Mono-*N*-glycosidation of β-Cyclodextrin − Synthesis of 6-(β-Cyclodextrinylamino)-6-deoxy-D-galactosides and of *N*-(6-Deoxy-β-cyclodextrinyl)-*galacto*-azepane

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Keywords: Reductive amination / Cyclodextrins / Glycosidation / Molecular modelling

An easy route to 6-oxogalactose and to 6-oxogalactosides through an oxidation reaction mediated by galactose oxidase allowed the *N*-glycosidation of 6-amino-6-deoxy-β-cyclodextrin by reductive amination in the presence of $NaBH_3CN$ in DMSO. When methyl α-D- and *p*-nitrophenyl α- and β-D-*galacto*-hexodialdo-1,5-pyranosides (**1**, **2** and **3**, respectively) were used as substrates, 6-(β-cyclodextrinylamino)-6-deoxy-D-galactopyranosides were obtained in 25–50% yields. The same was true in the cases of 6^{II}-oxolactose **4** and of 6^{II}-oxomelibiose **5**, for which the anomeric carbon was shown to be unreactive in complexation with DMSO. This reaction was also successfully applied in water with an oxidized polygalactomannan **6**. The behaviour of *galacto*-hexodialdo-1,5-pyranose **7** was different, since further intramolecular cyclisation and reduction resulted in a *N*-(6-deoxy-β-cyclodextrinyl)-*galacto*-azepane in good yield. Molecular modelling was also used to explain the course of this reaction. Calculations indicated that the formation of the *cis* D-form of the intermediate *N*-β-CD imine of **7** was strongly preferred.

Introduction

The synthesis of monoglycosylated cyclodextrins is very important because these compounds, more soluble in water than their native counterparts, offer better opportunities for drug encapsulation applications. In addition, the complexation properties of CDs are not modified by the monoglycosidation to any great extent. Although methods involving protection-deprotection steps have allowed the preparation of α- and β-6-*O*-glucopyranosyl-cyclomaltoheptaose from CDs,[1−4] most previously published syntheses have used 6-*O*-monotosyl-β-cyclodextrin (β-CD-*O*-Ts) as starting material. This easily available compound[5] has been efficiently converted into 6-amino-6-deoxy-β-cyclodextrin (β-CD-NH_2).[6] Thioglycosidation has been achieved by treatment of β-CD-*O*-Ts with sodium salts of thioglycosides.[7] Similarly, the action of amino sugars on β-CD-*O*-Ts has afforded N-glycosylated CDs,[8] while numerous works have also been devoted to the *O*-glycosylation of CD. Unfortunately, none of the methods described was selective enough to achieve the synthesis of glycosylated CD in good yields. This was particularly true for direct acid-catalysed glycosidation of unprotected CD in organic solvents, which produces a complex mixture of polyglycosylated derivatives.[9] Despite the selectivity usually displayed by enzymes, the same was true when CDs were used as acceptors in transglycosylation reactions mediated by glycoside hydrolases.[10−15] A very small amount of a mixture of mono- and diglucosylated CD was also produced by the action of cyclodextrin glucanotransferase (CGTase) on potato starch.[16] Curiously, the reductive amination approach to the synthesis of *N*-glycosylated CDs has not been widely studied; only one paper on this topic is to be found in the literature. This work describes the synthesis of β-CD modified with a *N*-polyol chain by treatment of β-CD-NH_2 with glucose.[17] Meanwhile, *N*-glycopyranosylated CD can be synthesized via reductive amination with *glyco*-hexodialdo-1,5-pyranoses and β-CD-NH_2 as substrates. The former compounds are readily available through the oxidation of galactose and galactosides in the presence of dioxygen and galactose oxidase.[18−21] The aim of this work was therefore to evaluate the potential of this approach for the synthesis of 6-(β-cyclodextrinylamino)-6-deoxy-D-galactopyranosides (*N*-galactosyl-CDs).

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Results and Discussion

All the *galacto*-hexodialdo-1,5-pyranosides used in this work were prepared enzymatically by the method outlined in Scheme 1.[19]

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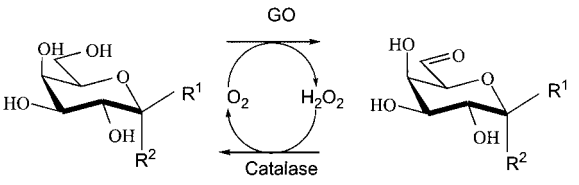
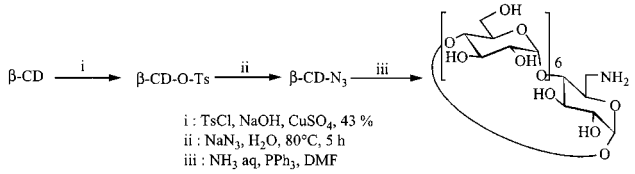


1 R^1 = H, R^2 = OCH_3
2 R^1 = H, R^2 = O-*p*-nitrophenyl
3 R^1 = O-*p*-nitrophenyl, R^2 = H
4 R^1 =4-O-glucosyl, R^2 = H
5 R^1 = H, R^2 = 6-O-glucosyl
6 R^1 = H, R^2 = polygalactomannan
7 R^1 = OH or H, R^2 = H or OH

Scheme 1. Enzymatic synthesis of 6-oxogalactosides

β-CD-NH_2 was synthesized from unprotected β-CD by the reactions shown in Scheme 2.[5,6,22,23]



Scheme 2. Three-step synthesis of β-CD-NH_2[5,6]

The amino-reduction reactions, carried out in the presence of sodium cyanoborohydride as a reductive agent and the products that would normally be expected from 6-oxogalactosides **1**−**7**, are described in Scheme 3.

In previous experiments we had used water as solvent at pH 9. Under these conditions, the expected products were indeed produced when the anomeric positions were protected (the cases of **8**, **9** and **10**), but complex mixtures were obtained when a free anomeric position was present (the cases of **11**, **12** and **14**). An interesting property of DMSO lies in its complex formation with hydroxy groups. For this reason, the rate of the mutarotation phenomenon is considerably lowered in DMSO. By taking advantage of this situation, one could expect to reduce the reactivity of the anomeric carbon, thus preventing the undesirable amino-reduction at this position. Furthermore, the separation of the reaction products from boron salts in water was not easy, whereas by performing amino-reduction in anhydrous DMSO, it was quite easy to precipitate all the saccharides at the end of the reaction by adding acetone. The solid, obtained after filtration and further washings with acetone, is free of boron salts.

