



Iridium-catalyzed diacylmethylation of tyrosine and its peptides with sulfoxonium ylides†

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Pyridyloxy-directed Ir(III)-catalyzed diacylmethylation of protected tyrosines was achieved with alkyl and (hetero)aryl sulfoxonium ylides, furnishing tyrosine-based unnatural amino acids in good yields. Furthermore, the late stage exemplification of the strategy was successfully accomplished in tyrosine-containing dipeptides, tripeptides and tetrapeptides in moderate yields. This methodology is distinguished by its site-selectivity, tolerance of sensitive functional groups, scalability, and retention of the chiral configuration for tyrosine motifs.

Unnatural amino acids (UAAs) prepared by site selective manipulation or conjugation of an amino acid with bioactive molecules have demonstrated remarkable applications in drug optimization and drug delivery studies.¹ Such designer amino acids possess unique structural features that foster peptide interactions with biological targets, leading to the discovery of new therapeutic modalities.² In addition, introducing UAAs at specific sites in a protein can fine-tune its properties and functions as per the desired requirement.³ Thus, the applications of functionalized peptides and peptidomimetics as molecular probes and therapeutics have increased manifold, leading to a growing need for developing novel strategies for chemical modification of amino acids with structural diversity.⁴

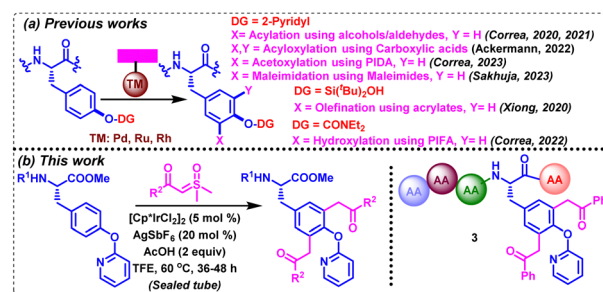
Tyrosine (Tyr) is a proteinogenic amino acid that is ubiquitous in both natural and synthetic bioactive molecules, including endomorphine,⁵ levothyroxine,^{6a} KN-62,^{6b} OF4949-I,^{6c} and mycoecyclosin.^{6d} Due to the presence of an amphiphilic phenolic side chain, it has been involved in varied biochemical processes, exhibiting a significant role in protein–protein and protein–ligand interactions. Its susceptibility to other functionalities by post-translational modifications in the human body

further highlight the need for investigating such selectively modified tyrosines in bioactive peptides and proteins. In spite of the low natural abundance of tyrosine, it is found to be embedded in distinct biological environments in most of the proteins.⁷ Notably, a handful of eye-catching metal-catalyzed and metal-free strategies have evolved over the years, aiming at functionalizing proximal and distal C–H bonds in varied amino acids, including tyrosine.^{8,9} In this realm, Xiong, Correa and Ackerman have intensified their efforts recently towards the selective functionalization of tyrosine and tyrosine-containing oligopeptides by employing a variety of directing groups (DGs) (Scheme 1a). For example, Xiong's group reported silanol-mediated C₃Ar–H olefination in tyrosine and tyrosine-containing peptides with olefins under Pd-catalysis.^{10a} Correa's group achieved pyridyloxy-directed late-stage C₃Ar–H acylation and acetoxylation of Tyr-containing oligopeptides with alcohols/aldehydes and (diacetoxy-iodo)benzene (PIDA) under Pd- and Ru-catalyzed conditions, respectively.^{10b–d} In addition, the same group achieved C₃Ar–H hydroxylation by carbamate-directed Ru-catalyzed activation with PhI(OCOCF₃)₂.^{10e} Ackermann's group employed carboxylic acids for the late-stage diacyloxylation of tyrosine-containing peptides under electro-catalyzed conditions.^{10f} Recently, our group has also disclosed a Rh(II)-catalyzed strategy for the C₃Ar–maleimidation of tyrosines and tyrosine-containing oligopeptides with maleimides.^{10g} Interestingly, sulfoxonium ylides that are often employed for

