Using aza-Proline for the Assembly of a Melanostatin aza-Peptide Derivative

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Introduction

Due to their biochemical nature, bioactive peptides possess a short biological half-life and thus are generally not suitable to be used as pharmaceuticals. By incorporating aza-amino acid residues in biologically active peptides, the stability and bioavailability of peptide drugs are increased as a result of resistance towards peptidases.[1,2] The replacement of α-carbons with nitrogen atoms is known to increase the acidity of the amino group, which allows the establishment of stronger hydrogen bonds than the ones formed by proteinogenic amino acids.[2] This may also result in improved activity and selectivity of aza-peptides.[3] Moreover, the α-nitrogen atom can interchange between planar and pyramidal geometries in a dynamic manner,[4] making aza-amino acids very useful for the design of secondary structures in peptides and proteins.[2,4]

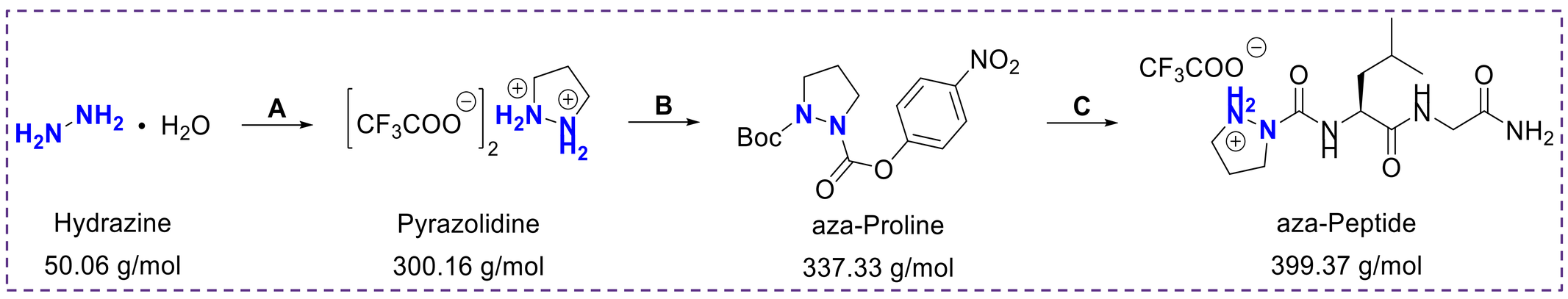
Melanostatin is an endogenous hypothalamic neuropeptide with the potential to be used as a treatment for Parkinson's disease.[5] Pharmacologically, this peptide binds to dopamine D_2 receptors (D_2R) and acts as a positive allosteric modulator (PAM), decreasing the amount of dopamine needed to activate them.[5] Since the D_2R are involved in the aetiology of Parkinson's disease, the development of potent PAM for these receptors is considered to be a good alternative to reduce the dependence on levodopa therapy, which has major side effects.

Objective

In this work, it is reported the preparation of aza-proline and its incorporation in Melanostatin neuropeptide for the assembly of the correspondent aza-peptide derivative. The main goal of this project is to study the influence of the aza-amino acid residue on the biological half-life and PAM activity of Melanostatin.

Synthesis

To assemble the Melanostatin aza-peptide derivative (scheme 1), we started with the synthesis of activated aza-proline from hydrazine, which has similar properties to ammonia. Next, we proceeded with the coupling of aza-proline with methyl L-Leucylglycinate dipeptide, followed by the conversion of the *C*-terminal ester into a primary amide.



Scheme 1. *Synthetic route used for the assembly of the Melanostatin aza-peptide derivative. Reactants and conditions:* ***(A)*** *i) [90%] Et_3N (2 eq.), Boc_2O (2 eq.), DCM. ii) [87%] 1,3-dibromopropane (1.5 eq.), NaOH 50% (aq.), catalytic Et_4NBr, toluene (90 ºC).* ***(B)*** *iii) [100%] TFA (30 eq.), DCM. iv) [97%] Et_3N (4 eq.), Boc_2O (1 eq.), DCM. v) [86%] Et_3N (1 eq.), para-nitrophenyl chloroformate (2 eq.), DCM (0 ºC).* ***(C)*** *vi) [42%] Et_3N (3 eq.), L-Leu-Gly-OMe • HCl (1 eq.), DCM. vii) [94%] NH_3 (7 M) in MeOH. viii) [100%] TFA (30 eq.), DCM.*

Conclusion

This work focused on the synthesis of a Melanostatin aza-derivative using aza-proline as a proline surrogate. A reliable and synthetic route was developed for the preparation of activated aza-proline from hydrazine in 5 reaction steps, with 68% global yield. Then, the aza-peptide was successfully assembled by peptide coupling with a global yield of 28%. The critical step of this synthetic route was the coupling of activated aza-proline with the dipeptide L-Leu-Gly-OMe, which requires further optimization.

This aza-peptide will be evaluated through pharmacological and biological assays to study, respectively, its potency and efficacy as PAM of D_2R and to assess its cytotoxicity. As such, this project is expected to further appraise the application of aza-peptides in the development of novel peptide pharmaceuticals, while studying an alternative treatment for Parkinson's disease.

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