Liposomes Can Achieve Enantioselective C–C Bond Formation of an α-Amino Acid Derivative in Aqueous Media

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Supporting Information

ABSTRACT: We first report that a highly enantioselective C–C bond formation reaction was achieved with liposomes in aqueous media. Alkylation of N-(diphenylmethylene)glycine tert-butyl ester (DMGBE) with benzyl bromide was conducted in the presence of cetyltrimethylammonium bromide micelles, resulting in a high conversion of DMGBE but little enantiomeric excess (e.e.) of the product. The same reaction was then carried out in 1,2-dioleoyl-sn-glycero-3-phosphocholine liposome suspensions, where the e.e. values were high (at least 90 % (S)), indicating that the liposome membranes can behave as the promoter of the enantioselective reaction. Changing the type of lipid to 1,2-dipalmitoyl-sn-glycero-3-phosphocholine to form a more ordered bilayer membrane lowered the reaction conversion but still maintained high e.e. %, that is, >90 (S), regardless of lipid chirality. It is indicated that multiple interactions between the DMGBE intermediate and lipid molecules promoted the migration of the intermediate into the interior of the membrane, whose bottom side (Si face) could be free for alkylation. These results suggest that liposomes can promote and regulate the alkylation of amino acid derivatives.

1. INTRODUCTION

Phase-transfer catalyst (PTC) has been developed as a powerful tool in synthetic organic reactions. The major advantages in the use of PTC are high yield and enantioselectivity; furthermore, PTC can be used to synthesize both natural and artificial amino acids (Scheme 1). As an example of enantioselective alkylation of a prochiral glycine derivative (1) (N-(diphenylmethylene)glycine tert-butyl ester (DMGBE)) and benzyl bromide (2), the designed PTCs produced both 3S and 3R. In this reaction, (i) an inorganic base (e.g., NaOH) promotes the deprotonation of 1 at the organic solvent–water interface, (ii) the ion pair formation migrates the intermediate of 1 to the organic phase, and (iii) alkylation is conducted (Scheme S1). Because of the poor solubilities of 1 and PTC, the reaction requires an organic phase (e.g., toluene, CH₂Cl₂). To develop a green sustainable chemical process, metal-free, base/acid-free, and organic-solvent-free systems are desired. However, highly selective reactions have rarely been achieved.

Self-organized nanoarchitectures (such as micelles, vesicles, nanotubes, etc.) are generated by noncovalent interactions between amphiphilic molecules in aqueous media. They have attracted much attention in various fields. It has also been reported that the interface of reverse micelles was shown to increase the enantioselectivity of catalytic hydrogenation. Although there are several reports about the asymmetric synthesis of amino acid derivatives using amphiphilic catalysts as PTC, the use of organic solvents as reaction media still needs to be studied. To construct a hydrophobic environment in water, liposomes, spherical bilayer vesicles containing highly ordered hydrophobic–hydrophilic interfaces, can be utilized (Figure 1).

Our previous report showed that the pseudo-interphase of liposomes was able to act as a platform for reactant localization. At the self-assembly interface, organic synthesis, such as the Diels–Alder reaction, can be conducted without the use of organic solvent. It is, therefore, expected that the liposome membrane interface can also be utilized as an organic-solvent-free interface for chemical reactions. In addition, the physicochemical properties of membranes, in other words, the microscopic environment formed on the liposome or micelle surfaces, can be characterized using fluorescent probes. Particularly, the lower critical aggregation concentration (CAC) of phospholipid (<10⁻⁹ M) suggests that it possesses a relatively rigid interface (less hydrophilic), as compared to that of the micelle systems (CAC: ~10⁻⁴ M) (Figure 1c). It is, therefore, expected that the self-assembly (in aqueous media) can act like a PTC without the use of any organic solvent.

In this study, asymmetric alkylation of 1 with 2 was carried out in the presence of self-assemblies (micelle and liposome). Herein, the amphiphiles, including the quaternary ammonium group, were employed as a component of self-assembly:

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phospholipid (DOPC and DPPC), 1,2-dioleoyl-3-trimethylammonium bromide (DOTAP) for liposomes, and cetyltrimethylammonium bromide (CTAB) for micelles (see Figure S1 for amphiphile’s chemical structure). Because both the reactants 1 and 2 showed quite poor solubility in water, their localization could be limited to the interior or the surface of the self-assembled membranes in aqueous media. This is the first report of a metal-free and organic-solvent-free asymmetric synthesis of α-amino acid with high enantioselectivity (Scheme 2) using the self-assembly surface as the reaction media.

