in methanol (100 mL) for 8 h. Catalyst was removed by filtration through hyflo supercel, concentrated, and crystallized from THF–benzene.

***trans*-2,2-Dimethyl-3-phenyl-4-[*p*-(β-pyrrolidinoethoxy)phenyl]-7-hydroxychroman (7).** A mixture of 1 (4.57 g, 0.01 mol), finely powdered KOH (12.8 g, 0.22 mol), diethylene glycol (120 mL), and hydrazine hydrate (1.0 mL) was heated at 240 °C for 30 min under a dry N_2 atmosphere, cooled, poured over sodium dithionate solution (1.4 g in 1600 mL of H_2O), and acidified with the minimum amount of concentrated HCl. The solid that separated was collected by filtration, dried, and purified by column chromatography (basic alumina, 1% MeOH/$CHCl_3$).

***trans*-2,2-Dimethyl-3-phenyl-4-[*p*-[β-(diethylamino)ethoxy]phenyl]-7-methoxychroman Hydrochloride (8).** A mixture of 6 (0.9 g, 2.5 mmol), 2-(diethylamino)ethyl chloride hydrochloride (0.43 g, 2.5 mmol), anhydrous K_2CO_3 (8 g), and dry acetone (60 mL) was refluxed for 14 h, cooled, and filtered. Acetone was distilled off, and the residual oil was taken into ethyl acetate, washed with water, dried (Na_2SO_4), and concentrated. The oily residue was purified by filtration through basic alumina (benzene) to give 8 free base (0.8 g), which was converted to its hydrochloride salt.

2,3′-Dihydroxy-4-methoxybenzophenone (16). A mixture of *m*-methoxyphenol (12.4 g, 0.1 mol), *m*-hydroxybenzoic acid (13.8 g, 0.1 mol), and $SnCl_4$ (100 mL) was refluxed for 8 h, cooled, poured over crushed ice (500 g), and extracted with ethyl acetate; the organic extract washed with $NaHCO_3$ solution and then with water until neutral, dried (Na_2SO_4), and concentrated. The residual oil was chromatographed over silica gel (5% EtOAc/benzene) to give 16, recrystallized from benzene–hexane: IR (KBr) 3400, 1620, 1580 cm^{-1}.

3-Phenyl-4-(*m*-acetoxyphenyl)-7-methoxycoumarin (18). A solution of 16 (10.7 g, 0.043 mol) and phenylacetic acid (5.84 g, 0.043 mol) in Ac_2O (20 mL) and NEt_3 (9 mL) was refluxed under anhydrous conditions for 10 h. The reaction mixture was diluted with ethanol, and the solid thus obtained was collected by filtration, washed thoroughly with ethanol, dried, and crystallized from benzene–hexane: IR (KBr) 1770, 1710, 1600 cm^{-1}.

Similarly, 28 was obtained from 26 and *p*-hydroxyphenylacetic acid.

2,2-Dimethyl-3-phenyl-4-(*m*-hydroxyphenyl)-7-methoxychromene (19). To a stirred solution of MeMgI, prepared from 27.97 g of CH_3I (0.197 mol) and 4.74 g of Mg (0.197 g-atom) in dry ether (200 mL), was added dropwise a solution of 18 (13.0 g, 0.033 mol) in dry THF (250 mL). The reaction mixture was refluxed for 4 h, cooled, and decomposed with the minimum amount of concentrated HCl. THF was distilled off, and the residual oil was taken in ethyl acetate, washed with water until neutral, dried (Na_2SO_4) and concentrated. Residual oil was chromatographed over silica gel (1% EtOAc/C_6H_6) to give 19: IR (KBr) 3350, 2950, 1605 cm^{-1}; ^1H NMR δ 1.38 (s, 6 H, $2CH_3$), 3.21 (nh, 1 H, O*H*), 3.61 (s, 3 H, OCH_3), 6.1–7.1 (m, 12 H, Ar*H*).

2,2-Dimethyl-3-phenyl-4-[*m*-(β-pyrrolidinoethoxy)phenyl]-7-methoxychromene (20). Alkylation of 19 with *N*-(2-chloroethyl)pyrrolidine hydrochloride as described for 8 and its subsequent conversion to the hydrochloride salt gave the desired compound.

