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PAPER

The donor–acceptor biphenyl platform: A versatile chromophore for the engineering of highly efficient two-photon sensitive photoremovable protecting groups†

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Different photoremovable protecting groups in the *o*-nitrobenzyl, phenacyl, and 2-(*o*-nitrophenyl)propyl series with a donor–acceptor biphenyl backbone, known to display excellent two-photon absorption cross-sections, were investigated in order to develop efficient two-photon sensitive photoremovable protecting groups. The 2-(*o*-nitrophenyl)propyl series was a more versatile platform to increase the two-photon sensitivity of photoremovable protecting groups, leading to the *p*-alkoxy and *p*-bisalkylamino-4-nitro-[1,1′-biphenyl]-3-yl)propyl derivatives: PENB and EANBP respectively. Those two photoremovable protecting groups are to date the best caging groups for two-photon excitation at 800 and 740 nm respectively, offering attracting perspectives in chemical biology.

Introduction

The search for probes possessing suitable properties for an efficient and controlled photolytic release of biomolecules (uncaging) has been thoroughly developed in the last few decades leading to an impressive panel of different photoremovable protecting groups.[1] The uncaging of biomolecules requires several well-defined properties which include a rapid and efficient photolytic release of the biomolecule, an irradiation wavelength which should be compatible with the biological environment (preferably UV-Visible or visible excitation wavelengths), the formed side product should not interfere with the ongoing photolytic reaction and should be non-toxic for the biological system. Lastly, the biomolecule modified by the photoremovable group should be inert to the targeted biological system and remain freely soluble in aqueous media. None of the published probes satisfy fully all these properties at once, but one has to keep in mind that the requirements for biological applications are fairly different according to their issues. A more recent outcome for the uncaging of biomolecule is to use two-photon excitation (TPE).[2] Indeed, TPE can intrinsically provide fine spatio-temporal control during a photochemical cleavage. In such a photophysical process a chromophore can reach an excited state not only by the absorption of a single photon of energy $E = h\nu$, but also by the simultaneous absorption of two photons of half energy $E' = h\nu/2$. The interaction between the electric field linked to the electromagnetic wave of the excitation light (E) and the polarisable electronic cloud of a conjugated molecule induces a charge redistribution that induces a modification of the molecular dipole moment (μ).[3] The latter is usually described as a development of increasing powers of the electric field E, as described in eqn (1):

$$\mu = \mu_0 + \alpha E + \beta E^2 + \gamma E^3 + \ldots \quad (1)$$

The static dipole moment of the molecule is μ_0, when $\alpha \cdot E$ represents the effects related to the linear polarisability. βE^2 and γE^3 are related to the first and second order molecular non-linear optical properties, respectively. Noticeably the theory shows that the two-photon absorption phenomenon is related to the imaginary part of the cubic hyperpolarisability γ. The excitation probability of a molecule P is in this case proportional to the square of the light intensity, δ_a being the two-photon absorption cross-section as in eqn (2) (The two-photon absorption cross-section δ_a is often mentioned as σ_2 in most chemical papers):

$$P = 1/2 \times \delta_a I^2 \quad (2)$$

This quadratic dependence of P *versus* I is crucial for the spatial localisation of this non-linear optical phenomenon. The excitation will occur only where the light intensity is maximum, typically at the focal point of an optical system and only using a pulsed laser as excitation source to take benefit of the high peak power of the pulses. δ_a is generally expressed in Göppert-Mayer unit (GM) in honour of Maria Göppert-Mayer who first predicted theoretically this phenomenon in 1931 (1 GM =

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10^{-50} cm^4 s photon^{-1} molecule^{-1}).[4] In addition to an intrinsic fine 3D localisation of the excitation, this nonlinear process is obtained with low energy wavelength (typically with IR light for molecules which absorb classically in the UV). This induces limited phototoxicity for cells, tissues or organs, and an increased penetration depth (tissues transparency windows from 700 to 1000 nm),[5] for example, in highly scattering tissues such as brain slices. Most of the photoremovable groups described for one photon uncaging have been tested for their two-photon excitation properties, leading, overall, to rather disappointing results in terms of two-photon uncaging efficiencies defined by the uncaging action cross-section ($\delta_a\Phi_u$) also expressed in GM. In a seminal article,[6] the group of Tsien and colleagues has initiated this theme by describing brominated 7-hydroxycoumarin-4-ylmethyl caged carboxylic acid derivatives possessing improved two-photon uncaging action cross-sections over previously described chromophores. Two-photon uncaging of neurotransmitters is of particular interest in neurobiology for a better control of *in vivo* synaptic signalling and this has prompted the synthesis of different two-photon sensitive caged glutamate (MNI-Glu,[7] DMNPB-Glu,[8] PMNB-Glu[9]) and caged γ-aminobutyric acid derivatives (CDNI-GABA[10] and N-DCAC-GABA[11]). Nevertheless, none of the described chromophores displayed satisfactory efficiencies at 800 nm, excitation wavelength which corresponds to standard Ti:sapphire lasers justifying continuous efforts to improve the two-photon uncaging cross-sections at this wavelength.[12] In this article we investigate the connection of different photoremovable protecting groups with a donor–acceptor biphenyl backbone, known to display excellent two-photon absorption cross-sections, to form potential probes for two-photon uncaging of biomolecules.

