Synthesis of Oligonucleotide Glycoconjugates Using Sequential Click and Oximation Ligations

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Oligodeoxyribonucleotide glycoconjugates bearing two trivalent glycoclusters have been synthesized by two alternative methods based on solid-supported oximation of aminooxy functionalized oligonucleotides with glycoclusters constructed by click chemistry. In more detail, the trivalent glycoclusters (**5** and **6**) bearing three sugar pendants were first assembled by treating a 4-[tri-*O*-propargylpentaerythrityloxy]benzaldehyde scaffold with methyl 6-azido-6-deoxyglycopyranoside under the click reaction conditions. Two phosphoramidite reagents containing a phthaloyl protected aminooxy function, viz., 2-cyanoethyl *N*,*N*-diisopropylphosphoramidites derived from 3-[3,5-bis(phthalimidoxymethyl)phenoxy]propanol (**12**) and 5-(4,4′-dimethoxytrityl)-1,2-dideoxy-1-*C*-(2-phthalimidoxyethyl)-*β*-D-*erythro*-pentofuranose (**16**), were synthesized and incorporated as branching units in appropriate places of the oligonucleotide chains. On using **12**, the phthaloyl protections of the branching unit were removed and two identical glycoclusters were attached via oxime linkage to the 5′-terminus of the support-bound oligonucleotide chain. With branching unit **16**, the phosphoramidite coupling and the oximation were carried out alternately, allowing introduction of two dissimilar trivalent glycoclusters close to the 3′-end of the oligonucleotide chain. The products (**20**, **26**) were released and deprotected by ammonolysis and purified by HPLC chromatography.

INTRODUCTION

Oligonucleotide-based drugs show promise as a novel form of chemotherapy, although several thresholds must still be overcome on the way to applicable nucleic acid therapeutics (*1–14*). Poor cellular uptake, in particular, limits the applicability of both antisense oligonucleotides and siRNA. A possible way to combat this drawback is to conjugate the therapeutic oligonucleotide to an agent expected to exhibit cell-type organ specificity (*15–20*). The oligonucleotide conjugate, hence, is enriched on the cell-surface and internalized by endocytosis. It is well-known that carbohydrate−protein interactions play a major role in recognition of cells by pathogens (*21–23*). These interactions are multipodal; high-affinity binding is achieved by multiple simultaneous interactions, a phenomenon known as a glycocluster effect (*24–26*). Usually, multiantennary carbohydrates anchored to cell membranes recognize extracellular proteins (*27–29*), but the opposite is also possible. Trivalent *N*-acetylgalactosamine conjugate of oligodeoxynucleotide methylphosphonate (*30*) and phosphorothioate (*31*), tetravalent galactose conjugates of oligodeoxynucleotides (*32, 33*), and monovalent galactose conjugates of oligodeoxynucleotide phosphorothioates (*34*) have all shown enhanced uptake in liver via the galactose-specific asialoglycoprotein receptor-mediated endocytosis. It has also been shown that a transmembrane glycoprotein CD44 recognizes hyaluronic oligomers (*35*) and glycoconjugation may be exploited to target antigenic peptides to dendritic cells (*36*). Accordingly, glycotargeting appears to be a feasible approach (*37*) and development of reproducible methods for convenient preparation of various types of oligonucleotide glycoconjugates appears worthwhile. Workable chemistry is a prerequisite for extensive biological studies.

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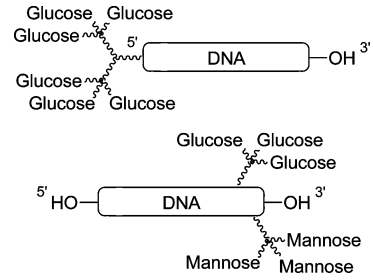

Figure 1. Oligonucleotide glycoconjugates prepared.

Solid-phase oximation, click chemistry, and phosphoramidite coupling are the methods most extensively applied to preparation of oligonucleotide glycoconjugates on a solid support (*19, 38, 39*). Apart from coupling of some aminoglycosides (*40, 41*) and trigalactosylated tetrahydroxycholane (*42*) by phosphoramidite chemistry, the approaches described so far allow attachment of mono- or disaccharides along the oligonucleotide chain using either nucleosidic (*43–45*) or non-nucleosidic (*46–49*) branching units or their conjugation to the internucleosidic phosphodiester linkages (*50*). The examples of oligonucleotide conjugates bearing a multiantennary terminal glycocluster are limited to prepration of a spruce-like tetramannosyl 5′-conjugate (*51*), a trivalent 5′-terminal conjugate containing three different sugars (*52*), and a 3′-cyclodextrin conjugate (*53*). High-affinity binding to membrane lectins may, however, require simultaneous interaction with even more than three or four appropriately situated sugar ligands. For this reason, we now report a protocol for solid-phase synthesis of an oligonucleotide conjugate bearing a hexavalent cluster at the 5′-terminus (Figure 1). In addition, we describe a protocol for attachment of various prefabricated trivalent clusters at desired sites of the oligonucleotide chain. Both methods are based on preparation of aldehyde functionalized trivalent clusters in

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solution by click chemistry and attachment of these clusters to the support-anchored oligonucleotide by oximation.

EXPERIMENTAL PROCEDURES

General Methods and Materials. CH_2Cl_2, 1,4-dioxane, and MeCN were dried over 4 Å molecular sieves, THF over 3 Å sieves. Diethyl ether was dried over sodium. Triethylamine was dried by consecutive distillations from ninhydrin and potassium hydroxide, and then stored over CaH_2. The NMR spectra were recorded at 500 MHz. The chemical shifts are given in ppm using internal TMS as a reference. Appropriate 2D NMR methods (COSY, HSQC, and HMBC) were used for peak assignment. The mass spectra were recorded using EI and ESI ionization methods. C18 RP HPLC analyses of oligonucleotide conjugates were performed using Thermo ODS Hypersil (250 × 4.6 mm, 5 μm) analytical column, a gradient elution from 0% to 50% MeCN in 0.05 mol L^{-1} NH_4OAc (aq) buffer over 30 min (conjugates **20**, **23**, and purified **26**) or 60 min (conjugates **25** and crude **26**), flow rate 1 mL min^{-1}, and detection at 260 nm. For purifications, Thermo ODS Hypersil (250 × 10 mm, 5 μm) column and flow rate 3 mL min^{-1} were used.

