# A Novel Receptor for Amino Acid Derivatives

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A novel macrobicycle featuring a thiourea as a carboxylate binding site, and amide functionality to provide further hydrogen-bonding interactions with suitable guests is prepared; the ability of this novel macrobicycle to bind simple amino acid derivatives is investigated.

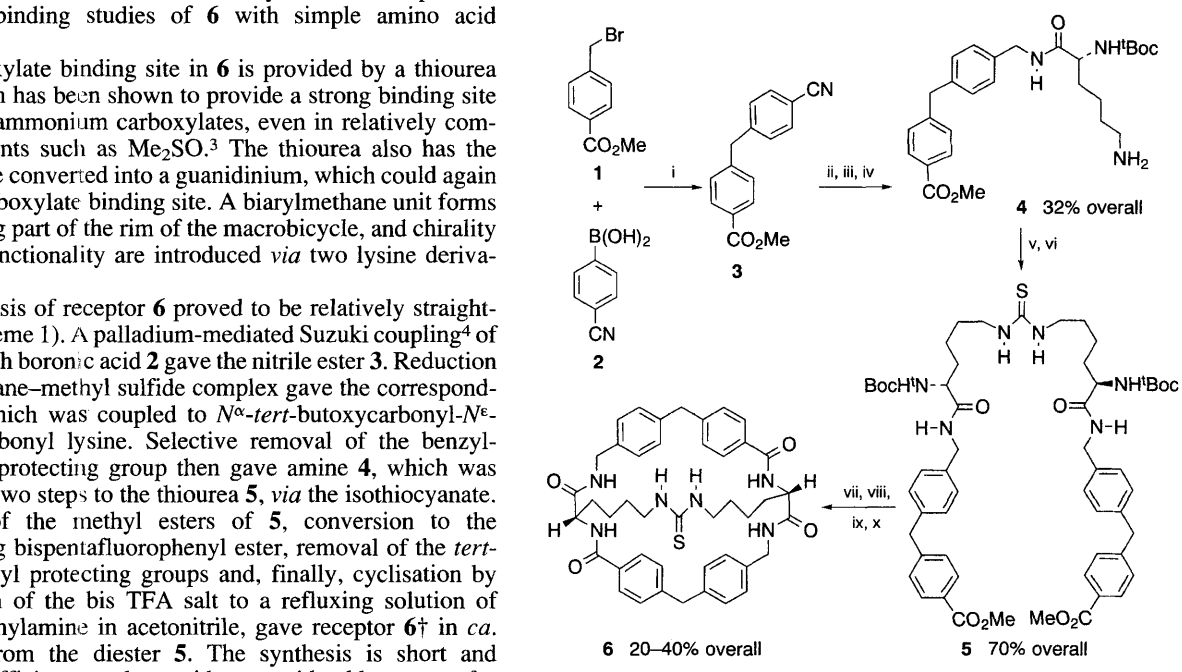


Considerable progress has been made in recent years in the design and synthesis of novel receptors for a range of organic substrates.[1] As part of our own efforts to develop novel receptors,[2] we set out to prepare a range of macrobicycles that feature a specific binding site for carboxylate functionality at the base of the cavity (represented schematically in Fig. 1). By incorporating additional amide functionality around the rim of such a macrobicyclic structure, we hoped to provide further hydrogen-bonding sites, suitably preorganised to interact with guests, such as amino acid derivatives, bound within the macrobicyclic cavity. In this paper, we describe the straightforward synthesis of such a macrobicycle **6**, and report on preliminary binding studies of **6** with simple amino acid derivatives.

The carboxylate binding site in **6** is provided by a thiourea moiety, which has been shown to provide a strong binding site for tetraalkylammonium carboxylates, even in relatively competitive solvents such as $Me_2SO$.[3] The thiourea also has the potential to be converted into a guanidinium, which could again serve as a carboxylate binding site. A biarylmethane unit forms the rigidifying part of the rim of the macrobicycle, and chirality and amide functionality are introduced *via* two lysine derivatives.

The synthesis of receptor **6** proved to be relatively straightforward (Scheme 1). A palladium-mediated Suzuki coupling[4] of bromide **1** with boronic acid **2** gave the nitrile ester **3**. Reduction of **3** with borane–methyl sulfide complex gave the corresponding amine which was coupled to $N^{\alpha}$-*tert*-butoxycarbonyl-$N^{\varepsilon}$-benzyloxycarbonyl lysine. Selective removal of the benzyloxycarbonyl protecting group then gave amine **4**, which was converted in two steps to the thiourea **5**, *via* the isothiocyanate. Hydrolysis of the methyl esters of **5**, conversion to the corresponding bispentafluorophenyl ester, removal of the *tert*-butoxycarbonyl protecting groups and, finally, cyclisation by slow addition of the bis TFA salt to a refluxing solution of diisopropylethylamine in acetonitrile, gave receptor **6**† in *ca.* 30% yield from the diester **5**. The synthesis is short and reasonably efficient, and provides considerable scope for variation of the building blocks, which should allow access to a range of related macrobicycles.

