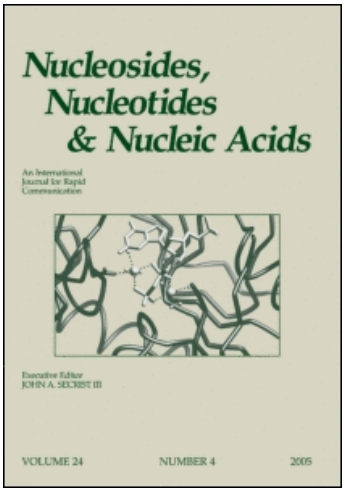

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SYNTHESIS OF AN EPOXIDE DERIVATIVE OF THYMIDINE AS A POTENTIAL ENZYME INHIBITOR

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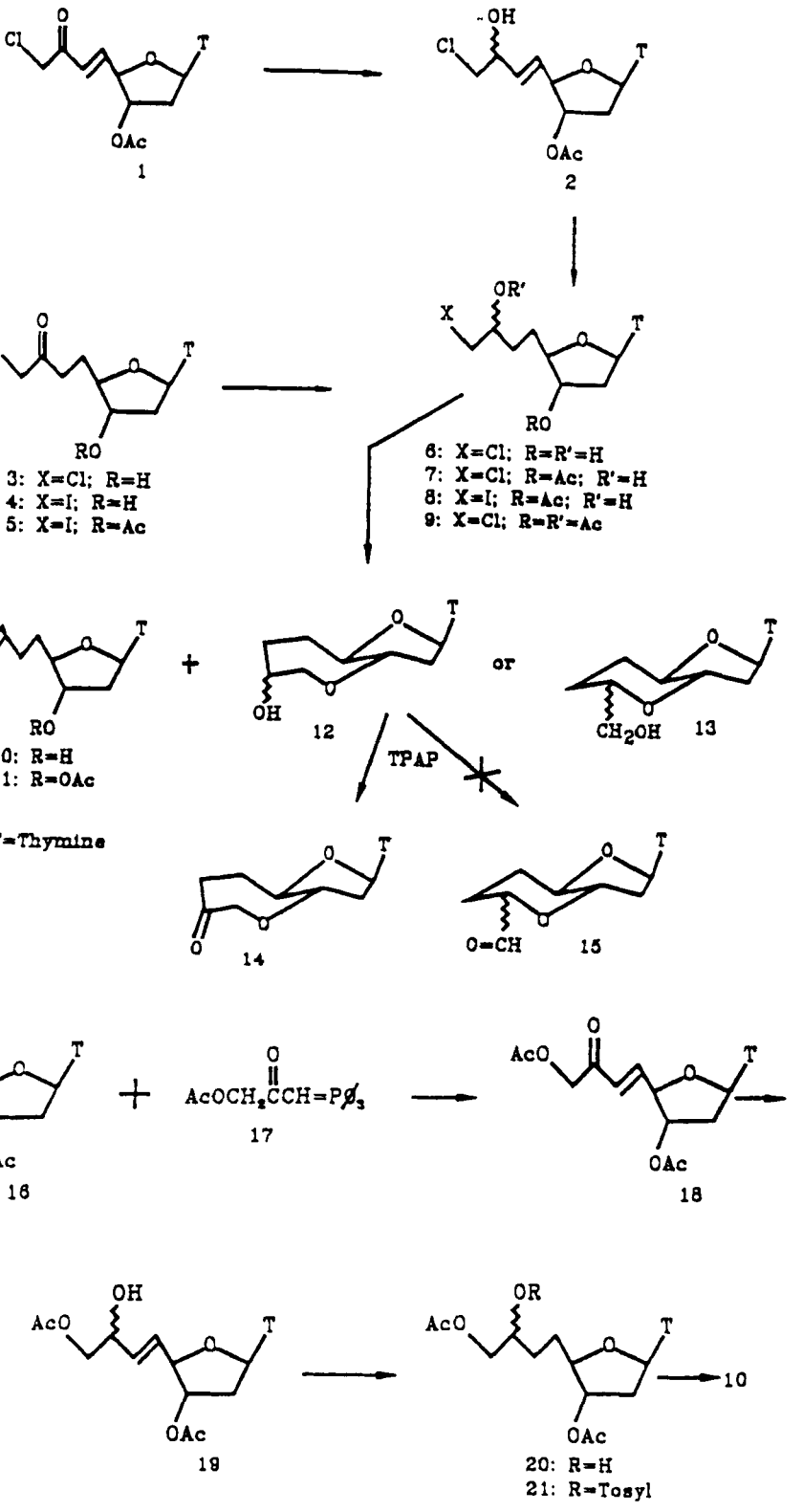
Summary. Attempts to prepare 1-[7,8-anhydro-2,5,6-trideoxy-α-L-lyxo-(and β-D-ribo)-octofuranosyl]thymine (**10**) by treatment of halohydrins **6-9** with sodium hydride in DMF or sodium methoxide in methanol gave mixtures of the epoxides **10** or **11** and the 3',8'-anhydronucleoside **12**. The structure of **12** was confirmed by oxidation to the cyclic ketone **14**. The successful synthesis of **10** involved a Wittig reaction on the thymidine-5'-aldehyde **16** to give the unsaturated ketoacetate **18** which was reduced in two steps to the diacetate **20**. The 7'-*O*-tosyl derivative **21** upon treatment with sodium methoxide in chloroform gave the pure epoxide **10** which was marginally toxic to L1210 cells in culture (I_{50}=25 μM) and demonstrated borderline *in vivo* activity (24% ILS) against P388 murine leukemia.

