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Photoredox-catalyzed *C*-glycosylation of peptides with glycosyl bromides

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ABSTRACT

Glycosyl radicals, produced under mild photoredox conditions, show unique utility in the preparation of C-linked glycoconjugates. We herein report the construction of *C*-glycosidic bonds on α,β-dehydroalanine (DHA) of peptides with easily available glycosyl bromides as glycosyl radical precursors under highly anomeric control, leading to *C*-glycosylation modifications of peptides. This method not only has outstanding functional group compatibility, but also is feasible in near-physiological conditions (pH ~ 7 and temperature $T \leq 37$ °C in aqueous media).

With the rapid development of peptide drugs, the modification of bioactive peptides has attracted wide attention of scientists in the field of chemical biology and synthetic chemistry [1–4]. Methods to directly and post-translationally modify peptides or proteins is difficult by conventional two-electron chemical approaches since it also needs to maintain good biocompatibility and site/chemoselectivity simultaneously under the reaction conditions [5]. As an alternative, radical chemistry, particularly by mild photoredox-catalyzed single electron transfer (SET) processes, has been proven to be a powerful tool for subsequent transformation and modification of various peptide side chains or proteins [6–10]. α,β-Dehydroalanine (DHA), as one of the most common unnatural amino acids, exist in various ribosome synthesized and post-translational modified peptides and other naturally occurring peptides [11]. Serine (Ser) or cysteine (Cys) residues used as precursors can effortlessly introduce DHA into complex peptide and protein sites through chemical or enzymatical methods [12]. Due to contain α,β-unsaturated carbonyl motif, DHA shows dramatic chemical reactivity as an acceptor of electrophilic or nucleophilic radicals [13,14]. Furthermore, the addition of a radical to DHA produces α-amino radical intermediates can be stabilized through captodative effect of amino and carbonyl groups [15]. This radical addition reactions are used to introduce various functional groups into peptides and proteins through single electron transfer (SET) process (Scheme 1a) [16–20].

Glycosylation is one of the most common and important post-translational modifications of proteins in organisms, which controls the localization, function, activity, lifespan, and diversity of proteins in tissues and cells [21,22]. Therefore, diversified glycosidic linkages are the basis for the functional regulation of glycoproteins. The relatively rare *C*-glycosidic bonds are generally more resistant to acid, base, as well as deglycosylase, than the prevalent *N/O*-glycosidic bonds. Therefore, *C*-glycosylation has received intensive attentions [23–26] and this strategy was also widely used in the improvement of glycopeptide drugs [27–30]. Radical glycosylation of peptides mainly focuses on aromatic amino acids (Trp and Phe) [31–33], glycine [34–36] and alkyne/alkene modified amino acids and peptides [37,38], while the glycosylation of DHA motifs is rarely reported. Kessler and Metzler-Nolte groups reported that nBu$_3$SnH/AIBN-mediated radical addition of glycosyl bromides to DHA derivatives for the synthesis of glycopeptides [39,40]. The Beckwith and co-workers performed *C*-glycosylation of DHAs under nBu$_3$SnCl/NaBH$_3$CN/UV conditions (Scheme 1b) [41]. These pioneering works have some limitations, such as the use of toxic tin reagents, the need for heating or UV to initiate the reactions, and poor chemo- and stereoselectivities.

Recently, our group constructed the *C*-glycosidic bond of glycosyl bromides with aromatic heterocycles and alkynyl bromides, respectively, under the photoredox-catalyzed conditions (Scheme 1c) [42,43]. Encouraged by our previous works, as well as aforementioned radical-based *C*-glycosylation of peptides and proteins, we aim at *C*-glycosylation of DHA in peptides with glycosyl bromides as glycosyl radical precursors under photoredox catalytic conditions (Scheme 1d) [44,45].

