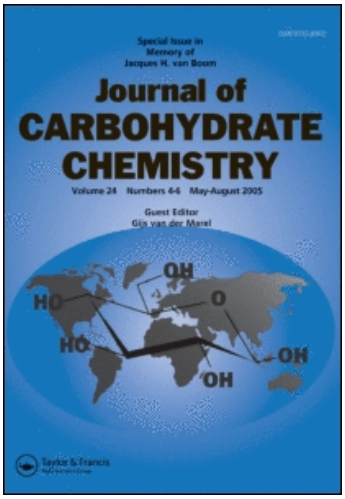

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Synthetic Studies Toward Gangliosides and Their Analogs: Synthesis of Appropriately Protected Core Oligosaccharides as Construction Blocks

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SYNTHETIC STUDIES TOWARD GANGLIOSIDES AND THEIR ANALOGS: SYNTHESIS OF APPROPRIATELY PROTECTED CORE OLIGOSACCHARIDES AS CONSTRUCTION BLOCKS[+]

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ABSTRACT

2-(Trimethylsilyl)ethyl 2,3,6,2'-tetra-O-acetyl-3'-O-benzyl-6'-O-benzyloxymethyl-β-D-lactoside (9) was synthesized starting from acetobromolactose (1) via 2-(trimethylsilyl)ethyl β-D-lactoside (3). Compound 9 was converted to the trisaccharide derivative 17 after coupling with 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide (11). Coupling of 17 with acetobromogalactose (19) gave the tetrasaccharide 20.

INTRODUCTION

As more and more biological functions of gangliosides[2] are being revealed, their laboratory synthesis is becoming increasingly stimulating and rewarding. The fact that their total synthesis in the labo-

[+]Synthetic studies on sialoglycoconjugates, Part 2. For Part 1, see ref. 1.

ratory involves some difficult steps in itself is sufficient to make the work fascinating for chemists.

Gangliosides display diverse structural characteristics with varying degrees of complexity. Reports concerning their total chemical synthesis have so far been limited to only a few in number.[3] In the present paper we describe the synthesis of some oligosaccharides in their potential form for further conversion to the desired gangliosides or their analogs.

RESULTS AND DISCUSSION

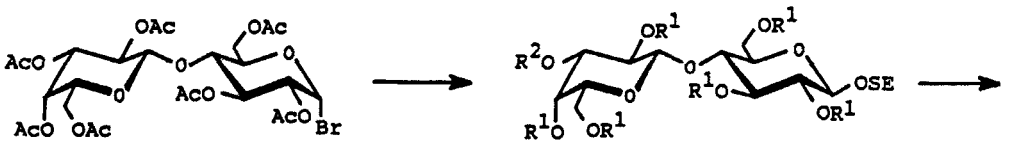
Of the various approaches available for the preparation of oligosaccharides we have largely adopted the stepwise construction technique to build target molecules. D-Lactose has, however, remained as our base material. The various glycosyl donors and acceptors reported here have therefore been designed to fulfil the structural requirements of our target molecules. Compounds 9, 17 and 20, suitable for further modification to gangliosides G_{M3}, G_{M2}, G_{M1}, etc. respectively, were synthesized starting from 2-(trimethylsilyl)ethyl β-D-lactoside[4] (3).

Compound 3 was obtained as fine crystals from 2-(trimethylsilyl)-ethyl hepta-O-acetyl-β-D-lactoside (2) by Zemplen deacetylation. Compound 2[4] was prepared from the easily obtainable crystalline aceto-bromolactose[5] (1) by coupling with 2-(trimethylsilyl)ethanol under Koenigs-Knorr conditions[6] in the presence of $AgClO_4$ (one equiv) and Ag_2CO_3 in dichloromethane at 25 °C. Complete stereospecificity was observed resulting in the desired 1,2-*trans* glycoside in over 60% yield. When $AgClO_4$ was used in only catalytic amounts, formation of the 1,2-ortho ester was preponderant. It is therefore reasonable to assume that (i) formation of the well accepted cyclic oxocarbonium ion intermediate which eventually leads to the 1,2-*trans* glycoside is effected via a glycosyl perchlorate (cf 4) and (ii) the strongly nucleophilic ClO_4^- ion forms an ion pair with the oxocarbonium ion thus stabilizing and enhancing the possibility of the acceptor reacting at the anomeric position to give the desired glycoside. Glycosides 12, and 20 were also prepared with ease under similar conditions in high

yields. Use of one equivalent of the perchlorate was not essential in the glycosidation of 9 with 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide (11) due to the presence of the phthalimido substituent in the latter compound.

