Original Article

An embodiment to the greener synthesis of amino acid pyrophosphates

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Abstract

A new approach for the green synthesis of amino acid pyrophosphates with excellent yield is described. This novel approach lay foundation for the exploration of amino acid pyrophosphates which are key supplements and are biologically useful compounds. Pyrophosphates find immense application as food additives, metal cleaners, water softener and as supplements like, Tetrasodium pyrophosphate. Use in clinical research for Osteoporosis, Alendronate finds application. Thymine pyrophosphate is biologically active and works in body with Vitamin B1.Ferric pyrophosphate is administered for patients suffering with iron deficiency which leads to disease like anaemia.Since amino acids are the building block of proteins in our body, amino acids pyrophosphates find extensive usage and commercially viable processes may remain indispensable for application to industrial production. Method of preparation is simple using conventional debenzylation technique and salt formation with almost quantitative yields with high purity. Synthesized products are identified by proton, carbon, and phosphorous NMR. The main benefit of this procedure is that it doesn't require delicate, expensive, or harmful chemicals. Amino acids are very useful in meals, medications, cosmetics, and other items As a result, the synthesis of -amino acids has long been a key field of study, and several synthetic preparation methods have been created to date.

Keywords: Amino Acids, Pyrophosphates, Green Synthesis, L-Alanine, L-Phenyl Alanine.

1 INTRODUCTION

Amino acids are the primary building blocks of proteins our body is made of. These proteins are derived from amino acids that are 20 in number. Amino acids are organic compounds composed of nitrogen, carbon, hydrogen and oxygen, along with a variable side chain group, associated to differentiate them from others. Nine out of them are considered essential amino acids based on inability of our body to synthesize them. These are required specifically for the synthesis of neurotransmitters, hormones and proteins for the growth of our body [1]. As a result, they may be taken as a body supplement to facilitate healthy metabolic activity. Namely, these are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Now, since the body cannot produce these essential amino acids, they are taken through diets or supplements [2]. Plant-based foods like Soy, quinoa and buckwheat contain all nine essential amino acids, making them complete protein sources. Have been observed to play a vital role for functions such as protein synthesis, tissue repair and nutrient absorption. Some may also prevent muscle loss and improve mood, sleep, athletic performance and weight loss. Phenyl alanine known for being precursor for the neurotransmitters tyrosine, dopamine, epinephrine and norepinephrine. Has an integral role in the structure and function of proteins and enzymes and the production of other amino acids. Valine is one of three branched-chain amino acids, meaning it has a chain branching off to one side of its molecular structure. It helps stimulate muscle growth and regeneration and is involved in energy production, providing people recovery from fatigue.

Essential amino acid deficiencies can negatively impact nervous, reproductive, and immune and digestive systems. Another class of compounds “Pyrophosphates or diphosphates” play a vital role in body repair and healthy strong bones [3]. Pyrophosphates are found in ATP and other nucleotide triphosphates, which are very important in biochemistry. They are good complexing agents for metal ions (such as calcium and many transition metals) and have many uses in industrial chemistry. Pyrophosphate is the first member of an entire series of polyphosphates. Various diphosphates are used as emulsifiers, stabilisers, acidity regulators, raising agents, sequestrants, and water retention agents in food processing [4].

Phosphonates and bisphosphonates are chemically stable analogs of phosphates and pyrophosphates. Phosphate and pyrophosphate groups play numerous vital roles in the biochemistry of living organisms, and consequently, phosphonates and bisphosphonates constitute an important class of bioisosteres for medicinal chemists and chemical biologists [5][6]. The

usefulness of this class of molecules has been highlighted by the COVID-19 pandemic. Phosphonate nucleotide analogs, such as Tenofovir and Cidofovir, can inhibit SARS-CoV-2 RNA polymerase [7] and may thus represent a much needed therapeutic weapon against this viral threat. Fosfomycin is another example of a clinically relevant phosphonate. Used primarily for urinary tract infections, fosfomycin is a bactericidal antibiotic that inhibits UDP-N-acetylglucosamine enolpyruvyl transferase, the enzyme responsible for the first committed step in peptidoglycan biosynthesis [8]. Bisphosphonates, on the other hand, are particularly useful as inhibitors of enzymes involved in isoprenoid biosynthetic pathways. These compounds act by mimicking the pathway substrates that contain a pyrophosphate moiety. The pyrophosphate functionality promotes the solubility of these substrates and provides an efficient leaving group for condensation reactions [9].