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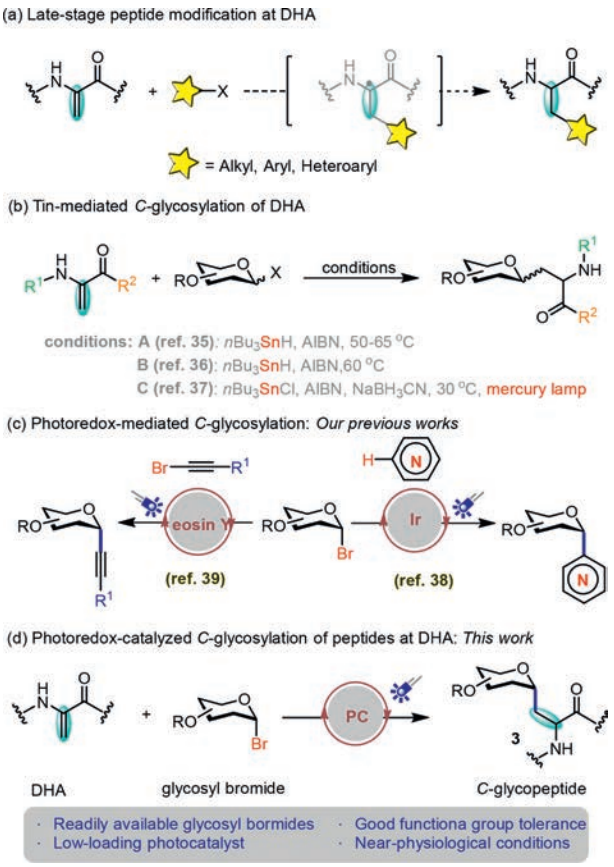
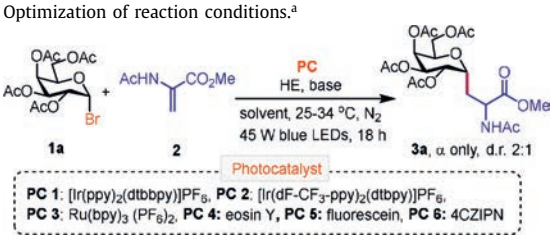


Scheme 1. Modification of DHA and construction of *C*-glycosidic bond. (a) Late-stage peptide modification at DHA. (b) Tin-mediated *C*-glycosylation of DHA. (c) Our previous works of *C*-glycosylation. (d) Photoredox-catalyzed *C*-glycosylation of DHA in peptides. PC = photocatalyst.

We initiated our studies by choosing galactose-derived bromide **1a** and α,β-dehydroalanine **2** as the model substrates under various photoredox conditions. Some representative results are exhibited in Table 1 (For comprehensive reaction condition optimization, see Supporting information). Based on our previous works about *C*-glycosylation [42,43] and Gagné group's report on intermolecular addition of glycosyl halides to alkenes [46], the coupling of galactosyl bromide **1a** and DHA **2** proceeded successfully to provide *C*-glycoalanine **3a** in an 88% NMR yield (56% isolated yield), with acetonitrile as the solvent, $[Ir(ppy)_2(dtbbpy)]PF_6$ (**PC 1**, 2 mol%) as the photocatalyst (PC), Hantzsch ester (HE) as the reductive quencher and Cs_2CO_3 as the acid scavenger under the irradiation of 45 W blue LEDs (Table 1, entry 1). Other photocatalysts, such as $[Ir(dF\text{-}CF_3\text{-}ppy)_2(dtbpy)]PF_6$ (**PC 2**), $Ru(bpy)_3(PF_6)_2$ (**PC 3**), Eosin Y (**PC 4**), fluorescein (**PC 5**) were evaluated, but lower yields were obtained in all cases (entries 2–5). Solvents, such as 1,4-dioxane, tetrahydrofuran (THF) and dichloromethane (DCM), were also tested and no better result was achieved (entries 6–8). When Cs_2CO_3 was replaced by K_2CO_3, the yield decreased to 43% (entry 9). Delightfully, the isolated yield was improved to 80% when the 2 equiv. of HE was used (entry 10). As expected, no reaction was observed in the absence of blue light or the photocatalyst (entries 11 and 12). It is worth mentioning that *C*-glycoalanine **3a** was produced with only α configuration at anomeric position and 2:1 diastereomeric ratio (*dr*) at α position of alanine in all cases.

