Hydrolytic activity of histidine-containing octapeptides in water identified by quantitative screening of a combinatorial library†

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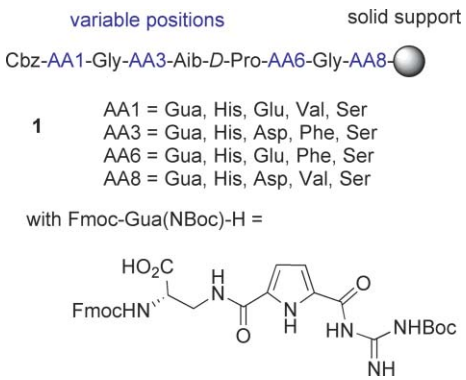
A quantitative on-bead screening of a combinatorial octapeptide library revealed catalysts with hydrolytic activity in water. Histidine is an essential amino acid but the catalytic activity as well as the substrate binding affinity is also dependent on the sequence of the octapeptide.

Introduction

Nature's use of proteins as catalysts has initiated a long standing interest in the development of small-peptide based catalysts. However, initial attempts to mimic *e.g.* the active sites of proteases such as chymotrypsin solely based on small peptides were not successful.[1] Only in the last few years has metal-free catalysis[2] with small peptides been achieved for a variety of reactions, including among others oxidation reactions,[3] aldol reactions,[4] Michael additions,[5] and acyl transfer reactions.[6] One major limitation of these oligopeptide-catalysts is that in general they only function in aprotic organic solvents such as chloroform or toluene but not under more competitive protic conditions. For example, Miller uses oligopeptides with *N*-alkylated histidines as catalysts for acyl transfer reactions.[6a–6c] Their use, however is limited to organic solvents and they do not function under protic conditions. Also the His-Ser containing peptideosteroids developed by Davis *et al*. only show hydrolytic activity in acetonitrile.[6d] Only a few examples for peptide-based catalysis in aqueous solutions have been reported so far. For instance, catalytically active peptide fragments such as His-Ser incorporated into dendrimers show esterase activity in water as was reported by Reymond *et al*.[7] However, even in these cases the reaction takes place within the core of the dendrimer, which provides an efficient shielding of the bound substrate against the aqueous environment.[8] Examples of non-dendrimer or non-polymer based small peptides with hydrolytic activity in water are lacking. In this context we report here that small histidine-containing octapeptides **1**, which were identified from the screening of a combinatorial library, show efficient esterase-activity in aqueous buffer solution.[9,10]

Results and discussion

Library **1** was previously developed by us and tested for phosphate ester hydrolysis in water.[10] For each of the four variable positions five different amino acids were used in the split–mix-protocol, giving rise to a total library size of 625 members. For these five amino acids different combinations of six proteinogenic amino acids (His, Ser, Glu, Asp, Phe, Val) as well as an artificial arginine analogue Gua[11] were used (Scheme 1). This choice of amino acids was based on the idea that the polar amino acids (His, Glu, Asp, Ser) would function as catalytically active residues or help in structuring the oligopeptide (*e.g. via* ion pair formation between the arginine analogue and Glu or Asp). The two unpolar amino acids Phe and Val were expected to provide additional stacking or hydrophobic interactions with the bound substrate. Details on the synthesis of library **1** as well as the application of this library to identify catalysts for phosphate ester hydrolysis are already described elsewhere.[10] In that study we found that octapeptides containing histidine and the unnatural arginine analogue Gua were the most potent catalysts within the library, achieving rate enhancements of up to a factor of 175 over the uncatalyzed background reaction.



Scheme 1 Oligopeptide library **1**

We now tested the same library for its capacity to hydrolyze the pyrene ester **2**.[7c] The catalytic activity of the individual octapeptides within the library was probed using an on-bead fluorescent assay by incubating the library with the pyrene ester **2**. Efficient hydrolysis of the ester in **2** leads to a significant increase in the fluorescence intensity. We first probed a pooled mixture of the library members to determine suitable conditions for the assay which would indicate <5% of active beads (Fig. 1, middle). The optimized conditions ([**2**] = 0.2 mM, 17.4 mM BisTris-buffer, pH 6) were then used to screen each member of the entire library quantitatively using a fluorescence spectrometer with a microtiter plate reader.

By this means, we were able to determine quantitative kinetic data for each of the 625 libraries members. This is in contrast to

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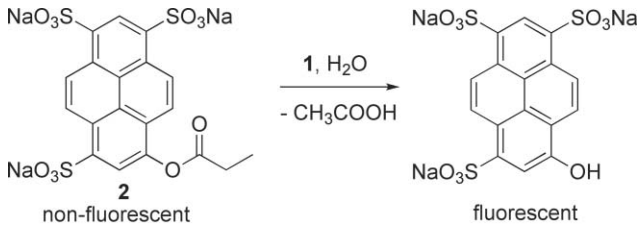

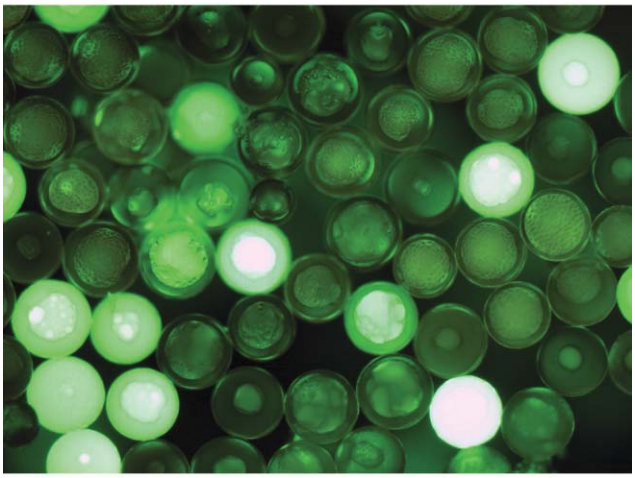

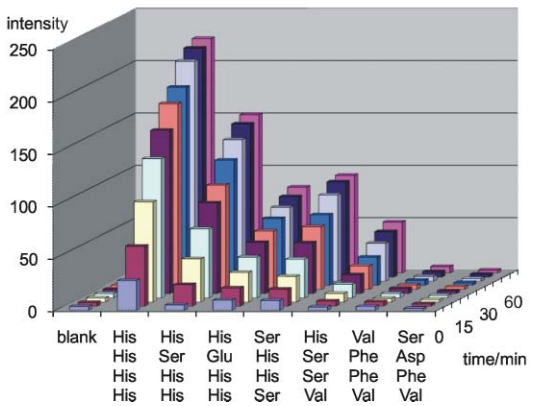

Fig. 1 The hydrolysis of **2** (0.2 mM in 17.4 mM BisTris-buffer, pH 6, 25 °C) leads to an increase in fluorescence intensity at 530 nm. In a qualitative on-bead screening of peptide library **1** active catalysts show up as highly fluorescent beads (middle). With the individual library members obtained spatially separated, quantitative kinetic measurements of all library members are also possible directly on-bead using a microtiter plate reader (bottom, selected examples of octapeptides ranging from highly active to non-active, amino acids listed from top to bottom AA1, AA3, AA6, AA8).

most other combinatorial approaches reported so far, in which only qualitative screenings have been performed, or at best semi-quantitative estimates from the different colouring of individual beads (similar to Fig. 1, middle) could be obtained. Hence, those library screenings only revealed a yes/no-answer whereas our approach used here allows us to directly obtain some quantitative structure–activity relationships from the on-bead screening (Fig. 1, bottom). Also, our previous screening of library **1** for phosphate ester hydrolysis was based on a purely optical inspection of beads therefore it did not provide quantitative data.

