Synthesis and characterization of peptidyl-cyclodextrins dedicated to drug targeting

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We have synthesized and investigated 8 peptidyl-cyclodextrin adducts in order to validate the general concept of drug-targeting. The adducts are composed of a β- or γ-cyclodextrin part and a peptidic moiety consisting of the neuropeptide Substance P or one of its shorter derivatives, Substance $P_{4–11}$. All these new compounds were fully characterized by dedicated NMR experiments and by mass spectrometry. The inclusion properties of the cyclodextrin moiety were also preserved as demonstrated by NMR experiments. Antibodies raised against β-, γ-cyclodextrin and Substance P, respectively, recognized the corresponding moiety in all prepared peptidyl-cyclodextrin derivatives.

Introduction

The continuing challenge of using cyclomaltosaccharides (cyclodextrins or CDs) for solubilization and drug targeting has led to the preparation of a wide variety of chemically modified derivatives[1] in order to improve the properties of the host molecules. A possible approach as far as pharmaceutical applications are concerned would be to combine size specificity of cyclodextrins with the recognition signal of bioactive molecules. Grafting bioactive peptides onto cyclodextrins may provide new vectors carrying signal molecules for targeting purposes. Owing to the large variety of potential cellular and tissular receptors, peptides appear as the most versatile class of compounds suitable for grafting to cyclodextrins.

Many publications describe the grafting of amino acids (mono- or per-substitution) on cyclodextrins. The structures of mono-6-peptidylamido-β- and -γ-cyclodextrin in solution have been investigated and it has been shown that for some amino acids strong intramolecular self-inclusion complexes are formed in aqueous solution.[2,3] Per-6-peptidylamido-6-deoxycyclomaltoheptaose derivatives have been investigated also.[4] However, little work has been devoted to large biologically active peptides. *N*-(Leu-enkephalinyl)-6-amido-6-deoxycyclomaltoheptaose[5,6] has been synthesized and represented as a new class of target-directed transporters, whose biological properties have not been investigated. Direct grafting of a hormone molecule,[7] *i.e.* the enkephalin analogue DPDPE (a cyclic peptide corresponding to the Tyr^1-Cys^2[S-S]Cys^3-[p-I]Phe^4-Gly^5 sequence), to βCD significantly reduced receptor affinity and selectivity. Recently, the C-terminal tetrapeptide amide gastrin and the related heptapeptide were grafted on βCD using mono- and per-substitution.[8] The spacer enhanced the receptor recognition process but per-substitution led to strong interference with the biological properties. The inclusion capacity of these derivatives was not investigated.

Here we describe the grafting on β- and γ-cyclodextrin of substance P[9] (SP, an 11-amino-acid peptide) and of one of its shorter derivatives, substance P 4–11 ($SP_{4–11}$) corresponding to the 8 C-terminal amino acids of SP. SP **1** is an active neuropeptide of the tachykinin family, and corresponds to the Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2 sequence.[9] It is involved in a large range of biological processes including nociception, smooth muscle contraction, exocrine gland secretions,[9,10] *etc.* The $SP_{4–11}$ (Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2) **2** derivative exhibits similar biological activities,[11] but possesses a single reactive amino function, which will be of importance for the synthesis of cyclodextrin adducts. All biological effects are due to the interaction of the peptide with a transmembrane protein, the NK-1 receptor,[12,13] which is coupled to the G-proteins.

We report here the synthesis of eight new peptidyl-cyclodextrin adducts, corresponding to the two peptides SP and $\mathbf{SP_{4–11}}$ and two β- and γ-CDs grafted at different positions on the SP backbone:† *N*-[L-(prolylamido)-6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose]-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **5a**, *N*-[L-(prolylamido)-6^I-deoxy-6^I-(succinylamido)cyclomaltooctaose]-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **5b**, *N*-[L-(arginylamido)-6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose]-Pro-N^ε-[trifluoroacetyl-L-(lysylamido)]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **6a**, *N*-[trifluoroacetyl-L-(arginylamido)]-Pro-N^ε-[L-(lysylamido)-6^I-deoxy-6^I-(succinylamido)-cyclomaltoheptaose]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **7a**, *N*-[L-(arginylamido)-6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose]-Pro-N^ε-[L-(lysylamido)-6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **8a**, *N*-[L-(arginylamido)-6^I-deoxy-6^I-(succinylamido)-cyclomaltooctaose]-Pro-N^ε-[trifluoroacetyl-L-(lysylamido)]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **6b**, *N*-[trifluoroacetyl-L-(arginylamido)]-Pro-N^ε-[L-(lysylamido)-6^I-deoxy-6^I-(succinylamido)cyclomaltooctaose]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **7b** and *N*-[L-(arginylamido)-6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose]-Pro-N^ε-[L-lysylamido-6^I-deoxy-6^I-(succinylamido)cyclomaltooctaose]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 **8b**. For the sake of clarity, we will use here the following usual names: βCD-$SP_{4–11}$ **5a**, γCD-$SP_{4–11}$ **5b**, βCD-(Arg^1)-SP **6a**, βCD-(Lys^3)-SP **7a**, di-(βCD)-(Arg^1, Lys^3)-SP **8a**, γCD-(Arg^1)-SP **6b**, γCD-(Lys^3)-SP **7b** and di-(γCD)-(Arg^1, Lys^3)-SP **8b** as displayed in Chart 1. All these new compounds were fully characterized by dedicated NMR experiments and by high-resolution mass spectrometry. Their inclusion properties towards hydrophobic guests were evaluated by NMR experiments. Since these different compounds were

† Names given for compounds **4a/b**–**8a/b** in this paper are non-systematic.

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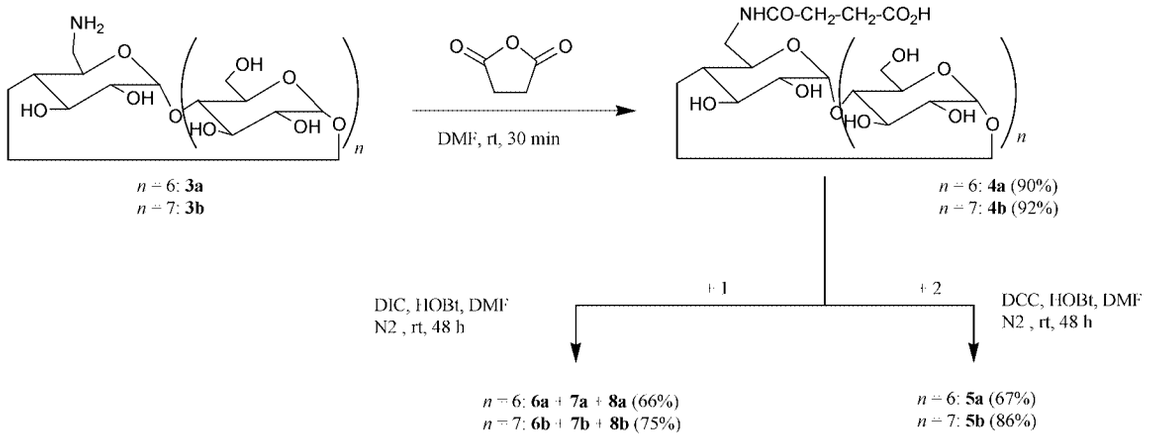


Scheme 1

supposed to target biological receptors, we also investigated the structural changes potentially induced in substance P by grafting CDs. For this purpose, specific antibodies directed against either substance P or CD moieties were used as molecular probes to estimate conformational modifications.

Results and discussion

Synthesis

The chemical substitution should neither preclude the recognition of the signal by the receptor nor the inclusion of the drug in the cavity of the cyclodextrin moiety. For these reasons, the following synthesis strategy was used:

(i) The free hydroxy groups of βCD or γCD cores should be retained since they may play a key role[14] in the stabilization of the inclusion complexes.

(ii) Since the recognition pattern of the peptide towards its receptor is localized at the C-terminal extremity of the peptide and is identified as the Phe[7]-X-Gly[9]-Leu[10]-Met[11]-NH_2 sequence,[11] the grafting of the SP and its derivative SP_{4-11} on the cyclodextrins should therefore involve the N-terminal amino acid in order to maintain the biologically relevant C-terminal moiety unaffected.

(iii) A spacer between the cyclodextrin and peptide moieties should be used to reduce the possible conformational and steric hindrance effects induced by the grafting of the peptide onto the cyclodextrin.

(iv) In the case of **1**, to obtain in one pot the largest number of analogue derivatives useful for pharmacological investigations, the amino functions were deliberately not protected. The various derivatives were further separated by HPLC and identified by both NMR and mass spectrometry.

The synthetic pathway to peptidyl-cyclodextrin adducts **5**–**8** involved the preparation of 6^I-amino-6^I-deoxycyclomaltoheptaose **3a** and 6^I-amino-6^I-deoxycyclomaltooctaose **3b** (Scheme 1). These β- and γ-cyclodextrin derivatives were synthesized in three steps from their respective parent cyclodextrins as already described.[2] Nucleophilic addition of **3a** and **3b** to succinic anhydride in *N*,*N*-dimethylformamide (DMF) at room temperature for 30 min afforded **4a** (90% yield) and **4b** (92% yield), respectively. The chemical structures of compounds **4a** and **4b** were confirmed by elemental analysis, NMR spectra and mass spectrometry (see Experimental section). It should be noted that observation of a new amide signal at δ 7.7 (^{1}H NMR, d_6-DMSO) is consistent with a successful addition reaction, without any formation of succinic ester (cyclodextrin dimer) as demonstrated by electrospray ionization mass spectrometry (ESI-MS) and ^{1}H NMR. Synthesis on a solid-phase, according to standard procedures,[15] afforded SP **1** and SP_{4-11} **2** in good yields after purification by HPLC. TOCSY and NOESY ^{1}H NMR experiments were performed in d_6-DMSO, and experimental NMR data obtained with **1** and **2** were consistent with literature values.[16]

