Carbohydrate Research, 184 (1988) 77–85
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF URIDINE 5′-(2-ACETAMIDO-2,4-DIDEOXY-4-FLUORO-α-D-GALACTOPYRANOSYL) DIPHOSPHATE AND URIDINE 5′-(2-ACETAMIDO-2,6-DIDEOXY-6-FLUORO-α-D-GLUCOPYRANOSYL) DIPHOSPHATE*

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(Received September 22nd, 1987; accepted for publication, November 2nd, 1987)

ABSTRACT

Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside was converted into its 4-*O*-(methylsulfonyl) derivative (**2**) by treatment with methanesulfonyl chloride in pyridine. Displacement of the methylsulfonyloxy group of **2** with fluoride ion afforded benzyl 2-acetamido-3,6-di-*O*-benzyl-2,4-dideoxy-4-fluoro-α-D-galactopyranoside, which on hydrogenolysis, followed by acetylation, furnished 2-acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-D-galactopyranose. Treatment of this and of 2-acetamido-1,3,4-tri-*O*-acetyl-2,6-dideoxy-6-fluoro-D-glucopyranose with trimethylsilyl trifluoromethanesulfonate in 1,2-dichloroethane at ~50° afforded the 4-deoxy-4-fluoro- or the 6-deoxy-6-fluoro-oxazolines (**5**) and (**11**), respectively. Reaction of **5** and **11** with dibenzyl phosphate in 1,2-dichloroethane produced the α-linked dibenzyl phosphate derivatives **6** and **12**, respectively. Catalytic hydrogenation of **6** provided 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-4-fluoro-α-D-galactopyranosyl phosphate (**7**), and that of **12** gave 2-acetamido-3,4-di-*O*-acetyl-2,6-dideoxy-6-fluoro-α-D-glucopyranosyl phosphate (**13**). Coupling of **7** and **13** with uridine 5′-monophosphomorpholidate in dry pyridine at ~37°, followed by *O*-deacetylation, furnished the title compounds, respectively, isolated and characterized as their respective dilithium salts.

INTRODUCTION

Sugar nucleotides, such as UDP-D-GalNAc and UDP-D-GlcNAc are commonly employed as substrate for *N*-acetyl-D-galactosaminyl- and *N*-acetyl-D-glucosaminyl-transferases, respectively. Interestingly, these sugar nucleotides also act as

*Synthetic Studies in Carbohydrates, Part LXI. For Part LX, see ref. 1. This investigation was supported by Grant No. GM-31425 from the National Institute of General Medical Sciences, National Institutes of Health, U.S. Public Health Service.

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0008-6215/88/$ 03.50

substrate for *N*-acetyl-α-D-hexosaminyl-phosphatetransferases. For example, UDP-D-GalNAc has been employed for the transfer of 2-acetamido-2-deoxy-α-D-galactopyranosyl phosphate to the appropriate, endogenous macromolecular acceptor[2], and UDP-D-GlcNAc has also been widely utilized as a donor-substrate for the transfer of 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate to O-6 of the α-D-mannopyranosyl residues of complex D-*manno*-oligosaccharide[3] under catalysis by the enzyme *N*-acetyl-α-D-glucosaminyl-phosphate transferase (EC 2.7.8.17; "GlcNAc-*P*-transferase"), which is known to be involved in lysosomal-enzyme targeting.

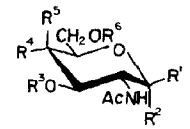
Thus, in an effort to gain more insight into the specificities of those enzymes, we initiated a program for the synthesis of some suitably modified sugar nucleotides. Such nucleotides analogs could prove useful in a variety of biochemical studies including: (a) understanding the binding-site requirements of the aforementioned transferases, (b) possible differentiation of phospho- and monosaccharide-transferases, and (c) inhibition studies of such transferases.

In this context, nucleotide analogs bearing fluorine atoms would be of particular interest, because of the similarities in bond length and polarization between C–F and C–OH groups[4–6]. We describe herein the synthesis of two such modified analogs.

