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Photoinduced decarboxylative radical addition reactions for Late Stage Functionalization of peptide substrates

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Abstract: Photoredox chemistry has greatly stimulated the application of radical based transformations in medicinal chemistry and early drug discovery in recent years. Carboxylate groups have been identified as traceless leaving groups that can be converted to radical intermediates capable of undergoing 1,4-conjugate addition reactions to Michael acceptors. Here, we show the successful C-terminal derivatization of small peptide substrates by using this methodology in a parallel synthesis setting. Finally, we outline a general strategy for the γ-homologation of several drugs derived from α-amino acids in a late stage functionalization (LSF) approach.

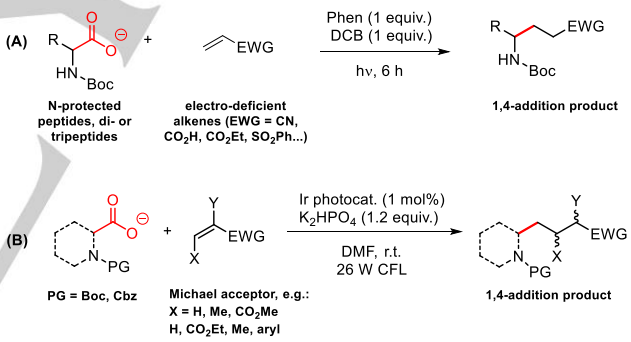
Introduction

1,4-Conjugate addition of carbon nucleophiles to electron-deficient alkenes is one of the most versatile methods for the formation of C-C bonds. A classic example is the Michael reaction, in which a CH-acidic Michael donor reacts with an α,β-unsaturated carbonyl compound as acceptor under basic conditions. [1] Tremendous efforts have been invested over decades into this reaction leading to the development of traceless activation groups (TAGs) like cuprates, [2] boronic acids, [3] and Grignard[4] reagents as carbon nucleophiles.

While these developments enabled the use of a structurally diverse set of carbon nucleophiles, acceptors or nucleophiles containing sensitive chemical functionalities still remained largely out of scope.[5,6] This fact also hampered the use of Michael-type additions in derivatizations of more complex molecules and intermediates in drug discovery at an advanced stage (late stage functionalization, LSF), an approach that was recently shown to be versatile for efficient exploration of chemical and property space. [7] For instance, substrates like peptides do not tolerate strongly basic reaction conditions required for the deprotonation of the Michael donor.

A breakthrough towards the use of peptide substrates in 1,4-conjugated additions was accomplished by Yoshimi *et al.* in 2009, taking advantage of photoredox catalysis as an easy entry into radical chemistry.[8] Initially, Yoshimi reported the decarboxylative reduction of *N*-Boc-α-amino acids mediated by phenanthrene and 1,4-dicyanobenzene, in the presence of a thiol as the hydrogen donor. [9] Realizing the potential of the radical intermediate generated via oxidative decarboxylation, the Yoshimi group expanded this chemistry to radical 1,4-conjugate additions of *N*-Boc-α-amino acids to a variety of electron-deficient alkenes.[10] In this case, the radical intermediate, formed after oxidative decarboxylation of the starting material, readily reacts as a Michael donor, avoiding the need for direct organometallic activation or propagation. In 2016, Yoshimi further expanded the scope of *N*-protected amino acid precursors and introduced *N*-acryloyl amino acid esters and peptides as Michael acceptors.[11]

Similarly, in 2014, MacMillan *et al.* reported radical 1,4-conjugate additions of readily available carboxylic acids and *N*-protected α-amino acids to electrophilic alkenes, using an iridium-based photoredox catalyst.[12] In 2017, this photoinduced decarboxylative 1,4-addition was further expanded and applied to the macrocyclization of peptides with the report of the synthesis of the macrocyclic somatostatin analog COR005[13] by an intramolecular decarboxylative radical addition as the key step.[14]



Scheme 1. Overview on important published earlier work in the field

Even though peptide drugs play a major role in several therapeutic approaches,[15] these are to our knowledge the most important examples applying photoredox catalyzed 1,4-additions to Michael acceptors for the C-terminal derivatization of peptides.[16]

We now envisioned the potential of this visible light-mediated decarboxylative radical 1,4-addition as a late stage function-alization strategy for peptidic substrates. Our main focus was directed towards establishing and using this methodology in an industrial parallel synthesis setup.

