4-Halovinyl- and 4-Ethynyl-4-deformylpyridoxal Derivatives and Related Analogues as Potentially Irreversible Antagonists of Vitamin B_6

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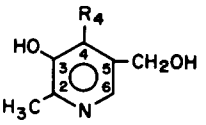
Analogues of pyridoxal bearing α- and β-chlorovinyl, β-bromovinyl, butadienyl, acetyl, and 1-butenyl groups in place of the formyl group have been synthesized by subjecting 3,α^5-di-*O*-benzylpyridoxal to appropriate Wittig or Grignard reactions. Similar methods yielded one-carbon homologues of pyridoxal and pyridoxol. Synthesis of the 4-ethynyl analogue of pyridoxal was achieved by dehalogenating blocked β-halovinyl derivatives. The substituted vinyl and the ethynyl analogues were found to be active as inhibitors of mouse mammary adenocarcinoma cells grown in cell culture at an ID_{50} of 10^{-5}–10^{-6} M. The inhibitory activity of the 4-ethynyl analogue could be partially reversed by pyridoxal. This analogue was found to inhibit pyridoxal phosphokinase, and its 5′-phosphate was likewise found to be a potent noncompetitive inhibitor of pyridoxine-P oxidase.

In the course of our studies dealing with the synthesis of analogues of vitamin B_6, we have extensively explored modification of the 4 position.[1-4] Among the various modifications of this position, replacement of the 4-formyl group of pyridoxal with an isosteric 4-vinyl group resulted in a vitamin B_6 antagonist (1, R_4 = CH=CH_2) that in several systems is more effective than the "classical" antagonist, 4-DOP[3] (1, R_4 = CH_3). Nevertheless, this



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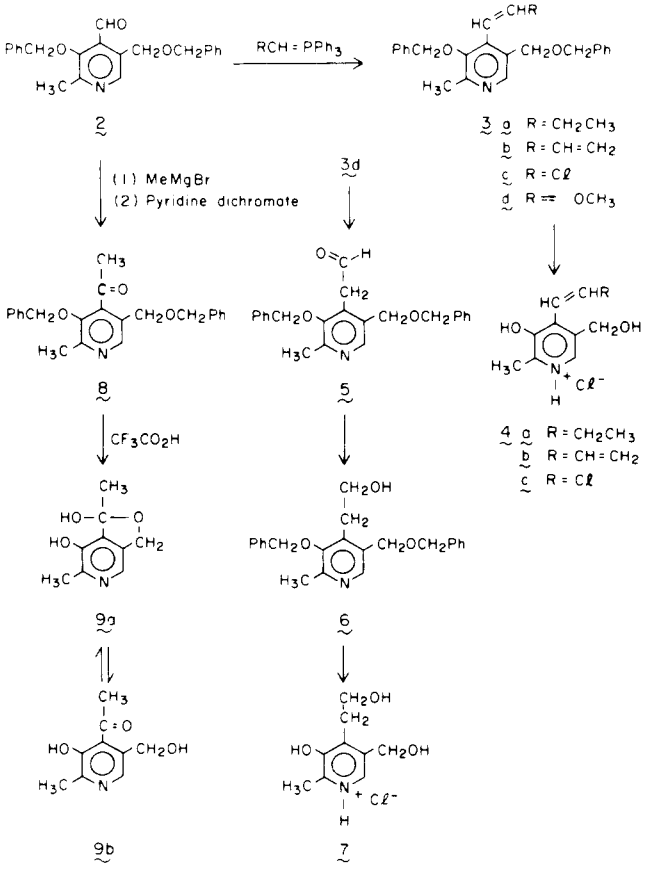
analogue could be reversed by vitamin B_6. In mice, it was also found to exhibit potent convulsing activity, which precluded its further investigation for tumor chemotherapy.[3]

In the present paper, we report other modifications of the 4 position, carried out in the hope of obtaining an antagonist with a different type of organ specificity, less reversibility by the vitamin, or both. To this end, we have synthesized 4-halovinyl, 4-butadienyl, and 4-ethynyl analogues of vitamin B_6. In addition, we have extended the 4-hydroxymethyl group by one carbon atom and replaced the 4-formyl group with an acetyl group, giving α^4-methylpyridoxal. In all of these syntheses, 3,α^5-di-*O*-benzylpyridoxal (**2**) served as the key intermediate (Scheme I).

Application of the Wittig reaction to **2** gave the β-substituted vinyl derivatives **3** as cis and trans isomers. The 1-butenyl (**3a**), butadienyl (**3b**), and chlorovinyl (**3c**) derivatives were deblocked with either HCl or trifluoroacetic acid to give the target analogues **4a–c**. Mild treatment of the β-methoxyvinyl analogue **3d** with HCl resulted in the blocked homopyridoxal derivative **5**, which was reduced to **6** and deblocked to the one-carbon pyridoxol homologue **7**. The corresponding two-carbon homologue was prepared earlier.[2]

The 4-formyl group of pyridoxal was replaced with an acetyl group (**9a** ⇌ **9b**) by reaction of the 4-aldehyde **2** with MeMgBr and oxidation of the resulting alcohol to the methyl ketone **8**. The deblocked material was shown to be in the hemiketal form **9a**. In D_2O solution, however, the presence of both the cyclic **9a** and the straight-chain **9b**, in approximately equimolar amounts, is shown by NMR spectroscopy. Under the same conditions, pyridoxal has been found to exist entirely in the hemiacetal form.[5,6] The preference of the methyl ketone to exist in the open form may be related to steric effects preventing the

