# p-Nitrophenylalanine

## Table of Contents





## summary


p-Nitrophenylalanine (pN-Phe) is a synthetic derivative of the amino acid phenylalanine, characterized by the presence of a nitro group (NO,) at the para position of its phenyl ring. With the molecular formula C‰H•€N,O„, pN-Phe plays a crucial role in various biochemical applications due to its unique chemical properties, which enhance its reactivity and interaction with biological systems.[1][2] This compound has garnered attention in fields such as protein engineering, drug development, and immunology, marking its significance in both research and industry.

The synthesis of pN-Phe can be achieved through several methods, including conventional nitration techniques and modern enzymatic approaches that utilize engineered microbes for more sustainable production.[3][4] Its incorporation into proteins has shown promise for developing novel therapeutics and improving vaccine efficacy, particularly in the context of autoimmune diseases and cancer immunotherapy.[4] Furthermore, pN-Phe's potential applications extend to the pharmaceutical industry, where it serves as an intermediate in drug synthesis, such as the migraine treatment Zolmitriptan.[5][6]

Despite its promising applications, p-nitrophenylalanine is not without risks. It poses significant health hazards, including toxicity upon ingestion and potential irritation from skin or respiratory exposure.[7][8] These safety concerns necessitate careful handling in laboratory and industrial settings, underlining the importance of appropriate safety measures and personal protective equipment.

As research into p-nitrophenylalanine continues to evolve, its role in enhancing immune responses and addressing challenges such as antibiotic resistance remains a focal point, showcasing the compound's multifaceted utility in advancing medical science and biotechnology.[4][9][10]

# Chemical Structure

p-Nitrophenylalanine, a derivative of the amino acid phenylalanine, possesses a molecular formula of C9H10N2O4 and is commonly represented as O2NC6H4CH2CH(NH2)CO2H · H2O.[1][11] Its structure features two primary components: a benzyl side chain and an alpha-amino group (-NH,) attached to a central carbon, known as the ±carbon. This ±carbon is also bonded to a carboxyl group (-COOH) and a hydrogen atom, leading to the characteristic features of phenylalanine, which includes its hydrophobic benzyl group, an aromatic phenyl ring (C†H…) linked to a methylene group (-CH,-).[12]

The presence of the nitro group (NO2) at the para position of the phenyl ring significantly influences the chemical properties of p-nitrophenylalanine, imparting unique reactivity and interactions within biological systems.[2] The hydrophobic nature of the benzyl side chain contributes to the stabilization of protein structures through À-À interactions and stacking with other aromatic residues.[12]

Moreover, the physical state of p-nitrophenylalanine at room temperature is solid, with a molecular weight of approximately 228.20 g/mol.[13][14] The compound's linear formula emphasizes its nitroaromatic functional groups, which are known to enhance the chemical and biological properties of various polypeptides, thus broadening its potential applications in biochemical research and industry.[2]

# Synthesis

p-Nitrophenylalanine (pN-Phe) is synthesized through various methods, with one of the most common approaches involving the nitration of L-phenylalanine. This method typically utilizes mixed acids to introduce a nitro group at the para position of the phenyl ring, resulting in high yields and shorter production times compared to traditional synthesis routes[3][15].

## Conventional Synthesis Methods

The nitration method can be enhanced by specific modifications to the reaction conditions. For instance, dissolving L-phenylalanine in concentrated sulfuric acid before the nitration process has been shown to improve yield outcomes significantly[16]. Furthermore, the process can be optimized by manipulating reaction parameters, such as temperature and time, to achieve better efficiency[3][17].

## Enzymatic and Microbial Synthesis

Recent advancements in biocatalysis have introduced enzymatic and microbial methods as viable alternatives to conventional synthesis. These approaches typically operate under milder conditions, which can lead to fewer by-products and a more sustainable production process. For example, researchers have engineered bacteria to reroute metabolic pathways for the production of pN-Phe, allowing the microbe to synthesize non-native compounds through the introduction of recombinant DNA[4][18]. This innovative method showcases the potential for microbial fermentation techniques in the synthesis of complex amino acids, thereby providing an environmentally friendly option for producing pN-Phe[19].

## Applications of Synthesized p-Nitrophenylalanine

The synthesized p-nitrophenylalanine is recognized for its unique nitro group, which enhances its reactivity and makes it a valuable building block in organic synthesis, particularly in the pharmaceutical industry. It is utilized in the synthesis of various drugs, including Zolmitriptan, which is used to treat migraines[5][6]. The compound's ability to serve as an intermediate in drug synthesis underscores its importance in medicinal chemistry[20][21].

# Applications

## Immunological Applications

Para-nitro-L-phenylalanine (pN-Phe) is recognized for its immunostimulatory properties, particularly when incorporated as a surface residue on proteins. It has shown potential in breaking immune self-tolerance, making it a candidate for therapeutic applications in autoimmune diseases and cancer immunotherapy[4]. The ability of pN-Phe to enhance immune responses towards self-proteins that are often upregulated in diseased cells is crucial, as generating a robust immune response against these antigens can be challenging due to the tolerance exhibited by CD4+ T helper cells[4].

## Vaccine Development

pN-Phe has significant implications in the development of engineered bacterial vaccine vectors. These vectors have the potential to immunize against both native and heterologous antigens, leveraging the immunological benefits of pN-Phe to enhance the immune response[4]. Notably, vaccines utilizing engineered bacterial vectors have reached phase III clinical trials, although some trials have faced challenges related to efficacy rather than safety[4]. Non-pathogenic commensal bacteria, such as Lactobacilli, are also being explored as vaccine carriers due to their high safety profile and capability to elicit mucosal immune responses, despite some limitations concerning the immunogenicity of heterologous antigens[4].

