Chitosan Derivatives Bearing Pendant Cyclodextrin Cavities: Synthesis and Inclusion Performance

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ABSTRACT: A new synthetic route to β-cyclodextrin-linked chitosan was developed. This was based on the preparation of a monosubstituted β-cyclodextrin (β-CD) derivative possessing a reducing sugar on the primary face followed by its reductive amination. The CD−polysaccharide was fully characterized in terms of chemical integrity and purity by high-resolution NMR and light scattering. The formation of inclusion complexes was investigated by NMR spectroscopy using *tert*-butylbenzoic acid and (+)-catechin as model guests. Inclusion properties of the grafted β-CDs were shown to be similar to those of native β-CD in terms of complex geometry and affinity constant. These results confirm that the pendant β-cyclodextrin preserves its conformation and its complexing characteristics.

Introduction

Polymer delivery systems have been used for various applications in therapeutics as well as in agriculture and foods.[1] Such delivery devices have many advantages over conventional systems especially for medicines. As a drug is injected or absorbed, its plasma level increases until a maximum and then rapidly decreases. Above a certain level the drug is toxic whereas below another one, it is inefficient. Polymer drug carriers allow a controlled release of a bioactive substance over a long period of time and with a constant level in the plasma. Such systems thus provide clues for the sustained release of bioactive molecules.[2] These polymer delivery devices are usually hydrogels, which may be bioerodible.[3] Biopolymers and various synthetic degradable polymers have been used for the preparation of such materials. Among these compounds, naturally occurring polysaccharides are very attractive. These polyhydroxyl derivatives may form physical gels under specific conditions,[4] and their functional groups permit various chemical modifications and/or cross-linkings. In the continuing challenge to improve the properties of such delivery systems, our objective was to modify chitosan, a biocompatible and biodegradable polysaccharide, by introducing cyclodextrins (CDs) and to investigate the inclusion properties of this CD−polymer by NMR spectroscopy. Cyclodextrins are water-soluble cyclic oligosaccharides which can include various guest molecules into their hydrophobic cavity, allowing the solubilization, stabilization, and transport of hydrophobic drugs.[5] Moreover, the choice of chitosan is important for biomedical applications since it is a bioactive macromolecule. In addition, the existence of primary amine groups on the C-2 position allows specific modifications on the repeat unit. In addition, the polymer itself can be easily cross-linked to be processed in gel, capsule, or membrane with a controlled porosity. Thus, grafting CD molecules onto chitosan may lead to a molecular carrier exhibiting promising properties owing to the cumulative effects of size specificity and transport properties of cyclodextrins and polymer matrix. The synthesis of such CD derivatives of chitosan has been recently reported.[6,7] The synthetic route was based on the coupling of a mono 2-*O*-(formylmethylated)-α-CD or β-CD derivative with chitosan with M_w = 40 000 or 60 000. In terms of cyclodextrin inclusion complexes, the introduction of a guest molecule in the cavity classically takes place from the wider secondary hydroxyl groups side although the other situation may be also encountered, depending on the guest. It has been shown that steric hindrance effects due to substitution of cyclodextrin could result in an important decrease of the association constant of complexes.[8] In view of these data, our strategy consisted in the monofunctionalization of β-cyclodextrin on the primary face in order to specifically attach it on chitosan from the side which is "less involved" in the inclusion of guests.

Results and Discussion

1. Synthesis of CD−Chitosan. Carbodiimide-mediated coupling reaction and reductive amination are typical examples of reactions which can be used for covalent attachment of substrates to the 2-amino functions of chitosan. Our first approach to the target CD−chitosan was based on an amidation reaction of chitosan with a monosubstituted β-CD derivative bearing a free carboxylic acid function (6^I-succinylamido-6^I-deoxycyclomaltoheptaose)[9] using the water-soluble carbodiimide 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). However, under these conditions, β-CD derivatives of chitosan were obtained with very low substitution degrees (DS). We next turned our attention to the reductive amination procedure, involving the preparation of an aldehyde-containing CD derivative. As shown by Yalpani et al.,[10] facile conversions of chitosan can be achieved by this method using any aldehydo or keto sugar.

In view of these data, our strategy for the synthesis of CD−chitosan involved a suitable synthesis of a CD derivative possessing a reducing sugar **4** (Scheme 1) and a subsequent reductive amination. The reducing sugar was also expected to act as a hydrophilic spacer between the CD cavities and the polymer. The synthesis of key compound **4** was based on the carbodiimide-mediated coupling of 6^I-amino-6^I-deoxycyclomaltoheptaose (**1**) with the intermediate 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranuronic acid (**2**). The β-cyclodextrin derivative **1** was synthesized in three steps from the parent