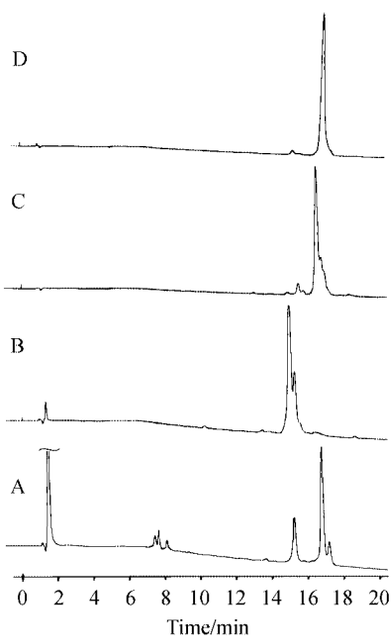
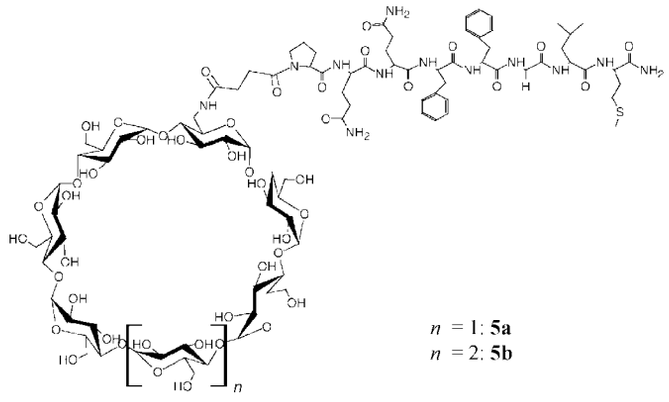
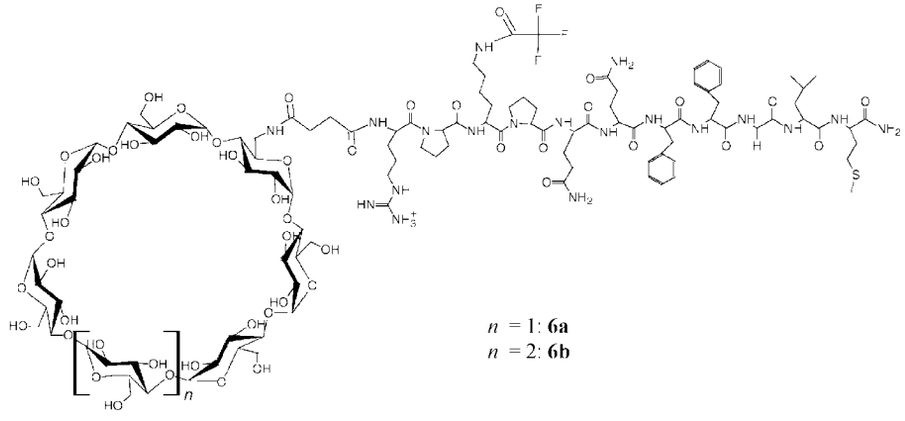


Fig. 1 HPLC chromatograms obtained for the product mixture of peptidyl-βCD (A) and its separated components: **8a** (B), **7a** (C) and **6a** (D).

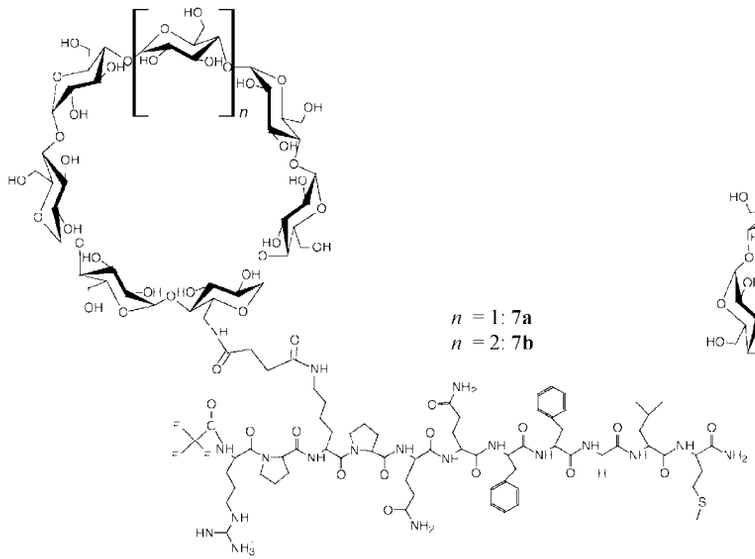
4a and **4b** were further allowed to react with **2** under standard peptidic coupling conditions [*N*,*N*′-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt) in dry DMF], resulting in 67% and 86% yields of βCD-SP_{4-11} **5a** and γCD-SP_{4-11} **5b**, respectively (Scheme 1). The final materials were purified by high-performance liquid chromatography (water–CH_3CN, gradient elution). Evidence of the grafting was demonstrated with high-resolution mass spectrometry (HRMS) and ^{1}H NMR as illustrated later. Under the same experimental conditions, SP **1** was treated with **4a** and **4b** without the protecting amino group strategy (Scheme 1). Since **1** has two reactive amino functions, the reaction may lead to a mixture of the three different coupling adducts: βCD-(Arg^1)-SP **6a**, βCD-(Lys^3)-SP **7a**, di-βCD-(Arg^1, Lys^3)-SP **8a** and γCD-(Arg^1)-SP **6b**, γCD-(Lys^3)-SP **7b**, di-γCD-(Arg^1, Lys^3)-SP **8b**, respectively. Final purification was achieved by HPLC (water (+1% TFA)–CH_3CN (+0.4% TFA), gradient elution from 100:0 to 50:0 in 40 min) and afforded pure βCD-(Arg^1)-SP **6a** (8% yield), βCD-(Lys^3)-SP **7a** (28% yield), di-βCD-(Arg^1, Lys^3)-SP **8a** (10% yield), and γCD-(Arg^1)-SP **6b** (7% yield), γCD-(Lys^3)-SP **7b** (29% yield) and di-γCD-(Arg^1, Lys^3)-SP **8b** (10% yield), respectively. HPLC chromatograms obtained for the product mixture of peptidyl-



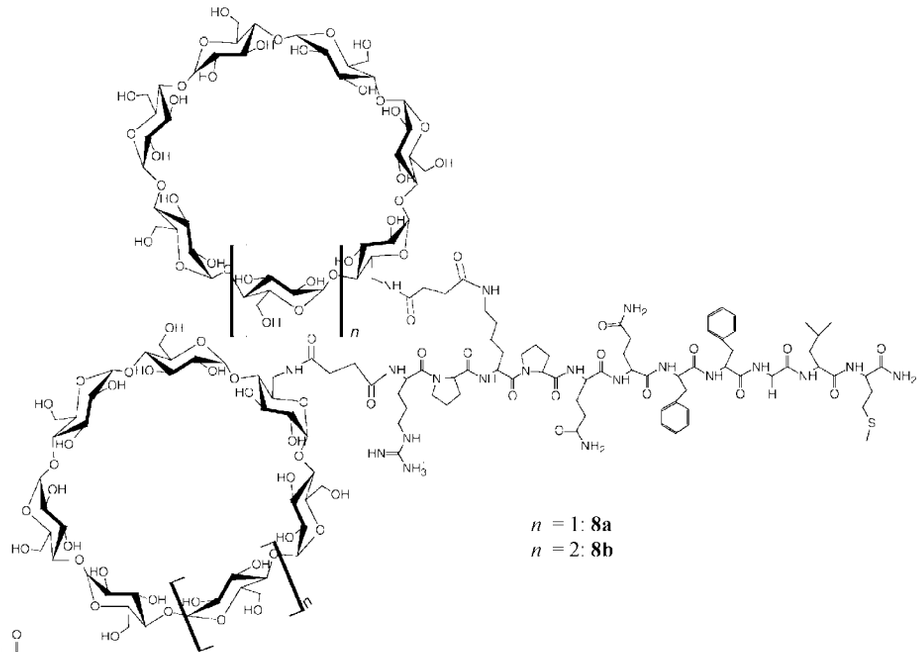
n = 1: **5a**
n = 2: **5b**



n = 1: **6a**
n = 2: **6b**



n = 1: **7a**
n = 2: **7b**



n = 1: **8a**
n = 2: **8b**

Chart 1

Table 1 Accurate mass data obtained by high-resolution ESI-MS performed on **5**–**8**

Compound	$[M + Na]^+$	$[M + H + Na]^{2+}$	Measured mass	Exact mass
5a	$C_{92}H_{140}N_{12}NaO_{46}S$		2203.863	2203.8603
5b	$C_{98}H_{150}N_{12}NaO_{51}S$		2365.904	2365.9131
6a	$C_{111}H_{170}F_3N_{19}NaO_{50}S$		2681.100	2681.0914
6b	$C_{117}H_{180}F_3N_{19}NaO_{55}S$		2843.154	2843.1543
7a	$C_{111}H_{170}F_3N_{19}NaO_{50}S$		2681.100	2681.0914
7b	$C_{117}H_{180}F_3N_{19}NaO_{55}F_3S$		2843.145	2843.1443
8a		$C_{155}H_{245}N_{20}NaO_{85}S$	1900.754	1900.7541
8b		$C_{167}H_{265}N_{20}NaO_{95}S$	2062.805	2062.8069

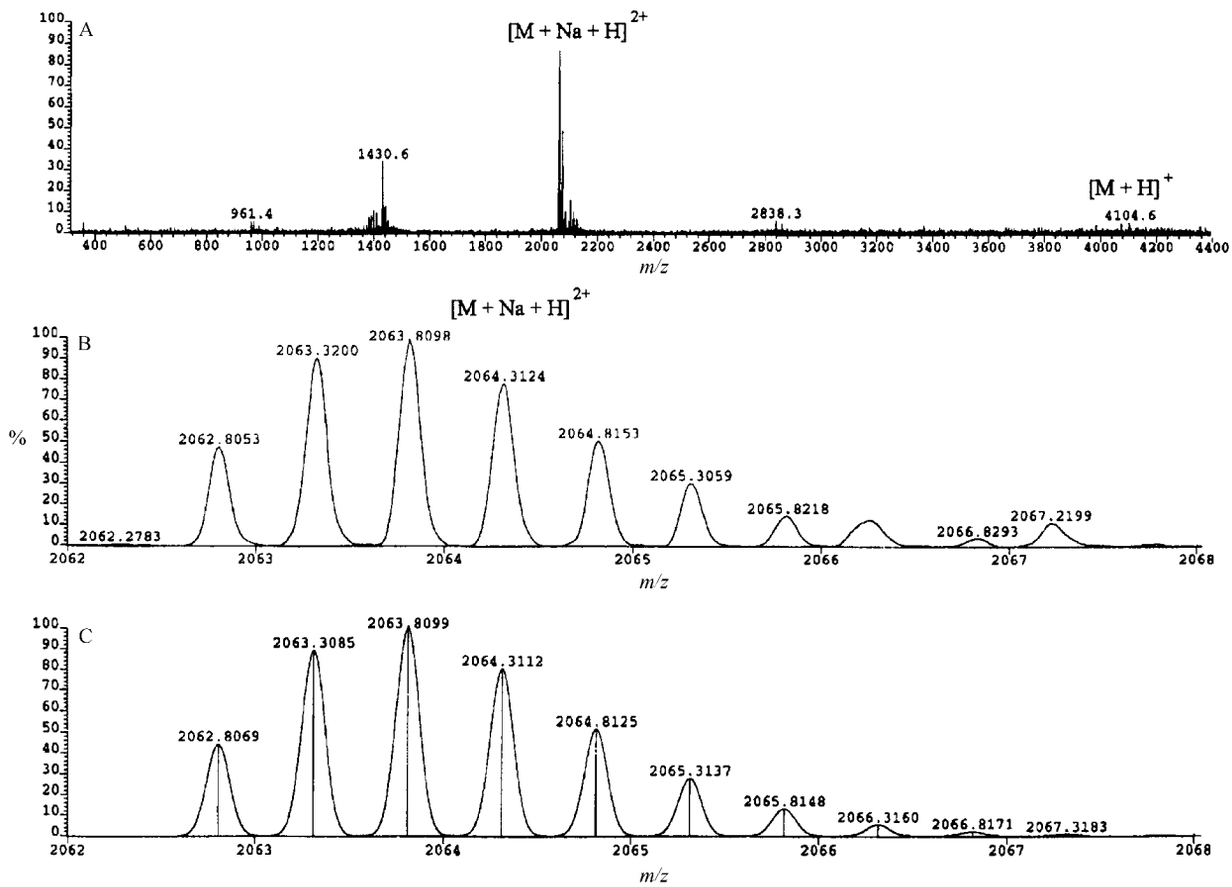


Fig. 2 ESI-MS spectrum obtained for **8b** (A) and isotope profile measured by ESI-HRMS at $m/z = 2063$ (B) and calculated (C) for $C_{167}H_{265}N_{20}NaO_{95}S$ $[M + Na + H]^{2+}$.

