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A Host–Guest Supramolecular Complex with Photoregulated Delivery of Nitric Oxide and Fluorescence Imaging Capacity in Cancer Cells

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Abstract: Herein we report the design, preparation, and properties of a supramolecular system based on a tailored nitric oxide (NO) photodonor and a rhodamine-labeled β-cyclodextrin conjugate. The combination of spectroscopic and photochemical experiments shows the absence of significant interchromophoric interactions between the host and the guest in the excited states. As a result, the complex is able to release NO under the exclusive control of visible light, as unambiguously demonstrated by direct detection of this transient species through an amperometric technique, and exhibits the typical red fluorescence of the rhodamine appendage. The supramolecular complex effectively internalizes in HeLa cancer cells as proven by fluorescence microscopy, shows a satisfactory biocompatibility in the dark, and induces about 50% of cell mortality upon irradiation with visible light. The convergence of all these properties in one single complex makes the present host–guest ensemble an appealing candidate for further delevopment of photoactivatable nanoscaled systems addressed to photostimulated NO-based therapy.

Keywords: cyclodextrins · fluorescence · nitric oxide · phototherapy · supramolecular chemistry

Introduction

Nitric oxide (NO) is one of the most appealing and studied molecules in the fascinating realm of the biomedical sciences.[1] Besides its pivotal role in the maintenance and bioregulation of vital functions including neurotransmission, hormone secretion, and vasodilatation in living bodies,[2] this ephemeral free radical has recently stimulated an upsurge of interest because of its promising antioxidant, antibacterial, and anticancer activity.[3–5] These exciting discoveries have made the development of new strategies and methods for NO delivery a hot topic[6] with the intriguing prospect to tackle important diseases, especially cancer.[7] The biological effects of NO have been shown to be highly site-, concentration-, and dosage-dependent, creating a complex role for this molecule in opposing beneficial and deleterious events.[8] This dichotomy has made the light-controlled NO donors much more appealing than those based on spontaneous thermolysis for potential use in nanomedicine.[9] Light is in fact the most elegant and finely tunable external input for the non-invasive introduction of therapeutic agents in a desired biological environment, mimicking an "optical syringe" with an exquisite spatiotemporal control.[10] In addition to providing fast reaction rates, light triggering represents a "biofriendly" and easily manipulated reactant, and offers the great benefit of not affecting physiological parameters such as temperature, pH, and ionic strength, a fundamental requisite for biomedical applications.[10]

Due to its half-life of approximately 5 seconds in tissues, its very small size, its lack of charge, and its lipophilic character, NO is capable to diffuse some micrometers (40–200 μm) in the cellular environment. Therefore, this radical species offers the advantage to confine its reactivity in the restricted region of space where it has been photogenerated. Furthermore, the NO radical is able to attack biological substrates of different nature like the plasma membrane,[11] the mitochondria,[12] and the cell nucleus,[13] thus representing a multitarget cytotoxic agent and avoiding problems of multidrug resistance encountered with several conventional anticancer drugs. Finally, since the NO release from NO photodonors is independent from O_2 availability, the NO-photostimulated therapy can potentially very well complement the photodynamic therapy, in which the phototoxicity mechanism is entirely dependent on the presence of oxygen,[14] at the onset of hypoxic conditions.

The cell-penetrating property of the NO photodonors is another indispensable prerequisite to be fulfilled in NO-based therapy. This issue has inspired an intense research activity devoted to develop biocompatibile vehicles.[6a,9a] Cy-

