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Visible light-activatable cyclodextrin-conjugates for the efficient delivery of nitric oxide with fluorescent reporter and their inclusion complexes with betaxolol†

 Mimimorena Seggio,^a Sara Payamifar,^{‡ab} Aurore Fraix,^a Eszter Kalydi,^{cd} Petr Kasal,^e Ovidio Catanzano,^{id f} Claudia Conte,^{id g} Fabiana Quaglia,^{id *g} and Salvatore Sortino,^{id *a}

This contribution reports the design, synthesis, photochemical properties and drug inclusion capability of two novel β -cyclodextrin (β CD) conjugates, **β CD-NBFNO1** and **β CD-NBFNO2**, covalently integrating an N-nitroso amino-nitro-benzofurazan in the primary and secondary hydroxyl rims of the β CD scaffold, respectively through flexible spacers of different length. Both β CD conjugates are water-soluble and release nitric oxide (NO) under the input of either blue or green light, with quantum yields Φ_{NO} (blue) = 0.13, 0.31 and Φ_{NO} (green) = 0.007, 0.013 respectively, the former representing the largest values ever reported for nonmetal-containing NO donors activatable by visible light. The good contrast between the fluorescence green emission of the chromogenic moiety after and before the NO release permits the easy and in real-time quantification of the amount of NO generated, without the addition of external fluorescent agents. Despite the presence of the appendages, these β CD derivatives are also able to complex betaxolol, a β -blocker drug widely used for the reduction of the intraocular pressure, with binding constants $K_b = 500 \pm 50$ and $1100 \pm 100 \text{ M}^{-1}$, respectively, without affecting the photochemical performances. In view of the well-known vasodilator properties of NO, the present β CD derivatives represent intriguing candidates for biopharmaceutical research studies addressed to combined therapeutic ocular applications.

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Introduction

The design of molecules able to generate nitric oxide (NO) has received increasing attention, especially over the last decade.^{1,2} This is due to the multifaceted role NO plays in a number of physiological processes including neurotransmission, vasodilatation

and hormone secretion,³ and its superb antioxidant, anticancer and antibacterial activity.⁴ NO has shown vasodilator effects on eye vasculature⁵⁻⁷ and wound healing effect on injured corneal surface opening new therapeutic perspectives in ophthalmology.⁸⁻¹⁰ This scenario has given a boost to the development of compounds able to release NO under physiological conditions as potential therapeutics to fight a variety of diseases.^{1,2,11,12}

The strict dependence of the biological effects of NO by its dosage and location¹³ has made NO-photodons (NOPD) more appealing than spontaneous NO releasers due to the superb spatiotemporal accuracy that light triggering offers.¹⁴⁻¹⁸ In particular, NOPD activation by environmental light can be very suited for supplying NO for therapeutic purposes.^{19,20} Unfortunately, most NOPD have poor water solubility requiring specific formulations for their delivery to the anterior segment of the eye.

Among the strategies available to tackle this issue, the use of cyclodextrins (CDs) used for decades to increase the water-solubility of lipophilic drugs still offers room to innovate. CDs are cyclic oligosaccharides, well-known for their capability to complex, stabilize and solubilize guest compounds.²¹⁻²⁵ CD solubilizing activity is strictly related to their aptitude to form

^a PhotoChemLab, Department of Drug and Health Sciences, University of Catania, Catania, Italy. E-mail: ssortino@unicat.it

^b Organic Chemistry Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran

^c CycloLab Ltd., Illatos út 7, H-1097 Budapest, Hungary

^d Department of Pharmacognosy, Semmelweis University, H-1085 Üllői út 26, Budapest, Hungary

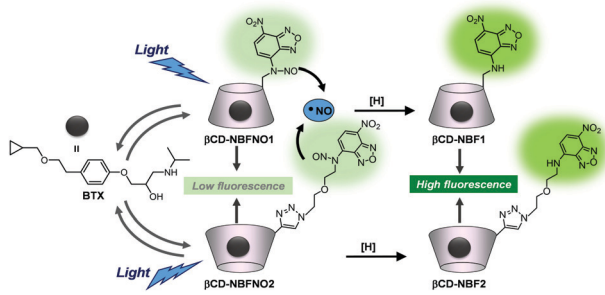
^e Department of Organic Chemistry, Charles University in Prague, Hlavova 8/8 128 43, Prague 2, Czech Republic

^f Institute for Polymers, Composites and Biomaterials, CNR, Via Campi Flegrei 34, 80078 Pozzuoli, Napoli, Italy

^g Drug Delivery Laboratory, Department of Pharmacy, University of Napoli Federico II, Via Domenico Montesano 49, 80131, Napoli, Italy. E-mail: quaglia@unicat.it

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‡ S. P. carried out the work at the University of Catania during her stage from the IASBS.



