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# Synthesis and characterization of mannosyl mimetic derivatives based on a β-cyclodextrin core

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The synthesis of branched β-cyclodextrins substituted with mannosyl mimetic derivatives at one primary hydroxy group is described. It was shown that the self-inclusion phenomenon observed for the target compounds in water did not preclude the inclusion properties of the cyclodextrin moiety.

## Introduction

Cyclodextrins (CDs) are hydrosoluble torus-like cyclic oligosaccharides composed of 6, 7 or 8 glucopyranose units. CDs are able to accommodate small guest molecules in their hydrophobic cavity, which has enabled them to act as drug carriers. Intensive efforts have been made to design modified CDs that are capable of delivering drugs to specific target sites. For example, CDs glycosylated on either primary hydroxy groups [1–5] or both primary and secondary hydroxy groups [6] have been prepared and investigated to assess their binding characteristics to sugar-specific receptors.

Sugar-specific receptors are known to be important in the interactions between the HIV-1 envelope glycoprotein gp120 and a macrophage in the early stages of infection. A complex multistep process is involved in which the gp120 binds to the receptor CD4 and co-receptors CXCR4 and CCR5 of a macrophage. Owing to the inhibition properties showed by mannans in such interactions,[7] *N*-glycans present on the highly glycosylated gp120 [8] are believed to play an important part in viral infection.

This work reports the synthesis of the novel glycosylated CDs **1** and **2** as potential inhibitors of the recognition events involved in HIV infection with a view to studying any structure–activity relationships which may emerge. Such glycosylated CDs are envisaged to interfere with or completely inhibit sugar–sugar interactions between gp120 and a macrophage (Fig. 1). The glycosidic component of the novel compounds was specifically chosen to mimic the mannose type oligosaccharides that are attached to the polypeptide backbone of gp120. A further feature of the design of the new compounds was to introduce a peptide spacer between the mannose moiety and the CDs to mimic the gp120 polypeptide backbone.

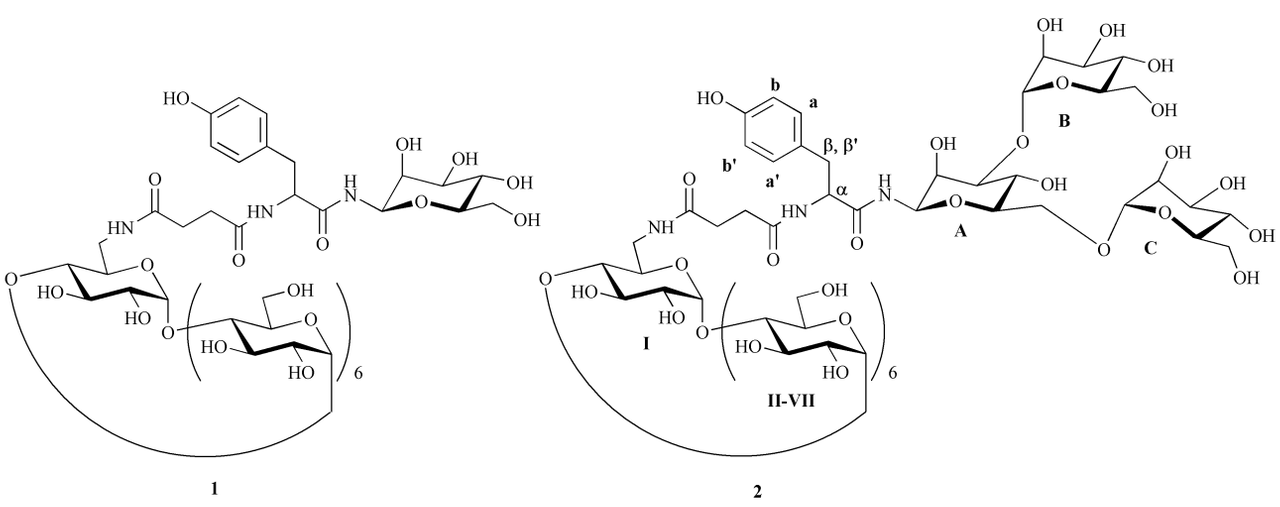
The route to the targeted glycosylated CDs **1** and **2** involved the design and synthesis of three building blocks, namely, 2,3,4,6-tetra-*O*-benzoyl-β-D-mannopyranosyl amine **3**, 2,4-di-*O*-benzoyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl amine **4** and *N*-6$^{I}$-deoxy-(L-tyrosinyl-amido)-6$^{I}$-succinylamidocyclomaltoheptaose **5** (Fig. 2).

The route to the target compounds **1** and **2** involved peptide type coupling reactions between *N*-6$^{I}$-deoxy-(L-tyrosinyl-amido)-6$^{I}$-succinylamidocyclomaltoheptaose **5** and the respective glycosylamines 2,3,4,6-tetra-*O*-benzoyl-β-D-mannopyranosyl amine **3** and 2,6-di-*O*-benzoyl-3,4-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl amine **4**. All of the final products and building blocks were fully characterized by NMR spectroscopy and high-resolution mass spectrometry. The inclusion properties of **1** and **2** towards hydrophobic guests were evaluated by NMR to establish that the chemical substitution of a cyclodextrin by a "signal" molecule should neither preclude the inclusion of a drug of interest nor impair the recognition of a "signal" at the receptor level. This would imply that the cavity of the modified cyclodextrin should remain vacant for the transportation of a drug.

We herein demonstrate that the self-inclusion phenomenon occurs in aqueous solution and that such conditions do not preclude the formation of an inclusion complex.

## Results and discussion

The target compounds were designed with the intention that chemical modification of the hydroxy groups of the cyclodextrin moiety should preclude neither the recognition of the signal at the receptor nor the inclusion for a drug into the cavity of the cyclodextrin. Moreover the final compounds were




1 2

**Fig. 1** Structures of glycosylated targets CDs **1** and **2**.

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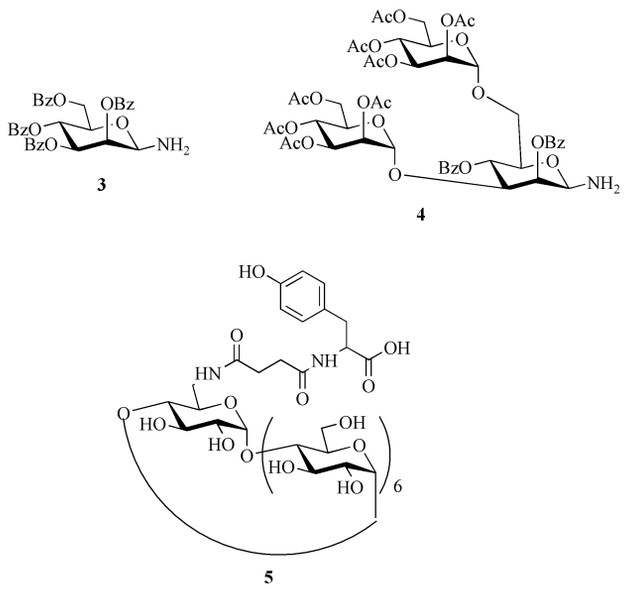


**Fig. 2** Structures of building blocks **3**, **4** and **5**.

required to bear a dedicated probe to assist future biological evaluations. Therefore, the following synthesis strategy was used:

Tyrosine was chosen as a classical probe since a specific iodination process by $^{125}$I is well known;[9]

A hydrophobic spacer between the cyclodextrin and adduct moieties was chosen to reduce possible steric hindrance effects induced by attaching a glycosidic moiety onto the cyclodextrin;[10]

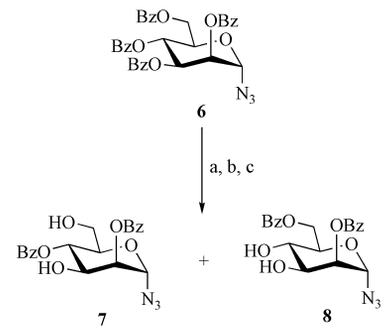
Only monosubstitution of the cyclodextrin moiety by the **3** and **4** building blocks was considered important since it is known that in some cases hyper-branched cyclodextrins exhibit a substantial decrease in the inclusion capacity.[5]

### Synthesis of mannopyranosylamine derivatives 3 and 4

2,3,4,6-Tetra-*O*-benzoyl-β-D-mannopyranosyl amine **3** was prepared by catalytic hydrogenation of the known 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl azide **6**.[11] In keeping with the observations of Chida *et al.*,[12] a rapid anomerization was found to occur to give the desired β-D-mannopyranosyl amine in 80% yield along with a 10% yield of the α-anomer. The same result was obtained starting from the more difficult to prepare β-azide analog.[11] Complete inversion of configuration at the 1-carbon atom of the mannose residue was observed by NOESY.

