Electronic Supplementary Information

Gramicidin S-inspired Antimicrobial Cyclodextrin to Disrupt Gram-negative and Gram-positive Bacterial Membranes

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Azide CD acylates 2-6

Preparation of azide γ-CD propanoate **3** was described. The other derivatives **4-6** were prepared similarly. The acetate **2** was synthesized as described in the lliterature.[1]

azide γ-CD propanoate 3

Octakis-6-azido-γ-CD **1** (44.3 mg, 9.64×10^{-5} mol) was reacted with propanoic anhydride (1.61 g, 1.23×10^{-2} mol) and dimethylaminopyridine (37.6 mg, 3.08×10^{-4} mol) in dry pyridine (5 cm^3) at r.t. for 3 days. Propanoic anhydride (0.80 g, 6.17×10^{-3} mol) was added and the reaction mixture was stirred for more 3 days and concentrated *in vacuo*. To the residue, ethyl acetate was added followed by washing with 0.5 M aq. HCl and sat. $NaHCO_3$,

and the organic layer was dried over Na_2SO_4. Silica gel column chromatography (hexane/EtOAc) gave the propanoate **3** (152 mg, 65.6%):: ^{1}HNMR (400 MHz, $CDCl_3$): δ 1.09-1.14 (48H, CH_3), 2.20-2.44 (32H, -CH_2-), 3.58 (8H, dd, *J* 4.0, 14.0, CD-H6), 3.74 (8H, CD-H6), 3.81 (8H, t, *J* 9.0, CD-H4), 3.95 (8H, CD-H5), 4.76 (8H, dd, *J* 3.5, 10.0, CD-H2), 5.16 (8H, d, *J* 3.5, CD-H1), 5.32 (8H, t, *J* 9.0, CD-H3), IR (KBr):2106.85, Found: C, 48.18; H, 6.10; N,13.80%. Calcd for C96H136N24O48: C, 48.16; H, 5.73; N, 14.04%.

azide γ-CD butanoate 4

^{1}HNMR (400 MHz, $CDCl_3$): δ 0.91-0.98 (48H, CH_3), 1.59-1.65 (32H, CH_3-$\underline{CH_2}$-CH_2-), 2.13-2.42 (32H, CH_3-CH_2-$\underline{CH_2}$-), 3.57 (8H, dd, *J* 4.0, 14.0, CD-H6), 3.59-3.82 (16H, CD-H4,6), 3.95 (8H, CD-H5), 4.73 (8H, dd, *J* 3.5, 10.0, CD-H2), 5.17 (8H, d, *J* 3.5, CD-H1), 5.34 (8H, t, *J* 9.5, CD-H3), IR (KBr):2105.89, MS (ESI): m/z 2641.1328 $[M+Na]^+$, Found: C, 51.67; H, 6.84; N,12.37%. Calcd for C112H168N24O48+CH_3COCH_3: C, 51.60; H, 6.55; N, 12.56%.

azide γ-CD 2-methylpropanoate 5

^{1}HNMR (400 MHz, $CDCl_3$): δ 1.09-1.19 (96H, CH_3), 2.48-2.56 (16H, CH), 3.51 (8H, d, *J* 13.5, CD-H6), 3.76 (8H, d, *J* 13.5, CD-H6), 3.95 (16H, CD-H4,5), 4.78 (8H, dd *J* 4.0, 9.5, CD-H2), 5.18 (d, 8H, *J* 4.0, CD-H1), 5.30-5.34 (8H, CD-H3), IR (KBr):2105.89, MS (ESI): m/z 2641.1353 $[M+Na]^+$, Found: C, 51.30; H, 6.79; N, 12.60%. Calcd for C112H168N24O48+0.6H_2O: C, 51.16; H, 6.49; N, 12.78%.

