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Synthesis and Peptide Binding Properties of a C_2 Symmetric Macrobicycle

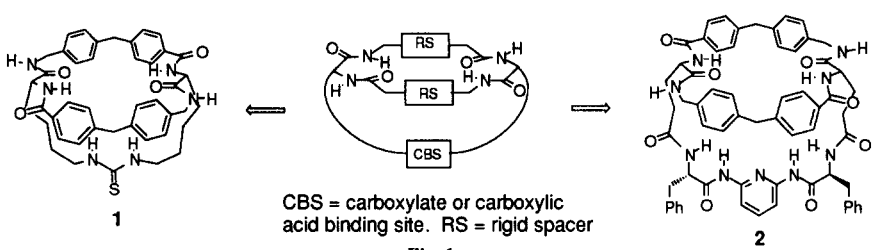
Christopher P. Waymark,[a] Jeremy D. Kilburn[a*] and Iain Gillies[b]

a Department of Chemistry, University of Southampton, Southampton, SO17 1BJ, UK

b Chemical Development Laboratory, Wellcome Foundation, Temple Hill, Dartford, Kent, DA1 5AH

Abstract: A novel macrobicycle featuring an amidopyridine unit as a carboxylic acid binding site, and amide functionality to provide further hydrogen bonding interactions with suitable guests has been prepared. The ability of this novel macrobicycle to bind peptide derivatives has been investigated.

We recently described a novel bicyclic receptor **1**, which employed a thiourea unit at the base of a C_2 symmetric cavity to bind the carboxylate anion of various amino acid derivatives.[1,2] Amide functionality was incorporated around the rim of the cavity to provide further hydrogen bonding interactions with the guest. In the preceding paper we have described the synthesis of a related macrobicycle which features an amidopyridine unit as a potential carboxylic acid binding site in a C_2 symmetric cavity.[3] A study of the solution conformation of that macrobicycle revealed a preference for a conformation with the amidopyridine unit inverted away from the cavity, which is unfavourable for binding of amino acids or peptidic guests. In this paper we report the synthesis of a larger C_2 symmetric receptor **2**, again with an amidopyridine unit as a carboxylic acid binding site, which shows strong binding to amino acid and dipeptide derivatives in chloroform solution.

