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Influence of steric effects in solid-phase aza-peptide synthesis

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ABSTRACT

Aza-peptides are peptide analogs with potential applications as drug candidates. However, due to difficulties associated with the synthesis of these compounds, information regarding their bioactivity is very limited. Herein, we identify steric hindrance as one reason for the slowness of the aza-peptide bond formation reaction. The steric effect of the side group of amino acids in their coupling with the semicarbazide moiety in the synthesis of a model peptide, H-AA-AzAla-Phe-NH_2, was studied and quantified using COMU as a coupling reagent. Characterization of the role of this structural factor in aza-peptide bond synthesis is essential for outlining a new and efficient synthesis protocol.

Natural peptides are often unsuitable for therapeutic use because of their rapid metabolism in blood.[1,2] As a result, many types of peptidomimetics have been designed to overcome this pharmacological disadvantage, while retaining or even enhancing the biological effects of the parent peptides.[2,3] Among these peptidomimetics are aza-peptides, which contain amino acid analogs where the C^{α} atom is replaced by a nitrogen atom.[4] Although this replacement provides sterically similar peptide analogs, these peptidomimetics lack chirality and possess reduced backbone flexibility.[5] These specific conformational properties make aza-peptides an attractive tool for the design of drugs whose effectiveness is dependent on their recognition by the target sites.[6,7]

However, due to complications associated with the chemical synthesis of aza-amino acid precursors as well as the aza-peptide bond, these peptidomimetics have been studied only occasionally.[3,8–13] The low yields for aza-peptide synthesis, using conventional peptide synthesis protocols, can be explained by the significantly lower nucleophilicity of the nitrogen atom of the *N*-terminal amino group in the aza-amino acid residue when compared with the corresponding amino acid nitrogen atom.[14,15] However, this fact has not been generally recognized, as many attempts have been made to use conventional peptide chemistry protocol for aza-peptide bond synthesis.[9–11,16–18]

Recently, we reported the applicability of various conventional SPPS amino acid activators for aza-peptide bond synthesis.[19] For this purpose, kinetic investigations were made into aza-peptide bond formation in the case of a model aza-peptide, H-Ala-AzAla-Phe-NH_2, utilising various coupling reagents. The reaction rates and yields were compared, and it was found that the rate of this reaction was correlated with the leaving group ability in the activated Fmoc-alanine molecule, quantitatively modeled by the acidity of the activator. Following this trend, oxyma-based activators COMU[20] and PyOxim[21] led to nearly complete aza-peptide bond formation, but the reaction time was approximately 30 times longer than in the case of conventional peptide synthesis.

However, in parallel with the impact of the leaving group acidity that seems to quantify polar effects, it is possible that steric hindrance of the activated amino acid, by analogy with the classical nucleophilic substitution reaction in ester molecules,[22] may also affect the rate of the aza-peptide bond formation reaction; this hypothesis has been confirmed in this report. Quantification of the role of polar and steric effects in this reaction is important for the systematic design of new types of activators needed to increase the reactivity of amino acids for efficient acylation of the semicarbazide moiety in aza-peptide bond synthesis.

The importance of the steric effect in substitution reactions at the carbonyl group has been formulated in the pioneering work of Taft,[23] and since then several experimental procedures for the quantification of this influence have been proposed.[22,24,25] In this work, we analyze the influence of the steric effect on the rate of aza-peptide formation in the model compound H-AA-AzAla-Phe-NH_2, where AA stands for various bulky amino acids linked to the aza-dipeptide *via* an aza-amino acid bond.

For this study, the resin-bound aza-dipeptide H-AzAla-Phe-NH_2 **3** (Scheme 1) was synthesized (see ESI for details). Thereafter, the kinetics of the reactions of various activated amino acids **2** with the model aza-dipeptide **3** were investigated under conditions defined

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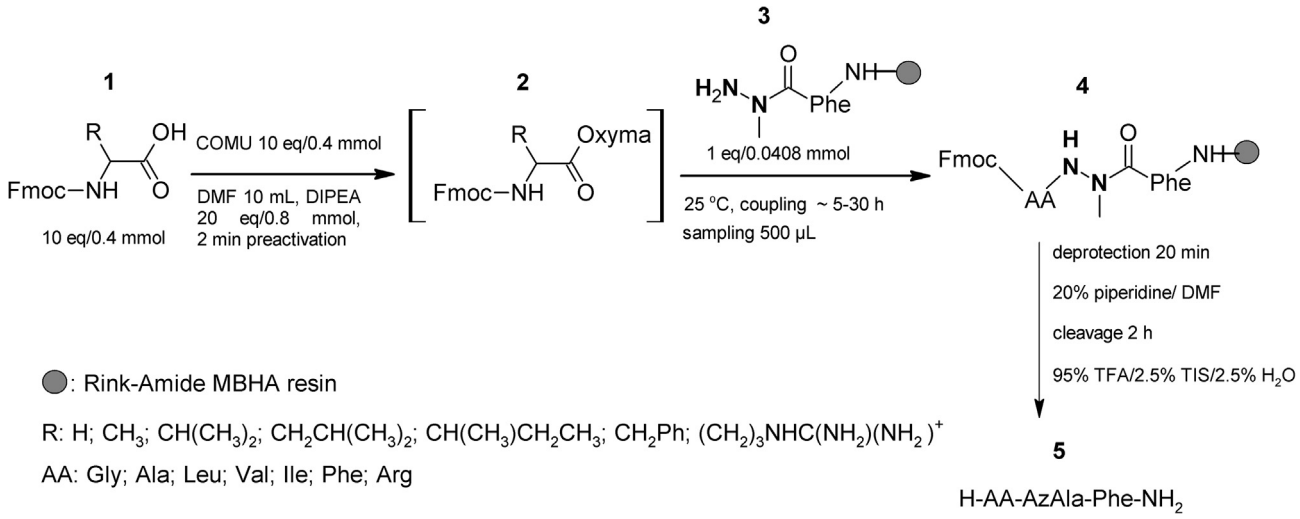

