N-formylation of amino acid esters and peptides via peroxide mediated decarboxylative C-N coupling with α-keto acids

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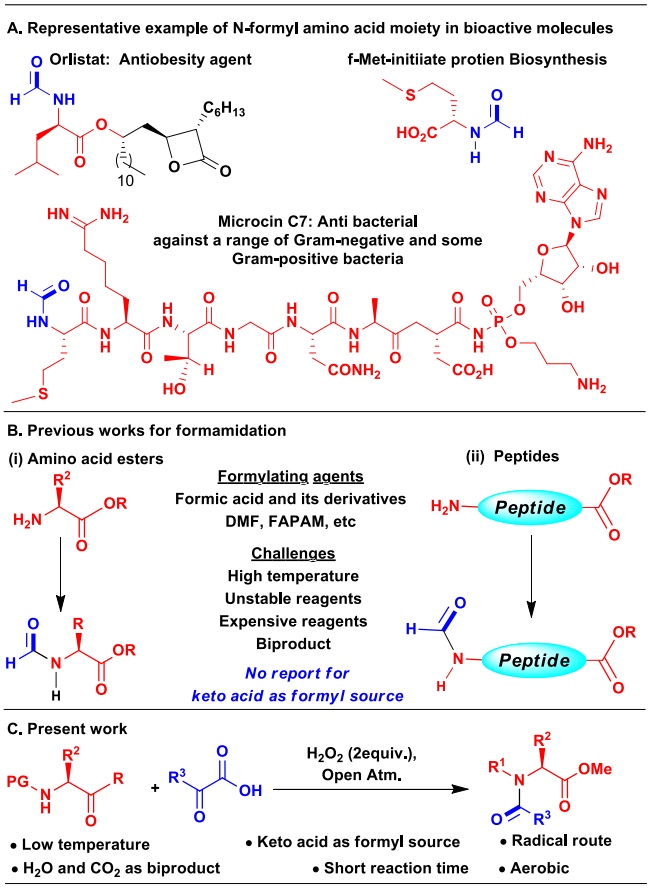
Synthesis of N-formyl amino acids and peptides, that play a crucial role in protein biosynthesis and for the development of antimicrobial peptides, faces many challenges including harsh reaction condition, biproduct management etc. Reported here, is an efficient peroxide mediated N-formylation of amino acid ester derivatives via radical decarboxylative coupling with glyoxylic acids where only water and carbon dioxide forms as biproduct. H_2O_2 works well with N-substituted while TBHP was successful for the N-formylation unprotected amino acid esters and oligopeptides. The methodology was extended for the synthesis of bioactive N-formyl methionine and f-MLP. The library of synthesised N-ferrocenyl as well as free N-formyl amino acid and peptide derivatives might serve as hybrid material for medicinal and electrochemical applications

Introduction

Amide bond formation and their reactions have emerged as a state of art approach for the construction of synthetically and biologically relevant peptide-therapeutics and peptidomimetics.[1] Many modern antimicrobial peptides (AMPs) frequently encompasses N- formyl amino acid moiety, that plays a pivotal role in innate immunity of the host by selective peptide binding and activating the enzyme.[2] Notably, N-formyl methionine (f-Met), a protein biosynthesis initiator in bacteria and a chemotactic agent for neutrophils, is present in antibacterial heptapeptide Microcin C, peptidyl tRNA and its mimic N-formyl methionine-leucine-phenyl alanine(f-MLP).[3] Additionally, formamide moiety is prevalent in natural products and pharmaceuticals such as orlistat,[4] leucovorin,[5] formoterol[6] and other chemotherapeutic agents[7] (Scheme 1A). Also, formamide serves as valuable feedstock for the synthesis of N-heterocycles,[8] isocyanides,[9] formamidines,[10] and as key intermediates in Vilsmeier–Haack reaction.[11] Existing methods for the N-formamidation of amino acids and peptides specifically employs formic acid[12] or its derivatives including ammonium formate, triethyl formate, trichloro phenyl formate, acetyl formate etc.[13] Other reagents includes imidazole in DMF [14] and formyloxyacetoxyphenylmetane (FAPM).[15] Nevertheless, challenge in using elevated temperature, coupling agents, expensive or sensitive reagents, and difficult to remove biproducts always urges to discover alternative approaches. In this context, Minisci reaction, a decarboxylative oxidative coupling of alpha ketoacids with heterocycles,[16] is getting attention owing to the low toxicity, easy handling, high stability, cost efficiency and functional group tolerance.[17]

