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Intramolecular conformational control in a cyclic peptide composed of alternating L-proline and substituted 3-aminobenzoic acid subunits†

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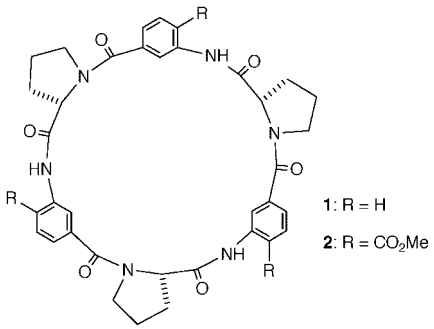
Methoxycarbonyl groups in the 4-position of the aromatic subunits strongly influence the conformational behaviour and the receptor properties of cyclic peptides composed of alternating 3-aminobenzoic acid and L-proline

We recently showed that cyclic hexapeptides composed of alternating natural amino acids and 3-aminobenzoic acid subunits bind cations and anions with high affinity.[1,2] Cation–π interactions with the aromatic subunits of such cyclopeptides generally result in inclusion of cations into the shallow dish-shaped receptor cavity.[3] By contrast, anions are only bound when they are able to form hydrogen bonds to the peptide amide groups. This latter interaction induces a receptor conformation in which all NH groups of the peptide point towards the cavity centre.[4] In the absence of suitable anions, the amide groups are able to rotate more freely. We speculated that suitable substituents on the peptide subunits would also restrict amide rotation. Such an approach may have the additional advantage that the anion complexation could be completely prevented by locking the NH groups in an orientation unsuitable for interactions with these guests. Here we report our first results in this direction.

Inspired by the work of Hamilton and coworkers who have shown that methoxycarbonyl groups can be used for the conformational control in oligoanthranilamides,[5] we introduced these substituents at the 4-position of the aromatic subunits of **1**.



Methoxycarbonyl groups are able to form hydrogen bonds to adjacent NH protons and thus induce an amide orientation parallel to the aromatic rings. They also cause the NH protons to point away from the cavity centre and make the amides less available for anion complexation.

Peptide **2** was synthesised from the commercially available 2-aminoterephthalic acid 1-methyl ester by following a procedure similar to that used for **1**.[1] Whereas **1** possesses a simple ^{1}H NMR spectrum that represents an averaged C_3-symmetrical structure, the spectrum of **2** is more complicated [Fig. 1(a) and (c)]. Identical protons of **2** give two signals in most cases, and even the methyl ester signal is split. Since the spectrum is not significantly affected by varying the concentration of **2** in the region 2–0.2 mM, an intermolecular association of the peptide at these concentrations is unlikely. Instead, the spectrum more probably represents a non-symmetrical conformation of **2** or different slowly interconverting peptide conformers. Temperature-dependent ^{1}H NMR spectroscopy of **2** in $C_2D_2Cl_4$ shows that at 120 °C the flexibility of the peptide is still somewhat restricted. The rigidity of **2** is certainly caused by the effects of the additional methoxycarbonyl substituents. The unusually large downfield shift of the NH protons in the ^{1}H NMR spectrum of **2** in $CDCl_3$ at δ 11.1 [for **1** δ(NH) 9.2] and the strong N–H vibration band at 3303 cm^{-1} in the FTIR spectrum in $CDCl_3$ indicate that these groups are involved in hydrogen bonds. The crystal structure of **2** monohydrate (Fig. 2) shows that, as predicted, these hydrogen bonds are formed between the amide NH groups and the neighbouring methoxycarbonyl groups.‡ The overall peptide conformation in this structure is non-symmetrical, presumably caused by the water molecule, with one aromatic subunit tilted away from the others. As a result, one would expect a weak cation affinity of **2**.

Nevertheless, a significant upfield shift of the guest protons is observed upon addition of quaternary ammonium salts such as *n*-butyltrimethylammonium picrate (BTMA$^+$ picrate) to solutions of **2** in $CDCl_3$. This shift is generally interpreted in terms of an inclusion of the cation into a receptor cavity which brings the guest protons in close proximity to the aromatic subunits.[3] The spectrum of the peptide is also affected in the presence of the cation. On increasing the guest concentration, the spectrum becomes simpler until, after addition of 4 equivalents of BTMA$^+$ picrate to a 2 mM solution of **2** in $CDCl_3$, it represents a symmetrical conformation [Fig. 1(b)]. Complex formation

