a calibration curve obtained with standard solutions of the pure reference compounds in 0.01 M Tris-HCl buffer (pH 9.0) and MeCN (2%). The curve for an analytical experiment carried out with a 0.01 M concentration of the racemic epoxide **8** is also shown in Figure 1. The preparative incubations were stopped after ca. 50% conversion (GC). The unreacted epoxide **8** was immediately extracted from the cooled incubation mixture with hexane (3 × 20 mL) with vigorous shaking for 5 min. This treatment transferred all epoxide into the organic phase, while the diol **13** remained in the aqueous phase. The organic phase was dried, evaporated under atmospheric pressure, and subjected to Kugelrohr distillation [70 °C (0.5 mm)] to yield the pure epoxide **8**, $[\alpha]^{30}_{D}$ −68° (*c* 2.0, $CHCl_3$).

The aqueous phase remaining after the extraction of **8** was concentrated to dryness under reduced pressure and then extracted with hot AcOEt (3 × 20 mL). The organic phase was dried, evaporated and subjected to Kugelrohr distillation [110 °C (0.5 mm)] to give pure diol **13**, $[\alpha]^{30}_{D}$ +53° (*c* 3.0, $CHCl_3$). Both unreacted epoxide **8** and produced diol **13** were obtained in ca. 70% yield, with respect to the racemic starting material. Blank experiments carried out with pure racemic epoxide **8** and boiled microsomes showed that no spontaneous hydrolysis occurred even at the longest incubation times.

Determination of Enantiomeric Excess of 8. In the ^{1}H NMR spectrum of the racemic epoxide **8** (4.1 mg, $CDCl_3$), after addition of tris[3-((heptafluoropropyl)hydroxymethylene)-(+)-camphorato]europium(III) [$Eu(hfc)_3$] (7.4 mg) the doublet of Me at C-5 was shifted and split into two doublets at δ 2.65 and 2.89. A 1000-Hz spectral width for 8192 data points was used, as the better compromise between folding and digitalization. Both the integral and height values of the signals were evaluated on several spectra of the same experiment. Very good agreement was obtained from the mean values of the two methods, the latter being preferred for ease of evaluation. The maximum error was never higher than 2%.

The spectrum of the epoxide **8** [5.2 mg + 9.4 mg of $Eu(hfc)_3$] recovered from enzymatic hydrolysis after 50% conversion, showed only the doublet at δ 2.89. In order to evaluate the sensitivity of the determination of ee, 25 μL of a solution of racemic **8** (4.3 mg) and $Eu(hfc)_3$ (8.0 mg) in $CDCl_3$ (1.0 mL) was added to the same sample. This corresponded to the addition of 1% of L-**8** and produced no clearly detectable signal for L-**8** in the spectrum. However a second addition of 25 μL of the same solution produced a signal three times more intense than the noise. This provided sure evidence for an ee of at least 96% of the epoxide **8** recovered from the partial enzymatic hydrolysis.

Determination of Enantiomeric Excess of 13. The same procedure as for **8** was applied to the diacetyl derivative **15** obtained from **13** as described above. The addition of $Eu(hfc)_3$ (32.5 mg for 5.0 mg of racemic **15**) shifted and split the doublet of Me-5 into two doublets at δ 3.81 and 3.54. The spectrum of **15** obtained from the 50% conversion enzymatic product showed only the doublet at δ 3.81. A sensitivity control by addition of racemic **15** showed again an ee of at least 96%.

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Registry No. **1**, 75041-67-7; **2**, 75041-68-8; **3**, 75041-69-9; **4**, 108266-29-1; **5**, 108266-30-4; **6**, 108266-31-5; **7**, 108167-77-7; **8**, 108167-78-8; D-**8**, 102790-72-7; **13**, 108167-79-9; L-**13**, 102790-77-2; **14**, 108167-80-2; **15**, 108167-81-3; **16**, 108167-82-4; **17**, 108167-83-5; MEH, 9048-63-9.

A Novel Two-Step Conversion of 3′,5′-Di-*O*-tosylthymidine to 5′-Amino-5′-deoxythymidine Analogues with Inversion of the 3′-Hydroxyl Group

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The reaction of 3′,5′-di-*O*-tosylthymidine (**1a**) with methylamine at 35 °C gave a 75% yield of 2,5′-(methylimino)-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (**4a**). A similar reaction of **1a** with ammonia in Me_2SO at 78 °C gave a 41% yield of 2,5′-imino-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (**4b**). Hydrolysis of **4a** and **4b** in 1 N sodium hydroxide gave 1-[2,5-dideoxy-5-(methylamino)-β-D-*threo*-pentofuranosyl]thymine (**5a**) and the corresponding 5-amino analogue **5b**. Proposed intermediates in the conversion of **1a** to **4a** and **4b** are 2,3′-anhydro-1-[2-deoxy-5-*O*-(*p*-tolylsulfonyl)-β-D-*threo*-pentofuranosyl]thymine (**2a**) and the aminopyrimidine nucleosides **3a** (R = Me, H).