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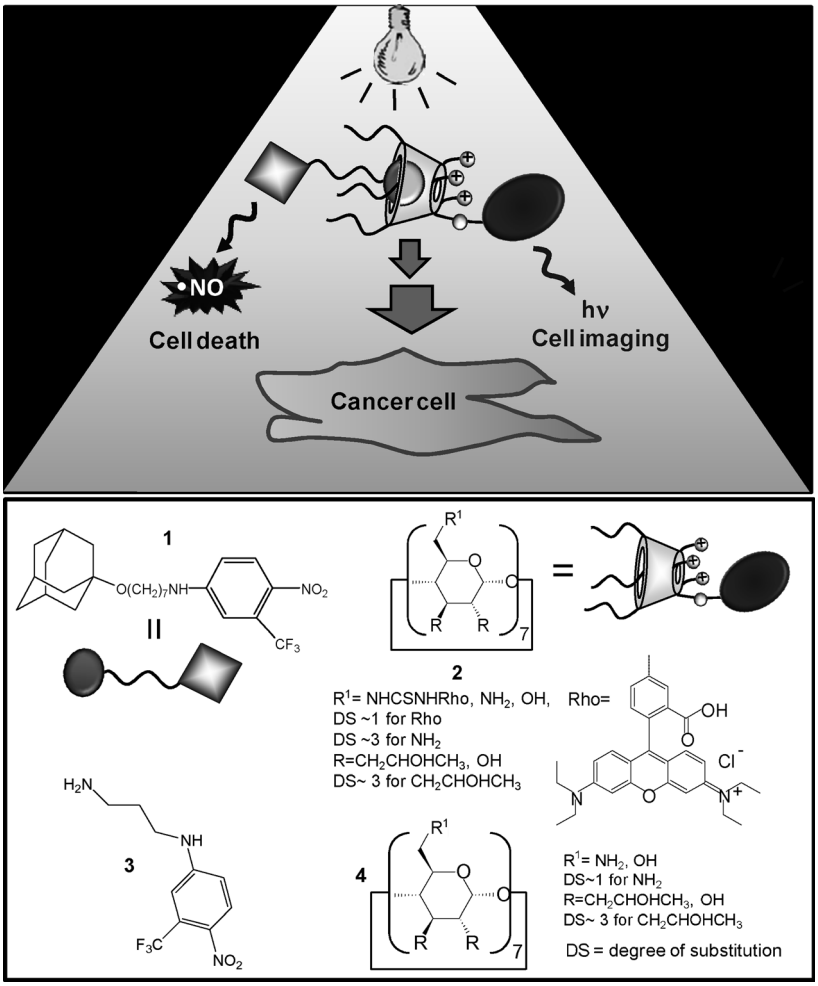
clodextrins (CDs) comprise a family of biocompatible cyclic oligosaccharide nanocages, made of α-D-glucopyranose units joined in a circular way to form a ring with a hydrophilic exterior and a hydrophobic cavity, that are able to host a range of substrates.[15] Although the chemistry of CDs and their implementation as carriers of "conventional" drugs have been greatly developed during the last thirty years,[16] the suitability of CDs as carriers for photoactivatable therapeutic compounds has been only recently object of attention.[17] Due to their hydrophobic cavity, natural CDs can host a variety of photosensitive agents by supramolecular interactions.[18] However, in most cases the low binding constants between unmodified CDs and guest molecules are a major limitation of these systems as bio-carriers. This drawback makes the modification of the CD structure strictly necessary in view of actual applications.[19] CDs can be selectively mono- or perfacially modified with a plethora of substituents which can 1) elongate the cavity or make it flexible, thus modifying its binding capacity, 2) alter the hydrophilic characteristics, 3) introduce charged groups that will enable cell penetration, and 4) enable them to form conjugates with other functional molecules.[20]

In the last years we have achieved a number of NO-photoreleasing nanoassemblies,[9a] including nanoparticles,[21] thin films[22], and molecular conjugates.[23] In the framework of this ongoing interest, we report herein the design, preparation, and properties of a multifunctional, water-soluble host–guest supramolecular complex based on a tailor-made NO photodonor and a novel fluorescent β-CD conjugate. We demonstrate that such a complex internalizes in living cells, can be easily localized therein by fluorescence microscopy, exhibits a good biocompatibility in the dark, releases NO under the exclusive control of visible-light stimuli and, as result, induces an appreciable level of photomortality in tumor cell lines.

Results and Discussion

In search of a viable strategy to construct a stable, non-covalent photoactivable inclusion complex able to display the convergence of all the above properties, we have designed a molecular ensemble consisting of the photoresponsive components **1** and **2** (Scheme 1).

The photoactive guest **1** integrates a commercial nitroaniline derivative and an adamantane appendage, joined together by an alkyl spacer, within the same molecular skeleton. We have demonstrated that such a nitroaniline derivative is a suitable NO photodonor as it satisfies several prerequisites for biological applications.[21a,24] In this chromophoric unit, the twisted conformation of the nitro group with respect to the aromatic plane is crucial for the NO photorelease. Similarly to other nitroaromatics having the same conformation, the mechanism of NO release involves an initial nitro-to-nitrite photorearrangement followed by cleavage of the O-NO bond.[25] However, it has been shown that incorporation of this chromophore within constrained media, such as either a β-CD cavity[26] or densely packed vesicles,[27] can lead to a partial planarization of the nitro group with consequent drastic modification of the NO photoreleasing properties. This finding spurred us to devise compound **1** in which the adamantane moiety, a very effective guest for β-CD,[28] is expected to have a much more pro-



Scheme 1. Schematic of the photoresponsive host–guest supramolecular complex and molecular structures of the compounds used in this work.

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nounced preference for the CD cavity of the host than the nitroaniline chromophore.[29] As a consequence, the NO photoreleasing unit should locate outside the CD cage, thus preserving the suitable conformation for the NO photodelivery (see below).

