HETEROCYCLES, Vol. 67, No. 1, 2006, pp. 135 - 160. © The Japan Institute of Heterocyclic Chemistry
Received, 2nd March, 2005, Accepted, 15th April, 2005, Published online, 19th April, 2005. COM-05-S(T)6

SYNTHESIS OF SUBSTITUTED PYRIMIDINE HYDRAZINE ACIDS (PHA) AND THEIR USE IN PEPTIDE RECOGNITION

Stefan Miltschitzky, Veronika Michlova, Stefan Stadlbauer, and Burkhard Koenig*

Department of Organic Chemistry, University of Regensburg, Universitaetsstrasse 31, 93051 Regensburg, Germany

Dedicated to Prof. Barry Trost on the occasion of his 65th birthday

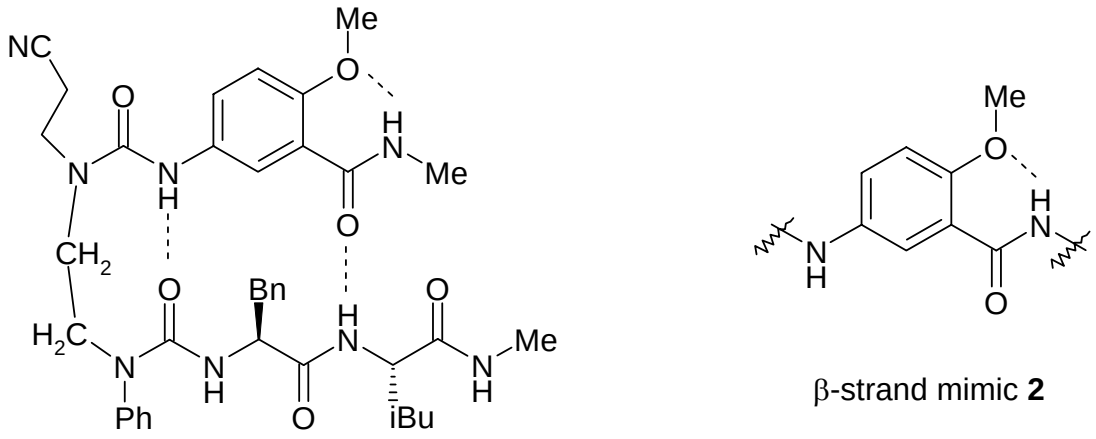
Abstract - Substituted pyrimidine-hydrazine-acids (PHA) were prepared from orotic acid in five synthetic steps and high yields. Their geometry of hydrogen bond acceptor and donor sites make them suitable for the molecular recognition of peptide β-sheets. In non-protic solvents the PHA unit emits at around 420 nm after irradiation at 281 nm. The emission intensity decreases upon peptide binding and signals the binding event. Peptides consisting of PHAs and natural amino acids or a turn structure motif were prepared. The investigation of the intramolecular binding pattern by NMR spectroscopy revealed the expected interaction of the PHA and peptide β-sheet.

Introduction

Interactions between peptide β-sheets are of importance for protein quaternary structure formation, protein-protein interaction, and protein aggregation. They are involved in important biological processes and the development of diseases - ranging from cancer and AIDS, to anthrax and Alzheimer´s.[1,2] Small synthetic molecules that mimic surface epitopes on proteins are a potential source of novel ligands for use in drug and vaccine design.[3] Over the last years peptidomimetics have attracted interest from both organic and medicinal chemists. In the biological, chemical, and pharmaceutical areas they offer advantages over physiologically active peptides, which as active substances are crucial for the organism and may lead to severe side effects.[4] There is much current interest in the preparation of peptides and peptidomimetics

with a well-defined conformation.[5] These conformationally constrained peptides are useful to probe protein folding and to mimic peptide structures.

Since 1992, Nowick *et al.* have reported the development of peptidomimetic templates, which have been termed molecular scaffolds.[6] These templates have oligourea structure and reassemble, in some ways, β-turns. Early in 1996, Nowick described an artificial antiparallel β-sheet **(1**), in which a 5-amino-2-methoxybenzamide β-strand mimic **(2**) and a diurea molecular scaffold stabilize a β-sheet structure in an attached peptide strand (Scheme 1).[7]



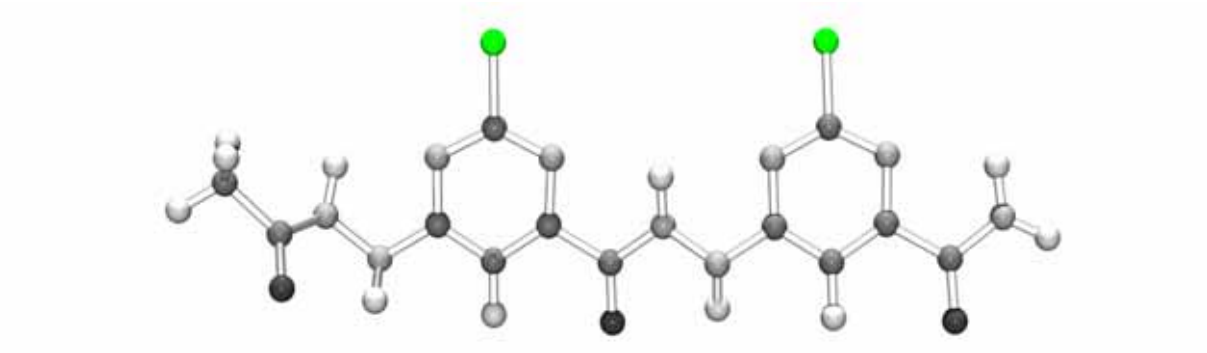
Scheme 1: Nowick´s artificial antiparallel β-sheet (**1**) and β-strand mimic (**2**)

Results and Discussions

We report in this paper the synthesis of substituted pyrimidine-hydrazine-acids (PHA), and their inter- and intramolecular peptide binding properties as determined by NMR spectra and emission changes.

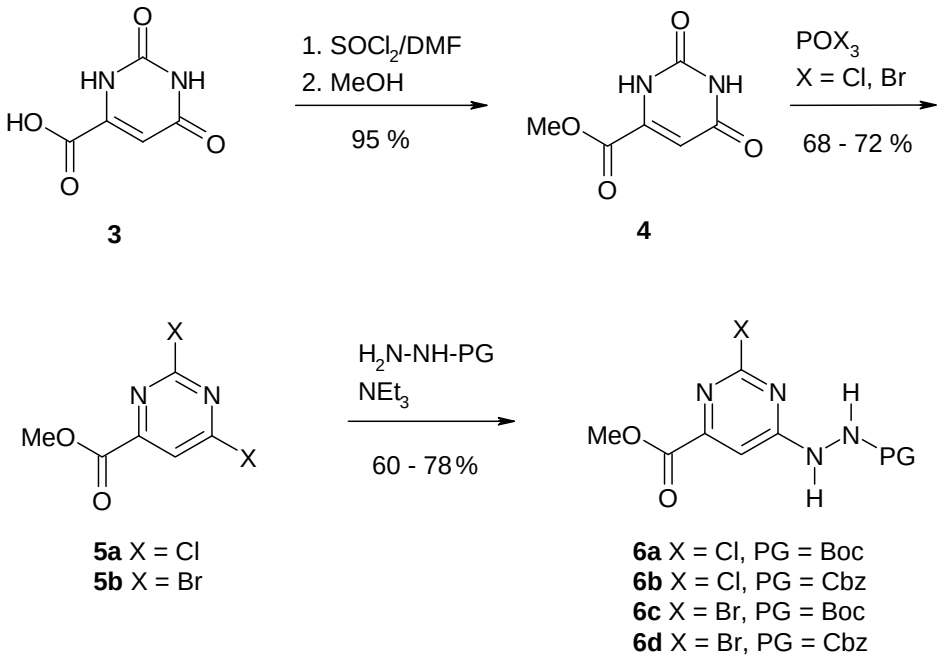
We envisioned 6-hydrazinopyrimidine-4-carboxylic acids as a building block for extended β-strand mimics. Molecular modelling calculations on force field level (MMFF, program package Spartan)[8] were carried out to explore the conformational preferences of PHA. The calculations show that oligomers of PHA adopt a linear conformation required to be complementary to peptide β-sheets.

The hydrazine provides the right pattern of hydrogen bond donor and acceptor geometry complementary to extended peptide β-sheets and allows the formation of an intramolecular hydrogen bond. This should keep the pyrimidine ring of PHA oligoamides in a planar arrangement.



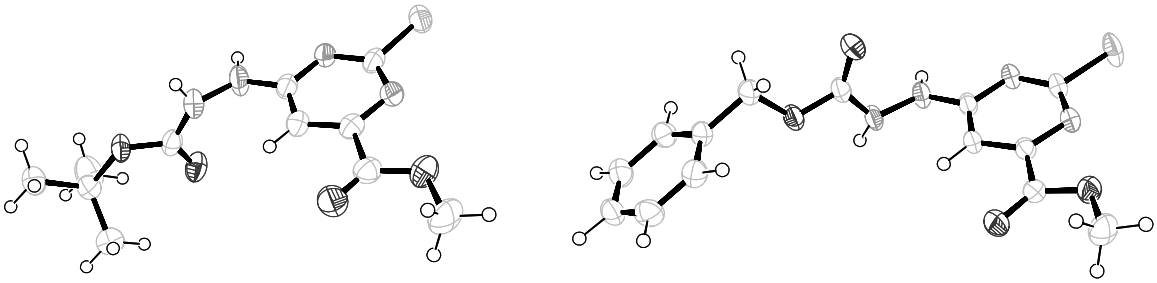
Scheme 2: Low energy conformation of a PHA dimmer as calculated by force field method

Starting material for PHA derivatives is the commercial available orotic acid **(3)**. The acid is converted into the ester with methanol according to a literature procedure,[9] followed by a halogenation with $POCl_3$[9] or $POBr_3$, respectively. The substitution of a halogen atom by Boc- or Cbz-monoprotected hydrazine takes place selectively in 6-position. The yields of the substitution reaction are typically between 60 and 95 %.



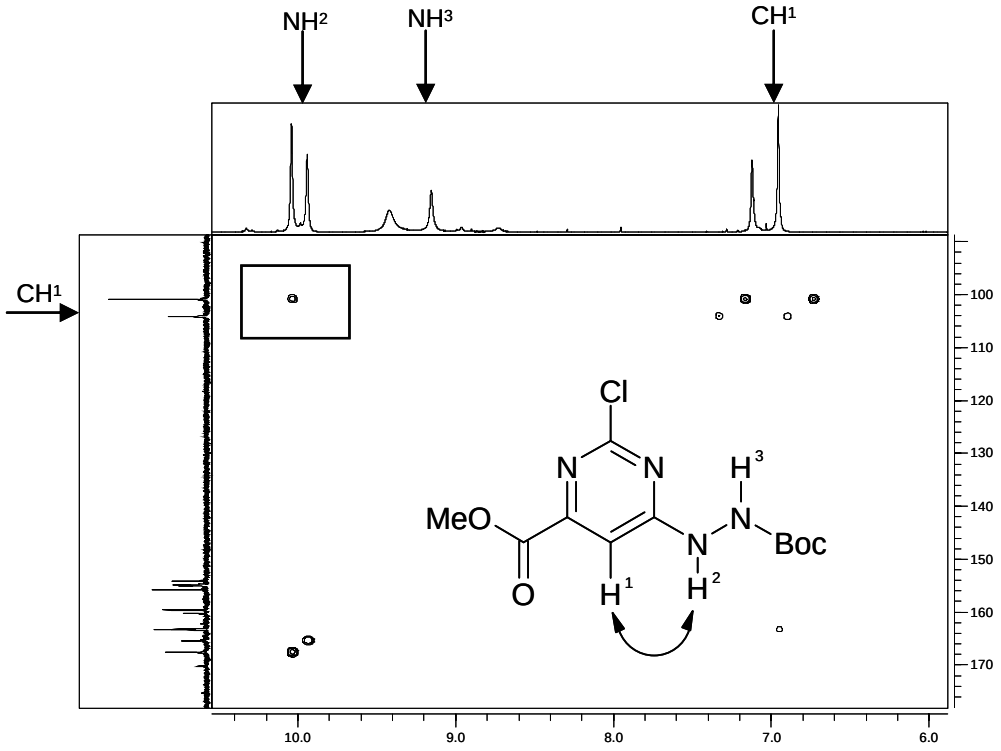
Scheme 3: Synthesis of the PHA parent structure

The selective substitution of the chlorine and bromine was investigated by Yamanaka *et al.* in detail.[10] Computational and experimental studies both showed a dependency on the substituent in 4-position. Acceptors, such as the methyl ester guide substitution in 6-position, while a methoxy groups yields selective substitution in 2-position. The X-Ray structure analysis of **6a** and **6b** and the HMBC spectrum of **6a** confirm the expected substitution pattern.



Scheme 4: Structures of **6a** (left) and **6b** (right) in the crystal

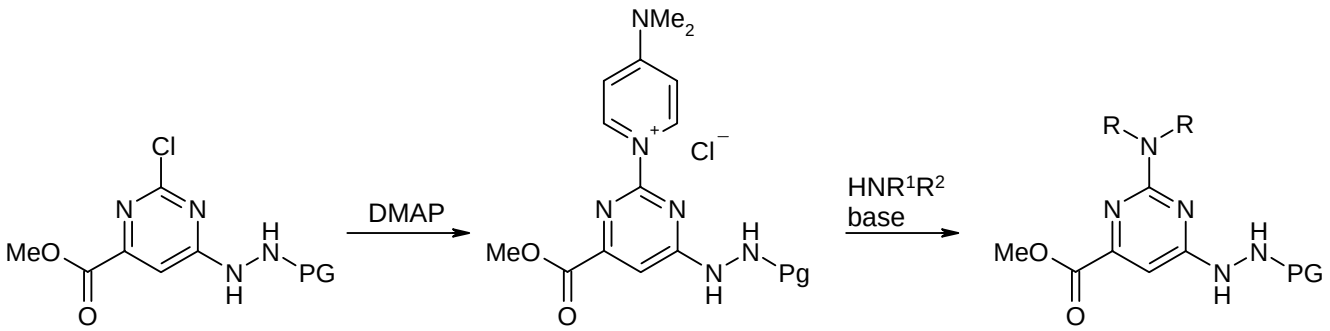
The HMBC spectra of an 8 mM solution in deuterated DMSO at room temperature of **6a** shows a sharp cross peak between the protons on hydrazine nitrogen NX and on carbon CH1, supporting the structure of the expected compound. The double set of signals arises from the *cis*- and *trans*-isomers of the Boc protecting group.[11]



Scheme 5: HMBC-spectrum of **6a** in deuterated DMSO.

