1 H), 3.04 (sextet, J = 4.5 Hz, 1 H), 2.75 (dd, J_1 = 10 Hz, J_2 = 13.5 Hz, 1 H), 2.17 (s, 1 H), 1.91 (comp mult, 2 H), 1.72 (comp mult, 3 H) ppm. ^{13}C NMR: δ 140.6, 140.4, 139.2, 129.2, 128.4, 128.3, 128.1, 127.6, 126.4, 126.1, 68.9, 43.0, 39.4, 29.3, 23.4 ppm. ^{13}C NMR of the cis isomer: δ 140.5, 140.2, 138.6, 129.0, 128.9, 128.5, 128.2, 127.4, 126.2, 126.0, 67.5, 42.7, 39.2, 28.3, 21.8 ppm.

2,3:6,7-Dibenzobicyclo[3.2.2]nona-2,6-diene (3).[1,2] Crystalline *trans*-4-benzyl-1-tetralol (14.05 g) was added slowly and in small portions to 150 mL of well-stirred concentrated H_2SO_4 at room temperature (ca. 25 °C). The crystals appeared to liquefy in the reaction mixture, which turned red, and tan solid material formed at the top of the stirred mixture. After all of the 4-benzyl-1-tetralol had been added (during about 30 min), the mixture was stirred an additional 10 min. Hexane (75 mL) was added; the solid dissolved in it. The mixture was transferred to a separatory funnel and the layers were separated. The acid layer was extracted with two 25-mL portions of hexane, which were then added to the other hexane layer. The hexane solution was washed with saturated sodium bicarbonate solution, dried over 4 Å molecular sieves, and stripped of solvent under reduced pressure at room temperature. The white solid residue, 12.53 g, amounted to 96% of the theoretical amount of slightly impure 2,3:6,7-dibenzobicyclo[3.2.2]-nona-2,6-diene (3), mp 60–68 °C. This product was dissolved in hexane and passed through a column of silica gel. The hexane was removed and the residual solid was recrystallized from methanol to afford 11.17 g (86%) of pure material, mp 75–77 °C.[1,2] Repeated crystallization of a small amount of this material from hexane afforded an X-ray-quality crystal; the X-ray analysis of this compound will be reported separately.[9] ^{1}H NMR: δ 7.09 (m, 8 H), 3.73 (br d, 1 H), 3.13 (br s, 3 H), 2.1 (br mult, 4 H) ppm. ^{13}C NMR: δ 143.9, 143.3, 141.2, 135.9, 131.3, 128.0, 126.6, 126.3, 125.7, 125.4, 47.4, 41.1, 37.5, 30.9, 26.3 ppm. MS [*m/e* (relative intensity)]: 220 (M^+, 100), 205 (22), 192 (84), 128 (21), 105 (23).

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Chemical Synthesis of Stereospecifically Labeled Pyridoxamine 5′-Phosphate

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The vitamin B_6 phosphate dependent enzymes are an important class of enzymes that catalyze a wide variety of biological transformations involved in the metabolism of amino acids and amines, such as transamination, decarboxylation, racemization, β- and γ-elimination, and substitution.[1] Although the catalytic roles of vitamin B_6 phosphate are amazingly versatile, its catalytic functions rely primarily on the ability of this cofactor to act as an electron sink, temporarily storing the electrons that are later used for the cleavage and/or formation of covalent bonds. While the aldehyde form of this cofactor, pyridoxal 5′-phosphate (PLP), is the most common form for vitamin B_6 dependent enzymes, the amine form of this coenzyme, pyridoxamine 5′-phosphate (PMP), has also been shown to play an important role in the reactions mediated by transaminases.[2] Since transaminases act by a ping-pong mechanism, the oscillation of the coenzyme between the aldehyde and amine forms occurs simultaneously with the substrate's alternation between an amino and a keto acid. Although both PLP and PMP are now perceived as the standard coenzymes for the metabolism of amino acids, they have also been demonstrated to be involved in glycogen phosphorylation[3] and a sugar deoxygenation process,[4] respectively. Studies of the catalysis of vitamin B_6 phosphate linked enzymes have shown a remarkable stereochemical uniformity in which the bond cleavage and/or formation, with a few exceptions, always take places at the *si* face of C-4′ in the substrate–cofactor complex.[5] Such stereochemical consistency is expected to be maintained by an enzyme that adheres to the well-established vitamin B_6 phosphate cofactor chemistry. Thus, a study directed at elucidating the stereochemical course of the reaction catalyzed by this class of enzymes may provide unique mechanistic insights that are not available from other experimental approaches.

Crucial to this stereochemical analysis is the availability of the (4′*S*)- and (4′*R*)-[4′-$^3H_1$]PMP coenzymes. While the 4′*S*-labeled pyridoxamine can be obtained by incubating pyridoxal and glutamate with apoaspartate aminotransferase in tritiated buffer and the 4′*R*-labeled epimer can be prepared from [4′-$^3H_1$]pyridoxal by an identical procedure,[6] this enzymatic method is laborious and is limited to microscale preparation. Although an asymmetric reduction of an L-threonine–pridoxal–metal complex with sodium borohydride to make chirally labeled pyridoxamine is also known,[7] this chemical method leads to products of low enantiomeric purity. In order to circumvent these problems, we have developed a reaction sequence producing the requisite stereospecifically labeled PMP with high chiral purity and satisfactory chemical yield. The detailed pathway and a full account of the experimental procedures involved are described in this paper.

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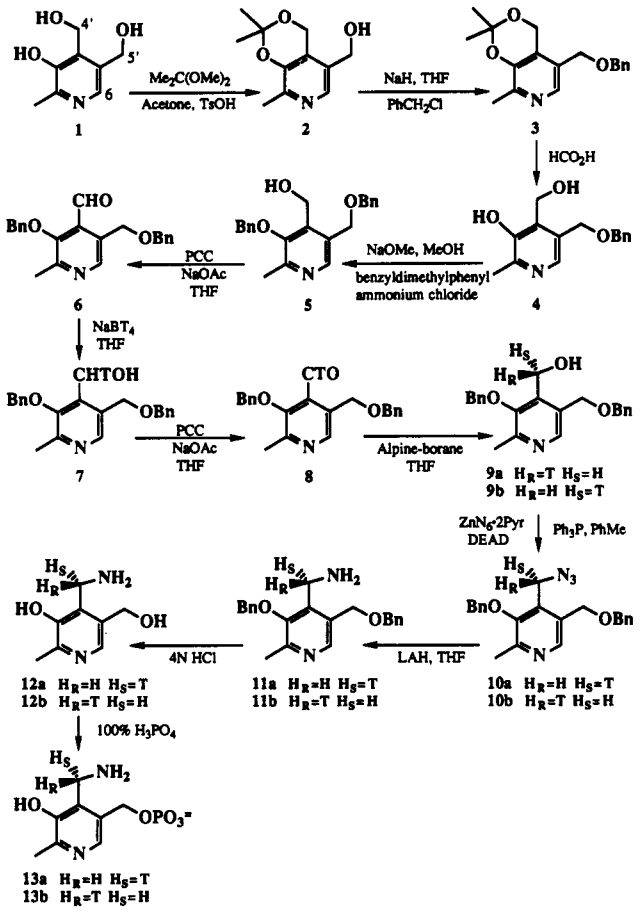
Scheme I



The first phase of our synthesis called for the construction of the key intermediate 8 which bears a tritium label at C-4′. Preparation of this compound starting with the isopropylidenylation of pyridoxine (1)[8] is summarized in Scheme I. On the basis of a procedure reported by Korytnyk and Ikawa,[9] the C-5′ hydroxyl moiety of the resulting 2 could be protected by benzylation, allowing the C-3 phenolic group to be derivatized later by reacting 3 with formic acid followed by benzyldimethylphenylammonium chloride that was prepared separately from *N,N*-dimethylaniline and benzyl chloride.[10] Upon treatment with PCC,[11] [^{3}H]sodium borohydride, and PCC in sequence, compound 5 was converted, via a tritiated pyridoxine 7 (3.12 mCi/mmol), to the desired pyridoxal derivative 8. Since a kinetic isotope effect of 5.71 had been observed for the PCC oxidation of a deuterated primary alcohol at 30 °C,[12] a tritium isotope effect of 12.3 is expected for the oxidation of 7 to 8 based on the Swain–Schaad relationship,[13] $K_H/K_T = (K_H/K_D)^{1.44}$. Such a large isotope effect projects a substantial rate discrimination on the C–H versus C–T bond cleavage and thereby predicts a full retention of tritium in the oxidized product. As anticipated, most of the radioactivity was indeed found to be retained in the isolated pyridoxal 8 (3.0 mCi/mmol).

