Nucleoside Conjugates. 6. Synthesis and Comparison of Antitumor Activity of 1-β-D-Arabinofuranosylcytosine Conjugates of Corticosteroids and Selected Lipophilic Alcohols[1]

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Five new P^1-(steroid-21-yl)-P^2-(1-β-D-arabinofuranosylcytosin-5′-yl)pyrophosphates (ara-CDP-steroids), five 1-β-D-arabinofuranosylcytosine 5′-*O*-(alkyl)phosphates (ara-CMP-alkyl esters), and two P^1-(alkyl)-P^2-(1-β-D-arabinofuranosylcytosin-5′-yl)pyrophosphate (ara-CDP-alkyl esters) have been prepared and evaluated against L1210 lymphoid leukemia in culture and in mice ($C_3D_2F_1$/J). These include ara-CDP-11-deoxycorticosterone (**6a**), ara-CDP-cortisone (**6c**), ara-CDP-corticosterone (**6d**), ara-CDP-cortexolone (**6e**), and ara-CDP-prednisone (**6g**), ara-CMP hexadecyl ester (**7a**), ara-CMP 1-cyclohexylmethyl ester (**7b**), ara-CMP 1-adamantylmethyl ester (**7c**), ara-CMP 2-(1-adamanthyl)ethyl ester (**7d**), ara-CMP 2-chloroethyl ester (**7e**), ara-CDP hexadecyl ester (**9a**), and ara-CDP 1-cyclohexylmethyl ester (**9b**). The in vitro antitumor results indicated that ara-CDP-steroids were as active as the previously reported ara-CMP-steroids and that ara-CMP and ara-CDP-alkyl esters were less growth inhibiting than ara-CDP-steroids and ara-C. However, the in vivo antitumor results indicated that ara-CDP-steroids were generally less effective than the previous monophosphate derivatives. Among them ara-CDP-corticosterone (**6d**) and the known ara-CDP-cortisol (**6b**) showed greater efficacy than ara-C with ILS value of 152% and 209%, respectively, at the optimal dose of 40 and 80 (mg/kg)/day for 9 days, while that of ara-C was 138% at the optimum dose of 9.2 (mg/kg)/day. Generally, ara-CMP alkyl esters (**7a–e**), given ip to the L1210 leukemic mice, were found to be toxic and ineffective. However, ara-CDP hexadecyl ester (**9a**) showed marginal activity (ILS, 38%). These preliminary results support the thesis that the ara-C conjugates of this type may require a lipophilic and naturally occurring moiety for improved efficacy.

After initial attempts to improve the efficacy of 1-β-D-arabinofuranosylcytosine (ara-C)[2] by utilizing lipophilic alkyl esters[3] and amides[4] as molecular depots of the parent nucleoside, recent studies have been carried out with ara-C-corticosteroid[5–10] (**1**, **2**, **6b**, **6f**) and ara-C phospholipid[11–16] (**3**) conjugates linked by a phosphodiester or pyrophosphate diester bond (Figure 1). In an attempt to enhance cellular uptake of ara-CMP, lipophilic long-chain 5′-(alkyl phosphate) esters (**7a**) of ara-C have also been reported.[17] These lipophilic phosphate esters of ara-C are resistant to cytidine deaminase and serve as prodrugs of ara-C or ara-CMP.

In order to further investigate this extremely promising class of prodrugs, the study herein was undertaken with the aims of evaluating the effect of changing the nature of the conjugate linkages and defining structure–activity relationships in the lipophilic moieties of ara-C conjugates. This paper describes the synthesis of five new ara-CDP-steroids (11-deoxycorticosterone, cortisone, corticosterone, cortexolone, and prednisone), five ara-CMP alkyl esters, and two ara-CDP alkyl esters and compares their biological activities with those of the previous ara-C conjugates.

Chemistry. 21-Phosphonocorticosteroids (**5**) were prepared by condensation of the steroid with 2-cyanoethyl phosphate in the presence of DCC[18] or by reaction of 21-iodocorticosteroid[19,20] with phosphoric acid.[21] The latter was found to be a preferred method because of a shorter reaction time and an easy separation of the product by crystallization.

(1) Presented in part: See "Abstracts of Papers", Second Chemical Congress of th North American Continent, Las Vegas, NV, Aug 1980; American Chemical Society: Washington, DC, 1980; MEDI 84. See also "Book of Abstracts"; 183rd National Meeting of the American Chemical Society, Las Vegas, NV, March 1982; American Chemical Society: Washington, DC, 1982, MEDI 78.

(2) Abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; ara-CMP, 1-β-D-arabinofuranosylcytosine 5′-monophosphate; ara-CDP-steroid, P^1-(steroid-21-yl)-P^2-(1-β-D-arabinofuranosylcytosin-5′-yl)pyrophosphate; ara-CMP alkyl ester, 1-(β-D-arabinofuranosyl)cytosine 5′-*O*-(alkyl) phosphate; ara-CDP alkyl ester, P^1-(alkyl)-P^2-(1-β-D-arabinofuranosylcytosin-5′-yl)pyrophosphate; DCC, *N*,*N*′-dicyclohexylcarbodiimide; ara-CMP morpholidate, 1-β-D-arabinofuranosylctyosine 5′-monophosphoromorpholidate; ip, intraperitoneally.