βCD and its separated components **6a**, **7a** and **8a** are displayed in Fig. 1. Similar chromatograms are obtained for the γCD derivatives. At this stage, ^{1}H NMR and ESI-MS revealed that the mono-CD-SP adducts **6a**–**b** and **7a**–**b** were substituted at the other primary amino site of the SP backbone with a trifluoroacetamido group. Introduction of this protecting group occurred during the coupling reaction between the last free amino group of the peptide and the TFA salts from the HPLC purification step of the native peptide **1**. This phenomenon could explain the relatively low yields of the final compounds **6**–**8**. However, the TFA amido group can be quantitatively and easily removed by treatment with piperidine in DMF (20 : 80, *v* : *v*) for 24 h at room temperature.

The chemical structures of βCD-(Arg^1)-SP **6a**, βCD-(Lys^3)-SP **7a**, di-βCD-(Arg^1, Lys^3)-SP **8a** and γCD-(Arg^1)-SP **6b**, γCD-(Lys^3)-SP **7b** and di-γCD-(Arg^1, Lys^3)-SP **8b** were assessed using high-resolution ^{1}H NMR and ESI-MS (high- and low-resolution).

ESI-MS investigations of the peptidyl-cyclodextrin adducts 5–8

Electrospray ionization mass spectrometry in positive mode was used to investigate the chemical structures of **5**–**8**. Mass spectra yielded mainly singly and doubly charged ions for each analogue. The mass resolution was sufficient to identify the charge states of the different ions from the spacing observed for the stable isotope profiles, as shown in Fig. 2 (**8b** as example). The main accurate ESI-HRMS data are displayed in Table 1. Measurements of the molecular masses allowed the assignment of all derivatives and established the presence of a trifluoroacetamido group for **6a**,**b** and **7a**,**b** in positive mode. The negative mode confirmed these structures with the presence of $[M - H]^-$ and $[M - 2H]^{2-}$ ions.

NMR investigations of the peptidyl-cyclodextrin adducts 5–8

The structural NMR analysis was performed in d_6-DMSO and demonstrated that the purified final samples were free of any included by-product or reagent. TOCSY experiments were performed (mixing time 80 ms) to elucidate the primary sequence by referring to the spectra of native peptides **1** and **2** and native cyclodextrins **4a** and **4b**, respectively. The degree of substitution was shown by digital integration of the NMR signals arising from the peptide and cyclodextrin moieties. This study revealed the following stoichiometries: 1 : 1 for **5a**,**b**, **6a**,**b** and **7a**,**b**, and 1 : 2 for **8a**,**b**.

Since the spectra are relatively complex owing to the lack of molecular symmetry of the cyclodextrin moiety (Fig. 3), a complete analysis was obtained from stepwise identification of the eight final compounds by COSY, and successive RELAY and NOESY experiments. Being located in a very specific domain, anomeric and amide protons were used as starting points for stepwise assignment of the cyclodextrin and peptidic moieties, respectively.[17] All the resonances of the peptide component of conjugates including the succinyl spacer were identified. However, since the resonances related to the glucosidic units show strong overlaps, only the substituted glucosidic units were unambiguously assigned.

For **6–8**, the grafting site positions (N-Arg^1) or (N$^\varepsilon$-Lys^3) were demonstrated by means of NOESY experiments allowing 'through-space' connection of the cyclodextrin-amide signal to the succinic methylenic protons and, further, to the amide protons of the amino acid involved in the coupling. As an example, Fig. 4 displays partial contour plots of TOCSY and NOESY experiments performed on **6a** in d_6-DMSO. These experiments confirm the attachment of SP to the 6^I-amino-βCD derivative through the succinic spacer by evidencing dipolar interactions (indicating spatial proximities). Indeed, in the partial NOESY contour plot, each amide proton NH(Arg^1) and NH(βCD) yields two dipolar cross-peaks at δ 8.05 and 7.55, one arising from protons of its own moiety [*i.e.*, NH(Arg^1) with H$^\alpha$, at δ 4.52, or NH(βCD) with H^6 and H$^{6'}$, at δ 3.25 and 3.61] and one from the neighbouring methylene group of the succinic moiety δ at 2.42, highlighting the amino acid involved in the grafting. The same NMR strategy was applied to the other peptidyl-cyclodextrin adducts. The main results are displayed in Table 2 and also revealed a grafting (N-Arg^1) position for **6b** and a grafting (N$^\varepsilon$-Lys^3) position for **7a** and **7b**. In the case of the derivatives **8a** and **8b**, three types of cross-peaks were present as expected (data not shown), evidencing the presence of two cyclodextrin moieties grafted simultaneously at the N-Arg^1 and N$^\varepsilon$-Lys^3 positions.

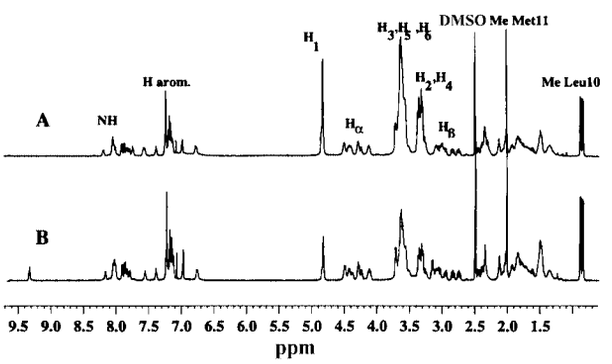


Fig. 3 ^{1}H NMR spectra (310 K; 500.13 MHz; 2.5 mmol dm^{-3} in d_6-DMSO) of **8a** (A) and **6a** (B).

Table 2 Chemical shifts of amide protons of compounds **6–8** exhibiting inter-residue NOEs with succinic protons at δ 2.42

Compound	δ NH$_{CD}$ (ppm)	δ NH$_{Arg1}$ (ppm)	δ εNH$_{Lys3}$ (ppm)
6a	7.55	8.05	—
6b	7.57	8.01	—
7a	7.55	—	7.75
7b	7.57	—	8.04
8a	7.55	8.05	7.75
8b	7.57	8.01	8.04

Since these different compounds were supposed to target biological receptors, we have also investigated in water the conformational changes induced in the SP and SP$_{4-11}$ backbones by grafting cyclodextrins. From TOCSY and NOESY data recorded in water and by comparison with the results obtained with **1** and **2** alone, all the resonances of the peptide components of **5–8** were assigned. Conversely, the cyclodextrin signals of **5–8** overlap dramatically in water and unambiguous assignment of the signals of the subunits was not possible. Besides the inter-residue sequential NOEs (CHαi/NHi + 1) obtained for compounds **5–8** in water at 310 K, we have not observed any other type of inter-residue NOE specific to an ordered conformation like a β- or γ-type turn or an α-helical conformation. Analysis of the temperature dependence of the chemical shifts of the NH protons showed the same slope as those of the natives **1** and **2**.[16] It is reasonable to postulate that, unlike natives **1** and **2**, the peptidic moieties of **5–8** do not show any ordered conformation in water. It seems that the synthesis of peptido-cyclodextrins did not induce marked conformational modifications of the peptidic moieties in the SP or SP$_{4-11}$ series. However, for **7a** and **8a**, NOEs are observed between protons of the H^3–H^5 area of cyclodextrin and aromatic protons of a phenylalanine residue, indicating the formation of an intramolecular inclusion complex in aqueous solution. The self-inclusion process[2,3] is a well described phenomenon in the cyclodextrin domain. For example, previous NMR results from our laboratory showed that 6^I-deoxy-6^I-phenylalanylamidocyclomaltheptaose forms a very strong self-inclusion complex in solution, the cavity being fully occupied by the aromatic moiety of the amino acid, precluding formation of other inclusion

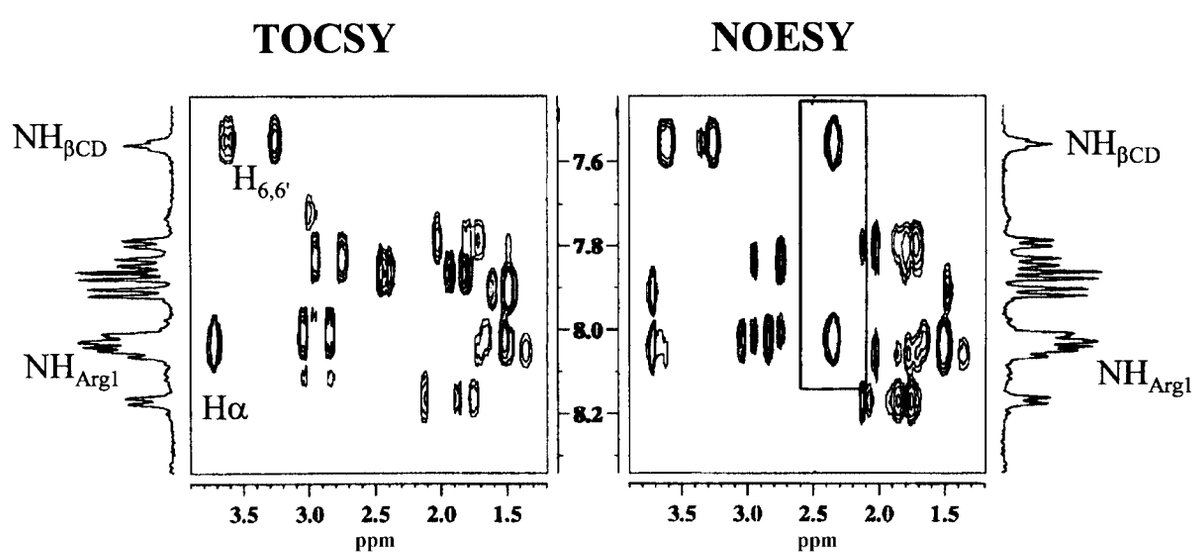


Fig. 4 Partial contour plots (310 K; 500.13 MHz; d_6-DMSO) of TOCSY and NOESY experiments (80 and 200 ms mixing time at 10 and 18 dB attenuation, respectively) performed on samples containing 2.5 mmol dm^{-3} of **6a**. Area of inter-residue C$_\alpha$Hi/NHi + 1 cross-peaks.

complexes. The self-inclusion process is weaker for **6a**–**8a** than for 6^I-deoxy-6^I-phenylalanylamidocyclomaltheptaose since it was also shown that the presence of spacer inhibited this phenomenon. For γCD analogues, we did not observe any interactions between aromatic protons and protons of the cyclodextrin cavity. These results are consistent with literature data[2] since interactions of the aromatic moiety of the phenylalanine in the cavity of γCD carrier are clearly weaker than with the βCD analogue, suggesting a much looser fitting of the aromatic ring in the larger cavity.

It was therefore necessary to evaluate the inclusion properties of **5**–**8** towards hydrophobic guests by the same technique to confirm that the self-inclusion process does not inhibit the inclusion capacity of these derivatives. Moreover, immunoassays using specific antibodies raised against the peptidic or cyclodextrin moiety are expected to confirm the weak conformational modifications observed by NMR in aqueous solution.

