

Supplementary information

Host-Guest Strategy to Potently Camouflage And Restore the Activity And Toxicity of Drugs Affecting Bacterial Growth and Viability

Julien Gravel, Claude R. Elie, Michèle Khayat and Andreea R. Schmitzer

Département de Chimie, Université de Montréal, Montréal, Canada

Université de Montréal, 2900 Édouard Montpetit, CP 6128, succ. Centre ville, Montréal, Québec, Canada.

E-mail: ar.schmitzer@umontreal.ca

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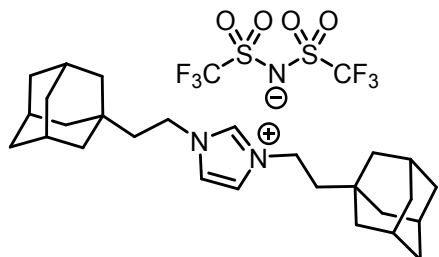
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Materials

All chemicals were purchased from Sigma-Aldrich and were used without further purification.

Synthesis

1,3-bis(2-(adamantan-1-yl)ethyl)imidazolium bis(trifluoromethylsulfonyl)imide (**1**):



The syntheses and characterization of **1** are already reported in the literature.¹

Biological assays

Preparation of the samples in the minimal inhibitory concentration (MIC) assays

To preserve supramolecular complexation, every sample was prepared in a water/MeOH solution, the solvents were evaporated and 1 mL of DMSO was used to dissolve compounds only prior to the MIC experiment.

Preparation of the **1**:(**β CD**)₂ complex: **1** (8.4 mg, 0.012 mmol, 1 eq.) was dissolved in 1 mL of MeOH and was added to 1 mL of water containing the **β CD** (28.3 mg, 0.025 mmol, 2 eq.). The solution was then diluted to 5 mL with MeOH. Aliquots of 1 mL were separated in 5 vials and were dried under vacuum.

Preparation of **1**:(**β CD**)₂:**CG**: **1** (8.4 mg, 0.012 mmol, 1 eq.) and **CG** (4.5 mg, 0.025 mmol, 2 eq.) were dissolved in 1 mL of MeOH and the mixture was added to 1 mL of water containing the **β CD** (28.3 mg, 0.025 mmol, 2 eq.). The solution was then diluted to 5 mL with MeOH. Aliquots of 1 mL were separated in 5 vials and were dried under vacuum.

MIC assays

5 mL of lysogeny broth (LB) medium was inoculated with *B. thuringiensis* (*HD73* strain). The precultures were grown overnight at 37°C under stirring and resuspended in 75 mL of a fresh LB medium. The cultures were grown at 37°C until optical density reached 0.5 at 600 nm (O.D.₆₀₀ = 0.5) and then rediluted in fresh LB medium to an O.D.₆₀₀ = 0.1. Each well of a 96 wells plate was filled with 185 μ L of bacterial culture, 10 μ L of MiliQ water and 5 μ L of DMSO (for the untreated bacteria) or 5 μ L of compounds in DMSO solution, as the final volume in each well was 200 μ L and the concentration in DMSO 2.5% (v/v). Every experiment was performed in two sets of quintuplicates. The MICs were determined as the minimal concentration at which no bacterial growth was detected by optical density. The plates were stirred in a thermostated incubator at 37°C and the O.D.₆₀₀ was monitored at t = 0, 2, 5, 10 and 24 h.

(a) In the first assay, *B. thuringiensis* was incubated at 37°C with **1** (concentrations ranging from 2 μM to 32 μM).

(b) Control assay: *B. thuringiensis* was incubated at 37°C with **βCD** (concentrations ranging from 4 μM to 64 μM).

(c) Control assay: *B. thuringiensis* was incubated at 37°C with **CG** (concentrations ranging from 4 μM to 64 μM).

(d) Control assay with the compounds **βCD** and **CG** (**βCD:CG**): *B. thuringiensis* was incubated at 37°C with the same concentrations as above of **βCD** and **CG**.

(e) Control assay with the compounds **1** and **CG** (**1:CG**): *B. thuringiensis* was incubated at 37°C with the same concentrations as above of **1** and 2 equivalents of **CG** (with respect to **1**).

(f) In the assay with the compounds **1** and **βCD** (**1:(βCD)₂**): *B. thuringiensis* was incubated at 37°C with the same concentrations as in (a) of **1** and 2 equivalents of **βCD** (with respect to **1**).

(g) In the competitive assay with the compound **1** and **βCD** versus **CG** (**1:(βCD)₂:CG**): *B. thuringiensis* was incubated at 37°C with the same concentrations as in (a) of **1** and 2 equivalents of **βCD** and **CG** (with respect to **1**).

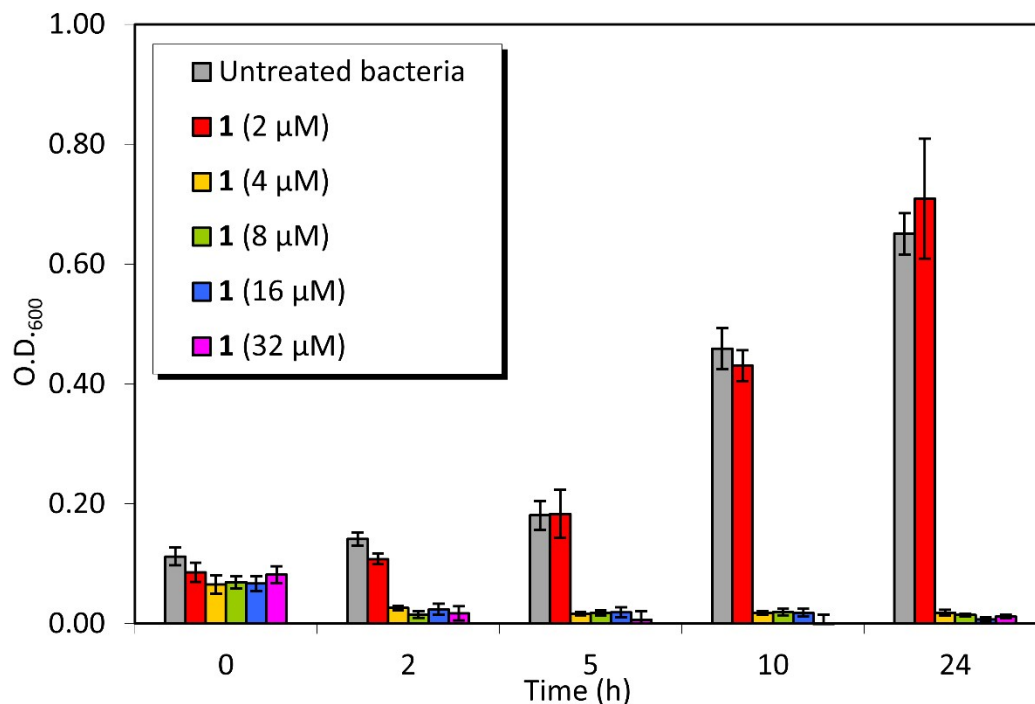


Figure S1. Dose-dependent growth inhibition of *B. thuringiensis* (HD73) by **1**. Error bars show standard deviation.

