Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

Design, synthesis, and supramolecular properties of bis-crown ether modified peptides

Normand Voyer[a]; Johanne Roby[a]; Denis Deschênes[a]; Julie Bernier[a]
[a] Département de chimie, Université de Sherbrooke, Sherbrooke, Québec, Canada

To cite this Article Voyer, Normand , Roby, Johanne , Deschênes, Denis and Bernier, Julie(1995) 'Design, synthesis, and supramolecular properties of bis-crown ether modified peptides', Supramolecular Chemistry, 5: 1, 61 — 69
To link to this Article: DOI: 10.1080/10610279508029888
URL: http://dx.doi.org/10.1080/10610279508029888

SUPRAMOLECULAR CHEMISTRY, Vol. 5, pp. 61–69
Reprints available directly from the publisher
Photocopying permitted by license only

Design, synthesis, and supramolecular properties of bis-crown ether modified peptides†

NORMAND VOYER*, JOHANNE ROBY, DENIS DESCHÊNES and JULIE BERNIER

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

†*Dedicated to Professor Donald J. Cram on the occasion of his 75th birthday.*

(Received June 20, 1994)

The synthesis of peptides bearing two benzo-18-crown-6 side chains at different positions is reported. These peptides were designed to undergo specific ligand induced conformational changes using the cooperative complexation of difunctional guests in between the two crown moieties. Binding studies show that the peptides indeed complex selectively Cs^+ and linear α,ω-diammonium alkanes using the simultaneous action of the two distant crown ethers. The complexation of Cs^+ resulted in a specific conformational change in the backbone of peptide 2, the best Cs^+ binder, that switches from a β-sheet to a β-turn structure. However, peptides 1 and 3 did not change conformation upon complexing Cs^+ since they have their binding side chains already preorganized for complexation.

INTRODUCTION

Many biologically important hormones are short, linear, and highly flexible peptides. One quintessential feature of these molecules is their capacity to adopt different active conformations depending on the environment and/or on the recognition of specific ligands. These induced conformational changes serve to regulate and control many cellular functions of living organisms. A detailed understanding of those molecular events is therefore required for the development of peptide based therapeutics[1] and *de novo* designed proteins.[2]

On the other hand, these types of biological processes are the perfect source of inspiration for supramolecular chemists involved in the development functional molecular devices, switches, and sensors.[3] Hence, we sought to take advantage of the conformational behavior of peptidic molecules to construct such molecules.[4] In particular, we report here the development of crown ether modified peptidic molecules with conformational properties that can be modulated by the cooperative binding of specific effectors as illustrated schematically in Figure 1.[5,6]

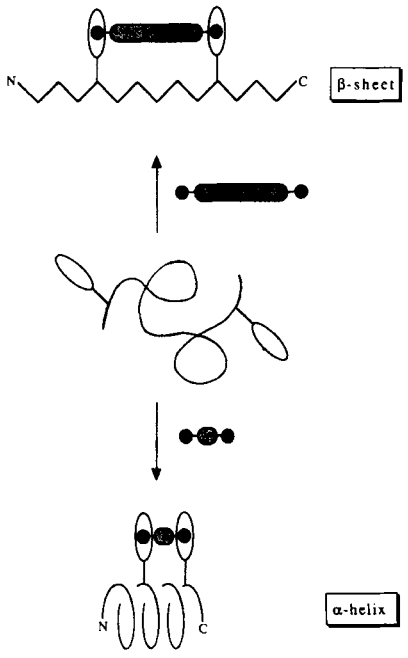


Figure 1 Present working hypothesis: the cooperative complexation of difunctional guests by a flexible bis-crown ether peptide induces a specific conformation such as an α-helix or a β-sheet that orients the binding side chains in a complementary fashion to the guest geometry.

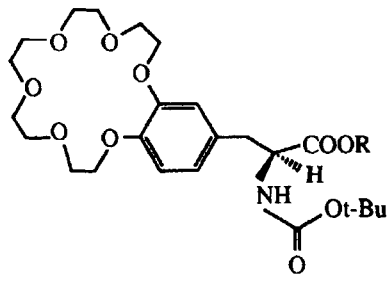
Design

Peptides 1–3 were designed in a way that under specific conformations such as α-helix and β-sheet, the two crown rings can be organized in a cooperative manner to

*To whom correspondence should be addressed.

N-t-BOC-Ala-Ala-Ala-**CE**-Ala-**CE**-Ala-CONH-nC_3H_7 **1**
N-t-BOC-Ala-Ala-**CE**-Ala-Ala-**CE**-Ala-CONH-nC_3H_7 **2**
N-t-BOC-Ala-**CE**-Ala-Ala-Ala-**CE**-Ala-CONH-nC_3H_7 **3**
CE = 3,4-(18-Crown-6)-L-Phenylalanine

N-t-BOC-Ala-Ala-**Phe**-Ala-Ala-**Phe**-Ala-Ala-CONH-nC_3H_7 **4**



5 R= H
6 R= CH_3

H_3N^+-$(CH_2)_n$-NH_3^+ • 2 Picrate- **7a-h** (n=2-9)

complex complementary difunctional ligands. The peptides are composed of five L-alanines and two 18-crown-6 derivatives of L-phenylalanine. Alanine was chosen because it can be accommodated easily in α-helices, β-sheets and even turn structures. Also, in addition to their important biological activity,[7] alanine rich peptides have been used extensively in structural studies.[8] On the other hand, the crown amino acid may be prepared readily taking advantage of the catechol function of the available L-DOPA. The crown ether residues of peptides **1**–**3** have been separated systematically by one, two and three alanines respectively. Using the schematic representations of the two most important peptide secondary structures, the α-helix and the β-sheet, it can be seen in Figure 2 that only under a sheet conformation that the two crown moieties of peptide **1** are oriented for cooperative binding and distanced by 6–7Å. Therefore, it is anticipated that the binding by peptide **1** of a difunctional ligand with the proper complementary geometry should induce and stabilize its β-sheet conformation. However, with peptide **2**, only under an α-helix structure that the crown units are organized for binding and spaced by 4.5Å. Thus, the helical conformation should then be predominant in the presence of the proper ligands. Finally, the binding sites of peptide **3** can be oriented for cooperative complexation in both forms. This time the crown rings are separated by 6Å and 13–14Å for the helix and the sheet structures respectively. Hence, the backbone conformation of **3** could be modulated, in principle, upon binding difunctional guests of complementary shapes such as α,ω-diammonium alkanes (**7a**–**7h**). On the other hand, it is also important to note that the crown rings in peptides **1**–**3** may be well organized for cooperative binding under several types of turn structures. Finally, peptide **4** is an analogue of **2** that lacks the two crown rings and it will

