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Highly efficient and fast pre-activation cyclization of the long peptide: Succinimidyl ester-amine reaction revisited

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ABSTRACT

A new method for the pre-activation cyclization of a long peptide is described. The approach involves the formation of a pre-activated succinimidyl ester species in advance of amidation, which completely eliminates the potentially troublesome amine end-capping side reaction. The cyclization reactions proceed with high efficiency and fast reaction kinetics for the long peptide with 25 residues. The exploration and large-scale preparation of synthetic cyclic peptides should become more accessible and feasible with this approach. This method has a potential to be further applied for the synthesis of much longer and more complex cyclic peptides.

Molecules with cyclic topology are not only attractive from an aesthetic point of view but also offer a number of functional advantages over their linear counterparts. Peptides, in particular, are significantly affected by the topological effects of cyclization.[1,2] Cyclic peptides are resistant to proteolytic degradation and are conformationally constrained. Such conformational rigidity helps to minimize the entropic penalty associated with the target binding and better mimics the stabilized native state of a folded protein.[3] For these reasons, cyclic peptides have been considered promising high molecular weight drug candidates.[4,5] Moreover, cyclic peptides have shown interesting characteristics as building blocks for self-assembly.[6–8] Cyclic peptides enable the formation of self-assembled peptide nanostructures with stable secondary structures, unusually high thermostability, and well-controllable morphological features.

Peptides are among the largest cyclic molecules. Owing to the advent of various cyclization reaction chemistries, the preparation of relatively low molecular weight cyclic peptides is generally straightforward.[1,2,9–14] However, when it comes to the synthesis of large cyclic peptides, it is notoriously problematic. Peptides or proteins can also be cyclized by biological methods; however, but they are limited in making nonpolar, amphiphilic, and chemically modified peptides.[15,16] Because the sizes and compositions of peptides used for chemical cyclization differ from one literature to another, there is no clear definition of the size requirements for efficient peptide cyclization. Surveying the literature reveals that peptides used for cyclization are typically no more than 20–25 amino acids long. In our experience, peptide cyclization reaction becomes very difficult, if not impossible, with peptides longer than approximately 20–30 amino acids. Therefore, the development of a novel reaction and methodology for the facile cyclization of long peptides is indispensable for developing highly functional molecules and materials based on peptides.

Both ends of a peptide should lie in close proximity for intramolecular cyclization to occur, as this would otherwise result in intermolecular oligomerization. To prevent this intermolecular reaction, peptide cyclization is usually performed under high dilution or pseudo-high dilution conditions.[17] Intramolecular cyclization reactions are generally much slower than intermolecular reactions.[18] The probability that both ends lie in close enough proximity to react decreases exponentially as the size of the peptide increases. Hence, the synthesis of long cyclic peptides is known to be very difficult.[1]

Amide bond formation between the ends of a protected peptide fragment is one of the most common methods for cyclization. One problem is that most coupling reagents that are used in amide formation are degraded during prolonged cyclization reactions.[19] The cyclization of a 20–30 residue peptide often requires overnight to several days of reaction time. Worse still, the nucleophilic amines of long peptides are more likely to be end-capped than those of small peptides by coupling reagents during the slow cyclization and carboxyl activation steps.[19,20] Guanidino formation is the most common example of end-capping that permanently terminates