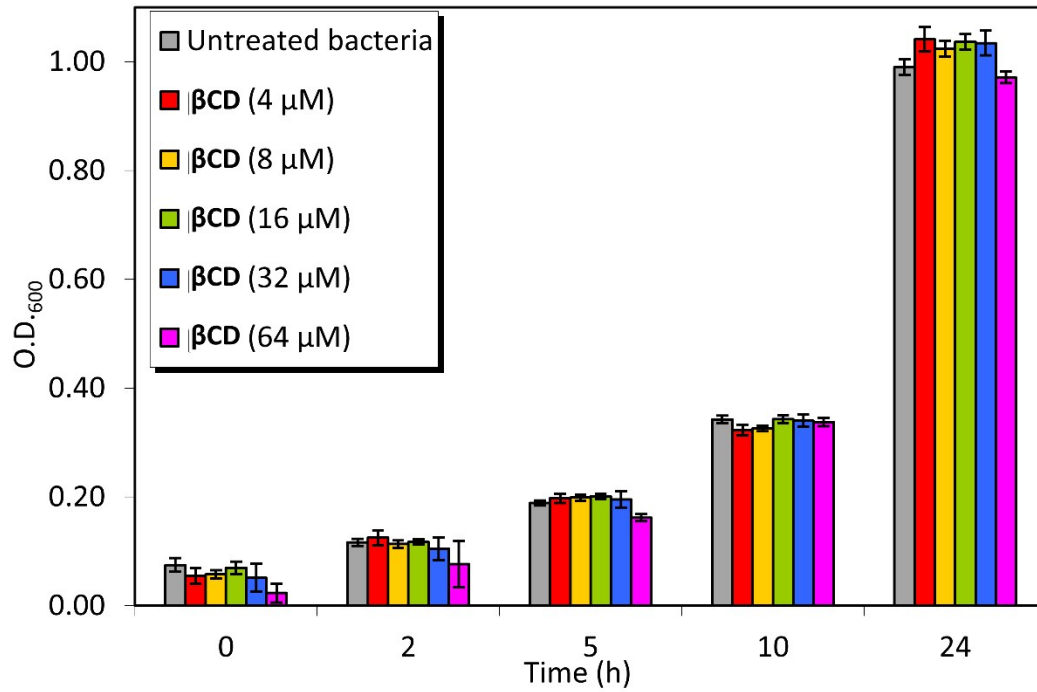


Figure S2. Dose-dependent growth inhibition of *B. thuringiensis* (HD73) by β CD. Error bars show standard deviation.

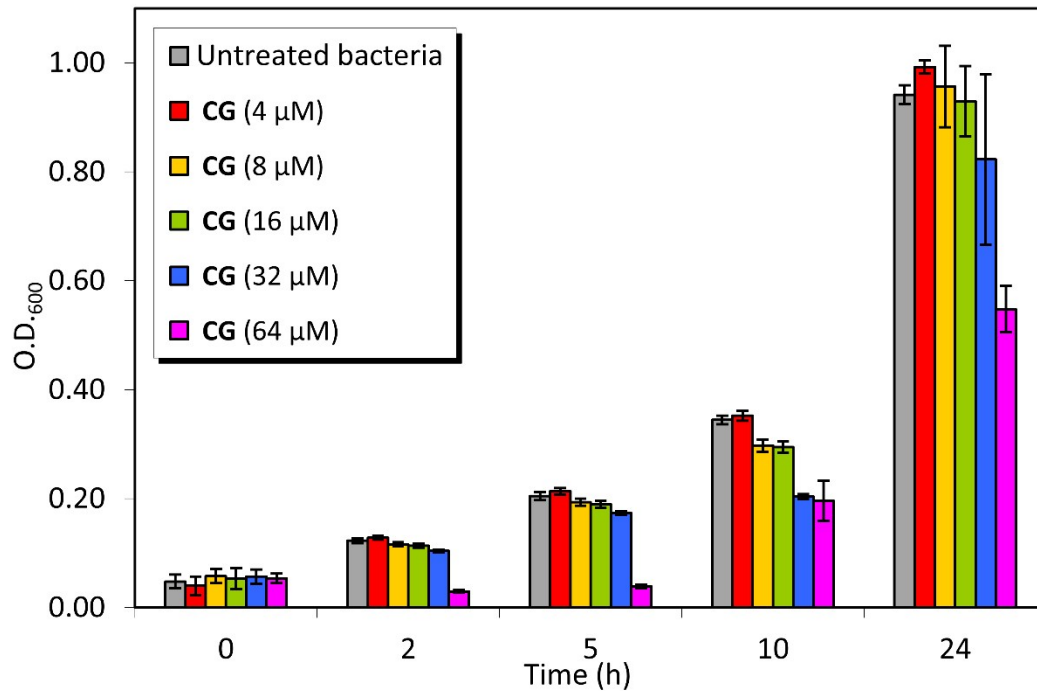


Figure S3. Dose-dependent growth inhibition of *B. thuringiensis* (HD73) by CG. Error bars show standard deviation.