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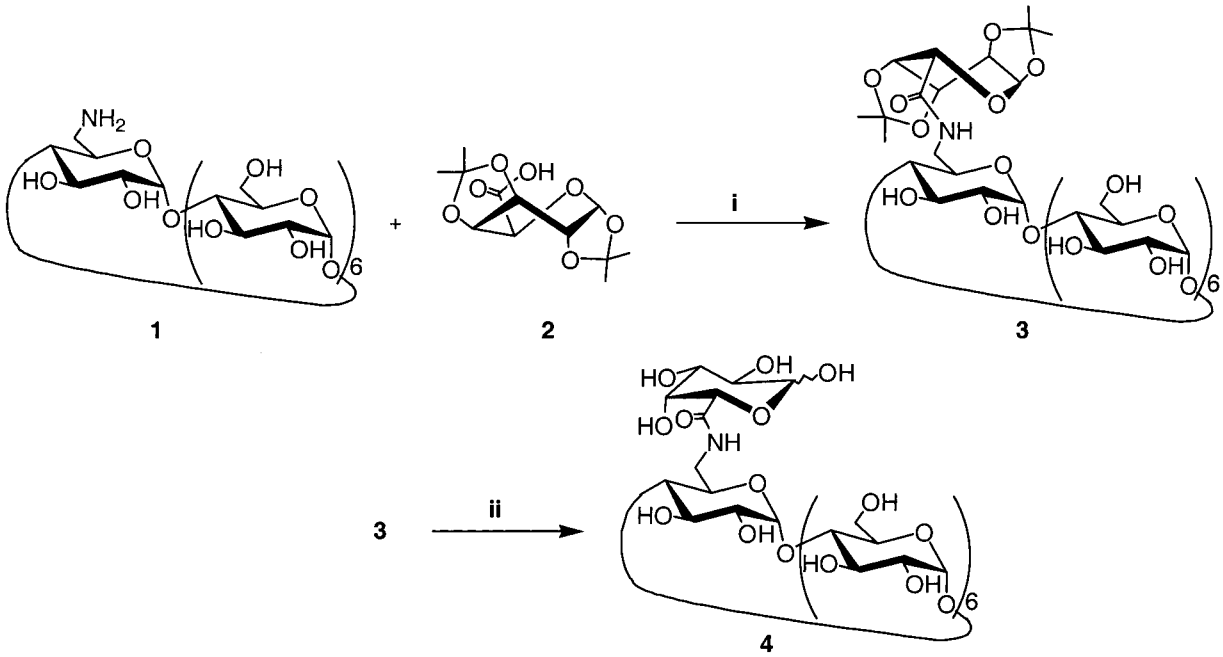
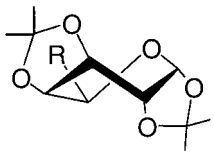
Scheme 1. Reagents: (i) DIC, HOBt, DMF, RT, 82%; (ii) CF_3CO_2H−water, RT, 55%



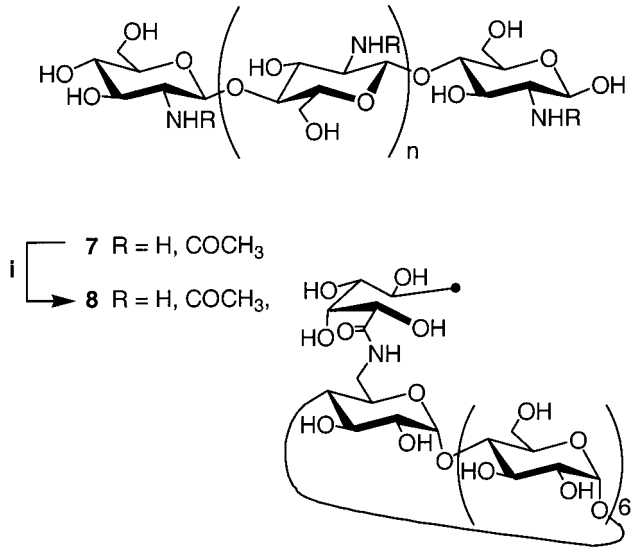
cyclodextrin following a published procedure.[11] Compound **2** was readily produced by oxidation of the primary hydroxyl group of 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose (**5**) using Nilsson's conditions[12] (PDC, *tert*-BuOH, Ac_2O) followed by saponification of the *tert*-butyl ester **6**. Reaction of monoaminocyclodextrin (**1**) with uronic acid (**2**) using the *N*,*N*′-diisopropylcarbodiimide/hydroxybenzotriazole (DIC−HOBt) procedure in anhydrous *N*,*N*-dimethylformamide resulted in the formation of **3** in 82% yield. Quantitative removal of the isopropylidene groups by treatment with aqueous trifluoroacetic acid and subsequent purification by HPLC (95:5 water/MeOH) afforded the target CD derivative **4** in 55% yield.



5 R = CH_2OH
6 R = $CO_2{}^tBU$
2 R = CO_2H

The chemical structure of the key intermediate **4** was assessed using high-resolution NMR (^1H and ^{13}C) and mass spectrometry with electrospray infusion mode. The NMR analysis was performed in deuterium oxide. In this solvent, the uronamide residue of **4** was shown to behave like the free reducing sugar D-galacturonic acid[13] as it presents as a mixture of α- and β-pyranose forms (α:β ≈ 1/1). The monosubstitution of β-cyclodextrin was shown by digital integration of the NMR signals arising from the anomeric protons of the α- and β-pyranose forms of the galacturonamide and cyclodextrin moieties. Since the spectrum is relatively complex owing to the lack of molecular symmetry of the cyclodextrin moiety, a complete analysis was obtained from stepwise identification of protons of **4** by COSY and successive RELAY experiments. Being located in a very specific

Scheme 2. Reagents: (i) Cyclodextrin Derivative 4, $NaCNBH_3$, Aqueous CH_3CO_2H/MeOH (pH 5), RT, 64 %



spectral domain, anomeric protons were used as a starting point for stepwise assignment of the different sugar moieties.

Having the aldehydo CD derivative **4** in hand, the last step involved its introduction onto chitosan using the reductive amination procedure as reported in the literature.[10] Chitosan **7** dissolved in a mixture of aqueous acetic acid and methanol was reacted with **4** (0.1 equiv per monomer unit) in the presence of sodium cyanoborohydride to provide CD−chitosan **8** in 64% yield (Scheme 2).

