# 4-Halovinyl- and 4-Ethynyl-4-deformylpyridoxal Derivatives and Related Analogues as Potentially Irreversible Antagonists of Vitamin B6

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Analogues of pyridoxal bearing $\alpha$- and $\beta$-chlorovinyl, $\beta$-bromovinyl, butadienyl, acetyl, and 1-butenyl groups in place of the formyl group have been synthesized by subjecting 3,$\alpha^5$-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 $\beta$-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 B6, 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 B6 antagonist (1, $R_4$ = CH=CH2) that in several systems is more effective than the "classical" antagonist, 4-DOP[3] (1, $R_4$ = CH3). Nevertheless, this
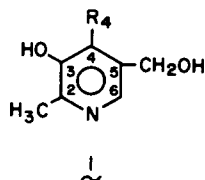


analogue could be reversed by vitamin B6. In mice, it was also found to exhibit potent convulsing activity, which precluded its further investigation for tumor chemotherapy.[3]

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 B6. In addition, we have extended the 4-hydroxymethyl group by one carbon atom and replaced the 4-formyl group with an acetyl group, giving $\alpha^4$-methylpyridoxal. In all of these syntheses, 3,$\alpha^5$-di-*O*-benzylpyridoxal (2) served as the key intermediate (Scheme I).

Application of the Wittig reaction to 2 gave the $\beta$-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 $\beta$-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 D2O 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**
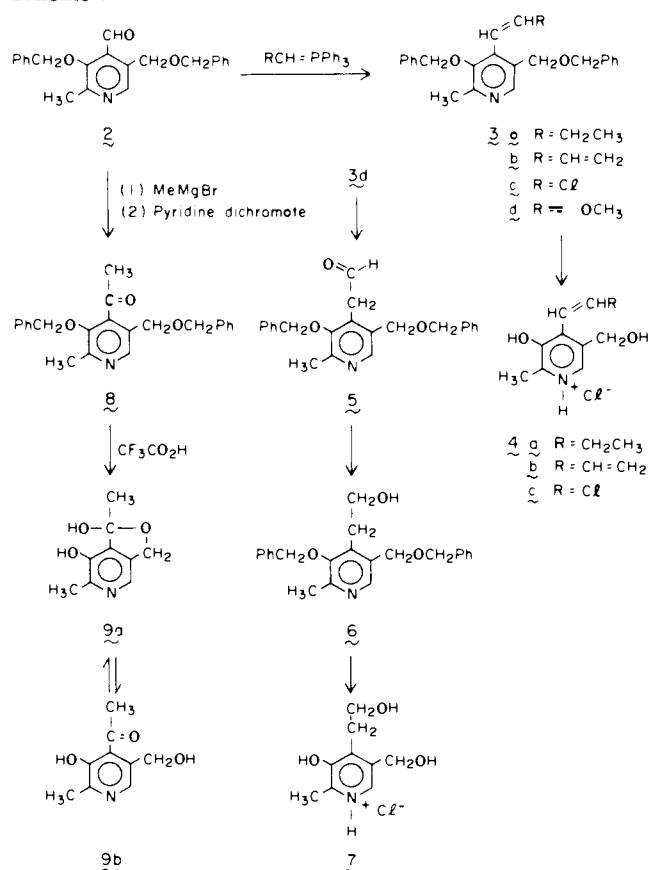


methylcarbonyl group from assuming coplanarity with the pyridine ring, as has been pointed out by Karpeisky et al.[7] for the 5'-phosphate derivative of 9b.

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 AgNO3, giving a sparingly soluble Ag complex[9] of 12, which was isolated by filtration and decomposed by treatment with NaCN.

