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Luminescent pyrimidine hydrazide oligomers with peptide affinity

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Abstract—The modular synthesis of pyrimidine oligohydrazides and their peptide binding ability are reported. Ethylene glycol substituents ensure water solubility of the compounds. The pattern of hydrogen bond donors and hydrogen bond acceptors resembles the functionalities of a peptide backbone, and intramolecular hydrogen bonds restrict conformational mobility. The pyrimidine heterocycles show emission at 423 nm if either excited with light of 320 nm or by a FRET process from a nearby Trp residue. This property is useful for the luminescent detection of interactions with peptides and proteins.

1. Introduction

Non-biological oligomers with defined secondary or tertiary structure[1–3] provide templates for the design of molecules that interact with protein surfaces and compete for protein–protein[4,5] or protein–membrane interactions. Recent examples include synthetic mimics of α-helical surface binding domains,[6] β-peptides[7] that fold into well-defined three-dimensional structures, turn mimics[8] that align peptides or peptidomimetic chains which form β-sheet structures.

Peptide β-sheet mimics[9,10] typically consist of heterocyclic or heteroaromatic units that ensure an overall planar orientation (Scheme 1). Nowick used an ornithine residue with the β-strand-mimicking *ortho*-hydroxy-4-amino benzoic acid (Hao)[11] to extensively probe the intramolecular induction of sheet conformations into small natural peptides (**1**),[12] and more recently, to investigate side-chain interactions[13] or the preference of homo- versus heterochiral binding of β-sheets.[14] In earlier work, oligomers of 5-amino-2-methoxybenzoic acid and 5-hydrazino-2-methoxybenzoic acid were investigated.[15] Oligoamides of a pyrazol amino acid (**2**) were used by Schrader and Kirsten[16] and König and co-workers.[17] Schrader showed that small oligoamides of this type intercept peptide aggregation[18] and interact with the KLVFF nucleation sequence for the pathogenic aggregation of the Alzheimer's peptide.[19] Bartlett introduced unsaturated cyclohexenone @-tide[20] β-strand peptidemimetics (**3**). Modified pyrrols were used as β-sheet mimics based on α,β-dehydroamino acids (MOPAS, **4**)[21] and combined with Gellman's turn[22] to study the intramolecular interaction with peptides of natural amino acids. However, most heterocyclic peptide β-sheet mimics are not water soluble, which limits investigations. Exceptions are aminopyrazole hybrid receptors[19] or 5-amino-2-methoxybenzamide templates.[23]

The interaction of peptide β-sheet mimics with peptides or proteins is typically monitored by NMR techniques or luminescent labeling is required. An exception is Bartlett's @-tide β-strand peptidemimetic.[20] The circular dichroism (CD) signature of the @-unit changes with its conformation in organic and aqueous solvents and, therefore, is useful as a quantitative measure of the @-tide association and folding processes.[24]

We report here the synthesis of substituted oligomers of pyrimidine hydrazino carboxylic acids (PHAs; general structure **5**) that are water-soluble and signal peptide binding by changes of their luminescent properties. Their photophysical properties allow fluorescence resonance energy transfer from a nearby Trp peptide residue to confirm binding processes.

Keywords: Peptide mimics; Pyrimidine; Hydrazine acid; Luminescence; FRET.
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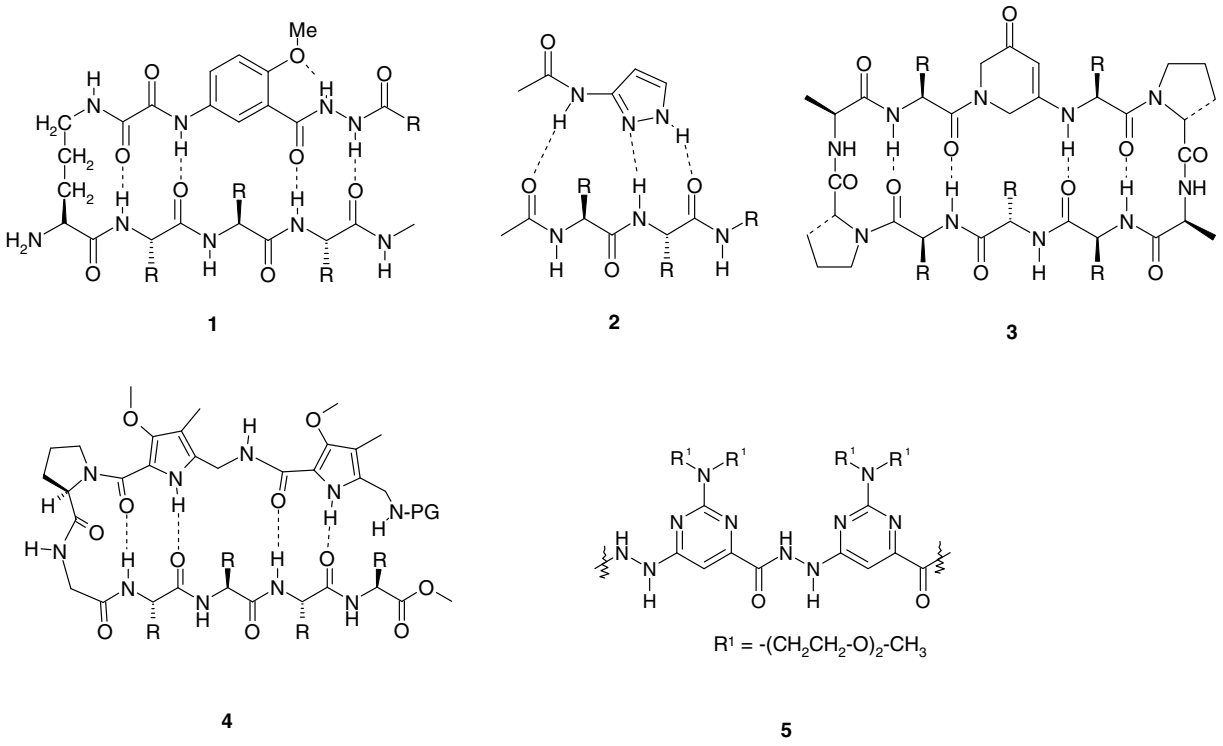


Scheme 1. Structures of heterocyclic peptide β-sheet mimics.