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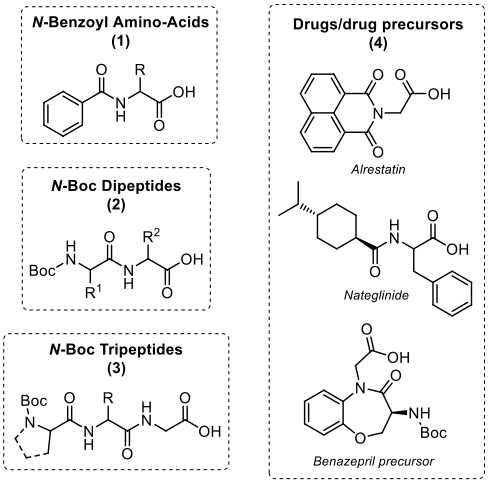


Figure 1. General structures of studied Michael donor precursors (R, R_1, R_2 side chains of biologically relevant amino acids).

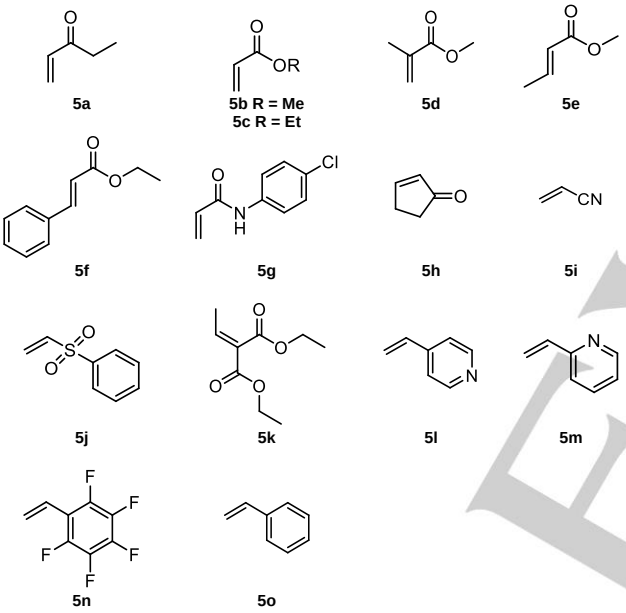


Figure 2. Scope of Michael acceptors.

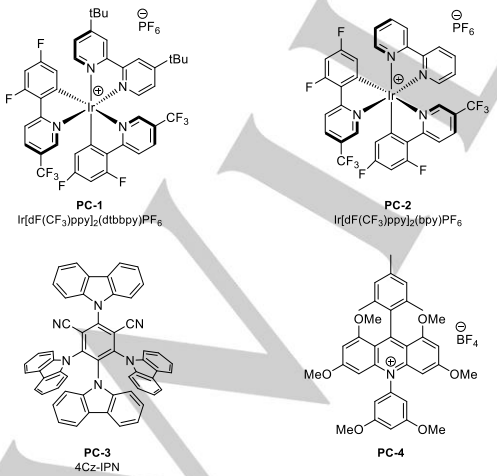
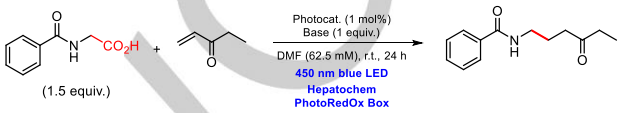


Figure 3. Relevant Ir-based[17] and organic photocatalysts[18,19] Abbreviations: ppy, 2-phenylpyridyl; dtbbpy, 4,4'-di-tert-butyl-2,2'-bipyridyl; bpy, 2,2'-bipyridyl; Cz, carbazole.

