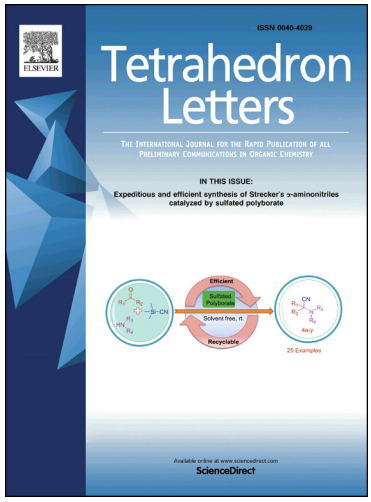


Improved Synthesis of Unnatural Amino Acids for Peptide Stapling

Bo Li, Jie Zhang, Yongjuan Xu, Xiaoxiao Yang, Li Li

PII: S0040-4039(17)30581-6
DOI: http://dx.doi.org/10.1016/j.tetlet.2017.05.007
Reference: TETL 48900

To appear in: *Tetrahedron Letters*

Received Date: 7 April 2017
Revised Date: 3 May 2017
Accepted Date: 4 May 2017

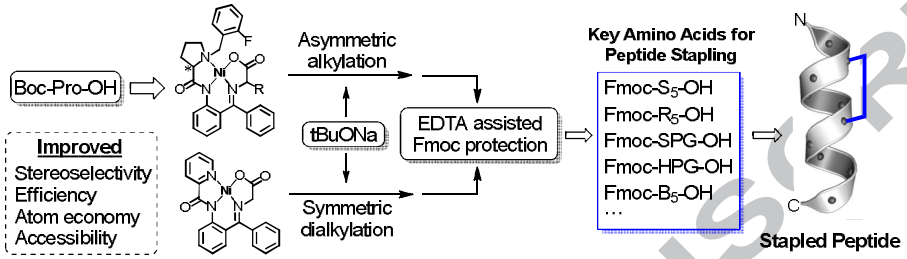
Please cite this article as: Li, B., Zhang, J., Xu, Y., Yang, X., Li, L., Improved Synthesis of Unnatural Amino Acids for Peptide Stapling, *Tetrahedron Letters* (2017), doi: http://dx.doi.org/10.1016/j.tetlet.2017.05.007

Graphical Abstract

Improved Synthesis of Unnatural Amino Acids for Peptide Stapling

Bo Li, Jie Zhang, Yongjuan Xu, Xiaoxiao Yang*, Li Li*

Leave this area blank for abstract info.



Tetrahedron Letters
journal homepage: www.elsevier.com

Improved Synthesis of Unnatural Amino Acids for Peptide Stapling

Bo Li, Jie Zhang, Yongjuan Xu, Xiaoxiao Yang * and Li Li*

Beijing Key Laboratory of Active Substances Discovery and Drugability Evaluation, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China.

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Stapled peptide
Unnatural amino acids
Asymmetric synthesis
Ni(II)-complex

ABSTRACT

The procedures for the synthesis of various α-alkenyl and alkyne amino acids were systematically optimized in light of enhancing atom economy, reducing hazardous reagent usage, and simplifying workup. By starting with Boc-Pro-OH and coupling with EDCI/DMAP followed by alkylation, chiral auxiliary was synthesized with high yield and enantioselectivity. For alkylation of the chiral complex, tBuONa was found and proved by quantitative calculation to be superior to tBuOK in generating more nucleophilic enolate salt, thereby can significantly enhance yield under room temperature. Final Fmoc protection was also dramatically facilitated in one-pot sequential manner by adding EDTA-2Na as the Nickel chelator. Synthesis of α-bisalkenyl amino acid was also accomplished by achiral complex approach with high yield and efficacy. Accordingly, five most commonly used N-Fmoc protected α-alkenyl and alkynyl amino acids were synthesized and characterized.

* Corresponding authors. Tel.: +86-10-63165251; e-mail: yangxx@imm.ac.cn (X. Yang), annaleelin@imm.ac.cn (L. Li)

The research in stapled peptide has been under the spotlight of peptide based drug discovery in recent years and has successfully emerged its potential in the clinical trial of p53 stapled peptide.[1] Such peptides represent potential therapeutics against intracellular targets previously thought to be unreachable[1a]. The all-hydrocarbon staple could endue the stapled peptide with significant improvement in the stability of secondary structure, binding affinity, and metabolic stability[2]. The most prevalent strategy to achieve single[3] or double hydrocarbon stapled peptide[4] as well as "stitched peptide" [2] is the olefin metathesis between α-alkenyl Alanine derivatives (Figure 1, **1a**-**1e**) or with additional α-bisalkenyl substituted glycine (Figure 1, **1g**). Alternative strategies including stapling by Husigen click reaction[5] and photo-induced thiol-yne click reaction[6] are facilitated by α-alkyne amino acids (Figure1, **1f**). Apparently, these unnatural α-alkyl amino acids are essential for those described approaches.

