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Antagonists of Vitamin B_6. Simultaneous and Stepwise Modification of the 2 and 4 Positions[1]

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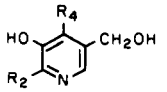
Methods for the simultaneous and stepwise modification of the 2 and 4 positions of vitamin B_6 have been developed and have been applied to the synthesis of several analogues of this vitamin. 3,α^5-*O*-Dibenzylpyridoxol was converted to its *N*-oxide and was rearranged to an α^2-hydroxy derivative with $(CF_3CO)_2O$. The 2,4-bis(hydroxymethyl) intermediate was oxidized (MnO_2) to the 2,4-dialdehyde, which was converted by a Wittig reaction with triphenylmethylphosphorane to the 2,4-divinyl derivative. Removal of the benzyl groups with acid gave the 2,4-divinylpyridoxol analogue, which was phosphorylated in the 5′ position to give the cofactor analogue. The 2-CH_3 in the known vitamin B_6 antagonists, 4-deoxypyridoxol and 4-vinylpyridoxal, was similarly modified to CH_2OH, CHO, and $CH{=}CH_2$. Modifications of the 2 position in the vitamin B_6 antagonists are expected to be associated with changes in selectivity for enzymes in various tissues without a concomitant loss of biological activity, because of the well-established bulk tolerance in this position. Active analogues are expected to undergo, in vivo, 5′-phosphorylation, which is probably a prerequisite for their antagonist activity. Some of the compounds (e.g., the 2,4-divinyl analogue) have substantial growth-inhibitory activity for cultured mouse mammary adenocarcinoma. In contrast to that of the parent compounds, this activity was only partially reversed by pyridoxal.

Potent analogues of vitamin B_6[2] have been obtained by modifying its 4 position.[3] Notable examples of such analogues are 4-deoxypyridoxine (1, $R_2 = R_4 = CH_3$; 4-DOP) and 4-vinyl-4-deformylpyridoxal (1, $R_4 = CH{=}CH_2$; $R_2 = CH_3$; 4-vinylpyridoxal, 4-VPAL).[5] These analogues have been of interest as potential anticancer agents and as reactants for investigating the active sites of various enzymes.[4] More recently we have described analogues modified in the 2 position, such as 2-vinylpyridoxine (1, $R_2 = CH{=}CH_2$; $R_4 = CH_2OH$), which was shown to be a potent inhibitor of cultured mouse mammary adenocarcinoma cells; this effect was not reversed by pyridoxal.[9] Other modifications in the 2 position could be considered, particularly since a certain bulk tolerance in this position has been observed, with respect to enzymes requiring pyridoxal phosphate.[3,6] Antagonists that have both the 2 and 4 positions modified with reactive groups could potentially cross-react within the active site. Since the 5-hydroxymethyl is unchanged, they have the structural feature that is needed in order to be substrates of pyridoxal



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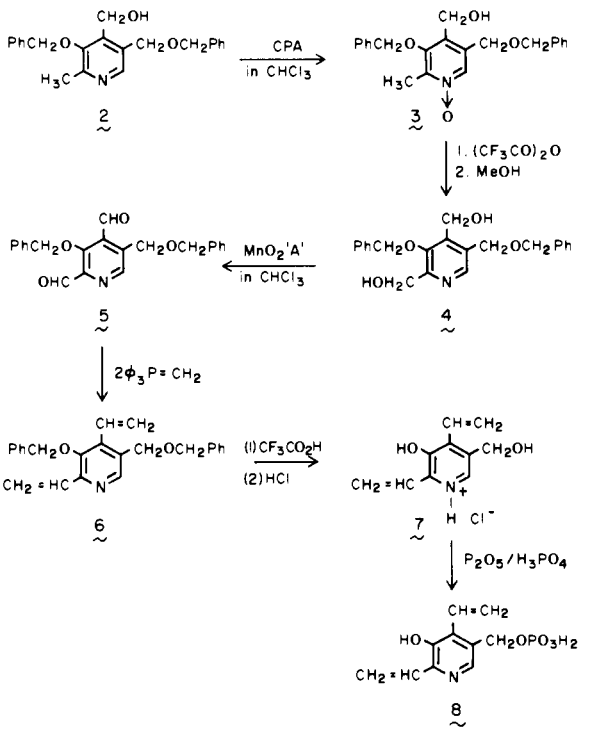
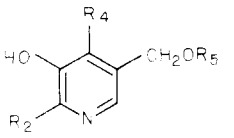
phosphokinase. This is a very important consideration for in vivo activity, since only the phosphorylated analogues are effective inhibitors of pyridoxine-P oxidase[7] and can therefore compete for the cofactor site of various enzymes dependent on pyridoxal-P.[6] As a consequence, it appeared attractive to introduce a vinyl group into the 2 position

