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OXAZOLINE SYNTHESIS OF 1,2-*trans*-2-ACETAMIDO-2-DEOXYGLYCOSIDES. GLYCOSYLATION WITH 2-METHYL-(3,4,6-TRI-*O*-ACETYL-1,2-DIDEOXY-α-D-GALACTOPYRANO)-[2′,1′:4,5]-2-OXAZOLINE*

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ABSTRACT

The glycosylating activity of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-galactopyrano)-[2′,1′:4,5]-2-oxazoline has been tested in reaction with partially protected saccharides having free primary or secondary hydroxyl groups or with hydroxy amino acids. 3-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-*N*-benzyloxycarbonyl-L-serine benzyl ester (**3**), 6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactopyranose (**5**), *p*-nitrophenyl 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (**7**), 6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-glucose (**9**), and 3-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-glucose (**11**) were synthesized in high yield.

INTRODUCTION

Synthesis of oligosaccharides containing 2-acetamido-2-deoxy-D-galactopyranosyl residues is of great interest for structural investigations of fragments of glycoproteins and glycolipids of animal origin, and also as substrates for the study of enzymic systems.

For the synthesis of oligosaccharides of this type, we have used the oxazoline method of glycosylation whose advantage was demonstrated previously in our laboratory[2–4] and by others [5–7].

RESULTS AND DISCUSSION

2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-galactopyrano)-[2′,1′:4,5]-2-oxazoline (**1**) was first obtained by Fletcher and co-workers[8], as a by-product of the

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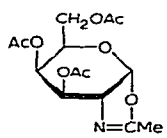
acetylation of 2-acetamido-2-deoxy-D-galactose in the presence of a large amount of zinc chloride, in a yield of 26%. The general method developed in this laboratory[9] gave the oxazoline **1** from 2-acetamido-2-deoxy-D-galactose in 59% yield, without purification of the intermediate glycosyl chloride. The oxazoline **1** was homogeneous in thin-layer chromatography in several solvents on alumina or silica gel, but its optical rotation differed from those reported previously[8]. The structure of **1** was confirmed by n.m.r. and by the i.r. spectrum, which showed the presence of a C=N bond (1670 cm^{-1}) and the absence of a NHCO group (no absorption at 1500–1600 cm^{-1}). The chemical properties also substantiated the high purity of the product. In the presence of *p*-toluene-sulfonic acid, the oxazoline **1** gave, with methanol, the well-known methyl glycoside[10] (**2**) in nearly quantitative yield (*cf.* with the 52% yield of ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside obtained[8] in the reaction of **1** with ethanol). Finally, the structure and purity of the oxazoline **1** prepared in our laboratory was supported by the high yields of the resultant disaccharides.

In all cases, the glycosylation was performed with a slight excess of **1** for 15–20 min at 70–80° in dry toluene (or in a mixture of nitromethane–toluene for **6**) in the presence of a catalytic amount of *p*-toluene-sulphonic acid (at pH 3–4), *i.e.* in the standard conditions described earlier[2,3].

The high reactivity of the oxazoline **1** was shown in the glycosylation of *N*-benzyloxycarbonyl-L-serine benzyl ester. The corresponding glycoside (**3**) was obtained in a yield of 75%, whereas the Koenigs–Knorr reaction gave a 49% yield for this compound[11].



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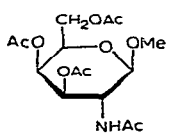

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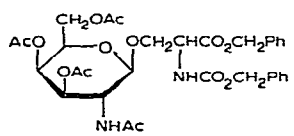

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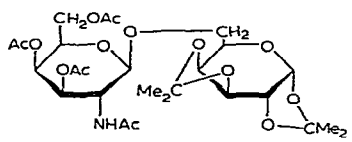

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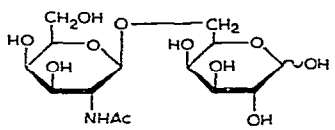

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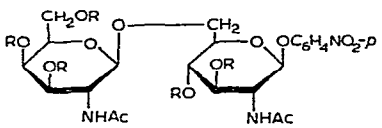
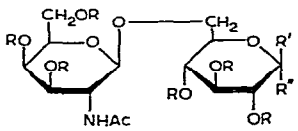
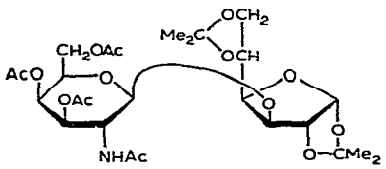