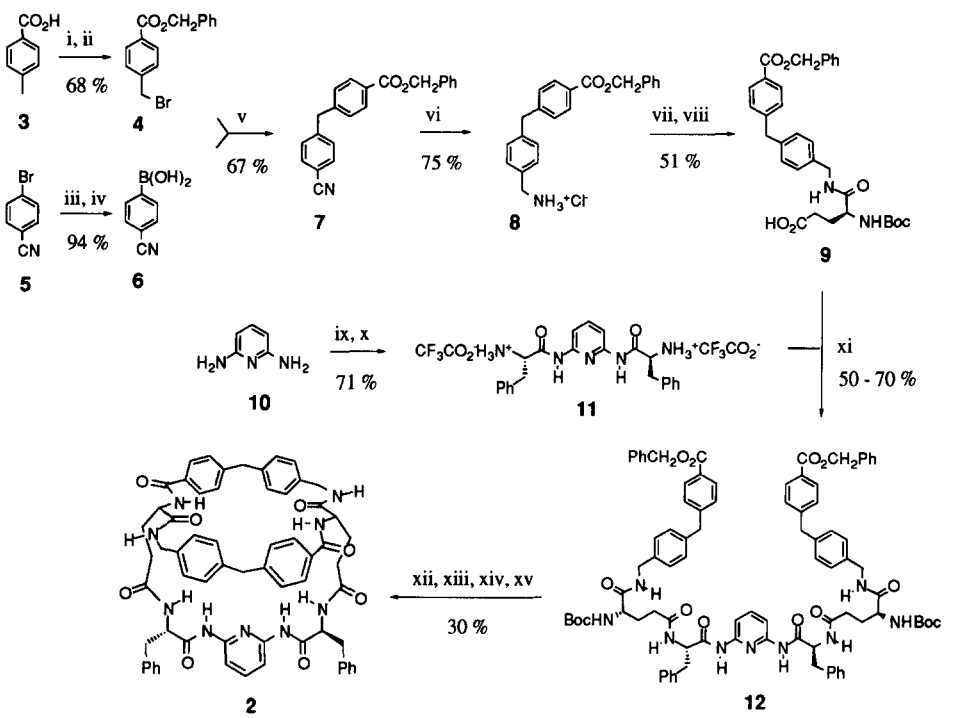


1 CBS = carboxylate or carboxylic acid binding site. RS = rigid spacer **2**

Fig. 1

The synthesis of receptor **2** is outlined in Scheme 1, and follows the same strategy used for the synthesis of the previously described macrobicycles,[1,3] with a double intramolecular macrocyclisation of a suitably activated precursor as the critical step. Receptor **2** uses the same biarylmethane unit as the rigidifying unit in the rim of the structure as used previously in receptor **1**,[1] and was again constructed by a Suzuki coupling[4] of boronic acid **6** and a bromide **4**. Reduction of the resulting nitrile **7** gave the amine **8** which was coupled with *tert*butoxycarbonyl *L*-glutamic acid γ-allyl ester, followed by removal of the allyl protecting group to give acid

9. Coupling of diaminopyridine with *tert*butoxycarbonyl *L*-phenylalanine and removal of the amine protecting groups gave the bis(trifluoroacetate) salt **11** which was then coupled with acid **9** to give the protected macrocylisation precursor **12**. The benzyl esters were successfully hydrogenolysed (selective alkaline hydrolysis of the corresponding dimethyl ester had failed in earlier studies, due to competitive cleavage of the amide bonds of the amidopyridine unit), and the resulting diacid was converted to the bis(pentafluorophenyl ester). Removal of the amine protecting groups with trifluoroacetic acid and, finally, slow addition of the resulting bis(trifluoroacetate salt) to a solution of diisopropylethylamine, in acetonitrile, gave the desired macrobicycle **2** in ~30 % overall yield from **12**.



Reagents: i, Br_2, AIBN; ii, $PhCH_2OH$, *p*TsOH, toluene, reflux; iii, *n*BuLi, THF, -100 °C; iv, $(MeO)_3B$; v, $Pd(Ph_3P)_4$, DME, Na_2CO_3; vi, $BH_3.Me_2S$, THF; vii, *tert*butoxycarbonyl *L*-glutamic acid γ-allyl ester, DCC, HOBT, DIPEA, DMF; viii, $Pd(Ph_3P)_4$, pyrrolidine, CH_2Cl_2; ix, *tert*butoxycarbonyl *L*-phenylalanine, PyBOP, DIPEA, DMF; x, 50 % TFA, CH_2Cl_2; xi, PyBOP, DIPEA, DMF; xii, 10 % Pd/C, NH_4CO_2H, DMF; xiii, C_6F_5OH, DCC, DMAP, THF; xiv, 50 %, TFA, CH_2Cl_2; xv, syringe pump addition to DIPEA, CH_3CN, 70 °C, final concentration 1.75 mmolar.

SCHEME 1

Macrocycle **2** was obtained in essentially optically pure form, as determined by amino acid analysis,[5] and a 2D roesy spectrum revealed no nOe's from the pyridine ring protons to the rim of the macrocycle, suggesting that the pyridine ring is not inverted into the macrocyclic cavity, as was found for the related structure described in the preceding paper.[3]

Binding studies with macrocycle **2** were carried out with a series of substrates in deuteriochloroform, using a standard NMR titration experiment, and analysing the resultant binding curves using linear regression methods.[7,8] The results are presented in table 1.

Table 1. Binding of **2** and Peptide Substrates in $CDCl_3$ Solution.

