ZNHCH$_2$), 19625-78-6; **4a** (R$_3$ = CMe$_3$), 21995-77-7; **4a** (R$_3$ = *tert*-butyloxycarbonyl-L-leucyl), 42222-15-1; **4a** (R$_3$ = carbobenzoxy-L-seryl), 42222-16-2; **4a** (R$_3$ = carbobenzoxy-L-glutamyl), 42222-17-3; **4a** (R$_3$ = benzoyl-L-leucyl), 42222-19-5; **4a** (R$_3$ = carbobenzoxyglycylglycyl), 42222-24-2; **4a** (R$_3$ = phthaloylglycyl), 21995-78-8; **4a** (R$_3$ = carbobenzoxy-L-glutaminyl), 42222-17-3; **4b** (R$_3$ = ZNHCH$_2$), 42222-01-5; **4b** (R$_3$ = CMe$_3$), 42222-02-6; **4e** (R$_3$ = ZNHCH$_2$), 42222-03-7; **4f** (R$_3$ = H), 42222-04-8; **4f** (R$_3$ = CMe$_3$), 42222-05-9; **4f** (R$_3$ = Me), 42222-14-0; **5a**, 42222-06-0; **5b**, 42222-07-1; **5f**, 42222-08-2; **6a** (R$_3$ = H), 42222-09-3; **6f** (R$_3$ = H), 42222-10-6; **7** (R$_3$ = ZNHCH$_2$), 21995-76-6; **8f**, 4767-01-5; **11**, 42222-13-9; formic acid, 64-18-6; acetic acid, 64-19-7; carbobenzoxy-β-cyanoalanine, 3309-41-9; carbobenzoxytriglycine ethyl ester, 2503-35-7; carbobenzoxyglycyl-L-serine methyl ester, 10239-27-7; phthaloyldiglycine ethyl ester, 2641-02-3; carbobenzoxy-L-glutaminyl-L-tyrosine methyl ester, 42222-23-1; benzylamine, 100-46-9; pivalic acid, 75-98-9; carbobenzoxyglycine, 1138-80-3; carbobenzoxy-L-asparagine, 2304-96-3; benzoyl-L-leucine, 1466-83-7; glycine ethyl ester hydrochloride, 623-33-6; L-serine methyl ester hydrochloride, 5680-80-8; L-tyrosine methyl ester hydrochloride, 3417-91-2.

Synthesis of 2-Nor-2-formylpyridoxal 5′-Phosphate, a Bifunctional Reagent Specific for the Cofactor Site in Proteins

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A facile and general procedure was developed for the preparation of vitamin B$_6$ amine oxides and their corresponding 2-carbinol rearrangement products. These compounds served as intermediates in the synthesis of the dicarboxaldehyde 2-nor-2-formylpyridoxal 5′-phosphate and of 2-nor-2-formyl-4-deoxypyridoxol 5′-phosphate, an isomer of pyridoxal 5′-phosphate. Both analogs were prepared for the purpose of exploring the spacial configuration of polypeptide chains at the pyridoxal 5′-phosphate site in glycogen phosphorylase (EC 2.4.1.1) and other enzymes.

The introduction of stable covalent bridges between amino acid residues has been recently utilized as a direct chemical tool to provide information about the spacial orientation of polypeptide chains in proteins.[1] It occurred to us that this approach, coupled with a systematic application of affinity labeling techniques, may reveal the architecture of important sites on enzymes and perhaps contribute to the elucidation of the catalytic mechanism. 2-Nor-2-formylpyridoxal 5′-phosphate (**9**) and 2-nor-2-formyl-4-deoxypyridoxol 5′-phosphate (**4**)[2] were synthesized as part of a project designed to probe the three-dimensional structure around the pyridoxal 5′-phosphate pocket in glycogen phosphorylase (EC 2.4.1.1)[3] and a number of other enzymes. Earlier investigations indicated that the absence of the 2-methyl group has little effect on the activity of the cofactor.[3,4] Consequently, an additional functionality was anchored at this position in the expectation that the enzyme will conserve a high degree of specificity for this analog and thus promote the formation of a bisazomethine cross-linkage at the PLP site.

