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Molecular flavin catalysts for C–H functionalisation and derivatisation of dehydroamino acids†

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In nature, the isoalloxazine heterocycle of flavin cofactors undergoes reversible covalent bond formation with a variety of different reaction partners. These intermediates play a crucial role *inter alia* as the signalling states and in selective catalysis reactions. In the organic laboratory, covalent adducts with a new carbon–carbon bond have been observed with photochemically excited flavins but have, so far, only been regarded as dead-end side products. We have identified a series of molecular flavins that form adducts resulting in a new C–C bond at the C4a-position through allylic C–H activation and dehydroamino acid oxidation. Typically, these reactions are of radical nature and a stepwise pathway is assumed. We could demonstrate that these adducts are no dead-end and that the labile C–C bond can be cleaved by adding the persistent radical TEMPO leading to flavin regeneration and alkoxyamine-functionalised substrates. Our method allows for the catalytic oxidation of dehydroamino acids (16 examples) and we show that the acylimine products serve as versatile starting points for diversification. The present results are envisioned to stimulate the design of further catalytic reactions involving intermediates at the flavin C4a-position and their reactivity towards metal complexes or other persistent organic radicals. Our method for dehydrobutyrine derivatisation is orthogonal to the currently used methods (*i.e.*, nucleophilic attack or radical addition) and offers new perspectives for peptide natural product diversification.

Introduction

The isoalloxazine core of flavins has a strong tendency to form covalent adducts with several reagents.[1] In nature (Fig. 1A), flavin adenine dinucleotide (FAD, **1**) and flavin adenine mononucleotide (FMN) reversibly form covalent C–S bonds in the C4a-position with cysteine in light, oxygen, and voltage (LOV) photoreceptors to yield covalent intermediate **2**.[2] In a second example from flavoenzymes, the reduced cofactor $FADH_2$ (**3**) reacts with oxygen to yield C4a-hydroperoxide **4** which is involved in oxygenation reactions.[3] Besides these well-studied types of intermediates, the reversible formation of a carbon–carbon single bond at the flavin's C4a-position is less common. In the organic laboratory, Hemmerich *et al.* (Fig. 1B) have reported the photochemical decarboxylation of phenylacetic acid by quinoid lumiflavin derivative **5**, which results in the clean formation of a C4a-benzyl flavin **6**.[4,5] The authors expanded on these findings and could observe that a number of organic substances undergo photochemical reactions yielding covalent C–C bonds in the C4a-position. One example is compound **7** which resulted from the reaction with 2,5-dimethyl-2,4-hexadiene.[6] Related C4a-benzyl adducts have also been observed by photochemical oxidation of benzylamines.[7] Such flavin species with a new covalent C–C bond at the C4a-position were often found to be unstable when exposed to air.

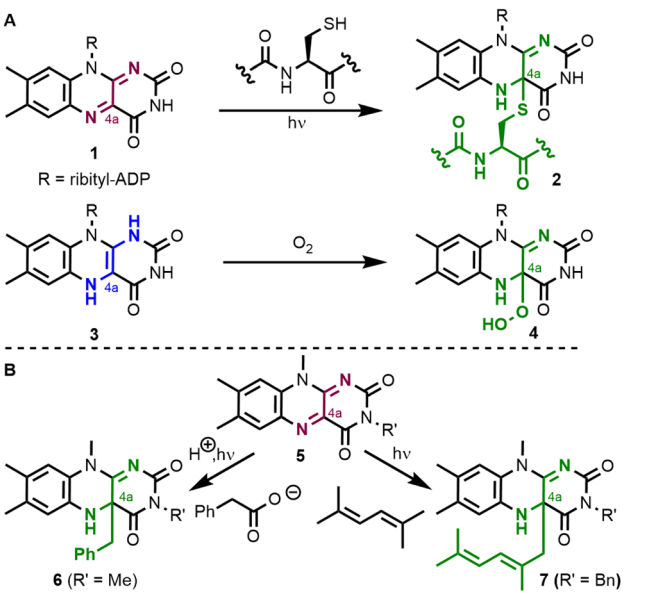

Fig. 1 Covalent adducts of molecular flavins in the C4a-position. Oxygen and sulfur heteroatom–carbon bonds are formed in LOV domains and oxygenating flavoenzymes (A) while C–C bonds are known from photochemical oxidations (B).

