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ARTICLE

o-Nitrobenzyl photoremovable groups with fluorescent uncaging report.

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o-Nitrobenzyl (*o*-NB) derivatives are the most widely applied photoremovable groups for the study of dynamic biological processes. By introducing different substituents to the benzylic position we were able to generate a fluorescent signal upon irradiation. This signal originates from the formation of nitrosoketone by-product able to achieve a keto-enol tautomerism leading to pi-conjugated α-hydroxystilbene derivatives. Those latter *o*-NB caging groups are able to directly monitor the uncaging event by the release of a detectable fluorescent side-product.

Introduction

Biological processes are very complex phenomena ruled by series of precise spatio-temporal events. To reveal intimate mechanism of these phenomena, cellular activity needs to be precisely controlled and tuned with the help of orthogonal tools. During the last decade, light has become one of the major orthogonal triggers[1-4] which was initially developed for neuroscience[1-6] and used today in many fields of biology, such as genetics or embryology.[1-4,7] Chemical photo-triggers provide the capability to rapidly cause the initiation of wide range of dynamic biological processes. This latter strategy uses light irradiation to induce a photolytical reaction, leading to the release of chemically or biologically active compounds. During the last two decades, the challenge was to overcome the dilemma that only high energy light can induce photochemical reactions. One strategy to lower phototoxicity within the domain of one-photon excitation process is to tailor the caging groups with extended π-conjugation and introduce heteroatoms and functional groups in the ring system so that larger dipole change can be generated when being excited.[1-4, 8-9]

Therefore, blue light sensitive photoremovable groups have been reported in coumarin,[10-12] cinnamate[13-18] orthonitrophenethyl[19-24] and orthonitrobenzyl[25-27] series. The *o*-nitrobenzyl (*o*-NB) derivatives have been the most widely applied photoremovable protecting groups (PPG) for the study of dynamic biological processes.[1-4,8-9] However, this type of PPGs and the uncaging secondary product exhibit similar weak brightness leading to light induced triggering of the studied responses without the possibility of quantification of the biological effector delivery.

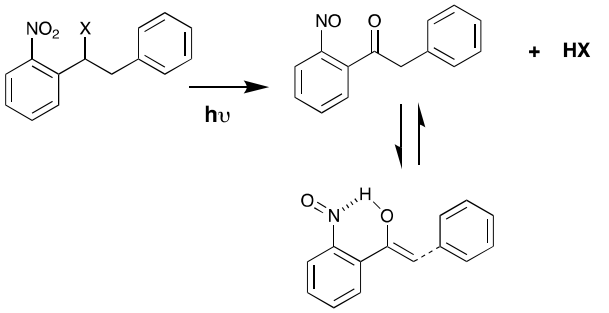
It would be very advantageous to monitor the uncaging event, for example, by the emergence of a fluorescent signal (e.g. Optical reporting). Except for cinnamate[13-18] type and thiochromone-type[28-31] photoremovable groups, the development of optical reporters of uncaging has only attracted little attention.[32,33] Interestingly, these two last PPGs have been designed to release a fluorophore as a side product.[13-18,28-31] In this work, we propose a new caging group designed to allow direct monitoring of the uncaging event by the release of an easily detectable fluorescent side product in the *o*-NB series by using 1-(2-nitrophenyl)-2-phenylethan-1-ol PPGs.

Results and Discussion

Design.

Based on the photolytical release mechanism of the *o*-NB PPG leading to the formation of a nitrosoketone derivative,[9] we decided to introduce, benzyl substituents in the benzylic position of this class of PPGs. This new class of PPGs should lead to the formation of a nitrosoketone derivative able to achieve a keto-enol tautomerism to generate a conjugated α-hydroxystilbene derivative.

We postulate that this later conjugated compound could lead to a fluorescent chromophore (Scheme 1).



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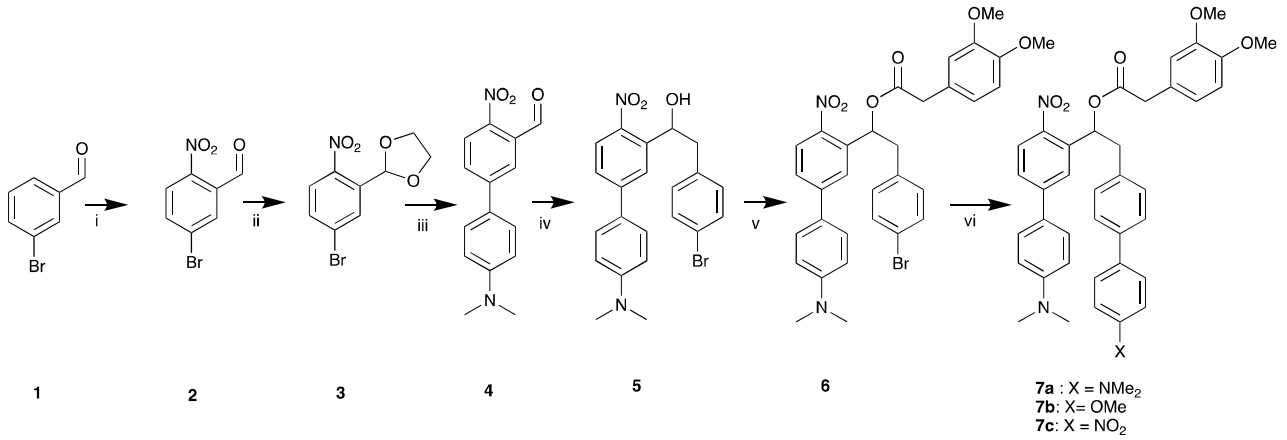
Scheme 1: Proposed photolytic reaction for aryl substituted (1-(2-nitrophenyl) ethyl) derivatives