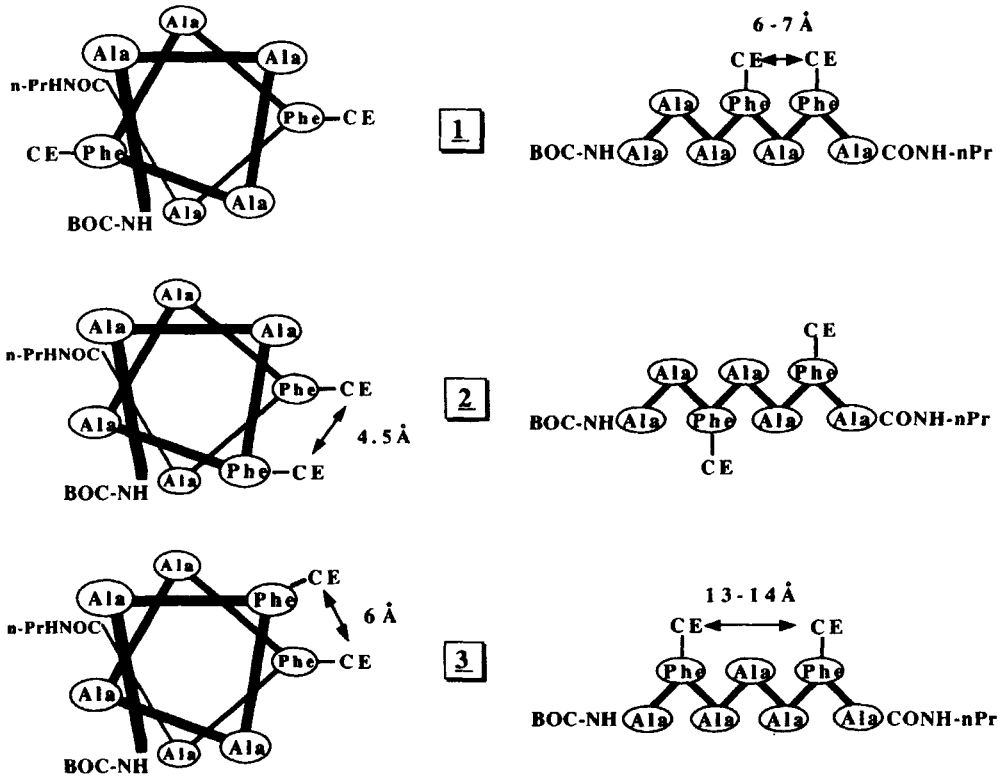


Figure 2 Schematic representations of the α-helix (axial projection)[9] and the β-sheet conformations of peptides **1**–**3**.

serve as a control to demonstrate that the binding processes do not involve the peptidic chains of **1**–**3**.

RESULTS AND DISCUSSION

Synthesis

Peptides **1**–**3** were synthesized by the solid phase method on a p-nitrophenyl oxime resin[10] using BOC protected amino acids following the procedure shown on Figure 3. The amino acids were coupled for 1 h using 3 equivalents of their hydroxybenzotriazole activated ester.[11] Each coupling was monitored for completion by the ninhydrin test[12] and performed twice when necessary to obtain a completely negative test. Peptides were cleaved quantitatively from the oxime resin by a 4 hour treatment with a freshly prepared 0.5M solution of n-propylamine in chloroform.[13] Crude peptide n-propylamides were purified by crystallization in a $CHCl_3$/MeOH mixture or by HPLC to obtain analytically pure peptides. Peptides **1**–**3** were obtained in 86, 65 and 57% yields respectively and were characterized by FAB mass spectrometry and ^1H NMR spectroscopy. The necessary N-t-BOC-18-crown-6 L-phenylalanine **5** was prepared in four steps with a 27% overall isolated yield from L-DOPA following the procedure described in Figure 4. The crown ethers **5** and **6** were optically pure by ^1H NMR using $Eu(hfc)_3$ as chiral shift reagent.[14]

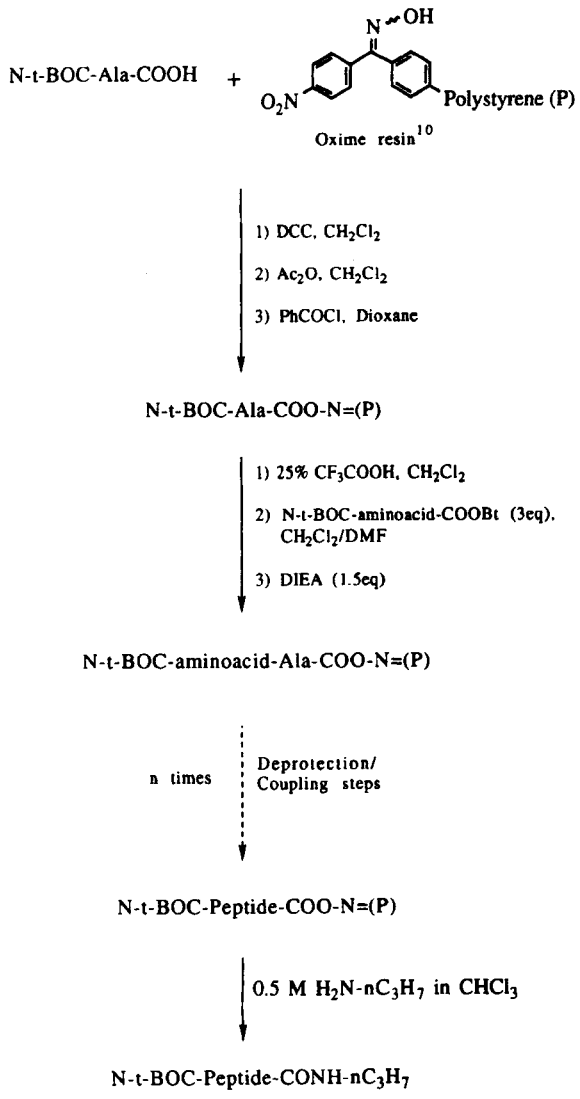


Figure 3 Synthesis of peptides **1**–**4** using the oxime resin.[10] See the Experimental Section for more details.

Complexation properties

Before investigating the conformational changes of peptides **1**–**3** that could be induced by specific recognition processes, we studied their binding abilities towards several mono- and difunctional guests. In particular, we were interested to demonstrate that the two distant crown ether side chains could act in a cooperative fashion to bind difunctional molecules. Indeed, if the complexation phenomena do not involve the two binding sites, then they should not lead to important and specific conformational changes.

The complexing ability of the bis-crown peptides **1**–**3** and the monomeric crown ether analogue **6** towards several metal ions and the diammoniums **7a**–**7h** was investigated using the picrate extraction method.[15,16] The applicability of this technique to establish the relative binding ability of mono- and bis-crown ether compounds towards these guests has been demonstrated previously.[17] The results are reported in Tables 1 and 2.