Having established the optimized reaction conditions, we began to test the reactivity of various glycosyl bromides with α,β-dehydroalanine **2**. As shown in Scheme 2, D-galactose-, D-glucose-, D-mannose-, D-fucose- and D-arabinose-derived bromides underwent this reaction smoothly to give *C*-glycopeptide **3a**-**3i** with

Table 1
Optimization of reaction conditions.[a]



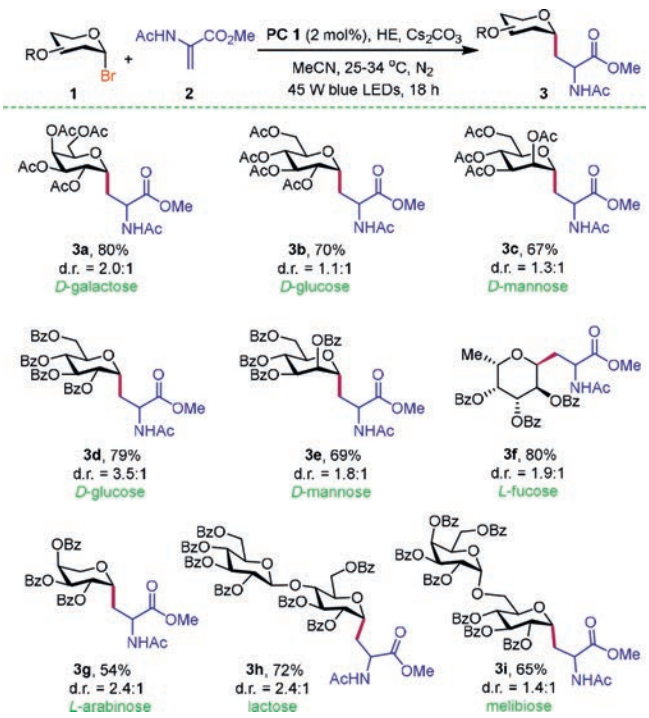
Entry	PC	Base	Solvent	Yield (%)[b]
1	**PC 1**	Cs_2CO_3	MeCN	88 (56)[c]
2	**PC 2**	Cs_2CO_3	MeCN	72
3	**PC 3**	Cs_2CO_3	MeCN	54
4	**PC 4**	Cs_2CO_3	MeCN	Trace
5	**PC 5**	Cs_2CO_3	MeCN	Trace
6	**PC 1**	Cs_2CO_3	1,4-Dioxane	58
7	**PC 1**	Cs_2CO_3	THF	46
8	**PC 1**	Cs_2CO_3	DCM	29
9	**PC 1**	K_2CO_3	MeCN	43
10[d]	**PC 1**	Cs_2CO_3	MeCN	(80)[c]
11[e]	**PC 1**	Cs_2CO_3	MeCN	0
12	–	Cs_2CO_3	MeCN	0

[a] Reaction conditions: a solution of **1a** (0.2 mmol), **2** (0.24 mmol), **PC** (2 mol%), HE (0.3 mmol) and Cs_2CO_3 (0.3 mmol) in MeCN (2 mL) was irradiated by 45 W blue LEDs for 18 h. The *dr* was determined by ^{1}H NMR analysis of crude reaction mixtures.
[b] Yields were based on ^{1}H NMR analysis of the crude product using 1,2-dibromoethane as an internal standard.
[c] Isolated yields.
[d] 0.4 mmol of HE (2.0 equiv.) was used.
[e] In dark.

moderate to good yields (54%–80%) and α-stereoselectivity. In addition, disaccharide-derived bromides (such as lactose and melibiose) could also be used as the coupling couplers and the *C*-glycosylation products **3h** and **3i** were generated in satisfactory yields (72% and 65% respectively). The α-configuration was carefully established by ^{1}H NMR analysis based on coupling constants [47,48].

