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Highly Efficient and Practical Synthesis of 3,6-Branched Oligosaccharides

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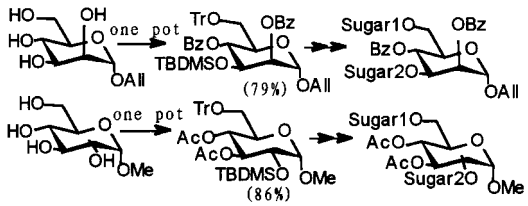
ABSTRACT



A one-pot formation of the 3- and 6-OH differentially protected sugar synthon was described. A mannopyranosyl pentasaccharide and a glucopyranosyl hexasaccharide were prepared employing this new finding.

The oligosaccharide chains of glycoproteins play a significant role in cell recognition and signal transduction during numerous biological processes.[1] Among those oligosaccharides present at cell surface, many have been found to have branched-chain structures.[2] For example, the 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannopyranosyl structure is a feature common to all *N*-linked oligosaccharides while 3,6-di-*O*-(*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl structure is the characteristic of elicitor-active *β*-glucan and antitumor polysaccharide schizophyllan, sceroglucan and lentinan.[3] As a result, the efficient preparation of this group of oligosaccharides has been a major focus in carbohydrate chemistry not only because of their biological functions but also because of their unique branched chain structure.[4] In pursuing the preparation of bioactive oligosaccharide, here we explore a general and practical method for the facile synthesis of 3,6-disubstituted sugar chains.

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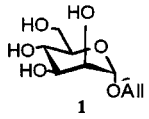
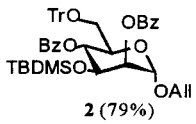
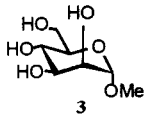
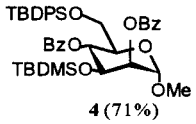
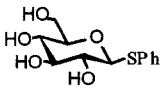
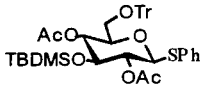
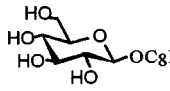
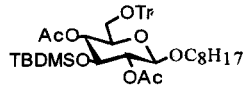
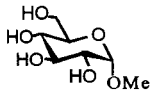
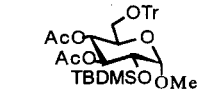
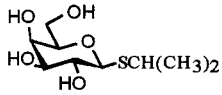
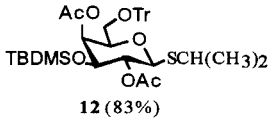
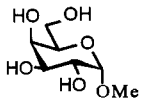
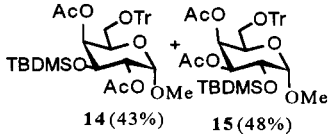
Our strategy involves a one-pot regioselective synthesis of 2,4-di-*O*-acyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl glycoside, exemplified by the preparation of corresponding α-D-mannopyranoside **2**. In this protocol, allyl α-D-mannopyranoside **1** was subjected to the following three sequential reactions in one-pot: (1) treatment of **1** with 1.25 equiv of trityl chloride and catalytic amount of 4-(dimethylamino)-pyridine (DMAP) in pyridine at 80 °C; (2) regioselective silylation on C-3 with 1.1 equiv of *tert*-butyldimethylchlorosilane (TBDMSCl) and 2 equiv of imidazole at room temperature; and (3) benzoylation on C-2 and C-4 with 2.5 equiv of BzCl at 50 °C.[5] One column separation gave allyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-α-D-mannopyranoside (**2**) in an isolated yield of 79%. The higher reactivity of the 3-OH in mannopyranoside is not unexpected since the 2-OH is in the sterically hindered axial position, and the 4-OH is generally known to be the least reactive. We were gratified to find that the new method was also effective for other sugar derivatives.[6] For example, *β*-D-glucopyranoside **5**, **7** and *β*-D-galactopyranoside **11** were transformed into the corresponding 3,6-disubstituted compound **6**, **8**, and **12**, respectively, in good to excellent yields. On the other hand, when the aforementioned one-pot reaction was applied to the α-D-glucopyranoside **9**, 2-selective silylation was given leading to methyl 3,4-di-*O*-acetyl-2-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-α-D-glucopyranoside **10** in high yield (86% after column purification), while the same reaction for methyl α-D-galactopyranoside **13** generated a regio-isomeric mixture of **14** and **15** (91% yield in total). It is worth to note that changing 6-*O*-trityl to 6-*O*-*tert*-butyldiphenylsilyl, as in **3**, afforded 3,6-disubstituted mannopyranoside **4** in good yield (71%).[7]