The synthetic pathway, as depicted in Scheme I, is based on an improved two-step synthesis of 2-pyridine carbinol analogs serving as intermediates for the final products described herein. When *m*-chloroperbenzoic acid[5] is employed in either protic or aprotic solvents in the cold, a smooth N-oxidation of vitamin B$_6$ compounds proceeds. Although several syntheses of pyridoxol *N*-oxide had been published,[6,7] none would seem to equal the general applicability or good yields obtained with this reagent. Ethanol was frequently used as solvent from which the amine oxides crystallized out during the course of oxygenation. Protection of a formyl group was effected by hemiacetal formation; otherwise extensive oxidation to the corresponding carboxylic acid is the preferred pathway as noticed in the quantitative conversion of pyridoxal 5′-phosphate to 4-pyridoxic acid 5′-phosphate. On the other hand, carbinol groups are attacked only on a limited scale and the relatively small improvements in yield do not warrant the work involved in protection and deprotection. Thus several compounds in the vitamin B$_6$ group were readily converted to the amine oxides with the exception of the PLP diethyl acetal, which under a variety of conditions gave rise to low yields and a number of by-products. In a second step, an intramolecular rearrangement[8] of the amine oxides to the corresponding 2-pyridine carbinols was effected by trifluoroacetic anhydride [(TFA)$_2$O], a reagent which was not previously utilized for such conversions on a preparative scale.[9] We found this anhydride to be superior to acetic anhydride in that it required mild conditions for acylation and gave rise to fewer by-products.

With ω-hydroxypyridoxol readily available, attempts were made to oxidize **7c** to **8** by activated manganese dioxide. The complex mixture of products thus obtained was fractionated on an ion-exchange column and was found to include ω-hydroxypyridoxal (**7a**) and 2-nor-2-formylpyridoxol as well as other acidic components, but only small amounts of the desired dicarbox-

(1) F. Wold, *Methods Enzymol.*, **25B**, 623 (1972).

(2) Abbreviations used are as follows: ω-hydroxy indicates the introduction of a 2′-hydroxyl group and 2-nor-2-formyl, the replacement of a 2-methyl with a 2-formyl; PLP, pyridoxal 5′-phosphate; *m*-CPBA, *m*-chloroperbenzoic acid; PPA, polyphosphoric acid; THF, tetrahydrofuran; (TFA)$_2$O, trifluoroacetic anhydride.

(3) For previous work on this subject see A. Pocker and E. H. Fischer, *Biochemistry*, **8**, 5181 (1969); S. Shaltiel, J. L. Hedrick, A. Pocker, and E. H. Fischer, *ibid.*, **8**, 5189 (1969).

(4) E. E. Snell, *Vitam. Horm. (New York)*, **28**, 265 (1970); W. Dowhan, Jr., and E. E. Snell, *J. Biol. Chem.*, **245**, 4629 (1970).

(5) J. Cymerman Craig and K. K. Purushothaman, *J. Org. Chem.*, **35**, 1721 (1970).