Results and Discussion

We started our studies with the photoinduced radical 1,4-addition of the model compound *N*-benzoyl glycine **1a** to ethyl vinyl ketone **5a** under reported reaction conditions,[10] affording addition product **6aa** with a 40% yield (Table 1, entry 1). In an attempt to improve these results, we screened several photocatalysts and bases (Table 1), leading to slightly higher yield with catalyst **PC-2** (44%, Table 1, entry 2). Therefore, we decided to use both **PC-1** and **PC-2** during our further studies. The reactions were performed in DMF to avoid any solubility problems with peptidic substrates. Nevertheless, in our general experience ACN and DMF are the most suitable solvents for performing photochemical reactions.

Table 1. Evaluation of test system I:



1a **5a** **6aa**

Entry	Photocatalyst	E (P*/P$^-$) (V)	E (P/P$^-$) (V)	Base	Yield (%)
1	**(PC-1)**	+ 1.21	- 1.37	Cs_2CO_3	40
2	**(PC-2)**	+ 1.32	- 1.37	Cs_2CO_3	44
3	**(PC-3)**	+ 1.65	- 0.82	Cs_2CO_3	18
4	**(PC-4)**	+ 1.35	- 1.04	Cs_2CO_3	21
5	**$Ru(bpz)_3(PF_6)_2$**	+ 1.45	-0.80	Cs_2CO_3	0
6	(PC-1)	-	-	**K_2HPO_4**	25
7	(PC-1)	-	-	**K_3PO_4**	34
8	(PC-1)	-	-	**CsF**	32
9	(PC-1)	-	-	**Na_2CO_3**	16
10	(PC-1)	-	-	**DBU**	10

[a] Isolated yields, bpz: 2,2'-bipyrazine

The mechanism of the described transformation (shown in Scheme 2) requires the oxidation of a carboxylate ion and subsequent loss of CO_2 to form the radical intermediate A in the first SET step.[10] Typically carboxylate ions have redox potentials between +0.97V to +1.27V (vs SCE)[20] thus only strongly oxidizing photocatalysts in the excited state can be expected to be effective for the desired conversion.

In the second SET step the reduction of an α-acyl radical intermediate (EWG = acyl) is required; published redox potentials of related derivatives are at -0.6 V vs. SCE.[21] In our investigations only photocatalysts with E(P/P$^-$) ≤ -0.82 V were able to complete the catalytic cycle (Table 1: entries 3 and 5). Best yields were obtained when using inorganic salts as a base to deprotonate the *N*-protected amino acid. On the contrary, the organic base DBU only delivered 10% yield.

Parallelization of the studied photochemical reactions was enabled by the use of the PhotoRedoxBox® photoreactor.[22] Depending on the chosen scale up to 32 reactions can be carried out simultaneously in this device. We usually chose a 0.25 to 0.5 mmol scale to perform the reactions. After completion of the reaction, the samples were simply filtered on a robotic platform and directly submitted to our automated purification and isolation process using HPLC chromatography with autonomous peak detection (via UV and MS), fraction collection and subsequent lyophilization and reformatting (including aliquoting for analysis). We only report isolated yields after these fully automated