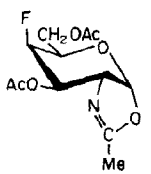
RESULTS AND DISCUSSION

Esterification of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**1**) with methanesulfonyl chloride in pyridine afforded, in 75% yield, the crystalline 4-*O*-(methylsulfonyl) derivative **2**. Treatment of **2** with anhydrous tetrabutylammonium fluoride in boiling acetonitrile, followed by column chromatography purification, gave in 59% yield crystalline benzyl 2-acetamido-3,6-di-*O*-benzyl-2,4-dideoxy-4-fluoro-α-D-galactopyranoside (**3**), the ^{1}H-n.m.r. spectrum of which contained signals in support of the overall structure expected. This was further confirmed by its ^{19}F-n.m.r. spectrum which exhibited a doublet of triplets at ϕ 220.07 (J 50.2, 27.7, and 27.9 Hz), attesting for the presence of an axially-disposed fluorine atom at C-4 (see Experimental section).

Catalytic hydrogenolysis of the benzyl groups of **3**, in glacial acetic acid and



1 R'=R^5=H ; R^2=OBn; R^3=R^6=Bn; R^4=OH
2 R'=R^5=H ; R^2=OBn; R^3=R^6=Bn; R^4=OMs
3 R'=R^4=H ; R^2=OBn; R^3=R^6=Bn; R^5=F
4 R', R^2=OAc, H ; R^3=R =OAc; R^4=H; R^5=F



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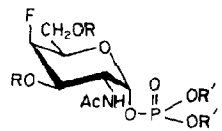
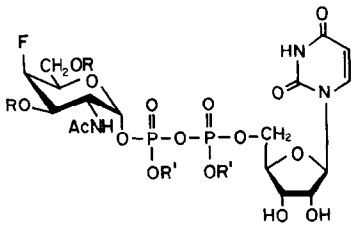
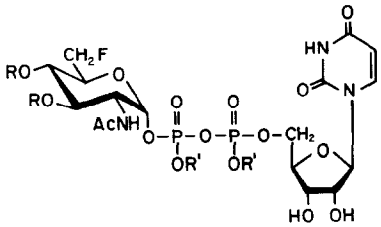


6 R=Ac, R'= Bz
7 R=Ac, R'=H

in the presence of 10% Pd–C, followed by acetylation (1:2 acetic anhydride–pyridine) of the crude product, gave in 87% yield, after silica gel column chromatography, crystalline 2-acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-D-galactopyranose (**4**), the ^{19}F- and ^{1}H-n.m.r. spectra of which were in accord with the structure assigned. Compound **4** was converted into its corresponding D-*galacto-pyrano*-oxazoline **5** by the procedure of Nakabayashi *et al.*[7]. Oxazoline **5** was obtained in 85% yield as a yellowish syrup, the ^{1}H-n.m.r. spectrum of which showed a low-field doublet (δ 6.10, J 7 Hz), characteristic for H-1 on this type of oxazoline ring[8]. This doublet experienced a further splitting (~2 Hz), presumably a result of long-range coupling[9] with the fluorine atom at C-4. The acetyl-group methyl protons, and the oxazoline-methyl-group protons were accounted for by the signals at δ 2.25–2.10.



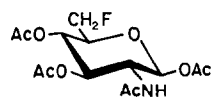
8 R=Ac, R'=H
9 R=H, R'=Li^+



14 R=Ac, R'=H
15 R=H, R'=Li^+

2-Acetamido-1,3,4-tri-*O*-acetyl-2,6-dideoxy-6-fluoro-D-glucopyranose[10] (**10**) was similarly converted into oxazoline **11**, the ^{1}H-n.m.r. spectrum of which, also, contained a low-field doublet (δ 6.00, J 7 Hz) attributable to H-1. The ^{19}F-n.m.r. spectrum of **11** showed a sextet at ϕ 231.63 (J 47.3 and 22.0 Hz) which was compatible with the presence of F-6 (see Experimental section). In the ^{19}F-n.m.r. spectrum of **5**, however, a doublet of triplets occurred at ϕ 217.64 with spacings of 52.1, 22.9, 29.0, and 2.1 Hz. The first three spacings could reasonably be attributed to the coupling of F-4 with H-4, H-5, and H-3, respectively, whereas the small coupling (2.1 Hz) observed is more likely due to a long-range coupling of F-4 with H-1.

