Solid phase synthesis of a prototype of a new class of biomimetic receptors for anionic carbohydrates

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A solid phase synthesis for a new biomimetic receptor **1** was developed. First binding studies show that **1** binds anionic carbohydrates such as glucose-1-phosphate and cAMP with association constants in the lower millimolar range in 20% buffered water in DMSO using both polar and apolar interactions.

Introduction

Molecular recognition of carbohydrates by proteins plays a vital role for cell–cell interactions. For example, selectins interact with Sialyl-Lewis X on the surface of leucocytes and hence mediate leucocyte adhesion in inflammation processes.[1] Also bacterial pathogens use protein–carbohydrate interactions for infections of host cells. Artificial receptors that target carbohydrates can help us better understand the underlying molecular recognition events of these biological processes and might also find interesting applications *e.g.* as sensors[2] or drug candidates in the future. Therefore, the supramolecular chemistry of carbohydrates has attracted much attention since Aoyama *et al.* reported the first example of an artificial carbohydrate receptor in 1988.[3] However, whereas a variety of artificial receptors has been developed since then that allow carbohydrate complexation in organic solvents,[4] only very few systems have been reported so far that form stable complexes with carbohydrates in more polar solutions.[5] Carbohydrates are extremely challenging substrates for recognition in aqueous solvents, as their dominant functionality (the OH group) is difficult to distinguish from water molecules which will also interact with any binding site present in the receptor. Only the simultaneous interaction of a receptor with the OH groups as well as the small apolar patches provided by the sugar framework and any other more distinct functional group present in the sugar (carboxylate, phosphate *etc.*) will allow the formation of a stable complex even in aqueous solvents. In this context, we present here the solid phase synthesis of a first prototype (**1**) of a new class of biomimetic receptors for anionic carbohydrates.

Nature uses a combination of both polar and apolar interactions for carbohydrate binding. In carbohydrate binding proteins, often aromatic amino acid residues such as phenyl alanine or tryptophan are found to interact with the CH framework of the sugar, whereas polar residues such as asparagine, serine, lysine or especially arginine form (ionic) H-bonds with the OH groups.[6] Based on the same principle binding interactions[7] we designed a new prototype of a carbohydrate receptor (**1**) shown in Fig. 1. Two dipeptide moieties, Ser-Phe, are attached to an aromatic scaffold (black) together with an arginine analogue, which has improved binding affinity for oxoanions in aqueous solvents compared to a normal arginine.[8,9] As found in carbohydrate binding proteins the receptor could allow for both apolar (Phe) and polar interactions (Ser, Arginine analogue) with a sugar.

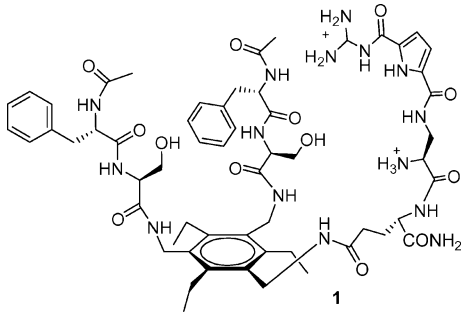

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Fig. 1 A new biomimetic host **1** for carbohydrate binding in aqueous solvents. As found in carbohydrate binding proteins the receptor allows for both apolar (Phe) and polar interactions (Ser, Arginine analogue) with a sugar. The glutamate residue is needed as an attachment point for the resin during the solid phase synthesis of **1**.

Results and discussion

Receptor **1** was synthesized using a solid-phase strategy as shown in Scheme 1. MBHA resin was functionalized with Fmoc-Glu(O*t*Bu)-OH using PyBOP [= 1*H*-benzotriazol-1-yloxy tris(pyrrolidino)phosphonium hexafluorophosphate] in dimethyl formamide (DMF) as the coupling reagent. After deprotection of the Fmoc-group, the bis-Cbz protected arginine analogue **2** was attached (Fmoc = fluorenylmethyloxycarbonyl, Cbz = benzyloxycarbonyl). The *tert*-butyl ester group in the glutamate side chain was then cleaved with trifluoroacetic acid (TFA) and the resulting free carboxylic acid **3** was reacted with the bis-Boc protected aromatic template **4** using the same coupling conditions as before. A negative Malachite green test indicated complete conversion (= absence of carboxylic acid groups) under the experimental conditions. The two Boc-groups on the aromatic template were then cleaved with TFA. The two peptide arms were attached using a standard Boc-protocol. First, Boc-Ser(OBz)-OH was coupled onto the aromatic template. The N-terminal Boc group was cleaved with 50% TFA in dichloromethane and Ac-Phe-OH was attached. In all cases a negative Kaiser test ensured complete conversion. Finally, receptor **1** was cleaved from the resin using 10% trifluoromethane sulfonic acid (TFMSA) in TFA. Under these conditions the Cbz protecting groups on the arginine analogue and the OBz group on the serine side chains