3,6-Di-*O*-(α-D-mannopyranosyl)-β-D-mannopyranosyl amine **5** was synthesised according to a modified procedure recently described by Bundle *et al.*:[13] a 3,6-di-*O*-unprotected mannoside acceptor was obtained simultaneously along with its 3,4-di-*O*-unprotected analogue in one step from the corresponding 1-*O*-protected mannopyranosyl derivative, which was glycosylated using the trichloroacetimidate method[14] to afford a mixture of a mannotrioside and mannodioside.

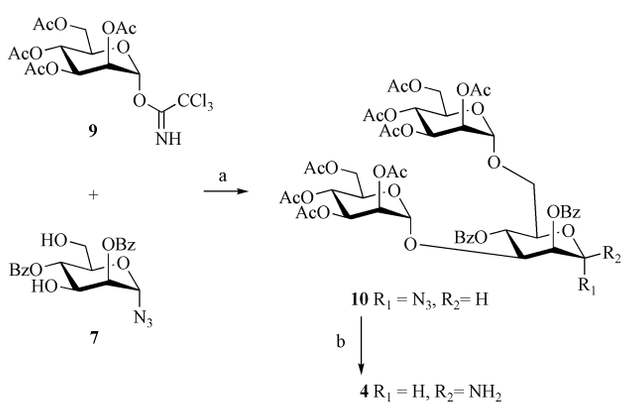
2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl azide **6** was first deprotected by Zemplèn methanolysis to give the corresponding α-D-mannopyranosyl azide, which was then reacted with triethyl orthobenzoate in the presence of *p*-toluenesulfonic acid as a catalyst. The 2,3–4,6-di-orthoester intermediate thus obtained was hydrolysed with aqueous trifluoroacetic acid (1 : 9). As expected, the opening of the 2,3-cyclic orthoester to the axial ester–equatorial alcohol[15] was highly regioselective affording the 2-benzoate exclusively. In contrast, the 4,6-orthoester opened without any pronounced regioselectivity to give a mixture of the corresponding 4- and 6-benzoates, namely, 2,4-di-*O*-benzoyl-α-D-mannopyranosyl azide **7** and 2,6-di-*O*-benzoyl-α-D-mannopyranosyl azide **8** in 43% and 37% yields respectively (Scheme 1). Both compounds were readily separated by flash chromatography.



**Scheme 1** Synthesis of acceptor **7**. *Reagents*: (a) MeONa, MeOH; (b) triethyl orthobenzoate, CSA, $CH_3CN$, 2 h; (c) $CF_3CO_2H$ 90%, $CH_3CN$, 10 min, 39% of **7** (3 steps).

Glycosylation of the two free hydroxy groups of the acceptor **7** was achieved in one step in the presence of a catalytic amount of trimethylsilyl triflate (TMSOTf). The primary hydroxy group was first glycosylated at −40 °C by the addition of 1.5 equivalents of the known trichloroacetimidate donor **9**.[16] It is notable that at this temperature, no glycosylation of the secondary hydroxy group was detected even when two equivalents of the donor were used. Without isolation, the disaccharide thus formed was extended to a trisaccharide by the addition of 1.5 equivalents of the donor **9** at room temperature thus affording the desired 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl azide **10** in high yield (82%).

Unfortunately, attempts to reduce the azido group to the desired amine by catalytic hydrogenation with Pd/C were unsuccessful in our hands. Treatment of the trisaccharide **10** with 1,3-propanedithiol as reducing agent[17] in the presence of Hünig's base afforded 3,6-di-*O*-(α-D-mannopyranosyl)-β-D-mannopyranosyl amine **4** in 37% yield (Scheme 2).

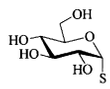
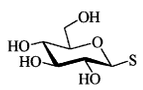
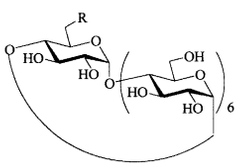
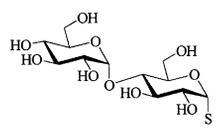
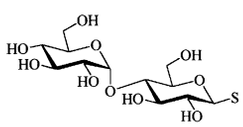


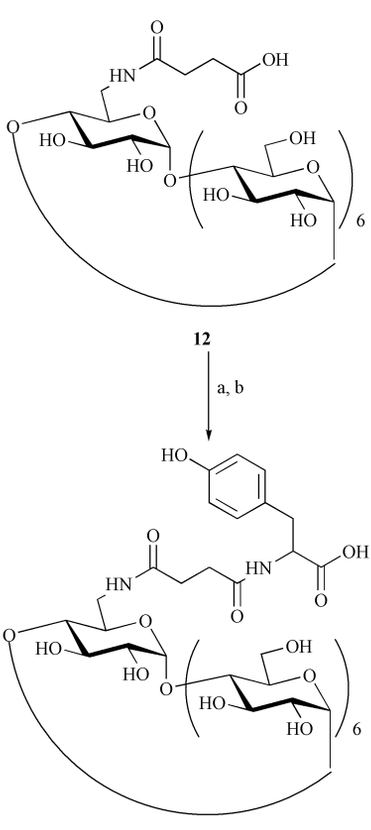
**Scheme 2** Synthesis of **4**. *Reagents*: (a) TMSOTf, $CH_2Cl_2$, −40 °C then rt, 82%; (b) *N*,*N*-diisopropylethylamine, 1,3-propanedithiol, MeOH, 37%.

### Synthesis of L-tyrosinyl-β-cyclodextrin 5

6$^{I}$-Deoxy-6$^{I}$-succinylamidocyclomaltoheptaose **12** was prepared from the known 6$^{I}$-deoxy-6$^{I}$-aminocyclomaltoheptaose[18] by nucleophilic addition on succinic anhydride as has been already described.[19] L-Tyrosine methyl ester, freshly obtained from the commercially available L-tyrosine methyl ester hydrochloride, was attached to **12** by a peptide link using *N*,*N*′-diisopropylcarbodiimide (DIC) and *N*-hydroxysuccinimide (NHS) to give **13** in high yield (97%). Transformation of the ester group into the corresponding acid was performed in aqueous NaOH to afford the desired L-tyrosinyl-cyclomaltoheptaose derivative **5** (Scheme 3). It should be pointed out that this

**Table 1** Solubilities in water of native β-cyclodextrin, thio-glucosylated and mannosylated derivatives

| Compounds | | Solubility at 20 °C/mmol $L^{-1}$ |
|---|---|---|
| β-Cyclodextrin | | 15 |
| | R =  | 327 |
| | R =  | 23 |
|  | R =  | 549 |
| | R =  | 23 |
| **1** | | 505 |
| **2** | | 620 |



**12**

a, b



**5**

**Scheme 3** Synthesis of L-tyrosinyl-β-cyclomaltoheptaose derivative **5**. *Reagents*: (a) DIC, NHS, L-tyrosine methyl ester hydrochloride, *N*,*N*-diisopropylethylamine, DMF, 72 h, rt, 97%; (b) NaOH, $H_2O$, 3 h, rt, 97%.

basic treatment is required to be effected with no more than 5 equivalents of NaOH to avoid epimerization of the amino acid.

### Synthesis of target glycosylated cyclodextrins 1 and 2

The L-tyrosinyl-β-CD **5** was reacted with each of the mannopyranosyl amine derivatives **3** and **4** under standard coupling conditions (DIC and hydroxybenzotriazole (HOBt)) to give the monomannosyl-β-CD **14** and trimannosyl-β-CD **15**, which were readily isolated from the reaction mixture by a "precipitation" procedure. The monomannosyl-β-CD **14** was obtained in high yield (99%) with sufficient purity to be used in the next step without further purification. Nevertheless, high-performance liquid chromatography (HPLC: using a $H_2O/CH_3CN$ gradient elution; $t_r$ of 23.43 min) of an analytical sample was undertaken to characterize **14**. Zemplèn methanolysis of crude **14** gave the desired final monoglycosylated β-CD **1** in quantitative yield, which was purified by HPLC ($H_2O/CH_3CN$, gradient elution, $t_r$ of 10.39 min). In contrast, a similar "precipitation" procedure was unsuccessful in achieving comparable purity of the protected trimannosyl-β-CD **15**. Therefore, purification of crude **15** was undertaken by HPLC ($H_2O/CH_3CN$, gradient elution; $t_r$ of 23.84 min). Moreover, deprotection of **15**, following the Zemplèn procedure, proved to be much longer than expected.