Conversion of 8 to the stereospecifically labeled pyridoxine (**9a/9b**) was accomplished by the reduction of 8 with Alpine-Borane (Aldrich).[14] When *S*-Alpine-Borane was used in the reduction, this well-established reducing agent converted 8 to **9a** with tritium labeling at the 4′-H_R locus. Replacing the reducing agent with *R*-Alpine-Borane afforded **9b** as the product, bearing tritium at the 4′-H_S position. In order to assess the stereospecificity of this reduction, identical reactions were performed with Alpine-Borane on a deuterated sample (**17**) that was prepared from **6** as delineated in Scheme II. Acylation of the resulting products **18a** and **18b** with (*S*)-(+)-α-acetoxyphenylacetic acid gave the mandelic esters **19a** and **19b**, respectively, suitable for ^{1}H NMR analysis.[15] Since the protons of interest (4′-H_R and 4′-H_S) in **19a/19b** are now diastereotopic due to the neighboring chiral acyl substituent, they are well resolved at δ 5.33 and 5.20.[16] The diminishing of the high-field signal (δ 5.20) from isomer **19a** and the low-field signal (δ 5.33) from isomer **19b** permitted an unambiguous assignment of these resonances to the protons at 4′-H_R and 4′-H_S, respectively. On the basis of the integration of the C-4′ methylene signals, the stereospecificity of this reduction was estimated to be greater than 94.5%, translating to an 89% enantiomeric excess. Similar results were also obtained by analyzing the ^{1}H NMR spectra of the corresponding camphanic esters **20a** and **20b**.[17] Interestingly, the separation of the 4′-H_R and 4′-H_S signals of **20a** and **20b** at δ 5.38 and 5.32, respectively, is observed without the addition of a lanthanide shift reagent. This may be ascribed to the restricted rotation of the camphanic ester bond locking the C-4′ methylene group into a diastereotopic environment.[18] Thus, no shift reagent is required to achieve the desired separation of resonances.

The key step in the second stage of this synthesis is the displacement of the C-4′ oxygen moiety by an amine equivalent, converting **9a/9b** to the corresponding pyridoxamine derivatives. In order to monitor the stereochemical course and determine the stereospecificity of such a substitution, testing of the possible conditions was performed on the deuterated samples **18a** and **18b**. As depicted in Scheme II, upon treatment with mesyl chloride/triethylamine followed by sodium azide/15-crown-5,[19] **18a/18b** were successfully transformed, via the mesylate intermediates **22a/22b**, to the azide derivatives **21a/21b**. Reduction of the azide moiety by lithium aluminum hydride led to the desired pyridoxamines **24a** and **24b**. However, this route was complicated by the formation of chlorides **23a/23b** as coproducts under the mesylation conditions. Although this problem is easily remedied by running the mesylation reaction at room temperature overnight to facilitate the complete transformation of the labile mesylates (**22a/22b**) to the chlorides (**23a/23b**), the enantiomeric purity of the azide products was vitiated by the double inversion of configuration at C-4′ resulting from two consecutive S_N2 substitutions. An attempt to execute

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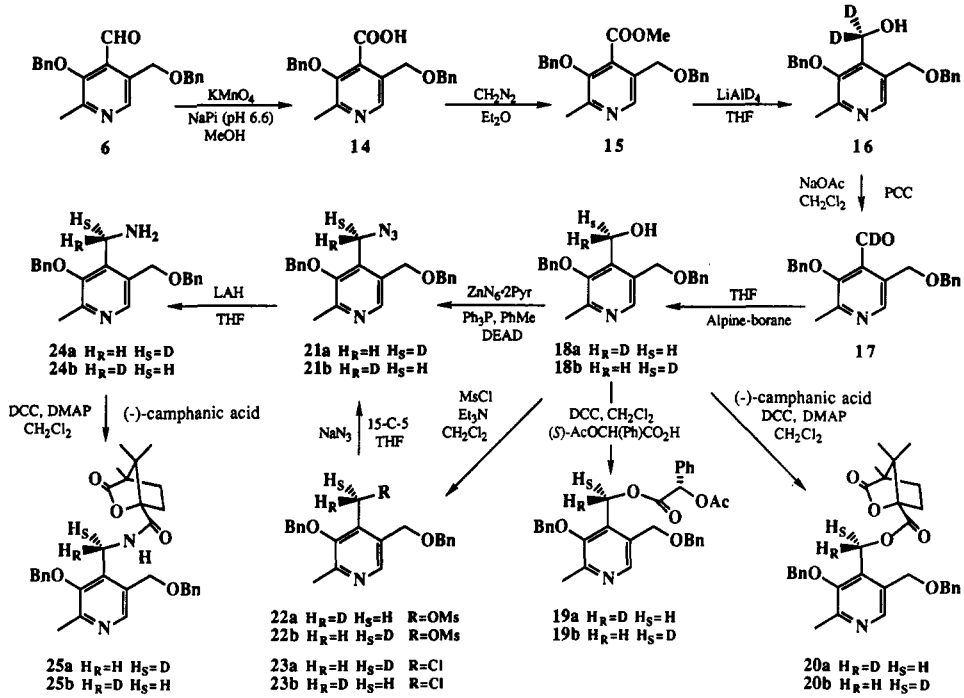
Scheme II



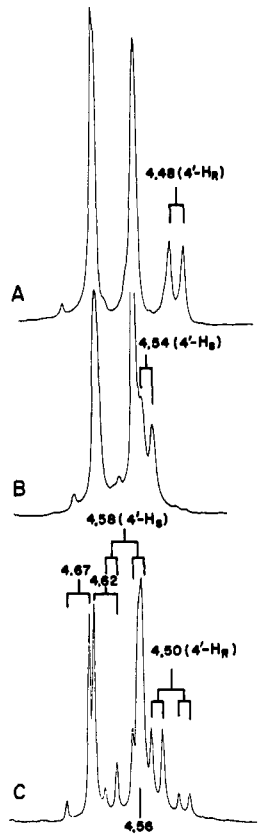


Figure 1. ^{1}H NMR spectra showing the C-4′ methylene region of 4′-*N*-camphanyl-3,5′-*O*-dibenzylpyridoxamine: (A) compound **25a**, (B) compound **25b**, (C) unlabeled standard. The AB quartet at δ 4.67 and 4.62 and the broad singlet at δ 4.56 in spectrum C are the resonances of the benzylic hydrogens of the benzyl protecting groups. The 4′-H signal of isomers **25a** and **25b**, shifted upfield by 0.02 (spectrum A) and 0.04 ppm (spectrum B), respectively, is due to the isotope substitution effects.[23] Although the 4′-H_S signal of **25b** is partially overlapped with the adjacent resonance at δ 4.56, the disappearance of the 4′-H_R signal clearly indicates the high degree of enantiomeric purity of this sample.

this conversion through a single inversion using triphenylphosphine, DEAD, and diphenyl phosphorazidate[20] was also futile. Fortunately, a zinc azide/bis(pyridine)[21] mediated Mitsunobu substitution[22] effectively converted **18a/18b** to the corresponding azide **21a/21b** in excellent yield. The desired pyridoxamine derivatives (**24a/24b**) were then obtained by hydride reduction of the azides. The enantiomeric excess of both samples, as revealed by ^{1}H NMR analysis of their camphanamide derivatives (**25a/25b**),[17] was found to be greater than 89% (Figure 1). It is evident that the Mitsunobu substitution step is fully stereospecific and proceeds with clean inversion. Following the same strategy, the tritiated pyridoxines **9a** and **9b** were subjected to the identical reaction sequence with full expectation of retention of stereochemical integrity (Scheme I). The resulting pyridoxamines **11a** and **11b**, upon acidic hydrolysis and phosphorylation,[24] were readily converted to the (4′*S*)- and (4′*R*)-[4′-^{3}H]PMP (**13a** and **13b**), respectively.

