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Original Article

On-resin Intramolecular Chemoselective Oxime Bond Formation to Cyclic Peptides

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Abstract

The synthesis of a peptide containing an oxime bridge performed on solid-phase is described. The strategy used takes advantage of selective acidolytic removal of Boc and acetal protecting while minimally cleaving a PAL anchor, as well as compatibility of PEG-PS resin supports with aqueous conditions for oxime formation.

Key words: cyclic peptide, oxime bond, on-resin, intramolecular bridge.

Introduction

Due to the frequency of helical secondary structures in peptide and proteins, considerable effort has been directed toward design and synthesis of different bridges stabilizing helical structures (1, 2). Synthetic helical peptides have been achieved through the incorporation of covalent or non-covalent linkages between constituent amino acid side chains. Examples include salt bridges (3), lactams (4), disulfide bridges (5), hydrophobic effects (6), metal ligation between natural (7) and unnatural amino acids (8). Usually substantial helix stabilization has been achieved when the tether was positioned between *i* and *i+4* or *i+7* residues in the peptide backbone.

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Materials and Methods

All reagents and chemicals used were of analytical grade.

The present focus concentrates in the synthesis of *consolidated ligands*, which combine in the same molecule peptide sequences recognized by SH2 and SH3 domains (i.e., Pro-Val-Tyr-Glu-Asn-Val and Pro-Pro-Ala-Tyr-Pro-Pro-Pro-Pro-Val-pro, respectively), and exhibit enhanced affinities and specificities towards dual SH (32) Abelson Kinase (9, 10). For first generation consolidated ligands, binding sequences were connected by a flexible linker, e.g., Glyn. With the goal to further improve their efficacies, several second generation consolidated ligands with a more rigid linker, e.g., Alan, and optionally including an intramolecular lactam bridge "lock", were designed (figure 1) and synthesized(11).

Probably due to lactam bridge step, the synthesis of this peptide was troublesome by the earlier attempts (12). To overcome these difficulties, we successfully performed on resin intramolecular oxime bridge, using regioselective reaction between an aldehyde and an aminoxy partner, with a model sequence from the original 32 residue structure which includes an 11 residue peptide containing an *i* to *i+7* bridge to connect the side chains of Glu and Lys(Figure 2).

