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Three novel anabaenopeptins from the cyanobacterium Anabaena sp.

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1. Introduction

Anabaenopeptins are a group of cyclic hexapeptides, which are characterized by a 19-membered peptide ring derived from the cyclization of the C-terminal amino acid carboxyl to the primary ϵ -amine of the N-terminus-lysine. The α -amine of the lysine is linked through an ureido bridge to the side chain amino acid. Twenty of twenty-three anabaenopeptins (anabaenopeptins A-I.¹⁻⁵ T,⁶ and KT864,⁷ ferintoic acids A and B,⁸ oscillamides B, C,⁹ and Y,¹⁰ nodulapeptins A and B,¹¹ and schizopeptin 791¹²) described to date from cyanobacteria, contain p-lysine and have an L-stereochemistry for the other amino acids. Three compounds, brunsvicamides A-C,¹³ recently reported from *Tychonema* sp. posses L-lysine and L-N-MeTrp moieties and are closely related in structure to a group of six marine sponge metabolites, konbamide,¹⁴ keramamides A¹⁵ and L¹⁶ mozamides A and B¹⁷ and psymabamide A.¹⁸ Psymabamide A contains D-lysine and L-N-MeTrp although isolated from a marine sponge. Anabaenopeptins possess inhibitory activity to several proteolytic enzymes^{4–6,10,12} and protein phosphatases.⁹ In the present study, we describe three novel anabaenopeptins that possess N-methyl glycine instead of the common N-methyl alanine or N-methyl homotyrosine in most of the other known anabaenopeptins. The compounds were isolated from the crude extract of the cultured cyanobacterium Anabaena sp. (strain NZ-3-1) by repeated chromatography on open-column RP-18, Sephadex LH-20 and reversed-phase HPLC column. The structure elucidation of the new compounds is discussed.

ABSTRACT

Three new cyclic peptides, anabaenopeptins NZ825, NZ841, and NZ857, were isolated from the hydrophilic extract of the cultured cyanobacterium *Anabaena* sp. The planar structure of the compounds was determined by homonuclear and inverse-heteronuclear 2D-NMR techniques as well as high-resolution mass spectrometry. The absolute configuration of the asymmetric centers was studied using Marfey's method for HPLC. This is the first report of anabaenopeptins that contain *N*-methyl glycine instead of the common *N*-methyl alanine. The incorporation of *N*-methyl glycine into the cyclic portion of the compounds results in their appearance as a mixture of two, equally stable, conformers, instead of the one distinct conformer in anabaenopeptins that contain *N*-methyl alanine or *N*-methyl homotyrosine. The three compounds were tested for inhibition of serine proteases and found to be not active.

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The NMR spectra of three compounds (1-3) recovered from the HPLC separations exhibited duplication of most of the proton and carbon signals. The duplication of the spectra was evident in several deuterated solvents with no change in the ratio (almost 1:1) of the duplicated signals. Comparison of the NMR and mass spectra of three compounds revealed that they are oxidation homologs of phenyl to phenol derivatives. Heating of compound 2 in pyridine- d_5 from RT to 340 K resulted in almost coalescence of the doubled signals and upon cooling to RT the spectrum returned to its initial shape (see Supplementary data). On the basis of these results it was concluded that the compounds exist in solution as a mixture of two conformers of almost equal energies. DMSO- d_6 was selected as the solvent for structure elucidation since it gave the best signal separation. Comparison of the chemical shifts of the proton and carbon signals of the two conformers in 1-3 revealed that the biggest chemical shift differences are concentrated around the N-Me glycine moiety (see Tables 1–3 and Tables 1a–3a in Supplementary data).

The less polar compound, anabaenopeptin NZ825 (1) was isolated as a glassy material with a molecular weight of 825 mass units. The molecular formula of **1** was determined as $C_{45}H_{59}N_7O_8$ by HR MALDI TOF mass measurements, indicating 20 elements of unsaturation within the molecule. The analysis of the NMR data was difficult and was performed simultaneously for the two conformers of each amino acid. For the clarity of the discussion, only the data for the major isomer (see Table 1) will be presented and discussed. The data of the minor isomer is presented in Supplementary data (see Table 1a). The peptide nature of the compound was suggested on the basis of the ca. 1:1 ratio between the carbonyl and α -carbons in the ¹³C NMR spectrum and between the amide and α -protons in the ¹H NMR spectrum. The distribution of the





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proton signals at the aromatic region suggested that 1 contain only mono-substituted phenyl rings. The number of the aromatic moieties couldn't be estimated by integration of the signals because of the overlap of their signals with amide protons. Analysis of the aromatic region of the ¹³C NMR spectrum revealed three quaternary sp²-carbons (141.5, 141.3, and 137.5 ppm) of mono-substituted phenyl moieties, thus suggesting the existence of three such moieties in **1**. Five amide protons ($\delta_{\rm H}$ 8.87 d, 8.55 d, 6.78 d, 6.47 d, and 6.25 d ppm) and a single *N*-methyl moiety ($\delta_{\rm H}$ 2.80 s ppm) suggested that the peptide is composed of six amino acids. This information was not obvious from the number of carbonyl carbons, in the ¹³C NMR spectrum, since some of them, from different amino acids and different isomers, overlapped. Analysis of the H-H COSY and TOCSY experiments allowed the assignment of six fragments (see Table 1 and Table 1a in Supplementary data): two NH-CH-CH₂-CH₂ moieties, a CH₂ moiety, a NH-CH-CH(CH₃)CH₂CH₃, a NH-CH-CH₂-CH₂-CH₂-CH₂-CH₂-NH moiety, and a NH-CH-CH₂ moiety, which account for 40 of the 59 protons of **1**. The correlations from the HMQC spectrum allowed the assignment of the carbons signals to these fragments. Analysis of the correlation map of the HMBC experiment (with the aid of the HMQC correlations) allowed the assignment of the three mono-substituted phenyl rings and their attachment to the aliphatic moieties as well as the attachment of the α -carbons to the carbonyl carbons. This procedure established the two homophenylalanine and one phenylalanine moieties (see Table 1). The remaining three fragments were extended to N-methyl glycine, isoleucine, and lysine moieties in a similar fashion (see Table 1). The diastereotopic nature of the lysine 6,6'-protons and the single ε -amide proton suggested that the lysine side chain is part of a cyclic peptide such as that found in all anabaenopeptins.^{1–18} HMBC correlation of the NH of Hph¹ with the carbonyl of NMe-Gly, of the NMe of NMe-Gly with the carbonyl of Hph², of the NH of Hph² with the carbonyl of Ile, of the NH of Ile with the carbonyl of lysine, and of ε -NH of lysine with the carbonyl of Hph¹, established the structure of cyclic portion of 1. The NOE correlations from a ROESY experiment reinforced the latter assignment (see Table 1). The attachment of the final Phe unit to the cyclic peptide through the uredio carbonyl was accomplished through the HMBC correlations of the ureido carbonyl with lysine-α-NH and Phe-NH and the mutual NOE correlations of Phe H-2 and NH with Lys H-2 and α -NH. Such correlations are characteristic of the ureido bridge.¹² Extended acid hydrolysis of anabaenopeptin NZ825 (to improve the yield of ureido moiety hydrolysis) and derivatization with Marfey's reagent,¹⁹ followed by HPLC analysis, demonstrated the L-stereochemistry of the isoleucine, phenylalanine, and homophenylalanine residues and the D-stereochemistry the lysine residue.

Anabaenopeptin NZ841 (2) was isolated as amorphous white solid. HR MALDI TOF MS measurements of anabaenopeptin NZ841 revealed a pseudo-molecular ion adduct (MNa⁺) at m/z 864.4336, corresponding to a molecular formula of C45H59N7O9Na. The latter molecular formula suggested that 2 contain additional oxygen relative to **1**. Inspection of the ¹H and ¹³C NMR spectra of **2** revealed that the difference (relative to 1) is in the aromatic regions of the spectra. On the basis of the aromatic protons chemical shifts, the additional acidic phenol proton signal, the aromatic carbons chemical shifts, and the additional phenolic carbon, we could propose that one of the phenyl moieties was converted to p-hydroxyphenyl (see Table 2). Full assignment of the NMR data proved this assumption and assigned the new amino acid as homotyrosine (see Table 2). HMBC correlation of the NH of Hph with the carbonyl of NMe-Gly, of the NMe, H-2a and H-2b of NMe-Gly with the carbonyl of Hty, of the NH of Hty with the carbonyl of Ile, of the NH of Ile with the carbonyl of lysine and NOE correlation between H-2 of Hph and ϵ -NH of lysine established the planar structure of the cyclic portion of 2.

The HMBC correlation of the ε -NH of lysine with the carbonyl carbon at $\delta_{\rm C}$ 171.2 established the latter as the carbonyl of Hph. Similarly, HMBC and NOE correlations as in **1**, established the ureido bridge and concluded the structure elucidation of **2**. Marfey's analysis for HPLC,¹⁹ of **2**, established the L-stereochemistry of the isoleucine, phenylalanine homophenylalanine, and homotyrosine, and the D-stereochemistry of the lysine residue.

