Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

pH-dependent ion permeability control of a modified amphotericin B channel through metal complexation

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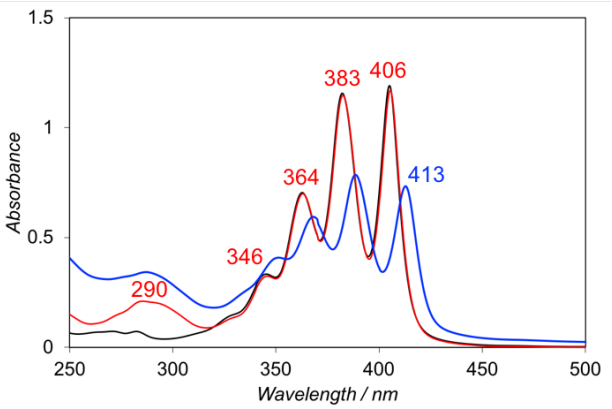

Fig. S1 UV-vis spectra of bpy-AmB (red) and AmB (black) in MeOH ([bpy-AmB or AmB] = 10 μM), and bpy-AmB in POPC liposome at pH 9.0 (blue).

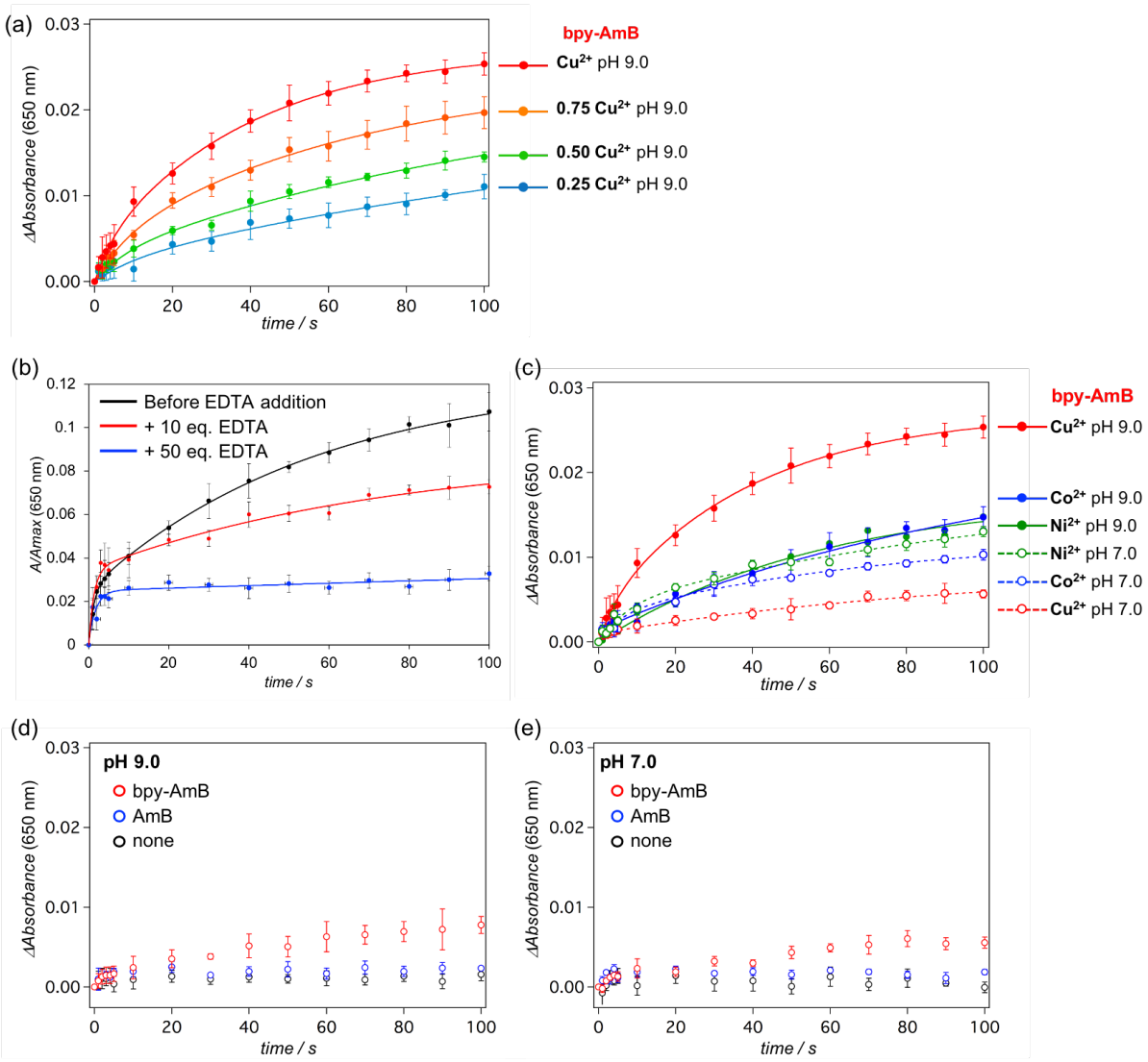

