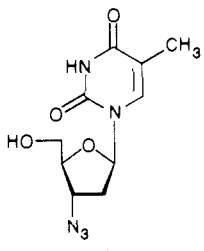
1-(3-Cyano-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (Cyanothymidine): Synthesis and Antiviral Evaluation against Human Immunodeficiency Virus

Colin W. Greengrass,*,† David W. T. Hoople,† Stephen D. A. Street,† Fiona Hamilton,† Michael S. Marriott,† Jon Bordner,‡ Angus G. Dalgleish,§ Hiroaki Mitsuya,∥ and Samuel Broder∥

Pfizer Central Research, Sandwich, Kent, CT13 9NJ, United Kingdom, Pfizer Central Research Laboratories, Groton, Connecticut 06340, Clinical Research Centre, Harrow, Middlesex, United Kingdom, and National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892. *Received February 1, 1988*

1-(3-Cyano-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (cyanothymidine) (**3a**) has been prepared by an unambiguous route starting from D-xylose. The relative and absolute stereochemistry of **3a** and its anomeric isomer **9** have been confirmed by NOE experiments and by X-ray diffraction analysis. In antiviral tests vs HIV **3a** was shown to be inactive, a surprising result in view of a preliminary disclosure claiming potent anti-HIV activity. The activity previously assigned to **3a** is believed to be due to contamination of that sample with the known antiviral nucleoside analogue **5b**.

The etiologic agent of the acquired immunodeficiency syndrome (AIDS) is the human immunodeficiency virus (HIV), a retrovirus.[1] An essential step in the replicative cycle of all retroviruses is the synthesis of DNA from viral RNA and this is accomplished by using the viral enzyme, reverse transcriptase. Inhibition of this enzyme is the mechanism of action of certain antiretroviral nucleoside analogues such as 3′-azido-3′-deoxythymidine[2] (AZT) (**1**) and 2′,3′-dideoxycytidine (**2**).[3] These compounds are able



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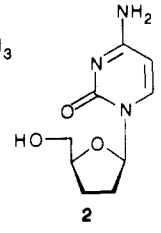

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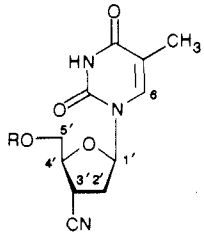
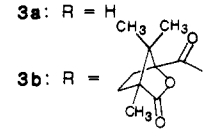


3a: R = H

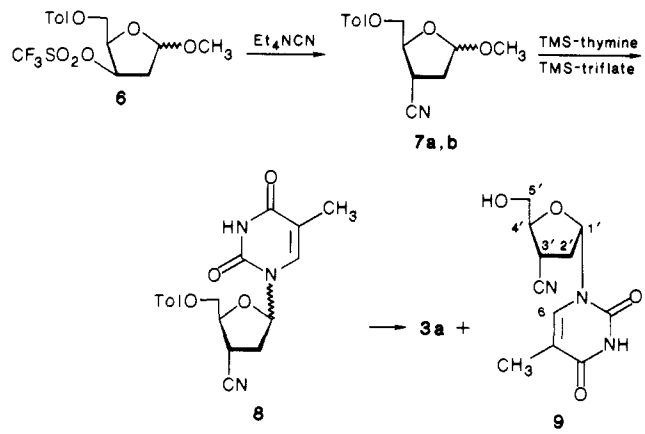


3b: R =

to enter cells and are converted by cellular enzymes to their triphosphates, which are potent competitive inhibitors of reverse transcriptase. Since these analogues do not have the 3′-hydroxyl group of the natural substrates, DNA chain elongation is precluded.

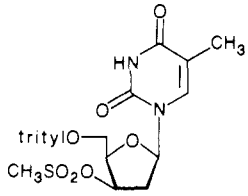
As part of an investigation of nucleoside analogues as potential anti-HIV agents for AIDS therapy, we have studied novel thymidine derivatives having various 3′-substituents which, like azide, would block DNA chain elongation. The 3′-cyano group was a substituent of particular interest since it is electronically similar to hydroxyl and azide (inductive effect *F* values:[4] CN +0.51; OH +0.29; N_3 +0.30) and has low steric bulk (molar refractivity values: CN 6.33; OH 2.85; N3 10.2). Thus one could speculate that 1-(3-cyano-2,3-dideoxy-β-D-*eryth*o-pentofuranosyl)thymine (**3a**) might be a substrate for intracellular kinases and the resulting triphosphate would inhibit reverse transcriptase. In this study we report the unambiguous synthesis of **3a** and study its action as a potential antiretroviral agent. Since completing this work several preliminary communications describing alternative chemical syntheses[5] of **3a** have been reported, but full biological evaluation has not been detailed.

