Synthesis of a new artificial host for the binding of dipeptides in water

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An artificial peptide receptor **5** was prepared by a simple procedure. Initial binding studies (UV titrations) in buffered water showed preferential complexation of *N*-acetyl-dipeptide carboxylates containing alanine in the C-terminal position in comparison with simple amino acids, other dipeptides and two tripeptides.

Introduction

The search for artificial peptide receptors is an interesting field of research in supramolecular chemistry.[1] Artificial peptide receptors might be of interest as sensors, to interfere with biological peptide recognition or as drug candidates. However, for such applications the host has to bind the peptide in aqueous media. This makes the development of peptide receptors even more challenging as often in supramolecular chemistry directed H-bonds are used for substrate binding. The strength of such polar host–guest interactions unfortunately decreases rapidly with increasing polarity of the solvent.[2] Purely H-bonded assemblies are in general not stable in water. Peptide complexation by artificial hosts can only be achieved in combination with additional noncovalent interactions such as hydrophobic interactions[3] or ion pair formation.[4] Metal complexes have also been used in this context to allow complexation of histidine-rich peptides, for example.[5] We are currently exploring the use of ion pair formation for complexation of negatively charged peptides in polar solvents.[6] For this purpose we have introduced guanidiniocarbonyl pyrroles[7] as an efficient oxoanion binding site some while ago.[8]

We present here the prototype of a new dicationic host **5**, which, as we show, efficiently binds dipeptide carboxylates in water with association constants $\approx 2\text{–}5 \times 10^3\ M^{-1}$. Host **5** contains a lysine attached to the guanidiniocarbonyl pyrrole cation, as this combination showed up favorably in screening experiments with solid phase bound combinatorial libraries of peptide receptors.[6] Furthermore, **5** contains a serine to enhance solubility in aqueous solution, and a naphthyl group for potential additional hydrophobic interactions.

Results and discussion

The synthesis of receptor **5** is outlined in Scheme 1. First, commercially available (L)-*N*-2-naphthylserinamide (**1**) was coupled to (L)-Boc-Lys(Cbz)-OH using PyBOP in DMF as the coupling reagent. The α-amino group in **2** was then deprotected with TFA in quantitative yields. The free amine was then directly coupled to *N*-Boc-5-guanidinocarbonyl pyrrole (**3**),[9] again using PyBOP standard coupling conditions. The fully protected precursor **4** was thus obtained in 68% yield over both steps. Deprotection

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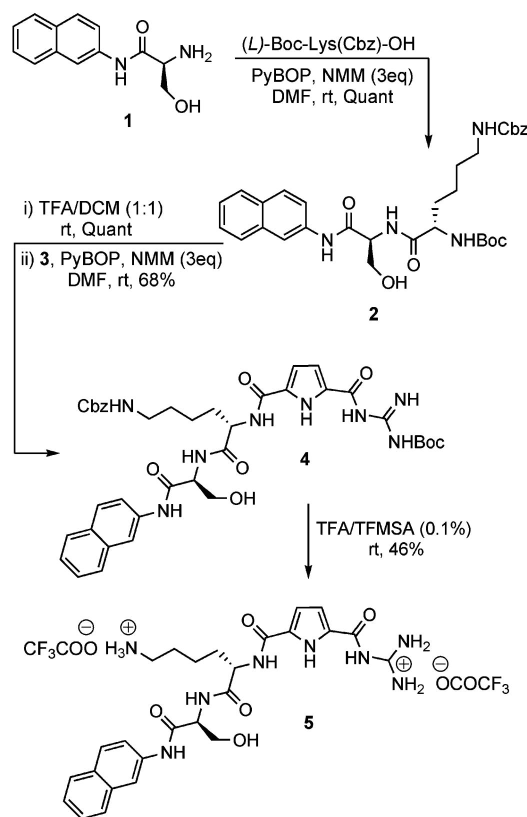

Scheme 1 Synthesis of host compound **5**.

