ACS APPLIED MATERIALS & INTERFACES

Research Article

Cite This: ACS Appl. Mater. Interfaces 2018, 10, 5365−5372

www.acsami.org

Supramolecular Chemotherapy: Carboxylated Pillar[6]arene for Decreasing Cytotoxicity of Oxaliplatin to Normal Cells and Improving Its Anticancer Bioactivity Against Colorectal Cancer

Qi Hao,[†,∥] Yueyue Chen,[‡,§,∥] Zehuan Huang,[†] Jiang-Fei Xu,[†] Zhiwei Sun,*[,‡,§] and Xi Zhang*[,†]

[†]Key Lab of Organic Optoelectronics & Molecular Engineering, Department of Chemistry, Tsinghua University, Beijing 100084, P. R. China

[‡]Department of Toxicology and Sanitary Chemistry, School of Public Health, and [§]Beijing Key Laboratory of Environmental Toxicology, Capital Medical University, Beijing 100069, P. R. China

Supporting Information

ABSTRACT: We have successfully demonstrated that the host−guest complex of carboxylated pillar[6]arene with oxaliplatin (OxPt) exhibits low cytotoxicity toward normal cells and displays higher anticancer bioactivity against colorectal cancer cells than OxPt itself. Owing to higher binding affinity of carboxylated pillar[6]arene with spermine (SPM) than that with OxPt, the encapsulated OxPt can be thoroughly released from its host−guest complex by the competitive replacement with SPM. This supramolecular chemotherapy works well both in vitro and in vivo for SPM-overexpressed cancers, such as colorectal cancer. Compared to OxPt itself, the anticancer bioactivity of this host−guest complex is further improved by about 20%. Such an improvement results from the combined effect of controlled release of OxPt from its host−guest complex and simultaneous consumption of SPM by carboxylated pillar[6]arene. It is anticipated that this supramolecular strategy may be extended to other clinical anticancer drugs for decreasing their severe side effects and improving their anticancer bioactivity, thus enriching the realm of supramolecular chemotherapy.

KEYWORDS: *supramolecular chemotherapy, host−guest chemistry, pillar[n]arene, controlled drug release, anticancer bioactivity*

1. INTRODUCTION

Chemotherapy is currently among the most effective anticancer therapeutic modalities in clinical treatments.[1,2] However, many chemotherapeutic agents suffer from substantial drawbacks including poor water-solubility, high cytotoxicity, and low therapeutic specificity among normal cells and cancer cells, which leads to severe side effects and greatly impedes the therapeutic efficacy of anticancer drugs.[3−5] Over the past few decades, great efforts have been devoted to exploiting a variety of drug delivery systems such as micelles,[6−11] liposomes,[12−14] vesicles,[15,16] and other nanocarriers[17−26] for increasing the bioavailability of anticancer drugs and reducing their side effects.

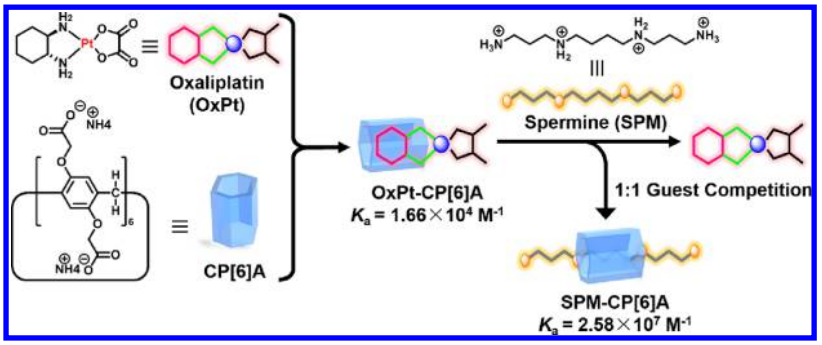
In our previous article, we have proposed a concept of supramolecular chemotherapy, which is aimed to employ a supramolecular approach to reduce the cytotoxicity of anticancer drugs to normal cells and improve their anticancer bioactivity against cancer cells.[27,28] As a proof of concept, we utilized oxaliplatin (OxPt)[29,30] as a clinical anticancer drug encapsulated in cucurbit[7]uril (CB[7])[31−34] to form a host−guest complex (OxPt−CB[7]). As expected, the cytotoxicity of OxPt to normal cells was significantly reduced by encapsulation of OxPt within CB[7]. Interestingly, OxPt−CB[7] exhibited higher anticancer bioactivity than OxPt itself to colorectal cancer cells which can overexpress spermine (SPM). SPM is a well-documented cancer biomarker overexpressed in some types of cancers such as colorectal and lung cancers, and it is essential for cancer cell growth and proliferation.[35−37] Hence, the improvement of anticancer bioactivity is rationalized by the combination of controlled release of OxPt from its host−guest complex and simultaneous consumption of SPM by CB[7]. Nevertheless, the binding affinities of OxPt with CB[7] and SPM with CB[7] are quite close to each other. The competitive replacement of OxPt from OxPt−CB[7] by SPM is a concentration-dependent process. Due to this reason, OxPt cannot be completely released from its host−guest complex unless SPM is 30-fold higher than the concentration of OxPt−CB[7].

To deal with the above-mentioned problem, we need to choose a macrocyclic host which displays higher binding affinity with SPM than that with OxPt. Pillar[*n*]arenes, composed of *n* repeating hydroquinone units connected by methylene bridges at the para positions, are a new kind of macrocyclic hosts in supramolecular chemistry.[38,39] Owing to the rigidity in the

Received: December 29, 2017
Accepted: January 22, 2018
Published: January 22, 2018

DOI: 10.1021/acsami.7b19784
ACS Appl. Mater. Interfaces 2018, 10, 5365−5372

Scheme 1. Schematic Illustration of Supramolecular Chemotherapy Based on Host−Guest Complexation between OxPt and CP[6]A, and the Thorough Release of OxPt from CP[6]A via Competitive Binding with SPM



molecular structure and the convenience in chemical functionalization, pillar[*n*]arenes are endowed with considerable capacity for binding various guest molecules and constructing diverse supramolecular systems.[40−43] Among this pillar[*n*]arene family, carboxylated pillar[6]arene (CP[6]A) has been demonstrated to possess good water-solubility and biocompatibility.[44−48] Considering that CP[6]A has a unique structural advantage over CB[7] for its negatively charged rims, we hypothesized that CP[6]A could exhibit higher binding affinity with positively charged SPM than that with OxPt because of their multiple electrostatic interactions.

In this article, we describe an effective system of supramolecular chemotherapy using CP[6]A for more efficient drug release (Scheme 1). To this end, we could employ CP[6]A to encapsulate OxPt within its cavity to form a host−guest complex of OxPt−CP[6]A, thus reducing the cytotoxicity of OxPt to normal cells. In the SPM-overexpressed colorectal cancer microenvironments, OxPt would be released from OxPt−CP[6]A through competitive replacement by SPM, thus recovering its anticancer bioactivity. Since SPM is positively charged at a physiological pH, negatively charged CP[6]A would exhibit higher binding affinity with SPM than that with OxPt; therefore OxPt would be thoroughly released from the OxPt−CP[6]A complex. Simultaneously, consumption of intracellular SPM by CP[6]A could further improve the anticancer bioactivity of OxPt−CP[6]A in our supramolecular chemotherapy system. We envisioned that such improvement of this supramolecular strategy can be achieved both in vitro and in vivo.