Experimental

General procedures

All chemicals were purchased from Sigma–Aldrich, Alfa Aesar or Fluka in analytical grade. The NPE-ATP was purchased from Jena Bioscience. 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 Zorbax C18 column (4.6, 250 mm) or an Acclaim C18 column (4.6, 250 mm) were used for HPLC analysis. Absorption spectra were recorded on a UVIKON XS spectrometer. Microwave reactions were performed in a Biotage Initiator EXP EU at 2.45 GHz.

Synthesis

1-(4′-Hydroxy-[1,1′-biphenyl]-4-yl)ethanone (1). A mixture of 1-(4-bromophenyl)ethanone (298 mg, 1.5 mmol), 4-hydroxyphenylboronic acid (310 mg, 2.25 mmol), K_2CO_3 (518 mg, 3.75 mmol), Bu_4NBr (489 mg, 1.5 mmol), and $Pd(OAc)_2$ (catalytic) in EtOH (10 mL) and water (5 mL) was heated under microwave conditions at 150 °C for 10 min. Water (100 mL) was added and the aqueous phase was extracted by EtOAc (200 mL). Purification by flash chromatography using heptane/EtOAc 8/2 in vol. gave 170 mg of the title compound in 54% yield.

^{1}H NMR (400 MHz, MeOD): δ (ppm) = 2.64 (s, 3H), 6.90 (d, J = 8.8 Hz, 2H), 7.57 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H), 8.04 (d, J = 8.8 Hz, 2H).

2-Bromo-1-(4′-hydroxy-[1,1′-biphenyl]-4-yl)ethanone (2). A mixture of 1-(4′-hydroxy-[1,1′-biphenyl]-4-yl)ethanone (160 mg, 0.755 mmol) and $CuBr_2$ (286 mg, 1.28 mmol) in anhydrous ethyl acetate (15 mL) was stirred at reflux for 19 h. Water (100 mL) was added. The aqueous phase was extracted by EtOAc (2 × 200 mL). Purification by flash chromatography using heptane/EtOAc 8/2 in vol. gave 124 mg of the title compound in 56% yield.

^{1}H NMR (400 MHz, MeOD): δ (ppm) = 4.67 (s, 3H), 6.91 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 8.07 (d, J = 8.4 Hz, 2H).

1-(4′-(Bis(2-(2-methoxyethoxy)ethyl)amino)-[1,1′-biphenyl]-4-yl)ethanone (3). A mixture of 4-iodo-*N*,*N*-bis(2-(2-methoxyethoxy)ethyl)aniline (827 mg, 1.95 mmol), 4-acetylphenylboronic acid (213 mg, 1.30 mmol), K_2CO_3 (254 mg, 1.95 mmol), Bu_4NBr (1.42 g, 2.92 mmol), and $Pd(OAc)_2$ (catalytic) in EtOH (10 mL) and water (5 mL) was heated under microwave conditions at 150 °C for 10 min. Water (100 mL) was added and the aqueous phase was extracted by EtOAc (200 mL). Purification by flash chromatography using gradient elution of heptane/EtOAc 1/1 in vol. gave 674 mg of the title compound in 80% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.54 (s, 3H), 3.30 (s, 6H), 3.44–3.80 (m, 16H), 6.73 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.91 (d, J = 8.4 Hz, 2H).

