Articles
pubs.acs.org/acschemicalbiology

In Vivo Light-Driven DNA Binding and Cellular Uptake of Nucleic Acid Stains

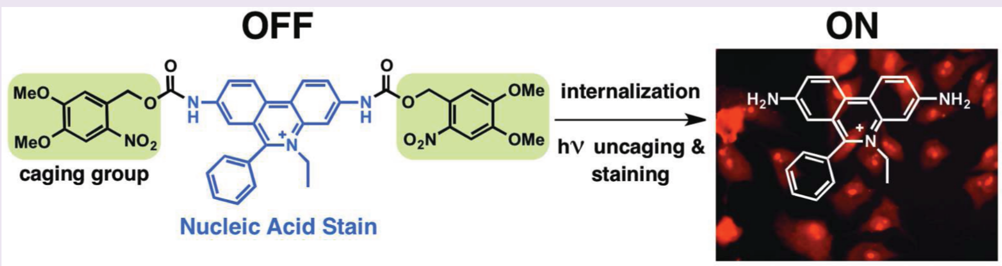
Mateo I. Sánchez,[†] José Martínez-Costas,[‡] Francisco Gonzalez,[§,∥] María A. Bermudez,[§] M. Eugenio Vázquez,*[,†] and José L. Mascareñas*[,†]

[†]Departamento de Química Orgánica and [‡]Departamento de Bioquímica y Biología Molecular, Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares, Unidad Asociada al CSIC, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

[§]Departamento de Fisiología, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

[∥]Servicio de Oftalmología, Complejo Hospitalario Universitario de Santiago de Compostela, 15706 Santiago de Compostela, Spain.

Supporting Information



ABSTRACT: Chemical derivatization of nucleic stains such as ethidium bromide or DAPI with tailored, photoresponsive caging groups, allows for "on demand" spatiotemporal control of their *in vivo* nucleic acid binding, as well as for improving their cellular uptake. This effect was particularly noteworthy for a nitro-veratryloxycarbonyl-caged derivative of ethidium bromide that, in contrast with the parent stain, is effectively internalized into living cells. The activation strategy works in light-accessible, therapeutically relevant settings, such as human retinas, and can even be applied for the release of active compounds in the eyes of living mice.

The development of nucleic-acid-targeted drugs continues to be a major research endeavor at the interface between chemistry and biomedicine. A large variety of DNA-binding agents have been developed, and many of them have found application as drugs or as DNA stains.[1−5] Unfortunately, many of these molecules present selectivity and toxicity problems that seriously restrict their therapeutic potential. In this context, derivatizing these molecules with photolabile appendages that confer advantageous physicochemical features while providing for external control of their activity might open important therapeutic avenues.[6−8] Needless to say, the use of light-activated compounds in photodynamic therapy might greatly benefit from current technical advances that make it possible to irradiate almost any tissue in the human body.[9]

DAPI (4′,6-diamidino-2-phenylindole, **1**; Figure 1) and ethidium bromide (EtBr, 3,8-diamino-5-ethyl-6-phenyl-phenanthridinium bromide, **2**) are among the best known nucleic acid stains[10−14] and have found wide and important applications in molecular and cell biology. Ethidium bromide binds nucleic acids by intercalation and has been extensively used for detecting double-stranded DNA (dsDNA), mainly in gel electrophoresis assays. However, it cannot be used for direct staining of cellular nucleic acids, as it is poorly internalized. In contrast, the blue-fluorescent DAPI can be used to stain cell nuclei and, unlike the non-specific EtBr, prefers to bind A/T-rich DNA sequences, inserting into their narrow minor groove.

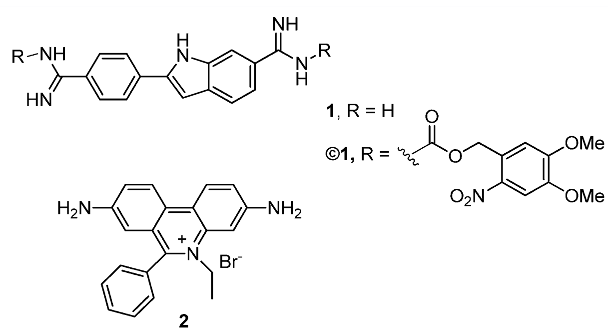


Figure 1. Structures of DAPI (**1**), its caged derivative (**©1**), and ethidium bromide (**2**).