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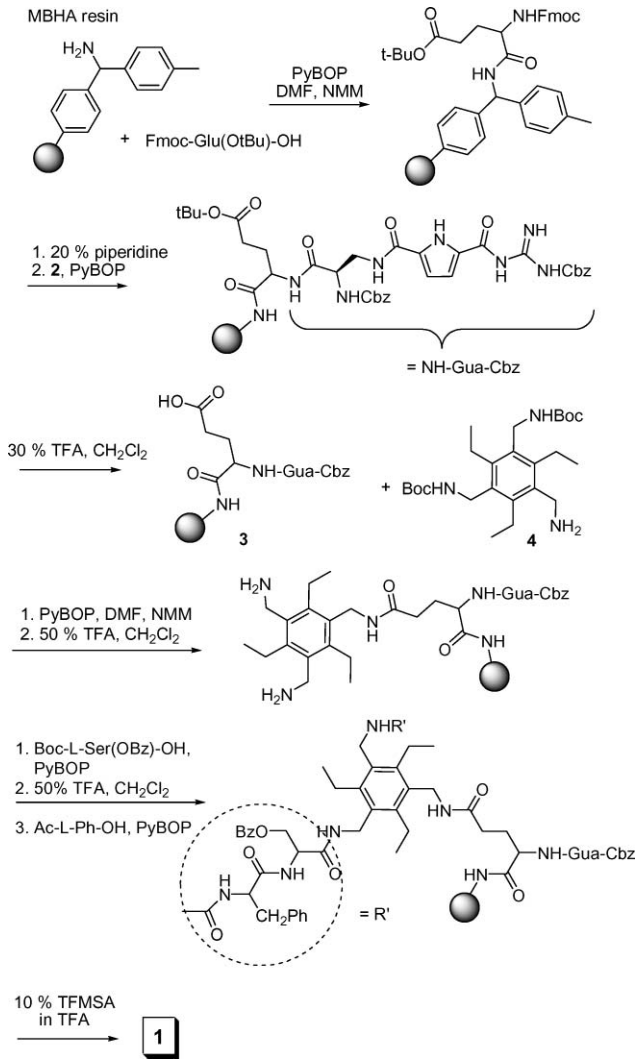
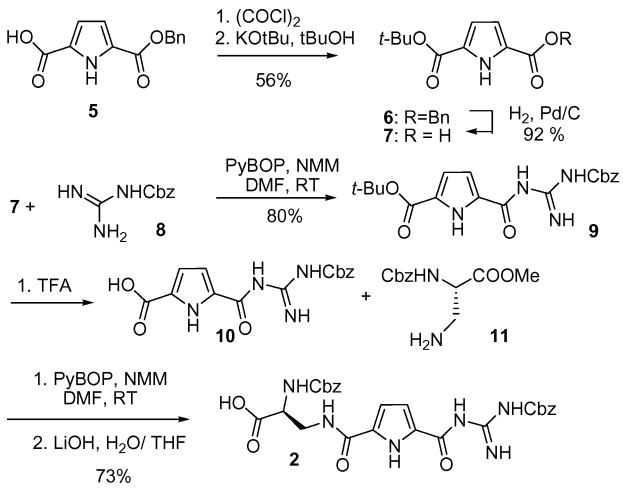


Scheme 1 Solid phase synthesis of receptor **1** on MBHA resin.

are also cleaved as we could show in separate control experiments. The receptor could be isolated by precipitation with diethyl ether from the concentrated cleavage solution. Lyophilisation with HCl finally provided the chloride salt of receptor **1**.