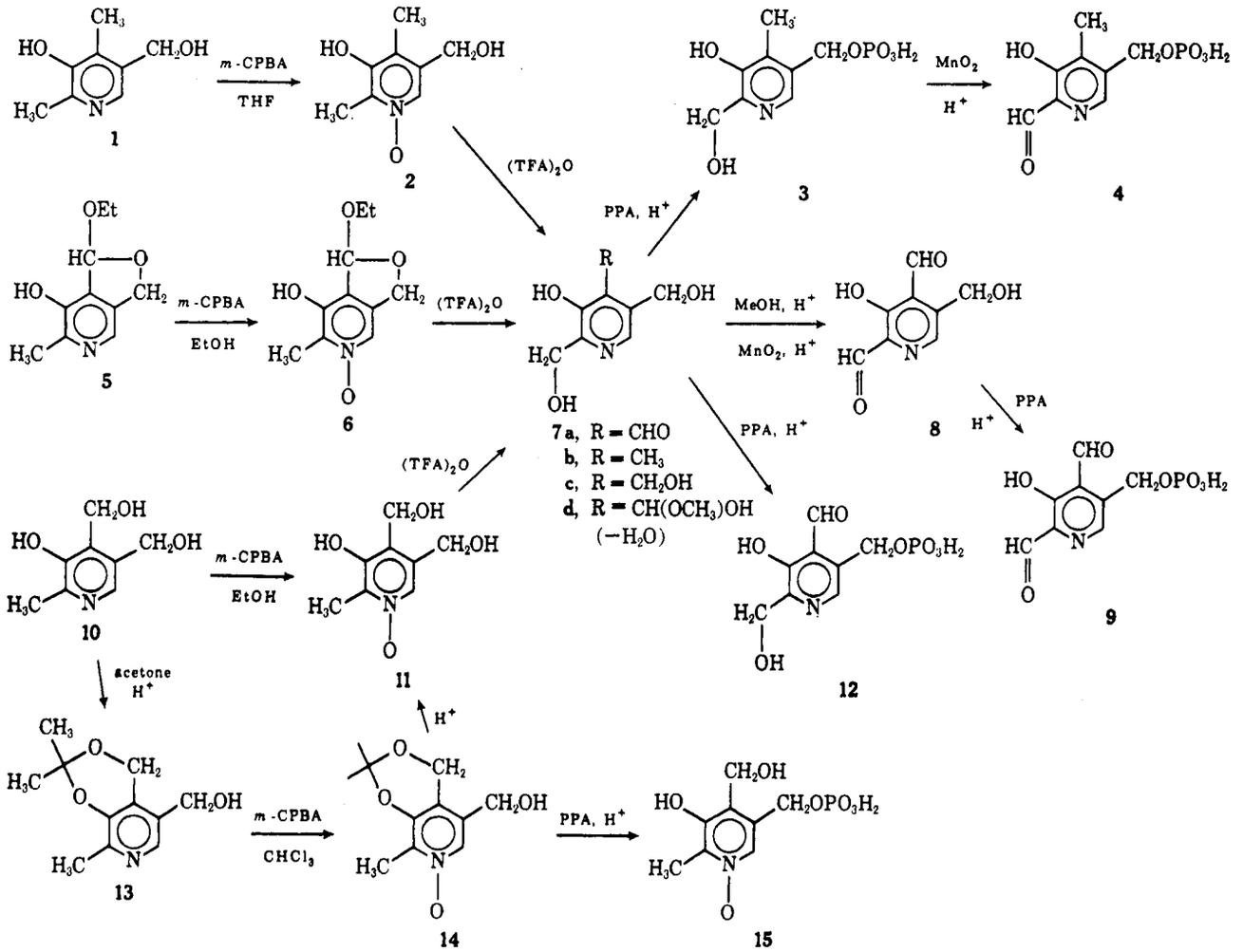
(6) T. Sakuragi and F. A. Kummerow, *J. Org. Chem.*, **24**, 1032 (1959).

(7) G. R. Bedford, A. R. Katritzky, and H. M. Wuest, *J. Chem. Soc.*, 4601 (1963).

(8) S. Oae, *Tetrahedron*, **20**, 2677 (1966).

(9) Trifluoroacetic anhydride was previously employed on a preparative scale in a Beckmann rearrangement [see W. D. Emmons, *J. Amer. Chem. Soc.*, **79**, 6522 (1957)] and in mechanistic studies of N–O bond cleavage of amine oxides [T. Koenig, *ibid.*, **88**, 4045 (1966)].

SCHEME I



aldehyde. Consequently, **8** was obtained by the selective oxidation of ω-hydroxypyridoxal methyl hemiacetal (**7d**) followed by acid hydrolysis. Phosphorylation of the 5-hydroxymethyl function was effected with polyphosphoric acid by well-established procedures[10,11] to afford **9**.

A similar synthesis gave 2-nor-2-formyl-4-deoxypyridoxol 5′-phosphate **4**, a cofactor analog possessing a divergent orientation of reactive groups around the pyridine ring. Likewise, the preparation of ω-hydroxypyridoxal 5′-phosphate (**12**), previously obtained *via* an elaborate procedure,[12] is readily accomplished starting from **7a** (unpublished results). This approach constitutes a general route for the conversion of 2-methylpyridoxol analogs to their 2-formyl derivatives and opens the way to further modifications of this position.

Verification of the structure of the new compounds proceeded in a number of ways. Whereas 3-pyridinols display in general only one absorption band at acid pH, the amine oxides consistently show two strong bands, as illustrated in Table I. Therefore, ultraviolet spectra served as a diagnostic tool to differentiate the amine oxides from their precursors as well as from their 2-carbinol derivatives. Moreover, the course of N-oxidation can be conveniently monitored by following the marked hypsochromic shift (*ca.* 20 nm) of the $\pi-\pi^*$ transition which occurs in ethanolic solution; the contribution of the reagent *m*-CPBA is minimal. Aromatic amine oxides are known to possess low acidic dissociation constants and are consequently singled out from all other intermediates by migration in an electrical field and on cationic exchangers.