Table 1. Regioselective Tritylation and Silylation on Monosaccharide

entry	substrate	product (isolated yield)
1	 **1**	 **2** (79%)
2	 **3**	 **4** (71%)
3	 **5**	 **6** (75%)
4	 **7**	 **8** (65%)
5	 **9**	 **10** (86%)
6	 **11**	 **12** (83%)
7	 **13**	 **14** (43%) **15** (48%)

These 3,6- or 2,6-differentially protected carbohydrates are very useful intermediates for oligosaccharide synthesis.

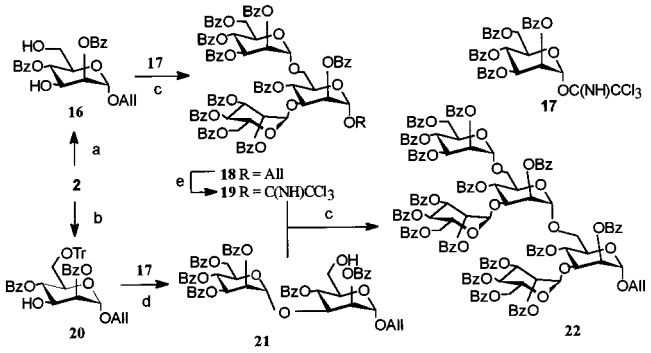
(5) Typical reaction procedure is as following: To a solution of **1** (7 g, 31.8 mmol) in pyridine (65 mL) was added 1.25 equiv of TrCl and 30 mg of DMAP. The mixture was stirred at 80 °C for 16 h, then cooled to 0 °C, added 2 equiv of imidazole. Finally, 1.1 equiv of TBDMSCl in DMF (5 mL) was added portion in portion during 2 h. The mixture was stirred at room temperature overnight, then a premixed BzCl (2.5 equiv) and pyridine (5 mL) was added. Let the reaction mixture stirred at 50 °C overnight, then poured into ice-cold water, extracted with EtOAc. The organic phase was concentrated to dryness with the help of toluene. The residue was subjected to column chromatography on silica gel with petroleum ether/EtOAc as the eluent (12/1) to give **2** (19.7 g, 79%). Similarly, using Ac_2O (3 equiv) instead of BzCl in the aforementioned acylation step furnished the corresponding acetylated derivatives smoothly.

(6) We cannot give an unbeatable explanation for this regioselectivity. We found that the orientation of anomeric oxygen or sulfur atom is critical to the reaction outcomes. Generally, α-D-manno-, *β*-D-gluco- and *β*-D-galactopyranosides gave 3,6-disubstituted products. Interestingly, α-D-glucopyranosides generated 2,6-disubstitutes while α-D-galactopyranosides gave 2,6- and 3,6-disubstituted mixtures.

