Molecular Crystals and Liquid Crystals

ISSN: 1542-1406 (Print) 1563-5287 (Online) Journal homepage: http://www.tandfonline.com/loi/gmcl20

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To cite this article: Sebastien Piant, Alexandre Specht, Boris Zemelman, Attila Losonczy & Frédéric Bolze (2016) Two-photon sensitive photolabile protecting groups: From molecular engineering to nanostructuration, Molecular Crystals and Liquid Crystals, 627:1, 56-65, DOI: 10.1080/15421406.2015.1137119

To link to this article: http://dx.doi.org/10.1080/15421406.2015.1137119

Published online: 13 May 2016.

MOL. CRYST. LIQ. CRYST.
2016, VOL. 627, 56–65
http://dx.doi.org/10.1080/15421406.2015.1137119

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Two-photon sensitive photolabile protecting groups: From molecular engineering to nanostructuration

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ABSTRACT

General molecular engineering rules for the optimization of two-photon sensitive cages are presented and examples for nitrobenzyl, indole and nitrophenethyl platforms are highlighted. The efficiency of electron donor and acceptor groups in dipolar structures and the length of the conjugated system in the photolabile protecting group on two-photon uncaging efficiency will be discussed, as well as the emergence of symmetrical platform based on *bis*-electron donor or on quadrupolar architectures. We will then present our first results on the nanostructuration of donor-acceptor systems based on nitrophenetyl platform using diethyleneglycol or pentaerythritol cores.

KEYWORDS

Two-photon absorption; caging group; nanostructuration; neurosciences

1. Introduction

The study of dynamic biological process is the key to understand the mechanism of action of physiological events. In neuroscience or embryology, physiological pathways follow strict pattern, with a very precise spatiotemporal activation and or inhibition. To study these phenomena, tools that would be able to perturb selectively physiological process with high spatiotemporal control became necessary. Thus, photolabile protecting groups, also known as caging group, appeared 30 years ago with Kaplan's works on caged ATP [1] to reveal activation of Na:K pump. The principle of these caging groups is based on a photolabile protecting group linked by the way of a covalent bond to a physiological ligand. In this state the resulting system (photolabile protecting group-ligand, or caged ligand) is inert and can't induce a physiological response [2, 3]. After light irradiation, provoking the cleavage of the bond linking the two protagonists, the active uncaged biomolecule is released and can then induce a physiological biological response (Figure 1).

An irradiation in the near-UV, inducing one-photon excitation (OPE), is classically used to trigger this photo-release. However in biological tissues, photon scattering and absorption limit the action depth to submillimeter, and allow only a two-dimensional spatial control, the excitation occurring on the entire light pathway. These two limitations can be overcome by the use of a non-linear phenomenon, two-photon excitation (TPE). Indeed, this non-linear

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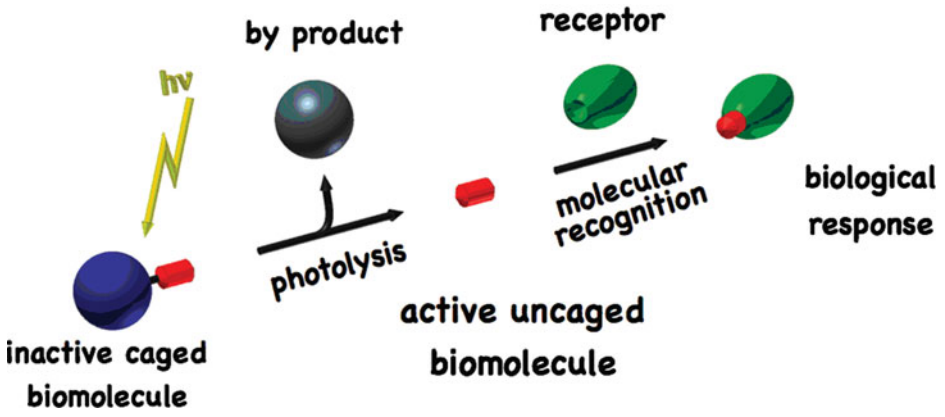


Figure 1. Principle of uncaging.

absorption technique allows a fine 3D spatial control of the triggering. The probability of the two-photon excitation is proportional to the squared light intensity, and thus occurs only at the focal point of an optical system with a pulsed laser as light source, allowing high peak power for each pulse, while offering a low average power [4] (Figure 2). In addition, the wavelength of the light used for TPE is twice the one used in OPE, and is typically ranging in the IR region instead of in the UV one. The IR light is in the living tissue transparency window (700–1100 nm), limiting absorption and scattering by diffusing living tissues; these combined two phenomena providing deeper penetration in living tissue and allowing excitation as deep as few mm in optimized conditions [5, 6].