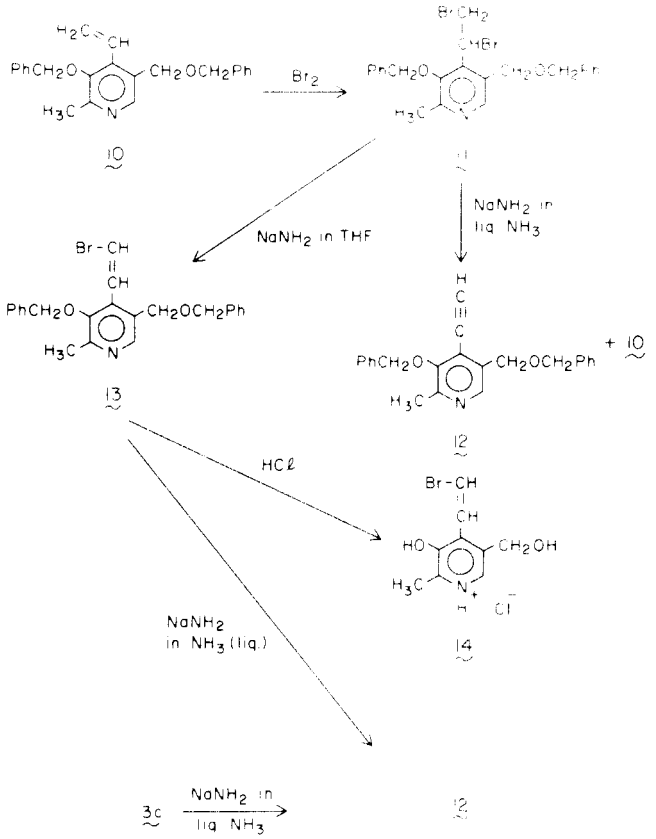
Scheme I



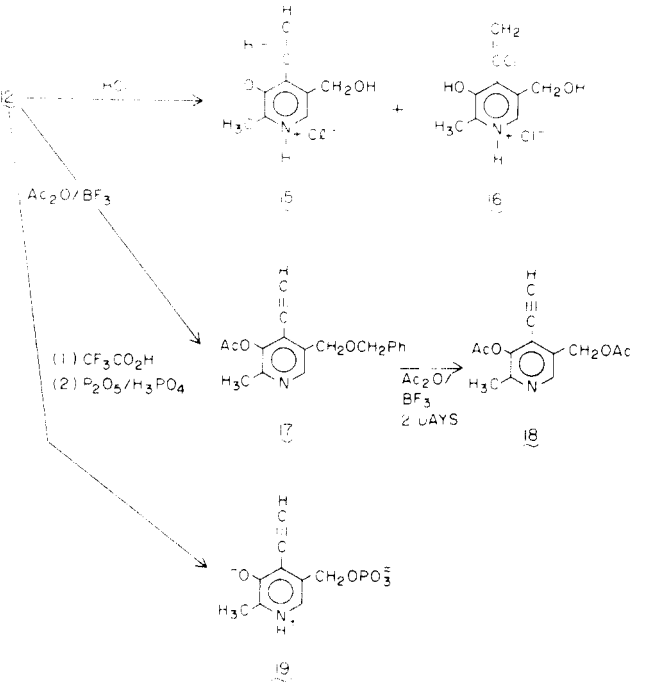
methylcarbonyl group from assuming coplanarity with the pyridine ring, as has been pointed out by Karpeisky et al.[7] for the 5′-phosphate derivative of **9b**.

Initially, synthesis of the 4-ethynyl analogue of pyridoxal was attempted by the same approach as we described earlier for synthesis of the 5-ethynyl analogue,[8] namely, addition of bromine to the vinyl group in **10** and subsequent dehydrobromination of the resulting dibromide **11** (Scheme II). This procedure gave the vinyl compound **10** as the major product, and only a minor amount of the desired 4-ethynyl derivative **12** was obtained. When the dehydrobromination was carried out in THF in the presence of small amounts of ammonia, the desired 4-ethynyl derivative predominated, but there was still a substantial proportion of **10**. Since the two products could not be cleanly separated by TLC, the mixture was treated with alcoholic $AgNO_3$, giving a sparingly soluble Ag complex[9] of **12**, which was isolated by filtration and decomposed by treatment with NaCN.

Scheme II



Scheme III



A more satisfactory synthesis of **12** was achieved in two steps. Monodehydrobromination of **11** proceeded smoothly, giving **13**, when $NaNH_2$ in THF was used and ammonia was completely excluded. Acid hydrolysis gave the completely deblocked compound **14**. The low coupling constants of the α and β vinyl protons indicate that **14** is a cis isomer. This makes a quasi-trans configuration of the starting material probable, assuming trans elimination of HBr. When the blocked β-bromide was treated with sodium amide in liquid ammonia, the ethynyl derivative **12** was obtained in excellent yield. The same is true of the analogous reaction with the β-chloro analogue **3c**, a procedure described earlier.[10]

It was thought that the unusual course of the elimination reaction of the dibromide **11** could be changed by preparing the *N*-oxide of the compound and then attempting an elimination reaction. Only a poor yield of the ethynyl derivative was obtained, however, on treatment of the *N*-oxide of **11** with $NaNH_2$ in liquid ammonia (see Experimental Section).

The formation of the vinyl derivative as the major product in the dehydrobromination reaction was surprising, although not without precedent. Dehydrobromination of 1,2-dibromostyrene with $LiNH_2$ in liquid ammonia gave a mixture of styrene and phenylacetylene.[11] In the absence of liquid ammonia, $LiNH_2$ effected a single dehydrobromination, yielding β-bromovinylstyrene. This result has been interpreted as the formation of a carbene by an α-dehydrobromination reaction:[11]

$$ArCHBrCH_2Br + NH_2^- \rightarrow Ar\ddot{C}CH_2Br + NH_3 + Br^-$$

The resulting product from the carbene would depend on the reaction conditions. In our case, the carbene derived from the dibromo compound **11** in the presence of ammonia adds on a hydride derived from ammonia, producing the vinyl derivative **10**. In the absence of ammonia, a shift of hydride from the β-carbon produces the β-bromovinyl derivative **13**. In the presence of some ammonia, the carbene formed undergoes dehydrobromination, yielding the acetylenic derivative **12** as the main product. Electron-withdrawing substituents, such as the 4-substituted pyridine, would be expected to favor the formation of the carbene intermediate, and this possibility may explain our divergent results in the synthesis of the 5-ethynyl analogue described earlier[8] and the 4-ethynyl analogue described here.