Now, in this screening for ester hydrolysis the quantitative time-course measurements provide quantitative kinetic data on-bead. Ester **2** is efficiently hydrolyzed within a few hours in aqueous buffer solution by a fraction of the library members (Fig. 1, bottom). The sequence analysis of the results from the on-bead screening reveals that all active catalysts contain histidine as an essential amino acid. Similar observations have also been reported from other groups.[12] The most active catalyst under these conditions, octapeptide **1a**, carries four histidines at the four variable positions. However, as shown below, this does not mean that the hydrolysis of peptides with less than four histidines is less efficient but rather reflects differences in the substrate binding affinity. This is different from other studies,[12] in which catalysis was not assisted by substrate binding. Octapeptides without histidine but with for example Val, Phe, Phe, Val in positions AA^1, AA^3, AA^6 and AA^8, respectively, show no activity (Fig. 1, bottom). Interestingly, in contrast to the previously reported phosphate hydrolysis by this library,[10] the artificial arginine analogue Gua did not show up in the most potent catalysts. In contrast, the best catalysts for phosphate ester hydrolysis were only averagely active in this screening. Obviously, the two different reactions are catalysed in a different way by these octapeptides.

Selected octapeptides (Table 1) were then resynthesized on Rink-amide resin using standard Fmoc-solid phase peptide synthesis. Besides the most active peptide with four histidine residues (**1a**) another three peptides with just three histidines but an additional flanking carboxylate (Asp, Glu) or OH-group (Ser), as well as one peptide with just two histidines and two serines, were chosen (all amino acids that are commonly found in the active sites of enzymes). These peptides were tested for their catalytic activity in aqueous solution ([**2**] = 0.2 mM, 30 mol % octapeptide **1**, 17.4 mM BisTris buffer, pH 6, 25 °C). Initial rates v_0 were obtained from conversion–time-profiles (Fig. 2). The kinetic studies confirmed the results from the quantitative on-bead experiments. Octapeptides such as **1a** or **1b** efficiently catalyze the hydrolysis of **2** in water. However, the actual catalytic activity is not only dependent on the number of histidine residues within the peptide but also depends on the sequence of the octapeptide. Peptides with the same number of histidines but in a different sequence show different catalytic activity (compare, *e.g.*, **1b** with **1c** and **1d**). Imidazole (a commonly used control in such studies) was also tested under the same conditions (30 mol-% relative to substrate **2**) but showed only a very small rate enhancement compared to the background reaction (second order rate constant $k_{im} = 0.5\ M^{-1}\ min^{-1}$).

The most efficient peptide **1a** within the library was then studied in more detail. A Lineweaver–Burk analysis of the saturation kinetics at pH 6.0 (Fig. 3) provided the following kinetic parameters for octapeptide **1a**: $k_{cat} = 0.019\ min^{-1}$, $K_m = 0.17$ mM and $k_{cat}/K_m = 112\ M^{-1}{\cdot}min^{-1}$. Hence, octapeptide **1a** is an efficient catalyst for the hydrolysis of **2**, with a significant rate enhancement relative to the uncatalyzed background reaction ($k_{cat}/k_{uncat} = 345$). Compared to imidazole, octapeptide **1a** is also a much better catalyst ($(k_{cat}/K_m)/k_{im} = 224$). Even per histidine residue the catalysis by **1a** is still 56 times more efficient ($(k_{cat}/K_m)/k_{im}/His = 56$).

Octapeptide **1a** also shows catalytic turn-over. Even 10 mol-% of **1a** are sufficient to hydrolyze the substrate completely. The addition of EDTA (equimolar amounts to octapeptide **1**) has no

Table 1 Selected octapeptides **1** used to study the hydrolysis of **2** (0.2 mM) in free solution (17.4 mM BisTris buffer, pH 6) and initial rates v_0 (in M·min^{-1}) in the presence of 30 mol-% catalyst at 25 °C

1 = Cbz-AA^1-Gly-AA^3-D-Pro-Aib-AA^6-Gly-AA^8-NH_2

Catalyst	AA^1	AA^3	AA^6	AA^8	$v_0 \cdot 10^7$ min M^{-1}
1a	His	His	His	His	8.09
1b	His	His	His	Ser	5.92
1c	His	His	His	Glu	3.41
1d	Asp	His	His	His	3.15
1e	Ser	His	His	Ser	1.48
imidazole					0.17
background					0.11

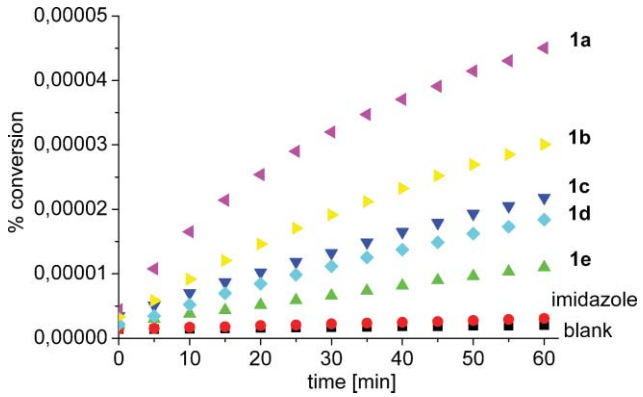

Fig. 2 Concentration–time-plot for the hydrolysis of **2** (0.2 mM, 17.4 mM aqueous BisTris-buffer, pH 6.0) in the presence of 30 mol-% of different octapeptides **1**.

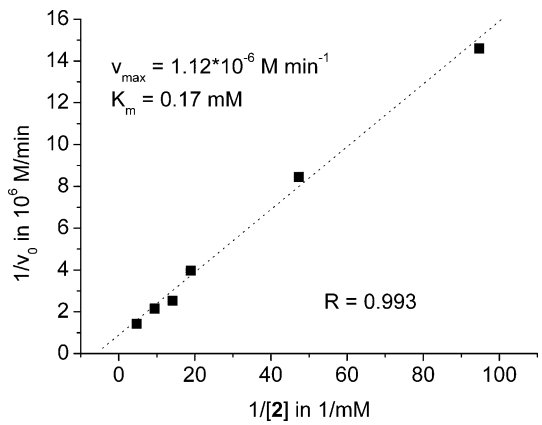

Fig. 3 Lineweaver–Burk plot for the hydrolysis of **2** in the presence of octapeptide **1a** (0.06 mM) in 17.4 mM aqueous BisTris-buffer at pH 6.0 at 25 °C.

effect on the rate of hydrolysis, showing that metal ions are not involved.

