Solid-Phase Synthesis of Multivalent Glycoconjugates on a DNA Synthesizer

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Carbohydrate−oligonucleotide conjugates and glycodendrimers were synthesized utilizing a DNA synthesizer. The synthesis of multivalent glycoconjugates on solid-phase allows custom tailoring of their structure to the requirements of biological assays within hours, as opposed to traditional approaches that require weeks or months of work in the laboratory. Therefore it will become much easier to investigate carbohydrate−protein interactions and optimize for objectives such as the receptor-mediated targeting of antisense oligonucleotides.

INTRODUCTION

Many recognition events in biology are based on multivalent carbohydrate protein interactions (*1*). Numerous examples from recent years demonstrate that molecules capable of presenting multiple carbohydrates in an appropriate way to viruses, bacteria, and toxins can efficiently target or inhibit them (*2*). Tremendous inhibition properties have been achieved, when structurally well defined, monodisperse glycoconjugates matched the carbohydrate receptors of bacterial AB5 toxins. These toxins recognize their host cells through the simultaneous interaction of their five carbohydrate-binding proteins with gangliosides on human cell surfaces. Critical information from the X-ray crystallographic structure of AB5 toxins led to the syntheses of pentameric glycoconjugates with optimized spacer lengths that exhibited binding at a level 10^7-fold higher than that for single ligands (*3*, *4*). However, in most cases the exact structure of receptors for biologically active carbohydrates are unknown, making the screening of glycoconjugates with different structures of special interest. Recently, the chemotactic response of *Escherichia coli* to galactose-functionalized oligomers was optimized by tuning the valency of these galactose arrays (*5*); in another study, the inhibition of influenza viruses by sialic acid-conjugated dendritic polymers with various backbone structures was investigated (*6*).

Numerous attempts have been made to utilize carbohydrate−protein interactions to enhance the cellular uptake and the targeting of drug delivery vehicles by coating them with carbohydrates ligands (*7*). Currently, only a few examples are known in which carbohydrate ligands have been used to increase the uptake of antisense oligonucleotides (*8*). Carbohydrate-coated structures have been used to achieve the receptor-mediated endocytosis of antisense oligonucleotides by either complexation with mannosylated polylysine (*9*) or chemical ligation to galactosylated polylysine (*10*). However, a drawback in the synthesis of multivalent glycoconjugates is the complexity of their synthesis and the difficulty of their purification (*11*). This becomes even more problematic if only biocompatible connections are allowed (*12*). Recently, the synthesis of oligosaccharides and simple glycoconjugates was facilitated by their automated assembly on solid phase (*13*). The automated synthesis of multivalent glycoconjugates on solid phase would allow their custom tailoring to the requirements of biological assays within hours, whereas solution phase strategies require weeks or months of work in the laboratory.

In the course of an ongoing antisense project with macrophages in our laboratory, it became of interest to synthesize oligonucleotides with mannosylated dendrons for receptor-mediated endocytosis. Therefore a flexible methodology to attach multivalent carbohydrate structures to oligonucleotides that allows for easy modification of the bouquet of presented carbohydrates was necessary. The most appealing way to synthesize such hybrid molecules involved the use of a consistent type of chemistry enabling the preparation of the hybrid as a whole instead of synthesizing two separate moieties using distinct carbohydrate and nucleic acid methodologies. In previous work a DNA synthesizer has been used to assemble dendritic structures that present multiple DNA strands (*14*, *15*) or dyes (*16*). Branched phosphoramidites as well as mannose-containing phosphoramidites have been described in the literature for different purposes (*17*−*21*). Therefore, it was reasonable to try and complete the whole synthesis of our oligonucleotides with mannosylated glycodendrons on the DNA synthesizer. Herein we demonstrate that a standard DNA synthesizer may be used for the small-scale assembly of a variety of multivalent glycoconjugates with biological relevance, which may or may not contain DNA fragments.

RESULTS AND DISCUSSION

The DNA synthesizer is an ideal tool for the assembly of complex, multivalent glycoconjugates from versatile components, such as spacer units, branching points, or terminal carbohydrates. Therefore it should be possible to select the combination, number, and arrangement of building blocks to create a variety of structures. Originally, DNA synthesizers were designed to assemble oligonucleotides from cyanoethyl phosphoramidites on solid supports (*22*). Numerous functional groups have since been introduced in the middle or at the end of the oligonucleotide by adapting the desired functionality into an appropriate phosphoramidite (*23*). Utilizing this approach, the synthesis of mannose, galactose, and branched functional phosphoramidites is described, followed by the