It should also be pointed out that the aldehyde groups of 6-oxogalactosides exist mainly as hydrates in water solution.[19,24] In anhydrous DMSO, the equilibrium is shifted to the more reactive aldehyde forms.[24] These, known for their instability,[25] had sufficient lifetimes at the concentrations used (0.1 mol/L) for our reactions. We therefore decided to use DMSO and to optimize the reaction conditions with this solvent. The best conditions found, resulting in complete or maximum consumption of β-CD-NH_2, smaller numbers and lower concentrations of by-products and thus maximum yields of expected products **8**−**10**, were shown to be 1 equivalent of β-CD-NH_2 for 5

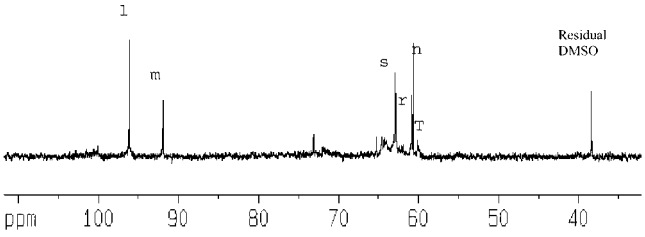
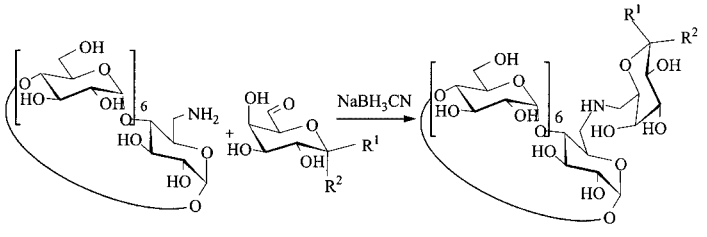
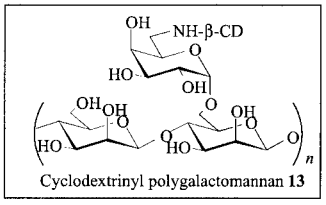


Figure 1. ^{13}C NMR spectrum (solvent D_2O) of the amino-reduction mixture obtained under the following conditions: β-CD-NH_2 (1 equivalent), pseudoracemic mixture of D-[1-^{13}C]- and L-[6-^{13}C]-6-oxogalactopyranoses (10 equivalents), $NaBH_3CN$ (50 equivalents) in [D_6]DMSO. λ: β-D-[1-^{13}C]-galactose, μ: α-D-[1-^{13}C]-galactose, ν: β-L-[6-^{13}C]-galactose, ρ: α-L-[6-^{13}C]-galactose, σ: galactitol, T: *N*-(6-*d*eoxy-β-cyclodextrinyl)-*galacto*-azepane



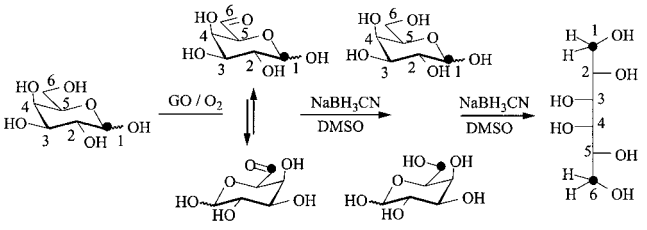


8 R^1 = H, R^2 = OCH_3
9 R^1 = H, R^2 = O-*p*-nitrophenyl
10 R^1 = O-*p*-nitrophenyl, R^2 = H
11 R^1 = 4-O-glucosyl, R^2 = H
12 R^1 = H, R^2 = 6-O-glucosyl
13 R^1 = H, R^2 = polygalactomannan
14 R^1 = OH or H, R^2 = H or OH

Scheme 3. Reductive amination with β-CD-NH_2 in the presence of 6-oxogalactosides **1**−**7**

equivalents of 6-oxo-D-galactopyranoside and 50 equivalents of sodium cyanoborohydride.[26]

In the case of free anomeric compounds (**11**, **12**, **14**), 10 equivalents of oxidized saccharide were used. The reason for using such large excesses of aldehyde lies in the fact that the rate of carbonyl reduction in DMSO looked similar to that of the intermediate imine. We obtained experimental evidence of this situation when we tried to follow the kinetics of the amino-reduction by ^{13}C NMR spectroscopy in the presence of a pseudoracemic mixture of D-[1-^{13}C]-and L-[6-^{13}C]-6-oxogalactopyranoses (prepared from D-[1-^{13}C]-galactopyranose, see Scheme 4) and of β-CD-NH_2. The corresponding spectra showed the presence of large amounts of a pseudoracemic mixture of D-[1-^{13}C]-and L-[6-^{13}C]-6-galactose and of [1-^{13}C]-galactitol (see Figure 1). The reaction mixtures also contained degradation products of 6-oxogalactosides and probably of their condensation with β-CD-NH_2. Previous attempts to purify *N*-galactosyl-CD by gel permeation chromatography or on silica gel columns after acetylation did not give satisfactory results. In fact, the purification of compounds **8**−**12** was achieved by preparative HPLC on a 1 m long (o.d. 2.5 cm) C18 silica column with water/acetonitrile as an eluent.



Scheme 4. Concurrent carbonyl reduction of D-[1-^{13}C]- and L-[6-^{13}C]-6-oxogalactopyranose by $NaBH_3CN$ in DMSO

The structures of products **8**−**12** were then established by one- and two-dimensional ^1H and ^{13}C NMR spectra (COSY, HCCOR, HOHAHA). Figure 2, for example, shows the ^1H NMR spectrum of compound **8**, which shows characteristic CH_2-N and CH anomeric resonances at 2.5−3 ppm and 4.81 ppm, respectively, thus confirming the 6,6′-*N*-glycosidic linkage. The ^{13}C NMR spectrum was also completely consistent with structure **8**. However, the loss of symmetry of the *N*-galactosyl-CD ring made the attribution of each resonance difficult (see Exp. Sect.). When the reaction was performed with 6^{II}-oxolactose **4** or 6^{II}-oxomelibiose **5**, the free anomeric carbons, partially protected by the DMSO complexation, were not reactive enough to undergo a second amino-reduction reaction, so the structures of the corresponding *N*-glycosyl-CDs **11** and **12** were confirmed by the presence of characteristic α and β anomeric proton and carbon resonances (see Exp. Sect.).

