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NUCLEOSIDES LXXXIX. SYNTHESIS OF 1-(2-CHLORO-2-DEOXY-α- AND -β-D-ARABINOFURANOSYL)CYTOSINES*

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ABSTRACT

1-(2-Chloro-2-deoxy-β-D-arabinofuranosyl)cytosine (**16**) and its α anomer (**18**) were synthesized by direct condensation of 3,5-di-*O*-acetyl-2-chloro-2-deoxy-α-D-arabinofuranosyl bromide with trimethylsilylated N^4-acetylcytosine in the absence of catalyst. A new and convenient method for the synthesis of 1,3,5-tri-*O*-acetyl-2-chloro-2-deoxy-α-D-arabinofuranose from methyl 3,5-di-*O*-benzyl-α,β-D-ribofuranoside is described.

INTRODUCTION

The finding that 1-(2-deoxy-2-fluoro-β-D-arabinosyl)cytosine (2′-F-Ara-C) has a growth inhibitory effect on the suspension culture[1] of L1210 mouse leukemia suggested the synthesis of the 2′-chloro analog of arabinosylcytosine. On the basis of many reports[2] from our own laboratory as well as others on pyrimidine nucleoside transformations, it is clear that the *direct* introduction of a halogeno group in the 2′-"up" (arabino) configuration on a preformed nucleoside cannot be achieved by presently-available methods. This conclusion is based upon the ease with which the 2-carbonyl group of the pyrimidine moiety participates in any displacement reactions. The synthesis of 2′-F-Ara-C was achieved[1] by condensation of an appropriate 2-deoxy-2-fluoroarabinofuranosyl halide with a trimethylsilylated cytosine; however, the preparation of the sugar is cumbersome and the overall yields are low. We therefore devoted our efforts to the development of a practical synthesis of a suitably protected 2-deoxy-2-chloroarabinofuranosyl sugar amenable to condensation reactions to give nucleosides.

The most direct approach to the synthesis of chlorodeoxy sugars seemed to be that used originally by Lee and Nolan[3,4] in which a combination of triphenyl-

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phosphine and carbon tetrachloride converts primary and secondary alcohols into their corresponding chlorides under neutral and relatively mild conditions. We now report the successful application of this method to the synthesis of a 2-chloro-2-deoxy-arabinofuranosyl sugar and its subsequent conversion into 2′-Cl-Ara-C. Purine nucleosides containing a 2′-chloro-2′-deoxy-D-arabinofuranosyl moiety have been reported[5].