Investigation of inclusion properties of peptidyl-cyclodextrin adducts

At this stage, it must be kept in mind that the synthesized products will be used as 'molecular shuttles'. To demonstrate the inclusion properties of the peptidyl-cyclodextrin adducts, we studied the inclusion of indomethacin in βCD-(Lys^3)-SP **7a** by NMR. It should be noted that a self-inclusion occurs in this case as demonstrated before. A large number of organic molecules are known to form inclusion complexes with cyclodextrins.[14] For this purpose, indomethacin was selected since its inclusion in cylodextrins[18] has been extensively studied by NMR. The molecular structure of this pertinent compound is shown in Fig. 5. It is well established that indomethacin forms a 1:1 inclusion complex with βCD and that the association constant[18] is 760 (±30) $dm^3\ mol^{-1}$. This compound was also selected on the basis of its aromatic character, which induces large variations in chemical shifts of proton NMR lines upon inclusion.[19] First, one-dimensional spectra of **7a** in the absence and in the presence of the potential guest were compared. Since the spectra are extremely complex, the inclusion complex was demonstrated through the spatial proximities of protons of the host and guest molecules. This can be achieved by investigation of the dipolar interactions using two-dimensional NOESY with a WATERGATE solvent-suppression sequence. As found in many other situations regarding inclusion complexes in water, a 200 ms mixing time was selected.

A typical example is shown in Figs. 5A and 5B for **7a** alone and in the presence of one molar equivalent of indomethacin, at pH 8. All non-diagonal peaks are indicative of spatial proximities between protons. Complex formation was demonstrated by the presence of strong dipolar cross-peaks between protons of **7a** (located in the area spectrum of H^3 and H^5 of the cyclodextrin moiety as shown in Fig. 5A) and protons of the *para*-substituted aromatic ring of indomethacin (signals labelled with * in the Fig. 5B at δ 7.6 and 7.7, respectively). No other cross-peak was observed between the protons of indomethacin and/or of the peptidic part of **7a**, thus precluding interactions of indomethacin with the peptide. NMR data are similar to the case of inclusion of indomethacin in the natural βCD,[18] suggesting that the two inclusion complexes have the same conformation. It was therefore concluded that the peptide moiety weakly influenced complex formation. These results are in agreement with those derived from the conformational study of the peptidyl-cyclodextrin adducts in water. Cross-peaks between protons of the cavity of cyclodextrin (δ 3.5–4.0) and protons of the phenylalanine moiety (δ 7.15–7.25) decreased in the presence of indomethacin (Fig. 5B). **7a** formed an intramolecular inclusion complex in aqueous solution but the interactions were clearly much weaker than for the 6^I-phenylalanylamido-6^I-deoxycyclomaltoheptaose[2] analogue, suggesting a much looser fitting of the aromatic group in the cavity which does not inhibit the inclusion capacity of **7a**. No binding constant was measured with **7a** owing to the complexity of the spectra recorded for the different mixtures of **7a**: indomethacin. However, careful studies of the NOE cross-peak intensities and of the variations in chemical shifts experienced indicate that the binding constant of the peptidyl-cyclodextrin adduct **7a** is globally preserved with indomethacin. From this result it was legitimately inferred that the other adducts retain their inclusion properties, especially when the complex formation involves the secondary hydroxy-group rim of the cyclodextrins.

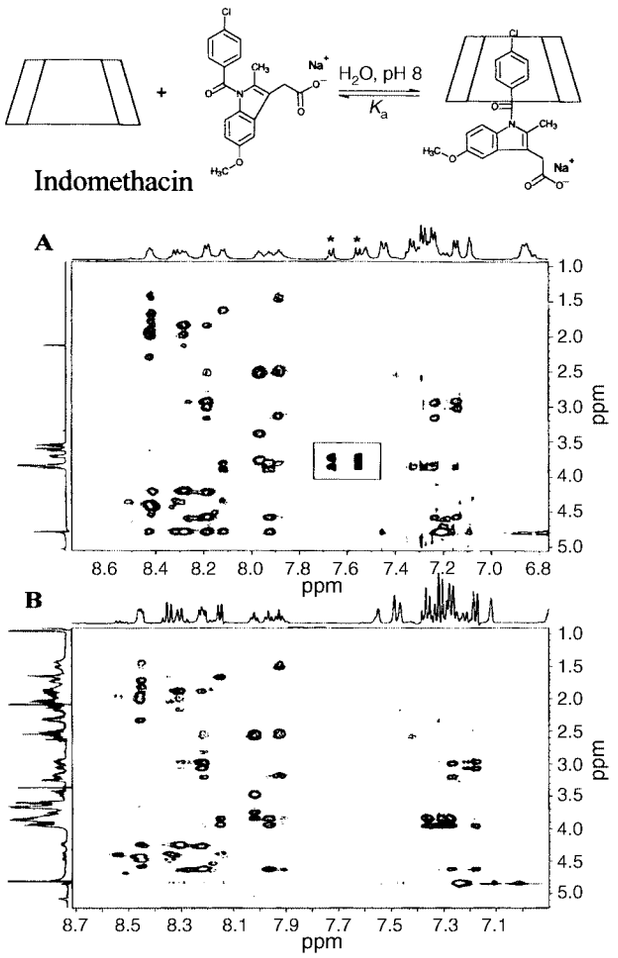


Fig. 5 Partial contour plots of WATERGATE-NOESY (2.5 mmol dm^{-3} in H_2O–D_2O 90:10; 298 K; 500.13 MHz; 200 ms mixing time) performed on **7a** in the presence of one mole equivalent of indomethacin (A) and alone (B). ^1H NMR spectrum of βCD is given as reference (A).

Immunoassays

For these bimolecular compounds to be efficient as 'molecular vehicles', each component must retain a complementary property, *i.e.* the inclusion properties of the cyclodextrin moiety and the ability of SP **1** to bind its specific receptor. As demonstrated above, CD–SP derivatives are fully active to entrap host molecules. The evaluation of the capacity of these compounds to bind to a biological receptor requires either an *in vitro* model of cultured cells transfected with the NK1 receptor or *in vivo* administration of these compounds and evaluation of physiological or morphological parameters. None of these experiments would be straightforward. As an easier alternative, we used specific antibodies directed against the carboxyterminal moiety, *e.g.* the 5 last residues of SP,[20] which has been shown to bind the receptor. Although these antibodies are not supposed to mimic the real interactions of SP with the receptor, they can

Table 3 Relative cross-reactivity (CR) (%) obtained with **1**,**2** and **5**–**8** taking **1** as 100% reference (anti-SP polyclonal antibodies)

Compound	B/B_0 50% (nmol dm^{-3})	CR (%)
1	0.101	100
2	0.126	79
5a	0.113	88
5b	0.103	97
6a	0.144	70
6b	0.116	86
7a	0.180	55
7b	0.180	55
8a	0.093	107
8b	0.098	102

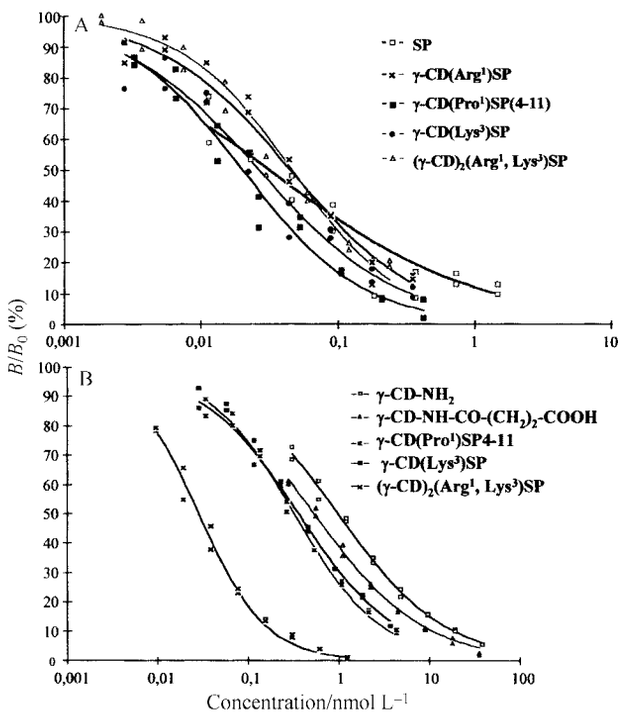


Fig. 6 Enzyme immunoassay titration curves for γCD derivatives **3b**–**8b** using anti-SP (A) and anti-γCD (B) antibodies.

efficiently detect structural modifications which can therefore impair proper binding of these new adducts to the receptor. It was also interesting to obtain the same information on cyclodextrin structure, with the goal of confirming the accessibility of the cavity, through the interactions of specific anti-β[21] or anti-γCD[22] polyclonal antibodies with these new compounds. For example, as shown in Fig. 6, competitive enzymic immunoassays performed using either anti-SP or anti-γCD antibodies show good recognition of **5b**–**8b**. The same strategy was applied to **5a**–**8a** and the results are expressed in terms of sensitivity at B/B_0 50% and in terms of percentage of cross-reactivity (CR) (Tables 3 and 4). Discrete modifications of SP immunoreactivity (as revealed by a small decrease in CR in Table 3) occurred but are considered to affect only minimally the interactions of these molecules with the NK1 receptor. Compounds **5**–**8** displayed higher CR (*i.e.*, greater immunoreactivity) than the references **3a** and **3b** (Table 4). Addition of peptidyl moieties to compounds **3a** or **3b** remarkably enhanced the affinity of binding to anti-β and anti-γCD antibodies. These new compounds can be considered as 'mimetic' analogues of the immunogen since, for the production of antibodies, haptens **3a** and **3b** were covalently linked to bovine serum albumin using glutaraldehyde.[21,22]

The SP immunoassay was used to compare the stability of the new adducts with natural SP in either blood or culture medium. All compounds obviously appeared stable in culture medium. Interestingly, the new CD–SP products were largely protected against enzymic degradation in plasma, exhibiting a half-life close to 6 h compared with less than 10 min for SP (data not shown). This probably results from steric hindrance introduced by coupling one or two cyclodextrins to the SP backbone, thus limiting enzyme action. This stability is of the greatest importance for drug targeting. According to this experiment, it can be assumed that CD–SP conjugates would not be significantly degraded *in vivo* before reaching their cellular targets. On the other hand, the bioavailability of these CD–SP conjugates could be investigated in the near future using the specific immunoassays directed against the CD and SP moieties in order to study the effect of the addition of CD(s) on the fate and behaviour of SP. The preliminary results described in the present publication strengthen our interest in developing such compounds, which are currently being evaluated for binding in an *in vitro*-cultured cell model and after *in vivo* administration to rats.

These experiments will provide further information on the potential use of these new compounds dedicated to drug targeting.