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Both the formation of covalent C–C adducts at the C4a-position and presumably also their reaction with O_2 are typically radical pathways. We wondered whether the formal homolysis step would allow using flavin C–C adducts as a reservoir for carbon-centred substrate radicals. Following this rationale, the adducts should also react with other radical species besides O_2 and we were particularly interested in the reaction with the persistent radical TEMPO. On the substrate side, we focused on the activation of allylic C–H positions in olefins and the oxidation of dehydroamino acids. Radical addition reactions of the latter substrates are a perfect tool for the diversification of peptide natural products,[8] which are of high pharmaceutical relevance.[9] The typical protocol relies on radical generation and addition to a dehydroamino acid's β-position.[10] An iridium-mediated generation of heteroatom-stabilized radicals from dimethylanilines and their subsequent addition to a short peptide **8** yielding conjugate **9** serves as a representative example here (Fig. 2A).[11] The orthogonal approach, which relies on the oxidation of a dehydroamino acid and its subsequent intermolecular reaction is, however, much less explored. This reactivity was found in PCET-type[12] oxidation of dehydrophenylalanine **10** (BDFE = 456 kJ mol^{-1}) with Ag_2O to an intermediate radical **11**, which reacts with TEMPO at the β-position yielding alkoxyamine product **12** (Fig. 2B).[13] Related TEMPO-functionalisation of peptides has also been accomplished by dechalcogenation.[14]

We wondered whether such functionalisation of dehydroamino acids could be performed catalytically with molecular flavins.[15] While their use as photo-oxidants is well-established,[16] we were in particular interested in using covalent intermediates at the flavin's C4a-position as stabilised substrate radicals (Fig. 2C).

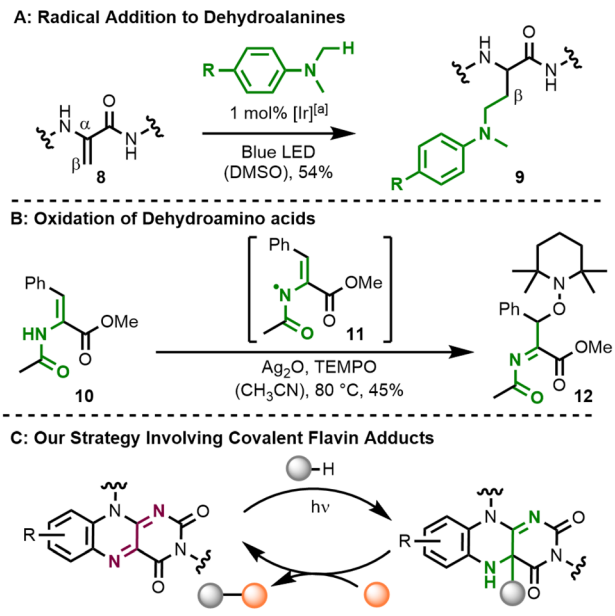


Fig. 2 Radical functionalisation of dehydroamino acids. (A) The addition of C-centred radicals to dehydroalanine-containing peptides allows access to conjugates, which contain diverse substituents (in the R-position) such as drug compounds. (B) Silver(I)oxide was found to be a capable base and oxidant for PCET-activation of dehydroamino acids. (C) Our concept for the use of flavin catalysts as substrate oxidants. [a] Iridium catalyst: [Ir(dF(CF$_3$)ppy)$_2$(dtbbpy)](PF$_6$).

Results and discussion

We first prepared a series of molecular flavins which contain electron-withdrawing substituents.[17] Flavin synthesis starts with a sequence of S_NAr-reaction and nitro group reduction to *ortho*-diaminoaryls **13** (Fig. 3). The isoalloxazine core is then formed by a two-step reaction involving oxidation of monobutyl barbituric acid followed by its condensation with diamines **13**. We prepared flavins with a methyl carboxylate (**14**) and methyl carboxamide (**15**) in the C6-position. For comparison reasons we also prepared the C7-methyl carboxylate **16** and the parent alkyl-substituted isoalloxazine **17**. This choice of substitution pattern was based on the possibility of forming a stabilising intramolecular hydrogen bond in semiquinone **18**, which has been observed in similar cases.[18]

