Organic LETTERS

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pubs.acs.org/OrgLett

5-Methylisoxazole-3-carboxamide-Directed Palladium-Catalyzed γ-C(sp^3)−H Acetoxylation and Application to the Synthesis of γ-Mercapto Amino Acids for Native Chemical Ligation

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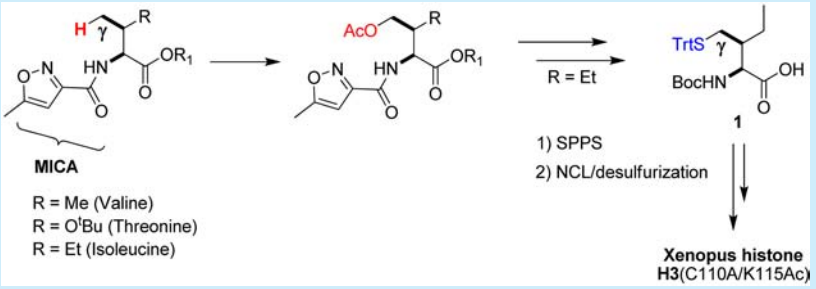
Supporting Information



ABSTRACT: Palladium-catalyzed acetoxylation of the primary γ-C(sp^3)−H bonds in the amino acids Val, Thr, and Ile was achieved using a newly discovered 5-methylisoxazole-3-carboxamide directing group. The γ-acetoxylated α-amino acid derivatives could be easily converted to γ-mercapto amino acids, which are useful for native chemical ligation (NCL). The first application of NCL at isoleucine in the semisynthesis of a *Xenopus* histone H3 protein was also demonstrated.

Recent years have witnessed tremendous advances in the development of methodologies for selective functionalization of inert C(sp^3)−H bonds using transition-metal catalysts.[1] The success is largely attributed to the use of the so-called directing groups or auxiliaries, which can bond and deliver the metal atom to a particular C−H bond for its activation via cyclometalation.[2] The transformational simplicity of direct C(sp^3)−H functionalization endows it with great potential in organic synthesis, and recent advances have helped to establish it as a viable methodology in the synthesis of complex organic compounds such as natural products and pharmaceutics.[3] Another interesting application is the synthesis of certain nonproteinogenic "unnatural" amino acids through the direct functionalization of C(sp^3)−H bonds in simple amino acids.[4,5] Among the many uses of unnatural amino acids, one is ascribed to native chemical ligation (NCL), which in its original form utilizes an N-terminal Cys residue of one peptide to mediate ligation with another thioester peptide to form an Xaa-Cys peptide bond at the ligation junction.[6] As an extension of NCL, many unnatural β or γ-mercapto amino acids have been prepared and used as NCL mediators for ligation at non-Cys junctions through a ligation−desulfurization strategy which was first introduced by Dawson et al.[7,8] Despite the great utility of these mercapto amino acids for NCL, their synthesis using existing methods is often very tedious and inefficient due to ramifications in the steps of carbon framework construction, introduction of functional groups, and protection and deprotection maneuvers.[9] New and more efficient methods to prepare these mercaptoamino acids continue to be well sought after. An attractive route to the synthesis of these mercapto amino acids would be via direct functionalization of their natural amino acid counterparts; however, so far this has been realized only for amino acids with "activated" C−H bonds on the side chain,[8a,m,n,p,q,s] such as Asp and Glu of which the side-chain COOH when in its ester form makes the respective β and γ-C−H bonds more acidic for direct functionalization.[8m,n,p] For example, a β-HS-Asp analogue can be synthesized stereoselectively in three steps,[8m] but this cannot work for "inactivated systems" in amino acids with inert β and γ-C−H bonds. For these systems, the aforementioned transition-metal catalyzed C−H activation presents a conceptually attractive way to access their thiol-modified derivatives. Figure 1 shows such a synthetic strategy for certain γ-mercapto-α-amino acids.