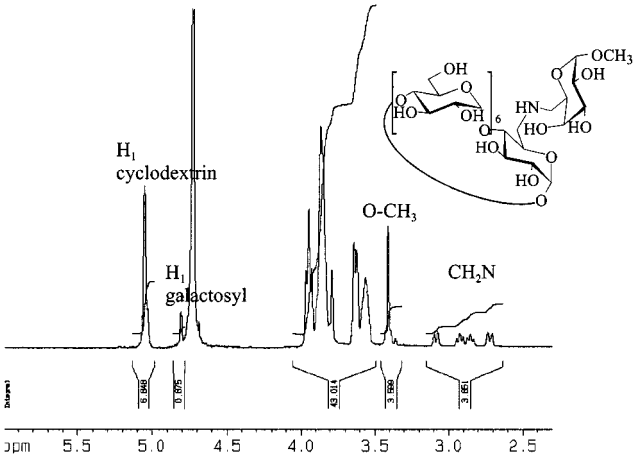


Figure 2. ^1H NMR spectrum of 6-(β-cyclodextrinylamino)-6-deoxy-methyl-α-D-galactopyranoside **8**

The generality of the method was also demonstrated with polysaccharides, since galactose oxidase was able to oxidise the polygalactomannan obtained from the gum of *Ceratonia siliqua*. The structure of this 310000 Dalton polymer was made up of a β-1,4-polymannan chain substituted with α-1,6 galactosyl units (see Scheme 3). The ratio between mannose and galactose units was 4. In this case, the amino-reduction was performed in water at pH 8. Assaying of the 6-oxogalactosyl units by means of the Nelson colorimetric method[27] showed that only 20% of galactose residues had reacted. Fortunately, the yield of the amino-reduction was 100%, thus affording a polysaccharide modified with 80 *N*-β-cyclodextrinyl units for every 1900 monosaccharide residues.[26] Comparison between the proton NMR spectra of

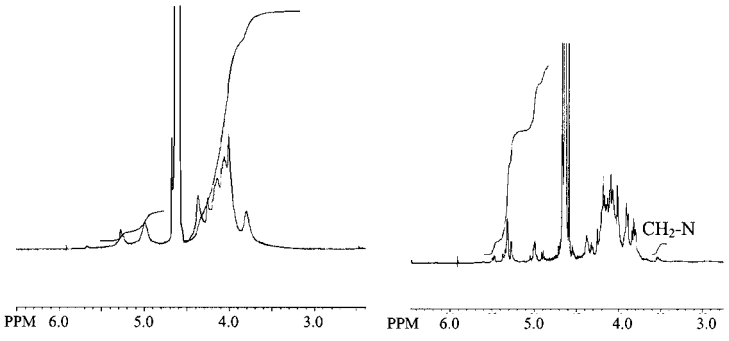


Figure 3. Proton NMR spectra (solvent D_2O) of the native polygalactomannan from the gum of *Ceratonia siliqua* and the *N*-cyclodextrinyl one **13** obtained after amino-reduction of the former

the native polymer and that of the modified one confirmed this result qualitatively (presence of CH_2-N) and quantitatively (see Figure 3).

Amino-reduction with galactose dialdehyde **7** was a special case, since the products of the reaction were not the two expected diastereoisomeric forms of *N*-galactosyl-β-CD **14**. Instead, a single compound, *N*-(6-deoxy-β-cyclodextrinyl)-*galacto*-azepane **15** was synthesized, as revealed by a careful analysis of proton and carbon NMR spectra (see Figure 4 and Figure 5). The former shows the presence of three CH_2N groups, consistently with the structure of **15**. In fact, **14** was an intermediate that had undergone a further intramolecular cyclisation followed by a reduction step (see Scheme 5). Similar one-pot cyclisation-reduction strategies, producing *gluco*-, *galacto*- and *manno*-azepanes, have been described previously.[28−42] The same reaction has also been reported when starting from protected 6-oxoglycosides (prepared in several steps from norbornyl derivatives) in the presence of benzylamine to yield *N*-benzyl *glyco*-azepanes.[43] Our results indicate that the enzymatic synthesis of 6-oxogalactose, which avoids any need for hydroxy group protection, allows a short synthesis of **15** by use of the readily available β-CD-NH_2. Because of the particular

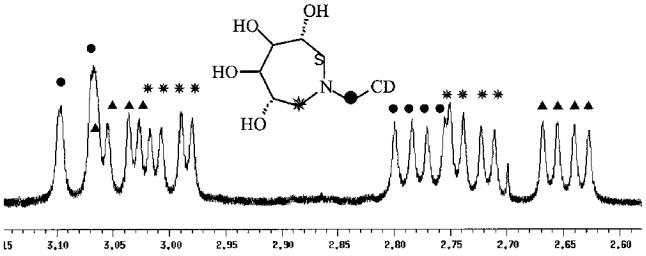


Figure 4. *N*-CH_2 proton resonances of azepane **15** (solvent D_2O, the whole ^1H spectrum is shown in Figure 5); λ: CD-CH_2, Y: diastereotopic *pro S*,*R*-CH_2, σ: diastereotopic *pro R*,*S*-CH_2

