Rapid Chemical Synthesis of Sugar Nucleotides in a Form Suitable for Enzymatic Oligosaccharide Synthesis

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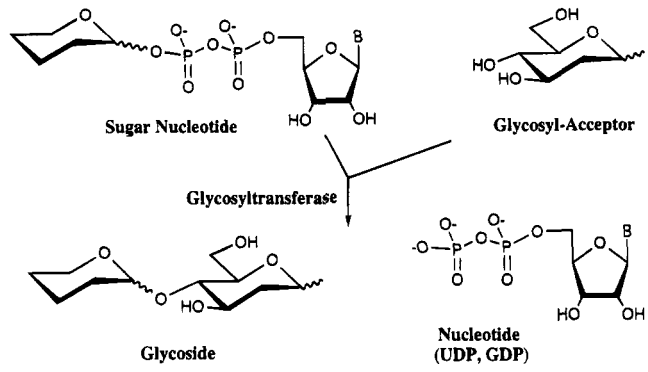
Enzyme-assisted oligosaccharide synthesis is becoming increasingly attractive since it obviates the need for many of the cumbersome protection–deprotection and chemical glycosylation steps that are required in a classical chemical approach.[2] Both glycosidases, running in reverse, and glycosyltransferases have been used extensively. The use of glycosyltransferases is generally preferred since these are the enzymes which assemble the natural oligosaccharides and hence are highly stereo- and regiospecific.[2]

The general reaction catalyzed by a glycosyltransferase is shown in Scheme 1 which summarizes the three key components essential for incorporation of a glycosyltransferase step into a synthetic scheme: (1) An acceptor oligosaccharide substrate must be available, which is usually chemically synthesized. (2) An activated sugar donor (sugar–nucleotide) must be available, which always contains a glycosyl phosphate. This is either prepared chemically or through enzyme-recycling systems.[2] (3) The appropriate glycosyltransferase must be available. In the past, the availability of these enzymes has severely limited their use, but now over 19 glycosyltransferases have been cloned[3] and they are therefore becoming increasingly accessible.

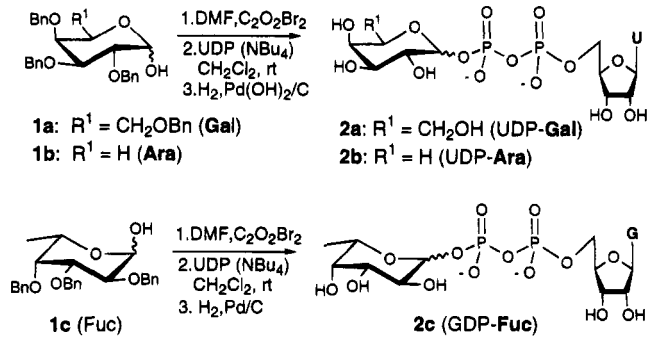
Sugar nucleoside diphosphates are the most common donors in animal systems where UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc, UDP-Xyl, UDP-GlcA, GDP-Man, and GDP-Fuc are extensively used. Of these, the first seven are α (axial)-linked while GDP-Fuc is β (equatorial)-linked. By contrast, very little is known about either the anomeric configuration or the identity of the base in glycosyl donors from plants and bacteria. The chemical synthesis of each of the mammalian sugar nucleotides has been reported.[2] Published procedures have involved the multistep synthesis of the required pyranosyl phosphate, the activation of either the pyranosyl phosphate or the nucleotide 5′-monophosphate (usually as the morpholidate), and subsequent formation of the pyrophosphate linkage. The coupling step alone often requires several days, and the yields are in the range 25–50%. The product sugar nucleotides require extensive purification by ion-exchange chromatography or HPLC. Analogs of the natural sugar nucleotides can be very chemically labile and can decompose during the purification.[2b,4]

We report here a simple and rapid synthesis of sugar nucleoside diphosphates which proceeds under neutral

Scheme 1



Scheme 2



conditions and involves the direct reaction of readily accessible per-*O*-benzylglycopyranosyl bromides with organic-soluble tetrabutylammonium salts of UDP and GDP.[5] These organic salts are readily prepared from the commercial sodium salts by passage through a column of Dowex-50 (H^+) in water and neutralization of the eluate with aqueous tetrabutylammonium hydroxide[6] until the pH was raised to between 5 and 7 (the pH is not critical), followed by lyophilization of the resulting aqueous solution to yield white powders. No decomposition occurred during this process as monitored by ^{31}P-NMR.

Reaction of the per-*O*-benzylated pyranosyl bromides (produced from **1a-c** by reaction with oxalyl bromide in dichloromethane[7] in the presence of DMF, 0.5 h, rt) yielded directly the glycopyranosyl pyrophosphates within 2–5 h (CH_2Cl_2, rt) which were purified by chromatography on Iatrobead silica gel.[8] Hydrogenolysis of the benzyl ethers (atm H_2, 20% $Pd(OH)_2/C$, MeOH, 3–6 h, rt) then yielded the sugar nucleotides **2a-c** (Scheme 2). No reduction of the bases was observed during the benzyl group removal. Further purification can be accomplished, if desired, on DEAE using a linear gradient (0.05–0.3 M) ammonium bicarbonate.[9] The ammonium salts of the product sugar nucleotides were obtained as white powders after neutralization of the product-containing fractions from the DEAE column with Dowex

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(2) (a) Ichikawa, Y.; Look, G. C.; Wong, C.-H. *Anal. Biochem.* **1992**, *202*, 215. (b) Klaffke, W. *Carbohydr. Eur.* **1994**, *10*, 9. (c) Khan, S. H.; Hindsgaul, O. In *Molecular Glycobiology: Frontiers in Molecular Biology Series*; Fukuda, M., Hindsgaul, O., Eds.; Oxford University Press: Oxford, UK, 1994; p 206.

