Potential formation of intramolecular inclusion complexes in peptido-cyclodextrins as evidenced by NMR spectroscopy

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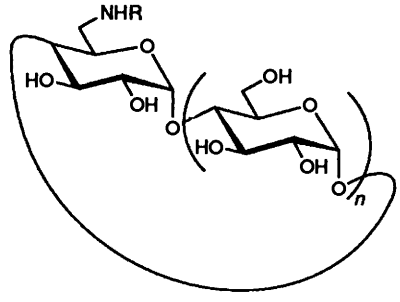
Investigations of the structure of β- and γ-cyclodextrin† derivatives in solution obtained by grafting amino acids or peptides are presented. These compounds are models for vectorization-dedicated molecular carriers. It is shown that for some amino acids, strong intramolecular self-inclusion complexes are formed in aqueous solution. This process strongly depends upon the nature and position of the pertinent amino acid in the peptide sequence. Two dimensional NMR experiments are used in conjunction with competition with external guests to evidence and estimate the strength of these auto-inclusion complexes.

Attempts to optimize the performances of cyclodextrins and derivatives for the transport of bio-active drugs has opened the field of new vector-carrying molecules dedicated to the recognition of specific biological receptors. These transporters are dedicated to binding to the final receptor through their 'signal' moiety and hence to concentrate the included drug at the level of the organ tissue to be processed. Owing to the large variety of potential cellular or tissular receptors, peptides appear as the most versatile class of compounds suitable for grafting to cyclodextrins.[1] Chemical substitution of the cyclodextrin by a 'signal' molecule should obviously neither preclude the inclusion of the drug of interest nor impair the recognition of the 'signal' at the receptor level. This implies that the cavity of the modified cyclodextrin must remain vacant for the transportation of the drug of interest. Fulfilling these requirements requires a careful design of the chemical structure of the final compound. We demonstrate here that investigations of model compounds can be highly informative in this respect. It was indeed suspected that synthetic derivatives obtained by grafting L- or D-phenylalanine onto 6-amino-6-deoxycyclomaltoheptaose exhibit peculiar behaviour in solution as observed by NMR spectroscopy.[2] The large spectral dispersion of the ^1H NMR spectra in D_2O was tentatively explained by the formation of intramolecular self-inclusion complexes. Similar assumptions were proposed by others[3] on the basis of NMR data for *N*-formyl-L-Phe derivatives of cyclomaltoheptaose. Increasingly, no large spectral dispersion was observed in the L-Phe derivatives of 2,6-dimethyl-β-cyclodextrin[4] nor in the permethylated derivative. This observation suggests that the free hydroxy groups play a key role in the self-inclusion process. For this reason we have decided to consider only natural unmodified cyclodextrins as carriers for amino acids and peptides. This choice is clearly more convenient in terms of bio-compatibility, the methylated derivatives exhibiting a high hemolytic character which is absent in the compounds to be described below.[5] We present here evidence for the reality of such self-inclusion complexes and the influence of the nature and position of amino acid residues on this process. For the present purposes, the following β- and γ-cyclodextrin derivatives (Fig. 1) have been synthesized and purified.

Compound **1** derived from glycine will be considered here as a reference as any observed spectral dispersion can arise from the substitution effect only. In addition to the phenylalanine derivatives **2a** and **b**, compounds **3** and **4** will serve to investigate



$n = 6$ R = Glycyl **1**
= L-Phenylalanyl **2a**
= D-Phenylalanyl **2b**
= L-Tyrosyl **3**
= L-Tryptophanyl **4**
= L-Leucyl **5**
= Glycyl-L-phenylalanyl **6**
= L-Phenylalanyl-glycyl **7**
= Glycyl-L-tyrosyl **8**
= L-Tyrosyl-glycyl **9**

$n = 7$ R = L-Phenylalanyl **10**
= L-Tryptophanyl **11**
= CH_2CH_2NH-L-Tryptophanyl **12**

Fig. 1 General structure of compounds investigated in this study. Unless indicated otherwise, amino acids are L-forms.

the possible effects of the polarity or of the bulkiness of the aromatic side-chain. Compound **5** will finally be considered as representative of aliphatic amino acids. Compounds **6–9** will be considered in the last section to investigate the possible role of the position of the amino acid residue on the self-inclusion process. Finally, compounds **10**, **11** and **12** will serve to study the possible effect of the size of the cavity.

Synthesis

Mono(6-amino-6-deoxy)cyclomaltoheptaose **a** was obtained in three steps from the parent cyclodextrin as described elsewhere.[6] It should be emphasized that the compound may contain up to 15% β-cyclodextrin which was removed by ion-exchange chromatography. We describe here the synthesis of mono(6-amino-6-deoxy)cyclomaltooctaose **b** under control of the inclusion process. The mono(6-*O*-*p*-tosyl-6-deoxy)cyclomaltooctaose is indeed very difficult to obtain in a pure form since γ-cyclodextrin does not form a complex with tosyl derivatives. Conversely, the molecular structure of the strong 1:1 inclusion complex formed between naphthalene derivatives

† β-Cyclodextrin = cyclomaltoheptaose. γ-Cyclodextrin = cyclomaltooctaose.

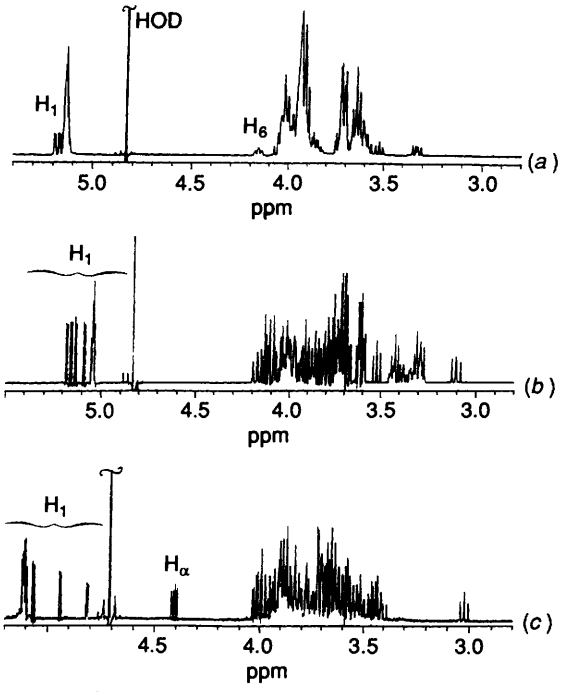


Fig. 2 Partial ^{1}H NMR spectra (298 K; 500 MHz; D_2O; 10 mmol dm^{-3} samples; pH 4.5) of (*a*) glycyl, (*b*) L-phenylalanyl and (*c*) L-tryptophanyl derivatives of β-cyclodextrin (compounds **1**, **2b** and **4**, respectively)

