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Sequence-Dependent Binding of Dipeptides by an Artificial Receptor in Water

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Abstract: An artificial dipeptide receptor (**1**) was designed and observed to bind the deprotonated dipeptide Ac-D-Ala-D-Ala-OH in buffered water with $K = 33\,100\,\text{M}^{-1}$, whereas other dipeptides such as Ac-Gly-Gly-OH or Ac-D-Val-D-Val-OH were bound less efficiently, by factors of more than 10 ($K < 3000\,\text{M}^{-1}$). The efficient binding and the pronounced sequence selectivity are the result of a combination of strong electrostatic contacts and size-discriminating hydrophobic interactions. To provide such a combination, a guanidiniocarbonylpyrrole cation was attached to a novel cyclotribenzylene-substituted alanine derivative **5**, to provide a hydrophobic bowl-shaped cavity just large enough to bind a methyl group but not any larger alkyl chains, thus causing the receptor to prefer alanine to valine. We describe the synthesis of **1** and the evaluation of its complexation properties in UV and fluorescence titration studies.

Keywords: artificial receptors · guanidinium cations · molecular recognition · peptides · supramolecular chemistry

Introduction

Artificial receptors that selectively bind to a given peptide sequence are of considerable interest not only for study of the underlying principles of such molecular recognition events but also for the construction of biosensors or as tools with which to probe cellular processes.[1,2] Only a very few artificial receptors that allow the complexation of an oligopeptide in aqueous solvents (e.g., under physiological conditions) exist.[3] Quite often, however, the substrate selectivities of these receptors cannot be predicted a priori, especially when they have been identified by the screening of combinatorial libraries,[4] so the design of a specific receptor for a given peptide sequence still remains an open and challenging task. We recently designed a receptor that was capable of binding dipeptides in aqueous solvents with association constants up to $K_{ass} = 50\,000\,\text{M}^{-1}$, but this receptor was not able to distinguish between different dipeptides, since the interactions between receptor and substrate were limited to the amide backbone of the dipeptide.[5] While the receptor did therefore prefer dipeptides over amino acids, the selectivity between dipeptides of different sequences was only modest, since complexation did not invoke any direct interactions with the amino acid side chains.[6] The receptor had a modest preference for large and bulky amino acids over smaller ones—Val-Val, for example, was bound better than Ala-Ala, which was in turn bound better than Gly-Gly—but this trend in affinities most probably just reflected the overall hydrophobic characters of the corresponding dipeptides. In general, hydrophobic interactions simply increase with the areas of the interacting surfaces or residues.[7]

We now present here the synthesis and the binding properties of an artificial receptor **1** (Figure 1) specifically designed to bind alanine-containing dipeptide carboxylates. Such dipeptide sequences are of considerable biological

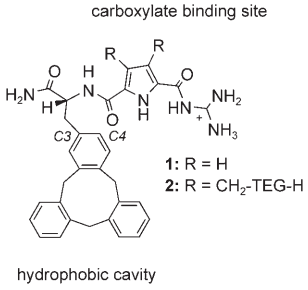

Figure 1. Design of an artificial receptor **1** with a preference for alanine-containing dipeptide carboxylates.

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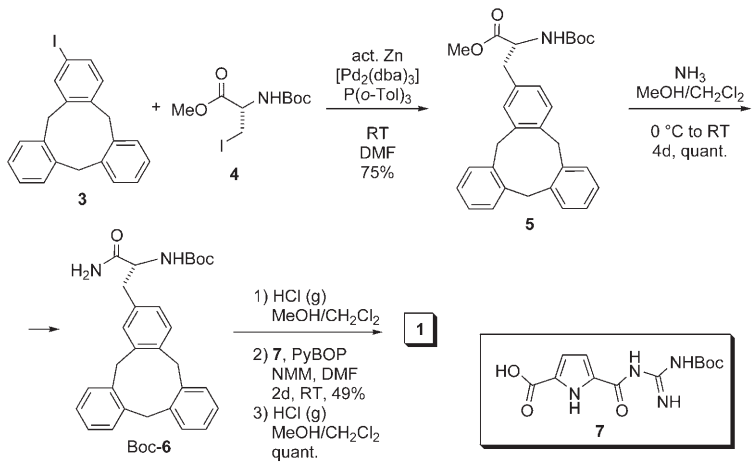
relevance: the dipeptide sequence D-Ala-D-Ala, for example, represents the C-terminal part of a bacterial peptidoglycan and plays an important role in cell wall maturation in Gram-positive bacteria, whilst also being the target of the vancomycin group antibiotics,[8] which bind to this dipeptide sequence through a combination of hydrogen bonds and electrostatic and hydrophobic interactions. Our newly designed receptor **1** is capable of selectively binding D-Ala-D-Ala even in water, with an association constant of $K_{ass} = 33\,100\,M^{-1}$, in preference to other dipeptides such as D-Val-D-Val ($K_{ass} < 3000\,M^{-1}$). The selectivity is most probably the function of a size-discriminating cyclotribenzylene cavity that provides a shallow hydrophobic pocket large enough only for an alanine methyl group.[9]



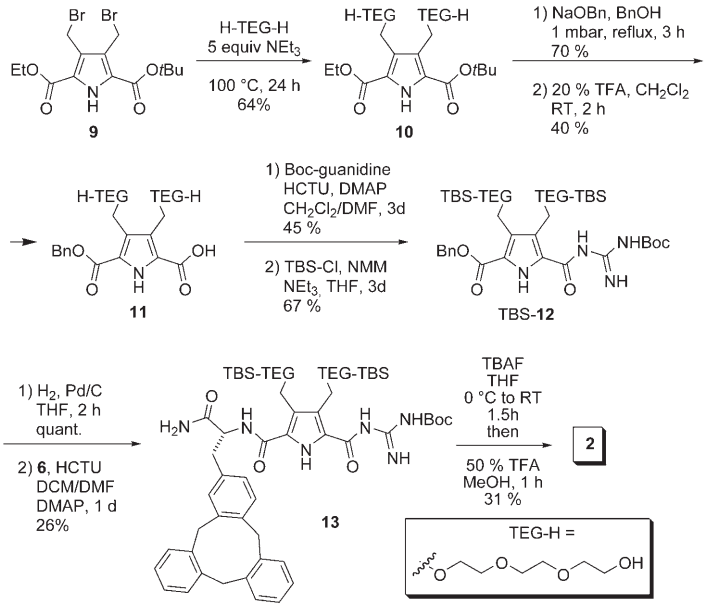
Scheme 1. Synthesis of receptor **1**.

Results and Discussion

Design and synthesis of the receptor: The design of **1** was based on some general features taken from the natural antibiotic Vancomycin.[8] In vancomycin a protonated secondary amine serves as a cationic anchor point for the dipeptide's carboxylate, and several hydrogen bonds to the peptide backbone, together with hydrophobic interactions, then further strengthen the complex.[10] In addition, several aromatic rings form a rather rigid aromatic cavity in which the dipeptide is embedded. This provides the selectivity for alanine, as no larger alkyl chains fit into this bowl-shaped structure, so the efficient binding features of Vancomycin are due to a combination of electrostatic interactions (H-bonds and charge interactions) and a size-selecting aromatic cavity. We have now developed an artificial receptor **1** (Figure 1) by following the same guidelines.[11] Receptor **1** consists of two major building blocks: a carboxylate binding site attached to a size-selecting aromatic cavity. As the binding site for the dipeptide's carboxylate we used a cationic guanidiniocarbonylpyrrole moiety.[12] This modified guanidinium cation has already proven useful in the context of peptide recognition even in water.[13,14] To mimic at least a part of the aromatic cavity formed by Vancomycin's several aromatic rings we chose a cyclotribenzylene unit. This moiety provides a hydrophobic bowl just large enough to accommodate a methyl but not any other alkyl group,[15,16] thus hopefully providing a selectivity for alanine.[17] Molecular mechanics calculations (see below) confirmed that receptor **1** should be able to bind D-Ala-D-Ala preferentially.

