Visible Light Promoted Stereoselective C(sp^3)-H Glycosylation for the Synthesis of *C*-Glycoamino Acids and *C*-Glycopeptides

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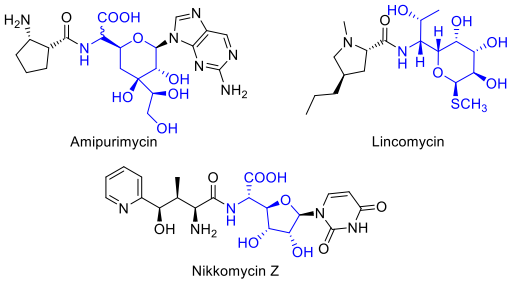
Abstract: Glycosylative modification of peptides could improve the pharmacological properties of peptide drugs and deliver them efficiently to the target sites. Compared with *O*-/*N*-glycosides, *C*-glycosides exhibit more metabolic stability. We here disclose the first example of visible-light promoted and Cu-catalyzed stereoselective *C*-glycosylations. The mild reaction conditions are compatible with various carbohydrate substrates, including a series of mono monosaccharides and disaccharide, and is amenable to the synthesis of a wild variety of *C*-glycoamino acids and *C*-glycopeptidomimetics with good yields and excellent stereoselectivities. The dual-functional photocatalyst formed in situ via coordination of glycine derivatives and chiral phosphine Cu

complex could not only catalyze the photoredox process but also control the stereoselectivity of glycosylation reaction.

Peptides exhibit high receptor affinity and low toxicity compared to small molecule drugs.[1] However, the therapeutic potential of peptides is severely hampered by their low bioavailability and poor pharmacokinetic (PK) properties, as they are often metabolized by numerous proteases and peptidases.[2] Different strategies have been explored to overcome these obstacles, and one of the most promising strategies is glycosylation. Furthermore, incorporation of glycoamino acid could provide carbohydrate-binding sites into bioactive peptides, which would further improve the pharmacodynamic (PD) properties of peptides in a drug-like manner.[3] In this context, during the past few decades, *O*-/*N*-glycosylations have been widely applied as the main glycosylative modification ways in peptide synthesis. However, *O*-/*N*--glycosyl peptides are chemical and enzymatic unstable under physiological conditions, which potentially limits the utility of *O*/*N*-glycopeptides as in drug discovery.[4]

In contrast to the fragile linkage between the *O*/*N* atoms of peptides and glycosyl groups, the *C*-glycoside linkage is more capable to tolerate acids, bases, and enzymatic hydrolysis.[5] Notably, some *C*-glycosylated peptides and amino acids received significantly improvement in biological activities compared to their naturally occurring analogs.[6] In comparison to the well-studied *O*/*N*-glycosylation of amino acids or native peptides,[7] the *C*-glycosylations of amino acids or peptides is much more difficult and lacks of sufficient methodologies.[8] The reported examples of *C*-glycosylation of peptide were restricted to the couplings either with indole motif in tryptophan residue[9] or with the preinstalled highly reactive groups (alkenyl, alkynyl, or bromide).[10] The Pd-catalyzed stereoselective C(sp^3)-H glycosylation of amino acids were disclosed. However, the harsh

conditions were poorly compatible with peptides and oligosaccharides substrates,[9a,11] and the sugar donors were limited in glycals.[9a]

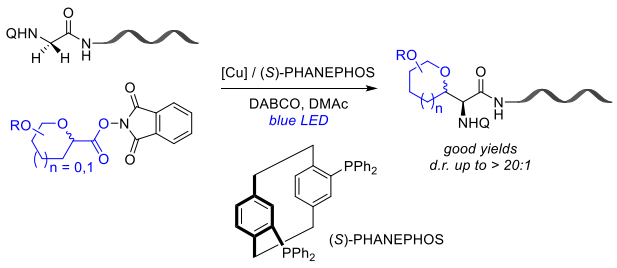


Scheme 1. Representative drugs containing C-glycosyl glycine moiety or its derivatives