***cis*-2,2-Dimethyl-3-phenyl-4-(*m*-hydroxyphenyl)-7-methoxychroman (21).** Catalytic hydrogenation of 19 (100 mg) over 10% Pd/C (50 mg) in methanol at 60 psi for 8 h on workup gave 21 as a solid, recrystallized from benzene/hexane.

***cis*-2,2-Dimethyl-3-phenyl-4-[*m*-(β-pyrrolidinoethoxy)phenyl]-7-methoxychroman (22).** Alkylation of 21 with *N*-(2-chloroethyl)pyrrolidine hydrochloride and its subsequent conversion to the hydrochloride salt following the procedure described for 8 gave 22.

***trans*-2,2-Dimethyl-3-phenyl-4-[*m*-(β-pyrrolidinoethoxy)phenyl]-7-methoxychroman (11).** Isomerization of 22 with *n*-BuLi in Me_2SO following the procedure described for 5 gave 11.

Registry No. 1, 31477-60-8; 2, 51423-20-2; 3, 51423-18-8; 4, 84394-05-8; 5, 84394-06-9; 6, 57897-46-8; 7, 84394-36-5; 8, 78994-27-1; 8·HCl, 84394-07-0; 9, 78994-28-2; 9·HCl, 84394-08-1; 10, 84394-28-5; 10·HCl, 84394-09-2; 11, 84394-29-6; 11·HCl, 84394-10-5; 12, 84394-30-9; 12·HCl, 84394-11-6; 13, 84394-37-6; 16, 84394-12-7; 18, 84394-13-8; 19, 84394-14-9; 20, 84394-26-3; 20·HCl, 84394-15-0; 21, 84394-16-1; 22, 84394-27-4; 22·HCl, 84394-17-2; 23, 84394-18-3; 24, 84394-19-4; 25, 84394-31-0; 25·HCl, 84394-20-7; 26, 131-57-7; 27, 156-38-7; 28, 84394-21-8; 29, 84394-22-9; 30, 84394-32-1; 30·HCl, 84394-23-0; 31, 84394-24-1; 32, 84394-33-2; 32·HCl, 84394-25-2; 33, 84394-35-4; 34, 84394-34-3; 34·HCl, 84416-61-5; 35, 53996-41-1; 2-(diethylamino)ethyl chloride hydrochloride, 869-24-9; *m*-methoxyphenol, 150-19-6; *m*-hydroxybenzoic acid, 99-06-9; phenylacetic acid, 103-82-2; *N*-(2-chloroethyl)pyrrolidine hydrochloride, 7250-67-1.

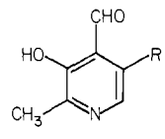
Phosphonate Analogues of Pyridoxal Phosphate with Shortened Side Chains

Chi-Neng A. Han,[1] Chuzo Iwata,[2] and David E. Metzler*

Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50011. *Received June 25, 1982*

A phosphonate analogue of pyridoxal 5′-phosphate containing a 5-phosphonomethyl group and its monoethyl and diethyl esters have been prepared. Except for the diethyl ester, the compounds appear to bind into the active site of aspartate aminotransferase. However, they lack detectable catalytic activity with this enzyme and with glutamate decarboxylase of *Escherichia coli*. The phosphonomethyl analogue bound to aspartate aminotransferase does react slowly with substrates, as determined by spectrophotometric observations; the monomethyl ester reacts about 20 times less rapidly. Because of the stability of the phosphonate linkage, these compounds may be useful as modifying reagents for various proteins.

The phosphate group of pyridoxal 5′-phosphate (1) is



1, R = $CH_2OPO_3H^-$
5, R = CH_3

necessary for the binding of this coenzyme to the enzymes for which it is essential. Because it is also possible that the phosphate group has a direct function in enzymic catalysis or that it plays an essential structural role in enzymes, the study of analogues of 1 with modified side chains in the 5-position is of interest.[3-8] Additional in-

(1) Taken in part from the Ph.D. Dissertation of Chi-Neng Han, Iowa State University, 1978.

(2) Present address, Osaka University, Faculty of Pharmaceutical Sciences, 1-6, Yamada-oka, Suita, Osaka, 565, Japan.

