The Synthesis of 3-Deazaorotidine and Related Nucleoside Derivatives of 4-Hydroxy-2-pyridone (1,2)

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Condensation of 6-carbethoxy-4-hydroxy-2-pyridone or a silyl derivative of 5-carbomethoxy-4-hydroxy-2-pyridone with 2′,3′,5′-tri-*O*-benzoyl-D-ribofuranosyl halide has provided the 3-deaza analogs of orotidine and uridine-5-carboxylic acid. The corresponding amides have also been prepared in view of their possible structural relationship to 1-β-D-ribofuranosyl nicotinamide. Tri-*O*-benzoyl-3-deazauridine was treated with *N*-bromosuccinimide to give, after deblocking, 3-bromo-4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone. The anomeric configuration of these nucleosides was confirmed by pmr spectroscopy.

The important role of orotidine 5′-phosphate as a biosynthetic precursor of uridine (3) and the discovery of a 5-carboxyuracil nucleoside as a new group of antifungal antibiotics (the polyoxins) (4) suggested the preparation of 3-deaza analogs of these pyrimidine systems. The successful synthesis of 3-deaza analogs of uridine, cytidine and 2′-deoxy and arabinosyl derivatives (5) gave a new type of nucleoside analog which demonstrated significant biological potency (6). The present report describes studies of the synthesis of 1-β-D-ribofuranosyl 5 and 6 substituted 4-hydroxy-2-pyridone.

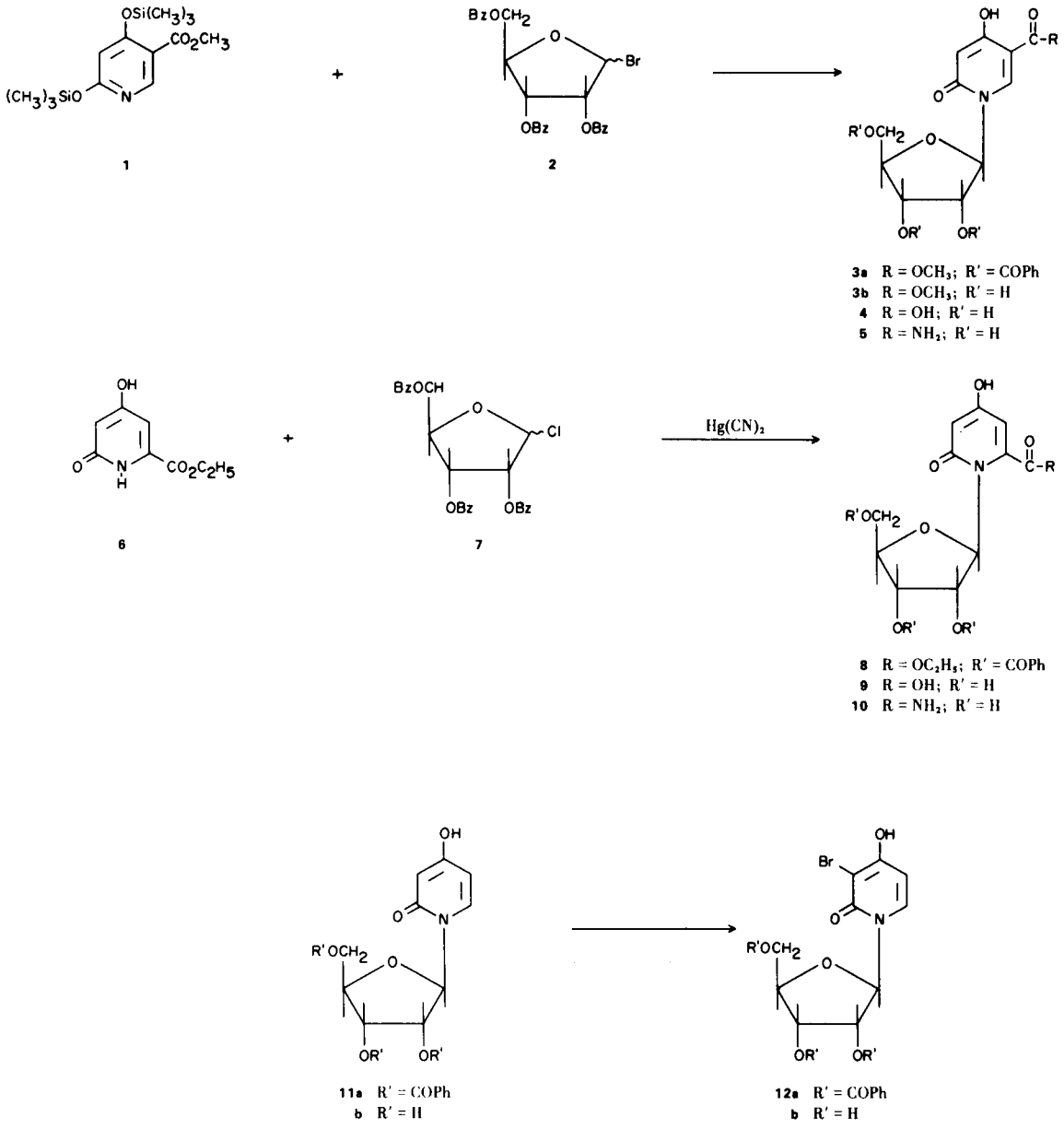
Condensation of the 2,4-bistrimethylsilyloxy derivative (**1**) of 5-carbomethoxy-4-hydroxy-2-pyridone with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**2**) in acetonitrile at room temperature for one week gave a 48% yield of 5-carbomethoxy-4-hydroxy-1-(2,3,5-tri-*O*-benozyl-β-D-ribofuranosyl)-2-pyridone (**3a**) after column chromatographic purification. A recent coupling of the same sugar (**2**) with 5-carbethoxy uracil proceeded in 30% yield *via* the mercuric cyanide method (7). While this work was in progress (5), a brief communication appeared (8) reporting the coupling of a mercury salt of 5-carbethoxy-4-hydroxy-2-pyridone and **7**. However, no uv or nmr spectroscopic characterization of this product was given. Treatment of **3a** with methanolic sodium methoxide gave 5-carbomethoxy-3-deazauridine (**3b**) which was saponified to give 4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone-5-carboxylic acid (**4**) in 63% yield. Treatment of **3a** with methanolic ammonia afforded 4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone-5-carboxamide (**5**) in quantitative yield.

