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An Asymmetric Synthesis of L-Pyrrolysine

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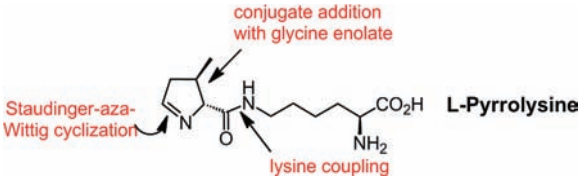
ABSTRACT



An efficient asymmetric synthesis of the 22nd amino acid L-pyrrolysine has been accomplished. The key stereogenic centers were installed by an asymmetric conjugate addition reaction. A Staudinger/aza-Wittig cyclization was used to form the acid-sensitive pyrroline ring. Pyrrolysine was synthesized in 13 steps in 20% overall yield.

Pyrrolysine is the 22nd genetically encoded amino acid.[1] It consists of a (4*R*,5*R*)-4-methyl-5-carboxypyrroline ring linked to the ε-nitrogen of L-lysine.[2,3] Pyrrolysine was identified by X-ray crystallography, when it was first observed in the structure of *Methanosarcina barkeri* monomethylamine methyltransferase.[1] It has been hypothesized that this unique amino acid plays an important role in methane production in some archaeal species.[4]

Pyrrolysine is encoded by an in-frame UAG codon, which is nonterminating, and its incorporation is mediated by a dedicated tRNA and cognate tRNA synthetase.[5] The mechanism of pyrrolysine incorporation into proteins offers a platform for developing new protein labeling methods.[6] Small molecules, such as fluorophores,[6a] biotin,[5e] ubiquitin,[6b] and oligosaccharides,[5e] have been used for labeling pyrrolysine and pyrrolysine-surrogate residues.[7] Although pyrrolysine has been exploited for protein labeling, an understanding of its biosynthesis,[5e,8] evolutionary purpose, and distribution in the proteome is incomplete. New synthetic methods that provide sufficient quantities of this novel amino acid would facilitate an understanding of pyrrolysine biochemistry.

Pyrrolysine is a synthetic target.[2,9] One reported chemical synthesis[2] involves the coupling of (4*R*,5*R*)-4-methylpyrroline-5-carboxylic acid to lysine. While this innovative route affords the desired product, the yield is modest (9% overall). Moreover, we and others[5a,9] have found that the reactions were irreproducible. We therefore sought to devise an alternative synthetic route.

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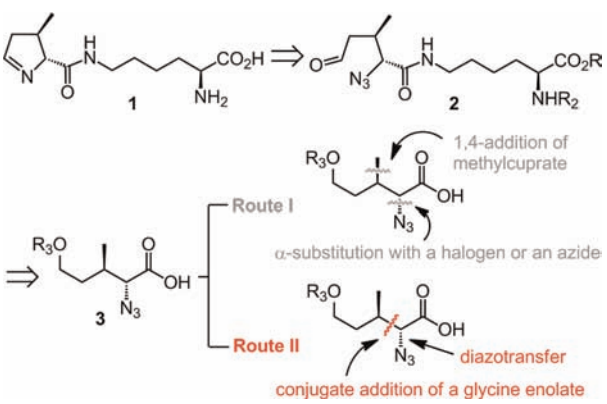
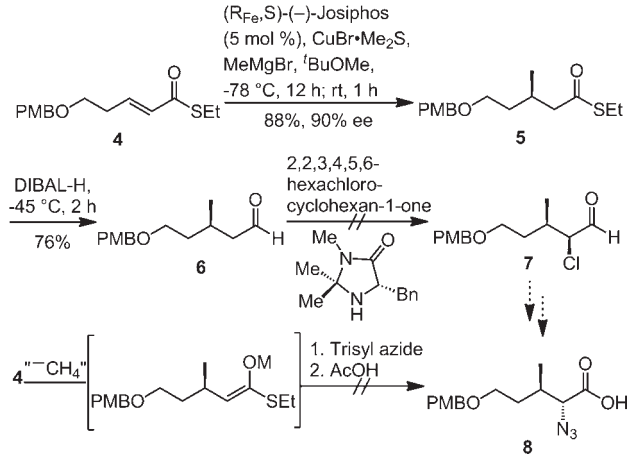
Scheme 1. Retrosynthesis of Pyrrolysine



