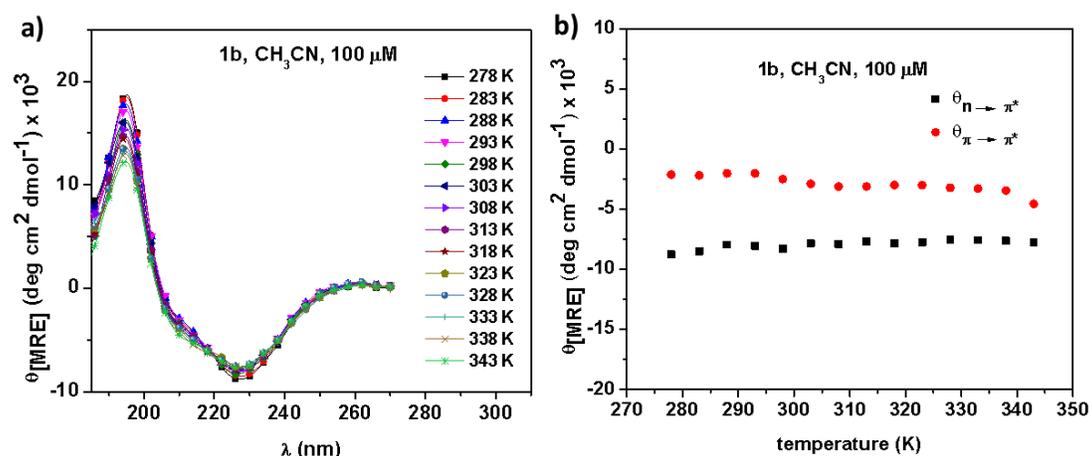


Figure S28. Temperature-dependent (278 – 343 K, 100 μM) CD spectra of Moc[AFA]G-OMe, **1b** in CH₃CN: a) θ_{MRE} vs λ ; b) $\theta_{n \rightarrow \pi^* \text{MRE}}$ and $\theta_{\pi \rightarrow \pi^* \text{MRE}}$ vs temperature.

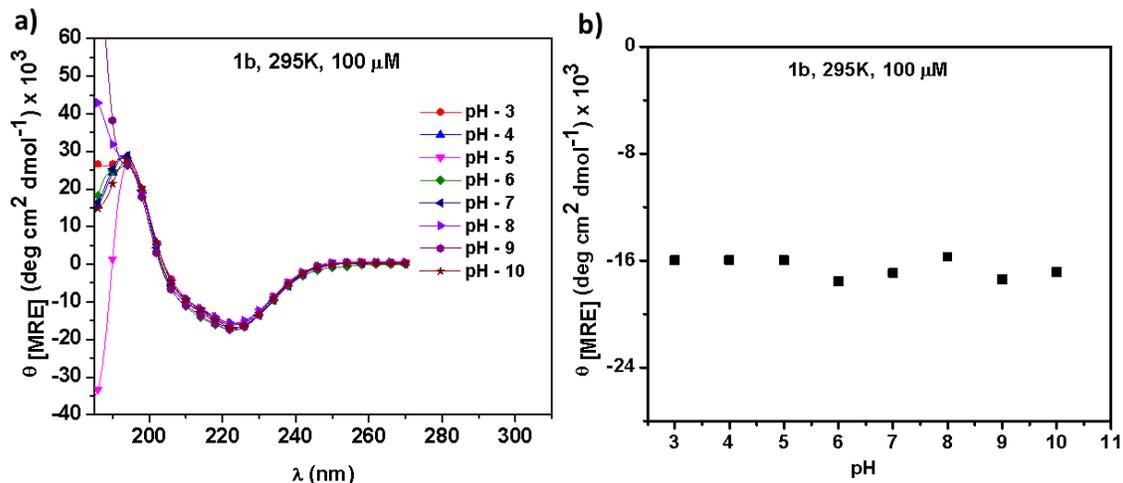


Figure S29. a) pH-dependent CD spectra of Moc[AFA]G-OMe, **1b**. pH – 3 – 4 (30 mM acetate buffer, 295 K), pH – 5 - 7 (30 mM phosphate buffer, 295 K), pH – 8 - 10 (30 mM carbonate buffer, 295 K). b) Plot of $\theta_{n \rightarrow \pi^*}$ MRE vs pH. The peptide concentration is 100 μM.

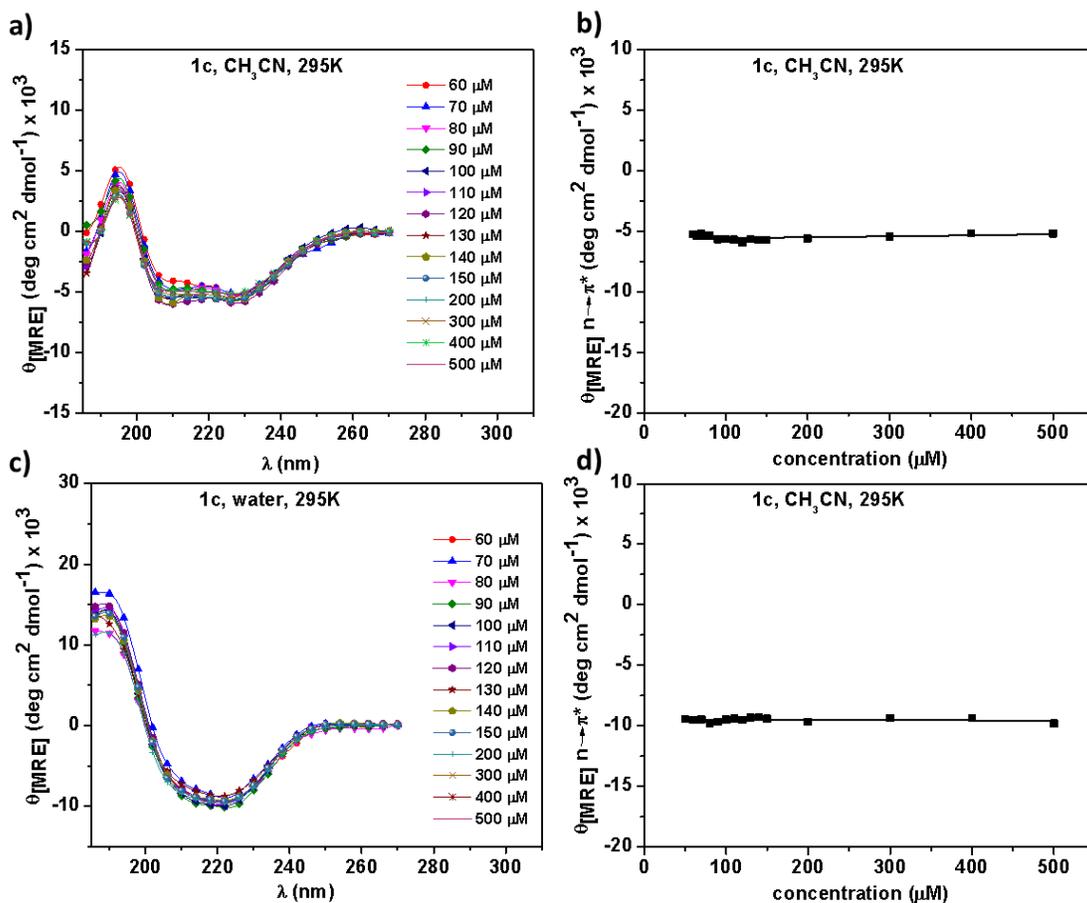


Figure S30. CD spectral plots of a), c) θ_{MRE} (mean molar residue ellipticity) vs λ_{nm} and b), d) $\theta_{n \rightarrow \pi^*}$ MRE vs concentration of Moc[GFA]A-OMe, **1c** in CH₃CN (295 K) and water (295 K) respectively.

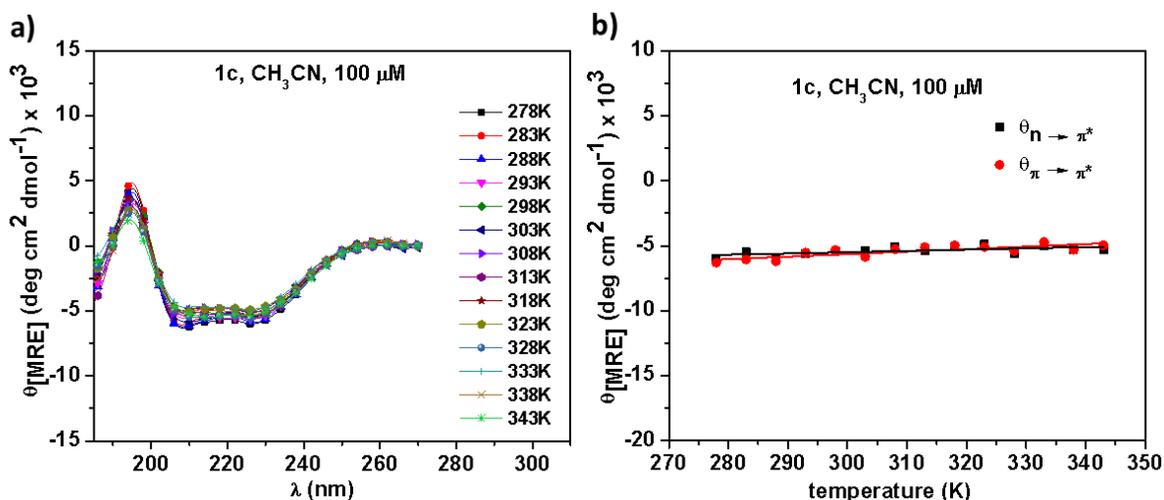


Figure S31. Temperature-dependent (278 – 343 K, 100 μM) CD spectra of Moc[GFA]A-OMe, **1c** (in CH_3CN): a) θ_{MRE} vs λ_{nm} ; b) $\theta_{\text{n} \rightarrow \pi^*}$ and $\theta_{\pi \rightarrow \pi^*}$ vs temperature.

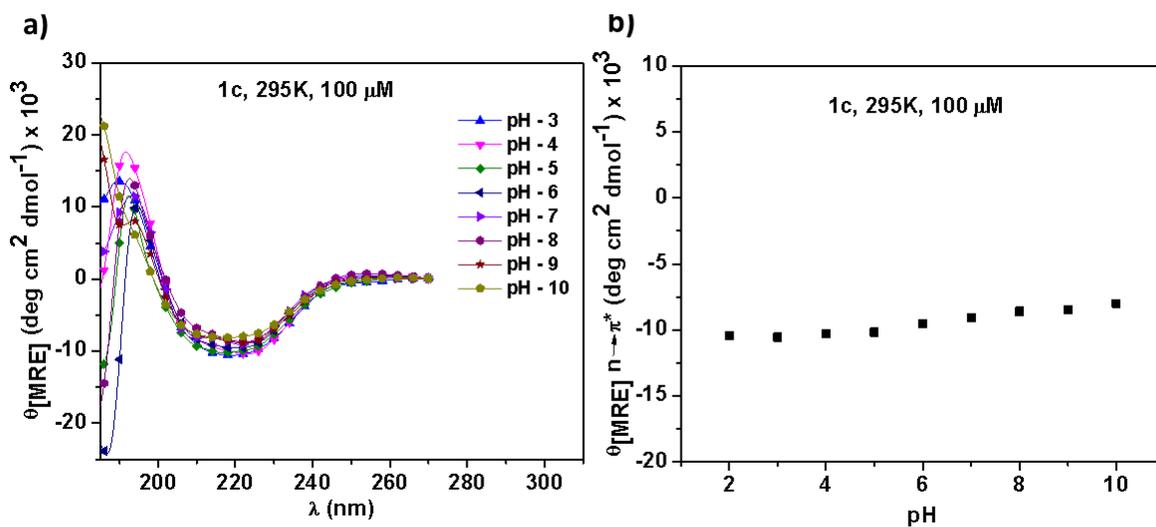


Figure S32. a) pH dependent CD spectra of Moc[GFA]A-OMe, **1c**. pH – 3–4 (30 mM acetate buffer), pH – 5–7 (30 mM phosphate buffer), pH – 8–10 (30 mM carbonate buffer). b) Plot of $\theta_{\text{n} \rightarrow \pi^*}$ vs pH. The peptide concentration is 100 μM .