The most polar compound, anabaenopeptin NZ857 (3) was isolated as a glassy solid with a pseudo-molecular ion adduct (MNa^+) at m/z 880.4268 in the HR MALDI TOF MS measurement. On the basis of this mass measurement the molecular formula of 3 was determined as C₄₅H₅₉N₇O₁₀ suggesting that anabaenopeptin NZ857 (3) has extra oxygen relative to anabaenopeptin NZ841 (2). This finding was also evident from 1D 1 H and 13 C NMR spectra of 3, which suggested the presence of one phenyl and two *p*-hydroxyphenyl moieties (see Table 3). Assignment of the NMR data of **3** proved this assumption and assigned the new amino acid as homotyrosine (see Table 3). HMBC connectivities between Hty1-NH and NMe-Gly carbonyl, Hty2-NH, and Ile carbonyl, Ile-NH and Lys carbonyl and the NOE correlations between Hty¹ H-2 and Lys-E-NH and of Hty¹ H-2 and NMe-Gly H-2b established the *cyclo*-(Hty¹-*N*Me-Gly-Hty²-Ile-Lys- ε -NH) structure of the cyclic portion of **3**. The HMBC correlation of NMe-Gly NMe and H-2a,2b and the carbonyl carbon at $\delta_{\rm C}$ 171.3 established the



Figure 1. Comparison of the *gauche* interactions of the different staggered rotamers around the CN bond of (a) *N*-methyl alanine/phenylalanine versus (b) *N*-methyl glycine.