In the course of preparing analogues of 5′-(bromoacetamido)-5′-deoxythymidine (BAT), a compound with demonstrated anticancer activity,[1-6] a quantity of 5′-deoxy-5′-methylaminothymidine was required as an intermediate. This amine has been prepared[2] in near-quantitative yield by reaction of 5′-*O*-tosylthymidine with methylamine at 35 °C. When this reaction was carried out with 3′,5′-di-*O*-tosylthymidine (**1a**) present as a contaminant, the previously unreported 2,5′-iminonucleoside[7] **4a** was isolated as a byproduct (Scheme I). It was subsequently determined that **4a** could be obtained in 75% yield starting with

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(7) The *Chemical Abstracts* names for **4a** and **4b** for the 1967–1971 index period and the preferred names in accord with the IUPAC rules of organic nomenclature are, respectively, as follows: (6*R*,8*R*,9*R*)-6,7,8,9,10,11-hexahydro-8-hydroxy-3,11-dimethyl-6,9-epoxy-2*H*-pyrimido[1,2-*a*][1,3]diazocin-2-one and (6*R*,8*R*,9*R*)-6,7,8,9,10,11-hexahydro-8-hydroxy-3-methyl-6,9-epoxy-2*H*-pyrimido[1,2-*a*][1,3]diazocin-2-one. The current *Chemical Abstracts* names for **4a** and **4b** are as follows: [6*R*-(6α,8α,9α)]-6,7,8,9,10,11-hexahydro-8-hydroxy-3,11-dimethyl-6,9-epoxy-2*H*-pyrimido[1,2-*a*][1,3]diazocin-2-one and [6*R*-(6α,8α,9α)]-6,7,8,9,10,11-hexahydro-8-hydroxy-3-methyl-6,9-epoxy-2*H*-pyrimido[1,2-*a*][1,3]diazocin-2-one.

0022-3263/87/1952-2892$01.50/0

Scheme I

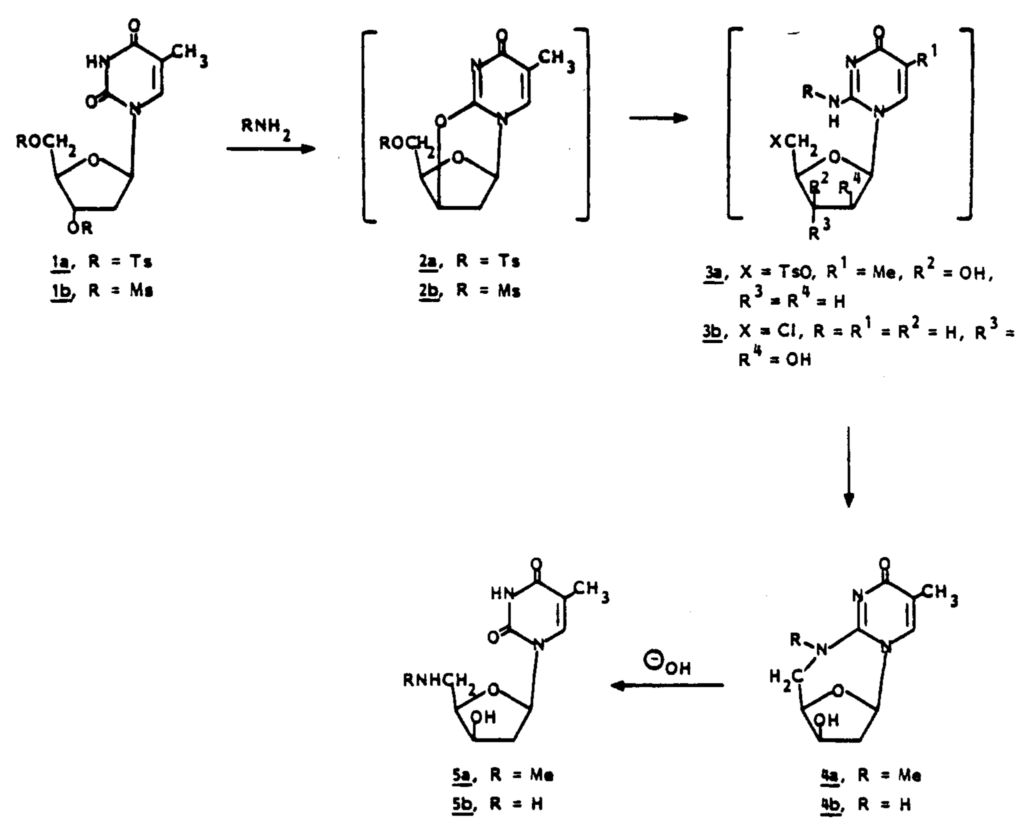
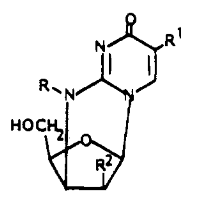