(7) Selected ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): (**19**) *δ* 3.84 (dd, 1 H, J < 1, J = 9.2 Hz, H-6a), 4.15 (dd, 1 H, J = 6.3, J = 11.0 Hz, H-6b), 4.30−4.37 (m, 2 H), 4.48−4.58 (m, 5 H), 4.73 (dd, 1 H, J = 3.4, J = 9.8 Hz, H-3), 5.10 (d, 1 H, J = 1.2 Hz, H-1), 5.38−5.41 (m, 2 H, H-1, H-2), 5.69−5.73 (m, 2 H, H-2, H-3), 5.90−5.95 (m, 2 H), 6.01 (t, 1 H, J = 10.8 Hz, H-4), 6.07 (t, 1 H, J = 10.0 Hz, H-4), 6.12 (t, 1 H, J = 10.0 Hz, H-4), 6.59 (d, 1 H, J = 1.5 Hz, H-1), 7.00−8.13 (m, 50 H, Ph), 9.00 (s, 1 H, N*H*). (**21**) *δ* 2.89 (bs, H, O*H*), 3.75−3.80 (m, 2 H, 2 H-6), 4.00 (ddd, 1 H, H-5), 4.07 (ddd, 1 H, H-5′), 4.18−4.25 (m, 1 H, CH_2═CH−CH_2), 4.35 (dd, 1 H, $J_{5',6'a}$ = 3.6, $J_{6'a,\ 6'b}$ = 12.3 Hz, H-6′a), 4.44−4.50 (m, 1 H, CH_2═CH−CH_2), 4.61 (dd, 1 H, $J_{5',6'b}$ = 2.4 Hz, H-6′b), 4.70 (dd, 1 H, $J_{2,3}$ = 3.5, $J_{3,4}$ = 9.8 Hz), 5.15 (d, 1 H, $J_{1,2}$ = 1.4 Hz, H-1), 5.23−5.36 (m, 3 H, H-2 and CH_2═CH−CH_2), 5.41 (d, 1 H, $J_{1',2'}$ = 1.7 Hz, H-1′), 5.65−5.69 (m, 2 H, H-2′, H-3′), 5.72 (t, 1 H, $J_{3,4}$ = 10 Hz, H-4), 5.89 (m, 1 H, CH_2═C*H*-CH_2), 6.02 (t, 1 H, $J_{3',4'}$ 10.0 Hz, H-4′), 7.19−8.25 (m, 30 H, Ph). (**22**) *δ* 3.44 (bd, 1 H, H-6), 3.82 (dd, 1 H, H-6), 4.01 (dd, 1 H, H-6), 4.14 (ddd, 1 H, H-5), 4.20−4.53 (m, 12 H), 4.58 (dd, 1 H, H-6), 4.67 (dd, 1 H, H-3), 4.73 (dd, 1 H, H-3), 4.78 (d, 1 H, H-1), 5.17−5.19 (m, 2 H, H-1 and one proton of CH_2═CH−CH_2−), 5.21 (d, 1 H, H-1), 5.29−5.32 (m, 3 H, H-1, H-2, and one proton of CH_2═CH−CH_2−), 5.37 (d, 1 H, H-1), 5.44 (dd, 1 H, H-2), 5.57 (dd, 1 H, H-2), 5.65 (dd, 1 H, H-3), 5.69−5.74 (m, 2 H, H-2, H-3), 5.82 (dd, 1 H, H-2), 5.85−6.01 (m, 4 H, H-3, 2 H-4, CH_2═C*H*-CH_2), 6.04 (t, 1 H, H-4), 6.07 (t, 1 H, H-4), 6.08 (t, 1 H, H-4), 7.18−8.35 (m, 80 H, Ph). (**27**) *δ* 0.88 (t, 3 H, CH_3), 1.25−1.35 (bs, 10 H, 5 CH_2), 1.45−1.55 (m, 2 H, CH_2), 2.00, 2.02, 2.02, 2.03, 2.04, 2.06, 2.09, 2.09, 2.09 (7 s, 27 H, 9 CH_3CO), 3.40 (dt, 1 H, one proton of OCH_2), 3.55−3.68 (m, 5 H, H-5^B, H-5^C, H-3^A, H-6a^A, H-6b^A), 3.80−3.90 (m, 3 H, H-3^C, H-5^A, one proton of OCH_2), 4.03 (dd, 1 H, J = 2.2, J = 12.4 Hz, H-6a^B/H-6a^C), 4.10 (dd, 1 H, J = 2.1, J = 12.3 Hz, H-6a^C/H-6a^B), 4.25−4.32 (m, 3 H, J = 8.1 Hz, H-1^A, H-6b^B, H-6b^C), 4.50 (d, J = 8.1 Hz, H-1^C), 4.55 (s, 2 H, PhCH_2), 4.57 (d, 1 H, J = 8.1 Hz, H-1^B), 4.69 (t, 1 H, J = 9.7 Hz, H-4^A), 4.88−5.00 (m, 3 H, H-2^{A,B,C}), 5.06 (t, J = 9.7 Hz, H-4^C), 5.09 (t, 1 H, J = 9.7 Hz, H-4^B), 5.18 (t, 1 H, J = 9.5, H-3^B), 7.21−7, 33 (m, 5 H, Ph). (**30**) *δ* 0.88 (t, 3 H), 1.24−1.27 (m, 10 H), 1.40−1.50 (m, 2 H), 1.94 (s, 3 H), 1.95 (s, 3 H), 1.96 (bs, 6 H), 1.98 (s, 3 H), 1.99 (s, 6 H), 2.01 (s, 9 H), 2.02 (s, 3 H), 2.03 (s, 3 H), 2.08 (s, 3 H), 2.09 (2 s, 6 H), 2.13 (s, 3 H), 2.14 (s, 3 H), 2.23 (s, 3 H), 2.24 (s, 3 H), 3.40 (dt, 1 H, one proton of OCH_2), 3.50−3.55 (m, 1 H), 3.56−3.72 (m, 6 H), 3.72−3.77 (m, 1 H), 3.78−3.91 (m, 5 H), 3.96 (dd, 1 H), 4.05 (dd, 1 H), 4.09−4.40 (m, 3 H), 4.43−4.58 (m, 6 H), 4.62−4.75 (m, 3 H), 4.86−4.94 (m, 2 H), 4.95−5.05 (m, 4 H), 5.10−5.22 (m, 6 H). Selected ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 95.48, 99.95, 100.24, 100.38, 100.75, 100.89 (6 C-1), 168.55, 168.73, 168.81, 169.20 (2 C), 169.24, 169.28, 169.39, 169.44 (2 C), 170.13 (2 C), 170.20, 170.24, 170.49, 170.60, 170.63, 171.10, 171.17 (19 CH_3CO).

