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2′-Azido-2′,3′-dideoxythymidine: synthesis and crystal structure of a 2′-substituted dideoxynucleoside

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Summary

1-(2-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (2′-N_3ddThd) was synthesized from 1-(5-*O*-trityl-2,3-anhydro-β-D-*lyxo*furanosyl)thymine by two different procedures. Method A prepared the title compound by opening of the oxirane ring with $LiEt_3BH$ followed by mesylation of the 2′-hydroxyl function, introduction of the 2′-azido substituent and deblocking of the 5′-function. In method B nucleophilic opening of 3′-deoxy-5′-*O*-(tert-butyldimethylsilyl)-5-methyl-2,2′-anhydrouridine was carried out with sodium azide in hexamethylphosphoramide in the presence of benzoic acid. Single X-ray crystallographic studies indicated a solid state conformation ($^3T_2$), which was opposite to that of the A form of AZT ($^2T_3$) but closely resembled that of 1-(2-fluoro-2,3-dideoxy-β-D-*erythro*pentofuranosyl)thymine (2′-FddThd) ($^3T_2$) and of 3′-azido-2′,3′-dideoxy-2,6-diaminopurine riboside (3′-N_3ddDAP) ($^3T_2$). Whereas the latter displayed significant inhibitory activity against human immunodeficiency virus (HIV) replication, 2′-FddThd and 2′-N_3ddThd were essentially inactive.

Dideoxynucleosides, 2′-substituted; 2′-AZT; Activity anti-HIV; AIDS; Solid state conformation

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Introduction

HTLV-III/LAV, now called human immunodeficiency virus (HIV), has been identified as the etiologic agent of AIDS (Barré-Sinoussi et al., 1983; Gallo et al., 1984). This has in turn stimulated attempts at developing new agents that would be effective in the treatment of this disease (for a review see De Clercq, 1989). Based on the anti-HIV activity reported for 3′-azido-2′,3′-dideoxythymidine (AzddThd, AZT, 6) and its mechanism of action (Mitsuya et al., 1985), most strategies have focused on the design and synthesis of 2′,3′-dideoxynucleoside analogues. Among the pyrimidine dideoxynucleosides, the most potent and selective anti-HIV agents are AzddThd (6) (Mitsuya et al., 1985), 2′,3′-dideoxycytidine (DDC, 7) (Mitsuya and Broder, 1986); 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T, 8) (Baba et al., 1987; Balzarini et al., 1987), 3′-fluoro-2′,3′-dideoxythymidine (FddThd, 9) (Herdewijn et al., 1987; Balzarini et al., 1988) and 3′-fluoro-2′,3′-dideoxy-5-chlorouridine (FddC1Urd, 10) (Balzarini et al., 1989a,b; Van Aerschot et al., 1989) (Fig. 1).

Dideoxynucleoside analogues with a 2′-substituent have not been accredited with appreciable anti-HIV activity except for some derivatives with a 2′-fluorine in the "*ara*"-configuration (Van Aerschot et al. (1989); Martin et al. (1990); Watanabe et al. (1990); Sterzycki et al. (1990)). In contrast with the very potent anti-HIV compound FddThd, its regio-isomer 2′-fluoro-2′,3′-dideoxythymidine (2′-FddThd, 11) (Van Aerschot et al., 1989) is only marginally active. Likewise,

