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Total Chemical Synthesis of RNF4 by Sequential Native Chemical Ligation: C-To-N Versus N-To-C Strategies

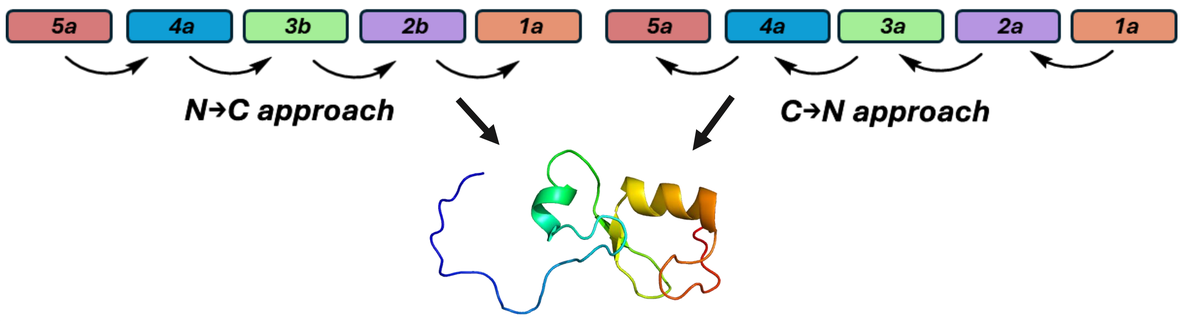
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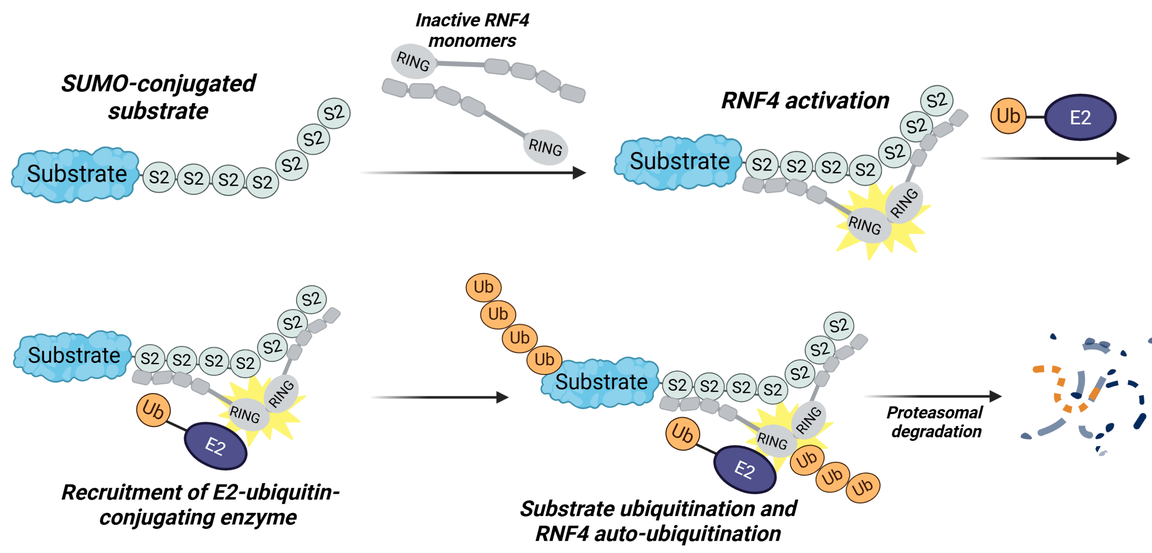
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1 Abstract