The key intermediate for the synthesis of **1** (Scheme 1) was the nonnatural amino acid **5**, obtained from a Pd-catalyzed Negishi coupling between racemic (*P*/*M*)-iodocyclotribenzylene **3**[18] and the zinc derivative of D-iodoalanine (**4**).[19] We modified Jackson's initial protocol, which gave yields of about 40%, by increasing the reaction scale and by using a prolonged reaction time, thanks to which we were able to improve the yield significantly, to an excellent 75%. Because of the planar chirality of the iodocyclotribenzylene **3**, which was used as a racemic mixture, the amino acid derivative **5** was obtained as a mixture of two diastereoisomers, denoted *P*/D and *M*/D, respectively, with the configuration of the stereogenic center of the amino acid described by D/L nomenclature and the configuration of the cyclotribenzylene unit by *P*/*M* descriptors. These two diastereomers differ only in the absolute curvature of the cyclotribenzylene bowl, as the ring flip at room temperature is rather slow,[15b,20] or looked at another way, the cyclotribenzylene unit can be viewed as being attached to the amino acid either through C3 or C4 in the two diastereomers (see Figure 1). This produces only a very small difference in the three-dimensional structures of the two diastereomers, and the diastereomeric (*P*,*M*)/D mixture of **5** accordingly could not be separated even by HPLC on a chiral column. We therefore used the diastereomeric mixture (*P*,*M*)/D-**5** in the synthesis of receptor **1**, since modeling studies also suggested that the binding affinity should not be affected significantly by the absolute configuration of the cyclotribenzylene unit (see below). The ester group in **5** was then transformed into the amide by treatment with ammonia, and after deprotection with HCl the free amine **6** was coupled with the pyrrolecarboxylic acid (CBS) **7** in the presence of PyBOP in DMF to give the *N*-Boc-protected receptor **8**. Removal of the Boc group with HCl provided the free cationic receptor (*P*,*M*)/D-**1** (chloride salt).

Additionally, a triethyleneglycol-substituted analogue **2** was also synthesized in the hope that the triethyleneglycol chains would increase the solubility of this receptor in water and hence facilitate the binding studies later on. Receptor **2** was synthesized as shown in Scheme 2. In the first step the dibromide **9** was treated with triethyleneglycol (TEG) in the



Scheme 2. Synthesis of the triethyleneglycol-substituted receptor **2**.

presence of triethylamine to provide the TEG-substituted diester **10**, which was isolated from the reaction mixture directly by RP18-MPLC. In this way the yield could be significantly increased over that of the aqueous workup described by us previously (64 versus 46%, respectively).[21] Transesterification of the ethyl ester to the benzyl ester **10b** was achieved with sodium benzylate in benzyl alcohol under reduced pressure to remove the liberated EtOH. When we tried to cleave the *t*Bu ester in **10b** by a standard method (20% TFA in CH_2Cl_2 at RT) we also observed significant decomposition of the product before all starting material had reacted, resulting in low overall yields, and so we limited the reaction time to 2 h, when decomposition was just starting according to TLC monitoring. In this way the free acid **11** was obtained in a yield of 40%, together with 35% of reisolated and reusable starting material. The free acid group in **11** was then coupled with Boc-protected guanidine in the presence of 1*H*-benzotriazolium-1-[bis(dimethylamino)methylene]-5-chloro-hexafluorophosphate-1,3-oxide (HCTU) as the coupling reagent.

Because of increasing difficulties with the isolation of the TEG-substituted products we decided to protect the two alcohol functions as silyl ethers. In the presence of an excess of NMM (4-methylmorpholine) and triethylamine, TBS-Cl was added in portions with frequent TLC monitoring to provide the optimal reaction time needed for the maximum yield of the O-diprotected product (without N-protection of the pyrrole NH), and in this way the disilylated product TBS-**12** could be obtained in 67% yield. Hydrogenolysis of the benzyl ester and subsequent coupling of the resulting free carboxylic acid with **6** gave the protected receptor **13**, which could be easily purified by flash chromatography on silica gel. TBS removal with TBAF and Boc removal with TFA provided the free cationic triethyleneglycol-substituted receptor **2**, again as a mixture of the two (*P*,*M*)/D diastereoisomers. Separation of the two diastereomers was attempted by MPLC purification of **13**, of the *N*-Boc-protected precursor Boc-**2** (after TBAF cleavage of the TBS ethers), and of the free receptor **2**, but no conditions to separate the two isomers could be found either by silica-MPLC or by RP18-HPLC, and all subsequent studies were consequently carried out with the (*P*,*M*)/D-diastereoisomeric mixtures of receptors **1** or **2**.

Binding studies: We first investigated the complexation properties of the unsubstituted receptor **1** by UV and fluorescence titration studies in aqueous buffer solution.[22] For these experiments, samples of **1** were purified by preparative HPLC before use. Control experiments confirmed that up to a concentration of [**1**] $\approx 8\cdot10^{-5}$ M ([bis-tris buffer] = 0.032 M in 10% DMSO in water) the UV absorption of the pyrrole band at $\lambda_{max} = 300$ nm displayed linear Lambert–Beer behavior (Figure 2), but at higher concentrations of **1**

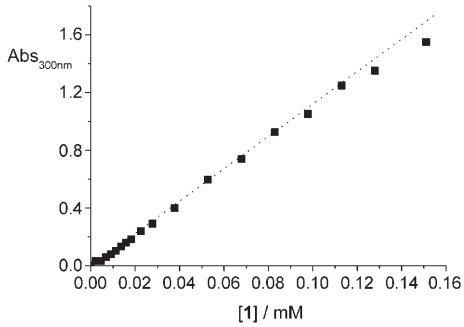


Figure 2. Comparison of the experimentally observed absorption of receptor **1** at 300 nm with the theoretical prediction according to the Lambert–Beer rule. The deviation at larger concentrations (>0.1 mM) indicates the start of self-aggregation of receptor **1**.

the solution became foamy and a significant deviation from linearity was observed, most probably due to the start of self-aggregation of the receptor. Binding studies were therefore performed in water with 10% DMSO (added to increase the solubility of the receptor) at a receptor concentration of [**1**] $\approx 3\cdot10^{-5}$ M to prevent any interference due to self-aggregation of **1**.

Upon addition of Ac-D-Ala-D-Ala-OH (**14**) to a solution of **1** at pH 6.1 (3 mM bis-tris buffer[23]) a significant decrease in the absorbance of the pyrrole band was observed (Figure 3). From this binding isotherm an association constant of $K = 33\,100\ M^{-1}$ ($\log K = 4.52$) was calculated by a nonlinear curve-fitting procedure taking dilution into account (estimated error in K is $\pm 30\%$).[24] The 1:1 complex stoichiometry was determined by a Job plot extracted from the titration.[25] The high affinity of receptor **1** for this dipeptide Ac-D-Ala-D-Ala-OH (**14**) was independently confirmed by fluorescence titration under the same conditions with monitoring of the decrease in intensity at 331 nm

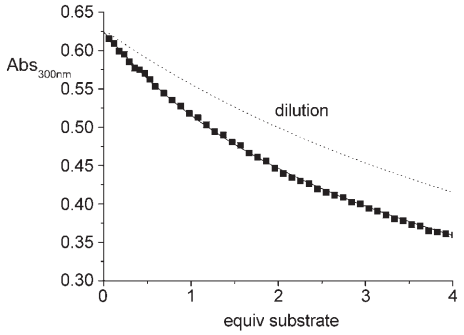


Figure 3. Binding isotherm ($\log K = 4.52$) as obtained from a UV titration of **1** with Ac-D-Ala-D-Ala-OH (**14**) in aqueous buffer solution with monitoring of the decrease in absorbance of **1** at $\lambda = 300$ nm. The solid line represents the curve fitting a 1:1 complexation, whereas the dotted line indicates the expected absorbance change due to simple dilution of the sample if no complexation were to take place.

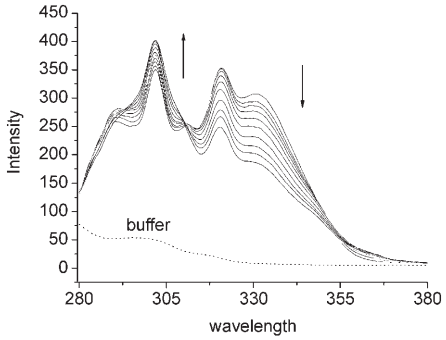


Figure 4. Fluorescence titration of **1** with Ac-D-Ala-D-Ala-OH (**14**) in aqueous buffer. The data were analyzed through the decrease in intensity at $\lambda = 331$ nm to minimize any interference by the background fluorescence of the buffer (·····).