azide γ-CD 3-methylbutanoate 6

^{1}HNMR (400 MHz, $CDCl_3$): δ 0.92-0.97 (96H, CH_3), 2.00-2.27 (40H, $(CH_3)_2\underline{CHCH_2}$), 2.41 (8H, dd, J 5.0, 15.0, $(CH_3)_2CH\underline{CH_2}$), 3.60 (dd, 8H, *J* 4.0, 13.5, CD-H6), 3.76 (16H, CD-H4,6), 3.97 (8H, CD-H5), 4.70 (8H, dd, *J* 3.5, 10.5, CD-H2), 5.17 (8H, d, *J* 3.5, CD-H1), 5.38 (8H, t, J 9.5, CD-H3), IR (KBr):2105.89, MS (ESI): m/z 2865.3850, $[M+Na]^+$, Found: C, 54.14; H, 7.48; N, 11.39%. Calcd for C128H200N24O48+H_2O+CH_3COCH_3: C, 53.90; H, 7.18; N, 11.52%.

Amino CD acylates

Preparation of amino γ-CD propanoate **8** was described. The other derivatives **7** and **9-14** were prepared similarly.

amino γ-CD propanoate 8

A reaction solution was prepared by dissolving octakis(6-azido-2,3-propanoyl)-γ-CD **3** (48.3 mg, 2.02×10^{-5} mol) in DMSO–H_2O (5:1) (4.8 cm^3) containing *N*-Boc-propargylamine (31.4 mg, 2.02×10^{-4} mol, 10 mol eq. to the CD), $CuSO_4$ $5H_2O$ (4.1 mg, 0.8 mol eq.), and sodium ascorbate (40.2 mg, 10 mol eq.). After MW heating (100°C, 30 min), ethyl acetate was added followed by washing with 5% aq. EDTA. Silica gel column chromatography ($CHCl_2$/MeOH) gave the click reaction product (65.3 mg, 89.0%). Deprotection of the Boc group with TFA f gave the desired product **8** in 88.5%:

^{1}H NMR (400 MHz, DMSO-d_6): δ 0.80-1.10 (48H, $\underline{CH_3}$-CH_2-), 2.16-2.32 (32H, CH_3-$\underline{CH_2}$), 3.61 (8H, CD-H4), 3.99-4.06 (16H, -$\underline{CH_2}$-NH_3^+), 4.39 (8H, CD-H5), 4.65-4.72 (24H, CD-H2,6), 5.29-5.35 (16H, CD-H1,3), 8.11 (8H, triazole CH), 8.29(24H, -NH^{3+}), ^{13}CNMR (100 MHz, DMSO-d_6): δ 8.6, 8.7 ($\underline{CH_3}$-CH_2-), 26.6 (CH_3-$\underline{CH_2}$), 33.9 (-$\underline{CH_2}$-NH_3^+), 49.5 (CD-6), 69.6 (CD-5), 69.72 (CD-2,3), 76.2 (CD-4), 96.0 (CD-1), 126.2 (CHtriazole), 140.3 (Ctriazole), MS (MALDI): m/z 2856.2243 $[M+Na]^+$, 2872.1800 $[M+K]^+$, Found: C, 40.63; H, 5.30; N,10.84%. Calcd for C120H176N32O48+8CF_3COOH +16H_2O: C, 40.48; H, 5.40; N, 11.11%.

amino γ-CD acetate 7

^{1}H NMR (400 MHz, DMSO-d6): δ 1.90-2.10 (48H, CH_3), 3.58 (8H, CD-H4), 3.90-4.20 (16H, -$\underline{CH_2}$-NH_3^+), 4.36 (8H, CD-H5), 4.55-4.80 (24H, CD-H2,6), 5.28 (8H, CD-H3), 5.38 (8H, CD-H1), 8.11 (8H, triazole CH), 8.41(24H, -NH^{3+}), ^{13}CNMR (100 MHz, DMSO-d6): δ 20.4 (CH_3), 33.9 (CH_2), 49.4 (CD-6), 69.6 (CD-5), 69.7 (CD-2,3), 76.2 (CD-4), 95.9 (CD-1), 117.0 ($CF_3\underline{CO}$), 126.2 (CHtriazole), 140.3 (Ctriazole), 158.8 (CF_3), 169.4, 169.9 (CO), MS (MALDI): m/z 2611.0267 $[M+H]^+$, 2633.0036 $[M+Na]^+$, Found: C, 39.19; H, 4.75; N, 11.97%. Calcd for C104H144N32O48+8CF_3COOH+7H_2O: C, 39.50; H, 4.59; N, 11.97%.