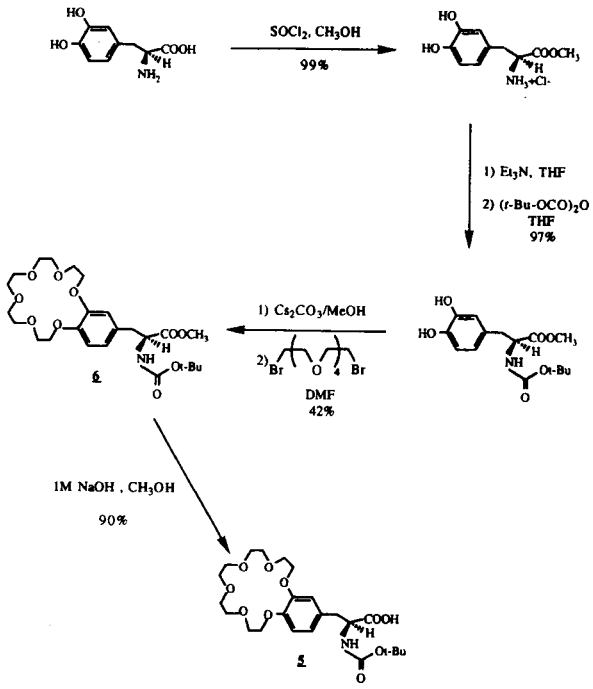


Figure 4 Synthesis of the N-t-BOC protected crown ether amino acid **5** from L-DOPA.

Table 1 Binding ability of peptidic receptors **1**-**3** and crown ether **6** towards different metal ions

	$K_a X10^{-8}$ (M^{-1})			
Substrate	**6**	**1**	**2**	**3**
Na^+	0.04	0.10	0.25	0.25
K^+	1.61	0.54	1.59	0.36
Rb^+	0.11	0.18	0.49	0.13
Cs^+	0.11	0.78	11.5	1.28

Metal ions

When the guest ion is known to form only a 1:1 complex with an 18-crown-6 host (e.g. Na^+ and K^+) no enhancement and even a decrease in the binding ability is observed when using the bis-crown ether peptides instead of the monomeric analogue **6**. The lower binding ability of peptides **1** and **3** toward K^+ can be explained by the unfavorable electrostatic interactions created by the binding of a second K^+ ion to the same receptor. However, in the case of Rb^+ and especially Cs^+ who form 2 to 1 "sandwich" complexes with two 18-crown-6 rings,[18] peptides **1**, **2**, and **3** demonstrate a much stronger binding ability than the single crown ether **6**. This high binding affinity of the bis-crown peptides for Cs^+ can be rationalized by the cooperative and simultaneous action of the two distant crown ethers to complex strongly Cs^+ ions. It has to be noted that no binding could be detected in control experiments with peptide **4**, the analogue of **2** without the crown ethers. On the other hand, the fact that receptors **1**–**3** do not bind Cs^+ to the same extent demonstrates that their peptidic backbone plays a functional role in the recognition processes. Among the three peptides, peptide **2** exhibits the highest affinity for Cs^+ and its selectivity for this ion compare well with the one of other flexible and rigid bis- or multiple benzo-18-crown-6 derivatives reported in the literature.[19] Interestingly, the distance between the crown rings of **2** under its α-helical conformation is estimated to 4.5Å and this value is very close to the one found in the crystal structure of the $(18\text{-crown-6})_2 \cdot Cs^+$ complex (around 4.2Å).[20] This seems to suggest that the helical conformation of peptide **2** is induced and stabilized in the presence of Cs^+. However, as discussed below, the conformational studies do not support the helical structure in this case.

Table 2 Binding ability of peptidic receptors **1**-**3** and crown ether **6** towards the primary diammonium guests **7a**-**h**

		$K_a X10^{-8}$ (M^{-1})			
Substrate $H_3N^+(CH_2)_nNH_3^+$		**6**	**1**	**2**	**3**
7a	n=2	0.32	2.56	21.8	6.75
7b	n=3	0.32	4.42	9.00	4.79
7c	n=4	0.23	2.88	9.22	9.58
7d	n=5	0.34	4.44	11.5	6.42
7e	n=6	0.08	10.3	41.6	11.6
7f	n=7	0.20	28.5	82.1	31.8
7g	n=8	0.25	55.7	113.0	52.5
7h	n=9	0.64	63.8	205.0	133.0

α,ω-Diammonium alkanes

The recognition ability of the peptidic receptors **1**–**3** as well as the crown ether **6** towards linear alkyl diammoniums was also investigated by the picrate extraction method modified specifically for this type of difunctional guests.[16] The results are reported in Table 2 and were calculated assuming the formation of 1:1 complexes. As for Cs^+, all peptides demonstrated a high binding ability towards diammonium cations as compared to the crown ether analogue **6**. The bis-crown receptors are especially selective for the longer ones (**7e**–**7h**).

The K_a values obtained range between 10^8 and 10^{10} M^{-1} for the receptors **1**–**3** while they are about constant at 10^7 M^{-1}, as expected, for **6**. Also, no binding could be detected in control experiments using peptide **4** lacking the two crown rings. Thus, the high values of association constant observed for the flexible receptors **1**–**3** can also be attributed in these cases to the cooperative action of the two distant crown ether side chains to bind the difunctional substrates in an intramolecular complementary fashion. As for Cs^+, the bis crown peptide **2** having the ligand modified residues separated by two alanines showed the best binding ability towards the difunctional ligands. In addition, as it can be seen in Table 2, there are important differences observed in the binding ability of peptides **1**–**3** with the diammonium guests. It is possible that these differences result from the favourable or unfavourable conformational changes associated with the molecular recognition processes. On the other hand, the different binding ability noted here supports the conclusions drawn with the metal ions that the peptidic chain of receptors **1**–**3** plays a functional role and does not act like a passive linker between the two crown ethers. The fact that the longest and the most flexible ligands are bound more tightly than the shorter ones might be explained in terms of their greater adaptability. A similar binding tendency have been reported previously with the same guests by rigid receptors.[16,21] However, even though the longer substrates are bound more tightly, it is noteworthy that the bis-crown peptide **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 probably a more favourable conformation to bind **7a** than for the slightly longer difunctional substrates **7b**, **7c**, and **7d**.

Several more rigid bis-azacrown ethers have been reported to accomplish shape selectivity for primary alkyl diammonium cations.[22] However, the complexation results reported herein constitute one of the first example[23], to our knowledge, of *flexible* molecular receptors possessing high binding affinity and shape selectivity with difunctional substrates of different geometry.

Conformational studies

We have used circular dichroism spectropolarimetry (hereafter CD) to determine if the complexation of specific ligands could induced conformational changes in peptides **1**–**3**. Studies were made in 1,2-dichloroethane (DCE) since chloroform is too absorbant at the wavelengths useful to study peptidic conformations (190–250nm). The guests used were Cs^+ (acetate and perchlorate) and the primary diammoniums as their dichloride salt. The complexes were formed by a solid-liquid extraction of the solid guests by the bis-crown peptides.