Fig. S2 Ca^{2+}-influx assays of (a) bpy-AmB depending on the equivalence ratio of bpy-AmB to Cu^{2+} ion, (b) bpy-AmB in the presence of Cu^{2+} ion at pH 9.0 after the addition of EDTA, (c) bpy-AmB in the presence of Cu^{2+}, Co^{2+} or Ni^{2+} ion at pH 9.0 and 7.0, and (d) bpy-AmB and AmB in the absence of metal ion at pH 9.0, and (e) pH 7.0.

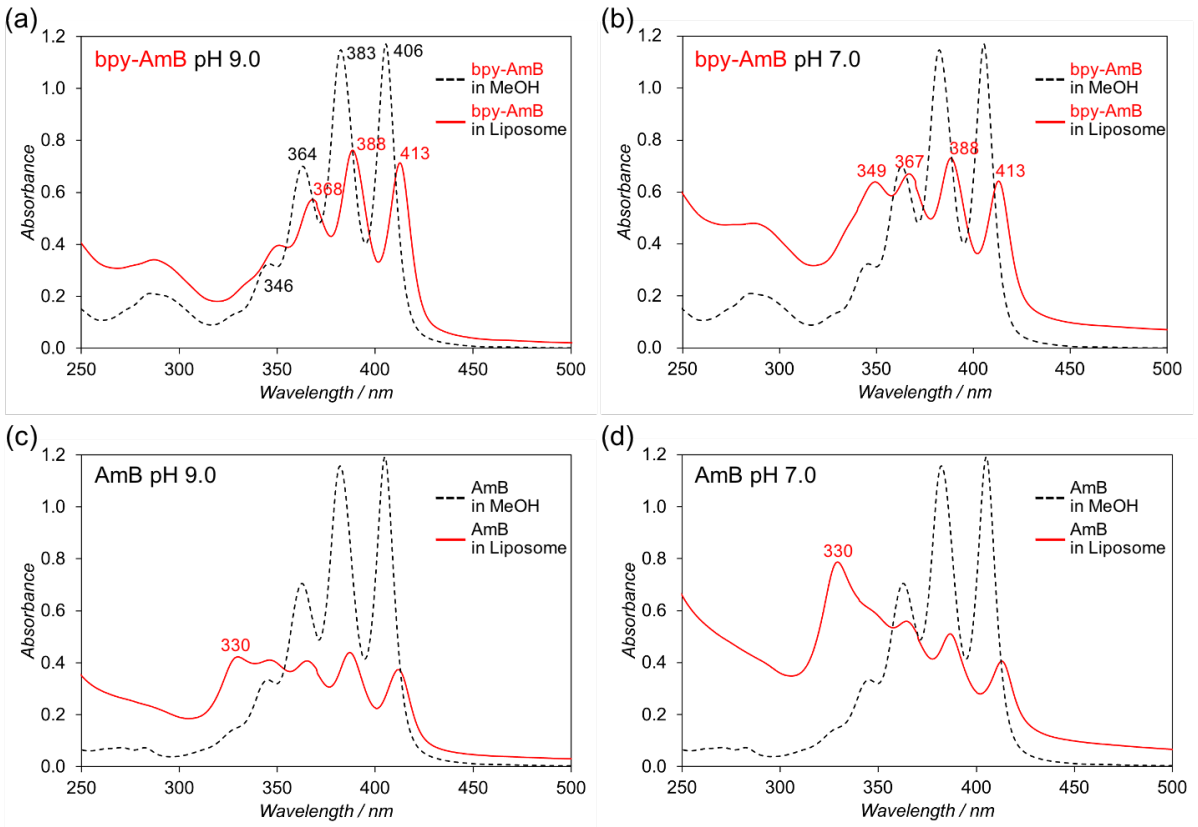

Fig. S3 UV-vis spectra of (a) bpy-AmB at pH 9.0, (b) bpy-AmB at pH 7.0, (c) AmB at pH 9.0, and (d) AmB at pH 7.0 in POPC liposome suspension in the absence of metal ions.

