Acyclic Cucurbit[n]uril-Type Molecular Containers: Influence of Glycoluril Oligomer Length on their Function as Solubilizing Agents

Supporting Information

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R = CH$_2$CH$_2$CH$_2$SO$_3$Na
D$_2$O, 125 MHz
1,4-dioxane as internal reference
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**Figure S19.** $^1$H NMR recorded (400 MHz, 20 mM NaD2PO4, pD = 7.4) for 1b at different concentrations (0.1 mM – 20 mM) for self-association study.

**Figure S20.** Plot of chemical shift of 1b versus [1b]. The solid line represents the best non-linear fitting of the data to a two-fold self-association model with $K_s = 92$ M$^{-1}$. 


Figure S21 $^1$H NMR recorded for 2b at different concentrations (0.1 mM to 190 mM) (400 MHz, 20 mM NaD$_2$PO$_4$, pH = 7.4) for self-association study.

Figure S22. Plot of chemical shift of 2b versus [2b] The solid line represents the best non-linear fitting of the data to a two-fold self-association model with $K_s = 6$ M$^{-1}$. 
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**Figure S28.** Plot of chemical shift of 3a versus [3a]. The solid line represents the best non-linear fitting of the data to a two-fold self-association model with $K_s = 3 \text{ M}^{-1}$. 
Procedure to measure the solubility of containers 1-3. Excess amount of container was stirred in deuterated sodium phosphate buffer (20 mM, pD = 7.4). The suspended mixture was magnetically stirred at room temperature for 12 h. During this period, the pD value of the solution was monitored and adjusted back to 7.4 if it changed. The mixture was then filtered and diluted (usually 10-fold). The $^1$H NMR spectrum of the supernatant was measured (400 MHz) after addition of 1,3,5-benzenetricarboxylic acid (usually 1.00 mM) as internal standard. The signal for the internal standard resonates at 8.35 ppm (s, 3H). Diagnostic signals for the containers were also integrated. From the ratio of the integrals of the internal standard relative to the drug resonances, and the concentration of reference, the concentration of the containers can be calculated.

Procedure to measure the solubility of drugs with Host 1 – 3. Excess amount of drug was added into a solution of host (1 – 3) of known concentration in deuterated sodium phosphate buffer (20 mM, pD = 7.4). The suspended mixture was magnetically stirred at room temperature for 6 h. During this period, the pD value of the solution was monitored and adjusted back to 7.4 if it changed. The mixture was then filtered. The $^1$H NMR spectrum of the supernatant was measured (400 MHz) with 1,3,5-benzenetricarboxylic acid (1.00 mM) as internal standard. The resonance for the internal standard appears at 8.35 ppm (s, 3H). Diagnostic signals for the dissolved drug were also integrated. From the ratio of integrals of the internal standard relative to the resonances for the drug, and the known concentration of internal standard allows calculation of the concentration of the drug.
**Figure S29.** Phase solubility diagram of $\beta$-estradiol with different hosts in sodium phosphate buffer (20 mM, pH 7.4, RT). Data points shown in red were not used in the linear fit.

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Figure S47. $^1$H NMR recorded for camptothecin with 3b (10 mM) (400 MHz, 20 mM NaD$_2$PO$_4$, pD 7.4, RT, 1,3,5 benzenetricarboxylic acid as reference).
Sample Error Analysis of Calculation for $K_a$. The $K_a$ values were obtained from equation 1 and the uncertainty of $K_a$ values was related to the intrinsic solubility values ($S_0$) and the slopes of the linear fit of the phase solubility diagrams (Slope). Here we give a sample calculation of the error analysis used to determine the uncertainty associated with the $K_a$ value for 1b•Camptothecin.

$$K_a = \frac{Slope}{S_0 \cdot (1 - Slope)}$$ (1)

**Step 1 – Determination of the uncertainty of the intrinsic solubility of the water-insoluble drug and slopes of the linear fit of the phase-solubility diagrams.** The slope values were obtained from the linear fit of the phase solubility diagrams and the uncertainty of the slope values were obtained as the standard deviation. The intrinsic solubility of the water-insoluble drug was measure with $^1$H NMR for three times. The mean value of the samples was used as the $S_0$ value and the standard deviation of the samples was used as the uncertainty of $S_0$.