The overall efficiency of one photon excited cages can be express in a similar way than the brightness of a fluorophore ($\varepsilon.\Phi_{fluo}$) by the product of the molar absorption coefficient and the photochemical uncaging reaction quantum yield ($\varepsilon.\Phi_u$); alike, the two-photon uncaging action cross-section can be defined by $\delta_u = \delta_a.\Phi_u$, where ε is the molar extinction coefficient, Φ_{fluo} the fluorescence quantum yield, δ_a the two-photon absorption cross-section and Φ_u the photochemical uncaging reaction quantum yield. δ_u is generally expressed in Göpper-Mayer unit ($1GM = 10^{-50}.cm^{-4}.s.photon^{-1}.molecule^{-1}$). Classical cages were used first with TPE, but with low two-photon uncaging action cross-section [7]. To allow a wide development of this technique, the design of new photolabile protecting groups specifically engineered for TPE was necessary.

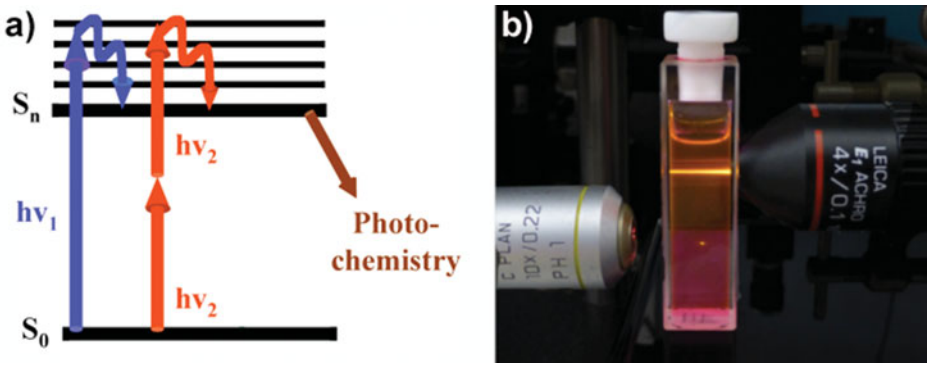


Figure 2. One vs two-photon excitation; principle (a) and application in cuvette (b): classical excitation with UV light with fluorescence along the light pathway (up) and two-photon excitation inducing fluorescence only at the focal point (down).

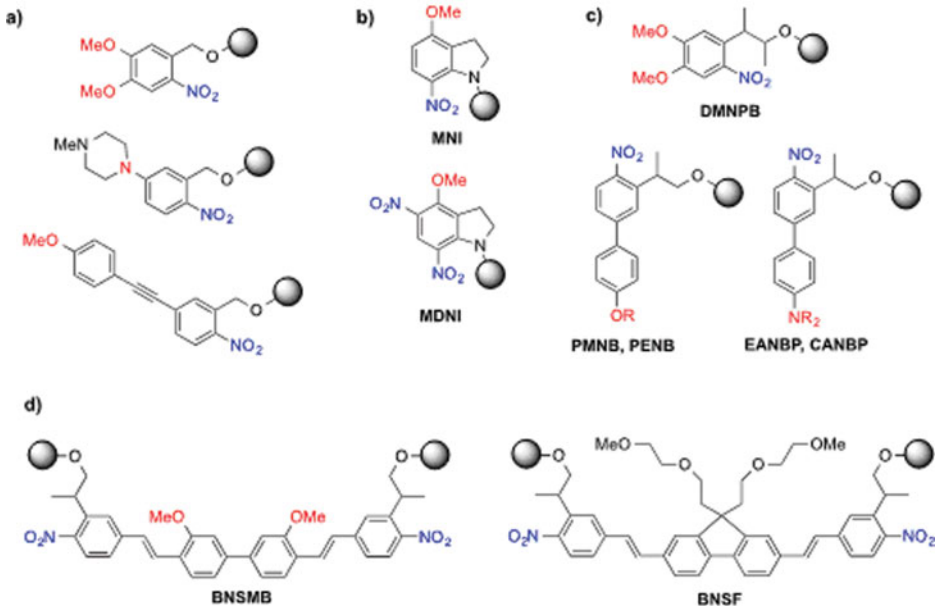
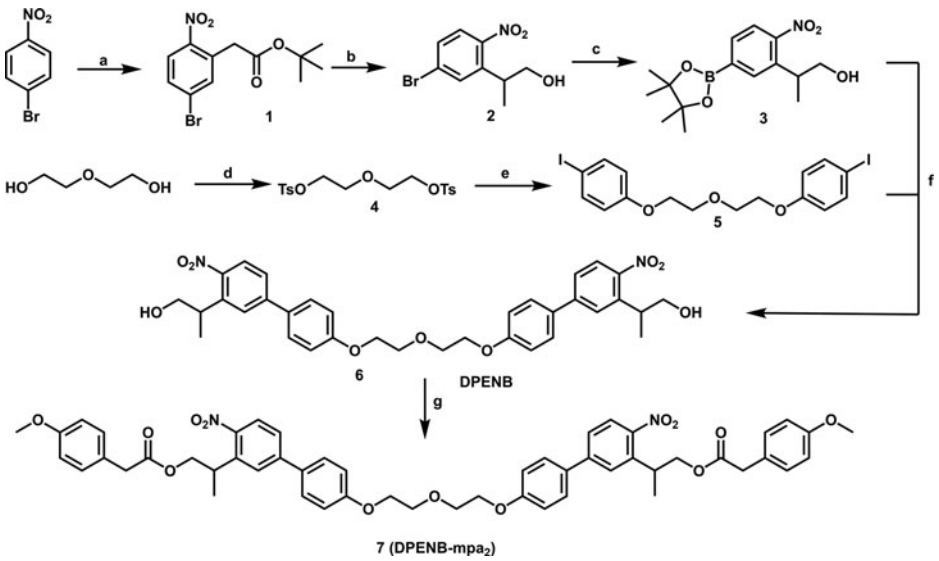