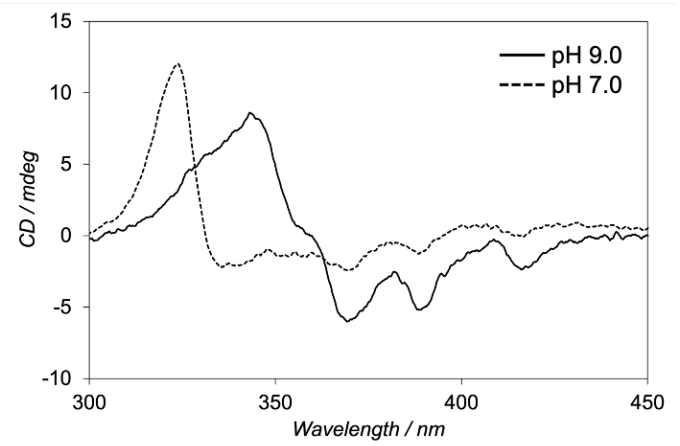

Fig. S4 CD spectra of AmB in POPC liposome at pH 9.0 and pH 7.0 (final concentration of the mixture: [bpy-AmB] = 10 μM and [phospholipid] = 1 mM).

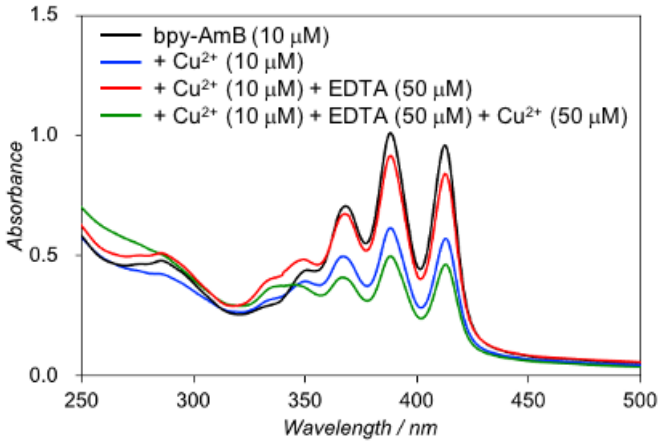

Fig. S5 Reversible changes in UV-vis spectra of bpy-AmB in POPC liposome suspension (pH 9.0) after subsequent addition of Cu^{2+} and EDTA.

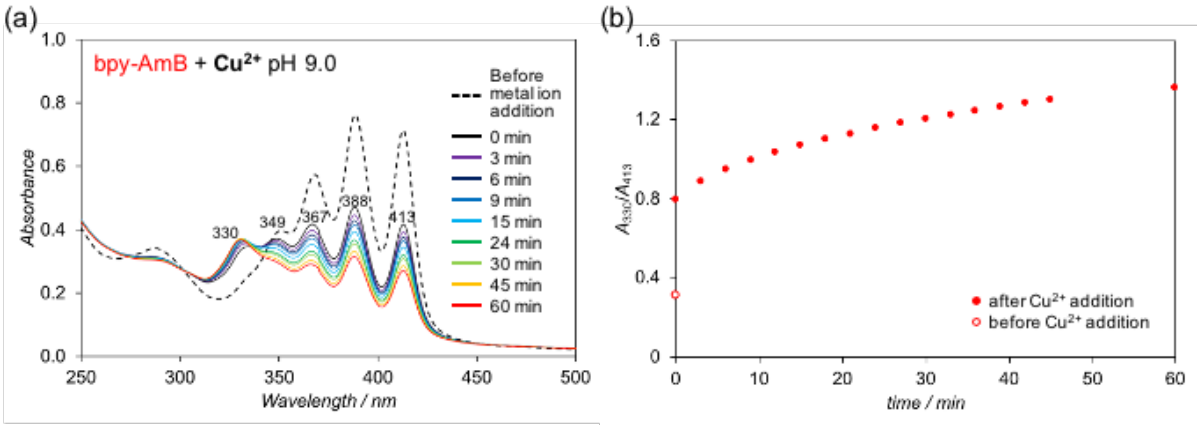

Fig. S6 (a) Time course UV-vis spectra of bpy-AmB after the addition of Cu^{2+} ion at pH 9.0 (final concentration of the mixture: [bpy-AmB] = 10 μM, [Cu^{2+}] = 10 μM, [phospholipid] = 1 mM), and (b) the absorbance ration at 330 nm (aggregated form) and 413 nm (monomeric form) calculated based on Fig. S5 (a).

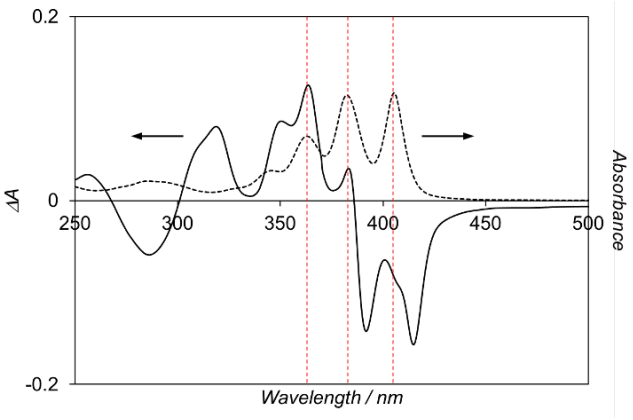

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Fig. S7 The UV-vis spectrum of bpy-AmB in MeOH (monomeric form) (broken line), and the difference spectrum obtained by the subtraction of spectra presented in Fig. 3b (before and after the addition of Cu^{2+} ion) (solid line).

