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Electronic Supplementary Information

Synthesis of Antimicrobial Cyclodextrins Bearing Polyarylamino and Polyalkylamino Groups via Click Chemistry for Bacterial Membrane Disruption

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1. Synthesis of Compounds

General

^{1}H NMR spectra were recorded at 30 °C on a Bruker AVANCE400Plus Nanobay. Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass measurements were conducted using a JEOL JMS-S3000 spectrometer. A Biotage Initiator EXP microwave reactor was used for the Huisgen reactions.

Benzylamino derivative 3

A reaction solution was prepared by dissolving octaazido–γ-CD derivative **2** (52.2 mg, 3.49×10^{-5} mol) in DMSO–H_2O (10:1) (10 cm^3) containing *N*-benzyl-*N*-2-propynylamine (53 mg, 1.25 mol eq. to the azide group), $CuSO_4$ $5H_2O$ (6.7 mg, 0.1 mol eq.), and sodium ascorbate (69.2 mg, 1.25 mol eq.). After MW heating (120 °C, 20 min), water (50 dm^3) was added and the solution lyophilized. The residue was washed with acetone and 10% aq. NH_3 and then lyophilized to give **3** (71.8 mg, 77%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 5.12 (H1), 7.22 (Hphenyl), 7.73 (Htriazole), MS (MALDI): m/z 2659.3 $[M + H]^+$, 2780.9 $[M + Na]^+$, Found: C, 53.82; H, 5.93; N,15.51%. Calcd for C128H161N32O32+10.1 H_2O: C, 54.12; H, 6.39; N, 15.78%.

Phenylpentylamino derivative 5

A reaction solution was prepared by dissolving octaazido–γ-CD derivative **2** (200 mg, 1.34 × 10^{-4} mol) in DMSO–H_2O (10:1) (22 cm^3) containing *N*-phenylpentyl-*N*-2-propynylamine (297 mg, 1.25 mol eq. to the azide group), $CuSO_4$ $5H_2O$ (26.8 mg, 0.1 mol eq.), and sodium ascorbate (265 mg, 1.25 mol eq.). After MW heating (120 °C, 90 min) and filtration, the solution was concentrated in vacuo. The residue was then washed with acetone and 10% aq. NH_3 and lyophilized to give **5** (361 mg, 87%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ 1.21-1.48 (H_{CH2}), 5.09 (H1), 7.11-7.21 (Hphenyl), 7.73 (Htriazole), MS (MALDI): m/z 3144.6 $[M + K]^+$, Found: C, 60.17; H, 7.01; N,12.96%. Calcd for C160H224N32O32+7.4H_2O: C, 59.29; H, 7.43; N, 12.83%.

Cyclohexylmethylamino derivative 6

A reaction solution was prepared by dissolving octaazido–γ-CD derivative **2** (200 mg, 1.34 × 10^{-4} mol) in DMSO–H_2O (10:1) (22 cm^3) containing *N*-cyclohexylmethyl-*N*-2-propynylamine (250 mg, 1.5 mol eq. to an azide group), $CuSO_4$ $5H_2O$ (26.8 mg, 0.1 mol eq.), and sodium ascorbate (265 mg, 1.25 mol eq.). After MW heating (120°C, 10 min) treatment followed by filtration, the solution was concentrated in vacuo. The residue was washed with acetone and 10% aq. NH_3 and lyophilized to give **6** (312 mg, 86%): ^{1}HNMR (400 MHz, DMSO-d6): δ 0.78-1.59 (Hcyclohexyl), 2.27-2.22(H_{CH2}-cyclohexyl), 5.09 (H1), 7.73 Htriazole), MS (MALDI): m/z 2744.7 $[M + K]^+$, Found: C, 54.47; H, 7.35; N,15.04%. Calcd for C128H210N32O32+7.5H_2O: C, 54.09; H, 7.91; N, 15.77%.

Cyclopentylmethylamino derivative 8

A reaction solution was prepared by dissolving per-2,3-acetylated γ-CD octaazide **11** (50 mg, 2.30 × 10^{-5} mol) in DMSO–H_2O (26:3) (2.6 cm^3) containing *N*-Boc-*N*-cyclopethylmethyl-*N*-2-propynylamine (55 mg, 1.25 mol eq. to the azide group), $CuSO_4$ $5H_2O$ (4.57 mg, 0.1 mol eq.), and sodium ascorbate (45.2 mg, 1.25 mol eq.). After MW heating (120°C, 10 min), ethyl acetate was added followed by washing with 5% aq. EDTA. Silica gel column chromatography (hexane/ethyl acetate) gave the click reaction product (60.1 mg, 64.2%). Deprotection of the Boc group with TFA followed by removal of the acetyl groups with NaOMe–MeOH gave the desired product **8** in (76.7%): ^{1}H NMR (400 MHz, DMSO-d6): δ 1.15, 1.48, 1.55, 1.72, 2.09 (Hcyclopentyl), 2.85 (H_{CH2}-cyclopentyl), 5.10 (H1), 8.08 Htriazole), MS (MALDI): m/z 2594.4702 $[M + H]^+$, 2616.4516 $[M + K]^+$, Found: C, 44.65; H, 5.89; N,11.34%. Calcd for C120H192N32O32+Na+2.6H_2O+10.3TFA: C, 44.25; H, 5.48; N, 11.74%.