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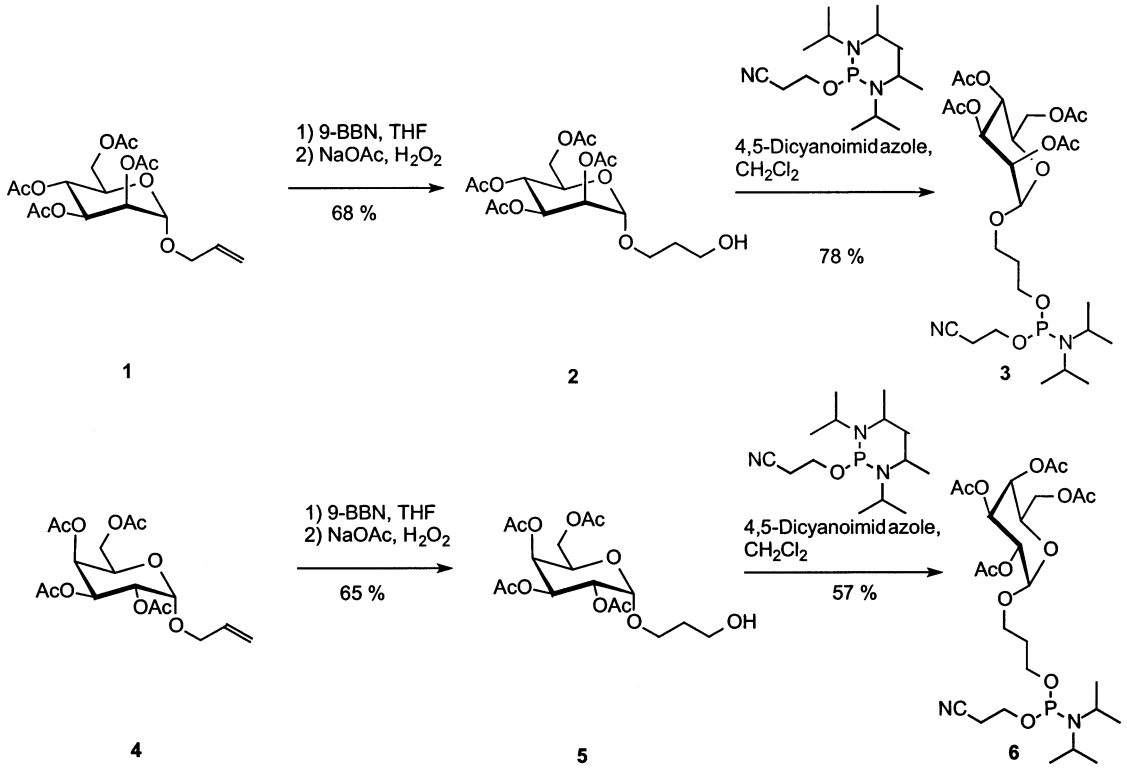
Scheme 1. Synthesis of Carbohydrate Phosphoramidites



assembly of an oligonucleotide with mannosylated glycodendron and other glycoconjugates.

Phosphoramidite Syntheses. The mannose- and galactose-derivatized phosphoramidites, **3** and **6**, respectively, were synthesized by hydroboration of the acetylated allyl glycosides, **1** and **4**, and subsequent reaction with 2-cyanoethyl tetraisopropylphosphordiamidite (Scheme 1). The hydroboration was performed with 9-BBN to achieve a stereoselective anti-Markownikoff addition of the borane (*24*). Nonbasic conditions were required to retain the four ester protecting groups on the carbohydrate. Therefore, the normal hydroboration workup procedure was replaced with aqueous 3 M NaOAc and 30% H_2O_2; less than 1 equiv of 9-BBN was used to avoid base formation by hydrolysis of excess 9-BBN. Best results for the synthesis of phosphoramidites such as **3** and **6** were achieved by a modification of a recently published procedure with 2-cyanoethyl tetraisopropylphosphordiamidite and 4,5-dicyanoimidazole (*25*). Only 0.1 equiv of 4,5-dicyanoimidazole were used to avoid the formation of side products, in which both diisopropylamino groups reacted with the alcohol donor. Furthermore, the suggested aqueous workup was eliminated, and the reaction products were directly purified with dichloromethane over basic alumina, which was made less polar by increasing the Brokmann factor (*26*), so that the use of reactive solvents such as methanol could be avoided.

Branched functional phosphoramidite **11** was synthesized by selective extension and protection of two hydroxyl groups of 1,1,1-trihydroxymethylethane and its transformation into a phosphoramidite (Scheme 2). After allylation of two hydroxyl groups of 1,1,1-trihydroxymethylethane, the remaining third hydroxyl group was protected with the UV-active benzoyl group to facilitate reaction monitoring. The bis allyl ether **8** was hydroborated as described earlier with less than 1 equiv of 9-BBN followed by workup with 3 M NaOAc/30% H_2O_2 to afford the diol **9**. The sterically hindered diol **9** was protected with 4,4′-dimethoxytrityl chloride (DMTCl) using a procedure with DMTCl, NH_4ClO_4, and 2,4,6-trimethylpyridine, thereby generating in situ the active protection reagent 4,4′-dimethoxytrityl perchlorate (*27*). After debenzoylation with sodium methoxide in a THF/methanol mixture, the resulting alcohol **10** was reacted with 2-cyanoethyl tetraisopropylphosphordiamidite and 4,5-dicyanoimidazole to yield the branched phosphoramidite **11**, which was purified with basic alumina.

Spacer functionalized phosphoramidites and triple branched functional phosphoramidites are required in addition to the phosphoramidites described above to vary the spacer length and multiplicity of the targeted multivalent glycoconjugates. The previously known spacer phosphoramidite **12** and the triply branched phosphoramidite **13** meet these requirements perfectly (Chart 1) (*17, 28*). Following a synthetic approach different from that previously reported, we synthesized **12** by mono DMT-protection of triethylene glycol and subsequent reaction with 2-cyanoethyl tetraisopropylphosphordiamidite and 4,5-dicyanoimidazole. The triply branched phosphoramidite **13** was synthesized following the procedure for the branched phosphoramidite **11** starting with pentaerythritol triallyl ether instead of 1,1,1-trihydroxymethylethane.