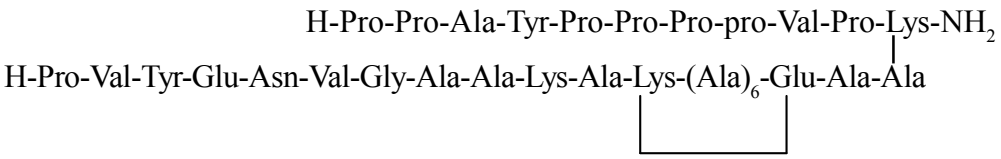


Fig. 1: consolidated ligand

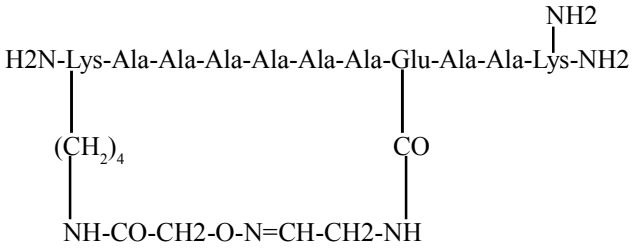
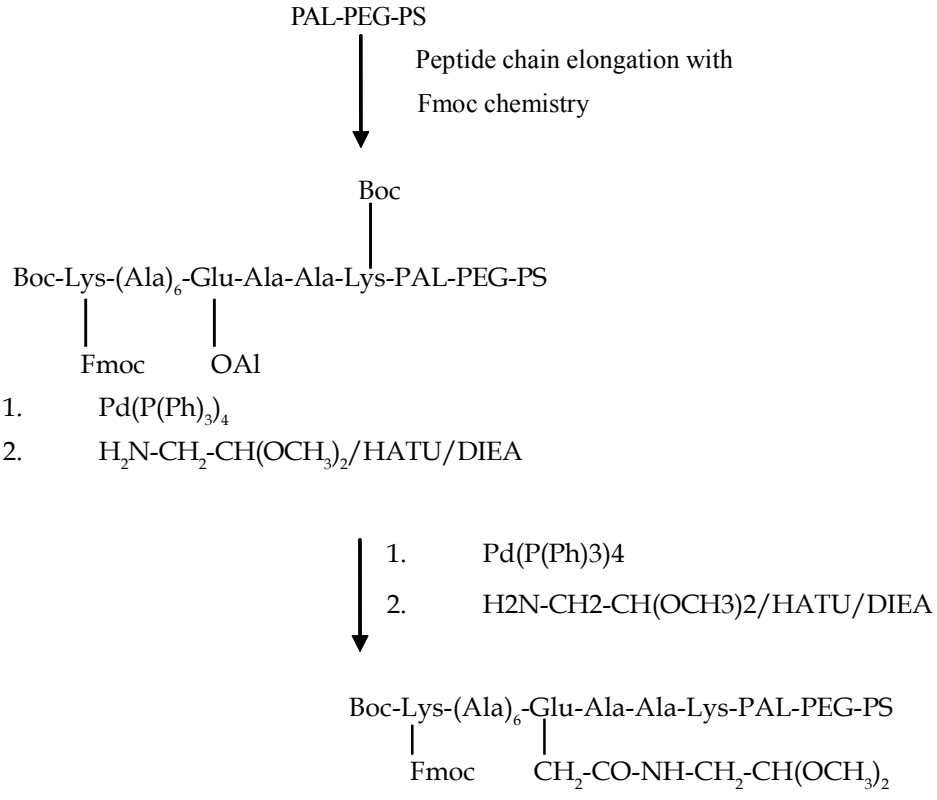
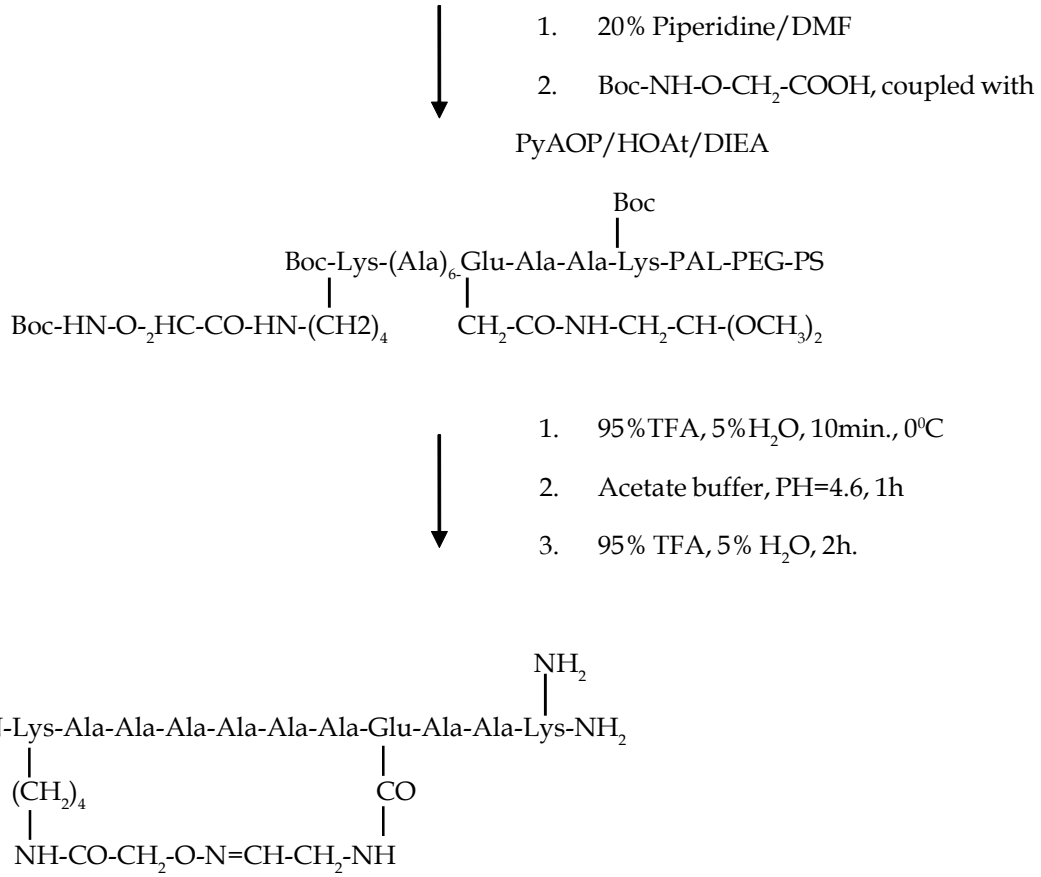


Fig. 2: 11 residue peptide with an oxime bridge

In the strategy depicted in scheme 1, we assume that 1) it is possible to optimize a TFA treatment condition which will be strong enough to remove Boc and acetal protection and thus, generate both aldehyde and aminoxy simultaneously without cleaving of peptide-PAL bond, 2)it was possible to perform on-resin intramolecular bridge by oxime bond without any acylation step.





