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Synthesis of UDP-6-Deoxy- and -6-Fluoro-D-galactoses and their Enzymatic Glycosyl Transfer to Mono- and Biantennary Carbohydrate Chains

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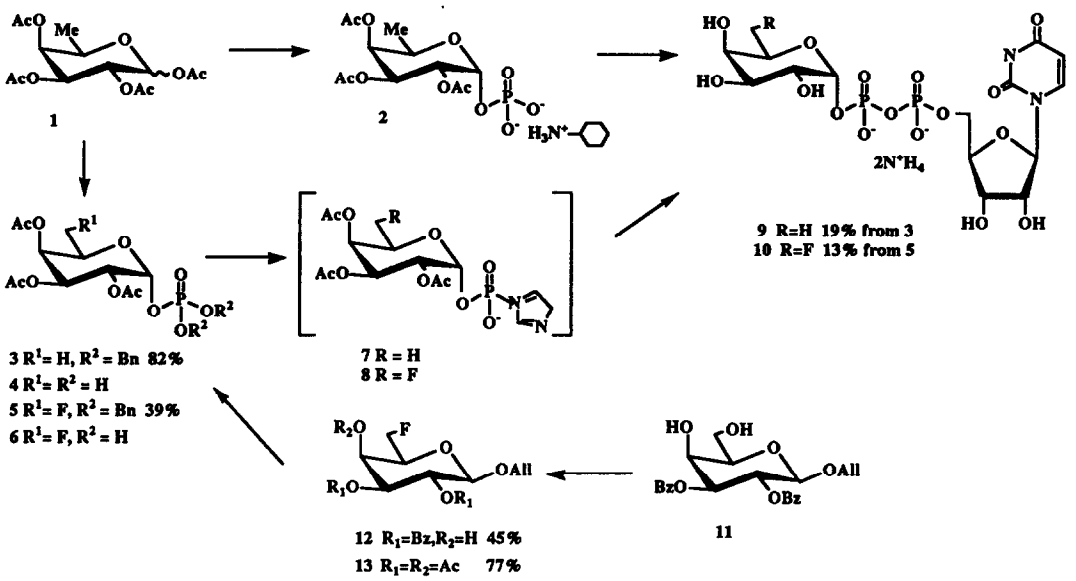
Abstract: Glycosylation with chemically prepared UDP-6-deoxy-D-galactose and its 6-fluoro analog using bovine (1-4)-β-D-galactosyltransferase was demonstrated to be effective enough for practical purpose. A biantennary pentasaccharide having two 6'-deoxylactosamine residues was synthesized.

The carbohydrate ligands are expected as drugs of new generation and many such ligands have been synthesized. Enzymatic approach for synthesizing those ligands is accelerated by recent success of cloning and expression of glycosyltransferases.[1,2] Because of the intrinsic substrate specificities,[3] enzymatic methods have been thought to be applicable only to the synthesis of natural oligosaccharides. Recent reports, however, showed that even unnatural sugar analogues could be transferred by some glycosyltransferases,[4] thereby suggesting that enzymatic method can provide the oligosaccharide mimics and is also applicable for modification of the carbohydrate moiety in glycoprotein. Recent studies on glycoproteins, such as erythropoietin and tissue plasminogen activator, showed that the structure of the carbohydrate moiety affected their activities *in vivo*.[5] Remodeling or modification with the sugar analogue should be available for changing the biological response. For example, substitution of the galactose residue with its analogue might block the hepatic clearance.[6] Here we report the enzymatic synthesis of 6'-modified lactosaminyl moiety in mono- and biantennary sugar chain. This approach can be used for the modification of glycoprotein.

The 6-modified UDP-D-galactoses were synthesized in two routes, (i) *via* the cyclohexylammonium glycosylphosphate **2**, and (ii) *via* glycosylphosphoric imidazolides, **7** and **8**. The former conventional method[7] was used for the synthesis of UDP-6-deoxy-D-galactose **9**. The product was purified on columns of anion exchange resin and charcoal after hydrolysis of excess UDP with phosphatase. The latter method has an advantage in large scale preparation, because no column purification is necessary in the intermediary steps. The 6-deoxy analogue **9** was synthesized alternatively from peracetylated 6-deoxy-D-galactose **1** as follows. Selective *O*-deacetylation at C-1 with hydrazine acetate and successive treatment with n-BuLi and dibenzylphosphoryl chloride gave the α-phosphate **3** exclusively. The debenzylated phosphate **4** obtained by hydrogenolysis (Pd/C) of **3** was converted with carbonyldiimidazole into the imidazolide **7**, which in turn was coupled with UMP to give **9**.[8]

