

# An Oxazetidine Amino Acid for Chemical Protein Synthesis by Rapid, Serine-Forming Ligations

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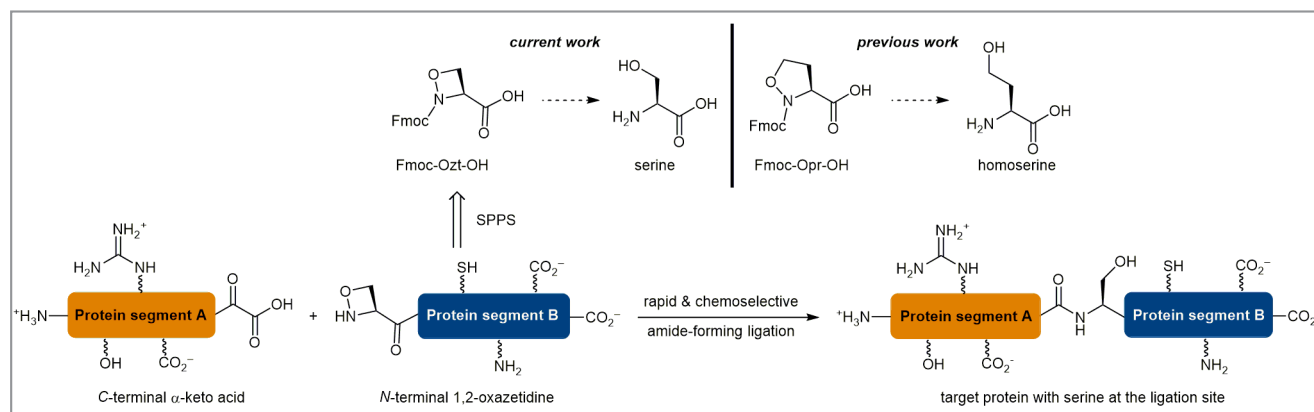
The development of chemical ligation reactions has revolutionized modern chemical biology. Among these techniques, the native chemical ligation (NCL) of thioesters and N-terminal cysteines – reported by Kent and co-workers over twenty years ago (see the original *Nat. Chem.* article for references) – has been one of the greatest advances in protein synthesis; still, however, many synthetic targets remain out of reach. In order to identify more general and complementary protein ligation reactions, numerous groups have pursued the development of novel methods and ligation partners, including the group of Professor Jeffrey Bode at the ETH Zürich (Switzerland). Professor Bode said: “In our own effort to provide a valuable alternative, we identified the reaction between C-terminal  $\alpha$ -keto acids and N-terminal hydroxylamines (KAHA ligation) to be robust and chemoselective.”

The KAHA ligation with oxaproline (Opr, see Scheme 1) as hydroxylamine proved to be an excellent alternative to the NCL, and the group prepared numerous proteins with this ligation. However, Professor Bode commented that the reaction presents some drawbacks. “The primary products of the KAHA ligation with Opr are desipeptides, and the amino acid formed at the ligation site is a non-canonical homoserine residue,” explained Professor Bode. “The reaction rate of the KAHA is suitable for the preparation of small and medium-sized proteins but may not be sufficient when moving to larger or more challenging targets where only micromolar concentrations of

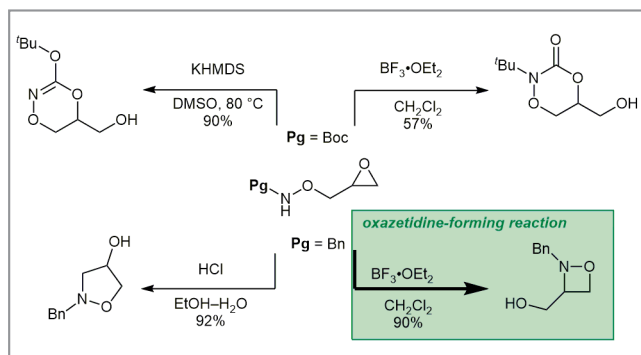
the reactants are present.” Through the synthesis of several proteins of up to 184 residues, the first two drawbacks were shown to be almost always negligible and sometimes even advantageous. However, the third – reaction kinetics – remains a concern for the Zürich based researchers, especially when the ligation of folded proteins or very hydrophobic segments is attempted.

In order to provide a KAHA protein ligation that both affords canonical amino acid residues and operates at a faster rate, the group sought to prepare an Fmoc-protected oxazetidine amino acid (Fmoc-Ozt-OH). Professor Bode said: “In our eyes, this compound had not only the potential to ligate giving rise to a natural amino acid (Ser), but also to react more rapidly because of the ring strain of the four-membered ring. However, this simple answer generated even more questions than the ones we were trying to address: will the oxazetidine ring be stable? Will it undergo KAHA ligation when exposed to  $\alpha$ -keto acids? If so, will the reaction be faster? But first of all: how to prepare the oxazetidine amino acid?”