The photoactive host **2** consists of a β-CD scaffold which has been ad hoc modified at both the upper and the lower rim. The rationale behind the design of compound **2** was that 1) the peripheral amino groups, which are expected to be protonated at physiological pH, should assist in cell penetration, owing to their positive charges, and ensure an excellent solubility in aqueous media, 2) the isopropyl termini should further promote the binding with the guest through cooperative hydrophobic interchain interactions involving the alkyl spacer of **1**, and 3) the rhodamine fluorogenic unit is expected to facilitate the localization of the supramolecular system in living cells by fluorescence microscopy, without affecting the NO photoreleasing properties of the guest.

The absorption and fluorescence spectra of **2** in aqueous solution are dominated by the rhodamine chromophore and exhibit maxima at 558 and 586 nm, respectively (Figure 1,

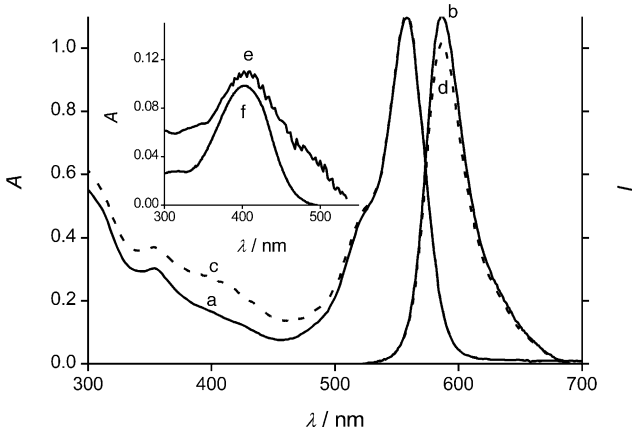

Figure 1. Absorption and fluorescence emission spectra of **2** (10.8 μM, $\lambda_{exc}=525$ nm, isosbestic point) in 10 mM phosphate buffer (pH 7.4) in the absence (curves a and b) and in the presence (curves c and d) of **1** (10 μM). The inset shows the difference absorption spectrum between sample c and a (curve e) and absorption spectrum of the model compound **3** (curve f). $T=25\,°C$, cell path = 1 cm.

curves a,b). The fluorescence quantum yield for this compound was $\Phi_f=0.21$. This value is close to that reported for rhodamine B under identical experimental conditions ($\Phi_f=0.31$),[30] thereby accounting for a CD conjugate with satisfactory emission properties.

Compound **1** is completely insoluble in aqueous media. However, it becomes soluble in an aqueous solution of **2** as a consequence of the formation of a supramolecular complex. This is proven by the appearance of an additional absorption contribution in the region below 500 nm, which is due to the nitroaniline chromophore (Figure 1, curve c). The presence of this unit is better visible in the difference spectrum between the sample containing both components and that containing **2** alone (inset in Figure 1, curve e). These absorption features are very similar to those exhibited by the model compound **3**, which is completely water soluble (inset in Figure 1, curve f). Our previous work demonstrated that the energy of the visible absorption band of the nitroaniline unit is very sensitive to both microenvironment polarity and changes in the geometry of the nitro group.[27] In this view, the absence of any shift in the absorption maximum of **1** with respect to **3** suggests a binding mode in which the NO photodonor is mainly exposed to a water pool while the adamantane unit occupies the CD cavity, as illustrated in Scheme 1. Based on the absorbance values of the NO photodonor at 403 nm and of the rhodamine label at 558 nm, and the related extinction coefficients at the same wavelengths (ca. $10000\,M^{-1}cm^{-1}$ and $100000\,M^{-1}cm^{-1}$, respectively), an nearly complete complexation between **1** and **2** with a 1:1 stoichiometry can be estimated. In view of the low concentrations of the components (ca. 10 μM), this finding accounts for a high binding constant ($K_a>10^5\,M^{-1}$). Taking into account that the K_a values for adamantane and unfunctionalized β-CD are on the order of $10^4\,M^{-1}$,[28] we believe that the effective binding process might stem from cooperative intracavity interactions involving the adamantane moiety and hydrophobic interchain interactions involving the alkyl spacer of **1** and the isopropyl termini of **2**. Such an efficient complexation of the active guest is a fundamental requisite for its intracellular delivery (see below).