Condensation of oxazolines **5** and **11** with dibenzyl phosphate in dry 1,2-dichloroethane, for 24 h at room temperature, followed by purification of the crude product mixtures by preparative t.l.c. (solvent *A*), afforded the α-linked phosphate



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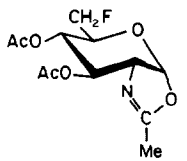

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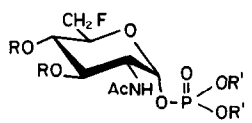

12 R=Ac, R'= Bzl
13 R=Ac, R'=H

derivatives **6** and **12**, respectively. That both **6** and **12** adopted the α-D configuration at their anomeric centers was clearly evidenced by their ^{13}C-n.m.r. spectra. Thus, in the ^{13}C-n.m.r. spectrum of **6**, C-1 resonated as a doublet at δ 97.23 ($J_{C\text{-}1,P}$ 7 Hz), whereas in that of **12** it was observed as a doublet at δ 96.45 ($J_{C\text{-}1,P}$ 7 Hz). This is in agreement with the finding of Khorlin *et al.*[11] and Warren *et al.*[12] that this procedure indeed yields the thermodynamically controlled α-D rather than the kinetically controlled β-D anomer. It is also noteworthy that, in the ^{13}C-n.m.r. spectrum of **6**, the signal for C-4 was shifted downfield, and occurred as a doublet at δ 86.29 ($J_{C\text{-}4,F\text{-}4}$ 186.6 Hz; *cf.* Kováč and Glaudemans[13]). On the other hand, C-6 of compound **12** resonated as a doublet at δ 81.06 ($J_{C\text{-}6,F\text{-}6}$ 176.4 Hz) because of substitution with a fluorine atom.

Catalytic hydrogenolysis of the benzyl groups of **6** and **12** in methanol, and in the presence of 10% Pd–C, furnished 2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-4-fluoro-α-D-galactopyranosyl phosphate (**7**), and 2-acetamido-3,4-di-*O*-acetyl-2,6-dideoxy-6-fluoro-α-D-glucopyranosyl phosphate (**13**), in 88 and 94% yield, respectively. Both of compounds **7** and **13** were then condensed with uridine 5′-monophosphomorpholidate according to the literature procedure[14]. However, attempts to purify the crude product mixtures, at this stage, by preparative t.l.c. (solvent *B*) resulted in partial *O*-deacetylation, as evidenced by ^{1}H-n.m.r. spectroscopy. Therefore, both crude product mixtures (containing **8** and **14**) were directly *O*-deacetylated in aqueous NaOH to afford the title sugar-nucleotides **9** and **15**, respectively, which were isolated as their lithium salts, and had ^{1}H-n.m.r. spectra in agreement with the expected overall structures.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at 25–26° with a Perkin–Elmer 241 polarimeter. I.r. spectra were recorded with a Perkin–Elmer 297 instrument, and u.v. spectra with a Perkin–Elmer Lambda 4A UV/VIS spectrophotometer at 25°. All n.m.r. spectra were recorded at ~25°, ^{1}H-n.m.r. with a Varian EM-390 instrument operating at 90 MHz, the peaks (δ) being expressed from the Me_4Si signal. ^{13}C-N.m.r. and ^{19}F-n.m.r. spectra were recorded with a Varian XL-100 instrument at 25.2 and 94.1 MHz, respectively, the positions of the peaks (δ or φ) being expressed from Me_4Si or $CFCl_3$ signals, respectively. T.l.c. was conducted on aluminum sheets, precoated with 0.2-mm layers of Silica Gel 60F-254 (E. Merck Darmstadt, Germany); the components were located by exposure to u.v. light or spraying the plates with 5% H_2SO_4 in ethanol (or both) and heating; phosphorus-containing compounds were sprayed with the molybdate reagent[15] and warmed to ~35°. Preparative t.l.c. was conducted on 20 × 20 cm glass sheets precoated with 1000-μm layers of silica gel (Analtech, New York, U.S.A.); the components were located by exposure to u.v. light. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). The following