Scheme 2: Synthesis of substituted 4'-nitro-[1,1'-biphenyl]-4-amine *o*-NB at the benzylic position.

i) HNO_3/H_2SO_4, 0°C, 3h, 70% ii) ethylene glycol, toluene, 165°C, 4h, 100% iii) 4-(dimethylamino)phenyl boronic acid, K_2CO_3, Bu_4NBr, $Pd(OAc)_2$, ethanol/water, 160°C, 15 min, *p*-TsOH, acetonitrile/water/dichloromethane, 80°C, 3h, 88% iv) bromobenzylzinc bromide, THF, -78°C, overnight, 80% v) 2-(3,4-dimethoxyphenyl) acetic acid, DMAP, diisopropylcarbodiimide, dichloromethane, 0°C, 3h, 68% vi) K_2CO_3, ethanol/water/toluene, $Pd(PPh_3)_4$, 80°C, 45 min for **7a** dimethylamino phenyl boronic acid, 55% , for **7b** 4-methoxy phenyl boronic acid, 46%, for **7c** 4-nitro phenyl boronic acid, 58%

To achieve our goal, we selected blue light sensitive chromophore in the *o*-NB series. The N, N-dimethyl-4'-nitro-[1,1'-biphenyl]-4-amine core was selected since this chromophore showed long wavelength absorption (around 400 nm) induced by the introduction of electron-donating groups to promote the internal charge transfer (ICT) and good two-photon absorption cross-sections induced by a push-pull donor-acceptor biphenyl system.

Synthesis.

The substituted *o*-NB derivatives based on a N, N-dimethyl-4'-nitro-[1,1'-biphenyl]-4-amine core were synthesized following the sequence of reactions shown in Scheme 2. Commercially available 3-bromobenzyladehyde **1** was nitrated in *ortho* position using a mixture of concentrated nitric and sulfuric acids leading to 5-bromo-2-nitrobenzaldehyde **2** with 70 % yield. The aldehyde function of this latter was protected, using ethylene glycol, leading quantitatively to the dioxolane **3**. The biphenyl derivative **4** was obtained in 75 % yield after a Suzuki-Miyaura cross-coupling reaction[34] between the dioxolane **3** and 4-(dimethylamino) phenylboronic acid followed by the deprotection of the dioxolane to obtain compound **4.** The 2-(4-bromophenyl)-1-(4'-(dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl) ethan-1-ol key intermediate **5** was obtained in 85 % yield by the reaction of freshly prepared 4-bromobenzyl zinc bromide[35] with aldehyde **4**. However, intermediate **5** could not be separated from 1,2-bis(4-bromophenyl) ethane obtained by homocoupling as a side product at this step. 3,4-Dimethoxyphenylacetic acid (MPAA) was then grafted to this first *o*-NB substituted PPG **5** using N, N'-diisopropyl carbodiimide (DIC) with catalytic amount of N, N'-dimethylaminopyridine (DMAP) in order to get access to the pure ester **6** in 68 % yield. Of note, MPAA coupling was chosen in this study instead of biologically relevant acid (e.g. Glutamate, GABA, …) to easily quantify the uncaging efficacy by HPLC analysis. Finally, the bromoaryl derivative **6** was used in order to increase the conjugation of this system with electron donating or electron withdrawing groups using Suzuki-Miyaura cross-coupling reaction leading to the formation of compounds **7a-c** in 46-58 % yields.

Photophysical and Photochemical characterization

The photophysical properties of compounds **6** and **7a-c** (100 μM) were studied in a 1/1 (v/v) mixture of phosphate buffer (pH 7.4, 0.1 mM) in acetonitrile. All compound showed a similar absorbance peak at 413 nm with absorption coefficients of 7750 $M^{-1}cm^{-1}$ due to the 4,4'-amino-nitro-biphenyl system. Interestingly each compound shows a very weak fluorescence (in the blue-green region, $\Phi < 0.2$ %) before irradiation. The photoinduced liberation of MPAA from **6** and **7a-c** was monitored by UV-Visible spectroscopy and HPLC.

Photolysis was carried out by irradiation of samples (between 60 and 135 μM) at 405 nm (using a LUMOS 43 LED source from Atlas Photonics Inc.) in an acetonitrile/phosphate buffer (pH 7.4, 0.1 mM) mixture (1/1, v/v). The isosbestic points at 360 nm and 485 nm for **6** (see supporting information), at 387 nm and 480 nm for **7a** (see supporting information) and at 370 and 495 nm for **7b** (Figure 1A) indicate that a clean photochemical reaction occurred leading to stable photoproducts. However, the absence of isosbestic points for **7c** (Figure S25) indicates a much complex photochemical behavior for this later compound. An almost quantitative (≥ 95 %) release of MPAA was measured by HPLC for the photo-conversion of **6** and **7a-c**. The hydrolytic stability was also explored by HPLC in acetonitrile /phosphate buffer (pH 7.4, 0.1 mM) mixture (1/1, v/v) at room temperature. No hydrolysis was observed after 24 h for **6** and **7a-c**. The postulated mechanism for the photo-induced liberation of a