2. EXPERIMENTAL SECTION

2.1. Materials. All the chemical reagents were commercially available and used as supplied without further purification (Supporting Information).

2.2. Job Plot Experiment. The Job plot method based on UV−vis spectroscopy was employed to determine binding stoichiometry between OxPt and CP[6]A. In this method, the total amount of OxPt and CP[6]A was held at a constant concentration of 2.0×10^{-5} M with their mole fractions varied from 0 to 1.0. A series of sample solutions were prepared in 20 mM phosphate buffer solution (pH 7.4). Their UV−vis spectra were recorded on a Hitachi UH-4150 spectrophotometer at 37.0 °C. The path length of the quartz spectrophotometric cuvette was 10.0 mm.

2.3. Isothermal Titration Calorimetry. Isothermal titration calorimetry (ITC) experiments were conducted on a Microcal VP-ITC device at a setting temperature of 37.0 °C. All the involved sample solutions were prepared in 20 mM phosphate buffer solution (pH 6.8 or 7.4). Before the titration, the solutions of OxPt (1.5 mM) or SPM (1.0 mM) was sucked in the injection syringe, whereas the solution of CP[6]A (0.10 mM) was filled in the sample cell. Each sample solution was degassed prior to titration. The titration device was programmed to perform the first injection of 5 μL and 28 sequential injections of 10 μL with a 300 s interval between two injections. The titration data were recorded and fitted by one set of sites binding model to obtain detailed thermodynamic parameters.

2.4. ^{1}H NMR Titration Experiment. ^{1}H NMR titration spectra for competitive replacement of OxPt from the OxPt−CP[6]A complex by SPM were recorded on a JOEL JNM-ECA400 apparatus (400 MHz) at room temperature. All these sample solutions were prepared in 20 mM phosphate buffer D_2O solution (pD 7.4). In this experiment, the concentration of OxPt−CP[6]A remained at 3.0 mM. Then, a solution of SPM (50 mM) was added into OxPt−CP[6]A according to different molar ratios.

2.5. UV−Vis Titration Experiment. UV−vis absorption spectroscopy was performed on a Hitachi UH-4150 spectrophotometer equipped with a temperature control unit. Temperature of each test was set at 37.0 °C, and the cuvette filled with sample solution was heated in a sample cell for 10 min. The scan wavelength ranged from 200 to 340 nm and scan speed was 600 nm/min. The path length of the quartz cuvette was 2.0 mm. The concentration of OxPt−CP[6]A was kept at 0.20 mM. UV titration experiments were performed by adding increasing amounts of SPM (0−2.0 equiv) into the solution of OxPt−CP[6]A. The release percentage of OxPt from OxPt−CP[6]A was calculated by absorbance at 307 nm, and more details were included in the Supporting Information.

2.6. Cell Culture. NCM460 and HCT116 cells were purchased from the American Type Culture Collection (ATCC, Rockville MD) and cultured in the Roswell Park Memorial Institute 1640 (RPMI1640) containing 10% fetal bovine serum (Corning, USA) and 1% penicillin/streptomycin. Cells grew as a layer and were detached upon confluence using a medium. Then, cells were centrifuged, and the supernatant was discarded. Next, cells were suspended in serum-supplemented RPMI1640 at a concentration of 1.0×10^4 cells/mL. Cells were cultured at 37.0 °C in a humid atmosphere of 5% CO_2−95% air.

2.7. Evaluation of Cytotoxicity. Cytotoxicity of free OxPt, CP[6]A, OxPt−CP[6]A, OxPt−2CP[6]A, OxPt−3CP[6]A, and OxPt−4CP[6]A to NCM460 and HCT116 cells were determined by Cell Counting Kit 8 (CCK-8) assays in 96-well cell culture plates. All the sample solutions were sterilized by filtration together with a pressure cooker before tests. NCM460 or HCT116 cells were seeded at a density of 1.0×10^4 cells/well in a 96-well plate and incubated for 24 h for attachment. Cells were then incubated with above-mentioned compounds at a series of concentrations for 24 h. After washing the cells with RPMI1640, 10% CCK-8 solution (5 mg/mL) was added to each well. After 4 h incubation at 37.0 °C, absorbance of the formazan product was measured at 450 nm using a spectrophotometer (Bio-Rad model 680). Untreated cells in media were used as a control. All experiments were carried out with five replicates. The IC_{50} (50% growth inhibition concentration) values for OxPt and OxPt−2CP[6]A in a colorectal cancer HCT116 cell line were obtained by GraphPad Prism 5.

DOI: 10.1021/acsami.7b19784
ACS Appl. Mater. Interfaces 2018, 10, 5365−5372

2.8. Human SPM Synthase ELISA Kit. Specimen requirement: colorectal cancer HCT116 cells treated with 50.0, 25.0, and 12.5 μM of OxPt, CP[6]A, OxPt−CP[6]A, and OxPt−2CP[6]A for 24 h, respectively. Expression levels of intracellular SPM synthase (SPMS) were determined according to instructions.

2.9. Animals and Tumor Models. Male and female BALB/c nude mice (4 weeks old, ∼20 g body weight) were purchased from Beijing HFK Bioscience Co., Ltd. (Beijing, China) and maintained in a pathogen-free environment under controlled temperature (24.0 °C). Animal care and handling procedures were in agreement with the guidelines evaluated and approved in the Guide for the Care and Use of Laboratory Animals. Study protocols involving animals were approved by the Animal Care and Use Committee in Beijing Vital River Laboratory Animal Technology Co., Ltd. All the nude mice were injected subcutaneously in the right flank region with 200 μL of cell suspension containing 5.0×10^6 HCT116 cells. Then, tumors were allowed to grow to about 100 mm^3 before experimentation. The tumor volume was calculated as (tumor length) × (tumor width)^2/2.

2.10. Evaluation of in Vivo Antitumor Efficacy. The tumor-bearing nude mice were randomly divided into following three groups (n = 8): control, OxPt, and OxPt−2CP[6]A. When the mean tumor volume reached about 100 mm^3, this day was set as day 0. Nude mice were administered intravenously with 0.9% NaCl, free OxPt, and OxPt−2CP[6]A at the same dose of 10 mg OxPt/kg twice weekly for three weeks. Tumor volume and body weights were measured every two days. The tumor inhibition study was stopped on the 21st day.

2.11. Statistical Analysis. All experiments were carried out at least in triplicate, and the results were expressed as means ± SD. One-way analysis of variance (ANOVA) was used for analysis. The differences of statistics were considered significantly at $*p < 0.05$ using SPSS 17.0.