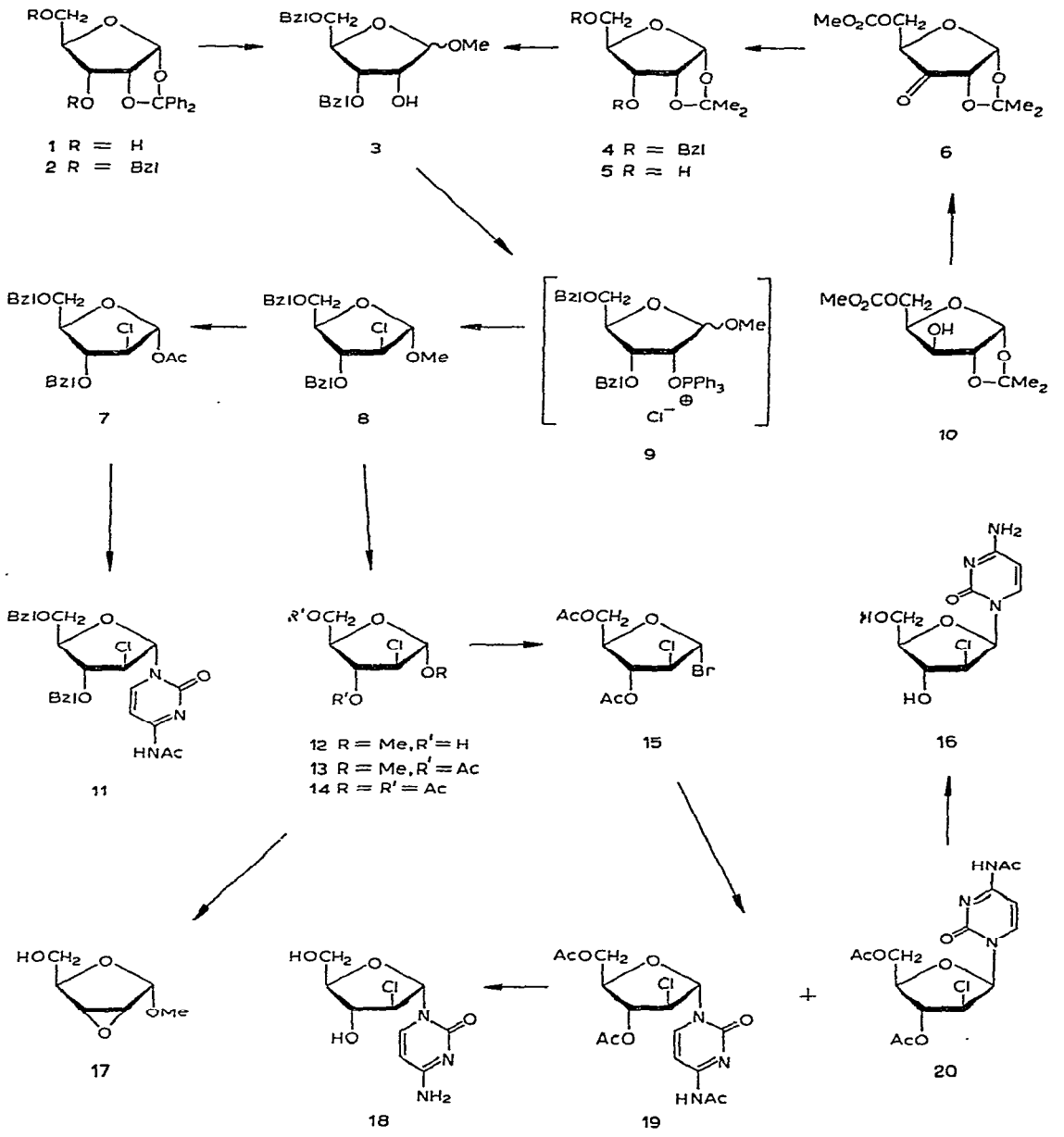
RESULTS AND DISCUSSION

Our first attempt to obtain a ribofuranoside with the 2-OH selectively unblocked made use of 1,2-diphenylmethylidene-α-D-ribofuranose[6] (**1**). Benzylation of **1** afforded 3,5-di-*O*-benzyl-1,2-diphenylmethylidene-α-D-ribofuranose[7] (**2**) in high yield. Treatment of **2** with a catalytic amount of sulfuric acid in methanol afforded an anomeric mixture of methyl 3,5-di-*O*-benzyl-D-ribofuranoside (**3**). Easier access to this key intermediate (**3**) was achieved from the known[8] 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-xylofuranose* (**10**) by oxidation to give 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-pentofuranos-3-ulose (**6**), followed by reduction with sodium borohydride, in a manner analogous to that of Sowa[9], to give 1,2-*O*-isopropylidene-α-D-ribofuranose (**5**) in good overall yield. Compound **5** was also prepared according to the method of Brimacombe and Mofti[10] from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. Benzylation of **5** gave 3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (**4**), which was methanolyzed into **3** in almost quantitative yield.

The n.m.r. spectrum of **3** showed a signal for the anomeric proton at δ 4.87 as a singlet. Close examination of this signal, however, revealed a slight shoulder that could not be resolved. In order to determine whether this product was an anomeric mixture (which could be expected due to the acidic reaction conditions), **3** was debenzylated by hydrogenation in the presence of 10% palladium-on-charcoal to give the corresponding deblocked methyl α,β-D-ribofuranoside. The n.m.r. spectrum of the isolated reaction product in dimethyl sulfoxide-d_6 displayed two anomeric signals for H-1; a doublet at δ 4.72 ($J_{1,2}$ 4 Hz) for the α anomer, and a singlet at δ 4.62 for the β anomer (lit.[11]: δ 4.77, $J_{1,2}$ 4 Hz for α and δ 4.62 for β anomer). In addition, the relative intensity of the two methoxyl signals at δ 3.28 (α) and 3.23 (β) (lit.[11]: δ_α 3.31, δ_β 3.27) indicated an approximate ratio of β to α anomer of 4:1.

Chlorination of **3** was achieved by treatment with carbon tetrachloride and triphenylphosphine[3,4] in acetonitrile at reflux for several days. By following the reaction by t.l.c., it was found that whereas the ionic intermediate **9** was formed almost immediately, subsequent nucleophilic attack by chloride ions on C-2 occurred very slowly. Appreciable decomposition took place during the reaction, and the yields of methyl 3,4-di-*O*-benzyl-2-chloro-2-deoxy-α-D-arabinofuranoside (**8**), therefore, did not exceed 38%. In view of the fact that the anomeric mixture **3** contained mostly the

*This compound is commercially available from Pfanstiehl Labs. Inc., Waukegan, Ill. 60085.