2. Characterization of CD−Chitosan. The chemical integrity and purity of the final product **8** were checked by high-resolution ^1H NMR and light scattering. The NMR analysis was performed in D_2O/DCl (pD 3.5) and demonstrated that **8** was free of any byproduct (see Figure 1). Digital integration of the NMR signals arising from the anomeric protons of chitosan and cyclodextrin gave a substitution degree of approximately

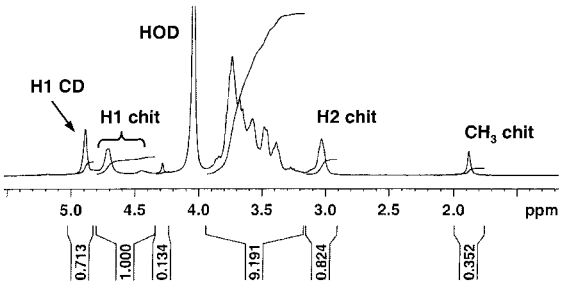


Figure 1. ^{1}H NMR spectrum (400 MHz, 80 °C, 6 mg mL^{-1} in D_2O/DCl, pD 3.5) of CD−chitosan **8**.

10%, indicating that the grafting of the CD residues proceeded quasi-quantitatively, at least for low degrees of grafting.

From dynamic light scattering, it was proved that no aggregate is formed; only one relaxation time is observed corresponding to **8**. CD−chitosan was examined for its polymeric characteristics by static light scattering using the usual procedure. The weight-average molecular weight M_w, second virial coefficient A_2, and radius of gyration R_g were obtained from the Zimm diagram established in dilute regime and were compared to the values found for initial chitosan **7**[14] (see Figure 2). The value for A_2 was found lower for CD−chitosan **8** than for nonmodified chitosan **7**; the CD grafting thus seems to decrease slightly the solubility of the polymer in the usual good solvent of chitosan (0.3 M CH_3COOH/0.05 M CH_3COONa). A large increase in the molecular weight, M_w, is observed for **8**. This in fact corresponds to a CD grafting degree of approximatively 12%, which is in very good agreement with the value obtained by NMR. Moreover, this result provides clear evidence of the covalent attachment of the CD molecules onto chitosan and indicates that no degradation of the polymer backbone occurs during the grafting reaction. The same conclusion was previously drawn in the case of the reductive amination of aldehydes with long alkyl chains by chitosan.[15] Concerning the radius of gyration, a slight increase is observed for **8**, which may indicate an increase in the persistence length of the derivative with respect to that of initial chitosan having the same contour length.

3. Inclusion Properties. The inclusion ability of CD−chitosan **8** was investigated by NMR spectroscopy using 4-*tert*-butylbenzoic acid (**9**) as a model guest, in a first step. It is well documented that benzoic acid and its derivatives form 1:1 inclusion complexes with cyclodextrin, the carboxylic group being closer to the smaller rim.[16] In the case of 4-*tert*-butylbenzoic acid, it was shown from ROE data and due to the bulkiness of the 4-*tert*-butyl group that inclusion proceeds through the wider side of β-CD (see Figure 3).[17] Unlike its benzoate anion homologue, compound **9** is sparingly soluble in water, but its solubility is clearly improved by inclusion in β-CD. Figure 4 shows the partial 400 MHz ^{1}H NMR spectra of native β-CD in the absence and in the presence of 4-*tert*-butylbenzoic acid (**9**). Inclusion in β-CD is evidenced by modifications of the ^{1}H NMR spectrum of the host molecule.[18] Under these conditions, only shifts of the signals were observed. In all cases, no new peak appeared which could be assigned to the pure complex. This observation implies that complexation is a dynamic process, i.e., the included molecule is in fast exchange (relative to the NMR time scale) between the "free" and "bound" states. It is observed here that guest

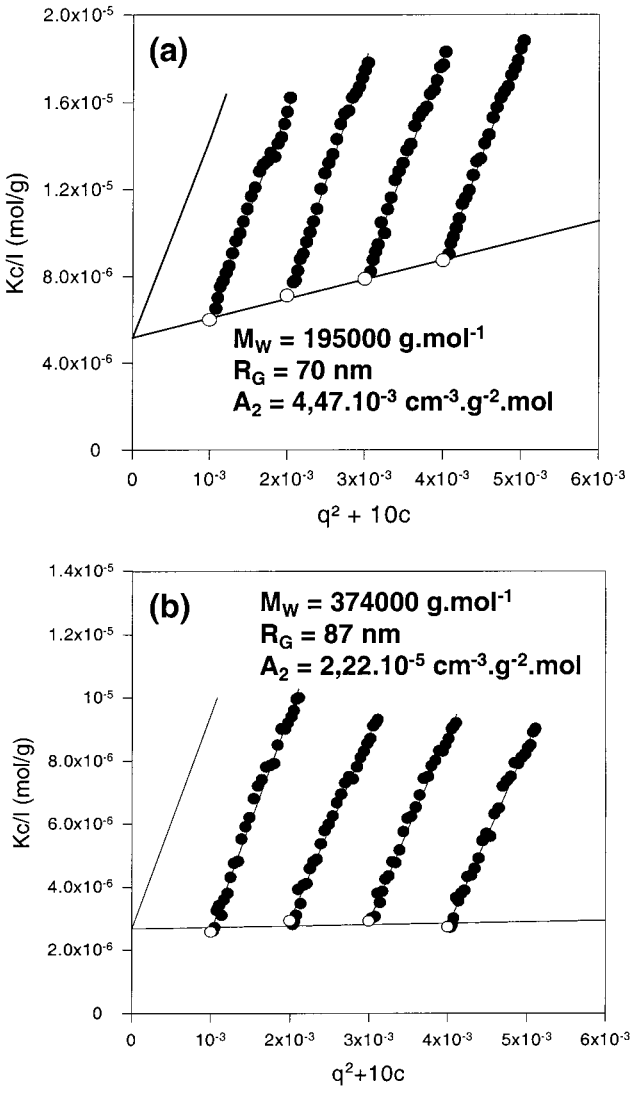


Figure 2. Zimm plots obtained for (a) chitosan **7** and (b) CD−chitosan **8** at T = 25 °C. The solvent is a 0.3 M CH_3COOH/0.05 M CH_3COONa solution. Points (○) are extrapolations to zero wave vector, and solid lines represent best fits.