However, the X-Ray structure of **6a** does not show the assumed intramolecular hydrogen bond of the proton on hydrazine nitrogen and the pyrimidin nitrogen. The temperature dependence of the ^{1}H NMR chemical shift of the NH^2 proton (-5.15 ppb/K) in 10 mmol solution $CDCl_3$ reflects its state of hydrogen bonding.

The course of the subsequent substitution in 2-position with *N*-nucleophiles depends on the halogen atom. Chlorine substitution occurs only when activated with DMAP *in situ*.



Scheme 6: Proposed mechanism of the substitution of 2-chlorine atoms by *N*-nucleophiles

The DMAP displaces the chlorine atom as a nucleophile forming the charged intermediate (**7**). The amine attacks in the subsequent step the more electrophilic carbon C2 under basic conditions. The amine is nucleophile and base, but an excess of 10 equivalents is used. In case of NEt_3 as an auxiliary base, still 5 equivalents of the nucleophile are required. Although the second step of the reaction releases the DMAP, stoichiometric amounts are required to achieve good yields. The highest yields were obtained in the presence of 1.2 equivalents of DMAP, 2 equivalents of NEt_3 and 8 equivalents of amine (see EXPERIMENTAL). The bromine derivative is, as expected, more reactive than the chlorine substituted compound. No activation or addition of base is required for the substitution. In the presence of DMAP and NEt_3 however, complete conversion requires a 5-fold excess of amine. Best yields were obtained by using 2 equivalents of NEt_3 and a 10-fold excess of amine (see EXPERIMENTAL). Table 1 summarizes the different compounds either derived from **6a** (method A) or **6c** (method B). In general, the more nucleophilic secondary amines give higher yields than primary amines. The hydrazine protecting group (PG) does not influence the yield of the substitution reaction.

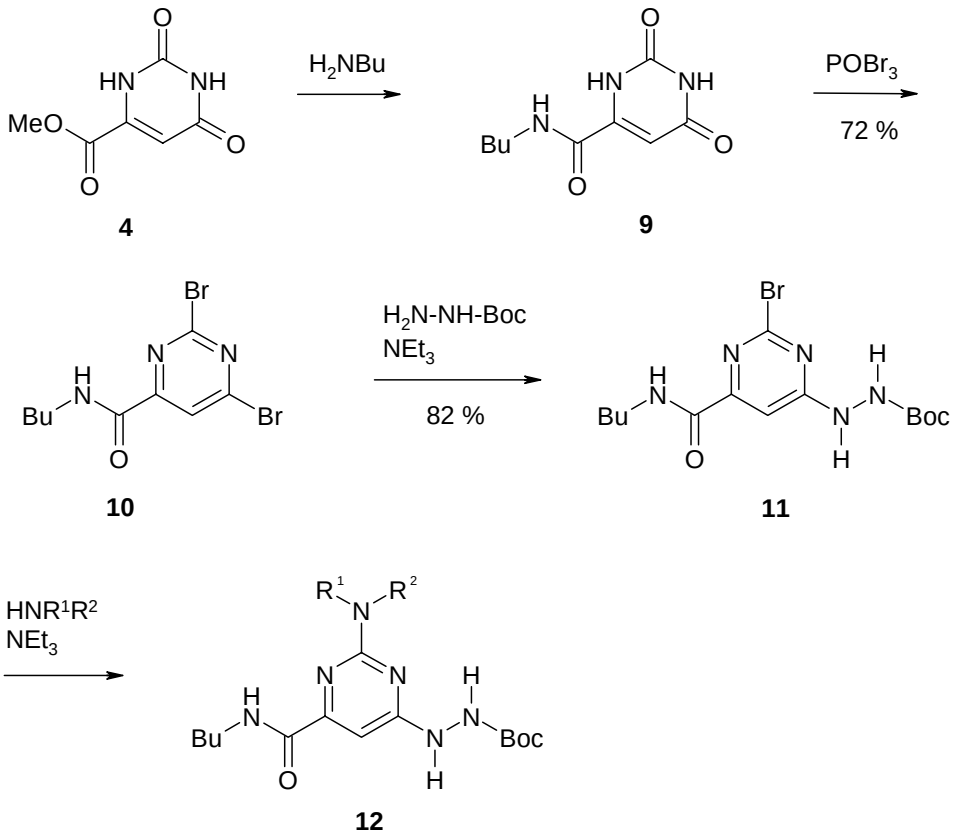
PHA	**Amine**	**PG**	**Method**	**Yield** (corrected*) **[%]**
8a	$HNEt_2$	Boc	A	42
8a	$HNEt_2$	Boc	B	74
8b	$HNEt_2$	Cbz	B	80
8c	H_2NBu	Boc	B	13 (26)

* Yield of the reaction adjust to conversion in brackets

Table 1: Synthesized derivatives (**8**)

To avoid a side reaction by aminolysis of the ester, **4** was converted into the butylamide **(9**) according to a literature procedure[12] followed by bromination and subsequent substitution in 6- and 2-position with Boc-

protected hydrazine and amines. The amidic hydrogen atom allows the formation of another intramolecular hydrogen bond to the pyrimidine nitrogen. Table 2 summarizes the results of the Br-substitution in 2-position of compound **(11)** with several amines.



Scheme 7: Synthesis of compounds **(12)**

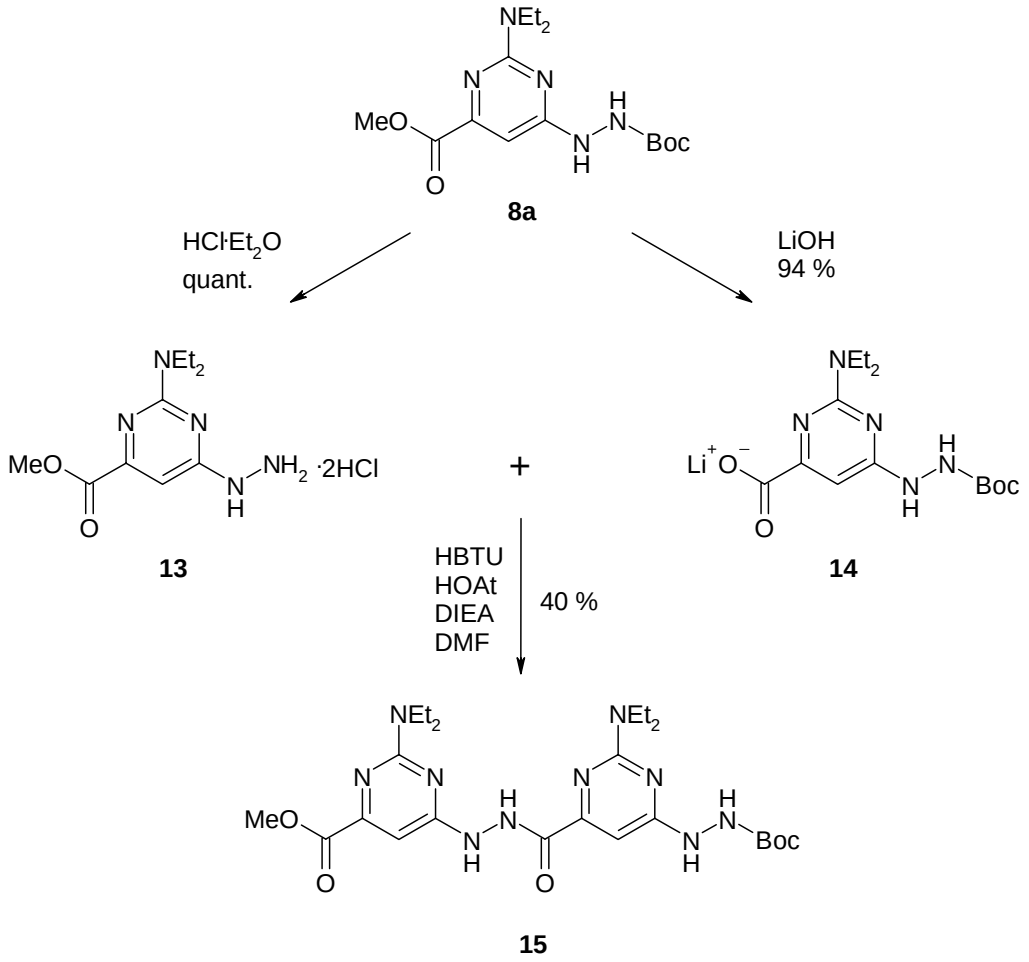
Entry	**Amine**	**Yield** (corrected*) **[%]**
12a	H_2NBu	68
12b	$HNEt_2$	82
12c	Morpholine	67
12d	18-aza-crown-6	56
12e	2-(2-Methoxy-ethoxy)-ethylamine	33 (68)

* Reaction yield adjust to conversion given in brackets

Table 2: Synthesized derivatives **(12)**

A more extended peptide **(15)** was prepared to show the complementary structure and binding affinity of PHA oligomers to peptide β-sheet structures. Cleaving off the ester and the Boc-group of **8a** under basic

and acidic conditions gives the acid (**13**) and amine (**14**), respectively. A coupling of (**13**) and (**14**) under standard peptide coupling conditions yields dipeptide (**15**) in moderate yield.[13]



Scheme 8: Synthesis of dipeptide (**15**)

All synthesized PHA derivatives appear as mixtures of *cis*- and *trans*-isomers of the carbamate protecting groups in the ^{1}H NMR spectra. The pyrimidine ring and the hydrazine protons do not show tautomerisation. Compounds (**8**, **12** and **15**) emit around 420 nm when irradiated at 281 nm. The halogenated compounds (**5**, **6**, **10**, and **11**) have no luminescence. The emission intensity of solutions of the compounds decreases from less polar (e.g. $CHCl_3$) to polar aprotic solvents (e.g. MeCN). Polar protic solvents, such as H_2O, quench the emission completely. The intensity of emission changes with hydrogen bonds to PHA. Therefore, fluorescence titration is a tool to monitor the binding process between PHA and β-sheet peptides in aprotic solvents.

The mono PHA (**8a**) provides three binding sites as acceptor-donor-acceptor (ADA) motif, while bis PHA **(15)** can form up to five (ADADA) intermolecular hydrogen bonds with a complementary binding partner. Top and bottom face of an *N*- and *C*-terminal protected tripeptide differ in their hydrogen bond

donor and acceptor pattern. While the top face can form four hydrogen bonds (DADA), the bottom face has only three (ADA). An *N*- and *C*-terminally protected tetrapeptide offers five hydrogen binding sites (ADADA) at the top face and four (DADA) on the bottom face.

The binding affinities of the PHA derivatives (**8a**) and (**15**) to small peptides of natural amino acids were determined by ^{1}H NMR spectra and fluorescence titrations in $CDCl_3$ and $CHCl_3$. Binding constants were determined by non-linear fitting of chemical induced shifts (CIS) of several protons[14] in the NMR spectrum and of the decrease of emission intensity. Self-associations of the peptides and the PHA (**8a** K_{11} = 17 L/mol, **15** K_{11} = 28 L/mol) were determined independently and were taken into account. Emission intensities corrected for dilution during the titration are used. The stoichiometry of the complexes was determined according to Job´s method of continuous variations.[15] Table 3 summarizes the results of our binding studies.

Entry	PHA	Peptide	Stoichiometry	K_a [L/mol][a]
1	**8a**	Ac-Phe-Val-Leu-OMe	1:1	101
2	**15**	Boc-Phe-Ala-Val-Leu-OMe	1:1	78
3	**15**	Boc-Phe-Gly-Val-Leu-OMe	2:1	2742
				15381

[a]All binding constants have errors of approx. ± 10 %.

Table 3: K_a values determined from NMR and fluorescence titrations in $CDCl_3$ and $CHCl_3$

The affinity of **8a** to the tripeptide is 101 L/mol. Figure 3 shows the CIS of the acetyl singlet during titration. By formation of three cooperative hydrogen bonds with PHA **8a** the binding of the peptide´s top face should be favoured (see Figure 1).

