Electronic Supplementary Information

A water-soluble pillar[5]arene as a new carrier for an old drug

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Materials and Methods. Amikacin disulfate salt **(1)** was obtained from Sigma-Aldrich. The strain of *Staphylococcus aureus* used in the microbiological studies was purchased from the American Type Culture Collection (ATCC 29213, LGC Promochem, Milan, Italy). The other chemicals were reagent grade and were used without any further purification.

General Experimental Methods. ^{1}H NMR spectra were recorded at room temperature in D_2O, at 500 MHz. The solvent residual peak (δ = 4.65 ppm) was used as an internal standard for ^{1}H NMR spectra. Deca-carboxylatopillar[5]arene **WP5** was synthesized according to a literature procedure.[1] NOESY spectra were recorded on a spectrometer equipped with a pulse-field gradient probe, using a 300 ms mixing time, 16 transients for each increment (256 in total) and a relaxation time of 3 s. NMR titration studies were carried out at a fixed **WP5** concentration (1 mM) and samples were routinely prepared by dissolving solid **WP5** in a D_2O phosphate buffer solution (Na_2HPO_4/NaH_2PO_4 50 mM, pH 7.2). A stock solution of amikacin ([**1**] = 10 mM) was, in turn, prepared by using the above-mentioned 1 mM **WP5** buffered solution as a convenient solvent so as, during the titration, the host concentration did not vary upon addition of increasing aliquots of the guest. The association constant was calculated by a nonlinear regression method using the WinEQNMR[2] program. The stoichiometry of the complex was determined according to the Job's method of continuous variations. To this end, samples were prepared by mixing aliquots of host (2 mM) and guest (2 mM) solution in phosphate buffer (Na_2HPO_4/NaH_2PO_4 50 mM in D_2O, pH 7.2) in such a way the total concentration was kept constant ([H] + [G] = 2 mM) whereas the molar fraction of the host (χ_{WP5}) in the resulting solutions increased from 0.0 to 1.0.

Emission titration experiments.

UV/Vis absorption spectra were taken on a Jasco V-560 spectrophotometer. For steady-state luminescence measurements, a Jobin Yvon-Spex Fluoromax 2 spectrofluorimeter was used, equipped with a Hamamatsu R3896 photomultiplier. Spectra were corrected for photomultiplier response using a program purchased with the fluorimeter. The absorption and luminescence data were fitted with the SPECFIT program, [SPECFIT, Spectrum Software Associates, R. A. Binstead, Chapel Hill, 1996.]. Data interpolation was carried out assuming a 1:1 complexation model.

Determination of the minimum inhibitory concentration (MIC). MIC is the lowest concentration of an antimicrobial agent able to prevent the growth of a bacterial strain. In

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1 T. Ogoshi, M. Hashizume, T.-A. Yamagishi and Y. Nakamoto, *Chem. Commun*., 2010, **46**, 3708.
2 M. J. Hynes, *J. Chem. Soc., Dalton Trans*., 1993, 311.

order to determine the MIC of amikacin, cultures of *S. aureus* ATCC 29213, at a final inoculum of approximately 10^5–10^6 bacteria per mL, were inoculated with increasing aliquots of amikacin ranging between 0.25 and 64 μg/mL. The MIC_{90}, *i.e.* the lowest concentration of the drug required to prevent 90% of the microbial growth, of amikacin was found to be 4 μg/mL. Subsequent antibacterial assays were routinely carried out at a sub-MIC concentration, namely 2.5 μg/mL, to assess the efficacy of the pillararene-based guest transport system.

