Journal of Medicinal Chemistry

Volume 17, Number 3 March 1974

Synthesis and Biological Activity of 9-β-D-Arabinofuranosyladenine Cyclic 3′,5′-Phosphate and 9-β-D-Arabinofuranosylguanine Cyclic 3′,5′-Phosphate

A. Mohsin Mian, Richard Harris, Robert W. Sidwell, Roland K. Robins, and Tasneem A. Khwaja*

ICN Pharmaceuticals, Incorporated, Nucleic Acid Research Institute, Irvine, California 92664. *Received July 16, 1973*

8,2′-Anhydro-8-hydroxy-9-β-D-arabinofuranosylpurine cyclic 3′,5′-phosphates (Va,b) have been synthesized by intramolecular cyclization of the corresponding 8-hydroxy-2′-*O*-tosyl-9-β-D-ribofuranosylpurine cyclic 3′,5′-phosphates (IVa,b). A reductive cleavage of anhydro derivatives Va,b with H_2S followed by Raney nickel desulfurization provided 9-β-D-arabinofuranosyladenine cyclic 3′,5′- (VIIa, c-ara-AMP) and 9-β-D-arabinofuranosylguanine cyclic 3′,5′-phosphate (VIIb, c-ara-GMP). c-Ara-AMP is cytotoxic to KB and HeLa cells in culture and exhibits significant antiviral activity against herpes simplex type 1 and type 2 infections. c-Ara-GMP is cytotoxic to KB, Sarcoma 180, and HeLa cells. Unlike c-ara-AMP, c-ara-GMP did not exhibit any significant antiviral activity.

The chemistry and biological properties of 9-β-D-arabinofuranosyladenine (ara-A) have been comprehensively reviewed.[1,2] Ara-A has been shown to be a potent inhibitor of a variety of DNA viruses[3] *in vivo* and exhibits carcinostatic activity against a number of experimental tumors in mice.[4] The antiviral and anticancer activity of ara-A is possibly due to its inhibition of DNA synthesis.[5,6] Ara-A is metabolized to the corresponding 5′-triphosphate (ara-ATP) which in turn is a potent inhibitor of DNA-polymerase and cytidine diphosphate reductase.[5,6] Ara-ATP is a substrate as well as competitive inhibitor of adenylyl cyclase isolated from *Escherichia coli*.[7] Ara-A is also incorporated into DNA where it acts as a chain terminator;[8] it is also reported to be incorporated into the 3′ terminus of transfer ribonucleic acids.[1] The effectiveness of ara-A as a drug may be limited due to its low solubility (0.5 mg/ml at 25°) and rapid deamination by adenosine deaminase.[4] Unlike ara-A, 9-β-D-arabinosylguanine (ara-G) is not a naturally occurring nucleoside and is available only *via* a multistep chemical synthesis.[9] Brink and LePage[10] have reported the inhibition of DNA synthesis in the TA3 ascites tumor in BAF_1 mice by ara-G. However, no further studies with ara-G have been reported due to the relative unavailability of the compound.

The biological resistance to treatment by purine and pyrimidine antimetabolites can be ascribed to high levels of a deaminase[4] or lack of enzymatic conversion to phosphorylated nucleosides.[11-13] The problem could be overcome by the use of nucleoside 5′-phosphates. However, nucleotide molecules at physiological pH carry two negative charges and in general[14] are not capable of passing through the cellular membrane. The ability of exogenous adenosine cyclic 3′,5′-phosphate (c-AMP) to exert specific biological effects of c-AMP on intact cells suggests that the cyclic 3′,5′-phosphates could cross the cell membrane.[15] The growth regulatory[16,17] function of c-AMP, its ability to inhibit tumor growth[18,19] *in vitro* and *in vivo*, and the recent evidence that guanosine cyclic 3′,5′-phosphate (c-GMP) could induce cellular proliferation[20,21] suggested the synthesis of arabinofuranosylnucleoside cyclic 3′,5′-phosphates as potential antitumor and antiviral agents.