2. Results and discussion

2.1. Synthesis

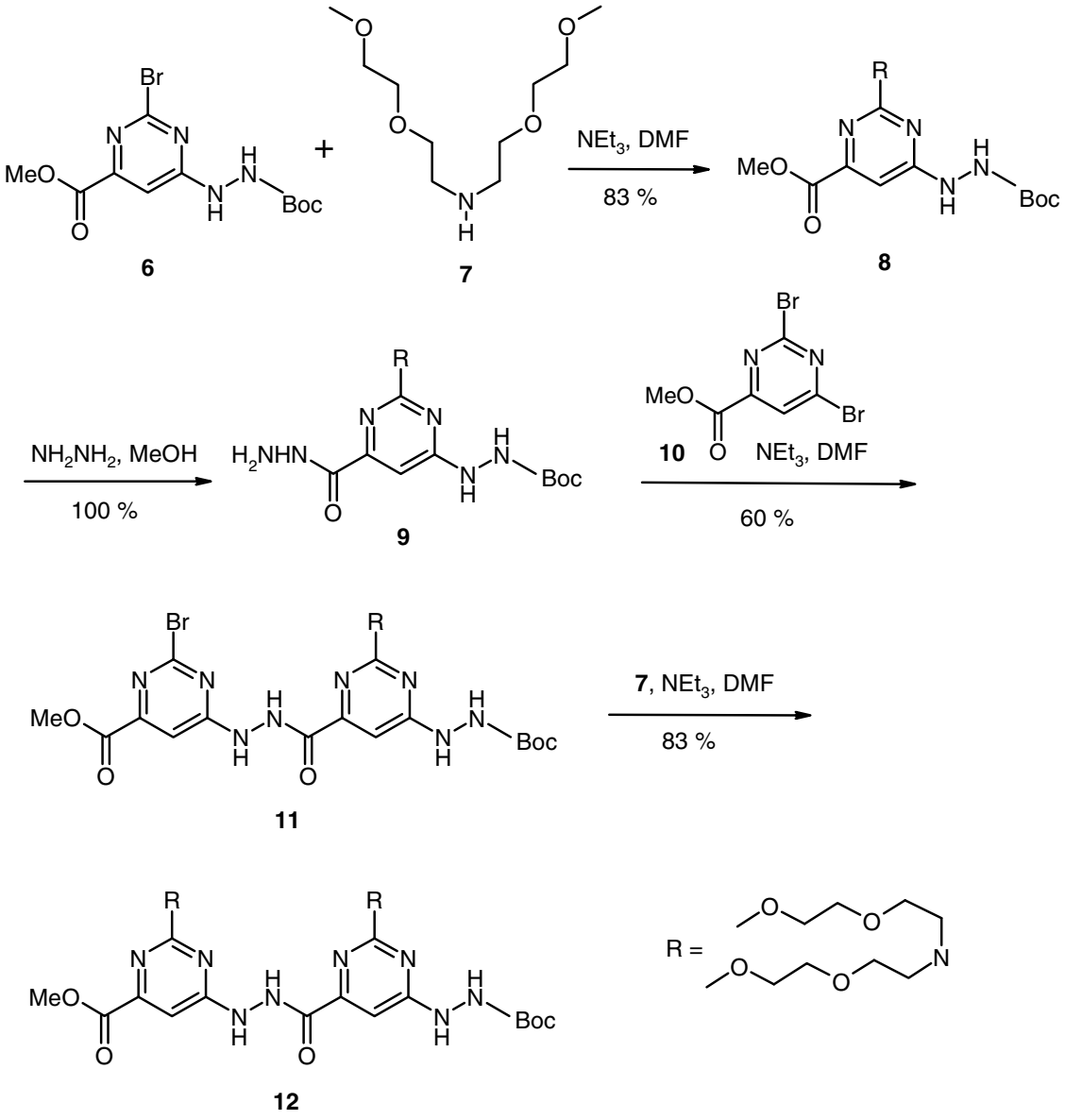
The structure of the pyrimidine hydrazino carboxylic acid (PHA) building block is related to Nowick's 5-hydrazino-2-methoxybenzoic acid. The carbonyl group and the hydrazine moiety provide a hydrogen bond donor and acceptor pattern complementary to a peptide main chain. The intramolecular hydrogen bond of a hydrazine N–H proton to the arene methoxy group in **1** is replaced by a contact to the pyrimidine nitrogen atoms in structure **5**. The proposed conformation of the hydrazine moiety was observed in the X-ray structure analysis of compounds **19**-*Z* and **19**-Boc (see Supporting information). The PHA unit is readily available in large amounts starting from orotic acid. The general synthesis of the pyrimidine heterocycle and its incorporation into turn structures by peptide coupling were recently reported.[25] However, these compounds are not water soluble and the yields of peptide coupling for oligomerization were unsatisfactory. Therefore, an alternative synthetic approach was developed.

Bis-[2-(2-methoxy-ethoxy)-ethyl]-amine **7** was synthesized in three steps and 80% overall yield according to a known procedure.[26] The previously prepared pyrimidine **6** was reacted with **7** to give compound **8** in 83% yield. For the extension to PHA oligoamides, compound **8** was converted quantitatively into the corresponding hydrazide **9**. For the coupling reaction with another PHA unit we choose a regioselective bromine substitution reaction. Hydrazine **9** displaced selectively the bromine atom in position 4 of dibromo pyrimidine **10** to give bis-PHA **11** in 60% yield. Displacement of the remaining bromo substituent by amine **7** gave the highly water-soluble bis-PHA **12** in 85% yield; 51% overall yield for the three steps starting from **8** (Scheme 2).

Next, the method was extended to prepare PHA tri- (**13b**) and tetramers (**14b**). Compound **12** was converted into the corresponding hydrazide in quantitative yield, reacted with dibromo pyrimidine **10** (45%), and treated with amine **7** to give tris-PHA **13b** in 80% yield. The reaction sequence was repeated, starting from **13b**, to give tetra-PHA **14b** in 21% overall yield (Scheme 3).

2.2. Spectroscopic investigations

The emission quantum yields of several substituted PHAs were measured in different solvents with quinine bisulfate as reference to explore variations of the photophysical properties. The effect of the solvent on the emission is similar for all compounds showing decreasing quantum yields with higher polarity. Compounds having electron-donating groups in position 2, such as secondary (**18**) and tertiary amines (**15**–**17**), show higher emission quantum yields than compounds bearing halides, such as chlorine (**19**) and bromine (**6**), in this position. Table 1 summarizes the properties. Compound **20** shows the highest quantum yield due to the aromatic Trp side chain, which acts as an antenna chromophore (vide infra). Extension of the PHA chromophore does not increase the quantum yield. The bis-PHA **16** shows a quantum yield of 8% in $CHCl_3$, which is similar to compound **15**-OMe with 10% quantum yield and one PHA chromophore.



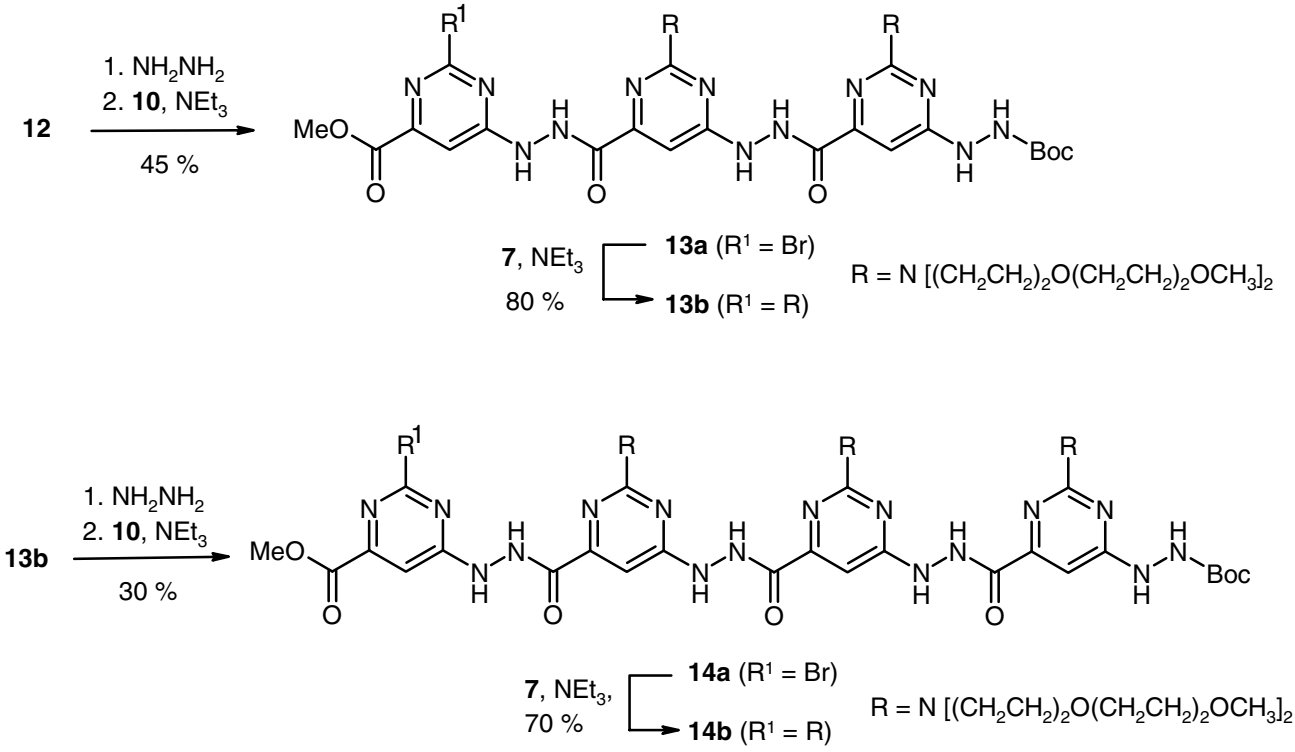
Scheme 2. Synthesis of bis-PHA **12**.

