Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

Lipid-membrane-incorporated arylboronate esters as agents for boron neutron capture therapy

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Experimental Section

Materials

Pinacol, phenylboronic acid (**4**), 2-naphthaleneboronic acid and 2,6-dimethylphenylboronic acid (**5**) were purchased from Tokyo Chemical Industries Co., Ltd (Tokyo, Japan). 4,4,5,5-Tetramethyl-2-phenyl-1,3,2-dioxaborolane (**1**) was purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan). 4,4,5,5-Tetramethyl-2-(naphthalen-2-yl)-1,3,2-dioxaborolane (**2**) and 2-(2,6-dimethylphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**3**) were synthesised as described previously.[S1,S2] 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were obtained from NOF Corp. (Tokyo, Japan) and Avanti Polar Lipids, Inc. (Alabaster, AL, USA), respectively.

Preparation of lipid membrane-incorporated guest molecules (LMIGs)

Solutions of DMPC or DPPC (4.00×10^{-6} mol) and compounds **1**–**3** in chloroform (0.1 mL) were dried under a steady stream of nitrogen at ambient temperature. The compositions of the mixtures were as follows: [**1** or **3**]/[DMPC] = 0–300 mol%, [**2**]/[DMPC] = 0–100 mol% and [**3**]/[DPPC] = 0–200 mol%. The mixtures were treated with D_2O (1.0 mL) and agitated on a vortex mixer for 1 min. To resulting multilamellar vesicles were subjected to eight freeze-thaw cycles and extruded eleven times (LiposoFast-Basic; Avestin Inc., Ottawa, Canada) through two stacked polycarbonate membranes (pore size 50 nm) to afford unilamellar vesicles. The final lipid concentration was 4.0 mM. DMSO (0.4 mM) was added to the samples for ^{1}H NMR spectroscopy as an internal standard so that the concentrations could be determined from the peak intensities.

UV-vis Absorption Spectra

UV-vis spectra were recorded using a UV-3600PC spectrophotometer (Shimadzu Corp., Kyoto, Japan). All of the experiments were performed at 25 °C using a 1 mm cell in D_2O. We prepared D_2O solutions of DMPC or DPPC liposomes in the absence of the guest molecules, with DMPC or DPPC concentrations that were the same as those of the LMIGs. By subtracting the spectrum of the DMPC or DPPC liposome solution from that of the LMIG solution, we obtained the absorption spectra of all of the LMIGs by subtracting the light scattering signals of the DMPC or DPPC liposomes.

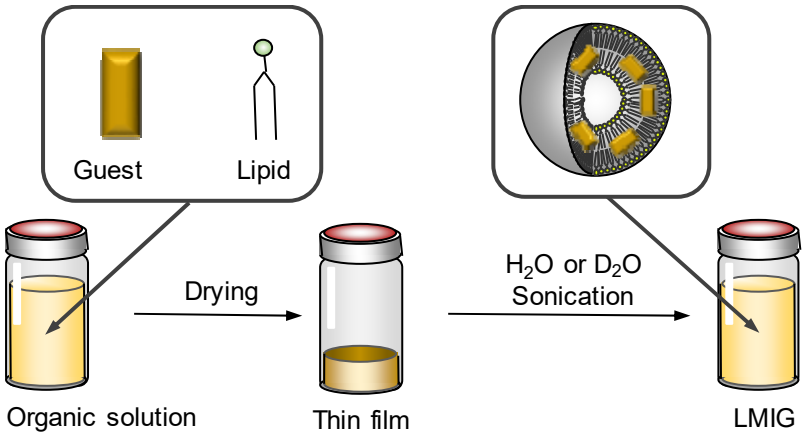
^{1}H NMR spectroscopy

^{1}H NMR data were recorded on a Varian 400-MR (400 MHz) spectrometer (Varian Associates, Inc.; Palo Alto, CA, USA). All of the experiments were performed at 25 °C in D_2O containing DMSO (0.4 mM), which was adopted as an internal standard.