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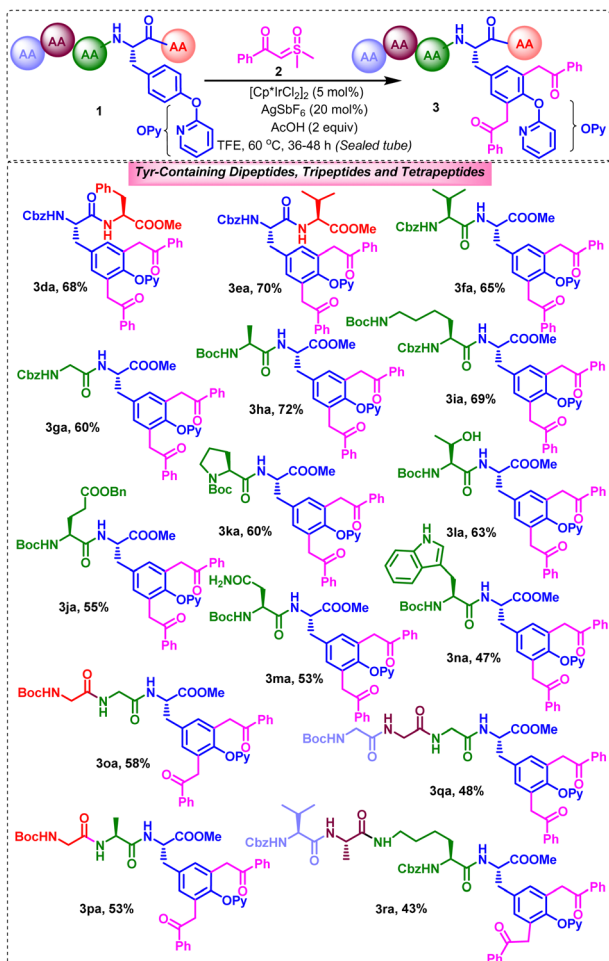
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Scheme 1 C₃Ar–H functionalization of Tyr- and Tyr-containing peptides via DG-assisted TM-catalyzed C–H activation.





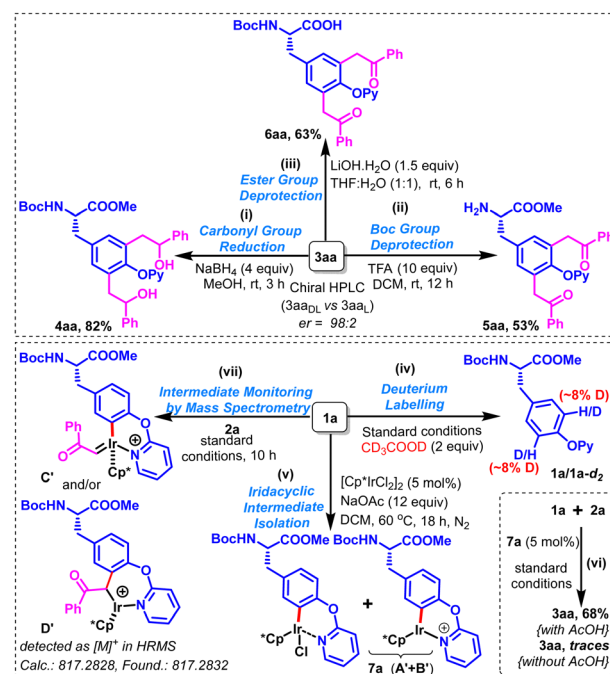
Scheme 3 Late-stage Tyr-containing peptide diacylmethylation.

(1k), Boc-L-Thr-L-Tyr(OPy)-OMe (1l), Boc-L-Asn-L-Tyr(OPy)-OMe (1m), and Boc-L-Trp-L-Tyr(OPy)-OMe (1n), with 2a produced their respective expected dibenzoylmethylated Tyr-containing dipeptides (3da, 3ea, 3fa, 3ga, 3ha, 3ia, 3ja, 3ka, 3la, 3ma, and 3na) in 47–72% yields (Scheme 3). Among these, 3ka was obtained as a mixture of rotamers as usually observed for proline-containing dipeptides. Notably, the strategy is equally workable for *o*-pyridyl tyrosine-containing dipeptides possessing tyrosine at the C- or N-terminus ends and Cbz or Boc protecting groups at the N-side or at the appended side chain functionalities, albeit in 36–48 h. Gratifyingly, this strategy works reasonably well for the late stage difunctionalization of tyrosine-containing tripeptides Boc-Gly-Gly-L-Tyr(OPy)-OMe (1o) and Boc-Gly-L-Ala-L-Tyr(OPy)-OMe (1p) and tetrapeptides Boc-Gly-Gly-Gly-L-Tyr(OPy)-OMe (1q) and Cbz-L-Val-L-Ala-L-Lys (*N*²-Boc)-L-Tyr(OPy)-OMe (1r) with 2a, producing their corresponding bis(benzoylmethyl)-appended Ty-containing tripeptides (3oa, 3pa) and tetrapeptides (3qa, 3ra) in 53–58% and 43–48% yields, respectively.

