Synthesis of a Cellobiosylated Dimer and Trimer and of Cellobiose-Coated Polyamidoamine (PAMAM) Dendrimers to Study Accessibility of an Enzyme, Cellodextrin Phosphorylase

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To examine the accessibility of the enzyme cellodextrin phosphorylase (CDP) towards multivalent cluster carbohydrates, the cellobiosylated dimer **10** and trimer **12**, as well as the cellobiose-coated PAMAM dendrimers **14**, **16**, **18**, **20** and **22**, with four, eight, sixteen, thirty-two and sixty-four cellobiose units at the outer surface of PAMAM dendrimers, respectively, have been synthesized for the first time and used as acceptor substrates for the enzyme CDP. It was found that CDP was able to transfer a glucosyl moiety from glucose-1-phosphate (Glc-1-P) into these synthesized cluster cellobiosylated glycoconjugates and cellobiose-coated PAMAM dendrimers, which were thus acceptor substrates for CDP. It was found that the ability of CDP to interact with smaller cellobiosylated glyconjugates and with PAMAM dendrimers containing up to eight cellobiose units was similar to that seen with cellobiose. However, this capability of CDP was somewhat lessened with the PAMAM dendrimer containing sixteen cellobiose moieties and dramatically decreased towards PAMAM dendrimers with thirty-two and sixty-four cellobiose units. This might be due to their steric bulk, CDP enzyme no longer being able to hold them properly on its active site.

Introduction

Phosphorylase-mediated enzymatic synthesis in carbohydrates is well documented.[1] Cellodextrin phosphorylase (CDP), a β-1,4-oligoglucan orthophosphate glucosyltransferase,[2] has been found to be very effective for the synthesis of derivatives of cellooligosaccharides, requiring cellobiose or various β-glucosides (laminaribiose, phenyl-β-D-glucoside, etc.) as the smallest substrate for the elongation of the sugar chain to form a new β-1,4-glucosyl linkage.[2] Study on CDP to date is limited to the application of monovalent cellobiosylated substrate.

Multivalent carbohydrate ligands are expected to be very promising in terms of carbohydrate-protein interactions. To explore the effect of multivalency, carbohydrate-based dendrimers[3,4] have been synthesized and the importance of multivalent sugar residues has been described.[4]

Poly(amidoamine) (PAMAM) dendrimers,[5] on the other hand, are highly branched macromolecules containing amino groups on their surfaces. Their unique structural shapes, as well as their cytotoxic natures,[6] have encouraged scientists to produce PAMAM dendrimer-based synthetic macromolecules for biochemical, biophysical and medicinal applications.[6−8] It has been demonstrated that the synthetic persubstituted PAMAM dendrimers are able to improve drug delivery systems.[6] Similarly, sugar-persubstituted PAMAM dendrimers[8] exhibited binding affinities towards carbohydrate binding proteins several times higher than those of monovalent ligands.

Syntheses of persubstituted PAMAM dendritic sugar balls containing glucosyl,[9a,9b] glucoaminosyl,[9a,9b] lactones of lactose[9c] and maltose[9c] moieties have been described by Okada and co-workers.[9] Lindhorst et al.[10] and Cloninger et al.[11] have reported mannosyl-persubstituted glycoPAMAM dendrimers,[10,11] whereas Roy et al.[8,12] have synthesized mannosyl-,[12a] lactosyl-,[12b] sialosyl-[8] and T-antigen-persubstituted[12c] glycoPAMAM dendrimers by coupling with the respective glycosyl donors and the free amines of the PAMAM dendrimers. Glucosyl-, lactosyl-, and sialosyl-persubstituted aminoxy PAMAM dendrimers have also been reported.[13]

There is, however, no report on cellobiose-persubstituted PAMAM dendrimers. So far, only Lindhorst et al.[10] have reported a triantennary cellobioside, obtained by coupling of an isothiocyanate derivative of cellobiose with tris(aminoethyl)amine.

The cluster effects of carbohydrates for the recognition of proteins are well established.[4,8,12c] However, the effect of the population density of carbohydrates on recognition of enzymes as substrates has not been well studied. Enzymatic chain elongation of carbohydrates on dendrimers is considered to be useful in preparation of bioactive glycoconjugates, so it is important to examine the accessibility of the enzyme towards the multivalent cluster carbohydrates.

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Since CDP, obtained from *Clostridium thermocellum*,[14] was found to be very useful for the synthesis of cellooligosaccharides[14] through the use of cellobiose as the smallest substrate, we focussed on synthesizing cellobiosylated dimer, trimer and cellobiose-coated PAMAM dendrimers as model substrates to examine the ability of the enzyme CDP to interact with those multivalent cellobiosylated glycoconjugate clusters.

PAMAM dendrimers of generations 0, 1, 2, 3 and 4 contain 4, 8, 16, 32 and 64 amino groups on their surfaces, respectively. To examine the effectiveness of the enzyme CDP, a cellobiosylated dimer and trimer, as well as cellobiose-coated PAMAM dendrimers possessing 4, 8, 16, 32 and 64 cellobiose units at the PAMAM dendrimer outer surface, were synthesized by conjugation of dendrimer free amine with the free carboxylic acid moiety attached at the reducing end of cellobiose in the presence of BOP[15] reagent and used as acceptor substrates for that enzyme.

To the best of our knowledge, this report describes for the first time both the syntheses of multivalent cellobiosylated glycoconjugates and the ability of the enzyme CDP to interact with those synthesized cluster cellobiosylated glycoconjugates.[16]

Results and Discussion

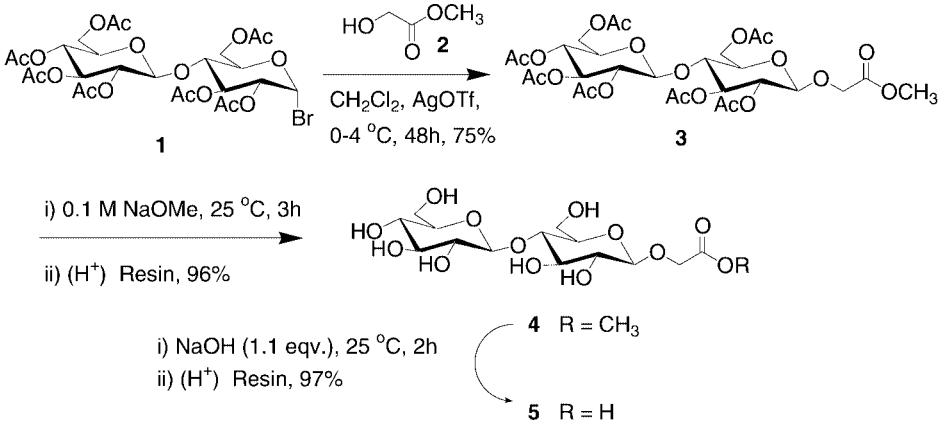
Synthesis

The strategy adopted for the syntheses of the desired cellobiose-coated PAMAM dendrimers of generations 0, 1, 2, 3 and 4 was firstly to synthesize carboxymethyl β-D-cellobioside (**5**), with a free carboxylic acid attached at the reducing end of cellobiose through the smallest possible spacer. Methoxycarbonylmethyl β-D-cellobioside **5** was thus synthesized for the first time, as described in Scheme 1.