amino γ-CD butanoate 9

^{1}H NMR (400 MHz, DMSO-d6): δ 0.88 (48H, t, J 7.0), 1.40-1.62 (32H), 2.08-2.46 (32H), 3.20-3.85, 3.85-4.25, 4.25-4.50, 4.50-4.90 (56H), 5.20-5.50 (16H), 8.11 (8H), 8.39 (24H), ^{13}CNMR (100 MHz, DMSO-d6): δ 13.2, 17.4, 33.9, 35.0, 49.4, 69.5, 96.0, 126.1, 140.4, 171.6, 172.2,
MS (MALDI): m/z 3081.4785 $[M+Na]^{+}$, 3097.4893 $[M+K]^{+}$, Found: C, 43.74; H, 5.69; N, 10.34%. Calcd for C136H208N32O48+8CF_3COOH+10H_2O+DMSO: C, 43.73; H, 5.77; N, 10.60%.

amino γ-CD 2-methylpropanoate 10
^{1}H NMR (400 MHz, DMSO-d6): δ 0.50-1.30 (96H), 2.30-2.70 (16H), 3.70-4.30, 4.30-5.10, 5.10-5.80 (72H), 7.80-8.30 (8H), 8.30-9.10 (24H),
^{13}CNMR (100 MHz, DMSO-d6): δ 17.9, 18.5, 32.9, 33.7, 49.7, 69.2, 95.3, 126.7, 140.1, 174.2, 175.4, MS (MALDI): m/z 3081.5070 $[M+Na]^{+}$, Found: C, 44.60; H, 5.69; N, 10.64%. Calcd for C136H208N32O48+8CF_3COOH+7H_2O: C, 44.55; H, 5.66; N, 10.94%.

amino γ-CD 3-methylbutanoate 11
^{1}H NMR (400 MHz, DMSO-d6): δ 0.65-1.10 (96H), 1.80-2.40 (48H), 3.20-3.80 (8H), 3.80-4.30 (16H), 4.30-5.10 (32H), 5.10-5.80 (16H), 8.11 (8H), 8.40 (24H), ^{13}CNMR (100 MHz, DMSO-d6): δ 22.0, 24.6, 33.8, 42.0, 50.0, 70.0, 76.6, 96.0, 126.2, 140.2, 158.6, 171.3, MS (MALDI): m/z 3305.7083 $[M+Na]^{+}$, Found: C, 47.31; H, 6.13; N, 10.25%. Calcd for C152H240N32O48+8CF_3COOH+3.5H_2O: C, 47.38; H, 6.03; N, 10.52%.

amino γ-CD 12
The product (15.6 mg, 4.57×10^{-6} mol) obtained from click reaction of azide CD acetate **2** and *N*-Boc-propargylamine was deprotected with $NaOCH_3$-CH_3OH and followed by trifluoroacetic acid to give **12** (13 mg) quantitatively.
^{1}H NMR (400 MHz, DMSO-d_6): δ 3.15-3.40 (16H, CD-H2,4), 3.68 (8H, t, J 9.0, CD-H3)), 3.95 (16H, -$\underline{CH_2}$-NH_3^{+}), 4.03 (8H, CD-H5), 4.30-4.43 (16H, CD-H6), 5.10 (8H, CD-H1)), 6.07-6.13 (16H, CD-OH), 8.02 (8H, triazole CH), 8.36 (24H, -NH^{3+}), ^{13}CNMR (100 MHz, DMSO-d_6): δ 33.8 (CH_2), 49.6 (CD-6), 69.8 (CD-5), 72.0 (CD-2), 72.2 (CD-3), 82.2 (CD-4), 101.7 (CD-1), 117.1 ($CF_3\underline{CO}$), 125.8 (CHtriazole), 140.2 (Ctriazole), 158.8(CO), MS

