Preference of amino acid residues in the synthesis of cyclic peptides

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Abstract

Many cyclic peptides show promising biological activities, such as anticancer, antibacterial, antiviral, antifungal, anti-inflammatory, and anti-clotting or anti-atherogenic properties. Synthetic cyclic peptides with a stable backbone conformation can be tools for mapping the biologically active conformation of peptide sequences and may thus represent an intermediate step in the path from polypeptide to peptidomimetic. Moreover, their constrained geometry enables conformational investigations in epitope/pharmacophore studies, which provide a predictable conformation of diverse functionalities around a core, whose flexibility depends on the ring size. However, cyclohexapeptides are constrained cyclic peptides whose synthesis is considered difficult. It investigated a methodology based on on-resin head-to-tail cyclization by anchoring the side chain of an amino acid. In this study, linear and cyclic hexapeptides, cyclo(D-Ser-Lys-Xaa-Lys-Xaa'-Lys) (Xaa and Xaa'= Gly, D-Pro or D-Ala), were synthesized, and full geometry optimizations were performed using the molecular mechanics (MMFF) and semi-empirical molecular orbital method.

Keywords; cyclization, cyclohexapeptide, molecular orbital calculations, MMFF, PM3

1. Introduction

Cyclic peptides are important tools in medicinal chemistry because they exhibit a reduced conformational flexibility compared with their linear precursors, which results in improved metabolic stability and possibly enhanced biological activity, receptor selectivity and bioavailability[1-5]. Moreover, their constrained geometry enables conformational investigations in epitope/pharmacophore studies[6, 7], which provide a predictable conformation of the diverse functionalities around a core, whose flexibility depends on the ring size.

This study was approach to the cyclic peptide libraries features a solid phase-based formation of head-to-tail cyclic peptides with on-resin ring closure[8-10]. In this strategy, at least the initial resin-linked residue can be fixed at a position. To provide greater structural diversity for the range of cyclic peptides, pseudopeptides, and peptide mimetics, the preference of amino acid residues was explored[11-13].

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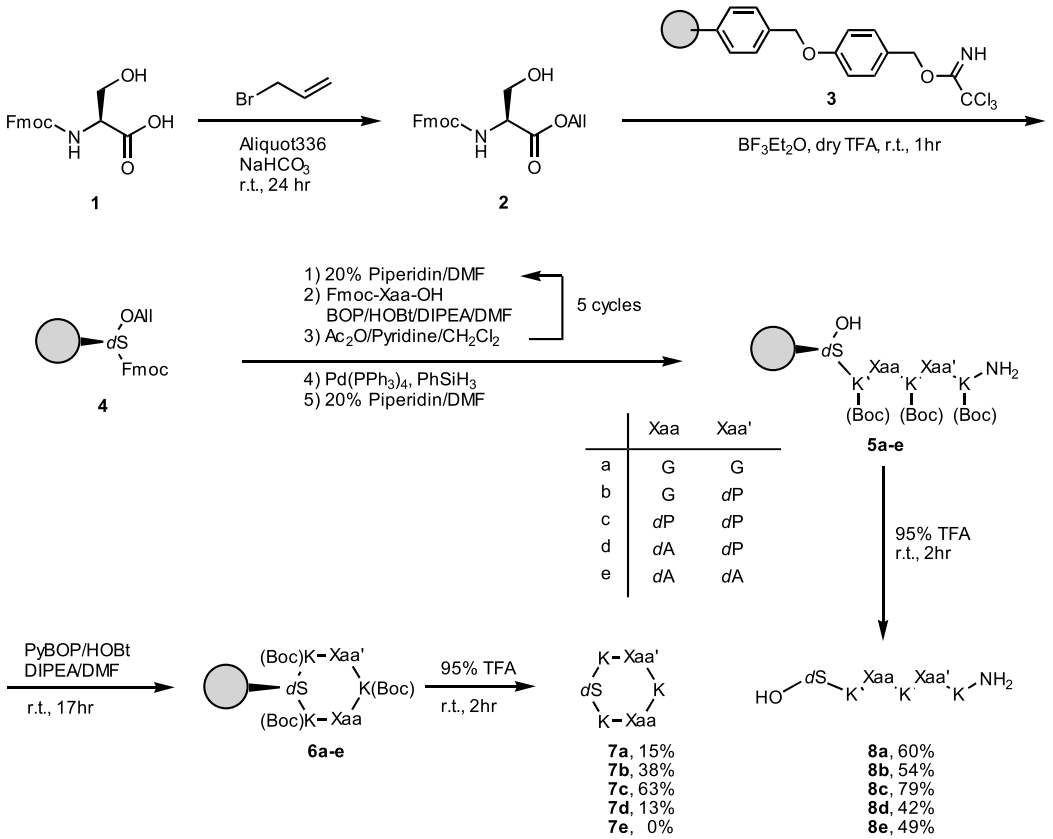
Cyclic peptides were first synthesized using classical methods of peptide chemistry in a solution[14]. Following the development of solid-phase peptide synthesis (SPPS), there were reports on the successful synthesis of head-to-tail cyclized peptides using solid-phase support[15-17]. An excellent review article covers the present state of the art synthesis of cyclic peptides on solid-phase carriers.