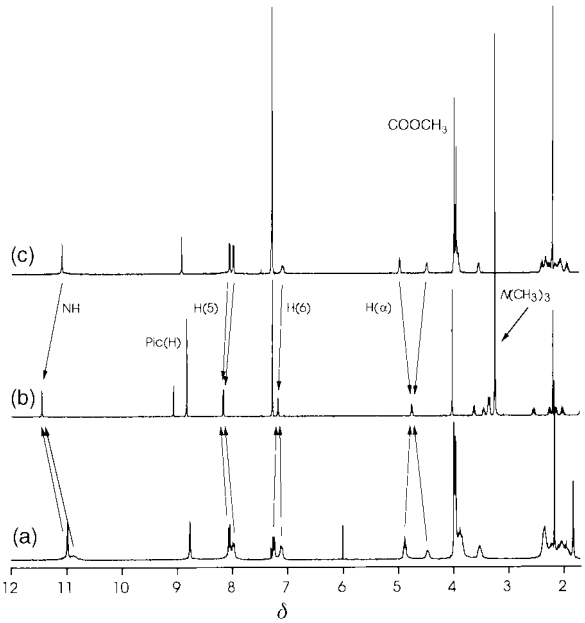


Fig. 1 ^{1}H NMR spectra of **2** (a) in $C_2D_2Cl_4$ (60 °C), (c) in $CDCl_3$ (25 °C) and (b) after addition of 4 equiv. of BTMA$^+$ picrate in $CDCl_3$ (25 °C).

† Electronic supplementary information (ESI) available: synthesis, IR and NMR data for **2**. See http://www.rsc.org/suppdata/cc/b0/b000568l/

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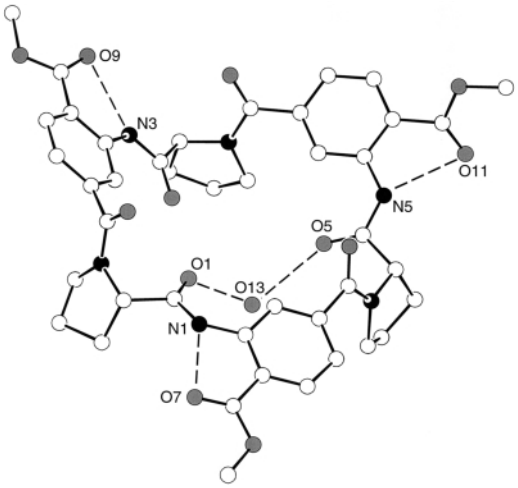

Fig. 2 Molecular structure of **2**·H_2O. Selected interatomic distances (Å): N1··O7 2.677(2), N3··O9 2.656(2), N5··O11 2.903(3).

obviously causes a shift of the conformational equilibrium of **2**. The NOESY NMR spectrum shows strong NOE effects between NH and H(α), which confirms that the NH groups are oriented towards the methoxycarbonyl substituents. As yet, we have not been able to obtain crystals of the **2**·BTMA$^+$ complex. However, **2** crystallises from acetone with one solvent molecule per peptide unit. X-Ray crystallography reveals that the solvent molecule is located inside the peptide cavity (Fig. 3). Moreover, the peptide conformation in this structure is more symmetrical than in **2**·H_2O. The three aromatic subunits are all tilted into the same direction with all hydrogen bonds between NH and the methoxycarbonyl substituents retained. These results indicate that suitable guest molecules can induce a symmetrical peptide conformation well suited for guest binding when they are included into the cavity of **2**. The NMR spectroscopic results demonstrate that certain cations induce a similar conformation in solution. This mechanism of complex formation is therefore consistent with an 'induced-fit'.

The upfield shift of the BTMA$^+$ protons in the presence of **2** can be used to quantitatively determine the complex stability by NMR titrations.[6] When the shifts of the cation protons of BTMA$^+$ picrate were followed in the titration, a stability constant K_a was obtained that is almost an order of magnitude larger than that of the corresponding complex of **1** (Table 1). This significant increase of cation complex stability can be attributed to the conformational rigidity of **2**.