The bis-Cbz protected arginine analogue **2** that was needed for the synthesis of **1**, was synthesized according to Scheme 2. Starting from pyrrole dicarboxylic acid mono benzyl ester **5**, the free carboxylic acid group was transformed into the *tert*-butyl ester **6** *via* the acyl chloride and subsequent reaction with KO*t*Bu in *t*BuOH. After hydrogenolysis of the benzyl ester, mono-Cbz protected guanidine **8** was attached to the free carboxylic acid group in **7** using PyBOP in DMF as the coupling reagent. The *tert*-butyl ester in **9** was then cleaved with TFA and the resulting pyrrole carboxylic acid **10** was reacted with the azaserine derivative **11**.[8b] Hydrolysis of the methyl ester group using LiOH gave the desired bis-Cbz protected guanidinocarbonyl pyrrole building block **2**.

After having successfully established a solid phase protocol for the synthesis of **1**, some first preliminary binding studies were performed using UV titrations in 20% buffered water (10 mM bis-tris buffer, pH = 6.0) in DMSO.[10] Samples of **1** were purified using semipreparative HPLC before use. To a solution of receptor **1** (50 μM) aliquots of a 1.25 mM stock solution of various anionic



Scheme 2 Synthesis of bis-Cbz protected arginine analogue **2**.

carbohydrates as well as methyl phosphate and acetate were added and the UV spectrum was recorded after each addition. The decrease in the absorbance of the pyrrole moiety at 300 nm can be used to calculate the association constant using a non-linear curve fitting for a 1 : 1-complexation (Fig. 2).[11] The molar absorption coefficients ε of both receptor and substrate were determined from independent dilution studies and used as constants for the curve fitting. Furthermore, the absorbance changes due to dilution of the sample during the titration was taken into account. The formation of 1 : 1-complexes was confirmed by Job plots. As the data in Table 1 show, receptor **1** is indeed capable of binding anionic carbohydrates such as uronic acids or sugar phosphates as well as the more complex substrates AMP or cAMP with association constants in the lower millimolar range under these aqueous conditions. A comparison with simple anions such as methyl phosphate or acetate as well as between different epimeric sugars or regioisomeric substrates reveals that the sugar part indeed influences the binding constant. Most likely the actual affinity is determined by a complex interplay of steric effects of the substrate, solvation of the anion and its basicity as well as any

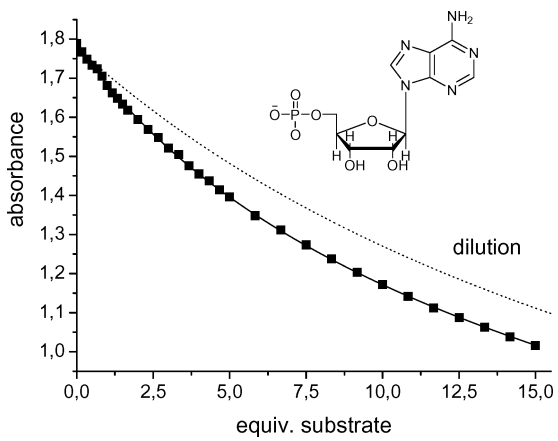


Fig. 2 UV-Titration of receptor **1** with AMP in aqueous DMSO at pH = 6. The decrease in absorbance at 300 nm can be used to calculate the binding constant. The solid line represents the curve fitting for a 1 : 1-complexation (the dotted line is the expected change in absorbance due to the dilution of the sample if no binding occurred).

Table 1 Association constants K_{ass} [in M^{-1}] determined for the binding of anionic substrates by host **1** in aqueous solvents

Substrate	K_{ass}/M^{-1} [a]
AMP	2800
cAMP	2400
Galactose-1-phosphate	3400
Glucose-1-phosphate	3000
Glucose-6-phosphate	≈600
Glucoronic acid	1100
Galacturonic acid	≈700
Methyl phosphate	3800
Acetate	n.d.[b]

[a] UV titration at pH = 6 (10 mM bis-tris buffer in 20% water in DMSO, $[host]_0 = 50$ μM), error estimated to be ±25%. Average value of at least two independent measurements. [b] No complex formation could be detected under these conditions.

additional secondary interactions with the sugar moiety besides simple ion pairing. Unfortunately, no clear straightforward trend is obvious from the data, besides the fact that phosphates are in general bound better than carboxylates which however is a general feature of guanidinium cations.

