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8-Aza-7-deaza-2′,3′-dideoxyadenosine: Synthesis and Conversion into Allopurinol 2′,3′-Dideoxyribofuranoside

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4-Amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**5**) was synthesized from 8-aza-7-deaza-2′-deoxyadenosine (**1**). Benzoylation of the 4-amino group of **1** followed by 4,4′-dimethoxytritylation of the 5′-hydroxyl function gave **2b**. Barton deoxygenation of the phenoxythiocarbonyl compound **3** afforded **4a** and yielded **5** after removal of the protecting groups. The *N*-glycosylic bond stability of **5** to acid was higher than that of 2′,3′-dideoxyadenosine (ddA). Compound **5** showed no appreciable activity against human immunodeficiency virus. It was converted into allopurinol 2′,3′-dideoxyribofuranoside (**6**) by adenosine deaminase but at a lower rate than the conversion of ddA into ddI.

Keywords——8-aza-7-deaza-2′,3′-dideoxyadenosine; allopurinol 2′,3′-dideoxyribofuranoside; pyrazolo[3,4-*d*]pyrimidine; Barton deoxygenation; *N*-glycosylic bond stability; anti-HIV activity; adenosine deaminase

2′,3′-Dideoxynucleosides can be metabolized to become potent inhibitors of deoxyribonucleic acid (DNA) synthesis on a ribonucleic acid (RNA) template catalyzed by the enzyme reverse transcriptase.[1] They are currently used as chemotherapeutic agents for the treatment of acquired immunodeficiency syndrome (AIDS).[2] Among the series of purine 2′,3′-dideoxynucleosides, 2′,3′-dideoxyadenosine (ddA) completely protects H9 cells against human immunodeficiency virus (HIV) infection at a concentration of 10 μM.[3] However, there are problems with these antiviral active compounds: as 2′,3′-dideoxynucleoside triphosphates are inhibitors of both viral and cellular DNA polymerases, the drug can be cytotoxic to the host cell. On the other hand, the dideoxynucleosides can be deactivated by cellular metabolizing enzymes. As a consequence, interest has been directed to structurally modified compounds which behave differently than the parent purine 2′,3′-dideoxynucleosides.

Recently, we have synthesized 4-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1**), which is an isostere of the DNA constituent, 2′-deoxyadenosine.[4] We now report on the deoxygenation of **1** leading to the corresponding 2′,3′-dideoxynucleoside **5**, which was also converted into **6** by adenosine deaminase on a preparative scale.

At the first step of the reaction route, the 4-amino group of **1** was benzoylated to give **2a** (with transient silyl-protection of the hydroxyl groups). This was followed by 4,4′-dimethoxytritylation of the 5′-hydroxyl group to yield compound **2b**.[5] As *O*-phenoxythiocarbonyl derivatives of nucleosides are particularly useful for Barton deoxygenation[6] the 3′-hydroxyl group of compound **2b** was converted into the thiono ester **3**.

Deoxygenation of **3** in toluene with tri-*n*-butyltin hydride in the presence of 2,2′-azobis(2-methylpropionitrile) (AIBN) afforded **4a**. The protecting groups of **4a** were removed stepwise. Removal of the 4-benzoyl group was carried out in $MeOH/NH_3$. Compound **4b** was obtained as a colorless foam after chromatographic purification. Subsequent treatment of **4b** with

TABLE I. ^{13}C-NMR Chemical Shifts of 2′-Deoxy- and 2′,3′-Dideoxynucleosides[a)]

	C-3 (C-8)	C-3a (C-5)	C-4 (C-6)	C-6 (C-2)	C-7a (C-4)	C-1′	C-2′	C-3′	C-4′	C-5′
1	132.8	100.4	157.3	155.7	153.6	84.1	38.0	71.0	87.5	62.3
3	133.0	104.7	158.1	155.1	153.1	84.3	40.2	82.7	84.7	63.8
4a	132.9	104.3	158.0	154.8	152.8	84.6	30.4	27.2	80.1	66.0
4b	132.9	100.4	157.9	156.1	153.7	84.2	30.4	27.2	79.9	66.0
5	133.0	100.3	158.1	156.1	153.6	84.4	30.4	27.4	81.7	64.3
6	135.2	106.1	157.3	148.4	152.3	84.6	30.7	27.3	82.2	64.2
ddA	139.1	119.2	156.1	152.5	148.9	84.5	31.8	25.8	81.8	63.0

a) Spectra were measured in $(Me)_2SO$-d_6; purine numbering in parenthesis.