Antibacterial activity assessment. *S. aureus* ATCC 29213 was grown at 37 °C in a Mueller Hinton broth (MHB) prior to use. The microorganisms were stored in a Trypti-case Soy broth (TSB), containing 20% (v/v) glycerol, at –80 °C. Four different MHB samples containing amikacin (2.5 μg/mL) and increasing amounts of **WP5** (0.0, 0.5, 2.0 and 4.0 equiv., respectively) were prepared and inoculated with a fresh culture of *S. aureus* up to a final concentration of 10^6 bacteria per mL. Following overnight incubation in a shaker incubator (350 rpm) operating at 37 °C, the bacterial growth was monitored by an automated turbidometry analyzer (Bioscreen C Labsystems). Ten replicates of bacterial cultures containing each of the aforementioned **WP5/**amikacin ratios were incubated in honeycomb plates for 48 h at 37 °C under continuous shaking. Read-outs of the bacterial culture optical density at 540 nm (OD_{540}) were periodically taken at 15 min. time intervals. Bacterial growth was monitored in terms of OD_{540}, for an incubation time of 48 h. After 2, 4 and 8 hours of incubation, the counts of viable bacteria in the treated microbial samples were estimated by a colony formation assay. The number of colony-forming units (CFU) was counted and expressed as percentage of CFU mL^{-1}.

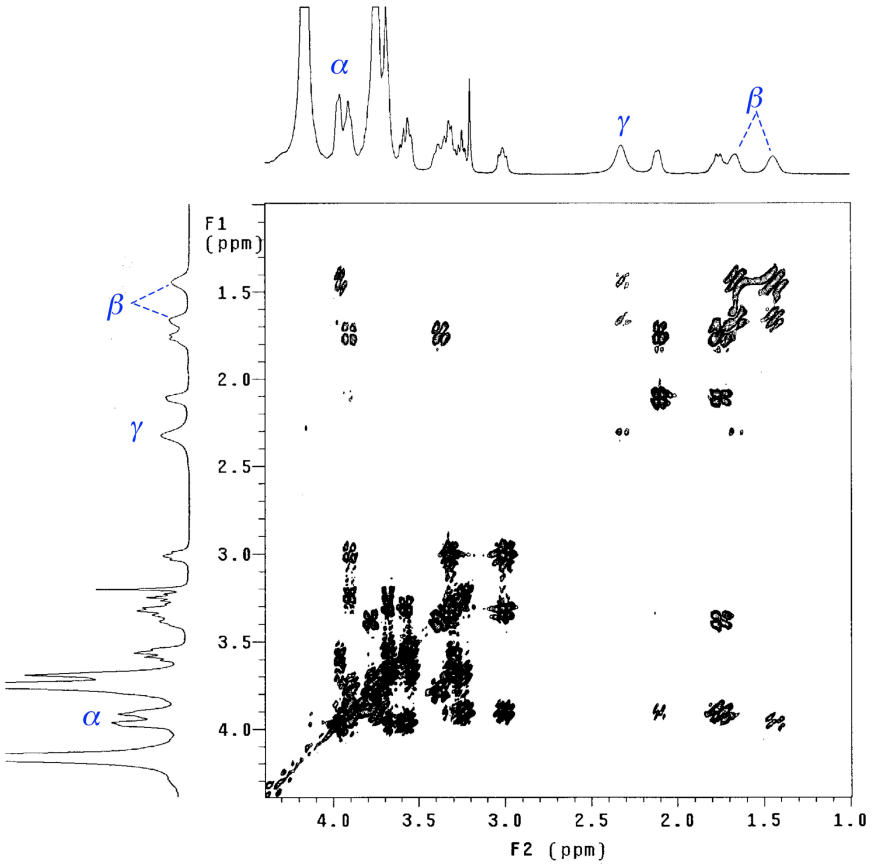

Fig. S1. Section of the COSY spectrum (500 MHz, 298 K, D_2O) of a solution of **WP5** and **1** (15.0 mM each).

