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Propargylamine Amino Acids as Constrained N^{ε}-Substituted Lysine Mimetics

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INTRODUCTION

Lysine (Lys) is an essential amino acid playing a **crucial role in a plethora of biologically active proteins and peptides**, due to its primary amine moiety connected to a hydrophobic alkyl side chain. Naturally, Lys undergoes **various reversible post-translational modifications** (e.g. methylations), which affect the functioning of proteins such as histones.[1]

Therefore, N^{ε}-alkylated and constrained Lys derivatives (e.g. **1-8a-e**, **Figure 1**) have been developed for application in peptides targeting these proteins, such as peptide inhibitors of methylated lysine reader proteins.[2] In this context, we developed an efficient synthetic pathway towards **lysine analogues rigidified in their side chain** by an internal alkyne functionality and decorated with various **substituents at the ε-amine** (**9**).[3] Solution syntheses of this type of lysine mimetics were only reported for aza-Lys-D-Phe dipeptides (Lubell Group) and racemic α-CF3 substituted propargylglycine (Osipov Group), both protected at their *N*- and *C*-termini.[4,5]

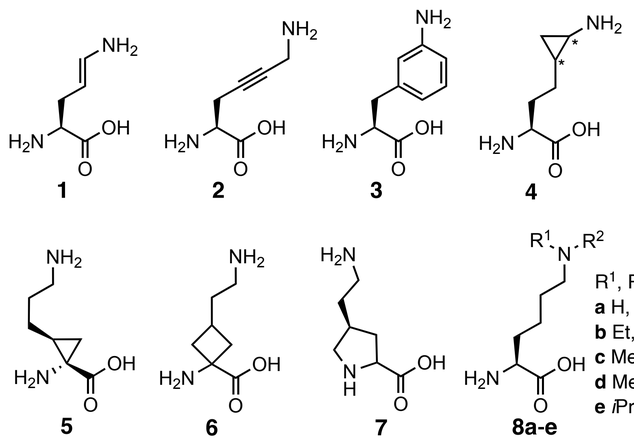
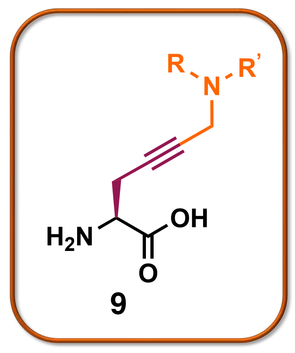
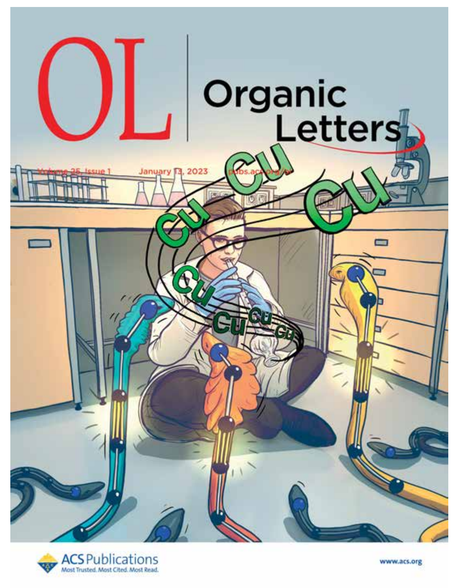
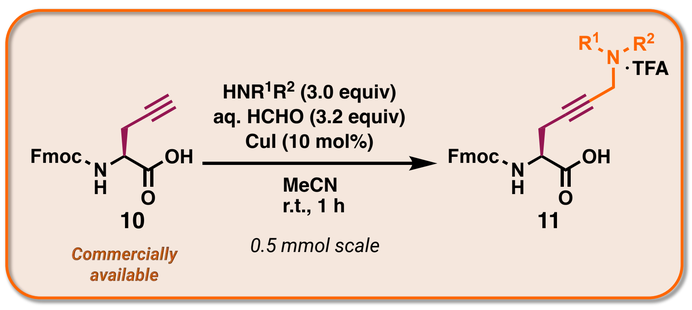


Figure 1. Examples of reported constrained and alkylated lysine derivatives.