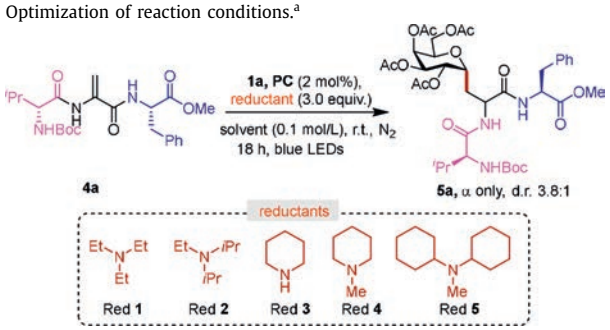
After succeeding in *C*-glycosylation of α,β-dehydroalanine **2** with glycosyl bromides, we turned our attention to *C*-glycosylation of DHA-containing peptides. First, we chose DHA-containing tripeptide **4a** as the model substrate. Unfortunately, the coupling of tripeptide **4a** with glycosyl bromide **1a** under our newly established optimized conditions in Table 1 resulted in a complicated reaction mixture and the desired *C*-glycopeptide **5a** could not be identified. Therefore, we had to re-optimize the reaction conditions (Table 2). When Cs_2CO_3 was replaced by Et_3N, the target *C*-glycopeptide **5a** was obtained in 27% with only α configuration at anomeric position and 3.8:1 *dr* at α position of alanine (entry 1). Given that Et_3N can serve as the reductive quencher and the acid scavenger, the dosage of Et_3N increased to 3.0 equiv. without the addition of HE and the yield of **5a** was improved to 56% (entry 2). Different reductants were tested (entries 3–6) and DIPEA (Red **2**) gave the best result. Other solvents, such as 1,4-dioxane, THF and dimethyl sulfoxide (DMSO), were not superior to MeCN (entries 7–9). Subsequently, photocatalysts, such as $[Ir(dF\text{-}CF_3\text{-}ppy)_2(dtbpy)]PF_6$ (**PC 2**), $Ru(bpy)_3(PF_6)_2$ (**PC 3**), Eosin Y (**PC 4**), and 4CzIPN (**PC 6**), were also explored, but none of them showed better catalytic efficiency than **PC 1** (entries 10–13). The control experiments showed that the blue light, photocatalyst and reductant were all indispensable for this reaction (entries 14–16).

After determining the optimized conditions for *C*-glycosylation of DHA-containing peptides, we then investigated substrate scopes of glycosyl bromides and DHA-containing peptides. As shown in Scheme 3a, a variety of glycosyl bromides were coupled with DHA-containing tripeptide **4a**. Bz-protecting D-galactose-derived bromide could also under this *C*-glycosylation to give the *C*-



Scheme 2. Scope of glycosyl bromides. Reaction conditions: A solution of **1** (0.2 mmol), **2** (0.24 mmol), **PC 1** (2.0 mol%), HE (0.4 mmol) and Cs_2CO_3 (0.3 mmol) in MeCN (2 mL) was irradiated by 45 W blue LEDs for 18 h, isolated yields. The *dr* values were determined by ^{1}H NMR analysis of crude reaction mixtures.