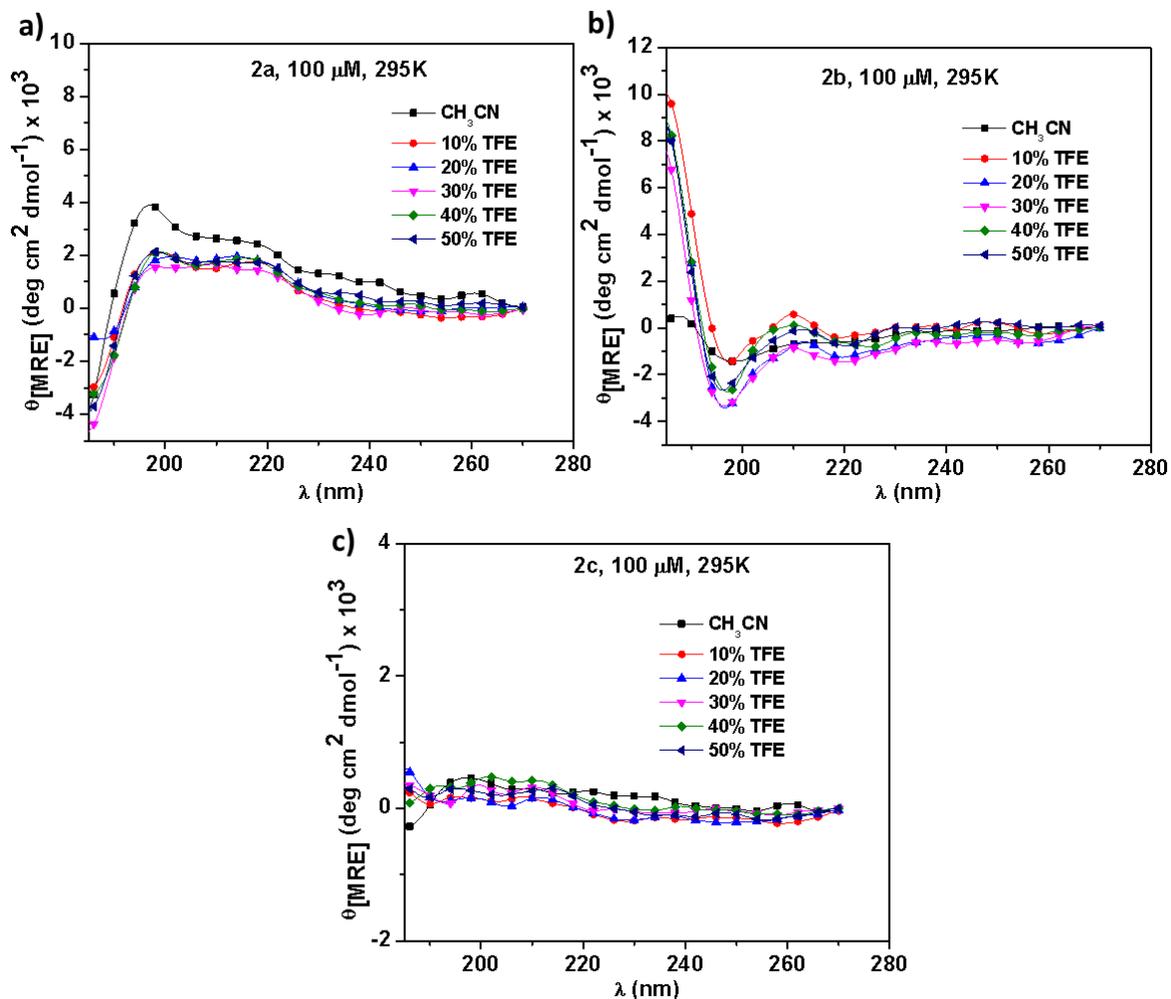


Figure S33. CD spectra of 1363 analogues a) **2a**, b) **2b** and c) **2c** at 0-50% TFE in CH_3CN . Increasing the % of helix-inducing solvent TFE does not impose helicities, showing that these 1363 model peptides truly lack any helical order. The peptide concentration is 100 μM at 295 K.

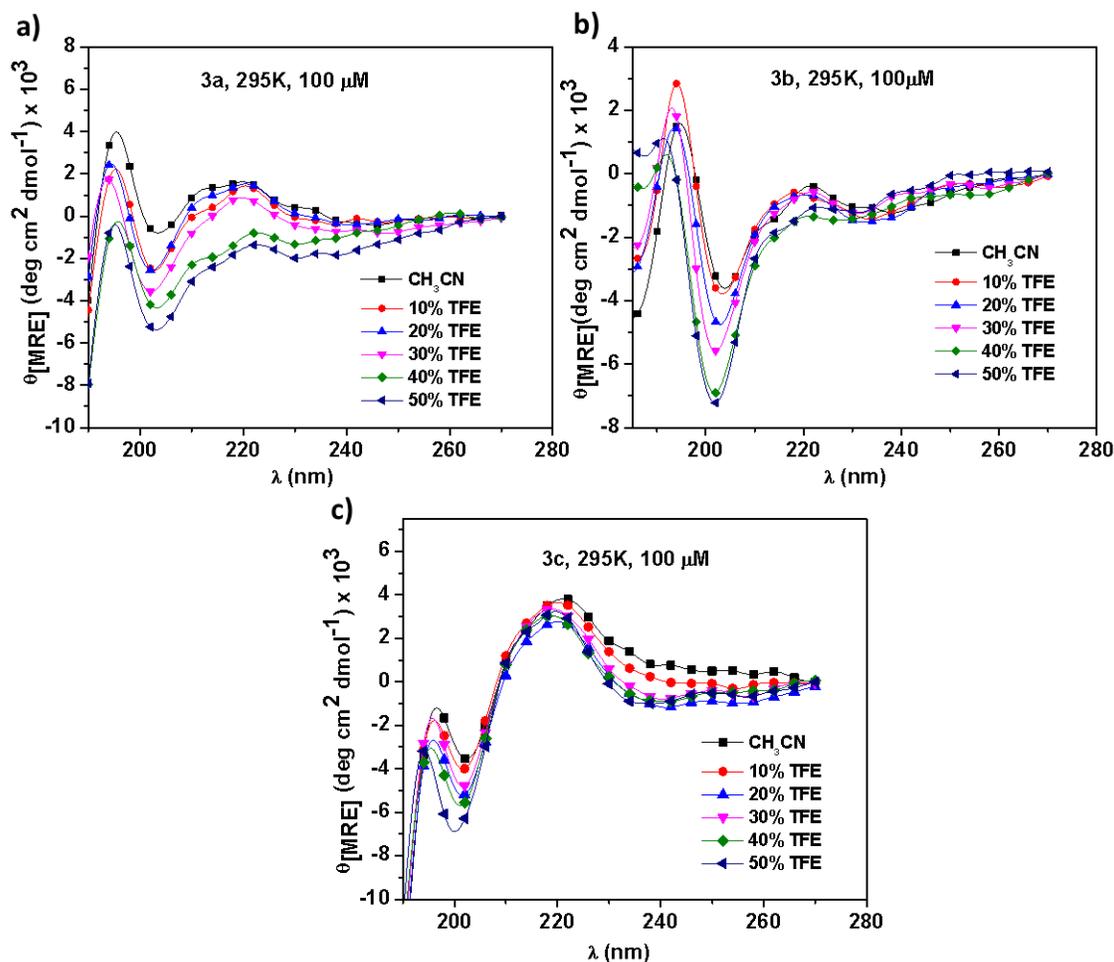


Figure S34. CD spectra of acyclic linear tetrapeptide analogues a) **3a**, b) **3b** and c) **3c** at 0-50% TFE in CH₃CN. Increasing the % of helix-inducing solvent TFE does not impose helicities, showing that these tetrapeptides truly lack any helical order. The peptide concentration is 100 μM at 295 K.

Table S6. Theoretical AGADIR helicity prediction of different sequences.

| No | Sequence | AGADIR* predicted % helicities |
|----|----------|--------------------------------------|
| 1 | GFAG | 0.08 |
| 2 | AFAG | 0.38 |
| 3 | GFAA | 0.18 |
| 4 | GFGG | 0.01 |
| 5 | AFGG | 0.06 |

*Serrano *et.al.* *Nat. Struct. Mol. Biol.* **1994**, *1*, 399-409

Table S7. Comparison of $\theta_{n \rightarrow \pi^*}$ and thermal ellipticity coefficient ($\Delta\theta/\Delta K$) values of the current single α -helical turn models Moc[GFA]G-OMe (**1a**) and Moc[AFA]G-OMe (**1b**) with corresponding values for earlier reported models:

| No | Sequence | Constraint | $\theta_{n \rightarrow \pi^*}$ $\times 10^3$ | $\Delta\theta/\Delta K$ | Ref. |
|----|---|--------------------------------|---|-------------------------|------|
| 1 | Moc-[G F A] G -OMe (1a) | HBS + Moc | -25.3 | -48 | a |
| 2 | Moc-[A F A] G -OMe (1b) | HBS + Moc | -16.9 | -16 | a |
| 3 | [YLL]G-OMe | HBS N- and C-terminal extended | ~-22 | - | b |
| 3 | EF-hand+La ³⁺ | calmodulin + La ³⁺ | ~-22 | ~-57 | c |
| 4 | [K A A AD]lactam | SC-SC x covalent | ~-13.5 | ~-144 | d |
| | [K A R AD]lactam | SC-SC x covalent | ~-12.8 | ~-150 | e |
| 5 | Ala-rich | SC-SC x covalent | ~-16 | - | f |

References.

- a. Prabhakaran et al. present work
b. Alewood et al. *Angew. Chem. Int. Ed.*, **2009**, *48*, 5675.
c. Baldwin et al., *Proc. Natl. Acad. Sci. U.S.A.*, **2002**, *99*, 15416.
d. Fairlie et al., *J. Am. Chem. Soc.*, **2005**, *127*, 2974.
e. Fairlie et. al., *J. Am. Chem. Soc.*, **2005**, *127*, 2974.
f. Fairlie et al., *Angew. Chem. Int. Ed.*, **2014**, *53*, 6965.

Table S8. The effect of solvent H-bonding interactions and ionic groups on the excitonic transition energies ($\lambda_{n \rightarrow \pi^*}$ and $\lambda_{\pi \rightarrow \pi^*}$ nm) of the peptide chromophores in **1a**, **1b**, **1c**.

| | 1a | | | 1b | | | 1c | | | |
|--------------------|--|--|---------------------------------|--|--|---------------------------------|--|--|---------------------------------|---------------------------------|
| | $\lambda_{\pi \rightarrow \pi^*(\parallel)}$ | $\lambda_{\pi \rightarrow \pi^*(\perp)}$ | $\lambda_{n \rightarrow \pi^*}$ | $\lambda_{\pi \rightarrow \pi^*(\parallel)}$ | $\lambda_{\pi \rightarrow \pi^*(\perp)}$ | $\lambda_{n \rightarrow \pi^*}$ | $\lambda_{\pi \rightarrow \pi^*(\parallel)}$ | $\lambda_{\pi \rightarrow \pi^*(\perp)}$ | $\lambda_{n \rightarrow \pi^*}$ | $\lambda_{n \rightarrow \pi^*}$ |
| CH ₃ CN | 209.8 | 194.2 | 227.8 | 207.4 | 194.8 | 226.4 | 195.2 | 208.4 | 226.2 | 218.0 |
| water | 209.4 | 193.2 | 223.0 | 208.1 | 193.2 | 222.6 | 194.4 | 208.4 | 222.2 | - |
| pH - 7 | 208.6 | 193 | 222.8 | 208.0 | 193.2 | 223.2 | 193.4 | 209.8 | 221.6 | - |

Note: the relatively large blue shifts in $\lambda_{n \rightarrow \pi^*}$ values upon H-bonding and ionic interactions with solvent.

Table S9. Comparison of $\theta_{n \rightarrow \pi^* \text{MRE}}$ intensities for the α -helically coupled peptide transitions in the 13₇3 turn analogues (**1a**, **1b**, **1c**) and for their corresponding acyclic linear tetrapeptide analogues (**3a**, **3b**, **3c**).

| | | $\theta_{n \rightarrow \pi^* \text{MRE}}$ in different solvents | | |
|-----------|------------------------|---|------------------------|------------------------|
| cpd. | constraint | CH ₃ CN | water | pH 7 |
| | | observed | observed | observed |
| 1a | 13₇3 | -13.46 | -22.95 (1.71) | -25.25 (1.88) |
| 3a | acyclic | 1.62 | 1.94 (1.2) | 1.99 (1.23) |
| 1b | 13₇3 | -8.30 | -17.02 (2.05) | -16.91 (2.04) |
| 3b | acyclic | -0.36 | -0.74 (1.94) | 1.06 (2.94) |
| 1c | 13₇3 | -5.62 | -9.02 (1.61) | -9.07 (1.61) |
| 3c | acyclic | 3.82 | 4.16 (1.09) | 4.34 (1.14) |

The $\theta_{n \rightarrow \pi^* \text{MRE}}$ intensities consistently enhance upon H-bonding and ionic interactions of the ordered coupled peptide chromophores with solvents. **Numbers in parenthesis denote the x fold enhancement in $\theta_{n \rightarrow \pi^* \text{MRE}}$ intensities upon solvent change.**

4.4. FTIR analysis of 2a-c:

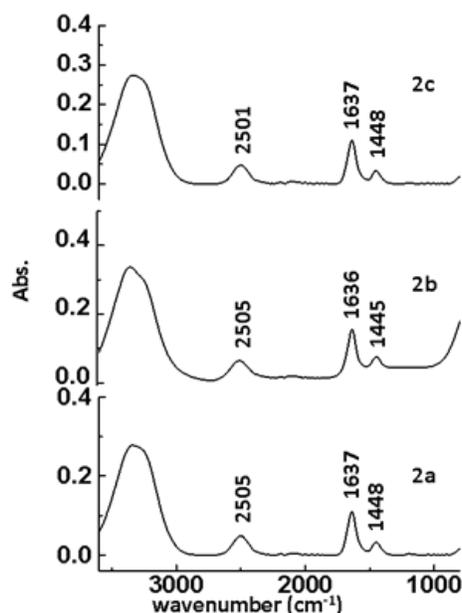


Figure S35. The full-range FTIR spectra (H₂O, 2 mM, 295 K) of N-Moc deprotected analogues **2a-c**.