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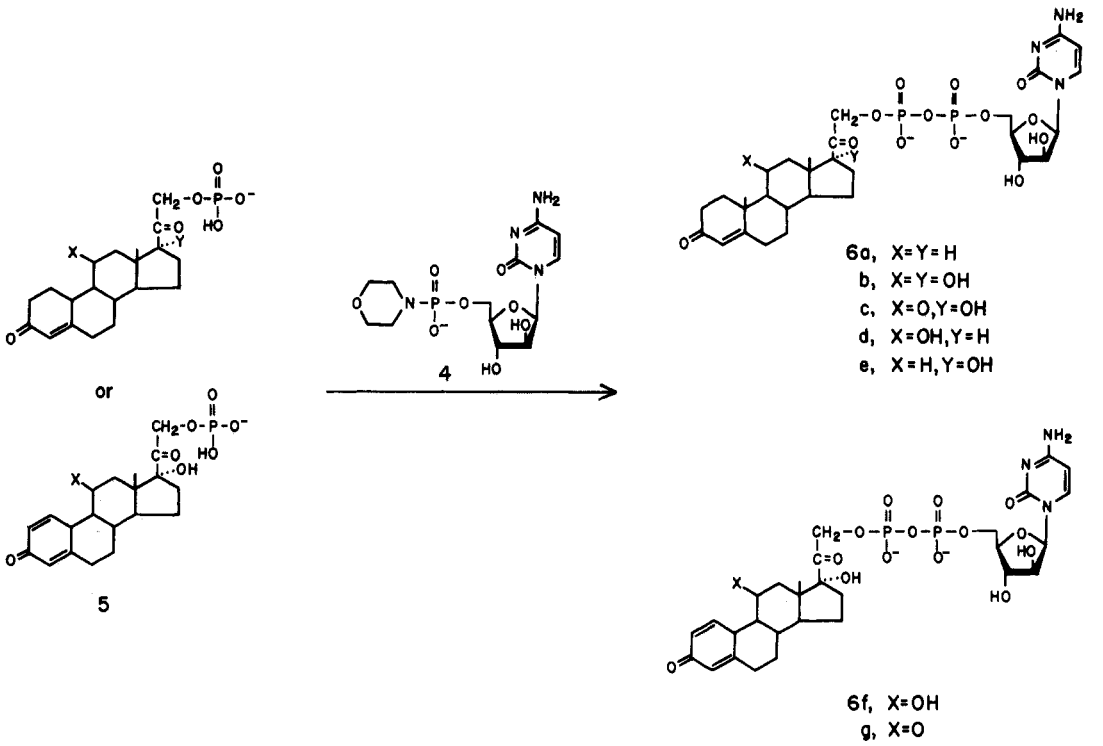
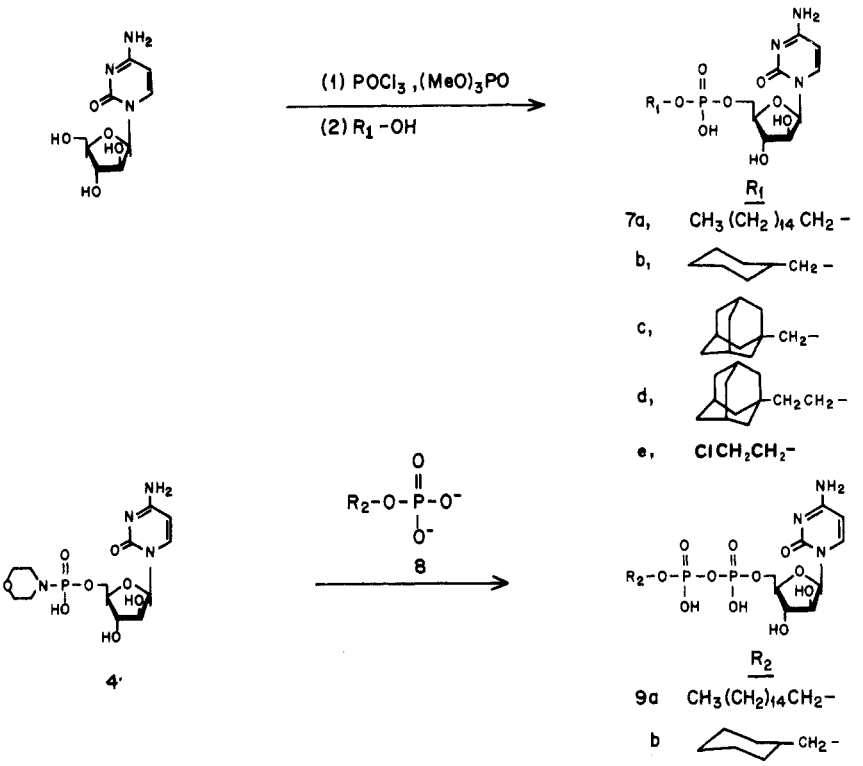
Scheme I. Synthesis of ara-CDP-steroids



Scheme II. Synthesis ara-CMP and ara-CDP Alkyl Esters



ara-CDP-cortisol (**6b**) and ara-CDP-prednisolone (**6f**) have been prepared previously.[10] The new conjugates **6a,c–e,g** were prepared in an analogous manner by condensation of ara-CMP morpholidate (**4**) and the 21-phosphonocorticosteroid **5** in pyridine at room temperature for 5 days (method A) (Scheme I).

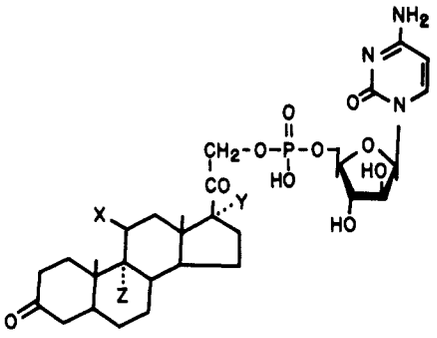
ara-CMP alkyl esters **7a–e** were prepared by two methods. The first method (B) was a continuous operation involving reaction of ara-C with $POCl_3$ in trimethyl phosphate followed 4 h later by quenching with the appropriate anhydrous alcohol and storing at 0–5 °C for 2 days[7,17] (Scheme II). The second method (C) was the condensation of N^4,2′,3′-triacetylara-CMP[6] with 2 molar equiv of the appropriate alcohol in the presence of DCC and pyridine at room temperature for 2 days.[6] The acetyl groups were then removed in 2 N NH_3–MeOH. Conjugate **7a** prepared by method C had chromatograph mobilities in various solvents and IR and UV spectra that were identical with those of the same compound prepared previously by method B.[17]

ara-CDP alkyl esters **9a,b** were prepared by condensation of ara-CMP morpholidate and the alkyl phosphate