Scheme I



of 4-VPAL and 4-DOP. Initially, we decided to obtain an analogue in which the 4-formyl and 2-methyl groups of pyridoxal were replaced with vinyl groups, as in **7** (Scheme I) and its 5′-phosphate **8**. The starting intermediate in

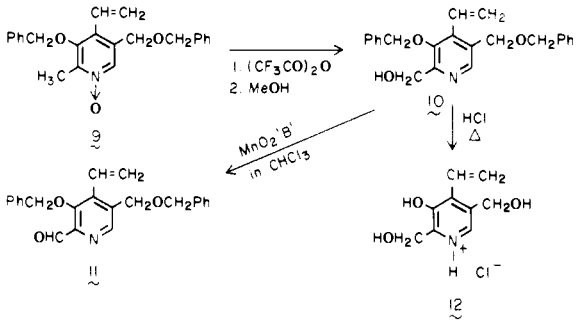
Table I. Inhibitory Activity of 4-Vinyl Analogues Modified in the 2 Position



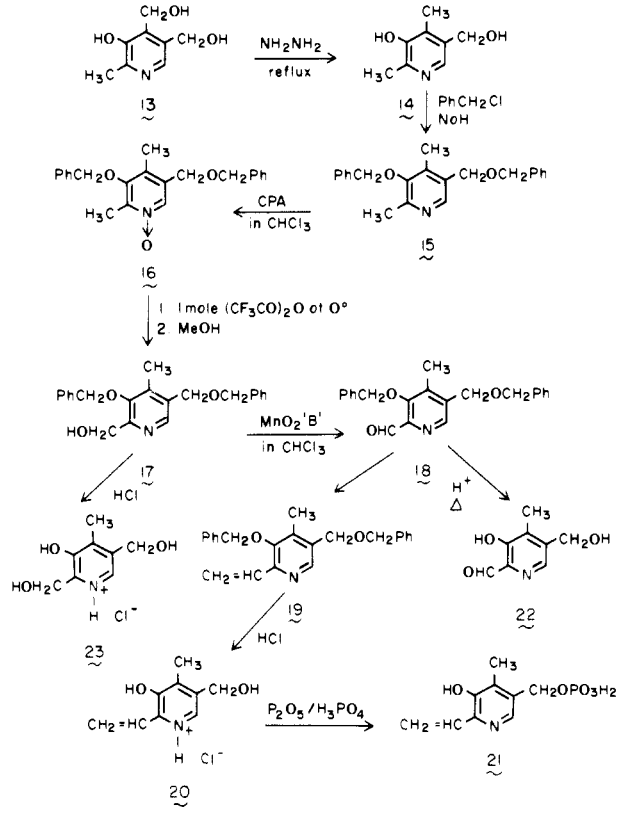
Compd	R_2	R_4	R_5	ID_{50}, M (TA3 cells)[a]	Reversal with PAL (ID_{50})[b]
4-VPAL	CH_3	$CH=CH_2$	H	2.5×10^{-9}	Complete
2-VPAL	$CH=CH_2$	CH_2OH	H	9.4×10^{-6}	None
7	$CH=CH_2$	$CH=CH_2$	H	6.8×10^{-6}	Partial (3.2×10^{-5} M)
8	$CH=CH_2$	$CH=CH_2$	PO_3H_2	No inhibn at 10^{-4}	
12	CH_2OH	$CH=CH_2$	H	No inhibn at 10^{-4}	

[a] Pyridoxal at 10^{-7} M to sustain minimal growth. [b] Pyridoxal at 10^{-5} M.

Scheme II



Scheme III

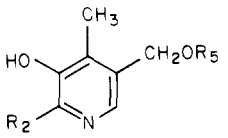


these syntheses was 3,5-di-*O*-benzylpyridoxine (**2**),[3,8] which was converted to the 2-hydroxymethyl derivative **4** in excellent yield by taking advantage of the ease with which its *N*-oxide **3** rearranges with trifluoroacetic anhydride. This sequence of reactions has been applied previously and represents a general and convenient method for modifying the 2-methyl group in vitamin B_6.[9,10] Oxidation of **4** with MnO_2 gave a poor yield of the dialdehyde **5**, and hence a better method of synthesis for this key intermediate was devised, as will be discussed in this paper. A Wittig reaction gave the divinyl intermediate **6**, which was deblocked with HCl to **7** and subsequently phosphorylated with polyphosphoric acid to **8**. A stepwise procedure for synthesis of the key intermediate **5** and other analogues starts with the known 3,5-dibenzyl-4-vinyl analogue,[5] which was N-oxidized to **9** with *m*-chloroperbenzoic acid (Scheme II) and converted to the 2-hydroxymethyl derivative **10**. Oxidation of the latter now gave a satisfactory yield of the 2-aldehyde derivative **11**, which was then converted to the intermediate **6**. Besides offering an improved yield, the stepwise procedure has made available other 2-modified vitamin B_6 analogues, such as the 2-hydroxymethyl analogue **12**.