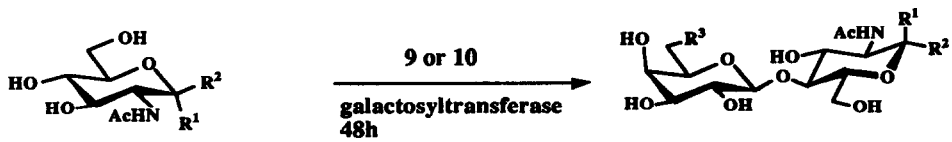


UDP-6-fluoro-D-galactose **10**[9] was also synthesized by the second method via **5**, **6**, and **8** from peracetylated 6-deoxy-6-fluoro-D-galactose **13**, which was obtained by selective fluorination of the galactopyranoside **11**. The phosphorylation step proceeded stereoselectively to give an α-phosphate predominantly. The contaminated β-phosphate of **8** (8%) was removed by silica-gel column chromatography.

Table 1. Transfer Rate[10] of Galactose Analogues

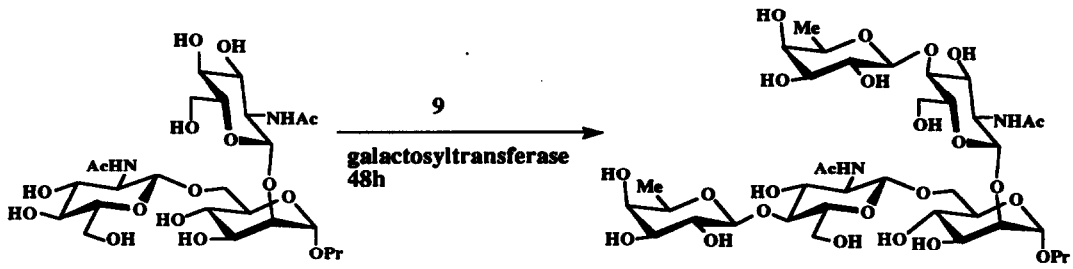
Donor		Relative Rate
UDP-Gal		100.0
UDP-6deoxyGal	**9**	1.3
UDP-6FGal	**10**	0.2

The susceptibilities of both 6-modified analogues, **9** and **10**, toward UDP-galactose-*N*-acetyl-D-glucosamine-(1-4)-β-D-galactosyltransferase are shown in Table 1. The relative transfer rates were estimated by the initial velocities measured by amount of UDP formed under the optimized conditions.[10] Use of HEPES buffer instead of Tris-HCl increased the velocity remarkably and the conversion rate was accerelated in the presence of phosphatase.[11] The lactosamine analogues, **16** and **17**, were synthesized enzymatically using *N*-acetyl-D-glucosamine **14** and methyl *N*-acetyl-β-D-glucosaminide **15** as acceptors and **9** and **10** as donors, respectively, under the following typical conditions. In HEPES buffer (pH 7.0), 196 μmoles of **14** and 137 μmoles of **9** were incubated with 4 units of the galactosyltransferase and 10 units of alkaline phosphatase. 6'-Deoxylactosamine **16**[12] was obtained as a sole product in 30% yield based on the donor**9**. Similar glycosylation of **15** with **10** yielded 6'-deoxy-6'-fluorolactosamine **17**[13] as a sole product in 59% yield based on the acceptor.



14 R^1, R^2=OH , H
15 R^1=H; R^2 = OMe

16 R^1, R^2=OH , H; R^3=H
17 R^1=H; R^2 = OMe; R^3= F



18

19

6-Deoxy-D-galactosyl residue could be also introduced to a branched carbohydrate chain,*i.e.* biantennary glycan **18**[14] having two GlcNAc residues at the nonreducing terminal, in the same manner as described above using 3 molar equivalents of **9**. After 48 hours, a single product was observed on TLC and was obtained in 55% yield based on the acceptor. Proton NMR spectral data of the synthesized pentasaccharide **19**[15] indicate that two 6-deoxy-D-galactose residues were attached to two GlcNAc residues through β-linkage.

Modification of the glycan moiety in glycoproteins are expected to change their biological properties as shown in the recent progress of glycobiology. This paper showed the first example of simultaneous enzymatic introduction of two modified D-galactosyl residues to the branched trisaccharide. This methodology should be useful not only for the synthesis of the modified oligosaccharides having such biological activities as inhibitory activity toward glycosyltransferase,[16] but also for that of the modified glycoproteins.

