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SYNTHESIS OF 9-(3-DEOXY-3-*C*-METHYL-β-D-XYLOFURANOSYL)-ADENINE: A BRANCHED-CHAIN SUGAR ANALOG OF CORDYCEPIN

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ABSTRACT

Reaction of methyl 2,3-anhydro-5-*O*-trityl-β-D-ribofuranoside (**4**) with methylmagnesium chloride gave a mixture of methyl 3-chloro-3-deoxy-5-*O*-trityl-β-D-xylofuranoside (**5**) and methyl 3-deoxy-3-*C*-methyl-5-*O*-trityl-β-D-xylofuranoside (**6**). Removal of the trityl group from **6** gave methyl 3-deoxy-3-*C*-methyl-β-D-xylofuranoside (**9**), which, on treatment with *p*-nitrobenzoyl chloride, gave methyl 3-deoxy-3-*C*-methyl-2,5-di-*O*-(*p*-nitrobenzoyl)-β-D-xylofuranoside (**10**). The glycosyl chloride (**15**) derived from **10** reacted with 6-(benzamido)chloromercuripurine to give 6-(benzamido)-9-[3-deoxy-3-*C*-methyl-2,5-di-*O*-(*p*-nitrobenzoyl)-D-xylofuranosyl]purine (**16**). Alkaline methanolysis of **16** produced 9-(3-deoxy-3-*C*-methyl-α-D-xylofuranosyl)-adenine (α-**1**) and 9-(3-deoxy-3-*C*-methyl-β-D-xylofuranosyl)adenine (β-**1**) in the ratio of 1:4. Treatment of **6** with benzoyl chloride gave methyl 2-*O*-benzoyl-3-deoxy-3-*C*-methyl-5-*O*-trityl-β-D-xylofuranoside (**17**). The trityl group was removed from **17** to give methyl 2-*O*-benzoyl-3-deoxy-3-*C*-methyl-β-D-xylofuranoside (**18**), which was converted into methyl 2,5-di-*O*-benzoyl-3-deoxy-3-*C*-methyl-β-D-xylofuranoside (**19**). When the glycosyl halide (**20**) obtained from **19** reacted with 6-(benzamido)chloromercuripurine, 6-(benzamido)-9-(2,5-di-*O*-benzoyl-3-deoxy-3-*C*-methyl-D-xylofuranosyl)-purine (**21**) was produced. Ammonolysis of **21** gave α-**1** and β-**1** in the ratio of 1:16.

INTRODUCTION

3′-Deoxyadenosine (cordycepin), a biologically active nucleoside, is rapidly converted *in vivo* into biologically inactive 3′-deoxyinosine by the action of adenosine deaminase[1]. On the other hand, 3′-*C*-methyladenosine is relatively inert to deamination by adenosine deaminase[2]. It was, therefore, of considerable interest to determine the stability of the branched-chain sugar analog of cordycepin, namely, 9-(3-deoxy-3-*C*-methyl-β-D-xylofuranosyl)adenine* (**1**), toward adenosine deaminase.

For the synthesis of **1**, a suitable derivative of the hitherto unknown 3-deoxy-3-*C*-methyl-D-xylofuranose was needed. A method that has been used for preparing

*The synthesis of the 3′-*C* epimer of **1**, namely, 9-(3-deoxy-3-*C*-methyl-β-D-ribofuranosyl)adenine, by a method quite different from that recorded here has been reported[3].

branched-chain, deoxy sugars involves the reaction of Grignard reagents with sugar epoxides[4]. This approach has been used mostly with pyranoses; for these, the position of nucleophilic attack on the epoxide (*trans*-diaxial ring-opening)[5] is more predictable than for furanoses. However, a convenient starting-point for the present work was found in the readily available α and β anomers (**2** and **3**) of methyl 2,3-anhydro-D-ribofuranoside[6], nucleophilic attack on which has been observed to occur mainly[7] at C-3; the trityl group was chosen to protect the 5-hydroxyl group. The reaction of methyl 2,3-anhydro-5-*O*-trityl-β-D-ribofuranoside[8] (**4**) with an excess of methylmagnesium chloride in ether, under reflux, was sluggish, and maximum utilization of the starting material required several days. Chromatography of the crude reaction-product on silica gel gave unchanged starting-material (**4**, 15%), methyl 3-chloro-3-deoxy-5-*O*-trityl-β-D-xylofuranoside (**5**, 16%), and methyl 3-deoxy-3-*C*-methyl-5-*O*-trityl-β-D-xylofuranoside (**6**, 50%). For characterization, the branched-chain sugar **6** was treated with *p*-nitrobenzoyl chloride in pyridine, to give methyl 3-deoxy-3-*C*-methyl-2-*O*-(*p*-nitrobenzoyl)-5-*O*-trityl-β-D-xylofuranoside (**7**). [The chloro sugar **5**, formed by the attack of Cl^- on the epoxide, was characterized as its 2-*O*-benzoyl derivative (**8**).] Removal of the trityl group from **6** afforded methyl 3-deoxy-3-*C*-methyl-β-D-xylofuranoside (**9**) which, on treatment with *p*-nitrobenzoyl chloride in pyridine gave methyl 3-deoxy-3-*C*-methyl-2,5-di-*O*-(*p*-nitrobenzoyl)-β-D-xylofuranoside (**10**).