Formation of the glycosyl bromide 11 from its acetate precursor is noteworthy. When the latter, namely 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-D-galactopyranose (ca. 1:3 mixture of α and β) prepared from D-galactosamine hydrochloride by the method of Lemieux et al.[7], was treated with a mixture of acetic anhydride and hydrogen bromide in glacial acetic acid solution (28%, w/v), the crystalline α-bromide 11 was obtained instead of the frequently described β-isomer.[3] Under these conditions the D-glucosamine derivative gives the corresponding β-bromide.[7] The anomeric configuration of the halide 11 was unambiguous from its ^{1}H NMR spectrum (δ 6.69, d, $J_{1,2}$ = 3.3 Hz).

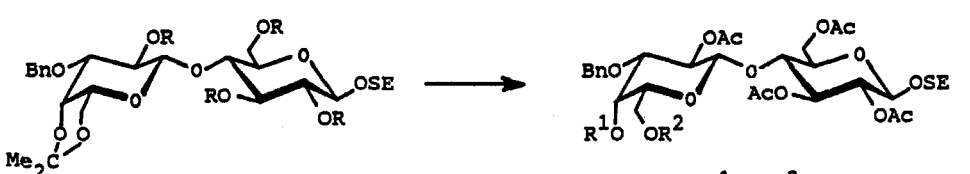
SCHEME 1



1

2 $R^1 = R^2 = Ac$
3 $R^1 = R^2 = H$
4 $R^1 = H$, $R^2 = Bn$
5 $R^1 = Ac$, $R^2 = Bn$

SE = $CH_2CH_2Si(CH_3)_3$
Bn = CH_2Ph

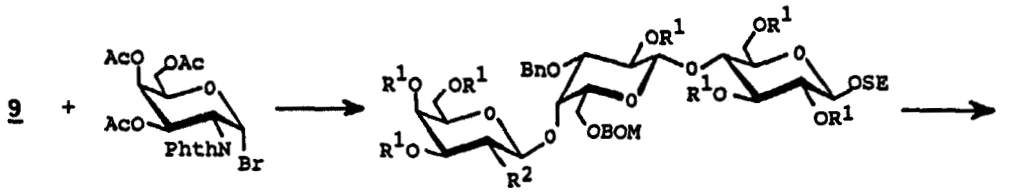


6 R = H
7 R = Ac

8 $R^1 = R^2 = H$
9 $R^1 = H$, R^2 = BOM
10 $R^1 = Ac$, R^2 = BOM

BOM = CH_2OCH_2Ph

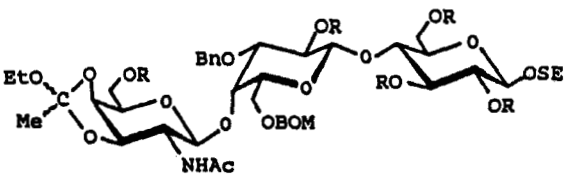
SCHEME 2



11

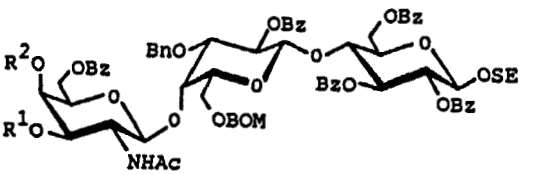
12 R^1 = Ac, R^2 = NPhth
13 R^1 = H, R^2 = NPhth
14 R^1 = H, R^2 = NHAc

NPhth = phthalimido

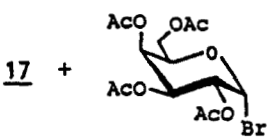


15 R = H
16 R = Bz

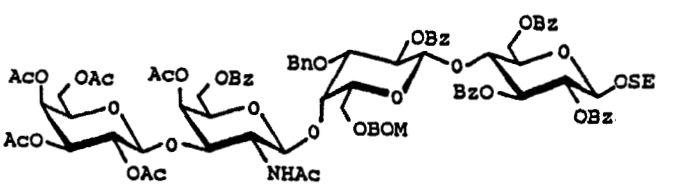
Bz = COPh



17 R^1 = H, R^2 = Ac
18 R^1 = R^2 = Ac



19



20

Paulsen and Bunsch[8] also obtained the α-bromide (11) in a similar preparation but the melting point of their product was considerably lower than that of ours.