Glycine is the basic skeleton of α-amino acids. Direct glycosylating the C(sp^3)-H of glycine residue in peptide could provide an opportunity to modify the peptide backbone with sugar motifs, which would be very interesting in peptide drug discovery.[3,4] Notably, the *C*-glycosylated glycine moiety or its derivatives are constituents of natural products and possess remarkable antibiotic activities (Scheme 1).[12] Wang and co-workers recently disclosed a very interesting approach for synthesis of *C*-glycosylated glycine under photo catalytic conditions through the nonestereoselective addition of glycosyl radical to imine.[13] In the reaction, the conformation of glycosyl radical was conserved and gave the addition product in nearly 1:1 mixture of diastereomers. Thus far, with the complex peptide and densely functionalized carbohydrate substrates, control both the stereocenters of two coupling partners in the reaction is quite challenging. To date, the established *C*-glycosylations of glycine was either lack of stereocontrol[13] or poorly tolerated with peptide substrates.[9c,13]

The visible light induced radical coupling has emerged as an important pathway for C-C bond formation. The good functional group tolerance renders a significant strategy for chemoselective biomolecule modifications. In recent years, a series of *C*-glycosylation strategies have been

reported.[14a-c] Notably, in the reactions, the stereoselectivities of the *C*-glycosylations were dominantly determined by the conformations of the glycosyl radical intermediates.[14d-h] We recently reported a photo induced Cu catalyzed asymmetric C(sp^3)-H alkylation of glycine derivatives, in which the conformations of radical coupling products were fully controlled by chiral catalyst.[15] We envisioned that by using this strategy, the stereoselective *C*-glycosylation of glycine could possibly achieve, in which one of the vicinal two stereocenters is determined by chiral Cu catalyst and the other conserves the conformation of glycosyl radical.[14a-b] In line with our interests on photoinduced late-stage modification of peptide,[16] we here report the first example of visible-light promoted Cu-catalyzed stereoselective C(sp^3)-H glycosylation of glycine residue in complex peptide substrates. It is worth noting that our reaction is compatible with various functional groups and carbohydrate substrates, including disaccharide, and is amenable to the synthesis of a wild variety of *C*-glycoamino acids and *C*-glycopeptidomimetics with good yields and excellent stereoselectivities (Scheme 2).

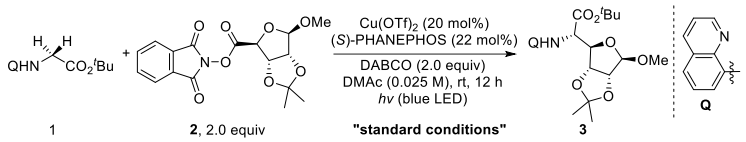


Scheme 2. Visible-light-promoted enantioselective C(sp^3)-H glycosylation of peptide.

In the initial study, we selected quinolinyl-8-glycinate ester **1** as model substrate. The quinoline group in **1** would coordinate to a Cu salt together with a chiral phosphorous ligand, and in situ generate a new chiral Cu complex.[15a] The complex could not only mediate the intramolecular photoredox process but also control the stereoselectivity in the coupling reaction. Gratifyingly,

under blue LED irradiation, the reaction between **1** and ribose-derived *N*-hydroxyphthalimide (NHP) ester **2** (prepared according to reported methods[13b]) proceeded smoothly to form the C(sp^3)-H glycosylation product **3** in 80% ^{1}H NMR yield and > 20:1 d.r. in the presence of 20 mol% of $Cu(OTf)_2$, 22 mol% of (*S*)-PHANEPHOS, 2.0 equiv of DABCO, and using DMAc (0.025 M) as solvent (Table 1, entry 1, standard conditions, see the Supporting Information for details). Without light, $Cu(OTf)_2$, or (*S*)-PHANEPHOS, the coupling product was not observed (entries 2-4). The control reaction lacking external basic additives (DABCO) also provided product **3**, albeit with a lower yield of 30% (entry 5). We surmised that the basic phthalimide anion generated from decarboxylative process could also promote the reaction (entry 5). Substituting $Cu(OTf)_2$ with other cupric or cuprous catalysts led to dramatic decrease of yields and stereoselectivities, and only $Cu(MeCN)_4PF_6$ gave acceptable result (entry 6).

Table 1. Optimization of the reaction conditions.