The data on first sight suggest that simply the number of histidine residues determines the catalytic activity. However, most likely both substrate binding as well as catalysis is affected by the actual sequence of the oligopeptide. We therefore studied the influence of the sequence of octapeptides **1** on their catalytic activity systematically with a set of six peptides each containing two histidines and two serines (**1e–1j**). This subset of peptides was chosen as the combination His-Ser is not only a common motive in enzymes but also has been identified in other peptide based catalysts (*e.g.* in dendrimers).[7] For each of the six octapeptides saturation kinetics were measured under the same conditions as outlined above for **1a**, and the kinetic parameters were determined from Lineweaver–Burk plots. The data are summarized in Table 2.

Table 2 Selected octapeptides **1** used to study the sequence dependence of the hydrolysis of **2** in solution (17.4 mM BisTris buffer, pH 6)

1 = Cbz-AA^1-Gly-AA^3-D-Pro-Aib-AA^6-Gly-AA^8-NH_2

	AA^1	AA^3	AA^6	AA^8	k_{cat}[a]	K_m[b]	k_{cat}/K_m[c]
1a	His	His	His	His	0.019	0.17	112
1e	Ser	His	His	Ser	0.0087	1.00	9
1f	Ser	Ser	His	His	0.0049	0.71	7
1g	His	Ser	His	Ser	0.0162	1.51	11
1h	Ser	His	Ser	His	0.0019	0.42	5
1i	His	Ser	Ser	His	0.0064	1.13	6
1j	His	His	Ser	Ser	0.0035	0.48	7

[a] in min^{-1}; [b] in mM; [c] in (M min)$^{-1}$

The most reactive octapeptide within this series with two histidines and two serines (**1g**) is nearly as active as octapeptide **1a** in terms of k_{cat} (k_{cat} = 0.019 min^{-1} *vs.* 0.016 min^{-1}, respectively). However, substrate binding by **1g** is much less efficient (by a factor of 9: K_m = 1.51 *vs.* 0.17 mM respectively), leading to the overall decreased catalytic efficiency as expressed by k_{cat}/K_m. Nevertheless, in terms of the reaction rate k_{cat} the two histidines in **1g** are as efficient as the four histidines in **1a**. This clearly shows that the histidine residues in **1a** are not only catalytically active but also assist in substrate binding (probably by charge interactions between positively charged histidinium residues and the anionic substrate and/or by aromatic stacking interactions).

Furthermore, inspection of the data in Table 2 clearly shows that the rate enhancement of the octapeptides is also sequence dependant. Among the six octapeptides **1e–1j** with two histidines and two serines each, the k_{cat} values differ significantly. The most striking difference is between **1g** and **1h** in which the sequence of the amino acids at the variable positions is simply reversed (His, Ser, His, Ser *versus* Ser, His, Ser, His, respectively). The two peptides differ in their corresponding k_{cat} values by more than a factor of 8. Also, substrate binding depends on the sequence, but to a smaller extent. The K_m values differ by a factor of 3.6 at most. Interestingly, peptides with high affinity for the substrate such as **1h** or **1j** are poor catalysts whereas the most active peptide in this series, **1g**, has the lowest substrate affinity. This reverse trend between k_{cat} and K_m is responsible for the overall small difference of only a factor of two in catalytic efficiency (k_{cat}/K_m).

Compared to our previous results on phosphate hydrolysis the octapeptides identified here as catalysts for ester hydrolysis are more efficient (k_{cat} being larger by one order of magnitude roughly). This most likely reflects the increased chemical reactivity of carboxylic esters compared to phosphates. More interestingly however is the already mentioned altered efficiency profile among the library. Peptides that were most active for phosphate ester hydrolysis are only averagely active for the ester hydrolysis studied here. And vice versa, peptides such as **1a** which are most efficient with respect to ester **2** were only slightly active in the phosphate hydrolysis studied before.[10] This suggests that for phosphate hydrolysis substrate binding or TS stabilization as provided by the

arginine analogue Gua (or Arg in natural peptides) is much more important than for hydrolysis of pyrene ester **2**. The negatively charged phosphate requires efficient charge compensation by interaction with a cationic residue (Gua, Arg) before attack of a nucleophile takes place. This charge assisted substrate binding and/or activation is obviously not needed for hydrolysis of the neutral carboxylic acid ester group in **2**.

Conclusion

In conclusion, we have shown here that small histidine containing oligopeptides show efficient esterase activity even in water, with a rate enhancement of more than 10^2 compared to both the uncatalyzed background reaction as well as catalysis by imidazole alone. The quantitative screening of a combinatorial library revealed that the catalytic activity is sequence dependent as was also confirmed in subsequent studies with selected peptides in solution. The rate enhancement k_{cat} is not just simply correlated with the number of histidines, as the histidine residues also assist in substrate binding. Furthermore, even for peptides with the same number of histidine residues, both catalytic activity (k_{cat}) and substrate affinity (K_m) show a pronounced dependence on the sequence of the peptide. We are currently further exploring octapeptides **1** and their catalytic activities with respect to their detailed mode of action and extending the library screening to other substrates.

Experimental

Solvents were dried and distilled under argon before use. All other reagents were used as obtained. Melting points were measured in open end glass capillary tubes and are uncorrected. ^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 MHz spectrometer. The chemical shifts are reported relative to the deuterated solvents. Peaks assignment is based on DEPT studies and comparison with literature data. ESI- and HR-mass spectra were recorded on a micrOTOF from Bruker Daltonik. Analytical HPLC was run on a Supelcosil LC18 (Supelco) 5 µm, (25 cm × 4.6 mm) column.

The solid phase bound library **1** was synthesized as described previously.[10]

General procedure for peptide synthesis on Rink-amide resin

The resin (300 mg, 0.74 mmol/g, 0.22 mmol, 1.0 eq) was swollen in DMF (10 mL) for one hour. The solvent was removed by suction filtration and then the Fmoc-protecting group on the resin was removed by addition of DMF containing 20% piperidine (10 mL), shaking for 30 minutes each time and removal of the cleavage solution with suction filtration. This step was repeated once again. Afterwards the resin was washed with DMF (5 × 10 mL) to remove any remaining traces of piperidine. For the coupling of the first amino acid the resin was suspended in DMF (10 mL) which contained 3% NMM (300 µL). The Fmoc-protected amino acid (0.55 mmol, 2.5 eq) and PyBOP (290 mg, 0.55 mmol, 2.5 eq) were added, and the reaction mixture was shaken for 5 to 6 hours until a negative Kaiser-test confirmed quantitative coupling. The solvent was removed by suction filtration, and the resin was washed with DMF (3 × 10 mL). Then the Fmoc-protecting group was removed as described before. The presence of free amino groups could be detected with a positive Kaiser test. Each additional amino acid was then coupled as described before using 2.5 equivalents of the Fmoc-protected amino acid and 2.5 equivalents of PyBOB. The last amino acid was coupled as the N-Cbz-derivative.