Table S10. The list of peptides/proteins in literature with predominant α -helical conformations and their corresponding $\tilde{\nu}_{\text{C=O}}$ for the amide I region in different solvent systems.

| peptides/proteins | medium | wave number (cm ⁻¹) |
|--|-----------------------------|---------------------------------|
| Myoglobin ^a | water | 1653 |
| Ac-AAKAAY-NH ₂ ^b | hydrated film | 1648 |
| | water | 1650 |
| Ac-AAAKAAAAKAAAAY-NH ₂ ^b | 40% TFE | 1650 |
| | water | ~1645 |
| Ac-AAAKAAAAKAAAAY-NH ₂ ^b | 40% TFE | ~1645 |
| | water | ~1647 |
| VRRFAWWAFLRR-NH ₂ ^c | 40% TFE | ~1647 |
| | Ethanol | ~1659 |
| VRRFPYYPFLRR-NH ₂ ^c | DPC (dodecylphosphocholine) | ~1650 |
| | Ethanol | ~1659 |
| | DPC (dodecylphosphocholine) | ~1651 |

a - *Crit. Rev. Biochem. Mol. Biol.*, **1995**, 30, 95-120

b - *Biochemistry*, **2005**, 44, 369-376

c - *Biochim. Biophys. Acta*, **2006**, 1758, 1596-1608

4.5. Theoretical calculation:

4.5.1. Table S11. a) List of ROE cross peaks and their corresponding % intensities (40% CDCl₃ in CD₃CN) for the major conformers of Moc[GFA]G-OMe (**1a**), Moc[AFA]G-OMe (**1b**) and Moc[GFA]A-OMe (**1c**). b) Consideration of distance constraint based on the ROE intensities.

a) ROE cross peaks for the major conformers

| 1a | | 1b | | 1c | |
|--|---------------|--|---------------|--|---------------|
| cross peaks | % intensities | cross peaks | % intensities | cross peaks | % intensities |
| H ^α Ala ₃ ...Hprp ^c | 22 | H ^N Ala ₃ ...H ^N Phe ₂ | 2 | H ^α Ala ₃ ...Hprp ^c | 230 |
| H ^α Gly ₁ ...Hprp ^a | 12 | H ^α Ala ₁ ...H ^N Phe ₂ | 9 | H ^β Ala ₄ ...Hprp ^c | 22 |
| H ^α Gly ₄ ...Hprp ^c | 70 | H ^α Gly ₄ ...Hprp ^c | 80 | H ^α Ala ₄ ...Hprp ^b | 67 |
| H ^α Gly ₄ ...Hprp ^b | 130 | H ^α Gly ₄ ...Hprp ^b | 140 | H ^α Gly ₁ ...H ^N Phe ₂ | 16 |
| H ^α Gly ₄ ...Hprp ^c | 43 | H ^α Gly ₄ ...Hprp ^c | 48 | H ^α Gly ₁ ...H ^β Phe ₂ | 49 |
| H ^α Gly ₄ ...Hprp ^b | 23 | H ^α Gly ₄ ...Hprp ^b | 14 | H ^α Gly ₁ ...Hprp ^a | 33 |
| H ^α Ala ₃ ...H ^α Gly ₄ | 14 | H ^α Phe ₂ ...H ^N Ala ₃ | 5 | | |
| | | H ^β Phe ₂ ...H ^N Ala ₃ | 17 | | |
| | | H ^α Ala ₃ ...Hprp ^c | 24 | | |
| | | H ^α Ala ₃ ...H ^α Gly ₄ | 9 | | |
| | | H ^α Ala ₁ ...Hprp ^a | 21 | | |

H^αAla₃...H^βAla₃ cross peak intensity is 100%

| b) relative intensities | distance (Å) | types of interactions |
|-------------------------|--------------|-----------------------|
| 40 - 100 | 3 | strong |
| 10 - 39 | 4 | medium |
| 9 - 1 | 5 | weak |
| <1 | 6 | very weak |

Note: The % intensities were calculated relative to the H^αAla₃...H^βAla₃ TOCSY/ROE cross peak intensity, which was taken as 100%.

4.5.2. Explanation for the number of ROE crosspeaks observed in 1a and 1b:

The four ROE cross peaks that we have assigned for major conformers of **1b**, but not for **1a**, are all between Phe₂ and Ala₃. They are:

1. H^NF₂...H^NA₃,
2. H^NA₃...H^αF₂, H^NA₃...H^βF₂ and
3. H^NF₂...H^αA₁

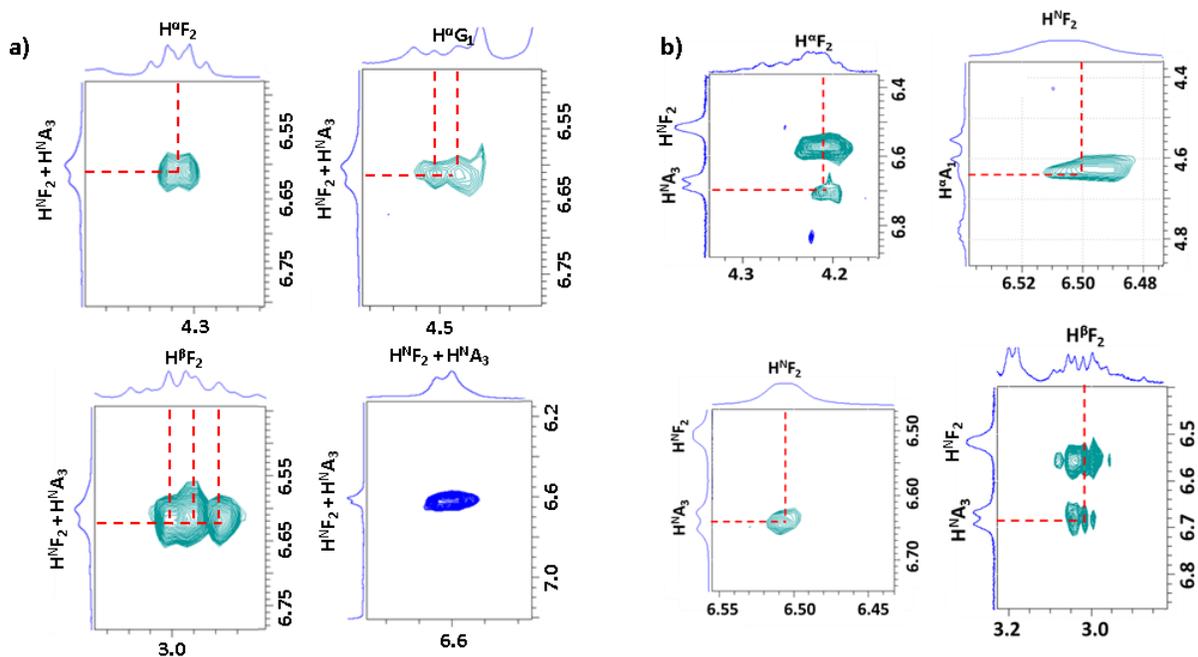


Figure S36. a) The four distinct regions of ROESY spectra associated with the Phe₂ and Ala₃ present in **1a**. b) The set of 4 ROEs associated with the Phe₂ and Ala₃ present in **1b**.

These ROE cross peaks are unambiguously observed in **1b**. Hence, we report them for **1b**, in the manuscript.

Indeed, all these 4 cross peaks are observed in **1a** as well. But in **1a**, the NH signals of Phe₂ and Ala₃ come merged at ~6.61 ppm (**Supporting Information, page S48, Figure S8 a**) in the major conformer. So, their cross peaks also come merged together. Although we observe all these four cross peaks here also **1a** (as in **1b**), we consider it incorrect to unambiguously assign these cross peaks to the corresponding ROE cross peaks in **1a** – because of the merger of the two NH signals of Phe₂ and Ala₃ in it. So, we abstained from assigning or reporting these ROE cross peaks for **1a**.

So, we have been cautious in assigning ROE cross peaks and have not taken the benefit of doubt between TOCSY and the ROESY cross peaks for both the Phe₂ and Ala₃ spin systems in **1a**. Hence, we do not include these cross peaks in the structure of **1a** in the manuscript. Notice that the energy minimized structures are still identical despite the non-inclusion of this ROE for **1a**.

4.5.3. Table S12. Comparison of inter-carbonyl orientations between peptide groups in HBS-constrained 1373 analogues (**1a**, **1b** and **1c**), with those in classical α -helices made from corresponding sequences (built with Discovery Studio).

| | $\text{O}\cdots\overset{\text{O}}{\text{C}}'$ (Å) | $\text{O}\cdots\text{C}'=\text{O}$ \angle_{le} (deg) | $\text{C}'=\text{O}\cdots\text{C}'=\text{O}$ torsion (deg) |
|--|--|--|---|
| | | $\text{O}_{i+1} \rightarrow \text{C}'_{i+2}$ | |
| Natural α-helix | 2.78 | 98.65 | -166.98 |
| 1a | 3.50 | 105.32 | -177.11 |
| 1b | 3.68 | 127.26 | -156.66 |
| 1c | 3.54 | 112.89 | -172.89 |
| | | $\text{O}_{i+2} \rightarrow \text{C}'_{i+3}$ | |
| Natural α-helix | 2.78 | 97.5 | 162.71 |
| 1a | 3.41 | 101.69 | -170.72 |
| 1b | 3.82 | 98.70 | 166.52 |
| 1c | 3.64 | 124.33 | -154.77 |
| | | $\text{O}_{i+1} \rightarrow \text{C}'_{i+3}$ | |
| Natural α-helix | 3.98 | 131.83 | -155.01 |
| 1a | 5.15 | 133.86 | -172.12 |
| 1b | 5.41 | 125.19 | -162.15 |
| 1c | 5.32 | 159.29 | 175.94 |

4.5.4. Energy minimized structures of minor conformers of 1a,b:

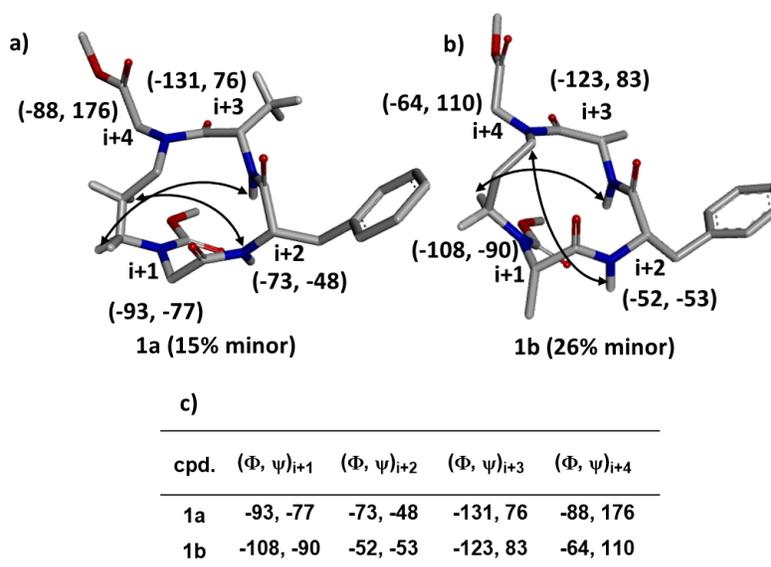


Figure S37. Energy minimized structures of minor conformers of a) **1a** and b) **1b**. c) The list of (Φ, ψ) values from the energy-minimized structure of the minor conformers.

Note: **1a**, which contains two highly flexible Gly residues (at $i+1^{\text{st}}$ and $i+4^{\text{th}}$ positions) has an additional such (third) conformer (8%) – whose structure we are unable to characterize due to its low relative population and hence the lack of any corresponding spectral signals, especially in the 2D NMR. It can at best be speculated that the third rotamer could be one of the kinetic minima reported by Broussy et al. (*Org. Biomol. Chem.* **2018**, *16*, 459-471; *Tetrahedron Lett.* **2015**, *56*, 2456-2459), where there is flipping of one or more of the peptide planes.

4.6 Supporting images of ^1H and ^{13}C NMR spectra.

Figure S38. ^1H NMR (400 MHz) of Boc-Gly-Phe-Ala-Gly-OMe (**3a**) in DMSO- d_6 (10 mM).

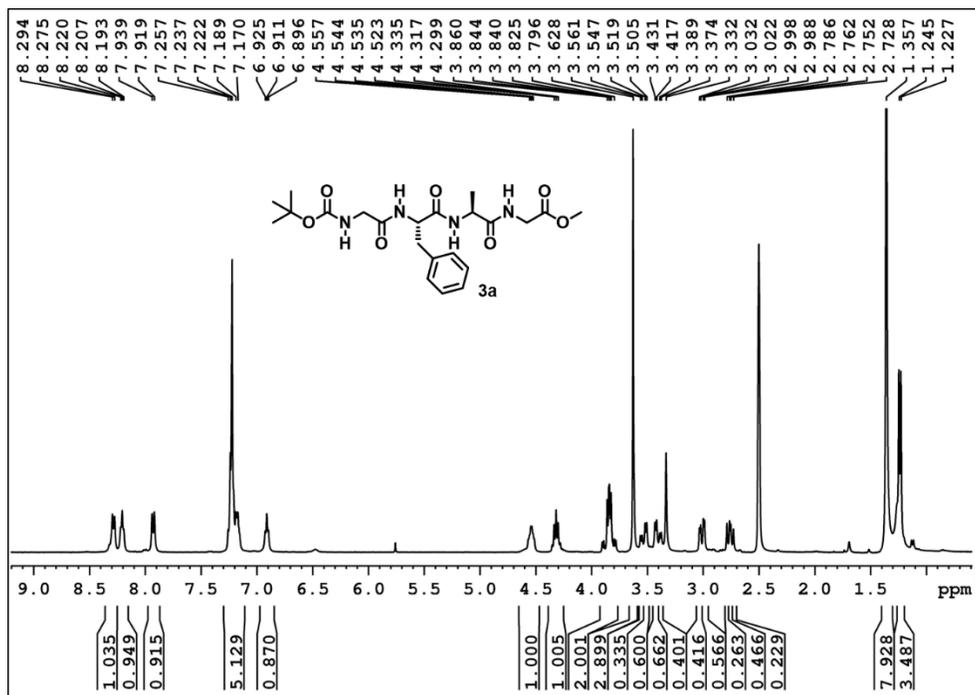


Figure S39. ^{13}C NMR (100 MHz) of Boc-Gly-Phe-Ala-Gly-OMe (**3a**) in DMSO- d_6 (60 mM).

