Photodeamination to quinone methides in cucurbit[*n*]urils: potential application in drug delivery

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We demonstrate a proof of principle for a new approach in the development of a drug delivery system. Positively charged prodrug (phenol) can form stable inclusion complex with CB[7], which enables more efficient delivery of the prodrug. After photochemical transformation (photoactivation) inside the complex, an active drug quinone methide (QM) is formed and released from the complex, since it is neutral molecule and forms less stable complex with CB[7].

One of the important objectives in drug development is the optimization of the ADME (absorption, distribution, metabolism, and excretion) properties of the system. In the *lead* optimization for a potential drug candidate, it is important to maximize the therapeutic agent release at the desired anatomical site and the maintenance of the drug concentration within the therapeutic range for a desired duration. Many compounds with very high pharmaceutical activity are never developed into drugs due to their poor ADME properties. However, some problems, such as poor solubility in water or low stability under certain physiological conditions, can be circumvented by developing drug delivery systems through advancement of conventional drug formulation techniques.[1] Supramolecular drug delivery host-guest systems based on non-covalent interactions offer new possibilities for the design of novel drug delivery methods.[2] One approach to supramolecular drug delivery is encapsulation of the drug inside a macrocyclic host, in which the drug molecule is protected from reactive species in the aqueous environment. Much progress has already been made by exploring different types of supramolecular host-guest interactions in drug formulations.[2,3]

Cucurbit[n]urils (CB[n]s) are a family of macrocyclic compounds composed of different number of glycouril units (Scheme 1 and Fig. S1 in the ESI).[4] The inner cavity of CB[n]s is characterized by low polarity and polarizability, whereas the two portals are decorated with carbonyl groups that act as Lewis bases with encapsulated guests.[5] Therefore, CB[n]s are ideal hosts for hydrophobic guests bearing positive charges.[6] Contrary to cyclodextrins of comparable size, binding is generally characterized by much higher affinity constants provided the guest has a positive charge.[7] Encapsulation of guests in the CB[n]s offers special physico-chemical properties such as increase of the guest's conjugated acid pK_a, changes in photophsyical properties, higher guest solubility and protection of the guest from reactivity with species outside of the complex, allowing for the development of applications including sensing, logic gates and molecular machines.[8] Moreover, the low cytotoxicity of CB[n]s,[9] the ability of this macrocycle to cross cell membranes[10] and to solubilise drugs in physiological media[11] allowed for the development of novel systems for drug delivery using CB[n] as drug carriers.[12] CB[n]s can be used for the delivery of atenolol, glibenclamide, memantine and paracetamol,[13] topotecan,[14] some steroids[15] and doxorubicin.[16] One property of drug delivery systems of pivotal importance is the control of the uptake and release of guest drug molecules.[17] CB[n]s show promise in this respect,[18] such as through modulation of guest molecules pK_a upon binding.[19] Enhanced antiproliferative action and lower cytotoxicity of drugs in the presence of CB[n]s has been demonstrated for platinum organometallic complexes,[20] doxorubicin,[21] temozolomide,[22] or camptothecin.[23] Furthermore, photochemical stimuli have been used for drug release from cucurbit[n]urils.[24] However, photochemical generation of a drug in the CB[n] inclusion complex from the prodrug has hitherto not been reported. Herein we

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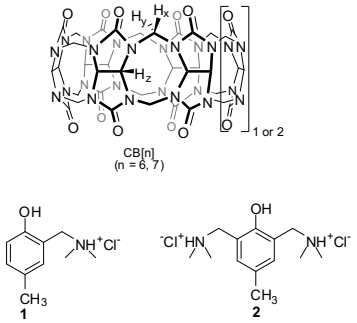
Electronic Supplementary Information (ESI) available: general experimental procedures, protonation constants for prototropic forms for **1** and **2**, UV-vis spectra of **1** and **2** in the presence of CB[6] and CB[7], NMR titration experiments, NOESY spectra, molecular modeling data, isothermal microcalorimetric titration data, laser flash photolysis data and antiproliferative tests. See DOI: 10.1039/x0xx00000x