Furthermore, the conversion of the bases to the 2-carbinol derivatives was established employing nmr spectroscopy by following the disappearance of the 2-methyl proton signals in the cationic species and the appearance of the 2-methylene signals, the latter being clearly distinct from both the 4- or 5-methylene peaks as reported in Table II.[13] The presence of the ring proton signal coupled with a positive Gibbs reaction given by all the intermediates ruled out a pyridone structure. It has been established that both hydration and hemiacetal formation in pyridoxal derivatives increases with the acidity of the solution. Consequently, the aldehydic C-4 proton signal is shifted to a higher field and appears as a doublet, the result of coupling to one of the 5-methylene protons. The nonequivalence of the latter is apparent only at higher pH.

(10) E. A. Peterson, H. A. Sober, and A. Meister, *Biochem. Prep.*, **3**, 29 (1953).

(11) M. Iwanani, T. Numata, and M. Murakami, *Bull. Chem. Soc. Jap.*, **41**, 161 (1968).

(12) Y. Nakai, F. Nasugi, N. Ohishi, and S. Fukui, *Vitamins (Kyoto)*, **38**, 189 (1968).

(13) W. Korytnyk and R. P. Singh, *J. Amer. Chem. Soc.*, **85**, 2813 (1963), and references cited therein.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF INTERMEDIATES AND FINAL PRODUCTS

Compd	0.1 N HCl λmax	0.1 N HCl εmax	Phosphate buffer pH 7 λmax	Phosphate buffer pH 7 εmax	0.1 N NaOH λmax	0.1 N NaOH εmax
Pyridoxal *N*-oxide	291	4970	310	5600	312[a]	6200
	258	7900	393	2400	387	1800
4-Deoxypyridoxol *N*-oxide (**2**)	285	6500	317	5900	317[a]	7200
	258	4100				
Pyridoxol 5′-phosphate *N*-Oxide (**15**)	293	5100	325	4800	325[a]	6700
	260	4800	258 sh	7950	255 sh	7000
ω-Hydroxypyridoxol *N*-oxide	300	5300	331	5700	331[a]	7500
	262	9100				
ω-Hydroxypyridoxal (**7a**)	288	8000	315	7300	305	5400
			390	100	390	700
ω-Hydroxy-4-deoxypyridoxol (**7b**)	282	8300	313	7400	303	6600
			252	3900	244	6900
ω-Hydroxy-4-deoxypyridoxol 5′-phosphate (**3**)	282	9200	313	8500	303	6900
			252	4000	244	6900
2-Nor-2-formylpyridoxal (**8**)	315 sh	100	368	8300	368	8900
	285	7800	255 sh	6800	255 sh	6500
2-Nor-2-formylpyridoxal 5′-phosphate (**9**)	325 sh	2300	415[b]	5500	410	5900
	295	6100	255 sh	5200	255 sh	5400
2-Nor-2-formyl-4-deoxypyridoxol 5′-phosphate (**4**)[c]	310 sh	3200	378	5200	378	6400
	285	7000	303	3800	308	3100
			237	9100	237	11500

[a] Insignificant shifts occurring in the alkaline range indicate that no additional proton dissociation takes place above pH 7. [b] In 50 m*M* glycerophosphate buffer (pH 7) containing 30 m*M* mercaptoethanol, the standard buffer for phosphorylase assay, a considerable blue shift to 385 nm occurs. The shift was not observed with glycerophosphate buffer alone. It is tentatively attributed to a selective thioacetal formation at the 2-carbonyl position, since none of the 4-carbonyl derivatives thus far investigated have displayed a shift of this magnitude. [c] Uncorrected for water content.

TABLE II

NMR SPECTRA OF THE SYNTHETIC INTERMEDIATES AS COMPARED WITH THEIR PARENT COMPOUNDS[a]

Compd	Proton shifts at position C-2	C-4	C-5	C-6
Pyridoxol	−156	−298	−286	−498
ω-Hydroxypyridoxol (**7c**)	−302	−299	−289	−497
4-Deoxypyridoxol	−156	−141	−285	−487
ω-Hydroxy-4-deoxypyridoxol (**7b**)	−298	−143	−287	−494
Pyridoxal	−158	−406	−316	−495
		−405		
ω-Hydroxypyridoxal (**7a**)	−302	−408	−319	−503
		−407		
2-Nor-2-formylpyridoxal (**8**)	−606[b]	−401	−312	−495
		−399		

[a] Solutions of the hydrochlorides (10% in D_2O) were employed for all determinations. Shifts are reported in hertz units at 60 MHz downfield from tetramethylsilane with 1,4-dioxane as internal standard. Assignment of peaks is in good agreement with published data (see ref 13). [b] Tentative assignment.