(Figure 4).[26] Analysis of the binding isotherm gave a binding constant of $K = 39\,800\ \text{M}^{-1}$ ($\log K = 4.60$).

As already mentioned above, the solubility of receptor **1** is limited by its self-aggregation to rather low concentrations (<0.1 mM), so no binding studies at higher concentrations—as might be needed for NMR titrations, for example—were possible. This was our initial reason for also synthesizing the TEG-substituted receptor **2**, its solubility in water being much better. A UV titration in pure water (without any added DMSO) was therefore performed ([bis-tris buffer] = 4 mM, pH 5.8)[27] and provided a similar affinity of $\log K = 4.14$ for dipeptide **14**. Unfortunately, though, gelation of the solution occurred when we tried to perform NMR binding studies with the TEG-substituted receptor **2** at mM concentrations (Figure 5), thus preventing any further investigation by NMR spectroscopy. This is most probably due to the amphiphilic nature of **2**: gelation might be caused by self-aggregation and stacking of the nonpolar cyclotribenzylene units, together with the attached hydrophilic TEG groups, which are capable of strongly interacting with water molecules.[28] No further experimental binding studies could thus be performed and nor could any other structural data besides the

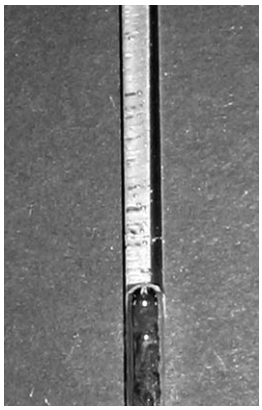


Figure 5. At mM concentrations receptor **2** caused gelation of an aqueous solution during attempts to perform NMR titrations in D_2O.

already described UV and fluorescence titration results be obtained.[29]

However, the formation of a 1:1 complex between receptor **2** and dipeptide **14** could also be confirmed by ESI-MS experiments (Figure 6). Whereas the unsubstituted receptor **1** did not give any signals due to complex formation in the ESI-MS, the TEG-substituted receptor **2** allowed the detection of the complex in the negative ion mode.

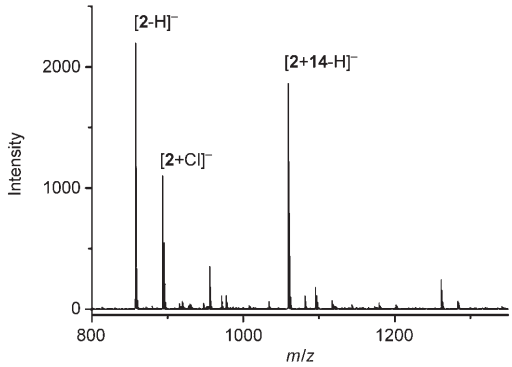


Figure 6. ESI-MS spectrum (negative ion mode) of a 1:1 mixture of **2** and **14** in water. Besides the signals for the receptor, an intense signal at m/z 1059.50 a.u. indicates the formation of a 1:1 complex.

Substrate selectivity: The binding studies had so far confirmed that receptor **1** is indeed capable of strong binding to the dipeptide Ac-D-Ala-D-Ala-OH (**14**) in buffered water, with a binding affinity even larger than that observed with our previously reported dipeptide receptor.[5] Whereas the latter receptor preferred larger and more hydrophobic residues, resulting in a greater affinity for Val-Val than for Ala-Ala, it was believed that our new receptor **1** should now show the opposite binding affinity, due to the size-selecting hydrophobic cavity, so various other dipeptides in which one or both of the alanines had been exchanged for glycine or valine were studied in order to examine the substrate selectivity of **1**. The results obtained from UV titration studies

under the same conditions as described above are summarized in Table 1 and are shown in Figure 7.

Table 1. Calculated binding constants for the complexation of various dipeptides by receptor **1** obtained from UV titrations in buffered water.

Dipeptide	K[a]	log$K$
Ac-D-Ala-D-Ala-OH (**14**)	33100	4.52
Ac-L-Ala-L-Ala-OH (*ent*-**14**)	30900	4.49
Ac-D-Ala-D-Lac-OH (**15**)	18600	4.27
Ac-D-Ala-Gly-OH (**16**)	4911	3.69
Ac-Gly-D-Ala-OH (**17**)	4260	3.63
Ac-D-Ala-D-Val-OH (**18**)	5020	3.70
Ac-D-Val-D-Ala-OH (**19**)	6300	3.80
Ac-Gly-Gly-OH (**20**)	2900	3.47
Ac-D-Val-D-Val-OH (**21**)	–	<3.0
Ac-L-Ala-OH (**22**)	–	<3.0

[a] K in M^{-1}, estimated error limit in $K < \pm 30\%$.

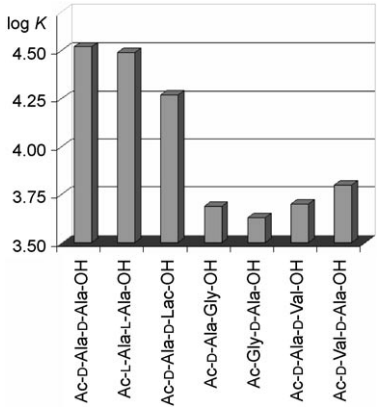


Figure 7. Binding constants (K_{ass}) for complex formation between receptor **1** and various dipeptides in buffered water.

Inspection of the data in Figure 7 and Table 1 shows that receptor **1** indeed favors alanine-containing peptides such as D-Ala-D-Ala (**14**) or its enantiomer L-Ala-L-Ala (*ent*-**14**) over dipeptides with other side chains. The receptor does not show any stereoselectivity between Ac-D-Ala-D-Ala-OH (**14**) and Ac-L-Ala-L-Ala-OH (*ent*-**14**), although this should not be unexpected, at least from the results of modeling studies (see below). However, a significant drop in affinity is observed if Ala is exchanged for Val: compound **14** is bound about six times more strongly than D-Val-D-Ala (**19**) or D-Ala-D-Val (**18**), for example, whilst binding of D-Val-D-Val (**21**) was not even detectable by UV titration under these conditions, suggesting a value of log$K = 3.0$, similarly to the situation with simple amino acids. Receptor **1** thus has an affinity for D-Ala-D-Ala over 10 times larger than that for D-Val-D-Val. Obviously, the large isopropyl side chain in valine is too bulky and does not allow direct favorable interactions with the shallow hydrophobic cyclotribenzylene cavity of the receptor. The binding affinity also drops when alanine is exchanged for glycine: **14** is bound about eight times more strongly than D-Ala-Gly (**16**) or Gly-D-Ala (**17**), for example, and again about ten times more strongly than Gly-Gly (**20**). This most probably reflects the loss of any hydrophobic interactions and the increasing flexibility of the glycine-rich dipeptides in relation to their alanine counterparts. Interestingly, the depsipeptide Ac-D-Ala-D-Lac-OH (**15**) (Lac = lactate) is also bound (log$K = 4.3$), though slightly less efficiently than Ac-D-Ala-D-Ala-OH (**14**) or Ac-L-Ala-L-Lac-OH (*ent*-**14**). This could be due to the exchanging of an attractive hydrogen bond with the amide NH of the dipeptide in the complex for a repulsive dipole interaction with the ester oxygen of the depsipeptide; similar effects in artificial systems have been observed before both by us and by others.[30] The same effect is also responsible for the reduced affinity of the antibiotic Vancomycin to this depsipeptide sequence in resistant bacterial strains.[31]

Molecular modeling studies: The binding studies in aqueous buffer confirmed that receptor **1** indeed shows sequence-dependant dipeptide binding, preferring alanine over valine or glycine. Hence, even though hydrophobic interactions in general increase simply with the areas of the interacting surfaces, the use of the size-discriminating shallow cyclotribenzylene cavity in **1** allowed the hydrophobic interactions to be restricted to small alkyl residues. As no further structural data could be obtained experimentally because of solubility problems we turned to molecular mechanics calculations (Macromodel V8.0, Amber* force field, GBSA water solvation).[32] A Monte Carlo conformational search with 100000 steps was performed until the resulting energy-minimized structure was found several times, and this structure was then further subjected to a MD simulation at 300 K for 100 ps. The obtained complex structure is shown in Figure 8. The dipeptide **14** is bound in a conformation that allows for ion pairing with the guanidinium cation of receptor **1** and hydrogen-bond formation between the backbone amides. Furthermore, the N-terminal methyl group is directly situated above the cyclotribenzylene bowl, which should allow for favorable hydrophobic interactions between those two groups in water, so substrates lacking this methyl group (Gly) or possessing a too bulky group (Val) that does not fit into the aromatic bowl are bound less efficiently. Their binding constants are all around $K \leq 10^3\,M^{-1}$, similar to a value expected for simple ion pairing between an amino acid carboxylate and our guanidiniocarbonylpyrrole moiety.[5]