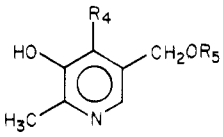
Removal of the benzyl blocking groups in **12** was associated with problems (Scheme III). When HCl was used as described by us earlier for the corresponding 4-vinyl derivative,[3] the α-chlorovinyl analogue **16** was the main reaction product, and only a very low yield of the desired ethynyl derivative **15** could be isolated. Among milder deblocking procedures, reaction with triphenylmethyl fluoroborate[12] was tried and was found to remove only the 3-*O*-benzyl group from **12**. Acetolysis[13] of **12** was more successful and was carried out in two steps, leading to the isolation of **17** and **18**. Finally, deblocking with trifluoroacetic acid was tried and was found to give a satisfactory yield of the target compound **15**, which was phosphorylated to the 5′-phosphate **19**. These last two synthetic experiments have already been described.[10]

The ethynyl proton in the blocked derivative **12** resonates at δ 3.68 ($CDCl_3$ from Me_4Si), as expected for a negatively substituted ethynyl compound. In the deblocked compound **15**, however, there is a shift of almost 1 ppm to a lower field (δ 4.65 ppm in D_2O from Me_4Si). This shift suggests an intramolecular H-bonding, as has been found in the case of *o*-ethynylphenol.[14]

Biological Activity. Compounds synthesized in the present study were tested as inhibitors of the growth of mouse mammary adenocarcinoma (TA3) cells in cell culture in Eagle's medium with a minimal amount of pyridoxal (1×10^{-7} M) to sustain growth. Inhibition data, compared with those for 4-DOP and 4-VPAL, are summarized in Table I. All of the new analogues were found to be considerably less potent than either 4-DOP or 4-VPAL.

The decreased activity in the 4-vinyl series may be related to the effect of the bulkiness of the group. Thus

Table I. Inhibitory Activity of 4-Modified Analogues[a]



Compd	R_4	R_5	ID_{50} (TA3 cells), M
4-DOP	$-CH_3$	H	6.8×10^{-8}
4-VPAL	$-CH=CH_2$	H	2.5×10^{-9}
3a	$-CH=CHEt$	H	1.5×10^{-5}
3b	$-CH=CHCH=CH_2$	H	1.5×10^{-5}
3c	$-CH=CHCl$	H	3×10^{-6}
16	$-CCl=CH_2$	H	9.5×10^{-6}
14	$-CH=CHBr$	H	1.2×10^{-5}
9	$-C=O(CH_3)$	H	No inhibn at 10^{-4}
15	$-C\equiv CH$	H	8×10^{-6}
19	$-C\equiv CH$	PO_3H_2	3.8×10^{-6}

[a] See ref 17 for conditions of testing.

the two chlorovinyl analogues **3c** and **16** are more potent than the bromovinyl derivative **14**. The ethynyl analogue **15** and its 5′-phosphate **19** were found to be about equally active. The ethynyl analogue **15** was also tested in the complete medium containing approximately 10^{-5} M pyridoxal. Under these conditions, its ID_{50} was 9×10^{-5} M, whereas the inhibitory effects of 4-DOP and 4-VPAL were completely reversed. We do not have reversal data for other compounds in this series. The ethynyl analogue extends the series of vitamin B_6 antagonists that have been found to be either partially[15] or not at all[16] reversed by pyridoxal.

The 4-ethynyl phosphate analogue was found to react irreversibly with apoaspartate aminotransferase, and this reaction has been studied in some detail spectrophotometrically.[10] We have also studied the inhibition of pyridoxine-P oxidase by this analogue. The Lineweaver–Burk plot (Figure 1) follows noncompetitive kinetics. With a K_I value of 0.55 μM, it is as potent as the 4-vinyl analogue of pyridoxal 5′-phosphate, which was found to be the most potent competitive inhibitor of pyridoxine-P oxidase. The unphosphorylated compound is probably a substrate of pyridoxal phosphokinase, and hence it is likely that the inhibitory effects on the cell may be due to inhibition of the oxidase.

It should be mentioned that introduction of the ethynyl group into the 2 or 5 position of pyridoxol has resulted in only marginally active antagonists,[8,16] supporting our hypothesis of in vivo phosphorylation of the 4-ethynyl analogue. When **15** was tested as an inhibitor for the phosphorylation of pyridoxal by pyridoxal phosphokinase, the K_I value was found to be 125 μM, with noncompetitive kinetics. This value is somewhat lower than the K_m value of 283 μM for pyridoxal but is substantially higher than the K_I value found for its 4-vinyl analogue (K_I = 22.5 μM), which was found to be a competitive inhibitor for the enzyme as well as a substrate.[17] Enzymatic inhibition studies have been carried out as described earlier.[17]

Experimental Section

Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within ±0.04% of the theoretical values. TLC (silica gel) was used routinely as described earlier.[18] IR spectra were determined with a Perkin-Elmer 457 spectrophotometer and NMR spectra with a Varian A-60A instrument, using 8–15% solutions in $CDCl_3$, Me_2SO-d_6, or D_2O; positions of peaks are expressed in cps units from Me_4Si, or from dioxane (δ 3.70), as an internal standard. Peaks are assigned on the basis of previous work.[19]

3,α^5-Di-*O*-benzyl-4-(1-butenyl)-4-deformylpyridoxal [4-(1-Butenyl)-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine] (3a). The procedure described by us earlier[3,16] for the Wittig reaction was applied to 3,α^5-di-*O*-benzylpyridoxal (**2**) (200 mg, 0.58 mmol), using propyltriphenylphosphonium bromide (400 mg, 1.03 mmol), and gave the 4-(1-butenyl) derivative **3a** of the corresponding 4-deformyl compound as an oil: yield, 164 mg (76%); NMR ($CDCl_3$, isomers) 4-(CH_3CH_2CH=CH) 45, 51, 52, 60, 61, 68 (from both isomers); 4-(CH_3CH_2CH=CH) 100–143 (multiplet); 4-CH=CH 342–400 (complex), 2-CH_3 151, 3 × CH_2 269, 270, 272, 273, 286, 2 × C_6H_5 444, 6-H 503, 507; IR ν_{max}^{neat} 1585 cm^{-1} (C=C); UV λ_{max} (95% alcohol) 254 nm. The oil was dissolved in ether, and methanolic HCl was added. The hydrochloride was crystallized from methanol–ether: mp 131–132 °C; NMR ($CDCl_3$) 4-(CH_3CH_2CH=CH) 52–70 (multiplet); 4-(CH_3CH_2CH=CH) 105–120 (multiplet); 2-CH_3 162, 3 × CH_2 278, 280, 292, 2 × C_6H_5 447, 6-H 518, 509; IR ν_{max}^{KBr} 1610 cm^{-1} (C=C); UV λ_{max} (95% alcohol) 255 nm. Anal. ($C_{25}H_{28}NO_2Cl$) C, H, Cl.