β anomer, it was surprising to find that all of product **8** was obtained as the pure α anomer, as ascertained from its n.m.r. spectrum.

Compound **8** was treated at room temperature for 30 min with a freshly prepared mixture of acetic anhydride containing 0.1% of sulfuric acid and the reaction was followed on silica gel t.l.c. in petroleum ether–acetone (5:1, v/v). Only under such carefully controlled conditions could **8** be converted into the 1-*O*-acetyl derivative (**7**),

which was characterized by the n.m.r. spectrum of the solution in chloroform-*d*; δ (p.p.m.) 7.26 (10 H, s, aromatic-H), 6.25 (1 H, s, H-1), and 1.98 (3 H, s, acetyl-CH_3). When **7** was condensed with trimethylsilylated N^4-acetylcytosine in 1,2-dichloroethane with tin(IV) chloride as catalyst[12], the only nucleoside isolated (in 40% yield) was the α anomer (**11**), as indicated by the n.m.r. spectrum of the solution in chloroform-*d*; δ (p.p.m.) 6.11 (a singlet for H-1'). Compound **11**, presumably N^4-acetyl-1-(3,4-di-*O*-benzyl-2-chloro-2-deoxy-α-D-arabinofuranosyl)cytosine, was not investigated further.

Since the use of a Lewis acid as catalyst appeared to favor the formation of an α-D nucleoside, it was decided to transform the methyl riboside **8** into a glycosyl halogenide which, we hoped, would react with trimethylsilylated N^4-acetylcytosine without the need for a catalyst. Treatment of **7** or **8** with hydrogen bromide under various conditions gave very complex mixtures due probably to the cleavage of the benzyloxy blocking groups. This problem was circumvented by the use of acetyl blocking groups.

Debenzylation of **8** to give methyl 2-chloro-2-deoxy-α-D-arabinofuranoside (**12**) was achieved, in nearly quantitative yield, by hydrogenation in the presence of 10% palladium-on-charcoal at 2.3 atm. As additional proof for the anomeric configuration of **8**, **12** was treated with a slight excess of sodium methoxide in methanol at reflux to yield methyl 2,3-anhydro-α-D-ribofuranoside[8,13] (**17**) as the sole product of the reaction. Epoxide **17** was compared with authentic samples of both α and β anomers of this riboside[8,13] and found to be identical with the α anomer by n.m.r. spectroscopy and by chromatography.

Methyl 3,5-di-*O*-acetyl-2-chloro-2-deoxy-α-D-arabinofuranoside[14] (**13**) was obtained in excellent yield by treating **12** with acetic anhydride and sodium acetate. Compound **13**, in turn, was converted into 1,3,5-tri-*O*-acetyl-2-chloro-2-deoxy-α-D-arabinofuranose (**14**) by a modification of the method of Kuszmann and Vargha[14]. Treatment of the triacetate (**14**) with hydrogen bromide in dichloromethane at 0° afforded 3,5-di-*O*-acetyl-2-chloro-2-deoxy-α-D-arabinosyl bromide (**15**), as a light-yellow syrup. Evidence for the α configuration of **15** was obtained from its n.m.r. spectrum (in chloroform-*d*) in which the H-1 signal appeared at δ 6.5 as a singlet. The lack of coupling between H-1 and H-2 is characteristic of a 1,2-*trans* relationship in furanose derivatives[15] and hence **15** is the α anomer.

Reaction of **15** in various solvents with an excess of trimethylsilylated N^4-acetylcytosine gave a mixture of N^4-acetyl-1-(3,5-di-*O*-acetyl-2-chloro-2-deoxy-α-D-arabinofuranosyl)cytosine (**19**) and N^4-acetyl-1-(3,5-di-*O*-acetyl-2-chloro-2-deoxy-β-D-arabinofuranosyl)cytosine (**20**). This anomeric mixture of nucleosides was readily separated from minor by-products by column chromatography on silica gel (70–230 mesh) in chloroform–methanol (20:1, v/v) as a solvent. Final separation of the anomeric mixture (**19** and **20**) was achieved by column chromatography on fine silica gel (<230 mesh) with petroleum ether (30–60°)–chloroform–ethyl acetate (1:1:4, v/v) as the eluent. Both isomers crystallized from a mixture of chloroform and diethyl ether.