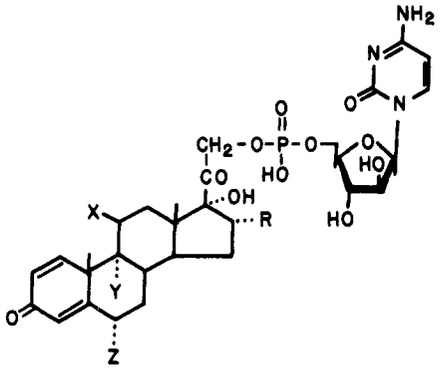
Table I. 1-β-D-Arabinofuranosylcytosine Conjugates

no.	compd	mp, °C	method	yield, %	formula[a]	UV_{max}, nm (ε × 10^{-3}) pH 7.0	0.1 N HCl	0.1 N NaOH
6a	ara-CDP-11-deoxycorticosterone	212–216	A	9.3	$C_{30}H_{41}N_3O_{13}P_2 \cdot 2NH_4 \cdot 5H_2O$[b]	245 (21.9)	246 (20.8)	242 (21.1)
6c	ara-CDP-cortisone	211–215	A	12.0	$C_{30}H_{39}N_3O_{15}P_2 \cdot 2NH_4 \cdot 4.5H_2O$	246 (20.2)	248 (19.7)	244 (20.9)
6d	ara-CDP-corticosterone	210–213	A	28.7	$C_{30}H_{41}N_3O_{14}P_2 \cdot 2NH_4 \cdot 4H_2O$	243 (22.3)	245 (19.8)	243 (21.9)
6e	ara-CDP-cortexolone	208–212	A	8.2	$C_{30}H_{41}N_3O_{14}P_2 \cdot 2NH_4 \cdot 5.5H_2O$	246 (22.1)	247 (20.1)	246 (22.0)
6g	ara-CDP-prednisone	210–215	A	7.3	$C_{30}H_{37}N_3O_{15}P_2 \cdot 2NH_4 \cdot 6H_2O$	244 (21.4)	250 (19.8)	242 (21.5)
7a	ara-CMP hexadecyl ester[c]	159–164	C	15.3	$C_{25}H_{46}N_3O_8P \cdot 0.5H_2O$	273 (15.4)	276 (17.9)	269 (14.7)
7b	ara-CMP 1-cyclohexylmethyl ester	190–195	B	33.4	$C_{16}H_{26}N_3O_8P \cdot H_2O$	273 (17.7)	276 (18.3)	270 (17.2)
7c	ara-CMP 1-adamantylmethyl ester	191–196	B	32.8	$C_{20}H_{30}N_3O_8P$	274 (16.1)	277 (19.8)	270 (15.8)
7d	ara-CMP 2-(1-adamantyl)ethyl ester	189–194	B	15.2	$C_{21}H_{31}N_3O_8P \cdot NH_4$	272 (17.8)	276 (17.9)	268 (17.2)
7e	ara-CMP 2-chloroethyl ester	182–187	B	32.0	$C_{11}H_{17}ClN_3O_8P \cdot 0.5H_2O$	274 (16.0)	277 (18.7)	270 (13.6)
			C	43.4				
9a	ara-CDP hexadecyl ester	188–193	D	9.5	$C_{25}H_{47}N_3O_{11}P_2 \cdot H_2O$	276 (18.6)	278 (22.4)	272 (17.9)
9b	ara-CDP 1-cyclohexylmethyl ester	192–197	D	12.0	$C_{16}H_{27}N_3O_{11}P_2 \cdot 4H_2O$	278 (20.3)	281 (23.8)	273 (19.3)

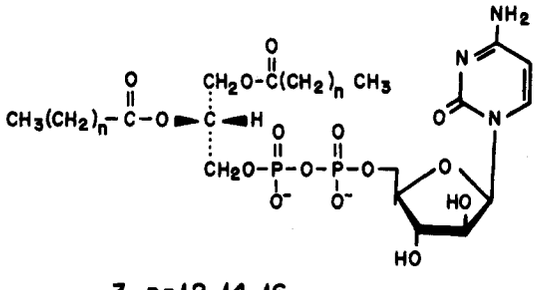
[a] Analyses for C, H, N, and P for all compounds listed are within ±0.4% of the calculated values unless noted otherwise. [b] P: calcd, 7.38; found, 6.76. [c] Also reported in ref 17.



1 X=OH, =O, Y=H, OH, Z=H, F



2 X=OH, =O, Y=H, F, Z=R=H, CH_3



3 n=12, 14, 16

Figure 1. Structure of ara-C-corticosteroid and ara-C-phospholipid conjugates.

8 in pyridine at room temperature for 5 days according to the previously reported procedure.[10] The phosphates 8 were prepared in good yield by phosphorylating the appropriate alcohols with a mixture of $POCl_3$, pyridine, CH_3CN, and H_2O.[22]

The conjugates were separated on a DE-52 (formate or acetate) column using a HOAc, triethylammonium formate, or NH_4OAc gradient in H_2O, 50% EtOH, or $CHCl_3$–MeOH–H_2O (2:3:1) depending on their acidity and lipophilicity as described in the Experimental Section. Physical and UV data for the conjugates are shown in Table I.

Biological Results. In Vitro Studies. Growth inhibition of L1210 lymphoid leukemia cells in culture by compounds listed in Table II was measured by Trypan blue exclusion from the viable cells.[6,23] Most of ara-CDP-steroids **6c–e,g** were more active (ED_{50} = 0.02–0.09 μM) than ara-C (ED_{50} = 0.19 μM) but slightly less active than ara-CMP (ED_{50} = 0.05 μM) except conjugate **6e**. The latter was the most potent conjugate (ED_{50} = 0.02 μM) among the ara-CDP-steroids. ara-CMP alkyl esters **7a–e** showed less activity (ED_{50} = 0.31–16.5 μM) than both ara-C and ara-CMP (Table II). The ED_{50}'s of these conjugates show a decrease in antiproliferative activity with decreasing rate of hydrolysis of the phosphodiester bond by phosphodiesterase I (EC 3.1.4.1).

ara-CDP alkyl esters **9a,b** show slightly greater activity (ED_{50} = 4.30–5.60 μM) than the corresponding ara-CMP derivatives (ED_{50} = 7.00–16.5 μM). However, they were less active than ara-C or ara-CMP. Their superior activity to those of the corresponding ara-CMP derivatives appeared to be due to their sensitivity to hydrolysis by phosphodiesterase I (Table II).

The in vitro results indicate that ara-CDP-steroids are as active as the previously reported ara-CMP-steroids (**1**, **2**)[5–7] and that the ara-C conjugates of lipophilic alcohols are less growth inhibiting than the conjugates of steroids.