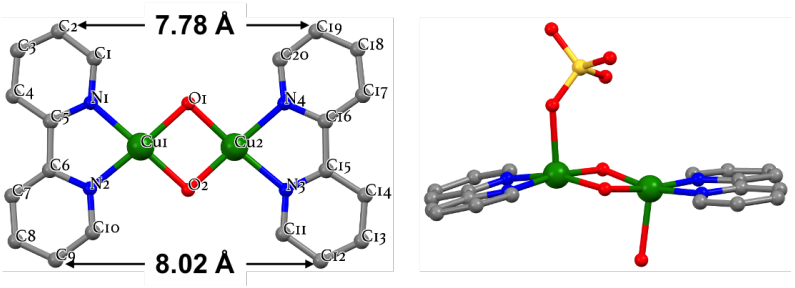


Fig. S8 The crystal structure of $[Cu_2(bpy)_2(H_2O)(OH)_2(SO_4)]\bullet 4H_2O$.[1]

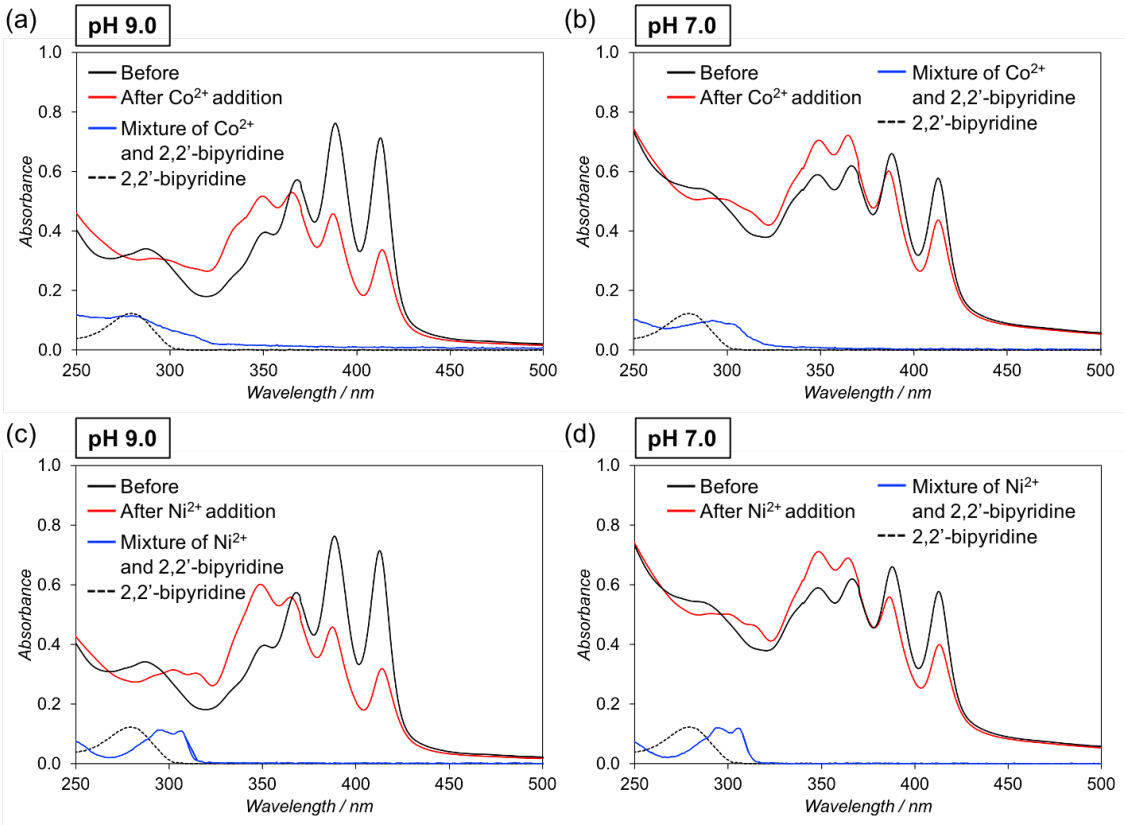


Fig. S9 UV-vis spectra of bpy-AmB in POPC liposome before and after the addition of Co^{2+} ion at (a) pH 9.0 and (b) pH 7.0, and after the addition of Ni^{2+} ion (c) at pH 9.0 and (d) at pH 7.0. UV-vis spectra of the mixture of Co^{2+} or Ni^{2+} ion and 2,2'-bypyridine are also shown ([M^{2+}] = [2,2'-bypyridine] = 10 μM).