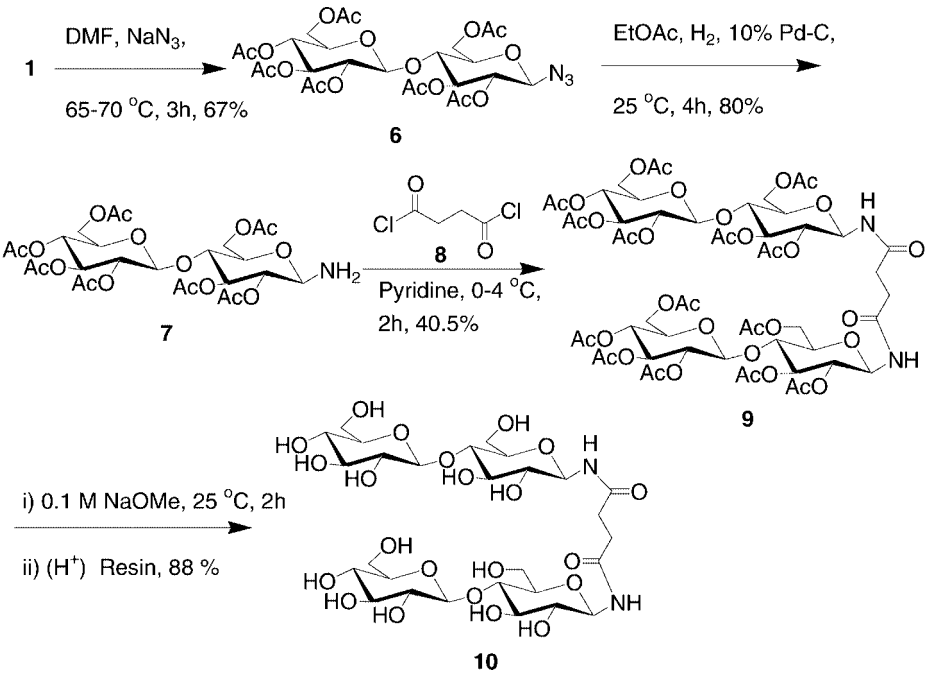
Treatment of the known hepta-*O*-acetyl-α-D-cellobiosyl bromide (**1**)[17] and the commercially available methyl glycolate (**2**) in the presence of silver triflate afforded the desired methoxycarbonylmethyl hepta-*O*-acetyl β-D-cellobioside (**3**) in 75% yield. Zemplen de-*O*-acetylation of compound **3** afforded methyl ester **4** in 96% yield. Finally, hydrolysis of methyl ester **4** with 1.1 equivalents of NaOH afforded the desired carboxylic acid **5** in 97% yield. Compounds **3**, **4** and **5** were confirmed by their ^{1}H NMR and ^{13}C NMR spectra as well as by their FAB and high-resolution ESI mass spectra. The assignment of compound **5** was based on its HMBC spectrum. The β-glycosidic linkages of compound **5** were confirmed by the presence of characteristic peaks at δ = 4.44 ppm (J = 7.9 Hz) for H-1 and 4.45 ppm (J = 7.9 Hz) for H-1′ in the ^{1}H NMR spectrum and peaks at δ = 102.5 ppm for C-1 and 103.0 for C-1′ in the ^{13}C NMR spectrum.

Besides cellobiose-coated PAMAM dendrimers, the divalent cellobiose derivative **10**, with a heteroatom attached at the reducing end of cellobiose, and the triantennary cellobiose derivative **12** were designed and synthesized as the smallest possible multivalent acceptor substrates for the enzyme cellodextrin phosphorylase.

The desired β-D-cellobiosyl dimer **10** was obtained in four steps, starting from the known hepta-*O*-acetyl-α-D-cellobiosyl bromide[17] (**1**) as shown in Scheme 2. Firstly, the known hepta-*O*-acetyl-β-D-cellobiosyl azide[17] (**6**) was synthesized by treatment of **1** with sodium azide at 65−70 °C in anhydrous DMF for 3 h, and was obtained in 67% yield. Both the ^{1}H and the ^{13}C NMR spectra of **6** were similar to those reported.[17a] The azido group of compound **6** was reduced to provide the desired amine **7** in 80% yield by catalytic hydrogenation on 10% Pd-C in ethyl acetate as solvent. The free amine **7** was then treated with succinoyl chloride (**8**) in anhydrous pyridine at 0 °C for 2 h to afford the peracetylated β-D-cellobiosylamine dimer **9** in 74% yield. Deacetylation of compound **9** by Zemplen's method afforded the desired cellobiosyl dimer **10** in 88% yield. Compounds **7**, **8**, **9** and **10** were characterized by their ^{1}H and ^{13}C NMR spectra as well by their FAB and high-resolution ESI mass spectra. The characteristic peaks at δ = 4.43 ppm (J = 7.9 Hz) for H-1 and 4.90 ppm (J = 9.2 Hz) for H-1′ in the ^{1}H NMR spectrum of compound **10**, as well as peaks at δ = 79.1 ppm for C-1 (anomeric *C*−NH−CO) and 102.5 ppm for C-1′ in the ^{13}C NMR spectrum, confirmed its β-glycosidic linkages. The ^{13}C NMR spectroscopic data for the anomeric β-*C*-NHCO in compound **10** were satisfac-



Scheme 1. Synthesis of carboxymethyl β-D-cellobioside (**5**)



Scheme 2. Synthesis of *N*,*N*-bis(β-D-cellobiosyl)succinamide (**10**)

torily consistent with values reported for other sugar derivatives.[18] Compound **10** was further verified by gel-permeation HPLC, showing only a single peak with a retention time of 20.969 min, as shown in Table 1. The conditions for gel-permeation HPLC have been described in a general procedure for the synthesis of cellobiose-coated PAMAM dendrimers of generations 0, 1, 2, 3 and 4.

Table 1. Retention times (min) of gel-permeation HPLC chromatograms

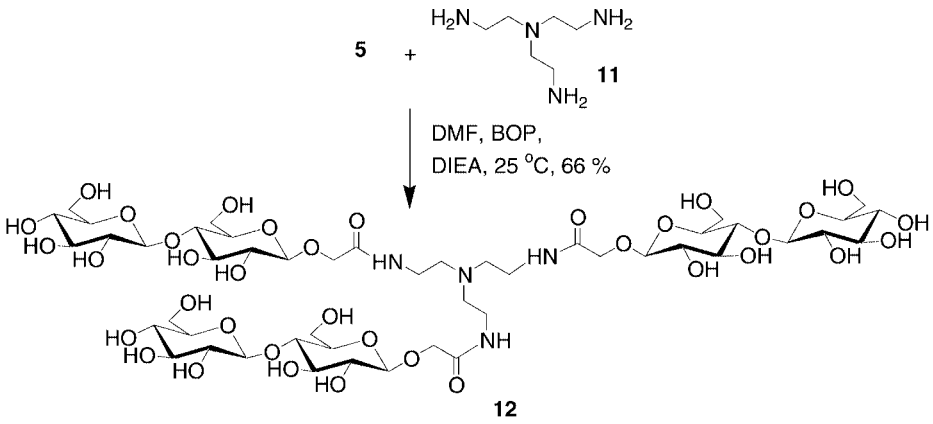
Compounds	Retention time of products (min)
Compound **10**	20.97
Compound **12**	20.49
Compound **14**	19.94 (22.21)[a]
Compound **16**	18.78 (20.60)[a]
Compound **18**	17.49 (19.38)[a]
Compound **20**	16.51 (18.20)[a]
Compound **22**	15.02 (17.02)[a]

[a] Values in parentheses are the retention times (min) for starting PAMAM Dendrimers