Nickel catalysed electrochemical decarboxylative N-formylation of amines using glyoxylic acid developed by Lin *et.al* failed with bulky amines.[18] Wu's group introduced H_2O_2 promoted decarboxylative *N*-formylation of amines with glyoxylic acid[19] where benzylamines and alkylamines showed mediocre reactivity (Scheme 1B). Although, decarboxylative coupling of amino acids to other radical acceptors[20] are reported, to the best of our knowledge decarboxylative coupling of α-ketoacids to amino acids has not yet been reported. On that note, we report a simple and efficient methodology for N-formylation of amino acids and peptides using glyoxylic acid as formyl equivalent mediated by peroxides through decarboxylative coupling (Scheme 1C).



Scheme 1 N-formyl amino acid in selected bioactive molecules, background and present work

Result and Discussions

To validate our assumption, N-benzyl-L-alanine methyl ester **(1a)** and glyoxylic acid (**2a**) was taken as the model substrate. The reaction was carried out with **1a** (1 equiv.) and **2a** (2 equiv.) at room temperature for 1 hour in the presence of 2 equiv. of hydrogen peroxide in 1ml of dimethyl sulfoxide (DMSO) as solvent under open atmosphere yielding N-benzyl-N-formyl-L-alanine methyl ester **(3a)** in 53% as mixture of two rotamers in the ratio 1: 0.76 (Table 1 entry 1). The structure of **3a** was confirmed by ^1H, ^{13}C NMR and HRMS analysis. The existence of dynamic rotamers were confirmed by recording the proton nmr spectrum in DMSO-d_6 where the conformer ratio was changed to 1: 0.92.

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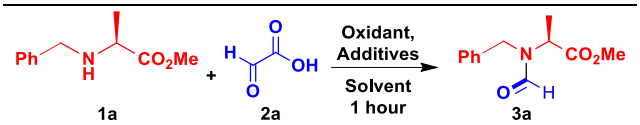
Table 1. Optimization of reaction conditions.