Following our design principle, we directly started with ester-modified flavin **14** and interrogated the photochemical allylic activation of cycloheptadiene (BDE for allylic C–H bond is 347.3 kJ mol^{-1})[19] as a model substrate. Indeed, when irradiating a solution of flavin and diene in CD_2Cl_2 under inert conditions in a J Young NMR tube, we observed the clean formation of covalent adduct **19** (as a set of two diastereomers), which has a newly established C–C bond at the C4a-position (Fig. 4A). This compound was characterized by 2D-NMR and HR-ESI (see ESI† for details). Upon contact with air, quinoid flavin **14** was regenerated. We then studied whether the new C–C bond is weak enough to be cleaved upon the addition of a persistent radical and treated adduct **19** with TEMPO under inert conditions (Fig. 4B). Again, we observed the formation of quinoid flavin **14** but we could also detect the stoichiometric release of alkoxyamine-functionalised diene **20** in the NMR tube (Fig. 4C).

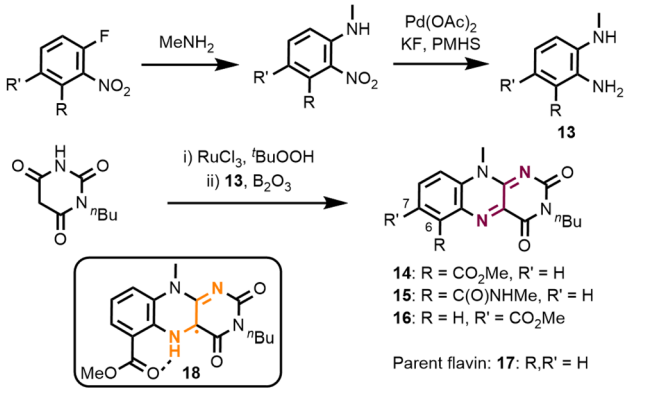


Fig. 3 Flavin catalyst synthesis. For reaction conditions and yields of diamines **13**, see the ESI.† Flavin synthesis: 2.5 equiv. *N*-butyl barbituric acid, 3 mol% $RuCl_3 \cdot (H_2O)_3$, 5.0 equiv. ^{t}BuOOH (CH_2Cl_2/H_2O), r.t., 22 h; then 1.0 equiv. B_2O_3 (AcOH), r.t., o/n. Yields for this two-step procedure: 59% (**14**), 42% (**15**), and 33% (**16**). PMHS: poly(methylhydrosiloxane).

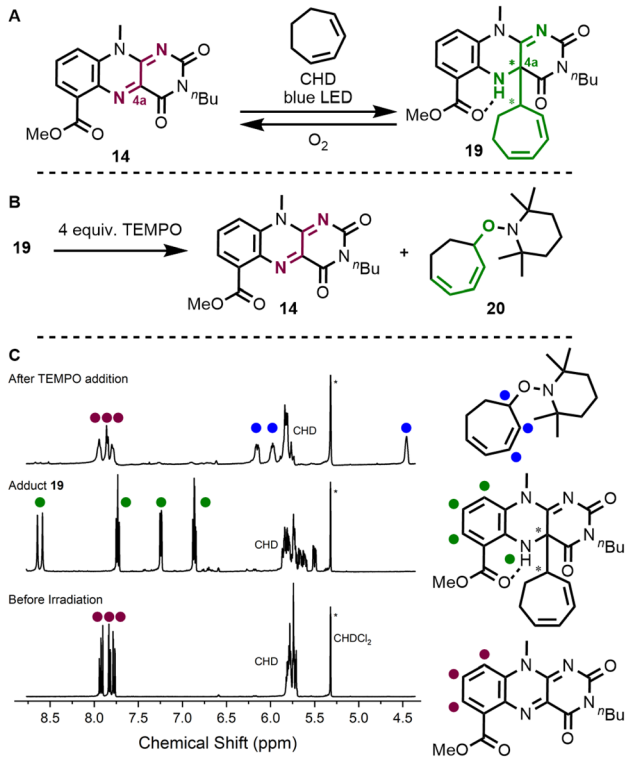


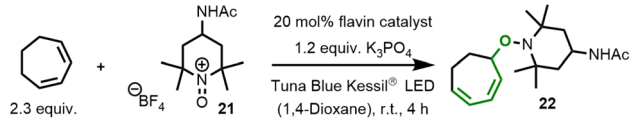
Fig. 4 Studies of covalent adduct formation. The reaction of flavin **14** with cycloheptadiene (CHD) was found to liberate quinoid flavin when the adduct is exposed to air (A). When conducted under inert conditions, the weak C–C bond at the C4a-position can also be cleaved by addition of TEMPO (B and C).