A further improvement of the binding efficiency and even more importantly substrate selectivity might be achieved by varying the amino acids in the two side arms of the receptor. For this purpose a combinatorial approach is the most useful one.[12] This however requires a solid phase synthesis on a resin that swells in aqueous solvents such as TentaGel and hence allows subsequent screening of the library in aqueous solvents directly on the bead. For this purpose the side chains need to be deprotected without cleaving the receptors from the resin. We therefore tested whether the synthetic scheme described in Scheme 1 can also be used on TentaGel resin. Preliminary studies showed however that the conditions used to deprotect the side chains (10% TFMSA in TFA) led to a complete decomposition of the TentaGel resin. In the NMR of the cleaved-off product intense signals for polyethylene glycol fragments at around $\delta = 5.6$ were observed. Obviously, the TentaGel resin is much more acid sensitive compared to the standard MBHA resin used. TentaGel survives a treatment with TFA alone but these conditions are not acidic enough to cleave the Cbz protecting groups. We therefore attached compound **2** onto TentaGel-NH_2-resin and tested different concentrations of TFMSA in TFA. No decomposition and no cleavage of **2** from the resin was observed upon treatment with 0.1% TFMSA in TFA whereas any higher concentration of TFMSA already caused a significant cleavage of **2** from the resin. To determine the time needed to achieve complete deprotection of the side chains using 0.1% TFMSA in TFA time dependent control studies under HPLC control were performed. They showed that after three hours at r.t. the starting material was completely deprotected.

This could also be confirmed by NMR studies. For this purpose aliquots were taken from the cleavage solution after certain time periods. The TFA was removed under vacuum and the residue was treated with diethyl ether. The resulting solid was then dissolved in DMSO-d6 and the NMR spectrum was measured. After 30 min of treatment of the resin-bound bis-Cbz-protected **2** with 0.1% TFMSA in TFA the Cbz-group on the α-amino groups had been cleaved off whereas the Cbz-group on the guanidino moiety required longer reaction times of >120 min to disappear completely (Fig. 3). Hence, a three hour treatment at r.t. assures complete deprotection of the side chains of the resin-bound compound without causing decomposition of the resin or cleavage of the product from the resin.

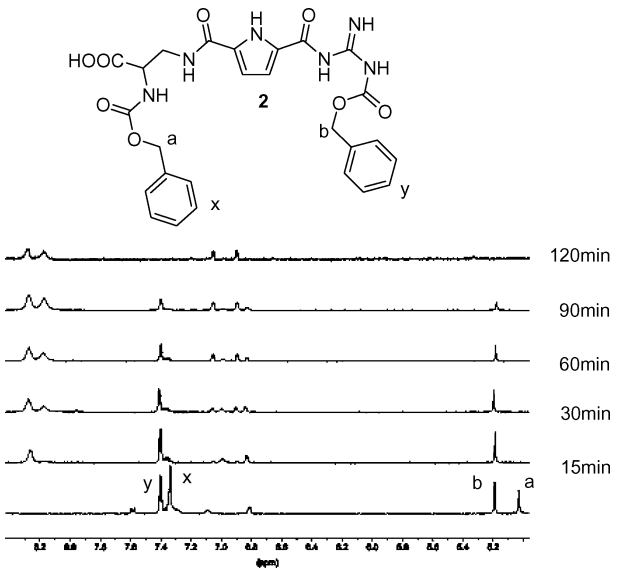


Fig. 3 Deprotection of **2** with 0.1% TFMSA in TFA under NMR control (DMSO-d6). After 15 min the Cbz group on the α-amino group starts to disappear whereas the Cbz-group on the guanidino group requires much longer reaction times of >120 min.

Conclusions

In conclusion, we have presented here the successful solid phase synthesis of the biomimetic carbohydrate host **1**, that can make use of the same kind of binding interactions found also in natural systems (a combination of apolar and polar interactions). Preliminary binding studies showed that **1** indeed binds anionic sugars with low millimolar affinity in aqueous DMSO proving the principal usefulness of the general receptor design. With the necessary synthetic protocols now at hand, a combinatorial approach can be used in future work to improve both affinity and selectivity of such receptors by optimization of the amino acids in the two side arms of **1**. Such work is currently in progress and will be reported in due course.

Experimental

Solvents were dried and distilled before use. All experiments were run in oven-dried glassware. The compounds were dried in high vacuum over phosphorus pentoxide at room temperature overnight unless otherwise stated. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 or 600 spectrometer. The chemical shifts are reported relative to the deuterated solvents. The EI-mass spectra were recorded on a Finnigan MAT 90, the ESI- and HR-mass spectra were recorded on a Finnigan MAT 900 S.