CD studies with the α,ω-diammonium guests

In DCE at 25 °C, the free peptides **1**–**3** adopt a β-sheet conformation as characterized by a negative band around 215–220 nm and a positive band between 190 and 200 nm (Figure 5).[24] Their conformation is quite stable even at 55 °C. Since picrate anion is too absorbing between 190 and 250 nm, the preparation of the diacetate salts was attempted but amorphous heavily hydrated unsuitable materials were obtained. By contrast, the bis-chloride salts of **7a**–**7h** were easily prepared using a dry HCl solution in dioxane. Treatment of the peptide DCE solutions with the solid diammonium dichlorides did not lead to significant conformational changes. It is possible that no extraction and complexation occurred between the bis chloride salts of **7a**–**7h** and the bis-crown peptides since the chloride is a hard and tightly bound anion that is not a good counterion for complexation experiments. This would explained the lack of conformational changes noted in these cases. More experiments have to be made with different anions to corroborate these hypotheses.

CD studies with Cs^+

Upon addition of Cs^+, the conformation of peptides **1** and **3** remained almost unchanged (Figure 5a and c). This conformational behavior is not unexpected since **1** and **3** exist mainly in a β-sheet conformation where the two crown side chains are already preorganized[3e,25] to complex Cs^+ (Figure 2). Therefore, no conformational changes are required to bind Cs^+. By contrast, the conformation of **2** changed dramatically upon complexation of Cs^+ (Figure 5b). This result is also not unexpected.

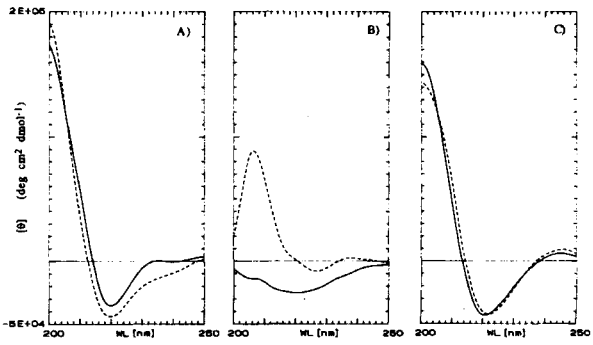


Figure 5 Circular dichroism curves of peptides **1**(a), **2**(b), and **3**(c) free (solid lines) and in presence of Cs^+ (dashed lines) in DCE.

Indeed, under a β-sheet conformation the two crown units of the free peptide are not oriented properly to form a cooperative complex with Cs^+. Hence, a major conformational reorganization is required to orient the binding sites appropriety to form a "sandwich" complex with Cs^+. The structure of **2** switches from a β-sheet to a β-turn structure characterized in CD by the appearance of a positive band at 207 nm and a smaller negative band at 227 nm (Figure 5b). This type of curve is similar to the one observed for type II β-turn structures.[26] However, it is risky to propose the definite structure of a β-turn on the sole basis of CD.[27] Interestingly, the β-turn conformation induced in **2** by the complexation of Cs^+ is in contradiction with our original prediction (Figure 2). This result can be explained by the fact that the predicted α-helical form is not stable enough with peptides shorter than 12 amino acids.[28] Nevertheless, there is an important conformational change in peptide **2** induced by the recognition of Cs^+ ions. The change induced is specific to Cs^+ since the complexation of Na^+ and K^+ did not lead to significant changes in the backbone conformation of the three peptides (Figure 6a).

Furthermore, the use of Cs^+ perchlorate instead of the acetate induced the same conformation in **2** demonstrating that the supramolecular phenomenon observed is due to the specific recognition of Cs^+ and not to non specific interactions with the anions (Figure 6b). It is noteworthy that the bis-crown peptide **2** who has the binding side chains not properly oriented under a β-sheet form is a better Cs^+ binder than the "*preorganized*" receptors **1** and **3**.[3e,25] This is probably due to the highly favorable conformation of the peptidic chain of **2** when bound to Cs^+ as compared to the free form. Indeed, the supramolecular β-turn structure of Cs^+•**2** is even stable in a strong hydrogen bonding solvent like methanol *(Figure 6c)*.

In order to determine the precise structure of the Cs^+•peptide **2** complex, we have started some ^1H NMR studies in $CDCl_3$. Preliminary results confirm that there are important conformational changes in **2** upon recognition of Cs^+ as indicated by the shifting and the sharpening of the amides and αCH resonances (Figure 7)

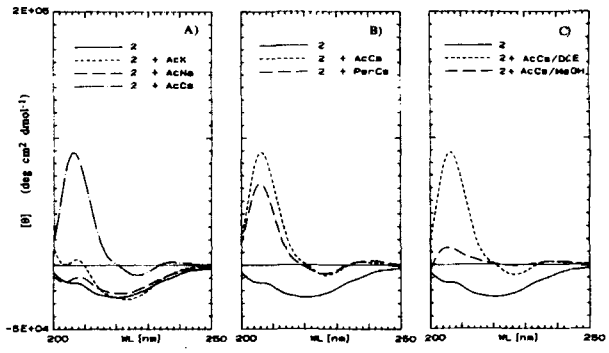


Figure 6 Circular dichroism curves of peptide **2** in DCE: a) free and in the presence of K^+, Na^+, and Cs^+ acetates (Ac); b) free and with Cs^+ acetate (AcCs) and perchlorate (PerCs), and; c) free and with Cs^+ acetate (AcCs) in DCE and MeOH.

However, heavy overlaps of several resonances precluded so far the detailed structure elucidation of the complex.

CONCLUSION

We have reported the synthesis of three bis-crown ether modified peptides designed to undergo ligand induced conformational changes. We have shown that the two distant crown side chains act in a cooperative fashion to complex selectively difunctional guests such as Cs^+ and the longer α,ω-diammonium alkanes. The binding selectivities observed are impressive taking into account the flexibility of the receptors and the geometrical similarity of the guests studied. On the other hand, the differences observed in the binding ability of the bis-crown peptides **1**–**3** indicate that their peptidic chain plays a functional role in the recognition phenomena. For instance, it was demonstrated that the "non preorganized" receptor **2** is a better Cs^+ binder than the "preorganized" analogues **1** and **3** probably because the complexation of Cs^+ induces a highly favorable conformation in the backbone of **2**.

Only the addition of Cs^+, known to bind in between two 18-crown-6 moieties, induced a conformational change in peptide **2**. But, contrarily to the original prediction, the conformation induced in **2** by the complexation of Cs^+ is not an α-helical structure but rather a β-turn structure. This result is of great interest knowing that this type of structures plays a dominant role in biological recognition phenomena.[29] More studies are therefore required to determine the detailed structure of the supramolecular complex and to be able to predict accurately the possible conformation of the bis-crown ether peptides when complexed with specific guests. Finally, the results reported constitute also the groundwork of the development of novel peptide based molecular switches able to respond selectively to specific chemical signals. Work along these lines is in progress.