4-DOP (**14**) served as the starting material for another series of 2,4-modified analogues. It is readily obtainable from pyridoxol **13** by the hydrazine reduction procedure[3,11] (Scheme III). Benzylation of **14** with benzyl chloride in the presence of NaH gave the dibenzyl derivative **15**. The remaining steps of the synthesis were performed in a manner similar to that in Scheme II. Rearrangement of the *N*-oxide **15** at room temperature, however, gave not only the desired **17** but also its isomer **2**. When the reaction was carried out at 0 °C and quenched with methanol just before completion, the desired product **17** could be obtained in good yield. The latter was oxidized to the 2-aldehyde **18**, which was subjected to the Wittig reaction to yield **19**, and the benzyl groups were hydrolyzed to give the 2-vinyl analogue **20**. Phosphorylation (polyphosphoric acid) gave the cofactor analogue **21**. The blocking groups were hydrolyzed at different stages of the synthesis, thus yielding various analogues of 4-DOP in which the 2 position was modified. Accordingly, hydrolysis of **18** gave the 2-aldehyde **22**, which was also converted to the 2-thiosemicarbazone analogue, because of the well-known anticancer activity of thiosemicarbazones of 2-aldehydopyridines, an activity related to their inhibition of nucleotide diphosphate reductase.[13] The 2-hydroxymethyl derivative **23** has also been obtained. The synthesis of this analogue by a somewhat different procedure has been reported.[10]

Biological Activity. Compounds synthesized in this study were tested as inhibitors of the growth of mouse mammary adrenocarcinoma (TA3) cells grown in cell culture in Eagle's medium with minimal amount of pyridoxal (1×10^{-7} M) to sustain growth. Inhibition data are summarized in Table I for the 4-vinyl series of analogues, and in Table II for the 4-deoxy series. Compounds were also tested in the complete medium containing approximately 10^{-5} M pyridoxal. Under these conditions, the activity of 4-VPAL and 4-DOP was completely reversed. Although the 2-vinyl analogue was less inhibitory than either 4-VPAL or 4-DOP, its growth-inhibitory activity was not reversed. On the other hand, the intro-

Table II. Inhibitory Activity of 4-DOP Analogues Modified in the 2 Position



Compd	R_2	R_5	ID_{50}, M (TA3 cells)[a]	Reversal with PAL (ID_{50}, M)[b]
4-DOP (14)	CH_3	H	6.8×10^{-8}	Complete
20	$CH{=}CH_2$	H	3×10^{-6}	Partial (3.2×10^{-5})
21	$CH{=}CH_2$	PO_3H_2	3.8×10^{-5}	Partial (1.8×10^{-4})
22	CHO	H	6×10^{-5}	Partial (1.6×10^{-4})
Thiosemicarbazone of **22**	$CH{=}NNHC(=S)NH_2$	H	2.7×10^{-6}	None
Oxime of **22**	CH=NOH	H	40% inhibn at 10^{-4}	
23	CH_2OH	H	No inhibn at 10^{-4}	

[a] Pyridoxal at 10^{-7} M to sustain minimal growth. [b] Pyridoxal at 10^{-5} M.

duction of a second vinyl group into the 2 position, as in **7**, somewhat improved the inhibitory activity, although that activity was partially reversed by pyridoxal. The phosphate of this compound (**8**) had no activity at 10^{-4} M, probably because of decreased permeability. Replacement of the 2-methyl group with a 2-hydroxymethyl as in **12**, representing a relatively minor change, abolished the inhibitory activity of the parent compound.