Table 1		
NMR data ^a of a	anabaenopeptin NZ825	(1) major conformer

$\begin{array}{ $	Position	$\delta_{\rm C/N}$, mult. ^b	δ_{H} , mult., J (Hz)	LR H–C Correlations ^c	Selected NOE correlations ^d
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Hph ¹ 1	171.1 s		Hph ¹ -2, Lys-ε-NH	
	2	53.1 d	4.10 ddd, 2.7,9.0,11.5	Hph ¹ -4,4'	Lys-ε-NH, Hph ¹ -3,3′,4,4′NH
4 32 27 248 m Hph ⁵ ,6' Hph ¹ -4,7,7' 66' 128.4 (1/2) 7.7 (1/2) Hph ¹ -4,7,7' 7.7 128.4 (1/2) 7.22 m (1/2) Hph ¹ -4,7,7' 8 126.6 d 7.20 m (1/2) Hph ¹ -4,7,7' 8 126.6 d 7.20 m (1/2) Hph ¹ -4,7,7' 8 126.6 d 7.20 m (1/2) Hph ¹ -4,7,7' 8 126.6 d 7.20 m (1/2) Hph ¹ -4,7,7' 8 126.6 d 7.20 m (1/2) Hph ¹ -4,7,7' 7 128.4 (1/2) 7.60 (1.50 m (1/2), 10 m (1/2), 1	3	33.6 t	2.15 m, 1.90 m		Hph ¹ -2
5 141.5 * 141.5 * 141.4 * 66° 128.4 d (x.2) 7.17 m (x.2) 110 ** 110 ** 7.7 128.4 d (x.2) 7.20 m 110 ** 110 ** 8 126.6 d 7.20 m 110 ** 110 ** NH 161 d 8.5 d.8.0 NMe-Gly-22, Hpl ¹⁻ 2,3',4',NMe-Gly-23',4',NMe-Gly-23',A',NMe-Gly-23',A',NMe-Gly-23',A',NMe-Gly-23',A',NMe-Gly-23',NMe NMe-Gly 2.6 ho 3.6 d.1 f.0 NMe-Gly-22, NMe Hpl ¹⁻ -MH, Hpl ⁵⁻ 2, 3',4', NMe-Gly-23',NMe NMe 3.0 d.1 f.0 NMe-Gly-22, NMe Hpl ¹⁻ -MH, Hpl ⁵⁻ 2, 3',4', NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NMe-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gly-23',NME-Gl	4	32.2 t	2.48 m	Hph ¹ -6,6'	Hph ¹ -2
66 128.4 ($1/2$) 7.17 m (2) Hpl ⁻¹ .4(3 7.7 128.4 ($1/2$) 7.20 m ($3/2$) Hpl ⁻¹ .4(3 8 126.6 d 7.20 m ($3/2$) Hpl ⁻¹ .4($3/2$) NH 161.6 8.55 d.8.0 Hpl ⁻¹ .4($3/2$) NMe-Gly 1 160.0 s Me-Gly 2.2, Hpl ⁻¹ .4)H Hpl ⁻¹ .4($3/2$) 2 5 5.6 d.16.0 Me-Gly 2.2, MMe-Gly -2.2, NMe Hpl ⁻¹ .4($3/2$) NMe 106.1 s Hpl ⁻¹ .4($3/2$) Hpl ⁻¹ .4($3/2$) Hpl ⁻¹ .4($3/2$) 2 43.0 q 4.65 d.dt Hpl ⁺² .3.3, MMe-Gly-2.2, NMe Hpl ⁻¹ .4($3/2$) 2 43.3 d 4.65 d.dt Hpl ⁺² .3.3, MMe-Gly-2.2, NMe Hpl ⁻¹ .4($3/2$) 3 2.25 r 1.88 m (2) Hpl ⁻¹ .4($3/2$) Hpl ⁻² .3.3, ($4/2, 7/2$ Hpl ⁻² .2 4 12.61 d 7.20 m (2) Hpl ⁻⁴ .4/4 $^{3/2}$ Hpl ⁻² .3.3, ($4/2, 7/2$ Hpl ⁻² .3.3, ($4/2, 7/2$ 8 12.61 d 7.20 m (2) Hpl ⁻² .4/4 $^{3/2}$ Hpl ⁻² .3.3, ($4/2, 7/2$ 16 12.84 d (2) 7.23 m (2.2) Hpl ⁻² .4/4 $^{3/2}$	5	141.5 s		Hph ¹ -4,4',7,7'	
77 128.44 (x^2) 723 m (x^2) 16^{12} $16^{11}-2, 16^{11}-2, 3/, 4/, NMe-Gly-2D, NMe}$ NH 1161 d 8.55 0, 8.0 $Me-Gly-2X, Hp^{1-NH}$ $Me-Gly-2X, Hp^{1-NH}$ NMe-Gly-10 500 t $360, 160$ $Me-Gly-2X, Hp^{1-NH}$ $Me-Gly-Me$ $Me-Gly-2X, Hp^{1-NH}$ 2^{12} $360, 160$ $Me-Gly-2X, Hp^{1-NH}$ $Me-Gly-2X, Hp^{1-NH}$ $Hp^{1-NH}, MMe-Gly-2a$ NMe $360, 160$ $Me-Gly-2X, Hp^{1-NH}$ $Hp^{1-NH}, MMe-Gly-2a$ $Hp^{1-NH}, MMe-Gly-2a$ NMe $300, 150$ 128.46 $Me-Gly-2X, Me-Gly-2X, Me$ $Hp^{1-NH}, MP-Gly-2a$ 100 $325, 10$ $360, 60$ $Hp^{1-NH}, MP^{1-2}, 3/, 4/, MMe-Gly-2a$ $Hp^{1-NH}, Hp^{1-2}, 3/, 4/, MMe-Gly-2a$ 2 $493, 4$ $455, 60, 60$ $Hp^{1-2}, 3/, 4/, MMe-Gly-2a$ $Hp^{1-1}, 2/, 4/, MMe-Gly-2a$ 4 $123, 7$ $226, 10$ $168, 70, 10$ $Hp^{1-2}, 3/, 4/, MMe-Gly-2a$ 4 $124, 1/2, 2/2, 10$ $100, 10, 1/2, 10$ $100, 1/2, 1/2, 10$ $Hp^{1-2}, 3/, 4/, 1/2, 1/2, 1/2, 1/2, 1/2, 1/2, 1/2, 1/$	6,6′	128.4 d (×2)	7.17 m (×2)	Hph ¹ -4,4′,8	
8 NH126.6 d720 mHph ¹ -6.6'Hph ¹ -2.3, 1.4, YAMe-Gly-2.BAMANH161.08.55 d. 8.0Nme-Gly-2.7, Hph ¹ -NHMme-Gly-2.7, Hph ¹ -NH20 25.0.16.0.0, 16.0Nme-Gly-2.7, Hph ¹ -NHMme-Gly-2.8NMe106.1 sNme-Gly-2.2, Ihph ¹ -NHMme-Gly-2.2, Ihph ¹ -NHMme-Gly-2.2, Ihph ¹ -NH171.2 sHph ² Hph ² -3, 37, 44, NMe-Gly-2.2, Ihph ¹ -NHHph ² -3, 37, 44, NMe-Gly-2.8243.04.55 ddtHph ² -3, 37, 44, IAMe-Gly-2.8Hph ² -3, 37, 44, IAMe-Gly-2.8243.02.27 inHph ² -3, 37, 44, IAMe-Gly-2.8Hph ² -3, 37, 44, IAMe-Gly-2.8243.02.27 inHph ² -3, 37, 44, IAMe-Gly-2.8Hph ² -3, 37, 44, IAMe-Gly-2.8332.5 i1.88 m(2H)Hph ² -3, 37, 44, IAMe-Gly-2.8Hph ² -3, 37, 44, IAMe-Gly-2.8412.5 i1.28 M (CH)Hph ² -3, 37, 44, IAMe-Gly-2.8Hph ² -3, 37, 44, IAMe-Gly-2.851.43 s1.88 m(2H)Hph ² -3, 37, 44, IAMe-Gly-2.8Hph ² -3, 37, 44, IAMe-Gly-2.851.43 s1.28 M (CH)7.27 m(2H)Hph ² -4, 7761.28 A (CH)7.23 m (CH)Hph ² -3, 37, 44, IAMe-Gly-2.87.712.84 (CH)7.27 m (CH)Hph ² -3, 37, 44, IAMe-Gly-2.881.26 1d7.27 m (CH)Hph ² -3, 37, 44, IAMe-Gly-2.891.28 A (CH)7.27 m (CH)Hph ² -3, 37, 44, IAMe-Gly-2.811.28 A (CH)7.27 m (CH)Hph ² -3, 37, 44, IAMe-Gly-2.811.28 A (CH)1.29 m (CH)Hph ² -3, 3	7,7′	128.4 d (×2)	7.23 m (×2)		
NH Ibit d 8.55 d. 8.0 Hph ¹ -2, Hph ¹ -NI Hph ¹ -2, Jph ¹ -NI Hph ² -2, Jph ² -NI	8	126.6 d	7.20 m	Hph ¹ -6,6′	
NMe-Gy-11600 s1600 sMe-Gy-2XMe-Gy-NHeMe-Gy-NMeMe-Gy-NMe2540 t3.60 d, 16.0NMe-Gy-22', NMeHph ¹ -NH, Hph ² -2Mm-Gy-22', NMeHph ² -NH, Hph ² -2NMe-Gy-21100 tsNMe-Gy-22', NMe-Gy-22', NMeHph ² -3, 3', Me-Gy-22', NMe-Gy-22', NMe-Gy-22', NMe-Gy-22', NMe-Gy-22', NMe-Gy-22', NMe-Gy-22', NMe-Gy-23', MP-Gy-22', NMe-Gy-23', MP-Gy-23', MP-Gy-22', NMe-Gy-23', MP-Gy-23', MP-Gy-24', MP-	NH	116.1 d	8.55 d, 8.0		Hph ² -2, Hph ¹ -2,3',4,4',NMe-Gly-2b,NMe
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	NMe-Gly 1	169.0 s		<i>N</i> Me-Gly-2,2', Hph ¹ -NH	
$\begin{array}{ c c c } & 150 & 150 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160 & 160$	2a	54.0 t	3.60 d, 16.0	NMe-Gly-NMe	NMe-Gly-NMe
NMe Class MmeCity-22' Mph ¹ -NH, NMe-Gity-2a, NMe-Gity-22, NMe Hph ² 1 T12 s Hph ² -33, MMe-Gity-22, NMe Hph ² -33, MMe-Gity-22, NMe 2 493 d 465 ddt Hph ² -33, MMe-Gity-22, NMe 3 325 t 188 m (2H) Hph ² -3, MMe-Gity-22, NMe 4 2.56 Hph ² -46' Hph ² -2 5 141.3 s Hph ² -3, MA(7,7) Hph ² -4, 4/8 66' 128.4 (v2) 723 m (v2) Hph ² -6, 6' Hph ² -2, 3/1 le-2 7.7 128.4 d (v2) 723 m (v2) Hph ² -6, 6' Hph ² -2, 3/1 le-2 8 161 d 80 d. Hph ² -7, 7 Hph ² -14, 4/8 7.8 161 d 80 d. Hort Hph ² -14, 18 1 161 d 80 d. Hort Hph ² -14, 18 2 56 d 106 m He-2, 4, 18 Hph ² -14, 18 3 3.56 d 105 m He-2, 4, 19 He-2, 4, 19 4 12.8 d 6.78 d, 7.1 He-2, 3, 1 He-2, 3, 4, 1, 19, 5-2, -NH 5	2b		4.55 d, 16.0		Hph ¹ -NH, Hph ² -2
N 106.1 s N 106.1 s Hpl ² 1 T12.2 s Hpl ² -33, MAC-Gly-2, 2, NMe 3 3.25 t 1.88 m (2H) Hpl ² -3, 3, MAC-Gly-2, 2, NMe 4 2.25 t 1.88 m (2H) Hpl ² -2, 3, 3, 44, 7, 7 4 2.26 m Hpl ² -2, 3, 3, 44, 7, 7 6 128.4 d (v2) 7, 17 m (v.2) Hpl ² -4, 4, 8 7, 7 128.4 d (v2) 7, 23 m (v.2) Hpl ² -7, 7 7, 7 128.4 d (v.2) 7, 23 m (v.2) Hpl ² -7, 7 8 126.1 d 2, 20 m Hpl ² -7, 7 8 126.1 d 2, 20 m Hpl ² -7, 7 8 126.1 d 2, 20 m Hpl ² -7, 7 8 126.1 d 100 m Hpl ² -7, 7 8 126.1 d 100 m He-2, 3, 10 m 9 1, 45 m He-2, 44, 4 Hpl ² -2, 3, 9, He-2, 10 m 1 105 m, 1, 45 m He-2, 44, 4 He-2, 3, 44, Hy He-2, 44, Hy He, 1, Hy He, 1,	NMe	C 34.0 q	2.80 s	NMe-Gly-2,2'	Hph ¹ -NH, NMe-Gly-2a
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		N 106.1 s			
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Hph ² 1	171.2 s		Hph ² -3 3' NMe-Glv-2 2' NMe	
