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Note

Chemical transformation of sugar nucleotides: acetylation of uridine 5′-(α-D-glucopyranosyl diphosphate)

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The comprehensive review of sugar nucleotide chemistry published in 1973 by Kochetkov and Shibaev[1] contains 525 references. Of these, only 8 deal with the chemical modification of these molecules. We are unaware of any further work since that time. The cause is not lack of interest in transformations of this important class of molecules; it is, rather, the lack of solvents suitable for carrying out the necessary chemistry. We report here the acetylation of UDP-Glc [uridine 5′-(α-D-glucopyranosyl diphosphate)] using two different approaches to the solubility problem. In the first, we extend the discovery of Beaucage and Ogilvie[2] that addition of tetrabutylammonium fluoride to tetrahydrofuran greatly increases the solubility of nucleosides in that solvent. We find that that effect extends itself to nucleotides and sugar nucleotides. In the second, we exploit the fact that a number of polar molecules show enhanced solubilities in organic solvents in the presence of lithium salts[3–6]. We have succesfully acetylated UDP-Glc by both approaches, and also report complete assignment of both the ^{1}H- and ^{13}C- n.m.r. spectra, including the acetyl groups, of the peracetylated molecule [(UDP-Glc)Ac_6].

Beyond the solubility problem, we have encountered only one complication, cleavage of the sugar nucleotide to UMP and (presumably) the sugar 1,2-cyclic phosphate[7]. We have observed this decomposition only in the lithium salt system, never in the presence of Bu_4NF. Qualitative observations suggest that the decomposition is promoted by increased solubility of the sugar nucleotide (for instance, in DMF as opposed to THF) and by lithium chloride as opposed to lithium acetate. We have observed no corresponding cleavage of the acetylated sugar nucleotide.

These observations may be rationalized along the following lines: the cleavage occurs *via* nucleophilic attack of the 2-hydroxyl group of glucose at the beta-phosphorus atom. It is prevented in the Bu_4NF system by close association of the bulky Bu_4N^+ ion with the phosphate groups; it is promoted by LiCl because chloride ions form a tight, relatively inactive ion-pair with the acylation catalyst[8], 4-dimethylaminopyridine;

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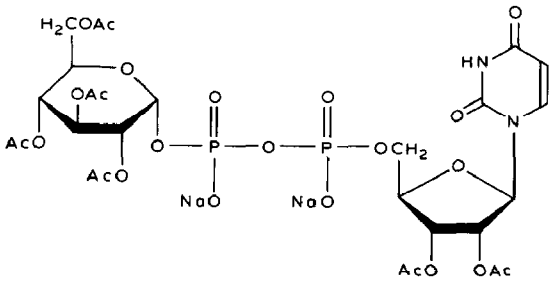
and it is promoted by greater solubility of the sugar nucleotide because of competition between the cleavage and acetylation reactions.

Comparison of the n.m.r. data for UDP-Glc and (UDP-Glc)Ac_6 shows no striking changes, except for those expected from acetylation. The conformations of the two molecules are evidently similar.



UDP-D-GlcAc$_6$

EXPERIMENTAL

Spectroscopy. — All n.m.r. experiments were performed with a Bruker 500-MHz spectrometer. Reproductions of the ^{1}H- and ^{13}C-n.m.r. spectra are given in ref. 9. Signal assignment were made by homonuclear and heteronuclear COSY experiments. Assignments of the acetyl groups presented some difficulties. Application of the H,C-COLOC technique, as previously used[10], failed to reveal several of the carbonyl carbon–ring-proton couplings (see ref. 11). All of the couplings were detected by selective excitation of the carbonyl groups and inverse detection according to the sequence described by Bermel *et al.*[12], Fig. 1. Assignment of the carbonyl groups allowed assignment of the ^{1}H-methyl group resonances and this, in turn, gave the ^{13}C-methyl group assignments by conventional H,C-COSY.

Acetylation of UDP-Glc with tetrabutylammonium fluoride. — *N*-Methylimidazole (2 g) was dissolved in 10 mL of a M Bu_4NF solution in tetrahydrofuran (Aldrich). Disodium UDP-Glc dihydrate (100 mg, 0.155 mmol, Sigma) was added and partially dissolved. The solution was cooled in an ice-bath, causing some precipitation. Acetic anhydride (3.2 g) was then added dropwise with mixing. The mixture was stirred for 1 h and the homogeneous solution kept for 18 h at 37°. The tetrahydrofuran was removed by rotary evaporation at 35° to give a yellow syrup to which water (~2 mL) was added and the mixture was applied to a column (27 × 1 cm, 20–50 mesh), of polystyrene Bio Beads SM-2 (XAD4)[13] equilibrated with water. The column had been previously thoroughly washed with 95% EtOH until the absorbance at 254 nm was zero. The acetylated UDP-Glc adhered to the column; the excess reactants were washed out with water (~300 mL). The acetylated UDP-Glc was eluted with 95% EtOH (~300 mL) and the EtOH removed by rotary evaporation. The remaining yellow syrup contained the Bu_4N^+ salt of (UDP-Glc)Ac_6 and excess Bu_4NF. The latter was removed by absorbing the (UDP-Glc)Ac_6 on a column of DEAE cellulose (30 × 1.5 cm) equilibrated with 40%