2. RESULTS AND DISCUSSION

2.1. Alkylation of DMGBE with Benzyl Bromide in Aqueous Media. In the alkylation of 1 with 2 using PTC, inorganic bases (NaOH, KOH) have been used to promote the deprotonation of 1.24 In the presence of amphiphilic assemblies and NaOH, the alkylation reactions of 1 with 2 were conducted (Table 1, Figure 2). In the presence of tetrabutylammonium hydrogensulfate (TBAH in CH2Cl2−water solvent: entry I) and CTAB (micelle in water: entry III), product 3 was obtained with a higher conversion but small enantioselectivity (Table 1). The values of both conversion and enantiomeric excess (e.e.) of the reaction with TBAH in water were very low (entry II), clearly suggesting that the organic solvent−water interface is necessary for this reaction. In the presence of the CTAB micelle, the hydrophobic environment of the self-assembly can be utilized as an alternative to organic solvent, wherein the value of e.e. was very low (e.e.% < 10). In contrast, the reaction conducted with the DOPC liposome resulted in a high enantioselectivity (3S, e.e.% > 90: entry IV). Such differences in the values of conversion and e.e. could be provided by the

![Image of Scheme 1](https://example.com/scheme1.png)

**Figure 1.** Structures of liposome (a) and micelle (b). Comparison of the membrane rigidity (c): s, solid-ordered phase (1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposome); l, liquid-disordered phase (1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) liposome).

**Scheme 2.** Alkylation of 1 with 2 in the Presence of Liposomes

| entry | amphiphile (assembly) | medium               | conv. (%) | e.e. (%) (S−R) |
|-------|-----------------------|----------------------|-----------|----------------|
| I     | TBAH                  | water/CH2Cl2         | 94 ± 2    | −3 ± 3         |
| II    | TBAH                  | water                | 17 ± 6    | −6 ± 7         |
| III   | CTAB (micelle)        | water                | 95 ± 2    | 5 ± 3          |
| IV    | DOPC (liposome)       | water                | 62 ± 5    | 97 ± 1         |

Table 1. Conversion and e.e. of the Alkylation Reaction of 1 with 2 in the Presence of Self-Assemblies*

*Unless otherwise notified, all reactions were carried out in water at room temperature (r.t.) for 24 h. [1] = 1.0 mM, [2] = 12 mM, [amphiphiles] = 10 mM, and [NaOH] = 0.3 M. TBAH in water does not form any particular assembly. Independent experiments were conducted at least three times to calculate the values of conversion and e.e.
membrane structure: CTAB as the micelle and DOPC as the lipid bilayer.

2.2. Reaction Site at the Membrane Interface. To estimate the possible reaction site in the liposome, 1 and 2 were independently incorporated into self-assemblies, and then, the suspensions were mixed to initiate the reaction (Table 2, Figure 2).

Table 2. Reaction at the Membrane Interior or Intermembrane

| entry | amphiphile (assembly) | medium | conv. (%) | e.e. (%) (S−R) |
|-------|-----------------------|--------|-----------|----------------|
| V<sup>a</sup> | CTAB (micelle) | water | 92 ± 4 | 4 ± 2 |
| VI<sup>b</sup> | DOPC (liposome) | water | 56 ± 8 | 92 ± 2 |
| VII<sup>c</sup> | DOPC | water | <2 | n.d. |
| VIII<sup>d</sup> | DOPC | water | 11 ± 2 | >90 |

<sup>a</sup>1-Adsorbed CTAB micelle and 2-adsorbed CTAB micelle suspensions were mixed to initiate the reaction. <sup>b</sup>1 was added to 2-adsorbed DOPC liposome suspension to initiate the reaction. <sup>c</sup>1-Adsorbed DOPC liposome and 2-adsorbed DOPC liposome suspensions were mixed to initiate the reaction. <sup>d</sup>The reaction was conducted for 9 h at r.t. *Not detected.

