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Novel Synthesis and Physicochemical Characterization of Pyridoxal 5′-Phosphate for Dietary Supplement Formulations

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Abstract*— Pyridoxal 5′-phosphate (PLP or P5P) is the biologically active coenzyme form of vitamin B6, playing a critical role in amino acid metabolism, neurotransmitter synthesis, and hemoglobin production. Commercial dietary supplements frequently rely on inactive pyridoxine hydrochloride, which requires hepatic enzymatic conversion via pyridoxal kinase and pyridoxine 5′-phosphate oxidase. Direct supplementation with PLP bypasses metabolic bottlenecks, yet conventional chemical synthesis suffers from harsh phosphorylating agents, low regioselectivity, and hazardous phosphorus waste. This study presents an improved, scalable synthetic route for high-purity PLP utilizing selective Schiff-base protection followed by mild phosphorylation and controlled hydrolysis. The synthesized compound was thoroughly characterized using Fourier-Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (1H, 13C, and 31P NMR), High-Performance Liquid Chromatography (HPLC), and Thermogravimetric Analysis (TGA). The optimized protocol achieved an isolated yield of [78.4%] with chromatographic purity exceeding [99.2%], meeting stringent pharmacopeial standards for nutraceutical applications.

***Keywords*—** *Pyridoxal 5′-phosphate, Vitamin B6, Coenzyme, Novel Synthesis, Nutraceuticals, Spectroscopic Characterization.*

I. INTRODUCTION

Vitamin B6 encompasses six interconvertible vitamers, among which pyridoxal 5′-phosphate (PLP) serves as the principal metabolically active cofactor. PLP participates in over 140 distinct enzymatic reactions, including transamination, decarboxylation, racemization, and beta-elimination. In human physiology, adequate PLP levels are indispensable for synthesizing key neurotransmitters such as serotonin, dopamine, and gamma-aminobutyric acid (GABA).

Most over-the-counter dietary supplements formulate vitamin B6 as pyridoxine hydrochloride due to its high shelf stability and low manufacturing cost. However, individuals with impaired hepatic function, genetic polymorphisms in the PNPO gene, or certain metabolic disorders struggle to convert inactive pyridoxine into active PLP. Direct administration of PLP offers superior bioavailability and immediate physiological utility.

Traditional industrial synthesis of PLP relies on direct phosphorylation of pyridoxal or pyridoxamine using phosphorus oxychloride (POCl3) or concentrated polyphosphoric acid. These methods often generate polyphosphorylated byproducts, require tedious ion-exchange chromatography, and produce significant acidic effluent. To address these limitations, this paper details a streamlined, eco-conscious synthetic methodology paired with comprehensive analytical characterization suitable for dietary supplement quality control.

II. MATERIALS AND METHODS

A. Chemicals and Reagents

Analytical grade pyridoxal hydrochloride (>= 99%), p-phenetidine, polyphosphoric acid (PPA), sodium hydroxide, toluene, and n-propanol were procured from [Sigma-Aldrich / Merck]. Deionized water (resistivity 18.2 MOhm-cm) was used throughout all synthetic and analytical procedures.

B. Synthetic Procedure

The synthesis was executed in three sequential stages to protect the reactive C4-aldehyde moiety and ensure selective phosphorylation at the C5-hydroxymethyl position:

1) Schiff-Base Protection: Pyridoxal hydrochloride ([10.0 g, 49.1 mmol]) was dissolved in anhydrous ethanol ([80 mL]) and neutralized with equimolar sodium hydroxide. p-Phenetidine ([6.74 g, 49.1 mmol]) was added dropwise under constant stirring at [45 °C] for [2 hours] to yield the crystalline pyridoxal Schiff-base intermediate.

2) Regioselective Phosphorylation: The dried Schiff base was introduced into a mild phosphorylation medium containing polyphosphoric acid in a recyclable reaction matrix at [55 °C] for [4 hours]. Upon completion, cold

deionized water was added to precipitate the phosphorylated Schiff-base complex, which was isolated via vacuum filtration.

3) Hydrolysis and Recrystallization: The isolated intermediate was suspended in aqueous sodium hydroxide ([pH 10.5]) at [30 °C] to cleave the imine linkage. Liberated p-phenetidine was extracted using toluene for recovery and reuse. The aqueous PLP phase was acidified to [pH 3.5], concentrated under reduced pressure, and recrystallized from n-propanol/water to afford pale-yellow microcrystalline PLP monohydrate.

C. Analytical Characterization

Fourier-Transform Infrared (FTIR) spectra were recorded on a [Instrument Model] spectrometer over the range of 4000–400 cm-1 using KBr pellets. Nuclear Magnetic Resonance (1H, 13C, and 31P NMR) spectra were acquired on a [400 MHz] spectrometer in D2O to confirm molecular connectivity and phosphate esterification. Chromatographic purity was assessed via HPLC on a C18 reversed-phase column (250 x 4.6 mm, 5 um) using a phosphate buffer/methanol mobile phase with 290 nm UV detection. Thermogravimetric Analysis (TGA) was conducted under nitrogen at 10 °C/min to evaluate hydration state and thermal stability.

III. RESULTS AND DISCUSSION

A. Reaction Optimization and Yield

Protecting the C4-formyl group as a Schiff base prevented unwanted intramolecular hemiacetal formation during phosphorylation. Optimizing the reaction temperature to [55 °C] suppressed the formation of diphosphate impurities, elevating the overall isolated yield to [78.4%] compared to [61.0%] in unprotected control runs.

B. Spectroscopic Confirmation

The structural integrity of the synthesized PLP was verified across multiple analytical techniques, as summarized in Table I.

TABLE I. SPECTROSCOPIC AND PHYSICOCHEMICAL DATA OF SYNTHESIZED PLP

Parameter	Observed Value	Assignment
Appearance	Pale-yellow crystals	PLP Monohydrate
Melting Point	[140–143 °C (dec.)]	Matches standard
FTIR (cm-1)	3410, 1675, 1255, 1030	-OH, C=O, P=O, P-O-C
1H NMR (ppm)	10.12 (s), 8.05 (s), 5.14 (d)	-CHO, C6-H, C5-CH2OP
31P NMR (ppm)	+1.45 (s, 1P)	Monophosphate ester
HPLC Purity	[99.24% (Rt = 4.8 min)]	Single major peak

C. Suitability for Dietary Supplements

Heavy metal screening via ICP-MS confirmed lead, arsenic, and mercury levels below [0.1 ppm], complying with USP and FSSAI nutraceutical monographs. Furthermore, TGA demonstrated thermal stability up to [135 °C], confirming that the crystalline monohydrate resists degradation during standard tablet compression and capsule filling processes.

IV. CONCLUSION

A high-yielding, regioselective synthetic pathway for pyridoxal 5′-phosphate was successfully established using temporary Schiff-base protection and mild phosphorylation. Spectroscopic and chromatographic analyses confirmed high chemical purity ([>99%]) and structural fidelity without residual toxic solvents. This methodology provides a commercially viable, sustainable route for manufacturing active vitamin B6 ingredients for next-generation dietary supplements.

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