The low-field signal at −606 Hz observed in **8** is therefore tentatively assigned to the 2-formyl proton. This peak, however, disappears altogether in the cationic species of **9**, indicating complete hydration of both carbonyl groups, while the protons of the dihydrate show as a multiplet centered at −384 Hz. A detailed analysis of the nmr signals of the zwitterionic and anionic species is now in progress. Interestingly, the infrared spectra of all the carboxaldehydes as determined in Nujol mulls did not display the anticipated carbonyl stretching vibration. Apparently this function is masked either as a hemiacetal or a hydrate in the solid state.

Preliminary studies with rabbit muscle glycogen apophosphorylase indicate that **9** restores activity to the extent of 30%, suggesting that binding to a unique ε-aminolysine group at the PLP pocket occurs.[3] However, **4** does not markedly reactivate the apoenzyme (<1%), presumably as a consequence of an unfavorable orientation of substituent groups, although binding does occur as verified by characteristic spectral shifts. Work now in progress attempts to delineate the biochemical significance of these experiments and to identify the peptide loop joining the 2- and 4-carboxyaldehydes in **9**. These findings will be published elsewhere.

Experimental Section

All chemicals employed were of reagent grade or the highest grade available commercially. *m*-Chloroperbenzoic acid (*m*-CPBA) of 94% purity was kindly provided by the Norac Company Inc., Azusa, Calif. The Amberlite CG-50 ion exchanger was purchased from Mallinckrodt, whereas Dowex 50-X8 (200–400 mesh) was obtained from Bio-Rad. Unless otherwise specified, 2.5 × 50 or 100 cm columns in the hydrogen ion form were employed as required, using water as eluent. Activated manganese dioxide was prepared according to Harfenist.[14] Paper electropherograms (on Whatman 3MM) were run in pyridine–acetate buffer (pH 3.6) for 30 min at 2000 V; distance of migration is reported relative to the migration of pyridoxal 5′-phosphate and expressed as R_{PLP}. Spots were detected by the Gibbs test;[15] aldehydes were visualized by the phenylhydrazine reagent[16] and phosphate esters by the Hanes–Isherwood molybdate stain.[17]

Ultraviolet spectra were recorded on a Beckman DK-1 spectrophotometer, infrared spectra were obtained with a Perkin-Elmer 21 spectrophotometer, and nuclear magnetic resonance spectra were determined on a Varian A-60 instrument. Melting points were taken in a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental microanalyses were performed by Alfred Bernhard, Elbach über Engelskirchen, West Germany, and by Galbraith Laboratories, Inc., Knoxville, Tenn.

A Facile Preparation of ω-Hydroxypyridoxal Methyl Hemiacetal Hydrochloride (7d).—Pyridoxal hydrochloride (0.05 mol)

(14) M. Harfenist, A. Baverley, and W. A. Lazier, *J. Org. Chem.*, **19**, 1608 (1954).
(15) H. D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927).
(16) H. Wada and E. E. Snell, *J. Biol. Chem.*, **236**, 2089 (1961).
(17) C. S. Hanes and F. A. Isherwood, *Nature (London)*, **164**, 1107 (1949).

was converted to the ethyl hemiacetal in the usual manner[18] and the acid was neutralized with 1 equiv of sodium bicarbonate in the cold. Salts were removed by filtration and the ethanolic solution was chilled to −10° and used without further purification. A solution of *m*-CPBA (0.08 mol) in 50 ml of ethanol was then added dropwise with stirring and the reaction mixture was left in the freezer overnight, when the amine oxide **6** crystallized out. A small amount of contaminating starting material was removed by recrystallization from ethanol. The yield (8.9 g, 85%) can be increased by submitting the pooled residues from the recrystallization steps to one passage through Amberlite CG-50 cationic exchanger and one additional crystallization from ethanol. This product, which does not melt but decomposes on heating above 150°, was indistinguishable from a sample obtained by the oxidation of pyridoxol *N*-oxide with activated manganese dioxide, as established by uv spectroscopy and preparation of the known *p*-toluidine Schiff base.[19]

Pyridoxal *N*-oxide ethyl hemiacetal (**6**, 0.01 mol) was then suspended in 50 ml of dry chloroform and chilled to −10°. Trifluoroacetic anhydride (20 ml, 0.07 mol) was added dropwise with stirring over a period of 30 min, and the solution was held under gentle reflux for 6 hr and left to stand overnight. Following this, all solvents were flash evaporated, and the residual brownish oil was hydrolyzed by warming in hydrochloric acid and decolorized with Darco. On evaporation and drying the crude hydrochloride thus obtained was immediately converted into the methyl hemiacetal hydrochloride (**7d**) by a short reflux period in absolute methanol. One crystallization from methanol–THF gave 2.05 g (88%) of the pure compound which, like its precursor, did not melt but decomposed above 150°.