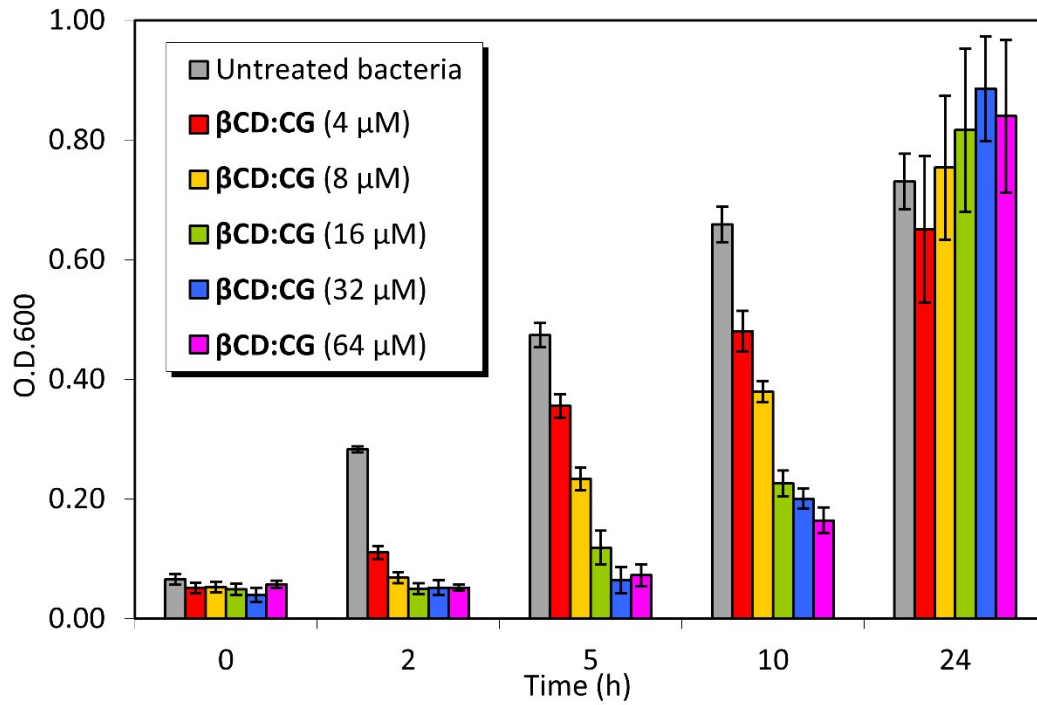


Figure S4.

Figure S4. Dose-dependent growth inhibition of *B. thuringiensis* (HD73) by β CD:CG. Error bars show standard deviation.

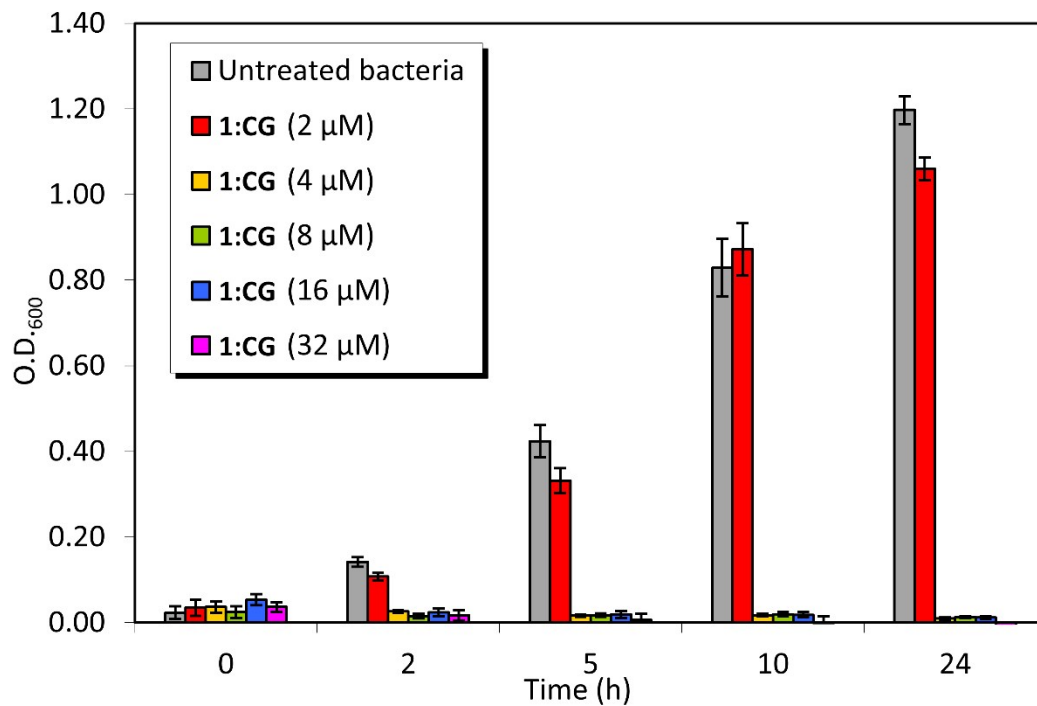


Figure S5. Dose-dependent growth inhibition of *B. thuringiensis* (HD73) by **1** in the presence of 2 equivalents of **CG**. Concentrations are shown with respect to **1**. Error bars show standard deviation.

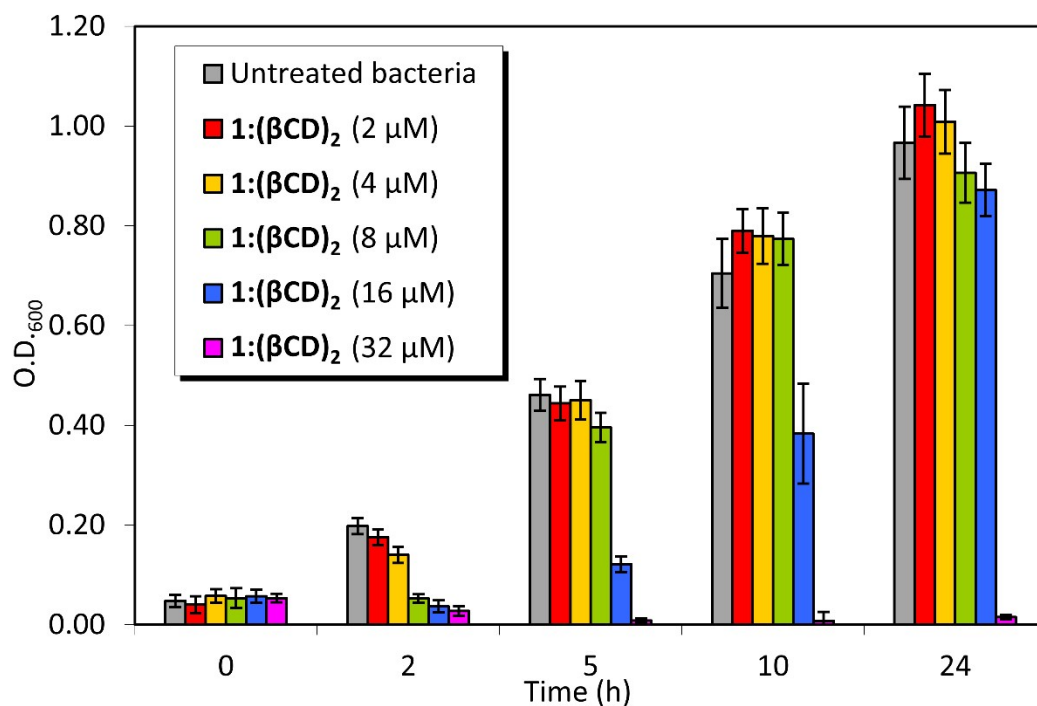


Figure S6. Dose-dependent growth inhibition of *B. thuringiensis* (HD73) by 1:(β CD)₂. Concentrations are shown with respect to 1. Error bars show standard deviation.