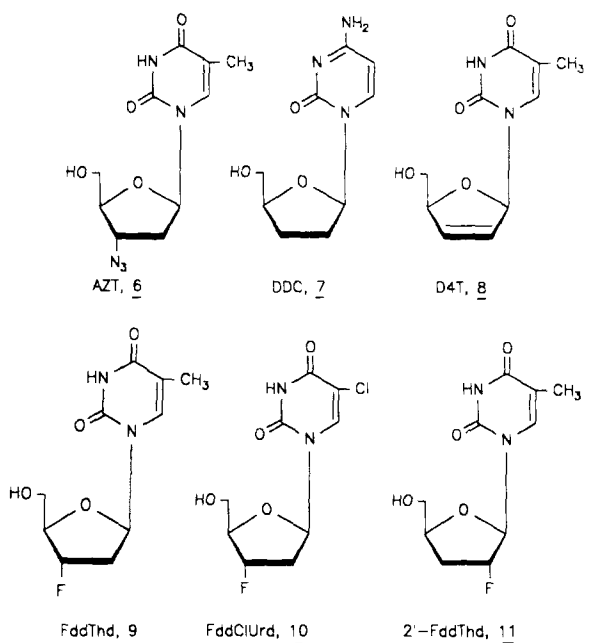

Fig. 1. Structures of the most selective 2′,3′-dideoxynucleosides in the pyrimidine series (6–10) and of the inactive regio-isomer 11.

the doubly substituted 2′,3′-difluoro-2′,3′-dideoxythymidine (Herdewijn and Van Aerschot, 1989) is devoid of anti-HIV activity. We now report on the synthesis, crystal structure, and anti-HIV-1 activity of 2′-azido-2′,3′-dideoxythymidine (2′-AZT, 5), the 2′-regio-isomer of AZT.

Materials and Methods

Synthesis of the compounds

Melting points were determined with a Büchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Beckman UV 5230 spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were determined with a JEOL FX 90A spectrometer with tetramethylsilane as internal standard (s=singlet; d=doublet; t=triplet; m=multiplet; br=broad signal). Electron impact mass spectra (70 eV) were recorded on a AEI-MS12 mass spectrometer. Elemental analysis was carried out by Dr Rodzinski at the Institute für Organische Chemie, Stuttgart. Precoated Merck silica gel F254 plates were used for TLC. Column chromatography was performed on Merck silica gel (0.063–0.200 mm). Pyridine was dried by distillation after it had been refluxed on potassium hydroxide for 24 h. Tetrahydrofuran was distilled from CaH_2. Lithium triethylborohydride was bought from Janssen Chimica. Acetic acid was distilled on chromium trioxide.

Antiviral test procedure

The HTLV-III_B strain of HIV-1 was used throughout all experiments. The viruses were prepared from the culture supernatant of HTLV-III_B-infected MT-4 cells. The antiviral assays were based on the protection of HIV-infected MT-4 cells against virus-induced cytopathogenicity (Balzarini et al., 1988, 1989a). They were run in parallel with the cytotoxicity assays, which were based on the toxicity of the compounds for uninfected MT-4 cells.

Results

Chemistry

1-(3-Deoxy-2-O-methanesulfonyl-5-O-triphenylmethyl-β-D-threo-pentofuranosyl)thymine (3)

To a solution of 1.4 g (2.90 mmol) of 1-(5-*O*-triphenylmethyl-2,3-anhydro-β-D-*lyxo*-pentofuranosyl)thymine (Codington et al., 1962) (1) in anhydrous THF, were added 9 ml of a 1 M solution of lithium triethylborohydride in THF. Reaction and workup were done as described (Webb et al., 1988). After extraction, the organic phase was purified on silica gel by flash chromatography ($CHCl_3$-MeOH 98:2). The appropriate fractions were pooled, evaporated and coevaporated with pyridine.

The oily residue was dissolved in 40 ml anhydrous pyridine, cooled on an ice bath and 1.5 ml methanesulfonyl chloride was added. After 2 h incubation at ambient temperature the mixture was evaporated and the residue was partitioned between $CHCl_3$, and a 5% aqueous $NaHCO_3$. The water phase was extracted once more with $CHCl_3$ and purification by silica gel column chromatography ($CHCl_3$-MeOH 99:1) afforded 1.30 g (2.31 mmol, 80%) of the title product as a foam.