(MALDI): m/z 1960 $[M+Na]^+$, 1976 $[M+K]^+$, Found: C, 35.56; H, 4.79; N, 14.65%. Calcd for $C72H112N32O32+8CF_3COOH+8H_2O$: C, 35.30; H, 4.58; N, 14.97%.

amino α-CD propanoate 13

^{1}H NMR (500 MHz, DMSO-d_6): δ 0.96-1.01 (36H, $\underline{CH_3}$-CH_2-), 2.18-2.32 (24H, CH_3-$\underline{CH_2}$), 3.66 (8H, CD-H4), 4.02 (12H, -$\underline{CH_2}$-NH_3^+), 4.55 (8H, CD-H5), 4.62 (6H, CD-H2), 4.77 (6H, CD-H6), 5.37 (6H, CD-H1), 5.50 (16H, CD-H3), 8.08 (8H, triazole CH), 8.44 (24H, -NH^{3+}), ^{13}CNMR (125 MHz, DMSO-d_6): δ 8.8 ($\underline{CH_3}$-CH_2-), 26.7 (CH_3-$\underline{CH_2}$), 33.8 (-$\underline{CH_2}$-NH_3^+), 49.8 (CD-6), 69.2 (CD-5), 70.0 (CD-2,3), 77.1 (CD-4), 96.3 (CD-1), 126.6 (CHtriazole), 140.3 (Ctriazole), 172.4, 173.3 (CO), MS (MALDI): m/z 2125.9314 $[M+H^+]$, 2147.9155 $[M+Na]^+$ 2163.8894 $[M+K]^+$, Found: C, 42.12; H, 5.37; N, 11.16%. Calcd for $C90H132N24O36+6CF_3COOH+6H_2O$: C, 41.98; H, 5.18; N, 11.52%.

amino β-CD propanoate 14

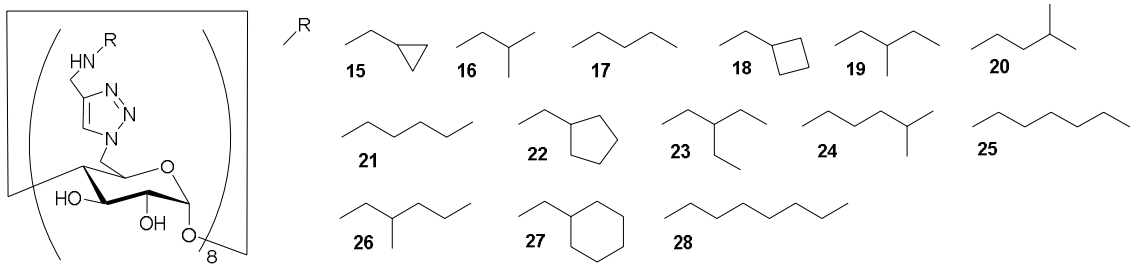
^{1}H NMR (500 MHz, DMSO-d_6): δ 0.98-1.01 (42H, $\underline{CH_3}$-CH_2-), 2.17-2.33 (28H, CH_3-$\underline{CH_2}$), 3.60 (7H, CD-H4), 3.97-4.06 (14H, -$\underline{CH_2}$-NH_3^+), 4.44 (7H, CD-H5), 4.61-4.67 (21H, CD-H2,6), 5.32-5.38 (14H, CD-H1,3), 8.08 (7H, triazole CH), 8.40 (21H, -NH^{3+}),
^{13}CNMR (125 MHz, DMSO-d_6): δ 8.6, 8.8 ($\underline{CH_3}$-CH_2-), 26.7 (CH_3-$\underline{CH_2}$), 33.8 (-$\underline{CH_2}$-NH_3^+), 49.7 (CD-6), 69.4, 69.8 (CD-2,3,5), 76.5 (CD-4), 96.0 (CD-1), 126.3 (CHtriazole), 140.3 (Ctriazole), 172.4, 173.3 (CO),
MS (MALDI): m/z 2502.0633 $[M+Na]^+$ 2518.0413 $[M+K]^+$,
Found: C, 43.12; H, 5.02; N, 11.83%. Calcd for $C105H154N28O42+7CF_3COOH+2H_2O$: C, 43.31; H, 5.24; N, 11.46%.