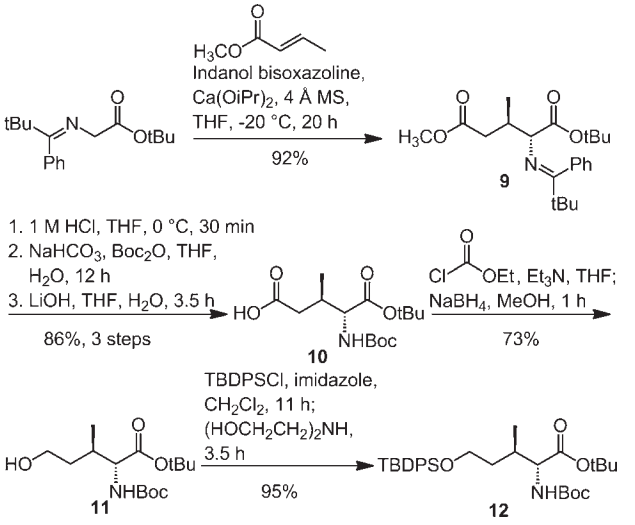
A drawback of the reported pyrrolysine synthesis is that it entails exposure of the sensitive carboxypyrroline ring to strongly acidic conditions, during both imine formation and lysine coupling. We focused on generating the pyrroline ring in the penultimate step of the synthesis. In this way, we hoped to avoid reactions that could lead to pyrroline ring degradation. Specifically, we envisioned forming the cyclic imine from azido aldehyde **2** via a tandem Staudinger/aza-Wittig reaction[10] (Scheme 1). Azido aldehyde **2** could be obtained from coupling of **3** with a selectively protected lysine derivative. We considered two different approaches to compound **3**: an asymmetric conjugate addition of a methylcarbanion followed by α-substitution ("Route I") or a Michael addition with a glycine enolate ("Route II").

Scheme 2. Attempts To Synthesize Pyrrolysine Precursors via Asymmetric Methylcarbanion 1,4-Addition and α-Substitution



We pursued these strategies in parallel. For Route I, we anticipated the methyl group of pyrrolysine precursor **3** could be installed by conjugate addition to a compound such as **4**. The Feringa group has developed efficient catalysts for this type of transformation.[11] Accordingly, the Josiphos catalyst gave rise to the 1,4-addition of methyl Grignard to α,β-unsaturated thioester **4** (Scheme 2). Attempts to functionalize the α-position with an electrophilic azide source or by an asymmetric α-halogenation[12] and subsequent nucleophilic displacement with an azide[13] did not afford the desired products. A more significant drawback of this approach was our observation that the most robust α-halogenation methods[14] were incompatible with the thioester required for the Josiphos-catalyzed reaction. As a result, the conjugate addition and the selective halogenation reactions demanded different carbonyl derivatives (thioester versus aldehyde), thereby necessitating multiple changes in oxidation state.

Scheme 3. Pyrroline Precursors via Michael Addition



The roadblocks encountered pursuing Route I prompted us to focus on Route II. In this approach, we planned to install the pyrroline stereochemistry by carrying out Michael addition of a glycine enolate in the presence of a chiral catalyst. Calcium ion promoted conjugate addition of *N*-(*tert*-butylphenylmethylidene)glycine *tert*-butyl ester to methyl crotonate produced **9**[15] in 92% yield with 99% enantiomeric excess and greater than 99:1 diastereomeric excess (Scheme 3). This reaction was not only stereoselective and efficient but also robust. These favorable

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attributes were preserved even when the reactions were conducted on a medium scale (50−100 mmol).

While the Schiff base was necessary for the high stereoselectivity attained,[16] the imine was prone to hydrolysis upon exposure to acid or base. Accordingly, the *tert*-butylphenyl imine **9** was hydrolyzed, and the unmasked amine was immediately exposed to di-*tert*-butyl dicarbonate. We anticipated the *tert*-butyl carbamate could be removed later concomitantly with *tert*-butyl ester hydrolysis. The methyl ester was then saponified to afford **10**. The three-step interconversion of protecting groups was executed in an overall yield of 86%. The series of transformations proceeded with no loss of stereochemical integrity, as revealed by X-ray crystallography (Figure 1). The structure of acid **10** confirmed it had the expected absolute stereochemistry.

At this point in the synthesis, we recognized that differentiation of the two carboxy termini would be crucial for accessing lysine-coupled azido aldehyde **2** (Scheme 1). To this end, the carboxylic acid was transformed to a mixed anhydride, which was reduced with sodium borohydride to afford hydroxy *tert*-butyrate **11** in 73% yield. The resulting primary hydroxyl group was converted to a silyl ether to mitigate side reactions with a free hydroxyl group. The silyl ether **12** was generated under mildly basic conditions as these avoided lactone formation. To minimize the formation of a disiloxane byproduct that coeluted with the product during chromatography, the reaction was quenched under nonaqueous conditions. Using these precautions, compound **12** could be readily obtained.