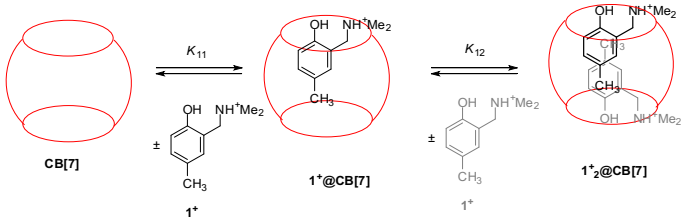
demonstrate a different approach in the development of a drug delivery system where the prodrug@CB[7] complex affords the desired solubilisation and transport properties, while the active drug is formed through a photochemical process followed by drug release, which has the potential for spatial and temporal control of the release. As prodrug candidates (guests, G), we chose phenol derivatives **1** and **2** (Scheme 1) which are positively charged molecules that are anticipated to form stable complexes with CB[n]s. In photochemical deamination reactions **1** and **2** deliver quinone methides (QMs).[25] QMs are reactive intermediates that induce alkylation of biologically important molecules including amino acids,[26] proteins[27] and DNA,[28] and therefore, exert anticancer activity.[29] Furthermore, QMs are neutral species (not bearing charge) which should form less stable inclusion complexes with CB[n]s, and therefore, dissociate from the hosts.



Scheme 1 Structures of host molecules (H) cucurbit[n]urils CB[6] and CB[7] and cresol guest molecules (G) **1** and **2**.

The cresol derivatives **1** and **2** were prepared as described in the literature.[25] Their complexation with CB[6] and CB[7] was probed by UV-vis, and NMR spectroscopy, as well as by isothermal titration calorimetry (ITC). UV-vis spectroscopy revealed the complexation of **1** and **2** with CB[6] or CB[7] when the spectra were measured at pH values close to the pK_a of **1** and **2**. (for pK_a values see Schemes S1, S2 and Table S1 in the ESI; for the UV spectra see Fig. S2 and S3). This result is expected since CB[n]s usually increase the pK_a of the conjugated acid of complexed molecules by up to 2-3 orders of magnitude.[30] However, the changes in the UV-vis spectra were too small to determine binding isotherms (Figs. S2 and S3 in the ESI), which were obtained from ITC measurements.

The ITC experiments were conducted at different pH values to show how the binding ability of **1** and **2** depended on the different prototropic forms (Scheme S1 and S2 in the ESI). In near-neutral and acidic solution, at pH < 8.5 for **1**, and pH < 5.9 for **2**, the guests are in the monocationic (**1**$^+$) and dicationic form (**2**$^{2+}$), respectively, so they are expected to bind to CB[n]s with the highest affinity constants possible. The fit of the binding isotherm for the complex formation of **1**$^+$ with CB[7] at pH 2.47 required the inclusion of the 1:1 and 1:2 (host:guest) equilibria (Fig. 1 and Scheme 2). The formation of the 1:1 complex (K_{11}) is enthalpically and entropically favored and the equilibrium constant for this complex (5.3 ± 0.3) × 10^4 M^{-1} is higher than for the 1:2 complex (K_{12}) (9 ± 1) × 10^3 M^{-1}, where complexation of the guest is entropically favored but enthalpically disfavored (Table S2 in the ESI). The binding of neutral (zwitterionic) **1zw** at pH 9.83 is much weaker as expected with the loss of the positive charge on the guest (Fig. S4 and Table S2 in the ESI). The binding of **2**$^{2+}$ with CB[7] is much weaker than for **1**$^+$, with equilibrium constants of 600 ± 200 M^{-1} and 22 ± 6 M^{-1} (Fig. S5 and Table S2 in the ESI) The binding of **1**$^+$ and **2**$^{2+}$ with CB[6] was attempted but the heat evolution is too low for the determination of binding constants using ITC.



Scheme 2

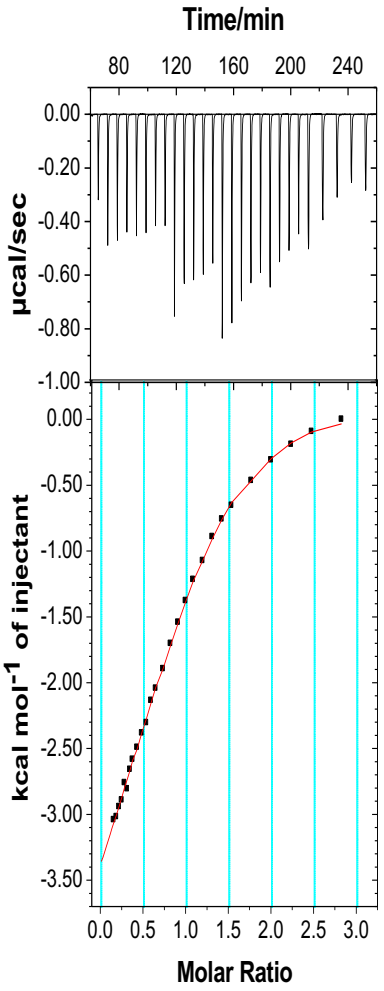


Fig 1. ITC titration of CB[7] (0.1 mM) with **1**$^+$ in H_2O at pH = 2.47 in the presence of phosphate buffer (0.1 M) and NaCl (0.1 M) at 25 °C. Top: raw ITC data; Bottom: dependence of successive enthalpy changes per mol of titrant on the **1**$^+$:CB[7] ratio. The calculated fit is shown as a red line.

