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Efficient synthesis of pyruvate ketals of carbohydrates☆

Geetanjali Agnihotri and Anup Kumar Misra*

Medicinal and Process Chemistry Division, Central Drug Research Institute, Chattar Manzil Palace, Lucknow 226001, UP, India

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Abstract—An efficient method for the preparation of pyruvate ketals of carbohydrates has been developed using *N*-iodosuccinimide or 1,3-dibromo-5,5-dimethylhydantoin as activator and methyl 2,2-di(ethylthio)propionate. The method has been applied for the preparation of a pyruvate ketal-containing trisaccharide present in the bacterial cell-wall of *Mycobacterium smegmatis*.

1. Introduction

Pyruvate ketals are present in many lipopolysaccharides of bacterial origin, in capsular polysachharides,[1] and also in glycolipids isolated from fish nerve fibres.[2] As a result of the unique structural features including the presence of a negative charge on the carboxyl functional group and the chiral centre, pyruvate ketals influence immunological specificity and patterns of immunological cross reactivity and are therefore recognized as immunodominant structural features of polysaccharides which play an important role in cell–cell recognition processes.[3–8] It has also been proved that hexopyranosides containing pyruvic acid ketals are very useful tools for immunochemical studies of *Klebsiella* polysaccharides.[5,6] Therefore, efficient chemical syntheses of pyruvate ketal-containing oligosachharides are essential for their use in immunochemical studies.

Most commonly, pyruvate ketals are present as 4,6-ketals of hexose residues.[9] However they also occur as 1,4-dioxolanes formed either from *cis*-axial-equatorial[10,11] or *trans*-diequatorial hydroxyl groups.[12] Pyruvate ketals can be synthesized by direct condensation of a pyruvate ester with a diol in the presence of a Lewis acid, but this is less preferred because of the electron-withdrawing effect of the adjacent carboxylate group.[13–15] Therefore, several indirect methods for the acetalization have been introduced including condensation with pyruvate derivatives[16,17] or generation of the carboxylate group by oxidation of a suitable precursor.[18–21] However, these procedures are not generally applicable due to low yields or sensitivity of glycosides under the reaction conditions. Earlier reports of the preparation of pyruvic ketals by the reaction between diols and methyl pyruvate dialkyl dithioacetal,[22,23] activated by methyl triflate, dimethyl(methylthio)sulfonium trifluoromethane sulfonate (DMTST), nitroso tetrafluoroborate ($NOBF_4$) or SO_2Cl_2-trifluoromethanesulfonic acid are limited because the yields or the stereochemical outcome of these reactions was not satisfactory. Therefore, the development of a convenient and efficient methodology for the synthesis of pyruvate ketals of carbohydrates is desirable and herein, we report an expedient protocol for the synthesis of pyruvate ketal derivatives and its application for the synthesis of a pyruvate ketal-containing trisaccharide related to the glycolipid found in *Mycobacterium smegmatis*. The *N*-Iodosuccinimide (NIS) and trifluoromethanesulfonic acid combination is a well known glycosyl promoter used as a thioglycoside activator for the preparation of several oligosaccharides.[24] We envisioned that NIS might efficiently activate pyruvate dithioketal in the presence of a carbohydrate diol to furnish pyruvate ketals.

In a set of initial experiments, methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside was treated with methyl 2,2-di(ethylthio)propionate[23] in the presence of NIS-TfOH in a variety of solvents and reaction conditions. The use of 2.0 equiv of methyl 2,2-di(ethylthio)propionate and 3.8 equiv of NIS and 0.3 equiv of TfOH in anhydrous acetonitrile furnished an excellent yield of the pyruvate ketal (Scheme 1). Reduction of the proportion of methyl 2,2-di(ethylthio)propionate and NIS produced

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* Corresponding author. Tel.: +91 522 2612411 18; fax: +91 522 2623938; e-mail: akmisra69@rediffmail.com

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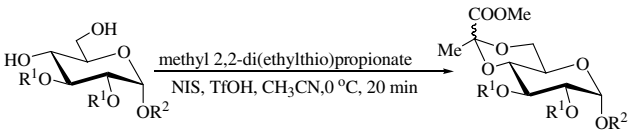


Scheme 1. Synthesis of pyruvate ketals using NIS-TfOH as the thiophilic activator.