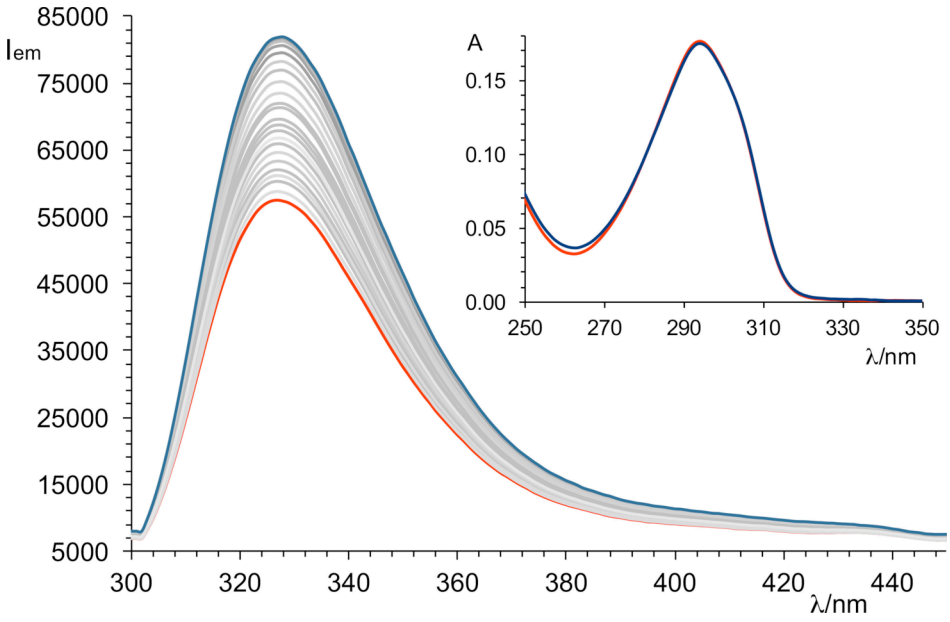

Fig. S2. Emission titration spectra (λ_{exc} = 280 nm) of **WP5** (1.10×10^{-5} M) upon addition of increasing amounts of **1** (in aqueous solution at room temperature). The inset shows the absorption spectra of **WP5** in the absence of **1** (blue curve) and at the end of titration (red curve).

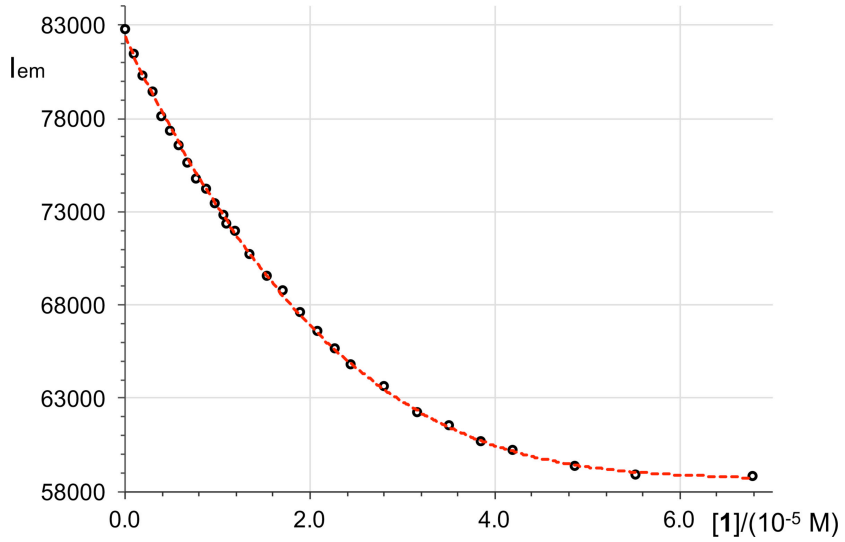

Fig. S3. Emission intensity changes of **WP5** (λ = 327 nm) upon addition of **1**. The dashed red line was obtained from a non-linear curve-fitting.

