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Note

Use of 2-methyl-(3,6-di-*O*–acetyl-1,2,4-trideoxy-4-fluoro-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline as a glycosyl donor. Synthesis of benzyl 2-acetamido-6-*O*-(2-acetamido-2,4-dideoxy-4-fluoro-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranoside*

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In our efforts to synthesize modified oligosaccharides for use as substrates in studies related to glycosidase and glycosyltransferases[2,3], some suitably modified oligosaccharide oxazolines were required. Various procedures for the synthesis of glycopyrano-oxazolines have previously been described[4–6], but most of those methods seem to have certain limitations, particularly when applied to oligosaccharides[7]. However, a recent procedure described by Nakabayashi *et al.*[7] appeared to be promising in so far as it tends to alleviate a number of difficulties thus far encountered. In this method, for example, it is neither a prerequisite to utilize the relatively inaccessible β-acetate of the 2-acetamido-2-deoxy-D-glycose residue, nor is it necessary to prepare a glycosyl halide as a precursor for the desired oxazoline. Therefore, it seemed advantageous to adopt this procedure for our projected syntheses and, as an example of its versatility, we describe herein the synthesis of 2-methyl-(3,6-di-*O*-acetyl-1,2,4-trideoxy-4-fluoro-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**6**). To the best of our knowledge, **6** was hitherto unknown and, by the synthesis of benzyl 2-acetamido-6-*O*-(2-acetamido-2,4-dideoxy-4-fluoro-β-D-glucopyranosyl)-2-deoxy-α-D-galactopyranoside (**8**), we illustrate its utility for introducing a 2-acetamido-2-deoxy-β-D-glucopyranosyl group bearing a fluorine atom at C-4.

For the synthesis of peracetate **4** (a precursor of **6**), benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-galactopyranoside[8] (**1**) was treated with diethylamino-

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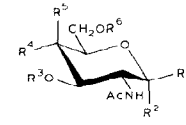
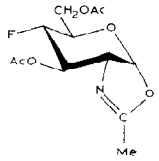
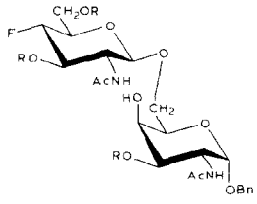


1 $R^1 = R^4 = H, R^2 = OBn, R^3 = R^6 = Bn, R^5 = OH$
2 $R^1 = R^5 = H, R^2 = OBn, R^3 = R^6 = Bn, R^4 = F$
3 $R^1, R^2 = H, OH, R^3 = R^5 = R^6 = H, R^4 = F$
4 $R^1, R^2 = H, OAc, R^3 = R^6 = Ac, R^4 = F, R^5 = H$
5 $R^1 = R^4 = R^6 = H, R^2 = OBn, R^3 = Ac, R^5 = OH$



6



7 R = Ac
8 R = H

sulfur trifluoride (Et_2NSF_3) in dry Diglyme, under conditions analogous to those previously described[9], to give, after column chromatographic purification on silica gel, crystalline **2**. Hydrogenolysis of the benzyl groups of **2** in glacial acetic acid in the presence of 10% palladium-on-charcoal gave crude 2-acetamido-2,4-dideoxy-4-fluoro-α,β-D-glucopyranose (**3**), which was not characterized but directly acetylated (1:2 acetic anhydride–pyridine) to afford crystalline 2-acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-α,β-D-glucopyranose (**4**).

A mixture of **4** and trimethylsilyl trifluoromethanesulfonate in 1,2-dichloroethane was stirred for 24 h at ~50° in an atmosphere of nitrogen. After the usual processing[7], the crude product mixture was subjected to column chromatography on silica gel to afford oxazoline **6**. As a test of its glycosylating capability, **6** was allowed to react with diol **5** for 24 h at ~75° in 1,2-dichloroethane and in the presence of *p*-toluenesulfonic acid monohydrate to afford disaccharide **7**, the ^{1}H-n.m.r. spectrum of which was in agreement with the overall structure assigned (see Experimental section). Zemplén transesterification of **7** furnished, in 75% yield, disaccharide **8**.