1-(4′-(Bis(2-(2-methoxyethoxy)ethyl)amino)-[1,1′-biphenyl]-4-yl)-2-bromoethanone (4). A mixture of 1-(4′-(bis(2-(2-methoxyethoxy)ethyl)amino)-[1,1′-biphenyl]-4-yl)ethanone (233 mg, 0.56 mmol), diisopropylethylamine (166 μL, 0.95 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (257 μL, 1.12 mmol) in anhydrous CH_2Cl_2 (15 mL), were stirred at room temperature for 4 h. A saturated $NaHCO_3$ solution (100 mL) was added. The aqueous phase was extracted by CH_2Cl_2 (2 × 200 mL). The organic phases were combined and evaporated. The crude product was dissolved in anhydrous CH_2Cl_2 (15 mL) and bromine (29 μL, 0.56 mmol) was added at 0 °C. After 1 h at room temperature, a saturated $NaHCO_3$ solution (100 mL) was added. The aqueous phase was extracted by CH_2Cl_2 (2 × 200 mL). Purification by flash chromatography using gradient elution of heptane/EtOAc: 1/1 to pure EtOAc gave 190 mg of the title compound in 68% yield.

^{1}H NMR (400 MHz, $CDCl_3$): 3.41 (s, 6H), 3.51–3.70 (m, 16H), 4.48 (s, 2H), 6.92 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 8.4 Hz, 2H).

General procedure for coupling and deprotection of *N*-Boc-GABAs in the phenacyl series. The bromide caged precursors **2** or **4** (0.35 mmol) were dissolved in dry benzene (15 mL), then *N*-Boc-GABA (146 mg, 0.7 mmol) and 1,8-diazabicycloundec-7-ene (DBU) (63 μL, 0.42 mmol). The solution was further stirred for 19 h at room temperature. A saturated solution of $NaHCO_3$ was then added. The mixture was extracted with

dichloromethane (200 mL), and the protected caged-GABAs were purified by silica gel chromatography using a mixture of heptane/EtOAc: 6/4 or 1/1 to pure EtOAc as eluent for the synthesis of **2** or **4** respectively. Finally the protected caged GABAs were dissolved in anhydrous CH_2Cl_2 (8 mL) and TFA (6 mL). After 1 min at room temperature under argon the solution was evaporated to yield 90 mg (60%) and 190 mg (86%) of the TFA salts of **5** and **6** respectively.

pHBP-GABA (5). ^{1}H NMR (400 MHz, Acetone-d_6): δ (ppm) = 2.17 (t, J = 7.2 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H), 3.93 (t, J = 7.2 Hz, 2H), 5.50 (s, 2H), 6.99 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 7.77 (d, J = 8.4 Hz, 2H), 8.02 (d, J = 8.4 Hz, 2H).

^{13}C NMR (100 MHz, Acetone-d_6): δ (ppm) = 28.1, 35.4, 51.4, 71.5, 121.2, 131.5, 133.5, 133.6, 135.6, 137.4, 151.4, 163.6, 176.9, 197.2.

MS (ESI-ACI): m/z = 314.2 [M + H].

pABP-GABA (6). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 2.17 (m, 2H), 2.61 (m, 2H), 3.12 (m, 2H), 3.36 (s, 6H), 3.50-3.63 (m, 16H), 5.31 (s, 2H), 6.75 (d, J = 8.8 Hz, 2H), 7.47 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.0 Hz, 2H), 7.84 (d, J = 8.0 Hz, 2H)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 30.8, 39.2, 51.0, 59.1, 66.3, 68.4, 70.6, 72.0, 112.1, 125.9, 126.6, 128.2, 128.5, 131.1, 148.1, 149.3, 172.2, 192.0.

MS (ESI-ACI): m/z = 517.2 [M + H].

Synthesis of alkoxy-4-nitro-[1,1′-biphenyl]-3-yl)propan-1-ol (8a–c). 2-(5-Iodo-2-nitrophenyl)propan-1-ol[13] (507 mg, 1.66 mmol) was suspended in dry toluene (30 mL) under argon. Tetrakistriphenylphosphine palladium (200 mg, 0.19 mmol) was then slowly added to the mixture and stirred until it was fully dissolved. Na_2CO_3 in water (20 mL) was then added. The mixture was then heated at 110 °C during 30 min. 4-(2-(2-Methoxyethoxy)ethoxy)phenylboronic acid,[14] 3-methoxyphenylboronic acid or 2-methoxyphenylboronic acid (3.29 mmol) was dissolved in ethanol (3 mL) and dropwise added to the mixture. The reaction was stirred at 110 °C and followed by TLC. After reaction, the mixture was cooled to room temperature, diluted with a saturated NaCl solution, and extracted with ethyl acetate. The organic layer was then washed with water and dried on $MgSO_4$ and the solvent was removed under vacuum. The crude product was purified on column chromatography (SiO_2; cyclohexane/AcOEt 2 : 8 v/v). PENB-OH (**8a**) (320 mg, 46%), mMNB-OH (**8b**) (434 mg, 90%) and oMNB-OH (**8c**) (448 mg, 91%) were obtained as slight yellow viscous oils.