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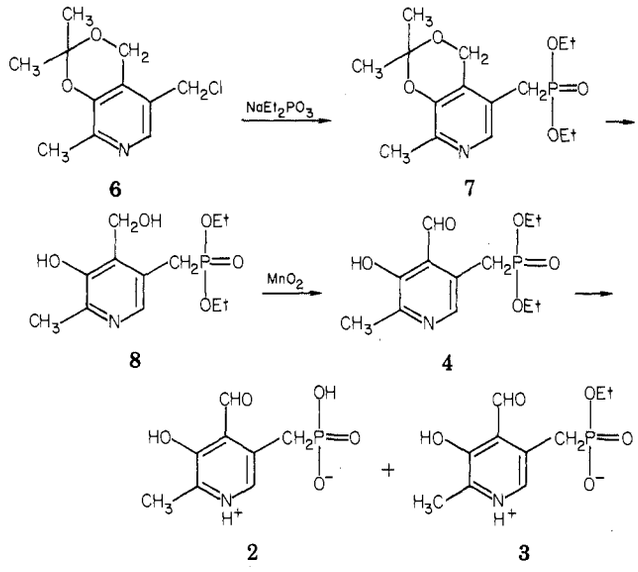
Scheme I



terest is provided by the fact that **1** itself inhibits many enzymes.[9] Analogues of **1** also can be expected to inhibit enzymes, but they may have substantially different specificity than does **1**. Compounds with a single negative charge in the side chain may bind at different sites[10] than does **1**. Analogues with longer or shorter side chains than **1** may bind more or less tightly. Some analogues, such as the phosphonate **2**, will be resistant to the action of phosphatases and may be of value for in vivo studies. In all cases, Schiff bases formed by **1** or its analogues can be reduced with sodium borohydride to fix a fluorescent or isotopic label onto a target protein.

In this report we describe the synthesis (Scheme I) and some chemical and biochemical characteristics of [(4-formyl-3-hydroxy-2-methyl-5-pyridyl)methyl]phosphonic acid (**2**), an analogue of **1** in which the 5-position side chain has been replaced by a phosphonomethyl group, and of the mono- and diethyl esters **3** and **4**.

Chemical Properties. The NMR spectra of compounds **2**–**4** and **6**–**8** (Scheme I) are all consistent with the assigned structures. Mass spectra obtained by fission fragment ionization confirmed the expected masses of **2** and **3**. UV absorption spectra are similar to those of 5-deoxypyridoxal (**5**) and of **1**. From the changes in these spectra with pH, it was possible to compute the stepwise acid dissociation constants (as pK values) by using the method of Nagano.[11] These pK values are given in Table I along with those of several related compounds. Both **2** and **3** form Schiff bases with amino acids. Spectra and formation constants have been reported elsewhere.[3]

Reactivities with Enzymes. Furbish et al.[3] could detect no coenzymatic activity for **2** with aspartate aminotransferase. We have investigated this compound more extensively and have also tested **3** and **4** with the same enzyme. All three compounds are inactive when preincubated at pH 8.2 at a concentration of 10^{-3} M with 10^{-4} M apoenzyme for 20 min and then diluted to 10^{-7}–10^{-8} M for assay. Compound **2** was also without detectable activity at pH 6.5 and 7.5. Nevertheless, **2** and **3** appear to bind specifically at the active site of the enzyme. When **2** (3.6×10^{-5} M) was mixed with excess (5.5×10^{-5} M) apoenzyme at pH 8.3, the absorption band of free **2** at 395 nm shifted to 357 nm, giving a stable spectrum within 3–5 min. A small shoulder was also present at 424 nm. Similarly, the monoethyl ester **3**, over a period of about 40 min, was bound with a shift in peak position from 391 to 364 nm and development of a shoulder of 413 nm. For both compounds, the major band showed a positive circular dichroism with $\Delta A/A$ equal to 1.0×10^{-3}, in good agreement with the results of Furbish et al. for **2**.[3] The weaker absorption bands giving rise to the shoulders had a weak negative CD. These results are similar to those observed[14] for **1** and for its monoethyl or monomethyl esters, all of which give single bands at 363–365 nm with a positive CD and $\Delta A/A$ of about 2.0×10^{-3}. In contrast, the absorption band of the diethyl ester **4** did not shift upon incubation with apoenzyme, and there was no CD.