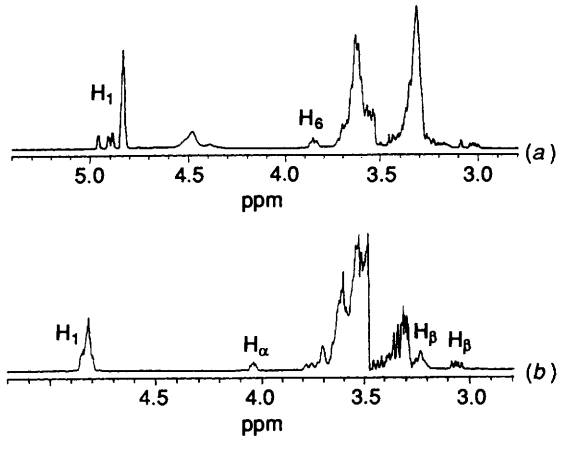


Fig. 3 Partial ^{1}H NMR spectra (298 K; 500 MHz; 10 mmol dm^{-3} samples; pH 4.5) of (*a*) glycyl (*b*) L-phenylalanyl derivatives of β-cyclodextrin(hydrochlorides) in [$^2H_6$]DMSO

and γ-cyclodextrin suggests that naphthalenesulfonyl chloride can be used to control the substitution of γ-cyclodextrin. The mono(6-*O*-naphthalenesulfonyl-6-deoxy)cyclomaltooctaose was indeed obtained in good yield. This compound was prepared by the reaction of dry γ-cyclodextrin with three equiv. of naphthalenesulfonyl chloride in dry pyridine for 3 h. Note that the concentration of γ-cyclodextrin must be lower than 2×10^{-2} mol dm^{-3} to avoid polysubstitution. Mono(6-*O*-naphthalenesulfonyl-6-deoxy)cyclomaltooctaose was converted into mono(6-azido-6-deoxy)cyclomaltooctaose by reaction with lithium azide in water. This compound was reduced smoothly by treatment with triphenylphosphine in *N*,*N*-dimethylformamide (DMF) and addition of concentrated ammonium hydroxide. Mono(6-amino-6-deoxy)cyclomaltooctaose **b** was purified by ion-exchange chromatography as described before. The mono(6-*O*-naphthalenesulfonyl-6-deoxy)cyclomaltooctaose was quantitatively converted into mono(*N*-aminoethylene-6-amino-6-deoxy)cyclomaltooctaose **c** by nucleophilic substitution with diaminoethane as solvent.

The pertinent amino acid was *N*-protected by the fluoren-9-ylmethoxycarbonyl (FMOC)[7] group and grafted to cyclodextrin derivatives **a**, **b** or **c** using the 1,3-dicyclohexylcarbodiimide–hydroxybenzotriazole (DCC–HOBT)[8] procedure in anhydrous DMF at 0 °C. Direct grafting of *N*-FMOC-L-tryptophan onto monoaminocyclodextrins failed and the coupling was hence performed in two steps: the carboxyl group of the *N*-protected amino acid was first activated using the DCC–hydroxysuccinimide procedure[9] and then coupled to the monoaminocyclodextrin in DMF. In both cases, the crude material was purified from small amounts of unreacted aminocyclodextrins by ion-exchange chromatography in DMF–water (2:1 v/v) and deprotected with piperidine in DMF. The final material was purified by ion-exchange chromatography, converted into the hydrochloride (for β-cyclodextrin compounds) and freeze-dried. The chemical and optical purity was checked by ^{1}H NMR. The results obtained with D- and L-phenylalanine show that the entire process is racemization free.

Results and discussion

A comparison of the structural properties of β-CD derivatives in the free base and ammonium forms has shown that the protonation process does not modify the general conclusions.[2] In the following all β-CD derivatives will be investigated as the protonated forms since these compounds are more soluble in water as the corresponding free bases. γ-CD derivatives exhibit the same behaviour but they are less stable at even slightly acidic pH. This behaviour is related to the lower chemical stability of γ-CD itself. All γ-CD derivatives were therefore investigated at neutral or slightly basic pH. In all cases, the working pH will be indicated in the experimental conditions and in the Figure captions.

Cyclomaltoheptaose derivatives

Effect of the nature of the amino acid. *Observation of the proton NMR spectra in deuterium oxide and in DMSO.*—The partial NMR spectra of the three pertinent amino acid derivatives (compounds **1**, **2a** and **4**) in D_2O are displayed in Fig. 2 with the region of anomeric protons since the latter is highly representative of the reduction of the seven-fold symmetry. In this Figure, a number of resonances are indicated. Assignments were performed using two-dimensional high resolution experiments as described elsewhere.[10]

Besides the case of the glycine derivative **1** for which only weak inequivalence is observed as expected from the substitution effect and of the L-Leu derivative **5** (spectrum not shown), all other compounds display a considerable spectral dispersion. Compounds **2a**, **2b** and **3** display similar spectra with almost complete separation of anomeric protons. A number of non-aromatic protons are shifted upfield by as much as 1 ppm relative to the value found in the glycine derivative. Conversely, when NMR spectra are collected using [$^2H_6$]DMSO as solvent, this spectral dispersion collapses and only weak inequivalences are retained. This is displayed in Fig. 3 for the glycine and phenylalanine derivatives. Very similar spectra, as far as the cyclodextrin moiety is concerned, are obtained from all other samples. Since DMSO has a strong solvating power for the cyclodextrin cavity, it is known to preclude the formation of inclusion complexes. This behaviour is the first clue to the possible inclusion of the aromatic rings in the cavity of the cyclodextrin in aqueous solutions. Compound **4** from L-tryptophan exhibits a peculiar behaviour as several anomeric protons show considerable upfield shifts. At the present time, the leucine derivative **5** seems to be more close to the glycine analogue. It should be emphasized, however, that the aliphatic side-chain is not expected to induce as important shifts as the

Table 1(*a*) Scalar coupling constants of the amino acid moieties measured for the peptido-cyclodextrins in D_2O and [$^2H_6$] DMSO compared with the free amino acids in water

Solvent	$J_{\alpha-\beta}$/Hz	$J_{\alpha-\beta'}$/Hz
Compound **2a**		
D_2O	5.0	10.5
DMSO	5.4	7.9
Free amino acid in D_2O	5.2	8.0
Compound **2b**		
D_2O	6.0	10.1
DMSO	5.4	7.9
Free amino acid in D_2O	5.2	8.0
Compound **3**		
D_2O	4.7	11.0
DMSO	5.5	8.0
Free amino acid in D_2O	5.5	7.6
Compound **4**		
D_2O	4.9	9.1
DMSO	5.1	8.6
Free amino acid in D_2O	5.3	7.4
Compound **5**		
D_2O	7.2	7.2
DMSO	7.0	7.0
Free amino acid in D_2O	5.9	8.0

Table 1(*b*) Rotamer populations about the C_α–C_β bond (derived from coupling constants) in the amino acid moieties of the peptido-cyclodextrins in D_2O and [$^2H_6$]-DMSO compared with values obtained for the free amino acids in water