One of the major research efforts in this laboratory involves the synthesis of nucleosides containing a chemically reactive function attached through a spacer to the 5'- position of the sugar in the hope that such a function properly positioned could react with the binding site of enzymes involved in the biosynthetic pathway to nucleic acids.[1-8] It has been determined that a reactive group one and three atoms removed from the 5'- carbon should be in the proper position to react with the monophosphate binding sites. For a nucleoside diphosphate, it should be ideally one, three, or five atoms removed.[1] The choice of reactive function for reaction under *in vivo* conditions is critical. The group must be stable enough to reach the enzyme binding site unchanged and reactive enough to interact with functional groups at the binding site. In addition, the reactive nucleoside must have properties which allow it to be transported into cells. An example of a chemically reactive nucleoside which fits these criteria is 5'-bromoacetamido-5'-deoxythymidine (BAT)[4-6] which has a reactive function spaced three atoms from the 5'- carbon. BAT has been found to be cytotoxic to H.Ep.-2 and L1210 cells in culture and produced 71% ILS in the P388 mouse leukemia screen. The epoxide **10**, the synthesis of which is described here[9] was selected based on the recognized chemical reactivity of the epoxide group and its presence in structures with demonstrated antitumor activity.[10,11]



An initial attempt to prepare **10** involved reduction of the chloroketone **3**[3] with sodium borohydride to give the chlorohydrin **6** as a 1:1 mixture of 7′ epimers. Treatment of **6** with sodium hydride in DMF gave a mixture (53:47) of the epoxide **10** and an isomeric by-product postulated to be **12** or **13**. Although this mixture of products was not separable by silica gel chromatography, the product ratio could be determined by HPLC or ^{1}H NMR spectral data. If the leaving group at the 8′- position is displaced with the 3′- alkoxide anion, the product would be the 7-membered ring **12**. Compound **12** could also form by attack of the 3′-alkoxide anion on C-8′ of the epoxide **10**. A similar attack of the 3′-alkoxide anion on C-7′ of the epoxide would give the 6-membered ring **13**. The similar rf. values for **10** and **12** or **13** would not allow separation by chromatography; however, treatment of the mixture with 1N HCl resulted in reversion of **10** to the chlorohydrin **6** which could be separated from the stable 3′-ether by preparative TLC. Oxidation of this 3′- ether with tetra-*n*-propylammonium perruthenate gave only the ketone **14**. None of the aldehyde **15** which would have resulted from oxidation of **13** could be detected. The structure of **14** was confirmed by ^{1}H NMR spectral data and borohydride reduction of **14** to **12**, thus identifying the 3′- ether by-product as the 7-membered ring **12**. This reduction gave a 3:2 ratio of epimers of **12**, indicating a slightly greater preference of the borohydride for one side of the carbonyl group. The major epimer formed in this reduction was the same as the major epimer formed from **6**.

The ^{1}H NMR spectrum of **12** revealed a 9:1 ratio of C-7′ epimeric alcohols. Since the chlorohydrin **6** was a 1:1 mixture of C-7′ epimers, a possible explanation for the unequal epimer ratio in **12** could be unequal ease of displacement of the chloride in **6** by the 3′-alkoxide anion in each of the epimers due to differences in steric environment and hydrogen bonding in each of the epimers. A second possible mechanism is displacement of the chloride by the 7′-oxygen to give the epoxide **10** as a 1:1 mixture of epimers. The preferential formation of one epimer of **12** could then be explained by selective attack of the 3′-alkoxide anion of **10** on only one of the diastereomers of **10**. The possibility that **10** could isomerise in the presence of base was demonstrated by treatment of the epoxide **10** (obtained from **21** as described below) with sodium hydride in DMF to give a 3:1 mixture of **10** and **12**. The **12** from this isomerization was determined by NMR to be a 7:3 mixture of C-7′ epimers, the major one being the same as that formed from **7**.

In an attempt to avoid formation of **12**, the 3′-*O*-acetylchloroketone **1**[3] was reduced with sodium borohydride to give the unsaturated chlorohydrin **2** which was further reduced catalytically to give the monoacetylated chlorohydrin **7**. Treatment of **7** with sodium hydride in DMF gave a low yield of impure **11**. A major by-product in this reaction was **12** which could have resulted from partial hydrolysis of the 3′- acetyl group of **7** or **11**. Attempts to improve the yield of **11** from this reaction were unsuccessful.

The effect of the leaving group on this ring closure was investigated by treatment of **3** with sodium iodide in acetone to give the iodomethylketone **4**[3] which was then acetylated to give

5. Compound **5** was also prepared from the 3′-*O*-acetate of **3** and sodium iodide. Reduction of **5** with sodium borohydride gave the iodohydrin **8** which, upon treatment with sodium hydride in DMF, gave a low yield of a mixture of **11** and **12**.

The synthetic route which provided pure epoxide **10** was modeled after a procedure described by Kim and Szarek.[13] A Wittig reaction of 3′-*O*-acetylthymidine-5′-aldehyde (**16**)[14] with [(acetoxymethyl)-carbonyl]methylenetriphenylphosphorane (**17**)[13] gave the unsaturated ketoacetate **18**. Borohydride reduction of the carbonyl group of **18** gave the unsaturated alcohol **19** which was hydrogenated in the presence of palladium on carbon to give **20**. Tosylation of **20** gave **21** which was treated with sodium methoxide in chloroform to give as the sole product the epoxide **10** as a mixture of epimers. Although separation of these epimers was not possible by standard column chromatography techniques, partial separation using a graphitized carbon HPLC column indicated an approximate 1:1 mixture of epimers. The UV spectra of these peaks were identical as expected.

The possibility that the sodium methoxide-chloroform combination might be crucial for exclusive formation of epoxide was investigated by conversion of **6** to the 3′,7′-diacetate **9**. Treatment of **9** with sodium methoxide in chloroform again gave a mixture (82:18) of **10** and **12**.

These results indicate that the leaving groups should be at the 7′- position rather than at the 8′-position to obtain pure epoxide. Molecular models show that displacement of a leaving group at the 7′- position by the 3′- alkoxide anion involves slightly more steric strain than a similar displacement of a leaving group at the 8′- position. Exclusive formation of **12** from isomerization of **10** with sodium hydride-DMF lends further evidence for preferential attack at the 8′-position. The possibility that the tosyl leaving group might be a significant factor in the direction of ring closure was not determined.

Although no further attempts were made to optimize formation of **12** from the halo intermediates **6-9**, careful avoidance of excess base may be a significant factor in limiting isomerization of **9** to **11**.

