DOI: 10.1002/cbic.200900035

Synthesis and Structure–Activity Correlation of a Brunsvicamide-Inspired Cyclopeptide Collection

Thilo Walther,[a, b] Steffen Renner,[b] Herbert Waldmann,*[a, b] and Hans-Dieter Arndt*[a, b]

The brunsvicamides are modified cyclopeptides from cyanobacteria, cyclised through the ε-amino group of a D-lysine unit. They are functionalised with urea groups and show potent carboxypeptidase inhibitory activities. In order to unravel the structural parameters that determine their activities, a collection of brunsvicamide analogues with varied amino acid structures and stereochemistries was synthesised by a combined solution- and solid-phase approach. Biochemical investigation of the compound collection for carboxypeptidase A inhibition revealed that the presence of D-lysine and L-isoleucine in the urea section is important for inhibition. It was found that brunsvicamide A is a substrate-competitive inhibitor of carboxypeptidase A. These findings are in agreement with the substrate specificity of the enzyme and were rationalised by computational studies, which showed the high relevance of the lysine stereochemistry for inhibitory activity.

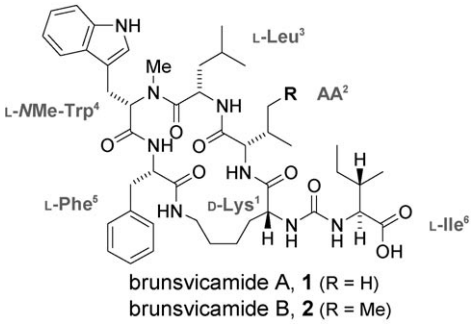
Introduction

Natural products (NPs) can be regarded as evolutionarily selected and therefore biologically relevant small molecules endowed with the ability to interact with multiple biological targets.[1] Due to this biological prevalidation, the structural frameworks underlying the multiple NP classes can be considered "privileged".[2] In turn, NPs continue to stimulate investigations into compound library development for chemical biology[3] and medicinal chemistry research.[4]

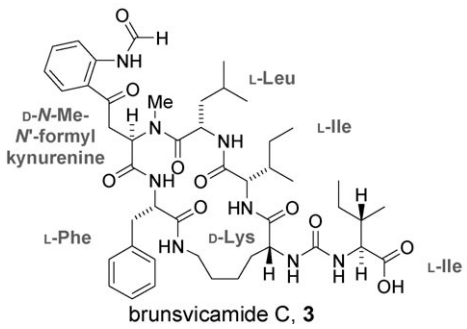
A prolific class of producers of bioactive NPs with novel structures are cyanobacteria (formerly called "blue–green algae"), which are quite diverse prokaryotes that live by photosynthesis.[5] Cyanobacteria produce a large variety of secondary metabolites that are believed to function as defence agents,[6] and probably improve survival chances in their particular ecological niche. Interestingly, many of these compounds are structurally diversified peptides, especially cyclopeptides. The spectrum of the activities of these compounds reaches from immunosuppressive through antibiotic, toxic and cytostatic action to inhibition of specific enzymes. Consequently, such cyanobacterial secondary metabolites (CSMs) have become an inspiring natural source of new pharmacologically active molecules with potential therapeutic applications.[7]

Earlier reports suggested that several cyanobacterial cyclopeptides arise from nonribosomal peptide synthesis pathways,[8] in line with the occurrence of nonproteinogenic amino acids and noncanonical linkages in their structures. However, recent contributions have provided evidence that they can also originate from ribosomal peptide synthesis—in the cases of the aerucyclamides or microviridin, for example.[9] It can therefore be speculated that the pathway depends on the individual compound, and that both variants might even coexist.

The brunsvicamides (BVAs) A–C (**1**–**3**;) were recently isolated from *Tychonema* sp.[10] Five amino acids form the cyclic backbones of the these branched hexapeptides through a D-lysine side chain. The sixth amino acid is found linked to a urea moiety at the α-amino group of the D-lysine. The BVAs belong to a group of structurally related cyclic peptides that are generally classified as "anabaenopeptin-type" (or "-like") peptides.



brunsvicamide A, **1** (R = H)
brunsvicamide B, **2** (R = Me)



brunsvicamide C, **3**

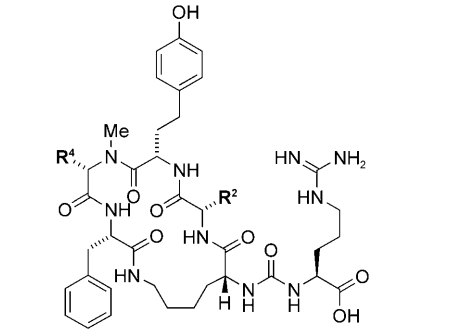
[a] *T. Walther, Prof. Dr. H. Waldmann, Dr. H.-D. Arndt*
Technische Universität Dortmund, Fakultät Chemie
Otto-Hahn-Strasse 6, 44221 Dortmund (Germany)

[b] *T. Walther, Dr. S. Renner, Prof. Dr. H. Waldmann, Dr. H.-D. Arndt*
Max-Planck-Institut für Molekulare Physiologie
Otto-Hahn-Strasse 11, 44227 Dortmund (Germany)
Fax: (+49) 231-133-2498;
E-mail: hans-dieter.arndt@mpi-dortmund.mpg.de
herbert.waldmann@mpi-dortmund.mpg.de

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.200900035.

To date, 41 such anabaenopeptin-type peptides have been isolated from different natural sources.[11] All examples retrieved from cyanobacteria feature a D-lysine unit, whereas anabaenopeptin-type peptides isolated from other sources typically incorporate an L-lysine component. All of them, however, have a urea moiety, which is known as a surrogate for an amide bond in peptidomimetics.[12] In addition, they all share a common *N*-methylated residue at position four in their amino acid sequences.

Anabaenopeptin-type molecules show a broad spectrum of biological activities. The oscillamides B (**4**) and C (**5**), for instance, were found to inhibit protein phosphatase types 1 and



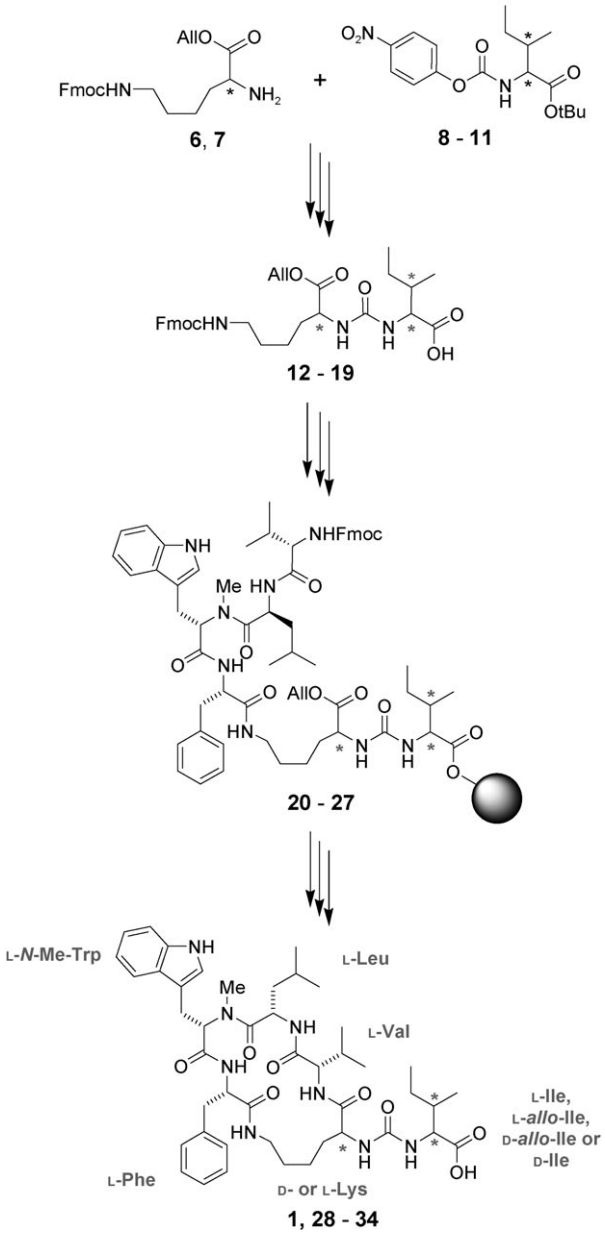
oscillamide B, **4** (R^2 = $(CH_2)_2$-S-CH_3, R^4 = CH_3)
oscillamide C, **5** (R^2 = CHMeEt, R^4 = $(CH_2)_2$-C_6H_4-OH)

2A.[13] Several anabaenopeptins have been shown to inhibit carboxypeptidases (CPDs).[14] CPDs are zinc-containing carboxy-*exo*-peptidases[15] that process a variety of peptides or proteins that play crucial roles in immunological reactions/allergic responses and inflammation, as well as in blood coagulation and thrombosis.[16]

The BVAs have been found to inhibit human carboxypeptidases A and B.[17] Moreover, the NP isolates interfered with the function of tyrosine phosphatase B from *Mycobacterium tuberculosis* (MptpB) at micromolar concentrations.[10] In general, protein phosphatases act in concert with protein kinases to control the state of protein phosphorylation, and thereby regulate enzymatic activity and modulate interactions between proteins.[18] We have recently reported the correct stereochemistry of BVA A (**1**) from a solid-phase-based total synthesis.[19] Here we give a detailed account on the synthesis and biochemical activity of BVA A and of a focused library of 17 analogues. The biological activities of the synthetic material and the isolate were shown to differ markedly. Notably, a 1000-fold loss in potency was caused by the inversion of one stereogenic centre. Computational docking studies allowed us to rationalise the results from the enzyme inhibition data.

Results and Discussion

In order to determine the correct stereochemistry of BVA A, eight stereochemical isomers of BVA A (Scheme 1, **1**, **28**–**34**; see Table 1 for a detailed structural assignment) were synthesised by both solution and solid-phase methods. Four stereoisomeric 4-nitrophenyl carbamates (**8**–**11**) were each treated with L- and D-Lys(Fmoc)OAll (**6**, **7**) to yield eight orthogonally



Scheme 1. Synthesis of brunsvicamide A (**1**) and seven stereochemical analogues (**28**–**34**). For the stereochemical assignments see Table 1.

protected urea building blocks, which were transformed into the free acids **12**–**19**. Treatment of 2-chlorotrityl chloride resin with the purified urea building blocks reliably gave access to the solid-phase-bound ureas. Peptide chain elongation was performed, and cleavage of the resulting allyl esters **20**–**27** was followed by on-resin ring-closure. The solid-phase-bound BVA analogues were then released from the solid support and purified by HPLC. BVA A (**1**) and the seven stereoisomers **28**–**34** were subjected to biochemical testing.