	Rotamer (%)		
Solvent	gt	tg	gg
Compound **2a**			
D_2O	12	88	0
DMSO	25	50	25
Free amino acid in D_2O	26	48	26
Compound **2b**			
D_2O	14	84	0
DMSO	24	53	23
Free amino acid in D_2O	26	53	21
Compound **3**			
D_2O	18	82	0
DMSO	25	50	25
Free amino acid in D_2O	25	50	25
Compound **4**			
D_2O	18	64	18
DMSO	20	59	21
Free amino acid in D_2O	22	45	33
Compound **5**			
D_2O	44	49	14
DMSO	41	41	18
Free amino acid in D_2O	29	52	19

aromatic amino acids, even if self-inclusion is present and more sophisticated experiments will be required. To allow a clear distinction between inter- and intra-molecular complexes, the NMR spectra of all compounds have been collected at several concentrations (in the range 0.5–25 mmol dm^{-3}) keeping the pH and temperature constant. No important variations of the basic features of the NMR spectra could be observed although the L-tryptophan derivative exhibits the largest variations. At the present stage, it seems reasonable to postulate that all aromatic derivatives form intramolecular inclusion complexes in solution. The possible implications of such a process for larger peptides encouraged us to peform complementary experiments to provide clear evidence of the presence (or absence) of self-inclusion complexes. Before using more sophisticated approaches, it is worth considering the conformational properties of the amino acid moiety.

Conformational properties of the amino acid moiety in water and DMSO.—The most readily available NMR parameter concerns the conformation about the C_α–C_β bond. It is however important to note that, in the case of the present derivatives, H_α and H_β are not always clearly visible on the NMR spectra precluding an accurate analysis of coupling constants. This difficulty was solved by the use of two dimensional NMR experiments (phase sensitive COSY or double-quantum correlations) or by selective excitation transfers.[11] The pertinent coupling constants were thus derived for all derivatives in D_2O and DMSO and converted into rotamer populations.[12] The results are collected in Table 1(*a*) and (*b*). For comparison purposes, the values found in free amino acids or in random-coiled peptides are reported as well.

It is clear from these Tables that derivatives of phenylalanine (**2a**, **2b**) and L-tyrosine (**3**) exhibit extremely different behaviour in water and DMSO. In the former case, the tg rotamer is by far the most populated and taking into account the accuracy of the relation between coupling constants and rotamer populations, it can be concluded that the C_α–C_β bond is locked in a single position. Conversely, in DMSO solution, the conformational equilibrium between the three rotamers is restored and the derived populations are similar to those encountered for the free amino acid in water. In derivatives **4** and **5** (from L-tryptophan and L-leucine, respectively) such a strong difference is not encountered and conformational equilibrium is retained in all cases, whatever the nature of the solvent. If, as expected, the strong conformational strain observed in derivatives **2a**, **2b** and **3** is related to a self-inclusion process, compounds from L-leucine and L-tryptophan do not experience this effect. Additional proofs will hence require the use of more dedicated experiments to assess the reality of this self-inclusion.

Use of dipolar interactions.—A more convincing proof for the reality of self-inclusion is expected from the direct observation of spatial proximities between protons of the amino acid side-chain and protons located in the internal cavity of the cyclodextrin. This type of information can be obtained by showing dipolar interactions (nuclear Overhauser effects) between the relevant protons. The use of the classical two dimensional NOESY[13] sequence for this purpose is hampered by the unfavourable correlation time of these compounds at the concentrations and temperatures used. This leads to very weak effects even for spatially close protons. This problem has been solved by the use of the ROESY experiment.[14] In this case, dipolar interactions are expressed in the much weaker rotating magnetic field. The benefits of this type of two dimensional experiment have already been stressed for medium-sized molecules as encountered here.[15,16] However, as has been demonstrated,[17] this type of experiment has two drawbacks: the angular dispersion due to the chemical shift distribution and the indirect transfer (HOHAHA effect) can provide artefacts. Under these conditions, it was considered that the presence of a cross-peak between protons located on two different moieties of the molecule (*e.g.* the amino acid and cyclodextrin parts) was indicative of possible spatial proximity between the relevant signals but the distance between the two protons could not be determined. Although ROESY experiments have been carried out on all compounds, only two of them will be considered here for the sake of brevity. Fig. 4 shows a complete contour plot of a ROESY experiment performed at 600 MHz on **3** in H_2O.

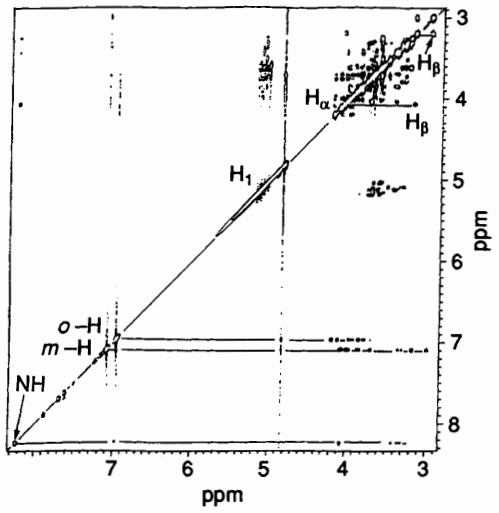


Fig. 4 Complete contour plot of a ROESY experiment (spin lock time: 350 ms; attenuation: 22 dB, 512 scans per time-increment) performed at 600 MHz on **3** (10 mmol dm^{-3}; pH 4.5) in H_2O at 298 K. The intense signal of the solvent at 4.83 ppm was suppressed by presaturation (1 s at low power).

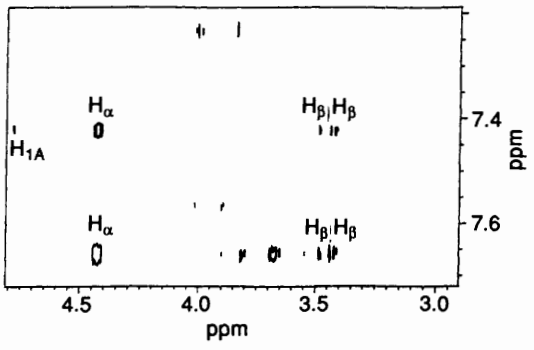


Fig. 5 Partial contour plot of a ROESY experiment (spin lock time: 350 ms; attenuation: 22 dB, 512 scans per time-increment) performed at 500 MHz on **4** (10 mmol dm^{-3}; pH 4.5) in D_2O at 298 K. Horizontal scale: cyclodextrin region; vertical scale: aromatic region.

