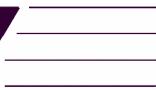


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Supporting Information

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Photoinduced Intramolecular Macrocyclization Reaction between a Bpa and a Met Residue in a Helical Peptide: 3D-Structures of the Diastereomeric Products Formed

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Peptide Characterization

General

Thin-layer chromatography was performed on Merck Kieselgel 60/F₂₅₄ precoated plates with the following solvent systems: (1) 9:1 CHCl₃ / EtOH; (2) 6:2:2 1-BuOH / AcOH / H₂O; (3) 7:1 toluene / EtOH.

Fmoc-L-Met-Aib-OMe

Fmoc-L-Met-OH (8 g, 21.5 mmol) was dissolved in anhydrous CH₂Cl₂ and kept at 0 °C. Then, HOAt (2.9 g, 21.5 mmol) and EDC·HCl (4.3 g, 22.5 mmole) were added to the solution. Then, HCl·H-Aib-OMe (3.3 g, 21.5 mmol) was added, followed by 4.4 ml (43 mmol) of NMM (N-methylmorpholine). After stirring the mixture at room temperature for 24 hours, CH₂Cl₂ was evaporated under reduced pressure. The oily residue was dissolved in EtOAc and washed with 10% KHSO₄, H₂O, 5% NaHCO₃, H₂O, dried over anhydrous Na₂SO₄, and evaporated to dryness under reduced pressure. The product was crystallized from EtOAc/petroleum ether. Yield 93%; m.p.= 99-100 °C; R_{f1} = 0.85, R_{f2} = 0.95, R_{f3} = 0.80; [α]_D²⁰ = -18.0 ° (c = 0.5, MeOH); IR(KBr) 3300, 1742, 1693, 1659, 1539 cm⁻¹; ¹H-NMR (CDCl₃, 250 MHz): δ 7.77 (m, 2H, Fmoc CH ar.), 7.60 (m, 2H, Fmoc CH ar.), 7.36 (m, 4H, Fmoc CH ar.), 6.70 (s, 1H, Aib NH); 5.55 (d, 1H, Met NH), 4.39 (m, 3H, 1H Met αCH and 2H Fmoc CH₂), 4.24

(m, 1H, Fmoc CH⁹), 3.73 (s, 3H, OMe), 2.58 (m, 2H, Met γ CH₂), 2.03 (s, 3H, Met ϵ CH₃), 2.14-2.02 (m, 2H, Met β CH₂), 1.55 (s, 3H, Aib CH₃), 1.54 (s, 3H, Aib CH₃).

Fmoc-Aib-L-Met-Aib-OMe

Fmoc-Aib-OH (2.6 g, 8.0 mmol) was dissolved in anhydrous CH₂Cl₂ and kept at 0 ° C. Then, HOAt (1 g, 8.0 mmol) and EDC·HCl (1.6 g, 8.14 mmol) were added to the solution. Then, H-L-Met-Aib-OMe (2 g, 8.0 mmol) (obtained by deprotection of Fmoc-L-Met-Aib-OMe in a mixture of 70:30 acetonitrile/diethylamine) was added, followed by 0.88 ml of NMM (9.22 mmol). After stirring the mixture at room temperature for 24 hours, CH₂Cl₂ was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with 10% KHSO₄, H₂O, 5% NaHCO₃, H₂O, dried over anhydrous Na₂SO₄, and evaporated to dryness under reduced pressure. The product was crystallized from EtOAc/petroleum ether. Yield 83%; m.p.= 161-162 ° C; R_{f1} = 0.65, R_{f2} = 0.90, R_{f3} = 0.70; [α]_D²⁰ = -38.6 ° (c = 0.5, MeOH); IR (KBr) 3332, 1748, 1713, 1659, 1533 cm⁻¹; ¹H-NMR (CDCl₃, 250 MHz): δ 7.78 (m, 2H, Fmoc CH ar.), 7.55 (m, 2H, Fmoc CH ar.), 7.41-7.32 (m, 6H, 4H Fmoc CH ar. and 2H 2NH), 5.16 (s, 1H, NH Aib), 4.44 (m, 3H, 1H Met α CH and 2H Fmoc CH₂), 4.20 (m, 1H, Fmoc CH⁹), 3.67 (s, 3H, OMe), 2.59 (m, 2H, Met γ CH₂), 2.04 (s, 3H, Met ϵ CH₃), 2.09-2.03 (m, 2H, Met β CH₂), 1.52 (s, 6H, Aib 2CH₃), 1.49 (s, 6H, Aib 2CH₃).

Fmoc-(Aib)₂-L-Met-Aib-OMe

Fmoc-Aib-OH (1 g, 3.3 mmol) was dissolved in anhydrous CH₂Cl₂ and kept at 0° C. Then, HOAt (0.5 g, 3.3 mmol) and EDC·HCl (0.7 g, 3.5 mmol) were added to the solution. Then, H-Aib-L-Met-Aib-OMe (1 g, 3.3 mmol) (obtained by deprotection of Fmoc-Aib-L-Met-Aib-OMe in a 70:30 mixture of acetonitrile/diethylamine) was added, followed by 0.36 ml of NMM (3.3 mmol). After stirring the mixture at room temperature for 24 hours, CH₂Cl₂ was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with 10% KHSO₄, H₂O, 5% NaHCO₃, H₂O, dried over anhydrous Na₂SO₄, and evaporated to dryness under reduced pressure. The product was crystallized from CH₂Cl₂ / petroleum ether. Yield 80%; m.p.= 182-183 ° C; R_{f1} = 0.50, R_{f2} = 0.90, R_{f3} = 0.65; [α]_D²⁰ = -13.3 ° (c = 0.5, MeOH); IR(KBr) 3317, 1732, 1700, 1662, 1531 cm⁻¹; ¹H-NMR (CDCl₃, 250 MHz): δ 7.77 (m, 2H, Fmoc CH ar.), 7.58 (m, 2H, Fmoc CH ar.), 7.38 (m, 6H, 4H Fmoc CH ar. and 2H 2NH), 6.37 (s, 1H, Aib NH), 5.13 (s, 1H, Aib NH), 4.56 (m, 3H, 2H Fmoc CH₂ and 1H Met α CH), 4.21 (t, 1H, Fmoc CH⁹), 3.65 (s, 3H, OMe), 2.51 (m, 2H, Met γ CH₂), 2.32 (m, 1H, Met β CH₂), 2.03-2.01 (m, 1H, Met β CH₂), 2.02 (s, 3H, Met ϵ CH₃), 1.50-1.41 (m, 18H, Aib 6CH₃).