Table I. Apparent pK Values for Phosphonate Analogues of Pyridoxal Phosphate and Related Compounds

compd	pK values	source
1 (pyridoxal phosphate)	3.62, 6.10, 8.33	ref 12
2	4.07, 6.76, 9.20	this study
3	3.91 8.21	this study
4	3.03 7.00	this study
5 (5-deoxypyridoxal)	4.15 8.05	ref 12
8	4.53 8.59	this study

When either **2** or **3** bound to the apoenzyme was treated at pH 8.3 with solid sodium borohydride, the absorption band shifted to 325 nm. This reduced enzyme was precipitated with trichloroacetic acid, and the precipitate was collected and redissolved in 6 M guanidine hydrochloride. The chromophore remained quantitatively with the redissolved protein. However, while **4** was reduced by borohydride, it was not bound to the precipitated protein. When **1** was added to a solution of the reduced complex of **2** or **3** with apoenzyme, the coenzyme peak remained at 390 nm, the position for free **1**. However, when **1** was added to borohydride-treated apoenzyme, the spectrum of native aspartate aminotransferase was regenerated. We conclude that in the reduced complex of **2** and **3**, the coenzyme site had indeed been blocked.

The complexes of **2**, **3**, and the ethyl and methyl esters of **1** with apoenzyme were titrated to lower pH values with 2 M acetate buffer, pH 5.4. For **2**, as reported by Furbish et al., the absorption band shifted to 420 nm as in the native enzyme with a pK of about 7 (estimated as 6.1 by Furbish et al.). However, as with other analogues containing a single negative charge in the side chain,[3] the complexes of **3** and the ethyl ester of **1** underwent no change in spectrum when the pH was lowered to 6.

In an assay[3] capable of detecting 1% of the activity of the native enzyme, **2**–**4** were devoid of activity with apoaspartate aminotransferase. However, to detect the presence of a very small amount of activity, changes in the absorption species of the reconstituted enzymes were observed following the addition of the substrate glutamate. Most of the native enzyme is instantly converted by glutamate to the pyridoxamine phosphate form. Likewise, bound **2** is converted within less than 2 min to a form absorbing at 325 nm, presumably the corresponding amine

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form. However, the bound monoethyl ester 3 reacted very slowly, and we assume that the crystalline sample used by Furbish et al. must have contained 3 as well as 2. After a small rapid drop in the original absorption band, the remaining bound 3 was converted to a form absorbing at 318 nm with an apparent first-order rate constant of 6.7×10^{-4} s^{-1}. In addition, there was a small transient rise in absorption at 430 nm. Ethyl and methyl esters of 1 behaved similarly, forming products absorbing at 315 nm with apparent rate constants of 10×10^{-4} and 25×10^{-4} s^{-1}, respectively.[14] In all three of these cases, there was no circular dichroism in the absorption bands of the products, and it appears that the amine forms are completely dissociated from the protein as was observed by Furbish et al.[3] for the 5-carboxyethyl analogue.

When bound 2 was allowed to react with the inhibitor α-methylaspartate (34 mM), the shoulder at 430 nm was doubled in height and the band at 370 nm decreased, changes indicating a partial conversion to the bound Schiff base of 2 with the inhibitor (the "external" Schiff base).[15] A weak negative circular dichroism ($\Delta A/A = -0.75 \times 10^{-3}$) was observed in the 430-nm band. However, with 3 and with the ethyl ester of 1, there was no increase in the 430-nm band, indicating a nearly complete lack of the external Schiff base. When DL-*erythro*-β-hydroxyaspartate[16] at a 4 mM concentration was added to bound 2, a stable spectrum appeared within 2 min with strong bands at 430 and at 325 nm, a weaker band at 370 nm, and a small shoulder at about 490 nm, presumably the quinonoid intermediate of transamination.[15] Thus, 2 behaves somewhat like 1, but the equilibrium mixture of species present on the enzyme contains a smaller fraction of quinonoid form and a much higher fraction of the 430-nm form than for 1. When bound 3 was reacted with β-hydroxyaspartate, a small peak appeared at about 400 nm and decayed to the final equilibrium spectrum over many hours. Thus, conversion of 2 to its ethyl ester 3 greatly slows down the reaction. The same is true for the ethyl ester of 1, although in this instance no transient 500-nm absorption band appears.[14]

Apo-glutamate decarboxylase from *Escherichia coli* also binds 2 to give an inactive complex. The apparent dissociation constant for the complex at pH 4.6 was estimated as $9.0 \pm 1 \times 10^{-4}$ M, about the same as that reported by O'Leary[17] for 5-nitrosalicylaldehyde and at least 1000 times higher than that for 1.