to give host **5** proved to be more difficult than expected. Standard deprotection of the Cbz-group by hydrogenolysis and subsequent Boc deprotection with TFA only caused decomposition. Different conditions were tried [*e.g.* 10% Pd/C in MeOH at rt or 50 °C, or in THF at 30 °C with AcOH (cat.)] but no conditions were found which provided **5** in acceptable yields. The use of strong organic acids, such as TFMSA, is reported to remove different protecting groups in a peptide at the same time (*e.g.* Tos and Cbz).[10] Therefore, we tested a mixture of TFA and TFMSA for the deprotection of both the Cbz-protected amine and the Boc-protected guanidine simultaneously.[11] The reaction time and the amount of TFMSA must be controlled in order to avoid decomposition of the products, probably generated by cleavage of the naphthylamide bond. After some attempts with different amounts of TFMSA (2–0.1%) in TFA, the best conditions were finally found using 0.1%

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Table 1 Binding constants of **5** with various carboxylates in buffered water (20% DMSO, pH = 6.0)

Substrate	K_{ass} [a]
Ac-L-AlaOH (**6**)	n.d.[b]
Ac-L-PheOH (**7**)	800
Ac-L-Ala-L-AlaOH (**8**)	4800
Ac-L-Ala-L-PheOH (**9**)	—[c]
Ac-D-Phe-L-AlaOH (**10**)	5000
Ac-L-Phe-L-AlaOH (**11**)	1000
Ac-D-Phe-L-PheOH (**12**)	1850
Ac-L-Phe-L-PheOH (**13**)	2300
Ac-D-Ala-D-AlaOH (**14**)	2000
Ac-D-Ala-D-ValOH (**15**)	1000
Ac-D-Val-D-AlaOH (**16**)	4300
Ac-D-Val-D-ValOH (**17**)	2800
Ac-L-Ala-L-Ala-L-GluOH (**18**)	2600
Ac-L-Ala-L-Phe-L-GluOH (**19**)	1500

[a] K in M^{-1}, estimated error limit in $K < \pm 25\%$. [b] n.d. = binding not detectable. [c] Precipitation occurred during titration.

of TFMSA in TFA for 5 h. But even then, this procedure gave **5** only in moderate yields of 46%, requiring RP18-chromatography to purify receptor **5** from the decomposition byproducts.

To probe the complexation properties of **5** in solution, we performed UV-titration studies[12] in 20% DMSO in buffered water with various *N*-acetylated amino acids, dipeptides and tripeptides as substrates (Table 1). The 20% DMSO content was needed to ensure the solubility of the peptide guests in solution. Aliquots of a stock solution of the substrate ($[\text{guest}]_0 = 1.5$ mM) were added to a solution of the receptor (5 mM Bis-Tris buffer, pH = 6.0, $[\mathbf{5}]_0 = 0.04$ mM). The UV spectrum was recorded after each addition. As the absorbance of the pyrrole moiety at $\lambda = 297$ nm decreases upon complex formation (Fig. 1, top); this change in the spectrum can be used to determine the binding constants. Of course, dilution of the sample during titration has to be taken into account. Analysis of the data was performed using the Specfit/32 software program from Spectrum Software Associates with nonlinear least-square fitting according to a 1 : 1 association model. This 1 : 1 stoichiometry was confirmed by an independent Job plot under the same conditions (Fig. 1, bottom).[13]

First, we evaluated the binding of **5** for amino acids Ac-L-AlaOH (**6**) and Ac-L-PheOH (**7**), finding no affinity or only weak binding ($K < 1000$ M^{-1}). We then tested the dipeptides **8–17** as substrates, which contain D-Val, both enantiomers of Ala and enantiomers of Phe. The dipeptides were expected to bind more efficiently to receptor **5** than simple amino acids, because their length should allow them to interact more strongly due to additional binding sites within the complex (*e.g.* H-bonds between the backbone amides, hydrophobic contacts). Dipeptide **9** did not give reliable results, as it has a very low solubility and precipitation occurred during the titration. The other dipeptides were indeed bound more strongly than simple amino acids. Among the various dipeptides tested, the highest association constants were found for substrates **8**, **10** and **16** ($K > 4000$ M^{-1}). All three dipeptides have Ala in the C-terminal position.[14] The comparison of dipeptides **10** and **12**, as well as **14** and **15**, suggests that alanine in the C-terminal position is preferred over more bulky amino acids such as valine or phenylalanine. Host **5** shows some modest enantioselectivity (*cf.* dipeptides **8** and **14**) as well as diastereoselectivity (at least in the case of dipeptides **10** and **11**).