3. RESULTS AND DISCUSSION

3.1. Host−Guest Complexation between OxPt and CP[6]A. ^{1}H NMR spectroscopy was employed to investigate host−guest complexation between OxPt and CP[6]A at physiological pH (7.4). As shown in Figure 1, compared with free OxPt, all the proton peaks (a−e) of OxPt displayed significant upfield shifts in the presence of an equivalent amount of CP[6]A because of the shielding effect of the electron-rich cavity of CP[6]A, suggesting the formation of a host−guest complex of OxPt−CP[6]A. Subsequently, binding stoichiometry of this complex was studied by the Job plot

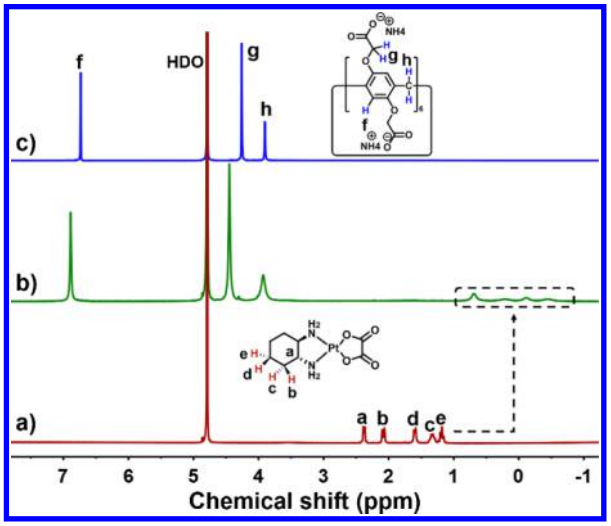


Figure 1. ^{1}H NMR spectra (400 MHz, 298 K) of (a) OxPt, (b) OxPt−CP[6]A, and (c) CP[6]A at a concentration of 3.0 mM in the 20 mM phosphate buffer solution (pD 7.4).

method using UV−vis spectroscopy. Figure 2a shows the recorded UV−vis absorption spectra of the mixture of CP[6]A and OxPt at different molar ratios from 10:0 to 0:10. By plotting the difference in absorption at 290 nm against molar fraction of OxPt (X_{OxPt}), the Job plot for CP[6]A and OxPt was obtained as shown in Figure 2b. It is clear that the mole fraction of OxPt at the maximum of the Job curve is 0.5, indicating that CP[6]A encapsulates OxPt in 1:1 binding stoichiometry.

To quantitatively study the binding affinity of the host−guest complexation between CP[6]A and OxPt, an ITC experiment was carried out to determine the binding constant (K_a). To this end, OxPt was titrated into CP[6]A in the phosphate buffer solution of pH 7.4 at 37.0 °C, and then, the titration curve was fitted by one set of sites binding model. As shown in Figure 3, the binding constant of the host−guest complexation between CP[6]A and OxPt was calculated to be 1.66×10^4 M^{-1}.

3.2. Cytotoxicity of OxPt to Normal Cells Reduced by Encapsulating OxPt into CP[6]A. We wondered if CP[6]A could reduce the cytotoxicity of OxPt through the host−guest complexation. To answer this question, in vitro cell experiments were conducted to evaluate cell viabilities of colorectal normal cells (NCM460) on exposure of a series of concentrations of OxPt, CP[6]A, and OxPt−CP[6]A for 24 h. As shown in Figure 4, it was clearly observed that CP[6]A displayed almost nontoxicity to colorectal normal cells at low concentrations, and it displayed only a little bit cytotoxicity when the dosage was as high as 100 μM, suggesting that CP[6]A is relatively biocompatible. In contrast, OxPt exhibited high cytotoxicity to colorectal normal cells with concentration dependence. Interestingly, via host−guest complexation, the cytotoxicity of OxPt−CP[6]A (1:1 complex) to colorectal normal cells was significantly reduced. Considering the moderate binding affinity of CP[6]A toward OxPt, we further investigated the influence of molar ratio of OxPt to CP[6]A on reducing the cytotoxicity of OxPt. When the molar ratio of OxPt to CP[6]A approached 1:2, the cytotoxicity of OxPt−2CP[6]A to normal cells was further reduced. However, once the molar ratio reached 1:3 or 1:4 (corresponding to OxPt−3CP[6]A or OxPt−4CP[6]A), the cytotoxicity was increased because of high excess of CP[6]A, which is consistent with the reports about in vitro cytotoxicity of CP[6]A in the literature.[45] Therefore, the molar ratio of 1:2 (OxPt−2CP[6]A) is the optimized condition in term of its low cytotoxicity for further study.

3.3. Host−Guest Complexation between SPM and CP[6]A. As mentioned previously, we envisioned that SPM, a biomarker overexpressed in colorectal cancer cells, might possess an advantageous capability of binding with CP[6]A than OxPt because of the positive charge distribution along the SPM. To demonstrate this point, ^{1}H NMR spectroscopy studies and ITC experiment were performed to investigate the host−guest complexation between CP[6]A and SPM. As shown in Figure 5, upon addition of 1.0 equiv of CP[6]A, the proton peaks (i−m) of SPM showed significant upfield shifts, indicating the formation of the host−guest complex of SPM−CP[6]A. To evaluate its binding affinity, an ITC experiment was conducted at 37.0 °C by titrating SPM into CP[6]A in the phosphate buffer solution of pH 7.4. As shown in Figure 6, an abrupt change was observed when the molar ratio of SPM to CP[6]A reached 1:1, indicating that the binding stoichiometry of the SPM−CP[6]A complex is 1:1. The titration curve was then fitted by one site of sites binding model, and the binding constant of SPM−CP[6]A was calculated to be 2.58×10^7 M^{-1}. This high binding affinity

DOI: 10.1021/acsami.7b19784
ACS Appl. Mater. Interfaces 2018, 10, 5365−5372

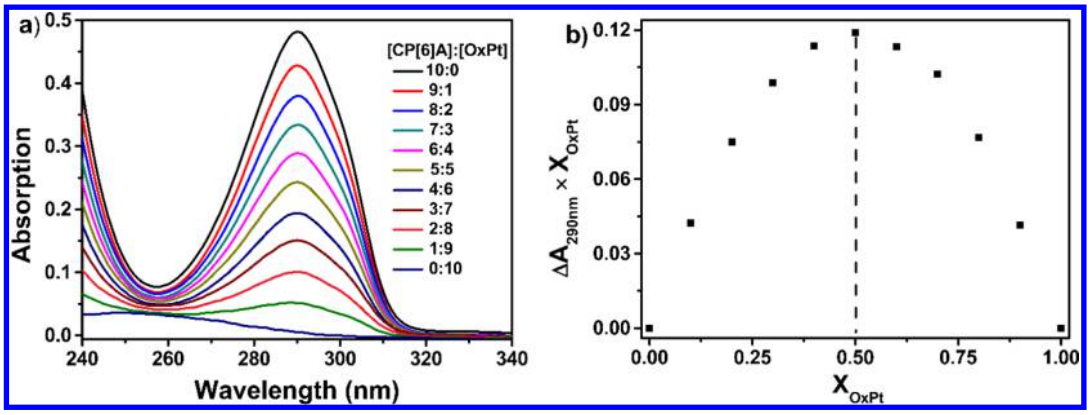


Figure 2. (a) UV−vis spectra of the mixture of CP[6]A and OxPt in different molar ratios at a constant total concentration of 2.0×10^{-5} M. (b) Job plot for CP[6]A and OxPt by plotting the difference in absorbance at 290 nm against the mole fraction of OxPt.