Assembly of Phosphoramidites. With the mannose, galactose, spacer, and branched phosphoramidites in hand, all the building the blocks for the synthesis of multivalent glycoconjugates on a DNA synthesizer are complete and model compounds can be assembled. Initially, the coupling parameters for each novel phosphoramidite needed to be optimized. Several simple structures were synthesized with different reaction times and flow parameters to optimize the coupling conditions for both the new carbohydrate presenting phosphoramidites and the branched phosphoramidites **3**, **6**, and **11**. The purity of the desired products was monitored by MALDI-TOF MS. It revealed that, when used in the synthesizer, all the functional phosphoramidites we prepared were much

Scheme 2. Synthesis of Branched Phosphoramidites

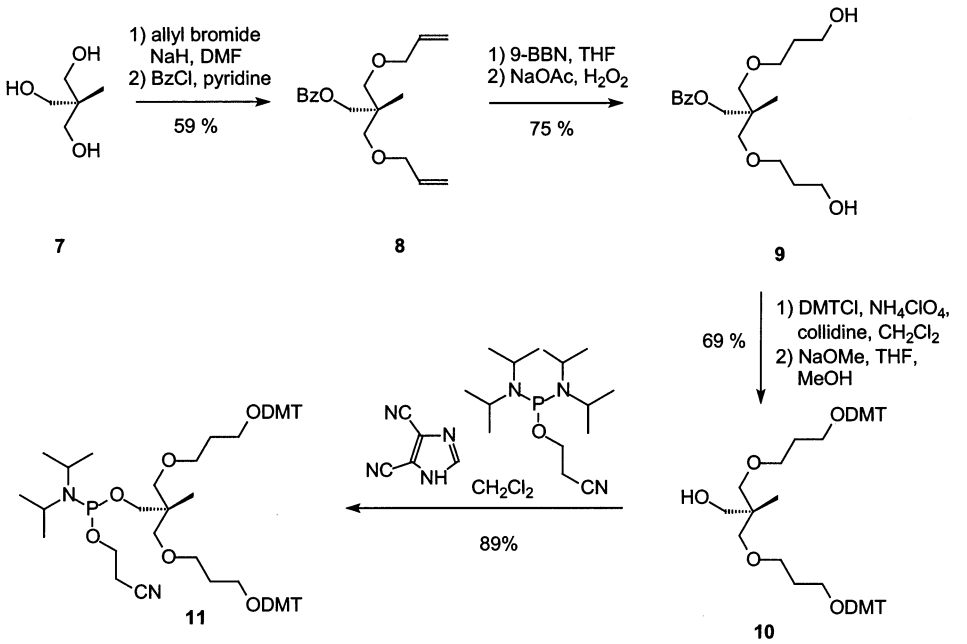
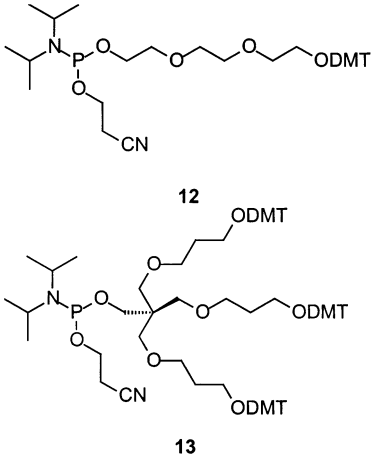


Chart 1. Branched and Spacer Phosphoramidites.



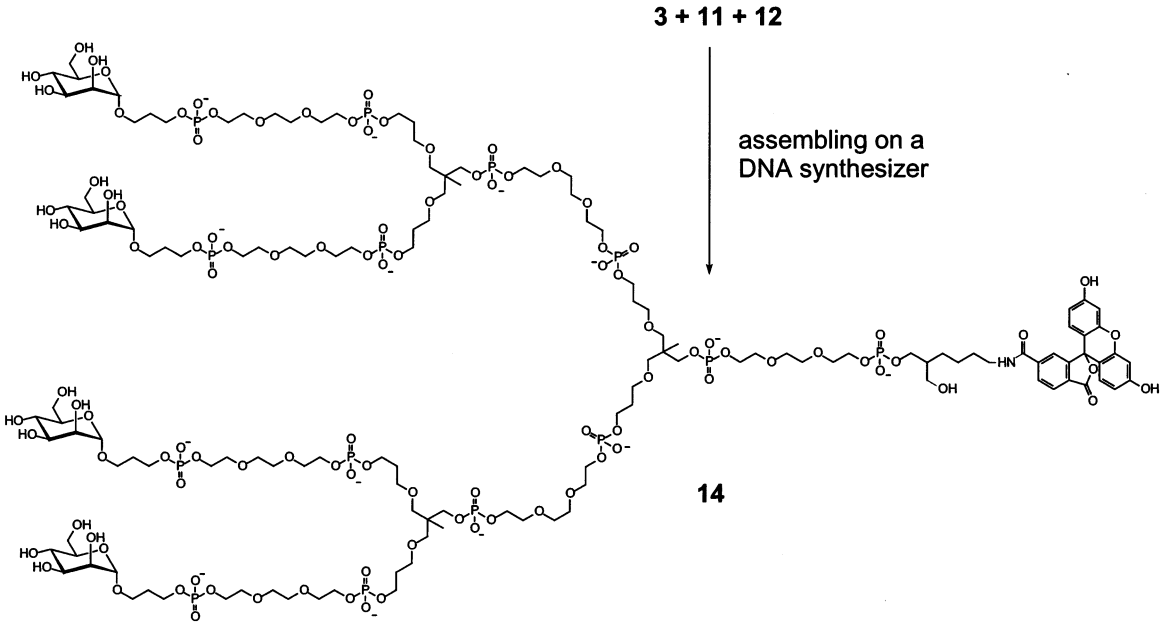
less reactive than the phosphoramidites of the four nucleotides adenosine, guanosine, cytosine, and thymidine, and that a simple increase in the normal reaction time was not sufficient to achieve complete coupling. Instead it was found that best results were obtained when the phosphoramidite−activator mixture was regularly renewed over the solid support every 1 or 2 min over a period of 6 or 18 min, respectively. Details about the use of this procedure on an Expedite 8909 synthesizer can be found in the Supporting Information.