4-(1-Butenyl)-4-deformylpyridoxal [4-(1-Butenyl)-3-hydroxy-2-methyl-5-pyridinemethanol] (4a) Hydrochloride. A solution of the blocked 4-(1-butenyl) compound **3a** (100 mg, 0.26 mmol) in 4 N HCl (15 mL) was heated on a steam bath for 20 h. Excess HCl and benzyl alcohol were removed by repeated evaporation with water, and the residue was crystallized from alcohol: yield 40 mg (69%); mp 140–142 °C dec; NMR (D_2O, cis and trans isomers) 4-(CH_3CH=CH) 42, 73; 4-(CH_3CH_2CH=CH), 90, 122; 2-CH_3 153, 5-CH_2 281, 280; 4-(CH_3CH_2CH=CH) 365–390; 4-(CH_3CH_2CH=CH) 445–450, 6-H 489, 490; IR ν_{max}^{KBr} 1655 cm^{-1} (C=C); MS, the molecular peak agreed with the molecular weight of the compound. Anal. ($C_{11}H_{16}NO_2Cl$) C, H.

3,α^5-Di-*O*-benzyl-4-(1,3-butadienyl)-4-deformylpyridoxal [4-(1,3-Butadienyl)-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine] (3b). 3,α^5-Di-*O*-benzylpyridoxal (500 mg, 1.44 mmol) was allowed to react with triphenyl-2-propenylphosphonium bromide, ($Ph_3P^+CH_2CH$=CH_2)Br^- (900 mg, 2.66 mmol), giving the 4-butadienyl compound **3b** as an oil: yield 320 mg (55%); NMR ($CDCl_3$) 2-CH_3 151, 3 × CH_2 270, 274, 289, –CH=CHCH=CH_2 300–420 (highly complex), 2 × C_6H_5 445, 448, 6-H, 509; IR ν_{max}^{neat} 1590 cm^{-1} (C=C); UV λ_{max} (95% alcohol) 273 nm. The oil (25 mg, 0.066 mmol) was taken up in anhydrous ether (1 mL), and enough ethereal HCl was added to convert the free base into its hydrochloride. After removal of excess HCl, the hydrochloride of **3b** was crystallized from alcohol–ether: yield 20 mg (73%); mp 118–121 °C; NMR ($CDCl_3$) 2-CH_3 155, 3 × CH_2 275, 281, 297, –CH=CHCH=CH_2 320–400 (highly complex), 2 × C_6H_5 455, 6-H 515; IR ν_{max}^{KBr} 1610 cm^{-1} (C=C). Anal. ($C_{25}H_{26}NO_2Cl$) C, H.

4-(1,3-Butadienyl)-4-deformylpyridoxal [4-(1,3-Butadienyl)-3-hydroxy-2-methyl-5-pyridinemethanol] (4b). The

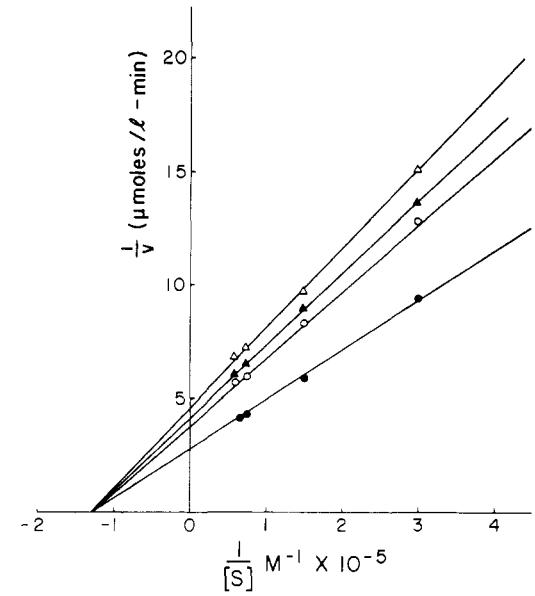


Figure 1. Lineweaver–Burk plots for inhibition by the 4-ethynyl 5′-phosphate analogue **19** of the oxidation of pyridoxol 5′-phosphate catalyzed by pyridoxine-P oxidase: no inhibitor (●), 0.1 (○), 0.33 (▲), and 0.66 μM (△) of **19**.

blocked compound **3b** (290 mg, 0.78 mmol) was taken up in CF_3COOH (15 mL), and the solution was refluxed for 20 h. The acid was evaporated under vacuum, leaving a mixture of compounds, which was separated on a silica gel column, using $CHCl_3$–MeOH (9:1) as the eluent. The fractions containing the desired compound were combined and were evaporated to a solid residue. The compound was extracted with $CHCl_3$ (to remove the silica), and the $CHCl_3$ was evaporated. The product was converted into its hydrochloride with alcoholic HCl, and the hydrochloride was crystallized from alcohol: yield 60 mg (34%); mp 155–157 °C dec; NMR (D_2O) 2-CH_3 158, 5-CH_2 278, –CH=CHCH=CH_2 310–420 (highly complex), 6-H 494; IR ν_{max}^{KBr} 1630 cm^{-1} (C=C); UV λ_{max} (0.1 N HCl) 296 nm (ϵ 9.55 × 10^3); UV λ_{max} (0.1 N NaOH) 315 nm (ϵ 6.48 × 10^3); UV λ_{max} (pH 7) 245 nm (ϵ 1.11 × 10^4), 334 (5.3 × 10^3); MS, the molecular peak ion was in agreement with the molecular weight of the hydrochloride. Anal. ($C_{11}H_{14}NO_2Cl$) C, H, Cl.

4-(2-Chlorovinyl)-4-deformylpyridoxal [4-(2-Chloroethenyl)-3-hydroxy-2-methyl-5-pyridinemethanol] (4c). 3,α^5-Di-*O*-benzyl-4-(2-chlorovinyl)-4-deformylpyridoxal (**3c**) (100 mg, 0.26 mmol) was dissolved in 3 N HCl (10 mL), and the solution was heated on a steam bath for 20 h. Excess HCl and benzyl alcohol were removed by evaporation under vacuum, and the residue was crystallized from ethanol: yield 32 mg (52%); mp 201–203 °C dec; the trans form was the major product; NMR (D_2O) 2-CH_3 155, 5-CH_2 282, 4-CH=C*H*Cl 407, 421.5; 4-C*H*CHCl 437.5, 452 (J = 14.5 Hz, trans), 6-H 491; IR ν_{max}^{KBr} 3340 (-OH), 1580 (C=C), 965 cm^{-1} (C=C trans); UV λ_{max} (0.1 N HCl) 291 nm; UV λ_{max} (0.1 N NaOH) 346 nm. Anal. ($C_9H_{11}NO_2Cl_2$) C, H, Cl.