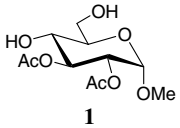
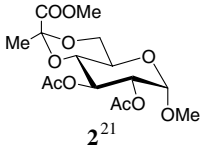
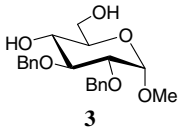
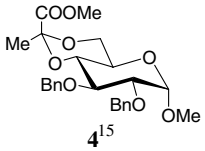
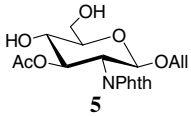
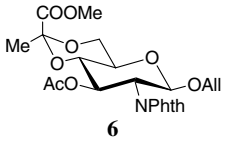
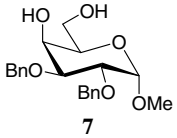
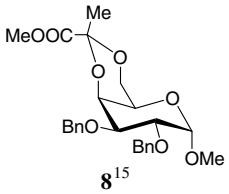
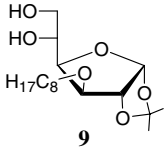
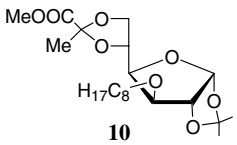
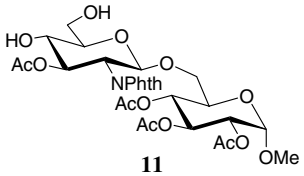
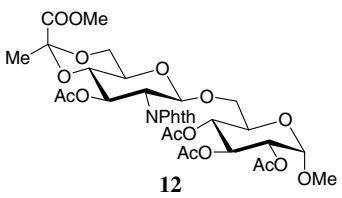
a sluggish reaction which did not reach completion even after 24 h. Under similar reaction conditions, a series of pyruvate ketal derivatives of mono-, di- and tri-saccharides were synthesized (Table 1). Most of the protecting groups and the inter-glycosidic bond remained unaffected.

In order to develop the pyruvate ketal formation using a cheaper thiophilic activator, a series of parallel reactions were carried out using 1,3-dibromo-5,5-dimethylhydantoin (DBDH). Recently, DBDH has been found to act as thiophilic activator for converting dithioacetals to the corresponding aldehydes as well as *O*,*O*-acetals.[25] Earlier, DBDH had been used as a free radical brominating agent[26] and as a source of active bromonium ions.[27] Therefore, it was reasoned that DBDH might act as an economically convenient thiophillic activator.

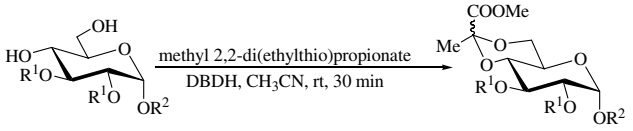
Treatment of methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside with methyl 2,2-di(ethylthio)propionate in the presence of DBDH in a variety of solvents and reaction conditions showed that the use of 2.0 equiv of methyl 2,2-di(ethylthio)propionate and 2.5 equiv of DBDH in acetonitrile under anhydrous conditions furnished excellent yields of the pyruvate ketals (Scheme 2 and Table 1).

NIS-TfOH activated formation of 4,6-*O*-pyruvate ketal using methyl 2,2-di(ethylthio)propionate, is more stereoselective than DBDH activation, only a single isomer (*S*-isomer) being formed (Table 1). The configurations of the pyruvate ketals were confirmed by the ^{13}C-chem-

Table 1. Synthesis of pyruvate ketals using DBDH or NIS-TfOH

Entry	Substrate	Product	DBDH		NIS	
			Yield (%)	*S*/*R*	Yield (%)	*S*/*R*
a	 **1**	 **2**[21]	72	2:1	69	*S*
b	 **3**	 **4**[15]	73	1:1	71	2:1
c	 **5**	 **6**	68	2:1	68	*S*
d	 **7**	 **8**[15]	70	2:3	70	1:2
e	 **9**	 **10**	58	—	55	—
f	 **11**	 **12**	61	2:1	63	*S*