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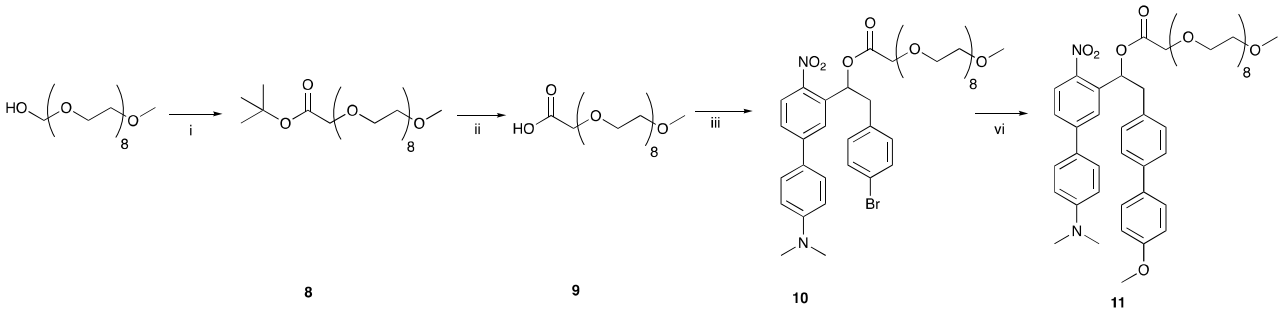
nitrosoketone derivative (adapted from the literature described mechanism) should be able to achieve a keto-enol tautomerism to generate a conjugated α-hydroxystilbene derivative stabilized by intramolecular hydrogen bond between the nitrogen from the nitroso group and the H of the enol group.

In order to characterize the formation of the nitroso photoproducts (see Scheme 1) an FT-IR study was undertaken using compound **7b** in acetonitrile. The IR spectra of this product before and after irradiation (Figure S28 and S29) show main differences in the 1300-1500 cm^{-1} region. The band at 1465 cm^{-1} which can be attributed to the 19a vibration of NO_2 group (according to Wilson notation[36]) is decreasing while a band is appearing at 1446 cm^{-1} (attributed to the 19b vibration of NO group[37]) and at 1372 cm^{-1} (which could be attributed to an enol form signature.[38]



8 9 10 11

Scheme 3: Synthesis of **11**: a PEGylated version of the *o*-nitrobiphenyl methoxy derivative.

i) NaH, *tert*-butylbromoacetate, THF, 0°C, 2h, 87%; ii) CF_3COOH/dichloromethane, 3h, room temperature, 100%; iii) **5**, DMAP, diisopropylcarbodiimide, dichloromethane, 0°C, 48%; vi) 4-methoxyphenyl boronic acid, K_2CO_3, ethanol/toluene/water, $Pd(PPh_3)_4$, 80°C, 45 min, 30%.

A weak absorption can be detected at 3175 cm^{-1} could also indicate an enol O-H bond vibration.

To confirm the keto-enol tautomerism, a ^{1}H-NMR study was undertaken using compound **6** in deuterated acetonitrile CD_3CN at respectively 267 and 405 μM. After respectively 85% and 80% cleavage, the NMR spectra nicely showed the release of the MPAA (see supporting information) together with 3 major sub-products based on the ^{1}H dimethyl-amino signals. More interestingly, in a concentration dependent manner, 3 new NMR signals together with 2 new signals were detected respectively between 4-5.2 ppm and 9.5-10 ppm (Figure S31 and S32). The singlet at 4.1 ppm is in good agreement with the CH_2 of the nitroso-keto sub-product. And the two singlets (at 4.87 ppm and 5.19 ppm) together with the two singlets (at 9.69 ppm and 9.97 ppm) are also in good agreement with the ^{1}H expected signals for respectively the Alkene =CH and the OH signals of the enols sub-products (cis and trans).

After having confirmed the formation of a keto-enol tautomerism in the uncaging sub-product, we assumed that the elongated conjugated system of the α-hydroxystilbene should induce a strong enhancement of the UV-vis absorption and emission properties. In order to study the photoinduced changes in fluorescence, emission spectra were recorded for irradiated samples (100 μM, 405 nm) in an acetonitrile/phosphate buffer (pH 7.4, 0.1 mM) mixture (1/1, v/v). For all four compounds, the fluorescence emission is very weak before photocleavage (with a maximum at 504, 489, 526 and 480 nm for **6**, **7a, 7b** and **7c** respectively). After complete photoconversion, compounds **6** and **7a** showed a moderate increase in their emission intensity (x 40 and x 32 for **6** and **7a** respectively). In contrast, for **7b** an intense red shifted (526 nm) emission was observed with more than 200 times increase in the fluorescence Intensity (Figure 1B). Of note, this later emission band seems to indicate that the increase of the conjugation length of the system and substitution with strong electron donating groups is able to generate a red-shifted emission band upon photocleavage. Finally, compound **7c** showed during irradiation a more complex fluorescent behavior presumably due to the photodegradation of the photolytical by-product leading first to an increase followed by a decrease in the fluorescence intensity (Table S4).

All photochemical and photophysical properties of compounds **6** and **7a-c** are summarized in Table 1.

Biological evaluation of the uncaging fluorescent reporting property on Hela Cells

Since compound **7b** showed the highest photoinduced fluorescence linear increase and the most red-shifted fluorescent emission upon irradiation, we decided to use this later compound to evaluate the possibility to monitor the uncaging events by fluorescence recording on cell culture. For this reason, we synthesized compound **11,** a PEGylated version of the *o*-nitrobiphenyl methoxy derivative **7b**. The product was synthesized (Scheme 3) in 4 steps starting from an octaethylene glycol monomethyl ether which was reacted with *tert*-butyl-bromoacetate to form **8** which was then deprotected to afford compound **9**. This latter was reacted with **5** followed by a Suzuki coupling to give **11** in 30% yield.