The stannylene oxide mediated selective etherification of 3 to give 2-(trimethylsilyl)ethyl 3'-O-benzyl-β-D-lactoside (4) (Scheme 1) could be achieved in 75% yield after column chromatography. Selective monosubstitution in this compound was confirmed from the low field shift of the remaining secondary carbon bound ring protons and the coupling constants of various protons in the ^{1}H NMR spectrum of its acetate (5) as compared with the spectrum of compound 4. Thus, the H-3' signal for compound 5 appeared at 3.48 ppm as a characteristic double of doublets having spacings of 10.3 and 3.3 Hz. Formation of the 4',6'-O-isopropylidene derivative 6, on condensation of 2,2-dimethoxypropane with 4, also supports the structure of compound 4. The O-deisopropylidenation of 7 to yield 2-(trimethylsilyl)ethyl 2,3,6,2'-tetra-O-acetyl-3'-O-benzyl-β-D-lactoside 8 was done using 90% aqueous acetic acid without affecting the trimethylsilylethyl group. Selective benzyloxymethylation of 6'-OH in the diol 8 could be effectively carried out in the presence of N,N-diisopropylethylamine in dichloromethane to yield 2-(trimethylsilyl)ethyl-2,3,6,2'-tetra-O-acetyl-3'-O-benzyl-6'-O-benzyloxymethyl-β-D-lactoside (9). Ether substitutions at the C-3' and C-6' positions of the glycosyl acceptor 9 assure a clean reaction at its 4'-OH in glycosidations to follow. The ^{1}H NMR spectrum of 12 showed signals characteristic of those of the starting materials 9 and 11. That the new glycosidic bond formed in this coupling reaction is indeed β was confirmed after its partial deprotection to 14 and will be discussed later.

As demonstrated in the preparation of the trisaccharide 12 by coupling 9 with the glycosyl bromide 11, 9 can be employed as a suitable acceptor for conversion to other higher saccharides in combination with appropriate glycosyl donors by substitution at 4'-OH. Moreover, since chain extension at the C-3' position of 9 and 10 is possible, these compound can be used for the synthesis of ganglioside G_{M3}.

Compound 12 was converted to the trisaccharide acceptor 17 in four steps in about 46% overall yield (Scheme 2). Zemplen deacetyla-

tion followed by successive treatments with hydrazine monohydrate and acetic anhydride gave the partially protected trisaccharide 14. Three doublets in the ^{1}H NMR spectrum of 14 appeared at δ 4.30, 4.33 and 4.39 with spacings of 8.1, 8.1 and 8.4 Hz respectively and are attributable to H-1, H-1' and H-1", and consistent with the β glycosidic linkages in the trisaccharide.

With a view to selectively benzoylating the hydroxyl groups of 14, this latter compound was treated with 2.1 equiv of benzoyl chloride in anhydrous pyridine at subambient temperatures (up to -60 °C). This, however, only gave four monobenzoylated derivatives, presumably, the 3"-O-, 6"-O-, 2-O- and 6-O-monobenzoates (in decreasing order of their yields), instead of the desired 6,6"-di-O-benzoate. This inference was drawn from the results obtained in the partial benzoylation of 3 and 4 under similar conditions.[1] The structures of these monobenzoates were, however, not further investigated.

In an alternative approach, compound 14 was converted to the acceptor 17 in three steps as follows. Treatment of 14 with triethyl orthoacetate[9] gave the 3",4"-O-2-(ethoxy)ethylidene derivative (15) along with its 4",6"-O-analog in about a 4:1 ratio (TLC). Without further purification this mixture was treated with benzoyl chloride in pyridine at -25 °C and the resulting product on treatment with aqueous acetic acid followed by purification on a silica gel column afforded the required acceptor 17 having a free hydroxyl group at C-3" position. The structure of 17 was confirmed from the ^{1}H NMR spectra of 17 and its acetate 18. 17 as well as 18 correspond to the core trisaccharide of gangliosides G_{M2} and G_{D2}.

Tetrasaccharide 20 was obtained, as described earlier, by coupling the trisaccharide acceptor 17 with acetobromogalactose (19) (Scheme 2). Its structure and stereochemistry were confirmed by ^{1}H as well as ^{13}C NMR spectroscopy. Details are provided in the Experimental Section.

This tetrasaccharide after the introduction of the sialic acid(s) unit at 3'-O-position shall form the complete sugar skeleton of gangliosides G_{M1} and G_{D1b}.