The ratio of α to β anomer obtained varied considerably depending on the solvent used in the condensation reaction. In the more polar solvent, such as acetonitrile, the ratio of α to β anomer was ~1:1. In 1,2-dichloroethane, the ratio was ~1:3. The best results were obtained with dichloromethane (~1:6).

These ratios were determined by n.m.r. spectroscopy of the mixtures prior to the final chromatographic separation. The H-1′ signal for the β anomer (**20**) in $CDCl_3$ appears as a doublet at δ 6.23 ($J_{1',2'}$ 3.5 Hz) at slightly lower field than that for the α anomer which appears at δ 6.00 ($J_{1',2'}$ ~1.0 Hz). The predominant formation of β anomer (**15**→**20**) as the polarity of the solvent is decreased may be explained* in part by a corresponding increase of the SN2 character of the reaction leading to an inversion of the anomeric configuration at C-1.

The free nucleosides, 1-(2-chloro-2-deoxy-α-D-arabinofuranosyl)cytosine (**18**) and 1-(2-chloro-2-deoxy-β-D-arabinofuranosyl)cytosine (**16**) were obtained as their crystalline hydrochloride salts by de-blocking **19** or **20** in methanol saturated with HCl. Basic conditions for de-blocking were avoided in order to obviate the possible formation of a 2′,3′-anhydronucleoside (and subsequently a 2,2′-anhydronucleoside) under these conditions. The close similarity of the u.v. spectra of nucleosides **16** and **18** to that of cytidine showed that glycosylation had occurred only at N-1 of the pyrimidine.

Preliminary studies** on the activity of these nucleosides against mouse leukemia cells *in vitro* showed that the ID_{50} of 2′-chloro-Ara-C (**16**) after 96 hours incubation with L5178Y and P815 leukemic cells is of the same order of magnitude as exhibited by 1-β-D-arabinofuranosylcytosine [Ara-C] and by 2′-fluoro-Ara-C. The α anomer (**18**) is essentially inactive against these cell-culture systems.

EXPERIMENTAL

General. — Melting points were determined with a Thomas–Hoover capillary apparatus. N.m.r. spectra were obtained on a Varian A-60 spectrometer with tetramethylsilane as reference. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. T.l.c. was performed on microscope slides covered with a layer of Merck Silica gel GF_{254}. The substances were detected either by u.v.-absorption or by spraying and charring with 20% ethanolic sulfuric acid. Unless specified otherwise, column chromatography was performed on "Merck Silica Gel type 60" (70–230 mesh).

3,5-Di-O-benzyl-1,2-diphenylmethylidene-α-D-ribofuranose[7] (**2**). — 1,2-Diphenylmethylidene-α-D-ribofuranose[6] (**1**, 9.5 g) was heated and stirred for 2 h at 80° in benzyl chloride (75 ml) containing powdered potassium hydroxide (15 g) in suspension. After the suspension was cooled, ether (300 ml) was added, and the mixture was washed with water (3 × 100 ml). The organic layer was separated, dried (sodium

*For a discussion of the possible role of solvents in the stereochemical control of nucleoside products see p. 21 in Ref. 16.

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sulfate), filtered, and evaporated *in vacuo*. The remaining, slightly-yellow oil was dissolved in cyclohexane (100 ml) and crystallization occurred almost immediately. After being kept at 0° for several hours, the colorless crystals were filtered off to give **2** (13.8 g, 92%). Recrystallization from cyclohexane (100 ml) gave 13.2 g of analytically pure material, m.p. 105–106°; lit.[7]: m.p. 105–106°.

Anal. Calc. for $C_{32}H_{30}O_5$: C, 77.11; H, 6.11. Found: C, 77.69; H, 5.94.

