Pergamon

Tetrahedron Letters, Vol. 36, No. 19, pp. 3409-3412, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)00494-7

Synthesis and Binding Properties of a Peptide Receptor

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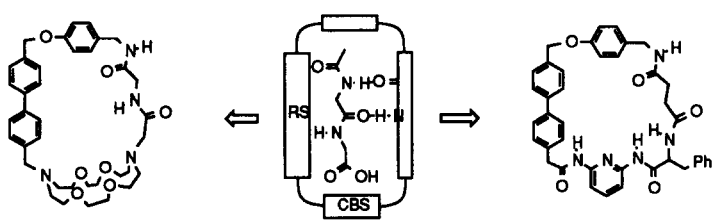
Abstract: Macrocyclic receptor **2**, with an amidopyridine unit as the binding site for carboxylic acid functionality and amide functionality to provide additional hydrogen bonding sites, has been synthesised. Preliminary binding studies with **2** and various peptidic guests are described.

With the aim of developing synthetic receptors for peptides[1] we set out to prepare macrocycles which feature a specific binding site for the carboxylate or carboxylic acid terminus of such 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 (represented schematically in fig. 1).



1 **2**

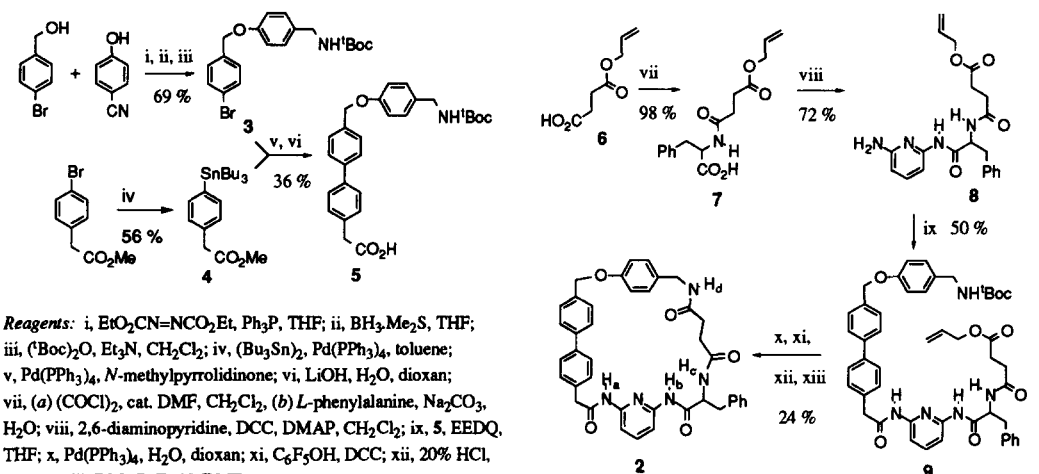
CBS = carboxylic acid/carboxylate binding site; RS = rigid spacer

Fig. 1

We recently reported the synthesis of macrocycle **1**, with a specific binding site for the potassium salt of the carboxylate terminus of a peptidic guest provided by a diazacrown ether, and described preliminary binding studies for this macrocycle.[2] The synthesis of macrocycle **1** required the formation of the biaryl bond as the macrocyclic ring closing step, since all attempts to close the ring *via* amine, or amide bond formation, failed. In this paper we describe the synthesis of a closely related macrocycle **2**, with an amidopyridine unit as the binding site for carboxylic acid functionality.[3] This synthesis has been achieved with a more conventional ring closure, forming an amide bond. Preliminary binding studies indicate that **2** is able to bind to simple dipeptides with some selectivity.

The synthetic route to macrocycle **2** is outlined in Scheme 1. Thus, synthesis of bromide **3** followed the previously reported sequence.[2] A palladium catalysed coupling of bromide **3** with stannane **4**[4] followed by methyl ester hydrolysis then gave acid **5**. Opening of succinic anhydride with allyl alcohol gave monoallylester **6** which was coupled to *L*-phenyalanine to give acid **7** in essentially quantitative yield. Diaminopyridine was

successively coupled with acid **7** and then with acid **5** in moderate yields to give the protected cyclisation precursor **9**. Removal of the allyl group using $Pd(Ph_3P)_4$ and aqueous dioxan, activation of the carboxylic acid as the pentafluorophenyl ester and removal of the *tert*butyloxycarbonyl protecting group with HCl/dioxan gave the amine hydrochloride salt, which was cyclised at high dilution (5 mmolar) in DMF, with DMAP/triethylamine, to give the final macrocycle **2**. The last four steps from **9** to macrocycle **2** were best carried out without purification of the intermediates and gave **2** in 20 - 25 % yield over four steps.[5]