Scheme 1. Model aza-peptide H-AA-AzAla-Phe-NH_2 synthesis.

by the conventional SPPS protocol. The amino acids **1** were chosen so that the substituent (R) attached to the α carbon did not contain a protecting group. COMU was selected as the amino acid activator because oxyma-based activators gave the best yields of aza-peptide bond formation in our previous study.[19]

Kinetic experiments were carried out under pseudo-first order conditions using a tenfold excess of amino acid relative to the number of resin-bound reaction sites. At appropriate times, aliquots were taken from the reaction mixture during the coupling reaction and analyzed *via* HPLC (see ESI for details). The process was described using a first-order rate equation:

$$Y = e^{-kobst} + Y_\infty \quad (1)$$

where k_{obs} is the observed first-order rate constant, t is the reaction time, and Y_∞ is the plateau value that is reached at the end of the acylation reaction. Parameter Y characterizes the tripeptide **4** formation process and was calculated using the dipeptide and tripeptide peak areas (*S*) from the same chromatographic run:

$$Y = \frac{S_{\text{dipeptide}}}{S_{\text{dipeptide}} + S_{\text{tripeptide}}} \quad (2)$$

The observed first-order rate constants (k_{obs}) and acylation reaction yields ($1 - Y_\infty$) were determined in DMF at 25 °C. The kinetic curves obtained under these conditions are shown in Fig. 1, and the results of the kinetic experiments are summarized in Table 1. From the reaction yields given in Table 1, it can be seen that practically complete conversion of the dipeptide into the tripeptide was achieved using COMU as a coupling reagent. Therefore, COMU may be applicable for the synthesis of various aza-peptide sequences, if appropriate reaction times are used.

Table 1
Kinetic study of aza-peptide bond formation *via* the reaction of Fmoc-AA-OH with the semicarbazide group of the resin-bound H-AzAla-Phe residue in DMF using COMU as an activator at 25 °C. Eq. (1) was used to calculate the k_{obs} and yield ($1 - Y_\infty$) values using Graphpad 5 software.

AA	k_{obs}, min^{-1}	Yield	Ξ[a]
Gly	0.0330 ± 0.006	0.89 ± 0.07	0.00
Ala[19]	0.0217 ± 0.001	0.99 ± 0.01	1.28
Leu	0.0117 ± 0.001	0.99 ± 0.03	2.59
Val	0.0018 ± 0.0002	0.89 ± 0.03	3.67
Ile	0.0012 ± 0.00006	0.94 ± 0.02	4.19
Phe	0.0209 ± 0.001	0.91 ± 0.05	2.94
Arg	0.0160 ± 0.002	0.92 ± 0.042	2.34

[a] Graph shape index. The parameter is calculated from the molecular graph structure of the amino acid side chain.[26]

It can be seen from Table 1 that the rate of the acylation reaction, characterized by the rate constant k_{obs}, depends on the structure of the side group of the amino acid. More explicitly, Gly reacts almost 1.5 times faster than Ala and 2.8-fold faster than Leu. Coupling reactions with Val and Ile occur nearly 6.5- to 28-fold slower than in the case of the former amino acids. This difference is

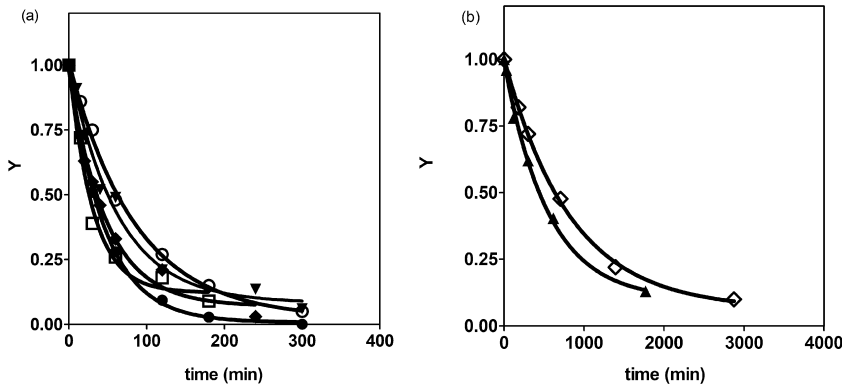

Fig. 1. Time evolution of the reactions between resin-bound H-AzAla-Phe and COMU-activated (a) Fmoc-Ala-OH (●), Fmoc-Gly-OH (□), Fmoc-Leu-OH (○), Fmoc-Phe-OH (◆), Fmoc-Arg(Pft)-OH (▼); (b) Fmoc-Ile-OH (◇), Fmoc-Val-OH (▲) during aza-peptide bond synthesis at 25 °C in DMF.