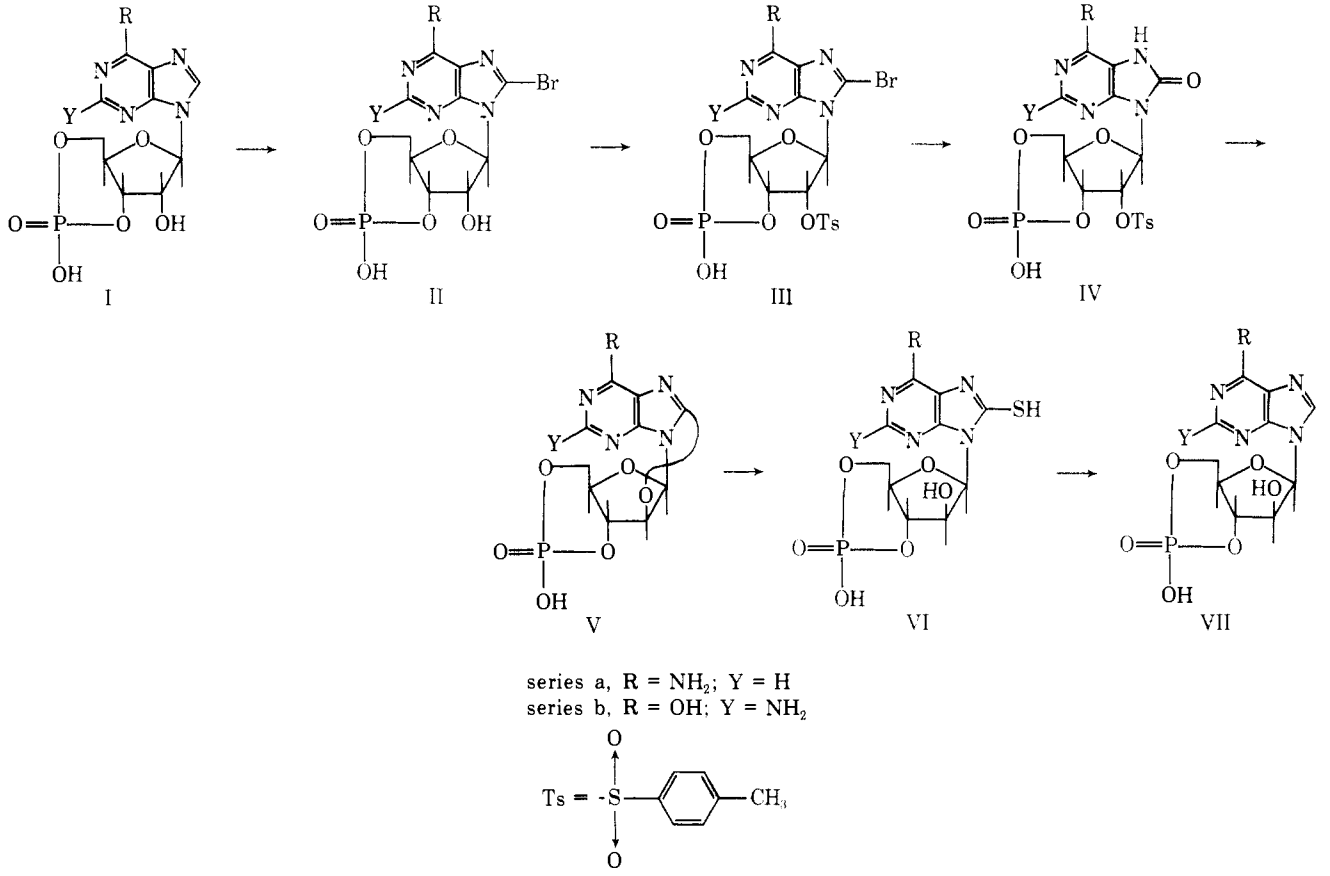
The synthesis of 9-β-D-arabinofuranosyladenine cyclic 3′,5′-phosphate[22] (c-ara-AMP, VIIa) *via* reductive cleavage of 8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosyladenine cyclic 3′,5′-phosphate (Va) has been reported in a preliminary communication. We now report the full experimental details for the synthesis of c-ara-AMP and report that the same method[23] can be successfully applied to the synthesis of 9-β-D-arabinofuranosylguanine cyclic 3′,5′-phosphate (VIIb) as well.

An aqueous suspension of c-GMP (Ib) was brominated[24] to obtain 8-bromoguanosine cyclic 3′,5′ phosphate (IIb) in over 90% yield. Compound IIb was treated with *p*-toluenesulfonyl chloride in aqueous dioxane to provide crystalline 8-bromo-2′-*O*-tosylguanosine cyclic 3′,5′-phosphate (IIIb) in 82% yield. Compound IIIb was refluxed with sodium acetate in acetic acid at 125° for 45 hr to give 8-hydroxy-2′-*O*-tosylguanosine cyclic 3′,5′-phosphate (IVb) in 85% yield. Treatment of IVb with anhydrous sodium acetate in DMF at 125° for 3 hr resulted in intramolecular displacement[25] of the 2′-*O*-tosyl group (disappearance of the tosyl band at 1179 cm^{-1}) to obtain 8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosylguanine cyclic 3′,5′-phosphate (Vb). The important intermediate Vb was characterized by its ultraviolet absorption spectrum[25] and the elemental analysis. The anomeric proton of Vb was a characteristic doublet centered at δ 6.4 confirming[23] the arabinose configuration due to the 8,2′-anhydro linkage. The same procedure has now also been applied to the synthesis of 8,2′-anhydro-8-hydroxy-9-β-D-arabinofuranosyladenine cyclic 3′,5′-phosphate (Va) and is preferred over that described earlier (Scheme I).[23]

Compound Vb was suspended in DMF and heated in a stainless steel bomb in the presence of liquid H_2S (anhydrous conditions) at 120° for 20 hr to provide 8-mercapto-9-β-D-arabinofuranosylguanine cyclic 3′,5′-phosphate (VIb) in 44% yield. Compound VIb was characterized by

*Address correspondence to this author at the LAC/USC Cancer Center, Advanced Therapeutics Program, Los Angeles, Calif. 90033.