Table S1 UV-bands for bpy complexes

Complex	λ / nm (ε / $M^{-1}cm^{-1}$)	Solvent	Reference
bipyridine	233 (10,200), 280 (13,300)	aqueous solution	2
$[Co(bpy)]^{2+}$	245 (10,500), 295 (16,000), 304 (16,000)	aqueous solution	2
$[Co(bpy)_2]^{2+}$	293	–	3
$[Co(bpy)_3]^{2+}$	292, 301	MeOH	
$[Ni(bpy)]^{2+}$	244 (11,200), 294.5 (16,700), 305.5 (18,700)	aqueous solution	2
$[Ni(bpy)_3]^{2+}$	245 (32,000), 296 (42,000), 318 (40,000)	aqueous solution	2

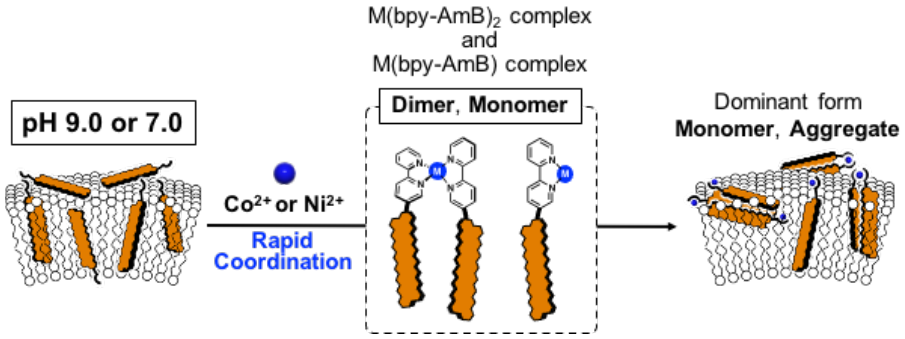
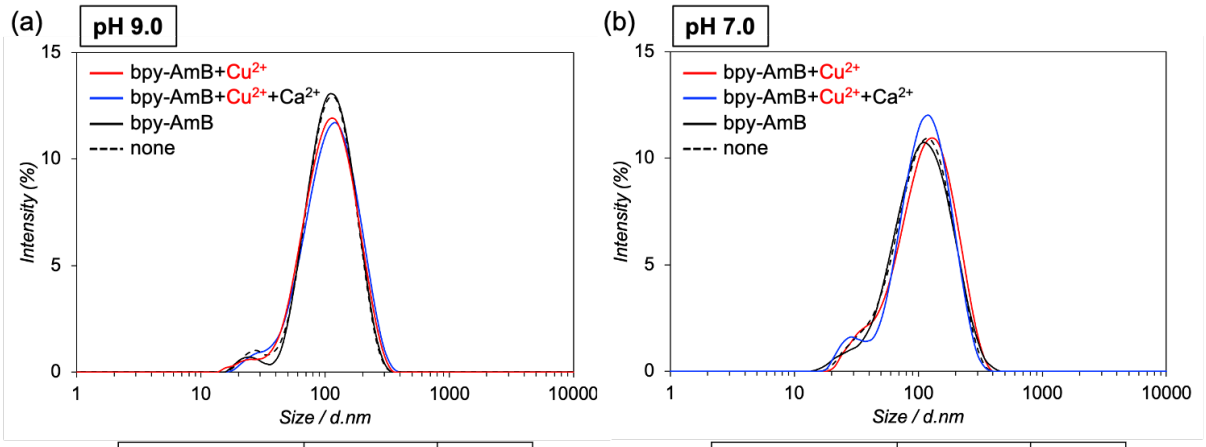


Fig. S10 Schematic representation of the changes in the molecular association state of bpy-AmB triggered by Co^{2+} or Ni^{2+}coordination.



Sample (pH 9.0)	$Z_{AVE.}$ Diameter (nm)	PdI
bpy-AmB+Cu^{2+}	94.6	0.20
bpy-AmB+Cu^{2+}+Ca^{2+}	96.5	0.20
bpy-AmB	95.9	0.19
none	94.3	0.19

Sample (pH 7.0)	$Z_{AVE.}$ Diameter (nm)	PdI
bpy-AmB+Cu^{2+}	96.6	0.23
bpy-AmB+Cu^{2+}+Ca^{2+}	93.1	0.22
bpy-AmB	92.5	0.23
none	90.5	0.22

Fig. S11 DLS measurements of liposomes containing bpy-AmB before and after the addition of Cu^{2+} and Ca^{2+} at (a) pH 9.0 and (b) pH 7.0 (final concentration of the mixture: [bpy-AmB] = 10 μM, [Cu^{2+}] = 10 μM, [Ca^{2+}] = 0.5 mM, [phospholipid] = 1 mM).