Fmoc-L-Bpa-(Aib)₂-L-Met-Aib-OMe

Fmoc-L-Bpa-OH (0.3 g, 0.61 mmol) was dissolved in anhydrous CH₂Cl₂ and kept at 0 ° C. Then, HOAt (0.08 g, 0.61 mmol) and EDC·HCl (0.13 g, 0.64 mmol) were added to the solution. Then, H-(Aib)₂-L-Met-Aib-OMe (0.26 g, 0.61 mmol) [obtained by deprotection of Fmoc-(Aib)₂-L-Met-Aib-OMe in a 70:30 mixture of acetonitrile/diethylamine] was added, followed by 0.066 ml of NMM (0.61 mmol). After stirring the mixture at room temperature for 24 hours, CH₂Cl₂ was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with 10% KHSO₄, H₂O, 5% NaHCO₃, H₂O, dried over anhydrous Na₂SO₄, and evaporated to dryness under reduced pressure. The product was crystallized from EtOAc/petroleum ether. Yield 90%; m.p.= 115-117 ° C; R_{f1} = 0.60, R_{f2} = 0.95, R_{f3} = 0.65; [α]_D²⁰ = -9.8 ° (c = 0.5, MeOH); IR(KBr) 3319, 1727, 1717, 1660, 1578 cm⁻¹; ¹H-NMR (CDCl₃, 250 MHz): δ 7.78-7.30 (m, 19H, 8H Fmoc CH ar., 9H Bpa CH ar. and 2H 2NH), 6.71 (s, 1H, Aib NH), 6.59 (s, 1H, Aib NH), 5.94 (d, 1H, Bpa NH), 4.52-4.10 (m, 5H, 2H 2 α CH, 2H Fmoc CH₂ and 1H Fmoc CH⁹), 3.68 (s, 3H, OMe), 3.31-2.95 (m, 2H, Bpa CH₂), 2.57 (m, 2H, Met γ CH₂), 2.36 (m, 1H, Met β CH₂), 2.21 (m, 1H, Met β CH₂), 2.09 (s, 3H, Met ϵ CH₃), 1.53-1.42 (m, 18H, Aib 6CH₃).

Boc-Aib-L-Bpa-(Aib)₂-L-Met-Aib-OMe (SH)

Boc-Aib-OH (91 mg, 0.45 mmol) was dissolved in anhydrous CH₂Cl₂ and kept at 0° C. Then, HOAt (61 mg, 0.45 mmol) and EDC·HCl (90 mg, 0.47 mmol) were added to the solution. Then, H-L-Bpa-(Aib)₂-L-Met-Aib-OMe (300 mg, 0.45 mmol) [obtained by deprotection of Fmoc-L-Bpa-(Aib)₂-L-Met-Aib-OMe in a 70:30 mixture of acetonitrile/diethylamine] was added, followed by 0.049 ml of NMM (0.45 mmol). After stirring the mixture at room temperature for 24 hours, CH₂Cl₂ was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with 10% KHSO₄, H₂O, 5% NaHCO₃, H₂O, dried over anhydrous Na₂SO₄, and evaporated to dryness under reduced pressure. The product was crystallized from EtOAc/petroleum ether. Yield 74%; m.p.= 99-101 ° C; R_{f1} = 0.65, R_{f2} = 0.90, R_{f3} = 0.65; [α]_D²⁰ = -10.5 ° (c = 0.5, MeOH); IR(KBr) 3310, 1742, 1661, 1578 cm⁻¹; ¹H NMR (600 MHz, CDCl₃): δ 7.88 (s, 1H, Aib³ NH), 7.81 (d, 2H, Ar-CH, ring 1), 7.79 (d, 2H, Ar-CH *ortho*, ring 2), 7.63 (m, 1H, Ar-CH *para*, ring 2), 7.57 (d, 1H, Met⁵ NH), 7.52 (m, 2H, Ar-CH *meta*, ring 2), 7.49 (s, 1H, Aib⁶ NH), 7.32 (d, 2H, Ar-CH, ring 1), 7.11 (s, 1H, Aib⁶ NH), 6.59 (d, 1H, Bpa² NH), 4.92 (s, 1H, Aib¹ NH), 4.45 (m, 1H, Met αCH), 4.32 (m, 1H, Bpa αCH), 3.71 (s, 3H, OCH₃), 3.26 (m, 2 H, Bpa βCH₂), 2.62 (m, 2 H, Met γCH₂), 2.40 and 2.20 (2m, 2H, βCH₂), 2.14 (s, 3 H, Met εCH₃), 1.61, 1.58 and 1.54 (3s, 9H, 3 Aib βCH₃), 1.53-1.50 (m, 6H, 2 Aib βCH₃), 1.47 and 1.42 (2s, 6H, 2 Aib βCH₃), 1.36 (s, 9H, Boc CH₃), 1.34 (s, 3H, Aib βCH₃).

Laser Flash Photolysis

Acetonitrile (MeCN; Caledon HPLC grade) was used as received from the supplier.

Laser flash photolysis experiments employed the pulses from a Lambda-Physik Compex 120 excimer laser filled with F₂/Kr/Ne mixtures (248 nm; ca. 20 ns; ca. 100 mJ) or a Lumonics TE-861M excimer laser filled with Xe/HCl/H₂/He (308 nm, 15 ns, ca. 40 mJ), and a Luzchem Research mLFP-111 laser flash photolysis system, modified as described previously.^{S1} Samples were contained in 7 x 7 mm Suprasil quartz cells sealed with rubber septa, at concentrations adjusted so as to produce absorbances between 0.2 to 0.9 at the laser wavelength. Each solution was deaerated with argon or dry nitrogen until constant lifetimes were achieved. Quenchers (1,3-cyclohexadiene or Boc-L-Met-OMe) were added as aliquots of standard solutions in MeCN.

Transient decay rate constants were calculated by non-linear least squares analysis of the absorbance-time profiles using the Prism 5.0 software package (GraphPad Software, Inc.), after importing the raw data from the Luzchem mLFP software. Quenching rate constants (“*k_Q*”) were calculated by linear or non-linear least-squares analysis of decay rate-concentration data (at least 7 points) that spanned as large a range in transient decay rate as possible. Errors are quoted as twice the standard deviation obtained from the least-squares analyses.

Preparative Photolysis of SH

SH (17 mg) was dissolved in 25 mL acetonitrile (0.8 mM). The solution was divided into five equal portions, which were each placed in quartz tubes (12 mm diameter) and deaerated with a fine stream of argon for 10 minutes. The tubes were sealed with rubber septa and irradiated in parallel for 40 minutes in a Rayonet® photochemical reactor (Southern New England Ultraviolet Co.) equipped with 12 × 350 nm lamps. The progress of the photolysis reaction was monitored by LC-MS [Waters 2960 HPLC system, equipped with a HyPurity Aquastar column, 150 × 4.6 mm, 3 micron particle size, Thermo Electron Corp.; Micromass Quattro Ultima triple-quadrupole mass spectrometer, using electrospray ionization (in positive ion mode)]. The elution rate was 1 mL/min using the solvent program below (linear gradient).