In the 4-deoxypyridoxol series (Table II), modifications in the 2 position decreased the inhibitory activity to a variable extent. Introduction of the vinyl group into the 2 position resulted in inhibitors **20** and **21**, the activity of which was only partially reversed by the vitamin. The 2-vinyl analogue **20** was found to be a substrate of pyridoxal phosphokinase. This was shown qualitatively by using ATP-γ-^{32}P according to a method described earlier. The enzyme was also inhibited by this analogue, K_I being 68.1 μM. The kinetics of inhibition were noncompetitive.[17]

As in the 4-vinyl series, replacement of the methyl group with a hydroxymethyl function as in **23**, a seemingly minor modification, abolished the inhibitory activity. The same thing is true for the introduction of the formyl group, as in **22**. It has been previously reported that an extension of the 5-hydroxymethyl group in 4-DOP also resulted in a loss of inhibitory activity.[12] The thiosemicarbazone derivative of **22** had an inhibitory activity comparable to that of the 2-vinyl analogue, but the inhibition was not reversed by pyridoxal to any extent and may likely owe its inhibitory activity to its being a nucleotide diphosphate reductase inhibitor.[13]

Thus the introduction of the vinyl group into the 2 position of the well-established vitamin B_6 antagonists 4-DOP and 4-VPAL decreases their inhibitory activity somewhat but makes that activity only partially reversible by the vitamin, suggesting that we are dealing with a separate class of inhibitory agents. A complete or partial lack of reversibility may be due either to the inhibition of other metabolic pathways in addition to those involving vitamin B_6 or to the irreversible nature of the inhibition of vitamin B_6 enzymes by these analogues. It is known that 2- and 4-vinylpyridines react readily with nucleophiles, such as the SH group in cysteine. As will be recalled, 4-VPAL was found to be a very potent convulsing agent in mice,[5] thus precluding its further exploration in the chemotherapy of cancer. Hence it would be of interest to investigate the organ specificity of these new vinyl analogues.

Experimental Section

Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. TLC (silica gel) was used routinely as described earlier.[14] 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, or D_2O; positions of peaks are expressed in hertz from Me_4Si, or from dioxane (δ 3.70), as an internal standard. Peaks are assigned on the basis of previous work.[15]

The mouse mammary adenocarcinoma cells (TA3) were grown in stationary tube cultures in RPMI 1640 medium containing 10% horse serum. An inoculum of 50 000 cells in 1 ml of medium was supplemented with 1 ml of medium containing the compound to be tested. The tubes were incubated in an upright position for 3 days, and growth was estimated by protein assay. The growth in controls varied from six- to tenfold. Every concentration was tested in five tubes each. For compounds found to be inhibitory, the tests were repeated at least twice. Variation between different tests was within ±10% for the 50% inhibitory concentration.

3,α^5-*O*-Dibenzylpyridoxol *N*-Oxide [2-Methyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]-4-pyridinemethanol 1-Oxide (3)]. A solution of *m*-chloroperbenzoic acid (1.35 g, 6.6 mmol, 85% purity) in $CHCl_3$ (15 ml) was added to a stirred solution of 3,α^5-*O*-dibenzylpyridoxol (**2**, 1.74 g, 5 mmol) in $CHCl_3$ (10 ml) during 10 min. The reaction mixture was left stirring at room temperature for another 30 min and was then diluted with $CHCl_3$ and shaken with 10% Na_2SO_3 (to destroy the peracid), followed by 5% $NaHCO_3$ solution. After drying (Na_2SO_4), the $CHCl_3$ solution was evaporated, and the *N*-oxide **3** was crystallized from acetone (mp 149–151 °C, 1.72 g, yield 95%): NMR ($CDCl_3$) 145 (2-CH_3), 280, 282, 297 ($4CH_2$), 448, 450 ($2C_6H_5$), 494 (C_6H); ir ν_{max}^{KBr} 1130 cm^{-1} (N → O). Anal. ($C_{22}H_{23}NO_4$) C, H, N.

3,α^5-*O*-Dibenzyl-α^2-hydroxypyridoxol [3-(Phenylmethoxy)-5-[(phenylmethoxy)methyl]-2,4-pyridinedimethanol (4)]. The *N*-oxide **3** (1.44 g, 3.9 mmol) was taken up in dry CH_2Cl_2 (10 ml), and the solution was cooled in an ice bath. Trifluoroacetic anhydride (0.6 ml, 4 mmol, 99%) was added during 5 min. The reaction mixture was stirred for 1 h at room temperature, giving 3,α^5-*O*-dibenzyl-α^2,α^4-bis(trifluoroacetyl)pyridoxol (NMR of the reaction mixture: 274–284 ($3CH_2$), 324–329 ($2CH_2$), 440–443 (2- and 4-CH_2, $2C_6H_5$), 522 (C_6H)). Now MeOH (10 ml) was added to hydrolyze the trifluoroacetyl groups. The solvent was evaporated, and the material was taken up in $CHCl_3$ and shaken with 10% $NaHCO_3$ solution, washed with water, dried ($MgSO_4$), filtered, and evaporated to a solid. The α^2-hydroxy compound **4** was crystallized from hot acetone (mp 80–82 °C, 1.40 g, yield 97%): NMR (Me_2SO-d_6) 281, 287, 292, 303 ($5CH_2$), 443, 446 ($2C_6H_5$), 510 (C_6H). Anal. ($C_{22}H_{23}NO_4$) C, H, N.