As depicted in Fig. 3a, total deprotection of **15** was not achieved under standard conditions and gave a mixture of the desired **2** and the monobenzoylated intermediate **16**. Two main peaks can be observed at $m/z$ = 1904 and $m/z$ = 2008 corresponding to $[M_2 + Na]^+$ and $[M_{16} + Na]^+$ respectively even after ten days of reaction (Fig. 3b).

Despite monitoring by mass spectrometry, no reactions conditions could be found to achieve complete deprotection. The reaction mixture containing the target trimannosyl-β-CD **2** was contaminated by 10% of **16**, probably benzoylated on the fourth position of the mannopyranosyl unit **A**.

Purification of **2** (39% yield) was finally achieved by HPLC ($H_2O/CH_3CN$, gradient elution; $t_r$ of 7.52 min). Structure elucidation of **1** and **2** was achieved using NMR and ESI-MS (high and low resolution).

### Solubility in water of 1 and 2

The solubility in water at 20 °C was determined for compounds **1** and **2** (Table 1), and is compared with the solubility data for native β-cyclodextrin and some 6-*S*-glycosyl-6-thio derivatives of β-CD.[20] In the latter case, it can be seen that branched derivatives with the β-anomeric configuration exhibit a much lower solubility in water compared with the corresponding α-anomer. Conversely, the derivatives **1** and **2** with the β-anomeric configuration exhibit a much higher solubility in water even when they bear a hydrophobic spacer between the cyclodextrin and glycosyl moieties.

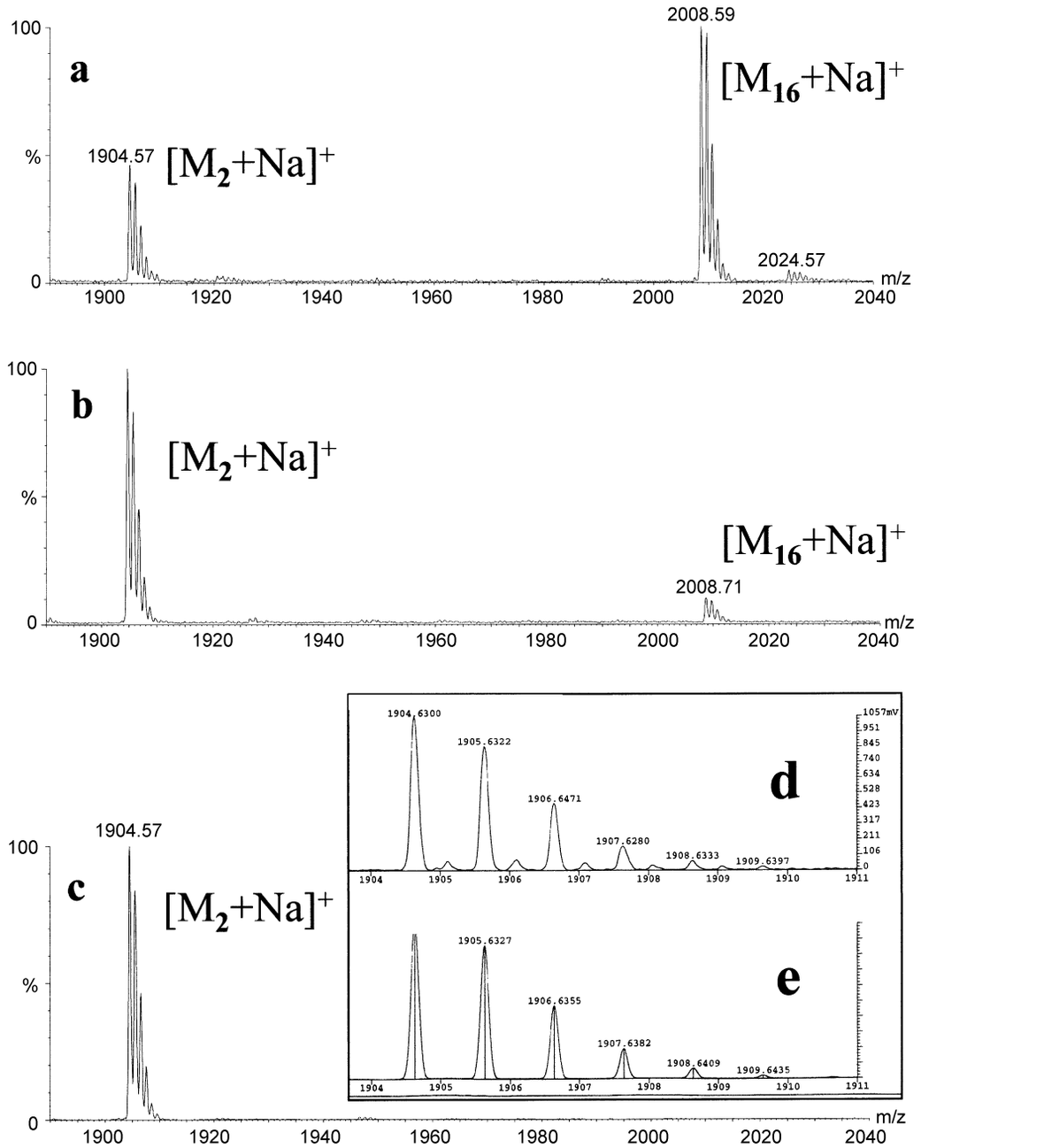



**Fig. 3** Partial ESI-MS spectra of crude **2** following 3 h (a) and 10 days (b) of deprotection reaction conditions and HPLC purified **2** (c). Isotope profile measured by ESI-HRMS at *m/z* = 1904 (d) and calculated (e) for $C_{75}H_{115}N_3NaO_{53}$.

### NMR investigations of 1 and 2

The structural elucidation of the final compounds by NMR was performed in $d_6$-DMSO and $D_2O$ and demonstrates that the purified samples were free of any included by-products or reagents. Since the spectra are relatively complex owing to the lack of molecular symmetry of the cyclodextrin moiety, a complete analysis was obtained by stepwise identification of both of the final compounds by COSY and successive RELAY, HMQC, HMBC and NOESY experiments. Being located in a highly specific domain, anomeric and amide protons were used as starting points for stepwise assignment. All the resonances of the spacer and glycosylated moieties (**A**, **B**, and **C**) were identified. However since the resonances relating to the glycosidic units (**I–VII**) show strong overlaps, only the substituted glycosidic unit (**I**) was unambiguously assigned. It should be pointed out that the NMR structural analysis revealed no evidence of epimerisation of the $H_{1A}$ and $H_\alpha$ protons of the mannosyl and amino acid moieties respectively, potentially induced by the several peptide type coupling reactions employed. The mannosyl, amino acid and cyclodextrin moieties were sequenced by T-ROESY.[21]

Since the different compounds were intended to target biological receptors, we also investigated the conformation of **1** and **2** in water. Each of the compounds displayed a considerable spectral dispersion in $D_2O$. Conversely when NMR spectra were collected using $d_6$-DMSO as solvent, the spectral dispersion collapsed. DMSO is well known to preclude the formation of an inclusion complex. Also, the self-inclusion process in water is a well-known phenomenon in cyclodextrin chemistry. To investigate these phenomena, a comparison was made of T-ROESY spectra of **2** in $D_2O$ and $d_6$-DMSO respectively (Fig. 4). A large number of cross-peaks between the aromatic protons of tyrosine and presumably the protons $H_3$–$H_5$ of cyclodextrin are observed in water (Fig. 4b). A quite different situation is encountered in DMSO (Fig. 4a) such that only dipolar interactions are observed between aromatic protons ($H_a$, $H_b$) and $H_\alpha$ and $H_\beta$ of tyrosine moiety.