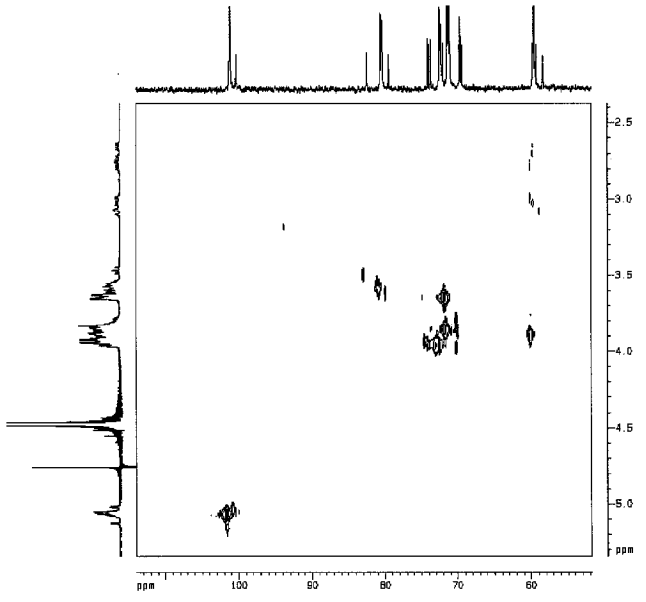


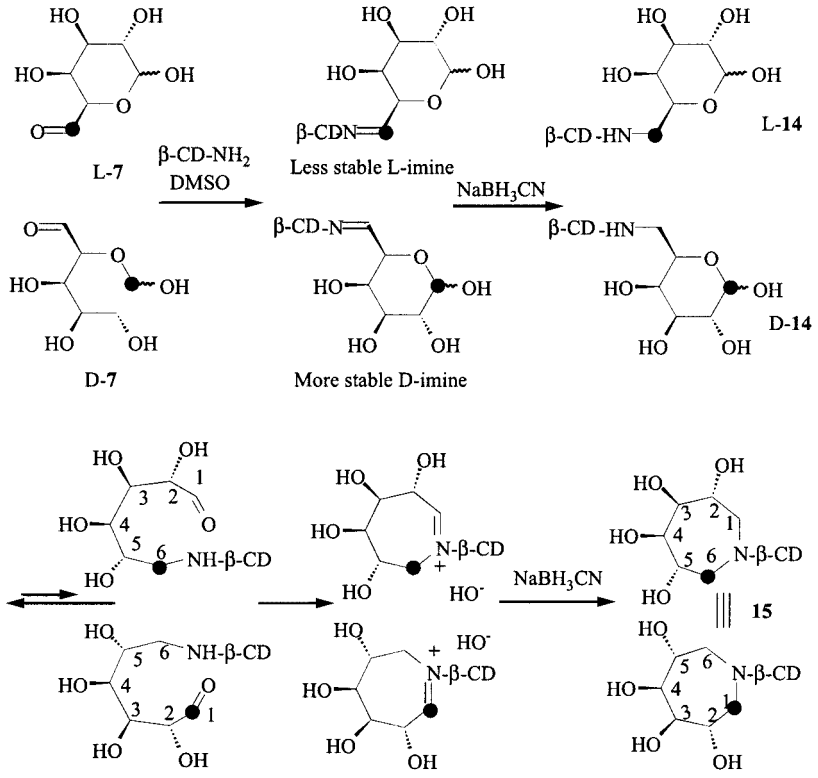
Figure 5. NMR proton-carbon correlation of azepane **15** (solvent D_2O)

configuration of the *galacto*-azepane cycle of **15,** the same isomer of this compound is obtained whether starting from either the D or the L enantiomer of **7** (see Scheme 5). The same was true when starting from the pseudo-racemic mixture of D-[1-^{13}C]- and L-[6-^{13}C]-6-oxogalactose, which gave the corresponding azepane labelled with a ^{13}C at the *pro-S*,*R* diastereotopic methylene group. In the ^{13}C spectrum of the reaction mixture thus obtained (see Figure 1), the resonance at 61.2 ppm corresponds to the latter *N*-methylenic group.

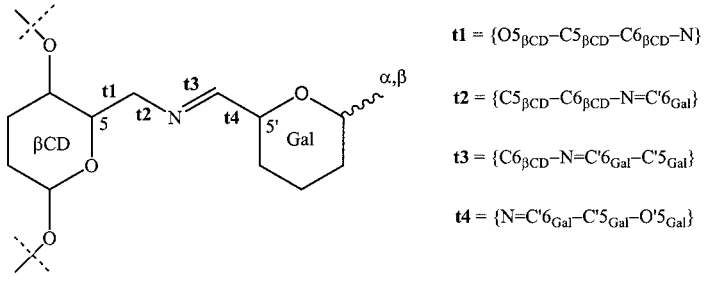
In order to obtain a better understanding of the mechanism of the amino-reduction reaction and of the structure of the CD-azepane **15**, we carried out a molecular modelling study. The intermediate D- and L-imines (see Scheme 5) implicated in the determining step of the process were regarded as representative of the transition state. The relative calculated energies for the best conformations of the stereoisomers of the imines α-D-, β-D-, α-L-, β-L-*N*-Gal-CD, were 11.4, 0.0, 9.3 and 8.4 kcal/mol, respectively (see Figure 6).

Because of the permanent exchange between α and β enantiomers, the only chemically observed differentiation concerns the D and L configurations. This means that the best D solution (β-D) had significantly lower energy than all other solutions (about 10 kcal/mol, see above). The best L solution obtained (β-L, 8.4 kcal/mol, see above) indicated a high level of stereoselection induced by the CD for the D-imine form. It must be emphasized that the final energies were very sensitive to hydrogen bond networks and that many manual reorientations of OH bonds were necessary in order to obtain the lowest-energy solutions. Figure 7 presents the best D and L optimized molecules.

The general shapes were quite similar, with the galactosyl ring covering the narrowest part of the truncated cone of β-CD to optimize the hydrogen bond network between most hydroxy groups of the former and the primary hydroxy groups of the latter. As a consequence, the β-CD showed a slight loss of symmetry (see upper representations in Figure 7). In addition, this picture showed that the D- and the L-galactosyl rings had opposite orientations to accommodate their configurations to the primary hydroxy oxygen atoms of β-CD. In both cases, equatorial galactosyl hydroxy groups were involved in H bonds, yielding almost 'symmetric' networks. Five H bonds were identified for the βD and βL enantiomers as well ($O1_{Gal}-O6_{Glc(C)}$; $O2_{Gal}-O6_{Glc(E)}$; $O2_{Gal}-O6_{Glc(F)}$; $O3_{Gal}-O6_{Glc(G)}$; $N_{Gal}-O6_{Glc(B)}$ and $O1_{Gal}-O6_{Glc(F)}$; $O1_{Gal}-O6_{Glc(E)}$; $O2_{Gal}-O6_{Glc(C)}$, $O3_{Gal}-O6_{Glc(C)}$; $O4_{Gal}-O6_{Glc(B)}$ respectively). The values of *t*1, *t*2, *t*3 and *t*4 are reported in Figure 6 (a) for the best α-D, β-D, α-L and β-L conformations. Value t1 mainly governed the relative positions between β-CD and the galactosyl unit. Thus, with *t*1 = 159° and 68°, the β-D and α-L *N*-Gal-CD diastereoisomers of **14** had *tg* and *gt* orientations, respectively, for the glucosyl ring (A) linked to the galactosyl residue. Low values of *t*3 corresponded to the *cis* conformation of the N=C bond for these β-(D or L) forms, while *trans* conformations (*t*3 ≈ 180°) were deduced for α-(D or L) ones.