Entry	Substrate	$-\Delta G_{assoc}$ (kJ mol^{-1})	Entry	Substrate	$-\Delta G_{assoc}$ (kJ mol^{-1})
1.	Phenyl acetic acid	14.2	10.	^{t}Boc *L*-valine	12.6
2.	Cbz glycine	16.3	11.	^{t}Boc *D*-valine	10.7
3.	Cbz β-alanine	16.5	12.	^{t}Boc *L*-serine	17.6
4.	Cbz *L*-alanine	15.6	13.	^{t}Boc *D*-serine	14.8
5.	Cbz *D*-alanine	17.6	14.	Cbz glycinyl-*L*-serine	19.2
6.	^{t}Boc *L*-phenylalanine	14.6	15.	Cbz glycinyl-*D*-serine	15.4
7.	^{t}Boc *D*-phenylalanine	15.8	16.	Cbz *L*-alanyl-*L*-alanine	14.6
8.	Cbz *D*-phenylalanine	17.6	17.	Cbz *D*-alanyl-*D*-alanine	17.7
9.	Ac *D*-phenylalanine	19.5	18.	Cbz β-alanyl-*L*-alanine	19.1
			19.	Cbz β-alanyl-*D*-alanine	22.8

Titration of receptor **2** with phenylacetic acid (entry 1) gave $-\Delta G_a$ = 14.2 kJ mol^{-1} with a downfield shift of the amidopyridine NH signal in the ^{1}H NMR, but with no significant changes to the rest of the signals for **2**. This is consistent with the anticipated binding of the carboxylic acid functionality by the amidopyridine unit, and gives a good indication of the strength of this interaction, in this system, against which to compare the binding of the other substrates. Binding of amino acid derivatives (entries 1 - 13) generally showed improved binding over phenylacetic acid. For alanine and phenylalanine substrates (entries 4 - 9) there is a small preference for the *D*-amino acid, but there is an interesting reversal of this selectivity for the valine and serine substrates (entries 10 - 13). For the *D*-phenylalanine substrates, binding was significantly improved on decreasing the size of the *N*-protecting group (entries 7 - 9), and the poor binding of the valine substrates (entries 10, 11) may be attributed to steric demands of the side-chain, while the serine substrates (entries 12, 13) showed substantially better binding, suggesting a positive interaction between the hydroxymethyl moiety of the substrate and the receptor. This is good evidence for supposing that the substrates are bound within the cavity as hoped, and not on the outside where steric demands would be less important.

Dipeptide substrates did not show substantially better binding, as compared with the single amino acid derivatives, until the β-alanylalanine substrates were used (entries 18, 19). The receptor **2** again showed a preference for the *D*- substrate (entry 19), and both the β-alanylalanine substrates were significantly better bound than the corresponding alanine substrates (entries 4, 5) or the alanylalanine substrates (entries 16, 17).

In conclusion we have synthesised a novel C_2 symmetric macrobicycle which shows selectivity in the binding of peptides with a carboxylic acid terminus. While binding appears to be dominated by the carboxylic acid - amido pyridine interaction, further binding interactions must account for the observed binding selectivities.[9] Further studies are now underway to determine the structure of these host-guest complexes.

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5. Receptor **2** was characterised by ^{1}H and ^{13}C NMR, HRMS, IR and amino acid analysis.
6. Amino acid analysis (hydrosylation of **2** and conversion of the amino acids so formed into their methyl ester *N*-pentafluoropropionyl derivatives was followed by separation of the *D* and *L* isomers on a Chirasil-*L*-Val capillary column) showed that **2** contained >95 % *L*-phenylalanine and >90 % *L*-glutamic acid.
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8. In a typical experiment, 10 µl aliquots of the chosen substrate (0.02 M in $CDCl_3$) were added to receptor **2** (0.6 ml of a 3 mM solution in $CDCl_3$) and the ^{1}H NMR spectrum recorded after each addition, monitoring the movement of the amidopyridine NH signal Receptor **2** does not appear to dimerise or aggregate at the concentrations used in these titrations as adjudged by a simple dilution experiment. The binding data was analysed using Wilcox's Hostest 5 software (Wilcox, C. S.; Glagovich, N. M. University of Pittsburgh, USA, © C. S. Wilcox and the University of Pittsburgh, 1993).
9. The observed selectivities provide good circumstantial evidence that the additional binding interactions are provided within the receptor cavity. Attempts to confirm this by measuring intermolecular nOe's between 1:1 mixtures of receptor **2** and guests have so far been hampered by loss of resolution of the guest signals in the ^{1}H NMR.

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