Scheme I



its ultraviolet absorption spectrum which is typical of 8-mercaptoguanosine.[24] This reductive cleavage of the 8,2′-anhydro linkage has already been described in the adenine series,[23,26] but this is the first example of its use in the synthesis of arabinofuranosylguanine derivatives. The use of intermediates Va and Vb for the synthesis of a variety of 8-substituted arabinofuranosylpurine cyclic 3′,5′-phosphates will be reported elsewhere.

Compound VIb (like VIa) was desulfurized with Raney nickel to constitute the first reported synthesis of 9-β-D-arabinofuranosylguanine cyclic 3′,5′-phosphate (c-ara-GMP, VIIb) in 48% yield. Compound VIIb moved as a homogeneous ultraviolet-absorbing component in several thin-layer systems and on paper electrophoresis (phosphate buffer, pH 7.2, and borate buffer, pH 9.2) had the same mobility as c-GMP (Ib). The structure of c-ara-GMP was also confirmed by its ultraviolet absorption spectrum, elemental analysis, and pmr spectrum (determined in D_2O) in which the anomeric proton was a doublet located at δ 6.15.

Biological Activity. c-Ara-AMP was a substrate for c-AMP phosphodiesterase[27,28] but did not activate c-AMP-dependent protein kinases. In cell culture studies c-ara-AMP at concentrations of 100 μg/ml caused 50% inhibition of growth and HeLa and KB tumor cell lines. The results of *in vitro* antiviral activity of ara-A and c-ara-AMP against type 1 and type 2 herpes simplex infections in KB cells are described in Table I. It can be seen that c-ara-AMP at concentrations of 3.2–10 μg/ml showed moderate to good inhibition of herpesviruses and at these concentrations the drug showed no cytotoxicity to the host cells. Limited *in vivo* experiments also suggest that c-ara-AMP has approximately the same antiviral activity as ara-A at markedly lower dosage.† This along with the observation of LePage and Hersh[28] that c-ara-AMP can enter the intact cell and is not deaminated, and the suggestion of Hughes and Kimball[29] that c-ara-AMP may independently inhibit mammalian DNA polymerase, makes this compound a strong candidate for further studies which will be described elsewhere.†

c-Ara-GMP, like c-ara-AMP, is also a substrate for phosphodiesterase;‡ however, unlike the latter, it did not exhibit any appreciable antiviral activity *in vitro*. As shown in Table II c-ara-GMP was cytotoxic to S-180, KB, and HeLa cell lines indicating its potential as an antitumor agent.

Experimental Section

Thin-layer chromatography was run on Merck cellulose F plates and silica gel F-254 plates developed with either solvent system A (isopropyl aocohol–concentrated ammonium hydroxide–water, 7:1:2, v/v), solvent system B (1-butanol–acetic acid–water, 5:2:3, v/v), or solvent system C (acetonitrile–0.2 *M* aqueous ammonium chloride, 7:3, v/v). Ultraviolet spectra were recorded on Cary-15 spectrophotometer and infrared spectra were determined on Perkin-Elmer Model 257 spectrophotometer. Proton magnetic resonance studies were run with a Hitachi Perkin-Elmer R-20A spectrometer with DSS as internal reference. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Paper electrophoresis was performed at 1500 V with Whatman No. 3 chromatographic paper using phosphate (pH 7.3) and acetate (pH 4) buffers.

8-Bromo-2′-*O*-*p*-toluenesulfonyladenosine Cyclic 3′,5′-Phosphate (IIIa). Compound IIa[30] (40.9 g, 100 mmol) was dissolved in 1 *N* NaOH (250 ml) and to the stirred solution *p*-toluenesulfonyl chloride (76.26 g, 0.4 mol in 600 ml of dioxane) was added during a period of 15 min. The reaction solution was maintained at room temperature (with stirring) for 4 hr and the resulting mixture was poured over ice–water (5 l.) with strong stirring. The precipitated material was filtered, washed with ice-cold water (2 l.), and dried under suction. The dried precipitate was suspended in chloroform (800 ml), stirred vigorously, filtered, and finally washed with ex-

†R. W. Sidwell, L. B. Allen, J. H. Huffman, T. A. Khwaja, and R. K. Robins, manuscript in preparation.

‡T. A. Khwaja, J. P. Miller, and R. K. Robins, unpublished results.

Table I. Relative *in Vitro*[a] Antiherpesvirus Activity of 9-β-D-Arabinofuranosyladenine Cyclic 3',5'-Phosphate and 9-β-D-Arabinofuranosyladenine

Drug concn, μg/ml	c-Ara-AMP			Ara-A		
	Visible cytotoxicity, %	% inhibition of cytopathic effect		Visible cytotoxicity, %	% inhibition of cytopathic effect	
		Type 1 herpesvirus	Type 2 herpesvirus		Type 1 herpesvirus	Type 2 herpesvirus
1000	90	100	100	90	100	100
320	75	100	100	75	100	100
100	50	100	100	25	100	82
32	13	100	87	0	82	45
10	0	28	67	0	75	12
3.2	0	12	32	0	31	12
1.0	0	0	0	0	31	12

[a]Test run in disposable micro tissue culture plates according to the methods described previously: R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971). KB cells in culture were treated with varying amounts of drug 10–20 min postviral inoculation. The inhibition of viral cytopathic effect was determined microscopically 3 days after viral infection.

cess of chloroform (3 l.). The white powdery residue was dried in a vacuum desiccator over P_2O_5 overnight to obtain 55.6 g (94%) of IV as a chromatographically homogeneous material. The ir spectrum showed a strong band at 1179 cm^{-1} corresponding to the arylsulfonyl absorption. In the pmr spectrum the anomeric proton (determined in D_2O) was centered at δ 5.82 as a singlet. On paper electrophoresis in phosphate buffer (pH 7.2) it moved with a single charge like c-AMP: uv λ_{max} (H_2O) 266 mμ (ε 15,100), λ_{max} (pH 1) 264 mμ (ε 17,700), λ_{max} (pH 11) 266 mμ (ε 15,200). *Anal.* ($C_{17}H_{17}N_5O_8PSBr$) C, H, N.

