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New macrobicyclic receptors for amino acids

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Abstract

Two new receptors, **9** and **13**, have been prepared, utilising a pyridyl aryl ether unit in the rim of the macrobicyclic structure, and the crystal structures for both of these receptors are reported. The presence of the pyridyl unit provides additional hydrogen bonding functionality in comparison to earlier structures of this type, and **9** is an effective receptor for *N*-acetyl L-asparagine carboxylate in CH_2Cl_2. © 2000 Elsevier Science Ltd. All rights reserved.

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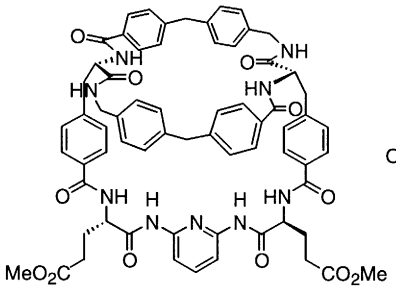
The development of synthetic receptors for biological substrates, particularly for peptides and amino acids, continues to be an active area of research.[1] We have been successful in developing receptors for amino acids and peptidic substrates using a range of receptor structures and in particular using C_2 symmetric macrobicyclic receptors, such as **1**[1a] and **2**,[2] featuring a specific carboxylic acid, or carboxylate, binding site at the base of a bowl-shaped cavity.[3]



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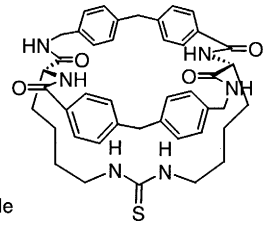

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Macrobicycle **1**, for example, was found to be a selective receptor for the dipeptide Cbz L-Ala-L-Ala-OH,[1a] while macrobicycle **2** was found to have the unusual property of binding *N*-acetyl L-amino acid carboxylate salts (specifically alanine and phenylalanine) with the acetyl amide in the *cis* configuration.[2a] A common feature of these receptors[1a,2,3] has been the use of a biarylmethane unit to

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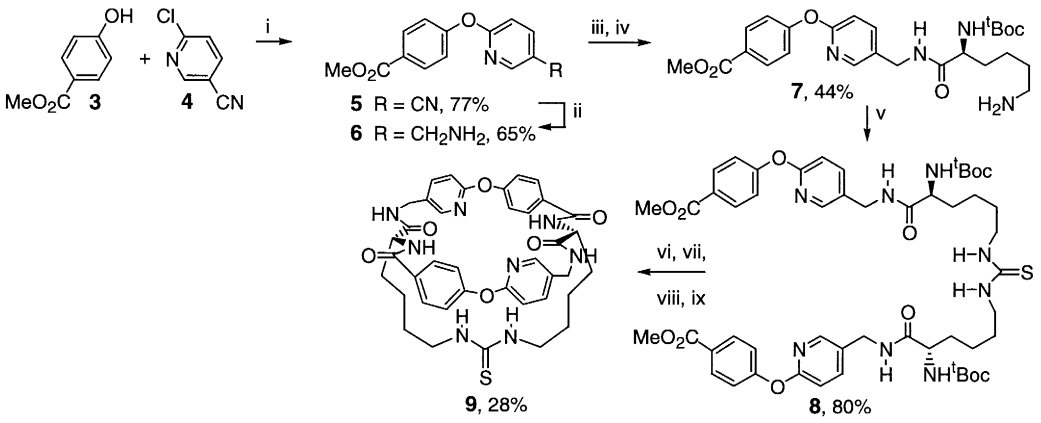
give a relatively rigid and well-defined, open rim to the bowl-shaped cavities. We wished to incorporate additional hydrogen bonding functionality, which might in turn lead to more selective binding properties, but without dramatically altering the gross structure of these receptors. We also wished to develop an efficient route to such structures without the C_2 symmetry, since this would greatly increase the number of accessible macrobicyclic receptors of this type. In this paper we describe the successful and straightforward synthesis of two new macrobicycles, **9** and **13**, closely related to macrobicycle **2**, but with one or both of the biarylmethanes now replaced by a pyridyl aryl ether. We also describe the crystal structures of **9** and **13**, the first crystal structures of this class of macrobicyclic receptor, and preliminary binding studies using receptor **9**.

