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A New Strategy for the Synthesis of the Core Trisaccharide of Asparagine-Linked Sugar Chains

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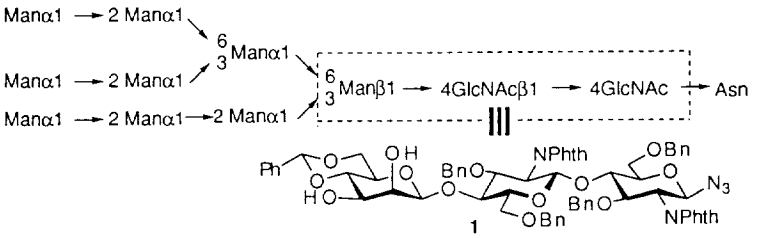
Abstract: The core trisaccharide common to all asparagine-linked glycoprotein oligosaccharides was synthesized using two novel processes: 1) regioselective acetylation using lipase, and 2) inversion of the C-2 hydroxyl group of the glucose residue in a glucosyl chitobiose derivative to produce the corresponding mannosyl chitobiose derivative.

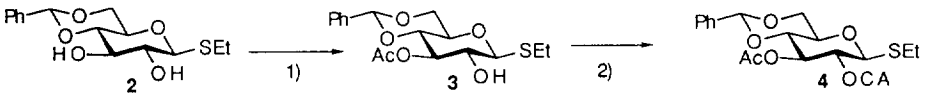
All N-linked glycoprotein oligosaccharides share one common core structure - the β1-4-linked D-mannosyl chitobiose unit.[1] This trisaccharide is naturally an important intermediate for the synthesis of complex and high-mannose type oligosaccharides.[2] Therefore, a great deal of effort by a number of laboratories has been devoted to the synthesis of this core trisaccharide.[3] The structure of this trisaccharide contains a β-mannoside bond, and stereoselective β-mannoside bond formation is one of the most difficult challenges in synthetic carbohydrate chemistry today.[4] In this paper we demonstrated a highly efficient approach to core trisaccharide **1**.



Our synthetic strategy includes two novel processes: 1) regioselective acetylation using lipase, and 2) inversion of the C-2 hydroxyl group of the glucose residue in a glucosyl chitobiose derivative to produce the corresponding mannosyl chitobiose derivative.

The synthesis of thioglucoside donor **4** by means of the chemoenzymatic method is shown in scheme 1. For the regioselective acetylation of the compound **2**[5] by the trans esterification activity of lipase, we screened 13 different commercially available lipases.[6] Some of the results are shown in Table 1. The regioselectivity of this reaction was dependent on the microbial source of lipase. We found that only the use of Lipase Amano AK™ (from *Pseudomonas fluorescens*)[7] resulted in

completely regioselective acetylation at the C-3 position. The isolated yield of compound **3**[8] was 92%. We were able to perform the same reaction on a multi-gram scale, enjoying the same yield and regioselectivity found during screening. Chloroacetylation of **3** provided the glucosyl donor **4**[8] in 92% yield. It is noteworthy that all the products described thus far were purified by simple recrystallization, thereby avoiding column chromatography.



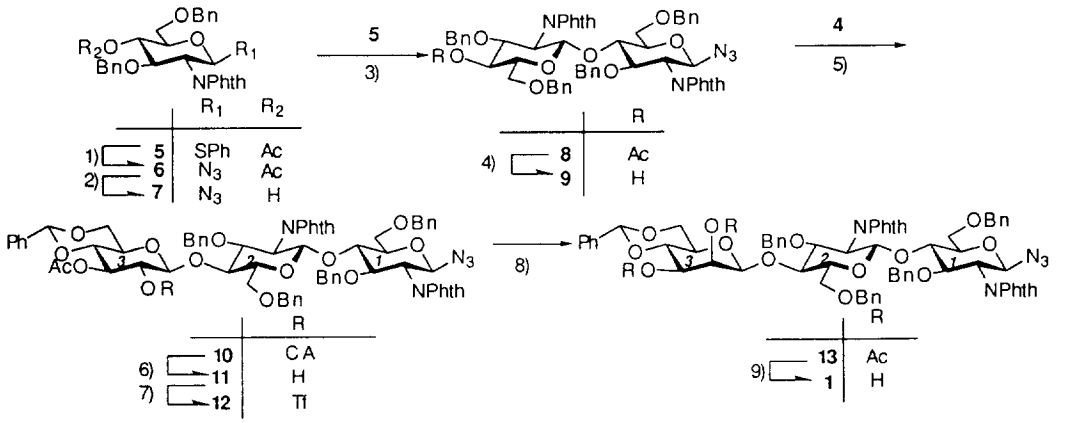
Conditions: (1) Lipase , Vinyl acetate , 45 °C, 92 %;(2) Chloroacetic anhydride, Pyridine, CH_2Cl_2 , 92 %.

