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SYNTHESIS BY MACROCYCLIZATION OF PEPTIDE IN PRESENCE OF PHOSPHATE-BUFFERED SALINE (PBS) USING NOVEL METHOD

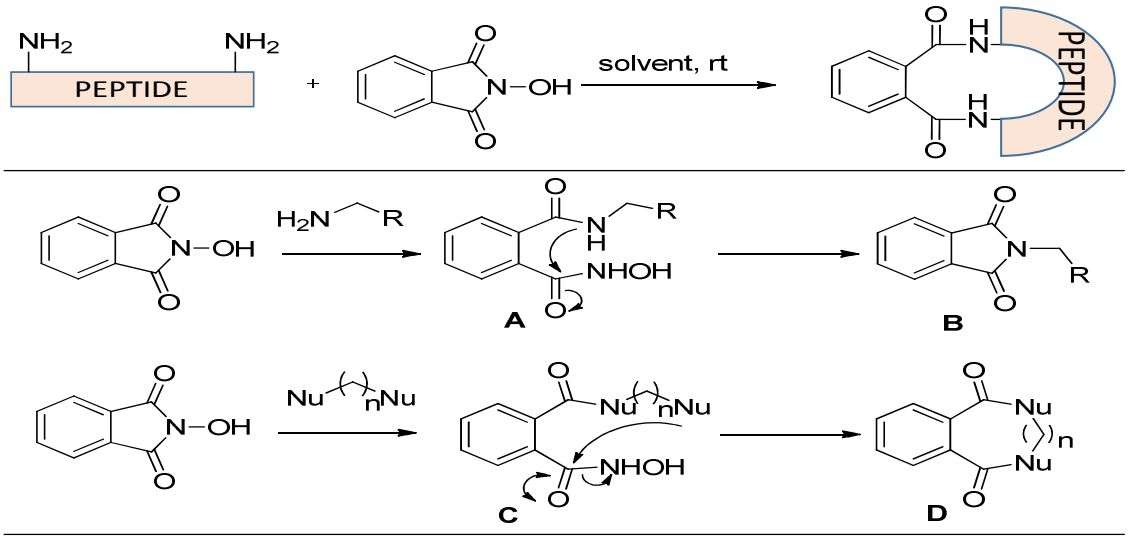
Abhijit V. Bachute[1]* and Santosh B. Gaikwad[2]

[1]*Department of Chemistry, Sambhaji Kendre Mahavidyalaya, Jalkot, Latur, MS, India-413532*
[2]*Department of Chemistry, Late Pundalikrao Gawali Arts and Science College, Shirpur (Jain), Washim, MS, India-444505*
***Corresponding Author:** *abhijitbachute327@gmail.com*

ABSTRACT
In this research article, we have introduced novel way for synthesis by peptide macrocyclization of lysine pair using side chain macrocyclization method in presence of phosphate-buffered saline (PBS). All the characterization are ongoing in laboratory because of future work. This method gives good yield of product.

KEYWORD: Macrocyclization, peptide, buffer, PBS.

INTRODUCTION
Methods for generating macrocyclic peptides, and their applications, have attracted considerable interest owing to the peculiar conformational and molecular recognition properties[i]. A number of cyclic peptides are used for their medicinal purpose such as antibacterial vancomycin, antifungal, immunosuppressant and anticancer therapies[ii]. Cyclization of peptides produces elevated metabolic stability and may lead to increased specificity or resemblance due to the changed conformational restrictions[iii]. Cell permeability of peptides can be modulated by cyclization extending the scope to drug delivery[iv]. Peptide macrocyclization is known for stabilizing the α-helices and shows further utility in inhibiting intracellular or extracellular protein-protein interactions *in vivo*[v]. Cyclic peptides also find utility as inhibitors of HIV fusion[vi] and as selective activators of enzymes involved in diabetes[vii].
Macrocyclization of peptides has been achieved by ring closing metathesis and organic molecules/reagents which can react selectively with a particular amino acid residue. Recently, a highly chemo-selective strategy for peptide cyclization has been developed which involves treating linear unprotected dicysteine-containing peptides with R, R′-dibromoxylenes, leading to a stable bis-thioether linkage[viii]. Growing interest for synthetic tools suitable for peptide macrocyclization has been apparent in last one decade. We hypothesized that lysine pairs can offer suitable functionalities for macrocyclization, if the method is enabled by transamidation through N-hydroxyphthalimide (**Scheme 1**).



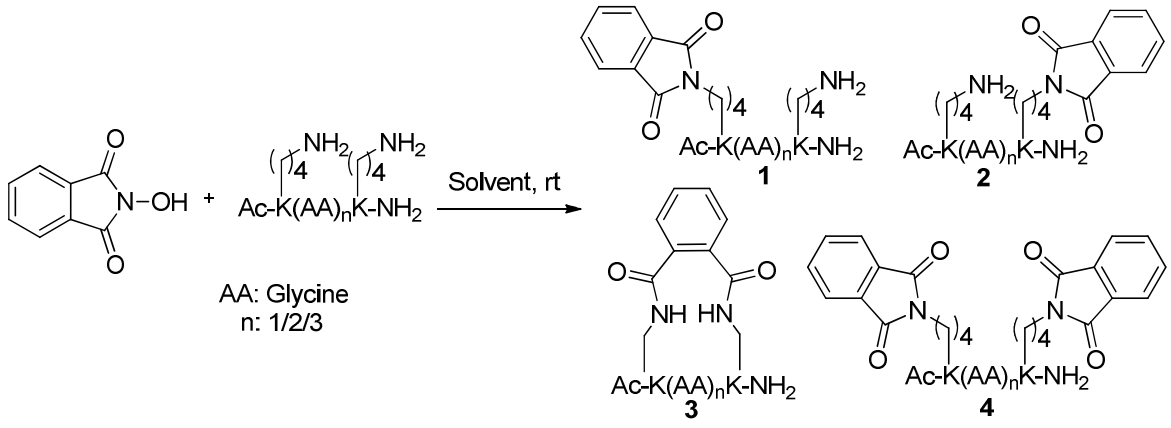
Scheme 1. *Hypothesis for macrocyclization of peptides using Lys pairs.*