Scheme 1 Structures of β CD-NBFNO1, β CD-NBFNO2, their respective fluorescent photoproducts β CD-NBF1, β CD-NBF2 formed after NO photorelease, and BTX used as guest molecule.

supramolecular complexes with a guest molecule while covalent modification of the CDs ring through functionalization of the primary and/or secondary hydroxyl groups with suitable appendages has been much less explored. The integration of photoresponsive units into the CDs scaffold is of great interest as recently proven by a variety of photoresponsive CD-based nanoconstructs with potential phototherapeutic applications.^{26,27} Several NOPD have been supramolecularly combined with CDs derivatives.²⁸ In contrast, only limited examples of NO photodonor covalent conjugates with CDs are reported to date.^{29,30} In the context of ocular diseases, the covalent modification of the CD with NOPD could bring the benefit of obtaining a functional NO-releasing scaffold whilst, at the same time, maintaining the macrocycle capacity for encapsulation of small therapeutics in a combinatory therapeutic approach.^{29–32}

In this contribution, we provide a proof of concept of this approach by synthesising two novel β CD conjugates, β CD-NBFNO1 and β CD-NBFNO2, integrating an N-nitroso amino-nitro-benzofurazan (NBF-NO) within the primary and secondary rim of the β CD scaffold, respectively through flexible spacers of different length (Scheme 1). The N-nitroso amino-nitro-benzofurazan photoresponsive unit was selected since it represents the non-metal based NO releaser generating NO with the highest quantum yield upon excitation with Vis light developed so far.³³ Concomitantly to NO release, this compound forms the highly fluorescent non-nitrosated derivative as the sole stable photoproduct, representing an optical reporter useful for the NO detection even at the naked eye. Despite the excellent photochemical performances, NBF-NO has poor solubility in an aqueous medium, which severely limits its ocular application in the absence of any carrier system. After an in depth characterization of the photophysical properties of the β CD conjugates, we explore their host-guest complexation properties with betaxolol (BTX), a well-known β -blocker drug used against glaucoma for the reduction of intraocular pressure.^{34,35}

Experimental section

Materials

All reagents (Sigma-Aldrich, Alfa Aesar, Molar Chemicals Kft and Cyclolab) were of high commercial grade and were used

without further purification. All solvents used (from Carlo Erba) were spectrophotometric grade.

Synthetic procedures

All β CD conjugates were synthesized according to the procedures reported in ESI.†

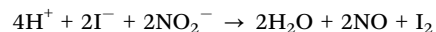
Instrumentation

¹H-NMR spectra were recorded on a Varian VXR-600 at 600 MHz. UV-Vis spectra absorption and fluorescence emission spectra were recorded with a JascoV-560 spectrophotometer and a Spex Fluorolog-2 (mod. F-111) spectrofluorimeter, respectively, in air-equilibrated solutions, using quartz cells with a path length of 1 cm. Fluorescence lifetimes were recorded with the above fluorimeter equipped with a TCSPC Triple Illuminator. The samples were excited with a pulsed diode excitation source (Nanoled) at 455 nm, the decays were monitored at 550 nm, and ethanol solution itself was used to register the prompt at 455 nm. The system allowed a time-resolution > 200 ps. The multiexponential fit of the fluorescence decay was obtained by the following equation:

$$I(t) = \sum \alpha_i \exp(-t/\tau_i)$$

Absorption spectral changes were monitored by irradiating the sample in a thermostated quartz cell (1 cm path length, 3 mL capacity) under gentle stirring, using a continuum laser with $\lambda_{\text{exc}} = 405$ nm, 20 mW, and $\lambda_{\text{exc}} = 532$ nm, ca. 100 mW, and having a beam diameter of ca. 1.5 mm.