3,α^5-Di-*O*-benzyl-4-(2-methoxyvinyl)-4-deformylpyridoxal [4-(Methoxy-1-propenyl)-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine] (3d). Methoxymethyltriphenylphosphonium chloride (1.025 g, 3 mmol) was suspended in THF (8 mL, distilled over $LiAlH_4$), and 3 mmol of phenyllithium (2 M, 70:30 benzene–ether solution) was added dropwise from a hypodermic syringe. After stirring for 1 h, ylide formation was complete. Next 0.70 g (2 mmol) of **2** in THF (2 mL) was added, and the reaction mixture was stirred for 4 h. After filtration and evaporation, the oily residue was extracted with ether. The ether extracts were evaporated to a small volume, and some triphenylphosphine oxide crystallized. The crystals were filtered off, and the concentrated ether solution was applied to a silica gel column (BIO-SIL "A", 100–200 mesh), which was then eluted with a 2:3 mixture of ethyl acetate and chloroform. Fractions containing **3d** (TLC, R_f 0.68 in 3:7 ethyl acetate and chloroform) were evaporated to a yellow oil (0.5 g, 67%), which was characterized by NMR ($CDCl_3$) as a mixture of cis and trans isomers: 2CH_3 151 and 149; OCH_3 215; 5-C*H*$_2$OCH$_2$ 276–271; 3-OCH_2 290 and 291; 4-CH=C*H*-OCH_3 373 (d, J = 7 Hz, cis), 453 (d, J = 13 Hz, trans).

3,α^5-Di-*O*-benzyl-α^4-homopyridoxal [2-Methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)-4-pyridineethanal] (5) Hydrochloride. Compound **3d** (500 mg) was dissolved in 10 mL of 2 N HCl. Some acetone was added, and the mixture was kept at 50 °C for 1 h. After evaporation under vacuum, the residue was crystallized from ethyl acetate: yield 300 mg (57%); mp 124 °C; NMR ($CDCl_3$) 2-CH_3 169, 5-CH_2Ph 271, 4-CH_2 238 (s, br), 3-O-CH_2Ph 296, phenyls 439 and 442, C_6-H 507.

3,α^5-Di-*O*-benzyl-α^4-homopyridoxol [2-Methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)-4-pyridineethanol] (6) Hydrochloride. The aldehyde **5** (100 mg) was added to $NaBH_4$ (140 mg) in ethanol (90%, 3 mL), and the mixture was stirred for 15 min. After addition of acetone and some additional stirring, the reaction mixture was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in ether and extracted with water. The ether layer was dried, and some ethereal HCl was added, resulting in precipitation of the hydrochloride of **6**: yield 90 mg (90%); mp 154 °C; NMR ($CDCl_3$) 2-CH_3 163, 3-OCH_2Ph and 5-CH_2OCH_2Ph 300, 285, and 278, 4-C*H*$_2$OH 224–242 and 175–188 (br), phenyls 444, 440, C_6-H 502. Anal. ($C_{23}H_{26}NO_3Cl$) C, H, N, Cl.

α^4-Homopyridoxol [3-Hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridineethanol] Hydrochloride (7). A solution of **6** (40 mg) in 2 N HCl (3 mL) was refluxed for 4 h. After evaporation to dryness, the residue was crystallized from acetone, yielding 18 mg (82%) of **7**: mp 161 °C (from acetone–methanol); NMR (Me_2SO-d_6) 2-CH_3 159, 4-CH_2CH_2OH 222 (t, J = 6 Hz), 180 (t, J = 6 Hz), 5-CH_2OH 283, C_6-H 489. Anal. ($C_9H_{24}NO_3Cl$) C, H, N, Cl.

3,α^5-Di-*O*-benzyl-α^4-methylpyridoxal [1-[2-Methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)-4-pyridyl]-1-ethanone] (8). To a solution of 3,α^5-di-*O*-benzyl-α^4-methylpyridoxol[2] (0.50 g) in CH_2Cl_2 (25 mL) was added pyridine dichromate (1 g), and the reaction mixture was stirred at room temperature for 15 h. The solvent was then evaporated, and the product was extracted several times with ethyl acetate. The extract was washed several times with water, dried ($MgSO_4$), and evaporated to an oil (0.48 g, 96%), which was crystallized from ethyl acetate and ethanol: mp 143–144 °C; IR ν_{max}^{KBr} 1715 cm^{-1}; NMR ($CDCl_3$) 2-CH_3 146, 4-$COCH_3$ 166, 3 × CH_2 273, 296, 2 × C_6H_5 438, C_6-H 472. Anal. ($C_{23}H_{23}NO_3$) C, H.

α^4-Methylpyridoxal [1-[3-Hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridyl]-1-ethanone] (9a). A solution of **8** (0.30 g) in trifluoroacetic acid (15 mL) was refluxed for 15 h. The acid was removed in vacuo, but attempted crystallization of the salt of **9a** was not successful. The product was dissolved in water, and the solution was passed through an Amberlite CG-50 column to convert the product to the free base, which was then treated with ethereal HCl. The hydrochloride was crystallized from ethanol–ether: yield 62 mg (31%); NMR (D_2O) CH_3 (hemiketal) 105, CH_3 (ketone and 2-CH_3) 247, 249, CH_2O 295, 308, C_6H 471, 477; UV λ_{max} (0.1 N HCl) 288 nm; UV λ_{max} (0.1 N NaOH) 248, 302, 335 nm; UV λ_{max} (pH 7) 256, 320 nm. Anal. ($C_9H_{32}NO_3$-Cl-H_2O) C, H, N.