References

S1 S. Morandi, E. Caselli, A. Forni, M. Bucciarelli, G. Torre and F. Prati, *Tetrahedron: Asymmetry*, 2005, **16**, 2918–2926.

S2 Z. Wang, J. Sun and X. Jia, *J. Polym. Sci., Part A: Polym. Chem.*, 2014, **52**, 1962–1969.



Scheme S1. Premixing method used for the preparation of the LMIGs.

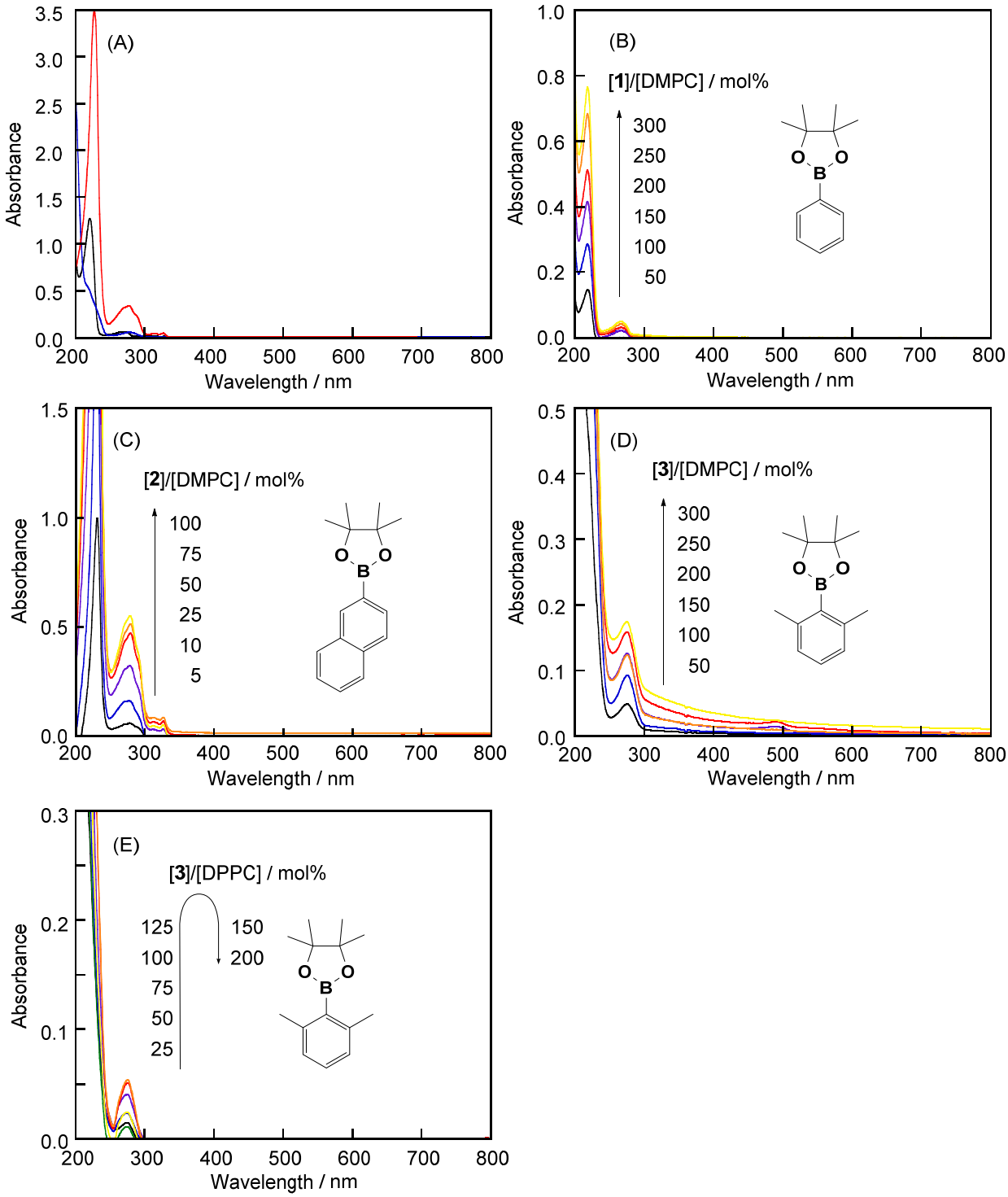


Fig. S1 UV-vis absorption spectra of (A) **1** (black line), **2** (red line) and **3** (blue line) in hexane ([**1**–**3**] = 0.1 mM), (B) LMI**1** {[**1**]/[DMPC] = 50 (black line), 100 (blue line), 150 (purple line), 200 (red line), 250 (orange line) and 300 (yellow line) mol%, [DMPC] = 0.25 mM}, (C) LMI**2** {[**2**]/[DMPC] = 5 (black line), 10 (blue line), 25 (purple line), 50 (red line), 75 (orange line) and 100 (yellow line) mol%, [DMPC] = 1.0 mM}, (D) LMI**3** consisting of DMPC {[**3**]/[DMPC] = 50 (black line), 100 (blue line), 150 (purple line), 200 (red line), 250 (orange line) and 300 (yellow line) mol%, [DMPC] = 0.25 mM} and (E) LMI**3** consisting of DPPC {[**3**]/[DPPC] = 25 (black line), 50 (blue line), 75 (purple line), 100 (red line), 125 (orange line), 150 (yellow line) and 200 (green line) mol%, [DPPC] = 0.25 mM}. The absorption spectra (B–E) were obtained by subtracting the light scattering from the DMPC liposomes and were measured at 25 °C (1 mm cell).