Direct monitoring of NO release in solution was performed by amperometric detection (World Precision Instruments), with an ISO-NO meter, equipped with a data acquisition system, and based on direct amperometric detection of NO with short response time (< 5 s) and sensitivity range 1 nM–20 μ M. The analogue signal was digitalized with a four-channel recording system and transferred to a PC. The sensor was accurately calibrated by mixing standard solutions of NaNO₂ with 0.1 M H₂SO₄ and 0.1 M KI according to the reaction:



Irradiation was performed in a thermostated quartz cell (1 cm path length, 3 mL capacity) using the continuum laser with $\lambda_{\text{exc}} = 405$ nm or 532 nm. NO measurements were carried out under stirring with the electrode positioned outside the light path in order to avoid NO signal artefacts due to photoelectric interference on the ISO-NO electrode.

Fluorescence and photodecomposition quantum yields

Fluorescence quantum yields (Φ_f) were determined using optically-matched solutions at the excitation wavelength of conjugates and Fluorescein NaOH 0.1 M as reference ($\Phi_{\text{ref}} = 0.95$)³⁶ through the following equation:

$$\Phi_f = \Phi_{f(s)} (I/I_{(s)})$$

where $\Phi_{f(s)}$ is the fluorescence quantum yield of the standard; I and $I_{(s)}$ are the areas of the fluorescence spectra of compounds

and standard, respectively; absorbance at the excitation wavelength was less than 0.1 in all cases.

Photodecomposition quantum yield (Φ_{NO}) was determined at $\lambda_{\text{exc}} = 405$ nm and 532 nm within the 20% transformation of the conjugates by using the following equation

$$\Phi_{\text{NO}} = [C]V/t(1-10^{-A})I$$

where $[C]$ is the concentration of phototransformed $\beta\text{CD-NBFNO1}$ or $\beta\text{CD-NBFNO2}$, V is the volume of the irradiated sample, t is the irradiation time, A is the absorbance of the sample at the excitation wavelength and I the intensity of the excitation light. The concentration of the phototransformed conjugates was determined spectrophotometrically, by taking into account the absorption changes at 385 nm and the related $\Delta\epsilon$ at the same wavelength. I was calculated by potassium ferrioxalate actinometry.

Dark stability tests

The stability of both conjugates in the dark was evaluated by recording the absorption spectra of water solution of each compound in thermostated baths at different temperatures. The percent of decomposition was evaluated by the changing of the absorption spectra, taking into account the molar extinction coefficients of the starting compounds and their respective non-nitrosate derivatives.

Host-guest complexation experiments with BTX

To 2 ml of an aqueous solution of BTX (1.4×10^{-3} M), different volumes of either $\beta\text{CD-NBFNO1}$ or $\beta\text{CD-NBFNO2}$ stock solutions (6×10^{-3} M) were added. After the addition of each aliquot, the solutions were stirred for 5 min, and the absorption spectra were recorded. The spectral changes were evaluated by subtracting the absorption of the same concentration of conjugates in neat water. The absorbance changes were then plotted as a function of the concentration of the conjugates according to the Benesi-Hildebrand equation (*vide infra*).

Results and discussion

$\beta\text{CD-NBFNO1}$ and $\beta\text{CD-NBFNO2}$ show excellent solubility in water up to *ca.* 10^{-2} M. Fig. 1 shows the UV-Vis spectroscopic properties of aqueous solutions of the two derivatives and, for the sake of comparison, those of the non-nitrosated βCD conjugates $\beta\text{CD-NBF1}$ and $\beta\text{CD-NBF2}$.

The absorption spectra of $\beta\text{CD-NBFNO1}$ and $\beta\text{CD-NBFNO2}$ (Fig. 1A) exhibit a dominant absorption at *ca.* 380 nm and a shoulder at 475 nm. The main absorption is blue-shifted by more than 90 nm as compared to that of the non-nitrosated analogues (see Fig. 1B) as a consequence of the loss of the "charge transfer" character due to the presence of the electron-withdrawing NO group at the amino functionality. Note that the shoulder in the visible region is much more intense if compared with that observed for the same chromogenic unit non-linked to the βCD scaffold.³³ In principle one may think that such absorption can be due to the presence of impurities of