3,α^5-*O*-Dibenzyl-2-formyl-2-norpyridoxal [3-(Phenylmethoxy)-5-[(phenylmethoxy)methyl]-2,4-pyridinedicarboxaldehyde (5)]. Compound **4** (1.0 g, 2.9 mmol) was taken up in dry $CHCl_3$ (50 ml). To this solution was added MnO_2 "A" (12 g, prepared from $KMnO_4$ according to the method of Attenburrow et al.[16]), and the suspension was stirred at room temperature for 2 days. TLC showed the formation of dialdehyde (major) and monoaldehyde. (Continuing the stirring for 2 more days did not increase the proportion of the dialdehyde.) The reaction mixture was filtered with Celite filter aid and the MnO_2 residue was washed with hot $CHCl_3$ several times until the

washings gave a negative phenylhydrazine test. The $CHCl_3$ solution was evaporated to 1 ml and applied to a silica gel column. The dialdehyde (mp 72–74 °C, 550 mg, yield 55%) and monoaldehyde (mp 90–94 °C, 300 mg, yield 30%) were eluted with benzene–ether: NMR of the dialdehyde ($CDCl_3$) 283, 298, 314 ($3CH_2$), 449 ($2C_6H_5$), 501 (C_6H), 523 (2CHO); ir ν_{max}^{KBr} 1690, 1715 cm^{-1} (C=O). Anal. ($C_{22}H_{19}NO_4$) C, H, N.

3,α^5-O-Dibenzyl-2,4-divinyl-4-de(hydroxymethyl)-2-norpyridoxol [2,4-Diethenyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]pyridine (6)] and Its Hydrochloride. The dialdehyde **5** (720 mg, 2 mmol) was allowed to react with methylenetriphenylphosphorane liberated from the phosphonium salt (2 g, 5.6 mmol) as described earlier,[5] giving the divinyl compound **6** (492 mg, yield 68%); NMR ($CDCl_3$) 279, 280, 294 ($3CH_2$), 325–390 (CH_2=CH), 440–444 (highly complex, CH=CH_2), 450–453 ($2C_6H_5$), 506 (C_6H); ir ν_{max}^{neat} 1580 cm^{-1} (C=C). The free base **6** was converted into its hydrochloride by treatment with ethereal HCl (mp 152–154 °C; yield 86%). Anal. ($C_{24}H_{24}ClNO_2$) C, H, N.

2,4-Divinyl-4-de(hydroxymethyl)-2-norpyridoxol (4,6-Diethenyl-5-hydroxy-3-pyridinemethanol) Hydrochloride (7). The blocked vinyl intermediate **6** (100 mg, 0.28 mmol) was refluxed with CF_3COOH (10 ml) for 15 h. The acid was removed by evaporation under vacuum. Methanol was added, and the solution was evaporated again to dryness. The divinyl compound was purified by column chromatography (silica/$CHCl_3$–EtOAc) and the material was converted into its hydrochloride **7** by treatment with alcoholic HCl. The hydrochloride was crystallized from alcohol–ether (mp 156–158 °C dec, 37 mg, yield 64%): NMR (D_2O) 285 (5-CH_2), 340–440 ($2CH_2$, highly complex), 494 (C_6H). Anal. ($C_{10}H_{12}ClNO_2$) C, H.

2,4-Divinyl-4-deformyl-2-norpyridoxal 5′-Phosphate [4,6-Diethenyl-5-hydroxy-3-pyridinemethanol 3-(Dihydrogen phosphate) (8)]. The divinyl derivative **7** was phosphorylated with polyphosphoric acid as described earlier,[17] giving the 5′-phosphate (mp 234–242 °C, 23 mg, yield 36%); ir ν_{max}^{KBr} 3350 (OH), 1590 cm^{-1} (C=C). Anal. ($C_{16}H_{12}NO_5P\cdot H_2O$) C, H, N.

3,α^5-O-Dibenzyl-α^2-hydroxy-4-vinyl-4-de(hydroxymethyl)pyridoxol [4-Ethenyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]-2-pyridinemethanol (10)]. The blocked *N*-oxide **9** (890 mg, 2.46 mmol) was taken up in dry CH_2Cl_2 (3 ml), and the solution was cooled in an ice bath. To this was added $(CF_3CO)_2O$ (1 ml), and the mixture was left stirring for 45 min at room temperature. The reaction vessel was cooled in ice, and MeOH (5 ml) was added. The solvents were evaporated, and the residue was taken up in ethyl acetate, treated with 10% $NaHCO_3$ solution, and washed with water. It was dried over anhydrous $MgSO_4$, filtered, and evaporated to an oil, giving **10** (820 mg, yield 95%): NMR ($CDCl_3$) 274, 275, 281, 291 ($4CH_2$), 332–370 (4-CH_2=CH), 398–440 (4-CH=CH_2), 444 ($2C_6H_5$), 503 (C_6H); ir ν_{max}^{KBr} 1655 cm^{-1} (C=C). It was converted to the hydrochloride (mp 138–140 °C, crystallized from alcohol–ether). Anal. ($C_{23}H_{24}ClNO_3$) C, H.