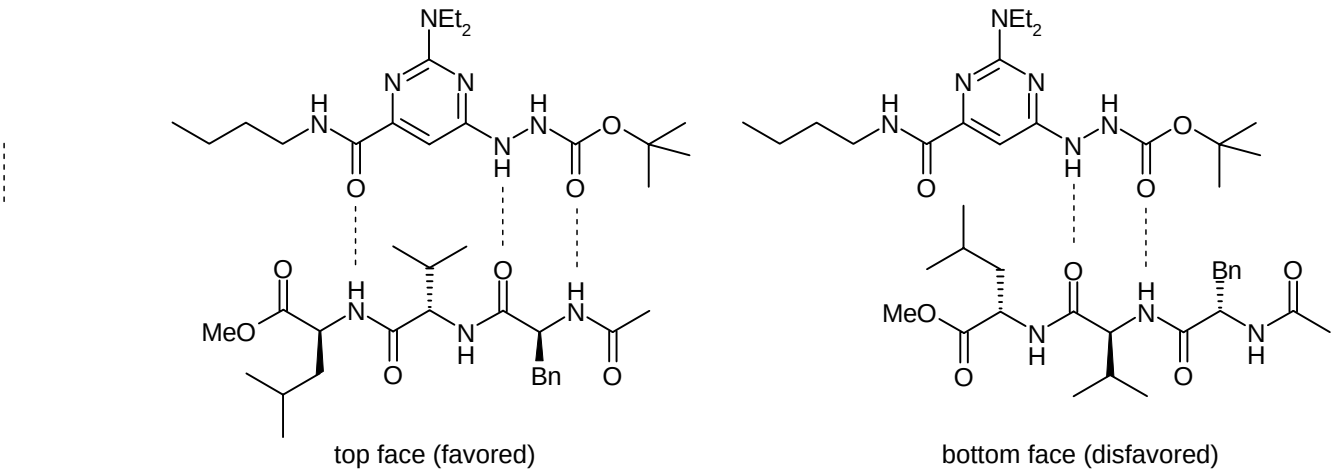


Figure 1: A 1:1 complex between **8a** and Ac-Phe-Val-Leu-OMe

The binding affinity determined for **15** to the tetrapeptide Boc-Phe-Ala-Val-Leu-OMe is in the same order of magnitude as the binding of **8a** to a tripeptide, although an additional hydrogen bond formation should be possible between **15** and the tetrapeptide. Figure 2 summarizes the possible 1:1 binding motifs between **15** and Boc-Phe-Ala-Val-Leu-OMe.

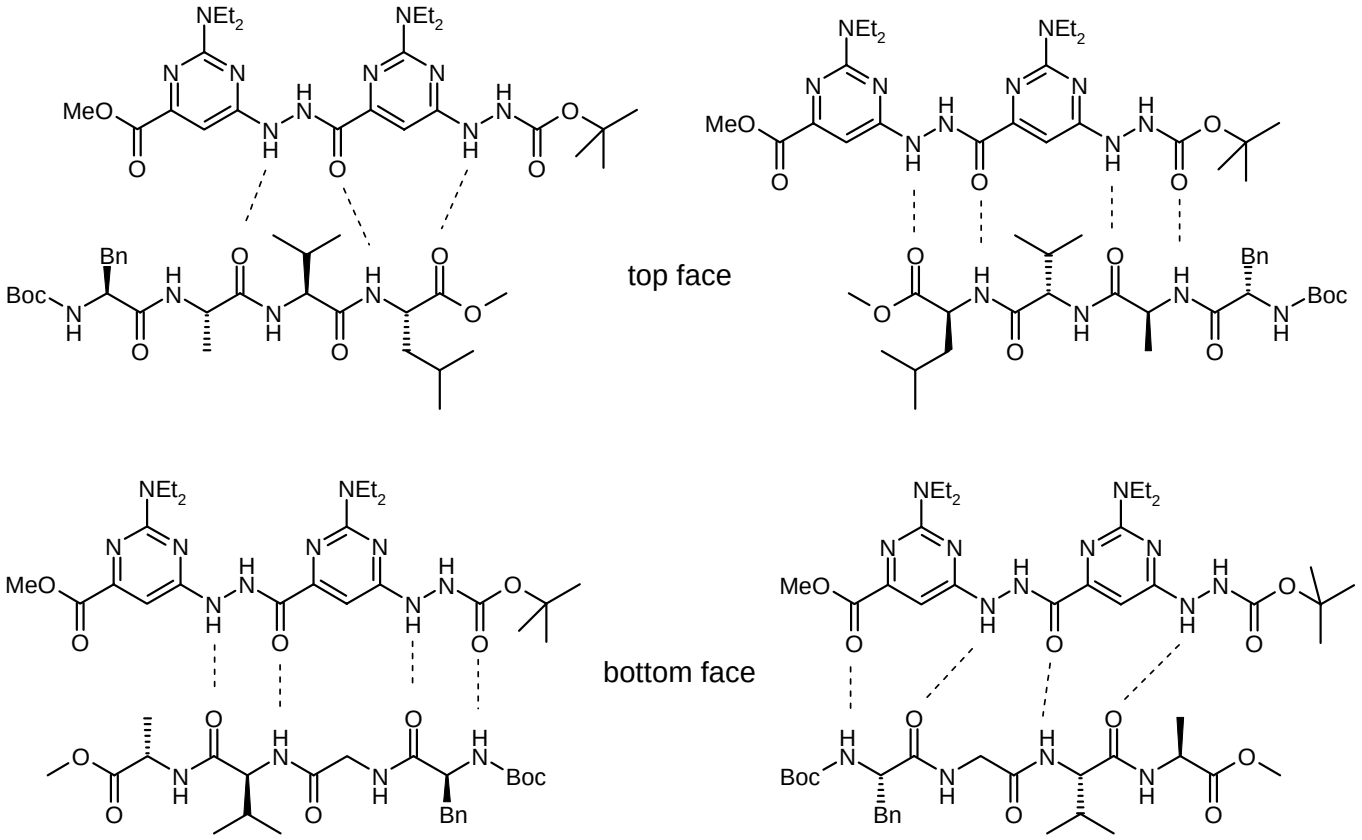


Figure 2: Possible 1:1 complexes between **15** and Boc-Phe-Ala-Val-Leu-OMe

For steric reasons, the binding motifs containing Boc protecting group on different ends might be favoured. The observed CIS and the calculated binding affinity result from an average value of the experiment. The different aggregates are presumably in a fast dynamic equilibrium and NMR cannot distinguish between them. All efforts to co-crystallize **8a** and **15** with a peptide giving a picture of the binding motif failed so far.

Figures 3 and 5 illustrate that a plateau in CIS or a minimum of emission is reached after the addition of 1 equivalent of the peptide to a solution of PHA. The ^{1}H NMR Job´s plot of **8a** and Ac-Phe-Val-Leu-OMe (Figure 4) shows a maximum at x = 0.5, confirming a 1:1 binding motif.

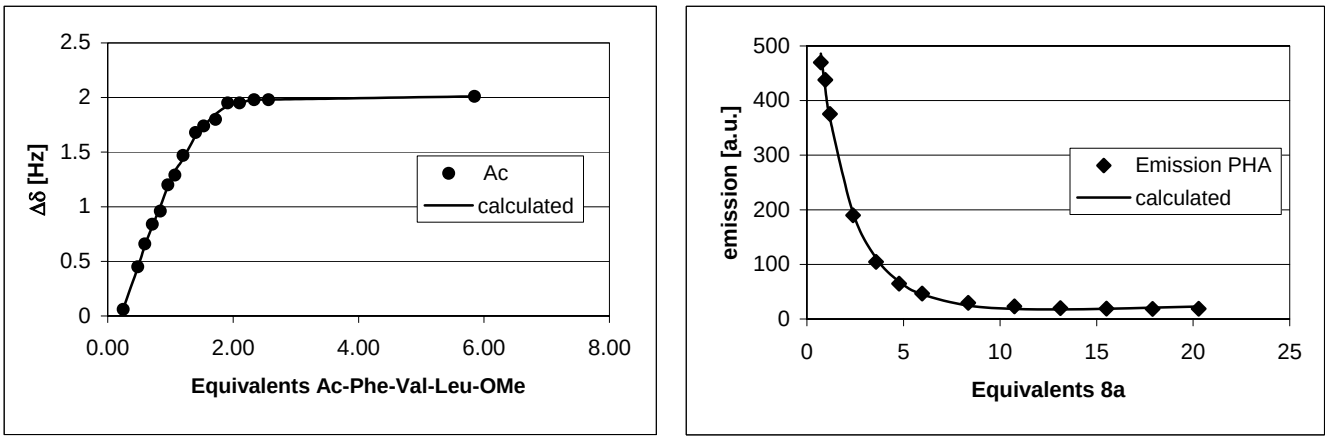


Figure 3: NMR titration curve (left) and fluorescence titration curve (right) of Ac-Phe-Val-Leu-OMe with compound (**8a**) in $CDCl_3$, c_0 = 10 mmol/L, observing CIS of the acetyl hydrogen resonance and the emission intensity at 423 nm (excitation at 280 nm)

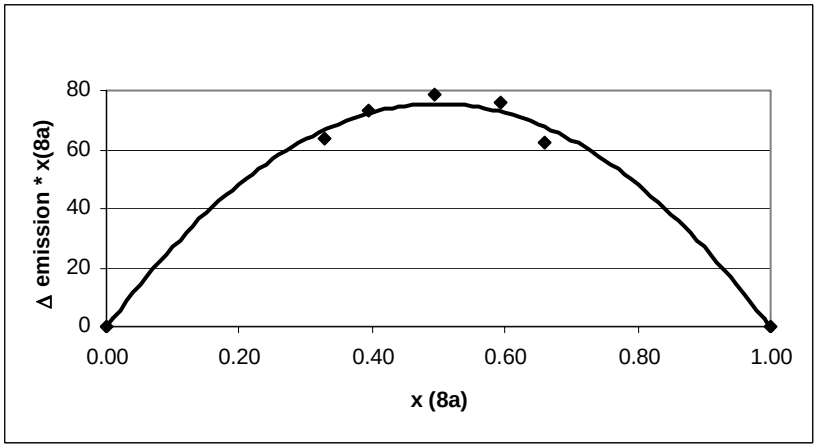


Figure 4: ^{1}H NMR job-plot of **8a** and Ac-Phe-Val-Leu-OMe

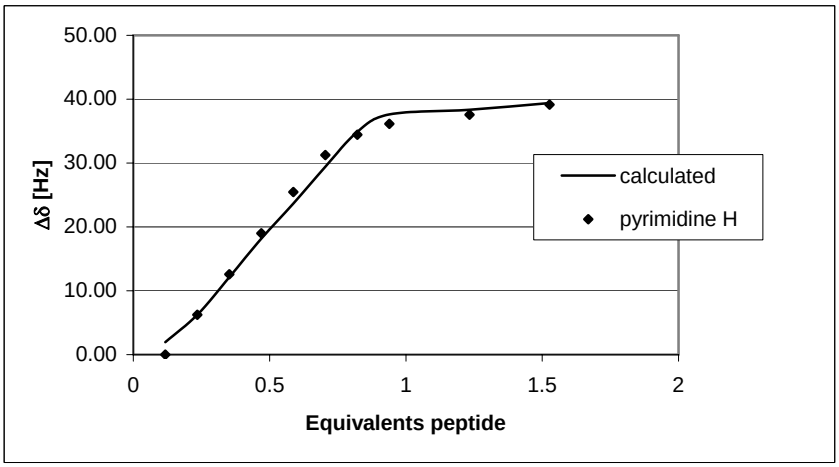


Figure 5: NMR spectral titration curve of Boc-Phe-Ala-Val-Leu-OMe with compound (**15**) in $CDCl_3$, c_0 = 6.0 mmol/L, monitoring CIS of the pyrimidine hydrogen resonance.

The titration of **15** with Boc-Phe-Gly-Val-Leu-OMe gives a different picture. A slight chance in the peptide sequence from Ala to Gly in position 2 leads to a significant increase of the binding affinity and change of the aggregate stoichiometry. The NMR spectra titration curve indicates two subsequent binding processes.