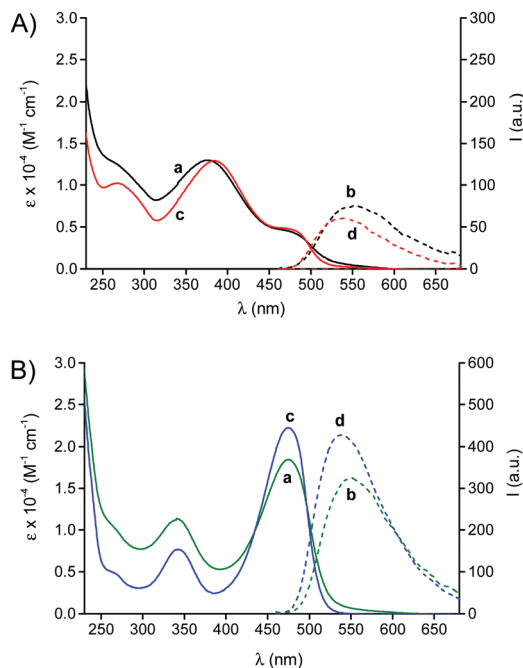


Fig. 1 (A) Absorption and fluorescence emission spectra ($\lambda_{\text{exc}} = 450$ nm) of aqueous solutions of $\beta\text{CD-NBFNO1}$ (a and b) and $\beta\text{CD-NBFNO2}$ (c and d). (B) Absorption and fluorescence emission spectra ($\lambda_{\text{exc}} = 450$ nm) of aqueous solutions of denitrosated $\beta\text{CD-NBF1}$ (a and b) and $\beta\text{CD-NBF2}$ (c and d). Fluorescence emission spectra were carried out with optically matched solutions of all compounds at the excitation wavelength. $T = 25$ °C.

non-nitrosate derivative. However, chromatographic analysis (see ESI†) and the photolysis carried out upon irradiation of this band with green light (*vide infra*) ruled out this hypothesis. In contrast, such a shoulder can be due to either intra or intermolecular non-covalent interaction between the N-nitroso appendages and the βCD moiety. The presence of this absorption, even in very dilute solutions, make the former hypothesis the more likely.³⁷

Analogously to what already observed for **NBF-NO**, the presence of the nitroso group has a significant effect on the emission properties. $\beta\text{CD-NBFNO1}$ and $\beta\text{CD-NBFNO2}$ exhibited fluorescence emission in the green region (Fig. 1A) but with quite low quantum yields, being $\Phi_{\text{f}} = 0.026$ and 0.018, respectively, that are values *ca.* 4 and 7-fold smaller than those of the analogues non-nitrosated derivatives ($\Phi_{\text{f}} = 0.10$ and 0.13) (Fig. 1B).

The broad absorption band and the quite large extinction coefficient of the conjugates make possible their excitation in the visible region, even in a reduced concentration range. Fig. 2A shows the absorption and fluorescence emission spectral changes observed upon blue light excitation of an air-equilibrated solution of $\beta\text{CD-NBFNO1}$. They show the bleaching of the main absorption at 377 nm and the formation of a new, intense absorption band at 475 nm. The spectral evolution is also characterized by the presence of 3 isosbestic points, accounting for a quite clean photochemical process. Note that, the spectrum observed at the end of the photolytic process is identical to that of