Synthesis of receptor 1

MBHA resin (100.0 mg, 1.3 mmol g^{-1}, 0.13 mmol) was swollen in DMF for 1 h. The resin was treated with Fmoc-Glu(O*t*Bu)-OH

(128 mg, 0.33 mmol, 2.5 eq.) and PyBOP (169 mg, 0.33 mmol, 2.5 eq.) in DMF (10 ml) containing 3% *N*-methylmorpholine (NMM) for 20 h. The Fmoc group was removed by treatment with piperidine in DMF (20%) for 30 min. The attachment of the bis-Cbz protected arginine analogue **2** was performed under related conditions: 2.5 eq. **2** (179 mg, 0.33 mmol), 2.5 eq. PyBOP (169 mg, 0.33 mmol) and DMF (10 ml) containing 5% NMM with a reaction time of 20 h. The coupling step was repeated to ensure quantitative coupling. In the next step the resin was treated with 50% TFA in CH_2Cl_2 (= DCM; 10 ml) for 30 min to remove the side chain protecting group of the glutamic acid. The resulting free carboxylic acid functions were coupled with the template **4** by the use of the following procedure: 1.5 eq. template (89.9 mg, 0.2 mmol) and 2.5 eq. of PyBOP (169 mg, 0.33 mmol) in DMF (10 ml) containing 5% NMM. To remove the Boc protecting groups of the template **4** the resin was treated with 40% TFA in DCM (10 ml) for 30 min. The resulting free amino functions were coupled with Boc-L-Ser(OBz)-OH (192 mg, 0.65 mmol, 5 eq.) by using PyBOP (339 mg, 0.65 mmol, 5 eq.) in DMF (10 ml) as the coupling reagent containing 3% NMM. The Boc protecting groups of the amino acid were removed by treatment with 50% TFA in DCM (10 ml) for 30 min. In the last coupling step the free amino functions of the amino acid were coupled with Ac-Phe-OH (135 mg, 0.65 mmol, 5 eq.) again by using PyBOP (339 mg, 0.65 mmol, 5 eq.) in DMF (10 ml) containing 3% NMM. The resin was washed thoroughly with DCM, methanol, diethyl ether and further DCM to remove traces of DMF. With the treatment of a TFA–TFMSA mixture (90 : 10) for 3 h the Cbz- and OBz-protecting groups were removed and the product was cleaved from the solid report. The solvent was evaporated and the remaining oil was treated with dry diethyl ether. To obtain the hydrochloride salt, the colorless solid was dissolved in water (40 ml), acidified with hydrochloric acid (0.1 N, 4 ml), and lyophilized. This step was repeated twice. Yield: 0.025 g, 0.021 mmol, 16%; ^{1}H-NMR (600 MHz, [D$_6$]DMSO, 27 °C): δ = 1.05 (m, 9H, C*H*$_3$), 1.73 (m, 6H, ac-C*H*$_3$), 1.83 (m, 1H, glu-C*H*$_2$), 1.93 (m, 1H, glu-C*H*$_2$), 2.18 (m, 2H, glu-C*H*$_2$), 2.66 (br s, 6H, C*H*$_2$), 2.79 (m, 2H, phe-C*H*$_2$), 2.92 (m, 2H, phe-C*H*$_2$), 3.55 (m, 4H, ser-C*H*$_2$), 3.59 (m, 1H, C*H*$_2$), 3.79 (m, 1H, C*H*$_2$), 3.79 (m, 1H, glu-C*H*), 3.98 (m, 1H, ser-C*H*), 3.98 (m, 1H, C*H*), 4.29 (br s, 6H, C*H*$_2$-NH), 4.51 (m, 2H, phe-C*H*), 6.93 (br s, 1H, pyrrole-C*H*), 7.04 (br s, 1H, pyrrole-C*H*), 7.04 (m, 2H, N*H*$_2$), 7.18 (m, 6H, phe-C*H*), 7.62 (br s, 2H, ser-N*H*), 7.88 (br s, 1H, glu-N*H*), 8.12 (br s, 2H, phe-N*H*), 8.23 (br s, 4H, gua-N*H*$_2$), 8.26 (br s, 3H, N*H*$_3$), 8.69 (m, 1H, N*H*), 10.96 (br s, 1H, N*H*), 12.39 (br s, 1H, pyrrole-N*H*); HR-MS (ESI pos.): calcd for $C_{58}H_{80}N_{15}O_{13}$ + H^+: 1194.6055, found 1194.606.