Time / min	1% formic acid in water	1% formic acid in acetonitrile
0	95	5
25	5	95
27	5	95

Photolysis for 40 minutes resulted in the gradual disappearance of the starting hexapeptide (**SH**) and the concomitant formation of two major photoproducts (**A** and **B**) with roughly equal HPLC peak areas, both of which were isomeric with **SH**. The contents of the 5 tubes were recombined and the solvent was removed under vacuum at ambient temperature to yield a pale yellow solid.

The reaction mixture was subjected to flash column chromatography (silica gel, 95:5 CH₂Cl₂/EtOH solvent mixture as eluant) to yield, in order, the starting hexapeptide **SH**, and the photoproducts **A** and **B**.

Photoproduct A

m.p.= 161-163 °C; R_{f1} = 0.50; R_{f2} = 0.95; R_{f3} = 0.55; [α]_D²⁰ = + 22.3° (c = 0.5, MeOH); IR (KBr): 3308, 1739, 1659, 1534 cm⁻¹; ¹H NMR (600 MHz, CDCl₃): δ 7.81 (s, 1H, Aib³ NH), 7.66 (d, 1H, Met⁵ NH), 7.47 (s, 1H, Aib⁶ NH), 7.45 (d, 2H, Ar-CH *meta*, ring 2), 7.38 (d, 2H, Ar-CH ring 1), 7.32 (m, 2H, Ar-CH *ortho*, ring 2), 7.27 (m, 1H, Ar-CH *para*, ring 2), 7.13 (s, 1H, Aib⁴ NH), 7.06 (d, 2H, Ar-CH, ring 1), 6.72 (d, 1H, Bpa² NH), 5.79 (s, 1H, Aib¹ NH), 4.01 (2H, Met α-CH and Bpa α-CH), 3.73 (s, 3H, OCH₃), 3.65 (m, 1 H, Met ε-CH₂), 3.20 (d, 1 H, Bpa β-CH₂), 3.04 (d, 1 H, Met ε-CH₂), 2.60 (m, 1 H, Bpa β-CH₂), 2.01 (4H, Met β-CH₂ and Met γ-CH₂), 1.53 (s, 3H, 1 Aib β-CH₃), 1.51 (9H, 3 Aib β-CH₃), 1.50 and 1.49 (2s, 6H, 2 Aib β-CH₃), 1.43 (s, 3H, 1 Aib β-CH₃) 1.42 (s, 9H, Boc CH₃), 1.41 (s, 3H, 1 Aib β-CH₃).

Photoproduct B

m.p.= 182-184°C ; R_{f1} = 0.50; R_{f2} = 0.95; R_{f3} = 0.55; [α]_D²⁰ = + 38.4° (c = 0.5, MeOH); IR (KBr): 3308, 1740, 1660, 1533 cm⁻¹; ¹H NMR (600 MHz, CDCl₃): δ 7.82 (s, 1H, Aib³ NH), 7.62 (d, 1H, Met⁵ NH), 7.53 (s, 1H, Aib⁶ NH), 7.49 (d, 2H, Ar-CH *ortho*, ring 2), 7.35 (m, 2H, Ar-CH ring 1), 7.32 (m, 2H, Ar-CH *meta*, ring 2), 7.26 (m, 1H, Ar-CH *para*, ring 2), 7.14 (s, 1H, Aib⁴ NH), 7.06 (d, 2H, Ar-CH, ring 1), 6.73 (d, 1H, Bpa² NH), 5.07 (s, 1H, Aib¹ NH), 4.24 (m, 1H, Met α-CH), 3.96 (m, 1H, Bpa α-CH), 3.74 (s, 3H, OCH₃), 3.56 and 3.40 (2m, 2 H, Met ε-CH₂), 3.11 and 2.62 (2m, 2 H, Bpa β-CH₂), 2.06 and 1.93 (2m, 4H, Met γ-CH₂), 1.85 and 1.65 (2m, 4H, Met β-CH₂), 1.59-1.58 (6H, 2 Aib β-CH₃), 1.54 (3H, 1 Aib β-CH₃), 1.53 (3H, 1 Aib β-CH₃), 1.49 (s, 9H, Boc CH₃), 1.46, 1.42, 1.40 and 1.33 (4s, 6H, 2 Aib β-CH₃).

X-Ray Diffraction

Colorless crystals of the product **A** arising from the Yang photocyclization on Boc-Aib-L-Bpa-(Aib)₂-L-Met-Aib-OMe were grown as a monohydrate by slow evaporation from a methanol solution. Diffraction data were collected on a Philips PW1100 four-circle diffractometer, using graphite-monochromated CuKα radiation. Unit cell parameters were obtained by least-squares refinement of the angular settings of 48 carefully centered reflections in the 12 – 18° θ range. Intensities were corrected for Lorentz and polarization effects, not for absorption.

The structure was solved by direct methods of the SIR 2002 program.^{S2} Refinement was carried out by least-squares procedures on *F*², using all data, by application of the SHELXL-97 program,^{S3} with all non-H atoms anisotropic, and allowing the positional parameters and the anisotropic displacement parameters of the non-H atoms to refine at alternate cycles. The macrocycle resulting from the photocrosslinking of the Bpa and Met side chains is partly disordered. Specifically, the Met C^β and C^γ

atoms were refined on two sets of positions (atoms C5B – C5G and C5B' – C5G') with population parameters of 0.55 and 0.45, respectively. In addition, the phenyl ring external to the macrocycle shows librational disorder and was refined on two sets of positions, namely atoms labeled C202 to C207 with 0.55 population parameter, and atoms labeled C212 to C217 with 0.45 population parameter. Restraints were applied to the bond distances, bond angles, and anisotropic displacement parameters of the atoms belonging to the disordered parts. A planarity restraint was also imposed to each of the phenyl rings. H-atoms on the cocrystallized water molecule were located on a ΔF map and they were not refined. The remaining H-atoms were calculated at idealized positions and refined using a riding model. Crystal data and structure refinement parameters are listed in Table S1. Relevant torsion angles and H-bond parameters are reported in Tables S2 and S3, respectively.