N-hydroxyphthalimide is known to react with amine and leads to formation of final product **B** through an intermediate **A**. First step of this reaction is fast but the **A** to **B** is the slow step, so we can get an advantage of opportunity to trap an intermediate **A** by another nucleophile which is seating at close proximity. In such scenario attack of the second nucleophile should be faster than the formation of five membered ring. This can be done by playing around reaction parameters such as pH, concentration of reaction, and changing the positioning of two appropriate nucleophiles. Peptide macrocyclization can be done by this method which has number of applications and the area has been developed by ring closing metathesis and by using small organic molecules.

MATERIALS AND METHODS:

All the chemicals are purchased from Sigma-Aldrich and used without purification. Melting and boiling points of compounds are confirmed using digital calibration apparatus. All the peptides are purified using preparative HPLC.

RESULTS AND DISCUSSION:



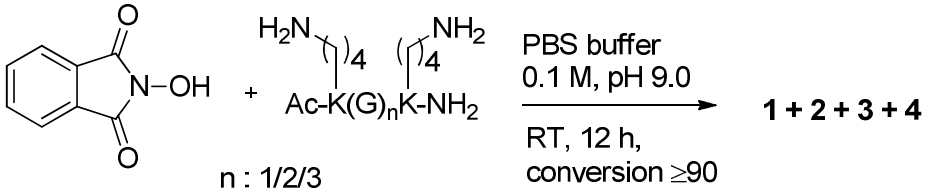
Scheme: *General synthetic approach for peptide microcyclization of product* ***1, 2, 3*** *and* ***4***

Table 1. Optimization of reaction conditions

S. No.	Solvent	Time (h)	Observation	Conversion (%)[a]
1	DMF	24	Mono-labeled	70
3	H_2O	24	Mono-labeled	80
5	Aq. buffer[b] pH 7.0	12	Mono- and Bis-labeled	80
7	Aq. buffer[b] pH 7.8	12	Mono- and Bis-labeled	90
9	Aq. buffer[b] pH 8.3	12	Mono- and Bis-labeled	90

[a] *Conversions are calculated by HPLC.* [b] *Phosphate buffer (0.1 M).*

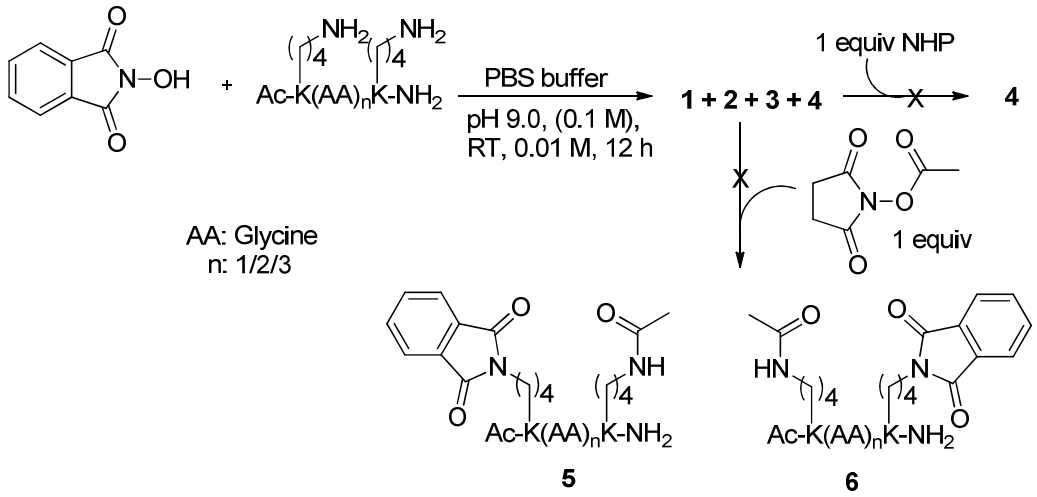
Since we know the nucleophilicity of ε-amine is very poor in pH range 7-8. So we need to work at higher pH range. We have done the reaction at pH 9.0 and got the mono-labeled product more than 90% conversion by HPLC.



Scheme 2. *Reaction at higher pH*

Since monophthalimidated product and cyclized products are having same molecular weight we have to confirm by some additional experiments. For confirmation of cyclization we can add excess of N-hydroxyphthalimide. If cyclized product is formed then another amine functionality can react with NHP and will end up with bis-labeled product. An alternative for this, we can add better electrophile which can react with unreacted amine moiety and can shows the mass difference.

After addition of excess of NHP instead of getting bis-labeled we got hydrolysed product that is acid formation in above conditions. With N-Succinimidyl Ester (NHS ester) starting material remains as such which kind of suggest that both the lysines are unreactive because of they are involved in macrocyclization.



Scheme 3. *Confirmatory experiments for cyclization*.

From **Scheme 3** we can conclude that possibility of product **1** and **2** is excluded whereas from mass spectra bis-labeled product **4** also is not formed. The cyclized product need to be analysed properly by ^{13}C and ^{1}H NMR. These modified peptides are purified by using preparative HPLC.

CONCLUSION:

In conclusion we developed a method for peptide macrocyclization. Achieving good isolated yield and characterization is ongoing in our lab. This will be a novel protocol for side-chain peptide macrocyclization of Lysine pair which can find further applications in proteomics.

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