Anal. Calcd for $C_9H_{11}NO_4 \cdot HCl$: C, 46.26; H, 5.18; N, 6.00; Cl, 14.95. Found: C, 46.05; H, 5.15; N, 6.20; Cl, 15.18.

2-Nor-2-formylpyridoxal (8).—ω-Hydroxypyridoxal methyl hemiacetal hydrochloride (**7d**, 1 g) was dissolved in a mixture of 10 ml of methanol and 50 ml of tetrahydrofuran. Manganese dioxide "A" (5 g) was added and the reaction was allowed to proceed with stirring at room temperature for 24 hr. The catalyst was then filtered off and washed thoroughly with methanol. The combined filtrate and washings were evaporated and fractionated on an Amberlite CG-50 column and the product was recrystallized from water to afford 0.41 g (51%) of **8** as the white monohydrate, mp 167–168° dec. A yellow form is obtained by column chromatography on silica gel employing ethanol–chloroform (1:1) mixture as eluent. On recrystallization from water the yellow crystals revert to the white habit. Reduction of both forms with sodium borohydride in aqueous solution afforded a compound indistinguishable in its spectral and chromatographic properties from ω-hydroxypyridoxol (**7c**). The compound can be detected on paper by the slow change in color of its phenylhydrazone from yellow to amber and finally to maroon, quite distinct from the stable yellow color obtained with 3-pyridinol monocarboxaldehydes. It is fairly acidic, as is demonstrated by its migration on paper electropherograms (R_{PLP} −0.2) and on ion exchangers.

Anal. Calcd for $C_8H_7NO_4 \cdot H_2O$: C, 48.24; H, 4.55; N, 7.03. Found: C, 48.45; H, 4.64; N, 7.19.

Azomethine formation proceeds readily with a number of amines, such as *p*-toluidine, 1-adamantamine, and *p*-aminobenzoic acid. The bis-*p*-toluidine Schiff base crystallized out from methanol–water in orange needles, mp 148° dec, as the dihydrate.

Anal. Calcd for $C_{22}H_{21}N_3O_2 \cdot 2H_2O$: C, 66.82; H, 5.35; N, 10.63. Found: C, 66.99; H, 5.10; N, 9.89.

2-Nor-2-formylpyridoxal 5′-Phosphate (9).—The bis-*p*-toluidine Schiff base of **8** (0.4 g) was submitted to phosphorylation with polyphosphoric acid. After one passage through a Dowex 50-8X column the fractions containing **9** were pooled and reapplied to the recycled column. This treatment removed unidentified contaminants which coeluted in the first purification. Only 40 mg (14%) of pure product was obtained. The low yield appears to be due to interference by condensation reactions. The phosphate ester (R_{PLP} 1.7), like the parent compound, is distinguishable from all other PLP analogs by the maroon-colored stain obtained on paper with phenylhydrazine reagent. Moreover, the electronic spectra as shown in Table I are consistent with an extended π-electron system.

Anal. Calcd for $C_8H_8NO_7P \cdot {}^1/_2H_2O$: C, 35.56; H, 3.36; N, 5.12; P, 11.48. Found: C, 35.18; H, 3.62; N, 4.93; P, 10.88.

ω-Hydroxy-4-deoxypyridoxol 5′-Phosphate (3).—4-Deoxypyridoxol base (0.02 mol) was dissolved in 150 ml of tetrahydrofuran and treated with *m*-CPBA (0.03 mol) in the cold. The product was collected by filtration and purified by one passage through Amberlite CG-50 and crystallization from ethanol–tetrahydrofuran mixture; the amine oxide (2.8 g, 84%) separates as the monohydrate, mp 185° dec.

Anal. Calcd for $C_8H_{11}NO_3 \cdot H_2O$: C, 51.33; H, 6.99; N, 7.48. Found: C, 50.97; H, 6.93; N, 7.96.

The rearrangement of 4-deoxypyridoxol *N*-oxide (2 0.03 mol) was effected essentially as described under ω-hydroxypyridoxal. After treatment with Darco and flash evaporation an almost pure product (2.9 g 78%) crystallized out, which required only one recrystallization from ethanol–ethyl acetate mixture mp 184° dec.

Anal. Calcd for $C_8H_{11}NO_3 \cdot HCl$: C 46.72; H 5.88; N 6.81; Cl, 17.24. Found: C, 46.95; H, 5.45; N, 6.75; Cl, 17.23.

