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Note

Rapid glycosylations under extremely mild acidic conditions. Use of ammonium salts to activate glycosyl phosphites via *P*-protonation

Fumiko Matsumura, Shiro Tatsumi, Natsuhisa Oka, Takeshi Wada *

Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Bioscience Building 702, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan

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ABSTRACT

Trifluoromethanesulfonic acid salts of tertiary amines were employed as extremely mild acidic activators for rapid glycosylations. Glycosyl phosphite triesters bearing an acid-labile 4,4′-dimethoxytrityl (DMTr) group for transient protection worked as glycosyl donors effectively in the presence of the activators to afford the corresponding disaccharides in good yields without loss of the DMTr group.

Oligosaccharides and their conjugates with other biomolecules (glycoconjugates) play important roles in a wide variety of biological processes and extensive studies are currently in progress to clarify the whole picture.[1–3] Oligosaccharides and glycoconjugates with defined structures are indispensable for such studies.[4–6] Chemical synthesis is a major approach to obtain these materials and advantageous because it can provide pure materials in sufficient quantities at relatively low costs. Oligosaccharides and glycoconjugates containing chemical modifications at defined sites are also available by this approach and used as probes.[7] In addition to the applications to glycobiology, chemically synthesized fragments of these carbohydrates and their modified analogues have been applied to therapeutic studies.[8,9]

Many of oligosaccharides and glycoconjugates have rather complex structures. The complexity comes from the diversity of monosaccharides, which have multiple hydroxy groups and can be connected to each other at different positions through α- or β-glycosyl bonds. To synthesize these compounds, the development of efficient glycosylation reactions that can be performed under mild reaction conditions is strongly required so that a variety of orthogonal protecting groups can be used.[4] With this background, we sought to develop a glycosylation reaction that could be performed under extremely mild acidic conditions and found that salts of tertiary amines consisting of less-nucleophilic components were useful for rapid glycosylations using glycosyl phosphite triesters as glycosyl donors. The results of this study are described in this paper.

Glycosyl phosphite triesters are one of the most reactive glycosyl donors developed so far and have been applied to the synthesis of a wide variety of oligosaccharides and glycoconjugates.[10] Since the early reports on their use as glycosyl donors,[10a–c] strong Lewis acids, typically trimethylsilyl trifluoromethanesulfonate (TMSOTf), 10a,b have been used for their activation. On the other hand, some research groups have reported that the glycosyl phosphites can be activated under mild acidic conditions.[10g–j] For example, Hashimoto et al. have reported that glycosyl phosphites can be activated by 2,6-*tert*-butylpyridinium iodide (DTBPI) to give the corresponding O-glycosylation products in good yields.[10g] However, the relatively long reaction time would be a bottleneck for the synthesis of rather complex oligosaccharides and glycoconjugates. As they have described, the activation of glycosyl phosphites by DTBPI generates the corresponding glycosyl iodides as active intermediates. The resultant glycosyl iodides have modest reactivity to alcohols and react to afford the final glycosylation products typically within 24–48 h under standard reaction conditions.[10g] We anticipated that the reactions of the glycosyl phosphites would be much faster if they are activated only by *P*-protonation under mild acidic conditions in the absence of any nucleophilic catalysts. It has been reported that glycosyl phosphite triesters can be activated via *P*-protonation by using strong Brønsted acids, such as TfOH and Tf_2NH,[10d,k] but it was not clear if the glycosyl phosphites would work as glycosyl donors effectively with a much weaker Brønsted acid, such as tertiary ammonium salts, without any nucleophilic catalysts (Fig. 1).

* Corresponding author. Tel./fax: +81 4 7136 3612.
E-mail address: wada@k.u-tokyo.ac.jp (T. Wada).

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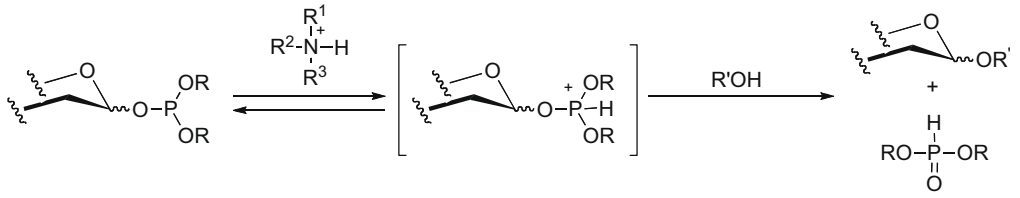
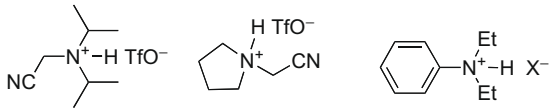


Figure 1. Glycosylation with tertiary ammonium salts via *P*-protonated glycosyl phosphites.