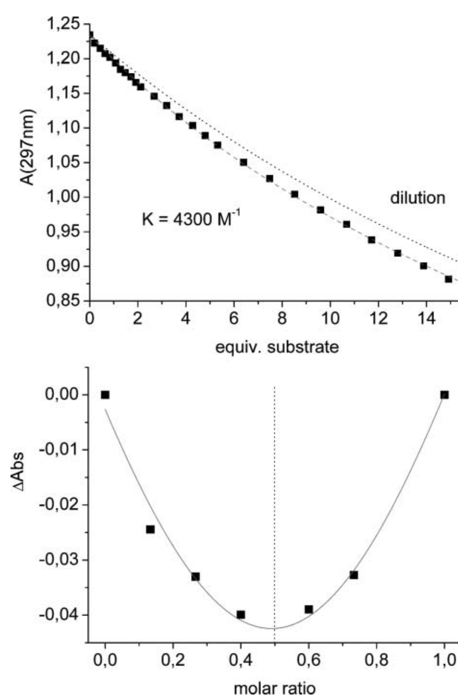


Fig. 1 Binding isotherm for the complexation of Ac-D-Val-D-AlaOH (**16**) by receptor **5** as obtained from a UV titration in 20% DMSO in buffered water at pH = 6.0 and 20 °C (dotted line = expected UV change due to simple dilution without complex formation). The Job plot confirms the 1 : 1 binding stoichiometry.

Two tripeptide carboxylates (**18** and **19**) were also studied for their binding properties. Their affinity was in the same range as observed for the dipeptides, even though they should allow for even more pronounced binding interactions with host **5** compared to the dipeptides. One possible reason is that these substrates are already too long and flexible, so that the entropic costs upon complex formation outweigh any additional interactions between host and substrate.

With these initial binding results from the UV titrations at hand, we decided to perform complementary studies to confirm complex formation between receptor **5** and the dipeptide carboxylates. Specifically, we carried out mass spectrometric as well as NMR studies with the complex between **5** and Ac-D-Val-D-AlaOH (**16**), because this complex showed one of the highest association constants in the UV titrations. Complex formation between **5** and dipeptide **16** was indeed supported by mass spectrometry. An ESI-MS experiment (positive ion mode) of a 1 : 1 mixture in methanol showed a distinct signal for a 1 : 1 complex between **5** and Ac-D-Val-D-AlaOH (**16**) at $m/z = 767$ (Fig. 2), as well as an intense peak at $m/z = 537$, which corresponds to the free host **5**.

^{1}H NMR studies (in DMSO-d_6) also provided support for complex formation between **5** and **16** (Na^+ salt). In a 1 : 1 mixture, small but distinct complexation-induced shift (CIS) changes could be observed relative to the spectra of the two individual components. For example, the signals of one of the pyrrole CHs and the guanidinium amide NH shifted downfield; the broad signal of the four guanidinium $(NH_2)_2$ was split into two downfield-shifted signals, and the broad signal of the pyrrole NH in the free host sharpened and also shifted downfield in the complex. A small downfield shift was also observed for the Ala

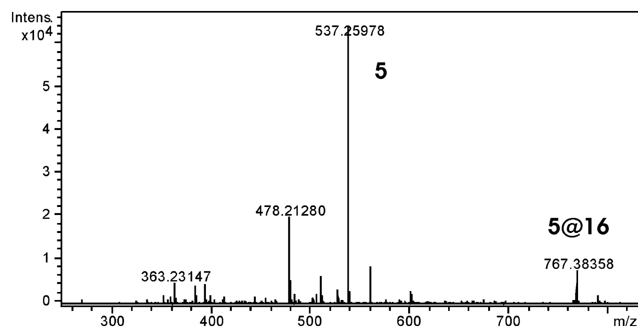


Fig. 2 ESI-MS spectrum (positive ion mode) from a 1 : 1 mixture of **5** and **16** in MeOH.