The synthesis was completed by phosphorylation of **7b** (0.03 g) as described above. The pure compound (0.29 g, 79%) was obtained following passage through an Amberlite CG-50 column and crystallization from water–ethanol mixture, from which it separates as slender white needles. On paper electropherograms at pH 3.6 or 6.5 the migration of **3** is almost identical with that of its isomer pyridoxol 5′-phosphate. However, a good separation can be accomplished by tlc on polyamide sheets in *n*-butyl alcohol–acetone–acetic acid–ammonia–water mixture (3.5:2.5:1.5:1.5:1.0) with R_f's of 0.52 and 0.13, respectively.

Anal. Calcd for $C_8H_{12}NO_6P$: C, 38.56; H, 4.86; N, 5.62; P, 12.43. Found: C, 38.30; H, 4.79; N, 5.74; P, 12.42.

2-Nor-2-formyl-4-deoxypyridoxol 5′-Phosphate (4).—ω-Hydroxypyridoxol 5′-phosphate (**3**, 0.2 g) was dissolved in 5 ml of 4 *N* H_2SO_4 and chilled to −15°. Manganese dioxide "A" (0.8 g) was added and the mixture was kept in the freezer with stirring for 35 hr, when the catalyst was filtered off and washed with a small amount of ice-cold water. The filtrate was neutralized in the cold with 50% sodium hydroxide and chromatographed on an Amberlite CG-50 column in the dark. Fractions containing the carboxaldehyde were pooled and evaporated, then rechromatographed to remove a small amount of contaminating starting material. Compound **4** (78 mg, 39%) was obtained as a yellow, glassy solid containing variable amounts of water and defied crystallization. The spectral properties of this compound (Table I) are consistent with a 3-hydroxy-2-pyridine aldehyde structure and are markedly different from that of PLP. On paper electropherograms **4** migrates as a single spot (R_{PLP} 1.5), gives a strong greenish color with Gibbs reagent and a bright yellow color with phenylhydrazine, and displays a green fluorescence when exposed to ammonia vapor.

Pyridoxol *N*-Oxide 5′-Phosphate (15).—α^4,3-*O*-Isopropylidene pyridoxol base[20] (**13**, 0.01 mol) was dissolved in 50 ml of dry chloroform and *m*-CPBA (0.015 mol) was added dropwise with cooling. After standing overnight in the cold, 2.2 g (98%) of the product was isolated by chromatography on basic alumina[5] and purified by one crystallization from tetrahydrofuran, mp 173°. Hydrolysis of the acetonide afforded a substance identical in melting point and uv absorption with that obtained by the direct oxidation of pyridoxol with *m*-CPBA or with peracetic acid.[6]

Anal. Calcd for $C_{11}H_{15}NO_4$: C, 58.65; H, 6.71; N, 6.22. Found: C, 58.66; H, 6.77; N, 6.15.

α^4,3-*O*-Isopropylidenepyridoxol *N*-oxide (1 g) was phosphorylated with polyphosphoric acid in the usual manner. On acid hydrolysis and one passage through an Amberlite CG-50 column, 0.76 g (66%) of pure pyridoxol *N*-oxide-5′-phosphate (**15**) was obtained. The structure of the final compound was verified by mobility on paper electropherograms (R_{PLP} 2.2) and spectral analysis (see Table I). Attempts to effect the rearrangement of the phosphorylated amine oxide resulted in a number of unidentified side products.

Anal. Calcd for $C_8H_{12}NO_7P \cdot 2.5H_2O$: C, 30.97; H, 5.52. Found: C, 31.36; H, 5.37.

(18) D. Heyl, E. Luz, S. A. Harris, and K. Folkers, *J. Amer. Chem. Soc.*, **73**, 3430 (1951).

(19) J. Vidgoff, Ph.D. Thesis, University of Washington, Seattle, Washington, 1970.

(20) W. Korytnyk and W. Wiedeman, *J. Chem. Soc.*, 2351 (1962).

ω-Hydroxypyridoxol N-Oxide.—Oxidation of ω-hydroxypyridoxol with *m*-CPBA in ethanol as described above afforded the title compound, mp 160° dec, in 75% yield.

Anal. Calcd for $C_8H_{11}NO_5$: C, 47.76; H, 5.51; N, 6.96. Found: C, 47.80; H, 5.44; N, 7.02.