3 32.5 t 188 m (2H) Hph ² -2, 4 Hph ² -2, 4 4 31.2 t 2.74 m Hph ² -6,67 Hph ² -2, 3,34,47,77 5 141.3 s Hph ² -2, 4/4,8 Hph ² -2, 4/4,8 7,7 128.4 d (x.2) 7.23 m (x.2) Hph ² -2, 7,7 8 126.1 d 7.00 m Hph ² -2, 7,7 8 16.1 d 8.87 d, 8.0 Hph ² -6,67 NH 116.1 d 8.87 d, 8.0 Hph ² -2,7,7 8 126.1 d 1.00 m Hph ² -2,3,9, He-2 16 17.2 s Ie-2,3, Hph ² -NH Ie-2,3, Hph ² -NH 2 35.6 d 1.60 m IIe-2,45.6 IIe-2,3 4 24,7 t 1.05 m, 1.45 m IIe-2,3, Hph ² -NH IIe-2,3, Hph ² -NH 5 10.6 q 0.72 t, 7.2 IIe-44' IIe-2,3, Hph ² -NH IIIe-2,3, Hph ² -NH 5 15.2 q 0.91 d, 6.8 IIe-1, Hys-2,3,3' IIIe-2,3, Hph ² -NH IIIe-2,3, Hph ² -NH 6 15.2 q 0.91 d, 6.8 IIIe-1, Hys-2,3,3' IIIe-2,3, Hph ² -NH IIIe-2,3, Hph ² -NH 7 12.8 d, 12.7 5 m, 3.46 m IIIe-2,3, Hph ²	2	49.3 d	4.65 ddt	Hph ² -3.3'.4	Hph ¹ -NH, Hph ² -3.3',4.4', <i>N</i> Me-Gly-2b
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	3	32.5 t	1.88 m (2H)		Hph ² -2
1 2.56 m Har A Ha Har A Ha </td <td>4</td> <td>31.2 t</td> <td>2.74 m</td> <td>Hph²-6.6'</td> <td>Hph²-2</td>	4	31.2 t	2.74 m	Hph ² -6.6'	Hph ² -2
5 141.3 s International organizational organizati			2.56 m		-
66' 128 4 d (×2) 7.7 m (×2) $hph^2 - 4.4' s^{1/2}$ 7.7' 128 4 d (×2) 7.23 m (×2) $hph^2 - 4.4' s^{1/2}$ 7.7' 128 4 d (×2) 7.23 m (×2) $hph^2 - 4.4' s^{1/2}$ 8 126.1 d 7.20 m $hph^2 - 6.6'$ NH 116.1 d 887 d.8.0 Ile-2 11 17.2 s s Ile-2,3, Hph^2 - NH Ile-2,3, Hph^2 - NH 2 58.8 d 397 t.8.0 Ile-6 Hph ² - 4.7 i 3 35.6 d 1.60 m Ile-2,3, Hph^2 - NH Ile-2 4 24.7 t 1.05 m, 1.45 m Ile-2,4.4' Ile-2,3 6 152 q 0.91 d.6.8 Ile -2,4.4' Ile-2,3 112 N 12.8 d 6.7 d.7.1 Ile-NH, Lys-2,3.3' Ile-2,3.4', Lys-2,a-NH 12 y 1 172.2 s Ile-15 m Ile-NH Ile-2,3.4', Lys-2,a-NH 12 y 1 172.4 s Ile-NT Ile-NH Ile-2,3.4', Lys-2,a-NH 12 s 5 m Ile-NT Ile-NH Ile-2,3.4', Lys-2,a-NH 12 s 3 a 37 t 13 a 5 m Ile-N Ile-NH 2 s - NH<	5	141.3 s		Hph ² -3.3'.4.4'.7.7'	
7.7 $128.4 d(\times 2)$ $723 m(\times 2)$ $Hph^2-7.7$ 8 $126.1 d$ $720 m$ $Hph^2-6/6$ $Hph^2-2,3.3$, Ile-2 NH 116.1 d $877 t, 8.0$ Ile-2,3, Hph^2-NH $Hph^2-2,3.3$, Ile-2 11 $172.8 s$ $162.3, Hph^2$ -NH $Hph^2-2,3.3$, Ile-2 2 $58.8 d$ $397 t, 8.0$ Ile-2,3,5.6 Ile-2 4 $24.7 t$ $105 m, 145 m$ Ile-2,4.4' Ile-2,3 5 $106 q$ $0.72 t, 7.2$ Ile-4.4' Ile-2,3 6 $15.2 q$ $0.91 d, 6.8$ Ile-2,4.4' Ile-2,3 NH $112.8 d$ $6.78 d, 7.1$ Ile-2,3 Ile-2,3 2 $54.7 d$ $38 m$ Ile-NH, Ilys-2,3.3' Ile-2,3 2 $54.7 d$ $38 m$ Ile-NH Ile-2,3 4 $20.4 t$ $108 m, 118 m$ Ile-NH Ile-S 4 $20.4 t$ $108 m, 118 m$ Ile-NH Ile-S 6^{-N} $38.3 t$ $2.75 m, 3.46 m$ Hph-3.2' Ile-NH, Ile-NH, Ile-NH, Ile-NH 4^{-N} $106.7 d$ $7.2 m$ <	6,6′	128.4 d (×2)	7.17 m (×2)	Hph ² -4,4',8	
s 126.1 d 720 m $Hph^2-6.6'$ $Hph^2-2,3.3', Ile-2$ NH 116.1 d 8.87 d. 8.0 Ile-2,3, Hph ²⁻ NH $Hph^2-2,3.3', Ile-2$ le 172.8 s Ile-23, S Ile-2,3, Hph ²⁻ NH $Hph^2-2,3.3', Ile-2$ 2 58.8 d 3.97 t. 8.0 Ile-2,3, Hph ²⁻ NH Ile-2,3, Hph ²⁻ NH 3 35.6 d 1.60 m Ile-2,3, Hph ²⁻ NH Ile-2,3, Hph ²⁻ NH 4 24.7 t 1.05 m. 1.45 m Ile-2,5.6 Ile-2,3 5 10.6 q 0.72 t. 7.2 Ile-4.4' Ile-2,3 Ile-2,3,4,1 tys-2,α-NH 6 15.2 q 0.91 d. 6.8 Ile-2,4.4' Ile-2,3,4,1 tys-2,α-NH 10.8 1.52 m Ile-NH, Lys-2,3.3' Ile-2,3,4,4, tys-2,α-NH 4 2.5 m Ile-NH Lys-2,3.3' Ile-2,3,4,4, tys-2,α-NH 4 2.04 t 1.08 m. 1.18 m Lys-6 Ile-NH 5 1.08 m.1 m Ile-NH Lys-5 Ile-NH 6 38.1 t 2.75 m.3.46 m Ile-2,3.3',NH Ile-S 6 38.1 t 2.75 m.3.46 m Phe-2,3.3',NH Ilys-2, NH, Ile-NH <	7.7′	128.4 d (×2)	7.23 m (×2)	Hph ² -7'.7	
NH 161 d 8.87 d. 8.0 Hph ² -2,3,7, Ile-2 Ile 1 172.8 s Ile-23, Aph ² -NH Hph ² -NH, Ile-4,5,6, Lys-2, α -NH 2 58.8 d 3.97 t, 8.0 Ile-24,5,6 Hph ² -NH, Ile-4,5,6, Lys-2, α -NH 3 35.6 d 1.60 m Ile-2,4,5,6 Ile-2,4,5,6 4 24.7 t 1.05 m, 1.45 m Ile-2,5,6 Ile-2,3 5 10.6 q 0.72 t, 72 Ile-4,4' Ile-2,3 6 15.2 q 0.91 d, 6.8 Ile-2,4.4' Ile-2,3 7 12.8 d 6.78 d, 7.1 Ile-2,3 Ile-2,3,4.4, Lys-2, α -NH 1ys 1 17.2 s Ile-NH Ile-NH Ile-NH Ile-NH 2 5.4 7 d 3.88 m Ile-NH Ile-S Iles-S 3 1.5 t 1.54 m Ile-NH Ile-NH Iles-S Iles-S 4 2.04 t 1.08 m, 11.8 m Iles-N Iles-S Iles-S Iles-S 6 8.8 t 2.75 m, 3.46 m Iles-2,3.3',NH Iles-2,3.4',NH Iles-NH Iles-2,3.3',NH Iles-2,3.3',NH Iles-2,3.3',6,6' Iles-2,3.3',6,6'	8	126.1 d	7.20 m	Hph ² -6,6'	
	NH	116.1 d	8.87 d, 8.0	r v	Hph ² -2,3,3', Ile-2
1 12.5 3 102.5 1m² - M1 2 58 d 3.97 t, 8.0 Ile-6 Hph²-NH, Ile-4.5.6, Lys-2,α-NH 3 35.6 d 1.60 m Ile-2.4.5.6 Ile-2 4 24.7 t 1.05 m, 145 m Ile-2.5.6 Ile-2 5 10.6 q 0.72 t, 7.2 Ile-4.4' Ile-2.3 6 15.2 q 0.91 d, 6.8 Ile-2.4.4' Ile-2.3 NH 112.8 d 6.78 d, 7.1 Ile-2.4.4' Ile-2.3 1ys 1 172.2 s Ile-NH Ile-2.3.3' Ile-2.3 2 54.7 d 3.88 m Lys-q-NH Ile-2.3 3 31.5 t 1.54 m Ile-NH Lys-q-NH 3 31.5 t 1.54 m Ile-NH Lys-q-NH 4 20.4 t 1.08 m, 1.18 m Lys-6 Ile-NH 5 28.6 t 1.35 m Lys-6 Lys-7 e-NH 109.5 d 7.14 m Lys-2,3.3',MH Lys-2, Phe-2,NH, Ile-NH 2 53.9 d 4.35 m Phe-2,3.3',AG' Lys-a-NH, Phe-3.3',NH 3 37.7 t 2.88 m, 2.	Ile 1	172 8 s		lle_2.3 Hpb ² _NH	
2 36 at 160 m 11e-24,5,6 4 24,7 t 105 m, 145 m 11e-25,5 6 11e-2,3 5 10.6 q 0.72 t, 7.2 11e-2,4/4' 11e-2,3 6 15.2 q 0.91 d, 6.8 11e-2,4/4' 11e-2,3,4/4, 1ys-2,a-NH 1ys 1 172.2 s 11e-7,3,4/4, 1ys-2,a-NH 11e-2,3,4/4, 1ys-2,a-NH 1ys 1 172.2 s 11e-NH 11e-2,3,3' 2 54.7 d 388 m 12ys-a-NH 3 3.15 t 1.54 m 11e-NH 1ys-a-NH 4 20.4 t 1.08 m, 1.18 m 12ys-6 1.50 m 4 20.4 t 1.08 m, 1.18 m 1ys-5 1.54 m 5 28.6 tt 1.35 m 1.95 m 1.95 m 6 38.3 t 2.75 m, 3.46 m 1.95 m 1.95 m 6 3.9 d 4.35 m 1.95 m 1.95 m 6 3.9 d 4.35 m 1.95 m 1.95 m 7 116.7 d 6.47 d, 7.2 Phe-2,3.3',NH 1.95 m 7 1.95 m 1.95 m 1.95 m 1.95 m	2	58.8 d	3 97 t 8 0	lle-6	Hph ² -NH Ile-456 Jvs-2 a-NH
4247 t1.05 m, 145 m11e 2,3611e-25106 q0.72 t, 7.211e-4.4'11e-2,3615.2 q0.91 6,6811e-2,44'11e-2,3NH112.8 d6.78 d, 7.111e-2,3,44', 1ys-2, α -NH1ys 1172.2 s11e-NH, 1ys-2,3,3'254.7 d3.88 m11e-NH331.5 t1.54 m11e-NH420.4 t1.08 m, 1.18 m11e-NH528.6 t1.35 m1.50 m420.4 t1.08 m, 1.18 m1ys-6638.3 t2.75 m, 3.46 m1ys-61.18 m116.7 d6.47 d, 7.21ys-2Phe 1173.6 s1ys-2, Phe-2,NH, 1le-NH253.9 d4.35 mPhe-2,3,3',NH33.77 t2.88 m, 2.98 mPhe-2,3,3', 733.77 t2.88 m, 2.98 mPhe-2,3,3', 75.5'129.3 d (x2)7.15 d, 8.0 (x2)Phe-3,3', 76.6'128.4 d (x2)7.20 m (x2)Phe-3,3', 76.6'128.4 d (x2)7.20 m (x2)Phe-3,3', 76.6'128.4 d (x2)7.10 mPhe-5,5'NH116.2 d6.25 d, 8.2Phe-2,3,3', 1ys-2, α-NHUreido CO157.1 sPhe-2,5 d, 8.2Phe-NH	3	35.6 d	160 m	lle-2 4 5 6	11pii -111, 11C-4, 5, 6, Ly3-2, 0-111
410.6 q0.72 t, 7.210.2 s, 010.2	4	24.7 t	105 m 145 m	lle-2,5,6	Ile-2
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	5	10.6 a	0.72 t 7.2	lle-4 4'	ne z
NH 112.8 d 6.78 d, 7.1 He-2,3,4,4', Lys-2,α-NH Lys 1 172.2 s 16.7 d 3.88 m Lys-α-NH 2 54.7 d 3.88 m Lys-α-NH 3 31.5 t 1.54 m Ile-NH 4 20.4 t 1.08 m, 118 m Lys-6 5 28.6 t 1.35 m Lys-6 1.18 m 1.18 m Lys-5 6 38.3 t 2.75 m, 3.46 m Lys-5 ε-NH 109.5 d 7.14 m Hph ¹ -2 α-NH 116.7 d 6.47 d, 7.2 Lys-2, NH, Ile-NH 2 53.9 d 4.35 m Phe-2,3.3', NH 2 53.9 d 4.35 m Phe-2,3.3', OH 3 3.7.7 t 2.88 m, 2.98 m Phe-2,3.3', OH 5.5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3/3'.7 6.6' 7.4 m 10.52 m Phe-2,3.3', CH 5.5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3/3'.7 6.6' 7.4 m 10.52 m Phe-2,3.3', Lys-2,α-NH 1.62 d	6	15.0 q	0.91 d 6.8	lle-2.4.4/	lle-2.3
