ChemComm

A synthetic receptor for the Cbz-L-Ala-L-Ala-OH dipeptide sequence

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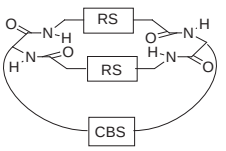
Received (in Liverpool, UK) 28th April 1999, Accepted 26th May 1999

A novel bowl-shaped macrobicyclic receptor has been prepared and is a particularly strong and selective receptor for Cbz-L-Ala-L-Ala-OH ($-\Delta G_{ass}$ = 25 kJ mol^{-1} at 293 K in $CDCl_3$).

The development of synthetic receptors for biologically relevant substrates such as peptides[1] continues to be a fertile area of research. The bacterial cell wall precursor peptide L-Lys-D-Ala-D-Ala-OH represents a particularly interesting target because a selective receptor[2] for this peptide sequence might lead to mimics for the vancomycin family of antibiotics.[3] We have described several systems designed to act as receptors for peptides specifically with a free carboxylic acid terminus[1a,1e,4] and in particular we have prepared a series of receptors **1** which



CBS = carboxylate binding site
RS = rigid spacer

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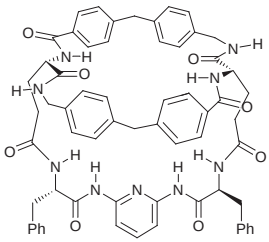

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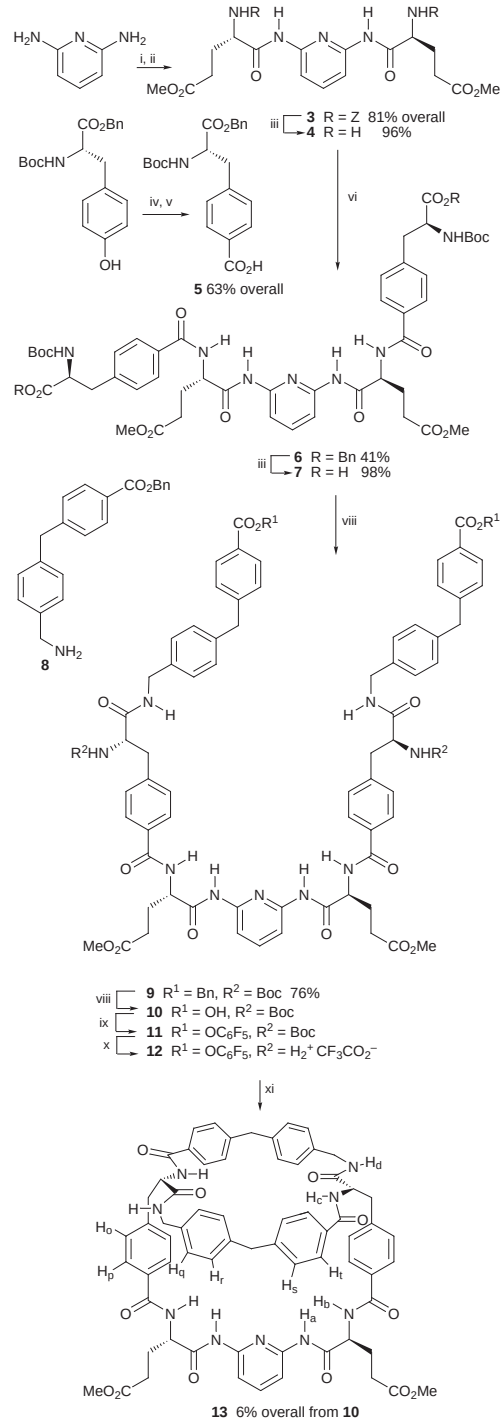
feature a specific binding site for the carboxylic acid terminus of peptide guests at the base of a bowl-shaped cavity.[4] Thus we have described macrobicycle **2**, which incorporates a diamidopyridine unit to serve as a carboxylic acid binding site and is an effective receptor for certain dipeptides, in particular for Cbz-β-Ala-D-Ala-OH, in chloroform solution.[4b] Despite the encouraging binding selectivities found for **2**, NMR and molecular modelling studies revealed it to be a highly flexible molecule, particularly in the portion derived from glutamic acid and phenylalanine, which links the diamidopyridine unit and the rim of the cavity. Thus **2** is far from preorganised for optimal binding of peptide guests. Here we describe a new bowl-shaped macrobicyclic receptor **13**, with increased rigidity in comparison to **2**, which has proved to be a particularly strong and selective receptor for Cbz-L-Ala-L-Ala-OH.

