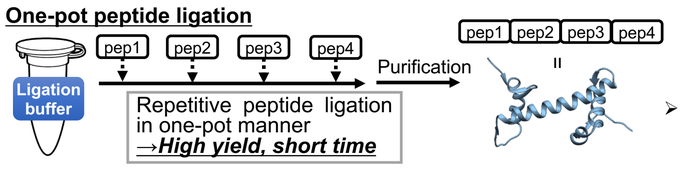
Direction-Switching One-Pot Peptide Ligation with Orthogonal Cysteinyl Protection

Koki Nakatsu[1]; Fumika Yoshitomi[2]; Hiroki Onoda[3]; Reia Shikimachi[3]; Kyohei Arita[3]; Akimitsu Okamoto[4]; Hiroshi Murakami[2]; Gosuke Hayashi[2]
([1]*University of Cambridge,* [2]*Nagoya University,* [3]*Yokohama City University,* [4]*The University of Tokyo*)
https://doi.org/10.17952/38EPS.2026.P042

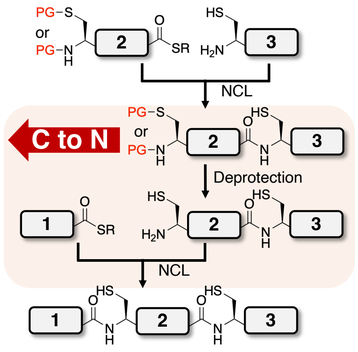
Introduction / Background

One-pot peptide ligation



C-to-N peptide ligation

➢ **N-terminal cysteine** is protected by a protecting group

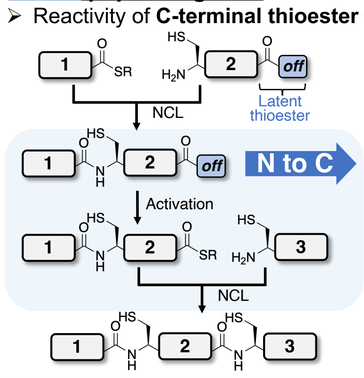


N-terminal cysteinyl protecting groups used in one-pot peptide ligation.

Thz … D. Bang, et al. *Angew. Chem. Int. Ed.* **2004**
Acm … S. K. Maity, et al. *Angew. Chem. Int. Ed.* 2016.
Tfacm … S. Tang, et al. *Angew. Chem. Int. Ed.* 2015.
PPQZ … S. Wang, et al. *J. Am. Chem. Soc.* 2022.
Dobz … J. Li, et al. *Org. Biomol. Chem.* 2014.
Fmoc … A. Kar, et al. *Angew. Chem. Int. Ed.* 2020.

N-to-C peptide ligation

➢ Reactivity of **C-terminal thioester surrogate** is controlled.

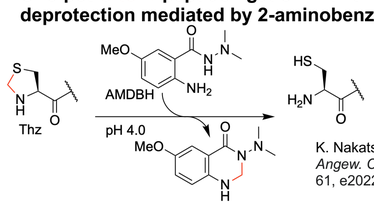


1. Kinetically Controlled Ligation
e.g.) Alkyl & Phenyl Thioester
D. Bang et al. *Angew. Chem. Int. Ed.* **2006**.

2. Activatable Thioester
e.g.) hydrazide → pyrazole
Flood, D. T et al. *Angew. Chem. Int. Ed.* **2018**.

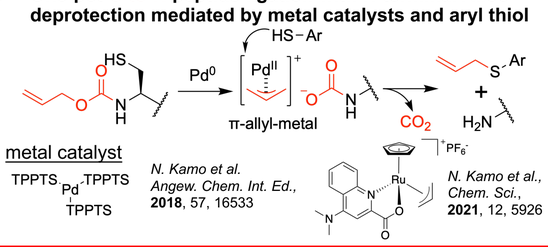
3. Caged Thioester
eg) SEAlide+NV
Aihara, K. et al. *Org. Lett.* **2016**.