Experimental section

All solvents and materials were Reagent, Spectro, or HPLC grade quality purchased commercially and used without any further purification except for DMF which was distilled from $MgSO_4$ onto 4Å molecular sieves.

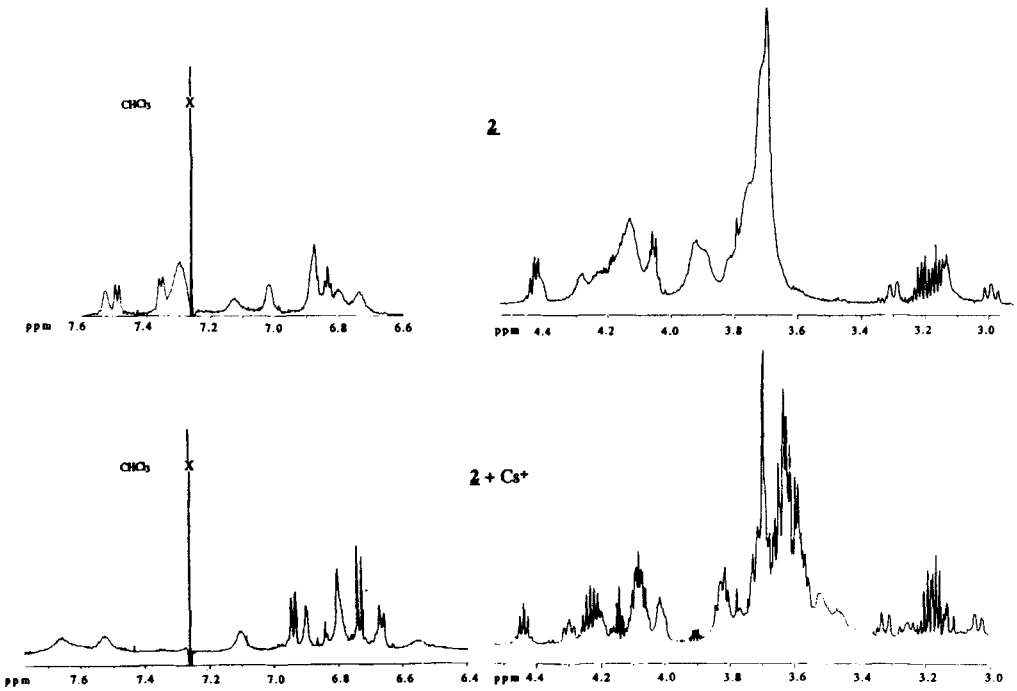


Figure 7 Portions of the 600 MHz ^1H NMR of peptide **2** free (top) and in the presence of Cs^+ (bottom) in $CDCl_3$. The conformational changes induced by the complexation of Cs^+ are noted by important shifts in the positions of the amide and α-carbon protons.

Melting points were determined on a Reichert Hot Stage apparatus and are uncorrected. Mass spectral assays (ms, m/e) were performed using a VG Micromass ZAB-1F spectrometer. FAB mass spectra (glycerol matrix) were recorded on a VG-Autospec Q spectrometer and were performed at the Regional Center of Mass Spectrometry, Université de Montréal. Optical rotations were measured at 25°C on a Perkin Elmer 241 polarimeter. Proton NMR spectra were recorded on a 300 MHz Bruker WM-300 instrument except for the 600 MHz spectra that were obtained on a Bruker AMX-600 at the National Research Council, Ottawa, Canada. Chemical shifts are reported in δ values (ppm) relative to DMSO or chloroform as internal standard. Abbreviations used are m: multiplet, s: singulet, d: doublet, t: triplet, q: quartet, qn: quintuplet. Circular dichroism studies were performed using a Jasco J-710 spectropolarimeter. The absorbance measurements were recorded on a Hewlett-Packard HP-8452 Diode Array Spectrophotometer.

Chromatographic separations were made using Merck Kieselgel 60 silica gel (230–400 mesh ASTM). Analytical and preparative reversed phase HPLC were performed on Vydac C_{18} columns with gradients of H_2O/0.1% TFA and CH_3CN/0.1% TFA solutions. Thin layer chromatography (TLC) was performed using silica gel Kieselgel 60 plates. The developed plates were visualized directly with ninhydrin or with ninhydrin after exposure to HCl in the case of BOC-protected molecules. The following solvent mixtures (v/v) used were either chloroform-methanol-acetic acid (85:10:5) or chloroform-methanol (9:1).

N-t-BOC 3,4-(18-crown-6)-L-phenylalanine methyl ester 6

Under Argon, N-t-BOC 3,4-dihydroxy-L-phenylalanine methyl ester[30] (44 g, 142 mmol) and cesium carbonate (46.23 g, 142 mmol) were dissolved in 600 mL of dry degased methanol. The mixture was gently warmed on a water bath to 45°C until complete dissolution. Methanol was then evaporated to dryness under reduced pressure at 45°C. DMF (50 mL) was added and evaporated under high vacuum at the same temperature to remove the residual methanol. The green product obtained was dissolved in 300 mL of DMF and transferred in a 3 L three-neck flask containing 1L of DMF under Argon at 60°C to generate a deep green solution. A 300 mL DMF solution of dibromo pentaethylene glycol[31] (51.7 g, 142 mmol) was then added dropwise over a 1 h period. The resulting solution was stirred at 60°C overnight. The brown suspension was evaporated under high vacuum at 45°C. The residue was taken up in dichloromethane and washed 3 times with a 5% $NaHCO_3$ and 3 times with H_2O then dried with $MgSO_4$. After filtration and evaporation, the crude brown oil was purified by flash chromatography on SiO_2 using first CH_2Cl_2 then CH_2Cl_2 with 2 and 4% methanol. Trituration with petroleum ether/ ether (10/1) gave the desired crown ether as a white powder in a 42% isolated yield. m. p.: 91-3°C; $[\alpha]^{23}D$ = +5.5° (c= 1, MeOH); ^1H NMR ($CDCl_3$): 1.40 (s, 9H); 3.01 (m, 2H); 3.68 (s, 3H); 3.63–3.78 (m, 12H); 3.88 (m, 4H); 4.10 (m, 4H); 4.52 (m, 1H); 4.98 (d, 1H, J=7.9 Hz); 6.61–6.72 (m, 2H); 6.80 (m, 1H); FAB MS ($C_{25}H_{39}O_{10}N$): 536 (M+ Na^+); 513 (M + H^+)

N-t-BOC 3,4-(18-crown-6)-L-phenylalanine 5:

The above ester (16.36 g, 32 mmol) was suspended in 50 mL of 1M NaOH solution at 0 °C and stirred at room temperature for 1.5h. The clear alkaline solution was washed with ether, then acidified with ice cold 1M HCl to pH 4 producing a milky solution. The aqueous layer was extracted with 3 times with 300 mL of CH_2Cl_2. The organic phase was washed with water, dried with $MgSO_4$, filtered and evaporated to give a yellowish oil that was triturated three times with ether to give the acid as a white fluffy solid.

