Supporting Information

Structure of Bimolecular Complex between Amphotericin B and Ergosterol in Membrane is Stabilized by Face-to-Face Van der Waals Interaction with their Rigid Cyclic Cores

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1. GENERAL METHODS

AmB and cholesterol were purchased from Nacalai Tesque (Kyoto, Japan). Ergosterol was from Tokyo Kasei (Tokyo, Japan) and palmitoylleoylphosphatidylcholine (POPC) was from Avanti Polar Lipid Inc. (Alabaster, AL) or NOF corp. (Tokyo, Japan). All other chemicals were obtained from standard venders.

All reaction was carried out under an atmosphere of argon. For polyene compounds, all reactions were carried out under low light condition because of its light sensitivity. Thin-layer chromatography (TLC) was performed on a glass plate precoated with silicagel (Merck Kieselgel 60 F254). Column chromatography was performed with silica gel 60N (KANTO chemical co., inc., particle size 0.100-0.210 mm) or silica gel 60 (Merck, particle size 0.063-0.200 mm, 60-230 mesh). Solution NMR spectra were recorded on VNS600 (Agilent), ECA-500 and ECS-400 spectrometer (JEOL). MS was measured on a LTQ-Orbitrap XL (Thermo Quest).

2. Dilution experiments for choosing a proper spin system for interpretation of $^{13}C\{^{19}F\}$ REDOR results

It is important to choose a proper spin system upon interpreting REDOR results. Here, we consider two kinds of systems; a ^{13}C-^{19}F two spin system and ^{19}F-^{13}C-^{19}F three spin system. As we reported previously (ref. 24a), AmB-AmB orientation under the present REDOR conditions was considered to be mostly parallel since intermolecular dipole interaction was observed between the headgroups (14-F and C41) but not between the head (14-F) and tail groups (C40). Thus, in this study we assumed that neighboring AmB-AmB pair was directed in a parallel manner.

In order to investigate the effect of the 2nd ^{19}F atom in a 3 spin system on the distance measurements by $^{13}C\{^{19}F\}$REDOR experiments, we prepared the bilayer membrane composed of nonlabeled-AmB/14-F-AmB/^{13}C-Erg/POPC = 0.5:0.5:1/9. Assuming that nonlabeled-AmB and 14-F-AmB are similarly distributed in the AmB-Erg assembly, the possible arrangements of AmB/Erg/AmB in a parallel AmB-AmB orientation can be categorized into four patterns with an equal probability of 25% (Figure S1). If both of the AmB molecules are close to ^{13}C- Erg within the range of 5-7 Å, the maximum dephasing effect of the ^{13}C- Erg signal should reach 0.56 (75% of 0.75) at around 15 ms even under the diluted conditions since AmB-Erg pairs in panels A, B, and C cause the dephasing of ^{13}C signal of Erg. On the other hand, if one AmB molecule is close to Erg and the other is not (over 8 Å), the pairs in patterns **A** and **B** (not **C**) of Figure S1 cause the dephasing. The latter case agrees with the experimental result of the dilution experiment that was saturated at 0.37, which is 50% of the values (0.75) with the undiluted F-AmB (Figure S2). These observations imply that the REDOR data can be interpreted as a two spin system upon estimating the interatomic distance of the nearest $^{13}C/^{19}F$ pair. The effect of the 2nd nearest ^{19}F

atom on the REDOR curves in 3 spin systems is summarized in Figure S3, which suggests that if the nearest AmB-Erg pair is close as compared with the distances of the other pair(s), the shortest ^{13}C-^{19}F distance can be determined with reasonable accuracy.

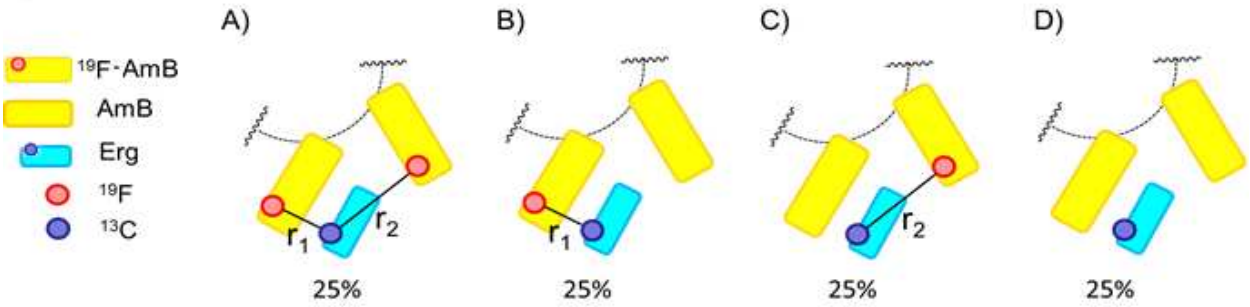


Figure S1. Possible alignments of AmB and Erg for ^{19}F-AmB/AmB/Erg complexes in the dilution experiment with probability of occurrence in the parallel AmB/Erg/AmB orientation.

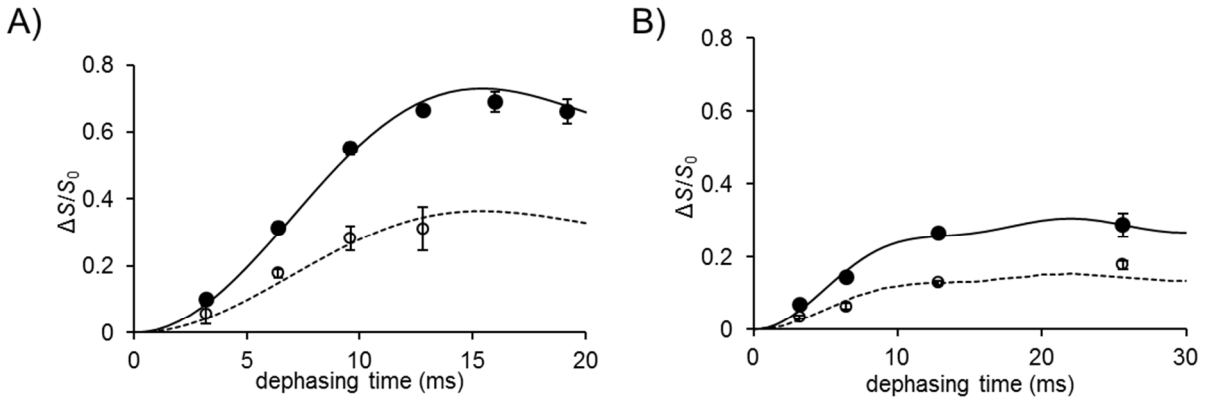


Figure S2. The dilution experiments for 14-F-AmB/4-^{13}C-Erg (A) and 14-F-AmB/26,27-$^{13}C_2$-Erg (B). The experimental dephasing rate for $^{13}C\{^{19}F\}$REDOR using F-AmB/AmB/^{13}C-Erg/POPC=0.5/0.5/1/9 (○) and F-AmB/^{13}C-Erg/POPC=1/1/9 (●). The solid lines present the best fit simulation curves to undiluted experiment (The detail was described in the main text.), and the dashed lines show the half values of the undiluted ones, where the solid lines were simply reduced along the y-axis by 50%.

The REDOR dephasing curves for ^{19}F-^{13}C-^{19}F three spin systems (Figure S3) indicate that the second nearest ^{19}F atom does not significantly influence the dephasing effect of the closest ^{13}C-^{19}F pair, if ^{13}C-^{19}F distance from the second ^{19}F exceeds 10 Å, in the range of dephasing time of the present experiments (Figures 2 and 3 in the manuscript). As shown in Figure 3S, some of three spin systems with closer ^{13}C-^{19}F distances such as 6.6 and 7.6 Å nicely reproduce the undiluted experimental data with a ^{19}F-^{13}C-^{19}F angle ranging from 40^{o} to 140^{o}; please note that the initial part of the curves (0-10 ms) can be fitted better with a longer ^{13}C-^{19}F distance (6.6 Å) if the first and second nearest ^{13}C-^{19}F distances are close (Figure S3**A**). However, in the dilution experiment, these closer $^{13}C/^{19}F$ combination cannot reproduce the experimental data (Figure S3**B**). On the other hand, if the second nearest ^{19}F atom resides relatively far from the first nearest one (over 1.5 times), the theoretical curves can reproduce the experimental results in both undiluted and diluted three spins (Figure S3, **C** and **D**); when the difference in the two ^{13}C-^{19}F distances exceeds 2.0 times, the REDOR curve of the three spin systems become very close to those of the two spin systems shown in Figures 2 and 3 in the manuscript.

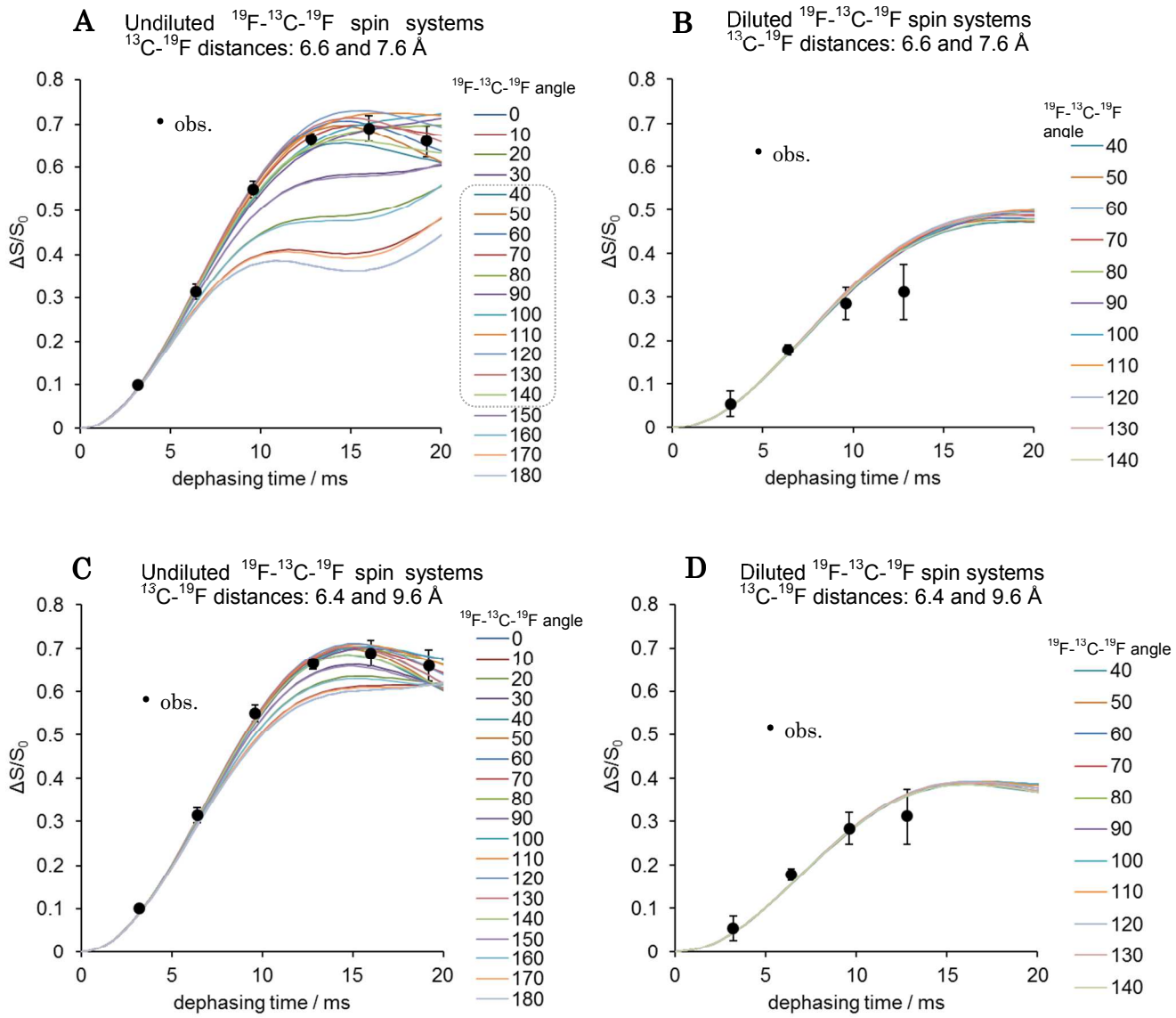


Figure S3. Three spin $^{13}C\{^{19}F\}$REDOR curves in undiluted and diluted experiments for evaluating effects of the second nearest ^{19}F atom on the REDOR curves in ^{19}F-^{13}C-^{19}F three spin systems. Effect of the second ^{19}F atom at 9.4 Å (1.5 times of the nearest distance) apart from the observed ^{13}C is relatively small (bottom) as compared with the case of the ^{19}F atom at 7.6 Å (1.15 times) shown in the top. Change in a ^{19}F-^{13}C-^{19}F angle from 40° to 140° does not significantly affect the REDOR curve shape.