Assembly of Glycodendrimer 14. The first glycoconjugates targeted was glycodendrimer **14** (Scheme 3). A solid support tagged with 6-fluorescein was used, to obtain fluorescently tagged glycoconjugates that will facilitate monitoring within biological assays. Since the Applied Biosystems Expedite 8909 synthesizer allows the assignment of letters to certain reagents together with corresponding reaction protocols, the automated synthesis became as simple as entering a "XVWVWV" code into the synthesizer computer. Typically the codes X, W, and V were assigned to reagent bottles with phosphoramidites **3**, **11**, and **12**, respectively, together with their appropriate reaction times and flow parameters. It is possible to cleave glycodendrimer **14** from the solid support and deprotect it under normal oligonucleotide deprotection conditions, for example with aqueous ammonia, but it is more convenient to use standard carbohydrate deprotection conditions. The only protecting group used, acetate ester, becomes volatile methyl acetate, if sodium methoxide in methanol is used for the deprotection. The glycodendrimer **14** was characterized by ^{1}H NMR spectroscopy and MALDI-TOF mass spectroscopy.

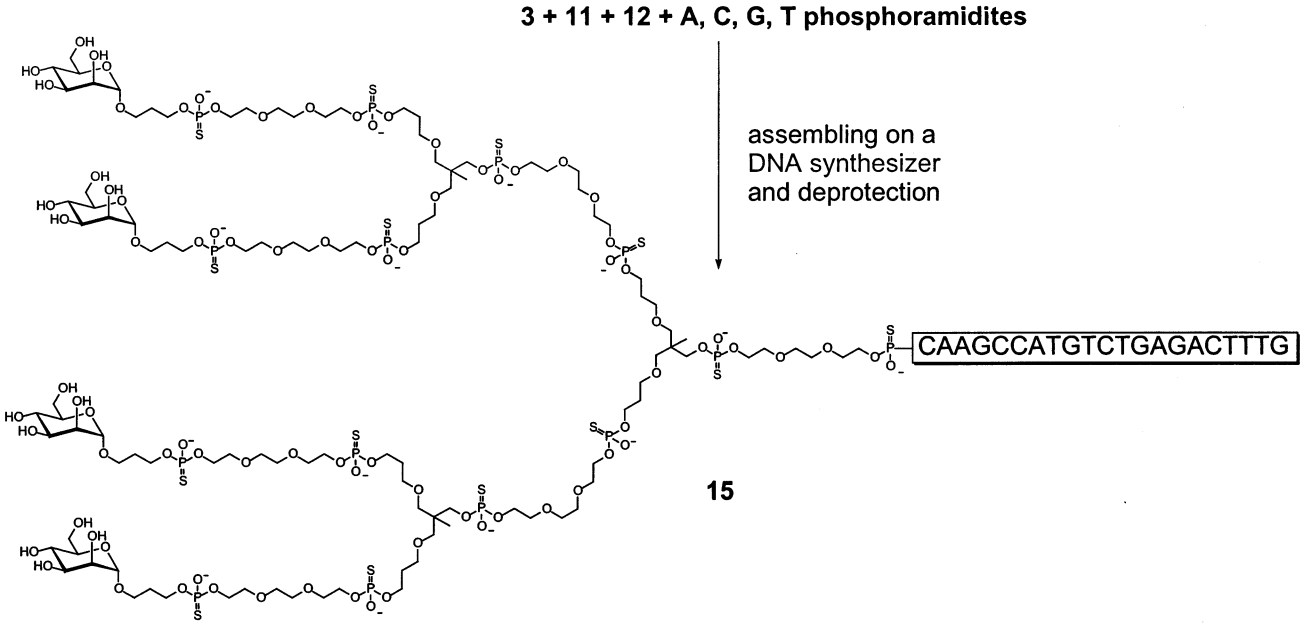
Assembly of Oligonucleotide−Glycodendron Conjugate 15. The thioated oligonucleotide (5-CAAGCCATGTCTGAGACTTTG) coded for the antisense inhibition of inducible nitric oxide synthase (*29*) with mannosylated glycodendron **15** was grown divergently in the same manner as **14** on a standard guanosine-support (Scheme 4). The standard oxidizing reagent iodine, which yields oligonucleotides with phosphate backbone, was replaced by the sulfurization reagent 3*H*-1,2-benzodithiole-3-one 1,1-dioxide to obtain a phosphorthioate backbone that increases the stability against nucleases. The deprotection was done with aqueous ammonia/methylamine 1:1 at 40 °C (*30*). The oligonucleotide−glycodendron conjugate **15** was purified by an ethanol precipitation and subsequently characterized by MALDI-TOF mass spectroscopy and gel electrophoresis. The sterically bulky glycodendron causes delayed elution in gel electrophoresis, and **15** behaves like a 38mer though it only has 34 negative charges. The formation of duplexes of **15** with complementary oligonucleotide strands was not hindered, due to the good solubility properties of the glycodendron. A melting point of 44.2 °C (50 mM NaCl) was measured for the hybridization product of **15** with a complementary DNA strand with two short PEG chains at its 5′ end, compared to 44.1 °C (50 mM NaCl) for the unmodified DNA strands.

Assembly of Cluster Glycoside 16. Galactosylated cluster glycosides were used in the first example of the exponential affinity enhancement of carbohydrate−protein interactions through multiple contacts by multivalent glycoconjugates, also known as cluster effect (*31*, *32*). Using the galactose phosphoramidite **6**, the triple branched phosphoramidite **13**, and the spacer phosphoramidite **12**, it is easy to use the DNA synthesizer to assemble such cluster glycosides with variable spacer

Scheme 3. Synthesis of Mannosylated Glycodendrimer 14



Scheme 4. Synthesis of Thioated Antisense Oligonucleotide with Mannosylated Glycodendron 15



lengths. The negatively charged backbone may increase inhibition potency of antisense-oligonucleotide−glycodendron conjugates in some cases (*33*) and may obstruct structural investigations with the presented cluster glycosides. In such cases it is possible to avoid the negative charges, by using methyl phosphoramidites instead of cyanoethyl phosphoramidites, though in this case the resulting structures will be mixtures of diastereomers with neutral methylphosphonate backbone. The cluster glycoside **16** with phosphate backbone was synthesized as a model compound and characterized by MALDI-TOF and ^{1}H NMR (Scheme 5). As was the case for the syntheses of **14** and **15**, this synthesis was carried out on a 1 μmol scale. This scale is normally sufficient to meet the amount of glycoconjugate needed for in vitro biological investigations.