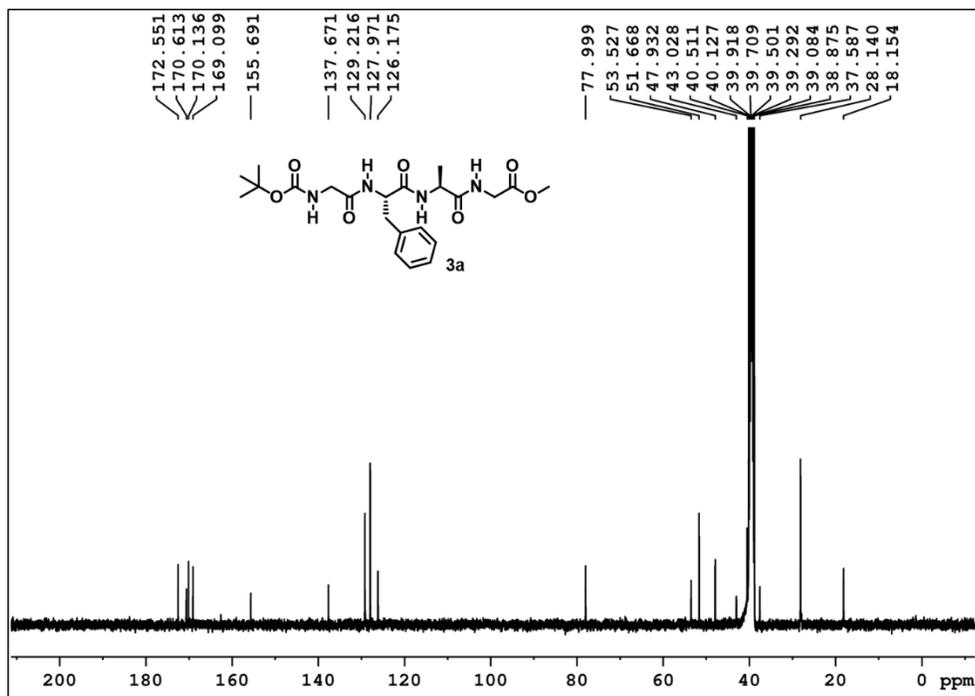


Figure S40. ¹H NMR (400 MHz) of Boc-Ala-Phe-Ala-Gly-OMe (**3b**) in DMSO-d₆ (10 mM).

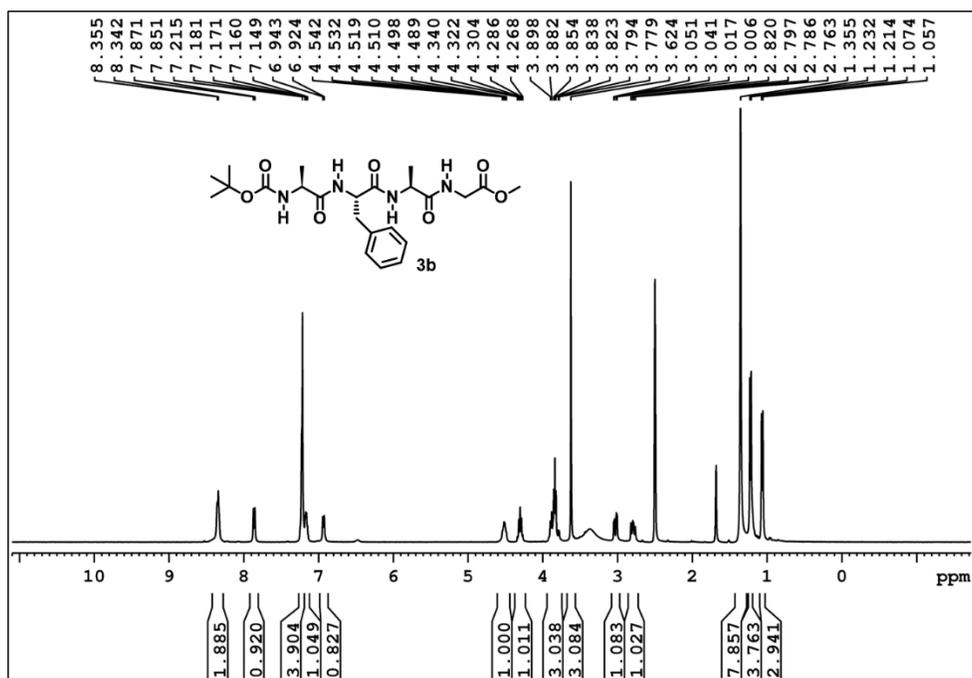


Figure S41. ¹³C NMR (100 MHz) of Boc-Ala-Phe-Ala-Gly-OMe (**3b**) in DMSO-d₆ (60 mM).

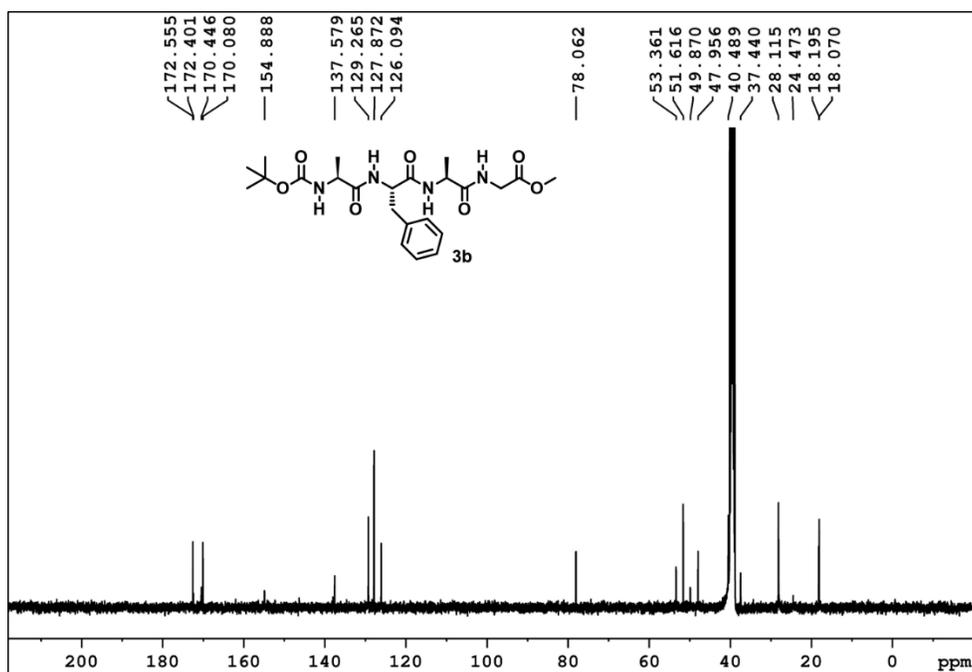


Figure S42. ^1H NMR (400 MHz) of Boc-Gly-Phe-Ala-Ala-OMe (**3c**) in CDCl_3 (10 mM).

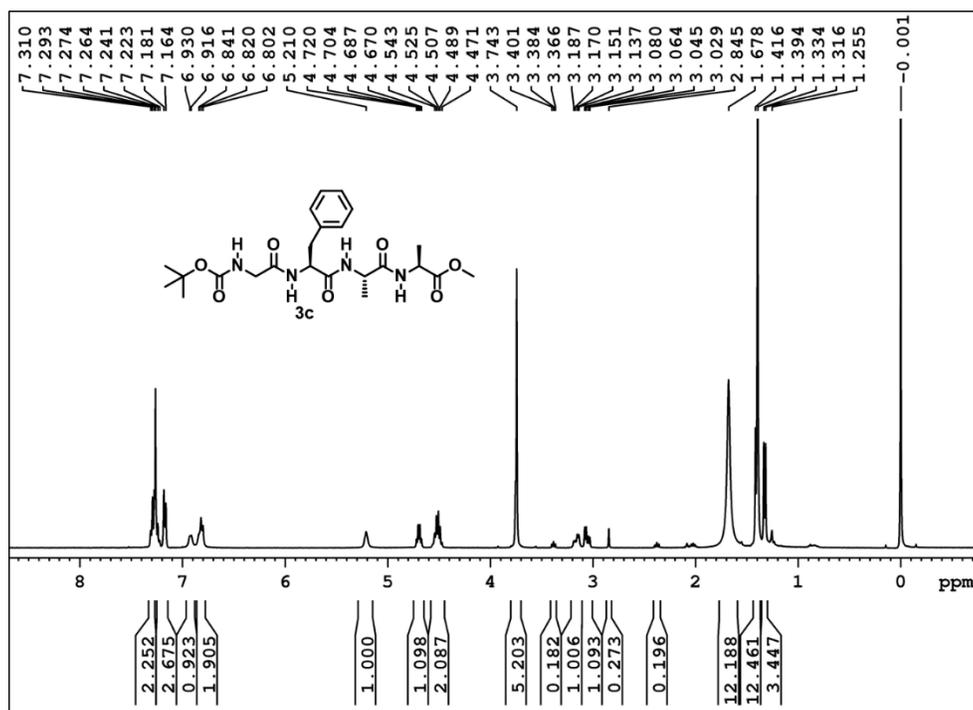


Figure S43. ^{13}C NMR (100 MHz) of Boc-Gly-Phe-Ala-Ala-OMe (**3c**) in CDCl_3 (60 mM).

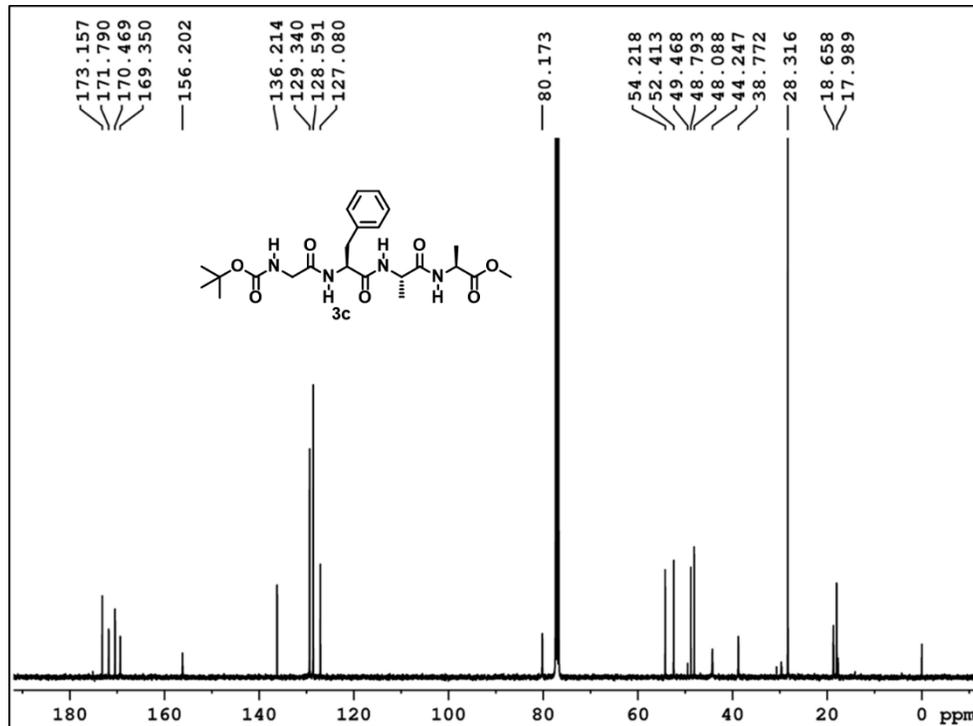


Figure S44. ¹H NMR (400 MHz) of **10a** in CDCl₃ (10 mM).

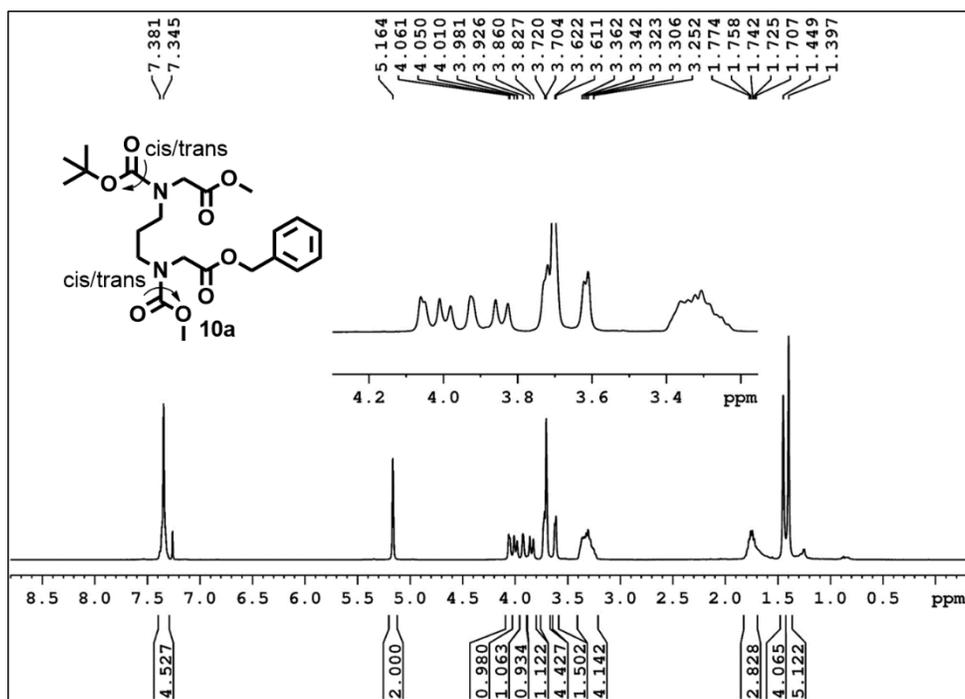


Figure S45. ¹³C NMR (100 MHz) of **10a** in CDCl₃ (60 mM).

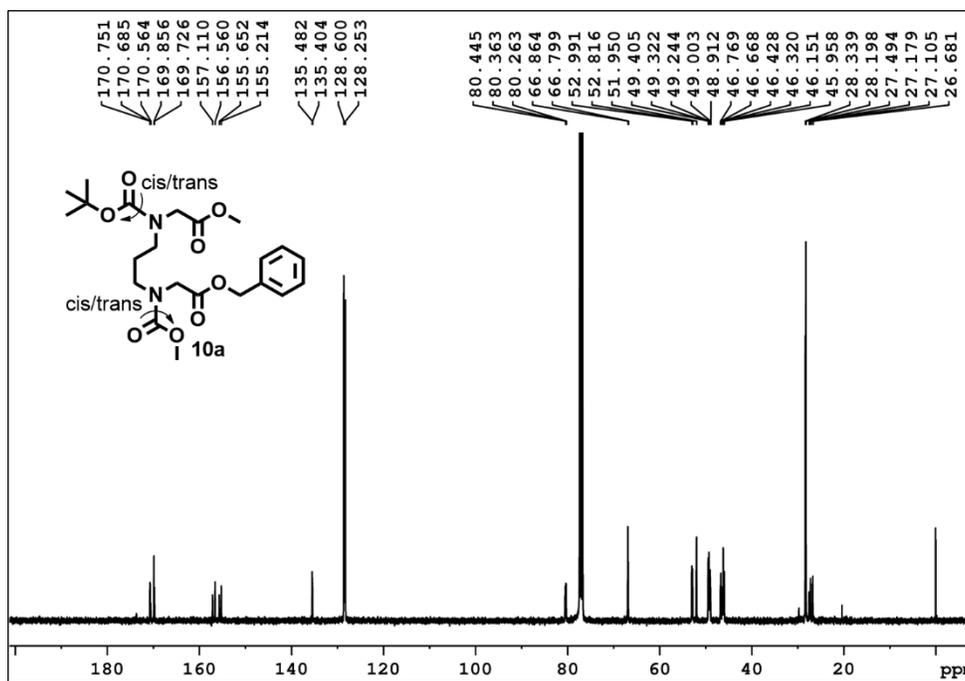


Figure S46. ¹H NMR (400 MHz) of **10b** in CDCl₃ (10 mM).

