Supplementary Information

Concealing the Taste of the Guinness World's Most Bitter Substance by a Synthetic Nanocontainer

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Experimental Section

Materials and Instruments

The nanocontainer molecule CB[7] was prepared according to a previously published method.[1] Denatonium benzoate was purchased from JK Chemical (Shanghai) and used without further purification. All samples were prepared with ultrapure water (18 MΩ·cm). A Thermo LTQ Orbitrap XL equipped with an ESI/APcI multiprobe was used to acquire ESI-MS spectra. A Bruker 600 MHz NMR spectrometer was used to record the NMR spectra. A Malvern Microcal PEAQ ITC was used to conduct all ITC experiments. The modelled structures of the complexes demonstrated in this investigation were calculated by energy-minimizations using DFT (B3LYP/6-31G(d)) level of theory within the Gaussian09 Rev. D01 package program, which were conducted at the computing facility of Aix Marseille Université, France.

Animals

Forty adult female C57BL/6 mice (Animal Facility, University of Macau, Macau) were maintained on a 12:12 light-dark cycle and at an ambient temperature of 20 ± 2°C with *ad* libitum access to water and food. **All animal experiments were conducted in accord with the ethical guidelines of the Institute of Chinese Medical Sciences, University of Macau, and the protocol was approved by the Animal Ethics Committee, University of Macau**.

^{1}H NMR titration

^{1}H NMR titration experiments of DB with CB[7] were performed in D_2O at room temperature. A 1 mM DB solution was prepared. 0.5 equiv., 1.1 equiv. and 2.1 equiv. CB[7] was dissolved in the prepared DB solution, respectively. These solutions were characterized by using ^{1}H NMR spectroscopy (NS = 128 times).

Electrospray Ionization-Mass Spectrometry (ESI-MS)

CB[7] (0.2 mM) and DB (0.1 mM) in H_2O was filtered (0.25 μm) and then employed for ESI-MS determination. ESI-MS analytical parameters: ionization spray voltage 4.5 kV, capillary temperature 275 °C, and sheath gas pressure (N_2) 5 arb.

Isothermal titration calorimetry (ITC)

DB solution (3.0 mM) and CB[7] solution (0.2 mM) were prepared respectively with degassed ultrapure water for the ITC experiments. Solutions were degassed by centrifuge (12000rpm, 10

min) before the titration. CB[7] solution (0.2 mL) was added into the sample cell and DB solution (0.04 mL) was loaded in the syringe for titration. The experiment was conducted by titrating 19 aliquots of DB solution at T = 25.0 ^{o}C at the time intervals of 150 s and stirring speed of 750 rpm. The first injection of 0.4 µL DB was ignored to eliminate the diffusion effects from the syringe on the calorimetric cell. The subsequent injections were carried out at a constant volume of 3.0 µL DB. The obtained raw data were further analyzed by the built-in software of MicroCal PEAQ ITC Analysis after subtraction of the blank data (dilution of DB in water). The ‘‘one set of sites’’ mathematic model was applied to analyze the thermodynamic parameters.

Two-bottle Preference Test

Drinking tubes for measuring fluid intakes were made of 50 mL conical centrifuge tubes (Falcon®), a sizeable rubber stopper and a stainless-steel tip with a small hole from which the mice could lick fluids. Before TBP test, the mice were housed in individual cages for a week with two tubes of MilliQ water for being accustomed to have two tips to drink water. After that, the mice were treated with one tube containing MilliQ water and the other one containing different concentrations of DB (100, 300, 500 µM) in the absence or in the presence of CB[7].

Two tubes have paralleled position with the same angle of tip. Each solution was tested for 48 h. To avoid the side preference caused by the unaltered position, the positions of two tubes were switched after 24 h of each 48-hour test. Measurements of 24-hour fluid intakes of individually caged mice were recorded before exchanging tube’s position. Body weights were recorded after the TBP test within 2 weeks to evaluate if the intakes would cause systemic toxicity on mice.

Data analyses

In TBP test, average daily (24-hour) fluid intakes were calculated for each mouse for each tested solution. Preference (%) = [average daily tested solution intake / average daily total (tested solution + MilliQ water) intake] × 100%. The significance of preference or avoidance of a taste solution in the TBP test was determined by comparing the solution and water intake using paired t-tests. The data for each tested group were analyzed using one-way ANOVA.