We have recently demonstrated that caging of the amidinium moieties of DAPI and other bisbenzamidinium DNA binders with suitable photoresponsive protecting groups suppresses their DNA binding.[15] Importantly, irradiation with UV light

Received: December 6, 2011
Accepted: May 2, 2012
Published: May 3, 2012

removes the caging groups and regenerates the parent active compounds, thus allowing the control of the *in vitro* DNA binding activity of these molecules by external stimuli (UV light). We were then challenged to demonstrate that such conditional activation strategy could also be used in living cells and tissues, a process that should be easily monitored by the increased fluorescence of these molecules upon staining of the cell nuclei. In addition, we were also intrigued by the possibility of extending the caging strategy to other DNA binders lacking amidinium groups, such as ethidium bromide (EtBr).

Herein we demonstrate that both DAPI (**1**) and EtBr (**2**) can be efficiently caged using photolabile groups and that their nucleic acid binding ability can be restored in living cells and in therapeutically relevant tissues such as retinas, in a *spatially* and *temporarily* controlled manner. We also show that it is even possible to release the stains in the eyes of living mice by using an external irradiation source.

■ RESULTS AND DISCUSSION

As expected from the relatively low pK_a of the amidines and their propensity to act as leaving groups, the caging groups of DAPI derivative ©**1**[15] can be readily removed in the presence of dsDNAs using a standard gel transilluminator lamp as irradiation source (λ = 300−375 nm, Supplementary Figure S1). For the *in vivo* assays, we incubated independent samples of Vero cells[16] with 5 μM ©**1** for 30 min and monitored their evolution by fluorescence microscopy. Initial irradiation experiments were carried out using the transilluminator, but the UV light source from the microscope could also effectively photolyze ©**1**. This allowed an easy monitoring of the changes in fluorescence emission of the dye inside the cells in real time. Remarkably, the fluorescence emission is initially relatively weak and diffuse, but apparently more intense on the perimeter (membrane) of the cells. Over time it becomes progressively concentrated in the cell nuclei, while growing fainter at the cell edges. Indeed, after 20 min under the microscope, the edges become barely distinguishable and only the nuclei are observed (Supplementary Video S1). Control experiments with liposomes confirm that, as expected, ©**1** is basically non-emissive, and addition of DAPI to a liposome solution results in a marked increase in its emission intensity (Supporting Information, page S12). Therefore it can be safely stated that the fluorescence observed in the membrane is due to uncaged DAPI.

In order to separate the effects of the uncaging and cellular redistribution, Vero cells were incubated with ©**1** in PBS for 30 min and irradiated for approximately 3 min in the transilluminator (enough to uncage most of the stain). Comparison of micrographs obtained immediately after the irradiation and after 5 min of incubation confirms that the uncaged dye requires some time to accumulate and stain the cell nuclei (Supporting Information, page S17). All of these results are consistent with the presence of the hydrophobic caged DAPI (©**1**) in the cytoplasmic membrane and probably also in other cell locations. Upon irradiation, photolysis of ©**1** releases the fluorogenic DAPI, which is highly emissive in the hydrophobic phospholipid matrix. However, the uncaged DAPI is very hydrophilic (ACD/Laboratories LogD (pH 7.4) = −1.03)[17,18] and is therefore driven out of the membrane and freely diffuses toward the cell nuclei, finally forming a stable, fluorescent DNA complex (Figure 2).