Figure 3. Formula of cages discussed in this work: dissymmetrical Donor-Acceptor architecture a) nitrobenzyl [9]; b) nitro-indole [11]; c) nitrophenetyl [10] plateforms; d) symmetrical architecture [12].

2. Results and discussion

Tsien's group described the first caged ligand with a TPE uncaging cross section $\delta_u \geq 1$GM derived from bromohydroxycoumarin [8]. Other platforms were developed for example from the *ortho*-nitrobenzyl moiety by Jullien's group [9], *ortho*-nitrophenethyl by Goeldner's team [10] and nitro-indole by Ellis-Davies's group, [11] and showed moderate two-photon uncaging action cross-sections for neurotransmitters.

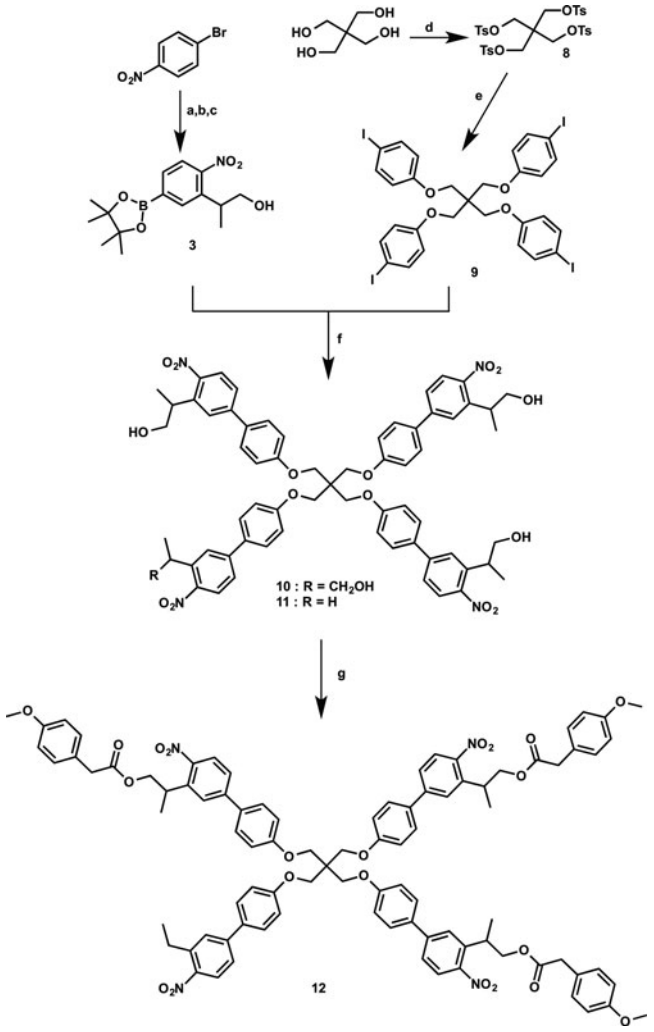
Starting from these molecules, classical molecular engineering was performed to improve their properties (Figure 3). The donor-acceptor (D-A) platform is ubiquitous in phototriggering, even for OPE cages. The modification of the electron donating or withdrawing groups efficiency yielded to MDNI for the indole platform (addition of a second nitro group) [11], and various nitrobenzyl derivatives with enhanced δ_u (with amine groups instead of methoxy one) [9]. The other key parameter in TPA is the length of the conjugated system. Thus by increasing the length of conjugation, two-photon uncaging efficiency can be improved, in the nitrobenzyl series as well as in the nitrophenetyl series. Goeldner's group particularly worked on the extension of the π system to improve two-photon absorption in nitrophenetyl series. PENB for example gave tremendous two photons uncaging parameters, 3.2 GM (740 nm) with the introduction of one supplementary phenyl ring to a classical cage and 11 GM (800 nm) for EANBP with replacement of the oxygen atom by a nitrogen atom acting as better electron donating group. Centro-symmetrical systems were also explored with quadrupolar structure (i.e. A-D-D-A) or D-D systems centered on a fluorenyl core, such as BNSF [12] which have a δ_u (800 nm) = 5 GM. However, BNS due to its extended hydrophobic conjugated π system didn't showned a good solubility in aqueous media (0.1 mM) and didn't lead to a quantitative release of neurotransmitters (120% instead of 200% for a complete release). Another way to increase the concentration gap obtained by uncaging and to modulate the pharmacokinetics of such caged molecule would be nanostructuration around dendrimeric architectures. In