A large number of cross-peaks between the aromatic protons of tyrosine and protons of the cyclodextrin is observed. As all protons of the cyclodextrin have been assigned previously, it is obvious that the aromatic protons of the phenol ring experience interactions with protons located in the cavity of the cyclodextrin (virtually only protons 3 and 5 are involved in these interactions). This observation fully supports the reality of the intramolecular self-inclusion complex for this compound. The two derivatives obtained from L- and D-phenylalanine (**2a, b**) exhibit similar behaviour and the same conclusions can be drawn, although the glucose units involved in the interactions differ between all three compounds indicating slightly different orientations of the aromatic ring in the cavity as shown by used one dimensional NOE difference experiments.[18]

A quite different situation is encountered with the L-tryptophan derivative **4**. Fig. 5 shows the corresponding ROESY contour plot. In this case, a limited number of dipolar interactions are observed. Furthermore, they do not involve proximities between the aromatic protons of the indole ring and protons located in the cavity of the cyclodextrin torus. A sharp cross-peak indeed shows spatial proximity between H_2 of the indole ring and one anomeric proton of the cyclodextrin. This implies that the indole moiety is not included in the cavity.

ROESY experiments performed on the L-leucine derivative **5** failed to show any correlation between the aliphatic side-chain and the cavity indicating no or extremely weak inclusion in this case. From the present ROESY experiments, the following conclusions can be drawn. (*i*) Compounds **2a**, **2b** and **3** from L-phenylalanine, D-phenylalanine and L-tyrosine, respectively, show evidence for the presence of intramolecular self-inclusion complexes in aqueous solution. This is in agreement with the observed spectral dispersion as induced by the strong ring currents created by the aromatic rings as well as with the conformational constraint at the C_α–C_β bond as evidenced by the analysis of scalar coupling constants. (*ii*) Compound **4** from L-tryptophan does not show self-inclusion, but rather it appears that the indole ring has a specific orientation outside the cavity. The corresponding induced ring currents support the large spectral dispersion of anomeric protons. (*iii*) Compound **5** from L-leucine does not show inclusion of the side-chain.

These conclusions imply that in the former case (**2a**, **2b**, **3**) the cavity of β-cyclodextrin is permanently occupied and that in the case of **4** and **5** it remains vacant. This general concept can be supported by competition experiments dedicated to determining whether the cavity is occupied or vacant.

Competition experiments.—Attempts to include external guests in the cavity of the title compounds had two main goals, first to investigate the potential vacancy of this cavity and secondly to estimate the strength of the self-inclusion complex. The external guest was selected according to the following criteria: (*i*) the guest must have a high affinity for the cavity of β-cyclodextrin and (*ii*) the NMR spectrum of the guest should not superimpose that of the hosts. Sodium anthraquinone-2-sulfonate (ASANa) fulfils these requirements since it interacts strongly with cyclodextrins as already reported[19] and its NMR spectrum is limited to the 8.5–7.8 ppm domain.

Addition of ASANa to any of the cyclodextrin derivatives induced shifts of almost all signals of the potential host. This cannot be considered as a proof for inclusion since the complementary charges of the hosts and guest assume some contribution of ionic interactions between these molecules. The complex nature of the NMR spectra precludes a classical analysis of induced shifts and the use of ROESY experiments was preferred. It is indeed expected that if the external guest can be accommodated in the cavity, dipolar interactions will be observed as already shown in the previous section. For this purpose, equimolar solutions of the potential host and of ASANa were subjected to ROESY experiments under strictly identical conditions and the results compared with those obtained in the absence of the competing guest.

With compound **3**, no cross-peaks between the protons of ASANa and those of the cavity of the cyclodextrin derivative are observed. Conversely, the aromatic protons of the amino acid display a large number of well defined cross-peaks with protons 3 and 5 of selected glucose units. Furthermore, these cross-peaks are identical in number intensity and position to those observed in the absence of ASANa indicating clearly that the added guest cannot enter the cyclodextrin cavity, the latter being already fully occupied by the aromatic moiety of the amino acid. This observation implies that the self-inclusion complex is very strongly stabilized. The two compounds **2a** and **2b** exhibit similar behaviour and the same conclusions can be drawn. Conversely, in the case of the L-tryptophan derivative, a large number of well resolved dipolar interactions are evidenced between the protons of ASANa and those of the cavity. The cavity is therefore vacant and can be occupied by the competitive guest. The dipolar interactions between the indole ring and protons of the external part of the cyclodextrin are retained in the presence of ASANa indicating that the peculiar orientation of the indole ring is still present in the complex with ASANa.

As a conclusion to this section, it appears that the combination of ROESY, NOE difference and competition experiments provides a clear picture of the potential formation of intramolecular inclusion complexes in this series. It can be

Table 2(*a*) Scalar coupling constants of the L-phenylalanyl moiety measured for **10** in D_2O at various temperatures

T/K	$J_{\alpha-\beta}$/Hz	$J_{\alpha-\beta'}$/Hz
283	4.5	9.7
293	5.0	9.1
303	5.8	9.0
313	6.1	9.0

Table 2(*b*) Rotamer populations at the C_α–C_β bond of the L-phenylalanyl moiety measured for **10** in D_2O at various temperatures

	Rotamers (%)		
T/K	gt	tg	gg
283	14	71	15
293	19	62	19
303	28	62	10
313	31	62	7

concluded that both derivatives of phenylalanine lead to strong intramolecular self-inclusion complexes in water as postulated from the NMR spectra. Similarly, a self-inclusion complex was evidenced in the case of the L-tyrosine derivative. The presence of the hydroxy group on the aromatic ring does not preclude the inclusion of this moiety. Conversely, and probably for steric reasons, the indole ring of L-tryptophan does not self-include in the cyclodextrin cavity which is left vacant and prone to accommodate external guests. This conclusion differs from the preliminary assumption based on the simple consideration of the chemical shift dispersion. This proves that a simple observation of NMR spectra might not be sufficient to ascertain inclusion processes. More complex and dedicated experiments should be performed to provide proof for the presence or absence of intramolecular complexes.

Effect of the position of the amino acid residue in a peptide sequence on the formation of intramolecular self-inclusion complexes. We have observed in the previous section that when phenylalanine or tyrosine is grafted onto mono(6-amino-6-deoxy)-β-cyclodextrin, self-inclusion complexes are formed. Our purpose being to graft more complex peptides, it is of considerable importance to determine whether this behaviour depends upon the position of the aromatic residue in the peptide sequence. In order to get information about this potential problem, compounds **6**–**9** (see Fig. 1) have been synthesized. In compounds **7** and **9**, the aromatic amino acid is in a situation similar to that described with the single amino acid derivatives. Conversely, in **6** and **8**, glycine is used as a spacer.

As emphasized previously, the simple observation of the NMR spectra should not be considered as indicative of self-inclusion processes. However a lesser spectral dispersion is observed in **6** and **8** where glycine is used as a spacer between the aromatic amino acid and the cyclodextrin.

The general strategy used for the previous compounds will be applied to the four dipeptide derivatives, namely the comparison of ROESY experiments in the absence and in the presence of ASANa (data not shown).