4-(Tri-*O*-propargylpentaerythrityloxy)benzaldehyde (2). To a solution of tri-*O*-propargyl pentaerythritol (*54*) (**1**, 0.50 g, 2.0 mmol) in dry THF (10 mL), 4-hydroxybenzaldehyde (0.29 g, 2.4 mmol) and triphenylphosphine (0.63 g, 2.4 mmol) were added. The mixture was cooled to 0 °C and diisopropylazodicarboxylate (DIAD, 0.50 mL, 2.4 mmol) was added dropwise. The mixture was stirred at 70 °C for 10 h and evaporated to dryness. Crude product was purified by silica gel chromatography (30% ethyl acetate in petroleum ether) to yield 0.38 g (53%) of **2.** ^{1}H NMR 500 MHz, $CDCl_3$): δ_{ppm} 9.88 (s, 1H, CHO), 7.82 (m, 2H, Ar), 7.02 (m, 2H, Ar), 4.14 (d, 6H, J = 2.4 Hz, 3 × CH_2CCH), 4.08 (s, 2H, CH_2OAr), 3.66 (s, 6H, 3 × CH_2O), 2.40 (t, 3H, J = 2.4 Hz, 3 × CH_2CCH); ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm} 190.8, 164.2, 131.9, 129.9, 115.0, 79.8, 74.3, 68.5, 67.1, 58.8, 44.7; HRMS (EI): M^+ $C_{21}H_{22}O_5$ requires 354.1467, found 354.1455.

Glucoside Cluster (5). Compound **2** (64 mg, 0.18 mmol) was dissolved in 1,4-dioxane (1.3 mL) together with methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-glucopyranoside (**3**, 310 mg, 0.90 mmol). $CuSO_4$ as 0.05 mol L^{-1} aqueous solution (36 μL 1.8 μmol) and sodium ascorbate as 0.1 mol L^{-1} aqueous solution (0.90 mL, 0.090 mmol) were added. The mixture was stirred at 50 °C for 4.5 h and then at room temperature overnight. The volatiles were evaporated and the residue was dissolved in ethyl acetate and washed twice with brine. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography (5% MeOH in CH_2Cl_2) to yield 140 mg (56%) of **5**. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 9.89 (s, 1H, CHO), 7.83 (m, 2H, Ar), 7.64 (s, 3H, 3 × CH triazole), 6.95 (m, 2H, Ar), 5.47 (m, 3H, 3 × H-3 Glc), 4.88−4.81 [m, 9H, 3 × (H-1, H-4, and H-2 Glc)], 4.58 (s, 6H, 3 × OCH_2C═), 4.56 (dd, 3H, J = 2.4 and 14.4 Hz, 3 × H-6a Glc), 4.38 (dd, 3H, J = 8.3 and 14.4 Hz, 3 × H-6b Glc), 4.17 (m, 3H, 3 × H-5 Glc), 3.99 (s, 2H, CH_2OAr), 3.56 (s, 6H, 3 × CCH_2O), 3.09 (s, 9H, 3 × OCH_3), 2.10 (s, 9H, 3 × CH_3), 2.07 (s, 9H, 3 × CH_3), 2.01 (s, 9H, 3 × CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm} 190.7, 170.1, 169.8, 169.8, 163.9, 145.0, 131.9, 129.9, 124.1, 114.7, 96.5, 70.6, 69.9, 69.7, 68.5, 67.7, 67.0, 64.8, 55.3, 50.5, 45.0, 20.6, 20.6; HRMS (ESI): $[M + Na]^+$ $C_{60}H_{79}N_9NaO_{29}$ requires 1412.4882, found 1412.4879.

Mannoside Cluster (6). was synthesized from **2** (64 mg, 0.18 mmol) and methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranoside (*55*) (**4**, 310 mg, 0.90 mmol) as described for **5** to yield 142 mg (57%) of **6**. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 9.90 (s, 1H, CHO), 7.83 (m, 2H, Ar), 7.65 (s, 3H, 3 × CH triazole), 6.93 (m, 2H, Ar), 5.32 (dd, 3H, J = 3.4 and 10.0 Hz, 3 × H-3 Man), 5.21 (dd, 3H, J = 1.6 and 3.4 Hz, 3 × H-2 Man), 5.14 (m, 3H, 3 × H-4 Man), 4.62 (d, 3H, J = 1.6 Hz, 3 × H-1 Man), 4.59−4.56 [m, 9H, 3 × (H-6a Man and OCH_2C═)], 4.36 (dd, 3H, J = 9.1 and 14.3 Hz, 3 × H-6b Man), 4.15−4.11 (m, 3H, 3 × H-5 Man), 3.96 (s, 2H, CH_2OAr), 3.54 (s, 6H, 3 × CCH_2O), 3.04 (s, 9H, 3 × OCH_3), 2.14 (s, 9H, 3 × CH_3), 2.11 (s, 9H, 3 × CH_3), 2.00 (s, 9H, 3 × CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm} 190.7, 170.1, 169.8, 169.7, 163.9, 144.8, 131.9, 130.0, 124.1, 114.8, 98.4, 69.4, 69.1, 68.7, 68.6, 67.5, 67.0, 64.8, 55.1, 50.9, 45.0, 20.8, 20.8, 20.6; HRMS (ESI): $[M + H]^+$ $C_{60}H_{80}N_9O_{29}$ requires 1390.5062, found 1390.5033.

Dimethyl 5-[3-(4-Methoxytrityl)propoxy]isophthalate (8). Dimethyl 5-hydroxyisophthalate (**7**, 2.10 g, 10.0 mmol) and 3-(4-methoxytrityl)propan-1-ol [prepared as corresponding tritylated propanediol (*56*)] (3.78 g, 10.8 mmol) were dissolved in dry THF (70 mL) and triphenylphosphine (2.85 g, 10.8 mmol) was added. The mixture was cooled to 0 °C and diethylazodicarboxylate (DEAD, 1.68 mL, 10.8 mmol) in dry THF (7 mL) was added dropwise. The reaction was allowed to proceed at room temperature for 19 h, followed by addition of triphenylphosphine (0.53 g, 2.0 mmol) and DEAD (0.31 mL, 2.0 mmol) and the stirring was continued for 2 h. The mixture was evaporated to dryness and the residue was purified by silica gel chromatography (25% ethyl acetate in petroleum ether) to yield 4.14 g (77%) of **8** as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 8.27 (m, 1H, Ar), 7.71 (br d, 2H, Ar), 7.43 (m, 4H, MMTr), 7.30 (m, 2H, MMTr), 7.25 (m, 4H, MMTr), 7.20 (m, 2H, MMTr), 6.79 (m, 2H, MMTr), 4.19 (t, 2H, J = 6.2 Hz, CH_2OAr), 3.94 (s, 6H, 2 × $COOCH_3$), 3.77 (s, 3H, OCH_3), 3.30 (t, 2H, J = 6.0 Hz, MMTrOCH_2), 2.07 (tt, 2H, J = 6.0 and 6.2 Hz, $CH_2CH_2CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm} 166.2, 159.0, 158.5, 144.6, 135.8, 131.7, 130.3, 128.4, 127.7, 126.8, 122.8, 119.8, 113.0, 86.2, 65.5, 59.5, 55.2, 52.4, 29.8; HRMS (ESI): $[M + Na]^+$ $C_{33}H_{32}NaO_7$ requires 563.2046, found 563.2043.