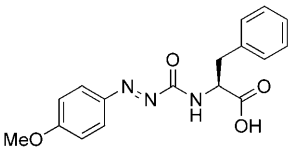
Investigation of phosphatase inhibition

Synthetic BVA A (**1**) and its stereoisomers **28**–**34** were each studied for inhibition of MptpB. However, in contrast with the

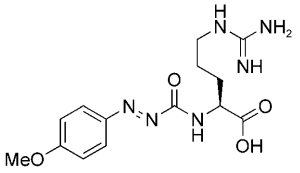
material retrieved by isolation,[10] neither synthetic **1** nor **28**–**34** gave any measurable inhibition of MptpB up to a concentration of 100 μM with the substrates pNPP[20] or DiFUMP[21] (see the Supporting Information for details). Currently we reason that an impurity might be responsible for the inhibitory activity of the material isolated from the producing organism.

Investigation of CPD inhibition

On the other hand, synthetic BVA A (**1**) inhibited the activities of carboxypeptidases A and B (CPDA/B) as reported earlier, and a detailed investigation was initiated. The inhibition of bovine CPDA and porcine CPDB, which are both homologues of human CPDs,[22] by the synthetic BVAs **1** and **28**–**34** was investigated by an absorbance assay based on *N*-(4-methoxyphenylazoformyl)phenylalanine (**35**, AAF-Phe-OH) and -arginine (**36**, AAF-Arg-OH) as substrates.[23] The reaction rates were determined from the slopes of the absorbance change at 355 nm at ten different concentrations of inhibitor and related to controls in the absence of the inhibitor.



35



36

Compound **1**, the NP with D-lysine and L-isoleucine, proved to be the most potent inhibitor tested (see Table 1). Structure–activity correlation indicates that L stereochemistry is clearly favoured for the amino acid at position 6 (AA6) off the urea. The stereochemistry of the lysine is of highest importance for inhibitory activity (**1**: $IC_{50}=5$ nM, **31**: $IC_{50}=5$ μM). The findings are consistent with the known specificity of CPDA for substrates that bear large aliphatic or aromatic amino acid residues with the L configuration at their C termini.

The inhibitory activities of **1** and **28**–**34** towards CPDB were significantly lower (**1**: 88 nM, **28**: 480 nM; see Table 1). These experimental findings correspond with the known specificity of CPDB for substrates carrying basic amino acid residues at their C termini. The difference in CPDA inhibitory activity between **1** and **31**—three orders of magnitude—is a promising starting point for future library design, and it prompted us to undertake a systematic study by alanine and serine scans. The amino acid residues were varied to yield analogues that each differ in only one position of the original sequence. Essential interactions or steric hindrance between inhibitor and enzyme can typically be identified by this method.[24]

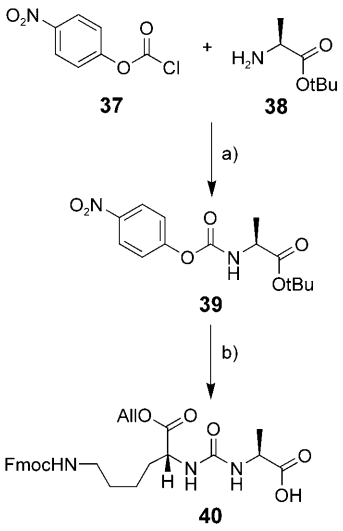
Alanine and serine scan

We chose the BVA A (**1**) sequence as the basic scaffold and varied the isoleucine to L- or D-alanine and the amino acid residues AA2, AA3 and AA5 (see Scheme 1 for the numbering) to alanine or serine. The *N*-Me-tryptophan was varied to *N*-Me-alanine or *N*-Me-serine. The synthesis of the necessary urea building blocks started with the reaction between 4-nitrophenyl chloroformate (**37**) and L-alanine *tert*-butyl ester (**38**) to yield 4-nitrophenyl carbamate **39**, which was purified by column chromatography (Scheme 2).[25] Upon addition of Hünig's base to carbamate **39**, an intense yellow coloration indicated the in situ formation of the isocyanate,[26] which was treated with D-Lys(Fmoc)OAll to give the orthogonally protected[27] urea building block. Column chromatography removed residual 4-nitrophenol, and this was directly followed by *tert*-butyl ester cleavage to yield the free acid **40**. We obtained the L-Ala urea **40**

Table 1. Inhibition of bovine CPDA and porcine CPDB by BVA A (**1**) and analogues (**28**–**34**, **54**–**63**).

Compound	Amino acid sequence/residue number						IC_{50}	
	1	2	3	4	5	6	CPDA (μM)	CPDB (μM)
1[a,b]	D-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	L-Ile	0.0050 ± 0.0001	0.088 ± 0.006
28[b]	D-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	L-*allo*-Ile	0.0077 ± 0.0001	0.480 ± 0.004
29[b]	D-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	D-*allo*-Ile	17.1 ± 0.5	> 50
30[b]	D-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	D-Ile	27.1 ± 0.6	> 50
31[b]	L-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	L-Ile	5.4 ± 0.1	> 50
32[b]	L-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	L-*allo*-Ile	> 50	> 50
33[b]	L-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	D-*allo*-Ile	> 50	> 50
34[b]	L-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	D-Ile	> 50	> 50
54[c]	D-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	L-Ala	0.102 ± 0.007	–
55[c]	D-Lys	L-Val	L-Leu	L-NMe-Trp	L-Phe	D-Ala	5.7 ± 0.1	–
56[c]	D-Lys	L-Val	L-Leu	L-NMe-Trp	L-Ala	L-Ile	0.0066 ± 0.0002	–
57[c]	D-Lys	L-Val	L-Leu	L-NMe-Trp	L-Ser	L-Ile	0.0079 ± 0.0003	–
58[c]	D-Lys	L-Val	L-Leu	L-NMe-Ala	L-Phe	L-Ile	0.0048 ± 0.0002	–
59[c]	D-Lys	L-Val	L-Leu	L-NMe-Ser	L-Phe	L-Ile	0.0289 ± 0.0005	–
60[c]	D-Lys	L-Val	L-Ala	L-NMe-Trp	L-Phe	L-Ile	0.0083 ± 0.0002	–
61[c]	D-Lys	L-Val	L-Ser	L-NMe-Trp	L-Phe	L-Ile	0.0076 ± 0.0004	–
62[c]	D-Lys	L-Ala	L-Leu	L-NMe-Trp	L-Phe	L-Ile	0.0087 ± 0.0003	–
63[c]	D-Lys	L-Ser	L-Leu	L-NMe-Trp	L-Phe	L-Ile	0.0225 ± 0.0015	–

[a] Brunsvicamide A. [b] Variation of the three stereocentres in the urea section. [c] Alanine and serine scan.



Scheme 2. Representative synthesis of alanine-containing building blocks. a) Pyridine (2 equiv), CH_2Cl_2, RT, 16 h, 51%; b) i: EtN(*i*Pr)$_2$ (2 equiv), CH_2Cl_2, RT, 16 h, 74%; ii: TFA (50%), CH_2Cl_2, RT, 3 h, quant.

and its D-Ala-containing isomer in 38% and 50% overall yields, respectively.

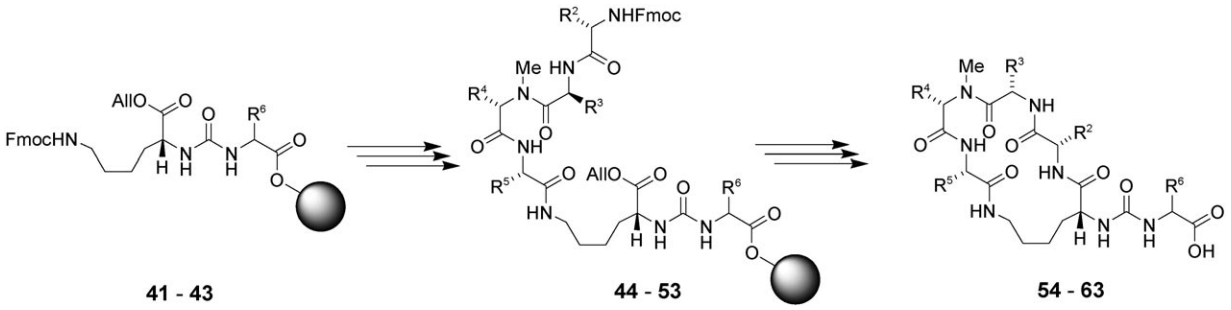
Treatment of 2-chlorotrityl chloride resin with the purified urea building blocks, followed by capping with methanol, gave access to the solid-support-bound ureas **41**–**43** (Scheme 3). After a 16 h reaction time a loading of 0.6 mmol g^{-1} was determined (UV-based Fmoc determination).[28] For the extension of the peptide chain, solid-phase peptide synthesis (SPPS) was performed, with use of IRORI™ MacroKans with radio frequency identification (RFID) technology[29] for split-mix synthesis. The Fmoc groups were removed with DMF/piperidine. The peptide couplings were performed by use of either DIC/HOBt or HATU/HOAt for the coupling to the *N*-Me-amino acid residues. In each case Kaiser or chloranil tests[30] were performed to check for complete conversion. The allyl esters **44**–**53** were cleaved with Pd(PPh$_3$)$_4$ in the presence of phenylsulfinate as a scavenger.[19,27,31] Removal of the N-terminal Fmoc groups was followed by macrolactam formation on resin by use of DIC/HOBT in DMF. The crude products were released with TFA and purified by silica gel column chromatography (with dichloromethane/methanol/formic acid as eluent) and preparative reversed-phase HPLC purification (MeOH/H_2O). For compounds **57**, **59**, **61** and **63**, column chromatography was followed by deprotection of the *tert*-butyl protected serine by treatment with trifluoroacetic acid. For final purification preparative reversed-phase HPLC (MeOH/water) was used. Compounds **54**–**63** were obtained on solid support with overall yields from 5–16% based on the loading of ureas **41**–**43**.