➢ **One-pot C-to-N peptide ligation with thiazolidine (Thz) deprotection mediated by 2-aminobenzamide derivative**



K. Nakatsu et al. *Angew. Chem. Int. Ed.* **2022**, 61, e202206240

➢ **One-pot C-to-N peptide ligation with N-terminal Alloc deprotection mediated by metal catalysts and aryl thiol**

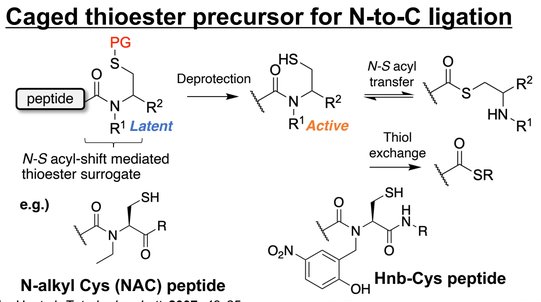


N. Kamo et al. Angew. Chem. Int. Ed., **2018**, 57, 16533

N. Kamo et al., Chem. Sci., **2021**, 12, 5926

These orthogonal methods enable only C-to-N ligation, not N-to-C ligation.

Caged thioester precursor for N-to-C ligation



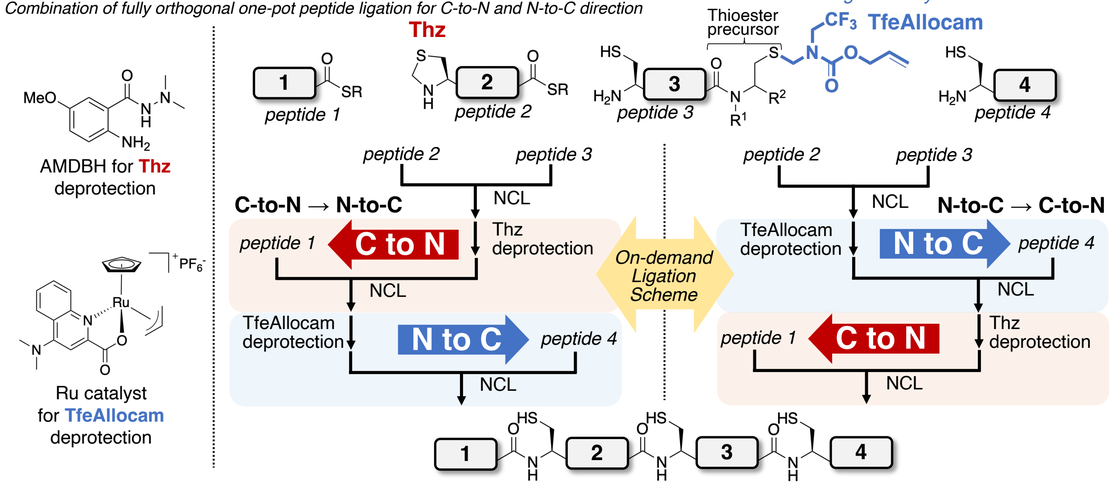
Hojo, H. et al. *Tetrahedron Lett.* **2007**, *48*, 25.

V. P. Terrier et al. *Chem. Sci.* **2016**, *7*, 339.

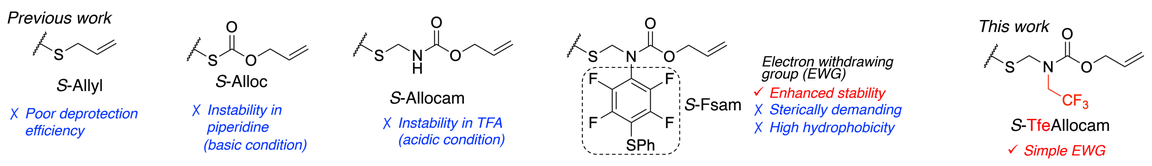
Concept

This work … Direction-Switching One-pot Peptide Ligation

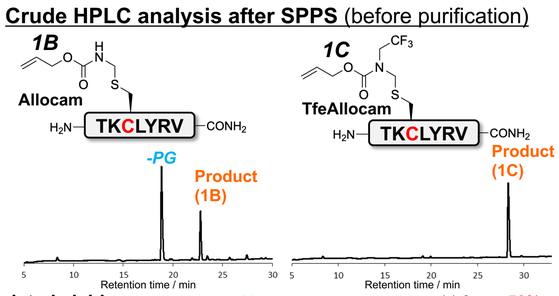
- ✓ *A new S-allylic protecting group compatible with chemical protein synthesis*
- ✓ *Combination of fully orthogonal one-pot peptide ligation for C-to-N and N-to-C direction*