1,2-O-Isopropylidene-α-D-ribofuranose (**5**). — 1,2-*O*-Isopropylidene-5-*O*-methoxycarbonyl-α-D-xylofuranose[8] (**10**) (41.3 g) was dissolved in dichloromethane (400 ml) and ruthenium dioxide (1.5 g) was added. The suspension was cooled in an ice-bath and stirred vigorously. An aqueous solution of sodium metaperiodate (53.5 g in 300 ml of water) was added. After 45 min, potassium carbonate (1.0 g) was added, and the solution was allowed to warm to room temperature. After ~16 h, additional sodium metaperiodate (3.0 g) and potassium carbonate (1.0 g) were added. When the oxidation was completed [as judged by t.l.c. in 1:1 (v/v) ethyl acetate–chloroform, R_F of **10** 0.50, R_F of product 0.35], 2-propanol (25 ml) was added and stirring continued for 0.5 h. The reaction mixture was filtered through Celite and the organic layer was separated. The aqueous layer was extracted with dichloromethane (3 × 200 ml), and the organic extracts were combined, dried (sodium sulfate), and evaporated to a semi-crystalline solid (35.5 g). This was dissolved in aqueous ethanol (175 ml of ethanol diluted with 75 ml of water) and cooled in an ice-bath. Sodium borohydride (3.4 g) was added and the stirred solution allowed to come to room temperature. After 16 h, additional sodium borohydride (1.0 g) was added and the solution was warmed on a steam-bath until reduction was complete. The solution was cooled and residual sodium borohydride decomposed with AG 50 (H^+) ion-exchange resin. IR-45 (OH^-) ion-exchange resin (25 ml) was then added and stirring continued for 0.5 h. After filtration and evaporation, the residue was dried by azeotropic distillation with methanol (200 ml) and then shaken with dichloromethane (500 ml). The suspension was filtered through Celite, and the filtrate was dried (sodium sulfate) and evaporated to give a colorless syrup (26.3 g). Crystallization from benzene–diethyl ether provided **5** (16.2 g), m.p. 86–87.5°; lit.[17]: m.p. 86–87°; n.m.r. and t.l.c. behavior was identical to that of the authentic material. Concentration of the mother liquor provided additional material (3.7 g, m.p. 85–86.5°, total yield 63%). The residual syrup was shown by t.l.c. to contain **5** plus the reduced form of **6** and a compound remaining at the origin (presumably D-ribose).

3,5-Di-O-benzyl-1,2-O-isopropylidene-α-D-ribofuranose (**4**). — A solution of **5** (12 g) in benzyl chloride (100 ml) containing powdered potassium hydroxide (20 g) in suspension was heated at 80° for 2 h with rapid stirring. The reaction mixture was allowed to reach room temperature, ether (300 ml) was added, and the mixture was washed with water (3 × 100 ml). The organic layer was separated, dried (sodium sulfate), filtered, and evaporated in high vacuum. The remaining syrup (23 g) was chromatographically homogeneous and was used without further purification for the next step. A small amount was distilled to give analytically pure **4**, (b.p.$_{0.025}$ 178–182°) as a colorless syrup; n.m.r. data (chloroform-*d*): δ 7.36 (10 H, 2 s, benzyl H), 5.81

(1 H, d, $J_{1,2}$ 4 Hz, H-1), 3.5–4.8 (9 H, m, H-2,3,4,5- and -CH_2-benzyl), and 1.62 and 1.39 (6 H, 2 s, CH_3 of Ip).

Anal. Calc. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.08. Found: C, 71.25; H, 7.12.

Methyl 3,5-di-O-benzyl-D-ribofuranoside (**3**). — *From* **2**. Compound **2** (41 g) was dissolved in dry methanol (750 ml) containing conc. sulfuric acid (1 ml) and the solution was heated to reflux for 2 h. The solution was cooled and sodium hydrogencarbonate (7 g) was added with rapid stirring to neutralize the acid. The mixture was evaporated, redissolved in chloroform (200 ml), and the inorganic material was removed by filtration. The clear solution was evaporated to a syrup and **3** was separated from benzophenone by chromatography on silica gel (500 g) using benzene–ethyl acetate (9:1, v/v) as eluent. Chromatographically homogeneous **3** was thus obtained as a clear syrup (25.5 g, 93%), which was used directly for the next step.

From **4**. Compound **4** (9 g) was dissolved in dry methanol (100 ml) containing conc. sulfuric acid (1 drop), the solution was heated to reflux for 3 h and then cooled. Sodium hydrogencarbonate was added with stirring to neutralize the acid, the mixture was evaporated to dryness, redissolved in ether (100 ml), and the inorganic material was removed by filtration. After evaporation of the ether, chromatographically homogeneous **3** (8.3 g) was obtained and used directly for the next step. A small amount was distilled to give analytically pure **3**, b.p.$_{0.025}$ 185–190° as a clear syrup; n.m.r. data (chloroform-*d*): δ 7.30 (10 H, s, benzyl H), 4.87 (1 H, s, H-1), 3.4–4.7 (9 H, m, H-2,3,4,5 and -CH_2 benzyl), 3.28 (3 H, s, –OCH_3), and 3.0 (1 H, broad s, –OH).