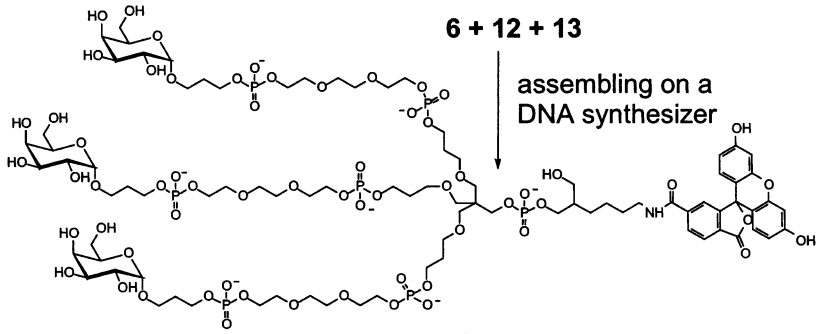
CONCLUSION

In conclusion, a methodology for the facile, fast, and efficient synthesis of oligonucleotides with glycodendrons for receptor-mediated endocytosis was presented. This approach also allowed the synthesis of high molecular-weight, multivalent glycoconjugates, such as glycodendrimers and cluster glycosides. The large glycoconjugates prepared are highly soluble in water and present potential high affinity ligands for carbohydrate binding proteins. The syntheses of multivalent glycoconjugates on a DNA synthesizer allows the custom-tailoring of their structure to meet the requirements of biological assays and affords the desired samples within hours, while normal approaches to the synthesis of multivalent glycoconjugates take weeks or months of work in the laboratory. Therefore, it should be possible to generate biological results much faster and optimize for instance the affinity of antisense oligonucleotides fitted with glycodendrons to their target cells.

EXPERIMENTAL SECTION

General Procedures. For the preparation of Brockmann type II or III alumina, 3% or 6% water was added

Scheme 5. Synthesis of Galactosylated Cluster Glycoside 16



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to basic alumina type I, respectively, and the resulting material was agitated for 10 min and equilibrated overnight. Vanillin (2.5 g) in 5% H_2SO_4 in ethanol (100 mL) was used as the staining reagent for phosphoramidites. Simple alcohols and unsaturated compounds were stained with basic $KMnO_4$-staining reagent [$KMnO_4$, (3 g), K_2CO_3 (20 g) in 300 mL water plus 5% (w/v) aqueous NaOH (5 mL)]. Carbohydrate-containing compounds were stained with 5% H_2SO_4 in ethanol. Dimethoxytrityl-containing compounds were monitored by UV light 254 nm. MALDI-TOF spectra were measured on a Voyager DE MALDI-TOF mass spectrometer from Perseptive Biosystems using 3-hydroxy picolinic acid (HPA) as a matrix [0.7 M HPA, 0.07 M ammonium citrate dibasic, acetonitrile/water 1:1]. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded on Bruker VBAMX-400 or DRX-500 spectrometers. Melting points were measured on a Lambda 35 UV spectrometer from Perkin-Elmer with PTP 6 Peltier unit.

Oligonucleotide Synthesis. Assembly of the phosphoramidites was done on an Expedite 8909 synthesizer from Applied Biosystems with 1*H*-tetrazole as activator. All synthesizer reagents and controlled glass pore (CPG) supports were obtained from Glen Research. A 1 μmol 500 Å 3-(6-fluorescein)-LCAA-CPG (LCAA: Long Chain Alkylamine) was used for fluorescein-labeling of **14** and **16**, and 1 μmol G-1000 Å-LCAA-CPG support was used for the synthesis of **15**. Optimized long coupling procedures were used for the phosphoramidites **3**, **6**, **11**, **12**, and **13**. A short coupling procedure with 16 μmol 0.06 M reagent/1 μmol support in five equal injections over 6 min and a longer coupling procedure with 26 μmol 0.06 M reagent/1 μmol support in eight equal injections over 18 min was used for the introduction of the more sterical hindered branched phosphoramidites **11** and **13**, as well as for the extension of branched structures. Both procedures use a larger phosphoramidite excess than normal 6 μmol 0.06 M reagent/1 μmol support to achieve a better than 98% coupling yield.

3-Hydroxypropyl 2,3,4,6-Tetra-*O*-acetyl α-D-mannopyranoside (2). Allyl 2,3,4,6 tetra-*O*-acetyl α-D-mannopyranoside (**1**) (1.43 g, 3.68 mmol) was dissolved in dry THF (40 mL). 9-BBN (7 mL, 3.5 mmol) was added, and the solution was stirred for 1 day at room temperature. Ice (5 g) and 3 M NaOAc (3.5 mL) were added followed by 30% hydrogen peroxide (3.5 mL) added slowly while stirring at 0 °C in an ice bath. The solution was stirred for 8 h and then quickly saturated with K_2CO_3 at 0 °C. The phases were carefully separated and the aqueous phase washed twice with THF. The combined organic layers were filtered, dried over $MgSO_4$, filtered, concentrated, and purified over silca gel with ethyl acetate/cyclohexane 2:1 to yield **2** as white amorphous solid (1.01 g, 68%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.23 (dd, *J* 3.2 Hz and *J* 9.9 Hz, 1H), 5.18 (dd≈t, *J* 9.5 Hz, 1H), 5.15 (dd, *J* 1.8 Hz, *J* 3.1 Hz, 1H), 4.74 (d, *J* 1.6 Hz, 1H), 4.19 (dd, *J* 12.3 Hz, *J* 5.4 Hz, 1H), 4.03 (dd, *J* 12.1 Hz, J 2.5 Hz, 1H), 3.93 (m, 1H), 3.77 (m, 1H), 3.66 (t, *J* 6.1 Hz, 2H), 3.50 (m, 1H), 2.07, 2.02, 1.97, 1.91 (each s, each 3H), 1.77 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 170.8, 170.1, 169.9, 169.8, 97.6, 69.5, 69.1, 68.3, 66.1, 65.1, 62.5, 59.3, 32.0, 20.9, 20.8, 20.7, 20.6 ppm. Anal. Calcd for $C_{17}H_{26}O_{11}$: C, 50.24; H, 6.45. Found: C, 49.99; H, 6.49.

