analytical sample: mp 220° dec; $\lambda_{max}^{pH\,1}$ 330 (ϵ 21,800), $\lambda_{max}^{pH\,11}$ 317 (21,600), $\lambda_{max}^{pH\,7}$ 330 nm (23,200). *Anal.* ($C_9H_{11}N_2O_7PS$) C, H, N.

Ammonium 1-β-D-Arabinofuranosyl-4-methylthio-2-pyrimidone 3′,5′-Cyclic Phosphate (5). To a soln of **4** (0.322 g, 1 mmole) in MeOH–H_2O (5 ml, 8:2) was added concentrated NH_4OH dropwise until pH 11. MeI (1 ml) was added, and the soln stirred at room temp for 5 hr, then kept at 5° for 12 hr. Crystalline NH_4I was removed by filtration and the filtrate concentrated to dryness *in vacuo*. The residue was dissolved in H_2O (25 ml) and applied to a column of S & S DEAE-cellulose. Following elution with triethylammonium bicarbonate as described above, the appropriate fractions were combined and concentrated to dryness *in vacuo*. H_2O (50 ml) was added and removed *in vacuo*. This process was repeated. The resulting solid was dissolved in H_2O (20 ml) and applied to a column of Dowex 50W-X8 (NH_4^+) and the column developed with H_2O. The eluate was concentrated to dryness and the residue crystallized from EtOH for analysis: yield, 0.165 g, 47%; mp 245° dec; $\lambda_{max}^{pH\,11}$ 303 (ϵ 14,900), $\lambda_{max}^{pH\,7}$ 303 nm (15,150). *Anal.* ($C_{10}H_{16}N_3O_7PS$) C, H, N.

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Pyrimidine Nucleosides. 5. Syntheses of Carcinostatic Halogenocyclonucleosides

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Some derivatives of 2,2′-anhydro-ara-C (III), carcinostatic nucleosides, were prepared: 5-bromo (IIa), 5-iodo (IIb), and 5-fluoro (IIc) derivatives were prepared by heating the corresponding ribonucleosides with a partially hydrolyzed phosphorus oxychloride in ethyl acetate. Acyl derivatives (IVa,b) of IIa were obtained directly by bromination of 2,2′-anhydro-ara-C with bromine and an acid anhydride (Ac_2O, $(PhCO)_2O$), which were converted to 1-β-D-arabinofuranosyl-2-amino-5-bromo-4-imino-1,4(2*H*)-dihydropyrimidine (VII) with ammonia in MeOH. Hydrogenation of anhydronucleosides was also reported. All of 2,2′-anhydro-ara-C derivatives were markedly active against leukemia L1210 in mice, but 6-hydroxy-2′,6-anhydro-ara-C (IX) prepared from 5-iodo-ara-C displayed only a weak activity.

1-β-D-Arabinofuranosylcytosine has been used clinically in the treatment of acute leukemias and lymphomas.[1] However, the compound is deaminated very rapidly to inactive 1-β-D-arabinofuranosyluracil.[2] In a short communication,[3] the present authors reported that 2,2′-cyclo-arabinofuranosylcytosine (abbreviated to cyclo-ara-C) (III), which was resistant to cytidine deaminase of mouse kidney, was the most active (against L1210) and the least toxic among the antitumor agents tested.

This finding prompted us to study other pyrimidine cyclonucleosides. In view of the reported biological activities of some halogenated pyrimidine deoxyribonucleosides,[4] 5-halogenated derivatives of III were of interest. This paper deals with the preparation and preliminary biological testing of these compounds.

By conventional halogenation of nucleosides, *viz.*, bromination by bromine water, iodination by iodine monochloride,[5] iodine with iodic acid,[6] or iodine in the pres-

ence of nitrous acid,[7] III failed to give any of the desired halogenocyclonucleosides. But, bromination of III by bromine in the presence of an acid anhydride such as Ac_2O (or benzoic anhydride) afforded 3′,5′-di-*O*-acyl-5-bromocyclo-ara-C (IVa, IVb).

It was difficult to obtain an unprotected 5-halogenated cyclo-ara-C, since alkaline hydrolysis of IVa or IVb was accompanied by cleavage of the anhydro linkage. Treatment of IVa with methanolic ammonia at 5°, for example, afforded 1-β-D-arabinofuranosyl-2-amino-5-bromo-4-imino-1,4(2*H*)-dihydropyrimidine (VII). Catalytic hydrogenation of IVa (or IVb) with 5% Pd/C in hydrogen atmosphere gave the 3′,5′-diester of III (VIa or VIb) in good yield.