UV (MeOH) λ_{max} 232 and 266 nm; ^{1}H NMR ($CDCl_3$) δ: 1.71 (s, 3H, CH_3), 2.25–3.0 (m, 2H, H-3′,H-3″), 2.94 (s, 3H, CH_3SO_2), 3.37 (t, 2H, J=3.3 Hz, H-5′, H-5″), 4.26 (m, 1H, H-4′), 5.35 (m, 1H, H-2′), 6.09 (d, 1H, J=4.4 Hz, H-1′), 7.15–7.6 (m, 16H, trityl and H-6) ppm; ^{13}C NMR ($CDCl_3$) δ: 12.1 (CH_3), 33.5 (C-3′), 38.0 (CH_3SO_2), 64.2 (C-5′), 76.0 (C-2′), 77.9 (C-4′), 84.5 (C-1′), 109.8 (C-5), 135.9 (C-6), 150.3 (C-2), 163.7 (C-4) ppm + trityl signals.

1-(2-Azido-2,3-dideoxy-5-O-triphenylmethyl-β-D-erythro-pentofuranosyl)thymine (4)

The foam 3 obtained in the previous preparation (1.3 g, 2.31 mmol) was dissolved in 75 ml dimethylformamide, and 2 g of sodium azide was added. The mixture was heated at 80°C for 8 h and evaporated. The residue was partitioned between $CHCl_3$ and a 5% aqueous $NaHCO_3$. The organic phase was flash purified on silica gel ($CHCl_3$) to yield 1.1 g of a light yellow oil containing small impurities.

UV ($CHCl_3$) λ_{max} 266 nm; ^{1}H NMR ($CDCl_3$) δ: 1.46 (d, 3H, J=1 Hz, CH_3), 1.75–2.05 (m, 1H, H-3′), 2.14–2.52 (m, 1H, H-3″), 3.29 (dd, 1H, $J_{5',5''}$=11 Hz, $J_{4',5'}$=3.5 Hz, H-5′), 3.59 (dd, J=11 Hz and 2.2 Hz, H-5″), 4.25–4.5 (m, 2H, H-2′, H-4′), 5.85 (d, 1H, J=1.7 Hz, H-1′), 7.1–7.5 (m, 15H, trityl), 7.63 (d, J=1 Hz, H-6), 9.21 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ: 11.9 (CH_3), 31.1 (C-3′), 63.3 (C-5′), 66.1 (C-2′), 80.1 (C-4′), 87.3 (Ph_3C), 90.3 (C-1′), 110.5 (C-5), 134.7 (C-6), 150.1 (C-2), 163.7 (C-4) ppm + trityl signals.

1-(2-Azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)thymine (5)

Method A: The oil 4 (1.1 g) was dissolved in 100 ml 80% aqueous acetic acid and heated for 30 min at 100°C. The mixture was evaporated, coevaporated with toluene, dissolved in methanol and adsorbed on silica gel by evaporation. The product-containing residue was put on top of a silica gel column (40 g) which was eluted with $CHCl_3$-MeOH (99:1 followed by 96:4) to afford 470 mg (1.76 mmol, 76% from 3) of 5 as a light yellow oil. Crystallization from acetone-hexane yielded 255 mg of analytically pure material. For the determination of anti-HIV activity the product was recrystallized twice from the same solvent mixture affording, finally, 152 mg (0.57 mmol).

mp 167–168°C [lit. 167–169°C (Warshaw and Watanabe, 1990)] $[\alpha]_D^{20}$ -43.5 (-c 0.92, DMSO); UV (MeOH) λ_{max} 268 nm (ϵ=10050); MS m/z 267 (M^+), 225 (M-N_3), 142 (M-B), 126 (B+H), 114 (142-N_2, 100); ^{1}H NMR (DMSO-d_6) δ: 1.78 (d, 3H, J=0.8 Hz, CH_3), 1.94–2.44 (m, 2H, H-3′,H-3″), 3.54–3.78 (m, 2H,H-5′,H-5″), 4.07–4.32 (m, H-4′), 4.35–4.43 (m, H-2″), 5.20 (t, 1H, J=5 Hz, 5′-OH), 5.75 (d, 1H, J=2.2 Hz, H-1′), 7.86 (d, 1H, J=0.8 Hz, H-6) ppm; ^{13}C NMR (DMSO-d_6) δ: 12.2 (CH_3), 30.3 (C-3′), 61.2 (C-5′), 65.5 (C-2′), 81.0 (C-4′), 89.0 (C-1′), 108.8