*PENB-OH (**8a**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.79 (d, 1H), 7.58 (d, 1H), 7.45 (m, 3H), 6.98 (d, 3H), 4.14 (m, 2H), 3.85 (m, 4H), 3.65 (m, 6H), 3.50 (m, 4H), 3.31 (s, 3H), 1.30 (t, 3H).

^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) = 159.0, 148.4, 144.9, 138.8, 131.1, 128.1, 125.9, 124.6, 124.6, 114.8, 99.2, 71.5, 70.4, 70.2, 70.1, 69.3, 67.2, 58.3, 36.1, 17.3.

*mMNB-OH (**8b**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.86 (d, J = 8.4 Hz, 1H), 7.66 (dd, J = 8.4 Hz and 1.8 Hz, 1H), 7.42 (t, J = 8.1 Hz, 1H), 7.17 (d, J = 8.1 Hz, 1H), 7.17 (d, J = 7.2 Hz, 1H), 7.11 (s, 1H), 6.97 (dd, J = 8.1 Hz and 1.8 Hz, 1H), 3.84–3.89 (m, 5H), 3.66 (m, 1H), 1.39 (d, J = 6.9 Hz, 3H).

^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) = 160.0, 149.5, 145.6, 140.6, 138.8, 130.1, 126.9, 125.8, 124.8, 119.8, 113.6, 113.4, 67.9, 55.4, 36.4, 17.5.

*oMNB-OH (**8c**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.84 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 1.2 Hz, 1H), 7.53 (d, J = 8.4 Hz and 1.8 Hz, 1H), 7.38–7.44 (m, 1H), 7.34 (dd, J = 7.8 Hz and 1.2 Hz, 1H), 7.02–7.10 (m, 2H), 3.83–3.85 (m, 5H), 3.64–3.68 (m, 1H), 3.50 (m, 1H), 1.37 (d, J = 6.9 Hz, 3H).

^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) = 156.8, 149.4, 143.8, 138.3, 131.0, 130.4, 129.9, 128.9, 128.7, 124.5, 121.5, 111.9, 68.3, 56.0, 36.9, 18.0.

General procedure for coupling and deprotection of *N*-Boc-Glu-O*t*Bu in the 2-(*o*-nitrophenyl)-propyl series. The caged alcohols **8a–c** (0.5 mmol) were dissolved in dry CH_2Cl_2 (15 mL), then *N*-Boc-Glu-O*t*Bu (150 mg, 0.5 mmol), DCC (110 mg, 0.55 mmol) and DMAP (6 mg) was added at 0 °C. The solution was further stirred for 19 h at room temperature. A saturated solution of $NaHCO_3$ was then added. The mixture was extracted with dichloromethane (200 mL), and the protected caged-Glutamates were purified by silica gel chromatography using a mixture of heptane/EtOAc 8 : 2 in vol. as eluent. Finally the protected caged glutamates were dissolved in anhydrous CH_2Cl_2 (8 mL) and TFA (6 mL). After 5 h at room temperature under argon the solution was evaporated to yield the caged glutamates **9a–c**.

*PENB-Glu (**9a**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 7.79 (d, 1H), 7.64 (m, 4H), 7.00 (m, 2H), 4.25 (m, 4H), 3.85 (m, 1H), 3.70 (m, 8H), 3.50 (m, 3H), 2.55 (br, 2H), 2.07 (m, 2H), 1.30 (m, 3H).

^{13}C NMR (400 MHz, DMSO-d_6): δ (ppm) = 173.5, 160.3, 149.7, 145.9, 138.8, 132.1, 129.5, 127.0, 126.3, 125.8, 116.0, 99.2, 72.3, 71.2, 70.8, 70.5, 70.4, 69.5, 68.6, 59.2, 55.2, 53.8, 34., 30.4, 25.8, 17.9.

*mMNB-Glu (**9b**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.73 (d, J = 8.4 Hz, 1H), 7.57 (s, 1H), 7.42 (d; J = 8.1 Hz, 1H), 7.28–7.34 (m, 1H), 7.4–7.11 (m, 1H), 6.88 (d; J = 8.4 Hz, 1H), 4.11–4.19 (m; 2H), 3.69–3.81 (m; 5H), 2.52 (br, 2H), 2.05–2.14 (m, 2H), 1.24–1.30 (m, 3H), 0.85–0.92 (m, 1H).

^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) = 173.7, 173.2, 160.0, 149.0, 145.5, 140.3, 137.9, 130.0, 126.7, 125.9, 124.8, 119.7, 113.7, 113.1, 68.8, 55.2, 53.7, 32.9, 30.0, 25.6, 17.3.