After successful synthesis the peptide was cleaved off the resin. To remove all traces of DMF the resin was thoroughly washed with CH_2Cl_2 (3 × 10 mL), methanol (3 × 10 mL), diethyl ether (10 mL) and then with CH_2Cl_2 again (2 × 10 mL). The resin was dried under vacuum and then treated with a mixture of TFA with 5% dry CH_2Cl_2 (10 mL) at room temperature. After 2 to 3 hours, in which the resin turned dark red, the solvent was removed by suction filtration. The resin was washed with a fresh portion of the cleavage solution (10 mL). The combined cleavage and washing solutions (brown to dark red) were concentrated by solvent evaporation under vacuum to a volume of 2–3 mL. Then 30 mL of dry diethyl ether were added which caused the precipitation of a colorless solid. The solid was isolated by suction filtration using a Büchner funnel and then washed with dry diethyl ether (20 mL). The precipitate was dissolved in water (10 mL), then dilute HCl (0.1 M, 2 mL) was added and the mixture was lyophilized to obtain the peptide as a colorless powder. The peptides were analytically pure (>98%) as identified from NMR and HPLC analyses and used as such for the kinetic analyses.

CbzNH-*L*-His-Gly-*L*-His-*D*-Pro-Aib-*L*-His-Gly-*L*-His-NH_2 (1a)

Yield: 268 mg (0.27 mmol, 77%); mp 131 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ_H(ppm): 1.24 (s, 3H, C*H*$_3$-Aib), 1.25 (s, 3H, C*H*$_3$-Aib), 1.80 (m, 2H, C*H*$_2$-Pro), 1.89 (m, 1H, C*H*$_2$-Pro), 2.05 (m, 1H, C*H*$_2$-Pro), 2.93 (m, 4H, C*H*$_2$-His), 3.18 (m, 4H, C*H*$_2$-His), 3.71 (m, 6H, C*H*$_2$-Gly, C*H*$_2$-Pro), 4.37 (m, 1H, NHC*H*), 4.43 (m, 1H, NHC*H*), 4.51 (m, 2H, NHC*H*), 4.81 (m, 1H, NHC*H*), 4.98 (m, 2H, C*H*$_2$-Ph), 7.31 (m, 11H, C*H*-Ph, N*H*$_2$, C*H*-His), 7.64 (d, J = 8.12 Hz, 1H, N*H*), 7.88 (d, J = 7.61 Hz, 1H, N*H*), 7.93 (t, 1H, N*H*-Gly), 8.09 (t, 1H, N*H*-Gly), 8.35 (m, 2H, N*H*), 8.95 (d, J = 8.07 Hz, 4H, C*H*-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.58 (*H*H$_2$-Pro), 24.75 (*H*H$_3$-Aib), 24.92 (*H*H$_3$-Aib), 25.75 (*H*H$_2$-Pro), 26.32 (*H*H$_2$-His), 26.72 (*H*H$_2$-His), 27.05 (*H*H$_2$-His), 28.83 (*H*H$_2$-His), 41.99 (*H*H$_2$-Gly), 42.43 (*H*H$_2$-Gly), 47.28 (*H*H$_2$-Pro), 50.22 (*H*H-His), 51.80 (*H*H-His), 52.42 (*H*H-His), 53.66 (*H*H-His), 56.04 (H_q-Aib), 60.42 (*H*H-Pro), 65.71 (*H*H$_2$-Ph), 116.86 (*H*H-His), 117.06 (*H*H-His), 117.34 (*H*H-His), 118.27 (*H*H-His), 127.59 (*H*H-Ph), 127.87 (*H*H-Ph), 128.39 (*H*H-Ph), 129.11 (H_q-His), 129.58 (H_q-His), 129.62 (H_q-His), 129.94 (H_q-His), 133.75 (*H*H-His), 133.81 (*H*H-His), 133.90 (*H*H-His), 136.78 (H_q-Ph), 155.99 (H_q-Bn), 168.89 (H_q), 168.95 (H_q), 169.05 (H_q), 170.77 (H_q), 170.82 (H_q), 171.85 (H_q), 172.49 (H_q), 174.84 (H_q); IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3298.64, 3146.29, 3033.48, 2861.84, 2634.29, 1672.95, 1542.77, 1432.85, 1255.43, 1203.36, 1134.90, 835.99, 799.35, 722.21, 628.68; HR-MS (pos. ESI): m/z calcd. for $C_{45}H_{57}N_{17}NaO_{10}^+$ [M + Na]$^+$: 1018.437, found: 1018.436.

CbzNH-*L*-His-Gly-*L*-His-*D*-Pro-Aib-*L*-His-Gly-*L*-Ser-NH_2 (1b)

Yield: 253 mg (0.27 mmol, 76%); mp 112 °C; ^{1}H NMR ((500 MHz, DMSO-d6) δ_H(ppm): 1.23 (s, 3H, C*H*$_3$-Aib), 1.29 (s, 3H, C*H*$_3$-Aib), 1.80 (m, 2H, C*H*$_2$-Pro), 1.92 (m, 1H, C*H*$_2$-Pro), 2.06 (m, 1H, C*H*$_2$-Pro), 2.93 (m, 3H, C*H*$_2$-His), 3.12 (m, 3H, C*H*$_2$-His), 3.23 (m, 2H, C*H*$_2$-Ser), 3.68 (m, 6H, C*H*$_2$-Gly, C*H*$_2$-Pro), 4.21