**Table 2** Rotamer populations about the $C_\alpha$–$C_\beta$ bond derived from coupling constants, in the tyrosine moiety of **2** (in $D_2O$, $d_6$-DMSO and $D_2O$ in the presence of ASANa respectively) and comparisons with values obtained for the free amino acid in water

| Derivative | Rotamer (%) | | |
|---|---|---|---|
| | *gt* | *tg* | *gg* |
| Free tyrosine in $D_2O$ | 25 | 50 | 25 |
| **2** in $D_2O$ | 28 | 66.9 | 5.4 |
| **2** in DMSO | 25.7 | 55.6 | 18.7 |
| **2** + ASANa in $D_2O$ | 24.6 | 55.7 | 19.7 |

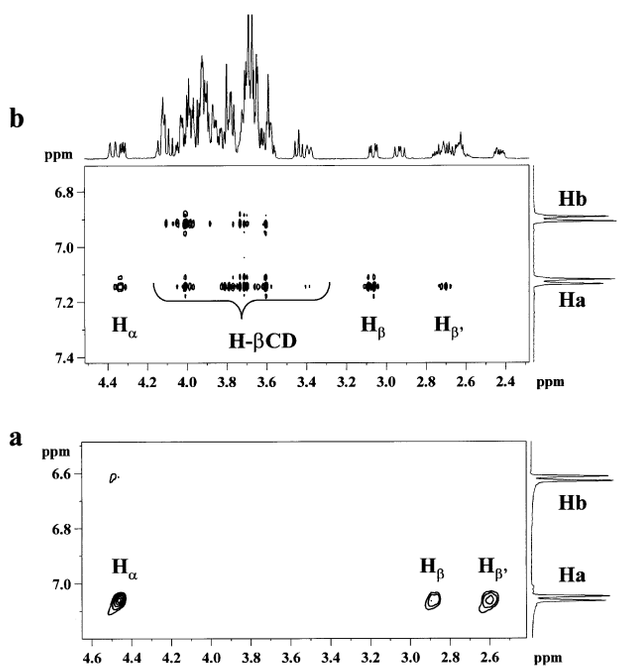



**Fig. 4** Partial T-ROESY contour plots (200 ms of spin lock at 22 dB attenuation) for **2** (10 mM, 300 K, 500.13 MHz) (a) in $d_6$-DMSO and (b) in $D_2O$.

The investigation was extended to study the conformation about the $C_\alpha$–$C_\beta$ bond of the amino acid moiety in water and in DMSO. The pertinent coupling constants were collected in both solvents, converted into rotamer populations[10] and compared to the values found for the free amino acid (Table 2). A significant but relatively weak conformational strain is observed for **2** in water. In DMSO solution, the conformational equilibrium between the three rotamers is partially restored. At this stage, it can be concluded that the phenol part of the amino acid is included in the cyclodextrin cavity when in water but is expelled from the same cavity when in DMSO. Similar results were obtained from parallel experiments with the compound **1**. In keeping with the well-known self-inclusion phenomenon in the cyclodextrin field, we have already shown that *N*-6$^{I}$-deoxy-6$^{I}$-tyrosinylamidocyclomaltoheptaose forms a very strong self-inclusion complex in water precluding formation of other inclusion complexes.[10] However, the self-inclusion process seems to be weaker for **1** and **2** when compared with *N*-6$^{I}$-deoxy-6$^{I}$-tyrosinylamido-cyclomaltoheptaose due to inhibition caused by the spacer.

### Investigation of inclusion properties of the glycosylated cyclodextrins 1 and 2

It was therefore necessary to evaluate the inclusion properties of **1** and **2** towards hydrophobic guests to confirm that the self-inclusion process does not inhibit the inclusion capacity of these derivatives. To demonstrate the inclusion properties of **2**, we studied the formation of the inclusion complex with sodium anthraquinone-2-sulfonate (ASANa) by NMR. The molecular structure of ASANa is shown in Fig. 5. It is well established that ASANa forms a 1 : 1 inclusion complex with β-CD and that it can be used as a shift reagent for the NMR studies of cyclodextrins.[22] An apparent binding constant of 350 $M^{-1}$ was estimated. It should be pointed out that this value is lower than the average value (490 $M^{-1}$) extrapolated to 721 different guest : β-CD inclusion complexes.[23]

One dimensional NMR spectra of **2** in the absence and in the presence of ASANa as guest molecule were compared (Fig. 5). It can be seen that ASANa induces shifts consistent with its inclusion.

Moreover, the rotamer populations of the $C_\alpha$–$C_\beta$ bond of the amino acid moiety of **2** in the presence of ASANa and water are similar to those observed for **2** alone in DMSO (Table 2). This result suggests that the phenol group is expelled from the cyclodextrin cavity in either ASANa and water or in DMSO alone.

From these results, it was concluded that **1** and **2** formed an intramolecular inclusion complex in aqueous solution but the interactions were clearly much weaker than for the *N*-6$^{I}$-deoxy-6$^{I}$-tyrosinylamidocyclomaltoheptaose analogue.[10] This suggests a much looser fitting of the aromatic group in the cyclodextrin cavity which does not inhibit the inclusion capacity of **1** and **2**, especially when the complex formation involves the secondary hydroxy group rim of the cyclodextrin.

In conclusion, we report here the total synthesis of mannosyl mimetic derivatives based on the β-cyclodextrin core. Both of the compounds were fully characterized by NMR spectroscopy and mass spectrometry. The NMR study showed that even in the presence of a succinic spacer the cavity of the cyclodextrin is occupied by the phenol group of the tyrosine moiety. However it was demonstrated that this self-inclusion process did not preclude the inclusion properties of the branched β-cyclodextrins. We are currently investigating the inhibition properties of the target molecules on the interactions between gp120 and receptors and co-receptors using dedicated biological models.

## Experimental

### General methods

Optical rotations were measured with a JASCO DIP-370 digital polarimeter, using a sodium lamp ($\lambda$ = 589 nm) at 20 °C. All NMR experiments were performed at 300.13 and 500.13 MHz using Bruker DMX300 and DRX500 spectrometers equipped with a Z-gradient unit for pulsed-field gradient spectroscopy. $Me_4Si$ was used as an external standard and calibration was performed using the signal of the residual protons or of the carbon of the solvents as a secondary reference. Measurements were performed at 300 K with careful temperature regulation. The length of the 90° pulse was approximately 7 μs. 1D NMR spectral data were collected using 16K data points. 2D experiments were run using 1K data points and 256 time increments. The phase sensitive (TTPI) sequence was used and processing resulted in a 1K × 1K (real–real) matrix. Details concerning experimental conditions are given in the figure captions.

Low resolution electrospray mass spectra were obtained on a hybrid quadrupole/time-of-flight (Q-TOF) instrument, equipped with a pneumatically assisted electrospray (Z-spray) ion source (Micromass). High resolution mass spectra were recorded in positive mode on a ZabSpec TOF (Micromass, UK) tandem hydrid mass spectrometer with EBETOF geometry. The compounds were individually dissolved in water–$CH_3CN$ (1 : 1) 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 range of 4000–100 Da. The mass spectra were collected as continuum profile data. Accurate mass measurement was achieved using polyethylene glycol as internal

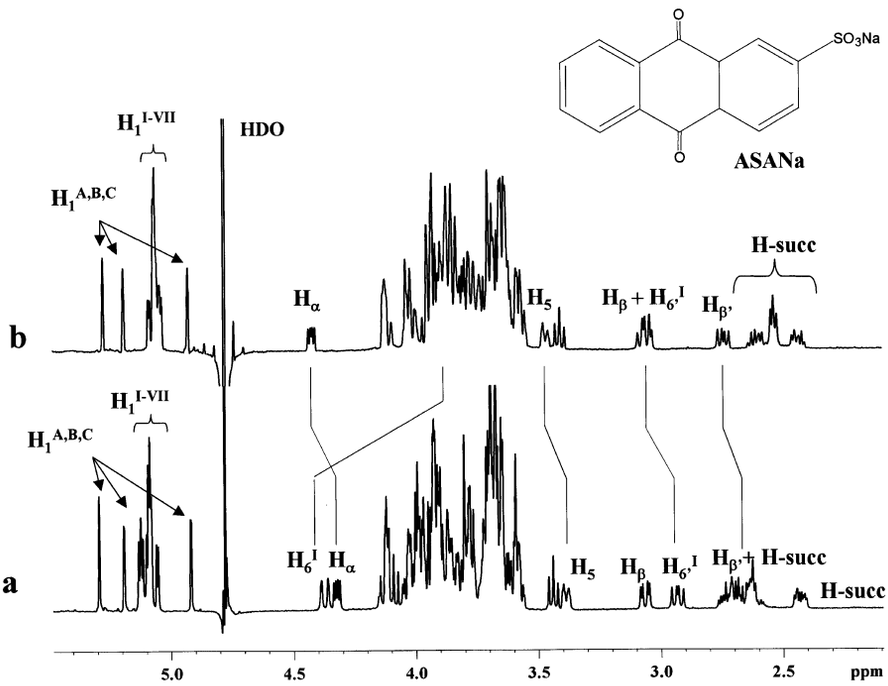



**Fig. 5** Partial $^1$H NMR spectra (300 K, 500.13 MHz, 10 mM in $D_2O$) of **2** (a) alone and (b) in the presence of ASANa (15 mM).

reference masses with a resolving power set to a minimum of 10 000 (10% valley).