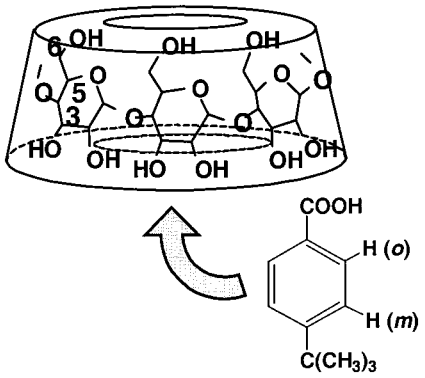


Figure 3. Model for the inclusion process of 4-*tert*-butylbenzoic acid (**9**) in β-cyclodextrin in aqueous solution.

9 induces large shifts in the NMR signals of the H-3 and H-5 protons located in the cavity and, to a lesser extent, the H-6 and H-1 protons, which clearly demonstrates the formation of an inclusion complex. Large shift changes are also experienced with CD−chitosan **8** upon addition of excess **9** followed by removal of the nondissolved particles by centrifugation (see Figure 5a). Digital integration of the NMR signals arising from the

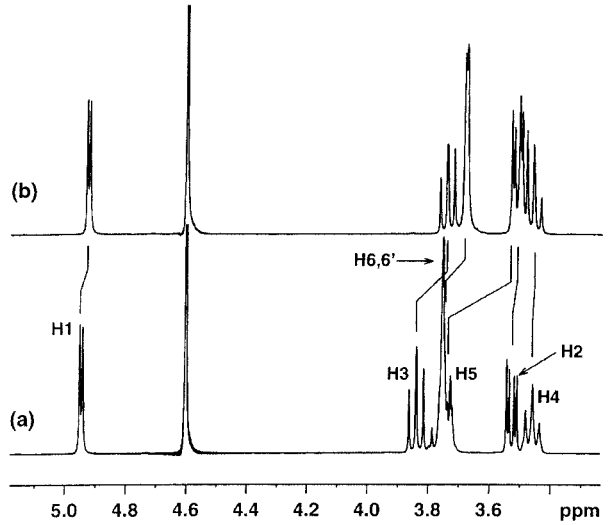
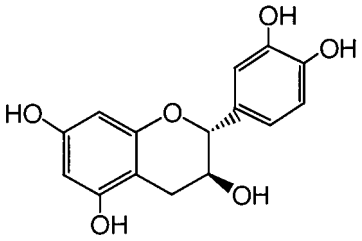


Figure 4. Partial ^{1}H NMR spectra (400 MHz, 30 °C, D_2O/DCl, pD 3) of (a) β-CD (10 mM) and (b) a mixture of β-CD (6 mM) and 4-*tert*-butylbenzoic acid (**9**) (4 mM).

aromatic and anomeric protons of **9** and cyclodextrin, respectively, indicates a **9**/CD molar ratio of about 0.8. This suggests possible selective interaction of **9** with the CD cavities grafted onto chitosan. Furthermore, it can be noticed that for the anomeric protons only those of cyclodextrin show shift variations. However, owing to the complexity of the NMR spectrum (spectral overcrowding in the 3.0−4.0 ppm region), a clear observation of the shifts experienced by the H-3 and H-5 CD protons, which are the most prone to be affected by the inclusion process, is impossible. These important features can be observed using dedicated experiments based upon stepwise magnetization transfers from anomeric protons.[19] For this purpose, 1D TOCSY experiments[20] were carried out using various mixing times (T_m) from 10 to 100 ms. Figure 5b shows the partial 1D TOCSY spectra obtained with the same samples as depicted in Figure 5a, using a mixing time of 40 ms. In this example, the region of the CD anomeric protons shown in Figure 5a by an arrow was selectively excited, and the magnetization was further transferred to the H-2 and H-3 protons. It is clear from the present data that the H-3 protons experience large shifts upon addition of guest **9** as a result of the formation of an inclusion complex. More direct evidence for the complex formation can be derived from the observation of dipolar interactions (nuclear Overhauser effects) between protons of compound **9** and the grafted CDs. For this purpose, 2D T-ROESY experiments[21] which are shown to provide the most sensitive approach to the structural analysis of cyclodextrin inclusion complexes were performed. Figure 6 displays partial T-ROESY contour plots obtained with the **9**/CD−chitosan **8** and the **9**/native β-CD mixtures. In the case of the latter system, dipolar contacts (indicating spatial proximities) are observed between the meta protons of guest **9** and the cavity protons (H-3 and H-5) of β-CD, on one hand, and between the ortho protons of **9** and the H-5 and H-6 protons of β-CD, on the other. The present data are thus in good agreement with the inclusion model shown by Figure 3. The same conclusion can be drawn for the **9**/CD−chitosan **8** from the T-ROESY spectrum.