Reagents: i, $EtO_2CN{=}NCO_2Et$, Ph_3P, THF; ii, $BH_3.Me_2S$, THF; iii, $(^tBoc)_2O$, Et_3N, CH_2Cl_2; iv, $(Bu_3Sn)_2$, $Pd(PPh_3)_4$, toluene; v, $Pd(PPh_3)_4$, *N*-methylpyrrolidinone; vi, LiOH, H_2O, dioxan; vii, (*a*) $(COCl)_2$, cat. DMF, CH_2Cl_2, (*b*) *L*-phenylalanine, Na_2CO_3, H_2O; viii, 2,6-diaminopyridine, DCC, DMAP, CH_2Cl_2; ix, **5**, EEDQ, THF; x, $Pd(PPh_3)_4$, H_2O, dioxan; xi, C_6F_5OH, DCC; xii, 20% HCl, dioxan; xiii, DMAP, Et_3N, DMF.

SCHEME 1

Macrocycle **2** was obtained as a racemate as a result of complete racemisation of the phenyalanine unit,[6] presumably during the slow coupling (2 days) of diaminopyridine to acid **7**. The ^1H NMR of **2** (in $CDCl_3$) gave a well resolved spectrum which could be fully assigned with the help of 2D NMR experiments. A 2D ROESY spectrum revealed no nOe's between protons on opposite sides of the macrocyclic ring, suggesting that **2** exists in an open conformation in $CDCl_3$ solution, as desired.

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.

Substrate	$-\Delta G_{assoc}$ (kJ mol^{-1})	Substrate	$-\Delta G_{assoc}$ (kJ mol^{-1})
Phenyl acetic acid	11.5	Cbz *L*-phenylalanine	14.2/13.7
Cbz glycine	14.4	^{t}Boc *L*-alanine	14.2/13.7
Cbz β-alanine	12.9	Cbz *L*-alanyl-*L*-alanine	16.0/14.6
Cbz *L*-alanine	15.8/15.2	Cbz β-alanyl-*L*-alanine	19.4/16.2
Cbz *D*-alanine	15.6/15.2		

[a]Where two figures are reported these refer to the binding energies for the two diastereomeric complexes formed - see text.

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. Titration with phenylacetic acid gave $-\Delta G_a = 11.5$ kJ mol^{-1} which places an upper limit on the strength of the amidopyridine-carboxylic acid interaction. N-Benzyloxy-carbonyl β-alanine ($-\Delta G_a = 12.9$ kJ mol^{-1}) showed a modest increase in binding over phenylacetic acid, while N-benzyloxycarbonyl glycine ($-\Delta G_a = 14.4$ kJ mol^{-1}) showed a more pronounced increase.

When the racemic receptor was titrated with homochiral peptide substrates two distinct diastereomeric complexes were immediately evident in the ^1H NMR, and the well separated signals for NH_b of the two complexes (and to a lesser extent for NH_c and NH_d) could be conveniently followed throughout the titration experiment. Analysis of this data[7,8] gave estimates[9] of the two binding constants for the diastereomeric complexes. Titration of **2** with N-benzyloxycarbonyl L-alanine gave $-\Delta G_a = 15.8$ and 15.2 kJ mol^{-1} for the two diastereomeric complexes (which was essentially mirrored, as expected, by titration with N-benzyloxy-carbonyl D-alanine) and titration with N-benzyloxycarbonyl L-phenylalanine gave $-\Delta G_a = 14.2$ and 13.7 kJ mol^{-1} for the two diastereomeric complexes.

Titration of **2** with the dipeptide N-benzyloxycarbonyl L-alanyl-L-alanine showed no significant increase in binding over simple amino acid derivatives for either of the two diastereomeric complexes formed, but titration of **2** with N-benzyloxycarbonyl β-alanyl-L-alanine gave two significantly different binding constants for the two diastereomeric complexes ($-\Delta G_a = 19.4$ and 16.2 kcal mol^{-1}, $\Delta\Delta G = 3.2$ kJ mol^{-1}, representing a binding enantioselectivity of ~ 80 : 20, although the sense of the enantioselectivity cannot be determined from these experiments).