In Vivo Studies. Previous studies[5–10] from this laboratory indicated that the efficacy of ara-C-corticosteroid conjugate was more apparent in in vivo studies than in in vitro. Accordingly, we have evaluated the compounds described herein against L1210 lymphoid leukemia in mice. The effects of ara-C and its conjugates of steroids and lipophilic alcohols against ip implanted L1210 lymphoid leukemia in $C_3D_2F_1/J$ are shown in Table III. The procedure was in accord with National Cancer Institute protocol,[24] with the exception that the inoculum in our studies contained 1 × 10^6 cells instead of 1 × 10^5 cells. Under our

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Table II. Correlation of in Vitro Cytotoxicity of ara-CMP and ara-CDP Esters with Cleavage by Phosphodiesterase I

no.	compd	concn, μM, for 50% loss of viability at 72 h (ED_{50})	phosphodiesterase I, % of hydrolysis at 24-h incubation
6a	ara-CDP-11-deoxycorticosterone	0.16	100[a]
6c	ara-CDP-cortisone	0.08	100[a]
6d	ara-CDP-corticosterone	0.09	100[a]
6e	ara-CDP-cortexolone	0.02	100[a]
6g	ara-CDP-prednisone	0.09	100[a]
7a	ara-CMP hexadecyl ester	16.5	0
7b	ara-CMP 1-cyclohexylmethyl ester	7.00	0
7c	ara-CMP 1-adamantylmethyl ester	1.25	9
7d	ara-CMP 2-(1-adamantyl)ethyl ester	0.31	25
7e	ara-CMP 2-chloroethyl ester	3.13	2
9a	ara-CDP hexadecyl ester	4.30	80
9b	ara-CDP 1-cyclohexylmethyl ester	5.60	80
	ara-C	0.19	
	ara-CMP	0.05	
	ara-CMP + corticosterone-21-P	0.08	

[a] Complete hydrolysis after 30-min incubation.

Table III. Antitumor Activity against Intraperitoneally Inoculated L1210 Lymphoid Leukemic Mice[a]

no.	compd	treatment schedule (qd)	active dose range,[b] [mg (μmol)/kg]/day	optimal dose,[c] [mg (μmol)/kg]/day	wt change,[d] g/mouse, on day 8	survival times, days: range	survival times, days: median (T/C)[e]	% ILS[f]	30-day survivors
	ara-C	1–5	5.00 (20.6)–80.0 (32.9)	9.2 (38.0)	−2.66	12–42	15.5/9.5	63	1
		1–9	5.00 (20.6)–20.0 (82.2)	9.2 (38.0)	−4.56	21–30	25.0/10.5	138	1
6a	ara-CDP-11-deoxycorticosterone	1–5		20.0 (28.0)	−3.73	14–15	14.0/11.5	22	0
6b	ara-CDP-cortisol	1–9	20.0 (26.8)–60.0 (80.4)	40.0 (53.6)	+0.94	18 to >45	34.0/11.0	209	4
6c	ara-CDP-cortisone	1–5	40.0 (53.7)–60.0 (80.6)	40.0 (53.7)	−0.78	14–18	15.0/11.0	36	0
6d	ara-CDP-corticosterone	1–5	20.0 (27.3)–80.0 (109)	60.0 (82.0)	−0.45	15–28	19.5/10.0	95	0
		1–9	40.0 (54.7)–80.0 (109)	80.0 (109)	−1.95	19 to >45	26.5/10.5	152	2
6e	ara-CDP-cortexolone	1–5	20.0 (27.3)–60.0 (82.0)	60.0 (82.0)	−2.15	13–18	16.0/12.0	33	0
6f	ara-CDP-prednisolone	1–9	20.0 (26.8)–60.0 (80.5)	40.0 (53.6)	−0.49	18 to >45	24.5/11.0	123	2
6g	ara-CDP-prednisone	1–5	20.0 (26.9)–80.0 (108)	40.0 (53.8)	−0.33	12–17	16.0/11.0	46	0
		1–9	40.0 (53.8)–60.0 (80.7)	60.0 (80.7)	−2.21	13–43	22.5/11.0	105	1
7a	ara-CMP hexadecyl ester	1–5		20.0 (36.5)	−4.75	8–10	9.0/11.5	−22	0
7b	ara-CMP 1-cyclohexylmethyl ester	1–5		40.0 (95.4)	−0.67	10–12	10.5/11.0	−5	0
7d	ara-CMP 2-(1-adamantyl)ethyl ester	1–5		40.0 (82.4)	+0.87	12–14	13.0/11.0	18	0
9a	ara-CDP hexadecyl ester	1–5	20.0 (32.5)–60.0 (97.6)	40.0 (65.0)	+2.92	11	11.0/8.0	38	0

[a] $C_3D_2F_1/J$ mice in groups of six (av wt 25 g) were inoculated ip with 1×10^6 cells of L1210 lymphoid leukemia. Treatment (ip) was initiated 24 h after tumor inoculation. Animals were observed daily until death or 45 days. [b] Dose producing an increase in life span ≥25% over the controls. [c] Dose producing greatest increase in life span. [d] Weight change for the control studies (52 mice) averaged +0.99 ± 2.63 (SD) g/mouse. [e] Calculated from the survivors according to the NCI protocols (ref 24). Median survival day of 52 mice was 10.56 ± 1.16 (SD) days. [f] Percent increase in life span: (T/C − 1) × 100.

experimental conditions, the active dose range found for ara-C, which produced an increase in life span (ILS) ≥ 25% over the control, was 5.00–80.0 (mg/kg)/day. When the optimum dose of 9.2 mg (38 μmol)/kg/day of ara-C was given ip daily for five and nine successive days, the ILS values obtained were 63% and 138%, respectively. Previously we reported that ara-CDP-cortisol (**6b**) and ara-CDP-prednisolone (**6f**) showed greater efficacy than ara-C at their respective optimal doses on a five-day treatment schedule.[10] The present work with the new ara-CDP-steroids showed that only ara-CDP-corticosterone (**6d**) produced a greater efficacy (ILS, 95%) than ara-C with a five-day treatment at the optimum dose. Conjugates **6c,e,g** showed a marginal activity (ILS, 33–46%). The more active conjugates were also given to the leukemic mice for 9 days. They exhibited better efficacy with the extended treatment. However, only **6b** and **6d** showed greater efficacy than ara-C and maximum ILS values obtained were 209% and 152%, respectively.