Supporting References

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Table S1. Crystal data and structure refinement parameters for mc140b (photoproduct A monohydrate)

Empirical formula	$C_{43} H_{62} N_6 O_{10} S \times H_2O$	
Formula weight	873.06	
Temperature	293(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P2 ₁ 2 ₁ 2 ₁	
Unit cell dimensions	a = 12.533(2) Å	$\alpha = 90^\circ$
	b = 15.944(3) Å	$\beta = 90^\circ$
	c = 24.989(5) Å	$\gamma = 90^\circ$
Volume	4993.5(16) Å ³	
Z	4	
Density (calculated)	1.161 Mg/m ³	
Absorption coefficient	1.062 mm ⁻¹	
F(000)	1872	
Crystal size	0.35 × 0.25 × 0.15 mm ³	
Theta range for data collection	3.54 to 58.93°.	
Index ranges	-1 ≤ h ≤ 13, 0 ≤ k ≤ 17, 0 ≤ l ≤ 27	
Reflections collected	4403	
Independent reflections	4350 [R(int) = 0.0418]	
Completeness to theta = 58.93°	99.8 %	
Absorption correction	None	
Refinement method	Full-matrix-block least-squares on F ²	
Data / restraints / parameters	4350 / 170 / 623	
Goodness-of-fit on F ²	0.882	
Final R indices [I > 2σ(I)]	R ₁ = 0.0744, wR ₂ = 0.1882	
R indices (all data)	R ₁ = 0.1716, wR ₂ = 0.2304	
Extinction coefficient	0.00027(14)	
Largest diff. peak and hole	0.273 and -0.245 e.Å ⁻³	

Table S2. Selected torsion angles [°] for mc140b (photoproduct **A** monohydrate)

C01-OU-C0-N1	-177.2(13)
OU-C0-N1-C1A	-179.7(8)
N1-C1A-C1-N2	-28.0(12)
C1A-C1-N2-C2A	-179.2(8)
C1-N2-C2A-C2	-53.0(12)
N2-C2A-C2B-C2G	-176.4(8)
C2A-C2B-C2G-C2D1	-102.9(10)
C2A-C2B-C2G-C2D2	77.8(10)
C2E1-C2Z-C201-C5E	90.0(13)
C2E2-C2Z-C201-C5E	-87.0(13)
N2-C2A-C2-N3	-37.2(11)
C2A-C2-N3-C3A	-172.9(8)
C2-N3-C3A-C3	-53.6(11)
N3-C3A-C3-N4	-30.3(12)
C3A-C3-N4-C4A	-177.5(8)
C3-N4-C4A-C4	-59.4(12)
N4-C4A-C4-N5	-24.0(13)
C4A-C4-N5-C5A	178.1(9)
C4-N5-C5A-C5	-65.0(12)
N5-C5A-C5B-C5G	-176.1(15)
N5-C5A-C5B'-C5G'	90(2)
C5A-C5B-C5G-S5	178.6(12)
C5A-C5B'-C5G'-S5	162.0(16)
C5B-C5G-S5-C5E	82.9(16)
C5B'-C5G'-S5-C5E	-173(2)
C5G-S5-C5E-C201	-144.2(9)
C5G'-S5-C5E-C201	-110.7(15)
N5-C5A-C5-N6	-34.7(13)
C5A-C5-N6-C6A	178.8(10)
C5-N6-C6A-C6	46.3(15)
N6-C6A-C6-OT	41.8(14)
C6A-C6-OT-CT	-175.1(13)

Table S3. Intra- and intermolecular H-bond parameters [\AA and $^\circ$] for mc140b (photoproduct A monohydrate)

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N3-H3...O0	0.86	2.27	3.082(10)	158.3
N4-H4...O1	0.86	2.33	3.128(10)	154.9
N5-H5...O2	0.86	2.09	2.924(9)	163.3
N6-H6...O3	0.86	2.28	2.981(11)	139.2
N1-H1...O5#1	0.86	2.12	2.956(9)	163.5
N2-H2...O1W	0.86	2.12	2.958(11)	164.0
O1W-H1W...O4#1	0.78	1.90	2.665(13)	166.7
O1W-H2W...OU	0.85	2.30	3.150(17)	177.1
O201-H201...O1#2	0.82	2.22	2.972(11)	153.3

Symmetry transformations used to generate equivalent atoms:

#1 $-x+1/2, -y+1, z-1/2$ #2 $x+1/2, -y+1/2, -z+1$

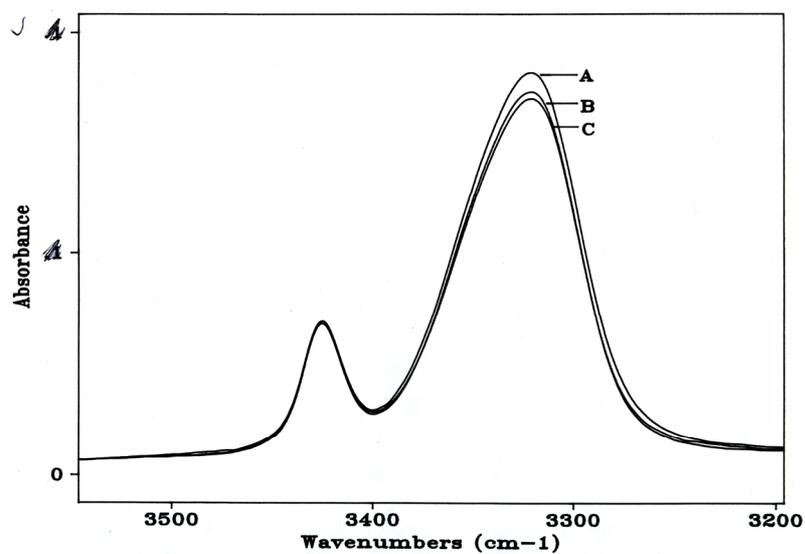


Figure S1. FT-IR absorption spectra (3500 – 3200 cm⁻¹ region) of **SH** in CDCl₃ solution as a function of peptide concentration (A: 10 mM; B: 1 mM; C: 0.1 mM).

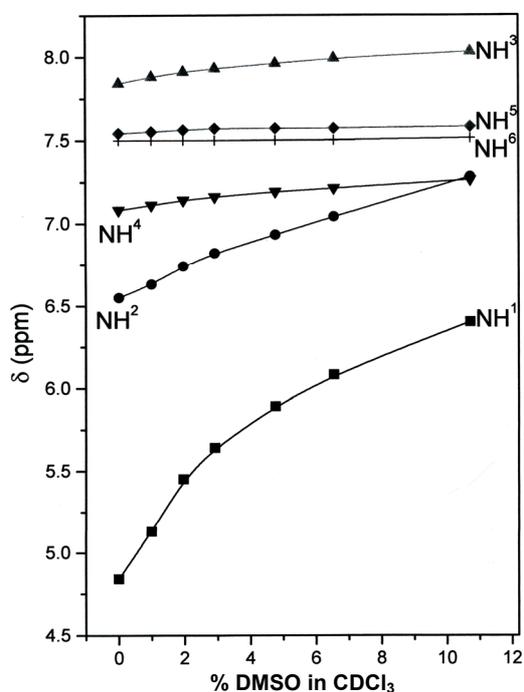


Figure S2. Plot of NH proton chemical shifts in the ¹H NMR spectrum of **SH** as a function of increasing percentages (v/v) of DMSO-*d*₆ added to the CDCl₃ solution. Peptide concentration: 10 mM.