5-Bromo (IIa), 5-iodo (IIb), and 5-fluoro (IIc) cyclo derivatives were alternatively prepared by a previously reported[8,9] direct cyclization reaction from the respective 5-halogenocytidines, which were easily prepared by the usual method, except for 5-fluorocytidine (Ic). Ic was synthesized in a manner similar to that described by Fox, *et al.*[10] 2′,3′,5′-Tri-*O*-benzoyl-5-fluorouridine, a key intermediate for the preparation of Ic which was already obtained by the mercuri procedure,[11] was alternatively prepared by the trimethylsiyl method[12] from 2,4-bis(trimethylsiloxy)-5-fluoropyrimidine with tribenzoyl-D-ribofuranosyl bromide.

Thus, cyclization was performed by heating Ia, Ib, or Ic with a partially hydrolyzed phosphorus oxychloride in a small volume of ethyl acetate. The separation of anhydro compounds (IIa, IIb, and IIc) was achieved by ion-exchange chromatography. The structural assignments rest upon the ultraviolet spectral properties and combustion value. 2,2′-Anhydro structures of these compounds were confirmed by the downfield shifts of the H-2′ signal in nmr, characteristic of the 2,2′-cyclonucleoside structure (Scheme I).

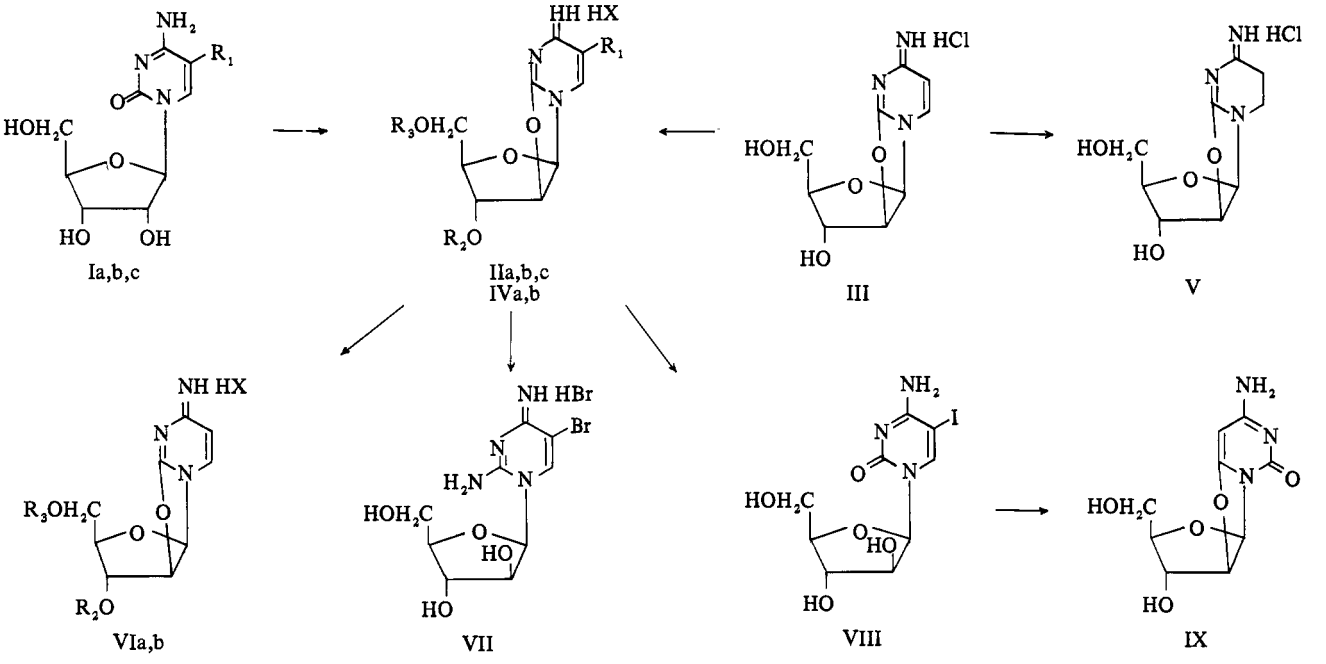
IIa and IIb were then subjected to catalytic hydrogenation with 5% Pd/C in hydrogen atmosphere to afford III, but a prolonged hydrogenation gave a mixture of III and 5,6-dihydro compound. Hydrogenation of III for 6 hr under the same conditions gave 5,6-dihydro-cyclo-ara-C (V).