Binding studies with receptor **6** were carried out with various acylated amino acids[5] as the tetrabutylammonium salts (see Table 1). Initially, we tried to determine binding constants by conventional NMR titration experiments, with deuteriochloroform as solvent.[6] This approach was hindered by the fact that the various NH signals we tried to monitor during the titration shifted into the aromatic region of the NMR spectrum.‡ Instead, we were able to determine binding constants by extracting the guests from aqueous solution into a chloroform solution of the receptor, by analogy with the picrate extraction method developed by Cram.[7] Thus, the tetrabutylammonium salts were partitioned between water and chloroform, both in the absence of receptor **6**, and with a known quantity of receptor **6** in the chloroform layer. Aliquots of the aqueous and chloroform layers were taken and the quantities of the tetrabutylammonium salts present in each aliquot were determined by integration of signals for the tetrabutylammonium salts in the $^1H$ NMR relative to a known quantity of dioxane. Partition and extraction




**Scheme 1** *Reagents and conditions*: i, $Pd(PPh_3)_4$, $Na_2CO_3$, DME; ii, $BH_3$·$SMe_2$, THF; iii, $N^{\alpha}$-*tert*-butoxycarbonyl-$N^{\varepsilon}$-benzyloxycarbonyl-L-lysine, 1-hydroxybenzotriazole hydrate, DCC, THF; iv, $H_2$, Pd, C; v, thiophosgene, $K_2CO_3$, $CHCl_3$, reflux; vi, **4**, pyridine, reflux; vii, LiOH, $H_2O$, dioxane; viii, $C_6F_5OH$, 1-hydroxybenzotriazole hydrate, DCC, cat. DMAP, THF; ix, TFA, $CH_2Cl_2$, (1 : 1, *v/v*); x, syringe pump addition of the bis(TFA salt) to a refluxing solution of diisopropylethylamine (3.0 equiv.) in MeCN, 7 mmol $dm^{-3}$ final concentration

**Table 1** Association constants for receptor **6** with various tetrabutylammonium carboxylates in $CDCl_3$

| Substrate (tetrabutylammonium salt) | $K_a$/mol$^{-1}$ |
|---|---|
| *N*-Ac-Glycine | 68 600 |
| *N*-Ac-L-Alanine | 16 900 |
| *N*-Ac-D-Alanine | 14 600 |
| *N*-Ac-L-Phenylalanine | 22 000 |
| *N*-Ac-D-Phenylalanine | 13 300 |
| *N*-Ac-L-Glutamine | 11 100 |
| *N*-Ac-L-Asparagine | 9 600 |
| $N^{\alpha}$-Ac-L-Histidine | 5 800 |
| $N^{\alpha}$-Ac-L-Lysine | 130 000 |

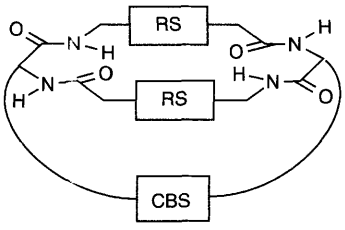



**Fig. 1** CBS = carboxylate binding site
RS = rigid spacer

**Fig. 1** CBS = carboxylate binding site, RS = rigid spacer

coefficients for each guest could thus be determined allowing calculation of association constants following the analysis described by Cram[7] and elsewhere.[8] This analysis assumes 1 : 1 complexation, which was confirmed by a Job plot[8] for the L-phenylalanine derivative, and assumes that none of the receptor **6** is extracted from the chloroform layer into the aqueous layer. None of receptor **6** could be detected in the $^1$H NMR spectra of the aqueous layers. The partition and extraction experiments were each carried out five times to obtain consistent results, and dilution experiments confirmed that neither the receptor **6**, nor any of the guests, were dimerising or aggregating to a significant extent at the concentrations used in the binding experiments.