Yield: 90%; m. p.: 96–98°C; $[\alpha]^{23}D$= +15.5° (c= 1, MeOH); ^1H NMR ($CDCl_3$):1.40 (s, 9H,); 3.01 (d, 2H); 3.63–3.73 (m, 12H); 3.78–3.92 (t, 4H); 3.92–4.22 (m, 4H); 4.48 (broad m, 1H); 5.20 (d, 1H, J= 7.9 Hz); 5.42 (broad s, 1H); 6.68–6.80 (m, 3H); MS ($C_{26}H_{41}O_{11}N$) Exact mass: calc.=499.2417, obt.=499.2405; FAB MS: 522 (M+Na^+); 499 (M^+).

N-t-BOC-L-alanine-Resin:

N-t-BOC-L-alanine (2.8 g, 14.8 mmol, 5 eq.) dissolved in dichloromethane (25 mL) and activated with DCC at 0 °C for 30 min was mixed with 5 g (2.95 mmol) of p-nitrophenyloxime resin[10] (subs. level=0.59 mmol/g) previously swollen in dichloromethane in a reaction vessel bearing an opening at the top, a glass fritted disc and a stopcock at the bottom. The resulting suspension was stirred on a rotary shaker overnight at 25°C. The next day, the resin was filtered, carefully washed as follow: 3×100 mL DMF, 3×100 mL MeOH, 3×100 mL DMF, 3×100 mL MeOH, and suction dried. The resin was then acetylated using acetic anhydride (5 mL) and diisopropyl ethylamine (0.5mL) in DMF for 1 h and benzoylated with benzoyl chloride (10 mL) in dioxane (50 mL) for 1h. Washing and drying as described above gave the resin used in the next step. The substitution level after deprotection with TFA was 0.35 mmol/g by the quantitative ninhydrin test.[32]

N-t-BOC-Peptides-Resin:

The peptides were prepared using 5 g of the above resin. Deprotection of the N-t-BOC group was performed by treating the resin with a 25% TFA solution in dichloromethane for 30 min. The washing steps in all cases were as follows: 3×100 mL DMF, 3×100 mL MeOH, 3×100 mL DMF, 3×100 mL MeOH. N-t-BOC

protected amino acids (3 eq.) were activated at 0°C for 30 min in dichloromethane using DCC and HOBt[11] and coupled in DMF for 1h in the presence of DIEA (1 eq.). Completion of the coupling reactions was monitored by the ninhydrin test.[12]

N-t-BOC-Peptide-n-propylamides:[13]
The peptides were cleaved from the resin by a 4 h treatment using a freshly prepared 0.5 M solution of n-propylamine in chloroform. The suspension was filtered and the filtrate was evaporated to give the crude peptides **1**–**4** that were crystallized in a chloroform-methanol mixture then purified by reverse phase HPLC.

N-t-BOC-Ala-Ala-Ala-CE-Ala-CE-Ala-nC_3H_7 1
Yield: 86%; FAB MS ($C_{61}H_{96}O_{21}N_8$): M + H^+ = 1278, M + Na^+ = 1300; ^1H NMR (DMSO-D_6): 0.78 (t, 3H), 1.08–1.28 (m, 15H), 1.35 (s, 9H), 2.58–2.80 (m, 2H), 2.80–3.14 (m, 4H), 3.42–3.62 (m, 24H), 3.62–3.78 (m, 8H), 3.82–4.10 (m, 9H); 4.10–4.30 (m, 4H), 4.32–4.52 (m, 2H), 6.62–6.90 (m, 6H), 6.92 (d, 1H, J-7.9 Hz), 7.70–8.18 (m, 7H).

N-t-BOC-Ala-Ala-CE-Ala-Ala-CE-Ala-nC_3H_7 2
Yield: 65%; FAB MS ($C_{61}H_{96}O_{21}N_8$): M + H^+ = 1278, M + Na^+ = 1300; ^1H NMR (DMSO-D_6): 0.78 (t, 3H), 1.08–1.40 (m, 15H), 1.35 (s, 9H), 2.57–2.82 (m, 2H), 2.82–3.14 (m, 4H), 3.40–3.62 (m, 24H), 3.62–3.78 (m, 8H), 3.82–4.10 (m, 9H); 4.12–4.29 (m, 4H), 4.32–4.52 (m, 2H), 6.60–6.92 (m, 6H), 6.94 (d, 1H, J-7.9 Hz), 7.65–8.19 (m, 7H).

N-t-BOC-Ala-CE-Ala-Ala-Ala-CE-Ala-nC_3H_7 3
Yield: 57%; FAB MS ($C_{61}H_{96}O_{21}N_8$): M^+ = 1278, M + Na^+ = 1300; ^1H NMR (DMSO-D_6): 0.76 (t, 3H), 1.10–1.25 (m, 15H), 1.36 (s, 9H), 2.58–2.81 (m, 2H), 2.81–3.12 (m, 4H), 3.43–3.60 (m, 24H), 3.60–3.79 (m, 8H), 3.82–4.11 (m, 9H); 4.11–4.30 (m, 4H), 4.34–4.55 (m, 2H), 6.63–6.87 (m, 6H), 6.92 (d, 1H, J-7.9 Hz), 7.66–8.15 (m, 7H).

N-t-BOC-Ala-Ala-Phe-Ala-Ala-Phe-Ala-Ala-nC_3H_7 4
Yield: 73%; FAB MS ($C_{44}H_{65}O_{10}N_9$): M + H^+ = 880, M + Na^+= 902; ^1H NMR (DMSO-D_6): 0.78 (t, 3H), 1.02–1.28 (m, 18H), 1.35 (s, 9H), 2.68–2.82 (m, 2H), 2.82–3.18 (m, 4H), 3.62–3.78 (m, 8H), 3.82–3.94 (m, 1H); 4.08–4.28 (m, 5H), 4.40–4.51 (m, 2H), 6.93 (d, 1H, J=7.9 Hz), 7.10–7.28 (m, 10H), 7.70–8.10 (m, 8H).

Complexation experiments:

Metal ions:
The binding ability of the receptors was determined by the picrate extraction technique following a described procedure.[15,16] Metal picrate solutions were prepared in doubly distilled deionized water using the metal picrates.[33] In a typical experiment, 0.5 mL (0.75 mL for Cs^+) of the metal picrate solution (1.5mM, except for Cs^+= 1mM which was less soluble) was mixed in a 6 mL centrifuge tube with 0.5 mL of the receptor chloroform solution (1.5 mM in crown unit). The tube was capped, centrifuged for 10 sec, and stirred vigourously on a vortex mixer for 20 min. The solution was then centrifuged 10 min at 1500 rpm. Three aliquots of 75 µL were withdrawn with a syringe and diluted to 1 mL with acetonitrile. The picrate absorbance was measured and used in the calculations. Triplicate experiments were performed and were reproducible within ±5%. The K_a values reported are the averages of the three separate runs.