**Scheme II**

**Scheme III**
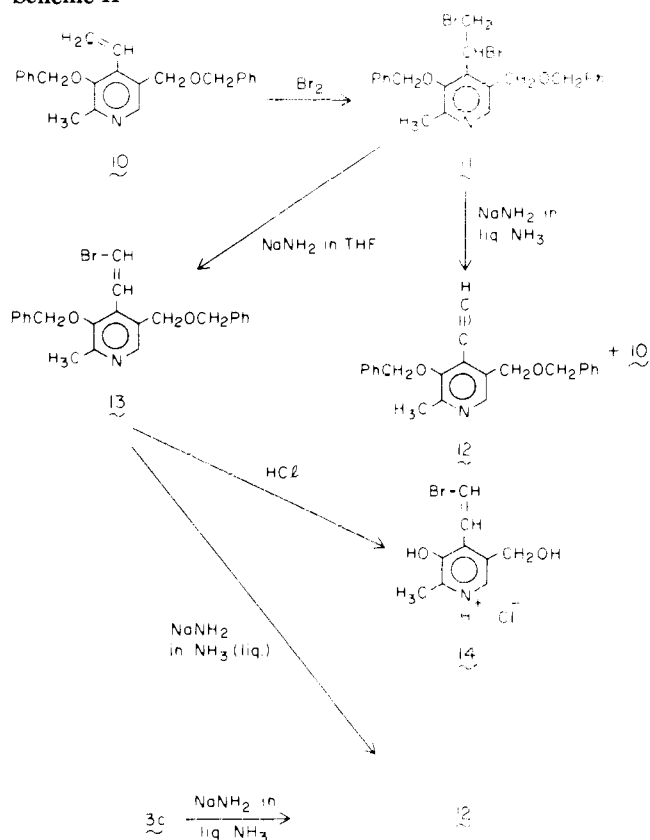
A more satisfactory synthesis of **12** was achieved in two steps. Monodehydrobromination of **11** proceeded smoothly, giving **13**, when $NaNH_2$ in THF was used and ammonia was completely excluded. Acid hydrolysis gave the completely deblocked compound **14**. The low coupling constants of the $\alpha$ and $\beta$ 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 $\beta$-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 $\beta$-chloro analogue **3c**, a procedure described earlier.[10]
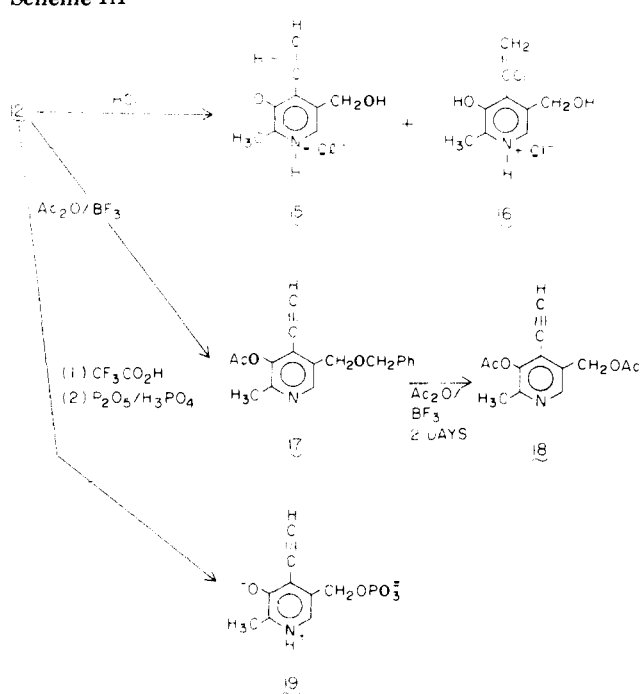
It was thought that the unusual course of the elimination reaction of the dibromide **11** could be changed by preparing the $N$-oxide of the compound and then attempting an elimination reaction. Only a poor yield of the ethynyl derivative was obtained, however, on treatment of the $N$-oxide of **11** 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 $\beta$-bromovinylstyrene. This result has been interpreted as the formation of a carbene by an $\alpha$-dehydrobromination reaction:[11]

$$ArCHBrCH_2Br + NH_2^- \rightarrow Ar\ddot{C}CH_2Br + NH_3 + Br^-$$
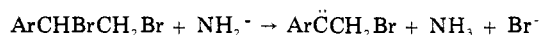
The resulting product from the carbene would depend on the reaction conditions. In our case, the carbene derived from the dibromo compound **11** in the presence of ammonia adds on a hydride derived from ammonia, producing the vinyl derivative **10**. In the absence of ammonia, a shift of hydride from the $\beta$-carbon produces the $\beta$-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 $\alpha$-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 $\delta$ 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 ($\delta$ 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 ($1 \times 10^{-7}$ 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 | R₄ | R₅ | ID₅₀ (TA3 cells), M |
|---|---|---|---|
| 4-DOP | -CH₃ | H | $6.8 \times 10^{-8}$ |
| 4-VPAL | -CH=CH₂ | H | $2.5 \times 10^{-9}$ |
| 3a | -CH=CHEt | H | $1.5 \times 10^{-5}$ |
| 3b | -CH=CHCH=CH₂ | H | $1.5 \times 10^{-5}$ |
| 3c | -CH=CHCl | H | $3 \times 10^{-6}$ |
| 16 | -CCl=CH₂ | H | $9.5 \times 10^{-6}$ |
| 14 | -CH=CHBr | H | $1.2 \times 10^{-5}$ |
| 9 | -C=O(CH₃) | H | No inhibn at $10^{-4}$ |
| 15 | -C≡CH | H | $8 \times 10^{-6}$ |
| 19 | -C≡CH | PO₃H₂ | $3.8 \times 10^{-6}$ |