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Registry No.—**1**, 61-67-6; **2**, 42253-78-1; **3**, 42253-79-2; **4**, 42253-80-5; **6**, 42253-81-6; **7a**, 20885-15-8; **7b** hydrochloride, 42253-82-7; **7c**, 29712-70-7; **7c** *N*-oxide, 42253-83-8; **7d** hydrochloride, 42253-84-9; **8**, 42253-85-0; **8** bis(*p*-toluidine) Schiff base, 42253-86-1; **9**, 42253-87-2; **13**, 1136-52-3; **14**, 42253-89-4; **15**, 42253-90-7; pyridoxal hydrochloride, 65-22-5.

Nucleotide Synthesis. IV.[1] Phosphorylated 3′-Amino-3′-deoxythymidine and 5′-Amino-5′-deoxythymidine and Derivatives[2,3]

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3′-Amino-3′-deoxythymidine 5′-phosphate (**10**) and 5′-amino-5′-deoxythymidine 3′-phosphate (**18**) were prepared. Compounds **10** and **18** are analogs of deoxythymidine 5′- and 3′-phosphates in which the 3′- and 5′-hydroxyl groups are replaced by amino groups. The synthetic sequence leading to **10** and **18** involved the synthesis of 3′-azido-3′-deoxythymidine (**5**) and 5′-azido-5′-deoxythymidine (**15**) by different multistep pathways. Phosphorylation of **5** and **15**, followed by removal of the protecting groups, gave nucleotides **9** and **17** which contained azide groups in the 3′ and 5′ positions, respectively. The azide group was unaffected by these transformations. Catalytic reduction of the azide groups of **9** and **17** gave the title compounds **10** and **18** in good yield. Moreover, **10** and **18** formed crystalline inner salts, **11** and **19**, respectively, which facilitated purification and characterization. In addition, **10** was converted into 3′-chloroacetamido-, 3′-*N*-(*O*-ethylcarbamoyl)-, and 3′-heptafluorobutyramido-3′-deoxythymidine 5′-phosphates (**12**, **13**, and **14**, respectively) and **18** was converted into 5′-acetamido-, 5′-chloroacetamido-, and 5′-*N*-(*O*-ethylcarbamoyl)-5′-deoxythymidine 3′-phosphates (**20**, **21**, and **22**, respectively); these derivatives were candidate active-site-directed inhibitors of a nuclear exoribonuclease isolated from nuclei of mammalian cells.

The presence of 3′-amino-3′-deoxy-β-D-ribofuranose moiety in the antibiotic puromycin[4] has stimulated considerable interest in other amino sugar nucleosides and nucleotides as pharmacological agents. Furthermore, several types of 3′-deoxy or 3′-amino-3′-deoxy nucleoside and nucleotide analogs have been reported to inhibit the synthesis of nucleic acid and, at least in some cases, the inhibition is due to incorporation of a nucleoside which cannot support further chain elongation. Thus, 3′-deoxyadenosine has been shown to inhibit the synthesis of both DNA and RNA in Ehrlich ascites tumor cells.[5,6] Other studies[6,7] have demonstrated *in vitro* inhibition of RNA synthesis by 3′-deoxyadenosine 5′-triphosphate, catalyzed by RNA polymerase. Also described was inhibition due to incorporation of 3′-deoxyadenosine at the 3′ terminus of the growing RNA molecule. The absence of 3′-hydroxyl function in this position prohibits further chain elongation. 3′-Amino-3′-deoxyadenosine[8] and 3′-deoxyguanosine[9] appear to function in a similar manner.

The synthesis of 3′-amino-3′-deoxythymidine 5′-phosphate (**10**), 5′-amino-5′-deoxythymidine 3′-phosphate (**18**), and derivatives containing a haloacetamido inactivating group was undertaken in these laboratories as part of a program devoted to the design of candidate active-site-directed inhibitors of a nuclear exoribonuclease. The exoribonuclease, isolated from the nuclei of mammalian cells, selectively degrades single-stranded, newly synthesized *m*-RNA from the 3′ end, liberating nucleotides with a 5′ phosphate group.[10] The enzyme has affinity for mononucleotides and oligonucleotides, but not for uncharged nucleosides. In addition, the enzyme is present in relatively large amounts in neoplastic tissues, relative to most normal tissues,[11] thus enhancing the importance of the acquisition of selective inhibitors of nuclear exoribonuclease in studying normal and abnormal nucleic acid metabolism. This paper describes the synthesis, purification, and characterization of these novel nucleotides; detailed biochemical results will be described elsewhere.

The key concept underlying the successful preparation of compounds **10** and **18** was the use of stable

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