solvent systems (v/v) were used for chromatography: (*A*) 10:10:1 chloroform–ether–methanol, and (*B*) 7:1:2 2-propanol–conc. NH_4OH–water. Organic solutions were generally dried with anhydrous $MgSO_4$. Acetonitrile was distilled from P_2O_5 immediately before use, pyridine was dried over KOH, and 1,2-dichloroethane was dried over 4A molecular sieves. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave, Madison, New Jersey, 07940, U.S.A.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(methylsulfonyl)-α-D-glucopyranoside (**2**). — To an ice-cold and stirred solution of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside[6] (**1**; 11.0 g, 22.4 mmol) in dry pyridine (100 mL) was added methanesulfonyl chloride (7 mL, 89 mmol) dropwise, and the stirring was continued for 24 h at ~4°. The mixture was then slowly poured into vigorously stirred ice–water, and the resulting solid filtered off and dissolved in dichloromethane (400 mL). The solution was successively washed with cold 1% HCl and water, dried, and evaporated to a small volume. Addition of ether caused the crystallization of **2** (9.5 g, 75%), m.p. 168–170°, $[\alpha]_D^{26}$ +98° (*c* 0.8, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.20 (m, 15 H, arom.), 5.70 (d, 1 H, NH), 4.80 (d, 1 H, *J* ~4 Hz, H-1), 2.80 (s, 3 H, CH_3), and 1.80 (s, 3 H, NAc).

Anal. Calc. for $C_{30}H_{35}NO_8S$: C, 63.25; H, 6.19; N, 2.46. Found: C, 62.96; H, 6.31; N, 2.35.

Benzyl 2-acetamido-3,6-di-O-benzyl-2,4-dideoxy-4-fluoro-β-D-galactopyranoside (**3**). — To a solution of **2** (9.0 g, 15.8 mmol) in dry acetonitrile (120 mL) was added anhydrous tetrabutylammonium fluoride (60 g, 231 mmol), and the mixture was boiled for 72 h in an atmosphere of dry N_2. It was then cooled to room temperature, and poured into vigorously stirred ice–water to give a solid, which was filtered off and dissolved in dichloromethane. The solution was repeatedly washed

TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS[a]

Carbon atom	*Compound*		
	[b]	**6**	**12**
C-1	103.6	97.21 (7)	96.45 (7)
C-2	70.9	47.71 (5.5)	54.94 (8)
C-3	71.6 (18.3)	67.97 (18.0)	69.97
C-4	89.5 (185.6)	86.29 (186.6)	67.48 (6.3)
C-5	73.7 (17.1)	69.41 (18.6)	70.50 (23.5)
C-6	59.9 (4.9)	61.89 (6.2)	81.06 (176.4)
CH_3CO		20.73, 22.73	20.60, 22.75
$CH_2C_6H_5$		70.13	70.27

[a]For solutions in $CDCl_3$ with Me_4Si as the internal standard. Aromatic and carbonyl resonances are not shown. Where aplicable, $^2J_{C,OP}$, $^3J_{C,COP}$, and J_{C-F} in Hz are shown in parenthesis. [b]Methyl 4-deoxy-4-fluoro-β-D-galactopyranoside[10]. The chemical shifts and coupling constants for the last-mentioned compound are included for comparison.

with water, dried, and evaporated to a small volume, applied to a column of silica gel, and eluted with 8:1 (v/v) chloroform–acetone. Evaporation of the fractions corresponding (t.l.c. 3:1 chloroform–acetone) to the product yielded a solid, which crystallized from dichloromethane–ether to afford **3** (4.6 g, 59%), m.p. 182–185°, $[\alpha]_D^{26}$ +121° (*c* 1.0, chloroform); ^{19}F-n.m.r. ($CDCl_3$): ϕ 220.07 ($J_{F\text{-}4,H\text{-}4}$ ~ 50.4, $J_{F\text{-}4,H\text{-}5}$ ~27.7, $J_{F\text{-}4,H\text{-}3}$ ~27.9 Hz); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30–7.20 (m, 15 H, arom.), 5.40 (d, 1 H, NH), 4.90 (d, 1 H, *J* 3 Hz, H-1), and 1.85 (s, 3 H, NAc).