The epoxide **10** was found to be marginally cytotoxic to L1210 cells in culture[15] (I_{50}=25 μM) and exhibited little cytotoxicity to H.Ep.-2 cells[16] (I_{50}=80 μM). *In vivo* tests of **10** against P388 leukemia[17] in mice also showed borderline activity with 24% increase in life span at a dose of 200 mg/kg. The epoxide **10** was found to have a half-life of 5.5 days in pH 7 buffer. The rate of decomposition was followed by HPLC.

Experimental Section

All evaporations were carried out *in vacuo* with a rotary evaporator or by short-path distillation into a dry ice-acetone-cooled receiver under high vacuum. Analytical samples were

normally dried *in vacuo* over P_2O_5 at room temperature for 16 h. Analtech precoated (250-μm) silica gel GF plates were used for TLC analyses; the spots were detected by irradiation with a Mineralight and by charring after spraying with saturated $(NH_4)_2SO_4$. All analytical samples were TLC homogeneous. Melting points were determined with a Kofler Heizbank apparatus unless otherwise specified. Purifications by preparative TLC were carried out using 2 mm Brinkman silica gel plates. Purifications by flash chromatography[18] were carried out on Merck Silica gel 60 (230-400 mesh) using the slurry method of column packing. The UV absorption spectra were determined in 0.1 N HCl (pH 1), pH 7 buffer, and 0.1 N NaOH (pH 13) with a Cary 17 spectrophotometer or with a Perkin-Elmer Lambda 9 spectrometer: the maxima are reported in nanometers (ε x 10^{-3} M^{-1} cm^{-1}). The ^1H NMR spectra and the ^{13}C NMR spectra with tetramethylsilane as an internal reference were determined with a Nicolet NT 300NB spectrometer operating at 300.635 MHz and 75.602 MHz, respectively. Chemical shifts (δ) quoted in the case of multiplets were measured from the approximate center. The mass spectral data were obtained with a Varian-MAT 311A mass spectrometer in the electron impact (EI) or fast atom bombardment (FAB) mode. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within $\pm$0.4% of the theoretical values. HPLC analyses were carried out with a Hewlett-Packard HP 1084B liquid chromatograph with a Waters Associates μBondapak C_{18} column (3.9 mm x 30 cm) with UV monitoring (254 nm). The solvent system used was a gradient of 10% to 90% acetonitrile in water.

1-[3-*O*-Acetyl-8-chloro-2,5,6,8-tetradeoxy-β-D-*ribo*-(and α-L-lyxo)-oct-5-enofurano-syl]thymine (2). A solution of 1[3] (1.32 g, 3.70 mmol) in anhydrous ethanol (50 mL) was chilled in a dry ice-acetone bath, treated with a solution of sodium borohydride (140 mg, 3.7 mmol) in 14 mL of EtOH, stirred at ambient temperature for 5 min, adjusted to pH 5 with 1N HCl, and evaporated to dryness *in vacuo*. The residue was partitioned between water and EtOAc. The EtOAc layer was dried over $MgSO_4$ and evaporated to dryness *in vacuo*. A $CHCl_3$ solution of the residue was flash columned on 125 g of silica gel using $CHCl_3$-MeOH (98:2) as eluting solvent. The product, a mixture of diastereomers, was obtained as a syrup: yield 1.05 g (79%); R_f 0.54, $CHCl_3$-MeOH (9:1); MS (FAB) *m/z*, 359 $(M + H)^+$. ^1H NMR ($CDCl_3$) δ 9.75 (s, 1, H-3, exchanges with D_2O), [7.22 (q, H-6, $^4J_{6,CH_3}$=1.3 Hz) and 7.20 (q, H-6, $^4J_{6,CH_3}$=1.3Hz)](1H), [6.31 (ψt, H-1′, $J_{1',2'}$=5.6 Hz) and 6.28 (ψt, H-1′, $J_{1',2'}$=5.5 Hz)](1H), 6.06-5.98 (m, 1, H-5′, $J_{5',6'}$=15.5 Hz), 5.94-5.87 (m, 1, H6′), 5.14 (m, 1, H-3′), 4.51 (m, 1, H-4′), 4.49 (m, 1, H-7′), 3.68-3.62 (m, 1, H-8′b), 3.59-3.53 (m, 1, H-8′a), 3.22 (br s, 1,7′-OH, exchanges with D_2O), 2.46-2.38 (m, 1, H-2′b), 2.33-2.23 (m, 1, H-2′a), 2.12 (s, 3, CH_3CO_2-), 1.93 (d, 3, 5-CH_3). This material was used without further purification for the preparation of 7.

1-[3-*O*-Acetyl-2,5,6,8-tetradeoxy-8-iodo-β-D-erythro-octofuranos-7-ulos-1-yl]thymine (5). Method A (from 4). A mixture of 4[3] (256 mg, 0.63 mmol) in 30 mL of Fritz-Schenk

reagent[19] (2M Ac_2O in EtOAc containing p-toluenesulfonic acid) was stirred for 1.5 h at 25 °C and poured into 250 mL of a mixture of ice and saturated $NaHCO_3$ solution. The ethyl acetate layer was washed repeatedly with saturated $NaHCO_3$ until neutral, then it was washed with H_2O, dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The residue, a syrup, crystallized on standing and was recrystallized from ethyl acetate-cyclohexane to give **5** as a white solid: yield 187 mg (66%); mp 132-133 °C; R_f 0.59, $CHCl_3$-MeOH (9:1); UV λ_{max} (ε x 10^{-3}); at pH 1, 266 nm (9.95); at pH 7, 266 nm (9.82); at pH 13, 266 nm (7.48); MS (FAB) *m/z* 325 (sugar)$^+$, 451 (M + 1)$^+$. ^1H NMR (Me_2SO-d_6) δ 11.30 (s, 1, H-3, exchanges with D_2O), 7.48 (q, 1, H-6, $^4J_{6,CH_3}$=1.2 Hz), 6.12 (dd, 1, H-1′, $J_{1',2'a}$=6.1 Hz, $J_{1',2'b}$=8.5 Hz), 5.03 (m, 1, H-3′), 4.07 (s, 2, H-8′), 3.85 (m, 1, H-4′), 2.85-2.72 (m, 2, H-6′), 2.49-2.41 (m, 1, H-2′b), 2.22-2.14 (m, 1, H-2′a), 2.06 (s, 3, CH_3CO_2-), 1.96-1.84 (m, 2, H-5′), 1.81 (d, 3, 5-CH_3). *Anal.* calcd for $C_{13}H_{17}N_3O_7$: C, 47.70; H, 5.23; N, 12.83. Found: C, 47.45; H, 5.42; N, 13.20.