Glycosyl phosphites with an acid-labile 4,4′-dimethoxytrityl (DMTr) group at the *O*-6 position (**5**, **6**) were synthesized as glycosyl donors for this study via regioselective tritylation of the *O*-6-position of reducing sugars (**1**,[11] **2**[12]) in the presence of silver nitrate and subsequent *O*-1 phosphitylation (Scheme 1). The DMTr group is routinely used for transient protection of primary hydroxy groups in nucleic acid synthesis[13] for its advantages, such as the regioselective protection of primary hydroxy groups, stability under various reaction conditions, acid lability for facile deprotection under mild acidic conditions, and the strong UV–vis absorption of the resultant DMTr cation, which is used for quantitation.[14] Despite these advantages, the DMTr group has been rarely used for the synthesis of oligosaccharides,[11,15] mainly because of its incompatibility with the common glycosylation conditions using strong Brønsted or Lewis acids. The development of glycosylation reactions which proceed rapidly under mild acidic conditions would allow the synthesis of oligosaccharides using more extensive protecting groups than those currently used; the DMTr group would be representative of acid-labile protecting groups. We also expected that the bulky DMTr group at the 6-position might work as an α-directing group in glycosylation reactions.[11,16]

Trifluoromethanesulfonic acid salts of tertiary amines (Fig. 2, **7**–**9**[17]) were employed as activators for the following reasons: (1) the activators must have a mild but sufficient acidity to protonate the phosphorous atom of phosphite triesters and must be less-nucleophilic so that the formation of stable N-glycosides, such as pyridinium glycosides,[18] would be avoided. The suitable acidity and extremely low nucleophilicity of **7** and **8** have already been demonstrated by using them to activate *P*-stereogenic diastereopure phosphoramidite derivatives via protonation to give the corresponding phosphite derivatives in a stereospecific manner.[17b,19] The *N*,*N*-diethylanilinium salt **9** was also employed because of its modest acidity and low nucleophilicity. (2) The counter anion must also be less nucleophilic. TfO^- would be a suitable counter anion because the resultant glycosyl trifluoromethanesulfonates have been reported to be extremely reactive even at −78 °C and act as the corresponding oxocarbenium cations at ambient temperatures.[20] An *N*,*N*-diethylanilinium salt **10** containing a nucleophilic iodide anion was used as a control.

Glycosylation reactions of appropriately protected monosaccharides **11** and **12** were then carried out by using the glycosyl donors (**5**, **6**) and the activators (**7**–**10**) (Table 1). The glycosylations of the sterically less-hindered **11** were completed within 5–10 min when the less-nucleophilic activators **7**–**9** were used and the desired



7 **8**

9: $X^- = TfO^-$
10: $X^- = I^-$

Figure 2. Tertiary ammonium salts containing less-nucleophilic triflate anion (**7**–**9**) or highly-nucleophilic iodide anion (**10**).

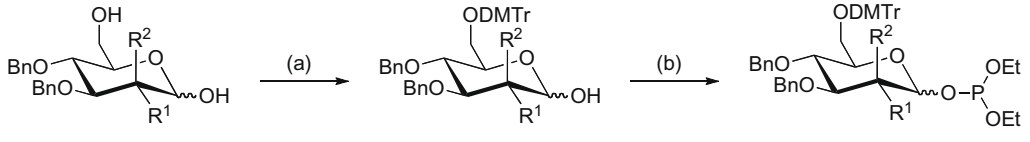
disaccharides **13** and **14** were isolated in good to excellent yields (entries 1–5 and 7). The reactions were slightly slower in polar solvents (entries 3 and 4) than in less polar CH_2Cl_2 (entry 2). In sharp contrast, the glycosylation proceeded very slowly when promoted by *N*,*N*-diethylanilinium iodide **10** due to the formation of the glycosyl iodide intermediate **19**, though the α-selectivity was excellent as reported in the literature (entry 6).[10g] The formation of the glycosyl iodide **19** from **5** and **10** was also confirmed by ^1H NMR.[21] Removal of the *O*-6-DMTr group of the fully protected disaccharides **13** and **14** was achieved by using 3 vol % dichloroacetic acid in CH_2Cl_2 within 2 min in the presence of Et_3SiH as a scavenger of the liberated DMTr cation[22] to afford the detritylated disaccharides **16**[23] and **17**,[24] respectively, virtually quantitatively (entries 5 and 7). In the cases of the sterically more-hindered glycosyl acceptor **12**, the reaction was completed within 5 min by using the activator **8** (entry 8), whereas it was sluggish when the activator **9** was used (entry 9). The resultant disaccharide **15**[11] was detritylated to afford **18**[25] in modest yields (entries 8 and 9). Only the α-isomer of **18** was obtained in both of these reactions most likely due to the α-directing effect of the *O*-6-DMTr group.