Anal. Calc. for $C_{29}H_{32}FNO_5$: C, 70.57; H, 6.53; N, 2.84. Found: C, 70.39; H, 6.58; N, 2.77.

*2-Acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-*D*-galactopyranose* (**4**). — A solution of **3** (3.0 g, 61 mmol) in glacial acetic acid (60 mL) was shaken under H_2 at 345 kPa for 24 h at room temperature in the presence of 10% Pd–C (3.0 g). The suspension was filtered through a bed of Celite, the solid was thoroughly washed with glacial acetic acid, and the filtrate and washings were combined and evaporated under diminished pressure. Several portions of toluene were added to and evaporated from the syrupy residue, which was mixed with 1:2 (v/v) acetic anhydride–pyridine (120 mL) and kept for 24 h at room temperature. The solution was evaporated under diminished pressure, and the residue coevaporated with three added portions of toluene. The crude product was purified by column chromatography with solvent *A* as eluent, to afford, after crystallization from dichloromethane–ether–hexane, **4** (1.83 g, 87%), m.p. 137–139°, $[\alpha]_D^{26}$ +115° (*c* 1.0, chloroforom); ^{19}F-n.m.r. ($CDCl_3$): ϕ 219.18, ($J_{F\text{-}4,H\text{-}4}$ ~50.5, $J_{F\text{-}4,H\text{-}5}$ ~25.2, $J_{F\text{-}4,H\text{-}3}$ ~26.7 Hz); ^{1}H-n.m.r. ($CDCl_3$): 5.85 (d, 1 H, NH) and 2.20–1.90 (s, 12 H, 3 OAc and NAc).

Anal. Calc. for $C_{14}H_{20}FNO_8$: C, 48.14; H, 5.77; N, 4.01. Found: C, 47.96; H, 5.87; N, 3.90.

*2-Methyl-(3,6-di-*O*-acetyl-1,2,4-trideoxy-4-fluoro-α-*D*-galactopyrano)-[2,1-*d*]-2-oxazoline* (**5**). — A solution of **4** (1.0 g, 2.9 mmol) in dry 1,2-dichloroethane (40 mL) containing trimethylsilyl trifluoromethanesulfonate (1.0 mL, 4.5 mmol) was stirred in an atmosphere of dry N_2 for 44 h at ~50°. It was then neutralized with triethylamine, and purified in a column of silica gel with solvent *A* (containing 0.1% triethylamine) as the eluent. On evaporation, fractions corresponding (t.l.c. solvent *A*) to the major product afforded syrupy **5** (0.74 g, 85%), $[\alpha]_D^{26}$ +71° (*c* 0.7, chloroform), ν_{max}^{film} 1750 (OAc) and 1650 cm^{-1} (C=N); ^{19}F-n.m.r. ($CDCl_3$): ϕ 217.6 ($J_{F\text{-}4,H\text{-}4}$ ~52.1, $J_{F\text{-}4,H\text{-}5}$ ~22.9, $J_{F\text{-}4,H\text{-}3}$ ~29.0, $J_{F\text{-}4,H\text{-}1}$ ~2.1 Hz); ^{1}H-n.m.r. ($CDCl_3$): δ 6.10 (dd, 1 H, $J_{1,2}$ ~7.0, $J_{F\text{-}4,H\text{-}1}$ ~2.0 Hz, H-1) and 2.25–2.10 (s, 9 H, 2 OAc and CH_3–C=N).