3. $^{13}C\{^{19}F\}$REDOR at −30 °C to examine static disorder of AmB and Erg in membrane

We evaluated the effect of the mobility of Erg on the distance calculation, and carried out the $^{13}C\{^{19}F\}$REDOR experiments using 14-F-AmB/4-^{13}C-ergostrol/POPC=1/1/9 at −30 °C. The obtained dephasing rates at the low temperature were not greatly different from those obtained at 38 °C, indicating that the exchange of AmB-Erg pairing hardly occurred during REDOR experiment as described in main text. Then, to evaluate the molecular motion in membrane, we calculated the REDOR dephasing under the conditions that the ^{13}C- and ^{19}F-spins move to a certain extent in the membrane-bound complexes.

The first assumption is that the AmB and Erg molecules in channel complex is virtually immobilized as the most stable structure at −30 °C. Second is that the average intermolecular distance between AmB and Erg molecules under the VDW contact with their rigid polyene and alicyclic motifs is the smallest at −30 °C while the molecular motion at 38 °C is always in the direction away each other, and the existence probability of atoms follows the 3D Gaussian distribution having its center at the most stable position. In this case, when the position of the ^{13}C atom is supposed to be immobilized at the (x, y, z)= (0, 0, 0) and the most stable position of the fluorine was defined as (r_{static}, 0, 0), the existence probability of the ^{19}F atom at R'(x, y, z) can be expressed according to the Gaussian function as follows (x≥r_{static}):[1]

$$\mathrm{P(x,y,z)} = \frac{p(x,y,z)}{\int_{x=r_{static}}^{\infty}\int_{y=0}^{\infty}\int_{z=0}^{\infty} p(x,y,z)dzdydz} \qquad \text{[eq.1]}$$

$$p(\mathrm{x,y,z}) = \frac{1}{(2\pi\sigma)^{\frac{3}{2}}} exp\left\{-\frac{(x-r_{static})^2+y^2+z^2}{2\sigma^2}\right\}$$

The averaged dipole coupling $D_{ave.}$ in hertz between ^{13}C and ^{19}F in motion can be calculated by eq.2 where γ_I and γ_S are the respective ^{13}C and ^{19}F gyromagnetic ratios and μ_0 is permittivity of vacuum.

$$D_{ave.} = \int_{x=r_{static}}^{\infty} \int_{y=0}^{\infty} \int_{z=0}^{\infty} \left\{ \left(\frac{\gamma_I \gamma_S \ \ \mu_0}{8\pi^2 (x^2+y^2+z^2)^{\frac{3}{2}}} \right) \times P(x,y,z) \right\} dz dy dx \qquad \text{[eq.2]}$$

The dephasing values at 38 °C and −30 °C well agreed with the theoretical curve for the ^{19}F-^{13}C dipole of 107.7 and 125.3 Hz, respectively (Figure S3), indicating that r_{static} is 6.1 Å that is the shortest ^{19}F-^{13}C distance during motion, and the standard deviation σ is approximately 0.4 Å. This result suggests that the ^{19}F atom in motion stays largely in the range of 1.0 Å, and average distance of ^{19}F from ^{13}C is 6.4 Å, which is a better distant constraint for molecular dynamics calculation for the AmB-Erg complex at 38 °C (Figure 4).

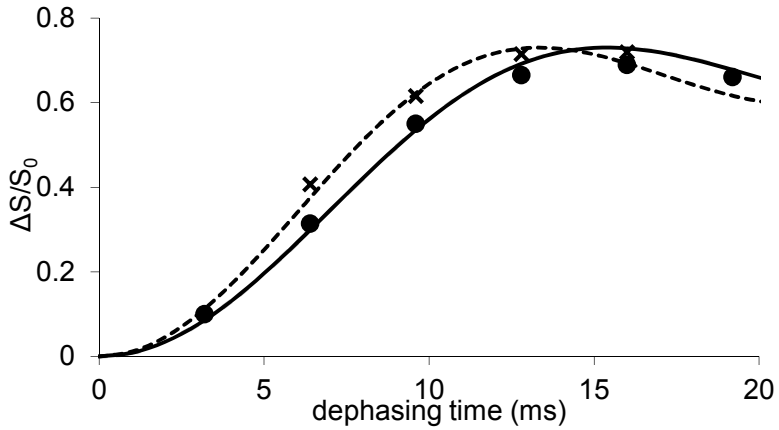


Figure S4. Experimental $^{13}C\{^{19}F\}$REDOR dephasing values ($\Delta S/S_0$) for the C4 signal of Erg at 38 °C (●) and −30 °C (×) obtained from 14-F-AmB/4-^{13}C-ergostrol/POPC=1/1/9. The simulation curves for the ^{13}C–^{19}F dipole of 107.7 (solid lines) and 125.3 Hz (dash line) (assuming that 70% of Erg interact in parallel manner).

4. **NMR experiments to estimate the ratios of Erg-bound AmB and of AmB-bound Erg**

The ^{19}F NMR spectra of a 14-F-AmB/Erg/POPC mixture were recorded at the air temperature of 30 °C and −25 °C under magic angle spinning and dipolar decoupling (DD-MAS) to estimate the fractional amount of AmB that forms assemblies (Figure S5). Judging from the peak areas of the broad and sharp components, the fraction of assembly-forming AmB with low mobility that shows similarly broad peaks to those at -25°C is roughly estimated to be 70-90% of the total AmB. In the spectrum of a AmB/26,27-$^{13}C_2$-Erg/POPC mixture, the ^{13}C signal from 26/27-$^{13}C_2$ of the bound Erg was somewhat separated from that of the unbound form. The peak integration allowed us to estimate the ratio of bound and unbound Erg to be 1:3.3. Thus, the fraction of AmB bound to Erg in the complex was estimated to be 26-33%. These results reveal that not many Erg molecules bind to the AmB assemblies. As we reported previously (Refs. 24a and 24e), however, high contents of Erg markedly increase the mobility of AmB assemblies, which extremely complicates the interpretation of the REDOR data. For REDOR experiments, therefore, we adopted the present conditions where AmB could be treated as a static state.

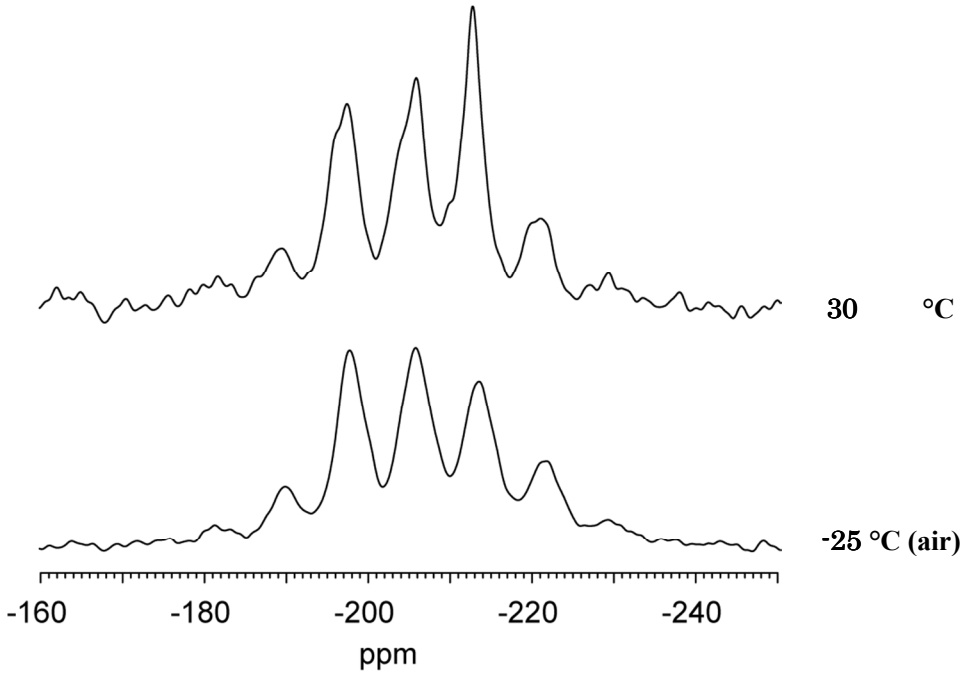


Figure S5. ^{19}F NMR spectra of 14-F-AmB/Erg/POPC=1/1/9. Spectra were recorded at the air temperature of 30°C (top) and -25°C (bottom). ^{19}F NMR experiments were carried out on 400 MHz AVANCE400 (Bruker, Karlsruhe, Germany). The spectra were recorded with the temperature-controlling air set at 30 °C and −25 °C using H/F/X MAS probe with a 4 mm spinner module. The MAS frequency was 3 kHz and regulated to ±1 Hz. A spin-echo pulse sequence was used with the 90° pulse width for ^{19}F was 5.5 μs. The recycle delay was 5 s, and the sweep width was 75 kHz. The TPPM ^{1}H decoupling (40 kHz) was applied during spin-echo and acquisition. The sharp component of the spectrum at 30 °C can be assigned as highly mobile AmB molecules. Base on the area integrations of peaks, the static AmBs that cause REDOR dephasing comprise 70-85% of the total AmB.