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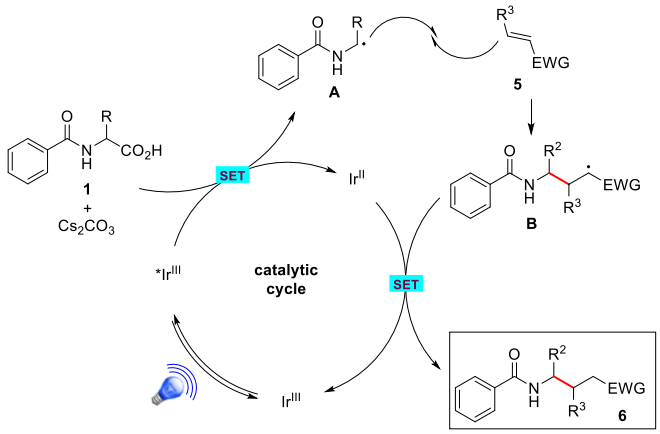
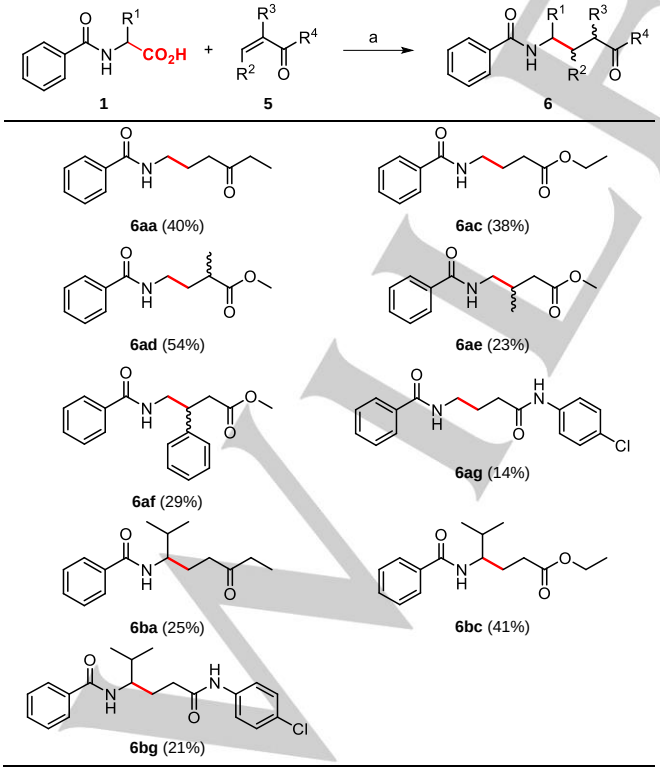
purification and isolation steps; therefore, we consider any yields > 40% satisfactory.[23]



Scheme 2. Proposed mechanism for the discussed decarboxylate alkylation.

With a suitable set of conditions in hand we proceeded to investigate the scope of this transformation. Functionalization of *N*-benzoyl glycine **1a** with a selected set of Michael acceptors afforded the corresponding coupling products in acceptable yields up to 54%. (see Scheme 3). The best yields were obtained with acrylate derivatives **5c** and **5d**, which have the highest electrophilic reactivities among the selected Michael acceptors.[24] Less reactive Michael acceptors such as **5e** or **5g** finally led to decreased reaction efficiency (23% and 14% respectively). Unreacted starting material (amino acid, peptide) was often observed together with the decarboxylated amino acid derivative as side product. These compounds were only observed by LCMS and not isolated.

Scheme 3. Functionalization of *N*-benzoyl-α-amino acid derivatives **1**: Michael acceptors scope (I).