Coupling with suitably protected mono or disialic acid residues at the desired positions of compounds derived from 9, 17 and 20 fol-

lowed by introduction of the suitable ceramide moiety shall be the subject matter of subsequent articles in this series.

EXPERIMENTAL

General Procedures. Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Specific rotations were determined with a Union PM-201 polarimeter, and ^{1}H NMR spectra were recorded at 270 MHz with a JEOL JNM-GX270 spectrometer. Column chromatography was conducted using silica gel (Wako Co.; 200 mesh) and was accomplished with the solvent systems (v/v) specified. Concentration and evaporations were conducted *in vacuo*.

2-(Trimethylsilyl)ethyl Hepta-O-acetyl-β-D-lactoside (2). A mixture of acetobromolactose[5] (1, 20 g, 28.7 mmol) and powdered molecular sieves (4 A, 10 g) in abs dichloromethane (150 mL) was stirred for several h at 25 °C and then added to a stirred mixture (stirred for several h in the dark) of silver perchlorate (1 molar equiv), silver carbonate (1 molar equiv), 2-(trimethylsilyl)ethanol (2.5 molar equiv) and powdered molecular sieves (4 A, 15 g) in abs dichloromethane (75 mL), and the stirring was continued for 9 h at 25 °C in the dark. Solids were then separated by filtration through a celite-pad and the residue was washed with dichloromethane. The combined filtrate and washings on concentration gave a colorless syrup which on silica gel (250 g) column chromatography using 8:1 and 3:1 n-hexane-ethyl acetate as successive eluants afforded 2 as a colorless glass (13.2 g, 63%): $[\alpha]_D$ -11.5° (c 0.5, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.83-1.03 (m, 2H, CH_2SiMe_3), 1.97, 2.03, 2.04, 2.05, 2.06, 2.12 and 2.15 (7s, 21H, 7 AcO), 3.52-3.63 (m, 2H, one of the $OCH_2CH_2SiMe_3$ and H-5), 3.80 (t, 1H, $J_{3,4}$ = 9.2 Hz and $J_{4,5}$ = 9.5 Hz, H-4), 3.87 (near t, 1H, $J_{5',6'}$ = 7.0 Hz, H-5'), 3.94 (m, 1H, the remaining one H of the $OCH_2CH_2SiMe_3$), 4.02-4.18 and 4.42-4.52 (2m, 4H, H-6 and H-6'), 4.47 (2d, $J_{1,2}$ = $J_{1',2'}$ = 7.7 Hz, H-1 and H-1'), 4.88 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 4.95 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, $J_{3',4'}$ = 3.3 Hz, H-3'), 5.11 (dd, 1H, H-2'), 5.19 (t, 1H, H-3), and 5.35 (near d, 1H, H-4').

Anal. Calcd for $C_{31}H_{48}O_{18}Si$ (736.79): C, 50.54; H, 6.57. Found: C, 50.27; H, 6.61.

2-(Trimethylsilyl)ethyl β-D-Lactoside (3). Compound 2 (13.0 g, 17.63 mmol) in abs methanol was deacetylated by Zemplen's method to give 3 as crystals (7.76 g, quantitative): mp 186-187 °C; $[\alpha]_D$ -12.5° (c 0.5, methanol); ^{1}H NMR (CD_3OD) δ 0.87-1.08 (m, 2H, $C\underline{H}_2SiMe_3$), 3.55-3.65 and 3.91-4.01 (2m, 2H, $OC\underline{H}_2CH_2SiMe_3$), and 4.27 and 4.33 (2d, 2H, J = 8.1 and 7.0 Hz, H-1 and H-1').

Anal. Calcd for $C_{17}H_{34}O_{11}Si$ (442.53): C, 46.14; H, 7.74. Found: C, 45.91; H, 7.65.