For the case of DOPC liposome, a negligible amount of 3 was obtained, suggesting that both 1 and 2 are isolated by each membrane and the reaction at the membrane–membrane interface hardly occurred (entry VII). In contrast, a mixing of 1 and 2-preadsorbed DOPC liposome resulted in 3S, indicating that the hydrophobic–hydrophilic interface of the liposome can act as its reaction site (entry VI). In the case of a CTAB micelle system, a mixing of 1 and 2, which were independently preadsorbed into the CTAB micelles, successfully conducted the reaction although the e.e. value was quite low (entry V). Because of the high CAC value of CTAB, the membrane surface of its micelle could show a dynamic nature, wherein the CTAB molecules continuously associate and dissociate with each other, and the entrapped reactants can then be in contact with the micellar interface. Such a dynamic nature of the CTAB micelles leads to racemic products as well as TBAH in organic solvent–water systems. On the contrary, the liposome membrane itself showed an advantage in enantioselectivity. Table 3 shows the comparison of enantioselectivity in the alkylation of 1 with 2 in the presence of the reported PTCs.<sup>2,3,15</sup> As a tendency, the PTC possessing a rigid structure shows a high enantioselectivity (PTC-1, PTC-2, PTC-4, and PTC-5). Although self-assembled materials are classified as soft matter, we demonstrated the enantioselective alkylation of 1 with 2 using liposome membranes as soft interface. A high e.e. value was obtained at an earlier stage of the reaction in the presence of the DOPC liposome (entry VIII). Particularly, zwitterionic DOPC assemblies showed high e.e. values, indicating that the migration of the reaction intermediate (step (ii)) into the interior of the liposome was assisted by its interaction with the lipid headgroup, which restricts α-carbon of 1 attacking 2 existing in the membrane.

2.3. Evaluation of the Polar Environment Constructed on the Membrane Surface. Liposomes (DOPC, DPPC, and DOTAP) were found to have higher enantioselectivities than the CTAB micelle (Table 4). The enhancement of the reaction at the surface of the liposome membrane can be related to the microscopic environment. Particularly, the membrane polarity, evaluated by Laurdan,<sup>17,22,23</sup> could be a key parameter to understand the surroundings of 1 and 2. In brief, a spectrum of Laurdan includes multiple emission peaks depending on its surrounding, and the obtained spectrum can be deconvoluted to understand the balance of the existing hydrophobic and hydrophilic environments. As shown in Figure 3, one is from Laurdan embedded in the hydrophilic area (green lines: membrane in disordered phase<sup>23</sup>) and the other is from the hydrophobic area (blue lines: membrane in ordered phase<sup>23</sup>). DOPC contains unsaturated alkyl chains (fluid phase at r.t.<sup>23</sup>),

![Figure 2. Chemical structures of amphiphiles: 4, TBAH; 5, DOPC; and 9, CTAB (bromide salt). High-performance liquid chromatography (HPLC) for the reaction in the presence of 4 (water–CH₂Cl₂, water), 5 (liposome), and 9 (micelle). For the retention times of 1, 2, 3R, and 3S, see Figure S2 and ref 1.](image-url)

![Table 3. Yield and e.e. of the Alkylation of 1 with 2 in the Presence of PTC](table-3)

| entry | catalyst<sup>e</sup> | medium | yield (%) | e.e. (%) (S−R) |
|-------|----------------------|--------|-----------|----------------|
| IX<sup>a</sup> | TBAH | water/CH₂Cl₂ | 78 | |
| X<sup>b</sup> | PTC-1 | water/hexane | 75 | −66 |
| XI<sup>c</sup> | PTC-2 | water/hexane | 85 | 64 |
| XII<sup>d</sup> | PTC-3 | water/hexane | 34 | −21 |
| XIII<sup>e</sup> | PTC-4 | water/hexane | 73 | −79 |
| XIV<sup>f</sup> | PTC-5 | water/hexane | 98 | −99 |

<sup>a</sup>See ref 15 for detailed information. <sup>b</sup>See ref 2 for detailed information. <sup>c</sup>See ref 3 for detailed information. <sup>d</sup>See ref 1 for detailed information. <sup>e</sup>See Figure S4 for chemical structures.