Sanford[10] and Yu[11] demonstrated early examples of Pd-catalyzed selective oxygenation of inert C(sp^3)−H bonds in simple organic compounds using monodentate nitrogen-based

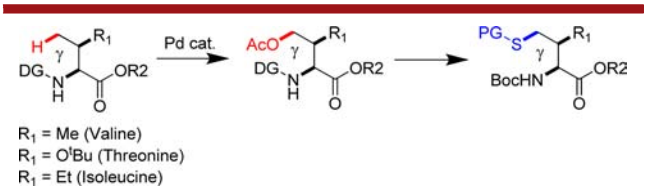


Figure 1. Pd-catalyzed direct acetoxylation of γ-CH$_3$-containing α-amino acid derivatives and conversion to γ-mercapto amino acids. DG = directing group.

Received: April 21, 2016
Published: May 24, 2016

DOI: 10.1021/acs.orglett.6b01160
Org. Lett. 2016, 18, 2696−2699

directing groups. Based on the pioneering work of Daugulis on removable bidentate 8-aminoquinoline (AQ) and picolinamide (PA) directing groups for C−H activation,[12] Corey first showed AQ-directed β-acetoxylation in amino acid substrates.[13] Subsequently, both AQ and PA as well as other directing groups have been used for the β and γ-acetoxylation of carboxylic acid and alkylamine substrates including some amino acids.[14] We realized that such a method for inert C−H acetoxylation would be useful to prepare the otherwise difficult-to-prepare γ-mercaptoamino acids. As the reaction occurs on the γ-C, it would avoid the challenges involved in obtaining the desired chiral configuration at the β-carbon of threonine or isoleucine when using conventional synthetic methods. γ-Mercaptoisoleucine would be a particularly difficult target for the traditional methods, and likely for this reason it has not been reported thus far for use in NCL.

Herein, we show that our newly discovered 5-methyl isoxazole-3-carboxamide (MICA) auxiliary group[15] can be used to direct palladium-catalyzed C−H acetoxylation on the γ-carbon of Val, Thr, and Ile. Moreover, we show that the acetoxylated α-amino acids can be easily converted to γ-mercaptoamino acids, which are useful mediators of NCL for protein synthesis.

Built on the initial success of our MICA directing group for C−C bond formation,[15] we prepared MICA-protected Val, Thr, and Ile esters for use as substrates for direct Pd-catalyzed γ-C acetoxylation. Indeed, when these MICA-amino acid esters were treated with the oxidizing agent $PhI(OAc)_2$ (2.5 equiv) in toluene (0.25 M) in the presence of the catalyst $Pd(OAc)_2$ (0.1 equiv) and AcOH (1 equiv) at 90 °C for 24−36 h, the acetoxylation products were obtained in very good yields (Figure 2). Similar to C−C bond formation,[15] the predominant

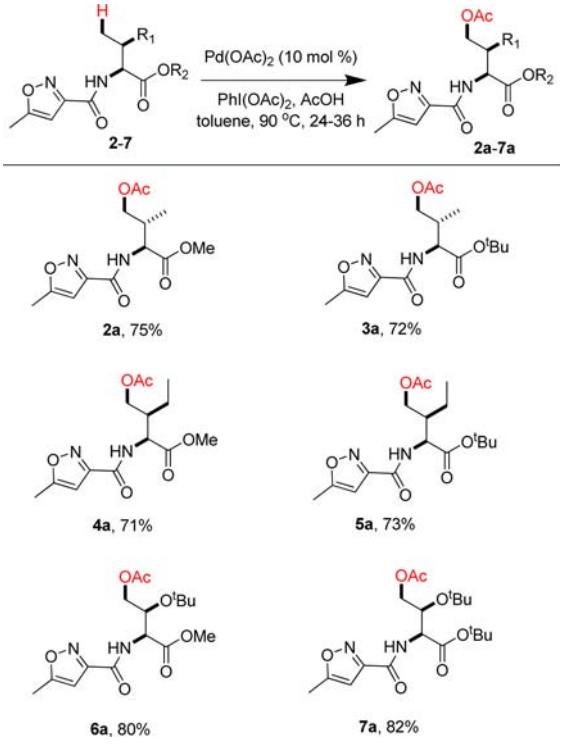
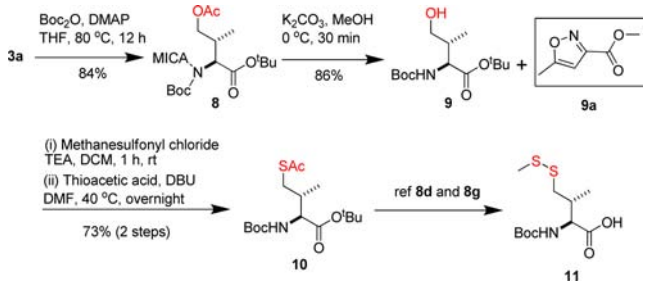