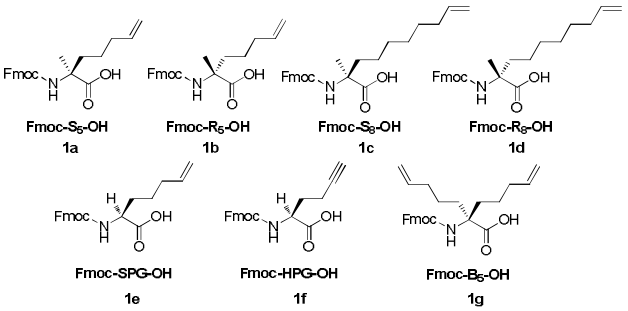
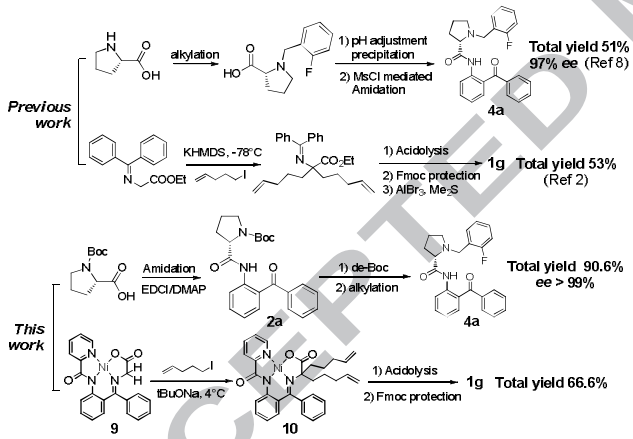
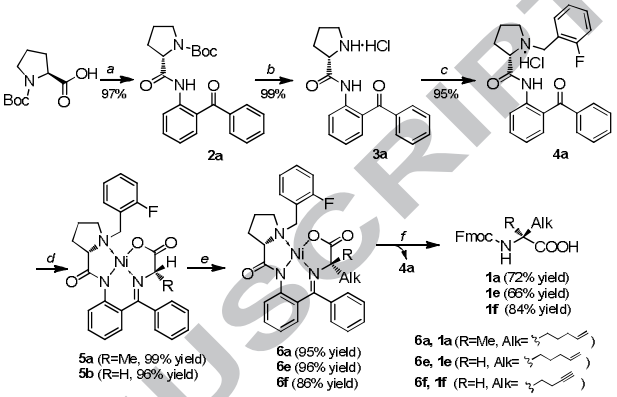


Figure 1. Unnatural amino acids commonly involved in synthesis of stapled peptides.



Scheme 1. Synthesis strategies for compound **4a** and **1g**

During our undergoing stapled peptide research, we found these amino acids were of high price and some of them were even not commercially available. To tackle this, upon studying of literature, glycine/alanine derived chiral Ni(II)-complex auxiliary provided an accessible starting point to access asymmetric synthesis of α-alkyl amino acids[7]. However, the reported procedure[8] for the synthesis of the preferable chiral auxiliary (*S*)-N-(2-benzoylphenyl)-1-(2-fluorobenzyl)-pyrrolidine-2-carboxamide (2-FBPB, **4a**) required methylsulfonylchloride (MsCl) which was a tightly regulated violent toxic reagent to achieve efficient coupling. Furthermore, the hydrophilicity of the benzylproline intermediate had to be isolated by isoelectric precipitation and column purification was required for each workup step which made the whole process quite tedious for large scale preparation in the lab. After alkylation of the Ni(II) complex Schiff bases, most reported procedures require separation of the free amino acid by ion-exchange before N-protection with Fmoc group[8-9]. On the other hand, for the synthesis of **1g**, reported procedure of bis-alkylation of *N*-diphenylmethylene-glycine ethyl ester involved tedious protection strategy and low-temperature condition[2]. To establish a more feasible synthesis, the synthesis of the chiral complex and asymmetric alkylation process were modified and optimized. The synthesis of the α,α-disubstituted amino acid **1g** was also achieved by introducing achiral complex analogously (Scheme 1).