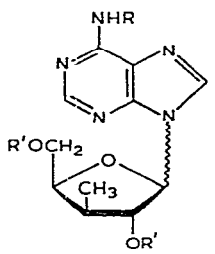
Proof that nucleophilic attack of methylmagnesium chloride on the anhydro sugar **4** had occurred at C-3, not at C-2, was obtained from n.m.r.-spectral measurements on **9** and its derivatives. Resonances assignable to the anomeric protons of **6**, **9**, and **10** showed $J_{1,2}$ of 2.0, 2.5, and 0 Hz; these values are low enough to indicate that the protons at C-1 and C-2 are *trans* to each other[9]. Attack of the Grignard reagent at C-2 would have led to a product having its C-1 and C-2 protons *cis*, an arrangement which would not be compatible with the low coupling-constants observed. Similarly, a singlet for H-1 (τ 4.93) in the n.m.r. spectrum of **8** is evidence that its C-1 and C-2 protons are *trans*, and indicates that the epoxide was opened by attack of Cl^- at C-3, and not at C-2.

In contrast to the reaction of the β-D anomer **4** with methylmagnesium chloride, the reaction of methyl 2,3-anhydro-5-*O*-trityl-α-D-ribofuranoside (**11**) was rapid. At 5°, utilization of the starting material **11** was complete, and no change in composition of the reaction mixture was observed, by t.l.c., after 1.5 h. Chromatography of the crude product on silica gel gave methyl 3-chloro-3-deoxy-5-*O*-trityl-α-D-xylofuranoside (**12**, 36%), methyl 3-deoxy-3-*C*-methyl-5-*O*-trityl-α-D-xylofuranoside (**13**, 48%), and a third component*.

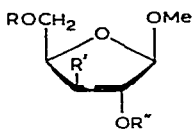
*Although this component was not completely characterized, its n.m.r. spectrum was consistent with its formulation as methyl 2-deoxy-2-*C*-methyl-5-*O*-trityl-α-D-ribofuranoside. For example, the multiplet at τ 7.7 and the doublet at τ 9.05 are assignable to the -CH(CH_3) moiety. The resonance for the anomeric proton, a doublet at τ 5.37 ($J_{1,2}$ 2.0 Hz) indicates a probable *trans* disposition of the protons at C-1 and C-2 which could obtain only if the nucleophilic attack by methylmagnesium chloride had occurred at C-2 of **11**.

For characterization, **12** was converted into the crystalline 2-*O*-*p*-nitrobenzoyl derivative (**14**). The structures proposed for **12**, **13**, and **14** were confirmed by their n.m.r. spectra.

The methyl β-glycoside **10** was converted into 3-deoxy-3-*C*-methyl-2,5-di-*O*-(*p*-nitrobenzoyl)-α-D-xylofuranosyl chloride (**15**). Reaction of **15** with 6-(benzamido)-chloromercuripurine[10] produced 6-benzamido-9-[3-deoxy-3-*C*-methyl-2,5-di-*O*-(*p*-nitrobenzoyl)-D-xylofuranosyl]purine (**16**), which was isolated by chromatography on silica gel and was chromatographically homogeneous by t.l.c. in several solvent systems. However, when the protecting groups in **16** were removed in methanolic sodium methoxide, two nucleosides were obtained in the ratio of ~2 to 3 (according to n.m.r. spectroscopic results). The products, which were obtained pure by chromatography on silica gel, were shown by a variety of physical measurements to be the α and β anomers, respectively, of 9-(3-deoxy-3-*C*-methyl-D-xylofuranosyl)adenine (α-**1** and β-**1**). In order to obtain a more objective measure of the ratio of anomers produced, a sample of the crude mixture of products was saponified, and the resultant products were separated on t.l.c. plates of silica gel. The u.v. absorption of eluates of appropriate zones showed the ratio of products to be α-**1**:β-**1** = 1:4. The larger proportion of α anomer obtained was caused by inadvertent losses of β anomer due to band overlap with impurities during the chromatographic purification of **16**. The production of such a large proportion of the α anomer was unexpected. Previously, the reactions of 6-(benzamido)chloromercuripurine with glycosyl halides having a group on C-2

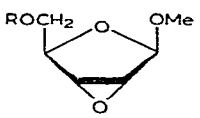


1 R = R' = H (α and β)
16 R' = pNBz, R = Bz (α and β)
21 R = R' = Bz (α and β)

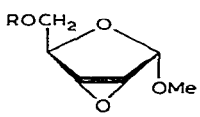


5 R = Tr, R' = Cl, R" = H
6 R = Tr, R' = Me, R" = H
7 R = Tr, R' = Me, R" = pNBz
8 R = Tr, R' = Cl, R" = Bz
9 R = R" = H, R' = Me