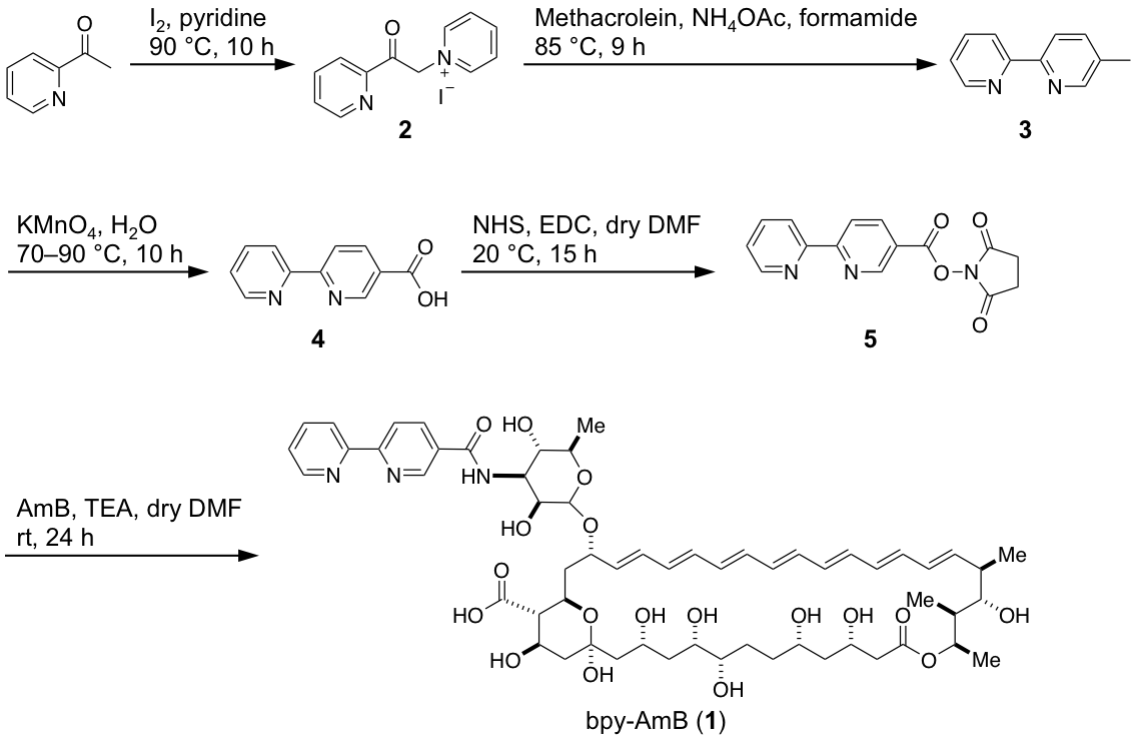
Materials

Phospholipids were obtained from Avanti Polar Lipids. Reagents were purchased from Wako, TCI and Sigma-Aldrich and used without further purification. $[(bpy)Cu(\mu\text{-}OH)]_2(SO_4)$[4,5] and $[Cu(bpy)]SO_4 \bullet 2H_2O$[6,7] were synthesized according to the literature methods.

Physical Measurements

UV-vis absorption spectra were measured with a JASCO V-630. CD spectra were recorde with a JASCO J-820. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM-ECA600 or JEOL JNM-ECS400 spectrometer. Electrospray ionization-mass spectrometry was recorded on a Bruker Daltonics micrOTOF. Dynamic light scattering was performed using a Zetasizer Nano ZS (Malvern).

Synthesis of bpy-AmB (1)



Scheme S1 Synthesis of bpy-AmB (**1**).

1-(2-pyridylacetyl)pyridinium iodide (2): Compound **2** was synthesized by the literature method.[8] Yield: 13.1 g (72%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 6.51 (2H, s), 7.82-7.85 (1H, m), 8.08 (1H, d, J = 7.7 Hz), 8.14 (1H, td, J = 7.5, 1.7 Hz), 8.27 (2H, t, J = 6.2 Hz), 8.73 (1H, t, J = 7.8 Hz), 8.87 (1H, d, J = 3.7 Hz), 9.00 (2H, d, J = 5.5 Hz).

5-metyl-2, 2'-bipyridine (3): Compound **3** was synthesized by the literature method.[8] Yield: 1.99 g (98%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.41 (3H, s), 7.30-7.32 (1H, m), 7.67 (1H, dd, J = 8.1, 2.0 Hz), 7.84 (1H, td, J = 8.1, 1.7 Hz), 8.32 (1H, d, J = 8.1 Hz), 8.41 (1H, d, J = 8.4 Hz), 8.53 (1H, s), 8.69 (1H, d, J = 4.7Hz).

2,2'-bipyridinyl-5-carboxylic acid (4): Compound **4** was synthesized by the literature method.[8] Yield: 1.69 g (76%). ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.49-7.50 (1H, m), 8.00 (1H, d, J = 7.7 Hz), 8.34 (1H, td, J = 7.5, 1.7 Hz), 8.38-8.49 (3H, m), 8.71 (1H, d, J = 4.3 Hz), 9.13 (1H, d, J = 1.2 Hz).

