Synthesis of Glycosyl Phosphates Using the Fraser-Reid Activation

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Received December 19, 1990

Glycosyl phosphates are biologically important, both as intermediates in metabolism and as constituents of cell walls. Polymers of glycosyl phosphates are an immunologically active part of the capsule or cell wall of several microorganisms. A convenient synthetic route to this important class of compounds would be useful. Although enzymatic syntheses appear attractive in principle, they are now practical only in the galactose series. The enzymes involved in formation of most sugar phosphates catalyze equilibria unfavorable to the sugar 1-phosphates, although galactokinase (EC 2.7.1.6) catalyzes the direct phosphorylation at the anomeric center by ATP and is thermo-

(3) Kennedy, J. F.; White, C. A. Bioactive Carbohydrates, Ellis Horwood Ltd., West Sussex, 1983.
Table I. Stereochemistry of Formation of Tetraphenylglucosyl Dibenzyl Phosphate 2

<table>
<thead>
<tr>
<th>solvent</th>
<th>halonium* (equiv)</th>
<th>DBP (equiv)</th>
<th>time (h)</th>
<th>α/β yield (%)</th>
<th>conversion (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeCN</td>
<td>NBS</td>
<td>1.0</td>
<td>8</td>
<td>0.20 72</td>
<td>90</td>
</tr>
<tr>
<td>MeCN</td>
<td>NBS</td>
<td>1.1</td>
<td>4</td>
<td>0.35 38</td>
<td>51</td>
</tr>
<tr>
<td>EtO</td>
<td>NBS</td>
<td>1.1</td>
<td>24</td>
<td>0.55 54</td>
<td>83</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>NBS</td>
<td>1.3</td>
<td>16</td>
<td>0.80 27</td>
<td>31</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>IL₂ClO₄</td>
<td>1.1</td>
<td>24</td>
<td>0.61 40</td>
<td>64</td>
</tr>
<tr>
<td>MeCN</td>
<td>IL₂ClO₄</td>
<td>1.3</td>
<td>4</td>
<td>0.15 50</td>
<td>79</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>IL₂ClO₄</td>
<td>1.3</td>
<td>4</td>
<td>0.29 55</td>
<td>73</td>
</tr>
</tbody>
</table>

*IL₂ClO₄ = I-collidine)Cl₂O₄. The anomic ratios were estimated by NMR spectroscopy on the crude reaction mixture. The yields were determined after purification by flash chromatography. The conversion is calculated on the basis of unreacted 1.

dynamically favorable. For preparation of sugar 1-phosphates other than galactose 1-phosphates, especially of unnatural or modified sugars, chemical synthesis is therefore presently preferred over enzymatic synthesis. The main problem is to control the stereochemistry of the anomeric center. The α anomer is generally the most important biologically, although β anomers are interesting as analogues or inhibitors.

In this paper, we outline a synthesis of sugar 1-phosphates based on the recent developments by Fraser-Reid and co-workers in the chemistry of 4-pentenyl glycosides. Under appropriate conditions, the pentenylxy chain of these compounds can be converted to an alkoxy or hydroxyl group by oxidation. In the mechanism proposed for these reactions, the pentenylxy chain is attacked electrophilically by halonium ion. This attack generates an oxonium ion leaving group at the anomeric center. This group can be displaced by the oxygen of a nucleophile (eq 1, PG = protecting group, Nu = H₂O, MeOH, HO-sugar). We have applied this reaction to the synthesis of protected sugar 1-phosphates (eq 1, Nu = OP(OBn)₂).

Fraser-Reid et al. have observed that the α/β ratio depends upon reaction conditions. We undertook several experiments with a readily available representative of the 4-pentenyl glycosides. 4'-pentenyl 2,3,4,6-tetra-O-benzylglucoside (Scheme I). Table I summarizes our results.

When a mixture of the 4-pentenyl 2,3,4,6-tetra-O-benzylglucoside (1) and dibenzyl phosphate, DBP, was allowed to react with NBS or iodonium dicollidine perchlorate, TLC showed the formation of a new product that gave a positive spot with a phosphorus-sensitive stain. After purification by flash chromatography, the anomeric protons of the product were clearly distinct from the other protons, the α anomer as a doublet of doublets at δ 5.97 (J = 6.7 and 3.2 Hz) and the β anomer as a triplet at δ 5.24 (J = 6.7 Hz). Both protons had the characteristic carbon-phosphorus coupling constant J_CoP = 6.7 Hz. In the proton-decoupled 13C NMR spectra, both C1 and C2 exhibited characteristic carbon-phosphorus coupling constants between carbon and phosphorus, J_CoP = 4.2 Hz and J_CoP = 6.7 Hz. Experiments using 13C DEPT NMR spectroscopy show the presence of two benzylic carbons coupled with phosphorus (J = 3.5 Hz).