In summary, rapid glycosylations were achieved with glycosyl phosphites as glycosyl donors and less-nucleophilic trifluoromethanesulfonic acid salts of tertiary amines as activators. The absence of nucleophilic catalysts was essential for rapid reactions. Glycosyl donors having an acid-labile DMTr group were compatible with the present reaction conditions, indicating that a wide range of acid-labile substrates and protecting groups can be used in this method.

1. Experimental

1.1. General methods

IR spectra were recorded on a JASCO FT/IR-480 Plus spectrophotometer. NMR spectra were recorded on a Varian Mercury 300. ^1H



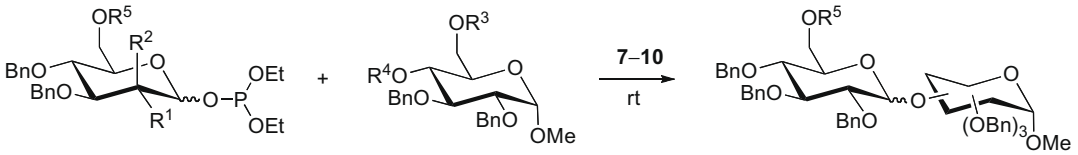
1: R^1 = OBn, R^2 = H
2: R^1 = H, R^2 = OBn

3: R^1 = OBn, R^2 = H, 81%
4: R^1 = H, R^2 = OBn, 88%

5: R^1 = OBn, R^2 = H, 77% (α:β = 58:42)
6: R^1 = H, R^2 = OBn, 55% (α:β = 93:8)

Scheme 1. Synthesis of glycosyl donors **5** and **6**. Reagents and conditions: (a) DMTrCl, Et_3N, $AgNO_3$, THF–DMF (1:1, v/v); (b) $(EtO)_2PCl$, Et_3N, CH_2Cl_2.

Table 1
Glycosylation reactions using glycosyl phosphites **5**, **6**, and acidic activators **7**–**10**



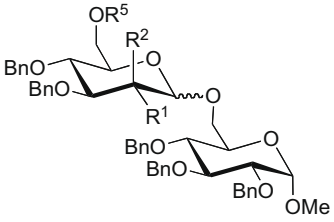
5: R^1 = OBn, R^2 = H, R^5 = DMTr
6: R^1 = H, R^2 = OBn, R^5 = DMTr

11: R^3 = H, R^4 = OBn
12: R^3 = OBn, R^4 = H

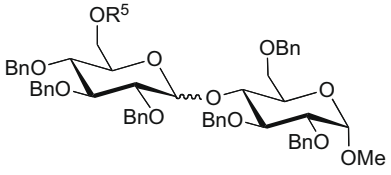
DCA, Et_3SiH → **13**–**15**: R^5 = DMTr; **16**–**18**: R^5 = H

Entry	Substrate		Activator	Time	Solvent	Isolated yield of **13**–**15**/% (α:β)[a]		Isolated yield of **16**–**18**/% (α:β)[a]	
1[b]	**5**	**11**	**7**	5 min	CH_2Cl_2	**13**	73 (5:1)	**16**	—
2[c]	**5**	**11**	**8**	5 min	CH_2Cl_2	**13**	83 (4:1)	**16**	—
3[c]	**5**	**11**	**8**	10 min	CH_3CN	**13**	81 (2:3)	**16**	—
4[c]	**5**	**11**	**8**	10 min	1,4-Dioxane	**13**	82 (6:1)	**16**	—
5[b]	**5**	**11**	**9**	5 min	CH_2Cl_2	**13**	91 (5:1)	**16**	86[f] (5:1)
6[b]	**5**	**11**	**10**	120 h	CH_2Cl_2	**13**	25 (α)	**16**	—
7[b]	**6**	**11**	**9**	5 min	CH_2Cl_2	**14**	71[d]	**17**	70[f] (2:1)
8[e]	**5**	**12**	**8**	5 min	CH_2Cl_2	**15**	30 (α)	**18**	58[g] (α)
9[e]	**5**	**12**	**9**	1 h	CH_2Cl_2	**15**	—	**18**	43[g] (α)

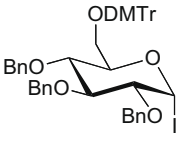
[a] Anomeric ratios were determined by ^{1}H NMR.
[b] Glycosyl donor/acceptor/activator molar ratio = 2/1/2.
[c] Glycosyl donor/acceptor/activator molar ratio = 1/0.8/2.
[d] Anomeric ratio was not determined.
[e] Glycosyl donor/acceptor/activator molar ratio = 2/1/4.
[f] Yield from **11**.
[g] Yield from **12** without purification of **15**.