Scheme 5. Mechanism of the formation of azepane **15** obtained in the amino-reduction of (D,L)-6-oxogalactoside in the presence of CD-NH_2; λ: 99 % ^{13}C enriched



	αD-GalCH=NCD	βD-GalCH=NCD	αL-GalCH=NCD	βL-GalCH=NCD
E (kcal/mol)	***11.4***	***0.0***	***9.3***	***8.4***
t1 (°)	131	159	95	68
t2 (°)	-65	-57	62	81
t3 (°)	176	1	179	3
t4 (°)	-106	179	167	150

Figure 6. Schematic representation of imine intermediate; relative energies and *t*1 to *t*2 essential conformational parameters are given

Energy calculations were also performed on the *N*-Gal-CD isomers of **14**. The relative energies of the most probable conformers for the α-D, β-D, α-L and β-L **14** isomers were 5.8, 0.0, 0.2 and 3.4 kcal/mol, respectively (conformations not shown). Contrary to the observations made on the intermediate imines, the most likely conformers for L and D isomers were not distinguishable in terms of energy (insignificant difference of 0.2 kcal/mol between β-D- and α-L-Gal-CD). The central values of *t*2 and *t*3 (not reported here) seemed to correspond to specific adaptations between β-CD and galactosyl units. Both solutions had three hydrogen bonds, consistently with the similar calculated energies. This result confirmed that the imine formation is implicated as the limiting step of the synthesis of azepane **15**. Furthermore, our calculations on the relative energies of conformers of diastereoisomeric imines showed a highly preferred reaction pathway via the D-*cis* form.

In conclusion, we have shown that 6-*N*-monoglycosylation of β-cyclodextrins can be achieved in good yields by means of the reductive amination approach with 6-deoxy-6-amino-β-cyclodextrin and various 6-oxogalactosides. When this reaction was performed in the presence of 6-oxogalac-

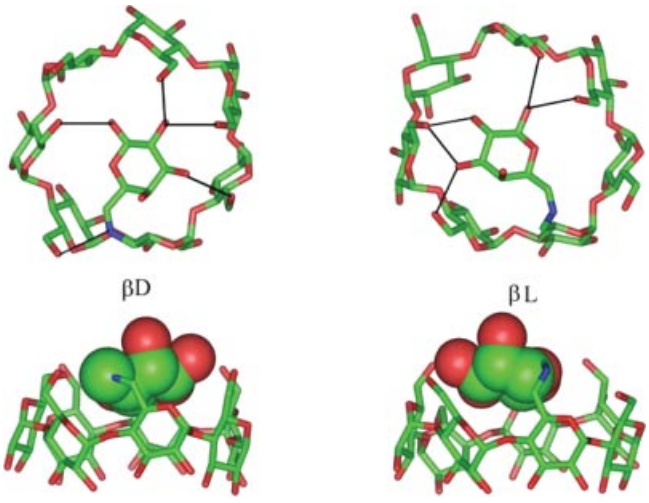


Figure 7. Representations of imine intermediate isomers; β-D (left) and β (right) are the lowest-energy conformers for D- and L-*N*-galactosyl-β-CD imines; upper images represent the galactosyl ring covering the narrowest part of the truncated cone of β-CD; thin lines are hydrogen bonds between equatorial hydroxyl groups of the galactosyl part and some primary hydroxy groups of glucosyl residues; lower representations are perpendicular views of upper ones; CPK are used for the galactosyl part to visualise its occupancy at the entrance of the β-CD hole; hydrogen atoms are omitted for clarity

tose (galactose dialdehyde), a further cyclisation, resulting in *N*-(6-deoxy-β-cyclodextrinyl)-*galacto*-azepane in good yields, was observed. Molecular modelling calculations carried out on the relative energies of intermediate D- and L-*cis* and D- and L-*trans* imines of this last reaction showed a highly preferred pathway via the D-*cis* form.

Experimental Section

General Procedures: The chemicals supplied by Sigma were used without further purification. Deuterium oxide was purchased from Eurisotop (isotopic purity 99.9 %). Labelled galactose [1-^{13}C] was supplied by Omicron Biochem. The course of the reactions was followed by HPLC (Asahipak NH2P50−4E column with water as eluent at a flow rate of 0.5 mL/min with detection by an Evaporated Light Scattering Detector) and by ^{1}H NMR spectroscopy at 500 MHz (Bruker AX500 spectrometer). The complete analysis of the ^{1}H and ^{13}C NMR resonances and the subsequent structure assignments were achieved by use of standard 1D and 2D sequences (COSY HH, HCOOR correlations and HOHAHA experiments) and by comparison with previously published data.[44] The spectra were recorded with a Bruker AX 500 spectrometer operating at 500 MHz for ^{1}H (solvent D_2O, chemical shifts in ppm quoted from the resonance of DMSO at 2.50 ppm) and 125 MHz for ^{13}C (solvent D_2O, chemical shifts in ppm quoted from the resonance of DMSO at 39.5 ppm).

Synthesis of 6-Oxogalactosides 1−6: The enzymatic oxidation was performed by an already described procedure.[19]

Synthesis of β-CD-NH_2: This compound was prepared by previously known procedures[6] by reduction of 6-deoxy-6-azido-β-CD, this in turn being obtained from the readily available β-CD-*O*-Ts.[5] Yields obtained for these compounds, NMR parameters and optical rotations are given below.

β-CD-*O*-Ts: $[\alpha]_D$ = +115.3 (*c* = 0.5, DMSO, 20 °C), m.p. 193 °C (decomp.). 43 % yield. ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 7.75 (d, 8 Hz, 2 H, H_{ar}), 7.45 (d, 8 Hz, 2 H, H_{ar}), 6.7 (m, OH-2 and OH-3), 4.85 (d, *J* = 5 Hz, 7 H, H-1), 4.1−4.5 (m, 14 H, H-6-OTs and H-6′-OTs and OH-6), 3.1−3.8 (m, 28 H, H-3, H-4, H-5, H-2), 2.45 (s, 3 H, Me) ppm. ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 145 (C_{ar}), 133 (C_{ar}), 130 (2 CH_{ar}), 128 (2 CH_{ar}), 103 (C-1), 102 (C-1), 83 (C-4), 82 (C-4), 74−72 (C-2, C-3, C-5), 60 (C-6), 59 (C-6 modified unit), 22 (Me) ppm.

The above β-CD-*O*-Ts (300 mg, 0.23 mmol, 1 equiv.) was added to a solution of NaN_3 (160 mg, 2.46 mmol, 10.7 equiv.) in water (3 mL). The mixture was stirred for 5 hours at 80 °C. The 6-deoxy-6-azido-β-CD was precipitated by addition of acetone (50 mL) and filtered.