Elemental analyses were performed at the Service de Microanalyse de l'Université de Champagne-Ardennes in Reims, France. The samples were previously dried under vacuum for one week.

Preparative HPLC was carried out with a Waters Prep LC 4000 System chromatograph fitted with an evaporative light scattering detector PL-ELS 1000 (Polymer Laboratories) and a Prevail C-18 column (5 μm, 22 × 250 mm). Thin-layer chromatography was performed on E. Merck glass plates silica gel sheets (silica gel $F_{254}$) followed by charring with vanillin. Column chromatography was performed on Kieselgel (E. Merck 230–400 mesh).

### 2,3,4,6-Tetra-*O*-benzoyl-β-D-mannopyranosyl amine 3

Pd/C (100 mg) was added to a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl azide **6**[11] (3.5 g, 5.63 mmol) in EtOH (200 ml). The mixture was stirred in a hydrogen atmosphere (20 bar) for 24 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was purified by flash chromatography (EtOAc–hexane 1 : 1 v/v) to give compound **3** (2.65 g, 80%) as a white powder (Found: C, 68.29; H, 4.84; N, 2.42. $C_{34}H_{29}NO_9$ requires C, 68.57; H, 4.87; N, 2.35%); $[\alpha]_D$ −116° (*c* 1, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.09–7.22 (m, 20 H, $OCOC_6H_5$), 5.96 (t, 1 H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 5.90 (dd, 1 H, $J_{1,2}$ 0.8 Hz, $J_{2,3}$ 3.18 Hz, H-2), 5.63 (dd, 1 H, H-3), 4.77 (d, 1 H, H-1), 4.72 (dd, 1 H, $J_{5,6}$ 2.7 Hz, $J_{6,6'}$ 11.8 Hz, H-6), 4.44 (dd, 1 H, $J_{5,6'}$ 4.4 Hz, H-6′), 4.12 (ddd, 1 H, H-5), 2.18 (s, 2 H, $NH_2$); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 166.16, 165.53, 165.41 and 165.36 ($OCOC_6H_5$), 133.45–128.20 ($OCOC_6H_5$), 82.49 (C-1), 73.31, 72.84, 71.45 and 66.72 (C-2, C-3, C-4 and C-5), 63.08 (C-6); *m*/*z* (ES-HRMS) 618.1747 ([M + Na]$^+$ $C_{34}H_{29}NNaO_9$ requires 618.1740).

### 2,4-Di-*O*-benzoyl-α-D-mannopyranosyl azide 7 and 2,6-di-*O*-benzoyl-α-D-mannopyranosyl azide 8

Sodium methoxide (65 ml, 1 M in MeOH) was added to a solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl azide **6**[11] (10 g, 16.1 mmol) in dry methanol (180 ml). The mixture was stirred overnight, neutralized with acidic resin (Amberlite IR 120) and then filtered. After concentration in *vacuo*, the residue was dissolved in water and washed twice with EtOAc. Then, the aqueous layer was freeze-dried to afford the α-D-mannopyranosyl azide (3 g, 90%). To a suspension of α-D-mannopyranosyl azide (3 g, 14.6 mmol) in dry $CH_3CN$ (150 ml) were added triethyl orthobenzoate (15.2 ml, 66.95 mmol) and camphor-10-sulfonic acid (640 mg, 2.75 mmol). The reaction mixture was stirred for 2 h at room temperature. Triethylamine (1.5 ml) was added and the solution was evaporated to dryness. The residue was dissolved in $CH_3CN$ (150 ml) and a solution of 90% trifluoroacetic acid–water (9.4 ml) was added. The mixture was stirred at room temperature for 10 min. The solution was then diluted with toluene (100 ml) and evaporated. The residue was dissolved in $CH_2Cl_2$ (50 ml) and the solution was successively washed with saturated aq. $NaHCO_3$ (2 × 20 ml), and water (20 ml). The organic layer was dried over $Na_2SO_4$, filtered and concentrated *in vacuo* to give a syrup from which the products were separated by flash chromatography (EtOAc–hexane 3 : 7 v/v). Compound **7** (2.89 g, 43.5%) was the first to be eluted followed by compound **8** (2.47 g, 37.1%). Data for **7**: (Found: C, 57.41; H, 4.69; N, 9.73 $C_{20}H_{19}N_3O_7$ requires C, 58.11; H, 4.60; N, 10.16%); $[\alpha]_D$ +68° (*c* 0.5, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.12–7.19 (m, 10 H, $OCOC_6H_5$), 5.57 (t, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.55 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.33 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.34 (dd, 1 H, H-3), 4.06 (ddd, 1 H, $J_{5,6}$ 2.7 Hz, $J_{5,6'}$ 3.9 Hz, H-5), 3.81 (dd, 1 H, $J_{6,6'}$ 11.5 Hz, H-6), 3.71 (dd, 1 H, H-6′), 3.49 (s, 1 H, OH), 3.01 (s, 1 H, OH); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 166.89 and 165.74 ($OCOC_6H_5$), 133.54–128.34 ($OCOC_6H_5$), 87.44 (C-1), 72.56 (C-2), 72.22 (C-5), 69.48 (C-4), 67.54 (C-3), 61.00 (C-6); *m*/*z* (ES-HRMS) 436.1123 ([M + Na]$^+$ $C_{20}H_{19}N_3NaO_7$ requires 436.1121); data for **8**: (Found: C, 57.93; H, 4.51; N, 9.92 $C_{20}H_{19}N_3O_7$ requires C, 58.11; H, 4.60; N, 10.16%); $[\alpha]_D$ +59° (*c* 1, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.09–7.22 (m, 10 H, $OCOC_6H_5$), 5.49 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.25 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.78 (dd, 1 H, $J_{6,6'}$ 11.4 Hz, $J_{5,6}$ 3.3 Hz, H-6), 4.56 (dd, 1 H, H-6′), 4.10 (dd, 1 H, $J_{3,4}$ 10.09 Hz, H-3), 3.99 (m, 2 H, H-4, H-5); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 166.84 and 165.83 ($OCOC_6H_5$), 133.36–128.29 ($OCOC_6H_5$), 87.50 (C-1), 72.60 (C-5), 71.83 (C-2), 69.08 (C-3), 67.16 (C-4), 63.17 (C-6); *m*/*z* (ES-LRMS) 431 ([M + $NH_4$]$^+$ $C_{20}H_{23}N_4O_7$ requires 431).