***N*-hydroxysuccinimide 2,2'-bipyridine-5-carboxylate (5):** Compound **4** (141 mg, 0.7 mmol), NHS (81.3 mg, 0.71 mmol), and EDC (138.6 mg, 0.72 mmol) were dissolved in 1.5 mL of DMF under nitrogen atmosphere and the mixture was stirred at 20 °C for 15h. The DMF was evaporated under reduced pressure and the product was purified by column chromatography (silica, gradient from 100% $CHCl_3$ to EtOAc/$CHCl_3$ 1:1 v/v) to obtain **5** (144.4 mg, 71%) as a white powder. Yield: 144.4 mg (71%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.96 (4H, s), 7.41 (1H, td), 7.89 (1H, t), 8.53-8.50 (2H, m), 8.61 (1H, d), 8.74 (1H, dd), 9.37 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.1, 161.0, 160.9, 154.4, 151.1, 149.5, 138.8, 137.3, 125.1, 122.4, 121.1, 120.8, 25.7. Elemental analysis (%) calcd for $C_{15}H_{11}N_3O_4 \cdot 0.5H_2O$: C 58.82, H 3.95, N 13.72; found: C 58.72, H 3.61, N 13.55.

bpy-AmB (1): Compound **5** (34.6 mg, 0.12 mmol) and AmB (56.7 mg, 0.06 mmol) were dissolved in 3 mL of dry DMF under nitrogen atmosphere. Triethylamine was added to the solution until pH of 8 was obtained. The orange reaction mixture was stirred at room

temperature for 24 h. The DMF and trimethylamine were evaporated under reduced pressure and the product was purified by column chromatography (silica, chroloform/methanol/ 28%NH_{3aq} 10:6:1 v/v) to obtain **1** as a yellow powder. Yield: 28.4 mg (41.8%). ESI-MS calcd. for $C_{58}H_{79}N_3O_{18}[M+Na]^+$ 1128.53, found: 1128.54; calcd for $C_{58}H_{79}N_3O_{18}[M+2Na–H]^+$ 1150.51, found: 1150.50. Elemental analysis (%) calcd for $C_{58}H_{79}N_3O_{18}\cdot 2H_2O\cdot NH_3$: C 59.38, H 7.20, N 4.75; found: C 60.09, H 7.48, N 4.83.

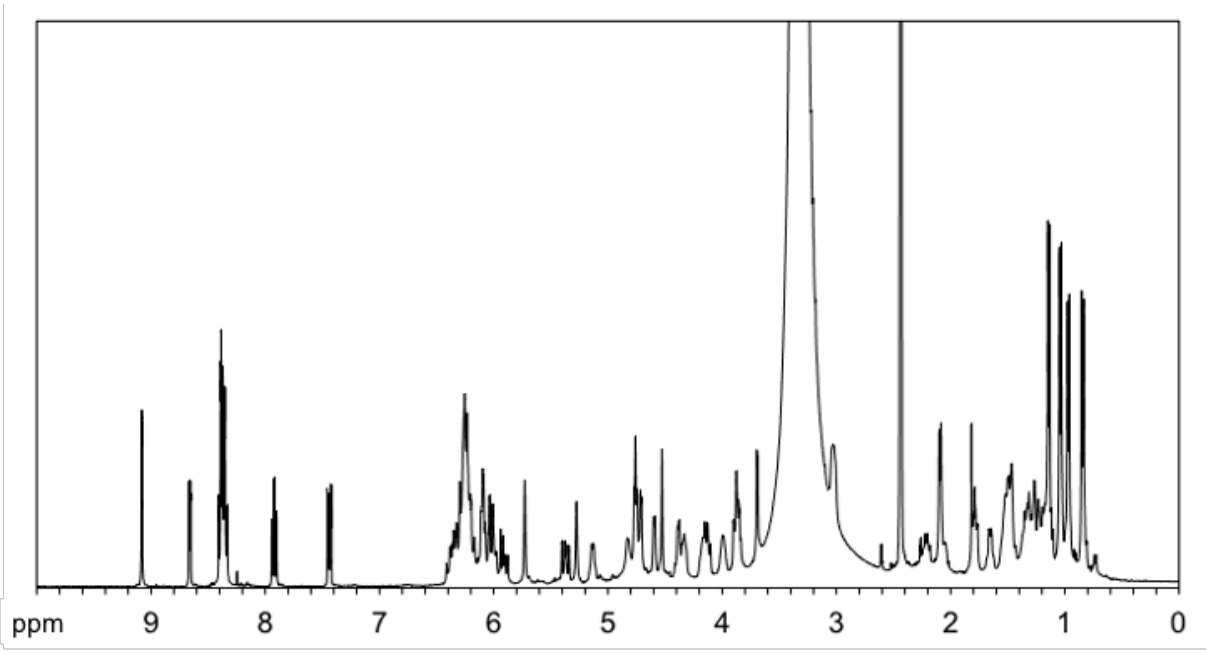


Fig. S12 ^{1}H NMR spectrum of bpy-AmB (400 MHz, DMSO-d_6)