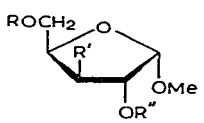
10 R = R" = pNBz, R' = Me
17 R = Tr, R' = Me, R" = Bz
18 R = H, R' = Me, R" = Bz
19 R = R" = Bz, R' = Me



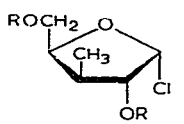
3 R = H
4 R = Tr



2 R = H
11 R = Tr



12 R = Tr, R' = Cl, R" = H
13 R = Tr, R' = Me, R" = H
14 R = Tr, R' = Cl, R" = pNBz



15 R = pNBz
20 R = Bz

Tr = trityl
Bz = benzoyl
pNBz = *p*-nitrobenzoyl

capable of participation *via* a cyclic carbonium ion have only given an appreciable proportion of the *cis* product when the reaction was catalyzed with titanium tetrachloride[11]. The production of the large proportion of α-**1** can be ascribed to the diminished neighboring-group participation provided by the 2-*O*-*p*-nitrobenzoyl group compared to that provided by the 2-*O*-benzoyl protecting-group usually employed in the synthesis of purine nucleosides by the mercury method*. To test this, compound **6** was benzoylated, to give methyl 2-*O*-benzoyl-3-deoxy-3-*C*-methyl-5-*O*-trityl-β-D-xylofuranoside (**17**). Removal of the 5-*O*-trityl group by treatment with acid, followed by benzoylation, gave methyl 2,5-di-*O*-benzoyl-3-deoxy-3-*C*-methyl-β-D-xylofuranoside (**19**). Transformation of **19** into 2,5-di-*O*-benzoyl-3-deoxy-3-*C*-methyl-D-xylofuranosyl chloride (**20**) was accomplished in the usual way, and reaction of **20** with 6-(benzamido)chloromercuripurine led to 6-benzamido-9-(2,5-di-*O*-benzoyl-3-deoxy-3-*C*-methyl-D-xylofuranosyl)purine (**21**). Ammonolysis of **21** with methanolic ammonia gave a mixture of α-**1** and β-**1**; however, in this case, the mixture was composed of 94 percent of the β anomer and only 6 percent of the α anomer. This result clearly shows that the *p*-nitrobenzoyl group is an important factor in the formation of the higher proportion of α anomer in the first condensation.

In experiments with adenosine deaminase, it was determined that β-**1** is inert to the action of the enzyme under conditions that cause complete conversion of adenosine and 3′-deoxyadenosine into the corresponding hypoxanthine derivatives. It was also found that β-**1** is not an inhibitor of adenosine deaminase.

EXPERIMENTAL

General. — Melting points were determined on a micro hot-stage, and are corrected. Thin-layer chromatography (t.l.c.) was performed on Silica Gel G (Analtech) plates, and column chromatography on silica gel (100–200 mesh; J. T. Baker). The zones on t.l.c. plates were made visible by spraying with a solution of 100 mg of 1,3-naphthalenediol and 1 ml of phosphoric acid in 100 ml of ethanol, and then heating the plates on a steam cone. Another system used for the detection of nucleosides consisted in spraying the plates with a 1% solution of *tert*-butyl hypochlorite in cyclohexane, removing the excess of the reagent by heating on a steam cone for 1.5 min, and observing the zones after spraying with aqueous 2% starch–2% potassium iodide. Petroleum ether refers to the fraction boiling at 30–60°. N.m.r. spectra were recorded with a Varian A-60 or HA-100 spectrometer, with chloroform-*d* as the solvent unless otherwise noted.

*Methyl 2,3-anhydro-5-*O*-trityl-α-*D*-ribofuranoside* (**11**). — To a solution of **2** (10 g; 69 mmoles) in dry pyridine (100 ml) was added chlorotriphenylmethane (19.1 g; 69 mmoles), and the mixture was stirred for 9 days at 25°. Ice (~15 g) was added, and, after 0.5 h, the mixture was evaporated, and the residue was extracted

*It was noted earlier that the α:β anomer ratio is a function of the nature of the 2-acyloxy group in the Hilbert–Johnson synthesis of pyrimidine nucleosides[12].

with ether (650 ml). The extract was washed successively with three 120-ml portions of cold 5% hydrochloric acid, three 120-ml portions of saturated sodium hydrogen carbonate, and water until neutral, and evaporated; the residual solid (26 g) was chromatographed on a column of silica gel (1.3 kg) with 99:1 benzene–ethyl acetate. Fractions containing only the product (R_F 0.37 on t.l.c. in 19:1 benzene–ethyl acetate) were combined and evaporated, giving a total of 19.5 g (73%) of **11**; m.p. 131–133.5°, $[\alpha]_D$ +11° (*c* 1, chloroform).

Anal. Calc. for $C_{25}H_{24}O_4$: C, 77.30; H, 6.23. Found: C, 77.03; H, 6.20.