Anal. Calc. for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.84; H, 7.15.

Methyl 3,5-di-O-benzyl-2-chloro-2-deoxy-α-D-arabinofuranoside (**8**). — Compound **3** (5 g) was dissolved in acetonitrile (25 ml) and tetrachloromethane (5 ml), both previously dried over molecular sieves. Triphenylphosphine (10 g) was then added and, after the initial exothermic reaction had stopped, 7 ml of solvent was distilled off. The mixture was then heated to reflux for 2 days, during which time the solution slowly turned black. After the solution had been cooled, methanol (10 ml), and after another 15 min water–acetone (100 ml, 3:2, v/v) was added. The solution was then extracted with petroleum ether (b.p. 30–60°) (3 × 50 ml) and the combined extracts were dried (sodium sulfate). After filtration, the clear solution was evaporated to a syrup which contained mainly **8**. If, during evaporation, triphenylphosphine oxide crystallized, the syrup was redissolved in 1:1 petroleum ether–diethyl ether and the crystals were removed by filtration. Chromatographically homogeneous **8** (2 g, 38%) was obtained by column chromatography on silica gel (100 g) with benzene–ethyl acetate (20:1, v/v) as eluent. The clear syrup was used in the next step without further purification; n.m.r. data (chloroform-*d*): δ 7.30 (10 H, s, benzyl H), 5.01 (1 H, d, $J_{1,2}$ <1 Hz, H-1), 4.0–4.8 (7 H, m, H-2,3,4 and -CH_2-benzyl), 3.60 (2 H, d, $J_{4,5}$ 4 Hz, H-5), and 3.32 (3 H, s, –OCH_3).

Anal. Calc. for $C_{20}H_{23}ClO_4$: C, 66.20; H, 6.39; Cl, 9.77. Found: C, 66.30; H, 6.38; Cl, 9.84.

Methyl 2-chloro-2-deoxy-α-D-arabinofuranoside (**12**). — Compound **8** (10.8 g)

was dissolved in ethanol (150 ml) and 10% palladium-on-charcoal (200 mg) was added to the solution. Hydrogenation was conducted in a Parr hydrogenator at an initial pressure of 2.3 atm. The hydrogen uptake was completed after 5 h. The catalyst was then removed by filtration through Celite, and thoroughly washed with ethanol. After complete evaporation of the filtrate, chromatographically homogeneous **12** (5.4 g, 99%) was obtained as a clear syrup and used directly for the next step; n.m.r. data (chloroform-*d*): δ 4.95 (1 H, broad s, H-1), 3.6–4.3 (5 H, m, H-2,3,4,5), 3.5 (2 H, very broad s, 2 OH), and 3.38 (3 H, s, $-OCH_3$).

Anal. Calc. for $C_6H_{11}ClO_4$: C, 39.46; H, 6.07; Cl, 19.42. Found: C, 39.53; H, 6.07; Cl, 19.35.

*Methyl 3,5-di-*O*-acetyl-2-chloro-2-deoxy-α-*D*-arabinofuranoside*[14] (**13**). — Compound **12** (5.4 g) was stirred overnight at room temperature in acetic anhydride (100 ml) with anhydrous sodium acetate (500 mg). The mixture was evaporated *in vacuo* and the residue was dissolved in ether (100 ml) and extracted with water (2 × 50 ml). The organic layer was then separated, dried (sodium sulfate), and evaporated to give chromatographically homogeneous **13** (7.58 g, 96%) as a clear syrup, which was used without further purification for the next step; n.m.r. data (chloroform-*d*): δ 5.16 (1 H, m, H-3), 5.09 (1 H, s, H-1), 4.0–4.5 (4 H, m, H-2,4,5), 3.42 (3 H, s, $-OCH_3$), and 2.11 (6 H, s, 2 $-COCH_3$).

*1,3,5-Tri-*O*-acetyl-2-chloro-2-deoxy-α-*D*-arabinofuranose*[14] (**14**). — Compound **13** (7.58 g) was dissolved at room temperature in acetic anhydride (45 ml) and conc. sulfuric acid (10 drops) was added. The reaction was followed by t.l.c. with 5:1 (v/v) petroleum ether–acetone as solvent. After 30 min, **13** had completely disappeared. The reaction mixture was poured into ice–water (200 ml), and the aqueous suspension was extracted with chloroform (2 × 100 ml). The combined organic extracts were washed free of acid with a saturated sodium hydrogencarbonate solution and dried (sodium sulfate). After evaporation, minor colored impurities were removed by column chromatography on silica gel (150 g) with 9:1 (v/v) benzene–ethyl acetate as eluent. After evaporation of the appropriate fractions, chromatographically homogeneous **14** (8.14 g, 97%) was obtained as a clear syrup and used as such for the next step; n.m.r. data (chloroform-*d*): δ 6.29 (1 H, s, H-1), 5.17 (1 H, m, H-3), 4.1–4.5 (4 H, m, H-2,4,5), and 2.1 (9 H, 2 s, 3 $COCH_3$).