Method B (from 3). A mixture of **3**[3] (100 mg, 0.32 mmol) in 10 mL of Fritz-Schink reagent (see above) was stirred for 1 h and poured into a mixture of ice and saturated $NaHCO_3$ (50 mL). The ethyl acetate layer was washed with saturated $NaHCO_3$ until neutral, then washed with H_2O, dried over $MgSO_4$ and evaporated *in vacuo* to give the crude 3′-*O*-acetate of **3** as a glass. A solution of this glass (53 mg) and anhydrous sodium iodide (68 mg, 0.45 mmol) in anhydrous acetone (10 mL) was stirred at ambient temperature for 0.5 h, then filtered, and evaporated to dryness *in vacuo*. A solution of the residue in ethyl acetate was washed with H_2O, dried over $MgSO_4$, and evaporated *in vacuo* to give a syrup which crystallized on standing: yield 54 mg (39%). This product was identical to that described in A above.

1-[8-Chloro-2,5,6,8-tetradeoxy-β-D-ribo-(and α-L-lyxo)-octofuranosyl]thymine (6). A solution of **3**[3] (248 mg, 0.78 mmol) in 50 mL of anhydrous EtOH was chilled in a dry ice-acetone bath while a solution of sodium borohydride (29.5 mg, 0.78 mmol) in 3 mL of EtOH was quickly added. After 15 min, the resulting solution was neutralized carefully with 1N HCl and evaporated to dryness *in vacuo*. The residue obtained was purified by preparative TLC using $CHCl_3$-MeOH (9:1) as solvent. A MeOH extract of the product band was evaporated to give a crystalline solid. Recrystallization from EtOH gave a 1:1 mixture of diastereomers: yield 92 mg (37%); mp 142-143 °C; R_f 0.32, $CHCl_3$-MeOH (9:1); UV λ_{max} (ε x 10^{-3}) at pH 1, 267 nm (9.64); at pH 7, 267 (9.44); at pH 13, 267 (7.58); MS (FAB) *m/z* 319 (M + H)$^+$; ^1H NMR (Me_2SO-d_6) δ 11.28 (br s, 1, H-3, exchanges with D_2O), 7.38 (s, 1, H-6), 6.12 (t, 1, H-1′, $J_{1',2'}$=6.6 Hz), 5.24 (d, 1, 3′-OH, $J_{3',3'\text{-OH}}$=4.2 exchanges with D_2O), 5.08 and 5.06 (two partially overlapping doublets, 1, 7′-OH, $J_{7',7'\text{-OH}}$=4.2 exchanges with D_2O), 4.03 (m, 1, H-3′), 3.64 (m, 2, H-4′, H-7′), 3.53 (m, 2, H-8′), 2.20-1.99 (m, 2, H-2′), 1.79 (s, 3, CH_3), 1.73-1.38 (m, 4, H-5′, H-6′). *Anal.* calcd for $C_{13}H_{19}ClN_2O_5$: C, 48.98; H, 6.01; N, 8.79. Found: C, 49.15; H, 6.34; N, 8.91.

1-[3-*O*-Acetyl-8-chloro-2,5,6,8-tetradeoxy-β-D-ribo-(and α-L-lyxo)-octofuranosyl]-thymine (7). A solution of **2** (1.06 g, 3 mmol) in EtOH (100 mL) containing 500 mg of 30% palladium-on-carbon catalyst was hydrogenated at ambient temperature and atmospheric pressure, filtered, and evaporated to dryness *in vacuo*. A $CHCl_3$ solution of the syrup was purified by preparative TLC on 8 silica gel plates which were developed in $CHCl_3$-MeOH (9:1). The product, a mixture of diastereomers, was obtained as a syrup: yield 531 mg (49%); R_f 0.54, $CHCl_3$-MeOH (9:1); MS (EI) *m/z* 235 (sugar)$^+$, 360 (M)$^+$; ^{1}H NMR ($CDCl_3$) δ 9.41 (br s, 1, H-3, exchanges with D_2O), 7.18 (q, 1, H-6, $^4J_{6,CH_3}$=1.3 Hz), [6.25 (ψt, H-1′, $J_{1',2'}$=5.5 Hz), 6.22 (ψt, H-1′, $J_{1',2'}$=5.5 Hz)] (1H), 5.05 (m, 1, H-3′), 4.02 (m, 1, H-4′), 3.87 (m, 1, H-7′), 3.65-3.59 (m, 1, H-8′b), 3.54-3.47 (m, 1, H-8′a), 2.69 (br s, 1, 7′-OH, exchanges with D_2O), 2.45-2.36 (m, 1, H-2′b), 2.26-2.15 (m, 1, H-2′a), 2.11 (s, 3, CH_3CO_2-), 1.94 (d, 3, 5′-CH_3), 1.92-1.62 (m, 4, H-5′, H-6′); ^{13}C NMR ($CDCl_3$) δ 170.46 (CH_3-$\underline{C}O_2$-), 163.47 (C-4), 150.30 (C-2), 134.79 and 134.76 (C-6), 111.69 and 111.63 (C-5), 84.88 and 84.83 (C-1′, $^1J_{C,H}$=170 Hz), 84.18 and 83.79 (C-4′), 76.58 and 76.44 (C-3′), 70.86 (C-7′), 50.14 and 50.11 (C-8′), 36.82 (C-2′), 30.43, 30.15, 29.94, 29.75 (C-5′, C-6′), 20.99 (CH_3), 12.64 (5-CH_3). This product was sufficiently pure for the preparation of **11** and **12**.