Scheme I



Chemistry

The synthesis of **3a** was attempted initially by nucleophilic displacement of the known methanesulfonate[6] (**4**)



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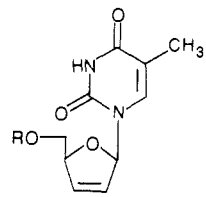

5a: R = trityl
b: R = H

with cyanide. However, under various conditions (NaCN or Et_4NCN in THF or DMF) the major product, formed in high yield, was the 2′,3′-didehydro nucleoside (**5a**). Thus the following alternative strategy was employed, Scheme I.

The known trifluoromethanesulfonate[7] (**6**), prepared from D-xylose, was reacted with tetraethylammonium cyanide[8] in acetonitrile to give an anomeric mixture of the

† Pfizer Central Research, Sandwich, U.K.
‡ Pfizer Central Research Laboratories, Groton, CT.
§ Clinical Research Centre.
∥ National Institutes of Health.

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Table I. NOE Data for **3a** and **9** in $(CD_3)_2CO$ at 300 MHz

	observed NOE (%)									
	3a					**9**				
multiplet irradiated	H-1′	H-3′	H-4′	CH_2-5′	H-6	H-1′	H-3′	H-4′	CH_2-5′	H-6
H-1′		0	3.6	0	4.6		3.0	0	0	5.2
H-3′	0		1.7	0	3.5	3.2		3.5	1.0	0
H-4′	3.8	5.6		4.7	0	0	2.6		4.5	4.3
CH_2-5′	0	1.9	13.3		0.8	0	2.9	11.3		0
H-6	3.3	3.1	0	0		4.7	0	3.7	0	

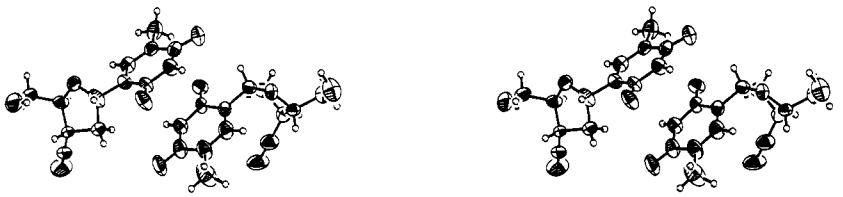

Figure 1. Stereoplot for compound **9**.

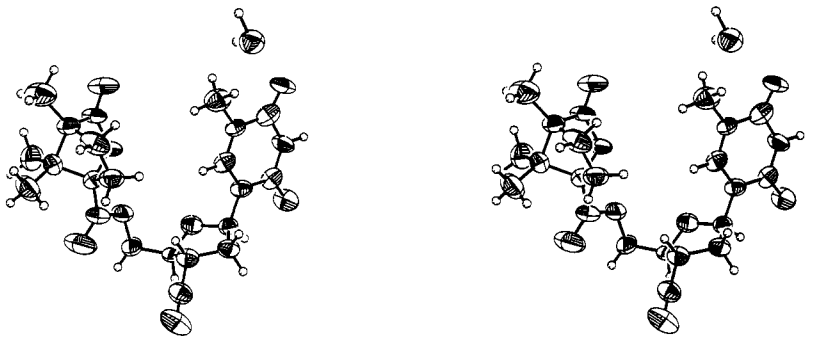

Figure 2. Stereoplot for compound **3b**.

3′α-nitrile derivatives (**7a,b**) (38%), which were separable by chromatography. Coupling of either anomer or a mixture with thymine using the trimethylsilyl triflate method of Vorbrüggen[9] gave a 1:1 anomeric mixture of protected nucleosides (**8**) (45%), which was not separated at this stage. Deprotection of the mixture followed by chromatographic purification provided the target cyano nucleoside (**3a**) (33%) and its anomer (**9**) (32%).