According to the previously detailed analysis, it was demonstrated that the cavity is fully occupied in the case of the Gly-L-Phe derivative and remains vacant in the L-Phe-Gly isomer. Identical conclusions (data not shown) can be drawn for the corresponding tyrosine derivatives. It can hence be concluded that the presence of another amino acid on the *N*-terminal side of tyrosine or phenylalanine does not affect the formation of a strong intramolecular inclusion complex. Conversely, insertion of glycine as a spacer between β-cyclodextrin and the aromatic amino acid totally precludes self-inclusion.

Cyclomaltooctaose derivatives

In order to get a deeper insight into the importance of the size of the cavity, a number of amino acyl derivatives of cyclomaltoheptaose were prepared as described in the Experimental section. These compounds were investigated in aqueous solution as the corresponding cyclomaltoheptaose analogues, but as the free bases as already emphasized.

The different behaviour of phenylalanyl and tryptophanyl residues in cyclomaltoheptaose derivatives leads us to select these two amino acids to investigate the role of the cavity size. It could indeed be expected that the tryptophanyl derivative could form a self-inclusion complex in cyclomaltooctaose owing to the wider cavity.

The ^1H NMR spectrum of the L-phenylalanyl derivative in D_2O shows a spectral dispersion which is much lower than in the corresponding β-cyclodextrin derivative **2**. However, the ROESY experiment provides evidence of dipolar contacts between aromatic protons of the Phe moiety and protons from the cavity of the cyclodextrin.

Note that NMR spectra of compound **10** have been collected at several concentrations (in the 1–15 mmol dm^{-3} range) keeping both pH and temperature constant; no large variations in the NMR spectra could be observed. Conversely, the effects of variation in temperature, keeping pH and concentration constant, were very important. When the temperature was increased, most of the signals were shifted downfield and the NMR spectrum displayed a better resolution. But the most affected NMR parameter concerns the conformation around the C_α–C_β bond. The pertinent coupling constants were derived for all temperatures in D_2O and converted into rotamer populations [Table 2(*a*) and (*b*)].

Note from Table 2 that at a lower temperature the tg rotamer is the most populated and it can be concluded that the C_α–C_β bond is locked in a single position. Conversely, at a higher temperature conformational equilibrium between both rotamers is restored. Finally, attempts to perform competition experiment leads to the following conclusion: the cavity is not vacant, but could be occupied by the competitive guest as ASANa.

This study confirms that the aromatic moiety of phenylalanine is also self-included in the cavity of the cyclomaltooctaose carrier. However, interactions are clearly much weaker than with the β-cyclodextrin analogue suggesting a much looser fitting of the aromatic ring in the cavity.

Concerning the tryptophan derivative, the results obtained differ slightly from those obtained with the β-CD analogue. No important variations in the NMR spectra could be observed with several concentrations. Moreover, the conformation around the C_α–C_β bond was not affected by temperature variations. The coupling constant derived for compound **11** in D_2O and converted into rotamer populations were similar to those obtained for compound **4**: for both compounds, no conformational strain was observed. Addition of any guest to compound **11** leads to the same results obtained for the β-cyclodextrin analogue: the cavity was vacant and could be occupied by the guest. But although the spectral dispersion is similar to that obtained with compound **4**, especially if anomeric protons are considered, the ROESY experiment displays spatial proximities between proton H_2 of the indole ring and protons H_5 or H_6 of the cyclomaltooctaose. Furthermore, a weak interaction between H_7 of the indole ring and one anomeric proton of the cyclodextrin implies that the indole moiety is not included in the cavity and suggests that the tryptophan derivative is located near the primary side.

Finally, insertion of a spacer between the cyclodextrin and the tryptophan moiety also resulted in a complete suppression of these interactions. Moreover, compound **12** exhibits a strong amphiphilic character since the NMR signals of spectrum obtained in water appeared as broad lines.

Experimental

General procedures and instrumentation

The β- and γ-cyclodextrins obtained from Roquette Freres and Wacker Companies, respectively, were freeze-dried before synthesis. Toluene-*p*-sulfonyl chloride (TsCl) and naphthalenesulfonyl chloride (NsCl) were recrystallized from light petroleum, DCC was purified in ethyl acetate immediately before use.

All NMR experiments were performed using Bruker AMX500 or AMX600 spectrometers operating at 500.13 and 600.13 MHz, respectively, using standard pulse programs from the Bruker library. In all cases, the length of the 90° pulse was *ca.* 6 μs. 1D NMR spectra were collected using 16 K data points. All two dimensional experiments were acquired using 2 K data points and 256 time increments. In all experiments, a total relaxation delay of 1.5 s (taking the acquisition time into account) was allowed. Scalar correlations (COSY, Relay and Double-quantum correlation experiments) were processed in the absolute value mode after zero-filling resulting in a 1 K × 1 K (real points) data matrix. For dipolar correlations (ROESY experiments), the phase sensitive (TPPI) sequence was used and processing resulted in a 1 K × 1 K (real–real) matrix. All samples derived from β-cyclodextrin were used as hydrochlorides. The probe temperature was carefully controlled within 0.1° by means of a Haake exchange device. Chemical shifts are given in ppm downfield from external tetramethylsilane (TMS). D_2O and [$^2H_6$]DMSO were obtained from Euriso-Top (France). Elemental analyses were performed at the Service Central de Microanalyses of CNRS, Lyon, France. The samples were dried under high-vacuum. Mass spectrometry was performed on a SCIEX spectrometer with electrospray infusion mode. IR spectra obtained on a Perkin-Elmer 1725X spectrometer are reported in cm^{-1}. Unless indicated otherwise, all solutions in D_2O were adjusted to pH 4.5 or 7.5 (uncorrected meter reading) for β- and γ-CD derivatives, respectively.

Synthesis

6-Amino-6-deoxycyclomaltooctaose b. 6-*Naphthalenesulfonyl-6-deoxycyclomaltooctaose*.—Dry γ-cyclodextrin (1 g, 0.771 mmol) was dissolved in pyridine (60 cm^3) and NsCl (524 mg, 2.3 mmol) was added. The reaction mixture was stirred for 3 h at room temperature. The reaction was stopped by addition of 10 cm^3 of water. After evaporation of solvent under reduced pressure, the mixture was poured into 250 cm^3 of acetone and the crude product was precipitated. This was isolated by filtration and recrystallization in hot water provided a pure sample of 6-naphthalenesulfonyl-6-deoxycyclomaltooctaose (195 mg, 28%). TLC [NH_4OH (6%)–EtOH–BuOH; 5:5:1 v/v/v] R_f = 0.65 (Found: C, 46.5; S, 2.5; H, 5.9. Calc. for $C_{58}H_{86}O_{42}S$: C, 46.8; S, 2.1; H, 5.8%); δ_H\{500 MHz; [$^2H_6$]DMSO (after addition of 5% D_2O)\} 8.2–7.5 (ArH), 4.78 (grafted 1-H), 4.95–4.85 (other 1-H), 4.30 and 4.45 (grafted 6,6'-H) *m/z* 1488 [M + H]$^+$.