5-[3-(4-Methoxytrityl)propoxy]-1,3-phenylenedimethanol (9). Compound **8** (4.14 g, 7.66 mmol) was dried over P_2O_5, dissolved in dry diethyl ether (80 mL), and cooled to 0 °C. Lithium aluminum hydride (0.87 g, 23 mmol) was slowly added and stirring was continued for 2.5 h at room temperature. The excess hydride was destroyed by careful addition of water and the solids were filtered. The product containing filtrate was diluted with ethyl acetate and washed with brine. The organic layer was dried with Na_2SO_4 and evaporated to dryness to yield 3.50 g (94%) of **9** as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 7.42 (m, 4H, MMTr), 7.29 (m, 2H, MMTr), 7.25 (m, 4H, MMTr), 7.20 (m, 2H, MMTr), 6.91 (br s, 1H, Ar), 6.79 (br s, 2H, Ar), 6.77 (m, 2H, MMTr), 4.63 (s, 4H, 2 × CH_2OH), 4.12 (t, 2H, J = 6.2 Hz, CH_2OAr), 3.77 (s, 3H, OCH_3), 3.27 (t, 2H, J = 6.0 Hz, MMTrOCH_2), 2.05 (tt, 2H, J = 6.0 and 6.2 Hz, $CH_2CH_2CH_2$), 1.90 (br s, 2H, 2 × OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm} 159.5, 158.4, 144.7, 142.7, 135.9, 130.4, 128.4, 127.7, 126.8, 117.4, 113.0, 112.2, 86.2, 65.2, 64.9, 59.8, 55.2, 29.9; HRMS (ESI) $[M + Na]^+$ $C_{31}H_{32}NaO_5$ requires 507.2147, found 507.2169.

5-[3-(4-Methoxytrityl)propoxy]-1,3-phenylenebis(methyleneoxyphthalimide) (10). To a solution of **9** (3.50 g, 7.22 mmol) in dry THF (80 mL) *N*-hydroxyphthalimide (2.35 g, 14.4 mmol) and triphenylphosphine (3.77 g, 14.4 mmol) were added. The mixture was cooled to 0 °C and diethylazodicarboxylate (DEAD, 2.24 mL, 14.4 mmol) in dry THF (10 mL) was added dropwise. The mixture was stirred for 20 h

at room temperature and evaporated to dryness. Crude product was purified by silica gel chromatography (1% MeOH in CH_2Cl_2) to yield 3.01 g (53%) of **10** as white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 7.71 (m, 8H, Pht), 7.44 (m, 4H, MMTr), 7.31 (m, 2H, MMTr), 7.26−7.16 (m, 7H, MMTr and Ar), 7.12 (br d, 2H, Ar), 6.78 (m, 2H, MMTr), 5.18 (s, 4H, 2 × CH_2ON), 4.19 (t, 2H, J = 6.2 Hz, CH_2OAr), 3.76 (s, 3H, CH_3), 3.27 (t, 2H, J = 6.0 Hz, MMTrOCH_2), 2.06 (tt, 2H, J = 6.0 and 6.2 Hz, $CH_2CH_2CH_2$); ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm} 163.4, 159.2, 158.4, 144.7, 136.0, 135.4, 134.4, 130.3, 128.9, 128.4, 127.7, 126.7, 123.5, 122.7, 116.5, 113.0, 86.2, 79.4, 65.0, 59.8, 55.2, 29.9; HRMS (ESI) $[M + Na]^+$ $C_{47}H_{38}N_2NaO_9$ requires 797.2475, found 797.2512.

3-[3,5-Bis(phthalimidoxymethyl)phenoxy]propanol (11). Compound **10** (3.01 g, 3.88 mmol) was suspended in 80% aqueous acetic acid (40 mL) and stirred for 4.5 h. The mixture was evaporated to dryness, and the residue was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted four times with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel chromatography (4−5% MeOH in CH_2Cl_2) to yield 1.61 g (82%) of **11** as white powder. ^{1}H NMR [500 MHz, $(CD_3)_2SO$]: δ_{ppm} 7.86 (s, 8H, Pht), 7.22 (br s, 1H, Ar), 7.15 (br d, 2H, Ar), 5.16 (s, 4H, 2 × CH_2ON), 4.58 (t, 1H, J = 5.2 Hz, OH), 4.08 (t, 2H, J = 6.4 Hz, CH_2OAr), 3.56 (dt, 2H, J = 5.2 and 6.2 Hz, CH_2OH), 1.87 (tt, 2H, J = 6.2 and 6.4 Hz, $CH_2CH_2CH_2$); ^{13}C NMR [125 MHz, $(CD_3)_2SO$]: δ_{ppm} 163.6, 159.1, 136.4, 135.3, 129.0, 123.7, 122.8, 116.4, 79.3, 65.3, 57.7, 32.5; HRMS (ESI): $[M + Na]^+$ $C_{27}H_{22}N_2NaO_8$ requires 525.1274, found 525.1305.

3-[3,5-Bis(phthalimidoxymethyl)phenoxy]propyl 2-cyanoethyl *N,N*-Diisopropylphosphoramidite (12). Compound **11** (290 mg, 0.578 mmol) was dried over P_2O_5 and suspended in dry 1,4-dioxane (3 mL). Dry triethylamine (287 μL, 2.07 mmol) and 2-cyanoethyl *N,N*-diisopropylphosphonamidic chloride (141 μL, 0.632 mmol) were added under nitrogen. The mixture was stirred for 2.5 h and then subjected directly to a silica gel column. Elution with 1% triethylamine and 29% petroleum ether in ethyl acetate yielded 284 mg (70%) of **12** as white powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 7.81 (m, 4H, Pht), 7.73 (m, 4H, Pht), 7.21 (m, 1H, Ar), 7.16 (br d, 2H, Ar), 5.17 (s, 4H, 2 × CH_2ON), 4.15 (t, 2H, J = 6.1 Hz, CH_2OAr), 3.91−3.76 (m, 4H, 2 × $POCH_2$), 3.61 [m, 2H, 2 × $CH(CH_3)_2$], 2.64 (t, 2H, J = 6.5 Hz, CH_2CN), 2.10 (m, 2H, $CH_2CH_2CH_2$), 1.19 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.17 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm} 163.4, 159.2, 135.4, 134.4, 128.9, 123.5, 122.7, 117.7, 116.5, 79.5, 64.7, 60.1 [d, $J(^{13}C, ^{31}P)$ = 17.6 Hz], 58.4 [d, $J(^{13}C, ^{31}P)$ = 19.2 Hz], 43.0 [d, $J(^{13}C, ^{31}P)$ = 12.5 Hz], 30.9 [d, $J(^{13}C, ^{31}P)$ = 7.2 Hz], 24.6 (m), 20.4 [d, $J(^{13}C, ^{31}P)$ = 7.1 Hz]; ^{31}P NMR (200 MHz, $CDCl_3$): δ_{ppm} 147.7; HRMS (ESI): $[M + Na]^+$ $C_{36}H_{39}N_4NaO_9P$ requires 725.2353, found 725.2371.

