

Supporting Information for

Step-wise and pre-organization induced synthesis of a crossed alkene-bridged nisin Z DE-ring mimic by ring-closing metathesis

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Experimental section

General

Unless stated otherwise chemicals were obtained from commercial sources and used without further purification. DIPEA was distilled consecutively from ninhydrin and KOH. Dry solvents were obtained as peptide grade solvents from Biosolve (Valkenswaard, The Netherlands) and stored over molecular sieves (4Å). NMR spectra were recorded on a Varian Gemini-300 (300 MHz) or on an INOVA-500 (500 MHz). ¹H NMR chemical shift values are given in ppm relative to TMS. Peak assignments are based on COSY, TOCSY and ROESY experiments. Electrospray ionization mass spectrometry (EI-MS) was carried out using a Shimadzu LC-MS QP-8000 single quadrupole benchtop mass spectrometer coupled to a QP-8000 data system. High resolution mass spectra (HR-MS) were measured on a Micromass LCT mass spectrometer, with pentaphenylalanine as reference. MS/MS-spectra were analyzed on a Micromass Quattro Ultima or a Micromass Q-TOF mass spectrometer. MALDI-TOF analysis was performed on a Kratos Axima CFR apparatus, with bradykinin(1-7) as external reference and α-cyano-4-hydroxycinnamic acid as matrix. *R_f* values were determined by thin layer chromatography (TLC) on Merck precoated silica gel 60F₂₅₄ (0.25 mm) plates. Spots were visualized with UV quenching, ninhydrin or TDM/Cl₂.¹ Column chromatography was performed on silica gel 60 (70-230 mesh). Analytical HPLC was performed on a Shimadzu HPLC system (SPD-10A VP) coupled to an evaporative light scattering detector (PL-

ELS 1000, Polymer Laboratories) or a UV/VIS detector operating at 220/254 nm. Analytical HPLC runs were performed on an Alltech Adsorbosphere XL C8 column (90Å pore size, 5µm particle size, 0.46 × 25 cm) at a flow of 1 mL/min using a linear gradient of buffer B (100% in 25 min) from 100% buffer A (buffer A: 0.1% TFA in H₂O; buffer B: 0.085% TFA in CH₃CN/H₂O 95:5 v/v). Preparative HPLC runs were performed on an Alltech Adsorbosphere XL C8 column (90Å pore size, 10µm particle size, 2.2 × 25 cm) at a flow of 11.5 mL/min using a linear gradient of buffer B (100% in 40 min) from 100% buffer A (buffer A: 0.1% TFA in H₂O; buffer B: 0.085% TFA in CH₃CN/H₂O 95:5 v/v). Azido acids were synthesized according to the method described by Lundquist and Pelletier.² The hydroxysuccinimide esters were synthesized as described by Anderson *et al.*³

Computational modeling

The modeling experiments were performed with MacroModel 7.0⁴ on a SiliconGraphics O₂ workstation using the organic builder and the peptide builder in the grow mode. MMFF was used as forcefield.^{5a} Structure minimization was performed on a Silicon Graphics Origin 200 Server and molecular mechanics calculations were performed with the next settings:^{5b-d} MMFF (planar N's), PRCG, CCrit 0.01 kJ/molÅ, Iterations > max. Finally, a conformational search was carried out starting with the minimized structure using a Monte Carlo run which generated 1000 structures (all appropriate single bonds will become variable, all double bonds, amide bonds and ester bonds will become

constrained, potential chiral centers will be set and flexible rings will be opened). The goal of conformational searching was to locate the low-energy conformations of the structure of interest. The settings of a standard conformational search: Monte Carlo Multiple Minimum (MCMM); Number of Steps: 1000; Solvent: chloroform.^{5e} After each MCMM step, the structure was again minimized (with the same settings as above).