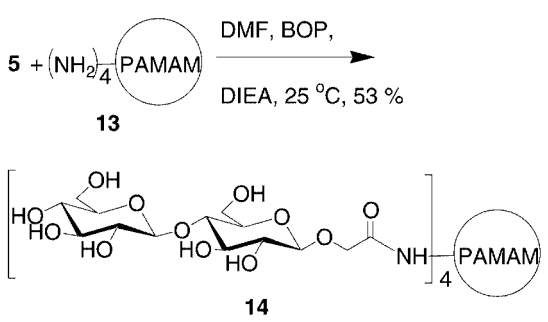
Cellobiosyl trimer **12** was synthesized in one step (Scheme 3) by coupling of **5** with commercially available tris-(aminoethyl)amine (**11**) in anhydrous DMF at 25 °C for 16 h in the presence of BOP[15] {[(benzotriazol-1-yloxy-tris-(dimethylamino)]phosphonium hexafluorophosphate}, and was obtained in 64% yield. The selection of BOP for coupling was based on its previous usefulness even in the synthesis of complex carbohydrate derivatives.[19] Compound **12** was purified by column chromatography on silica gel by use of 2:1 and 1:1 acetonitrile/water as eluent, followed by passage of the solvent through a Sep-Pak Plus environmental cartridge (Waters, USA), and was assigned by its ^{1}H NMR and ^{13}C NMR spectra and also by its high-resolution ESI mass spectra. Compound **12** was further checked by gel-permeation HPLC and showed only a single peak with a retention time of 20.490 min. The characteristic peaks at δ = 4.51 ppm (J = 8.0 Hz) for H-1 and 4.60 ppm (J = 7.8 Hz) for H-1′ in the ^{1}H NMR spectrum as well as peaks at δ = 102.7 ppm for C-1, 102.9 ppm for C-1′ and 172.2 ppm for NH*C*OCH$_2$ in the ^{13}C NMR spectrum confirmed the presence both of the β-glycosidic linkages and of the amide bond, respectively.

Cellobiose-coated PAMAM dendrimer of generations 0, 1, 2, 3, and 4 were each synthesized in one step, as outlined in Schemes 4−8.

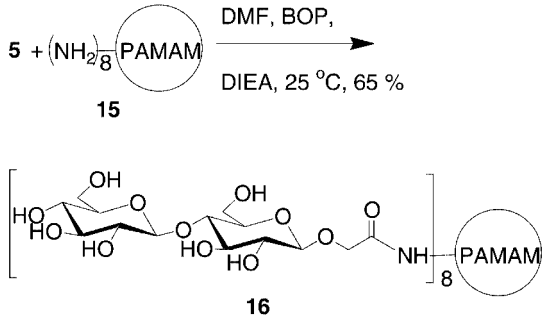
Commercially available methanolic solutions of PAMAM dendrimers of generations 0 (**13**), 1 (**15**), 2 (**17**), 3 (**19**) and 4 (**21**) were coupled with **5** (2 equivalents per amine unit) in anhydrous DMF at 25 °C for 2−21 h. The products were purified by passage through a mixed ion (50−50 cation and anion) exchange resin column and obtained in 53−76% yields. The desired cellobiose-coated PAMAM dendrimers **14**, **16**, **18**, **20** and **22** were further verified by gel-permeation HPLC, only a single peak being found in the gel-permeation HPLC chromatogram for each product. The retention times of the products are summarized in Table 1 and were readily distinguishable from the retention times of the corresponding starting PAMAM dendrimers. The synthesized PAMAM dendrimers **14**, **16**, **18**, **20** and **22** were assigned by the presence of characteristic peaks in their ^{1}H NMR and ^{13}C NMR spectra. MALDI-TOF mass spectra showed masses of 2046.6±1.0 [M + H]$^+$ for compound **14** (molecular mass of **14**: 2044.8), 4505±5 [M + Na]$^+$ for compound **16** (molecular mass of **16**: 4485.9), 9397±7 [M + Na]$^+$for compound **18** (molecular mass of **18**: 9368.1),



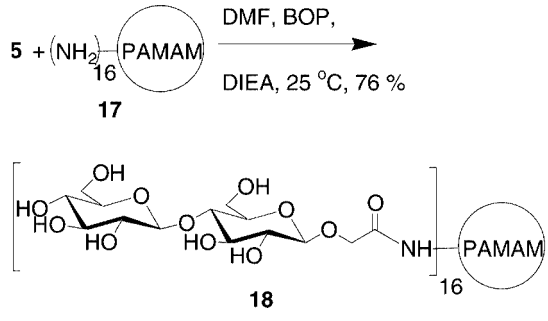
Scheme 3. Synthesis of tris(aminoethyl *N*-carbonylmethyl β-D-cellobiosyl)amine **(12)**



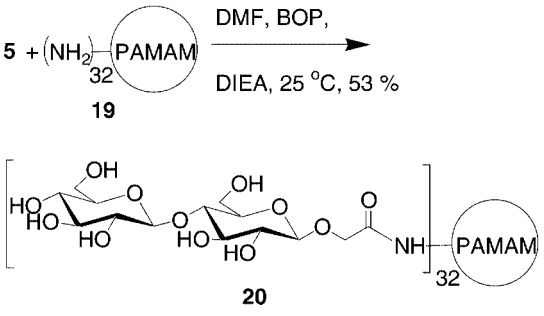
Scheme 4. Synthesis of tetrameric β-D-cellobiosyl PAMAM dendrimer **14**



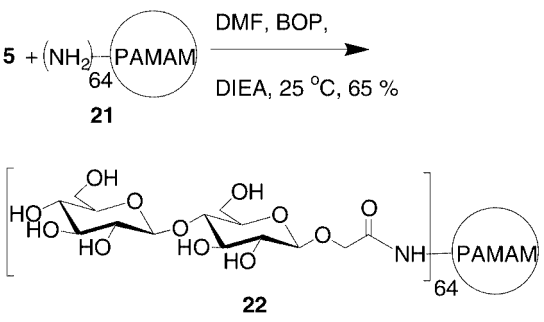
Scheme 5. Synthesis of octameric β-D-cellobiosyl PAMAM dendrimer **16**



Scheme 6. Synthesis of hexadecameric β-D-cellobiosyl PAMAM dendrimer **18**



Scheme 7. Synthesis of 32-meric β-D-cellobiosyl PAMAM dendrimer **20**



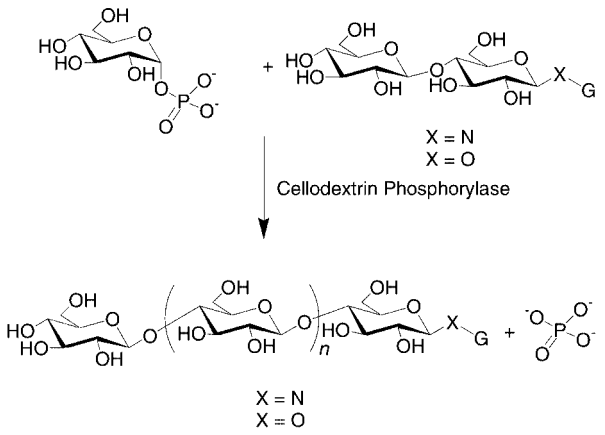
Scheme 8. Synthesis of 64-meric β-D-cellobiosyl PAMAM dendrimer **22**

and 19166±15 $[M + Na]^+$ for compound **20** (molecular mass of **20**: 19132). The MALDI-TOF method failed to obtain the mass spectrum of compound **22** (molecular mass of **22,** $C_{1518}H_{2656}O_{892}N_{250}$: 38661).