α,ω-diammonium alkanes:[16]
The picrate solution of the diamines (0.5 mM) were prepared *in situ* using the freshly distilled diamines and crystallized picric acid in doubly distilled deionized water. The receptor chloroform solutions for these experiments had a concentration of 0.35 mM in crown unit. The extraction experiments were done as above using 0.5 mL of the picrate and receptor solutions.

Circular dichroism studies:
Peptide solutions (0.5 mM) were prepared in 1,2-dichloroethane (DCE). The peptides were dried under high vacuum for several hours before use. Measurements were made using a 0.1 mm pathlength that allows the recording as low as 200 nm with DCE solutions. First, the curve of the free peptide was recorded. Then, for the complexation, the thoroughly dried solid guests (stored over P_2O_5 several days) were added to the peptide solutions in a slight excess and vortex for 15 min. The suspensions were filtered into the cell and the CD curves measured. The data reported are the molar ellipticities, [θ], in mdeg cm^2 $dmol^{-1}$. The dichloride salts of the diamines **7a**–**7h** were prepared by their precipitation from ether with a 4N HCl solution in dioxane. The solid dichlorides were filtered, triturated with ether, and dried under vacuum over P_2O_5 for several hours prior to their use.

ACKNOWLEDGEMENTS

Financial support from the NSERC of Canada, the FCAR Fund of Québec, and the Université de Sherbrooke is gratefully acknowledged. We thank Dr. M. Evans (Regional Center for Mass Spectrometry, Université de Montréal) for obtaining the mass spectra and S. Gagné and J.-R. Brisson (NRC, Ottawa) for performing the 600 MHz NMR spectra. We are also indebt-

ed to Dr. Henry Wolfe, Jr from Sterling Drug Co., Collegeville, PA for technical assistance in the peptide synthesis. N. V. is a recipient of a Young Investigator Award from the Fondation de l'Université de Sherbrooke for 1993–1994.

REFERENCES AND NOTES

1 a) Kaiser, E.T. *Trends Biochem. Sci.* **1987**, *123*, 305; b) Gupta, A.; Bharadwaj, A.; Chauhan, V.S.; *J. Chem. Soc. Perkin Trans. II*, **1990**, 1911; c) Moser, R.; Klauser, S.; Leist, T.; Langen, H.; Epprecht, T.; Gutte, B.; *Angew. Chem. Intl. Ed. Engl.* **1985**, *24*, 719.

2 Mutter, M.; Herspeger, R.; *Angew. Chem. Intl. Ed. Engl.* **1990**, *29*, 185; b) DeGrado, W.F.; *Adv, Protein Chem.* **1988**, *39*, 51 and references cited therein; c) Richardson, J.S.; Richardson, D.C.; *Trends Biochem. Sci.* **1989**, *14*, 304.

3 a) Stoddart; J.F. in *Frontiers in Supramolecular Organic Chemistry and Photochemistry*, Schneider, H.-J.; Dürr, H. (Eds), VCH Publishers, Weinheim, FRG, 1991, p. 251; b) Lehn, J.-M., *ibid*, p. 1; c) Stinson, S.C.; *Chem. Eng. News* **1990**, *26*, July 16; d) Schanze, K.S.; Sauer, K.; *J. Am. Chem. Soc.* **1988**, *110*, 1180: e) Cram, D.J.; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1009; f) Lehn, J.-M., *ibid*, **1988**, *27*, 89; g) Rebek, J., Jr., *ibid*, **1990**, *29*, 245.

4 a) Voyer, N.; Guérin, B.; *Tetrahedron* **1994**, *50*, 989; b) Voyer, N.; Maltais, F.; *Adv. Mater.* **1993**, *5*, 568; c) Voyer, N.; Guérin, B.; *J. Chem. Soc. Chem. Commun.* **1992**, 1253; d) Voyer, N.; *J. Am. Chem. Soc.* **1991**, *113*, 1818.

5 For preliminary accounts see: a) Voyer, N.; Deschênes, D.; Bernier, J.; Roby, J.; *J. Chem. Soc. Chem. Commun.* **1992**, 134; b) Voyer, N.; Roby, J.; *Tetrahedron Lett.*, **1991**, *32*, 331.

6 For model studies on peptide secondary structure stabilization using unnatural side chain recognition interactions or synthetic templates see: a) Ghadiri, M.R.; Soares, C.; Choi, C.; *J. Am. Chem. Soc.* **1992**, *114*, 4000 and 825; b) Ruan, F.; Chen, Y.; Itoh, K.; Sasaki, T.; Hopkins, P.B.; *J. Org. Chem.* **1991**, *56*, 4347; c) Ruan, F.; Chen, Y.; Hopkins, P.B.; *J. Am. Chem. Soc.* **1990**, *112*, 9403; d) Ghadiri, M.R.; Fernholz, A.K.; *J. Am. Chem. Soc.* **1990**, *112*, 9633; e) Ghadiri, M.R.; Choi, C. *ibid*, **1990**, *112*, 1630; f) Imperiali, B.; Kapoor, T.M.; *Tetrahedron* **1993**, *49*, 3501; g) Dado, G.P.; Gellman, S.H.; *J. Am. Chem. Soc.* **1993**, *115*, 12609; h) Diaz, H.; Tsang, K.Y.; Choo, D.; Kelly, J.W.; *Tetrahedron* **1993**, *49*, 3533; i) Kemp, D.S.; Curran, T.P.; Boyd, J.D.; Allen, T.J.; *J. Org. Chem.* **1991**, *56*, 6683; j) Berthet, M.; Yordanov, S.; Sonveaux, E.; *Makromol. Chem., Rapid Commun.* **1986**, *7*, 205; k) Berthet, M.; Sonveaux, E.; *Biopolymers* **1986**, *25*, 189; l) Nowick, J.S.; Powell, N.A.; Martinez, E.J.; Smith, E.M.; Noronha, G.; *J. Org. Chem.* **1992**, *57*, 3763; see also ref. 2a.

7 a) Parry, D.A.D. in *Fibrous Protein Structure*, Academic Press, New York, 1987, p. 142; b) Yang, D.S.C.; Sax, M.; Chakrabartty, A.; Hew, C.L.; *Nature*, **1988**, *333*, 232.