The ^{13}C-n.m.r. spectrum of **8** was in accord with the structure proposed (see Table I). Thus, whereas the resonance at δ 96.30 could reasonably be assigned to C-1 carrying the α-benzyloxy group, that at δ 101.6 was assigned to the β-anomeric carbon atom at the interglycosidic linkage. That C-6 was the site of glycosylation was clearly evidenced by the occurance of its signal at low field (δ 68.5) by comparison to that normally observed for C-6 of the parent benzyl α-D-glycoside (see Table I). The signal at δ 90.1 (J ~179 Hz) could only arise from C-4′ bearing the fluorine atom (*cf.* the analogous assignment in the spectrum of a 4-deoxy-4-fluoro-D-glucose derivatives[10]).

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at 25–27° with a Perkin–Elmer 241 polarimeter. Thin-layer chromatography (t.l.c.) was conducted on aluminum sheets, precoated with 0.2-mm layers of Silica gel 60F-254 (E. Merck,

TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS (δ) AND C–F COUPLING CONSTANTS (Hz)[a] FOR DISACCHARIDE **8** AND SELECTED MONOSACCHARIDES[b]

Compd.	*Residue or group*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*CH_3CO*
[c]	α-D-Gal*p*NAcOBn	96.08	49.59	67.19	67.55	71.34	60.53	22.49
[d]	β-D-Glc*p*NAcOMe	101.56	55.41	74.21	70.44	76.74	60.88	22.96
[e]	4-F-D-Glc			75.02	90.29	72.27		
8	α-D-Gal*p*NAcOBn	96.30	49.69	67.15	67.68	69.51	68.47	23.0
	4-F-β-D-Glc*p*NAc	101.55	55.20	71.71	90.11	73.70	60.0	22.6
			(8.4)	(18.2)	(179.3)	(24.2)		

[a]In hertz in parentheses. [b]For a solution in ($^2H_3$)Me_2SO with Me_4Si as the internal standard. Carbonyl and aromatic resonances are not shown. [c]Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside[12]. [d]Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside[12]. [e]4-Fluoro-4-deoxy-D-glucose[9]. The n.m.r. data for the last three compounds are included for comparison purposes.

Darmstadt, Germany); the components were located by exposure to u.v. light, or by spraying the plates with 5% H_2SO_4 in ethanol (or both), and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). I.r. spectra were recorded with a Perkin–Elmer 297 instrument. All n.m.r. spectra were recorded at ~25°; ^{1}H-n.m.r. spectra with a Varian EM-390 instrument operating at 90 MHz, the peaks (δ or ϕ) being expressed from the signal of internal tetramethylsilane. ^{13}C-N.m.r. and ^{19}F-n.m.r. spectra were recorded with a Varian XL-100 at 25.2 and 94 MHz respectively, the positions of the peaks (δ or ϕ) being expressed from the signals of tetramethylsilane or trichlorofluoromethane, respectively. Organic solutions were generally dried with anhydrous Na_2SO_4. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, New Jersey 07940.

*Benzyl 2-acetamido-3,6-di-*O*-benzyl-2,4-dideoxy-4-fluoro-α-*D*-glucopyranoside* (**2**). — A solution of **1** (0.90 g, 1.8 mmol) in dry Diglyme (4 mL) was slowly introduced into a cold (~0°, bath) and stirred solution of diethylaminosulfur trifluoride (1 mL, 7.3 mmol) in dry 2-methoxyethyl ether (4 mL). The mixture was allowed to warm gradually to room temperature, and the stirring continued for an additional 1 h at room temperature. T.l.c. (1:1 chloroform–ethyl acetate) revealed the disappearance of **1** and the presence of a major product, faster-migrating than **1**. The mxiture was cooled (0°, bath), and methanol (20 mL) was cautiously added, to destroy the excess of reagent. The mixture was evaporated to a yellowish syrup which was subjected to column chromatography. Elution with 3:1 (v/v) chloroform–ethyl acetate gave a solid that crystallized from ether–hexane to afford **2** (0.5 g, 55%), m.p. 148–150°, $[\alpha]_D^{25}$ +135° (*c* 0.5, chloroform); ^{19}F-n.m.r. ($CDCl_3$): ϕ −193.5 (dd, $J_{F\text{-}4,H\text{-}4}$ 50.3, $J_{F\text{-}4,H\text{-}3}$ 14.6 Hz)*; ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.20 (m, 15 H, arom.) and 1.90 (s, 3 H, NAc).