In order to produce a more rigid variant of our bowl-shaped receptors, we chose to link the bisarylmethane-derived rim used previously with a tyrosine derived aromatic unit, and a glutamic acid residue so that ultimately the side-chain methyl ester could be functionalised to give a water-soluble receptor.

The synthesis of macrobicycle **13** follows the strategy for the synthesis of **2** and related macrobicycles, with a double intramolecular cyclisation of a suitably activated acyclic precursor **12** as the key step (Scheme 1). Assembly of the cyclisation precursor then requires coupling together of the various amino acid building blocks around the carboxylic acid binding site, in this case diaminopyridine. Thus Cbz-L-glutamic acid γ-methyl ester was converted to the corresponding acid fluoride, using cyanuric fluoride and pyridine,[5] and coupled to diaminopyridine, which had been pretreated with *N*,*O*-bis-(trimethylsilyl)acetamide[6] to give **3** in 81% yield. Removal of the Cbz-protecting groups to yield the corresponding diamine **4** was readily achieved with Pd/C/H_2. Acid **5** was prepared by a



Scheme 1 *Reagents and conditions*: i, *N*,*O*-bis(trimethylsilyl)acetamide; ii, Cbz-L-Glu(OMe)-F, TBAF; iii, Pd, C, H_2, MeOH; iv, Tf_2O, pyridine, CH_2Cl_2; v, $Pd(OAc)_2$, $Ph_2P(CH_2)_3PPh_2$, KOAc, CO, DMSO, 65 °C, then 1 M HCl; vi, **5**, EDC, HOBT, DMAP, DMF; vii, **8**, EDC, HOBT, DMAP, DMF; viii, Pd, C, H_2, DMF; ix, F_5C_6OH, EDC, DMAP, DMF–THF (1:3 v/v); x, 10% TFA, CH_2Cl_2; xi, slow addition to a refluxing solution of Pr^i_2NEt in MeCN.

Table 1 Binding constants (K_{ass}) and free energies of complexation ($-\Delta G_{ass}$) for the 1:1 complexes formed between macrobicycle **13** and various substrates, in $CDCl_3$ at 20 °C

Entry	Substrate	K_{ass}[a]/M^{-1}	$-\Delta G_{ass}$/kJ mol^{-1}
1	Phenylacetic acid	2100 ± 100	18.6 ± 0.1
2	Cbz-L-Ala-OH	5300 ± 500	20.9 ± 0.3
3	Cbz-D-Ala-OH	800 ± 70	16.3 ± 0.3
4	Cbz-β-Ala-L-Ala-OH	2500 ± 130	19.0 ± 0.1
5	Cbz-L-Ala-L-Ala-OH	33000 ± 3200	25.3 ± 0.3
6	Cbz-D-Ala-D-Ala-OH	4500 ± 300	20.5 ± 0.2
7	Cbz-Gly-L-Ala-OH	1800 ± 150	18.3 ± 0.2
8	Cbz-Gly-D-Ala-OH	3100 ± 370	19.6 ± 0.3

[a] Errors were estimated from the quality of the fit of the experimental data to the calculated, by carrying out several titration experiments and by monitoring the shift of several protons (H_a, H_o, H_r) to obtain several estimates of K_{ass} and averaging the values obtained.

palladium catalysed carbonylation of the triflate derived from Boc-L-tyrosine benzyl ester.[7] In the presence of potassium acetate[8] and the bidentate dppp ligand,[9] the carbonylation worked well to give the desired acid **5** in 66% yield after aqueous acid work-up. Acid **5** was then coupled to diamine **4** using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole hydrate (HOBT), and the resulting diester **6** was debenzylated to give the diacid **7**, which in turn was coupled with the previously described amine **8**[4b] to give the protected cyclisation precursor **9**. Debenzylation of **9** and formation of the bispentafluorophenyl ester was followed by removal of the Boc protecting groups to give the bisamine salt **12**. Slow addition of **12** to a refluxing solution of Pr^i_2NEt in MeCN gave the desired macrobicycle **13**, but in only 6% yield overall from diacid **10**, after purification by column chromatography. The yield of the final cyclised product was disappointing and in previous syntheses of related compounds[4] the analogous sequence typically yielded ~30% of macrobicycle, but in the present case the low yield is probably attributable to the greater rigidity of the precursor and of the resulting macrobicycle.