*2-Acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-4-fluoro-α-*D*-galactopyranosyl dibenzyl phosphate* (**6**). — To a stirred solution of oxazoline **5** (0.52 g, 1.7 mmol) in dry 1,2-dichloroethane (25 mL), was added dibenzyl phosphate (0.64 g, 2.3 mmol). The mixture was stirred in an atmosphere of dry N_2 for 24 h at room temperature. It was then evaporated to a small volume and purified by preparative t.l.c. with solvent *A* as the irrigant to afford syrupy **6** (0.31 g, 32%), $[\alpha]_D^{26}$ +76° (*c* 1.5,

chloroform); ν_{max}^{film} 3020 (C–H, arom.), 1740 (OAc), 1670 (amide), 1270 [$(RO)_3$–P–], and 750 cm^{-1} (P–O–CH_2); ^{19}F-n.m.r. ($CDCl_3$): ϕ 216.5 ($J_{F\text{-}4,H\text{-}4}$ ~50.2, $J_{F\text{-}4,H\text{-}5}$ ~23.6, $J_{F\text{-}4,H\text{-}3}$ ~29.5, $J_{F\text{-}4,H\text{-}1}$ ~2.0 Hz); ^{1}H-n.m.r. ($CDCl_3$): δ 7.4 (m, 10 H, arom.), 5.70 (dd, 1 H, $J_{1,2}$ ~3.0, $J_{1,P}$ ~6 Hz, H-1), and 2.10–1.95 (s, 6 H, 2 OAc), and 1.82 (s, 3 H, NAc); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{26}H_{31}FN_2O_{10}P$: C, 55.03; H, 5.51; N, 2.47. Found: C, 54.97; H, 5.68; N, 2.48.

*Dilithium uridine 5'-(2-acetamido-2,4-dideoxy-4-fluoro-α-*D*-galactopyranosyl) diphosphate* (**9**). — A slow stream of H_2 was bubbled for 1 h at room temperature into a stirred mixture of **6** (0.2 g, 0.08 mmol) and Pd–C (0.1 g) in methanol (5 mL). The suspension was filtered (a bed of Celite), the solid was washed thoroughly with methanol, and the filtrate and washings were combined and evaporated (<35°) to give a solid, which was dissolved in a small volume of methanol. Addition of ether caused the precipitation of **7** (0.12 g, 88%), amorphous, $[\alpha]_D^{26}$ +103.5° (*c* 1.8, methanol); ^{1}H-n.m.r. (CD_3OD): δ 5.60 (dd, 1 H, $J_{1,2}$ ~3, $J_{1,P}$ ~7 Hz, H-1), 2.10 (s, 6 H, OAc), and 2.00 (s, 3 H, NAc).

To a stirred solution of **7** (0.12 g, 0.31 mmol) in dry pyridine (6 mL) was added uridine 5'-monophosphomorpholidate (0.21 g, 0.31 mmol), and the stirring was continued in an atmosphere of dry N_2 for 24 h at 37°. It was then evaporated (<35°), and several portions of toluene were added to, and evaporated from, the residue which was purified by preparative t.l.c. with solvent *B* as the irrigant to afford a solid (0.15 g, containing **8**). Examination by ^{1}H-n.m.r. spectroscopy revealed that partial *O*-deacetylation had occurred. The residue was therefore taken up in water (5 mL) and treated with M NaOH (1 mL), and stirred for 1 h at room temperature. The base was neutralized with Dowex 50W-X4 ($C_5H_4N^+$) cation-exchange resin, the suspension filtered, and the resin thoroughly washed with water. The filtrate and washings were combined and evaporated to a small volume (the pH being adjusted to 7.5–8.0 by the addition of M LiOH). Addition of 1:4 (v/v) methanol–acetone precipitated **9** (0.09 g, 67%) as a hydroscopic solid $[\alpha]_D^{26}$ +43° (*c* 0.3, water); ν_{max}^{KBr} 3250 (OH), 1680 (C=O), 1250 (–P–O–), 1120 (P=O), and 920 cm^{-1} (P–O–C); $\lambda_{max}^{H_2O}$ 261.6 nm; ^{1}H-n.m.r. (D_2O): δ 7.82 (d, 1 H, $J_{5,6}$ ~8.0 Hz, H-6), 5.86 (d, 1 H, $J_{1',2'}$ 4 Hz, H-1'), 5.50 (dd, 1 H, $J_{1,2}$ ~3.0, $J_{1,P}$ ~9 Hz, H-1), and 2.00 (s, 3 H, NAc).