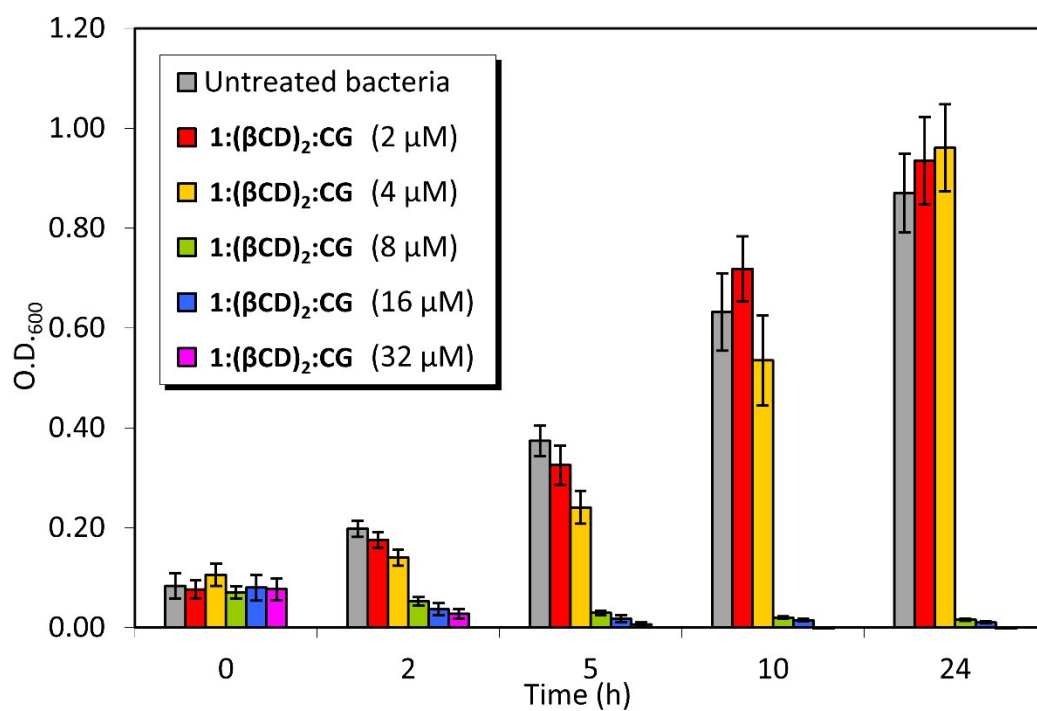


Figure S7. Dose-dependent growth inhibition of *B. thuringiensis* (HD73) by 1:(β CD)₂:CG. Concentrations are shown with respect to 1. Error bars show standard deviation.

Preparation of the samples for the LIVE/DEAD® BacLight™ Bacterial Viability assay

β CD (1:(β CD)₂): 1 (8.4 mg, 0.012 mmol, 1 eq.) was dissolved in 2 mL of MeOH and was added to 2 mL of water containing the **β CD** (56.6 mg, 0.050 mmol, 4 eq.). The solution was then diluted to 5 mL with MeOH. Aliquots of 1 mL were separated in 5 vials and were dried under vacuum. **1:(β CD)₂:CG): 1** (8.4 mg, 0.012 mmol, 1 eq.) and **CG** (11.2 mg, 0.062 mmol, 5 eq.) were dissolved in 2 mL of MeOH and was added to 2 mL of water containing the **β CD** (56.6 mg, 0.062 mmol, 4 eq.). The solution was then diluted to 5 mL with MeOH. Aliquots of 1 mL were separated in 5 vials and were dried under vacuum. 1 mL of DMSO was used to dissolve compounds only prior to the experiment.

Fluorescence spectroscopy

Calibration of the LIVE/DEAD® BacLight™ Bacterial Viability Assay

B. Thuringiensis HD73 was inoculated in 1 mL of fresh LB which was grown over night with agitation at 37°C. An aliquot of 200 μ L of the preculture was inoculated in 60 mL of fresh LB which was grown with agitation (200 rpm) at 37°C for 4 hours. Bacteria were centrifuged at 1000 \times g for 10 minutes at room temperature and the pellet were resuspended in 5 mL of 0.9% (w/w) NaCl solution. 1 mL of the bacterial suspension was added to 20 mL of 0.9% (w/w) NaCl solution for the quantification of the alive bacteria; 1 mL of the bacterial suspension was added to 20 mL of 70% (v/v) *i*PrOH in 0.9% (w/w) NaCl for quantification of dead bacteria. The suspensions were then incubated for 1 hour at 37°C with manual stirring every 15 minutes. Samples were centrifuged at 1000 \times g for 10 minutes at room temperature and were resuspended in 0.9% (w/w) NaCl and washed once by centrifugation. The pellets were resuspended each in 8 mL of 0.9% (w/w) NaCl solution and their OD₆₀₀ was measured (as in *Membrane integrity assay*). The OD₆₀₀ was then adjusted to the lowest of the samples from the *Membrane integrity assay* and the calibration was performed as followed: an aliquot of 100 μ L of Live/Dead bacteria were prepared in 0:100, 10:90, 50:50, 90:10 and 100:0 ratios and was transferred to a 96-well flat-bottom microplate in triplicate. The nucleic acid stains SYTO9 and propidium iodide from the LIVE/DEAD® BacLight™ Bacterial Viability Kit (L7012) were prepared as followed: 1 μ L of each were diluted in 1 mL of 0.9% (w/w) NaCl. Aliquots of 100 μ L of this solutions were mixed with the samples and was incubated in the dark for 15 minutes. Fluorescence was measured using a *Tecan Infinite M200* microplate reader with $\lambda_{ex}/\lambda_{em}$ set at 480/515 nm for the SYTO9 fluorescence and $\lambda_{ex}/\lambda_{em}$ set at 490/625 nm for propidium iodide fluorescence. Calibration was repeated for every *Membrane integrity assay* experiments.