3-(2,3,4,6-Tetra-*O*-acetyl α-D-mannopyranosyloxy)-propyl (2-Cyanoethyl) (*N*,*N*-Diisopropyl)phosphoramidite (3). To a solution of **2** (410 mg, 1.01 mmol) in dry dichloromethane were added 2-cyanoethyl tetraisopropylphosphordiamidite (0.45 mL, 1.4 mmol) and 4,5-dicyanoimidazole (12 mg, 0.1 mmol). The reaction mixture was stirred for 2 h and then directly purified by column chromatography on Brockmann Type III alumina with dichloromethane as the eluent to yield **3** as colorless viscous liquid (480 mg, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.34−5.24 (m, 2H), 5.22 (m, 1H), 4.80 (m, 1H), 4.28 (m, 1H), 4.08 (dd, *J* 12.3 Hz, *J* 2.4 Hz, 1H), 3.99 (m, 1H), 3.89−3.52 (m, 8H), 2.63 (t, *J* 11.4 Hz, 2H), 2.14, 2.09, 2.02, 1.98 (each s, each 3H), 1.90 (m, 2H), 1.18, 1.16 (each d, *J* 2.0 Hz, each 6H) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 170.6, 169.9, 169.8, 169.6, 117.5, 97.6, 69.5, 69.0, 68.3, 66.0, 64.9 (d, *J* 6.0 Hz), 62.3, 59.9 (d, *J* 17.5 Hz), 58.2 (d, *J* 9.8 Hz), 42.9 (d, *J* 12.3 Hz), 30.8 (d, *J* 6.8 Hz), 24.6, 24.5, 24.5, 24.4, 20.8, 20.7, 20.6, 20.6, 20.3 (d, *J* 7.2 Hz) ppm. ^{31}P NMR ($CDCl_3$, 400 MHz): δ 146.6 ppm. Anal. Calcd for $C_{26}H_{43}N_2O_{12}P$: C, 51.48; H, 7.14; N, 4.62. Found: C, 51.52; H, 7.18; N, 4.65.

3-(2,3,4,6-Tetra-*O*-acetyl α-D-galactopyranosyloxy)-propyl (2-Cyanoethyl) (*N*,*N*-Diisopropyl)phosphoramidite (6). To a solution of **5** (386 mg, 0.950 mmol) in dry dichloromethane (20 mL) were added 2-cyanoethyl tetraisopropyl phosphordiamidite (0.44 mL, 1.39 mmol) and 4,5-dicyanoimidazole (14 mg, 0.2 mmol). The reaction mixture was stirred for 2 h and directly purified by column chromatography on Brockmann type III alumina using dichloromethane as eluent, to yield **6** as colorless syrup (330 mg, 54%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.41 (m, 1H), 5.31 (dd, *J* 10.6 Hz, *J* 3.4 Hz, 1H), 5.11−5.05 (m, 2H), 4.20 (m, 1H), 4.11−4.01 (m, 2H), 3.88−3.45 (m, 8H), 2.61 (dt, *J* 6.3 Hz, *J* 1.8 Hz, 2H), 2.10, 2.05, 2.01, 1.95 (each s, each 3H), 1.87 (m, 2H), 1.16−1.13 (m, 12H) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 170.4, 170.3, 170.2, 170.0, 117.6, 96.3, 68.2, 68.1, 68.0, 67.6, 65.1 (d, *J* 3.6 Hz), 61.6, 60.2 (t, *J* 18.0 Hz), 58.3 (dd, *J* 19.5 Hz, *J* 7.9 Hz), 43.1 (dd, *J* 2.3 Hz, *J* 12.5 Hz), 31.1 (t, *J* 7.5 Hz), 24.6, 24.5, 20.8, 20.8, 20.7, 20.7, 20.4 (d, *J* 7.0 Hz) ppm. ^{31}P NMR ($CDCl_3$, 400 MHz): δ 146.6 ppm. Anal. Calcd for $C_{26}H_{43}N_2O_{12}P$: C, 51.48; H, 7.14; N, 4.62. Found: C, 51.17; H, 6.97; N, 4.27.