Interestingly, the emissive properties of the host **2** were only little influenced by the presence of the guest **1** in the complex (Figure 1, curves b and d), thereby offering a great advantage for its easy mapping in living cells by fluorescence microscopy (see below). This result is in line with the binding mode suggested in Scheme 1, which implies a distance between the two chromophores long enough to avoid any potential intramolecular quenching by photoinduced electron transfer.[31]

The most convenient methodology to demonstrate the generation of NO from the supramolecular complex is the direct and real-time monitoring of this radical species. To this end, we have used an ultrasensitive NO electrode, which directly detects NO, with a sensitivity in the nanomolar concentration range, by an amperometric technique.[32] The results illustrated in Figure 2A provide unambiguous evidence that the complex is stable in the dark but supplies NO upon illumination with $\lambda_{exc}=420$ nm. The release process is strictly dependent on the external light input, as confirmed by the linear NO photodelivery that promptly stops when the light is turned off and restarts when the illumination is turned on again.

Figure 2B shows the changes in the electronic absorption spectrum of the complex observed upon light irradiation. It can be noted that bleaching occurs in the corresponding absorption region of the NO photodonor (around 400 nm), whereas only negligible changes are observed in the band of the rhodamine chromophore (around 558 nm). This photobleaching accounts for a photochemical reaction involving exclusively the guest **1** and is in excellent agreement with

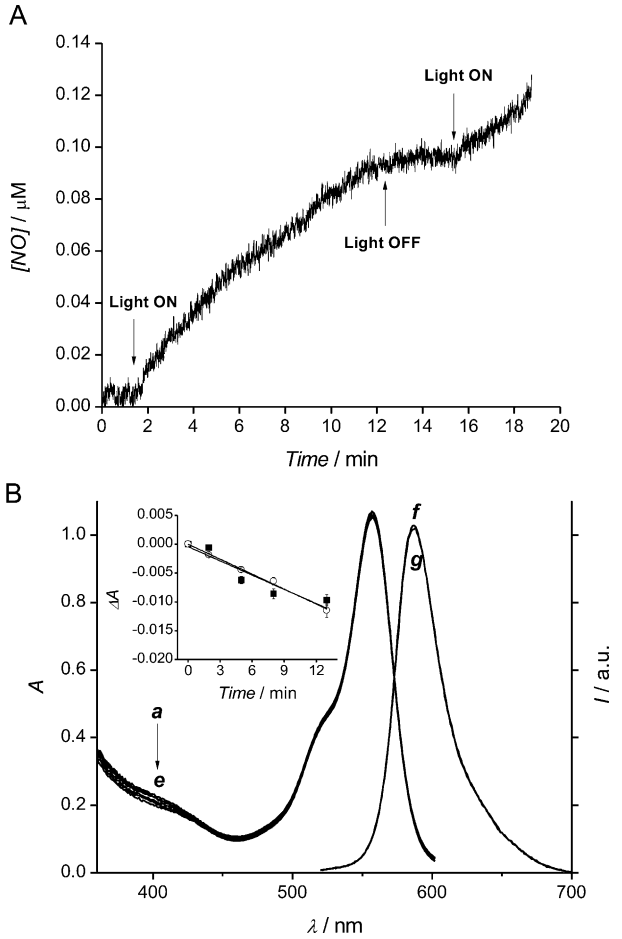

Figure 2. A) NO release profile observed upon irradiation with monochromatic light at $\lambda = 420$ nm of the complex of **1** with **2** (ca. 10 μM) in 10 mM phosphate buffer (pH 7.4) at 25 °C. B) Absorption spectral changes observed upon light irradiation at $\lambda > 400$ nm, from 0 min (curve a) to 40 min (curve e), of the complex under the same conditions as in panel A. Fluorescence emission spectra of the supramolecular complex before (curve f) and after (curve g) photolysis ($\lambda_{exc} = 525$ nm). The inset shows the kinetic profiles, monitored at $\lambda = 400$ nm, and the related linear fittings, observed in the case of the complex of **1** with either **2** (○) or with the model compound **4** (■).

the photochemical pathway leading to NO release previously proposed in the case of the single NO photodonor unit.[21a] The notion that the rhodamine fluorophore is not involved in any photochemical process was also confirmed by the almost complete preservation of the fluorescence spectrum observed after the photolysis experiments (Figure 2B, curves f and g).

To investigate whether the rhodamine unit influences the photoconversion rate of **1**, we carried out comparative experiments with a supramolecular complex between **1** and the model compound **4** (see Scheme 1). In this case, the β-CD scaffold bears both the amino and isopropyl appendages but, in contrast to **2**, lacks of the rhodamine fluorophore. As shown in the inset in Figure 2, the kinetic profiles for the photobleaching exhibit basically the same slopes, thereby excluding any relevant effect of the rhodamine fluorophore on the photoreactivity of the NO photodonor.