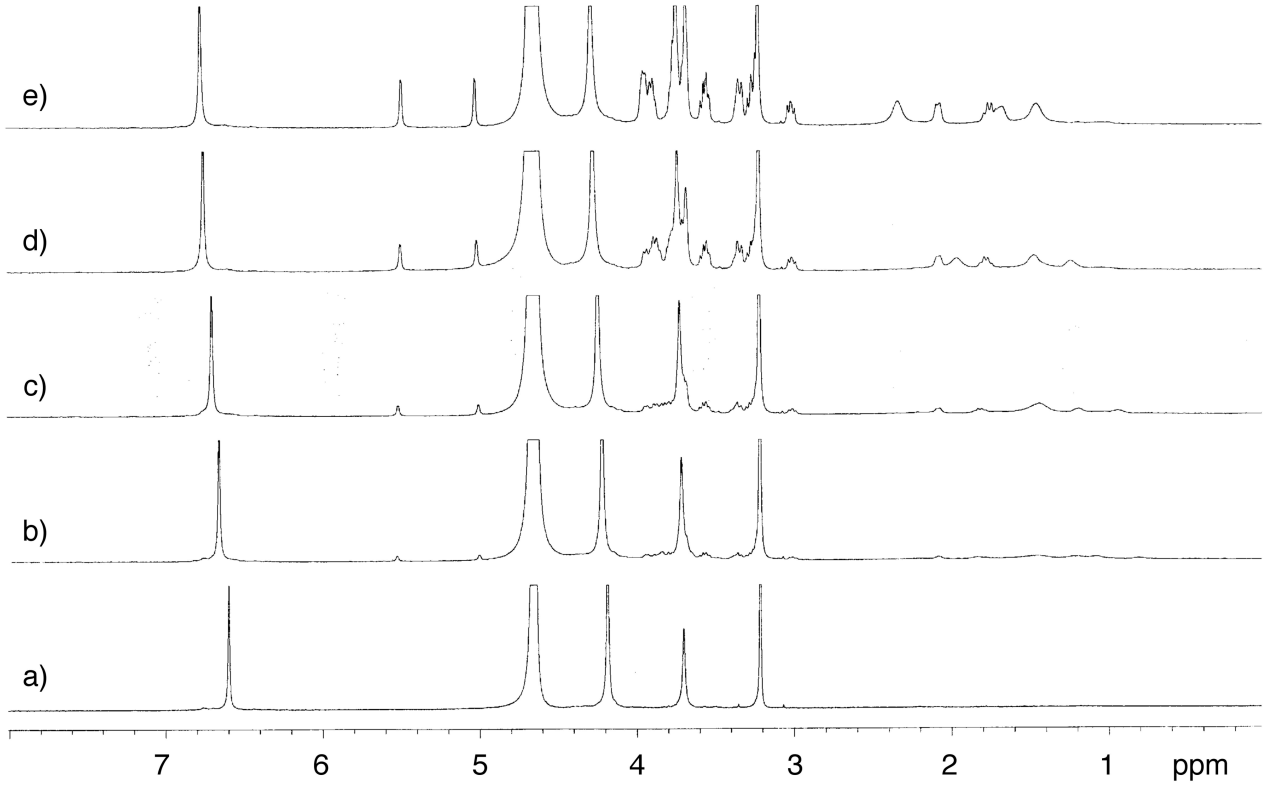

Fig. S4. ^{1}H NMR spectra (500 MHz, 298 K, D_2O Na_2HPO_4/NaH_2PO_4 50 mM, pH 7.2) of a 1.0 mM solution of **WP5** upon titration with **1**: a) 0.0 mM; b) 0.29 mM; c) 0.57 mM; d) 1.23 mM and e) 2.06 mM.