S3).
showing the disordered phase as dominant (Figure 3a). On the other hand, the fluorescence spectrum of DPPC (Figure 3b), which only contains saturated alkyl chains (gel phase at r.t. 23), showed that the ordered phase was dominant. Lower GP$_{340}$ values (GP$_{340} < -0.2$, Figure 3d) indicated the presence of liquid-disordered (l_d) phase, whereas higher GP$_{340}$ values (GP$_{340} > 0.327$) indicated the presence of solid-ordered (s_o) phase. Our previous report also revealed that the hydrophobic molecules could adsorb onto the disordered-phase liposomes (e.g., DOPC liposome), indicating that the interaction between the hydrophobic reactants and the liposomes was stronger with DOPC than that with DPPC. In the presence of the DPPC liposome, the conversion value was lower than that with other liposomes (entry XV). Unadsorbed reactants (in aqueous phase) were less reactive in the bulk aqueous solution (cf., entry II). The effects of the addition of CTAB and dilauryldimethylammonium bromide (DDAB) on the membrane properties and the phase state of the liposome were also investigated. The DOPC liposome modified with CTAB and DDAB showed slight changes in the GP$_{340}$ values, whereas the liposomes modified with CTAB and DDAB resulted in similar values of conversion and e.e. (entries XVI and XVII). Importantly, the analysis of Laurdan can distinguish the vesicles and micelles. The micelles show a lower GP$_{340}$ value (GP$_{340} < -0.7$), and their less hydrophobic environment could not produce enantioselectivity. By comparing the peak area ratio of the disordered phase ($A_d$) and the ordered phase ($A_o$), a phase state of each self-assembly can also be revealed. The area ratio values ($A_o/A_d$) of DOPC liposome, DPPC liposome, and CTAB micelle were 0.16, 6.6, and 0.05, respectively. On the basis of these data, an ordered phase has $A_o/A_d > 2.5$, a disordered phase has $0.3 > A_o/A_d > 0.1$, and a micelle phase has $A_o/A_d < 0.1$. DDAB, a bilayer-forming amphiphile, also showed a higher conversion but an opposite enantioselective-

### Table 4. Conversion and e.e. Values in the Presence of Self-Assemblies in Aqueous Media

| Entry | Amphiphile (Assembly) | Medium | Conv. (%) | e.e. (%) (S−R) |
|-------|----------------------|--------|-----------|----------------|
| XV    | (L)-DPPC (liposome)  | water  | 45 ± 7    | 94 ± 1         |
| XVI   | DOPC/CTAB = 90:10 (liposome) | water  | 66 ± 5    | 96 ± 1         |
| XVII  | DOPC/DDAB = 90:10 (liposome) | water  | 54 ± 7    | 96 ± 2         |
| XVIII | DOPC liposome        | water  | 61 ± 3    | 97 ± 2         |
| XIX   | DDAB (liposome)$^b$  | water  | 90 ± 3$^b$| −49 ± 13$^b$  |

$^a$See ref 25 for detailed information. $^b$See ref 26.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Fluorescence spectra of Laurdan in each self-assembly: DOPC liposome (a), DPPC liposome (b), and CTAB micelle (c). GP$_{340}$ values of each self-assembly (d). a. DPPC liposome, b. DOPC liposome, c. DOPC/CTAB = 90:10 liposome, d. DOPC/DDAB = 90:10 liposome, e. DOTAP liposome, f. CTAB micelle.
The $GP_{340}$ value of the DDAB liposome was similar to that of the CTAB micelle, indicating that the more polar environment ($GP_{340} < -0.4$) of the liposome contributed to produce 3S as the product. These results suggest that the enantioselectivity of the reaction in the presence of self-assemblies could be controlled by the membrane polarity.

2.4. Discussion of the Enantioselectivity of the Reaction Mechanism in the Presence of Liposomes. According to Kitamura et al., a successful strategy for the design of PTC involves the following components: (1) quaternary ammonium, (2) rigid naphthyl group, and (3) a flexible alkyl chain (PTC-5). Particularly, flexible alkyl chains increase the polarity around the ammonium group, which enables the enolate to access the reaction pocket (see ref 1 and Figure S5). A series of works by Maruoka et al. have reported the PTCs that can provide (enantiomer of L-DPPC) was used to form a liposome. As a result, the $Re$ face of the intermediate of 1 can be masked with the lipid headgroup, and then, the alkylation was conducted mainly at the $Si$ face.