NH as well as the Ala methyl group of guest **16**. Unfortunately, no reliable quantitative analysis of the changes in the NMR spectra was possible, preventing any more detailed structure analysis or the determination of a binding constant by NMR.

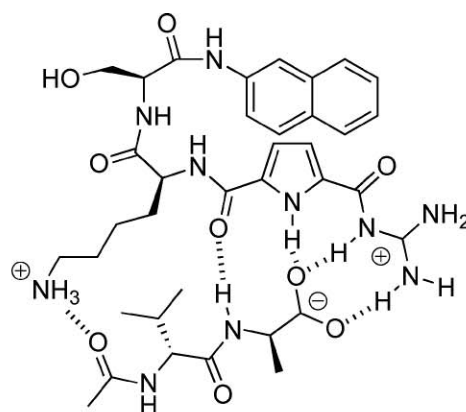
Therefore, a possible structure of the complex between host **5** and dipeptide **16** was calculated using molecular mechanics calculations (Macromodel 8.0, Amber* force field, GB/SA water solvation).[15] A Monte Carlo conformational search with 25 000 steps revealed the energy-minimized structure shown in Fig. 3. The naphthyl ring of **5** π-stacks with the guanidiniocarbonyl pyrrole cation.[16] The carboxylate of **16** is bound in the same bidentate fashion by the acyl guanidinium cation as observed previously for other systems, and the lysine interacts with the N-terminal acetyl group of the substrate. The alanine methyl group is in close proximity to the naphthyl ring, which might explain why dipeptides with bulkier amino acids in this position have lower affinities.

Conclusions

In conclusion, we report here the synthesis of a new prototype of a bis-cationic host **5** by a simple procedure. Host **5** binds dipeptides with a free carboxylate in aqueous buffer solution with millimolar affinities and some preference for Ala in the C-terminal position. We are currently extending the host design by using more rigid linkers between the naphthyl amide and the guanidiniocarbonyl pyrrole. This should further increase complex stability by inducing a more extended conformation in host **5** and thereby facilitating more pronounced hydrophobic contacts with the substrate.

Experimental

Reaction solvents were dried and distilled under argon before use. All other reagents were used as obtained from BAChem, Acros, GL Biochem and Lancaster. Flash column chromatography were run on ICN silica (0.032–0.063 nm) from Biomedicals GmbH or on medium pressure flash system (MPLC, CombiFlash®, Companion™, Isco Inc.) with a prepacked silica gel cartridge (RP-18 Reverse Phase 4.3 g from RediSep). 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. Peak assignment is based on DEPT studies and comparison with literature data. ESI- and HR-mass spectra were recorded on a microTOF from Bruker Daltonik instrument.



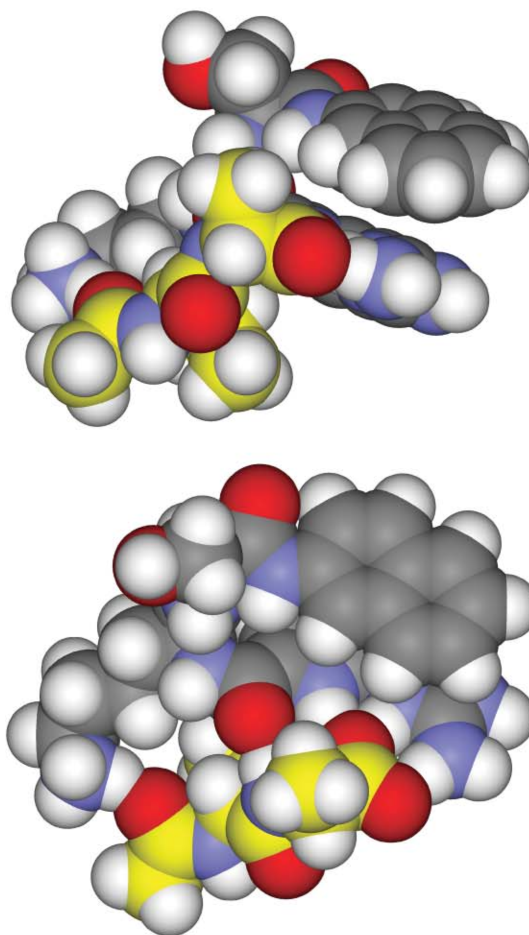


Fig. 3 Schematic representation (top) and energy-minimized structure (middle and bottom) of the 1 : 1 complex between **5** (grey) and **16** (yellow) according to force field calculations.