Synthesis of the diester 6

To a solution of the pyrrole dicarboxylic acid mono benzyl ester **5** (5.0 g, 20.4 mmol) in dichloromethane (40 ml) and catalytic amounts of DMF was slowly added a solution of oxalylchloride (5.2 g, 61.17 mmol) in dry dichloromethane (10 ml). The reaction mixture was stirred for one hour at room temperature and afterwards the solvent was evaporated. The resulting yellow powder was suspended in dry *tert*-butanol (75 ml), heated to 40 °C and to the solution was added KO*t*Bu (3.74 g, 30.58 mmol). The reaction mixture was stirred for another 2 h at 40 °C and then the solvent was evaporated. The slightly brown powder was suspended in dichloromethane (100 ml) and washed with a sodium hydrogen carbonate solution (3 × 50 ml) and water (1 × 50 ml). Again the organic solvent was evaporated and the crude product was purified by column chromatography (SiO_2, hexane–ethyl acetate = 8 : 2 + 1% triethylamine) yielding **6** as a slightly yellow solid. Yield: 3.45 g, 11.4 mmol, 56%; mp: 69 °C; FT IR (KBr) ν [cm^{-1}] = 3326 [m], 2976 [w], 1704 [s], 1550 [m], 1448 [m], 1368 [m], 1280 [s], 1161 [s], 1119 [s], 1007 [m], 851 [m]; ^{1}H-NMR (400 MHz, $CDCl_3$, 27 °C): δ = 1.43 (s, 9H, *t*Bu), 5.2 (s, 2H, benzyl-C*H*$_2$), 6.66–6.68 (m, 1H, pyrrole-C*H*), 6.75–6.76 (m, 1H, pyrrole-C*H*), 7.24–7.28 (m, 5H, benzyl-C*H*); ^{13}C-NMR (100 MHz, [D$_6$]DMSO, 27 °C): δ = 27.1 (*t*Bu, *C*H$_3$), 65.5 (benzyl-*C*H$_2$), 80.8 (*C*$_q$, *t*Bu), 114.9 (pyrrole-*C*H), 115.5 (pyrrole-*C*H), 125.5 (*C*$_q$), 127.8, 128.0, 128.4 (benzyl-*C*H), 136.1 (*C*$_q$), 159.1, (*C*$_q$, *C*OO benzyl), 159.6 *C*$_q$, *C*OO *t*Bu); HR-MS (ESI pos.): calcd for $C_{17}H_{19}NO_4$: 301.131, found for $C_{17}H_{19}NO_4$ + Na^+: 324.120.

Synthesis of the free carboxylic acid 7

A mixture of the diester **6** (710 mg, 2.36 mmol) and 10% Pd/C (71 mg) in methanol (20 ml) was vigorously stirred for 5 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a celite pad and washed with methanol. The combined filtrates were evaporated to give the free carboxylic acid **7** as a colorless solid, which was dried *in vacuo.* Yield: 460 mg, 2.18 mmol, 92%; mp: 162 °C; FT IR (KBr) ν [cm^{-1}] = 3421 [m], 3309 [s], 2971 [m], 1708 [s], 1683 [s], 1556 [w], 1458 [s], 1359 [m], 1275 [s], 1157 [s], 1127 [m], 815 [s]; ^{1}H-NMR (400 MHz, $CDCl_3$, 27 °C): δ = 1.59 (s, 9H, *t*Bu), 6.67–6.68 (m, 1H, pyrrole-C*H*), 6.96–6.98 (m, 1H, pyrrole-C*H*), 10.34 (br s, 1H, pyrrole-N*H*); ^{13}C-NMR (100 MHz, [D$_6$]DMSO, 27 °C): δ = 27.9 (*t*Bu, *C*H$_3$), 80.6 (*C*$_q$, *t*Bu), 114.9 (pyrrole-*C*H), 114.9 (pyrrole-*C*H), 127.2 (*C*$_q$), 127.5 (*C*$_q$), 159.2 (*C*$_q$, *C*OO *t*Bu), 161.3 (*C*$_q$, *C*OOH); HR-MS (ESI neg.) calcd for $C_{10}H_{13}NO_4$ (= M − H): 210.077, found 210.077.