2.4.2. Phosphatidyl Group. Phospholipids (DOPC, DPPC) are zwitterionic molecules, whereas DOTAP and CTAB are in the form of alkylammonium salt in self-assembly. The effect of the phosphate group can be discussed using the DOTAP liposome. As shown in Table 4, the DOTAP liposome, as well as the DOPC liposome, also produced 3S with a higher e.e. value. The carbonyl group can act as a hydrogen-bond donor; thus, the speculated interactions between 1 and DOTAP molecules are electrostatic (at headgroup), hydrophobic (at membrane interior), and hydrogen-bond (at carbonyl group) interactions. It is assumed that the counterion (chloride) of the DOTAP molecule forms an ion pair with sodium cation in the reaction step (i) and this ion pair migrates the intermediate of 1 into the interior of the membrane in step (ii).

2.4.3. Carbonyl Group. DDAB, tetraalkylammonium bromide with two C12 chains, is also known to form a vesicular (liposome) structure. Interestingly, 3R was obtained as the major product in the presence of the DDAB liposome, whereas the DOPC/DDAB = 90:10 liposome showed e.e. > 96% (3S). Focusing on the polar environment of the membrane, the characteristic of the DDAB liposome was quite similar to that of the CTAB micelle; in contrast, the DOTAP liposome was similar to the DOPC liposome (Figure 3). Because of the lack of carbonyl group, the lateral association of DDAB molecules could be weaker than that of the DOTAP-type lipids; meanwhile, the membrane could be less rigid. Although details must be investigated for the reaction mechanism with the DDAB liposome, it is expected that the $Re$ face could be free after the interaction with the DDAB liposome.

As a summary, a supposed interaction mechanism of the reaction in the presence of the DOPC liposome is shown in Figure 4. In this study, although an excess amount of amphiphiles was used as compared to that of the reactants, we demonstrated that the liposome conducted the alkylation of 1 with 2 in aqueous media (without any organic solvent) and then obtained a high enantioselectivity.

### 3. CONCLUSIONS

Liposomes were shown to be potentially useful as a platform for the asymmetric synthesis of $\alpha$-amino acid derivatives. Localization of the reactants was determined to be an important factor for regulating the reaction and its chiral selectivity. In the liposome membrane, a planar hydrophobic interior faces the ionized headgroup, wherein the ion pair formation of the reactant and lipid headgroup could promote the phase transfer of the reaction intermediate into the membrane interior. In a general aspect, a chiral selectivity in an asymmetric reaction is provided by a chiral environment, which is usually originated from the chirality of catalyst. At the same time, we demonstrated that the liposomes provided a chiral selectivity of the product independent of the lipid chirality, suggesting that the self-assembled membrane structure could contribute to provide a chiral environment for asymmetric reaction in aqueous media. It is expected that the liposome membrane
systems can be utilized as a novel reaction medium for safe and clean organic synthesis processes.

4. EXPERIMENTAL SECTION

4.1. Materials. DPPC and DOPC were purchased from Avanti Polar Lipid (Alabaster, AL). CTAB, sodium hydroxide, and benzyl bromide were purchased from Wako Pure Chemicals (Osaka, Japan). DDAB, TBAH, and DMGBE were purchased from Tokyo Chemical Industries (Tokyo, Japan). All of these chemicals were used without further purification.

4.2. Preparation of Liposomes. A chloroform solution containing lipids was dried in a round-bottom flask by evaporation under vacuum. The obtained lipid thin film was dissolved in chloroform again, and the solvent was evaporated. The lipid thin film was kept under high vacuum for at least 3 h and then hydrated with distilled water at r.t. The liposome suspension was frozen at −80°C and thawed at 50°C to enhance the transformation of small vesicles into larger multilamellar vesicles (MLVs). This freeze–thaw cycle was performed five times. The MLVs were used to prepare large unilamellar vesicles (LUVs) by extruding the MLV suspension 11 times through two layers of polycarbonate membranes with a mean pore diameter of 100 nm using an extruding device (Liposofast; Avestin Inc., Ottawa, Canada).