The antitumor activity of ara-CDP-steroids (**6b,d,f**) were also compared with that of ara-CMP-steroids (**1**, **2**) (Figure 2). The five-day treatments of the leukemic mice with ara-CDP-cortisol (**6b**), ara-CDP-corticosterone (**6d**), and ara-CDP-prednisolone (**6f**) gave the maximum ILS values of 86%, 95%, and 116%, respectively, while those of the corresponding monophosphates were 181%, 200%, and 89%. When the monophosphate derivatives, ara-CMP-cortisol, ara-CMP-corticosterone, and ara-CMP-prednisolone, were given for 9 days, the maximum ILS values obtained were 244%, 306%, and 175%, respectively. The monophosphate derivatives exhibited a greater efficacy (17–110% more) than the diphosphate except ara-CMP-prednisolone with a five-day treatment, as indicated by the results. ara-CMP-corticosterone was twofold more active than the corresponding diphosphate derivative (**6d**). Generally, ara-CMP alkyl esters **7a–e** were toxic. The results obtained with **7a,b** and **7d** are listed in Table III. Some of the mice treated with **7a** and **7b** died before the control, thus giving negative ILS values. However, the diphosphate derivative **9a** showed a marginal activity (ILS, 38%).

Enzymatic Hydrolysis. Enzymatic hydrolysis of the ara-CDP-steroids with purified phosphodiesterase I (EC 3.1.4.1), 5′-nuceotidase (EC 3.1.3.5) from *Crotalus adamanteus*, and acid phosphatase (EC 3.1.3.2) from wheat germ showed that the products were the 21-phosphono-

corticosteroid and ara-CMP. The latter was further hydrolyzed to ara-C during a 24-h incubation period by 5′-nucleotidase and acid phosphatase. The 21-phosphonocorticosteroid was also hydrolyzed further to the steroid by acid phosphatase during 24-h incubation. For example, when ara-CDP-prednisolone (**6f**) was incubated with phosphodiesterase I, 50% of the conjugate was hydrolyzed to ara-CMP and prednisolone 21-monophosphate with 5 min of incubation and the hydrolysis was completed within 30 min. However, 50% of the conjugate was hydrolyzed after a 1-h incubation with acid phosphatase and the hydrolysis was completed within 3 h. ara-CMP alkyl esters were found to be quite resistant to enzymatic hydrolysis by the enzymes tested except ara-CMP 2-(1-adamantyl)-ethyl ester, which was 25% hydrolyzed during 24-h incubation with phosphodiesterase I (Table II). However, ara-CDP alkyl esters were 80% hydrolyzed by phosphodiesterase I during 24-h incubation. All conjugates were found to be resistant to enzymatic hydrolysis by bacterial alkaline phosphatase (EC 3.1.3.1). When ara-CDP-steroids were incubated with normal human serum at 37 °C for 24 h, approximately 40% of each conjugate remained intact. For example, ara-CDP-cortisol (**6b**) was 62% hydrolyzed, the final mixture being the conjugate (24% in molar ratio), ara-CMP (22%), 1-β-D-arabinofuranosyluracil (16%) and cortisol (38%).

Resistance to Cytidine Deaminase. Under the experimental condition using a crude preparation of cytidine deaminase (EC 3.5.4.5) from a human liver,[25] all conjugates remained intact during a 24-h incubation period at 37 °C.

Discussion

Since corticosteroids such as prednisolone and prednisone have been used in combination with other anticancer agents in the treatment of human lymphoid leukemias and lymphomas[26] and since they also exerted synergistic effects in combination therapy,[27] the previous ara-CMP-steroids[5-8] and the new ara-CDP-steroids were synthesized.

The in vitro antiproliferative activity of ara-CDP-steroids against L1210 lymphoid leukemia in culture showed that the conjugates were as active as the corresponding monophosphate derivative.[5-9] However, ara-CDP-steroids were found to be generally less effective than the corresponding monophosphate derivative against ip implanted L1210 lymphoid leukemia in mice (Figure 2). Since the hydrolysis product, a mixture of ara-C and the steroid, was reported to be inactive against L1210 leukemia in mice,[7] differences in the in vivo activity between the mono- and diphosphate derivatives might be attributable to their differences in the rate of enzymatic hydrolysis of the phosphate bonds. For example, when ara-CMP-corticosterone was incubated with phosphodiesterase I, the hydrolysis was complete within 6-h incubation,[7] while completion of hydrolysis of the corresponding diphosphate (**6d**) required only 30 min.

In contrast to these results, ara-CMP alkyl esters, which were resistant to the enzymatic hydrolysis, were found to be very toxic and ineffective as prodrugs of ara-C in L1210 leukemia mice. Besides sensitivity to hydrolysis of the phosphate bond, there might be other reasons for the significant activity found for ara-CMP-steroids and ara-CDP-steroids, since ara-CDP hexadecyl ester displayed only marginal activity (Table III) in spite of its sensitivity to phosphodiesterase I (Table II). Thus, the conjugates

