Late-stage functionalization of glucosides and aminoglycosides to combat bacterial resistance

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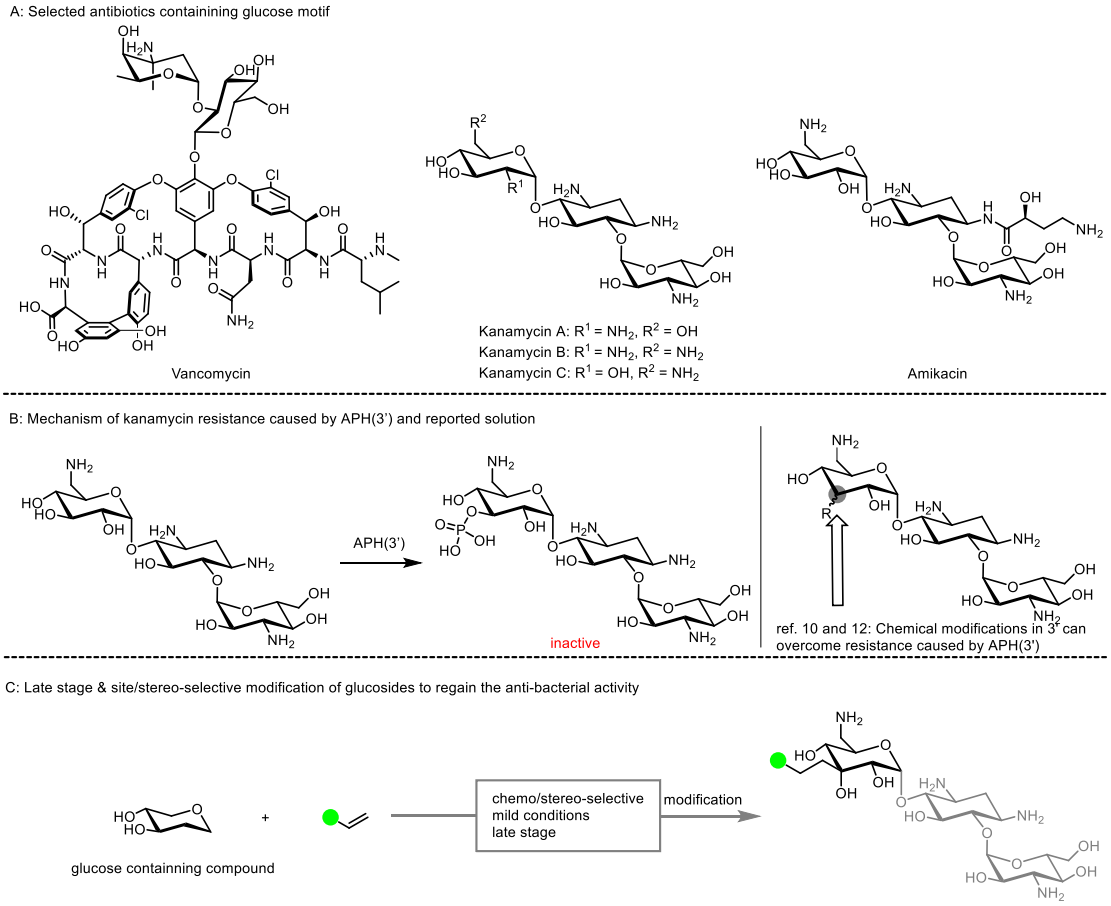
Abstract: The rapid emergence of resistant bacteria and the scarcity of antibiotic pipeline has been a persistent threat to the global health. To expand the antibiotic pipeline, we have focused our strategy by revitalizing the current antibiotic via novel site-specific derivatization. Aminoglycosides (AGs), once considered effective therapeutics for treating clinical infections, are now seeing limited use due to drug-induced toxicity and AG-resistant bacteria. Challenges in synthesis and modifications of AGs impedes their potential for re-gaining efficacy. Here we discuss a photoredox catalyzed late-stage modification of glucosides including AGs. Our chemistry can selectively install an alkyl group at the 3- position of unprotected glucoside with a broad substrate scope and high yields. Among the products, we characterized a series of compounds that can overcome drug resistance caused by APH (3') and one of them achieved 30-fold potency compared to kanamycin against sensitive strains. The chemistry we describe here opens a new avenue for discovering new AGs antibiotics that overcome drug resistance and may also serve as the basis for understanding their SARs.