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8. **9**(diammonium salt): $[\alpha]_D$+49.3°(c=0.4, H_2O); ^1H NMR [D_2O] δ 7.98(d, $J_{5,6}$=8.3Hz, H-6), 6.00(d, H-5), 5.98(d, $J_{1',2'}$=5.3Hz, H-1'), 5.57(dd, $J_{1'',2''}$=3.6Hz, $J_{1'',P}$=6.6Hz, H-1''), 3.93(dd, $J_{3'',4''}$=3.3Hz, $J_{2'',3''}$=10.2Hz, H-3''), 3.83(d, H-4''), 3.76(ddd, $J_{2'',P}$=3.0Hz, H-2''), 1.23(d, $J_{5'',6''}$=6.6Hz, H-6''); ^{31}P NMR [D_2O] δ(H_3PO_4, ext. std.) -10.55, -12.05(each d, $J_{P,O,P}$=19.8Hz). Neg. FABMS: m/z 549.0527 [M-H]$^-$, calcd. 549.0523 for $C_{15}H_{23}O_{16}N_2P_2$.
9. **10**(diammonium salt): $[\alpha]_D$+50.5°(c=0.2, H_2O); ^1H NMR [D_2O] δ 7.98(d, $J_{5,6}$=8.3Hz, H-6), 6.00(d, H-5), 5.98(d, $J_{1',2'}$=4.6Hz, H-1'), 5.66(dd, $J_{1'',2''}$=3.6Hz, $J_{1'',P}$=6.6Hz, H-1''), 4.65(broad d, $J_{6'',F}$=45.9Hz, H-6''); ^{31}P NMR [D_2O] δ (H_3PO_4, ext. std.) -10.44, -12.10(each d, $J_{P,O,P}$=19.8Hz). Neg. FABMS: m/z 567.0440 [M-H]$^-$, calcd. 567.0429 for $C_{15}H_{22}O_{16}N_2FP_2$.
10. Initial velocities were determined by measuring the rate of UDP formation. The reaction mixtures contained 60 mM HEPES, pH 7.0, 20 mM GlcNAc, 150 μM donor **9** or **10**, 10 mM $MnCl_2$, 25units phosphatase (EC 3.1.3.1), 7 milli-units of galactosyltransferase (EC 2.4.1.22, SIGMA, bovine milk) in a total volume of 100 μl .(For UDP-Gal the same reaction except 0.07 milli-units of galactosyltransferase was used) The reaction mixtures were incubated at 37°C. The incubation times were varied to limit donor consumption to 15%. During the reaction liberated UDP was hydrolysed to uridine. After stopping the reaction, uridine formed was quantitated by HPLC (Asahipak GS320, 200 mM phosphate, pH 3.0).
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12. **16**: ^1H NMR [D_2O] δ(TPS) 5.19(broad s, H-1'a), 4.72(d, $J_{1'b,2'}$=6.7Hz, H-1'b), 4.42(d, $J_{1,2}$=8.0Hz, H-1), 2.04(s, Ac), 1.25(d, $J_{5,6}$=6.5Hz, H-6). Pos. FABMS: m/z 390.1375 [M+Na]$^+$, calcd.390.1377 for $C_{14}H_{25}O_{10}NNa$.
13. **17**: ^1H NMR [D_2O] δ(TPS) 4.57(d, $J_{1',2'}$=7.8Hz, H-1'), 4.50(d, $J_{1,2}$=8.5Hz, H-1), 4.05(d, $J_{3',4'}$=3.5Hz, H-4'), 3.99(dd, $J_{5,6a}$=2.0Hz, $J_{6a,6b}$=13.0Hz, H-6a), 3.89(dd, $J_{5,6b}$=5.2Hz, H-6b), 3.82-3.78 (m, 2H, H-2,3), 3.74(t, 1H, $J_{4,5}$=$J_{3,4}$=9.7Hz, H-4), 3.74(dd, $J_{2',3'}$=9.3Hz, H-3'), 3.68-3.65(m,1H, H-5), 3.62(dd, H-2'), 3.56(s, OMe), 2.09(s, Ac). Pos. FABMS: m/z 400.1607 [M+H]$^+$, calcd.400.1619 for $C_{15}H_{27}O_{16}NF$.
14. Synthesis of **18** will be described elsewhere. ^1H NMR [D_2O] δ(TPS) 4.56(d, J=7.6Hz, GlcNAc anomeric), 4.53(d, J=7.9Hz, GlcNAc anomeric); ^{13}C NMR [D_2O] δ(TPS) 102.3, 100.5, 97.5 (three anomeric carbons).
15. **19**: ^1H NMR [D_2O] δ(TPS) 4.49, 4.45(each d, each 1H, J=7.8Hz, GlcNAc-anomeric x 2), 4.35-4.33(broad d, 2H, Gal-anomeric x 2), 1.98, 1.95(each s, each 3H, Ac x 2), 1.57-1.52(m, 2H, $OCH_2\underline{CH_2}CH_3$), 0.86(t, 3H, J=7.6Hz, $OCH_2CH_2\underline{CH_3}$). Pos. FABMS: m/z 921.3940 [M+H]$^+$, calcd. 921.3928 for $C_{37}H_{65}O_{24}N_2$.
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