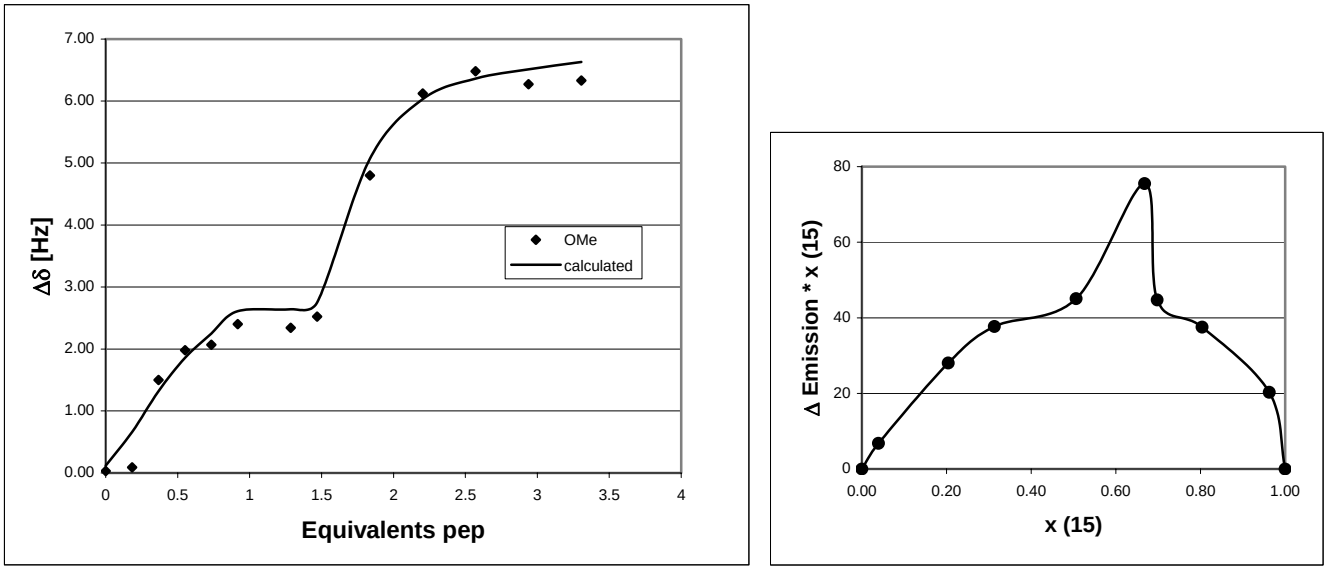
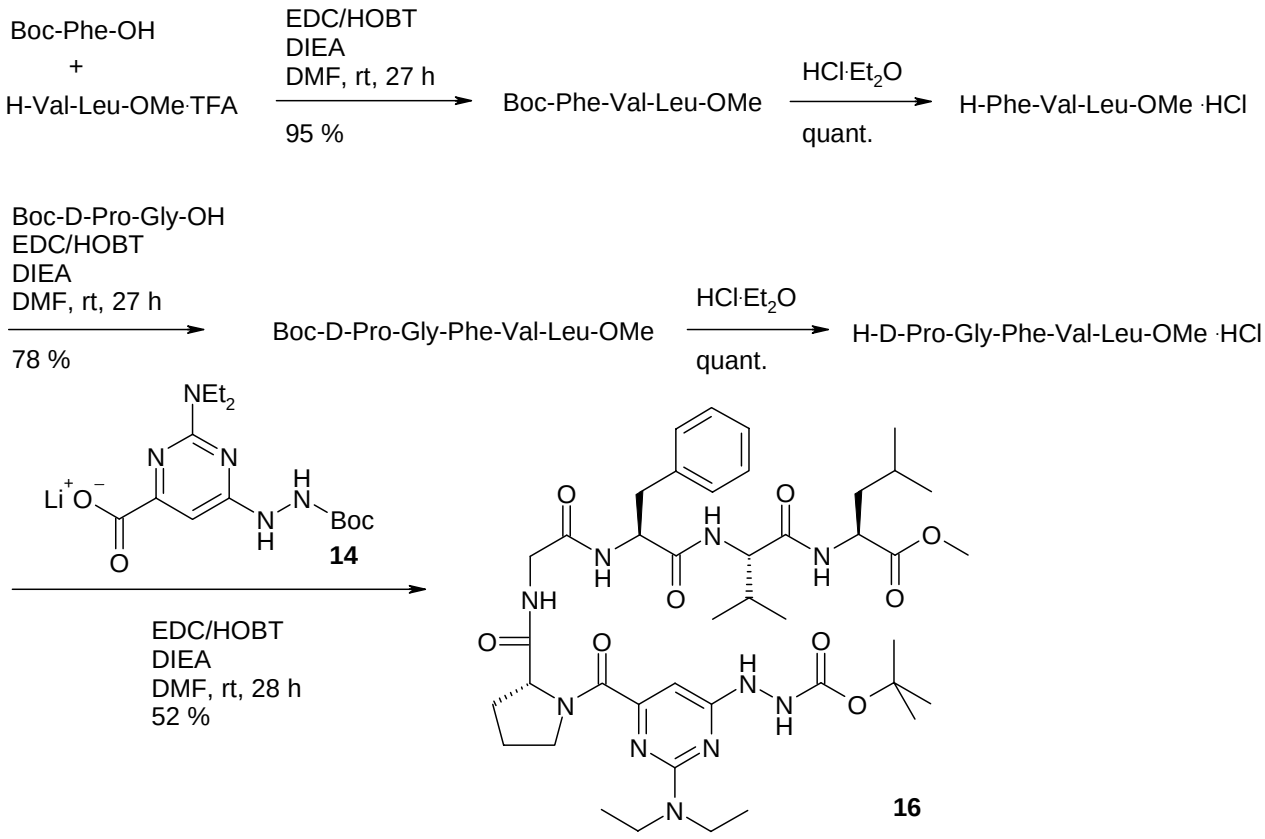


Figure 6: NMR spectral titration curve (left; CIS of the methyl ester group) and emission Job´s plot analysis (right; emission intensity at 423 nm,excitation at 280 nm)) of Ac-Phe-Val-Leu-OMe with compound (**15**) in $CDCl_3$, c_0 = 9.5 mmol/L.

The first part of the NMR titration curve is identical in shape to the titrations of **8a** with the tripeptide Boc-Phe-Val-Leu-OMe or **15** with the tetrapeptide Boc-Phe-Ala-Val-Leu-OMe, respectively. Addition of one equivalent **15** to Boc-Phe-Gly-Val-Leu-OMe saturates the CIS. Addition of a second equivalent of **15** leads to a further CIS of the methoxy resonance signal. For the first binding process an affinity constant of K_{11} = 2742 L/mol is derived, while the subsequent binding with K_{12} of 15381 L/mol indicates a highly cooperative assembly process. We cannot provide a structure for the complex assembly, but the exchange of alanine for glycine in the tripeptide sequence allows almost free rotation around the C-C and the C-N bond in the glycine residue.[16]

To avoid higher aggregates and arrive at a more defined PHA – peptide interaction a short β-turn fragment incorporating PHA was prepared. Scheme 9 shows the synthesis. Extension of Gellman´s β-turn fragment Gly-D-Pro[17] by H-Phe-Leu-Val-OMe required 1.1 equivalents of EDC and HOBt and 2 equivalents of Huenig´s Base. The product precipitates from DMF solution after the addition of water. Lithium salt (**14**) and the deprotected pentapeptide couple to **16** in moderate yields.



Scheme 9: Synthesis of the β-turn fragment (**16**)

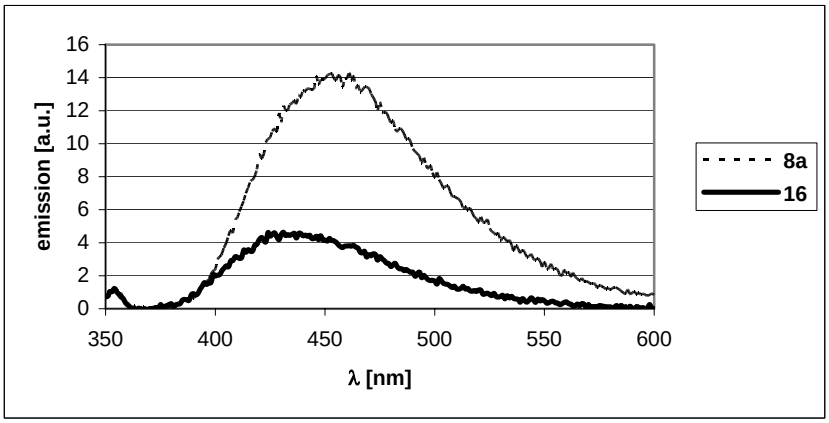


Figure 7: Emission spectra of (**8a**) and **(16**) in a 8 mM solution in acetonitrile

Figure 7 shows the emission intensity of equally concentrated solutions of **8a** and **16** (8 mM) in acetonitrile. The significant decreased emission intensity of **16** in comparison to **8a** indicates an intramolecular binding process of PHA and peptide.

NMR spectra in 10 mM deuterated DMSO solution show only one conformer for (**16**) in solution. Analysis of the observed intramolecular NOE contacts in deuterated DMSO clearly confirm a β-turn structure as depicted in Figure 8.

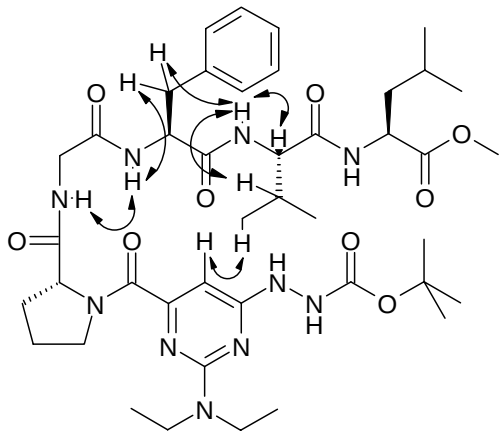
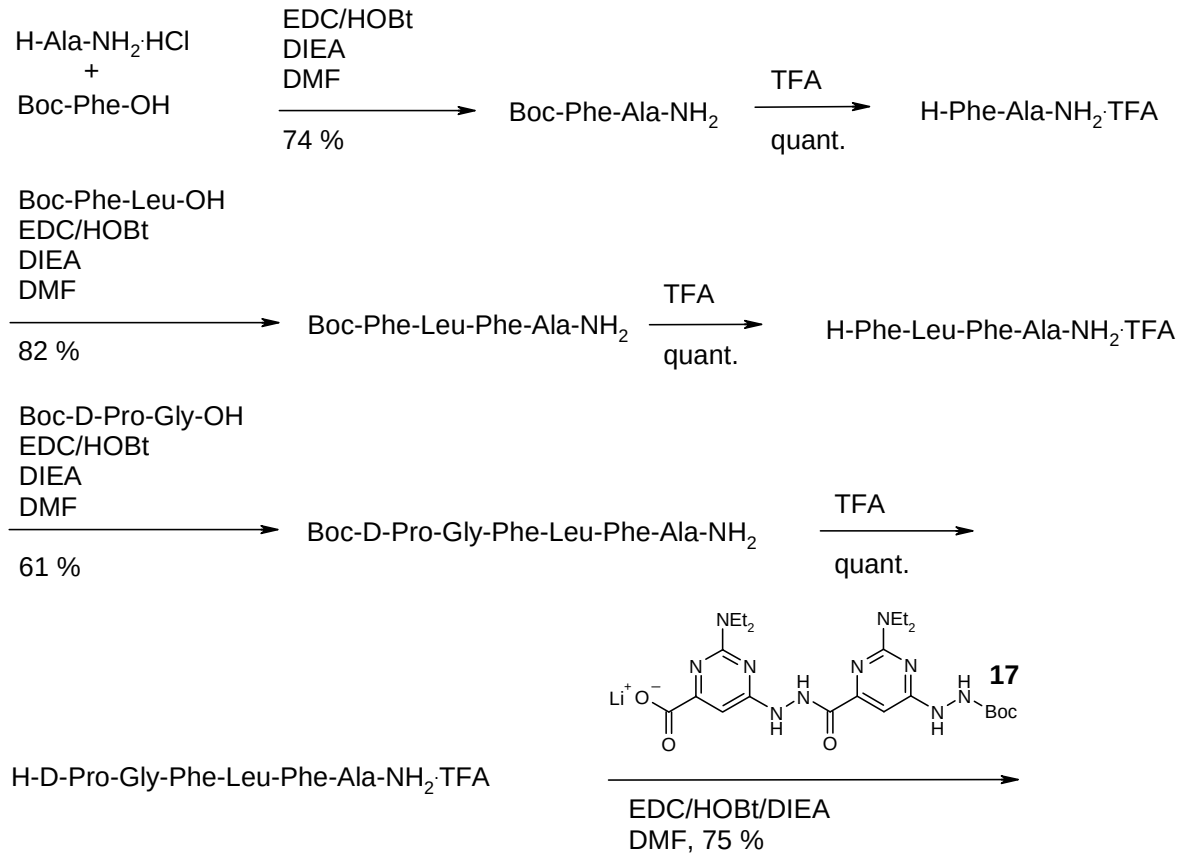
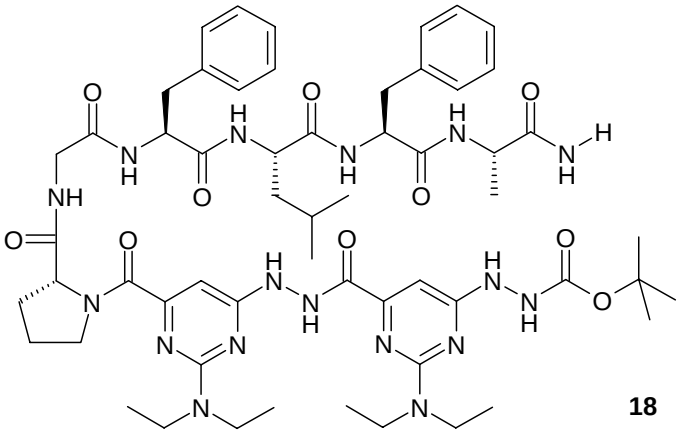


Figure 8: NOE contacts found in the analysis of compound (**16**)

ROESY experiments revealed numerous short-range and long-range NOEs. The NOE contact between the pyrimidine proton and the Val-*i*-Pr-residue indicates a folded conformation. Due to signal overlap and broad NH signals no further NOE contacts of PHA and peptide are observable.[18]

A more extended β-turn fragment was prepared incorporating dipeptide (**15**). The β-turn fragment Gly-D-Pro was extended by H-Phe-Leu-Phe-Ala-NH_2 and coupling of the deprotected hexapeptide with the lithium carboxylate (**17**) gave **18** in good yield.





Scheme 10: Synthesis of the β-turn fragment (**18**)

Figure 9 shows the analysis of the intramolecular NOE contacts of compound (**18**) in deuterated DMSO solution. The NOE contacts between the *N*-terminal PHA unit and the *C*-terminal amino acid residue indicate that the interaction of heterocycles and peptide chain propagates the turn. The proton on the hydrazine of the terminal PHA unit shows a contact to the methyl group and the C_α proton of alanine to the CH_2 group of the phenylalanine. In both cases, signal overlap prohibits distinction of the diasterotopic protons of the CH_2 group in the phenylalanine and leucine side chain. The NOE contact of a PHA proton and the glycine amide proton confirms the turn structure. Overall, eight NOE contacts support a solution structure of **18** as shown in Figure 9.