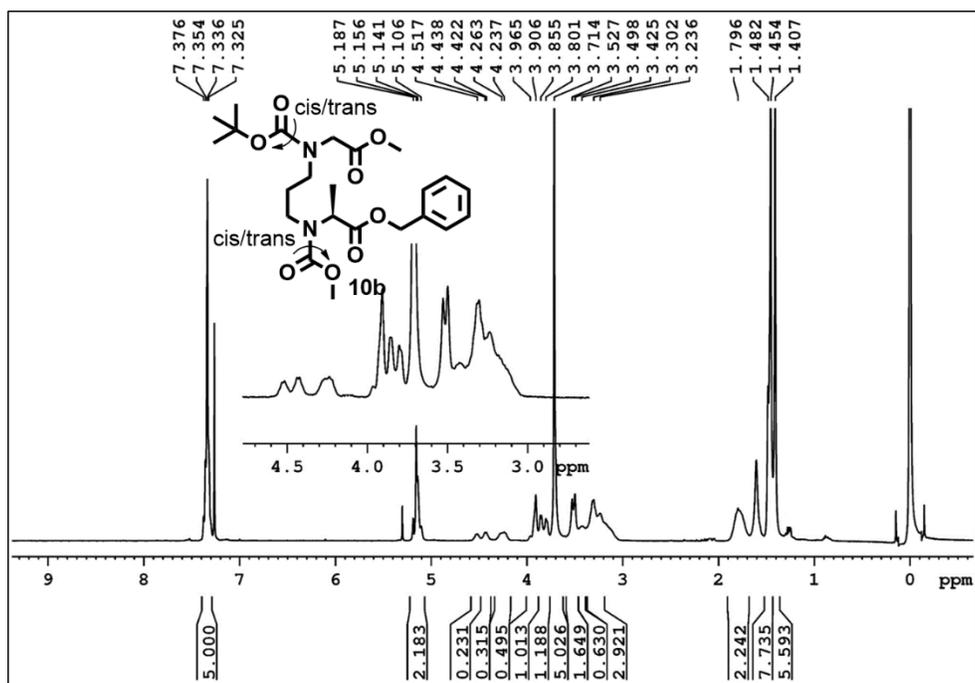


Figure S47. ¹³C NMR (100 MHz) of **10b** in CDCl₃ (60 mM).

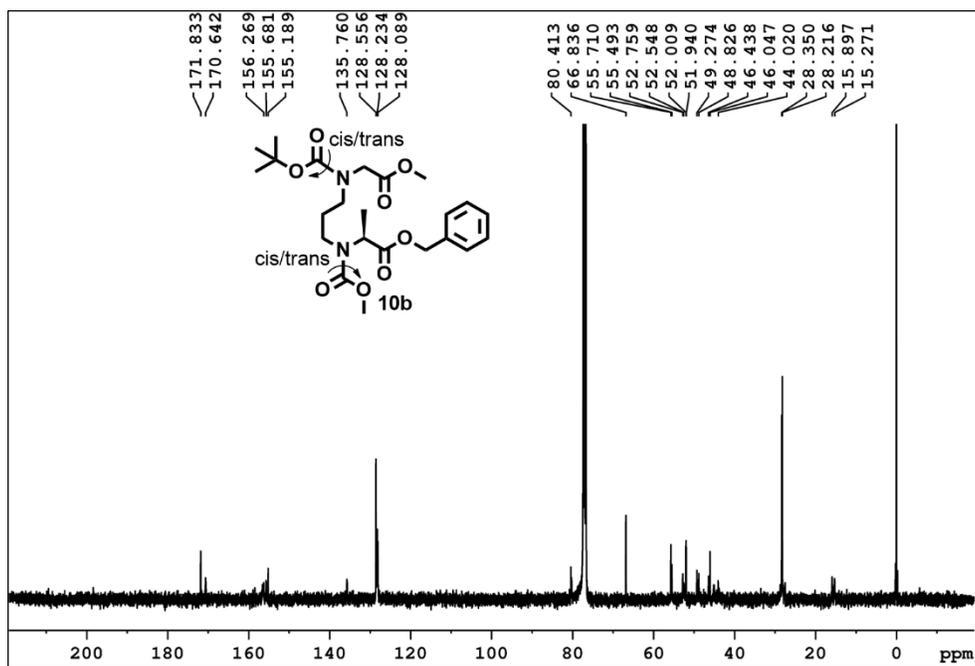


Figure S48. ^1H NMR (400 MHz) of **10c** in CDCl_3 (10 mM).

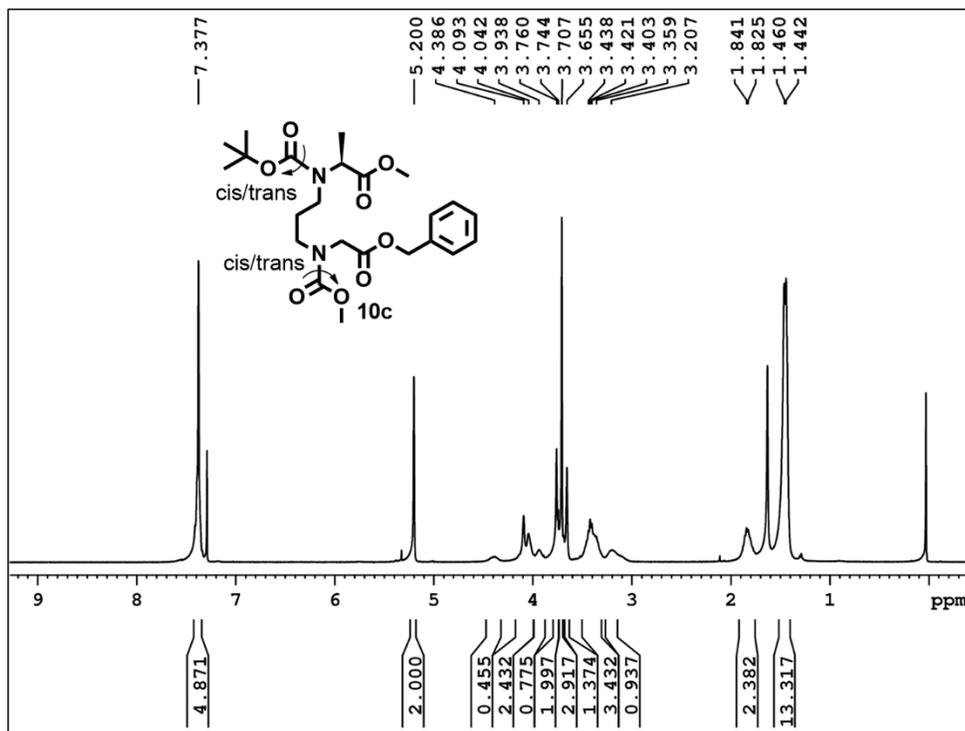


Figure S49. ^{13}C NMR (100 MHz) of **10c** in CDCl_3 (60 mM).

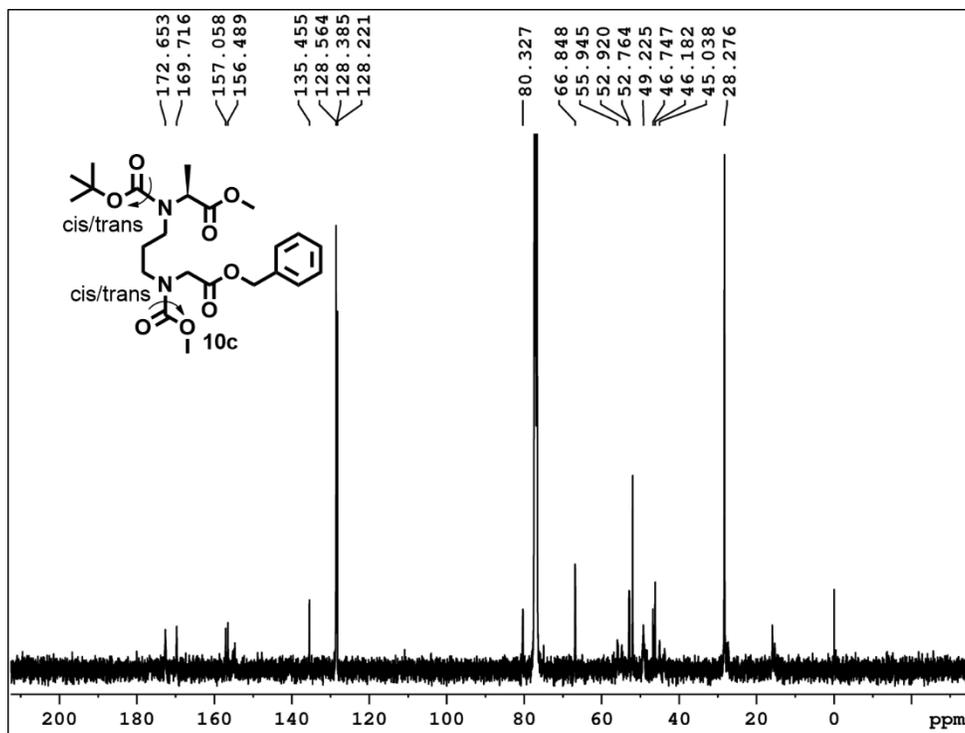


Figure S50. ¹H NMR (400 MHz) of **12a** in CDCl₃ (10 mM).

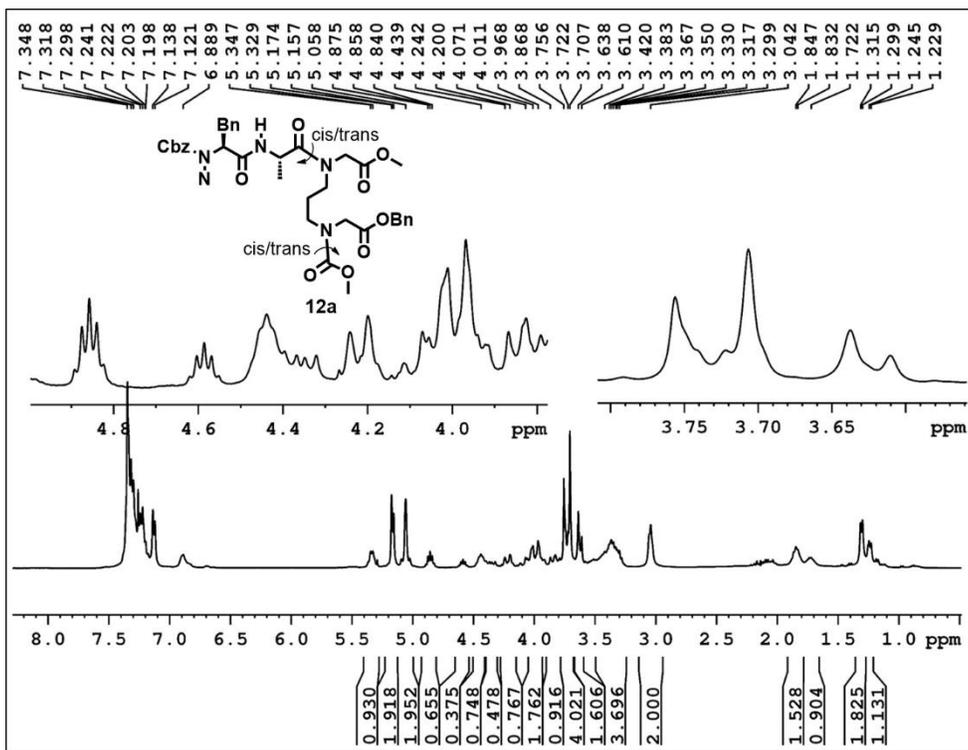


Figure S51. ¹³C NMR (100 MHz) of **12a** in CDCl₃ (60 mM).

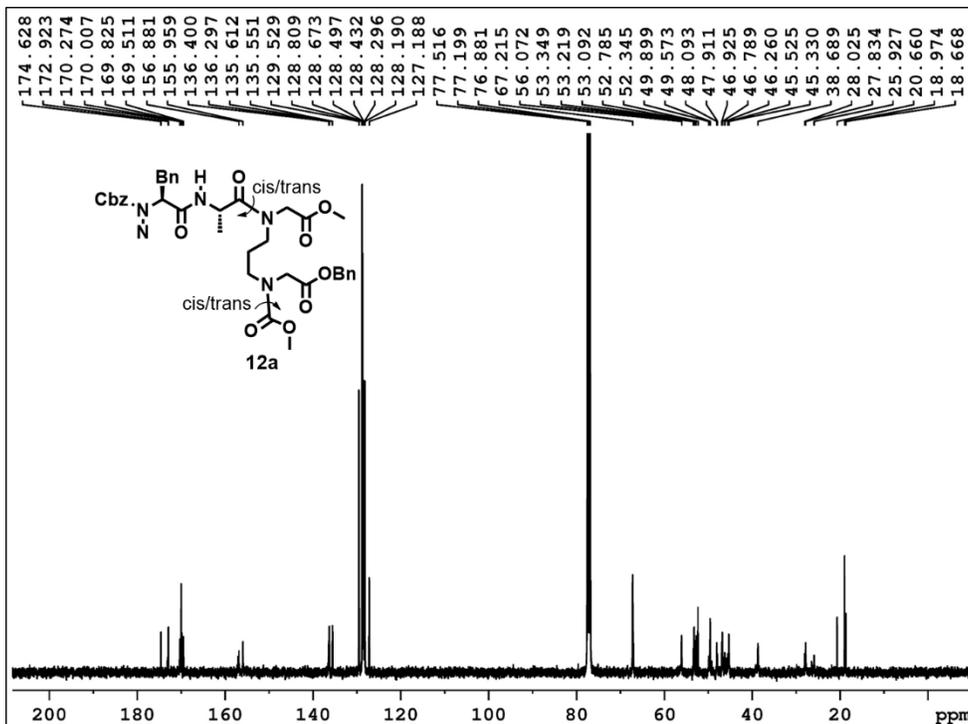


Figure S52. ¹H NMR (400 MHz) of **12b** in CDCl₃ (10 mM).