The modeling studies also reproduced the observed lack of stereoselectivity with regard to the two dipeptides D-Ala-D-Ala and L-Ala-L-Ala, as well as the nearly identical binding features of the two *P*/D and *M*/D-diastereoisomers of receptor **1**. The linkage between the cyclotribenzylene moiety and the rest of the receptor is a C–C single bond, allowing for easy rotation, so the bowl can adjust to the individual position of the alanine methyl group; the calculated structures for the two complexes of the two diastereomers of receptor **1** with the dipeptide **14**, for example, differ only in a tilt of the cyclotribenzylene cavity relative to the alanine methyl group and are energetically virtually identical within the error limit of the calculation ($\Delta E < 2\,kJ\,mol^{-1}$), so no significant difference in binding affinity are to be expected,

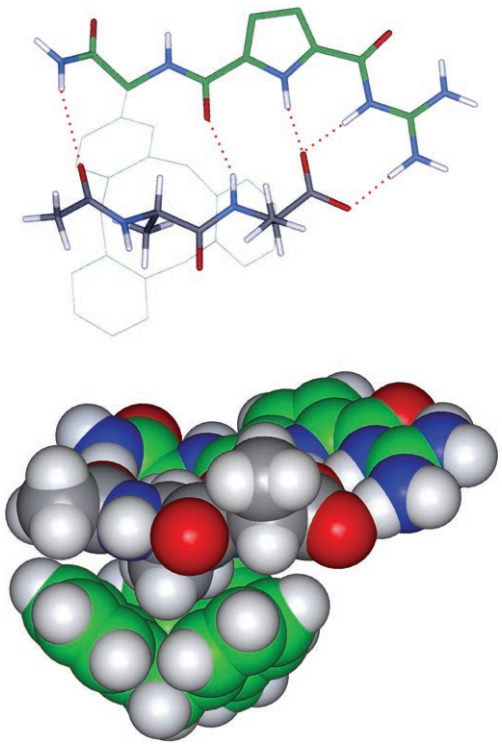


Figure 8. Energy-minimized structure for the complex formed between receptor **1** (green) and the dipeptide D-Ala-D-Ala **14** (gray) from the top, highlighting the hydrogen bonds (red dotted lines), and from the side, showing the interaction between the methyl group and the cyclotribenzylene cavity.

at least from these calculations. The flexibility of the receptor and the resulting structural similarity of the two diastereoisomers is also most probably the reason for the failure, mentioned above, to accomplish any separation by chromatography. Nevertheless, even with this diastereomeric mixture of receptor **1**, sequence-dependant binding of dipeptides in aqueous solution with a selectivity of up to a factor of 10 is possible.

Conclusion

In conclusion, we have demonstrated here that an artificial receptor (**1**) for D-Ala-D-Ala can be rationally designed by the same binding principles as found in the natural antibiotic Vancomycin: strong electrostatic interactions in combination with a size-selecting shallow hydrophobic cavity determine the binding features of receptor **1**. Even the rather unspecific and nondirectional hydrophobic interactions can hence be used to discriminate between related substrates such as D-Ala-D-Ala and D-Val-D-Val. A more deliberate use of hydrophobic interactions might be a promising approach to improve the performance of supramolecular receptors for aqueous solvents in the future.

Experimental Section

General remarks: Solvents were dried and distilled under argon before use. All other reagents were used as commercially obtained. ^{1}H and ^{13}C NMR shifts are reported relative to the deuterated solvents. Peak assignments are based on either DEPT or 2D NMR studies and/or comparison with literature data. IR spectra were recorded with samples prepared as tablets (KBr) or with ATR technique. Melting points are uncorrected.

***N*-Boc-(*P*/*M*)-D-Ctb-OMe (5)**: A suspension of zinc (900 mg, 13.8 mmol) and 1,2-dibromoethane (58 μL, 675 μmol) in dry DMF (1.5 mL) was stirred and heated to 110 °C with a heat gun under argon five times. Chlorotrimethylsilane (18 μL, 142 μmol) was added and the suspension was stirred for 30 min at room temperature. A solution of *N*-Boc-D-iodoalanine methyl ester (**4**, 741 mg, 2.25 mmol) in dry DMF (1.0 mL) was added by syringe and the reaction mixture was stirred for 1 h at room temperature. Iodocyclotribenzylene **3** (Ctb)[17] (893 mg, 2.25 mmol) and then [Pd_2(dba)$_3$] (60 mg, 66 μmol) and tri-*o*-tolylphosphine (66 mg, 217 μmol) were added and the reaction mixture was stirred at room temperature for 10 min. After addition of further DMF (1.5 mL) the mixture was stirred for 2 h, AcOEt (75 mL) was added, and the solid was separated by filtration and washed with AcOEt. The organic filtrate was washed with H_2O (2×50 mL) and brine (50 mL) and the solvent was removed in vacuo. The brown solid was purified by flash column chromatography to afford **5** (791 mg, 1.68 mmol, 75 %) as a white solid. M.p. 157–158 °C; R_f = 0.47 (CH_2Cl_2/AcOEt 25:1 + 1 % NEt_3); ^{1}H NMR (300 MHz, $CDCl_3$; since **5** is a mixture of two diastereomers, each of which exists in the form of two conformational isomers about the carbamate protecting group, up to four sets of signals can be observed for each proton in the NMR): δ = 1.36, 1.41, 1.45, 1.48 (4×s, 9H; C(CH_3)$_3$), 2.96–3.05 (m, 2H; benzyl-CH_2), 3.60, 3.66, 3.73, 3.75 (4×s, 3H; CO_2CH_3), 3.69–3.78 (m, 3H; benzyl-C*H*H), 4.45–4.55 (m, 1H; C*H*), 4.84–4.89 (m, 3H; benzyl-CH*H*), 6.83–7.44 ppm (m, 11H; aryl-C*H*); ^{13}C NMR (75 MHz, $CDCl_3$; since **5** is a mixture of two diastereomers, each of which exists in the form of two conformational isomers about the carbamate protecting group, up to four signals can be observed for each carbon in the NMR): δ = 28.2, 28.3, 30.9 (C-(CH_3)$_3$), 36.8, 37.1, 37.2 (benzyl-CH_2), 52.1, 52.2 (*C*H; CO_2CH_3), 126.9, 127.0, 127.8, 127.9, 129.9, 130.0, 130.1, 130.2, 130.3, 130.9, 131.0, 134.4, 134.5, 138.1, 138.2, 139.2, 139.3, 139.4, 139.5, 139.6 (aryl-*C*), 172.3 ppm (*C*=N; *C*=O); IR (ATR): $\tilde{\nu}$ = 3336 (bm), 2982 (bw), 1738 (m), 1692, 1593, 1527 (s), 1488, 1444 (w), 1364 (m), 1251 (w), 1168 (m), 1061, 1018, 746 (w), 719 (s), 630 (w), 612, 605 cm^{-1} (s); MS (EI): *m*/*z* (%): 471 (5) $[M]^+$, 415 (10) $[M-C_4H_{10}]^+$, 397 (10), 354 (75), 312 (20), 283 (90), 191 (30), 179 (75), 88 (35), 57 (100); HR-MS: *m*/*z*: calcd for $C_{30}H_{33}NO_4$: 471.241; found: 471.240 $[M]^+$; elemental analysis calcd (%) for $C_{30}H_{33}NO_4$ (471.6): C 76.41, H 7.05, N 2.97; found: C 76.32, H 7.00, N 3.01.