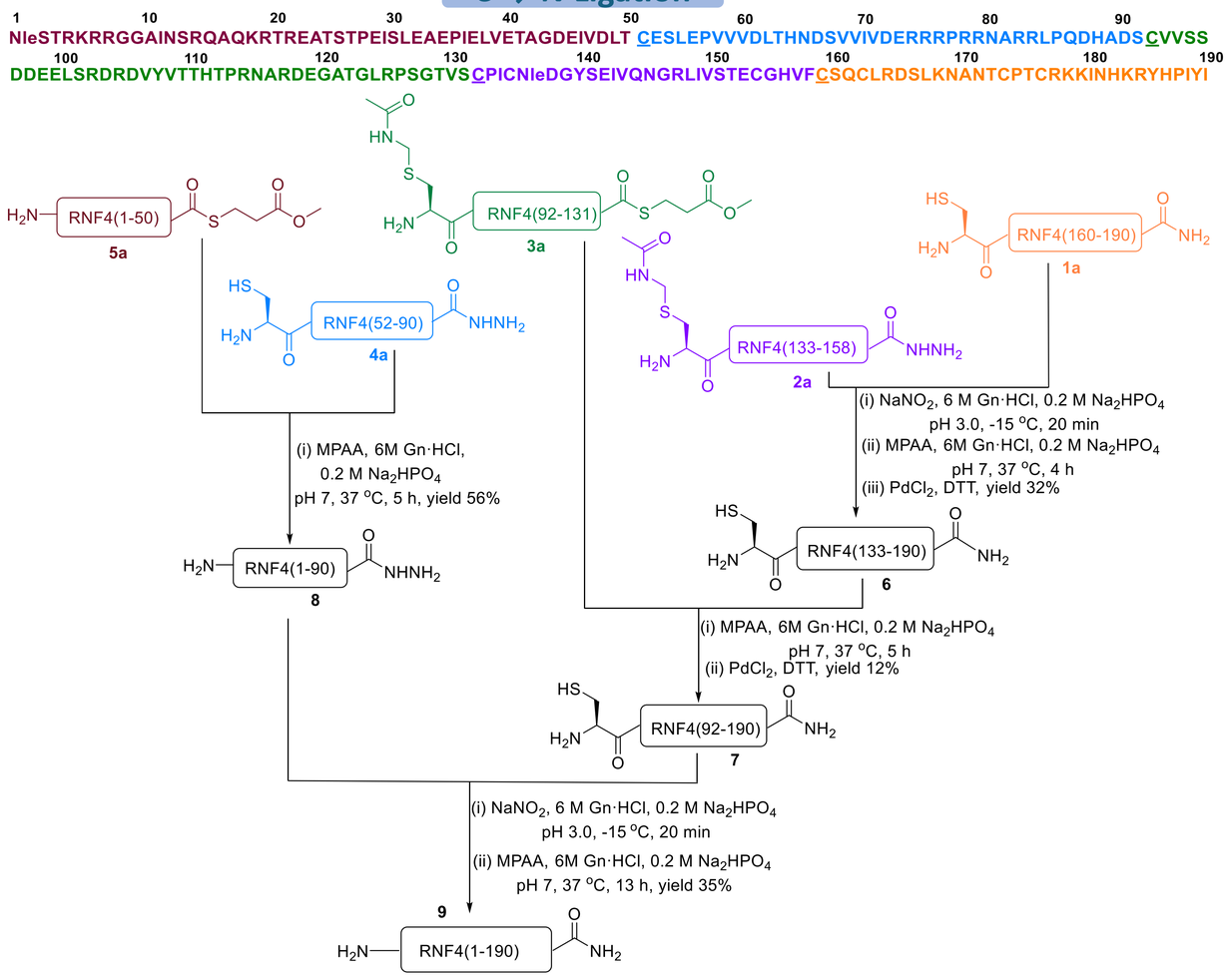
RNF4 is a RING-type E3 ubiquitin ligase. It targets polySUMOylated proteins for ubiquitination and subsequent proteasomal degradation. Chemical synthesis of RNF4 provides new opportunities to study its structure. It also enables deeper investigation of its biological function, including its role in degrading the oncoprotein PML-RARα in acute promyelocytic leukemia (APL). Importantly, this synthetic access allows the generation of site-specifically modified RNF4 variants bearing defined post-translational modifications (PTMs). To achieve this, we performed a total chemical synthesis of RNF4 via sequential native chemical ligation (NCL). Since RNF4 contains nine cysteine residues, we assembled it stepwise from five peptide fragments. Two synthetic strategies were explored: a convergent C → N ligation approach and a linear N → C ligation. The C → N strategy required Acm protection of cysteine residues, which increased deprotection and purification steps, whereas the N → C approach avoided cysteine protection, and further simplified the workflow. Overall, this work provides a reliable synthetic platform for RNF4 and enables future preparation of PTM-defined RNF4 variants for future biological studies.