Image Interaction Model methods Interaction Interaction Lys 1 172.2 s Ile-NH, Lys-2.3,3' Lys-α-NH 2 547 d 3.88 m Lys-α-NH 3 31.5 t 1.54 m Ile-NH 150 m 150 m Lys-α-NH 4 20.4 t 1.08 m, 1.18 m Lys-6 5 28.6 t 1.35 m Lys-5 1.18 m Lys-5 Lys-5 e-NH 109.5 d 7.14 m Hph ¹ -2 a-NH 116.7 d 6.47 d, 7.2 Lys-7 Lys-7 Phe 1 173.6 s Phe-2,3.3', NH Lys-7 Lys-7-NH, Phe-3,3', NH 3 37.7 t 2.88 m, 2.98 m Phe-3,3', 66' Phe-2,3.3', 66' 5.5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3', 7 Phe-2,3.3', 66' 5.5' 128.4 d (×2) 7.20 m (×2) Phe-6, 6 Phe-2,3.3', Lys-2,α-NH 6.6' 128.4 d (×2) 7.20 m (×2) Phe-6, 6 Phe-2,3.3', Lys-2,α-NH MH 116.2 d 6.25 d, 8.2 Phe-4, 6', 6 Phe-2,3.3', Lys-2,α-NH <td< td=""><td>NH</td><td>112.8 d</td><td>678 d 71</td><td>110 2, 1, 1</td><td>IIe-2.3.4.4/ Lys-2.α-NH</td></td<>	NH	112.8 d	678 d 71	110 2, 1, 1	IIe-2.3.4.4/ Lys-2. α -NH
Lys 1 722 s Ide-NH, Lys-2,3,3' 2 54.7 d 3.88 m Lys-α-NH 3 31.5 t 1.54 m Ile-NH 4 20.4 t 1.08 m, 118 m Lys-6 5 2.86 t 1.35 m Lys-6 1.8 m Lys-5 Lys-6 e-NH 09.5 d 7.14 m Hph ¹ -2 α -NH 109.5 d 7.14 m Hph ¹ -2 α -NH 109.5 d 7.14 m Hph ² -2,3,3',NH γ -NH 106.7 d 6.47 d, 7.2 Lys- α -NH, Phe-3,3',NH Phe 1 7.7 t 2.88 m, 2.98 m Phe-2,3,3',OF 3 3.7.7 t 2.88 m, 2.98 m Phe-2,3,3',G6' 4 137.5 s Phe-2,3,3',G6' Phe-2,3,3',G6' 5.5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,4',7 6.6' 128.4 d (×2) 7.20 m (×2) Phe-6,6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-4,6,6 7 115.2 d 6.25 d, 8.2 Phe-5,5' NH 1		112.0 u	0.70 4, 7.1		iie 2,5, i, i , by 2,0 i iii
2 54,7 d 3.88 m Lys-α-NH 3 1.5 t 1.54 m Ile-NH 150 m 1.50 m 4 20.4 t 1.08 m, 118 m 5 28.6 t 1.35 m 1.18 m Lys-6 6 38.3 t 2.75 m, 3.46 m ε-NH 109.5 d 7.14 m α-NH 116.7 d 6.47 d, 7.2 Phe 1 173.6 s Phe-2,3.3',NH 2 53.9 d 4.35 m 7 5.39 d 4.35 m 7 129.3 d (×2) 7.15 d, 8.0 (×2) 7 125.9 d 7.15 d, 8.0 (×2) 7 16.2 d 7.17 m 7 16.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH	Lys 1	172.2 s		lle-NH, Lys-2.3,3'	
3 31.5 t 1.54 m Ile-NH 1.50 m 1.50 m 1.50 m 4 20.4 t 1.08 m, 1.18 m Lys-6 5 28.6 t 1.35 m Lys-5 6 38.3 t 2.75 m, 3.46 m Lys-5 ε-NH 109.5 d 7.14 m Hph ¹ -2 α-NH 116.7 d 6.47 d, 7.2 Lys-2, Phe-2,NH, Ile-NH Phe 1 173.6 s Phe-2,3,3',NH Lys-α-NH, Phe-3,3',NH 3 37.7 t 2.88 m, 2.98 m Phe-2,3,3',66' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6,6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH Ureido CO 157.1 s Phe-1 Phe-NH, Lys-α-NH	2	54.7 d	3.88 m		Lys-a-NH
4 20.4 t 1.08 m, 118 m 5 28.6 t 1.35 m Lys-6 1.8 m 1.8 m 1.8 m 6 38.3 t 2.75 m, 3.46 m Lys-5 e-NH 109.5 d 7.14 m Hph ¹ -2 α-NH 116.7 d 6.47 d, 7.2 Lys-2, Phe-2,NH, Ile-NH Phe 1 73.6 s Phe-2,3,3',NH Lys-α-NH, Phe-3,3',NH 2 53.9 d 4.35 m Phe-3,3' 3 37.7 t 2.88 m, 2.98 m Phe-2,55' Phe-2,3',6,6' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 Phe-3,3', Lys-2,α-NH 7 125.9 d 7.17 m Phe-5,5' Phe-2,3,3', Lys-2,α-NH Wreido CO 157.1 s 6.25 d, 8.2 Phe-NH, Lys-α-NH Phe-2,3,3', Lys-2,α-NH	3	31.5 t	1.54 m	lle-NH	
4 20.4 t 1.08 m, 1.18 m 5 28.6 t 1.35 m Lys-6 1.18 m 1.18 m 1.18 m 6 38.3 t 2.75 m, 3.46 m Lys-5 ε-NH 109.5 d 7.14 m Hph ¹ -2 α-NH 116.7 d 6.47 d, 7.2 Lys-2, Phe-2,NH, Ile-NH Phe 1 173.6 s Phe-2,33',NH Lys-α-NH, Phe-3,3',NH 3 37.7 t 2.88 m, 2.98 m Phe-2,5,5' Phe-2, 4 137.5 s Phe-2,33',6,6' Phe-2, 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 Phe-2, 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 Phe-2, 7 125.9 d 7.17 m Phe-5,5' Phe-2, NH 116.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH Phe-2, Ureido CO 157.1 s Phe-1 Phe-Y Phe-NH, Lys-α-NH			1.50 m		
5 28.6 t 1.35 m Lys-6 1.18 m 1.18 m 1.18 m 6 38.3 t 2.75 m, 3.46 m Lys-5 ε-NH 109.5 d 7.14 m Hph ¹ -2 α-NH 116.7 d 6.47 d, 7.2 Lys-2, Phe-2,NH, Ile-NH Phe 1 173.6 s Phe-2,33',NH Lys-α-NH, Phe-3,3',NH 2 53.9 d 4.35 m Phe-2,5,5' Phe-2,3,3',6,6' 3 37.7 t 2.88 m, 2.98 m Phe-2,5,5' Phe-2 4 137.5 s Phe-2,3,3',6,6' Phe-2,3,3',6,6' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-7,3', Lys-2,α-NH Ureido CO 157.1 s Phe-1 Phe-NH, Lys-α-NH	4	20.4 t	1.08 m, 1.18 m		
6 38.3 t 2.75 m, 3.46 m Lys-5 ε-NH 109.5 d 7.14 m Hph ¹ -2 α-NH 116.7 d 6.47 d, 7.2 Lys-2, Phe-2,NH, Ile-NH Phe 1 73.6 s Phe-2,3,3',NH Lys-3,3',NH 2 53.9 d 4.35 m Phe-2,5,5' Lys-α-NH, Phe-3,3',NH 3 37.7 t 2.88 m, 2.98 m Phe-2,5,5' Phe-2,1,3',6,6' 4 137.5 s Phe-2,3,3',6,6' Phe-2,3,3',6,6' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-7,9,7 Ureido CO 157.1 s Phe-1 Phe-NH, Lys-α-NH	5	28.6 t	1.35 m		Lys-6
b 38.3 f 2.75 m, 3.46 m Lys-5 ε-NH 109.5 d 7.14 m Hph ¹ -2 α-NH 116.7 d 6.47 d, 7.2 Lys-2, Phe-2,NH, Ile-NH Phe 1 173.6 s Phe-2,33',NH 2 53.9 d 4.35 m Phe-3,3' 3 37.7 t 2.88 m, 2.98 m Phe-2,5,5' 4 137.5 s Phe-2,3,3',6,6' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH	C	20.2 t	1.18 m		Inc. 5
E-NH109.5 d7.14 mHpfl -2 α -NH116.7 d6.47 d, 7.2Lys-2, Phe-2,NH, Ile-NHPhe 1173.6 sPhe-2,3,3',NH253.9 d4.35 mPhe-3,3'337.7 t2.88 m, 2.98 mPhe-2,5,5'4137.5 sPhe-2,3,3',6,6'5,5'129.3 d (\times 2)7.15 d, 8.0 (\times 2)Phe-3,3',76,6'128.4 d (\times 2)7.20 m (\times 2)Phe-6',67125.9 d7.17 mPhe-5,5'NH116.2 d6.25 d, 8.2Phe-2,3,3', Lys-2, α -NHUreido CO157.1 sPhe-Can and the second		38.3 t	2.75 m, 3.46 m		Lys-5
α-NH H6.7 d 6.47 d, 7.2 Lys-2, PHe-2,NH, He-NH Phe 1 173.6 s Phe-2,33',NH 2 53.9 d 4.35 m Phe-3,3' 3 37.7 t 2.88 m, 2.98 m Phe-2,3,3',6,6' 4 137.5 s Phe-2,3,3',6,6' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH	E-INH	109.5 d			HpH -2
Phe 1 173.6 s Phe-2,3,3',NH 2 53.9 d 4.35 m Phe-3,3' Lys-α-NH, Phe-3,3',NH 3 37.7 t 2.88 m, 2.98 m Phe-2,5,5' Phe-2,3,3',6,6' 4 137.5 s Phe-2,3,3',6,6' Phe-2,3,3',6,6' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH	a-inn	110.7 u	0.47 u, 7.2		Lys-2, Pile-2, Nr, Ile-Inr
2 53.9 d 4.35 m Phe-3,3' Lys-α-NH, Phe-3,3',NH 3 37.7 t 2.88 m, 2.98 m Phe-2,5,5' Phe-2 4 137.5 s Phe-2,3,3',6,6' Phe-2,3,3',6,6' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH	Phe 1	173.6 s		Phe-2,3,3',NH	
3 37.7 t 2.88 m, 2.98 m Phe-2,5,5' Phe-2 4 137.5 s Phe-2,3,3',6,6' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH	2	53.9 d	4.35 m	Phe-3,3′	Lys-α-NH, Phe-3,3′,NH
4 137.5 s Phe-2,3,3',6,6' 5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH	3	37.7 t	2.88 m, 2.98 m	Phe-2,5,5′	Phe-2
5,5' 129.3 d (×2) 7.15 d, 8.0 (×2) Phe-3,3',7 6,6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-NH, Lys-α-NH Ureido CO 157.1 s Phe-NH, Lys-α-NH	4	137.5 s		Phe-2,3,3',6,6'	
6.6' 128.4 d (×2) 7.20 m (×2) Phe-6',6 7 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-2,3,3', Lys-2,α-NH Ureido CO 157.1 s Phe-NH, Lys-α-NH	5,5′	129.3 d (×2)	7.15 d, 8.0 (×2)	Phe-3,3',7	
/ 125.9 d 7.17 m Phe-5,5' NH 116.2 d 6.25 d, 8.2 Phe-2,3,3', Lys-2,α-NH Ureido CO 157.1 s Phe-NH, Lys-α-NH	6,6'	128.4 d (×2)	7.20 m (×2)	Phe-6',6	
NH 110.2 α 6.25 α, 8.2 Phe-2,3,3', Lys-2,α-NH Ureido CO 157.1 s Phe-NH, Lys-α-NH		125.9 d	7.17 m	Phe-5,5'	
Ureido CO 157.1 s Phe-NH, Lys-α-NH	INFI	110.2 U	0.25 U, 8.2		riie-2,3,3', Lys-2,a-infi
	Ureido CO	157.1 s		Phe-NH, Lys-α-NH	