HeLa cells were incubated 5 min with a 1 μM solution of **11**. The cells were irradiated with 365 nm light from an EL6000 lamp (see supporting information) of a Leica SPE microscope. A confocal image was taken each 5 min for 15 min of continuous irradiation (the experiment was stopped after 15 min due to phototoxicity of

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the UV irradiation). A clear increase in the detected fluorescence intensity was observed upon irradiation (See Figure S33). Of note, HeLa cells incubated 35 min with a 1 μM solution of **11** showed only weak fluorescence intensity. A quantitative analysis of the fluorescence intensity was performed on 5 cells showing a linear increase in the fluorescence intensity for the first 15 min and reaching a plateau (Figure 2). Therefore, we could follow the uncaging event of this new type of *o*-nitrobenzyl PPGs by the increase of fluorescence signal on cell.

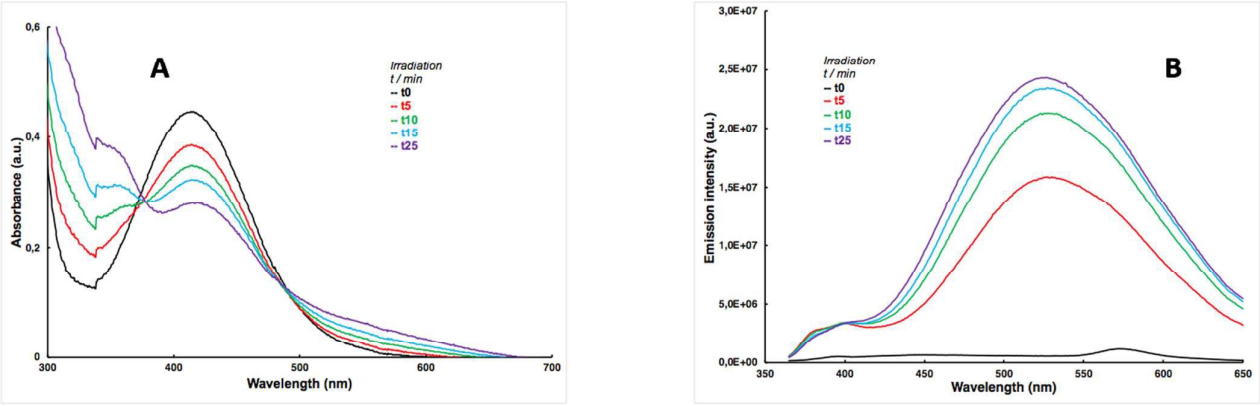

Figure 1: Variation of UV absorbance (A) and fluorescence emission (B) after irradiation at 405 nm of 55 µM solution (Acetonitrile/PBS 1:1 in vol.) of **7b**.

Compound*	λ_{em}(nm)	$I_0 x10^8$	Average % of photocleavage**	Average** I_{full} $x10^8$	I_{full}/I_0
6	504	0.5254	95	21.006	40
7a	489	4.4211	93	143.233	32
7b	526	1.0296	97	214.721	208
7c	480	0.3409	83	-***	-***

Table 1: Variation of fluorescence intensity ratio and emission wavelength of derivatives **6** and **7a-c.**

* 100µM solutions, ** Average of 3 separate irradiation times, *** Not calculated due to the high bleaching of the sub-product.

Conclusions

There is a sizeable need for photoremovable groups able to generate an easy way to quantify their side-products, in particular to be able to monitor in real time the light induced concentration jump of a given biological effector. This leads us to synthesize a new class of *o*-nitrobenzyl photoremovable groups able to generate a fluorescent side-product. Those latter compounds were designed in order to produce after photoreaction a nitrosoketone by-product able to achieve a keto-enol tautomerism leading to a conjugated α-hydroxystilbene product. A 1,2-addition of a the Bromoaryl organozinc halides to the 4'-(dimethylamino)-4-nitro-[1,1'-biphenyl]-3-carbaldehyde followed by Miyaura-Suzuki cross coupling reactions were used in order to synthesize biphenyl substituted (1-(2-nitrophenyl) ethyl) photoremovable groups. Interestingly each synthesized *o*-NB PPG shows a very weak fluorescence signal before irradiation. To be able to quantify the photorelease of carboxylic acid functions a chromophoric 3,4-dimethoxyphenylacetic acid was coupled to our *o*-NB PPGs. Except for the *p*-nitrobiphenyl derivate **7c**, an almost quantitative (≥ 93%) release of MPAA was measured by HPLC after complete photo-conversion for each photolytical precursor of MPAA. More interestingly, all those compounds showed an interesting fluorescent signal increase induced by the photolytical reaction. In particular, the *p*-methoxy biphenyl derivative **7b** showed a 200 time increase in the fluorescent green (526 nm) emission intensity after full photocleavage. Therefore, a water-soluble version of this later compound has been successfully used for *in vitro* cell imaging and real time monitoring of the uncaging event. Our future studies will focus on the use of this strategy to the development of more efficient visible light sensitive photoremovable groups in the *o*-NB serie[8] to be able to follow the uncaging events simultaneously by fluorescence and with a physiological event (for example induced by the photorelease of a neurotransmitter).