Reagents and conditions: (**a**) 2-aminobenzophenone, EDCI, DMAP, DCM, reflux, 12h; (**b**) HCl/EtOAc, rt, 2h. (**c**) 2-fluorobenzyl bromide, K_2CO_3, acetonitrile, 60~70°C,8h; (**d**) Gly, $Ni(NO_3)_2{\cdot}6H_2O$, KOH, MeOH, 65°C, 1.5h; (**e**) From **5a**: iodoalkene, tBuONa, DMF, rt, 0.5h.; From **5b**: iodoalkene or bromoalkyne, NaOH, acetonitrile, r.t, 0.5h; (**f**) *i*) $3mol{\cdot}l^{-1}$ HCl, MeOH, reflux; *ii*) EDTA-2Na, Fmoc-OSu, Na_2CO_3, H_2O/ acetonitrile, r.t., 16h.

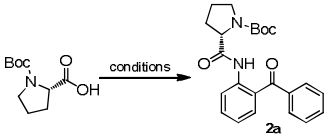
Scheme 2. Synthesis of Fmoc protected α-alkenyl and α-alkyne amino acids

For the synthesis of chiral Ni(II)-complex, Boc-Pro-OH was coupled with (2-aminophenyl)phenylmethanone to give the relatively hydrophobic intermediate which could be purified by simple extraction and washing with $1mol.L^{-1}$ HCl aqueous solution. Considering low nucleophilicity of the amino group of the (2-aminophenyl)-phenylmethanone, various coupling reagents were screened for their efficiency. We found the readily available EDC/DMAP combination could achieve ideal yield comparable with MsCl/N-methylimidazole (Table 1, entry 11). Upon removing Boc group by HCl/EtOAc, alkylation by 2-fluorobenzyl bromide and complexation with $Ni(NO_3)_2{\cdot}6H_2O$ and Glycine or Alanine were carried out according to the reported procedure [9b,10]. Therefore, the chiral auxiliary **5a** and **5b** were synthesized with 87% and 89% yields from Boc-Pro-OH with feasible reagents and easy workup procedure (Scheme 1). The optical purity of **4a** and its enantiomer **4b** were confirmed (*ee* >99%) by chiral HPLC chromatography.

As for the alkylation step, glycine complex **5b** could be deprotonated by sodium hydroxide at room temperature and successively reacted with alkyl bromide in good yield and diastereoselectivity in the solvent of CH_3CN. However, due to the lower acidity of second α-proton of **5a**, the established method utilized four equivalents of NaOH as the base under heating with four equivalents of alkyl bromide. It could be presumed that more active alkyl halide and stronger base might be needed to achieve better enolization under milder condition. In order to lower the excess of base and alkyl halides, reaction temperature, and time consumption, initially, we transform the alkyl bromide to the more active alkyl iodide by simply heating alkyl bromide with NaI in acetone. Next, we screened several typical bases at 1.2 equivalents in 30min reaction time at room temperature. As shown in Table 2, tBuONa was found to get nearly quantitative yield in the synthesis of **6a**. Other solvents including CH_3CN, THF, Dioxane, tBuOH and DCM were also

tested to accompany with tBuONa but gave inferior results. To the best of our knowledge, this protocol improved the yield by 33% in the alkylation step and significantly reduced reaction time and enhanced atom economy compared to the reported synthesis[8]. To investigate the dramatic difference between tBuONa and tBuOK, their solubility in DMF was determined to be 0.043g/ml for tBuONa and 0.067g/ml for tBuOK by ^{1}H-NMR using TMS as the internal standard[11]. Since the concentrations of these tertbutyl bases used in the synthesis were within the range of tested solubility, it may not be the essential cause of the difference. It could be presumed that sodium with smaller atom size than potassium may induce less steric hindrance resulted from forming ion cluster of enolate salt[12] of **5b**, and sodium could form more stable enolate intermediate than potassium, thereby enhance the yield and stereoselectivity of the S_N2 reaction with alkyl halides.