*Methyl 2,3-anhydro-5-*O*-trityl-β-*D*-ribofuranoside* (**4**). — The tritylation of 10.29 g (70 mmoles) of compound **3** was performed as described for the tritylation of **2**. Recrystallization of the product (13.4 g) obtained after chromatography from benzene–petroleum ether gave 12.1 g (45%) of **4**; it had m.p. 131–133°, $[\alpha]_D$ −62.2° (*c* 1, chloroform) [lit.[8] m.p. 127°, $[\alpha]_D$ −59° (chloroform)], R_F 0.59 on t.l.c. in 19:1 benzene–ethyl acetate.

Anal. Calc. for $C_{25}H_{24}O_4$: C, 77.30; H, 6.23. Found: C, 77.70; H, 6.13.

*Reaction of methyl 2,3-anhydro-5-*O*-trityl-β-*D*-ribofuranoside* (**4**) *with methylmagnesium chloride.* — A stirred solution of **4** (16 g, 41 mmoles) in benzene (50 ml) and ether (500 ml) was treated with M methylmagnesium chloride (100 mmoles) in ether. The mixture was boiled under reflux for 15 h, an additional 50 mmoles of methylmagnesium chloride was added, and heating was continued for a further 20 h. T.l.c. in 4:1 benzene–ethyl acetate then showed zones at R_F 0.8 (blue-green; **4**), 0.45 (maroon; methyl 3-chloro-3-deoxy-5-*O*-trityl-β-D-xylofuranoside, **5**), and 0.3 (pink-tan, methyl 3-deoxy-3-*C*-methyl-5-*O*-trityl-β-D-xylofuranoside, **6**). An additional 50 mmoles of methylmagnesium chloride was added, and refluxing was continued for a total of 140 h. The reaction mixture was poured into a stirred mixture of ammonium chloride (160 g), ice–water (900 ml), and ether (200 ml), and the aqueous layer was extracted with four 400-ml portions of ether. The extracts were combined, washed successively with 10% ammonium chloride (400 ml), saturated sodium hydrogen carbonate (300 ml), and two 300-ml portions of water, and evaporated to a syrup (17 g) which was chromatographed on a column of silica gel (1.3 kg) with 19:1 benzene–ethyl acetate. The chromatography yielded 2.5 g of unreacted anhydro derivative (**4**), 2.8 g (19%) of **5** as a glass, and 8.0 g (57%) of **6** as a glass having $[\alpha]_D$ −20° (*c* 1, chloroform); n.m.r. data: τ 5.19 (doublet, H-1, $J_{1,2}$ 2.0 Hz).

Anal. Calc. for $C_{26}H_{28}O_4$: C, 77.20; H, 6.98. Found: C, 76.76; H, 7.20.

The 3-deoxy-3-chloro sugar (**5**, 1.1 g, 2.7 mmoles) was benzoylated with benzoyl chloride in pyridine in the usual way, to give 1.4 g of syrupy product. Crystallization from 1:4:1 benzene–ether–petroleum ether gave pure methyl 2-*O*-benzoyl-3-chloro-3-deoxy-5-*O*-trityl-β-D-xylofuranoside (**8**): m.p. 163–165°; $[\alpha]_D$ −10° (*c* 1, chloroform); R_F 0.51 (yellow-gray) on t.l.c. in benzene; n.m.r. data: τ 4.92 (singlet, H-1) and 4.56 (singlet, H-2).

Anal. Calc. for $C_{32}H_{29}ClO_5$: C, 72.65; H, 5.52; Cl, 6.70. Found: C, 72.68; H, 5.45; Cl, 6.67.

*Methyl 3-deoxy-3-*C*-methyl-2-*O*-(*p*-nitrobenzoyl)-5-*O*-trityl-β-*D*-xylofuranoside*

(7). — A solution of 700 mg (1.7 mmoles) of **6** in 15 ml of pyridine was treated with 371 mg (2.0 mmoles) of *p*-nitrobenzoyl chloride, and the mixture was stirred at 25°. T.l.c. (9:1 benzene–ethyl acetate) showed that the reaction was complete within 0.5 h. The mixture was then treated with ice-water (0.5 ml) and stirred for 1 h; the pyridine and water were removed under diminished pressure, and the residue was extracted with ether (50 ml); the extract was washed successively with two 15-ml portions of 4% hydrochloric acid, two 30-ml portions of saturated sodium hydrogen carbonate, and six 30-ml portions of water, and evaporated, and the residue (800 mg) was chromatographed on silica gel (20 g) with 99:1 benzene–ethyl acetate. Fractions containing the desired product were combined and evaporated. The yield was 800 mg (85%) of **7** as a glass having $[\alpha]_D$ −15.4° (*c* 1, chloroform); R_F 0.62 on t.l.c. in 19:1 benzene–ethyl acetate; n.m.r. data: τ 4.82 (doublet, H-2, $J_{2,3}$ 2.2 Hz) and 4.92 (singlet, H-1).

Anal. Calc. for $C_{33}H_{31}NO_7$: C, 71.59; H, 5.64; N, 2.53. Found: C, 71.86; H, 5.89; N, 2.55.