Results: Design of A New Allylic Thiol Protecting Group



Crude HPLC analysis after SPPS (before purification)



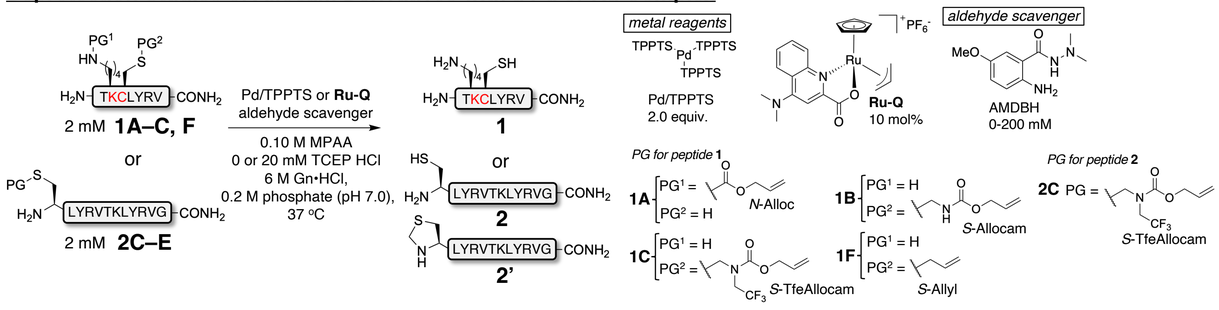
Isolated yields 4.7 mg, 18% 14.6 mg, 52%

Stability of S-Allylic Cysteinyl Protecting Groups of Peptides

	remaining yield/%		
peptide	**TFA cocktail[a] (4 h)**	**20% piperidine[b] (24 h)**	**NCL buffer (24 h)[c]**
1B (*S*-Allocam)	15%	<2%	46%
1C (*S*-TfeAllocam)	>98%	83%	>98%

[a] TFA cocktail: TFA/TIPS/H_2O = 95/2.5/2.5 at r.t.
[b] 20% piperidine: piperidine/DMF = 20/80 (v/v) at r.t.
[c] NCL buffer: 6 M Gn·HCl, 0.2 M phosphate (pH 7.0), 100 mM MPAA, 20 mM TCEP·HCl at 37 ° C.

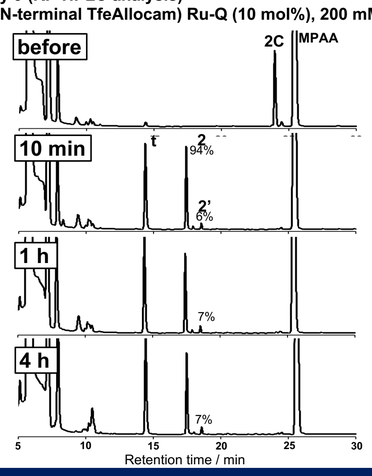
Deprotection Studies of Allocam Derivatives with a Pd or Ru Complex