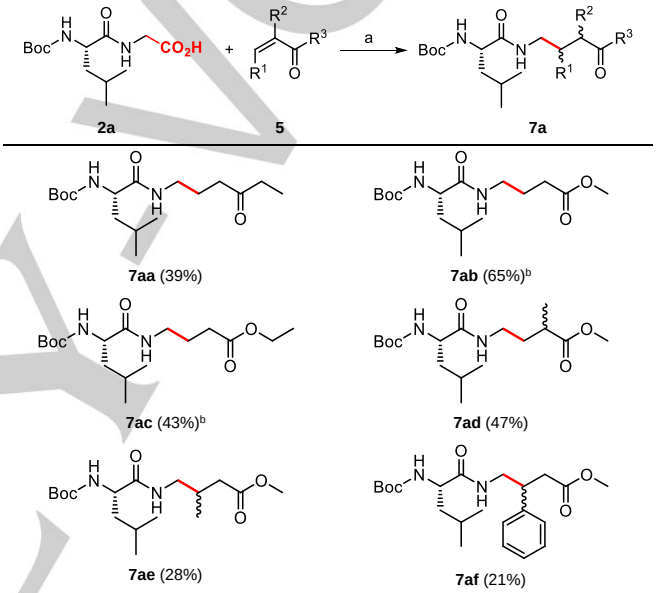


[a] Reactions conditions: PC-1 (1 mol%), *N*-benzoyl-α-amino acid **1** (1.5 equiv.), Cs_2CO_3 (1.5 equiv.), Michael acceptor **5** (1 equiv.), DMF, r.t., 24 h, Hepatochem photoreactor (λ = 450 nm).

When using α-substituted amino acids, such as *N*-benzoyl valine, we found that the introduction of an alkyl side chain led to slightly lower yields (**6ba**, **6bc** and **6bg**). As expected for reactions at a radical center, in these cases the chiral center in the starting material was racemized under the reaction conditions (see supporting information for epimerization studies with dipeptide **2g** and **ent-2g**).[10]

We then proceeded to assess the applicability of this method to the functionalization of dipeptides, using Boc-Leu-Gly-OH **2a** as radical precursor and our initial set of Michael acceptors (Scheme 4). In general, the reactions proceeded smoothly, with **PC-2** as catalyst delivering slightly better yields than **PC-1** for the substrates tested (see product **7ab** in scheme 4).

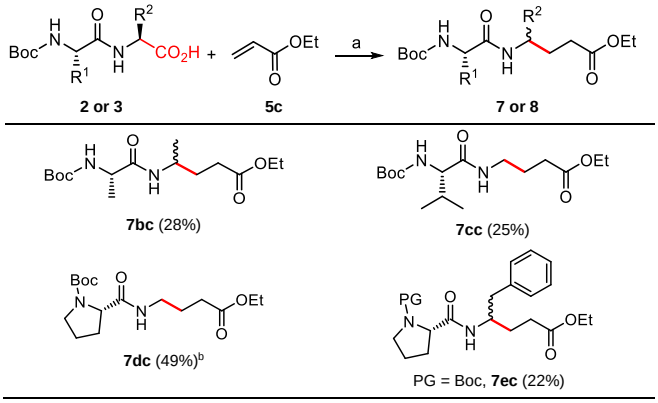
Scheme 4. Functionalization of Boc-Leu-Gly-OH **2a**: Michael acceptors scope (I).



[a] Reactions conditions: PC-1 (1 mol%), Boc-Leu-Gly-OH **2a** (1.5 equiv.), Cs_2CO_3 (1.5 equiv.), Michael acceptor **5** (1 equiv.), DMF, r.t., 24h, Hepatochem photoreactor (λ = 450 nm); [b] PC-2 (1 mol%) , **2**a (1.0 equiv.), and Penn PhD photoreactor[25] (λ = 450 nm) were used for this reaction.

The results obtained (cf. scheme 4) prompted us to stay with the highly reactive ethyl acrylate **5c** as Michael acceptor for further reactivity assessment of various di- and tripeptides **2** or **3**. We were pleased to see that some of the tested reactions proceeded with improved yields > 40% for these larger substrates (Scheme 5).

Scheme 5. Functionalization of various di- and tripeptides **2** and **3** with ethyl acrylate **5c**.



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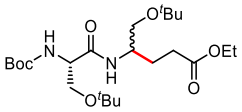
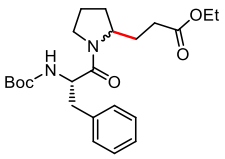
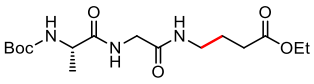
PG = Cbz, **7fc** (78%)[b]



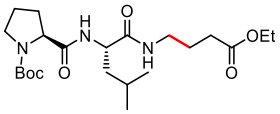
7gc (32%)



7hc (52%)[b]