Remarkably, it could be used to synthesize either homo- or hetero-trisaccharide core structure from the same key intermediate such as **2**. For example, the mannose di-*O*-substituted derivative **2** can be used readily as a precursor for the synthesis of polymanans (Scheme 1).[8] Thus, compound **2** was treated with 90% trifluoroacetic acid (TFA) to give diol **16** in 91% yield. Coupling of **16** with trichloroacetimidate **17** (2.1 equiv) in anhydrous CH_2Cl_2 using TMSOTf as catalyst gave trisaccharide **18** in 85% yield. Deallylation on **18** with $PdCl_2$ (2 equiv) and NaOAc (4 equiv) in 90% aqueous acetic acid, followed by C-1 Schmidt activation with trichloroactonitrile, furnished trisaccharide donor **19** in 80% yield (two steps). Convergently, compound **2** was treated with tetrabutylammonium fluoride in tetrahedrofuran (THF) to afford 3-OH derivative **20** in 66% yield. As shown in Scheme 1, assembly of the pentamannose core structure **22** was achieved through two glycosylation steps: (1) glycosylation of trichloroacetimidate **17** with acceptor **20** was accomplished in 40 min using TMSOTf (0.08 equiv) as the catalyst at −15 °C, more TMSOTf (0.9 equiv)[9] was added into this reaction mixture, and then it was stirred at room temperature for 4 h to afford disaccharide acceptor **21** (80%); (2) coupling of trisaccharide donor **19** with **21** under the same reaction conditions as described in the preparation of **18** finished the pentasaccharide (**22**) in 85% yield.

Scheme 1. Synthesis of Penta-Mannose **22**[a]

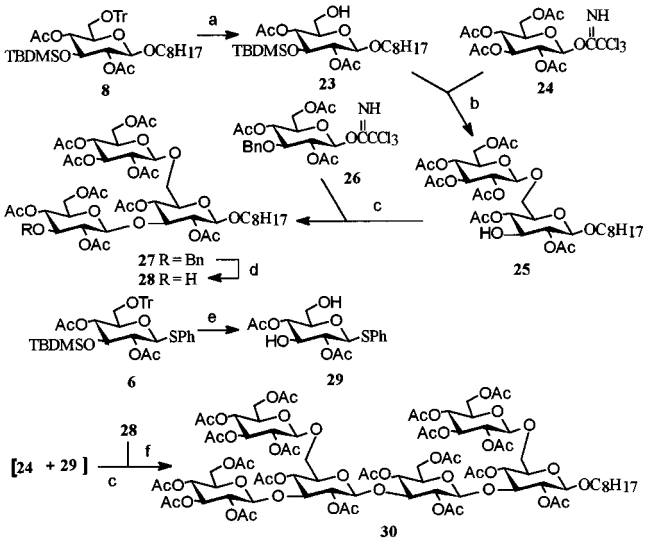


[a] (a) 90% TFA, 91%. (b) TBAF, THF, 66%. (c) TMSOTf, CH_2Cl_2, 0 °C, 85% for both **18** and **22**. (d) TMSOTf, CH_2Cl_2, 0 °C, then excess TMSOTf or TFA, 80%. (e) 90% HOAc, NaOAc, $PdCl_2$; Cl_3CCN, DBU, CH_2Cl_2, 80% (two steps).