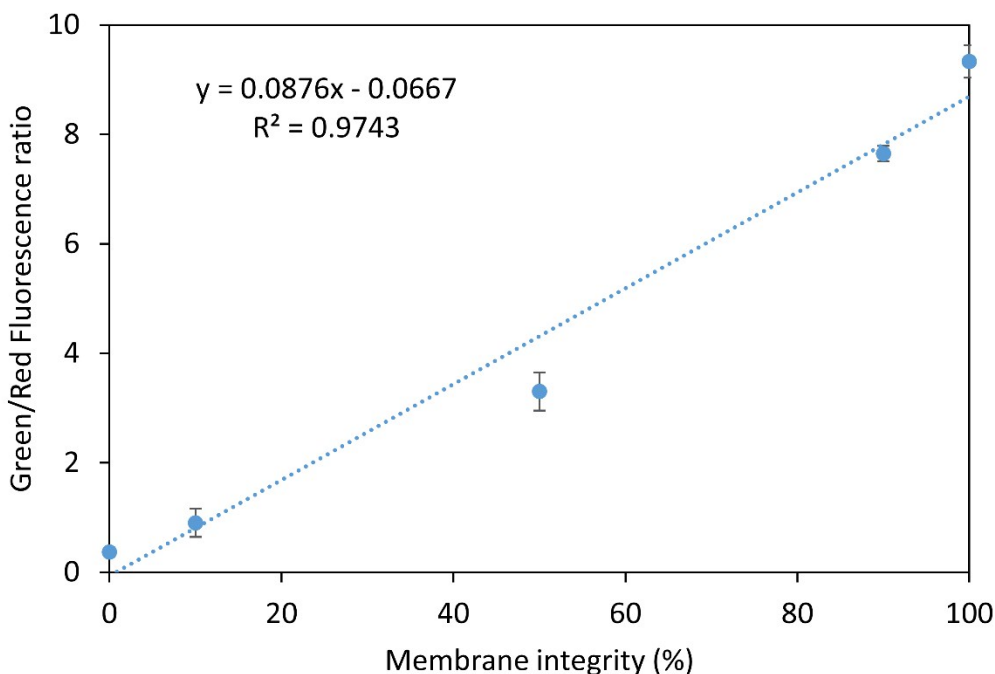


Figure S8. LIVE/DEAD® BacLight™ Bacterial Viability calibration curve for *B. Thuringiensis* HD73. Error bars show standard deviation.

Membrane integrity assay of *B. thuringiensis* HD73

Bacteria were prepared as described in *Calibration of the LIVE/DEAD® BacLight™ Bacterial Viability Assay* where 250 μ L of bacterial suspension was added to 5 mL of the following samples diluted in 0.9% (w/w) NaCl with a maximum of 1% of DMSO: β CD (80 μ M), CG (100 μ M), **1** (4 μ M, MIC), **1** (20 μ M, 5 \times MIC), **1:(β CD)₂** (20 μ M and 80 μ M, respectively) or **1:(β CD)₂:CG** (20 μ M, 80 μ M and 100 μ M, respectively). The suspensions were then incubated for 1 hour at 37°C with manual stirring every 15 minutes. Samples were centrifuged at 1000 \times g for 10 minutes at room temperature and were resuspended in 0.9% (w/w) NaCl and washed once by centrifugation. The pellets were resuspended each in 2 mL of 0.9% (w/w) NaCl and their OD₆₀₀ were measured (as well as *Calibration of the LIVE/DEAD® BacLight™ Bacterial Viability Kit* samples). OD₆₀₀ was then adjusted to the lowest of the samples and all samples were transferred to a 96-well flat-bottom microplate in triplicate. Aliquots of 100 μ L of the nucleic acid stains, prepared as in *Calibration of the LIVE/DEAD® BacLight™ Bacterial Viability Assay*, were mixed with the samples and were incubated in the dark for 15 minutes. Fluorescence was measured using *Tecan Infinite M200* microplate reader with $\lambda_{\text{ex}}/\lambda_{\text{em}}$ set at 480/515 nm for the SYTO9 fluorescence and $\lambda_{\text{ex}}/\lambda_{\text{em}}$ set at 490/625 nm for propidium iodide fluorescence. This assay was repeated thrice. Results are reported in *Membrane integrity (%)* using the calibration curve obtained.

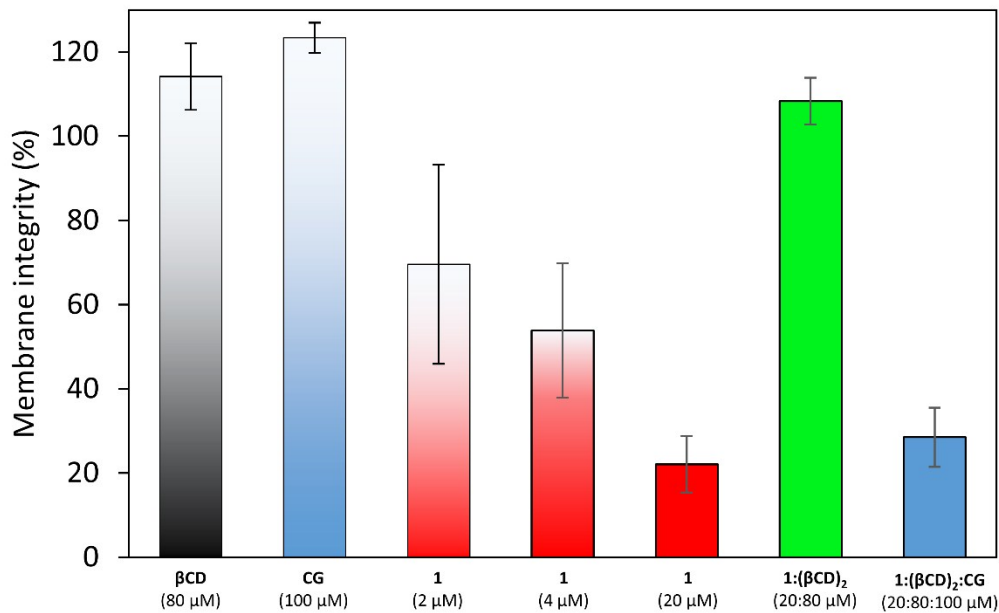


Figure S9. Results of the membrane integrity assays of *B. thuringiensis*. Error bars show standard.

Fluorescence microscopy

Membrane integrity assay of *B. Thuringiensis HD73*

B. Thuringiensis HD73 was inoculated in 5 mL of fresh LB containing 125 μ L of 20% (w/w) glucose which was grown overnight with agitation at 37°C. 1 mL of the bacterial preculture was inoculated in 9 mL of fresh LB containing 175 μ L of 20% (w/w) saccharose. Aliquots of 400 μ L were distributed in 3 sterile 8-well chambered coverglasses and were incubated at 37°C for 24 hours. Each well was rinsed twice with 200 μ L of PBS (1.44 g Na₂HPO₄, 0.24 g KH₂PO₄, 0.2 g KCl and 8 g NaCl per L, pH = 7.4) followed by a slight agitation. Aliquots of 400 μ L of the following samples diluted in PBS with 0.8% (v/v) of DMSO in triplicate were added in PBS, β CD (80 μ M), CG (100 μ M), 1 (20 μ M), 1:(β CD)₂ (20 μ M and 80 μ M, respectively) or 1:(β CD)₂:CG (20 μ M, 80 μ M and 100 μ M, respectively). The chambered coverglasses were incubated at 37°C for 3 hours. Each well was rinsed with 200 μ L of PBS followed by a slight agitation. Aliquots of 200 μ L of the nucleic acid stains, prepared as in *Calibration of the LIVE/DEAD® BacLight™ Bacterial Viability Kit*, were mixed with the samples and were incubated at room temperature in the dark for 15 minutes. Each well was rinsed with 200 μ L of PBS followed by a slight agitation and 200 μ L of PBS was added. The fluorescence was observed at 63X using a *Leica DMI6000 B* microscope equipped with a *DFC360 FX* digital camera.