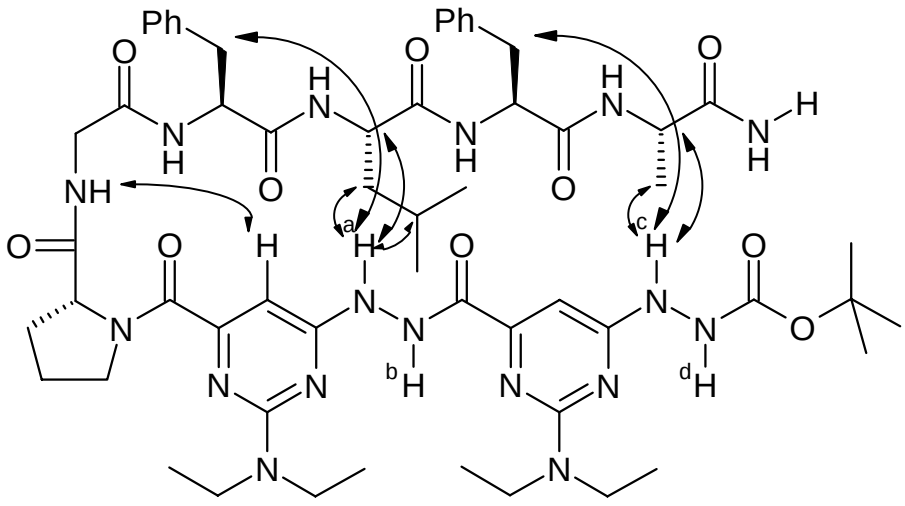


Figure 9: NOE contacts found in the analysis of compound (**18**)

Conclusion

We have reported the synthesis of substituted pyrimidine-hydrazine-acids (PHA) from orotic acid. Standard peptide coupling procedures couple or incorporate the heterocyclic hydrazine acids into peptides of natural amino acids. Their geometry of hydrogen bond acceptor and donor sites make them suitable for complementary interaction with peptide β-sheets. An interesting feature is their luminescence in non-polar solvents, which changes upon peptide binding. PHA´s are simple dipeptide mimics, with a build in luminescence chemosensor monitoring the local polarity of the environment and interaction with adjacent peptide chains.

EXPERIMENTAL

General Methods: Melting points were determined on a Tottoli micromelting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 K. Chemical shifts (δ) are reported in ppm downfield from internal TMS. Emission spectra were recorded on a Cary Eclipse spectrophotometer. Thin layer chromatographic (TLC) analyses were performed on silica gel 60 F-254 with a 0.2 mm layer thickness. Preparative chromatography columns were packed with silica gel Geduran SI 60. Mass spectra were recorded on a Finnigan MAT TSQ 7000 (ESI) and on a Finnigan MAT 95 (HRMS).

X-Ray Structures: Crystallographic data for the structures (**6a**) and (**6b**) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 267816 & 267817. Copies of the data can be obtained, free of charge, on application to CDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

^{1}H-NMR and Emission Titrations: A solution of the PHA molecule (c = 15 mmol/L) was added in portions via a microsyringe to a solution of a peptide (c = 1 mmol/L). Volume and concentration changes were taken into account for the analysis of the binding event.[14]

Job´s Plot: Equimolar solutions (0.1 mM) of PHA molecule and peptide were prepared and mixed in various amounts. Emission spectra of the mixtures were recorded, and the emission intensities were analyzed by Job´s method modified for emission results.[15]

General procedure for the substitution of a halogen atom by a protected hydrazine (GP1): To a solution of the halogenated pyrimidine (1 mmol) in DMF (1 mL/mmol) was added triethylamine (1.1 mmol), followed by a solution of the protected hydrazine (1.1 mmol) in DMF (2 mL/mmol). The solution was stirred at 60 °C until complete conversion of the pyrimidine (12-24 h). The reaction mixture was allowed to cool to rt, diluted with water, and extracted with ethyl acetate. The organic phases were

dried over Na_2SO_4, evaporated, and then concentrated under reduced pressure. If necessary, the residue was purified by column chromatography (mixtures of hexanes and ethyl acetate) to afford the products.

General procedure for the substitution of a halogen atom by an amine (GP2): To a solution of the brominated pyrimidine (1 mmol) in DMF (2 mL/mmol) was added the amine (10 mmol). The solution was stirred at 60 °C until complete conversion of the pyrimidine was observed (18-36 h). The reaction mixture was allowed to cool to rt, diluted with ethyl acetate, and washed with brine. The organic phases were dried over Na_2SO_4, evaporated, concentrated under reduced pressure and purified by column chromatography (mixtures of hexanes and ethyl acetate) to afford the products.

2,6-Dibromopyrimidine-4-carboxylic acid methyl ester (5b): Orotic acid methyl ester (**4**) (0.4 g, 2.35 mmol) was dissolved in acetonitrile (5 mL) and $POBr_3$ (3.37 g, 11.8 mmol) was added. The reaction mixture was stirred for 3 h under reflux. The resulting yellow solution was allowed to cool to rt, poured into 20 mL of crushed ice water, and extracted with ethyl acetate. The combined extracts were dried over Mg_2SO_4, and evaporated off. The residue was recrystallized from ethyl acetate/hexanes (3:1) to give **5b** as brown crystalline needles (541 mg, 78 %).

mp: > 185 °C (decomp). IR (KBr disk): 3116 cm^{-1}, 3069, 1729, 1549, 1517, 1439, 1314, 1233, 1103, 957, 784, 730. ^{1}H-NMR (DMSO-d_6): δ: 3.94 ppm (s, 3 H, OCH_3), 8.33 (s, 1 H, PHA-H). ^{13}C-NMR (DMSO-d_6): δ: 53.4 ppm (+; HMBC: OCH_3), 124.8 (+; HMBC: PHA-H), 150.8 (C_{quat}), 155.0 (C_{quat}), 157.0 (C_{quat}), 161.8 (C_{quat}; HMBC: -CO-OCH_3). UV (MeCN): λ_{max} (log ε): 283 nm (4.664). MS *m/z* (%): 313.9 [$M+NH_4^+$] (100), 296.9 [MH^+] (15).

Methyl 6-(*N*'-*tert*-butoxycarbonylhydrazino)-2-chloropyrimidine-4-carboxylate (6a): Compound (**5a**) (1.32 g, 6.38 mmol) was allowed to react with triethylamine (1.06 mL, 0.78 g, 7.73 mmol) and Boc-hydrazine (1.02 g, 7.73 mmol) following **GP1** (hexanes/ethyl acetate 2:1, R_f = 0.30) to yield **6a** (1.17 g, 60 %) as a colorless powder.

mp: 151-152 °C. IR (KBr disk): 3264 cm^{-1}, 3230, 3109, 2982, 1735, 1609, 1446, 1263, 1159, 993, 754. ^{1}H-NMR ($CDCl_3$): δ: 1.39-1.41 ppm (m, 9 H, Boc-CH_3), 3.85 (s, 3 H, O-CH_3), 6.61 (s, 1 H, NH), 7.33 (s, 1 H, PHA-H), 7.77 (br, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ: 28.1 ppm (+; HMQC: Boc-CH_3), 53.4 (+; HMQC: OCH_3), 82.8 (C_{quat}; HMBC: Boc), 102.1 (+; HMQC: PHA-H); 155.1 (C_{quat}), 156.6 (C_{quat}), 160.5 (C_{quat}), 163.8 (C_{quat}; HMBC: C=O ester), 168.0 (C_{quat}). UV/VIS (MeCN): λ_{max} (log ε): 244 nm (4.860), 301 (4.517). MS *m/z* (%): 622.1 [$2M+NH_4^+$] (1), 605.2 [$2M+H^+$] (2), 303.2 [MH^+] (100), 247.1 [MH^+-C_4H_8] (8), 203.1 [MH^+-Boc] (2). Anal. Calcd for $C_{11}H_{15}N_4O_4Cl$: C: 43.6; H: 5.0; N: 18.5; Found: C: 43.9; H: 5.0; N: 17.9.

Methyl 6-(*N*'-benzyloxycarbonylhydrazino)-2-chloropyrimidine-4-carboxylate (6b): Compound (**5a**) (228 mg, 1.10 mmol) was allowed to react with triethylamine (128 µL, 122 mg, 1.21 mmol) and

Cbz-hydrazine (201 mg, 1.21 mmol) following **GP1** (hexanes/ethyl acetate 3:2, $R_f = 0.25$) to yield **6a** (280 mg, 75 %) as a colorless powder.

mp: 94-96 °C. IR (KBr disk): 3264 cm^{-1}, 3116, 2956, 1729, 1597, 1561, 1443, 1267, 1203, 978, 754. ^{1}H-NMR ($CDCl_3$): δ: 3.98 ppm (s, 3 H, O-CH_3), 5.21 (s, 2 H, CH_2-Ar), 6.78 (br s, 1 H, N-H), 7.31-7.60 (m, 7 H, 5 Ar-H + PHA-H + N-H). ^{13}C-NMR ($CDCl_3$): δ: 53.3 ppm(+; HMQC: OCH_3), 68.4 (-; HMQC: CH_2 Bzl), 102.2 (+; HMQC: PHA-H), 128.3 (+; HMQC: Ar-H), 128.7 (+; HMQC: Ar-H), 135.0 (C_{quat}, Ar), 156.1 (C_{quat}), 156.7 (C_{quat}), 160.6 (C_{quat}), 163.6 (C_{quat}; HMBC: C=O ester), 168.2 (C_{quat}). UV/VIS (MeCN): λ_{max} (log ε): 234 nm (4.689), 300 (4.360). MS *m/z* (%): 337.1 [MH^+] (100), 228.9 [MH^+-$PhCH_2OH$] (7), 202.9 [MH^+-Cbz] (22), 188.1 [MH^+-Cbz-NH] (29). Anal. Calcd for $C_{14}H_{13}N_4O_4Cl$: C: 49.9; H: 3.9; N: 16.6; Found: C: 49.8; H: 4.2; N: 16.5.

Methyl 2-bromo-6-(*N*'-*tert*-butoxycarbonylhydrazino)pyrimidine-4-carboxylate (6c): Compound (**5b**) (164 mg, 0.55 mmol) was allowed to react with triethylamine (91 µL, 87 mg, 0.66 mmol) and Boc-hydrazine (87 mg, 0.66 mmol) following **GP1** (hexanes/ethyl acetate 1:1, $R_f = 0.54$) to yield **6a** (123 mg, 64 %) as a colorless powder.

mp: 227-228 °C. IR (KBr disk): 3228 cm^{-1}, 3105, 2981, 1734, 1603, 1445, 1259, 1159, 752. ^{1}H-NMR (DMSO-d_6, 363 K): δ: 1.44 ppm (s, 9 H, Boc-CH_3), 3.89 (s, 3 H, OCH_3), 7.36 (s, 1 H), 8.92 (bs, 1 H), 9.71 (s, 1 H). ^{13}C-NMR (DMSO-d_6): δ = 28.2 ppm (+; HMQC: Boc-CH_3), 53.5 (+; HMQC: OCH_3), 83.0 (C_{quat}; HMBC: Boc), 102.7 (+; HMQC: PHA-H), 152.1 (C_{quat}), 155.0 (C_{quat}; HMBC: C=O Boc), 156.3 (C_{quat}), 163.7 (C_{quat}; HMBC: C=O ester), 167.1 (C_{quat}). UV (MeCN): λ_{max} (log ε): 235 nm (4.973), 303 (4.654). MS *m/z* (%): 340.0 [MH^+] (100). HRMS Calcd for $C_{11}H_{15}N_4O_4Br$: 346.0277; Found: 346.0275±0.0002.

Methyl 6-(*N*'-benzyloxycarbonylhydrazino)-2-bromopyrimidine-4-carboxylate (6d): Compound (**5b**) (836 mg, 2.83 mmol) was allowed to react with triethylamine (470 µL, 343 mg, 3.39 mmol) and Cbz-hydrazine (563 mg, 3.39 mmol) following **GP1** (ethyl acetate/hexanes 2:3, $R_f = 0.22$) to yield **6a** (839 mg, 78 %) as a colorless powder.

mp: 94-95 °C. IR (KBr disk): 3354 cm^{-1}, 3270, 2958, 2930, 1721, 1691, 1591, 1512, 1376, 1287, 1211, 1121, 995, 968, 773, 743, 695. ^{1}H-NMR ($CDCl_3$): δ: 3.92 ppm (s, 3 H, OCH_3), 5.16 (s, 2 H, CH_2), 7.05-7.58 (m, 7 H, Ar-H, PHA-H, NH), 8.60 (br s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ: 53.5 ppm (+, OCH_3), 68.4 (-, CH_2), 102.4 (+, PHA-H), 127.3 (+, Ar-H), 127.7 (+, Ar-H), 134.1 (C_{quat}, Ar), 150.8 (C_{quat}), 155.2 (C_{quat}, C=O Cbz), 162.5 (C_{quat}), 162.6 (C_{quat}), 165.8 (C_{quat}). UV (MeCN): λ_{max} (log ε): 301 nm (4.644). MS *m/z* (%): 380.9 [MH^+] (100). Anal. Calcd for $C_{14}H_{13}N_4O_4Br$: C: 44.1; H: 3.4; Br: 21.0; N: 14.7; O: 16.8; Found: C: 44.6; H: 3.4; N: 14.9.

Methyl 6-(N'-tert-butoxycarbonylhydrazino)-2-diethylaminopyrimidine-4-carboxylate (8a): Method A: A solution of **6a** (117 mg, 0.39 mmol), DMAP (59 mg, 0.48 mmol) and diethylamine (80 µL, 57 mg,

0.78 mmol) in DMF (6 mL) is stirred for 1 d at 40 °C. The red solution is diluted with ethyl acetate (8 mL) and washed with brine (2x15 mL). The organic extract is dried over $MgSO_4$, evaporated under reduced pressure and the residue purified by column chromatography (hexanes/ethyl acetate 3:1, R_f = 0.22) to afford **8a** (55 mg, 42 %) as a colourless powder.

