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UDP-*N*-ACETYL-5-THIO-GALACTOSAMINE IS A SUBSTRATE OF LACTOSE SYNTHASE

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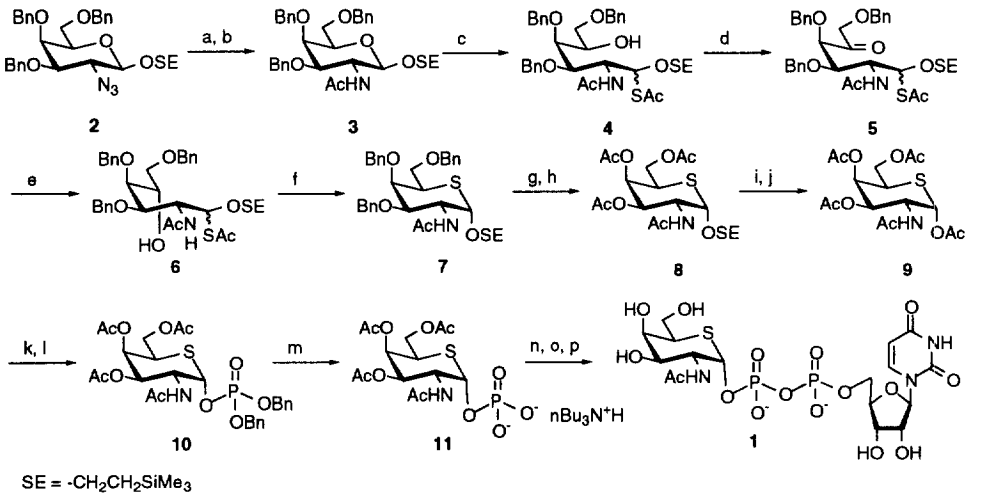
Abstract: Uridine 5'-(*N*-acetyl-5-thio-galactosaminyl diphosphate) (UDP-5SGalNAc) was synthesized from an *N*-acetylgalactosamine derivative via ring opening-recyclization approach. UDP-5SGalNAc was active as a donor substrate for lactose synthase, the complex of galactosyltransferase and lactalbumin, giving the disaccharide mimic (5SGalNAcβ[1→4]GlcNAc) which has a sulfur in the ring of the non-reducing sugar. The initial rate of the formation of the disaccharide mimic was 0.23 % that for the natural disaccharide (GalNAcβ[1→4]GlcNAc). © 1997 Elsevier Science Ltd.

Glycosidase-resistant oligosaccharide mimics are potentially useful as research tools in glycobiology. It is further advantageous for such mimics to be constructed in vitro by enzymes. In this context, one of the authors have disclosed that a galactosyltransferase (GalT) catalyzes transfer of 5-thio-galactose, the ring sulfur analog of galactose, from uridine 5'-(α-D-5-thio-galactopyranosyl diphosphate) (UDP-5SGal) to *N*-acetylglucosamine (GlcNAc).[1] 5'-Thio-*N*-acetyl-lactosamine thus synthesized was galactosidase resistant. The purpose of this study is to extend the scope of this methodology to another oligosaccharide.

Do and coworkers[2] have recently found that lactose synthase (EC 2.4.1.22), the complex of GalT (EC 2.4.1.38) and lactalbumin, catalyzes transfer of *N*-acetyl-galactosamine (GalNAc) from UDP-GalNAc to GlcNAc to give GalNAcβ(1→4)GlcNAc. This disaccharide is included in luteinizing and thyroid-stimulating glycoprotein hormones and is functional in the circulation of these hormones.[3] Thus the mimic of this disaccharide has a potential to modify the blood level of these hormones. In hope of obtaining a hydrolase-resistant disaccharide mimic, we examined the lactose synthase catalyzed transfer of *N*-acetyl-5-thio-galactosamine (5SGalNAc)[4] from UDP-5SGalNAc to GlcNAc. Synthesis of UDP-5SGalNAc was performed based on the ring opening-recyclization approach.[5]

UDP-5SGalNAc (**1**) was synthesized as shown in Scheme 1. Though the ring opening of 2-

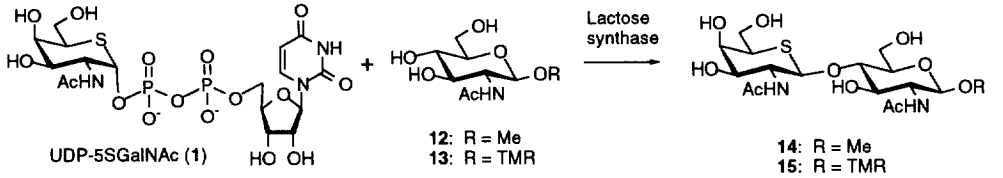
trimethylsilylethyl 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**2**) with dimethylboron bromide (Me_2BBr)[6] was unsuccessful, the same reaction for the acetamido derivative **3** gave the acyclic compound **4** in a good yield as a 3:2 mixture of diastereomers. It should be noted that the ring opening of the galactopyranoside derivative afforded only a moderate yield of acyclic product.[6] Inversion of configuration at C-5 position of the compound **4** was performed by the consecutive oxidation-reduction to give the L-*altro* derivative **6** with a diastereomer excess (d.e.) of 55 %. The corresponding d.e. for the reduction of a *gluco* type 5-ulose is 92 %,[5a] exemplifying importance of the configuration at C-4 in the 5-ulose reduction. De-*S*-acetylation and subsequent recyclization of the compound **6** occurred simultaneously[5b] under Mitsunobu conditions to give 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-5-thio-α-D-galactopyranoside (**7**). Interestingly, the β-anomer was not obtained at all. Deprotection of the benzyl groups of the compound **7** was carried out by Birch reduction and subsequent acetylation gave the acetate **8**. Deprotection of the trimethylsilylethyl group with trifluoroacetic acid and subsequent acetylation gave 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-5-thio-α-D-galactopyranose (**9**). Compound **9** was transformed into UDP-5SGalNAc[7] in a basically the same manner as described for UDP-5SGlc (**1**).[1]

