

# Nucleoside Conjugates V: Synthesis and Biological Activity of 9-( $\beta$ -D-Arabinofuranosyl)adenine Conjugates of Corticosteroids

CHUNG IL HONG\*, ALAN J. KIRISITS, ALEXANDER NECHAEV, DAVID J. BUCHHEIT, and CHARLES R. WEST

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**Abstract** □ Eight 5'-(steroid-21-phosphoryl)-9-( $\beta$ -D-arabinofuranosyl)-adenines (IV–XI) have been prepared and evaluated against L1210 lymphoid leukemia in culture. These include the 9-( $\beta$ -D-arabinofuranosyl)adenine conjugates of hydrocortisone (IV), cortisone (V), corticosterone (VI), cortexolone (VII), 11-deoxycorticosterone (VIII), prednisolone (IX), prednisone (X), and dexamethasone (XI). Conjugates IV, IX, X, and XI inhibited the *in vitro* growth of L1210 lymphoid leukemia cells by 50% (ED<sub>50</sub>) at a concentration of 2.3–7.8  $\mu$ M, while 9-( $\beta$ -D-arabinofuranosyl)adenine (vidarabine, I) and its 5'-monophosphate (II) each showed ED<sub>50</sub> value of 30  $\mu$ M. All of the conjugates were enzymatically hydrolyzed to the corresponding steroid and II, the latter undergoing further hydrolysis to I, by phosphodiesterase I, 5'-nucleotidase, and acid phosphatase. However, these conjugates were resistant to hydrolysis by alkaline phosphatase and adenosine deaminase.

**Keyphrases** □ 9-( $\beta$ -D-arabinofuranosyl)adenine—conjugates with corticosteroids, synthesis, antileukemic activity □ Corticosteroids—conjugates with 9-( $\beta$ -D-arabinofuranosyl)adenine, synthesis, antileukemic activity □ Antileukemic agents—potential, 9-( $\beta$ -D-arabinofuranosyl)adenine—corticosteroid conjugates, synthesis, L1210 screen

The therapeutic effectiveness of 9-( $\beta$ -D-arabinofuranosyl)adenine (vidarabine, I) as an antitumor and antiviral agent is limited by poor solubility in water and by its metabolism to the less active 9-( $\beta$ -D-arabinofuranosyl)hypoxanthine by adenosine deaminase (1). In an attempt to overcome these problems, a variety of analogues and prodrugs of I have been synthesized (2–6). In an effort to develop nucleoside conjugates as potential antitumor and antiviral agents, we reported the synthesis and antitumor activity of 1-( $\beta$ -D-arabinofuranosyl)cytosine conjugates of corticosteroids, linked by a phosphodiester bond (7–9). Most of the conjugates showed superior antitumor activity and reduced toxicity based on 1-( $\beta$ -D-arabinofuranosyl)cytosine (cytarabine) content, and were resistant to enzymatic deamination by cytidine deaminase. Furthermore, the antiproliferative effects of corticosteroids on experimental cell systems (10, 11) and their sensitivity even in many nonlymphoid experimental and human tumors, including brain tumors (12–14), have been well demonstrated. In view of the success of the conjugation with corticosteroids, we synthesized 9-( $\beta$ -D-arabinofuranosyl)adenine conjugates of corticosteroids linked through a naturally occurring phosphodiester bond. The advantages of this type of conjugate are (a) increased water solubility, (b) resistance to enzymatic deamination by adenosine deaminase, (c) biological activities from the steroid moiety after release from the conjugate, (d) increased lipophilicity, and (e) a possible target-specific carrier role by the steroid. This paper describes the synthesis and antitumor activity of eight I–corticosteroid conjugates.

## EXPERIMENTAL<sup>1</sup>

**9-( $\beta$ -D-Arabinofuranosyl)adenine 5'-Monophosphate (II)**—This compound was prepared by a previously published procedure (15) with some modification in the isolation procedure. To a cooled mixture (0°C) of 4 mL (44 mmol) of phosphorus oxychloride, 3.9 mL (48 mmol) of pyridine, and 10 mL of acetonitrile was added 0.5 mL of water in a dropwise manner, and the mixture was stirred at 0°C for 15 min, and then, 2.67 g (10 mmol) of I was added and the suspension was stirred at 0–5°C for 3 h. The solution was poured into 200 mL of ice water and neutralized to pH 7.0 with concentrated ammonium hydroxide solution. The mixture was then evaporated to dryness, and the residue was dissolved in 200 mL of water. The aqueous solution was applied to an AG1-X8 (formate) column (4 × 25 cm) prepacked in water. The column was eluted with water (500 mL) and then with 0.5 M formic acid (1000 mL). The 0.5 M formic acid eluate (first 600 mL) was evaporated to dryness, the residue was treated with acetone, and the resulting material removed by filtration and washed with acetone to give 2.62 g (76%) of II as a white solid. The chromatographic mobilities in various solvents and the IR spectrum were identical with those of authentic II; TLC:  $R_f$ (A) 0.21,  $R_f$ (B) 0.22,  $R_f$ (C) 0. Enzymatic hydrolysis with 5'-nucleotidase (EC 3.1.3.5) gave I.