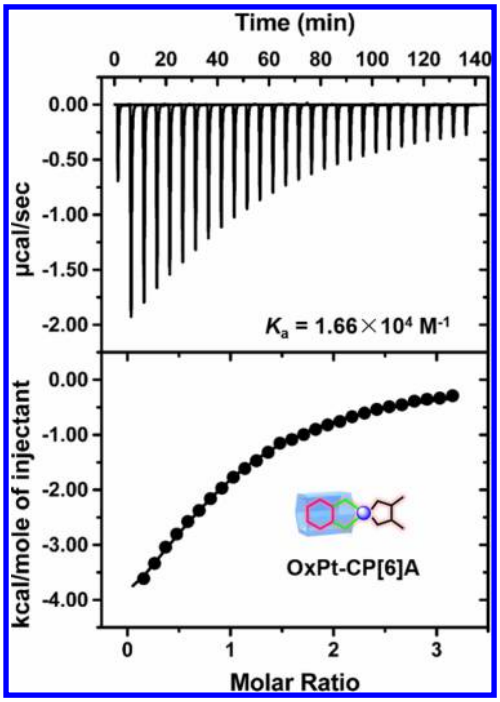


Figure 3. ITC titration curve and fitted data of host−guest complexation of OxPt−CP[6]A in the 20 mM phosphate buffer solution (pH 7.4) at 37.0 °C.

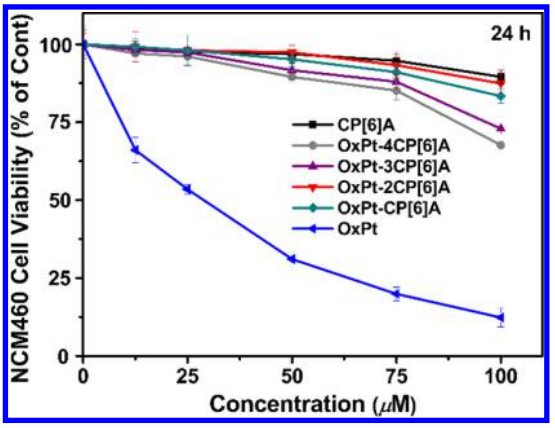


Figure 4. In vitro cytotoxicity of OxPt−CP[6]A in different molar ratios and comparison with OxPt in NCM460 normal cells. Graphs show the OxPt concentration response on cell survival after treatment for 24 h. Data shown are mean ± SD from $n = 5$. *$p < 0.05$ compared to the nontreated cell control group, one-way ANOVA.

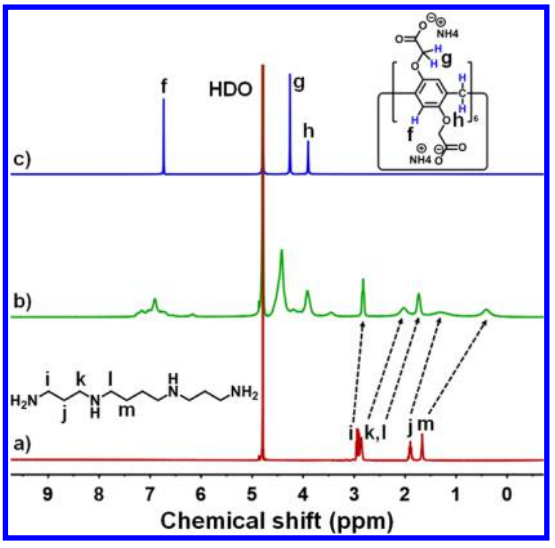


Figure 5. ^{1}H NMR spectra (400 MHz, 298 K) of (a) SPM, (b) SPM−CP[6]A, and (c) CP[6]A at a concentration of 3.0 mM in the 20 mM phosphate buffer solution (pD 7.4).

results from their strong multiple electrostatic interactions.[49,50] By comparison with the binding constant of OxPt−CP[6]A (1.66×10^4 M^{-1}), such a high binding constant would endow SPM with an advantage to competitively bind with CP[6]A for thoroughly releasing OxPt from the OxPt−CP[6]A complex.

3.4. Competitive Replacement of OxPt from the OxPt−CP[6]A Complex by SPM in Vitro. To test if OxPt could be efficiently released from the OxPt−CP[6]A complex by competitive replacement with SPM in vitro, ^{1}H NMR titration experiments were conducted to characterize the process of competitive binding at physiological pH (7.4). A series of spermine varying from 0.25 to 1.50 equiv was quantitatively added into OxPt−CP[6]A (3.0 mM). As shown in Figure 7, with the increase of SPM, the proton peaks (a−e) related to OxPt gradually shifted downfield and became clear. Comparing the proton peaks of free OxPt with those observed after the addition of 1.0 equiv SPM to OxPt−CP[6]A, two sets of the proton peaks related to free OxPt were well-matched (black dash lines), suggesting that OxPt was released from the OxPt−CP[6]A complex. Moreover, the signals of the protons on SPM (i−m) were the same as the signals (blue dash lines)

DOI: 10.1021/acsami.7b19784
ACS Appl. Mater. Interfaces 2018, 10, 5365−5372

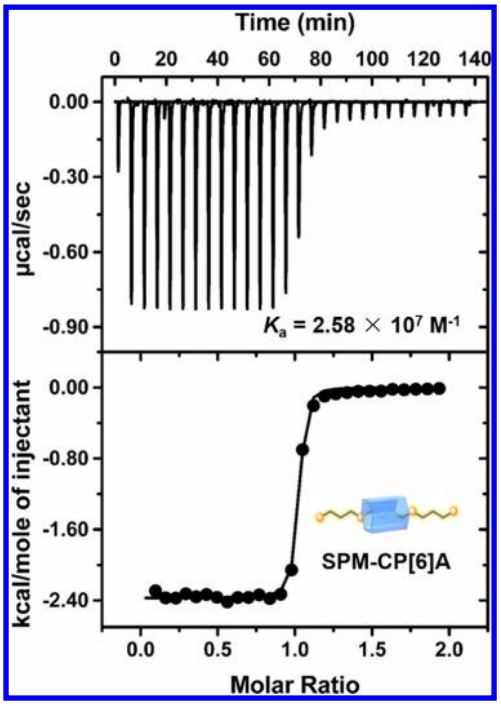


Figure 6. ITC titration curve and fitted data of the host–guest complexation of SPM–CP[6]A in the 20 mM phosphate buffer solution (pH 7.4) at 37.0 °C.