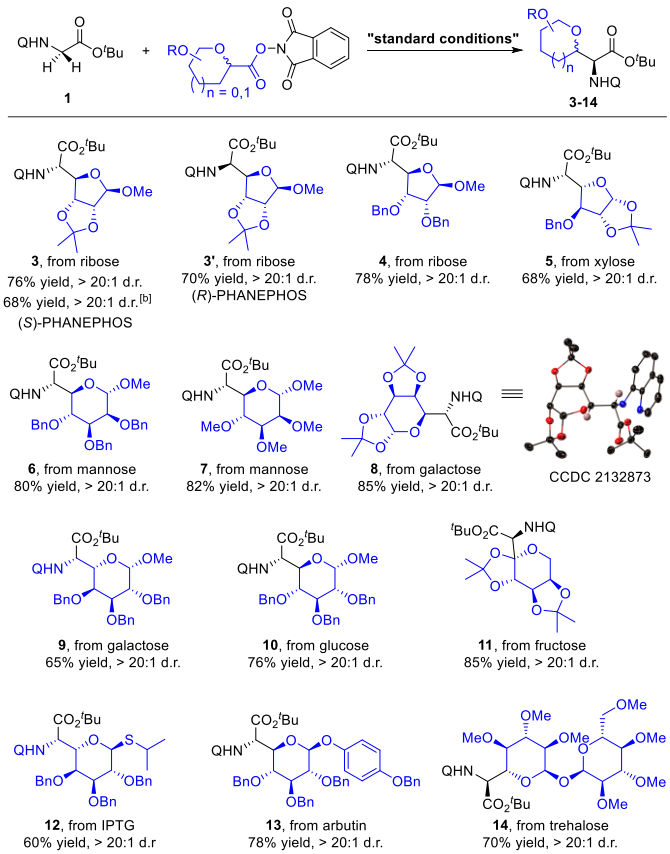


entry[a]	change from the "standard conditions"	yield [%][b]	d.r.[c]
1	**standard conditions**	**80 (76)**	**> 20:1**
2	without *hv*	0	--
3	without $Cu(OTf)_2$	0	--
4	without (*S*)-PHANEPHOS	0	--
5	without DABCO	30	> 20:1
6	$Cu(CH_3CN)_4PF_6$ instead of $Cu(OTf)_2$	35	> 20:1

[a] 0.05 mmol scale. [b] ^{1}H NMR yield based on **1** with 1,3,5-trimethoxybenzene as the internal standard, isolated yield in parentheses. [c] D.r. was determined by ^{1}H NMR analysis.

With the optimal reaction conditions established (Table 1, entry 1), we next investigated the scope of the coupling reaction. As shown in Table 2, glycosyl NHP esters derived from various monosaccharides, including ribose, xylose, mannose, galactose, glucose, and fructose all could

Table 2. Substrate scope with respect to saccharide-derived *N*-hydroxyphthalimide esters.[a]



[a] Reaction conditions: glycine derivative **1** (0.1 mmol, 25.8 mg), saccharide-derived NHP ester (0.2 mmol, 2.0 equiv), $Cu(OTf)_2$ (20 mol%, 7.2 mg), (*S*)-PHANEPHOS (22 mol%, 12.7 mg), DABCO (0.2 mmol, 2.0 equiv, 22.4 mg), DMAc (4 mL), room temperature, argon atmosphere, 12 h and under blue LED. **6-14** were obtained at -10 °C. Isolated yields base on **1** after chromatographic purification. D.r. was determined by ^{1}H NMR analysis. [b] 4 mmol after 18 h.

participate in this reaction smoothly and afforded the desired products in 65-85% yields and >20:1 d.r. (**3-11**). The bioactive sugar derivatives, such as isopropyl-β-D-thiogalactopyranoside (IPTG) and arbutin were also competent substrates (**12** and **13**). Importantly, disaccharide (trehalose) was compatible with the coupling conditions, and gave the glycoamino acid **14** in 70% yield and excellent stereoselectivity (> 20:1 d.r.). Furthermore, the reaction could be conducted in 4 mmol