TABLE 1
Atomic coordinates ($x10^4$) and equivalent isotropic thermal parameters with ESDs of the refined parameters in parentheses

Atom	$B_{eq} = {}^4/_3 \Sigma_i\Sigma_j\beta_{ij}a_i \cdot a_j$			
	x	y	z	$B_{eq}(Å^2)$
N1	3519(3)	1771(2)	3931(1)	2.74(4)
C2	3421(4)	1235(2)	4789(1)	2.98(4)
O2	3480(3)	152(1)	4886(1)	4.20(4)
N3	3234(3)	2009(2)	5524(1)	3.12(4)
C4	3128(3)	3246(2)	5487(1)	2.67(4)
O4	2946(3)	3821(2)	6206(1)	3.50(3)
C5	3261(3)	3736(2)	4557(1)	2.82(4)
C6	3443(4)	2994(2)	3839(1)	2.85(4)
C7	3157(4)	5061(2)	4439(2)	3.92(6)
C1′	3731(3)	959(2)	3118(1)	2.63(4)
C2′	1922(3)	360(2)	2889(2)	2.84(4)
N3′	2258(3)	−852(2)	2489(1)	3.41(4)
N4′	2337(3)	−1607(2)	3124(1)	3.34(4)
N5′	2382(4)	−2333(2)	3664(2)	4.67(5)
C3′	1178(3)	1170(2)	2134(2)	3.23(5)
C4′	2851(4)	1587(2)	1624(2)	3.14(5)
O4′	4248(2)	1624(1)	2340(1)	2.96(3)
C5′	2738(4)	2776(2)	1138(2)	3.88(6)
O5′	2131(3)	3668(1)	1764(1)	4.59(4)

(C-5), 135.6 (C-6), 150.3 (C-2), 163.8 (C-4) ppm; *anal calcd.* for $C_{10}H_{13}N_5O_4$: C, 44.94; H, 4.90; N, 26.21. Found: C, 44.69; H, 4.85; N, 26.17.

Method B: Compound 5 was also synthesized independently by nucleophilic opening of 3′-deoxy-5′-*O*-(tert-butyldimethylsilyl)-5-methyl-2,2′-anhydrouridine with sodium azide in hexamethylphosphoramide in the presence of benzoic acid (data not shown). This route is similar to that described in the course of this work by Warshaw and Watanabe (1990).

Single crystal X-ray analysis

Colorless prismatic crystal obtained at room temperature from an aqueous solution, dimensions ~0.50 × 0.30 × 0.25 mm. STADI4 computer-controlled four-circle diffractometer, graphite-monochromated Mo $K\alpha$ radiation, $\omega/2\theta$ scan technique ($2\theta_{max}$ = 50°, $0 \le h \le 9$, $0 \le k \le 13$, $-17 \le l \le 17$). Cell dimensions by least-squares refinement of the setting angles of 24 reflections with $20° < 2\theta < 30°$, spacegroup $P2_12_12_1$ from systematic absences $0h0$ for h odd, $0k0$ for k odd and $00l$ for l odd. Four standard reflections (042, 126, 231, 410) monitored after every 120 minutes showed no significant decrease in intensity per hour. 2398 Reflections measured, 1230 unique reflections (R_{int}=0.012) of which 1008 were considered observed [$F > 4\theta$ (F)]. Two reflections (011, 013) badly affected by extinction were eliminated. Lorentz-polarisation corrections, no absorption corrections, scattering factors [Cromer and Waber, 1974; Stewart et al., 1965 (for H)]. Initial attempts to resolve the structure with *MULTAN82* (Main et al., 1982) resulted in the well-known 'chicken-wire' E-maps. Modification of the default input parameters did not