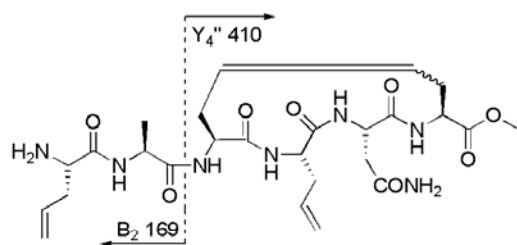
References and Notes

1. E. von Arx, M. Faupel and M.J. Brugger, *J. Chromatogr.*, 1976, **120**, 224.
2. J.T. Lundquist, IV and J.C. Pelletier, *Org. Lett.*, 2001, **3**, 781.
3. G.W. Anderson, J.F. Zimmerman and F.M. Callahan, *J. Am. Chem. Soc.*, 1964, **86**, 1839.
4. F. Mohamadi, N.C.J. Richards, W.C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson and W.C. Still, *J. Comput. Chem.*, 1990, **11**, 440.
5. (a) Halgren, *J. Comput Chem.*, 1996, **17**, issues 5 and 6. (will contain five articles introducing MMFF94 as a good force field for biopolymers (peptides and proteins) and many organic molecules); (b) RCG: Conjugate gradient minimization using the Polak-Ribiere first derivative method with restarts every 3N iterations. Should not find saddle points. Best general minimization method for energy minimization. BatchMin code for carrying out this method is highly vectorized for efficient operation on vector hardware: E. Polak and G. Ribiere, *Revue*

Francaise Informat. Recherche Operationelle, 1969, **16**, 35; (c) Convergence criterion - energy, movement or gradient. Default is gradient (first derivative RMS) convergence (criterion = 0.01 kJ/molÅ); (d) Iterations/stop, sets the maximum number of iterations BatchMin will use to energy minimize a structure; (e) W.C. Still, A. Tempczyk, R.C. Hawley and T. Hendrickson, *J. Am. Chem. Soc.*, 1990, **112**, 6127 (This solvent model provides a volume-based continuum model (the GB/SA model) for the electrostatic (polarization) component).

Scheme 1. Chemical structures of peptides **8**, **9**, and **17**.

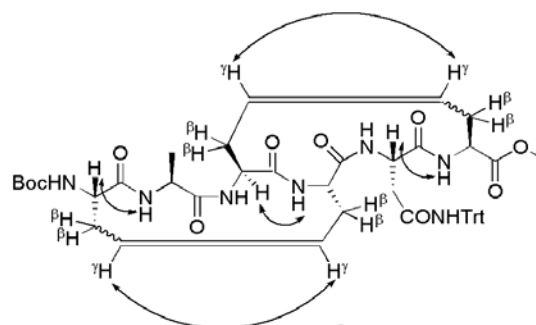
MS/MS fragmentation pattern of **8**:



8

cyclo(3-6)-H-Alg¹-Ala²-Alg³-Alg⁴-Asn⁵-Alg⁶-OMe

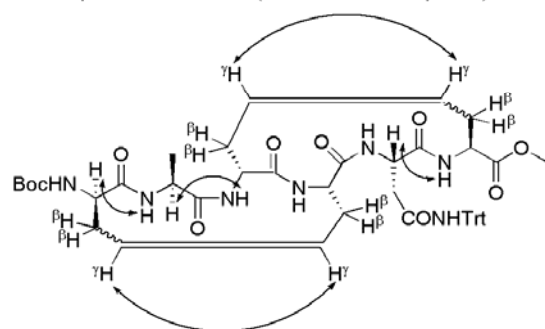
Proton-proton interactions (ROESY/TOCSY spectra) of **9**:



9

bicyclo(1-4/3-6)-Boc-Alg¹-Ala²-Alg³-Alg⁴-Asn(Trt)⁵-Alg⁶-OMe

Proton-proton interactions (ROESY/TOCSY spectra) of **17**:



17

bicyclo(1-4/3-6)-Boc-D-Alg¹-Ala²-D-Alg³-Alg⁴-Asn(Trt)⁵-Alg⁶-OMe

Figure 1. TOCSY spectrum of bicyclic peptide **9**. The regions that are expanded in the panels A, B and C correspond to Figure 5A upper panel, Figure 5A middle panel and Figure 5B, respectively.