*Methyl 3-deoxy-3-*C*-methyl-β-*D*-ribofuranoside* (**9**). — A solution of 4.0 g (9.9 mmoles) of **6** in methanol (150 ml), acetic acid (30 ml), and water (20 ml) was boiled under reflux for 5.5 h, cooled, and concentrated under diminished pressure; several 100-ml portions of methanol were then added to and distilled from the residue to remove the excess of acetic acid. The residue was chromatographed on a column of silica gel (200 g) with 1:1 benzene–ethyl acetate. After removal of triphenylmethanol, fractions containing the product (R_F 0.45; t.l.c. on silica gel with ethyl acetate) were obtained. These were combined and evaporated to give 1.2 g (75%) of syrupy methyl 3-deoxy-3-*C*-methyl-β-D-ribofuranoside. On cooling, the syrup yielded crystalline **9** having m.p. 49–50°; $[\alpha]_D$ −12° (*c* 1, chloroform); n.m.r. data: τ 5.17 (doublet, H-1, $J_{1,2}$ 2.5 Hz), 5.70 (multiplet, H-4), 6.04 (quartet, H-2, $J_{2,3}$ 7 Hz), 6.34 (doublet, H-5,5'), 6.53 (singlet, -OMe), 7.65 (multiplet, H-3), and 8.87 (doublet, 3-Me, $J_{3,3\text{-Me}}$ 7 Hz).

Anal. Calc. for $C_7H_{14}O_4$: C, 51.84; H, 8.70. Found: C, 51.87; H, 8.54.

*Methyl 3-deoxy-3-*C*-methyl-2,5-di-*O*-(p-nitrobenzoyl-β-*D*-ribofuranoside* (**10**). — A solution of 1.0 g (6.16 mmoles) of compound **9** in dry pyridine (58 ml) was treated with 3.4 g (18.5 mmoles) of *p*-nitrobenzoyl chloride, and stirred for 3 h at 25°. Water (5 ml) was added, and stirring was continued for 45 min at 25°. The pyridine and water were removed under diminished pressure, and the residue was extracted with chloroform (200 ml). The extract was washed successively with three 25-ml portions of cold 5% hydrochloric acid, saturated sodium hydrogen carbonate solution, and water, dried (magnesium sulfate), and evaporated to an oil (3 g). The oil was chromatographed on a column of silica gel (200 g) with 99:1 benzene–ethyl acetate, and early fractions yielded 2.0 g (89%) of the desired product as an oil. Crystallization of the oil from benzene (2 ml) and ether (15 ml) gave 1.6 g of **10** having m.p. 93.5–95°; $[\alpha]_D$ −3° (*c* 1, chloroform); R_F 0.4 (t.l.c. on silica gel with 19:1 benzene–ethyl acetate); n.m.r. data: τ 4.80 (doublet, H-2, $J_{2,3}$ 2.2 Hz), 5.02 (singlet, H-1), 5.58 (multiplet, H-4 and H-5), 6.78 (singlet, OMe), 7.70 (multiplet, H-3), and 8.93 (doublet, 3-Me, $J_{3,3\text{-Me}}$ 7.5 Hz).

Anal. Calc. for $C_{21}H_{20}N_2O_{10}$: C, 54.78; H, 4.38; N, 6.09. Found: C, 54.54; H, 4.43; N, 6.15.

*Reaction of methyl 2,3-anhydro-5-*O*-trityl-α-*D*-ribofuranoside* (**11**) *with methylmagnesium chloride.* — A solution of 1.0 g (2.6 mmoles) of **11** in 50 ml of ether was cooled and treated with 1.6M methylmagnesium chloride (10 ml). A white solid was precipitated immediately, and no change in the composition of the mixture was noted (t.l.c.) after 1.5 h. After 20 h at 25°, the mixture was poured into a mixture of ammonium chloride (20 g) in ice–water (50 ml) and ether (50 ml). The aqueous layer was separated, and extracted with ether (50 ml). The extracts were combined and evaporated, and, on t.l.c. with 9:1 benzene–ethyl acetate, the residue (1 g) showed zones at R_F 0.54 (blue-green, methyl 3-chloro-3-deoxy-5-*O*-trityl-α-D-xylofuranoside, **12**); 0.39 (yellow-pink, methyl 3-deoxy-3-*C*-methyl-5-*O*-trityl-α-D-xylofuranoside, **13**); and 0.26 (maroon). Chromatography on a column of silica gel (40 g) with 39:1 benzene–ethyl acetate gave 400 mg (36%) of **12** as an oil; n.m.r. data: τ 4.95 (doublet, H-1, $J_{1,2}$ 4.0 Hz) and 5.71 (triplet, H-2).

A sample (290 mg, 680 μmoles) of **12** in 15 ml of pyridine was treated with 290 mg (1.2 mmoles) of *p*-nitrobenzoyl chloride in the usual way. The crude product crystallized from benzene–petroleum ether, and gave 230 mg (59%) of methyl 3-chloro-3-deoxy-2-*O*-(*p*-nitrobenzoyl)-5-*O*-trityl-α-D-xylofuranoside (**14**) having m.p. 169–171°; $[\alpha]_D^{25}$ +90° (*c* 1, chloroform); n.m.r. data in benzene-d_6: τ 4.68 (doublet, H-1, $J_{1,2}$ 4.5 Hz) and 4.30 (triplet, H-2).