6 R = Ac
7 R = H



8 R = Ac, R' = OAc, R" = H
9 R = H, R' + R" = H,OH



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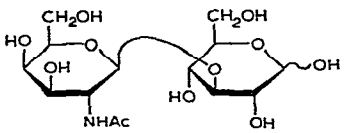

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Under the conditions just described, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose was condensed with the oxazoline **1** to give 6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose[12] (**4**) in 81% yield. The *O*-isopropylidene groups were conveniently removed by the action of 90% aqueous trifluoroacetic acid[13], without cleavage of the glycosidic bond, whereas the treatment with dilute sulfuric acid caused extensive cleavage[12]. After catalytic deacetylation, crystalline 6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactopyranose[12,14] (**5**) was obtained in high yield.

In the same manner, the oxazoline **1** was condensed with *p*-nitrophenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside and with 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose to give, respectively, *p*-nitrophenyl 2-acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**6**) in 71% yield and 6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (**8**) in 74% yield. After de-*O*-acetylation of **6** and **8**, the disaccharides (**7**) and (**9**) respectively, were obtained in crystalline form.

Condensation of the oxazoline **1** with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose under the same conditions gave 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**10**) in 52% yield. Because of the well-known lability of the (1 → 3) linkage under alkaline conditions[15], **10** was conveniently *O*-deacetylated with triethylamine in methanol. For the removal of the *O*-isopropylidene groups, treatment with 90% trifluoroacetic acid at room temperature or with hot 60% aqueous acetic acid was found to cleave the glycoside bond to a great extent, but 60% aqueous trifluoroacetic acid at room temperature gave satisfactory results, merely a trace of disaccharide being cleaved, and the disaccharide 3-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-glucose (**11**) was obtained in crystalline form in 77% yield starting from **10**.

The complete hydrolysis of the synthetic disaccharides **5**, **7**, **9**, and **11** by 2-acetamido-2-deoxy-β-D-glucosidase (B1 form) from boar epididymis[16] (E. C. 3.2.1.30) firmly established the β configuration and the ring size of the 2-acetamido-2-deoxy-D-galactopyranosyl residue. The β-D configuration was also established by n.m.r. spectroscopy (doublets at δ 4.5–4.6 p.p.m., $J_{1,2}$ 8–9 Hz).

Comparison of this method of synthesis of oligosaccharides containing a 2-acetamido-2-deoxy-β-D-galactopyranosyl residue with the other known procedures used for this purpose[12,14,17] shows the advantages of the oxazoline method.

After this work had been completed[1], a similar preparation of the oxazoline **1**, which was used for condensation with benzyl alcohol and with 1,2,3-tri-*O*-benzoyl-α-D-galactopyranose, was described[18].

EXPERIMENTAL

General. — Melting points were measured with a Boëtius apparatus and are corrected. Optical rotations were determined with a Perkin–Elmer Model 141 polar-

imeter at 20–25°. N.m.r. spectra were recorded with a JEOL JNM 4H-100 spectrometer with tetramethylsilane as internal standard. The i.r. spectrum was obtained with a Perkin–Elmer Model 237 spectrometer. Thin-layer chromatography (t.l.c.) was performed with Silica Gel LS 5/40, the spots being detected with methanolic sulfuric acid, and column chromatography with Silica Gel L 40/100 (Lachema, Czechoslovakia). Paper chromatography (p.c.) was performed on Filtrak Niederschlag FN-3 paper with 4:1:2 (v/v) butanol–ethanol–water unless otherwise stated. The spots on p.c. were detected by treatment with aniline hydrogen phthalate (for reducing sugars) or with methanolic sodium hydroxide (for *p*-nitrophenyl glycosides). All the compounds described were homogeneous on t.l.c. or p.c. Evaporations were performed at 30–40° *in vacuo*.