The position of glycosidation was assigned as N_1 based on the similarity of the uv spectra of the nucleosides and their corresponding bases, (5) the base stability of the nucleosides, (9) and the method of synthesis. The β configuration was expected from the method of synthesis (5) and supported by the small coupling constant ($J_{1'-2'}$ = 1.5 Hz) (10) of the peak corresponding to the anomeric proton in the nmr spectrum of **5**.

The difficulty of direct condensation of glycosyl halides with pyrimidines substituted with electron withdrawing groups in the 6-position next to the desired site of glycosidation has been documented (11). As might have been expected in the case of 6-carbethoxy-4-hydroxy-2-pyridone (**6**) (15), very poor yields were obtained by application of the silyl, (5,12) fusion, (13) and mercuric oxide-mercuric chloride (14) method of nucleoside coupling. However, condensation of 6-carbethoxy-4-hydroxy-2-pyridone (**6**) and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (**7**) in the presence of mercuric cyanide (7) afforded a 71% yield of 6-carbethoxy-4-hydroxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyridone (**8**) after silica gel column chromatographic purification. Treatment of **8** with methanolic ammonia gave 3-deazauridine-6-carboxamide (**10**) in 98% yield. Saponification of **8** occurred in alcoholic sodium ethoxide to give 4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone-6-carboxylic acid (3-deazaorotidine) (**9**) directly, after passage of the product through an acidic resin.