(m, 2H, NHC*H*), 4.37 (m, 1H, NHC*H*), 4.51 (m, 1H, NHC*H*), 4.83 (m, 1H, NHC*H*), 4.98 (m, 2H, CH_2-Ph), 7.13 (s, 2H, NH_2), 7.32 (m, 8H, C*H*-Ph, C*H*-His), 7.62 (d, J = 8.07 Hz, 1H, N*H*), 7.74 (d, J = 8.13 Hz, 1H, N*H*), 7.97 (m, 2H, N*H*), 8.33 (m, 2H, N*H*), 8.95 (m, 3H, C*H*-His), 14.25 (s, 3H, N*H*-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.65 (*H*H_2-Pro), 24.74 (*H*H_3-Aib), 25.15 (*H*H_3-Aib), 26.14 (*H*H_2-Pro), 26.35 (*H*H_2-His), 27.07 (*H*H_2-His), 28.82 (*H*H_2-His), 41.97 (*H*H_2-Gly), 42.38 (*H*H_2-Gly), 47.33 (*H*H_2-Pro), 50.20 (*H*H), 52.23 (*H*H), 53.65 (*H*H), 55.41 (*H*H), 56.10 (H_q-Aib), 60.58 (*H*H-Pro), 61.61 (*H*H_2-Ser), 65.71 (*H*H_2-Ph), 117.03 (*H*H-His), 117.09 (*H*H-His), 117.36 (*H*H-His), 127.60 (*H*H-Ph), 127.88 (*H*H-Ph), 128.41 (*H*H-Ph), 129.13 (H_q-His), 129.55 (H_q-His), 129.80 (H_q-His), 133.81 (*H*H-His), 133.86 (*H*H-His), 133.90 (*H*H-His), 136.80 (H_q-Ph), 155.98 (H_q-Bn), 168.98 (H_q), 169.07 (H_q), 170.73 (H_q), 170.79 (H_q), 172.01 (H_q), 172.32 (H_q), 174.52 (H_q); IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3297.69, 3150.15, 3039.26, 2927.41, 2878.24, 2634.29, 1671.02, 1542.77, 1432.85, 1254.47, 1202.40, 1134.90, 835.99, 799.35, 721.25, 627.72; HR-MS (pos. ESI): m/z calcd. for $C_{42}H_{55}N_{15}NaO_{11}^+$ [M + Na]$^+$: 968.410, found: 968.410.

CbzNH-*L*-His-Gly-*L*-His-*D*-Pro-Aib-*L*-His-Gly-*L*-Glu-NH$_2$ (1c)

Yield: 298 mg (0.30 mmol, 86%); mp 136 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ_H(ppm): 1.23 (s, 3H, CH_3-Aib), 1.27 (s, 3H, CH_3-Aib), 1.77 (m, 3H, CH_2-Pro, CH_2-Glu), 1.98 (m, 3H, CH_2-Pro, CH_2-Glu), 2.24 (m, 2H, CH_2-Pro, CH_2-Glu), 2.93 (m, 2H, CH_2-His), 3.14 (m, 3H, CH_2-His), 3.24 (m, 1H, CH_2-His), 3.77 (m, 6H, CH_2-Gly, CH_2-Pro), 4.19 (m, 2H, NHC*H*), 4.40 (m, 2H, NHC*H*), 4.83 (m, 1H, NHC*H*), 4.97 (m, 2H, CH_2-Ph), 7.31 (m, 10H, C*H*-Ph, C*H*-His, NH_2), 7.62 (d, J = 8.35 Hz, 1H, N*H*), 7.81 (d, J = 7.77 Hz, 1H, N*H*), 7.87 (d, J = 7.96 Hz, 1H, N*H*), 7.94 (t, 1H, N*H*-Gly), 8.34 (m, 2H, N*H*), 8.95 (m, 3H, C*H*-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.61 (*H*H_2-Pro), 24.68 (*H*H_3-Aib), 25.06 (*H*H_3-Aib), 25.87 (*H*H_2-Pro), 26.35 (*H*H_2-His), 27.06 (*H*H_2-His), 27.31 (*H*H_2-Glu), 28.84 (*H*H_2-His), 30.20 (*H*H_2-Glu), 41.95 (*H*H_2-Gly), 42.37 (*H*H_2-Gly), 47.32 (*H*H_2-Pro), 50.16 (*H*H), 52.07 (*H*H), 52.50 (*H*H), 53.64 (*H*H), 56.05 (H_q-Aib), 60.43 (*H*H-Pro), 65.70 (*H*H_2-Ph), 117.07 (*H*H-His), 117.10 (*H*H-His), 117.34 (*H*H-His), 127.60 (*H*H-Ph), 127.87 (*H*H-Ph), 128.39 (*H*H-Ph), 129.09 (H_q-His), 129.54 (H_q-His), 129.84 (H_q-His), 133.82 (*H*H-His), 133.90 (*H*H-His), 133.94 (*H*H-His), 136.78 (H_q-Ph), 155.96 (H_q-Bn), 168.92 (H_q), 168.99 (H_q), 170.70 (H_q), 170.78 (H_q), 172.46 (H_q), 173.20 (H_q), 173.92 (H_q), 173.95 (H_q), 174.74 (H_q); IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3297.68, 3152.08, 3038.30, 2931.27, 2878.24, 2640.07, 1670.05, 1541.81, 1437.67, 1254.47, 1202.40, 1134.90, 835.03, 799.35, 721.25, 628.68; HR-MS (pos. ESI): m/z calcd. for $C_{44}H_{57}N_{15}NaO_{12}^+$ [M + Na]$^+$: 1010.420, found: 1010.422.

CbzNH-*L*-Asp-Gly-*L*-His-*D*-Pro-Aib-*L*-His-Gly-*L*-His-NH$_2$ (1d)

Yield: 236 mg (0.24 mmol, 69%); mp 114 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ_H(ppm): 1.25 (s, 3H, CH_3-Aib), 1.26 (s, 3H, CH_3-Aib), 1.82 (m, 2H, CH_2-Pro), 1.90 (m, 1H, CH_2-Pro), 2.05 (m, 1H, CH_2-Pro), 2.68 (m, 1H, CH_2-Asp), 2.94 (m, 3H, CH_2-His), 3.13 (m, 4H, CH_2-His, CH_2-Asp), 3.67 (m, 6H, CH_2-Gly, CH_2-Pro), 4.21 (m, 1H, NHC*H*), 4.36 (m, 1H, NHC*H*), 4.43 (m, 1H, NHC*H*), 4.50 (m, 2H, NHC*H*), 5.02 (m, 2H, CH_2-Ph), 7.34 (m, 10H, C*H*-Ph, NH_2, C*H*-His), 7.66 (d, J = 7.56 Hz, 1H, N*H*), 7.85 (d, J = 7.77 Hz, 1H, N*H*), 7.92 (t, 1H, N*H*-Gly), 7.97 (d, J = 7.89 Hz, 1H, N*H*), 8.09 (d, J = 8.17 Hz, 1H, N*H*), 8.23 (t, 1H, N*H*-Gly), 8.27 (s, 1H, N*H*), 8.95 (m, 3H, C*H*-His), 14.25 (s, 3H, N*H*-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.58 (*H*H_2-Pro), 24.78 (*H*H_3-Aib), 24.90 (*H*H_3-Aib), 25.74 (*H*H_2-Pro), 26.22 (*H*H_2-His), 26.68 (*H*H_2-His), 28.8 (*H*H_2-His), 36.29 (CH_2-Asp), 42.10 (*H*H_2-Gly), 42.39 (*H*H_2-Gly), 47.28 (*H*H_2-Pro), 50.28 (*H*H), 51.45 (*H*H), 51.76 (*H*H), 52.37 (*H*H), 56.03 (H_q-Aib), 60.54 (*H*H-Pro), 65.71 (*H*H_2-Ph), 116.86 (*H*H-His), 117.03 (*H*H-His), 117.27 (*H*H-His), 127.77 (*H*H-Ph), 127.88 (*H*H-Ph), 128.38 (*H*H-Ph), 129.13 (H_q-His), 129.57 (H_q-His), 129.90 (H_q-His), 133.73 (*H*H-His), 133.79 (*H*H-His), 133.87 (*H*H-His), 136.76 (H_q-Ph), 156.01 (H_q-Bn), 168.97 (H_q), 170.77 (H_q), 171.50 (H_q), 171.80 (H_q), 171.89 (H_q), 172.42 (H_q), 174.76 (H_q); IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3311.18, 3152.08, 3037.34, 2882.09, 2639.11, 1671.98, 1541.81, 1432.85, 1249.65, 1203.36, 1135.87, 835.99, 799.35, 722.21, 627.72; HR-MS (pos. ESI): m/z calcd. for $C_{43}H_{55}N_{15}NaO_{12}^+$ [M + Na]$^+$: 996.405, found: 996.406.