5-(4,4′-Dimethoxytrityl)-3-*O-tert*-butyldimethylsilyloxy-1,2-dideoxy-1-C-(2-phthalimidoxyethyl)-β-D-erythro-pentofuranose (14). 5-(4,4′-Dimethoxytrityl)-3-*O-tert*-butyldimethylsilyloxy-1,2-dideoxy-1-*C*-(2-hydroxyethyl)-β-D-*erythro*-pentofuranose (*40*) (**13**, 1.64 g, 2.84 mmol), *N*-hydroxyphthalimide (0.557 g, 3.42 mmol), and triphenylphosphine (0.896 g, 3.41 mmol) were dissolved in dry THF (40 mL). Diethylazodicarboxylate (DEAD, 573 μL, 3.68 mmol) was added dropwise and the mixture was stirred for 89 h at room temperature. After evaporation, the residue was dissolved in CH_2Cl_2 and washed three times with saturated $NaHCO_3$. The combined aqueous layers were extracted with CH_2Cl_2, and then the organic layers were combined, dried with Na_2SO_4, and evaporated. Crude product was purified by silica gel chromatography (0.1% triethylamine and 3% MeOH in CH_2Cl_2) to yield 1.90 g (93%) of **14**. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 7.82 (m, 2H, Pht), 7.73 (m, 2H, Pht), 7.44 (m, 2H, DMTr), 7.33 (m, 4H, DMTr), 7.27 (m, 2H, DMTr), 7.18 (m, 1H, DMTr), 6.82 (m, 4H, DMTr), 4.40−4.31 (m, 3H, CH_2ON and H-1), 4.25 (m, 1H, H-3), 3.88 (m, 1H, H-4), 3.79 (s, 6H, 2 × OCH_3), 3.07 (m, 2H, H-5), 2.08 (m, 2H, CH_2CH_2ON), 1.94 (m, 1H, H-2a), 1.74 (m, 1H, H-2b), 0.84 [s, 9H, $C(CH_3)_3$], 0.01 (s, 3H, CH_3Si), −0.02 (s, 3H, CH_3Si); ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm} 163.6, 158.4, 145.0, 136.2, 134.4, 130.1, 128.9, 128.2, 127.7, 126.6, 123.5, 113.0, 86.4, 85.9, 76.1, 75.2, 74.3, 64.3, 55.2, 41.3, 34.2, 25.8, 18.0, −4.6, −4.8; HRMS (ESI) $[M + Na]^+$ $C_{42}H_{49}NNaO_8Si$ requires 746.3125, found 746.3147.

5-(4,4′-Dimethoxytrityl)-1,2-dideoxy-1-*C*-(2-phthalimidoxyethyl)-β-D-erythro-pentofuranose (15). Triethylamine trihydrofluoride (409 μL, 2.51 mmol) was added to a solution of **14** (1.21 g, 1.67 mmol) in dry THF (15 mL). The mixture was stirred at room temperature for 24 h, after which another portion of triethylamine trihydrofluoride (408 μL, 2.50 mmol) was added. The reaction was allowed to proceed for another 24 h, followed by addition of triethylamine (200 μL, 1.43 mmol) and evaporation. The crude product was purified by silica gel chromatography (0.1% triethylamine and 3% MeOH in CH_2Cl_2) to yield 0.538 g (53%) of **15**. ^{1}H NMR (500 MHz, $CDCl_3$): δ_{ppm} 7.82 (m, 2H, Pht), 7.73 (m, 2H, Pht), 7.42 (m, 2H, DMTr), 7.32 (m, 4H, DMTr), 7.27 (m, 2H, DMTr), 7.19 (m, 1H, DMTr), 6.82 (m, 4H, DMTr), 4.42−4.29 (m, 4H, CH_2ON, H-1, and H-3), 3.91 (m, 1H, H-4), 3.79 (s, 6H, 2 × OCH_3), 3.23 (dd, 1H, J = 4.6 and 9.6 Hz, H-5a), 3.08 (dd, 1H, J = 5.9 and 9.6 Hz, H-5b), 2.10−2.05 (m, 3H, CH_2CH_2ON and H-2a), 1.90−1.84 (m, 2H, H-2b and OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ_{ppm} 163.6, 158.5, 144.9, 136.1, 134.5, 130.1, 128.9, 128.2, 127.8, 126.8, 123.5, 113.1, 86.1, 85.8, 75.9, 75.3, 74.7, 64.7, 55.2, 40.9, 34.2; HRMS (ESI): $[M + Na]^+$ $C_{36}H_{35}NNaO_8$ requires 632.2261, found 632.2268.