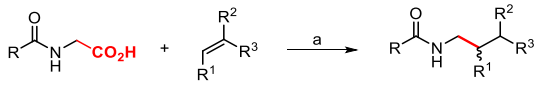
8ac (49%)[b]



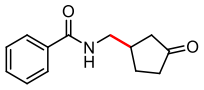
8bc (19%)

[a] Reactions conditions: PC-1 (1 mol%), dipeptide **2** or tripeptide **3** (1.5 equiv.), Cs_2CO_3 (1.5 equiv.), ethyl acrylate **5c** (1 equiv.), DMF, r.t., 24 h, Hepatochem photoreactor (λ = 450 nm); [b] PC-2 (1mol%) , **2** or **3** (1.0 equiv.), and Penn PhD photoreactor (λ = 450 nm) were used for this reaction.

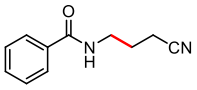
Scheme 6. Functionalization of *N*-benzoyl glycine **1a** and Boc-Leu-Gly-OH **2a**: Michael acceptors scope (II).



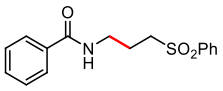
1a or 2a **5** **6 or 7**



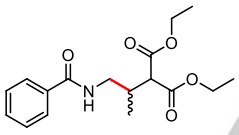
6ah (23%)



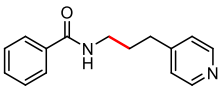
6ai (9%)



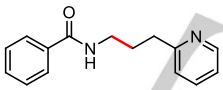
6aj (28%)



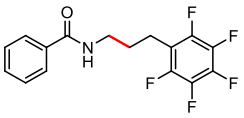
6ak (53%)



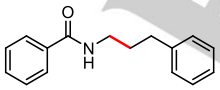
6al (46%)



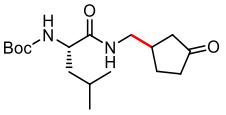
6am (30%)



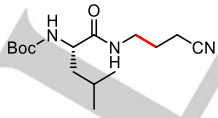
6an (7%)



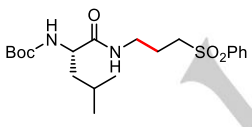
6ao (11%)



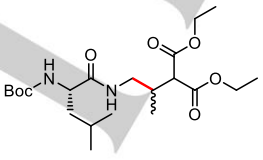
7ah (34%)



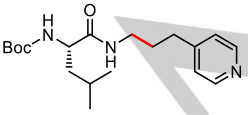
7ai (14%)



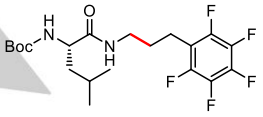
7aj (53%)



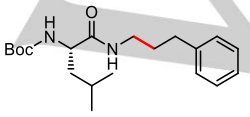
7ak (58%)



7al (37%)



7an (20%)



7ao (6%)

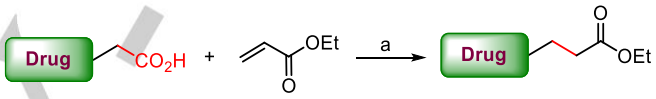
[a] Reactions conditions: PC-2 (1 mol%), *N*-benzoyl glycine **1a** or Boc-Leu-Gly-OH **2a** (1.5 equiv.), Cs_2CO_3 (1.5 equiv.), Michael acceptor **5** (1 equiv.), DMF, r.t., 24h, Hepatochem photoreactor (λ = 450 nm).

Although the use of the Cbz protecting group (exemplified for the Pro-Gly-OH dipeptide) led to more efficient reactions in comparison with Boc (Scheme 5, entries **7ec** *vs* **7fc**), we preferred Boc-derivatives for our studies due to their better commercial availability.