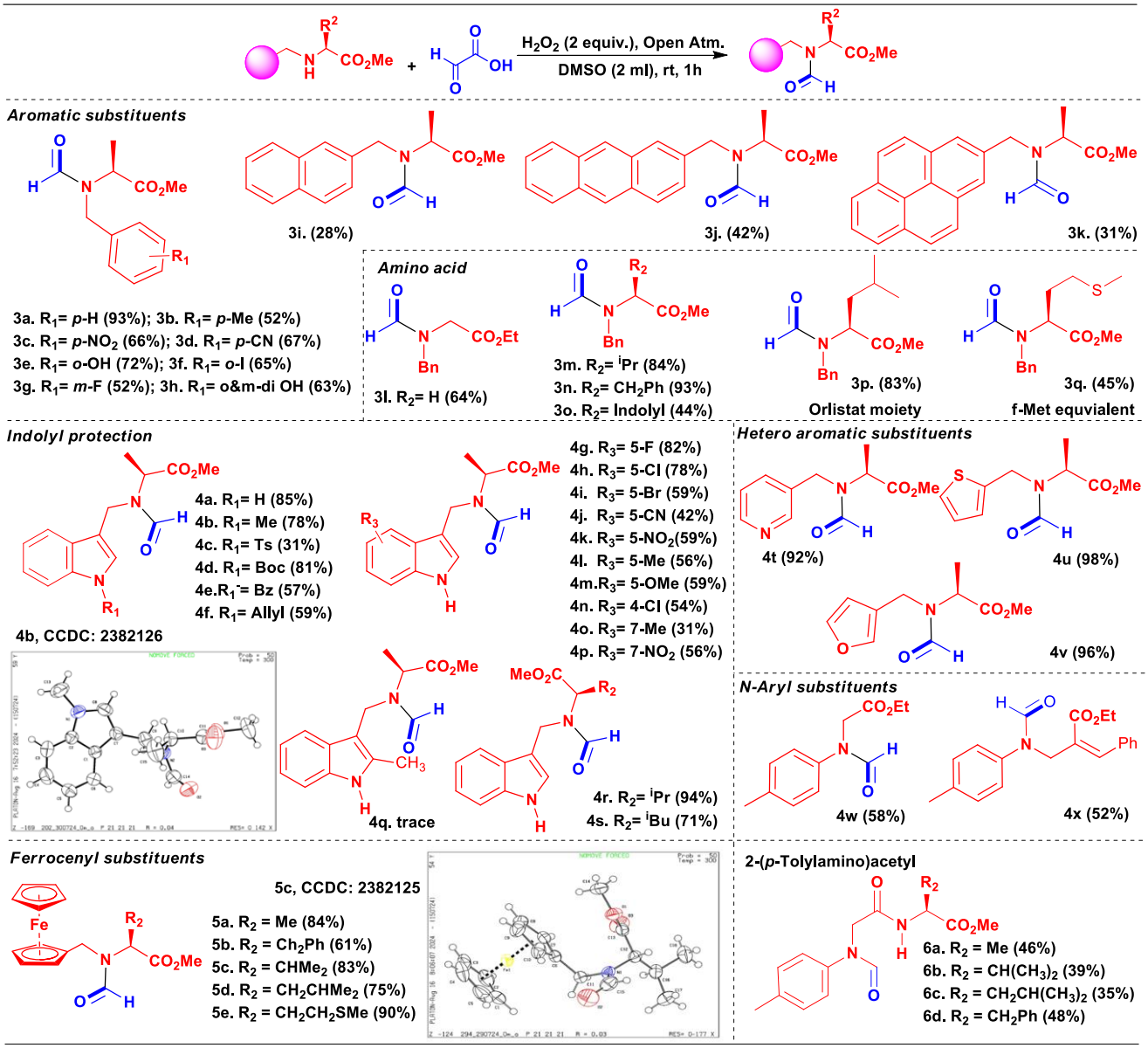
S. No	Oxidant (equiv.)	Additives (equiv.)	Solvent (ml)	Yield [a] (%) 3a
1	H_2O_2(2)	Nil	DMSO (1)	53
2	**H_2O_2(2)**	**Nil**	**DMSO (2)**	**93**
3	H_2O_2 (1)	Nil	DMSO (2)	49
4	H_2O_2(2)	Nil	DMSO (2)	44[b]
5	TBHP (2)	Nil	DMSO (2)	52
6	TBHP (2)	Nil	DMSO (2)	89[c]
7	H_2O_2(2)	K_2CO_3 (0.1)	DMSO (2)	24
8	H_2O_2(2)	K_2CO_3 (1)	DMSO (2)	61
9	H_2O_2(2)	NaOAc (0.1)	DMSO (2)	16
10	H_2O_2(2)	NaOAc (1)	DMSO (2)	69
11	H_2O_2 (2)	Benzoic acid (0.1)	DMSO (2)	70
12	H_2O_2 (2)	Benzoic acid (1)	DMSO (2)	42
13	H_2O_2 (2)	Acetic acid (0.1)	DMSO (2)	37
14	H_2O_2 (2)	Acetic acid (1)	DMSO (2)	19
15	H_2O_2 (2)	$BF_3.OEt_2$ (0.1)	DMSO (2)	53
16	H_2O_2 (2)	$BF_3.OEt_2$ (1)	DMSO (2)	0
17	H_2O_2(2)	Nil	DMSO (2)	80[d]
18	H_2O_2(2)	Nil	DMSO (2)	68[e]

Reaction conditions: **1a** (1 equiv.), **2a** (1 equiv.) under open atmosphere. [a] Yield after purification; [b] 1.5 equiv. of glyoxylic acid; [c] 12hr; [d] Ar atmosphere; [e] O_2 atmosphere.