A general method for the SPPS of cyclic peptides containing an Asp residue in their sequence was proposed by Roverro et al[10]. The synthesis was performed using the starting form of an Asp-OFm residue linker to PAM-resin through its β-carboxylic acid group. After the linear precursor was synthesized using the Boc/benzyl strategy and the Boc protecting group on the N-terminal amino function was removed using TFA, the C-terminal carboxyl group of the Asp residue was selectively deprotected with piperidine, and cyclization was easily accomplished using the BOP method[18]. The final Hartree-Fock (HF) deprotection of the side chains of the trifunctional residues with concomitant cleavage from the resin produced the cyclic peptide as a single product of good purity in a reasonable quantity. This approach was used for the first reported synthesis of "head-to-tail" cyclic peptide libraries[19].

This paper describes a systematic study



Scheme 1

of the synthesis of cyclohexapeptides by on-resin head-to-tail cyclization using the Fmoc/Boc/OAll three-dimensional protection scheme (Scheme 1).

A series of cyclic hexapeptides, cyclo(D-Ser-Lys-Xaa-Lys-Xaa'-Lys) (Xaa and Xaa' = Gly, D-Pro or D-Ala), were selected as the model peptides. In the theoretical frame work of cyclic reactions, the reactivity differences in a series of cyclic hexapeptides can often be analyzed and predicted by using molecular mechanics (MMFF) and semi-empirical molecular orbital theory. Therefore, I applied this theory to the reactivity of the selected cyclic hexapeptides.

2. Results and Discussion

The use of a three-dimensional protection scheme enables the orthogonal deprotection of the C- and N-termini for the subsequent on-resin head-to-tail cyclization. The syntheses were performed by anchoring Fmoc-D-Ser-OAll **(2)** to the resin through its side chain. The D-Ser side chain was bound to the Trichloroacetimidate Wang resin **(3)**, which was selected because of the versatility of its linker to further exploit the cyclopeptide libraries that contained different functional amino acids. The liner hexpeptides Fmoc-Lys-Xaa-Lys-Xaa'-Lys-D-ser(resin)-OAl **(5a-e)** were synthesized using BOP/HOBt/DIPEA as the coupling. To avoid a nucleophilic attack of the free amino function, which would lead to by-products, the C-terminal carboxyl function must be deprotected before the last Fmoc removal. Then, allyl deprotection was performed using $Pd(PPh_3)_4$ in $CHCl_3$/AcOH/NMM under anhydrous conditions. The treatment of the resin with 20% piperidine in DMF caused the liner peptides **8a-e** to be anchored to the solid support and deprotected at the C- and N-termini. Cyclization was performed using PyBOP/HOBt/DIPEA as the coupling. Cleavage from the resin provided the cyclohexpeptides **7a-e** (Scheme 1). Cyclohexpeptides **7a-e** were characterized using the reverse-phase high-performance