Scheme 1. Synthesis of DPENB-mpa$_2$. a) Potassium *tert*-butoxide, *tert*-butylchloroacetate, DMF, 24°C, 2 h, 99%. b) 1. NaH, CH_3I, THF, 24 h RT; 2. DIBAL-H, THF, 30 min, 0°C, 67%. c) bis(pinacolato)diboron, AcOK, Pd(dppf)Cl_2, DMSO, 80°C, 15 h, 53%. d) TsCl, Et_3N, CH_3CN, RT, 24 h, 88%. e) K_2CO_3, iodophenol, CH_3CN, 100°C, 24 h, 65%. f) K_2CO_3, TBAB, Pd(OAc)$_2$, EtOH H_2O (2:1), microwave 170°C, 15 min, 38%. g) 4-methoxyphenylacetic acid, DCC, DMAP, DCM, 15 h, RT, 44%.

this way and inspired by dendrimerization of fluorophores [13], we focused our investigation on the dendrimerization of one of the phototrigger we developed in our laboratory, the PENB scaffold (Fig. 3). PENB is preferred to EANBP despite a lower δ_u because the phenolic oxygen is more easily functionalizable than the nitrogen of EANBP. First, a non-conjugated dimer was prepared to compare it to the conjugated dimer BNSF. It was developed around a diethyleneglycol central core. A convergent synthesis was used to prepare it (Scheme 1). The nitro-containing part of the molecule **3** was prepared as described in the literature [14]. To build the second part **5**, a diethyleneglycol bis-tosylate was prepared in two steps from diethyleneglycol. The diethyleneglycol chain was chosen for its solubility in water and easy functionalization. This bis-tosylate was then reacted with 4-iodophenate to obtain the central core **5**. A double-suzuki coupling was then used to link the two parts to give the photolabile protecting groups **6**. From **6**, several ligands can be linked with the caging group. 4-Methoxyphenylacetic acid (mpa) was first chosen because the efficiency of the photochemical reactions can be easily monitored by UV-vis spectroscopy and can give rapidly results. The photochemistry will be studied in another study on more relevant biologically molecule such as glutamate, but the studies are more complicated due to the necessity of derivatization for glutamate analysis.

The second system envisioned had a tetrameric structure; the same nitro-containing moiety **3** was used like for dimers, but the central core used was a pentaerythritol derivative. The synthesis pathway is similar to the dimer one, as depicted in Scheme 2. The precursors **9** was obtained in two steps from pentaerythritol. The tetra-Suzuki coupling was problematic, due to the low yield of the mono-coupling step and the many possibilities of side reactions. The desired product **10** was obtained only as traces, and the major product isolated was a deformylated analog **11**, in 11% yield. Mpa was then conjugated on the pseudo-tetramer **11**, in order to obtain **12** in 10% yield.