Once we demonstrated the possibility of *in vivo* UV-controlled DNA interaction with a minor groove binder, we

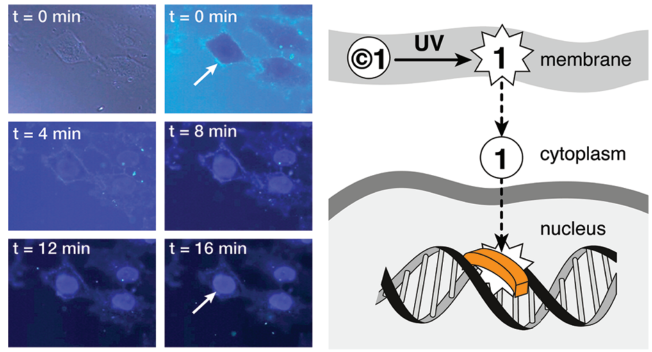
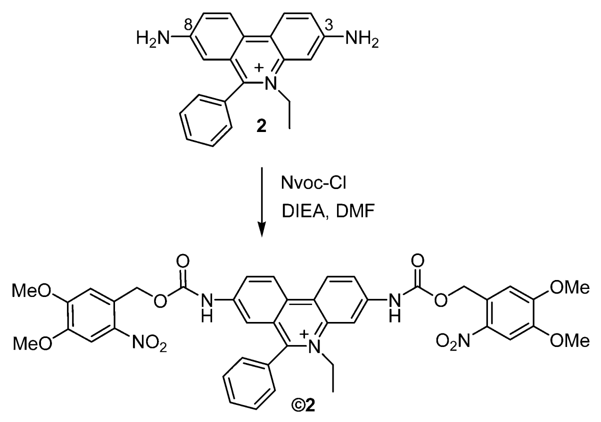


Figure 2. (Left) Fluorescence microscopy of Vero cells incubated with 5.0 μM ©**1** showing the fluorescence changes inside the cells (t = 0, brightfield and fluorescence; t = 4−16 min, fluorescence); arrows highlight the brighter areas. (Right) Schematic representation of the proposed activation dynamics of caged DAPI, which is, at least in part, stored in the cell membrane. Initial background fluorescence at t = 0 most probably results from non-specific interaction between uncaged DAPI and the hydrophobic polymeric Mowiol 4−88 matrix used as coverslip mounting solution.

sought to extend this strategy to EtBr (**2**), which binds DNA by intercalation and lacks the amidinium groups of DAPI. Despite its wide use as an *in vitro* DNA stain, EtBr has very limited value for *in vivo* experiments due to its poor cell membrane permeability. We envisioned that masking the amino groups of EtBr with the non-polar Nvoc caging group might not only interfere with its DNA binding but also improve the internalization properties of the stain by increasing its hydrophobic character. Therefore the bis-Nvoc phenanthridyl derivative ©**2** was prepared by treatment of the commercial EtBr with nitro-veratryloxycarbonyl (Nvoc) chloride under basic conditions (Scheme 1).

Scheme 1. Synthesis of Nvoc-Caged Ethidium Bromide ©2



As expected, ©**2** did not show any fluorescence in the presence of a short dsDNA oligonucleotide, and competition experiments with uncaged EtBr confirmed that ©**2** is unable to bind DNA. Irradiation of a solution of ©**2** in Tris-HCl buffer, 100 mM NaCl, pH 7.5, with UV light (standard transilluminator lamp, λ = 300−375 nm) promoted the cleavage of the photolabile groups, thus regenerating the active EtBr intercalator (**2**). Importantly, this can be achieved in the presence of DNA, leading to a large increase (60-fold) in the emission intensity at 600 nm, which is consistent with the intercalation of the released EtBr into the DNA (Figure 3).

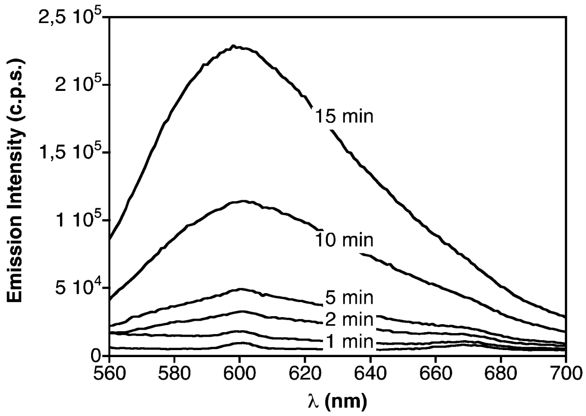


Figure 3. Fluorescence emission spectra of a 0.8 μM solution of ©**2** in Tris-HCl buffer at increasing irradiation times, in the presence of a hairpin ds-oligonucleotide. No further increase in fluorescence was observed after approximately 15 min of irradiation.