13: R^1 = OBn, R^2 = H, R^5 = DMTr
14: R^1 = H, R^2 = OBn, R^5 = DMTr
16: R^1 = OBn, R^2 = H, R^5 = H
17: R^1 = H, R^2 = OBn, R^5 = H



15: R^5 = DMTr
18: R^5 = H



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NMR spectra were obtained at 300 MHz with tetramethylsilane (TMS) (δ 0.0) as an internal standard in $CDCl_3$. ^{13}C NMR spectra were obtained at 75.5 MHz with $CDCl_3$ as an internal standard (δ 77.0) in $CDCl_3$. ^{31}P NMR spectra were obtained at 121.5 MHz with 85% H_3PO_4 (δ 0.0) as an external standard. ESI mass spectra were recorded on an Applied Biosystems QSTAR. All the reactions were conducted under an inert atmosphere. Silica gel column chromatography was carried out using Kanto Silica Gel 60 N (40–50 μm). Analytical TLC was performed on Merck Kieselgel 60-F_{254} plates.

1.2. 6-*O*-Dimethoxytrityl-2,3,4-tri-*O*-benzyl-D-glucopyranose (3)

Et_3N (0.70 mL, 5.0 mmol) was added to a solution of **1**[11] (0.49 g, 1.1 mmol), silver nitrate (0.17 g, 1.0 mmol), and DMTrCl (0.34 g, 1.0 mmol) in dry THF–DMF (1:1, v/v, 2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 50 min and warmed to rt. After stirring for 20 min, silver nitrate (0.020 g, 0.12 mmol) and DMTrCl (0.036 g, 0.11 mmol) were added, and the reaction mixture was stirred for 100 min at rt. Then additional silver nitrate (0.035 g, 0.20 mmol) and DMTrCl (0.067 g, 0.20 mmol) were added, and the reaction mixture was stirred for 40 min at rt. Then the reaction was quenched by the addition of MeOH (5 mL). The mixture was diluted with $CHCl_3$ and filtered. The filtrate was then washed with satd aq $NaHCO_3$ (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography [hexane–AcOEt–Et_3N (70:30:1, v/v/v)] on silica gel to afford **3**[11] (0.66 g, 81%).

1.3. 6-*O*-Dimethoxytrityl-2,3,4-tri-*O*-benzyl-D-mannopyranose (4)

Et_3N (0.42 mL, 3.0 mmol) was added to a solution of **2**[12] (0.46 g, 1.0 mmol), silver nitrate (0.18 g, 1.05 mmol), and DMTrCl (0.36 g, 1.05 mmol) in dry THF–DMF (1:1, v/v, 2 mL) at 0 °C. The reaction mixture was stirred for 20 min at 0 °C, and the reaction was quenched by the addition of MeOH (5 mL). The mixture was diluted with CH_2Cl_2 (20 mL) and filtered. The filtrate was then washed with satd aq $NaHCO_3$ (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography [hexane–AcOEt–Et_3N (30:10:0.4–20:10:0.3, v/v/v)] on silica gel to afford **4** (0.67 g, 88%). Colorless form; IR (KBr, cm^{-1}): 3434, 3061, 3031, 2931, 1607, 1583, 1509, 1454, 1363, 1301, 1251, 1177, 1155, 1098, 913, 829, 738, 698, 584; ^{1}H NMR ($CDCl_3$): δ 7.58–6.68 (m, Ar), 5.30 (s, αH-1), 5.22–4.25 (m, βH-1, CH_2Ph), 4.19–3.20 (m, αH-2–6, βH-2–6, β-OH, OMe), 2.83 (d, α-OH); ^{13}C NMR ($CDCl_3$): δ 158.3 (C), 158.2 (C), 145.0 (C), 138.7 (C), 138.5 (C), 138.4 (C), 138.2 (C), 138.1 (C), 138.0 (C), 136.4 (C), 136.3 (C), 136.0 (C), 130.1 (CH), 130.2 (CH), 130.3 (CH),