6-*Azido-6-deoxy-cyclomaltooctaose*.—6-Naphthalenesulfonyl-6-deoxycyclomaltooctaose (1.5 g, 1.00 mmol) was dissolved in 100 cm^3 of water and LiN_3 (0.6 g, 12.25 mmol) was added. The solution was stirred for 4 h at 90 °C. The solvent was removed under reduced pressure and the mixture was poured into 100 cm^3 of acetone. The crude material was isolated by filtration, washed with acetone and boiled ethanol. We obtained 980 mg (74%) of a pure sample, TLC (DMF–BuOH–H_2O; 2:1:1 v/v/v); R_f = 0.80 (Found: C, 43.5; N, 3.0; H, 6.1. Calc. for $C_{48}H_{79}N_3O_{39}$: C, 43.6; N, 3.15; H, 6.0%); ν_{max}/cm^{-1} 2100 (N_3); δ_H(500 MHz; D_2O) absence of aromatic signals, 5.20–5.15 (1-H), 3.75–3.60 (2- and 4-H); 4.10–3.80 (3-, 5- and 66'-H); *m/z* 1322 [M + H]$^+$.

6-*Amino-6-deoxycyclomaltooctaose*.—6-Azido-6-deoxycyclomaltooctaose (0.64 g, 0.484 mmol) was dissolved in 20 cm^3 DMF. To this solution, triphenylphosphine (507, 1.93 mmol) dissolved in 10 cm^3 of DMF was added dropwise. The solution was stirred at room temperature for 1 h and cooled at 0 °C. 15 cm^3 of aq. ammonia (20%) was added and the solution was stirred at room temperature for 18 h. The solvents were removed under reduced pressure. When the residual solid was dissolved in 100 cm^3 of water, copious amounts of a white precipitate resulted. After filtration, the aqueous solution was concentrated under reduced pressure and then poured into 100 cm^3 of acetone. The filtration provided the crude compound (550 mg, 90%). It was dissolved in 5 cm^3 of water and layered into a lewatit SP1080H$^+$ column (3 × 25 cm prepared with water) and eluted with 500 cm^3 of water and then with 100 cm^3 of aq. ammonia (6%). The pure compound appeared in ammonia fractions. These fractions were evaporated almost to dryness and lyophilized to yield 482 mg (77%) of 6-amino-6-deoxycyclomaltooctaose. TLC (BuOH–DMF–H_2O; 2:1:1 v/v/v); R_f = 0.2 (Found: O, 47.95; N, 2.0; H, 6.7. Calc. for $C_{48}H_{81}NO_{39}$: O, 47.8; N, 2.2; H, 6.6%); δ_H(500 MHz; D_2O) 5.25 (grafted 1-H); 5.20–5.15 (other 1-H); *m/z* 1296 [M + H]$^+$, 649 [M + 2H]$^{2+}$.

***N*-Aminoethylene-6-amino-6-deoxycyclomethooctaose c.** 6-Naphthalenesulfonyl-6-deoxycyclomaltooctaose (1.02 g, 8.06 mmol) was dissolved in 7 cm^3 of 1,2-diaminoethane. The mixture was stirred at room temperature for 18 h. Most of the diaminoethane was removed under reduced pressure to give a thick syrup which was poured into 500 cm^3 of acetone. The crude material precipitated and was filtered, washed with acetone and dried. This product was dissolved in 5 cm^3 of water and layered into a lewatit SP1080 H$^+$ column (4 × 50 cm prepared with water) eluted with 500 cm^3 of water and then with 200 cm^3 of aq. ammonia (6%). The basic fractions were evaporated to near dryness and the syrup was poured into 300 cm^3 of acetone. The pure compound (850 mg, 79%) was filtered, washed with acetone and dried. TLC (NH_4OH–BuOH–EtOH; 5:1:5 v/v/v); R_f = 0.26; *m/z* 1339 [M + H]$^+$ (Calc. for $C_{50}H_{85}N_2O_{39}$ 1338). Owing to its highly surfacting properties, this compound aggregates in solution resulting in extremely broad NMR signals.

Peptidocyclodextrins. N-*FMOC-amino acid*.—The amino acid (5.33 mmol) was dissolved in 20 cm^3 of water. To this solution, 1 equiv. of sodium hydrogen carbonate, 20 cm^3 of acetone and 1 equiv. of fluoren-9-ylmethyloxycarbonyl-*O*-succinimide ester (FMOC-*O*-Su) were added. The suspension was stirred at room temperature until the mixture was clear. Acidification of the solution with 35 cm^3 of aq. $KHSO_4$ led to the precipitation of the *N*-FMOC-amino acid. After filtration, the sample was washed with water and dried. We have obtained the following pure samples: *N*-fluoren-9-ylmethoxycarbonylglycine (95% yield); *N*-fluoren-9-ylmethoxycarbonyl-L-phenylalanine (93% yield); *N*-fluoren-9-ylmethoxycarbonyl-D-phenylalanine (95% yield); *N*-fluoren-9-ylmethoxycarbonyl-L-tyrosine (88% yield); *N*-fluoren-9-ylmethoxycarbonyl-L-tryptophan (77% yield); *N*-fluoren-9-ylmethoxycarbonyl-L-leucine (91%); *N*-fluoren-9-ylmethoxycarbonylglycyl-L-phenylalanine (96% yield); *N*-fluoren-9-ylmethoxycarbonylglycyl-L-tyrosine (76% yield); TLC[BuOH–AcOH (60%); 6:4, v/v] R_f 0.9; ^1H NMR spectra were performed in [$^2H_6$]DMSO and were in agreement with pure samples without free amino acid.

Grafting route.—General procedure. DCC (0.35 mmol) and hydroxybenzotriazole (0.35 mmol) were dissolved in 5 cm^3 of

dry DMF. The solution was stirred at 0 °C under nitrogen for 15 min. 1 Equiv. of *N*-FMOC-amino acid and 1 equiv. of amino cyclodextrin dissolved in 15 and 10 cm^3 of dry DMF, respectively, were added dropwise. The mixture was stirred at 0 °C, under nitrogen atmosphere, for 15 h. The solvent was removed under reduced pressure and the residual syrup was poured into 100 cm^3 of acetone. The white precipitate obtained was filtered, washed with acetone and dried. The crude material was dissolved in a mixture of 2 cm^3 of DMF and 4 cm^3 of water and layered onto a column of lewatit SP1080H$^+$ (10 × 1 cm prepared in DMF–water 2:1, v/v) and eluted with 100 cm^3 of DMF–water (2:1, v/v). The fraction containing the pure sample was processed as described for the crude compound.

6-[N-(*Fluoren-9-ylmethoxycarbonyl*)*glycylamido*]-6-*deoxycyclomaltoheptaose*.—Yield 86%; R_f 0.25 [BuOH–AcOH (60%); 6:4, v/v] (Found: C, 45.05; H, 6.3; N, 3.6; O, 45.5. Calc. for $C_{59}H_{97}N_2O_{44}$: C, 45.08; H, 6.30; N, 3.02; O, 45.70%).