The β configuration of **8**, **9** and **10** was verified by the pmr spectrum of **10**. The anomeric proton signal appeared as a sharp singlet thus proving the *trans* H_1'-H_2' orienta-

REACTION SCHEME



tion (10). The uv spectra, base stability and method of synthesis support glycosidation at N_1.

In order to alter the hydrogen-bonding properties (p*K*, *enol-keto* tautomerism, etc.) of 3-deazauridine (**11b**), 3-deazauridine tribenzoate (**11a**) was treated with *N*-bromosuccinimide in chloroform. This resulted in formation of 3-bromo-4-hydroxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyridone (**12a**) as expected (16). This product was debenzoylated with methanolic sodium methoxide to give 3-bromo-3-deazauridine (**12b**) in 50% yield. The position of bromination was verified by the pmr spectrum of **12b** which exhibited a clean AX system with J_{5-6} = 8 Hz for the pyridine region thus demonstrating loss of *m* coupling from H_5-H_3 as well as loss of the H_3 peak.

The biological properties of these compounds are under study.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns block and are uncorrected. Nmr spectra were determined on a Varian A-60 instrument with tetramethylsilane or sodium 5,5-dimethyl-5-sila-

pentanesulfonate as internal standard. Uv spectra were determined on a Beckman DK-2 instrument. Evaporations were accomplished using a Büchler rotating evaporator under reduced pressure (aspirator or vacuum pump). Optical rotations were determined on a Perkin-Elmer model 141 digital readout polarimeter. Thin layer chromatography (tlc) was run on glass plates coated with Silic AR-7GF (Mallinckrodt Chemical Works) using chloroform:acetone (8:2).

5-Carbomethoxy-4-hydroxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyridone (**3a**).

A solution of (**2**) [prepared (17) from 8.80 g. (0.0175 mole) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose] in 150 ml. of dry acetonitrile was added to the trimethylsilyl derivative **1** [prepared (5) from 2.92 g. (0.0175 mole) of 5-carbomethoxy-4-hydroxy-2-pyridone (18). The solution was protected from moisture and allowed to stand for seven days at room temperature and then was evaporated to dryness. The residue was treated with 50 ml. of 85% aqueous ethanol and boiled on a steam bath for five minutes. This solution was evaporated to dryness and the resulting dark solid foam dissolved in 35 ml. of chloroform and applied to a column (1¼″ diameter, 170 g.) of silica gel (dry packed). The column was washed with 4000 ml. of chloroform and tlc showed all of the unreacted sugar had been eluted. Elution was then begun with chloroform:acetone (8:2); the eluate was evaporated to produce 5.1 g. (47.7%) of a white solid foam.

Anal. Calcd. for $C_{33}H_{27}NO_{11}$: C, 64.60; H, 4.43; N, 2.28. Found: C, 64.72; H, 4.51; N, 2.09.

5-Carbomethoxy-4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone (**3b**).

A solution of 3.0 g. (0.0049 mole) of **3a** and 2.0 g. of sodium methoxide in 150 ml. of methanol was refluxed for one hour. The mixture was neutralized by stirring with Dowex 50W-X 12 (H^+) (100-200 mesh). The resin was removed by filtration, the filtrate was evaporated to dryness, and the residue triturated thoroughly in dry ethyl acetate. The crude product, 0.94 g. (64%) was collected by filtration and a small sample recrystallized from methanol for analysis, m.p. 193.5-194°; $[\alpha]_D^{27}$ + 22.9° (*c*, 1, water); uv max (*p*H 1) 259.5; 230 nm (ε, 8470; 27,800) 280 nm sh (ε, 3870), (*p*H 11) 233.5 nm (ε, 44,600) 268 nm sh (ε, 3440), (methanol) 261.5; 232 nm (ε, 7890; 29,200) 283 nm sh (ε, 4010); nmr (DMSO-d_6) δ 3.83 (s, 3, OCH_3), 6.01 (d, 1, $J_{1'-2'}$ = 1.5 Hz, $H_{1'}$), 5.73 (s, 1, H_3), 9.18 (s, 1, H_6).