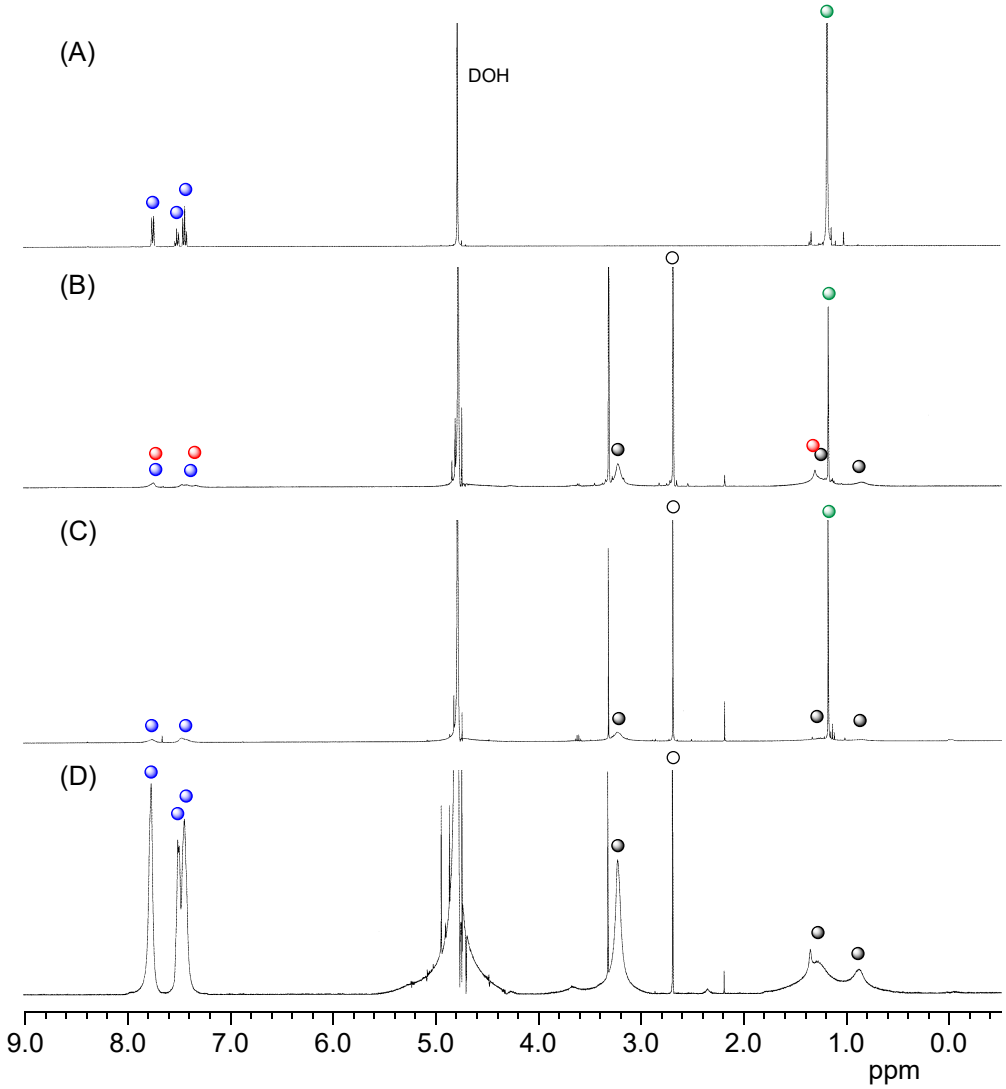


Fig. S2 Complete ^{1}H NMR spectra (400 MHz, D_2O, 25 °C) of (A) a mixture of **4** and pinacol ([**4**] = [pinacol] = 13.0 mM), LMI**1** (B) immediately and (C) one week after its preparation ([**1**]/[DMPC] = 100.0 mol%, [DMSO] = 0.4 mM), and (D) a mixture of liposome and **4** [**4**]/[DMPC] = 300 mol%. ([DMPC] = 2.0 mM, [DMSO] = 0.4 mM). (●: **1**, ●: **4**, ●: pinacol, ●: DMPC lipid in the small aggregates and ○: DMSO).