5-Halogenated cyclo-ara-C was easily hydrolyzed by weak base to the corresponding 5-halogeno-ara-C. Thus IIb was converted to 5-iodo-ara-C (VIII) by treatment with 1 *M* triethylammonium bicarbonate at 50° for 1 hr. The melting point and the uv spectral characteristics of VIII were in agreement with that of authentic compound prepared by published method.[13]

VIII was transformed to 2′,6-anhydro-6-hydroxy-ara-C (IX) which was of interest since it had a similar structure to biologically important III. This transformation was accomplished by heating VIII with an excess of sodium *tert*-butoxide in a mixture of *tert*-BuOH and DMSO (similar conditions to Lipkin's method[14] for the preparation of 5′,6-anhydro-6-hydroxycytidine). The structure of VIII was confirmed by comparison of the melting point (276° dec) and uv spectrum with published data.[15] In this reaction, another product which was not yet characterized was obtained.

Biological Activity. Antitumor activity of the compounds was examined by the methods previously reported.[16] Groups of six male and female BDF_1 mice weighing 20 ± 2 g were

Scheme I



	R_1	R_2	R_3
Ia	Br		
b	I		
c	F		
IIa	Br	H	H
b	I	H	H
c	F	H	H
IVa	Br	Ac	Ac
b	Br	PhCO	PhCO
VIa	H	Ac	Ac
b	H	PhCO	PhCO

Table I. Effect of Pyrimidine Cyclonucleosides against L1210 Leukemia

Compd	Dose, mg/kg per day	% ILS over controls[a]	Activity[b]
Ara-C	100	50	+++
III	100	76	+++
IIa	100	68	+++
IIb	100	58	+++
IIc	30[c]	56	+++
IVa	100	75	+++
IVb	100	65	+++
VIb	100	63	+++
V	100	42	+++
IX	100	18	+

[a]Length of survival time in control groups was 7–9 days. [b]+ = $P < 0.05$; +++ = $P < 0.001$. P was the value for the difference between treated and control groups for the indicated ILS. [c]Only IIc was injected ip at 30 mg/kg per day once daily for 5 days, starting 24 hr after transplantation.

used, 1×10^5 cells of L1210 leukemia were implanted intraperitoneally (ip). The compound to be tested was injected ip at 100 mg/kg per day once daily for 5 days, starting 24 hr after transplantation. Antitumor activity was evaluated by the per cent of increase in life-span (ILS). Activity was graded as follows: – = $P > 0.05$; + = $P < 0.05$; ++ = $P < 0.01$; and +++ = $P < 0.001$, where P was the value for the difference between treated and control groups for the indicated ILS.

Results of the evaluation of the cyclo derivatives of ara-C are given in Table I. The 2′,-6-cyclo derivative IX, in contrast to III, produced only a weak ILS. The 5,6-dihydro compd V showed antitumor activity similar to ara-C but inferior to cyclo-ara-C (III).[3] 5-Halogeno (IIa, IIb) and its acyloxy (IVa, IVb) and 3′,5′-dibenzoyloxy (VIb) derivatives were active against L1210 leukemia among the compounds tested but were not superior to III. IIc was markedly active at a low dose (30 mg/kg per day). Higher doses of IIc are being examined; low toxicity of the compounds is expected. Details of the results and the activity of IIc at higher doses will be reported in the near future.

Experimental Section

All melting points were uncorrected. Nmr spectra in D_2O were obtained on a Varian A-60 instrument, internal standard being DSS. R_f(A) stands for the R_f value in solvent A. Solvents used were: A, *n*-BuOH–H_2O, 86:14; B, *n*-BuOH–AcOH–H_2O, 5:2:3.

2′,3′,5′-Tri-*O*-benzoyl-5-fluorouridine. A suspension of 4.9 g (37.6 mmoles) of 5-fluorouracil in 17.3 ml of hexamethyldisilazane containing 1.73 ml of chlorotrimethylsilane was refluxed by heating in an oil bath at 160–165° for 2.5 hr under anhydrous conditions. The excess hexamethyldisilazane and chlorotrimethylsilane were distilled off at 90° (oil bath at 165°) under atmospheric pressure. The oily residue showed strong bands at 1255, 1045, and 850 cm^{-1} (ir), indicating the presence of the $(CH_3)_3SiO-$ group. The yield was 10.37 g.