From a synthetic standpoint, the formation of alkoxyamine **20** reminded us of the known reactivity of alkenes towards nitroxyl radicals[20] and oxoammonium cations,[21] which is typically very low with disubstituted olefins. We wondered, whether our flavin activation method might offer a way to catalyse these reactions and found that a flavin-mediated method with TEMPO as a reactant and oxidant indeed leads to product formation (Table 1, entry #1). When using Bobbitt's salt (**21**) instead of TEMPO, only one equivalent of the reagent is required since the oxoammonium salt not only acts as an oxidant (E_{mp} [nitroxo$^+$/nitroxyl$^\bullet$] = +0.65 V *vs.* Ag/AgCl (+0.61 V *vs.* SCE)),[22] but also liberates the persistent radical reaction partner. Potassium phosphate is added as a base to trap the released fluoroboric acid (entry #2). The parent flavin RFTA(Me) performed worse (entry #3) and all control experiments verified that a photochemically excited flavin is required (entries #4 and #5).

With these encouraging results in hand, we investigated whether flavin catalysis would also allow the oxidative TEMPO-functionalisation of more complex substrates such as dehydroamino acids (*c.f.* Fig. 2). We chose dehydrobutyrine **23** as a model substrate and indeed, the formation of acylimine **24** was achieved.

Here, the use of TEMPO (3.0 equiv.) was found to result in better yields when compared to Bobbitt's salt **21** (Table 2, entries #1 and #2). The carboxamide flavin **15**, C7-substituted

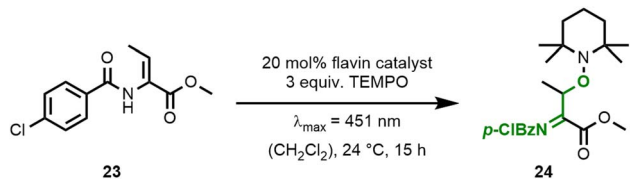
Table 1 Catalytic C–H activation and alkoxyamine formation with a molecular flavin and Bobbitt's salt **21** as limiting reagent



Entry	Catalyst	Change of conditions	Yield[a,b]
#1	**14**	TEMPO instead of **21**	27%[c]
#2	**14**	—	42% (37%)[d]
#3	RFTA(Me)[e]	—	17%
#4	**14**	No irradiation	n.d./n.d.[c]
#5	None	—	n.d./n.d.[c]

[a] Determined by NMR *vs.* internal standard. [b] n.d. = no product formation was detected. [c] TEMPO (without a base) was used instead of Bobbitt's salt **21** leading to product **20**. [d] Isolated yield. [e] (−)-*N*-3-Methyl riboflavin tetraacetate.

Table 2 Flavin catalysts for oxidative β-functionalisation of dehydrobutyrine substrate **23**



Entry	Catalyst	Change of conditions	Yield[a,b]
#1	**14**	—	60%[c]
#2	**14**	**21** instead of TEMPO	6%[d]
#3	**15**	—	15%
#4	**16**	—	35%
#5	**17**	—	43%
#6	**14**	With 10 equiv. H_2O	<5%
#7	**14**	No irradiation	n.d./n.d.[d]
#8	None	—	n.d./n.d.[d]

[a] Determined by NMR *vs.* internal standard. [b] n.d. = no product formation was detected. [c] Acylimine **24** slowly hydrolyses during purification by column chromatography and a significantly reduced isolated yield (14%) was obtained. [d] Reagent **21** (1.0 equiv. together with K_3PO_4 as a base) was used instead of TEMPO leading to product **24^{NHAc}** with an acetamide substituent in the piperidine's 4-position.

ester **16**, and parent flavin **17** all resulted in lower yields (entries #3–#5). Since catalysis product **24** is sensitive towards hydrolysis, the addition of water (10 equiv.) to the reaction mixture resulted in only a trace amount of product (entry #6). The control reactions (entries #7 and #8) confirmed that the reaction is driven by a photochemically excited flavin catalyst.