Scheme 1: Stepwise synthesis of on-resin cyclization by oxime bond

The starting point was a PAL-PEG-PS resin (0.17 meq/g), on a Pioneer peptide synthesis system and used standard Fmoc-strategy with HBTU/HOBt/DIEA. To perform selective deprotection, Glu was introduced as Fmoc-Glu(OAl)-OH and the N-terminal lysine was introduced as Boc-Lys (Fmoc)-OH. A 4-fold molar excess of activated amino acids were used for all the couplings.

Results and Discussion

To valid our strategy, the stability of peptide-PAL bond to the TFA treatment conditions, in terms of concentration, temperature and reaction time, used for the Boc deprotection and the demasking of aldehyde was investigated. The resin stability was tested with a model tripeptide Ala-Gly-Ala synthesized using Fmoc strategy on PAL-PEG-PS resin. The conditions 95% TFA containing 5% H2O at 00C for 10 min. used to remove an acetal protection revealed that the cleavage of the peptide form the resin could be low enough (~15%). The removal of Boc group was also tested with this treatment and was found completed by comparison with the standard Boc removing conditions, i.e.; 50%TFA/DCM, 25 min., 250C.

The next steps were manually carried out in a syringe containing a fritted. The selective deprotection of the side chain glutamic acid was performed with palladium(13). The ã-COOH (1 eq.) activated by HATU/DIEA (10 eq. / 11 eq.) and treated with aminoacetaldehyde-dimethylacetal (10 eq.) to generate the masked glycinal on the side chain glutamic acid. The Nå –Fmoc group of the N-terminal lysine was removed with piperidine in DMF for 20 min. and aminooxyacetic acid (Aoa) was introduced as Boc-Aoa-OH and coupled with it. The peptide resin was treated with 95% TFA containing 5% H2O at 00C for 10 min. to free aldehyde and aminoxy fractions simultaneously. Following several water washings, 0.1 M acetate buffer at pH 4.6 was used to

form on resin bridge via an oxime bond. The peptide resin was washed with H2O and subjected to final cleavage with 95% TFA/H2O for 2h.The filterate was collected and concentrated and cold ether was added. The precipitated peptide was washed several times with ether and dried to get the crude peptide in 84% yield. It was characterized by HPLC and two peaks appeared with retention times (tR) of 10.27 and 10.81 respectively (figure 3). The identity of these HPLC peaks was confirmed by MALDI-TOF analysis, tR 10.27min., m/z 1067.65 (calcd 1067.10); tR 10.81 min., m/z 1067. 65 (calcd 1067.10), and they are likely due to syn- and anti-forms of the oxime bond (14).

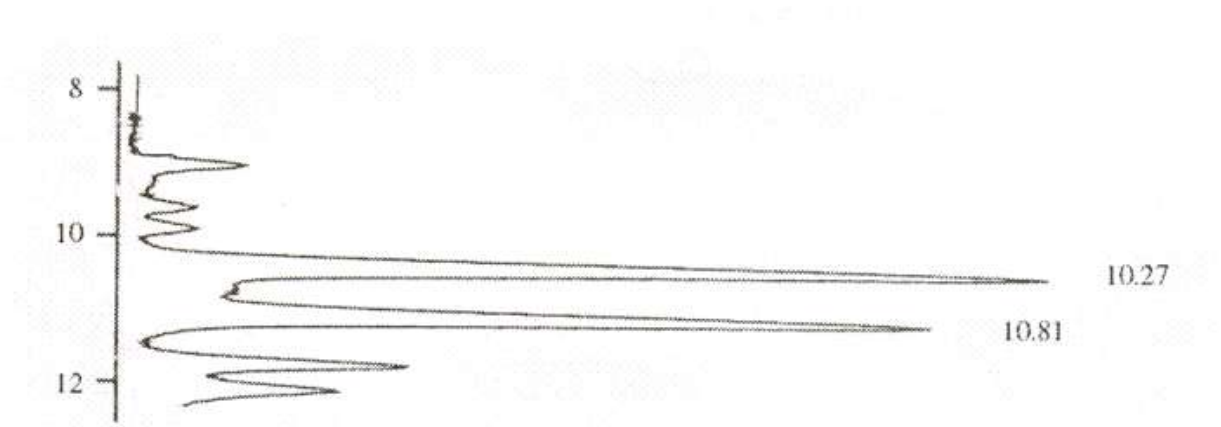


Fig. 3: Analytical HPLC profile of crude peptide. A: 0.1% TFA in water, B: 0.1% TFA in CH_3CN. Gradient: 5-20% B in 20min., Flow rate 1.2ml/min.