5-(4,4′-Dimethoxytrityl)-1,2-dideoxy-1-*C*-(2-phthalimidoxyethyl)-β-D-erythro-pentofuranosyl-3-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) (16). 2-Cyanoethyl *N,N,N′,N′*-tetraisopropylphosphordiamidite (365 μL, 1.15 mmol) was added to a solution of **15** (0.538 g, 0.884 mmol) in dry MeCN (1 mL), followed by addition of tetrazole as 0.45 mol L^{-1} solution in MeCN (2.0 mL, 0.90 mmol). After 80 min, the mixture was evaporated to dryness, saturated $NaHCO_3$ was added, and the product was extracted twice with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4 and evaporated to yield 0.618 g (86%) of **16**. ^{1}H NMR (500 MHz, CD_3CN): δ_{ppm} 7.83 (m, 4H, Pht), 7.49−7.46 (m, 2H, DMTr), 7.37−7.30 (m, 6H, DMTr), 7.25−7.21 (m, 1H, DMTr), 6.91−6.86 (m, 4H, DMTr), 4.42 (m, 1H, C*H*HON), 4.38−4.32 (m, 3H, CH*H*ON, H-1, and H-3), 4.01 (m, 1H, H-4), 3.84−3.67 (m, 8H, $POCH_2$ and 2 × OCH_3), 3.60 (m, 2H, H-2), 3.15 (m, 1H, H-5a), 3.05 (m, 1H, H-5b), 2.66 (t, 1H, J = 6.0 Hz, C*H*HCN), 2.55 (t, 1H, J = 6.0 Hz, CH*H*CN), 2.24−2.00 [m, 3H, $CH(CH_3)_3$ and CH_2CH_2ON], 1.93−1.85 [m, 1H, $CH(CH_3)_3$], 1.19−1.03 (m, 12H, 4 × CH_3); ^{31}P NMR (200 MHz, CD_3CN): δ_{ppm} 147.2; HRMS (ESI): $[M + Na]^+$ $C_{45}H_{52}N_3NaO_9P$ requires 832.3339, found 832.3311.

Synthesis of Oligodeoxyribonucleotide 5′-Glycoconjugate (20). The oligodeoxyribonucleotide **18** was synthesized on an Applied Biosystems 392 DNA synthesizer on 1.0 μmol scale using commercial 1000 Å CPG-succinyl-thymidine support and conventional phosphoramidite chemistry. Standard DNA coupling protocol was used except for a prolonged coupling time for phosphoramidite **12** (600 s, 0.13 mol L^{-1} solution in dry MeCN). After the oligonucleotide assembly, the support-bound material was removed from the synthesizer

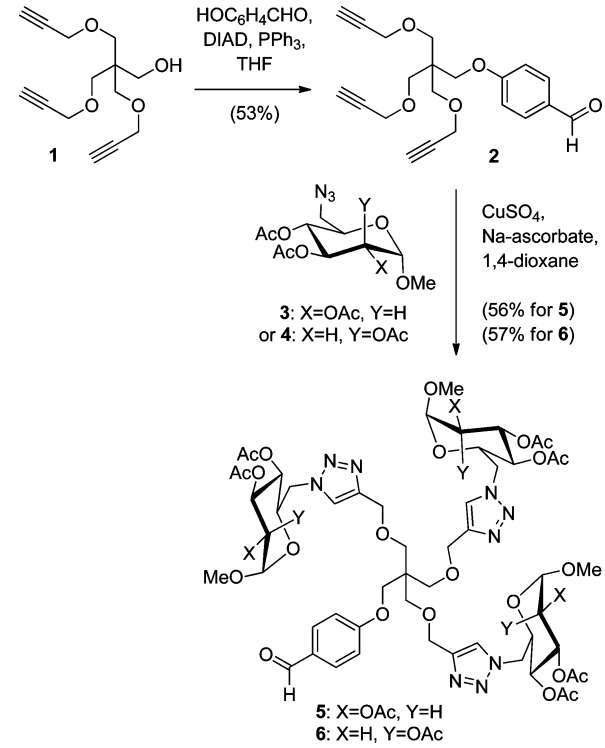
and the phthaloyl protections were removed by 0.5 mol L^{-1} hydrazinium acetate treatment; i.e., the support was suspended in a mixture of $NH_2NH_2 \cdot H_2O$, pyridine, and AcOH (0.124/4/1 v/v/v) for 30 min, washed with pyridine and MeCN, and dried. An aliquot of the support **19** (6.9 mg, 0.21 μmol) was transferred to microcentrifuge tube, and the glucoside cluster **5** was coupled to the aminooxy groups by adding the glycocluster as a 0.08 mol L^{-1} solution (55 μL, 4.2 μmol) in MeCN to the support. The mixture was shaken at ambient temperature for 24 h, filtered, washed with MeCN, and dried. To ensure the completeness of the reaction, oximation was repeated twice. The unreacted aminooxy groups were capped with acetone (1 h), and the support was dried. The oligodeoxyribonucleotide glycoconjugate was released from the support and deprotected with concentrated ammonia (33% aqueous NH_3, 16 h at 55 °C). The crude product was evaporated, dissolved in 30% aqueous MeCN, and purified by C18 RP HPLC. The authenticity of the conjugate **20** was verified by MS (ESI) spectroscopy: required 6817.2, found 6816.5.

Synthesis of Oligodeoxyribonucleotide 3′-Glycoconjugate (26). The general procedures were as described for **20**. Phosphoramidite **16** (0.11 mol L^{-1} solution in dry MeCN) was coupled to CPG-succinyl-thymidine support using a prolonged coupling time of 1200 s and 6 extra detritylations. The support-bound material **22** was removed from the synthesizer, and the phthaloyl protection was removed by hydrazinium acetate treatment as described for **20**. The support (18.8 mg, 0.56 μmol) was transferred to microcentrifuge tube, and the mannoside cluster **6** was coupled to the aminooxy group by adding the glycocluster as a 0.07 mol L^{-1} solution (80 μL, 5.7 μmol) in MeCN to the support. The mixture was shaken at ambient temperature for 24 h, filtered, washed with MeCN, and dried. Oximation with the glycocluster was repeated twice, followed by acetone treatment (1 h). The support **23** was dried and reloaded to the DNA synthesizer. The oligonucleotide chain assembly was continued by coupling of a thymidine residue (two consecutive couplings, 600 s each) and phosphoramidite **16** (three consecutive couplings, 1200 s each). The support-bound **24** was again removed from the synthesizer, the phthaloyl protection was removed, and the glucoside cluster **5** was coupled three times as a 0.09 mol L^{-1} solution (60 μL, 5.1 μmol) in MeCN to the support as described above. After acetone treatment (1 h) and drying, the support **25** was reloaded to the DNA synthesizer for the assembly of the remaining chain. Two consecutive couplings were again performed with the adjacent thymidine residue, whereas the rest of the chain was assembled using standard DNA coupling protocol. The oligodeoxyribonucleotide glycoconjugate was released from the support and deprotected with concentrated ammonia (33% aqueous NH_3, 16 h at 55 °C). The crude product was evaporated, dissolved in 30% aqueous MeCN, and purified by C18 RP HPLC. The authenticity of the conjugate **26** was verified by MS (ESI) spectroscopy: required 7599.6, found 7599.0.