We were intrigued by the improved activity of ester-modified flavin **14** and went on to study similarities and differences when compared to RFTA(Me). Both quinoid flavins show relatively similar spectroscopic properties (Fig. 5A, see ESI† for details): λ^{abs}_{max} = 442 nm, $E(S_0 \leftarrow S_1)$ = 246 kJ mol^{-1} (EtOH, r.t.), and $E(S_0 \leftarrow T_1)$ = 205 kJ mol^{-1} (EtOH, 77 K) (**14**) *vs.* λ^{abs}_{max} = 448 nm,

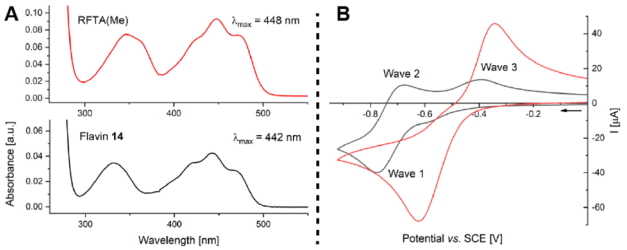


Fig. 5 Studies of flavin **14** by UV/Vis spectroscopy and cyclic voltammetry. (A) Absorption spectra of RFTA(Me) (top, red) and flavin **14** (bottom, black) in CH_2Cl_2 (0.1 mM). (B) Cyclic voltammetry of flavin **14** in 0.1 M $TBAPF_6/CH_3CN$ with a scan rate of 0.5 V s^{-1} (in black) and in the presence of 0.4 equiv. AcOH (in red).

$E(S_0 \leftarrow S_1) = 244$ kJ mol^{-1} (EtOH, r.t.), and $E(S_0 \leftarrow T_1) = 205$ kJ mol^{-1} (EtOH, 77 K) (RFTA(Me)). Cyclic voltammetry, however, revealed characteristic differences: The *N*3-alkylated flavin RFTA(Me) shows a simple cyclic voltammogram with a reversible reduction process ($E_{1/2} = -0.86$ V *vs.* SCE, see ESI† for details).[23] Under analogous conditions, flavin **14** shows two separate oxidation waves (waves 2 and 3) and a half-wave potential of $E_{1/2} = -0.72$ V *vs.* SCE for the reversible reduction. We rationalise this observation by a significantly faster protonation of the immediately formed anion $\mathbf{14}^{\bullet-}$ to the neutral radical **18**, which is reasonable based on the favoured intramolecular hydrogen bonding. This neutral semiquinone **18** is easier to reduce than quinoid flavin **14** and, therefore, it is converted to hydroquinoid $\mathbf{18}^-$ in wave 1. Reoxidation of $\mathbf{14}^{\bullet-}$ (wave 2) and $\mathbf{18}^-$ (wave 3) then occur separately. In order to prove this hypothesis, we accelerated the protonation step by measuring cyclic voltammograms in the presence of acetic acid.[24] Indeed, this resulted in the detection of only one oxidation wave, which corresponds to the oxidation of $\mathbf{18}^-$ (Fig. 5B, see ESI† for details).

The flavin-catalysed method was successfully applied to the oxidative modification of a variety of dehydroamino acid substrates (Fig. 6), which also do not show any uncatalysed reactivity under our conditions with either TEMPO or Bobbitt's salt (see ESI† for attempted conversion of a dehydrobutyrine substrate to acylimine **25**). Owing to the decreased electrophilicity of the acylimine amides compared to ester-derived product **24**, the former are significantly less reactive towards water hydrolysis and their isolation is straightforward. Similar results were observed when increasing the size of the alkyl chain on the substrate's C–C double bond or the amide (products **25–28**). The acylamides **29–31** from amides consisting of two amino acid residues were also successfully formed. We found that our method is not limited to benzamide substrates and the readily available Boc- and Cbz-protected products **32–34** were obtained in reasonable yields as well. Aromatic amino acid side chains such as phenylalanine (**35**) or protected histidine (**36**) and tyrosine (**37**) are also tolerated. Dehydroamino acid amides are significantly more reactive compared to the analogous esters, which allowed the selective formation of acylimine **25** with only negligible amounts of ester **38** in a competition experiment. The same preference was observed in an intramolecular setting,