TABLE II. Kinetic Data of *N*-Glycosyl Bond Hydrolyses of ddA and Compound **5**[a)]

	.1 N HCl		0.1 N HCl		0.01 N HCl	
	$\tau/2$ (min)	k (min^{-1})	$\tau/2$ (min)	k (min^{-1})	$\tau/2$ (min)	k (min^{-1})
5	0.83	0.85	20.4	0.033	280	0.0025
ddA	—	—	1.9	0.363	31.5	0.022

a) Determined at 25 °C; nucleoside concentration was 170 μM.

acetic acid gave 4-amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine (**5**).

Table I shows the carbon-13 nuclear magnetic resonance (^{13}C-NMR) chemical shifts of compounds **5** and **6**, as well as their precursors and 2′,3′-dideoxyadenosine. The assignments of the ^{13}C-NMR signals of the sugar moiety of the 2′,3′-dideoxynucleosides **5**, **6**, and that of ddA were made on the basis of two dimensional [^{1}H, ^{13}C] correlation spectroscopy recently carried out on similar 2′,3′-dideoxyguanosine derivatives.[7)] It showed that the C-1′ and C-4′ signals are reversed compared to the corresponding 2′-deoxynucleosides and that the C-3′ signal is located upfield from the C-2′ signal.

The stability of the *N*-glycosylic bond of 2′,3′-dideoxynucleosides is related to the pharmacodynamics, as this bond can be cleaved by cellular enzymes with loss of the chemotherapeutic activity. We have determined the stability of this linkage to proton-catalyzed hydrolysis and compared the hydrolysis rates of compound **5** and 2′,3′-dideoxyadenosine. Hydrolysis experiments were carried out at 25 °C at three different concentrations of hydrochloric acid. Under these conditions the nucleobase was released from both compounds. In order to get quantitative data the decrease of the ultraviolet (UV) absorbance was monitored at 258 nm for both dideoxynucleosides. From time-absorbance plots the first order hydrolysis constants (k) and half life times ($\tau/2$) were determined according to the equation

$$k = 1/t \ln(E_0 - E_\infty)/(E_t - E_\infty)$$

The data are summarized in Table II. They indicate that compound **5** is about 10 times more stable than the parent ddA.

Compound **5** was deaminated by adenosine deaminase (0.25 μg ADA/ml) from calf intestine mucosa more slowly than ddA. The reaction was followed at 275 nm (nucleoside concentration was 100 μM) by measuring the decrease of the UV absorbance. A half life of 160 min was obtained for **5**, whereas ddA showed a value of only 2 min. Nevertheless,