Analytical HPLC was run on a Supelcosil LC18 (Supelco) 5 μm, (25 cm × 4.6 mm) column. Gua = guanidiniocarbonyl pyrrole.

Synthesis of (2-naphthyl)-L-Ser-L-Lys(Cbz)-NHBoc (2)

A solution of (L)-N^2-Boc-N^6-Cbz-lysine (330 mg, 0.87 mmol, 1 eq), PyBOP (452 mg, 0.87 mmol, 1 eq.) and N-methylmorpholine (NMM) (0.29 mL, 2.60 mmol, 3 eq.) in 6 mL of dry DMF was stirred for 20 min at rt. Afterwards, (L)-N-2-naphthylserinamide

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(**1**) (200 mg, 0.87 mmol, 1 eq.) was added and the solution was stirred at rt overnight. Then it was poured onto 50 mL of water and the suspension was stirred at 0 °C for 2 h. The precipitate was filtered and washed several times with water. The residue was lyophilized, obtaining 515 mg (quant.) of **2** as a pale brown solid: mp = 106–109 °C; ^{1}H NMR (DMSO-d$_6$, 400 MHz): δ = 1.45–1.25 (m, 4H, Lys 2CH$_2$), 1.39 (s, 9H, C(CH$_3$)$_3$), 1.57–1.47 (m, 1H, Lys CHC*H$_2$*), 1.68–1.59 (m, 1H, Lys CHC*H$_2$*), 3.03–2.89 (m, 2H, Lys C*H$_2$*NH), 3.76–3.65 (m, 2H, Ser CH$_2$), 3.99–3.89 (m, 1H, Lys CH), 4.48–4.44 (m, 1H, Ser CH), 5.00 (s, 2H, PhC*H$_2$*), 5.06 (t, 1H, OH, J = 5.4 Hz), 7.01 (d, 1H, Lys N*H*COOC(CH$_3$)$_3$, J = 7.4 Hz), 7.21 (br t, 1H, Lys N*H*COOBn, J = 5.5 Hz), 7.36–7.29 (m, 5H, Ph), 7.42–7.37 (m, 1H, naphthyl H$_6$ or H$_7$), 7.48–7.44 (m, 1H, naphthyl H$_6$ or H$_7$), 7.65 (br d, 1H, naphthyl H$_3$, J = 9.0 Hz), 7.85 (s, 1H, Ser NH), 7.88–7.78 (m, 3H, naphthyl H$_4$, H$_5$ and H$_8$), 8.30 (d, 1H, naphthyl H$_1$, J = 1.6 Hz), 10.05 (s, 1H, naphthyl NH); ^{13}C NMR (DMSO-d$_6$, 100 MHz): δ = 22.8 (Lys CH$_2$), 26.0 (Lys CH$_2$), 28.3 (C(*C*H$_3$)$_3$), 31.2 (Lys CH$_2$), 40.05 (Lys CH$_2$), 54.9 (Lys CH), 55.9 (Ser CH), 61.5 (Ser CH$_2$), 65.6 (Ph*C*H$_2$), 79.5 (*C*(CH$_3$)$_3$), 116.0 (naphthyl CH), 120.2 (naphthyl CH), 125.3 (naphthyl CH), 127.0 (naphthyl CH), 127.4 (naphthyl CH), 127.8 (naphthyl CH), 127.9 (C$_4$ Ph), 128.2 (naphthyl CH), 128.7 (C$_2$ and C$_3$ Ph), 130.2 (naphthyl Cq), 133.4 (naphthyl Cq), 136.0 (naphthyl Cq), 137.2 (C$_1$ Ph), 156.8 (2NHCOO), 163.4 (Lys CONH), 169.3 (Ser CONH); MS (ESI$^+$) m/z = 615 [M + Na]$^+$, 1207 [2M + Na]$^+$; HR-MS (ESI$^+$) m/z = 615.2788 (calculated for ^{12}C$_{32}$H$_{40}$N$_4$NaO$_7$: 615.2789).