3,α^5-Di-*O*-benzyl-4-(1,2-dibromoethyl)-4-deformylpyridoxal [4-(1,2-Dibromoethyl)-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine] (11). A solution of **10** (500 mg, 1.45 mmol) in dry CCl_4 (4 mL) was cooled in an ice bath. A solution of bromine (300 mg, 1.89 mmol) in dry CCl_4 (1 mL) was added to the solution of **10** dropwise during 15 min. The mixture was stirred for another 30 min. The CCl_4 was evaporated, and the residue was dried to an oil: yield 800 mg (91%); NMR ($CDCl_3$) 2-CH_3 156, 4-$CHBrCH_2Br$ 227–357 (highly complex and analogous to the NMR spectrum of styrene dibromide); 3 × CH_2 273, 280, 2 × C_6H_5 447, 450 (splitting), 6-H 499; UV λ_{max} (95% alcohol) 499 nm. The free base **11** was converted into its hydrochloride by the addition of ethereal HCl. After excess HCl was removed, the compound was crystallized from acetone–ether: mp 122–123 °C dec; NMR ($CDCl_3$) 2-CH_3 154, 4-$CHBrCH_2Br$ 229–358 (highly complex), 3 × CH_2 275, 280, 282, 2 × C_6H_5 448, 6-H 501; UV λ_{max} (95% alcohol) 285 nm. Anal. ($C_{23}H_{24}NO_2Br_2Cl$) C, H, halogens.

3,α^5-Di-*O*-benzyl-4-(2-bromovinyl)-4-deformylpyridoxal [4-(2-Bromoethenyl)-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine] (13). Fresh $NaNH_2$ was prepared according to the method of Vaughn et al.[20] Liquid NH_3 (25 mL) was collected in a three-necked flask cooled in a mixture of dry ice and Me_2CO. Then $Fe(NO_3)_2$ (2 mg) was added, followed by Na (104 mg), with magnetic stirring, moisture being excluded. After 1 min, the blue color of the solution faded and was completely discharged by the passage of dry air. Na (115 mg) was added in small pieces during 10 min. After the mixture became gray, 3 mg of aniline was added as a catalyst. The solution of $NaNH_2$ thus obtained was cooled and vigorously stirred for 1 h, and a solution of the dibromo compound **11** (505 mg, 1 mmol) in Et_2O (anhydrous, 30 mL) was added dropwise during that time. Stirring was continued for another 2 h. The sodium acetylide analogue formed was hydrolyzed by the addition of concentrated NH_4OH (20 mL), followed by H_2O. Excess $NaNH_2$ and NH_4OH were decomposed by careful addition of dilute HCl while cooling with a mixture of dry ice and EtOH. Extraction with Et_2O (three times), washing the extract with H_2O, and drying gave an oil, **13**: yield 305 mg (72%); NMR ($CDCl_3$) 2-CH_3 151, 3 × CH_2 270, 274, 287, -CH=CH- (under benzyl peaks, and integration showed an additional 2 protons), 2 × C_6H_5 446, 6-H 497; IR ν_{max}^{neat} 1605 cm^{-1} (C=C); UV 262 nm. Addition of ethereal HCl to an ethereal solution of the oily **13** (40 mg, 0.09 mmol) resulted in formation of the hydrochloride: yield 32 mg (73%); mp 136–138 °C dec; NMR ($CDCl_3$) 2-CH_3 167, 3 × CH_2 282, 284, 300, 2 × C_6H_5 448, -CH=CH- (obscured by the benzyl peaks), 6-H 515; IR ν_{max}^{KBr} 1590 cm^{-1} (C=C); UV λ_{max} (95% alcohol) 260 nm. Anal. ($C_{23}H_{23}NO_2BrCl$) C, H, halogens.

4-(2-Bromovinyl)-4-deformylpyridoxal [4-(2-Bromoethenyl)-3-hydroxy-2-methyl-5-pyridinemethanol] (14). The dibenzyl derivative **13** (200 mg, 0.47 mmol) was taken up in 3 N HCl (15 mL), and the mixture was heated on a steam bath for 18 h. Excess HCl and benzyl alcohol were removed by repeated evaporation under vacuum, and the product **14** was crystallized from methanol–ether: yield 82 mg (62%); mp 198–200 °C dec; NMR (D_2O) 2-CH_3 156, 5-CH_2 283, CH=CHBr 422, 438, 439, 448, 6-H 492; IR ν_{max}^{KBr} 1525 cm^{-1} (C=C); UV λ_{max} (95% alcohol) 297 nm. Anal. ($C_9H_{11}NO_2BrCl$) C, H, halogens.

3,α^5-Di-*O*-benzyl-4-ethynyl-4-deformylpyridoxal [4-Ethynyl-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine] (12). A solution of 3,α^5-di-*O*-benzyl-4-(2-bromovinyl)-4-deformylpyridoxal (**13**, 424 mg, 1 mmol) in anhydrous ether (10 mL) was added dropwise to sodium amide in liquid ammonia (3 mmol, prepared as described for **13**), which was cooled in a mixture of dry ice and alcohol, and was kept stirring overnight. The rest of the work-up was performed exactly as described for **13**. The yield was 280 mg (81%); NMR and IR spectra were indistinguishable from those of the sample of **12** obtained earlier.[10]

$AgNO_3$ Complex of 12. The mixture of the 4-ethynyl (**12**) and 4-vinyl (**10**) compounds obtained by dehydrobromination of the dibromide **11** with $NaNH_2$ in liquid NH_3 was taken up in alcohol, and alcoholic silver nitrate solution was added. The $AgNO_3$ complex of the 4-ethynyl compound came out of the solution as a solid, whereas the complex of the 4-vinyl compound was soluble in alcohol. The solution was filtered, and the solid complex was crystallized from alcohol: NMR [Me_2SO-d_6; Na salt of 3-(trimethylsilyl)-1-propanesulfonic acid as internal standard] 2-CH_3 142, 3 × CH_2 265, 278, 303, 2 × C_6H_5 423, 434, 6-H 492. Anal. ($C_{23}H_{20}N_2O_5Ag_2$) C, H, N.

3,α^5-Di-*O*-benzyl-4-ethynyl-4-deformylpyridoxal (12) from Its $AgNO_3$ Complex. To a solution of the solid $AgNO_3$ complex of the 4-ethynyl compound (50 mg, 0.08 mmol) in water (10 mL) was added NaCN (20 mg), and the mixture was heated on a steam bath for about 1 h. The water was evaporated, and the product was extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried over anhydrous $MgSO_4$, filtered, and evaporated, giving oily **12**: yield 20 mg (72%); NMR and IR spectra of the compound were identical with those of the authentic sample.

