Enantioselective complexation of excitatory amino acid derivatives by chiral, cage-like C_3-symmetrical receptors

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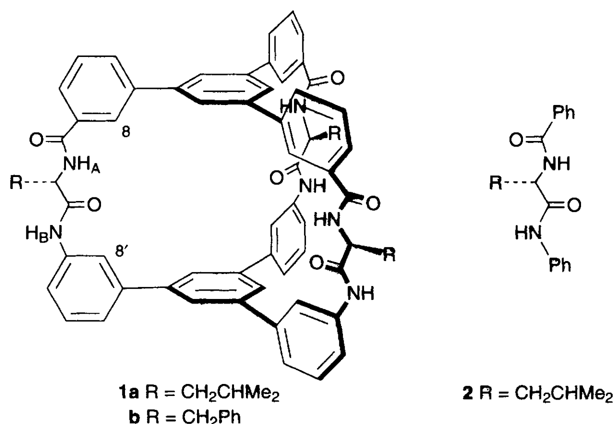
Chiral, cage-like C_3-symmetrical receptors are prepared in a short, modular synthesis and found to be able to enantioselectivly recognise *N*-Z-Glu in both titration and solubilisation studies.

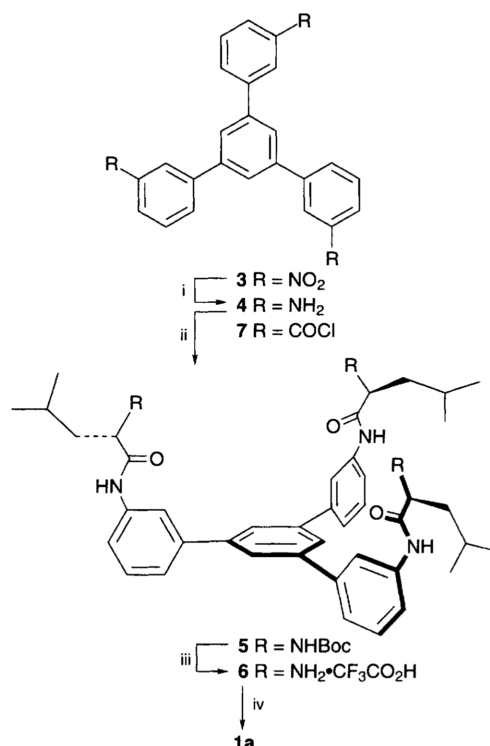
Nature has evolved selective binding sites which are characterised by a large degree of encapsulation of the targeted substrate. We have taken this lesson to heart in the design of cage-like receptors[1] which are internally functionalised with H-bonding sites.[2] Receptors of this kind should only select guests complementary with respect to both H-bonding as well as size and shape.[3] Here we report on the synthesis of receptors **1a** and **1b** and show their selective binding properties of amino acid derivatives using H-bonding in aprotic solvents.[4]

The target compounds **1a** and **1b** contain 1,3,5-triarylbenzene units as both 'floor' and 'ceiling' of a cavity. These units are linked by three amino acid spacers through peptide bonds, which are the potential H-bonding sites. The amino acid spacers also introduce chirality which is translated through an overall twist to the molecule's interior, creating the potential for enantioselective recognition.

Receptor **1a** was synthesised *via* a short route and in a modular fashion from trinitro derivative **3**[5] and acyl halide **7**,[6] (Scheme 1). Receptor **1b** and reference compound **2** were synthesised following similar protocols. Molecular modelling of **1a** suggests that the molecule has an open, non-collapsed cavity.† ^{1}H NMR spectroscopic data are consistent with this picture.