Conclusion

This strategy provides an elegant way to introduce an on-resin intramolecular bridge as oxime bond using an aqueous-compatible polymer support and the different stability to TFA between Boc and acetal protection on the hand and peptide-PAL bond on the other hand. The cyclization step was carried out without activation, by using a chemoselective coupling of a C-electrophile on an aldehyde and a N-nucleophile on an aminoxy. Moreover, the process demanded only commercially available products.

Abbreviations used

SH2, Src homology type 2; SH3, Src homology type 3; PAL, [5-(4-Fmoc-aminomethyl-3,5-dimethoxy phenoxy) valeric acid]; PEG-PS, polyethylene glycol-polystyrene; TFA, trifluoroacetic acid; Boc, ter-butyloxycarbonyl; OAl, allyl; DIEA, N,N-diisopropylethylamine; Fmoc, N-(9-fluorenyl)methoxycarbonyl; HATU, O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; HOBt, 1-hydroxybenzotriazole; PyAOP, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; MALDI-TOF, matrix assisted laser desorption/ionization –time-of –flight.

References

1. Blackwell, H.E.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* 1998, 37, 3281-3284.
2. Creighton, T. E. *Proteins: Structures and Molecular Properties*, 2nd ed.; Freeman: New York, 1984, 182-188.
3. Scholtz, J. M.; Qian, H.; Robbins, V. H.; Baldwin, R. L. *Biochemistry* 1993, 32, 9668-9676 and references therein.
4. Phelan, J. C.; Skelton, N. J.; Braisted, A. C.; Medowell, R. S. *J. Am. Chem. Soc.* 1997, 119, 455-460 and references therein.
5. (a) Jackson, D. Y.; King, D. S.; Chmielewski, J.; Singh, S.; Schultz, P. G. *J. Am. Chem. Soc.* 1991, 113, 9391-9392. (b) Rueping, M.; Jaun, B.; Seebach, D.; *J. Chem. Soc. Chem. Commun.* 2000, 2267-2268.
6. Albert, J. S.; Hamilton, A. D. *J. Am. Chem. Soc.* 1995, 34, 984-990.
7. (a) Ghadiri, M. R.; Fernholz, A. K. *J. Am. Chem. Soc.* 1990, 112, 9633-9635. (b) Todd, R. J.; Van Dam, M. E.; Casimiro, D.; Haymorer,

B. L.; Arnold, F. H.; *Proteins: Struct., Funct., Genet.* 1991, 10, 156-161. (c) Kelso, M. J.; Hoang, H. N.; Appleton, T. G.; Fairlie, D. P.; *J. Am. Chem. Soc.* 2000, 122, 10488-10489.
8. (a) Ruan, F.; Chen, Y.; Hopkins, P. B. *J. Am. Chem. Soc.* 1990, 112, 9403-9404. (b) Gilbertson, S. R.; Wang, X.; J. Org. Chem. 1996, 61, 434-435.
9. Cowburn, D., Zheng, J., Xu, Q., and Barany, G. *J. Biol. Chem.* 1995, 270, 26738-26741.
10. Xu, Q., Cowburn, D., Zheng, J., Barany, G., Lett. Pept. Sci.1996, 3, 31-36.
11. Chen, L., Xu, Q., Cowburn, D., ., Zheng, J., Barany, G., *Peptides for the new Millennium (Proceedings of the 16th American Peptide Symposium,* Fields, G. B.; Tam, J. P. and Barany, G. (Eds) Minneapolis, MN, 1999, 579-580.
12. Jaya T. Varkey, David Cowburn and George Barany.; *Peptides-The wave of the future (proceedings of the second international and 17th American peptide Symposium,* Lebel, M., and Houghten, R. A. (Eds.), San Diego, CA, 2001, 222-223.
13. Lloyd-Williams, P., Jou, G., Albercio, F., and Giralt, E. Tetrahedron Letters, 1991, 89, 87-90.
14. Lang, I., Donze, N., Garrouste, P., Dummy, P., Mutter, M., J. Pep. Sci., 1998, 4, 72-80.

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