Existing literature survey revealed the importance of phosphate compunds, both in drugs and as supplements in diet. Available synthesis employs use of phosphate salt preparation using phosphoric acid or ion exchange methods using inorganic phosphate salts. The usage of inorganic phosphates pose problems related to assay and toxicity pertaining to available molecular structure, thereby causing potential risk of purity, required for the launch of the drug product or supplement [10] [11].

Here in this study, an improved synthesis using protected pyrophosphates and its in-situ generation as stable pyrophosphates that form salts with available amino groups of amino acids based on their basicity and reactivity, in turn. Use of Palladium on carbon as a catalyst in catalytic quantity provides methodology adopted for the synthesis of amino acid pyrophosphates.

The reaction is conducted in water and post reaction completion, washed with organic solvent, which is immiscible with water to eliminate the by-product. This aqueous layer is treated with addition of an alcoholic solvent to isolate the product in molar yield and characterization done by NMR, IR and Phosphorous NMR. L-Alanine and L-Phenyl Alanine were subjected to reaction with protected pyrophosphates in water and debenzylated to obtain pure essential amino acid pyrophosphate salts.

2 Experimental Procedure

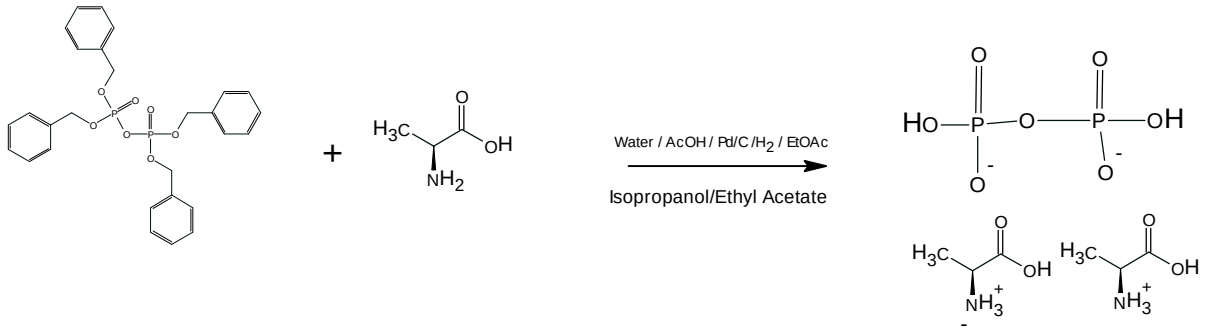
All reagents were purchased from Sigma Aldrich and solvents from laboratory grade. All chemical are used without further purification. Pyrophosphoric acid tetrabenzyl ester, 2-Amino-3-phenylpropanoic acid, 2-Aminopropionic acid, Acetic acid and Ethyl acetate, Isopropanol, and distilled water were used. Palladium on carbon catalyst was made available from Monarch Catalyst. Thin layer chromatography is also used for monitoring the reaction.