CbzNH-*L*-Ser-Gly-*L*-His-*D*-Pro-Aib-*L*-His-Gly-*L*-Ser-NH$_2$ (1e)

Yield: 257 mg (0.29 mmol, 82%); mp 134 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ_H(ppm): 1.24 (s, 3H, CH_3-Aib), 1.29 (s, 3H, CH_3-Aib), 1.79 (m, 2H, CH_2-Pro), 1.92 (m, 1H, CH_2-Pro), 2.06 (m, 1H, CH_2-Pro), 2.92 (m, 1H, CH_2-His), 3.11 (m, 2H, CH_2-His), 3.24 (m, 1H, CH_2-His), 3.71 (m, 10H, CH_2-Gly, CH_2-Pro, CH$_2$-Ser), 4.07 (m, 1H, NHC*H*), 4.20 (m, 2H, NHC*H*), 4.49 (m, 1H, NHC*H*), 4.76 (m, 1H, NHC*H*), 5.02 (m, 2H, CH_2-Ph), 7.29 (m, 9H, C*H*-Ph, C*H*-His, NH_2), 7.71 (d, J = 7.95 Hz, 1H, N*H*), 7.96 (t, 1H, N*H*-Gly), 8.07 (m, 1H, N*H*), 8.24 (m, 2H, N*H*), 8.39 (m, 1H, N*H*), 8.95 (m, 2H, C*H*-His), 14.39 (s, 2H, N*H*-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.62 (*H*H_2-Pro), 24.85 (*H*H_3-Aib), 25.01 (*H*H_3-Aib), 26.13 (*H*H_2-Pro), 26.20 (*H*H_2-His), 28.80 (*H*H_2-His), 42.07 (*H*H_2-Gly), 42.31 (*H*H_2-Gly), 47.31 (*H*H_2-Pro), 50.29 (*H*H), 52.19 (*H*H), 55.35 (*H*H), 56.09 (H_q-Aib), 57.13 (*H*H), 60.69 (*H*H-Pro), 61.61 (*H*H_2-Ser), 61.74 (*H*H_2-Ser), 65.66 (*H*H_2-Ph), 117.10 (*H*H-His), 117.32 (*H*H-His), 127.79 (*H*H-Ph), 127.87 (*H*H-Ph), 128.38 (*H*H-Ph), 129.07 (H_q-His), 129.70 (H_q-His), 133.76 (*H*H-His), 133.87 (*H*H-His), 136.85 (H_q-Ph), 156.05 (H_q-Bn), 168.97 (H_q), 169.06 (H_q), 170.71 (H_q), 170.89 (H_q), 171.95 (H_q), 172.19 (H_q), 174.42 (H_q); IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3308.29, 3152.08, 3039.26, 2931.27, 2882.09, 2635.25, 1671.98, 1541.81, 1437.67, 1249.65, 1203.36, 1134.90, 1081.87, 835.99, 800.31, 722.21, 627.72; HR-MS (pos. ESI): m/z calcd. for $C_{39}H_{53}N_{13}NaO_{12}^+$ [M + Na]$^+$: 918.383, found: 918.383.

CbzNH-*L*-Ser-Gly-*L*-Ser-*D*-Pro-Aib-*L*-His-Gly-*L*-His-NH$_2$ (1f)

Yield: 254 mg (0.28 mmol, 81%); mp 162 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ_H(ppm): 1.23 (s, 3H, CH_3-Aib), 1.26 (s, 3H, CH_3-Aib), 1.81 (m, 2H, CH_2-Pro), 1.92 (m, 1H, CH_2-Pro), 2.05 (m, 1H, CH_2-Pro), 2.98 (m, 2H, CH_2-His), 3.18 (m, 2H, CH_2-His), 3.68 (m, 10H, CH_2-Gly, CH_2-Pro, CH$_2$-Ser), 4.07 (m, 1H, NHC*H*), 4.25 (m, 1H, NHC*H*), 4.42 (m, 1H, NHC*H*), 4.49 (m, 1H, NHC*H*), 4.57 (m, 1H, NHC*H*), 5.02 (m, 2H, CH_2-Ph), 7.32 (m, 9H, C*H*-Ph, C*H*-His, NH_2), 7.80 (d, J = 7.71 Hz, 1H, N*H*), 7.89 (m, 1H, N*H*), 8.02 (t, 1H, N*H*-Gly), 8.17 (t, 1H, N*H*-Gly), 8.28 (d, J = 4.97 Hz, 1H, N*H*), 8.97 (m, 2H, C*H*-His), 14.40 (s, 4H, N*H*-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.50

(HH$_2$-Pro), 24.53 (HH$_3$-Aib), 25.21 (HH$_3$-Aib), 25.76 (HH$_2$-Pro), 26.71 (HH$_2$-His), 28.81 (HH$_2$-His), 41.90 (HH$_2$-Gly), 42.38 (HH$_2$-Gly), 47.31 (HH$_2$-Pro), 51.67 (HH), 52.32 (HH), 53.29 (HH), 56.03 (H_q-Aib), 57.21 (HH), 60.31 (HH), 61.24 (HH$_2$-Ser), 61.76 (HH$_2$-Ser), 65.65 (HH$_2$-Ph), 116.82 (HH-His), 117.09 (HH-His), 127.82 (HH-Ph), 127.88 (HH-Ph), 128.39 (HH-Ph), 129.60 (H_q-His), 129.86 (H_q-His), 133.72 (HH-His), 133.86 (HH-His), 136.88 (H_q-Ph), 156.06 (H_q-Bn), 168.92 (H_q), 170.54 (H_q), 170.67 (H_q), 170.80 (H_q), 171.76 (H_q), 172.69 (H_q), 174.56 (H_q), 174.80 (H_q); IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3310.21, 3152.08, 3044.09, 2931.27, 2882.09, 1672.95, 1541.81, 1431.89, 1257.36, 1203.36, 1134.90, 836.95, 800.31, 722.21, 627.72; HR-MS (pos. ESI): m/z calcd. for $C_{39}H_{54}N_{13}O_{12}^+$ [M + H]$^+$: 896.401, found: 896.401.