It was thus straightforward to use 1H NMR spectroscopy to determine the anomeric ratio of the product (Table I). This ratio appeared to be independent of the anomic composition of the starting material, since the anomeric mixture was constant (α/β(α + β) = 0.6) in the starting material and varied widely in the product. Moreover, the anomeric composition of the recovered starting material was almost unchanged (α/β(α + β) = 0.55).

As expected, the solvent influenced the anomic ratio. The selectivity could be reversed by changing from acetonitrile to methylene chloride. The halonium reagent also influenced the anomic ratio. Iodonium dicollidine per-
chlorate gave a smaller fraction of the α anomer than NBS. The results with the iodonium salt and the fact that a prolonged reaction time increased the proportion of the α anomer suggest that the β anomer is a kinetic product that slowly equilibrates to the α anomer. When 2 was kept in a CDCl₃ solution, no equilibration could be detected by 1H NMR even after one week. The phosphate group in 2 can be selectively deprotected by Pd/C-catalyzed hydrogenation in the presence of cyclohexylamine (2 equiv; MeOH, 1 h, room temperature). Further hydrogenation gave the cyclohexylammonium salt of glucose 1-phosphate.

Because we wished to develop practical synthetic routes to sugar nucleotides in general and to UDP-GlcNAc in particular, we investigated the phosphorylation of 4-pentenyl glycosides derived from glucosamine. The N-phthalimidoglucosamine derivative 3 was obtained as described, except that introduction of the 4-pentenolxy chain was achieved more efficiently using the Hamada procedure (Scheme II). Under the conditions of this scheme, 3 yielded exclusively the β-2-N-phthalimidom-3,4,6-tri-O-acetylglicosyl 1-phosphate 4. The β stereochemistry was established by 1H NMR spectroscopy, on the basis of the coupling constants (J = 8.1 Hz and Jhcop = 7.6 Hz). On the basis of the examples provided by 2 and 4, we believe that the Fraser-Reid methodology provides convenient access to protected glycosyl l-phosphates. The generality of the method remains to be established through further examples.

### Experimental Section

**General.** Reagents and solvents were reagent grade and used as received; CH₂Cl₂ and MeCN were distilled from CaH₂ and Ba(OH)₂ from sodium benzoquinone ketyl. TLC analyses were performed on glass plates with UV fluorescent indicator (Merck, Silica gel 60 F₂₅₄) and were stained with a mixture of p-anisaldehyde, acetic acid, sulfuric acid, and ethanol (5.5:32:7.5:2.00) or with the Dittmer-Lester reagent for phospho compounds. Flash chromatography employed 40-63 pm of silica (Merck). lH NMR spectra were obtained at 300 and 500 MHz, 13C at 75.45 MHz, and 31P at 121.49 MHz. Molecular sieves (4 A, Aldrich) were dried in an oven at 180 °C. I and 3 were prepared as described with slight modifications for 3 (see text).