Acceptor Activities of Cellobiosylated Dimer and Trimer and Cellobiose-Coated PAMAM Dendrimers

The following experiment was designed for the determination of the substrate specificities of cellodextrin phosphorylase with use of the multivalent cellobiosylated glycoconjugates described above as acceptor substrates. The en-

zymatic reaction in general is described in Scheme 9, where "G" represents other groups present in the molecule.



Scheme 9. Enzyme reaction

During the CDP reaction, Glc-1-P was quantified by the phosphoglucomutase/glucose-6-phosphate dehydrogenase method.[16] The reaction mixture, containing aqueous glucose-1-phosphate, aqueous substrate and a 50 mM MOPS buffer solution of CDP, was incubated at 37 °C. At certain intervals from 0 h to 120 h, aliquots were removed and the remaining Glc-1-P was quantified by the above method, followed by measurement of the absorbance at 340 nm. The percentage of remaining Glc-1-P was calculated in each case and plotted against time (h), and the results are summarized in Figure 1.

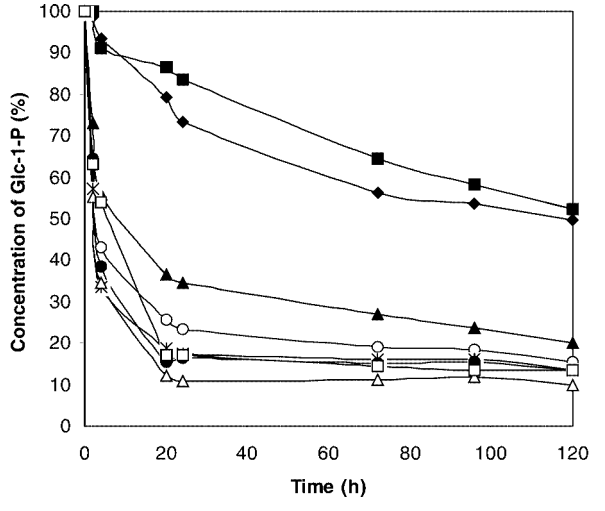


Figure 1. Time course of the CDP reaction with cellobiosylated dimer and trimer and cellobiose-coated PAMAM dendrimers. (◆) 64-mer (**22**); (■) 32-mer (**20**); (▲) 16-mer (**18**); (○) octamer (**16**); (*) tetramer (**14**); (●) trimer (**12**); (△) dimer (**10**) and (□) cellobiose

Figure 1 clearly indicates that the enzyme cellodextrin phosphorylase was able to transfer glucosyl moieties from glucose-1-phosphate to the synthesized cellobiosylated dimer **10** and trimer **12** and to the cellobiose-coated PAMAM dendrimers containing four (**14**), eight (**16**), 16 (**18**), 32 (**20**) and 64 (**22**) cellobiose units, and hence that these were acceptor substrates for that enzyme. It was found that the accessibility of CDP towards smaller cellobiosylated glyconjugates and up to eight cellobiose units containing PAMAM dendrimers as substrates was not significantly different than cellobiose. However, the accessibility of CDP was slowly decreased toward sixteen cellobiose-coated PAMAM dendrimer **16** and dramatically decreased towards thirty-two **20** and sixty-four **22** cellobiose units containing PAMAM dendrimers. This might be due to their sterically hindered sizes, enzyme CDP no longer being able to hold them properly on its active site. The rates for 32-cellobiose-coated PAMAM dendrimer **20** and 64-cellobiose-coated PAMAM dendrimer **22** were practically identical, indicating that the critical difference in the accessibility of CDP was found only between 16 and 32 cellobiosyl units.

From this study, we conclude that the smaller cellobiosylated glyconjugates and the PAMAM dendrimers containing up to eight cellobiose units are, thanks to their sterically less bulky sizes, readily available to the active site of CDP for transfer of the glucosyl residue from Glc-1-P, relative to their sterically more bulky PAMAM dendrimer homologues with sixteen, thirty-two and sixty cellobiose units (**18, 20** and **22**). Further evaluation of the results should be possible once the three-dimensional structure of CDP has been determined.

Experimental Section

General Methods: All reactions were carried out under nitrogen in oven-dried glassware. Methyl glycolate, silver triflate, and PAMAM dendrimers of generations 0, 1, 2, 3 and 4 were purchased from Sigma, Aldrich Chemicals (USA). Anhydrous CH_2Cl_2 and anhydrous DMF were purchased from Wako Chemicals (Japan). Diisopropylethylamine was purchased from Nacalai Tesque Chemicals (Japan) and was used directly without further distillation. Anhydrous sodium chloride and sodium dihydrogen phosphate were purchased from Nacalai Tesque Chemicals (Japan). Thin-layer chromatography was performed on 60 F_{254} plates (E. Merck) and results were viewed by charring with 5% sulfuric acid in methanol. Silica gel 60 (230−400 mesh) was used for column chromatography. Gel permeation HPLC was performed on a TSKGel G2000 SWXL (7.8 mm I.D. × 30 cm) HPLC column purchased from TOSOH, Japan. Detection was at 220 nm and 50 mM sodium dihydrogen phosphate buffer of pH 7.0 containing 0.3 M NaCl per liter was used as eluent, with a flow rate of 0.5 mL/min. Phosphoglucomutase and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemicals (USA). Cellodextrin phosphorylase used in this study was obtained from *Clostridium thermocellum*.[14]

^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C with a Bruker DRX 600 NMR instrument. Proton NMR shifts are reported in ppm with use of TMS (δ = 0 ppm) as reference standard for $CDCl_3$ solutions and δ = 4.70 ppm for D_2O solutions. Carbon NMR shifts were reported in ppm with use of δ = 77.0 ppm as reference standard for $CDCl_3$ solutions. ^{13}C NMR spectra for D_2O solutions were measured by fixing of the HOD peak at δ = 4.70 ppm followed by measurement of the ^{13}C NMR spectra, so carbon NMR shifts for D_2O solutions are reported in ppm without any reference standards. FAB and ESI mass spectra were measured

with a Bruker APEX II 70e mass instrument. MALDI-TOF mass spectra were recorded with a Bruker REFLUX II mass instrument using retinoic acid as matrix. A Beckman DU 650 Spectrophotometer, USA, was used for spectrophotometric measurements.