1-[3-*O*-Acetyl-2,5,6,8-tetradeoxy-8-iodo-β-D-ribo-(and α-L-lyxo)-octofuranosyl]thymine (8). A solution of **5** (409 mg, 0.91 mmol) in anhydrous EtOH (25 mL) was chilled in an ice bath while a solution of sodium borohydride (34 mg, 0.91 mmol in 3.4 mL of anhydrous EtOH) was added. The solution was allowed to rise to ambient temperature, stirred for 10 min, then neutralized with solid carbon dioxide, and evaporated to dryness *in vacuo*. The residue was resolved by preparative TLC on 4 silica gel plates which were developed in $CHCl_3$-MeOH (9:1). The product (**8**) (R_f 0.73) was obtained as a glass: yield 212 mg (47%); MS (FAB) *m/z* 453 (M + 1)$^+$.

1-[3,7-Di-*O*-acetyl-8-chloro-2,5,6,8-tetradeoxy-β-D-ribo-(and α-L-lyxo)-octofuranosyl]-thymine (9). A solution of **6** (169 mg, 0.53 mmol) in anhydrous pyridine (10 mL) at 0 °C was treated with acetic anhydride (433 mg, 4.24 mmol), kept 20 h at ambient temperature, and poured into 50 mL of ice and water. The mixture was extracted with chloroform (2 x 50 mL) and the extract washed successively with cold dilute H_2SO_4 and water, dried over $MgSO_4$, and evaporated to dryness *in vacuo* to give a syrup: yield 202 mg (955); R_f0.79, $CHCl_3$-MeOH (9:1); MS (FAB) *m/z* 403 (M + 1)$^+$; ^{1}H NMR ($CDCl_3$) δ 8.86 (br s, 1, H-3), [7.14 (q, H-6, $^4J_{6,CH}$=1.3 Hz), 7.12 (q,H-6, $^4J_{6,CH_3}$=1.3 Hz), 7.12 (q, H-6, $^4J_{6,CH}$=1.3 Hz)] (1H), 6.23 (dd, 1, H-1′, $J_{1',2'}$=8.3 Hz), 5.08 (m, 1, H-7′), 5.01 (m, 1, H-3′), 3.97 (m, 1, H-4′), 3.68-3.56 (m, 2, H-8′$_b$), 2.45-2.36 (m, 1, H-2′$_a$), 2.21 (s, 6, 2 $C\underline{H}_3CO_2$-s), 1.96 (m, 3, 5-CH_3), 1.94-1.61 (m, 4, H-5′, H-6′). This material was used without further purification for reaction with sodium methoxide.

Reaction of 9 with sodium methoxide. This reaction was carried out exactly as described for the reaction of **21** with sodium methoxide using 54 mg (0.13 mmol) of **9** to give 17 mg (45%) of a mixture found by HPLC to contain 82% of **10** and 18% of **12**.

1-[7,8-Anhydro-2,5,6-trideoxy-α-L-lyxo-(and β-D-ribo)-octofuranosyl]thymine (10). A solution of **21** (441 mg, 0.82 mmol) in 40 mL of anhydrous chloroform and 20 mL of anhydrous benzene was evaporated to dryness *in vacuo*. The residue was dried for 1 h at 0.07 nm before it was dissolved in 40 mL of anhydrous chloroform. The resulting solution was chilled in an ice bath, treated with sodium methoxide (110 mg, 2.05 mmol), stirred for 20 h in a Dewar of ice, and then kept at ambient temperature for 4 h. The reaction solution (pH 8) was treated with additional sodium methoxide (44 mg, 0.82 mmol) and stirred for 20 h in a Dewar of ice. While still in the cold, the mixture was diluted with enough anhydrous methanol to give a clear solution. The solution was then carefully neutralized with glacial acetic acid and evaporated to dryness *in vacuo*. A solution of the residue in $CHCl_3$-MeOH (95:5) was applied to a flash column of 25 g of silica gel. The column was eluted with the same solvent system, and the product fraction evaporated to dryness *in vacuo* to give a crystalline solid (135 mg, 72%); mp 141 °C; R_f 0.40, $CHCl_3$-MeOH (95:5); MS (EI), *m/e* 157 $(sugar)^+$, 282 $(M^+)^+$, UV λ_{max} (ϵ x 10^{-3}) at pH 1, 267 nm (9.75); at pH 7, 266 (9.62); at pH 13, 267 (7.45). $_1$H NMR (Me_2SO-d_6) 11.29 (s, 1, H-3, exchanges with D_2O), [7.39 (q, H-6, $^4J_{6,CH_3}$=1.1 Hz), 7.37 (q, H-6, $^4J_{6,CH_3}$=1.0 Hz)] (1H), 6.12 (ψt, 1, H-1′, $J_{1',2'}$=7.0 Hz), 5.25 (d, 1, 3′-OH, $J_{3',3'\text{-OH}}$=4.5 Hz, exchanges with D_2O), 4.05 (m, 1, H-3′), 3.66 (m, 1, H-4′), 2.92 (m, 1, H-7′), 2.68 (m, 1, H-8′b), 2.45 (m, 1, H-8′a), 2.22-2.13 (m, 1, H-2′b), 2.07-1.99 (m, 1, H-2′a), 1.79 (s, 3, 5-CH_3), 1.78-1.42 (m, 4, H-5′, H-6′); ^{13}C NMR (Me_2SO-d_6) δ 163.53 (C-4), 150.30 (C-2), 135.88 and 135.83 (C-6), 109.70 and 109.69 (C-5), 83.15 and 83.13 (C-1′, $^1J_{C,H}$=160 Hz), 72.97 and 72.94 (C-3′), 51.30 and 51.11 (C-7′, $^1J_{C,H}$=172 Hz), 46.14 and 46.07 (C-8′, $^1J_{C,H}$=174 Hz), 38.29 (C-2′), 29.30 and 29.21 (C-6′), 28.51 and 28.39 (C-5′) and 12.03 (5-CH_3). Absence of **11** in this product was confirmed by HPLC. Further HPLC analysis of **10** on a Hypercarb Shandon graphitized carbon column using acetonitrile-water (95:5) resulted in partial separation of the approximately 1:1 mixture of epimers. The UV spectra of these peaks were identical. *Anal.* calcd for $C_{13}H_{18}N_2O_5$·0.1 H_2O: C, 54.96; H, 6.46; N, 9.86. Found: C, 55.10; H, 6.81; N, 9.50.