Table I. ^{1}H NMR Coupling Constants (Hz)

compd	$J_{1',2'b}$	$J_{1',2'a}$	$J_{2'a,2'b}$	$J_{2'b,3'}$	$J_{2'a,3'}$	$J_{3',4'}$	$J_{4',5'b}$	$J_{4',5'a}$	$J_{5'a,5'b}$	other
1a	6.9		3.9	7.0	3.2					
2a	3.9	0	13.0	1.0	1.0	1.0	3.9	7.1	10.5	
4a	8.2	2.4	13.9	10.5	6.3	6.4	2.8	1.7	14.3	$J_{3',3'\text{-OH}} = 4.0$
4b	8.2	2.7	13.8	10.5	6.8	6.6	1.6	2.6	13.9	$J_{3',3'\text{-OH}} = 4.0$ $J_{5'b\text{-NH}} = 4.5$
5a	8.5	2.6	14.6	5.2	0	3.3	5.2	6.3	12.5	
5b	8.6	2.6	14.4	5.2	0	3.2	5.9	6.5	13.0	

pure **1a**. The structure of **4a** is based on elemental analysis and UV, ^{1}H NMR, and mass spectral data. The UV spectrum of **4a** shows absorption peaks at 248 and 270 nm (0.1 N HCl) and at 229 and 270 nm (pH 7). This spectrum is very similar to that reported for the known 2,3′-imino-nucleoside **6a**, which absorbs at 244 and 270 nm (pH 0)



6a, R = R^1 = Me, R^2 = H
6b, R = R^2 = H, R^1 = Me
6c, R^1 = H, R^2 = OH

and at 228 and 269 nm (pH 6.9).[8] The ^{1}H NMR spectrum of **4a** (Figure 1; Table I) is consistent with the structure assigned. The H-1′ resonance appears as a doublet of doublets (dd), showing a narrow ($J_{1',2'a}$ = 2.4 Hz) and a wide ($J_{1',2'b}$ = 8.2 Hz) spacing, and the signals for H-2′a and H-2′b are widely separated multiplets with δ 1.65 (ddd) and 2.68 (ddd). These values indicate that the 3′-hydroxyl group is in the "up" configuration, consistent with the findings of Horton and Sakata[9] who observed that ^{1}H

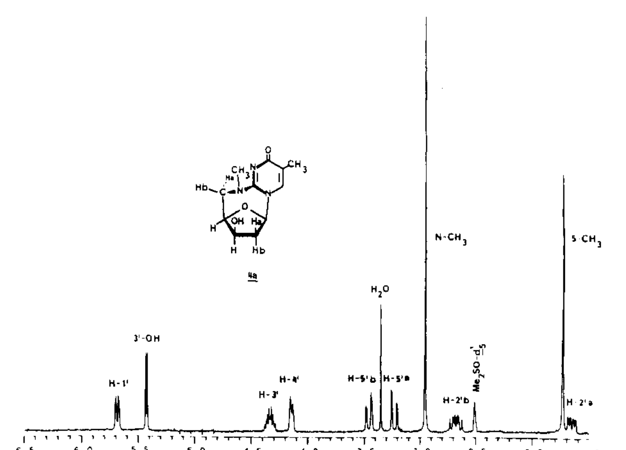


Figure 1.

NMR spectra of various purine and pyrimidine 2′-deoxy-nucleosides with H-1′ and H-3′ cis to each other in a furanose ring display a doublet of doublets for H-1′ and wide separation of the H-2′a and H-2′b signals. These ^{1}H NMR patterns for H-1′, H-2′a, and H-2′b are exactly the reverse of the situation observed for 2′-deoxynucleosides such as thymidine and 9-(2-deoxy-α-D-*arabino*-hexofuranosyl)-adenine with H-1′ and H-3′ trans to each other.[10,11] These

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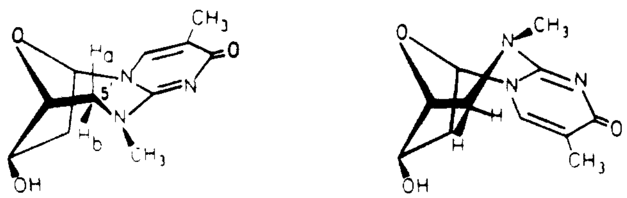
trans nucleosides show a pseudotriplet for H-1′, $J_{1',2'a} \cong J_{1',2'b} \cong 6.6$–$7.0$ Hz, and little separation of the signals for H-2′a and H-2′b.