Solvent polarity affects the emission intensity and the maximum emission wavelength. PHA **15**-NEt_2 exhibits an emission maximum at 437 ± 1 nm in $CHCl_3$, which gradually red shifts with increasing solvent polarity. The emission intensity decreases drastically in the presence of water in MeCN solution (Fig. 1). A similar observation is made in the case of the water-soluble PHA **13b**. The emission intensity decreases (quantum yields: 9.1 in $CHCl_3$; 1.1 in water) and the wavelength of maximum intensity shifts to the red with increasing polarity of the solvent (Fig. 2).

The PHA emission is sensitized by Trp. Titration of Fmoc-Trp(Boc)-OH (1.21 μM) with 7 equiv of **15**-NH-Bu in MeCN and excitation of the mixture at 280 nm result in a decrease of the Trp emission at 320 nm, while the PHA emission around 430 nm appears (Fig. 3).

2.3. Binding studies

As structural peptide β-sheet mimics, PHAs are expected to interact with peptides and proteins. The previously reported[25] detailed spectroscopic investigation of a PHA-containing peptide β-turn structure showed the proposed complementary binding of the peptide main chain and PHA groups (see Supporting information for data). To confirm the peptide–PHA interaction motif for ethylene glycol appended PHA structures reported in this work, compound **12** was titrated with Z-Lys(Boc)-Gly-OMe (see Supporting information for data). Only the hydrazine hydrogen atoms next to the pyrimidine show a chemically induced shift upon addition of the peptide, which suggests their involvement in the binding process. The weak affinity prevents a detailed spectroscopic determination of the binding motif in intermolecular aggregates or solvents competing for hydrogen binding.



Scheme 3. Synthesis of trimeric and tetrameric PHAs **13** and **14**.

To show their peptide binding ability, the affinity of compounds **15**-NH-Bu, **12**, and **13b** to the tetradecapeptide somatostatin (SRIF)[27] was investigated. The peptide is a potent regulator of biological functions.[28] For our experiments we used commercially available ampoules of CuraMed® containing SRIF-x-acetate. Figure 4 shows the amino acid sequence of SRIF, which contains a Trp residue in the β-turn region. Residues Trp 8 and Lys 9 are crucial for the peptides' biological activity.[29]

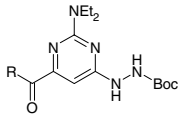
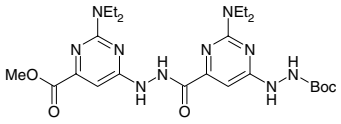
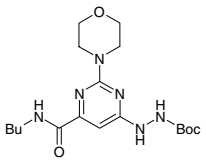
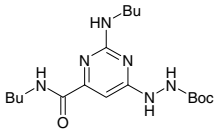
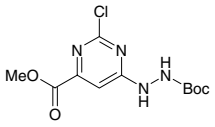
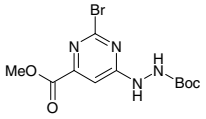
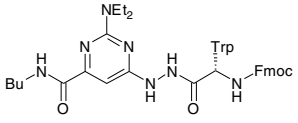
The titration of SRIF with **15**-NH-Bu in acetonitrile was monitored by emission spectroscopy. At different ratios of both compounds, the sample is excited at 281 nm and the quenching of the Trp emission is recorded. In addition, at each titration point the PHA moiety is excited at 320 nm to monitor its spectroscopic change upon peptide binding. Figure 5 summarizes the titration results.

The emission titration of SRIF with **15**-NH-Bu in acetonitrile shows an emission intensity increase of **15**-NH-Bu with an increase of the receptor concentration if either excited at 281 or 320 nm. Saturation is observed in the presence of approximately 7 equiv. Simultaneously the emission (excitation at 281 nm) is quenched with increasing amounts of PHA added, reaching saturation after the addition of 8 equiv PHA. This indicates a FRET process between the Trp of SRIF and the binding PHA.[30] Affinity constants were calculated by non-linear fitting of the emission intensity of Trp8 and **15**-NH-Bu.[31] Assuming a 1:1 binding motif, an affinity constant of $3.4 \times 10^3 \pm 0.2$ L/mol is derived from the Trp quenching curve. As SRIF contains only one Trp, the observed FRET requires donor and acceptor in close proximity (less than 10 nm),[32] and the affinity of the PHA is moderate, the 1:1 motif is likely and its assumption is justified. Job's plot[33] analysis confirmed the expected 1:1 stoichiometry (Fig. 6).

Next, the PHA–SRIF binding process was studied in buffered water using compounds **12** and **13b**, which are soluble under physiological conditions. A 0.01 M phosphate buffer (PBS, pH 7.2) was used as solvent (see Supporting information for the fluorescence titration of **12**, **13b**, and SRIF with selective excitation of Trp8 at 280 nm). The emission changes of PHA in the presence of the peptide are similar to the effects in MeCN, but less pronounced. Quenching of the Trp emission indicates an energy transfer to the PHA, but emission of this chromophore is not effective in the protic solvent. Direct excitation of PHA (at 392 nm) leads to an emission increase in the presence of the peptide. Multiple binding sites on the peptide can lead to many different PHA–SRIF aggregates, which are in dynamic equilibrium and cannot be distinguished. We therefore abstain from deriving apparent binding constants from the titration data. However, the larger initial slope of the emission decrease for **13b** suggests increased affinity to SRIF with extension of the oligoamide chain being similar.