**Step 2 – Determination of the uncertainty of $K_a$.** We propagated the above uncertainty with $K_a$ using equation 1 and equations 2 – 5 (Bevington “Data Reduction and Error Analysis for the Physical Sciences”, McGraw-Hill, New York, 1969, page 61 – 62, eq. 4 – 11). Equation 2 delivers the uncertainty associated with the weighted product (x) of two values (u and v) (e.g. $x = \pm a \ u \ v$). Similarly, equation 3 delivers the uncertainty for dividing two numbers (e.g. $x = \pm (a \ u) / v$). We make the assumption that the fluctuations in u and v are not correlated ($\sigma_{uv} = 0$) which when substituted into equations 2 and 3 delivers equation 4. Equation 5 delivers the uncertainty for addition and subtraction ($x = u \pm v$).

$$\frac{\sigma_x^2}{x^2} = \frac{\sigma_u^2}{u^2} + \frac{\sigma_v^2}{v^2} + 2 \frac{\sigma_{uv}}{uv}$$ (2)

$$\frac{\sigma_x^2}{x^2} = \frac{\sigma_u^2}{u^2} + \frac{\sigma_v^2}{v^2} - 2 \frac{\sigma_{uv}}{uv}$$ (3)

$$\frac{\sigma_x^2}{x^2} = \frac{\sigma_u^2}{u^2} + \frac{\sigma_v^2}{v^2}$$ (4)

$$\sigma_x^2 = \sigma_u^2 + \sigma_v^2$$ (5)

In this calculation, we break the calculation into three parts: 1) Subtracting (1 – Slope), 2) Multiplying ($S_0 \cdot (1 – Slope)$), and 3) Dividing Slope with the results in the previous part. From equation 5, we determined that $\sigma_{\text{Slope}} = \sigma_{1 - \text{Slope}}$. From equation 4 we can obtain the uncertainty of $S_0 \cdot (1 – Slope)$ (equation 6).

$$\frac{\sigma_{S_0(1-\text{Slope})}^2}{S_0(1 – Slope)^2} = \frac{\sigma_{S_0}^2}{S_0^2} + \frac{\sigma_{\text{Slope}}^2}{(1 – Slope)^2}$$ (6)

Meanwhile, from equation 4 we are able to calculate the uncertainty of $K_a$ from equation 7.

$$\frac{\sigma_{K_a}^2}{K_a^2} = \frac{\sigma_{\text{Slope}}^2}{Slope^2} + \frac{\sigma_{S_0(1-\text{Slope})}^2}{S_0^2 (1 – Slope)^2}$$ (7)

Substitution equation 6 into equation 7 gives equation 8.

$$\frac{\sigma_{K_a}^2}{K_a^2} = \frac{\sigma_{\text{Slope}}^2}{Slope^2} + \frac{\sigma_{S_0}^2}{S_0^2} + \frac{\sigma_{\text{Slope}}^2}{(1 – Slope)^2}$$ (8)
Substitution of $S_0 \ (54 \ (\pm \ 3.9) \times 10^{-6} \ M)$, Slope $(0.54 \ (\pm \ 0.0032))$ and $K_a \ (2.2 \times 10^4 \ M^{-1})$ into equation 8 gives the $\sigma_{Ka} = 1.6 \times 10^3 \ M^{-1}$.

**Binding Model (Self-association) implemented in Scientist™**

// Micromath Scientist Model File
// self-association model for NMR
IndVars: conctot
DepVars: Deltaobs
Params: Ka, Deltasat, Deltazero
Ka = concBound/(concFree*concFree)
concTot=concFree + concBound/2
Deltaobs = Deltazero + (Deltasat - Deltazero) * (1/2*concBound/concTot)
//Constraints
0 < Ka
0 < concFree < concTot
0 < concBound < concTot
***

**Binding Model (Self-association) for Global Fitting implemented in Scientist™**

// Micromath Scientist Model File
IndVars: ConcHost
DepVars: CSA, CSB, CSC
Params: Ka, CSAzero, CSAsat, CSBzero, CSBsat, CSCzero, CSCsat
Ka = ConcHG/(ConcHfree*ConcGfree)
ConcHost=ConcHfree+ConcHG
0.0002=ConcGfree+ConcHG
CSA = CSAzero + ((CSAsat-CSAzero)*(ConcHG/0.0002))
CSB = CSBzero + ((CSBsat-CSBzero)*(ConcHG/0.0002))
CSC = CSCzero + ((CSCsat-CSCzero)*(ConcHG/0.0002))
0<ConcHfree<ConcHost
0<ConcGfree<0.0002