The yield was found to be increasing up to 93% with increasing the amount of DMSO to 2 ml (Table 1, entry 2). The reaction was repeated by changing the stoichiometry of H_2O_2 to 1 equiv. and the product formation was decreased to 49% (Table 1, entry 3). Attempting the reaction using 1.5 equiv. of glyoxylic acid decreased the formation of **3a** to 44% (Table 1, entry 4). Other solvents, except 1,4-dioxane (88% of **3a**), gave inferior results **(ESI).** Oxidizing agents like $K_2S_2O_8$, $(NH_4)_2S_2O_8$, $Na_2S_2O_8$ and *m*-CPBA were completely failed to form the product, even though Oxone gave 4% product **(ESI).** TBHP yielded **3a** in 52%, on extending the reaction time to 12hr, the yield was increased to 89% (Table 1, entry 5 & 6). These investigations led us to finalize the solvent, oxidant and reagent stoichiometry. Comparing the effect of various inorganic and organic bases indicate that rate of product formation gets diminished with the addition although yield became elevated with increasing the stoichiometries of bases. (Table 1, entries 7 to 10). A reverse effect was observed with acid as additives where the yield got improved with lowering the stoichiometry (Table 1, entries 11-16). These findings suggest that the decarboxylative formylation is slow in both acidic and basic condition. Finally, we executed the reaction under argon and oxygen atmosphere (Table 1, entry 15 & 16) which gave the desired product in 80% and 68% yield respectively. A detailed optimization is available in the electronic supplementary information. Further the reaction was scaled up to 5.17 mmol and the yield of **3a** obtained was 87% (ESI).

Encouraged by the optimum condition, we sought to explore the substrate scope with different N-substituted amino acid esters. Initially we have expanded the substrate scope to various substituted benzyl, napthalenyl, anthracenyl and pyrenyl protected alanine methyl esters. Electronic effects of the substituents on benzene ring was negligible as the corresponding products **3a-3h** were obtained in 93- 63% yields. Remarkably, N-napthalenyl, anthracenyl and pyrenyl substituted alanine methyl esters gave the corresponding N-formyl products **(3i-k)** in albeit in moderate yields presumably because of the steric effect. We further explored our substrate scope towards other amino acid esters. N-Benzyl glycine ethyl ester, valine and phenyl alanine gave the desired product **(3l, 3m & 3n)** in very good yields while tryptophan **(3o)** gave 44% of desired product. N- Benzyl leucine methyl ester **(3p)** shows good reactivity towards formylation, whereas methionine methyl ester **(3q)** gave lower yield. Notably, N-formyl leucine moiety is a part of clinically running obesity drug orlistat, whereas N-formyl methionine (f-Met)[21] is structurally relevant moiety available in Microcine-C7 heptapeptide and f-MLP tripeptide.[22] We have extended this methodology for the synthesis of N-formyl-N-indolyl amino acid esters **(4a-4q)**. Simple indolyl protected alanine methyl ester **(4a)** gave the desired product in 85% of yield whereas N-Me **(4b)** gave78% of product. Electron withdrawing N-tosyl indole **(4c)** gave lower yield whereas N-Boc **(4d)** indolyl substituent afforded the corresponding product in 78%. The structure of **4b** was confirmed by single crystal XRD analysis **(ESI).** N-benzyl **(4e)** and N-allyl **(4f)** indolyl protected alanine methyl ester show almost same reactivity towards formamide synthesis. Substituents at 4, 5 and 7 position of indole aromatic ring underwent N-formylation in excellent to moderate yield **(4g-4p)**. Electron donating group such as Me and OMe at 5th position is well tolerated for this reaction condition offered **4l** and **4m** in 56% and 59% respectively. Unfortunately, Indolyl with 5-OBn substitution failed to give product under standard conditions. However, indole having 4-Cl **(4n)**, 7-Me **(4o)** and 7-NO_2 **(4p)** substituents gave the desired product in 54%, 31% and 56% respectively. Meanwhile, the formylation yields were remarkably affected by 2 -substituted indolyl amino acid derivatives as 2-methyl **(4q),** which gave only trace amount of desired product, confirmed by HRMS, whereas 2- phenyl substituted indolyl derivative failed to undergo formylation because of the steric effect. Indolyl protected valine and leucine gave **(4r)** and **(4s)** 94% and 71% respectively. Interestingly, pyridinyl, thiophenyl and furanyl protected alanine methyl esters were tolerated the condition and gave **(4t-4v)** in 92%, 98 % and 96% yields respectively. The above results reaffirm the reduced steric effect exerted by the five membered rings. Interestingly, N-tolyl glycine methyl ester gave **4w** in 58% compared to the N-benzyl substitution whereas an allyl aryl amine gave **4x** in 52%. Furthermore, ferrocene bioconjugate of amino acids/peptide are widely accepted for their fascinating Furthermore, ferrocene bioconjugate of amino acids/peptide are widely accepted for their fascinating structural properties henceforth are used as biosensors, anti-microbial, and in biomedical applications. [21] The ferrocenyl N-formyl amino acid bioconjugates are yet to be unravelled.