4,8-Dioxa-6-[(benzoyloxy)methyl]-6-methylnona-1,10-diene (8). 1,1,1-Trihydroxymethylethane (12.4 g, 103.5 mmol) and allyl bromide (17.9 mL, 206.8 mmol) were dissolved in DMF (60 mL), and NaH (12.0 g, 327 mmol) was added at 0 °C over 1 h. The solution was stirred for 12 h. Ice and brine (400 mL) were added at 0 °C, and the solution was extracted five times with cyclohexane (50 mL each). The combined cyclohexane fractions were dried over $MgSO_4$ and purified over silica gel using a toluene → toluene/ethyl acetate 10:1 gradient, to obtain the bis-allyl ether as a colorless viscous liquid. The syrup was dissolved in pyridine (100 mL), and benzoyl chloride (10 mL) was added at 0 °C over 10 min. The solution was stirred overnight. Methanol (5 mL) was added at 0 °C to quench the reaction, and the solution was poured into ice−water (300 mL). Dichloromethane was added, the phases were separated, and the aqueous phase was extracted twice with dichloromethane. The combined organic phases were dried ($MgSO_4$), concentrated, and purified over silica gel with cyclohexane/ethyl acetate 9:1 to yield **8** as colorless viscous liquid (18.6 g, 59%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.02 (d, *J* 8.2 Hz, 2H), 7.53 (t, *J* 7.4 Hz, 1H), 7.43 (t, *J* 7.4 Hz, 2H), 5.85 (m, 2H), 5.26 (m, 2H), 5.12 (m, 2H), 4.27 (s, 2H), 3.96 (t, *J* 1.5 Hz, 2H), 3.95 (t, *J* 1.5 Hz, 2H), 3.41 (s, 4H), 1.08 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 166.4, 135.0, 132.9, 130.5, 129.6, 128.4, 116.5, 72.7, 72.3, 67.5, 40.3, 17.5 ppm. Anal. Calcd for $C_{18}H_{24}O_4$: C, 71.03; H, 7.95. Found: C, 70.70; H, 7.96.

4,8-Dioxa-6-[(benzoyloxy)methyl]-6-methylnonane-1,11-diol (9). To a solution of **8** (5.85 g, 19.2 mmol) in dry THF (40 mL) was added 9-BBN (75.7 mL, 37.8 mmol), and the solution was stirred for 3 d at room temperature. Ice (10 g) and 3 M NaOAc (38 mL) were added followed by 30% hydrogen peroxide (37.5 mL) added slowly while stirring at 0 °C in an ice bath. The solution was stirred for 6 h and then quickly saturated with K_2CO_3 at 0 °C. The phases were carefully separated, and the aqueous phase was washed twice with THF. The combined organic layers were filtered, dried over $MgSO_4$, filtered, concentrated, and purified over silica gel with dichloromethane/methanol 50:3 to yield **9** as colorless viscous liquid (4.89 g, 75%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.02 (d, *J* 8.3 Hz, 2H), 7.56 (t, *J* 7.4 Hz, 1H), 7.46 (t, *J* 7.4 Hz, 2H), 4.24 (s, 2H), 3.73 (t, *J* 5.5 Hz, 4H), 3.58 (t, *J* 5.5 Hz, 4H), 3.39 (s, 2H), 3.38 (s, 2H), 2.77 (s, 2H), 1.78 (m, 4H), 1.02 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 166.1, 133.0, 129.6, 128.5, 73.7, 70.2, 67.5, 61.3, 40.1, 31.8, 17.8 ppm. Anal. Calcd for $C_{18}H_{28}O_6$: C, 63.51; H, 8.29. Found: C, 63.15; H, 8.31.

1,11-Bis(4,4′-dimethoxytrityloxy)-4,8-dioxa-6-(hydroxymethyl)-6-methylnonane (10). The diol **9** (2.0 g, 5.9 mmol) was dissolved in dry CH_2Cl_2 (100 mL). 2,4,6-Trimethylpyridine (3.16 mL, 23.8 mmol), NH_4ClO_4 (5.43 g, 15.9 mmol), and 4,4′-dimethoxytrityl chloride (5.38 g, 15.9 mmol) were added, and the solution was stirred for 5 d. For workup, the solution was washed with saturated aqueous $NaHCO_3$ and water, dried (Na_2SO_4), filtered through a alumina Brockmann III pad, concentrated, and dried in high vacuum. For debenzoylation, the residue was dissolved in THF (50 mL), and NaOMe in methanol (0.1 M, 20 mL) was added. The solution was stirred overnight, concentrated, redissolved in CH_2Cl_2 and washed with brine (2×), dried (Na_2SO_4), concentrated, and purified over silica gel with toluene/ethyl acetate 9:1 to yield **10** as colorless viscous liquid (3.42 mg, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.47−7.43 (m, 4H), 7.36−7.16 (m, 14H), 6.86−6.83 (m, 8H), 3.81 (s, 12H), 3.54 (t, *J* 6.5 Hz, 4H), 3.48 (d, *J* 5.8 Hz, 2H), 3.36 (d, *J* 8.9 Hz, 2H), 3.29 (d, *J* 8.9 Hz, 2H), 3.15 (t, *J* 6.2 Hz, 4H), 2.84 (t, *J* 5.7 Hz, 1H), 1.86 (m, 4H), 0.78 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 158.7, 145.6, 136.9, 130.3, 128.5, 128.1, 127.0, 113.3, 86.1, 75.8, 69.7, 69.1, 60.6, 55.5, 40.7, 30.6, 17.8 ppm. Anal. Calcd for $C_{60}H_{64}O_{10}$: C, 76.25; H, 6.83. Found: C, 75.85; H, 7.18.