In a second step, we quantitatively investigated the inclusion performances of CD−chitosan **8** using water-soluble (+)-catechin **(10)** as a guest molecule. Catechins



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are plant extracts which are very attractive owing to their numerous biological functions, including antioxidation, deodorization, antivirus, and cancer inhibiting properties.[22] However, these phenolic compounds are bitter, brown, and easily oxidized and hence difficult to

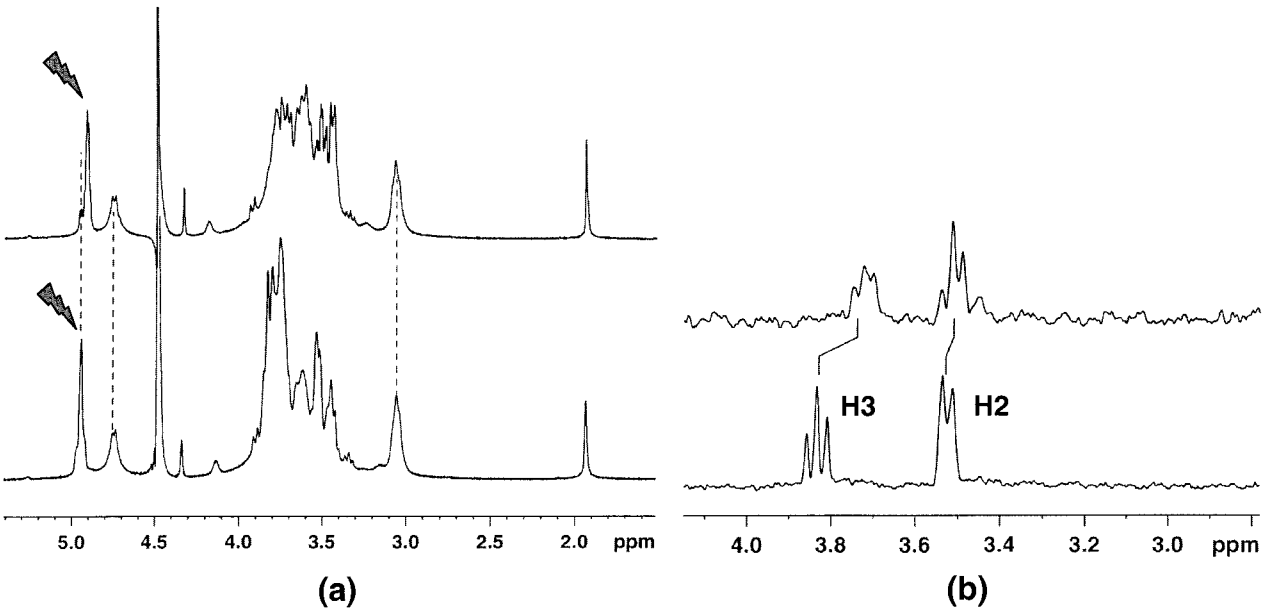

Figure 5. Partial ^{1}H NMR spectra (400 MHz, 40 °C) of CD−chitosan **8** 6 mg mL^{-1} in D_2O/DCl (pD 3.5) alone (bottom scan) and in the presence of about 0.8 mol equiv (with respect to the grafted CDs) of 4-*tert*-butylbenzoic acid (**9**) (top scan). (a) Normal 1D proton experiments; (b) 1D TOCSY experiments with a 40 ms mixing time.

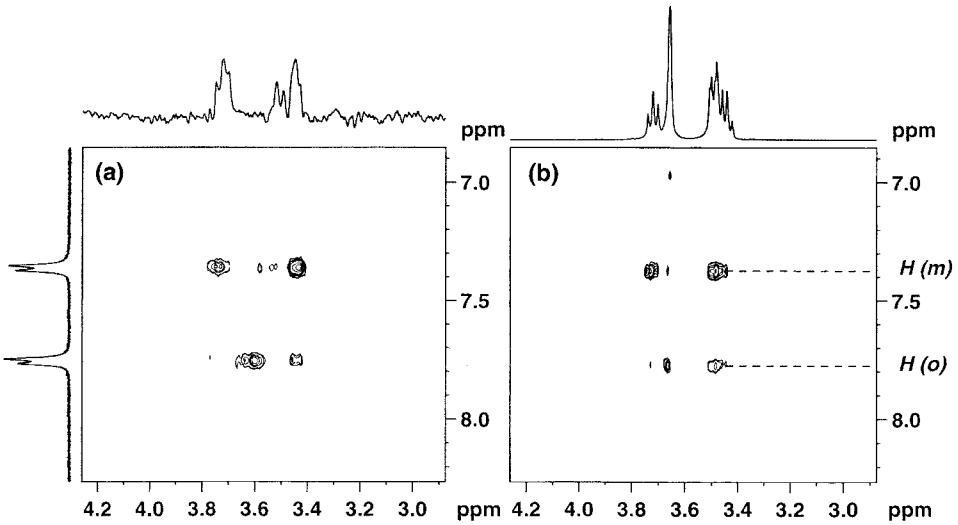

Figure 6. Partial contour plots of T-ROESY experiments (400 MHz, 30 °C, D_2O/DCl, pD 3−3.5) performed on the **9**/CD−chitosan **8** and **9**/native β-CD mixtures using spin-lock times of 200 and 300 ms at 21 dB attenuation, respectively. (a) **8** (6 mg mL^{-1}) in the presence of about 0.8 mol equiv (with respect to the grafted CDs) of **9**; (b) β-CD (6 mM) in the presence of **9** (4 mM). On the top axis are shown the partial projection 1D TOCSY spectrum (T_m = 65 ms) of **8** in the presence of **9** and the partial ^{1}H NMR projection spectrum of β-CD in the presence of **9**.