Since the overall yield of this synthesis is nearly 20% and the enantiomeric excess of the product is greater than 89%, the procedures described in this paper should be a compelling alternative to the classic enzymatic method for the preparation of stereospecifically labeled pyridoxamine coenzyme. The ready availability of chirally tritiated PMP will certainly facilitate the stereochemical analysis of vitamin B_6 phosphate dependent enzymes.[25]

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Experimental Section

Melting points are uncorrected. The NMR assignments labeled with an asterisk (*) may be interchangeable. Flash chromatography[26] was performed in columns of various diameters with J. T. Baker (230–400 mesh) silica gel by elution with the solvents reported. Analytical TLC was carried out on Merck silica gel 60 G-254 plates (25 mm), while preparative thin-layer chromatography was performed by using Analtech cellulose MN300 plates (250 mm) and developed with the solvents mentioned. TLC spots were visualized either with UV light or by dipping the plates into the staining solutions of vanillin/ethanol/sulfuric acid (1:98:1) or phosphomolybdic acid (7% ethanolic solution) and then heating them. The drying agent used in the routine workup was anhydrous magnesium sulfate. Radioactivity was measured by liquid scintillation counting, and Ecoscint A (National Diagnostics, Manville, NJ) was used as scintillation cocktail. Tritiated sodium borohydride was purchased from Amersham (Arlington Heights, IL), and Alpine-Borane was obtained from Aldrich. Solvents, unless otherwise specified, were reagent grade and distilled once prior to use. It should be noted that the tritium-containing compounds prepared in this work were not submitted to elemental analysis or exact mass measurement due to the possibility of radioactive contamination. However, satisfactory analytical results were obtained with the corresponding unlabeled or deuterium-labeled analogues.

3,4′-*O*-Isopropylidenepyridoxine (2).[8] To a suspension of pyridoxine (1) (8.2 g, 48.6 mmol) and 2,2-dimethoxypropane (100 mL) in dry acetone (150 mL) was added *p*-toluenesulfonic acid (33.4 g, 194.2 mmol) which had been dried at 130 °C under reduced pressure for 6 h prior to use. This mixture was stirred for 16 h, and the resulting dark-brown solution was then neutralized with sodium bicarbonate and concentrated in vacuo. The oily residue was mixed with water and extracted with methylene chloride. The organic extracts were combined, washed with water, dried, and concentrated. The desired product (2) was recrystallized from ethanol/ethyl ether, mp 110–111 °C (lit.[9] mp 110–111 °C). The yield was 83%: ^{1}H NMR ($CDCl_3$) δ 7.78 (1 H, s, 6-H), 4.91 (2 H, s, 5′-H's), 4.52 (2 H, s, 4′-H's), 2.34 (3 H, s, 2-Me), 1.52 (6 H, s, isopropylidene Me's); ^{13}C NMR ($CDCl_3$) δ 147.5 (C-3), 146.1 (C-2), 138.5 (C-4), 129.7 (C-5), 126.0 (C-6), 99.8 (ketalic C), 60.0 (C-5′), 58.6 (C-4′), 24.8 (isopropylidene Me's), 18.2 (2-Me).

3,4′-*O*-Isopropylidene-5′-*O*-benzylpyridoxine (3).[9] THF (40 mL) was added to NaH (3.26 g, 135.8 mmol) at 0 °C under an argon atmosphere. To this suspended mixture was added 3,4′-*O*-isopropylidenepyridoxine (2) (7.0 g, 33.5 mmol) which was dissolved in 100 mL of THF. The resulting mixture was refluxed for 30 min, and a significant amount of precipitate accumulated during the reflux. After cooling to room temperature, benzyl chloride (8.0 mL, 69.5 mmol) was introduced dropwise, and the resulting mixture was refluxed again for 4 h. The reaction was quenched carefully by adding ice-cold water to the viscous mixture at 0 °C and then mixing with a saturated ammonium chloride solution followed by extraction with methylene chloride. The organic extracts were collected, washed with brine, dried, and concentrated to give a dark brown oil. The crude product was purified by flash chromatography (10% ethyl acetate/hexane) to afford compound 3 as an oil in 92% yield: ^{1}H NMR ($CDCl_3$) δ 7.95 (1 H, s, 6-H), 7.37–7.23 (5 H, m, Ar H's), 4.86 (2 H, s, 5′-H's), 4.47 (2 H, s, 4′-H's)*, 4.41 (2 H, s, PhCH_2O)*, 2.41 (3 H, s, 2-Me), 1.54 (6 H, s, isopropylidene Me's); ^{13}C NMR ($CDCl_3$) δ 148.2, 146.1, 139.8, 137.6, 128.5, 127.9, 126.3, 126.1 (Ar C's), 99.7 (ketalic C), 72.2 (PhCH_2O), 67.4 (C-5′), 58.7 (C-4′), 24.6 (isopropylidene Me's), 18.5 (2-Me).

5′-*O*-Benzylpyridoxine (4). To a solution of 5′-*O*-benzyl-3,4′-*O*-isopropylidenepyridoxine (3) (8.0 g, 26.8 mmol) in distilled water (15 mL) was added 15 mL of 98% formic acid. The mixture was stirred at 50 °C for 24 h. The solution was then adjusted to pH 7 with saturated sodium bicarbonate solution. After neutralization, the reaction mixture was extracted several times with methylene chloride. The combined organic extracts were dried and concentrated to give a dark-brown solid. The crude product was purified by flash chromatography (60% ethyl acetate/hexane) to give compound 4 as a white solid, mp 116–117 °C (lit.[9] mp 117–118 °C). The yield was 94%: ^{1}H NMR ($CDCl_3$) δ 8.43 (1 H, br s, OH), 7.72 (1 H, s, 6-H), 7.35–7.24 (5 H, m, Ar H's), 5.02 (2 H, s, 5′-H's), 4.42 (2 H, s, 4′-H's)*, 4.35 (2 H, s, PhCH_2O)*, 2.40 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 152.1, 148.1, 139.0, 137.5, 131.2, 128.5, 128.2, 128.0, 127.9 (Ar C's), 72.2 (PhCH_2O), 67.7 (C-5′), 60.4 (C-4′), 18.3 (2-Me).

3,5′-*O*-Dibenzylpyridoxine (5). A solution of benzyldimethylphenylammonium chloride[9] (9.8 g, 39.6 mmol) in dry methanol (30 mL) was added to a solution of sodium methoxide that was freshly prepared by mixing sodium (1.14 g, 49.6 mmol) and dry methanol (30 mL) at 0 °C under an argon atmosphere. To this mixture was then added a solution of 5′-*O*-benzylpyridoxine (4) (6.6 g, 25.5 mmol) in methanol (80 mL). After being stirred at room temperature for 20 min, the above mixture was transferred to a separatory funnel and added, over a period of 30 min, to a two-neck round-bottom flask which contained 500 mL of hot toluene. During the addition, gentle heat was provided so that the volatile material (methanol) was slowly distilled via a condensor to a receiving flask. The distillation was continued until less than 200 mL of solvent was left in the reaction flask. The mixture was cooled, and the excess toluene was decanted. The oily residue was dissolved in a saturated ammonium chloride solution followed by extraction with methylene chloride. The combined organic extracts were dried and concentrated. The crude product was purified by flash chromatography (30% ethyl acetate/hexane) to afford compound 5 in 83% yield: mp 68–69 °C (lit.[9] mp 64-69 °C); ^{1}H NMR ($CDCl_3$) δ 8.22 (1 H, s, 6-H), 7.49–7.31 (10 H, m, Ar H's), 4.95 (2 H, s, 5′-H's), 4.71 (2 H, s, 4′-H's), 4.63 (2 H, s, PhCH_2O), 4.61 (2 H, s, PhCH_2O), 3.59 (1 H, br s, OH), 2.53 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 154.0, 151.9, 145.2, 141.7, 137.1, 136.7, 131.1, 128.7, 128.4, 128.2, 128.1 (Ar C's), 76.7 (PhCH_2O), 72.9 (PhCH_2O), 68.4 (C-5′), 56.1 (C-4′), 19.7 (2-Me).