8-Hydroxy-2'-*O*-*p*-toluenesulfonyladenosine Cyclic 3',5'-Phosphate (IVa). Sodium acetate (32.8 g) in acetic acid (800 ml) and compound IIIa (24.0 g, 40 mmol) were added to a solution of acetic anhydride (80 ml). The solution was kept under reflux (absence of moisture) for 3.75 hr (bath temperature 125°); the reaction mixture was cooled and evaporated to dryness under reduced pressure. The residue was evaporated by MeOH, EtOH, and dioxane to remove the last traces of acetic acid and then suspended in a mixture of MeOH (600 ml) and EtOH (400 ml). The solid lumps in the suspension were broken and the precipitated material was filtered (200 ml of MeOH was used for washing). The dried material gave one homogeneous spot on chromatography in several systems: yield 22.61 g; uv λ_{max} (pH 1) 290 mμ, λ_{max} (pH 11) 272.5 and 309.5 mμ.

The acetylated product IVa (2.168 g, 4 mmol) as obtained in the last step was dissolved in methanolic ammonia (60 ml) and the solution was left at room temperature for 90 hr. Then the solution was evaporated under reduced pressure to dryness. The residue was suspended and triturated with EtOH (40 ml) and left in refrigerator overnight. The next morning the precipitated material was filtered, washed with EtOH (20 ml), and dried to obtain 1.7 g of IVa. The filtrate and the washings were evaporated under reduced pressure and the residue was crystallized from MeOH–EtOH to obtain another 65 mg of the product: total yield 1.765 g (85.3%); uv λ_{max} (pH 1) 265 and 288 mμ, λ_{max} (pH 11) 282 mμ.

8,2'-Anhydro-9-β-D-arabinofuranosyl-8-hydroxyadenine Cyclic 3',5'-Phosphate (Va). Compound IVa (1.551 g, 3 mmol) was dissolved in methanolic ammonia (50 ml) and the solution maintained in a bomb for 6 hr at 80°. The reaction products were adsorbed in a silica acid column (Mallinkrodt, 100 mesh, 2.7 × 70 cm). The column was eluted with chloroform (400 ml), followed by MeOH–chloroform (1:1, v/v). The product Va appeared in the second peak and the corresponding fractions were collected and evaporated. The residue was dissolved in MeOH (40 ml), cooled to obtain the product as a crystalline ammonium salt, and filtered (440 mg), and the filtrate was acidified with 2 *N* HCl to *ca.* pH 2 when another crop of Va (0.16 g) was obtained as free acid: total yield 0.60 g (59.3%). The ir spectrum showed no absorption band corresponding to an arylsulfonyl group at 1179 cm^{-1}. In the pmr spectrum (determined in DMSO–NaOD) the anomeric proton appeared as a doublet centered at δ 6.5 indicating an arabinose configuration of the 2'-anhydro linkage. The 2' proton was a triplet located at δ 5.95; uv λ_{max} (pH 1) 259 and 286 mμ (sh), λ_{max} (pH 11) 257 mμ. *Anal.* ($C_{10}H_{10}N_5O_6P$) C, H, N.

9-β-D-Arabinofuranosyl-8-mercaptoadenine Cyclic 3',5'-Phosphate (VIa). Method I. Compound Va (1.30 g, 4 mmol) was suspended in DMF (15 ml) and pyridine 2 (2 ml), followed by liquid H_2S (40 ml) in a bomb. The contents were kept at 100° (bath temperature) for 16 hr, cooled, and then evaporated to dryness under vacuum. The residue was adsorbed on a silicic acid (Mallinkrodt, 100 mesh) column (5 × 40 cm). The column was eluted with chloroform (950 ml), followed by a mixture of chloroform and MeOH (1:1, v/v). There were two major uv-absorbing peaks; the fractions corresponding to the first peak were collected and evaporated to obtain 900 mg of white powder (VIa) and recrystallized from MeOH–EtOH to obtain 835 mg (57.7%) in three different crops. A portion was dissolved in water and the aqueous solution was acidified to pH 2 to obtain the crystalline free acid of VIa which was dried at 80° for 4 hr under vacuum: uv λ_{max} (pH 1) 244 mμ (ε 10,620) and 308 (26,170), λ_{max} (pH 11) 294 mμ (ε 23,970). Pmr (in D_2O) showed the anomeric proton as a characteristic doublet centered at δ 7.0. *Anal.* ($C_{10}H_{12}N_5O_6PS \cdot H_2O$) C, H, N.