ical shift of the ketal methyl carbon. The 4,6-*O*-pyruvate ketals of disaccharide **11** and trisaccharide **16** were synthesized exclusively as single diastereomers applying NIS-TfOH as promotor. However, in the case of benzylated derivatives, 2,3-di-*O*-benzyl-α-D-glucopyranoside **3** and 2,3-di-*O*-benzyl-α-D-galactopyranoside **7,** diastereomeric mixtures of the pyruvate ketals were isolated. Following a similar reaction protocol, the 5,6-*O*-pyruvate ketal of 3-*O*-octyl-α-D-glucofuranoside **10** was formed in high yield. The configuration of the ketal carbon was assigned using ^{13}C NMR spectroscopy by comparison with those previously reported.[19,28,29] For 4,6-*O*-(1-carboxymethyl)ethylidene-D-glucopyranoside derivatives **2**, **4**, **6**, **12** and **17** (Table 1, Scheme 3) the methyl group of the pyruvate ketal appeared at 24–26 ppm for the *S*-isomers and at 17–19 ppm for the *R*-isomers of the ketal carbon. In the case of D-galacto derivative **8**, the methyl group showed peaks at 26 ppm, for the major *R*-isomer and at 20.4 ppm for the minor *S*-isomer of the ketal carbon.[19,29] In the case of the five-membered pyruvate ketal **10**, there was no significant difference between the chemical shifts of the two diastereomers, as reported earlier.[30] The significantly higher diastereoselectivity with NIS and TfOH can be explained by assuming that under acidic conditions, the thermodynamically less stable isomers rearranged to the thermodynamically favoured ones which were finally isolated as the sole or major products. In the case of 4,6-*O*-ketals, the thermodynamically favoured isomers were formed, with an axial methoxycarbonyl group (*S*-isomers for D-Glcp and *R*-isomers for D-Galp derivatives).[15]



Scheme 2. Synthesis of pyruvate ketals using DBDH as the thiophilic activator.

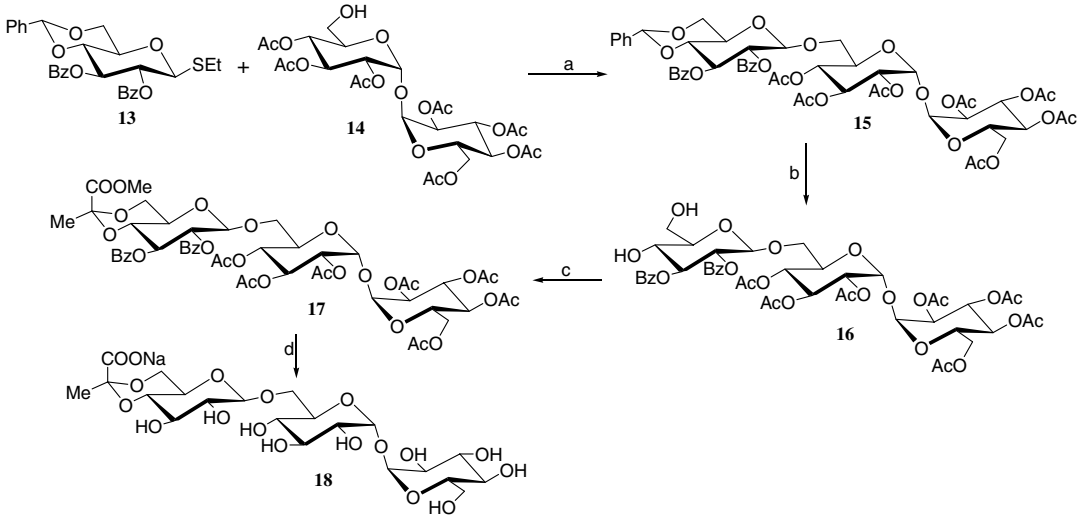
1.1. Typical experimental protocol for the synthesis of pyruvate ketals using NIS-TfOH

To a solution of methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside (1; 360 mg, 1.29 mmol) in dry acetonitrile (10.0 mL) was added methyl 2,2-di(ethylthio)propionate (404 mg, 1.92 mmol) and N-iodosuccinimide (1.1 g, 4.8 mmol) and the mixture was stirred at 0 °C for 10 min. To the reaction mixture, trifluoromethanesulfonic acid (35 μL, 0.4 mmol) was added and the reaction mixture was stirred at 0 °C for a further 20 min. After completion of the reaction (TLC), satd $Na_2S_2O_3$ was added, then the solvent was evaporated followed by dilution with dichloromethane. The organic solution was washed with satd $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (5:1) to give pure pyruvate acetal 2 (322 mg, 69%).[31]

