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Synthesis of Glycosyl Phosphates Using the Fraser-Reid Activation

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Glycosyl phosphates are biologically important, both as intermediates in metabolism[1] and as constituents of cell walls.[2-5] Polymers of glycosyl phosphates are an immunologically active part of the capsule or cell wall of several microorganisms.[4,5] A convenient synthetic route to this important class of compounds would be useful. Although enzymatic syntheses[6] 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,[7] although galactokinase (EC 2.7.1.6) catalyzes the direct phosphorylation at the anomeric center by ATP and is thermo-

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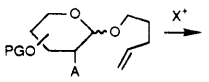
Table I. Stereochemistry of Formation of Tetrabenzylglucosyl Dibenzyl Phosphate 2

solvent	halonium[a] (equiv)	DBP (equiv)	time (h)	$\alpha/\alpha + \beta$[b]	yield (%)[c]	conversion (%)[d]
MeCN	NBS	1.0	8	0.20	72	90
MeCN	NBS	1.1	4	0.35	38	51
Et_2O	NBS	1.1	24	0.55	54	83
CH_2Cl_2	NBS	1.3	16	0.80	27	31
CH_2Cl_2	NBS	1.1	24	0.61	40	64
MeCN	IL_2ClO_4	1.3	4	0.15	50	79
CH_2Cl_2	IL_2ClO_4	1.3	4	0.29	55	73

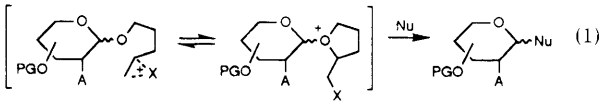
[a] IL_2ClO_4 = I(collidine)$_2^+ClO_4^-$. [b] The anomeric ratios were estimated by NMR spectroscopy on the crude reaction mixture. [c] The yields were determined after purification by flash chromatography. [d] The conversion is calculated on the basis of unreacted 1.

dynamically favorable.[7] For preparation of sugar 1-phosphates other than galactose 1-phosphate, especially of unnatural or modified sugars, chemical synthesis is therefore presently preferred over enzymatic synthesis.[8] 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.[9]

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.[10-13] Under appropriate conditions, the pentenyloxy chain of these compounds can be converted to an alkoxy or hydroxyl group by oxidation. In the mechanism proposed for these reactions, the pentenyloxy 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 = H_2O, MeOH, HO-sugar). We have applied this reaction to the synthesis of protected sugar 1-phosphates (eq 1, Nu = $OP(OBn)_2$).

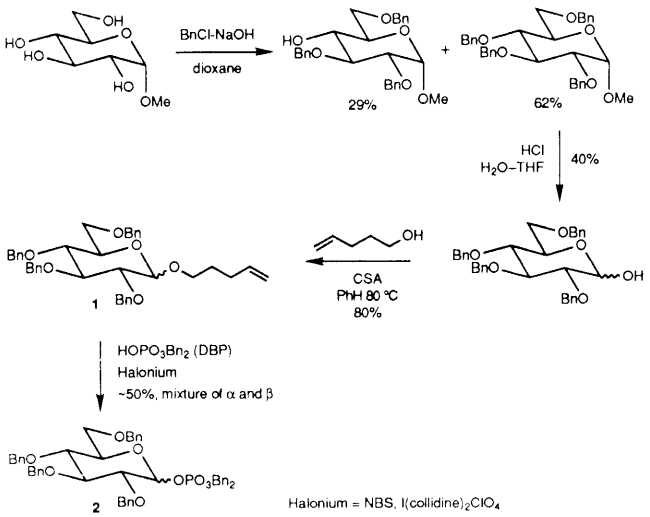


A = OAc, NHAc, OPG, NHPG
X+ = Halonium

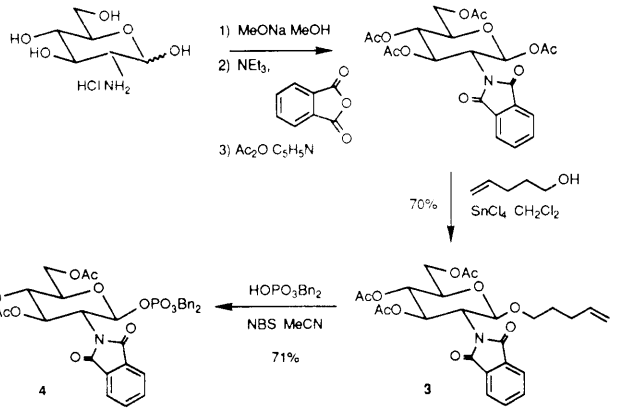


Fraser-Reid et al. have observed that the α-β ratio depends upon reaction conditions. We undertook several experiments with a readily available representative of the 4-pentenyl glycosides,[12-14] 4'-pentenyl 2,3,4,6-tetra-*O*-benzylglucoside (Scheme I). Table I summarizes our results.