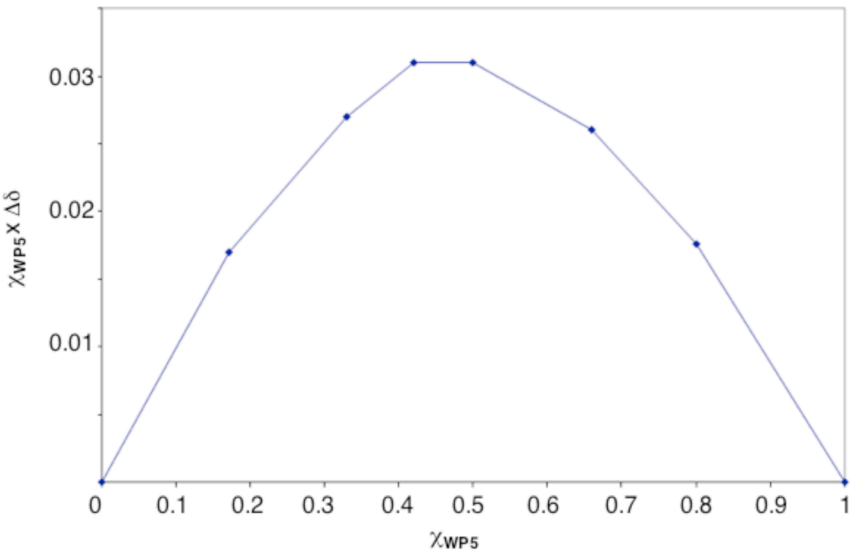

Fig. S5. Job plot of the pillararene-amikacin host-guest complex showing the 1:1 stoichiometry between **WP5** and **1**. Complexation induced shifts ($\Delta\delta$) of the **WP5** ArH resonances are plotted against the molar fraction of **WP5**.

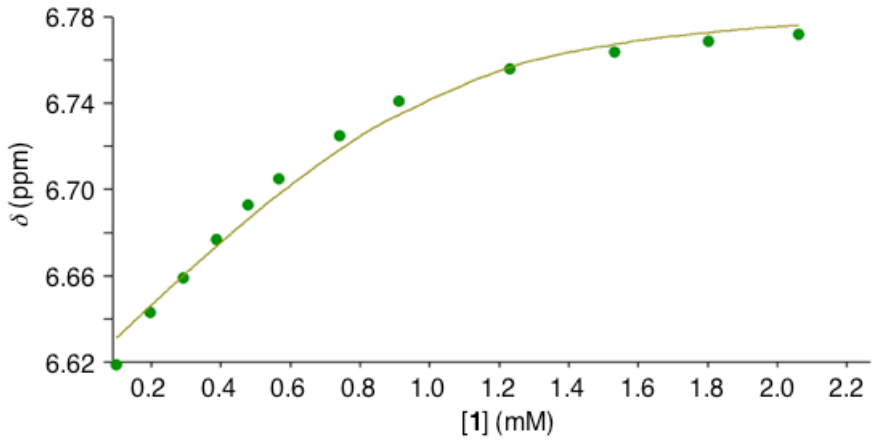

Fig. S6. The non-linear curve-fitting of the ^{1}H NMR titration experiment (500 MHz, 298 K, D_2O Na_2HPO_4/NaH_2PO_4 50 mM, pH 7.2) of a 1 mM buffered solution of **WP5** upon varying the concentration of **1** from 0.099 to 2.06 mM.