1.2. Typical experimental protocol for the synthesis of pyruvate ketals using DBDH

To a solution of methyl 2,3 di-*O*-acetyl-α-D-glucopyranoside (1; 360 mg, 1.29 mmol) in anhydrous acetonitrile (10.0 mL) was added methyl 2,2-di(ethylthio)-propionate (540 mg, 2.58 mmol) and 1,3-dibromo-5,5-dimethylhydantoin (DBDH, 925 mg, 3.23 mmol) and the mixture was stirred at room temperature for 30 min. After completion of the reaction (TLC), triethylamine was added and the solvent was evaporated followed by dilution with dichloromethane. The organic solution was washed with satd $Na_2S_2O_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (5:1) to give a diastereomeric mixture of 2 (336 mg, 72%).

This methodology was applied to the synthesis of a pyruvate ketal-containing trisaccharide related to the



Scheme 3. Reagents and conditions: (a) *N*-iodosuccinimide, TfOH, CH_2Cl_2–toluene (1:1), 4 Å MS, −30 °C, 30 min, 73%; (b) $HClO_4$–SiO_2, CH_3CN, rt, 30 min, 95%; (c) methyl 2,2-di(ethylthio)propionate, *N*-iodosuccinimide, TfOH, CH_3CN, 0 °C, 20 min, 55%; (d) MeONa, MeOH, rt, 5 h, then five drops of H_2O, rt, 12 h, 86%.

cell-wall glycolipid of *M. smegmatis*. The trisaccharide, β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→1)-D-α-Glc*p* is known to have antigenicity.[3–5,32,33] Furthermore, the pyruvate ketal is supposed to provide the serological specificity to the immunodominant oligosaccharide structure.

The synthesis of the pyruvylated trisaccharide is presented in Scheme 3. Glycosylation of ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside **13**, prepared from D-glucose in five steps, with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→1)-2,3,4-tri-*O*-acetyl-α-D-glucopyranose **14**,[34] prepared from α-D-glucopyranosyl-(1→1)-α-D-glucopyranose (trehalose) in three steps, using *N*-iodosuccinimide and trifluoromethanesulfonic acid gave 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→6)-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→1)-2,3,4-tri-*O*-acetyl-α-D-glucopyranose **15** in 73% yield.[35] Removal of the benzylidene acetal from **15** using perchloric acid supported on silica gel ($HClO_4$–SiO_2) furnished 2,3-di-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→1)-2,3,4-tri-*O*-acetyl-α-D-glucopyranose **16**[36] which on pyruvylation using methyl 2,2-di(ethylthio)propionate and *N*-iodosuccinimide in the presence of trifluoromethane sulfonic acid gave 2,3-di-*O*-benzoyl-4,6-[(*S*)-(1-carboxymethyl)ethylidene]-β-D-glucopyranosyl)-(1→6)-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→1)-2,3,4-tri-*O*-acetyl-α-D-glucopyranose **17**.[37] Signals at δ 1.49 (s, 3H) for the ketal methyl (CCH_3) in the ^{1}H NMR and at δ 99.6 (CCH_3) and 25.5 (CCH_3) in the ^{13}C NMR spectra of compound **17** suggested the *S*-configuration by comparison with literature reports. Saponification of compound **17** using sodium methoxide gave 4,6-[(*S*)-(1-sodium carboxylate)ethylidene]-β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→1)-D-α-Glc*p* **18**.[38]

In conclusion, a high yielding reaction protocol with good stereoselectivity for the synthesis of 4,6- as well as 5,6-*O*-pyruvate ketals has been developed. These convenient and easy to perform reaction conditions have been applied to the synthesis of a trisaccharide pyruvate ketal related to the cell-wall glycolipid found in *M. smegmatis*.