*3,5-Di-*O*-acetyl-2-chloro-2-deoxy-α-*D*-arabinofuranosyl bromide* (**15**). — Compound **14** (600 mg) was dissolved in dry dichloromethane (20 ml) and the solution was saturated with dry hydrogen bromide gas under cooling at 0°. A slow stream of the gas was maintained until no trace of starting material remained, as could be determined by t.l.c. with 5:1 (v/v) petroleum ether–acetone as solvent. The solution was then evaporated *in vacuo* (bath temperature <35°). The residual syrup was dissolved in toluene twice (2 × 100 ml) and evaporated each time *in vacuo* to remove acetic acid. Chromatographically homogeneous **15** was thus obtained as a slightly colored syrup which was used without delay for the condensation step; n.m.r. data (chloroform-*d*): δ 6.5 (1 H, s, H-1), 5.17 (1 H, d, $J_{3,4}$ 3.5 Hz, H-3), 4.72 (1 H, s, H-2), 4.15–4.70 (3 H, m, H-4 and 2 H-5), 2.14, and 2.07 (6 H, 2 s, 2 $COCH_3$).

N^4-Acetyl-1-(3,5-di-O-acetyl-2-chloro-2-deoxy-α- and -β-D-arabinofuranosyl)-cytosine (**19**, **20**). — Compound **15**, freshly prepared from **14** (600 mg), was dissolved in acetonitrile (10 ml, previously dried over molecular sieves), and the solution was added to trimethylsilylated N^4-acetylcytosine[18] (prepared from 450 mg of N^4-acetylcytosine in hexamethyldisilazane at reflux). The clear mixture was then stirred under rigorously dry conditions at room temperature, and the course of the reaction was followed to completion by t.l.c. with 20:1 (v/v) chloroform–methanol. After 50 h, methanol (1 ml) was added and the resulting suspension was stirred for 15 min. The solid was removed by filtration through Celite, and the filter cake was washed thoroughly with chloroform. The clear filtrate, which contained mainly the anomeric nucleosides **19** and **20** was then evaporated *in vacuo* to a foam. A mixture containing only **19** and **20** was obtained by column chromatography on silica gel (125 g) with 14:1 (v/v) chloroform–methanol as the eluent. The two anomers were separated by column chromatography on "Merck Silica Gel 60" (<230 mesh, 50 g) with 1:1:4 (v/v) petroleum ether–chloroform–ethyl acetate as eluent. Slight pressure had to be employed to maintain a reasonable elution speed. The first nucleoside to be eluted was **19**. After evaporation of all fractions containing this anomer, the solid residue was dissolved in chloroform (2 ml) and ether (10 ml) was added; **19** crystallized almost immediately to yield 160 mg (20%) of fine, colorless needles, m.p. 95–99° (dec.), n.m.r. data (chloroform-*d*): δ 7.70 (1 H, d, $J_{5,6}$ 7.5 Hz, H-6), 7.40 (1 H, q, $J_{5,6}$ 7.5 Hz, $J_{NH,H-5}$ 1.5 Hz, H-5), 6.00 (1 H, d, $J_{1',2'}$ ~1 Hz, H-1'), 5.20 (1 H, m, H-3'), 4.2–4.8 (4 H, m, H-2',4',5'), 2.27 (3 H, s, *N*-acetyl), 1.90, and 2.10 (6 H, 2 s, 2 $OCOCH_3$). (Although satisfactory carbon analysis could not be obtained for this compound, it was suitable for subsequent reactions).

Anal. Calc. for $C_{15}H_{18}ClN_3O_7$: C, 46.46; H, 4.68; Cl, 9.14; N, 10.84. Found: C, 45.39; H, 4.79; Cl, 8.95; N, 10.58.