Table 4 Relative cross-reactivity (CR) (%) obtained with **3**–**8** taking **3a** and **3b** as 100% reference (with anti-β and anti-γCD polyclonal antibodies, respectively)

Compound	B/B_0 50% (nmol dm^{-3})	CR (%)
3a	0.90	100
3b	0.98	100
4a	0.12	750
4b	0.08	1225
5a	0.025	3600
5b	0.062	1580
6a	0.025	3600
6b	0.053	1849
7a	0.022	4090
7b	0.032	3062
8a	0.0046	19 565
8b	0.0064	15 312

Experimental

General methods and instrumentation

β- and γ-Cyclodextrins were from Roquette Frères (Lestrem, France) and Wacker (München, Germany), respectively, and were freeze-dried before synthesis. Toluene-*p*-sulfonyl chloride (TsCl) was recrystallized from light petroleum (distillation range 40–60 °C). Lithium azide was from Kodak. DCC was purified by recrystallization in ethyl acetate immediately before use. All other reagents and anhydrous solvents were from Fluka and were used without further purification. Indomethacin (sodium salt) was a generous gift from Prof. D. Wouessidjewe from the University J. Fourier of Grenoble (France). TLC was performed on silica gel 60 plates (E. Merck, Darmstadt) followed by charring with 10% H_2SO_4. Semi-preparative HPLC was carried out with a Waters Delta Prep 3000 chromatograph equipped with a light-scattering evaporative detector (LSED) and a μBondapak C_{18}-bonded silica column, using a linear gradient of CH_3CN (+0.04% TFA) in water (+0.1% TFA) from 0:100 to 50:50 in 50 min at 10 cm^3 min^{-1}. Electrospray mass spectrometry analysis was performed in positive and negative mode in a ZABSpec TOF (Micromass, UK) tandem hybrid mass spectrometer with EBETOF geometry. The compounds were individually dissolved in a mixture of 1:1 water–CH_3CN at a concentration of 10 μg cm^{-3} and then infused into the electrospray ion source at a flow rate of 10 mm^3 min^{-1} at 60 °C. The mass spectrometer was operated at 4 kV whilst scanning the magnet at a typical mass range of 4000–100 U. The mass spectra were collected as continuum profile data. Accurate mass measurement was achieved using poly(ethylene glycol) as

internal reference masses with a resolving power set to a minimum of 10 000 (10% valley). All MS data were processed and plotted using OPUS® software on a digital alpha workstation. Elemental analysis was performed at the Service Central de Microanalyses of CNRS (Vernaison, France). The samples (cyclomaltoheptaose and cyclomaltooctaose derivatives) were previously dried under vacuum for 48 h in the presence of P_2O_5.

^{1}H NMR experiments were performed at 500.13 MHz using a Bruker DRX500 spectrometer equipped with a Z-gradient unit for pulsed-field-gradient spectroscopy. In all cases, the samples were prepared in deuterium oxide and d_6-DMSO (Euriso-Top, Saclay, France) and measurements were performed at 298 K with careful temperature regulation. The length of the 90° pulse was approximately 7 μs. 1D NMR spectra were collected using 16K data points. Chemical shifts are given relative to external tetramethylsilane (δ 0) and calibration was performed using the signal of the residual protons of the solvent as a secondary reference. 2D experiments were obtained using the pulse programs available from the Bruker library. These two-dimensional experiments were run using 2K data points and 256 time increments. The phase-sensitive (TPPI) sequence was used and processing resulted in a 1K*1K (real–real) matrix. Details concerning experimental conditions are given in the Figure captions. All NMR data were processed and plotted using the UXNMR program (Bruker Analytische Messtechnik) on a Silicon Graphics workstation.

Immunological titration

Competitive enzyme immunoassay (EIA) was performed as described elsewhere.[20] 96-Well microtiter plates (Immunoplate Maxisorb, Nunc, Denmark) were coated with mouse monoclonal antirabbit immunoglobulins in order to ensure separation between the free and the bound moieties of the enzymic tracer during the immunological reaction. Before use, the plates were washed with 10 mmol dm^{-3} phosphate buffer (pH 7.4) containing 0.05% Tween 20. Phosphate buffer, pH 7.4, was obtained by dissolving 71 mg of anhydrous Na_2HPO_4 and 60 mg of anhydrous NaH_2PO_4 in 100 mL of ultrapure water. The total volume of the immunological reaction was 0.15 cm^3, each component (enzymic tracer, diluted rabbit polyclonal antisera and cyclodextrin standard) being added in a 0.05 cm^3 volume. CD-AChE[21,22] enzyme conjugates with β- or γ-derivative were used at a concentration of 5 Ellman units cm^{-3} while SP-AChE tracer was used at 2 Ellman units cm^{-3}. The working dilution for the different rabbit bleedings was previously determined by performing serial dilution experiments.

After an 18 h incubation at 277 K, the plates were washed and the enzyme activity of the bound immunological complex was revealed by addition of 0.2 cm^3 of enzymic substrate and chromogen (Ellman's reagent) to each well. After 2 h of gentle shaking in the dark at room temperature, the absorbance at 414 nm in each well was measured automatically. Results are given in terms of $100*B.B_0^{-1}$ as a function of the dose, B and B_0 representing the bound enzyme activity in the presence and absence of competitor, respectively. A linear log-logit transformation was used to fit the standard curve. Serial-dilution curves were performed with the different synthesized compounds and compared with the reference curves obtained with either SP or βCD or γCD. All experiments were done in duplicate and quadruplicate for B_0.

Stability of 6–8

Either **1** or **6**–**8** at a 1 μmol dm^{-3} final concentration was added to 2 cm^3 of either culture medium or freshly collected rat blood samples. The mixtures were incubated at 310 K and aliquots (0.1 cm^3) were collected at the following times: 5, 10, 15, 20, 25, 30 min; 1, 2, 3, 6, 8, 18, and 24 h. Proteins were immediately precipitated by addition of 0.4 cm^3 of cold methanol. After centrifugation, the supernatants were dried under vacuum before reconstitution with assay buffer and immunoassay.

Synthesis

Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH$_2$ (SP) 1. Substance P was solid-phase synthesized according to standard procedures[15] by using benzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate (BOP) and HOBt as coupling reagents, in a Milligen 9050 pepsynthesizer apparatus. N-terminal fluoren-9-ylmethoxycarbonyl (Fmoc) and side-chain-protected amino acid (Neosystem) and polystyrene rink amide resin (Novasyn PR 500, Novabiochem), with a loading capacity of 0.3–0.6 mmol g^{-1}, were used. The synthesis was performed with 5 molar equivalents of amino acids and coupling reagents. Cleavage and deprotecting of the peptide was performed using K reagent (2.5 g of phenol, 2.5 cm^3 water, 2.5 cm^3 of thioanisole and 1.25 cm^3 of ethane-1,2-dithiol in 50 cm^3 of TFA) with the following ratio: 25 cm^3 of reagent K *vs.* 1 g of resin-bound peptide. Resin was stirred for 2 h at room temperature, filtered, and washed with TFA. The filtrate was concentrated under reduced pressure to 5 cm^3. The crude material was precipitated in 50 cm^3 of diethyl ether and centrifuged. The pellet was resuspended in diisopropyl ether and recentrifuged. The final pellet was dissolved in 10 cm^3 of water–CH_3CN–AcOH (3:2:5 *v*:*v*:*v*) and freeze-dried. Purification by semi-preparative HPLC (t_R 17.6 min) provided a pure sample of SP **1** (0.1 g, 0.074 mmol). ESI-HRMS *m*/*z* 1347.7360 (Calc. for $C_{63}H_{99}N_{18}O_{13}S$ [M + H]$^+$). Measured *m*/*z*, 1347.736; δ_H (500.13 MHz; d_6-DMSO) 8.13 (NH_{Arg1}), 8.22 (NH_{Lys3}), 8.17 (NH_{Gln5}), 7.80 (NH_{Gln6}), 7.89 (NH_{Phe7}), 8.07 (NH_{Phe8}), 8.09 (NH_{Gly9}), 7.94 (NH_{Leu10}), 7.90 (NH_{Met11}), 4.16 ($H\alpha_{Arg1}$), 4.44 ($H\alpha_{Pro2}$), 4.43 ($H\alpha_{Lys3}$), 4.31 ($H\alpha_{Pro4}$), 4.12 ($H\alpha_{Gln5}$), 4.16 ($H\alpha_{Gln6}$), 4.47 ($H\alpha_{Phe7}$), 4.51 ($H\alpha_{Phe8}$), 3.73 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 1.79 ($H\beta_{Arg1}$), 1.92, 1.85 ($H\beta_{Pro2}$), 1.71, 1.54 ($H\beta_{Lys3}$), 1.93, 1.84 ($H\beta_{Pro4}$), 1.86, 1.75 ($H\beta_{Gln5}$), 1.79, 1.71 ($H\beta_{Gln6}$), 2.97, 2.74 ($H\beta_{Phe7}$), 3.05, 2.85 ($H\beta_{Phe8}$), 1.5, 1.48 ($H\beta_{Leu10}$), 1.94, 1.82 ($H\beta_{Met11}$); other protons: Arg1: 1.71 (Hγ), 3.14 (Hδ), 7.72 (NHε); Pro2: 2.12 (Hγ), 3.69, 3.46 (Hδ); Lys3: 1.41 (Hγ), 1.55 (Hδ), 2.77 (Hε), 6.8 (NH); Pro4: 2.017 (Hγ), 3.62, 3.54 (Hδ); Gln5: 2.12 (Hγ), 7.2 (NHε); Gln6: 2.04 (Hγ), 7.2 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.62 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.45 (Hγ), 2.02 (H_{Me}).

Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH$_2$ (SP$_{4–11}$ 2). Compound **2** was prepared using the solid-phase synthesis procedure as previously described for the synthesis of **1**. Compound **2** (0.1 g, 0.103 mmol): HPLC t_R 18.2 min; ESI-MS *m*/*z* 965.4 (Calc. for $C_{46}H_{67}N_{11}O_{10}S$). Measured *m*/*z*, 966.2 [M + H]$^+$; δ_H (500.13 MHz; d_6-DMSO) 8.35 (NH_{Gln5}), 8.09 (NH_{Gln6}), 7.92 (NH_{Phe7}), 8.11 (NH_{Phe8}), 8.06 (NH_{Gly9}), 7.92 (NH_{Leu10}), 7.88 (NH_{Met11}), 3.82 ($H\alpha_{Pro4}$), 4.25 ($H\alpha_{Gln5}$), 4.18 ($H\alpha_{Gln6}$), 4.49 ($H\alpha_{Phe7}$), 4.50 ($H\alpha_{Phe8}$), 3.72 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 2.10, 1.77 ($H\beta_{Pro4}$), 1.74, 1.84 ($H\beta_{Gln5}$), 1.79, 1.69 ($H\beta_{Gln6}$), 2.96, 2.75 ($H\beta_{Phe7}$), 3.04, 2.84 ($H\beta_{Phe8}$), 1.61 ($H\beta_{Leu10}$), 1.95, 1.81 ($H\beta_{Met11}$); other protons: Pro4: 1.71 (Hγ), 3.62, 3.54 (Hδ); Gln5: 2.05 (Hγ), 7.2 (NHε); Gln6: 2.01 (Hγ), 7.2 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.48 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.47 (Hγ), 2.02 (H_{Me}).

6^I-Deoxy-6^I-(succinylamido)cyclomaltoheptaose 4a. Succinic anhydride (0.057 g, 0.57 mmol) dissolved in dry dimethylformamide (DMF, 1.5 cm^3) was added to a solution of freeze-dried 6^I-amino-6^I-deoxycyclomaltoheptaose **3a** (0.615 g, 0.543 mmol) in dry DMF (10 cm^3). The reaction mixture was stirred for 30 min at room temperature under nitrogen. After evaporation off of most of the solvent under reduced pressure, the residual syrup was poured into acetone (150 cm^3). The crude product was isolated by filtration, washed with acetone and dried under vacuum for 48 h in the presence of P_2O_5.