3,5′-*O*-Dibenzylpyridoxal (6). To a mixture of pyridinium chlorochromate (6.2 g, 28.8 mmol), sodium acetate (4.6 g, 5.6 mmol), and powdered 3-Å molecular sieves (5 g) in dry methylene chloride (100 mL) at 0 °C under argon was added pyridoxine 5 (5.0 g, 14.3 mmol) which was dissolved in methylene chloride (10 mL). After 2 h of stirring at room temperature, an equal volume of anhydrous ether was poured into the reaction mixture, and the resulting mixture was stirred for an additional 30 min. The solution was then filtered through silica gel which was washed extensively with ether. The combined filtrates were evaporated to dryness to give the desired product as a white solid in 94% yield: mp 70–71 °C (lit.[9] mp 72 °C); ^{1}H NMR ($CDCl_3$) δ 10.40 (1 H, s, C*H*O), 8.64 (1 H, s, 6-H), 7.37–7.25 (10 H, m, Ar H's), 4.96 (2 H, s, 5′-H's), 4.82 (2 H, s, PhCH_2O), 4.63 (2 H, s, PhCH_2O), 2.60 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 191.8 (C-4′), 154.4, 154.0, 144.8, 137.8, 135.3, 132.5, 131.4, 128.9, 128.8, 128.5, 127.8, 127.7 (Ar C's), 78.0 (PhCH_2O), 73.2 (PhCH_2O), 67.4 (C-5′), 19.4 (2-Me); high-resolution FAB-MS calcd for $C_{22}H_{22}NO_3$ $(M + 1)^+$ 348.1600, found 348.1589.

[4′-$^3H_1$]-3,5′-*O*-Dibenzylpyridoxine (7). An ampule containing [^{3}H]sodium borohydride (100 mCi, specific activity >9.5 Ci/mmol) was opened in a glovebag under an argon atmosphere. The radioactive reducing agent was dissolved in THF (5 mL) and transferred to a 100-mL round-bottom flask. The vial was rinsed with more THF, and this wash was added to the reaction flask that was chilled to −20 °C. To this solution was added pyridoxal 6 (3.5 g, 10.1 mmol) dissolved in THF (50 mL) at −20 °C. The resulting mixture was allowed to react at that temperature for 20 min followed by the addition of nonradioactive $NaBH_4$ (700 mg, 18.5 mmol) which was suspended in 5 mL of THF. After stirring for an additional 2 h at room temperature, the reaction was quenched with acetone, and the solvent was evaporated in vacuo. The residue was diluted with water and extracted with methylene chloride. The combined organic extracts were dried and concentrated. Upon purification by flash chromatography (20% ethyl acetate/hexane), compound 7 was obtained in 86% yield with a specific activity of 3.12 mCi/mmol. The ^{1}H NMR spectrum of this compound is identical with that of the unlabeled pyridoxine 5.

[4′-$^3H_1$]-3,5′-*O*-Dibenzylpyridoxal (8). According to the same procedure used in the synthesis of pyridoxal 6, 2.47 g of pyridinium chlorochromate (11.5 mmol), 1.88 g of sodium acetate (22.9 mmol),

(26) Still, W. C.; Mitra, A.; Khan, M. *J. Org. Chem.* **1978**, *41*, 2923.

and 2.0 g of 3-Å molecular sieves were mixed under nitrogen in 20 mL of freshly distilled methylene chloride to oxidize 2.0 g of alcohol **7** (5.73 mmol). The reaction was complete in 1 h. The resulting dark solution was diluted with 20 mL of ether and filtered through a silica gel column. The product was purified by flash chromatography (15% ethyl acetate/hexane) to give compounds **8** as a white solid in 92% yield. The specific activity of the purified product was 3.0 mCi/mmol. The ^{1}H NMR spectrum of this compound is identical with that of the unlabeled pyridoxal **6**.

(4′*R*)-[4′-^{3}H$_1$]-3,5′-*O*-Dibenzylpyridoxine (9a) and (4′*S*)-[4′-^{3}H$_1$]-3,5′-*O*-Dibenzylpyridoxine (9b). A solution of tritiated pyridoxal **8** (700 mg, 2.0 mmol) in THF (5 mL) was added via cannula to a THF solution of *S*-Alpine-Borane (0.5 M in THF solution, 4.84 mL) at 0 °C under an argon atmosphere. This mixture was kept at 0 °C, and the reaction was monitored by TLC. It was generally finished within 15 min. Enough propanal (ca. 45 μL) to quench the excess reducing reagent was added until the release of hydrogen gas subsided. After stirring for an additional 10 min, the solvent was evaporated in vacuo. The residual oil was dissolved in 4 mL of anhydrous ether, cooled to 0 °C, mixed with ethanolamine (146 μL, 2.42 mmol), and stirred for 15 min at 0 °C. The resulting white precipitate was removed by filtration and washed with ether. The combined organic filtrates were extracted with brine, dried, concentrated, and purified by flash chromatography (20% ethyl acetate/hexane). Compound **9a** was isolated in 85% yield with a specific activity of 2.9 mCi/mmol. When *R*-Alpine-Borane was used as the reducing reagent, the same reaction procedure led to the formation of **9b** in 82% yield with a specific activity of 3.0 mCi/mmol. The ^{1}H NMR spectra of these compounds are identical with that of the pyridoxine **5**.

(4′*S*)-[4′-^{3}H$_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl Azide (10a) and (4′*R*)-[4′-^{3}H$_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl Azide (10b). Zinc azide/bis(pyridine) complex (ZnN_6·2Py, 197.5 mg, 0.643 mmol) which was prepared from zinc nitrate, sodium azide, and pyridine according to a procedure of Viaud and Rollin[21] was added to a mixture of pyridoxine **9a** (300 mg, 0.86 mmol) and triphenylphosphine (450 mg, 1.72 mmol) in anhydrous toluene (12 mL). To this stirred mixture at room temperature was added DEAD (300 mg, 1.72 mmol) dropwise, causing a slightly exothermic reaction. Stirring was continued until complete consumption of **9a** (less than 30 min) was observed. The heterogeneous mixture was filtered over a Celite pad, concentrated in vacuo, and purified by flash chromatography (20% ethyl acetate/hexane) to afford the pure azide **10a** in 92% yield: IR (neat) 3031, 2868, 2095 (N_3), 1454, 1209, 1069 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.29 (1 H, s, 6-H), 7.46–7.30 (10 H, m, Ar H's), 4.91 (2 H, s, 5′-H's), 4.58 (2 H, s, PhCH_2O), 4.57 (2 H, s, PhCH_2O), 4.44 (2 H, s, 4′-H's), 2.59 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 153.8, 152.3, 145.7, 137.5, 136.4, 136.3, 131.0, 128.8, 128.6, 128.5, 128.0, 127.9 (Ar C's), 76.2 (PhCH_2O), 72.7 (PhCH_2O), 67.4 (C-5′), 44.8 (C-4′), 20.0 (2-Me). Compound **9b** was similarly converted to **10b** in 87% yield. The ^{1}H and ^{13}C NMR spectra of **10b** are identical with those of **10a**.