Acknowledgements

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References and notes

1. Lindberg, B. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 279–318.
2. Araki, S.; Abe, S.; Yamada, S.; Satake, M.; Fujiwara, N.; Kon, K.; Ando, S. *J. Biochem.* **1992**, *112*, 461–469.
3. Dudman, W. F.; Heidelberger, M. *Science* **1969**, *164*, 954–955.
4. Lew, J. Y.; Heidelberger, M. *Carbohydr. Res.* **1976**, *52*, 255–258.
5. Rao, A. S.; Liao, J.; Kabat, E. A.; Osserman, E. F.; Haeboe, M.; Nimmich, W. *J. Biol. Chem.* **1984**, *259*, 1018–1026.
6. Thayer, W. R.; Bazic, C. M.; Camphausen, R. T.; McNeil, M. *J. Clin. Microbiol.* **1990**, *28*, 714–718.
7. Gray, J. X.; Rolfe, B. G. *Mol. Microbiol.* **1990**, *4*, 1425–1432.
8. Hotter, G. S.; Scott, D. B. *J. Bacteriol.* **1991**, *173*, 851–859.
9. Hirase, S. *Bull. Chem. Soc. Jpn.* **1957**, *30*, 68–79.
10. Choy, Y. M.; Dutton, G. G. S. *Can. J. Chem.* **1974**, *52*, 684–687.
11. Dutton, G. G. S.; Karunaratane, D. N. *Carbohydr. Res.* **1984**, *134*, 103–114.
12. Gorin, P. A. J.; Mazurek, M.; Duarte, H. S.; Duarte, J. H. *Carbohydr. Res.* **1981**, *92*, C1–C4.
13. Liptak, A.; Szabo, L. *J. Carbohydr. Chem.* **1989**, *8*, 629–644.
14. Valeshek, I. E.; Shakhova, M. K.; Mineav, V. A.; Samokhvalov, G. I. *Zh. Obshch. Khim.* **1974**, *44*, 1161–1164.
15. Hiruma, K.; Tamura, J.; Horito, S.; Yoshimura, J.; Hashimoto, H. *Tetrahedron* **1994**, *50*, 12143–12158.
16. Zeigler, T.; Eckhardt, E.; Herold, G. *Liebigs Ann. Chem.* **1992**, 441–451.
17. Zeigler, T.; Eckhardt, E.; Neumann, K.; Birault, V. *Synthesis* **1992**, 1013–1017.
18. Gorin, P. A. J.; Ishikawa, T. *Can. J. Chem.* **1967**, *45*, 521–532.
19. Garegg, P. J.; Lindberg, B.; Kvarnstrom, I. *Carbohydr. Res.* **1979**, *77*, 71–78.
20. Collins, P. M.; McKinnon, C.; Manro, A. *Tetrahedron Lett.* **1989**, *30*, 1399–1400.
21. Aspinell, G. O.; Ibrahim, I. H.; Khare, N. K. *Carbohydr. Res.* **1990**, *200*, 247–256.
22. Liptak, A.; Szabo, L. *Carbohydr. Res.* **1988**, *184*, C5–C8.
23. Liptak, A.; Bajza, I.; Kerekgyarto, J.; Hajko, J.; Szilagyi, L. *Carbohydr. Res.* **1994**, *253*, 111–120.
24. (a) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313–4316; (b) Veenemann, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.
25. Misra, A. K.; Madhusudan, S. K. *Carbohydr. Res.* **2005**, *340*, 497–502.
26. Eguchi, H.; Kawaguchi, H.; Yoshinaga, S.; Nishida, A.; Nishiguchi, T.; Fujisaki, S. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1918–1921.
27. Chassaing, C.; Handrechy, A.; Langlois, Y. *Tetrahedron Lett.* **1997**, *38*, 4415–4416.
28. Zeigler, T.; Eckhardt, E.; Herold, G. *Tetrahedron Lett.* **1992**, *33*, 4413–4416.
29. Gorin, P. A. J.; Mazurek, M.; Duarte, H. S.; Iacomini, M.; Duarte, J. H. *Carbohydr. Res.* **1982**, *100*, l–15.
30. Hiruma, K.; Tamura, J.; Hashimoto, H. *Carbohydr. Res.* **1996**, *282*, 299–306.
31. Spectral data of novel pyruvate ketal-containing carbohydrate derivatives:
Methyl 2,3-di-O-acetyl-4,6-O-[(S)-(1-methoxycarbonyl)-ethylidene]-α-D-glucopyranoside **2**: Yellow oil; $[\alpha]_D^{25}$ +97.1 (*c* 1.0, $CHCl_3$); IR (neat): 2931, 2363, 1594, 1353, 1053, 766 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.42 (t, $J = 9.6$ Hz, 1H), 4.86 (d, $J = 3.3$ Hz, 1H), 4.83 (dd, $J = 9.9$, 3.3 Hz, 1H), 4.03 (dd, $J = 10.8$, 4.8 Hz, 1H), 3.82 (s, 3H, $COOCH_3$), 3.67–3.61 (m, 1H), 3.53–3.46 (m, 2H), 3.38 (s, 3H, OCH_3), 2.07, 2.06 (2s, 6H, $COCH_3$), 1.50 (s, 3H, CCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.6, 170.4, 169.9, 99.6, 97.9, 74.4, 71.7, 69.3, 65.7, 62.0, 55.7,

