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SYNTHESIS OF 2'-O-SUBSTITUTED ß-D-Gal-(1→4)-ß-D-GlcNAc-1-O-Bn AS SPECIFIC ACCEPTORS FOR α-L-(1→3) FUCOSYLTRANSFERASES

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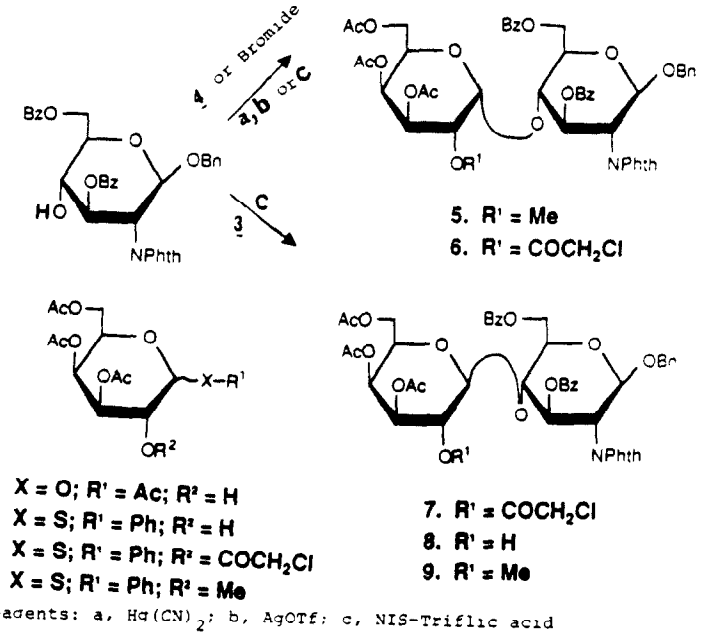
Abstract: The synthesis of 2'-O-methyl lactosamine-ß-OBn and 2'-O-α-L-fucopyranosyl lactosamine-ß-O-Bn were accomplished through the use of a key glycosyl donor, phenyl 3,4,6-tri-O-acetyl-2-O-chloroacetyl-1-thio-α-D-galactopyranoside, with benzyl 3,6-di-O-benzoyl-2-deoxy-2-phthalimido-ß-D-glucopyranoside as an acceptor.

α-L-Fucosyltransferase which transfers L-fucose from GDP-L-fucose to 2-acetamido-2-deoxy-D-glucose or D-glucose has been detected in various sources[1-5]. This class of enzyme is also responsible for the accumulation of highly fucosylated polylactosamine compounds that are associated with various human cancers[6-9]. One such enzyme involved in the synthesis of X-determinant α-L-Fuc-(1→3)-[ß-D-Gal(1→4)]-ß-D-GlcNAc is α-L-(1→3)-fucosyl-transferase. At least seven α-L-(1→3)-fucosyltransferases[10] have been suggested in different mammalian sources on the basis of their specificity and biochemical properties. Thus, the availability of compounds capable of acting as acceptors for a single enzyme, even in the presence of other related enzymes, would be of particular importance in the study of fucosyltransferases. We have recently shown that our synthetic compound, 2'-O-methyl-N-acetyllactosamine acts as a specific acceptor for α-L-(1→3)-fucosyltransferase from human serum[11] and this activity has been explored as a potential tumor marker in the sera and saliva of various cancer patients[12,13]. Recently, Oriol et al.[14] reported on the use of oligosaccharides containing a hydrophobic aglycon as acceptors for glycosyltransferase in a simplified assay method using reverse phase SEP-PAK C_{18} cartridges. Our recent studies[15] have shown that 2-O-Me-Galß1→3GlcNAcß-OBn is a better acceptor for α-L-(1→4)-fucosyltransferase. Recently, Glick et al.[16] reported a unique α-L-(1→3)-fucosyltransferase activity from human neuroblastoma (CHP 134) cells which displayed a requirement for the Fucα1→2Galß1→4GlcNAc unit. In order to conduct further substrate specificity studies on this type of α-L-(1→3)-fucosyltransferase, we report the synthesis of 2'-O-methyl- (11) and 2'-O-α-L-fucopyranosyl Galß1→4 GlcNAcß-OBn (15) employing phenyl 3,4,6-tri-O-acetyl-2-O-chloro-

acetyl-1-thio-α-D-galactopyranoside (3) as an effective glycosyl donor.