HPLC monitoring of the photolysis reaction showed that the process generates Nvoc-monoprotected temporary intermediates, but both caging groups are fully removed after 18−20 min of irradiation (Supporting Information, page S10). Control experiments with a mixture of specifically synthesized C3 and C8 Nvoc-monoprotected EtBr showed that these compounds are not fluorescent in the presence of DNA, despite retaining some DNA binding affinity (Supporting Information, pages S9−S10). Therefore, uncaging of both groups is required for good DNA binding and for DNA-dependent fluorescent staining.

Once the photocontrolled DNA binding of ethidium was confirmed, we studied whether the presence of the hydrophobic appendage could improve the cell transport properties of the dye. Therefore, independent samples of Vero cells were treated in parallel with 12.5 μM caged ©**2** and 20.0 μM EtBr (**2**) and examined by fluorescent microscopy after 30 min (excitation filter 530−550 nm/emission filter 590 nm). As shown in Figure 4A/C, in both cases the cells were essentially non-fluorescent, and only a very faint emission could be detected in the case of EtBr. However, UV irradiation of the cells incubated with ©**2** (10 min) resulted in a very significant increase in the emission intensity, a result that must be correlated with photorelease of ethidium bromide from the inactive derivative ©**2** (Figure 4B). Remarkably, staining is

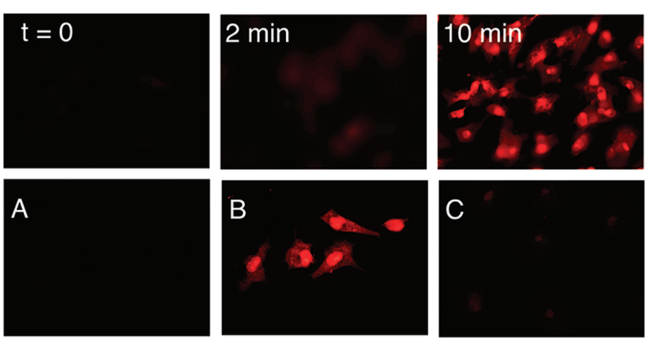


Figure 4. (Top) Fluorescence microscopy of Vero cells (12.5 μM ©**2**) at increasing irradiation times. (Bottom) 12.5 μM ©**2**, after 30 min incubation:(A) before irradiation and uncaging; (B) after uncaging by UV irradiation for 15 min; (C) control experiment with 20.0 μM of ethidium bromide **2** after 30 min incubation.

mainly localized in the nucleoli, as demonstrated by colocalization experiments with DAPI and mitotracker dyes (for further information see the Supporting Information, pages S14 and S16).

These results suggest that, in contrast to the parent phenanthridinium **2**, the designed photoresponsive derivative ©**2** seems to efficiently cross cell membranes, accumulating inside the cell in a latent form, until irradiation with UV light releases the active intercalator, which binds and stains nucleolar nucleic acids. A control experiment at increasing irradiation times revealed a progressive increase in the fluorescence emission from the cells, reaching a maximum after approximately 10 min of irradiation (Figure 4, top). It is probable that some monocaged EtBr derivatives are also present, but cannot be monitored by fluorescence imaging. The use of a more potent illumination source should warrant a more rapid and efficient generation of the active binder.

In addition to temporal activation, the caging strategy should also allow for the spatial control of the interaction. To demonstrate that, we prepared a cell monolayer on a 100 mm tissue culture plate (Figure 5A) and irradiated it through a

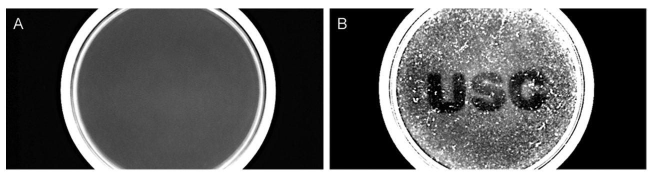


Figure 5. (A) cell monolayer in the culture plate before irradiation; (B) same culture after irradiation through a cardboard cutout.

cardboard cutout of our university acronym, USC. After 15 min of irradiation through the mask, we looked at the culture through a gel imaging system, observing the expected cellular photopatterning only for the cell populations that were irradiated (Figure 5B). Therefore, derivative ©**2** is a light-responsive, transport-efficient version of ethidium bromide and should represent a significant addition to the as yet short arsenal of nucleic acid stains that are effective *in vivo*.

