Regeneration of Sugar Nucleotide for Enzymatic Oligosaccharide Synthesis: Use of Gal-1-Phosphate Uridyltransferase in the Regeneration of UDP-Galactose, UDP-2-Deoxygalactose, and UDP-Galactosamine

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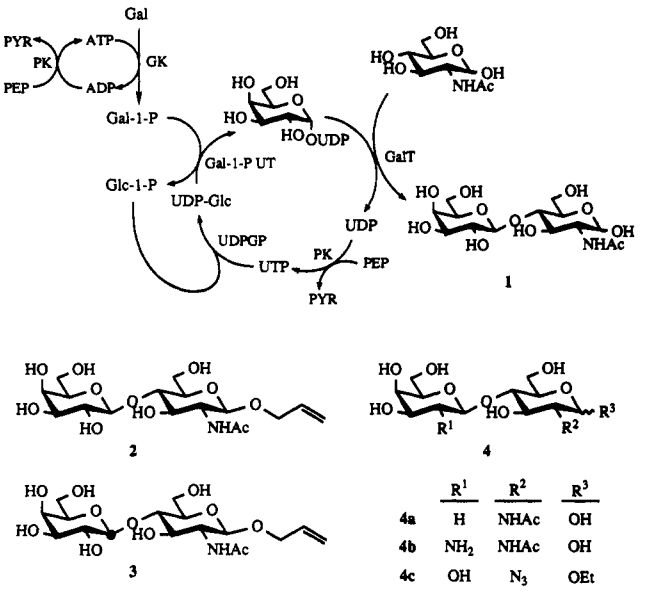
Summary: A new multiple enzymatic system containing galactose-1-phosphate uridyltransferase suitable for the regeneration of UDP-Gal, UDP-2-deoxy-Gal, and UDP-galactosamine for the synthesis of *N*-acetyllactosamine and analogs has been developed.

Glycosyltransferase-mediated oligosaccharide synthesis is of current interest in synthetic carbohydrate chemistry.[1] Although the enzymatic method is regio- and stereoselective and does not require multiple protection and deprotection steps, it is still hampered by the inavailability of transferases, the problem of product inhibition in stoichiometric reactions, and the tedious preparation of the donor substrate's sugar nucleotides when large-scale processes are needed. In order to overcome such drawbacks, multiple enzymatic systems have been developed for practical syntheses of *N*-acetyllactosamine[2] (LacNAc) and sialyl LacNAc[3] with in situ cofactor regeneration, in which the sugar nucleotides are continuously regenerated from readily available monosaccharides. Here we report a new one-pot enzymatic synthesis of lactose and *N*-acetyllactosamine derivatives using β1,4galactosyltransferase (EC 2.4.1.22, GalT) combined with galactokinase (EC 2.7.1.6, GK) and galactose-1-phosphate uridyltransferase (EC 2.7.7.12, Gal-1-P UT) reactions, in which galactose 1-phosphate (Gal-1-P) generated in situ from galactose with GK and ATP is converted to UDP-Gal via reaction with UDP-Glc catalyzed by Gal-1-P UT (Scheme I).

The disaccharide units, Galβ1, 4GlcNAc, or Galβ1,4Glc, are most often found in oligosaccharide chains and are also core structures of several biologically important carbohydrates.[4] Development of practical routes to such disaccharides and related structures may provide a new entry to novel saccharides. Since GalT has been proven to have

Scheme I



	R^1	R^2	R^3
4a	H	NHAc	OH
4b	NH_2	NHAc	OH
4c	OH	N_3	OEt

a broad specificity in terms of donor and acceptor,[5] the use of GalT combined with GK and Gal-1-P UT may be suitable for the construction of such disaccharides, if both GK and Gal-1-P UT also accept unnatural substrates and convert them to UDP-Gal analogs acceptable for GalT.

The previous one-pot synthesis of LacNAc involved the use of UDP-Glc pyrophosphorylase (UDPGP) and UDP-Gal 4-epimerase (UDPGE) for the regeneration of UDP-Gal.[2] In this UDPGE-dependent system, Glc-1-P is generated in situ from Glc-6-P[2] via phosphoglucomutase-catalyzed reaction, and Glc-6-P is prepared from glucose with hexokinase.[6] UDP-Glc is then converted to UDP-Gal catalyzed by UDPGE; the equilibrium of this reaction is in favor of UDP-Glc. The system described here, however, uses GK and Gal-1-P UT ($5/U, Sigma), whose synthetic utilities have not been well explored for the regeneration of UDP-Gal derivatives.

GK phosphorylates galactose in the presence of ATP and has been used by the Whitesides group[7] for the preparation of UDP-GalNAc. Since the enzyme is specific for galactose-related compounds and does not phosphorylate glucose derivatives, we take advantage of this strict specificity for the in situ generation of Gal-1-P and analogs.

Gal-1-P UT catalyzes the exchange reaction of Gal-1-P and UDP-Glc to produce UDP-Gal and Glc-1-P.[8] The enzyme also accepts GalN-1-P as a substrate and gives the corresponding UDP derivative, which is known to be a donor substrate for GalT.[7]

The multienzyme system started with galactose (Gal), *N*-acetylglucosamine (GlcNAc), phospho(enol)pyruvate (PEP),[6] and catalytic amounts of Glc-1-P, ATP, and UDP.