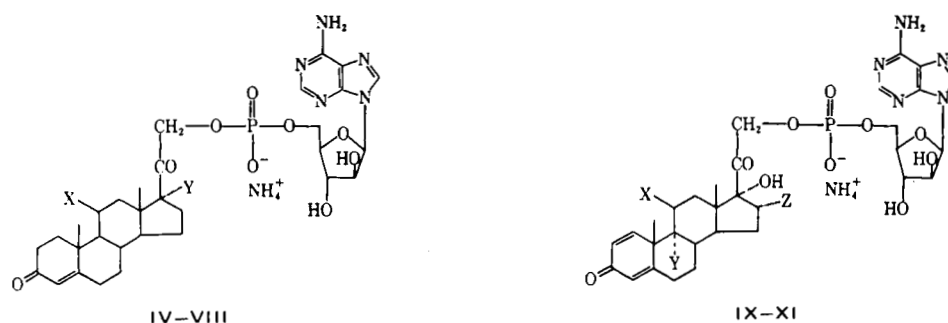
**N<sup>6</sup>,2',3'-Triacetyl-9-( $\beta$ -D-arabinofuranosyl)adenine 5'-Monophosphate (III)**—A mixture of 1.04 g (3 mmol) of dried II, 15 mL of acetic anhydride, and 30 mL of anhydrous pyridine was stirred at room temperature for 18 h, and then 10 mL of water was added to the ice-cooled mixture. After being stirred at room temperature for 2 h, the mixture was evaporated to dryness, and the last traces of water were removed by coevaporation 3 times with pyridine. The syrupy material was used in the next step without further purification.

TLC showed mainly one spot:  $R_f$ (A) 0.21,  $R_f$ (B) 0.23, and  $R_f$ (C) 0.44 (streak). The UV spectrum of the pyridine-free compound taken in 50% ethanol showed a maximum at 270.5 nm with a shoulder at 257 nm. The UV maximum was shifted to 277.5 and 290 nm in 0.1 M HCl and 0.1 M NaOH, respectively.

**5'-(Prednisone-21-phosphoryl)-9-( $\beta$ -D-arabinofuranosyl)adenine (X)**—Compound III, prepared by acetylation of 1.04 g (3 mmol) of II with acetic anhydride (15 mL) and pyridine (30 mL) as described above, was stirred with 2.16 g (6 mmol) of prednisone and 2.45 g (12 mmol) of *N,N'*-dicyclohexylcarbodiimide in 150 mL of anhydrous pyridine at room temperature for 2 d. The mixture was evaporated to dryness, and then the final traces of pyridine were removed by coevaporation with toluene (10 mL). The residue was treated with 100 mL of 50% ethanol, and the insoluble urea was removed by filtration. The filtrate was evaporated to dryness, and the residual material was stirred with 100 mL of 2 M methanolic ammonia at room temperature overnight. The solvent was removed by evaporation and the residue was dissolved in 100 mL of 50% ethanol, and then the solution was applied to a DE-52 (acetate) column (3.5 × 26 cm) prepacked in 50% ethanol. The material was then eluted

<sup>1</sup> Melting points were determined in capillary tubes using a Mel-Temp apparatus and are uncorrected. UV absorption spectra were obtained on a Beckman Acta V spectrophotometer. IR spectra were recorded on a Perkin-Elmer 297 IR spectrophotometer. <sup>1</sup>H-NMR spectra were obtained with a Varian EM-390 spectrometer using tetramethylsilane as internal standard. AG1-X8 (formate, Bio-Rad), diethylaminoethyl cellulose (DE-52, Whatman), and cellulose powder (CC-31, Whatman) were used for column chromatography. Evaporation was performed *in vacuo* at 30°C. TLC was performed on glass plates coated with a (0.25-mm layer of silica gel PF-254 (Brinkman) and on polygram sil G UV 254 plates (Brinkman) using the following solvent systems: (A) isopropyl alcohol–water–concentrated ammonium hydroxide (7:2:1), (B) ethanol–0.5 M ammonium acetate, pH 7.5 (5:2), and (C) chloroform–methanol–water–acetic acid (25:15:4:2). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Table I—Physical Data and Elemental Analysis for the Conjugates