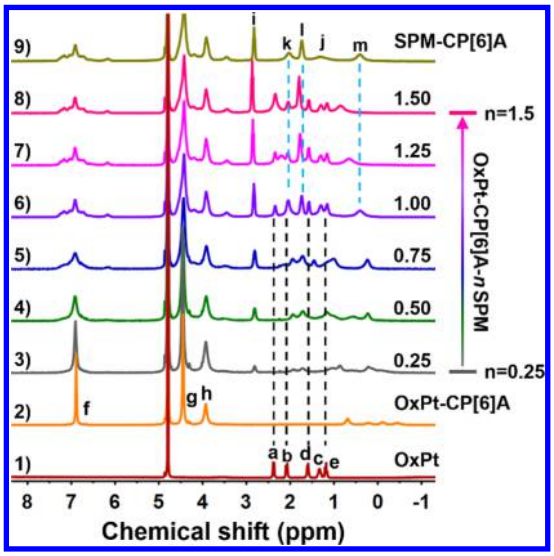


Figure 7. ^{1}H NMR spectra (400 MHz, 298 K) of (1) OxPt (3.0 mM), (2) OxPt–CP[6]A (3.0 mM), and OxPt–CP[6]A (3.0 mM) with the addition of (3) 0.25, (4) 0.50, (5) 0.75, (6) 1.00, (7) 1.25, and (8) 1.50 equiv of SPM, and (9) SPM–CP[6]A (3.0 mM) in 20 mM phosphate buffer solution (pD 7.4).

corresponding to the SPM–CP[6]A, suggesting that SPM occupied the host cavity of CP[6]A. These results indicate a complete release of OxPt from OxPt–CP[6]A by an equal amount of SPM because the binding affinity of SPM with CP[6]A is three orders of magnitude higher than that of OxPt with CP[6]A.

3.5. Anticancer Bioactivity Improved by the OxPt–CP[6]A Complex in Vitro and in Vivo. To investigate if the anticancer bioactivity of OxPt–CP[6]A could be recovered in a SPM-overexpressed cancer microenvironment, a colorectal cancer HCT116 cell line which can overexpress SPM was chosen to perform cytotoxicity experiments in vitro. As shown in Figure 8, we evaluated cell viabilities of colorectal cancer

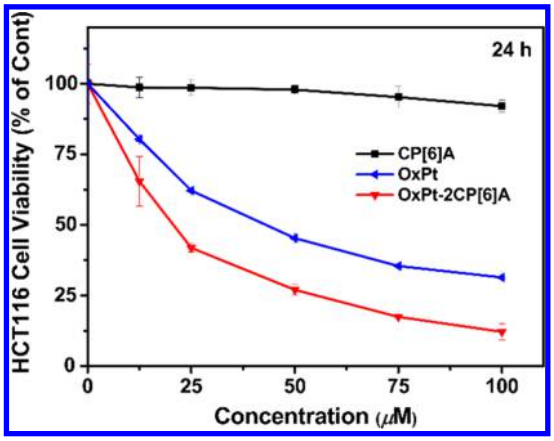


Figure 8. In vitro cytotoxicity of OxPt–2CP[6]A and its comparison with OxPt and CP[6]A in a colorectal cancer HCT116 cell line. Graphs show OxPt concentration response on HCT116 cell survival after treatment with free OxPt, CP[6]A, and OxPt–2CP[6]A for 24 h. Data shown are mean ± SD from n = 5. *p < 0.05 compared to the nontreated cell control group, one-way ANOVA.

HCT116 cells treated with OxPt, CP[6]A, and OxPt–2CP[6]A at a series of concentrations for 24 h. It was observed that OxPt, as an anticancer drug, displayed considerable anticancer bioactivity against cancer cells, whereas CP[6]A displayed almost noncytotoxicity to cancer cells. Very importantly, OxPt–2CP[6]A exhibited higher anticancer bioactivity than OxPt itself with an increased amount of about 20%. In addition, the IC_{50} values of OxPt and OxPt–2CP[6]A were calculated to be about 23.3 and 13.7 μM in HCT116 cells, respectively, indicating that the anticancer bioactivity of OxPt–2CP[6]A to colorectal cancer cells is higher than that of free OxPt. In view of that CP[6]A is almost noneffective for killing cancer cells; the above-mentioned results indicate that the anticancer bioactivity of OxPt–CP[6]A against colorectal cancer HCT116 cells is recovered and notably improved in vitro.

What could be the reason for the improvement of the OxPt–CP[6]A complex on its anticancer performance? According to our previous reports about supramolecular chemotherapy,[27,28] the mechanism behind the improved anticancer bioactivity of the OxPt–CP[6]A complex could be resulted from the combined effect of the controlled release of OxPt and simultaneous consumption of SPM by CP[6]A. It is widely known that colorectal cancer cells can overexpress SPM, and it is noteworthy that SPM is indispensable for cancer cell growth and proliferation.[51,52] It should be pointed out that OxPt kills cancer cells by cross-linking DNA and arrest cancer cells in the S phase of the cell cycle,[30,53,54] whereas SPM is overexpressed within cancer cells mainly in the S phase for assisting DNA replication and fast cell proliferation.[55,56] Thus, consumption of SPM would interfere DNA replication in the S phase and inhibit cell growth and proliferation. If the intracellular concentration of SPM is decreased, more SPM synthase (SPMS) will be expressed to assist the production of more needed SPM as a feedback.[57,58] To demonstrate the hypotheses about this mechanism, we evaluated the expression levels of SPMS by an ELISA kit in a colorectal cancer HCT116 cell line. As shown in Table 1, the expression levels of SPMS in the cases

DOI: 10.1021/acsami.7b19784
ACS Appl. Mater. Interfaces 2018, 10, 5365–5372

Table 1. Expression Levels of SPMS Induced by Different Treatments in HCT116 Cells for 24 h[a]

	concentration of SPMS in HCT116 cells(pg/mL)		
sample	50.0 μM	25.0 μM	12.5 μM
OxPt−2CP[6]A	918.4 (±0.4)*	797.6 (±0.2)*	653.2 (±0.1)*
OxPt−CP[6]A	759.4 (±0.2)*	695.1 (±0.4)*	620.1 (±0.1)*
OxPt	415.1 (±0.1)	313.2 (±0.2)	201.7 (±0.2)
CP[6]A	563.7 (±0.3)	478.5 (±0.1)	408.3 (±0.5)
Control		368.9 (±0.2)	

[a]Data shown are mean ± SD from n = 4; *p < 0.05 versus vehicle treated control, one-way ANOVA.

treated with OxPt−CP[6]A and OxPt−2CP[6]A were both much higher than that with free OxPt and CP[6]A, indicating that the biosynthetic pathway of SPM within cancer cells may be activated because of the consumption of SPM by CP[6]A. In other words, controlled release of OxPt and consumption of SPM by CP[6]A could happen simultaneously, thus leading to a higher anticancer activity of OxPt−2CP[6]A than free OxPt. Therefore, the combined effect of recovery of the anticancer bioactivity of OxPt and simultaneous consumption of SPM should be the reason behind the improved anticancer bioactivity of the host−guest complex of OxPt with CP[6]A.