Anal. Calc. for $C_{32}H_{28}ClNO_7$: C, 66.95; H, 4.92; Cl, 6.18; N, 2.44. Found: C, 66.83; H, 4.92; Cl, 6.19; N, 2.39.

Further elution of the chromatographic column gave 500 mg (48%) of methyl 3-deoxy-3-*C*-methyl-5-*O*-trityl-α-D-ribofuranoside (**13**) which, after recrystallization from ether, afforded 330 mg of **13**; m.p. 118–120°, $[\alpha]_D$ +111° (*c* 1, chloroform); n.m.r. data: τ 5.03 (doublet, H-1, $J_{1,2}$ 4.5 Hz), 5.72 (multiplet, H-4), 5.98 (doublet, H-2, $J_{2,3}$ 7.0 Hz), 6.50 (singlet, -OMe), 6.90 (octet, H-5,5'), 7.78 (multiplet, H-3), and 9.10 (doublet, 3-Me, $J_{3,3\text{-Me}}$ 7.0 Hz).

Anal. Calc. for $C_{26}H_{28}O_4$: C, 77.20; H, 6.98. Found: C, 77.36; H, 7.08.

*3-Deoxy-3-*C*-methyl-2,5-di-*O*-(p-nitrobenzoyl)-α-D-xylofuranosyl chloride* (**15**). — To a solution of 1.8 g (3.9 mmoles) of **10** in 25 ml of acetic acid, cooled to 10°, were added acetyl chloride (5 ml) and 54 ml of acetic acid presaturated at 10° with hydrogen chloride. After 4 h at 25°, the solution was evaporated under diminished pressure, and four 25-ml portions of dry toluene were added to and distilled from the residue under diminished pressure, to give a partly crystalline solid; this was a mixture of 3-deoxy-3-*C*-methyl-2,5-di-*O*-(*p*-nitrobenzoyl)-α-D-xylofuranosyl chloride and 1-*O*-acetyl-3-deoxy-3-*C*-methyl-2,5-di-*O*-(*p*-nitrobenzoyl)-β-D-xylofuranose [n.m.r. data in benzene-d_6: τ 3.43 (singlet, H-1), 4.73 (doublet, H-2, $J_{2,3}$ 2.0 Hz), and 8.29 (singlet, acetyl Me)]. The mixture was treated with 100 ml of ether presaturated with hydrogen chloride at 0° and containing 5 ml of acetyl chloride, and was then kept in a stoppered flask for 3 h at 25°. The mixture was evaporated under diminished pressure, and four 25-ml portions of dry toluene were added to and distilled from the residual

solid under diminished pressure, to give 1.8 g of **15** having m.p. 153–161° (transition); $[\alpha]_D$ +153° (*c* 1, chloroform); n.m.r. data: τ 3.38 (doublet, H-1, $J_{1,2}$ 4.0 Hz) and 4.73 (quartet, H-2).

Anal. Calc. for $C_{20}H_{17}ClN_2O_9$: C, 51.68; H, 3.69; Cl, 7.63; N, 6.03. Found: C, 51.40; H, 3.99; Cl, 7.60; N, 5.73.

*6-(Benzamido)-9-[3-deoxy-3-*C*-methyl-2,5-di-*O*-(*p*-nitrobenzoyl)-α(and β)-*D*-xylofuranosyl]purine* (**16**). — Xylene (~100 ml) was distilled from a suspension of 6-(benzamido)chloromercuripurine (1.8 g; 3.9 mmoles) in xylene (350 ml). The mixture was cooled, a suspension of **15** (1.8 g; 3.9 mmoles) in dry xylene (50 ml) was added, and the stirred mixture was boiled under reflux until a clear solution was obtained. The solution was cooled, whereupon a solid separated; additional solid separated after the addition of 400 ml of petroleum ether. The mixture was cooled, and the solid was filtered off and dissolved in warm chloroform (500 ml). The solution was successively washed with four 50-ml portions of 30% potassium iodide solution and two 50-ml portions of water, and evaporated, to give 2.8 g of a partly solid residue. The product was chromatographed on a column of silica gel (70 g) with 4:1 chloroform–ethyl acetate, and, after removal of a small amount of more-mobile impurities, fractions were obtained that afforded 1.8 g (69%) of amorphous 6-(benzamido)-9-[3-deoxy-3-*C*-methyl-2,5-di-*O*-(*p*-nitrobenzoyl)-α(and β)-D-ribofuranosyl]purine; R_F 0.59 on t.l.c. in 3:1 ethyl acetate–benzene; $[\alpha]_D$ +33° (*c* 1, chloroform).

Anal. Calc. for $C_{32}H_{25}N_7O_{10}$: C, 57.57; H, 3.78; N, 14.68. Found: C, 57.30; H, 3.70; N, 14.66.