The enantiopurity of one of the products 3aa (3aa_L) was ascertained by comparing its HPLC profile with that of the corresponding racemic product [3aa_{DL}; obtained in 86% yield from racemic Boc-DL-Tyr(OPy)-OMe (1a_{DL})]. The high

enantiomeric ratio (>98:2) observed in the HPLC profile of 3aa_L indicates that almost no racemization is observed at the chiral center during the product formation (ESI⁺). The chemical applicability of the synthesized Tyr-based UAAs was demonstrated by performing the reduction of the two carbonyl groups with sodium borohydride in methanol to afford the corresponding bis(2-hydroxy-2-phenylethyl)-functionalized tyrosine derivative (4aa) in 82% yield as a mixture of diastereomers (Scheme 4i). Furthermore, deprotection of *N*-Boc and ester groups using TFA/DCM and LiOH/THF:H₂O produced their respective *N*- and *C*-side deprotected derivatives (5aa & 6aa) in 53% & 63% yields, respectively (Scheme 4ii and iii). It is worth mentioning that attempted deprotection of the *o*-pyridyl directing group from 3aa (or 3ba/3ca) by using the standard two-step protocol, involving the *N*-methylation with MeOTf/DCM followed by hydrogenation with Pd(OH)₂-C/HCOONH₄/MeOH or Na/MeOH could not succeed as messy mixtures were obtained in each case. To gain insights into the reaction mechanism, a few investigations were carried out. A very low H/D scrambling (approx. ~8%) was observed by performing deuterium labelling experiments on 1a under the standard conditions using 2 equivalents of deuterated acetic acid, suggesting the non-reversible nature of metalation/deutero(proto)demetalation of C₃Ar/C₅Ar-H bond(s) (Scheme 4iv). Interestingly, a pale yellow semi-solid (7a) was obtained in high yield by performing a stoichiometric reaction between 1a and [Cp*IrCl₂]₂ at 60 °C for 18 h; the detailed spectroscopic investigations of 7a suggested that it could possibly be an equimolar mixture of two six-membered iridacyclic intermediates (A' + B') (Scheme 4v). Notably, 3aa was obtained in 68% yield by carrying out the standard reaction between 1a and 2a

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Scheme 4 Chemical applicability of 3aa and mechanistic studies.



using a catalytic amount of **7a** with acetic acid, while traces of **3aa** were visible on TLC in the absence of acetic acid (Scheme 4vi). These observations suggested **7a** to be a viable intermediate, and a crucial role of acetic acid in the described transformation. Furthermore, real-time standard reaction monitoring through mass spectrometry indicated the presence of **C'** and **D'** in the reaction mixture (Scheme 4vii).

On the basis of the literature studies¹² and our investigation, a plausible mechanism is proposed (Scheme S1, ESI†). The reaction could be believed to proceed by AgSbF₆-mediated dissociation of [Cp*IrCl₂]₂ to monomeric active [Cp*IrCl(SbF₆)] species, which undergoes pyridyl-aided C_{3Ar}-H metalation in **1** to furnish a six-membered iridacyclic intermediate **B** via **A**. Subsequently, the coordination of sulfoxonium ylide **2** to the iridium center in **B** generates Ir-carbene complex **C** along with the extrusion of DMSO, which is followed by its regioselective migratory insertion between the C_{3Ar}-Ir bond generating a seven-membered iridacyclic intermediate **D**. Thereafter, protonolysis of the C_{3Ar}-acylmethyl group followed by re-coordination of Ir(III) with C_{5Ar}-H probably via a reversible rollover cyclometalation gives **E**, which upon repeated coordination of another molecule of sulfoxonium ylide with the iridium center initiates a second catalytic pathway to furnish **3aa**, along with the regeneration of the active Ir(III) catalyst for the next catalytic cycle.

In conclusion, we have developed a Ir(III)-catalyzed strategy for C_{3Ar}/C_{5Ar}-difunctionalization of *N*-protected *O*-pyridyloxy tyrosines with electron-rich and deficient sulfoxonium ylides to furnish a series of diacylmethylated tyrosine-based unnatural amino acids in moderate-to-good yields. The strategy was successfully employed for the late-stage difunctionalization of tyrosine-containing dipeptides, tripeptides, and tetrapeptides in reasonable reactivity. Isolation of a stable iridacyclic intermediate and the successful deprotection of the C- and N-side protecting groups are the added highlights of the presented work.

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Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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