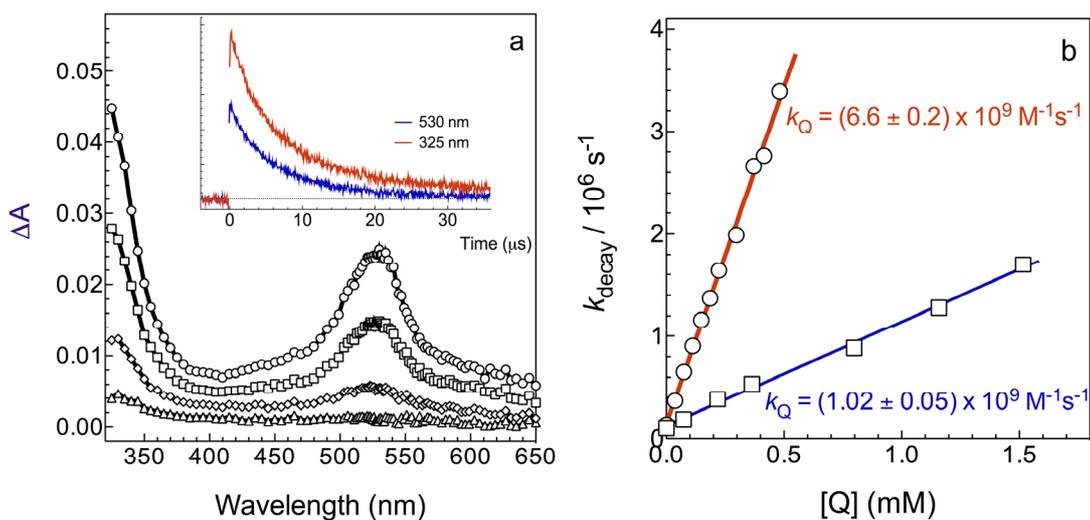


Figure S3. (a) Transient absorption spectra recorded by 308 nm laser flash photolysis of a deoxygenated solution of Boc-L-Bpa-OMe (5.9 mM) in acetonitrile, 0.3 – 0.8 μs (\circ), 2.9 – 4.2 μs (\square), 9.4 – 12.7 μs (\diamond), and 28 – 34 μs (\triangle) after the laser pulse; the inset shows representative transient decay traces, recorded at monitoring wavelengths of 530 nm and 325 nm. The spectrum and lifetime is almost indistinguishable from that of 4-methylbenzophenone under similar conditions.^{S4} (b) Plots of the pseudo-first order decay rate constant (k_{decay}) of the 525 nm absorption of Fig. S3a vs. the concentration of added 1,3-cyclohexadiene (red) and Boc-L-Met-OMe (blue) at 25 °C; the solid lines are the best linear least squares fits of the data to the equation $k_{\text{decay}} = k_0 + k_Q[Q]$. The value of k_Q obtained with the diene as quencher verifies that the 525 absorption in Fig. S3a is due to the triplet state of Boc-L-Bpa-OMe, while that obtained with Boc-L-Met-OMe verifies that the triplet is quenched (bimolecularly) by the methionine residue in Boc-L-Met-OMe.

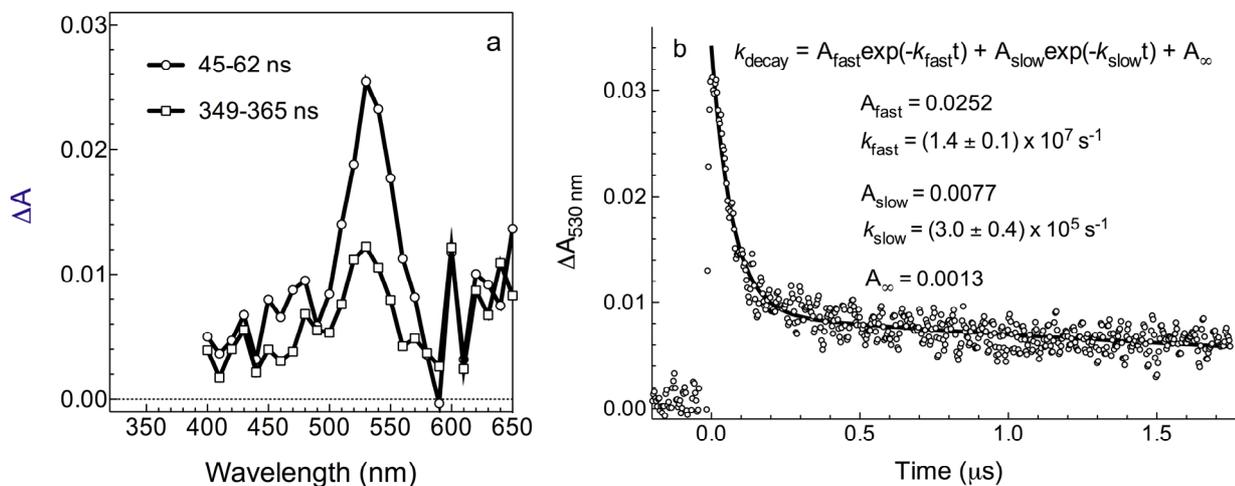


Figure S4. (a) Transient absorption spectra recorded by 248 nm laser flash photolysis of a deoxygenated solution of **SH** (58 μM) in acetonitrile, 45 - 62 ns (\circ) and 349 - 365 ns (\square) after the laser pulse. (b) Representative transient decay trace, recorded at a monitoring wavelength of 530 nm; the solid line shows the non-linear least squares fit of the data to a two-phase exponential decay ($k_{\text{decay}} = A_{\text{fast}}e^{-k_{\text{fast}}t} + A_{\text{slow}}e^{-k_{\text{slow}}t} + A_{\infty}$), with A_{∞} constrained to a value of $A_{\infty} = 0.0013$ (determined in experiments carried out on longer time-scales). The best fit parameters are shown in the figure (errors as $\pm 2\sigma$).

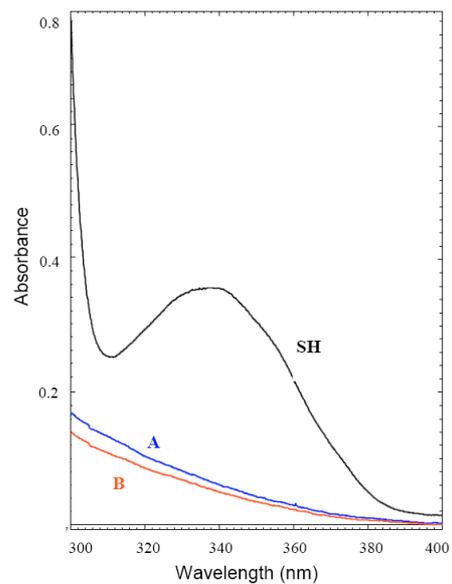


Figure S5. Near-UV absorption spectra (300 – 400 nm region) of **SH**, **A**, and **B** in acetonitrile solution. Peptide concentration: 1 mM.