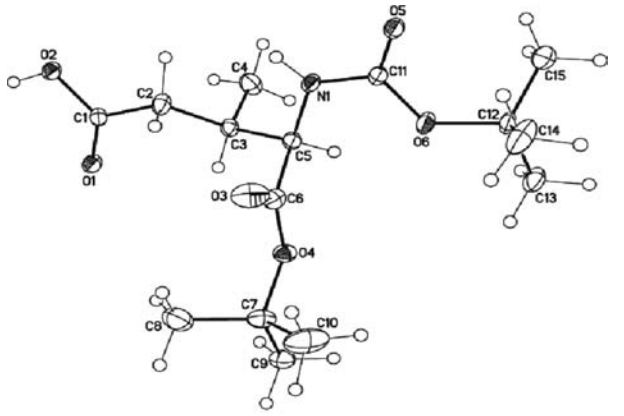
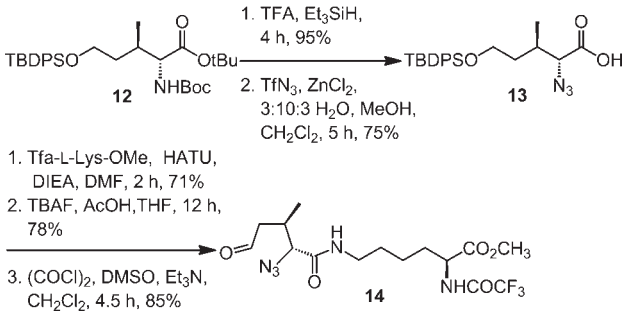


Figure 1. The stereochemistry generated in the production of imine **9** was preserved through multiple transformations (Scheme 3) as revealed by X-ray structure crystallographic analysis of acid **10**.

Each functional group in ester **12** (Scheme 4) is poised for manipulation to access compound **14**, the precursor for the tandem sequence. As anticipated, the *tert*-butyl ester and carbamate protecting groups could both be removed in a single step. The azide group was installed using a Zn^{2+}-mediated diazotransfer from triflyl azide,[17] which afforded coupling partner **13** in 75% yield over two steps.[18] The precursor to the pyrroline ring, α-azido carboxylic acid **13**, was appended to the epsilon nitrogen of *N*-trifluoroacetamidyl lysine methyl ester.[2] The silyl protecting group was cleaved, and the resulting primary alcohol was converted to an aldehyde by the Swern oxidation.[19] This sequence provided compound **14** as the substrate for the Staudinger/aza-Wittig reaction.

Scheme 4. Generating the Staudinger/aza-Wittig Substrate



Pyrroline **15** was generated using an efficient sequence that minimized purification steps (Scheme 5). The reduction of the azide moiety was effected using polystyrene-supported triphenylphosphine. The putative iminophosphorane underwent cyclization to afford protected pyrrolysine derivative **15**. Solution-phase triarylphosphine reagents have been used previously to execute tandem Staudinger reduction and intramolecular aza-Wittig reactions.[20] The popularity of this strategy is gaining, presumably because of the efficiency and simplicity it affords for generating even highly sensitive imines such as **15**. The use of an immobilized phosphine to effect the transformation offers an additional advantage. It circumvents the need for chromatography, which can be a major benefit in preparing sensitive imines. To generate the final pyrrolysine product, we removed the protecting groups at the N- and C-termini[2] of pyrroline **15** to afford the lithium salt of L-pyrrolysine **16** in 20% overall yield.

The sequence described is high yielding and stereoselective. It affords L-pyrrolysine in 13 steps and 20% overall yield. Through our examination of multiple strategies for

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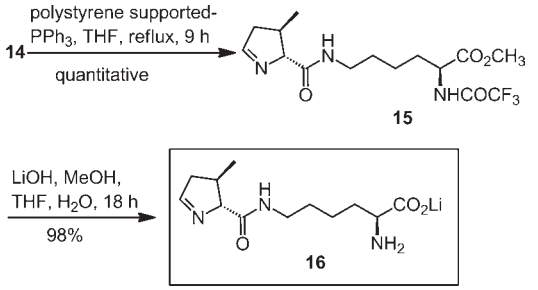
Scheme 5. Tandem Staudinger/aza-Wittig and Protecting Group Removal



the installation of the two stereogenic centers of pyrrolysine, we identified the Ca^{2+}-mediated conjugate addition reaction as an efficient process for generating pyrroline precursors. Another feature of our route is the gentle conditions offered by a polymer-supported phosphine to effect the Staudinger/aza-Wittig cyclization. We anticipate that this mild transformation can be used to generate pyrrolysine analogs. Our route will also be valuable for synthesizing isotopically labeled pyrrolysine to explore the prevalence of this intriguing amino acid in microorganisms and to carry out proteomic analyses.

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Supporting Information Available. Experimental procedures and full characterization of compounds **9**–**15** and associated intermediate compounds and X-ray crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.