Keywords: photocatalysis, carbohydrates alkylation, Late-Stage modification, Aminoglycosides resistance

The discovery and application of antibiotics are undoubtedly the greatest milestone in drug development in human history and have saved millions of lives from otherwise fatal infectious diseases. However, the rapid emergence of antibiotics resistance driven by the abusive use of antibiotics both in medicine and in agriculture has greatly threatened the public health.[1] The drug-resistant bacteria, especially those gram-negative super-bugs are particularly troubling as they are insensitive to many clinically used antibiotics. Despite considerable efforts from academia and pharmaceutical industry, the development of novel antibiotics has slowed down tremendously.[2] In the face of this tremendous challenge, one of the most promising solutions is to pursue novel functionalization of the old-generation antibiotics and thereby revitalizes their antibacterial activity against these resistant strains.[3] Glucoside and aminoglycoside are common structural motifs found in many antibiotics, such as vancomycin, kanamycin, and amikacin (Figure 1A).[4-6] The two latter compounds belongs to an important class of board spectrum antibiotic known as aminoglycosides (AGs),[7, 8] and were often used to treat gram-negative bacillary infections until people noticed their distinct toxicological profiles.[9] Furthermore, the emergence of resistant strains also reduces the clinical use of AGs.[10] A major resistance mechanism from these superbugs is the expression of O-phosphotransferases (APH), enzymes that catalyze the transfer of the γ-phosphate from ATP to the antibiotics, which results in their inactivation. Specifically, APH (3') phosphorlate most of the common AGs (including kanamycins, neomycins, paromomycins, neamine, ribostamycin, geneticin, and paromamine)at the 3'-OH group (Figure 1B).[11] Tsuchiya has reported that chemical modification at the 3'-OH position of kanamycin blocked the phosphorylation by APH, allowing the modified drug to overcome the APH resistance mechanism and regained antibacterial activity.[12]

Figure 1. Proposed late-stage 3'-modification of aminoglycosides to overcome APH (3') caused bacterial resistance.

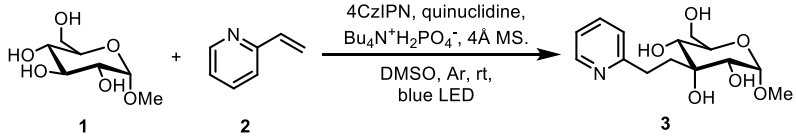


However, chemical manipulations of AGs and other carbohydrates are often challenged by the presence of numerous OH groups, which render the synthetic strategy to heavily rely on protecting groups. To improve the synthetic efficiency, protecting-group-free(or minimally protected) modification of carbohydrates has attracted significant interest.[13-23] Radical chemistry, especially photoredox-mediated hydrogen atom transfer (HAT) process, has shown to be a powerful new tool of C-C bond formation with broad functional groups tolerance.[24-26] MacMillan group has reported a pioneering example, in which they achieved selective alkylation of 6-C-H bond on a 1,2,3,4-protected pyranoside via a tetrabutylammonium dihydrogen phosphate catalyzed HAT mechanism.[27] Hydrogen bond between the hydroxyl group and the $H_2PO_4^-$ anion was thought to accelerate the HAT process. Subsequently, Minnaard[28] and Taylor[29, 30] group reported two modified protocols respectively, in which they achieved different selectivity using the same Ir catalyst and HAT reagent. Very recently, the photoredox-mediated HAT process was innovatively applied to synthesize rare sugars by Wendlandt group.[31] A mechanism involving hydrogen-atom abstraction and hydrogen-atom donation mediated by quinuclidine and adamantane thiol was established, and tetrabutylammonium p-chlorobenzoate acted as an accelerator in the HAT process. Inspired by these findings, we envisioned that this powerful strategy might be applicable to the late-stage diversification of biologically important targets. Specifically, if we can broaden the scope of alkylating reagent, *in casu* AGs, that would allow for the access to novel chemical/biological space and combat drug-resistant pathogens, or to even reduce their toxicity. In this article, we reveal a new str-ategy to modify the C-H bond of unprotected glucoside using unactivated alkenes through a photoredox-mediated HAT process. By subjecting the resulting 3-alkylated AGs to