Synthesis of (2-naphthyl)-L-Ser-L-Lys(Cbz)-Gua-NHBoc (4)

The Boc-protected amine **2** (500 mg, 0.84 mmol, 1 eq.) was dissolved in a 1 : 1 mixture of TFA–dry dichloromethane (DCM) (6 mL TFA, 6 mL dry DCM), and stirred at rt for 1 h. Then, the excess of TFA and the solvent were removed *in vacuo*, and the oily residue was lyophilized, obtaining 512 mg (quant.) of a pale brownish solid. It was used in the next step without further purification. A solution of *N*-Boc-5-guanidinocarbonyl pyrrole (**3**) (335 mg, 0.84 mmol, 1 eq.), PyBOP (439 mg, 0.84 mmol, 1 eq.) and NMM (0.28 mL, 2.53 mmol, 3 eq.) in 8 mL of dry DMF was stirred for 20 min at rt. Afterwards the free amine (512 mg, 0.84 mmol, 1 eq.) was added and the solution was stirred at rt overnight. Then it was poured onto 50 mL of water and the suspension was stirred at 0 °C for 2 h. The precipitate was filtered, washed several times with water and lyophilized. The residue was purified by flash chromatography (SiO$_2$, EtOAc–MeOH 99 : 1), obtaining 441 mg (68%) of **4** as a white solid; mp = 140–142 °C; ^{1}H NMR (DMSO-d$_6$, 400 MHz): δ = 1.46–1.28 (m, 4H, Lys 2CH$_2$), 1.46 (s, 9H, C(CH$_3$)$_3$), 1.69–1.62 (m, 1H, Lys CHC*H$_2$*), 1.82–1.77 (m, 1H, Lys CHC*H$_2$*), 3.01–2.97 (m, 2H, Lys C*H$_2$*NH), 3.72 (br s, 2H, Ser CH$_2$), 4.52–4.46 (m, 2H, Ser CH and Lys CH), 4.98 (s, 2H, PhC*H$_2$*), 5.04 (t, 1H, OH, J = 5.3 Hz), 6.85 (br s, 2H, pyrrole CH), 7.21 (br t, 1H, Lys N*H*COOBn, J = 5.5 Hz), 7.34–7.28 (m, 5H, Ph), 7.42–7.38 (m, 1H, naphthyl H$_6$ or H$_7$), 7.48–7.44 (m, 1H, naphthyl H$_6$ or H$_7$), 7.65 (dd, 1H, naphthyl H$_3$, J = 8.8 Hz, J = 1.9 Hz), 7.87–7.80 (m, 3H, naphthyl H$_4$, H$_5$ and H$_8$), 8.14 (d, 1H, Ser NH, J = 7.6 Hz), 8.31 (s, 1H, naphthyl H$_1$), 8.47 (d, 1H, Lys NH, J = 7.4 Hz), 8.60 (br s, 1H, guanidino NH), 9.30 (br s, 1H, guanidino NH), 10.04 (s, 1H, naphthyl NH), 10.90 (br s, 1H, guanidino NH), 11.50 (br s, 1H, pyrrole NH); ^{13}C NMR (DMSO-d$_6$, 100 MHz): δ = 23.4 (Lys CH$_2$), 28.2 (C(*C*H$_3$)$_3$), 29.5 (Lys CH$_2$), 31.7 (Lys CH$_2$), 40.5 (Lys CH$_2$), 53.8 (Lys CH), 56.4 (Ser CH), 61.9 (Ser CH$_2$), 65.8 (Ph*C*H$_2$), 82.5 (*C*(CH$_3$)$_3$), 113.9 (pyrrole CH), 114.3 (pyrrole CH), 116.1 (naphthyl CH), 120.5 (naphthyl CH), 125.5 (naphthyl CH), 127.2 (naphthyl CH), 127.8 (naphthyl CH), 128.1 (naphthyl CH), 128.2 (C$_4$ Ph), 128.4 (naphthyl CH), 129.0 (C$_2$ and C$_3$ Ph), 129.0 (pyrrole Cq), 130.5 (naphthyl Cq), 133.8 (naphthyl Cq and pyrrole Cq), 136.5 (naphthyl Cq), 137.6 (C$_1$ Ph), 156.9 (2NHCOO), 158.7 (pyrrole *C*ONH), 160.8 (pyrrole *C*ONH), 169.6 (Lys CONH), 172.8 (Ser CONH); MS (ESI$^+$) m/z = 771 [M + H]$^+$, 793 [M + Na]$^+$; HR-MS (ESI$^+$) m/z = 771.346 (calculated for ^{12}C$_{39}$H$_{47}$N$_8$O$_9$: 771.346).