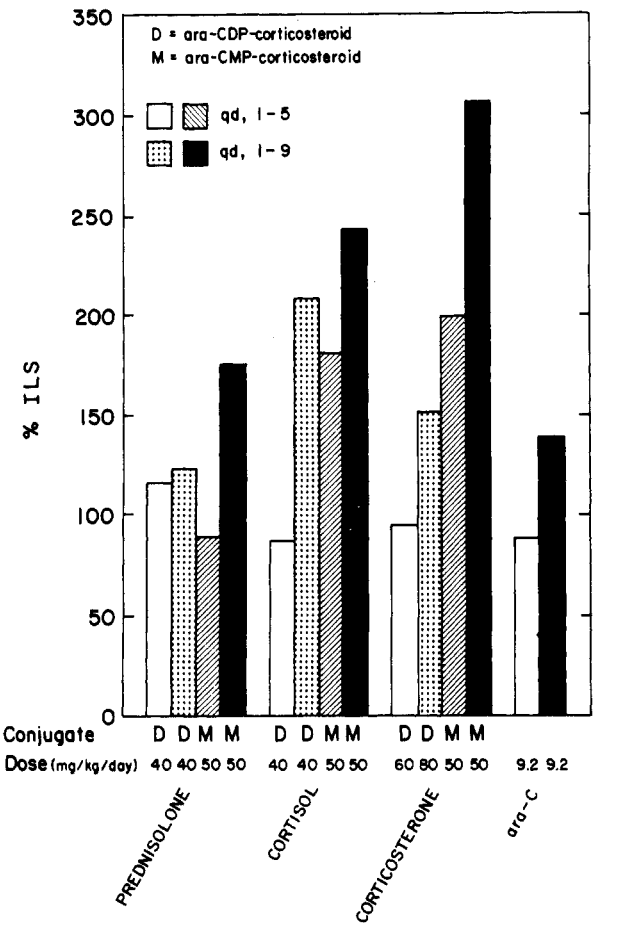

Figure 2. Comparison of ara-CMP- and ara-CDP-steroids on survival of ip inoculated L1210 leukemic mice.

of this type seem to require that the possible carrier moiety is lipophilic and that the phosphate bond is moderately sensitive to phosphoester hydrolyses. Even though the ip injection of ara-CMP alkyl esters was toxic and inactive, it has been reported recently that ara-CMP alkyl esters of 16–20 carbon numbers in alkyl moiety are used as orally active prodrugs of ara-C.[28]

In conclusion, ara-CDP-steroids are ara-C conjugates of corticosteroids with improved water solubility[10] over previous monophosphate derivatives. They displayed significant antitumor activity against ip implanted L1210 lymphoid leukemia in mice with an extended treatment schedule (qd 1–9). These results demonstrate the potential interest of the conjugates in cancer chemotherapy. Further work will be necessary to evaluate additional potential advantages of these conjugates.

Experimental Section

Synthesis. 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 infrared spectrophotometer. NMR spectra were obtained with a Varian EM-390 spectrometer using Me_4Si as an internal standard. AG1-X8 (formate, Bio-Rad), diethylaminoethylcellulose (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

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systems: (A) *i*-PrOH–H_2O–concentrated NH_4OH (7:2:1), (B) EtOH–0.5 M NH_4OAc, pH 7.5 (5:2), and (C) $CHCl_3$–MeOH–H_2O–HOAc (25:15:4:2). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. When analyses are reported only by the element symbols, results were within ±0.4% of the theoretical values.

1-β-D-Arabinofuranosylcytosine 5′-Monophosphoromorpholidate (4). This compound was prepared by condensation of ara-CMP (3.23 g, 10 mmol) with morpholine (3.4 mL, 40 mmol) in the presence of DCC (8.25 g, 40 mmol) by using the published procedure.[10,11] The yield of ara-CMP morpholidate as its *N*,*N*′-dicycohexyl-4-morpholinecarboxamidinium salt was 6.72 g (98.0%). This compound was dried in vacuo over P_2O_5 at 100 °C for 24 h before the condensation reaction.

General Method for the Preparation of 21-Phosphonocorticosteroid (5). (A) By Condensation with 2-Cyanoethyl Phosphate. Steroid (10 mmol) was condensed with 2-cyanoethyl phosphate, prepared from 2-cyanoethyl phosphate barium salt dihydrate (6.45 g, 20 mmol), in the presence of DCC (16.5 g, 80 mmol) and anhydrous pyridine by using the published procedure[18] with some modification[10] of the separation of the product. The yield was 70%.

(B) By Reaction of a 21-Iodocorticosteroid[19] with Phosphoric Acid. 21-Iodocorticosteroids were prepared by refluxing the corticosteroid 21-*O*-*p*-toluenesulfonate and 1.3 equiv of NaI in Me_2CO by using the published procedure.[21] The 21-iodocorticosteroid (8.5 mmol) in CH_3CN (100 mL) was then mixed with a solution of 85% phosphoric acid (2 mL, 34 mmol) and Et_3N (12 mL) in CH_3CN (125 mL) and the mixture was refluxed for 4 h. The solvent was removed in vacuo and the residue was dissolved in MeOH. The methanolic solution was adjusted to pH 11 with 1 N NaOH in MeOH and the white solid was filtered. The filtrate was evaporated to dryness and the gummy residue was dissolved in MeOH (30 mL). After addition of Me_2CO (500 mL) to the solution, the Na salt of 21-phosphonocorticosteroid separated as a white solid. The product was collected, washed with Me_2CO, and dried. The yield was 70%.

***P*^{1}-(Corticosteron-21-yl)-*P*^{2}-(1-β-D-arabinofuranosylcytosin-5′-yl)pyrophosphate (ara-CDP-corticosterone, 6d). Method A.** A dried mixture of 1.28 g (3 mmol) of corticosterone 21-monophosphate and 1.37 g (2 mmol) of ara-CMP morpholidate *N*,*N*′-dicyclohexyl-4-morpholinecarboxamidinium salt (4) in 200 mL of anhydrous pyridine was stirred at room temperature for 5 days. The reaction mixture was then evaporated to dryness and the residue was dissolved in 100 mL of H_2O and 10 mL of HOAc. After being stirred at room temperature for 1 h, the mixture was extracted with a total of 200 mL of Et_2O in three portions and the aqueous layer was evaporated to dryness. The residue was then dissolved in 100 mL of H_2O and the solution was applied to a DE-52 ($HCOO^-$) column (250 g, 5 × 20 cm), prepacked in H_2O. The column was then eluted with a linear gradient of 0–0.5 M triethylammonium formate (pH 7.0) (1000 mL each). Fractions (20 mL) between 1580 and 1660 mL were pooled and passed through a Dowex 50W-×8 (H^+) column (100 g). The column was eluted further with H_2O and the eluate containing the product was evaporated to dryness followed by treating with Me_2CO. The white solid was filtered, washed with Me_2CO, and dried in vacuo over P_2O_5 at room temperature overnight: yield 420 mg (28.7%). The analytical sample (as NH_4 salt) was prepared by passing the product (100 mg) through a cellulose column (100 g, 2.5 × 40 cm) with solvent A: mp 210–213 °C slowly dec; TLC (silica gel), R_f(A) 0.42, R_f(B) 0.85, R_f(C) 0.58; IR (KBr) 3350–3100 (br), 2920 (CH), 1715 (C=O), 1640 (C=O, C=C, C=N), 1410, 1230 (P=O), 1110, 1050 cm^{-1} (POC).