Anal. Calcd. for $C_{12}H_{15}NO_8$: C, 47.84; H, 5.02; N, 4.65. Found: C, 47.89; H, 4.96; N, 4.50.

4-Hydroxy-1-(β-D-ribofuranosyl)-2-pyridone-5-carboxylic Acid (**4**).

A solution of 0.30 g. (0.001 mole) of **3b** in 10 ml. of 0.2 *N* sodium hydroxide was heated in an oil bath at 70° for one hour and then evaporated to dryness. The residue was dissolved in 3 ml. of water and applied to a column (1 x 9 cm) of Dowex 50W-X12 (H^+) (100-200 mesh). The column was eluted with water and 7 ml. fractions were collected. Fractions 3 to 15 were pooled and evaporated to dryness. The residue was recrystallized from methanol to yield 0.18 g. (63%) of **4**; m.p. darkens 205-210°; $[\alpha]_D^{27}$ + 17.5° (*c*, 1, water); uv max (*p*H 1) 259; 227; 208 nm (ε, 8050; 29,150; 27,250) 283 nm sh (ε, 4450), (*p*H 11) 226 nm (ε, 32,500) 257 nm sh (ε, 6050), (water) 281; 221 nm (ε, 3800; 31,000) 251 nm sh (ε, 6400); nmr (DMSO-d_6) δ 6.01 (d, 1, $J_{1'-2'}$ = 2 Hz, $H_{1'}$).

Anal. Calcd. for $C_{11}H_{13}NO_8$: C, 46.00; H, 4.56; N, 4.87. Found: C, 45.83; H, 4.49; N, 4.80.

5-Carboxamido-4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone (**5**).

To 100 ml. of methanol presaturated with ammonia at -10° was added 1.0 g. (0.0017 mole) of **3a**. The mixture was sealed and allowed to stand at room temperature for five days. The resulting solution was evaporated and the residue was triturated thoroughly with dry ethyl acetate to give 0.45 g. (100%) of crude material which was recrystallized from methanol to give a sample for analysis, m.p. 245-246° dec.; $[\alpha]_D^{27}$ + 17.3 (*c*, 1, water); uv max (*p*H 1) 255; 225; 210 nm (ε, 7980; 26,100; 24,800) 278 nm sh (ε, 3650), (*p*H 11) 234 nm (ε, 44,700), (methanol) 230 nm (ε, 29,000) 280; 255 nm sh (ε, 2940; 6730); nmr (DMSO-d_6) δ 6.01 (d, 1, $J_{1'-2'}$ = 3 Hz, $H_{1'}$).

Anal. Calcd. for $C_{11}H_{14}N_2O_7$: C, 46.15; H, 4.93; N, 9.79. Found: C, 46.26; H, 5.15; N, 9.55.

6-Carbethoxy-4-hydroxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyridone (**8**).

A mixture of 0.366 g. (0.002 mole) of 6-carbethoxy-4-hydroxy-2-pyridone (**6**) (15) and 1.0 g. (0.004 mole) of mercuric cyanide in 200 ml. of nitromethane was protected from moisture and heated under reflux with stirring until 40 ml. of nitromethane were collected in a Dean-Stark trap. To this mixture was added, dropwise, a solution of 1.92 g. (0.004 mole) of **7** (20) in 10 ml. of nitromethane. The mixture was then heated at reflux for three hours, cooled to room temperature and evaporated to dryness. The residue was extracted with 100 ml. of hot chloroform. The chloroform solution was washed twice with 50 ml. of 30% aqueous potassium iodide, twice with 50 ml. of water, dried (sodium sulfate) and evaporated to dryness. The resultant dark residue was dissolved in 10 ml. of chloroform and applied to a column (1 cm diameter, 45 g.) of silica gel (dry packed) which was washed with chloroform. The first fractions containing sugar were discarded and the fractions containing nucleoside were pooled and evaporated to a pale yellow solid foam, 0.89 g. (71%).