Scheme 2. Synthesis of TPENB-mpa$_4$: a)Potassium *tert*-butoxide, *tert*-butylchloroacetate, DMF, 24°C, 2 h, 99%. b) 1. NaH, CH_3I, THF, 24 h RT; 2. DIBAL-H, THF, 30 min, 0°C, 67%. c) bis(pinacolato)diboron, AcOK, Pd(dppf)Cl_2, DMSO, 80°C, 15 h, 53%. d) TsCl, Et_3N, CH_3CN, RT, 24 h, 30%. e) iodophenol, NaOH, DMF, 160°C, 24 h,71%. f) K_2CO_3, TBAB, Pd(OAc)$_2$, EtOH H_2O (2:1), microwave 160°C, 45 min, 13%. g) 4-methoxyphenylacetic acid, DCC, DMAP, DCM, 15 h, RT, 10%.

The photophysical and phototochemical properties of **DPENB-mpa$_2$** (**7**) and **TPENB-mpa$_3$** (**12**) were determined and are presented in table 1, as well as parent molecules (monomers) EANBP, PENB and conjugated dimer BNSF for comparison.

First, we have to notice that the dimer and the tetramer have low absorptivity. We expected additive ε, in the 20 000 $M^{-1}.cm^{-1}$ range for the dimer and around 40 000 $M^{-1}.cm^{-1}$ for the pseudo-tetramer. The hydrophobic biphenyl moiety certainly induces π-stacking and the flexibility of diethyleneglycol central core cannot circumvent this interaction. A second observation is that **TPENB-mpa$_3$** has the double of ε than **DPENB-mpa$_2$**, which suggest a π-stacking by couple, as well as a red shifted absorption of 13 nm. Unfortunately, both systems showed poor release of the desired substrate, around 80%, similar to the one of the reference monomer (PENB 90%). A hypothesis would be than after the first release of the mpa moiety

Table 1. Photochemical and photophysical properties of biphenyl caged in the 2-(o-nitrophenyl)propyl series

Compound	λ_{max} (nm)	ε (M^{-1} cm^{-1})	Expected % photorelease[a]	Experimental % photorelease
EANBP-GABA [10][b]	397	7500	/	95
BNSF-Glu [12]	415	63 960	200	130
PENB-Glu [10][b]	317	9900	/	90
(7) DPENB-mpa_2[c]	312	12 600	180	80
(12) TPENB-mpa_3[c]	335	25 000	270	70

[a]According to monomer efficiency, [b]in water, [c]in water-acetonitrile (1/1 in vol.).

and the formation of a vinylic derivative, the interaction between the two monomers is reinforced by the loss of steric hindrance due to mpa, limiting the release of the second substrate by the apparition of competing photochemistry pathways, not favorable to the release.

3. Conclusion

In summary, avoiding π-stacking is a necessity to keep the efficiency of each monomeric unit in a multi-assembly of cages, but it can be hard to rigidify the scaffold without losing in water solubility. Mpa used to determine photochemical parameters reinforce hydrophobicity and is favorable to π-stacking interaction. Coupling **DPENB** (**6**) or **TPENB** (**11**) with hydrophilic neurotransmitters, like glutamate, will create repulsive charges that could be very helpful to avoid this phenomenon. PolyAMidoAMine (PAMAM) polymers seem to be a potential candidate of a new central core. Indeed, steric hindrance and long chains could be sufficient to keep away each monomeric unit. Moreover, PAMAM have a great solubility in water and many of its derivatives and generations are commercial.

4. Materials and methods

General procedure

All chemicals were purchased from Sigma–Aldrich, Alfa Aesar or Fluka (analytical grade). An Agilent MM-ESI-ACI-SQ MSD 1200 SL spectrometer or an Agilent LC-MS Agilent RRLC 1200SL ESI QTof 6520 were used for ESI analysis. ^{1}H NMR and ^{13}C NMR were run at 300 or 400 and 75 or 100 MHz, respectively. Coupling constants (J) are quoted in Hz and chemical shifts (δ) are given in parts per million (ppm) using the residue solvent peaks as reference relative to TMS. A Phenomenex C18 column (4.6, 250 mm) was used for HPLC analysis. Absorption spectra were recorded on a UVIKON XS spectrometer. Microwave reactions were performed in Anton Paar Monowave 300.

Synthesis

***Tert*-butyl 2-(5-bromo-2-nitrophenyl)acetate (1)**: To a stirred solution of *ter*-BuOK (17.4 g, 155 mmol) in anhydrous DMF (50 mL) was added through a cannula a solution of 4-Bromonitrobenzene (5.00 g, 24.75 mmol) and *tert*-butyl chloroacetate (5.47 mL, 38.5 mmol) in DMF (50 mL) and the reaction was carried out for 2 hours under argon at room temperature. 5% HCl (50 mL) was poured into the mixture at 0°C. The mixture was then extracted with AcOEt, the extract dried over Na_2SO_4 and the combined extracts were evaporated to afford a brown oil solid (8.34 g, 100%).