Macrocycle **13** gave a well-resolved ^1H NMR spectrum in $CDCl_3$ which could be fully assigned with the help of 2D NMR experiments. Binding studies on macrobicycle **13** were therefore carried out with a number of Cbz-protected alanine derived amino acids and dipeptides in $CDCl_3$, using a standard ^1H NMR titration experiment, monitoring the shift of various signals, and analysing the resultant binding curves (Table 1).[10] In each binding experiment a 1:1 binding stoichiometry has been assumed which was generally supported by the good fit of the measured data to the theoretical model, on analysis. In each titration experiment significant downfield shifts of the signal for NH_a were observed ($\Delta\delta$ = 0.3–0.8 ppm), consistent with a strong association between the carboxylic acid and the amidopyridine moiety. Addition of corresponding methyl esters to a solution of **13** led to no significant changes in the ^1H NMR spectrum for **13**, further confirming the importance of the carboxylic acid–amidopyridine interaction in the observed binding. The addition of all carboxylic acid substrates, with the exception of phenylacetic acid (Table 1, entries 2–8), also led to large downfield shifts of the signals for NH_c ($\Delta\delta$ = 0.4–0.9 ppm) and addition of the dipeptide substrates (entries 5–8) led to large downfield shifts of NH_d ($\Delta\delta$ = 0.1–0.5 ppm) implicating these NHs in hydrogen bonding with the respective substrates. (The signal for NH_b was obscured by the aromatic region and could not be accurately monitored.) Significant shifts in the signals for the aromatic protons were also observed. In particular, addition of substrates incorporating an L-Ala-OH moiety (entries 2, 4, 5 and 7) led to an upfield shift of ArH_p ($\Delta\delta$ = 0.1–0.2 ppm) and a downfield shift of ArH_t ($\Delta\delta$ = 0.1– 0.3 ppm), whereas addition of substrates incorporating D-Ala-OH (entries 3, 6 and 8) gave much smaller shifts of ArH_p and ArH_t, but significant upfield shifts of both ArH_q and ArH_r ($\Delta\delta$ = 0.1–0.2 ppm).

The association constants for the various guests studied indicate that the receptor, while able to bind a range of substrates, is a particularly strong receptor for Cbz-L-Ala-L-Ala-OH ($-\Delta G_{ass}$ = 25.3 kJ mol^{-1}). The receptor is very selective for this substrate as evidenced by the fact that Cbz-L-Ala-L-Ala-OH is bound > 4 kJ mol^{-1} more strongly than Cbz-D-Ala-D-Ala-OH and ~7 kJ mol^{-1} more strongly than Cbz-Gly-L-Ala-OH. The latter result is particularly notable since the difference in binding energies (~7 kJ mol^{-1}) is the consequence of replacing a single proton with a methyl group at the second residue of the dipeptide (Gly → L-Ala), and is probably a consequence of the methyl group establishing a stabilising interaction with the bisarylmethane unit in the rim of the macrobicycle—which is evidenced by the large upfield shift of the signal for the methyl group ($-\Delta\delta \approx 0.7$ ppm) on complexation with macrobicycle **13**.[11]

Thus the increased rigidity, and hence preorganisation, introduced into the structure of macrobicycle **13** has provided a stronger and considerably more selective receptor than the previously described macrobicycle **2** and, whereas the latter was a better receptor for β-alanyl-D-α-amino acid substrates, macrobicycle **13** is selective for dipeptides derived from two α-amino acids, and in particularly for the significant L-Ala-L-Ala-OH sequence.

We thank the EPSRC for a studentship for P. H. and Dr Tobias Braxmeier and Dr Herbert Röttele (Karlsruhe University) for carrying out additional NMR experiments.

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11 In view of the selectivity of macrobicycle **13** for Cbz-L-Ala-L-Ala-OH over Cbz-D-Ala-D-Ala-OH (Table 1, entries 5 and 6), and for Cbz-L-Ala-OH over Cbz-D-Ala-OH (entries 2 and 3), the preference for Cbz-Gly-D-Ala-OH over Cbz-Gly-L-Ala-OH (entries 7 and 8) is somewhat surprising. The origin of this selectivity is unclear at this stage and will need to be examined in further studies.

Communication 9/03449H