^a Carried out on a Bruker Avance 400 spectrometer.

^b Multiplicity and assignment from HMQC experiment.

^c Determined from HMBC experiment, ${}^{n}J_{CH}=8$ Hz, recycle time 1 s.

^d By ROESY experiment.

latter as the carbonyl of Hty². On the basis of relative height of the carbon signal at $\delta_{\rm C}$ 171.3 it was also assigned as the carbonyl of the major conformer of Hty¹. Similar HMBC and NOE correlations as in **1** and **2**, established the ureido bridge and concluded the structure elucidation of **3**. Marfey's analysis for HPLC,¹⁹ of **3**, established the L-stereochemistry of the isoleucine, phenylalanine, and homotyrosine, and the D-stereochemistry of the lysine residue.

Anabaenopeptins NZ825, NZ841, and NZ857 appear as a mixture of two conformers. As described above the difference between the two conformers, in **1–3**, appears to be around the NMe-Gly moiety. The latter moiety differs from the corresponded moiety in other anabaenopeptins that usually contain at the same position *N*Me-Ala, *N*Me-Trp, or *N*Me-Hty. Comparison of the *gauche* interactions

of the different staggered rotamers around the CN bond of the *N*-methylated amino acid (see Fig. 1) revealed that for all amino acids except glycine the trans rotamer has two *gauche* interactions while the two *cis* rotamers has three *gauche* interactions, leading to a single conformer of the pentapeptide ring. In the case of glycine, one *cis* and the *trans* rotamers have one *gauche* interaction each and the second *cis* rotamer has two *gauche* interactions, leading to two energetically almost equal rotamers and as a consequence two conformers of the pentapeptide ring of compounds **1–3**. Comparison of the protons and carbons chemical shifts of both conformers in compounds **1–3** as well as the NOE interactions (see Tables 1–3) revealed that the pairs of conformers in **1–3** are similar but not identical. In **1–3**, the major conformer is the *cis* conformer characterized by the NOE correlation of Hph¹-NH and *N*Me-Gly-H-2b

Table 2

NMR data^a of anabaenopeptin NZ841 (2) major conformer

Position	$\delta_{\rm C/N}$, mult. ^b	$\delta_{\rm H}$, mult., J (Hz)	LR H–C/H–N Correlations ^c	Selected NOE correlations ^d
Hph 1	171.2 s		Lys-E-NH	
2	53.3 d	4.07 ddd, 2.8,8.9,11.5	Hph-NH	Lys-E-NH, Hph-4,6,6′,NH
3	34.0 t	2.12 m, 1.90 m		Hph-NH, NMe-Gly-2b
4	31.8 t	2.48 m, 2.46 m	Hph-6,6'	Hph-2,NH, NMe-Gly-2b
5	141.5 s	715 (2)	Hph-3',4,4',7,7'	
6,6°	129.4 d (×2)	$7.15 \text{ m}(\times 2)$		
7,7	128.5 (I (×2)	7.25 III (×2)	Hpli-6,6',7',7	прп-6,6
o NH	126.0 u 116.1 d	8.61 d 8.0	прії-о,о	Htv-2 Hpb-2344'66' NMe-Clv-222b NMe Lvs-6 s-NH
INIT	110.1 u	8.01 u, 8.0		11ty-2, 11pii-2, 3, 4, 4, 0, 0, 1 mite-Giy-2a, 20, mite, 193-0, c-mit
NMe-Gly 1	169.1 s		NMe-Gly-2a,2b, Hph-NH	
2a	54.2 t	3.56 d, 16.0	NMe-Gly-NMe	Hph-NH, NMe-Gly-NMe(s)
26	6.244	4.57 d, 16.0		Hph-3,3',NH, Hty-2, NMe-Gly-NMe(w)
INIVIE	C 34.1 q N 106.1 s	2.79 \$	/NMe-GIY-2a,2D	Hpn-NH, Hty-2(W), 3',4', NME-GIY-2a(S),2D(W)
Hty 1	171.4 s		NMe-Gly-2a,2b,NMe, Hty-2	
2	49.3 d	4.64 m	Hty-3',4,4',NH	Hty-3,3',4,4',6,6',NH, NMe-Gly-2b,NMe, Hph-NH
3	33.0 t	1.90 m, 1.80 m	Hty-NH	Hty-2,NH
4	30.4 t	2.50 m, 2.63 m	Hty-6,6′	Hty-2,NH
5	131.3 s		Hty-3',4,4',7,7'	
6,6′	129.4 d (×2)	7.02 d, 8.0 (×2)	Hty-6',6,7,7'	Hty-2,4, NMe-Gly-2b
7,7′	115.3 d (×2)	6.66 d, 8.0 (×2)	Hty-6,6′,7′,7	Hty-8-OH
8	155.7 s		Hty-6,6′,7,7′	
NH	129.0 d	8.89 d, 8.0		Ile-2,6, Hty-2,3,3',4,4'
8-OH		9.18 br s		Hty-7,7′
lle 1	172.8 s		Hty-NH, Ile-2,3	
2	56.8 d	3.95 t, 8.0	Ile-3,6	Hty-NH, Ile-4,4',5,6
3	35.7 d	1.60 m	Ile-2,4,4′,5,6,NH	
4	24.9 t	1.03 m, 1.45 m	Ile-2,3,5,6	Ile-2
5	10.7 q	0.75 t, 7.2	Ile-3,4,4′	lle-2
6	15.3 q	0.91 d, 6.8	Ile-3,4,4′	Hty-NH, Ile-2,3
NH	114.4 d	6.80 d, 6.8		Ile-2,4,5, Lys-2,α-NH
Lys 1	172.2 s		Ile-NH, Lys-2	
2	54.8 d	3.86 m	Lys-a-NH	Lys-a-NH
3	31.6 t	1.52 m, 1.48 m	Lys-α-NH	
4	20.5 t	1.07 m, 1.18 m	Lys-6′	
5	28.2 t	1.35 m		Lys-6
6	38.4 t	2.73 m, 3.47 m		Lys-5, Php-NH
ε-NH	109.5 d	7.13 m		Hph-2,NH
α-NH	116.7 d	6.44 d, 8.5		Lys-2, Phe-2,NH, Ile-NH
Phe 1	173.7 s		Phe-2,3,3',NH	
2	54.0 d	4.32 m	Phe-3,3',NH	Phe-3,3',5,5',NH
3	37.7 t	2.98 m, 2.88 m	Phe-5,5',NH	Phe-2,NH
4	137.6 s		Phe-2,3,3',6,6'	
5,5′	129.4 d (×2)	7.15 m (×2)	Phe-6',6	Phe-2,NH
6,6′	128.4 d (×2)	7.25 m (×2)	Phe-6,6',7',7	
7	126.6 d	7.17 m	Phe-5,5'	
NH	116.2 d	6.26 d, 8.0		Phe-2,3,3',5,5', Lys-α-NH
Ureido CO	157.1 s		Phe-NH, Lys-α-NH	
Ureido CO	157.1 s		Phe-NH, Lys-α-NH	

