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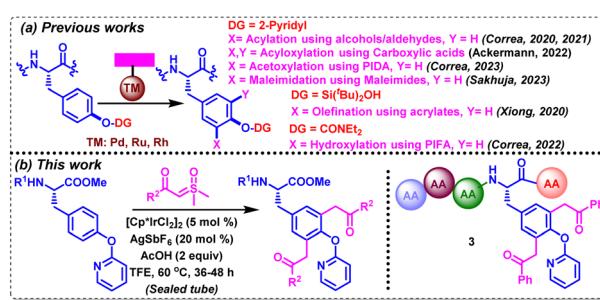
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Pyridyloxy-directed Ir(III)-catalyzed diacetylmethylation of protected tyrosines was achieved with alkyl and (hetero)aryl sulfoxonium ylides, furnishing tyrosine-based unnatural amino acids in good yields. Furthermore, the late stage exemplification 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 (UAs) 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 UAs 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 mycocyclosin.^{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



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

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o-acylmethylation, and annulation of variedly decorated (hetero)arenes, have not been explored for the $C_{Ar}-H$ acylmethylation of any aromatic amino acids.^{11a,b} We aimed to functionalize a tyrosine scaffold with such safe carbene precursors *via* transition-metal-catalyzed C–H bond activation with the aid of an appropriate directing group.

In the backdrop of the aforementioned discussion, we herein disclose a Ir^{III} -catalyzed pyridyloxy-directed methodology for the synthesis of $C3_{Ar}/C5_{Ar}$ -diacylmethylated tyrosines and tyrosine-containing oligopeptides using a variety of alkyl and (hetero)aryl sulfoxonium ylides in moderate-to-good yields (Scheme 1b).

We performed our optimization studies with Boc-L-Tyr(OPy)-OMe (**1a**) and 2-(dimethyl(oxo)-1*o*-sulfanylidene)-1-phenylethan-1-one (**2a**) as model substrates under Ir -catalyzed conditions (Table 1 and Table S1, ESI[†]). The use of PivOH (2 equiv.) as a co-additive with $AgSbF_6$ (20 mol%) with catalytic amounts of $[Cp^*IrCl_2]_2$ initiated the stoichiometric reaction between the model substrates in DCE at 60 °C to afford a mixture of mono- and diacylmethyl functionalized products (**3aa** & **3aa'**) in 18% and 12% yields, respectively (Table 1, entry 1). Delightfully, the usage of 3 equivalents of sulfoxonium ylide (**2a**) dramatically elevated the yield of **3aa** to 58% along with a slight increase in the yield of **3aa'** to 22% (Table 1, entry 2). Substitution of PivOH with ADA produced **3aa** in 64% yield along with 14% of **3aa'**, while its replacement with AcOH furnishes **3aa** in 72% yield along with very small (non-isolable) amounts of **3aa'** (Table 1, entries 3–4). Our solvent screening studies suggested that this transformation is highly solvent-dependent (Table S1, ESI[†]), and the target difunctionalized product could be regioselectivity obtained in 88% yield using TFE as a solvent (Table 1, entry 5). Catalyst loading modulation had no significant effect on the product yield (Table S1, ESI[†]). It is worth mentioning that increasing the temperature for the aforementioned optimized reaction to 80 °C produced detrimental effects on the yield of **3aa** as a few other minor side products were visible on TLC (Table 1, entry 6).

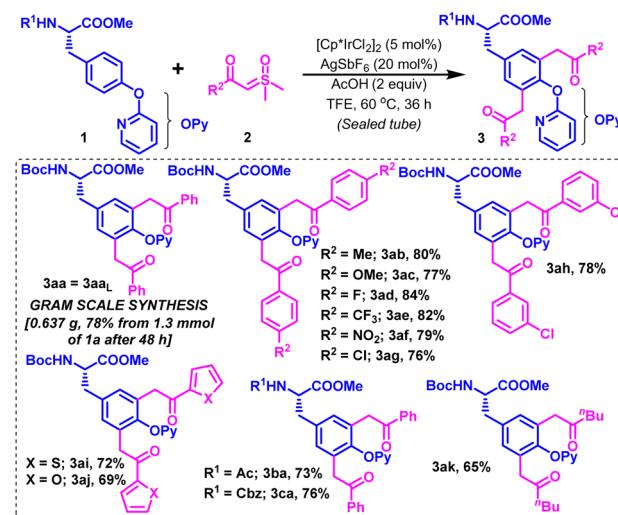
Table 1 Selective optimization^a of the reaction conditions