During the titration a shift of the Trp emission maximum is observed. At low concentrations of **12**, SRIF exhibits an emission maximum at 358 ± 1 nm. It gradually red-shifts with increasing concentration of **12**, and finally levels off at 393 ± 1 nm. The wide range of emission maxima of Trp residues in proteins is attributed to differences in the microenvironment and its effect on the

Table 1. Quantum yields of PHAs in chloroform, acetonitrile, and methanol

Compound	Quantum yields[a] (%)		
	$CHCl_3$	MeCN	MeOH
 **15-OMe** R=OMe **15-NHBu** R=NH-Bu **15-NEt$_2$** R=NEt$_2$	10.0 (R = OMe); 10.0 (R = NEt_2)	2.1 (R = OMe); 2.0 (R = NEt_2)	
 **16-Boc**	8.1	2.1	0.9
 **17-Boc**	6.6	5.2	2.0
 **18-Boc**	2.8	2.8	2.3
 **19-Boc**	1.0		
 **6-Boc**	1.0		
 **20**	20.7		

[a] Quinine bisulfate was used as emission standard to determine pseudo-absolute quantum yields; concentration, 4×10^{-5} M.

excited indole ring of Trp. For example, a fluorescence red shift occurs as the microenvironment surrounding Trp residues changes from non-polar to polar.[34] The observed emission changes in SRIF indicate higher polarity of the Trp environment upon interaction with **12**.

The proposed linear structures of **12** and **13b** should reflect in higher binding affinities toward peptides and proteins with β-sheet structures if compared to unstructured or helical peptides. To test the hypothesis, the binding of **12** and **13b** to the protein concavalin A (ConA) was investigated. ConA is a lectin isolated from jack beans.[35] Its three-dimensional structure was determined at a resolution of 1.75 Å and later refined to 1.2 Å.[36] The lectin monomer (see Supporting information for figure) features a 'jelly roll' fold architecture. It is made up largely of three antiparallel β-sheets. The lectin dimer is termed the 'canonical

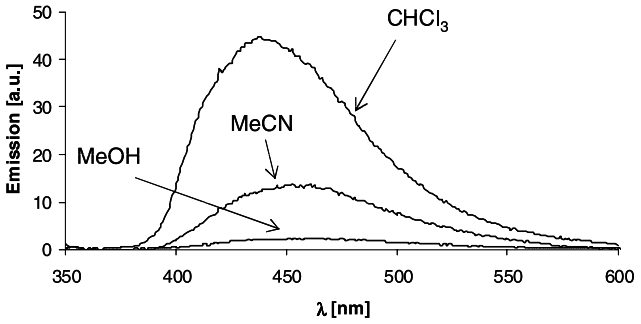


Figure 1. Emission spectra of **15**-NEt_2 [12 μM] in different solvents (excitation at 330 nm).

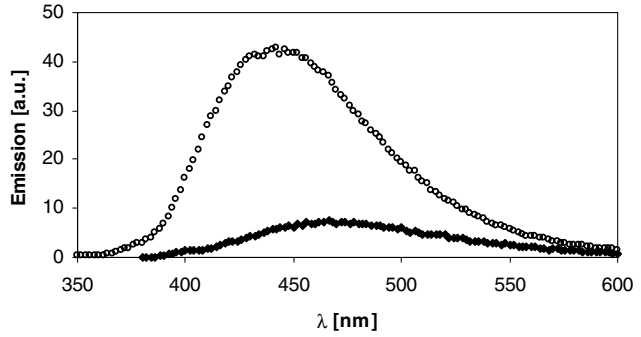


Figure 2. Emission spectra of **13b** in a 1×10^{-5} M solution of $CHCl_3$ (circle) and H_2O (dotted).

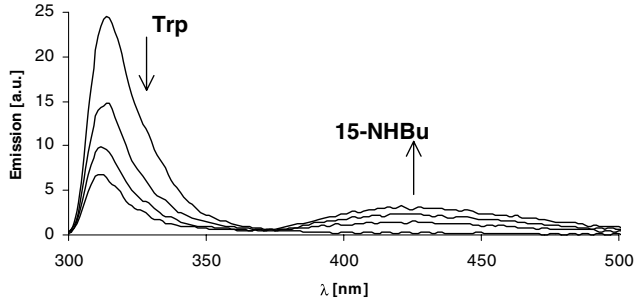


Figure 3. Emission changes upon titration of F_{moc}-Trp(Boc)-OH (1.21 μM) with **15**-NH-Bu (0–7 equiv) in MeCN (see Supporting information for UV spectra of the compounds).

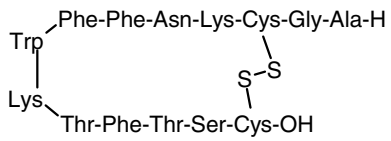


Figure 4. Amino acid sequence of the tetradecapeptide somatostatin (SRIF).

dimer', which is characterized by a large 12-stranded β-sheet resulting from the antiparallel side-by-side alignment of the two six-stranded back sheets (see Supporting information for figure). Two such canonical dimers associate with the center parts of their back sheets in a perpendicular manner forming the tetramer. Depending on the concentration of denaturing urea one can affect a specific structure of ConA.[37] The denaturation equilibrium displays a three-state mechanism involving a structured monomeric state (2.7 M urea) between native tetrameric and unfolded monomeric state (>8 M urea). In addition, ConA was denatured in the presence of trypsin following a modified method of Burger and Noonan[38] to eliminate most structural elements of the peptide. Depending on the state the number of accessible extended β-sheets of ConA varies. With increasing concentration of urea or in the presence of trypsin, the number of β-sheet regions decreases leading to an unfolded peptide strand. When enzymatically digested, ConA remains with almost no distinct secondary and tertiary structure. The CD spectra (see Supporting information for data) clearly show the peptides' secondary structure under different conditions. The native tetramer (data not shown) and the dissociated monomer in 2.7 M urea show the characteristics of β-sheet structures. Both spectra are different from that in 8 M urea and from trypsinized ConA. ConA denatured by 8 M urea may still have some extended β-sheet substructures, while these are clearly eliminated in the case of trypsinized ConA.

The affinity of PHA **12** to ConA in the presence of different concentrations of urea and after trypsin digestion was investigated in PBS buffer by emission titration. ConA has eight Trp residues per monomer located both on the surface and in the core of the peptide. They can be excited at 280 nm and show emission around 340 nm in PBS. The extended β-sheet structures of ConA lead to many different binding motifs and possible stoichiometries of aggregates. Therefore, no apparent binding constants are derived from the titration data. The initial decrease of Trp emission, which depends on the efficiency of energy transfer from Trp to PHA, is used as indicator of protein binding ability of the PHA compound (see Supporting information for data). Comparison of the native protein and the partly unfolded ConA (2.7 M urea) with denatured (8 M urea) or digested ConA reveals a decreased affinity with diminished β-sheet structure content. The emission response of ConA upon titration with **13b** is similar (see Supporting information for data). Although a quantitative correlation of PHA binding affinity with the extent of available β-sheet structure is not possible due to the complexity of the system, the qualitative correlation becomes apparent.