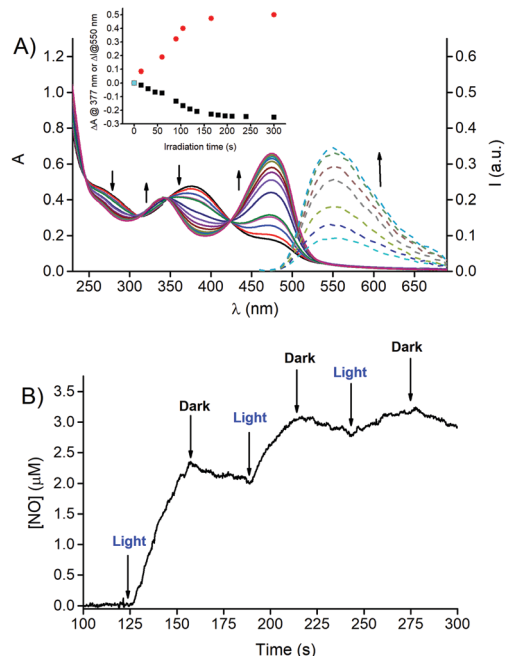


Fig. 2 (A) Absorption (solid lines) and fluorescence emission, ($\lambda_{\text{exc}} = 424$ nm, isosbestic point) (dotted lines) spectral changes observed upon exposure of an aqueous solution of $\beta\text{CD-NBFNO1}$ ($35 \mu\text{M}$) at $\lambda_{\text{exc}} = 405$ nm (ca. 20 mW cm^{-2}) for time intervals from 0 to 300 s. The arrows indicate the course of the spectral profile with the illumination time. The inset shows the different absorbance changes at $\lambda = 377$ nm (■) and fluorescence changes at $\lambda = 550$ nm (●), respectively. (B) NO release profile observed for an aqueous solution of $\beta\text{CD-NBFNO1}$ ($35 \mu\text{M}$) upon alternate cycles of irradiation ($\lambda_{\text{exc}} = 405$ nm, ca. 20 mW cm^{-2}) and dark. $T = 25$ °C.

the non-nitrosated conjugate $\beta\text{CD-NBF1}$ (compare spectra a in Fig. 1A and B) showing the typical charge transfer band at 475 nm of the amino-nitro-benzofurazan chromogenic unit.³⁸

Besides, the evolution of the fluorescence emission spectra observed upon irradiation shows a significant restoring of the green emission with $\lambda_{\text{max}} = 550$ nm, typical of the amino-nitro-benzofurazan fluorophore (Fig. 2A).³⁸ These findings clearly account for the NO photorelease from $\beta\text{CD-NBFNO1}$ and the concomitant formation of the non-nitrosated fluorophore that act as suitable fluorescent reporter to follow the NO uncaging in real time. The inset of Fig. 2A shows a very good agreement between the evolution of the absorption and fluorescence changes as a function of the irradiation time and indicates that the photolysis was complete within ca. 2 min of irradiation. This accounts for a very effective photochemical reaction as confirmed by the high of the quantum yield for the NO photorelease, $\Phi_{\text{NO}} = 0.13$, a value very close to that observed for the non-water-soluble NBF-NO ($\Phi_{\text{NO}} = 0.15$).³³ NO release was demonstrated by the direct detection of this radical species using an ultrasensitive NO electrode. Fig. 2B shows that NO is promptly released upon illumination, of the aqueous solution of $\beta\text{CD-NBFNO1}$, stops in the dark and restarts once the light source is switched on again.

Fig. 3A and related inset show that $\beta\text{CD-NBFNO2}$ exhibited a similar photobehavior with an even higher quantum yield for the NO photorelease, $\Phi_{\text{NO}} = 0.31$, which represents the largest

value among those reported for any organic NOPD activatable in the *Vis* range.

Interestingly, the restoring of the emission of the optical reporter is visible even at naked eye (Fig. 3A) and gives easily readable information about the NO generated. The NO photo-release measured by amperometric monitoring was then related to the increase of the fluorescence emission for both compounds. As shown in Fig. 3B, we found a very good correlation between the concentration of NO liberated by both compounds upon light stimuli and the increase of the fluorescence intensity of the related optical reporters.

It needs to be stressed that the remarkable values of Φ_{NO} found for both conjugates permit the generation of a considerable amount of NO without the need of long irradiation times, which in some cases can be deleterious to cells. According to the literature, aniliny radical derivatives formed after the homolytic N–NO photocleavage evolves to stable photoproducts by H-transfer from the solvent medium. Since in neat water, like in our case, this process is thermodynamically not feasible,