To further validate the inhibitory effect of the OxPt−CP[6]A complex on tumor growth in vivo, HCT116 tumor-bearing nude mice were randomly divided into three groups as follows: a control group with 0.9% NaCl, a group treated with free OxPt (10 mg/kg), and a group treated with OxPt−2CP[6]A (equivalent to 10 mg/kg of OxPt dose). As shown in Figure 9a, the tumor volume in the control group treated with 0.9% NaCl increased about sixfold over the original state after 21 days. After treatment with free OxPt by intravenous injection, the growth of HCT116 tumors was slightly slowed. It was inspiring that OxPt−2CP[6]A exhibited a remarkable inhibitory effect on tumor growth than free OxPt (*p < 0.05). In the meantime, the body weights of mice in each group were evaluated as a measure of systemic toxicity. As shown in Figure 9b, treatment with free OxPt resulted in a significant loss in body weight of the mice over the time because of its severe side effects. However, in the case of mice treated with OxPt−2CP[6]A, the body weights of mice remained almost the same as that of the control group, suggesting that OxPt−2CP[6]A displays lower side effect compared to free OxPt in vivo. These results together confirm that the improved anticancer bioactivity and reduced systemic toxicity of OxPt complexed with CP[6]A are indeed achieved in the mouse model in vivo.

4. CONCLUSIONS

In summary, we have successfully demonstrated that carboxylated pillar[6]arene is an appropriate macrocyclic host for encapsulation of OxPt in supramolecular chemotherapy. Owing to the higher binding affinity of carboxylated pillar[6]arene toward SPM than OxPt, OxPt can be thoroughly released from its host−guest complex via competitive replacement with SPM. More importantly, simultaneous consumption of SPM by carboxylated pillar[6]arene can result in further improvement of anticancer bioactivity by about 20%. This supramolecular chemotherapy works well both in vitro and in vivo for SPM-overexpressed colorectal cancers. Besides carboxylated pillar[6]arene, other kinds of negatively charged macrocyclic hosts may be utilized in supramolecular chemotherapy. It is anticipated that this supramolecular strategy may be extended to other clinical anticancer drugs for decreasing their severe side effects and improving their anticancer bioactivity, thus enriching the realm of supramolecular chemotherapy.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.7b19784.

Materials and preparation of OxPt−CP[6]A complex; competitive replacement of OxPt by SPM monitored by UV−vis spectroscopy; binding constant of the OxPt−CP[6]A complex and the SPM−CP[6]A complex at pH 6.8; cell viability of HCT116 on exposure to the host−guest complex of OxPt with CP[6]A at other three molar ratios; and tumor morphology of HCT116 tumor-bearing nude mice (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: zwsun@ccmu.edu.cn (Z.S.).
*E-mail: xi@mail.tsinghua.edu.cn (X.Z.).

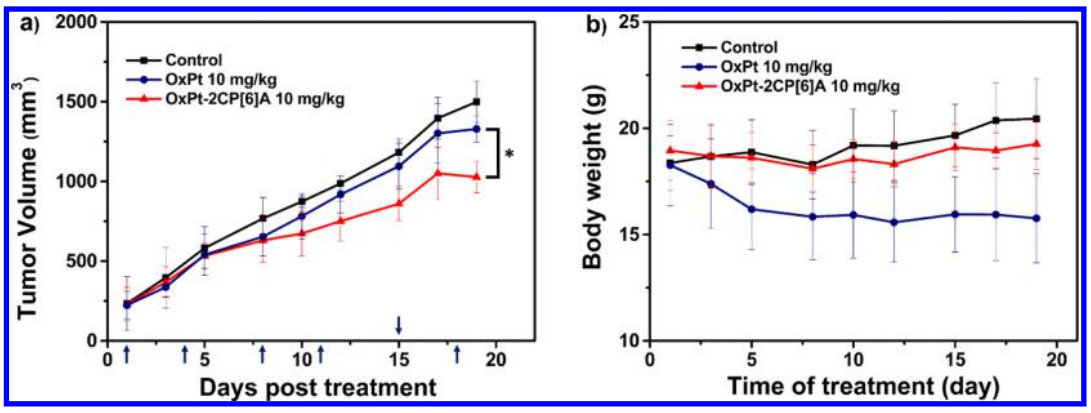


Figure 9. In vivo antitumor efficacy of OxPt−2CP[6]A in a mice tumor model. HCT116 tumor-bearing nude mice were treated with free OxPt and OxPt−2CP[6]A at a dose of 10 mg/kg twice weekly for 3 weeks. Arrows indicate intravenous injection days. Graphs show the effects of treatment on (a) tumor volume changes of the mice in three groups (n = 8) and (b) body weight changes of the mice in three groups. Data shown are mean ± SD from n = 8; *p < 0.05.

DOI: 10.1021/acsami.7b19784
ACS Appl. Mater. Interfaces 2018, 10, 5365−5372

ORCID

Xi Zhang: 0000-0002-4823-9120

Author Contributions

‖Q.H. and Y.C. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was financially supported by the National Natural Science Foundation of China (21434004, 91527000) and the Tsinghua University Scientific Initiative Research Program.

■ REFERENCES

(1) Chabner, B. A.; Roberts, T. G., Jr. Chemotherapy and the War on Cancer. *Nat. Rev. Cancer* **2005**, *5*, 65−72.

(2) Kasi, P. M.; Grothey, A. Chemotherapy Maintenance. *Cancer J.* **2016**, *22*, 199−204.

(3) Love, R. R.; Leventhal, H.; Easterling, D. V.; Nerenz, D. R. Side Effects and Emotional Distress During Cancer-Chemotherapy. *Cancer* **1989**, *63*, 604−612.

(4) Corrie, P. G. Cytotoxic Chemotherapy: Clinical Aspects. *Medicine* **2008**, *36*, 24−28.

(5) Alcindor, T.; Beauger, N. Oxaliplatin: A Review in the Era of Molecularly Targeted Therapy. *Curr. Oncol.* **2011**, *18*, 18−25.

(6) Kataoka, K.; Harada, A.; Nagasaki, Y. Block Copolymer Micelles for Drug Delivery: Design, Characterization and Biological Significance. *Adv. Drug Delivery Rev.* **2001**, *47*, 113−131.

(7) Cabral, H.; Kataoka, K. Progress of Drug-Loaded Polymeric Micelles into Clinical Studies. *J. Controlled Release* **2014**, *190*, 465−476.

(8) Lukyanov, A. N.; Torchilin, V. P. Micelles from Lipid Derivatives of Water-Soluble Polymers as Delivery Systems for Poorly Soluble Drugs. *Adv. Drug Delivery Rev.* **2004**, *56*, 1273−1289.

(9) Rapoport, N. Physical Stimuli-Responsive Polymeric Micelles for Anti-Cancer Drug Delivery. *Prog. Polym. Sci.* **2007**, *32*, 962−990.

(10) Liu, D. H.; Ding, J. X.; Xu, W. G.; Song, X. F.; Zhuang, X. L.; Chen, X. S. Stereocomplex Micelles Based on 4-Armed Poly(Ethylene Glycol)-Polylactide Enantiomeric Copolymers for Drug Delivery. *Acta Polym. Sin.* **2014**, 1265−1273.