The ^1H NMR of a 1:1 complex between **2** and N-benzyloxycarbonyl β-alanyl-L-alanine (7.5 mM in $CDCl_3$) also showed the most significant differences between the diastereomeric complexes so formed (as compared to 1:1 complexes with any other substrate). Thus NH_b shifted downfield (relative to uncomplexed **2**) from 8.0 ppm to 8.84 and 8.33 ppm respectively for the two diastereomeric complexes. NH_c shifted downfield from 6.25 ppm to 6.84 and 6.52 ppm respectively, and NH_d shifted downfield from 6.0 ppm to 6.30 and 6.16 ppm respectively. The position of the signal for NH_a, however, again appeared to be unaffected by the addition of the substrate. Essentially all other signals in the ^1H NMR were separated and could be assigned to the two diastereomeric complexes. A 2D ROESY spectrum of the 1:1 complex did not, unfortunately, reveal any intermolecular crosspeaks to help define the position of the bound substrate in the cavity.

In conclusion we have synthesised a simple macrocyclic receptor which shows surprising selectivity in the binding of some peptidic guests, demonstrating that the basic macrocyclic design is suitable for peptide binding. The structure of the complex between **2** and the various guests studied remains undefined, but it is clear that the acid functionality of the guest binds to the amidopyridine *via* NH_b, and further hydrogen bonding interactions enhance the binding, particularly for the best substrate N-benzyloxycarbonyl β-alanyl-L-alanine. More detailed information regarding the structure of the complex awaits the synthesis of **2** in homochiral form, and further binding studies.

We thank the SERC for a studentship to SSF, Dr G Calder (Rowett Research Institute, Aberdeen) for carrying out the amino acid analysis on **2**, and Professor C S Wilcox (Pittsburgh University, USA) for kindly providing us with his Hostest 5 program.

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5. Selected data for **2**: ^{1}H NMR (360 MHz, $CDCl_3$) δ: 8.02 (1H, s, NH_b), 7.91 (1H, d, J = 8 Hz, pyrH), 7.78 (1H, d, J = 8 Hz, pyrH), 7.70 (2H, d, J = 8 Hz, ArH), 7.65 (1H, t, J = 8 Hz, pyrH), 7.62 (2H, d, J = 8 Hz, ArH), 7.41 (2H, d, J = 8 Hz, ArH), 7.36 (2H, d, J = 8 Hz, ArH), 7.28 (1H, s, NH_a), 7.16 - 7.26 (3H, m, Ph), 7.13 (2H, d, J = 6 Hz, Ph), 7.04 (2H, d, J = 9 Hz, ArH), 6.79 (2H, d, J = 9 Hz, ArH), 6.34 (1H, d, J = 8 Hz, NH_c), 5.98 (1H, t, J = 5 Hz, NH_d), 5.30 (2H, s, CH_2O), 4.62 (1H, m, $C\underline{H}CH_2Ph$), 4.40 (1H, dd, J = 5, 14 Hz, $C\underline{H}_AH_BNH$), 4.05 (1H, dd, J = 5, 14 Hz, $CH_A\underline{H}_BNH$), 3.83 (1H, d, J= 17 Hz, $C_6H_4C\underline{H}_AH_BCO$), 3.77 (1H, d, J = 17 Hz, $C_6H_4CH_A\underline{H}_BCO$), 3.17 (1H, dd, J = 7, 14 Hz, $CHC\underline{H}_AH_BPh$), 2.96 (1H, dd, J = 8, 12 Hz, $CHCH_A\underline{H}_BPh$), 2.52 (1H, m, $C\underline{H}_AH_BCH_2$), 2.35-2.21 (3H, m, $CH_A\underline{H}_BC\underline{H}_2$). $C_{40}H_{38}N_5O_5$ (M+H) requires m/z 668.2873.
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 ~51.3 % *L*-phenylalanine and 48.7 % *D*-phenylalanine.
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8. In a typical experiment, 10 µl aliquots of the chosen substrate (0.1 M in $CDCl_3$) were added to receptor **2** (0.6 ml of a 5 mM solution in $CDCl_3$) and the ^{1}H NMR spectrum recorded after each addition. In general we monitored the movement of NH_b, NH_c and NH_d although problems of resolution meant that only the data for NH_b was consistently reliable. 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.) which has been developed to deal with complex binding equilibria including racemic binding.[7]
9. The quoted values for ΔG_{assoc} were estimated to have standard deviations in the range 0.5 - 0.9 $kJmol^{-1}$ indicating that there is genuine enantioselective binding for the dipeptide substrates. The magnitude of these enantioselectivities will be more accurately determined once a homochiral synthesis of **2** has been completed.

(*Received in UK* 10 *February* 1995; *revised* 16 *March* 1995; *accepted* 17 *March* 1995)