Cyclobutylmethylamino derivative 9

A reaction solution was prepared by dissolving per-2,3-acetylated γ-CD octaazide **11** (30.8 mg, 1.42

× 10^{-5} mol) in DMSO–H_2O (15:4) (3.8 cm^3) containing *N*-Boc-*N*-cyclobutylmethyl-*N*-2-propynylamine (32.1 mg, 1.25 mol eq. to the azide group), $CuSO_4$ $5H_2O$ (7.2 mg, 0.2 mol eq.), and sodium ascorbate (53.8 mg, 2.5 mol eq.). After MW heating (120 °C, 30 min), ethyl acetate was added followed by washing with 5% aq. EDTA. Silica gel column chromatography (CH_2Cl_2/methanol) gave the click reaction product (44.5 mg, 79.3%). Deprotection of the Boc group with TFA followed by removal of the acetyl groups with NaOMe–MeOH gave the desired product **9** in (86.6%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ 1.68-1.87, 2.01-2.02, 2.53-2.61 (Hcyclobutyl), 2.94 (H_{CH2}-cyclobutyl), 5.10 (H1), 8.07 Htriazole), MS (MALDI): m/z 2482.8240 $[M + H]^+$, 2504.7950 $[M + Na]^+$, Found: C, 41.44; H, 5.45; N, 11.60%. Calcd for $C_{112}H_{176}N_{32}O_{32}$+Na+11.5 H_2O +11TFA: C, 41.39; H, 5.50; N, 11.70%.

Cyclopropylmethylamino derivative 10

A reaction solution was prepared by dissolving per-2,3-acetylated γ-CD octaazide **11** (50 mg, 2.30 × 10^{-5} mol) in DMSO–H_2O (5:1) (2.4 cm^3) containing *N*-Boc-*N*-cyclopropylmethyl-*N*-2-propynylamine (48.1 mg, 1.25 mol eq. to the azide group), $CuSO_4$ $5H_2O$ (91.2 mg, 2.5 mol eq.), and sodium ascorbate (9.16 mg, 0.2 mol eq.). After MW heating (120 °C, 30 min), ethyl acetate was added followed by washing with 5% aq. EDTA. Silica gel column chromatography (CH_2Cl_2/methanol) gave the click reaction product (70.4 mg, 80.0%). Deprotection of the Boc group with TFA followed by removal of the acetyl groups with NaOMe–MeOH gave the desired product **10** in (73.1%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ 0.29-0.31, 0.54-0.56 1.00 (Hcyclopropyl), 2.81 (H_{CH2}-cyclobutyl), 5.10 (H1), 8.08 Htriazole), MS (MALDI): m/z 2370.6610 $[M + H]^+$, 2392.6400 $[M + Na]^+$, Found: C 40.52, H 5.25, N 11.80%. Calcd for $C_{104}H_{160}N_{32}O_{32}$ + 10 H_2O + 11 CF_3COOH: C 40.35, H 5.19, N 12.14%.

2. Bacteria

Staphylococcus aureus FDA 209P, *Bacillus subtilis* 168, *Escherichia coli* K12 strain W3110, and *Salmonella typhimurium* LT2 were used in this study. *S. aureus* cells were grown at 37 °C in a nutrient broth. *B. subtilis* cells were grown at 37 °C in a medium containing 1% polypepton, 0.5% yeast extract, and 0.5% NaCl (pH was adjusted to 7 by adding NaOH). *E. coli* and *S. typhimurium* cells were grown at 37 °C in a minimal salt medium supplemented with 1% polypepton.

3 Minimum inhibitory concentrations (MICs)

MICs were determined using the liquid microdilution method with serially diluted (twofold) CDs. Cells (1 × 10^4) were cultured at 37 °C for 20 h in Mueller–Hinton broth (0.1 cm^3) containing a CD

using a 96-well microtiter plate. MIC was determined as the lowest concentration of the CD at which cells were unable to grow.

4 K^+ efflux and cell viability

Cells were harvested in an exponential phase of growth, washed twice with buffer (100 mM choline chloride/50 mM Mops-Tris, pH 7.2) and suspended in this buffer at 2×10^9 cells cm^{-3}. The cells were then incubated with CD at 37 °C for 30 min, and the final volume of the cell suspension was 1 cm^3. The viability of the cells was determined by counting the colonies. 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. The remaining cell suspension was centrifuged, and the amount of K^+ in the supernatant was measured using a K^+-selective electrode. Melittin (10 μM) and polymyxin B (200 μg cm^{-3}) were used to determine the 100% level of K^+ efflux from *S. aureus* and *E. coli*, respectively. To disrupt the outer membrane structure of the *E. coli* cells, they were treated with 150 mM Tris-HCl (pH 7.2)/1 mM EDTA at 37 °C for 2 min.

5 Hemolytic activity

Rabbit erythrocytes were suspended in a buffer (150 mM NaCl/10 mM Hepes-NaOH, pH 7.4) at a final concentration of 0.5% hematocrit. After incubation with a CD at 37 °C for 30 min, the hemolysis was estimated by measuring the absorbance at 540 nm. Lysolecithin (50 μM) was used to determine the 100% level of hemolysis.