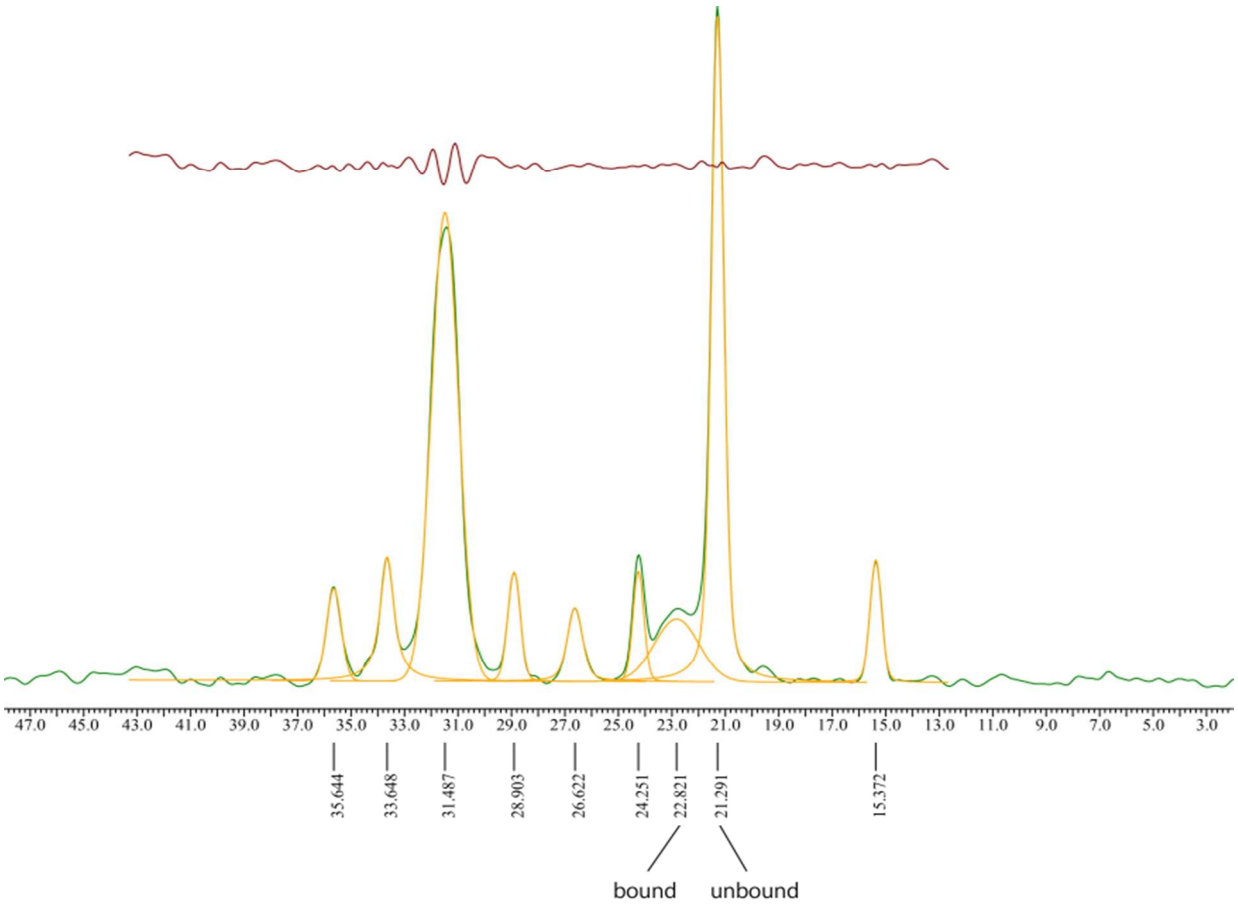


Figure S6. DDMAS spectra of ^{13}C-Erg to estimate the fraction of AmB-bound Erg.

The ^{13}C NMR spectrum was recorded under the same conditions as those for the REDOR experiments. DD-MAS spectrum of 14-F AmB/26,27-13C2-Erg/POPC=1/1/9. Spectrum was recorded using a CMX300 (Agilent Technologies, Santa Clara, CA, USA) equipped with 5 mm MAS probe. The MAS frequency was 5 kHz and regulated to ±1 Hz. The temperature of VT gas was set to 30 °C. The 90° pulse width for ^{13}C was 4 μs. The recycle delay was 10 s, and the sweep width was 30 kHz. The TPPM ^{1}H decoupling (50 kHz) was applied during acquisition.Base on the area integrations of deconvolution of peaks, the fraction of Erg bound to AmB that cause REDOR dephasing was estimated to comprise of 23% of the total Erg.

5. Molecular simulations to deduce Cholesterol-AmB interactions as compared with those of Erg-AmB

We carried out the molecular simulations for a AmB-cholesterol (Cho) complex with the same parameters as those for Erg (Figure 4) to examine the difference between Erg-AmB and Cho-AmB interactions (Figure S7). In the parallel orientation in panels **a** and **b**, the B ring of Erg comes closer to the macrocycle face of AmB as compared with Cho. In the antiparallel pairs in panels **c** and **d**, there is no prominent difference between Erg and Cho although the 7-axial hydrogen of Cho resides very close to the polyhydroxy chain of AmB, which may destabilize their complex (ref. 36).

a. Erg-AmB in parallel **b**. Cho-AmB in parallel **c**. Erg-AmB in antiparallel **d**. Cho-AmB in antiparallel

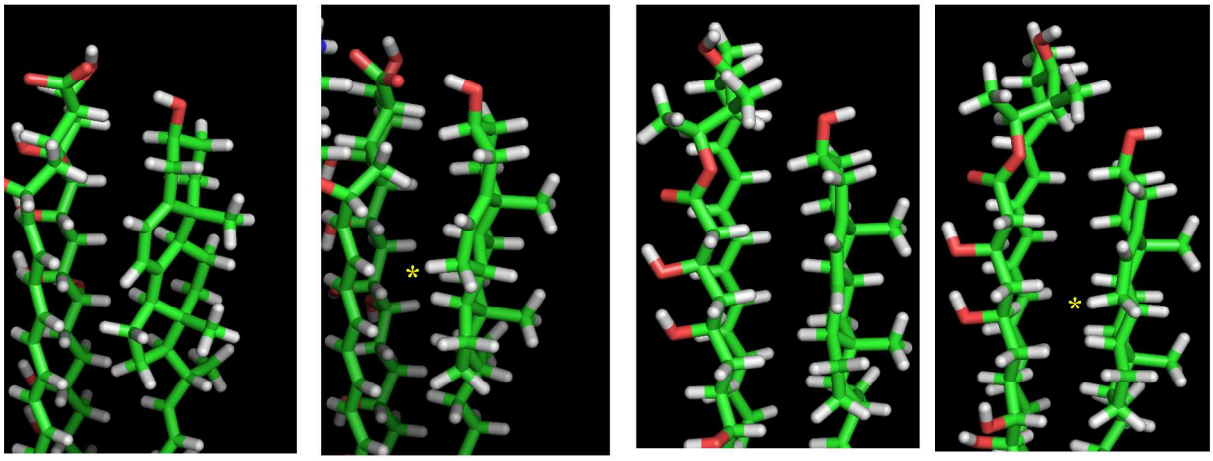
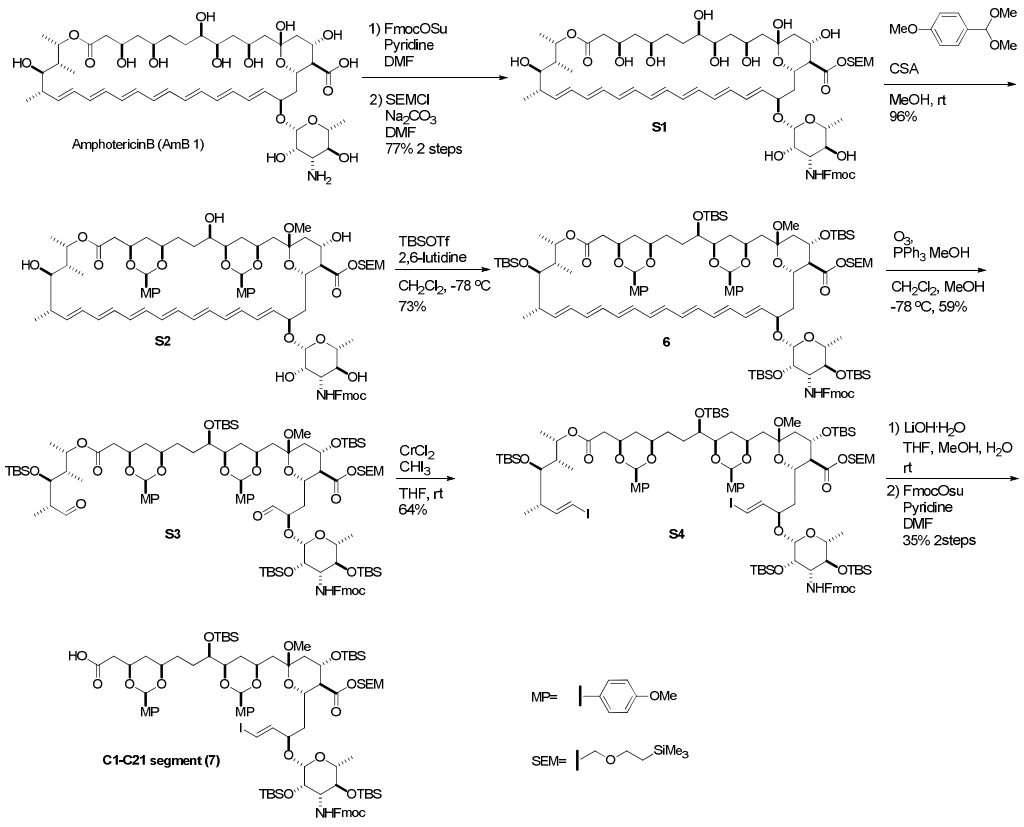


Figure S7. Conformational search for cholesterol (Cho)-AmB complexes **b** and **d** in comparison with Erg-AmB cases **a** and **c**. The simulations were carried out in the same way as those for Erg-AmB complexes shown in Figure 4. *Axial hydrogen at the C7 position of Cho.

6. Synthesis of 32-^{19}F-Amphotericin B



Scheme S1. Synthesis of C1-C21 segment

Conversion of AmB (1) to S1. To a stirred solution of AmB (**1**) (3.0 g, 3.25 mmol) in DMF (33 mL) was added FmocOSu (1.64 g, 4.88 mmol) and Pyridine (1.57 ml, 19.5 mmol). After stirred at room temperature for 15 h, the reaction was then poured into diethyl ether (400 ml). The resulting yellow precipitate was filtered and washed with diethyl ether (100 ml). The yellow powder was placed under vacuum prior to the next reaction. The yellow solid was dissolved in DMF (33 ml) and the solution was cooled to 0 ^{o}C. Sodium carbonate (396 mg, 3.43 mmol) was added to the solution and it was stirred for 1 hour. SEMCl (0.64 ml, 3.57 mmol) was added to the reaction mixture, and it was stirred for 1 hour at 0 ^{o}C. The reaction mixture was then poured into diethyl ether (800 ml). The resulting yellow precipitate was filtered and washed with diethyl

ether (100 ml). The crude was purified via flash chromatography (SiO_2; $CHCl_3$:MeOH 6:1) to yield **S1** as a yellow powder (3.19 g, 77% for 2 steps).

R_f = 0.49 ($CHCl_3$/MeOH = 6/1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (2H, d, *J* = 7.8 Hz, Fmoc), 7.73 (2H, dd, *J* = 7.1, 4.6 Hz, Fmoc), 7.41 (2H, t, *J* = 7.1 Hz, Fmoc), 7.32 (2H, m, Fmoc), 6.04-5.87 (13H, m, heptaene), 5.44 (1H, dd, *J* = 14.7, 10.1 Hz, H33), 5.19 (2H, m), 4.73 (2H, m), 4.54 (1H, dd, *J* = 22.7, 5.5 Hz), 4.35-4.42 (2H, m), 4.16-4.28 (5H, m), 4.04 (2H, m), 3.36-3.71 (7H, m), 3.04-3.19 (4H, m), 2.27 (1H, m), 2.17 (1H, *J* = 6.4 Hz), 2.01 (1H, t, *J* = 10.5 Hz), 1.91 (2H, m), 1.72 (1H, m), 1.49-1.62 (5H, m), 1.22-1.44 (1H, 5H, m), 1.16 (3H, d, *J* = 4.6 Hz, H6'), 1.10 (3H, d, *J* = 6.4, H38), 1.03 (3H, d, *J* = 6.0 Hz, H40), 0.91 (3H, d, *J* = 7.3, H39), -0.04 (9H, s, SEM); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.46, 170.76, 144.13, 144.09, 140.87, 137.00, 136.38, 134.03, 133.86, 133.48, 133.39, 132.72, 132.69, 132.51, 132.43, 132.26, 132.07, 131.38, 129.09, 129.07, 127.82, 127.27, 125.65, 125.55, 120.24, 97.56, 97.46, 96.92, 88.30, 77.21, 74.81, 73.90, 73.77, 73.66, 73.58, 69.60, 69.50, 69.44, 69.33, 69.15, 67.87, 67.75, 66.85, 66.45, 66.33, 65.87, 65.41, 65.23, 57.28, 57.21, 57.16, 46.88, 44.85, 42.25, 42.20, 37.03, 35.25, 35.21, 29.19, 29.15, 18.68, 18.30, 17.66, 17.17, 12.24, −1.13; MS (ESI) *m*/*z* calcd for $C_{68}H_{97}NO_{20}Si[M+Na^+]$ 1298.6271, found: 1298.6281