Methoxycarbonylmethyl Hepta-*O*-acetyl-β-D-cellobioside (3): A mixture of hepta-*O*-acetyl β-D-cellobiosyl bromide (**1**, 1.5 g, 2.15 mmol), methyl glycolate (**2**, 0.18 mL, 2.33 mmol) and molecular sieves (4 Å, 1 g) in CH_2Cl_2 (20 mL) was stirred at 0−4 °C for 30 min. Silver triflate (600 mg, 2.33 mmol) was then added. The reaction mixture was stirred at 0−4 °C for 48 h and then diluted with CH_2Cl_2 (20 mL). The insoluble solids were separated by filtration through a Celite bed and washed twice with CH_2Cl_2 (10 mL × 2). The combined organic solution was washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel column (22 × 3 cm). Elution with ethyl acetate/hexane (1:1, 2:1 and 4:1) afforded **3** as white solid. Yield 1.15 g (75%); silica gel TLC R_f = 0.38 (ethyl acetate/hexane, 2:1). ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 1.98−2.13 (7s, 21 H, OCOCH_3), 3.54−3.62 (m, 1 H), 3.63−3.69 (m, 1 H), 3.74 (s, 3 H, COOCH_3), 3.80 (t, *J* = 9.8 Hz, 1 H), 3.99−4.13 (m, 2 H), 4.25 (d, *J* = 1.0 Hz, 2 H, OCH_2-COOCH_3), 4.36 (dd, *J* = 12.5, *J* = 4.5 Hz, 1 H), 4.51 (d, *J* = 8.0 Hz, 1 H, H-1), 4.53 (dd, *J* = 12.0, *J* = 2.1 Hz, 1 H), 4.61 (d, *J* = 7.8 Hz, 1 H, H-1′), 4.88−4.98 (m, 2 H), 5.06 (t, *J* = 9.9 Hz, 1 H), 5.14 (t, *J* = 9.4 Hz, 1 H) and 5.21 (t, *J* = 9.3 Hz, 1 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): δ = 20.5−20.8 (OCO*C*H_3), 52.0 (COO*C*H_3), 61.5 (C-6′), 61.6 (C-6), 65.1 (O*C*H_2COOCH_3), 67.8 (C-4), 71.2, 71.6, 72.0, 72.1, 72.8, 72.9, 75.3, 100.0 (C-1), 100.7 (C-1′) and 169.0−170.5 (O*C*OCH$_3$) ppm. MS (FAB): *m*/*z* = 709.29 [M + H]$^+$ ($C_{29}H_{41}O_{20}$ requires 709.2185), ESI-MS: *m*/*z* = 731.1997 [M + Na]$^+$ ($C_{29}H_{40}O_{20}Na$ requires 731.2005).

Methoxycarbonylmethyl β-D-Cellobioside (4): NaOMe solution (0.1 N, 5 mL) was added to a solution of **3** (1 g, 1.41 mmol) in anhydrous MeOH/$CHCl_3$ (5:1, 6 mL). The reaction mixture was stirred at 25 °C for 3 h and neutralized with Dowex 50-WX8 (H^+) cation-exchange resin. The solution was filtered and the solvents were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (15 × 3 cm). Elution with methanol/ethyl acetate (1:1 and 2:1) afforded **4** as a white foam. Yield 560 mg (96%); silica gel TLC R_f = 0.58 (methanol/ethyl acetate, 2:1). ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 3.19−3.26 (m, 1 H), 3.29−3.35 (m, 2 H), 3.37−3.44 (m, 2 H), 3.46−3.51 (m, 1 H), 3.52−3.61 (m, 2 H), 3.62−3.67 (m, 1 H), 3.69 (s, 3 H, COOCH_3), 3.70−3.76 (m, 1 H), 3.80−3.90 (m, 2 H), 4.23−4.40 (m, 2 H), 4.42 (d, *J* = 7.9 Hz, 1 H, H-1) and 4.46 (d, *J* = 9.5 Hz, 1 H, H-1′) ppm. ^{13}C NMR (150 MHz, D_2O, 25 °C): δ = 51.5 (COO*C*H_3), 58.8 (C-6′), 59.5 (C-6), 65.1 (O*C*H_2COOCH_3), 68.4 (C-4), 71.7, 72.1, 73.1, 73.8, 74.4, 74.9, 77.3, 100.9 (C-1), 101.5 (C-1′) and 171.3 (*C*O-OCH$_3$) ppm. MS (FAB): *m*/*z* = 415.15 [M + H]$^+$ ($C_{15}H_{27}O_{13}$ requires 415.1452), ESI-MS: *m*/*z* = 437.1267 [M + Na]$^+$ ($C_{15}H_{26}O_{13}Na$ requires 437.1265).

Carboxymethyl β-D-Cellobioside (5): NaOH (16 mg, 0.4 mmol) in H_2O (100 mL) was added to a solution of **4** (160 mg, 0.386 mmol) in MeOH/H_2O (3:1, 3 mL). The reaction mixture was stirred at 25 °C for 2 h and then neutralized with Dowex 50-WX8 (H^+) cation-exchange resin. The solution was filtered and the solvents were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (6 × 3 cm). Elution with methanol/ethyl acetate (2:1) and methanol afforded **5** as a white solid. Yield 150 mg (97%); silica gel TLC R_f = 0.21 (methanol/ethyl acetate, 2:1). ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 3.21−3.27 (m, 1 H, H-2′), 3.31−3.38 (m, 2 H, H-2, H-5′), 3.39−3.47 (m, 2 H, H-5, H-6′), 3.48−3.54 (m, 1 H, H-6′), 3.55−3.63 (m, 2 H, H-6, H-4), 3.64−3.70 (m, 1 H, H-6), 3.72−3.78 (m, 1 H, H-4′), 3.82−3.87 (m, 1 H, H-3), 3.88−3.92 (m, 1 H, H-3′), 4.06 (d, *J* = 15.6 Hz, 1 H, OCH_2COOH), 4.24 (d, *J* = 15.6 Hz, 1 H, OCH_2COOH); 4.44 (d, *J* = 7.9 Hz, 1 H, H-1), 4.45 (d, *J* = 7.9 Hz, 1 H, H-1′) ppm. ^{13}C NMR (150 MHz, D_2O, 25 °C): δ = 60.3 (C-6′), 60.9 (C-6), 68.7 (O*C*H_2COOH), 69.8 (C-4), 73.3, 73.5, 74.5, 75.2, 75.9, 76.4, 78.9, 102.5 (C-1), 103.0 (C-1′) and 177.4 (*C*OOH) ppm. MS (FAB): *m*/*z* = 423.11 [M + Na]$^+$. ESI-MS: *m*/*z* = 423.1116 [M + Na]$^+$ ($C_{14}H_{24}O_{13}Na$ requires 423.1109).

Hepta-*O*-acetyl-β-D-cellobiosyl Azide (6): NaN_3 (184 mg, 2.83 mmol) was added to a solution of **1** (1.8 g, 2.57 mmol) in anhydrous DMF (10 mL). The reaction mixture was stirred at 65−70 °C for 3 h. The insoluble materials were filtered through a Celite bed and washed twice with ethyl acetate (10 mL × 2). The combined organic solution was washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residual DMF was removed by co-evaporation with toluene (10 mL × 3). The residue was purified by column chromatography on a silica gel column (20 × 3 cm). Elution with ethyl acetate/hexane (1:1 and 2:1) afforded **6** as white solid: yield 1.14 g (67%); silica gel TLC R_f = 0.66 (ethyl acetate/hexane, 2:1). The ^{1}H and ^{13}C NMR spectra of compound **6** were identical with those reported.[17a]