**2,4-Di-*O*-benzoyl-3,6-di-*O*-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl azide 10**

TMSOTf (15 μl, 0.083 mmol) was added to a stirred solution of **7** (192 mg, 0.46 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate **9**[16] (336 mg, 0.70 mmol) in dry $CH_2Cl_2$ (10 ml) under argon at −40 °C. Stirring was continued at −40 °C for 30 min. The mixture was allowed to warm to room temperature, after which a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate **9** (336 mg, 0.70 mmol) and TMSOTf (15 μl, 0.083 mmol) in dry $CH_2Cl_2$ (5 ml) was added. The reaction mixture was stirred at room temperature for 30 min, neutralized with triethylamine and concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc–hexane 5.5 : 4.5 v/v) to afford the trisaccharide **10** (408 mg, 82%) (Found: C, 53.15; H, 5.12; N, 3.56 $C_{48}H_{55}N3O_{25}$ requires C, 53.68; H, 5.12; N, 3.91%); $[a]_D$ +50° (*c* 0.73, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.10–7.41 (m, 10 H, $OCOC_6H_5$), 5.63 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4A), 5.59 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1A), 5.35 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2A), 5.30 (dd, 1 H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 10.1 Hz, H-3C), 5.20 (dd, 1 H, $J_{1,2}$ 1.6 Hz, H-2C), 5.18 (t, 1 H, H-4C), 5.04–5.01 (m, 2 H, H-3B, H-4B), 4.97 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1B), 4.82 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2B), 4.76 (d, 1 H, H-1C), 4.36 (dd, 1 H, H-3A), 4.26 (ddd, 1 H, $J_{5,6}$ 5.7 Hz, $J_{5,6'}$ 2.8 Hz, H-5A), 4.12–3.84 (m, 7 H, H-5B, H-5C, H-6A, H-6B, H-6′B, H-6C, H-6′C), 3.62 (dd, 1 H, $J_{6,6'}$ 10.8 Hz, H-6′A), 2.06, 2.05, 2.03, 1.99, 1.92, 1.87, 1.81 and 1.77 (8 s, 24 H, $OCOCH_3$); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 170.45, 169.74, 169.58, 169.45 and 168.99 ($OCOCH_3$), 165.70 and 165.01 ($OCOC_6H_5$), 133.77–128.48 ($OCOC_6H_5$), 99.26 (C-1B), 97.26 (C-1C), 87.31 (C-1A), 74.36 (C-3A), 71.46 (C-5A), 71.17 (C-2A), 69.30 (C-5B), 69.14 (C-2C), 68.99 (C-2B), 68.90 (C-3C), 68.44 (C-5C), 68.06 (C-3B or C-4B), 67.96 (C-4B), 66.40 (C-6A), 65.74 (C-3B or C-4B, C-4C), 62.19 (C-6B, C-6C), 20.56 and 20.35 ($OCOCH_3$); *m/z* (ES-LRMS) 1091 ($[M + NH_4]^+$ $C_{48}H_{59}N_4O_{25}$ requires 1091).

**2,4-Di-*O*-benzoyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl amine 4**

A solution of the trisaccharide **10** (3.5 g, 3.26 mmol), *N*,*N*-diisopropylethyl amine (7 ml, 41 mmol) and 1,3-propanedithiol (15 ml, 148 mmol) in dry MeOH (100 ml) was stirred at room temperature for 4.5 h. The mixture was concentrated *in vacuo* and the residue purified by flash chromatography (EtOAc–hexane 4 : 1 v/v) to afford the desired amine **4** (1.23 g, 37%) (Found: C, 54.34; H, 5.40; N, 1.36 $C_{48}H_{57}NO_{25}$ requires C, 55.01; H, 5.44; N, 1.33%); $[a]_D$ −16° (*c* 0.95, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.20–7.40 (m, 10 H, $OCOC_6H_5$), 5.75 (dd, 1 H, $J_{1,2}$ 0.9 Hz, $J_{2,3}$ 2.5 Hz, H-2A), 5.55 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4A), 5.39 (dd, 1 H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 10.1 Hz, H-3C), 5.27 (dd, 1 H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.5 Hz, H-2C), 5.24 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4C), 5.15 (t, 1 H, $J_{4,5}$ 10.1 Hz, H-4B), 5.03 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-3B), 4.94 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1B), 4.85 (dd, 1 H, H-2B), 4.80 (d, 1 H, H-1C), 4.39 (m, 1 H, H-5B), 4.33 (dd, 1 H, $J_{5,6}$ 4.8 Hz, $J_{6,6'}$ 15.7 Hz, H-6B or H-6C), 4.24 (dd, 1 H, H-3A), 4.19–3.98 (m, 4 H, H-5C, H-6B or H-6C, H-6′B, H-6′C), 3.91 (dd, 1 H, $J_{5,6}$ 6.12 Hz, $J_{6,6'}$ 10.6 Hz, H-6A), 3.81 (ddd, 1 H, $J_{5,6'}$ 2.2 Hz, H-5A), 3.60 (dd, 1 H, H-6′A), 2.13, 2.05, 2.02, 2.00, 1.99, 1.80 and 1.77 (7 s, 24 H, $OCOCH_3$); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 170.58, 169.74 and 168.96 ($OCOCH_3$), 166.16 and 165.07 ($OCOC_6H_5$), 133.56–128.50 ($OCOC_6H_5$), 99.37 (C-1B), 97.57 (C-1C), 82.73 (C-1A), 77.03 (C-3A), 73.82 (C-5A), 72.67 (C-2A), 69.33, 69.03, 68.45 and 68.14 (C-4A, C-2B, C-3B, C-5B, C-2C and C-5C) 67.20 (C-6A), 65.97 and 65.90 (C-4B and C-4C), 62.35 and 62. 19 (C-6B and C-6C), 20.72, 20.41 and 20.28 ($OCOCH_3$); *m/z* (ES-HRMS) 1070.3120 ($[M + Na]^+$ $C_{48}H_{57}NNaO_{25}$ requires 1070.3117).

***N*-6$^I$-Deoxy-(L-tyrosinylamido methyl ester)-6$^I$-succinylamido-cyclomaltoheptaose 13**

A solution of compound **12** (1 g, 0.81 mmol), DIC (630 μl, 4.06 mmol) and NHS (470 mg, 4.08 mmol) in dry DMF (12 ml) was stirred for 2 h at room temperature under argon. A solution of L-tyrosine methyl ester hydrochloride (290 mg, 1.25 mmol) and *N*,*N*-diisopropylethylamine (700 μl, 4.02 mmol) in dry DMF (3 ml) was prepared 30 min before use and then added to the reaction mixture. After 72 h of stirring, DMF was removed *in vacuo*. The residual syrup was dissolved in DMF (5 ml) and stored in a refrigerator for 2 h to allow the precipitation of the urea derivative. After filtration through a Millex-GS filter, the filtrate was concentrated to dryness. The subsequent residue was suspended in acetone (50 ml) and stirred for 20 min to give a precipitate which was filtered and washed several times with acetone to afford compound **13** (1.11 g, 97%) as a white powder. $[a]_D$ +109° (*c* 1, $H_2O$); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 9.23 (s, 1 H, OH-Tyr), 8.24 (d, 1 H, $J_{NH,\alpha}$ 7.4 Hz, NH-Tyr), 7.63 (t, 1 H, $J_{NH,6I} = J_{NH,6I'}$ 6.1 Hz, NH-βCD); $^1$H NMR (300 MHz, $D_2O$): $\delta$ 6.90 (d, 2 H, $J_{a,b}$ 8.2 Hz, H-a, H-a′), 6.74 (d, 2 H, H-b, H-b′), 4.91 (m, 7 H, H-1I-VII), 4.22–4.17 (m, 2 H, H-6I, H-α), 3.95–3.19 (m, 43 H, H-2I-VII, H-3I-VII, H-4I-VII, H-5I-VII, H-6II-VII, H-6′II-VII, $OCH_3$), 2.72 (dd, 1 H, $J_{5,6}$ 9.5 Hz, $J_{6,6'}$ 15.4 Hz, H-6′I), 2.68–2.61 (m, 2 H, H-β, H-β′), 2.57–2.18 (m, 4 H, 2 × $CH_2$); $^{13}$C NMR (75 MHz, $D_2O$): $\delta$ 175.12, 173.81 and 172.95 (NHCO), 155.65, 130.17, 128.42 and 115.62 ($C_6H_4OH$), 102.45, 102.20 and 102.04 (C-1I-VII), 84.01 (C-4I), 81.41, 81.10 and 80.76 (C-4II-VII), 73.39, 73.13, 72.47 and 72.26 (C-2I-VII, C-3I-VII, C-5I-VII), 60.25 and 60.02 (C-6II-VII), 55.40 (C-α), 53.17 ($OCH_3$), 40.54 (C-6I), 37.72 (C-β), 31.43 and 31.19 (2 × $CH_2$ Succ); *m/z* (ES-LRMS) 1433 ($[M + Na]^+$ $C_{56}H_{86}N_2NaO_{39}$ requires 1433).