53.0, 25.6 (CCH_3), 21.2, 21.0; ESI-MS: *m/z* 385.2 [M+Na]; Anal. Calcd for $C_{15}H_{22}O_{10}$ (362): C, 49.72; H, 6.12. Found: C, 49.48; H, 6.30.

Allyl 3-O-acetyl-2-deoxy-4,6-O-[(S)-(1-methoxycarbonyl)-ethylidene]-2-phthalimido-β-D-glucopyranoside **6**: yellow oil; $[\alpha]_D^{25}$ +17.7 (*c* 1.0, $CHCl_3$); IR (neat): 3452, 2918, 2364, 1748, 1448, 1116, 1057, 748 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.87–7.74 (m, 4H, aromatic protons), 5.73–5.62 (m, 2H), 5.41 (d, *J* = 8.4 Hz, 1H), 5.10–5.04 (m, 1H), 4.27–4.21 (m, 2H), 4.14 (dd, *J* = 10.8, 3.9 Hz, 1H), 4.02 (dd, *J* = 12.9, 6.3 Hz, 1H), 3.86 (s, 3H, $COOCH_3$), 3.80–3.74 (m, 1H), 3.72–3.68 (m, 1H), 3.66–3.61 (m, 2H), 1.96 (s, 3H, $COCH_3$), 1.54 (s, 3H, CCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.1, 134.4, 133.6, 131.9, 123.9, 118.1, 99.6, 97.9, 75.6, 70.5, 70.4, 70.2, 66.1, 65.4, 55.6, 52.9, 25.6 (CCH_3), 20.9; ESI-MS: *m/z* 498 [M+Na]; Anal. Calcd for $C_{23}H_{25}NO_{10}$ (475): C, 58.10; H, 5.30. Found: C, 57.82; H, 5.58.

1,2-O-Isopropylidene-5,6-O-(1-methoxycarbonyl)ethylidene]-3-O-octyl-α-D-glucofuranose **10**: yellow oil; $[\alpha]_D^{25}$ −18.2 (*c* 1.0, $CHCl_3$); IR (neat): 2933, 2363, 1595, 1351, 1084, 670 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.86 (dd, *J* = 9.9, 3.3 Hz, 1H), 4.54–4.52 (m, 1H), 4.44–429 (m, 1H), 4.28–4.22 (m, 2H), 4.14 (t, *J* = 5.4 Hz, 1H), 3.88 (dd, *J* = 12.0, 3.3 Hz, 1H), 3.79 (s, 3H, OCH_3), 3.63–3.58 (m, 1H), 3.58–3.54 (m, 1H), 1.59, 1.56 (2s, 6H, $C(CH_3)_2$), 1.50 (s, 3H, CCH_3), 1.31–128 (m, 12H), 0.90 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 111.8, 105.3, 100.1, 82.5, 82.1, 80.6, 73.6, 70.7, 69.1, 31.8, 29.7, 29.2, 26.8, 26.3, 26.0, 22.6 (CCH_3), 14.0; ESI-MS: *m/z* 439 [M+Na]; Anal. Calcd for $C_{21}H_{36}O_8$ (416): C, 60.56; H, 8.71. Found: C, 60.31; H, 8.83.