^{1}H NMR titrations and NOESY experiments were conducted to verify the stoichiometries obtained for the complexes with ITC measurements and to obtain structural information about

these complexes (for details see Tables S3-S10 in the ESI). In the NMR titration of CB[6] with **1**$^+$ at pH 5.6, no changes were observed in the NMR spectra (Fig. S6 and Table S5 in the ESI), but NOE interactions between H-atoms of **1**$^+$ and CB[6] were observed (Figs. S7 and S8 in the ESI). The cavity of CB[6] is too small to accommodate **1**$^+$ (Fig. S9 in the ESI) which is in line with the lack of changes observed in the NMR spectra. However, the NOE data suggests the formation of an exclusion complex (**1**$^+$**·CB[6]**) where the positive charge of **1**$^+$ interacts with the portals of CB[6]. The behavior is similar for the larger guest **2**$^{2+}$ (Fig. S10 in the ESI) where the NOESY spectra (see Fig. S11 in the ESI) are consistent with the formation an exclusion complex **2**$^{2+}$**·CB[6]**.

The ^{1}H NMR spectra measured in the titration of CB[7] with **1**$^+$ at pH 2.63 (pD 3.04) (Fig. 2, Figs. S12 - S14 and Table S6 in the ESI) indicates that the exchange on the ^{1}H NMR timescale between free and complexed guest is fast because no distinct resonances for the protons of the free and bound guest were observed. The continuously shifting peaks are a measure of the average environment that the free and complexed guest experiences. These shifts shown that upon binding to CB[7], the protons of the N(**CH$_3$**)$_2$ are deshielded, whereas Ar-**CH$_3$** protons are shielded. Phenyl ring protons are also shielded in the complex, but a significant line broadening takes place, so their chemical shifts are not available. However, discernible shifting signals of the methyl groups indicate that the exchange is fast. The trend of the signal changes suggests that the N(**CH$_3$**)$_2$ protons in **1**$^+$ are above the CB[7] rim and form electrostatic interactions with the portals bearing carbonyl groups. On the contrary, Ar-**CH$_3$** and Ar-**H** protons are in the cavity and experience the shielding effect of the CB[7] carbonyl groups. These trends in the spectra are consistent with the formation of an inclusion complex **1**$^+$**@CB[7]** and this assignment is supported by literature precedent for the inclusion complexes of small molecules.[31] Formation of the inclusion complex was further supported by a NOESY spectrum (see Fig. S15 and Table S7 in the ESI).

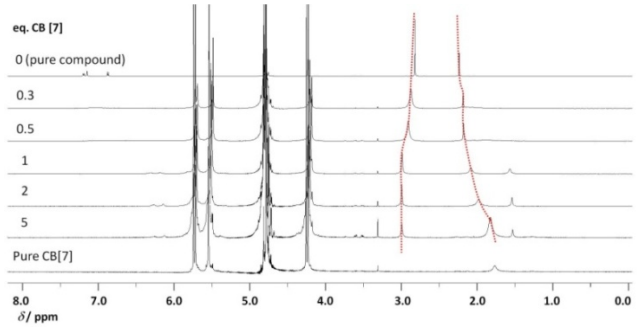


Fig 2. ^{1}H NMR spectra of **1**$^+$ (6 mM) in D_2O, in the presence of NaCl (0.1 M), buffered with D_3PO_4 and NaD_2PO_4 (0.1 M) to pH = 2.63, in the presence of different concentrations of CB[7]. The spectra from top to bottom correspond to pure **1**$^+$ and increasing concentrations of CB[7] (0.32-200 mM), while the bottom spectrum corresponds to neat CB[7] (3.5 mM) in buffered D_2O (dependence of the chemical shift of the N(**CH$_3$**)$_2$ and Ar-**CH$_3$** protons on concentration of CB[7] are shown in Figs. S16 and S17).