(4′*S*)-[4′-^{3}H$_1$]-3,5′-*O*-Dibenzylpyridoxamine (11a) and (4′*R*)-[4′-^{3}H$_1$]-3,5′-*O*-Dibenzylpyridoxamine (11b). The azide **10a** (150 mg, 0.4 mmol) dissolved in dry THF (3 mL) was added dropwise to a solution of lithium aluminum hydride (565 μL, 1 M in THF) at −30 °C under argon. The resulting solution was gradually warmed to 0 °C and was kept at that temperature until the reaction was complete (2 h). The reaction was quenched at −30 °C by adding a few drops of wet THF (10% H_2O) solution. After the evolution of gas ceased, a few drops of 0.5 N sodium hydroxide were added and vigorous stirring was continued for 15 min. To this reaction mixture was then added ethanol (10 mL), followed by magnesium sulfate. The resulting mixture was stirred for another 10 min before being filtered through a pad of Celite. The organic filtrates were combined and concentrated. The residual oil was purified by flash chromatography (50% ethyl acetate/hexane) to give **11a** in 88% yield: ^{1}H NMR ($CDCl_3$) δ 8.21 (1 H, s, 6-H), 7.46–7.26 (10 H, m, Ar H's), 4.91 (2 H, s, 5′-H's), 4.57 (2 H, s, PhCH_2O), 4.56 (2 H, s, PhCH_2O), 3.83 (2 H, s, 4′-H's), 2.56 (3 H, s, 2-Me), 1.90 (2 H, br s, NH_2); ^{13}C NMR ($CDCl_3$) δ 153.6, 151.9, 145.9, 144.4, 137.5, 136.6, 130.1, 128.8, 128.6, 128.5, 128.1, 128.0, 127.9 (Ar C's), 75.9 (PhCH_2O), 72.7 (PhCH_2O), 68.1 (C-5′), 37.7 (C-4′), 19.9 (2-Me). Compound **11b** was prepared from **10b** by the same procedure in 85% yield. Its ^{1}H and ^{13}C NMR spectra are identical with those of **11a**.

(4′*S*)-[4′-^{3}H$_1$]Pyridoxamine (12a) and (4′*R*)-[4′-^{3}H$_1$]-Pyridoxamine (12b). Compound **11a** (50 mg, 14.4 μmol) was refluxed with 4 N hydrochloric acid (5 mL) for 24 h. The solution was adjusted to pH 8 by the addition of saturated sodium bicarbonate solution and was then evaporated to dryness. Purification by preparative TLC (cellulose MN300) using a developing solution of 1-propanol/ammonia/water (6:3:1) gave the desired product **12a** in 95% yield: ^{1}H NMR (D_2O) δ 7.60 (1 H, s, 6-H), 4.69 (2 H, s, 5′-H's), 4.29 (2 H, s, 4′-H's), 2.42 (3 H, s, 2-Me); ^{13}C NMR (D_2O) δ 164.3 (C-3), 152.2 (C-2), 136.0 (C-4), 133.1 (C-5), 131.1 (C-6), 62.5 (C-5′), 39.8 (C-4′), 20.5 (2-Me). Compound **12b** was prepared from **11b** by the same procedure in 90% yield. Its ^{1}H and ^{13}C NMR spectra are identical with those of **12a**.

(4′*S*)-[4′-^{3}H$_1$]Pyridoxamine 5′-Phosphate (13a) and (4′*R*)-[4′-^{3}H$_1$]Pyridoxamine 5′-Phosphate (13b). The pyridoxamine **12a** (10 mg, 28.6 μmol) was mixed with 10 times its weight of anhydrous phosphoric acid (100 mg), and the resulting mixture was heated at 100 °C for 24 h. After cooling to room temperature, 9 volumes of absolute ethanol was slowly added with stirring to quench the reaction. The white precipitate that formed was collected by filtration and washed successively with absolute ethanol and ether. It was then dissolved in a minimal amount of water, and the pH of the solution was adjusted to 7 with a concentrated ammonia solution. Upon purification by preparative TLC (cellulose, developed in propanol/NH_3/H_2O (6:3:1)), the labeled pyridoxamine 5′-phosphate (**13a**) was obtained in 74% yield. The analytical sample was further purified by HPLC equipped with a Whatman Partisil 10 SAX column (an anion-exchange column) which was eluted with a linear gradient of 0–120 mM potassium phosphate buffer (pH 6.6). The desired PMP has a retention time of 11.5 min with a flow rate of 1 mL/min: ^{1}H NMR (D_2O) δ 8.31 (1 H, s, 6-H), 5.21 (2 H, d, J = 6.5, 5′-H's), 4.53 (2 H, s, 4′-H's), 2.81 (3 H, s, 2-Me); ^{13}C NMR (D_2O) δ 163.7 (C-3), 146.0 (C-2), 135.8 (C-5, d), 134.0 (C-4), 125.1 (C-6), 62.9 (C-5′), 37.6 (C-4′), 16.4 (2-Me). Compound **13b** was prepared from **12b** by the same procedure in 70% yield. Its NMR spectra are identical with those of **13a**.

2-Methyl-3-(benzyloxy)-5-((benzyloxy)methyl)pyridine-4-carboxylic Acid (14). A solution of pyridoxal **6** (1.0 g, 2.88 mmol) in methanol (8 mL) was diluted with an equal volume of a 5% aqueous sodium phosphate (monobasic) solution (pH 6.6). The oxidation was initiated by the addition, with vigorous stirring, of an aqueous potassium permanganate solution (6 mL, 1 M) at room temperature.[27] The reaction was quenched 1 h later by addition of a saturated solution of sodium sulfite (pH 9) until the purple color was fully discharged. The pH of the resulting brown suspension was adjusted to 3 with cold dilute HCl to dissolve the colloidal MnO_2. The reaction mixture was then extracted with ether, and the organic extracts were combined, concentrated, and purified by flash chromatography (40% ethyl acetate/hexane). The desired product was isolated as a white solid in 82% yield: mp 171–172 °C; ^{1}H NMR ($CDCl_3$) δ 8.37 (1 H, s, 6-H), 7.37–7.27 (10 H, m, Ar H's), 5.09 (2 H, s, 5′-H's), 4.63 (2 H, s, PhCH_2O), 4.51 (2 H, s, PhCH_2O), 2.51 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 165.8 (C-4′), 151.2, 149.2, 143.2, 136.9, 135.0, 134.4, 130.0, 129.1, 128.8, 128.5, 128.1, 127.9 (Ar C's), 77.3 (PhCH_2O), 73.5 (PhCH_2O), 66.3 (C-5′), 15.1 (2-Me); high-resolution FAB-MS calcd for $C_{22}H_{22}NO_4$ $(M + 1)^+$ 364.1549, found 364.1535.

2-Methyl-3-(benzyloxy)-4-(methoxycarbonyl)-5-((benzyloxy)methyl)pyridine (15). The diazomethane used in this esterification was generated by dropwise addition of Diazald (Aldrich) (5.0 g, 23 mmol, in 45 mL of ether) into a preheated (70 °C) potassium hydroxide solution (5.0 g, in 8 mL of water and 10 mL of 95% ethanol). The resulting diazomethane was co-distilled with ether and collected in a chilled receiving flask (Mini Diazald apparatus from Aldrich). The solution of diazomethane in ether was then added slowly through a plastic funnel to a stirred methanol solution of acid **14** (800 mg, 2.2 mmol) at 0 °C. Addition was continued until the solution turned yellow. More diazomethane was added, if necessary, to keep the solution yellow for at least 10 min. The reaction mixture was then evaporated in vacuo, and the crude product was purified by flash chromatography (20% ethyl acetate/hexane). Compound **15** was isolated

(27) Abiko, A.; Roberts, J. C.; Takemasa, T.; Masamune, S. *Tetrahedron Lett.* **1986**, 4537.

as a white solid in 89% yield: mp 133–134 °C; ^{1}H NMR ($CDCl_3$) δ 8.30 (1 H, s, 6-H), 7.42–7.28 (10 H, m, Ar H's), 4.96 (2 H, s, 5′-H's), 4.58 (2 H, s, PhCH_2O), 4.51 (2 H, s, PhCH_2O), 3.74 (3 H, s, OMe), 2.55 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 166.2 (C-4′), 153.8, 149.6, 144.0, 137.3, 136.2, 134.6, 129.4, 128.4, 128.3, 128.2, 127.8, 127.7 (Ar C's), 76.3 (PhCH_2O), 72.2 (PhCH_2O), 67.4 (C-5′), 58.7 (OMe), 18.3 (2-Me); high-resolution FAB-MS calcd for $C_{23}H_{24}NO_4$ (M + 1)$^+$ 378.1705, found 378.1678.

