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First Application of the Combined *o*-NPS N$^{\alpha}$-Protection/Carpino's Acylfluoride C$^{\alpha}$-Activation Methods to the SPPS of Very Hindered Peptide Sequences

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Introduction

We developed a novel, improved dyad of α-amino protection/α-carbonyl activation procedures in SPPS. This combination demonstrated to be very appealing, in particular with the sterically hindered, C$^{\alpha}$-tetrasubstituted α-amino acids. The *ortho*-nitrophenylsulfenyl (*o*-NPS) α-amino protection (Figure 1), which cannot generate the chirally dangerous and poorly reactive 5(4H)-oxazolone intermediate, was used in conjunction with the Carpino's highly efficient α-aminoacyl fluoride (Figure 1) C-activation approach [1].

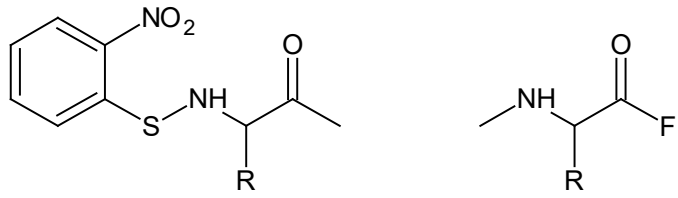

Fig. 1. Chemical structures of the derivatives o-NPS-α-aminoacyl (left) and α-aminoacyl fluoride (right) moieties.

Results and Discussion

In the course of our experiments, we modified gradually the spatial congestion of the -NH_2 moiety of the amino substrates along with the properties of the reaction conditions, in particular the coupling efficacy of the carboxyl component.

We first SPPS synthesized the pentapeptide Fmoc-(Aib)$_4$-L-Ala-OH using the Carpino's procedure, specifically with Fmoc-Aib-F and the Fmoc-L-Ala-SASRIN α-amino acid resin. This method is confirmed to be *highly efficient*.

Then, by employing the same SPPS procedure as above, we made an attempt to prepare the *much more hindered* tetrapeptide sequence -[D-(αMe)Val]$_4$-, using five equivalents of Fmoc-D-(αMe)Val. In this case, the synthesis essentially *failed* and the desired final compound was *not* found.

In the third step, our synthetic target was the tripeptide *o*-NPS-L-(αMe)Phg-Aib-D-(αMe)Phg-O*t*But by *solution* methods. We utilized o-NPS-Aib-F and (separately) the two enantiomeric *o*-NPS-(αMe)Phg-F. The α-amino group of the nucleophile was preactivated with BTSA [N,O-*bis*-(trimethylsilyl)-acetamide]. The final product was obtained in excellent (92%) yield after chromatographic purification.

We *initially* applied the *o*-NPS protection to *SPPS* for the production of the longer hexapeptide -L-Ala-(Aib)$_4$-L-Ala- by use of the *milder* Carpino's activation *HATU/HOAt* [2] method, Fmoc-L-Ala-SASRIN, and *o*-NPS-Aib-OH. The expected product was generated in a *very limited* percentage.

Our next step was the *first* application of the *o*-NPS-/-CO-F dyad to *SPPS* [in the synthesis of the pentapeptide sequence -L-Ala-(Aib)$_3$-L-Ala-]. Here, we took advantage of Fmoc-L-Ala-SASRIN and

o-NPS-Aib-F. Satisfactorily, the *largely major* peak in HPLC is that corresponding to the target product.

Finally, we prepared by SPPS *the very much congested* (at its *N*-terminus) tripeptide -[L-(αMe)Phg]$_2$-L-Ala-OH. To this end, we exploited *o*-NPS-L-(αMe)Phg-F and BTSA (N,O-bis(trimethylsilyl)acetamide). The HPLC and mass spectrometry results are *extremely* promising (88% overall yield).

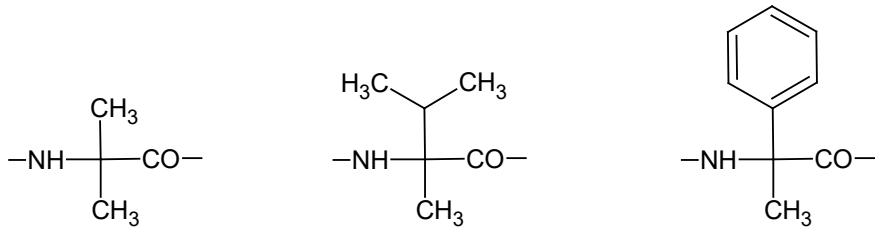

Fig. 2. Chemical structures of the C^{α}-tetrasubstituted α-amino acids used.

References

1. Carpino, L.A., Sadat-Aalaee, D., Chao, H.G., and DeSelms R.H. *J. Am. Chem. Soc.* **112**, 9651-9652 (1990), http://dx.doi.org/10.1021/ja00182a041
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