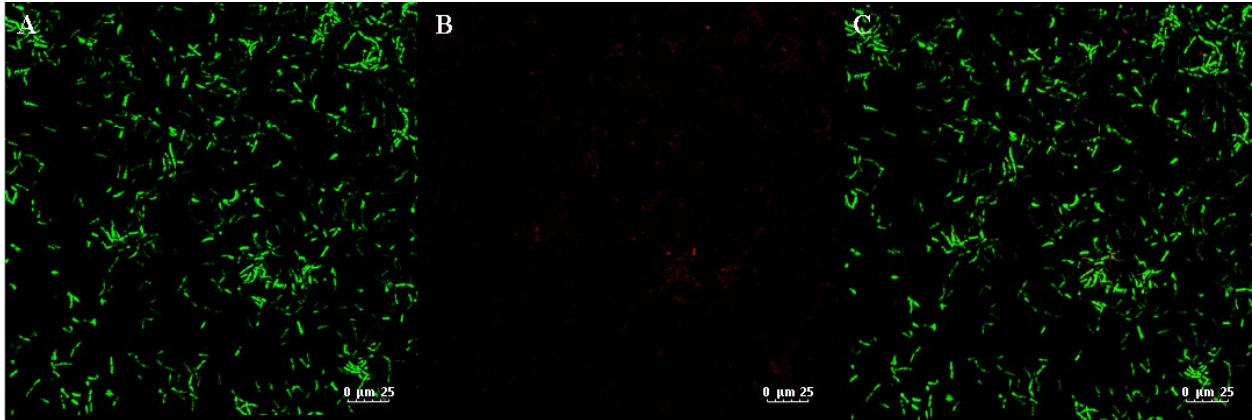


Figure S10. Fluorescence microscopy of *B. thuringiensis* stained by BacLight™ stains after incubation with NaCl 0.9%. A) Green fluorescence. B) Red fluorescence C) Merge of A and B.

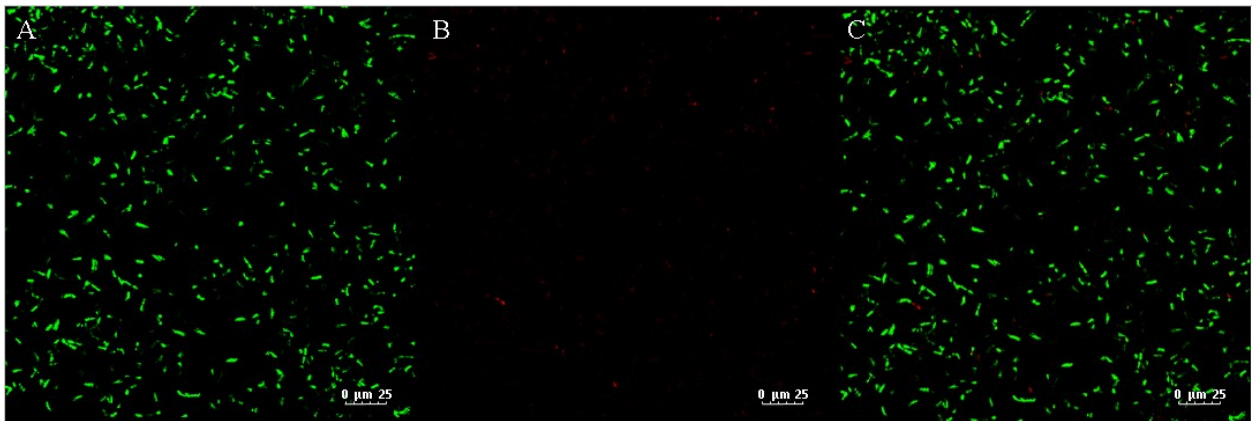


Figure S11. Fluorescence microscopy of *B. thuringiensis* stained by BacLight™ stains after incubation with β CD (80 μ M). A) Green fluorescence. B) Red fluorescence C) Merge of A and B.

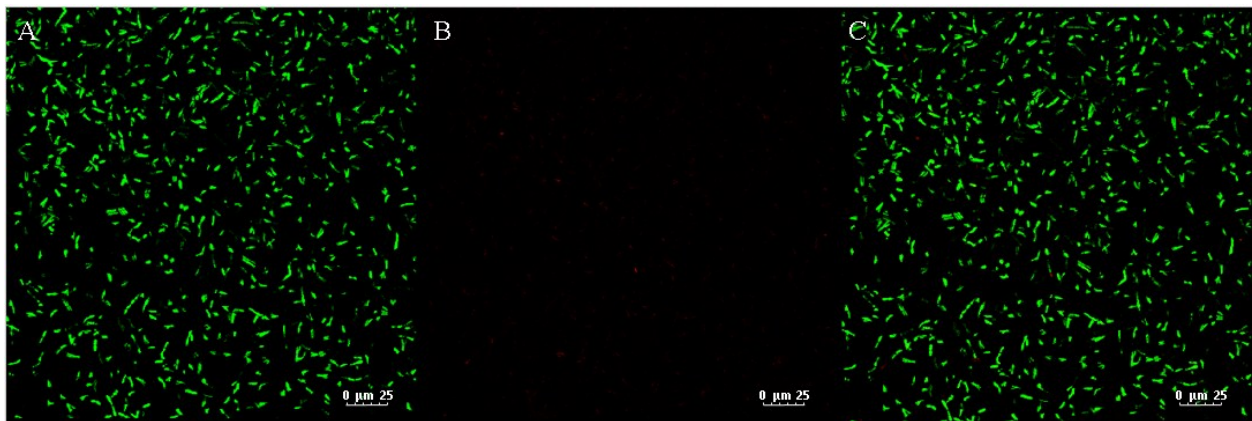


Figure S12. Fluorescence microscopy of *B. thuringiensis* stained by BacLight™ stains after incubation with CG (100 μ M). A) Green fluorescence. B) Red fluorescence C) Merge of A and B.