3. Conclusion

PHA chromophore excitation is possible by direct light absorption (at 382 nm) or a FRET process utilizing a nearby Trp, but the PHA emission is largely quenched in protic solvents. PHA oligomers mimic the hydrogen bond pattern of a peptide structure in β-sheet conformation and therefore interaction with peptides was expected. Previous investigations confirmed this for non-polar solvents ($CDCl_3$) and small peptides. With a newly established synthetic route water-soluble PHAs became available, which were

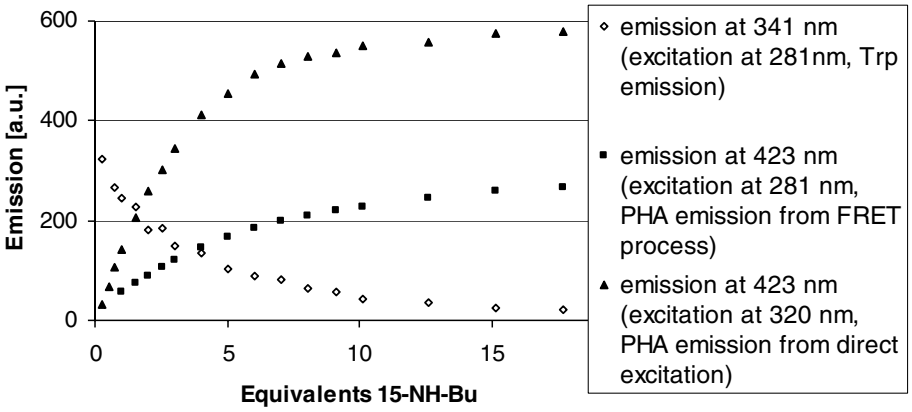


Figure 5. Emission titration of a 7.4×10^{-5} M solution of SRIF in acetonitrile with compound **15**-NH-Bu.

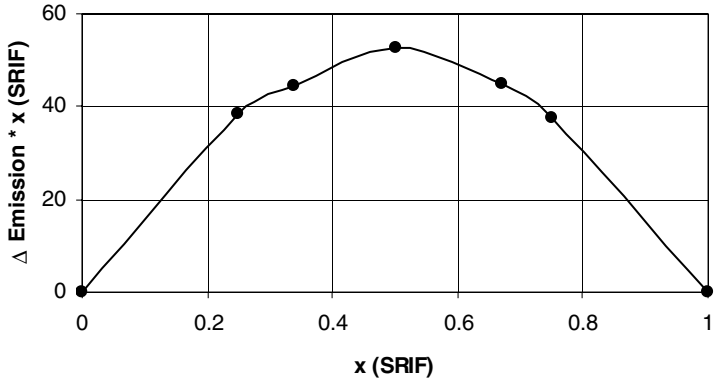


Figure 6. Job's plot analysis of SRIF and **15**-NH-Bu (c 1.5×10^{-4} mol/L).

extended to di-, tri-, and tetramers. Although the emission intensity of the water-soluble PHAs is diminished in polar solvents, they can serve as luminescent peptide probes. In the presence of the Trp containing tetradecapeptide somatostatin a FRET from the peptides Trp to bound PHA is observed. At the same time, binding to the peptide leads to an increase in PHA emission intensity if excited directly at 382 nm, due to polarity changes in the proximity of the peptide. The affinity of the compounds to the protein ConA depends on the amount of available β-sheets. Denaturation or digestion, which destroys β-sheet secondary structural elements, is indicated by diminished binding affinities of the PHA compounds. In summary, the efficient and modular synthesis of the PHA building block and its oligomers, and their luminescent properties make the compounds suitable for the construction of emitting probes for the recognition of peptides and protein β-sheet structural elements.

4. Experimental

The synthesis of compounds **6**, **15**-OMe, **16**–**20** was reported previously.[25] All ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$ at 300 K with TMS as internal reference at 300 or 75.5 MHz, respectively, if not stated otherwise. Somatostatin CuraMed is a product of Delta-Select (www.deltaselect.de).

4.1. Methyl-2-{bis-[2-(2-methoxy-ethoxy)-ethyl]-amino}-6-(*N'*-*tert*-butoxycarbonyl-hydrazino)-pyrimidine-4-carboxylate (8)

A solution of compound **6** (1.4 g, 4.09 mmol), **7** (1.8 g, 8.20 mmol), and Et_3N (621 mg, 6.15 mmol) in DMF (20 mL) was stirred at 60 °C for 12 h. The solvent was removed in vacuum, the residue was dissolved in EtOAc, and the organic phase was washed with water, brine, and dried over Na_2SO_4. After removal of the solvent at reduced pressure, the crude product was purified by column chromatography (CH_2Cl_2–MeOH 1:50, $R_f = 0.3$) to afford compound **8** as a yellow oil (1.25 g, 80%). Three hundred milligrams of compound **6** was recovered. IR (neat): 3300 cm^{-1}, 3012, 2933, 1734, 1590, 1520, 1251, 1216, 1099, 756, 667. ^{1}H NMR: δ 1.44 (s, 9H, Boc-CH_3), 3.35 (s, 6H, OCH_3), 3.48–3.51 (m, 4H, CH_2), 3.57–3.63 (m, 8H, CH_2), 3.79 (s, 4H, CH_2), 3.86 (s, 3H, $COOCH_3$), 6.56 (s, 1H, Het–H), 6.70 (s, 2H, NH). ^{13}C NMR: δ 28.2, 48.0, 52.6, 59.0, 69.1, 70.2, 72.0, 81.7, 92.8, 155.8, 161.4, 166.3. UV (MeCN): λ_{max} ($\log \varepsilon$): 336 nm (4.647) MS (ESI, MeOH + 10 mmol/l NH_4Ac): m/z (%) = 488.4 [MH^+] (100). Anal. Calcd for $C_{21}H_{37}N_5O_8$: C, 51.4; H, 7.7; N, 14.4. Found: C, 51.1; H, 7.3; N, 14.2.

4.2. *tert*-Butyl-*N*′-(2-{bis-[2-(2-methoxy-ethoxy)-ethyl]-amino}-6-hydrazinocarbonyl-pyrimidin-4-yl)-hydrazine carboxylate (9)

A solution of compound **8** (1.5 g, 3.07 mmol) and $NH_2NH_2{\cdot}H_2O$ (938 mg, 18.4 mmol) in methanol was refluxed for 1 h. After removal of the solvent and excess hydrazine in vacuum, the crude product **9** was isolated in quantitative yields (1.49 g) and used in the next step without further purification. IR (neat): 3019 cm^{-1}, 1734, 1592, 1507, 1216, 1158, 758, 669. ^1H NMR: δ 1.43 (s, 9H, Boc-CH_3), 3.34 (s, 6H, OCH_3), 3.49–3.51 (m, 4H, CH_2), 3.55–3.62 (m, 8H, CH_2), 3.73–3.75 (m, 4H, CH_2), 4.06 (s, 2H, NH_2), 6.69 (s, 1H, Het–H), 6.91 (s, 2H, NH), 8.85 (s, 1H, NH). ^{13}C NMR: δ 28.2, 48.4, 59.1, 69.2, 70.3, 72.0, 81.6, 90.4, 155.9, 160.4, 164.3. UV (MeCN): λ_{max} ($\log\varepsilon$): 328 (4.751). MS (ESI, MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 488.3 [MH^+] (100). Anal. Calcd for $C_{20}H_{37}N_7O_7$: C, 49.3; H, 7.7; N, 20.1. Found: C, 48.9; H, 7.5; N, 20.1.