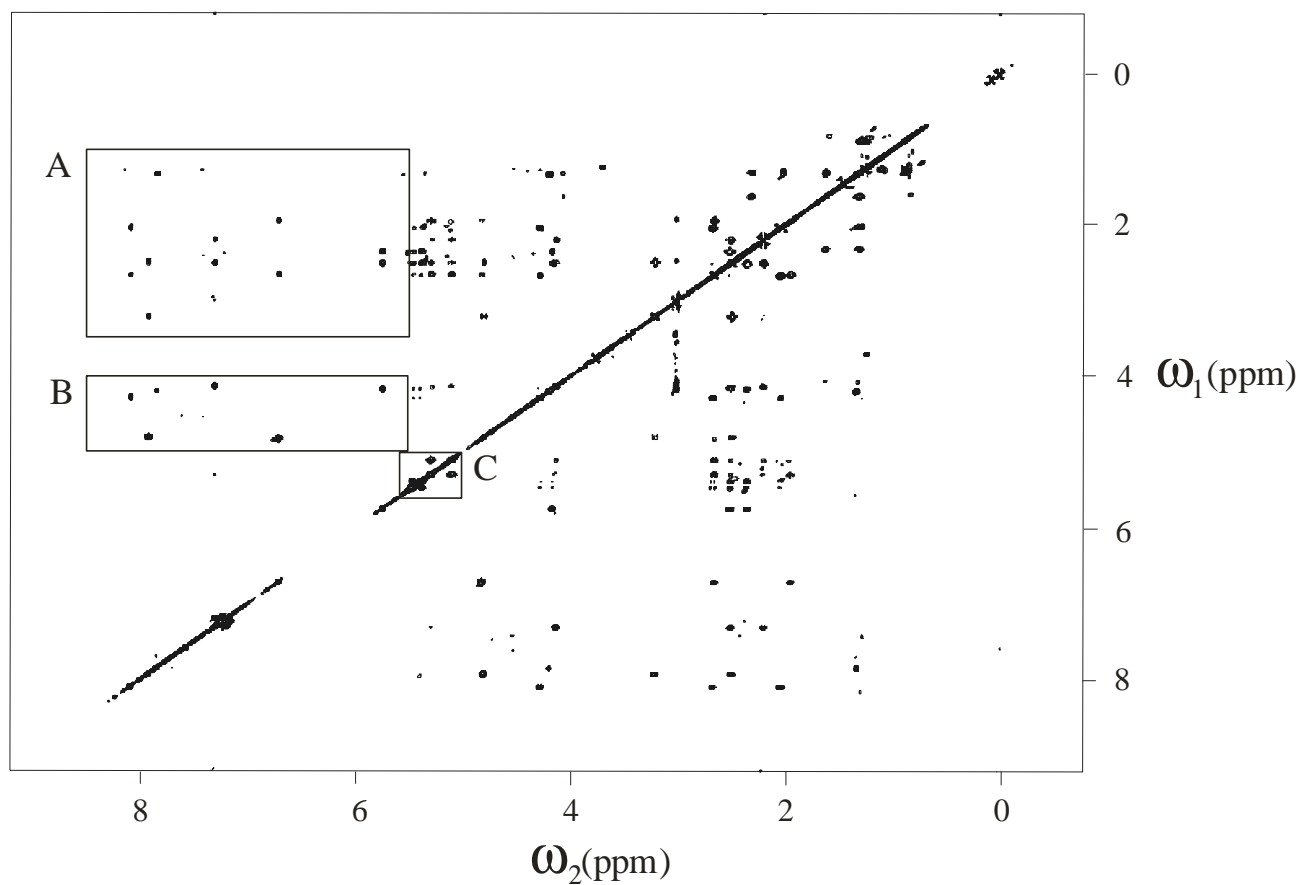


Figure 2. Expansion of the TOCSY spectrum of bicyclic peptide **17** showing the $C\beta H/C\alpha H/NH$ cross-peaks.