(11) Song, W.; Tang, Z.; Li, M.; Lv, S.; Sun, H.; Deng, M.; Liu, H.; Chen, X. Polypeptide-Based Combination of Paclitaxel and Cisplatin for Enhanced Chemotherapy Efficacy and Reduced Side-Effects. *Acta Biomater.* **2014**, *10*, 1392−1402.

(12) Yeh, M.-K.; Chang, H.-I.; Cheng, M.-Y. Clinical Development of Liposome-Based Drugs: Formulation, Characterization, and Therapeutic Efficacy. *Int. J. Nanomed.* **2012**, *7*, 49−60.

(13) Allen, T. M.; Cullis, P. R. Liposomal Drug Delivery Systems: From Concept to Clinical Applications. *Adv. Drug Delivery Rev.* **2013**, *65*, 36−48.

(14) Mu, L.-M.; Ju, R.-J.; Liu, R.; Bu, Y.-Z.; Zhang, J.-Y.; Li, X.-Q.; Zeng, F.; Lu, W.-L. Dual-Functional Drug Liposomes in Treatment of Resistant Cancers. *Adv. Drug Delivery Rev.* **2017**, *115*, 46−56.

(15) Duan, Q.; Cao, Y.; Li, Y.; Hu, X.; Xiao, T.; Lin, C.; Pan, Y.; Wang, L. pH-Responsive Supramolecular Vesicles Based on Water-Soluble Pillar[6]arene and Ferrocene Derivative for Drug Delivery. *J. Am. Chem. Soc.* **2013**, *135*, 10542−10549.

(16) Palivan, C. G.; Goers, R.; Najer, A.; Zhang, X.; Car, A.; Meier, W. Bioinspired Polymer Vesicles and Membranes for Biological and Medical Applications. *Chem. Soc. Rev.* **2016**, *45*, 377−411.

(17) Hamidi, M.; Azadi, A.; Rafiei, P. Hydrogel Nanoparticles in Drug Delivery. *Adv. Drug Delivery Rev.* **2008**, *60*, 1638−1649.

(18) Angelos, S.; Khashab, N. M.; Yang, Y.-W.; Trabolsi, A.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I. pH Clock-Operated Mechanized Nanoparticles. *J. Am. Chem. Soc.* **2009**, *131*, 12912−12914.

(19) Brown, S. D.; Nativo, P.; Smith, J.-A.; Stirling, D.; Edwards, P. R.; Venugopal, B.; Flint, D. J.; Plumb, J. A.; Graham, D.; Wheate, N. J. Gold Nanoparticles for the Improved Anticancer Drug Delivery of the Active Component of Oxaliplatin. *J. Am. Chem. Soc.* **2010**, *132*, 4678−4684.

(20) Feng, X.; Lv, F.; Liu, L.; Tang, H.; Xing, C.; Yang, Q.; Wang, S. Conjugated Polymer Nanoparticles for Drug Delivery and Imaging. *ACS Appl. Mater. Interfaces* **2010**, *2*, 2429−2435.

(21) Sahoo, B.; Devi, K. S. P.; Banerjee, R.; Maiti, T. K.; Pramanik, P.; Dhara, D. Thermal and pH Responsive Polymer-Tethered Multifunctional Magnetic Nanoparticles for Targeted Delivery of Anticancer Drug. *ACS Appl. Mater. Interfaces* **2013**, *5*, 3884−3893.

(22) Huang, P.; Wang, D.; Su, Y.; Huang, W.; Zhou, Y.; Cui, D.; Zhu, X.; Yan, D. Combination of Small Molecule Prodrug and Nanodrug Delivery: Amphiphilic Drug-Drug Conjugate for Cancer Therapy. *J. Am. Chem. Soc.* **2014**, *136*, 11748−11756.

(23) Zhang, Q.; Jiang, Q.; Li, N.; Dai, L.; Liu, Q.; Song, L.; Wang, J.; Li, Y.; Tian, J.; Ding, B.; Du, Y. DNA Origami as an in Vivo Drug Delivery Vehicle for Cancer Therapy. *ACS Nano* **2014**, *8*, 6633−6643.

(24) Webber, M. J.; Langer, R. Drug Delivery by Supramolecular Design. *Chem. Soc. Rev.* **2017**, *46*, 6600−6620.

(25) Sun, Q.; Zhou, Z.; Qiu, N.; Shen, Y. Rational Design of Cancer Nanomedicine: Nanoproperty Integration and Synchronization. *Adv. Mater.* **2017**, *29*, 1606628.

(26) Qin, H.; Ding, Y.; Mujeeb, A.; Zhao, Y.; Nie, G. Tumor Microenvironment Targeting and Responsive Peptide-Based Nanoformulations for Improved Tumor Therapy. *Mol. Pharmacol.* **2017**, *92*, 219−231.

(27) Chen, Y.; Huang, Z.; Xu, J.-F.; Sun, Z.; Zhang, X. Cytotoxicity Regulated by Host−Guest Interactions: A Supramolecular Strategy to Realize Controlled Disguise and Exposure. *ACS Appl. Mater. Interfaces* **2016**, *8*, 22780−22784.

(28) Chen, Y.; Huang, Z.; Zhao, H.; Xu, J.-F.; Sun, Z.; Zhang, X. Supramolecular Chemotherapy: Cooperative Enhancement of Antitumor Activity by Combining Controlled Release of Oxaliplatin and Consuming of Spermine by Cucurbit[7]uril. *ACS Appl. Mater. Interfaces* **2017**, *9*, 8602−8608.

(29) Wiseman, L. R.; Adkins, J. C.; Plosker, G. L.; Goa, K. L. Oxaliplatin: A Review of Its Use in the Management of Metastatic Colorectal Cancer. *Drugs Aging* **1999**, *14*, 459−475.

(30) Arango, D.; Wilson, A. J.; Shi, Q.; Corner, G. A.; Arañes, M. J.; Nicholas, C.; Lesser, M.; Mariadason, J. M.; Augenlicht, L. H. Molecular Mechanisms of Action and Prediction of Response to Oxaliplatin in Colorectal Cancer Cells. *Br. J. Cancer* **2004**, *91*, 1931−1946.

(31) Macartney, D. H. Encapsulation of Drug Molecules by Cucurbiturils: Effects on Their Chemical Properties in Aqueous Solution. *Isr. J. Chem.* **2011**, *51*, 600−615.

(32) Cao, L.; Hettiarachchi, G.; Briken, V.; Isaacs, L. Cucurbit[7]uril Containers for Targeted Delivery of Oxaliplatin to Cancer Cells. *Angew. Chem., Int. Ed.* **2013**, *52*, 12033−12037.

(33) Barrow, S. J.; Kasera, S.; Rowland, M. J.; del Barrio, J.; Scherman, O. A. Cucurbituril-Based Molecular Recognition. *Chem. Rev.* **2015**, *115*, 12320−12406.

(34) Kuok, K. I.; Li, S.; Wyman, I. W.; Wang, R. Cucurbit[7]uril: An Emerging Candidate for Pharmaceutical Excipients. *Ann. N.Y. Acad. Sci.* **2017**, *1398*, 108−119.