Compound **20**, which was eluted next, was first obtained as an amorphous solid after evaporation of the proper fractions. This substance crystallized slowly after addition of a small amount of ether to afford **20** (175 mg, 22%) as colorless crystals, m.p 175–177°; n.m.r. data (chloroform-*d*): δ 8.0 (1 H, d, $J_{5,6}$ 8 Hz, H-6), 7.53 (1 H, d, $J_{5,6}$ 4 Hz, H-5), 6.23 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-1'), 5.30 (1 H, d, $J_{3',4'}$ 2 Hz, H-3'), 4.87 (1 H, d, $J_{1',2'}$ 3.5 Hz, H-2'), 4.2–4.6 (3 H, m, H-4',5'), 2.30 (3 H, s, *N*-acetyl), 2.14, and 2.12 (6 H, 2 s, 2 $OCOCH_3$).

Anal. Calc. for $C_{15}H_{18}ClN_3O_7$: C, 46.46; H, 4.68; Cl, 9.14; N, 10.84. Found: C, 46.25; H, 4.82; Cl, 9.06; N, 10.66.

A better overall yield (65%) of **19** and **20** was obtained in dichloromethane as solvent by the reaction of the glycosyl halide **15** (5.90 g) with trimethylsilylated N^4-acetylcytosine (from 6.12 g of the unblocked base). In this case, the ratio of β to α anomer was ~6:1, as could be judged by n.m.r. spectroscopy. This mixture was directly unblocked (see subsequent paragraphs) to give the pure β anomer **16** (2.2 g, 37%) as the hydrochloride, after a few recrystallizations.

1-(2-Chloro-2-deoxy-α-D-arabinofuranosyl)cytosine hydrochloride (**18**). — Compound **19** (100 mg) was dissolved in methanol (4 ml, previously dried over molecular

sieves), and the solution cooled to 0° was saturated with hydrogen chloride gas. The reaction mixture was stored at room temperature, and the course of the reaction was followed on t.l.c. with 4:1 (v/v) chloroform–methanol as solvent. After complete deblocking (about 30 min), the solution was evaporated until crystals appeared. Ethanol (1 ml) was then added. The mixture was chilled and **18** was filtered off. The mother liquor was evaporated to dryness, and the remaining solid redissolved in methanol (1 ml). Ethanol (1 ml) was then added and, after cooling, a second crop of crystals was filtered off to give **18** as the hydrochloride salt, 51 mg (66%), m.p. 220–223° (dec.),$[\alpha]_D^{24}$ +6° (*c* 0.8, water); n.m.r. data (deuterium oxide): δ 7.96 (1 H, d, $J_{5,6}$ 8 Hz, H-6), 6.23 (1 H, d, $J_{5,6}$ 8 Hz, H-5), 6.06 (1 H, d, $J_{1',2'}$ 4.5 Hz, H-1'), 4.59 (1 H, m, H-2'), 4.41 (2 H, m, H-3',4'), and 3.84 (2 H, m, H-5').

Anal. Calc. for $C_9H_{12}ClN_3O_4 \cdot HCl$: C, 36.26; H, 4.40; Cl, 23.79; N, 14.10. Found: C, 36.32; H, 4.45; Cl, 23.84; N, 13.95.

*1-(2-Chloro-2-deoxy-β-*D*-arabinofuranosyl)cytosine hydrochloride* (**16**). — Compound **20** (100 mg) was treated with methanolic hydrogen chloride as described for **18**. The reaction mixture was evaporated to a crystalline solid which was resuspended in boiling methanol (1 ml). After the suspension had been cooled, the colorless crystals were filtered off to give **16** as the hydrochloride salt (55 mg, 71%), m.p. 239–241° (dec.), $[\alpha]_D^{24}$ +125° (*c* 1.2, water); n.m.r. data (deuterium oxide): δ 8.11 (1 H, d, $J_{5,6}$ 8 Hz, H-6), 6.33 (1 H, d, $J_{1',2'}$ 5.5 Hz, H-1'), 6.29 (1 H, d, $J_{5,6}$ 8 Hz, H-5), 4.71 (1 H, t, $J_{1',2'} = J_{2',3'} = 5.5$ Hz, H-2'), 4.39 (1 H, t, $J_{2',3'} = J_{3',4'} = 5.5$ Hz, H-3'), 4.06 (1 H, m, H-4'), and 3.91 (2 H, m, H-5').

Anal. Calc. for $C_9H_{12}ClN_3O_4 \cdot HCl$: C, 36.26; H, 4.40; Cl, 23.79; N, 14.10. Found: C, 35.92; H, 4.56; Cl, 23.54; N, 13.82.

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