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Modified Synthesis and Binding Properties of a Peptide Receptor

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Abstract: Macrocyclic receptor 9, has been prepared in homochiral form. The receptor shows selectivity for certain dipeptides, and most notably a strong preference for N-Cbz-β-alanyl amino acids over N-Cbz-β-alanyl lactic acids. © 1997, Elsevier Science Ltd. All rights reserved.

We recently described the synthesis of macrocycle 9 in racemic form.¹ Macrocycle 9 incorporates a diamidopyridine unit as a specific binding site for the carboxylic acid terminus of a peptidic guest, amide functionality to provide hydrogen bonding with the backbone of the guest, and a suitable rigid spacer to hold the macrocycle open. When racemic macrocycle 9 was titrated with homochiral peptide substrates two distinct diastereomeric complexes were formed and a single titration experiment gave estimates of the binding constants for the two diastereomeric complexes, indicating that the macrocycle could bind peptides with some selectivity although the sense of this selectivity could not be determined directly from these experiments. We now wish to describe a modified synthesis which provides macrocycle 9 as a single enantiomer and has allowed us to probe the binding selectivity more fully.

The synthesis of homochiral macrocycle 9 was similar to the original synthesis of the racemic material and ultimately involved coupling and cyclisation of the two fragments 1 and 6 (Scheme). However, the synthesis of amine 1 has been modified in order to avoid racemisation of the phenylalanine unit. Thus diaminopyridine was coupled to one equivalent of N-^tBoc-*L*-phenylalanine and, after removal of the *tert*butyloxycarbonyl protecting group, coupling to the acid chloride of the monoallyl ester of succinic acid gave homochiral 1 directly and without any need to protect the other pyridylamine functionality. The previously described synthesis of acid 6 was improved by carrying out the Stille coupling² of stannane 3 with bromide 4 using Pd(PPh₃)₂Cl₂ as catalyst and toluene as solvent, to give ester 5 in 63% yield (in the earlier synthesis Pd(PPh₃)₄ and N-methylpyrrolidinone as solvent gave 5 in only 40% yield). The stannane 3 could itself be produced more efficiently (76%) than previously (56%) by carrying out the conversion from the bromide 2 with an excess of hexabutylditin in a concentrated solution in toluene.³ The coupling of amine 1 and acid 6 was best achieved via the acid fluoride $7.^4$ The resulting protected cyclisation precursor 8 was carried through the previously described procedure to give the macrocyclic product in ~30% yield over four steps.



Our previous studies with racemic 9 showed that the receptor bound *N*-Cbz- β -alanyl alanine with enhanced binding compared to simple *N*-Cbz-amino acids or phenylacetic acid. Titration of homochiral 9 with a range of *N*-Cbz- β -alanyl amino acids (see table) has allowed us to determine binding data for these substrates by conventional analysis of the shift of NH_b in the ¹H NMR monitored throughout the titration.⁵ Whereas titration of such guests with the racemic macrocycle had led to the formation of two diastereomeric complexes, clearly distinguishable in the ¹H NMR, we now observed formation of a single diastereoisomeric complex, allowing determination of binding constants for each enantiomer of the various guests, and confirming that no racemisation had occurred in the modified synthesis of 9. As before, in each titration experiment significant downfield shifts of NH_b were observed with no apparent shift of NH_a, consistent with a strong association between the carboxylic acid and the amidopyridine moiety, presumably involving NH_b and not NH_a. From the binding data it is clear that 9 shows enantioselectivity for N-Cbz- β -alanyl-L-amino acids compared to the corresponding N-Cbz- β -alanyl-D-amino acids (with the greatest observed selectivity (68% e.e.) for N-Cbz- β -alanyl valine).

Substrate	$-\Delta G_{assoc}$ (kJ mol ⁻¹)	Substrate	$-\Delta G_{assoc}$ (kJ mol ⁻¹)
Phenyl acetic acid	11.5 ^a		
N-Cbz glycine	14.4 ^a	N-Cbz β -alanyl-L-phenylalanine	16.2 ± 0.4
N-Cbz β-alanine	12.9 ^a	N-Cbz β -alanyl-D-phenylalanine	14.0 ± 0.5
N-Cbz L-alanyl-L-alanine	18.4 ± 0.7	N-Cbz β -alanyl-L-valine	16.8 ± 0.6
N-Cbz D-alanyl-D-alanine	16.5 ± 0.3	N-Cbz β-alanyl-D-valine	12.7 ± 0.2
N-Cbz β -alanyl-L-alanine	19.9 ± 0.7	N-Cbz β -alanyl-L-lactate	13.1 ± 0.3
N-Cbz β-alanyl-D-alanine	16.6 ± 0.7	N-Cbz β -alanyl-D-lactate	11.2 ± 0.2

Table. Binding of 9 and Peptide Substrates in CDCl₃ Solution.

^aData obtained previously with racemic macrocycle 9.¹

We have also measured the binding of the ester linked N-Cbz- β -alanyl lactic acids as substrates for receptor 9, effectively replacing a hydrogen bond donor substituent (NH) with a hydrogen bond acceptor substituent (O-lone pair) in the guest structure.

$$\begin{array}{ccc} H & H \\ Ph & O_{H} & N & CO_{2}H \\ O & O & CH_{3} \end{array}$$

N-Cbz-\beta-alanyl-L-alanine



N-Cbz-\beta-alanyl-L-lactate

The binding of these ester substrates was substantially lower than the corresponding N-Cbz- β -alanyl alanines, strongly suggesting that a hydrogen bond from the guest NH to the receptor side wall is a key interaction in the binding of the latter. This result is also of interest in view of recent observations that certain bacteria have mutated their cell wall precursor structure from a terminal *D*-Ala-D-Ala-CO₂H sequence to a *D*-Ala-*D*-Lac-CO₂H sequence, thus providing immunity to conventional antibacterials such as the natural *D*-Ala-CO₂H receptor vancomycin.^{6,7}

Thus, we have been able to establish that macrocycle 9 is an effective receptor for dipeptides with a carboxylic acid terminus, and shows some selectivity, particularly for N-Cbz- β -alanyl-L-amino acids. Although the observed enantioselectivity is not as great as has been observed in other peptide binding systems,⁸ it is surprisingly good given the flexibility of the receptor and the minimal chirality which it incorporates. Evidence from the binding studies suggests that the binding of such substrates involves interaction of the carboxylic acid of the substrates with the amidopyridine unit (with a strong hydrogen bond

to NH_b) and involves a hydrogen bond interaction with the amide NH of the substrate.

Molecular modelling studies to further elucidate the mode of binding of macrobicycle 9 generated a range of low energy structures for the 1:1 complexes with no clear trends to indicate specific binding interactions and showed that, as expected, the macrocycle is a fairly flexible structure.⁹ This flexibility may also explain the lack of observable intermolecular nOe's in detailed 2-D NMR studies (NOESY and ROESY) carried out on 1:1 mixtures of macrocycle 9 and various substrates.

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