The initial assignment of stereochemistry at C-1′ in **3a** and **9** was made by using nuclear Overhauser effect (NOE) studies as follows. In both compounds H-4′ was assumed to be α-orientated, below the plane of the deoxyribose ring, as defined by the starting material and method of synthesis. In one isomer irradiation of H-4′ produced a strong NOE (3.6%) at H-1′, suggesting that H-1′ and H-4′ are cis orientated: this isomer was therefore assigned structure **3a**. In the other isomer irradiation of H-4′ produced a strong NOE (4.3%) at H-6 of the thymine base, but no NOE at H-1′ was observed. This result indicates that the base and H-4′ are cis orientated and supports the assignment of structure **9**. Complete NOE data reported in Table I are fully in accord with these assignments of configuration.

It was of interest to confirm these stereochemical assignments by X-ray analysis. Crystals of **9** proved suitable for this study, but derivatization of **3a** was necessary to produce satisfactory crystals, thus crystalline derivative **3b** was produced by esterification of **3a** with (*l*)-(*S*)-(−)-camphanic acid. X-ray analysis confirmed the above stereochemical assignments and the absolute stereochemistry of **3b** by reference to the known chirality of (−)-camphanic acid. Figures 1 and 2 are stereoviews of **9** and **3b**, respectively. In summary these studies confirm that **3a** has the target structure in which the base is β-orientated as in natural thymidine and the 3′-cyano substituent replaces the 3′α-hydroxyl group of thymidine. In contrast, isomer **9** has the base in the unnatural α-configuration.

Recent X-ray studies of **1**[10-12] show that two conformations are present in the crystal asymmetric cell. The conformation of the deoxyribose ring may be defined by using the pseudorotation concept.[13] One conformer has pseudorotation phase angle $P = 174°$, corresponding to the commonly found C2′-endo,C3′-exo conformation, whereas the other form has $P = 213°$, corresponding to the unusual C3′-exo,C4′-endo conformation. Though it has been suggested[10] that the latter higher energy conformation may represent the biologically active form of **1**, it has been alternatively proposed[11] that the two crystalline conformations may reflect different intermolecular interactions related to crystal packing. Interestingly, X-ray studies of **2**[14] reveal that this antiretroviral nucleoside also adopts the C3′-exo,C4′-endo conformation ($P = 207°$). In contrast to these findings, **3b** adopts a C4′-exo conformation ($P = 51.7°$) that though energetically favored[15] is infrequently observed in nucleosides. The torsion angles about the glycosyl bond and the C4′–C5′ bond of **3b** are within the

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Table II. Comparative Activity of **3a** and **1** as Inhibitors of HIV Replication

HIV isolate	cell line	ED90,[a] μg/mL 3a	1	5b
CBL-1	C8166[b]	>100	0.1	ND
RF	C8166[b]	>100	1.0	1.0
HTLF III_B	ATH8[c]	>2.5[d]	<0.25	<0.25

[a] Effective dose of compound achieving 90% protection of cells against the cytopathic effect of HIV. [b] Laboratory of A.G.D. [c] Laboratory of H.M. and S.B. [d] At 25 μg/mL it was not possible to assess the antiviral activity of **3a** owing to cytotoxicity in infected and control cells. This was probably due to DMSO, which was used as a diluent (final concentration, 2.5% v/v).

commonly found ranges (χ = 236°, γ = 52.7°, respectively). Though these conformational observations of **3b** are of general interest, it is not appropriate to extrapolate conclusions regarding the conformation of the parent nucleoside **3a** since the bulky 5′-ester group of **3b** is likely to influence crystal packing and hence the molecular conformation.

Results and Discussion

3a was tested for anti-HIV activity in permissive human T-cell lines using **1** as the reference standard in all tests, Table II. Two cell lines and three isolates of HIV were used, but in all test systems **3a** was ineffective in protecting the cells from the progressive effects of HIV infection. As expected, **1** was highly active in all tests. In addition, **3a** was also found inactive vs Moloney murine Leukemia virus in the standard XC overlay assay[16] using **1** as the standard (data not shown).