CbzNH-*L*-His-Gly-*L*-Ser-*D*-Pro-Aib-*L*-His-Gly-*L*-Ser-NH$_2$ (1g)

Yield: 301 mg (0.34 mmol, 96%); mp 155 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ_H(ppm): 1.25 (s, 3H, CH_3-Aib), 1.30 (s, 3H, CH_3-Aib), 1.84 (m, 2H, CH_2-Pro), 1.96 (m, 1H, CH_2-Pro), 2.07 (m, 1H, CH_2-Pro), 2.91–3.28 (m, 4H, CH_2-His), 3.54–3.93 (m, 10H, CH_2-Gly, CH_2-Pro, CH$_2$-Ser), 4.24 (m, 2H, NHCH), 4.40 (m, 1H, NHCH), 4.53 (m, 1H, NHCH), 4.65 (m, 1H, NHCH), 5.01 (m, 2H, CH_2-Ph), 7.27 (m, 9H, CH-Ph, CH-His, NH_2), 7.67 (d, J = 8.29 Hz, 1H, NH), 7.87 (d, J = 7.92 Hz, 1H, NH), 7.98 (t, 1H, NH-Gly), 8.16 (d, J = 7.07 Hz, 1H, NH), 8.24 (t, 1H, NH-Gly), 9.00 (m, 2H, CH-His), 14.42 (s, 4H, NH-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.53 (HH$_2$-Pro), 24.71 (HH$_3$-Aib), 25.22 (HH$_3$-Aib), 26.10 (HH$_2$-Pro), 27.03 (HH$_2$-His), 28.84 (HH$_2$-His), 41.81 (HH$_2$-Gly), 42.31 (HH$_2$-Gly), 47.35 (HH$_2$-Pro), 52.14 (HH), 53.20 (HH), 53.69 (HH), 55.28 (HH), 56.08 (H_q-Aib), 60.35 (HH), 61.35 (HH$_2$-Ser), 61.63 (HH$_2$-Ser), 65.69 (HH$_2$-Ph), 116.98 (HH-His), 117.12 (HH-His), 127.60 (HH-Ph), 127.86 (HH-Ph), 128.40 (HH-Ph), 129.44 (H_q-His), 129.74 (H_q-His), 133.78 (HH-His), 133.88 (HH-His), 136.80 (H_q-Ph), 155.96 (H_q-Bn), 168.85 (H_q), 168.89 (H_q), 169.73 (H_q), 170.54 (H_q), 170.72 (H_q), 171.96 (H_q), 172.56 (H_q), 174.55 (H_q); IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3310.21, 3150.15, 3061.44, 2936.09, 2882.09, 2635.25, 1672.95, 1541.81, 1436.71, 1256.40, 1203.36, 1135.87, 1082.83, 1054.87, 836.95, 800.31, 722.21, 627.72; HR-MS (pos. ESI): m/z calcd. for $C_{39}H_{54}N_{13}O_{12}^+$ [M + H]$^+$: 896.401, found: 896.401.

CbzNH-*L*-Ser-Gly-*L*-His-*D*-Pro-Aib-*L*-Ser-Gly-*L*-His-NH$_2$ (1h)

Yield: 284 mg (0.32 mmol, 91%); mp 158 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ_H(ppm): 1.31 (s, 3H, CH_3-Aib), 1.35 (s, 3H, CH_3-Aib), 1.77 (m, 2H, CH_2-Pro), 1.93 (m, 1H, CH_2-Pro), 2.06 (m, 1H, CH_2-Pro), 2.90 (m, 2H, CH_2-His), 3.07 (m, 1H, CH_2-His), 3.18 (m, 1H, CH_2-His), 3.65 (m, 10H, CH_2-Gly, CH_2-Pro, CH$_2$-Ser), 4.04 (m, 1H, NHCH), 4.13 (m, 1H, NHCH), 4.24 (m, 1H, NHCH), 4.50 (m, 1H, NHCH), 4.73 (m, 1H, NHCH), 5.00 (m, 2H, CH_2-Ph), 7.30 (m, 9H, CH-Ph, CH-His, NH_2), 7.54 (d, J = 6.75 Hz, 1H, NH), 7.98 (m, 1H, NH), 8.13 (m, 1H, NH), 8.23 (t, 1H, NH-Gly), 8.35 (m, 1H, NH), 8.96 (m, 2H, CH-His), 14.41 (s, 4H, NH-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.39 (HH$_3$-Aib), 24.62 (HH$_2$-Pro), 25.48 (HH$_3$-Aib), 26.18 (HH$_2$-Pro), 26.65 (HH$_2$-His), 28.79 (HH$_2$-His), 42.01 (HH$_2$-Gly), 42.53 (HH$_2$-Gly), 47.22 (HH$_2$-Pro), 50.17 (HH), 51.64 (HH), 56.05 (H_q-Aib), 56.37 (HH), 57.17 (HH), 60.47 (HH), 61.06 (HH$_2$-Ser), 61.76 (HH$_2$-Ser), 65.67 (HH$_2$-Ph), 116.77 (HH-His), 117.36 (HH-His), 127.80 (HH-Ph), 127.88 (HH-Ph), 128.39 (HH-Ph), 128.93 (H_q-His), 129.63 (H_q-His), 133.73 (HH-His), 133.76 (HH-His), 136.86 (H_q-Ph), 156.07 (H_q-Bn), 168.72 (H_q), 168.96 (H_q), 169.05 (H_q), 170.88 (H_q), 171.03 (H_q), 171.77 (H_q), 172.41 (H_q), 174.77 (H_q); IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3310.21, 3149.22, 3039.19, 2938.45, 2882.09, 1672.95, 1541.81, 1431.35, 1255.11, 1203.36, 1135.98, 836.95, 800.31, 722.21, 627.72; HR-MS (pos. ESI): m/z calcd. for $C_{39}H_{54}N_{13}O_{12}^+$ [M + H]$^+$: 896.401, found: 896.401.