Entry	Co-additive	Solvent	Yield ^b of 3aa (%)	Yield ^b of 3aa' (%)
1 ^c	PivOH (2 equiv.)	DCE	18	12
2	PivOH (2 equiv.)	DCE	58	22
3	ADA (2 equiv.)	DCE	64	14
4	AcOH (2 equiv.)	DCE	72	<10
5	AcOH (2 equiv.)	TFE	88	Traces
6 ^d	AcOH (2 equiv.)	TFE	72	Traces

^a Reaction conditions: the reactions were carried out with **1a** (0.187 mmol) and **2a** (0.561 mmol) with $[Cp^*IrCl_2]_2$ in the presence of additive/Co-additive as indicated in a solvent (2 mL) at 60 °C for 36 h in a sealed tube. ^b Isolated yields. ^c **2a** (0.187 mmol). ^d Temperature 80 °C (minor additional spots on TLC were observed).

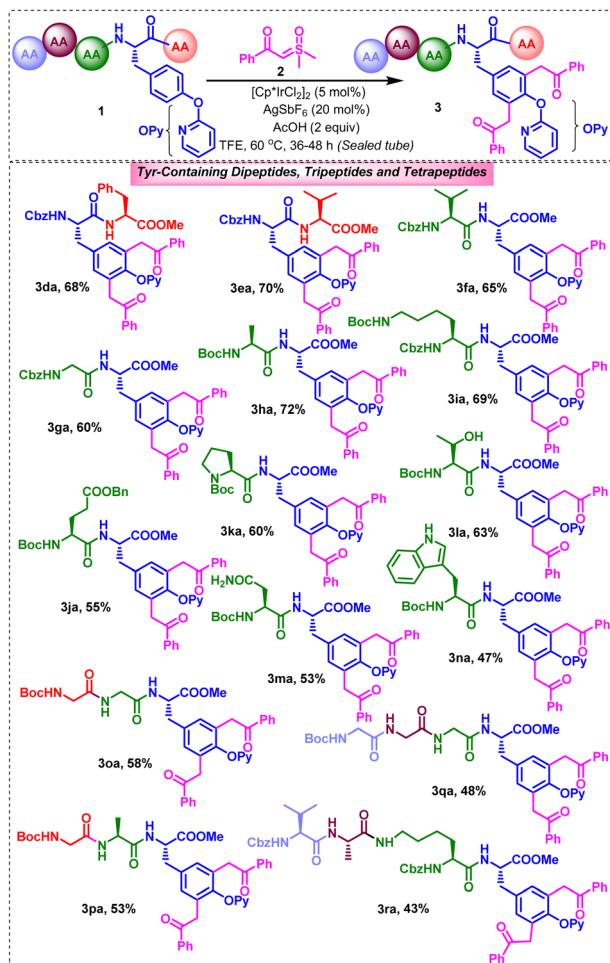
The scope of this optimized Ir -catalyzed $C_{Ar}-H$ diacylmethylation of Boc-protected tyrosine (**1a**) was next examined with variedly decorated alkyl and (hetero)aryl carbonyl sulfoxonium ylides (**2b–k**) (Scheme 2). Sulfoxonium ylides substituted with electron-donating groups [**2b** ($R^2 = 4$ -MeC₆H₄) and **2c** ($R^2 = 4$ -OMeC₆H₄)] and strong electron-withdrawing groups [**2d** ($R^2 = 4$ -FC₆H₄), **2e** ($R^2 = 4$ -CF₃C₆H₄)] and **2f** ($R^2 = 4$ -NO₂C₆H₄)] reacted smoothly with **1a** under the described conditions to furnish their corresponding diacylmethylated products **3ab–3af** in 77–84% yields (Scheme 2). Slight variation in the reactivity of *para*- and *meta*-substituted chlorophenyl carbonyl sulfoxonium ylides was noticed, producing their respective products (**3ag** & **3ah**) in 76% and 78% yields, respectively. Gratifyingly, the reaction was workable with (hetero)aryl carbonyl sulfoxonium ylides [**2i** ($R^2 = 2$ -thienyl) and **2j** ($R^2 = 2$ -furyl)] in reasonable reactivity to comfortably accomplish dithienylmethylation and difurylmethylation at the C3/C5 positions in protected tyrosine (**1a**), affording **3ai** and **3aj** in 72% and 69% yields, respectively. The $C-H$ diacylmethylation of **1a** also proceeded with moderate ease by using alkyl carbonyl sulfoxonium ylide [**2k** ($R^2 = ^n$ Bu)], delivering the desired product **3ak** in 65% yield. Interestingly, the presence of other *N*-protecting groups, such as Ac, and Cbz (substrates **1b–c**) was well-tolerated on the *N*-side of tyrosine esters under the optimized conditions, producing their relevant diacylmethyl functionalized Tyr-based UAAs (**3ba–ca**) in 73–76% yields. The strategy was scalable on a 1.3 mmol scale to afford **3aa** in 78% yield under the standard conditions in 48 h (Scheme 2).