The finding that **3a** is inactive vs HIV contrasts earlier reports[17] that it was a potent antiviral agent. The sample of **3a** used in the previously reported work was obtained in low yield via lithium cyanide reaction of **4**[18]; however, in our hands the major product of this preparation (after deprotection) was the known olefin (**5b**), which had potent anti-HIV activity in our test systems (Table II). 2′,3′-Unsaturated analogues of nucleosides are known to inhibit HIV replication in vitro;[19] thus, it is probable that the activity assigned to **3a** resulted from contamination with **5b**, a potent inhibitor of HIV replication.[20]

Two explanations can be suggested to rationalize the inactivity of a nucleoside analogue such as **3a** as an inhibitor of HIV replication. Firstly, the nucleoside triphosphate may not be formed in cells. 3′-Amino-3′-deoxythymidine appears[21] to be such a compound: the triphosphate is a potent inhibitor of isolated reverse transcriptase, but the nucleoside itself has only slight antiretroviral activity. Formation of a nucleoside triphosphate requires that following entry into cells the nucleoside serves as a substrate for the set of enzymes catalyzing triphosphorylation: these enzymes have a demanding structure–activity relationship for unnatural substrates. A second possibility is that although a triphosphate may be produced, it is not an inhibitor of reverse transcriptase. In this context it is worth noting that studies[22] of reverse transcriptase inhibition by nucleoside triphosphates show a restricted SAR of 3′-modification. Further work would be needed to clarify whether one of these possibilities, or features of both, explains the inactivity of **3a**.

Experimental Section

Chemistry. Melting points were determined on a Büchi apparatus in glass capillary tubes and are uncorrected. Spectroscopic data for all compounds were recorded on Perkin-Elmer 197 (IR), Perkin-Elmer 141 (optical rotation), and Nicolet QE300 (NMR) instruments and were consistent with assigned structures. Silica gel TLC was performed on 60F-254 precoated plates (Merck) and column chromatography was conducted on silica gel (Merck, 230–400 mesh). Where elemental analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical values. Accurate mass data were obtained on a VG 7070E instrument under FAB conditions in glycerol/thioglycerol.

3-Cyano-2,3-dideoxy-1α- and -1β-*O*-methyl-5-*O*-(4-methylbenzoyl)-D-*erythro*-pentofuranoside (7a,b). A solution of 2-deoxy-1-*O*-methyl-5-*O*-(4-methylbenzoyl)-3-*O*-[(trifluoromethyl)sulfonyl]-D-*threo*-pentofuranoside[7] **6** (5.62 g, 0.014 mol) in dry acetonitrile (40 mL) at 0 °C was treated with tetraethylammonium cyanide (3.9 g, 0.025 mol) and the solution was stirred for 2 h at 0 °C. The mixture was kept at room temperature overnight and evaporated to dryness and the residue was dissolved in H_2O (50 mL) and CH_2Cl_2 (50 mL). Separation of the organic layer, followed by drying and evaporation to dryness in vacuo, gave a light brown oil (3.6 g). Silica gel chromatography in hexane/ether (8:2) with gradient elution to hexane/ether (7:3) gave both anomers **7a,b** separately as colorless oils (the anomeric stereochemistry was not assigned).

Anomer i: 750 mg (19%); R_f (Et_2O–hexane, 1:1) 0.32; IR (CH_2Cl_2) 2250 cm^{-1} (CN); ^1H NMR ($CDCl_3$) δ 2.31–2.53 (m, 2 H, 2 H-2), 2.44 (s, 3 H, CH_3), 3.31 (s, 3 H, OCH_3), 3.28–3.40 (m, 1 H, H-3), 4.42–4.53 (m, 2 H, 2 H-5), 4.58–4.62 (m, 1 H, H-4), 5.12–5.14 (m, 1 H, H-1), 7.27–7.30 and 7.99–8.01 (m, 4 H, C_6H_4).

Anomer ii: 740 mg (19%); R_f (Et_2O–hexane, 1:1) 0.25; IR (CH_2Cl_2) 2240 cm^{-1} (CN); ^1H NMR ($CDCl_3$) δ 2.29–2.58 (m, 2 H, 2 H-2), 2.44 (s, 3 H, CH_3), 3.02–3.09 (m, 1 H, H-3) 3.43 (s, 3 H, OCH_3), 4.47–4.62 (m, 3 H, H-4 and 2 H-5), 5.18–5.20 (m, 1 H, H-1), 7.27–7.28 and 7.94–8.00 (m, 4 H, C_6H_4).