Synthesis of 9

A mixture of the *tert*-butyl ester **7** (1.75 g, 8.28 mmol), PyBOP (4.30 g, 8.28 mmol) and *N*-methyl morpholine (1 ml) was stirred in DMF (10 ml) at room temperature for 30 min. Cbz-Guanidine **8** (2.60 g, 13.47 mmol) was added and the resulting solution stirred overnight. The red solution was hydrolyzed with water (20 ml) and extracted three times with diethyl ether (40 ml). The combined organic layers were extracted two times with water (50 ml), dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography (SiO_2, hexane–ethyl acetate = 8 : 2) yielding **9** as a colorless solid. Yield: 2.57 g, 6.65 mmol, 80%; mp: 158 °C; FT IR (KBr) ν [cm^{-1}] = 3395 [s], 3372 [s], 2977 [m], 1736 [s], 1705 [m], 1636 [s], 1535 [m], 1158 [s], 1275 [s], 1036 [s], 825 [m]; ^{1}H-NMR (400 MHz, $CDCl_3$, 27 °C): δ = 1.56 (s, 9H, *t*Bu), 5.19 (s, 2H, benzyl-C*H*$_2$), 6.77–6.78 (m, 1H, pyrrole-C*H*), 6.85–6.86 (m, 1H, pyrrole-C*H*), 7.32–7.37 (m, 5H, benzyl-C*H*), 8.71 (br s, 1H, N*H*), 9.8 (br s, 1H, pyrrole-N*H*); ^{13}C-NMR (100 MHz, [D$_6$]DMSO, 27 °C): δ = 27.8 (*t*Bu, *C*H$_3$), 66.2 (benzyl-*C*H$_2$), 80.9 (*C*$_q$, *t*-Bu), 114.8 (pyrrole-*C*H), 114.8 (pyrrole-*C*H), 127.8, (benzyl-*C*H), 127.9 (*C*$_q$), 128.3 (*C*$_q$), 159.2 (*C*$_q$, *C*OO *t*Bu); HR-MS (ESI pos.): calcd for $C_{19}H_{22}N_4O_5$ + H^+: 387.159, found 387.168.

Synthesis of the pyrrole carboxylic acid 10

A solution of the mono-Cbz protected arginine analogue **9** (2.57 g, 6.65 mmol) in trifluoroacetic acid (10 ml) and dichloromethane (10 ml) was stirred for 3 h at room temperature. The trifluoroacetic acid was evaporated *in vacuo* yielding **10** as a colorless solid. Yield: 2.20 g, 6.64 mmol, 99%; mp: 198 °C; FT IR (KBr) ν [cm^{-1}] = 3355 [s], 3329 [s], 3237 [m], 3142 [m], 2978 [m], 1755 [s], 1696 [s], 1651 [s], 1548 [m], 1351 [s], 1288 [m], 1233 [m]; ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 5.18 (s, 2H, benzyl-CH_2), 6.88 (br s, 1H, pyrrole-C*H*), 7.01 (br s, 1H, pyrrole-C*H*), 7.40–7.41 (br s, 5H, benzyl-C*H*), 9.00 (br s, 1H, N*H*), 9.54 (br s, 1H, pyrrole-N*H*), 12.08 (s, 1H, COO*H*); ^{13}C-NMR (100 MHz, [D_6]DMSO, 27 °C): δ = 67.6 (benzyl-*C*H_2), 115.2 (pyrrole-*C*H), 115.2 (pyrrole-*C*H), 128.1 (benzyl-*C*H), 128.4 (C_q), 128.5 (C_q), 155.4 (C_q, pyrrole-*C*ONH), 161.1 (C_q, *C*OOH); HR-MS (ESI pos.): calcd for $C_{15}H_{14}N_4O_5$ + H^+: 331.094, found 331.099.