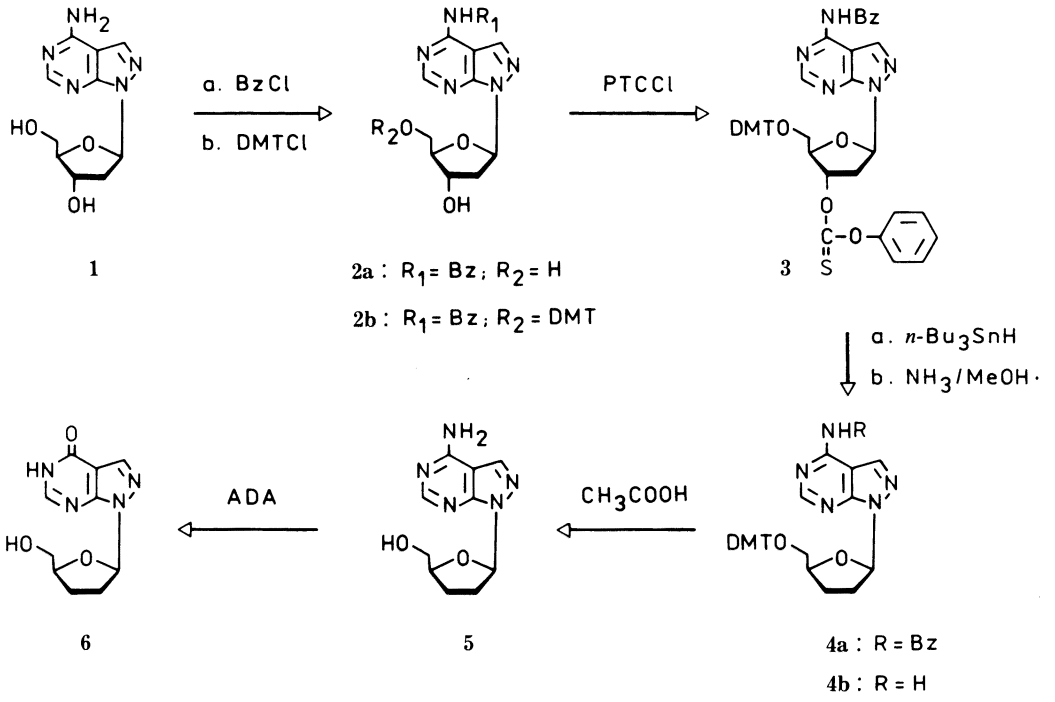

Chart 1

preparative scale conversion of **5** was possible, giving allopurinol 2′,3′-dideoxyribofuranoside (**6**) in almost quantitative yield. The dideoxynucleoside **5** showed no appreciable activity against HIV.[8] It is not clear whether cellular phosphorylation of **5** is restricted, or its triphosphate is not able to inhibit reverse transcriptase.

Experimental

Melting points were determined on a Linström apparatus (Wagner & Munz, München, FRG) and are not corrected. NMR spectra were recorded on a Bruker AC-250 spectrometer, and data are given as δ-values in ppm relative to TMS as an internal standard. Chemical shifts are positive when downfield from the appropriate standard. UV spectra were recorded on a Hitachi-150-20 spectrophotometer (Hitachi, Japan). Thin-layer chromatography (TLC) was performed on silica gel SIL G-25 UV_{254} plates (Macherey-Nagel, FRG). Flash chromatography was performed with Silica gel 60H (Merck, FRG) at 0.9 bar. Solvent systems: (A) CH_2Cl_2–EtOAc, 95:5; (B) CH_2Cl_2–acetone, 7:3; (C) CH_2Cl_2–acetone, 8:2; (D) CH_2Cl–MeOH, 9:1. Adenosine deaminase (E.C. 3.5.4.4, calf intestine) was a product of Boehringer Mannheim (FRG). Elemental analysis were performed by Microanalytisches Labor Beller (Göttingen, FRG).

4-Benzoylamino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-3′-*O*-phenoxythiocarbonyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (3)——Compound **2b** (200 mg, 0.3 mmol) in anhydrous acetonitrile (4 ml) was reacted with phenylchlorothionocarbonate (82 μl, 0.6 mmol) at room temperature for 16 h in the presence of 4-(dimethylamino)pyridine (90 mg, 0.75 mmol). After evaporation of the solvent, the residue was applied to a 12 × 2 cm column (Silica gel 60H, solvent A) and purified by flash chromatography. Isolation of the material of the main zone yielded **3** (150 mg, 63%) as a colorless foam. UV (MeOH) λ_{max} nm (ε): 236 (42100), 275 (18500). TLC (silica gel, A) *Rf* 0.4. ^{1}H-NMR [$(Me)_2SO$-d_6] δ: 3.26 (2H, m, H-C(5′)), 3.69, 3.70 (6H, 2s, 2 OCH_3), 4.45 (1H, m, H-C(4′)), 5.98 (1H, m, H-C(3′)), 8.45 (1H, s, H-C(3)), 8.78 (1H, s, H-C(6)), 11.72 (1H, s, NH). *Anal.* Calcd for $C_{45}H_{39}N_5O_7S$: C, 68.08; H, 4.95; N, 8.82; S, 4.04. Found: C, 68.09, H, 5.12; N, 8.68; S, 4.06.

4-Benzoylamino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-5′-*O*-(4,4′dimethoxytriphenylmethyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidine (4a)——AIBN (15 mg, 0.1 mmol) and tri-*n*-butyltin hydride (150 μl, 0.55 mmol) were added to a solution of compound **3** (200 mg, 0.25 mmol) in dry toluene (7 ml), and the mixture was stirred for 1 h at 80 °C under nitrogen. After evaporation of the solvent, the residue was subjected to flash chromatography (Silica gel 60H, A) and

compound **4a** (120 mg, 75%) was obtained as a colorless foam. UV (MeOH) λ_{max} nm (ε): 236 (33300), 275 (16000). TLC (silica gel, A) *Rf* 0.3. ^{1}H-NMR [$(Me)_2SO$-d_6] δ: 2.16 (2H, m, H-C(3′)), 2.49 (2H, m, H-C(2′)), 2.99 (2H, m, H-C(5′)), 3.65, 3.68 (6H, 2s, 2 OCH_3), 4.32 (1H, m, H-C(4′)), 6.69 (1H, m, H-C(1′)), 8.41 (1H, s, H-C(3)), 8.80 (1H, s, H-C(6)), 11.66 (1H, s, NH). *Anal.* Calcd for $C_{38}H_{35}N_5O_5$: C, 71.12; H, 5.49; N, 10.91. Found: C, 71.11; H, 5.59; N, 10.90.