The NMR titration data were processed by Thordarson's fitting program.[32] These experiments showed that **1**$^+$ and **2**$^{2+}$ form inclusion complexes with CB[7] with 1:1 and 1:2 H:G stoichiometries. For the titration of **1**$^+$ with CB[7] at pH 2.63, the best fit was obtained with a model assuming a 1:2 H:G stoichiometry, judged by the random distribution of residuals between the experimental and the calculated values (Fig. S16 in the ESI). However, errors on the values for the equilibrium constants recovered from the fitting routine were large. Therefore, the ^{1}H NMR data reveal the formation of two complexes, but these data could not be used for the quantification of the equilibrium constants and therefore we rely on the equilibrium constant values obtained by ITC. Analysis of the molecular geometry reveals that two guests can be accommodated in CB[7] (Fig. S9 in the ESI). Examples of CB[7] binding two guests are not common, and they were mostly related to inorganic cations acting as lids on the CB[7] portals.[4,33] In the titration experiment with zwitterionic **1zw** and CB[7] at pH 9.25 the same trend for the signal changes was observed when compared to the binding of **1**$^+$, indicating formation of an inclusion complex of **1zw** with CB[7] and the same stoichiometry 1:1 and 1:2 (Figs. S17-S19 and Table S8 in the ESI).

The titration with **2** and CB[7] was conducted at pH 2.51, 7.75 and 12.7 where the molecule is in dicationic **2**$^{2+}$, monocationic **2**$^+$, and neutral zwitterionic form **2zw**, respectively (Table S9 in the SI). In all cases, NMR titrations indicated complex formation. At pH 2.51 and low CB[7] concentration, the Ar-**CH$_3$** protons experienced deshielding, whereas at higher CB[7] concentration they are shielded (Figs. S20-S22 and Table S10 in the SI). This observation was rationalized by the formation of complexes with 1:1 and 1:2 (H:G) stoichiometries. The shielding effect is due to the formation of 1:1 inclusion complex, whereas deshielding results from the anisotropic effect of one aryl ring to the methyl group of the other molecule in the 1:2 complex.

Laser flash photolysis (LFP) experiments[34] using 266 nm excitation were conducted to monitor the formation of QMs in the presence of CB[n]s. The formation of the quinone methide **1QM** (Scheme 3), with a characteristic absorption maximum at 400 nm,[25] was observed for solutions of **1**$^+$ and **1zw** in the absence and presence of CB[7] (Fig. 3, for all LFP data see Figs. S23-S37 and Tables S11 and S12 in the ESI). The formation of the transient occurred within the laser pulse and the transient absorbance at the end of the laser pulse is a measure of the efficiency with which the QM is formed for samples that have the same absorbance at 266 nm. In the presence of CB[7], the efficiency for QM formation is about 20% lower than without CB[7]. QM formation is also observed in the presence of CB[6] but in this case the formation efficiency is the same with and without CB[6], which is in accordance with the conclusion that **1** and CB[6] cannot form the inclusion complex.

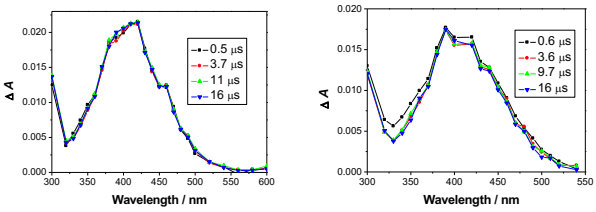
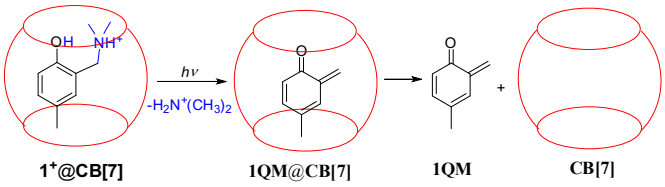


Fig 3. Transient absorption spectra of **1**$^{+}$ (c = 6.35 × 10^{-4} M) in CH_3CN-H_2O (1:9) at pH 4.41 without CB (left), and in the presence of CB[7] c = 6.79 × 10^{-4} M (right).



Scheme 3

The calculated distribution of species for the experimental conditions of the LFP experiments based on the K_{11} and K_{12} values determined by ITC (5 % **1**$^{+}$, 55% **1**$^{+}$**@CB[7]** and 40 % **1**$^{+}_2$**@CB[7]**) shows that more than 95% of **1** was complexed. The formation of the QM occurs within the time resolution of the LFP system (20 ns) and this time is too short for a relocation to occur of the excited state of **1**$^{+}$ from the complex to the aqueous phase. Therefore, if **1QM** was only formed in the aqueous phase, the transient signal after the laser pulse should have been 5% compared to the signal in the absence of CB[7]. The much larger signal in the presence of CB[7] shows that **1QM** is formed inside of the complex.