Method B: A solution of **6c** (200 mg, 0.58 mmol) and diethylamine (299 µL, 212 mg, 2.90 mmol) in DMF (4 mL) is stirred for 8 h at 80 °C. The solvent is evaporated and the crude product purified by column chromatography (hexanes/ethyl acetate 3:1, R_f = 0.22) to afford **8a** (146 mg, 74 %).

mp: 176-177 °C. IR (KBr disk): 3237 cm^{-1}, 2967, 2933, 1732, 1668, 1601, 1531, 1368, 1253, 1161. ^{1}H-NMR (DMSO-d_6): δ: 1.09 ppm (t, ^3J = 6.9 Hz, 6 H, CH_3), 1.25-152 (m, 9 H, Boc-CH_3), 3.54 (q, ^3J = 6.9 Hz, 4 H, CH_2), 3.81 (s, 3 H, OCH_3), 6.32 (s, 1 H, PHA-H), 8.48-9.17 (m, 2 H, N-H). ^{13}C-NMR (DMSO-d_6): δ: 13.1 ppm (+, CH_3), 27.9 (+, Boc-CH_3), 40.7 (-, CH_2), 52.2 (+, OCH_3), 78.8 (C_{quat}, Boc), 93.5 (+, PHA-H), 155.2 (C_{quat}), 155.6 (C_{quat}), 160.6 (C_{quat}), 164.3 (C_{quat}), 165.5 (C_{quat}). UV (MeCN): λ_{max} (log ε): 243 (5.316) nm, 329 (4.660). MS *m/z* (%): 701.5 [$2MNa^+$] (6), 340.0 [MH^+] (100)

Methyl 6-(*N*'-benzyloxycarbonylhydrazino)-2-diethylaminopyrimidine-4-carboxylate (8b): To a solution of **6d** (270 mg, 0.71 mmol) in DMF (5 mL) was added diethylamine (366 µL, 260 mg, 3.55 mmol). The reaction mixture was stirred for 16 h at 70 °C. The solution was diluted with ethyl acetate (7 mL) and washed with brine (2x10 mL). The organic phase washed dried over $MgSO_4$, evaporated under reduced pressure and purified by column chromatography (hexanes/ethyl acetate 3:2, R_f = 0.40) affording **8b** (212 mg, 80 %).

mp: 174-177 °C. IR (KBr disk): 3262 cm^{-1}, 2955, 1783, 1596, 1499, 1443, 1263, 1196, 976, 748. ^{1}H-NMR ($CDCl_3$): δ: 1.12 ppm (t, ^3J = 7.0 Hz, 6 H, NCH_2CH_3), 3.58 (q, ^3J = 6.6 Hz, 4 H, NCH_2CH_3), 3.89 (s, 3 H, OCH_3), 5.18 (s, 2 H, CH_2 Cbz), 6.51-6.72 (m, 3 H, PHA-H, NH), 7.22-7.38 (m, 5 H, Ar-H). ^{13}C-NMR ($CDCl_3$): δ: 13.1 ppm (+, NCH_2CH_3), 41.6 (-, NCH_2CH_3), 52.7 (+, OCH_3), 67.9 (-, CH_2 Cbz), 92.1 (+, PHA-H), 128.3 (+, Ar-H), 128.5 (+, Ar-H), 128.6 (+, Ar-H), 135.5 (C_{quat}, Ar), 156.0 (C_{quat}, C=O Cbz), 156.8 (C_{quat}), 161.1 (C_{quat}), 165.6 (C_{quat}), 166.0 (C_{quat}). UV (MeCN): λ_{max} (log ε): 246 nm (5.289), 341 (4.579). MS *m/z* (%): 374.0 [MH^+] (100).

Methyl 6-(*N*'-*tert*-butoxycarbonylhydrazino)-2-butylaminopyrimidine-4-carboxylate (8c): To a stirred solution of **6c** (100 mg, 0.29 mmol) in DMF (3 mL) was added butyl amine (71 µL, 53 mg, 0.72 mmol) and the reaction mixture was stirred for 18 h at 65 °C. The solution was diluted with ethyl acetate (5 mL) and washed with brine (3x8 mL). The organic extract was dried over Na_2SO_4, evaporated off and purified by column chromatography (hexanes/ethyl acetate 3:2, R_f = 0.14) to afford **8c** (13 mg, 13 %) as colorless powder and unreacted **6c** (49 mg, 0.14 mmol).

mp: 110-112 °C. IR (KBr disk): 3337 cm^{-1}, 2959, 2873, 1735, 1591, 1542, 1439, 1368, 1249, 1160, 1102, 778. ^{1}H-NMR ($CDCl_3$): δ: 0.92 ppm (t, ^3J = 7.2 Hz, CH_3), 1.24-1.58 (13 H, Boc-CH_3, CH_3CH_2,

$CH_3CH_2\mathit{CH_2}$), 3.36 (q, ^3J = 6.4 Hz, 2 H, $\mathit{CH_2}NH$), 3.93 (s, 3 H, OCH_3), 5.34 (br s, 1 H, NH), 6.55 (s, 1 H, NH), 6.65 (s, 1 H, PHA-H), 6.76 (br s, 1 H, NH). ^{13}C-NMR (DMSO-d_6): δ: 13.8 ppm (+, CH_3), 20.1 (-, $CH_3\mathit{CH_2}$), 28.2 (+, Boc-CH_3), 31.6 (-, $CH_3\mathit{CH_2}$), 36.9 (-, $CH_3CH_2\mathit{CH_2}$), 53.0 (+, OCH_3), 82.0 (C_{quat}, Boc), 93.2 (+, PHA-H), 155.6 (C_{quat}, 2 C), 162.4 (C_{quat}), 165.5 (C_{quat}), 166.7 (C_{quat}). UV (MeCN): λ_{max} (log ε): 244 (5.213) nm, 323 (4.552). MS *m/z* (%): 340.1 [MH^+] (100).

2,6-Dibromopyrimidine-4-carboxylic acid butylamide (10): Orotic acid butylamide (**9**) (1.65 g, 5.76 mmol) and $POBr_3$ (7.74 g, 28.8 mmol) were suspended in acetonitrile (15 mL). The reaction mixture was stirred for 3.5 h at 65 °C. The clear solution was allowed to cool to rt and poured on crushed ice (20 mL) and water (10 mL) and extracted with ethyl acetate (3x45 mL). The organic extract was washed with sat. $NaHCO_3$ solution and dried over Na_2SO_4, evaporated off affording **10** (466 mg, 72 %) in analytical pure form.mp: > 175 °C (decomp). IR (KBr disk): 3010 cm^{-1}, 2987, 2979, 1650, 1636, 1225, 675. ^{1}H-NMR (DMSO-d_6): δ: 0.88 ppm (t, 3 H; ^3J = 7.4 Hz, CH_3), 1.18-1.36 (m, 2 H, CH_3-$\mathit{CH_2}$), 1.40-1.57 (m, 2 H, $\mathit{CH_2}$-CH_2-NH), 3.27 (q, 2 H, ^3J = 6.7 Hz, $\mathit{CH_2}$-NH), 8.21 (s, 1 H, PHA-H), 9.02 (t, 1 H, ^3J = 6.8 Hz, N-H). ^{13}C-NMR (DMSO-d_6): δ = 13.5 ppm (+, CH_3), 19.4 (-, CH_3-$\mathit{CH_2}$), 30.8 (-, $\mathit{CH_2}$-CH_2-NH), 38.8 (-, $\mathit{CH_2}$-NH), 122.4 (+, PHA-H), 146.6 (C_{quat}), 150.1 (C_{quat}), 154.7 (C_{quat}), 160.1 (C_{quat}). UV (MeCN): λ_{max} (log ε): 276 nm (4.774). MS *m/z* (%): 333.7 [M+H^+] (100).

2-Bromo-6-[*N*'-(*tert*-butoxyhydroxymethyl)hydrazino]pyrimidine-4-carboxylic acid butylamide (11): 10 (350 mg, 1.04 mmol) was allowed to react with triethylamine (173 µL, 127 mg, 1.25 mmol) and Boc-hydrazine (165 mg, 1.25 mmol) following **GP1** (ethyl acetate/hexanes 3:5, R_f = 0.18) to yield **11** (331 mg, 82 %) as a colorless powder.

mp: 161-163 °C. IR (KBr disk): 3339 cm^{-1}, 3227, 2974, 2932, 1725, 1651, 1593, 1526, 1392, 1272, 1254, 1165. ^{1}H-NMR ($CDCl_3$): δ: 0.95 ppm (t, 3 H, ^3J = 7.3 Hz; HSQC: CH_3), 1.36-1.45 (m, 2 H; ROESY: CH_3CH_2), 1.47 (s, 9 3.43 (q, 2 H, ^{3}J = 6.7 Hz, $\mathit{CH_2}$-NH), 6.79 (s, 1 H; ROESY: ^{1}NH), 7.46 (s, 1 H; HSQC: PHA-H), 7.62 (bs, 1 H; ROESY: ^{2}NH), 7.77 (t, ^3J = 5.8 Hz, 1 H; HMBC: NHCO). ^{13}C-NMR (DMSO-d_6): δ: 13.7 ppm (+; HSQC: CH_3), 20.1 (-; HSQC: $CH_3\mathit{CH_2}$), 28.2 (+; HSQC: Boc-CH_3), 31.5 (-; HSQC: $CH_3CH_2\mathit{CH_2}$), 39.4 (-; HSQC: $\mathit{CH_2}NH$), 82.7 (C_{quat}; HMBC: Boc), 99.6 (+; HSQC: PHA-H), 150.8 (C_{quat}), 154.8 (C_{quat}; HMBC: C=O Boc), 161.7 (C_{quat}), 167.3 (C_{quat}). UV (MeCN): λ_{max} (log ε): 240 nm (5.227), 295 (4.709). MS *m/z* (%): 388.0 [MH^+] (88), 331.9 [MH^+ - C_4H_8] (100). Anal. Calcd for $C_{14}H_{22}N_5O_3Br$: C. 43.3; H: 5.7; N: 18.0; O: 12.4; Br: 20.6; Found: C: 43.6; H: 5.6; N: 17.9.

6-[*N*'-(*tert*-Butoxyhydroxymethyl)hydrazino]-2-butylaminopyrimidine-4-carboxylic acid butylamide (12a): 11 (142 mg, 0.36 mmol) was allowed to react with butylamine (357 µL, 263 mg, 3.60 mmol) following **GP2** (ethyl acetate/hexanes 1:1, R_f = 0.37) to give **12a** (93 mg, 68 %) as a yellow solid.

mp: 184-186 °C. IR (KBr disk): 3372 cm^{-1}, 3213, 2962, 1735, 1665, 1589, 1528, 1460, 1367, 1250, 1161, 1015, 771. ^{1}H-NMR ($CDCl_3$): δ: 0.73-0.97 ppm (m, 6 H, 2 CH_3), 1.32-1.66 (m, 17 H, Boc-CH_3, 4 CH_2), 3.32-3.45 (m, 4 H, 2 *CH_2*NH), 5.14 (br s, 1 H, NH), 6.73 (br s, 1 H, NH), 6.80 (s, 1 H, PHA-H), 7.09 (br s, 1 H, NH), 7.89 (br s, 1 H, NHCO). ^{13}C-NMR ($CDCl_3$): δ: 13.8 ppm (+, CH_3), 13.9 (+, CH_3), 20.0 (-), 20.1 (-) 28.2 (+, Boc-CH_3), 31.6 (-), 31.7 (-), 39.0 (-), 41.2 (-), 88.9 (C_{quat}, Boc), 91.0 (+, PHA-H), 155.7 (C_{quat}), 156.0 (C_{quat}), 161.3 (C_{quat}), 161.4 (C_{quat}), 163.8 (C_{quat}), 164.1 (C_{quat}). UV (MeOH): λ_{max} (log ε): 236 nm (5.197), 328 (4.460). MS *m/z* (%): 381.1 [MH^+] (100).

6-[N'-(tert-Butoxy-hydroxymethyl)hydrazino]-2-diethylaminopyrimidine-4-carboxylic acid butylamide (12b): To a solution of **11** (242 mg, 0.62 mmol) in DMF (3 mL) was added diethylamine (312 µL, 228 mg, 3.12 mg) and the solution was stirred for 16 h at 70 °C. The solution was diluted with ethyl acetate (10 mL) and washed with brine (2x20 mL). The organic extract was dried over $MgSO_4$ and evaporated off. The residue was purified by column chromatography (hexanes/ethyl acetate 1:2, R_f = 0.33) to afford **12b** (200 mg, 82 %).

mp: 165-167 °C. IR (KBr disk): 3226cm^{-1}, 2967, 2933, 1732, 1668, 1601, 1531, 1368, 1253, 1161. ^{1}H-NMR ($CDCl_3$): δ: 0.95 ppm (t, ^3J = 7.3 Hz, 3 H, CH_3 Bu), 1.17 (t, ^3J = 7.0 Hz, 6 H, CH_3 Et), 1.30-1.73 (m, 13 H, Boc-CH_3, CH_3*CH_2*, CH_3CH_2*CH_2*), 3.41 (q, ^3J = 6.7 Hz, *CH_2*NHCO), 3.57 (q, ^3J = 7.0 Hz, CH_2 Et), 6.68-6.77 (m, 2 H, NH), 6.91 (s, 1 H, PHA-H), 7.88 (br s, 1 H, NHCO). ^{13}C-NMR ($CDCl_3$): δ: 13.5 ppm (+, CH_3 Bu), 13.6 (+, CH_3 Et), 19.4 (-, CH_3*CH_2*), 27.9 (+, Boc-CH_3), 31.0 (-, CH_3CH_2*CH_2*), 38.2 (-, CH_2 Et), 38.5 (-, CH_2 Et), 40.7 (-, CH_2NHCO), 80.2 (C_{quat}, Boc), 98.3 (+, PHA-H), 155.6 (C_{quat}, C=O Boc), 158.6 (C_{quat}), 161.3 (C_{quat}), 163.5 (C_{quat}), 167.2 (C_{quat}). UV (MeCN): λ_{max} (log ε): 243 nm (5.316), 330 (4.645). MS *m/z* (%): 783.6 [2M+Na^+] (4), 761.7 [2M+H^+], 381.0 [M+H^+] (100), 324.9 [MH^+-C_4H_8] (32), 280.8 [MH^+-Boc] (48).