*These values are similar to those previously reported for a related 2-acetamido-2-deoxy-D-glucopyranose derivative[11].

Anal. Calc. for $C_{29}H_{32}FNO_5$: C, 70.57; H, 6.53; N, 2.84. Found: C, 70.37; H, 6.23; N, 2.83.

*2-Acetamido-1,3,6-tri-*O*-acetyl-2,4-dideoxy-4-fluoro-α,β-*D*-glucopyranose* (**4**). — A mixture of **2** (1.8 g, 3.5 mmol) and 10% Pd–C (1.8 g) in glacial acetic acid (30 mL) was shaken under H_2 at ~345 kPa for 48 h at room temperature. The suspension was filtered (a bed of Celite), the solid thoroughly washed with glacial acetic acid, and the filtrate and washings combined and evaporated under diminished pressure. The resulting syrup was treated with several added portions of toluene, followed by evaporation to afford **3**, a white solid which was not characterized, but directly mixed with 1:2 (v/v) acetic anhydride–pyridine (45 mL), and stirred for 24 h at room temperature. The acetic anhydride and pyridine were evaporated under diminished pressure and several portions of toluene were added to, and evaporated from the residue, which was applied to a column of silica gel and eluted with 8:1 (v/v) chloroform–acetone. Evaporation of the fractions gave a solid which crystallized from dichloromethane–ether–hexane to afford **4** (0.6 g, 64%), m.p. 183–185°, $[\alpha]_D^{25}$ +38° (*c* 0.6, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 2.20–2.00 (s, 9 H, 3 OAc) and 1.95 (s, 3 H, NAc).

Anal. Calc. for $C_{14}H_{20}FNO_8$: C, 48.14; H, 5.77; N, 4.01. Found: C, 48.19; H, 5.74; N, 3.91.

*2-Methyl-(3,6-di-*O*-acetyl-1,2,4-trideoxy-4-fluoro-α-*D*-glucopyrano)-[2,1-*d*]-2-oxazoline* (**6**). — A solution of **4** (0.2 g, 0.7 mmol) in dry dichloroethane (7 mL) containing trimethylsilyl trifluoromethanesulfonate (0.17 mL, 0.86 mmol) was stirred for 24 h at 50°, under an atmosphere of N_2. It was then processed[7] and the residue purified by column chromatography on silica gel using 10:10:1 (v/v) chloroform–ether–methanol (containing 0.1% by volume triethylamine) as the eluent to afford **6** (0.11 g, 69%), yellowish syrup, $[\alpha]_D^{26}$ −24° (*c* 0.6, chloroform), ν_{max}^{film} 1750 (C=O) and 1660 cm^{-1} (C=N); ^{1}H-n.m.r. ($CDCl_3$): δ 6.00 (d, 1 H, *J* 7 Hz, H-1), 5.65 (t, 1 H, *J* 5 Hz, H-2), 5.30 (t, 1 H, *J* 4 Hz, H-4), and 2.15–2.05 (s, 9 H, 2 OAc and N=CMe).

Anal. Calc. for $C_{12}H_{16}FNO_6$: C, 49.83; H, 5.57; N, 4.84. Found: C, 49.61; H, 5.65; N, 4.62.

*Benzyl 2-acetamido-6-*O*-(2-acetamido-3,6-di-*O*-acetyl-2,4-dideoxy-4-fluoro-β-*D*-glucopyranosyl)-3-*O*-acetyl-2-deoxy-α-*D*-galactopyranoside* (**7**). — To a stirred solution of **6** (0.6 g, 2.0 mmol) in dry 1,2-dichloroethane (10 mL) were aded **5** (0.51 g, 1.5 mmol) and 20mM *p*-toluenesulfonic acid in dry 1,2-dichloroethane (10 mL). The mixture was stirred for 24 h at ~75°, then cooled, made neutral with triethylamine (few drops), and evaporated to a syrup. The crude syrup was purified in a column of silica gel with 20:1 (v/v) chloroform–methanol as the eluent to give a solid which crystallized from ethyl acetate–methanol–ether to afford **7** (0.6 g, 61%), m.p. 232–235°, $[\alpha]_D^{25}$ +63° (*c* 0.2, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.33 (m, 5 H, arom.) and 2.10–1.90 (s, 15 H, 3 OAc and 2 NAc).

