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) benzylation 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 man-
nopyranoside **4** in good yield (71%).⁷

(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 Ac₂O (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-gluc- 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 ¹H NMR (400 MHz, CDCl₃, 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, NH). (**21**) δ 2.89 (bs, H, OH), 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, CH₂=CH-CH₂), 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, CH₂=CH-CH₂), 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 CH₂=CH-CH₂), 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, CH₂=CH-CH₂), 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,

Table 1. Regioselective Tritylation and Silylation on Monosaccharide

entry	substrate	product (isolated yield)
1		
2		
3		
4		
5		
6		
7		

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

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 CH₂=CH-CH₂), 5.21 (d, 1 H, H-1), 5.29–5.32 (m, 3 H, H-1, H-2, and one proton of CH₂=CH-CH₂), 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, CH₂=CH-CH₂), 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, CH₃), 1.25–1.35 (bs, 10 H, 5 CH₂), 1.45–1.55 (m, 2 H, CH₂), 2.00, 2.02, 2.02, 2.03, 2.04, 2.06, 2.09, 2.09, 2.09 (7 s, 27 H, 9 CH₃CO), 3.40 (dt, 1 H, one proton of OCH₂), 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 OCH₂), 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, PhCH₂), 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 OCH₂), 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 ¹³C NMR (CDCl₃, 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 CH₃CO).

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, com-

Scheme 1. Synthesis of Penta-Mannose **22**^a

^a (a) 90% TFA, 91%. (b) TBAF, THF, 66%. (c) TMSOTf, CH₂Cl₂, 0 °C, 85% for both **18** and **22**. (d) TMSOTf, CH₂Cl₂, 0 °C, then excess TMSOTf or TFA, 80%. (e) 90% HOAc, NaOAc, PdCl₂; Cl₃CCN, DBU, CH₂Cl₂, 80% (two steps).

pound **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 CH₂Cl₂ using TMSOTf as catalyst gave trisaccharide **18** in 85% yield. Deallylation on **18** with PdCl₂ (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 tetrahydrofuran (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.

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). FeCl₃ catalyzed

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

^a (a) FeCl₃·6H₂O, CH₂Cl₂, 90%. (b) TMSOTf, CH₂Cl₂, 0 °C, then excess TFA, 75%. (c) TMSOTf, CH₂Cl₂, 0 °C, 79%. (d) NaBrO₃, Na₂S₂O₄, EtOAc, 93%. (e) 90% TFA, 91%. (f) NIS, TMSOTf, CH₂Cl₂, 0 °C, 50.2%.

detritylation¹⁰ 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 CH₂Cl₂ 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/H₂O¹¹ 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 glycosylation¹² 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 hexagluco-pyranoside **30** in 50.2% yield as an amorphous solid.⁷

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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 hetero-trisaccharide 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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