The above results encouraged us to evaluate the performance of both caged DAPI (©**1**) and caged ethidium (©**2**) in a more stringent, therapeutically relevant setting. Therefore we incubated mice eye retinas with 5.0 μM ©**1** and 12.5 μM ©**2** (PBS buffer) and analyzed the resulting cellular staining before and after irradiation. Before irradiation, no staining was visible, whereas after irradiation both ©**1** and ©**2** were effectively uncaged in the retina, and the cell nuclei became fluorescent (Figure 6a,b; see also Supplementary Video S2). Importantly, the same results were reproduced with human retinas (Figure 6d,e), confirming that these compounds can be activated in the complex cellular environment of human tissues.

In a step forward, we also explored the possibility of *in vivo* activation of these molecules by direct external irradiation of the eyes in living mice. Therefore, mice were treated with caged DAPI (©**1**) using a femoral vein injection, and a transpupillar irradiation of one of their eyes was made. The mice were sacrificed, and the retinas were extracted and flat mounted for study. As expected, only the retina of the irradiated eyes showed a clear fluorescence emission, which was mostly concentrated in the vascular walls (Figure 6c), possibly because the blood-retinal barrier prevented most of the molecules from entering the retinal cells. This is a known phenomenon that occurs in healthy retinas; however, if the retinas present some

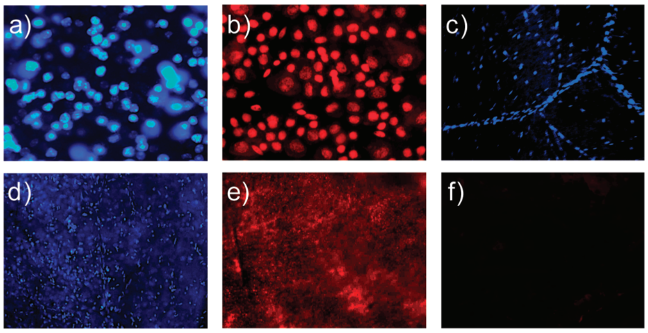


Figure 6. Fluorescence microscopy of mouse and human retinas. (a) Mouse retina incubated with 5.0 μM of **©1**, after ~5 min irradiation. (b) Mouse retina incubated with 12.5 μM **©2**, after ~10 min irradiation. (c) Retina extracted from a mouse previously injected with **©1** in the right femoral vein (100 μL, 5.0 μM) and transpupillary irradiated for ~10 min before sacrificing the animal; the nuclei of aligned cells forming a vessel image can be clearly observed. (d) Human retina incubated with 5.0 μM **©1**, after ~5 min irradiation. (e) Human retina incubated with 12.5 μM of **2**, after ~10 min irradiation. (f) Human retina incubated with 12.5 μM of **©2** before irradiation. Control, non-irradiation experiments were carried in all cases, and none of them showed traces of fluorescence. Images in panels a and b were acquired with 1 s exposure time, and images in panels c−f with 400 ms. All images with ISO 400 sensitivity.

degree of damage the compounds should be able to enter the cells.[19] These results confirm that the designed compounds can be selectively activated in the posterior segment of eyes of living animals using an external irradiation source and suggest the eye as a suitable initial target organ for future therapeutic intervention using this type of light-activatable compounds. Delivery of drugs in a minimally invasive, safe, and effective manner in the eye is still a major therapeutic challenge.[20] Given the well-established use of lasers in ophthalmology, the uncaging tactic should open new opportunities to treat specific eye diseases that require the selective release of active drugs in specific areas of the eye.[21,22]