Figure 2. MICA-directed acetoxylation of Val, Ile, and Thr. All of the reactions were carried out in a Teflon-sealed tube (see the Supporting Information for experimental details).

product for the L-valine substrates was the monoacetoxylated form (**2a** and **3a**) which was isolated in 75% and 72% yields for the methyl and *tert*-butyl esters respectively (Figure 2). It is noteworthy that acetoxylation occurred selectively on the pro-R γ-C of Val. For isoleucine, acetoxylation occurred exclusively on the primary γ-C (**4a** and **5a**). Acetoxylation of L-threonine substrates also yielded the desired products **6a** and **7a** in excellent yields. The *tert*-butyl and acetate groups from compounds **6a** and **7a** could be removed using orthogonal methods. This would provide amino acid derived, chemically differentiated 1,2-diols which have great synthetic value for the synthesis of complex natural products.

The γ-acetoxylated amino acids are precursors to γ-mercapto amino acid derivatives. Using conventional synthetic procedures as shown in Scheme 1, the γ-acetoxy-MICA-Val-O-tBu

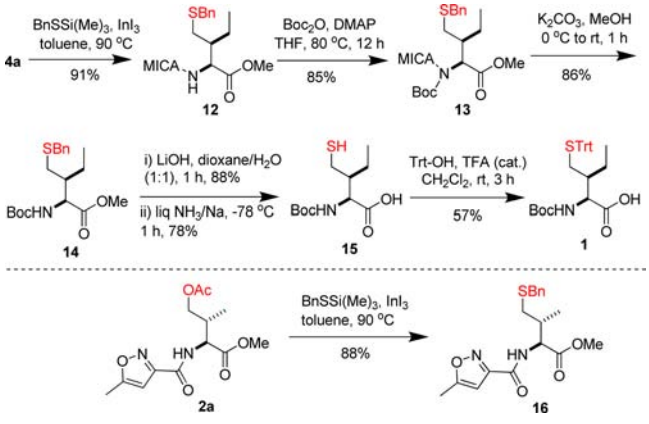
Scheme 1. Conversion of γ-AcO-valine to γ-Mercaptovaline Derivative by Conventional Procedures



ester was first converted to its Boc-protected form **9**, with recovery of the directing group, 5-methylisoxazole-3-carboxylate, as the methyl ester (**9a**) in two steps. After removal of the *O*-acetyl group, the exposed hydroxyl was mesylated, followed by treatment with thioacetate to furnish the nucleophilic substitution product **10**. Simple deprotection and reprotection procedures would then lead to Nα-Boc-Val(γ-$SSCH_3$)-OH **11**, which can be coupled in solid-phase peptide synthesis as the N-ter residue of a peptide for NCL.

Previously, Baba et al. developed a procedure to transform alkyl acetates to thioethers using indium triiodide catalyst.[16] We found that, using this procedure, the acetoxy group in the C−H functionalization products could also be directly converted to benzylthioether. As shown in Scheme 2, treatment of MICA-Ile(γ-OAc)-OMe **4a** with benzylthiotrimethylsilane and indium

Scheme 2. Conversion of γ-AcO-isoleucine to the γ-Mercaptoisoleucine Derivative Using a New Procedure