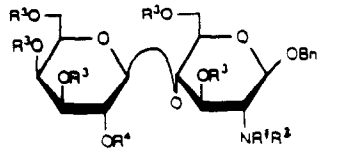
Our intial efforts were directed toward the synthesis and use of phenyl 3,4,6-tri-O-acetyl-2-O-methyl-1-thio-α-D-galactopyranoside (4) which was prepared by the treatment of 1,3,4,6-tetra-O-acetyl-α-D-galactopyranose[17] (1) with (phenylthio)trimethyl silane-trimethylsilyl triflate in 1,2-dichloroethane, and 3,4,6-tri-O-acetyl-2-O-methyl-α-D-galactopyranosyl bromide[17] as glycosyl donors to provide desired compounds. We have already shown that these glycosylating agents, containing the non-participating O-methyl group at C-2, after glycosylation with benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranoside[17] provided an α/ß product mixture of the



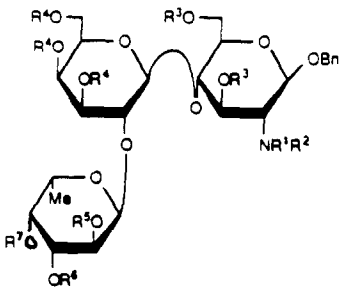
corresponding disaccharide which was separated by silica gel column chromatography. To our surprise the glycosyl donor 4 on reaction with benzyl 3,6-di-O-benzoyl-2-deoxy-2-phthalimido-ß-D-glucopyranoside[18] (acceptor) in the presence of N-iodosuccinimide and triflic acid[19] gave exclusively the α-disaccharide 5 $[\alpha]_D$ +82.5° ($CHCl_3$). An attempt at glycosylation with this donor 4 containing a non-participating substituent at C-2 using tris (4-bromophenyl) ammonium hexachloroantimonate[20] as the catalyst to give a ß-linked disaccharide was not successful in our hands. When the correspondent bromide of 4 was reacted with the same acceptor under $Hg(CN)_2$/aceto-

nitrile or AgOTf/methylene chloride conditions it also furnished the α-disaccharide 5 $[\alpha]_D$ +83.9° ($Hg(CN)_2$ method); +81.2 (from AgOTf method).

We next became interested in examining the glycosylating capability of 3 which was obtained from 1 in two steps. Thus, 1,3,4,6-tetra-O-acetyl-α-D-galactopyranose (1) on treatment with (phenylthio)trimethylsilane-trimethylsilyl triflate[21] gave the corresponding phenylthio compound 2 $[\alpha]_D$ +260.4° ($CHCl_3$) in 95% yield. Chloroacetylation[22] of 2 (chloroacetic anhydride-$NaHCO_3$-DMF) furnished 3 $[\alpha]_D$ +217.2° in 67% yield. It is noteworthy that glycosylation of benzyl 3,6-di-O-benzoyl-2-deoxy-2-phthalimido-ß-D-glucopyranoside with 3 in the presence of N-iodosuccinimide-triflic acid gave a mixture of the α- 6 (25%) and ß-anomer 7 (38%), respectively. De-O-chloroacetylation of 7 was found to proceed smoothly in a thiourea-pyridine-ethanol system to give the intermediate, key acceptor 8 in 65% yield for further modifications.



a → 10. R¹, R² = H, Ac; R⁴ = Me; R³ = Ac
a → 11. R¹, R² = H, Ac; R⁴ = Me; R³ = H



b → 12. R¹, R² = Phth; R³ = Bz; R⁴ = Ac; R⁵ = 4-MeOBn; R⁶, R⁷ = CMe_2
c → 13. R¹, R² = Phth; R³ = Bz; R⁴ = Ac; R⁵ = R⁶ = R⁷ = H
a → 14. R¹, R² = H, Ac; R³ = R⁴ = R⁵ = R⁶ = R⁷ = Ac
a → 15. R¹, R² = H, Ac; R³ = R⁴ = R⁵ = R⁶ = R⁷ = H

Reagents: a, MeOH-MeONa; b, $CHCl_3$-TFA-H_2O; c, NH_2-$NH_2.H_2O$/EtOH pyridine/acetic anhydride

Methylation of 8 with trimethyloxonium tetrafluoroborate-2,6-di-tert-butyl-4-methyl pyridine[23] in dichloromethane, gave the 2'-O-methyl derivative 9 $[\alpha]_D$ +33° ($CHCl_3$) in 60% yield. Conversion of 9 into 11 was carried out in 4 steps. [1. 0.02 M sodium methoxide-methanol (de-O-acylation). 2. NH_2-$NH_2.H_2O$/EtOH (phthalimido group removal). 3. Pyridine-acetic anhydride (N- and O-acetylation). 4. 0.02 M sodium methoxide-methanol de-O-acetylation]. The structural assignment of 11 was confirmed by ^{13}C-n.m.r. and f.a.b. mass spectroscopy[24].

Glycosylation between 8 and methyl 3,4-O-isopropylidene-2-O-(4-methoxybenzyl)-1-thio-ß-L-fucopyranoside[25] was also performed successfully and stereoselectively under cupric bromide-tetrabutylammonium bromide[26] condition to give the desired α-linked trisaccharide derivative 13 $[\alpha]_D$ - 13° ($CHCl_3$) in 64.5% yield after removal of the 4-methoxybenzyl and isopropylidene group from compound 12. Conventional transformation of 13 into 15 was achieved in 4 steps as described earlier for the preparation of 11 from 9. The ^{13}C-n.m.r. spectrum of 15 was in agreement with the structure assigned[24]. These benzyl glycosides compounds 11 and 15 were found to act as very good acceptors for α-L-(1→3) fucosyltransferase, even better than their parent compounds.