In compound **4a**, the C-5′ signals (the AB part of an ABX spin system) consist of two doublets of doublets with δ 3.23 (H-5′a) and 3.46 (H-5′b) ($J_{4',5'a} = 1.7$ Hz, $J_{4',5'b} = 2.8$ Hz, $J_{5'a,5'b} = 14.3$ Hz), which is a spin pattern characteristic of 2,5′-anhydronucleosides.[8] The coupling constants indicate that the dihedral angles defined by H-4′, H-5′a, and H-5′b are both about 60°.[12] A Dreiding molecular model of **4a** in the chair conformation (A) dihedral angles very



A

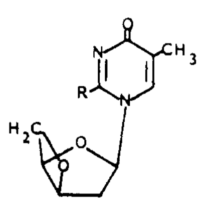
chair conformation

B

boat conformation

close to this predicted value. The boat conformation (B) for **4a** is ruled out, since a molecular model for B gives a dihedral angle between H-4′ and H-5′a close to 0°, and it follows that the coupling between these protons should be large ($J \cong 8$ Hz). The same arguments based on ^{1}H NMR data have been proposed for the chair conformation in the analogous seven-membered imino rings of 5′-deoxy-6,5′-iminothymidine[13] and 5′-deoxy-6,5′-imino-2′,3′-*O*-isopropylideneuridine.[14]

The relaxation of a proton may depend significantly on through-space dipolar coupling with a second set of protons, and its NMR signal intensity may be enhanced when the set of protons that assist in relaxation are saturated by irradiation. The phenomenon, termed the nuclear Overhauser effect (NOE), is highly dependent on the distance between protons.[15] The NOE experiment allowed us to make an unambiguous assignment of H-5′a and H-5′b as well as to further confirm the chair conformation (A) for the seven-membered ring in compound **4a** (Figure 1). Irradiation of the NCH_3 protons at δ 2.95 in **4a** gave 4.4% enhancement of H-5′b at δ 3.45, 0.5% enhancement of 3′-OH at δ 5.41, and no enhancement of H-5′a. In the chair conformation (A), the NCH_3 group is closer to H-5′b than to H-5′a; therefore, H-5′b would be expected to show a greater enhancement than H-5′a. The 0.5% enhancement of the 3′-OH further confirms the chair conformation as well as the up configuration of the 3′-OH, both requiring close proximity of the *N*-Me and 3′-OH. The presence of a 3′-OH doublet rules out the possibility of a cyclic ether structure such as **7a**.

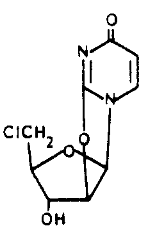


7a, R = NHMe
7b, R = OH

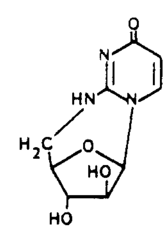
The mechanism of conversion of **1a** to **4a** is believed to involve formation of the anhydronucleoside **2a** followed by ring opening to give the 2-(methylamino)pyrimidine intermediate **3a** (R = Me), which can form **4a** by an intramolecular nucleophilic displacement of the tosyl group. Related nitrogen- and oxygen-bridged cyclonucleoside transformations have been widely studied.[16] Initial formation of **2a** is almost certain, based on reaction of **1a** with ammonia to give **2a** (see below) and the well-documented, high-yield conversion of the closely related **1b** to **2b** with ammonia,[17] sodium hydroxide,[18,19] or sodium benzoate.[18] Further attack of methylamine on **2a** is believed to occur at C-2, forming the 2-(methylamino)pyrimidine intermediate **3a** (R = Me). This assumption is based on the known reaction of **1b** or **2b** with aqueous base to give the cyclic ether **7b**.[19] Attack of hydroxide ion at C-2 opens the anhydro ring, and the resulting 3′-oxygen anion displaces mesyl to give **7b**. If the attack of base on **2b** displaced the mesyl group, formation of **7b** could not take place.