***tert*-Butyl *N*'-(6-butylcarbamoyl-2-morpholin-4-ylpyrimidin-4-yl)hydrazine carboxylate (12c):** Compound (**11**) (100 mg, 0.26 mmol) was allowed to react with 1,4,7,10,13-pentaoxa-16-aza-cyclo-octa-decane (225 µL, 225 mg, 2.58 mmol) following **GP2** (ethyl acetate/hexanes 1:1, R_f = 0.24) to give **12c** (68 mg, 0.17 mmol, 66 %) as a colourless, hygroscopic solid.

mp: 167-170 °C. IR (KBr disk): 3356 cm^{-1}, 3290, 3197, 2691, 1724, 1659, 1558, 1523, 1447, 1361, 1254, 1161, 1104, 1002, 875. ^{1}H-NMR ($CDCl_3$): δ: 0.95 ppm (t, ^3J = 7.3 Hz, 3 H, CH_3), 1.23-1.64 (m, 13 H, Boc-CH_3, CH_3*CH_2*, CH_3CH_2*CH_2*), 3.41 (dd, ^3J = 7.1 Hz, ^3J = 13.4 Hz, 2 H, *CH_2*NHCO), 3.75 (br s, 8 H, CH_2 morpholine), 6.61-6.82 (m, 3 H, PHA-H, 2 NH), 7.79 (t, ^3J = 5.6 Hz). ^{13}C-NMR ($CDCl_3$): δ: 13.8 ppm (+, CH_3), 20.2 (-, CH_3CH_2), 28.2 (+, Boc-CH_3), 31.7 (-, CH_3CH_2*CH_2*), 39.1 (-, CH_2NHCO), 44.3 (-, CH_2 morpholine), 66.8 (-, CH_2 morpholine), 81.8 (C_{quat}, Boc), 91.2 (+, PHA-H), 155.8 (C_{quat}, C=O Boc), 157.4 (C_{quat}), 160.7 (C_{quat}), 163.8 (C_{quat}), 166.7 (C_{quat}). UV (MeOH): λ_{max} (log ε): 242 nm (5.449), 328 (4.680). MS *m/z* (%): 395.1 [MH^+] (100), 296.8 [MH^+-Boc] (52). HRMS Calcd for

$C_{18}H_{30}N_6O_4$: 394.2329; Found: 394.2330±0.0004. Anal. Calcd for $C_{18}H_{30}N_6O_4$: C: 54.8; H: 7.7; N: 21.3; O: 16.2; Found: C: 54.8; H: 7.5; N: 21.5.

***tert*-Butyl *N*'-[6-butylcarbamoyl-2-(1,4,7,10,13-pentaoxa-16-azacyclooctadec-16-yl)pyrimidin-4-yl]hydrazine carboxylate (12d):** Compound (**11**) (145 mg, 0.37 mmol) and triethylamine (154 µL, 112 mg, 1.11 mmol) were dissolved in DMF (3 mL) and 1,4,7,10,13-pentaoxa-16-aza-cyclooctadecane (292 mg, 1.11 mmol) was added. The resulting solution was stirred for 36 h at 65 °C, diluted with ethyl acetate (5 mL) and washed with brine (2x10 mL). The organic extract was dried over $MgSO_4$, evaporated off and purified by column chromatography (ethyl acetate, $R_f = 0.17$) to afford **12d** (118 mg, 56 %).

mp: > 180 °C (decomp). IR (CH_2Cl_2): 3110 cm^{-1}, 2979, 1665, 1571, 1259, 902, 886. ^{1}H-NMR ($CDCl_3$): δ: 0.87 ppm (t, ^3J = 7.3 Hz, 3 H, CH_3), 1.21-1.72 (m, 13 H, Boc-CH_3, 2 CH_2), 3.32 (q, ^3J = 6.8 Hz, 2 H, *CH_2*NHCO), 3.41-3.94 (m, 24 H, CH_2 crown ether), 6.39-6.99 (m, 3 H, PHA-H, 2 NH), 7.84 (t, ^3J = 5.6 Hz, 1 H, NHCO). ^{13}C-NMR ($CDCl_3$): δ = 13.8 ppm (+, CH_3), 20.1 (-, CH_3CH_2), 28.2 (+, Boc-CH_3), 39.0 (-, CH_3CH_2*CH_2*), 48.8 (-, *CH_2*NHCO), 69.5 (-, CH_2 crown ether), 70.7 (-, CH_2 crown ether), 70.8 (-, CH_2 crown ether), 81.6 (C_{quat}, Boc), 90.1 (+, PHA-H), 155.8 (C_{quat}, Boc C=O), 157.4 (C_{quat}), 160.4 (C_{quat}), 164.1 (C_{quat}), 166.6 (C_{quat}). UV (MeCN): λ_{max} (log ε): 244 nm (5.255), 331 (4.578). MS *m/z* (%): 609.3 [M+K^+] (12), 593.3 [M+Na^+] (10), 571.4 [MH^+] (100). HRMS Calcd for $C_{26}H_{46}N_6O_8$: 570.3377; Found: 570.3379±0.0004.

***tert*-Butyl N'-{6-butylcarbamoyl-2-[2-(2-methoxyethoxy)ethylamino]pyrimidin-4-yl}hydrazine carboxylate (12e):** Compound (**11**) (110 mg, 0.28 mmol) was allowed to react with 2-(2-methoxy-ethoxy)ethylamine (361 µL, 334 mg, 2.80 mmol) following **GP2** (ethyl acetate, $R_f = 0.16$) to give **12e** (33 mg, 28 %) as a colourless powder and unreacted **11** (51 mg, 0.13 mmol).

mp: 88-89 °C. IR (KBr disk): 3436 cm^{-1}, 3376, 3242, 2963, 1730, 1670, 1590, 1569, 1522, 1440, 1257, 1149, 1091, 802. ^{1}H-NMR ($CDCl_3$): δ: 0.95 ppm (t, ^3J = 7.3 Hz, 3 H, CH_3), 1.36-1.46 (m, 11 H, Boc-CH_3, 2 CH_2), 1.53-1.63 (m, 2 H, CH_2), 3.36-3.43 (m, 5 H, OCH_3, CH_2), 3.55-3.65 (m, 8 H, 4 CH_2), 5.55 (br s, 1 H, NH), 6.74-6.81 (m, 2 H, PHA-H, NH), 7.00 (br s, 1 H, NH), 7.86 (br s, 1 H, NH). ^{13}C-NMR ($CDCl_3$): δ: 12.8 ppm (+, CH_3 Bu), 19.1 (-), 27.2 (+, Boc-CH_3), 30.6 (-), 38.1 (-), 40.1 (-), 58.0 (+, OCH_3), 69.0 (-), 69.3 (-), 70.9 (-), 80.8 (C_{quat}, Boc), 90.3 (+, PHA-H), 154.6 (C_{quat}, C=O Boc), 158.6 (C_{quat}), 160.2 (C_{quat}), 162.7 (C_{quat}), 166.1 (C_{quat}). UV (MeOH): λ_{max} (log ε): 242 nm (4.758). MS *m/z* (%): 427.1 [MH^+] (100), 853.6 [2M+H^+] (8).

Methyl 2-diethylamino-6-hydrazinopyrimidin-4-carboxylate bishydrochloride (13): Compound (**8a**) (53 mg, 0.16 mmol) is dissolved in ether saturated with HCl (2 mL) at 0 °C and stirred for 15 min. Removal of the solvent under reduced pressure gives **13** in quantitative yield (50 mg). The product is used without further purification.

mp: > 185 °C (decomp). IR (KBr disk): 3446 cm^{-1}, 2887, 1744, 1643, 1591, 1521, 1449, 1337, 1254, 1182, 1058. ^{1}H-NMR (DMSO-d_6): δ: 1.14 ppm (t, ^3J = 6.9 Hz, 6 H, CH_3), 3.64 (q, ^3J = 6.9 Hz, 4 H, CH_2), 3.86 (s, 3 H, OCH_3), 6.56 (s, 1 H, PHAt-H), 10.55 (br s, 5 H, NH). MS *m/z* (%): 240.0 [MH^+] (100).

Lithium 6-(*N*'-*tert*-butoxycarbonylhydrazino)-2-diethylaminopyrimidine-4-carboxylate (14): To a solution of **8a** (108 mg, 0.32 mmol) in a 4:1 mixture of acetone/water (6 mL) was added $LiOH{\cdot}2H_2O$ (13.4 mg, 0.32 mmol). The reaction mixture was stirred for 6 h at rt. The solvents are removed under reduced pressure to afford **14** (100 mg, 94 %) in almost quantitative yield. The salt is used without further purification.

mp: > 185 °C (decomp). IR (KBr disk): 3298 cm^{-1}, 2974, 1725, 1589, 1520, 1420, 1362, 1247, 1164, 1077, 859, 784. ^{1}H-NMR (DMSO-d_6): δ: 1.06 ppm (t, ^3J = 6.9 Hz, 6 H, CH_3), 1.42 (s, 9 H, Boc-CH_3), 3.51 (q, ^{3}J = 6.9 Hz, 4 H, CH_2), 6.33 (s, 1 H, PHA-H), 8.68 (bs, 1 H, NH), 8.95 (bs, 1 H, NH). ^{13}C-NMR (DMSO-d_6): δ: 13.8 ppm (+, CH_3), 29.5 (+, Boc-CH_3), 40.6 (-, CH_2), 78.9 (C_{quat}, Boc), 90.4 (+, PHA-H), 155.7 (C_{quat}, C=O Boc), 160.4 (C_{quat}), 166.9 (C_{quat}), 167.7 (C_{quat}), 174.6 (C_{quat}, C=O carboxylate). MS *m/z* (%): 326.0 [M-H^+] (100).

Methyl 6-{*N*'-[6-(*N*'-tert-butoxycarbonylhydrazino)-2-diethylaminopyrimidine-4-carbonyl]-hydrazino}-2-diethylaminopyrimidine-4-carboxylate (15): Lithium salt (**14**) (198 mg, 0.35 mmol), hydrazine dihydrochloride (**13**) (109 mg, 0.35 mmol), HOAt (57 mg, 0.42 mmol), HATU (160 mg, 0.42 mmol), and Huenig´s Base (238 µL, 181 mg, 1.40 mmol) are dissolved in dichloromethane (5 mL). The solution is stirred for 30 h at rt. The solvent is removed under reduced pressure and the yellow residue purified by column chromatography (ethyl acetate/hexanes 3:1, R_f = 0.68) to afford **15** (78 mg, 40 %) as a colorless powder.

mp: 154-156 °C. IR (KBr disk): 3257 cm^{-1}, 3388, 2974, 1720, 1604, 1583, 1548, 1474, 1435, 1130, 1259, 1165, 1122. ^{1}H-NMR (DMSO-d_6): δ: 0.81-1.22 ppm (m, 12 H, 4 CH_3), 1.28-1.54 (m, 9 H, Boc-CH_3), 3.34-3.78 (m, 8 H, 4 CH_2), 3.80 (s, 3 H, OCH_3), 6.15-6.70 (m, 4 H, 2 N-H, 2 PHA-H), 8.50-10.2 (m, br s, 2 H, N-H). ^{13}C-NMR (DMSO-d_6): δ: 12.7 ppm (+, CH_3), 13.1 (+, CH_3), 28.0 (+, Boc-CH_3), 40.8 (-, CH_2), 52.2 (+, OCH_3), 85.8 (C_{quat}, Boc), 121.6 (+, PHA-H), 129.5 (+, PHA-H), 152.3 (C_{quat}, C=O Boc), 160.5 (C_{quat}, C=O), 165.4 (C_{quat}, C=O); further signals could not be labelled. UV (MeCN): λ_{max} (log ε): 211 nm (5.483), 245 (5.519), 346 (4.933). MS *m/z* (%): 547.4 [MH^+] (100). HRMS Calcd for $C_{24}H_{38}N_{10}O_5$: 546.3027; Found: 546.3026±0.0004.

Methyl 2-(2-{2-[2-({1-[6-(*N*'-*tert*-butoxycarbonylhydrazino)-2-diethylaminopyrimidine-4-carbonyl]-pyrrolidine-2-carbonyl}amino)acetylamino]-3-phenylpropionylamino}-4-methylpentanoylamino)-3-methylbutyrate (16): Compound (**14**) (75 mg, 0.23 mmol), H-D-Pro-Gly-Phe-Val-Leu-OMe (131 mg, 0.23 mmol), EDC (45 µL, 39 mg, 0.25 mmol), HOBt (34 mg, 0.25 mmol), and Huenig´s Base (75 µL,

57 mg, 0.46 mmol) were dissolved in DMF (3 mL). The reaction mixture was stirred for 18 h at rt. At 0 °C, water (5 mL) was added to precipitate (**16**) (99 mg, 52 %) in analytical pure form.