^a Carried out on a Bruker Avance 400 spectrometer.

^b Multiplicity and assignment from HMQC experiment.

^c Determined from HMBC experiment, ${}^{n}J_{CH}$ =8 Hz, recycle time 1 s.

^d By ROESY experiment.

with Hph²-H-2 in **1**, Hph-NH and NMe-Gly-H-2b with Hty-H-2 in **2**, and Hty¹-NH with Hty²-NH and NMe-Gly-H-2b with Hty²-H-2 in **3**. The inhibitory activity of **1–3** was studied for four enzymes, the

The inhibitory activity of 1-3 was studied for four enzymes, the serine proteases elastase, chymotrypsin thrombin, and trypsin. Compounds 1-3 did not inhibit the four-serine proteases at $45.0 \ \mu g/m L^{20,21}$

2. Experimental section

2.1. General experimental procedures

High-resolution MS were recorded on an Applied Biosystems Voyager System 4312 instrument. UV spectra were recorded on a Kontron 931 plus spectrophotometer. Optical rotation values were obtained on a Jasco P-1010 polarimeter at the sodium D line (589 nm). NMR spectra were recorded on a Bruker ARX-500 spectrometer at 500.136 MHz for ¹H and 125.76 MHz for ¹³C and a Bruker Avance 400 spectrometer at 400.13 MHz for ¹H, 100.62 MHz for ¹³C, and 40.55 MHz for ¹⁵N. ¹H, ¹³C, DEPT, gCOSY, gTOCSY, gROESY, gHMQC, and gHMBC spectra were recorded using standard Bruker pulse sequences. HPLC separations were performed on an ISCO HPLC system (model 2350 pump and model 2360 gradient programmer) equipped with an Applied Biosystems Inc. diode-array detector and Merck-Hitachi HPLC system (model L-4200 UV–vis detector and model L-6200A Intelligent pump).

2.2. Cyanobacterial material

Anabaena sp., TAU strain NZ-3-1, was collected in Milford Sound, New Zealand on October 21, 1998. It was cultured in 20 L glass bottles containing a BG-11 medium.²² Cultures were continuously illuminated at an intensity of 100 μ ein/M²/s from fluorescent tubes

Table 3	
NMR data ^a of anabaenopeptin NZ857 (3) major conformed	r

Hty ¹ 1 171.3 s 2 53.2 d 4.05 ddd, 2.8,9.0,11.6 3 33.8 t 1.86 m 2.04 m	Hty ¹ -NH,6,6′, Lys-ε-NH Hty ¹ -NH
2 53.2 d 4.05 ddd, 2.8,9.0,11.6 3 33.8 t 1.86 m 2.04 m	Hty ¹ -NH,6,6′, Lys-ε-NH Hty ¹ -NH
3 33.8 t 1.86 m 2.04 m	Hty ¹ -NH
4 31.4 t 2.31 m Hty ¹ -6,6'	Hty ¹ -NH
2.35 m	Hty ¹ -NH
5 131.5 s Hty ¹ -4,4′7,7′	
6,6' 129.3 d (×2) 6.86 d, 8.0 (×2) Hty ¹ -6',6	Hty ¹ -2, NMe-Gly-NMe
7,7' 115.1 d (×2) 6.61 d, 8.0 (×2) Hty ¹ -7',7,8-OH 8 155.6 s Hty ¹ -6.6' 77',8-OH	Hty ¹ -8-OH
8-OH 911 s	Htv ¹ -77'
NH 8.53 d, 8.0	Hty ¹ -2,3,4,4', Lys- ε -NH, NMe-Gly-2b,NMe, Hty ² -NH
NMe-Gly 1 169.0 s Hty ¹ -NH, NMe-Gly-2a,2b	
2a 52.2 t 3.55 d, 16.4 NMe-Gly-NMe	NMe-Gly-NMe(w)
2b 4.54 d, 16.4	Hty ¹ -NH, Hty ² -2
NMe 37.1 q 2.78 s NMe-Gly-2a,2b	Hty ¹ -6,6',NH, Hty ² -2, <i>N</i> Me-Gly-2a(w)
Hty ² 1 171.3 s <i>N</i> Me-Gly-2a,2b,NMe	
2 49.3 d 4.62 m	Hty ¹ -NH, Hty ² -6,6',NH, Lys-ε-NH, NMe-Gly-2b,NMe
3 33.0 t 1.90 m, 1.84 m	Hty ² -NH
4 30.9 t 2.63 m Hty ² -3,3',6,6' 2.49 m	Hty ² -NH Hty ² -NH
5 1313 s Htv ² -4 4′ 77′	ity iti
6.6' 129.3 d (×2) 7.02 d. 8.0 (×2) Htv ² -4.4'.6'.6	Htv ² -2
7.7' 115.3 d (\times 2) 6.66 d, 8.0 (\times 2) Htv ² -6.6', 7', 7.8-OH	Hty ² -8-OH
8 155.7 s Hty ² -6,6,7,7,8-OH	2
ОН 9.16 s	Hty ² -7,7′
NH 8.87 d, 4.0	Hty ² -2,3,3',4,4' lle-2,3,6
lle 1 172.8 s Hty ² -NH, lle-2	
2 56.9 d 3.94 m lle-6	Hty ² -NH, Ile-5,6,NH
3 35.7 d 1.59 m lle-2,5,6	Hty ² -NH
4 24.9 t 1.44 m lle-2,5,6	
1.03 m	
5 10.8 q 0.78 t, 76 lle-4,4'	lle-2
6 15.2 q 0.91 d, 6.4 lie-2	lle-2, Hty ² -NH
NH 6.90 d, 8.8	lie-2, Lys-α-NH
Lys 1 172.2 s Ile-NH, Lys-2	
2 54.8 d 3.84 m	Lys-α-NH, Phe-NH
3 31.6 t 1.48 m (2H)	
4 20.5 t 1.24 m, 1.03 m	
5 28.6 t 1.35 m	
o NU 710 710 710 710 710 710 710 710 710 710	$Utu^1 2 Utu^2 2$
α-NH 6.40 m	Lys-2, Ile-NH, Phe-NH
Phe 1 173.8 s Phe-2.3.3'	
2 54.0 d 4.34 m Phe-3.3'	Lvs-q-NH. Phe-NH.5.5'
3 37.7 t 2.99 m Phe-2.5.5'	2,5 % 1.1., 1.1.0 1.1.,0,5
2.86 m	
4 137.6 s Phe-2,3,3',6,6'	
5,5' 129.4 d (×2) 7.16 d, 8.0 Phe-3,3',5',5,7	Phe-2
6,6' 128.4 d (×2) 7.26 m (×2) Phe-6',6	
7/ 126.6 d 7.20 m Phe-5,57	Lvc.g.NH
111 0.20 U, 0.2	Lys-u-infi
Ureido CO 157.0 s Phe-NH, Lys-NH	

^d Carried out on a Bruker Avance 400 spectrometer.

^b Multiplicity and assignment from HMQC experiment.

^c Determined from HMBC experiment, ^{*n*}J_{CH}=8 Hz, recycle time 1 s.

^d By ROESY experiment.

and aerated with 0.5% CO_2 in air (1 L/min) at an incubation temperature of 25 °C for 30–35 days.