6-Azido-6-deoxy-β-CD: $[\alpha]_D$ = +111.4 (*c* = 0.5, H_2O, 20 °C), m.p. 205 °C (decomp.). 98 % yield. ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 5.7 (OH-2 and OH-3), 4.8 (m, 7 H, H-1), 4.5 (m, OH-6), 3.8−3.5 (m, 28 H, H-3, H-5, H-6, H-6′), 3.3 (m, 14 H, H-4, H-2) ppm. ^{1}H NMR (500 MHz, D_2O): δ = 4.9 (C-1), 3.3−3.9 (H-2, H-3, H-4, H-5, H-6, H-6′) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 102.1 (C-1), 101.9 (C-1 modified unit), 82.4 (C-4 modified unit), 81.5 (C-4), 73.4−70.8 (C-3, C-2, C-5), 60.6 (CH_2OH), 51.4 (CH_2N_3) ppm.

The above 6-deoxy-6-azido-β-CD (270 mg, 233 μmol, 1 equiv.) was added to a solution of PPh_3 (103 mg, 393 μmol, 1.7 equiv.) in DMF (5 mL). Aqueous NH_3 (800 μL, 5.76 mmol, 24 equiv.) was then added. The mixture was stirred for 4 hours at room temperature. The β-CD-NH_2 precipitated by addition of acetone (50 mL) was filtered, washed three times with acetone (5 mL) and dried.

β-CD-NH_2: $[\alpha]_D$ = +129.6 (*c* = 0.5, H_2O, 20 °C), m.p. 203 °C (decomp.). 98 % yield. ^{1}H NMR (500 MHz, D_2O): δ = 4.8 (s, 7 H, H-1), 3.7−3.3 (m, 42 H, H-2, H-3, H-4, H-5, H-6, H-6′), 2.85 and 3.15 (CH_2N) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 102.7 (C-1), 83.7 (C-4 modified unit), 82.0 (C-4), 73.9−72.7 (C-2, C-3, C-5), 61.2 (CH_2OH), 41.7 (CH_2N) ppm.

Methyl 6-(β-Cyclodextrinylamino)-6-deoxy-α-D-galactopyranoside (8): The above $CDNH_2$ (226 mg, 200 μmol, 1 equiv.) was added to a solution of methyl α-D-*galacto*-hexodialdo-1,5-pyranoside (1 mmol, 5 equiv.) and $NaBH_3CN$ (620 mg, 10 mmol, 50 equiv.) in DMSO (10 mL). The mixture was stirred for 6 h at 60 °C. Saccharides were precipitated by addition of acetone. The precipitate was filtered, washed twice with acetone (50 mL) and twice with diethyl ether (50 mL) and dried. The obtained mixture was purified by preparative HPLC on an Amicon C18 column (20 × 1000 mm), flow rate 10 mL/min, H_2O/CH_3CN, 95:5 (50 mg of reaction mixture was introduced in 4 mL of eluent). The purity of the different fractions was checked by analytical HPLC on a Shodex Asahipak NH_2P50-E column (eluent: water 100 %, flow rate 1 mL/min, DDL). Yield 45 %.

Compound 8: $[\alpha]_D$ = +184 (*c* = 0.5, H_2O, 20 °C), m.p. 235 °C (decomp.). ^{1}H NMR (500 MHz, D_2O): δ = 5.05 (m, 7 H, H-1), 4.81 (d,1 H, *J* = 4.1 Hz, H-1 gal), 3.92 (dd, 1 H, H-2 Gal), 3.83 (dd, 1 H, H-3 Gal) 3.96−3.53 (m, 45 H, H-2, H-3, H-4, H-5, H-6, H-6′, Me) 3.13 (dd, 1 H, *J* ≈ 0 Hz, *J* = −12.7 Hz, CH_2N CD), 3.08 (dd, 1 H, *J* = −14.4 Hz, 8.1 Hz CH_2N), 3.00 (dd, 1 H, *J* = −12.7 Hz, 8.8 Hz, CH_2N CD) 2.88 (dd, 1 H, *J* ≈ 0 Hz, −14.4 Hz, CH_2N) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 102.5−101.9 (C-1

CD), 100.1 (C-1 Gal), 84.4−81.1 (C-4 CD), 73.6−72.3 (C-3,C-2,C-5 CD and C-5 Gal), 70.6 (C-3 Gal), 69.8 (C-4 Gal), 68.6(C-2), 60.9−60.7 (C-6), 55.9 (OMe), 49.8 and 49.5 (2 CH_2N) ppm. ES-MS (LC Quattro Micromass, ES +, triple quadrupoles, direct introduction): 1339 ($M + H^+ + 2Na^+ - H_2O$).

***p*-Nitrophenyl 6-(β-Cyclodextrinylamino)-6-deoxy-α-D-galactopyranoside (9) and β Anomer 10:** $CDNH_2$ (40 mg, 40 μmol, 1 equiv.) was added to a solution of *p*-NP-D-*galacto* hexodialdo-1,5-pyranoside (60 mg, 200 μmol, 5 equiv.) and $NaBH_3CN$ (120 mg, 2 mmol, 50 equiv.) in DMSO (2 mL). The mixture was stirred for 6 h at 60 °C. Saccharide precipitation was achieved by addition of acetone. The precipitate was filtered, washed twice with acetone (5 mL) and twice with diethyl ether (5 mL) and dried. The obtained mixture was purified by silica gel column chromatography (eluent: MeOH/$CHCl_3$/AcOH/H_2O, 30:60:3:5 to elute mono- and disaccharides and then pure MeOH to elute CD). Products **9** and **10** were obtained in 20 and 70 % yields.

Compound 9: $[\alpha]_D$ = +24 (*c* = 0.5, H_2O, 20 °C), m.p. 194 °C (decomp.). ^1H NMR (500 MHz, D_2O): δ = 8.25, 7.27 (pNP), 5.71 (1 H, *J* = 4.7 Hz, H-1 Gal), 5.05 (s, 7 H, H-1 CD), 4.18−3.13 (m, 44 H, H-2, H-3, H-4, H-5, H-6, H-6′) 2.86−2.63 (m, 4 H, 2 CH_2NH) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 160, 140, 123, 113 (pNP), 101.0 (C-1 CD), 97.7 (C-1 Gal), 79.8 (C-4 CD), 71.8−70.5 (C-3, C-2, C-5 CD, Gal), 59.6 (C-6 CD), 45.6, 40.9 (2 CH_2N) ppm. ES-MS (LC Quattro Micromass, ES+, triple quadrupoles, direct introduction): 1439 [$M + Na^+$].

