Electronic Supplementary Information

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

Hatsuo Yamamura, a Yuuki Sugiyama, a Kensuke Murata, a Takanori Yokoi, a Ryuji Kurata, a Atsushi Miyagawa, a Kenji Sakamoto, b Keiko Komagoe, b Tsuyoshi Inoue, b and Takashi Katsu b

a Graduate School of Engineering, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555 (Japan)
bGraduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University 1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530 (Japan)

E-mail: yamamura.hatsuo@nitech.ac.jp

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-$\gamma$-CD derivative 2 (52.2 mg, $3.49 \times 10^{-5}$ mol) in DMSO–H$_2$O (10:1) (10 cm$^3$) containing N-benzyl-N-2-propynylamine (53 mg, 1.25 mol eq. to the azide group), CuSO$_4$ 5H$_2$O (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): $\delta$ 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 C$_{128}$H$_{161}$N$_{32}$O$_{32}$+10.1 H$_2$O: 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–H₂O (10:1) (22 cm³) containing N-phenylpentyl-N-2-propynylamine (297 mg, 1.25 mol eq. to the azide group), CuSO₄ 5H₂O (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. NH₃ and lyophilized to give 5 (361 mg, 87%): ¹H NMR (400 MHz, [D₆]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+7.4H₂O: 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–H₂O (10:1) (22 cm³) containing N-cyclohexylmethyl-N-2-propynylamine (250 mg, 1.5 mol eq. to an azide group), CuSO₄ 5H₂O (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. NH₃ and lyophilized to give 6 (312 mg, 86%): ¹H NMR (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+7.5H₂O: 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–H₂O (26:3) (2.6 cm³) containing N-Boc-N-cyclopentylmethyl-N-2-propynylamine (55 mg, 1.25 mol eq. to the azide group), CuSO₄ 5H₂O (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%): ¹H NMR (400 MHz, DMSO-d₆): δ 1.15, 1.48, 1.55, 1.72, 2.09 (Hcyclopentyl), 2.85 (HCH₂-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+2.6H₂O+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–H₂O (15:4) (3.8 cm³) containing N-Boc-N-cyclobutylmethyl-N-2-propynylamine (32.1 mg, 1.25 mol eq. to the azide group), CuSO₄ 5H₂O (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 (CH₂Cl₂/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%): ¹H NMR (400 MHz, [D₆]DMSO): δ 1.68-1.87, 2.01-2.02, 2.53-2.61 (Hcyclobutyl), 2.94 (HCH₂-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 C₁₁₂H₁₇₆N₃₂O₃₂+Na+11.5 H₂O +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–H₂O (5:1) (2.4 cm³) containing N-Boc-N-cyclopropylmethyl-N-2-propynylamine (48.1 mg, 1.25 mol eq. to the azide group), CuSO₄ 5H₂O (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 (CH₂Cl₂/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%): ¹H NMR (400 MHz, [D₆]DMSO): δ 0.29-0.31, 0.54-0.56 1.00 (Hcyclopropyl), 2.81 (HCH₂-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 C₁₀₄H₁₆₀N₃₂O₃₂ + 10 H₂O + 11 CF₃COOH: 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 × 10⁴) were cultured at 37 °C for 20 h in Mueller–Hinton broth (0.1 cm³) 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 $2 \times 10^9$ 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 Heps-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.