Following the successful strategy used previously for the synthesis of all of our macrobicyclic receptors we aimed to prepare cyclisation precursors, which could ultimately undergo a double macrocyclisation, by coupling the modified biaryl portion to a suitably protected lysine derivative, followed by formation of a thiourea using the side-chain amino group of the lysines.

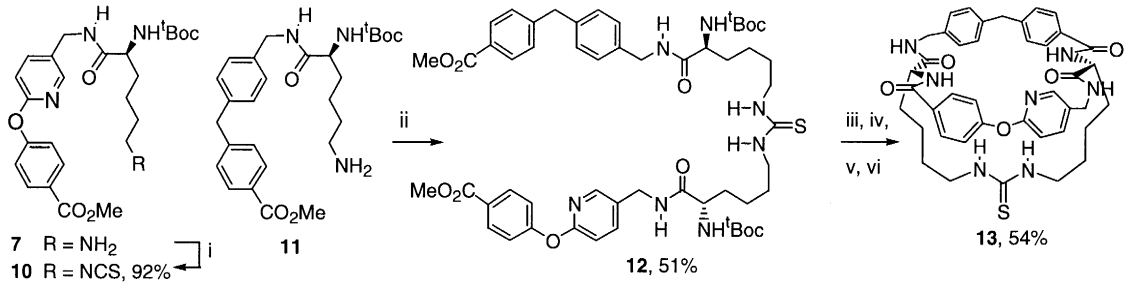
The desired modified biaryl amino acid derivative **6** was obtained by coupling[4] methyl 4-hydroxybenzoate, **3**, with chloropyridine **4**, to give the pyridyl aryl ether **5**, followed by reduction of the nitrile with H_2/Pd/C[5] (Scheme 1). Amine **6** was coupled to N^{α}-Boc-N^{ε}-Cbz-L-lysine, and the N^{ε}-Cbz protecting group was removed by hydrogenolysis. Treatment of the resulting free amine **7** with 0.5 equiv. of thiophosgene, using aqueous K_2CO_3 as base, led directly to the symmetric thiourea **8**, in good yield. Hydrolysis of the methyl esters, formation of the corresponding pentafluorophenyl esters and treatment with anhydrous TFA[6] in dry CH_2Cl_2 gave the desired cyclisation precursor. Cyclisation by slow addition of a solution of the precursor, in CH_3CN, to a refluxing solution of DIPEA in CH_3CN yielded the C_2 symmetric macrobicycle **9** in 28% yield.



Scheme 1. (i) NaH, DMSO, 120°C; (ii) H_2, Pd/C, MeOH, NH_3; (iii) N^{α}-Boc-N^{ε}-CBz-L-lys, HOBT, 1-(3-dimethylamino propyl)-3-ethyl carbodiimide (WSC), THF; (iv) H_2, Pd, C; (v) 0.5 equiv. $Cl_2C{=}S$, K_2CO_3, H_2O, $CHCl_3$, reflux; (vi) LiOH, H_2O, dioxane; (vii) C_6F_5OH, WSC, DMAP, THF; (viii) CF_3CO_2H, CH_2Cl_2, (1:1, v/v); (ix) $^{i}Pr_2EtN$, CH_3CN, reflux, high dilution

Alternatively, amine **7** could be converted to the isothiocyanate **10** using 1.0 equiv. of thiophosgene, again with aqueous K_2CO_3 as base (Scheme 2). Coupling with the previously described amine **11**,[2a] gave the unsymmetrical thiourea **12**. Now an identical sequence of hydrolysis of the methyl esters, formation of the corresponding pentafluorophenyl esters, removal of the Boc protecting groups with TFA, and cyclisation, gave macrobicycle **13** in a particularly good yield of 54%.