Scheme I



Scheme II



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,[15] TLC showed the formation of a new product that gave a positive spot with a phosphorus-sensitive stain.[16] 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 coupling constant J_{HCOP} = 6.7 Hz. In the proton-decoupled ^{13}C NMR spectra, both C1 and C2 exhibited characteristic carbon-phosphorus coupling constants between carbon and phosphorus, $J_{C1,P}$ = 4.2 Hz and $J_{C2,P}$ = 6.7 Hz. Experiments using ^{13}C 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 anomeric composition of the starting material, since the anomeric mixture was constant ($\alpha/(\alpha + \beta)$ = 0.6) in the starting material and varied widely in the product. Moreover, the anomeric composition of the recovered starting material was almost unchanged ($\alpha/(\alpha + \beta) \approx 0.55$).

As expected, the solvent influenced the anomeric ratio. The selectivity could be reversed by changing from acetonitrile to methylene chloride. The halonium reagent also influenced the anomeric ratio. Iodonium dicollidine per-

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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 $CDCl_3$ 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[17,18] 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,[13] except that introduction of the 4-pentenyloxy chain was achieved more efficiently using the Hamada procedure[19] (Scheme II). Under the conditions of this scheme, **3** yielded exclusively the β-2-*N*-phthalimido-3,4,6-tri-*O*-acetylglucosamine 1-phosphate **4**. The β stereochemistry was established by ^1H NMR spectroscopy, on the basis of the coupling constants (J = 8.1 Hz and J_{HCOP} = 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 1-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; CH_2Cl_2 and MeCN were distilled from CaH_2 and Et_2O 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.[16] Flash chromatography employed 40–63 μm of silica (Merck). ^1H NMR spectra were obtained at 300 and 500 MHz, ^{13}C at 75.45 MHz, and ^{31}P at 121.49 MHz. Molecular sieves (4 Å, Aldrich) were dried in an oven at 180 °C. **1** 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 of 4′-pentenyl tetrabenzylglucoside (120 mg, 0.19 mmol, 1 equiv) and dry acetonitrile (2 mL), were added successively dibenzyl phosphate (55 mg, 0.2 mmol, 1 equiv), and NBS (70 mg, 0.4 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%, $\alpha/(\alpha + \beta)$ = 0.2). The same procedure was applied using iodonium dicollidine perchlorate with these quantities: 4′-pentenyl tetrabenzylglucoside (210 mg, 0.34 mmol, 1 equiv), acetonitrile (4 mL), dibenzyl phosphate (128 mg, 0.45 mmol, 1.15 equiv), iodonium dicollidine perchlorate (188 mg, 0.4 mmol, 1.18 equiv), and a reaction time of 10 h. Compound **2** (135 mg, 50%, $\alpha/\alpha + \beta$ = 0.15) was again isolated as gum: ^1H NMR ($CDCl_3$) 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), 5.10 (br d, 2 H, J = 6.9 Hz), 5.04 (t, 2 H, J = 6.7 Hz), 4.92–4.67 (m, 5 H), 4.58–4.44 (m, 3 H), 3.91–3.45 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 138.5–137.5 (m, Ph), 128.60, 128.53, 128.31, 128.09, 128.04, 127.98, 127.90, 127.82 (Ph); β anomer 99.31 (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), 73.64 (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); ^{31}P NMR ($CDCl_3$) δ −4.15. Anal. Calcd for $C_{48}H_{49}O_9P$: C, 72.02, H, 6.12. Found: C, 71.62, H, 6.28.

2-*N*-Phthalimido-3,4,6-tri-*O*-acetylglucosyl Dibenzyl Phosphate (4). To a suspension of activated molecular sieves in a solution containing 4′-pentenyl 2-*N*-phthalimido-3,4,6-tri-*O*-acetylglucoside (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 ($CDCl_3$) 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 β-anomer, 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 (ddd, H5, J = 10.1, 4.4, 2.9 Hz), 2.75 (s, 3H), 2.03 (s, 3H), 1.85 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 134.63, 128.83, 128.78, 128.66, 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); ^{31}P NMR ($CDCl_3$) δ −5.28. Anal. Calcd for $C_{34}H_{34}O_{13}NP$: C, 58.73; H, 4.89, N, 2.01. Found: C, 58.60, H, 5.01, N. 1.95.

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Registry No. **1**, 134003-35-3; α-**2**, 82300-58-1; β-**2**, 38768-84-2; **3**, 124771-17-1; **4**, 88862-86-6.

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