*oMNB-Glu (**8c**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.72 (d, J = 8.2 Hz, 1H), 7.58 (s, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.26–7.37 (m; 2H), 6.95–7.04 (m, 2H), 4.05–4.25 (m, 2H), 3.68–3.88 (m; 5H), 2.40–2.62 (m, 2H), 2.09–2.15 (m, 2H), 1.28–1.31 (m; 3H), 0.82–0.92 (m, 1H).

^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm) = 174.0, 173.9, 156.8, 148.9, 144.0, 137.3, 131.0, 130.5, 129.8, 128.9, 128.6, 124.5, 121.5, 111.9, 69.7, 55.9, 33.3, 30.4, 25.7, 17.8, 17.7.

Quantification of GABA release

A chromophoric derivative was coupled to the GABA moiety in pHBP-GABA and pABP-GABA to quantify GABA release by HPLC.[15]

Synthesis. 1*H*-Benzo[1,2,3]triazol-1-yl-4-methoxybenzoate (4-methoxybenzoic [MBA] activated ester:

A mixture of 4-methoxybenzoic acid (200 mg, 1.31 mmol), PyBop (685 mg, 1.31 mmol) and diisopropylamine (230 μL, 1.9 mmol) in 10 mL of anhydrous DMF was stirred under argon at room temperature over night. Water (50 mL) was added and the organic compounds were extracted into EtOAc (50 mL). Purification by flash chromatography using heptane/EtOAc 1 : 1 in vol. gave 335 mg of a white powder in 95% yield.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.95 (s, 3H), 7.07 (d, J = 8.9 Hz, 2H), 7.42–7.57 (m, 3H), 8.10 (d, J = 8.2 Hz, 1H), 8.24 (d, J = 8.9 Hz, 2H).

General procedure for coupling GABA or caged GABA's to the 4-methoxybenzoate activated ester. A mixture of 1*H*-benzo [1,2,3]triazol-1-yl-4-methoxybenzoate (4 mg, 0.015 mmol) and GABA or caged GABAs (pHBP-GABA and pABP-GABA) (0.01 mmol) with DIEA (0.01 mmol) in anhydrous DMF, were stirred over night at room temperature. Purification by semi-preparative C-18 HPLC with a 250 × 10 BetaBasic-18 column, from Thermo Scientific, using elution at a flow rate of 4 mL min^{-1} with a linear gradient of acetonitrile in 0.1% TFA in water from 0 to 100% (v/v) over 30 min gave respectively pHBP-GABA-MBA, pABP-GABA-MBA and GABA-MBA after evaporation. Retention time: 19.3 min for the pHBP-GABA-MBA, 19.7 min for the pABP-GABA-MBA and 13.7 min for GABA-MBA.

GABA-MBA. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.96 (m, 2H), 2.48 (t, J = 6.1 Hz, 2H), 3.54 (m, 2H), 3.85 (s, 3H), 6.92 (d, J = 8.7 Hz, 2H), 7.73 (d, J = 8.7 Hz, 2H).

pHBP-GABA-MBA. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.10 (t, J = 6.4 Hz, 2H), 2.66 (t, J = 6.8 Hz, 2H), 3.61 (t, J = 7.2 Hz, 2H), 3.85 (s, 3H), 5.40 (s, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 8.8 Hz, 2H), 7.96 (d, J = 8.4 Hz, 2H).
MS (ESI-ACI): m/z = 314.2 [M + H].

pABP-GABA-MBA. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 2.06 (t, J = 6.4 Hz, 2H), 2.62 (t, J = 6.8 Hz, 2H), 3.15 (t, J = 7.2 Hz, 2H), 3.37 (s, 6H), 3.51–3.64 (m, 16H), 3.80 (s, 3H), 5.36 (s, 2H), 6.88 (d, J = 8.8 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.8 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H).
MS (ESI-ACI): m/z = 651.2 [M + H].

Released GABA quantification. The yield of released GABA-MBA from irradiated pHBP-GABA-MBA and pABP-GABA-MBA is determined by HPLC analysis. A 1.0 mL portion of a 50 μM solution of pHBP-GABA-MBA or pABP-GABA-MBA in phosphate buffer (50 mM) was irradiated at 315 or 365 nm respectively for pHBP-GABA-MBA or pABP-GABA-MBA, to ensure a complete conversion of the starting compound using a LUMOS 43 (Atlas Photonics Inc.). 200 μL of each irradiated solutions were analyzed by HPLC and compared to the HPLC calibration curve obtained by injection of 200 μL of 20–150 μM solutions of GABA-MBA in phosphate buffer.