RESULTS AND DISCUSSION

Synthesis of Glycoclusters (5,6). Previously (*54*) published tri-*O*-propargyl pentaerythritol (**1**) was used as a core molecule in the synthesis of trivalent glycoclusters (Scheme 1). First, an aldehyde functionality was introduced in the core to allow ligation of the clusters to aminooxy functionalized oligonucleotides. For this purpose, the free hydroxyl group of **1** was subjected to Mitsunobu reaction with 4-hydroxybenzaldehyde, which gave the desired scaffold **2** in 53% yield. Methyl 6-azido-6-deoxy-α-D-gluco- (**3**) and -α-D-mannopyranoside (**4**) (*55*) were used for construction of the clusters. Accordingly, copper(I)-catalyzed 1,3-dipolar cycloaddition (*57, 58*), the so-called click reaction, was applied to attach the sugarazide units to scaffold **2** to obtain glycoclusters **5** (56%) and **6** (57%). The advantage of clustering the sugars via the 6-position of the carbohydrate ring is that it allows variation of the aglycone moiety, which may in some cases affect the efficiency of lectin binding (*29*).

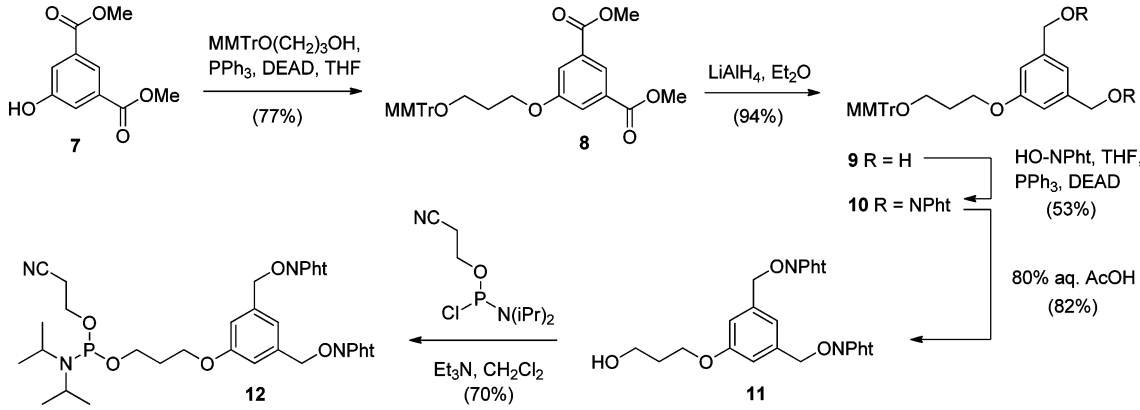
Scheme 1. Synthesis of Glycoclusters 5 and 6



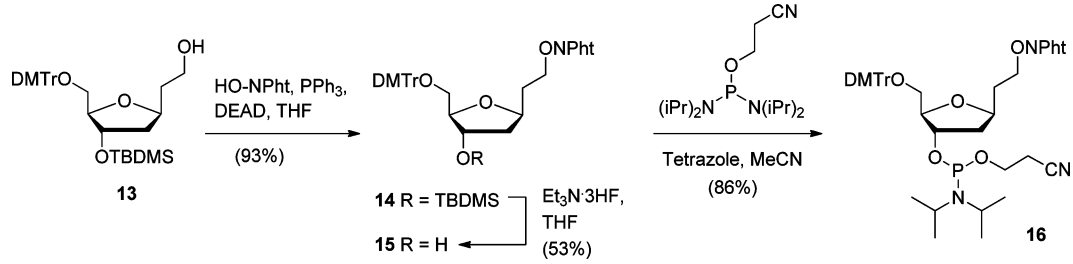
Synthesis of the 5′-Branching Unit (12). To enable conjugation of two trivalent glycoclusters to the 5′-end of an oligonucleotide, a phosphoramidite reagent bearing two phthaloyl-protected aminooxy functions (**12**, Scheme 2) was synthesized. This building block is compatible with conventional solid-phase oligonucleotide synthesis by the phosphoramidite strategy and it can be attached to the oligonucleotide chain by a somewhat elongated coupling cycle. Commercially available dimethyl 5-hydroxyisophthalate **7** was used as the starting material to which 4-methoxytrityl protected propanediol arm (prepared as corresponding tritylated propanediol (*56*)) was attached by Mitsunobu reaction (→ **8**, 77%). The methyl ester functions were then reduced to primary alcohols with lithium aluminum hydride to obtain **9** (94%). The phthaloyl protected aminooxy groups were generated by Mitsunobu reaction with *N*-hydroxyphthalimide (→ **10**, 53%). The 4-methoxytrityl protection was finally removed with acetic acid (→ **11**, 82%) and the exposed hydroxyl group was phosphitylated to give **12** (70%).

Synthesis of the Intrachain Branching Unit (16). To enable conjugation of trivalent clusters in desired positions within the oligonucleotide chain and, in particular, to allow introduction of dissimilar glycoclusters in different sites within the chain, a deoxyribose-derived C-glycosidic phosphoramidite reagent **16** (Scheme 3) bearing a phthaloyl protected aminooxy function was synthesized. Previously (*40*) prepared 5-(4,4′-dimethoxytrityl)-3-*O*-*tert*-butyldimethylsilyloxy-1,2-dideoxy-1-*C*-(2-hydroxyethyl)-β-D-*erythro*-pentofuranose (**13**) was used as a starting material. The primary hydroxyl group was converted to a phthaloyl protected aminooxy group by the Mitsunobu reaction to obtain **14** (93%). *tert*-

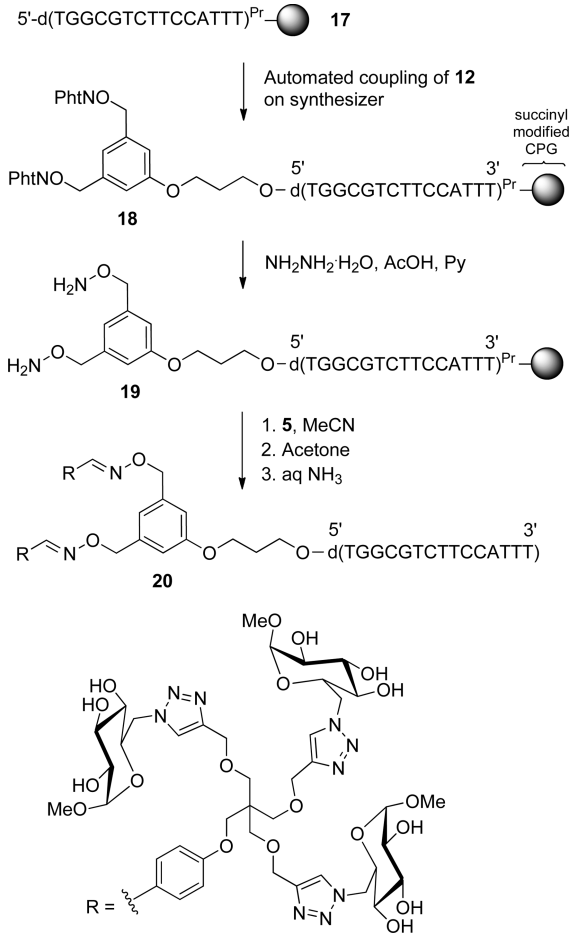
Scheme 2. Synthesis of the 5′ Branching Unit 12



Scheme 3. Synthesis of the Intrachain Branching Unit 16



Scheme 4. Synthesis of Oligonucleotide 5′-Glycoconjugate 20[a]



[a] Abbreviation Pr used for the fully protected oligodeoxyribonucleotide.