4.3. Methyl 6-{*N*′-[2-{bis-[2-(2-methoxy-ethoxy)-ethyl]-amino}-6- (*N*′-*tert*-butoxycarbonyl-hydrazino)-pyrimidine-4-carbonyl]- hydrazino}-2-bromo-pyrimidine-4-carboxylate (11)

A mixture of **9** (1.5 g, 3.07 mmol), **10** (996 mg, 3.38 mmol), and Et_3N (372 mg, 3.68 mmol) in DMF (15 mL) was stirred at 70 °C for 14 h. The solvent was removed in vacuum, the residue was dissolved in EtOAc, and the organic phase was washed with water, brine, and dried over sodium sulfate. After removal of the solvent in vacuum, the crude product was purified by column chromatography (CH_2Cl_2–MeOH 1:40, R_f = 0.25) to afford compound **11** as a yellow oil (1.06 g, 55%). One hundred and fifty milligrams of **9** was recovered. IR (neat): 3019 cm^{-1}, 1724, 1601, 1479, 1216, 1115, 770, 668. ^1H NMR: δ 1.43 (s, 9H, Boc-CH_3), 3.32–3.36 (m, 6H, OCH_3), 3.52 (m, 4H, CH_2), 3.61–3.67 (m, 6H, CH_2), 3.82 (s, 6H, CH_2), 3.97 (s, 3H, $COOCH_3$), 6.80 (s, 1H, Het–H), 6.76 (s, 1H, Het–H), 11.06 (s, 2H, NH). ^{13}C NMR: δ 28.3, 48.2, 53.6, 59.1, 61.6, 69.6, 70.2, 70.5, 72.0, 81.7, 152.5, 156.1, 158.7, 160.5, 164.4. UV (MeCN): λ_{max} ($\log\varepsilon$): 330 (5.163). MS (ESI, MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 704.3 [MH^+] (100). Anal. Calcd for $C_{26}H_{40}BrN_9O_9$: C, 44.5; H, 5.7; N, 17.9. Found: C, 44.2; H, 5.6; N, 18.3.

4.4. Methyl-2-{bis-[2-(2-methoxy-ethoxy)-ethyl]-amino}-6- {*N*′-[2-{bis-[2-(2-methoxy-ethoxy)-ethyl]-amino}-6-(*N*′-*tert*-butoxycarbonyl-hydrazino)-pyrimidine-4-carbonyl]- hydrazino}-pyrimidine-4-carboxylate (12)

A solution of **11** (1 g, 1.42 mmol), **7** (470 mg, 2.13 mmol), and Et_3N (172 mg, 1.7 mmol) in DMF (10 mL) was stirred at 82 °C for 14 h. After the solvent was removed in vacuum, the crude product was purified by column chromatography (CH_2Cl_2–MeOH 1:35, R_f = 0.3) to give compound **12** as a yellow oil (968 mg, 85%). Fifty milligrams of compound **11** was recovered. IR (neat): 3288 cm^{-1}, 3018, 2892, 1734, 1576, 1521, 1249, 1216, 1099, 757, 668. ^1H NMR: δ 1.42 (s, 9H, Boc-CH_3), 3.32-3.35 (m, 12H, OCH_3), 3.46–3.48 (m, 10H, CH_2), 3.56–3.63 (m, 14H, CH_2), 3.77–3.79 (m, 8H, CH_2), 3.83 (s, 3H, $COOCH_3$), 6.62 (s, 1H, Het–H), 6.74 (s, 1H, Het–H), 7.50 (s, 2H, NH), 9.68 (s, 1H, NH). ^{13}C NMR: δ 28.2, 48.1, 48.4, 52.6, 59.0, 69.1, 69.2, 70.2, 71.9, 81.5, 93.4, 155.5, 156.0, 160.5, 161.4, 166.0. UV (MeCN): λ_{max} ($\log\varepsilon$): 338 (5.204). MS (ESI, MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 843.6 [MH^+] (100), 422.3 [$M+2H^+$] (31), 394.3 [$M+2H^+-C_4H_8$] (10).

4.5. Compound 13b

A solution of **12** (1 g, 1.18 mmol) and $NH_2NH_2{\cdot}H_2O$ (360 mg, 7.08 mmol) in methanol (10 mL) was refluxed for 1 h. After removal of the solvent and excess hydrazine in vacuum, the hydrazide of compound **12** was obtained as crude product in quantitative yield and sufficient purity to be used in the next step. IR (neat): 3019 cm^{-1}, 1590, 1216, 759, 669. ^1H NMR: δ 1.41 (s, 9H, Boc-CH_3), 3.33 (s, 12H, OCH_3), 3.49–3.51 (m, 10H, CH_2), 3.58–3.67 (m, 14H, CH_2), 3.79–3.81 (m, 8H, CH_2), 4.11 (s, 2H, NH_2), 6.95–7.06 (m, 3H, Het–H), 8.20 (s, 1H, NH), 8.89 (s, 1H, NH), 10.36 (s, 2H, NH). ^{13}C NMR: δ 28.2, 48.6, 59.1, 69.3, 70.4, 72.0, 90.4, 156.1, 160.2, 160.5, 164.6. UV (MeCN): λ_{max} ($\log\varepsilon$): 333 (5.016). MS (ESI, MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 843.6 $[MH]^+$ (100). Anal. Calcd for $C_{35}H_{62}N_{12}O_{12}$: C, 49.9; H, 7.4; N, 19.9. Found: C, 49.3; H, 7.1; N, 20.0.

A mixture of the so prepared hydrazide of compound **12** (1 g, 1.19 mmol), **10** (385 mg, 1.31 mmol), and Et_3N (144 mg, 1.43 mmol) in DMF (15 mL) was stirred at 75–80 °C for 20 h. The solvent was removed in vacuum and the residue purified by column chromatography (CH_2Cl_2–MeOH 1:35, R_f = 0.2) to afford PHA trimer **13a** as a yellow oil (507 mg, 45%). One hundred milligrams of the hydrazide of compound **12** was recovered. IR (neat): 3019 cm^{-1}, 1477, 1216, 758, 669. ^1H NMR: δ 1.39–1.44 (m, 9H, Boc-CH_3), 3.31–3.36 (m, 12H, OCH_3), 3.51–3.58 (m, 14H, CH_2), 3.67–3.69 (m, 8H, CH_2), 3.83–3.93 (m, 8H, CH_2), 4.00 (s, 3H, CH_3), 6.94 (s, 2H, Het–H), 7.49 (s, 1H, NH), 7.96 (s, 1H, NH), 10.47 (s, 1H, NH), 10.69 (s, 1H, NH), 11.34 (s, 1H, NH). ^{13}C NMR: δ 28.2, 46.0, 48.5, 49.1, 53.7, 59.1, 69.5, 70.5, 72.0, 81.4, 94.1, 105.9, 152.6, 152.9, 156.0, 157.2, 157.7, 160.5, 164.9. UV (MeCN): λ_{max} ($\log\varepsilon$): 330 (5.260). MS (ESI, MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 1059.6 $[MH]^+$(100). Anal. Calcd for $C_{41}H_{65}BrN_{14}O_{14}$: C, 46.6; H, 6.2; N, 18.5. Found: C, 46.0; H, 6.4; N, 19.2.

A solution of **13a** (500 mg, 0.47 mmol), **7** (156 mg, 0.71 mmol), and Et_3N (57 mg, 0.56 mmol) in DMF (10 mL) was stirred at 85 °C for 18 h. After the solvent was removed in vacuum, the crude product was purified by column chromatography (CH_2Cl_2–MeOH 1:30, R_f = 0.2) to afford compound PHA trimer **13b** as a yellow oil (390 mg, 80%). Forty milligrams of **13a** was recovered. IR (neat): 3019 cm^{-1}, 1577, 1474, 1216, 758, 669. ^1H NMR: δ 1.42 (s, 9H, Boc-CH_3), 3.32–3.34 (m, 18H, OCH_3), 3.48–3.69 (m, 36H, CH_2), 3.84–3.90 (m, 16H, CH_2, CH_3), 6.98 (s, 3H, Het–H), 7.44

(s, 1H, NH), 10.52 (s, 5H, NH). ^{13}C NMR: δ 28.2, 48.2, 48.6, 52.8, 59.0, 69.5, 70.5, 72.0, 81.4, 156.1, 160.5, 161.3. UV (MeCN): λ_{max} (log ε): 338 (6.332). MS (ESI, MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 1199.0 $[MH]^+$ (100), 600 $[MH_2]^{2+}$ (28).