*Benzyl 2-acetamido-6-*O*-(2-acetamido-2,4-dideoxy-4-fluoro-β-*D*-glucopyranosyl)-2-deoxy-α-*D*-galactopyranoside* (**8**). — Compound **7** (0.43 g, 0.67 mmol) was

suspended in methanol (30 mL) containing M sodium methoxide (0.5 mL), and the mixture stirred at room temperature. The suspended **7** quickly dissolved, and within ~0.5 h crystallization ensued. The stirring was continued for 24 h at room temperature, the base neutralized with a few drops of glacial acetic acid, the mixture refrigerated for 2 h, and the crystalline mass filtered, and thoroughly washed with ice-cold ethanol, to afford **8** (0.3 g, 87%), m.p. 302–304°, $[\alpha]_D^{25}$ +102° (*c* 0.5, dimethyl sulfoxide); ^{19}F-n.m.r. (Me_2SO-d_6): ϕ −193 ($J_{F\text{-}4,H\text{-}4'}$ ~50, $J_{F\text{-}4,H\text{-}3}$ ~15 Hz) [the coupling of F-4 with H-5 (normally ~4.5 Hz)[13,14] was not resolved because of the broadness of the signal]; ^{1}H-n.m.r. (Me_2SO-d_6): δ 7.30 (s, 5 H, arom.), 1.85, and 1.80 (s, 6 H, 2 NAc); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{23}H_{33}FN_2O_{10}$: C, 53.48; H, 6.43; N, 5.42. Found: C, 53.65; H, 6.06; N, 5.33.

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REFERENCES

1 R. K. JAIN, S. A. ABBAS, AND K. L. MATTA, *Carbohydr. Res.*, 173 (1988) 299–304.
2 C. F. PISKORZ, S. A. ABBAS, AND K. L. MATTA, *Carbohydr. Res.*, 131 (1984) 257–263; K. L. MATTA, R. VIG, AND S. A. ABBAS, *ibid.*, 132 (1984) 119–126; S. A. ABBAS, C. F. PISKORZ, AND K. L. MATTA, *ibid.*, 167 (1987) 131–142.
3 R. MADIYALAKAN, S. YAZAWA, S. A. ABBAS, J. J. BARLOW, AND K. L. MATTA, *Anal. Biochem.*, 152 (1986) 22–28.
4 K. L. MATTA AND O. P. BAHL, *Carbohydr. Res.*, 21 (1972) 460–464.
5 R. U. LEMIEUX AND H. DRIGUEZ, *J. Am. Chem. Soc.*, 97 (1975) 4063–4068.
6 R. K. SRIVASTAVA, *Carbohydr. Res.*, 103 (1982) 286–292; M. A. NASHED, C. W. SLIFE, M. KISO, AND L. ANDERSON, *ibid.*, 58 (1977) c13–c16.
7 S. NAKABAYASHI, C. D. WARREN, AND R. W. JEANLOZ, *Carbohydr. Res.*, 150 (1986) c7–c10.
8 S. S. RANA AND K. L. MATTA, *Carbohydr. Res.*, 116 (1983) 71–81.
9 M. SHARMA AND W. KORYTNYK, *Tetrahedron Lett.*, (1977) 573–576; P. J. CARD, *J. Org. Chem.*, 48 (1983) 393–395.
10 V. WRAY, *J. Chem. Soc., Perkin Trans. 2*, (1976) 1598–1605.
11 L. HOUGH, A. A. E. PENGLIS, AND A. C. RICHARDSON, *Can. J. Chem.*, 59 (1981) 396–405.
12 S. A. ABBAS, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 112 (1983) 201–211.
13 L. PHILLIPS AND V. WRAY, *J. Chem. Soc., B*, (1971) 1618–1624.
14 A. A. E. PENGLIS, *Adv. Carbohydr. Chem. Biochem.*, 38 (1981) 195–285.