*2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-*D*-galactopyrano)-[2′,1′:4,5]-2-oxazoline* (**1**). — A suspension of dry 2-acetamido-2-deoxy-D-galactose (12.0 g) in acetyl chloride (180 ml) was saturated with dry gaseous hydrogen chloride at −10°. The mixture was kept for 24 h at room temperature in a well stoppered flask, and then evaporated. After two additions and evaporations of toluene, the chromatographically pure syrup was dissolved in dry acetonitrile (170 ml), and 2,4,6-trimethylpyridine (18 ml) and silver nitrate (9.0 g) were added with stirring. After 3 h, the reaction mixture was filtered and the filtrate evaporated. The semicrystalline residue was dissolved in dry benzene (100 ml) and the solution cooled for several hours at 5°. The collidine nitrate was removed by filtration and, after evaporation of the filtrate, the residual syrup was passed through a column of neutral alumina (Brockmann IV). Elution with ether gave **1** as a syrup which failed to crystallize (10.5 g, 59%), $[\alpha]_D$ +82° (*c* 1, chloroform); i.r. data: ν_{max}^{film} 1750 (acetate), 1670 cm^{-1} (C=N); n.m.r. data (chloroform-*d*): δ 6.0 (doublet, $J_{1,2}$ 6.7 Hz, H-1), 2.07–2.13 (acetates and C-CH_3).

Anal. Calc. for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25. Found: C, 51.3; H, 5.9; N, 4.3.

Fletcher and co-workers[8] reported $[\alpha]_D^{20}$ +25.5° (*c* 1.25, chloroform) with the same spectral characteristics, and Matta *et al.*[18] gave a value of $[\alpha]_D^{23}$ +25.8° (*c* 1, chloroform); no rationalization for the discrepancy in specific rotation is proposed at this time.

When **1** was treated with sulfuric acid in aqueous acetone at pH 3, it could no longer be detected after 10 min.

*Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranoside* (**2**). — The oxazoline **1** (670 mg) was dissolved in absolute methanol (20 ml) and a catalytic amount of *p*-toluenesulfonic acid was added. T.l.c. (in ethyl acetate) showed, after 2 h, complete conversion of the starting material into a single product. After neutralization with barium carbonate, filtration, and evaporation of the filtrate, the residual syrup was crystallized from ethanol to give **2** (620 mg, 84%), m.p. 216–217°, $[\alpha]_D$ −17° (*c* 1, chloroform); Tarasiejska and Jeanloz[10] reported the same constants.

*3-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranosyl)-*N*-benzyloxycarbonyl-*L*-serine benzyl ester* (**3**). — A solution of **1** (720 mg, 2.2 mmoles), *N*-

benzyloxycarbonyl-L-serine benzyl ester[19] (658 mg, 2.0 mmoles), and *p*-toluenesulfonic acid in dry toluene (5 ml) was heated at 80° for 30 min. After cooling and addition of one drop of pyridine, the dark solution was evaporated. The excess of **1** was hydrolyzed with sulfuric acid in aqueous acetone at pH 3 for 10 min at room temperature. After neutralization with resin AV-17 (CO_3^{2-}), filtration, and evaporation of the filtrate, the residual syrup was passed through a column of silica gel. Elution with (4:1) chloroform–acetone afforded a pure syrup which was crystallized from 2-propanol to give **3** as white needles (985 mg, 75%), m.p. 151–152°, $[\alpha]_D$ +12° (*c* 1, chloroform); lit.[11]: m.p. 152°, $[\alpha]_D^{20}$ +12° (*c* 1, chloroform).

6-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**4**). — This compound was prepared from **1** (2.4 mmoles) and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (2.0 mmoles) as described for the preparation of **3**. Elution with ethyl acetate, followed by crystallization from 2-propanol gave **4** in 81% yield, m.p. 141–142°, $[\alpha]_D$ −63° (*c* 1, chloroform); lit.[12]: m.p. 142–144°, $[\alpha]_D^{20}$ −62.5° (*c* 1, chloroform).