4.6. Compound 14b

A solution of **13b** (200 mg, 0.16 mmol) and $NH_2NH_2 \cdot H_2O$ (50 mg, 1.01 mmol) in methanol (5 mL) was refluxed for 1 h. After removal of the solvent and excess hydrazine in vacuum, the hydrazide of **13b** was obtained quantitatively and pure enough to be used for the next step. IR (neat): 3019 cm^{-1}, 1577, 1474, 1216, 758, 669. ^{1}H NMR: δ 1.41 (s, 9H, Boc-CH_3), 3.32–3.35 (m, 18H, OCH_3), 3.48–3.57 (m, 24H, CH_2), 3.69–3.70 (m, 12H, CH_2), 3.84–3.87 (m, 12H, CH_2), 7.18 (s, 1H, Het–H), 7.35 (s, 1H, Het–H), 7.57 (s, 1H, Het–H), 8.89 (s, 2H, NH), 10.61 (s, 4H, NH). ^{13}C NMR: δ 28.2, 48.5, 48.6, 59.0, 59.1, 69.5, 70.5, 72.0, 81.1, 159.2, 160.3, 160.6. UV (MeCN): λ_{max} (log ε): 337 (5.229). MS (ESI, DCM–MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 1199.0 $[MH]^+$ (33), 600.0 $[M+2H^+]$ (100). Anal. Calcd for $C_{50}H_{87}N_{17}O_{17}$: C, 50.1; H, 7.3; N, 19.9. Found: C, 49.6; H, 7.5; N, 19.9.

A mixture of the hydrazide of compound **13b** (200 mg, 0.16 mmol), **10** (54 mg, 0.18 mmol), and Et_3N (25 mg, 0.25 mmol) in DMF (7 mL) was stirred at 80–83 °C for 20 h. The solvent was removed in vacuum and the crude product was purified by column chromatography (CH_2Cl_2–EtOAc–MeOH 10:10:1, R_f = 0.2) to afford compound **14a**, as a yellow solid (63 mg, 30%). Twenty milligrams of the hydrazide of compound **13b** was recovered. IR (neat): 3019 cm^{-1}, 1577, 1476, 1216, 758, 669. ^{1}H NMR: δ 1.41 (s, 9H, Boc-CH_3), 3.33-3.35 (m, 18H, OCH_3), 3.46–3.49 (m, 22H, CH_2), 3.70–3.72 (m, 12H, CH_2), 3.86–3.92 (m, 14H, CH_2), 4.02 (s, 3H, OCH_3), 6.98 (s, 1H, Het–H), 7.55 (s, 1H, Het–H), 7.61 (s, 1H, Het–H), 8.02 (s, 1H, Het–H), 10.52 (s, 2H, NH), 10.68 (s, 2H, NH), 11.48 (s, 1H, NH). ^{13}C NMR: δ 28.2, 48.6, 53.7, 59.0, 59.0, 69.6, 70.5, 72.0, 81.4, 94.0, 152.6, 152.8, 153.6, 160.6, 165.0. UV (MeCN): λ_{max} (log ε): 333 (5.370). MS (ESI, DCM–MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 1415.0 $[MH]^+$ (13), 708.2 $[M+2H^+]$ (100). Anal. Calcd for $C_{56}H_{90}BrN_{19}O_{19}$: C, 47.6; H, 6.4; N, 18.8. Found: C, 47.4; H, 6.5; N, 18.9.

A solution of **14a** (63 mg, 0.044 mmol), **7** (15 mg, 0.067 mmol), and Et_3N (7 mg, 0.066 mmol) in DMF (4 mL) was stirred at 85 °C for 20 h. The solvent was removed in vacuum and the crude product was purified by column chromatography (CH_2Cl_2–EtOAc–MeOH 10:10:1, R_f = 0.25) to give compound **14b** as a yellow solid (42 mg, 70%). Eight milligrams of **14a** was recovered. IR (neat): 3019 cm^{-1}, 1776, 1545, 1473, 1216, 1101, 758. ^{1}H NMR: δ 1.41 (s, 9H, Boc-CH_3), 3.31–3.34 (m, 24H, CH_2, OCH_3), 3.47–3.49 (m, 16H, CH_2), 3.57–3.58 (m, 16H, CH_2), 3.69–3.72 (m, 16H, CH_2), 3.87–3.91 (m, 19H, CH_2, OCH_3), 6.68 (s, 1H, Het–H), 7.32 (s, 1H, Het–H), 7.59 (s, 2H, Het–H), 10.62 (s, 4H, NH). ^{13}C NMR: δ 28.2, 48.6, 59.0, 69.6, 70.5, 72.0, 81.2, 94.2, 165.6. UV (MeCN): λ_{max} (log ε): 338 (5.336). MS (ESI, MeOH + 10 mmol/l NH_4Ac): *m/z* (%) = 1554.1 $[MH]^+$ (6), 777.7 $[M+2H^+]$ (100). Anal. Calcd for $C_{66}H_{112}N_{20}O_{23}$: C, 51.0; H, 7.3; N, 18.0. Found: C, 50.8; H, 7.6; N, 18.0.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.05.003.

References and notes

1. Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893–4012.
2. Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180.
3. (Recent examples, see:) Schmitt, M. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2004**, *126*, 6848–6849; (b) Stone, M. T.; Fox, J. M.; Moore, J. S. *Org. Lett.* **2004**, *6*, 3317–3320; (c) Chen, F.; Zhu, N.-Y.; Yang, D. *J. Am. Chem. Soc.* **2004**, *126*, 15980–15981.
4. Yin, H.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4130–4163.
5. Choi, S.; Clements, D. J.; Pophristic, V.; Ivanov, I.; Vemparala, S.; Bennett, J. S.; Klein, M. L.; Winkler, J. D.; DeGrado, W. F. *Angew. Chem.* **2005**, *117*, 6843–6847. *Angew. Chem., Int. Ed* **2005**, *44*, 6685–6689.
6. (a) Horwell, D. C.; Howson, W.; Ratcliffe, G. S.; Willems, H. M. G. *Bioorg. Med. Chem.* **1996**, *4*, 33; (b) Ernst, J. T.; Becerril, J.; Park, H. S.; Yin, H.; Hamilton, A. D. *Angew. Chem.* **2003**, *115*, 553–557; . *Angew. Chem. Int. Ed.* **2003**, *42*, 535–539; (c) Yin, H.; Lee, G. I.; Sedey, K. A.; Rodriguez, J. M.; Wang, H. G.; Sebti, S. M.; Hamilton, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 5463; (d) Ernst, J. T.; Kutzi, O.; Debnath, A. K.; Jiang, S.; Lu, H.; Hamilton, A. D. *Angew. Chem.* **2002**, *114*, 288–291.
7. For a recent example of a β-peptide, see Huck, B. R.; Gellman, S. H. *J. Org. Chem.* **2005**, *70*, 3353–3362.
8. (Recent review:) Miltschitzky, S.; König, B. *Org. Prep. Procced. Int.* **2005**, *37*, 307–336.
9. Nowick, J. S. *Acc. Chem. Res.* **1999**, *32*, 287–296.
10. (For β-sheet models based on aliphatic amino acids, see) Gellman, S. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 717–725.
11. (a) Nowick, J. S.; Tsai, J. H.; Bui, Q.-C. D.; Maitra, S. *J. Am. Chem. Soc* **1999**, *121*, 8409–8410; (b) Nowick, J. S.; Chung, D. M.; Maitra, K.; Maitra, S.; Stigers, K. D.; Sun, Y. *J. Am. Chem. Soc.* **2000**, *122*, 7654–7661.
12. (a) Nowick, J. S.; Lam, K. S.; Khasanova, T. V.; Kemnitzer, W. E.; Maitra, S.; Mee, H. T.; Liu, R. *J. Am. Chem. Soc.* **2002**, *124*, 4972–4973; (b) Nowick, J. S.; Cary, J. M.; Tsai, J. H. *J. Am. Chem. Soc.* **2001**, *123*, 5176–5180.
13. Chung, D. M.; Dou, Y.; Baldi, P.; Nowick, J. S. *J. Am. Chem. Soc.* **2005**, *127*, 9998–9999.