Methyl (3-O-acetyl-2-deoxy-4,6-O-[(S)-(1-methoxycarbonyl)ethylidene]-2-phthalimido-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-α-D-glucopyranoside **12**: yellow oil; $[\alpha]_D^{25}$ +45.2 (*c* 1.0, $CHCl_3$); IR (neat): 3406, 2363, 1746, 1228, 1038, 770 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.28–8.12 (m, 4H, aromatic protons), 6.04 (t, *J* = 9.0 Hz each, 1H), 5.78 (d, *J* = 8.4 Hz, 1H), 5.64 (t, *J* = 9.9 Hz each, 1H), 5.12 (t, *J* = 9.6 Hz each, 1H), 5.02 (dd, *J* = 10.2, 3.6 Hz, 1H), 4.88 (d, *J* = 3.3 Hz, 1H), 4.58–4.50 (m, 2H), 4.18 (s, 3H), 4.17–4.08 (m, 3H), 4.05–4.00 (m 2H), 3.87–3.75 (m, 1H), 3.45 (s, 3H), 2.39, 2.32, 2.29, 2.22 (4s, 12H, 4 $COCH_3$), 1.90 (s, 3H, CCH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.2, 169.6 (2C), 169.3, 167.9 (2C), 134.3–123.7 (aromatic carbons), 99.5 (CCH_3), 99.3, 96.6, 79.4, 75.4, 74.2, 73.3, 70.6, 70.1, 69.2, 68.0, 55.7, 55.4, 55.1, 52.9, 55.3, 54.9, 25.5 (CCH_3), 20.9 (2C), 20.7 (2C); ESI-MS: *m/z* 760 [M+Na]; Anal. Calcd for $C_{33}H_{39}NO_{18}$ (737): C, 53.73; H, 5.33. Found: C, 53.60; H, 5.58.