Pure 6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose **4a** was obtained (0.602 g, 90%), R_f (DMF–*n*-BuOH–water 1:2:1) 0.66 (Calc. for $C_{46}H_{75}NO_{37}\cdot 2H_2O$: C, 43.46; H, 6.22%. Found: C, 43.57; H, 6.34); ESI-MS *m/z* 1256.6 (Calc. for $C_{46}H_{75}NNaO_{37}$ $[M + Na]^+$). Measured *m/z*, 1256.4; *m/z* 1272.7 (Calc. for $C_{46}H_{75}KNO_{37}$ $[M + K]^+$). Measured *m/z*, 1272.4; δ_H (500.13 MHz; D_2O) 5.05–5.12 (m, 7H), 4.05–3.8 (m, 20H), 3.72–3.6 (m, 13H), 3.4 (t, 1H), 3.29 (t, 1H), 2.55–2.7 (m, 4H); δ_C (125.77 MHz; D_2O) 176.4, 174.3, 101.7, 101.4, 82.9, 80.9, 72.91, 71.9, 71.6, 70.5, 60.1, 40.01.

6^I-Deoxy-6^I-(succinylamido)cyclomaltooctaose 4b. Succinic anhydride (0.050 g, 0.49 mmol) dissolved in dry dimethylformamide (DMF, 1.5 cm^3) was added to a solution of freeze-dried 6^I-amino-6^I-deoxycyclomaltooctaose **3b** (0.613 g, 0.470 mmol) in dry DMF (10 cm^3). The reaction mixture was stirred for 30 min at room temperature under nitrogen. After evaporation off of most of the solvent, the residual syrup was poured into acetone (150 cm^3). The crude product was isolated by filtration, washed with acetone, and dried under vacuum for 48 h in the presence of P_2O_5. Pure 6^I-deoxy-6^I-(succinylamido)cyclomaltooctaose **4b** was obtained (0.608 g, 92%), R_f (DMF–*n*-BuOH–water 1:2:1) 0.68 (Calc. for $C_{52}H_{85}NO_{42}\cdot 2H_2O$: C, 43.03; H, 6.27%. Found: C, 43.17; H, 6.35); ESI-MS *m/z* 1418.4 (Calc. for $C_{52}H_{85}NNaO_{42}$ $[M + Na]^+$). Measured *m/z*, 1418.5; *m/z* 1434.4 (Calc. for $C_{52}H_{85}KNO_{42}$ $[M + K]^+$). Measured *m/z*, 1434.3; δ_H (500.13 MHz; D_2O) 5.14–5.2 (m, 8H), 3.87–4.05 (m, 13H), 3.6–3.73 (m, 13H), 3.49 (t, 1H), 3.31 (dd, 1H), 2.6–2.7 (m, 4H); δ_C (125.77 MHz; D_2O) 179.5, 177.2, 104.0, 103.5, 84.6, 82.7, 75.3, 74.6, 74.0, 72.7, 62.5, 42.5, 39.3, 33.7, 32.7, 32.0.

***N*-[L-Prolylamido-6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose]-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ 5a.** DCC (0.380 g, 1.84 mmol) and HOBt (0.250 g, 1.85 mmol) were added successively to a solution of freeze-dried 6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose **4a** (0.227 g, 0.184 mmol) in dry DMF (8 cm^3). The mixture was stirred under nitrogen for 1 h at room temperature, and freeze-dried SP_{4-11} **2** (0.172 g, 0.178 mmol) as a solution in dry DMF (2 cm^3) was added. The mixture was stirred at room temperature for 48 h. The reaction was stopped by addition of water (0.2 cm^3) at 0 °C and the mixture was stirred at room temperature for 2 h. The dicyclohexylurea was removed by filtration. After evaporation off of most of the solvent, the residual oil was poured into diethyl ether (150 cm^3). The white precipitate was filtered off, washed with diethyl ether, dried under vacuum for 48 h in the presence of P_2O_5, and freeze-dried to give a solid which contained almost pure **5a** (261 mg, 67%). Final purification by HPLC (t_R 15.5 min) afforded **5a** as an amorphous white powder (Calc. for $C_{92}H_{140}N_{12}O_{46}S\cdot 4TFA\cdot 3H_2O$: C, 43.26, H, 5.94; N, 5.71%. Found: C, 43.71; H, 6.17; N, 5.81); ESI-HRMS *m/z* 2203.8603 (Calc. for $C_{92}H_{140}N_{12}NaO_{46}S$ $[M + Na]^+$). Measured *m/z*, 2203.863; *m/z* 2219.8342 (Calc. for $C_{92}H_{140}KN_{12}O_{46}S$ $[M + K]^+$). Measured *m/z*, 2219.840; δ_H (500.13 MHz; d_6-DMSO) 8.35 (NH_{Gln5}), 8.09 (NH_{Gln6}), 7.92 (NH_{Phe7}), 8.11 (NH_{Phe8}), 8.06 (NH_{Gly9}), 7.92 (NH_{Leu10}), 7.88 (NH_{Met11}), 3.82 ($H\alpha_{Pro4}$), 4.25 ($H\alpha_{Gln5}$), 4.18 ($H\alpha_{Gln6}$), 4.49 ($H\alpha_{Phe7}$), 4.50 ($H\alpha_{Phe8}$), 3.72 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 2.10, 1.77 ($H\beta_{Pro4}$), 1.74, 1.84 ($H\beta_{Gln5}$), 1.79, 1.69 $H\beta_{(Gln6)}$, 2.96, 2.75 ($H\beta_{Phe7}$), 3.04, 2.84 ($H\beta_{Phe8}$), 1.61 ($H\beta_{Leu10}$), 1.95, 1.81 ($H\beta_{Met11}$); other protons: Pro4: 1.71 (Hγ), 3.62, 3.54 (Hδ); Gln5: 2.05 (Hγ), 7.2 (NHε); Gln6: 2.01 (Hγ), 7.2 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.48 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.47 (Hγ), 2.02 (H_{Me}). CD moiety: δ 7.54 (t, $NH_{\beta CD}$), 4.88–4.93 (m, 7H), 3.55–3.70 (m, 26H, H^3, H^5 and H^6), 3.20–3.40 (m, 14H, H^2, H^4), 3.61 (dd, 1H, $H^6{}_{sub}$), 3.15 (dd, 1H, $H^6{}_{sub}$), 2.4 (m, 4H, $H^2{}_{succ}$).

***N*-[L-Prolylamido-6^I-deoxy-6^I-(succinylamido)cyclomaltooctaose]-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ 5b.** DCC (0.193 g, 0.9 mmol) and HOBt (0.126 g, 0.9 mmol) were added successively to a solution of freeze-dried 6^I-deoxy-6^I-(succinylamido)-cyclomaltooctaose **4b** (0.262 g, 0.188 mmol) in dry DMF (8 cm^3). The mixture was stirred under nitrogen for 1 h at room temperature, and freeze-dried SP_{4-11} **2** (0.167 g, 0.173 mmol) as a solution in dry DMF (2 cm^3) was added. The mixture was stirred at room temperature for 48 h. The reaction was stopped by addition of water (0.2 cm^3) at 0 °C and the mixture was stirred at room temperature for 2 h. The dicyclohexylurea was removed by filtration. After evaporation off of most of the solvent, the residual oil was poured into diethyl ether (150 cm^3). The white precipitate was filtered, washed with diethyl ether, dried under vacuum for 48 h in the presence of P_2O_5, and freeze-dried to give a solid which contained almost pure **5b** (0.351 g, 86%). Final purification by HPLC (t_R 15.2 min) afforded **5b** as an amorphous white powder (Calc. for $C_{98}H_{150}N_{12}O_{51}S\cdot 4TFA\cdot 3H_2O$: C, 43.26; H, 5.94; N, 5.71%. Found: C, 43.71; H, 6.17; N, 5.81); ESI-HRMS *m/z* 2365.9131 (Calc. for $C_{98}H_{150}N_{12}NaO_{51}S$ $[M + Na]^+$). Measured *m/z*, 2365.904. A residual peak (*m/z* 988.5 $[M + Na]^+$) was identified to be native SP_{4-11} (content < 5%); δ_H (500.13 MHz; d_6-DMSO) 8.35 (NH_{Gln5}), 8.09 (NH_{Gln6}), 7.92 (NH_{Phe7}), 8.11 (NH_{Phe8}), 8.06 (NH_{Gly9}), 7.92 (NH_{Leu10}), 7.88 (NH_{Met11}), 3.82 ($H\alpha_{Pro4}$), 4.25 ($H\alpha_{Gln5}$), 4.18 ($H\alpha_{Gln6}$), 4.49 ($H\alpha_{Phe7}$), 4.50 ($H\alpha_{Phe8}$), 3.72 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 2.10, 1.77 ($H\beta_{Pro4}$), 1.74, 1.84 ($H\beta_{Gln5}$), 1.79, 1.69 $H\beta_{Gln6}$), 2.96, 2.75 ($H\beta_{Phe7}$), 3.04, 2.84 ($H\beta_{Phe8}$), 1.61 ($H\beta_{Leu10}$), 1.95, 1.81 ($H\beta_{Met11}$); other protons: Pro4: 1.71 (Hγ), 3.62, 3.54 (Hδ); Gln5: 2.05 (Hγ), 7.2 (NHε); Gln6: 2.01 (Hγ), 7.2 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.48 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.47 (Hγ), 2.02 (H_{Me}). CD moiety: δ 7.54 (t, $NH_{\beta CD}$), 4.88–4.93 (m, 8H), 3.55–3.70 (m, 40H, H^3, H^5 and H^6), 3.20–3.40 (m, 16H, H^2, H^4), 3.60 (dd, 1H, $H^6{}_{sub}$), 3.12 (dd, 1H, $H^6{}_{sub}$), 2.40 (m, 4H, $H^2{}_{succ}$).

***N*-(Peptidylamido)-6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose 6a, 7a, 8a.** *General procedure*: Diisopropylcarbodiimide (DIC) (0.139 cm^3, 0.9 mmol) and HOBt (0.126 g, 0.9 mmol) were added successively to a solution of freeze-dried 6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose **4a** (0.244 g, 0.198 mmol) in dry DMF (8 cm^3). The mixture was stirred under nitrogen for 1 h at room temperature and a solution of freeze-dried SP **1** (0.121 g, 0.09 mmol) in dry DMF (2 cm^3) was added. The mixture was stirred at room temperature for 48 h. The reaction was stopped by addition of water (0.2 cm^3) at 0 °C and the mixture was stirred at room temperature for 2 h. After evaporation off of most of the solvent, the residual oil was poured into acetone (150 cm^3). The white precipitate was centrifuged, washed with acetone, dried under vacuum for 48 h in the presence of P_2O_5 and freeze-dried to give a crude mixture (0.283 g) of **6a**, **7a** and **8a**. Final purification by HPLC afforded **6a** (t_R 28.1 min; 0.015 g, 8%), **7a** (t_R 27.2 min; 0.068 g, 28%) and **8a** (t_R 24.25 min; 0.06 g, 10%).

