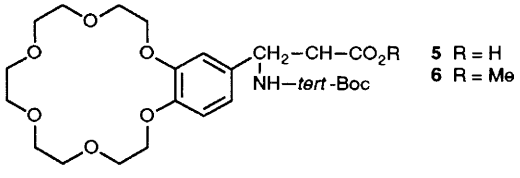
Geometrical Recognition Ability of Flexible Bis-crown Ether Peptidic Receptors

Normand Voyer,* Denis Deschênes, Julie Bernier, and Johanne Roby

Département de chimie, Université de Sherbrooke, Québec J1K 2R1, Canada

Three flexible peptidic receptors bearing two crown ethers at different positions are demonstrated to have a geometrical recognition ability towards longer substrates of a series of primary diammonium aliphatic guests.

Conformational modulation of peptidic molecules is an important regulating mechanism used by living systems.[1] For example, the binding and transport of ions by proteins are processes involved in numerous biological functions that are regulated by specific induced conformational changes.[1,2] However, the recognition steps involved in those phenomena are not well understood at the molecular level. To gain a better comprehension of the natural recognition and transport processes, we have adopted a biomimetic approach. In that regard, ligand modified amino acids could be valuable tools for the preparation of peptide-based biomimetic systems. For instance, in one aspect, we have demonstrated that a hexa-crown ether peptide properly designed forms a tubular artificial channel by stacking the crown side chains on top of each other.[3] That type of molecule possesses the basic requirements to mimic the transport properties of the natural ion channel proteins.[4,5] On the other hand, we have showed that octapeptides bearing two 18-crown-6 side chains complexed strongly and selectively Cs^+ ions by forming cooperative 2 : 1 'sandwich' complexes with the two ligand side chains organized in a complementary fashion to the guest shape.[6] Thus, it is conceivable that the backbone conformation of such bis-ligand modified peptides could then be modulated by the binding of difunctional substrates of different geometry as illustrated in Fig. 1. With this objective in mind, we now report the geometrical recognition ability of the bis-crown ether peptides **1–3** towards primary aliphatic diammonium substrates **7a–h**.



5 R = H
6 R = Me

N-tert-Boc-Ala-Ala-Ala-CE-Ala-CE-Ala-CONH-n-Pr

1

N-tert-Boc-Ala-Ala-CE-Ala-Ala-CE-Ala-CONH-n-Pr

2

N-tert-Boc-Ala-CE-Ala-Ala-Ala-CE-Ala-CONH-n-Pr

3

3,4-(18-Crown-6)-L-phenylalanine

CE

N-tert-Boc-Ala-Ala-Phe-Ala-Ala-Phe-Ala-CONH-n-Pr

4

H_3N^+-$(CH_2)_n$-NH_3^+·2 picrate$^-$

7a–h (n = 2–9)

Heptapeptides **1–3** are composed only of five L-alanines and two 18-crown-6 derivatives of L-phenylalanine. The choice of alanine is justified by the fact that it can be accommodated easily in any peptidic conformations due to its small side-chain methyl group. On the other hand, the crown ether amino acid **5** necessary for the synthesis can be prepared readily in four steps and 27% overall yield by taking advantage of the built-in catechol function of commercially available L-DOPA.[3,6] In peptides **1–3**, the crown ether residues are separated systematically by one, two and three alanines with one crown amino acid fixed at position 6 (from the *N*-terminal end). This was done to verify the effect of the relative positions of the ligand amino acids on the geometrical recognition ability. Indeed, in some cases, the conformation necessary to bring the crown ether side chains complementary to the guest's geometry may be more or less favourable depending on the distance between the crown ether modified residues. This phenomena should translate into a weaker or stronger binding ability towards diammonium aliphatic guests of specific geometries.