BVA A (**1**) and most analogues obtained from the alanine and serine scan (**54**–**63**) significantly affected the activity of CPDA at low nanomolar concentrations. The IC_{50} values (Table 1) indicate clearly that the D-lysine and L-isoleucine in the urea are essential for high activity on CPDA, while variations in the remaining amino acids did not exert any major influence. Only when the slightly polar serine was introduced at positions 2 and 4 were moderate decreases in efficiency observed (four- to sixfold). This suggests that none of these residues is critically involved in binding to the target enzyme. Hence they might be useful for synthetic replacement in the future—to tailor compound properties, for instance.

For the producing organism this apparent promiscuity may contribute to its ecological fitness, as the broadly active anabaenopeptin scaffold can be quickly adapted by mutation to defend against novel predators. Furthermore, the purpose for which these compounds are really produced in nature currently remains unclear, and it might be that broad activity against a whole collection of similar enzymes is a generally desirable feature in the producers' habitat—as it might also be for a good library scaffold. Future work will be necessary to clarify the ecological meaning of these findings.

Mode of inhibition

To determine the mode of inhibition of compound **1**, the initial rates of substrate conversion were measured as a function of substrate concentration at four fixed inhibitor concentrations (10 nM, 5 nM, 2.5 nM, and without **1**). The obtained rates were plotted as a function of substrate concentration for each inhibitor concentration. To these data the Michaelis–Menten equation[32] was fitted. This gave the apparent K_M (Michaelis–Menten constant) and V_{max} (maximum reaction rate with substrate saturation) values. The values of the reaction rate for any substrate concentration were calculated by use of the reciprocal Michaelis–Menten equation. Overlaying of the double-reciprocal plots yielded a pattern of lines clearly intersecting at the origin (Figure 1). It can therefore be assumed that **1** is a competitive inhibitor with respect to enzyme substrate (AAF-Phe-OH, **35**).[33]



Scheme 3. Synthesis of ten brunsvicamide analogues (**54**–**63**).

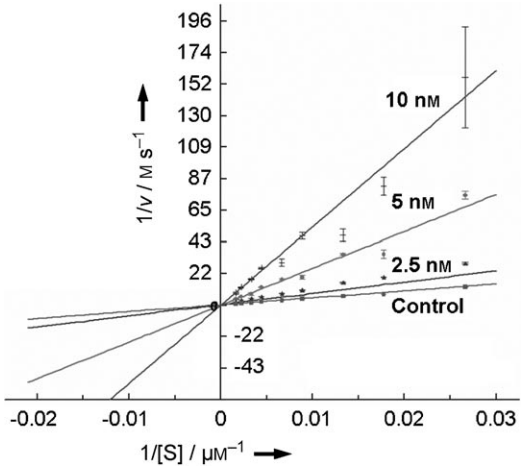


Figure 1. Double reciprocal plots for the effects of BVA A (**1**) on the substrate turnover of the CPDA-catalysed reaction.

Computational studies

The competitive mode of inhibition observed in the enzyme kinetics study indicated that the BVA binding sites are similar to those of natural peptide substrates. Furthermore, the increase in IC_{50} values from compounds **1** and **28**, through **54**, **31** and **55**, to **30** and **29**, which bear different amino acid residues at the free C terminus of BVA, is consistent with the reported substrate preference of CPDA, namely for large aliphatic C-terminal side chain residues with L stereochemistry.[15] Potential binding modes of the NP **1** in the CPDA peptide binding pocket were studied by means of computational docking simulations based on this rationale. Prior to the docking, an ensemble of low-energy conformations of the macrocycles was computed, because the docking algorithm was not able to treat them as flexible. Of the proposed binding modes, the best-scoring solutions out of the conformers within 10 kJ mol^{-1} of the global energy optimum were selected, in a manner similar to that used in a recent study on chondramide C.[34]

As in the case of the natural peptide substrate, in the best predicted binding mode of **1** the C-terminal carboxy group interacted with Asn144 and Arg145, and the isoleucine was located in the selectivity pocket for aliphatic and aromatic side chains. The urea coordinated to the zinc atom as an amide bond surrogate, which is inert to the hydrolytic activity of the enzyme (Figure 2).

We compared our docking results for **1** with a high-resolution crystal structure (PDB ID: 7CPA)[35] harbouring the inhibitor Cbz-Phe-Val-P(O)-Phe (**64**; Figure 2). Compound **64** is a noncovalent, tight-binding phosphonate inhibitor and a transition-state analogue of the CPDA-catalysed reaction. It binds to the active-site groove of CPDA, in which the terminal phenylalanine of **64** is clearly bound by the corresponding binding subsite (see Figure 2B, Site S1), which is the selectivity pocket of CPDA.[36]

The phosphonic ester group of **64** coordinates to the zinc at the CPDA active site, while the array of phenyl rings adopts a configuration that allows stabilizing aromatic–aromatic edge-to-face interactions from one ring to the next. Our overlay revealed similar binding modes for the two inhibitors **1** and **64** (Figure 2C). In particular, the C-terminal residues of both molecules—the zinc-coordinating urea and phosphonate groups—overlap well, as do the two residues directly adjacent. The remaining residues of the BVAs are not predicted to have any major contacts with the protein; this is consistent with the ex-

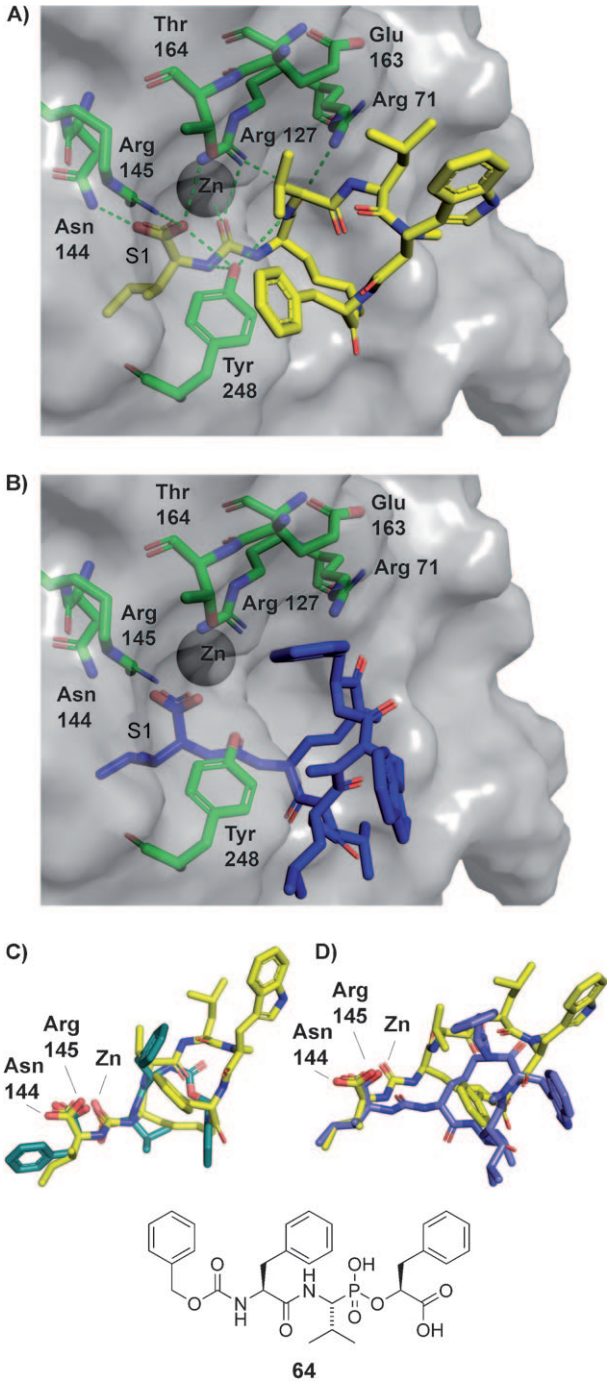


Figure 2. A) BVA A (**1**) docked into the active site of CPDA. B) The L isomer **31** docked into the active site of CPDA. C) Overlay of BVA A (**1**, in yellow) with phosphonate inhibitor **64** (cyan) bound to CPDA. D) Overlay of **1** (D-Lys, yellow) with isomer **31** (L-Lys, blue). Predicted key interactions with the CPDA protein are indicated; the chemical structure of inhibitor **64** is shown at the bottom.

perimental observation that their replacement by Ala or Ser is broadly tolerated.

The largest increase in IC_{50} for a single residue replacement was found in the case of **31** (from 5 nM to 5 μM), with an L- rather than a D-lysine in the NP. In order to rationalise the 1000-fold decrease in enzyme inhibition activity, **31** was also docked into the crystal structure. The highest-scoring docking solution out of the best conformations of **31** still had the C-terminal isoleucine in the selectivity pocket and the carboxy group that interacted with Asn144 and Arg145. The urea moiety was found to be more distant from the zinc than in the case of **1**; this indicates a weaker coordination of the zinc ion. The overlay revealed that because of the inverted stereochemistry of the lysine residues, the 19-membered ring binds to the binding site in an inverted orientation for **31** relative to **1** (Figure 2D). This model explains the remaining but largely decreased activity of the stereoisomeric compound **31**.

Conclusions

In summary, a series of analogues of the cyanobacterial cyclopeptide BVA A was prepared by effective solid-support-based total synthesis. Variations of the amino acid stereochemistries revealed the importance of the D-lysine and the L-isoleucine residues for the inhibitory activity of BVA A against carboxypeptidase A. Systematic scanning of the peptide scaffold by specific introduction of alanine or serine residues showed that only the residues adjacent to the urea are critical for protein binding. Our results relating to the structure–activity relationships in the BVAs are consistent with the available data for peptide substrate specificity of carboxypeptidases A and B, and could be rationalised well by means of computational docking studies. These findings should allow the development of more potent CPD inhibitors based on the BVA A structure and might lead to the generation of an expanded collection to be investigated in biological and biochemical assays.

Experimental Section

See the Supporting Information for general synthetic methods, procedures, characterisation of compounds **39** and **40** and details of the phosphatase inhibition assays. For characterisation data of compounds **1**, **6**--**19** and **28**--**34** see ref. [19].

