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Cucurbit[7]uril Inclusion Complexes of Platinum(II)-based Anticancer Drugs: Further Insight

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The ^{1}H NMR characterization of the encapsulation of bisplatinum complex CT-3610 by cucurbit[7]uril is reported. The formation of the intermediate monoinclusion complex can be easily detected in the ^{1}H NMR spectrum, and the pD dependence of the equilibrium has also been assessed. No significant differences were observed in cytotoxicity experiments between the free and encapsulated form of CT-3610 on A2780 and cisplatin resistant A2780 human ovarian cancer cell lines.

In the past 40 years, since the discovery of cisplatin (CDDP),[1] hundreds of new platinum(II) and platinum(IV)-based complexes have been synthesized and tested as anticancer drugs. Among them, carboplatin is currently used when treatment by cisplatin is poorly tolerated,[2] and oxaliplatin has been approved for the treatment of colorectal cancer in combination with 5-fluorouracil and leucovorin. All of these mononuclear platinum complexes have a similar mechanism of action, i.e., prevention of DNA transcription and replication leading to cellular apoptosis, developing drug resistance, and displaying severe dose-limiting toxicities.

Farrell and co-workers[3] synthesized a new generation of charged complexes (multinuclear platinum complexes) active at concentrations 10- to 100-fold lower than cisplatin and able in many cases to overcome drug resistance. Among them, BBR3464 reached Phase II clinical trials, but its development was discontinued due to unsatisfactory results which were ascribed to the extensive degradation by plasma proteins.

Recently, Wheate and co-workers[4–8] discovered that both toxicity and degradation of BBR3464 can be reduced by encapsulation inside the cucurbit[*n*]uril (Q[*n*]) macromolecules (Figure 1). Cucurbit[*n*]uril is a barrel-shaped molecule containing a hydrophobic cavity (Figure 1) and can be synthetized in a variety of sizes ($n = 5$, 6, 7, 8, and 10).[9] Encapsulation of reactive platinum complexes into cucurbit[7]uril protects them by steric hindrance from degradation, through either hydrolysis or attack by soft nucleophiles or various anions present in plasma.[10–14] Hence, cucurbiturils may represent an interesting drug delivery system.

CT-3610 (**1**, Figure 2) is an isosteric and equally charged dinuclear complex analog of BBR3464, where the central platinum atom has been replaced by an ethylenediamine group.

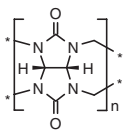

Figure 1. Chemical structure of (Q[*n*]).

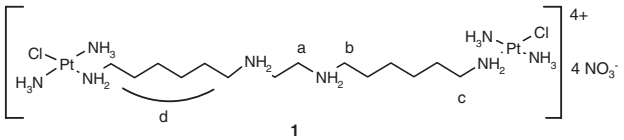

Figure 2. Chemical structure of bisplatinum complex CT-3610 (**1**).

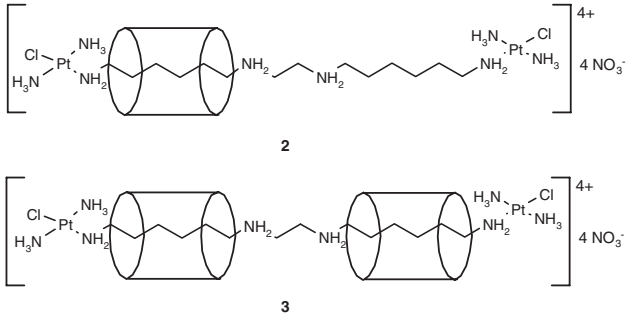

Figure 3. Chemical structures of mono-IC (**2**) and bis-IC (**3**).

In this work, we report the characterization studies of the encapsulation of **1** into cucurbit[7]uril (Q[7]) by NMR spectroscopy. Contrary to previous studies with BBR3464, we were able to characterize also the intermediate complex **2**, and we discovered a pD dependence of the complexation equilibrium.

First of all, we conducted a titration of **1** with Q[7] in D_2O solution directly in an NMR tube.[15] Two sets of peaks were observed at a Q[7]:**1** ratio lower than 0.5. The simultaneous presence of two sets of signals indicated that **1** binds Q[7] with slow exchange kinetics on the NMR time scale.[10] After 3 h of equilibration, no significant change in the molar ratios between each species present in the solution was observed, which is consistent with a fast reaction between **1** and Q[7].

At Q[7]:**1** molar ratios from 0.5 up to 1 three sets of signals were observed;[15] this was due to the simultaneous coexistence of three species: free CT-3610 (**1**), the monoinclusion complex (mono-IC) **2** and the final bisinclusion complex (bis-IC) **3**.

Even at Q[7]:**1** molar ratio of 2.5 about 10% residual **2** remained in solution, which could be ascribed to a thermodynamic additional cost to allow CT-3610 (**1**) to enter into the second Q[7] cavity, probably due to steric hindrance.

The inclusion complexes **2** and **3** were also obtained as a lyophilized powder from 1:1 and 2:1 ratio mixtures of Q[7] and **1** saline solutions (Figure 3), respectively.[15]

These complexes were prepared in saline solution because Q[7] is poorly soluble in pure aqueous solution.