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The UDP was converted into UTP with PK (EC 2.7.1.40) and PEP, and the UTP reacted with Glc-1-P catalyzed by UDPGP (EC 2.7.7.9) to produce UDP-Glc. The Gal-1-P UT-mediated exchange reaction of the UDP-Glc and Gal-1-P, generated from Gal and ATP in the presence of GK, gave UDP-Gal and Glc-1-P. The UDP-Gal was consumed by GalT to give LacNAc. The produced UDP was again converted to UTP, which reacted with the released Glc-1-P to generate UDP-Glc. A typical experimental procedure is as follows: A solution of GlcNAc (17.7 mg, 80 μmol), Gal (14.4 mg, 80 μmol), Glc-1-P (2.1 mg, 8 μmol), PEP trisodium salt (37.5 mg, 160 μmol), UDP disodium salt (3.80 mg, 8 μmol), ATP disodium salt (4.67 mg, 8 μmol), DTT (3.1 mg, 10 mM), Mg^{2+} (5 mM), K^+ (20 mM), cysteine (8 mM), PK (100 U), UDPGP (5 U), GK (5 U), inorganic pyrophosphatase (EC 3.6.1.1, PPase) (5 U), and Gal-1-P UT (5 U) in HEPES buffer (2 mL; 100 mM, pH 7.4) was stirred under nitrogen atmosphere for 4 days at room temperature. The reaction mixture was filtered, and the filtrate was concentrated. The residue was applied to a column of Bio Gel P-2 (1.5 × 90 cm) with water as the eluant. The disaccharide-containing fractions were pooled and lyophilized to give LacNAc (15 mg, 45%).

Using this multienzyme system, several other disaccharides (**2–4**) were prepared on 50 mg scales.[9] 2′-Deoxy-LacNAc (**4a**) was prepared from 2-deoxygalactose, *N*-acetylglucosamine, and a catalytic amoung of Glc-1-P. This compound was prepared previously[2c] from 2-deoxyglucose based on the UDPGE-dependent regeneration system.[2] The disaccharide **3** with ^{13}C-labeled at Gal C-1 was prepared from 1-^{13}C-galactose in 57% yield. Compound **3** was further converted to sialy Lewis x (NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc) for conformational study.[10]

In summary, we have demonstrated an efficient one-pot synthesis of LacNAc derivatives with in situ regeneration of sugar nucleotides (UDP-Gal, UDP-Glc, UDP-2-deoxy-Gal, and UDP-GlcN) combined with GK and Gal-1-P UT. Since this multienzyme system does not require the separate preparation of sugar 1-phosphates and sugar nucleotides, this procedure provides an alternative way for the synthesis of LacNAc and related disaccharides.

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(9) Compound **1** (D-galactopyranosyl-β(1,4)-2-acetamido-2-deoxy-D-glucopyranose): 45% yield. Both ^1H- and ^{13}C-NMR spectra are in good agreement with those of a commercially available LacNAc from Sigma. Compound **2** (allyl D-galactopyranosyl-β(1,4)-2-acetamido-2-deoxy-β-D-glucopyranoside): (61% yield): ^1H NMR (D_2O) δ 2.00 (3 H, s, NHAc), 3.50 (1 H, dd, J = 7.83, 9.96 Hz, H-2′), 3.52–3.57 (1 H, dd, J = 3.27, 10.00 Hz, H-3′), 3.80 (1 H, dd, J = 5.10, 12.20 Hz, H-6a), 3.89 (1 H, br d, J = 3.32 Hz, H-4′), 3.95 (1 H, dd, J = 2.02, 12.20 Hz, H-6b), 4.43 (1 H, d, J = 7.81 Hz, H-1′), 4.54 (1 H, d, J = 8.28 Hz, H-1); ^{13}C NMR (D_2O) δ 22.58, 49.29, 55.46, 60.47, 61.45, 68.97, 70.90, 71.38, 72.90, 75.18, 75.77, 78.83, 100.41, 103.28, 118.60, 133.73. Compound **3** (allyl D-1-^{13}C-galactopyranosyl-β(1,4)-2-acetamido-2-deoxy-β-D-glucopyranoside): (57% yield): ^1H NMR (D_2O) δ 2.00 (3 H, s, NHAc), 3.48–3.52 (1 H, m, H-2′), 4.43 (1 H, dd, $J_{H\text{-}1,H\text{-}2}$ = 8.32 Hz, $J_{H\text{-}1,13C\text{-}1}$ = 162.33 Hz, H-1′), 4.54 (1 H, d, J = 8.32 Hz, H-1). Compound **4a** (2-deoxy-D-galactopyranosyl-β(1,4)-2-acetamido-2-deoxy-D-glucopyranose): (36% yield). Both the ^1H NMR spectrum of its heptaacetate and the ^{13}C NMR spectrum of **4a** are in good agreement with those reported.[2a] Compound **4b** (2-amino-2-deoxy-D-galactopyranosyl-β-(1,4)-2-acetamido-2-deoxy-D-glucopyranose): (12%). The ^1H NMR spectrum of its acetate is in good agreement with that reported.[5f] Compound **4c** (ethyl D-galactopyranosyl-β(1,4)-2-azido-2-deoxy-D-glucopyranoside): In this case, DTT was eliminated since the 2-azido group was reduced to the corresponding amine in the presence of DTT (15% yield): ^1H NMR (D_2O) β anomer δ 1.22 (1 H, t, J = 7.80 Hz, OCH_2CH_3), 3.27 (1 H, dd, J = 8.33, 9.64 Hz, H-2), 4.40 (1 H, d, J = 7.81 Hz, H-1′), 4.55 (1 H, d, J = 8.24 Hz, H-1).

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