N-[L-(Arginylamido)-6^I-deoxy-6^I-(succinylamido)cyclomaltoheptaose]-Pro-N$^{\varepsilon}$-[N-trifluoroacetyl-L-(lysylamido)]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ **6a**.—ESI-HRMS: *m/z* 2681.0914 (Calc. for $C_{111}H_{170}F_3N_{19}Na_2O_{50}S$ $[M + Na]^+$). Measured *m/z*, 2681.100, *m/z* 1352.0406 (Calc. for $C_{111}H_{170}F_3N_{19}Na_2O_{50}S$ $[M + 2Na]^{2+}$). Measured *m/z*, 1352.042, *m/z* 1341.0496 (Calc. for $C_{111}H_{171}F_3N_{19}NaO_{50}S$ $[M + H + Na]^{2+}$). Measured *m/z*, 1341.050; δ_H (500.13 MHz; d_6-DMSO) 8.05 (NH_{Arg1}), 8.22 (NH_{Lys3}), 8.17 (NH_{Gln5}), 7.80 (NH_{Gln6}), 7.89 (NH_{Phe7}), 8.07 (NH_{Phe8}), 8.09 (NH_{Gly9}), 7.94 (NH_{Leu10}), 7.90 (NH_{Met11}), 4.16 ($H\alpha_{Arg1}$), 4.44 ($H\alpha_{Pro2}$), 4.43 ($H\alpha_{Lys3}$), 4.31 ($H\alpha_{Pro4}$), 4.12 ($H\alpha_{Gln5}$), 4.16 ($H\alpha_{Gln6}$), 4.47 ($H\alpha_{Phe7}$), 4.51 ($H\alpha_{Phe8}$), 3.73 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 1.79 ($H\beta_{Arg1}$), 1.92, 1.85 ($H\beta_{Pro2}$), 1.71, 1.54 ($H\beta_{Lys3}$), 1.93, 1.84 ($H\beta_{Pro4}$), 1.86, 1.75 ($H\beta_{Gln5}$), 1.79, 1.71 ($H\beta_{Gln6}$), 2.97, 2.74 ($H\beta_{Phe7}$), 3.05, 2.85 ($H\beta_{Phe8}$), 1.5, 1.48 ($H\beta_{Leu10}$), 1.94, 1.82 ($H\beta_{Met11}$); other protons: Arg1: 1.71 (Hγ), 3.14 (Hδ), 7.72 (NHε); Pro2: 2.12 (Hγ), 3.69, 3.46 (Hδ); Lys3: 1.41 (Hγ), 1.55 (Hδ), 2.77 (Hε), 8.33 (NH); Pro4: 2.017 (Hγ), 3.62, 3.54 (Hδ);

Gln5: 2.12 (Hγ), 7.2 (NHε); Gln6: 2.04 (Hγ), 8.33 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.62 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.45 (Hγ), 2.02 (H_{Me}). CD moiety: δ 7.55 (t, $NH_{\beta CD}$), 4.88–4.93 (m, 7H), 3.55–3.70 (m, 26H, H^3, H^5 and H^6), 3.20–3.40 (m, 14H, H^2, H^4), 3.64 (dd, 1H, $H^6{}_{sub}$), 3.17 (dd, 1H, $H^6{}_{sub}$), 2.4 (m, 4H, $H^2{}_{succ}$).

N-[N-Trifluoroacetyl-L-(arginylamido)]-Pro-N$^{\varepsilon}$-[L-(lysylamido)-6^{I}-deoxy-6^{I}-(succinylamido)cyclomaltoheptaose]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ **7a**.—ESI-MS *m/z* 2660.1 (Calc. for $C_{111}H_{171}F_3N_{19}O_{50}S$ $[M + H]^+$). Measured *m/z*, 2660.4; *m/z* 2681.1 (Calc. for $C_{111}H_{170}F_3N_{19}NaO_{50}S$ $[M + Na]^+$). Measured *m/z*, 2681.3; ESI-HRMS *m/z* 2681.014 (Calc. for $C_{111}H_{170}F_3N_{19}NaO_{50}S$) measured *m/z* 2681.100; *m/z* 1352.0406 (Calc. for $C_{111}H_{170}F_3N_{19}Na_2O_{50}S$ $[M + 2Na]^{2+}$). Measured *m/z*, 1352.042; *m/z* 1341.0496 (Calc. for $C_{111}H_{171}F_3N_{19}NaO_{50}S$ $[M + H + Na]^{2+}$). Measured *m/z*, 1341.058; δ_H (500.13 MHz; d_6-DMSO) 8.43 (NH_{Arg1}), 8.22 (NH_{Lys3}), 8.17 (NH_{Gln5}), 7.80 (NH_{Gln6}), 7.89 (NH_{Phe7}), 8.07 (NH_{Phe8}), 8.09 (NH_{Gly9}), 7.94 (NH_{Leu10}), 7.90 (NH_{Met11}), 4.16 ($H\alpha_{Arg1}$), 4.44 ($H\alpha_{Pro2}$), 4.43 ($H\alpha_{Lys3}$), 4.31 ($H\alpha_{Pro4}$), 4.12 ($H\alpha_{Gln5}$), 4.16 ($H\alpha_{Gln6}$), 4.47 ($H\alpha_{Phe7}$), 4.51 ($H\alpha_{Phe8}$), 3.73 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 1.79 ($H\beta_{Arg1}$), 1.92, 1.85 ($H\beta_{Pro2}$), 1.71, 1.54 ($H\beta_{Lys3}$), 1.93, 1.84 ($H\beta_{Pro4}$), 1.86, 1.75 ($H\beta_{Gln5}$), 1.79, 1.71 ($H\beta_{Gln6}$), 2.97, 2.74 ($H\beta_{Phe7}$), 3.05, 2.85 ($H\beta_{Phe8}$), 1.5, 1.48 ($H\beta_{Leu10}$), 1.94, 1.82 ($H\beta_{Met11}$); other protons: Arg1: 1.71 (Hγ), 3.14 (Hδ), 7.72 (NHε); Pro2: 2.12 (Hγ), 3.69, 3.46 (Hδ); Lys3: 1.41 (Hγ), 1.55 (Hδ), 2.77 (Hε), 7.75 (NH); Pro4: 2.017 (Hγ), 3.62, 3.54 (Hδ); Gln5: 2.12 (Hγ), 7.55 (NHε); Gln6: 2.04 (Hγ), 7.2 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.62 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.45 (Hγ), 2.02 (H_{Me}). CD moiety: δ 7.55 (t, $NH_{\beta CD}$), 4.88–4.93 (m, 7H), 3.55–3.70 (m, 26H, H^3, H^5 and H^6), 3.20–3.40 (m, 14H, H^2, H^4), 3.62 (dd, 1H, $H^6{}_{sub}$), 3.16 (dd, 1H, $H^6{}_{sub}$), 2.4 (m, 4H, $H^2{}_{succ}$).

N-[L-(Arginylamido)-6^{I}-deoxy-6^{I}-(succinylamido)cyclomaltoheptaose]-Pro-N$^{\varepsilon}$-[L-(lysylamido)-6^{I}-deoxy-6^{I}-(succinylamido)-cyclomaltoheptaose]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ **8a**.—ESI-MS *m/z* 3802.5 (Calc. for $C_{155}H_{244}N_{20}NaO_{85}S$ $[M + Na]^+$). Measured *m/z*, 3802.5; *m/z* 1912.2 (Calc. for $C_{155}H_{244}N_{20}Na_2O_{85}S$ $[M + 2Na]^{2+}$). Measured *m/z*, 1912.3; *m/z* 1901.8 (Calc. for $C_{155}H_{245}N_{20}NaO_{85}S$ $[M + H + Na]^{2+}$). Measured *m/z*, 1901.8; ESI-HRMS *m/z* 1911.7451 (Calc. for $C_{155}H_{244}N_{20}Na_2O_{85}S$ $[M + 2Na]^{2+}$). Measured *m/z*, 1911.738, *m/z* 1900.7541 (Calc. for $C_{155}H_{245}N_{20}NaO_{85}S$ $[M + H + Na]^{2+}$). Measured *m/z*, 1900.754; δ_H (500.13 MHz; d_6-DMSO) 8.05 (NH_{Arg1}), 8.22 (NH_{Lys3}), 8.17 (NH_{Gln5}), 7.80 (NH_{Gln6}), 7.89 (NH_{Phe7}), 8.07 (NH_{Phe8}), 8.09 (NH_{Gly9}), 7.94 (NH_{Leu10}), 7.90 (NH_{Met11}), 4.16 ($H\alpha_{Arg1}$), 4.44 ($H\alpha_{Pro2}$), 4.43 ($H\alpha_{Lys3}$), 4.31 ($H\alpha_{Pro4}$), 4.12 ($H\alpha_{Gln5}$), 4.16 ($H\alpha_{Gln6}$), 4.47 ($H\alpha_{Phe7}$), 4.51 ($H\alpha_{Phe8}$), 3.73 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 1.79 ($H\beta_{Arg1}$), 1.92, 1.85 ($H\beta_{Pro2}$), 1.71, 1.54 ($H\beta_{Lys3}$), 1.93, 1.84 ($H\beta_{Pro4}$), 1.86, 1.75 ($H\beta_{Gln5}$), 1.79, 1.71 ($H\beta_{Gln6}$), 2.97, 2.74 ($H\beta_{Phe7}$), 3.05, 2.85 ($H\beta_{Phe8}$), 1.5, 1.48 ($H\beta_{Leu10}$), 1.94, 1.82 ($H\beta_{Met11}$); other protons: Arg1: 1.71 (Hγ), 3.14 (Hδ), 7.72 (NHε); Pro2: 2.12 (Hγ), 3.69, 3.46 (Hδ); Lys3: 1.41 (Hγ), 1.55 (Hδ), 2.77 (Hε), 7.75 (NH); Pro4: 2.017 (Hγ), 3.62, 3.54 (Hδ); Gln5: 2.12 (Hγ), 7.55 (NHε); Gln6: 2.04 (Hγ), 7.2 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.62 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.45 (Hγ), 2.02 (H_{Me}). CD moiety: δ 7.55 (t, $NH_{\beta CD}$), 4.88–4.93 (m, 14H), 3.55–3.70 (m, 54H, H^3, H^5 and H^6), 3.20–3.40 (m, 28H, H^2, H^4), 3.61–358 (m, 2H, $H^6{}_{sub}$), 3.15–3.10 (m, 2H, $H^6{}_{sub}$), 2.4 (m, 8H, $H^2{}_{succ}$).

***N*-(Peptidylamido)-6^{I}-deoxy-6^{I}-(succinylamido)cyclomaltooctaose 6b, 7b, 8b.** *General procedure*. DIC (0.139 cm^3, 0.9 mmol) and HOBt (0.123 g, 0.9 mmol) were added successively to a solution of freeze-dried 6^{I}-deoxy-6^{I}-(succinylamido)cyclomaltooctaose **4b** (0.276 g, 0.198 mmol) in dry DMF (8 cm^3). The mixture was stirred under nitrogen for 1 h at room temperature and a solution of freeze-dried **1** (0.121 g, 0.09 mmol) in dry DMF (2 cm^3) was added. The mixture was stirred at room temperature for 48 h. The reaction was stopped by addition of water (0.2 cm^3) at 0 °C and the mixture was stirred at room temperature for 2 h. After evaporation off of most of the solvent, the residual oil was poured into acetone (150 cm^3). The white precipitate was centrifuged, washed with acetone, dried under vacuum for 48 h in the presence of P_2O_5 and freeze-dried to give a crude mixture (0.306 g) of **6b**, **7b** and **8b**. Final purification by HPLC afforded **6b** (t_R 15.5 min; 0.0125 g, 7%), **7b** (t_R 13.85 min; 0.074 g, 29%) and **8b** (t_R 12.5 min; 0.033 g, 10%).