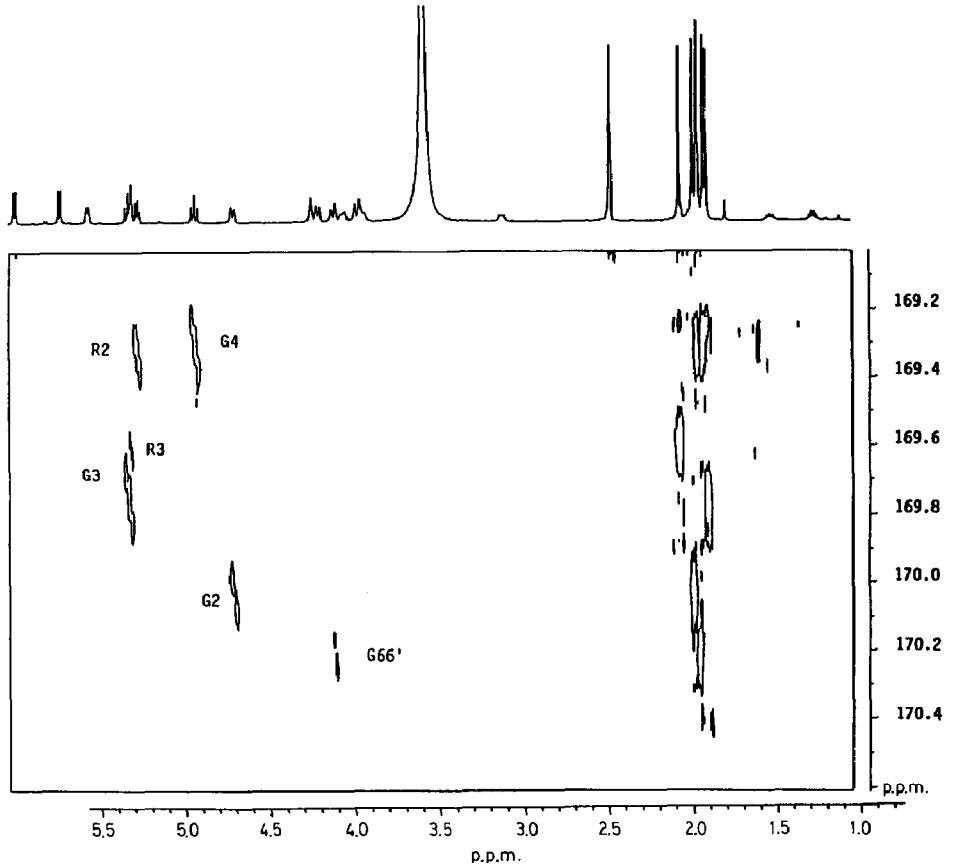


Fig. 1. 2-D spectrum using selective excitation of the carbonyl resonances of (UDP-Glc)Ac_6 according to ref. 12. The horizontal axis shows a portion of the proton spectrum; the vertical axis depicts the carbonyl region of the carbon spectrum. The three-bond correlations (ring protons) are labelled R for ribose and G for glucose. There are a number of artifacts in the two-bond correlation region (methyl protons), but the six major interactions are sufficiently clear.

EtOH. The column was first washed with ~200 mL of 40% EtOH to remove excess Bu_4NF. The UDP-$GlcAc_6$ was eluted with 0.1 M LiCl in 40% EtOH ~mL 200). Most of the EtOH was removed by rotary evaporation and the (UDP-Glc)Ac_6 desalted by adsorption on the polystyrene column as just described.

L.c. analysis of the resulting syrup on a C_{-18} column, using a linear gradient from aq. 0.1% H_3PO_4 to 80% MeCN with detection at 260 nm showed a single symmetrical peak. The f.a.b.-m.s. positive-ion spectrum of the dilithium salt gave peaks at 831 ($M + H^+$) and at 837 ($M + Li^+$). There was also a significant fragment at 501 ($M - GlcAc_4 + 2H^+$). Data for the ^1H- and ^{13}C-n.m.r. spectra are recorded in Tables I–III. The ^1H-n.m.r. spectrum showed that the (UDP-Glc)Ac_6 was still associated with ~10% Bu_4N^+, on a molar basis, but this impurity did not interfere with the spectral analysis. An analytically pure sample was obtained by the following alternative acetylation procedure.