1-(3-Cyano-2,3-dideoxy-5-*O*-(4-methylbenzoyl)-D-pentofuranosyl)thymine, α- and β-Anomers (8). A suspension of thymine (678 mg, 5.38 mmol), bis(trimethylsilyl)acetamide (4 mL 16.14 mmol), and a 1:1 mixture of anomers **7a,b** (740 mg, 2.69 mmol) in dry CH_3CN (15 mL) was stirred at reflux for 15 min. The resulting solution was cooled, treated with trimethylsilyl trifluoromethanesulfonate (776 mg, 3.5 mmol), and stirred at reflux for a further 3 h. After cooling, H_2O (50 mL) was added and the mixture extracted with EtOAc (2 × 50 mL). The combined EtOAc solutions were dried (Na_2SO_4) and evaporated to an oil. Silica gel chromatography (hexane to EtOAc gradient) then gave **8** as a mixture of anomers (1:1): 620 mg (62%); mp 122–125 °C; R_f (EtOAc) 0.58; IR (CH_2Cl_2) 2250 cm^{-1} (CN); ^1H NMR ($CDCl_3$) δ 1.75 and 2.00 (2 × s, 3 H, 5-CH_3), 2.45 (s, 3 H, CH_3), 2.53–3.05 (m, 2 H, 2 H-2′), 3.28–3.36 and 3.47–3.56 (2 × m, 1 H, H-3′), 4.52–4.95 (m, 3 H, 2H-5′ and H-4′), 6.03–6.08 and 6.16–6.20 (2 × m, 1 H, H-1′), 7.13 and 7.23 (2 × s, 1 H, H-6), 7.28–7.33 and

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7.94–7.98 (2 × m, 4 H, C_6H_4), 8.33 and 8.35 (2 × brs, 1 H, NH). Anal. ($C_{19}H_{19}N_3O_5$) C, H, N.

1-(3-Cyano-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (3a) and Its α-Anomer (9). A suspension of K_2CO_3 (0.56 g, 4.06 mmol) and 8 (1.0 g, 2.71 mmol) in MeOH (50 mL) was stirred at room temperature for 1.5 h, forming a clear solution. Acetic acid (3 mL) and toluene (30 mL) were then added and the solution was evaporated to dryness. Silica gel chromatography of the preadsorbed (MeOH) product in EtOAc with gradient elution to 5% MeOH gave anomers **3a** and **9** separately.

β-Anomer **3a**: 227 mg (33%); foam; R_f (EtOAc) 0.29; IR (film) 2250 cm^{-1} (CN); ^1H NMR (CD_3OD) δ 1.88 (d, 3 H, 5-CH_3, J = 1.1), 2.58 (ddd, 1 H, H-2a′, $J_{1',2a'}$ = 3.8, $J_{2a'}$ = 13.7, $J_{2a',3'}$ = 8.8), 2.74 (ddd, 1 H, H-2b′, $J_{1',2b'}$ = 7.3, $J_{2b',3'}$ = 9.5), 3.54 (q (apparent), 1 H, H-3′), 3.79 (dd, 1 H, H-5a′, $J_{5a',5b'}$ = 12.5, $J_{4',5a'}$ = 2.9), 3.95 (dd, 1 H, H-5b′, $J_{4',5b'}$ = 2.9), 4.25 (dt, 1 H, H-4′, $J_{3',4'}$ = 8.8), 6.17 (dd, 1 H, H-1′), 7.77 (q, 1 H, H-6). Anal. ($C_{11}H_{13}N_3O_4$) C, H, N. $[\alpha]_{365}$ +156°, $[\alpha]_{436}$ +72.4°, $[\alpha]_{546}$ +34.3°, $[\alpha]_{578}$ +29.2°, $[\alpha]_{589}$ +27.4° (c = 1%, MeOH, 25 °C). α-Anomer **9**: yield 220 mg (32%); mp 171–172 °C; R_f (EtOAc) 0.18; IR (film) 2250 cm^{-1} (CN); ^1H NMR (CD_3OD) δ 1.92 (d, 3 H, 5-CH_3, J = 1.1), 2.55 (ddd, 1 H, H-2a′, $J_{1',2a'}$ = 6.3, $J_{2a',2b'}$ = 13.5, $J_{2a',3'}$ = 8.7), 2.89 (ddd, 1 H, H-2b′, $J_{1',2b'}$ = 6.3, $J_{2b',3'}$ = 8.4), 3.46 (ddd, 1 H, H-3′), 3.66 (dd, 1 H, H-5a′, $J_{5a,5b'}$ = 12.4, $J_{4',5a'}$ = 3.5), 3.78 (dd, 1 H, H-5b′, $J_{4',5b'}$ = 3.5), 4.65 (dt, 1 H, H-4′, $J_{3',4'}$ = 7.3) 6.10 (t, 1 H, H-1′), 7.57 (q, 1 H, H-6). Anal. ($C_{11}H_{13}N_3O_4$) C, H, N. $[\alpha]_{365}$ −34.3°, $[\alpha]_{436}$ +20.3°, $[\alpha]_{546}$ +25.1°, $[\alpha]_{578}$ +23.6°, $[\alpha]_{589}$ + 23.5° (c = 1%, MeOH, 25 °C).