In conclusion, we have demonstrated that the Nvoc caging strategy can be applied to classic DNA intercalators such as EtBr. Since these molecules are nucleic-acid-dependent fluorescent probes, they provide an excellent opportunity for real-time monitoring of the activation process in living cells. For the Nvoc-DAPI (**©1**), we could observe the uncaging process and cell redistribution of the released stain in real time. Moreover, the caging strategy can be exploited not only for the "on demand" spatiotemporal control of the nucleic acid binding but also for fine-tuning the physicochemical properties of the substrates. In the case of the Nvoc-caged derivative **©2**, this allows for a rapid cell internalization, overcoming the poor cell transport ability of the parent ethidium bromide. Importantly, we have also demonstrated that this strategy can be successfully implemented in therapeutically relevant tissues such as the retina, it even being possible to activate the dyes with external irradiation in the eyes in living mice. To the best of our knowledge, our results provide the first evidence on the use of caged compounds in eyes and demonstrate that focalized release of drugs can be made with a minimally invasive technique, opening a new door for therapeutic and perhaps diagnostic applications.

■ METHODS

General. All reagents were acquired from commercial sources. Reactions were followed by analytical RP-HPLC with an Agilent 1100 series LC/MS using an Eclipse XDB-C18(4.6 mm × 150 mm, 5 μm) analytical column. Standard conditions for analytical RP-HPLC consisted of a linear gradient from 5% to 95% of solvent B for 30 min at a flow rate of 1 mL/min (solvent A: water with 0.1% TFA, solvent B: acetonitrile with 0.1% TFA). Compounds were detected by UV absorption at 220, 270, 304, and 330 nm. Final products were purified on a Büchi Sepacore preparative system consisting of a C-615 pump manager with two C-605 pump modules for binary solvent gradients, a C-660 fraction collector, and a C-635 UV Photometer. Purification was made using reverse phase of water/acetonitrile 0.1% TFA, using a prepacked preparative cartridge (150 mm × 40 mm) with reverse-phase RP_{18} silica gel (Büchi order no. 54863). The fractions containing the products were freeze-dried, and their identity was confirmed by ESI-MS(+). NMR spectra were recorded using Varian Mercury 300 or Bruker DPX 250 spectrophotometers and processed using the MestreNova v6.1.1-6384 suite (Mestrelab Research). ^{1}H NMR spectra were processed applying Global Spectrum Deconvolution (GSD).

Synthetic Procedure for 3,8-Bis({[(4,5-dimethoxy-2-nitrobenzyl)oxy]carbonyl}amino)-5-methyl-6-phenylphenanthridinium (Nvoc$_2$-ethidium, ©2. To a solution of commercially available ethidium bromide (**2**) (100 mg, 0.253 mmol) in 25 mL of DIEA/DMF (0.195 M) was added nitroveratryl chloride (209 mg, 0.759 mmol). The resulting mixture was stirred for 16 h under Ar and in the dark. After concentration, the residue was purified by preparative reverse-phase chromatography (Büchi Sepacore) (5 min isocratic 15% B, followed by linear gradient from 15% to 95% B during 30 min, and 10 min isocratic at 95% B). The combined fractions were concentrated and freeze-dried to obtain the desired product (**©2**) as a trifluoroacetic salt (77 mg, 0.086 mmol, 34%).

***In Vitro* Fluorescence Experiments with ©2.** A fluorescence cuvette containing a 0.82 μM solution of **©2** in Tris-HCl buffer 20 mM, 100 mM NaCl, pH 7.5, and 0.136 μM hairpin DNA oligonucleotide **AACGTT** was irradiated with UV light, and fluorescence emission spectra were recorded after different irradiation times. Hairpin oligonucleotide: **AACGTT**: GGC AAGCTT CGC TTTTT GCG AAGCTT GCC.