To ascertain the efficiency of this synthetic strategy, we next applied this method to the synthesis of branched gluco-hexasaccharide derivative **30** (see Scheme 2). $FeCl_3$ catalyzed detritylation[10] was carried out smoothly on octyl β-D-glucopyranoside **8** providing 6-OH acceptor **23** in 90% yield. Standard glycosylation of **23** with fully acetylated imidate **24** in CH_2Cl_2 followed by in situ hydrolysis with 90% TFA afforded 3-OH derivative **25** in a total yield of 75%. Coupling of disaccharide acceptor **25** with 3-*O*-benzylated Schmidt's reagent **26** gave trisaccharide **27**, which was debenzylated with sodium bromate/sodium dithionite in $EtOAc/H_2O$[11] generated trisaccharide acceptor **28** in 73% yield (two steps).

Treatment of **6** with 90% trifluoroacetic acid gave the core synthon **29** in 91% yield. Thus, one-pot glycosylation[12] was utilized in the final assembly as follows: (1) coupling of diol **29** with imidate **24** (2.05 equiv) was completed within 40 min using catalytic amount of TMSOTf (0.17 equiv) at 0 °C, providing the desired trisaccharide thioglucoside donor; (2) without purification, this reaction mixture was cooled to −15 °C, then trisaccharide acceptor **28** (1 equiv) was added, followed by addition of *N*-iodosuccinimide (NIS) (2 equiv) and TMSOTf (0.5 equiv). The reaction mixture was stirred at 0 °C for 2 h leading to the target octyl hexaglucopyranoside **30** in 50.2% yield as an amorphous solid.[7]

Scheme 2. Synthesis of Gluco-Hexasaccharide Derivative **30**[a]



[a] (a) $FeCl_3 \cdot 6H_2O$, CH_2Cl_2, 90%. (b) TMSOTf, CH_2Cl_2, 0 °C, then excess TFA, 75%. (c) TMSOTf, CH_2Cl_2, 0 °C, 79%. (d) $NaBrO_3$, $Na_2S_2O_4$, EtOAc, 93%. (e) 90% TFA, 91%. (f) NIS, TMSOTf, CH_2Cl_2, 0 °C, 50.2%.

(8) (a) Merritt, J. R.; Naiseng, E.; Fraser-Reid, B. *J. Org. Chem.* **1994**, *59*, 4443. (b) Grice, P.; Ley, S. V.; Pietruszka, T.; Osborn, H. M. I.; Priepke, H. W. M.; Warriner, S. L. *Chem. Eur. J.* **1997**, *3*, 431−440.

(9) We tried coupling reaction of **17** with **20** on small scale (around 100 mg of reactants) and disaccharide acceptor **21** was obtained in 4 h without adding extra TMSOTf. The same reaction condition was not suitable for larger reaction scale, thus more TMSOTf or trifluoroacetic acid was added into the reaction flask when TLC showed the first coupling reaction finished.

(10) Ding, X.; Wang, W.; Kong, F. *Carbohydr. Res.* **1997**, *303*, 445−448.

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(12) For "one-pot glycosylation" see (a) Ley, S. V.; Priepke, H. W. M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2292−2294. (b) Yamada, H.; Harada, T.; Miyazaki, H.; Takahashi, T. *Tetrahedron Lett.* **1994**, *35*, 3979−3982. (c) Zhang, Z.; Ollmann, I. R.; Ye, X. S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734−753. (d) Yu, B.; Yu, H.; Hui, Y.; Han, X. *Tetrahedron Lett.* **1999**, *40*, 8591−8594. (e) Yu, B.; Xie, J.; Deng, S.; Hui, Y. *J. Am. Chem. Soc.* **1999**, *121*, 12196−12197. (f) Cheung, M.-K.; Douglas, N. L.; Henzen, B.; Ley, S. V.; Pannecoucke, X. *Synlett.* **1997**, *3*, 257−260. (g) Green, L.; Hinzen, B.; Ince, S. J.; Langer, P.; Ley, S. V.; Warriner, S. L. *Synlett.* **1998**, *4*, 440−442.

In conclusion, a highly efficient and practical method was developed for the preparation of 3,6-branched oligosaccharides. It can be used to synthesize both homo- and heterotrisaccharide core structures which are used for the further assembly of advanced bioactive sugar chains. The use of sole acyl-protecting groups should simplify the synthetic procedure. More importantly, combination of this method with one-pot glycosylation may generate an efficient entry into more complex glycoconjugates.

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Supporting Information Available: Preparations and physical data for compounds **2**, **4**, **6**, **8**, **10**, **12**, **16**, **19**, **21**−**23**, **25**, and **27**−**30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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