Compound	X	Y	Z	Melting Point <sup>a</sup> , °C	Yield, %	Formula <sup>b</sup>	UV <sub>max</sub> , nm (ε × 10 <sup>-3</sup> )		
							H <sub>2</sub> O	0.1 M HCl	0.1 M NaOH
IV	OH	OH	—	200–205 dec	14	C <sub>31</sub> H <sub>41</sub> N <sub>5</sub> O <sub>11</sub> P·NH <sub>4</sub> ·3H <sub>2</sub> O	252 (22.5)	252 (22.7)	252 (22.1)
V	=O	OH	—	195–200 dec	16	C <sub>31</sub> H <sub>39</sub> N <sub>5</sub> O <sub>11</sub> P·NH <sub>4</sub> ·2.5H <sub>2</sub> O	252 (23.9)	253 (23.9)	252 (23.0)
VI	OH	H	—	192–197 dec	11	C <sub>31</sub> H <sub>41</sub> N <sub>5</sub> O <sub>10</sub> P·NH <sub>4</sub> ·3.5H <sub>2</sub> O	252 (21.1)	252 (21.2)	252 (21.2)
VII	H	OH	—	191–196 dec	21	C <sub>31</sub> H <sub>41</sub> N <sub>5</sub> O <sub>10</sub> P·NH <sub>4</sub> ·3H <sub>2</sub> O	253 (24.0)	250 (24.9)	251 (24.0)
VIII	H	H	—	187–192 dec	16	C <sub>31</sub> H <sub>41</sub> N <sub>5</sub> O <sub>9</sub> P·NH <sub>4</sub> ·2.5H <sub>2</sub> O	251 (24.1)	253 (24.8)	250 (24.2)
IX	OH	H	H	200–205 dec	21	C <sub>31</sub> H <sub>39</sub> N <sub>5</sub> O <sub>11</sub> P·NH <sub>4</sub> ·5H <sub>2</sub> O	256 (19.5)	254 (20.2)	255 (19.9)
X	=O	H	H	205–215 dec	39	C <sub>31</sub> H <sub>37</sub> N <sub>5</sub> O <sub>11</sub> P·NH <sub>4</sub> ·2.5H <sub>2</sub> O	253 (22.8)	252 (21.9)	254 (20.9)
XI	OH	F	CH <sub>3</sub>	190–195 dec	11	C <sub>32</sub> H <sub>40</sub> FN <sub>5</sub> O <sub>11</sub> P·NH <sub>4</sub> ·2H <sub>2</sub> O	251 (21.3)	251 (21.6)	250 (21.0)

<sup>a</sup> No distinct melting point. Slowly decomposed. <sup>b</sup> All compounds were analyzed for C, H, N, and P. Results were within ±0.4% of the theoretical values.

using a linear gradient of acetic acid in 50% ethanol (0–2.0 M, 1000 mL each). The fractions between 1250 and 1790 mL were pooled and evaporated to dryness. The residue was treated with acetone, the material removed by filtration, washed with acetone, and dried to give 798 mg (39%) of X. The analytical sample, as an ammonium salt was prepared as described previously (16), mp 205–215°C (slowly dec.). TLC *R<sub>f</sub>*(A) 0.75, *R<sub>f</sub>*(B) 0.71, *R<sub>f</sub>*(C) 0.65; IR (KBr): 3300–3200 br, 2940, 1720, 1690, 1650, 1600, 1400, 1230, 1080, and 1040 cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 0.49 (s, 3, CH<sub>3</sub>), 1.34 (s, 3, CH<sub>3</sub>), 1.00–3.00 (br m, 13), 3.98 (d, 1, *J* = 4 Hz, C'<sub>4</sub> H), 4.12 (d, 2, *J* = 4 Hz, C'<sub>5</sub> H), 4.30–5.10 (br m, 7), 5.99 (d, 1, *J* = 3 Hz, C<sub>4</sub> H), 6.08 (d, 1, *J* = 10 Hz, C<sub>2</sub> H), 6.28 (d, 1, *J* = 5 Hz, C'<sub>1</sub> H), 7.58 (d, 1, *J* = 10 Hz, C<sub>1</sub> H), 7.74 (br s, 2, NH<sub>2</sub>), 8.19 (s, 1, adenine C<sub>2</sub> H), and 8.25 (s, 1, adenine C<sub>8</sub> H). Table I lists the conjugates prepared in an analogous manner.