Butyldimethylsilyl protection was removed (→ **15**, 53%), and the hydroxyl group was phosphitylated to give **16** (86%).

Preparation of an Oligonucleotide 5′-Glycoconjugate (20). The synthesis of the oligonucleotide conjugate containing two trivalent glucopyranosyl clusters at the 5′-terminus was initiated by coupling phosphoramidite **12** to the 5′-hydroxy of otherwise fully protected support-bound oligonucleotide (**17**, Scheme 4). The support was removed from the synthesizer and the phthaloyl protections were removed from the solid-supported oligomer (**18**) by treatment with hydrazinium acetate in pyridine. The exposed aminooxy groups of **19** were then oximated with 20 equiv of the aldehyde functionalized glucoside cluster **5** in acetonitrile for 24 h. To ensure the efficiency of oximation, the treatment was repeated twice. The remaining

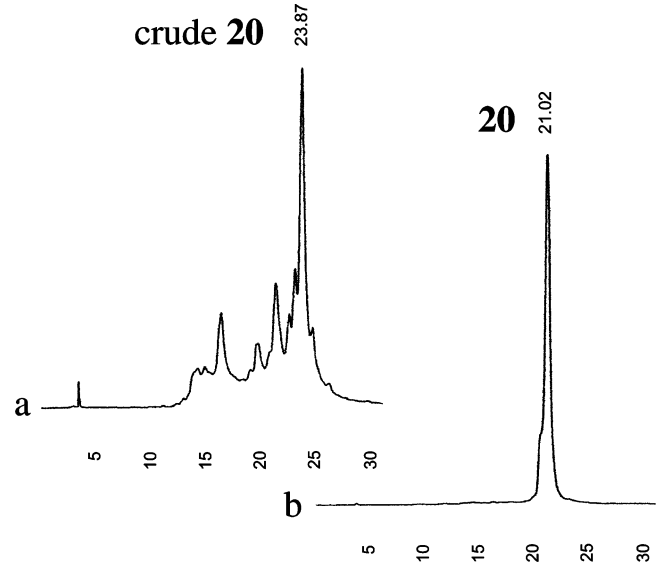
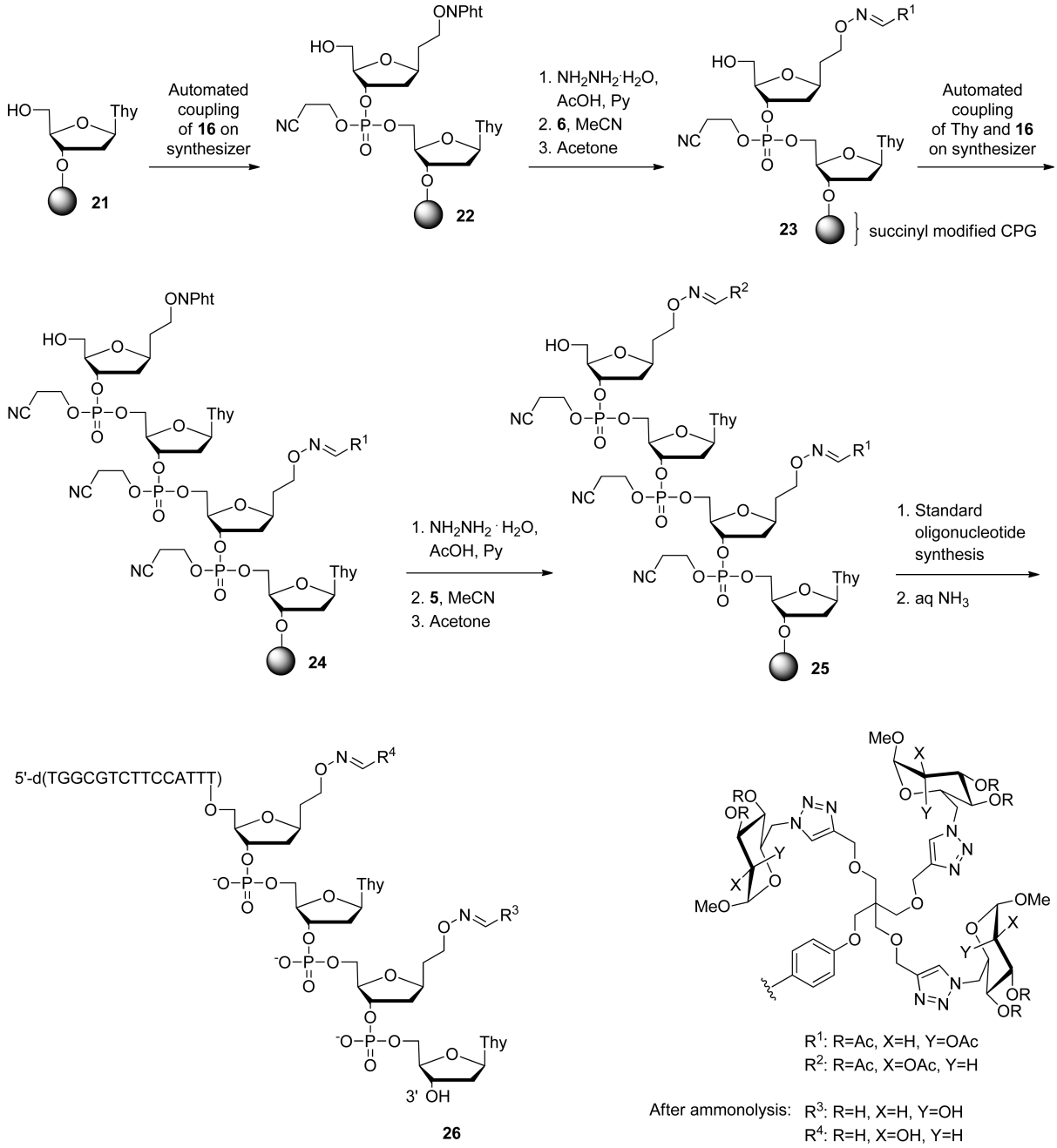


Figure 2. RP HPLC trace of the crude (a) and purified (b) oligonucleotide glycoconjugate **20**. For the chromatographic conditions, see experimental procedures.