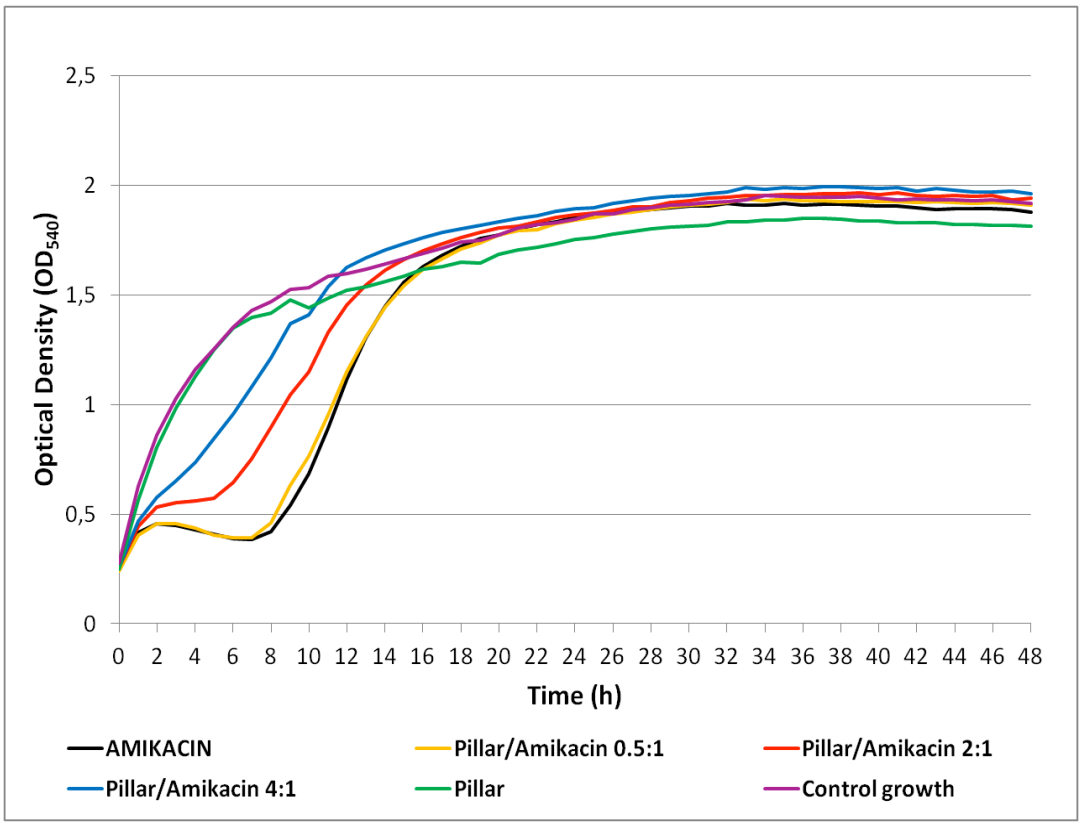

Fig. S7. Kinetics of the growth of *S. aureus* in a MHB medium, analyzed by measuring the optical density at 540 nm (OD_{540}) for an incubation time of 48 hours, inoculated with: amikacin (2.5 μg/mL), black trace; amikacin (2.5 μg/mL) and **WP5** (0.5 equiv., 1.6 μM), yellow trace; amikacin (2.5 μg/mL) and **WP5** (2.0 equiv., 6.4 μM), red trace; amikacin (2.5 μg/mL) and **WP5** (4.0 equiv., 12.8 μM), blue trace; **WP5** (12.8 μM), green trace and control culture, purple trace.

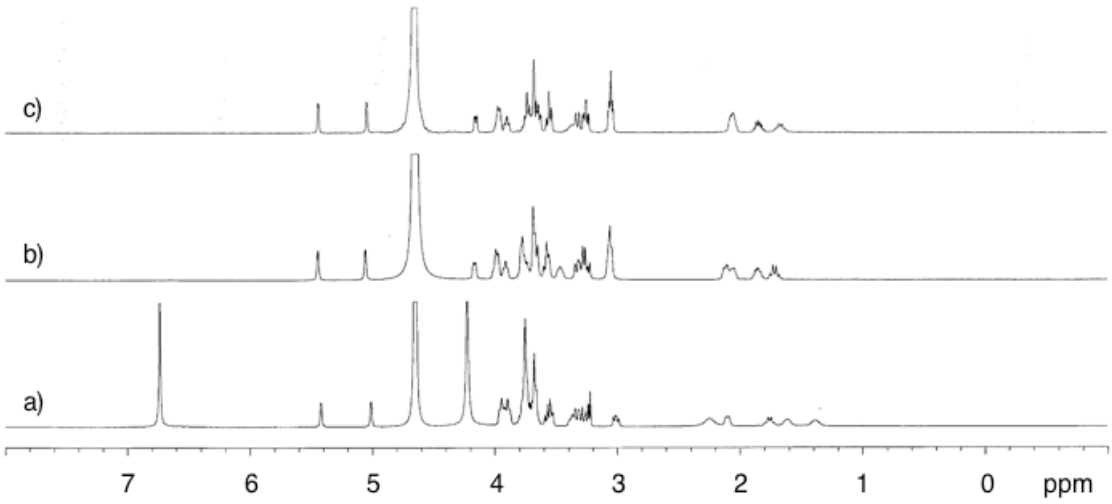

Fig. S8. ^{1}H NMR spectra (500 MHz, 298 K) of D_2O solutions of: a) [**WP5**] = 4 and [**1**] = 8 mM; b) [**WP5**] = 4 and [**1**] = 8 mM upon addition of an aqueous solution of 6 M DCl (2 μL) and c) [**1**] = 4 mM.