Discussion

Both the phosphate group and the dipolar ionic form of the pyridine ring of 1 are bound very specifically by ionic and hydrogen-bonding groups in the active sites of aspartate aminotransferase. Nevertheless, despite the shortened chain in the 5-position, phosphonate 2 binds into the active site of at least two pyridoxal phosphate requiring enzymes. Likewise, the monoethyl ester 3 and the corresponding ethyl ester of 1 bind. However, the diethyl ester 4, which has no negative charge on the side chain, does not bind. It is of interest that the analogues of 1 carrying a monoanionic side chain in the 5-position do not display a pK value in the pH 6–7 region when bound to apo-aspartate aminotransferase. For these same compounds, the amine form produced by transamination dissociates from the protein, a fact that may partially account for their very low catalytic activity. However, dissociation of the amine forms is slow compared to the 10 min required for the activity assay; the rates of turnover of the analogues in the active site must be much slower than the approximately 400 s^{-1} estimated for the native enzyme.[18a]

For enzymes, such as aspartate aminotransferase, that bind the coenzyme tightly, it is unlikely that 2 or 3 can serve as an effective antimetabolite in competition with 1. However, some enzymes bind the coenzyme loosely,[18b] and for these, 2 and 3 may be useful inhibitors. They might also be of value as inhibitors for various enzymes or receptors that do not normally react with 1 in vivo. Like 1, compound 2 is a general reagent for reaction with primary amines. Its Schiff base formation constant with valine[13] is about 3 times higher than that of 1, and its nonhydrolyzable nature would be advantageous.

Experimental Section

Diethyl [3-hydroxy-4-(hydroxymethyl)-2-methyl-5-pyridyl]phosphonate (8) was made in the following two ways: (1) α^4,3-*O*-Isopropylidene-α^5-pyridoxyl chloride (6) was prepared from the corresponding hydrochloride[7] by neutralization with saturated $NaHCO_3$ and extraction with methylene chloride. To a suspension of 6 (18.4 g, 81 mmol) and diethyl phosphite (12.0 g, 87 mmol) in anhydrous ether (240 mL) was added sodium metal (2 g, 87 mmol). After refluxing for 50 h, the mixture was filtered, and solvents were removed from the filtrate by evaporation. (2) The pyridoxyl chloride 6 (21.8 g, 97 mmol) was combined with triethyl phosphite (25 g, 150 mmol) and was refluxed for 1 h at 150 °C. After the solution was cooled, excess triethyl phosphite was removed under vacuum.

The crude product from either procedure was purified by vacuum distillation. A pressure of <0.3 mmHg is necessary to prevent excessive decomposition from the high pot temperature (185–190 °C at 0.3 mm). Method "1" gave 52% diethyl α^4-3-*O*-isopropylidene-α^5-pyridoxylphosphonate (7) after distillation: mass spectrum, m/z 329 ($M^{+\cdot}$), 271 ($M^{+\cdot} - CH_3COCH_3$), 243 (271 − CO), 215 (243 − C_2H_4), 106 [271 − P(O)$(OEt)_2$]; ^1H NMR (CCl_4) δ 1.18 (t, 6 H), 1.47 (s, 6 H), 2.24 (d, 3 H), 2.81 (d, 2 H), 3.94 (overlapping quartets, 4 H, 4.82 (d, 2 H), 7.77 (d, 1 H). Values are given for the center of each set. The 5′-CH_2 protons have a large ^{31}P–^1H splitting (22 Hz). A smaller ^{31}P–^1H splitting (~3 Hz) was observed for the 2-CH_3, 4′-CH_2, and 6-H signals.

A solution of 7 (1.6 g, 4.9 mmol) in 1 N HCl (20 mL) was heated on a steam bath for 15 min, and the cooled solution was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ solution was dried (Na_2SO_4) and evaporated. Recrystallization from benzene gave 0.9 g (60%) of 8: mp 128–129 °C, mass spectrum, m/z 289 ($M^{+\cdot}$), 271 ($M^{+\cdot} - H_2O$), 243 (271 − CO), 215 (243 − C_2H_4), 106 [271 − P(O) − $(OEt)_2$]. Anal. ($C_{12}H_{20}NO_5P$) C, H, N.