resolve this problem. The structure was resolved using a thymine skeleton as input model for the vector-search rotation-functions program *ORIENT* (Beurskens et al., 1987). The result of *ORIENT* was a well-oriented thymine fragment which had to be positioned with respect to the space group symmetry elements by strengthened translation functions using the automated program *TRADIR* (Doesburg and Beurskens, 1983). The outcome of *TRADIR* was a shifted well oriented thymine fragment which was used as input to *DIRDIF* (Beurskens et al., 1983) for further elucidation of the structure. *DIRDIF* revealed the position of all non-hydrogen atoms. A difference synthesis revealed the position of all hydrogen atoms. All hydrogen atoms were refined with fixed isotropic temperature factors 1.3 times that of the parent atom. All other atoms were refined anisotropically on F by full-matrix least-squares. The refinement converged at $R = 0.030$, $wR = 0.038$, $S = 1.248$. $w = 1/[\sigma(Fo)]^2$. 212 refined parameters, max. shift/e.s.d.=0.03, min. and max. electron density -0.152 and 0.112 e Å^{-3}. The number of reflections per refined variable was 1006/212 = 4.8. All calculations were performed on a PDP-11/73 microcomputer using *SDP/PDP* (Enraf-Nonius, 1982) and *PARST* (Nardelli, 1983). A *PLUTO* plot (Motherwell and Clegg, 1978) of the title compound with the atomic numbering scheme is shown in Fig. 3. The final fractional atomic coordinates are given in Table 1. Bond lengths, bond angles and some selected torsion angles are given in Table 2.

Anti-HIV-1 activity of 2′- or 3′-azido-ddThd and 2′- or 3′-fluoro-ddThd

The anti-HIV-1 activity and cytotoxicity of 2′-azido- and 2′-fluoro-ddThd were determined and compared with the antiviral and cytotoxic properties of their 3′-azido- and 3′-fluoro-substituted counterparts (Table 3). 2′-N_3ddThd synthesized according to method A as described in Materials and Methods proved 250-fold less inhibitory against HIV-1 replication than 3′-N_3ddThd, even after two recrystallisations.

However, a sample of 2′-N_3ddThd was also obtained following a completely different synthesis scheme, in which a potential contamination of 3′-azidothymidine is excluded. When evaluated for its antiviral properties, this preparation of 2′-N_3ddThd displayed no inhibitory effect against the replication of HIV at 500 μM. These observations point to the extreme care which should be taken when evaluating analogues of very active compounds where small impurities – inherent to the followed synthesis scheme – can provoke false results. The lack of anti-HIV-activity of 2′-N_3ddThd can be most likely explained by a lack of phosphorylation of the compound by cellular thymidine kinase, since phosphorylation by kinases is a prerequisite for the antiviral activity of dideoxynucleoside analogues. Indeed, both 2′-N_3ddThd and 2′-FddThd have little affinity for cellular dThd kinase as determined according to Balzarini et al., 1988 (data not shown).

TABLE 2
Bond lengths (Å), bond angles (°) and selected torsion angles (°) with ESDs in parentheses

Bond lengths (Å)			
N1-C2	1.373(3)	C1′-C2′	1.532(3)
N1-C6	1.378(3)	C1′-O4′	1.397(2)
N1-C1′	1.489(3)	C2′-N3′	1.494(3)
C2-O2	1.222(3)	C2′-C3′	1.517(3)
C2-N3	1.372(3)	N3′-N4′	1.245(3)
N3-C4	1.389(3)	N4′-N5′	1.124(3)
C4-O4	1.224(3)	C3′-C4′	1.511(3)
C4-C5	1.447(3)	C4′-O4′	1.458(3)
C5-C6	1.331(3)	C4′-C5′	1.506(3)
C5-C7	1.495(3)	C5′-O5′	1.417(4)
Bond angles (°)			
C2-N1-C6	121.3(2)	N1-C1′-C2′	110.2(2)
C2-N1-C1′	116.4(2)	N1-C1′-O4′	109.3(2)
C6-N1-C1′	122.4(2)	C2′-C1′-O4′	107.5(2)
N1-C2-02	122.4(2)	C1′-C2′-N3′	109.6(2)
N1-C2-N3	114.8(2)	C1′-C2′-C3′	102.0(2)
O2-C2-N3	122.9(2)	N3′-C2′-C3′	109.2(2)
C2-N3-C4	127.3(2)	C2′-N3′-N4′	110.1(2)
N3-C4-O4	119.9(2)	N3′-N4′-N5′	176.4(2)
N3-C4-C5	114.3(2)	C2′-C3′-C4′	103.7(2)
O4-C4-C5	125.9(3)	C3′-C4′-O4′	104.3(2)
C4-C5-C6	119.0(2)	C3′-C4′-C5′	116.9(2)
C4-C5-C7	118.5(2)	O4′-C4′-C5′	109.9(2)
C6-C5-C7	122.5(2)	C1′-O4′-C4′	110.9(2)
N1-C6-C5	123.4(2)	C4′-C5′-O5′	110.3(2)
Selected torsion angles (°)			
C2-N1-C1′-O4′	−165.7(2)		
C2′-C1′-O4′-C4′	4.1(2)		
O4′-C1′-C2′-C3′	−23.6(2)		
C1′-C2′-C3′-C4′	33.3(2)		
C2′-C3′-C4′-O4′	−31.7(2)		
C3′-C4′-O4′-C1′	17.4(2)		
C3′-C4′-C5′-O5′	52.8(3)		