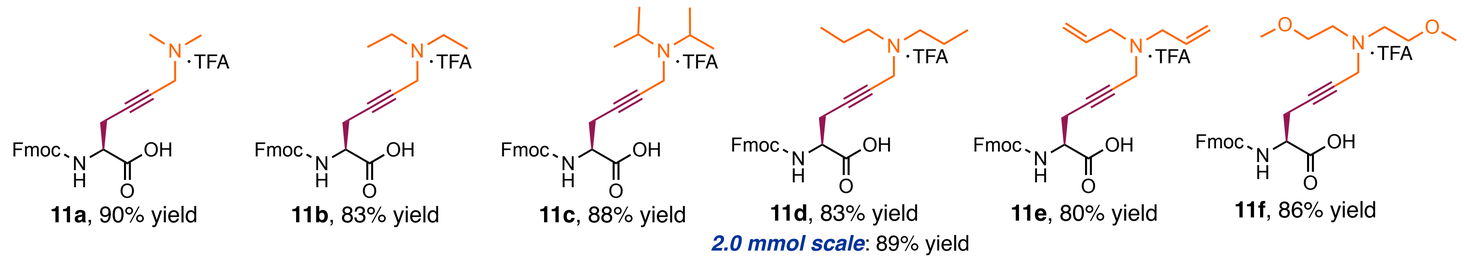




Copper(I)-catalyzed A³-reaction on Fmoc-Pra-OH



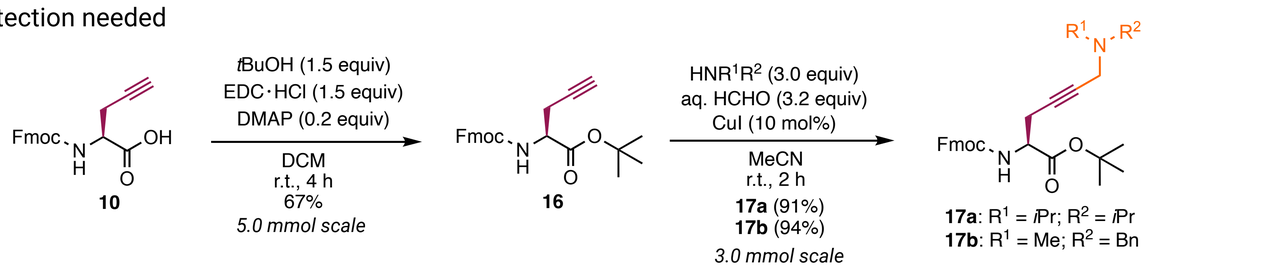
- **Fast conversions**
- Longer reaction times with sterically demanding amines
- Aqueous formaldehyde: limits allene formation
- Preformation of iminium: limits *N*-Fmoc removal
- **75-90% isolated yields** (RP chromatography)

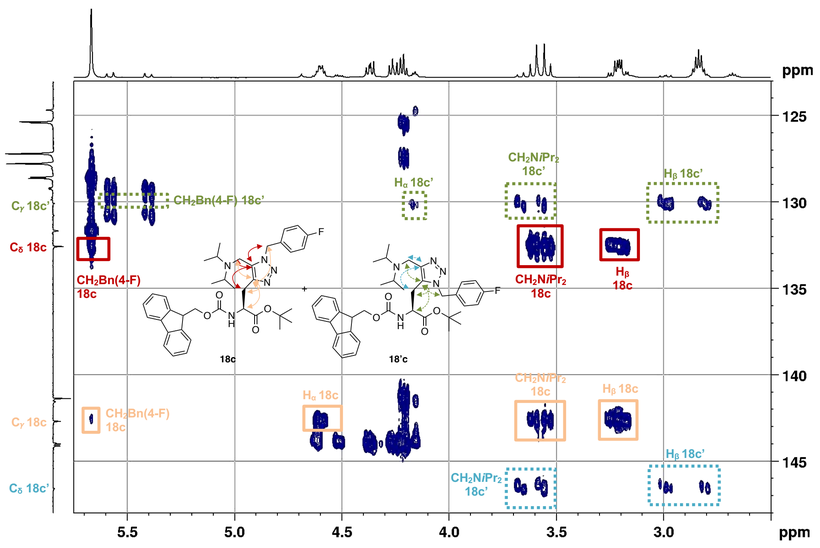


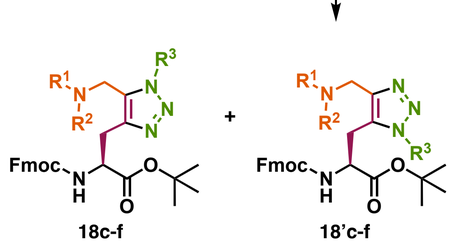
[a] 2 h reaction time. [b] Additional DIPEA (3.0 equiv) and CuI (1.5 equiv).