A mixture of this amount of the residue and 2′,3′,5′-tri-*O*-benzoyl-D-ribofuranosyl bromide, obtained from the reaction of 22.68 g (45.0 mmoles) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose with dry hydrogen bromide saturated in 130 ml of dichloromethane at 0° (1 hr at 0°, 15 min at room temperature), was heated at 150° for 1.5 hr, followed by heating at 150° (25 mm) for 4 hr, and then cooled. Dichloromethane (40 ml) was added to the dark mixture, followed by the addition of 2 ml of methanol which cleaved the silyl ether groups. The precipitate that formed was filtered off, and the product recrystallized from chloroform on being kept overnight in a refrigerator to give 6.16 g (28.47%) of 2′,3′,5′-tri-*O*-benzoyl-5-fluorouridine: mp 217–213°;[11] λ_{max} MeOH, 231, 268 nm.

3′,5′-Di-*O*-benzoyl-5-bromo-2,2′-anhydro-ara-C·HCl (IVb). III·HCl (0.3 g) was dissolved with stirring in a mixture of 1 g of benzoic anhydride and 1 ml of bromine. A crystalline mass deposited after about 20 min. After bromine was removed from the reaction mixture with bubbling N_2, the resulting solid was washed repeatedly with ether to remove the excess benzoic anhydride. The residue was dissolved in a small portion of EtOH and allowed to stand in a refrigerator to give a crystalline product: yield, 0.1 g (16.7%); mp 256–258° dec; λ_{max} ($\epsilon \times 10^{-3}$) MeOH, 237 (34.3), 281 nm (6.8). *Anal.* ($C_{23}H_{19}O_6N_3BrCl \cdot 0.5H_2O$) H, N; C: calcd, 52.88; found, 52.44.

3′,5′-Di-*O*-acetyl-5-bromo-2,2′-anhydro-ara-C·HBr (IVa). To a suspension of 1 g of III·HCl in 12 ml of Ac_2O was added 0.78 g of bromine and the resulting clear solution was allowed to stand at room temperature for 72 hr with stirring. The reddish solution was concentrated to dryness *in vacuo*, and the residue was treated with EtOH to give crystalline solid. Recrystallization from EtOH afforded an analytical sample (0.58 g, 32.35%): mp 147° (dec at 170°); λ_{max} ($\epsilon \times 10^{-3}$) MeOH, 237 (8.2), 280 nm (9.5). *Anal.* ($C_{13}H_{15}N_3O_6Br_2$) C; H: calcd, 3.23; found, 3.65, N: calcd, 8.99; found, 8.55.

3′,5′-Di-*O*-benzoyl-2,2′-anhydro-ara-C·HCl (VIb). A solution of 1.32 g of IVb in 50 ml of MeOH and 9 ml of AcOH containing 350 mg of 5% Pd/C which was pretreated with 5% AcOH was shaken in hydrogen atmosphere for 1 hr at room temperature. The catalyst was filtered off with aid of Celite. The filtrate and washings were combined and concentrated to dryness. The residue was crystallized from EtOH: yield, 1.05 g (92.0%); mp 268–269° dec; λ_{max} ($\epsilon \times 10^{-3}$) MeOH, 233 (6.0), 266 nm (18.2).

5,6-Dihydro-2,2′-anhydro-ara-C·HCl (V). A solution of 1 g of III in 40 ml of water containing 800 mg of 5% Pd/C was shaken in hydrogen atmosphere for 7 hr at room temperature. The catalyst was removed by filtration, and the pH of the filtrate was adjusted to pH 2.0 with 0.1 *N* HCl. The solution was concentrated to dryness at below 30°. The crystalline residue was triturated with 5 ml of MeOH and stored at 0° overnight. Recrystallization from MeOH gave 0.61 g (60.46%) of fine needles: mp 226–227° dec; λ_{max} ($\epsilon \times 10^{-3}$) H_2O, 253 nm (13.3); 1 *N* HCl, 253 nm (12.8); nmr (δ) 3.94 (multiplet, 2 H, C_6-H), 3.73 (multiplet, 2 H, C_5-H), 5.48 (d, 1 H, $C_{2'}$-H). *Anal.* ($C_9H_{14}N_3O_4Cl$) C, N; H: calcd, 5.35; found, 4.93.