Table II. Cytotoxicity of c-Ara-GMP to a Variety of Cell Lines in Culture[a]

Cell line	Concn in μg/ml required for 50% inhibition of the cell growth
KB	3.2
HeLa	10
H.Ep.-2	100
Sarcoma 180	1
RK-13	10

[a]See reference in footnote *a* of Table I.

Method II. Compound Va (7.7 g, 23.3 mmol) was suspended in DMF (110 ml) in a stainless steel bomb followed by addition of liquid H_2S (55 ml) in the absence of moisture. The reaction contents were maintained at 110° for 20 hr and cooled and then the precipitated residue was filtered with the help of EtOH (100 ml), washed with ether (100 ml), and dried under suction to obtain almost pure VIa as a white powdery material (5.39 g). The product was dissolved in H_2O (30 ml) and filtered. To the filtrate EtOH (30 ml) was added followed by acidification with 2 *N* HCl (pH <2) and the precipitated product was kept at room temperature for 1 hr and then filtered. The precipitate was washed with aqueous EtOH (5 ml, 50%) and then dried at 60° (0.1 mm pressure) for 4 hr to obtain 3.85 g of chromatographically homogeneous VIa. The filtrate on further acidification gave another crop of 0.20 g.

The original filtrate from the reaction mixture and the washings were mixed, evaporated, and adsorbed on a dry silicic acid column (4.5 × 18.0 cm). The column was eluted with 50% methanolic chloroform (150 ml), followed by 10% methanolic ammonia (10-ml fractions were collected). The fraction corresponding to pure VIa was collected and evaporated and the residue was dissolved in MeOH with the help of 2 *N* NH_4OH and precipitated by acidification to obtain 1.3 g of pure VIa: total yield 5.15 g (61.2%).

9-β-D-Arabinofuranosyladenine Cyclic 3',5'-Phosphate (VIIa). Compound VIa (3.04 g, 8 mmol) was dissolved in MeOH (250 ml) containing 10 ml of NH_4OH (2 *N*). To the solution Raney nickel catalyst (36 g wet weight) was added and the mixture was maintained under reflux (bath temperature 75°) for 18 hr. Then the catalyst was filtered through a Celite pad, washed with MeOH (100 ml containing 10 ml of 2 *N* NH_4OH). The filtrate and the washings were evaporated to dryness and the residue was dissolved in MeOH (30 ml) and a few drops of 2 *N* NH_4OH. The solution was filtered and acidified to precipitate the

product as free acid (2.05 g). The filtrate on concentration gave another crop of 240 mg: total yield 2.24 g (81%). It was recrystallized by dissolving in water and acidification and dried at 80° for 4 hr (1 mm pressure): uv λ_{max} (pH 1) 256 mμ (ε 15,200), λ_{max} (pH 11) 258 mμ (ε 15,400). *Anal.* ($C_{10}H_{12}O_6N_5P$) C, H, N.

8-Bromoguanosine Cyclic 3′,5′-Phosphate (IIb). Guanosine cyclic 3′,5′-phosphate (Ib, 21.95 g, 50 mmol) was suspended in H_2O (150 ml), and to the stirred suspension 50 ml of aqueous bromine (10 g, 125 mmol) was added in small portions. Then, the homogeneous reaction solution was stirred for another 2 hr and extracted with ether (2 × 200 ml). The aqueous layer was decolorized with Na_2SO_3 (~1–2 g) and neutralized with 2 *N* NaOH (pH ~8). To this solution, ethanol (twice the volume of the reaction mixture, ~600 ml) was added, and the precipitated material was kept in the freezer overnight. Then, the white precipitate was filtered, washed with ethanol and ether, and dried under suction to obtain compound IIb as white chromatographically homogenous powder (20.2 g, yield 90%). The pmr spectrum showed the anomeric proton (determined in DMSO-d_6) as a singlet centered at 5.71. On paper electrophoresis in phosphate buffer (pH 7.2) and borate buffer (pH 9.2) compound IIb moved like c-GMP: uv λ_{max} (pH 1) 263 mμ (ε 15,940) and 271 (sh, 13,230), λ_{max} (pH 11) 274 mμ (ε 13,200). *Anal.* ($C_{10}H_{10}N_5O_7PBrNa \cdot 1.1H_2O$) C, H, N.

8-Bromo-2′-*O*-*p*-toluenesulfonylguanosine Cyclic 3′,5′-Phosphate (IIIb). Compound IIb (22.3 g, 50 mmol) was dissolved in 1 *N* NaOH (100 ml) and to this stirred solution *p*-toluenesulfonyl chloride (38.13 g, 200 mmol in 480 ml of dioxane) was added dropwise (at a fast drop rate) during a period of 1 hr. The reaction mixture was stirred vigorously at room temperature for another 5 hr. Then the reaction mixture was poured over ice water (1.5 l.) and stirred vigorously for a period of 15 min, the precipitate was filtered, and the residual solid was washed with ice water (500 ml). The filtrate and the washings were combined and evaporated under vacuum to obtain a white residue which was coevaporated with ethanol and methanol. The product thus obtained was extracted with chloroform and ether exhaustively to remove *p*-toluenesulfonic acid, and the residual product was recrystallized from methanol to obtain compound IIIb as a white powder (24.8 g, 82%). Compound IIIb was chromatographically homogenous and on paper electrophoresis in phosphate buffer (pH 7.2) and borate buffer (pH 9.2) moved like c-GMP. In the pmr spectrum, the anomeric proton (determined in D_2O–NaOD) was centered at δ 5.71 as a singlet; uv λ_{max} (pH 1) 262 mμ (ε 16,028) and 275 (sh) (65,000), λ_{max} (pH 11) 270 mμ (ε 15,030). *Anal.* ($C_{17}H_{16}N_5O_9PSBrNa \cdot 2H_2O$) C, H, N.