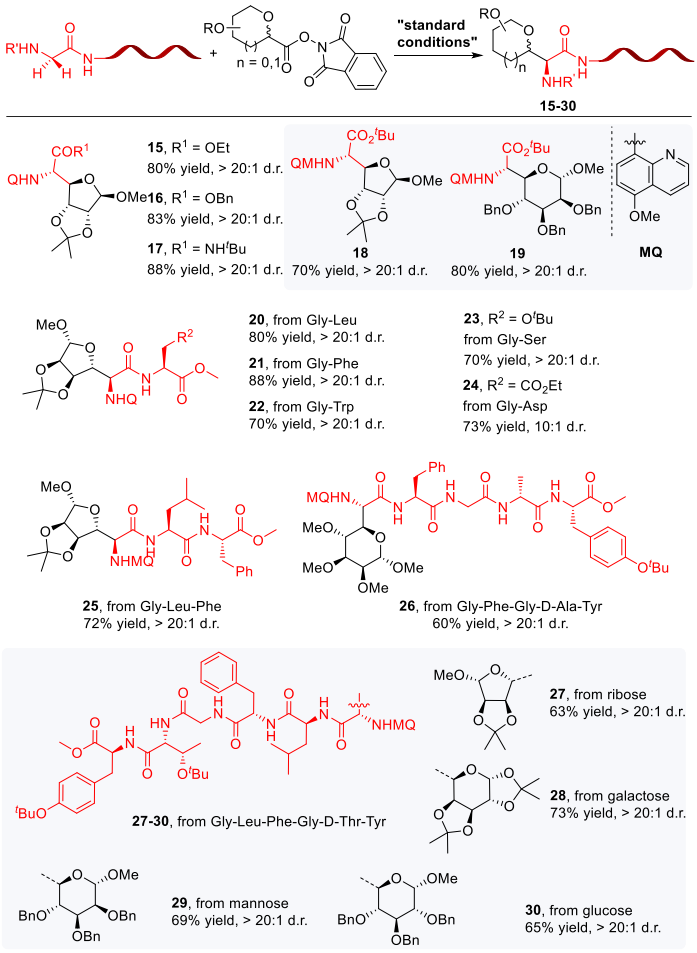
scales to provide the product in good yields and excellent stereoselectivity (**3**). The X-ray structure of **8** was consistent with previously report that the conformation glycosyl radical was conserved during the reductive elimination process.[14a,b] Due to the lack of anomeric effect preference, the stereochemical outcome of furanoses (ribose and xylose) derived radicals could be explained by a combination of stereoelectronic and steric effects. [13,14a,b] It should be noted that the stereochemistry of the product was primarily determined by the stereochemistry of chiral catalyst rather than the stereochemistry of sugar substrate (**3** versus **3'**).

Encouraged by above good performance, the scopes of glycine derivatives were evaluated (Table 3). This C(sp^3)-H glycosylation protocol was consistent with a variety of glycine derivatives, including different quinolinyl-8-glycinate esters (**15**, **16**), quinolinyl-8-glycinate amide (**17**) and 5-methoxyquinolinyl (MQ)-8-glycinate ester (**18**, **19**). A variety of dipeptides and tripeptides (Gly-Leu, Gly-Phe, Gly-Ser, Gly-Trp, Gly-Asp, and Gly-Leu-Phe) were prepared to test the regio- and chemoselectivity of the reaction. As shown in Table 3, the corresponding ribosyl peptides **20**-**25** were synthesized in good yields and excellent stereoselectivities (> 20:1 d.r.), and with other amino acid residues untouched.

Having established proof-of-concept with the above results, we became interested if our reactions could be applied in *C*-glycosylations of biologically important glycopeptidomimetics. Gratifyingly, the late-stage C(sp^3)-H mannosylations of pentapeptide (Gly-Phe-Gly-D-Ala-Tyr) derived substrates reacted smoothly, and the corresponding carbohydratepeptide conjugates were formed with good efficiency (**26**, 60%) and high d.r. (> 20:1) It was worth noting that the highly stereoselective *C*-glycosylations between hexapeptide (Gly-Leu-Phe-Gly-D-Thr-Tyr) substrate and different sugar NHP esters (derived from ribose, galactose, mannose, and glucose) were achieved