Whereas a dramatic increase of the cation complex stability was observed for **1** with iodide or tosylate anions,[1] the BTMA$^+$

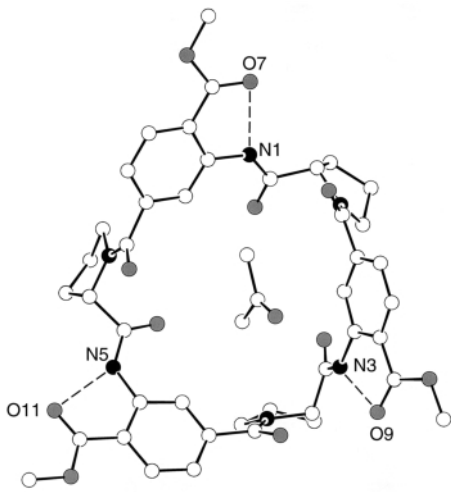

Fig. 3 Molecular structure of **2**·Me_2CO projected onto a plane through the three amide N atoms of **2** (tilt angles of the aromatic rings to this plane (°): at N1, +35; at N3, +57; at N5, +31). Selected interatomic distances (Å): N1··O7 2.668(3), N3··O9 2.692(3), N5··O11 2.678(2).

Table 1 BTMA$^+$ complex stabilities in $CDCl_3$ at 298 K (K_a = stability constant in M^{-1}, error limits of K_a < 20%; $\Delta\delta_{max}$ = maximum chemical shift in ppm; ΔG_H = Gibbs free energy of hydration of the anions in kJ mol^{-1})

	1		**2**		
Anion	K_a	$-\Delta\delta_{max}$	K_a	$-\Delta\delta_{max}$	$-\Delta G_H$
Picrate	1 260	0.70	10 800	0.54	197
Iodide	21 100	1.11	3 310	0.59	283
Tosylate	5 050 000	1.16	740	0.54	318

complex stabilities of **2** decrease when going from picrate to iodide and tosylate. A similar anion effect has also been reported for cation complexes of calixarenes.[7] Bartsch and coworkers have shown that the extraction efficiency of certain crown ether salt complexes correlates inversely with the hydration enthalpy of the anion.[8] In accordance with these findings, the stabilities of the BTMA$^+$ complexes of **2** decreases with increasing Gibbs free energy of hydration of the anion.[9] The dependence of the complex stabilities on the type of anion can therefore be attributed to an intrinsic property of the salts and not to possible peptide–anion interactions. Indeed, the FTIR spectrum of **2** is unaffected by the different anions, not even those that bind very strongly to **1**.

In summary, we have shown that the NH groups of **2** can be locked in a defined orientation by hydrogen bonds to methoxycarbonyl groups on the aromatic subunits. This results in a reduction of the conformational freedom of the cyclopeptide and in improved cation affinity as well as a complete loss of anion binding ability. Currently, we are investigating effects of other substituents. The fact that the conformation and hence the binding properties of these peptides can be influenced by non-covalent intramolecular interactions give them important advantages over many other artificial receptors.

S. K. thanks Professor G. Wulff, to whom this paper is dedicated on the occasion of his 65th birthday, for his generous support and Mrs D. Kubik for the preparative work.

Notes and references

‡ *Crystal data*: **2**·H_2O: $C_{42}H_{42}N_6O_{12}$·H_2O, M_r = 840.83, colourless prism, crystal size 0.44 × 0.54 × 0.58 mm, a = 13.2214(6), b = 17.0958(8), c = 18.0413(8) Å, U = 4077.9(3) Å^3, T = 100 K, orthorhombic, space group $P2_12_12_1$ (no. 19), Z = 4, D_c = 1.37 g cm^{-3}, μ = 0.10 mm^{-1}. Siemens SMART diffractometer, λ = 0.71073 Å. 44640 measured reflections, 15293 unique, 8260 with $I > 2.0\sigma(F_o^2)$. The structure was solved by direct methods and refined by full-matrix least squares on F^2 for all data with Chebyshev weights to R = 0.0595 [$I > 2\sigma(F_o^2)$], wR = 0.144 (all data), 553 parameters.

2·Me_2CO: $C_{42}H_{42}N_6O_{12}$·C_3H_6O, M_r = 880.89, colourless prism, crystal size 0.17 × 0.28 ×0.64 mm, a = 10.5607(6), b = 17.6047(10), c = 22.7991(13) Å, U = 4238.8(4) Å^3, T = 100 K, orthorhombic, space group $P2_12_12_1$ (no. 19), Z = 4, D_c = 1.38 g cm^{-3}, μ = 0.10 mm^{-1}. Siemens SMART diffractometer, λ = 0.71073 Å. 48668 measured reflections, 16532 unique, 9124 with $I > 2.0\sigma(F_o^2)$. Structure solution and refinement as above, R = 0.073 [$I > 2\sigma(F_o^2)$], wR = 0.172 (all data), 582 parameters.

CCDC 182/1564. See http://www.rsc.org/suppdata/cc/b0/b000568l/ for crystallographic files in .cif format.

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