Discussion

Chemistry

The *lyxo*-epoxide derivative of 5-methyluridine (1) was prepared in analogy with the synthesis of the uridine congener (Codington et al., 1962). Therefore 5-methyluridine, prepared by sugar-base condensation, was 5′-*O*-tritylated and mesylated. Controlled treatment with base afforded the 2′,3′-anhydro-derivative 1. Reaction of 1 with lithium triethylborohydride gave the 1-(3-deoxy-β-D-*threo*-pentofuranosyl)thymine analogue 2 (Webb et al., 1988) which was flash purified on silica gel. Treatment of the resulting foam with methanesulfonyl chloride afforded 3 in 80% yield. Nucleophilic displacement with sodium azide in hot dimethylformamide followed by detritylation with acetic acid yielded 82% of 5 as a light yellow oil. Crystallization, followed by two recrystallizations from acetone-hexane, afforded the analytical material for antiviral evaluation (Fig. 2). A sample

TABLE 3
Inhibitory effects of 2′- or 3′-azido-ddThd and 2′- or 3′-fluoro-2′,3′-dideoxythymidine derivatives on HIV-1 replication in MT-4 cells

Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	Selectivity index[c]
3′-N_3ddThd (AZT)[d]	0.003	4.81	1603
2′-N_3ddThd[e]	0.78	141	181
2′-N_3ddThd[f]	>500	>500	–
3′-FddThd[d]	0.001	0.197	197
2′-FddThd	54	>500	>10

[a]EC_{50}, or concentration required to inhibit HIV-induced cytopathogenicity in MT-4 cells.
[b]CC_{50}, or concentration required to reduce the viability of MT-4 cells by 50%.
[c]Selectivity index or CC_{50}/EC_{50} ratio.
[d]Data taken from Balzarini et al. (1989a).
[e]Sample obtained by method A after two recrystallisations.
[f]Sample obtained by method B.

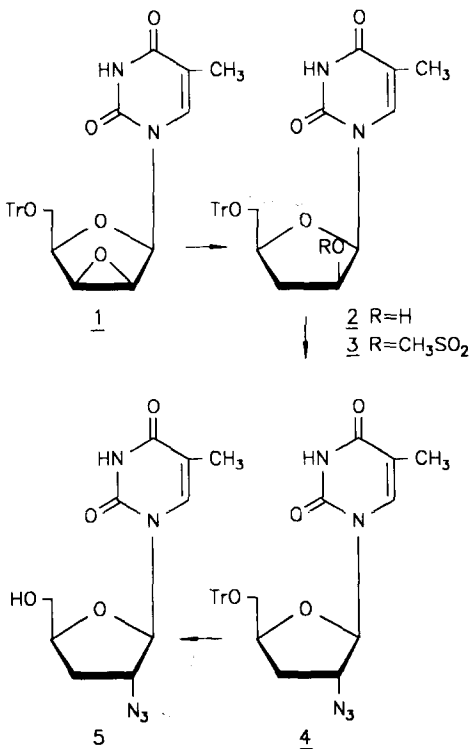

Fig. 2. Synthesis scheme of 2′-azido-2′,3′-dideoxythymidine (5).

of 2′-N_3ddThd was prepared by an alternative synthesis scheme, analogous to the scheme followed for the preparation of 2′-N_3ddThd by Warshaw and Watanabe (1990).