Anal. Calc. for $C_{17}H_{23}FLi_2N_3O_{16}P_2 \cdot 3\ LiOH \cdot 3\ H_2O$: C, 27.37; H, 3.91; N, 5.63. Found: C, 27.25; H, 3.75; N, 5.51.

*2-Methyl-(3,6-di-*O*-acetyl-1,2,6-trideoxy-6-fluoro-α-*D*-glucopyrano)-[2,1-*d*]-2-oxazoline* (**11**). — A solution of **10** (ref. 7; 0.6 g, 1.7 mmol) in dry 1,2-dichloroethane (20 mL) containing trimethylsilyl trifluoromethanesulfonate (0.5 mL, 2.6 mmol) was stirred in an atmosphere of dry N_2 for 44 h at ~53°. It was then neutralized with a little triethylamine, and purified by column chromatography on silica gel with solvent *A* (containing 0.1% triethylamine) as the eluent. On evaporation, the fractions corresponding to the major product afforded syrupy **11** (0.52 g, 99%), $[\alpha]_D^{25}$ +15.2° (*c* 2.1, chloroform); ν_{max}^{film} 1750 (OAc) and 1660 cm^{-1} (C=N); ^{19}F-

n.m.r. ($CDCl_3$): ϕ 231.63 ($J_{F\text{-}6,H\text{-}6a}$ ~47.3, $J_{F\text{-}6,H\text{-}6b}$ ~47.3, $J_{F\text{-}6,H\text{-}5}$ ~22.0 Hz); ^{1}H-n.m.r. ($CDCl_3$): δ 5.95 (d, 1 H, J 6.0 Hz, H-1), and 2.10–2.0 (s, 9 H, 2 OAc and N=C–CH_3).

2-Acetamido-3,4-di-O-acetyl-2,6-dideoxy-6-fluoro-α-D-glucopyranosyl dibenzyl phosphate (**12**). — To a stirred solution of **11** (0.52, 1.7 mmol) in dry 1,2-dichloroethane (25 mL) was added dibenzyl phosphate (0.64 g, 2.3 mmol), and the stirring was continued in an atmosphere of dry N_2 for 24 h at room temperature. The crude product was purified by preparative t.l.c. with solvent *A* as the irrigant to afford syrupy **12** (0.4 g, 39%), $[\alpha]_D^{25}$ +63° (*c* 2.0, chloroform); ν_{max}^{film} 3200 (C–H, arom.), 1740 (OAc), 1660 (amide), 1250 [$(RO)_3P{=}O$], and 725 cm^{-1} (P–O–CH_2); ^{19}F-n.m.r. ($CDCl_3$): ϕ 230.3 ($J_{F\text{-}6,H\text{-}6a}$ ~47.21, $J_{F\text{-}6,H\text{-}6b}$ ~47.21, $J_{F\text{-}6,H\text{-}5}$ ~22.9 Hz); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30–7.20 (m, 10 H, arom.), 6.20 (d, 1 H, NH), 5.71 (dd, 1 H, $J_{1,2}$ ~3.0, $J_{1,P}$ ~6.0 Hz, H-1), and 1.75 (s, 3 H, NAc); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{26}H_{31}FNO_{10}P$: C, 55.03; H, 5.51; N, 2.47. Found: C, 55.28; H, 5.75; N, 2.22.

Dilithium uridine 5′-(2-acetamido-2,6-dideoxy-6-fluoro-α-D-glucopyranosyl) diphosphate (**15**). — Compound **12** (0.14 g, 0.21 mmol) was hydrogenolyzed, exactly as described for **6** (to give **7**), to afford amorphous **13** (0.12 g, 94%), $[\alpha]_D^{25}$ +69° (*c* 1.0, methanol); ^{1}H-n.m.r. (CD_3OD): δ 5.45 (dd, 1 H, $J_{1,2}$ ~3, $J_{1,P}$ ~7 Hz, H-1), and 2.10–1.90 (s, 9 H, 2 OAc and NAc).