HPLC analysis was carried out on Acclaim Analytical SB-C18 (4.6 × 250 mm) column; elution was performed at a flow rate of 1 mL min^{-1} with a linear gradient of acetonitrile in 0.1% TFA in water from 0 to 100% (v/v) over 30 min.

Retention time: 22.9 min for pHBP-GABA-MBA, 23.0 min for pABP-GABA-MBA and 15.8 min for GABA-MBA.

Quantification of glutamate release

The glutamic acid formation was quantified by HPLC using a chromophoric derivative formed quantitatively after condensation with *o*-phthaldialdehyde and mercaptoethanol in a 50 mM Borate buffer pH 9.[16] The chromophoric adduct was detected by absorbance at 340 nm. HPLC retention time on a analytical SB-C18 Zorbax (4.6 × 250 mm) column using a 30 min linear gradient from 0 to 100% Acetonitrile in a 0.1% TFA water solution at 1 mL min^{-1} of the glutamate *o*-phthaldialdehyde adduct: 16.7 min.

One photon quantum yield determination

The quantum yields for the photoconversion of pHBP-GABA, pABP-GABA, PENB-Glu, mMNB-Glu and oMNB-Glu were determined by comparison with the photolysis of 1-(2-nitrophenyl)ethyl-ATP (NPE-ATP) (Φ_u = 0.63)[17] which was taken as reference in a phosphate buffer (0.1 mM, pH 7.4) at 25 °C. These compounds were tested at identical optical densities at the used irradiation wavelengths. Accordingly, mixtures of caged neurotransmitters and 0.2 mM of NPE-ATP reference were used. Those mixtures were photolyzed by continuous irradiation at 315 nm using a 1000 W Hg Lamp from Hanovia focused on the entrance slit of a monochromator, and aliquots were subjected to reversed-phase HPLC to determine the extent of the photolytic conversions. HPLC analysis was carried out on an Acclaim Analytical SB-C18 (4.6 × 250 mm) column; elution was performed at a flow rate of 1 mL min^{-1} with a linear gradient of acetonitrile in 0.1% TFA in water from 0 to 100% (v/v) over 30 min. Disappearances quantum yields Φ_d were calculated by considering the conversions up to 20%, in order to limit, as much as possible, errors due to undesired light absorption during photolysis.

Uncaging quantum yields are given by the following eqn (3):

$$\Phi_u = \Phi_d \times \text{photocleavage yield} \quad (3)$$

Two photon uncaging action cross-section determination

The two-photon uncaging action cross-section $\delta_a\Phi_u$ was determined by comparison with that of CouOAc (1.07 GM at 740 nm)[6] as a known reference, in the same illumination conditions. Solutions of caged neurotransmitters and CouOAc were irradiated during 10–40 min by a mode-locked titanium:sapphire laser (Tsunami, Spectra Physics, 100 fs, 80 MHz) at 740 nm. The measurements were performed at P = 250 mW, in the quadratic dependence range for such chromophore.[9] After irradiation, 80 μL of each solution is analyzed by HPLC to determine the extent of the photolytic conversions. HPLC analysis was carried out on an Acclaim Analytical SB-C18 Zorbax (4.6 × 250 mm) column; elution was performed at a flow rate of 1 mL min^{-1} with a linear gradient of acetonitrile in 0.1% TFA in water from 0 to 100% (v/v) over 30 min. The ratio between the slopes determined from the graphical representation of the photolytic conversion allows measurement of two-photon uncaging action cross-section (using CouOAc[6] as primary standard, for which the two-photon uncaging action cross-section of 1.07 at 740 nm is accurate within a factor of 2).