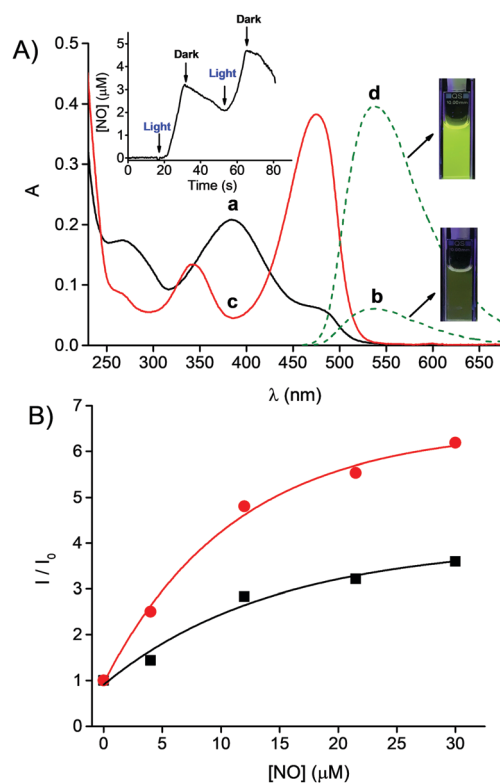


Fig. 3 (A) Absorption (solid lines) and fluorescence emission, ($\lambda_{\text{exc}} = 427$ nm, isosbestic point) (dotted lines) spectra changes observed before (a and b) and after the complete photolysis (c and d) at $\lambda_{\text{exc}} = 405$ nm (ca. 20 mW cm^{-2}) of aqueous solution of $\beta\text{CD-NBFNO2}$ ($16 \mu\text{M}$) and actual images of the solutions before (bottom) and after (top) the photolysis acquired upon excitation at $\lambda = 350$ nm. The inset shows the NO release profile observed for an aqueous solution of $\beta\text{CD-NBFNO2}$ ($16 \mu\text{M}$) upon alternate cycles of irradiation ($\lambda_{\text{exc}} = 405$ nm, ca. 20 mW cm^{-2}) and dark. (B) Correlation of the fluorescence increase observed upon photolysis of $\beta\text{CD-NBFNO1}$ (■) and $\beta\text{CD-NBFNO2}$ (●) and the concentration of NO photoreleased. I and I_0 represent the fluorescence intensities at the λ_{max} of emission after and before irradiation, respectively. $T = 25$ °C.

the high values observed for Φ_{NO} indicate a key role of the βCD scaffold as a reactant, providing a source of 14 easily abstractable H atoms and very close to the aniliny radical intermediate. Moreover, it should be noted that the absorption and emission spectral and time evolution observed upon irradiation were identical in the case of an N_2 -saturated solution (data not shown), suggesting that both the efficiency and nature of the photochemical reaction are not dependent by the presence of oxygen. This observation rules out the participation of a long-lived excited triplet state in the photodecomposition, suggesting a NO photodetachment occurring more likely from the short-lived excited singlet state. This hypothesis is supported well by (i) the negligible population of the excited triplet state reported for amino-nitro benzofurazan derivatives in polar solvents,³⁹ and (ii) the very short singlet lifetimes found for $\beta\text{CD-NBFNO1}$ and $\beta\text{CD-NBFNO2}$. As shown in Fig. 4, both conjugates exhibited a biexponential behavior with dominant components (*ca.* 80%) with $\tau = 2.30$ and 0.84 ns, respectively.

As discussed above, the absorption spectrum of both βCD conjugates shows a pronounced shoulder extending up to the green region, which is negligible in the case of NBF-NO .³³ These spectral features prompted us to investigate the photo-reactivity of the compounds upon excitation with green light. Therefore, aqueous solution of either $\beta\text{CD-NBFNO1}$ or $\beta\text{CD-NBFNO2}$ were irradiated at $\lambda_{\text{exc}} = 532$ nm. We observed changes in the absorption and fluorescence emission spectral profiles basically identical to those already observed under blue light stimuli (data not shown) although with lower photochemical efficiency. In fact, the values calculated for Φ_{NO} were = 0.007 and 0.013 for $\beta\text{CD-NBFNO1}$ and $\beta\text{CD-NBFNO2}$, respectively. Fig. 5 shows unambiguous evidence that NO release for both compounds can also be triggered by green light.

The photoreactivity dependence by the excitation wavelength is not surprising and may be due to the participation of upper excited singlet states populated with blue light as mediators of the photodecomposition route.

The stability of the conjugates was also evaluated in the dark at different temperatures and times. Fig. 6A shows moderate decomposition for both compounds (*ca.* 20%) at 25 °C, which is almost totally inhibited if samples are incubated for the same time at 4 °C. Moreover, incubation of the solution in a thermostated bath at a different temperature for 15 min each showed satisfactory stability up to 40 °C (Fig. 6B).