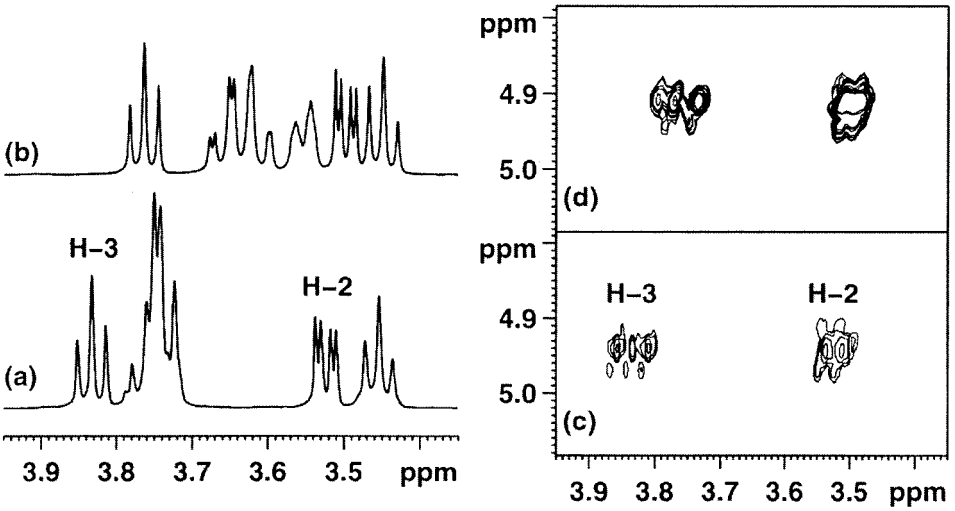

Figure 7. Comparison of the partial ^{1}H NMR spectra (500 MHz, 35 °C, D_2O/DCl, pD 4) of β-CD (2.9 mM) (a) in the absence and (b) in the presence of (+)-catechin **10** (9 mM) to the partial contour plots of one-step relay experiments from anomeric CD protons (500 MHz, 35 °C, D_2O/DCl, pD 4) performed on CD−chitosan **8** (CD concentration c_{CD} = 2.9 mM) (c) in the absence and (d) in the presence of (+)-catechin (9 mM).

use as medicine, cosmetic, or food additives. These problems might be solved by inclusion in cyclodextrins.[23] A similar strategy as presented for **9** was first used to evidence the inclusion of **10** in the CDs grafted on chitosan. Then, to assess the strength of the **10**/CD−chitosan inclusion complex, we examined the magnitude of the chemical shift changes for the **10**/CD−chitosan **8** and the **10**/native β-CD systems. As already indicated, a clear observation of the shifts experienced by the H-3 CD protons (which are the most prone to be affected by the inclusion process) of CD−chitosan requires the use of dedicated experiments based upon stepwise magnetization transfers from anomeric protons. For this, two-dimensional one-step relay experiments from anomeric protons allowing the identification of the H-2 and H-3 CD protons were used. Figure 7 displays the partial ^{1}H NMR spectra of native β-CD in the absence and in the presence of (+)-catechin and the partial contour plots of one-step relay experiments from CD anomeric protons performed under identical concentration conditions on CD−chitosan in the absence and in the presence of (+)-catechin. It is clearly observed that the H-3 protons of the free CDs and the grafted CDs experience the same shift. This suggests that although the β-CD molecule is bound to the chitosan polymer, its inclusion performances are completely retained. This was further confirmed by measuring the apparent association constant K of the inclusion complex. The stoichiometry and the association constant of the inclusion complex of β-CD with **10** could be derived by observing the changes in chemical shifts of the H-3 CD protons (see Figure 8).[8] Using the continuous variation technique, the stoichiometry of the complex was found to be 1:1 as described previously.[23] The average value for K derived from a numerical simulation of the experimental data[24] was found to be 2500 M^{-1} at 35 °C for the **10**/native β-CD system. This K value is the same order as those obtained by other authors (which were 2209 M^{-1} at 35 °C[23] and 2908 M^{-1} at 45 °C[25]). In the case of the CDs grafted on chitosan, the average value for K was found to be 2700 M^{-1} under identical conditions. This fully supports that the present method of CD grafting on chitosan leaves

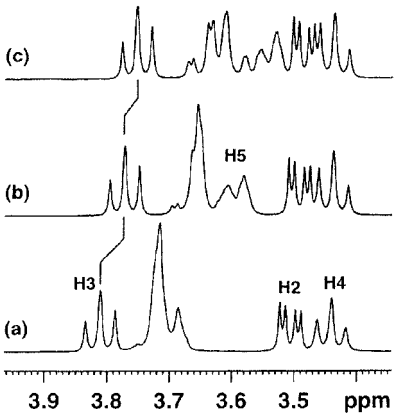

Figure 8. Partial ^{1}H NMR spectra of β-CD in the presence of guest **10** in D_2O at a temperature of 35 °C. (a) β-CD/**10** 3/0 mM; (b) β-CD/**10** 1.5/1.5 mM; (c) β-CD/**10** 1/2 mM.

the binding properties of the CD derivative unaffected relative to the original cyclodextrin.

In conclusion, a monosubstituted β-CD derivative possessing a reducing sugar on the primary face could be efficiently grafted on chitosan, yielding a new CD−polymer exhibiting the same inclusion properties toward hydrophobic guests as the native β-CD. Such polymers show promising properties for encapsulation and delivery of bioactive molecules owing to the cumulative effects of size specificity and transport properties of cyclodextrins and polymer matrix, respectively. The formation of chemical and physical gels from this CD−chitosan is under investigation and will be reported in the future.