We found that N-protected amino acids are bound in $CDCl_3$ by **1a**, judging from a strong downfield shift of the NH_A resonance of the receptor in the ^{1}H NMR spectrum, as well as from both up- and down-field shifts of its aromatic resonances.‡ A series of ^{1}H NMR titrations at constant host concentration showed only small differences in binding free energy between the various monoacids studied (Table 1, entries 2–5), and a similar association mode for them seems likely.§ The large downfield shift of the NH_A resonance suggests as the major binding mode a bidentate H-bonding of one spacer arm of **1a** to





Scheme 1 *Reagents and conditions*: i, H_2, Pd–C, DMF, 14 h, 94%; ii, *N*-(*tert*-butoxycarbonyl)-L-leucine monohydrate, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, cat. 4-(dimethylamino)pyridine, THF, 14 h, 60%; iii, CF_3CO_2H–CH_2Cl_2 1 : 1 (*v*/*v*), 1 h, 100%; iv, **7**, NEt_3, THF, 14 h, 10%

Table 1 Binding free energies§ (kcal mol^{-1}, 1 cal = 4.184 J) and, in brackets, calculated changes in chemical shift of host proton NH_A at saturation binding, determined by ^{1}H NMR titrations at 300 K^a

Entry	Host	Guest	$-\Delta G$ ($\Delta\delta_{sat}$) $CDCl_3$	$-\Delta G$ ($\Delta\delta_{sat}$) $CDCl_2CDCl_2$
1	**2**	*N*-Boc-Gly	2.1 (1.1)	
2	**1a**	*N*-Boc-Gly	2.9 (0.8)	
3	**1a**	*N*-Boc-L-Ser	2.9 (0.8)	
4	**1a**	*N*-Boc-L-Phe	2.6 (0.8)	
5	**1a**	*N*-Boc-L-Ala-L-Ala	2.8 (0.8)	
6	**1a**	*N*-Z-L-Asp	3.5 (1.1)	3.1 (1.3)
7	**1a**	Glutaric acid	3.2 (1.9)	2.7 (2.1)
8	**1a**	*N*-Z-L-Glu		3.5 (2.4)
9	**1a**	*N*-Z-D-Glu		2.5 (2.0)^b
10	**1a**	*N*-BuOCO-L-Glu		3.3 (2.5)
11	**1a**	*N*-BuOCO-D-Glu		2.7 (2.1)^c
12	**1b**	*N*-Z-L-Glu		3.2 (2.5)
13	**1b**	*N*-Z-D-Glu		2.1 (2.4)^d

^{a} [Host] = 5 mM (in entry 1) or 0.5–1.0 mM (in entries 2–13); [guest] = 0.5–50 mM; ^{b} $\Delta(\Delta G)$ enantioselectivity (kcal mol^{-1}), = 1.0. ^{c} 0.6. ^{d} 1.1.

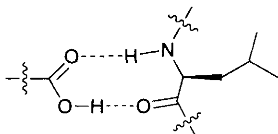


Fig. 1 Major interaction in the binding of amino acid derivatives to **1a** and **1b**

the CO_2H group of the substrate (Fig. 1), possibly complemented by a minor contribution from the carbamate group associating to a second spacer arm.

Consistent with the proposed binding mode, α,ω-dicarboxylic acids proved to be higher affinity guests. The excitatory amino acid derivative *N*-Z-L-Asp was complexed by **1a** in $CDCl_3$ with a binding free energy of 3.5 kcal mol^{-1} (Table 1, entry 6). From a 'reverse' titration experiment at varying concentration of **1a**, upfield shifts at saturation binding between 0.5 and 0.7 ppm were calculated for the α- and β-H-atoms of *N*-Z-L-Asp. These shifts support the notion that complexation takes place inside the cavity, especially since no such shifts were observed with 2,2-diphenylsuccinic acid, which is too large to fit inside the binding site.

Examining the interaction of **1a** with both enantiomers of *N*-Z-Glu in $CDCl_3$ showed that the ^1H NMR resonances of the L-enantiomer were dramatically broadened, whereas no such broadening was seen with the D-enantiomer. This is indicative of significant enantioselectivity in complexation, which due to the slow exchange could not be quantified. This problem was overcome by changing the solvent to $CDCl_2CDCl_2$. In this solvent, association energies were somewhat lower[7]§ (entries 6 and 7, Table 1) but ^1H NMR signals remained sharp enough throughout the titration for evaluation of the binding strength.‡ The enantioselectivity, *i.e.* the difference in stability between the two diastereoisomeric complexes formed between **1a** and the *N*-Z-Glu enantiomers, was $\Delta(\Delta G)$ = 1.0 kcal mol^{-1} (entries 8 and 9). Receptor **1b** showed similar binding behaviour to **1a**, although the measured association free energies were somewhat lower (entries 12 and 13). Replacing the Z by the smaller butoxycarbonyl group led to a reduced enantioselectivity (entries 10 and 11). The possible role of interactions between the carbamate moeity and the receptor was confirmed by a $^1H\{^1H\}$-ROESY spectrum of the complex between **1a** and *N*-Z-L-Glu, in which cross peaks between the Z CH_2 protons of the guest and the $(Me)_2CHCH_2$ protons of the receptor were observed.