1-β-D-Arabinofuranosyl-2-amino-5-bromo-4-imino-1,4(2*H*)-dihydropyrimidine·HBr (VII). IVa (1 g) was dissolved in 50 ml of MeOH presaturated with ammonia in an ice-cooled bath. The reaction mixture was allowed to stand overnight at 5°. The solvent and ammonia were evaporated *in vacuo*. The residue was dissolved in a small amount of MeOH and stored in a refrigerator, depositing white needles: 0.48 g (56.0%); mp over 300° (became dark at 196°); λ_{max} ($\epsilon \times 10^{-3}$) H_2O, 280 nm (16.7); 1 *N* HCl, 280 nm (16.9); 1 *N* NaOH, 290 nm (11.0). Crystallization from MeOH afforded an analytical sample. *Anal.* ($C_9H_{14}N_4O_4Br_2$) C, H; N: calcd, 13.93; found, 13.49.

5-Bromo-2,2′-anhydro-ara-C·HCl (IIa). 5-Bromocytidine (3 g) was dissolved in a mixture of a partially hydrolyzed phosphorus oxychloride (13.74 ml of $POCl_3$ and 2.69 ml of H_2O) and 14.5 ml of ethyl acetate and heated with stirring at 60° for 4.5 hr. The reaction mixture was evaporated, and the oily residue was dissolved in 50 ml of ice water. The cyclized product was isolated on a column of Dowex 50-W (acid form, 130 ml) by washing initially with water until free of ultraviolet-absorbing material, then eluting with 0.1 *M* pyridinum formate buffer (pH 4.5). The initial eluates (1.7 l.) were discarded, and 1.5 l. of the successive eluates was evaporated to dryness. The residue was dissolved in 25 ml of water and passed through the column of Diaion SA-11B (chloride form, 16 ml), followed by washing with water. The combined effluent and washings were concentrated to dryness. The residue was triturated with EtOH and crystallization took place. Colorless needles were obtained (1.13 g, 44.5%): mp 217 dec; λ_{max} ($\epsilon \times 10^{-3}$) H_2O, 235 (7.4), 282 nm (8.7); 1 *N* HCl, 235 (7.4), 282 nm (8.6); nmr (δ) 8.60 (s, 1 H, C_6-H), 6.70 (d, 1 H, $C_{1'}$-H), 5.66 nm (d, 1 H, $C_{2'}$-H). *Anal.* ($C_9H_{11}N_3O_4BrCl$) C, H, N.

5-Iodo-2,2′-anhydro-ara-C·HCl (IIb). 5-Iodocytidine hydrochloride (2.18 g) was dissolved in a mixture of a partially hydrolyzed phosphorus oxychloride (9.89 ml of $POCl_3$ and 1.94 ml of H_2O) and 10.45 ml of ethyl acetate and heated under stirring at 60° for 3 hr. The cyclized product was isolated as in the case of IIa. Two crops from the ethanolic solution afforded 1.08 g (51.67%): mp 184–186° dec; λ_{max} ($\epsilon \times 10^{-3}$) H_2O, 234 (8.6), 290 nm (6.5); 1 *N* HCl, 234 (8.5), 290 nm (6.3); R_f (A) 0.08, (B) 0.28; nmr (δ) 8.63 (s, 1 H, C_6-H), 5.59 (d, 1 H, $C_{2'}$-H). *Anal.* ($C_9H_{11}N_3O_4ICl$) C, H, N.

5-Fluoro-2,2′-anhydro-ara-C·HCl (IIc). 5-Fluorocytidine (1.05 g, 4 mmoles) was dissolved in a mixture of a partially hydrolyzed phosphorus oxychloride (3.80 ml of $POCl_3$ and 0.74 ml of H_2O) and 5.97 ml of ethyl acetate and heated under stirring at 70° for 4 hr. The reaction mixture was evaporated, and the oily residue was dissolved in 50 ml of ice water. A cyclized product was isolated on

a column of Dowex 50-W (acid form, 70 ml) by washing initially with water until free of uv-absorbing material, then eluting stepwise with 0.1 *M* and 0.3 *M* pyridinum formate buffer (pH 4.5). The initial eluates (2.8 l.) were discarded; 3.1 l. of the successive eluates was evaporated to dryness. The residue was dissolved in 25 ml of water and passed through the column of Diaion SA-11B (chloride form, 10 ml), followed by washing with water. The combined effluent and washings were concentrated to dryness. The residue was triturated with EtOH and crystallization took place. Colorless needles were obtained (0.61 g, 54.55%): mp over 260°; λ_{max} ($\epsilon \times 10^{-3}$) H_2O, 230 (9.1), 269 nm (11.2); 1 *N* HCl, 230 (10.1), 269 nm (12.0); nmr (δ) 8.37 (d, 1 H, C_6-H), 5.62 (d, 1 H, C_2-H). *Anal.* ($C_9H_{11}N_3O_4FCl$) C, H, N.