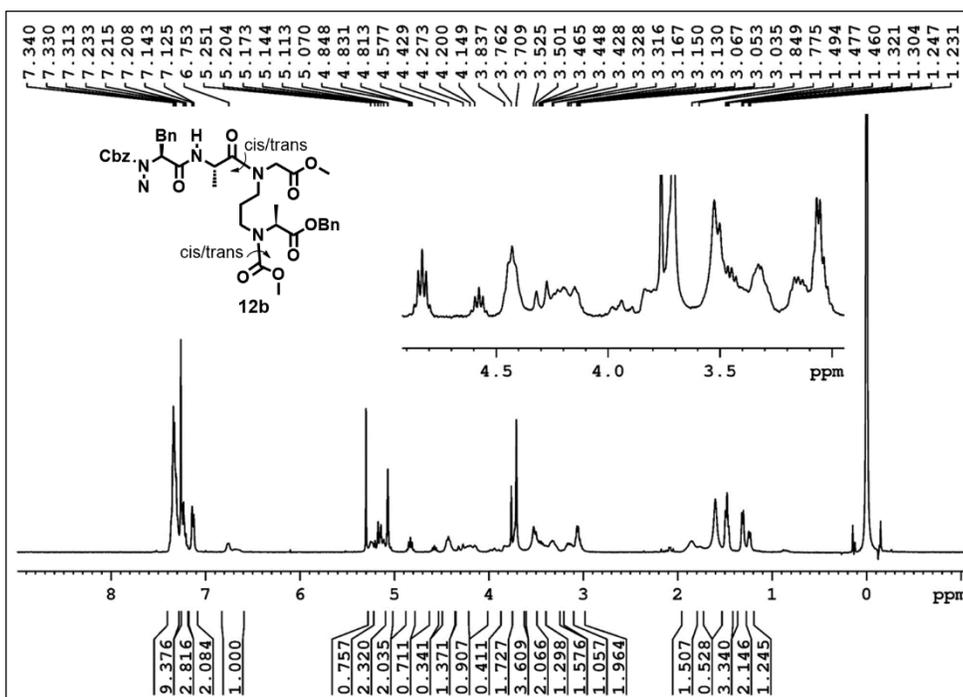


Figure S53. ¹³C NMR (100 MHz) of **12b** in CDCl₃ (60 mM).

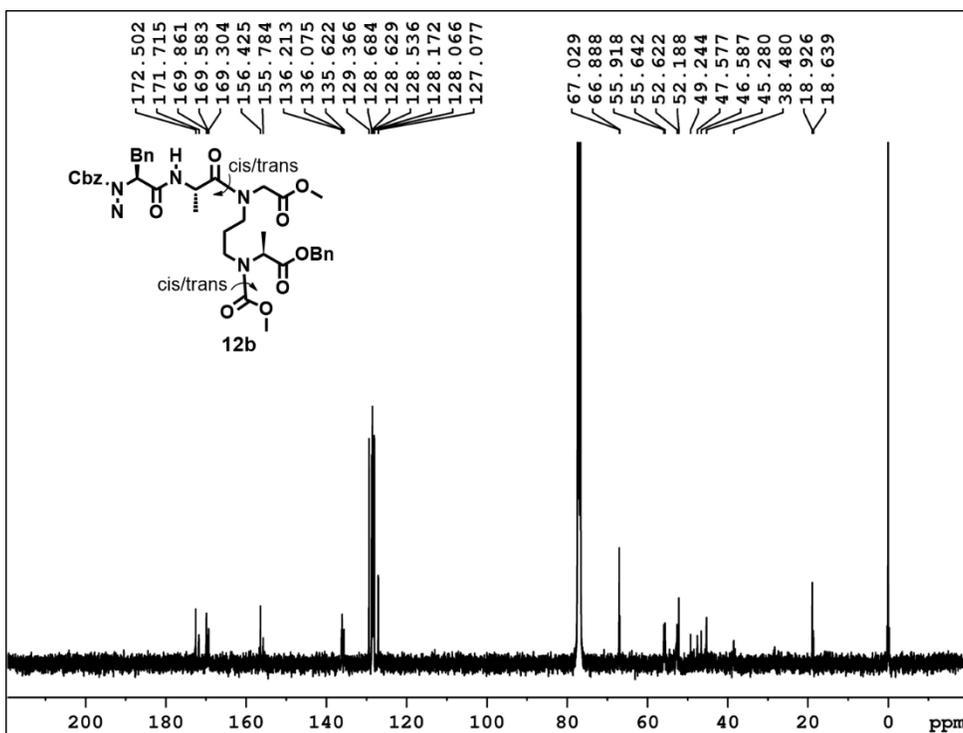


Figure S54. ¹H NMR (400 MHz) of **12c** in CDCl₃ (10 mM).

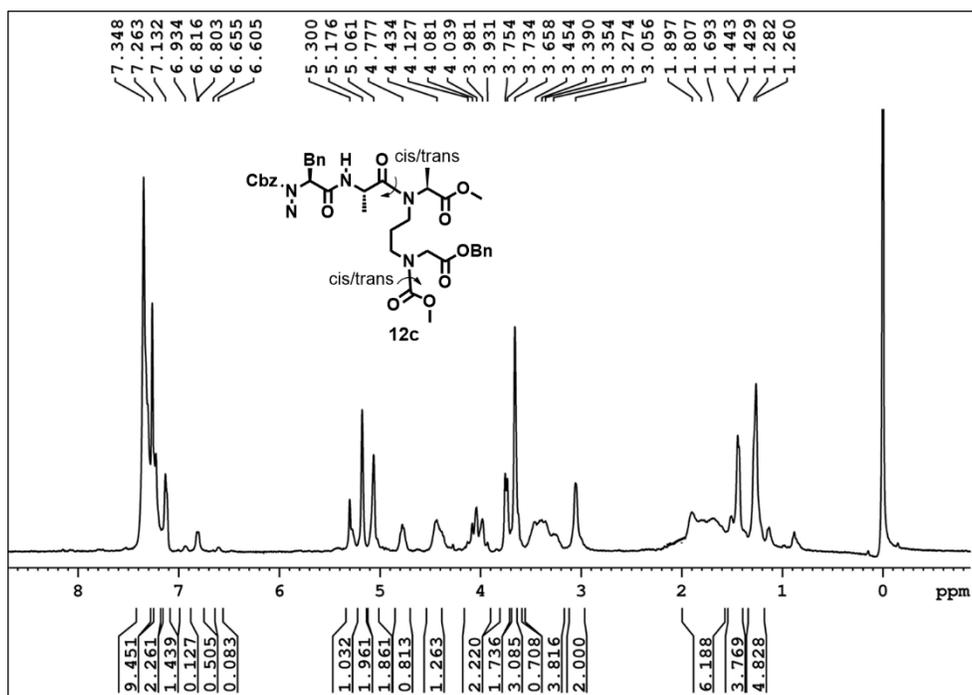


Figure S55. ¹³C NMR (100 MHz) of **12c** in CDCl₃ (60 mM).

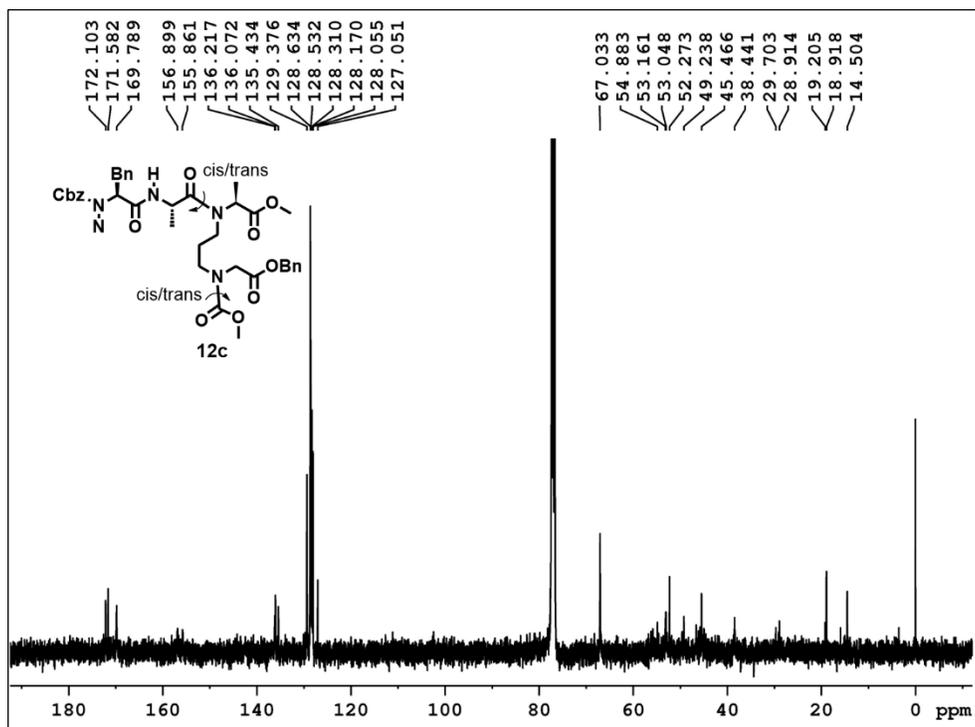


Figure S56. ^1H NMR (400 MHz) of **12d** in CDCl_3 (10 mM).

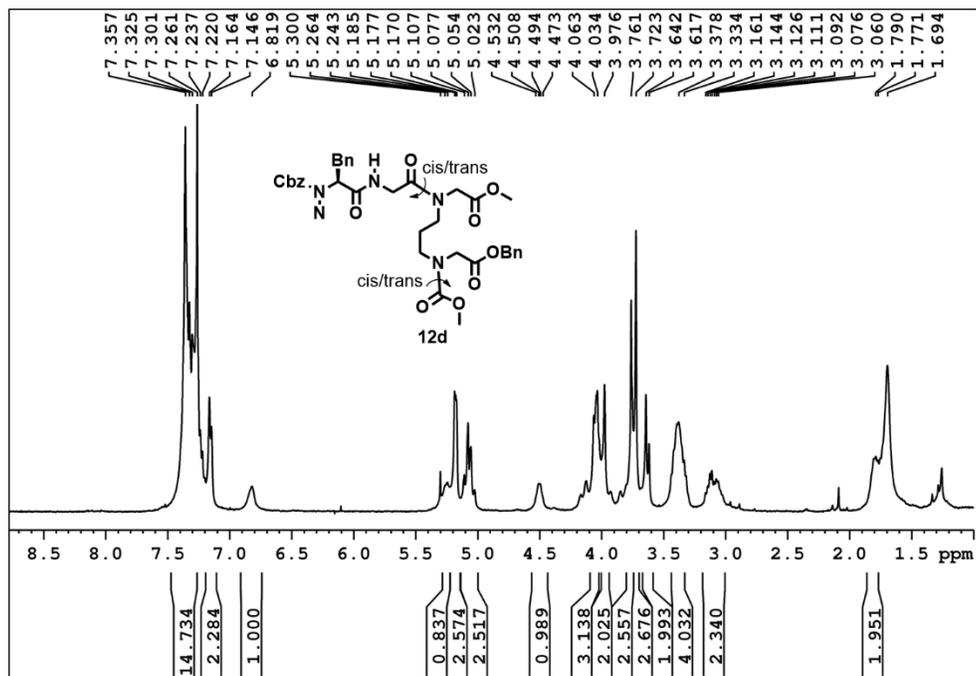


Figure S57. ^{13}C NMR (100 MHz) of **12d** in CDCl_3 (60 mM).

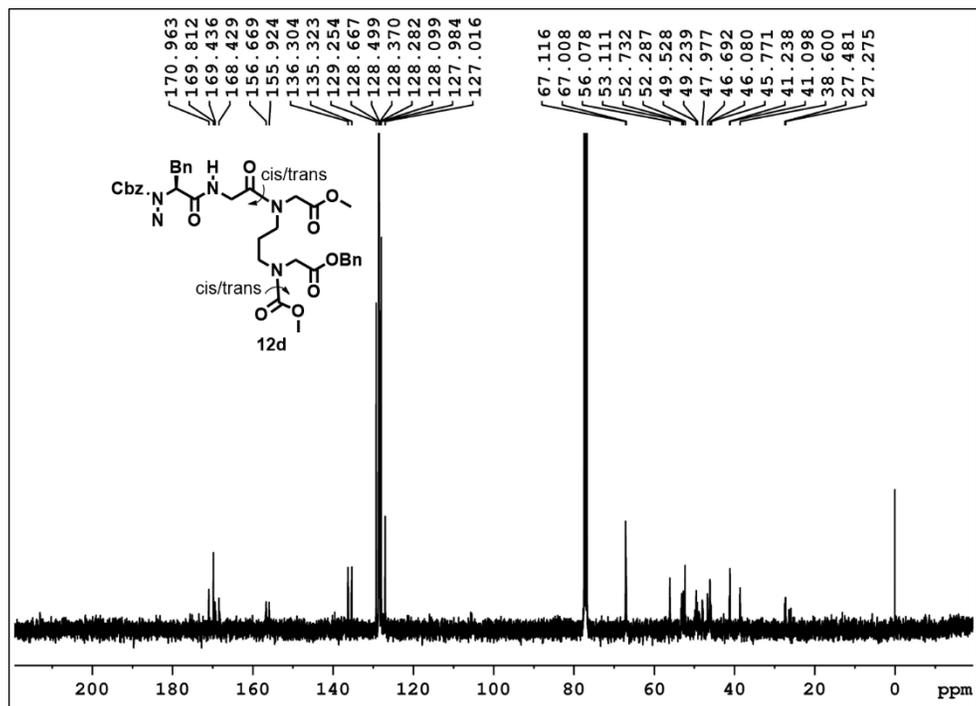


Figure S58. ¹H NMR (400 MHz) of **12e** in CDCl₃ (10 mM).