A similar mechanism involving the formation of the cyclic ether **7a** from **2a** and methylamine would appear to be reasonable. However, molecular models of **7a** indicate that the nitrogen of the methylamino group could not assume the appropriate configuration for a backside S_N2 attack on C-5′ without excessive bending of bonds. Also, at the temperature that reaction takes place (35 °C), the cyclic ether would be expected to be stable to base-catalyzed opening as demonstrated by the stability of **7b** to methoxide and refluxing 1 N sodium hydroxide.[19] The unusual stability of the tosyl group of **2a** to nucleophilic displacement is readily explained by the presence of the anhydro ring. This ring effectively blocks a backside displacement of the tosyl group, which for steric reasons must be oriented away from the anhydro ring.

Additional evidence for **3a** as an intermediate in this reaction is provided by Novotny et al.,[20] who reported the reaction of the 5′-chlorocyclouridine **8** with ethanolic am-



8



9

monia to give the isocytidine derivative **3b**, which on further treatment with ethanolic ammonia goes to the 2,5′-imino-bridged nucleoside **9**.

An initial attempt to prepare the unmethylated 2,5′-iminonucleoside **4b** by reaction of **1a** with ammonia at 35 °C resulted in formation of the anhydro intermediate **2a**, isolated in pure form in 68% yield. The poor solubility of **2a** in liquid ammonia may have prevented further reaction to give **4b**. The desired **4b** was obtained in 41% yield by heating **1a** with ammonia in Me_2SO at 78 °C. Treatment of the intermediate **2a** with ammonia in Me_2SO at 78 °C gave a 61% yield of **4b**.

The 2,5′-imino rings of **4a** and **4b** were hydrolyzed in 1 N sodium hydroxide at 70–80 °C to give low yields of

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1-[2,5-dideoxy-5-(methylamino)-β-D-*threo*-pentofuranosyl]thymine (**5a**) and the corresponding 5-amino analogue **5b**. Elemental analyses and spectral data (UV, MS, ^{1}H NMR) are consistent with the structures shown. The ^{1}H NMR of **5a** displays a doublet of doublets at δ 6.06 for H-1′ ($J_{1',2'a}$ = 2.6 Hz, $J_{1',2'b}$ = 8.5 Hz), and the signals for H-2′a and H-2′b are widely separated multiplets with δ 1.84 and 2.55, indicating a cis relationship between H-1′ and H-3′. The presence of a 3′-OH in the up configuration restricts rotation at C-5′, resulting in the C-5′ protons being displayed as two doublets of doublets with δ 2.78 and 2.84. The ^{1}H NMR of the isomeric 5′-deoxy-5′-(methylamino)-thymidine[2] in contrast displays a pseudotriplet at δ 6.13 for H-1′ ($J_{1',2'a}$ = 5.7 Hz, $J_{1',2'b}$ = 7.2 Hz), closely spaced multiplets for the H-2′ protons centered at δ 2.06 and 2.14, and a simple doublet for H-5′ with δ 2.68 ($J_{4',5'}$ = 5.4 Hz).

It is interesting to note that reported attempts to hydrolyze the isomeric 2,3′-imino-bridged nucleosides **6a** and **6b** in 7 N KOH at room temperature[8] or in refluxing 1 N sodium hydroxide[16a] to give the up 3′-amino-3′-deoxynucleosides were unsuccessful. Recently, Minamoto and co-workers[21] noted that similar 2,3′-imino-bridged uracil nucleosides with the general structure **6c** could be hydrolyzed in strong base to 1-[3-(arylamino)-3-deoxy-β-D-*lyxo*-furanosyl]uracils if the imino bridge of **6c** is substituted with an aryl group (R = phenyl, 4-methoxyphenyl). If R in this structure is alkyl or methylamino, hydrolysis of the imino bridge did not occur.

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 G(F) 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 flash chromatography[22] 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: the maxima are reported in nanometers ($\epsilon \times 10^{-3}$ M^{-1} cm^{-1}). The NMR spectra in Me_2SO-d_6 with tetramethylsilane as an internal reference were determined with a Nicolet NT 300NB spectrometer operating at 300.635 MHz. Chemical shifts (δ) quoted in the case of multiplets were measured from the approximate center. Where necessary, the chemical shift and coupling constant values (Table I; Experimental Section) for the non-first-order parts of the spectra were obtained from simulated spectra by employing the General Electric/Nicolet ITRACAL program for iterative analysis.[23] The NOE experiment was conducted in nondegassed solution. To minimize the effects of magnetic perturbations, eight fid's were acquired with the decoupler set at a desired frequency, and eight fid's were recorded with the decoupler off-resonance. The process was repeated until 1600 fid's had been accumulated for each experiment. Subsequent subtraction of the two spectra afforded the net enhancement. The mass spectral data were obtained with a Varian-MAT 311A mass spectrometer in the fast atom bombardment mode. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