1-(3-Cyano-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine, 5′-*O*-Camphanate (3b). A solution of **3a** (50 mg, 0.2 mmol) in dry pyridine (1 mL) was treated at 0 °C with (−)-camphanic acid chloride (Fluka) (70 mg, 0.32 mmol) and kept for 18 h at 0 °C. Water (20 mL) and CH_2Cl_2 (20 mL) were added and the mixture was neutralized (concentrated HCl). The CH_2Cl_2 layer was then washed with 5% aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated in vacuo to a white foam. Crystallization from EtOAc/hexane gave **3b** as a white solid: 62 mg (72% yield); mp 172–174 °C; R_f (EtOAc) 0.47; ^1H NMR ($CDCl_3$) δ 0.98 (s, 3 H, CH_3), 1.10 (s, 3 H, CH_3), 1.15 (s, 3 H, CH_3), 1.66–1.79, 1.97–2.12, and 2.45–2.54 (m, 4 H, camphanate 5-CH_2 and 6-CH_2), 1.95 (s, 3 H, 5-CH_3), 2.66–2.80 (m, 2 H, 2H-2′), 3.44–3.53 (m, 1 H, H-3′), 4.42–4.47 (m, 1 H, H-4′), 4.54–4.55 (m, 2 H, 2H-5′), 6.05–6.08 (m, 1 H, H-1′), 7.08 (s, 1 H, H-6), 8.33 (brs, 1 H, NH); accurate mass MH^+ 432.1758 (observed), $C_{21}H_{25}N_3O_7{\cdot}H^+$ requires 432.1769.

Single-Crystal X-ray Analyses. Representative crystals for compounds **9** and **3b** were surveyed and 1-Å data sets (maximum sin θ/λ = 0.5) collected on a Nicolet R3m/μ diffractometer. Compound **9** belonged to the orthorhombic space group $p2_12_12_1$ with a = 5.696 (2), b = 11.014 (4), and c = 38.84 (1) Å. The asymmetric unit contained two molecules with slightly different conformations. Compound **3b** belonged to the orthorhombic space group $p2_12_12_1$ with a = 6.316 (2), b = 9.269 (2), and c = 37.74 (1) Å. Atomic scattering factors were taken from the International Tables for X-Ray Crystallography.[23] All crystallographic calculations were facilitated by the SHELXTL[24] system. All diffractometer data were collected at room temperature by using copper Kα radiation (λ = 1.54178 Å).

Trial structures were obtained by direct methods. These trial structures refined routinely. Hydrogen positions were calculated wherever possible. The methyl hydrogens and the hydrogens on nitrogen and oxygen were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least-squares refinement were all less than 0.1 of their corresponding standard deviations. The final R index for compound **9** was 0.058 and 0.057 for compound **3b**. A final difference Fourier revealed no missing or misplaced electron density. Because compound **9** did not contain a suitable heavy atom, the absolute configuration could not be determined. The structure reported clearly shows the relative stereochemistry. Compound **3b** was a derivative of (−)-camphanic acid and contained a known chiral center. The structure reported for compound **3b** represents the absolute configuration of the camphanate ester of 1-(3-cyano-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine, which has analogous stereochemistry to the natural nucleosides. Tables III and IV summarize the crystal and refinement parameters of compounds **9** and **3b**, respectively.