RMN ^{1}H (400 MHz, $CDCl_3$): δ (ppm) = 7.99 (d, J = 8.8 Hz, 1H), 7.60 (dd, J = 1.0, 8.8 Hz, 1H), 7.51 (d, J = 1.0 Hz, 1H), 3.92 (s, 2H), 1.45 (s, 9H)

RMN ^{13}C (100 MHz, $CDCl_3$): δ (ppm) = 168.4, 136.1, 132.3, 131.4, 128.14, 126.6, 82.2, 40.7, 27.9

***Tert*-butyl 2-(5-bromo-2-nitrophenyl)propanoate** : *tert*-butyl-2-(5-bromo-2-nitrophenyl)acetate (7.74 g, 24.5 mmol) and sodium hydride (60% in oil, 1.00 g, 24.5 mmol) was dissolved in 80 mL of DMF. Methyl iodide (3.0 mL, 48.9 mmol) was then added dropwise at 0°C. The mixture was stirred at room temperature under argon for 24 h. The reaction was quenched with water and extracted with EtOAc. The extract was dried over Na_2SO_4 and the combined extracts were evaporated. The crude was purified by column chromatography (silica heptane EtOAc: 9/1 to 8/2 in vol.) to afford a yellowish solid (6.7 g, 83%).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm) = 7.83 (d, J = 8.8 Hz, 1H), 7.63 (d, J = 2.3 Hz, 1H), 7.55 (dd, J = 2.3, 8.8 Hz, 1H), 4.22 (q, J = 7.1 Hz, 1H), 1.58 (d, J = 7.1 Hz, 4H), 1.41 (s, 9H)

RMN ^{13}C (100MHz, $CDCl_3$): δ (ppm) = 171.6, 147.9, 137.7, 132.8, 130.9, 127.9, 126.3, 81.8, 27.8, 17.4.

HRMS (ESI) (m z) calcd. for [$C_{13}H_{16}BrNO_4Na$] 352.0160 found 352.01465.

***Tert*-butyl 2-(5-bromo-2-nitrophenyl)propan-1-ol (2)**: *tert*-butyl 2-(5-bromo-2-nitrophenyl)propanoate (2.0 g, 6.1 mmol) was dissolved in THF (20 mL) and stirred at 0°C. DIBAL-H 1.0 M in THF (18.2 mL, 18 mmol) was slowly added to the solution and the mixture was stirred 30 min at the same temperature. The solution was keep to 0°C and HCl 3 M (10 mL) was added dropwise. The crude solution was extract with EtOAc and purified by FLASH chromatography (silica gel heptane EtOAc, 8/2 in vol.) to obtain a dark oil (1.1 g, 67%).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm) = 7.64 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 2.4 Hz, 1H), 7.47 (dd, J = 8.4, 2.4 Hz), 3.78 (m, 2H), 3.53 (m, 1H), 1.31 (d, J = 7 Hz, 3H).

RMN ^{13}C (100MHz, $CDCl_3$): δ (ppm) = 149.4 140.5, 131.6, 130.4, 127.5, 125 .7, 67.6, 36.4, 17.4.

HRMS (ESI) (m z) calcd. for [$C_9H_{10}BrNO_3Na$] 281.9742 found 281.9724.

2-(2-Nitro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propan-1-ol (3): A mixture of *tert*-butyl 2-(5-bromo-2-nitrophenyl)propan-1-ol (800 mg, 3.08 mmol), bis(pinacolato)diboron (859 mg, 3.38 mmol), KOAc (368 mg, 3.75 mmol) and Pd(dppf)Cl_2 (169 mg, 0.23 mmol) in DMSO (40 mL) was heated to 85°C overnight. The reaction mixture was poor into 50 mL ice-water slush and extracted with ethyl acetate (3 × 100 mL) and dried over $MgSO_4$. After evaporation of solvent, crude product was purified by flash chromatography using gradient elution of Heptane / EtOAc: 9/1 to 8/2 in vol.to give 500 mg of 3 as black red oil (53%).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm) = 7.85 (d, J = 1.2 Hz, 1H), 7.74 (dd, J = 8.4, 1.2 Hz, 1H), 7.65 (d, J = 8.4 Hz, 1H), 3.80 (m, 2H), 3.41 (q, J = 6.8 Hz, 1H), 1.31–1.34 (m, 15 H).

RMN ^{13}C (100MHz, CDCl3): δ (ppm) = 162.6, 136.8, 134.4, 133.7, 133.6, 122.9, 84.5, 67.9, 36.5, 24.9, 17.6.