[4′-$^2H_2$]-3,5′-*O*-Dibenzylpyridoxine (16). A solution of ester **15** (650 mg, 1.72 mmol) in 4 mL of THF was slowly transferred via cannula to a suspension of lithium aluminum deuteride (108 mg, 2.57 mmol) in 4 mL of THF that was cooled to −78 °C under nitrogen. The mixture was allowed to warm to 0 °C and maintained at that temperature with stirring for 3 h. The excess hydride reagent was then quenched with ethyl acetate, followed by the addition of a few drops of 0.5 N NaOH. After 10 min of vigorous stirring, the resulting suspension was mixed with anhydrous magnesium sulfate and stirring was continued for another 20 min. This mixture was filtered through a pad of Celite, and the filter cake was washed thoroughly with ether and ethyl acetate. The combined filtrates were concentrated, and the desired product was purified by flash chromatography (30% ethyl acetate/hexane). Compound **16** was isolated in 93% yield: ^{1}H NMR ($CDCl_3$) δ 8.18 (1 H, s, 6-H), 7.45–7.27 (10 H, m, Ar H's), 4.90 (2 H, s, 5′-H's), 4.59 (2 H, s, PhCH_2O), 4.56 (2 H, s, PhCH_2O), 3.91 (1 H, s, OH), 2.49 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 154.0, 152.0, 145.2, 141.7, 137.1, 136.7, 131.0, 128.7, 128.4, 128.2, 128.1 (Ar C's), 76.7 (PhCH_2O), 73.0 (PhCH_2O), 68.4 (C-5′), 56.1 (very weak signal, C-4′), 19.7 (2-Me); high-resolution FAB-MS calcd for $C_{22}H_{22}{}^2H_2NO_3$ (M + 1)$^+$ 352.1913, found 352.1886.

[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridoxal (17). This compound was prepared from **16** (500 mg, 1.42 mmol) by the same procedure used in the synthesis of pyridoxal **6**. The desired product **17** was isolated in 93% yield: ^{1}H NMR ($CDCl_3$) δ 8.65 (1 H, s, 6-H), 7.36–7.25 (10 H, m, Ar H's), 4.95 (2 H, s, 5′-H's), 4.82 (2 H, s, PhCH_2O), 4.62 (2 H, s, PhCH_2O), 2.60 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 191.5 (very weak, t, J = 28.2, C-4′), 154.4, 154.0, 144.7, 137.9, 135.4, 132.4, 131.5, 129.0, 128.9, 128.5, 127.8, 127.7 (Ar C's), 78.0 (PhCH_2O), 73.2 (PhCH_2O), 67.5 (C-5′), 19.4 (2-Me).

(4′*R*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridoxine (18a) and (4′*S*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridoxine (18b). According to the same procedure used in the synthesis of **9a** and **9b**, pyridoxal **17** (180 mg, 517 μmol) was stereospecifically reduced by *S*-Alpine-Borane (3.22 mL, 0.5 M in THF, 1.61 mmol) at 0 °C under an argon atmosphere. After purification by flash chromatography (25% ethyl acetate/hexane), the desired product **18a** was obtained in 85% yield. Compound **18b** was similarly prepared from **17** (180 mg, 517 μmol) with *R*-Alpine-Borane in 82% yield: ^{1}H NMR ($CDCl_3$) δ 8.22 (1 H, s, 6-H), 7.49–7.31 (10 H, m, Ar H's), 4.96 (2 H, s, 5′-H's), 4.71 (1 H, s, 4′-H), 4.63 (2 H, s, PhCH_2O), 4.61 (2 H, s, PhCH_2O), 3.59 (1 H, br s, OH), 2.53 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 154.0, 151.9, 145.2, 141.7, 137.1, 136.7, 131.1, 128.7, 128.4, 128.2, 128.1 (Ar C's), 76.7 (PhCH_2O), 72.9 (PhCH_2O), 68.4 (C-5′), 56.1 (t, J = 23.0, C-4′), 19.7 (2-Me); high-resolution FAB-MS calcd for $C_{22}H_{23}{}^2H_1NO_3$ (M + 1)$^+$ 351.1834, found 351.1785. The spectra of **18b** are identical with those of **18a**.

(4′*R*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl (*S*)-(+)-*O*-Acetylmandelate (19a) and (4′*S*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl (*S*)-(+)-*O*-Acetylmandelate (19b). To a solution of (*S*)-(+)-α-acetoxyphenylacetic acid (53.4 mg, 0.275 mmol) in dry methylene chloride (4 mL) at room temperature were added alcohol **18a** (80 mg, 0.23 mmol) and 1,3-dicyclohexylcarbodiimide (56.7 mg, 0.275 mmol). The resulting mixture was stirred at room temperature for 2 h. After the precipitated urea was filtered off, the solvent was removed under reduced pressure, and the residue was purified by flash chromatography (10% ethyl acetate/benzene) to give **19a** as a colorless liquid. The yield was 81%: ^{1}H NMR ($CDCl_3$) δ 8.32 (1 H, s, 6-H), 7.55–7.30 (15 H, m, Ar H's), 5.88 (1 H, s, mandelic H), 5.33 (0.945 H, s, 4′-H_S), 5.20 (0.055 H, m, 4′-H_R), 4.73, 4.67 (1 H each, benzylic AB q, J = 11.0, 5′-H's), 4.42 (2 H, s, PhCH_2O), 4.43, 4.35 (1 H each, benzylic AB q, J = 11.3, PhCH_2O), 2.52 (3 H, s, 2-Me), 2.15 (3 H, s, OAc).[16] The enantiomeric purity of this sample was determined to be 89% (ee) based on the integrations of the 4′-H_S and 4′-H_R signals. ^{13}C NMR ($CDCl_3$): δ 170.2, 168.4 (C=O's), 153.3, 152.5, 144.7, 137.6, 136.1, 135.7, 133.4, 132.1, 129.5, 129.1, 128.9, 128.7, 128.6, 128.5, 128.1, 127.9, 127.8, 127.6 (Ar C's), 76.5 (PhCH_2O), 74.4 (PhCH_2O)*, 72.8 (mandelic α-C)*, 67.2 (C-5′), 58.1 (t, J = 23.6, C-4′), 20.7 (2-Me), 19.3 (OAc). Compound **19b** was obtained analogously from **18b** in 83% yield. The spectra of **19b** are the same as those of **19a** except for the integration of the ^{1}H NMR resonances at δ 5.33 (0.06 H, m, 4′-H_S) and 5.20 (0.94 H, s, 4′-H_R). The enantiomeric excess of this compound was determined to be 88%. High-resolution FAB-MS: calcd for $C_{32}H_{31}{}^2H_1NO_6$ (M + 1)$^+$ 527.2292, found 527.2282.