***N*-6$^I$-Deoxy-(L-tyrosinylamido)-6$^I$-succinylamidocyclomaltoheptaose 5**

A solution of compound **13** (1 g, 0.7 mmol) and 1 M NaOH (3.54 ml) in water was stirred at room temperature for 3 h. Then, the mixture was neutralized with acidic resin (Amberlite IR 120), filtered and evaporated to dryness. The residue was dissolved in water (10 ml) and freeze-dried to afford compound **5** (959 mg, 97%). $[a]_D$ +117° (*c* 1, $H_2O$); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 9.21 (s, 1 H, OH-Tyr), 8.11 (d, 1 H, $J_{NH,\alpha}$ 7.6 Hz, NH-Tyr), 7.64 (t, 1 H, $J_{NH,6I} = J_{NH,6I'}$ 6.1 Hz, NH-βCD); $^1$H NMR (300 MHz, $D_2O$): $\delta$ 6.99 (d, 2 H, $J_{a,b}$ 8.2 Hz, H-a, H-a′), 6.83 (d, 2 H, H-b, H-b′), 5.00–4.66 (m, 7 H, H-1I-VII), 4.33 (dd, 1 H, $J_{5,6}$ < 1 Hz, $J_{6,6'}$ 13.4 Hz, H-6I), 4.15 (t, 1 H, $J_{\alpha,\beta} = J_{\alpha,\beta'}$ 6.8 Hz, H-α), 4.06–3.41 (m, 39 H, H-2I-VII, H-3I-VII, H-4II-VII, H-5I-VII, H-6II-VII, H-6′II-VII), 3.32 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4I), 2.84–2.76 (m, 2 H, H-6′I and H-β), 2.68–2.45 (m, 4 H, 3 $CH_2$ Succ and H-β′), 2.31 (m, 1 H, 1 $CH_2$ Succ); $^{13}$C NMR (75 MHz, $D_2O$): $\delta$ 174.87, 174.61 and 173.98 (NHCO and COOH), 155.57, 130.09, 128.77 and 115.69 ($C_6H_4OH$), 102.61 and 102.19 (C-1I-VII), 84.13 (C-4I), 81.96, 80.99 and 80.85 (C-4II-VII), 73.48, 73.03 and 72.35 (C-2I-VII, C-3I-VII, C-5I-VII), 60.58 and 60.29 (C-6II-VII), 56.32 (C-α), 40.62 (C-6I), 38.03 (C-β), 31.75 and 31.56 (2 × $CH_2$ Succ); ES-LRMS $[M + H]^+$ *m/z* 1397 calcd. for $C_{55}H_{85}N_2O_{39}$, found: 1397.

***N*-6$^I$-Deoxy-(2,3,4,6-tetra-*O*-benzoyl-β-D-mannopyranosyl-L-tyrosinylamido)-6$^I$-succinylamidocyclomaltoheptaose 14**

A solution of compound **5** (1 g, 0.71 mmol), DIC (554 μl, 3.57 mmol) and HOBt (484 mg, 3.58 mmol) in dry DMF was stirred for 2 h at 0 °C. Then, a solution of 2,3,4,6-tetra-*O*-benzoyl-β-D-mannopyranosyl amine **3** (555 mg, 0.93 mmol) in dry DMF (5 ml) was added to the reaction mixture and stirred at room temperature for 72 h. DMF was removed *in vacuo* and the residue precipitated in diethyl ether. The precipitate was

removed by filtration and washed several times with diethyl ether to afford compound **14** (1.4 g, 99%) as a white powder (Found C, 49.65; H, 5.31; N, 2.42 $C_{89}H_{111}N_3O_{47}\cdot 10H_2O$ requires C, 49.60; H, 6.08; N, 1.95%); $[\alpha]_D$ +52° (*c* 1, DMF); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 9.06 (s, 1 H, OH-Tyr), 8.82 (d, 1 H, $J_{NH,1}$ 8.6 Hz, NH-Sugar), 8.02–7.28 (m, 22 H, $OCOC_6H_5$, NH-Tyr (8.00 ppm), NH-βCD (7.60)); $^1$H NMR (500 MHz, DMSO-$d_6$–$D_2O$ 9 : 1 v/v) $\delta$ 6.94 (d, 2 H, $J_{a,b}$ 8.6 Hz, H-a, H-a′), 6.88 (d, 2 H, H-b, H-b′), 5.84 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 5.80 (s, 1 H, H-1), 5.75 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 5.65 (d, 1 H, H-2), 4.79–4.76 (m, 7 H, H-1I-VII), 4.55–4.50 (m, 2 H, H-6, H-6′), 4.45–4.40 (m, 2 H, H-5, H-α), 3.65–3.30 (m, 40 H, H-2I-VII, H-3I-VII, H-4II-VII, H-5I-VII, H-6I-VII, H-6′II-VII), 3.20 (t, 1 H, $J_{3,4} = J_{4,5}$ 8.8 Hz, H-4I), 3.15 (dd, 1 H, $J_{5,6'}$ 6.6 Hz, $J_{6,6'}$ 14.0 Hz, H-6′I), 2.80 (dd, 1 H, $J_{\alpha,\beta'}$ 4.8 Hz, $J_{\beta,\beta'}$ 13.9 Hz, H-β), 2.60 (dd, 1 H, H-β′), 2.25–2.15 (m, 4 H, 2 × $CH_2$ Succ); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 172.71, 172.48 and 172.24 (NHCO), 166.11, 165.79 and 165.40 ($OCOC_6H_5$), 156.60, 134.55, 131.13, 130.31, 130.02, 129.69 and 115.59 ($OCOC_6H_5$ and $C_6H_4OH$), 102.80 (C-1I-VII), 84.14 (C-4I), 82.56 and 82.38 (C-4II-VII), 77.1 (C-1), 73.85, 73.26, 72.85, 71.38, 70.47 and 67.00 (C-2I-VII, C-3I-VII, C-5I-VII, C-2, C-3, C-4, C-5), 63.27 (C-6), 60.69 (C-6II-VII), 54.44 (C-α), 40.68 (C-6I), 37.69 (C-β), 31.42 (2 × $CH_2$ Succ); *m/z* (ES-HRMS) 1996.6256 ($[M + Na]^+$ $C_{89}H_{111}N_3NaO_{47}$ requires 1996.6286).

### *N*-6$^I$-Deoxy-(β-D-mannopyranosyl-L-tyrosinylamido)-6$^I$-succinylamidocyclomaltoheptaose 1

A solution of sodium methoxide (730 µl, 1 M in methanol) was added to a solution of compound **14** (110 mg, 0.055 mmol) in methanol/DMF (20 ml 1 : 1 v/v). The reaction mixture was stirred at room temperature for 24 h, neutralized with acidic resin (Amberlite IR 120) and filtered. Solvents were removed *in vacuo* and the resulting residue was dissolved in water. The aqueous layer was washed with $CH_2Cl_2$ (4 × 20 ml) and finally freeze-dried to afford the crude "mannosyl"-β-CD **1** (83 mg, 96%). Purification by high-performance liquid chromatography (water–$CH_3CN$, gradient elution from 100 to 0 in 40 min, $t_r$ of 10.39 min) gave the final product **1** (42 mg, 48%) (Found C, 42.21; H, 6.35; N, 3.01 $C_{61}H_{95}N_3O_{43}\cdot 10H_2O$ requires C, 42.14; H, 6.62; N, 2.41%); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 9.12 (s, 1 H, OH-Tyr), 8.12 (d, 1 H, $J_{NH,1}$ 9.0 Hz, NH-Sugar), 8.08 (d, 1 H, $J_{NH,\alpha}$ 8.4 Hz, NH-Tyr), 7.61 (t, 1 H, $J_{NH,6I} = J_{NH,6'I}$ 5.1 Hz, NH-βCD); $^1$H NMR (500 MHz, $D_2O$): $\delta$ 7.04 (d, 2 H, $J_{a,b}$ 8.1 Hz, H-a, H-a′), 6.82 (d, 2 H, H-b, H-b′), 5.19 (s, 1 H, H-1), 5.05–4.97 (7 d, 7 H, H-1I-VII), 4.30 (dd, 1 H, $J_{5I,6I} < 1$ Hz, $J_{6I,6'I}$ 14.0 Hz, H-6I), 4.32 (dd, 1 H, $J_{\alpha,\beta}$ 4.1 Hz, $J_{\alpha,\beta'}$ 8.4 Hz, H-α), 4.07–3.27 (m, 44 H, H-2I-VII, H-3I (t, 4.02 ppm, $J_{2I-3I} = J_{3I-4I}$ 9.5 Hz), H-3II-VII, H-4I (t, 3.36 ppm), H-4II-VII, H-5I-VII, H-6II-VII, H-6′II-VII, H-2, H-3, H-4, H-5, H-6, H-6′), 2.97 (dd, 1 H, $J_{\beta,\beta'}$ 13.9 Hz, H-β), 2.84 (dd, 1 H, $J_{5I,-6'I}$ 9.8 Hz, H-6′I) 2.69–2.54 (m, 4 H, H-β′, 3 $CH_2$ Succ), 2.35 (ddd, 1 H, $CH_2$ Succ); $^{13}$C NMR (75 MHz, $D_2O$): $\delta$ 175.53, 173.95 and 173.25 (NHCO), 155.47, 130.17, 129.42 and 115.55 ($C_6H_4OH$), 102.51 and 102.20 (C-1I-VII), 84.21 (C-4I), 81.39–80.72 (C-4II-VII), 78.07 (C-1), 73.42–66.65 (C-2I-VII, C-3I-VII, C-5I-VII, C-2, C-3, C-4, C-5), 61.19 and 60.43 (C-6II-VII, C-6), 56.54 (C-α), 40.63 (C-6I), 36.77 (C-β), 31.30 and 30.75 (2 × $CH_2$ Succ); *m/z* (ES-HRMS) 1580.5300 ($[M + Na]^+$ $C_{61}H_{95}N_3NaO_{43}$ requires 1580.5237).