Table 2
Optimization of reaction conditions.[a]



Entry	PC	Reductant	Solvent	Yield (%)[b]
1[c]	**PC 1**	HE/Red **1**	MeCN	27
2	**PC 1**	Red **1**	MeCN	56
3	**PC 1**	Red **2**	MeCN	77
4	**PC 1**	Red **3**	MeCN	Complicated
5	**PC 1**	Red **4**	MeCN	37
6	**PC 1**	Red **5**	MeCN	Trace
7	**PC 1**	Red **2**	1,4-Dioxane	58
8	**PC 1**	Red **2**	THF	60
9	**PC 1**	Red **2**	DMSO	Complicated
10	**PC 2**	Red **2**	MeCN	65
11	**PC 3**	Red **2**	MeCN	Trace
12	**PC 4**	Red **2**	MeCN	Trace
13	**PC 6**	Red **2**	MeCN	Complicated
14	**PC 1**	–	MeCN	0
15	**–**	Red **2**	MeCN	0
16[d]	**PC 1**	Red **2**	MeCN	0

[a] Reaction conditions: A solution of **4a** (0.2 mmol), **1a** (0.3 mmol), **PC** (2 mol%), reductant (0.6 mmol) in MeCN (2 mL) was irradiated by 45 W blue LEDs for 18 h.
[b] Isolated yields.
[c] 2.0 equiv. of HE and 1.5 equiv. of Et_3N were used.
[d] In dark.