(3) Schachter, H. In *Molecular Glycobiology: Frontiers in Molecular Biology Series*; Fukuda, M., Hindsgaul, O., Eds.; Oxford University Press: Oxford, UK, 1994; p 88.

(4) Gokhale, U. B.; Hindsgaul, O.; Palcic, M. M. *Can. J. Chem.* **1990**, *68*, 1063.

(5) Glycosyl bomides have previously been shown to react directly with tetrabutyl ammonium salts of phosphate esters, but not pyrophosphates; reviewed in ref 2a.

(6) Davisson, V. J.; Davis, D. R.; Dixit, V. M., Poulter, C. D. *J. Org. Chem.* **1987**, *52*, 1794.

(7) Spohr, U.; Lemieux, R. U. *Carbohydr. Res.* **1988**, *174*, 211.

(8) Compounds **2a** and **b** were purified using $CHCl_3$:MeOH:H_2O: concd aqueous NH_4OH 160:70:10:1 as solvent; **2c** was purified using $CHCl_3$:MeOH:H_2O:concd aqueous NH_4OH 120:70:10:1 as solvent.

(9) In the case of **2c**, the crude hydrogenation product was simply passed directly through a column of AG-50-Wx8 (sodium form), the eluate was lyophilized, and the residual white powder was used directly in the glycosyltransferase reaction.

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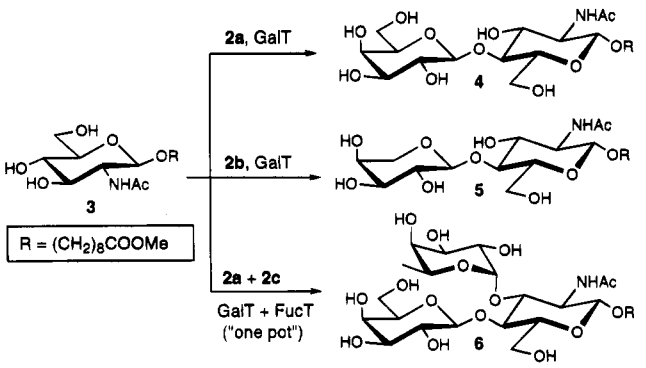
Scheme 3



50 (H^+), removal of the resin by filtration, and lyophilization. The yields of sugar nucleotides were modest, in the range 10–30% starting from the reducing sugars, and the α/β ratios varied between 1:1 and 3:1 depending on the pH of the initial titration, with lower pH favoring the α-anomer. *It is important to note, however, that glycosyltransferases are not significantly inhibited by the wrong anomers of the sugar nucleotide.*

To demonstrate the usefulness of this simple new method for the preparation of sugar nucleotides we used **2a-c**, prepared as described above, in the enzymatic synthesis of some oligosaccharides (Scheme 3). Incubation[10] of **2a** (containing 2 equiv of the active α-anomer) with the glycosyl acceptor **3** and commercial (Sigma) bovine β(1→4)galactosyltransferase (GalT, 0.5 u) gave the disaccharide **4** (4.1 mg) in quantitative yield[10,11] in 2 h. The reaction time was the same as that observed using the pure commercial α-anomer of UDP-Gal, indicating that there were no inhibitors of GalT present in the sample of **2a**.

The L-arabino-analog (**2b**, 2 equiv) of UDP-Gal reacted in the same manner but to yield the new disaccharide **5**[11] (6 mg) in 87% yield. Due to the slower rate of transfer of this analog, larger amounts of the GalT (15 u, 48 h) had to be used. Finally, the Le^X trisaccharide **6** was prepared in two enzymatic steps conducted simultaneously in one pot by combining **3** (1 mg), **2a** (2 equiv), **2c** (6 equiv), GalT (0.5 u), and a partially purified Lewis α(1-3/4) fucosyltransferase[10] (FucT, 0.1 u). The specificity of the enzymes requires the GalT to act first, producing *in situ* the disaccharide **4** which only then is a substrate for the FucT. After 4 days, transformation of **3** to **7** was complete. Oligosaccharides **5**–**7** were isolated on C-18 reversed-phase cartridges and characterized by NMR spectroscopy and FAB-mass spectrometry.[10,11]

In summary, sugar nucleotides have been produced for the first time by direct displacement of an anomeric leaving group with a nucleoside diphosphate. The resulting anomeric mixtures of sugar nucleotides thus obtained function perfectly well in glycosyltransferase-catalyzed oligosaccharide synthesis since the enzymes select the correct anomer as the donor substrate and are not appreciably inhibited by the other anomer. While the yields of sugar nucleotide can undoubtedly be improved by further systematic studies of the reaction and isolation protocols, they are similar to those obtained by classical pyrophosphate-bond formation. The merit of the direct displacement procedure, even in its present unoptimized form, lies in its simplicity and its speed.

Acknowledgment. The fucosyltransferase was a generous gift from M. M. Palcic and S. Gosselin. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

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(10) Enzymes, incubation conditions, and product isolation on C-18 cartridges were as previously described: (a) Palcic, M. M.; Heerze, L. D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 49. (b) Palcic, M. M.; Venot, A. P.; Ratcliffe, R. M.; Hindsgaul, O. *Carbohydr. Res.* **1989**, *190*, 1.

(11) NMR and MS data for compounds **4** and **6** produced in this work were identical to those previously reported[10b] for these known structures. Partial NMR (D_2O) data for **5**: ^1H δ 4.50 ($J_{1,2}$ = 8.0 Hz), 4.37 ($J_{1',2'}$ = 8.0 Hz) and ^{13}C 104.3 (C-1'), 102.0 (C-1); FAB MS M + H^+ = 524, M + Na^+ = 546.