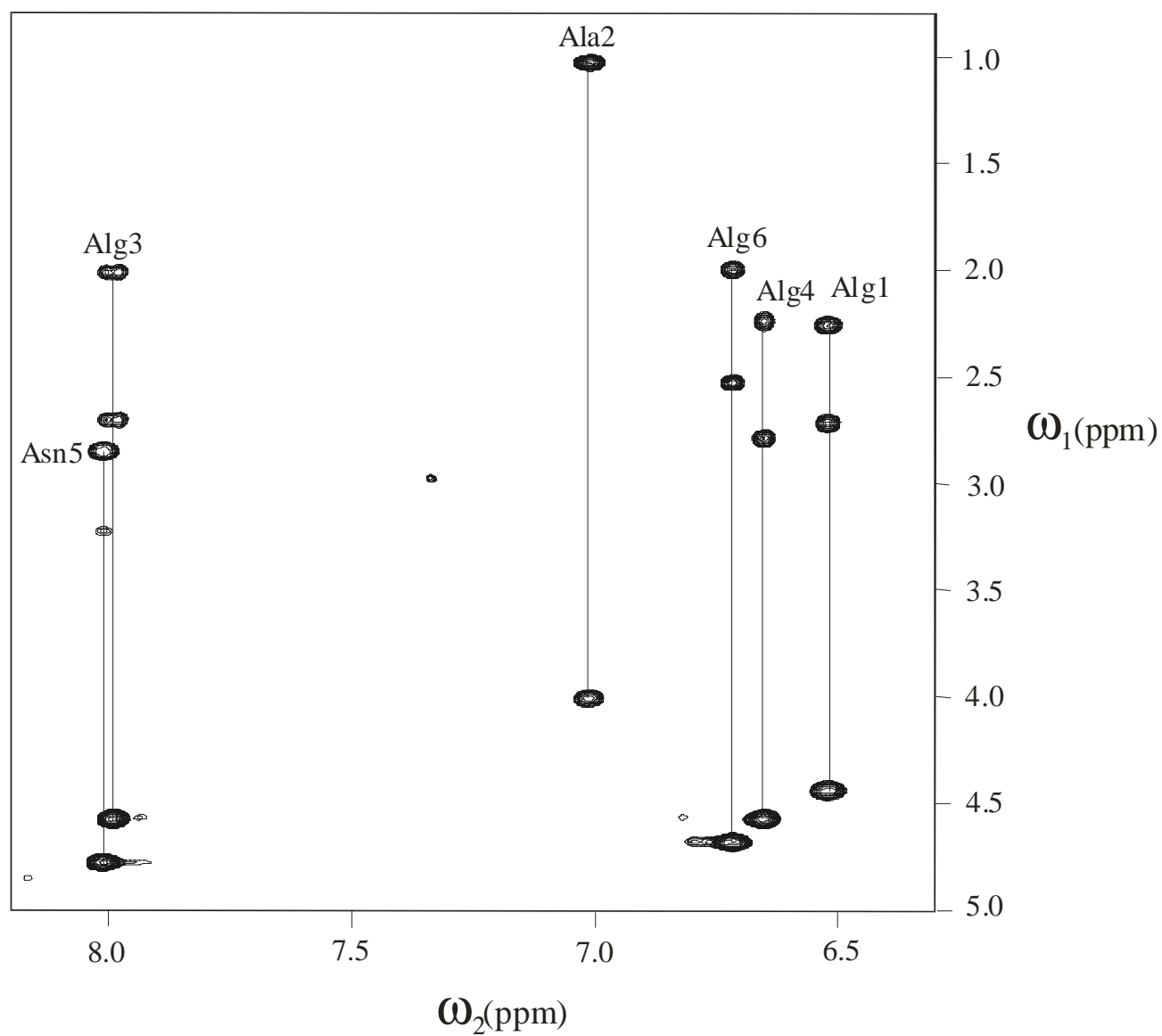


Figure 3. Expansion of the TOCSY spectrum of bicyclic peptide **17** showing the γ -proton connectivities of Alg4/D-Alg1 and D-Alg3/Alg6 which proves the bicyclic structure of **17**.