Acceptor (0.3 mM)	Relative activity in the α-L-(1→3) Fucosyltransferase Assay[11] Ovarian Cancer Sera [^{14}C] Fucose CPM	%	Ovarian Tumor Extract [^{14}C] Fucose CPM	%	Km (mM)
LacNAc	1615		5024		N.D.
2'-O-methyl LacNAc	1730	100	3977	100	1.33
2'-O-methyl LacNAcß-O-Bn (11)	5326	308	14924	375	0.44
2'-O-Fucosyl LacNAc	4379	100	18291	100	N.D.
2'-O-Fucosyl LacNAcß-O-Bn (15)	7352	168	42580	233	0.26

Assay: The incubation mixture contained 50 mM HEPES-NaOH, pH 7.5, 5 mM $MnCl_2$, 7 mM ATP, 3 mM NaN_3, 0.3 mM acceptor, 0.125 μCi of GDP-[U-^{14}C] Fuc (Sp.Act. 216 mCi/mmol) and enzyme [20 μl of serum or 20 μl of tumor extract (10 mg/ml)] in a total volume of 0.10 ml; the control incubation mixture had everything except the acceptor. At the end of incubation at 37°C for 18 h, the mixture was diluted with 1 ml water and passed through Dowex-1-Cl column (1 ml in a Pasteur pipet). The column was washed twice with 1 ml water; the breakthrough plus wash, which contained the [^{14}C] fucosylated acceptor, was collected in a scintillation vial and counted for radioactivity. Corrections were made by subtracting the radioactivity in the water eluates of the control incubation mixture from the corresponding test fractions. Km values were determined from the Lineweaver-Burk plots of enzyme activity (Incorporation of [^{14}C] fucose) as a function of acceptor concentration. We have also examined the acceptor capabilities of compound 11 and 15 for α-L-(1→3) fucosyltransferases from different sources using SEP-PAK C_{18} cartridge technique and their results will be published elsewhere.

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24. Data for compounds are given below: values of $[\alpha]_D$ measured at 25° ± 3° for solution a. $CHCl_3$ b. CH_3OH c. H_2O. Compound 2 $[\alpha]_D$ +260.4° (a); ^{1}H-n.m.r. ($CDCl_3$): δ 7.60-7.23 (m, 5 H, arom.), 5.67 (d, J ~ 4 Hz, 1 H, H-1), 2.17, 2.03 and 1.97 (each s, 3 H, OAc); Compound 3 $[\alpha]_D$ +217.2° (a); ^{1}H-n.m.r. ($CDCl_3$): δ 7.48-7.22 (m, 5 H, arom.), 5.93 (d, J ~ 4 Hz, 1 H, H-1), 4.10 (s, 2 H, CH_2Cl), 2.13, 2.01 and 1.97 (each s, 3 H, OAc); ^{13}C-n.m.r. ($CDCl_3$): δ 85.24 (C-1), 40.42 (CH_2Cl); Compound 4 $[\alpha]_D$ +235.2°; ^{1}H-n.m.r. ($CDCl_3$): δ 7.57-7.17 (m, 5 H, arom.), 5.83 (d, J ~ 4 Hz, 1 H, H-1), 3.46 (s, 3 H, OMe), 2.11, 2.00 and 1.92 (each s, 3 H, OAc); Compound 6 $[\alpha]_D$ +84.5° (a); ^{13}C-n.m.r. ($CDCl_3$): δ 96.71 (C-1), 96.47 (C-1'), 40.48 (CH_2Cl); Compound 7 $[\alpha]_D$ +36.5° (a); ^{13}C-n.m.r. ($CDCl_3$): δ 100.19 (C-1'), 97.15 (C-1), 40.36 (CH_2Cl); Compound 11 $[\alpha]_D$ -25.5° (b); ^{13}C-n.m.r. (CD_3OD): δ 104.73 (C-1'), 101.82 (C-1), 82.76 (C-2'), 80.68 (C-4), 61.42 (OMe) m/z: 488.4 $[M+1]^+$, 486 $[M-1]^-$; Compound 13 $[\alpha]_D$ -13° (a); ^{1}H-n.m.r. ($CDCl_3$): δ 2.03, 1.83 and 1.70 (each s, 3 H, OAc), 1.40 (d, J ~ 7 Hz, 3 H, CMe); Compound 15 $[\alpha]_D$ -83.2° (c); ^{13}C-n.m.r. (D_2O): δ 103.06 (C-1'), 102.78 (C-1"), 102.24 (C-1), 79.28 (C-2'), 78.91 (C-4), 18.12 (C-6"), m/z: 642.3 $[M+Na]^+$, 618 $[M-1]^-$.
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27. This publication is Part LXXXIV of Synthetic Studies in Carbohydrates, Part LXXXIII, see Jain, R.K.; Matta, K.L. Carbohydr. Res., 1991, accepted.