Anal. Calcd. for $C_{34}H_{29}NO_{11}$: C, 65.07; H, 4.66; N, 2.23. Found: C, 65.01; H, 4.64; N, 2.14.

4-Hydroxy-1-(β-D-ribofuranosyl)-2-pyridone-6-carboxylic Acid (3-Deazaorotidine) (**9**).

To a solution of 1.0 g. of sodium metal dissolved in 200 ml. of ethanol was added 3.27 g. (0.00522 mole) of **8** and the resultant mixture was refluxed for one hour. The tan precipitate was collected by filtration and washed with ethanol to yield 2.25 g. of crude 4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone-6-carboxylic acid sodium salt. A portion (0.30 g.) of this was dissolved in 3 ml. of water and applied to a column (1 x 25 cm) of Dowex 50W-X12 (H^+) (100-200 mesh) packed in water. The column was eluted with water and fractions (7 ml.) 4 through 20 were pooled and evaporated to dryness. The residue was recrystallized from methanol-ethyl ether to yield 0.053 g. (27%) of **9**, m.p. darkens above 200°; $[\alpha]_D^{27}$ -114° (*c*, 0.9, water); uv max (*p*H 1) 297 nm (ε, 5030), (*p*H 11) 296 nm (ε, 5980), (water) 297 nm (ε, 6620); nmr (DMSO-d_6) δ 5.52 (s, 1, $H_{1'}$).

Anal. Calcd. for $C_{11}H_{13}NO_8 \cdot H_2O$: C, 43.29; H, 4.96; N, 4.59. Found: C, 43.08; H, 4.84; N, 4.61.

6-Carboxamido-4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone (**10**).

To 100 ml. of methanol presaturated with ammonia at -10° was added 0.19 g. (0.0003 mole) of **8**. The mixture was sealed and allowed to stand at room temperature for five days. The resulting solution was evaporated to dryness and the residue was triturated thoroughly with dry ethyl acetate to give 0.085 g. (98%) of crude material. Recrystallization of this solid from methanol-water gave 0.070 g. (81%) of crystals, m.p. 197-200° dec., $[\alpha]_D^{27}$ -128.6° (*c*, 1, water); uv max (*p*H 1) 295; 217 nm (ε, 5440; 29,600), (*p*H 11) 310 nm (ε, 4580), (methanol) 302; 215 nm (ε,

4720; 29,900); nmr (DMSO-d_6) δ 5.57 (s, 1, H_1'), 7.85 and 8.13 two broad singlets exchangeable by deuterium oxide, $CONH_2$.

Anal. Calcd. for $C_{11}H_{14}N_2O_7$: C, 46.15; H, 4.93; N, 9.79. Found: C, 46.16; H, 4.74; N, 9.54.

3-Bromo-4-hydroxy-1-(β-D-ribofuranosyl)-2-pyridone (**12b**).

To a solution of 2.0 g. (0.0036 mole) of 4-hydroxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2-pyridone (**11a**) (5) in 100 ml. of dry chloroform was added 0.70 g. (0.0039 mole) of *N*-bromosuccinimide. The resultant solution was refluxed with stirring while protected from moisture for two hours and then was cooled overnight. The light yellow solution was washed with three 40 ml. portions of water, dried (sodium sulfate) and evaporated to a pale yellow foam, 2.26 g. This foam was dissolved in a minimum of chloroform and applied to a silica gel column (1 cm diameter 40 g., dry packed) and eluted with chloroform. Fractions (15 ml.) 17 to 25 were combined and evaporated to dryness to yield 0.94 g. (41%) of a white solid foam (**12a**), which was deblocked directly.