4.3. Evaluation of Membrane Polarities. Laurdan is sensitive to the polarity around the molecule itself, and its fluorescence properties enable us to evaluate the surface polarity of lipid membranes. The Laurdan emission spectra exhibit a redshift caused by dielectric relaxation. These spectra were measured with an excitation wavelength of 340 nm, and the general polarization ($GP_{340}$), the membrane polarity, was calculated as follows

$$GP_{340} = \frac{(I_{440} - I_{490})}{(I_{440} + I_{490})}$$

where $I_{440}$ and $I_{490}$ represent the fluorescence intensities of Laurdan at 440 and 490 nm, respectively. The total concentrations of lipid and Laurdan were 100 and 1 μM, respectively. The fluorescence spectrum of Laurdan was deconvoluted into two spectra using the software Peakfit (Systat Software Inc., San Jose, CA): one originates from the localization of Laurdan in an ordered membrane (ordered phase) and the other originates from the localization of Laurdan in a disordered membrane (disordered phase). By calculating the area below the spectrum originating from the ordered phase ($A_o$) and the area below the spectrum originating from the disordered phase ($A_d$), the area ratio of ordered phase to disordered phase in the actual vesicle sample ($A_o/A_d$) was determined.

4.4. Alkylation of DMGBE with Benzyl Bromide. DMGBE (0.3 mg, 1.0 μmol) was dissolved in pure water, and then, the water solution was mixed with liposome suspension and NaOH aqueous solution (10%) to obtain 1 mL of reaction solution. Benzyl bromide (1.5 μL, 12 μmol) was finally added to initiate the reaction (Scheme 1). The reaction solution was stirred at 500 rpm in r.t. for 24 h. The total concentrations of DMGBE, benzyl bromide, lipid, and NaOH were 1.0, 12, and 10 mM and 0.3 M, respectively. In the case of surfactants (CTAB, TBAH, etc.), an aqueous solution of the surfactant was added instead of the liposome suspension. The total concentrations were fixed to the same values as those of the condition of the reaction with liposomes.

4.5. HPLC Measurements of Reaction Solution. After the reaction was complete (24 h after the initiation of the reaction), the product (and reactant) was extracted to the organic solvent using the Bligh–Dyer method. In brief, 1 mL of the reaction solution was mixed with 2 mL of methanol and 1 mL of chloroform, resulting in a homogeneous, colorless, and transparent liquid. Then, 1 mL of chloroform and 1 mL of pure water were added to the solution to lead to phase separation. Centrifugation was performed (1400 rpm, 5 min) using a Tabletop Centrifuge KUBOTA S200 (Kubota, Tokyo, Japan) to complete the phase separation process.

After the extraction, the organic phase was moved to a round-bottom flask and chloroform was removed by evaporation. Diethyl ether (1 mL) was added to the flask, and 10 μL of it was taken and dissolved in 1 mL of mobile phase for HPLC analysis (Daicel Chiralpak IA, mobile phase was hexane/2-propanol = 99:1, flow rate = 0.5 mL/min). The HPLC analysis was done using Waters 1515 Isocratic HPLC Pump and Waters 2489 UV/visible Detector (Waters, Milford, MA).
to evaluate the conversion of the reaction and the e.e. The reproducibility of the reaction was good (at least $n \geq 3$ for each reaction system); the errors in the values of conversion and e.e. were less than 10% for all reactions carried out in this study. The trial number ($n$) of each reaction system is as follows: for I, II, III, and IV, $n \geq 5$; for XV, XVI, XVII, XVIII, XIX, XX, and XXI, $n \geq 4$; otherwise, $n \geq 3$.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.6b00479.

Reaction mechanism with PTC; chemical structures of amphiphiles and PTCS; HPLC chromatogram of 1, 2, V, VI, and VII; alkylation of 1 with 2 at the self-assembly interface in aqueous media; and space-filling model of a plausible transition state of 1 and PTC-6 (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. All authors contributed equally.

**Notes**

The authors declare no competing financial interest.

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