***N*-Boc-(*P*,*M*)-D-Ctb-NH_2 (Boc-6)**: A stream of ammonia was passed through a solution of **5** (900 mg, 1.91 mmol) in MeOH/$CHCl_3$ 2:1 (150 mL) for 1 h at 0 °C. This solution was stirred for 4 d at room temperature, during which ammonia was passed through the solution for 10 min at 0 °C once a day. After complete conversion (TLC monitoring) a strong stream of argon was passed through the solution for 1 h at room temperature to remove the ammonia. After removal of the solvent in vacuo, Boc-**6** (870 mg, 1.91 mmol, quant.) was obtained as a colorless solid. M.p. 268–269 °C; R_f = 0.48 (CH_2Cl_2/MeOH 20:1 + 0.1 % NEt_3); ^{1}H NMR (300 MHz, [D_6]DMSO; since Boc-**6** is a mixture of two diastereomers, each of which exists in the form of two conformational isomers about the carbamate protecting group, up to four sets of signals can be observed for each proton/carbon (see below) in the NMR): δ = 1.12, 1.18, 1.29, 1.31 (4×s, 9H; C(CH_3)$_3$), 2.56–2.85 (m, 2H; benzyl-CH_2), 3.59–3.71 (m, 3H; benzyl-C*H*H), 3.98–4.2 (m, 1H; C*H*), 4.86–4.95 (m, 3H; benzyl-CH*H*), 6.67, 6.76 (d, ^{4}J(H,H) = 7.95 Hz, 1H; N*H*), 6.93–7.44 ppm (m, 11H; aryl-C*H*); ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 28.3, 28.4 (C-(CH_3)$_3$), 35.9, 36.2, 36.4, 37.0, 37.1, 37.2 (benzyl-CH_2), 55.5, 55.6 (*C*H), 78.0, 78.1 (*C*(CH_3)$_3$), 126.7, 126.8, 126.9, 127.5, 127.8, 129.9, 130.0, 130.1, 130.2, 130.3, 130.6, 131.0, 136.6, 136.8, 137.8, 139.2, 139.4, 139.8, 139.9, 140.0 (aryl-*C*), 155.3, 155.4, 173.7, 173.9 ppm (*C*=O); IR (ATR): $\tilde{\nu}$ =

3343, 3188 (bw), 2955 (m), 2920 (s), 2850 (m), 2359 (w), 1659 (s), 1520, 1488, 1455, 1365, 1249, 1167, 1047, 744, 719 cm^{-1} (w); MS (ESI): *m/z* (%): 479 (100) $[M+Na]^+$, 401 (25), 357 (35), 312 (5); HR-MS: *m/z*: calcd for $C_{29}H_{32}N_2NaO_3Na$: 479.2311; found: 479.231 $[M+Na]^+$.

***N*-Boc-CBS-NH-(*P*/*M*)-D-Ctb-NH_2 (Boc-1)**: A stream of dry HCl was passed through a solution of Boc-**6** (557 mg, 1.22 mmol) in $CHCl_3$/MeOH 2:1 (75 mL) for 1 h at 0 °C. A stream of argon was then passed through the solution for 15 min at room temperature to remove the HCl. The solvent was removed in vacuo and the remaining solid (**6**) was dried in high vacuum. *N*-Boc-CBS-H (**7**, 360 mg, 1.22 mmol), PyBOP (632 mg, 1.22 mmol), and 4-methylmorpholine (1.25 mL, 11.4 mmol) were added, and the mixture was dissolved in DMF (25 mL) under argon and stirred for 2 d at room temperature. Brine (50 mL) was added to the yellowish solution and the mixture was extracted with chloroform (5×100 mL). The combined organic phase was washed with sat. $NaHCO_3$ (200 mL), H_2O (200 mL), and brine (200 mL) and dried in vacuo. The remaining yellow oil was purified by flash column chromatography, and the obtained oil was digerated in a few mL of dry diethyl ether and treated in an ultrasonic bath. The precipitating solid was crystallized from CH_2Cl_2 to afford Boc-**1** (379 mg, 600 μmol, 49%) as a white solid. M.p. 231 °C (decomp); R_f = 0.24 (CH_2Cl_2/acetone 5:1 + 1% NEt_3); ^1H NMR (300 MHz, [D$_6$]DMSO, T = 55 °C; since Boc-**1** is a mixture of two diastereomers, two sets of signals are observed for some protons/carbons): δ = 1.45 (s, 9H; C(CH_3)$_3$), 2.72–3.01 (m, 2H; benzyl-CH_2), 3.48–3.69 (m, 3H; benzyl-C*H*H), 4.45–4.66 (m, 1H; C*H*), 4.82–4.92 (m, 3H; benzyl-CH*H*), 6.80–7.43 (m, 13H; aryl-C*H*, pyrrole-C*H*), 7.53 (brs, 1H; N*H*), 8.42–8.50 (m, 1H; N*H*), 8.56 (brs, 1H; N*H*), 9.32 (brs, 1H; N*H*), 10.85 (brs, 1H; N*H*), 11.50 ppm (brs, 1H; N*H*); ^{13}C NMR (75 MHz, [D$_6$]DMSO, T = 55 °C): δ = 27.9 (C(CH_3)$_3$), 31.1, 35.8, 35.9, 36.1, 36.2, 36.4, 36.5, 36.6 (benzyl-CH_2), 53.5, 54.5 (*C*H), 112.9, 119.0, 119.1, 122.3, 126.6, 126.7, 126.8, 127.4, 127.7, 129.9, 130.0, 130.1, 130.2, 130.5, 130.6, 130.8, 136.5, 136.9, 137.8, 139.3, 139.5, 139.6, 139.7, 139.8, 139.9, 140.0 (pyrrole-*C*, aryl-*C*), 158.6, 158.7, 159.4, 159.5, 159.6, 173.4, 173.5 ppm (*C*=N, *C*=O); IR (ATR): $\tilde{\nu}$ = 3375 (bm), 2977 (bw), 1727 (w), 1673 (m), 1625, 1547 (s), 1474, 1446 (m), 1393, 1368 (w), 1292, 1241, 1146 (s), 1094, 839, 781, 753 (w), 721, 611 cm^{-1} (m); MS (ESI): *m/z* (%): 673 (30) $[M+K]^+$, 657 (100) $[M+Na]^+$, 567 (50), 537 (100), 321 (35), 312 (30), 280 (15), 258 (15); HR-MS: *m/z*: calcd for $C_{36}H_{38}N_6NaO_5Na$: 657.2802; found: 657.280 $[M+Na]^+$.

H-CBS-NH-(*P*,*M*)-D-Ctb-NH_2 (1): A stream of HCl was passed through a solution of Boc-**1** (107 mg, 169 μmol) in MeOH/$CHCl_3$ 2:1 (25 mL) for 1 h at 0 °C. Argon was then passed through this solution for 15 min and the solvent was removed in vacuo to afford **1** (92 mg, 161 μmol, 95%) as a white solid. For UV and fluorescence measurements parts of this white solid were purified by semipreparative RP18 HPLC. M.p. 176 °C (decomp); RP-HPLC (Supelcosil LC-18, 25 cm×10 mm, 5 μm): MeOH/H_2O 3:2 + 0.1% TFA, 4.0 mL min^{-1}, τ_R = 26.2 min; ^1H NMR (400 MHz, [D$_6$]DMSO; since **1** is a mixture of two diastereomers, two sets of signals are observed for some protons): δ = 2.65–2.92 (m, 2H; benzyl-CH_2), 3.60–3.70 (m, 3H; benzyl-C*H*H), 4.53, 4.64 (m, 1H; C*H*), 4.84–4.93 (m, 3H; benzyl-CH*H*), 6.81–7.42 (m, 13H; aryl-C*H*, pyrrole-C*H*), 7.59 (brs, 1H; N*H*), 8.43 (brs, 4H; NH_2), 11.7 (s, 1H; N*H*), 12.4 ppm (s, 1H; N*H*); IR (KBr): $\tilde{\nu}$ = 3393, 3322 (bs), 3184 (bm), 3015, 2977, 2923, 2878 (w), 1698, 1663 (s), 1558, 1475 (m), 1446, 1404 (w), 1282 (m), 1260, 1202, 1092, 748, 721 cm^{-1} (m); MS (ESI): *m/z* (%): 535 (100) $[M+H]^+$, 287 (30); HR-MS: *m/z*: calcd for $C_{31}H_{31}N_6O_3Na$: 535.2457; found: 535.244 $[M+Na]^+$.