Results and discussion

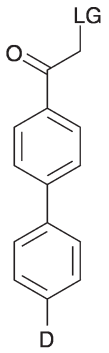
To optimize the two-photon uncaging action cross-section $\delta_a\Phi_u$ we have to improve the two-photon absorption process, linked to δ_a, as well as the efficiency of the photochemical reaction, related to Φ_u. As δ_a is related to the cubic hyperpolarisability γ, all the molecular engineering strategies classically used to optimize γ can be applied to the design of our new cages. Because of potential limitations for biological applications (*i.e.* the size and the aqueous solubility of the probe), we limited our investigations to non-centrosymmetric one-dimensional (1D) donor–acceptor (D–A) systems. (*i.e.* other geometries are possible, such as centrosymmetrical 1D A–A or A–D–D–A systems, see reference 12) For such systems it is well known that the optimization of γ requires an elongation of the conjugated π system and/or a modulation of the end groups D,A properties.[6,18] Previous works on the *o*-nitrobenzyl and *o*-nitrophenylbutyl series have pointed out the importance of these two parameters. Indeed Goeldner's group has described a caged glutamate based on the [3-(4,5-dimethoxy-2-nitrophenyl)-2-butyl] (**DMNPB**) cage, showing low two-photon uncaging action cross-section (0.17 GM at 720 nm) but high photocleavage efficiency (Φ_u = 0.26).[8] On the contrary Jullien's group has described a caged coumarin based on 5-(4-methoxyphenylethynyl)-2-nitrobenzyl cage which presents a low two-photon uncaging action cross-section (0.045 GM at 730 nm), the later being probably due to a too low efficiency for the photochemical reaction (Φ_u = 0.001), when δ_a was certainly improved by the introduction of a long conjugated donor–acceptor system.[19] We thus decided to design new cages based on the biphenyl central core ended by oxygen or nitrogen atoms as electron donors, and nitro or carbonyl groups as electron acceptors. We will describe here three caging platforms based on D–A system centred on a biphenyl core, successively the phenacyl series, the *o*-nitrobenzyl series and at last the *o*-nitrophenyl propyl series, deciphering unprecedented two-photon uncaging properties within the latter series.

Donor–acceptor biphenyl derivatives in the phenacyl series

In this series the caging platform is based on a biphenyl central core surrounded by a hydroxy or bis((bis-ethoxy)methoxy)amino electron donating group and the carbonyl function of the phenacyl group as electron acceptor (Scheme 1).

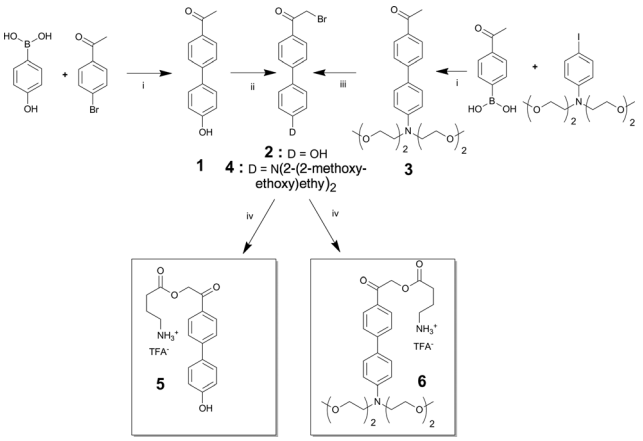
Synthetic pathways. The syntheses of two donor–acceptor biphenyl phenacyl cages: *p*-hydroxybiphenylacyl (**pHBP**) and *p*-bisalkyl-aminobiphenylacyl (**pABP**) are summarized in Scheme 2. The biphenyl moieties were obtained using Suzuki cross-coupling reactions with microwave activation[20] between 1-(4-bromophenyl)ethanone and 4-hydroxyphenylboronic acid or 4-iodo-*N*,*N*-bis(methoxyethyl)aniline and 4-acetylphenylboronic acid for the synthesis of **pHBP** or **pABP** cages respectively. The 1-(4′-hydroxy-[1,1′-biphenyl]-4-yl)ethanone (**1**) was converted to the corresponding α-bromoketone (**2**) by bromination with cupric bromide.[21]

However, 1-(4′-(bis(2-(2-methoxyethoxy)ethyl)amino)-[1,1′-biphenyl]-4-yl)-2-bromoethanone (**4**) was synthesized by treatment with bromine of the TBDMS silyl enol ether derivative of **3**. Protected GABA (*N*-α-*tert*-butoxycarbonyl-GABA) was



pHBP : D= OH
pABP : D=N(2-(2-methoxyethoxy)ethy)$_2$

Scheme 1 Structure of the donor–acceptor biphenyl derivatives in the phenacyl series: **pHBP** and **pABP**.



Scheme 2 Synthesis of pHBP-GABA (**5**) and pABP-GABA (**6**). i) K_2CO_3, Bu_4NBr, $Pd(OAc)_2$, $EtOH/H_2O$, microwave 150 °C, 10 min, ρ = 46–91%. ii) $CuBr_2$, AcOEt, Δ, 19h, ρ = 56%. iii) a) TBDMS-OTf, diisopropyl ethyl amine (DIEA), CH_2Cl_2, 4 h, RT. b) Br_2, CH_2Cl_2, 1 h, RT, ρ = 68%. iv) a) *N*-Boc GABA, DBU, benzene, 19 h, RT. b) TFA/CH_2Cl_2, 1 min, ρ = 60–86%.

grafted to the biphenyl α-bromoketones (**2**, **4**) to provide the new caged GABA analogues, **pHBP-GABA** (**5**) and **pABP-GABA** (**6**) respectively, after deprotection in acidic media.