2.3. Isolation procedure

The cultured, freeze-dried cells (230 g) were extracted with MeOH/H₂O (7:3) (×3) and then with MeOH/chloroform (1:1) (×3). The combined hydrophilic extract (A) was concentrated under reduced pressure to afford 56.5 g of crude extract. The crude extract was separated on an ODS (YMC-GEL, 120A, 4.4×6.4 cm) flash column with increasing amounts of MeOH in water. Fractions 5 and 6

(2:3 and 1:1 MeOH/H₂O) were combined (454.3 mg) and separated on a Sephadex LH-20 gel-filtration column with 1:1 CHCl₃/MeOH. Fractions 6–9 (94.9 mg) from the Sephadex LH-20 column were combined and subjected to a reversed-phase HPLC (YMC ODS-A 10 μ m, 250×20.0 mm, DAD at 210 nm, flow rate 5.0 mL/min) in 50:50 water (0.1% TFA)/acetonitrile to obtain pure compound **1**. Compound **1** (4.2 mg), 0.0018% yield based on the dry weight of the cyanobacteria cells, was eluted from the column with a retention time of 31.6 min. Fractions 3 and 4 of the flash chromatography (1:4 and 3:7 MeOH/H₂O) were combined (960.3 mg) and separated on a Sephadex LH-20 gel-filtration column with 1:1 CHCl₃/MeOH. Fractions 8 and 9 (139.5 mg), from the Sephadex LH-20 column were combined and subjected to a reversed-phase HPLC (YMC pack-C₈ 10 μ m, 250×20.0 mm, DAD at 238 nm, flow rate 5.0 mL/ min) in 65:35 water/acetonitrile to obtain semipure fraction 2. The semipure fraction was again subjected to a reversed-phase HPLC (YMC pack-C₈ 10 μ m, 250×20.0 mm, DAD at 238 nm, flow rate 5.0 mL/min) in 55:45 water (0.1% TFA)/acetonitrile to obtain pure compounds **2** and **3**. Compound **2** (10.5 mg), 0.0046% yield based on the dry weight of the cyanobacteria cells, was eluted from the column with a retention time of 23.2 min. Compound **3** (3.5 mg), 0.0015% yield based on the dry weight of the cyanobacteria cells, was eluted from the column with a retention time of 20.9 min.

2.3.1. Anabaenopeptin NZ825 (1)

Colorless glassy solid; $[\alpha]_{B}^{20}$ –6.5 (*c* 0.003, MeOH); UV (MeOH) λ_{max} (ε) 208 (13,800) nm; for ¹H and ¹³C NMR data see Table 1 and Table 1a in Supplementary data; HR MALDI TOF MS *m*/*z* 864.4036 [MK]⁺ (calcd for C₄₅H₅₉N₇O₈K, 864.4057).

2.3.2. Anabaenopeptin NZ841 (2)

Colorless glassy solid; $[\alpha]_D^{20}$ +4.5 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (ε) 215 (13,000) nm; for ¹H and ¹³C NMR data see Table 2 and Table 2a in Supplementary data; HR MALDI TOF MS *m*/*z* 864.4337 [MNa]⁺ (calcd for C₄₅H₅₉N₇O₉Na, 864.4266).

2.3.3. Anabaenopeptin NZ857 (**3**)

Colorless glassy solid; $[\alpha]_D^{20}$ +7.7 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (ε) 215 (7,500) nm; for ¹H and ¹³C NMR data see Table 3 and Table 3a in Supplementary data; HR MALDI TOF MS *m*/*z* 880.4269 [MNa]⁺ (calcd for C₄₅H₅₉N₇O₁₀Na, 880.4216).

2.4. Determination of the absolute configuration of the amino acids

Compounds 1-3 (0.5 mg) were dissolved in 6 M HCl (1 mL). The reaction mixture was then placed in a sealed glass bomb at 110 °C for 24 h. After removal of HCl, by repeated evaporation in vacuo, the hydrolyzate was resuspended in water (100 µL). A solution of (1-fluoro-2,4-dinitrophenyl)-5-L-alanine amide (FDAA) (4.2 mMol) in acetone (150 mL) and 1 M NaHCO₃ (20 μ L) was added to each reaction vessel and the reaction mixture was stirred at 40-45 °C for about 2 h. A 2 M HCl solution (10 μ L) was added to each reaction vessel and the solution was evaporated in vacuo. The N-[(-dinitrophenyl)-5-L-alanine amide]-amino acid derivatives, from hydrolyzates, were compared with similarly derivatized standard amino acids by HPLC analysis: Merck Chromolith performance RP-18e, 5 µm, 4.6×100 mm, flow rate: 3 mL/min, UV detection at 340 nm, linear gradient elution from 9:150 mM triethylammonium phosphate (TEAP) buffer (pH 3)/acetonitrile to 1:1 TEAP/acetonitrile within 90 min. The determination of the absolute configuration of each amino acid was confirmed by spiking the derivatized hydrolyzates with the derivatized authentic amino acids. HPLC analysis of Marfey's derivatives of 1: L-Ile, 23.0 min; L-Phe, 23.2 min; L-Hph, 27.4 min; and D-Lys, 29.9 min, of 2: L-Ile, 20.1 min; L-Phe, 20.3 min; L-Hph, 24.4 min; D-Lys, 27.6 min; and L-Hty, 30.4 min, and of **3** established: L-Ile, 22.4 min; L-Phe, 22.6 min;

p-Lys, 29.7 min; and L-Hty, 35.2 min. The relative intensity of the Phe derivative from compounds 1-3 was low although we used extended hydrolysis time (24 h) maybe due to the formation of 4-benzyloxazolidine-2,5-dione from the ureido bridge.

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Supplementary data

¹H, ¹³C NMR, COSY, TOCSY, ROESY, HMQC or HSQC, and HMBC spectra and NMR data tables of minor rotamers of anabaenopeptins NZ825 (**1**), NZ841 (**2**), and NZ857 (**3**), as well as, ¹H NMR spectra of anabaenopeptin NZ841 (**2**) in pyridine- d_5 at various temperatures, are available. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.08.015.

References and notes

- Harada, K.; Fujii, K.; Shimada, T.; Suzuki, M.; Sano, H.; Adachi, K.; Carmichael, W. W. Tetrahedron Lett. 1995, 36, 1511–1514.
- Fujii, K.; Harada, K.; Suzuki, M.; Kondo, F.; Ikai, Y.; Oka, H.; Carmichael, W. W.; Sivonen, K. In *Harmful and Toxic Algal Blooms*; Yasumoto, T., Oshima, Y., Fukuyo, Y., Eds.; Intergovernmental Oceanographic Commission of UNESCO: Paris, 1996; pp 559–562.
- Shin, H. J.; Matsuda, H.; Murakami, M.; Yamaguch, K. J. Nat. Prod. 1997, 60, 139–141.
- Itou, Y.; Suzuki, S.; Ishida, K.; Murakami, M. Bioorg. Med. Chem. Lett. 1999, 9, 1243–1246.
- Murakami, M.; Suzuki, S.; Itou, Y.; Kodani, S.; Ishida, K. J. Nat. Prod. 2000, 63, 1280–1282.
- Kodani, S.; Suzuki, S.; Ishida, K.; Murakami, M. FEMS Microbiol. Lett. 1999, 178, 343–348.
- Beresovsky, D.; Hadas, O.; Livne, A.; Sukenik, A.; Kaplan, A.; Carmeli, S. Isr. J. Chem. 2006, 46, 79–87.
- Williams, D. E.; Craig, M.; Holmes, C. F. B.; Andersen, R. J. J. Nat. Prod. 1996, 59, 570–575.
- 9. Sano, T.; Usui, T.; Ueda, K.; Osada, H.; Kaya, K. J. Nat. Prod. 2001, 64, 1052-1055.
- 10. Sano, T.; Kaya, K. Tetrahedron Lett. 1995, 36, 5933-5936.
- 11. Fujii, K.; Sivonen, K.; Adachi, K.; Noguchi, K.; Sano, H.; Hirayama, K.; Suzuki, M.; Harada, K. Tetrahedron Lett. **1997**, 38, 5525–5528.
- 12. Reshef, V.; Carmeli, S. J. Nat. Prod. 2002, 65, 1187-1189.
- Mueller, D.; Krick, A.; Kehraus, S.; Meher, C.; Hart, M.; Kuepper, F. C.; Saxena, K.; Prinz, H.; Schwalbe, h.; Janning, P.; Waldmann, H.; Koenig, G. M. *J. Med. Chem.* 2006, 49, 4871–4878.
- 14. Kobayashi, J.; Sato, M.; Murayama, T.; Ishibashi, M.; Walchi, M. R.; Kanai, M.; Shoji, J.; Ohizumi, Y. J. Chem. Soc., Chem. Commun. **1991**, 1050–1052.
- Kobayashi, J.; Sato, M.; Ishibashi, M.; Shigemori, H.; Nakamura, T.; Ohizumi, Y. Org. Bioorg. Chem. 1991, 2609–2611.
- 16. Schmidt, E. W.; Harper, M. K.; Faulkner, D. J. J. Nat. Prod. 1997, 60, 779-782.
- 17. Uemoto, H.; Yahiro, Y.; Shigemori, H.; Tsuda, M.; Takao, T.; Shimonishi, Y.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 6719–6724.
- Robinson, S. J.; Tenney, K.; Yee, D. F.; Martinez, L.; Media, J. E.; Valeriote, F. A.; vanSoest, R. W. M.; Crews, P. J. Nat. Prod. 2007, 70, 1002–1009.
- 19. Marfey, P. Carlsberg Res. Commun. 1984, 49, 591-596.
- 20. Grach-Pogrebinsky, O.; Sedmak, B.; Carmeli, S. Tetrahedron 2003, 59, 8329–8336.
- 21. Ploutno, A.; Carmeli, S. Tetrahedron 2005, 61, 575-583.
- Stainer, R. Y.; Kunisawa, M.; Mandel, M.; Cohen-Bazire, G. Bacteriol. Rev. 1971, 35, 171–205.