8-Hydroxy-2′-*O*-*p*-toluenesulfonylguanosine Cyclic 3′,5′-Phosphate (IVb). Compound IIIb (6 g, 10 mmol) was dissolved in acetic acid (600 ml), sodium acetate (anhydrous) (8.2 g, 100 mmol) was added, and the reaction mixture was heated at 125° (bath temperature) for 4.5 hr with vigorous stirring. The solution was cooled (room temperature) and evaporated to dryness under vacuum. The residue was evaporated from ethanol and methanol and dried under high vacuum at 60° for 18 hr. The dried yellow residue was extracted with methanol (3 × 100 ml) and the pale yellow residue was washed with ether and dried to obtain compound IVb (5.1 g, 85%). The analytical sample was prepared by preparative tlc on 2-mm silica gel GF glass plates developed in a MeOH–$CHCl_3$ (1:1, v/v) solvent system. The product was chromatographically homogeneous and on electrophoresis in phosphate buffer (pH 7.2) and borate buffer (pH 9.2) migrated like c-GMP. The anomeric proton (pmr spectrum in DMSO-d_6) was a signlet centered at δ 5.48; uv λ_{max} (pH 1) 231 mμ (ε 16,780), 248 (13,100), and 294 (10,050), λ_{max} (pH 11) 233 mμ (ε 16,070), 249 (10,085), and 278 (9920). *Anal.* ($C_{16}H_{17}N_5O_{10}PSNa \cdot 3H_2O$) C, H, N.

8,2′-Anhydro-9-β-D-arabinofuranosyl-8-hydroxyguanine Cyclic 3′,5′-Phosphate (Vb). Compound IVb (3.22 g, 6 mmol) was dissolved in dry DMF (250 ml) with the addition of 1,5-diazabicyclo[5.4.0]undec-5-ene (0.91 g, 6 mmol). To this solution, sodium acetate (4.929 g, 60 mmol) was added and the stirred reaction mixture heated (bath temperature) at 125–130° for 3 hr with constant stirring (a precipitate appeared after 30 min). Then the reaction mixture was cooled (room temperature) and filtered, and the gelatinous precipitate (R1) was washed with methanol (2 × 40 ml) and ether and dried to obtain 1.17 g of white powder. The methanol washings were combined with the DMF filtrate and evaporated to dryness, coevaporated with methanol (3 × 20 ml), and finally taken in methanol (~60 ml) and triturated with ether. The precipitated material was collected (centrifugation) and washed with ether. The yellow precipitate (5.4 g) was purified by column chromatography (dry Avicel microcrystalline cellulose column, 5 × 50 cm), and the product was developed with CH_3CN–H_2O (7:3, v/v). The appropriate fractions were combined and evaporated to obtain product Vb (750 mg).

The product R1 was further purified by preparative tlc [Anal Tech MN 300-F cellulose plates developed in a CH_3CN–H_2O (7:30, v/v) solvent system] to obtain pure compound Vb (570 mg): total yield 1.32 g (60%). The product was chromatographically homogeneous and on electrophoresis (pH 7.2 phosphate buffer and pH 9.2 borate buffer) moved like c-GMP. In the pmr spectrum (in DMSO-d_6–NaOD), the anomeric proton of compound Vb was a doublet centered at δ 6.4; uv λ_{max} (pH 1) 244 mμ (ε 12,360) and 286 (9520), λ_{max} (pH 11) 247 mμ (ε 12,100) and 270 (sh, m) (9250). *Anal.* ($C_{10}H_{10}N_5O_7P \cdot 0.5H_2O$) C, H, N.

9-β-D-Arabinofuranosyl-8-thioguanine Cyclic 3′,5′-Phosphate (VIb). Compound Vb (735 mg, 2 mmol) was suspended in dry DMF (15 ml) in a stainless steel bomb and heated with liquid H_2S (30 ml) at 115–120° for 18 hr with constant stirring. The reaction mixture was evaporated carefully under high vacuum and the residue coevaporated with methanol–ethanol. It was finally extracted with methanol (3 × 25 ml) and the methanol solution was triturated with ether to obtain a pale yellow product VIb (355 mg, 44%) which was chromatographically homogeneous. On paper electrophoresis in phosphate buffer (pH 7.2), it moved like c-GMP and in borate buffer (pH 9.2), it moved ahead of c-GMP; uv λ_{max} (pH 1) 285 (broad band) and 302 (sh) mμ, λ_{max} (pH 11) 290 mμ.