Hepta-*O*-acetyl-β-D-cellobiosylamine (7): A mixture of **6** (1.1 g, 1.66 mmol) and Pd-C (10%, 165 mg) in anhydrous ethyl acetate (10 mL) was stirred under H_2 at 25 °C for 4 h. The reaction mixture was filtered through a Celite bed and washed twice with ethyl acetate/ethanol (4:1, 10 mL × 2). The combined organic solution was evaporated under reduced pressure. The residue was purified by column chromatography on a silica gel column (15 × 3 cm). Elution with ethyl acetate followed by ethyl acetate/acetone (10:1 and 1:1) afforded **7** as white powder: yield 850 mg (80%); silica gel TLC R_f = 0.35 (ethyl acetate/acetone, 10:1). ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C): δ = 1.88−1.96 (m, 2 H, NH_2), 1.98−2.13 (7s, 21 H, OCOCH_3), 3.56−3.61 (m, 1 H), 3.63−3.68 (m, 1 H), 3.71 (t, *J* = 9.9 Hz, 1 H), 4.01−4.10 (m, 2 H), 4.11−4.20 (m, 1 H, H-1), 4.36 (dd, *J* = 12.5, *J* = 4.4 Hz, 1 H), 4.46 (dd, *J* = 12.6, *J* = 2.0 Hz, 1 H), 4.50 (d, *J* = 8.0 Hz, 1 H, H-1′), 4.74 (dd, *J* = 9.8, *J* = 9.0 Hz, 1 H), 4.92 (dd, *J* = 9.4, *J* = 8.0 Hz, 1 H), 5.07 (t, *J* = 12.5 Hz, 1 H), 5.14 (t, *J* = 9.3 Hz, 1 H), 5.22 (t, *J* = 9.2 Hz, 1 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): δ = 20.5−20.9 (OC-O*C*H_3), 61.6 (C-6′), 62.2 (C-6), 67.8 (C-4), 71.6, 71.9, 72.4, 72.7, 73.0, 73.7, 76.8, 84.7 (C-1), 100.8 (C-1′) and 169.0−170.5 (O*C*-OCH$_3$) ppm. MS (FAB): *m*/*z* = 636.28 [M + H]$^+$, ESI-MS: *m*/*z* = 636.2128 [M + H]$^+$ ($C_{26}H_{38}O_{17}N$ requires 636.2134).

***N*,*N*-Bis(hepta-*O*-acetyl-β-D-cellobiosyl)succinamide (9):** Compound **7** (465 mg, 0.73 mmol) was dissolved in anhydrous pyridine (3 mL) and the mixture was stirred at 0−4 °C for 15 min. A solution of succinoyl chloride (**8**, 44 mL, 0.4 mmol) in anhydrous $CHCl_3$ (1 mL) was then added dropwise and the reaction mixture was stirred at 0−4 °C for 2 h. The solution was evaporated to dryness and the residual pyridine was removed by co-evaporation with toluene (5 mL × 3). The residue was dissolved in ethyl acetate (20 mL) and washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel column (18 × 3 cm). Elution with ethyl acetate followed by ethyl acetate/acetone (10:1 and 1:1) afforded **9** as white foam. Yield 400 mg (40.5%); silica gel TLC R_f = 0.45 (ethyl acetate/acetone, 10:1). ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C):

δ = 1.98−2.13 (7s, 42 H, OCOCH_3), 2.33−2.42 (m, 2 H, NHCOCH_2), 2.46−2.57 (m, 2 H, NHCOCH_2), 3.62−3.71 (m, 4 H), 3.74 (t, *J* = 9.8 Hz, 2 H), 4.04 (dd, *J* = 12.4, *J* = 2.3 Hz, 2 H, H-1), 4.08−4.16 (m, 2 H), 4.37 (dd, *J* = 12.6, *J* = 4.5 Hz, 2 H), 4.45 (dd, *J* = 12.3, *J* = 1.9 Hz, 2 H), 4.50 (d, *J* = 7.9 Hz, 2 H, H-1′), 4.83 (t, *J* = 9.6 Hz, 2 H), 4.92 (dd, *J* = 9.3, *J* = 7.9 Hz, 2 H), 5.06 (t, *J* = 9.5 Hz, 2 H), 5.11−5.18 (m, 4 H), 5.26 (t, *J* = 9.2 Hz, 2 H), 6.32 (d, *J* = 9.2 Hz, 2 H, N*H*COCH$_2$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$, 25 °C): δ = 20.5−20.9 (OCO*C*H$_3$), 30.6 (NHCO*C*H$_2$), 61.6 (C-6), 61.8 (C-6′), 67.8 (C-4), 70.7, 71.6, 72.0, 72.1, 72.9, 74.5, 76.3, 78.1 (C-1), 100.7 (C-1′) and 169.0−171.7 (O*C*OCH$_3$) ppm. MS (FAB): *m*/*z* = 1353.32; ESI-MS: *m*/*z* = 1353.4248 $[M + H]^+$ ($C_{56}H_{77}O_{36}N_2$ requires 1353.4250).

***N*,*N*-Bis(β-D-cellobiosyl)succinamide (10):** NaOMe solution (0.1 N, 2 mL) was added to a solution of **9** (200 mg, 0.148 mmol) in anhydrous MeOH/$CHCl_3$ (4:1, 2.5 mL). The reaction mixture was stirred at 25 °C for 2 h and was then neutralized with Dowex 50-WX8 (H^+) cation-exchange resin. The solution was diluted with water and filtered to remove the cation-exchange resin. The filtrate was concentrated under reduced pressure and the residue was dissolved in water and re-precipitated with MeOH and filtered. The precipitates were dissolved in water and lyophilized to afford **10** as white foam. Gel-permeation HPLC of the product **10** showed only a single peak, with a retention time of 20.969 min: Detection at 220 nm. Yield 100 mg (88%). ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 2.50−2.64 (m, 4 H), 3.20−3.27 (m, 2 H), 3.30−3.37 (m, 4 H), 3.37−3.45 (m, 4 H), 3.53−3.62 (m, 6 H), 3.63−3.68 (m, 2 H), 3.69−3.77 (m, 2 H), 3.79−3.89 (m, 4 H), 4.43 (d, *J* = 7.9 Hz, 1 H, H-1), 4.90 (d, *J* = 9.2 Hz, 1 H, H-1′) ppm. ^{13}C NMR (150 MHz, D_2O, 25 °C): δ = 30.3 (NHCO*C*H$_2$), 59.8 (C-6), 60.6 (C-6′), 69.4 (C-4), 71.6, 73.1, 75.0, 75.5, 76.0, 76.3, 78.1, 79.1 (C-1), 102.5 (C-1′) and 176.0 (NH*C*OCH$_2$) ppm. MS (FAB): *m*/*z* = 765.30, ESI-MS: *m*/*z* = 765.2755 $[M + H]^+$ ($C_{28}H_{49}O_{22}N_2$ requires 765.2771).

Tris[2-({[(β-cellobiosyl)methyl]carbonyl}amino)ethyl]amine (12): A mixture of **5** (70 mg, 0.175 mmol), tris(aminoethyl)amine (**11**, 7.3 mL, 0.035 mmol) and diisopropylethylamine (61 mL, 0.35 mmol) in anhydrous DMF (3 mL) was stirred at 25 °C for 30 min, and BOP reagent (77 mg, 0.175 mmol) was then added. The reaction mixture was stirred at 25 °C for 16 h and was then diluted with ethyl acetate/methanol (3:1). The precipitates were separated by filtration, washed twice with ethyl acetate/methanol (3:1) and dissolved in water. The aqueous solution was concentrated under reduced pressure. The residue was purified by column chromatography on a silica gel column (8 × 1 cm) by elution with acetonitrile/water (2:1 and 1:1). The solvent was evaporated and the residue was dissolved in water, passed through a Sep-Pak Plus C18 environmental cartridge (Waters, USA) to remove insoluble solids, and finally lyophilized to afford **12** as a white foam. Gel-permeation HPLC of the product **12** showed only a single peak with a retention time of 20.490 min: detection at 220 nm. Yield 30 mg (66%); silica gel TLC R_f = 0.38 (2:1 acetonitrile-water). ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 2.62−2.72 (m, 6 H), 3.20−3.25 (m, 3 H), 3.26−3.37 (m, 12 H), 3.38−3.45 (m, 6 H), 3.48−3.52 (m, 3 H), 3.53−3.61 (m, 6 H), 3.62−3.68 (m, 3 H), 3.71−3.76 (m, 3 H), 3.81−3.84 (m, 3 H), 3.85−3.91 (m, 3 H), 4.18 (d, *J* = 15.6 Hz, 3 H), 4.29 (d, *J* = 15.7 Hz, 3 H), 4.42 (d, *J* = 7.9 Hz, 3 H, H-1) and 4.43 (d, *J* = 8.0 Hz, 3 H, H-1′) ppm. ^{13}C NMR (150 MHz, D_2O, 25 °C): δ = 36.9 (N*C*H$_2$CH$_2$NH), 52.5 (CONH*C*H$_2$CH$_2$NH), 60.3 (C-6), 61.0 (C-6′), 68.6 (O*C*H$_2$CO), 69.8 (C-4), 73.1, 73.5, 74.5, 75.3, 75.9, 76.4, 78.8, 102.7 (C-1), 103.0 (C-1′) and 172.3 (CH$_2$*C*ONH) ppm. MS ESI-MS: *m*/*z* = 1293.4932 $[M + H]^+$ ($C_{48}H_{85}O_{36}N_4$ requires 1293.4938).