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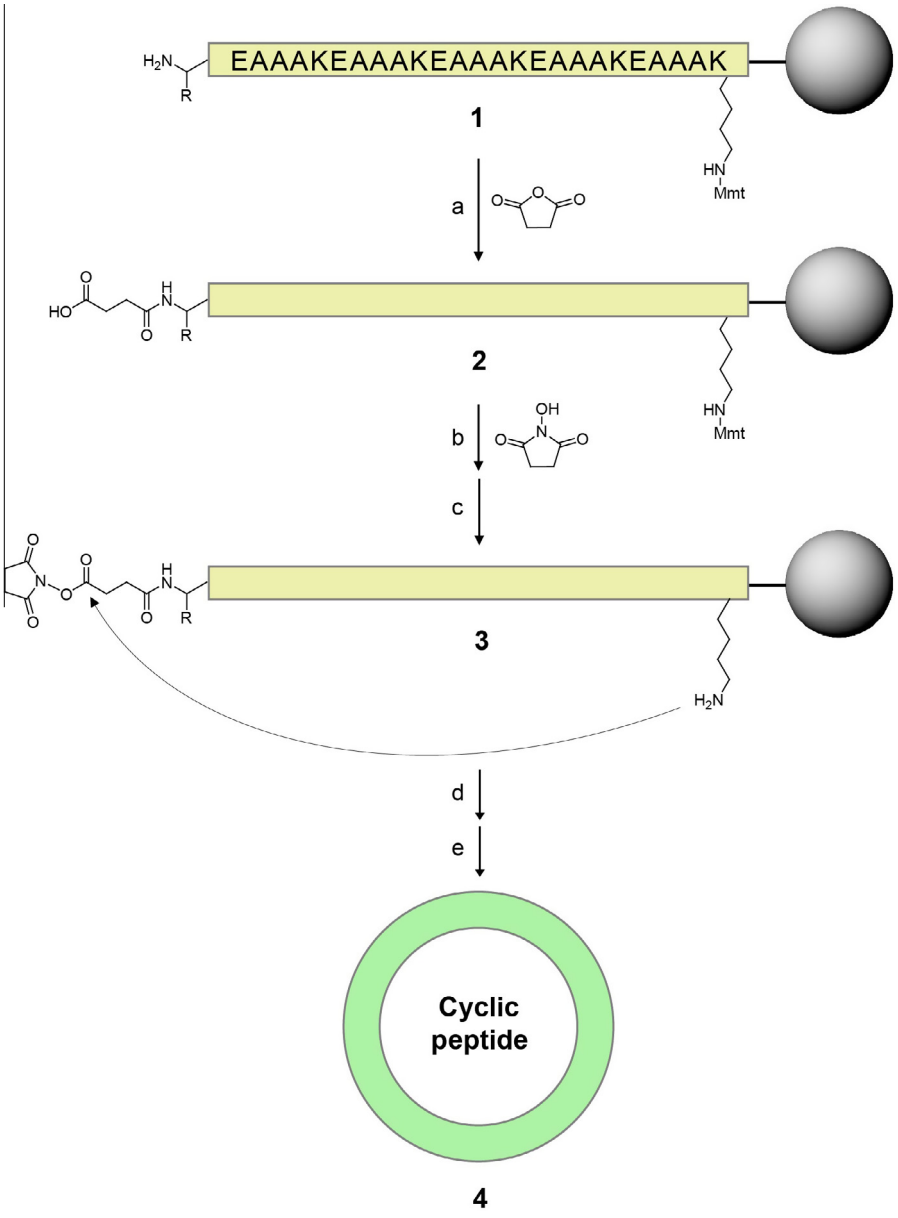

Figure 1. Synthetic strategy using pre-activated NHS esters for on-resin cyclization of cyclic peptides. (a) Succinic anhydride, DIPEA, NMP; (b) NHS, DIC, CH_2Cl_2; (c) AcOH/TFE/CH_2Cl_2 (1:2:7); (d) 2% DIPEA, NMP; (e) TFA/1,2-ethanedithiol/thioanisole (95:2.5:2.5).

amine reactivity. When long peptides are end-capped, they often exhibit similar retention times as the cyclized peptide in high performance liquid chromatography (HPLC) purification. Combined with many known and unidentifiable side products formed during this difficult and long process, the presence of end-capped peptides often necessitates multiple purification steps or renders the purification of the cyclized products almost impossible.

Amide bond formation in peptide synthesis is accomplished by using amino acid active esters, which can be included as pre-activated species or formed by in situ activation. *N*-Hydroxysuccinimide (NHS) is commonly used to activate carboxylic acids in amino acids and other biochemicals. Since its introduction five decades ago,[21,22] this method has been widely used in the syntheses of peptides and bioconjugates. At present, carboxyl activation by NHS ester formation is no longer the primary method of amide formation in peptide synthesis due to the advent of more efficient in situ carboxyl activation reagents, such as various uronium and phosphonium salts.[23–25] However, the NHS method is probably the most frequently used method for bioconjugation.[26] Compounds activated with NHS esters can be isolated and stored for long periods, which is why many compounds are commercially available in NHS ester-activated forms.

Here, we report the efficient cyclization of long peptides using pre-activated NHS esters of the protected peptide (Fig. 1). We hypothesized that forming the NHS ester in sequential steps, including coupling reagent removal, amine deprotection, and cyclization, should completely eliminate the problem of amine end-capping. The use of NHS for in situ activation and cyclization has been reported;[2] however, to our knowledge, this is the first time that a pre-activated NHS ester species has been used in peptide cyclization. Although simple, this new method enabled long-peptide cyclization reactions to proceed efficiently and quickly. This will make the exciting world of cyclic peptides more accessible and the large-scale production of cyclic peptides more feasible.

For cyclization, we used a relatively long peptide consisting of 25 amino acids. The reaction was performed on-resin using a