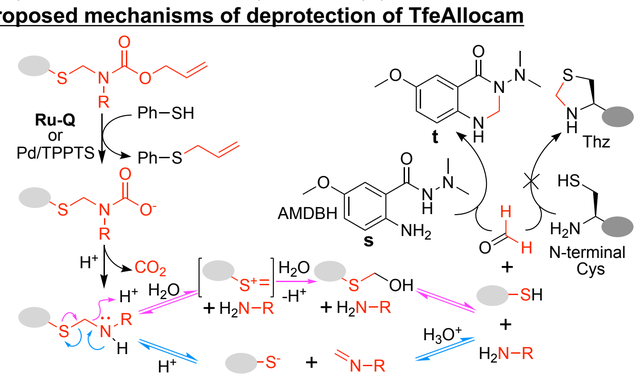
			conversion yields/% (Thz 2' formation yield/%)[a]		
entry	**peptide**	**additional deprotection conditions[a]**	**10 min**	**1 h**	**4 h**
1	**1A** (*N*-Alloc)	2.0 equiv. Pd/TPPTS, 20 mM TCEP·HCl	>98%		
2	**1B** (*S*-Allocam)	2.0 equiv. Pd/TPPTS, 20 mM TCEP·HCl	>98%		
3	**1C** (*S*-TfeAllocam)	2.0 equiv. Pd/TPPTS, 20 mM TCEP·HCl	>98%		
4	**1F** (*S*-Allyl)	2.0 equiv. Pd/TPPTS, 20 mM TCEP·HCl	N/D	N/D	
5	**1C** (*S*-TfeAllocam)	10 mol % **Ru-Q** b	96% (4%d)		
6	**2C** (*S*-TfeAllocam)	2.0 equiv. Pd/TPPTS, 20 mM TCEP·HCl	97% (3%)	84% (16%)	27% (73%)
7	**2C** (*S*-TfeAllocam)	10 mol % **Ru-Q** b	61% (39%)	9% (91%)	2% (98%)
8	**2C** (*S*-TfeAllocam)	2.0 equiv. Pd/TPPTS, 20 mM TCEP·HCl, AMDBH 200 mM	>98%	96% (4%)	95% (5%)
9	**2C** (*S*-TfeAllocam)	10 mol % **Ru-Q** b, AMDBH 200 mM	94% (6%)	93% (7%)	93% (7%)

[a] A standard deprotection condition is an aqueous buffer containing 0.10 M MPAA, 6 M Gn·HCl, and 0.2 M phosphate (pH 7.0) at 37 ° C.
[b] **Ru-Q** is [CpRu(QANMe$_2$)allyl]PF_6.
[c] Conversion yields calculated from the ratio of peak area of RP-HPLC analysis (220 nm).
[d] The yield is derived from a formaldehyde adduct of peptide **1**.

entry 9 (RP-HPLC analysis)
2C (N-terminal TfeAllocam) Ru-Q (10 mol%), 200 mM AMDBH