Table III. Single-Crystal X-ray Crystallographic Analysis of Compound **9**

A. Crystal Parameters	
formula	$C_{11}H_{13}N_3O_4$ (251.3)
crystallization medium	ethyl acetate and hexane
crystal size, mm	0.03 × 0.08 × 0.09
cell dimensions	a = 5.696 (2) Å
	b = 11.014 (4) Å
	c = 38.84 (1) Å
	α = 90.00°
	β = 90.00°
	γ = 90.00°
	V = 2437 (1) Å^3
space group	$P2_12_12_1$
molecules/unit cell	8
density obsd, g/cm^3	1.37
density calcd, g/cm^3	1.37
linear absorption factor, cm^{-1}	8.55
B. Refinement Parameters	
number of reflections	1492
nonzero reflections ($I > 3.0\sigma$)	1063
R index[a]	0.058
GOF[b]	1.92
scale factor	1.302 (2)
secondary extinction factor	none

[a] R index = $\sum||F_o| - |F_c||/\sum|F_o|$. [b] GOF = $[\sum w(F_o^2 - F_c^2)^2/(m - s)]^{1/2}$, where $w = [\sigma^2(F) + |g|F^2]^{-1}$ (g = 0.00000).

Table IV. Single-Crystal X-ray Crystallographic Analysis of Compound **3b**

A. Crystal Parameters	
formula	$C_{21}H_{25}N_3O_7{\cdot}H_2O$ (449.5)
crystallization medium	ethyl acetate and toluene
crystal size, mm	0.04 × 0.10 × 0.27
cell dimensions	a = 6.316 (2) Å
	b = 9.269 (2) Å
	c = 37.74 (1) Å
	α = 90.00°
	β = 90.00°
	γ = 90.00°
	V = 2209 (1) Å^3
space group	$P2_12_12_1$
molecules/unit cell	4
density obsd, g/cm^3	1.35
density calcd, g/cm^3	1.35
linear absorption factor, cm^{-1}	8.84
B. Refinement Parameter	
number of reflections	1354
nonzero reflections ($I > 3.0\sigma$)	1237
R index[a]	0.057
GOF[b]	1.51
scale factor	1.229 (2)
secondary extinction factor	none

[a] R index = $\sum||F_o| - |F_c||/\sum|F_o|$. [b] GOF = $[\sum w(F_o^2 - F_c^2)^2/(m - s)]^{1/2}$, where $w = [\sigma^2(F) + |g|F^2]^{-1}$ (g = 0.00000).

The refined structure was plotted by using the SHELXTL plotting package (Figures 1 and 2).

Antiviral Testing. C8166 Cells. C8166 cells, an HTLV-I infected human T cell line, were grown in RPMI +10% FCS. Freshly passaged cultures were infected with HIV-I, isolates RF, and CBL 1 and incubated at 37 °C for up to 7 days ± drug.[25] Syncytial formation was monitored on days 3–7, and scored–to ++++. Control cultures showed progressive formation of syncytia over the culture period as expected. **3a** was taken up at 1 mg/mL in RPMI and tested in the range 0.1 to 100 μg/mL.

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ATH8 Cells. The HIV-I cytopathic effect inhibition assay was performed as previously described.[2,3] Briefly, susceptible ATH8 cells (2×10^5) were pelleted, exposed to HIV-I (HTLV-III_B: 2,000 virus particles per cell) in the form of cell-free virions, resuspended in interleukin-2-containing medium, and cultured in the presence or absence of various concentrations of compounds. Control cells were similarly treated but not exposed to the virus. On day 7 in culture, the total viable target cells were counted in a hemocytometer under the microscope by the trypan blue dye exclusion method. Control-infected cultures untreated with drug were almost completely destroyed by the cytopathic effect of the virus. **3a** was tested in the range 0.25 to 25 μg/mL.

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Registry No. **3a**, 116195-58-5; **3b**, 118629-53-1; α-**6**, 118597-64-1; β-**6**, 118597-65-2; α-**7**, 118597-66-3; β-**7**, 118597-67-4; α-**8**, 118597-68-5; β-**8**, 118597-69-6; **9**, 116195-59-6; thymine, 65-71-4; (1*S*)-(−)-camphanoyl chloride, 39637-74-6.