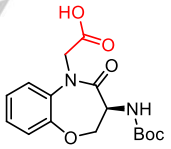
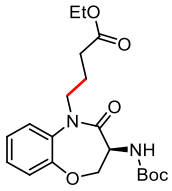
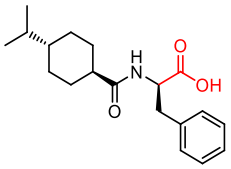
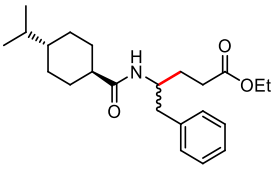
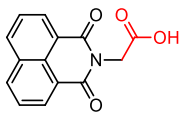
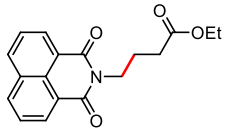
We then progressed to expand further the scope of Michael acceptors with *N*-benzoyl glycine **1a** and Boc-Leu-Gly-OH **2a** (Scheme 6) in combination with **PC-2** as photocatalyst.

In addition to classical Michael acceptor systems, our methodology also allows for the C-terminal modification of protected amino acids and dipeptides with vinyl substituted aryls. Reasonable yields were obtained with 4-vinylpyridine (**6al** and **7al**), and even simple styrene was able to react with *N*-benzoyl glycine **1a** in low yield (**6ao**). As expected, the highest yields were observed with the doubly activated Michael acceptor **5k**. However, using cyclopentenone **5h** as Michael acceptor led to only moderate yield (34%) [10]

Scheme 7. Application of methodology in LSF of established drugs Nateglinide (4b), Alrestatin (4c) or drug-precursor to benazepril (4a).



4 **5c** **9**

Entry	Drug	Product[a] (%)
1	 **4a**	 **9ac** (46%)[b]
2	 **4b**	 **9bc** (44%)
3	 **4c**	 **9cc** (4%)

[a] Reactions conditions: PC-2(1 mol%), drug **4** (1.5 equiv.), Cs_2CO_3 (1.5 equiv.), ethyl acrylate **5c** (1 equiv.), DMF, r.t., 24 h, Penn PhD photoreactor (λ = 450 nm); [b] 1 equiv. of benazepril precursor **4a** was used.

As late stage functionalization strategies are an emerging and important topic for medicinal chemistry[7], we looked for known amino acid derived drugs, which could undergo a C3 carbon homologation by the described protocol. We chose **4a**, a precursor of benazepril, Nateglinide **4b** and Alrestatin **4c** (Scheme 7) to test our optimized reaction conditions. While **4a** and **4b** reacted successfully with ethyl acrylate **5c**, we observed only minimal yield (4%) starting from **4c**. We hypothesize that this limitation is caused by the lower reactivity of the generated radical

due to the presence of the two carbonyl substituents in α-position of the nitrogen.

Conclusions

We have demonstrated that decarboxylative 1,4-conjugate additions are a valuable and broadly applicable synthetic strategy for the C-terminal modification of small peptide substrates. Compared to previous reports we have expanded the scope of Michael acceptors to acyclic unsaturated ketones and in particular less reactive acceptor systems - such as cinnamic acid ester and vinyl-substituted (hetero)aromatic systems.

The use of the Hepatochem PhotoRedOxBox® device and the robustness of the reaction conditions identified allowed us to establish this reaction type in a parallel setup and made the use of our automated purification and isolation protocols possible. This enables fast derivatizations of a wider range of substrates and allows the methodology to be employed in late stage functionalization of amino acid containing drugs and drug precursors, which was demonstrated for a precursor of benazepril, Nateglinide and Alrestatin.

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Keywords: 1,4-conjugate addition • photoredox • peptides • late stage functionalization • parallel syntheses

KEY TOPIC:
Photochemistry, Late Stage Functionalization

TABLE OF CONTENT TEXT:
In our contribution we showcase an application of photochemistry for Late Stage Functionalization (LSF) of amino acids and small peptides in an industrial medicinal chemistry environment. The radical intermediates generated under photochemical conditions from the C-terminal carboxylates undergo rapid 1,4-conjugate additions to a variety of Michael acceptors. The studied methodology is applicable to the functionalization of established drugs and drug precursors.

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