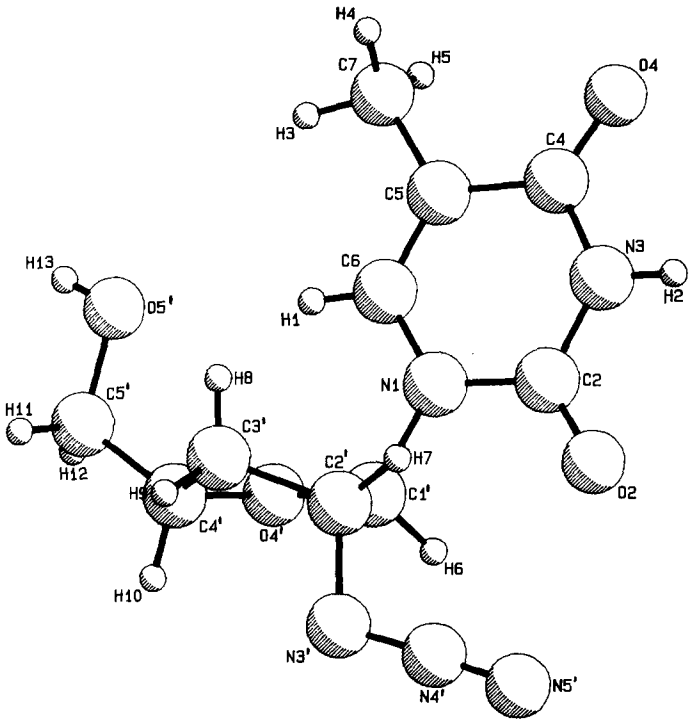

Fig. 3. Pluto plot of 5 with the atomic numbering scheme for X-ray analysis.

X-ray crystallographic studies and antiviral activity

1-(2-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine: $C_{10}H_{13}N_5O_4$,M_r = 267.24, orthorhombic, $P2_12_12_1$, a = 7.391(4), b = 11.202(6), c = 14.359(7)Å, V = 1189(1)Å^3, Z = 4, D_x = 1.493 Mg m^{-3}, graphite-monochromated Mo $K\alpha$ radiation, λ = 0.71073Å, μ = 0.1106 mm^{-1}, $F(000)$=560, T=290 K. Final R=0.030 for 1006 unique observed reflections. The N-glycosidic torsion angle χ has a value of $-165.7(2)°$ in the *anti* range. The sugar pucker is $^3T_2$ with P = 12(1)° and a maximum torsion angle ψ_m = 34(1)° and the C4′-C5′ conformation is *+sc* with γ = 52.8(3)°. The conformational parameters are in accordance with the IUPAC-IUB Joint Commission on Biochemical Nomenclature (1983) guidelines. No base-pair formation occurs in the crystal structure.

Having resolved the structure for 5, the conformations of both (azido substituted and fluorine-substituted) pairs of regio-isomers were compared with each other (Table 4). A close conformational similarity was found for the title compound and 2′-fluoro-2′,3′-dideoxythymidine (Everaert et al., 1990a). This latter compound has two separate molecules in its asymmetric unit with pseudorotation phase angles P of 12° and 8° and a ψ_m of 35° and 35°, respectively, compared to P = 12° and ψ_m = 34° for 5.