**Hydrolysis of the Conjugates with Barium Hydroxide**—The conjugate (0.01 mmol) and barium hydroxide (0.1 mmol) in 1 mL of water were heated at 90–95°C in a stoppered vial for 15 min. Aliquots (0.1 mL) were removed at the designated time, treated with Dowex 50 (H<sup>+</sup>) resin, and examined chromatographically in solvents A and B. Each band was extracted with 50% ethanol and quantitated by UV. Hydrolysis of the conjugate was completed in 5 min, and the products were the steroid 21-monophosphate and I. After standing at room temperature overnight, the steroid phosphate in the hydrolysate was further hydrolyzed to the corresponding steroid.

**Solubility in Water**—A mixture of the compound (20 mg) in 2 mL of water was vortex mixed at full speed at 25°C for 5 min and then filtered through a glass-fiber filter. The clear filtrate was quantitated by UV and the concentration was determined by using the molar extinction coefficient of the compound.

**Antiproliferative Activity In Vitro**—Compounds I, II, IV–XI, and mixtures of I with hydrocortisone, corticosterone, prednisolone, and prednisone were tested for growth-inhibitory activity against L1210 lymphoid leukemia in culture using the methodology described previously (8, 17).

**Determination of Resistance of Adenosine Deaminase**—Cytosolic adenosine deaminase (EC 3.5.4.4) of L1210 leukemia cells was prepared according to a published procedure (17). Assay of enzyme activity was performed in a UV spectrophotometer at 37°C in pH 7.3 phosphate buffer by determining the rate of change in absorbance at 265 nm, as previously described (17, 18). Activity of the enzyme with adenosine was 133.33 IU/L, and the protein concentration was 3.22 mg/mL. For assay of the conjugates, a mixture of compound (0.4 μmol) in 1 mL of water, 0.5 mL of enzyme preparation, and 0.5 mL of 0.18 M phosphate buffer (pH 7.5) was incubated at 37°C for 24 h. The mixture was then evaporated to a small volume and streaked on a TLC plate (0.1 × 20 × 20 cm), and the plate was developed with solvent A. The band matching with the conjugate was extracted with 50% ethanol and quantitated by UV. This was then further incubated with 5'-nucleotidase (EC 3.1.3.5)

from *Crotalus adamanteus* in 0.1 M Tris-HCl (pH 9.0) and 0.005 M magnesium sulfate at 37°C for 24 h. The products were separated by TLC and characterized and quantitated by UV as described previously (8).

**Enzymatic Hydrolysis**—Enzymatic cleavage of the phosphodiester bond of the conjugates was studied by incubating the compounds (5 μmol) with phosphodiesterase I (EC 3.1.4.1), 5'-nucleotidase (EC 3.1.3.5), acid phosphatase (EC 3.1.3.2), and alkaline phosphatase (EC 3.1.3.1) in appropriate buffer (final volume 1.0 mL) as described previously (8). Aliquots (0.1 mL) of the incubation mixtures at various lengths of time were streaked on TLC plates (0.05 × 10 × 20 cm) with authentic markers, and the plates were developed with solvent A. Each band was extracted with 50% ethanol and quantitated by UV.

## RESULTS AND DISCUSSION

**Chemistry**—The conjugates (IV–XI) were prepared by a method similar to that used for the preparation of the 1-(β-D-arabinofuranosyl)-cytosine conjugates (7–9). Phosphorylation of I with phosphorus oxychloride in the presence of water and pyridine in acetonitrile (15) afforded II in 76% yield. The latter compound was then acetylated with acetic anhydride in pyridine. Compound III showed one spot on TLC, and its observed UV maximum was 270.5 nm with a shoulder at 257 nm. Condensation of III with 2 molar equivalents of the various steroids in the presence of *N,N'*-dicyclohexylcarbodiimide and pyridine at room temperature for 2 d and the subsequent removal of the acetyl groups with 2 M methanolic ammonia gave the conjugates (IV–XI) in 10–40% yield after chromatography (Table I).

Table II—Effects of I, II, the Conjugates, and Mixtures of I and Steroids on the Viability of L1210 Lymphoid Leukemia Cells in Culture

Compound	ED <sub>50</sub> , μM <sup>a</sup>
I	30
II	30
IV	4.3
V	61
VI	12
VII	25
VIII	42
IX	2.3
X	4.1
XI	7.8
I and hydrocortisone	52.7
I and corticosterone	48
I and prednisolone	52
I and prednisone	100

<sup>a</sup> Concentration for 50% loss of viability at 72 h.