2-(Trimethylsily)ethyl 3'-O-Benzyl-β-D-lactoside (4). A mixture of 3 (7.5 g, 17.03 mmol) and powdered molecular sieves (4 A, 7.5 g) in dry benzene (75 mL) was stirred for 2 h at 80 °C and di-n-butyltin oxide (1.5 molar equiv) was added to it and stirring was continued for 5 h at 80 °C. This was followed by the addition of tetra-n-butylammonium bromide (0.5 molar equiv) and benzyl bromide (15 molar equiv). After heating the reaction mixture for another 80-90 min benzene was removed by evaporation. Excess benzyl bromide in the residual mixture was removed by repeated extraction with n-hexane. After adding dichloromethane the insolubles were removed by filtration through a layer of celite and washed with dichloromethane. The combined filtrate and washings on evaporation yielded a syrup which on purification on a silica gel (175 g) column using dichloromethane and 10:1 dichloromethane-methanol as successive eluants gave 4 as needles (6.8 g, 75%). The compound was recrystallized from dichloromethane: mp 185-185.5 °C; $[\alpha]_D$ +4.3° (c 0.5, methanol); ^{1}H NMR ($CDCl_3$+CD_3OD) δ 0.89-1.10 (m, 2H, $C\underline{H}_2SiMe_3$), 4.30, 4.35 (2d, 2H, $J_{1,2} = J_{1',2'} = 7.7$ Hz, H-1 and H-1'), 4.65-4.75 (2d, 2H, $C\underline{H}_2C_6H_5$) and 7.25-7.40 (m, 5H, $C_6\underline{H}_5$).

Anal. Calcd for $C_{24}H_{40}O_{11}Si$ (532.66): C, 54.12; H, 7.57. Found: C, 54.38; H, 7.52.

4 was further characterized as its hexaacetate 5: mp 52-53 °C; $[\alpha]_D$ +14.3° (c 0.6, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.83-1.02 (m, 2H, $C\underline{H}_2SiMe_3$), 2.01, 2.03, 2.04, 2.08, 2.10, 2.15 (6s, 18H, 6 AcO), 3.48 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, $J_{3',4'}$ = 3.3 Hz, H-3'), 3.50-3.62 (m, 2H, H-5 and one of the $OC\underline{H}_2CH_2SiMe_3$), 3.74 (t, 1H, $J_{3,4}$ = 9.2 Hz, H-4), 3.77 (near m, 1H, H-5'), 3.91 (m, 1H, the remaining one H of the $OC\underline{H}_2CH_2SiMe_3$), 4.11 (m, 3H, H-6a and H-6'), 4.36, 4.47 (2d, 2H, $J_{1,2} = J_{1',2'}$ = 8.1 Hz, H-1 and H-1'), 4.37, 4.68 (2d, 2H, $C\underline{H}_2C_6H_5$), 4.43 (dd, 1H, H-6b), 4.47 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.86 (dd, 1H,

$J_{2,3}$ = 9.5 Hz, H-2), 5.03 (dd, 1H, H-2'), 5.17 (t, 1H, H-3), 5.47 (near d, 1H, H-4'), and 7.24-7.37 (m, 5H, $C_6\underline{H}_5$).

2-(Trimethylsilyl)ethyl 2,3,6,2'-Tetra-O-acetyl-3'-O-benzyl-4',6'-O-isopropylidene-β-D-lactoside (7). A solution of 4 (6.60 g, 12.39 mmol) in dry N,N- dimethylformamide (DMF, 250 mL) was stirred at ambient temperature for 1 h in the presence of powdered Drierite (6.75 g). After cooling the mixture in an ice bath, 2,2-dimethoxypropane (15 molar equiv) was added and pH of the solution was brought to nearly 3 by the addition of p-toluenesulfonic acid monohydrate. During the continued stirring of the reaction mixture the temperature of the bath was allowed to come to about 15 °C. Stirring was stopped after 1 h and the reaction mixture was neutralized with Amberlite IR 410 (OH^-) resin. Filtration, evaporation of the solvent, and purification by silica gel (150 g) column chromatography using dichloromethane and 100:3 dichloromethane-methanol as successive eluants gave 6 as needles (6.18 g, 87%): mp 171.5-172.5 °C; $[\alpha]_D$ -13.5° (c 0.4, dichloromethane); ^{1}H NMR ($CDCl_3$+CD_3OD) δ 0.88-1.08 (m, 2H, $C\underline{H}_2SiMe_3$), 1.34, 1.46 (2s, 6H, $C(C\underline{H}_3)_2$), 4.07 (near d, 1H, $J_{3',4'}$ = 3.3 Hz, H-4'), 4.31, 4.45 (2d, 2H, $J_{1,2} = J_{1',2'}$ = 8.1 Hz, H-1 and H-1'), 4.63-4.74 (2d, 2H, $C\underline{H}_2C_6H_5$), and 7.25-7.36 (m, 5H, $C_6\underline{H}_5$).