To demonstrate the suitability of the supramolecular construct as a biocompatible vehicle with imaging and phototherapeutic capacities, we performed cell culture experiments with HeLa cancer cells. The cells were treated with the complex for three hours, harvested, and then analyzed by fluorescence microscopy. The presence of the CD derivative inside the cells can be easily revealed by the red fluorescence emission typical of the rhodamine label, while the location of the nuclear compartment is accomplished by staining the cells with the fluorescent DNA-binding dye Hoechst 33342. Figure 3A shows a well detectable level of intracellular red fluorescence within HeLa cells, confirming the ability of the CD conjugate to penetrate the cells. Figure 3B evidences nuclear staining of the same cells with Hoechst 33342, as revealed by the blue fluorescence emission of this dye. Figure 3C shows the corresponding merged image obtained by double fluorescence analysis. A close inspection of this image excludes the confinement of the CD derivative in the nuclear compartment of the HeLa cells, accounting for a localization mainly in the cytoplasm or a possible colocalization with mitochondria, in agreement with typical observations for rhodamine derivatives.[33]

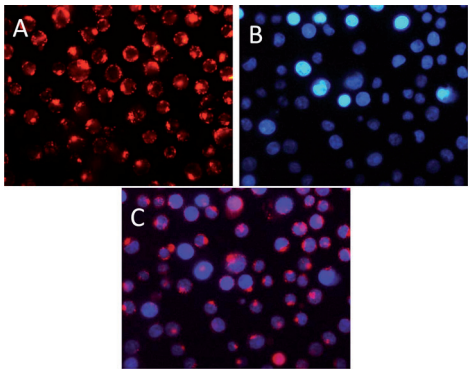

Figure 3. Fluorescence microscopy analysis of HeLa cells treated with the complex of **1** with **2** (ca. 10 μM) and stained with Hoechst 33342 dye in 10 mM phosphate buffer (pH 7.4). The cells were analyzed by fluorescence microscopy (40×) with a rhodamine emission filter (A) or a Hoechst emission filter (B). C) Merge of images shown in panels A and B.

To evaluate the effectiveness of this system to induce antitumor effects upon light irradiation, the cell cultures were treated for 3 hours with the complex and, for comparison, with the host **2** alone, and the samples were either kept in the dark or irradiated with visible light for 30 minutes. The results reported in Figure 4 show that both **2** and its complex with **1** exhibit a low level of cytotoxicity in the dark. By contrast, considerable cell mortality is observed under light excitation. It can be seen that the extent of cellular death observed for the complex is greater than that noted in the case of the host alone. This result provides clear-cut evidence for the capability of **2** to deliver **1** to the cell compartment, although we cannot exclude the possibility that the complex dissociates after cell internalization. The photoinduced cell mortality induced by **2** alone can be reasonably

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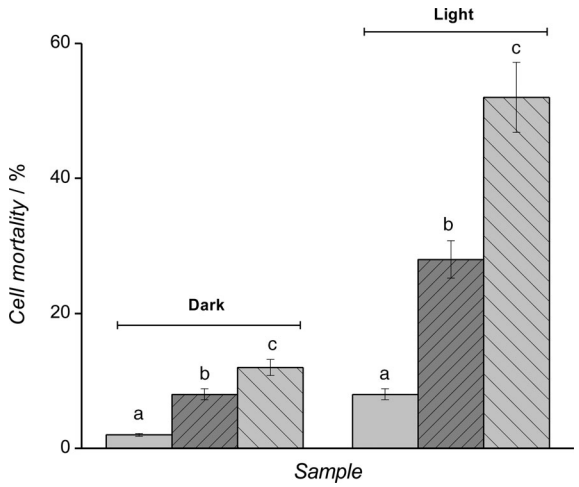


Figure 4. Cell mortality of HeLa cells in the dark or upon photoinduction in the absence of photoactive components (a), in the presence of **2** (10 μM) (b), and in the presence of the complex of **1** with **2** (c).

ascribed to the generation of reactive oxygen species (ROS) by the rhodamine dye, which has been reported to act as a photosensitizer although with low efficiency.[34]

Conclusions

We have presented here a novel multifunctional, photoresponsive supramolecular "Lego system" based on the effective formation of an inclusion complex between a tailor-made NO photodonor and an ad hoc devised CD conjugate carrying a rhodamine fluorophore. Both the guest and the host behave as independent photoactive centers in the supramolecular complex, as proven by the excellent preservation of their photochemical and photophysical properties. As a result, this nanoassembly exhibits the convergence of photoregulated release of NO and fluorescence imaging in one single nanostructure. We would like to highlight that, in contrast to non-photoresponsive compounds, the preservation of the photobehavior of independent components after their assembly is not a "trivial result". In most cases, the response to light of single or multiple photoactive units located in a confined space can be, in fact, considerably influenced, in both nature and efficiency, by the occurrence of competitive photoprocesses (i.e., photoinduced energy and/or electron transfer, hydrogen abstraction, nonradiative deactivation, etc.),[18,35] which preclude the final goal.