Scheme 5. Synthesis of Oligonucleotide 3′-Glycoconjugate 26



unreacted aminooxy groups were capped with acetone, after which the conjugate was released from the support and deprotected by standard ammonolysis (16 h, at 55 °C). The crude product was purified by RP HPLC (Figure 2) and analyzed by MS (ESI) spectroscopy. As seen from the HPLC trace, the desired conjugate **20** was the main product. In addition, an incompletely oximated conjugate, i.e., conjugate having only one sugar cluster attached, was detected in a minor amount. All in all, the method proved to be successful in the preparation of glycoconjugates bearing six sugars that may adopt a more or less parallel orientation in space.

Preparation of an Intrachain Oligonucleotide Glycoconjugate (26). Intrachain branching unit **16** was used for preparation of a glycoconjugate with two dissimilar glycoclusters attached close to the 3′-terminus of the oligonucleotide (**26**, Scheme 5). The first branching unit was coupled to CPG-succinyl-thymidine support after which the support (**22**) was removed from the synthesizer and the phthaloyl protection was removed by the hydrazinium acetate treatment discussed above. The exposed aminooxy group was oximated with 10 equiv of the mannoside cluster **6** in acetonitrile for 24 h. Oximation was repeated twice followed by capping with acetone. HPLC trace of the crude conjugate released by ammonolysis from an aliquot of support **23** can be seen in Figure 3. The support (**23**) was reloaded to the synthesizer and one thymidine unit and another branching unit **16** were coupled using consecutive couplings for both compounds to enhance the coupling efficiency. The support was again removed from the synthesizer and the phthaloyl protection was removed from the tetrameric structure (**24**) as before. The second oximation step was carried out three times using now the glucoside cluster **5**. After acetone capping, the support **25** was again reloaded to the synthesizer. The first thymidine residue following the branching unit was introduced by two consecutive couplings, while the rest of the oligonucleotide chain was assembled by standard DNA coupling protocol. The conjugate was released and deprotected by ammonolysis (16 h, at

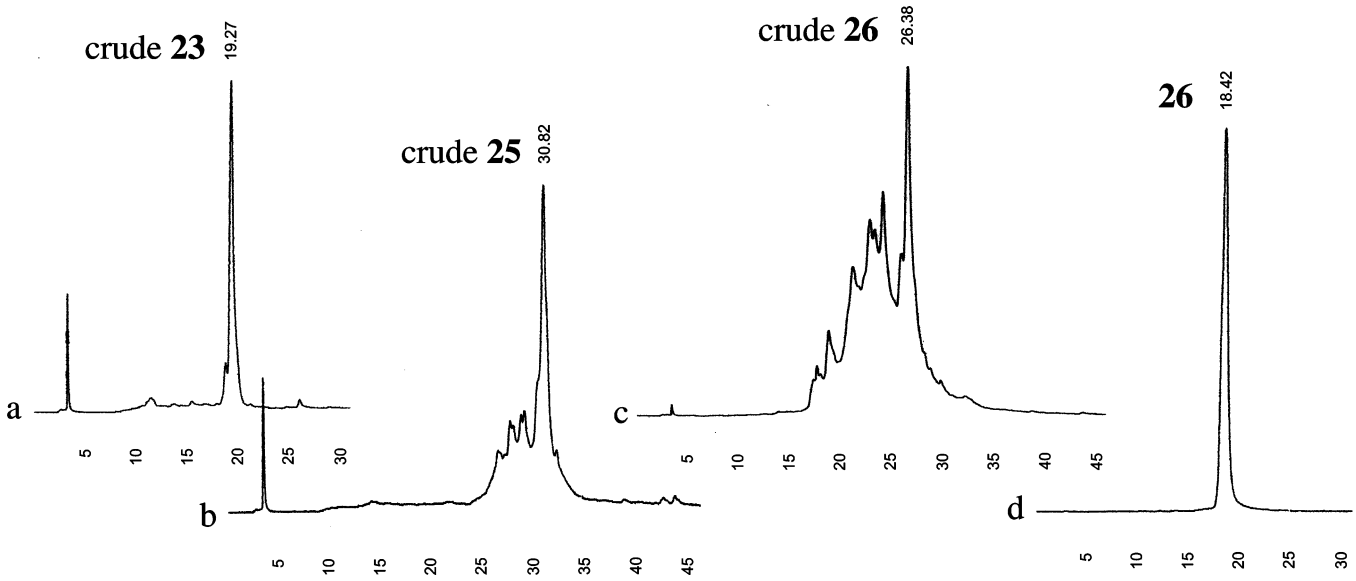


Figure 3. RP HPLC traces of the crude oligonucleotide glycoconjugates released by ammonolysis from aliquots of supports **23** (a), and **25** (b), and the HPLC trace of the crude (c) and purified (d) oligonucleotide glycoconjugate **26**. For the chromatographic conditions, see experimental procedures.

55 °C), followed by purification by RP HPLC (Figure 3) and analysis by MS (ESI) spectroscopy. Conjugate **26** was the main product although numerous side products could be detected, especially due to incomplete coupling of the second branching unit. Oxidation on the other hand seemed to proceed almost to completion on the basis of the HPLC traces of the crude intermediates released by ammonolysis from aliquots of supports **23** and **25** (Figure 3).

CONCLUSION

It has been shown that trivalent glycoclusters bearing a benzaldehyde moiety for subsequent conjugation (**5**, **6**) may be conveniently prepared from tri-*O*-propargylpentaerythritol in two steps by applying sequentially the Mitsunobu and click reactions. These clusters may rather efficiently be conjugated to solid supported oligonucleotides incorporating aminooxy functionalized branching units. On using a 5′-terminal 3,5-bis(aminooxymethyl)phenyl group (**12**) for branching, a conjugate bearing six sugars having a more or less parallel orientation is conveniently obtained. When a 2′-deoxyribose-derived aminooxyethyl *C*-glycoside (**16**) is inserted into the oligonucleotide chain, the prefabricated clusters may be attached to desired sites within the chain. This can be done in a stepwise manner, and hence, more than one type of clusters may be introduced.

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Supporting Information Available: NMR spectral data for **2**, **5**, **6**, **8**−**12**, **14**−**16**, and MS spectral data for **20** and **26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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