Conversion of S1 to S2. To a suspension of **S1** (5.9 g, 4.7 mmol) in MeOH (126 ml) was added *p*-methoxy benziliden dimethylacetal (13.1 ml, 78.1 mmol) and camphorsulfonic acid (127 mg, 0.50 mmol), and the mixture was stirred for 1.5 hours. The reaction mixture was quenched with sat. aq. sodium bicarbonate and extracted with ethyl acetate. The Organic layer was washed with brine and dried over sodium sulfate and concentrated in vacuo. The resulting solid was

purified via flash SiO_2 column chromatography (hexane/AcOEt, 5/1 then $CHCl_3$/MeOH, 10/1) to yield **S2** as a yellow powder. (6.82 g, 96%)

R_f = 0.75 ($CHCl_3$/MeOH, 6/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, *J* = 7.6 Hz), 7.61 (2H, m), 7.31-7.44 (6H, m, MP, Fmoc), 6.87 (4H, dd, *J* = 8.81, 6.01 Hz, MP), 6.05-6.35 (12H, m, H21-32), 5.82 (1H, dd, *J* = 5.8, 14.4 Hz, H20), 5.46 (1H, s, 3,5-MP), 5.43 (1H, dd, *J* = 14.5, 9.0 Hz, H33), 5.43 (1H, s, 9,11-MP), 5.39 (1H, d, *J* = 6.0 Hz, SEM), 5.28 (1H, d, *J* = 6.3 Hz, SEM), 4.60 (2H, m), 4.42 (2H, m), 4.20 (3H, m), 3.81-3.95 (2H, m), 3.80 (3H, s, MP), 3.78 (3H, s, MP), 3.61-3.76 (5H, m), 3.26-3.48 (3H, m), 3.08 (1H, m), 3.02 (3H, s, 13-OMe), 2.67 (1H, dd, J = 16.7, 5.4), 2.42 (1H, m), 2.33 (1H, m), 2.28 (1H, d, *J* = 7.6 Hz), 1.64-2.08 (11H, m), 1.35-1.61 (4H, m), 1.33 (3H, d, *J* = 6.0 Hz, H6'), 1.20 (3H, d, *J* = 6.4 Hz, H38), 1.12 (3H, d, *J* = 6.6 Hz, H40), 1.02 (3H, d, *J* = 7.2 Hz, H39), 0.01 (9H, s, SEM); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.35, 169.31, 159.87, 159.79, 158.06, 143.68, 141.28, 135.81, 134.05, 133.31, 133.10, 133.07, 133.04, 132.97, 132.83, 132.61, 132.34, 132.11, 131.91, 130.95, 130.90, 127.73, 127.42, 127.21, 127.10, 127.08, 125.06, 125.02, 119.99, 113.57, 113.53, 100.53, 100.02, 99.98, 97.15, 89.09, 79.99, 78.22, 77.20, 76.02, 73.41, 73.16, 72.54, 72.12, 70.02, 69.75, 68.19, 67.34, 66.85, 66.31, 56.40, 55.77, 55.25, 48.21, 47.10, 42.27, 40.92, 36.99, 32.66, 32.54, 28.03, 18.31, 18.07, 17.56, 17.12, 11.38, −1.47; MS (ESI) *m/z* calcd for $C_{85}H_{111}NO_{22}Si[M+Na^+]$ 1548.7265, found: 1548.7266.

Conversion of S2 to 6. To a solution of **S2** (9.0 g, 5.9 mmol) in CH_2Cl_2 was added 2,6-lutidine (34 ml, 295 mmol) and TBSOTf (13.9 ml, 76.7 mmol) at −50 °C. After stirred at −50 °C for 1 hour, the resulting solution was warmed to 0 ºC and it was stirred for 1 hour. The resulting

mixture was quenched with sat. aq. sodium bicarbonate and it was extracted with AcOEt. The organic layer was washed with 0.5 N HCl aq., sat. aq. sodium bicarbonate and brine and dried over sodium sulfate. The resulting solution was concentrated in vacuo and purified via flash chromatography (hexane/AcOEt, 10/1 to 5/1) to yield TBS ether **6** as a yellow solid (9.02 g, 73%).

R_f = 0.55 (hexane/AcOEt, 4/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (2H, d, *J* = 7.5 Hz, Fmoc), 7.59 (2H, m, Fmoc), 7.37-7.32 (6H, m, Fmoc, MP), 7.27 (2H, t, *J* = 7.5 Hz, Fmoc), 6.81 (4H, dd, *J* = 20.5, 8.5 Hz, MP), 6.22-6.01 (12H, m, H21-32), 5.73 (1H, dd, *J* = 14.5, 6.6 Hz, H20), 5.54 (1H, dd, *J* = 14.8, 9.2 Hz, 33H), 5.44 (1H, s, 3,5-MP acetal), 5.39 (1H, s, 9, 11-MP acetal), 5.32 (1H, d, *J* = 6.0 Hz, SEM), 5.24 (1H, d, *J* = 6.0 Hz, SEM), 4.90 (2H, d, *J* = 9.9 Hz), 4.40-4.46 (2H, m), 4.32-4.37 (1H, dd, *J* = 10.5, 6.6 Hz), 4,15-4.25 (3H, m), 3.85 (1H, m), 3.78 (6H, s, -OMe), 3.59-3.74 (3H, m,), 3.35 (1H, t, *J* = 9.5 Hz), 3.29 (1H, m), 3.02 (3H, s, -OMe), 2.61 (1H, dd, *J* = 17.4, 7.0 Hz, H16), 2.34 (2H, t, *J* = 10.5 Hz, H2), 2.20-2.52 (2H, m), 1.96 (1H, m), 1.75-1.84 (3H, m), 1.55-1.70 (5H, m), 1.23 (3H, d, *J* = 6.5 Hz, H6'), 1.20 (3H, d, *J* = 6.5 Hz, 38Me), 1.00 (3H, d, *J* = 6.5 Hz, 40Me), 0.95 (3H, d, *J* = 8.7 Hz, H39), 0.91, 0.90, 0.87, 0.83, 0.75(45H, s, TBS, SEM), 0.11-−0.14(41H, TBS, SEM); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.26, 169.71, 159.71, 159.61, 155.51, 144.02, 143.96, 141.36, 135.86, 133.63, 133.51, 133.32, 133.14, 132.74, 132.46, 132.20, 131.94, 131.73, 131.29, 131.21, 130.26, 127.62, 127.34, 126.96, 124.93, 119.89, 113.46, 113.25, 100.57, 100.17, 99.98, 97.89, 89.73, 80.21, 77.20, 75.29, 73.83, 73.44, 72.50, 72.34, 72.27, 72.20, 68.21, 68.78, 66.95, 66.66, 57.06, 56.06, 55.24, 47.99, 47.19, 43.03, 42.42, 40.66, 37.08, 36.01, 32.22, 31.84, 30.34, 28.91, 26.97, 26.08, 25.98, 25.75, 25.61, 18.84, 18.38, 18.31, 18.24, 18.11, 17.98, 17.82, 17.70, 14.02, 10.94, −1.44, −3.96, −4.03, −4.17, −4.26,

−4.39, −4.43, −4.56, −5.14, −5.33; MS (ESI) m/z calcd for $C_{115}H_{181}N_1O_{22}Si_6[M+Na^+]$ 2119.1589, found: 2119.1575

Conversion of 6 to S3. Compound **6** (3.0 g, 1.4 mmol) was dissolved in CH_2Cl_2 (375 ml) and MeOH (25 ml), and was cooled to −78 °C. Ozone was bubbled through the solution until a blue color persisted (~20 minutes) and then the excess ozone was bubbled out of the solution with a stream of argon. Triphenylphosphine (7.9 g, 30 mmol) was added and stirred for 3 hours. The resulting solution was concentrated in vacuo and purified via flash SiO_2 column chromatography (hexane:AcOEt, 20:1 to 3:1) to yield dialdehyde **S3** as a white solid (1.70 g, 59%).

R_f = 0.50 (hexane/AcOEt, 2/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.65 (1H, s, CHO), 9.49 (1H, d, *J* = 2.8 Hz, CHO), 7.75 (2H, d, *J* = 7.6 Hz, Fmoc), 7.59 (2H, d, *J* = 7.3 Hz, Fmoc), 7.41-7.27 (6H, m, Fmoc, MP), 7.27 (2H, t, *J* = 7.5 Hz, Fmoc), 6.82 (4H, dd, *J* = 20.5, 8.5 Hz, MP), 5.49 (1H, s, 3,5-MP acetal), 5.44 (1H, s, 9,11-MP acetal), 5.33 (1H, d, *J* = 6.0 Hz, SEM), 5.16 (1H, d, *J* = 6.0 Hz, SEM), 5.01 (1H, m), 4.49-3.60 (7H, m), 3.78, 3.77 (6H, s, -OMe), 3.33 (1H, m), 3.15 (3H, s, OMe), 2.67 (1H, dd, *J* = 15.3, 7.6 Hz), 2.51-2.39 (2H, m), 2.20 (1H, m), 1.96 (1H, m), 1.75-1.84 (3H, m), 1.55-1.70 (5H, m), 1.23 (3H, d, *J* = 6.5 Hz, H6'), 1.20 (3H, d, *J* = 6.5 Hz, 38Me), 1.00 (3H, d, *J* = 6.5 Hz, 40Me), 0.95 (3H, d, *J* = 8.7 Hz), 0.91, 0.90, 0.87, 0.86, 0.82, 0.75(45H, s, TBS, SEM), 0.10-−0.12 (41H, TBS, SEM); MS (ESI) *m*/*z* calcd for $C_{103}H_{169}N_1NaO_{24}Si_6[M+Na^+]$ 1995.0548, found: 1995.0515.

Conversion of S3 to S4. To a stirred mixture of $CrCl_2$ (2.50 g, 20.0 mmol) in dry THF (40 mL) was slowly added the THF (45 mL) solution of bis-aldehyde **S3** (1.70 g, 0.85 mmol) and

iodoform (2.0 g, 5.10 mmol) via canula. The resulting dark red slurry was stirred at room temperature for 11 hours and then quenched with aqueous sodiumu bicarbonate. The resulting green solution was extracted with diethyl ether. The Organic layer was dried over sodium sulfate and concentrated in vacuo. The resulting solid was purified via flash SiO_2 column chromatography (hexane/AcOEt, 1/0 to 5/1) to yield bisvinyl iodide **S4** as a white solid (1.20 g, 64%).