N-[L-(Arginylamido)-6^{I}-deoxy-6^{I}-(succinylamido)cyclomaltooctaose]-Pro-N$^{\varepsilon}$-[N-trifluoroacetyl-L-(lysylamido)]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ **6b**.—ESI-HRMS *m/z* 2843.1443 (Calc. for $C_{117}H_{180}F_3NaN_{19}O_{55}S$ $[M + Na]^+$). Measured *m/z*, 2843.145; *m/z* 1433.067 (Calc. for $C_{117}H_{180}F_3N_{19}Na_2O_{55}S$ $[M + 2Na]^{2+}$). Measured *m/z*, 1433.066; *m/z* 1422.0760 (Calc. for $C_{117}H_{181}F_3N_{19}NaO_{55}S$ $[M + H + Na]^{2+}$). Measured *m/z*, 1422.072; δ_H (500.13 MHz; d_6-DMSO) 8.01 (NH_{Arg1}), 8.22 (NH_{Lys3}), 8.17 (NH_{Gln5}), 7.80 (NH_{Gln6}), 7.89 (NH_{Phe7}), 8.07 (NH_{Phe8}), 8.09 (NH_{Gly9}), 7.94 (NH_{Leu10}), 7.90 (NH_{Met11}), 4.16 ($H\alpha_{Arg1}$), 4.44 ($H\alpha_{Pro2}$), 4.43 ($H\alpha_{Lys3}$), 4.31 ($H\alpha_{Pro4}$), 4.12 ($H\alpha_{Gln5}$), 4.16 ($H\alpha_{Gln6}$), 4.47 ($H\alpha_{Phe7}$), 4.51 ($H\alpha_{Phe8}$), 3.73 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 1.79 ($H\beta_{Arg1}$), 1.92, 1.85 ($H\beta_{Pro2}$), 1.71, 1.54 ($H\beta_{Lys3}$), 1.93, 1.84 ($H\beta_{Pro4}$), 1.86, 1.75 ($H\beta_{Gln5}$), 1.79, 1.71 ($H\beta_{Gln6}$), 2.97, 2.74 ($H\beta_{Phe7}$), 3.05, 2.85 ($H\beta_{Phe8}$), 1.5, 1.48 ($H\beta_{Leu10}$), 1.94, 1.82 ($H\beta_{Met11}$); other protons: Arg1: 1.71 (Hγ), 3.14 (Hδ), 7.72 (NHε); Pro2: 2.12 (Hγ), 3.69, 3.46 (Hδ); Lys3: 1.41 (Hγ), 1.55 (Hδ), 2.77 (Hε), 8.33 (NH); Pro4: 2.017 (Hγ), 3.62, 3.54 (Hδ); Gln5: 2.12 (Hγ), 7.2 (NHε); Gln6: 2.04 (Hγ), 7.2 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.62 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.45 (Hγ), 2.02 (H_{Me}). CD moiety: δ 7.57 (t, $NH_{\beta CD}$), 4.88–4.93 (m, 8H), 3.55–3.70 (m, 40H, H^3, H^5 and H^6), 3.20–3.40 (m, 16H, H^2, H^4), 3.60 (dd, 1H, $H^6{}_{sub}$), 3.15 (dd, 1H, $H^6{}_{sub}$), 2.40 (m, 4H, $H^2{}_{succ}$).

N-[N-Trifluoroacetyl-L-(arginylamido)]-Pro-N$^{\varepsilon}$-[L-lysyl-amido-6^{I}-deoxy-6^{I}-(succinylamido)cyclomaltooctaose]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ **7b**.—ESI-HRMS: *m/z* 2843.1443 (Calc. for $C_{117}H_{180}F_3N_{19}NaO_{55}S$ $[M + Na]^+$). Measured *m/z*, 2843.145; *m/z* 1433.0670 (Calc. for $C_{117}H_{180}F_3N_{19}Na_2O_{55}S$ $[M + 2Na]^{2+}$). Measured *m/z*, 1433.061; *m/z* 1422.0760 (Calc. for $C_{117}H_{181}F_3N_{19}NaO_{55}S$ $[M + H + Na]^{2+}$). Measured *m/z*, 1422.077; δ_H (500.13 MHz; d_6-DMSO) 8.43 (NH_{Arg1}), 8.22 (NH_{Lys3}), 8.17 (NH_{Gln5}), 7.80 (NH_{Gln6}), 7.89 (NH_{Phe7}), 8.07 (NH_{Phe8}), 8.09 (NH_{Gly9}), 7.94 (NH_{Leu10}), 7.90 (NH_{Met11}), 4.16 ($H\alpha_{Arg1}$), 4.44 ($H\alpha_{Pro2}$), 4.43 ($H\alpha_{Lys3}$), 4.31 ($H\alpha_{Pro4}$), 4.12 ($H\alpha_{Gln5}$), 4.16 ($H\alpha_{Gln6}$), 4.47 ($H\alpha_{Phe7}$), 4.51 ($H\alpha_{Phe8}$), 3.73 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 1.79 ($H\beta_{Arg1}$), 1.92, 1.85 ($H\beta_{Pro2}$), 1.71, 1.54 ($H\beta_{Lys3}$), 1.93, 1.84 ($H\beta_{Pro4}$), 1.86, 1.75 ($H\beta_{Gln5}$), 1.79, 1.71 ($H\beta_{Gln6}$), 2.97, 2.74 ($H\beta_{Phe7}$), 3.05, 2.85 ($H\beta_{Phe8}$), 1.5, 1.48 ($H\beta_{Leu10}$), 1.94, 1.82 ($H\beta_{Met11}$); other protons: Arg1: 1.71 (Hγ), 3.14 (Hδ), 7.72 (NHε); Pro2: 2.12 (Hγ), 3.69, 3.46 (Hδ); Lys3: 1.41 (Hγ), 1.55 (Hδ), 2.77 (Hε), 8.04 (NH); Pro4: 2.017 (Hγ), 3.62, 3.54 (Hδ); Gln5: 2.12 (Hγ), 7.2 (NHε); Gln6: 2.04 (Hγ), 7.2 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.62 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.45 (Hγ), 2.02 (H_{Me}). CD moiety: δ 7.57 (t, $NH_{\beta CD}$), 4.88–4.93 (m, 8H), 3.55–3.70 (m, 40H, H^3, H^5 and H^6), 3.20–3.40 (m, 16H, H^2, H^4), 3.62 (dd, 1H, $H^6{}_{sub}$), 3.15 (dd, 1H, $H^6{}_{sub}$), 2.40 (m, 4H, $H^2{}_{succ}$).

N-[L-(Arginylamido)-6^{I}-deoxy-6^{I}-(succinylamido)cyclomaltoheptaose]-Pro-N$^{\varepsilon}$-[L-(lysylamido)-6^{I}-deoxy-6^{I}-(succinylamido)cyclomaltooctaose]-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ **8b**.—ESI-MS: *m/z* 4102.6 (Calc. for $C_{167}H_{265}N_{20}O_{95}S$ $[M + H]^+$). Measured *m/z*, 4102.5; ESI-HRMS: *m/z* 2073.7979 (Calc. for $C_{167}H_{264}N_{20}Na_2O_{95}S$ $[M + 2Na]^{2+}$). Measured *m/z*, 2073.807; *m/z* 2062.8069 (Calc. for $C_{167}H_{265}N_{20}NaO_{95}S$ $[M + H + Na]^{2+}$). Measured *m/z*, 2062.805; δ_H (500.13 MHz; d_6-DMSO) 8.01 (NH_{Arg1}), 8.22 (NH_{Lys3}), 8.17 (NH_{Gln5}), 7.80 (NH_{Gln6}), 7.89 (NH_{Phe7}), 8.07 (NH_{Phe8}), 8.09 (NH_{Gly9}), 7.94 (NH_{Leu10}), 7.90 (NH_{Met11}), 4.16 ($H\alpha_{Arg1}$), 4.44 ($H\alpha_{Pro2}$), 4.43

($H\alpha_{Lys3}$), 4.31 ($H\alpha_{Pro4}$), 4.12 ($H\alpha_{Gln5}$), 4.16 ($H\alpha_{Gln6}$), 4.47 ($H\alpha_{Phe7}$), 4.51 ($H\alpha_{Phe8}$), 3.73 ($H\alpha_{Gly9}$), 4.31 ($H\alpha_{Leu10}$), 4.25 ($H\alpha_{Met11}$), 1.79 ($H\beta_{Arg1}$), 1.92, 1.85 ($H\beta_{Pro2}$), 1.71, 1.54 ($H\beta_{Lys3}$), 1.93, 1.84 ($H\beta_{Pro4}$), 1.86, 1.75 ($H\beta_{Gln5}$), 1.79, 1.71 ($H\beta_{Gln6}$), 2.97, 2.74 ($H\beta_{Phe7}$), 3.05, 2.85 ($H\beta_{Phe8}$), 1.5, 1.48 ($H\beta_{Leu10}$), 1.94, 1.82 ($H\beta_{Met11}$); other protons: Arg1: 1.71 (Hγ), 3.14 (Hδ), 7.72 (NHε); Pro2: 2.12 (Hγ), 3.69, 3.46 (Hδ); Lys3: 1.41 (Hγ), 1.55 (Hδ), 2.77 (Hε), 8.04 (NH); Pro4: 2.017 (Hγ), 3.62, 3.54 (Hδ); Gln5: 2.12 (Hγ), 7.2 (NHε); Gln6: 2.04 (Hγ), 7.2 (NHε); Phe7: 7.15–7.3 (5ArH); Phe8: 7.15–7.3 (5ArH); Leu10: 1.62 (Hγ), 0.87 (Hδ); Met11: 2.41, 2.45 (Hγ), 2.02 (H_{Me}). CD moiety: δ 7.57 (t, $NH_{\beta CD}$) 4.88–4.93 (m, 16H), 3.55–3.70 (m, 80H, H^3, H^5 and H^6), 3.20–3.40 (m, 32H, H^2, H^4), 3.60–3.55 (m, 2H, $H^6{}_{sub}$), 3.125–3.10 (m, 2H, $H^6{}_{sub}$), 2.40 (m, 8H, $H^2{}_{succ}$).

Preparation of inclusion complex between 7a and indomethacin. 7.3 mg (0.02 mmol) of indomethacin were dissolved in 4 cm^3 of water, and the pH of the solution was adjusted to 8 with 0.01 M NaOH. The solution was freeze-dried and re-dissolved in 4 cm^3 of water. **7a** (5.3 mg, 0.002 mol) was dissolved in 0.4 cm^3 of indomethacin mother solution (pH 8), and the resulting mixture (**7a**–indomethacin 1:1, pH 8) was lyophilized. After re-dissolution in 0.4 cm^3 of D_2O, ^1H NMR experiments were performed immediately to avoid indomethacin degradation. The same procedure was employed to realize a βCD–indomethacin complex as a reference sample.

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