HRMS (ESI) (m z) calcd. for [$C_{15}H_{22}BNO_5Na$] 330.1489 found 329.1522.

Oxybis(ethane-2,1-diyl)bis(4-methylbenzenesulfonate) (4): A mixture of triethylene glycol (10.0 g, 94.2 mmol), Tosyl chloride (44.9 g, 235.6 mmol), triehtylamine (32.7 mL, 235.6 mmol) in acetonitrile (100 mL) was stirred at r.t. overnight. Saturated $NaHCO_3$ was added, the mixture was extracted with ethyl acetate (3 × 20 mL), the organic phase was combined and washed with brine, after evaporation, a white solid was obtained (34.2 g, 87.6% yield).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm) = 7.75 (d, J = 8.4 Hz, 4H), 7.32 (d, J = 8.4 Hz, 4H), 4.06 (t, J = 4.6 Hz, 4H), 3.58 (t, J = 4.6 Hz, 4H), 2.42 (s, 6H).

4,4'-((Oxybis(ethane-2,1-diyl))bis(oxy))bis(iodobenzene) (5): A mixture of iodophenol (5.00 g, 22.7 mmol) and K_2CO_3 (3.2 g, 23.7 mmol) in MeCN (24 mL) was stirred for 30 min at 90°C, followed by addition of 4 (4.5 g, 10.9 mmol) in MeCN (36 mL). The mixture was stirred for another 24 h at 90°C, before being cooled to r.t. The precipitate is dissolved in a water EtOAc mixture and extracted with EtOAc (3 × 100 mL). The extract was dried over Na_2SO_4 and the combined extracts were evaporated. We obtained a white powder (3.62 g, 65%).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm): 7.52 (d, J = 8.9 Hz, 4H), 6.67 (d, J = 8.9 Hz, 4H), 4.09 (t, J = 4.8 Hz), 3.88(t, J = 4.8 Hz, 4 H).

HRMS (ESI) (m z) calcd. for $[C_{16}H_{16}I_2O_3Na]$ 532.9087 found 532.9078.

DPENB (6): A mixture of 3 (500 mg, 1.63 mmol), 5 (332 mg, 0.65 mmol), K_2CO_3 (270 mg, 1.95 mmol), and $Pd(OAc)_2$ (catalytic, 6% mol.) in EtOH (10 mL) and water (5 mL) was heated under microwave conditions at 160°C for 30 min. Water (100 mL) was added and organics were extracted into EtOAc (200 mL). The crude extract was purified by column chromatography on silica with 3/7 Heptane EtOAc in vol., to obtain a yellow oil (100 mg, 25%).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm) = 7.81 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 2.0 Hz, 2H), 7.5 (d, J = 8.4 Hz, 4H), 7.46 (m, J = 8.4 Hz, 2.0 Hz, 2 H), 7.0(d, J = 8.4 Hz, 4H), 4.21 (t, J = 5.0 Hz, 4H), 3.96 (t, J = 5.0 Hz, 4H), 3.8 (d, J = 6.5 Hz, 4H), 3.62 (q, J = 6.5 Hz, 2H), 1.34 (d, J = 6.8 Hz, 6H).

HRMS (ESI) (m z) calcd. for $[C_{34}H_{36}N_2O_9Na]$ 639.2319 found 639.2323.

DPENB-mpa$_2$ (7): 4-methoxyphenylacetic acid (6 mg, 36 μmol) and 5 (9 mg, 15 μmol) was dissolved in dry DCM (5 mL) and cool to 0°C. Next, DCC (9 mg, 44 μmol) and DMAP (cat. 1% mol.) was added to the mixture and stirred overnight. The mixture was purified by flash chromatography (1/1, Heptane Ethyl acetate) to obtain an orange oil (5.8 mg, 43.5%).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm) = 7.82 (d, J = 8.5 Hz, 2H), 7.46 (m, 8H), 7.01 (m, 8H), 6.71 (d, J = 8.5 Hz, 4H), 4.27(m, 4H), 4.22 (t, J = 4.6 Hz, 4H), 3.98 (t, J = 4.6 Hz, 4H), 3.83 (q, J = 6.7 Hz, 2H), 3.72 (s, 6H), 3.44 (s, 3H), 1.32 (d, 6.7 Hz, 6H).

HRMS (ESI) (m z) calcd. for $[C_{52}H_{52}N_2O_{13}Na]$ 935.3367 found 935.3373.

