

C-H ACTIVATION

Complex peptides made simple

Nature oxidizes biosynthetic intermediates into structurally and functionally diverse peptides. An iron-catalysed C-H oxidation mimics this approach in the lab, enabling chemists to synthesize structural analogues with ease.

Sean Bartlett and David R. Spring

Naturally occurring peptides exhibit an extraordinary range of properties and biological activities¹. Non-ribosomal peptides (NRPs) continue to represent an invaluable source of new small-molecule drugs², having already provided antibiotics such as the β -lactam, glycopeptide and lipopeptide families³. Advances in the bioengineering of NRP synthases⁴ and the availability of non-proteinogenic amino acids have expanded the compound pool available to

researchers in recent years, but chemical synthesis remains the tool of choice for the study of structural modifications on NRP derivatives nonetheless. Analogue synthesis is particularly laborious for structurally complex peptides when each one must be made from the ground up.

Nature has evolved to overcome this challenge by cutting down on the number of individual steps needed to access these final products⁵. In the case of NRPs, stereo- and regiospecific tailoring enzymes

generate structural and functional diversity through the late-stage modification of pluripotent intermediates⁶. Pluripotent compounds react selectively under different conditions to afford distinct products: for NRPs, these usually derive from oxidation, glycosylation, acylation or alkylation processes.

Pluripotency is the focus of increasing attention due to the importance of chemical diversity in modern drug discovery⁷. In theory, divergent chemical syntheses can