2-*tert*-Butyl 5-ethyl 3,4-bis(TEG-methyl)-1*H*-pyrrole-2,5-dicarboxylate (10): The dibromide **9** (1.8 g, 4.23 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and the solvent was removed in vacuo under argon to remove traces of moisture in the starting material. The remaining solid was dissolved in dry triethyleneglycol (50 mL), and NEt_3 (3 mL, 21.3 mmol) was added under argon. The solution was stirred for 24 h at 100 °C. After cooling down to room temperature the solution was diluted with H_2O (200 mL) and filtration over a RP18 column (100% H_2O to 100% MeOH + 0.1% TFA) was carried out. All fractions exhibiting absorption at 300 nm were collected and the solvent was evaporated. The remaining oil was purified with MPLC and **10** (1.53 g, 2.71 mmol, 64%) was obtained as a yellowish oil. MPLC (RediSep C-18, reverse phase, 130 g): 7 min H_2O + 0.1% TFA, then 18 min to MeOH/H_2O 2:1 + 0.1% TFA, then 20 min to MeOH + 0.1% TFA, 40 mL min^{-1}, τ_R = 30.6 min; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.32 (t, ^3J(H,H) = 7.0 Hz, 3H; $CO_2CH_2CH_3$), 1.53 (s, 9H; C(CH_3)$_3$), 3.43 (s, 2H; OH), 3.50–3.75 (m, 24H; CH_2OCH_2), 4.30 (q, ^3J(H,H) = 7.0 Hz, 2H; $CO_2CH_2CH_3$), 4.75 (s, 2H; pyrrole-CH_2), 4.78 (s, 2H; pyrrole-CH_2), 9.57 ppm (s, 1H; pyrrole-N*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.2 ($CO_2CH_2CH_3$), 28.2 (C(CH_3)$_3$), 60.9, 61.5, 62.5, 62.6, 69.07, 69.12, 70.2, 70.40, 70.43, 70.47, 70.49, 72.5 (CH_2OCH_2, pyrrole-CH_2, $CO_2CH_2CH_3$), 82.4 (*C*(CH_3)$_3$), 122.4, 124.3, 126.5, 127.4 (pyrrole-C_q), 159.7, 160.3 ppm (CO_2Et, CO_2tBu); IR (ATR): $\tilde{\nu}$ = 3441 (bw), 2920, 2865 (m), 1698 (s), 1455, 1365 (w), 1282, 1142, 1077 cm^{-1} (s); MS (ESI): *m/z* (%): 586 (55) $[M+Na]^+$, 530 (100) $[M-C_4H_8+Na]^+$; HR-MS: *m/z*: calcd for $C_{26}H_{45}NNaO_{12}Na$: 586.2839; found: 586.284 $[M+Na]^+$.

2-Benzyl 5-*tert*-butyl 3,4-bis(TEG-methyl)-1*H*-pyrrole-2,5-dicarboxylate (10b): Sodium (510 mg, 22.2 mmol) was treated with benzyl alcohol (40 mL) under argon at 0 °C. After complete conversion the solution was allowed to warm to room temperature and dropped by transfer cannula onto **10** (931 mg, 1.65 mmol). The resulting solution was stirred at 1.2 mbar and heated to reflux for 3 h. The solvent was removed by distillation at 0.1 mbar and the resulting oil was purified by MPLC to afford **10b** (720 mg, 1.15 mmol, 70%) as a yellowish oil. R_f = 0.56 (CH_2Cl_2/MeOH 5:1 + 0.1% TFA); MPLC (RediSep C-18 reverse phase, 43 g): 2 min MeOH/H_2O 1:1 + 0.1% TFA, then 33 min to MeOH + 0.1% TFA, 25 mL min^{-1}, τ_R = 28.2 min; ^1H NMR (400 MHz, $CDCl_3$): δ = 1.57 (s, 9H; C(CH_3)$_3$), 2.50 (s, 2H; O*H*), 3.48–3.72 (m, 24H; CH_2OCH_2), 4.80 (s, 4H; pyrrole-CH_2), 5.33 (s, 2H; CO_2CH_2Ph), 7.36–7.42 (m, 5H; aryl-C*H*), 9.58 ppm (brs, 1H; pyrrole-N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.3 (C(CH_3)$_3$), 61.6, 62.6, 62.7, 66.8, 70.3, 70.4, 70.5, 72.6 (CH_2OCH_2, pyrrole-CH_2, benzyl-CH_2), 82.6 (*C*(CH_3)$_3$), 122.1, 124.6, 126.6, 127.8 (pyrrole-C_q),128.4, 128.5, 128.7 (aryl-*C*H), 135.4 (aryl-C_q), 159.7, 160.2 ppm (CO_2Bn, CO_2tBu); MS (ESI): *m/z* (%): 648 (90) $[M+Na]^+$, 592 (100) $[M-C_4H_8+Na]^+$; HR-MS: *m/z*: calcd for $C_{31}H_{47}NNaO_{12}Na$: 648.2991; found: 648.2996 $[M+Na]^+$.

5-Benzyloxycarbonyl-3,4-bis(TEG-methyl)-1*H*-pyrrole-2-carboxylic acid (11): Compound **10b** (460 mg, 735 μmol) was dissolved in dry CH_2Cl_2 (4 mL) and the reaction mixture was cooled to −20 °C. TFA (1 mL) was added and the solution was allowed to warm to 5 °C and stirred for 2 h with frequent TLC monitoring. Although the conversion was not yet complete the reaction was stopped due to an appearing spot representing a by-product (R_f = 0.72). The mixture was cooled to −20 °C, and the solvent and the acid were removed under high vacuum. RP18 MPLC provided **11** (168 mg, 295 μmol, 40%) as a yellowish oil, together with reisolated **10b** (160 mg, 256 μmol, 35%). R_f = 0.36 (CH_2Cl_2/MeOH 5:1 + 0.1% TFA); MPLC (RediSep C-18 reverse phase, 43 g): 2 min H_2O + 0.1% TFA, then 21 min to MeOH + 0.1% TFA, 40 mL min^{-1}, τ_R = 16.2 min; ^1H NMR (400 MHz, $CDCl_3$): δ = 3.57–3.80 (m, 24H; CH_2OCH_2), 4.20 (brs, 2H; O*H*), 4.78 (s, 2H; pyrrole-CH_2), 4.86 (s, 2H; pyrrole-CH_2), 5.32 (s, 2H; CO_2CH_2Ph), 7.36–7.42 (m, 5H; aryl-C*H*), 10.0 ppm (brs, 1H; pyrrole-N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 61.7, 61.8, 62.7, 64.4, 67.1, 69.1, 69.9, 70.2, 70.4, 70.5, 70.6, 70.7, 72.67, 72.70 (CH_2OCH_2, pyrrole-CH_2, benzyl-CH_2), 122.7, 124.0, 125.9, 126.6 (pyrrole-C_q), 128.75, 128.79, 128.86 (aryl-*C*H), 135.3 (aryl-C_q), 159.9, 161.4 ppm (CO_2Bn, CO_2H); MS (ESI): *m/z* (%): 608 (40) $[M+K]^+$, 592 (100) $[M+Na]^+$, 470 (25); HR-MS: *m/z*: calcd for $C_{27}H_{39}NNaO_{12}Na$: 592.2365; found: 592.2370 $[M+Na]^+$.