2,2-Bis((tosyloxy)methyl)propane-1,3-diyl bis(4-methylbenzenesulfonate) (8): A mixture of pentaerytrithol (5.0 g, 36.73 mmol), tosyl chloride (42.0 g, 220.4 mmol), triethylamine (30.6 mL, 220.4 mmol) in acetonitrile (50 mL) was stirred at r.t. 48h. Saturated $NaHCO_3$ was added, the mixture was extracted with ethyl acetate (3 × 200 mL), and the organic phase was combined and evaporated. The crude was purified by flash chromatography (heptane EtOAc, 50% to 100%) to yield a yellowish powder (7.0 g, 25.3% yield).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm): 7.66 (d, J = 8.4 Hz, 8H), 7.34 (d, J = 8.4 Hz, 8H), 3.79 (s, 8H), 2.4 (s, 12H).

RMN ^{13}C (100MHz, CDCl3): δ (ppm): 145.7, 131.4, 130.2, 128.0, 65.63, 43.3, 21.7.

HRMS (ESI) (m z) calcd. for $[C_{33}H_{36}O_{12}S_4Na]$ 775.0987 found 775.0967.

4,4'-((2,2-Bis((4-iodophenoxy)methyl)propane-1,3-diyl)bis(oxy)) bis(iodobenzene) (9): A stirred mixture of pentaerythrityl tetratosylate (1.00 g, 1,33 mmol), 4-iodophenol (1.46 g, 6.64 mmol), and NaOH (0.26 g, 6.64 mmol) in DMF (20 mL) was heated at reflux for 24 h. The solution was then cooled, water (100 mL) was added, and the mixture was extracted twice with diethyl ether. The organic layers were combined, washed with water and brine, and dried

over Na_2SO_4. Removal of volatiles under reduced pressure left a residue that was purified by flash chromatography (silica, heptane (100%) to give 9 (890 mg, 0.94 mmol, 71%) as a white solid.

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm): 7.51 (d, J = 9.0 Hz, 8H), 6.65 (d, J = 9.0 Hz, 8H), 4.22 (s, 8H).

RMN ^{13}C (100MHz, $CDCl_3$): δ (ppm): 158.7, 138.5, 117.2, 83.6, 66.7, 44.9.

TPENB (11): A mixture of 9 (183 mg, 019 mmol), 3 (250 mg, 0.81 mmol), K_2CO_3 (160 mg, 1.16 mmol), tetrabutylammonium bromide (500 mg, 1.55 mmol) and $Pd(OAc)_2$ (cat. 0.03 mol%) in EtOH (10 mL) and water (5 mL) was heated under microwave conditions at 160°C for 45 min. Water (100 mL) was added and organics were extracted into EtOAc (200 mL). The crude extract was purified by column chromatography on silica with 3/7 Heptane EtOAc in vol., to obtain a brown oil (30.00 mg, 13%).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm): 7.82 (d, J = 8.5 Hz, 4H), 7.57 (bs, 4H), 7.52–7.44 (m, 12H), 7.05 (m, 8H), 4.47 (s, 4H), 4.45 (s, 4H), 3.80 (m, 8H), 3.62 (m, 4H), 1.34 (d, J = 6.8 Hz, 12H).

HRMS (ESI) (m z) calcd. for $[C_{64}H_{62}N_4O_{15}Na]$ 1149.4109 found 1149.4090.

TPENB-mpa$_3$ (12): 4-methoxyphenylacetic acid (21.5 mg, 129 μmol) and 11 (30 mg, 32.4 μmol) was dissolved in dry DCM (5 mL) and cool to 0°C. Next, DCC (16 mg, 77.7 μmol) and DMAP (cat. 1% mol) was added to the mixture and stirred overnight. The mixture was purified by flash chromatography (7/3, Heptane Ethyl acetate) to obtain 12 (4.6 mg, 10%).

RMN ^{1}H (400MHz, $CDCl_3$): δ (ppm): 7.82 (d, J = 8.4 Hz, 3H), 7.54 (d, J = 8.4 Hz, 3H), 7.46 (m, 14H), 7.04 (m, 14H), 6.71 (d, J = 8.9 Hz, 6H), 4.47 (s, 8H), 4.26 (m, 6H), 3.82 (m, 3H), 3.71 (s, 9H), 3.44 (s, 6H), 2.97 (q, J = 7.4 Hz, 2H), 1.32–1.30 (m, 12H).

HRMS (ESI) (m z) calcd. for $[C_{91}H_{86}N_4O_{21}Na]$ 1593.5682 found 1593.5639.

Quantification of mpa release

4-Methoxyphenylacetic acid (mpa) is a chromophoric moiety that absorb at 275 nm. From a benchmark curve build with several concentrations, quantity of mpa release was determined by HPLC.

Acknowledgment

HFSP is acknowledged for financial support (RGP0041/2012).

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