## Autoimmune Disease Research

Recent studies have demonstrated that immunization with pN-Phe-modified proteins, such as pN-Phe-TNF-±çan generate physiologically relevant antibody responses, suggesting that these modified epitopes could be effective in autoimmune disease contexts[4]. This strategy has been successfully extended to other disease-relevant antigens, including C5a, PDL1, and HER2, showing that pN-Phe can facilitate T-cell mediated activation and autoantibody production against these targets[4].

## Antibiotic Resistance and Pathogen Immunization

In light of increasing antibiotic resistance, the demand for innovative vaccine strategies that can effectively stimulate immune responses against pathogenic diseases has intensified. pN-Phe-enhanced vaccines represent a promising direction in this field, as they can help overcome the challenges associated with inducing immunity against specific antigens while ensuring safety through the use of attenuated bacterial vectors[4]. The selective targeting of mucosal antigen presenting cells by engineered bacterial vectors further enhances the potential for systemic protection against pathogens[4].

# Biological Significance

p-Nitrophenylalanine (pN-Phe) is a non-natural amino acid that plays a crucial role in protein engineering and biotechnology. Its unique properties make it a valuable building block for synthesizing modified proteins, enabling researchers to investigate the effects of various modifications on protein function and stability[22][23].

As an amino acid derivative, pN-Phe is incorporated into proteins to enhance or alter their characteristics, providing insights into protein structure and function. The stability of pN-Phe as a product is critical for successful application in biochemical pathways, as demonstrated by studies indicating that phenylalanine derivatives, including pN-Phe, exhibit substantial stability under metabolically active conditions in E. coli cultures[4].

Additionally, pN-Phe serves as a pharmaceutical ingredient, expanding its utility beyond research into practical applications in medicine and industry. Its incorporation into proteins can lead to new therapeutic agents and drug formulations, leveraging the unique properties conferred by its nitro group[23][19].

The exploration of pN-Phe and its metabolic derivatives, such as phenylpyruvate (pN-Pyr), is essential for understanding the biochemical interactions within cellular systems. Research shows that while pN-Phe remains stable, its counterpart pN-Pyr is comparatively unstable due to endogenous enzymatic activities, highlighting the importance of stability in biological processes[4]. Furthermore, the impact of pN-Phe on cell growth rates suggests minimal toxicity, making it an appealing candidate for further research in metabolic engineering and synthetic biology applications[4].

# Toxicity and Safety

p-Nitrophenylalanine is a yellow solid that poses several health and safety risks upon exposure. It is classified as toxic if ingested, with the potential to cause acute toxicity, which may manifest as headache, drowsiness, shortness of breath, nausea, methemoglobinemia, and in severe cases, unconsciousness. Notably, contact with skin can lead to irritation, and prolonged exposure may result in symptoms similar to those experienced from inhalation or ingestion.[7][8].

## Health Hazards

Inhalation of p-Nitrophenylalanine can trigger symptoms including headaches and nausea, while skin contact can cause irritation and potentially serious conditions with continued exposure.[8] Eye contact may lead to irritation and possible corneal damage, necessitating immediate medical attention.[8][24]. It is imperative to follow emergency procedures if exposure occurs, including flushing affected areas with water and seeking medical assistance promptly.[8]

## Fire and Reactivity Hazards

p-Nitrophenylalanine is a fire hazard, with toxic oxides of nitrogen potentially forming during combustion. It melts and burns, and care should be taken to avoid prolonged exposure to air and moisture, as it is sensitive to these conditions.[8][25]. The material is insoluble in water and has a flash point of 390°F, which indicates a need for caution in environments where ignition sources are present.[8][25].

## Personal Protective Measures

To minimize exposure risks, it is advised to handle p-Nitrophenylalanine with appropriate personal protective equipment (PPE), including gloves and face protection. Adequate ventilation is also crucial to avoid inhalation of dust or vapors.[26][27][28]. Furthermore, good industrial hygiene practices should be observed, such as washing hands after handling the substance and avoiding eating or drinking in areas where it is used.[29][30].

Given its hazardous nature, p-Nitrophenylalanine should be managed with care to ensure safety in laboratory and industrial settings.

# Research and Development

p-Nitrophenylalanine (p-NPA) has garnered significant interest in various fields of research due to its unique properties and applications. One notable area of application is in protein engineering, where p-NPA serves as a building block for synthesizing modified proteins. Researchers utilize this compound to study the effects of nitro groups on protein structure and function, which can lead to advancements in understanding protein behavior and interactions[9][10].

In drug development, p-NPA is being explored as a potential candidate for creating novel pharmaceuticals. Its unique chemical properties allow for targeted approaches in developing compounds aimed at specific enzymes or receptors involved in various disease pathways[9][10]. This has implications for treating a range of medical conditions and advancing therapeutic strategies.

Additionally, p-NPA is utilized in biochemical assays to measure enzyme activity and interactions, providing critical insights into metabolic processes. Its role in neurotransmitter research further underscores its importance, as studies involving p-NPA help elucidate the relationships between amino acids and neurotransmission, thereby contributing to a better understanding of neurological disorders[9][10][31].

Moreover, the incorporation of p-NPA into polymer matrices is being investigated within material science. This integration enhances the material properties for applications in coatings and adhesives, indicating a broad spectrum of utility for this amino acid derivative beyond biological systems[9][10].

As research progresses, efforts are being made to optimize methods for synthesizing higher amounts of nitrated proteins. This endeavor is aligned with the long-term goals of developing applications related to vaccines or immunotherapies, supported by notable funding initiatives such as the AIChE Langer Prize and the NIH Director's New Innovator Award[18]. The establishment of entities like Nitro Biosciences further illustrates the commitment to advancing the research and application of p-nitrophenylalanine[18].

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