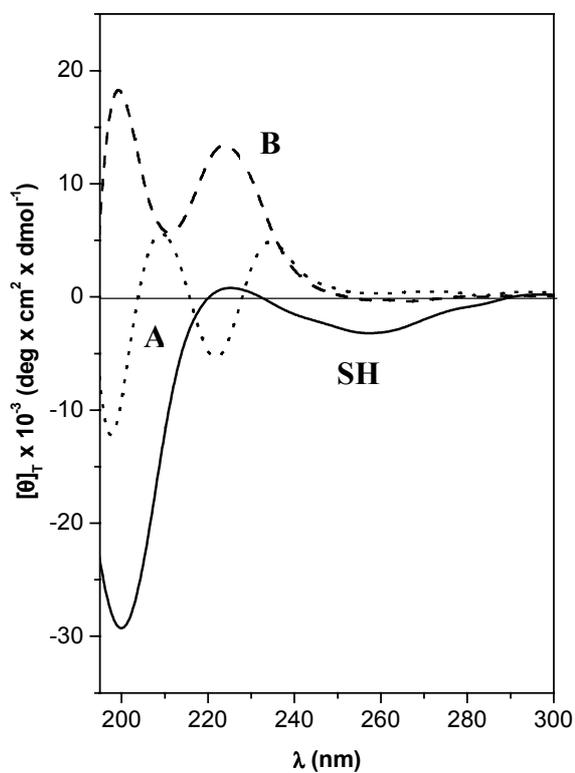


Figure S6. CD spectra (195 – 300 nm region) of **SH**, **A**, and **B** in 2,2,2-trifluoroethanol solution. Peptide concentration: 1 mM.

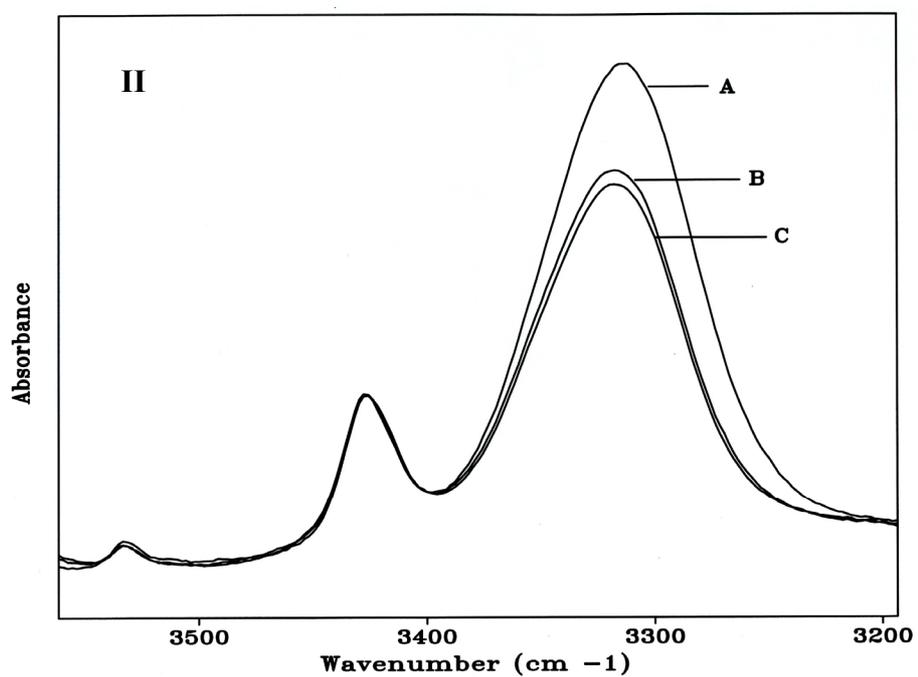
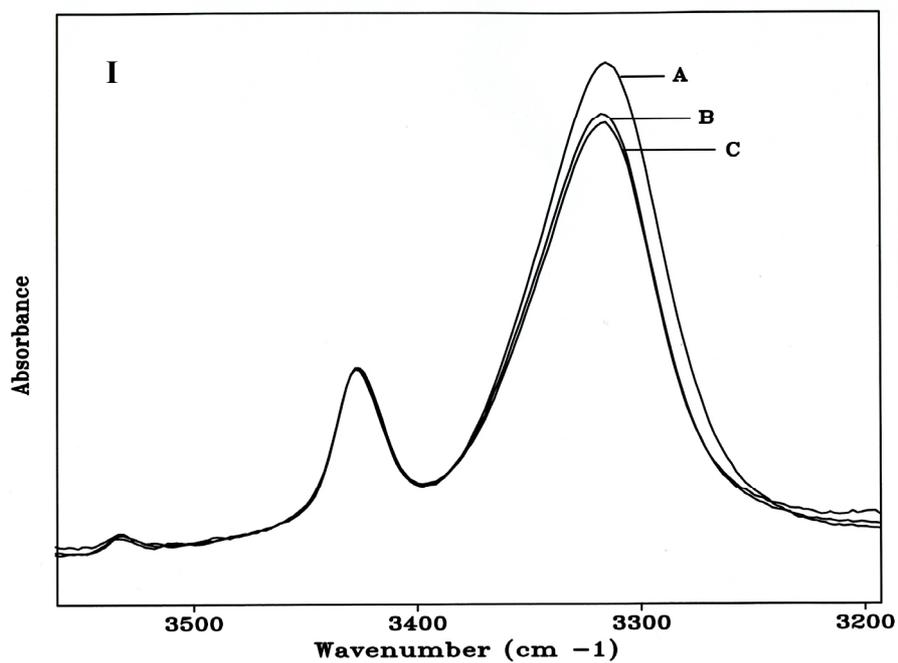


Figure S7. FT-IR absorption spectra (3500 – 3200 cm⁻¹ region) of **A** (I) and **B** (II) in CDCl₃ solution as a function of peptide concentration (A: 10 mM; B: 1 mM; C: 0.1 mM).