R_f = 0.62 (hexane/AcOEt, 5/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (2H, d, *J* = 7.6 Hz, Fmoc), 7.59 (2H, dd , *J* = 7.3 , 3.4 Hz, Fmoc), 7.41-7.27 (6H, m, Fmoc, MP), 7.27 (2H, t, *J* = 7.5 Hz, Fmoc), 6.83 (4H, dd, *J* = 20.5, 8.5 Hz, MP), 5.49 (1H, s, 3,5-MP acetal), 5.44 (1H, s, 9,11-MP acetal), 5.33 (1H, d, *J* = 6.0 Hz, SEM), 5.16 (1H, d, *J* = 6.0 Hz, SEM), 6. 45-6.53 (2H, m, H20, H33), 6.35 (1H, d, *J* = 14.7 Hz, H21), 6.02 (1H, d, *J* = 14.4 Hz, H32), 5.50 (1H, s, 3,5-MP acetal), 5.46 (1H, s, 9,11-MP acetal), 5.29 (1H, d, *J* = 6.0 Hz, SEM), 5.22 (1H, d, *J* = 6.2 Hz, SEM), 5.11 (1H, t, *J* = 6.4 Hz, H37), 4.83 (1H, d, *J* = 9.9 Hz, NH), 4.15-4.47 (8H, m), 3.79, 3.78 (6H, s, -OMe), 3.71 (2H, m), 3.63 (2H, m), 3.5 (1H, m), 3.19-3.35 (2H, m), 3.07 (3H, s, -OMe), 2.68 (1H, dd, *J* = 7.3, 15.6 Hz), 2.47 (1H, dd, *J* = 15.5, 5.9 Hz), 2.40 (1H, m), 2.29-2.28 (2H, m), 1.34-2.00 (20H, m), 1.23 (3H, d, *J* = 6.0 Hz, H6'), 1.15 (3H, d, *J* = 6.2 Hz, 38Me), 0.97 (3H, d, *J* = 6.6 Hz, 40Me), 0.93 (3H, 39H), 0.91, 0.88, 0.87, 0.83, 0.76 (45H, s, TBS, SEM), 0.08, 0.06, 0.05, 0.03, 0.02, 0.04, 0.06, 0.07, −0.11 (41H, TBS, SEM); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.21, 169.91, 159.70, 155.55, 150.14, 146.12, 143.95, 143.87, 141.36, 131.06, 127.62, 127.49, 127.26, 126.96, 124.89, 119.90, 113.41, 113.37, 100.62, 100.41, 100.30, 99.26, 89.53, 79.76, 79.57, 78.61, 77.20, 76.44, 75.99, 75.23, 73.85, 73.60, 73.25, 72.71, 72.35, 72.25, 71.58, 68.16, 67.36, 66.68, 66.61, 57.09, 55.26, 55.21, 47.97, 47.14, 43.69, 42.17, 41.36, 36.64, 31.68, 27.46, 26.01, 25.90, 25.72, 25.57, 18.74, 18.30, 18.17, 17.96, 17.69, 16.34, 14.12, 11.12,

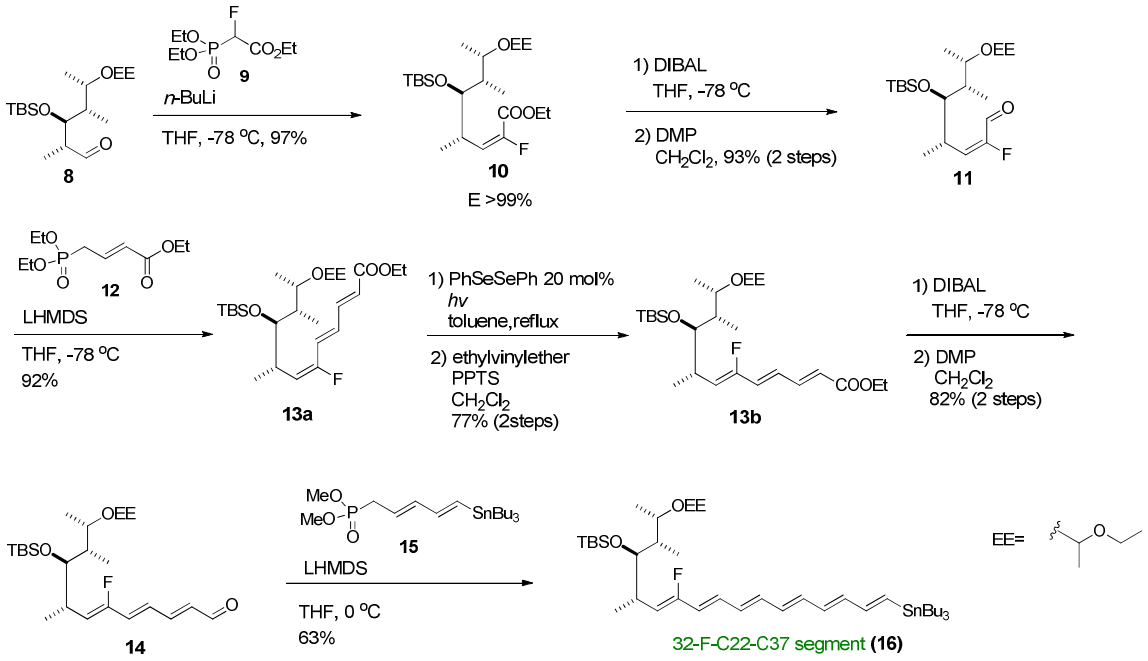
−1.35, −3.58, −3.94, −4.02, −4.05, −4.22, −4.28, −4.41, −4.60, −5.17, −5.31; MS (ESI) *m*/*z* calcd for $C_{103}H_{169}N_1NaO_{24}Si6[M+Na^+]$ 2242.8895, found: 2242.8877.

Conversion of S4 to C1-C21 segment (7). To a solution of bis-vinyl iodide **S4** (1.2g, 0.54 mmol) in THF (90 ml), H_2O (45 ml) and MeOH (45 ml) was added $LiOH \cdot H_2O$ (2.5 g, 59.4 mmol). After stirred at toom temprerature for 21.5 hours, the reaction mixture was quenched with sat. aq. ammonium chloride. The resulting mixture was extracted with diethyl ether and the organic layer was dried over sodium sulfate and concentrated in vacuo. The white solid was taken forward to the next step without further purification.

To a solution of the crude product in DMF (36 ml) was added pyridine (1.5 ml, 19 mmol) and FmocOSu (1.8 g, 5.4 mmol). After stirred for 5 hours, the mixture was quenched with sat. aq. ammonium chloride. The resulting mixture was extracted with diethyl ether and the organic layer was washed with brine. The organic layer was dried over sodium sulfate and concentrated in vacuo. The resulting solid was purified via flash SiO_2 column chromatography (hexane/AcOEt, 1/0 to 5/1) and gel filtered (JAIGEL-2H, $CHCl_3$, 4 ml/min) to yield C1-C21 segment **7** as a white solid (352 mg, 35% for 2 steps).

R_f = 0.65 (silica gel, ethyl acetate/hexane = 1/1); $[\alpha]_D^{25}$ +4.6 (*c* 0.57, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (2H, d, *J* = 7.6 Hz, Fmoc), 7.55 (2H, dd, *J* = 6.7, 5.0 Hz, Fmoc), 7.39-7.33 (6H, m, Fmoc, MP), 7.29 (2H, t, *J* = 7.5 Hz, Fmoc), 6.84 (4H, dd, *J* = 24.9, 8.5 Hz, MP), 6.50 (1H, dd, *J* = 14.5, 7.7 Hz, H20), 6.34 (1H, d, *J* = 14.5 Hz, H33), 5.51 (1H, s, -OMe), 5.46 (1H, s, -OMe), 5.29 (1H, d, *J* = 6.2 Hz, SEM), 5.22 (1H, d, *J* = 6.2 Hz, SEM), 4.84 (1H, d, *J* = 9.9 Hz, NH), 4.45 (0.5H, dd, *J* = 10.5, 6.6 Hz), 4.38 (1H, br), 4.35 (1H, dd, *J* = 10.5, 6.4 Hz), 4.16-4.30

(4H, m), 3.90 (1H, m), 3.82 (3H, m), 3.79, 3.38 (6H, s, -OMe), 3.71 (2H, q, *J* = 8.5 Hz), 3.62 (2H, tt, *J* = 9.9, 2.9 Hz), 3.31 (1H, t, *J* = 8.73 Hz), 3.24 (1H, m), 3.07 (3H, s, -OMe), 2.75 (1H, dd, *J* = 16.2, 7.0 Hz), 2.58 (1H, dd, *J* = 16.0.0, 5.9 Hz), 2.23-2.16 (2H, m), 1.95 (1H, br), 1.81-1.70 (4H, m), 1.57 (2H, m), 1.49 (2H, m), 1.21 (3H, d, *J* = 6.0 Hz, H6'), 1.00 (2H, t, *J* = 8.2 Hz, SEM), 0.88, 0.87, 0.82, 0.76 (36H, s, TBS), 0.05, 0.04, 0.02, 0.01, −0.05, −0.06, −0.08, −0.11 (33H, s, TBS, SEM): ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.20, 169.91, 159.86, 159.70, 155.60, 146.12, 143.93, 143.84, 141.36, 131.07, 130.82, 127.63, 127.48, 127.32, 126.97, 124.88, 119.91, 113.51, 113.37, 100.60, 100.55, 99.35, 89.53, 79.77, 79.52, 78.74, 77.21, 76.47, 73.86, 73.63, 72.80, 72.69, 72.36, 72.24, 68.16, 67.36, 66.72, 66.63, 57.34, 57.10, 55.27, 55.21, 47.98, 47.13, 42.99, 41.99, 40.52, 38.89, 36.37, 31.75, 31.67, 27.44, 26.00, 25.90, 25.72, 25.56, 18.73, 18.33, 18.16, 17.96, 17.69, −1.35, −3.58, −4.02, −4.24, −4.28, −4.41, −4.62, −5.18, −5.32; MS (ESI) *m*/*z* calcd for $C_{90}H_{142}INNaO_{21}Si_5[M+Na^+]$ 1862.7863, found:1862.7847.



Scheme 2. Synthesis of 32-F-C22-C37 segment

Conversion of 8 to 10. To a solution of triethyl-2-fluoro-2-phosphonoacetate **9** (2.6 g, 10.8 mmol) in THF (30 ml) was added dropwise *n*-BuLi at −78 ºC. After stirred at −78 ºC for 30 minutes, a solution of the aldehyde **8** (2.2 g, 6.37 mmol) in THF (30 ml) was added dropwise via canula. The reaction mixture was stirred in −78 ºC for 1 hour and then quenched with sat. aq. ammonium chloride. The mixture was extracted with diethyl ether and the organic layer was washed with brine and dried over sodium sulfate. The solution was concentrated in vacuo and purified by flash SiO_2 column chromatography (hexane/AcOEt 20/1 to 10:1) to furnish fluoro esther **10** as a colorless oil (2.7 g, 97%).

R_f= 0.65 (silica, hexane/AcOEt= 4/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.00 (0.5H, dd, *J* = 22.1, 10.76 Hz, H33), 5.91 (0.5H, dd, *J* = 22.1, 10.53 Hz, H33), 4.67 (1H, m, EE), 4.30 (2H, q, *J* = 7.10 Hz, OEt), 3.89 (1H, m, 37H), 3.67-3.41 (3H, m, EE, 35H, 34H), 1.86 (0.5H, ddd, *J* = 14.4, 7.1, 2.1 Hz, 35H), 1.92 (0.5H, ddd, *J* = 14.4, 7.1, 2.1 Hz, 35H), 1.35 (3H, t, *J* = 7.1 Hz, OEt), 1.28 (3H, d, 5.0 Hz, EE), 1.19 (3H, t, *J* = 6.9 Hz, EE), 1.08 (1.5H, d, *J* =6.4 Hz, 38H), 1.04 (1.5H, dd, *J* = 6.53, 3.21, 0.92 Hz, 40H), 1.02 (1.5H, d, *J* = 6.4 Hz, 38H) 0.93, 0.92 (9H, s, TBS), 0.88, 0.86 (3H, d, *J* = 2.8 Hz, 39H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.99, 160.94, 160.70, 160.66, 146.67, 146.66, 144.67, 144.63, 128.27, 128.24, 128.15, 128.10, 98.85, 98.23, 76.69, 76.67, 76.45, 76.44, 72.53, 71.18, 61.35, 61.31, 61.08, 60.01, 43.22, 42.22, 33.10, 33.06, 32.92, 32.89, 26.20, 26.13, 26.08, 20.99, 20.83, 18.43, 18.38, 16.04, 15.36, 15.31, 15.24, 14.44, 14.11, 13.87, 9.93, 9.77, −78 ºC 3.72, −78 ºC 3.98, −78 ºC 4.07; MS (ESI) *m*/*z* calcd for $C_{22}H_{43}FNaO_5Si$[M+Na^+] 457.2761, found: 457.2762.

Conversion of 10 to 11. To a solution of fluoro ester **10** (857 mg, 1.8 mmol) in THF (19 ml) was added DIBAL (1.0 M, 9.6 ml) at −78 °C via syringe. After stirred at −78 °C for 2 hours, the mixture was quenched with sat. aq. potassium sodium tartrate and sitirred for 5 hours. Then the solution was extracted with AcOEt. The Organic layer was washed with brine and dried over sodium sulfate and then concentrated in vacuo. The resulting alcohol was taken forward to the next step without further purification.