14. Chung, D. M.; Nowick, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 3062–3063.
15. (a) Nowick, J. S.; Holmes, D. L.; Mackin, G.; Noronha, G.; Shaka, A. J.; Smith, E. M. *J. Am. Chem. Soc.* **1996**, *118*, 2764–2765; (b) Smith, E. M.; Holmes, D. L.; Shaka, A. J.; Nowick, J. S. *J. Org. Chem.* **1997**, *62*, 7906–7907; (c) Nowick, J. S.; Pairish, M.; Lee, I. Q.; Holmes, D. L.; Ziller, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 5413–5424.
16. (a) Schrader, T.; Kirsten, C. *J. Chem. Soc., Chem. Commun.* **1996**, 2089–2090; (b) Schrader, T.; Kirsten, C. N. *J. Am. Chem. Soc.* **1997**, *119*, 12061–12068.
17. Rzepecki, P.; Gallmeier, H.; Geib, N.; Cernovska, K.; König, B.; Schrader, T. *J. Org. Chem.* **2004**, *69*, 5168–5178.
18. (a) Rzepecki, P.; Nagel-Steger, L.; Feuerstein, S.; Linne, U.; Molt, O.; Zadmard, R.; Aschermann, K.; Wehner, M.; Schrader, T.; Riesner, D. *J. Biol. Chem.* **2004**, *279*, 47497–47505; (b) Schrader, T.; Riesner, D.; Nagel-Steger, L.; Aschermann, K.; Kirsten, C.; Rzepecki, P.; Molt, O.; Zadmard, R.; Wehner, M. Patent application DE 102 21 052.7, 5/10/2002; (c) Rzepecki, P.; Wehner, M.; Molt, O.; Zadmard, R.; Schrader, T. *Synthesis* **2003**, 1815–1826.
19. Rzepecki, P.; Schrader, T. *J. Am. Chem. Soc.* **2005**, *127*, 3016–3025.
20. (a) Phillips, S. T.; Piersanti, G.; Ruth, M.; Gubernator, N.; van Lengerich, B.; Bartlett, P. A. *Org. Lett.* **2004**, *6*, 4483–4485; (b) Phillips, S. T.; Blasdel, L. K.; Bartlett, P. A. *J. Org. Chem.* **2005**, *70*, 1865–1871.
21. (a) Bonauer, C.; Zabel, M.; König, B. *Org. Lett.* **2004**, *6*, 1349–1352; (b) Kruppa, M.; Bonauer, C.; Michlová, V.; König, B. *J. Org. Chem.* **2005**, *70*, 5305–5308; (c) Bonauer, C.; König, B. *Synthesis* **2005**, 2367–2372.
22. Fisk, J. D.; Powell, D. R.; Gellman, S. H. . *J. Am. Chem. Soc.* **2000**, *122*, 5443–5447.
23. Junquera, E.; Nowick, J. S. *J. Org. Chem.* **1999**, *64*, 2527–2531.
24. Phillips, S. T.; Blasdel, L. K.; Bartlett, P. A. *J. Am. Chem. Soc.* **2005**, *127*, 4193–4198.
25. Miltschitzky, S.; Michlova, V.; Stadlbauer, S.; König, B. *Heterocycles* **2006**, *67*, 135–160.
26. Selve, C.; Ravey, J.-C.; Stebe, M.-J.; El Moudjahid, C.; Moumni, E. M.; Delpuech, J. J. *Tetrahedron* **1991**, *47*, 411–428.
27. Brazeau, P.; Vale, W.; Burgus, R.; Ling, N.; Butcher, M.; Rivier, J.; Guillemin, R. *Science* **1973**, *179*, 77.
28. In clinical studies, this substance was shown to suppress the release of growth hormone (GH) secretion from the anterior pituitary (a) Hall, R.; Besser, G. M.; Schally, A. V.; Coy, D. H.; Evered, D.; Goldie, D. J.; Kastin, A. J.; McNeilly, A. S.; Mortimer, C. H.; Phenekos, C.; Tunbridge, W. M. G.; Weightman, D. *Lancet* **1973**, *1*, 581; It inhibits the secretion of glucagon and insulin as well as gastrin (b) Yen, S. S. C.; Siler, T. M.; DeVane, G. W. *N. Eng. J. Med.* **1974**, *290*, 935.
29. (a) Brown, M.; Rivier, J.; Vale, W. *Endocrinology* **1976**, *98*, 336–343; (b) Rivier, J. E.; Brown, M.; Vale, W. *J. Med. Chem.* **1976**, *19*, 1010–1013.
30. The initial slopes of the emission titration curves at excitation at 281 nm are similar. The intensity of Trp8 emission is quenched to the same degree as the emission of PHA **12** increases resulting clearly from a FRET process. The selective excitation of **12** at 320 nm leads to a steeper slope of the emission enhancement, which indicates an additional effect of the peptide binding on the PHA emission.
31. (a) Schneider, H. J.; Kramer, R.; Simova, S.; Schneider, U. *J. Am. Chem. Soc.* **1988**, *110*, 6442; (b) Wilcox, C. S. In *Frontiers in Supramolecular Chemistry and Photochemistry*; Schneider, H. J., Duerr, H., Eds.; VCH: Weinheim, 1991.
32. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983.
33. Job, P. *Compt. rend.* **1925**, *180*, 928.
34. Burnstein, E. A.; Vedenkina, N. S.; Ivkova, M. N. *Photochem. Photobiol.* **1973**, *18*, 263.
35. Sumner, J. B. *J. Biol. Chem.* **1919**, *37*, 137.
36. (a) Hardman, K. D.; Agarwal, R. C.; Freiser, M. J. *J. Mol. Biol.* **1982**, *372*, 6388; (b) Parkin, S.; Rupp, B.; Hope, H. *Acta Crystallogr.* **1996**, *D52*, 1161.
37. Chatterjee, A.; Mandal, D. K. *Biochim. Biophys. Acta* **2003**, *1648*, 174; Pflumm, M. N.; Beychok, S. *Biochemistry* **1974**, *13*, 4982; Auer, H. E.; Schulz, T. *J. Pept. Protein Res.* **1984**, *24*, 569.
38. Burger, M. M.; Noonan, K. D. *Nature* **1970**, *228*, 512; Bosmann, H. B. *FEBS Lett.* **1972**, *22*, 97.