1-[3,8-Anhydro-2,5,6-trideoxy-β-D-ribo-(and α-L-lyxo)-octofuranosyl]thymine (12). Method A (from 6). A solution of **6** (275 mg, 0.86 mmol) in Me_2NCHO (10 mL) was treated with sodium hydride (50% dispersion in mineral oil) (83 mg, 1.72 mmol), stirred for 20 h at ambient temperature, neutralized in the cold with glacial acetic acid, and evaporated to dryness *in vacuo*. The residue was purified by flash chromatography on 25 g of silica gel using $CHCl_3$-MeOH (95:5) as the eluting solvent. An opaque solid was obtained: yield 225 mg (92%); R_f 0.46 $CHCl_3$-MeOH (9:1). HPLC showed a 56:44 ratio of **10** and **12**. ^{1}H NMR gave a similar ratio

(53:47) of **10** and **12**. A methanol solution of this mixture (225 mg) was acidified to pH 2 by the addition of 1*N* HCl. After 1 h, the solution was readjusted to pH 2 by addition of more 1*N* HCl. After an additional 3 h at ambient temperature, the solution was evaporated to dryness *in vacuo*. The residue was purified by flash chromatography on 25 g of silica gel using $CHCl_3$-MeOH (95:5) as eluting solvent. The product fraction was evaporated to give **12** as a solid: yield 77 mg (34% from the opaque solid); MS (FAB) *m/z* 283 $(M + 1)^+$. The ^1H NMR spectrum showed that **12** was a 9:1 mixture of epimers at C-7′. The ^1H NMR was similar to the analytical sample of **12** obtained by Method B and differed only in the ratio of C-7′ epimers.

Method B (from 10). A solution of **10** (282 mg, 1.00 mmol) in Me_2NCHO (10 mL) was treated with sodium hydride (60% dispersion in mineral oil, 80 mg, 2.00 mmol), stirred 20 h, neutralized with glacial acetic acid, and evaporated to dryness *in vacuo*. The residue was examined by HPLC and found to be a mixture of 77% **12** and 23% **10**. A solution of this mixture in methanol was acidified to pH 2 with 1*N* HCl and stirred at ambient temperature for 30 min to decompose the epoxide present. Purification of **12** was accomplished by preparative TLC on silica gel plates which were developed in $CHCl_3$-MeOH (9:1). The product (**12**, a 7:3 mixture of epimers) was obtained as a glass: yield 142 mg (47%); R_f 0.41, $CHCl_3$-MeOH (9:1); UV λ_{max} (ε x 10^{-3}) at pH 1, 267 nm (9.84); at pH 7, 267 (9.42); at pH 13, 267 (7.32); MS (FAB) *m/z* 157 (sugar)$^+$, 283 $(M + 1)^+$. *Anal.* calcd for $C_{13}H_{18}N_2O_5 \cdot 0.1$ MeOH$\cdot 1$ H_2O: C, 51.84; H, 6.77; N, 9.23. Found: C, 51.83; H, 6.37; N, 8.98.

Method C (from reduction of 14). A solution of **14** (16 mg, 0.06 mmol) in MeOH-EtOH (1:1) was treated with sodium borohydride (9 mg, 0.24 mmol) in EtOH (1 ml). The reaction solution was kept 16 h at ambient temperature, neutralized with Amberlite IR-120 (H^+) ion exchange resin, then filtered, and evaporated several times from MeOH to give **12** as a white solid: yield 14 mg (88%). The ^1H NMR spectrum showed that **12** was a 6:4 mixture of epimers at C-7′. The ^1H NMR of this product was similar to that of the analytical sample of **12**, differing only in ratio of C-7′ epimers.

The major epimer of 12. ^1H NMR (Me_2SO-d_6) δ 11.27 (brs, H-3, exchanges with D_2O), 7.36 (s, H-6), 6.07 (t, H-1′, $J_{1',2'}$=6.5 Hz), 4.84 (d, 7′-OH, $J_{7',OH}$=4.0 Hz, exchanges with D_2O), 4.06 (q, H-3′), 3.91 (m, H-7′), 3.82 (dd, H-8′a, $J_{8a',8b'}$=12.0 Hz, $J_{8a',7'}$=3.8 Hz), 3.63 (m, H-4′), 3.43 (dd, H-8′b, $J_{8b',7'}$=8.0 Hz), 2.20 (m, H-2′), 2.02 (m, H-5′a), 1.84 (m, H-6′a), 1.81 (s, CH_3), 1.73-1.46 (m, H-5′b, H-6′b); ^{13}C NMR (Me_2SO-d_6) δ 163.54 (C-4), 150.10 (C-2), 136.20 (C-6), 109.82 (C-5), 82.23 (C-1′), 81.82 (C-4′), 77.56 (C-3′), 73.66 (C-8′), 68.74 (C-7′), 37.03 (C-2′), 30.46 and 27.22 (C-5′ and C-6′), 11.98 (5-CH_3).

The minor epimer of 12. ^1H NMR (Me_2SO-d_6) δ 11.27 (brs, H-3, exchanges with D_2O), 7.36 (s, H-6), 6.04 (t, H-1′, $J_{1',2'}$=4.5 Hz), 4.87 (d, 7′-OH, $J_{7',OH}$=4.0 Hz, exchanges with D_2O), 4.11 (m, H-3′), 3.76 (m, H-7′), 3.57 (m, H-4′), 3.34 (m, H-8′a), 2.20 (m, H-2′), 2.02 (m, H-5′a),

1.84 (H-6'a), 1.81 (s, CH_3), 1.73-1.46 (m, H-5'b, H-6'b); ^{13}C NMR (Me_2SO-d_6) δ 163.54 (C-4), 150.10 (C-2), 136.11 (C-6), 109.74 (C-5), 82.27 (C-1'), 82.10 (C-4'), 78.64 (C-3'), 75.34 (C-8'), 68.23 (C-7'), 36.86 (C-2'), 30.01 and 24.58 (C-5' and C-6'), 12.00 (5-CH_3).