Cell Uptake Experiments. Vero cells were maintained in DMEM (Dulbecco Modified Eagle Medium) containing 10% of FBS (fetal bovine serum). The day before the cellular uptake experiments, cells were seeded in 12-well plates containing glass coverslips (15 mm). Cells were then washed 3 times in PBS and overlaid with 1 mL of fresh PBS with no serum added. Compound **©1** was added in order to obtain a final concentration of 5.0 μM, and in the case of **©2** 12.5 μM. Samples were incubated for 30 min at RT in the absence of light. For video recording and snapshots of Figure 2, after incubation the coverslips were mounted on glass slides with Mowiol 4−88 [100 mg mL^{-1} in 100 mM Tris-HCl pH 8.5, 25% glycerol, and 0.1% DABCO (as an antifading agent)]. Irradiation of selected samples was performed with a standard gel UV transilluminator (8 W, λ_{exc} 300−370 nm). Images were obtained with an Olympus DP-71 digital camera mounted on an Olympus BX51 fluorescence microscope (Olympus Corp.) equipped with a 360−370 nm excitation filter and 420 nm emission filter for the cells treated with **©1** and were further processed (cropping, resizing and global contrast and brightness adjustment) with Adobe Photoshop (Adobe Systems). In the case of **©2** we used a 530−550 nm excitation filter and a 590 nm emission filter.

Experiments with Retinas and with Live Mice. Animal retinas were obtained from 3-month-old wild type SV129 mice originally acquired from Charles River Laboratories. The animals were terminally anesthetized, the eyes were enucleated, the retinas were dissected from the eyecup, and flat mounts were prepared with the ganglion cell layer uppermost. Then the retinas were incubated with a solution of caged ethidium (**©2**, 12.5 μM in PBS buffer) or caged DAPI (**©1**, 5.0 μM, PBS buffer). The uncaging was performed with a standard gel UV transilluminator for **©2** (λ = 300−375 nm). In the case of **©1**, it was

dx.doi.org/10.1021/cb300100r | *ACS Chem. Biol.* 2012, 7, 1276−1280

found that the light of the microscope was intense enough to cleave the photolabile groups. The irradiated samples were observed in a fluorescence microscope equipped with a 530−550 nm excitation filter and a 590 nm emission filter for the cells treated with ©2 and a 360−370 nm excitation filter and 420 nm emission filter for the cells treated with ©1. The same procedure was followed in the case of a human retina obtained from a patient who underwent ocular evisceration because of a painful terminal glaucoma. Once the retina was removed from the eye, it was placed in DMEM containing 10% of FBS, 1% penicillin/streptomycin, and 1% glutamate and and cut into several pieces. Each piece was placed on a slide fluorescent microscope, and the incubation procedure described above was followed. Consent permission was obtained from the patient, and the procedure was authorized by the Ethical Committee for Clinical Research of the Xunta de Galicia.

For *in vivo* experiments the animals were anesthetized by intraperitoneal injection of 4% chloral hydrate (400 mg/kg body weight), and a drop of tropicamide solution (10 mg mL^{-1}) was instilled on the eyes to produce mydriasis. The right femoral vein was cannulated, and 0.1 mL of a solution of caged DAPI (©1, 5.0 μM in PBS) was injected. Then, the right eye was transpupillary irradiated (λ = 300 to 375 nm). Finally, the animals were sacrificed by cervical dislocation, the eyes were enucleated, and the retinas mechanically dissected out were mounted and viewed in the fluorescence microscope. All experiments involving animals were conducted according to the Bioethical Committee of our institution and adhered to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis, fluorescence spectroscopy, and further experiments with cells. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: joseluis.mascarenas@usc.es; eugenio.vazquez@usc.es.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are thankful for support given by the Spanish grants SAF2007-61015, SAF2010-20822-C02, CTQ2009-14431/BQU, Consolider Ingenio 2010 CSD2007-00006, and the Xunta de Galicia INCITE09 209 084PR, GRC2010/12, PGIDIT08CSA-047209PR. M.I.S. thanks the Spanish Ministry of Education for FPU Ph.D. fellowships.

■ REFERENCES

(1) Praveen, B. S., Reddy, S., Sonhdi, S. M., and Lown, J. W. (1999) Synthetic DNA minor groove-binding drugs. *Pharmacol.Ther. 84*, 1−111.

(2) Boer, D. R., Canals, A, and Coll, M. (2009) DNA-binding drugs caught in action: the latest 3D pictures of drug-DNA complexes. *J. Chem. Soc., Dalton Trans. 3*, 399−414.

(3) Strekowski, L., and Wilson, B. (2007) Noncovalent interactions with DNA: an overview. *Mutat. Res. 623*, 3−13.