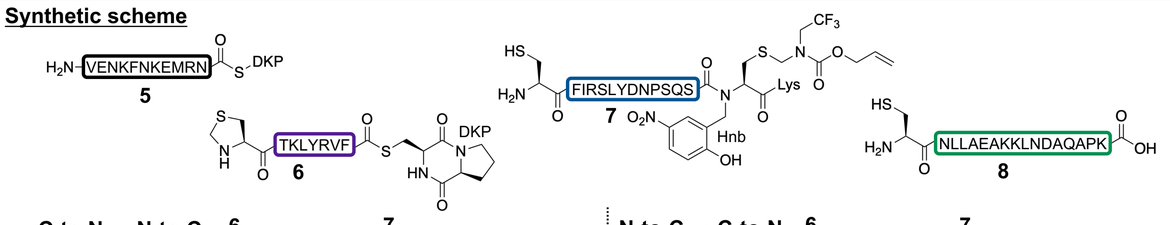


Proposed mechanisms of deprotection of TfeAllocam

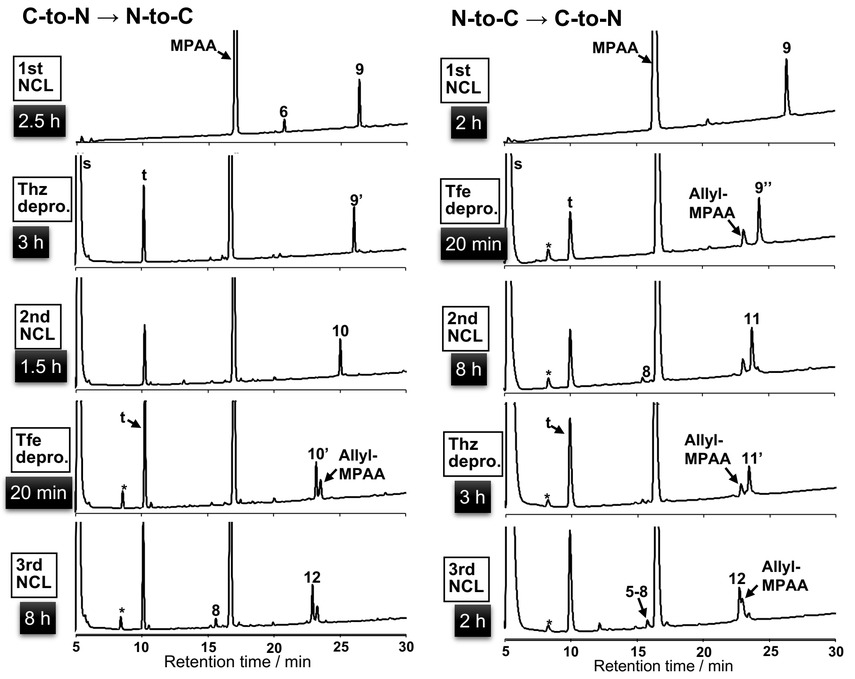


Results: Direction-Switching One-Pot Peptide Ligation

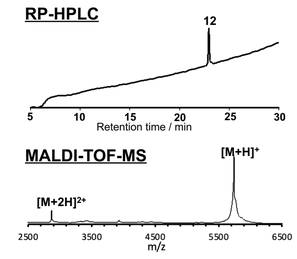
Synthetic scheme



RP-HPLC analysis of ligation reactions

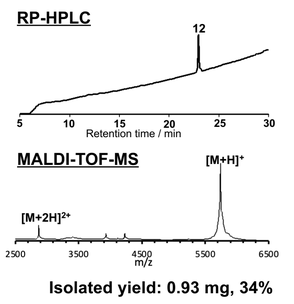


Ligation product 12 (C-to-N → N-to-C)



Isolated yield: 1.16 mg, 41%

Ligation product 12 (N-to-C → C-to-N)



Isolated yield: 0.93 mg, 34%

Both C-to-N → N-to-C and N-to-C → C-to-N assembly routes are possible using the same peptide segments, expanding flexibility in ligation-route design.

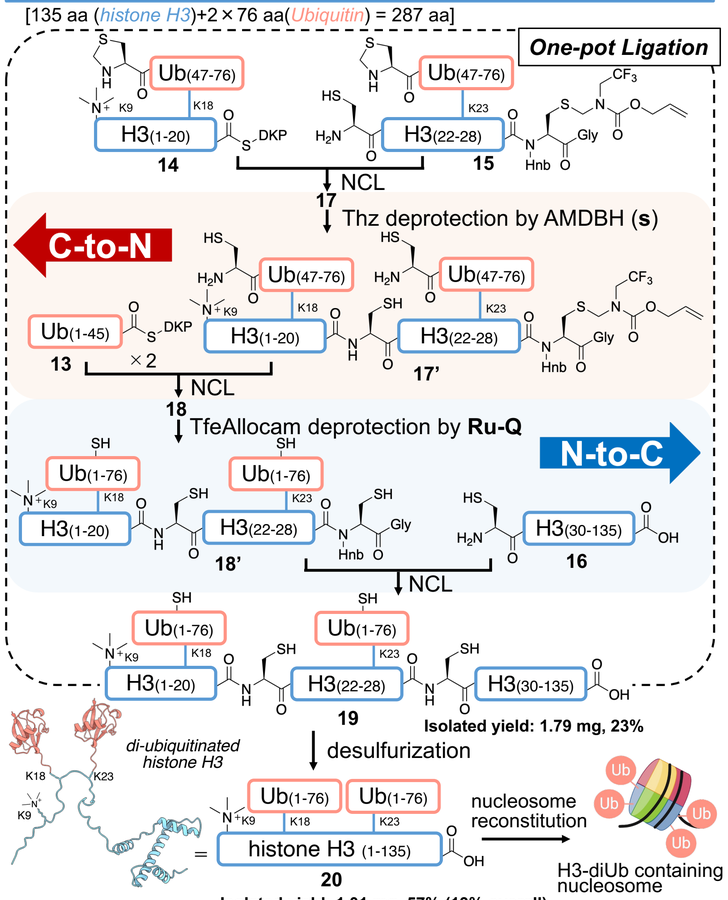
Results: Chemical Synthesis of Diubiquitinated Histone H3