Table 2 Substrate scope for the synthesis of substituted formamides



[a]Reaction condition: 1a (1 equiv.), 2a (1 equiv., 50% in water), H_2O_2 (2 equiv., 30% in water), DMSO (2mL), rt, Open atmosphere, 1hr, Isolated yield

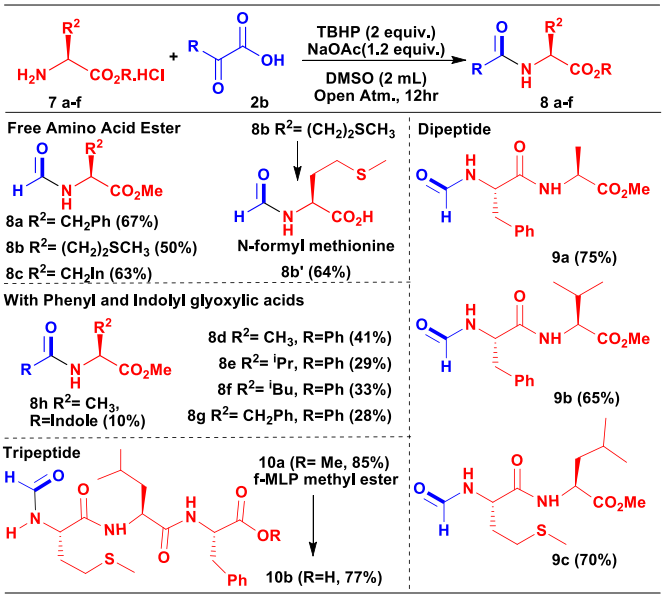
Interestingly, various ferrocenyl substituted amino acid derivatives underwent formylation effectively under optimized condition. The N-ferrocenyl substituted alanine, phenyl alanine, valine, leucine and methionine gave corresponding products **5a-5e** in 84%, 61%, 83%, 75% and 90% respectively. The structure of **5c** was confirmed by single crystal analysis. Regrettably, the reaction failed with proline and sarcosine methyl ester hydrochlorides, Boc, acetyl and benzoyl protected amino acid methyl esters **(ESI).** The selectivity of N-formylation amines over amides was verified using methyl 2-(2-(p-tolylamino) acetamido) propanoate where the N-formylation was selective towards secondary amine compared to peptide yielding **6a** in 46% yields. Similar reactivity was observed for valine, leucine and phenyl alanine ester derivatives and accomplished the formamides**(6b-d)** in 39%, 35% and 48% yields respectively.

Further, extending the substrate scope to free-amino acid ester hydrochlorides failed to undergo N-formylation under the optimized reaction condition. However, N-formylation of unprotected phenyl alanine methyl ester hydrochloride underwent N-formylation under the developed condition and yielded **8a** only in 12% even after keeping the reaction for 12hr.

Interestingly, the yield of **8a** was improved to 67% by replacing H_2O_2 with tertiary butyl hydroperoxide (70% TBHP in water) and by the addition of NaOAc (1.2 equiv.) to neutralize the acid salt (Table 3). (see optimization table in ESI). Extending this modified condition to methionine and tryptophan esters furnished the corresponding N-formylated product **8b** and **8c** in 50% and 63% in yield. Extending the modified condition to methionine and tryptophan esters furnished the corresponding N-formylated product **8b** and **8c** in 63% and 50% in yield (Table 3). **8b** on hydrolysis yielded N-formyl methionine (**8b'**), a protein biosynthesis initiator in bacteria.[21] Inspired by these results, we elaborated this methodology for N-benzoylation using benzoyl formic acid and yielded the respective products **8d-g**, in moderate yield. To our dismay, indole glyoxylic acid gave the amide