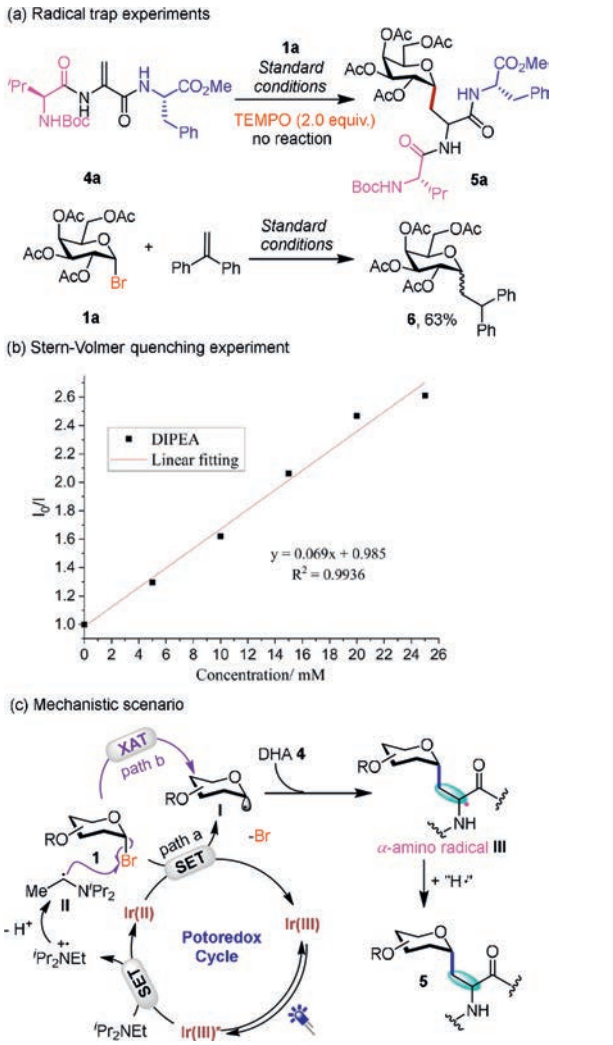
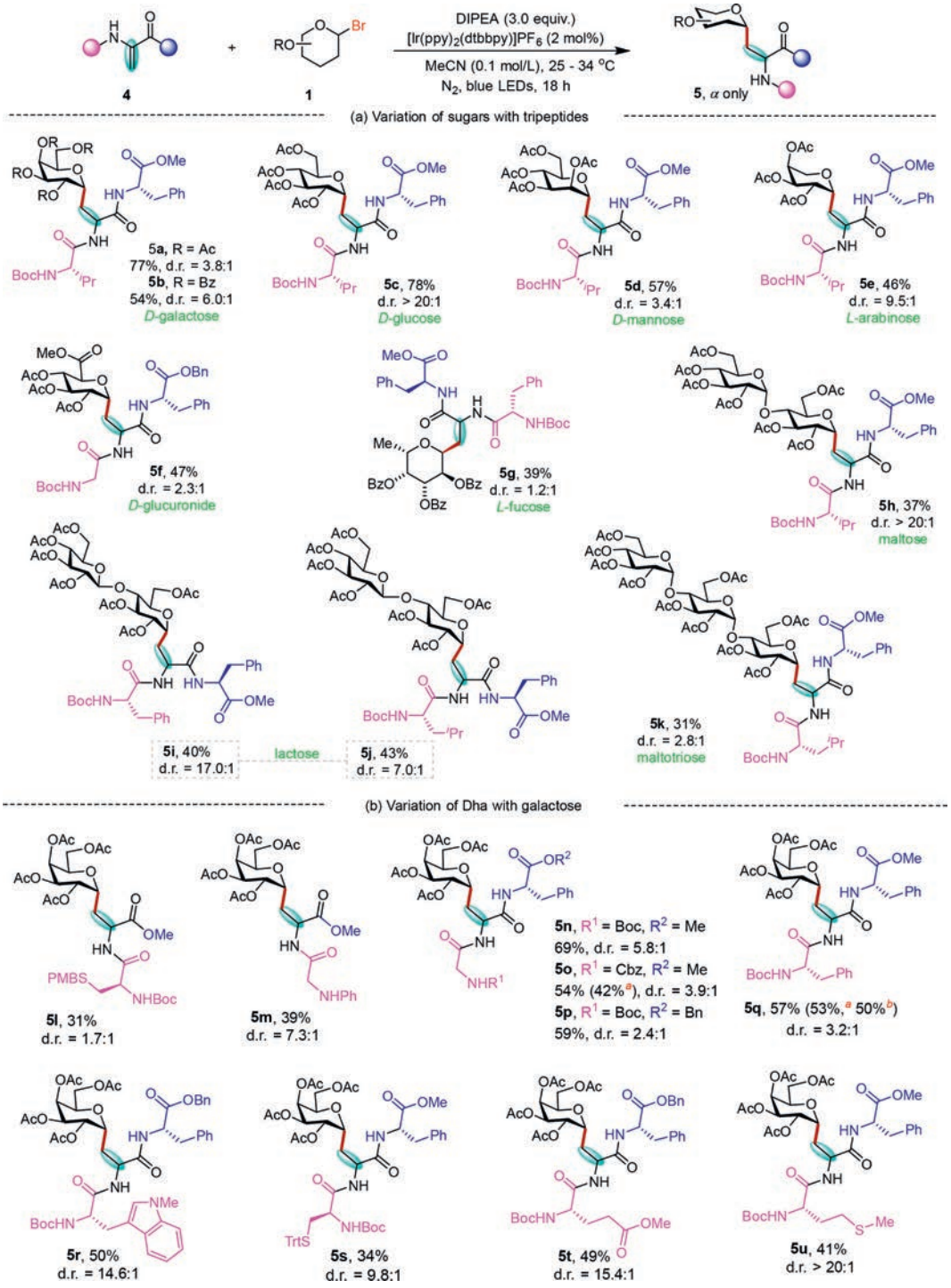


Fig. 1. Mechanism studies. (a) Radical trap experiments. (b) Stern–Volmer quenching experiment of the excited $[Ir(ppy)_2(dtbbpy)]PF_6$ (5×10^{-4} mol/L) with DIPEA. (c) Proposed mechanism.