(4) Pazos, E., Mosquera, J., Vázquez, M. E., and Mascarenas, J. L. (2011) DNA recognition by synthetic constructs. *ChemBioChem 12*, 1958−1973.

(5) Vázquez, M. E., Caamaño, A., and Mascareñas, J. L. (2003) From transcription factors to designed sequence-specific DNA-binding peptides. *Chem. Soc. Rev. 32*, 338−349.

(6) Deiters, A. (2010) Principles and applications of the photochemical control of cellular processes. *ChemBioChem 11*, 47−53.

(7) Lee, H.-M., Larson, D. R., and Lawrence, D. S. (2009) Illuminating the chemistry of life: Design, synthesis, and applications of "caged" and related photoresponsive compounds. *ACS Chem. Biol. 4*, 409−427.

(8) Caamaño, A. M., Vázquez, M. E., Martínez-Costas, J., Castedo, L., and Mascarenas, J. L. (2000) A light-modulated sequence-specific DNA-binding peptide. *Angew.Chem. Int. Ed. 39*, 3104−3107.

(9) Prasad, P. N. *Introduction to Biophotonics*, 1st ed.; Wiley-Interscience: New York, 2003.

(10) Wilson, W. D., Tanious, F. A., Barton, H. J., Jones, R. L., Fox, K., Wydra, R. L., and Strekowski, L. (1990) DNA sequence dependent binding modes of 4′,6-Diamidino-2-phenylindole (DAPI). *Biochemistry 29*, 8452−8461.

(11) Waring, M. J. (1965) Complex formation between ethidium bromide and nucleic acids. *J. Mol. Biol. 13*, 269−282.

(12) LePecq, J. B., and Paoletti, C. (1967) A fluorescent complex between ethidium bromide and nucleic acids. *J. Mol. Biol. 27*, 87−106.

(13) Vázquez, O., Sánchez, M. I., Martínez-Costas, J., Vázquez, M. E., and Mascareñas, J. L. (2010) Bis-4-aminobenzamidines: Versatile, fluorogenic A/T-selective dsDNA binders. *Org. Lett. 12*, 216−219.

(14) Vázquez, O., Sánchez, M. I., Mascareñas, J. L., and Vázquez, M. E. (2010) dsDNA-triggered energy transfer and lanthanide sensitization processes. *Chem. Commun. 46*, 5518−5520.

(15) Sánchez, M. I., Vázquez, O., Vazquez, M. E., and Mascareñas, J. L. (2011) Light-controlled DNA binding of bisbenzamidines. *Chem. Commun. 47*, 11107−11109.

(16) Yasumura, Y., and Kawakita, Y. (1963) Studies on SV40 in tissue culture - Preliminary step for cancer research "*in vitro*". *Nihon Rinsho 21*, 1201−1215.

(17) Bhal, S. K., Kassam, K., Peirson, I. G., and Pearl, G. M. (2007) The rule of five revisited: applying LogD in place of LogP in drug-likeness filters. *Mol. Pharmaceutics 4*, 556−560.

(18) Lusk, C. P., Blobel, G., and King, M. C. (2007) Nuclear pores allow small molecules (< 5 kDa) to freely diffuse across the nuclear envelop. Highway to the inner nuclear membrane: rules for the road. *Nat. Rev. Mol. Cell Biol. 8*, 414−420.

(19) Cunha-Vaz, J. G. (1997) The blood-ocular barriers: past, present, and future. *Doc. Ophthalmol. 93*, 149−157.

(20) Janoira, K. G., Gunda, S., Boddu, S. H., and Mitra, A. K. (2007) Novel approaches to retinal drug delivery. *Expert Opin. Drug Delivery 4*, 371−388.

(21) Thrimawithana, T. R, Young, S., Bunt, C. R., Green, C., and Alany, R. G. (2011) Drug delivery to the posterior segment of the eye. *Drug Discovery Today 16*, 270−277.

(22) Gomez-Ulla., F., Gonzalez, F., and Torreiro, M. G. (1998) Diode laser photocoagulation in idiopaticpolypoidalvasculopathy. *Retina 18*, 481−483.