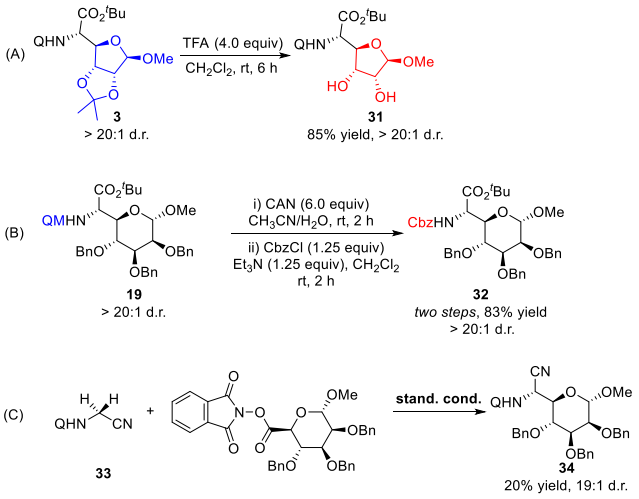
in uniformly good yields (**27-30**, 63-73%), which further highlight the generality of this method in modification of complex molecules. The deprotection (Scheme 3A) or protecting group transfer (Scheme 3B) of the C-glycosylation products proceeded smoothly under simple procedures in high yields, and did not erode the d.r. Moreover, the substrate with cyano group (**33**) also proceeded and afforded the coupling product **34** in 20% yield and 19:1 d.r.

Table 3. Substrate scopes with respect to glycine derivatives and peptides.[a]



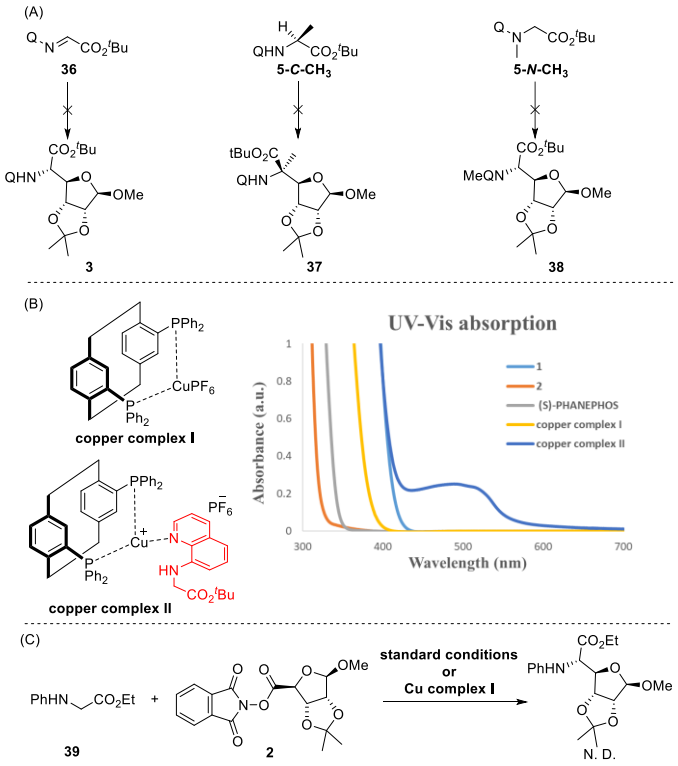
[a] Reaction conditions: glycine or peptide (0.1 mmol), saccharide-derived NHP ester (0.2 mmol, 2.0 equiv), $Cu(OTf)_2$ (20 mol%, 7.2 mg), (*S*)-PHANEPHOS (22 mol%, 12.7 mg), DABCO (0.2 mmol, 2.0 equiv, 22.4 mg), DMAc (4 mL), room temperature, argon atmosphere, 12 h and under blue LED. **19-30** were obtained at -10 °C. The

yields of **26**-**30** referred to 18 h. Isolated yields base on glycine derivative or peptide after chromatographic purification. D.r. was determined by ^{1}H NMR analysis.



Scheme 3. Synthetic applications.

In the mechanistic studies (see the Supporting Information for details), the radical trapping experiments with TEMPO (2,2,6,6-tetramethylpiperidin-1-oxyl) and BHT (2,6-di-tert-butyl-4-methylphenol) suggested that ribosyl radical was formed in the reaction system. The HRMS analysis of the original reaction mixture detected the formation of glycinate homo-coupling product (**35**), which evidenced the generation of glycinate radical. Several control experiments also were carried out and the results were shown in Scheme 4. Firstly, imine **36** failed to give the *C*-glycosylation product, revealing the Cu-mediated SET pathway likely involved rather than the two electron-oxidation process (Scheme 4A). Secondly, when **5-*C*-CH$_3$** and **5-*N*-CH$_3$** were used as substrates, corresponding products **37** and **38** were not detected due to the unfavorable deprotonation caused by the steric clash (Scheme 4A).[17] The results explained the exclusive mono glycosylations in all cases in Table 2 and 3.