5-Iodo-ara-C (VIII). IIb (100 mg) was dissolved in 15 ml of 1 *M* triethylammonium bicarbonate (pH 7.5) and heated at 50° for 40 min. The reaction mixture was evaporated and the residue was dissolved in 10 ml of water to adjust the pH to 2.0 with 0.1 *N* HCl. The product was isolated on a column of Dowex 50-W (ammonium form, 4 ml) by washing with water until free of uv-absorbing material. The effluent was concentrated to dryness, and the residue was crystallized from EtOH: yield, 57 mg (60.5%); mp 205° dec,[13] λ_{max} H_2O, 223, 295 nm; 1 *N* HCl, 310 nm.

2',6-Anhydro-6-hydroxy-ara-C (IX). VIII (3.0 g) was dissolved in a mixture of 70 ml of DMSO and 25 ml of *tert*-BuOH. To this solution was added a mixture of 20 ml of 1 *N* sodium *tert*-butoxide and 40 ml of DMSO under stirring at 60° and heating was continued for 2 hr. The pH of the reaction mixture was adjusted to 9.0 with Dowex 50-W (ammonium form, 40 ml), and the resin was filtered off. The filtrate was concentrated to 100 ml and passed through the column of Dowex 50-W (acid form, 140 ml) to absorb a cyclized product. Isolation was achieved by washing initially with water until free of uv-absorbing material, then eluting with 5% ammonium hydroxide (1.2 l.). The eluates were concentrated to a small volume to give pure crystals. Recrystallization from minimum amount of water afforded an analytical sample: yield, 0.986 g (50.30%); mp 276° dec; λ_{max} ($\epsilon \times 10^{-3}$) pH 7, 222 (11.14), 261.5 nm (14.1); pH 1, 266 (23.3); R_f (A) 0.12, (B) 0.32. *Anal.* ($C_9H_{11}N_3O_5$) C, H, N.

The combined effluent was concentrated to dryness, and the residue was repeatedly washed with ether and dissolved in a small amount of water to give white prisms: yield, 730 mg; mp 235–237° dec; λ_{max} H_2O, H^+, OH^-, 275 nm. This compound is not yet characterized.

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Indigogenic Phosphodiesters as Potential Chromogenic Cancer Chemotherapeutic Agents†

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Several indigogenic phosphodiesters of 5-iodo- (or nitro-) indol-3-yl 5'-nucleosides were prepared by coupling 5-substituted indol-3-yl phosphodichloridate with a suitably protected nucleoside. A pyrophosphate diester of 5-iodoindol-3-yl-5'-(5-fluorodeoxyuridine) was prepared by the reaction of *N*-acetyl-5-iodo-indol-3-yl phosphate and 3'-*O*-acetyl-5-fluorodeoxyuridine 5'-phosphodichloridate. These compounds were substrates for snake venom 5'-nucleotide phosphodiesterase, and the K_m and v_{max} values were measured by following the rate of indigo formation. Cytotoxicities of the phosphodiesters were tested on a rat mammary tumor (AC 33) and HeLa cell lines.

The synthesis of indigogenic nucleoside phosphodiesters as chromogenic substrates for the localization of nucleotide 5'-phosphodiesterase (E.C. 3.1.4.1.) activity in tissue has been reported from this laboratory.[1,2] The extension of this histochemical approach to the synthesis of chromogenic cancer chemotherapeutic agents has been illustrated recently.[3] The design principle is depicted in Scheme I.

The enzymatic activity can be measured by the liberated substituted indigo dye, and the anticancer activity assessed in a tissue culture study. While the use of simple nucleoside analogs depends on thymidine kinase for their actions, this approach depends on phosphodiesterase to deliver an antimetablite as the possible chemotherapeutic agent. These phosphodiesters therefore should be effective in tumors that

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