128.5–127.4 (m, CH), 126.5 (CH), 113.0 (CH), 93.5 (CH, αC-1), 92.6 (CH, βC-1), 85.7 (C), 85.6 (C), 83.0 (CH), 79.5 (CH), 75.8 (CH), 75.1–74.4 (m, CH, CH_2), 72.9 (CH_2), 72.7 (CH_2), 72.4 (CH_2), 72.1 (CH), 62.6 (CH_2), 62.2 (CH_2), 55.1 (CH_3); ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{48}H_{48}NaO_8^+$: 775.3247; found: 775.3241.

1.4. Diethyl 6-*O*-dimethoxytrityl-2,3,4-tri-*O*-benzyl-D-glucopyranosyl phosphite (5)

Et_3N (0.21 mL, 1.5 mmol) and diethyl chlorophosphite (0.080 mL, 0.55 mmol) were added to a solution of **3** (0.37 g, 0.50 mmol) in dry CH_2Cl_2 (5.0 mL) at rt and the mixture was stirred for 20 min at rt. The reaction was then quenched by the addition of satd aq $NaHCO_3$ (5 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (20 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography [hexane–ethyl acetate–pyridine (70:10:0.4, v/v/v)] on silica gel to give **5** (0.33 g, 77%, α:β = 58:42). Colorless foam: IR (KBr, cm^{-1}): 3061, 3011, 2929, 2835, 1607, 1582, 1508, 1454, 1388, 1356, 1300, 1250, 1176, 1149, 1071, 1028, 829, 698; ^1H NMR ($CDCl_3$): δ 7.53–6.74 (m, Ar), 5.72 (dd, $J_{1,2}$ = 2.7 Hz, J_{H-P} = 8.7 Hz, αH-1), 5.04–4.66 (m, βH-1, CH_2Ph), 4.35–4.29 (m, CH_2Ph), 4.06–3.22 (m, αH-2–H-6, βH-2–H-6, POCH_2CH_3, OMe), 1.30–1.16 (m, $POCH_2CH_3$); ^{31}P NMR ($CDCl_3$): δ 140.8 (s, β), 139.7 (s, α); ^{13}C NMR ($CDCl_3$): δ 97.2 (d, J_{C-P} = 15 Hz, βC-1), 91.2 (d, J_{C-P} = 15 Hz, αC-1); ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{52}H_{57}NaO_{10}^+$: 895.3582; found: 895.3590.

1.5. Diethyl 6-*O*-dimethoxytrityl-2,3,4-tri-*O*-benzyl-D-mannopyranosyl phosphite (6)

Synthesis of **6** from **4** (0.37 g, 0.50 mmol) was conducted following a protocol similar to that used for the synthesis of **5**. Purification by column chromatography [hexane–ethyl acetate–pyridine (70:10:0.4, v/v/v)] on silica gel to give **6** (0.24 g, 55%, α:β = 92:8). Colorless oil; IR (KBr, cm^{-1}): 3061, 3030, 2976, 2931, 2835, 1607, 1582, 1509, 1454, 1387, 1361, 1300, 1250, 1176, 1154, 1096, 1028, 986, 830, 699; ^1H NMR ($CDCl_3$): δ 7.55–6.70 (m, 28H, Ar), 5.64 (dd, 1H, $J_{1,2}$ = 1.8 Hz, J_{H-P} = 8.4 Hz, H-1), 4.90–4.63 (m, 5H, CH_2Ph), 4.28 (d, 1H, CH_2Ph), 4.23 (t, 1H, *J* = 9.9 Hz, H-4), 3.97–3.70 (m, 15H, H-2–H-5, POCH_2CH_3, OMe), 3.51 (dd, 1H, $J_{5,6}$ = 1.2 Hz, $J_{6,6}$ = 9.9 Hz, H-6), 3.28 (dd, 1H, $J_{5,6}$ = 3.9 Hz, $J_{6,6}$ = 9,9 Hz, H-6), 1.24–1.10 (q, 6H, $POCH_2CH_3$); ^{31}P NMR ($CDCl_3$): δ 139.1 (s); ^{13}C NMR ($CDCl_3$): δ 158.2 (C), 145.1 (C), 138.5 (C), 138.2 (C), 136.4 (C), 136.0 (C), 130.3 (CH), 130.1 (CH), 128.3–126.5 (m, CH), 113.0 (CH), 112.9 (CH), 91.7 (d, J_{C-P} = 11.8 Hz, CH, C-1), 85.5 (C), 79.2 (CH), 76.5 (d, J_{C-P} = 3.2 Hz, CH), 75.1 (CH_2), 74.7 (CH), 72.9 (CH), 72.6 (CH_2), 72.5 (CH_2), 62.1 (CH_2), 58.4 (m, CH_2), 55.1 (CH_3), 16.8 (d, J_{C-P} = 4.9 Hz, CH_3); ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{52}H_{57}NaO_{10}^+$: 895.3582; found: 895.3586.