To a solution of 100 ml. of methanol and 0.50 g. of sodium methoxide was added 0.94 g. (0.0015 mole) of **12a** and the mixture was sealed and allowed to stand at room temperature for six hours. The resultant solution was neutralized with Dowex 50W (H^+) resin and evaporated to dryness. The solid was triturated thoroughly in dry ethyl ether and collected by filtration to yield 0.24 g. (50%). A portion was recrystallized from methanol-ethyl acetate and gave crystalline **12b**, m.p. 90-93° dec., $[\alpha]_D^{27}$ + 54 (*c*, 1.0, water); uv max (*p*H 1) 292 nm (ε, 6680), (*p*H 11) 282; 262 nm (ε, 6340; 6200), (water) 283 nm (ε, 7000); nmr (DMSO-d_6) δ 5.95 (d, 1, $J_{1'-2'}$ = 3.0 Hz, H_1'), 5.80 (d, J_{5-6} = 8 Hz, H_5), 7.50 (d, 1, J_{6-5} = 8 Hz, H_6.

Anal. Calcd. for $C_{10}H_{11}NO_6Br \cdot 0.75\ H_2O$: C, 35.89; H, 4.06; N, 4.18; Br, 23.81. Found: C, 36.01; H, 3.88; N, 3.88; Br, 23.22.

REFERENCES

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(2) From the Ph.D. Dissertation of Bruce L. Currie, University of Utah, September, 1969.

(3) I. Lieberman, A. Kornberg and E. S. Simms, *J. Biol. Chem.*, **215**, 403 (1955).

(4) K. Isono, K. Asahi and S. Suzuki, *J. Am. Chem. Soc.*, **91**, 7490 (1969).

(5) M. J. Robins and B. L. Currie, *Chem. Commun.*, 1547 (1968); B. L. Currie, R. K. Robins and M. J. Robins, *J. Heterocyclic Chem.*, **7**, 323 (1970).

(6) M. J. Robins, B. L. Currie, R. K. Robins and A. Bloch, *Proc. Amer. Assoc. Cancer Res.*, **10**, 73 (1969).

(7) K. A. Watanabe and J. J. Fox, *J. Heterocyclic Chem.*, **6**, 109 (1969).

(8) P. C. Jain and N. Anand, *Indian J. Chem.*, **6**, 767 (1968).

(9) H. Pischel and G. Wagner, *Arch. Pharm.* (Weinheim), **300**, 602 (1967).

(10) R. U. Lemieux and J. W. Lown, *Can. J. Chem.*, **41**, 889 (1963).

(11) J. Pliml and M. Prystas, *Advan. Heterocyclic Chem.*, **8**, 115 (1967).

(12) L. Birkofer, A. Ritter and H. P. Kühlthau, *Angew. Chem. Intern. Ed. Engl.*, **2**, 155 (1963); T. Nishimura, B. Shimizu and I. Iwai, *Chem. Pharm. Bull.* Tokyo), **12**, 1471 (1964).

(13) M. J. Robins, W. A. Bowles and R. K. Robins, *J. Am. Chem. Soc.*, **86**, 1251 (1964) and references therein.

(14) E. Wittenburg, *Chem. Ber.*, **101**, 1095 (1968).

(15) W. Wislicenus and K. Schöllkopf, *J. Prakt. Chem.*, **95**, 269 (1917); H. Stetter and C. W. Schellhammer, *Chem. Ber.*, **90**, 755 (1957); H. Stetter and C. W. Schellhammer, *Ann. Chem.*, **605**, 58 (1957).

(16) C. R. Kolder and H. J. den Hertog, *Rec. Trav. Chim.*, **79**, 474 (1960).

(17) J. D. Stevens, R. K. Ness and H. G. Fletcher, Jr., *J. Org. Chem.*, **33**, 1806 (1968).

(18) This compound has been prepared by C. W. Noell in our laboratory using methyl acetonedicarboxylate in the Errera procedure (19) or can be obtained from the corresponding acid (19) using methanolic hydrogen chloride esterification.

(19) G. Errera, *Ber.*, **31**, 1682 (1898).

(20) N. Yung and J. J. Fox, *Methods Carbohyd. Chem.*, **2**, 109 (1963).