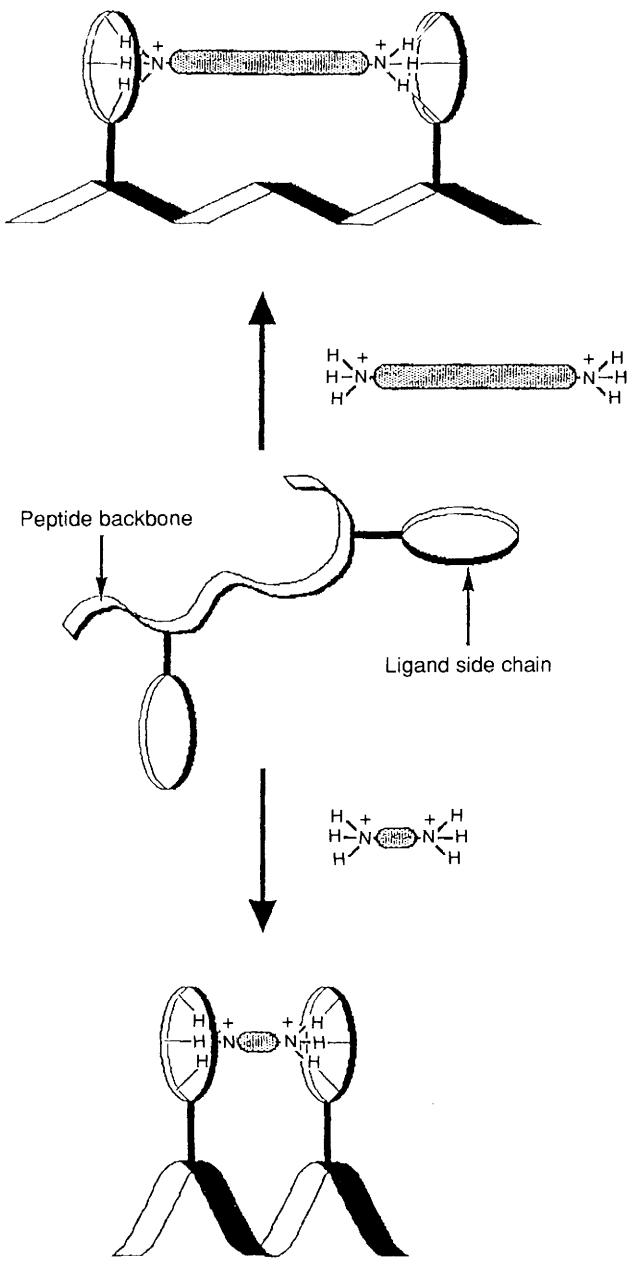


Fig. 1 Present working hypothesis: the cooperative complexation of difunctional guests by a flexible bis-crown ether peptide induces a specific conformation that organizes the ligand side chains in a complementary fashion to the substrate geometry as illustrated here with an α-helix and β-sheet conformations

The model peptides **1–3** were prepared by solid-phase peptide synthesis using the *p*-nitrophenyl oxime resin[7] as solid support. Amino acids protected with an α*N-tert*-(Boc = butoxycarbonyl) group were used and coupled for 1 h with three equivalents of their hydroxybenzotriazole activated ester.[8] The coupling steps were monitored for completion by the ninhydrin test[9] and doubly performed when necessary. The peptides were cleaved from the resin by a 10 min treatment with a 0.5 mol dm^{-3} n-propylamine solution in $CHCl_3$. The crude bis-crown ether peptides **1–3** were purified by crystallization from a $CHCl_3$–MeOH mixture followed by reverse-phase HPLC and obtained in 41, 54 and 38% yields, respectively.†

The recognition ability of the peptidic receptors **1–3** as well as the monomeric crown ether analogue **6** towards primary alkyl diammonium substrates was investigated by the picrate extraction method modified specifically for this type of difunctional guest.[10] The results are reported in Table 1 and Fig. 2 and were calculated assuming the formation of 1 : 1 complexes.‡ Compared to the monomeric crown ether analogue **6**, all peptides demonstrated a high binding ability towards diammonium cation substrates, especially with the longer ones **7e–7h**. The K_a values obtained range between 10^8 and 10^{10} dm^3 mol^{-1} for the receptors **1–3** while they are about constant, as expected, at 10^7 dm^3 mol^{-1} for the crown analogue **6**. Also, no binding could be detected in a control experiment using peptide **4**, an analogue of receptor **2** lacking the two crown ether rings. Thus, the high values of association constants observed for the flexible receptors **1–3** can be attributed to the cooperative action of the two crown ether side chains to bind the difunctional substrates in an intramolecular complementary fashion. Among the three peptidic receptors, **2**, having the ligand modified residues separated by