1.6. *N,N*-Diethylanilinium trifluromethanesulfonate (9)

Trifluoromethanesulfonic acid (1.8 mL, 20 mmol) was added dropwise to a stirred solution of *N,N*-diethylaniline (3.2 mL, 20 mmol) in dry Et_2O (5.0 mL) at 0 °C. The reaction mixture was kept under −30 °C until the formation of crystalline solid was observed. The resultant crystals were collected by filtration, washed with dry Et_2O (20 mL), and dried under vacuum to afford **9** (5.3 g, 89%) as a colorless solid; mp 65.5–66.0 °C; IR (KBr, cm^{-1}): 3502, 3043, 2987, 2687, 1628, 1495, 1277, 1244, 1167, 1030; ^1H NMR ($CDCl_3$): δ 10.3 (br, 1H, NH^+), 7.62–7.28 (m, 5H, Ar), 3.81–3.71 (m, 2H, CH_2CH_3), 3.58–3.44 (m, 2H, CH_2CH_3), 1.19 (t, 6H, *J* = 75 Hz); ^{13}C NMR ($CDCl_3$): δ 136.2 (C), 130.6 (CH), 122.2 (CH), 120.3 (q, *J* = 319 Hz), 54.4 (CH_2), 10.2 (CH_3); Anal. Calcd for $C_{11}H_{16}F_3NO_3S$: C, 44.14; H, 5.39; N, 4.68. Found: C, 44.08; H, 5.48; N, 4.52.

1.7. *N,N*-Diethylanilinium iodide (10)

A 57% (w/w) aqueous solution of hydrogen iodide (1.4 mL, 10 mmol) was added dropwise to a stirred solution of *N,N*-diethylaniline (1.6 mL, 10 mmol) in dry Et_2O (5.0 mL) at 0 °C. The resultant precipitate was collected by filtation, washed with dry Et_2O (20 mL), and dried under vacuum to afford **10** (1.9 g, 70%) as an off-white solid; IR (KBr, cm^{-1}): 3500, 2973, 2906, 2867, 2738, 2705, 2650, 2456, 1596, 1495, 1479, 1418, 1269, 1155, 1017; ^1H NMR ($CDCl_3$): δ 11.5 (br, 1H, NH^+), 7.84–7.82 (m, 2H, Ar), 7.62–7.30 (m, 3H, Ar), 3.83–3.71 (m, 2H, CH_2CH_3), 3.53–3.39 (m, 2H, CH_2CH_3), 1.31 (t, 6H, *J* = 72 Hz); ^{13}C NMR ($CDCl_3$): δ 136.0 (C), 130.5 (CH), 122.6 (CH), 54.1 (CH_2), 10.2 (CH_3); Anal. Calcd for $C_{10}H_{16}NI$: C, 43.34; H, 5.82; N, 5.05. Found: C, 43.15; H, 5.86; N, 4.80.

1.8. General protocol for glycosylation (13–15)

The acidic activator (**7**–**9** or **10**) was added to a solution (2.0 mL) of glycosyl donor (**5** or **6**) (0.10 mmol) and glycosyl acceptor (**11** or **12**) (0.050 mmol) at rt. The reaction mixture was stirred until either the glycosyl donor or the glycosyl acceptor was completely consumed (TLC). The reaction was then quenched by the addition of satd aq $NaHCO_3$ (5.0 mL), and the mixture was extracted with CH_2Cl_2 (25 mL). The organic layer was separated, washed with satd aq $NaHCO_3$ (25 mL × 3), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography [hexane–ethyl acetate (7:1 to 5:1, v/v)] on silica gel.