(4′*R*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl Camphanate (20a) and (4′*S*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl Camphanate (20b). To a solution of (−)-camphanic acid (25 mg, 126.3 μmol) in 0.5 mL of freshly distilled methylene chloride were added pyridoxine **18a** (30 mg, 85.7 μmol), 1,3-dicyclohexylcarbodiimide (26.6 mg, 129 μmol, in 1 mL of CH_2Cl_2), and a small amount of 4-(*N*,*N*-dimethylamino)pyridine. The reaction mixture was kept stirred at room temperature under argon for 45 min. The white precipitate that formed was removed by filtering through Celite and was washed twice with methylene chloride. The combined filtrates were concentrated under reduced pressure, and the crude product was purified by flash chromatography (25% ethyl acetate/hexane). Camphanate **20a** was isolated as a white solid in 90% yield: ^{1}H NMR ($CDCl_3$) δ 8.31 (1 H, s, 6-H), 7.45–7.32 (10 H, m, Ar H's), 5.38 (0.05 H, s, 4′-H_R), 5.32 (0.95 H, s, 4′-H_S), 4.92 (2 H, s, 5′-H's), 4.61 (2 H, s, PhCH_2O), 4.55 (2 H, s, PhCH_2O), 2.58 (3 H, s, 2-Me), 2.32–2.23 and 1.90–1.37 (4 H, m, camphanic CH_2's), 1.06, 0.95, 0.82 (3 H's each, s, camphanic Me's). The enantiomeric purity of this sample was determined to be 90% (ee) based on the integrations of the 4′-H_R and 4′-H_S signals. ^{13}C NMR ($CDCl_3$): δ 177.9, 167.2 (C=O's), 153.8, 152.5, 145.6, 137.6, 136.4, 135.3, 131.5, 128.7, 128.5, 128.4, 128.0, 127.9 (Ar C's), 76.1 (PhCH_2O), 72.7 (PhCH_2O), 67.5 (C-5′), 58.4 (t, J = 22.5, C-4′), 19.8 (2-Me), 90.9, 34.0, 30.7, 28.9, 25.7, 25.0, 16.6, 9.6 (camphanic C's). Compound **20b** was obtained from **18b** by the same procedure in 88% yield. The spectra of **20b** are the same as those of **20a** except for the integration of the ^{1}H NMR resonances at δ 5.38 (0.94 H, m, 4′-H_R) and 5.32 (0.06 H, s, 4′-H_S). The enantiomeric excess of this compound was determined to be 88%. High-resolution FAB-MS: calcd for $C_{32}H_{35}{}^2H_1NO_6$ (M + 1)$^+$ 531.2605, found 531.2594.

(4′*S*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl Azide (21a) and (4′*R*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4-yl Azide (21b). Preparation of azides **21a** and **21b** from the respective pyridoxines **18a** and **18b** was accomplished by a procedure similar to the one described for the synthesis of **10a** and **10b**. The ^{1}H and ^{13}C NMR spectra of these two compounds are identical and are similar to those of compounds **10a** and **10b**, except that the proton signal at δ 4.44 for **21a** and **21b** is integrated for only one proton and the carbon signal at δ 44.8 diminishes to a small triplet (J = 22.2). High-resolution FAB-MS: calcd for $C_{22}H_{22}{}^2H_1N_4O_2$ (M + 1)$^+$ 376.1884, found 376.1857.

(4′*R*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl Methanesulfonate (22a) and (4′*S*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl Methanesulfonate (22b). To a solution of pyridoxine **18a** (200 mg, 0.57 mmol) in dry methylene chloride (2.0 mL) at 0 °C was added freshly distilled triethylamine (158 μL, 1.75 mmol) followed by methanesulfonyl chloride (88.2 μL, 1.14 mmol). The mixture was stirred at 0 °C for 2 h. The reaction was quenched with water and extracted with methylene chloride. The combined organic extracts were dried and then concentrated in vacuo. The crude products were separated and purified by flash chromatography (10% ethyl acetate/hexane) to give compounds **22a** and **23a** in a 13:7 ratio. The combined yield was 80%: ^{1}H NMR ($CDCl_3$) δ 8.33 (1 H, s, 6-H), 7.47–7.28 (10 H, m, Ar H's), 5.30 (1 H, s, 4′-H), 4.92 (2 H, s, 5′-H's), 4.63 (2 H, s, PhCH_2O), 4.57 (2 H, s, PhCH_2O), 2.82 (3 H, s, OMs), 2.60 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 154.0, 152.5, 145.6, 137.4, 136.0, 133.7, 131.4, 128.7, 128.6, 128.5, 128.1, 128.0, 127.9 (Ar C's), 76.5 (PhCH_2O), 72.8 (PhCH_2O), 67.3 (C-5′), 62.0 (t, J = 22.5, C-4′), 37.2 (OMs), 19.9 (2-Me); high-resolution FAB-MS calcd for $C_{23}H_{25}{}^2H_1NO_5S$ (M + 1)$^+$ 429.1610, found 429.1589. Compounds **22b** was prepared from **18b** by the same procedure in 62% yield. Its ^{1}H and ^{13}C NMR spectra were identical with those of **22a**.

(4′*S*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl Chloride (23a) and (4′*R*)-[4′-$^2H_1$]-3,5′-*O*-Dibenzylpyridox-4′-yl Chloride (23b). To a solution of pyridoxine **18a** (200 mg, 0.57 mmol) in

dry methylene chloride (2.0 mL) at 0 °C was added freshly distilled triethylamine (158 μL, 1.75 mmol) followed by methanesulfonyl chloride (88.2 μL, 1.14 mmol). The mixture was stirred at room temperature for 8 h. The reaction was quenched with water and extracted with methylene chloride. The combined organic extracts were dried and then concentrated in vacuo. The crude product was purified by flash chromatography (10% ethyl acetate/hexane) to give compound **23a** as an oil in 71% yield: ^{1}H NMR ($CDCl_3$) δ 8.30 (1 H, s, 6-H), 7.50–7.34 (10 H, m, Ar H's), 4.97 (2 H, s, 5'-H's), 4.73 (2 H, s, PhCH_2O), 4.67 (1 H, s, 4'-H), 4.57 (2 H, s, PhCH_2O), 2.57 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 153.7, 151.5, 145.5, 138.2, 137.6, 136.3, 130.7, 128.6, 128.4, 128.2, 128.0, 127.8 (Ar C's), 76.0 (PhCH_2O), 72.7 (PhCH_2O), 67.1 (C-5'), 35.4 (t, J = 23.0, C-4'), 19.7 (2-Me); high-resolution FAB-MS calcd for $C_{22}H_{22}{}^2H_1NO_2Cl$ (M + 1)$^+$ 369.1495, found 369.1448. Compound **23b** was prepared from **18b** by the same procedure in 75% yield. Its ^{1}H and ^{13}C NMR spectra are identical with those of **23a**.

Conversion of Pyridoxyl Chlorides 23a and 23b to (4'*R*)-[4'-^{2}H$_1$]-3,5'-*O*-Dibenzylpyridox-4'-yl Azide (21b) and (4'*S*)-[4'-^{2}H$_1$]-3,5'-*O*-Dibenzylpyridox-4'-yl Azide (21a). To a solution of pyridoxyl chloride **23a** (100 mg, 0.23 mmol) in dry THF (4 mL) were added sodium azide (30 mg, 0.46 mmol) and 15-crown-5 (46.5 μL, 0.23 mmol) at room temperature under argon. The resulting mixture was heated to 70 °C and stirred at that temperature overnight. After dilution with water and extraction with methylene chloride, the organic extracts were combined and concentrated in vacuo. The crude azide was purified by flash chromatography (10% ethyl acetate/hexane) to give **21b** as a colorless liquid in 92% yield: IR (neat) 3031, 2868, 2095, 1454, 1209, 1069 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.29 (1 H, s, 6-H), 7.43–7.25 (10 H, m, Ar H's), 4.91 (2 H, s, 5'-H's), 4.58, 4.57 (2 H's each, s, PhCH_2O), 4.43 (1 H, s, 4'-H), 2.58 (3 H, s, 2-Me); ^{13}C NMR ($CDCl_3$) δ 153.8, 152.3, 145.6, 137.5, 136.4, 136.3, 131.0, 128.8, 128.6, 128.5, 128.0, 127.9 (Ar C's), 76.2 (PhCH_2O), 72.7 (PhCH_2O), 67.4 (C-5'), 44.8 (t, J = 22.2, C-4'), 20.0 (2-Me). Compounds **21a** was prepared from **23b** in 90% yield by the same procedure as described above. Its ^{1}H and ^{13}C NMR spectra are identical with those of **21b**.