Acetylation of compound 6 using acetic anhydride in pyridine gave crystals of 7 (7.99 g, quantitative): mp 90-92 °C; $[\alpha]_D$ +8.8° (c 0.5, dichloromethane); ^{1}H NMR($CDCl_3$) δ 0.87-1.03 (m, 2H, $C\underline{H}_2SiMe_3$), 1.37, 1.44 (2s, 6 H, $C(C\underline{H}_3)_2$), 2.03, 2.04, 2.08, 2.09 (4s, 12 H, 4 AcO) 3.41 (dd, 1H, $J_{2',3'}$ = 10.1 Hz, $J_{3',4'}$ = 3.6 Hz, H-3'), 4.10 (near d, 1H, H-4'), 4.12 (dd, 1H, H-6a), 4.28, 4.47 (2d, 2H, $J_{1,2} = J_{1',2'}$ = 8.1 Hz, H-1 and H-1'), 4.45 (dd, 1H, H-6b), 4.52-4.64 (dd, 2H, $C\underline{H}_2C_6H_5$), 4.88 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 5.17 (dd, 1H, H-2'), 5.18 (t, 1H, $J_{3,4}$ = 9.2 Hz, H-3), and 7.26-7.34 (m, 5H, $C_6\underline{H}_5$).

Anal. Calcd for $C_{35}H_{52}O_{15}Si$ (740.87): C, 56.74; H, 7.07. Found: C, 56.54; H, 7.12.

2-(Trimethylsilyl)ethyl 2,3,6,2'-Tetra-O-acetyl-3'-O-benzyl-6'-O-benzyloxymethyl-β-D-lactoside (9). A solution of 7 (7.75 g, 10.46 mmol) in aqueous acetic acid (150 mL, 90%, v/v) was stirred for 10 h at 45 °C. Concentration and coevaporation with toluene gave 8 as colorless glass (7.33 g, quantitative): $[\alpha]_D$ +2° (c 0.5, dichloromethane); ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 0.83-1.02 (m, 2H, $C\underline{H}_2SiMe_3$), 2.03,

2.04, 2.07, 2.09 (4S, 12H, 4 AcO), 4.35, 4.47 (2d, 2H, $J_{1,2} = J_{1',2'}$ = 7.7 Hz, H-1 and H-1'), and 7.28-7.36 (m, 5H, $C_6\underline{H}_5$). To a mixture of 8 (7.2 g, 10.28 mmol) and powdered molecular sieves (4 A, 7.5 g) in abs dichloromethane (150 mL) under nitrogen atmosphere and maintained at about 5 °C were added diisopropylethylamine (3 molar equiv) and benzyloxymethyl chloride (2 molar equiv) in succession and stirring was continued for 24 h at 25 °C. Another dose of the reagents was then introduced and the mixture was stirred for another 16 h. It was then filtered through a celite-bed and the residue was washed with dichloromethane. The combined filtrate and washings were then washed successively with dilute hydrochloric acid, aqueous sodium carbonate solution and water and was then dried (sodium sulfate). Solvent re moval yielded a light yellow oil which on column chromatography on silica gel (200 g) using 8:1 and 3:1 n-hexane-ethyl acetate as successive eluants gave 9 as a colorless glass (5.06 g, 60%): $[\alpha]_D$ +4.2° (c 0.5, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.82-1.01 (m, 2H, $C\underline{H}_2SiMe_3$), 2.01, 2.02, 2.03, 2.08 (4S, 12H, 4 AcO), 3.43 (dd, 1H, $J_{2',3'}$ = 10.2 Hz, $J_{3',4'}$ = 3.6 Hz, H-3'), 3.47-3.62 (m, 3H, H-5, H-5' and one of the two $OC\underline{H}_2CH_2SiMe_3$), 3.73 (t, 1H, $J_{3,4}$ = 9.2 Hz, H-4), 3.78, 3.89 (2dd, 2H, H-6') 3.88-3.97 (m, 1H, the remaining one H of the two $OC\underline{H}_2CH_2SiMe_3$), 4.03 (near d, 1H, H-4'), 4.10, 4.43 (2 dd, 2H, H-6), 4.30, 4.46 (2d, 2H, $J_{1,2} = J_{1',2'}$ = 8.1 Hz, H-1 and H-1'), 4.47-4.70 (4d, 4H, 2 $C\underline{H}_2C_6H_5$), 4.76-4.83 (2d, 2H, $OC\underline{H}_2Bn$), 4.87 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 5.09 (dd, 1H, H-2'), 5.16 (t, 1H, H-3), and 7.26-7.37 (m, 10H, $2C_6\underline{H}_5$).