Synthesis of host 5

The Boc-protected amine **4** (200 mg, 0.26 mmol, 1 eq.) was dissolved in 4 mL of TFA, and 4 μL (0.1%) of trifluoromethane-sulfonic acid (TFMSA) were added. The solution was stirred at rt for 5 h. Then, TFA and TFMSA were removed under reduced pressure (oil pump). The oil obtained was dried and lyophilized. The white solid residue was purified by MPLC (RP18 column, flow 40–20 mL min^{-1}, eluent: 20% MeOH + 0.1% TFA in H$_2$O + 0.1% TFA → 100% MeOH + 0.1% TFA), obtaining 91 mg (46%) of **5** as a white solid; mp = 220 °C (decomposition); ^{1}H NMR (DMSO-d$_6$, 400 MHz): δ = 1.45–1.38 (m, 2H, Lys CH$_2$), 1.59–1.54 (m, 2H, Lys CH$_2$), 1.70–1.63 (m, 1H, Lys CHC*H$_2$*), 1.85–1.78 (m, 1H, Lys CHC*H$_2$*), 2.79–2.77 (m, 2H, Lys C*H$_2$*NH), 3.73 (br d, 2H, Ser CH$_2$, J = 5.4 Hz), 4.61–4.50 (m, 2H, Ser CH and Lys CH), 5.10 (br s, 1H, OH), 6.93 (br s, 1H, pyrrole CH), 7.15 (br s, 1H, pyrrole CH), 7.42–7.38 (m, 1H, naphthyl H$_6$ or H$_7$), 7.49–7.45 (m, 1H, naphthyl H$_6$ or H$_7$), 7.63 (dd, 1H, naphthyl H$_3$, J = 8.8 Hz, J = 2.0 Hz), 7.67 (br s, 3H, NH$_3^+$), 7.88–7.79 (m, 3H, naphthyl H$_4$, H$_5$ and H$_8$), 8.26 (d, 1H, Ser NH, J = 7.4 Hz), 8.30 (s, 1H, naphthyl H$_1$), 8.49 (br s, 4H, guanidinium (NH$_2$)$_2$), 10.15 (s, 1H, naphthyl NH), 11.34 (br s, 1H, guanidinium NH), 12.56 (br s, 1H, pyrrole NH); ^{13}C NMR (DMSO-d$_6$, 100 MHz): δ = 22.6 (Lys CH$_2$), 26.5 (Lys CH$_2$), 31.1 (Lys CH$_2$), 53.1 (Lys CH), 56.1 (Ser CH), 61.5 (Ser CH$_2$), 113.9 (pyrrole CH), 115.3 (pyrrole CH), 115.9 (naphthyl CH), 120.2 (naphthyl CH), 125.3 (naphthyl CH), 127.0 (naphthyl CH), 127.4 (naphthyl CH), 127.7 (naphthyl CH), 128.8 (naphthyl CH and pyrrole Cq), 130.2 (naphthyl Cq), 131.8 (pyrrole Cq), 133.4 (naphthyl Cq), 136.0 (naphthyl Cq), 159.8 (pyrrole *C*ONH), 159.9 (pyrrole *C*ONH), 169.3 (Lys CONH), 172.2 (Ser CONH); MS (ESI$^+$) m/z = 537 [M − H]$^+$; HR-MS (ESI$^+$) m/z = 537.258 (calculated for ^{12}C$_{26}$H$_{33}$N$_8$O$_5$: 537.257); HPLC t_R = 4.68 min (100%); eluent: 50% MeOH + 0.1% TFA and 50% H$_2$O + 0.1% TFA, flow 1 mL min^{-1}, λ = 300 nm.

Acknowledgements

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