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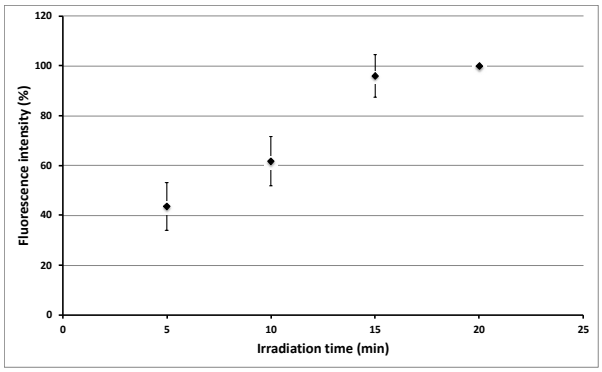

Figure 2: Fluorescence increase in function of irradiation time observed for Hela cells treated with 1 μM of 11.

Experimental

Nitro-3-bromo-benzaldehyde (2):

3-Bromobenzaldehyde (16.21 mmol, 0.35 mL) was dissolved in 15 mL of 98 % H_2SO_4. To this mixture, HNO_3 (52 mmol, 3.6 mL) was added dropwise under vigorous stirring at 0°C for 1h then at room temperature for 2h. The mixture was poured into ice water and extracted with ethyl acetate, the combined organic phases were dehydrated with $MgSO_4$ the crude product was purified over SiO_2 column using heptane/ethyl acetate (9/1) as eluent. The target compound **2** was isolated as pale-yellow crystals (2.6 g, 70 %, R_f = 0.31).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.41 (s, 1 H), 8.06 (s, 1H), 8.03 (d, ^{3}J(H,H) = 8.6 Hz, 1H), 7.88 (dd, ^{3}J(H,H) = 8.6 Hz, ^{4}J(H,H) = 2.2 Hz, 1H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 186.9, 148.2, 136.6, 132.8, 132.7, 129.7, 126.3 ppm.

MS(ESI): $[M+H]^+$ ($C_7H_5BrNO_3^+$) m/z Calcd: 229.93, m/z found: 229.82.

2-(5-Bromo-2-nitrophenyl)-1,3-dioxolane (3):

In 50 mL of anhydrous toluene, **2** (655 mg, 2.84 mmol) was dissolved and ethylene glycol (0.2 mL, 3.41 mmol) was added to the mixture with *p*-toluene sulfonic acid (55 mg, 0.284 mmol). The mixture was heated under reflux using a Dean-Stark apparatus. After 4h the reaction was done (monitored by TLC) and the solvent was evaporated. The mixture was extracted 3 times with dichloromethane and washed with water giving the target compound **3** as a light brown solid. (780 mg, 100 %, R_f = 0.52 AcOEt/heptane 1/9 in vol.).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.94 (s, 1H), 7.81 (d, ^{3}J(H,H) = 9 Hz, 1H), 7.63 (d, ^{3}J(H,H) = 9 Hz, ^{4}J(H,H) = 2.2 Hz, 1H), 6.48 (s, 1H), 4.05 (m, 4H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.5, 135.3, 132.7, 130.9, 127.9, 126.1, 99.1, 65.4 ppm.

MS(ESI): $[M+H]^+$ ($C_9H_8BrNO_4^+$) m/z Calcd: 273.96, m/z found: 273.84.

3'-(1,3-Dioxolan-2-yl)-N,N-dimethyl-4'-nitro-[1,1'-biphenyl]-4-amine:

In a microwave vial, 365 mg of **3** (1.33 mmol) with 264 mg of 4-(dimethyl amino) phenyl boronic acid (1.6 mmol), potassium carbonate (3.6 mmol) and tetrabutyl ammonium bromide (1.33 mmol) were dissolved in 13 mL of ethanol and 6.5 mL of water (2/1 v/v). The mixture was degassed 2 times (freeze-thaw cycles) then $Pd(OAc)_2$ (30 mg, 0.133 mmol) was added and the mixture was degassed 1 more time before heating under microwave radiations at 160°C for 15 minutes. The solvent was evaporated, and the residue was extracted with water and ethyl acetate and dried over $MgSO_4$. The solvent was evaporated under vacuum and the biphenyl acetal product was isolated as reddish-brown solid (418 mg, 85 %, R_f = 0.46 AcOEt/heptane 1/9 in vol.). The yield obtained is estimated since the side product (15 % homocoupling of boronic acid from ^{1}H NMR integration) has the same R_f as the target product, so at this step the product was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (d, ^{3}J(H,H) = 7.6 Hz, 1H), 7.97 (s, 1H), 7.63 (d, ^{3}J(H,H) = 9.6 Hz, ^{4}J(H,H) = 2.2 Hz, 1H), 7.56 (d, ^{3}J(H,H) = 8.4 Hz, 2H), 6.79 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 6.60 (s, 1H), 4.07 (m, 4H), 3.03 (s, 6H) ppm.

MS(ESI): $[M+H]^+$ ($C_{17}H_{19}N_2O_4^+$) m/z Calcd: 315.13, m/z found: 315.05.

4'-(Dimethylamino)-4-nitro-[1,1'-biphenyl]-3-carbaldehyde (4):

A solution of *p*-toluenesulfonic acid (2.61 g, 13.75 mmol) in acetonitrile (20 mL) and water (7 mL) was added to a solution of biphenyl aldehyde (432 mg, 1.375 mmol) in dichloromethane (8 mL). The mixture was then stirred at 80°C under reflux for 3h (monitored by TLC). After cooling to room temperature, the solvent was evaporated, and the residue was extracted with dichloromethane and washed with water. The crude product was purified over column to afford the target product **4** as a bright red solid (297 mg, 88 %, R_f = 0.3 AcOEt/heptane 15/85 in vol.).