**2,3,4,6-Tetra-O-benzylglucosyl Dibenzyl Phosphate (2).** To a suspension of activated molecular sieves (0.2 g; in a solution containing 4'-pentenyl 2-N-phthalimido-3,4,6-tri-O-acetylglucosyl dibenzyl phosphate (2 mg, 0.19 mmol, 1 equiv) and dry acetonitrile (2 mL), were added successively dibenzyl phosphate (55 mg, 0.22 mmol, 1 equiv) and NBS (70 mg, 0.44 mmol, 2 equiv). The mixture was stirred under argon at room temperature for 8 h. The suspension was filtered to remove the molecular sieves, concentrated in vacuo, and chromatographed (silica; eluent, petroleum ether-ethyl acetate (8:2 to 7:3)). Compound 2 was obtained as a gum (110 mg, 72%, α/β = 0.15) was again isolated as gum: 1H NMR (CDCl₃) δ 7.45-7.19 (m, 28 H), 7.19-7.08 (m, 2 H), 5.97 (dd, H1 α anomer, J = 6.7, 3.2 Hz), 5.24 (dd, H1 β-anomer, J = 6.7, 6.7 Hz), 5.18 (br d, 2 H, J = 6.9 Hz), 5.04 (t, 2 H, J = 6.7 Hz), 4.92-4.67 (m, 5 H), 4.54-4.44 (m, 3 H), 3.91-3.45 (m, 6 H), 13C NMR (CDCl₃) δ 138.5-137.5 (m, Ph), 128.60, 128.53, 128.31, 128.09, 128.94, 127.90, 127.82 (Ph); β anomer 99.51 (C1, d, J = 4.2 Hz), 84.55 (C3), 82.18 (C2, d, J = 6.7 Hz), 77.47 (C4), 75.82 (Bn), 75.71 (C5), 75.18 (Bn), 75.05 (Bn), 73.84 (Bn), 69.52 (BnOP, d, J = 3.5 Hz), 69.46 (BnOP, d, J = 3.5 Hz), 68.68 (C6); α anomer 95.93 (C1, d, J = 4.2 Hz), 81.34 (C3), 79.50 (C2, d, J = 6.7 Hz), 77.05 (C4), 75.80 (Bn), 75.25 (Bn), 73.69 (Bn), 73.24 (Bn), 72.73 (C5), 69.52 (BnOP, d, J = 3.5 Hz), 69.46 (BnOP, d, J = 3.5 Hz), 68.21 (C6). 31P NMR (CDCl₃) δ -4.15. Anal. Found: C, 71.62, H, 6.28.

**2-N-Phthalimidom-3,4,6-tri-O-acetylglicosyl Dibenzyl Phosphate (4).** To a suspension of activated molecular sieves in a solution containing 4'-pentenyl 2-N-phthalimido-3,4,6-tri-O-acetylglicosyl (166 mg, 0.33 mmol, 1 equiv) in dry acetonitrile (4 mL) were successively added dibenzyl phosphate (101 mg, 0.36 mmol, 1.1 equiv) and NBS (118 mg, 0.66 mmol, 2 equiv). The mixture was stirred under argon at room temperature for 11 h. The suspension was then filtered to remove the molecular sieves, concentrated in vacuo, and chromatographed (silica; eluent, petroleum ether-ethyl acetate (7:3)). Compound 4 was obtained as a white solid (162 mg, 71%, β only): 1H NMR (CDCl₃) δ 7.79-7.75 (m, 2 H), 7.70-7.66 (m, 2 H), 7.36-7.05 (m, 10 H), 6.13 (dd, H1, J = 8.1, 7.6 Hz), 5.88 (dd, H3, J = 10.2, 9.6 Hz), 5.21 (dd, H4, J = 10.1, 9.6 Hz), 5.02 and 4.97 (ABd, Bn, J = 12.0, 8.0 Hz), 4.82 and 4.72 (ABd, Bn, J = 12.0, 7.6 Hz), 4.45 (dd, H2, J = 10.2, 8.1 Hz), 4.32 (dd, H6 or 7, J = 12.8, 4.4 Hz), 4.14 (dd, H6 or 7, J = 12.8, 2.9 Hz), 4.00 (dd, H5, J = 10.1, 4.4, 2.9 Hz), 2.75 (s, 3H), 2.03 (s, 3H), 1.85 (s, 3H), 1.63 (s, 3H), 0.73 (s, 3H); 13C NMR (CDCl₃) δ 134.63, 128.83, 128.78, 128.06, 128.16, 127.69, 123.96, 94.31 (C1, d, J = 3.5 Hz), 72.83, 70.41 (C3, C4), 70.01 (Bn, d, J = 2.9 Hz), 69.74 (Bn, d, J = 3.5 Hz), 68.59 (C5), 61.82 (C6), 55.13 (C2, d, J = 4.9 Hz), 20.95, 20.89, 20.69 (3 Ac); 31P NMR (CDCl₃) δ -5.28. Anal. Found: C, 58.60, H, 5.01, N, 1.95.

**Acknowledgment.** This research was supported by the National Institutes of Health, Grant GM38889. P. Pale acknowledges support from NATO and from the CNRS, France.

**Registry No.** 1, 134005-35-3; α-2, 82300-58-1; β-2, 38768-84-2; 3, 124771-17-1; 4, 88862-86-6.