Scheme 1

Table 1. Trans esterification reaction of vinyl acetate into 3 by use of lipase of various origin

Starting sugar	Lipase[a,b]	Reaction time(h)[c]	%Conversion[d]	% of acylation in position C-2[d]	% of acylation in position C-3[d]
2	PS	5	100	6	94
2	AK	5	100	0	100
2	TOYO	2	100	19	81

[a] PS: *Pseudomonas cepacia* (Amano Pharmaceutical Co.,Ltd.), AK: *Pseudomonas fluorescens* (Amano Pharmaceutical Co.,Ltd.), TOYO:*Pseudomonas aeruginosa* (TOYOBO Co.,Ltd.). [b] The lipase was used as purchased; reaction conditions : sugar 0.5mmol, vinyl acetate 1ml, 45°C, 110rpm. [c] The reaction was continued until the TLC showed the disappearance of the starting material. [d] The ratio was determined by ^{1}H-NMR.



Conditions: (1) $TMSN_3$, NIS, TfOH, 88 % ; (2) NaOMe, MeOH / THF, 90 % ; (3) NIS, TfOH, 92 % ; (4) NaOMe, MeOH / THF, 86 % ; (5) NIS, TfOH, 78 % ; (6) Thiourea, $NaHCO_3$, 90 % ; (7) Tf_2O, Pyr., quant. ; (8) CsOAc, 18-Crown-6, 89 % ; (9) NaOMe, MeOH / THF, 93%.

Scheme 2

Scheme 2 shows construction of the trisaccharide and the inversion of the C-2 hydroxyl group of the glucose residue in a glucosyl chitobiose derivative to produce the corresponding mannosyl chitobiose derivative. Thiophenyl glucosamine derivative **5**[9] was chosen for syntheses of both **6** and chitobiose derivative **8**. Treatment of **5** with $TMSN_3$-NIS/TfOH[10] in CH_2Cl_2 at -40 °C, followed by deacetylation gave **7**[11] in 79% overall yield. Coupling of **5** and **7** using NIS/TfOH in CH_2Cl_2 at -20

^{o}C produced the chitobiose derivative **8**. Subsequent removal of the acetyl group at the C-4 position of **8** using NaOMe/MeOH at 0 ^{o}C gave the chitobiosyl acceptor **9** (overall yield 79%). Coupling of the aforementioned thioglucoside donor **4** and the disaccharide **9** using 5eq of NIS and 1eq of TfOH provided the trisaccharide **10**[8] as a single product in 78% yield based on the amount of **4**. The β-configuration of the newly formed glycosidic bond was confirmed by ^{1}H NMR spectroscopy. The observed coupling constant between H-1 and H-2 was 8.0 Hz, while that of α-glucosides is typically less than 5 Hz. Selective removal of the chloroacetyl group of **10** was achieved using thiourea and $NaHCO_3$ in CH_2Cl_2/EtOH at 70 ^{o}C. The resulting C-2 hydroxyl derivative **11** was treated with trifluoromethanesulfonic anhydride in dry pyridine at -20 ^{o}C to give compound **12**. The configuration at C-2 of the glucose residue in **12** was inverted via nucleophilic substitution using CsOAc and 18-crown-6[4n] in toluene under sonication at 40 ^{o}C. The mannosyl chitobiose derivative **13**[8] was obtained as a single product (89% yield from **11**). The coupling constants J_{1-2} and J_{2-3} in the ^{1}H NMR spectrum were ~1Hz and 3.5 Hz, respectively, supporting the β-mannosyl assignment. Deacetylation of **13** using NaOMe/MeOH in THF at -10 ^{o}C afforded our target compound - the 4,6-O-benzylidene-β-D-mannosyl-chitobiose derivative **1**[8]. This trisaccharide will be employed as the key synthon for the synthesis of complex type[3c] and high mannose type sugar chains.