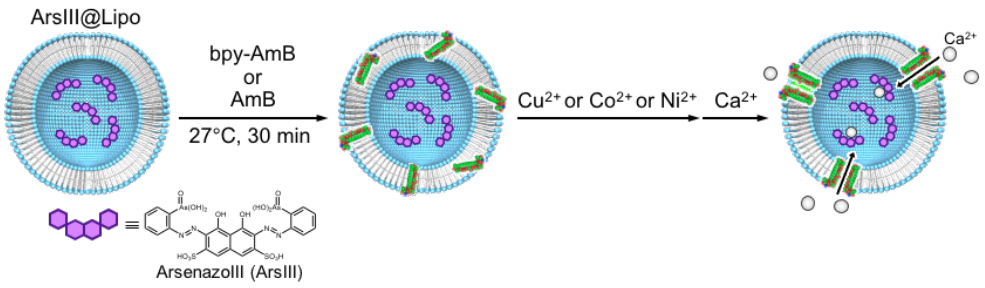
Preparation of ArsenazoIII entrapped Liposome (ArsIII@Lipo)

A chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (12 mM, 2 mL) was evaporated in a round-bottom flask, and the resulting lipid film was dried under vacuum for 6 h. The lipid film was hydrated with 2 mL of 2 mM ArsenazoIII in 20 mM Tris/HCl buffer (pH 9 or 7) at 30 °C for 15 h. The lipid suspension was sonicated at 30 °C for 30 min, and subjected to five freeze/thaw cycles and then extruded 21 times through a 100 nm polycarbonate membrane at 30 °C. The unentrapped ArsenazoIII was removed by size exclusion chromatography on a S-400 gel column equilibrated with 20 mM Tris/HCl buffer

(pH 9 or 7). The phospholipid concentration of ArsIII@Lipo was determined by the molybdenum blue method.

Ca^{2+} permeability assay

ArsIII@Lipo was diluted with 20 mM Tris/HCl buffer (pH 9 or 7) to adjust the final phospholipid concentration of 1 mM of phospholipids. The concentration of ArsenazoIII in ArsIII@Lipo (1 mM phospholipid) was 12.4 ± 0.5 μM. A DMSO solution of bpy-AmB or AmB (2 mM, 3 μL) was added to 600 μL of ArsIII@Lipo ([bpy-AmB] = [AmB] = 10 μM). The mixture was left at 27 °C for 30 min. Immediately after adding a solution of $CuCl_2$, $CoCl_2 \cdot 6H_2O$, or $NiCl_2 \cdot 6H_2O$ (2 mM, 3 μL), and a solution of $CaCl_2$ (100 mM, 3 μL) in 20 mM Tris/HCl buffer (pH 9 or 7) to the mixture ([metal ion] = 10 μM, [Ca^{2+}] = 0.5 mM), time-dependent change in the absorption at 650 nm derived from the formation of the Ca^{2+}-ArsIII complex was monitored at 27 °C for 100 seconds.



Time-course UV-vis spectra

A chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) (12 mM, 1 mL) was evaporated in a round-bottom flask, and the resulting lipid film was dried under vacuum for 6 h. The lipid film was hydrated with 2 mL of 20 mM Tris/HCl buffer (pH 9 or 7) at 30 °C. The lipid suspension was sonicated at 30 °C for 30 min. The suspension was extruded 21 times through a 100 nm polycarbonate membrane at 30 °C. The phospholipid

concentration of liposome solution was determined by the molybdenum blue method. Liposome solution was diluted with 20 mM Tris/HCl buffer (pH 9 or 7) to adjust the final phospholipid concentration of 1 mM. A DMSO solution of AmB or bpy-AmB (2 mM, 1 μL) was added to 200 μL of liposome solution ([bpy-AmB] = [AmB] = 10 μM). The mixture was left at 27 °C for 30 min. Immediately after adding a solution of $CuCl_2$, $CoCl_2 \cdot 6H_2O$, or $NiCl_2 \cdot 6H_2O$ (2 mM, 1 μL) in 20 mM Tris/HCl buffer (pH 9 or 7) to the mixture ([metal ion] = 10 μM), absorbance spectra were recorded at 27 °C. DLS measurements of liposomes containing bpy-AmB before and after the addition of Cu^{2+} and Ca^{2+} at pH 9.0 and pH 7.0 indicate that the addition of Cu^{2+} and Ca^{2+} do not induce liposomes deformation (Fig. S11).

References

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