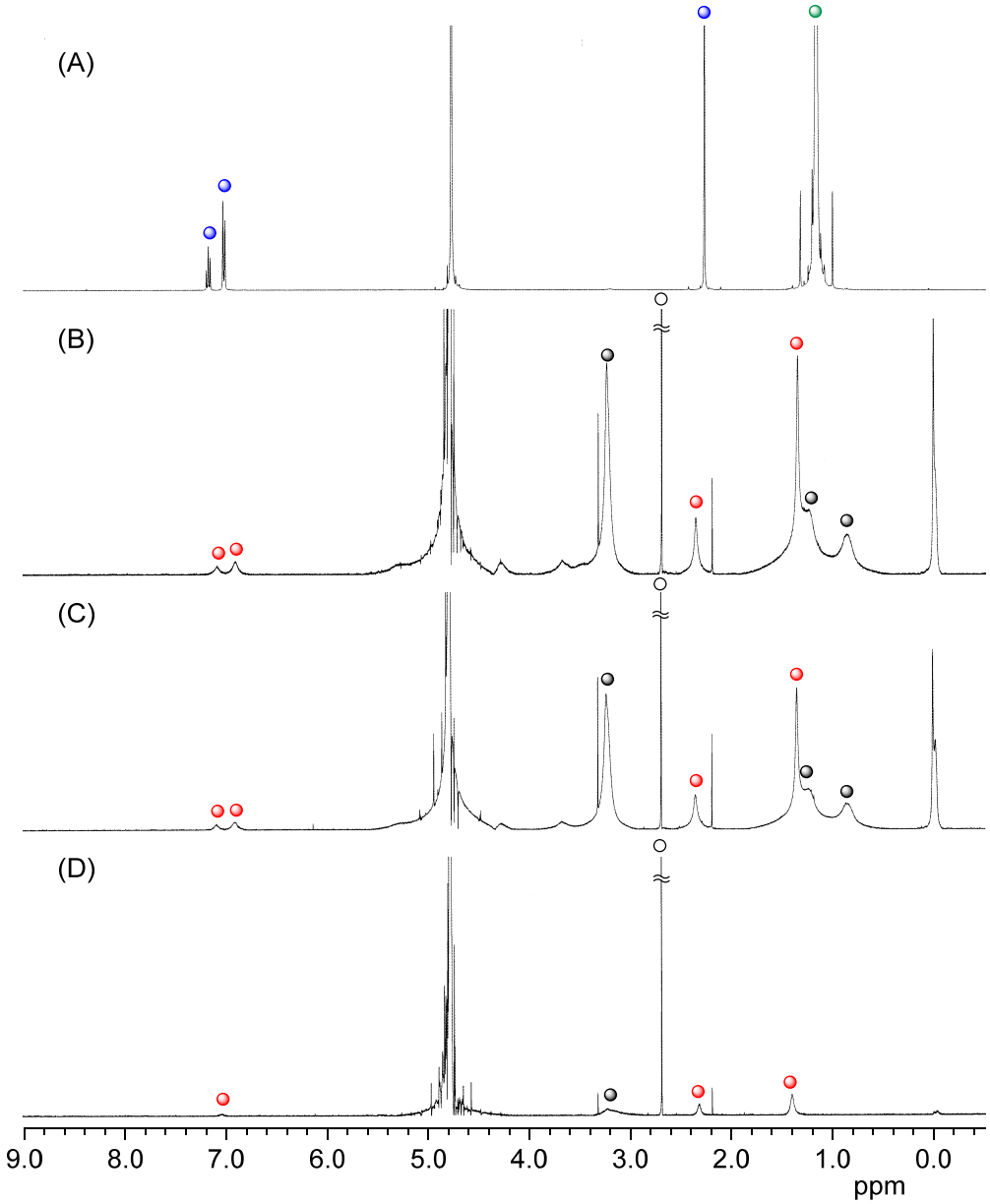


Fig. S3 Complete ^{1}H NMR spectra (400 MHz, D_2O, 25 °C) of (A) a mixture of **5** and pinacol ([**5**] = [pinacol] = 11.0 mM), LMI**3** consisting of DMPC (B) immediately and (C) 1 week after its preparation ([**3**]/[DMPC] = 100 mol%, [DMPC] = 2.0 mM and [DMSO] = 0.4 