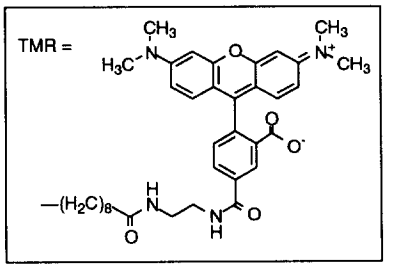


Scheme 1. (a) Lindlar cat., H_2 (1 atm), MeOH. (b) Ac_2O-Py, 81 % from **2**. (c) Me_2BBr (2 equiv), iPr_2NEt (2.5 equiv), AcSH (3 equiv), CH_2Cl_2, -78 °C, 62 %, S/R = 1.5/1. (d) $(COCl)_2$-DMSO, Et_3N, CH_2Cl_2, -78 °C, 89 %. (e) $Li(tBuO)_3AlH$ (2.6 equiv), Et_2O, 62 % (diastereomer 18 %). (f) Ph_3P (2 equiv), DEAD (2 equiv), benzene, 15 h, 61 %. (g) Na-liq. NH_3, THF. (h) Ac_2O-Py, 57 % from **7**. (i) TFA, CH_2Cl_2. (j) Ac_2O-Py, 63 % from **8**. (k) 25 % HBr-AcOH. (l) $AgOPO(OBn)_2$ (1 equiv), benzene, 90 °C, 10 min, 41 % from **9**. (m) 10% Pd/C, nBu_3N, H_2 (1 atm). (n) N,N'-carbonyldiimidazole, DMF. (o) Uridine 5'-monophosphate tri-n-butylammonium salt, DMF. (p) Et_3N-H_2O-MeOH (1:3:7), 18 % from **11**.

UDP-5SGalNAc (**1**) thus chemically synthesized was mixed with methyl *N*-acetyl-glucosaminide (**12**) and lactose synthase, and the mixture was incubated for 168 h at 37 °C.[8] TLC analysis indicated appearance of the disaccharide (**14**), which was confirmed by NMR and mass spectra.[9] The connectivity of the glycoside was deduced from a strong NOE between H-4 and H-1'. Tetramethylrhodamine labeled GlcNAc (**13**)[10] was used as an acceptor for the HPLC analysis of the rates of the reactions for both donors, UDP-5SGalNAc and UDP-GalNAc. The initial rate for UDP-5SGalNAc was 0.23 % that for UDP-GalNAc. Further, rate declination was eminent for UDP-5SGalNAc after 24 h, giving no more than 28 % conversion thereafter. The same reaction conditions in the absence of lactalbumin gave no disaccharide analogs.

In conclusion, we have found that UDP-5SGalNAc is a donor substrate of lactose synthase to give 5SGalNAcβ(1→4)GlcNAc, the mimic of a biologically important sequence. Thus the combination of UDP-5SGalNAc and lactose synthase is a potential tool that enables a modification of glycoproteins.





Scheme 2.

Acknowledgments

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References and Notes

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7. Spectral data for the compound **1**: ^{1}H NMR (270 MHz, D_2O) δ 8.01 (d, 1H, J = 8.2 Hz), 6.03-6.01 (m, 2H), 5.37 (dd, 1H, J = 2.7, 7.8 Hz), 4.44-4.23 (m, 7H), 3.94 (dd, 1H, J = 2.7, 10.7 Hz), 3.88-3.67 (m, 3H), 2.12 (s, 3H); ^{31}P NMR (109.25 MHz, D_2O) δ −10.41, −12.11 (J = 21.36 Hz).
8. Reaction conditions: **1** (4.8 mg), **12** (1.0 mg), GalT (2.5 U), α-lactalbumin (0.5 mg), alkaline phosphatase (50 U), $MnCl_2$ (5 mM) in 100 mM Na cacodylate (50 μL, pH 7.5), 37 °C, 22h. Additional **1** (1.0 mg) and GalT (2.5 U) were added, and the reaction continued for an additional 146 h. The raction mixture was lyophilized and purified with Iatrobeads ($CHCl_3$-MeOH-H_2O 65:35:1) to give 0.9 mg (47 %) of **14**.
9. Spectral data for the compound **14**: ^{1}H NMR (400 MHz, D_2O, 39 °C) δ 4.78 (d, 1H, J = 9.6 Hz), 4.45 (d, 1H, J = 8.4 Hz), 4.23 (t, J = 9.9 Hz), 4.21 (bs, 1H), 3.97 (dd, 1H, J = 2.2, 12.2 Hz), 3.85 (dd, 1H, J = 6.9, 11.3 Hz), 3.76 (dd, 1H), 3.75 (t, J = 8.4 Hz), 3.71 (dd, 1H, J = 7.0, 11.3 Hz), 3.67 (t, 1H, J = 8.5 Hz), 3.62 (t, 1H, J = 8.5 Hz), 3.60 (dd, 1H, J = 2.9, 9.9 Hz), 3.53 (s, 3H, OMe), 3.49-3.46 (m, 1H), 3.21 (dt, 1H, J = 1.5, 7.0, 7.0 Hz), 2.10, 2.07 (each s, each 3H); FAB Mass m/z 454.9 (M+H), 477.0 (M+Na).
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