CbzNH-*L*-His-Gly-*L*-Ser-*D*-Pro-Aib-*L*-Ser-Gly-*L*-His-NH$_2$ (1i)

Yield: 311 mg (0.35 mmol, 99%); mp 157 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ_H(ppm): 1.30 (s, 3H, CH_3-Aib), 1.35 (s, 3H, CH_3-Aib), 1.80 (m, 2H, CH_2-Pro), 1.95 (m, 1H, CH_2-Pro), 2.06 (m, 1H, CH_2-Pro), 2.92 (m, 2H, CH_2-His), 3.15 (m, 2H, CH_2-His), 3.51–3.83 (m, 10H, CH_2-Gly, CH_2-Pro, CH$_2$-Ser), 4.12 (m, 1H, NHCH), 4.27 (m, 1H, NHCH), 4.37 (m, 1H, NHCH), 4.47 (m, 1H, NHCH), 4.59 (m, 1H, NHCH), 4.99 (m, 2H, CH_2-Ph), 7.31 (m, 9H, CH-Ph, CH-His, NH_2), 7.57 (d, J = 6.87 Hz, 1H; NH), 7.65 (d, J = 8.29 Hz, 1H; NH), 7.89 (d, J = 8.48 Hz, 1H, NH), 7.94 (t, 1H, NH-Gly), 8.07 (d, J = 6.91 Hz, 1H, NH), 8.21 (t, 1H, NH-Gly), 8.35 (s, 1H, NH), 8.96 (m, 2H, CH-His), 14.37 (s, 4H, NH-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.28 (HH$_3$-Aib), 24.50 (HH$_2$-Pro), 25.60 (HH$_3$-Aib), 26.68 (HH$_2$-Pro), 27.01 (HH$_2$-His), 28.82 (HH$_2$-His), 41.71 (HH$_2$-Gly), 42.50 (HH$_2$-Gly), 47.24 (HH$_2$-Pro), 51.60 (HH), 53.28 (HH), 53.69 (HH), 56.02 (H_q-Aib), 56.35 (HH), 60.20 (HH), 61.07 (HH$_2$-Ser), 61.33 (HH$_2$-Ser), 65.70 (HH$_2$-Ph), 116.73 (HH-His), 117.09 (HH-His), 127.62 (HH-Ph), 127.87 (HH-Ph), 128.40 (HH-Ph), 129.46 (H_q-His), 129.63 (H_q-His), 133.73 (HH-His), 133.89 (HH-His), 136.79 (H_q-Ph), 155.96 (H_q-Bn), 168.91 (H_q), 168.97 (H_q), 169.56 (H_q), 170.49 (H_q), 171.01 (H_q), 171.74 (H_q), 172.72 (H_q), 174.87 (H_q); IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3310.21, 3153.98, 3032.77, 2936.24, 2882.09, 1672.95, 1541.81, 1429.98, 1261.22, 1203.36, 1130.12, 836.95, 800.31, 722.21, 627.72; HR-MS (pos. ESI): m/z calcd. for $C_{39}H_{54}N_{13}NaO_{12}^+$ [M + Na]$^+$: 918.383, found: 918.383.

CbzNH-*L*-His-Gly-*L*-His-*D*-Pro-Aib-*L*-Ser-Gly-*L*-Ser-NH$_2$ (1j)

Yield: 298 mg (0.33 mmol, 95%); mp 163 °C; ^{1}H NMR (500 MHz, DMSO-d6) δ_H(ppm): 1.36 (s, 3H, CH_3-Aib), 1.37 (s, 3H, CH_3-Aib), 1.82 (m, 2H, CH_2-Pro), 1.97 (m, 1H, CH_2-Pro), 2.09 (m, 1H, CH_2-Pro), 2.94 (m, 2H, CH_2-His), 3.13 (m, 2H, CH_2-His), 3.69 (m, 10H, CH_2-Gly, CH_2-Pro, CH$_2$-Ser), 4.19 (m, 1H, NHCH), 4.27 (m, 1H, NHCH), 4.38 (m, 1H, NHCH), 4.58 (m, 1H, NHCH), 4.85 (m, 1H, NHCH), 5.02 (m, 2H, CH_2-Ph), 7.27 (m, 9H, CH-Ph, CH-His, NH_2), 7.50 (d, J = 7.34 Hz, 1H, NH), 7.64 (d, J = 8.23 Hz, 1H, NH), 7.85 (d, H = 7.89 Hz, 1H, NH), 7.99 (t, 1H, NH-Gly), 8.30 (t, 1H, NH-Gly), 9.00 (m, 2H, CH-His), 14.42 (s, 4H, NH-His); ^{13}C NMR (126 MHz, DMSO-d6) δ_C(ppm): 24.50 (HH$_3$-Aib), 24.63 (HH$_2$-Pro), 25.52 (HH$_3$-Aib), 26.20 (HH$_2$-Pro), 27.01 (HH$_2$-His), 28.78 (HH$_2$-His), 41.89 (HH$_2$-Gly), 42.52 (HH$_2$-Gly), 47.19 (HH$_2$-Pro), 50.01 (HH), 53.61 (HH), 53.66 (HH), 55.35 (HH), 56.08 (H_q-Aib), 60.43 (HH), 61.22 (HH$_2$-Ser), 61.59 (HH$_2$-Ser), 65.70 (HH$_2$-Ph), 117.07 (HH-His), 117.37 (HH-His), 127.61 (HH-Ph), 127.87 (HH-Ph), 128.39 (HH-Ph), 128.91 (H_q-His), 129.47 (H_q-His), 133.74 (HH-His), 133.87 (HH-His), 136.78 (H_q-Ph), 155.94 (H_q-Bn), 168.60 (H_q), 168.86 (H_q), 169.05 (H_q), 170.63 (H_q), 170.80 (H_q), 171.97 (H_q), 172.32 (H_q), 174.51 (H_q);

IR: (KBr-pellet) $\tilde{\nu}$ [cm^{-1}] = 3310.21, 3151.45, 3054.02, 2933.59, 2882.09, 1672.95, 1541.81, 1433.23, 1259.22, 1203.36, 1132.77, 836.95, 800.31, 722.21, 627.72; HR-MS (pos. ESI): m/z calcd. for $C_{39}H_{54}N_{13}O_{12}^{+}$ $[M + H]^+$: 896.401, found: 896.400.

Screening of the library on bead

For the screening of the solid phase bound library, aliquots of each library member were placed in the well of a 96 titer plate. Then 45 μL of BisTris-buffer (20 mM, pH 6) and 5 μL of a stock solution of pyrene ester **2** (2 mM) in water were added. The well plate was gently shaken for two minutes to ensure mixing of the solutions. Then the fluorescence increase was followed over time using a Jasco fluorescence spectrometer FP-6500 equipped with a microtiter plate reader FMP-9633 (excitation wavelength 460 nm, emission wavelength 530 nm).

Screening of individual octapeptides in solution

For the kinetic studies in solution, 1.5 mL of solution was prepared containing a final concentration of 17.4 mM BisTris-buffer (pH 6), 0.2 mM pyrene ester **2** and 0.06 mM of the octapeptide (30 mol-% relative to **2**). The solution was vortexed in an eppendorf tube and then the fluorescence increase (excitation wavelength 460 nm, emission wavelength 530 nm) due to the hydrolysis of **2** was followed over time. The data were analysed using the initial rate method. For the Lineweaver–Burk analysis similar samples but with different ratios of octapeptide to substrate were prepared and analysed accordingly ([**1**] = 0.06 mM = const.; [**2**] was varied from 500, 250, 100, 75 and 50 to finally 25 mol-%).

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