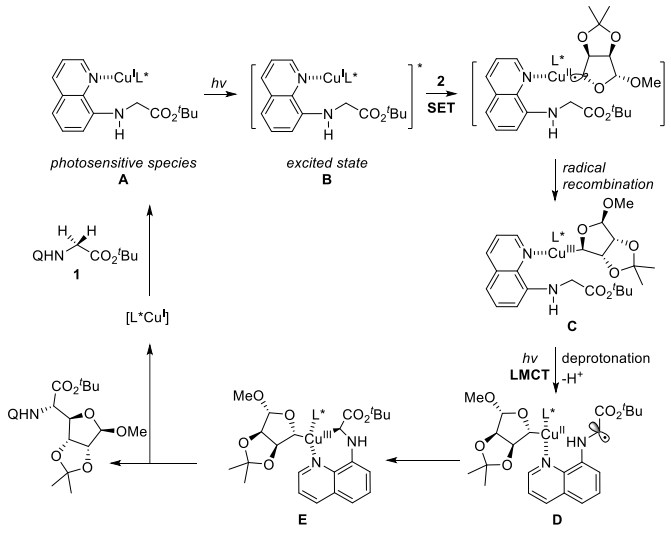


Scheme 4. Mechanistic studies.

To better understand the active Cu species that participated in photo-absorption and stereoselective control process, copper complex **I** and copper complex **II** were synthesized.[18] In the UV-Vis absorption studies, only copper complex **II** showed strong absorption in blue light region, which was in the range of the wavelengths used for the reaction (Scheme 4B). The results were consistent with the control reactions, that ethyl 2-(phenylamino) acetate (**39**) could not provide any glycosylation product under standard conditions or using Cu complex **I** as catalyst (Scheme 4C). Furthermore, the Stern-Volmer experiment and cyclic voltammetry experiments supported the oxidative quenching process between excited state of Cu complex **II** ($E_{p/2}^{red}$ [Cu^{II}/Cu^{I}*] = -1.901 V vs SCE in CH_3CN) and saccharide NHP esters ($E_P^{0/-1}$ (**2**) = -0.896 V vs SCE in CH_3CN) (see the Supporting Information). We surmised that the glycinate radical was generated via the intramolecular ligand-to-metal charge transfer (LMCT) of Cu complex **II**. We measured the steady-

state absorption of the reaction mixture, and the absorption spectrum was not changed over time in the absence of light (see the Supporting Information).[19] After continuous irradiation, the absorption band gradually bathochromic-shifted due to the photoreduction of Cu(III) to Cu(II) via LMCT. The quantum yield $\Phi = 0.024$ indicated a radical chain process might not involve in the reaction.

Based on the above mechanistic studies and previous research,[15,19] a plausible pathway was given in Scheme 5. Initially, photosensitive species **A** in situ forms via the coordination of [L*Cu^{I}] with glycinate ester **1**. Under blue LED irradiation, photosensitive species **A** is excited to its excited-state **B**. Then, the single electron transfer (SET) between **B** and NHP ester **2** occurs, followed by the generation of ribosyl radical with specific configuration. The recombination of ribosyl radical and Cu(II) gives Cu(III) complex **C**. The LMCT of complex **C** and deprotonation process generates *C*-centered radical **D**, which subsequently attacks copper center to form a chiral intermediate **E**. Finally, the stereoselectively reductive elimination afforded the *C*-glycosylation product.



Scheme 5. Plausible mechanism.

In conclusion, we reported the first example of visible-light-promoted and copper-catalyzed stereoselective C(sp^3)-H glycosylation. The mild reaction conditions are compatible with various

carbohydrate substrates, and is amenable to the synthesis of a wild variety of *C*-glycoamino acids and *C*-glycopeptidomimetics with good yields and excellent stereoselectivities. Give that the modification of peptides by introduction of carbohydrate moieties can significantly enhance the therapeutic behavior of peptide drugs, we anticipate that our protocol would have important impacts on the development of peptide drugs.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interests.

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