9-β-D-Arabinofuranosylguanine Cyclic 3′,5′-Phosphate (VIIb). Compound VIb (400 mg, 1 mmol) was dissolved in methanol (50 ml) and 2 *N* NH_4OH (5 ml). To this solution, Raney nickel catalyst (4 g wet wt) was added and the reaction carried out as described for VIIa (2 hr). The cooled reaction mixture was filtered and the residual catalyst washed with methanol (2 × 10 ml). The filtrate and the washings were combined and evaporated, and the residue was dissolved in H_2O (25 ml). The aqueous solution was passed through Dowex 50-X8 (100–200 mesh) Na^+ column (2 × 10 cm), and the appropriate fractions were combined and lyophilized to obtain compound VIIb as a white fluffy powder (176 mg, 48%). The analytical sample was prepared by preparative tlc using Anal Tech MN 300F cellulose glass plates developed with CH_3CN–H_2O (7:3, v/v). The product was chromatographically homogeneous and on paper electrophoresis in phosphate buffer (pH 7.2) and borate buffer (pH 9.2) it moved like c-GMP: uv λ_{max} (pH 1) 256 mμ (ε 10,387) and 275 (sh) (7400), λ_{max} (pH 7) 252 mμ (ε 11,150) and 275 (sh) (7434), λ_{max} (pH 11) 262 mμ (ε 10,280). The pmr spectrum (in D_2O) showed the anomeric proton as a doublet located at δ 6.15. *Anal.* ($C_{10}H_{11}N_5O_7PNa \cdot 4H_2O$) C, H, N.

References

(1) R. J. Suhaldonik, "Nucleoside Antibiotics," Wiley-Interscience, New York, N. Y., 1970, p 123.
(2) S. S. Cohen, *Prog. Nucl. Acid Res. Mol. Biol.* **5**, 1 (1966).
(3) F. M. Schabel, Jr., *Chemotherapy*, **13**, 321 (1968).
(4) D. L. Chao and A. P. Kimball, *Cancer Res.*, **32**, 1721 (1972).
(5) J. J. Furth and S. S. Cohen, *Cancer Res.*, **27**, 1528 (1967).
(6) J. L. York and G. A. LePage, *Can. J. Biochem.*, **44**, 19 (1966).
(7) P. J. Ortiz, *Biochem. Biophys. Res. Commun.*, **46**, 1728 (1972).
(8) A. Kornberg, *Science*, **163**, 1410 (1969).
(9) E. J. Reist and L. Goodman, *Biochemistry*, **3**, 15 (1964).
(10) J. J. Brink and G. A. LePage, *Cancer Res.*, **24**, 312 (1964).
(11) T. A. Khwaja and C. Heidelberger, *J. Med. Chem.*, **10**, 1066 (1967).
(12) R. W. Brockman, *Advan. Cancer Res.*, **7**, 129 (1963).
(13) I. C. Caldwell, J. F. Henderson, and A. R. P. Peterson, *Can. J. Biochem.*, **45**, 735 (1967).
(14) W. Plunkett, L. Lapi, P. Orritz, and S. S. Cohn, *Proc. Amer. Ass. Cancer Res.*, **14**, 32 (1973).
(15) G. A. LePage and E. M. Hersh, *Biochem. Biophys. Res. Commun.*, **46**, 1918 (1972).
(16) J. P. Jost and H. V. Rickenberg, *Annu. Rev. Biochem.*, **40**, 741 (1971).
(17) W. L. Ryan and M. L. Heidrick, *Science*, **162**, 1484 (1968).
(18) M. L. Heidrick and W. L. Ryan, *Cancer Res.*, **30**, 376 (1970).
(19) D. Gericke and P. Chandra, *Z. Physiol. Chem.*, **350**, 1469 (1969).
(20) J. W. Hadden, E. M. Hadden, M. K. Haddox, and N. D. Goldberg, *Proc. Nat. Acad. Sci. U. S.*, **69**, 3024 (1972).

(21) R. A. Long, G. L. Szekeres, T. A. Khwaja, R. W. Sidwell, L. N. Simon, and R. K. Robins, *J. Med. Chem.*, **15**, 1215 (1972).
(22) W. W. Lee, L. V. Fisher, and L. Goodman, *J. Heterocycl. Chem.*, 8, 179 (1971).
(23) T. A. Khwaja, R. Harris, and R. K. Robins, *Tetrahedron Lett.*, 4681 (1972).
(24) R. E. Holmes and R. K. Robins, *J. Amer. Chem. Soc.*, **86**, 1242 (1964).
(25) M. Ikehara and K. Muneyama, *J. Org. Chem.*, **32**, 3039 (1967).
(26) M. Ikehara, M. Kaneko, and Y. Ogiso, *Tetrahedron Lett.*, 4673 (1970).
(27) J. P. Miller, D. A. Shuman, M. B. Scholten, M. K. Dimmitt, C. M. Stewart, T. A. Khwaja, R. K. Robins, and L. N. Simon, *Biochemistry*, **12**, 1010 (1973).
(28) G. A. LePage and E. M. Hersh, *Biochem. Biophys. Res. Commun.*, **46**, 1918 (1972).
(29) R. G. Hughes, Jr., and A. P. Kimball, *Cancer Res.*, **32**, 1791 (1972).
(30) M. Ikehara and S. Uesugi, *Chem. Pharm. Bull.*, 17, 348 (1969).