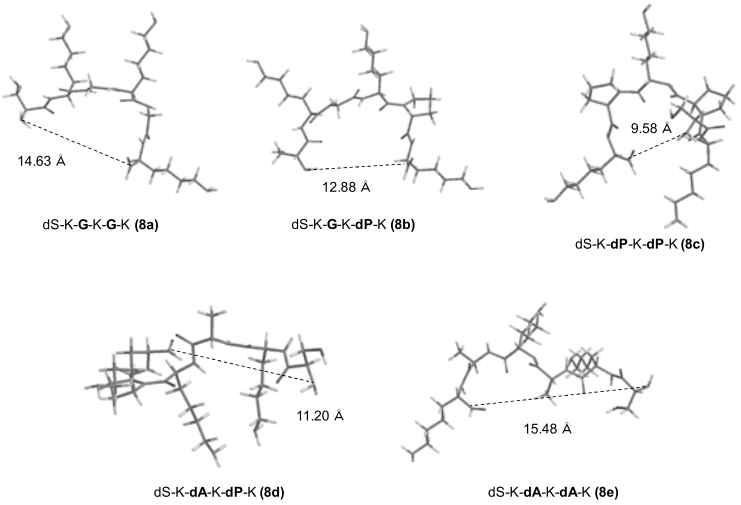


Fig. 1. PM3- calculated minimum energy conformations of hexapeptides.

liquid chromatography (RP-HPLC) and ninhydrin test. Cyclohexpeptides **7e** did not react in the RP-HPLC analysis.

To determine the reactivity differences in the cyclization, the minimum energy conformations of the cycloadduct reactions were calculated using molecular mechanics and semi-empirical molecular orbital method. A computational search of the hexapeptides was performed with the molecular modeling package Spartan '14 version 1.1.8[20] using an MC method, the MMF94 force field[21] and a semi-empirical quantum mechanical geometric optimization PM3[22] on a HP Z420 Workstation.

The PM3-calculated bond geometries and a graphical representation of hexapeptides **8a-e** are shown in Fig. 1.

In the PM3 calculation of the structures of hexapeptides **8e** containing D-Ala, the C- and N-termini distance is 15.48 Å. However, the C- and N-termini distance of hexapeptides **8c** containing D-Pro is 9.58 Å, which is shorter than that of **8e**. The energies of the MMFF and PM3 of the hexapeptides are provided in Table 1.

Table 1. MMFF- and PM3- calculated energies of cyclization.

Compound	MMFF		PM3	
	Energy[a]	ΔE[a]	Energy[a)]	ΔE[a]
7a	288.2	34.8	-1215.8	60.9
7b	397.6	73.7	-1264.2	32.7
7c	493.2	84.4	-1272.5	40.4
7d	397.6	29.7	-1319.5	-58.9
7e	333.7	-36.3	-1311.2	-41.1
8a	253.4	–	-1276.7	–
8b	323.9	–	-1296.9	–
8c	408.8	–	-1312.9	–
8d	367.9	–	-1260.6	–
8e	370.0	–	-1270.1	–

[a)] kJ/mol

The MMFF-and PM3-calculated minimum energies for hexapeptides **8c** and **8e** are 408.8 (MMFF), -1312.9 (PM3), 370.0 (MMFF) and -1270.1 (PM3) kJ/mol, respectively. The cyclohexapeptides **7c** and **7e** are 493.2 (MMFF), -1272.5 (PM3), 333.7 (MMFF) and -1311.2 (PM3) kJ/mol, respectively. Cyclization **7e** has higher ΔE (MMFF: -36.3 kJ/mol) and ΔE (PM3: -41.1 kJ/mol) than **8c**. For the cyclohexapeptides, the energy differences of the ΔE controlled reaction increase in the order of **8e** < **8c**, which is qualitatively consistent with the experimental results (see Scheme 1).

3. Conclusion

This study was describe a general method for the SPPS of "head-to-tail" cyclic peptide. The cyclization of Ala residues during the SPPS appears to be an underscored problem. The reaction barriers in the cycloadditions of **8e** are higher than those of **8c**, which indicates that the cycloadduct does not readily experience cycloadditions to produce hexapeptides. The present strategy is theoretically applicable to sequences that contain Ala residue.

4. References

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環状ペプチド合成におけるアミノ酸残基の影響

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キーワード：環化反応，環状ペプチド，分子軌道計算，MMFF，PM3

要　約

近年、生理活性を有する低分子化合物として、人工ペプチドが注目されている。その中でも環状ペプチドは、抗癌、抗バクテリア、抗ウイルスなどの生理活性が期待されている。しかしながら、環状ペプチド合成において、鎖状ペプチドにおけるアミノ酸残基が、環化反応時のコンフォメーションに影響を与え、環化反応が進行しないことが知られている。そこで、環化反応におけるアミノ酸残基の影響について、Head-to-tail 法によるヘキサペプチド環化反応を用い、合成的および計算化学的手法による検討を行った。