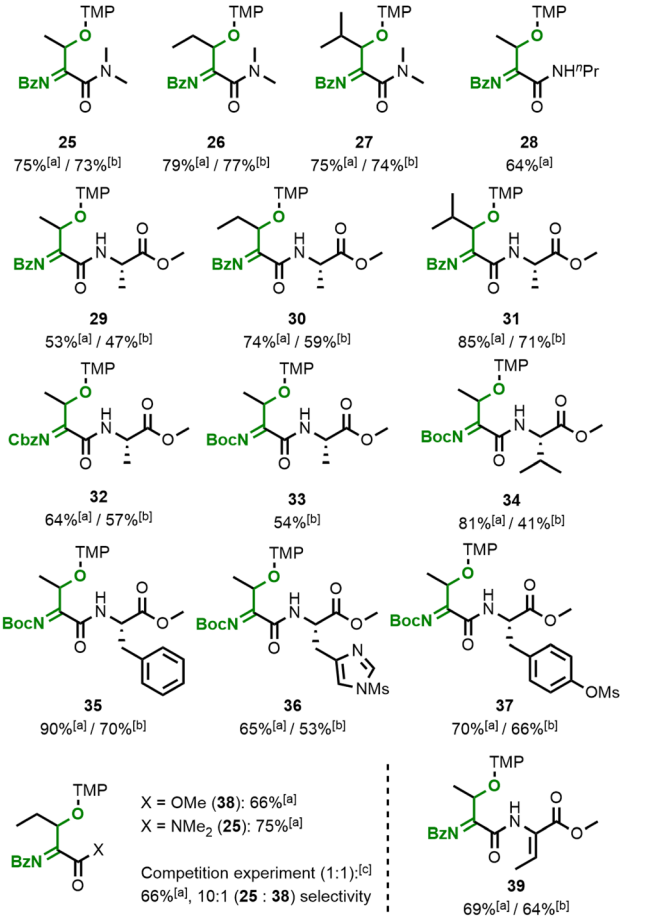


Fig. 6 Scope of the flavin-catalysed oxidative functionalisation of dehydroamino acids. Conditions: 20 mol% flavin **14**, 3.0 equiv. TEMPO, $\lambda_{max} = 451$ nm (CH_2Cl_2), 15 °C, 15 h. Compound **28** evaded isolation due to hydrolysis and the NMR yield of **33** could not be obtained due to overlapping signals. [a]: Determined by NMR *vs.* internal standard. [b]: Isolated yield. [c]: 2.0 equiv. TEMPO used. TMP = tetramethylpiperidine.

where mono-functionalised acylimine **39** was the only species we observed when using a substrate with two dehydrobutyrine residues.

We subsequently interrogated the mechanism of dehydroamino acid activation. Consistent with initial radical formation at the amide nitrogen position yielding an *N*-centered radical, we did not observe any conversion of *N*-methylated substrate (*E*)-**41** under standard catalysis conditions (see ESI†). When irradiating this amino acid substrate together with flavin **14** in a J Young NMR tube under the exclusion of air, we confirmed that neither *E*/*Z*-isomerisation[25] nor substrate conversion takes place (Fig. 7). This also holds true for the isomeric (*Z*)-**41**, which was also not converted under these conditions. In contrast, oxidation of both (*E*)- and (*Z*)-**40** results in radical formation at the dehydroamino acid's β-position. When we conducted such experiments in J Young NMR tubes with the exclusion of air and without TEMPO, we observed the formation of covalent flavin adducts within minutes and confirmed this by HR-ESI measurements. Careful inspection of

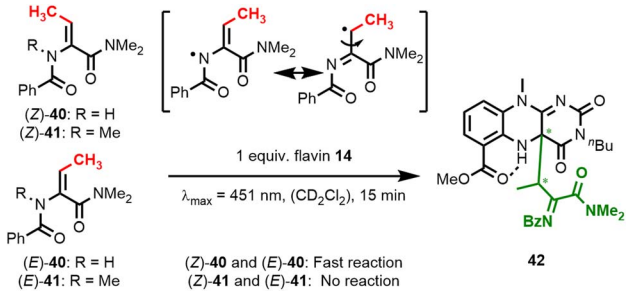


Fig. 7 Irradiation experiments with dehydrobutyrine substrates **40** and **41** in J Young NMR tubes under the exclusion of air.