Synthesis and Antitumor Activity of α- and β-2′-Deoxy-6-selenoguanosine and Certain Related Derivatives†

George H. Milne and Leroy B. Townsend*

Department of Biopharmaceutical Sciences and Department of Chemistry, University of Utah, Salt Lake City, Utah 84112.
Received August 17, 1973

Synthesis of the α and β anomers of 2′-deoxy-6-selenoguanosine (**2** and **4**) has been accomplished in good yield. Treatment of **2** and **4** with several alkyl halides under basic conditions has furnished the corresponding 2′-deoxy-6-alkylselenoguanosines. Treatment of the α and β anomers of 2-acetamido-6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl)purine (**5** and **1**) with sodium methoxide has provided the α and β anomers of 2-amino-6-methoxy-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine (**10** and **6**). Treatment of **5** and **1** with hydrogen in the presence of a Pd-on-C catalyst was followed by treatment with sodium methoxide to provide the α and β anomers of 2-amino-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine (**8** and **9**). Compounds **10** and **6** as well as **8** and **9** have also been prepared by an alternate route. The antitumor activity against leukemia L-1210 for the above compounds as well as certain related selenonucleosides prepared previously in this laboratory is discussed.

The antitumor activity[1,2] of 6-thioguanine (TG) is suggested to result from incorporation into DNA with ultimate cell death. It is of interest that TG has been recently isolated[3] from a fermentation broth of *pseudomonas* sp. C, H (HLR 186 B). Several tumor cell lines have developed[4] a resistance toward the chemotherapeutic effect of TG. A better therapeutic index and comparable antitumor inhibition have been reported[5] for 6-selenoguanine (SeG) and this prompted the first synthesis of 6-selenoguanosine (SeGR) in our laboratory.[6] A comparative study[7,8] (thio *vs.* seleno) has subsequently established that SeG and SeGR inhibit the growth of Sarcoma 180 ascites cells more effectively than the corresponding thionucleosides. The first synthesis of α- and β-2′-deoxy-6-selenoguanosine was accomplished in our laboratory[9] on the basis that both α- and β-2′-deoxy-6-thioguanosine had demonstrated[10] sufficient antitumor activity to be considered as candidates for clinical trials.

The initial route we envisaged for synthesizing selenonucleosides[9] in the deoxynucleoside area [2-amino-6-seleno-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**4**, β-2′-deoxy-6-selenoguanosine) and 2-amino-6-seleno-9-(2-deoxy-α-D-*erythro*-pentofuranosyl)purine (**2**, α-2′-deoxy-6-selenoguanosine)] was based on our successful preparation of SeGR by treatment of 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine with sodium hydrogen selenide in methanol at reflux temperature. However, synthesis of the starting materials [the α and β anomers of 2-amino-6-chloro-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine] presented some difficulties since treatment of the individual anomers (α and β) of 2-acetamido-6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl)purine[11] (**5** and **1**, respectively) with methanolic sodium methoxide at reflux temperature, which was required for a complete removal of the acyl group from the exocyclic 2-amino group, furnished a mixture of nucleosides in each instance. These nucleosides were separated by tlc and assigned the structures 2-amino-6-chloro-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine (minor component) and 2-amino-6-methoxy-9-(2-deoxy-D-*erythro*-pentofuranosyl)purine on the basis of a comparison of uv spectra with model compounds.[12,13] Since the desired nucleoside was found to be the minor component, this prompted us to initiate an alternate route for the synthesis of **2** and **4**.

Treatment of **1** with sodium hydrogen selenide in methanol effected a facile nucleophilic displacement of the 6-chloro group as evidenced by tlc and uv spectrum. The protecting groups were then removed with sodium methoxide in methanol at reflux temperature to furnish a bright yellow crystalline nucleoside which was characterized as β-2′-deoxy-6-selenoguanosine (**4**): pmr (DMSO-d_6) sharp singlet at δ 6.8 (exocyclic amino group), a triplet centered at δ 6.20 (peak width 14 Hz) (anomeric proton), and the characteristic pattern usually observed for the remaining protons of a 2-deoxyribofuranose moiety.[14] A similar procedure using **5** furnished a 46% yield of α-2′-deoxy-6-selenoguanosine (**2**). The pmr data obtained for **2** were found to be essentially the same as that observed for the β anomer **4**, except for the quartet at δ 6.2 (peak width 10.5 Hz) instead of a triplet which is generally characteristic[14] for the anomeric proton of a α-2′-deoxyribofuranoside. The synthesis of both anomers (α and β) of 2′-deoxy-6-selenoguanosine is of interest in view of a report[15] on the phosphorylation of both anomers of 2′-deoxy-6-thioguanosine. The β anomer of 2′-deoxy-6-thioguanosine has been converted to a nucleotide derivative by certain neoplastic and normal cells while the α anomer was more specific and although several neoplasms did afford the nucleotide derivative there was observed no phosphorylation in the normal tissues studied. A more recent

†This work was supported by Research Contract No. C-72-3710 and N01 CM 23710 with Drug Research and Development, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, U. S. Public Health Service.