[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 ID₅₀ was $9 \times 10^{-5}$ 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 B₆ 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 $\mu$M, 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 $\mu$M, with noncompetitive kinetics. This value is somewhat lower than the $K_m$ value of 283 $\mu$M for pyridoxal but is substantially higher than the $K_I$ value found for its 4-vinyl analogue ($K_I$ = 22.5 $\mu$M), 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 CDCl₃, Me₂SO-$d_6$, or D₂O; positions of peaks are expressed in cps units from Me₄Si, or from dioxane ($\delta$ 3.70), as an internal standard. Peaks are assigned on the basis of previous work.[19]

3,α⁵-Di-O-benzyl-4-(1-butenyl)-4-deformylpyridoxal [4-(1-Butenyl)-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine] (3a). The procedure described by us
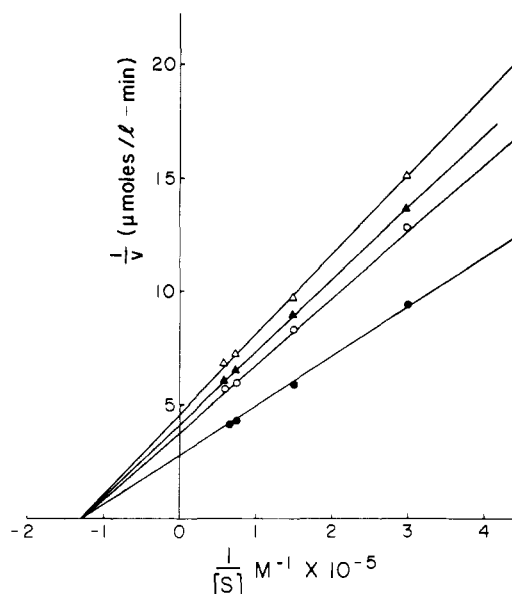


**Figure 1.** Lineweaver–Burk plots for inhibition by the 4-ethynyl 5′-phosphate analogue 19 of the oxidation of pyridoxol 5′-phosphate catalyzed by pyridoxine-P oxidase: no inhibitor (●), 0.1 (○), 0.33 (▲), and 0.66 $\mu$M (△) of 19.

earlier[3,16] for the Wittig reaction was applied to 3,α⁵-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 (CDCl₃, isomers) 4-(C*H*₃CH₂CH=CH) 45, 51, 52, 60, 61, 68 (from both isomers); 4-(CH₃C*H*₂CH=CH) 100–143 (multiplet); 4-CH=CH 342–400 (complex), 2-CH₃ 151, 3 × CH₂ 269, 270, 272, 273, 286, 2 × C₆H₅ 444, 6-H 503, 507; IR $\nu_{max}^{neat}$ 1585 cm⁻¹ (C=C); UV $\lambda_{max}$ (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 (CDCl₃) 4-(C*H*₃CH₂CH=CH) 52–70 (multiplet); 4-(CH₃C*H*₂CH=CH) 105–120 (multiplet); 2-CH₃ 162, 3 × CH₂ 278, 280, 292, 2 × C₆H₅ 447, 6-H 518, 509; IR $\nu_{max}^{KBr}$ 1610 cm⁻¹ (C=C); UV $\lambda_{max}$ (95% alcohol) 255 nm. Anal. (C₂₅H₂₈NO₂Cl) 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 (D₂O, cis and trans isomers) 4-(C*H*₃CH=CH) 42, 73; 4-(CH₃C*H*₂CH=CH), 90, 122; 2-CH₃ 153, 5-CH₂ 281, 280; 4-(CH₃CH₂C*H*=CH) 365–390; 4-(CH₃CH₂CH=C*H*) 445–450, 6-H 489, 490; IR $\nu_{max}^{KBr}$ 1655 cm⁻¹ (C=C); MS, the molecular peak agreed with the molecular weight of the compound. Anal. (C₁₁H₁₆NO₂Cl) C, H.