Diversification of the internal alkyne towards 1,2,3-triazoles

- Internal alkyne moiety: further functionalization via **ruthenium-catalyzed azide-alkyne cycloadditions** (RuAAC)
- Ester protection needed





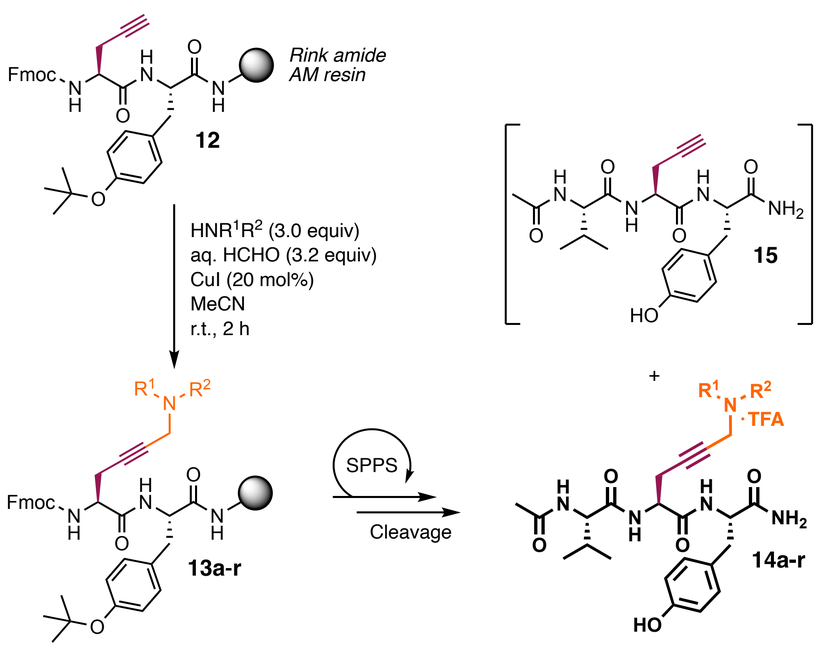


c: R^1 = *i*Pr; R^2 = *i*Pr; R^3 = $CH_2Ph(4\text{-}F)$; **18c**:**18'c** 82:18
d: R^1 = *i*Pr; R^2 = *i*Pr; R^3 = $CH_2(C{=}O)OEt$; **18d**:**18'd** 81:19
e: R^1 = Me; R^2 = Bn; R^3 = $CH_2Ph(4\text{-}F)$; **18e**:**18'e** 64:36
f: R^1 = Me; R^2 = Bn; R^3 = $CH_2(C{=}O)OEt$; **18f**:**18'f** 61:39

Mixture of two regioisomers: assigned by NMR through ^1H-^{13}C HMBC

Application of the A³-reaction during SPPS

- **Direct incorporation** in peptides of **11a-q** by Fmoc-based SPPS
- Diversification **during SPPS assembly**: pre-assembled **12**
  - Purification not jeopardized by less efficient conversions
  - Higher conversions: DMSO, 50 mol% CuI and extended reaction times



Code	HNR^1R^2 used for A³-coupling	Amount of **14a-r** (%) [c]	Amount of **15** (%) [c]
14a	Dimethylamine	71	23
14b	Diethylamine	n.d.[d]	n.d.[d]
14c	Di*iso*propylamine	91	1
14d	Dipropylamine	89	4
14e	Diallylamine	70	25
14f	Bis(2-methoxyethyl)amine	57	25
14g [a]	Piperidine	84	10
14h[a]	Morpholine	52	43
14i	*N*-Methylcyclohexylamine	90	4
14j	*N*-Methyl(tetrahydro-2*H*-pyran-4-yl)methanamine	89	5
14k	*N*-Methyl-*N*-(thien-2-yl-methyl)amine	66	26
14l	*N*-Methylbenzylamine	78	13
14m	4-Methoxy-*N*-methyl-benzylamine	83	10
14n	4-Fluoro-*N*-methyl-benzylamine	82	12
14o	*N*-Allylbenzylamine	62	34
14p [a]	Dibenzylamine	65	30
14q [b]	*Tert*-butyl sarcosinate hydrochloride	56	24

[a] Performed for 4h. [b] Additional DIPEA (3.0 equiv) and CuI (1.5 equiv). [c] Determined by analytical HPLC of the crude peptides. [d] Not determined due to overlap.

CONCLUSION & PERSPECTIVES

- **Efficient pathway toward *N*ε-alkylated propargylamine amino acids as lysine mimetics**
- **Synthetic accesibility by A³-reaction on Fmoc-protected building blocks and during solid phase assembly**
- **Additional functionalization of internal alkyne by the RuAAC towards 1,2,3-triazoles**
- **Diversification of azide substitutent: additional linkage point for peptide cyclizations or late-stage functionalization**

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[1] Wang, Z.; Cole, P. *Cell Chem. Biol.* **2020**, *27*, 953-969. [2] Lamb, K.; Bsteh, D. *et al. Cell Chem. Biol.* **2019**, *26*, 1365-1379. [3] Van holsbeeck, K.; Elsocht, M.; Ballet, S. *Org. Lett.* **2023**, *25*, 130-133. [4] Zhang, J.; Proulx, C. *et al. Org. Lett.* **2013**, *16* (1), 298-301. [5] Philippova, A. N.; Vorobyeva, D. *et al. Mendeleev Commun.* **2022**, *32* (2), 260-261.

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