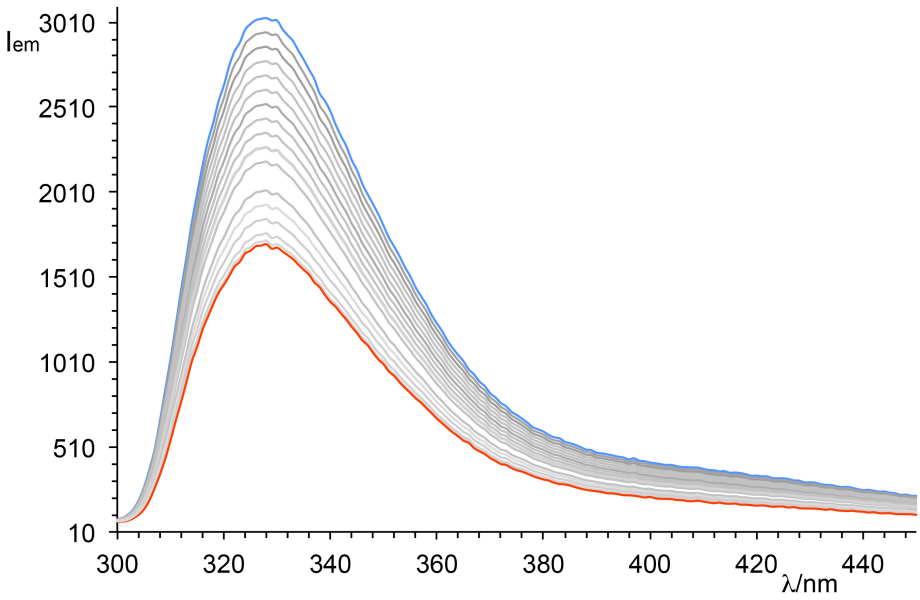

Fig. S9. Emission titration spectra (λ_{exc} = 280 nm) of **WP5** (1.20 × 10^{-5} M) upon addition of increasing amounts of **1** in a buffered aqueous solution (Na_2HPO_4/NaH_2PO_4 50 mM, pH 7.2).

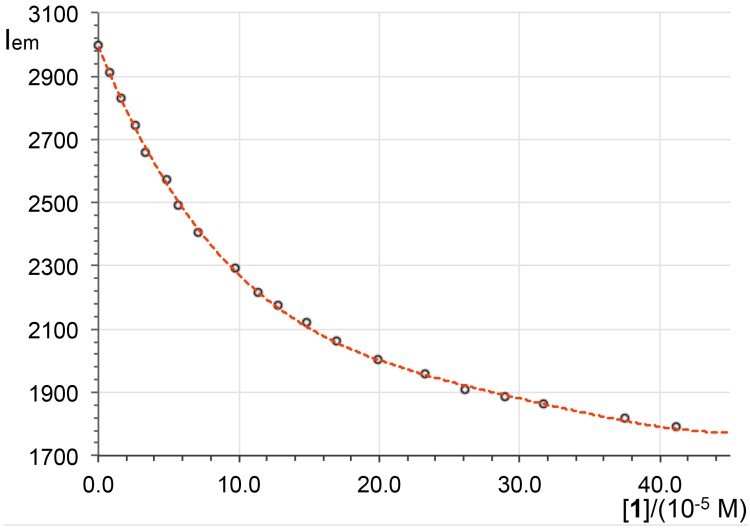

Fig. S10. Emission intensity changes of **WP5** (λ = 327 nm) upon addition of **1**. The dashed red line was obtained from a non-linear curve-fitting.