Diethyl [(4-Formyl-3-hydroxy-2-methyl-5-pyridyl)-methyl]phosphonate (4). To 8 (3.0 g, 10.3 mmol) in $CHCl_3$ (200 mL) was added activated MnO_2 (10 g).[19] The mixture was stirred in the dark at room temperature. When TLC (in acetone) indicated complete oxidation (12 h), the mixture was filtered, and the residue was washed with $CHCl_3$ until the washings were colorless. After evaporation on a rotary concentrator, the residue was purified by recrystallization (CH_2Cl_2–Skelley A, 1:6) or by chromatography on alumina (elution with chloroform): yield 53%; mp 70–73 °C; mass spectrum, m/z 287 ($M^{+\cdot}$), 259 ($M^{+\cdot}$ − CO), 231 (259 − CO or C_2H_4), 203 (231 − CO or C_2H_4), 123 [231 − P(O)$(OEt)_2$], 122 [231 − P(O)(OH)(OEt)]; ^1H NMR (D_2O, pD = 5.6) δ 1.26 (t, 6 H), 2.48 (d, 3 H), 3.74 (d, 2 H), 4.11 (overlapping quartets, 4 H), 7.66 (d, 1 H), 10.38 (s, 1 H). A small peak at δ 6.30 (0.1 H), representing the covalent hydrate of 4, was also present. Anal. ($C_{12}H_{18}NO_5P$) C, H, N.

Oxime of 4. To a solution of 4 (0.4 g, 1.4 mmol) in ethanol (50 mL) were added NH_2OH·HCl (0.35 g, 5 mmol) and sodium acetate (0.41 g, 5 mmol). The mixture was refluxed for 3 h and

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evaporated under reduced pressure. The residue was extracted with hot ethanol several times; the extract was concentrated to dryness, and the residue was recrystallized from ethanol, giving 0.25 g (59%) of the oxime of **4**, mp 227–228 °C. Anal. ($C_{12}H_{19}N_2O_5P$), C, H.

[(4-Formyl-3-hydroxy-2-methyl-5-pyridyl)methyl]phosphonic Acid (2) and Ethyl [(4-Formyl-3-hydroxy-2-methyl-5-pyridyl)methyl]phosphonate (3). A solution of **4** (1 g, 3.45 mmol) in 20% HCl (50 mL) was refluxed, hydrolysis being monitored by removal of small portions, dilution into 0.1 N HCl, and measurement of the UV–visible spectrum. Refluxing was continued for about 5 h until the absorbance ratio A_{296nm}/A_{339nm} attained its minimal value of about 1.8. The solution was concentrated under vacuum and applied to a 1.2 × 50 cm column of Dowex 50W-X4 (100–200 mesh) ion-exchange resin in the H^+ form and well washed with water. The column was eluted under nitrogen with water at a flow rate of 36 mL/h. The eluate was monitored at 280 nm. Compound **3** appeared in the second peak and compound **2** in the third peak detected in this way; selected fractions were concentrated under vacuum and lyophilized: yield 51 mg (40%) of **2** and 26 mg (20%) of **3**. Both **2** and **3** were crystallized from water–acetone. NMR (D_2O) of **2**: δ 2.38 (s, 3 H), 3.29 (d, 2 H), 7.5 (s, 1 H, broad), 10.13 (s, 1 H, broad). NMR (D_2O) of **3**: δ 1.09 (t, 3 H), 2.31 (d, 3 H), 3.22 (d, 2 H), 3.71 (sextet, 2 H), 7.41 (s, 1 H, broad), 10.10 (s, 1 H, broad). Mass spectra by fission fragment ionization for **4**, m/z 230 [$(M-1)^-$], 212 [$(M-1-H_2O)^-$], 202 [$(M-1-CO)^-$], 80 [$(PO_3H)^-$], 63 [$(PO_2)^-$], 49 [$(POH_2)^-$]; for **3**, m/z 258 [$(M-1)^-$], 230 [$(M-C_2H_5)^-$], 228 [$(M-1-C_2H_6)^-$], 212 [$(M-1-C_2H_5OH)^-$], 79 [$(PO_3)^-$], 63 [$(PO_2)^-$], 49 [$(POH_2)^-$]. Electrophoresis: both **2** and **3** migrated toward the anode at about the same rate and a little slower than **1** at pH 6.4. A trace impurity, detectable by fluorescence, migrated ahead of **2** and **3**. The IR spectra (KBr) of **4** and **8** show the three special peaks of phosphate or phosphonate groups at ca. 1245, 1030 (P–O–C), and 1160 (P–O–C) cm^{-1},[20] the aldehyde peaks of both **2** and **4** appear at 1661 cm^{-1}.