The C_2 symmetric compound **9** was recrystallised from MeOH and the non-symmetric compound **13** from DMSO/H_2O to provide X-ray quality crystals.[7] For both compounds the crystal structures (Fig. 1) reveal a large open cavity, as desired, with the sulphur of the thiourea pointing into the cavity. The



Scheme 2. (i) 1 equiv. $Cl_2C{=}S$, K_2CO_3, $CHCl_3$, reflux; (ii) **10**, K_2CO_3, H_2O, $CHCl_3$, reflux; (iii) LiOH, H_2O, dioxane; (iv) C_6F_5OH, WSC, DMAP, THF; (v) CF_3CO_2H, CH_2Cl_2, (1:1, v/v); (vi) $^{i}Pr_2EtN$, CH_3CN, reflux, high dilution

main difference in the two structures comes from the different orientations of aromatic rings in the biarylmethane fragment of **13** and the pyridyl aryl ether units with a more twisted arrangement for the latter, which leads to a more open rim in the case of **9**. For **9** the orientation of the thiourea, with the sulphur pointing into the cavity, is stabilised by a pair of hydrogen bonds from both thiourea NH's to the benzamide $C{=}O^3$ of an adjacent molecule of **9**. For **13**, the thiourea configuration is stabilised by an intramolecular hydrogen bond from the thiourea sulphur to N^4–H and one intermolecular hydrogen bond from thiourea N^1–H to the benzamide $C{=}O^3$ of an adjacent molecule of **13**. However, molecular modelling of these compounds and of **2**, and earlier binding studies on **2**, make it very clear that the thiourea, and the $(CH_2)_4$ chains that connect it to the macrobicyclic rim, are very flexible, allowing the thiourea to orientate itself into or out of the cavity with little energetic penalty when in solution, so that expected binding of amino acid derivatives within the cavity of the receptor can still be anticipated.

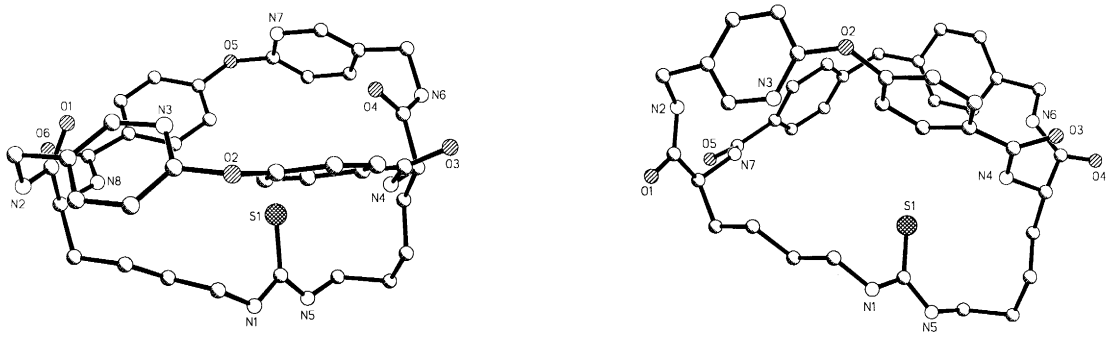


Fig. 1.

Some preliminary binding studies with macrobicycle **9** were carried out using UV titration experiments, monitoring the absorption at 249.5 nm, with the tetrabutyl ammonium salts of *N*-acetyl L-glutamine, *N*-acetyl L-asparagine and *N*-acetyl L-alanine as guests. Titration of **9**, in CH_2Cl_2, with glutamine or asparagine derivatives gave saturation curves with a good fit for the presumed 1:1 binding,[8] and binding constants of $8.7{\times}10^3$ (±20%) M^{-1} ($-\Delta G_{ass}$=22 kJ mol^{-1}) and $5.5{\times}10^4$ (± 15%) M^{-1} ($-\Delta G_{ass}$=27 kJ mol^{-1}), respectively. The 1:1 stoichiometry of binding for both of these substrates was confirmed by Job plots.[9] Titration of the alanine derivative, however, gave a complex titration curve, which indicated multiple binding equilibria, and a Job plot with this substrate confirmed that the binding was not a simple 1:1 stoichiometry. A likely explanation for these preliminary observations is that a number of modes of binding are possible for the *N*-acetyl L-alanine carboxylate salt (mirroring the previous observations[10] with the same substrate and macrobicycle **2**) whereas, for the asparagine substrate at least, incorporation of a pyridyl unit into the rim of the cavity provides additional hydrogen

bonding functionality, as originally hoped, and thus a stabilising interaction with the $CONH_2$ side-chain functionality of this guest leading to predominantly one mode of binding.