6-O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactopyranose (**5**). — Compound **4** (250 mg) was treated with 90% aqueous trifluoroacetic acid (5 ml) for 10 min at room temperature. The solution was evaporated and toluene was twice added and evaporated. The resultant solid was deacetylated with 0.05M methanolic sodium methoxide (10 ml), and the solution was neutralized with resin KU-2 (H^+) and evaporated. The resultant syrup was crystallized from dry methanol to give **5** (120 mg, 74%, calc. from **4**), m.p. 204–206°; $[\alpha]_D^{20}$ +44° (after 1 min and 24 h, *c* 0.5, water), R_{Glc} 0.31 (p.c.); lit.[12]: m.p. 204–205°;$_D$ [α] +38.5° (*c* 0.9, water); lit.[14]: m.p. 181–184°, $[\alpha]_D^{20}$ +9° (*c* 1.2, water).

2-Acetamido-2-deoxy-D-galactose and D-galactose were identified in the enzymic hydrolyzate of **5**.

p-*Nitrophenyl 2-acetamido-6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-3,4-di-O-acetyl-2-deoxy-β-D-glucopyranoside* (**6**). — A mixture of **1** (660 mg, 2.0 mmoles), *p*-nitrophenyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside[20] (640 mg, 1.5 mmoles), and *p*-toluenesulfonic acid in dry (1:1) nitromethane–toluene (10 ml) was heated for 15 min at 80°. After cooling, the product was filtered off, washed with toluene and ether, and recrystallized from methanol to give **6** (810 mg, 71%), m.p. 226–227°; $[\alpha]_D$ −28° (*c* 0.5, methanol); n.m.r. data (dimethyl sulfoxide-d_6): δ 7.73 (doublet, *J* 8 Hz, NH), 7.25 (doublet, *J* 8 Hz, NH), 5.46 (doublet, *J* 9 Hz, H_a GlcNAc), and 4.58 (doublet, *J* 9 Hz, H_a GalNAc).

Anal. Calc. for $C_{32}H_{41}N_3O_{18}$: C, 50.88; H, 5.47; N, 5.56. Found: C, 50.5; H, 5.5; N, 5.9.

p-*Nitrophenyl 2-acetamido-6-O-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside* (**7**). — Compound **6** (120 mg) was deacetylated with 0.05M methanolic sodium methoxide (8 ml) for 16 h at 5°. The product was collected, washed with cold methanol, and recrystallized from absolute methanol to give **7** (75 mg, 86%), m.p. 208–210° (dec.); $[\alpha]_D$ −41° (*c* 0.5, pyridine); $R_{p\text{-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside}}$ 0.36 (p.c. on FN-1 paper in 3:1:1 butanol–ethanol–water).

Anal. Calc. for $C_{22}H_{31}N_3O_{13}$: C, 48.43; H, 5.73; N, 7.70. Found: C, 48.05; H, 5.8; N, 7.4.

Enzymic hydrolysis of **7** yielded 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, and *p*-nitrophenol.

6-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose (**8**). — This compound was prepared from **1** (2.2 mmoles) and 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose (2.0 mmoles) as described for the preparation of **3**. Elution with ethyl acetate, followed by crystallization of the pure fraction from ethanol gave **8** in 74% yield m.p. 193–194°; $[\alpha]_D$ −12° (*c* 1, chloroform); n.m.r. data (chloroform-*d*): δ 6.21 (doublet, *J* 9 Hz, NH), 5.54 (doublet, *J* 8 Hz, H_a GlcNAc), and 4.52 (doublet, *J* 8 Hz, H_a GalNAc).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.06. Found: C, 49.5; H, 5.7; N, 2.25.

6-O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-D-glucopyranose (**9**). — Compound **8** (200 mg) was deacetylated as described for the preparation of **6**. Crystallization of the residue from absolute ethanol gave **9** (95 mg, 84%), m.p. 186–187° (dec.) $[\alpha]_D$ +36° (2 min) → +18° (24 h, equilibrium, *c* 0.5, water); R_{Glc} 0.38 (p.c.).

Anal. Calc. for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.57; N, 3.64. Found: C, 43.6; H, 6.9; N, 3.9.

Enzymic hydrolysis of **9** yielded 2-acetamido-2-deoxy-D-galactose and D-glucose.

3-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (**10**). — The oxazoline **1** (1.2 mmoles) and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (1.0 mmole) were condensed as described for **3**. After chromatography in (3:1) chloroform–acetone, **10** was obtained (52% yield) as a glassy solid that failed to crystallize, $[\alpha]_D$ −23° (*c* 1, chloroform).