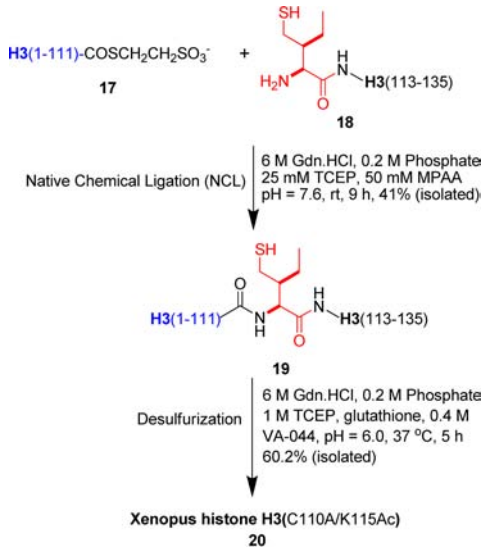
DOI: 10.1021/acs.orglett.6b01160
Org. Lett. 2016, 18, 2696−2699

triiodide in dry toluene at 90 °C for 5 h gave directly MICA-Ile(γ-SBn)-OMe **12** in 91% isolated yield. In a similar way, the acetoxylated Val product **2a** was also successfully converted to the benzylthioether form **16**. However, this procedure did not work for the acetoxylated Thr derivative **6a**, possibly due to the liability of the *tert*-butyl ether to the Lewis acid catalyst at elevated temperatures. Nevertheless, we can use the conventional method as shown in Scheme 1 for the preparation of the γ-mercaptothreonine derivative (Scheme S1).

We chose to proceed with MICA-Ile(γ-SBn)-OMe **12** to prepare the desired γ-mercapto-functionalized Ile as it has not been synthesized previously for NCL. After MICA was changed to Boc and the methyl ester was hydrolyzed, the benzyl thioether was deprotected with sodium in liquid ammonia to afford the free thiol compound **15**. The *S*-trityl group was then introduced to give Boc-Ile(γ-STrt)-OH **1**, which can then be coupled as the last amino acid in SPPS to give the γ-mercaptoisoleucyl peptide for NCL.

We then continued to demonstrate the native chemical ligation−desulfurization strategy for ligation at isoleucine in the semisynthesis of a histone protein, *Xenopus* H3 (Scheme 3). As

Scheme 3. Semisynthesis of *Xenopus* Histone H3(C110A/K115Ac)



for all histone proteins, H3 undergoes various post-translational modifications (PTMs) that regulate the dynamics and complexity of chromatin activity. Chemical synthesis of homogeneous histone H3 and its analogues should assist the ongoing efforts in elucidating and understanding the functional roles of these PTMs. Previously, our group has synthesized an H3 protein by using the thioacid capture ligation strategy.[17] In the current strategy, we designed the preparation for histone H3 **20** through the ligation between histone H3(1−111) **17**, which bears the MES thioester, and peptide H3(113−135) **18**, which has an N-terminal γ-mercapto-Ile.

The H3 C-terminal peptide H3(112−135) **18** with sequence H-**I*HAK**(Ac)**RVTIMPKDIQLARRIRGERA**-OH (**I*** denotes γ-HS-Ile) was synthesized manually by employing the standard fluorenylmethyloxycarbonyl (Fmoc) SPPS chemistry except for the last N-terminal amino acid which was added using Boc-Ile(γ-STrt)-OH. This synthetic peptide contains a Lys(Ac) at position 115. HPLC and ESI-MS analysis for the synthesis of compound **18** is shown in the Figures S1 and S2. The wild-type *Xenopus laevis* histone H3(**1−111**) MES thioester **17** was prepared through the use of the well-established intein splicing protocol. The purified protein thioester was analyzed by C4 analytical RP-HPLC and confirmed by ESI-MS analysis (Figures S4 and S5).

To synthesize *Xenopus* histone H3(C110A/K115Ac), the H3 C-terminal peptide **18** was reacted with H3(1−111)-MES **17** (Scheme 3). One milligram of H3(**1-111**)-MES thioester **17** and 1 mg of H3 C-terminal peptide **18** were dissolved in 1 mL of ligation buffer containing 6 M Gdn·HCl, 0.2 M phosphate, 25 mM TCEP, and 50 mM MPAA, pH 7.6. The ligation was done at room temperature for 9 h, and the reaction was monitored with C4 analytical HPLC (Figure S6). The ligation product was isolated by C4 semiprep HPLC and confirmed by ESI-MS. A total of 0.5 mg of ligation product was obtained (Figures S7 and S8).