3,α^5-O-Dibenzyl-2-formyl-4-vinyl-4-de(hydroxymethyl)-2-norpyridoxol [4-Ethenyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]-2-pyridinecarboxaldehyde (11)]. The alcohol **10** (500 mg, 1.38 mmol) was taken up in dry $CHCl_3$ (27 ml), and MnO_2 "B" was added. The reaction mixture was stirred for 14 h and then filtered with a Celite filter aid. The residue was washed with fresh warm $CHCl_3$, and the combined filtrate was evaporated to the aldehyde **11** (448 mg, yield 91%): NMR ($CDCl_3$) 278 (5-OCH_2), 299 (3-OCH_2), 333–368 (4-CH_2=CH), 394–430 (4-CH=CH_2), 443 ($2C_6H_5$), 501 (C_6H); ir ν_{max}^{KBr} 1710 (C=O), 1575 cm^{-1} (C=C). The base was converted to its hydrochloride (crystallized from alcohol–ether, mp 139–140 °C dec). Anal. ($C_{23}H_{22}ClNO_3$) C, H, Cl.

3,α^5-O-Dibenzyl-2,4-divinyl-4-de(hydroxymethyl)-2-norpyridoxol [2,4-Diethenyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]pyridine (6)]. The 4-vinyl-2-aldehyde **11** (100 mg, 0.25 mmol) was allowed to react with the ylide generated from methyltriphenylphosphonium bromide (200 mg, 0.56 mmol) as described earlier,[5] giving the divinyl derivative **6** (69 mg, yield 70%). Spectral data agreed with those of the sample prepared from the dialdehyde **5**.

α^2-Hydroxy-4-vinyl-4-deformylpyridoxal (4-Ethenyl-3-hydroxy-2,5-pyridinedimethanol) Hydrochloride (12). The blocked vinyl compound **10** (100 mg, 0.25 mmol) was refluxed with 3 N HCl (10 ml) for 20 h. Excess acid and benzyl alcohol were removed by repeated evaporation with water. The product was crystallized with some difficulty from an ethanol–acetone mixture (mp 142–144 °C dec, 28 mg, yield 51%): NMR (D_2O) 288, 293 ($2CH_2$), 337–362 (4-CH=CH_2), 398–429 (4-C*H*=CH_2), 495 (C_6H); ir ν_{max}^{KBr} 1650 cm^{-1} (C=C). Anal. ($C_9H_{12}ClNO_3$) C, H.

3,α^5-O-Dibenzyl-4-deoxypyridoxol [2,4-Dimethyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]pyridine (15)]. 4-Deoxypyridoxal, **14** (2 g, 11.8 mmol), was added to a stirred suspension of NaH [5 g of a 53% suspension in mineral oil, washed free of mineral oil with petroleum ether (bp 37–54 °C)] in DMF (30 ml, purified by distillation over CaH_2), while the reaction mixture was being stirred and heated to 65 °C and then gradually (for 45 min) cooled to 45 °C. The flask was cooled in ice, benzyl chloride (7 ml) was added dropwise, and the mixture was stirred overnight at 0 °C. After careful addition of water, the solution was extracted three times with petroleum ether. The extracts were dried (anhydrous $MgSO_4$) and were evaporated to an oil. The free base **15** was converted into its hydrochloride with ethereal HCl. The hydrochloride was crystallized from alcohol–ether (mp 152–154 °C, 3.1 g, yield 78%): NMR ($CDCl_3$) 130 (4-CH_3), 149 (2-CH_3), 267, 275, 289 ($3CH_2$), 441, 444 ($2C_6H_5$), 485 (C_6H). Anal. ($C_{22}H_{24}ClNO_3$) C, H.