^{1}H NMR (400 MHz, $CDCl_3$): δ =10.53 (s, 1H), 8.17 (d, ^{3}J(H,H) = 8 Hz, 1H), 8.07 (s, 1H), 7.86 (d, ^{3}J(H,H) = 7.2 Hz, ^{4}J(H,H) = 2.2 Hz, 1H), 7.6 (d, ^{3}J(H,H) = 8 Hz, 2H), 6.79 (d, ^{3}J(H,H) = 9.2 Hz, 2H), 3.03 (s, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 189.1, 151.2, 147.3, 146.3, 132.5, 129.3, 128.1, 125.9, 125.5, 124.5, 124.3, 112.4, 40.2 ppm.

MS(ESI): $[M+H]^+$ ($C_{15}H_{15}N_2O_3^+$) m/z Calcd: 271.01, m/z found: 270.99.

4-Bromobenzyl zinc bromide: Preparation:

LiCl (170 mg, 4 mmol) was added to a microwave reaction vial and dried with a flame for few minutes in the open air. This vial was left to cool down in a desiccator under vacuum. Air was used to back pressurize the desiccator and Zn powder (265 mg, 4 mmol) was added. The vial was heated again on a flame for few minutes in open air and left to cool down in a desiccator under vacuum, argon was added to back pressurize the desiccator. A stirring bar was added, and the vial was sealed, 3 mL of

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anhydrous THF was injected via the septum followed by 1,2-dibromoethane (10 μL, 0.1 mmol). The mixture was heated in a microwave oven at 85°C for 5 minutes. TMSCl (2.5 μL, 0.02 mmol) was added to the suspension and once again it was heated at 85°C for 5 minutes. 4-Bromobenzyl bromide (500 mg, 2 mmol) was then added as a solution in 2 mL THF and the vial was heated for 1h at 70°C. Iodometric titration indicated that the obtained solution of 4-bromobenzyl zinc bromide had a concentration of 0.4 M.

2-(4-Bromophenyl)-1-(4'-(dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl)ethan-1-ol (5):

In 10 mL of anhydrous THF, **4** (130 mg, 0.48 mmol) was dissolved. At -78°C, the solution of 4-bromobenzyl zinc bromide (3.6 mL, 1.44 mmol) was added dropwise and the mixture was stirred for 1h at -78°C and left to warm up to room temperature and stirred overnight. The reaction was quenched with 10% HCl and extracted with water and ethyl acetate and the crude compound was purified on column with heptane/AcOEt 85/15 in vol. as eluent to afford **5** as orange-red paste (210 mg, 80 %, R_f = 0.32 AcOEt/heptane 15/85 in vol.).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (d, ^{3}J(H,H) = 8 Hz, 1H), 8.01 (s, 1H), 7.59 (d, ^{3}J(H,H) = 9.6 Hz, ^{4}J(H,H) = 2.2 Hz, 1H), 7.55 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 7.48 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 7.23 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 6.79 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 5.59 (d, ^{3}J(H,H) = 10 Hz, 1H), 3.26 (d, ^{3}J(H,H) = 12.4 Hz, 1H), 3.03 (s, 6H), 2.8-2.86 (dd, ^{2}J(H,H) = 23, ^{3}J(H,H)= 13.6, 1H) ppm.

HRMS(ESI): $[M+H]^+$ ($C_{22}H_{22}N_2O_3^+$) m/z Calcd: 441.0703, m/z found: 441.0177.

2-(4-Bromophenyl)-1-(4'-(dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl)ethyl-2-(3,4-dimethoxyphenyl)acetate (6):

In 25 mL anhydrous dichloromethane, compound **5** (450 mg, 1.02 mmol) was dissolved and 2-(3,4-dimethoxyphenyl) acetic acid (300 mg, 1.53 mmol) was added along with 4-dimethyl aminopyridine DMAP (7 mg, 0.051 mmol) under argon. At 0°C, N, N'-diisopropyl carbodiimide DIC (0.24 mL, 1.53 mmol) was added dropwise and the reaction was stirred for 1h at 0°C, then for 2h at room temperature. The reaction was monitored by HPLC. The mixture was filtered on a glass-frit funnel and the filtrate was extracted with dichloromethane and water. To remove diisopropyl urea salts, the product is dissolved in cold acetonitrile. The crude product was purified over column with silica gel using 20/80 AcOEt/heptane in vol. as eluent to afford **6** as orange powder. (430 mg, 68 %, R_f = 0.38 AcOEt/heptane 20/80 in vol.)

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.1 (d, ^{3}J(H,H) = 8 Hz, 1H), 7.55 (d, ^{3}J(H,H) = 9.6 Hz, ^{4}J(H,H) = 2.2 Hz, 1H), 7.41 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 7.38 (s, 1H), 7.23 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 7.18 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 6.79 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 6.65-6.75 (m, 3H), 6.57 (d, ^{3}J(H,H) = 10 Hz, 1H), 3.85 (s, 3H), 3.75 (s, 3H), 3.52 (s, 2H), 3.32 (d, ^{3}J(H,H) = 12.4 Hz, 1H), 3.16 (s, 6H), 2.9-3.01 (dd, ^{2}J(H,H) = 23 Hz, ^{3}J(H,H) = 13.6 Hz, 1H) ppm.