TABLE I

^{1}H Chemical shifts (p.p.m.) and coupling constants (Hz)

	Proton						
Compound	*5*	*6*	*1'*	*2'*	*3'*	*4'*	*5'*
(UDP-Glc)Ac_6[a], δ	5.754	8.010	6.049	5.320	5.361	4.283	4.095, 3.982
J	8.2		6.7				
(UDP-Glc)[b], δ	5.991	7.964	6.002	4.403	4.378	4.306	4.271, 4.222
J			4.9				
Compound	*1''*	*2''*	*3''*	*4''*	*5''*	*6''*	
(UDP-Glc)Ac_6[a], δ	5.620	4.751	5.373	4.979	4.262	4.154, 4.027	
J	3.3	9.9	9.9	9.9	2.9	−12.4	
					1.9		
UDP-Glc[b], δ	5.621	3.556	3.793	3.483	3.915	3.875, 3.799	
J	3.5	9.8	9.2	10.1	4.4	−12.6	
					2.1		

[a] This paper, Me_2SO-d_6, Me_4Si internal reference, $J_{1'',P}$ 7.6, $J_{2',P}$ 1.4. [b] Lee and Sarma[14], D_2O, pH 8, Me_4NCl as internal reference, $J_{1'',P}$ 7.0, $J_{2'',P}$ 2.8.

TABLE II

^{13}C and ^{31}P chemical shifts (p.p.m.)

	Atom								
Compound	*2*	*4*	*5*	*6*	*1'*	*2'*	*3'*	*4'*	*5'*
(UDP-Glc)Ac_6[a]	150.79	163.18	103.04	141.01	85.26	72.43	71.35	81.74	64.42
UDP-Glc[b]	152.6	167.0	103.5	142.4	89.4	74.65	70.55	83.5	66.1
	Atom								
Compound	*1''*	*2''*	*3''*	*4''*	*5''*	*6''*	P_α	P_β	
(UDP-Glc)Ac_6[a]	91.29	70.22	69.67	67.90	67.35	61.37	−9.952[c]	−8.070[c]	
UDP-Glc[b]	96.75	72.3	73.75	70.0	73.6	61.2	−10.6[d]	−10.1[d]	

[a] This paper, Me_2SO-d_6, internal reference Me_2SO at 39.5. [b] London and Sherry[15], D_2O, pH 4, Me_4NCl internal reference at 56.2. The data, supplied by Dr. London, are the averages of the chemical shifts for the La and Lu complexes. [c] This paper, external reference, 85% H_3PO_4, see Gorenstein[16] for the sign convention, $J_{P,P}$ 17.7 Hz. [d] Rosner *et al.*[17], external reference, 85% H_3PO_4.

Acetylation of UDP-Glc using lithium salts. — All solvents were freshly distilled. Disodium UDP-Glc dihydrate (500 mg, ~0.8 mmol) was added to a stirred mixture of Li OAc· $2H_2O$ (1 g) 4-dimethylaminopyridine (0.1 g), pyridine (3 mL), and tetrahydrofuran (20 mL). The heterogeneous mixture was cooled on ice and then 10 mL of Ac_2O was added in portions. The mixture was stirred overnight at room temperature. The tetrahydrofuran was removed *in vacuo*. The residue was added to 200 mL of Et_2O in a separatory funnel. Water (4 mL) was added and the aq. phase was separated and washed

TABLE III

^1H and ^{13}C chemical shifts (p.p.m.) for the acetyl groups of (UDP-Glc)Ac_6 in Me_2SO-d_6

	Atom					
	2'	*3'*	*2''*	*3''*	*4''*	*6''*
^1H, CH_3	2.0066	2.1076	2.0219	1.9532	1.9671	1.9965
^{13}C, CH_3	20.221	20.479	20.548[a]	20.400	20.344	20.548[a]
^{13}C, CO	169.315	169.585	170.004	169.747	169.276	170.180

[a] Overlap

again with ether. The aq. phase was chromatographed on an XAD4 polystyrene column as already described. The EtOH wash contained the product as a mixed salt (sodium, lithium, dimethylaminopyridinium). The EtOH was removed *in vacuo*, the residue taken up in a small amount of water, and then applied to a small column of Dowex-50 in the Na^+ form. The eluate from this column was dried *in vacuo* at room temperature over P_4O_{10} to yield ~350 mg of pure material as a non-hydroscopic, colorless amorphous powder (40%); m.p. 190–193°, dec; $[\alpha]_D^{25}$ +73.8; $[\alpha]_{365}^{25}$ +266.9 (*c* 0.13, H_2O); ν_{max} 1250 (P=O), 1710 and 1770 cm−1 (C=O); λ_{max} 260 nm (ε 10 100).

Anal. Calc. for $C_{27}H_{34}N_2Na_2O_{23}P_2 \cdot 2H_2O$: C, 36.1; H, 4.26; N, 3.11; P, 6.90. Found: C, 36.3; H, 4.28; N, 3.29; P, 6.70.

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