(4'*S*)-[4'-^{2}H$_1$]-3,5'-*O*-Dibenzylpyridoxamine (24a) and (4'*R*)-[4'-^{2}H$_1$]-3,5'-*O*-Dibenzylpyridoxamine (24b). Compound **21a** and **21b** were converted to pyridoxamines **24a** and **24b**, respectively, by a procedure identical with that described above for the synthesis of **11a** and **11b**. The ^{1}H and ^{13}C NMR spectra of these two compounds are identical and are similar to the spectra obtained for compounds **11a** and **11b** except that the integration of the signal at δ 3.83 accounts now for only one proton in **24a** and **24b** and the carbon signal at δ 37.7 diminishes to a small triplet (J = 22.5). High-resolution FAB-MS: calcd for $C_{22}H_{24}{}^2H_1N_2O_2$ (M + 1)$^+$ 350.1924, found 350.1924.

4'-*N*-Camphanyl-(4'*S*)-[4'-^{2}H$_1$]-3,5'-*O*-dibenzylpyridoxamine (25a) and 4'-*N*-Camphanyl-(4'*R*)-[4'-^{2}H$_1$]-3,5'-*O*-dibenzylpyridoxamine (25b). To a solution of (−)-camphanic acid (45.4 mg, 0.23 mmol) in 0.5 mL of freshly distilled methylene chloride was added a solution of amine **24a** (40 mg, 0.115 mmol) and 1,3-dicyclohexylcarbodiimide (47.4 mg, 0.23 mmol) in 1 mL of methylene chloride. The resulting reaction mixture was stirred for 20 min at room temperature under an argon atmosphere. The desired product then was isolated by filtration of the reaction mixture through Celite and washing of the Celite pad twice with methylene chloride. The dried organic filtrates were concentrated in vacuo followed by flash chromatography (33% ethyl acetate/hexane) to give **25a** as a colorless liquid. The yield was 93%: ^{1}H NMR ($CDCl_3$) δ 8.22 (1 H, s, 6-H), 7.50–7.29 (11 H, m, Ar H's and NH), 4.90, 4.85 (1 H each, benzylic AB q, J = 10.9, 5'-H's), 4.67, 4.62 (1 H, benzylic AB q, J = 11.9, PhCH_2O), 4.56 (2 H, s, PhCH_2O), 4.48 (ca. 1 H, d, J_{NH} = 5.8, 4'-H_R), 2.56 (3 H, s, 2-Me), 2.50–2.40 and 1.93–1.56 (4 H, m, camphanic CH_2's), 1.06, 1.05, 0.76 (3 H's each, s, camphanic Me's);[23] ^{13}C NMR ($CDCl_3$) δ 178.0, 166.6 (C=O's), 153.9, 152.1, 145.8, 138.6, 137.3, 136.3, 130.8, 128.8, 128.6, 128.3, 128.1, 128.0 (Ar C's), 75.9 (PhCH_2O), 72.8 (PhCH_2O), 67.5 (C-5'), 58.1 (t, J = 21.8, C-4'), 19.7 (2-Me), 92.3, 55.2, 53.9, 34.0, 30.7, 16.6, 9.7 (camphanic C's). Compounds **24b** was converted to **25b** by a procedure similar to that described above for the synthesis of **25a**. Spectra of **25b** are identical with those obtained for **25a** except for the appearance of a new resonance at δ 4.54 (ca. 1 H, d, J = 5.8, 4'-H_S) and the disappearance of the 4'-H_R signal at δ 4.48–4.50 (Figure 1). High-resolution FAB-MS: calcd for $C_{32}H_{36}{}^2H_1N_2O_5$ (M + 1)$^+$ 530.2781, found 530.2766.

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Additions and Corrections

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Dale L. Boger,* Takayoshi Ishizaki, Paul A. Kitos, and Oranart Suntornwat. Synthesis of *N*-(*tert*-Butyloxycarbonyl)-CBI, CBI, CBI-CDPI$_1$, and CBI-CDPI$_2$: Enhanced Functional Analogues of CC-1065 Incorporating the 1,2,9,9a-Tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) Left-Hand Subunit.

Page 5825. Legend for Scheme II: (a) 2.0 equiv of (t-BuO_2C)$_2$O, dioxane, 95 °C, 3 h, 96%; (b) 1.2 equiv of *N*-bromosuccinimide, catalytic H_2SO_4, THF, −60 °C, 5 h, 98%; (c) 1.3 equiv of NaH, 3.0 equiv of 3-bromopropyne, 24 °C, 3 h, 99%; (d) 2.0 equiv of Bu_3SnH, 0.2 equiv of AIBN, benzene, 80 °C, 1 h; (e) 6 equiv of Me_2S–BH_3 THF, 0–25 °C, 3 h; 2 N NaOH, 30% H_2O_2, 0–25 °C, 1 h, 45 °C, 20 min, 62% from **12**; (f) 2.0 equiv of Ph_3P, 6 equiv CCl_4, CH_2Cl_2, 24 °C, 10 h, 99%; (g) 25% aqueous HCO_2NH_4/THF 2:15, 10% Pd/C, 0 °C, 2.5 h, 97%; (h) 3 equiv of NaH, THF, 0 to 24 °C, 2 h, 93%.

Page 5826. Legend for Scheme III: (a) For **21a**, 1.4 equiv of NaH, 1.4 equiv of **19**, THF–DMF (9:1), 24 °C, 12 h, 53%; for **21b**, 1.4 equiv of NaH, 1.6 equiv of **19**, DMF, 24 °C, 17 h, 73%; (b) for **22a**, 2.2 equiv of Bu_3SnH, 0.6 equiv of AIBN, benzene, 80 °C, 16 h, 75%; for **22b**, 2.2 equiv of Bu_3SnH, 0.2 equiv of AIBN, benzene, 80 °C, 12 h, 75%; (c) see text; (d) 1.3 equiv of NaH, 3.0 equiv of $BrCH_2CH{=}CH_2$, DMF, 24 °C, 1.5 h, 95%; (e) 1.3 equiv of NaH, 3.0 equiv of $BrCH_2CH{=}CMe_2$, DMF, 0 to 24 °C, 8 h, 94%; (f) for **23a**, O_3/O_2, CH_3OH, 0 °C, 10 min; Me_2S (excess), 0 to 24 °C, 4 h, 58%; for **23b**, O_3/O_2, CH_2Cl_2, −78 °C, 10 min; Me_2S (excess), −78 to 25 °C, 20 h, 88%; (g) see text.

Legend for Scheme IV: (a) 3 N anhydrous HCl/EtOAc, 24 °C, 20 min, 100%; (b) for **29**, 3 equiv of EDCI, 1.0 equiv of **27**, DMF, 24 °C, 8 h, 69%; for **30**, 3 equiv of EDCI, 1.0 equiv of **28**, DMF, 24 °C, 5 h, 78%; (c) for **7**, 3 equiv of NaH, THF–DMF (6:1), 0 °C, 1 h, 74%; for **8**, 2 equiv of NaH, THF–DMF (2:1), 0 °C, 1 h, 84%.

Page 5827. Legend for Scheme V: (a) 1.5 equiv of (−)-(*R*)-*O*-acetylmandelic acid, 1.7 equiv of EDCI, 0.1 equiv of 4-DMAP, CH_2Cl_2, 24 °C, 1 h, 81%; (b) 5 equiv of 4 N aq LiOH, CH_3OH–THF (2:3), 24 °C, 1 h, 97%; (c) 2.0 equiv of Ph_3P, 6.0 equiv of CCl_4, CH_2Cl_2, 24 °C, 10 h, 99%; (d) 25% aqueous HCO_2NH_4–THF (2:15), 10% Pd/C, 0 °C, 2.5 h, 97%; (e) see Scheme II, eq 1, and Scheme IV.