The host–guest complex internalizes in cancer cells, probably assisted by its cationic nature at physiological pH, can be easily mapped therein in view of its satisfactory fluorescence emission, and is able to induce about 50% cell photomortality. In this regard, it appears that the extent of the cellular death is not exclusively due to the light-triggered generation of NO but also to the involvement of ROS photogenerated by the rhodamine unit. Studies addressed to clarify this point deserve certainly attention.

The described experiments demonstrate the validity of our logical design and justify further research in this direction with the intriguing prospect to achieve a novel class of photoactivatable nanoscaled systems based on similar components that exhibit an improved photoaction. In this respect, we are currently working on the synthesis of rhodamine-labeled CD-based oligomers. The presence of multiple CD compartments should lead, in principle, to nanostructures able to accommodate a larger amount of NO photodispensers, with a consequential increase in both the light-harvesting properties and the reservoir of NO available. The results of this investigation will be reported in due course.

Experimental Section

Materials

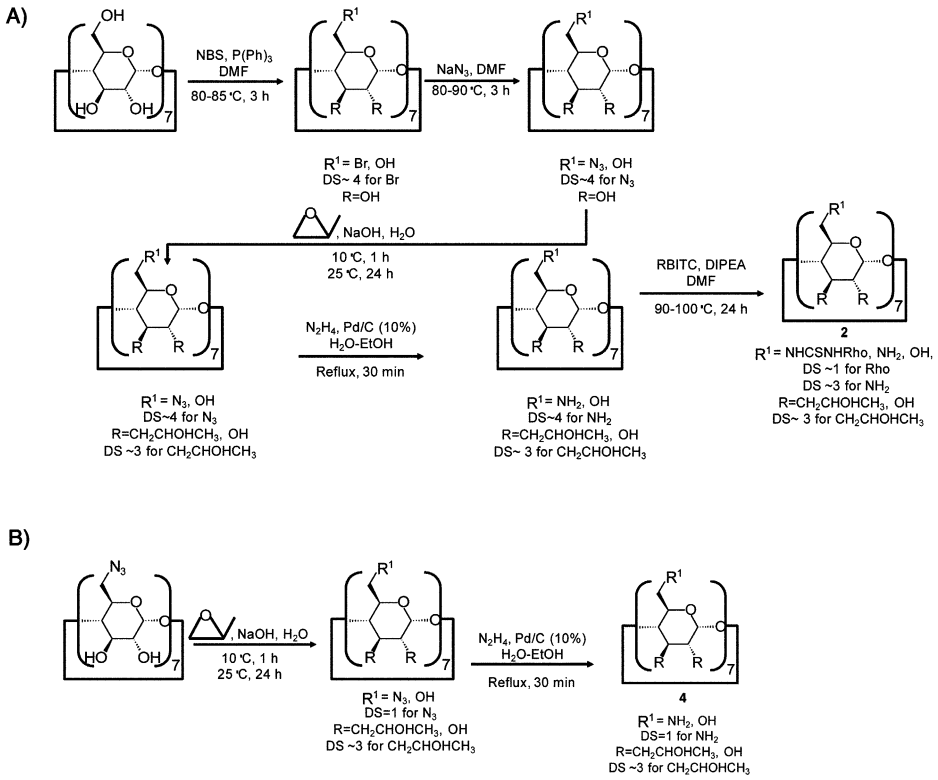
All cyclodextrin intermediates were synthesized by CycloLab (Budapest, Hungary). All other reagents were of the highest commercial grade available and used without further purification. All solvents used (from Carlo Erba) were analytical grade, and dried by conventional methods and distilled immediately prior to use. Slide-A-Lyzer Dialysis Casettes G2, with a cut-off MW of 2000 (Thermo Scientific), were used for dialyses. Thin layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel 60 F254 (Merck, Art. No.: 1.05554). Plates were developed in a saturated chamber of 1,4-dioxane/ammonium hydroxide (25%, 10:7 (*v*/*v*)), visualized by UV light at 254 nm and 366 nm, and charring with a solution of EtOH (96%)/H_2SO_4 (96%, 9:1) followed by heating at 105–110 °C. Phosphate buffer (10 mM, pH 7.4) was prepared with biological grade reagents and all solutions were prepared with nanopure water (18 MΩ).