8 a) Miick, S.M.; Martinez, G.V.; Fiori, W.R.; Todd, A.P.; Millhauser, G.L.; *Nature* **1992**, *359*, 653; b) Scholtz, J.M.; York, E.J.; Stewart, J.M.; Baldwin, R.L.; *J. Am. Chem. Soc.* **1991**, *113*, 5102; c) Merutka, G.; Stellwagen, E.; *Biochemistry* **1990**, *29*, 894; d) *ibid*, **1991**, *30*, 1591; e) Shoemaker, K.R.; Kim, P.S.; Marqusee, S.; York, E.J.; Chaiken, I.M.; Stewart, J.M.; Baldwin, R.L.; *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 1985; f) Jackson, D.Y.; King, D.S.; Chmielewski; Singh, S.; Schultz, P.G.; *J. Am. Chem. Soc.* **1991**, *113*, 9391; g) Marqusee, S.; Baldwin, R.L. *Proc.; Natl. Acad. Sci. USA* **1987**, *84*, 8898; h) Zier, A.; Mutter, M.; *Tetrahedron Lett.* **1994**, *35*, 1039.

9 Schiffer, M.; Edmundson, *Biophys. J.* **1967**, *7*, 121.

10 a) Kaiser, E.T.; *Acc. Chem. Res.* **1989**, *22*, 47; b) DeGrado, W.F.; Kaiser, E.T.; *J. Org. Chem.* **1982**, *47*, 3258; c) DeGrado, W.F.; Kaiser, E.T.; *J. Org. Chem.* **1980**, *45*, 1295.

11 König, W.; Geiger, R.; *Chem. Ber.* **1970**, *103*, 788.

12 Kaiser, E.; Colescott, R.L.; Bossinger, C.D.; Cook, P.I.; *Anal. Biochem.* **1970**, *34*, 595.

13 Voyer, N.; Lavoie, A.; Pinette, M.; Bernier, J.; *Tetrahedron Lett.* **1994**, *35*, 355.

14 Fraser, R.R. in *Asymmetric Synthesis*, Morisson, J.D. (Ed), Academic Press, New York, 1983, 173; see also ref. 6k.

15 Koenig, K.E.; Lein, G.M.; Stuckler, P.; Kaneda, T.; Cram, D.J.; *J. Am. Chem. Soc.* **1979**, *101*, 3553.

16 Smeets, J.W.H.; Visser, H.C.; Koats-Richters, V.E.M.; Nolte, R.J. M. *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 147.

17 a) Gokel, G.W.; Trafton, J.E. in *Cation Binding by Macrocyles*, Inoue, Y. and Gokel, G. W. (Eds.), Marcel Dekker Inc., New York, 1990, 253; b) Kikukawa, K.; He, G.-X.; Abe, A.; Goto, T.; Arata, R.; Ikeda, T.; Wada, F.; Matsuda, T.; *J. Chem. Soc, Perkin Trans. II* **1987**, 135; c) Kimura, K.; Sakamoto, H.; Koseki, Y.; Shono, T. *Chem. Lett.* **1985**, 1241.

18 a) Popov, A.I.; Lehn, J.-M. in *Coordination Chemistry of Macrocyclic Compounds*, Plenum Press, New York, USA, 1979, 537; b) Frensdorff, H.K.; *J. Am. Chem. Soc.* **1971**, *91*, 600; c) Kopolow, S.; Machacek, Z.; Takaki, U.; Smid, J.; *J. Macromol. Sci.-Chem.* **1973**, *A7*, 1015; d) Pedersen, C. J.; *J. Am. Chem. Soc.* **1967**, *89*, 7017.

19 Kopolow, S.; Hogen Esch, T.E.; and Smid, J.; *Macromolecules* **1973**, *6*, 133; see also ref. 17b and 18c.

20 Dawes, S. B.; Ward, D.L.; Huang, R.H.; Dye, J.L.; *J. Am. Chem. Soc.* **1986**, *108*, 3534.

21 Fages, F.; Desvergne, J.-P.; Kampke, K.; Bouas-Laurent, H.; Lehn, J.-M.; Meyer, M.; Albrecht-Gary, A.-M.; *J. Am. Chem. Soc.* **1993**, *115*, 3658.

22 a) Sutherland, I.O.; *Adv. Supramol. Chem.* **1990**, *1*, 65 and references cited therein; Hamilton, A.; Lehn, J.-M.; Sessler, J.L.; *J. Am. Chem. Soc.* **1986**, *108*, 5158.

23 Novel flexible receptors displaying important recognition abilities have been reported recently: Rebek, J. Jr.; *Angew. Chem. Intl. Ed. Engl.* **1990**, *29*, 245; b) Tjivikua, T.; Deslongchamps, G.; Rebek, J. Jr.; *J. Am. Chem. Soc.* **1990**, *112*, 8408; c) J.C.; Li, C.; Bott, S. G.; Atwood, J.L., Gokel, G.W.; *J. Am. Chem. Soc.* **1991**, *113*, 366; d) Galan, A.; Andreu, D.; Echavarren, A.M.; Prados, P.; de Mendoza, J.; *J. Am. Chem. Soc.* **1992**, *114*, 1511.

24 Walton, A.G.; *Polypeptides and Protein Structure*, Elsevier, New York, 1981, pp 180-185.

25 Cram, D.J.; Kaneda, T.; Helgeson, R.C.; Lein, G.M.; *J. Am. Chem. Soc.* **1979**, *101*, 6752.

26 a) Woody, R.W. in *Peptides, Polypeptides, and Proteins*, Blout, E.R.; Bovey, F.A.; Goodman, M.; Lotan, M. (Eds), Wiley & Sons. New York, 1974, 338; b) Imperiali, B.; Fisher, S.L.; Moats, R.A.; Prins, T.J.; *J. Am. Chem. Soc.* **1992**, *114*, 3182; c) Yang, J.T.; Wu, C.-S.C.; Martinez, H.M.; *Meth. Enzymol.* **1986**, *130*, 208 and references cited therein.

27 Percel, A.; Hollosi, M.; Foxman, B.M.; Fasman, G.D.; *J. Am. Chem. Soc.* **1991**, *113*, 9772.

28 Maser, F.; Bode, K.; Pillai, V.N.R.; Mutter, M.; *Adv. Polym. Sci.* **1984**, *65*, 177; see also ref. 8h.

29 Rose, G.D.; Gierasch, L.M.; Smith, J.A.; *Adv. Protein Chem.* **1985**, *37*, 1.

30 Banerjee, S.M.; Ressler, C.; *J. Org. Chem.* **1976**, *41*, 3056.

31 Van Keulen, B.J.; Kellogg, R.M.; Piepers, O.; *J. Chem. Soc. Chem. Commun.* **1979**, 285; see also ref. 4d.

32 Stewart, J.M.; Young, J.D.; *Solid Phase Peptide Synthesis*, 2nd Edition, Pierce Chem. Co., Rocford, IL, 1984, p. 105.

33 Coplan, M.A.; Fuoss, R.M.; *J. Phys. Chem.* **1964**, *68*, 1177.