8h in 10% yield while pyrrole glyoxylic acid, pyruvic acid and oxamic acid did not work under the above condition. Interestingly, this methodology worked for the N-formylation of di and tripeptides. N-Formylation of the methyl esters of dipeptides, phenyl alanine-alanine, phenyl alanine-valine and methionine-leucine afforded **9a, 9b** and **9c** in 75%, 65% and 70% yields respectively. Intrigued by these results synthesis of N-formyl methionyl-leucyl-phenylalanine **10b** (f-MLP), a chemotactic agent for leukocyte receptor and also a macrophage activator[22] was achieved in 77% yield by the formylation of the oligo peptide, followed by hydrolysis of **10a**.

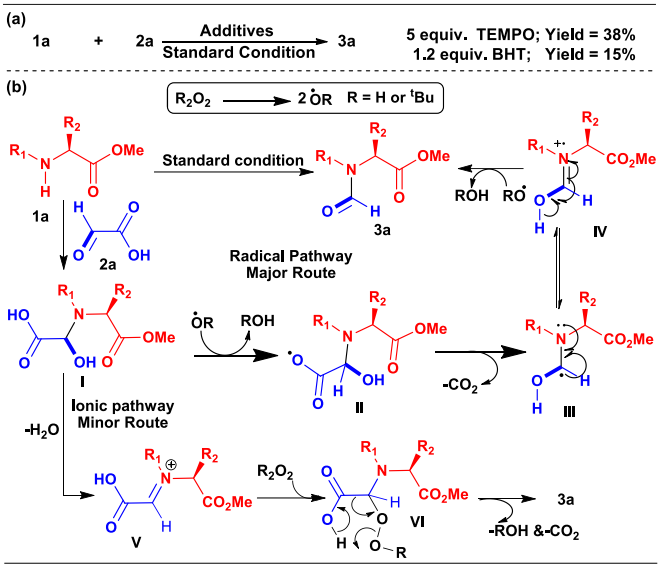
Table 3 Substrate scope with amino acids and peptides



[a]Reaction condition: 7 a-f (1 equiv.), 2b (1 equiv.), TBHP (2 equiv., 70% in water), NaOAc (1.2 equiv.) DMSO (2mL), rt, Open atmosphere, 12hr, Isolated yield based on 7 a-f.

Mechanistic investigation

To understand possible reaction mechanism several controlled experiments were performed (Scheme 2). Performing the reaction in presence of 5 equiv. radical scavenger 2,2,6,6-tetramenthyl-1-piperidinyloxy (TEMPO) yielded **3a** in 38% whereas 1.2 equiv. of BHT (butylated hydroxytoluene) quenched the reaction to 15% (more details can be found in ESI). Based on controlled experiments and previous literature reports, [17b, 19] we propose two different reaction mechanisms (Scheme 2). A major radical pathway initiated by the hydroxyl/tertiary butoxy radical formed from the homolysis of H_2O_2/TBHP. Intermediate **I** formed from **1a** and **2a** undergo a hydrogen radical transfer to form **II** and water/^{t}BuOH followed by the elimination of carbon dioxide generate radical **III.** The radical **III** gets stabilized by the lone pair on nitrogen to form iminium radical cation **IV.** A hydrogen radical abstraction by hydroxyl/tertiary butoxy radical to form **3a** and water/^{t}BuOH. Reaction with additives like acids and bases suggest that the role of imine formation through normal condensation (Table 1, entries 7 -16). So, in a minor pathway from Intermediate **I** forms an iminium cation **V** by losing water. Base promoted addition of peroxide to **V** followed by the removal of ROH/H_2O and CO_2 through a cyclic transition state generate **3a**.