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-L-alanyl)-L-valinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-phenylalanine] (54): Colourless powder; yield: 33 mg, 41 μmol, 10%; R_f=0.18 (CH_2Cl_2/MeOH 9:1+0.25% AcOH, *v/v*); m.p.: 217 °C; $[\alpha]_D^{20}=-128$ (*c*=0.7, MeOH); ^{1}H NMR (500 MHz, MeOD): δ=10.43 (s, 1H; NH-indole), 9.25 (d, *J*=8.7 Hz, 1H; NH-Phe), 8.34 (s, 1H; NH-Leu), 7.86 (d, *J*=8.2 Hz, 1H; NHε-Lys), 7.56 (d, *J*=7.8 Hz, 1H; 22-H), 7.36 (d, *J*=8.1 Hz, 1H; 25-H), 7.31–7.26 (m, 2H; 12-H, 14-H), 7.24–7.16 (m, 3H; 13-H, 11-H, 15-H), 7.12 (t, *J*=7.5 Hz, 1H; 24-H), 7.04 (t, *J*=7.5 Hz, 1H; 23-H), 6.89 (s, 1H; 19-H), 4.94 (d, *J*=11.1 Hz, 1H; H-17), 4.81 (brs, 1H; 8-H), 4.36–4.24 (m, 2H; 29-H, 41-H), 4.07 (s, 1H; 2-H), 3.86–3.75 (m, 2H; 6-H_2, 35-H), 3.52 (d, *J*=13.8 Hz, 1H; 18-H_2), 3.34 (d, *J*=12.8 Hz, 1H; 9-H_2), 3.05–2.91 (m, 2H; 6-H_2, 18-H_2), 2.86 (t, *J*=13.4 Hz, 1H; 9-H_2), 2.13–2.08 (m, 1H; 36-H), 2.06 (s, 3H; 27-H_3), 1.90–1.76 (m, 1H; 3-H_2), 1.77–1.64 (m, 1H; 3-H_2), 1.63–1.53 (m, 2H; 5-H_2), 1.51–1.42 (m, 1H; 4-H_2), 1.37 (d, *J*=7.3 Hz, 3H; 42-H_3), 1.42–1.16 (m, 2H; 31-H, 4-H_2), 1.10 (d, *J*=6.6 Hz, 3H; 37-H_3), 1.01 (d, *J*=6.5 Hz, 3H; 38-H_3), 0.41 (d, *J*=6.5 Hz, 3H; 32-H_3), 0.10 (d, *J*=6.4 Hz, 3H; 33-H_3), −0.55 ppm (t, *J*=12.3 Hz, 1H; 30-H_2); ^{13}C NMR (126 MHz, MeOD): δ=177.5, 175.7, 175.4, 174.3, 174.3, 172.6, 159.6, 139.4, 137.9, 130.3, 129.9, 128.7, 127.8, 124.7, 122.8, 120.3, 119.3, 112.8, 111.0, 63.2, 60.8, 56.5, 56.3, 49.9, 49.2, 40.1, 39.1, 38.9, 32.6, 31.5, 29.6, 29.1, 24.8, 23.5, 23.3, 21.7, 20.3, 20.2, 19.8, 18.7 ppm; IR: $\tilde{\nu}$=3294 (w), 2958 (w), 1633 (s), 1529 (m), 1455 (m), 1340 (w), 1222 (w), 1086 (w), 928 (w), 738 (m), 700 (w), 623 cm^{-1} (w); MS: *m/z* (%): 803.2 [*M*+H]$^+$; HR-MS (FAB): *m/z*: calcd for $C_{42}H_{58}N_8O_8$: 803.4450 [*M*+H]$^+$; found: 803.4473; HPLC-ESI: t_R=8.0 min.

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-D-alanyl)-L-valinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-phenylalanine] (55): Colourless powder; yield: 27 mg, 34 μmol, 8%; R_f=0.09 (CH_2Cl_2/MeOH 9:1+0.25% AcOH, *v/v*); m.p.: 214 °C; $[\alpha]_D^{20}=-130$ (*c*=1.4, MeOH); ^{1}H NMR (500 MHz, DMSO): δ=10.86 (s, 1H; NH-indole), 8.84 (d, *J*=8.9 Hz, 1H; NH-Phe), 8.39 (d, *J*=4.9 Hz, 1H; NH-Leu), 7.55 (d, *J*=7.9 Hz, 1H; 25-H), 7.35–7.33 (m, 1H; NHε), 7.31 (d, *J*=8.1 Hz), 7.25–7.08 (m, 5H; 11-H to 15-H), 7.05 (t, *J*=7.9 Hz, 1H. 24-H), 6.96 (t, *J*=7.3 Hz, 1H; 23-H), 6.93 (d, *J*=2.2 Hz, 1H; 19-H), 6.65 (brs, 1H; NH-Lys), 6.34 (brs, 1H; NH-Ile), 4.71 (dd, *J*=3.4, 11.1 Hz, 1H; 17-H), 4.61–4.52 (m, 1H; 8-H), 4.25–4.18 (m, 1H; 29-H), 3.90–3.79 (m, 2H; 2-H, 35-H), 3.77–3.69 (m, 1H; 41-H), 3.63–3.40 (m, 1H; 9-H_2), 3.24–3.18 (m, 1H; 18-H_2), 2.92–2.74 (m, 2H; 9-H_2, 18-H_2), 2.03–1.90 (m, 1H; 36-H), 1.93 (s, 3H; 27-H_3) 1.60–1.44 (m, 2H; 3-H_2), 1.45–1.33 (m, 3H; 5-H_2, 31-H), 1.31–1.18 (m, 1H; 4-H_2), 1.20–1.01 (m, 1H; 4-H_2), 1.12 (d, *J*=6.4 Hz, 3-H, 42-H_3), 0.94 (d, *J*=6.7 Hz, 3H; 37-H_3), 0.87 (d, *J*=6.5 Hz, 3H; 38-H_3), 0.86–0.83 (m, 1H; 30-H_2), 0.30 (d, *J*=6.6 Hz, 3H; 32-H_3), 0.16 (d, *J*=6.5 Hz, 3H; 33-H_3), −0.58 ppm (t, *J*=11.5 Hz, 1H; 30-H_2); ^{13}C NMR (126 MHz, MeOD): δ=175.9, 175.5, 174.4, 174.3, 172.7, 172.6, 159.7, 139.4, 137.9, 130.3, 129.9, 128.8, 127.8, 124.7, 122.8, 120.3, 119.3, 112.8, 111.1, 63.3, 60.7, 56.6, 56.6, 50.0, 49.3 ,40.1, 39.2, 39.0, 32.6, 31.6, 29.6, 29.2, 24.8, 23.5, 23.3, 21.8, 20.2, 20.1, 19.9, 19.2 ppm; IR: $\tilde{\nu}$=3294 (w), 2965 (w), 1633 (s), 1541 (m), 1454 (w), 1224 (w), 1084 (w), 1048 (w), 879 (w), 741 (w), 700 cm^{-1} (w); MS: *m/z* (%): 803.2 [*M*+H]$^+$; HR-MS (FAB): *m/z*: calcd for $C_{42}H_{58}N_8O_8$: 803.4450 [*M*+H]$^+$; found: 803.4471; HPLC-ESI: t_R=8.3 min.

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-alanine] (56): Colourless powder; yield: 31 mg, 40 μmol, 9%; R_f=0.28 (CH_2Cl_2/MeOH 9:1+0.25% AcOH, *v/v*); m.p.: 233 °C; $[\alpha]_D^{20}=-91$ (*c*=1.8, MeOH); ^{1}H NMR (500 MHz, MeOD): δ=7.61–7.56 (m, 1H; 16-H), 7.39–7.34 (m, 1H; 19-H), 7.15–7.08 (m, 1H; 18-H), 7.08–7.01 (m, 1H; 17-H), 7.00–6.96 (m, 1H; 13-H), 5.02 (d, *J*=11.2 Hz), 4.58 (d, *J*=7.1 Hz), 4.29 (d, *J*=11.9 Hz), 4.20 (s, 1H; 35-H), 4.03 (s, 1H; 2-H), 3.84–3.79 (m, 1H; 29-H), 3.78–3.71 (m, 1H; 6-H_2), 3.43 (d, *J*=15.3 Hz), 3.17 (t, *J*=13.3 Hz), 2.93 (d, *J*=13.6 Hz), 2.89 (s, 3H; 21-H_3), 2.02–1.94 (m, 1H; 30-H), 1.93–1.86 (m, 1H; 36-H), 1.85–1.74 (m, 1H; 3-H_2), 1.74–1.61 (m, 1H; 3-H_2), 1.61–1.41 (m, 4H; 37-H_2, 5-H_2, 4-H_2), 1.40–1.31 (m, 3*H;* 9-H_3), 1.26–1.13 (m, 2H; H-25, 37-H_2), 1.08 (t, *J*=13.0 Hz), 0.97–0.85 (m, 12H; 39-H_3, 38-H_3, 31-H_3, 32-H_3), 0.46–0.39 (m, 3H; 26-H_3), 0.09–0.03 (m, 3H; 27-H_3), −0.56 ppm (t, *J*=12.5 Hz, 1H; 24-H_2); ^{13}C NMR (126 MHz, MeOD): δ=176.2, 175.7, 175.1, 174.1, 172.0, 160.1, 138.0, 128.9, 124.8, 122.8, 120.4, 119.2, 112.9, 111.2, 63.5, 60.1, 59.1, 56.2, 50.2, 49.0, 40.0, 38.9, 38.7, 32.4, 31.4, 29.9, 29.8, 26.0, 24.8, 23.9, 23.3, 21.6, 20.1, 19.8, 19.2, 18.9, 16.6, 12.3 ppm; IR: $\tilde{\nu}$=3272 (w), 2971 (w), 1633 (s), 1547 (m), 1455 (w), 1258 (w), 1087 (w), 880 (w), 803 (w), 744 (w), 674 (w), 662 (w), 631 cm$^-$ (w)[1]; MS: *m/z* (%): 769.3 [*M*+H]$^+$; HR-MS (FAB): *m/z*: calcd for $C_{39}H_{60}N_8O_8$: 769.4607 [*M*+H]$^+$; found: 769.4624; HPLC-ESI: t_R=8.0 min.