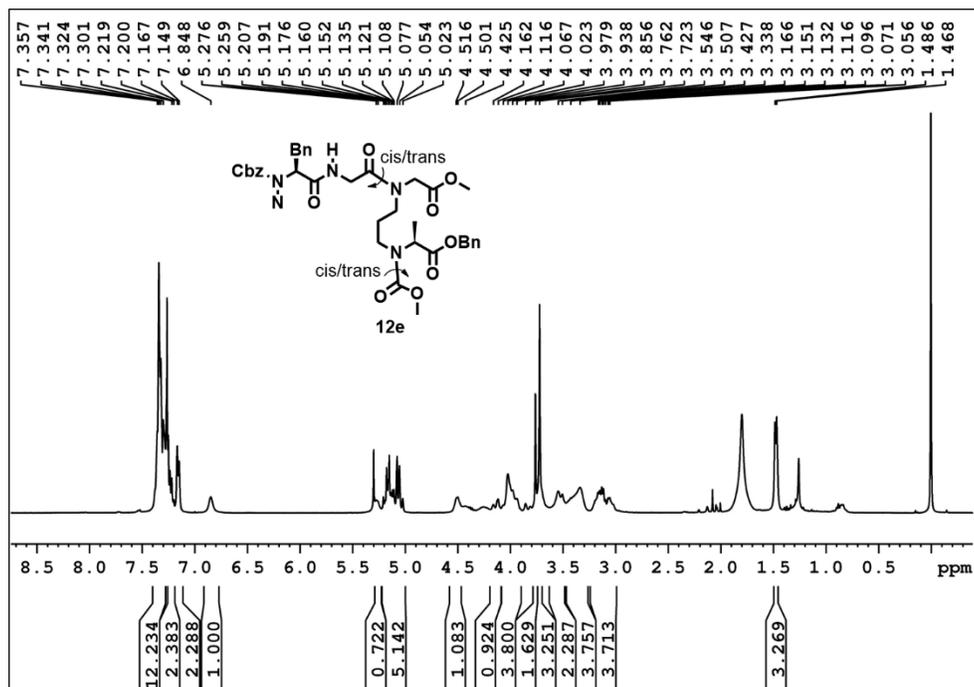


Figure S59. ¹³C NMR (100 MHz) of **12e** in CDCl₃ (60 mM).

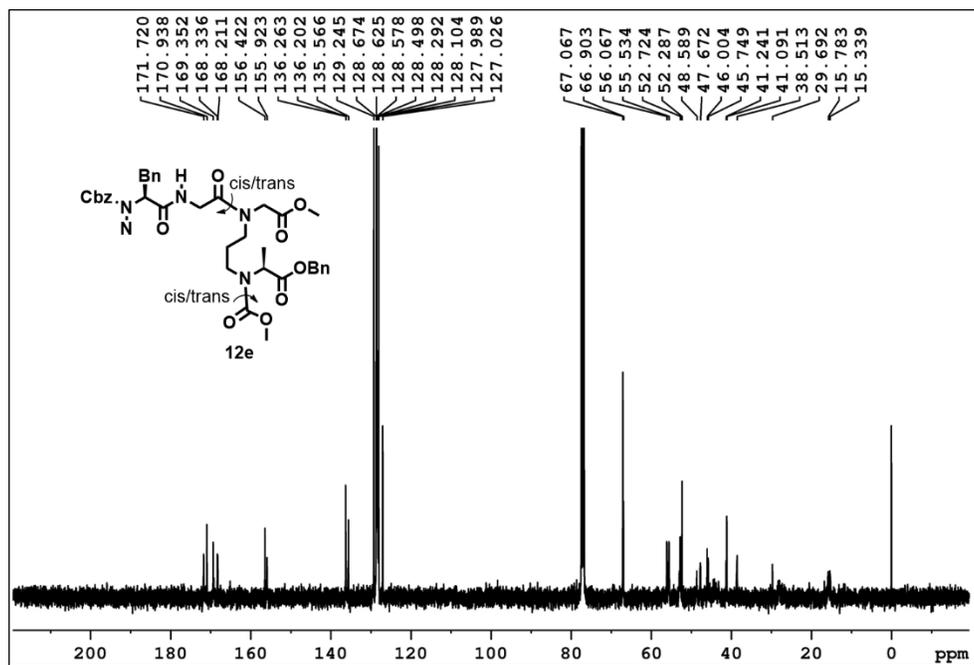


Figure S60. ^1H NMR (400 MHz) of **1a** in 40% CDCl_3 in CD_3CN (5 mM).

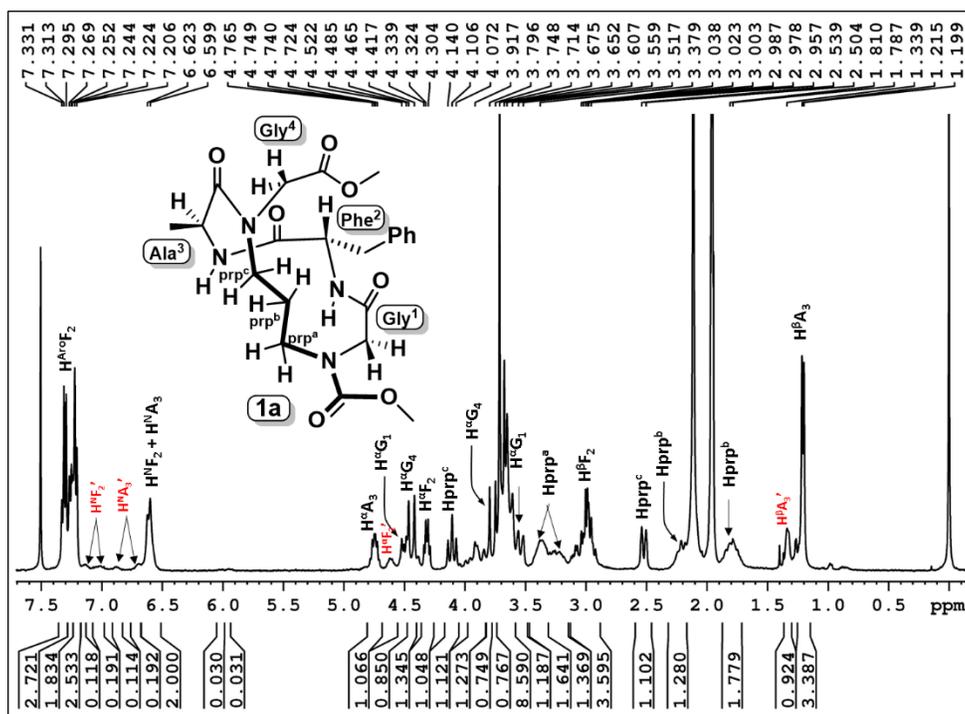


Figure S61. ^{13}C NMR (100 MHz) of **1a** in 40% CDCl_3 in CD_3CN (5 mM).

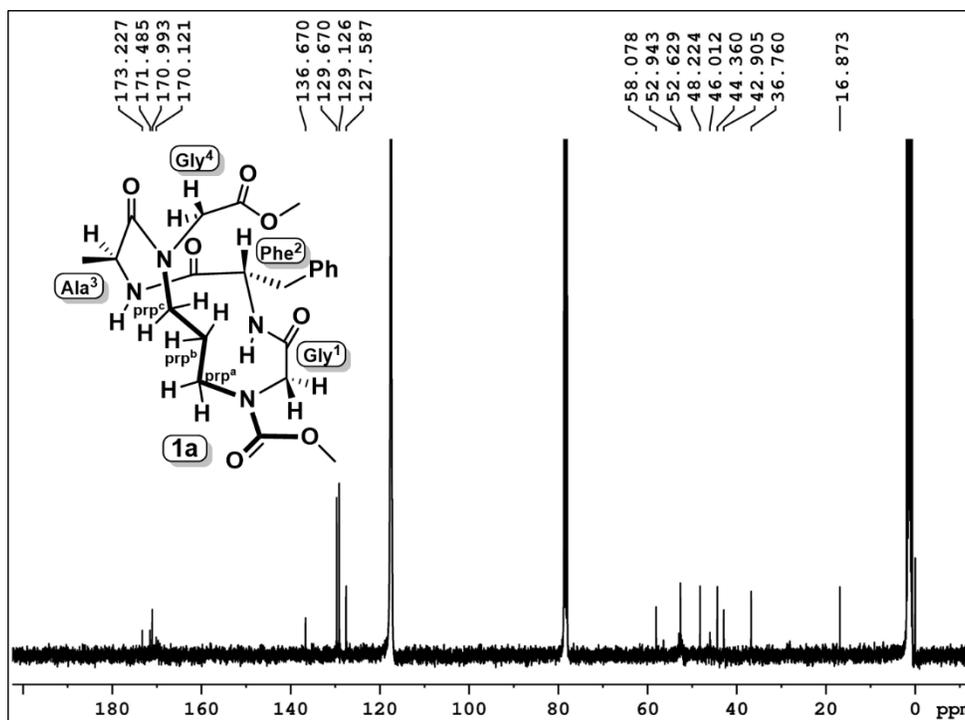


Figure S62. ^1H NMR (400 MHz) of **1b** in 40% CDCl_3 in CD_3CN (5 mM).

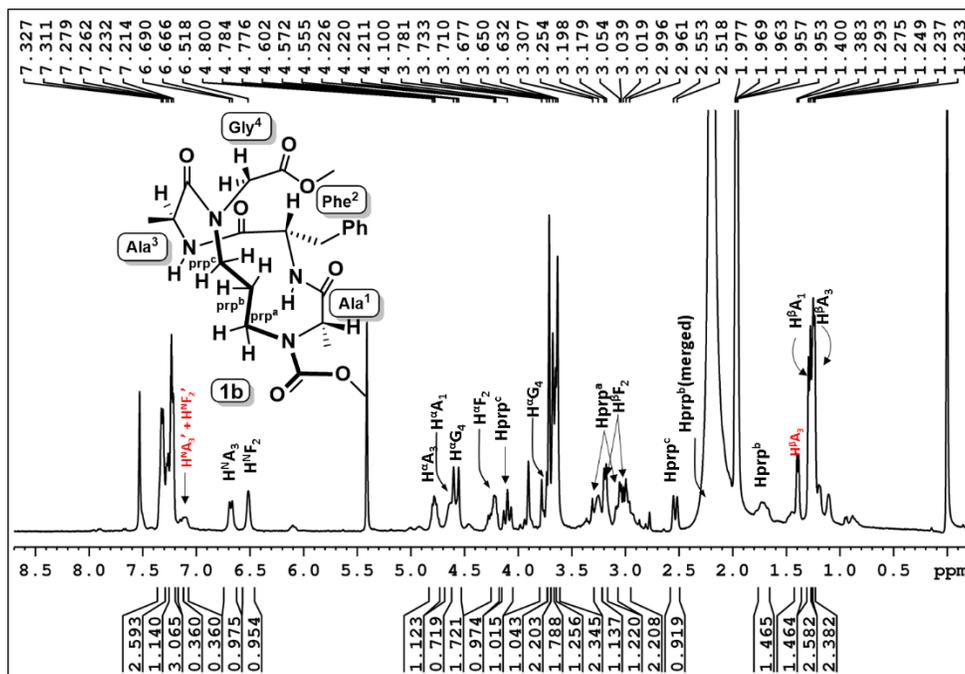


Figure S63. ^{13}C NMR (100 MHz) of **1b** in 40% CDCl_3 in CD_3CN (5 mM).