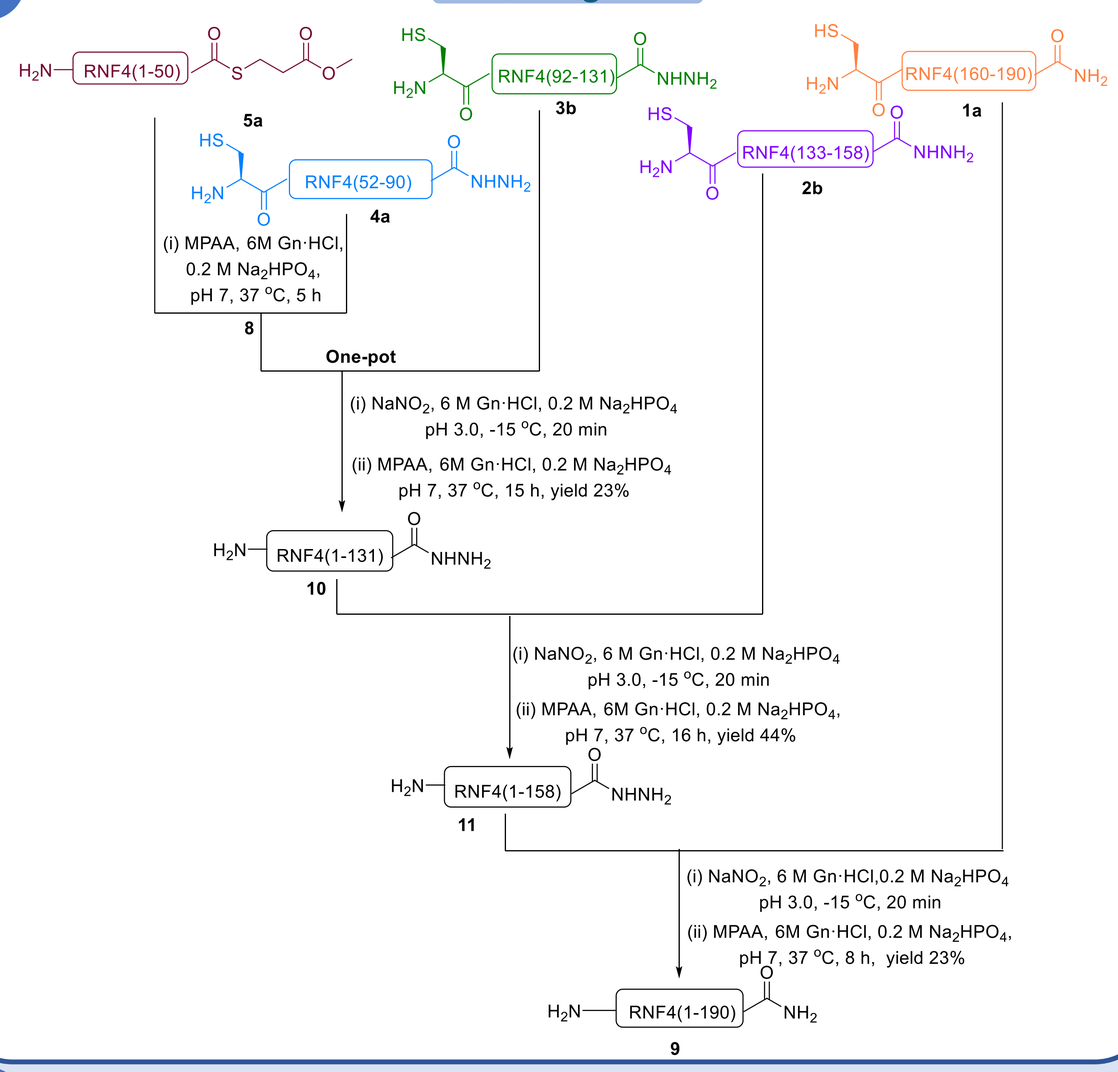
2 RNF4 SUMO-Targeted Ubiquitin Ligase



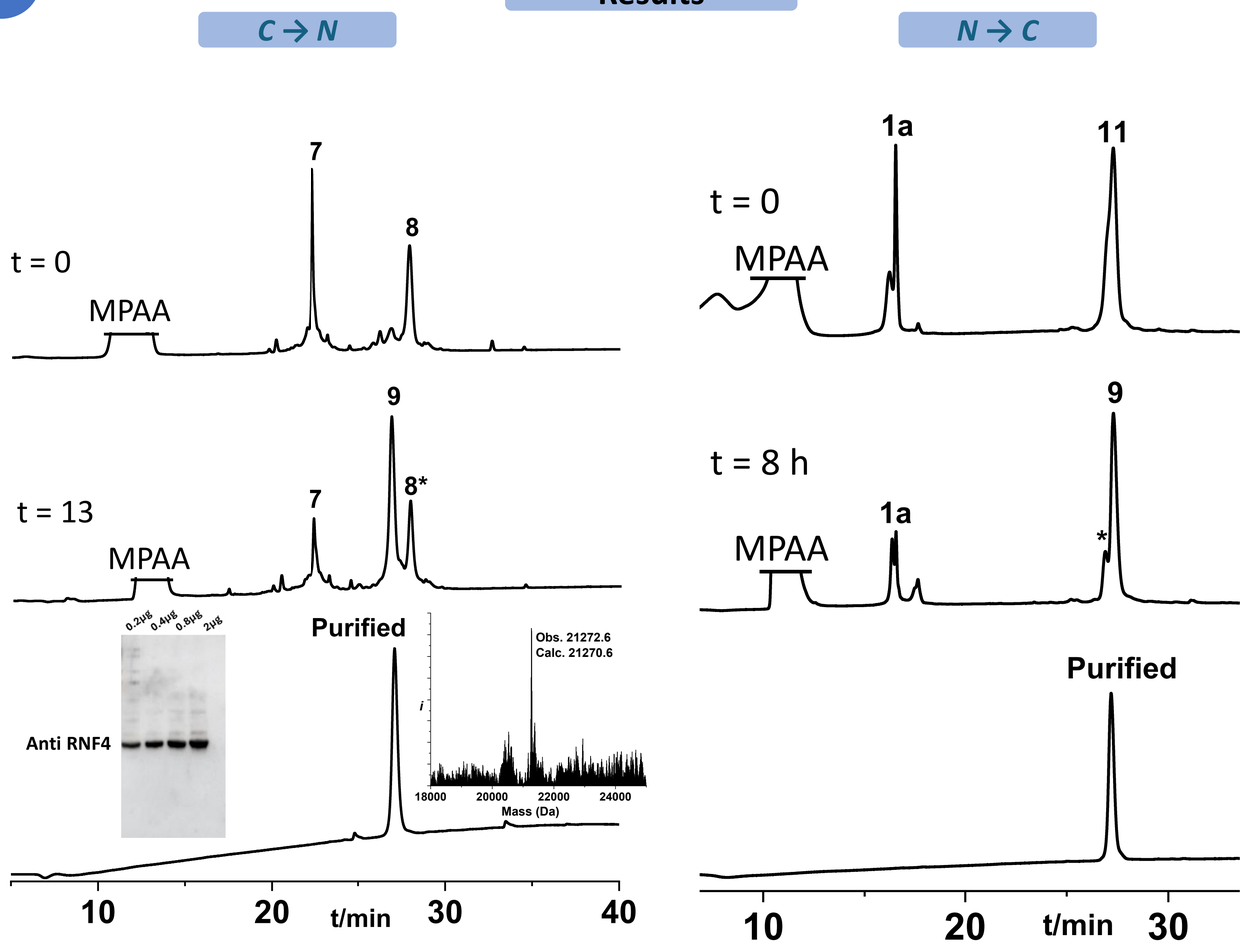
3 C → N Ligation



4 N → C Ligation



5 Results



6 Conclusion

1. We successfully achieved the **total chemical synthesis of full-length RNF4** via **native chemical ligation (NCL)**.
2. The C→N approach required cysteine protection/deprotection, additional purification steps, and a longer time - 3 weeks.
3. The N→C approach proceeded without cysteine protection, involved no purification steps a shorter workflow - 2 weeks.
4. The N→C ligation strategy not only enables efficient chemical synthesis of RNF4 but also provides a robust platform for the site-specific incorporation of future post-translational modifications, allowing systematic investigation of how defined PTMs regulate RNF4 activity, interactions, and cellular function.

7 Reference

1. Pallava, R.; **Bisher, S**.; Brik, A. *Total Chemical Synthesis of RNF4 by Sequential Native Chemical Ligation: C-to-N versus N-to-C Strategies.* J. Org. Chem. 2026

For further reading :

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3. Seenaiah, M.; Jbara, M.; Mali, S. M.; Brik, A. "Convergent Versus Sequential Protein Synthesis: The Case of Ubiquitinated and Glycosylated H2B." *Angewandte Chemie International Edition* **2015**, *54*, 12374–12378.

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