Furthermore, we attempted to exemplify the synthetic scope of the strategy towards the late stage functionalization of tyrosine in tyrosine-containing peptides (Scheme 3). Pleasingly, tyrosine-containing peptides underwent functionalization with sulfoxonium ylides in moderate-to-good reactivities. For example, the late stage C3/C5_{Ar}-difunctionalization of the dipeptides Cbz-L-Tyr(OPy)-L-Phe-OMe (**1d**), Cbz-L-Tyr(OPy)-L-Val-OMe (**1e**), Cbz-L-Val-L-Tyr(OPy)-OMe (**1f**), Cbz-Gly-L-Tyr(OPy)-OMe (**1g**), Boc-L-Ala-L-Tyr(OPy)-OMe (**1h**), Cbz-L-Lys(*N*^c-Boc)-L-Tyr(OPy)-OMe (**1i**), Boc-L-Glu(OBn)-L-Tyr(OPy)-OMe (**1j**), Boc-L-Pro-L-Tyr(OPy)-OMe



Scheme 2 $C3_{Ar}/5_{Ar}-H$ diacylmethylation of tyrosines with sulfoxonium ylides.





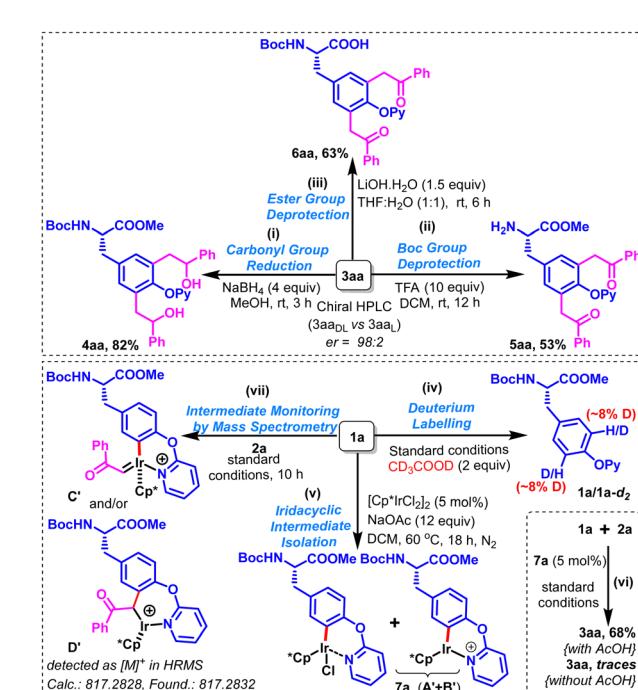
Scheme 3 Late-stage Tyr-containing peptide diacylmethylation.

(**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-L-Tyr(OPy)-OMe (**1q**) and Cbz-L-Val-L-Ala-L-Lys (N^{α} -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. $\sim 8\%$) 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 C_{3Ar}/C_{5Ar}-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**

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 AgSbF_6 -mediated dissociation of $[\text{Cp}^*\text{IrCl}_2]_2$ to monomeric active $[\text{Cp}^*\text{IrCl}(\text{SbF}_6)]$ species, which undergoes pyridyl-aided $\text{C3}_{\text{Ar}}\text{-H}$ metatlation 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 $\text{C3}_{\text{Ar}}\text{-Ir}$ bond generating a seven-membered iridacyclic intermediate **D**. Thereafter, protonolysis of the C3_{Ar} -acylmethyl group followed by re-coordination of $\text{Ir}(\text{III})$ with $\text{C5}_{\text{Ar}}\text{-H}$ 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 $\text{Ir}(\text{III})$ catalyst for the next catalytic cycle.

In conclusion, we have developed a $\text{Ir}(\text{III})$ -catalyzed strategy for $\text{C3}_{\text{Ar}}/\text{C5}_{\text{Ar}}$ -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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