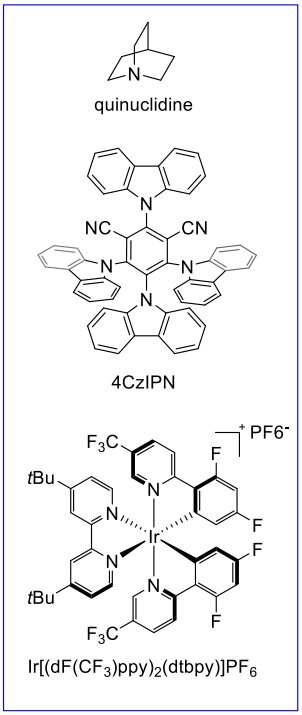
broad biological evaluations, we have identified candidate compounds regain activity against kanamycin-resistant strain.

To increase the diversity of the alkylating reagent, we commenced our study by utilizing α-methylglucoside **1** and an inactivated alkene **2** as model substrates for the optimization of the reaction conditions. As Active Pharmaceutical Ingredient (API) processes employ catalytic organic synthesis which provides cleaner processes and hence, greener chemistries, we decided to use organo-photo catalyst. After a systematic screening of all reaction parameters, we were delighted to obtain the desired glycoside **3** after irritation with blue LEDs in 92% yield using 4CzIPN (5 mol %), quinuclidine (10 mol %), tetrabutylammonium phosphate (50 mol %), and a 4 Å molecular sieves (100 mg) in DMSO (entry 1 in Table 1). In our proposal, the regioselectivity of the reaction may largely depend on the hydrogen bonds network between carbohydrate and $H_2PO_4^-$, and H_2O in the system may disturb the hydrogen bond network (entry 2 in Table 1). Otherwise, control experiments showed that the photocatalyst, quinuclidine, $Bu_4NH_2PO_4$, and blue light were essential for our reaction (entries 3-6 in Table 1). Further optimization showed that the other powerful photocatalyst $Ir[dF(CF_3)ppy)_2(dtbpy)]PF_6$ was less effective than 4CzIPN and 4-ClOBzBu_4N was less effective than $Bu_4NH_2PO_4$ (entries 7 and 8 in Table 1). Finally, increasing the concentration of quinuclidine and $Bu_4NH_2PO_4$ led to a decrease in yield. (entries 9-12 in Table 1).

Table 1. Effects of catalyst and additives.[a]