To a stirred solution of the alcohol in CH_2Cl_2 (20 ml) was added Dess-Martin periodinane (1.67 g, 3.94 mmol) at room temperature After stirred for 2 hours, the reaction mixture was quenched with sat. aq. sodium thiosulfate and it was extracted with AcOEt. Organic layer was washed with brine and dried over sodium sulfate and concentrated in vacuo. The crude was purified via flash SiO_2 column chromatography (hexane/AcOEt 1:0 to 20:1) to yield the aldehyde **11** as colorless oil (718 mg, 93% for 2 steps).

R_f= 0.62, 0.65 (hexane/AcOEt, 4/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (0.5H, d, *J* = 17.2 Hz, 31H), 9.67 (0.5H, d, *J* = 15.3 Hz, 31H), 6.24 (0.5H, dd, *J*=18.9, 11.6 Hz, 33H), 6.06 (0.5H, dd, *J* = 18.9, 11.5 Hz, 33H), 4.61 (2H, quin, *J* = 5.2 Hz, EE), 3.74 (1H, m, 37H), 3.57-3.37 (3H, m, EE, 35H), 3.36 (1H, m, 34H), 1.81 (1H, m, 34H), 1.23-1.80 (6H, m, EE, -OEt), 1.14 (3H, q, *J* = 6.6 Hz, EE), 1.07-1.04 (4.5H, m, 38,H 40H), 1.00 (1.5H, d, *J* = 6.2 Hz, 38H), 0.88, 0.87 (9H, s, TBS), 0.86, 0.85 (1H, d, *J* = 5.0 Hz, H39), 0.03, 0.02, 0.01, 0.00 (6H, s, TBS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.72, 182.54, 49, 182.29, 153.71, 153.63, 151.70, 151.62, 132.23, 131.11, 131.22, 131.10, 98.93, 98.74, 97.61, 77.39, 77.37, 75.87, 75.85, 72.90, 71.51, 61.75, 60.17, 59.77, 44.45, 42.62, 32.77, 32.73, 32.24, 32.20, 26.00, 25.90, 20.93, 20.47, 18.30, 18.18, 17.06, 16. 48, 16.46, 16.44, 15.72, 15.70, 15.15, 14.05, 11.45, 11.15, −3.80, −4.08, −4.18, −4.46; MS (ESI) *m*/*z* calcd for $C_{22}H_{43}FNaO_5Si$[M+Na^+] 413.2499, found: 413.2491.

Conversion of 11 to 13a (*E, E, E*). To a solution of unsaturated phosphonate **12** (181 mg, 0.72 mmol) in THF (5 ml) was added LiHMDS (1.0 M, 0.69 ml) at −78 ºC.After stirred at −78 ºC for 30 minutes, a solution of aldehyde **11** (181 mg, 0.72 mmol) in THF (5 ml) was added to the reaction mixture via canula and the resulting mixture was stirred at −78 ºC for 40 minutes. The reaction mixture was quenched with sat. aq. ammonium chloride and extracted with AcOEt. The organic layer was washed with brine and dried over sodium sulfate and concentrated in vacuo. The crude was purified via flash SiO_2 column chromatography (hexane/AcOEt, 1/0 to 20/1) to yield the unsaturated fluoro ester **13a** as yellow oil (162 mg, 92%).

R_f = 0.64 (hexane/AcOEt, 4/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 , 7.33 (1H, ddd, *J* = 11.5, 11.4, 1.3 Hz, 29H), 6.72-6.49 (2H, m, 30H, 31H), 6.00, 5.97 (1H, d, *J* = 8.45 Hz, 29H), 5.44, 5.32 (1H, dd, *J* = 20.8, 11.0 Hz, 33H), 4.67 (1H, m, EE), 4.22, 4.21 (2H, q, *J* = 7.1 Hz, -OEt), 3.83 (1H, m, 37H), 3.65-3.43 (3H, m, EE, H35), 2.67 (1H, m, 34), 1.92-1.82 (1H, m, H36), 1.32-1.17 (9H, m, EE, EE, -OEt), 1.10 (1.5H, t, *J* = 7.6 Hz, H38), 1.04-1.01 (4.5H, m, H38, H40), 0.93, 0.92 (9H, s, TBS), 0.88 (3H, t, *J* = 7.02 Hz), 0.06, 0.05 (6H, s, TBS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.51, 166.43, 155.40, 155.16, 153.00, 153.76, 143.09, 142.81, 127.33, 127.29, 127.00, 126.89, 126.85, 126.76, 126.43, 126.18, 123.24, 123.80, 119.01, 118.80, 118.45, 118.24, 99.08, 97.76, 77.52, 76.29, 73.222, 71.41, 61.60, 60.26, 60.20, 59.68, 53.26, 44.03, 42.50, 34.04, 33.97, 33.64, 33.56, 26.05, 25.96, 20.97, 20.58, 18.39, 18.21, 17.04, 16.17, 16.13, 15.00, 15.88, 15.21, 14.14, 14.01, 11.09, 10.90, −3.87, −4.05, −4.14, −4.38; MS (ESI) *m*/*z* calcd for $C_{26}H_{47}FNaO_5Si$[M+Na^+] 509.3074, found: 509.3087.

Conversion of 13a (*E, E, E*) to 13b (*Z, E, E*). To a solution of *E*-fuluro ester **13a** (247 mg, 0.43 mmol) in toluene (8.6 ml) was added diphenyl diselenide (27 mg, 0.09 mmol). Then the mixture was warmed to 100 ºC. After stirred for 21 hours under irradiation by a tungsten lamp, then the reaction mixture was cooled to room temperature and concentrated in vacuo. The resulting yellow oil was dissolved in CH_2Cl_2. Ethyl vinyl ether (0.85 ml) and PPTS (56 mg) was added to the solution and the mixture was stirred at room temperature for 2 hours. The resulting mixture was quenched with sat. aq. sodium bicarbonate and extracted with AcOEt. The organic layer was washed with brine, dried over sodium sulfate and concentrated in vacuo. The resulting yellow oil was purified via flash SiO_2 column chromatography (hexane/AcOEt, 1/0 to 20/1) to yield the *E*-unsaturated fluoro ester **13b** as a yellow oil (191 mg, 77%).

R_f= 0.64 (silica, hexane/AcOEt= 4/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (1H, m, H29), 6.52, 6.51 (1H, dd, *J* = 15.2, 11.46 Hz, H30), 6.22 (1H, dd, *J* = 25.9, 15.2 Hz, H31), 5.97, 5.96 (1H. d, *J* =15.2 Hz, H28), 5.08, 5.01 (1H, dd, *J* = 35.8, 9.7Hz, H33), 4.66 (1H, m, EE), 4.21 (2H, t, *J* = 6.6 Hz, -OEt), 3.89 (1H, m, H37), 3.65-3.42 (3H, m, EE, H35), 2.93 (1H, m, H34), 1.92, 1.85 (1H, m, H36), 1.30 (3H, t, *J* = 6.6 Hz, -OEt), 1.28 (3H, t, *J* =1.3 Hz, EE), 1.09 (1.5H, d, *J* = 6.3 Hz, H38), 1.02 (3H, d, *J* = 6.2 Hz, H40), 1.01 (1.5H, d, *J* = 6.2 Hz, H40), 0.92, 0.91 (9H, s, TBS), 0.87 (3H, d, *J* = 7.2 Hz, H39), 0.49, 0.02 (6H, s, TBS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.45, 155.57, 153.08, 142.82, 142.76, 130.94, 130.77, 130.69, 130.52, 125.85, 125.82, 125.65, 122.63, 122.50, 120.12, 119.96, 98.85, 97.88, 76.63, 76.40, 72.79, 71.22, 60.55, 60.13, 59.70, 43.14, 42.32, 32.75, 32.66, 25.99, 25.96, 20.80, 20.59, 18.27, 18.24, 16.30, 15.40, 15.22, 15.18, 14.70, 14.23, 14.10, 10.16, 10.08, −4.02, −4.09; MS (ESI) *m*/*z* calcd for $C_{26}H_{47}FNaO_5Si$[M+Na^+] 509.3074, found: 509.3087.

Conversion of 13b to 14. To a solution of *E*-fluoro-ester **13b** (86 mg, 0.18 mmol) in THF (1.8 ml) was slowly added DIBAL (0.7 ml) at −78 °C. After stirred for 1.5 hours at −78 °C, sat. aq. potassium sodium tartrate was added and stirred for 4 hours and then the solution was extracted with AcOEt. The organic layer was washed with brine and dried over sodium sulfate and then concentrated in vacuo. The resulting alcohol was taken forward to the next step without further purification.

To a solution of resulting alcohol in CH_2Cl_2 (1.7 mL) was added Dess-Martin periodinane (104 mg, 0.24 mmol) After stirred at room temperature for 2 hours, the reaction mixture was quenched with saturated aqueous sodium thiosulfate and extracted with AcOEt. The organic layer was washed with brine and dried over sodium sulfate and concentrated in vacuo. The crude was purified via flash SiO_2 chromatography (hexane/AcOEt, 1/0 to 20/1) to yield the aldehyde **14** as a colorless oil (60.9 mg, 82% for 2 steps).

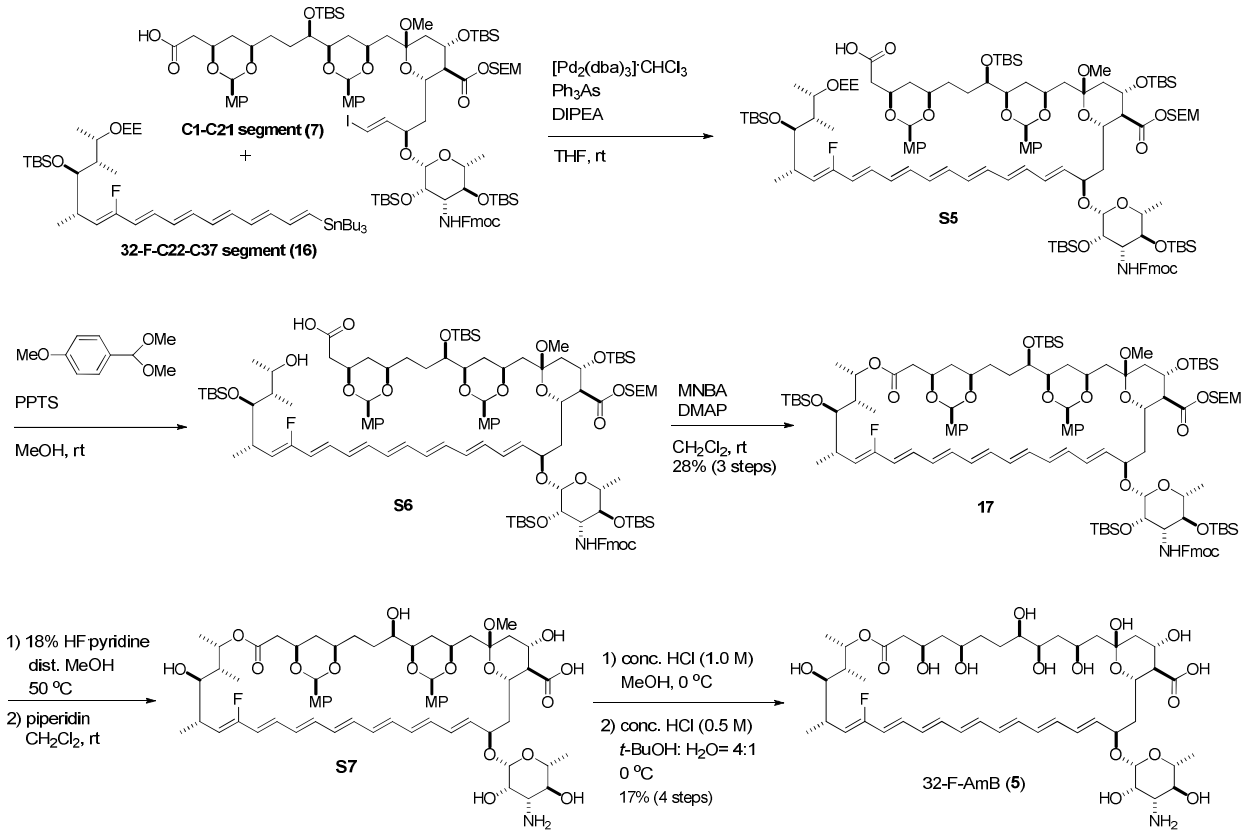
R_f = 0.62, 0.65 (hexane/AcOEt, 4/1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.49 (1H, d, *J* = 8.0 Hz), 7.06, 7.05 (1H, dd, *J* = 14.9, 11.2 Hz, H29), 6.56, 6.55 (1H, dd, *J* = 15.2, 11.2 Hz, H30), 6.28 (1H, dd, *J* = 25.7, 15.2 Hz, H31), 6.13 (1H, dd, *J* = 14.9, 8.0 Hz, H28), 5.12, 5.04(1H, dd, *J* = 35.7, 9.9 Hz, H33), 4.59 (1H, m, EE), 3.79 (1H, m, H37), 3.52 (1H, m, H35), 3.49-3.32 (2H, m, EE), 2.87 (1H, m, H34), 1.83, 1.78 (1H, m, H36), 1.21-1.08 (6H, m, EE), 1.01 (1.5H, d, *J* = 6.41 Hz, H38), 0.95 (4.5H, m, H38, H40), 0.84, 0.83 (9H, s, TBS), 0.80, 0.78 (3H, d, *J* = 4.6 Hz), −0.03, −0.05 (6H, s, TBS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.86, 155.39, 152.90, 149.96, 149.89, 132.65, 132.50, 132.47, 132.42, 132.22, 125.73, 125.70, 125.52, 125.48, 121.81, 121.64, 98.79, 97.78, 76.46, 76.19, 72.73, 71.93, 60.47, 60.04, 59.73, 59.62, 43.19, 42.31, 32.87, 32.80, 25.93, 25.90, 20.75, 20.53, 18.21, 18.17, 16.29, 15.39, 15.19, 15.13, 14.67, 14.14, 13.96, 10.14,