*9-(3-Deoxy-3-*C*-methyl-α-*D*-xylofuranosyl)adenine* (α-**1**) *and 9-(3-deoxy-3-*C*-methyl-β-*D*-xylofuranosyl)adenine* (β-**1**) *from* **16**. — A suspension of 1.5 g (2.2 mmoles) of **16** in methanol (75 ml) was treated with a solution of sodium methoxide (from 100 mg of sodium and 25 ml of methanol), and heated under reflux; complete dissolution occurred on boiling, and boiling was continued for 2 h. The solution was cooled and evaporated, the residue was dissolved in water (35 ml), the pH was adjusted to 7 with acetic acid, and the solution was successively washed with five 50-ml portions of ether and three 25-ml portions of chloroform, and freeze-dried, affording 850 mg of crude product.

A portion (500 mg) of the product was dissolved in 10 ml of water, and a solution of picric acid (400 mg) in boiling water (15 ml) was added. The mixture was cooled, and the resulting precipitate (560 mg) was collected. A portion (500 mg) of the picrate was decomposed portionwise with a total of 1.5 g of Dowex 2 X-8 (CO_3^{2-}) ion-exchange resin in water, the resin was filtered off, and the filtrate was freeze-dried. T.l.c. of the product in 6:4:1 chloroform–methanol–water showed zones at R_F 0.78 (β-**1**) and 0.67 (α-**1**). The product (350 mg) was chromatographed on a column of silica gel (100 g) with 9:1 ethyl acetate–methanol. Fractions containing only β-**1** were combined and evaporated, giving 130 mg (42%) of β-**1**. A sample (41 mg) was recrystallized from methanol by adding ether, to give 16 mg of pure β-**1**: m.p. 187–188°; $[\alpha]_D$ −34° (*c* 0.5, water); o.r.d. data: ϕ (nm) −300° (350), −2,500° tr (275), 0° (263), +1,100° pk (242), and 0° (234); n.m.r. data (deuterium oxide): τ 1.73 and

1.90 (singlets, H-4 and H-8), 4.16 (doublet, H-1′, $J_{1',2'}$ 6.9 Hz), 7.32 (multiplet, H-3′), and 8.76 (doublet, 3′-Me, $J_{3',3'-Me}$ 7.0 Hz; $\lambda_{max}^{H_2O}$ 260 nm (ε 14,200).

Anal. Calc. for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.70; N, 26.40. Found: C, 49.63; H, 5.48; N, 26.52.

After elution of several fractions containing a mixture of products, fractions containing only α-**1** were obtained. These were combined, and evaporated to give 81 mg (26%) of amorphous α-**1**; $[\alpha]_D$ +61° (*c* 0.5, water); o.r.d. data: ϕ (nm) +750° (350), +4,550° pk (272), 0° (260), −9,350° tr (219); n.m.r. data (deuterium oxide): τ 1.79, 1.99 (singlets, H-4 and H-8), 3.63 (doublet, H-1′, $J_{1',2'}$ 5.3 Hz), 7.43 (multiplet, H-3′), and 8.85 (doublet, 3′-Me, $J_{3',3'-Me}$ 7.5 Hz; $\lambda_{max}^{H_2O}$ 260 nm (ε 13,600).

Anal. Calc. for $C_{11}H_{15}N_5O_3$: C, 49.80; H, 5.70; N, 26.40. Found: C, 49.91; H, 5.69; N, 25.91.

Methyl 2-O-benzoyl-3-deoxy-3-C-methyl-5-O-trityl-β-D-xylofuranoside (**17**). — A solution of 1.5 g (3.7 mmoles) of **6** in 30 ml of pyridine was treated with benzoyl chloride (0.94 ml; 7.4 mmoles), and stirred for 1 h at 25°. The product was isolated in the usual way, and was crystallized from ether–petroleum ether, giving 1 g (47%) of **17**, m.p. 110–112°; $[\alpha]_D$ −23° (*c* 1, chloroform); R_F 0.56 on t.l.c. in 39:1 benzene–ethyl acetate.

Anal. Calc. for $C_{32}H_{32}O_5$: C, 77.93; H, 6.34. Found: C, 78.13; H, 6.23.

Methyl 2-O-benzoyl-3-deoxy-3-C-methyl-β-D-xylofuranoside (**18**). — A solution of 850 mg (1.7 mmoles) of **17** in acetic acid (5.95 ml), water (4.25 ml), and methanol (34 ml) was boiled under reflux for 16 h. The solution was cooled and evaporated, and methanol was added to and distilled from the product to remove residual acetic acid. The product was chromatographed on a column of silica gel (20 g) with 19:1 benzene–ethyl acetate, giving 389 mg (86%) of **18** as an oil; R_F 0.31 on t.l.c. in 4:1 benzene–ethyl acetate; $[\alpha]_D$ −0.2° (*c* 0.5, chloroform).

Anal. Calc. for $C_{14}H_{18}O_5$: C, 63.14; H, 6.81. Found: C, 63.19; H, 6.64.