A wide palette of approaches were considered by the authors of this study, including cycloadditions, electrocyclizations, intramolecular substitution reactions, and ring contractions. “The general conclusion of these attempts was that the thermodynamic price to be paid to close the 1,2-oxazetidine ring – the one with the desired substitution pattern! – was higher than expected,” said Professor Bode. “The most pro-



Scheme 1



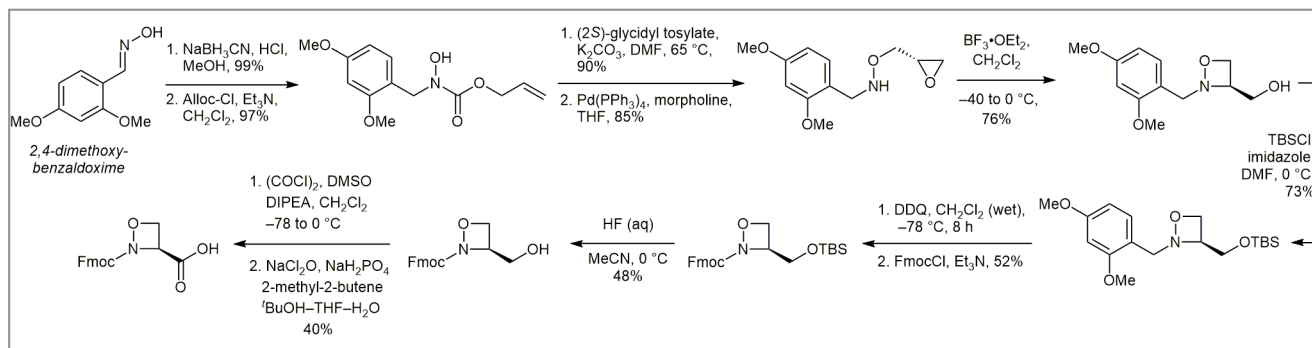
Scheme 2

missing route, intramolecular epoxide opening, proved to be a source of both excitement and disappointment,” continued Professor Bode. He explained: “Although we could successfully cyclize several substrates, we always obtained products that had the correct mass, and intriguing NMR spectra, but ultimately the wrong structure. After months of failures, we were elated to find a combination of N-protecting group and activation method that led finally to the oxazetidine ring (Scheme 2).”

Unfortunately, the benzyl substitution on the oxazetidine nitrogen atom had to be exchanged for another protecting group (ideally Fmoc) without breaking the N–O bond. This proved non-trivial, eventually requiring the use of an oxidatively cleavable amine-protecting group that necessitated careful experimentation and optimization. Professor Bode commented: “We were rewarded, however, by finding that the key cyclization proceeded in a stereospecific fashion, allowing us to prepare the oxazetidine in enantioenriched form. After some protecting group manipulations and oxidation state adjustments, we arrived at our target: Fmoc-Ozt-OH (Scheme 3).”

In Fmoc-protected form, the oxazetidine proved to be stable and easy to handle – surviving even cleavage from Rink-Amide resin under strongly acidic conditions to give peptide segments bearing the Fmoc-Ozt on their N-terminus. When deprotected and exposed to  $\alpha$ -keto acids, the ‘naked’ 1,2-oxazetidine displayed the postulated reactivity maintaining the selectivity of the KAHA ligation with Opr. “Although the unprotected oxazetidine is unstable – in marked contrast to its completely stable five-membered cousin 5-oxaproline – it can be used directly in serine-forming KAHA ligation under dilute, neutral conditions,” said Professor Bode, who concluded: “The utility of the new amino acid was shown by the three-fragment total chemical synthesis of S100A4, a protein involved in the metastasis process. This is a challenging target, made difficult by hydrophobic sequences and a tendency to aggregate. Only by performing the final ligation with the oxazetidine were we able to complete the synthesis of this target.”

*Matthew Farnish*



Scheme 3

## About the authors

*Dr. I. Pusterla*

**Ivano Pusterla** was born in 1985 in Morbio Inferiore (Switzerland). He completed his BSc (in 2007) and MSc (2009) in chemistry at ETH Zürich (Switzerland). After an industrial internship in medicinal chemistry at Novartis Pharma AG in Basel (Switzerland) and a postgraduate stay at the University of Zürich (Switzerland), he joined the research group of Professor Jeffrey W. Bode in 2010. His PhD thesis focused on the KAHA ligation and its applications. He received his PhD in 2014 and he is currently working as an R&D chemist in bioconjugation at Lonza AG.

*Prof. J. W. Bode*

**Jeffrey W. Bode** studied at Trinity University in San Antonio, TX (USA). Following doctoral studies at the California Institute of Technology (USA) and ETH Zürich (Switzerland) and postdoctoral research at the Tokyo Institute of Technology (Japan), he began his independent academic career at the University of California, Santa Barbara (USA) in 2003. He moved to the University of Pennsylvania (USA) as an associate professor in 2007 and to ETH Zürich (Switzerland) as a full professor in 2010. Since 2013, he is also a Principal Investigator and Visiting Professor at the Institute of Transformative Biomolecules (WPI-ITbM) at Nagoya University (Japan).