Thus the strategy of modifying the structure of our original macrobicycles has been successfully realised with efficient syntheses of **9** and **13**, and the novel macrobicycles have been characterised by X-ray crystallography. More detailed studies of the binding properties of both **9** and **13**, including detailed NMR studies of the 1:1 complexes, are now underway in our laboratory and will be reported in due course.

Acknowledgements

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5. Attempted reduction of nitrile **5** using borane, as successfully employed in the preparation of the original biarylmethane spacer used in macrobicycles **1** and **2**, led to cleavage of the pyridylaryl ether to give back the hydroxy benzoate.
6. We found that the use of rigorously dry TFA/CH_2Cl_2 was essential in the Boc deprotection step to avoid partial conversion of the thiourea moiety to the corresponding urea.
7. The intensity data were collected on a Nonius Kappa CCD area-detector diffractometer at the window of a rotating anode FR591 generator (Mo-Kα radiation, λ=0.71073 Å). The structures were solved by direct methods and refined on F^2 by full-matrix least-squares refinements. Full details have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 140813 (**9**) and CCDC 140814 (**13**). **9**: $C_{39}H_{42}N_8O_6S\cdots 2$ MeOH. T=293K, formula $C_{41}H_{50}N_8O_8S$, M=814.95, monoclinic, space group $P2_1$, Z=2, a=11.0792(2) Å, b=14.4360(3) Å, c=13.6271(3) Å, β=110.547(1)°, V=2040.86(7) Å^3, D_c=1.326 Mg/m^3, μ(Mo-Kα)=0.14 mm^{-1}. Colourless block, crystal size 0.70×0.35×0.35 mm, θ range: 2.0−25.2°, 99.9% coverage, 7395 unique data and 612 parameters, $R1[F^2>2\sigma(F^2)]$=0.042, *wR2*(all data)=0.111, Flack parameter=0.07(7). **13**: $C_{41}H_{45}N_7O_5S\cdots 3\ H_2O$. T=120K, formula $C_{41}H_{51}N_7O_8S$, M=801.95, monoclinic, space group $P2_1$, Z=4, a=9.0659(2) Å, b=17.6648(3) Å, c=26.1695(6) Å, β=98.6819(7)°, V=4143.0(1) Å^3, D_c=1.286 Mg/m^3, μ(Mo-Kα)=0.14 mm^{-1}. Colourless plate, crystal size 0.15×0.12×0.05 mm, θ range: 2.0−24.5°, 98.8% coverage, 13502 unique data and 1134 parameters, $R1[F^2>2\sigma(F^2)]$=0.061, *wR2*(all data)=0.133, Flack parameter=0.11(8).
8. Binding constants were calculated by fitting the titration data to a 1:1 binding isotherm using *NMRTit HG* software, kindly provided by Prof. C. A. Hunter, University of Sheffield. See: A. P. Bisson, C. A. Hunter, J. C. Morales, K. Young, *Chem. Eur. J.* **1998**, *4*, 845–851.
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10. With receptor **2**, *N*-acetyl L-alanine carboxylate salt (and the corresponding phenylalanine carboxylate salt) was bound predominantly within the bowl shaped cavity, utilising a carboxylate–thiourea interaction, but with the acetyl amide in the *cis* configuration (with the *cis* amide stabilised by two hydrogen bonds from the rim of the macrobicycle to the acetyl amide carbonyl). However detailed NMR studies (see Ref. 2a) revealed that there were additional modes of binding for these substrates, involving the *trans* configuration of the acetyl amide.