1.9. Methyl 6-*O*-(6-*O*-dimethoxytrityl-2,3,4-tri-*O*-benzyl-D-glucopyranosyl)- 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (13)

Colorless foam; IR (KBr, cm^{-1}): 3043, 2931, 1612, 1508, 1454, 1365, 1250, 1211, 1173, 1070, 1029, 830, 698; ^1H NMR ($CDCl_3$): δ 7.55–6.70 (m, Ar), 5.10–4.20 (m, CH_2Ph, αβH-1^{II}, αβH-1^{I}), 4.06–3.37 (m, αβH-2^{II}–αβH-$6a^{II}$, αβH-2^{I}–αβH-6^{I}, OMe), 3.25 (dd, $J_{5,6}$ = 3.9 Hz, $J_{6,6}$ = 10 Hz, βH-$6b^{II}$), 3.14 (dd, $J_{5,6}$ = 3.9 Hz, $J_{6,6}$ = 10 Hz, αH-$6b^{II}$); ^{13}C NMR ($CDCl_3$): δ 103.8 (CH, βC-1^{II}), 98.3 (CH, βC-1^{I}), 97.9 and 97.1 (CH, αC-1^{I}, αC-1^{II}); ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{76}H_{78}NaO_{13}^+$: 1221.5335; found: 1221.5329.

Anomeric ratios of **13** in Table 1 were determined by the integral ratios of the ^1H signals at δ 3.25 (βH-6^{II}) and δ 3.14 (αH-6^{II}).

1.10. Methyl 6-*O*-(6-*O*-dimethoxytrityl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (13α)

Colorless foam; IR (KBr, cm^{-1}): 3087, 3062, 3030, 3003, 2928, 2835, 1607, 1583, 1509, 1454, 1359, 1301, 1250, 1211, 1176, 1072, 1029, 829, 791, 698; ^1H NMR ($CDCl_3$): δ 7.55–6.70 (m, 43H, Ar), 5.08 (d, 1H, *J* = 3.3 Hz, H-1^{II}), 4.98–4.54 (m, 12H, CH_2Ph, H-1^{I}), 4.28 (d, 1H, *J* = 11 Hz, CH_2Ph), 4.02–3.60 (m, 15H, H-2^{II}–H-5^{II}, H-3^{I}–H-6^{I}, OMe), 3.46–3.36 (m, 5H, H-2^{I}, H-$6a^{II}$, OMe), 3.14 (dd, 1H, H-$6b^{II}$, $J_{5,6}$ = 3.9 Hz, $J_{6,6}$ = 10 Hz); ^{13}C NMR ($CDCl_3$): δ 158.3 (C), 145.0 (C), 138.8 (C), 138.7 (C), 138.6 (C), 138.3 (C), 138.1 (C), 136.3 (C), 135.9 (C), 130.2 (CH), 130.1 (CH), 128.4–126.6 (m, CH), 113.0 (CH), 112.9 (CH), 97.91 (CH, αC-1^{I} or αC-1^{II}), 97.1 (CH, αC-1^{I} or αC-1^{II}), 85.5 (C), 82.1 (CH), 81.8 (CH), 80.3 (CH), 80.1 (CH), 78.0 (CH), 77.7 (CH), 75.8 (CH_2), 75.7 (CH_2), 75.0 (CH_2), 74.8 (CH_2), 73.4 (CH_2), 72.3 (CH_2), 70.5 (CH), 70.4 (CH), 65.8 (CH_2), 62.1 (CH_2), 55.1 (CH_3), 55.0 (CH_3); ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{76}H_{78}NaO_{13}^+$: 1221.5335; found: 1221.5336.

1.11. Methyl 6-*O*-(6-*O*-dimethoxytrityl-2,3,4-tri-*O*-benzyl-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (14)

Colorless foam; IR (KBr, cm^{-1}): 3043, 3026, 2929, 1606, 1508, 1454, 1300, 1250, 1211, 1176, 1085, 1029, 829, 698; ^1H NMR ($CDCl_3$): δ 7.55–6.70 (m, 43H, Ar), 5.05–3.10 (m, 35H, CH_2Ph, αβH-1^{II}–αβH-$6a^{II}$, αβH-1^{I}–αβH-6^{I}, OMe); ^{13}C NMR ($CDCl_3$): δ 101.5 (CH, βC-1^{II}), 98.0, 97.7, 97.7 (CH, αC-1^{I} and αC-1^{II}); ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{76}H_{78}NaO_{13}^+$: 1221.5335; found: 1221.5336.

1.12. Methyl 4-*O*-(6-*O*-dimethoxytrityl-2,3,4-tri-*O*-benzyl-D-glucopyranosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranoside (15)

Synthesis of **15** from **5** (0.088 g, 0.10 mmol) and **12** (0.023 g, 0.050 mmol) was conducted following the general protocol for glycosylation. Purification by silica gel column chromatography [toluene–AcOEt–Et_3N (18:1:0.19 v/v)] and preparative thin layer chromatography [hexane–AcOEt–Et_3N (5:1:0.06–3:1:0.04 v/v/v)] afforded **15**[11] (0.018 g, 30%). The ^1H NMR data were identical to those reported in the literature.[11] We synthesized the detritylated disaccharide **18** without isolation of **15** as described above because of its poor chromatographic separation from some byproducts.