32. Ohta, M.; Pan, Y. T.; Laine, R. A.; Elbein, A. D. *Eur. J. Biochem.* **2002**, *269*, 3142–3149.
33. Saadat, S.; Ballou, C. E. *J. Biol. Chem.* **1983**, *258*, 1813–1818.
34. Szurmai, Z.; Kerékgyártó, J.; Harangi, J.; Lipták, A. *Carbohydr. Res.* **1987**, *164*, 313–325.
35. *2,3-Di-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→6)-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1→1)-2,3,4-tri-O-acetyl-α-D-glucopyranose* **15**: To a solution of compound **13** (1.28 g, 2.46 mmol) and compound **14** (1.0 g, 1.57 mmol) in anhydrous CH_2Cl_2: toluene (1:1; 20 mL) was added powdered 4 Å MS (3.0 g) and the mixture was stirred under argon for 1 h. *N*-Iodosuccinimide (580 mg, 2.58 mmol) was added to the reaction mixture at room temperature which was then cooled to −40 °C. Trifluoromethanesulfonic acid (22 μL, 0.25 mmol) was added to the reaction mixture and it was allowed to stir for 30 min at −40 °C. The reaction mixture was filtered through Celite and the filtrate was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$, water, dried (Na_2SO_4) and concentrated to a syrupy product. Column chromatography of the crude product over SiO_2 using hexane–EtOAc (2:1) afforded pure 15 (1.25 g, 73%) as glassy solid; $[\alpha]_D^{25}$ +105.4 (*c* 1.0, $CHCl_3$); IR (KBr): 2961, 2363, 1753, 1597, 1353, 1223, 1098, 766, 709 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.95–7.92 (m, 3H, aromatic protons), 7.49–7.26 (m, 12H, aromatic protons), 5.77 (t, *J* = 9.6 Hz each, 1H), 5.54 (s, 1H, CHC_6H_5), 5.46–5.37 (m, 3H), 5.05–4.97 (m, 3H), 4.93 (d, *J* = 3.6 Hz, 1H), 4.89–4.80 (m, 2H), 4.78 (t, *J* = 7.8 Hz, 1H), 4.43 (dd, *J* = 10.2, 4.8 Hz, 1H), 4.18 (t, *J* = 6.0 Hz, 1H), 4.14–4.04 (m, 2H), 3.98–3.81 (m, 4H), 3.68 (t, *J* = 4.8 Hz, 1H), 3.55–3.49 (m, 1H), 2.07, 2.03, 2.00, 1.97 (4s, 21H, $7COCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.2, 170.0, 169.9, 169.8, 169.6 (2C), 166.2, 166.1, 133.3–126.5 (aromatic carbons), 102.3 (PhCH), 101.8, 92.3, 91.9, 79.0, 72.3, 72.1, 70.7 (2C), 70.3 (3C), 69.5 (2C), 68.9, 68.7, 68.4, 67.1, 62.2, 20.9 (2C), 20.8 (3C), 20.7 (2C); ESI-MS: *m/z* 1072 [M+Na]; Anal. Calcd for $C_{53}H_{58}O_{25}$ (1095): C, 58.13; H, 5.34. Found: C, 58.10; H, 5.49.
36. Agnihotri, G.; Misra, A. K. *Tetrahedron Lett.* **2006**, *47*, 3653–3658.
37. *(2,3-Di-O-benzoyl-4,6-O-[(S)-(1-methoxycarbonyl)ethylidene]-β-D-glucopyranosyl)-(1→6)-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-(1→1)-2,3,4-tri-O-acetyl-α-D-glucopyranose* **17**: Prepared following the NIS-TfOH promoted general reaction protocol. Yield 55%; yellow oil; $[\alpha]_D^{25}$ +98.9 (*c* 1.0, $CHCl_3$); IR (neat): 1749, 1225, 1038, 713 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.96–7.34 (m, 10H, aromatic protons), 5.58 (t, *J* = 9.6 Hz each, 1H), 5.40–5.25 (m, 3H), 4.99–4.89 (m, 3H), 4.83–4.75 (m, 2H), 4.70 (d, *J* = 4.8 Hz, 1H), 4.17–4.11 (m, 2H), 4.02–3.96 (m, 2H), 3.92–3.87 (m, 2H), 3.83 (s, 3H, $COOCH_3$), 3.76–3.68 (m, 3H), 3.58–3.44 (m, 2H), 2.08, 2.06, 2.02, 2.00, 1.98, 1.96 (6s, 21H, $7COCH_3$), 1.49 (s, 3H, CCH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.4 (2C), 170.0, 169.8 (2C), 169.6 (2C), 165.7, 165.1, 133.7–128.7 (aromatic carbons), 102.1, 99.6 (CCH_3), 92.3, 91.8, 75.0, 72.2 (2C), 70.8, 70.3 (2C), 69.5 (2C), 68.8 (2C), 66.8, 66.7, 66.6, 65.2, 62.2, 52.9 ($COOCH_3$), 25.5 (CCH_3), 20.9 (3C), 20.8 (4C); ESI-MS: *m/z* 1113.3 [M+Na]; Anal. Calcd for $C_{50}H_{58}O_{27}$ (1090): C, 55.05; H, 5.36. Found: C, 54.87; H, 5.50.
38. *4,6-O-[(S)-(1-Sodium carboxylate)ethylidene]-β-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→1)-D-α-Glcp* **18**: A solution of compound **17** (400 mg, 0.36 mmol) in 0.05 M sodium methoxide in methanol (5.0 mL) was stirred at room temperature for 5 h. Then five drops of distilled water were added to the reaction mixture which was allowed to stir at room temperature for 12 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+), filtered and the filtrate was evaporated to dryness to give a glassy solid, which was again dissolved in distilled water and treated with Dowex 50W-X8 (Na^+) and concentrated. The crude product was purified through Sephadex LH-20 using 80% aqueous EtOH as eluant to give pure trisaccharide **18** as its sodium salt (185 mg, 86%); glassy solid; $[\alpha]_D^{25}$ +14 (*c* 1.0, H_2O); IR (KBr): 3760, 2375, 1595, 1352 cm^{-1}; ^1H NMR (300 MHz, D_2O): δ 5.12 (d, *J* = 3.3 Hz, 1H, H-1), 5.10 (d, *J* = 3.3 Hz, 1H, H-1′), 4.51 (d, *J* = 8.1 Hz, 1H, H-1″), 4.04–4.02 (m, 1H), 3.97 (d, *J* = 4.8 Hz, 1H), 3.84–3.77 (m, 5H), 3.72–3.71 (m, 2H), 3.68 (d, *J* = 4.5 Hz, 1H), 3.61–3.56 (m, 4H), 3.47 (t, *J* = 9.6 Hz, 1H), 3.42–3.36 (m, 3H), 1.41 (s, 3H); ^{13}C NMR (75 MHz, D_2O): δ 170.0, 102.1 (C-1″), 100.2 (ketal-*C*), 92.0 (2C), 74.6, 72.7, 71.5, 71.2 (2C), 70.9, 69.7 (2C), 68.4, 68.1, 67.4, 64.6, 62.9, 59.3 (2C), 23.4; ESI-MS: *m/z* 619.1 [M+Na]; Anal. Calcd for $C_{21}H_{33}NaO_{18}$ (596): C, 42.29; H, 5.58. Found: C, 42.05; H, 5.80.