3,α^5-Di-*O*-acetyl-4-(1-chlorovinyl)-4-deformylpyridoxal [3-Acetoxy-5-(acetoxymethyl)-4-(1-chloroethenyl)-2-methylpyridine] (16). Method A. The 4-ethynyl compound **12** (100 mg, 0.29 mmol) was taken up in 3 N HCl (10 mL), and the mixture was heated on a steam bath for about 20 h. The solution became very dark. TLC indicated the formation of several products. The major two spots were found to be 4-(1-chlorovinyl) (**16**) and 4-ethynyl (**15**) compounds. R_f values of these two compounds were approximately the same in several solvent systems, and separation was extremely difficult. Accordingly, the whole reaction mixture was acetylated with Ac_2O–pyridine, and the acetyl derivatives were separated by column chromatography, using silica–EtOAc. Only the acetyl derivative of the 4-(1-chlorovinyl) compound **16**, however, was obtained: yield 22 mg (27%); NMR ($CDCl_3$) 2 × CH_3C=O 124, 129, 2-CH_3 148, 5-CH_2 397, CH_2=C 396, 6-H 515; IR ν_{max}^{neat} 1785 1725 (C=O), 1620 (C=C), 3070 cm^{-1} (CH stretching of CH_2=C); UV λ_{max} (95% alcohol) 272 nm.

Method B. The 4-ethynyl compound **12** (100 mg, 0.29 mmol) was taken up in 5 N HCl (10 mL), and the solution was refluxed for 24 h. During the refluxing, the solution became very dark, and the compound underwent polymerization. The acid was removed by evaporation under vacuum. The dark residual mass was dissolved in alcohol, and the alcohol solution was decolorized with charcoal and filtered. The filtrate was evaporated to a solid, and the 4-(1-chlorovinyl) compound **16** was crystallized from alcohol–ether several times: yield 15 mg (22%); mp 152–153 °C dec; NMR (D_2O) 2-CH_3 156, 5-CH_2 280, CH_2=C– 414, 6-H 495; IR ν_{max}^{KBr} 3380 (OH), 1610 cm^{-1} (C=C); UV λ_{max} (0.1 N HCl) 293 nm (ϵ 1.27 × 10^4); UV λ_{max} (0.1 N NaOH) 318 nm (ϵ 5.3 × 10^3). Anal. ($C_9H_{11}NO_2Cl_2$) C, H.

3-*O*-Acetyl-α^5-*O*-benzyl-4-ethynyl-4-deformylpyridoxal [3-Acetoxy-4-ethynyl-2-methyl-5-(phenylmethoxymethyl)-pyridine] (17). The 4-ethynyl compound **12** (30 mg, 0.079 mmol) was taken up in Ac_2O (1 mL), and the mixture was stirred at 0 °C. The ether–BF_3 complex (1 mL), cooled to 0 °C, was added to the reaction flask, and the mixture was kept stirring at 4 °C for 12 h. The mixture was then poured into an ice–water mixture and left for a few minutes. The compound was extracted with ethyl acetate, and the ethyl acetate layer was washed with water, dried over anhydrous $MgSO_4$, filtered, and dried, giving an oil: yield 18 mg (70%); NMR ($CDCl_3$) CH_3C(=O)– 124, 2-CH_3 149, C≡CH 228, CH_2OCH_2 308, 210, C_6H_5 44, 6-H 505; IR ν_{max}^{neat} 2120 (C≡C), 1720 cm^{-1} (C=O).

3,α^5-Di-*O*-acetyl-4-ethynyl-4-deformylpyridoxal [3-Acetoxy-5-(acetoxymethyl)-4-ethynyl-2-methylpyridine] (18). Compound **17** (18 mg, 0.05 mmol), obtained by the method just described, was treated with the same acetolysis mixture but was kept at room temperature for 2 days. By the end of that time, the major product in the reaction mixture was 3,α^5-di-*O*-acetyl-4-ethynyl-4-deformylpyridoxal (**19**). Isolation of the compound was carried out as for **17**, giving an oil: yield 8 mg (62%); NMR (micro cell; $CDCl_3$) 2 × CH_3C(=O)– 125, 127, 2-CH_3 149, 5-CH_2 301, C≡CH 230, 6-H 510; IR ν_{max}^{neat} 2120 (C≡C), 1780, 1734 cm^{-1} (C=O).

α^5-*O*-Benzyl-4-ethynyl-4-deformylpyridoxal [4-Ethynyl-2-methyl-5-(phenylmethoxymethyl)-3-pyridinol]. Compound **12** (35 mg, 0.092 mmol) was taken up in CH_2Cl_2 (1 mL), $(C_6H_5)_3C^+BF_4^-$ (70 mg, 0.23 mmol) was added, and the reaction mixture was stirred overnight (12 h). The solvent was evaporated, and the product was purified by preparative TLC: yield 20 mg (77%); NMR ($CDCl_3$) 2-CH_3 149, C≡CH 228, CH_2 293, 5-CH_2 312, C_6H_5 445, 6-H 508; IR ν_{max}^{KBr} 3400 (OH), 2110 (C≡C), 1210 cm^{-1} (COC); UV λ_{max} (95% alcohol) 300 nm; the mass spectrum agreed with the molecular weight of the material.

3,α^5-Di-*O*-benzyl-4-(1,2-dibromoethyl)-4-deformylpyridoxal *N*-Oxide [4-(1,2-Dibromoethyl)-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine 1-Oxide]. 3,α^5-Di-*O*-benzyl-4-vinyl-4-deformylpyridoxal *N*-oxide[17] (100 mg, 0.27 mmol) was taken up in dry CCl_4 (5 mL), and the mixture was cooled in an ice bath. Bromine (60 mg, 0.33 mmol) in dry CCl_4 (0.5 mL) was added during 5 min. The reaction mixture was stirred for 45 min. Carbon tetrachloride was removed by evaporation, and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried over anhydrous $MgSO_4$, filtered, and evaporated, giving an oil: yield 110 mg (77%); NMR ($CDCl_3$) 2-CH_3 154, 3 × CH_2 290, 291, –$CHBrCH_2Br$ 320–200 (highly complex), 2 × C_6H_5 447, 452, 6-H 500. The oil (20 mg, 0.033 mmol) was taken up in anhydrous ether (1 mL), and ethereal HCl was added to convert the free base into its hydrochloride. The ether was evaporated under vacuum, and excess HCl was removed by repeated evaporation of the compound with fresh anhydrous ether. The hydrochloride was crystallized from acetone: yield 19 mg (89%); mp 103–105 °C dec; NMR ($CDCl_3$) 2-CH_3 158, 3 × CH_2 298, 294, 4-$CHBrCH_2Br$ 202–324 (complex), 2 × C_6H_5 448, 450, 6-H 506. Anal. ($C_{23}H_{24}NO_3BrCl$) C, H, halogens.