(35) Kingsnorth, A. N.; Lumsden, A. B.; Wallace, H. M. Polyamines in Colorectal Cancer. *Br. J. Surg.* **1984**, *71*, 791−794.

(36) Gerner, E. W.; Meyskens, F. L., Jr. Polyamines and Cancer: Old Molecules, New Understanding. *Nat. Rev. Cancer* **2004**, *4*, 781−792.

(37) Soda, K. The Mechanisms by Which Polyamines Accelerate Tumor Spread. *J. Exp. Clin. Cancer Res.* **2011**, *30*, 95.

(38) Ogoshi, T.; Kanai, S.; Fujinami, S.; Yamagishi, T.-a.; Nakamoto, Y. Para-Bridged Symmetrical Pillar[5]arenes: Their Lewis Acid Catalyzed Synthesis and Host−Guest Property. *J. Am. Chem. Soc.* **2008**, *130*, 5022−5023.

(39) Ogoshi, T.; Yamagishi, T.-a.; Nakamoto, Y. Pillar-Shaped Macrocyclic Hosts Pillar[n]arenes: New Key Players for Supramolecular Chemistry. *Chem. Rev.* **2016**, *116*, 7937−8002.

DOI: 10.1021/acsami.7b19784
ACS Appl. Mater. Interfaces 2018, 10, 5365−5372

(40) Strutt, N. L.; Forgan, R. S.; Spruell, J. M.; Botros, Y. Y.; Stoddart, J. F. Monofunctionalized Pillar[5]arene as a Host for Alkanediamines. *J. Am. Chem. Soc.* **2011**, *133*, 5668−5671.
(41) Yu, G.; Han, C.; Zhang, Z.; Chen, J.; Yan, X.; Zheng, B.; Liu, S.; Huang, F. Pillar[6]arene-Based Photoresponsive Host−Guest Complexation. *J. Am. Chem. Soc.* **2012**, *134*, 8711−8717.
(42) Cao, Y.; Hu, X.-Y.; Li, Y.; Zou, X.; Xiong, S.; Lin, C.; Shen, Y.-Z.; Wang, L. Multistimuli-Responsive Supramolecular Vesicles Based on Water-Soluble Pillar[6]arene and Saint Complexation for Controllable Drug Release. *J. Am. Chem. Soc.* **2014**, *136*, 10762−10769.
(43) Jiang, L.; Huang, X.; Chen, D.; Yan, H.; Li, X.; Du, X. Supramolecular Vesicles Coassembled from Disulfide-Linked Benzimidazolium Amphiphiles and Carboxylate-Substituted Pillar[6]arenes That Are Responsive to Five Stimuli. *Angew. Chem., Int. Ed.* **2017**, *56*, 2655−2659.
(44) Yu, G.; Xue, M.; Zhang, Z.; Li, J.; Han, C.; Huang, F. A Water-Soluble Pillar[6]arene: Synthesis, Host−Guest Chemistry, and Its Application in Dispersion of Multiwalled Carbon Nanotubes in Water. *J. Am. Chem. Soc.* **2012**, *134*, 13248−13251.
(45) Wheate, N. J.; Dickson, K.-A.; Kim, R. R.; Nematollahi, A.; Macquart, R. B.; Kayser, V.; Yu, G.; Church, W. B.; Marsh, D. J. Host−Guest Complexes of Carboxylated Pillar[*n*]arenes with Drugs. *J. Pharm. Sci.* **2016**, *105*, 3615−3625.
(46) Li, B.; Meng, Z.; Li, Q.; Huang, X.; Kang, Z.; Dong, H.; Chen, J.; Sun, J.; Dong, Y.; Li, J.; Jia, X.; Sessler, J. L.; Meng, Q.; Li, C. A pH Responsive Complexation-Based Drug Delivery System for Oxaliplatin. *Chem. Sci.* **2017**, *8*, 4458−4464.
(47) Shangguan, L.; Chen, Q.; Shi, B.; Huang, F. Enhancing the Solubility and Bioactivity of Anticancer Drug Tamoxifen by Water-Soluble Pillar[6]arene-Based Host−Guest Complexation. *Chem. Commun.* **2017**, *53*, 9749−9752.
(48) Zhou, J.; Yu, G.; Huang, F. Supramolecular Chemotherapy Based on Host−Guest Molecular Recognition: A Novel Strategy in the Battle against Cancer with a Bright Future. *Chem. Soc. Rev.* **2017**, *46*, 7021−7053.
(49) Geall, A. J.; Blagbrough, I. S.; Geall, A. J.; Taylor, R. J.; Earll, M. E.; Eaton, M. A. W. Synthesis of Cholesterol-Polyamine Carbamates: pKa Studies and Condensation of Calf Thymus DNA. *Chem. Commun.* **1998**, 1403−1404.
(50) Yang, K.; Pei, Y.; Wen, J.; Pei, Z. Recent Advances in Pillar[*n*]arenes: Synthesis and Applications Based on Host−Guest Interactions. *Chem. Commun.* **2016**, *52*, 9316−9326.
(51) Bloomfield, V. A. DNA Condensation by Multivalent Cations. *Biopolymers* **1997**, *44*, 269−282.
(52) Belting, M.; Borsig, L.; Fuster, M. M.; Brown, J. R.; Persson, L.; Fransson, L.-A.; Esko, J. D. Tumor Attenuation by Combined Heparan Sulfate and Polyamine Depletion. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 371−376.
(53) Raymond, E.; Faivre, S.; Woynarowski, J.; Chaney, S. Oxaliplatin: Mechanism of Action and Antineoplastic Activity. *Semin. Oncol.* **1998**, *25*, 4−12.
(54) Faivre, S.; Chan, D.; Salinas, R.; Woynarowska, B.; Woynarowski, J. M. DNA Strand Breaks and Apoptosis Induced by Oxaliplatin in Cancer Cells. *Biochem. Pharmacol.* **2003**, *66*, 225−237.
(55) Jänne, J.; Pösö, H.; Raina, A. Polyamines in Rapid Growth and Cancer. *Biochim. Biophys. Acta* **1978**, *473*, 241−293.
(56) Fuller, D. J. M.; Gerner, E. W.; Russell, D. H. Polyamine Biosynthesis and Accumulation during the G1 to S Phase Transition. *J. Cell. Physiol.* **1977**, *93*, 81−88.
(57) Pegg, A. E.; Poulin, R.; Coward, J. K. Use of Aminopropyltransferase Inhibitors and of Nonmetabolizable Analogs to Study Polyamine Regulation and Function. *Int. J. Biochem. Cell Biol.* **1995**, *27*, 425−442.
(58) Subhi, A. L.; Diegelman, P.; Porter, C. W.; Tang, B.; Lu, Z. J.; Markham, G. D.; Kruger, W. D. Methylthioadenosine Phosphorylase Regulates Ornithine Decarboxylase by Production of Downstream Metabolites. *J. Biol. Chem.* **2003**, *278*, 49868−49873.

DOI: 10.1021/acsami.7b19784
ACS Appl. Mater. Interfaces 2018, 10, 5365−5372