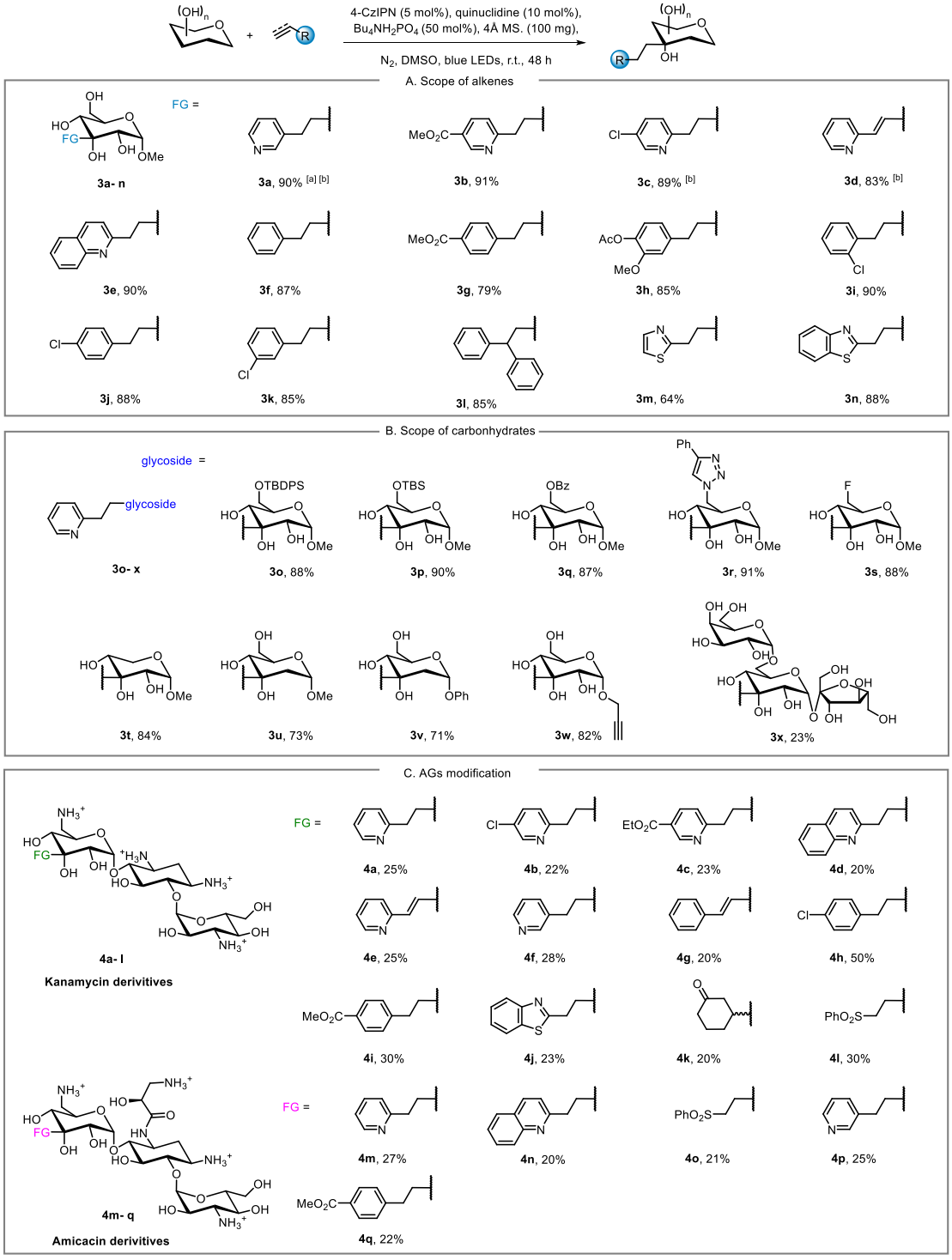
entry	variations from standard conditions	yield[b](%)
1	none	92%
2	no 4Å MS	83%
3	no 4CzIPN	0%
4	no Quinuclidine	0%
5	no $Bu_4NH_2PO_4$	0%
6	no Light	0%
7	$Ir[dF(CF_3)ppy)_2(dtbpy)]PF_6$ (1 mol %)	55%
8	4-ClOBzBu_4N (50 mol %)	55%
9	Quinuclidine (5 mol %)	48%
10	Quinuclidine (15 mol %)	42%
11	$Bu_4NH_2PO_4$ (25 mol %)	78%
12	$Bu_4NH_2PO_4$ (75 mol %)	63%



Standard conditions: **1** (0.2 mmol), **2** (2.0 equiv.), photocatalyst (5 mol %), quinuclidine (10 mol %), $Bu_4NH_2PO_4$ (50 mol %), 4Å molecular sieves (100 mg), DMSO (1.0 mL), 40 W blue LEDs (440 nm~445 nm), 48 h. [a] See figure s1 for more detailed mechanistic studies. [b]The isolated yields base on preparative HPLC is reported.