TABLE 4

Activity against HIV-1 in MT-4 cells and the most important conformational parameters of the compounds

Compound molecule[a]	EC_{50}, μM	Angle, degrees[g]				Conformation	[annotation]
		χ	γ	ψ_m	P		
3′-N_3ddThd[b]	0.003[c]						
A		−124.4 (3)	50.9 (4)	32.3	175	C2′-*endo*/C-3′-*exo*	$^2T_3$
B		−173.6 (4)	173.4 (3)	36.2	215	C3′-*exo*/C4′-*endo*	^1_3T
3′-FddThd	0.001[d]						
A		−138.4 (5)	50.2 (7)	36 (1)	164 (1)	C2′-*endo*	^2E
B		−159.6 (5)	52.8 (7)	32 (1)	169 (1)	C2′-*endo*/C3′-*exo*	$^2T_3$
2′-N_3ddThd	>500[e]						
		−165.7 (2)	52.8 (3)	34 (1)	12 (1)	C3′-*endo*/C2′-*exo*	$^3T_2$
2′FddThd	53[e]						
A		−155.2 (2)	55.1 (3)	35 (1)	12 (1)	C3′-*endo*/C2′-*exo*	$^3T_2$
B		−168.0	49.7 (3)	35 (1)	8 (1)	C3′-*endo*/C2′-*exo*	$^3T_2$
3′-N_3ddDAP[f]	0.3	−141.3 (2)	51.6 (3)	–	13.2	C3′-*endo*/C2′-*exo*	$^3T_2$

[a]Letters A and B refer to two independent molecules in the asymmetric unit.

[b]Structure as determined by Van Roey et al. (1989).

[c]Data taken from Balzarini et al. (1989a).

[d]Data taken from Balzarini et al. (1988).

[e]Data taken from Table 3.

[f]Structure and activity as determined by Robins et al. (1989).

[g]Estimated standard deviation (esd) in parentheses.

These 2′-substituted nucleosides have a C3′-*endo*/C2′-*exo* conformation but the 3′-substituted nucleosides adopt a C2′-*endo*/C3′-*exo* conformation as expected from energetic considerations (Saenger, 1984). Only molecule *B* of N_3ddThd (Van Roey et al., 1989) has a pseudorotation phase angle *P* of 215° compared to the almost identical pseudorotation phase angles of 175° for molecule *A* of N_3ddThd and 164 and 169° for molecule *A* and *B* of FddThd (Everaert et al., 1990b). Molecule *B* of N_3ddThd is the only one with a C4′-C5′ *ap* conformation with $\gamma = 173.4°$ compared to the C4′-C5′ *+sc* conformation for all other structures.

Both the 3′- and 2′-substituted nucleosides have a *N*-glycosidic torsion angle χ in the *anti* range with values between −124 and −174°. As expected for nucleosides with *anti*-oriented bases, the *+sc* conformer is stabilized by intramolecular C6-H...05′ 'hydrogen-bonding'. This non-classical bond can reasonably be described as a H-bond by satisfying the description established earlier (Taylor and Kennard, 1982), i.e. this H-bond favors the *+sc* conformer with *anti*-oriented base moieties over the two other possible staggered forms (*−sc* and *ap*). The C4′-C5′ *ap* conformation of molecule B of N_3ddThd is due to an H-bond between 05′-H of molecule *B* and 04 of molecule *A*.

These results seem to confirm the hypothesis of Van Roey et al. (1989) – at least for the pyrimidines – where they find a 3′-exo conformation to be a prerequisite to obtain an anti-HIV active nucleoside. However, this hypothesis does not hold for the purine series, as X-ray analysis of 3′-azido-2′,3′-dideoxy-2,6-diaminopurine riboside (3′-N_3ddDAP) revealed a 2′-exo conformation (Robins et al., 1989), close to the conformation found for both 2′-N_3ddThd and 2′-FddThd.

The pseudorotation phase angle (*P*) of 13.2° indeed renders 3′-N_3ddDAP with a $^3T_2$ conformation and can be compared to *P*=12° for 2′-N_3ddThd. However, in contrast to the latter, the former displays very high anti-HIV activity (EC_{50}=0.3 μM).

After this work was finished we became aware of the paper published by J.A. Warshaw and K.A. Watanabe [J. Med. Chem. 33:1663–1666 (1990)]. These authors likewise reported that 2′-N_3ddThd did not exhibit any significant anti-HIV activity in H9 cells.

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