Structures of the conjugates were verified by elemental analyses, UV, IR, and NMR spectroscopic methods, and chemical and enzymatic hydrolysis of the phosphodiester bond. The observed UV maxima in water for the conjugates were between 251–256 nm ( $\epsilon \sim 21,000$ ) (Table I), which were different from those for the steroids (237–242 nm) and I (259 nm). The strong IR absorption at 1720, 1690, 1650, and 1600  $\text{cm}^{-1}$  supported the presence of the steroid (C=O) and the purine ring (C=C, C=N), and the strong absorptions at 1230 and 1040  $\text{cm}^{-1}$  showed the presence of the phosphodiester bond (P=O, P—O—C). The presence of methyl groups of the steroids and the anomeric, C-2, and C-8 protons of the nucleoside (I) was also supported by the  $^1\text{H-NMR}$  spectra of the conjugates. Like the 1-( $\beta$ -D-arabinofuranosyl)cytosine conjugates (7–9), hydrolysis of the conjugates (IV–XI) by 0.05 M  $\text{Ba(OH)}_2$  yielded I and the corresponding steroid 21-monophosphate, which was further hydrolyzed to the steroid. When enzymatic hydrolysis with phosphodiesterase I (EC 3.1.4.1) was used, the products were II and the steroid.

The conjugates were soluble in water ( $\sim 5$  mg/mL). For example, solubilities of IV and IX were 6.69 and 6.49  $\mu\text{mol/mL}$  of water, respectively, while those of I and II were 3.38 and 13.2  $\mu\text{mol/mL}$  of water.

**Antiproliferative Activity In Vitro**—Growth inhibition of L1210 lymphoid leukemia cells in culture by the compounds listed in Table II was measured by Trypan blue exclusion from the viable cells (17). The concentration of each drug which resulted in 50% inhibition ( $\text{ED}_{50}$ ) of growth at 72 h is shown in Table II. The parent drugs, I and II, inhibited moderately the proliferation of L1210 cells in culture ( $\text{ED}_{50} = 30$   $\mu\text{M}$  each). Under the experimental conditions, only 11-deoxycorticosterone (among the steroids) used alone demonstrated an  $\text{ED}_{50}$  that was  $<100$   $\mu\text{M}$  (9). Mixtures of I and steroids showed an  $\text{ED}_{50}$  of 50–100  $\mu\text{M}$ . Among the conjugates, IX, X, and IV were the most potent inhibitors with  $\text{ED}_{50}$  values of 2.3, 4.1, and 4.3  $\mu\text{M}$ , respectively. The conjugates of dexamethasone (XI) and corticosterone (VI) were also active ( $\text{ED}_{50} = 7.8$  and 12.0  $\mu\text{M}$ , respectively); those of corticosterone (VII) and 11-deoxycorticosterone (VIII) were either as effective as I or II or nearly so. Only V was less inhibitory than the parent drugs. Thus, most of the conjugates we tested were found to be more inhibitory than I or II alone and mixtures of I and the steroids.

**Resistance to Adenosine Deaminase**—Using the adenosine deaminase (EC 3.5.4.4) from L1210 cells (17, 18), the observed  $K_m$  and  $V_{\max}$  for adenosine was 63.07  $\mu\text{M}$  and 4.92  $\mu\text{mol/L/min}$ , while those of I were 87.33  $\mu\text{M}$  and 1.32  $\mu\text{mol/L/min}$ . However, the conjugates remained intact under the same experimental condition. In fact, they were found to be resistant to the adenosine deaminase even during a 24-h incubation period at 37°C.

**Enzyme Hydrolysis**—Enzymatic hydrolysis of the conjugates with purified phosphodiesterase I (EC 3.1.4.1), 5'-nucleotidase (EC 3.1.3.5) from *C. adamanteus*, and acid phosphatase (EC 3.1.3.2) from wheat germ showed that the products were the steroid and II. The latter was further hydrolyzed to I during a 24-h incubation period. For example, when IX was incubated with phosphodiesterase I, 50% of the conjugate was hydrolyzed within 60 min. However, the conjugate was almost intact after 60-min incubation with both 5'-nucleotidase and acid phosphatase, and 50% of the conjugate remained intact at the end of a 24-h incubation. All conjugates were found to be resistant to enzymatic hydrolysis by bacterial alkaline phosphatase (EC 3.1.3.1).

Under the conditions of the experimental procedures, the conjugates show strong antiproliferative activity against L1210 in culture. Fur-

thermore, they are resistant to adenosine deaminase and water soluble. Thus, these findings will provide the bases for continued study of the effects of these conjugates *in vivo*. Moreover, as I is active in viral infections involving two groups of DNA viruses (herpes viruses and pox viruses) (19), the conjugates could be potential antiviral agents.

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