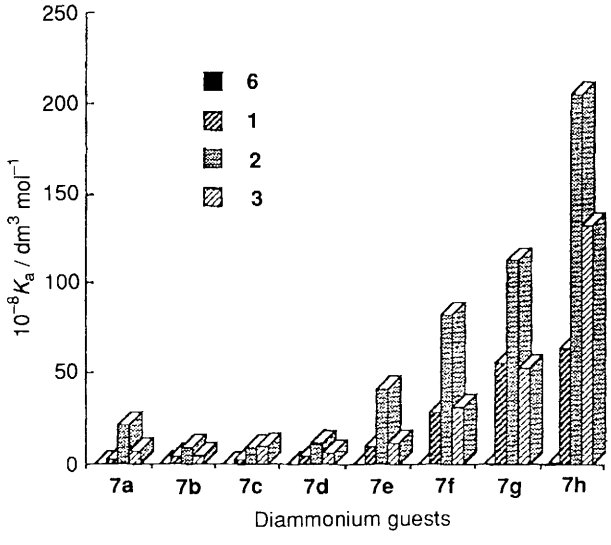


Fig. 2 Comparative binding ability of peptidic receptors **1–3** and crown analogue **6** towards the primary diammonium substrates **7a–h**

† Peptides **1–3** were characterized by ^1H NMR and FAB mass spectrometry. Details of the experimental procedures will be published elsewhere.

‡ In a typical experiment, 250 μl of a guest dipicrate aqueous solution (5×10^{-4} mol dm^{-3}) and 250 μl of a $CHCl_3$ solution of the host (1.75×10^{-4} mol dm^{-3} for **1–3** and 3.5×10^{-4} mol dm^{-3} for **6**) were mixed in a tube, stoppered, centrifuged 10 s, and shaken vigourously for 20 min. After 10 min of centrifugation, 75 μl of the organic layer was withdrawn, diluted to 1 ml with MeCN, and the picrate absorbance was recorded and used in the calculations.

Table 1 Association constants and free energy of complexation of primary diammonium alkyl substrates **7a–h** by the bis-crown ether peptides **1–3** and the monomeric crown ether analogue **6**[a]

Substrate $H_3N^+(CH_2)_nNH_3^+$		$K_a \times 10^{-8}$ dm^3 mol^{-1} ($-\Delta G^0$ in kcal mol^{-1})			
		6	**1**	**2**	**3**
7a	$n = 2$	0.32 (10.2)	2.56 (11.5)	21.8 (12.7)	6.75 (12.0)
7b	$n = 3$	0.32 (10.2)	4.42 (11.8)	9.00 (12.2)	4.79 (11.8)
7c	$n = 4$	0.23 (10.0)	2.88 (11.5)	9.22 (12.2)	9.58 (12.2)
7d	$n = 5$	0.34 (10.3)	4.44 (11.8)	11.5 (12.4)	6.42 (12.0)
7e	$n = 6$	0.08 (9.4)	10.3 (12.3)	41.6 (13.1)	11.6 (12.4)
7f	$n = 7$	0.20 (10.0)	28.5 (12.8)	82.1 (13.5)	31.8 (13.0)
7g	$n = 8$	0.25 (10.1)	55.7 (13.3)	113.0 (13.7)	52.5 (13.2)
7h	$n = 9$	0.64 (10.6)	63.8 (13.4)	205.0 (14.0)	133.0 (13.8)

[a] At 25 °C in $CHCl_3$ saturated with H_2O. Values are calculated assuming the formation of 1 : 1 complexes using the method given in ref. 10. Values reported are the averages of 10 independent runs reproducible within ±5%.

two alanines, showed the best binding ability. In addition, as can be seen from Fig. 2, there are important differences observed in the binding ability of peptides **1–3** with primary diammonium guests. It is possible that these differences result from the favourable or the unfavourable conformational changes associated with the molecular recognition processes. Furthermore, the different binding ability noted demonstrates that the peptidic chain of receptors **1–3** plays a functional role and does not act like a passive linker between the two ligands. The fact that the longest and the most flexible substrates are bound more tightly than the shorter ones might be explained in terms of their greater adaptability. A similar binding tendency has been reported previously with the same guests by rigid receptors.[10] However, even though the longer substrates are bound more tightly, it is noteworthy that peptidic receptor **2** exhibits some size selectivity towards the shortest guest, **7a**, over the ones having three, four and five methylene units, **7b–7d**. Again, this observation can be explained by the fact that the backbone of peptide **2** adopts a more favourable conformation to bind **7a** than for the slightly longer difunctional substrates **7b, c** and **d**.

While several more rigid bis-azacrown ethers have been reported to accomplish shape selectivity for primary alkyl diammonium cations,[11] the complexation results reported herein constitute one of the first examples,[12] to our knowledge, of *flexible* molecular receptors possessing high binding affinity and shape selectivity with difunctional substrates of different geometry. Work is currently underway to assess the binding ability of peptidic receptors **1–3** towards rigid difunctional guests as well as to study the conformational changes associated with the binding processes described by NMR and circular dichroism spectropolarimetry.

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