Synthesis of the protected arginine analogue 2-OMe

A mixture of the Cbz-protected guanidinocarbonyl pyrrole compound **10** (3.45 g, 6.65 mmol), PyBOP (3.46 g, 6.65 mmol) and *N*-methyl morpholin (4 ml) was stirred in DMF (10 ml) at room temperature for 30 min. The amino acid **11** (2.88 g, 9.98 mmol) was added and the resulting solution stirred overnight. The slightly yellow solution was hydrolyzed with water (20 ml) and extracted three times with diethyl ether (40 ml) and ethyl acetate (5 ml). The combined organic layers were extracted two times with water (50 ml), dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography (SiO_2, dichloromethane–methanol = 10 : 0.1 + 1% triethylamine) yielding the methyl ester of **12** as a colorless solid. Yield: 2.74 g, 4.85 mmol, 73%; mp: 176 °C; FT IR (KBr) ν [cm^{-1}] = 3277 [s], 3109 [m], 2958 [m], 1899 [s], 1544 [m], 1551 [s], 1387 [m], 987 [s], 852 [m]; ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 3.51 (m, 1H, CH_2), 3.61 (m, 1H, CH_2), 3.62 (s, 3H, COOMe), 4.28 (q, J = 5.2 Hz, 1H, C*H*), 5.15 (s, 2H, benzyl-CH_2), 5.25 (s, 2H, benzyl-CH_2), 6.90 (br s, 1H, pyrrole-C*H*), 7.01 (br s, 1H, pyrrole-C*H*), 7.46–7.5 (m, 10H, benzyl-C*H*), 7.84 (d, J = 8.2 Hz, 1H, N*H*), 8.62 (t, J = 5.9 Hz, 1H, N*H*), 8.88 (br s, 1H, N*H*), 9.48 (br s, 1H, pyrrole-N*H*); ^{13}C-NMR (100 MHz, [D_6]DMSO, 27 °C): δ = 45.8 (*C*H_2), 52.2 (*C*H_3), 53.8 (*C*H), 65.5 (*C*H_2-benzyl), 112.3 (pyrrole-*C*H), 112.3 (pyrrole-*C*H), 127–128.3 (benzyl-*C*H), 128.4 (C_q), 128.4 (C_q), 155.9 (C_q), 159.8 (C_q); HR-MS (ESI pos.): calcd for $C_{26}H_{28}N_6O_8$ + H^+: 565.196, found 565.197.

Synthesis of the bis-Cbz protected arginine analogue 2

A mixture of the methyl ester **2-OMe** (1.49 g, 3.44 mmol) and lithium hydroxide monohydrate (148 mg, 3.52 mmol) in THF–water mixture (10 ml, 4 : 1) was stirred for 3 h until complete hydrolysis. The organic solvent was evaporated and water (57 ml) and dichloromethane (150 ml) were added to the resulting solution. Acidification of the solution to a pH of 3–4 with 1 N hydrochloric acid produced **2** yielding as a colorless solid which was filtered and dried over phosphorus pentoxide. Yield: 1.89 g, 3.33 mmol, 99%; mp: >250 °C (decomposition); FT IR (KBr) ν [cm^{-1}] = 3389 [s], 3278 [s], 2765 [m], 1567 [s], 1222 [s], 987 [s], 889 [m], 852 [m]; ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C, pikrate-salt): δ = 3.02 (m, 1H, CH_2), 3.63 (m, 1H, CH_2), 4.23 (q, J = 5.2 Hz, 1H, C*H*), 5.02 (s, 2H, benzyl-CH_2), 5.23 (s, 2H, benzyl-CH_2), 6.84 (br s, 1H, pyrrole-C*H*), 6.99 (br s, 1H, pyrrole-C*H*), 7.33–7.57 (m, 10H, benzyl-C*H*), 7.59 (d, J = 8.2 Hz, 1H, N*H*), 8.54 (t, J = 5.7 Hz, 1H, N*H*), 9.29 (br s, 1H, N*H*), 9.76 (br s, 1H, N*H*), 12.23 (br s, 1H, pyrrole-N*H*); ^{13}C-NMR (100 MHz, [D_6]DMSO, 27 °C): δ = 45.8 (CH_2), 53.8 (*C*H), 65.5 (benzyl-*C*H), 112.3 (pyrrole-*C*H), 112.3 (pyrrole-*C*H), 127–128.3 (benzyl-*C*H), 128.4 (C_q), 128.4 (C_q), 155.9 (C_q), 159.8 (C_q), 171.89 (*C*OOH); HR-MS (ESI neg.): calcd for $C_{26}H_{26}N_6O_8$ (= M − H): 549.174, found 549.172.

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