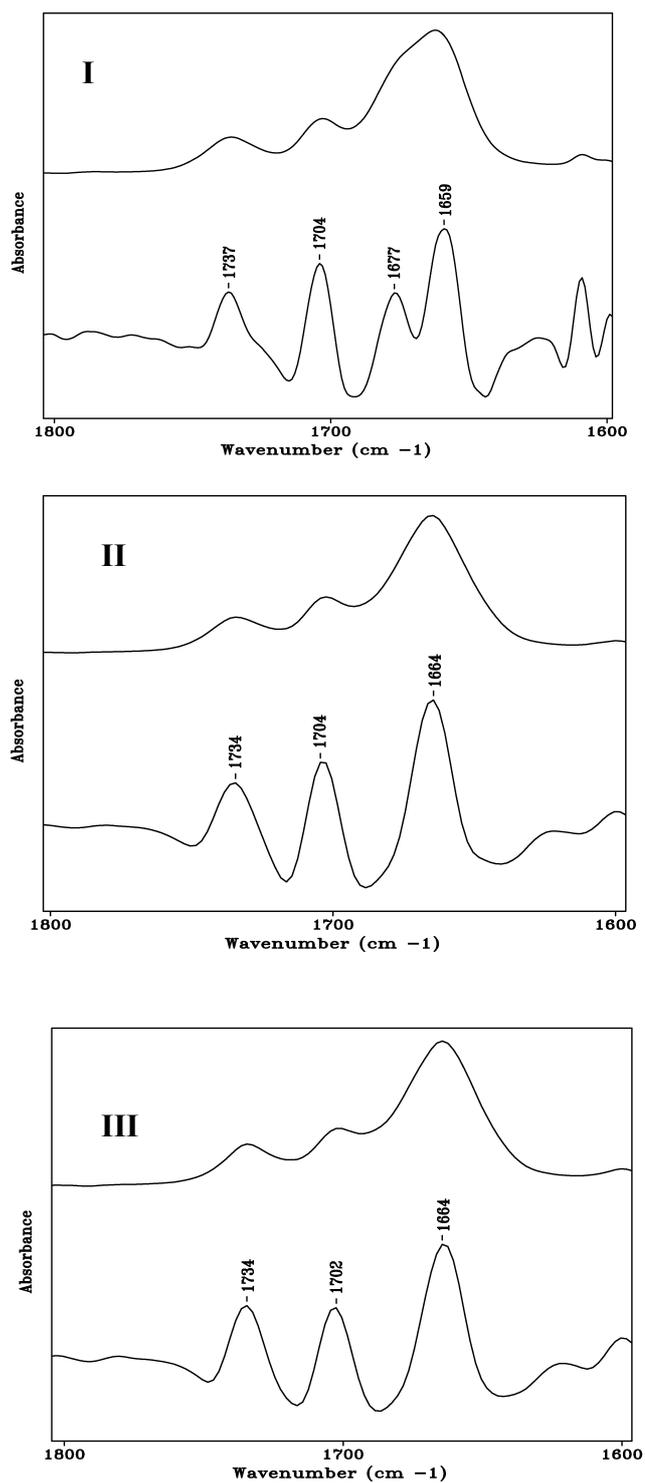


Figure S8. FT-IR absorption spectra and the corresponding inverted second derivatives (1800 – 1600 cm⁻¹ region) of SH (I), A (II), and B (III) in CDCl₃ solution. Peptide concentration: 1 mM.

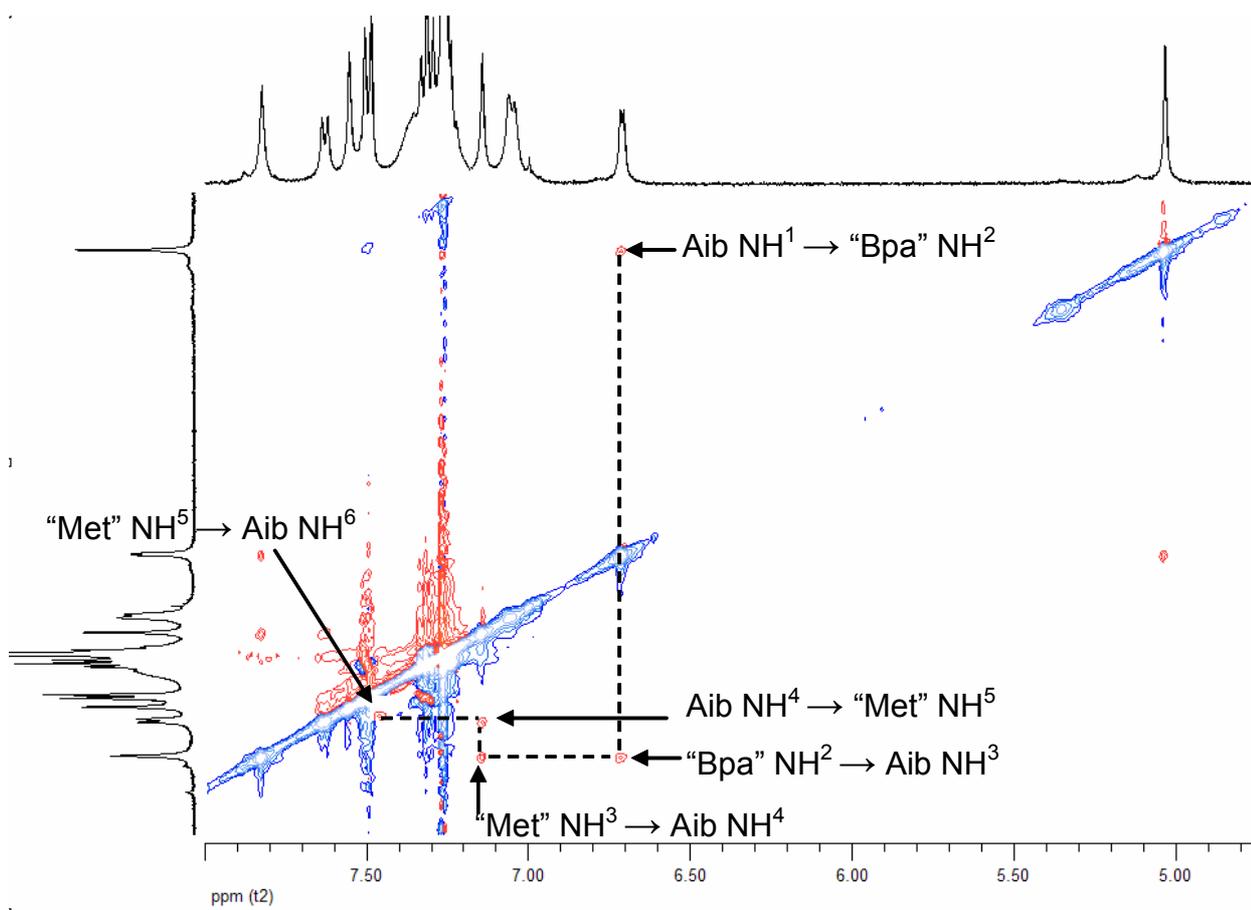


Figure S9. $\text{NH}_i \rightarrow \text{NH}_{i+1}$ sequential NOEs in the 600 MHz NMR spectrum of **B** in CDCl_3 solution. Peptide concentration: 1 mM.