In conclusion, we have developed a new synthetic route to trisaccharide **1**, which is an important intermediate in the synthesis of N-glycoside type oligosaccharides.[3c] The overall yield is high enough to make large scale preparation relatively easy and straightforward (we have already prepared several grams of trisaccharide **1** using this process).

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8. Physical data for key compounds are described below. $[\alpha]_D$ values was measured at 25 ^{o}C. ^{1}H and ^{13}C NMR spectra were measured on a UNITY 500 spectrometer in $CHCl_3$ and referenced to TMS. Signal assignments such as 1^3 stand for a proton or carbon at C-1 of sugar redidue 3. **1**; Rf 0.51 1:1 toluene-EtOAc;$[\alpha]_D$ +11.07^{o} (c 1.13, $CHCl_3$). ^{1}H δ 5.30 (d, 8.3Hz, 1^2), 5.16 (d, 9.5Hz, 1^1), 4.74 (s, 1^1), 3.94 (m, 2^3); ^{13}C δ 100.43 (1^3), 96.96 (1^2), 85.60 (1^1), 70.80 (2^3). **3**: mp 118-120 ^{o}C; Rf 0.48 in 2:1 toluene-EtOAc; $[\alpha]_D$ -67.30^{o} (c 1.03 $CHCl_3$); ^{1}H δ 5.23 (t, 9.3Hz, 3), 4.52 (d, 9.6Hz, 1), 3.65 (t, 9.7Hz, 4), 2.14(s, Ac), 1.33 (t, 7.6Hz, Me); ^{13}C δ 170.90, 101.51, 87.27 (1), 78.39 (4), 74.81 (3),72.21 (2), 20.95 (Ac). **4**; mp 157-159 ^{o}C; Rf 0.63 in 8:1 toluene-EtOAc; $[\alpha]_D$ -78.68^{o} (c 1.03 CH_2Cl_2); ^{1}H δ 5.36 (t, 9.8Hz, 3), 5.07 (t, 8.9Hz, 2), 4.61 (d, 10.1Hz, 1), 2.05 (s, Ac); ^{13}C δ169.93, 166.21, 101.49, 83.73 (1), 78.25 (4), 72.47 (3), 72.37 (2), 40.49 (CH_2Cl), 20.66 (Ac). **10**; Rf 0.26 10:1 toluene-EtOAc; $[\alpha]_D$ -16.9^{o} (c 1.0 CH_2Cl_2); ^{1}H δ 5.28 (d, 8.0Hz, 1^2), 5.18 (t, 9.5 Hz, 3^3), 5.16 (d, 9.5Hz, 1^1), 4.96(dd, 9.5Hz, 2^3), 4.74 (d, 8.0Hz, 1^3), 2.02 (s, Ac); ^{13}C δ 99.87 (1^3), 96.86 (1^2), 85.56 (1^1), 74.52 (2^3), 71.82 (3^2), 40,37 (CH_2Cl), 20.67 (Ac). **13**; Rf 0.32 5:1 toluene-EtOAc; $[\alpha]_D$ -0.38^{o} (c 1,06, $CHCl_3$). ^{1}H δ 5.47 (d, 3.5Hz, 2^3), 5.27 (d, 8.5Hz, 1^2), 5.15 (d,9.5Hz, 1^1),4.97 (dd, 10.5Hz, 3^3), 4.28 (s, 1^3), 2.17, 2.02 (s, Ac); ^{13}C δ 98.65 (1^3), 96.96 (1^2), 85.59 (1^1), 70.35 (3^3), 69.67 (2^3), 20.87, 20.71 (Ac).
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