Experimental Section

Materials and Methods. The chitosan used has a weight-average molecular weight M_w of 195 000; it is a commercial sample from PROTAN (Norway) with a degree of N-acetylation equal to 12%. It was purified by solubilization in aqueous AcOH and reprecipitation by NaOH at neutral pH. The polysaccharide was finally washed with deionized water and ethanol and then dried. The β-cyclodextrin was kindly supplied by Roquette Frères (Lestrem, France). 6^I-Amino-6^I-deoxycyclomaltoheptaose was obtained in three steps from the parent cyclodextrin as described elsewhere.[9] Other chemicals were purchased from Fluka (Buchs, Switzerland). Analytical thin-layer chromatography was performed on Merck (Darmstadt, Germany) 60 F_{254} silica gel plates followed by charring with ethanolic 10% H_2SO_4. Merck 60 (0.063−0.200 mm) silica gel was used for column chromatography. Preparative HPLC was carried out with a Waters chromatograph fitted with a refractometric detector and an Interchim C_{18}-bonded silica column. The CD derivative **4** was purified by elution with 95:5 water/methanol at 0.6 mL min^{-1}. (+)-Catechin (**10**) was purified by elution with 3:2 water/methanol at 1.2 mL min^{-1} in a first step and with 85:25 water/methanol at 1.2 mL min^{-1} in a second step. FAB and DCI mass spectra were measured on a Nermag R 1010C mass spectrometer. In the FAB mode, the primary beam consisted of Xe atoms, the samples were dissolved in glycerol, and the positive ions were separated and accelerated over a potential of 9 kV. Electrospray mass spectra were measured in the positive mode on a ZabSpec TOF (Micromass, UK) mass spectrometer. The CD derivative **4** was dissolved in methanol at a concentration of 0.1 mg mL^{-1} and infused into the electrospray ion source. The capillary voltage was set to 4 kV. Poly(ethylene glycol) was used for external calibration.

^{1}H NMR experiments were performed using Bruker DRX500, DRX400, and AC300 spectrometers operating at 500, 400, and 300 MHz, respectively. ^{13}C NMR spectra were recorded with Bruker DRX500, DRX400, and AC300 spectrometers operating at 125, 100, and 75 MHz, respectively. 1D NMR spectra were collected using 16K data points. All 2D experiments were acquired using 2K data points and 256 time increments. For dipolar correlations (T-ROESY experiments) the phase sensitive TPPI was used, and processing resulted in a 1K × 1K (real−real) matrix. Chemical shifts are given relative to external tetramethylsilane (TMS = 0 ppm), and calibration was performed using the signal of the residual protons or carbons of the solvent as a secondary reference. Deuterium oxide and dimethyl-d_6 sulfoxide were obtained from SDS (Vitry, France). Details concerning experimental conditions are given in the figure captions. Association constants for the formation of 1:1 complexes were determined using the following equation:[24]

$$[B]_t = \Delta\delta_{Aobs}/\Delta\delta_{Ac}([A]_t + (K(1 - \Delta\delta_{Aobs}/\Delta\delta_{Ac})) - 1) \quad (1)$$

where $[A]_t$ and $[B]_t$ are the total concentrations of the host and guest molecules, respectively. $\Delta\delta_{Aobs}$ represents the chemical shift difference (for a given proton) between free A (obtained in the absence of B) and the observed value in the presence of B, whereas $\Delta\delta_{Ac}$ represents the chemical shift difference between free A and the pure complex. The experimental data (corresponding to $[A]_t$, $[B]_t$, and $\Delta\delta_{Aobs}$) were processed using a multiparameter iterative fitting procedure contained in the SIMPLEX algorithm.[26]

Static and dynamic light-scattering measurements were both performed by means of a spectrometer fitted with an ionized argon laser source (Spectra Physics model 2020) operating at λ = 488 nm, an ALV-5000 correlator from ALV, Langen-FRG Instruments, a computer-controlled and stepping-motor-driven variable-angle detection system, and a temperature-controlled sample cell. The solutions were investigated in the polymer concentration range 10^{-4}−4 × 10^{-4} g mL^{-1} at the temperature T = 25 °C and in the 0.3 M CH_3COOH/0.05 M CH_3COONa solvent mixture. In static light scattering (SLS), one measures the angular dependence of the excess of scattered intensity $I(q)$ with respect to the solvent, where the scattering wave vector q is given by

$$q = (4\pi n/\lambda)\sin(\theta/2) \quad (2)$$

In eq 2, λ is the wavelength of light in the vacuum, n is the solvent refractive index, and θ is the scattering angle. In our experiments, the range of scattering angle covered is 20° < θ < 150°. Corrections to the absolute scattering intensities $I(q)$ (i.e., excess Rayleigh ratio) were made using a toluene sample reference. For a dilute polymer solution measured at a low scattering angle, $I(q)$ can be related to the weight-average molecular weight M_w, the second virial coefficient A_2, and the average radius of gyration R_g by

$$Kc/I(q,c) \approx 1/M_w[1 + q^2R_g^2/3] + 2A_2c \quad (3)$$

where $K = 4\pi^2n^2/(\lambda^4N_a)(dn/dc)^2$ with N_a, c, and dn/dc being Avogadro's number, the polymer concentration, and the refractive index increment, respectively. The value adopted for dn/dc was 0.195.[27]

Synthesis. *1,2;3,4-Di-O-isopropylidene-α-D-galactopyranuronic Acid (**2**).* To a solution of **5** (2 g, 7.68 mmol) in dry CH_2Cl_2 (48 mL), acetic anhydride (7.2 mL, 76.8 mmol), *tert*-butyl alcohol (14.4 mL, 153.6 mmol), and pyridinium dichromate (5.8 g, 15.4 mmol) were successively added. The resulting mixture was stirred under nitrogen at room temperature for 3 h. The mixture was then applied on a small silica gel pad with a 10 cm layer of Et_2O on the top of the gel in order to precipitate the chromium compounds. The latter were removed by filtration (elution with Et_2O), and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (elution with 7:3 cyclohexanes−Et_2O) to yield **6** as a white solid (1.8 g, 71%). To a solution of **6** in MeOH (15 mL), a 2 M aqueous NaOH solution (15 mL) was added dropwise at 0 °C. The mixture was left for 24 h at room temperature. The solution was subjected to an extractive

workup (extraction with CH_2Cl_2), and the organic phase was concentrated under reduced pressure, affording a white solid that contained almost pure **2** (1 g, 69%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm: 5.53 (H-1, d, $J_{1,2}$ 4.9); 4.65 (H-3, dd, $J_{2,3}$ 2.7, $J_{3,4}$ 7.8); 4.50 (H-4, dd, $J_{4,5}$ 2.2); 4.40 (H-2, dd); 4.19 (H-5, d); 1.44, 1.33, 1.29, 1.28 (CH_3). DCIMS: *m*/**z** 292 $[M + NH_4]^+$.