6-[N-(*Fluoren-9-ylmethoxycarbonyl*)-L-*phenylanylamido*]-6-*deoxycyclomaltoheptaose*.—Yield 90%; R_f 0.30 [BuOH–AcOH (60%); 6:4, v/v] (Found: C, 48.8; H, 6.2; N 2.6; O 41.6. Calc. for $C_{66}H_{98}N_2O_{41}$: C, 49.20; H, 6.20; N, 2.10; O, 41.60%).

6-[N-(*Fluoren-9-ylmethoxycarbonyl*)-L-*tyrosinamido*]-6-*deoxycyclomaltoheptaose*.—Yield 95%; R_f 0.30 [BuOH–AcOH (60%); 6:4, v/v] (Found: C, 49.5; H, 6.6; N, 2.5; O, 41.7. Calc. for $C_{66}H_{96}N_2O_{41}$: C, 50.10; H, 6.38; N, 2.26; O, 41.70%).

6-[N-(*Fluoren-9-ylmethoxycarbonyl*)-L-*leucinamido*]-6-*deoxycyclomaltoheptaose*.—Yield 97%; R_f 0.30 [BuOH–AcOH (60%); 6:4, v/v] (Found: C, 49.9; H, 6.8; N, 2.5; O, 41.9. Calc. for $C_{63}H_{98}N_2O_{40}$: C, 49.90; H, 6.60; N, 2.10; O, 41.70%).

6-[N-*Fluoren-9-ylmethoxycarbonyl*)-L-*glycyl*-L-*phenylalanyl-amido*]-6-*deoxycyclomaltoheptaose*.—Yield 96%; R_f 0.20 [BuOH–AcOH (60%); 6:4, v/v].

6-[N-*Fluoren-9-ylmethoxycarbonyl*)-L-*phenylalanylglycyl-amido*]-6-*deoxycyclomaltoheptaose*.—Yield 92%; R_f 0.20 [BuOH–AcOH (60%); 6:4 v/v].

6-[N-(*Fluoren-9-ylmethoxycarbonyl*)*glycyl*-L-*tyrosinamido*]-6-*deoxycyclomaltoheptaose*.—Yield 94%; R_f 0.35 [BuOH–AcOH (60%); 6:4, v/v].

6-[N-(*Fluoren-9-ylmethoxycarbonyl*)-L-*tyrosinylglycyl-amido*]-6-*deoxycyclomaltoheptaose*.—Yield 97%; R_f 0.35 [BuOH–AcOH (60%); 6:4 v/v].

6-[N-*Fluoren-9-ylmethoxycarbonyl*)-L-*phenylanylamido*]-6-*deoxycyclomaltooctaose*.—Yield 97%; R_f 0.35 [BuOH–AcOH (60%); 6:4, v/v].

^{2}H NMR data in DMSO were consistent with the expected structure and showed that starting materials were absent.

N-(*Fluoren-9-ylmethoxycarbonyl*)-L-*tryptophan*-O-*succinimide*.—FMOC-L-Trp (240 mg, 1 mmol) was dissolved in 20 cm^3 of ethyl acetate. 125 mg Hydroxysuccinimide was added and the mixture was stirred for 10 min and cooled at 0 °C. DCC (210 mg) dissolved in 20 cm^3 of ethyl acetate was added under nitrogen atmosphere. The mixture was stirred for 15 h at 0 °C and under nitrogen atmosphere. The white precipitate of dicyclohexylurea formed was eliminated by filtration and the solvent was removed under reduced pressure and the sample was dried under high vacuum and used without other purification. Yield 95%; R_f 0.35 [BuOH–AcOH (60%); 6:4, v/v]; NMR data: the ^{1}H NMR spectrum performed in [$^2H_6$]DMSO, showed the absence of free carboxyl groups and the presence of a singlet at 2.6 ppm owing to the methylene of the succinimide group. Upon activation, H_α of the amino acid moiety moved from 3.95 to 4.40 ppm.

Coupling procedure.—Amino cyclodextrin (0.26 mmol) was dissolved in 5 cm^3 of dry DMF under nitrogen atmosphere. 1 Equiv. of *N*-(fluoren-9-ylmethoxycarbonyl)-L-tryptophan-*O*-succinimide ester dissolved in 5 cm^3 of dry DMF was added. The mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and the residual syrup was poured into 50 cm^3 of acetone. The white precipitate was filtered, washed with acetone and dried. The crude material was dissolved in a mixture of 2 cm^3 of DMF and 4 cm^3 of water and layered into a lewatit SP1080H$^+$ column (10 × 1 cm prepared with DMF–water; 2 : 1, v/v) and eluted with 100 cm^3 of DMF–water (2 : 1, v/v). The solvent of the fraction was removed under reduced pressure and the pure sample was precipitated by addition of 50 cm^3 of acetone.

6-[N-*Fluoren-9-ylmethoxycarbonyl*)-L-*tryptophanamido*-6-*deoxycyclomaltoheptaose*.—*N*-protected amino acid: *N*-fluoren-9-ylmethoxycarbonyl-L-tryptophanaminocyclodextrin: 6-amino-6-deoxycyclomaltoheptaose; yield 87%; R_f 0.30 [BuOH–AcOH (60%); 6:4, v/v].

6-[N-(*Fluoren-9-ylmethoxycarbonyl*)-L-*tryptophanamido*]-6-*deoxycyclomaltooctaose*.—*N*-protected amino acid: *N*-fluoren-9-ylmethoxycarbonyl-L-tryptophan; aminocyclodextrin: 6-amino-6-deoxycyclomaltooctaose; yield 92%; R_f 0.35 [BuOH–AcOH (60%); 6:4 v/v].

6-[N-(*Fluoren-9-ylmethoxycarbonyl*)-L-*tryptophanamido*]-*aminoethylene*-6-*deoxycyclomaltooctaose*.—*N*-protected amino acid: *N*-fluoren-9-ylmethoxycarbonyl-L-tryptophanamino cyclodextrin: *N*-aminoethylene-6-amino-6-deoxycyclomaltooctaose. Yield 88%; R_f 0.35 [BuOH–AcOH (60%); 6:4, v/v].

In all cases, ^{1}H NMR spectroscopy in DMSO was used to provide evidence for the purity of compounds.

Deprotection of the amino group.—The *N*-protected compound (0.15 mmol) was dissolved in 10 cm^3 DMF. 10 cm^3 Piperidine was added to this solution. The mixture was stirred at room temperature for 1 h. The solvents were removed under reduced pressure and the crude material was precipitated by addition of 30 cm^3 of acetone, filtered, washed with acetone and dried. It was dissolved in 3 cm^3 water and layered into a lewatit SP1080H$^+$ column (10 × 1 cm prepared with water) and eluted with 50 cm^3 water and then by 10 cm^3 aq. ammonia (6%). The pure compound appeared in the ammonia fraction. This fraction was evaporated almost to dryness and lyophilized to afford the pure sample.