Enzyme Assay. The enzymatic activity of aspartate aminotransferase was assayed by direct spectrophotometric observation of oxaloacetate formation as described by Furbish et al.[3] Glutamate decarboxylase activity was assayed manometrically[21] at pH 4.6.

Acknowledgment. This work was supported in part by Research Grant AM-01549 from the United States Public Health Service. We thank Kevin Kelly for assistance and Frank H. Field and the Rockefeller University Mass Spectrometric Biotechnology Research Resource for the fission fragment ionization mass spectra.

Registry No. **2**, 26210-18-4; **3**, 84521-12-0; **4**, 84521-13-1; **4** (oxime derivative), 84521-14-2; **6**, 14142-90-6; **7**, 84521-15-3; **8**, 84537-05-3.

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Synthesis and Biological Activity of 5-(Trifluoromethyl)- and 5-(Pentafluoroethyl)pyrimidine Nucleoside Analogues

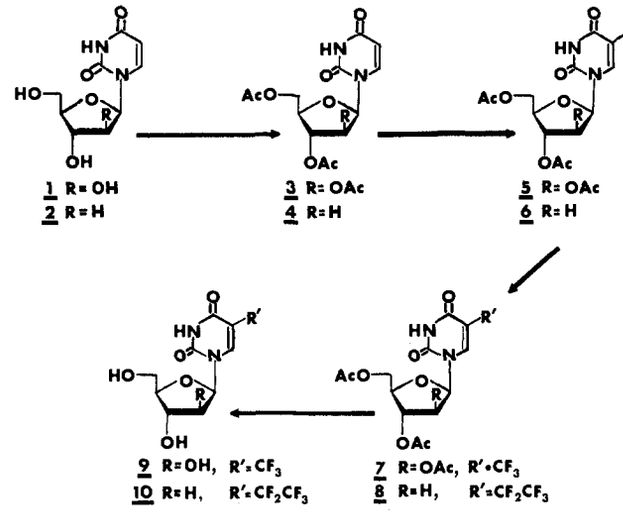
Tai-Shun Lin* and You-Song Gao[1]

Department of Pharmacology and Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510. *Received August 16, 1982*

Various 5-substituted perfluoroalkylpyrimidine nucleoside analogues have been synthesized, and their biological activity against L1210, S-180, Vero cells, and herpes simplex virus type 1 (HSV-1) was evaluated. The 5-trifluoromethyl derivatives, **7** and **9**, showed significant antiviral activity against HSV-1 with ED_{50} values of 7 and 5 μM, respectively. In addition, the unblocked nucleoside **9** was found to be about 64-fold less toxic to the host Vero cells and gave a favorable therapeutic index of 64 against HSV-1 in vitro.

Modifications of nucleosides in the sugar, the pyrimidine, or purine base, or both, have produced compounds with potent antiviral or anticancer activity. Arabinofuranosyl analogues of "normal" nucleoside with potent antiviral or anticancer activity include 1-β-D-arabinofuranosyladenine (*ara*-A), 1-β-D-arabinofuranosylcytosine (*ara*-C), and 1-β-D-arabinofuranosylthymine (*ara*-T).[2-5] In view of the biological activity of *ara*-T and other analogues of thymidine, a number of analogues of *ara*-T have been synthesized with varying substituents replacing the methyl moiety of *ara*-T, such as 1-β-D-arabinofuranosyl-5-ethyluracil,[6] 1-β-D-arabinofuranosyl-5-vinyluracil,[7] and 1-β-D-arabinofuranosyl-(*E*)-5-(2-bromovinyl)uracil.[8,9] Biological activity has also been obtained with arabinofuranosyluracil and arabinofuranosylcytosine substituted in the 5-position

Scheme I



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