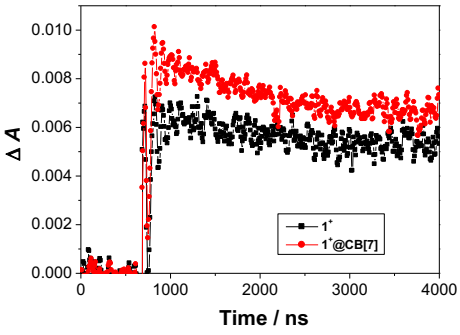


Fig 4. Decay of transient absorbance at 330 nm for **1**$^{+}$ (c = 6.35 × 10^{-4} M) in CH_3CN-H_2O (1:9) at pH 4.41 without CB[7] (black) and in the presence of CB[7] c = 6.79 × 10^{-4} M (red). The solutions were optically matched A_{266} = 0.37. For CB[7] k_{obs} = (1.0 ± 0.1) × 10^{6} s^{-1}.

The decay of QM is primarily taking place due to nucleophilic attack by H_2O.[25,29] The lifetimes of **1QM** in the absence and presence of CB[7] are the same at each pH studied (12-15 ms at pH 4.41 and 11 ms at pH 7.0, see table S11 in the ESI), indicating either that dissociation of **1QM** from CB[7] is faster than the **1QM** decay in water or that access of water to the CB[7] cavity is not impeded. The former possibility is supported by the analysis of the transient kinetics at short delays. Neutral **1QM** does not have the positive charge of precursor **1**$^{+}$ and we expect the binding dynamics of **1QM** to be faster in line with previous reports for the comparison of the complexation dynamics of neutral and charged guests with CB[n]s.[35,36] In the presence of CB[7], but not in water, we observed at short delays a weak transient between 310 and 380 nm (Fig. 3) with a lifetime of ca. 1 µs (Fig 4). We assign this fast decay to the exit kinetics of the QM from the inclusion complex (Scheme 3). Since QM is a polar species, it is plausible that its absorption spectrum exhibits solvatochromic shifts depending on solvent polarity, which is consistent with the assignment of the fast decay to the exit of **1QM** from CB[7]. Moreover, eliminated dimethylammonium molecule from **1**$^{+}$ is a charged species that is anticipated to bind to CB[7] and assist in the dissociation of **1QM** from the complex.

LFP experiments for **2**$^{2+}$ in the presence of CB[7] suggest that QM is also formed in the complex, although the complex **2@CB[7]** is less stable. However, QM formed from **2**$^{2+}$ contains a positive charge, so its dissociation from the complex is not anticipated (Scheme S3 in the ESI). Indeed, the fast decay at λ = 310-380 nm as seen for **1**$^{+}$ and CB[7], was not observed.

The supramolecular chemistry revealed here can rationalize the MTT tests on human cancer cell lines treated with **1** and **2**, CB[6], CB[7], or their mixtures (for the preliminary antiproliferative investigation on two human cancer cell lines see the ESI, Tables S13 and S14). The enhancement of antiproliferative activity observed with **1** (but not **2**) in the presence of CB[7] (but not CB[6]), upon irradiation only, is in agreement with the hypothesis that the inclusion complex **1@CB[7]** is formed and that upon irradiation the active drug **QM1** is released inside the cell where it leads to the biological effect. Our studies show the importance of determining the relevant host, CB[7] in this case, for the formation of the desired biologically active molecule, the **QM**, by studying the host-guest complexation. Encapsulation of the prodrug in CB[n]s and formation of the biologically active drug upon a trigger is anticipated to have important impact in the development of anticancer drugs by minimizing side effects caused by the pro-drug. Moreover, the concept could be applied to any drug that is unstable in physiological conditions and where photochemical formation from a prodrug and fast release from the supramolecular host at the desired destination could lead to a significant biological action with minimal side effects. Consequently, a large number of *leads* in the drug development that were disregarded in the preclinical testing due to poor ADME properties may eventually be developed into drugs.

In conclusion, the investigation of the QM formation in the inclusion complexes indicates the proof of concept that encapsulation of QM-precursor **1** (prodrug) enhances its transport to the cell and delivery to the site of action where photochemical reaction creates the active drug. The QM which does not bear positive charge leaves the CB[7] cavity, eliciting the desired biological effect.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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