Synthesis

Compound **1** and the model compound **3** were synthesized according to previously reported procedures.[21b,23a] 6-Tetradeoxy-6-triamino-6-rhodaminylthioureido-tri-O-(2-hydroxypropyl)cyclomaltoheptaose (**2**) was synthesized in five steps (see Scheme 2A). The key intermediate 6-tetrazido-6-tetradeoxycyclomaltoheptaose was synthesized by following the methods reported in the literature.[36a,b] The hydroxypropylation and the reduction steps were performed as reported by Malanga et al.[37]

The rhodaminylation was performed as follows: Rhodamine B isothiocyanate (RBITC, 0.37 g, 0.69 mmol) was dissolved in DMF (5 mL) and then added dropwise to a stirred solution of 6-tetramino-6-tetradeoxy-tri-*O*-(2-hydroxypropyl)cyclomaltoheptaose (0.91 g, 0.69 mmol) and *N,N*-diisopropylethylamine (DIPEA, 99%, 0.6 mL, 3.4 mmol) in DMF (30 mL). The solution was stirred at 90–100 °C for 24 h, cooled to 80 °C, and then the solvent was removed under reduced pressure ($T = 80$ °C). The crude product was dissolved in water (20 mL) and extracted with dichloromethane (3×100 mL). Subsequently, water was removed under reduced pressure ($T = 60$ °C) and the resulting material was dialyzed against deionized water. Freeze-drying yielded the target compound **2** as a violet powder (0.66 g, 52%); m.p.: 205–212 °C (dec.); IR (KBr): $\tilde{\nu} = 3349$, 2973, 2928, 1670, 1591, 1466, 1340, 1153, 1083, 1039, 946, 754, 607, 580 cm^{-1}; ^1H NMR (D_2O): $\delta = 1.13$–1.14 (m, 9H, hydroxypropyl-methyl-H), 1.29–1.36 (m, 12H, RBITC-methyl-H), 3.33–4.13 (m, 59H, H2, H3, H4, H5, H6, hydroxypropyl-methylene-H, hydroxypropyl-methyne-H, RBITC-methylene-H), 5.09–5.25 (d, 7H, H1) 6.76–7.39 (bs, 6H, aromatic-H), 8.00–8.04 (d, 1H, aromatic-H), 8.11–8–17 (d, 1H, aromatic-H), 8.45 ppm (m, 1H, aromatic-H); ESI-MS (MeOH, HP = 2-hydroxypropyl fragment): $[M_0+Na]^+ = 1826.77$; $[M_1+Na]^+ = 1768.73$; $M_1 = M_0 - HP$; $[M_2+Na]^+ = 1710.73$; $M_2 = M_1 - HP$; $[M_3+Na]^+ = 1884.82$; $M_3 = M_0 + HP$; $[M_4+Na]^+ = 1942.91$; $M_4 = M_3 + HP$.

The DS (degree of substitution) for HP and rhodamine was determined from the ^1H NMR data.



Scheme 2. Synthetic route for the preparation of the CD conjugate **2** (A) and the model compound **4** (B).

6-Monodeoxy-6-monoamino-tri-O-(2-hydroxypropyl)cyclomaltoheptaose (**4**) was synthesized in three steps (see Scheme 2B). The hydroxypropylation and the reduction steps were performed as reported by Malanga et al.[36] 6-Monodeoxy-6-monoazido-tri-O-(2-hydroxypropyl)cyclomaltoheptaose (60 g, 0.044 mol) was dissolved in a water/ethanol solution (400 mL, 1:1) at room temperature. Palladium on charcoal (6 g, 10% Pd content) was suspended in water (60 mL) at room temperature and added to the solution; the catalyst was washed-in with water (60 mL), then hydrazine carbonate (20 mL) was added, and the reaction mixture was heated to reflux. After 20 min, the reaction mixture was cooled to room temperature, the solids were filtered, and the volatiles were removed from the filtrate. Traces of hydrazine were removed with dry, activated H^+ ion-exchanger (30 g) in methanol. Subsequently, the ion-exchanger was removed by filtration, the filtrate was filtered through a membrane, and the solvent was removed to yield **4** as a white solid (49 g, 82%); m.p.: 202–205 °C (dec.); IR (KBr): $\tilde{\nu}$ = 3403, 2969, 2929, 1646, 1457, 1411, 1374, 1336, 1299, 1155, 1084, 1034, 948, 853, 757, 580 cm^{-1}; ^1H NMR (D_2O): δ = 1.13–1.14 (m, 9H, hydroxypropyl-methyl-H), 3.33–4.13 (m, 51H, H2, H3, H4, H5, H6, hydroxypropyl-methylene-H, hydroxypropyl-methyne-H), 5.09–5.25 ppm (d, 7H, H1).