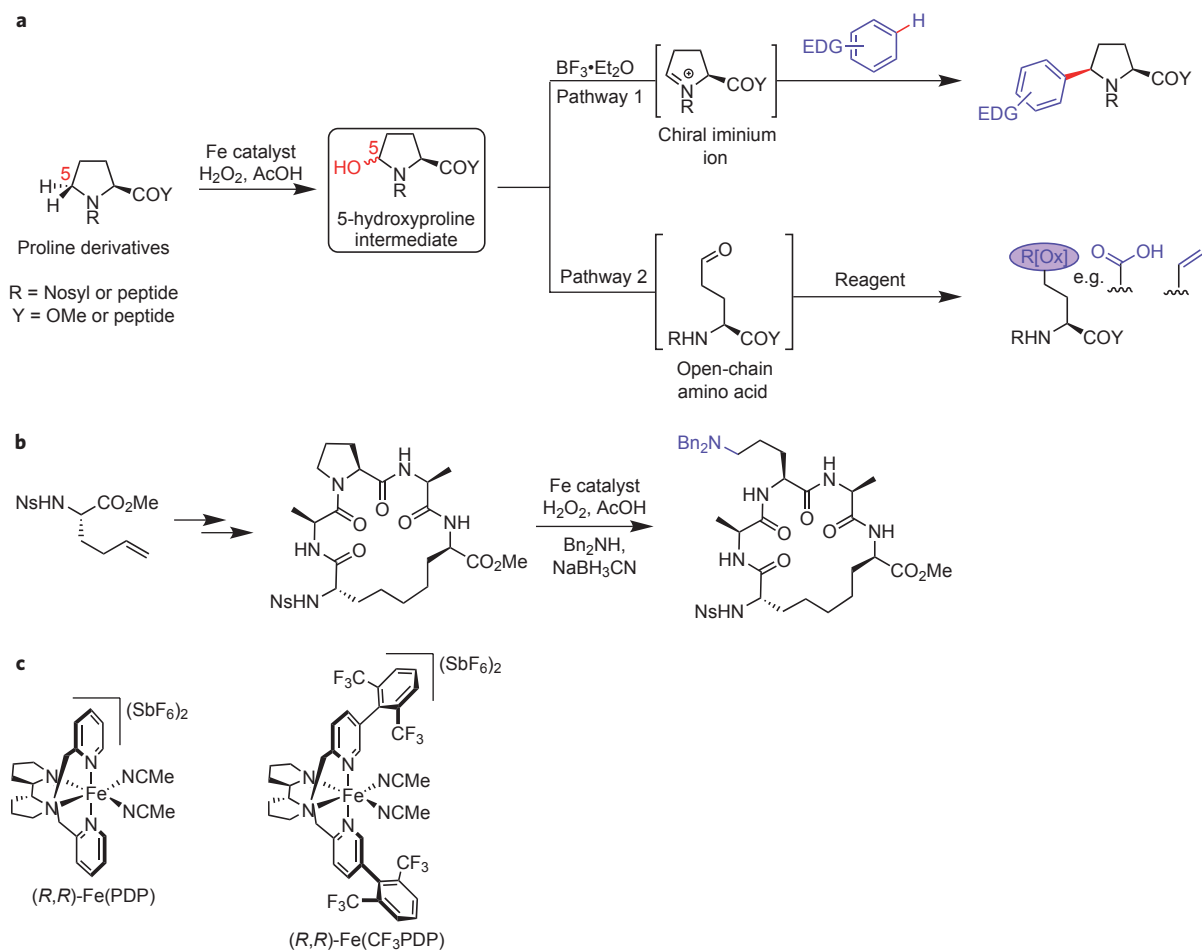


Figure 1 | Oxidative diversification of amino acids and peptides by small-molecule iron catalysis. **a**, Divergent reaction pathways of a pluripotent 5-hydroxyproline intermediate formed by an iron-catalysed C-H oxidation of a proline derivative. **b**, Exemplary synthesis of a macrocyclic analogue by means of a post-assembly C-H oxidation. **c**, Iron catalysts used in this work.

offer the best of both worlds by enabling the efficient generation of molecular complexity. However, as compounds become more and more complex, achieving selectivity in these systems requires the continued development of new and improved methods for synthesis. Pluripotency seems to be limited only by the tools we have to make use of it.

To this end, M. Christina White and co-workers at the University of Illinois at Urbana-Champaign⁸ report a remarkable iron-catalysed C–H oxidation for divergent peptide synthesis. This work draws on the combined strengths of nature's own oxidative diversification approach, Barton's pioneering Gif chemistry⁹, and strategic C–O bond formation¹⁰ to demonstrate the selective and catalytic oxidation of C–H bonds in the divergent synthesis of complex and medicinally relevant peptides.

The researchers observed that protected proline residues undergo stereoselective oxidation — effected by an iron catalyst, hydrogen peroxide and acetic acid — yielding the corresponding 5-hydroxyproline motif (Fig. 1). This transient hemiaminal is formed predominantly over other possible oxidation products due to a combination of structural and electronic factors unique to pyrrolidines and related ring systems. The pluripotent 5-hydroxyproline is then transformed selectively through one of two distinct reaction pathways. In one pathway, reaction of the tautomeric open-chain aldehyde affords linear amino acid derivatives; in the other, the reaction of an iminium species, formed by action of a Lewis acid, affords arylated prolines in a high diastereomeric ratio.

Building on this, the team demonstrated five separate transformations of a complex tripeptide: arylation, olefination, double oxidation, reduction and reductive amination. More impressive still is the elaboration of linear peptides into structurally and stereochemically complex macrocycles¹¹. Such compounds are massively underrepresented in

high-throughput screening collections, in part due to the synthetic challenge that they present. Enabled by this powerful method, the compounds described in this work would make an excellent starting point for library synthesis.

Where previously each macrocycle synthesis required five further steps from the tripeptide precursor, keeping the proline intact until the final step results in dramatic improvements. Preservation of the proline ring facilitates macrocyclization (a common stumbling block in synthesis) by bringing the ends of the linear peptide together in space. However, the real benefit now is that the proline motif opens the door to late-stage scaffold modification and analogue synthesis. In a process akin to the post-assembly modifications observed in nature, a single compound can be diversified into a collection of macrocycles. No longer does each macrocycle need to be synthesized individually, as many can be accessed from a shared late-stage intermediate. This development significantly reduces the effort needed to progress compounds through the pipeline and into the clinic.

Some substrates demonstrate low turnover under standard reaction conditions, and for these compounds it is necessary to increase the oxidant and acetic acid loadings to achieve comparable yields. Nevertheless, despite the common observation of some unreacted starting material and pyroglutamic acid side product, yields are consistently high throughout. Interestingly, the *para*-nosyl (nitrobenzenesulfonyl) protecting group, thought to be the origin of the *syn*-stereochemistry in arylated proline derivatives, is uniquely suited to this C–H oxidation. A proper understanding of the reaction mechanism will be needed to explain this observation and answer other more important questions regarding the active species and the precise catalyst–substrate interactions.

All parts of the molecular scaffold contribute to the biological activity

(and cell permeability) of macrocyclic compounds, but a full understanding of the relationship between structural modifications and molecular shape is still in development¹². Gratifyingly, the modular synthesis strategy developed by White and co-workers enables the systematic variation of diversity elements within the macrocycle core, and as such it is well-suited to shed light on the design of macrocycles and other small molecules with tailored molecular shape¹³.

Looking ahead, one envisages the design and incorporation of further amino acids amenable to this and other C–H oxidation processes in the search for small molecules that exhibit new biological activity. Moreover, as seen in the biosynthesis of the vancomycin scaffold, oxidative cross-linking of aryl side chains constrains a linear peptide into a rigidified cyclic architecture that is crucial for biological activity. In this context, it will be interesting to see this C–H oxidation strategy extended to the intramolecular cross-linking of aryl side chains and proline (and other amenable) residues. With these tools in hand, this work can realize a true platform technology in drug discovery. □

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