Compound **13** (90 mg, 0.23 mmol) was treated with uridine 5′-monophosphomorpholidate (0.21 g, 0.31 mmol) in a manner analogous to that described for **7** (to give **9**) to afford, after complete *O*-deacetylation of the partially *O*-deacetylated mixture (containing **14**), amorphous **15** (70 mg, 71%), $[\alpha]_D^{25}$ +33° (*c* 0.3, water); ν_{max}^{KBr} 3250 (OH), 1660 (amide), 1250 (–P–O–), 1120 (P=O), and 910 cm^{-1} (P–O–C); $\lambda_{max}^{H_2O}$ 261.6 nm; ^{1}H-n.m.r. (D_2O): δ 7.84 (d, 1 H, $J_{5,6}$ ~8 Hz), 5.88 (d, 1 H, $J_{1',2'}$ ~3.0 Hz), 5.40 (dd, 1 H, $J_{1,2}$ 3.0, $J_{1,P}$ ~7.0 Hz, H-1), and 2.0 (s, 3 H, NAc).

Anal. Calc. for $C_{17}H_{23}FLi_2N_3O_{16}P_2 \cdot 3$ LiOH: C, 29.50; H, 3.78; N, 6.07. Found: C, 29.02; H, 3.64; N, 5.73.

ACKNOWLEDGMENTS

The authors are grateful to Mr. Conrad F. Piskorz for his valuable technical assistance and to Mr. Jurij Potienko for recording the n.m.r. spectra.

REFERENCES

1 R. L. THOMAS, S. A. ABBAS, AND K. L. MATTA, *Carbohydr. Res.*, 183 (1988) 163–173.
2 J. BALSAMO, R. S. PRATT, AND J. LITTEN, *Biochemistry*, 25 (1986) 5402–5407.
3 M. L. REITMAN, L. LANG, AND S. KORNFELD, *Methods Enzymol.*, 107 (1984) 163–172.
4 N. F. TAYLOR AND P. W. KENT, *J. Chem. Soc.*, (1958) 872–875.
5 A. BEKOE AND H. M. POWELL, *Proc. R. Soc. London, Ser. A*, 250 (1959) 301–305; J. E. G. BARNETT, *Ciba Found Symp.*, 21 (1971) 95–115.
6 P. J. GAREGG AND H. HULTBERG, *Carbohydr. Res.*, 93 (1981) c10–c11.
7 S. NAKABAYASHI, C. D. WARREN, AND R. W. JEANLOZ, *Carbohydr. Res.*, 150 (1986) c7–c10.

8 M. A. NASHED, C. W. SLIFE, M. KISO, AND L. ANDERSON, *Carbohydr. Res.*, 82 (1980) 237–252; V. K. SRIVASTAVA, *ibid.*, 103 (1982) 286–292.
9 A. A. E. PENGLIS, *Adv. Carbohydr. Chem. Biochem.*, 38 (1981) 195–285.
10 M. SHARMA AND W. KORYTNYK, *Tetrahedron Lett.*, 6 (1977) 573–576.
11 A. YA. KHORLIN, S. E. ZURABYAN, AND T. S. ANTONENKO, *Tetrahedron Lett.*, 55 (1970) 4803–4804.
12 C. D. WARREN, A. HERSCOVICS, AND R. W. JEANLOZ, *Carbohydr. Res.*, 61 (1978) 181–196.
13 P. KOVÁČ AND C. P. J. GLAUDEMANS, *J. Carbohydr. Chem.*, 2(3) (1983) 313–327.
14 C. E. BULAWA AND C. R. H. RAETZ, *J. Biol. Chem.*, 259 (1984) 4846–4851.
15 J. C. DITTMER AND R. L. LESTER, *J. Lipid. Res.*, 5 (1964) 126–127.