2.1 Synthesis of Pyrophosphates

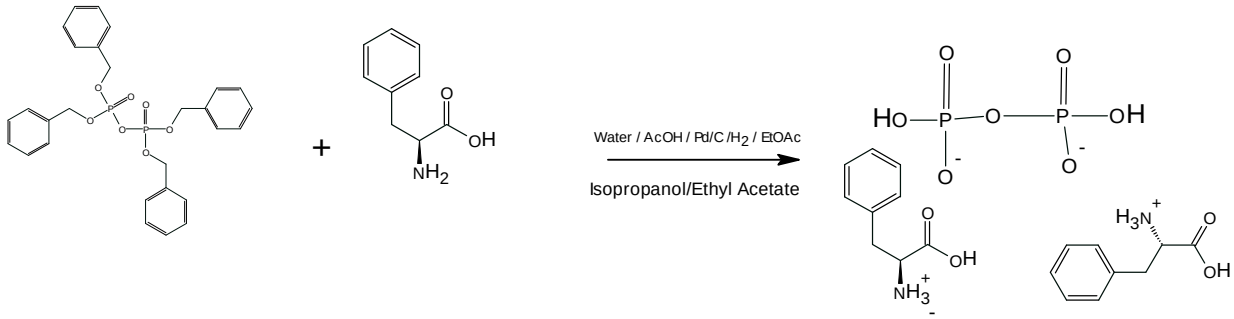
Charge L-Alanine (3.56 gm) to a Parr bottle containing water (40 ml) and charged Tetrabenzyl pyrophosphate (10.77 gm) followed by addition of Acetic acid (2.39 gm) and Ethyl acetate (10 ml). Charge Palladium on carbon (5%) 1.0 gm (50% wet) catalyst and subjected to hydrogenation at 5-10 kg pressure for around 10 hours at 25-35°C. Reaction monitored by TLC for absence of TBPP and the reaction mass is then filtered over hyflo bed. Layers separated and the entire mass is recovered to crystallize the product in a mixture of ethyl acetate and Isopropanol. Molar yield obtained as 90% (6.44 gm) and the product is purified by dissolution in water and precipitation with alcohol. Following this reaction procedure, L-Phenyl alanine pyrophosphate is synthesized from Charge L-Phenyl Alanine (3.3 gm). Characterization of synthesized product is done by NMR, and Phosphorous NMR.

3 Results and Discussion

The synthesis rotates around the generation of pyrophosphate moiety which reacts with amino acid to form amino acid pyrophosphate salt. In this study, L-Alanine and L-Phenyl alanine from the class of amino acids is under study and experiments were designed around for molar yields and high purity (Scheme 1). Molar yield obtained as 90 % and the product is purified by dissolution in water and precipitation with alcohol. Desired products are indentified by carbon, proton, and phosphorous NMR.



Tetrabenzyl Pyrophosphate L_Alanine L Alanine Pyrophosphate



Tetrabenzyl Pyrophosphate L_Phenyl Alanine L Phenyl Alanine Pyrophosphate

Scheme 1: Synthesis of amino acid pyrophosphates

Based on high throughput through synthesis and the various efficacy reported in available literature, provides an insight into development of these molecules for commercial interest as supplements or drugs. Pyrophosphates libraries will be enriched through the current research and will render the scientists develop molecules of interest to both academia and industry. Availability of tetrabenzyl pyrophosphate is key to this synthesis as it involves storage at sub zero temperatures and is observed to degrade with time and temperature.

Challenges are observed to contain the scale up at commercial scale due to high demand, especially to the need for elemental impurities, which are observed to occur due to phosphates, rendering leaching and imparting metals to the product. In order to tackle the problem, anti-chromos carbon may be used for treatment prior to crystallization of the compound of interest.

Identification of product by carbon, proton and phosphorous NMR of the products:

1-carboxyethan-1-aminium: 1H NMR (CdCl3, 400 MHZ): δ 3.756 (CH) ppm, 2.230 (CH3) ppm (Fig.1). 13C NMR (CdCl3, 400 MHZ): δ 172.1 ppm, 58.68 ppm, 17.414 ppm (Fig. 2). Phosphorous NMR: δ -10.974 ppm, -0.073 ppm.

1-carboxy-2-phenylethan-1-aminium: 1H NMR (CdCl3, 400 MHZ): δ 7.309 (m, CH) ppm, 4.145 (CH), 3.242 (CH2) ppm (Fig.4). 13C NMR (CdCl3, 400 MHZ): δ 172.0 ppm, 134.2 ppm, 129.3 ppm, 129.1 ppm, 127.8 ppm, 54.5 ppm, 35.7 ppm (Fig. 5). Phosphorous NMR: δ -10.962 ppm, -0.044 ppm (Fig. 6).