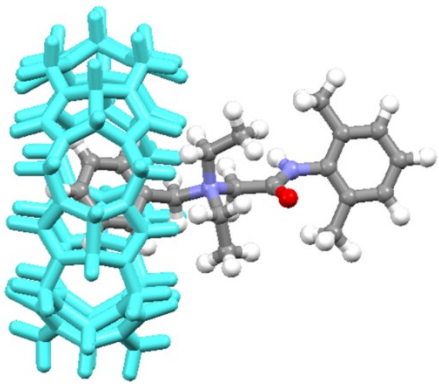

Fig. S1 Molecular modelling of the complex, with the benzyl group encapsulated in the cavity

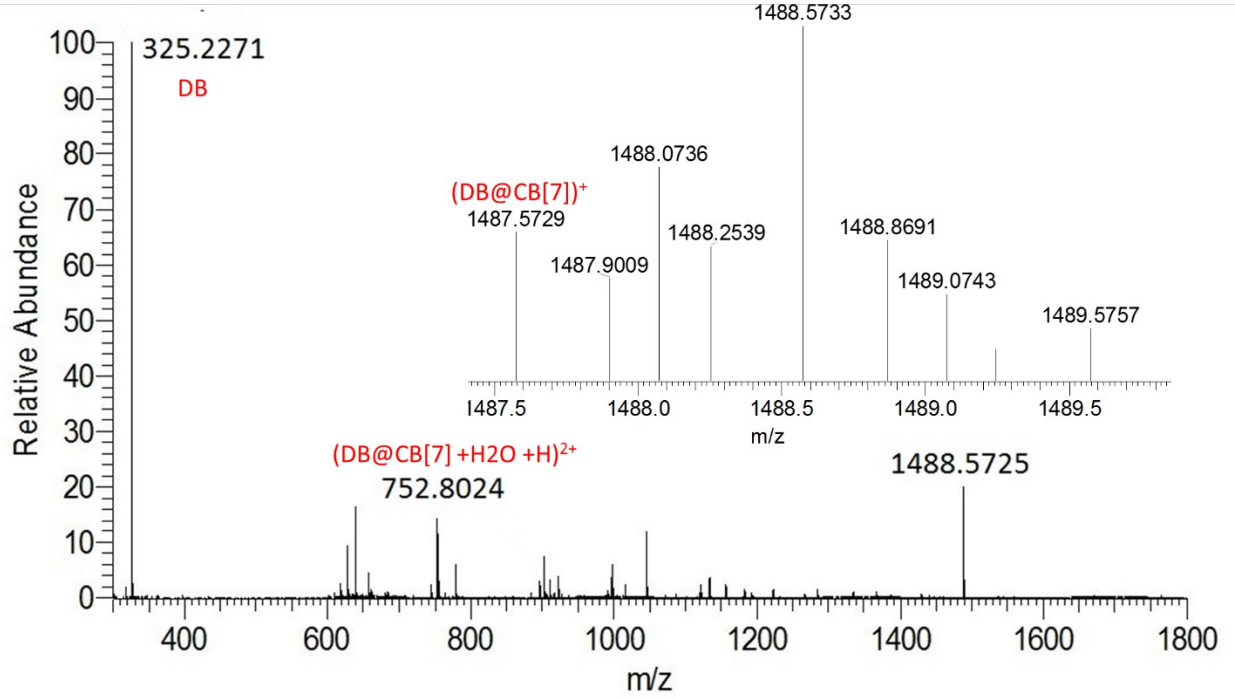

Fig. S2 ESI-MS spectrum of DB with CB[7]. The singlet charged peak at m/z = 1487.5729 is corresponding to $[DB@CB[7]]^+$ (calculated m/z = 1487.5715).

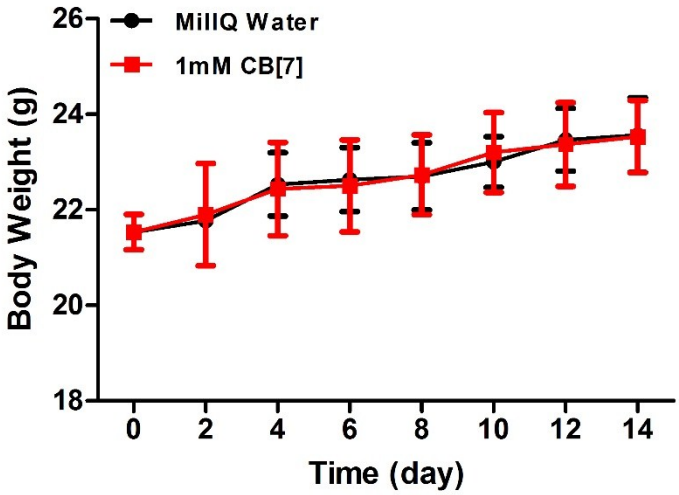

Fig. S3 The body weight of mice after exposure to MilliQ water and CB[7].

1.A. Day, A. P. Arnold, R. J. Blanch and B. Snushall, *J. Org. Chem.*, 2001, **66**, 8094-8100.