HMMS(ESI): $[M+H]^+$ ($C_{32}H_{32}N_2O_6Br^+$) m/z Calcd: 619.1443, m/z found: 619.1436

1-(4'-(Dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl)-2-(4'-methoxy-[1,1'-biphenyl]-4-yl)ethyl 2-(3,4-dimethoxyphenyl) acetate (7b):

In a microwave vial, 52 mg of **6** (0.084 mmol) with 15 mg of 4-methoxyphenyl boronic acid (0.1 mmol) and potassium carbonate (30 mg, 0.21 mmol) were dissolved in 2 mL of ethanol, 1 mL of water and 7 mL of toluene (2/1/7 in vol.). The mixture was degassed 2 times (freeze-thaw cycles) then $Pd(PPh_3)_4$ (5 mg, $4.2x10^{-3}$ mmol) was added and the mixture was degassed 1 more time before heating under microwave radiations at 80°C for 45 minutes. The solvents were evaporated, and the residue was extracted with ethyl acetate, washed with water and dried over $MgSO_4$. The solvent was evaporated under vacuum and the crude product was purified over column with silica gel using 40/60 AcOEt/heptane as eluent to afford product **7b** as reddish paste (25 mg, 46 %, R_f = 0.32 AcOEt/heptane 40/60 in vol.).

The same procedure was followed for the synthesis of **7a** (4-(dimethyl amino) phenyl boronic acid) yield 55% and **7c** (4-nitrophenyl boronic acid) yield 58%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (d, ^{3}J(H,H) = 8.4 Hz, 1H), 7.54 (d, ^{3}J(H,H) = 8.8 Hz, 2H), 7.52 (d, ^{3}J(H,H) = 7.6 Hz, 1H), 7.45 (d, ^{3}J(H,H) = 8.4 Hz, 2H), 7.31 (s, 1H), 7.19-7.24 (m, 4H), 6.98 (d, ^{3}J(H,H) = 9.8 Hz, 2H), 6.67-6.72 (m, 5H), 6.63 (d, ^{3}J(H,H) = 8.4 Hz, 1H), 3.86 (s, 3H), 3.77 (s, 3H), 3.70 (s, 3H), 3.52 (s, 2H), 3.39 (d, ^{3}J(H,H) = 14 Hz, 1H), 3.05-3.11 (dd, ^{2}J(H,H) = 22.8 Hz, ^{3}J(H,H) = 15.2 Hz, 1H), 3.02 (s, 6H) ppm.

HRMS(ESI): $[M+H]^+$ ($C_{39}H_{39}N_2O_7^+$) m/z Calcd: 647.2757, m/z found: 647.2767.

1-(4'-(Dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl)-2-(4'-(dimethylamino)-[1,1'-biphenyl]-4-yl)ethyl 2-(3,4 dimethoxyphenyl)acetate (7a):

This compound was obtained in 55% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.08 (d, ^{3}J(H,H)= 9.36 Hz, 1H), 7.52 (d, ^{3}J(H,H) = 9.6 Hz, ^{4}J(H,H) = 2.2 Hz, 1H), 7.46 (d, ^{3}J(H,H) = 7.6 Hz, 2H), 7.31 (s, 1H), 7.19-7.22 (m, 4H), 6.82 (d, ^{3}J(H,H) = 7.6 Hz, 2H), 6.68-6.71 (m, 5H), 6.63 (d, ^{3}J(H,H) = 8.8 Hz, 1H), 3.77 (s, 3H), 3.71 (s, 3H), 3.53 (s, 2H), 3.38 (d, ^{3}J(H,H) = 14.4 Hz, 1H), 3.05-3.11 (dd, ^{2}J(H,H) = 23.6 Hz, ^{3}J(H,H) = 13.6 Hz, 1H), 3.01 (s, 6H), 3.00 (s, 6H) ppm.

HRMS(ESI): $[M+H]^+$ ($C_{40}H_{42}N_3O_6^+$) m/z Calcd: 660.3073, m/z found: 660.3047.

1-(4'-(Dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl)-2-(4'-nitro-[1,1'-biphenyl]-4-yl)ethyl 2-(3,4-dimethoxyphenyl)acetate (7c):

This compound was obtained in 58% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.17 (d, ^{3}J(H,H) = 8.8 Hz, 2H), 7.96 (d, ^{3}J(H,H) = 9.6 Hz, 1H), 7.6 (d, ^{3}J(H,H) = 7.6 Hz, ^{4}J(H,H) = 2.2 Hz, 2H), 7.41 (d, ^{3}J(H,H) = 8.8 Hz, 1H), 7.38 (d, ^{3}J(H,H) = 8.8 Hz, 2H), 7.18 (d, ^{3}J(H,H) = 8 Hz, 2H), 7.13 (s, 1H), 7.09 (d, ^{3}J(H,H) = 6.8

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Hz, 2H), 6.56-6.63 (m, 5H), 6.54 (d, ^{3}J(H,H) = 8.4 Hz, 1H), 3.67 (s, 3H), 3.58 (s, 3H), 3.43 (s, 2H), 3.28 (d, ^{3}J(H,H) = 13.2 Hz, 1H), 2.97-3.02 (dd, ^{2}J(H,H) = 23.6 Hz, ^{3}J(H,H) = 13.6 Hz, 1H), 2.90 (s, 6H) ppm.

HRMS(ESI): $[M+H]^+$ ($C_{38}H_{36}N_3O_8^+$) m/z Calcd: 662.2502, m/z found: 662.2502.