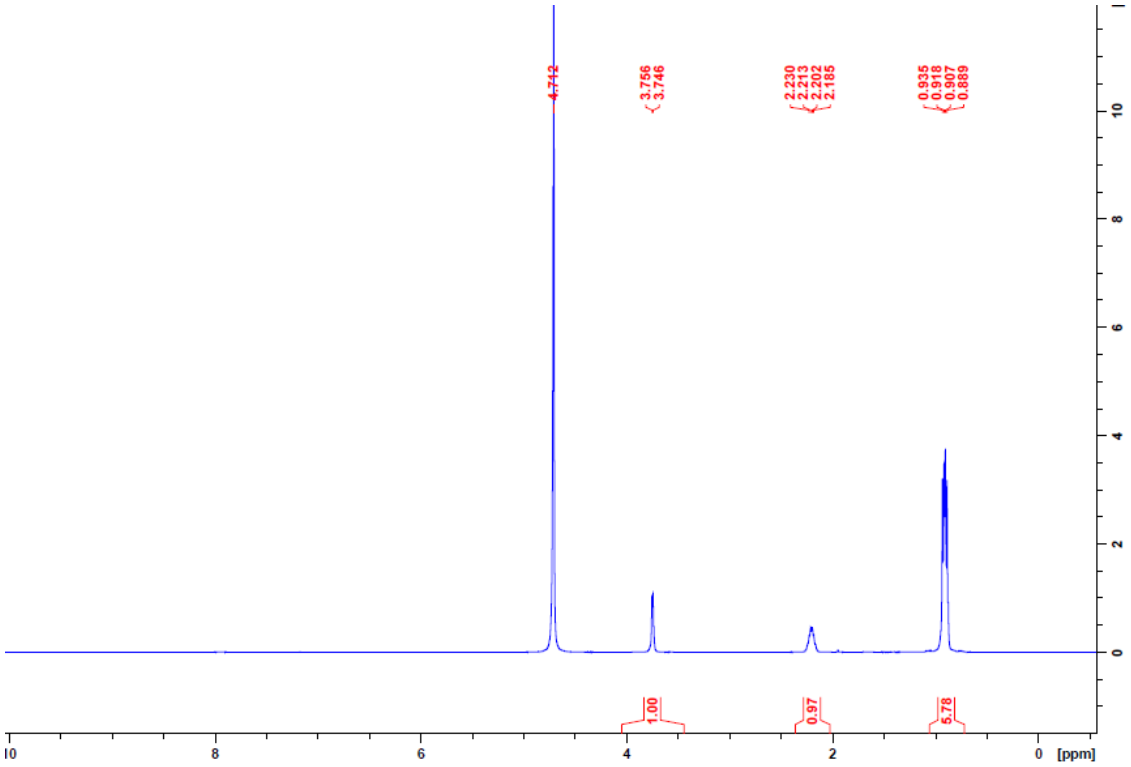


Figure 1. Proton NMR of L-Alanine Pyrophosphate

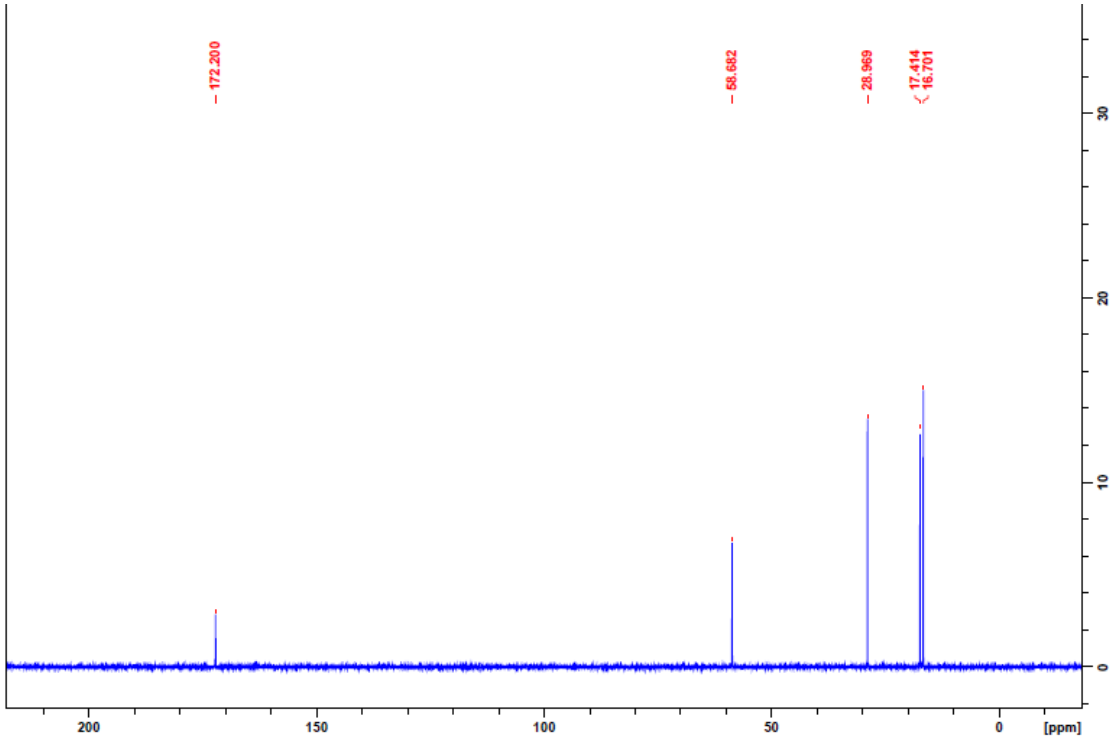


Figure 2. Carbon NMR of L-Alanine Pyrophosphate

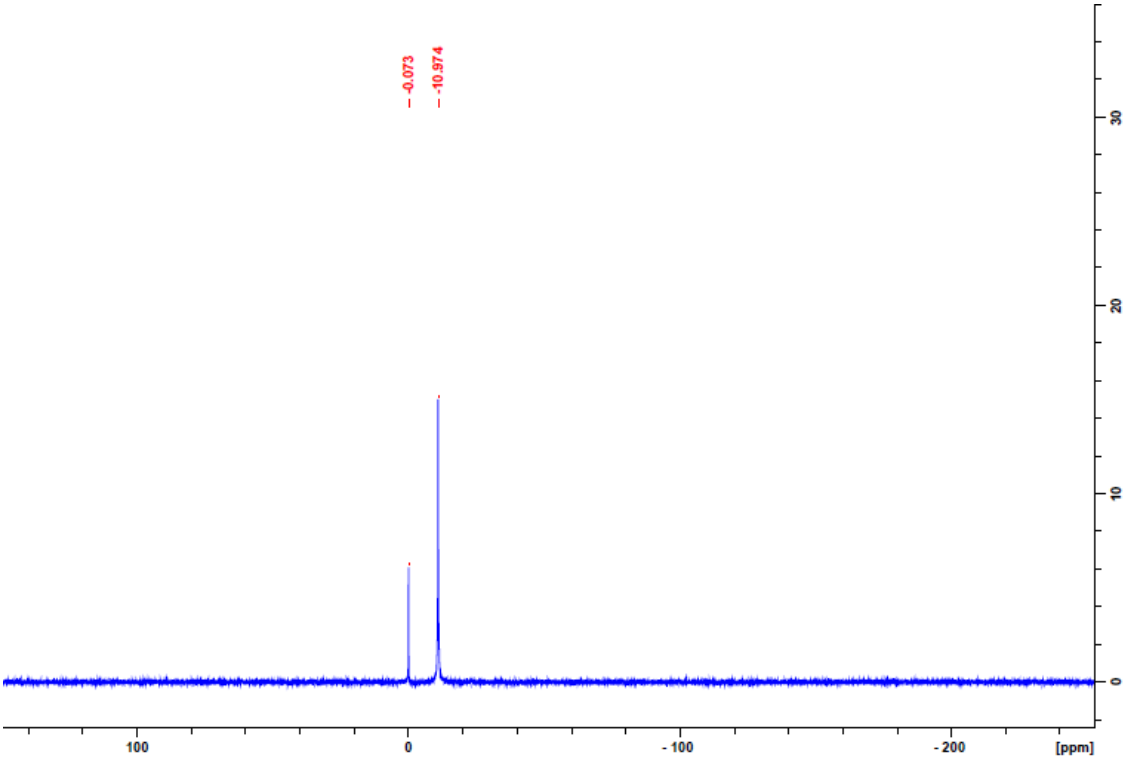


Figure 3. Phosphorous NMR of L-Alanine Pyrophosphate