Table I lists the conjugates prepared in an analogous manner.

1-β-D-Arabinofuranosylcytosine 5′-*O*-(1-Adamantylmethyl)phosphate (ara-CMP 1-Adamantylmethyl Ester, 7c). Method B. To a dried mixture of 730 mg (3 mmol) of ara-C and 15 mL of trimethyl phosphate was added 0.5 mL of $POCl_3$ at −10 °C. The mixture was then allowed to react at 0–5 °C for 4 h and then 2.5 g (15 mmol) of 1-adamantanemethanol was added. The mixture was then allowed to react at 0–5 °C for 2 days and poured into 250 mL of cold H_2O containing 1 g of $NaHCO_3$. The suspension was evaporated to a small volume and the residue was taken up in 500 mL of 50% EtOH. The solution was applied to a DE-52 (acetate) column (2.5 × 25 cm) and the column was eluted with a linear gradient of 0–1.0 HOAc in 50% EtOH (1 L each). Fractions between 360 and 860 mL were pooled and evaporated to dryness. The residue was treated with Me_2CO and the product was collected on a filter, washed with Me_2CO, and dried in vacuo over P_2O_5: yield 480 mg (32.8%), mp 191–196 °C slowly dec; TLC (silica gel), R_f(A) 0.58, R_f(B) 0.86, R_f(C) 0.70; IR (KBr) 3300–3100 (br), 2900 (CH), 1700 (C=O), 1600 (C=O, C=C, C=N), 1260 (P=O), 1185, 1015 cm^{-1} (POC).

1-β-D-Arabinofuranosylcytosine 5′-*O*-(2-Chloroethyl)phosphate (ara-CMP 2-Chloroethyl Ester, 7e). Method C. N^4,2′,3′-Triacetylara-CMP,[6] prepared by acetylation of 969 mg (3 mmol) of ara-CMP with 15 mL of Ac_2O in 30 mL of pyridine, was reacted with 2 mL of 2-chloroethanol and 2.5 g of DCC in 200 mL of pyridine at room temperature for 2 days. The excess DCC was then decomposed with 60 mL of H_2O and evaporated to dryness. The residue was treated with 100 mL of 50% EtOH, the insoluble urea was filtered off, and the filtrate was evaporated to dryness. The residue was then stirred in 100 mL of 2 N NH_3–MeOH at room temperature overnight. After evaporation of the solvent, the residue was taken up in 200 mL of 50% EtOH and the solution was applied to a DE-52 (acetate) column (2.5 × 25 cm) prepacked in 50% EtOH. The column was eluted with a linear gradient 0–1.0 N HOAc in 50% EtOH (1 L each). The fractions (20 mL) between 1520 and 1780 mL were pooled and evaporated to dryness. The residue was treated in Me_2CO and the solid was filtered and washed with Me_2CO. The dried product weighed 502 mg (43.4% yield), mp 182–187 °C slowly dec; TLC (silica gel), R_f(A) 0.49, R_f(B) 0.81, R_f(C) 0.42; IR (KBr) 3400–3100 (br), 2930 (CH), 1710 (C=O), 1670 (C=O, C=C, C=N), 1200 (P=O), 1065, 1030 cm^{-1} (POC).

Compound **7a** was prepared in an analogous manner.

Hexadecyl 1-Monophosphate (8, $R_2 = C_{16}H_{33}$). This compound was prepared by phosphorylation of 1-hexadecanol (4.85 g, 20 mmol) with a mixture of $POCl_3$ (8.06 mL), pyridine (7.74 mL), CH_3CN (20 mL), H_2O (1.0 mL), and $CHCl_3$ (15 mL) by using the published procedure[22] with the following modification on separation of the product. The reaction mixture was poured into ice–water (500 mL) and the aqueous suspension was extracted with $CHCl_3$ (3 × 100 mL). The $CHCl_3$ layer was then evaporated to dryness and the dried residue was treated with petroleum ether (bp 35–55 °C): yield 3.35 g (32%).

Cyclohexylmethyl 1-monophosphate (8, $R_2 = C_6H_{11}CH_2$) was prepared in an analogous manner.

***P*^{1}-(1-Hexadecyl)-*P*^{2}-(1-β-D-arabinofuranosylcytosin-5′-yl)pyrophosphate (ara-CDP Hexadecyl Ester, 9a). Method D.** A dried mixture 1.82 g (6 mmol) of hexadecyl 1-monophosphate and 2.05 g (3 mmol) of ara-CMP morpholidate *N*,*N*′-dicyclohexyl-4-morpholinecarboxamidinium salt (4) in 250 mL of anhydrous pyridine was stirred at room temperature for 5 days. The reaction mixture was then evaporated to dryness and the residue was dissolved in 100 mL of $CHCl_3$–MeOH–H_2O (2:3:1) followed by adding 10 mL of HOAc. After the mixture was stirred at room temperature for 1 h, the organic layer was separated out and the aqueous layer was extracted with $CHCl_3$ (2 × 50 mL). The combined organic layers were evaporated to dryness, and the residue was dissolved in $CHCl_3$–MeOH–H_2O (2:3:1). The solution was applied to a DE-52 (acetate) column (2.5 × 50 cm) prepacked in the same solvent. The column was eluted with a linear gradient of 0–1.0 M NH_4OAc made up in $CHCl_3$–MeOH–H_2O (2:3:1) (1 L each). The fractions 700–1000 mL were pooled and passed through a Dowex 50W-×8 (H^+) column (100 g). The column was eluted further with the same solvent and the eluate containing the product was evaporated to dryness. The residue was treated with Me_2CO and the solid was filtered off followed by washing well with Me_2CO before being dried in vacuo over P_2O_5: yield 180 mg (9.5%); mp 188–193 °C slowly dec; TLC (silica gel), R_f (A) 0.47, R_f(B) 0.90, R_f(C) 0.74; IR (KBr) 3400–3100 (br), 2920 (CH), 1730 (C=O), 1640 (C=O, C=C, C=N), 1190 (P=O), 1050 cm^{-1} (POC).