3,α⁵-Di-O-benzyl-4-(1,3-butadienyl)-4-deformylpyridoxal [4-(1,3-Butadienyl)-2-methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)pyridine] (3b). 3,α⁵-Di-O-benzylpyridoxal (500 mg, 1.44 mmol) was allowed to react with triphenyl-2-propenylphosphonium bromide, (Ph₃P⁺CH₂CH=CH₂)Br⁻ (900 mg, 2.66 mmol), giving the 4-butadienyl compound 3b as an oil: yield 320 mg (55%); NMR (CDCl₃) 2-CH₃ 151, 3 × CH₂ 270, 274, 289, –CH=CHCH=CH₂ 300–420 (highly complex), 2 × C₆H₅ 445, 448, 6-H, 509; IR $\nu_{max}^{neat}$ 1590 cm⁻¹ (C=C); UV $\lambda_{max}$ (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 (CDCl₃) 2-CH₃ 155, 3 × CH₂ 275, 281, 297, –CH=CHCH=CH₂ 320–400 (highly complex), 2 × C₆H₅ 455, 6-H 515; IR $\nu_{max}^{KBr}$ 1610 cm⁻¹ (C=C). Anal. (C₂₅H₂₆NO₂Cl) C, H.

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

blocked compound **3b** (290 mg, 0.78 mmol) was taken up in CF$_3$COOH (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$_2$O) 2-CH$_3$ 158, 5-CH$_2$ 278, –CH=CHCH=CH$_2$ 310–420 (highly complex), 6-H 494; IR $\nu_{max}^{KBr}$ 1630 cm$^{-1}$ (C=C); UV $\lambda_{max}$ (0.1 N HCl) 296 nm ($\epsilon$ 9.55 × 10$^3$); UV $\lambda_{max}$ (0.1 N NaOH) 315 nm ($\epsilon$ 6.48 × 10$^3$); UV $\lambda_{max}$ (pH 7) 245 nm ($\epsilon$ 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$_2$Cl) C, H, Cl.

**4-(2-Chlorovinyl)-4-deformylpyridoxal [4-(2-Chloroethenyl)-3-hydroxy-2-methyl-5-pyridinemethanol] (4c).** 3,$\alpha^5$-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$_2$O) 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 $\nu_{max}^{KBr}$ 3340 (–OH), 1580 (C=C), 965 cm$^{-1}$ (C=C trans); UV $\lambda_{max}$ (0.1 N HCl) 291 nm; UV $\lambda_{max}$ (0.1 N NaOH) 346 nm. Anal. (C$_9$H$_{11}$NO$_2$Cl$_2$) C, H, Cl.

**3,$\alpha^5$-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,$\alpha^5$-Di-*O*-benzyl-$\alpha^4$-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$_2$Ph 271, 4-CH$_2$ 238 (s, br), 3-O-CH$_2$Ph 296, phenyls 439 and 442, C$_6$-H 507.

**3,$\alpha^5$-Di-*O*-benzyl-$\alpha^4$-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$_2$Ph and 5-CH$_2$OCH$_2$Ph 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$_3$Cl) C, H, N, Cl.

**$\alpha^4$-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$_2$SO-$d_6$) 2-CH$_3$ 159, 4-CH$_2$CH$_2$OH 222 (t, $J$ = 6 Hz), 180 (t, $J$ = 6 Hz), 5-CH$_2$OH 283, C$_6$-H 489. Anal. (C$_9$H$_{24}$NO$_3$Cl) C, H, N, Cl.

**3,$\alpha^5$-Di-*O*-benzyl-$\alpha^4$-methylpyridoxal [1-[2-Methyl-3-(phenylmethoxy)-5-(phenylmethoxymethyl)-4-pyridyl]-1-ethanone] (8).** To a solution of 3,$\alpha^5$-di-*O*-benzyl-$\alpha^4$-methylpyridoxol[2] (0.50 g) in CH$_2$Cl$_2$ (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 $\nu_{max}^{KBr}$ 1715 cm$^{-1}$; NMR (CDCl$_3$) 2-CH$_3$ 146, 4-COCH$_3$ 166, 3 × CH$_2$ 273, 296, 2 × C$_6$H$_5$ 438, C$_6$-H 472. Anal. (C$_{23}$H$_{23}$NO$_3$) C, H.

**$\alpha^4$-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$_2$O) CH$_3$ (hemiketal) 105, CH$_3$ (ketone and 2-CH$_3$) 247, 249, CH$_2$O 295, 308, C$_6$H 471, 477; UV $\lambda_{max}$ (0.1 N HCl) 288 nm; UV $\lambda_{max}$ (0.1 N NaOH) 248, 302, 335 nm; UV $\lambda_{max}$ (pH 7) 256, 320 nm. Anal. (C$_9$H$_{32}$NO$_3$·Cl·H$_2$O) C, H, N.