Reaction of 7 with Sodium Hydride. A solution of **7** (129 mg, 0.36 mmol) in anhydrous Me_2NCHO (5 mL) was treated with sodium hydride (50% dispersion, 35 mg, 0.72 mmol), stirred for 20 h at ambient temperature, then filtered, and evaporated to dryness *in vacuo*. A solution of the residue in CH_2Cl_2 was neutralized with glacial acetic acid, washed with H_2O, dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The residue was purified by preparative TLC on 2 silica gel plates which were developed in $CHCl_3$-MeOH (9:1). The epoxide **11**, a 1:1 mixture of epimers at C-7', was obtained as a syrup: yield 29 mg (25%); R_f 0.61, $CHCl_3$-MeOH (9:1). ^1H NMR ($CDCl_3$) δ 7.15 (q) and 7.12 (q) (1, H-6, J_{6,CH_3}=1.1 Hz), 6.25 (m, 1, H-1'), 6.20-5.40 (brs, 1, NH, exchanges with D_2O), 5.03 (m, 1, H-3'), 4.02 (m, 1, H-4'), 2.99 (m, 1, H-7'), 2.80 (t) and 2.79 (t) (1, H-8'a, $J_{8'a,8'b}$=$J_{7',8'a}$=4.0 Hz), 2.54 (t) and 2.51 (t) (1, H-8'b, $J_{8'a,8'b}$=4.0 Hz, $J_{7',8'b}$=2.5 Hz), 2.45-2.38 (m, 1, H-2'a), 2.21-2.13 (m, 1, H-2b'), 2.11 (s, 3, CH_3CO_2-), 1.94 (brs, 1, 5-CH_3), 1.94-1.77 (m, 3, H-5'a, H-5'b, H-6'a), 1.68-1.51 (m, 1, H-6'b); ^{13}C NMR ($CDCl_3$) δ 170.39 ($CH_3\underline{C}O_2$-), 164.23 and 163.92 (C-4), 151.68 and 150.64 (C-2), 134.55 and 134.47 (C-6), 111.68 and 111.58 (C-5), 84.51 (C-1', $^1J_{C,H}$=169 Hz), 83.75 and 83.43 (C-4'), 76.42 and 76.31 (C-3'), 51.69 and 51.54 (C-7', $^1J_{C,H}$=171.7 Hz), 47.23 and 47.07 (C-8', $^1J_{C,H}$=174.7 Hz), 36.92 (C-2'), 30.26 and 30.17 (C-6'), 28.74 and 28.59 (C-5'), 20.98 (CH_3), 12.70 and 12.66 (5-CH_3). When this reaction was repeated, a 16% yield of **12** was isolated. No epoxide **11** could be detected.

Reaction of 8 with Sodium Hydride. To a solution of **8** (203 mg, 0.45 mmol) in anhydrous CH_2Cl_2 (10 mL) was added 21.6 mg of sodium hydride (50% dispersion in mineral oil, 0.45 mmol) and the resulting mixture stirred for 20 h at ambient temperature. Another 21.6 mg of the dispersion was then added. The mixture was stirred 20 h, diluted with 10 mL of MeOH-H_2O (1:1), taken to pH 7 with Amberlite IR-120 (H^+) ion exchange resin (200-400 mesh), and evaporated to dryness *in vacuo*. The residue was resolved by preparative TLC on 2 silica gel plates which were developed in $CHCl_3$-MeOH (9:1). A band with R_f 0.37 was extracted with MeOH and the solution evaporated to dryness to give 37 mg (20%) of deacetylated **8**. The band with R_f 0.52 was worked up to give 42 mg of impure product that was rechromatographed to give 28 mg of a TLC homogeneous solid; MS (FAB), m/z 282 $(M + 1)^+$. This material was found by ^1H NMR (Me_2SO-d_6) to be a 15:85 mixture of **10** and **12**, a 9:1 mixture of epimers.

1-[3,8-Anhydro-2,5,6-trideoxy-β-D-erythro-octofuranos-7-ulos-1-yl]thymine (14). A solution of **12** (130 mg, 0.43 mmol) in anhydrous CH_2Cl_2 (50 mL) containing Linde 4A Molecular Sieves (11.5 g) and *N*-methylmorpholine-*N*-oxide (81 mg, 0.69 mmol) was treated after 10 min with tetrapropylammonium perruthenate[12] (46 mg, 0.18 mmol). After an

additional 10 min the mixture was filtered, evaporated to 2 mL, and purified by preparative TLC using $CHCl_3$-MeOH (9:1) as developing solvent. Extraction of the product band with MeOH gave a crystalline solid: yield 60 mg. The analytical sample was obtained by recrystallization from methanol: yield 23 mg (19%); mp 253 °C; R_f 0.82 $CHCl_3$-MeOH (9:1); MS (FAB) m/z 281 $(M + 1)^+$. ^1H NMR (Me_2SO-d_6) δ 11.29 (s, 1, NH, exchanges with D_2O), 7.40 (s, 1, H-6), 6.16 (dd, 1, H-1′, $J_{1',2'b}$=2.6 Hz, $J_{1',2'a}$=8.8 Hz), 4.28 (d, 1, H-8′a, $J_{8'a,8b'}$=17.7 Hz), 3.99 (d, 1, H-8′b), 3.95 (m, 1, H-3′), 3.67 (m, 1, H-4′), 2.75 (dt, 1, H-6′a), 2.47-2.32 (m, 2, H-6′b, H-2′a), 2.24 (m, 1, H-2′b), 2.12 (m, 1, H-5′a), 1.79 (s, 3, CH_3), 1.67 (q, 1, H-5′b). *Anal.* calcd for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.76; N, 10.00. Found: C, 55.91; H, 6.13; N, 9.96.