Biochemical Studies. Growth-Inhibiting Assays in Cultured Cells. Compounds shown in Table II were screened for in vitro growth-inhibiting activity against L1210 lymphoid leukemia in culture by using the methodology described previously.[6,23]

Antitumor Activity in Vivo. Compounds shown in Table III were screened for in vivo antitumor activity against intra-

peritoneally implanted L1210 lymphoid leukemia in $C_3D_2F_1/J$ mice (C_3H/HEJ female × DBA/2J male mice, supplied by Jackson Labs) with following schedules qd 1–5 and qd 1–9 according to the procedures outlined in the NCI Protocol[24] with some modification.[5-7] Each drug was tested over a wide range of doses. The active dose ranges are those giving ILS values ≥ 25%. Optimum dose is a dose producing greatest increase in life span on a particular treatment schedule.

Enzymatic Hydrolysis. Enzymatic cleavage of the pyrophosphate diester bond of the conjugate by 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) was studied according to the procedures described previously.[6] Hydrolysis in normal human serum was also studied as described previously.[6]

Determination of Resistance to Cytidine Deaminase. Human liver cytidine deaminase (EC 3.5.4.5) was prepared from tissue which has been removed at autopsy according to the published procedure.[25] Assays for cytidine and ara-C were carried out spectrophotometrically at 290 nm.[25] The specific activity of the cytidine deaminase used in this study was 4.27×10^{-5} mU/mg of protein and protein concentration was 19.95 mg/mL. For assay of deamination of the conjugates, a mixture of compound (10 μmol), 0.2 mL of the enzyme preparation, and 0.8 mL of 0.1 M Tris-HCl (pH 8.0) was incubated at 25 °C for 24 h. During the incubation, aliquots (0.1 mL) were streaked on TLC plate (0.1 × 20 × 20 cm) followed by developing with solvent A. Each band was extracted with 50% ethanol and quantitated by UV. The band matching with the conjugate was further incubated with 5′-nucleotidase (EC 3.1.3.5) from *Crotalus adamanteus* (Sigma Chemical Co.) in 0.1 M Tris-HCl (pH 9.0) and 0.005 M $MgSO_4$ at 37 °C for 24 h, and the products were separated by paper chromatography and characterized and quantitated by UV as described previously.[6]

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Registry No. 4-*N*,*N*′-dicyclohexyl-4-morpholinecarboxamidine, 69467-87-4; **6a**, 93604-93-4; **6c**, 93644-70-3; **6d**, 93604-94-5; **6e**, 93604-95-6; **6g**, 93604-96-7; **7a**, 73532-89-5; **7b**, 93604-97-8; **7c**, 93604-98-9; **7d**, 93604-99-0; **7e**, 93605-00-6; **8** (R_2 = $C_{16}H_{33}$), 3539-43-3; **8** (R_2 = $C_6H_{11}CH_2$), 33026-79-8; **9a**, 93605-01-7; **9b**, 93605-02-8; ara-CMP, 7075-11-8; morpholine, 110-91-8; 2-cyanoethyl phosphate, 2212-88-6; 11-deoxy-21-iodocorticosterone, 20576-46-9; 21-iodocortisone, 5758-63-4; 21-iodocorticosterone, 35500-25-5; 21-iodocortexolone, 4470-79-5; 21-iodopregnisone, 55786-16-8; 1-adamantanemethanol, 770-71-8; N^4,2′,3′-triacetyl-ara-CMP, 71778-96-6; 2-chloroethanol, 107-07-3; corticosterone 21-monophosphate, 10589-81-8.

Synthesis, Stereochemistry, and Analgesic Activity of 4-Mono- and 4,4-Disubstituted 1,2,3,4,5,6-Hexahydro-2,6-methano-3-benzazocines

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The synthesis of 4-alkyl-, 4-aralkyl-, and 4-alkenyl-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocines is described together with some 4,4-disubstituted and 8-hydroxy derivatives. Evidence of the stereochemistry of the 4-substituent was from ^1H and ^{13}C NMR. In the 4-methyl series the equatorial epimer **1b** has a higher analgesic (hot-plate) potency than **1a**, and **10a**, **10c**, and **10f** are also good agonists. **5a** afforded analgesic properties without an antagonist component. Surprisingly **10d**, bearing an 8-OH function, was without analgesic activity, contrasting with the significant hot-plate activity exhibited by 1,2,3,4,5,6-hexahydro-3,5,6-trimethyl-2,6-methano-3-benzazocine. If the assumption is made that the more active enantiomorph in members of this series is configurationally related to (−)-morphine, then it may be that the enantiotopic edge in hexahydro-2,6-methano-3-benzazocines has a narrow steric requirement for analgesic responses.

In spite of the current interest in possible relationships between opioid peptides and synthetic analgesics related to morphine, much work remains to be done in the area of structure–activity relationships in analgesic series such as the 1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocines (6,7-benzomorphans).[1] Steric crowding about nitrogen[2] has been suggested as being important in the analgesic response of such compounds, and Belleau[3,4] and Kolb[5] have proposed that nonbonding electron directionality might influence activity both qualitatively and quantitatively.

Here, the synthesis and stereochemistry of a series of 4-substituted hexahydro-2,6-methano-3-benzazocines with and without an 8-position phenolic hydroxyl group are described, and the effect of steric crowding about nitrogen on analgesic response is explored. Alkyl, aralkyl, and alkenyl substitution α to nitrogen in the piperidine ring of benzomorphans does not unduly influence analgesic properties.

Chemistry. Key intermediates to our objective compounds (**1a–c**) were prepared by the conventional Grewe synthesis of 2,6-methano-3-benzazocines.[1] In the absence of an 11-methyl substituent, both the α-cis (**1a**) and β-trans (**1b**) epimers were isolated. The predominant α-cis epimer **1c** is formed when an 11-methyl group is present. These structures and their stereochemistry were established by examination of ^1H and ^{13}C NMR spectra.[6]

Insertion of alkyl, aralkyl, or alkenyl groups α to nitrogen in **1a**, **1b**, and **1c** may be effected by means of the mediation of the benzazocinium ion (**2**). Mercury(II) acetate oxidation of **1a** or **1b**, according to the method of Leonard,[7,8] afforded 50% of 1,2,5,6-tetrahydro-3,4,6-tri-

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