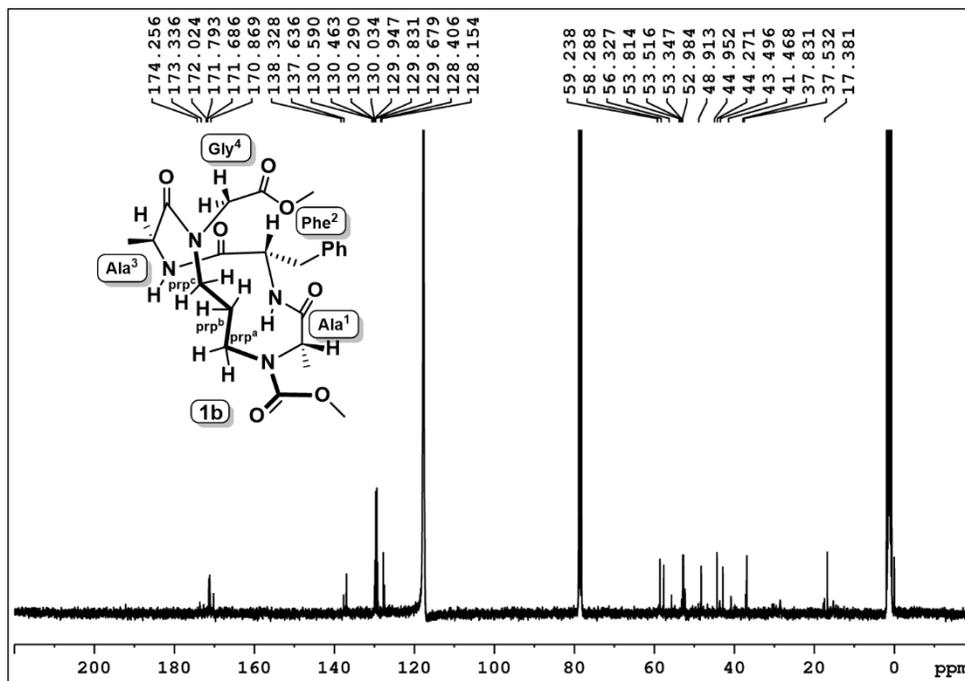


Figure S66. ¹H NMR (400 MHz) of **1d** in DMSO-d₆ (5 mM)

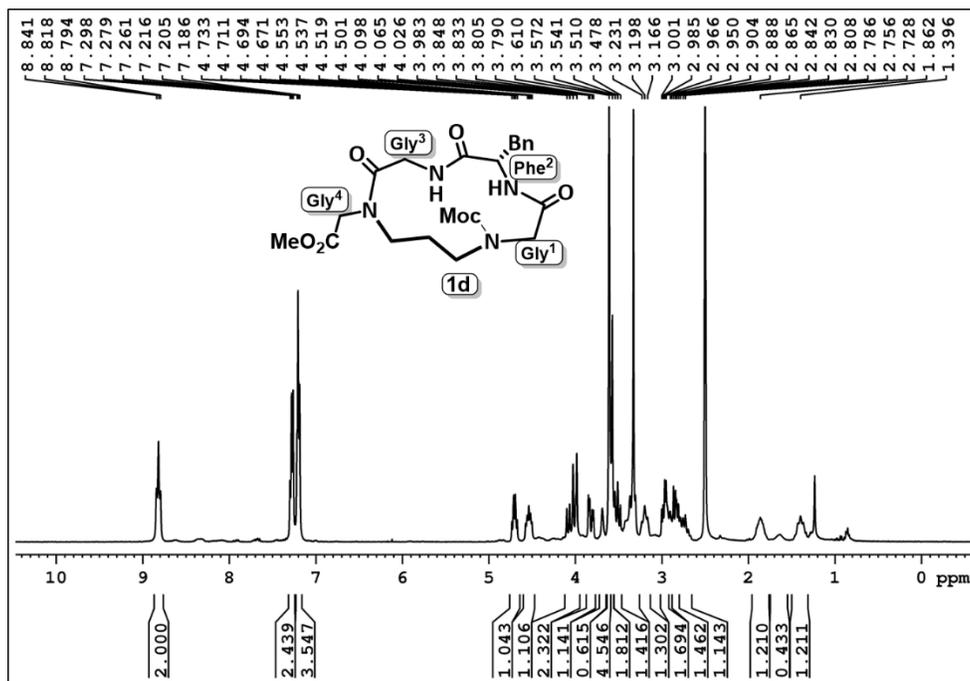


Figure S67. ¹³C NMR (100 MHz) of **1d** in DMSO-d₆ (5 mM)

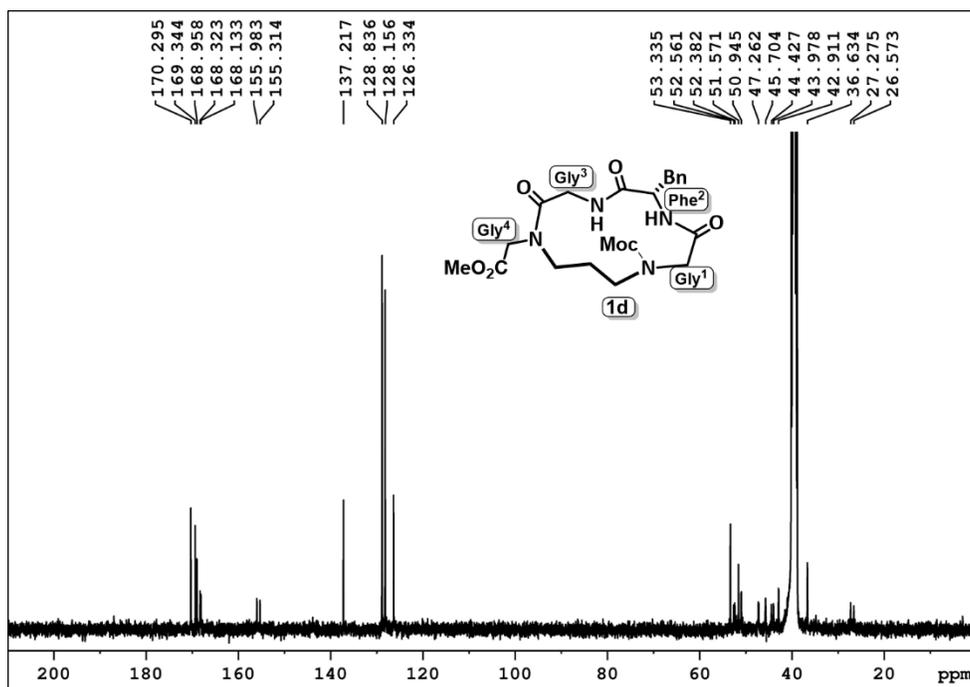


Figure S68. ^1H NMR (400 MHz) of **1e** in DMSO- d_6 (5 mM)

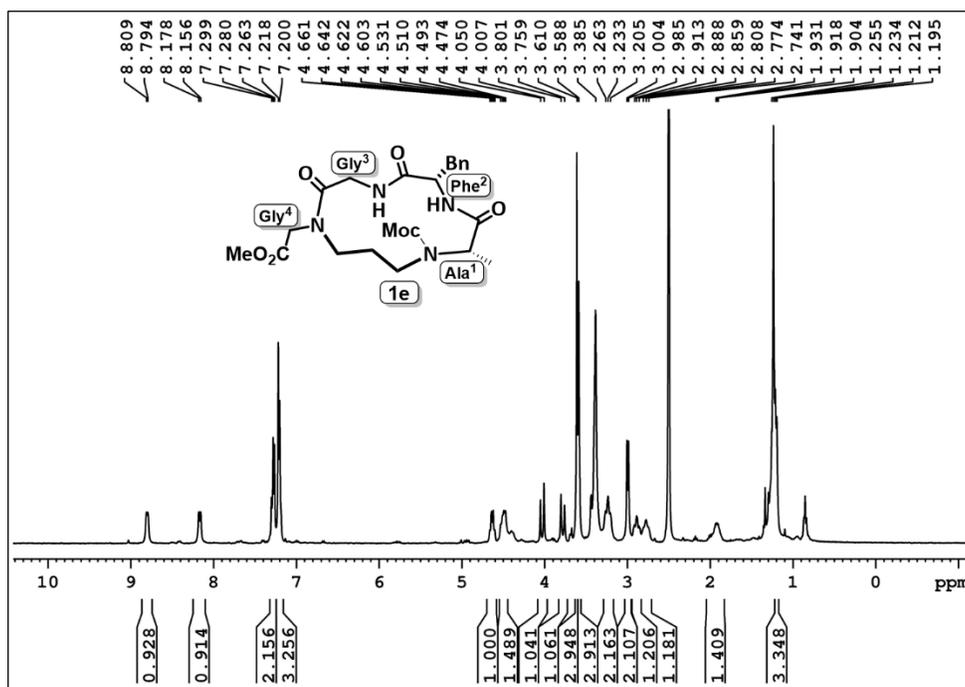


Figure S69. ^{13}C NMR (100 MHz) of **1e** in DMSO- d_6 (5 mM).

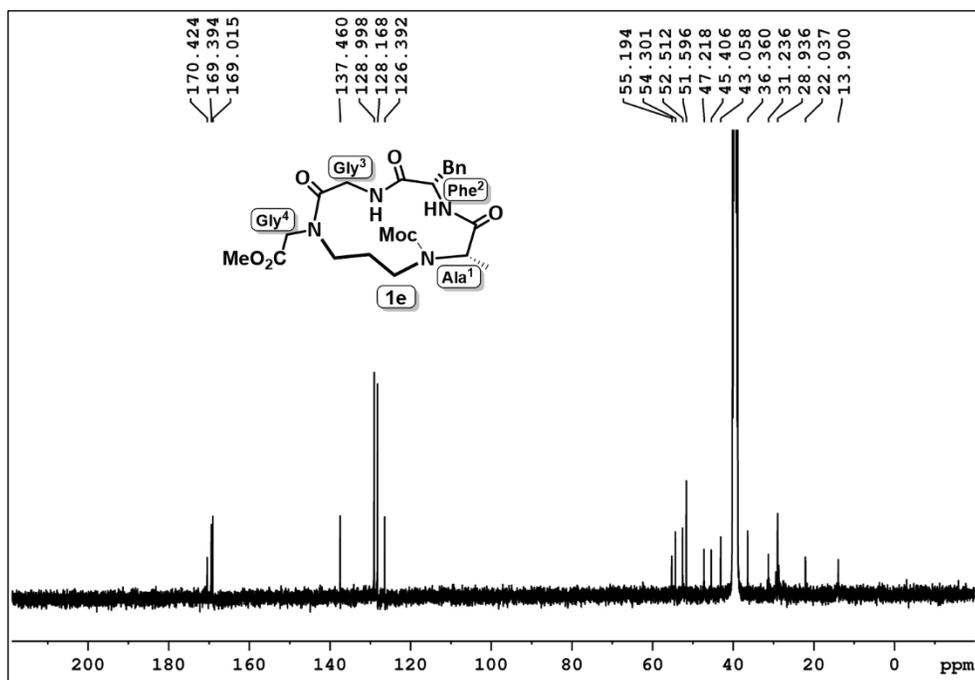


Figure S70. ¹H NMR (400 MHz) of **2a** in 10% D₂O in H₂O (5 mM)

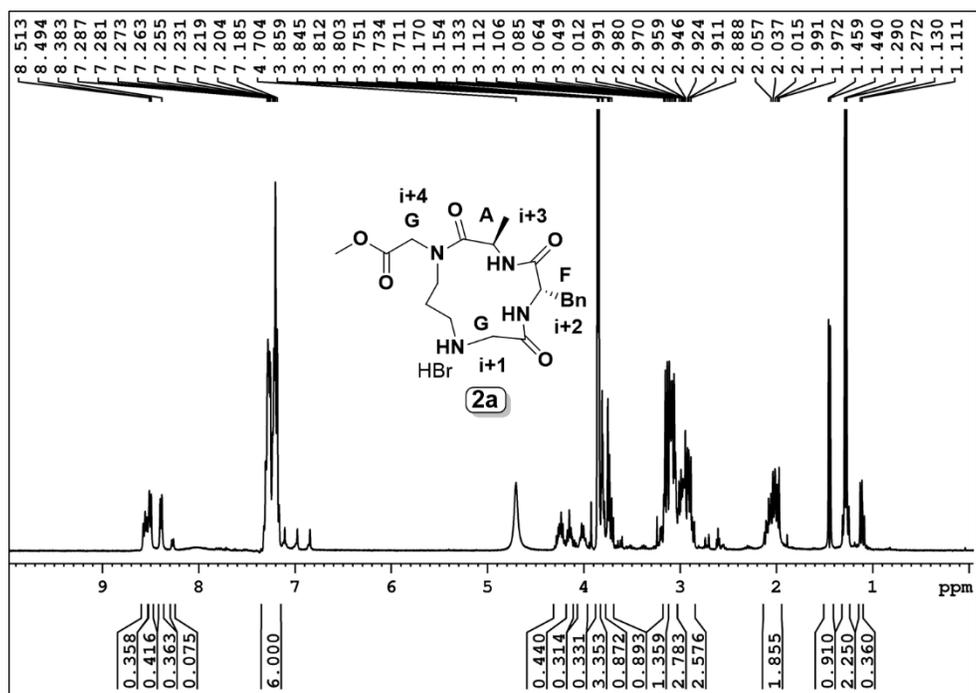


Figure S71. ¹H NMR (400 MHz) of **2b** in 10% D₂O in H₂O (5 mM)

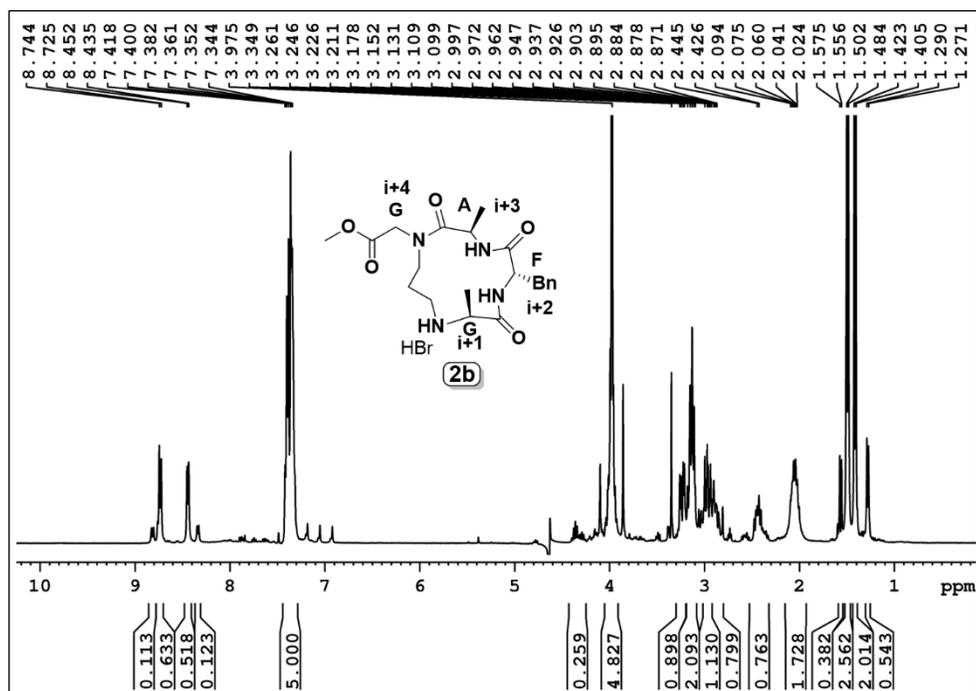


Figure S72. ¹H NMR (100 MHz) of **2c** in 40% CDCl₃ in CD₃CN (5 mM)