10.08, −4.08, −4.15; MS (ESI) m/z calcd for $C_{26}H_{47}FNaO_5Si[M+Na^+]$ 465.2812, found: 465.2810.

Conversion of 14 to 32-F-C22-C37 (16). Stanyl phosphonate **15**[2] (1.6 g, 3.4 mmol) was azeotropically dried via coevaporation with toluene and dissolved in THF (17 ml) and cooled to 0 ºC. LiHMDS (1.0 M, 3.2 mL) was added dropwise and stirred for 10 min. a solution of aldehyde **14** (743 mg, 1.68 mmol) was added dropwise via canula and the mixture was stirred at 0 ºC for 30 minutes. The reaction mixture was quenched with sat. aq. ammonium chloride and extracted with diethyl ether. The organic layer was washed with brine, dried over sodium sulfate and concentrated in vacuo. The crude was purified via florisil column chromatography (hexane/AcOEt, 1/0 to 30/1, 1% triethyl amine) to yield C22-C37 segment **16** as a yellow oil (820 mg, 63%).

R_f = 0.62, 0.65 (hexane/AcOEt, 4/1); $[\alpha]_D^{25}$+59.3 (c 1.14, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65-5.82 (10H, m, H22-31), 4.86, 4.80 (1H, dd, *J* = 36.7, 9.6 Hz, H33), 4.66 (1H, m, EE), 3.90 (1H, m, H37), 3.66-3.41 (3H, m, EE, H35), 2.90 (1H, m, H34), 1.93, 1.85 (1H, m, H36), 1.17-1.54 (18H, m, EE, $SnCH_2$ CH_2), 1.08 (1.5H, d, *J* = 6.3 Hz, H38), 1.02-0.94 (4.5H, m, H38, H40), 0.91-0.85 (24H, TBS, $Sn(CH_2)CH_2CH_3$), 0.84, 0.82 (3H, d, *J* = 7.0 Hz), 0.04, 0.03 (6H, s, TBS); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.51, 154.05, 146.76, 146.33, 136.43, 136.35, 136.32, 136.17, 136.13, 135.96, 135.72, 135.45, 134.84, 134.74, 134.65, 134.11, 134.05, 133.08, 132.01, 131.76, 131.72, 131.64, 128.83, 128.66, 123.48, 123.33, 123.24, 123.09, 116.178, 116.02, 98.99, 98.09, 76.91, 73.02, 71.38, 60.68, 60.21, 59.87, 42.98, 42.38, 32.63, 32.51, 29.14, 29.07, 29.03, 28.97, 28.93, 27.47, 27.20, 26.93, 26.12, 26.10, 20.95, 20.77, 18.37, 16.36, 15.40, 15.28, 14.83,

14.56, 14.11, 13.62, 10.43, 10.24, 10.11, 9.50, −3.87, −3.90, −3.95; MS (ESI) *m*/*z* calcd for $C_{41}H_{75}FNaO_3SiSn[M+Na^+]$ 805.4389, found: 805.4410.



Scheme S3. Synthesis of 32-F-AmB

Conversion of 7 to S5. Stanane **16** (147 mg, 0.19 mmol) and iodeolefin **7** (174 mg, 95 µmol) was azeotropically dried via coevaporation with toluene. To a solution of iodeolefin **7** and DIPEA (0.17 ml, 0.95 mmol) in THF (10 ml) was added stanane **16** in THF (5 mL), dibenzilidene acetone dipalladium (29 mg, 29 µmol) and triphenyl arsine (97 mg, 0.28 mmol) After stirred at room temperature for 4 hours, the resulting red solution was concentrated in

vacuo and purified via SiO_2 chromatography (hexane/AcOEt, 5:1 to $CHCl_3$/MeOH, 20/1) to yield the seco acid **S5** as a yellow solid (234 mg, impure).

Conversion of S5 to S6. To a solution of seco acid **S5** (234 mg) and *p*-methoxy benzealdehyde dimethyl acetal (1.85 ml) in MeOH (9.5 ml) was added PPTS (407 mg, 1.89 mmol) After stirred at room temperature for 3 hours, the reaction was quenched with aqueous sodium bicarbonate and extracted with diethyl ether. The organic layer was washed with brine, dried over sodium sulfate and concentrated in vacuo. The crude was purified via flash SiO_2 chromatography (hexane/AcOEt, 5:1 to $CHCl_3$/MeOH, 20/1) to yield the seco acid **S6** as a yellow solid (207 mg, impure).

Conversion of S6 to 17. To a solution of seco acid **S6** (207 mg) in CH_2Cl_2 (80 ml) was slowly added MNBA (202 mg, 0.59 mmol) and DMAP (110 mg, 1.17 mmol) in CH_2Cl_2 (80 ml) After stirred at room temperature for 4 hours, the reaction mixture was quenched with sat. aq. sodium bicarbonate and extracted with diethyl ether. The organic layer was washed with brine and dried over sodium sulfate. The resulting solution was concentrated in vacuo and purified via flash SiO_2 chromatography (hexane/AcOEt, 1/0 to 5/1) to yield the macrolactone **17** as a yellow solid. (55 mg, 28% for 3 steps)

R_f = 0.45 (silica gel, hexane/AcOEt=4/1); $[\alpha]_D^{26}$ +55.4613 (*c* 1.18 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ7.75 (2H, d, *J* = 7.5 Hz, Fmoc), 7.58 (2H, m, Fmoc), 7.40-7.36 (4H, m, Fmoc, MP), 7.33 (2H, d, *J* = 8.7 Hz, MP), 7.29 (2H, td, *J* = 7.5, 0.9 Hz, Fmoc), 6.80 (4H, dd, *J* = 21.7, 8.8 Hz, MP), 6.49 (1H, dd, *J* = 10.9, 15.1 Hz), 6.11-6.35 (9H, m, H21, 22, 23, 24, 25, 26, 27, 28,

29), 5.83 (1H, dd, *J* = 24.9, 15.2 Hz, H31), 15.79 (1H, dd, *J* = 14.7, 6.7 Hz, H20), 5.45 (1H, s, MP), 5.40 (1H, s, MP), 5.31 (1H, d, *J* = 6.0 Hz, SEM), 5.24 (1H, d, *J* = 6.0 Hz, SEM), 4.88 (1H, d, *J* = 9.9 Hz, NH), 4.78 (1H, t, *J* = 7.1 Hz), 4.66 (1H, dd, *J* = 36.0, 10.5 Hz), 4.52 (1H, t, *J* = 6.7 Hz, H19), 4.43 (1H, dd, *J* = 10.5, 6.6 Hz, Fmoc), 4.42 (1H, br, H1'), 4.35 (1H, dd, *J* = 10.5, 6.6 Hz), 4.16-4.24 (3H, m, Fmoc, H3, H15), 3.84 (2H, m, H3, 11), 3.78 (3H, s, -OMe), 3.77 (3H, s, -OMe), 3.63-3.75 (7H, m, H5, 8, 9, 17, 35, 3', 2'), 3.25-3.26 (2H, m, H4', H5'), 3.02 (3H, s, -OMe), 2.79 (1H, m, H34), 2.67 (1H, dd, *J* = 17.3, 6.0 Hz, H2a), 2.29-2.36 (2H, m, H2b, H16), 2.21 (1H, dd, *J* = 13.3, 4.6 Hz), 19.3-2.0 (2H, m), 1.74-1.89 (3H, m), 1.64-1.74 (2H, m), 1.50 (2H, br), 1.32 (1H, m), 1.17-1.29 (m), 1.00 (3H, d, *J* = 6.9 Hz, H38), 1.95 (3H, H40), 0.72-0.93 (53H, m, TBS, SEM, H39, H6'), 0.10-−0.147 (39H, m, TBS, SEM); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.18, 169.53, 159.64, 159.57, 155.44, 143.97, 143.90, 141.31, 136.24, 136.07, 135.13, 134.46, 134.02, 133.56, 132.16, 132.08, 131.72, 131.40, 131.20, 131.14, 130.36, 130.19, 129.14, 127.56, 127.52, 127.21, 126.92, 124.88, 123.21, 122.97, 121.84, 119.85, 113.39, 113.24, 100.49, 100.09, 99.93, 97.85, 89.69, 80.04, 77.20, 75.36, 75.11, 73.78, 72.99, 72.44, 72.40, 72.32, 69.57, 68.17, 68.03, 67.72, 66.92, 66.60, 60.31, 57.02, 56.01, 55.19, 55.13, 53.37, 47.96, 47.14, 42.98, 42.41, 40.29, 37.23, 37.23, 36.32, 31.96, 31.79, 31.53, 29.63, 26.79, 26.04, 26.93, 25.71, 25.58, 22.60, 20.98, 18.87, 18.81, 18.44, 18.33, 18.24, 18.17, 18.06, 17.93, 17.68, 14.15,14.08, 10.85, −1.38, −1.47, −3.53, −3.99, −4.07, −4.24, −4.31, −4.44, −4.57, −5.17, −5.37; MS (ESI) *m*/*z* calcd for $C_{115}H_{180}FNaO_3Si_6$[M+Na^+] 2137.1494, found: 2137.1497.

Conversion of 17 to S7. To a suspension of macrolactone **17** (112 mg, 53 μmol) in MeOH (2.7 mL) was added 18% HF-pyridine (2.1 ml) at room temperature. Then the mixture was warmed to 50 ^{o}C. After stirred at 50 ^{o}C for 40 hours, the mixture was poured into sat. aq. sodium

bicarbonate. The resulting mixture was extracted with AcOEt and organic layer was washed with 0.25 M HCl, sat. aq. sodium bicarbonate and brine. The extract was dried over sodium sulfate and concentrated in vacuo. The crude product was desalinized via ODS chromatography to yield yellow solid (83 mg).

To a solution of the resulting yellow solid (83 mg) in CH_2Cl_2 (6.0 ml) was added piperidine (160 µl, 1.6 mmol). After stirred at room temperature for 4 hours, the resulting solution was concentrated in vacuo and it was purified by flash SiO_2 chromatography ($CHCl_3$/MeOH, 20/1 to 0:1) to obtain compound **S7** as a yellow solid (78 mg, impure).