mp: 121 °C. IR (KBr disk): 3349cm^{-1}, 3024, 2995, 1754, 1670, 1591, 1364, 1275, 1161, 913, 898. ^{1}H-NMR (DMSO-d_6): δ: 0.74-0.95 ppm (m, 12 H; HSQC: CH_3 Leu, CH_3 Val), 1.04-1.18 (m, 6 H; HSQC: CH_3 PHA), 1.34-1.45 (m, 9 H; HSQC: Boc-CH_3), 1.46-1.53 (m, 1 H; COSY: CH_s Val), 1.54-1.70 (m, 2 H; COSY: CH_2 Pro), 1.75-1.85 (m, 2 H; COSY CH_2 Pro), 1.86-2.01 (m, 2 H; COSY: CH_2 Leu), 2.02-2.16 (m, 1 H; COSY: CH_s Leu), 2.70-2.89 (m, 1 H; HMBC: CH_2 Phe), 2.94-3.08 (m, 1 H; HMBC: CH_2 Phe), 3.41-3.81 (m, 11 H; COSY: $C_\delta H_2$ Pro, CH_2 PHA; HSQC: OCH_3, CH_2 Gly), 4.12-4.22 (m, 1 H; COSY: CH Pro), 4.24-4.33 (m, 1 H; COSY: CH Val), 4.38-4.47 (m, 1 H; COSY: CH Leu), 4.51-4.71 (m, 1 H; COSY: CH Phe), 5.99-6.24 (m, 1 H; HSQC: PHA-H), 7.10-7.29 (m, 5 H; HSQC: Ar-H), 7.79-7.91 (m, 1 H, NH), 7.92-8.00 (m, 1 H; NH), 8.04-8.11 (m, 1 H; NH), 8.14-8.24 (m, 1 H; NH), 8.30-8.38 (m, 1 H; NH), 8.45-8.55 (m, 1 H; NH). ^{13}C-NMR (DMSO-d_6): δ: 13.5 ppm (+; HMQC: CH_3 PHA), 18.5 (+; HMQC: CH_3 Leu), 18.7 (+; HMQC: CH_3 Leu), 19.5 (+; HMQC: CH_3 Val), 21.7 (+; HMQC: CH_3 Val), 23.2 (-; HSQC: CH_2 Pro), 24.6 (+; HSQC: CH_s Leu), 28.5 (+; HMQC: Boc-CH_3), 28.6 (+; HMQC; Boc-CH_3), 29.6 (-; HSQC: CH_2Pro), 31.1 (+; HSQC: CH_s Val), 40.2 (-; HSQC: CH_2 PHA), 40.7 (-; HSQC: CH_2 Leu), 41.8 (-; HSQC: CH_2 Pro), 42.5 (-; HSQC: CH_2 Gly), 50.7 (-; HSQC: CH_2 Phe), 52.2 (+; HSQC: OCH_3), 54.4 (+; HSQC: CH Val), 58.2 (+; HMBC: CH Phe), 60.7 (+; HSQC: CH Pro), 62.5 (+; HSQC: CH Leu), 79.4 (C_{quart}; HMBC: Boc), 116.7 (+; HSQC: PHA-H), 126.7 (+; HSQC: Ar-H), 128.5 (+; HSQC: Ar-H), 129.7 (+; HSQC: Ar-H), 138.2 (C_{quart}; HMBC: Ar), 156.8 (C_{quart}; HMBC: C=O Boc), 167.5 (C_{quart}), 168.4 (C_{quart}; HMBC: C=O Gly), 168.5 (C_{quart}; HMBC: C_{Het}-NEt_2), 169.3 (C_{quart}), 170.6 (C_{quart}; HMBC: C=O Phe), 170.7 (C_{quart}; HMBC: C=O Pro), 172.5 (C_{quart}; HMBC: C=O Leu), 172.7 (C_{quart}; HMBC: C=O Val). UV (MeOH): λ_{max} (log ε): 330 nm (6.961). MS *m/z* (%): 853.6 [MH^+] (100). HRMS Calcd for $C_{42}H_{65}N_{10}O_9^+$: 853.4936; Found: 853.4926±0.0006.

Lithium 6-{*N*'-[6-(*N*'-*tert*-butoxycarbonylhydrazino)-2-methylpyrimidine-4-carbonyl]hydrazino}-2-methylpyrimidine-4-carboxylate (17): To a solution of **15** (216 mg, 0.50 mmol) in a 4:1 mixture of acetone/water (8 mL) was added $LiOH{\cdot}2H_2O$ (20.6 mg, 0.50 mmol). The reaction mixture was stirred for 14 h under reflux. The solvents are removed under reduced pressure to afford **17** (202 mg, 95 %) in almost quantitative yield. The salt is used without further purification.

mp: 185 °C (decomp). IR (KBr disk): 3332 cm^{-1}, 2977, 1725, 1588, 1524, 1360, 1247, 1167. ^{1}H-NMR (DMSO-d_6): δ: 0.96-1.11 ppm (m, 12 H, CH_3), 1.44 (s, 9 H, Boc-CH_3), 3.38-3.55 (m, 8 H, CH_2), 6.39 (s, 1 H, PHA-H), 6.89 (s, 1 H, PHA-H), 8.24 (br s, 1 H, NH), 8.68 (br s, 1 H, NH), 8.87 (br s, 1 H, NH), 8.95 (br s, 1 H, NH). ^{13}C-NMR (DMSO-d_6): δ: 13.6 ppm (+, CH_3), 13.8 (+, CH_3), 29.5 (+, Boc-CH_3), 40.2 (-, CH_2), 40.6 (-, CH_2), 78.5 (C_{quat}, Boc), 90.4 (+, PHA-H), 98.7 (+, PHA-H), 155.7 (C_{quat}, C=O Boc), 160.4

(C_{quat}), 160.9 (C_{quat}), 164.7 (C_{quat}), 165.4 (C_{quat}), 166.9 (C_{quat}), 167.7 (C_{quat}), 169.6 (C_{quat}), 174.8 (C_{quat}, C=O carboxylate). MS *m/z* (%): 419.1 [M-H^+] (100).

Turn structure (**18)**: Compound (**17**) (43 mg, 0.10 mmol), D-Pro-Gly-Phe-Leu-Phe-Ala-NH_2.TFA (73 mg, 0.10 mmol), EDC (54 µL, 47 mg, 0.30 mmol), HOBt (41 mg, 0.30 mmol), and Huenig´s Base (65 µL, 50 mg, 0.40 mmol) were dissolved in DMF (4 mL). The reaction mixture was stirred for 15 h at rt. At 0 °C, water (5 mL) was added to precipitate (**18**) (87 mg, 75 %) in analytical pure form.

mp: 118 °C. IR (KBr disk): 3321 cm^{-1}, 2961, 2297, 1728, 1662, 1576, 1531, 1456, 1373, 1272, 1161, 1125, 1074, 779, 703. ^{1}H-NMR (DMSO-d_6, 290 K): δ: 0.61-0.64 ppm (m, 3 H; COSY: CH_3 Leu), 0.72-0.76 (m, 3 H; COSY: CH_3 Leu), 0.90-1.14 (m, 18 H; COSY: CH_3 PHA), 1.21 (d, ^{3}J = 7.05 Hz, 3 H; COSY: CH_3 Ala), 1.25-1.33 (m, 3 H; COSY: CH_2 Leu, CH_s Leu), 1.35-1.44 (m, 13 H; HMBC: Boc-CH_3; COSY: 2xCH_2 Pro), 1.76-1.87 (m, 1 H; COSY: CH_2 Pro), 2.06-2.13 (m, 1 H; COSY: CH_2 Pro), 2.75-2.85 (m, 1 H; NOESY: CH_2 Phe), 2.89-2.98 (m, 1 H; NOESY: CH_2 Phe), 3.02-3.10 (m, 2 H; NOESY: CH_2 Phe), 3.27-3.68 (m, 8 H; COSY: CH_2 PHA), 3.70-3.83 (m, 2 H; COSY: CH_2 Gly), 4.06-4.20 (m, 2 H; COSY: CH Leu, CH Ala), 4.35-4.55 (m, 3 H; COSY: CH Pro; NOESY: CH Phe, CH Phe), 6.29 (s, 1 H: HMBC: PHA-H), 6.99-7.37 (m, 13 H; HMBC: Ar-H, PHA-NH, Ala-NH_2), 7.70 (d, ^{3}J = 7.8 Hz, 1 H; COSY: NH Phe), 7.74-7.82 (m, 1 H; COSY: NH Ala), 7.85 (d, ^{3}J = 7.4 Hz, 1 H; NOESY: PHA-NH), 7.88-7.96 (m, 1 H; COSY: NH Phe), 7.97-8.00 (m, 1 H; COSY: NH Leu), 8.14-8.17 (m, 1 H; NOESY: PHA-NH), 8.93 (bs, 1 H; NOESY: PHA-NHN*H*), 10.09 (s, 1 H; NOESY: PHA-NHN*H*). ^{13}C-NMR (DMSO-d_6): δ: 15.7 ppm (+; HMQC: CH_3 Ala) 17.4 (+; HMQC: CH_3 Leu), 18.9 (+; HMQC: CH_3 Leu), 22.0 (+; HMQC: CH_3 PHA), 24.0 (-; HMQC: CH_2 Pro), 27.8 (-; HMQC: CH_2 Pro), 29.8 (+; HMBC: CH_s Leu), 31.2 (+; HMQC: Boc-CH_3), 33.7 (-; HMQC: CH_2 Pro), 36.7 (-; HMQC: CH_2 Phe), 37.4 (-; HMQC: CH_2 Phe), 39.4 (-; HMBC: CH_2 Leu), 47.4 (-; HMQC: CH_2 Gly), 50.1 (+; HMQC: CH Pro), 51.3 (-; HMQC: CH_2 PHA) 53.5 (+; HMQC: CH Phe), 57.4 (+; HMBC: CH Leu), 57.7 (+; HMQC: CH Ala), 60.3 (+; HMQC: CH Phe), 79.7 (C_{quat}; HMBC: Boc), 126.0 (+; HMBC: Ar-H), 127.7 (+; HMBC: Ar-H), 128.1 (+; HMBC: Ar-H), 129.1 (+; HMBC: Ar-H), 130.7 (+; HMBC: Ar-H), 137.3 (C_{quat}; HMBC: Ar), 137.5 (C_{quat}; HMBC: Ar). Further signals are not detectable. UV (MeOH): λ_{max} (log ε): 245 nm (3.275), 346 (1.730). UV (MeOH): λ_{max} (lg ε): 245 nm (3.275), 346 nm (1.730). MS *m/z* (%): 1186.8 [M+Na^+] (30), 1164.7 [MH^+] (80), 583.1 [M+$2H^+$] (100). HRMS Calcd for $C_{57}H_{82}N_{17}O_{10}^+$: 1164.6431; Found: 1164.6436±0.0005.

ACKNOWLEDGEMENTS

V. M. thanks the International Quality Network Medicinal Chemistry (IQN-MC; funded by the DAAD) for a postdoctoral stipend. We acknowledge support by the Graduiertenkolleg (GRK 760) Medicinal Chemistry and the Fonds der Chemischen Industrie.

REFERENCES AND NOTES

1. S. Maitra and J. S. Nowick, 'The Amide Linkage: Structural Significance in Chemistry, Biochemistry and Material Science,' ed. by A. Greenberg, C. M. Brenemann, and J. F. Liebman, John Wiley & Sons, New York, 2000, Chapter 15.

2. C. Petosa, R. J. Collier, K. R. Klimpel, S. H. Leppla, and R. C. Liddington, *Nature,* 1997, **385**, 833.

3. D. P. Fairlie, M. L. West, and A. K. Wong, *Curr. Med. Chem.,* 1998, **5**, 29.

4. For reviews, see: A. Gianni and T. Kolter, *Angew. Chem.*, 1993, **105**, 1303; *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1244; R. A. Wiley and D. H. Rich, *Med. Res. Rev.*, 1993, **13**, 327.

5. L. Regan and W. F. DeGrado, *Science,* 1988, **241**, 976; J. J. Osterhout, T. Handel Jr., G. Na, A. Toumadje, R. C. Long, P. J. Connolly, J. C. Hoch, W. C. Johnson, D. Live Jr., and W. F. DeGrado, *J. Am. Chem. Soc.*, 1992, **114**, 331.

6. J. S. Nowick, N. A. Powell, E. J. Martinez, E. M. Smith, and G. J. Noronha, *J. Org. Chem.*, 1992, **57**, 3763; J. S. Nowick, M. Abdi, K. A. Bellamo, J. A. Love, E. J. Martinez, G. Noronha, E. M. Smith, and J. W. Ziller, *J. Am. Chem. Soc.*, 1995, **117**, 89; J. S. Nowick, S. Mahrus, E. M. Smith, and J. W. Ziller, *J. Am. Chem. Soc.*, 1996, **118**, 1066.

7. J. S. Nowick, D. L. Holmes, G. Mackin, G. Noronha, A. J. Shaka, and E. M. Smith, *J. Am. Chem. Soc.*, 1996, **118**, 2764.

8. Wavefunction Inc., 18401 Von Karmann Ave., Suite 370, Irvine, CA 92612; J. J. P. Stewart, *J. Comput. Chem.*, 1989, **10**, 209.

9. H. Gershon, *J. Org. Chem.*, 1962, **27**, 3507.

10. M. Yukawa, T. Niiya, Y. Goto, T. Sakamoto, H. Yoshizawa, A. Watanabe, and H. Yamanaka, *Chem. Pharm. Bull.*, 1989, **37**, 2892.

11. Temperature dependent spectra show characteristic coalescence for *cis-trans* isomerisation of carbamates.

12. L. O. Ross, L. Goodman, and B. R. Baker, *J. Org. Chem.*, 1960, **25**, 1950.

13. Several conditions to form the amide bond have been tried, but the yield of the coupling reaction could not be improved.

14. H. J. Schneider, R. Kramer, S. Simova, and U. Schneider, *J. Am. Chem. Soc.*, 1988, **110**, 6442; C. S. Wilcox, 'Frontiers in Suplramolecular Chemistry and Photochemistry, ' ed. by H. J. Schneider and H. Duerr, VCH, Weinheim, 1991.

15. P. Job, *Comput. Rend.*, 1925, **180**, 928; M. T. Blanda, J. H. Horner, and M. Newcomb, *J. Org. Chem.*, 1989, **54**, 4626.

16. G. N. Ramachandran and V. Sasisekharan, *Adv. Protein Chem.*, 1968, **23**, 332.

17. F. A. Syud, H. E. Stanger, and S. H. Gellman, *J. Am. Chem. Soc.*, 2001, **123**, 8667; J. D. Fisk, D. R. Powell, and S. H. Gellman, *J. Am. Chem. Soc.*, 2000, **122**, 5443; T. S. Haque, J. C. Little, and S. H. Gellman, *J. Am. Chem. Soc.*, 1996, **118**, 6975.

18. Additional NOE contacts between the PHA unit and the peptide strand are expected, but not observed due to broad NH signals of the protons of the hydrazine. Resonances of NH protons of **16** appear shifted downfield of the analogous protons of the controls Boc-D-Pro-Gly-Phe-Val-Leu-OMe and **8a**. The analysis of the NH-C_αH coupling constants is not possible due to signal overlap.