Methyl 2,5-di-O-benzoyl-3-deoxy-3-C-methyl-β-D-xylofuranoside (**19**). — A solution of 325 mg (630 μmoles) of **18** in dry pyridine (10 ml) was treated with benzoyl chloride (177 mg; 145 μl; 1.26 mmoles), and the solution was stirred for 1 h at 25°. After being isolated in the usual way, the crude product was chromatographed on a column of silica gel (20 g) with 39:1 benzene–ethyl acetate. The yield of **19**, an oil, was 325 mg (72%); $[\alpha]_D$ −0.2° (*c* 1, chloroform); R_F 0.66 on t.l.c. in 19:1 benzene–ethyl acetate.

Anal. Calc. for $C_{21}H_{22}O_6$: C, 68.09; H, 5.99. Found: C, 68.56; H, 6.02.

2,5-Di-O-benzoyl-3-deoxy-3-C-methyl-α-D-xylofuranosyl chloride (**20**). — A solution of **19** (2 g; 5.35 mmoles) in acetic acid (25 ml) was added to 50 ml of acetic acid presaturated at 10° with hydrogen chloride. The solution was kept in a stoppered flask for 3 h at 25°. T.l.c. on alumina with 1:1 benzene–chloroform showed (iodine vapor) that no starting-material remained after 1 h. The solution was evaporated under diminished pressure, giving a residue which, by n.m.r. spectroscopy, consisted of 40% of 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-*C*-methyl-β-D-xylofuranose [τ 3.66 (singlet, H-1), and 7.96 (singlet, 1-*O*-acetyl protons); R_F 0.6 on t.l.c. on alumina with

9:1 benzene–chloroform], and 60% of chloride **20**. This mixture was dissolved in ether (100 ml) presaturated at 0° with hydrogen chloride, and the solution was kept in a tightly stoppered flask for 20 h at 25°. The solution was evaporated under diminished pressure, and three 50-ml portions of toluene were added to and evaporated from the residue under diminished pressure. The resulting, syrupy chloride **20** weighed 2 g, and had R_F 0.2 on t.l.c. on alumina with 9:1 benzene–chloroform; $[\alpha]_D$ +122° (*c* 1, chloroform); n.m.r. data: τ 3.42 (doublet, H-1), 4.83 (quartet, H-2, $J_{1,2}$ 4.4 Hz), and 8.72 (doublet, 3-Me, $J_{3,3\text{-Me}}$ 7.5 Hz).

*6-(Benzamido)-9-[2,5-di-*O*-benzoyl-3-deoxy-3-*C*-methyl-α(and β)-*D*-xylofuranosyl]purine* (**21**). — Xylene (~150 ml) was distilled from a suspension of 6-(benzamido)chloromercuripurine (2.53 g; 5.35 mmoles) in xylene (250 ml). The mixture was cooled to 20°, treated with a solution of **20** (2 g; 5.35 mmoles) in dry xylene (50 ml), and heated under reflux until boiling. Almost complete dissolution occurred and, after 1 h, the suspension was filtered, and the filtrate treated with petroleum ether (300 ml). The resulting precipitate was collected, and dissolved in chloroform (300 ml), and the solution was washed successively with four 50-ml portions of 30% potassium iodide and two 50-ml portions of water, and evaporated to give 2.7 g of crude product. Chromatography of this material on a column of silica gel (100 g) with 4:1 chloroform–ethyl acetate gave 2.35 g of amorphous 6-(benzamido)-9-[2,5-di-*O*-benzoyl-3-deoxy-3-*C*-methyl-α(and β)-D-xylofuranosyl]purine (**21**); $[\alpha]_D$ −41° (*c* 1, water); R_F 0.36 on t.l.c. with 3:1 chloroform–ethyl acetate.

Anal. Calc. for $C_{32}H_{27}N_5O_6$: C, 66.54; H, 4.71; N, 12.13. Found: C, 66.46; H, 4.82; N, 12.11.

*9-(3-Deoxy-3-*C*-methyl-α-D-xylofuranosyl)adenine* (α-**1**) *and 9-(3-deoxy-3-*C*-methyl-β-D-xylofuranosyl)adenine* (β-**1**) *from compound* **21**. — A solution of **21** (1.7 g; 2.95 mmoles) in methanol (50 ml) and liquid ammonia (55 g) was heated in a steel pressure-vessel for 20 h at 100°. The mixture was evaporated to dryness, the residue was dissolved in water (50 ml), and the solution was washed with eleven 40-ml portions of benzene (to remove benzamide), and freeze-dried. The residual solid (800 mg) was dissolved in methanol (6 ml) and, upon addition of ether (2.5 ml), 133 mg of β-**1** was obtained. The filtrate was evaporated, and the resulting oil was chromatographed on a column of silica gel (100 g) with 19:1 ethyl acetate–methanol. Early fractions of the eluate afforded 260 mg (total 393 mg; 50%) of β-**1**. After elution of several fractions containing both products, fractions that yielded 25 mg (3.3%) of α-**1** were obtained. The physical properties of α-**1** and β-**1** were identical with those of the products obtained from compound **16**.

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