1-[(E)-3,8-Di-*O*-acetyl-2,5,6-trideoxy-β-D-erythro-oct-5-eno-furanos-7-ulos-1-yl]-thymine (18). A solution of 2.5 mmol of **16**[14] and 774 mg (25 mmol) of **17**[13] in anhydrous MeCN (100 mL) was stirred 20 h at ambient temperature before it was evaporated to dryness *in vacuo*. The residue was purified by preparative TLC on silica gel plates using $CHCl_3$-MeOH (9:1) as the developing solvent. The residue thus obtained was further purified by flash chromatography on 80 g of silica gel using $CHCl_3$-MeOH (97:3) as eluting solvent. The product (**18**) was obtained as a glass: yield 751 mg (96%); R_f (0.16, 97:3 $CHCl_3$-MeOH); MS (FAB), m/z 381 $(M + 1)^+$; ^1H NMR (Me_2SO-d_6) δ 11.39 (s, 1, H-3, exchanges with D_2O), 7.59 (m, 1, H-6), 7.03 (dd, 1, H-5′, $J_{5',6'}$=16.1 Hz, $J_{4',5'}$=5.8 Hz), 6.35 (dd, 1, H-6′, $^4J_{4',6'}$=1.2 Hz), 6.25 (dd, 1, H-1′, $J_{1',2'a}$=6.2 Hz, $J_{1',2'b}$=8.2 Hz), 5.21 (m, 1, H-3′), 4.99 (s, 2, H-8′), 4.61 (m, 1, H-4′), 2.61-2.54 (m, 1, H-2′b), 2.32-2.25 (m, 1, H-2′a), 2.11 (s, 3, CH_3CO_2-), 2.09 (s, 3, CH_3CO_2-), 1.81 (m, 3, 5-CH_3). This material was suitable for preparation of **19**.

1-[(E)-3,8-Di-*O*-Acetyl-2,5,6-trideoxy-β-D-ribo-(and α-L-lyxo)-oct-5-enofuranosyl]-thymine (19). A solution of **18** (725 mg, 1.91 mmol) in anhydrous EtOH (25 mL) was chilled in an ice-EtOH bath and stirred while sodium borohydride (80 mg, 2.10 mmol) was added. After 5 min, the solution was carefully neutralized with 1*N* HCl and evaporated to dryness *in vacuo*. The residue was purified by flash chromatography on 80 g of silica gel using $CHCl_3$-MeOH (95:5) as eluting solution. The glassy product (517 mg, 71%) was found to be an 80:20 mixture of **19** and **20** by ^1H NMR in $CDCl_3$; R_f (0.22, 95:5 $CHCl_3$-MeOH); ^1H NMR ($CDCl_3$) (δ values for **19** only) δ 8.76 (br s, H-3, exchanges with D_2O), 7.20 (q, H-6, $^4J_{6,CH_3}$=1.1 Hz), 7.18 (q, H-6, $^4J_{6,CH_3}$=1.1 Hz), 6.31 (ψt, H-1′), 6.29 (ψt, H-1′), 5.99 (m, H-5′), 5.88 (m, H-6′), 5.11 (m, H-3′), 4.48 (m, H-7′,H4′), 4.25-4.00 (m, H-8′), 2.46-2.37 (m, H-2′b), 2.30-2.19 (m, H-2′a), 2.12 (s, CH_3CO_2-), 2.11 (s, CH_3CO_2-), 1.95 (m, 5-CH_3). This material was used without further purification for the synthesis of **20**.

1-[3,8-Di-*O*-acetyl-2,5,6-trideoxy-β-D-ribo-(and α-L-lyxo)-octofuranosyl]thymine (20). A solution of crude **19** (517 mg, 1.35 mmol) in ethyl acetate (50 mL) containing 30% palladium-on-carbon (175 mg) was hydrogenated at ambient temperature and atmospheric pressure until the uptake of hydrogen ceased (about 1 h), then filtered, and evaporated to

dryness *in vacuo*. The product, a 1:1 mixture of diastereomers, was obtained as a glass: yield 488 mg (94%); R_f 0.22, $CHCl_3$-MeOH (95:5); MS (FAB), *m/z* 485 $(M + 1)^+$; ^1H NMR ($CDCl_3$) δ 8.75 (br s, 1, H-3, exchanges with D_2O), 7.16 (m, 1, H-6), [6.25 (dd, H-1′, $J_{1',2'a}$=8.4, $J_{1',2'b}$=6.1 Hz), 6.23 (dd, H-1′, $J_{1',2'a}$=8.1, $J_{1',2'b}$=6.1 Hz)] (2H), 5.04 (m, 1, H-3′), 4.15 (m, 1, H-8′), 4.05-3.94 (m, 2, H-8′,H-4′), 3.94-3.88 (m, 1, H-7′ of one diastereomer), 3.73-3.66 (m, 1, H-7′ of the other diastereomer), 2.45-2.36 (m, 1, H-2′b), 2.25-2.16 (m, 1, H-2′a), 2.11 and 2.10 (two s, 6, $2CH_3CO_2$-'s), 1.95 (d, 3, 5-CH_3, $^4J_{6,CH_3}$=1.0 Hz), 1.92-1.60 (m, 4, H-5′, H-6′). This material was used without further purification for the synthesis of **21**.

1-[3,8-Di-*O*-Acetyl-2,5,6-trideoxy-7-*O*-p-tolylsulfonyl-β-D-ribo-(and α-L-lyxo)-octofuranosyl]thymine (21). To a cold (ice bath) solution of **20** (488 mg, 1.27 mmol) in anhydrous pyridine (25 mL) was added p-toluenesulfonyl chloride (242 mg, 1.27 mmol). The resulting solution was kept at 3 °C for 24 h. The presence of starting compound as observed by TLC prompted the addition of 242 mg more p-toluenesulfonyl chloride to the cold solution. After another 24 h in the cold, the solution, which still contained starting compound, was treated with 121 mg more p-toluenesulfonyl chloride. After another 24 h (73 h total) in the cold, the solution was evaporated to dryness *in vacuo*. A CH_2Cl_2 solution of the residue was washed with ice cold dilute H_2SO_4, then saturated with aqueous $NaHCO_3$, then water, dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The syrup obtained was purified by flash chromatography on 80 g of silica gel using $CHCl_3$-MeOH (97:3) as eluting solution. The fractions containing pure product gave 291 mg of TLC homogeneous material. The fractions containing contaminated product were worked up and recolumned to give 265 mg of pure material (total yield 556 mg, 81%); R_f 0.54, $CHCl_3$-MeOH (95:5); MS (FAB), *m/z* 539 $(M + 1)^+$. This material was used without further purification for the synthesis of **10**.

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