Compound 10: $[\alpha]_D$ = +91 (*c* = 0.5, H_2O, 20 °C), m.p. 186 °C (decomp.). ^1H NMR (500 MHz, D_2O): δ = 8.30, 8.20, 7.30, 7.25 (pNP), 5.18 (1 H, *J* = 7.6 Hz, H-1 Gal), 5.05 (s, 7 H, H-1 CD), 3.7−3.4 (m, 44 H, H-2, H-3, H-4, H-5, H-6, H-6′) 3.0−2.9 (m, 4 H, 2 CH_2NH) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 162.1, 142.2, 125.1, 116.1 (pNP), 102.5 (C-1 CD), 99.8 (C-1 Gal), 84.2−79.9 (C-4 CD), 73.3−68.3 (C-3,C-2,C-5 CD, Gal), 63.4−58.8 (C-6 CD), 50.2, 47.3 (2 CH_2N) ppm.

6^{II}-(β-Cyclodextrinylamino)-6^{II}-deoxy-D-lactose (11) and -melibiose (12): $CDNH_2$ (113 mg 100 μmol. 1 equiv.) was dissolved in DMSO (5 mL). The oxidized saccharides (1 mmol, 10 equiv) and $NaBH_3CN$ (310 mg, 5 mmol. 50 equiv) were then added. The mixture was stirred for 6 h at 60 °C. The saccharides precipitated on addition of acetone (100 mL) were filtered, washed twice with acetone (20 mL) and twice with diethyl ether (20 mL) and dried. The obtained mixture was purified by preparative HPLC on an Amicon C18 column (20 × 1000 mm), flow rate 10 mL/min, H_2O/ACN, 98:2 (25 mg of reaction mixture was introduced in 1.5 mL of eluent). The purity of the fractions was checked by analytical HPLC on a Shodex Asahipak NH_2P50-E column (eluent: water 100%, flow rate 1 mL/min, DDL). Products **11** and **12** were obtained in 15% yield.

Compound 11: $[\alpha]_D$ = +65 (*c* = 0.5, H_2O, 20 °C), m.p. 224 °C (decomp.). ^1H NMR (500 MHz, D_2O): δ = 5.2 (d, 0.4 H, *J* = 3.6 Hz, H−1α Glc), 5.05 (s, 7 H, H-1 CD), 4.63 (d, 0.6 H, *J* = 7.7 Hz, H-1β Glc), 4.43 (d, *J* = 7.6 Hz, 1 H, H-1Gal) 3.95 (H-6β Glc), 3.93 (H-5 CD), 3.91 (H-5α Glc), 3.90 (H-4 Gal), 3.88 (H-6α Glc), 3.87 (H-6 et H-2 CD), 3.83−3.82 (H-6′αβ Glc), 3.80 (H-3α Glc), 3.70 (H-5 Gal), 3.64 (H-3 CD), 3.63 (H-4β Glc), 3.62 (H-3β Glc), 3.60 (H-4α Glc), 3.59 (H-3β Gal), 3.58 (H-5β Glc), 3.56 (H-4 CD, H-2α Glc), 3.51 (H-2 Gal), 3.27 (H-2β Glc), 3.09−2.92 (m, *J* = 14.3 Hz, 4 H, 11.2 Hz, 8.6 Hz and 8.3 Hz, 2 CH_2NH) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 103.8 (C-1 Gal), 102.9 (C-1 CD), 96.8 (C-1 β Glc), 92.9 (C-1 α Glc), 82.1 (C-4 CD), 74.2−72.9 (C-3, C-2, C-5 CD, C Gal et C Glc), 61.4 (C-6 CD), 57.9, 56.7 and 55.6 (2 CH_2N) ppm. ES-MS (LC Quattro Micromass. ES + triple quadrupoles. direct introduction): 2658 ($M + 2Na^+$).

Compound 12: $[\alpha]_D$ = +161 (*c* = 0.5, H_2O, 20 °C), m.p. 230 °C (decomp.). ^1H NMR (500 MHz, D_2O): δ = 5.25 (d, *J* = 4 Hz, H-1α Glc), 5.05 (s, 7 H, H-1 CD), 4.94 (d, *J* = 4.0 Hz, 1 H, H-1 Gal), 4.66 (d, *J* = 7.8 Hz, H-1β Glc), 3.75−3.44 (m, 50 H, H-2, H-3, H-4, H-5, H-6, H-6′) 3.27−2.93 (m, 4 H, 2 CH_2NH) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 102.9 (C-1 CD), 99.3 (C-1 Gal), 97.3 (C-1β Glc), 93.4 (C-1α Glc), 82.2 (C-4 CD), 77.1−69.5 (C-3, C-2, C-5 CD, C Gal et C Glc), 61.4 (C-6 CD), 53.9, 49.9 (2 CH_2N) ppm. ES-MS (LC Quattro Micromass, ES+, triple quadrupoles, direct introduction): 2658 ($M + 2Na^+$).

6-(β-Cyclodextrinylamino)-6-deoxy-polygalactomannan (13): Galactose oxidase (EC, 1.1.3.9, 20 U) from *Dactylium dendroides* and catalase (EC, 1.11.1.6, 3 mg) from bovine liver were added to a suspension of polysaccharide (150 mg) from *Ceratonia siliqua* in water (150 mL). The mixture was saturated with oxygen, stirred for 72 hours at room temperature and then concentrated under reduced pressure. The obtained oxidized 6-oxogalactosyl-polysaccharide was suspended in water [40 mL; according to the Nelson method (see below), 20 % of galactosyl units were oxidized]. β-$CDNH_2$ (74 μmol, 83.8 mg) and 1.78 mL of a 5 mol/L $NaBH_3CN$ (8.88 mmol) in 1 mol/L sodium hydroxide solution were then added to this solution. The mixture was stirred for 196 hours at room temperature and dialysed against water with 10000 Da membranes. The Nelson oxidation test[27,45] was negative, thus showing complete amino reduction of the 6-oxogalactosyl units. The 6-(β-cyclodextrinylamino)-6-deoxy-polygalactomannan is therefore made up of 80 cyclodextrin units. The preparation of 100 mL of Nelson reagent was carried out by mixing the following components: K_2HPO_4 (2.8 g), Na,K tartrate (4 g), NaOH (1 M, 10 mL), $CuSO_4$ (0.8 g) and Na_2SO_4 (18 g), and that of arseno-molybdic reagent (used as a tracer) by mixing hexaammonium heptamolybdate tetrahydrate (2.5 g) in distilled water (45 mL), sulfuric acid (2.1 mL) and $AsO_4Na_2{\cdot}7H_2O$ (0.3 g) in distilled water (2.5 mL). The latter mixture was warmed at 37 °C for 48 h. Aliquots of 1, 1.5 and 2 mL of the reaction solution were mixed with 2 mL of the Nelson reagent and warmed at 100 °C for 20 minutes. The mixture was then refreshed quickly and 2 mL of arseno-molybdic reagent was added. This solution was diluted with 4 mL of distilled water and the absorbance was read at 520 nm. The calculation of the percentage of oxidized galactosyl residue was made by comparison with a standard calibration curve obtained for the oxidation of glucose.