With the optimized reaction conditions in hand, we investigated the scope of alkenes for the alkylation of α-methylglucoside (Scheme 1A). Given the prevalence of the pyridine moiety in natural products, pharmaceuticals, and chiral ligands, structurally diverse vinylpyridines were prepared and applied to this protocol. As shown in Scheme 1A, various substituted pyridinyl ethyl groups could be easily installed at

C-3 position in excellent yields (**3a-c**). To our delight, the reaction even tolerated 2-ethynylpyridine perfectly, forming the corresponding vinylpyridine **3d** in 83% yield, in which the versatile olefin bond can be further derivatized. In addition, glycoside with fused ring systems (**3e**) could also be produced in 90% yield. Remarkably, good to excellent yields and selectivities were obtained when we change the pyridyl ring to the phenyl ring (**3f-3k**). Neither electronic properties (**3g** vs. **3h**) nor positions (**3i-3k**) of



Scheme 1. Scope of C-3 functionalization of glucosides and aminoglycosides. Reaction were conducted at 0.2-mmol scale; the isolated yields base on preparative HPLC is reported. [a] The sugar to alkene ratio is 2:1. [b] Isolated in peracetylated form.

the substitutes influenced the reaction in any significant manner. The bulky 1,1-diphenylethylene was also a viable substrate and gave the desired product **3l** in good yield. Furthermore, other heterocycles like thiazole (**3m**) and benzothiazole (**3n**) ethyl modification could also be achieved under our conditions.

We then evaluated the scope of saccharides and glycans that can be used in this protocol (Scheme 1B). Using 2-vinylpyridine as a model olefin, we observed that numerous 6- functional groups were successfully accommodated, including different O- protecting groups (**3o-3q**), 1,2,3-triazole (**3r**), and fluorine (**3s**). To further test the adjacent effect on the reaction, methyl α-xylopyranoside, methyl, and phenyl 2-deoxy-α-glucoside were subjected to the standard conditions, and selective modification was smoothly achieved in good yield (**3t-3v**). With great functional group tolerance in C-1, C-2, and C-6 positions of our reaction, we next tried a substrate that beared a versatile ethynyl group in C-1. The anomeric ethynyl group survived surprisingly under the conditions (**3w**), which can be used as a Click partner in further applications. More complex glycans were subsequently evaluated to assess further the selectivity and functional group compatibility of the reaction conditions. Raffinose can be transformed selectively to **3x** in 23% yield and despite the presence of 11OH groups.

The most perceived application of our chemistry is the late-stage modification of biorelevant natural products and drugs. Clinical usage of AGs antibiotics is highly restricted because of the potential ototoxicity, nephrotoxicity, and drug resistance. One of the resistant mechanisms caused by APH (3') could be overcome by modifying the 3'- position, i.e., OH removal and OH fluorination.[12] Although the mechanism has been demonstrated for decades, further 3'-modification of AGs to regain activity to pathogen expressing APH (3') is limited by the lack of appropriate chemistry. With highly selective chemistry for glucoside modification in hand, we turned our attention to produce kanamycin and amikacin derivatives. In particular, Boc protected kanamycin and amikacin were subjected to the standard conditions using various alkene reagents. Removal of Boc group delivered the desired 3'- alkylating AGs (**4a-4q**) in acceptable yields.

Table 2. MICs of kanamycin derivatives (μM). [a] [b]

compound	*E. coli (pRSF-Duet)*	*E. coli*	*P.aeruginosa*	*E. hoshinae*	*S. aureus*	*E. faecalis*	*S. Saprop*
kanamycin	>1000.00	6.25	25.00	37.50	3.13	25.00	3.13
4d	>1000.00	>25.00	>25.00	>25.00	>25.00	25.00	1.56
4f	>1000.00	12.50	25.00	25.00	6.25	12.50	0.20
4h	250.00	3.13	12.50	25.00	3.13	12.50	0.01

[a] See table s1 for more detailed antibacterial activities of AGs derivatives. [b] The bacterial strains are *E. coli.* (pRSF-Duet), *E. coli.* (ATCC 25922), *P. aeruginosa.* (ATCC 27853), *E. hoshinae*. (ATCC 700323), *S. aureus.* (ATCC29213), *E. faecalis.* (ATCC 29212), *S. Saprop.* (ATCC BAA-750). pRSF-Duet is a E. coli. strain with over expressed APH (3').