Synthetic scheme

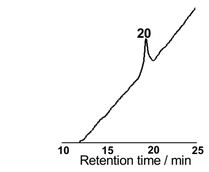
Ubiquitin: H-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIF$_{45}$ / AGKQLEDGRTLSDYNIQKESTLHLVLRLRGG$_{76}$-*(linked to histone H3)*

histone H3: H-ARTKQTARK(Me$_3$)STGGKAPRK*(Ubiquitin)*QL$_{20}$ / ATK*(Ubiquitin)*AARKS$_{28}$ / APATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFQRLVREIAQDFKTDLRF QSSAVMALQEASEAYLVGLFEDTNLAAIHAKRVTIMPKDIQLARRIRGERA$_{135}$-OH

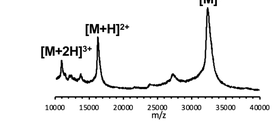
[135 aa (*histone H3*)+2 × 76 aa(*Ubiquitin*) = 287 aa]



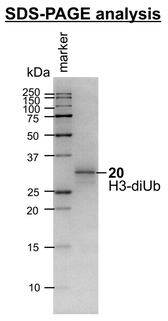
RP-HPLC profile after purification



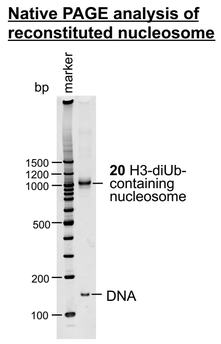
MALDI-TOF-MS spectrum



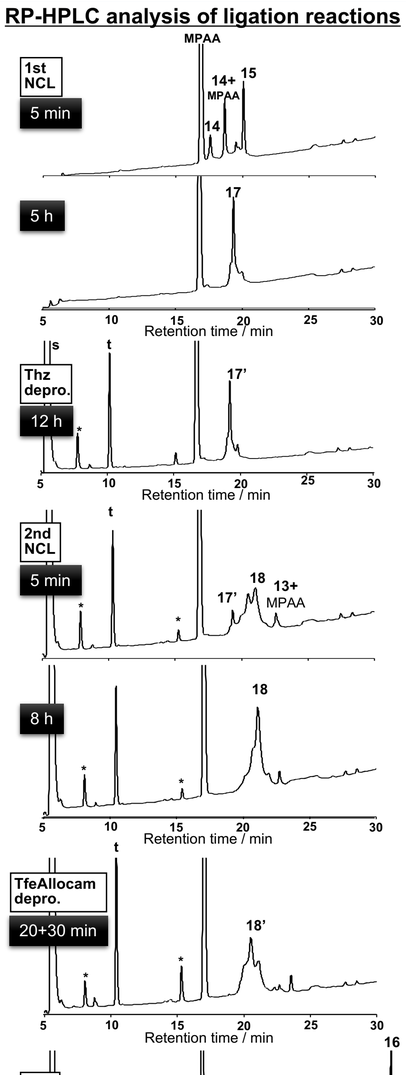
SDS-PAGE analysis



Native PAGE analysis of reconstituted nucleosome



RP-HPLC analysis of ligation reactions



Conclusions / Perspectives

- TfeAllocam provides robust cysteine protection and can be selectively removed under NCL-compatible conditions.
- Orthogonal Thz and TfeAllocam protection enables programmable direction-switching one-pot NCL.
- Alternative orthogonal systems may enable more efficient and versatile direction-switching ligation strategies.
- Final-product HPLC purification and byproduct accumulation remain challenges for further iterative ligation.

View the paper

Publication: Koki Nakatsu et al. Chemical Protein Synthesis via Direction-Switching One-Pot Peptide Ligation Enabled by Orthogonal Cysteinyl Protection. *J. Am. Chem. Soc.* 2026; 148 (4): 4622–4634. DOI: 10.1021/jacs.5c20510