***N*-(6-Deoxy-β-cyclodextrinyl)-*galacto*-azepane (15):** β-$CDNH_2$ (28 mg, 25 μmol, 1 equiv.) was added to a solution of *galacto*-hexodialdo-1,5-pyranoside (45 mg, D- and L-racemic mixture, 250 μmol, 10 equiv.) and $NaBH_3CN$ (78 mg, 1.25 mmol, 50 equiv.) in DMSO (1.5 mL). The mixture was stirred for 6 h at 60 °C. The precipitation of saccharides was then induced by addition of acetone. The precipitate was filtered, washed twice with acetone (5 mL) and twice with diethyl ether (5 mL) and dried. The obtained mixture was purified by preparative HPLC on an Amicon C18 column (20 × 1000 mm), flow rate 10 mL/min, H_2O/ACN, 96:4 (50 mg of reaction mixture was introduced in 4 mL of eluent). The purity of the fractions was checked by analytical HPLC on a Shodex Asahipak NH_2P50-E column (eluent: water 100 %, flow rate 1 mL/min, DDL). The product was obtained in 35 % yield.

Compound 15: $[\alpha]_D$ = +164 (*c* = 0.5, H_2O, 20 °C), m.p. 228 °C (decomp.). ^1H NMR (500 MHz, D_2O): δ = 5.13−5.05 (m, 7 H, *J* ≈ 3.8 Hz, H-1), 4.0−3.5 (m, 43 H, H-2,H-3,H-4,H-5,H-6,H-6′), 3.46 (dd, *J* = 9.5, 9.1 Hz, 1 H, H-4 of CD's glucosyl modified unit),

3.08 (dd, 1 H, $J \approx 1$, $J_{H6-H6'} = 15$ Hz, CH*H*NH-CD), 3.04 (dd, 1 H, $J = 4.5$, $J_{H6-H6'} = 13.8$ Hz, *pro-R*,*S*-CH*H*NH), 2.99 (dd, 1 H, $J = 4.8$, $J_{H6-H6'} = 13.8$ Hz, *pro-S*,*R*-CH*H*NH), 2.77 (dd, 1 H, $J = 7.5$, $J_{H6-H6'} = 15$ Hz, *pro-S*,*R*-CH*H*NH-CD), 2.73 (dd, 1 H, $J = 5.7$, $J_{H6-H6'} = 13.8$ Hz, CH*H*NH), 2.65 (dd, 1 H, $J = 6.3$, $J_{H6-H6'} = 13.8$ Hz, *pro-R*,*S*-CH*H*NH) ppm. ^{13}C NMR (125 MHz, D_2O): δ = 102.7 (C-1), 84.2 (C-4 modified unit), 81.8 (C-4), 75.7−71.1 (C-2, C-3, C-5 CHOH), 61.2 (C-6 *pro S*,*R*-CH_2N), 60.8 (*pro R*,*S*-CH_2N), 59.5 (CD-CH_2N) ppm. ES-MS (LC Quattro Micromass, ES+, triple quadrupoles. direct introduction): 1306.

Molecular Modelling: The molecular modelling was carried out on Silicon Graphics (SGI) computers with the Accelrys packages (San Diego, CA, USA). Molecular displays and energy minimisation were performed with InsightII, Biopolymer and Discover modules. The CFF91 forcefield[46] was used for all calculations. The stereoselectivity of the reaction was analysed according to the energies of diastereoisomers occurring along the chemical reaction (imine and amine step).Starting geometries and minimisation procedure. Basically, these molecules are made up of a β-cyclodextrin ring and a galactopyranosyl fragment linked together by a central linear part. The flexibility of the parts at each extremity is quite different from those of the central one because of rings (plus the cyclic constraint of β-CD), considerably restricting the motion of attached atoms. Optimized geometries were therefore used for the β-CD and galactosyl parts. For the central unit, all dihedrals defining the backbone were systematically scanned. Starting from symmetric β-CD forms with different primary hydroxy group orientations on glucopyranosyl rings (***tg***, ***gg*** or ***gt***), the branching of one NH_2 group yielded the ***gg*** preference that was used for initial β-CD constructions. Four optimized forms were also chosen for the galactopyranosyl ring: (α-D, α-L, β-D and β-L). The central part of the imine forms has four dihedrals defined as {$O5_{βCD}-C5_{βCD}-C6_{βCD}-N$} ***t*1**, {$C5_{βCD}-C6_{βCD}-N=C'6_{Gal}$} ***t*2**, {$C6_{βCD}-N=C'6_{Gal}-C'5_{Gal}$} ***t*3** and {$N=C'6_{Gal}-C'5_{Gal}-O'5_{Gal}$} ***t*4**. For steric reasons due to the vicinity of the β-CD, it was assumed that ***t*1** had initially three restricted domains around 180°, −60° and +60° values, so only these initial values were used. Because of the central double bond of ***t*3**, the *trans* and *cis* conformations (180° and 0° respectively) were tested for the initial construction. Finally, in the absence of obvious steric conflicts, ***t*2** and ***t*4** were scanned in steps of 10°. Thus, by starting from the four conformations of galactosyl fragments, all geometries defined with different values for ***t*1**, ***t*2**, ***t*3** and ***t*4** were built and corresponding instantaneous potential energies calculated. In the second series of calculations concerning the stereoisomers of **14**, the only difference lay in the absence of the $C6_{βCD}=N$ double bond. Consequently, the previous restriction of motion around this bond disappeared, thus necessitating another full scan of ***t*3** by steps of 10°.

General Minimisation Procedure: All probable conformations selected after this construction stage were refined with several minimisation procedures (10000 iterations) and a stopping criterion of final RMS fluctuations less than 0.001Å. All internal parameters were allowed to relax fully to decrease the potential energy. Manual reorientation of hydroxy groups was also performed to optimize the network of hydrogen bonds mainly responsible for significant energy lowering. This procedure (systematic scanning of the central dihedrals parameters and extensive minimisation procedures) resulted in an exhaustive conformational search.

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