2,2-Bis[5-(4,4′-dimethoxytrityloxy)-2-oxapentyl]-propyl (2-Cyanoethyl) (*N*,*N*-Diisopropyl)phosphoramidite (11). 10 (940 mg, 1.12 mmol) was dissolved in dry dichloromethane (10 mL). 2-Cyanoethyl tetraisopropyl phosphordiamidite (1.2 mL, 3.78 mmol) and 4,5-dicyanoimidazole (11.2 mg, 0.095 mmol) were added to the solution. The reaction mixture was stirred for 15 h and directly purified by column chromatography on Brockmann type II alumina using dichloromethane as eluent, to yield **11** as colorless viscous liquid (1.04 mg, 89%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.41−7.15 (m, 18H), 6.81−6.76 (m, 8H), 3.76 (s, 12H), 3.57−3.45 (m, 9H), 3.18 (s, 4H), 3.11 (t, *J* 6.3 Hz, 4H), 2.54 (t, *J* 6.7 Hz, 2H), 1.82 (m, 4H), 1.16−1.13 (m, 12H), 0.83 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 158.3, 145.4, 136.7, 130.1, 128.2, 127.7, 126.6, 117.7, 113.0, 85.7, 73.2, 68.6, 66.4 (d, *J* 16.3 Hz), 60.5, 58.2 (d, *J* 18.5 Hz), 55.2, 43.0 (d, *J* 13.3 Hz), 41.1 (d, *J* 8.2 Hz), 30.3, 24.7, 24.6, 20.3 (d, *J* 6.4 Hz), 17.3 ppm. ^{31}P NMR ($CDCl_3$, 400 MHz): δ 146.9 ppm. Anal. Calcd for $C_{62}H_{77}N_2O_{10}P$: C, 71.52; H, 7.45; N, 2.69. Found: C, 71.37; H, 7.61; N, 2.71.

Synthesis of Mannosylated Glycodendrimer 14. At the DNA synthesizer, 6 coupling cycles using the functional phosphoramidites in the sequence **12**, **11**, **12**, **11**, **12**, and **3** were applied to a 1 μmol 3′-(6-fluorescein) support. The first coupling step was done with 16 μmol 0.06 M reagent in five equal injections over 6 min, the second to fifth coupling steps were done with 26 μmol 0.06 M reagent in 8 equal injections over 18 min. The product was deprotected with 0.1 M NaOMe in MeOH at 40 °C over 3 h and neutralized with Dowex 50WX2 to obtain **14** as an amorphous orange solid (2.9 mg). ^{1}H NMR (d_4-MeOH, 500 MHz): δ 8.04 (m, 2H), 7.67 (s, 1H), 7.03 (d, *J* 9.2 Hz, 2H), 6.51 (m, 4H), 4.90 (s, 4H), 3.98−3.30 (m, 172H + MeOH), 1.90 (m, 20H), 1.65−1.30 (m, 7H), 0.97 (s, 9H) ppm. MALDI-TOF MS: *m*/*z* 4087.8 (Calcd for $C_{139}H_{255}NO_{107}P_{14}$: 4086.1).

Synthesis of Thioated Oligonucleotide with Mannosylated Glycodendron 15. The iNOS-sequence (5′-CAAGCCATGTCTGAGACTTTG-3′) was synthesized on a 1 μmol G-support at the DNA synthesizer using standard phosphoramidites. After the last coupling cycle, a sequence of six further coupling cycles using the functional phosphoramidites in the sequence **12**, **11**, **12**, **11**, **12**, and **3** was added. The first additional coupling step was done with 16 μmol 0.06 M reagent in five equal injections over 6 min, the second to sixth coupling steps were done with 26 μmol 0.06 M reagent in eight equal injections over 18 min. For each step the sulfurization reagent 3*H*-1,2-benzodithiole-3-one 1,1-dioxide was used to oxidize the intermediate phosphortriester. The product was deprotected with aqueous ammonia/aqueous methylamine (1:1; 1 mL) at 40 °C over 1 h and concentrated at the SpeedVac. The crude product was subjected to a standard ethanol precipitation to yield 151 OD of **15**. A MALDI-TOF MS spectrum was obtained after precipitation of a sample with 3 volume equivalents of 3 M NH_4-OAc with 0.01 M EDTA overnight at −20 °C. MALDI-TOF MS: *m*/*z* 10575.7 (calcd for $C_{316}H_{487}N_{77}O_{190}P_{34}S_{34}$: 10547.9).

The melting behavior of **15** with complementary DNA strand (5′-CAAAGTCTCAGACATGGCTTG-3′) was analyzed by UV spectrometry in 0.05 M aqueous NaCl from

20 °C to 70 °C. A melting point of 44.2 °C (50 mM NaCl, 2 × 0.2 OD) was observed for the hybridization of **15** with a complementary DNA strand with two short PEG chains at its 5′ end, compared to 44.1 °C (50 mM NaCl, 2 × 0.2 OD) for the unmodified DNA strands.

The mobility shift caused by the glycodendron, was monitored by gel electrophoresis using a 13% denaturing polyacrylamide gel (200 V, 45 min). The oligonucleotide was compared with thioated iNOS DNA (21 bases) and a 10 bp ladder. The iNOS oligonucleotide with mannosylated glycodendron behaved like a 38mer though it has only 34 negative charges.

Synthesis of Galactosylated Cluster Glycoside 16. At the DNA synthesizer, three coupling cycles using the synthesized phosphoramidites in the sequence **12**, **13** and **6** were applied to a 1 μmol 3′-(6-fluorescein) support. All coupling steps were done with 26 μmol 0.06 M reagent in eight equal injections over 18 min. The product was deprotected overnight with 0.06 M NaOMe in MeOH (1 mL) at 37 °C over 6 h and neutralized with Amberlite IR120 to obtain **16** as an amorphous orange solid (2.0 mg). ^{1}H NMR (d_4-MeOH, 500 MHz): δ 8.05 (m, 2H), 7.51 (s, 1H), 7.03 (d, J 9.3 Hz, 2H), 6.52 (m, 4H), 4.95 (s, 3H), 3.99−3.30 (m, 92H + MeOH), 1.92 (m, 12H), 1.65−1.30 (m, 7H) ppm. MALDI-TOF MS: m/z 2416.2 (calcd for $C_{87}H_{146}NO_{62}P_7$ 2414.90).

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Supporting Information Available: The synthesizer program for the Expedite 8909 DNA synthesizer. ^{1}H NMR for **14** and **16**. MALDI-TOF spectra **14**, **15**, and **16**. Gel electrophoresis and melting point diagram for **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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