glycopeptide **5b** in a 54% isolated yield. Other monosaccharides, such as D-glucose, D-mannose, L-arabinose, D-glucuronide and D-fucose, could also be coupled with DHA **4a** to provide the corresponding *C*-glycopeptides **5c**–**5g** in 39%–78% yields. Polysaccharide-derived bromides (maltose, lactose, and even maltotriose) could serve as the coupling partners providing *C*-glycosylation products **5h-5k** in moderate yields (31%–43%). We then explored *C*-glycosylation of various DHA-containing peptides with D-galactose-derived bromide **1a** (Scheme 3b). Dipeptide DHAs gave targeted compounds **5l** and **5m** with acceptable yields (31% and 39%, respectively). DHA-containing tripeptides (Gly-Ser-Phe) with different protecting strategies could smoothly couple with glycosyl bromide **1a** to give *C*-glycotripeptides **5n-5p** in yields of 54%–69%. This strategy was also applicable to DHA-containing peptides with various amino acid residues, such as Phe, Trp, Cys, Glu and Met. The desired *C*-glycotripeptides **5q-5u** could be constructed in 34%–57% yields. Notably, we attempted to use a mixed solvent of MeCN or 1,4-dioxane with phosphate pH 7.0 buffer, the products **5o** and **5q** were also obtained with yields of 42%, 53% and 50%, respectively. These mild and near-physiological conditions will be expected to provide valuable experience for *C*-glycosylation of complex biological peptides and proteins.

After the exploration of the substrate scopes, we then investigate the reaction mechanism. As shown in Fig. 1a, the addition



Scheme 3. Scope of glycosyl bromides and DHA-containing peptides. Reaction conditions: A solution of **4** (0.2 mmol), **1** (0.3 mmol), **PC 1** (2 mol%), DIPEA (0.6 mmol) in MeCN (2 mL) was irradiated by 45 W blue LEDs for 18 h, isolated yield. [a] 1.4-Dioxane/phosphate pH 7.0 buffer (3:1, 2 mL) instead of MeCN. [b] MeCN/phosphate pH 7.0 buffer (3:1, 2 mL) instead of MeCN.

of 2.0 equiv. of 2,2,6,6-tetramethyl-1-piperidyloxy (TEMPO) into the reaction mixture under standard conditions could inhibit the desired reaction completely. When DHA **4a** was replaced by 1,1-diphenylene (5 equiv.), the galactosyl radical-trapping product **6** was obtained in a 63% yield (Fig. 1a). These experiments indicated that the galactosyl radicals may participate in the reaction process. The Stern-Volmer quenching experiment supported that DIPEA was the quencher of the excited photocatalyst (Fig. 1b).

On the basis of the control experiments, as well as the literature precedents, a possible mechanism was proposed (Fig. 1c). An excited photocatalyst Ir(III)* is formed by the irradiation of photocatalyst Ir(III) with visible light. Ir(III)* is reductively quenched by DIPEA to give the corresponding DIPEA radical cation together with a low-valent Ir(II) complex [42,43]. Glycosyl bromide **1** is then reduced by Ir(II) to produce the glycosyl radical **I** along with the regeneration of photocatalyst Ir(III). Alternatively, based on Leonori's proposal [49], radical intermediate **II** generates radical **I** from glycosyl bromide **1** through the halogen-atom transfer (**XAT**) mechanism. Subsequently, the addition of radical **I** to DHA **4** forms a stable α-amino radical intermediate **III** [39–41]. This intermediate

III can abstract a hydrogen atom to give the *C*-glycopeptide product **5**.

In summary, we have proposed and implemented a strategy of synthesizing *C*-glycopeptides from α,β-dehydroalanine or DHA-containing peptides and glycosyl bromides under mild photocatalytic conditions. The main characteristics of this strategy include readily available the starting materials, simple experimental operation, outstanding functional group compatibility and near-physiological conditions (pH $\sim$ 7 and temperature $T \leq 37\,^{\circ}C$ in aqueous media). Peptides with different amino acid residues can serve as good coupling partners, enabling the site-specific *C*-glycosylation modification. 30 *C*-glycopeptide products were synthesized with moderate to good yields.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.109128.

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