3′,5′-Bis-*O*-(*p*-tolylsulfonyl)thymidine (1a). A solution of thymidine (10.0 g, 41.3 mmol) in anhydrous pyridine (200 mL) was cooled to −20 °C, treated with *p*-toluenesulfonyl chloride (23.6 g, 124 mmol), and allowed to stand at −20 °C for 4 days. Additional *p*-toluenesulfonyl chloride (7.88 g, 41.3 mmol) was added, and the solution was refrigerated (~4 °C) for 12 days and poured into a mixture of ice and saturated $NaHCO_3$ solution (1.5 L). The gummy precipitate was extracted into $CHCl_3$, and the extract was washed with aqueous $NaHCO_3$ and then H_2O and dried over $MgSO_4$. The evaporated extract was crystallized from 95% EtOH to give **1a**: yield 16.9 g (74%); mp 105–112 °C (Mel-Temp); UV (EtOH) [λ_{max}, nm ($\epsilon \times 10^{-3}$)] (pH 1) 227 (24.0), 264 (11.3), 273 sh (10.1), (pH 7) 226 (23.8), 264 (12.2), 273 sh (11.2), (pH 13) 227 (31.7), 264 (8.71), 273 sh (7.3); ^{1}H NMR δ 11.3 (br s, 1, H-3), 7.81 (d, 2, ortho H's of a tosyl ring, J = 8.2 Hz), 7.74 (d, 2, ortho H's of tosyl ring), 7.49 and 7.46 (2 d, 4, meta H's of two tosyl rings), (br s, 1 H-6), 6.05 (ψt, 1, H-1′), 5.02 (m, 1, H-3′), 4.20–4.04 (m, 3, H-4′, H-5′), 2.43 (s, 3, tosyl CH_3), 2.41 (s, 3, tosyl CH_3), 2.35 (m, 2, H-2′), 1.75 (s, 3, 5-CH_3); MS, m/z 551 $(M + 1)^+$. Anal. ($C_{24}H_{26}N_2O_9S_2$) C, H, N.

2,3′-Anhydro-1-[2-deoxy-5-*O*-(*p*-tolylsulfonyl)-β-D-*threo*-pentofuranosyl]thymine (2a). A solution of **1a** (4.00 g, 7.26 mmol) in liquid NH_3 (40 mL) was stirred at 35 °C in a glass-lined pressure vessel for 64 h and the excess NH_3 removed by volatilization. The residual solid was triturated with EtOH (25 mL), collected, dried, and crystallized from boiling MeOH (325 mL) with filtration and refrigeration to give pale yellow needles, which were collected, washed, and dried: yield 1.86 g (68%); mp 198 °C dec (lit.[24] mp 173–180 °C); UV (MeOH) [λ_{max}, nm ($\epsilon \times 10^{-3}$)] (pH 1) 228 (14.7), 254 (6.73), (pH 7) 228 (14.7), 254 (7.21), (pH 13) 228 (15.3), 254 (7.21); ^{1}H NMR δ 7.74 (d, 2, ortho H's of tosyl, J = 8.2 Hz), 7.49 (br s, 1, H-6), 7.44 (d, 2, meta H's of tosyl), 5.82 (d, 1, H-1′), 5.28 (br s, 1, H-3′), 4.43–4.38 (m, AB part of an ABM spin system, 2, H-4′, H-5′b), 3.88 (dd, M part of an ABM spin system, 1, H-5′a), 2.55 (dd, A part of an ABX spin system, 1 H-2′b), 2.49 (dt, B part of an ABX spin system, 1, H-2′a), 2.42 (s, 3, tosyl CH_3), 1.75 (s, 3, 5-CH_3); MS, m/z 379 $(M + 1)^+$. Anal. ($C_{17}H_{18}N_2O_6S$) C, H, N.

2,5′-(Methylimino)-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (4a). A solution of **1a** (1.56 g, 2.83 mmol) in liquid $MeNH_2$ (30 mL) was stirred in a glass-lined pressure vessel at 35 °C for 64 h. The excess $MeNH_2$ was evaporated and the residue in EtOH evaporated to dryness in vacuo. A solution of the residue in a minimum of 5:1 $CHCl_3$–MeOH was applied to a flash column of 80 g of silica gel and developed with the same solvent. The product fraction (R_f 0.5 in 5:1 $CHCl_3$–MeOH) was evaporated to dryness and the residue in EtOH again evaporated to dryness. A solution of the residue in boiling EtOH (10 mL) was filtered and allowed to crystallize slowly to give pure **4a**: yield 500 mg (75%); mp ca. 235 °C dec; UV (MeOH) [λ_{max}, nm ($\epsilon \times 10^{-3}$)] (pH 1) 214 (12.8), 247 (10.7), 272 (8.06 sh), (pH 7), 232 (26.2), 270 (5.46 sh), (pH 13) 232 (25.6), 270 (5.14 sh); ^{1}H NMR δ 7.55 (q, 1, H-6, J = 1.1 Hz), 5.68 (dd, 1, H-1′), 5.41 (d, 1, 3′-OH), 4.33 (m, 1, H-3′), 4.14 (dt, 1, H-4′), 3.45 (dd, B part of an ABX spin system, 1, H-5′b), 3.23 (dd, A part of an ABX spin system, 1, H-5′a), 2.95 (s, 3, NCH_3), 2.68 (ddd, 1, H-2′b), 1.73 (d, 1, 5-CH_3, J = 1.1 Hz), 1.66 (ddd, 1, H-2′a); MS, m/z 238 $(M + 1)^+$. Anal. ($C_{11}H_{15}N_3O_3$) C, H, N.