Benzyl 3,4-bis(TEG-methyl)-5-(Boc-guanidino)carbonyl-1*H*-pyrrole-2-carboxylate (12): Compound **11** (1.21 g, 2.12 mmol), Boc-guanidine (1.28 g, 6.63 mmol), HCTU (1.01 g, 2.44 mmol), and DMAP (609 mg, 4.98 mmol) were suspended under argon in dry CH_2Cl_2 (25 mL) and DMF was added until all components were dissolved. The solution was stirred for 3 d at room temperature, acetic acid (5 mL) was added, and the solvent was removed in vacuo. Filtration over a RP18 column (100% H_2O to 100% MeOH + 0.1% AcOH) was carried out, all fractions exhibiting absorption at 300 nm being collected and the solvent being evaporated. The remaining oil was purified by MPLC, and **12** (680 mg, 957 μmol, 45%) was obtained as a yellowish oil. MPLC (RediSep C-18

reverse phase, 130 g): 2 min MeOH/H_2O 2:1 + 0.1% TFA, then 25 min to MeOH + 0.1% TFA, 50 mL min^{-1}, τ_R = 13.8 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.51 (s, 9H; C(CH_3)$_3$), 1.82 (br s, 2H; O*H*), 3.11–3.73 (m, 24H; CH_2OCH_2), 4.80 (s, 2H; pyrrole-CH_2), 4.99 (s, 2H; pyrrole-CH_2), 5.32 (s, 2H; CO_2CH_2Ph), 7.32–7.43 (m, 5H; aryl-C*H*), 8.57 (s, 1H; N*H*), 9.10 (s, 1H; N*H*), 9.87 ppm (s, 2H; N*H*); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 28.2 (C(CH_3)$_3$), 61.6, 61.8, 62.6, 62.9, 66.7, 68.6, 69.4, 70.3, 70.5, 70.6, 70.8, 72.8, 72.9 (CH_2OCH_2, pyrrole-CH_2, benzyl-CH_2), 77.4 (C-(CH_3)$_3$), 121.3, 125.4, 128.3 (pyrrole-C_q), 128.59, 128.62, 128.8 (aryl-C*H*), 135.7 (aryl-C_q), 159.2, 160.4 ppm (*C*=O, *C*=N); MS (ESI): *m*/*z* (%): 733 (100) [*M*+Na]$^+$, 711 (70) [*M*+H]$^+$; HR-MS: *m*/*z*: calcd for $C_{33}H_{50}N_4NaO_{13}Na$: 733.3267; found: 733.3267 [*M*+Na]$^+$.

***N*-Boc-(TBS-TEG-CBS)-Bn (TBS-12)**: Compound **12** (510 mg, 717 μmol), TBS-Cl (217 mg, 1.44 mmol), and NEt_3 (300 mL, 2.13 mmol) were dissolved under argon in dry THF (30 mL) and the reaction mixture was stirred overnight at room temperature. As TLC indicated **12** to be still the major component of the solution, NMM and TBS-Cl were added gradually with further TLC monitoring. Mono- and disilylation can be monitored very well by TLC [silica gel, CH_2Cl_2/MeOH 10:1, R_f = 0.19 (**12**), 0.33 (mono-TBS adduct), 0.73 (TBS-**12**)]. After 3 d of stirring at room temperature and overall addition of ≈5 equiv of TBS-Cl and NMM the mixture was diluted with methyl *tert*-butyl ether (MTBE) (200 mL) and washed with sat. $NaHCO_3$ (200 mL), brine (200 mL), 5% AcOH in H_2O (80 mL), and brine (2×150 mL). The organic phase was dried with Na_2SO_4 and the solvent was removed in vacuo. Flash column chromatography (silica gel, cyclohexane/AcOEt 2:1) provided TBS-**12** (450 mg, 479 μmol, 67%) as a colorless oil. R_f = 0.73 (CH_2Cl_2/MeOH 10:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.047, 0.053 (2×s, 12H; Si-(CH_3)$_2$C(CH_3)$_3$), 0.879, 0.884 (2×s, 18H; Si(CH_3)$_2$C(CH_3)$_3$), 1.51 (s, 9H; OC(CH_3)$_3$), 3.52–3.76 (m, 24H; CH_2OCH_2), 4.78 (s, 2H; pyrrole-CH_2), 4.93 (s, 2H; pyrrole-CH_2), 5.33 (s, 2H; CO_2CH_2Ph), 7.32–7.43 (m, 5H; aryl-C*H*), 8.52 (s, 2H; N*H*), 9.86 ppm (s, 1H; N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ = −5.13, −5.11 (Si(CH_3)$_2$C(CH_3)$_3$), 18.5 (Si(CH_3)$_2$*C*(CH_3)$_3$), 26.1 (Si(CH_3)$_2$C(*C*H$_3$)$_3$), 28.2 (OC(*C*H$_3$)$_3$), 62.81, 62.84, 66.7, 69.1, 69.4, 70.59, 70.61, 70.7, 70.82, 70.84, 70.9, 72.77, 72.80 (CH_2OCH_2, pyrrole-CH_2, benzyl-CH_2), 83.2 (O*C*(CH$_3$)$_3$), 121.4, 126.1, 128.3 (pyrrole-C_q), 128.52, 128.55, 128.8 (aryl-C*H*), 130.0 (pyrrole-C_q), 135.7 (aryl-C_q), 154.6, 159.2, 160.4, 170.1 ppm (*C*=O, *C*=N); MS (ESI): *m*/*z* (%): 977 (4) [*M*+K]$^+$, 961 (100) [*M*+Na]$^+$, 939 (37) [*M*+H]$^+$; HR-MS: *m*/*z*: calcd for $C_{45}H_{79}N_4O_{13}Si_2$: 939.5177; found: 939.5179 [*M*+H]$^+$.

***N*-Boc-(TBS-TEG-CBS)-H (23)**: TBS-**12** (420 mg, 449 μmol) was dissolved in dry THF (25 mL) and a few mg of Pd/C were added. A stream of H_2 was passed through the solution for 2 h at room temperature, the catalyst was filtered off, and the solvent was evaporated to afford **23** (381 mg, 449 μmol, quant.) as a white wax. R_f = 0.28 (CH_2Cl_2/MeOH 10:1 + 0.1% AcOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.048, 0.051 (2× s, 12H; Si(CH_3)$_2$C(CH_3)$_3$), 0.879, 0.881 (2×s, 18H; Si(CH_3)$_2$C(CH_3)$_3$), 1.59 (s, 9H; OC(CH_3)$_3$), 3.52–3.76 (m, 24H; CH_2OCH_2), 4.90 (s, 2H; pyrrole-CH_2), 4.95 (s, 2H; pyrrole-CH_2), 9.03 (s, 1H; N*H*), 9.68 (s, 1H; N*H*), 10.64 (s, 1H; N*H*), 12.89 (s, 1H; N*H*), 15.84 ppm (s, 1H; CO_2H); ^{13}C NMR (100 MHz, $CDCl_3$; quaternary signals in ^1J, ^2J, and ^3J positions next to N do not appear, due to prolonged relaxation times): δ = −5.1 (Si(*C*H$_3$)$_2$C(CH$_3$)$_3$), 18.5 (Si(CH$_3$)$_2$*C*(CH$_3$)$_3$), 26.1 (Si(CH$_3$)$_2$C(*C*H$_3$)$_3$), 28.1 (OC(*C*H$_3$)$_3$), 62.8, 70.7, 70.9, 72.8 ppm (CH_2OCH_2, pyrrole-CH_2); MS (ESI): *m*/*z* (%): 893 (24) [*M*+2Na−H]$^+$, 871 (100) [*M*+Na]$^+$, 849 (22) [*M*+H]$^+$; HR-MS: *m*/*z*: calcd for $C_{38}H_{72}N_4NaO_{13}Si_2Na$: 871.45266; found: 871.45274 [*M*+Na]$^+$.