Bacteria. The microorganism *Bacillus subtilis* 168 cells, *Staphylococcus aureus* cells FDA 209P cells, *Escherichia coli* K12 W3110, *Salmonella* Typhimurium LT2, and *Pseudomonas aeruginosa* PAO1 cells were used.

Minimum inhibitory concentration (MIC). MICs against *B. subtilis*, *S. aureus*, *E. coli*, *S.* Typhimurium, and *P. aeruginosa* were determined by the liquid microdilution method, using serially diluted (two-fold) CDs. Cell (1

x 10^4) were cultured at 37 °C for 20 h in Mueller-Hinton broth (0.1 cm^3) containing CD in 96-well microtiter plate. The MIC was determined as the lowest concentration of CD at which cells were unable to grow.

SI Table 1. Minimum inhibitory concentration (MIC, μM) of the alkylamino-modified γ-CDs **15-28** against gram-positive *S. aureus* and *B. subtilis* strains and gram-negative *E. coli*, *S.* Typhimurium, and *P. aeruginosa* strains.[a]

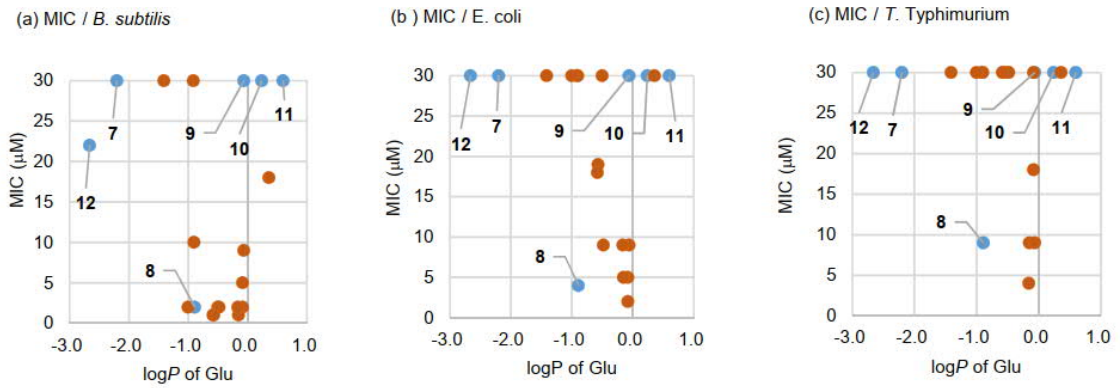


Compounds		Gram-positive strains		Gram-negative strains		
	log*P*[b]	*S. aureus*	*B. subtilis*	*E. coli*	*S.* Typhimurium	*P. aeruginosa*
15	-1.41	>39	>39	>39	>39	>39
16	-0.91	>39	>39	>39	>39	>39
17	-0.90	5	10	>39	>39	>39
18	-1.00	2	2	>38	>38	>38
19	-0.50	1	2	>38	>38	>38
20	-0.57	1	1	19	>38	>38
21	-0.48	1	2	9	>38	>38
22	-0.58	9	1	18	>36	>36
23	-0.08	2	2	5	18	18
24	-0.15	1	1	5	9	9
25	-0.06	5	9	9	9	5
26	-0.08	9	5	2	36	5
27	-0.16	4	2	9	4	18
28	0.36	35	18	>35	>35	.35

[a] MICs were determined by the liquid microdilution method.