From the results (Table 1) it is clear that the receptor binds simple acylated amino acids, and binding is dominated by the carboxylate–thiourea interaction. The glycine derivative is strongly bound, but binding is reduced for derivatives of alanine, phenylalanine, asparagine, histidine and glutamine suggesting that the amino acid sidechains are not well tolerated, presumably for steric reasons. The lysine derivative, however, is particularly strongly bound, suggesting a favourable interaction between the free amine residue and the macrobicycle, which overides any steric problems. Inspection of the $^1$H NMR spectra of 1 : 1 mixtures (10 mmol dm$^{-3}$ in $CDCl_3$) of receptor **6** and the various amino acid derivatives, revealed significant shifts of several proton signals with all of the three NH signals moving downfield by > 1 ppm, and in the case of the thiourea NH, by as much as 2.4 ppm. While there is no significant binding selectivity for the two alanine or phenylalanine enantiomers, the formation of diastereomeric complexes with these substrates is evidenced by significant differences in the $^1$H NMR of 1 : 1 complexes of receptor **6** and substrate, depending on the amino acid enantiomer being complexed. For the 1 : 1 complex of **6** with L-alanine, for example, the alanine methyl sidechain gives a signal at δ −0.01 (1.43 unbound), the signal for the alanine α proton appears at δ 1.91 (4.44 unbound) and that for the alanine NH at δ 5.15 (δ 7.10 unbound). Such significant upfield shifts, relative to those of the unbound substrate, clearly indicate that the L-alanine substrate is bound within the cavity as anticipated, with the α proton and methyl sidechain considerably shielded by the aromatic side walls of the receptor. For the 1 : 1 complex of **6** with D-alanine the alanine methyl sidechain gives a signal at δ 1.12 the signal for the alanine α proton appears at δ 4.21 and that for the alanine NH at δ 6.40. Clearly the bound D-alanine substrate is in a substantially different environment compared to the bound L-alanine substrate. Similarly, the 1 : 1 complex of **6** with the L-phenylalanine substrate showed dramatic upfield shifts of the signals for the benzylic, α and NH protons, as well as for the acetoxy methyl protons, of the L-phenylalanine substrate, while the D-phenylalanine substrate did not. In addition, intermolecular NOEs, from the α and benzylic protons of the L-phenylalanine substrate to the aromatic protons of the biarylmethane unit of the macrocycle, place this substrate within the cavity of the macrobicyclic receptor. While the clearly different modes of binding of L and D substrates do not result in significantly enantioselective binding, it suggests that a fuller understanding of the interactions involved in the diastereosomeric complex formation should allow the rational design of an enantioselective receptor, by incorporating features into the receptor which promote or block one of the binding modes.

In conclusion, we have developed a straightforward synthesis of a macrobicyclic receptor, which should allow for considerable structural variation in the future. Preliminary binding results indicate that the receptor can bind carboxylate salts as anticipated, with moderate selectivity for some amino acid derivatives.

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## Footnotes

† *Selected data* for **6**: $\delta_H$ ($CDCl_3$, 360 MHz) 7.60 (4H, d, *J* 8 Hz, ArH), 7.11 (4H, d, *J* 8 Hz, ArH), 7.05 (8H, d, *J* 8 Hz, ArH), 6.91 (2H, bs, NH), 6.56 (2H, bs, NHCS), 6.43 (2H, d, *J* 7 Hz, NH), 4.77 (2H, dd, *J* 8 and 14 Hz), 4.48 (2H, d, *J* 6 and 14 Hz), 4.0–3.8 (8H, m), 3.05 (2H, m), 2.26 (2H, m), 1.80 (2H, m), 1.37 (4H, m), 1.05–0.95 (4H, m); $\delta_C$ ($CDCl_3$) 183.0 (0), 171.6 (0), 168.5 (0), 146.3 (0), 140.1 (0), 136.7 (0), 131.7 (0), 129.6 (1), 129.2 (1), 128.9 (1), 127.8 (1), 54.4 (1), 43.6 (2), 42.9 (2) 41.6 (2), 30.5 (2), 30.1 (2), 20.8 (2); *m/z* (FAB) (M + H)$^+$ 745 (Found: M$^+$ 744.3477. $C_{43}H_{48}N_6O_4S$ requires 744.34578).

‡ It was possible to monitor various CH signals during the titration, but chemical shift changes (Δδ *ca.* 0.2 ppm) were smaller than for the NH signals, and resolution of the multiplets was troublesome. Resolution problems also meant that the lower limit for the concentration of the host in the NMR experiments was of the order of $5 \times 10^{-3}$ mol dm$^{-3}$. Because of this titration curves approached the 'infinite' limit with Weber's *p* value,[6,9] assuming $K_a$ values of $> 10^4$ mol$^{-1}$ (see Table 1), always being > 0.8, and no useful data could be extracted from these NMR experiments.

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