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-serine] (57): Colourless powder; yield: 40 mg, 47 μmol, 12%; R_f=0.34 (CH_2Cl_2/MeOH 9:1+0.25% AcOH, *v/v*); m.p.: 204 °C; $[\alpha]_D^{20}=-60$ (*c*=2.9, MeOH); ^{1}H NMR (500 MHz, DMSO) δ=10.93 (s, 1H; NH-indole), 8.52 (d, *J*=9.2 Hz, 1H; NH-Ser), 8.29 (d, *J*=5.2 Hz, 1H; NH-Leu), 7.57 (d, *J*=7.9 Hz, 1H; 25-H), 7.55–7.51 (m, 1H; NHε-Lys), 7.35 (d, *J*=8.1 Hz, 1H; 22-H), 7.08 (t, *J*=7.5 Hz, 1H; 24-H), 7.03 (d, *J*=2.1 Hz, 1H; 13-H), 6.99 (t, *J*=7.4 Hz, 1H; 23-H), 6.61–6.56 (m, 1H; NH-Val), 6.51–6.45 (m, 1H; NH-Lys), 6.32–6.27 (m, 1H; NH-Ile), 4.87–4.80 (m, 1H; 11-H), 4.45–4.36 (m, 1H; Ser-OH), 4.23–4.14 (m, 1H; 8-H), 4.01–3.93 (m, 1H; 23-H), 3.88–3.76 (m, 3H; 2-H, 29-H, 9-H_2), 3.60–3.49 (m, 1H; 6-H_2), 3.46–3.39 (m, 1H; 9-H_2), 3.38–3.31 (m, 1H; 12-H_2) 3.07–2.99 (m, 1H; 12-H_2), 2.87–2.79 (m, 1H; 6-H_2), 2.77 (s, 3H; 21-H_3), 1.89–1.79 (m, 1H; 30-H), 1.77–1.67 (m, 1H; 36-H), 1.63–1.50 (m, 2H; 3-H_2), 1.46–1.23 (m, 5H; 25-H, 5-H_2, 4-H_2, 37-H_2), 1.16–1.04 (m, 2H; 4-H_2, 37-H_2), 1.00–0.78 (m, 7H; 24-H_2, 31-H_3, 38-H_3, 39-H_3), 0.75 (d, *J*=6.8 Hz, 3H; 32-H_3), 0.35 (d, *J*=6.6 Hz, 3H; 26-H_3), 0.09 (d, *J*=6.5 Hz, 3H; 27-H_3), −0.59 ppm (t, *J*=12.2 Hz, 1H; 24-H_2); ^{13}C NMR (126 MHz, MeOD): δ=176.1, 176.0, 175.8, 174.8, 172.8, 172.7, 160.1, 138.1, 128.9, 124.9, 122.9, 120.4, 119.2, 112.9, 111.2, 64.5, 63.7, 60.1, 58.8, 57.4, 56.2, 49.1, 40.0, 38.9, 38.7, 32.2, 31.4, 30.1, 29.6, 26.1, 24.8, 23.9, 23.3, 21.9, 20.1, 19.8, 19.1, 16.5, 12.2 ppm; IR: $\tilde{\nu}$=3307 (w), 2964 (w), 1638 (s), 1543 (m), 1460 (m), 1341 (w), 1233 (w), 1088 (w), 744 (m), 678 cm^{-1} (w); MS: *m/z* (%): 785.2 $[M+H]^+$; HR-MS (FAB): *m/z*: calcd for $C_{42}H_{59}N_8O_9$: 785.4556 $[M+H]^+$; found: 785.4560; HPLC-ESI: t_R=8.2 min.

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-leucyl-L-*N*-methyl-alanyl-L-phenylalanine] (58): Colourless powder; yield: 24 mg, 33 μmol, 7%; R_f=0.29 (CH_2Cl_2/MeOH 9:1+0.25% AcOH, *v/v*); m.p.: 226 °C; $[\alpha]_D^{20}=-67$ (*c*=0.8, MeOH); ^{1}H NMR (500 MHz, DMSO) δ=8.85 (d, *J*=9.1 Hz, 1H; NH-Phe), 8.71 (d, *J*=4.2 Hz, 1H; NH-Leu), 7.41 (d, *J*=8.0 Hz, 1H; NHε-Lys), 7.23–7.04 (m, 5H; 11-H–15-H), 6.89 (d, *J*=5.2 Hz, 1H; NH-Val), 6.53 (d, *J*=8.1 Hz, 1H; NH-Lys), 6.26 (d, *J*=7.9 Hz, 1H; NH-Ile), 5.06–4.98 (m, 1H; 17-H), 4.72–4.63 (m, 1H; 21-H), 4.47–4.39 (m, 1H; 8-H), 3.96–3.82 (m, 3H; 33-H, 2-H, 27-H), 3.66–3.56 (m, 1H; 6-H_2), 3.55–3.47 (m, 1H; 9-H_2), 2.85–2.75 (m, 2H; 6-H_2, 9-H_2), 1.98–1.88 (m, 1H; 28-H), 1.76 (s, 3H; 19-H_3), 1.74–1.67 (m, 1H; 34-H), 1.68–1.61 (m, 1H; 23-H), 1.61–1.52 (m, 3H; 3-H_2, 22-H_2), 1.52–1.42 (m, 1H; 5-H_2), 1.42–1.28 (m, 4H; 35-H_2, 22-H_2, 5-H_2, 4-H_2), 1.27–1.20 (m, 1H; 4-H_2), 1.17–1.06 (m, 1H; 35-H_2), 1.09 (d, *J*=6.7 Hz, 3H; 18-H_3), 1.01 (d, *J*=6.7 Hz, 3H; 29-H_3), 0.95 (d, *J*=6.5 Hz, 3H; 24-H_3), 0.91 (d, *J*=6.6 Hz, 3H; 30-H_3), 0.88 (d, *J*=6.4 Hz, 3H; 25-H_3), 0.86–0.78 ppm (m, 6H; 36-H_3, 37-H_3); ^{13}C NMR (126 MHz, MeOD): δ=176.0, 174.4, 174.3, 174.2, 172.8, 160.2, 139.4, 130.4, 129.9, 127.7, 61.1, 59.1, 56.9, 56.8, 56.4, 49.2, 42.5, 40.2, 39.2, 38.7, 32.6, 31.6, 29.4, 28.6, 26.0, 26.0, 23.4, 23.4, 21.8, 20.6, 19.9, 16.6, 14.4, 12.3 ppm; IR: $\tilde{\nu}$=3307 (w), 2965 (w), 1633 (s), 1548 (m), 1454 (w), 1247 (w), 1086 (m), 1048 (m), 880 (w), 744 (w), 699 (w), 662 (w) cm^{-1}. MS: *m/z* (%): 730.3 $[M+H]^+$; HR-MS (FAB): *m/z*: calcd for $C_{37}H_{59}N_7O_8$ $[M+H]^+$: 730.4498; found: 730.4513; HPLC-ESI: t_R= 8.5 min.

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-leucyl-L-*N*-methyl-serinyl-L-phenylalanine] (59): Colourless powder; yield: 16 mg, 20 μmol, 6%; R_f=0.47 (CH_2Cl_2/MeOH 9:1+0.25% AcOH, *v/v*); m.p.: 219 °C; $[\alpha]_D^{20}=-46$ (*c*=1.3, MeOH); ^{1}H NMR (500 MHz, DMSO): δ=8.80 (d, *J*=9.1 Hz, 1H; NH-Phe), 8.68 (d, *J*=4.8 Hz, 1H; NH-Leu), 7.50 (d, *J*=7.1 Hz, 1H; NHε-Lys), 7.26–7.10 (m, 3H; 12-H, 13-H,14-H), 7.04 (d, *J*=7.2 Hz, 1H; 11-H, 15-H), 6.81 (d, *J*=6.5 Hz, 1H; NH-Val), 6.53 (d, *J*=6.9 Hz, 1H; NH-Lys), 6.30 (d, *J*=8.3 Hz, 1H; NH-Ile), 4.90–4.84 (m, 1H; 17-H), 4.67–4.62 (m, 1H; 21-H), 4.46–4.38 (m, 1H; 8-H), 4.02–3.97 (m, 1H; 33-H), 3.94–3.84 (m, 2H; 2-H, 27-H), 3.71–3.64 (m, 1H; 18-H_2), 3.62–3.56 (m, 1H; 6-H_2), 3.50–3.43 (m, 1H; 18-H_2), 3.42–3.36 (m, 1H; 9-H_2), 2.80 (d, 1H; 6-H_2), 2.72 (t, *J*=13.2 Hz), 1.98–1.90 (m, 1H; 28-H), 1.89–1.82 (m, 1H; 23-H), 1.80 (s, 3H; 19-H_3), 1.77–1.68 (m, 1H; 34-H), 1.64–1.54 (m, 3H; 3-H_2, 22-H_2), 1.48–1.30 (m, 5H; 5-H_2, 4-H_2, 35-H_2), 1.16–1.05 (m, 2H; 35-H_2, 22-H_2), 1.02–0.97 (m, 3H; 29-H_3), 0.94–0.79 ppm (m, 15H; 24-H_3, 25-H_3, 30-H_3, 36-H_3, 37-H_3); ^{13}C NMR (101 MHz, DMSO): δ=174.9, 173.3, 173.1, 171.4, 170.0, 158.2, 139.3, 129.7, 129.1, 126.9, 61.8, 58.9, 58.0, 57.5, 55.3, 54.8, 48.5, 44.4, 39.2, 38.5, 38.1, 32.7, 30.8, 29.2, 27.7, 25.5, 24.9, 24.4, 22.1, 21.1, 20.0, 19.8, 16.7, 12.5 ppm; IR: $\tilde{\nu}$=3264 (w), 2960 (w), 1644 (s), 1541 (m), 1457 (w), 1203 (w), 1050 (s), 1024 (s), 1005 (s), 822 (w), 760 (w), 700 cm^{-1} (w); MS: *m/z* (%): 746.2 $[M+H]^+$; HR-MS (FAB): *m/z*: calcd for $C_{37}H_{60}N_7O_9$ $[M+H]^+$: 746.4447; found: 746.4448; HPLC-ESI: t_R=7.9 min.