2,5′-Imino-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine (4b). (A) Reaction of 1a with NH_3. A solution of **1a** (6.00 g, 10.9 mmol), Me_2SO (50 mL), and liquid NH_3 (100 mL) was stirred in a glass-lined pressure vessel at 78 °C for 21 h. The reaction mixture was evaporated to dryness under high vacuum and the residue extracted with 6:1 $CHCl_3$–MeOH (15 mL). A solution of the evaporated extract in $CHCl_3$ (10 mL) was applied to a flash column of 125 g of silica gel prepared in 9:1 $CHCl_3$–MeOH and eluted with the same solvent. The product fraction was evaporated to give a solid (1.54 g), which was dissolved in MeOH (150 mL), stirred with Dowex 1X8 ($^-$OH) ion-exchange resin, filtered, and evaporated to dryness. Crystallization of the residue from hot MeOH (10 mL) gave pure **4b**: yield 396 mg; mp ca. 236 °C dec (Mel-Temp). Trituration of the evaporated mother liquor with EtOAc gave additional product: yield 624 mg; mp ca. 231 °C dec; total yield 41%; UV (MeOH) [λ_{max}, nm ($\epsilon \times 10^{-3}$)] (pH 1) 238 (8.88), 259 (8.34), (pH 7) 222 (26.5), 265 sh (4.72), (pH 13) 222 (26.2), 265 sh (4.88); ^{1}H NMR δ 7.48 (q, 1, H-6, J = 1.0 Hz), 7.17 (d, 1, NH, $J_{5'b}$ = 4.5 Hz), 5.66 (dd, 1, H-1′), 5.31 (d, 1, 3′-OH),

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4.30 (m, 1, H-3′), 4.14 (dt, 1, H-4′), 3.46 (ddd, B part of an ABMX spin system, 1, H-5′b), 3.13 (dd, A part of an ABMX spin system, 1, H-5′a), 2.72 (ddd, 1, H-2′b), 2.17 and 2.16 (d, 1, 5-CH_3 and ddd, 1, H-2′a); MS, m/z 224 $(M + 1)^+$. Anal. ($C_{10}H_{13}N_3O_3 \cdot 0.4H_2O$) C, H, N.

(B) Reaction of 2a with Ammonia. A stirred mixture of **2a** (1.00 g, 2.64 mmol), Me_2SO (10 mL), and liquid NH_3 (40 mL) was heated at 78 °C in a glass-lined stainless steel pressure vessel for 20 h. The reaction mixture was evaporated to dryness under high vacuum and an extract of the residue in 5:1 $CHCl_3$–MeOH (3 mL) applied to a flash column of 45 g of silica gel, which was then developed with the same solvent mixture. The product fraction was evaporated to dryness and the residue (617 mg) further purified as above on a second column of silica gel (80 g) to give crude **4b** (610 mg). A solution of this solid in MeOH (50 mL) was stirred with Dowex IX8 ($^-$OH) resin (2.0 g), filtered, and evaporated to a solid, which was triturated with EtOAc (1 mL), collected, and dried: yield 369 mg (61%); mp ca. 230 °C dec (Mel-Temp). The properties of this compound were identical with those described in A.