***Tert*-butyl 2-(2-methoxyoctaethoxy)acetate (8):**

To a stirred solution of octaethylene glycol monomethyl ester (250 mg, 0.65 mmol) in anhydrous THF (8 mL) was added NaH (40 mg, 1.62 mmol, 60 % dispersion in mineral oil) at 0°C. The solution was stirred for 30 minutes at 0°C, and *tertio*-butyl bromoacetate (0.18 mL, 1.17 mmol) was added dropwise over 2 minutes. The resulting solution was allowed to warm to room temperature and stirred for 2h. The reaction was quenched by a saturated solution of NH_4Cl and the aqueous phase was washed three times with dichloromethane and the combined organic extracts were dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was purified by a silica gel column chromatography using 100 % AcOEt then 98/2 AcOEt/MeOH (in vol.) as eluent to afford **8** (282 mg, 87 %, R_f = 0.32 AcOEt/MeOH 98/2 in vol.) as a yellowish oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.01 (s, 2H), 3.62-3.72 (m, 30H), 3.52-3.56 (m, 2H), 3.37 (s, 3H), 1.46 (s, 9H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.3, 83.5, 71.6, 70.1, 69.5, 68.1, 56.9, 31.7 ppm.

2-(2-Methoxyoctaethoxy)acetic acid (9):

272 mg of **8** was dissolved in 3 mL of CH_2Cl_2 and 1.5 mL of CF_3COOH (2/1 in vol.) and stirred for 3h. The solvent was evaporated and the remaining CF_3COOH was co-evaporated 3 times with CH_2Cl_2 to afford **9** in quantitative yield (240 mg, R_f = 0.33 AcOEt/MeOH 98/2 in vol.).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.1 (broad s, 1H), 4.17 (s, 2H), 3.62-3.77 (m, 30H), 3.54-3.58 (m, 2H), 3.38 (s, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3, 71.6, 70.1, 69.5, 68.1, 67.5, 56.9 ppm.

2-(4-Bromophenyl)-1-(4'-(dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl)ethyl-2-(2-methoxyoctaethoxy)acetate (10):

In 10 mL anhydrous dichloromethane, compound **5** (210 mg, 0.47 mmol) was dissolved and **9** (306 mg, 0.71 mmol) was added along with N, N'-dimethylaminopyridine DMAP (7 mg, 0.026 mmol) under argon. At 0°C, N, N'-diisopropyl carbodiimide DIC (0.11 mL, 0.71 mmol) was added dropwise and the reaction was stirred for 1h at 0°C, then for 2h at room temperature. The reaction was monitored by HPLC. The mixture was filtered on a glass-frit funnel and the filtrate was extracted with dichloromethane and water. To remove diisopropyl urea salts, the product is dissolved in cold acetonitrile. The crude product was purified over column with silica gel using 100% AcOEt then 98/2 AcOEt/MeOH in vol. as eluent to afford **10** (194 mg, 48%, R_f = 0.38 AcOEt/MeOH 98/2 in vol.) as an orange paste.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (d, ^{3}J(H,H) = 9.2 Hz, 1H), 7.54 (s, 1H), 7.38-7.48 (m, 4H), 7.14-7.23 (m, 3H), 6.76 (d, ^{3}J(H,H) = 7.6 Hz, 2H), 6.63 (d, ^{3}J(H,H) = 8.8 Hz, 1H), 4.06 (s, 2H), 3.49-3.72 (m, 32H), 3.34 (s, 3H), 3.31 (d, ^{2}J(H,H) = 18.6 Hz, 1H), 3.02-3.07 (dd, ^{2}J(H,H)= 21.3 Hz, ^{3}J(H,H)= 13.6 Hz, 1H), 3.01 (s, 6H) ppm.

MS(ESI): $[M+H]^+$ ($C_{41}H_{58}N_2O_{13}^+$) m/z Calcd: 865.30, m/z found: 865.43.

1-(4'-(Dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl)-2-(4'-methoxy-[1,1'-biphenyl]-4-yl)ethyl 2-(2-methoxyoctaethoxy)acetate (11):

In a microwave vial, 115 mg of **10** (0.135 mmol) with 25 mg of 4-methoxyphenyl boronic acid (0.162 mmol) and potassium carbonate (50 mg, 0.34 mmol) were dissolved in 3 mL of ethanol and 1.5 mL of water and 10 mL of toluene (2/1/7 in vol.). The mixture was degassed 2 times (freeze-thaw cycles) then $Pd(PPh_3)_4$ (8 mg, $6.75x10^{-3}$ mmol) was added and the mixture was degassed 1 more time before heating under microwave radiations at 80°C for 45 minutes. The solvent was evaporated, and the residue was extracted with water and dichloromethane and dried over $MgSO_4$. The solvent was evaporated under vacuum and the residue was purified over silica gel column to afford the product **11** as reddish paste (36 mg, 30%, R_f = 0.37 AcOEt/MeOH 98/2 in vol.).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (d, ^{3}J(H,H) = 8 Hz, 1H), 7.57 (dd, ^{3}J(H,H) = 10.4 Hz, ^{4}J(H,H) = 2.2 Hz, 1H), 7.48-7.53 (m, 5H), 7.40 (d, ^{3}J(H,H) = 9.6 Hz, 2H), 7.32 (d, ^{3}J(H,H) = 7.6 Hz, 2H), 6.97 (d, ^{3}J(H,H) = 7.6 Hz, 2H), 6.72-6.75 (m, 3H), 4.11 (s, 2H), 3.85 (s, 3H), 3.75 (s, 3H), 3.51-3.66 (m, 32H), 3.44 (d, ^{3}J(H,H) = 14 Hz, 1H), 3.12-3.19 (dd, ^{2}J(H,H)= 22 Hz, ^{3}J(H,H) = 12.7 Hz, 1H), 3.01 (s, 6H) ppm.

MS(ESI): $[M+H]^+$ ($C_{48}H_{65}N_2O_{14}^+$) m/z Calcd: 893.44, m/z found: 893.57.

Conflicts of interest

There are no conflicts to declare.

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***o*-Nitrobenzyl photoremovable groups able to generate a fluorescent uncaging side-product**.