Conversion of S7 to 5. To a solution of compound **S7** (78 mg) in MeOH (5.2 ml) was added conc. HCl (434 µl) at 0 ºC. After stirred for 20 minutes at 0 ºC, the reaction mixture was quenched with solid sodium bicarbonate (542 mg). The resulting suspension was filtered and the filtrate was concentrated in vacuo. To a solution of the resulting yellow solid in H_2O (1.1 ml) and *t*-BuOH (4.2 ml) was added conc. HCl (217 µl) at 0 ºC. After stirred for 5 hours at 0 ºC, the reaction mixture was directly charged onto ODS column (H2O/MeOH= 1/0→0/1). Further purification by HPLC yielded pure 32-F-AmB **5** as a yellow powder (8.5 mg, 17% for 4 steps).

R_f = 0.10 ($CHCl_3$/MeOH, 5/1); ^{1}H NMR (500 MHz, MeOH-d_4/DMSO-d_6, 5/1) δ 6.27-6.55 (8H, m, H22-30), 6.08 (1H, dd, *J* = 15.3, 8.5 Hz, H20), 6.17 (1H, dd, *J* = 15.3, 10.5 Hz, H21), 6.021 (1H, dd, *J* = 25.9, 15.3 Hz, H31), 5.16 (1H, m, *J* = 6.2, 3.01 Hz, H37), 4.73 (1H, dd, *J* = 10.7, 35.3 Hz, H33), 4.62 (1H, s, H1'), 4.35 (1H, m, H19), 4.38 (1H, m, H11), 4.37 (1H, m, H17), 4.24 (1H, brddd, *J* = 10.9, 4.5, 4.5 Hz, H15), 4.14 (1H, brdddd, *J* = 8.4, 8.4, 4.4, 4.4 Hz, H3) , 3.98 (1H, brs, H2'), 3.71 (1H, m, H5), 3.62 (1H, brd, *J* = 10.8 Hz, H9), 3.34 (1H, m, H5'),

3.32 (1H, m, H4'), 3.27 (1H, m, H35), 3.24 (1H, m, H8), 3.07 (1H, br, H3'), 2.82 (1H, m, H34), 2.24 (1H, dd, *J* = 16.5, 4.4 Hz, H2a), 2.28 (1H, m, 18a), 2.22 (1H, dd, *J* = 16.5, 8.02, H2b), 2.04 (1H, m, H14a), 1.99 (1H, m, h16), 1.88 (1H, m, H36), 1.69 (2H, m, 12ab), 1.68 (1H, m, 18b), 1.66 (1H, m, 10a), 1.61 (1H, m, 7a), 1.51-1.36 (6H, m, H4ab, H6ab, 7b, 10b), 1.27 (3H, d, *J* = 5.5 Hz, H6'), 1.21 (3H, d, *J* = 6.3 Hz, H38), 1.12 (3H, d, *J* = 6.4 Hz, H40), 1.02 (3H, d, *J* = 7.02 Hz, H39); ^{13}C NMR (100 MHz, MeOH-d_4/DMSO-d_6, 4/1) δ 176.54, 170.57, 156.04 (d, *J* = 243.6 Hz), 137.29, 135.81, 135.35, 134.96, 134.35, 134.20, 132.26, 131.62, 131.39, 129.62, 129.06, 127.85, 122.97, 97.38, 97.20, 77.55, 76.19, 74.42, 74.34, 74.03, 73.21, 70.66, 70.10, 69.68, 68.22, 66.64, 66.23, 65.97, 58.46, 55.90, 45.91, 44.73, 44.34, 42.44, 42.26, 39.7, 39.6, 35.09, 34.10, 29.44, 18.68, 17.85, 17.18, 12.04; MS (ESI) *m*/*z* calcd for $C_{47}H_{72}FNNaO_{17}$[M+Na^+] 964.4676, found: 964.4671.

7. Biological activity and sterol selectivity of 32-F-AmB.

METHODS

Hemolytic activity assay: Freshly collected human blood was centrifuged for 5 min at 1000*g*, and separated erythrocytes were washed three times by suspending in PBS buffer (pH 7.4). Sedimented erythrocytes were then resuspended with PBS to 100-fold volume of the original blood. Gradually diluted drug solutions (DMSO solution, 4 μL) were added to the erythrocyte suspensions (196 μL), and the suspensions were incubated with gently shaking at 38 °C. After 18 h, the suspensions were spun and the absorbance of the supernatant was determined at 450 nm by micro-plate reader (Molecular Devices). For a positive control, water was used instead of PBS buffer. As a negative control, 4 μL of DMSO was added instead of sample solution. From dose-response curves, the dosage that led to 50% hemolysis (EC_{50}) was determined.

Antifungal activity assay: Aspergillus niger was cultured in a GP liquid medium (2% glucose, 0.2% yeast extract, 0.5% polypeptone, 0.05% $MgSO_4$, and 0.1% KH_2PO_4) at 25 °C for 2 days. An aliquot of the broth was then spread onto a GP agar plate. The drugs dissolved in DMSO were spotted on paper disks of 8 mm in diameter. As a control, a disk containing only DMSO was also prepared. These paper disks were then placed on an agar plate containing *Aspergillus niger* mycelia. After cultivating at 25 °C for 83 hours, the diameter of the inhibitory zone on each paper disk was measured.

K^+ flux assays using ^{31}P NMR: Large unilamellar vecicles (LUV) were prepared according to methods reportedly by Hervé et al.[3] Briefly, 204 μmol of POPC and Ergosterol or Cholesterol (22.7 μmol) was dissolved in $CHCl_3$, and the mixture was evaporated to a thin film in a

round-bottom flask. After drying in vacuo for over 18 h, 3 mL of pH 4.5 buffer containing 0.4 M KH_2PO_4 and 1M EDTA in H_2O-D_2O 6:4 was added to the test tube. The lipid mixture was suspended in the buffer by vortexing and sonication. The resultant suspension was frozen at −20 °C and thawed at 60 °C four times to form large vesicles. After the sizing of the liposomes using Liposofast® by filtering 9 times through a polycarbonate filter of 200 nm pore size, 1 mL of the LUV suspension was diluted with 5 mL of 0.4 M K_2SO_4. Then, the LUV suspension was adjusted to pH 7.5 with KOH, and 750 μL of the suspension was mixed with 2 μL of 10 mM carbonyl cyanide-*p*-trifluoromethoxyphenyl hydrazone (FCCP) in ethanol. Then a DMSO solution of AmB or 32-F-AmB (6.39 μL) was added to the LUV suspension. After incubated for 3 hours at 24 °C, 550 μL of the LUV suspension was transferred to a 5 mm NMR glass tube and mixed with 4.4 μL of 100 mM $MnCl_2$. ^{31}P NMR spectrum was recorded at 25 °C on a JEOL ECS-400 spectrometer (^{31}P at 161.835 MHz) with ^{1}H-broad band decoupling.

Table S1. Hemolytic and antifungal assay results for AmB (**1**) and 32-F-AmB (**3**)

Compound	Haemolytic activity EC_{50} (μM)[a]	Antifungal activity (μg)[b]
AmB (**1**)	5.6	10
32-F-AmB (**5**)	6.5	10

[a] Against 1% human erythrocytes. [b] The minimal amount of samples on a paper disk that shows inhibitory zone on the culture of *Aspergillus niger*. The size of inhibition zone after 83 h is shown the Table below, which further confirm the comparable activity of 32-F AmB to that of natural AmB.

compounds	μg/disk	diameter of inhibition zone (cm)
AmB	30	1.8
	10	1.4
32-F AmB	30	2.1
	10	1.6

In order to evaluate the sterol selectivity, we assessed the K^+-permeabilizing activity of the derivative using artificial liposomes which comprise 10% Erg- or cholesterol-containing POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine). The liposomes possess a higher external K^+ concentration and trans-membrane pH gradient. Once K^+ influx into the liposomes takes place through ion channels formed across the membrane, H^+ leaks out of the liposome in the presence of proton-transporter FCCP, leading to a pH increase in liposome lumen. This pH change was monitored as chemical shift change of phosphate anions in ^{31}P NMR[3]; a down field signal of phosphate ions at δ 3.1 was ascribed to permeabilized liposomes, while an up-field signal at δ 1.2 was due to intact liposomes. Signals of phosphate ions outside the liposomes were quenched by paramagnetic Mn^{2+}. Accordingly, the channel formation of drug reduces the ^{31}P NMR peak at δ 1.2 and increases that at δ 3.1. Figure S8 shows the results of ion flux assays obtained from natural AmB and 32-F-AmB. These results demonstrated that 32-F-AmB elicited an all-or-none type K^+-flux in Erg-containing membrane, where two peaks were clearly seen at *δ* 1.2 and 3.1 resulting from intact and permeabilized liposomes, respectively. The all-or-none response reflects an instant pH increase in liposomes, thus implying the formation of ion channels with high conductance.[3] On the other hand, in the case of cholesterol-containing membrane, 32-F-AmB scarcely induced ion flux. These results admittedly demonstrate that 32-F-AmB retains ion-channel forming activity and sterol selectivity. From biological (Table S1) and K^+-permeabilizing activities (Figure S8), we concluded that 32-F-AmB forms the ion-permeable channel assemblies, which reproduce those of natural AmB.

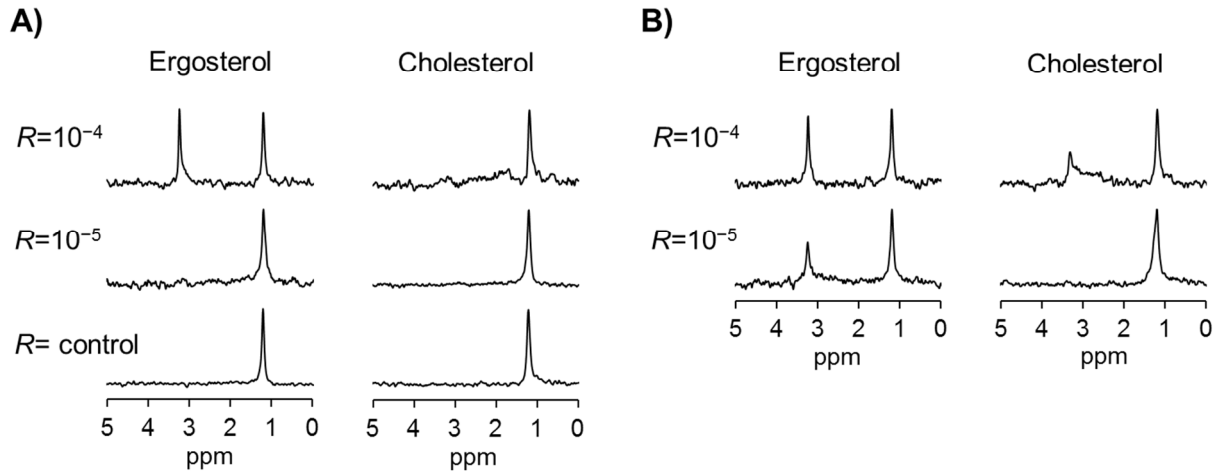


Figure S8. Membrane-permeabilizing activity of AmB and 32-F-AmB using ^{31}P NMR spectra of liposome-entrapped phosphate. AmB (A) and 32-F-AmB (B) were added to liposomes and incubated for 6 h. For control experiments, DMSO was added to each liposome instead of AmB solution (A, bottom). The lipid concentration of all the liposome suspensions was 12 mM. Liposomes were composed of 10% cholesterol-, or 10% Erg-containing POPC. The AmB/lipid ratios were 10^{-5} (lower) and 10^{-4} (upper). The peak around δ 1.2 corresponds to $H_2PO_4^-$ at pH 5.5 (intact liposomes) and that around δ 3.1 corresponds to HPO_4^{2-} at pH 7.5 (permeabilized liposomes). Signals between δ 1.2 and 3.1 are derived from liposomes with inside pH between 5.5 and 7.5.

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