Table 1. Coupling reagents screened for the synthesis of **2a** [a]



2a

Entry[b]	Coupling reagent[c]	Base[d]	Yield[e]
1	$SOCl_2$	-	77%
2	IBCF	DIPEA	81%
3	MsCl	N-Me-Imidazole	87%
4	HATU	DIPEA	57%
5	HBTU	DIPEA	Trace
6	HCTU	DIPEA	Trace
7	EDCI	DIPEA	ND
8	EDCI/HOBt	DIPEA	Trace
9	EDCI/Oxyma	DIPEA	ND
10	EDCI/HOAt	DIPEA	64%
11	EDCI/DMAP	DMAP	97%
12	EEDQ	-	ND
13	Phenylboronic acid	-	ND
14	PPh_3/I_2	-	ND
15	PPh_3/Hexachloroacetone	-	39%
16	PyAOP	DIPEA	67%

[a] All reactions were performed in dry DCM at 45°C for 12h;
[b] Boc-Proline(1.5eq.), *o*-Aminobenzophenone (1.0 eq.);
[c] Coupling reagent:Additive=1:1(1.5eq.)
[d] Base (1.5eq.)
[e] Isolated yield.

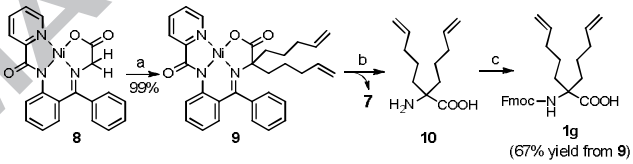
Table 2. Base screened for alkylation of Ala-Ni-FBPB complex [a]

Entry[b]	Base	Yield[c]
1	DBU	Trace
2	$CsCO_3$	ND
3	KOH	12%
4	MeONa	ND
5	NaH	20%
6	tBuOK	68%
7	tBuONa	95%

[a] Reactions were performed in dry DMF at 20°C for 30min;
[b] Base (1.2 equiv.), 5-iodine-1-pentene (1.2 equiv.);
[c] Isolated yield;

After acidolysis of the alkylated Ni(II) complex, due to the impediment of nickel cation to the Fmoc protection reaction, most reported procedures required separation of the free amino acid by ion-exchange. We found that by adding EDTA-2Na as nickel chelate agent, Fmoc protection could be facilitated neatly with a yield over 70% without tedious purification of the acidolysis product. With the optimized process in hand, we managed to synthesize other alkenyl and alkynyl amino acids **1c**, **1e** and **1f** accordingly. Likewise, **1b** was synthesized in 70% yield by the enantiomeric chiral auxiliary **4b** starting from Boc-*D*-Pro-OH following current protocol (see supplementary material for detail).

In addition to the synthesis of the α,α-disubstituted amino acids reported by Ellis *et al.*[13], Fmoc-B_5-OH (**1g**) was synthesized by introducing achiral glycine-Ni(II) complex[14] **8** for the first time (Scheme 3). Formation of nickel complex could not only activate α-carbon of the glycine through forming Schiff base, but also mask its carboxyl group, thereby distinguished from reported synthesis with low temperature and tedious workup procedrue[2]. Likewise, tBuONa was also found to be the optimal base to yield compound **9** from **8** in nearly quantitative yield. Amino acid **10** was released via acidolysis of **9**; 95% of the auxiliary *N*-(2-benzoylphenyl)picolinamide **7** could be efficiently recovered by extraction with dichloromethane. Finally, the Fmoc protection was also accomplished with the aid of EDTA-2Na to give the target compound **1g** (**Scheme 3**). In this way, the atom economy could be improved by over 100% compared with reported procedure [15].



Reagents and conditions: (**a**) 5-iodo-1-pentene **7**, tBuONa, DMF, r.t., 15min; (**b**) 3mol·l^{-1} HCl, MeOH, reflux, 30min; (c) EDTA-2Na, Fmoc-OSu, Na_2CO_3, H_2O/acetonitrile, r.t., 16h.
Scheme 3. Synthesis of Fmoc protected α-bisalkenyl amino acid.

Table 3. Yield and enantioselectivity of the target amino acids

Amino acid	Yield [a]	*ee* [b]
1a	67.2%	>99%
1b	69.8%	>99%
1e	60.2%	>99%
1f	69.1%	>99%
1g	64.4%	-

[a] Calculated based on Gly or Ala as the starting material.
[b] Determined by chiral HPLC

In summary, by systematically modification and optimization of the reaction process, a concise and efficient synthesis method for synthesis of the unnatural amino acids for peptide stapling was developed. It was much more preferable in the aspect of reagent and condition feasibility, workup procedure, yield, atom economy, and optical purity. Five most commonly used *N*-Fmoc protected α-alkenyl and alkynyl amino acids were synthesized (Table 3). We believe these methods would bring ease and efficiency to the stapled peptide research as well as the asymmetric synthesis of other unnatural amino acids.