Free-radical desulfurization was then performed to convert γ-mercaptoisoleucine112 at the ligation junction to isoleucine and also the native Cys110 to Ala. Initially, 0.5 mg of full-length H3 was dissolved in 300 μL of buffer containing 6 M Gdn·HCl, 0.2 M phosphate. Subsequently, 100 μL of 1 M TCEP, 5 μL of 100 mM reduced glutathione, and 20 μL of 0.4 M VA-044 were added sequentially. The final pH of the mixture was adjusted to 6.0. After 5 h reaction at 37 °C, another 20 μL of 0.4 M VA-044 was added and the reaction continued for another 3 h at 37 °C. The final desulfurization product was obtained after C4 semiprep HPLC purification and confirmed by ESI-MS. A total of 0.3 mg of final product H3(C110A/K115Ac) **20** was obtained (Figure 3).

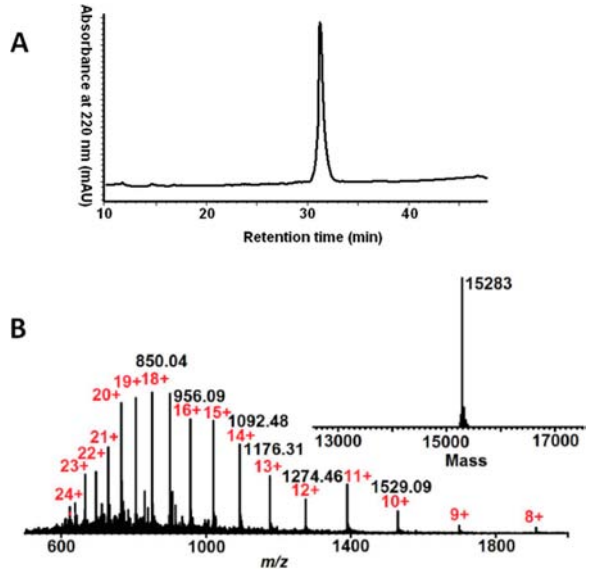


Figure 3. (A) C4 analytical HPLC of the final desulfurized product H3(C110A/K115Ac). Gradient: 0−80% buffer B in buffer A for 40 min. (B) Raw and deconvoluted ESI-MS of purified H3-(C110AK115Ac). Average mass: calcd 15280.7, obsd 15283.

In summary, we have shown that, in addition to the previously demonstrated C−C bond formation,[15] 5-methyl-isoxazole-3-carboxamide (MICA) can also be used to direct Pd-catalyzed acetoxylation of unactivated γ C(sp^3)−H bonds in several amino acid substrates (Val, Thr, and Ile). An excellent recent review on isoxazoles has documented metal-catalyzed reactions of isoxazoles.[18] Our work provides another new use of

DOI: 10.1021/acs.orglett.6b01160
Org. Lett. 2016, 18, 2696−2699

MICA, an isoxazole derivative, as a versatile bidentate directing group for Pd-catalyzed C−H functionization. The prepared γ-acetoxy-α-amino acid derivatives are precursors to the respective γ-mercapto-α-amino acids. This provides a direct way to modify the inert γ-CH_3 group of the related natural amino acids to access their thiol-modified analogues for NCL. We also demonstrated NCL using γ-mercaptoisoleucine in the semisynthesis of *Xenopus* histone H3 protein. Overall, our work provides the first example of practicing transition-metal catalyzed C−H activation in the synthesis of thiol-modified amino acids. In principle, the γ-acetoxy-α-amino acid derivatives can also be used as precursors to prepare many other biologically important compounds in medicinal chemistry and peptidomimetics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01160.

Experimental procedures and detailed characterization data of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is supported by a GAP grant from ETPL of A*star (ETPL-QP-19-06 to CFL).

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