General Procedure for the Synthesis of Cellobiose-Coated PAMAM Dendrimers of Generations 0, 1, 2, 3 and 4: A mixture of a methanolic solution of the PAMAM dendrimer of generation 0, 1, 2, 3 or 4, carboxymethyl β-D-cellobioside **5** (2 equivalents per free amine) and diisopropylamine (2 equivalents per carboxylic acid) in anhydrous DMF was stirred at 25 °C for 30 min, and BOP reagent (1 equivalent per carboxylic acid) was then added. The reaction mixture was stirred at 25 °C for 2−21 h and during that time − for generations 2, 3 and 4 − the products were precipitated. The mixture was diluted with ethyl acetate/methanol (3:1). The precipitates were separated by filtration, washed twice with ethyl acetate/methanol (3:1) and dissolved in water. The aqueous solution was concentrated under reduced pressure. The residue was dissolved in water and passed through a mixed ion (50−50 cation and anion) exchange resin column (8 × 1 cm) to remove the free amine-containing by-products, unchanged PAMAM dendrimer and unchanged free carboxylic acid. Elution with water, followed by evaporation of the desired fractions (confirmed by spotting on TLC plates and charring of those spots with 5% sulfuric acid in methanol) and finally lyophilization afforded **14**, **16**, **18**, **20** and **22** as white foam: yields 45−60 mg (53−76%). Compounds **14**, **16**, **18**, **20** and **22** were further verified by gel-permeation HPLC on a TSKGel G2000 SWXL (7.8 mm I.D. × 30 cm) HPLC column purchased from TOSOH, Japan and detection at 220 nm. 50 mM sodium dihydrogen phosphate buffer of pH 7.0 containing 0.3 M ^{1}$_4$I NaCl was used as eluent, with a flow rate of 0.5 mL/min. Gel-permeation HPLC showed only a single peak for each product. The retention times of the products are summarized in Table 1 and are readily distinguishable from the retention times of the corresponding starting PAMAM dendrimers.

Tetrameric β-D-Cellobiosyl-PAMAM Dendrimer (14): To a mixture of methanolic solution of PAMAM dendrimer, generation 0, **13** (20% wt. in methanol, 80μL, 0.031 mmol), **5** (100 mg, 0.25 mmol) in 4 mL of DMF, diisopropylethylamine (88 μL, 0.5 mmol) and BOP reagent (110 mg, 0.25 mmol) were added and the reaction mixture was stirred at 25 °C for 2 h. Purification followed by lyophilization afforded dendrimer **14** as a white foam. Gel-permeation HPLC of the product **14** showed only a single peak, with a retention time of 19.939 min: detection at 220 nm (Table 1). Yield 60 mg (53%). ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 2.32−2.44 (m, 7 H), 2.51−2.65 (m, 5 H), 2.71−2.84 (m, 10 H), 2.93−2.96 (d, *J* = 0.5 Hz, 3 H), 3.22−3.27 (m, 5 H), 3.31−3.38 (m, 16 H), 3.39−3.47 (m, 9 H), 3.49−3.55 (m, 4 H), 3.56−3.62 (m, 8 H), 3.63−3.70 (m, 4 H), 3.73−3.79 (m, 4 H), 3.82−3.95 (m, 9 H), 4.18 (d, *J* = 15.6 Hz, 4 H), 4.31 (d, *J* = 15.6 Hz, 4 H), 4.44 (d, *J* = 7.9 Hz, 4 H, H-1) and 4.46 (d, *J* = 7.8 Hz, 4 H, H-1′) ppm. ^{13}C NMR (150 MHz, D_2O, 25 °C): δ = 32.0, 36.2, 37.9, 38.0, 48.6, 49.4, 59.6 (C-6), 60.2 (C-6′), 67.6 (O*C*H$_2$CO), 69.1 (C-4), 72.3, 72.4, 72.7, 73.7, 73.9, 74.4, 75.1, 75.3, 75.5, 78.2, 101.8 (C-1), 102.0 (C-1′) and 171.4, 174.3 and 174.6 (CH$_2$*C*ONH) ppm. MS MALDI-TOF: *m*/*z* = 2046.6±1.0 $[M + H]^+$ ($C_{78}H_{137}O_{52}N_{10}$ requires 2045.8).

Octameric β-D-Cellobiosyl-PAMAM Dendrimer (16): To a mixture of methanolic solution of PAMAM dendrimer, generation 1, **15** (20% wt. in methanol) (115 μL, 0.016 mmol), **5** (100 mg, 0.25 mmol) in 4 mL of DMF, diisopropylethylamine (88 μL, 0.5 mmol) and BOP reagent (110 mg, 0.25 mmol) were added and the reaction mixture was stirred at 25 °C for 12 h. Purification followed by lyophilization afforded dendrimer **16** as a white foam. Gel-permeation HPLC of the product **16** showed only a single peak, with a retention time of 18.776 min: detection at 220 nm (Table 1). Yield 47 mg (65%). ^{1}H NMR (600 MHz, D_2O, 25 °C):

δ = 2.23−2.42 (m), 2.47−2.60 (m), 2.65−2.79 (m), 2.90−2.94 (m), 3.11−3.28 (m), 3.28−3.36 (m), 3.36−3.45 (m), 3.46−3.53 (m), 3.54−3.60 (m), 3.61−3.67 (m), 3.71−3.76 (m), 3.79−3.85 (m), 3.86−3.91 (m), 4.16 (d, *J* = 15.6 Hz, 8 H), 4.27 (d, *J* = 15.6 Hz, 8 H), 4.42 (d, *J* = 7.9 Hz, 8 H, H-1) and 4.43 (d, *J* = 8.0 Hz, 8 H, H-1′) ppm. ^{13}C NMR (600 MHz, D_2O, 25 °C): δ = 32.5, 32.6, 36.7, 36.9, 38.4, 38.6, 49.9, 51.2, 59.9 (C-6), 60.6 (C-6′), 68.1 (O*C*H_2CO), 69.5 (C-4), 72.7, 73.1, 74.1, 74.9, 75.5, 76.0, 78.5, 102.3 (C-1), 102.6 (C-1′) and 172.0, 174.4 and 175.0 (CH_2*C*ONH) ppm. MS MALDI-TOF: *m*/*z* = 4505±5 [M + Na]$^+$ ($C_{174}H_{304}N_{26}O_{108}$Na requires 4509).