mM), and (D) LMI**3** consisting of DPPC ([**3**]/[DPPC] = 100 mol%, [DPPC] = 2.0 mM and [DMSO] = 0.4 mM) (●: **3**, ●: **5**, ●: pinacol, ●: DMPC or DPPC lipid in the small aggregates and ○: DMSO).

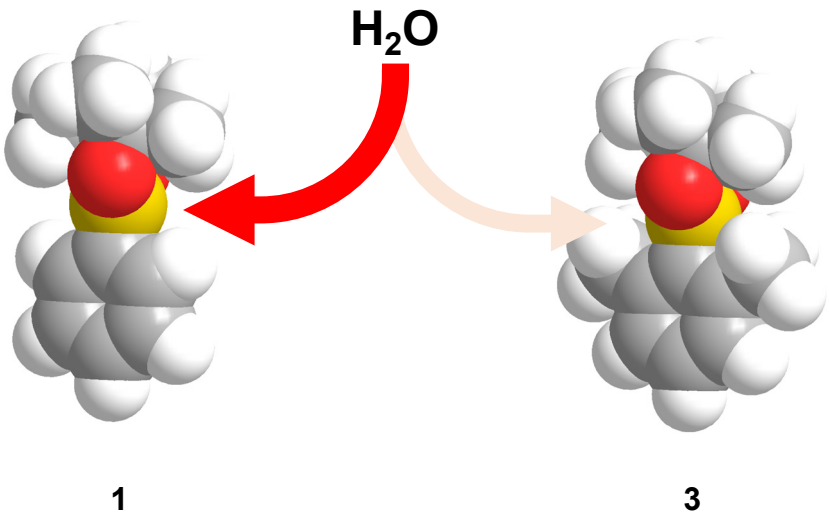


Fig. S4 Steric crowding around the boronate ester of **1** and **3.**