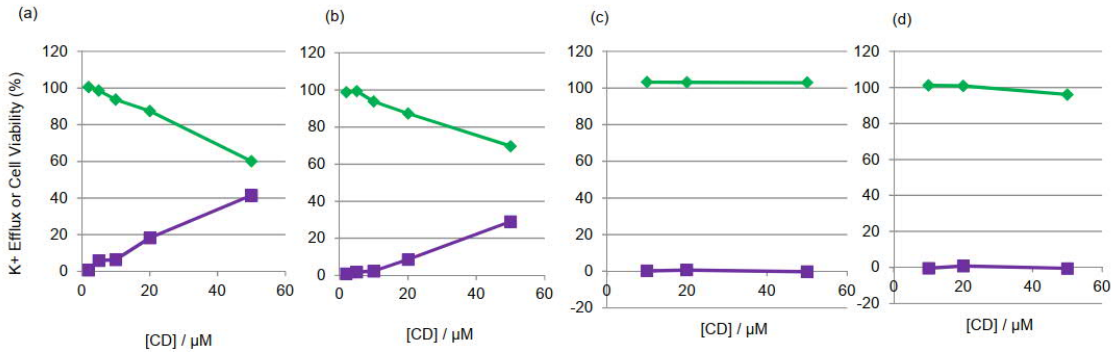
[b] Values of the corresponding substituted glucose.

SI Fig. 1 The relationship of the log*P* values of the amino acylates **7-12** and alkylamino derivatives **15-28** with their MICs against gram-positive *B. subtilis* (a) and gram-negative *E.coli* (b) and *S.* Typhimurium (c). Blue dots; amino acrylates **7-12**, orange dots; alkylamino derivatives **15-28**.



K^+ efflux and cell viability. Bacterial cells were washed twice with the buffer (100 mM choline chloride/50 mM Mops-Tris, pH 7.2) and suspended in this buffer at 2 x 10^9 cells cm^{-3}. The final volume of the cell suspension was 1 cm^3. The cells were incubated with CD at 37 °C for 30 min. After incubation, 0.1 cm^3 of the cell suspension was taken, diluted with physiological saline, and dispersed on an agar plate prepared with 1% polypepton, 0.5% yeast extract, 0.5% NaCl, and 1.5% agar (pH was adjusted by adding 1 M NaOH). The colonies were counted after standing overnight at 37 °C and the viability of the cells was determined. The remaining cell suspension was centrifuged, and the amount of K^+ in the supernatant was measured using a K^+-selective electrode. To determine the 100% level of K^+ efflux, melittin (10 μM for *S. aureus*) and polymixin B (200 μg cm^{-3}) were used.

SI Fig. 2 Dose-dependent curves for the K^+ efflux and cell viability of bacteria on addition of the butanoate **9** ((a) *S. aureus*, (b) *E.coli*) and native γ-CD **(**(c) *S. aureus* and (d) *E.coli*. Purple lines; K^+ efflux, green lines; cell viability. Cells were incubated with the CD derivative for 30 min at 37 °C. To determine the 100% level of K^+ efflux, melittin (10 μM for *S. aureus*) and polymixin B (200 μg cm^{-3} for *E. coli*) were used.



Hemolytic activity. Rabbit erythrocytes obtained from rabbit blood (NIPPON BIO-TEST LABORATORIES INC.) were suspended in buffer (150 mM NaCl/10 mM Hepes-NaOH, pH 7.4) at a final concentration of 0.5% hematocrit. After incubation with CDs at 37 °C for 30 min, hemolysis was estimated by measuring the absorbance at 540 nm. Lysolethicin (50 μM) was used to determine the 100% level of hemolysis.

Resistance development study of *S. aureus* and *E. coli* by multipassage treatment of CD 14.[2]

MIC values of CD **14**, fosfomycin and norfloxacin against *S. aureus* and *E. coli* were determined as described in experimental section. For each MIC experiment, the bacterial cells exposed to the sub-MIC concentration (1/2 of MIC at that particular passage) were re-grown to a logarithmic growth phase, and re-used for the subsequent passage's MIC measurement for the same antimicrobial agents. The process was repeated and the changes of MIC values were monitored.

References

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