Finally, we investigated the host-guest complexation ability of the βCD conjugates. As a prototype guest, we chose **BTX** for

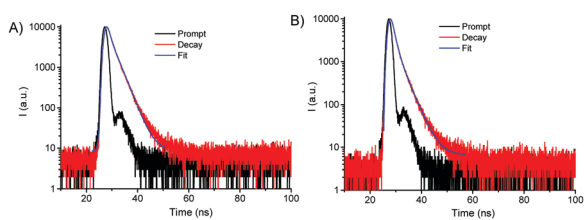


Fig. 4 Fluorescence decay and the related biexponential fitting of the aqueous solution of $\beta\text{CD-NBFNO1}$ (40 μM) (A) and $\beta\text{CD-NBFNO2}$ (B) recorded at $\lambda_{\text{exc}} = 455$ nm and $\lambda_{\text{em}} = 550$ nm.

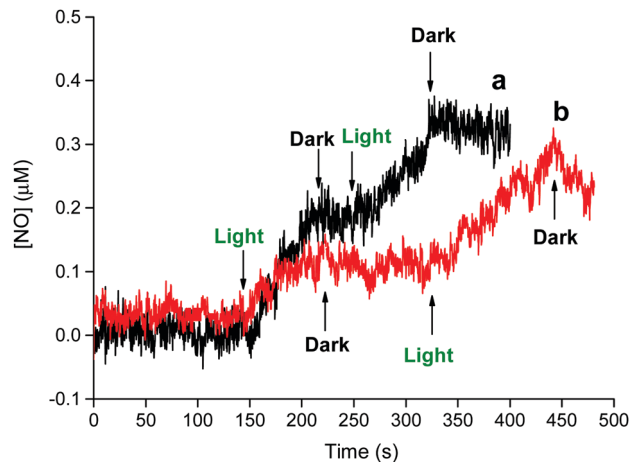


Fig. 5 NO release profiles observed for an aqueous solution of $\beta\text{CD-NBFNO1}$ (35 μM , a) and $\beta\text{CD-NBFNO2}$ (16 μM , b) upon alternate cycles of irradiation with green light ($\lambda_{\text{exc}} = 532$ nm, *ca.* 100 mW cm^{-2}) and dark.

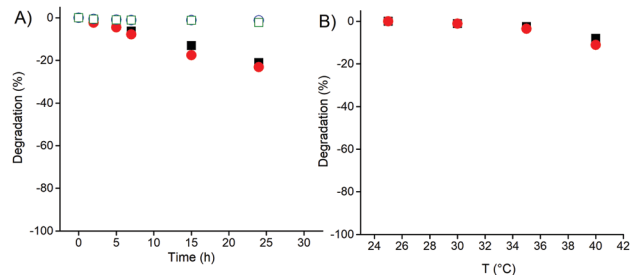


Fig. 6 (A) Dark stability of the aqueous solution of $\beta\text{CD-NBFNO1}$ (square) and $\beta\text{CD-NBFNO2}$ (circles) incubated in the dark at 25 °C (filled symbols) or 4 °C (open symbols) at different times. (B) Dark stability of the aqueous solution of $\beta\text{CD-NBFNO1}$ (square) and $\beta\text{CD-NBFNO2}$ (circles) incubated for 15 min at different temperatures.

the reasons motivated in the introductory part. Titration of an aqueous solution of **BTX** was then carried out using increasing amounts of either $\beta\text{CD-NBFNO1}$ or $\beta\text{CD-NBFNO2}$. **BTX** offers good spectroscopic requisite to follow the titration by UV-Vis absorption spectroscopy since its absorption maximum falls at 275 nm, a region in which both conjugates display small absorption. Fig. 7A and B show the absorption spectral changes in the **BTX** region observed upon the addition of the host molecules and after subtracting the same amount of hosts added to the same volume of water. We observed a hypochromic shift of the absorption band of **BTX** after addition of the βCD conjugates, according to typical host-guest encapsulation processes. The reciprocal of the absorbance changes at the absorption maximum was then plotted as a function of the reciprocal concentration of the host molecules, according to the Benesi-Hildebrand equation:⁴⁰

$$\frac{1}{\Delta A} = \frac{1}{K_{\text{ass}} \cdot \Delta \epsilon \cdot [\text{host}]} + \frac{1}{\Delta \epsilon}$$

where K_{ass} represents the association constants for the supramolecular host-guest process, $\Delta \epsilon$ is the difference of the molar