3,α^5-Di-*O*-benzyl-4-ethynyl-4-deformylpyridoxal *N*-Oxide [4-Ethynyl-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine 1-Oxide]. Sodium amide (39 mg, 1 mmol) in liquid NH_3 was prepared as already described and was cooled in a mixture of dry ice and alcohol. A solution of the dibromo *N*-oxide just described (90 mg, 0.172 mmol) in anhydrous ether (5 mL) was added to the sodium amide solution during 30 min. The reaction mixture was stirred overnight. Ammonium chloride solution was carefully added to the reaction vessel, and the products were extracted with ether. The ether solution was washed with water, dried over anhydrous Na_2SO_4, filtered, and evaporated, giving an oil. The oily ethynyl compound was purified by column chromatography (EtOAc–silica): yield 35 mg (69%); NMR ($CDCl_3$) 2-CH_3 140, C≡CH 227, 2 × CH_2 280, CH_2 311, 2 × C_6H_5 445, 6-H 502; IR ν_{max}^{neat} 2100 (C≡C), 1205 cm^{-1} (N→O); UV λ_{max} (95% alcohol) 233, 294 nm. The oil (30 mg, 0.08 mmol) was taken up in anhydrous ether (2 mL), and ethereal HCl was added. The solid hydrochloride separated out. Excess HCl was removed by evaporation under vacuum, and the hydrochloride was crystallized from acetone: yield 28 mg (84%); mp 140–142 °C; NMR ($CDCl_3$) 2-CH_3 151, 4-C≡C-H 230, 3 × CH_2 281, 282, 312, 2 × C_6H_5 448, 449, 6-H 508; IR ν_{max}^{KBr} 2110 (C≡C), 1200 cm^{-1} (N→O). Anal. ($C_{23}H_{22}NO_3Cl$) C, H, Cl.

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Mercapto Heterocyclic Carboxylic Acids, Analogues of 3-Mercaptopicolinic Acid[1]

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3-Mercaptopicolinic acid (3-MPA), a potent hypoglycemic agent in fasted rats, provided the impetus for substituting this compound with a 5-mercapto group (**1**), a 6-carboxyl group (**2**), and a 5-mercapto and 6-carboxyl group (**3**) and for replacing the pyridine ring with other heterocycles: quinoline (**4**), thiazole (**5**), pyrazine (**6**), isoquinoline (**7**), and indole (**8**). The methyl sulfoxide (**9**) and sulfone (**10**) of 3-MPA were also prepared. The new compounds 1–10, with the exception of 8, did not lower blood glucose levels in 48-h fasted rats. 8 was toxic at doses which were hypoglycemic.

3-Mercaptopicolinic acid (3-MPA), by inhibiting gluconeogenesis, produces hypoglycemia in a number of animal models.[2] 3,5-Dimercaptopicolinic acid (**1**), 3-mercapto-2,6-pyridinedicarboxylic acid (**2**), and 3,5-dimercapto-2,6-pyridinedicarboxylic acid (**3**) were prepared and evaluated biologically to determine how the hypoglycemic activity of 3-MPA would be affected by additional mercapto or carboxyl groups. The importance of the pyridine ring of 3-MPA was determined by replacing it with other heterocyclic rings: quinoline (**4**), thiazole (**5**), pyrazine (**6**), isoquinoline (**7**), and indole (**8**).

3-Methylsulfinylpicolinic acid (**9**) and 3-methylsulfonylpicolinic acid (**10**) were prepared to determine what effect altering the oxidation state of sulfur would have on hypoglycemic activity.

1, **2**, and **4** were prepared from bromo heterocyclic *N*-oxides (**11**) (Scheme I). Subjecting the intermediates **11** to the conditions of the Reissert–Kaufmann reaction[3] produced reasonable yields of products **12**. Treatment of **12** with the mercaptide ion generated from *p*-methoxybenzyl mercaptan (MBM)[4] gave the intermediates **13**. Subsequent alkaline hydrolysis and removal of the methoxybenzyl (MB) protecting group[2a] yielded respectively **14** and the desired mercapto acids **1**, **2**, and **4**. However, **3** could not be prepared by this route since neither 3,5-dibromo-2-cyanopyridine *N*-oxide (**11d**) nor methyl 3,5-dibromo-2-pyridinecarboxylate *N*-oxide (**11e**) (Table III) gave the Reissert–Kaufmann intermediate. The intermediate 1-methoxy compounds prepared by treatment of **11d** and **11e** with dimethyl sulfate reverted to **11d** and **11e** when allowed to react with aqueous cyanide.

Compound **3** was prepared from 3,5-dibromo-2,6-lutidine[5] which was oxidized to the diacid **15**. The diacid, in turn, was esterified to the diester **12d** and used as shown in Scheme I (**12d** → **13d** → **14d** → **3**).

An alternative synthesis involving attempted tetrazotization and sulfuration of 3,5-diamino-2,6-pyridinedicarboxylic acid (**17b**) or the corresponding diamino diester **17c** gave chiefly unchanged starting materials. These results were attributed to the poor solubilities of **17b** and **17c** in aqueous acid. Compounds **17b** and **17c** were prepared from diethyl 2,6-dimethyl-3,5-pyridinedicarboxylate. This ester was converted in turn to the 3,5-diamide **16a**,[6] to 3,5-diamino-2,6-lutidine (**16b**), and to 3,5-diacetamido-2,6-lutidine (**16c**). Oxidation of **16c** to 3,5-diacetamido-2,6-pyridinedicarboxylic acid **17a** and hydrolysis gave **17b** which was esterified to give **17c**. These intermediates are listed in Table I.

The thiazole analogue of the ethyl ester of 3-MPA was derived from ethyl 2-amino-4-thiazolecarboxylate (**18**).[7] This was brominated according to the method of Garreau[8] to give ethyl 2-amino-5-bromo-4-thiazolecarboxylate (**19a**). Deamination to give ethyl 5-bromo-4-thiazolecarboxylate (**19c**), reaction with MBM to give ethyl 5-*p*-methoxybenzylthio-4-thiazolecarboxylate (**19d**), and removal of the MB group were carried out as described in the Experi-