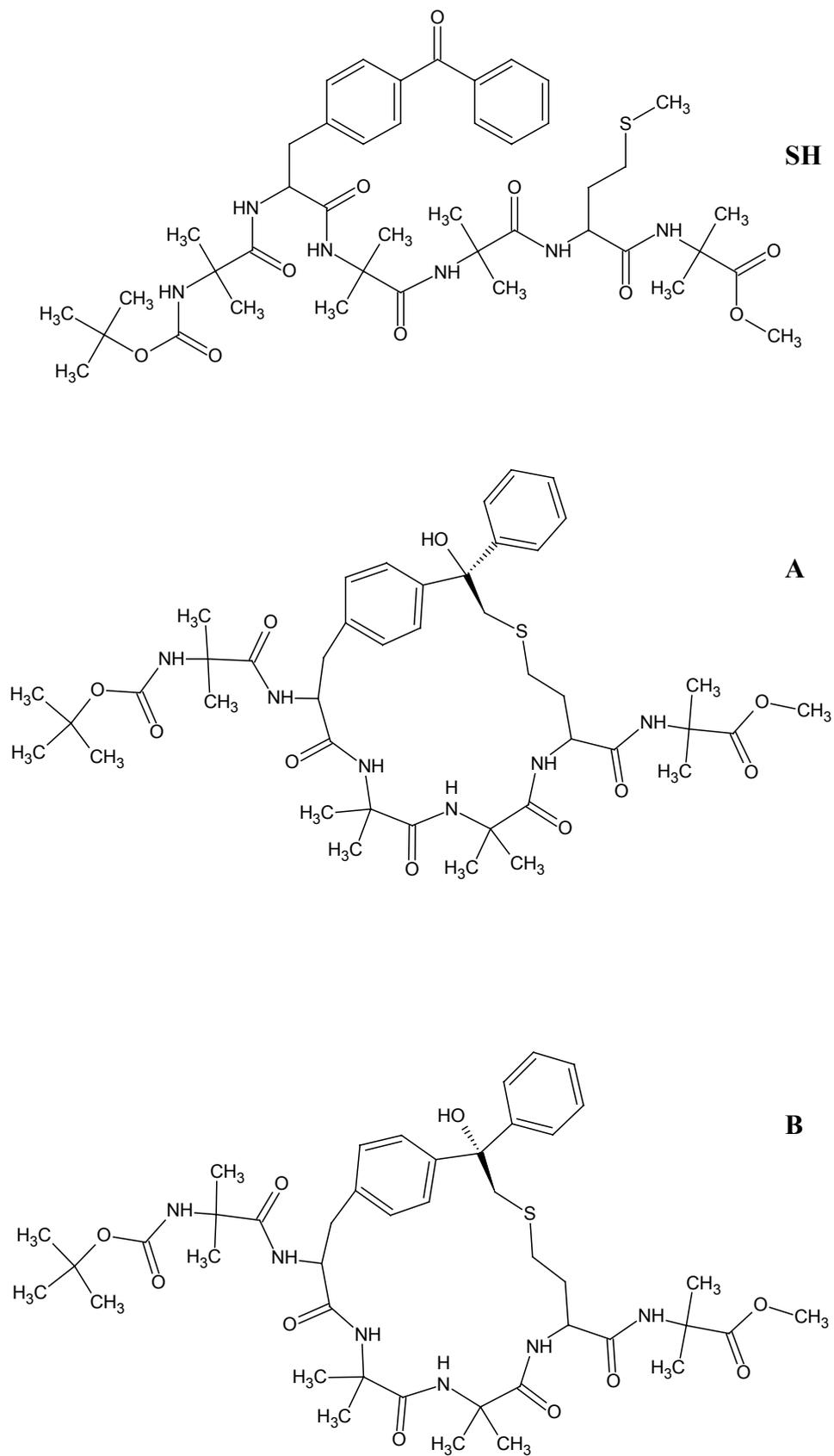


Figure S10. Chemical structures of SH, A, and B.

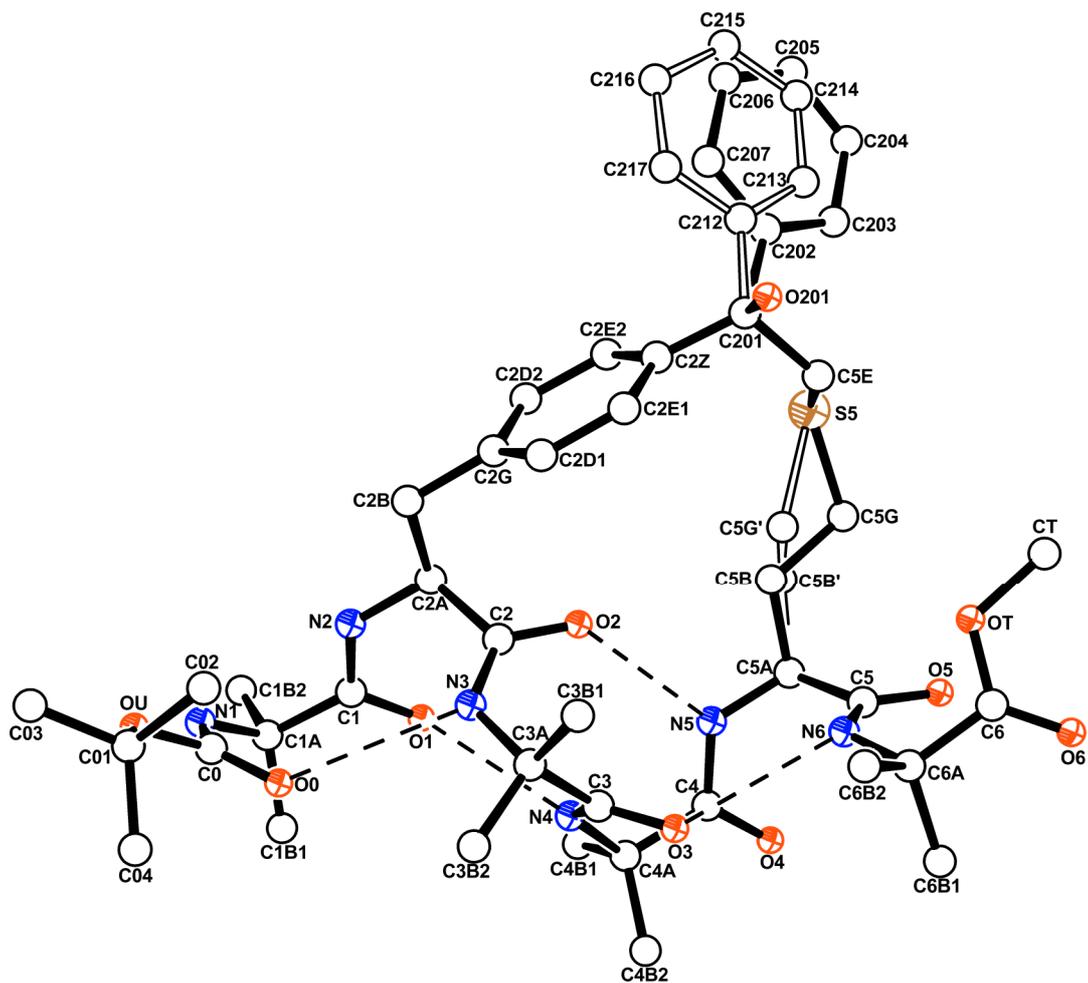


Figure S11. X-ray diffraction structure of **A** with atom numbering. The minor occupancy sites are indicated by “open” bonds.