4-Amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine (4b)——Compound **4a** (300 mg, 0.47 mmol) was stirred in MeOH (40 ml), saturated with NH_3, at 60 °C for 4 h. The solvent was evaporated off and the residue was subjected to flash chromatography (column 12 × 2 cm, Silica gel 60H, B) yielding **4b** as a colorless foam (200 mg, 81%). UV (MeOH) λ_{max} nm (ε): 260 (10150), 275 nm (11400). TLC (silica gel, C) *Rf* 0.25. ^{1}H-NMR [$(Me)_2SO$-d_6] δ: 2.16 (2H, m, H-C(3′)), 2.45 (2H, m, H-C(2′)), 2.99 (2H, m, H-C(5′)), 3.69, 3.70 (6H, 2s, 2 OCH_3), 4.25 (1H, m, H-C(4′)), 6.52 (1H, m, H-C(1′)), 7.74 (2H, s, NH_2), 8.06 (1H, s, H-C(3)), 8.24 (1H, s, H-C(6)). *Anal.* Calcd for $C_{31}H_{31}N_5O_4$: C, 69.26; H, 5.81; N, 13.03. Found: C, 69.13; H, 5.88; N, 12.87.

4-Amino-1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]-pyrimidine (5)——Compound **4b** (110 mg, 0.2 mmol) in 80% acetic acid (10 ml) was stirred at room temperature for 20 min. After evaporation of the solvent and coevaporation with H_2O (twice) the residue was dissolved in CH_2Cl_2 and purified by flash chromatography (silica gel, D). Compound **5** (40 mg, 85%) was obtained as a colorless solid. UV (MeOH) λ_{max} nm (ε): 260 (8900), 275 (10000). TLC (silica gel, D) *Rf* 0.4. ^{1}H-NMR [$(Me)_2SO$-d_6] δ: 2.11 (2H, m, H-C(3′)), 2.40 (2H, m, H-C(2′)), 3.36 (2H, m, H-C(5′)), 4.08 (1H, m, H-C(4′)), 4.75 (1H, m, OH-C(5′)); 6.45 (1H, dd, H-C(1′), *J*=6.9, 3.5 Hz), 7.75 (2H, s, NH_2), 8.14 (1H, s, H-C(3)), 8.18 (1H, s, H-C(6)). *Anal.* Calcd for $C_{10}H_{13}N_5O_2$: C, 51.06; H, 5.57; N, 29.77. Found: C, 50.96; H, 5.65; N, 29.80.

1-(2,3-Dideoxy-β-D-*glycero*-pentofuranosyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one ((Allopurinol 2′,3′-Dideoxyribofuranoside (6))——Compound **5** (60 mg, 0.25 mmol) dissolved in 1/15 M Na-phosphate buffer (pH 7.5, 5 ml) was incubated with adenosine deaminase (0.3 mg) at 25 °C for 2 h. The solvent was evaporated off and the residue applied to a 10 × 2 cm column Silica gel 60H, D). Isolation of the content of the main zone and crystallization from H_2O yielded **6** (50 mg, 84%) as colorless needles. mp 171 °C (H_2O). UV (MeOH) λ_{max} nm (ε): 251 (7700). TLC (silica gel, D) *Rf* 0.5. ^{1}H-NMR [$(Me)_2SO$-d_6] δ: 2.13 (2H, m, H-C(3′)), 2.40 (2H, m, H-C(2′)), 3.40 (2H, m, H-C(5′)), 4.09 (1H, m, H-C(4′)), 4.73 (1H, m, OH-C(5′)), 6.43 (1H, dd, H-C(1′), *J*=6.9, 3.5 Hz), 8.11 (1H, s, H-C(3)), 8.13 (1H, s, H-C(6)). *Anal.* Calcd for $C_{10}H_{12}N_4O_3$: C, 50.85; H, 5.12; N, 23.72. Found: C, 50.63; H, 5.16; N, 23.69.

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