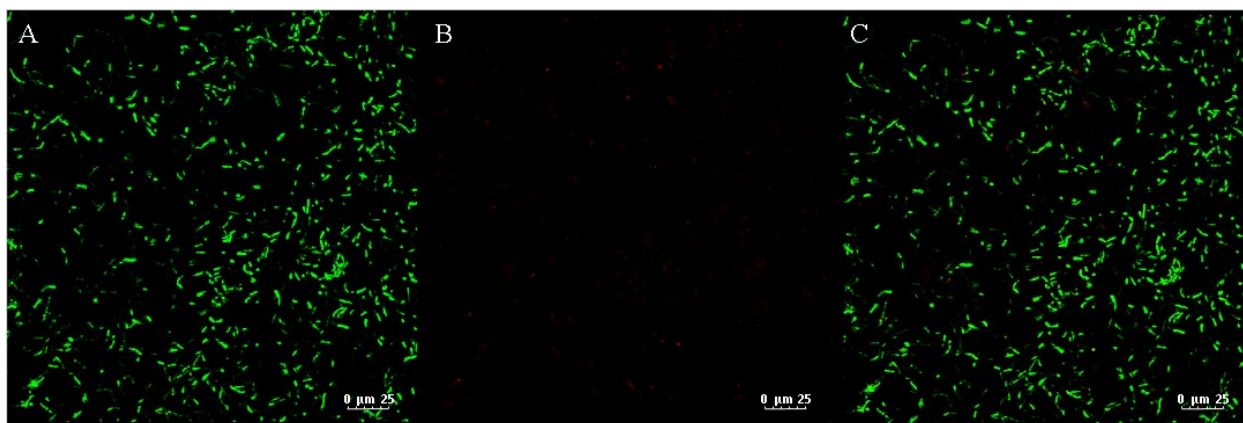


Figure S13. Fluorescence microscopy of *B. thuringiensis* stained by BacLight™ stains after incubation with β CD (80 μ M) and CG (100 μ M). A) Green fluorescence. B) Red fluorescence C) Merge of A and B.

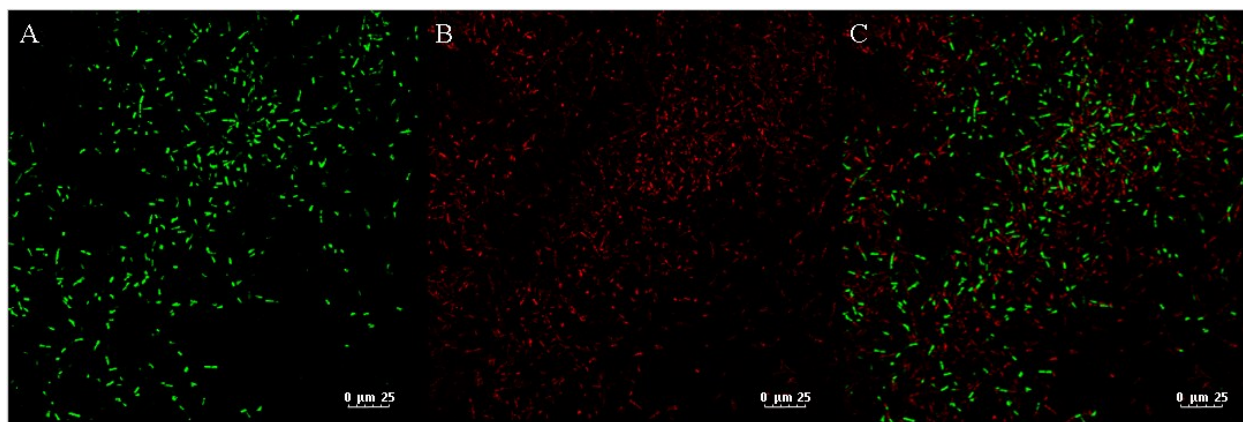


Figure S14. Fluorescence microscopy of *B. thuringiensis* stained by BacLight™ stains after incubation with **1** (20 μ M). A) Green fluorescence. B) Red fluorescence C) Merge of A and B.

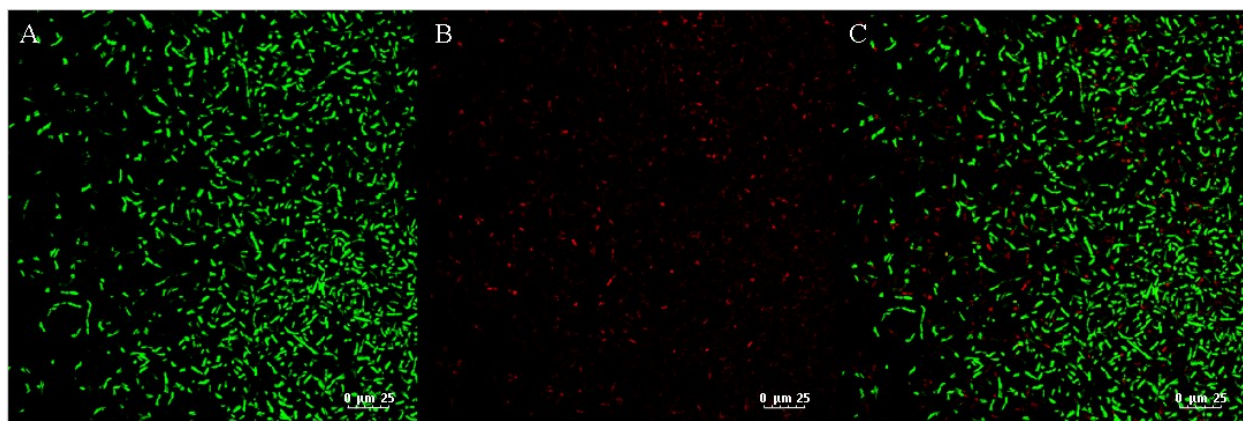


Figure S15. Fluorescence microscopy of *B. thuringiensis* stained by BaCLight™ stains after incubation with **1**:(β CD)₂ (20 μ M with respect to **1**). A) Green fluorescence. B) Red fluorescence C) Merge of A and B.

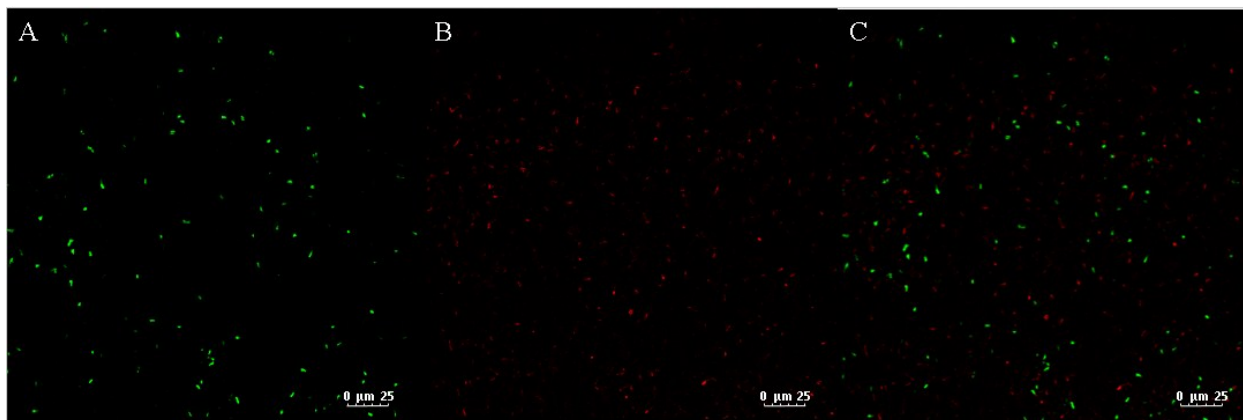


Figure S16. Fluorescence microscopy of *B. thuringiensis* stained by BaCLight™ stains after incubation with **1**:(β CD)₂:CG (20 μ M with respect to **1**). A) Green fluorescence. B) Red fluorescence C) Merge of A and B.