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-alanyl-L-*N*-methyl-tryptophyl-L-phenylalanine] (60): Colourless powder; yield: 56 mg, 70 μmol, 16%; R_f=0.28 (CH_2Cl_2/MeOH 9:1+0.25% AcOH, *v/v*); $[\alpha]_D^{20}=-122$ (*c*=1.8, MeOH); m.p.: 197 °C; ^{1}H NMR (500 MHz, MeOD): δ=10.35 (s; NH-indole), 9.29 (d, *J*=7.3 Hz, 1H; NH-Phe), 8.44 (br s, 1H; NH-Ala), 7.92 (d, *J*=7.4 Hz, 1H; NHε-Lys), 7.62 (d, *J*=7.2 Hz), 7.48 (br s, 1H; NH-Val), 7.35 (d, *J*=7.8 Hz), 7.31–7.17 (m, 11-H, 12-H, 13-H, 14-H, 15-H, 5H), 7.15–7.09 (m, 1H; 24-H), 7.08–7.02 (m, 1H; 23-H), 6.96 (s, 1H; 19H), 5.28 (d, *J*=11.2 Hz), 4.74 (d, *J*=9.5 Hz), 4.42–4.31 (m, 1H; 29-H), 4.29–4.20 (m, 1H; 38-H), 4.14–4.04 (m, 1H; 2-H), 3.89–3.78 (m, 1H; 6-H_2), 3.75 (d, *J*=6.1 Hz), 3.49 (d, *J*=13.8 Hz), 3.31–3.25 (m, 1H; 18-H_2), 3.05–2.95 (m, 2H; 18-H_2, 6-H_2), 2.89 (t, *J*=13.4 Hz), 2.11–2.03 (m, 1H; 33-H), 2.00 (s, 3H; 27-H_3), 1.96–1.82 (m, 2H; 39-H, 3-H_2), 1.79–1.70 (m, 1H; 3-H_2), 1.69–1.62 (m, 1H; 5-H_2), 1.61–1.53 (m, 1H; 5-H_2), 1.52–1.37 (m, 2H; 40-H_2, 4-H_2), 1.35–1.15 (m, 2H; 40-H_2, 4-H_2), 1.13–1.03 (m, 3H; 34-H_3), 1.03–0.87 (m, 9H; 38-H_3, 41-H_3, 42-H_3), 0.00 ppm (br s, 3H; 30-H_3); ^{13}C NMR (126 MHz, MeOD): δ=174.7, 174.5, 173.3, 172.8, 171.8, 159.0, 138.4, 137.0, 129.3, 128.7, 127.5, 126.6, 123.7, 121.6, 119.0, 117.9, 111.5, 109.9, 61.6, 59.9, 57.9, 55.8, 55.2, 45.1, 39.2, 38.1, 37.6, 31.5, 30.4, 28.2, 27.7, 24.9, 22.9, 20.7, 19.3, 18.7, 15.4, 14.6, 11.1 ppm; IR: $\tilde{\nu}$=3323 (w), 2972 (w), 1640 (s), 1554 (m), 1454 (w), 1086 (m), 880 (w), 741 (w), 672 cm^{-1} (w); MS: *m/z* (%): 803.2 $[M+H]^+$; HR-MS (FAB): *m/z*: calcd for $C_{42}H_{59}N_8O_8$: 803.4450 $[M+H]^+$; found: 803.4471; HPLC-ESI: t_R=8.1 min.

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-valinyl-L-serinyl-L-*N*-methyl-tryptophyl-L-phenylalanine] (61): Colourless powder; yield: 29 mg, 35 μmol, 8%; R_f=0.30 (CH_2Cl_2/MeOH 9:1+0.25% AcOH, *v/v*); m.p.: 210 °C; $[\alpha]_D^{20}=-89$ (*c*=2.0, MeOH); ^{1}H NMR (500 MHz, DMSO) δ=10.80 (s, 1H; NH-indole), 8.93 (d, *J*=8.8 Hz, 1H; NH-Phe), 8.50 (s, 1H; NH-Ser), 7.66–7.61 (m, 1H; NHε-Lys), 7.53 (d, *J*=7.9 Hz), 7.31 (d, *J*=8.1 Hz, 1H; 22-H), 7.24–7.05 (m, 6H; 11-H–15-H, NH-Val), 7.03 (t, *J*=7.5 Hz, 1H; 24-H), 6.99–6.91 (m, 2H; 23-H, 19-H), 6.53–6.47 (m, 1H; NH-Lys), 6.30–6.24 (m, 1H; NH-Ile), 5.18–5.12 (m, 1H; 17-H), 4.75–4.68 (m, 1H; 8-H), 4.55–4.47 (m, 1H; 29-H), 4.45–4.37 (br s, 1H; OH-Ser), 3.99–3.91 (m, 1H; 38-H), 3.92–3.84 (m, 2H; 32-H, 2-H), 3.68–3.59 (m, 1H; 6-H_2), 3.39–3.31 (m, 1H; 9-H_2), 3.16 (dd, *J*=5.7, 15.3 Hz), 3.01–2.92 (m, 1H; 30-H_2), 2.86–2.72 (m, 3H; 18-H_2, 6-H_2, 9-H_2), 2.35–2.30 (m, 1H; 30-H_2), 1.97–1.88 (m, 1H; 33-H), 1.87 (s, 3H; 27-H_3), 1.77–1.66 (m, 1H; 39-H), 1.65–1.55 (m, 2H; 3-H_2), 1.53–1.32 (m, 4H; 40-H_2, 5-H_2), 1.32–1.22 (m, 1H; 4-H_2), 1.22–1.14 (m, 1H; 4-H_2), 1.13–1.02 (m, 1H; 40-H_2), 0.98 (d, *J*=6.7 Hz, 3H; 34-H_3), 0.89 (d, *J*=6.7 Hz, 3H; 35-H_3), 0.87–0.77 ppm (m, 6H; 41-H_3, 42-H_3); ^{13}C NMR (126 MHz, MeOD): δ=176.4, 175.8, 174.6, 174.5, 174.3, 172.5, 160.1, 139.4, 138.0, 130.4, 129.9, 128.6, 127.8, 125.0, 122.8, 120.2, 119.1, 112.8, 111.1, 63.1, 61.7, 60.9, 58.8, 56.8, 56.4, 53.0, 40.2, 39.3, 38.6, 32.5, 31.7, 29.4, 28.9, 26.1, 24.2, 21.8, 20.3, 19.8, 16.5, 12.2 ppm; IR: $\tilde{\nu}$=3295 (w), 2959 (w), 1636 (s),

1544 (m), 1249 (w), 1083 (w), 741 (m), 700 cm^{-1} (w); MS: *m/z* (%): 820.2 $[M+H]^+$; HR-MS (FAB): *m/z*: calcd for $C_{42}H_{59}N_8O_8$: 819.4400 $[M+H]^+$; found: 819.4402; HPLC-ESI: $t_R = 8.2$ min.

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-alanyl-L-leucyl-L-*N*-methyl-tryptophyl-L-phenylalanine] (62): Colourless powder; yield: 47 mg, 58 μmol, 13%; $R_f = 0.22$ (CH_2Cl_2/MeOH 9:1 + 0.25% AcOH, *v/v*); m.p.: 203 °C; $[\alpha]_D^{20} = -118$ (*c* = 2.0, MeOH); ^{1}H NMR (500 MHz, DMSO): $\delta = 10.87$ (s, 1H; NH-indole), 8.71 (d, $J = 8.8$ Hz, 1H; NH-Phe), 8.45 (d, $J = 4.9$ Hz, 1H; NH-Leu), 7.56 (d, $J = 7.7$ Hz, 1 ArH, 22-H), 7.48 (d, $J = 7.4$ Hz, 1H; NHε-Lys), 7.32 (d, $J = 7.9$ Hz, 1 ArH, 25-H), 7.27–7.21 (m, 2 ArH, 12-H,14-H), 7.20–7.15 (m, 1 ArH, 13-H), 7.12–7.03 (m, 3H; 11-H, 15-H, NH-Val), 6.99–6.94 (m, 2 ArH, 23-H, 19-H), 6.44 (d, $J = 6.2$ Hz, 1H; NHα-Lys), 6.20 (d, $J = 8.3$ Hz, 1H; NHα-Ile), 4.81 (d, $J = 10.5$ Hz, 1H; 17-H), 4.56–4.48 (m, 1H; 8-H), 4.24 (br s, 1H; 29-H), 4.09–3.99 (m, 1H; 35-H), 3.96–3.83 (m, 2H; 39-H, 2-H), 3.67–3.54 (m, 1H; 6-H_2), 3.37–3.35 (m, 1H; 9-H_2), 3.14 (d, 1H; 19-H_2), 2.93–2.79 (m, 2H; 6-H_2, 19-H_2), 2.71 (t, 1H; 9-H_2), 1.96 (s, 3H; 27-H_3), 1.76–1.67 (m, 1H; 40-H), 1.61–1.50 (m, 2H; 3-H_2), 1.48–1.33 (m, 4H; 5-H_2, 41-H_2, 31-H), 1.31–1.16 (m, 2H; 4-H_2) 1.25 (d, 3H; 36-H_3, $J = 6.9$ Hz), 1.15–1.02 (m, 1H; 41-H_2), 0.94 (t, $J = 12.5$ Hz), 0.88–0.76 (m, 6H; 42-H_3, 43-H_3), 0.36 (d, $J = 6.4$ Hz, 3-H, 32-H_3), 0.11 (d, $J = 6.4$ Hz, 3H; 33-H_3), −0.58 ppm (t, $J = 11.6$ Hz, 1H; 30-H_2); ^{13}C NMR (126 MHz, MeOD): $\delta = 175.9$, 175.8, 175.7, 175.6, 174.3, 172.6, 160.3, 139.3, 137.9, 130.3, 130.0, 128.8, 127.7, 124.7, 122.8, 120.3, 119.2, 112.9, 111.1, 63.2, 59.2, 57.1, 56.0, 50.8, 48.9, 39.9, 39.6, 39.4, 38.5, 32.7, 29.4, 29.0, 26.0, 25.0, 23.5, 23.3, 21.5, 20.2, 17.3, 16.6, 12.3 ppm; IR: $\tilde{\nu} = 3307$ (w), 2971 (w), 1636 (s), 1551 (m), 1454 (w), 1086 (w), 879 (w), 741 (w), 699 (w), 662 cm^{-1} (w); MS: *m/z* (%): 817.2 $[M+H]^+$; HR-MS (FAB): *m/z*: calcd for $C_{43}H_{61}N_8O_8$: 817.4607 $[M+H]^+$; found: 817.4627; HPLC-ESI: $t_R = 8.3$ min.