The reaction mixtures were finally lyophilized and used for the subsequent NMR and biological studies.

In the literature, the guest molecules NMR behavior after encapsulation within the cucurbit[*n*]urils cavity has been

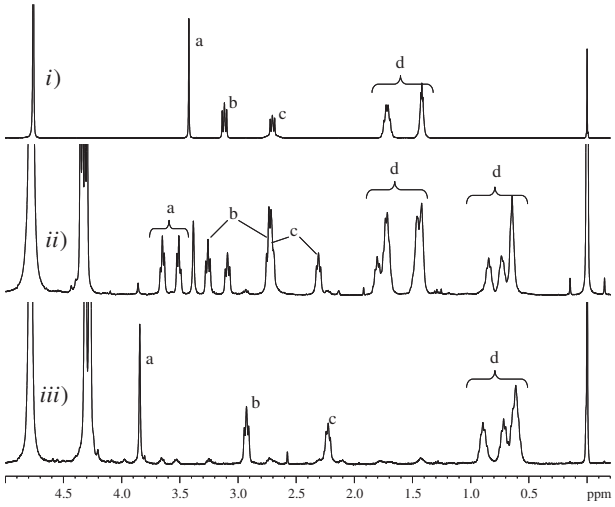

Figure 4. ^{1}H NMR spectra in D_2O 0.9% w/w NaCl of: *i*) CT-3610 (**1**); *ii*) mono-IC (**2**); and *iii*) bis-IC (**3**).

diffusely studied.[6,10,16–18] Full or partial encapsulation of platinum complexes is established through hydrophobic interaction within the cucurbituril cavity, while the portal regions are characterized by ion–dipole and dipole–dipole interactions.

In the ^{1}H NMR spectrum of lyophilized **2**, reconstituted with D_2O and directly used for the NMR experiment,[15] the resonance from the **a** methylene protons (Figure 4) was split into two triplet signals as a consequence of the loss of the molecular symmetry due to encapsulation of **1** within only one Q[7] (Figure 4). The higher downfield shift (0.27 ppm) of one triplet is consistent with the proximity of one of the **a** methylene to the Q[7] portal region, whereas the relatively modest downfield shift (0.13 ppm) of the other triplet is compatible with a larger distance from the portal region.

On the contrary, the upfield shifts of methylenes **d** (0.85 ppm), **c** (0.41 ppm), and **b** (0.34 ppm) revealed that these protons were located inside the cucurbit[7]uril cavity (Figure 4).[15]

In the ^{1}H NMR spectrum of bis-IC (**3**) in D_2O, molecular symmetry recovery produced a single signal for the **a** methylene protons (as in free **1**) with a downfield shift (0.47 ppm) consistent with protons in the portal region of Q[7]. On the other hand, the upfield shift (0.85 ppm) of the **d** methylenes and that of the **c** methylenes (0.49 ppm) were typical for protons deeply inside the Q[7] cavity (Figures 1 and 4). Finally, the ^{195}Pt resonance for the bis-IC (**3**) resulted in a downfield shift (17.8 ppm), which is consistent with a platinum inside the Q[7] cavity, as reported by Kemp.[6,15]

To investigate the effect of pD variations on the equilibrium between **3** and **2**, we carried out a titration of the bis-IC (**3**) (containing 30% of **2**), directly in the NMR tube with a DCl solution. The initial pD of the solution was 6.4. After each addition, the pD was measured and ^{1}H NMR spectra were recorded to evaluate[15] the amount of mono-IC (**2**). We observed that by lowering the pD the relative percentage of **2** increased[15] up to 50% at pD 1.4. The process was completely reversible since back titration with NaOD restored the initial equilibrium of the two species; moreover, at pD 9.4, a decrease in the relative percentage of **2** (7%) was detected.

Table 1. Cytotoxicity of CT-3610 (**1**) and CT-3610-Q[7] bis-IC (**3**) on the A2780 and A2780/CDDP human ovarian tumor cell lines

Time/h[a]	IC_{50}/nM			
	CT-3610 (**1**)		CT-3610/Q[7] (**3**)	
	A2780	A2780/CDDP	A2780	A2780/CDDP
1	0.9	32.9	1.5	48.5
24	0.8	7.2	1.6	7.1
120	0.6	11.9	0.8	13.3

[a]Drug exposure.

As recently reported by Wheate et al.[7] Q[*n*] encapsulation may differently affect metal complex behavior. Wheate showed that the encapsulation of BBR3464 into Q[7] resulted in a decreased antiproliferative activity in different tumor cell lines. Differently, the Q[7] bisadduct of BBR3571 (an analog of BBR3464 where the central platinum has been replaced by NH) showed IC_{50} values comparable to that of the free complex in a number of cisplatin sensitive and resistant cancer cell lines.

Analogously to BBR3571, (Table 1) the antiproliferative activity of bis-IC (**3**) is comparable to that of **1** in cisplatin sensitive (A2780) and resistant (A2780/CDDP) human ovarian tumor cell lines at all the assayed drug exposure times ranging from 1 to 120 h.

Further work has to be done to assess which multinuclear complex retains the best balance between complex stability and activity when encapsulated.

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