The enantioselective recognition of *N*-Z-Glu by **1a** was also apparent from solubilisation studies in $CDCl_3$–CCl_4 1 : 3, where both receptor **1a** and the two substrate enantiomers are nearly insoluble. When a mixture of solid **1a** and an excess of solid *N*-Z-L-Glu was briefly sonicated, a 1 : 1.1 (±0.1) host–guest complex was solubilised, whereas the same experiment with *N*-Z-D-Glu yielded hardly any detectable solubilisation of either host or guest. Solubilisation was also observed when an excess of racemic *N*-Z-Glu was employed.¶ Generally at least 80% of the receptor (1–4 mg ml^{-1} solvent) was solubilised as determined by a standard. The enantiomer ratio of the solubilised *N*-Z-Glu, as determined by gas chromatography on a chiral column after derivatisation,[8] was found to be ⩾5 : 1 in favour of the L-enantiomer, consistent with the titration results.

With their cage-like architecture, compounds **1a** and **1b** are selective receptors at moderate affinity. The combination of these two features makes them attractive starting points for the development of chiral stationary phases for chromatography with high separation factors.[9]

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Footnotes

† Monte Carlo conformational searches within MARCOMODEL ver. 5.0 (> 5000 steps) using the AMBER* force field and the GB/SA solvation model for $CHCl_3$ [for details, see ref. 4(*d*)], found only low-energy conformations with open cavities for **1a**. A few of them contained an intramolecular H-bond (7-membered ring) within one or more spacer arms. The ^1H NMR chemical shifts of the aromatic and amide protons of **1a** and **2** (*ca.* 1 mM, $CDCl_3$) are very similar, indicating no collapse of the cavity and no significant degree of intramolecular H-bonding.

‡ Dilution studies with both host and guests established that under titration conditions the host is monomeric and that the ^1H NMR spectrum of the guest is basically constant [$\Delta\delta$(NH) = 0.06 ppm between 1 and 21 mM]. Complexation induced shifts (ppm, + = downfield) for **1a** and *N*-Z-L-Glu at 80% saturation (Table 1, entry 8): +1.94 (NH_A), +0.59 (NH_B), −0.34 [H-C(8)] and +0.45 [H-C(8′)]. For comparison, **2** and *N*-Boc-Gly at 60% saturation binding (Table 1, entry 1): +0.71 (NH_A), +0.44 (NH_B), < 0.04 ppm (aromatic protons of **2**).

§ Apparent binding free energies obtained from fitting the titration data to a 1 : 1 model in the programme ASSOCIATE (ref. 10), based on the evaluation of several aromatic protons of the host and NH_A. Estimated error in ΔG: ±0.15 kcal mol^{-1}. The non-linear least-squares curve fitting of the experimental data as well as corresponding Job plots were not always fully in support of exclusive 1 : 1 host–guest complexation, due to additional weak external association. H-bonding association strength has been shown to both decrease [ref. 7(*a*)] and increase [ref. 7(*b*)] when changing from $CDCl_3$ to $CDCl_2CDCl_2$.

¶ A 1 : 1 mixture of pure crystalline enantiomers (an artificial conglomerate) (ref. 11), rather than crystals of racemic (±)-*N*-Z-Glu, prepared from (±)-glutamic acid, was used in the solubilisation studies. (±)-*N*-Z-L-Glu is a racemic compound (ref. 11) with a melting point 7–8 °C higher than that of the individual crystalline enantiomers and is much less efficiently solubilised.

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