Supplementary Material Available: X-ray data (coordinates, anisotropic temperature factors, distance, and angles) for compounds **3b** and **9** (16 pages). Ordering information is given on any current masthead page.

A Dihydropyridine Carrier System for Sustained Delivery of 2′,3′-Dideoxynucleosides to the Brain

Eduardo Palomino,[†] David Kessel,[‡,§] and Jerome P. Horwitz*[,†,‡]

Michigan Cancer Foundation and Departments of Pharmacology and Internal Medicine, Wayne State University School of Medicine, Detroit, Michigan 48201. *Received October 27, 1988*

The present study evaluates the utility of the dihydropyridine ⇌ pyridinium salt redox system for the specific delivery and sustained release of a model 2′,3′-dideoxynucleoside to the brain of mice as the initial effort in a search for agents that may prove effective in reversing the complicating neurological disorders of AIDS. The unsaturated nucleoside 2′,3′-didehydro-2′,3′-dideoxythymidine (**1**), which is effective in protecting ATH8 cells against the cytopathogenicity of HIV-1, was converted to the corresponding *N*-methyl-1,4-dihydronicotinate derivative, **4**, in three steps. The 5′-*O*-nicotinate ester, **2**, obtained by reaction of **1** with nicotinyl chloride, was converted in quantitative yield to the *N*-methylpyridinium salt **3** on treatment with MeI in acetone. Reduction of the latter with $Na_2S_2O_4$ gave **4** in 50% yield. Pseudo-first-order rate constants for the oxidation of **4** to **3** were observed in plasma ($k = 3.54 \times 10^{-5}$ s^{-1}) and in homogenates of mouse liver ($k = 9.2 \times 10^{-5}$ s^{-1}) and brain ($k = 8.85 \times 10^{-5}$ s^{-1}). None of the chemical delivery system **4** could be detected in the brain of female BDF/1 mice at 1 h postinjection. The peak level of **3** in the brain occurred at 3 h with a half-life of 25 h. Both **1** and *N*-methylnicotinic acid (trigonelline, **5**) were readily identified by HPLC in a brain homogenate derived from mice injected (25 mg/kg) with **4**. TLC showed a low level penetration of mouse brain by **1** (0.44 μg/g wet tissue) following injection of the corresponding labeled [*methyl*-^{3}H]-2′,3′-unsaturated nucleoside (25 mg/kg). The data indicate that **4** crosses the blood–brain barrier to be oxidized by cerebral tissue to the ionic structure **3**, which is "locked therein". The sustained local release of a 2′,3′-dideoxynucleoside, such as **1**, from a chemical delivery system (**4**) represents a potentially useful approach to the treatment of AIDS dementia complex.

A common and important cause of morbidity in patients with advanced stages of infection with human immunodeficiency virus type 1 (HIV-1) is AIDS dementia complex, a complicating neurological syndrome characterized by abnormalities in cognition, motor performance, and behavior.[1] There is evidence that AIDS dementia complex is caused either partially or wholly by direct HIV-1 brain infection and, further, that virus frequently invades the central nervous system (CNS) early in the course of systematic infection, even in the absence of symptoms.[2] Indeed, there is the possibility that the CNS serves as the major reservoir for HIV in the body.[3] Clearly a rationale exists for seeking antiviral drugs that can penetrate the blood–brain (BB) and blood–cerebrospinal fluid barriers (BCSFB). In this connection it may be noted that 3′-azido-2′,3′-dideoxythymidine (AZT) penetrates the BCSFB[4,5] and can, at least partly, reverse the neurological dysfunction due to HIV-1 in some patients.[6]

Balzarin et al.[7] have reported that AZT and the 2′,3′-unsaturated nucleoside 2′,3′-didehydro-2′,3′-dideoxythymidine (**1**) were equally effective in protecting ATH8 cells against the cytopathogenicity of HIV-1, but **1** had a higher in vitro chemotherapeutic index; i.e., it was less cytostatic and cytotoxic against ATH8 cells than AZT. Moreover, when evaluated for their inhibiting effects on the cytopathogenicity of HIV-1 in MT-4 cells, **1** was about 5 times more potent than 2′,3′-dideoxycytidine,[8] which is currently being evaluated for its therapeutic potential in

[†] Michigan Cancer Foundation.
[‡] Department of Internal Medicine.
[§] Department of Pharmacology.

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