3,α^5-O-Dibenzyl-4-deoxypyridoxol *N*-Oxide [2,4-Dimethyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]-pyridine 1-Oxide (16)]. A solution of *m*-chloroperbenzoic acid (722 mg, 3.55 mmol, on the basis of 85% purity of the reagent) in $CHCl_3$ (10 ml) was added to a stirred solution of 3,α^5-O-dibenzyl-4-deoxypyridoxol (free base obtained from the hydrochloride by treatment with 10% $NaHCO_3$ solution, 1 g, 3 mmol) in $CHCl_3$ (10 ml) during 15 min. The reaction mixture was left stirring for 40 min at room temperature. It was diluted with $CHCl_3$ and shaken with Na_2SO_4 (10%) and finally water. The $CHCl_3$ extract was dried (anhydrous Na_2SO_4) and was evaporated to a solid. The *N*-oxide **16** was crystallized from methanol–ether (mp 103–105 °C, 1.02 g, yield 97%): NMR ($CDCl_3$) 135 (4-CH_3), 152 (2-CH_3), 269, 271, 286 ($3CH_2$), 439, 441 ($2C_6H_5$), 483 (C_6H); ir ν_{max}^{KBr} 1125 cm^{-1} (N → O). Anal. ($C_{22}H_{23}NO_3$) C, H, N.

3,α^5-O-Dibenzyl-α^2-hydroxyl-4-deoxypyridoxol [4-Methyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]-2-pyridinemethanol (17)]. A solution of the *N*-oxide **16** (700 mg, 2 mmol) in CH_2Cl_2 (dried over $CaSO_4$, 5 ml) was cooled in an ice bath. Trifluoroacetic anhydride (99%, 0.3 ml, 2 mmol) was added dropwise during 10 min. The reaction mixture was stirred at 0 °C for 30 min. The reaction was quenched by the addition of MeOH (10 ml). TLC showed the presence of some starting material. On further keeping at 0 °C, the formation of the dibenzyl alcohol **2** was observed. The solvents were removed by evaporation under vacuum. The desired product was obtained by column chromatography (silica–EtOAc). The starting *N*-oxide (253 mg) was also recovered from the column. The α^2-hydroxy derivative **17** was crystallized from acetone three times to remove traces of the isomer **2** (mp 82–84 °C, 410 mg, yield 58%): NMR ($CDCl_3$) 134 (4-CH_3), 269, 271, 286 ($4CH_2$), 438, 440 ($2C_6H_5$), 400 (C_6H). Anal. ($C_{22}H_{23}NO_3$) C, H, N.

α^2-Hydroxy-4-deoxypyridoxol (3-Hydroxy-4-methyl-2,4-pyridinemethanol) Hydrochloride (23). The dibenzyl derivative **17** (120 mg, 0.34 mmol) was refluxed for 20 h with 4 N HCl (10 ml). Excess acid and benzyl alcohol were removed by evaporation under vacuum. The "isopyridoxol" **23** was crystallized from alcohol [mp 181–183 °C dec (lit.[10] mp 184 °C dec), 58 mg, yield 82%]: NMR (D_2O) 143 (4-CH_3), 288, 298 ($2CH_2$), 492 (C_6H). Anal. ($C_8H_{12}ClNO_3$) C, H, N.

3,α^5-Dibenzyl-2-formyl-4-deoxy-2-norpyridoxol [4-Methyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]-2-pyridinecarboxaldehyde (18)]. The alcohol **17** (200 mg, 0.57 mmol) was taken up in dry $CHCl_3$ (15 ml), and MnO_2 "B" (1.2 g) was added. The mixture was stirred at room temperature for about 20 h. The solution was filtered with a Celite filter aid, and the residue was washed several times with fresh $CHCl_3$. The combined filtrate was evaporated to a solid. The aldehyde was crystallized from hot ether (mp 62–68 °C, 187 mg, yield 94%): ir ν_{max}^{KBr} 1710 cm^{-1} (C=O). Anal. ($C_{22}H_{21}NO_3$) C, H, N.

2-Formyl-4-deoxy-2-norpyridoxol [3-Hydroxy-5-(hydroxymethyl)-4-methyl-2-pyridinecarboxaldehyde (22)]. The

blocked aldehyde **18** (70 mg, 0.2 mmol) was refluxed with trifluoroacetic acid (10 ml) for 17 h. It was evaporated to a dark oily compound. The desired product was purified by column chromatography (silica gel, eluted with ethyl acetate). The aldehyde **22** was crystallized from acetone–ether (mp 92–94 °C dec, 27 mg, yield 66%): NMR (acetone-d_6, Me_4Si as internal standard) 135 (4-CH_3), 286 (5-CH_2), 502 (C_6H), 599 (2-CHO); ir ν_{max}^{KBr} 1685 cm^{-1} (C=O); uv $\lambda_{max}^{0.1NHCl}$ 286 nm, $\lambda_{max}^{0.1NNaOH}$ 282 nm. Anal. ($C_8H_9NO_3 \cdot 0.5H_2O$) C, H, N.