The DS for HP was determined from the ^1H NMR data.

Instrumentation

UV/Vis absorption and fluorescence spectra were recorded with a Jasco V-560 spectrophotometer and a Fluorolog-2 (model F-111) spectrofluorimeter, respectively. Fluorescence images were taken with a Biomed fluorescence microscope (Leitz, Wetzlar, Germany). The ESI-MS experiments were performed using a Waters Q-TOF Premier instrument (Waters Corporation, MA, USA). Melting points were determined on a Büchi OP545 melting point apparatus and are uncorrected. ^1H- and ^{13}C NMR spectra were recorded on a Varian VXR-600 spectrometer at 400 or 600 MHz. IR spectra were recorded in KBr disks on a Nicolet 205 FTIR spectrometer. Photolysis experiments were performed in a thermostated quartz cell (1 cm path length, 3 mL capacity) by using a Rayonet photochemical reactor equipped with 8 RPR lamps, with an emission in the 380–480 nm range with a maximum at 420 nm in the presence of a 400 nm cut-off filter. The incident photon flux on quartz cuvettes was approximately 0.8×10^{15} quanta s^{-1}.

Sample Preparation

Compound **1** was dissolved in methanol and slowly evaporated to form a thin film. This film was then hydrated with an aqueous solution of either **2** or the model compound **4** in phosphate buffer at pH 7.4. The mixtures were stirred for 1 h at 50 °C, and then the final solutions were left to equilibrate at room temperature.

NO Detection

NO release was measured with a World Precision Instrument, ISO-NO meter, equipped with a data acquisition system, and based on direct amperometric detection of NO with short response time (< 5 s) and a sensitivity range of 1 nM–20 μM. The analog signal was digitalized with a four-channel recording system and transferred to a PC. The sensor was accurately calibrated by mixing standard solutions of $NaNO_2$ with 0.1 M H_2SO_4 and 0.1 M KI according to the reaction:

$$4\,H^+ + 2\,I^- + 2\,NO_2^- \rightarrow 2\,H_2O + 2\,NO + I_2$$

Irradiation was performed in a thermostated quartz cell (1 cm path length, 3 mL capacity) under gentle stirring by using the monochromatic radiation at 420 nm of the Fluorolog-2 as the light source. NO measurements were carried out with the electrode positioned outside the light path in order to avoid false-positive NO signals due to photoelectric interference on the ISO-NO electrode.

Cell Culture Experiments

HeLa cells were obtained from the American Type Culture Collection and propagated at 1:6 ratio using Dulbecco's modification of Eagle's Minimal Essential Medium supplemented with 10% FBS (Fetal Bovine Serum). The samples of cells treated with the different samples of either **2** or its complex with **1** were trypsinized, placed separately in spectrophotometric cuvettes, and either kept in the dark or irradiated with visible light in the photoreactor as described above. Before and after irradiation, 8×10^4 cells were placed in 96-well plates with 100 μL of phosphate buffer and incubated in the presence of a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS, Promega] and an electron coupling reagent, phenazine methosulfate (PMS dye, MTS, 20 μl/well). After further incubation for 1 h, the absorbance was read at 490 nm in a Labsystems Multiskan Bichromatic plate reader.The cell viability (%) was calculated according to the following equation:

$$\text{cell viability}\ (\%) = (A\ (\text{before Lamp})\text{-}(A\ (\text{after Lamp})/\text{-}A\ (\text{before Lamp})) \times 100$$

where A (before Lamp) denotes the absorbance of treated samples before light exposure and A (after Lamp) denotes the absorbance of treated samples after light exposure. The cell mortality (%) is the complement of cell viability.

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FULL PAPER

Supramolecular Chemistry

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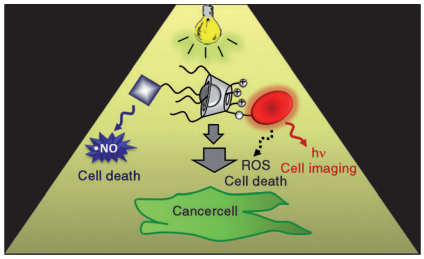
A Host–Guest Supramolecular Complex with Photoregulated Delivery of Nitric Oxide and Fluorescence Imaging Capacity in Cancer Cells



NO delivery today: A supramolecular host–guest complex, based on a rhodamine-labeled β-cyclodextrin conjugate and a tailored NO photodonor, effectively internalizes in cancer cells, can be easily mapped intracellularly due to its satisfactory red fluorescence emission, and induces about 50% of cellular death under the exclusive control of visible light stimuli.