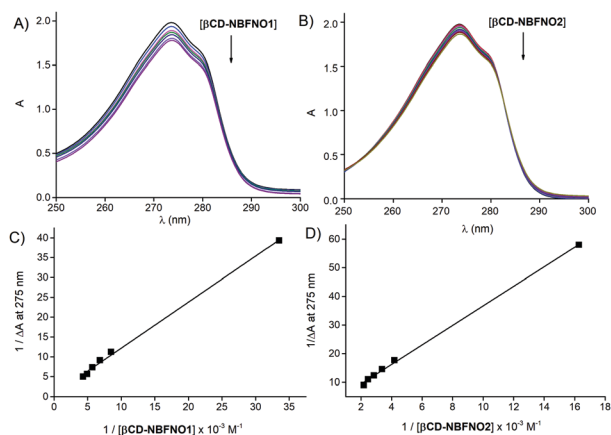


Fig. 7 Absorption spectral changes observed upon addition of different amounts of β CD-NBFNO1 from 28 μ M to 210 μ M (A) and β CD-NBFNO2 from 62 μ M to 500 μ M (B) to aqueous solutions of BTX (1.4 mM). The related double-reciprocal plots and the linear fitting of the data, according to the Benesi–Hildebrand equation, are reported for β CD-NBFNO1 (C) and β CD-NBFNO2 (D). $T = 25^\circ\text{C}$.

extinction coefficient between the free and complexed guest and [host] is the concentration of the β CD conjugates. As shown in Fig. 7C and D, we obtained very good linear plots in both cases and values of K_{ass} of $500 \pm 50 \text{ M}^{-1}$ and $1100 \pm 100 \text{ M}^{-1}$ for β CD-NBFNO1 and β CD-NBFNO2, respectively, were obtained from the intercept/slope ratio. The higher value observed for β CD-NBFNO2 is probably attributable to the longer flexible spacer between the CD scaffold and the chromogenic unit, which allows a better accommodation of the host within the hydrophobic cavity.

In order to be used in combination, one of the indispensable requisites for this host–guest system is that the encapsulation of BTX in the CD cavity does not affect the photochemical performances of the photoactivatable conjugates. Therefore, photolysis experiments on β CD-NBFNO1 and β CD-NBFNO2 were carried out in the presence of BTX. We observed that the presence of the guest molecule changes neither the nature nor the efficiency of the photoreactivity of both compounds, ruling out any intermolecular communication between the host and the guest through competitive photoinduced processes. This is not a trivial result for host–guest supramolecular complexes. In fact, it is known that the photoreactivity of the host and guest components can be remarkably influenced in efficiency nature or both upon complexation as result of their close proximity and the presence of specific interactions, steric constrains and reduced polarity.²⁷

Conclusions

We have designed, synthesized and characterized two novel photoactivatable β CD conjugates. These compounds show excellent water solubility, fairly good stability in the dark within 24 h at room temperature, and release of the biologically relevant NO under visible light stimuli. In particular, excitation with blue light leads to NO release with the highest quantum yield never observed for non-metal based NO photoreleaser

triggered by visible light. However, although with lower efficiency, both conjugates release NO even under stimuli of green light. The good fluorescence contrast between the highly fluorescent stable photoproducts and the poorly fluorescent starting compounds permits the formers to be excellent optical reporters for the easy detection of the NO generated, with the release process being followed even by the naked eye. Moreover, the reporter can be excited by using the same excitation wavelength used for NO uncaging, facilitating the real-time monitoring of NO, which is crucial given the transient nature of this diatomic radical, without requiring a double excitation source. It is also important to stress that, as extensively demonstrated in our recent studies on other nitroso-derivatives,⁴¹ these photoactivatable compounds do not show significant cytotoxicity in the dark in the concentration range used. Interestingly, functionalization of the CD scaffold with the photoactivatable moieties does not preclude the encapsulation guest as demonstrated by the association of both conjugates with the β -blocker BTX. In this regard, in view of the well-known vasodilator properties of NO, the present work may open intriguing prospects for biological studies on formulations for ocular application against glaucoma, addressed to explore the combinatory effect of BTX and NO, with this latter slowly released at physiological temperature but rapidly released upon environmental light. These studies are currently underway in our laboratories.

Conflicts of interest

We have no conflict of interests to declare.

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