Oxime of 22. To an aqueous solution of the aldehyde **22** (30 mg, 0.14 mmol) was added $NH_2OH \cdot HCl$ (15 mg, 0.2 mmol). The mixture, made basic with NaOAc, was heated for 4 h on a steam bath. The reaction mixture was then filtered and the precipitate was crystallized from alcohol (mp 200–202 °C, 25 mg, yield 77%): NMR (D_2O) 140 (4-CH_3), 288 (5-CH_2), 496, 503 (2-CH=N and C_6H). Anal. ($C_8H_{10}N_2O_3$) C, H, N.

Thiosemicarbazone of 22. The aldehyde **22** (25 mg, 0.12 mmol) was warmed with an ethanolic solution (5 ml) of thiosemicarbazide (13 mg, 0.14 mmol) for about 20 min. It was cooled and crystals separated out. The thiosemicarbazone was crystallized from hot methanol (mp 240–243 °C dec, 22 mg, yield 65%): ir ν_{max}^{KBr} 3420, 3310, 3156 cm^{-1} (NH bands). Anal. ($C_9H_{12}N_4O_2S$) C, H, N.

3,α^5-O-Dibenzyl-2-vinyl-4-deoxy-2-norpyridoxol [2-Ethenyl-4-methyl-3-(phenylmethoxy)-5-[(phenylmethoxy)methyl]pyridine (19)]. The aldehyde **18** (174 mg, 0.5 mmol) was condensed with triphenylphosphonium bromide (400 mg, 1 mmol) as described earlier,[5] giving the vinyl compound **19** (153 mg, yield 87%), which was converted to its hydrochloride. Anal. ($C_{23}H_{24}ClNO_2$) C, H.

2-Vinyl-4-deoxy-2-norpyridoxol (6-Ethenyl-5-hydroxy-4-methyl-3-pyridinemethanol) Hydrochloride (20). The blocking groups in **19** were removed in a manner analogous to the conversion of **18** to **22**. The target compound **20** was crystallized from MeOH–ethyl acetate (mp 208–210 °C dec, yield 82%): NMR (D_2O) 143 (4-CH_3), 286 (5-CH_2), 350–390 (2-CH=CH_2), 405–437 (2-CH=CH_2), 489 (C_6H). Anal. ($C_9H_{12}ClNO_2$) C, H, Cl.

2-Vinyl-4-deoxy-2-norpyridoxol 5′-Phosphate [6-Ethenyl-5-hydroxy-4-methyl-3-pyridinemethanol 3-(Dihydrogen phosphate) (21)]. The 2-vinyl compound **20** was phosphorylated with polyphosphoric acid as described earlier,[17] giving the target compound **21**, which was crystallized from water–acetone (mp 245–250 °C dec, yield 62%): ir ν_{max}^{KBr} 1610 (C=C), 1160 cm^{-1} (vs, POC). Anal. ($C_9H_{12}NO_5P \cdot 0.5H_2O$) C, H, N.

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Studies on Vitamin D (Calciferol) and Its Analogues. 10. Side-Chain Analogues of 25-Hydroxyvitamin D_3[1]

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A homologous series of side-chain analogues of 25-hydroxyvitamin D_3 (25-hydroxycholecalciferol) in which the length of the side chain is modified while maintaining its characteristic tertiary hydroxyl moiety has been synthesized. The following five analogues have been prepared and characterized: pentanor-25-OH-D_3 (**2a**), trinor-25-OH-D_3 (**2b**), dinor-25-OH-D_3 (**2c**), nor-25-OH-D_3 (**2d**), and homo-25-OH-D_3 (**2e**). Biological assays in vivo of intestinal calcium absorption and bone calcium mobilization in the chick of the five analogues revealed that the homo analogue **2e** exhibited a significant biological response relative to the –D (–vitamin D_3) control. Compared to the natural vitamin D_3, **2e** is as active in its ability to mobilize bone calcium and is about half as effective in stimulating intestinal calcium transport. The remaining analogues (**2a–d**) exhibited no significant activity in either assay, although the nor analogue **2d** was previously observed to exhibit antimetabolite activity.

By 1971 it had been shown that cholecalciferol (vitamin D_3, **1a**) must undergo two consecutive obligatory hydroxylations prior to the expression of its biological activities: first, a hydroxylation by a hepatic enzyme system to 25-hydroxyvitamin D_3 (25-OH-D_3, **1b**)[3] and finally hydroxylation of 25-OH-D_3 by a renal enzyme to 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3, **1c**].[4] 1α,25-Dihydroxyvitamin D_3 is the metabolite that is preferentially localized in the chromatin of the intestine[5] and is the most biologically active naturally occurring form of the vitamin