1-[2,5-Dideoxy-5-(methylamino)-β-D-*threo*-pentofuranosyl]thymine (5a). A stirred solution of **4a** (254 mg, 1.09 mmol) in 1 N NaOH (2.5 mL, 2.5 mmol) was heated in an oil bath at 70–75 °C for 20 h, adjusted to pH 8.5 with 1 N HCl, refrigerated, filtered, and evaporated to dryness under high vacuum. An EtOH (2 × 5 mL) extract of the residue was evaporated to an oil, which was purified on a flash column of 10 g of silica gel with MeOH as the eluting solvent. The product fraction was evaporated to dryness and further purified on a flash column of 45 g of silica gel with 20:10:1 $CHCl_3$–MeOH–NH_4OH as the eluting solvent. The product fraction was evaporated to dryness and the residue triturated with EtOAc (2 mL) to give a white powder, which was collected, washed with EtOAc, and dried at 56 °C: yield 64 mg (23%); mp 184 °C; UV (MeOH) [λ_{max}, nm ($\epsilon \times 10^{-3}$)] (pH 1) 266 (9.67), (pH 7) 266 (9.74), (pH 13) 266 (7.46); ^{1}H NMR δ 7.83 (q, 1, H-6, J = 1.0 Hz), 6.06 (dd, 1, H-1′), 4.22 (dd, 1, H-3′), 3.82 (dt, 1, H-4′), 2.84 (dd, B part of an ABX spin system, 1, H-5′b), 2.78 (dd, A part of an ABX spin system, 1, H-5′a), 2.55 (ddd, 1, H-2′b), 2.31 (s, 3, NCH_3), 1.84 (dd, 1, H-2′a), 1.77 (d, 3, 5-CH_3, J = 1.0 Hz); MS, m/z 256 $(M + 1)^+$. Anal. ($C_{11}H_{17}N_3O_4 \cdot 0.2H_2O$) C, H, N.

1-(5-Amino-2,5-dideoxy-β-D-*threo*-pentofuranosyl)thymine (5b). A stirred suspension of **4b** (585 mg, 2.62 mmol) and 1 N NaOH (6 mL) was heated in an oil bath at 80 °C for 11 h, adjusted to pH 8.5 with 1 N HCl, and evaporated to dryness in vacuo. The residue was evaporated with EtOH (2 × 25 mL) to remove H_2O. A solution of the residue in 20:10:1 $CHCl_3$–MeOH–NH_4OH (10 mL) was applied to a flash column of 125 g of silica gel and developed with the same solvent. The evaporated product was further purified on a second flash column of silica gel (45 g) to give, after evaporation of the product fraction, an oil, which was dissolved in 1:1 $CHCl_3$–EtOH (3 mL), filtered, and evaporated to an oil that solidified. The crystalline mass was triturated with $CHCl_3$, collected, washed with $CHCl_3$, and dried: yield 60 mg (9%); mp 190–195 °C (Mel-Temp); UV (MeOH) [λ_{max}, nm ($\epsilon \times 10^{-3}$)] (pH 1) 266 (9.41), (pH 7) 266 (9.33), (pH 13) 266 (7.20); ^{1}H NMR δ 7.82 (q, 1, H-6, J = 1.0 Hz), 6.06 (dd, 1, H-1′), 4.25 (dd, 1, H-3′), 3.69 (td, 1, H-4′), 3.44 (q, CH_2 of EtOH), 2.89 (dd, B part of an ABX spin system, 1, H-5′b), 2.83 (dd, A part of an ABX spin system, 1, H-5′a), 2.55 (ddd, 1, H-2′b), 1.84 (dd, 1, H-2′a), 1.76 (d, 3, 5-CH_3, J = 1.0 Hz), 1.06 (t, CH_3 of EtOH); MS, m/z 242 $(M + 1)^+$. Anal. ($C_{10}H_{15}N_3O_4 \cdot 0.2C_2H_5OH \cdot 0.7H_2O$) C, H, N.

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Stereochemical Studies of Polyols from the Polyene Macrolide Lienomycin

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Because the polyene macrolides are characterized by noncrystallinity and the presence of numerous chiral hydroxyl groups, elucidation of their stereochemistry has constantly been a challenging problem; to date the full stereochemistry of only amphotericin B is known. Taking lienomycin as an example, we have devised methods to determine the relative and absolute configurations of acyclic polyols. This has resulted in clarifying 10 of the 15 chiral centers in the aglycone.

Taking lienomycin, a polyene antibiotic with 15 chiral centers in the macrolactone ring, as an example, we have attempted to devise general approaches for determining the relative and absolute configurations of their polyol moieties. The method consists of (a) preparation of cleaved fragments by ozonolysis, etc.; (b) conversion of the 1,3-diol groups into 6-membered isopropylidenes to determine the relative configurations of *sec*-hydroxyl and *sec*-methyl groups; and (c) conversion of cleaved fragments into 6-membered hemiacetal dibenzoates to establish their absolute configurations by the dibenzoate chirality method. The absolute configurations of simpler fragments are determined by direct correlation with known or synthetic specimen.

The macrolide antibiotic lienomycin is produced by *Actinomyces diastatochromogenes* var. lienomycine[1] and