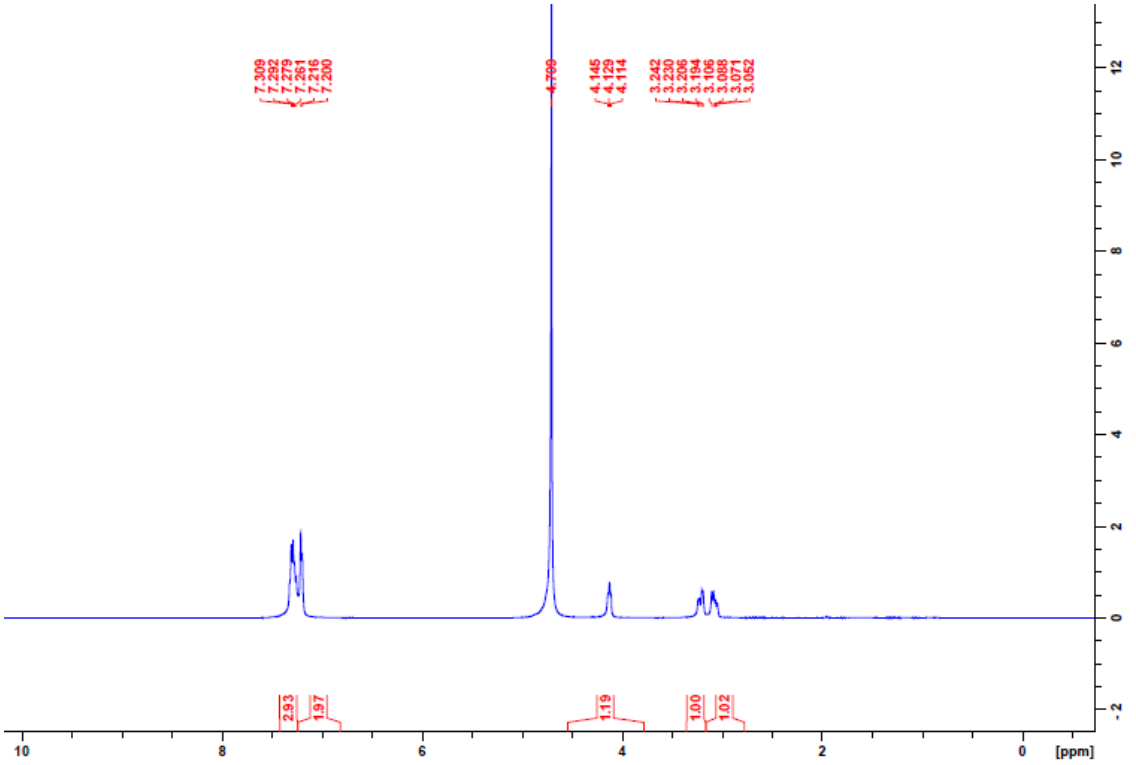


Figure 4. Proton NMR of L-Phenyl-Alanine Pyrophosphate

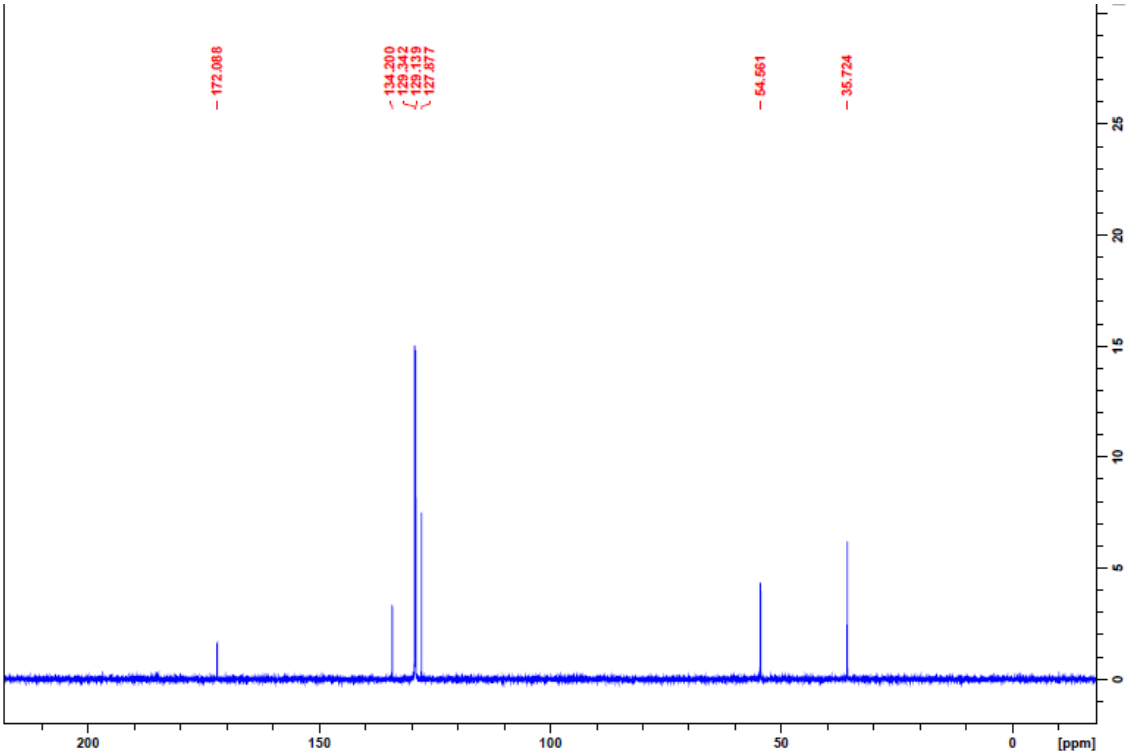


Figure 5. Carbon NMR of L-Phenyl-Alanine Pyrophosphate

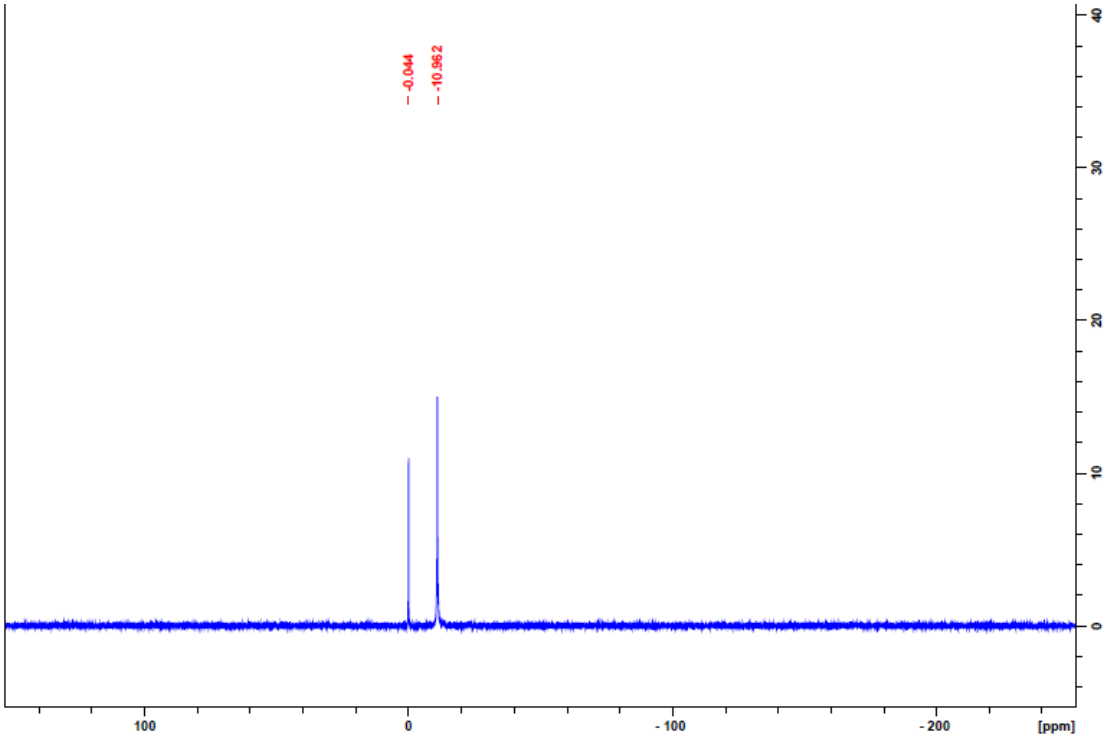


Figure 6. Phosphorous NMR of L-Phenyl-Alanine Pyrophosphate

4 Conclusion

We have developed a novel and green method for amino acid pyrophosphates synthesis under mild conditions. The main benefit of this procedure is that it doesn't require delicate, expensive, or harmful chemicals. These encouraging findings offer a technique for effective and eco-friendly amino acid synthesis.

Acknowledgement

Conflict of interest

Authors declare no conflict of interest.

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