Hemolytic activity

Hemolytic activity was tested on erythrocytes from human blood. Mixture samples were prepared as for the MIC assay. Fresh human red blood cells (blood type O) were centrifuged for 10 minutes at 200 \times g, washed three times with PBS, and diluted to a concentration of 2% (v/v) in PBS. Ten microliters of 2-fold serial dilutions of compounds solutions (**1**, **1**:(β CD)₂, **1**:(β CD)₂:CG or **CG**) in DMSO was added to 96-well plates, after which 190 μ L of erythrocyte suspension was added. After 1 or 24 h of incubation at 37°C with gentle shaking, the plates were centrifuged for 10 minutes at 200 \times g. A 50 μ L amount of supernatant from each well was transferred to a fresh plate, and the release of hemoglobin was monitored by measuring the absorbance at 405 nm. The values for 0% and 100% hemolysis were determined by incubating erythrocytes with PBS or

with 0.5% (v/v) Triton X-100. The hemolysis percentage was calculated using the following equation:

$$\text{hemolysis (\%)} = \frac{A - A_0}{A_{100} - A_0} \times 100\%$$

where A is the absorbance of supernatant of compounds solutions (**1**, $1:(\beta\text{CD})_2$, $1:(\beta\text{CD})_2:\text{CG}$ or **CG**), A_0 is the absorbance of supernatant with DMSO, and A_{100} is the absorbance of supernatant with 0.5% Triton X-100. Data are the mean of three separate experiments.

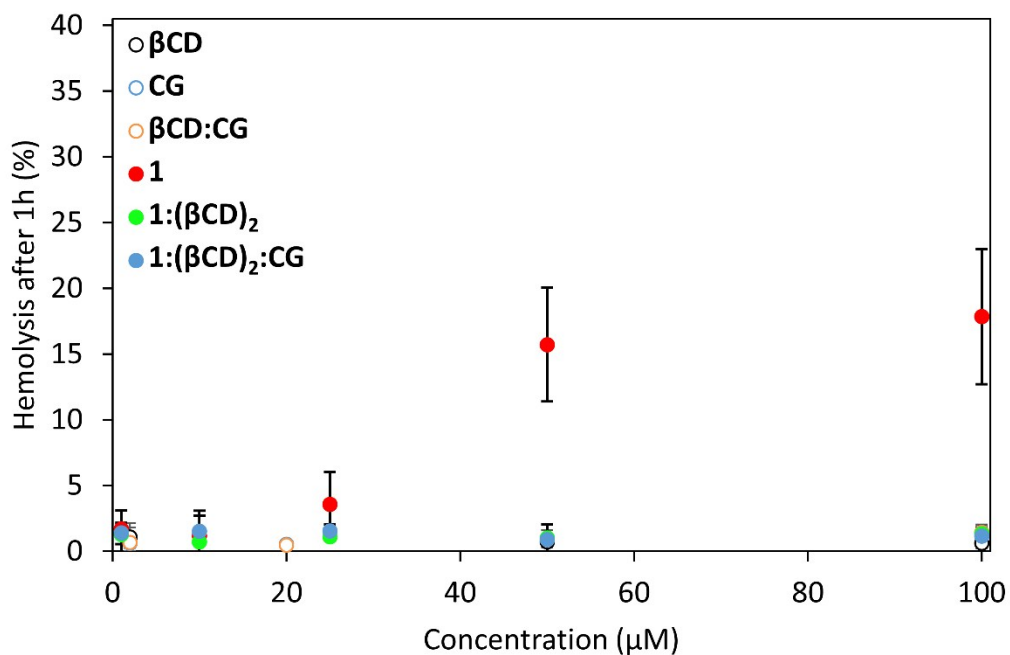


Figure S17. Hemolytic activity of **1**, βCD , **CG**, $1:(\beta\text{CD})_2$ and $1:(\beta\text{CD})_2:\text{CG}$ at various concentrations, with respect to **1**, after 1 hour of incubation.

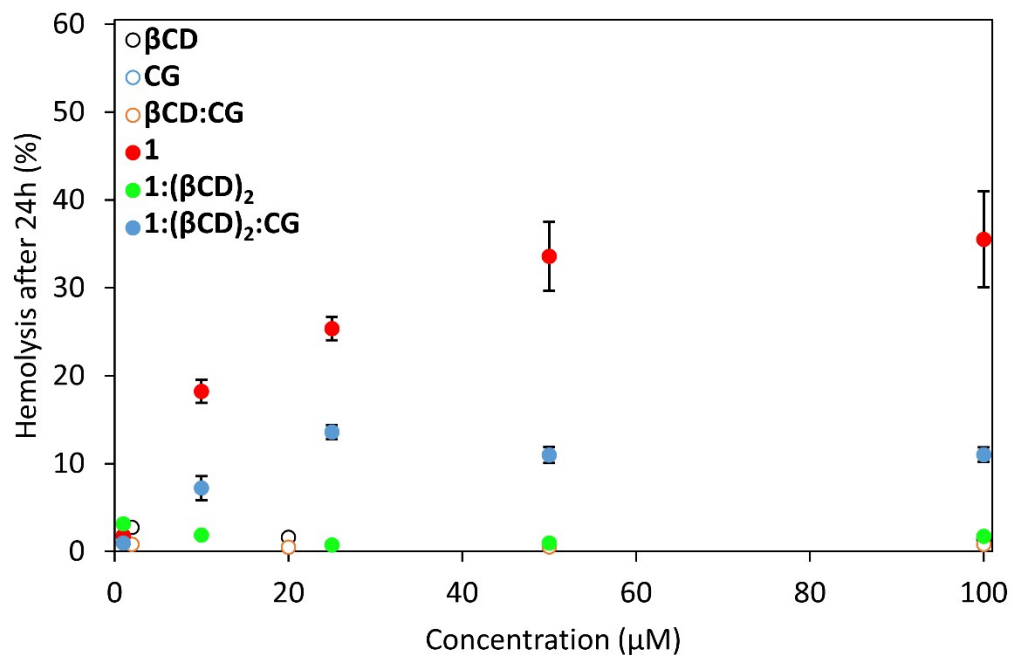


Figure S18. Hemolytic activity of **1**, **βCD**, **CG**, **1:(βCD)₂** and **1:(βCD)₂:CG** at various concentrations, with respect to **1**, after 24 hour of incubation.

Reference

1. J. Gravel and A. R. Schmitzer, *Supramolecular Chemistry*, 2014, **27**, 364-371.