1,5-Anhydro[D-lysyl-(N^{α}-oxamido-L-isoleucyl)-L-serinyl-L-leucyl-L-*N*-methyl-tryptophyl-L-phenylalanine] (63): Colourless powder; yield: 45 mg, 54 μmol, 12%; $R_f = 0.25$ (CH_2Cl_2/MeOH 9:1 + 0.25% AcOH, *v/v*); m.p.: 221 °C; $[\alpha]_D^{20} = -89$ (*c* = 2.2, MeOH); ^{1}H NMR (500 MHz, DMSO) $\delta = 10.86$ (s, 1H; NH-indole), 8.54 (m, 2H; NH-Phe, NH-Leu), 7.55 (d, $J = 7.7$ Hz, 1H; 22-H), 7.40 (d, $J = 8.0$ Hz, 1H; NHε-Lys), 7.32 (d, $J = 8.1$ Hz), 7.27–7.15 (m, 3H; 12-H, 14-H, 13-H), 7.09–7.02 (m, 3H; 11-H, 15-H, 24-H), 6.99–6.93 (m, 2H; 23-H, 19-H), 6.56 (d, $J = 6.1$ Hz, 1H; NH-Lys), 5.96 (br s, 1H; NH-Ile), 4.80–4.73 (m, 1H; 17-H), 4.58–4.50 (m, 1H; 8-H), 4.27–4.19 (m, 1H; 29-H), 4.04–3.97 (m, 1H; 35-H), 3.94–3.88 (m, 1H; 2-H), 3.86–3.78 (m, 1H; 39-H), 3.67–3.57 (m, 2H; 6-H_2, 36-H_2), 3.30–3.28 (m, 1H; 9-H_2), 3.14 (dd, $J = 2.9$, 14.7 Hz), 3.01–2.95 (m, 1H; 36-H_2), 2.89–2.80 (m, 2H; 6-H_2, 18-H_2), 2.68–2.61 (m, 1H; 9-H_2), 1.92 (s, 3H; 27-H_3), 1.81–1.72 (m, 1H; 40-H), 1.71–1.58 (m, 2H; 3-H_2), 1.58–1.40 (m, 3H; 5-H_2, 4-H_2), 1.39–1.33 (m, 1H; 41-H_2), 1.32–1.26 (m, 1H; 30-H_2), 1.26–1.15 (m, 1H; 4-H_2), 1.14–1.00 (m, 1H; 41-H_2), 0.99–0.87 (m, 1H; 30-H_2), 0.87–0.78 (m, 6H; 42-H_3, 43-H_3), 0.35 (d, 32-H_3, $J = 6.6$ Hz), 0.12 (d, $J = 6.6$ Hz), −0.59 ppm (d, $J = 21.7$ Hz); ^{13}C NMR (126 MHz, MeOD): $\delta = 176.4$, 175.8, 174.6, 174.5, 174.3, 172.5, 160.1, 139.4, 138.0, 130.4, 129.9, 128.6, 127.8, 125.0, 122.8, 120.2, 119.1, 112.8, 111.1, 63.1, 61.7, 60.9, 58.8, 56.8, 56.4, 53.0, 40.2, 39.3, 38.6, 32.5, 31.7, 29.4, 28.9, 26.1, 24.2, 21.8, 20.3, 19.8, 16.5, 12.2 ppm; IR: $\tilde{\nu} = 3295$ (w), 2932 (w), 1634 (s), 1537 (m), 1456 (m), 1226 (w), 1086 (w), 743 (m), 699 cm^{-1} (w); MS: *m/z* (%): 833.2 $[M+H]^+$; HR-MS (FAB): calcd $C_{43}H_{61}N_8O_9$: 833.4556 $[M+H]^+$; found: 833.4558; HPLC-ESI: $t_R = 8.6$ min.

Carboxypeptidase A inhibition assay: Bovine pancreatic CPD (Sigma) was dissolved at a concentration of 7 nM in Tris-HCl (1.0 M)/NaCl (0.5 M) buffer. Kinetic analysis at 37 °C with the substrate *N*-(4-methoxyphenylazoformyl)-Phe-OH at pH 7.5 in Tris-HCl (1.0 M)/NaCl (0.5 M)/NP-40 (0.025%)/DMSO (1%) and monitoring of the decrease in the AAF-Phe-OH absorption at 355 nm gave K_M and K_{cat} values of 272 (±49) μM and 5 s^{-1} ($K_{cat}/K_M = 18000$).

Carboxypeptidase B inhibition assay: Porcine pancreatic CPD (Sigma) was dissolved at a concentration of 7 nM in Tris-HCl (1.0 M)/NaCl (0.5 M) buffer. Kinetic analysis at 37 °C with the substrate *N*-(4-methoxyphenylazoformyl)-Arg-OH at pH 7.5 in Tris-HCl (1.0 M)/NaCl (0.5 M)/NP-40 (0.025%)/DMSO (1%) and monitoring of the decrease in the AAF-Arg-OH (Anisylazoformylarginine) absorption at 355 nm gave K_M and k_{cat} values of 203 (±20) μM and 5 s^{-1} ($K_{cat}/K_M = 24000$).

IC_{50} determination: The reaction volume was 100 μL, and the final substrate concentration was 150 μM (ca. 0.6 K_M). The reaction was started by the addition of AAF-Phe-OH (10 μL) to a solution (90 μL) containing the CPD, which had been incubated for 15 min with ten different concentrations from a twofold dilution series of inhibitors. Reaction rates were determined from the slope of the absorbance change at 355 nm and related to control values in absence of the inhibitor. IC_{50} values were measured in triplicate and calculated from linear extrapolations of reaction velocity as a function of the logarithm of concentration.

Computational studies: Conformations for the macrocycles were generated with the low mode conformational search method (LMOD) in Macromodel 9.1[37] with use of the MMFF forcefield[38] and GB/SA solvation; 35000 conformations were generated. An RMSD threshold of 0.25 Å for all ring atoms and the first atoms of the sidechains was used to identify unique conformations. All conformations with an energy of more than +50 kJ mol^{-1} relative to the global minimum were discarded. The remaining conformations were energy-minimised by use of the truncated Newton conjugated gradient (TNCG) option. The line search parameter was set from 1 to 0 to choose the direction of minimisation steps; default values were used for the remaining parameters. Convergence (RMSD gradient = 0.05 kJ mol^{-1} $Å^{-1}$) was reached after a maximum of 10000 iterations. Ligands were docked by use of GOLD version 4.0,[39] with the chemscore scoring function. The binding site was defined by a 20 Å sphere around the carbon in the carboxylic acid moiety of Glu72, coordinating the active site zinc atom. The genetic algorithm settings were set to "automatic" with an "autoscale factor" of 1. Ten solutions per input conformation were generated from Macromodel. For the NP **1**, the best-scoring docking pose was selected out of the conformers within 10 kJ mol^{-1} of the global minimum.

Abbreviations: $[\alpha]_D^{20}$: optical rotation at 20 °C and 589 nm; AAF-Arg-OH: *N*-(4-methoxyphenylazoformyl)phenylarginine; AAF-Phe-OH: *N*-(4-methoxyphenylazoformyl)phenylalanine; All: allyl; Aq.: aqueous (solution); BVA(s): brunsvicamide(s); Cbz: benzyloxycarbonyl; CPD: carboxypeptidase; CSM(s): cyanobacterial secondary metabolite(s); DIC: *N,N'*-diisopropyl carbodiimide; DiFUMP: difluoroumbelliferyl phosphate; DMF: *N,N'*-dimethylformamide; DMSO: dimethyl sulfoxide; Fmoc: fluorenylmethoxycarbonyl; GB/SA: generalised Born equation solvation solvent-accessible surface area; HATU: 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAt: 1-hydroxy-7-azabenzotriazole; HOBt: *N*-hydroxybenzotriazole; ESI: electrospray ionisation; HR-MS (FAB): high-resolution mass spectrometry (fast atom bombardment); K_{cat}: turnover number; K_M: Michaelis–Menten constant; LMOD: low mode conformational search method; MMFF: Merck molecular force field; MptpB: *M. tuberculosis* tyrosine phosphatase B; NP(s): natural product(s); NP-40: nonionic detergent; PDB: Protein Data Bank; pNPP: *p*-nitrophenol phosphate; RFID: radio frequency identification; RMSD: root mean square deviation; SPPS:

solid-phase peptide synthesis; TES: triethylsilyl; TFA: trifluoroacetic acid; TRIS: 2-amino-2-hydroxymethyl-propane-1,3-diol; TNCG: truncated Newton conjugated gradient; V_{max}: maximum velocity.

Acknowledgements

Funding by the Fonds der Chemischen Industrie, the Max-Planck-Society (to H.W.), and the Deutsche Forschungsgemeinschaft (Emmy-Noether young-investigator grant to H.-D.A.) was highly appreciated. This work was supported in part by the EU and the state of Nordrhein-Westfalen (ZACG Dortmund). T.W. acknowledges IMPRS Chemical Biology for financial support and thanks M. Riedrich for sharing freshly synthesised $Pd(PPh_3)_4$.

Keywords: combinatorial chemistry · inhibitors · natural products · peptidases · solid-phase synthesis

[1] R. Breinbauer, I. R. Vetter, H. Waldmann, *Angew. Chem.* **2002**, *114*, 3002–3015; *Angew. Chem. Int. Ed.* **2002**, *41*, 2878–2890.

[2] a) M. A. Koch, H. Waldmann, *Drug Discovery Today* **2005**, *10*, 471–483; b) A. Nören-Müller, I. Reis-Correa, Jr., H. Prinz, C. Rosenbaum, K. Saxena, H. J. Schwalbe, D. Vestweber, G. Cagna, S. Schunk, O. Schwarz, H. Schiewe, H. Waldmann, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10606–10611; c) M. Kaiser, S. Wetzel, K. Kumar, H. Waldmann, *Cell. Mol. Life Sci.* **2008**, *65*, 1186–1201.