6-*Glycylamido*-6-*deoxycyclomaltoheptaose* **1**.—Yield 84%; R_f 0.25 [BuOH–DMF–H_2O; 2:1:1, v/v/v) (Found: C, 40.3; H, 6.35; N, 2.4; O, 47.65. Calc. for $C_{44}O_{35}N_2H_{74}$: C, 40.60; H, 6.31; N, 2.40; O, 47.90); ν_{max}/cm^{-1} 1660 (CONH); *m*/*z* 1191 [M + H]$^+$.

6-L-*Phenylalanylamido*-6-*deoxycyclomaltoheptaose* **2**.—Yield 94%; R_f 0.45 [BuOH–DMF–H_2O; 2:1:1, v/v/v) (Found: C, 42.4; H, 6.1; N, 2.4; O, 44.5. Calc. for $C_{51}H_{80}N_2O_{35}$. C, 43.10; H, 6.40; N, 2.40; O, 44.75%); ν_{max}/cm^{-1} 1675 (CONH); *m*/*z* 1281 [M + H]$^+$

6-L-*Tyrosinamido*-6-*deoxycyclomaltoheptaose* **3**. Yield 94%; R_f 0.40 (BuOH–DMF–H_2O; 2:1:1, v/v/v) (Found: C, 44.1; H, 6.3; N, 2.4; O, 45.3. Calc. for $C_{51}H_{80}N_2O_{36}$: C, 43.82; H, 6.35; N, 2.40; O, 44.90%); ν_{max}/cm^{-1} 1675 (CONH); *m*/*z* 1297 [M + H]$^+$.

6-L-*Tryptophanylamido*-6-*deoxycyclomaltoheptaose* **4**.—Yield 96%; R_f 0.50 (BuOH–DMF–H_2O; 2:1:1, v/v/v); ν_{max}/cm^{-1} 1670 (CONH); *m*/*z* 1320 [M + H]$^+$.

6-L-*Leucylamido*-6-*deoxycyclomaltoheptaose* **5**.—Yield 85%; R_f 0.40 (BuOH–DMF–H_2O; 2:1:1, v/v/v) (Found: C, 41.4; H, 6.8; N, 2.2; O, 47.2. Calc. for $C_{48}H_{82}N_2O_{35}$ C, 41.40; H, 6.51; N, 2.29; O, 47.5%); ν_{max}/cm^{-1} 1670 (CONH); *m*/*z* 1247 [M + H]$^+$.

6-L-*Phenylalanylglycylamido*-6-*deoxycyclomaltoheptaose* **6**.—Yield 93%; R_f 0.45 (BuOH–DMF–H_2O; 2:1:1, v/v/v); ν_{max}/cm^{-1} 1660 (CONH); *m*/*z* 1338 [M + H]$^+$.

6-*Glycyl*-L-*phenylalanylamido*-6-*deoxycyclomaltoheptaose* **7**.—Yield 90%; R_f 0.30 (BuOH–DMF–H_2O; 2:1:1, v/v/v); ν_{max}/cm^{-1} 1660 (CONH); *m*/*z* 1338 [M + H]$^+$.

6-L-*Tyrosinylglycylamido*-6-*deoxycyclomaltoheptaose* **8**.—

Table 3 General behaviour of peptido-cyclodextrins in terms of the formation of intramolecular self-inclusion complexes

Compound	1	2a	2b	3	4	5	6
Self-inclusion	0	++	++	++	0	+	0
Compound	7	8	9	10	11	12	
Self-inclusion	++	0	++	+	0	0	

Yield 88%; R_f 0.45 (BuOH–DMF–H_2O; 2:1:1, v/v/v); ν_{max}/cm^{-1} 1665 (CONH); *m/z* 1354 $[M + H]^+$.

6-*Glycyl*-L-*tyrosinamido*-6-*deoxycyclomaltoheptaose* **9**.—Yield 90%; R_f 0.40 (BuOH–DMF–H_2O; 2:1:1, v/v/v); ν_{max}/cm^{-1} 1660 (CONH); *m/z* 1354 $[M + H]^+$.

6-L-*Phenylalanylamido*-6-*deoxycyclomaltooctaose* **10**.—Yield 74%; R_f 0.50 [BuOH–AcOH (60%); 6:4, v/v]; ν_{max}/cm^{-1} 1675 (CONH); *m/z* 1443 $[M + H]^+$.

6-L-*Tryptophanamido*-6-*deoxycyclomaltooctaose* **11**.—Yield 84%; R_f 0.60 [BuOH–AcOH (60%); 6:4, v/v); ν_{max}/cm^{-1} 1675 (CONH); *m/z* 1482 $[M + H]^+$.

6-L-*Tryptophanylamidoethyleneamino*-6-*deoxycyclomaltooctaose* **12**.—Yield 69%; R_f 0.60 [BuOH–AcOH (60%); 6:4, v/v]; ν_{max}/cm^{-1} 1675 (CONH); *m/z* 1525 $[M + H]^+$.

Conclusions

The capability of cyclodextrin derivatives to form intramolecular self-inclusion complexes is depicted in Table 3. The present study has shown that aromatic amino acids like tyrosine and phenylalanine are prone to form intramolecular self-inclusion complexes when grafted to the primary position of β-cyclodextrin. This behaviour is not affected by the presence of other residues at the *N*-terminal end. This observation implies that grafting of a longer peptide having tyrosine or phenylalanine as a *C*-terminal residue can result in a compound devoid of any use for targeting drugs since the cavity will not be free for inclusion of a drug. In addition, the fact that a part of the peptide is 'hidden' in the cavity can eventually preclude recognition of the pertinent signal by the receptor. An easy solution can be found by the incorporation of glycine as a spacer. Although devoid of any potential applications as vectors, the intramolecular complexes are of considerable interest as they provide pure inclusion complexes in solution. A more detailed analysis of the conformation of these systems can be expected from the combination of NMR and molecular modelling, using NMR data as constraints.[20] This analysis, requiring a complete determination of available NMR parameters such as coupling constants and spatial proximities, is currently in progress for all the presented peptidocyclodextrins. For this purpose, the determination of all torsion angles in the grafting region is of utmost importance. Since a number of the latter are not available from ^1H NMR, labelling with stable isotopes (*e.g.* ^{13}C and ^{15}N) at strategic positions is currently being performed. This approach is expected to provide an unambiguous picture of the conformation in the grafting region. Finally, comparison of the NMR data obtained with the phenylalanine derivatives grafted onto β-cyclodextrin and on the permethyl derivative can be highly informative. The observation that permethylation precludes the formation of intramolecular complexes stresses the key role of primary hydroxy groups. Further studies concerning the possible formation of stabilizing hydrogen bonds between one of these groups and the carbonyl are in progress.

Supplementary material

A complete assignment of the ^1H NMR spectra of compounds listed in this work is available from the authors upon request.

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