With the 3'- modified AGs (**4a-4q**) in hand, we then evaluated (Table 2, see Table s1 for detailed information). To test our hypothesis, an *E. coli.* strain with an overexpressed APH (3') gene via a pRSF-Duet vector was constructed. While the background *E. coli.* (ATCC 25922) showed a minimal inhibitory concentration (MIC) of 6.25 μM, the pRSF-Duet strain was resistant to kanamycin at >1000 μM (Table 2). Among our kanamycin derivatives, **4h** regained activity against the resistant strain with MICs of 250 μM. Besides, compound **4f** and **4h** showed a similar or even better activity against both gram negative and positive strains. Interestingly, MICs of compound **4h** against *Staphylococcus saprophyticus* are 10-30 folds more active than kanamycin. Together these MICs data clearly demonstrate that our 3'-

modification strategy of AGs can overcome drug resistance caused by APH (3') and retained activity against the sensitive strains. Modification and stereo-conversion at the 3'-position of AGs plays a vital role in combating APH (3') enzyme and expands the bioactive chemical space of AGs, which could be applied to produce novel AGs derivatives for MICs assay and SARs study. Further modification of AGs and detailed modes of action study is currently underway in our group.

In conclusion, we have unveiled an efficient strategy to install an inactivated alkene motif at the 3'-position of glucosides in a stereo/regio-selective fasion. Besides the excellent yields in mono-glucoside substrates, the chemistry also shows great selectivity on complicated raffinose and AGs, such as kanamycin and amikacin. These 3'- modified AGs were difficult to synthesis previously and never been achieved before. One of them, compound **4h** regained activity to a kanamycin-resistant *E. coli* strain and retained activity against the sensitive strains in the activity assay. The proof-of-concept experiments demonstrate that our chemistry show potential in late-stage modification of the glucoside containing natural products/drugs. This type of late stage modification strategies will likely meet the growing demands for the development of new antibiotics and further accelerate the study of their resistant mechanism, mode of action, and other potential biological applications in the near future.

Experimental procedures

Into a 10 mL reaction tube, glucoside (0.2 mmol, 1.0 equiv.), 4CzIPN (7.9 mg, 5 mol %), quinuclidine (2.2 mg, 10 mol %), $Bu_4NH_2PO_4$ (34.0 mg, 50 mol %) and 4Å Molecular sieve (vacuum-dried with heat gun, 5% m/v) were subsequently added. A PTFE magnetic stir bar and degassed DMSO (1.0 mL) were added. The reaction mixture was subjected to a three-cycle freeze-pump-thaw procedure under N2-atmosphere. Then olefin (0.4 mmol, 2.0 equiv.) was added through a glass syringe. The reaction tube was then placed 3 cm from a blue LED lamp and stirred at 300 rpm with a cooling fan. After 48 hours, the crude reaction mixture was lyophilized, and the remaining solid was dissolved in MeOH. To the reaction mixture, KPF_6 (20.2 mg, 1.1 equiv. based on $Bu_4NH_2PO_4$) was added in order to form $TBAPF_6$ to ease the removal of $Bu_4NH_2PO_4$, and then the solution was underwent a quick purification with flash column chromatography to get rid of 4Å Molecular sieve. The filtrate was then concentrated in vacuo for further purification (column chromatography, preparative HPLC or acetylation). See Supplemental Information for more detailed experimental procedures and characterization data for all products.

Conflict of interest

The authors declare that they have no conflict interest.

Acknowledgments

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Author contributions

Conceptualization, J.L. and X.W.; Methodology, T.G. and X.Y.; Investigation, T.G. and X.Y., H.C., and L.G.; Resources, L.G.; Writing – Original Draft, J.L. and X.W.; Writing – Review & Editing, T.G. and S.T.; Funding Acquisition, J.L. and X.W.; Supervision, J.L. and X.W.

Appendix A. Supplementary materials

Supplementary materials to this article can be found online at the Sci. Bull. Publications website.

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