Hexadecameric β-D-Cellobiosyl-PAMAM Dendrimer (18): To a mixture of methanolic solution of PAMAM dendrimer, generation 2, **17** (20% wt. in methanol) (127 μL, 0.0078 mmol), **5** (100 mg, 0.25 mmol) in 4 mL of DMF, diisopropylethylamine (88 μL, 0.5 mmol) and BOP reagent (110 mg, 0.25 mmol) were added and the reaction mixture was stirred at 25 °C for 14 h. Purification followed by lyophilization afforded dendrimer **18** as a white foam. Gel-permeation HPLC of the product **18** showed only a single peak, with a retention time of 17.489 min: detection at 220 nm (Table 1). Yield 56 mg (76%). ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 2.24−2.44 (m), 2.47−2.61 (m), 2.65−2.81 (m), 2.92 (s), 3.12−3.27 (m), 3.28−3.36 (m), 3.37−3.45 (m), 3.46−3.53 (m), 3.54−3.60 (m), 3.61−3.66 (m), 3.67−3.78 (m), 3.79−3.92 (m), 4.16 (d, 16 H, *J* = 15.6 Hz), 4.28 (d, 16 H, *J* = 15.6 Hz), 4.41 (d, 16 H, *J* = 7.9 Hz, H-1) and 4.42 (d, 16 H, *J* = 7.9 Hz, H-1′) ppm. ^{13}C NMR (150 MHz, D_2O, 25 °C): δ = 31.3, 32.6, 36.7, 36.9, 38.4, 38.6, 48.9, 51.2, 59.9 (C-6), 60.6 (C-6′), 68.1 (O*C*H_2CO), 69.5 (C-4), 72.7, 73.1, 74.1, 74.9, 75.5, 76.0, 78.5, 102.3 (C-1), 102.6 (C-1′) and 164.9, 172.0, 174.5 and 175.0 (CH_2*C*ONH) ppm. MS MALDI-TOF: *m*/*z* = 9397±7 [M + Na]$^+$ ($C_{366}H_{640}O_{220}N_{58}$Na requires 9391).

32-Meric β-D-Cellobiosyl-PAMAM Dendrimer (20): To a mixture of methanolic solution of PAMAM dendrimer, generation 3, **19** (20% wt. in methanol) (135 μL, 0.0039 mmol), **5** (100 mg, 0.25 mmol) in 4 mL of DMF, diisopropylethylamine (88 μL, 0.5 mmol) and BOP reagent (110 mg, 0.25 mmol) were added and the reaction mixture was stirred at 25 °C for 21 h. Purification followed by lyophilization afforded dendrimer **20** as a white foam. Gel-permeation HPLC of the product **20** showed only a single peak, with a retention time of 16.510 min: detection at 220 nm (Table 1). Yield 40 mg (53%). ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 2.20−2.41 (m), 2.48−2.59 (m), 2.60−2.90 (m), 2.92 (d, *J* = 0.4 Hz), 3.17−3.28 (m), 3.29−3.36 (m), 3.37−3.45 (m), 3.46−3.53 (m), 3.54−3.60 (m), 3.61−3.67 (m), 3.70−3.77 (m), 3.79−3.85 (m), 3.86−3.92 (m), 4.14 (d, 32 H, *J* = 15.6 Hz), 4.28 (d, 32 H, *J* = 15.6 Hz), 4.41 (d, 32 H, *J* = 7.9 Hz, H-1) and 4.42 (d, 32 H, *J* = 8.0 Hz, H-1′) ppm. ^{13}C NMR (150 MHz, D_2O, 25 °C): δ = 31.4, 32.7, 36.7, 36.9, 38.4, 38.6, 39.5, 48.9, 49.0, 51.3, 59.9 (C-6), 60.6 (C-6′), 68.1 (O*C*H_2CO), 69.5 (C-4), 72.7, 73.2, 74.1, 74.9, 75.5, 76.0, 78.5, 102.3 (C-1), 102.6 (C-1′) and 164.9, 172.0, 174.5, 174.6, 175.0, 175.2 and 177.3 (CH_2*C*ONH) ppm. MS MALDI-TOF: *m*/*z* = 19166±15 [M + Na]$^+$ ($C_{750}H_{1312}O_{444}N_{122}$Na requires 19155).

64-Meric β-D-Cellobiosyl-PAMAM Dendrimer (22): To a mixture of methanolic solution of PAMAM dendrimer, generation 4, **21** (10% wt. in methanol) (277 μL, 0.002 mmol), **5** (100 mg, 0.25 mmol) in 4 mL of DMF, diisopropylethylamine (88 μL, 0.5 mmol) and BOP reagent (110 mg, 0.25 mmol) were added and the reaction mixture was stirred at 25 °C for 16 h. Purification followed by lyophilization afforded dendrimer **22** as a white foam. Gel-permeation HPLC of the product **22** showed only a single peak, with a retention time of 15.016 min: detection at 220 nm (Table 1). Yield 50 mg (65%). ^{1}H NMR (600 MHz, D_2O, 25 °C): δ = 2.26−2.39 (m), 2.48−2.59 (m), 2.66−2.80 (m), 2.92 (s), 3.02−3.07 (m), 3.13−3.17 (m), 3.17−3.27 (m), 3.28−3.36 (m), 3.37−3.45 (m), 3.47−3.53 (m), 3.54−3.60 (m), 3.61−3.67 (m), 3.70−3.76 (m), 3.79−3.91 (m), 4.16 (d, 64 H, *J* = 15.7 Hz), 4.28 (d, 64 H, *J* = 15.7 Hz), 4.415 (d, 64 H, *J* = 7.9 Hz, H-1) and 4.425 (d, 64 H, *J* = 7.9 Hz, H-1′) ppm. ^{13}C NMR (150 MHz, D_2O, 25 °C): δ = 32.7, 36.7, 38.5, 38.6, 39.7, 49.0, 51.3, 59.9 (C-6), 60.6 (C-6′), 68.1 (O*C*H_2CO), 69.5 (C-4), 72.7, 73.2, 74.1, 74.9, 75.5, 76.0, 78.6, 102.3 (C-1), 102.6 (C-1′) and 172.0, 174.6, 174.6, 175.0 and 175.1 (CH_2*C*ONH) ppm.

Measurement of CDP Reaction on the Cellobiosyl Dendrimers: A mixture containing glucose-1-phosphate solution (40 mM, 25μL), cellobiose (40 mM, 25μL) solution and cellobiosylated glycoconjugates (20 mM of compound **10**, 13.3 mM of compound **12**, 10 mM of compound **14**, 5 mM of compound **16**, 2.5 mM of compound **18**, 1.25 mM of compound **20** and 0.625 mM of compound **22**) and a solution of the enzyme cellodextrin phosphorylase (0.15 U/mL, 50 μL) in MOPS buffer (pH 7.5, 50 mM) was incubated at 37 °C. Aliquots were removed (2 μL) at certain intervals from 0 h to 120 h and were diluted 50 times with MOPS buffer (pH 7.5, 50 mM) and heated at 100 °C for 10 min to stop the reaction. After keeping at 25 °C for 10 min, a solution of Glc-1-P quantification reagent[16] (100 μL) was added. The reaction mixture was incubated at 25 °C for 30 min and absorbance was measured at 340 nm to quantify Glc-1-P. A standard curve was obtained by measurement of absorbance at 340 nm using 50 μM, 100 μM, 150 μM, 200 μM, 250 μM and 300 μM solutions of Glc-1-P. The percentage of remaining glucose-1-phosphate was calculated and plotted against time (h), and the results are summarized in Figure 1.

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