Acknowledgments

We appreciate the financial support from the CAMS Innovation Funds for Medical Sciences (CIFMS, No. 2016-I2M-3-008 & No.2016-I2M-3-009) and Fundamental Research Funds for the Central Institutes (2015CX08).

Reference

1. (a) Walensky LD, Bird GH. *J Med Chem.* 2014; *57*: 6275; (b) Lau YH, De Andrade P, Wu Y, Spring DR. *Chem Soc Rev*. 2015; 44: 91; (c) Cromm PM, Spiegel J, Grossmann TN. *ACS Chem Biol*. 2015; 10: 1362; (d) Scott DE, Bayly AR, Abell C, Skidmore J. *Nat Rev Drug Discov*. 2016; 15: 533; (e) Araghi RR, Keating AE. *Curr Opin Struct Biol*. 2016; 39: 27.
2. Hilinski GJ, Kim YW, Hong J, Kutchukian PS, Crenshaw CM, Berkovitch SS, Chang A, Ham S, Verdine GL. *J Am Chem Soc.* 2014; *136*:12314.
3. Kim YW, Grossmann TN, Verdine GL. *Nat Protoc.* 2011; *6*:761.
4. Bird GH, Madani N, Perry AF, Princiotto AM, Supko JG, He X, Gavathiotis E, Sodroski JG, Walensky LD. *Proc Natl Acad Sci.* 2010;*107*:14093.
5. (a) Kawamoto SA, Coleska A, Ran X, Yi H, Yang CY, Wang S. *J Med Chem.* 2012; *55*:1137; (b) Cantel S, Isaad AleC, Scrima M, Levy JJ, DiMarchi RD, Rovero P, Halperin JA, D'Ursi AM, Papini AM, Chorev M. *J Org. Chem.* 2008; *73*:5663.
6. Tian Y, Li J, Zhao H, Zeng X, Wang D, Liu Q, Niu X, Huang X, Xu N, Li Z. *Chem Sci*. 2016; *7*:3325.
7. (a) Aceña JL, Sorochinsky AE, Soloshonok V. *Amino Acids* 2014; *46*:2047; (b) Sorochinsky AE, Aceña JL, Moriwaki H, Sato T, Soloshonok VA. *Amino Acids.* 2013; *45*, 691; (c) Sorochinsky AE, Aceña JL, Moriwaki H, Sato T, Soloshonok V. *Amino Acids.* 2013; *45*:1017.
8. Aillard B, Robertson NS, Baldwin AR, Robins S, Jamieson AG. *Org Biomol Chem.* 2014; *12*:8775.
9. (a) Yeo DJ, Warriner SL, Wilson AJ. *Chem Comm.* 2013; *49*:9131; (b) Soloshonok VA, Boettiger TU, Bolene SB. *Synthesis* 2008; *2008*: 2594.
10. Nádvorník M, Langer V, Jirásko R, Holčapek M, Weidlich T, Lyčka A, Popkov A. *Polyhedron* 2008; *27*:3477.
11. Excess amount of either tBuONa or tBuOK was desolved in same amout (1ml) of dry DMF until saturated at 25˚C. The suspension was centrifuged at 15000 rpm for 5min. The supernatent was drawn off and analysed by ^{1}H-NMR (Varian Mercury 400). The peak area of ^{1}H in the tBu group was mesured and concentration was caculated compared with TMS peak area using known concentration of TMS (0.5%).
12. Williard PG, Gene BC. *J Am Chem Soc* 1986; 108: 462.
13. (a) Ellis TK, Martin CH, Tsai GM, Ueki H, Soloshonok VA. *J Org Chem.* 2003; *68*:6208; (b) Ellis TK, Martin CH, Ueki H, Soloshonok VA. *Tetrahedron Lett.* 2003; *44*:1063.
14. Deng G, Wang J, Zhou Y, Jiang H, Liu H. *J Org Chem.* 2007; 72:8932.
15. Calculation based on compound **1g** as the final product, intermediate **8** and N-diphenylmethylene-glycine ethyl ester as the starting materials respectively. Atom economy of current process was 42% whereas atom economy of reported process[2] was 21% maximum (reagents involved in the ion-exchange process were not taken into account).

Research Highlights:

- Efficient synthesis of the unnatural amino acids for stapled peptide was developed.
- Synthesis strategy by chiral Ni(II) complex was modified and optimized.
- α-bisalkenyl amino acid was synthesized by achiral Ni(II) complex strategy.
- Reaction condition, yield, atom economy, and optical purity were optimized.