Anal. Calc. for $C_{26}H_{39}NO_{14}$: C, 52.96; H, 6.67; N, 2.38. Found: C, 53.2; H, 6.85; N, 2.45.

3-O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-D-glucopyranose (**11**). — Compound **10** (50 mg) was deacetylated in methanol (5 ml) with triethylamine (0.1 ml) at room temperature. The solution was evaporated, and the chromatographically pure residual syrup was treated for 5 h with 60% aqueous trifluoroacetic acid (3 ml) at room temperature. After several additions and evaporations of toluene, the solid material was crystallized from absolute methanol to give **11** (25 mg, 77%), m.p. 208–210°; $[\alpha]_D$ +78° (2 min) → +50° (24 h, equilibrium, *c* 0.2, water); R_{Glc} 0.60 (p.c.). Enzymic hydrolysis of this disaccharide, yielded 2-acetamido-2-deoxy-D-galactose and D-glucose.

Enzymic hydrolysis. — In a typical experiment, a mixture of substrate (0.5 mg) and 2-acetamido-2-deoxy-β-D-glucosidase from boar epididymis (E.C. 3.2.1.30, *ca.* 0.05 mg) was incubated under the optimum conditions of galactosaminidase activity[16] in citric acid–phosphate buffer, pH 3.8 (0.2 ml) for 10 h at 37°. The products released were determined by t.l.c. in chloroform–methanol (10:1 or 2:1) or by p.c

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REFERENCES

1 J. C. JACQUINET, S. E. ZURABYAN, AND A. YA. KHORLIN, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1973) 1889.
2 S. E. ZURABYAN, T. S. ANTONENKO, AND A. YA. KHORLIN, *Carbohyd. Res.*, 15 (1970) 21, and references cited therein.
3 T. S. ANTONENKO, S. E. ZURABYAN, AND A. YA. KHORLIN, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1970) 2766.
4 S. E. ZURABYAN, V. V. PIMENOVA, E. A. SHASHKOVA, AND A. YA. KHORLIN, *Khim. Prir. Soedin.*, (1971) 689.
5 L. M. LIKHOSHERSTOV, O. S. NOVIKOVA, AND V. A. DEREVITSKAYA, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1971) 652.
6 K. L. MATTA AND O. P. BAHL, *Carbohyd. Res.*, 21 (1972) 460.
7 R. IWAMOTO AND Y. IMANAGA, *Carbohyd. Res.*, 24 (1972) 133.
8 N. PRAVDIĆ, T. D. INCH, AND H. G. FLETCHER, JR., *J. Org. Chem.*, 32 (1967) 1815.
9 A. YA. KHORLIN, M. L. SHUL'MAN, S. E. ZURABYAN, I. M. PRIVALOVA, AND YU. L. KOPAEVICH, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1968) 2094.
10 Z. TARASIEJSKA AND R. W. JEANLOZ, *J. Amer. Chem. Soc.*, 80 (1958) 6325.
11 E. WERRIES AND E. BUDDECKE, *Hoppe-Seyler's Z. Physiol. Chem.*, 359 (1970) 1089.
12 A. J. ACHER AND D. SHAPIRO, *J. Org. Chem.*, 34 (1969) 2652.
13 J. E. CHRISTENSEN AND L. GOODMAN, *Carbohyd. Res.*, 7 (1968) 510.
14 K. MIYAI AND R. W. JEANLOZ, *Carbohyd. Res.*, 21 (1972) 57.
15 D. SHAPIRO, A. J. ACHER, AND E. S. RACHAMAN, *J. Org. Chem.*, 32 (1967) 3767.
16 G. V. VIKHA, E. D. KAVERZNEVA, M. L. SHUL'MAN, AND A. YA. KHORLIN, *Biokhimiya*, 37 (1972) 322.
17 D. SHAPIRO AND A. J. ACHER, *J. Org. Chem.*, 35 (1970) 229.
18 K. L. MATTA, E. A. JOHNSON, AND J. J. BARLOW, *Carbohyd. Res.*, 26 (1973) 215.
19 E. BAER AND J. MAURUKAS, *J. Biol. Chem.*, 212 (1955) 25.
20 T. OSAWA, *Carbohyd. Res.*, 1 (1966) 435.