**3,$\alpha^5$-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$_2$Br 227–357 (highly complex and analogous to the NMR spectrum of styrene dibromide); 3 × CH$_2$ 273, 280, 2 × C$_6$H$_5$ 447, 450 (splitting), 6-H 499; UV $\lambda_{max}$ (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$_2$Br 229–358 (highly complex), 3 × CH$_2$ 275, 280, 282, 2 × C$_6$H$_5$ 448, 6-H 501; UV $\lambda_{max}$ (95% alcohol) 285 nm. Anal. (C$_{23}$H$_{24}$NO$_2$Br$_2$Cl) C, H, halogens.

**3,$\alpha^5$-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$_2$CO. 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$_2$O (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$_4$OH (20 mL), followed by H$_2$O. Excess NaNH$_2$ and NH$_4$OH were decomposed by careful addition of dilute HCl while cooling with a mixture of dry ice and EtOH. Extraction with Et$_2$O (three times), washing the extract with H$_2$O, 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$_6$H$_5$ 446, 6-H 497; IR $\nu_{max}^{neat}$ 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$_6$H$_5$ 448, –CH=CH– (obscured by the benzyl peaks), 6-H 515; IR $\nu_{max}^{KBr}$ 1590 cm$^{-1}$ (C=C); UV $\lambda_{max}$ (95% alcohol) 260 nm. Anal. (C$_{23}$H$_{23}$NO$_2$BrCl) 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$_2$O) 2-CH$_3$ 156, 5-CH$_2$ 283, CH=CHBr 422, 438, 439, 448, 6-H 492; IR $\nu_{max}^{KBr}$ 1525 cm$^{-1}$ (C=C); UV $\lambda_{max}$ (95% alcohol) 297 nm. Anal. (C$_9$H$_{11}$NO$_2$BrCl) 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$_2$SO-$d_6$; Na salt of 3-(trimethylsilyl)-1-propanesulfonic acid as internal standard] 2-CH$_3$ 142, 3 × CH$_2$ 265, 278, 303, 2 × C$_6$H$_5$ 423, 434, 6-H 492. Anal. (C$_{23}$H$_{20}$N$_2$O$_5$Ag$_2$) 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$_2$O–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$_3$C=O 124, 129, 2-CH$_3$ 148, 5-CH$_2$ 397, CH$_2$=C 396, 6-H 515; IR $\nu_{max}^{neat}$ 1785 1725 (C=O), 1620 (C=C), 3070 cm$^{-1}$ (CH stretching of CH$_2$=C); UV $\lambda_{max}$ (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$_2$O) 2-CH$_3$ 156, 5-CH$_2$ 280, CH$_2$=C- 414, 6-H 495; IR $\nu_{max}^{KBr}$ 3380 (OH), 1610 cm$^{-1}$ (C=C); UV $\lambda_{max}$ (0.1 N HCl) 293 nm ($\epsilon$ 1.27 × 10$^4$); UV $\lambda_{max}$ (0.1 N NaOH) 318 nm ($\epsilon$ 5.3 × 10$^3$). Anal. (C$_9$H$_{11}$NO$_2$Cl$_2$) 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$_2$O (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$_3$C(=O)– 124, 2-CH$_3$ 149, C=CH 228, CH$_2$OCH$_2$ 308, 210, C$_6$H$_5$ 44, 6-H 505; IR $\nu_{max}^{neat}$ 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$_3$C(=O)– 125, 127, 2-CH$_3$ 149, 5-CH$_2$ 301, C=CH 230, 6-H 510; IR $\nu_{max}^{neat}$ 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$_2$Cl$_2$ (1 mL), (C$_6$H$_5$)$_3$C$^+$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$_6$H$_5$ 445, 6-H 508; IR $\nu_{max}^{KBr}$ 3400 (OH), 2110 (C=C), 1210 cm$^{-1}$ (COC); UV $\lambda_{max}$ (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$_2$Br 320–200 (highly complex), 2 × C$_6$H$_5$ 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$_2$Br 202–324 (complex), 2 × C$_6$H$_5$ 448, 450, 6-H 506. Anal. (C$_{23}$H$_{24}$NO$_3$BrCl) 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$_2$SO$_4$, 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$_6$H$_5$ 445, 6-H 502; IR $\nu_{max}^{neat}$ 2100 (C=C), 1205 cm$^{-1}$ (N→O); UV $\lambda_{max}$ (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$_6$H$_5$ 448, 449, 6-H 508; IR $\nu_{max}^{KBr}$ 2110 (C=C), 1200 cm$^{-1}$ (N→O). Anal. (C$_{23}$H$_{22}$NO$_3$Cl) 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 inter-

mediate 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-