Anal. Calcd for $C_{40}H_{56}O_{16}Si$ (820.96): C, 58.52; H, 6.88. Found: C, 58.76; H, 6.98.

9 on acetylation with pyridine-acetic anhydride gave 10 as a colorless foam: $[\alpha]_D$ +9.7° (c 0.9, dichloromethane). Its ^{1}H NMR resembled that of compound 9 except for the appearance of H-4' at δ 5.56 ($J_{3',4'}$ = 3.5 Hz).

2-(Trimethylsilyl)ethyl O-(3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1→4)-O-(2-O-acetyl-3-O-benzyl-6-O-benzyloxymethyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (12). As the glycosyl donor, 2,3,6-tri-O-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide (11) was prepared from D-galactosamine hydrochloride by the method of Lemieux et al.[7] as

fine needles: mp 179-180 °C; $[\alpha]_D$ +112.1° (c 0.6, dichloromethane); ^1H NMR ($CDCl_3$) δ 1.91, 2.08, and 2.17 (3s, 9H, 3 AcO), 4.13-4.27 (m, 2 H, H-6), 4.60 (t, 1 H, H-5), 4.83 (dd, 1H, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 11.7 Hz, H-2), 5.72 (m, 1 H, H-4), 6.53 (dd, 1H, H-3), 6.69 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), and 7.75-7.89 (2m, 4H, NPhth). Coupling of 9 (4.5 g, 5.48 mmol) and 11 (1.5 molar equiv) was carried out in the same manner as described for the preparation of compound 2, reaction time being 12 h at 25 °C. 12 was obtained as colorless glass after silica gel column chromatography using 8:1 and 2:1 n-hexane-ethyl acetate as successive eluants (5.56 g, 82% based on 9): $[\alpha]_D$ +5.5° (c 0.5, dichloromethane); ^1H NMR ($CDCl_3$) δ 0.81-1.03 (m, 2H, $C\underline{H}_2SiMe_3$), 2.01, 2.03, 2.14 (3s, 9H, 3 AcO), 2.04, 2.05 (2s, 12H, 4 AcO), 5.47 (near d, 1H, $J_{3'',4''}$ = 3.3 Hz, H-4, H-4"), 5.97 (dd, $J_{2''3''}$ = 11.7 Hz, H-3"), and 6.85-7.9 (m, 14H, NPhth, 2 $CH_2C_6\underline{H}_5$).

Anal. Calcd for $C_{60}H_{75}NO_{25}Si$ (1238.33): C, 58.20; H, 6.11; N, 1.13. Found: C, 58.47; H, 5.99; N, 1.10.

2-(Trimethylsilyl)ethyl O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-(1→4)-O-(3-O-benzyl-6-O-benzyloxymethyl-β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (14). Compound 12 (4.0 g, 4.04 mmol) was deacetylated by Zemplen's method and the product obtained was taken up in ethanol (80 mL) and hydrazine monohydrate (20 mL) was then added to it under a nitrogen atmosphere. The solution was then heated at 80 °C for 1.5 h. After removing ethanol by evaporation the residue was repeatedly coevaporated with methanol. The dry product thus obtained was then treated with acetic anhydride (20 mL) in dry methanol (200 mL) at room temperature for 1.5 h. The reaction mixture was then concentrated to dryness and was chromatographed on a column of silica gel using 100:3 dichloromethane-methanol as eluant to afford 14 as a colorless glass (1.94 g, 70.1% based on 12): $[\alpha]_D$ -10.2° (c 0.6, dichloromethane); ^1H NMR ($CDCl_3$+CD_3OD) δ 0.90-1.10 (m, 2H, $C\underline{H}_2SiMe_3$), 1.70 (s, 3H, AcN), 4.30, 4.33, 4.39 (3d, 3H, J = 8.1, 8.1, and 8.4 Hz, H-1, H-1' and H-1"), 4.55-4.91 (6d, 6H, 2 $C\underline{H}_2C_6H_5$ and $C\underline{H}_2OBn$), and 7.25-7.43 (m, 10H, 2 $CH_2C_6\underline{H}_5$).

Anal. Calcd for $C_{40}H_{61}NO_{17}Si$ (856.00): C, 56.13; H, 7.18; N, 1.64. Found: C, 56.40; H, 7.05; N, 1.63.