### *N*-6$^I$-Deoxy-(2,4-di-*O*-benzoyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranosyl-L-tyrosinylamido)-6$^I$-succinylamidocyclomaltoheptaose 15

A solution of the β-CD derivative **5** (800 mg, 0.57 mmol), DIC (455 µl, 2.93 mmol) and HOBt (396 mg, 2.93 mmol) in dry DMF (15 ml) was stirred for 2 h at 0 °C. Then, a solution of the trisaccharide **4** (560 mg, 0.53 mmol) in dry DMF (10 ml) was added and the reaction mixture was stirred for 72 h at room temperature. DMF was removed *in vacuo* and the residue added to water (200 ml) to give a suspension, which was stirred for 30 min. The mixture was filtered and the filtrate freeze-dried to afford the crude "high-mannosyl"-β-CD **15** (1.25 g, 90%) as a white powder. Purification was effected by high performance liquid chromatography (water–$CH_3CN$, gradient elution from 100 to 0 in 40 min, $t_r$ of 23.84 min) to give the pure β-CD derivative **15** (290 mg, 22%) (Found C, 46.92; H, 5.52; N, 2.50 $C_{103}H_{139}N_3O_{63}\cdot 10H_2O$ requires C, 47.12; H, 6.06; N, 1.60%); $[\alpha]_D$ +77° (*c* 1, $H_2O$–$CH_3CN$ 4 : 6 v/v); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 9.11 (s, 1 H, OH-Tyr), 8.85 (d, 1 H, $J_{NH,1A}$ 8.7 Hz, NH-Sugar), 8.14–7.50 (m, 12 H, $OCOC_6H_5$, NH-Tyr (7.98 ppm), NH-βCD (7.58 ppm)), 7.00 (d, 2 H, $J_{a,b}$ 8.0 Hz, H-a, H-a′), 6.60 (d, 2 H, H-b, H-b′), 5.80–3.25 (m, 91 H, OH-2 βCD, OH-3 βCD, OH-6 βCD, H-1I-VII, H-2I-VII, H-3I-VII, H-4I-VII, H-5I-VII, H-6I (3.58 ppm), H-6II-VII, H-6′I (3.28 ppm), H-6′II-VII, H-1A (5.74 ppm), H-1B-C, H-2A-C, H-3A-C, H-4A-C, H-5A-C, H-6A-C, H-6′A-C, H-α (4.53 ppm)), 2.80 (dd, 1 H, $J_{\alpha,\beta}$ 3.3 Hz, $J_{\beta,\beta'}$ 14.0 Hz, H-β), 2.59 (dd, 1 H, H-β′), 2.25–1.75 (m, 28 H, 2 × $CH_2$ Succ, $OCOCH_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 172.66, 172.50 and 172.00 (NHCO), 170.96, 170.57, 170.42 and 169.70 ($OCOCH_3$), 166.47 and 165.67 ($OCOC_5H_6$), 156.54, 134.61, 130.62, 130.28, 129.67, 129.50, 128.46 and 115.61 ($OCOC_6H_5$ and $C_6H_4OH$), 102.83 (C-1I-VII), 98.99 and 97.55 (C-1B, C-1C), 84.09 (C-4I), 82.55 and 82.39 (C-4II-VII), 77.59 (C-1A), 76.82, 73.85, 73.26, 72.86, 70.48, 69.75, 69.61, 69.23, 68.91, 68.64, 66.39 and 65.94 (C-2I-VII, C-3I-VII, C-5I-VII, C-2A-C, C-3A-C, C-4A-C, C-5A-C, C-6A (66.39 ppm)), 62.62 and 62.38 (C-6B, C-6C), 60.71 (C-6II-VII), 54.43 (C-α), 40.67 (C-6I), 37.81 (C-β), 31.54 and 31.38 (2 × $CH_2$ Succ), 21.39, 21.29, 21.08, 21.02 and 20.82 ($OCOCH_3$); *m/z* (ES-HRMS) 2448.7692 ($[M + Na]^+$ $C_{103}H_{139}N_3NaO_{63}$ requires 2448.7663).

### *N*-6$^I$-Deoxy-(3,6-di-*O*-α-D-mannopyranosyl-β-D-mannopyranosyl-L-tyrosinylamido)-6$^I$-succinylamidocyclomaltoheptaose 2

A solution of compound **15** (280 mg, 0.11 mmol) and sodium methoxide (87 mg, 1.61 mmol) in methanol (10 ml) and dry DMF (10 ml) was stirred at RT for ten days, then neutralized with acidic resin (Amberlite IR 120) and filtered. Solvents were removed *in vacuo*. The residue was dissolved in water and freeze dried. High-performance liquid chromatography (water–$CH_3CN$, gradient elution from 100 to 0 in 40 min, $t_r$ 7.52 mn) of the crude β-CD derivative thus obtained was performed to give final material **2** (85 mg, 39%) (Found C, 41.14; H, 6.55; N, 2.53 $C_{73}H_{115}N_3O_{53}\cdot 13H_2O$ requires C, 41.41; H, 6.66; N, 1.98%); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 9.10 (s, 1 H, OH-Tyr), 8.26 (d, 1 H, $J_{NH,1A}$ 9.1 Hz, NH-Sugar), 8.01 (d, 1 H, $J_{NH,\alpha}$ 8.9 Hz, NH-Tyr), 7.58 (t, 1 H, $J_{NH,6I} = J_{NH,6'I}$ 5.5 Hz, NH-βCD); $^1$H NMR (500 MHz, $D_2O$): $\delta$ 7.12 (d, 2 H, $J_{a,b}$ 8.3 Hz, H-a, H-a′), 6.89 (d, 2 H, H-b, H-b′), 5.30 (s, 1 H, H-1A), 5.20 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1B or H-1C), 5.13–5.05 (7 d, 7 H, H-1I-VII), 4.92 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1B or H-1C), 4.37 (dd, 1 H, $J_{5I,6I}$ 1.7 Hz, $J_{6I,6'I}$ 14.3 Hz, H-6I), 4.32 (dd, 1 H, $J_{\alpha,\beta}$ 3.8 Hz, $J_{\alpha,\beta'}$ 8.3 Hz, H-α), 4.15–3.37 (m, 56 H, H-2I-VII, H-3I-VII, H-4I (t, 3.44 ppm), H-4II-VII, H-5I-VII, H-6II-VII, H-6′II-VII, H-2A-C, H-3A-C, H-4A-C, H-5A-C, H-6A-C, H-6′A-C), 3.06 (dd, 1 H, $J_{\beta,\beta'}$ 14.3 Hz, H-β), 2.93 (dd, 1 H, $J_{5I,6'I}$ 10.3 Hz, H-6′I) 2.76–2.58 (m, 4 H, H-β′, 3 $CH_2$ Succ), 2.48 (ddd, 1 H, $CH_2$ Succ); $^{13}$C NMR (75 MHz, $D_2O$): $\delta$ 175.55, 174.00 and 173.20 (NHCO), 155.60, 130.22, 129.46 and 115.61 ($C_6H_4OH$), 102.65 (C-1I-VII, C-1B or C-1C), 98.81 (C-1B or C-1C), 84.26 (C-4I), 82.44–65.82 (C-2I-VII, C-3I-VII, C-4II-VII, C-5I-VII, C-1A (78.21 ppm), C-2A-C, C-3A-C, C-4A-C, C-5A-C), 65.41 (C-6A), 61.43–60.00 (C-6II-VII, C-6A-C), 56.51 (C-α), 40.67 (C-6I), 36.89 (C-β), 31.39 and 30.78 (2 × $CH_2$ Succ); *m/z* (ES-HRMS) 1904.6300 ($[M + Na]^+$ $C_{73}H_{115}N_3NaO_{53}$ requires 1904.6294).

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