***N*-Boc-(TBS-TEG-CBS)-NH-(*P*/*M*)-D-Ctb-NH$_2$ (13)**: Compound **23** (104 mg, 123 μmol), **6**·HCl (40 mg, 102 μmol), HCTU (90 mg, 218 μmol), and DMAP (88 mg, 720 μmol) were suspended under argon in dry CH_2Cl_2 (10 mL) and dissolved by addition of dry DMF, and the reaction mixture was stirred for 24 h at room temperature. The mixture was diluted with MTBE (150 mL) and washed with sat. $NaHCO_3$ (100 mL), brine (150 mL), $NaHSO_4$ (100 mL), and again brine (150 mL). To avoid Boc cleavage a few drops of NEt_3 were added. The solution was dried with Na_2SO_4 and the solvent was removed in vacuo. The crude product was purified by MPLC to afford **13** (32 mg, 26.9 mmol, 26%) as a clear oil. MPLC (RediSep silica gel, 12 g, deactivated with NEt_3): 1 min 2.5% MeOH in CH_2Cl_2, then 15 min to 5% MeOH in CH_2Cl_2, 30 mL min^{-1}, τ_R = 7.1 min; ^{1}H NMR (400 MHz, $CDCl_3$; since **13** is a mixture of two diastereomers, each of which exists in the form of two conformational isomers about the carbamate protecting group, up to four sets of signals can be observed for each proton/carbon): δ = 0.041, 0.044, 0.055, 0.058 (4×s, 12H; Si(CH_3)$_2$C(CH_3)$_3$), 0.873, 0.875, 0.886, 0.889 (4×s, 18H; Si(CH_3)$_2$C-(CH_3)$_3$), 1.518, 1.521 (2×s, 9H; OC(CH_3)$_3$), 2.58–4.92 (m, 37H; CH_2OCH_2, benzyl-CH_2, pyrrole-CH_2, C*H*), 5.53, 5.59, 6.45, 6.51 (4×s, 2H; N*H*$_2$), 6.86–7.10 (m, 5H; aryl-C*H*), 7.23–7.40 (m, 6H; aryl-C*H*), 8.41–9.13 (br m, 4H; N*H*), 10.10 ppm (s, 1H; N*H*); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = −5.1 (Si(*C*H$_3$)$_2$C(CH$_3$)$_3$), 18.5 (Si(CH$_3$)$_2$*C*(CH$_3$)$_3$), 26.1 (Si-(CH$_3$)$_2$C(*C*H$_3$)$_3$), 28.2 (OC(*C*H$_3$)$_3$), 36.5, 36.7, 37.0, 37.2, 37.3 (benzyl-*C*H$_2$), 54.8, 55.0 (*C*H), 62.4, 62.72, 62.77, 62.79, 62.80, 63.0, 68.3, 68.5, 68.6, 69.5, 69.8, 69.9, 70.2, 70.4, 70.5, 70.6, 70.83, 70.85, 72.6, 72.7, 72.78, 72.80 (CH_2OCH_2, pyrrole-CH_2), 122.6, 122.8, 124.6, 124.8, 125.9, 127.0, 127.1, 127.3, 128.2, 128.5, 129.9, 130.03, 130.06, 130.11, 130.20, 130.23, 130.4, 130.5, 131.3, 131.5, 135.9, 138.2, 138.3, 139.39, 139.48, 139.54, 139.6, 139.7, 139.8 (aryl-*C*), 158.3, 160.5, 160.7, 172.7, 173.5, 173.7, 174.4 ppm (*C*=O, *C*=N); IR (film): $\tilde{\nu}$ = 3388, 3268 (bm), 2952, 2928, 2858, 1772, 1683 (m), 1635, 1556, 1541 (s), 1447 (m), 1298, 1246, 1148, 1098 (s), 836 (m); MS (ESI): *m*/*z* (%): 1226 (1) [*M*+K]$^+$, 1210 (100) [*M*+Na]$^+$, 1188 (3) [*M*+H]$^+$; HR-MS: *m*/*z*: calcd for $C_{62}H_{94}N_6NaO_{13}Si_2Na$: 1209.631; found: 1209.633 [*M*+Na]$^+$.

***N*-Boc-(TEG-CBS)-NH-(*P*,*M*)-D-Ctb-NH$_2$ (Boc-2)**: Compound **13** (34 mg, 28.6 μmol) was dissolved in THF (10 mL) and the reaction mixture was cooled to 0°C. TBAF·3H_2O (20 mg, 63.0 μmol) was added and the solution was stirred for 1 h at 0°C. After the reaction mixture had been allowed to warm to room temperature and further stirred for 20 min, AcOH (0.2 mL) was added and the solvent was removed in vacuo. The crude product was purified by MPLC to afford Boc-**2** (16 mg, 16.7 μmol, 56%) as a clear oil. After attempts to separate the diastereomeric mixture by HPLC had failed, the obtained oil was directly converted into **2** (next reaction). MPLC (RediSep C-18 peverse phase, 2×4.3 g): 2 min MeOH/H_2O 1:1 + 0.1% AcOH, then 24 min to MeOH + 0.1% AcOH, 10 mL min^{-1}, τ_R = 18.2 min; RP-HPLC (Supelcosil LC-18, 25 cm×4.6 mm, 5μm): 5 min MeOH/H_2O 1:1 + 0.1% TFA, then 45 min to MeOH + 0.1% TFA, 1.5 mL min^{-1}, τ_R = 21.5, 22.1 min; MS (ESI): *m*/*z* (%): 997 (5) [*M*+K]$^+$, 981 (100) [*M*+Na]$^+$, 959 (6) [*M*+H]$^+$; HR-MS: *m*/*z*: calcd for $C_{50}H_{66}N_6NaO_{13}Na$: 981.4580; found: 981.4581 [*M*+Na]$^+$.

H-(TEG-CBS)-NH-(*P*,*M*)-D-Ctb-NH$_2$ (2): Boc-**2** (16 mg, 16.7 μmol) was dissolved in MeOH/TFA 1:1 (10 mL) and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo and the crude product was purified by MPLC. The obtained solid was lyophilized twice with HCl (0.1 M, 5 mL) to afford **2**·HCl (9 mg, 10.1 μmol, 60%) as a white solid. m.p. 107°C; MPLC (RediSep C-18 reverse phase, 2×4.3 g): 3 min MeOH/H_2O 3:2 + 0.1% TFA, then 35 min to MeOH + 0.1% TFA, 10 mL min^{-1}, τ_R = 11.8 min; RP-HPLC (Supelcosil LC-18, 25 cm×4.6 mm, 5μm): 5 min MeOH/H_2O 1:1 + 0.1% TFA, then 45 min to MeOH + 0.1% TFA, 1.5 mL min^{-1}, τ_R = 16.0, 16.6 min; ^{1}H NMR (600 MHz, CD_3OD; since **2** is a mixture of two diastereomers, two sets of signals are observed for some protons/carbons (see below)): δ = 2.90–3.83 (m, 31H, CH_2OCH_2; benzyl-CH_2, pyrrole-CH_2, C*H*), 4.34–5.01 (m, 6H; CH_2OCH_2, benzyl-CH_2, pyrrole-CH_2, C*H*), 6.63–7.46 ppm (m, 11H; aryl-C*H*); DEPT135 (150 MHz, CD_3OD): δ = 35.0, 36.9, 37.11, 37.15, 37.33, 37.35, 37.39 (benzyl-CH_2), 54.4, 55.9 (*C*H), 61.89, 61.91, 63.5, 69.1, 69.5, 70.4, 70.56, 70.63, 70.67, 70.74, 70.76, 70.89, 70.94, 70.97, 71.62, 71.65, 73.1, 73.3 (pyrrole-CH_2, CH_2OCH_2), 127.3, 127.46, 127.53, 127.59, 127.62, 127.8, 128.6, 129.0, 130.59, 130.63, 130.68, 130.76, 130.84, 130.99, 131.05, 131.09, 131.2, 131.8, 132.4 ppm (aryl-*C*H); IR (KBr): $\tilde{\nu}$ = 3431 (bs), 2922 (w), 2872 (w), 1700 (m), 1686 (m), 1637 (s), 1285 (m), 1076 (s), 723 cm^{-1} (m); MS (ESI): *m*/*z* (%): 881 (36) [*M*+Na]$^+$, 859 (100) [*M*+H]$^+$; HR-MS: *m*/*z*: calcd for $C_{45}H_{59}N_6O_{11}Na$: 859.4236; found: 859.4239 [*M*+Na]$^+$.

General procedure for UV and fluorescence titrations: Samples of the receptors were purified by preparative HPLC before use. A solution of the receptor (**1** or **2**, c = (2.5–7.6)×10^{-5} M, 10 mL) in bis-tris buffer (c = (1.1–3.3)×10^{-3} M, pH 5.77–6.29) was prepared and the pH was deter-

mined. A solution of the substrate ($c = (2.65–12.7)\times10^{-4}$ M) was prepared in the same buffer and adjusted to exactly the same pH (ΔpH 0.02) as the solution of the receptor with HCl or NaOH. The solution of the receptor (1.8–2.2 mL) was placed in a cuvette and the spectrum was measured. The solution of substrate was subsequently added in small steps (10–50 μL) with stirring and temperature control ($T = 25$ °C). After every addition a spectrum was recorded. To rule out the spectral changes being due simply to pH effects, the pH of the mixture was measured again after completion of addition: in all cases the pH had stayed constant during the course of the titration ($\Delta pH < 0.02$). A mathematical curve-fitting for a 1:1 complexation taking dilution into account was performed by use of the decrease in absorption of the pyrrole band of the receptor at $\lambda = 300$ nm for the UV titrations and $\lambda = 331$ nm for the fluorescence titration. The data were also analyzed over the whole spectral range with the aid of SpecFit analysis software. Both methods gave the same binding constants within the experimental error of the method, which is estimated to be ±30% in K.

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