2-(Trimethylsilyl)ethyl O-(2-Acetamido-4-O-acetyl-6-O-benzoyl-2-deoxy-β-D-galacopyranosyl)-(1→4)-O-(2-O-benzoyl-3-O-benzyl-6-O-benzyloxymethyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (17). To a stirred solution of 14 (1.5 g, 1.75 mmol) in abs toluene (75 mL) at 80 °C, triethyl orthoacetate (7 molar equiv) and p-toluenesulfonic acid (catalytic) were added and stirring was continued for 20min. Excess triethylamine was then added and after stirring for a few more minutes at room temperature it was concentrated to dryness. Powdered drierite (1.5 g) and dry pyridine (40 mL) were added to it and the mixture was stirred for 2 h at -25 °C. To this a previously stirred mixture of benzoyl chloride (1.63 mL, 8 molar equiv) and powdered drierite (4 A, 2 g) in dry pyridine (35 mL) maintained at the same temperature was added and stirring was continued for 24h. Methanol was then added to destroy the excess benzoyl chloride and after stirring for 15 min solids were separated on a celite-pad and washed with dichloromethane. The combined filtrate and washings were then transfered to a separating funnel and washed with dilute aqueous sodium carbonate solution and water successively. The organic layer was then dried (sodium sulfate) and concentrated to a light yellow foam. This was dissolved in aqueous acetic acid (80%, v/v, 150 mL) and was stirred for 30 min at room temperature. The solution was then concentrated to dryness by repeated coevaporation with toluene and the residue, on silica gel column chromatography using 8:1 and 2:1 n-hexane-ethyl acetate as successive eluants, gave 17 as a colorless foam (1.62 g, 65%, based on 14): $[\alpha]_D$ +3.0° (c 0.4, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.80-1.10 (m, 2H, $C\underline{H}_2SiMe_3$), 1.92, 2.17 (2s, 6H, NAc and OAc), and 7.17-8.25 (m, 35H, 2 $CH_2C_6\underline{H}_5$ and 5 Bz).

Anal. Calcd for $C_{77}H_{83}NO_{23}Si$ (1418.58): C, 65.20; H, 5.90; N, 0.99. Found: C, 65.50; H, 6.07; N, 1.00.

17 on acetylation with acetic anhydride in pyridine gave 18, a di-O-acetate confirming the presence of a free hydroxyl in 17: $[\alpha]_D$ +6.0° (c 1.2, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.80-1.10 (m, 2H, $C\underline{H}_2SiMe_3$), 1.85, 2.05, 2.19 (3s, 9H, AcN and 2 AcO), 5.85 (dd, 1H, $J_{2",3"}$ = 10.6 Hz, $J_{3",4"}$ = 3.3 Hz, H-3"), and 7.15-8.30 (m, 35H, 2 $CH_2C_6\underline{H}_5$ and 5 Bz).

2-(Trimethylsilyl)ethyl O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→3)-O-(2-acetamido-4-O-acetyl-6-O-benzoyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-O-(2-O-benzoyl-3-O-benzyl-6-O-benzyloxymethyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (20). This tetrasaccharide was prepared by coupling of the trisaccharide acceptor 17 with the glycosyl donor 19 by the same method as described for the preparation of 12. Compound 20 was obtained as a colorless glass after silica gel column chromatography using 8:1, 3:1 and 1:1 n-hexane-ethyl acetate as successive eluants. The product thus obtained was dissolved in 1,4-dioxane (5 mL) and was freeze dried to afford 20 as a snow-white powder (68 mg, 74.7%, based on the reacted 17): mp. 98-100 °C; $[\alpha]_D$ +2.46° (c 0.2, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.87-1.12 (m, 2H, CH_2SiMe_3), 1.92, 2.07, 2.14, 2.15, 2.17, 2.18 (6s, 18H, AcN and 5 AcO), 6.67-6.72 (d, 1H, NHAc), and 7.35-8.30 (m, 35H, 2 $CH_2C_6H_5$ and 5 Bz); ^{13}C NMR ($CDCl_3$) δ 100.6, 100.9, 101.4, 102.4 (four β anomeric carbons), and 165.7, 165.9, 166.2, 166.4, 166.6, 168.6, 168.9, 169.8, 170.7, 170.8, and 171.3 (eleven carbonyl carbons).

Anal. Calcd for $C_{91}H_{101}NO_{32}Si$ (1748.87): C, 62.50; H, 5.82; N, 0.80. Found: C, 62.76; H, 5.69; N, 0.84.

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