[3] For syntheses of NP-inspired and -derived compound collections from our laboratories see: a) B. Meseguer, D. Alonso-Diaz, N. Griebenow, T. Herget, H. Waldmann, *Angew. Chem.* **1999**, *111*, 3083–3087; *Angew. Chem. Int. Ed.* **1999**, *38*, 2902–2906; b) D. Brohm, S. Metzger, A. Bhargava, O. Müller, F. Lieb, H. Waldmann, *Angew. Chem.* **2002**, *114*, 319–323; *Angew. Chem. Int. Ed.* **2002**, *41*, 307–311; c) D. Brohm, N. Philippe, S. Metzger, A. Bhargava, O. Müller, F. Lieb, H. Waldmann, *J. Am. Chem. Soc.* **2002**, *124*, 13171–13178; d) O. Barun, S. Sommer, H. Waldmann, *Angew. Chem.* **2004**, *116*, 3258–3261; *Angew. Chem. Int. Ed.* **2004**, *43*, 3195–3199; e) S. Sommer, H. Waldmann, *Chem. Commun.* **2005**, 5684–5686; f) M. A. Sanz, T. Voigt, H. Waldmann, *Adv. Synth. Catal.* **2006**, *348*, 1511–1515; g) A. B. Garcia, T. Leßmann, J. D. Umarye, V. Mamane, S. Sommer, H. Waldmann, *Chem. Commun.* **2006**, 3868–3870; h) J. D. Umarye, T. Leßmann, A. B. Garcia, V. Mamane, S. Sommer, H. Waldmann, *Chem. Eur. J.* **2007**, *13*, 3305–3319; i) T. Leßmann, M. G. Leuenberger, S. Menninger, M. Lopez-Canet, O. Müller, S. Hümmer, J. Bormann, K. Korn, E. Fava, M. Zerial, T. U. Mayer, H. Waldmann, *Chem. Biol.* **2007**, *14*, 443–451; j) V. Mamane, A. B. Garcia, J. D. Umarye, T. Leßmann, S. Sommer, H. Waldmann, *Tetrahedron* **2007**, *63*, 5754–5767; k) S. Broussy, H. Waldmann, *J. Comb. Chem.* **2007**, *9*, 1138–1143; l) T.-S. Hu, R. Tannert, H.-D. Arndt, H. Waldmann, *Chem. Commun.* **2007**, 3942–3944; m) M. Scheck, M. A. Koch, H. Waldmann, *Tetrahedron* **2008**, *64*, 4792–4802; n) F. Wehner, A. Nören-Müller, O. Müller, I. Reis-Correa, Jr., A. Giannis, H. Waldmann, *ChemBioChem* **2008**, *9*, 401–405; o) H. Waldmann, V. Khedkar, H. Dückert, M. Schürmann, I. M. Oppel, K. Kumar, *Angew. Chem.* **2008**, *120*, 6975–6978; *Angew. Chem. Int. Ed.* **2008**, *47*, 6869–6872; p) S. Sommer, M. Kühn, H. Waldmann, *Adv. Synth. Catal.* **2008**, *350*, 1736–1750; q) N. Bisek, S. Wetzel, H.-D. Arndt, H. Waldmann, *Chem. Eur. J.* **2008**, *14*, 8847–8860.

[4] a) D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2007**, *70*, 461–477; b) D. J. Newman, *J. Med. Chem.* **2008**, *51*, 2589–2599.

[5] a) N. G. Carr, *Botanical Monographs (Oxford)* **1973**, *9*, 39–65; b) D. A. Bryant, *The Molecular Biology of Cyanobacteria*, Kluwer, Dordrecht, **1994**.

[6] a) M. Welker, H. von Doehren, *FEMS Microbiol. Rev.* **2006**, *30*, 530–563; b) K. Gademann, C. Portmann, *Curr. Org. Chem.* **2008**, *12*, 326–341.

[7] L. T. Tan, *Phytochemistry* **2007**, *68*, 954–979.

[8] a) E. Dittmann, B. A. Neilan, T. Börner, *Appl. Microbiol. Biotechnol.* **2001**, *57*, 467–473; b) B. S. Moore, *Nat. Prod. Rep.* **2005**, *22*, 580–593.

[9] a) E. W. Schmidt, J. T. Nelson, D. A. Rasko, S. Sudek, J. A. Eisen, M. G. Haygood, J. Ravel, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7315–7320; b) S. Sudek, M. G. Haygood, D. T. Youssef, E. W. Schmidt, *Appl. Environ. Microbiol.* **2006**, *72*, 4382–4387; c) N. Ziemert, K. Ishida, P. Quillardet, C. Bouchier, C. Hertweck, N. T. de Marsac, E. Dittmann, *Appl. Environ. Microbiol.* **2008**, *74*, 1791–1797; d) M. S. Donia, J. Ravel, E. W. Schmidt, *Nat. Chem. Biol.* **2008**, *4*, 341–343; e) N. Ziemert, K. Ishida, A. Liaimer, C. Hertweck, E. Dittmann, *Angew. Chem.* **2008**, *120*, 7870–7873; *Angew. Chem. Int. Ed.* **2008**, *47*, 7756–7759.

[10] D. Müller, A. Krick, S. Kehraus, C. Mehner, M. Hart, F. C. Küpper, K. Saxena, H. Prinz, H. Schwalbe, P. Janning, H. Waldmann, G. M. König, *J. Med. Chem.* **2006**, *49*, 4871–4878.

[11] a) K. Harada, K. Fujii, T. Shimada, M. Suzuki, H. Sano, K. Adachi, W. W. Carmichael, *Tetrahedron Lett.* **1995**, *36*, 1511–1514; b) D. E. Williams, M. Craig, C. F. B. Holmes, R. J. Andersen, *J. Nat. Prod.* **1996**, *59*, 570–575; c) K. Fujii, K. Sivonen, K. Adachi, K. Noguchi, H. Sano, K. Hirayama, M. Suzuki, K.-I. Harada, *Tetrahedron Lett.* **1997**, *38*, 5525–5528; d) E. W. Schmidt, M. K. Harper, D. J. Faulkner, *J. Nat. Prod.* **1997**, *60*, 779–782; e) H. J. Shin, H. Matsuda, M. Murakami, K. Yamaguchi, *J. Nat. Prod.* **1997**, *60*, 139–141; f) H. Uemoto, Y. Yahiro, H. Shigemori, M. Tsuda, T. Takao, Y. Shimonishi, J. i. Kobayashi, *Tetrahedron* **1998**, *54*, 6719–6724; g) M. Murakami, S. Suzuki, Y. Itou, S. Kodani, K. Ishida, *J. Nat. Prod.* **2000**, *63*, 1280–1282; h) V. Reshef, S. Carmeli, *J. Nat. Prod.* **2002**, *65*, 1187–1189; i) D. Beresovsky, O. Hadas, A. Livne, A. Sukenik, A. Kaplan, S. Carmeli, *Isr. J. Chem.* **2006**, *46*, 79–87; j) S. J. Robinson, K. Tenney, D. F. Yee, L. Martinez, J. E. Media, F. A. Valeriote, R. W. M. van Soest, P. Crews, *J. Nat. Prod.* **2007**, *70*, 1002–1009; k) S. Matthew, C. Ross, V. J. Paul, H. Luesch, *Tetrahedron* **2008**, *64*, 4081–4089; l) O. Grach-Pogrebinsky, S. Carmeli, *Tetrahedron* **2008**, *64*, 10233–10238.

[12] a) K. Burgess, D. S. Linthicum, H. Shin, *Angew. Chem.* **1995**, *107*, 975–977; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 907–909; b) J. Vagner, H. Qu, V. J. Hruby, *Curr. Opin. Chem. Biol.* **2008**, *12*, 292–296.

[13] T. Sano, T. Usui, K. Ueda, H. Osada, K. Kaya, *J. Nat. Prod.* **2001**, *64*, 1052–1055.

[14] a) Y. Itou, S. Suzuki, K. Ishida, M. Murakami, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1243–1246; b) P. Bjoerquist, M. Buchanan, M. Campitelli, A. Carroll, E. Hyde, J. Neve, M. Polla, R. Quinn (AstraZeneca AB, Sweden) WO 200503961, **2005**.

[15] D. W. Christianson, W. N. Lipscomb, *Acc. Chem. Res.* **1989**, *22*, 62–69.

[16] a) W. N. Lipscomb, N. Straeter, *Chem. Rev.* **1996**, *96*, 2375–2433; b) J. Vendrell, E. Querol, F. X. Aviles, *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol.* **2000**, *1477*, 284–298; c) D. H. Kim, *Mini-Rev. Med. Chem.* **2001**, *1*, 155–161.

[17] D. Müller, PhD Thesis, Universität Bonn (Germany), **2005**.

[18] D. Barford, A. K. Das, M.-P. Egloff, *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 133–164.

[19] T. Walther, H.-D. Arndt, H. Waldmann, *Org. Lett.* **2008**, *10*, 3199–3202.

[20] D. F. McCain, Z.-Y. Zhang, *Methods Enzymol.* **2002**, *345*, 507–518.

[21] J. Montalibet, K. I. Skorey, B. P. Kennedy, *Methods* **2005**, *35*, 2–8.

[22] a) A. Kilshtain-Vardi, M. Glick, H. M. Greenblatt, A. Goldblum, G. Shoham, *Acta Crystallogr. Sect. A* **2003**, *59*, 323–333; b) M. Adler, B. Buckman, J. Bryant, Z. Chang, K. Chu, K. Emayan, P. Hrvatin, I. Islam, J. Morser, D. Sukovich, C. West, S. Yuan, M. Whitlow, *Acta Crystallogr. Sect. A* **2008**, *64*, 149–157.

[23] W. L. Mock, Y. Liu, D. J. Stanford, *Anal. Biochem.* **1996**, *239*, 218–222.

[24] G. R. Marshall, *Tetrahedron* **1993**, *49*, 3547–3558.

[25] A. Boeijen, R. M. J. Liskamp, *Eur. J. Org. Chem.* **1999**, 2127–2135.

[26] J. A. W. Kruijtzer, D. J. Lefeber, R. M. J. Liskamp, *Tetrahedron Lett.* **1997**, *38*, 5335–5338.

[27] M. Schelhaas, H. Waldmann, *Angew. Chem.* **1996**, *108*, 2192–2219; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2056–2083.

[28] I. Coin, M. Beyermann, M. Bienert, *Nat. Protoc.* **2007**, *2*, 3247–3256.

[29] http://www.irori.com.

[30] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970**, *34*, 595–598.

[31] a) H. Kunz, H. Waldmann, *Angew. Chem.* **1984**, *96*, 49–50; *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 71–72; *Angew. Chem.* **1984**, *96*, 49–50; b) D. R. Coulson, *Inorg. Synth.* **1990**, *28*, 107–109; c) M. Honda, H. Morita, I. Nagakura, *J. Org. Chem.* **1997**, *62*, 8932–8936.

[32] L. Michaelis, M. L. Menten, *Biochem. Z.* **1913**, *49*, 333–369.

[33] R. A. Copeland, *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis*, 2nd ed., Wiley, New York, **2000**.

[34] H. Waldmann, T.-S. Hu, S. Renner, S. Menninger, R. Tannert, T. Oda, H.-D. Arndt, *Angew. Chem.* **2008**, *120*, 6573–6577; *Angew. Chem. Int. Ed.* **2008**, *47*, 6473–6477.
[35] H. Kim, W. N. Lipscomb, *Biochemistry* **1991**, *30*, 8171–8180.
[36] N. Abramowitz, I. Schechter, A. Berger, *Biochem. Biophys. Res. Commun.* **1967**, *29*, 862–867.
[37] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467.
[38] T. A. Halgren, *J. Comput. Chem.* **1996**, *17*, 490–519.
[39] G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol.* **1997**, *267*, 727–748.

Received: January 21, 2009
Published online on April 9, 2009