2D-NMR spectra and NOE-contacts corroborated our assignment of a covalent C–C adduct at the C4a-position (see ESI† for details). Flavin adduct **42** is formed as an almost equal mixture of two diastereomers. When adduct **42** is irradiated in the presence of TEMPO, quinoid flavin **14** is regenerated and acylimine **25** is released.

Acylimines are easily reduced to the corresponding amides by sodium borohydride, which leaves the TEMPO-functionalisation unaltered (Fig. 8A). Also, the relatively weak C–O bond in TEMPO-containing organic products allows subsequent transformations,[26,27] and we demonstrated the oxidation of alkoxyamine **43** to ketone **44** by *m*CPBA. All of the obtained acylimine products resemble reactive electrophiles and, therefore, offer the possibility for one-pot reactions leading to diversified amino acid products. Acylimine **28** was found to be unstable during chromatography with silica gel, but when subjected to a one-pot malonate addition sequence,[28] the formation of stable pyrrolidine-2,5-dione **45** was observed (Fig. 8B). We confirmed the relative stereochemistry in the five-membered ring by NOE-contacts. There are also literature protocols for the reduction of acylimines to the corresponding amides.[29] As a representative example for transforming the weak C–O bond in the alkoxyamine products, we performed a one-pot three-step sequence starting from dehydrobutyrine **23** with subsequent malonate addition and *m*CPBA oxidation to ketone **46** (Fig. 8C). These examples shall demonstrate that flavin catalysis is a powerful tool for the functionalisation of dehydroamino acid derivatives.

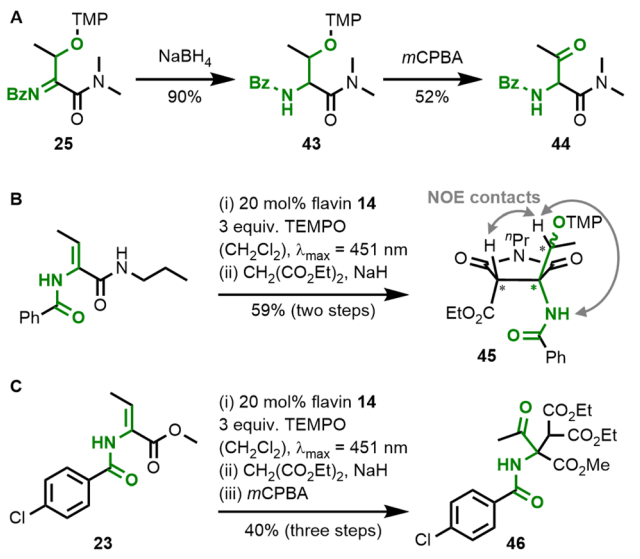


Fig. 8 Examples of synthetic one-pot modifications of dehydrobutyrine substrates in the β-position mediated by flavin catalysts: (A) reduction of acylimine **25** and subsequent TEMPO cleavage. (B) Two-step TEMPO-functionalisation and subsequent malonate addition. (C) Three-step reaction with oxidation of the alkoxyamine product to ketone **46**.

Conclusions

This proof-of-concept study of the reversible formation of covalent C–C bonds at the flavin's C4a-position demonstrates that such compounds can indeed be intermediates in catalytic cycles rather than only dead-end structures. In the presented transformations, flavin catalysis allows the functionalisation of dehydroamino acid substrates in the β-position. Such acylimine products offer a variety of different follow-up reactions and, therefore, our method is envisioned to be a valuable tool for amino acid diversification. Building on the general reactivity of flavin adducts at the C4a-position, we also expect other transformations to make use of such intermediates in the future. The dehydroamino acid derivatisation strategies may add to the toolbox of useful strategies in peptide natural product diversification.

Data availability

Original NMR datasets (FIDs) are available at Open Science-Framework at **https://osf.io/jyf9p/**.

Author contributions

A. R. performed and analysed the experiments. A. R. and G. S. designed the experiments and wrote the manuscript. G. S. supervised the project. A. W. performed and analysed the cyclic voltammetry measurements. All authors contributed to the analysis of data and reviewed the manuscript.

Conflicts of interest

There are no conflicts to declare.

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