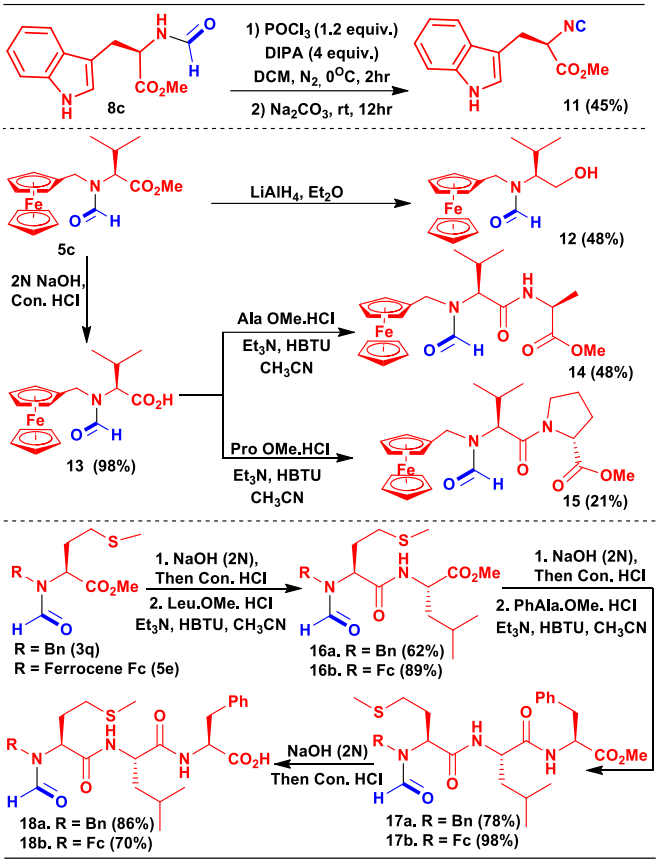


Scheme 2 Controlled experiments and Possible mechanism

Having manifested by the synthetic applications of N-formyl amino acids, we further demonstrated the functional group transformations of selected N-formyl amino acid esters and peptides. Isocyanides are considered as a strategic functional group in organic transformations[24], but also considered as a valuable pharmacophoric group in medicinal chemistry.[25] So, **8c** was converted to the corresponding isocyanide **11** which could be a precursor for (Z)-3-(2-Isocyanovinyl)-1H-indole, an indole antibiotic B371 precursor.[26] Additionally, N-Ferrocenyl-N-formyl valine ester hydrochloride **5c** was reduced with lithium aluminium hydride to the corresponding alcohol **12** (48%). **5c** was hydrolysed to yield the acid **13** in 98% which was further converted to the dipeptides **14** and **15** in 48% and 21% yields (Scheme 3). We have also demonstrated the application of current methodology for the synthesis of biorelevant N-formyl tripeptides, N-Formyl-methionine-leucyl-phenylalanine (f-MLP)[23] and its bioconjugate N-ferrocenyl-N-formyl-MLP starting from N-formyl methionine methyl ester **3q** and **5e** respectively by peptide coupling. To explore further the application of the ferrocene conjugated N-formamides, **5a-d,** preliminary photophysical and electro chemical studies were conducted. The absorption and florescence emission spectra were taken for one millimolar solutions in acetonitrile and the results are included in the ESI. Cyclic voltammetry (CV) was performed using a CHI660E electrochemical workstation with glassy carbon electrode (GCE), Ag/AgCl (1 M KCl) and platinum wire as the working, reference and counter electrodes respectively **(See ESI)** and observed that the redox potentials were similar to that of ferrocene. Further studies towards these aspects are underway.

Conclusion

In conclusion, a simple, convenient and mild method for the N-formylation of amino acids and peptides were achieved through metal and coupling reagent free condition where water and carbon dioxide as the easily removable biproducts. The developed methodology gives an access to a library of synthetically relevant N-formyl amino acid derivatives and peptides that can display interesting pharmaceutical and electrochemical applications. We have also applied this strategy for the synthesis of chemotactic agents N-formyl methionine and f-MLP as well as the corresponding ferrocene bioconjugates.



Scheme 3 Functional group conversion and synthesis of bioactive f-MLP analogue

Author Contributions

The authors confirm the following contributions to this paper: study conception and design, B. V.; Methodology, and experiments B. K., A.S. and B. T.; Manuscript preparation B. V. and B. K. All authors have given approval to the final version of manuscript.

Data availability

The data supporting this article has been included General information, Experimental procedure, ^{1}H, ^{13}C NMR, HRMS data, Crystallographic data, UV-Visible and CV data are provided as part of the Electronic supplementary information (ESI) †.

Conflicts of interest

The authors declare no competing financial interest.

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