*6^I-(1,2;3,4-Di-O-isopropylidene-α-D-galactopyranuronamido)-6^I-deoxycyclomaltoheptaose (**3**).* To a solution of **1** (0.4 g, 0.35 mmol) and **2** (0.126 g, 0.46 mmol) in dry DMF (20 mL), diisopropylcarbodiimide (DIC) (0.22 mL, 1.4 mmol) and hydroxybenzotriazole (HOBt) (0.095 g, 0.7 mmol) dissolved in DMF (2 mL) were successively added. The mixture was stirred under nitrogen at room temperature for 24 h. The reaction was stopped by addition of water (0.5 mL). After evaporation of most of the solvents, the residual oil was poured into acetone (200 mL). The white precipitate was filtered, washed with acetone, and dried to give pure **3** (0.4 g, 82%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ/ppm: 7.26 (NH, t, *J* 5.8); 5.86−5.68, 5.62−5.57 (OH-2, OH-3 CD); 5.56 (H-1′, d, $J_{1',2'}$ 4.9); 4.88, 4.85−4.81 (H-1 CD, d and m, $J_{1,2}$ 3.7); 4.62 (H-3′, dd, $J_{2',3'}$ 2.4, $J_{3',4'}$ 7.7); 4.47 (H-4′); 4.39 (H-2′); 4.52−4.45, 4.40−4.35 (OH-6 CD, m); 4.08 (H-5′, d, $J_{4',5'}$ 2.1); 3.72−3.18 (H-2, H-3, H-4, H-5, H-6a, H-6b CD); 1.44, 1.30, 1.29, 1.25 (CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ/ppm: 167.3 (CO); 108.4, 108.2 ($C(CH_3)_2$); 102.2, 102.0, 101.8, 101.3 (C-1 CD); 95.7 (C-1′); 83.7, 81.7, 81.5, 81.3, 81.2, 81.1, 80.7 (C-4 CD); 73.1−71.6 (C-2, C-3, C-5 CD); 71.1 (C-4′); 69.9 (C-2′, C-3′); 68.2 (C-5′); 59.9, 59.7, 59.3 (C-6 CD); 25.7, 24.6, 24.4 (CH_3). FABMS: *m*/**z** 1413 $[M + Na]^+$.

*6^I-(D-Galacturonamido)-6^I-deoxycyclomaltoheptaose (**4**).* Compound **3** (0.4 g, 0.29 mmol) was dissolved in a mixture of 99% trifluoroacetic acid (2 mL) and water (1 mL) and stirred at room temperature. When the reaction was complete (ca. 1 h), the solution was evaporated under reduced pressure. The crude residual material was purified by HPLC to afford **4** (0.20 g, 55%) as an amorphous powder after freeze-drying.

R_f 0.51 (1:1:2 DMF−H_2O-*n*-BuOH). ^{1}H NMR (D_2O, 500 MHz) δ/ppm: 5.24 (H-1′α, d, $J_{1',2'\alpha}$ 3.8); 4.97−4.94, 4.93, 4.9 (H-1 CD, m and d, $J_{1,2}$ 3.7); 4.51 (H-1′β, d, $J_{1',2'\beta}$ 7.9); 4.41 (H-5′α, d, $J_{4',5'\alpha}$ 1.4); 4.15 (H-4′α, dd, $J_{3',4'\alpha}$ 3.4); 4.11 (H-5′β, d, $J_{4',5'\beta}$ 1.4); 4.09 (H-4′β, dd, $J_{3',4'\beta}$ 3.4); 3.84−3.82, 3.81 (H-3 CD); 3.78−3.68 (H-5 CD); 3.84−3.67 (H-6 CD); 3.78 (H-3′α); 3.66 (H-2′α); 3.57 (H-3′β); 3.53, 3.53−3.51 (H-2 CD); 3.48−3.38, 3.32 (H-4 CD)°; 3.37 (H-2′β). ^{13}C NMR (D_2O, 125 MHz) δ/ppm: 104.7, 104.5, 104.3 (C-1 CD); 98.9 (C-1′β); 95 (C-1′α); 85.9, 84.1−83.8 (C-4 CD); 77.5 (C-5′β); 75.6 (C-3 CD); 74.7 (C-2 CD); 74.3 (C-5 CD); 74.2 (C-2′β); 73.3 (C-5′α); 72.4 (C-4′α); 71.8 (C-4′β); 70.7 (C-2′α), 63.2, 63.1, 62.8 (C-6 CD). ESI−HRMS: *m*/**z** 1332.4074 $[M + Na]^+$ (exact mass for $C_{48}H_{79}NO_{40}Na$: 1332.4076).

*CD−Chitosan (**8**).* To a solution of chitosan (0.2 g) in a mixture of 0.2 M aqueous CH_3CO_2H (14 mL) and MeOH (10 mL), a 0.1 M aqueous NaOH solution was added dropwise in order to adjust the pH to 5. Compound **4** (0.16 g, 0.122 mmol) was then added. After stirring at room temperature for 7 h, a solution of $NaCNBH_3$ (0.23 g, 3.66 mmol) in water (2 mL) was added, and the mixture was stirred overnight. The solution was filtered on Sartorius (Goettingen, Germany) membranes (pore size: 1.2 μm, then 0.45 μm) and then precipitated with 0.5 M aqueous NaOH. The precipitate was successively washed with water, 1:1 water−EtOH, and EtOH and dried to give chitosan−CD **8** (0.23 g, 64%).

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