1.13. General protocol for removal of DMTr groups (16–18)

A 3% (v/v) solution of dichloroacetic acid in CH_2Cl_2 (1.0 mL) and Et_3SiH (50 μL) was added to **13**–**15** (0.050 mmol) at rt. The reaction mixture was stirred for 2 min at rt and the reaction was quenched with the addition of satd aq $NaHCO_3$ (5.0 mL). The mixture was then extracted with CH_2Cl_2 (15 mL). The organic layer was washed with satd aq $NaHCO_3$ (15 mL), and the aqueous layer was back-extracted with CH_2Cl_2 (20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Anomeric ratios of the products were determined by ^1H NMR analysis of the crude mixtures. Purification by column chromatography [hexane–AcOEt, 2.5:1–2:1 (v/v)] on silica gel gave the detritylated disaccharides **16**–**18**.

1.14. Methyl 6-*O*-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (17α), methyl 6-*O*-(2,3,4-tri-*O*-benzyl-β-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (17β)

Spectral data for **17α**: ^1H NMR spectrum was in good agreement with the reported data.[24] IR (KBr, cm^{-1}): 3495, 3062, 3029, 2922, 1496, 1454, 1361, 1208, 1149, 1092, 1028, 698; ^1H NMR ($CDCl_3$): δ 7.45–7.15 (m, 30H, Ar), 5.01–4.47 (m, 14H, CH_2Ph, H-1^{II}, H-1^{I}), 3.98–3.31 (m, 15H, H-2^{II}–H-6^{II}, H-2^{I}–H-6^{I}, OMe), 1.85 (br, 1H, OH); ^{13}C NMR ($CDCl_3$): δ 138.5 (C), 138.4 (C), 138.3 (C), 138.2 (C), 138.1 (C), 138.0 (C), 128.5–127.5 (m, CH), 98.4 (CH, C-1^{II}), 97.8 (CH, C-1^{I}), 82.1 (CH), 79.9 (CH), 79.5 (CH), 77.5 (CH), 75.8 (CH_2), 75.1 (CH_2), 74.9 (CH_2), 74.7 (CH), 74.6 (CH), 73.3 (CH_2), 72.7 (CH_2), 72.2 (CH), 72.1 (CH_2), 69.6 (CH), 65.8 (CH_2), 62.2 (CH_2), 55.1 (CH_3); ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{55}H_{60}NaO_{11}^+$: 919.4028; found: 919.4015. Spectral data for **17β**: ^1H NMR spectrum was in good agreement with the reported data.[24] IR (KBr, cm^{-1}): 3505, 3026, 2921, 2883, 1500, 1453, 1365, 1070, 1022, 697; ^1H NMR ($CDCl_3$): δ 7.45–7.15 (m, 30H, Ar), 5.05–4.48 (m, 13H, CH_2Ph, H-1^{I}), 4.09–3.68 (m, 8H, H-3^{I}, H-5^{I}, H-$6a^{I}$, H-1^{II}, H-2^{II}, H-4^{II}, H-$6ab^{II}$), 3.53–3.21 (m, 8H, H-2^{I}, H-4^{I}, H-6^{I}, H-3^{II}, H-5^{II}, OMe), 2.15 (t, 1H, J = 7.0 Hz, OH); ^{13}C NMR ($CDCl_3$): δ 138.7 (C), 138.4 (C), 138.2 (C), 138.1 (C), 138.0 (C), 138.0 (C), 128.5–127.5 (m, CH), 101.5 (CH, C-1^{II}), 97.8 (CH, C-1^{I}), 82.2 (CH), 82.1 (CH), 79.7 (CH), 77.3 (CH), 75.7 (CH), 75.3 (CH_2), 74.8 (CH), 74.7 (CH_2), 73.8 (CH_2), 73.6 (CH), 73.6 (CH_2), 73.4 (CH_2), 71.7 (CH_2), 69.7 (CH), 68.4 (CH_2), 62.5 (CH_2), 55.1 (CH_3); ESIMS *m/z*: $[M+Na]^+$ calcd for $C_{55}H_{60}NaO_{11}^+$: 919.4028; found: 919.4022.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.03.009.

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