Photochemical and photophysical properties. The solubilities of **pHBP-GABA** (**5**) and **pABP-GABA** (**6**) at room temperature in phosphate buffer pH 7.4 (without any co-solvent) were 40 μM and 13 mM, respectively. The hydrolytic stability was explored by HPLC in phosphate buffer (pH 7.4) at room temperature; a half-time of 10 h was measured for the hydrolysis of **5** and **6**. The absorption maxima of **pHBP-GABA** and **pABP-GABA** are 313 and 369 nm with molecular extinction coefficients of 14800 and 18000 M^{-1} cm^{-1} respectively (Fig. 1). The photolytic release of GABA was analyzed by UV-visible spectroscopy and HPLC. Photolysis was carried out by irradiation of samples (around 50 μM), in phosphate buffer 50 mM pH 7.4, at 315 nm or 365 nm for **pHBP-GAGA** and **pABP-GABA** respectively.

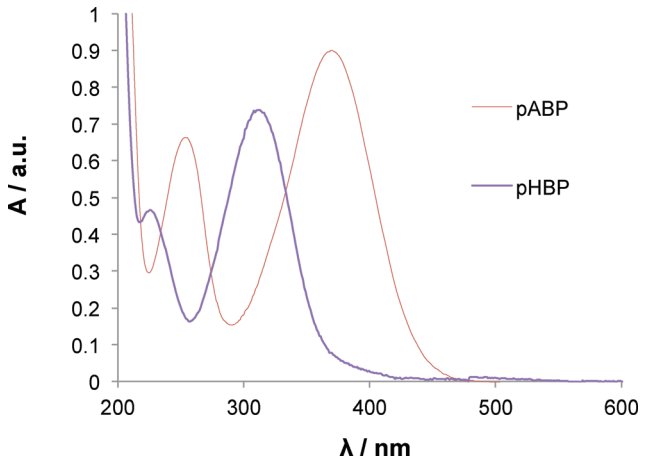


Fig. 1 UV spectra of donor–acceptor biphenyl chromophores in the phenacyl series at 50 μM in PBS pH = 7.4, 50 mM.

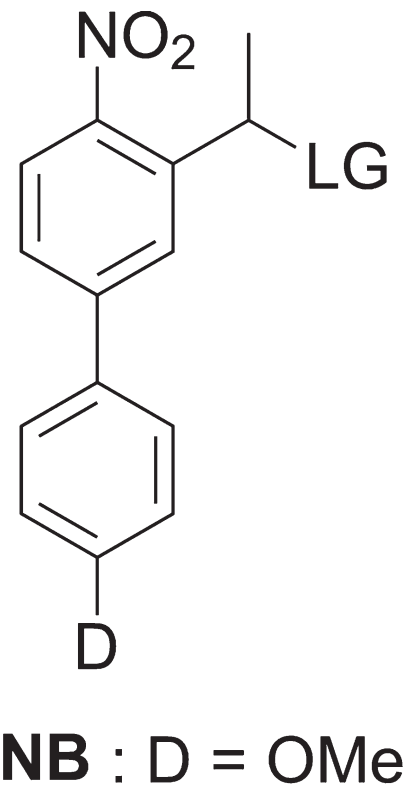
pHBP-GABA showed a new absorbance at 399 nm, whilst the initial absorbance at 313 nm gradually diminished, with the appearance of isobestic points (data not shown). Alternatively, **pABP-GABA** only showed a gradual diminution of the initial absorbance at 369 nm during irradiation.

To be able to quantify the released GABA by HPLC, our caged GABA derivatives **5** and **6** were coupled to an activated ester of 4-methoxybenzoic acid used as a chromophoric derivative (data not shown).[15] An almost quantitative (≥93%) formation of the GABA 4-methoxybenzamide derivative was measured by HPLC after complete photo-conversion of pHBP-GABA-MBA. However, only a 55% yield formation of the GABA 4-methoxybenzamide derivative was measured after complete photo-conversion of pABP-GABA-MBA, indicating that these functional groups induce some competitive photochemical pathways, which do not contribute to the photorelease of a carboxylic acid function. Quantum yields of the photolytic reactions for pHBP-GABA (**5**) and pABP-GABA (**6**) were determined by competition with the 2-(nitrophenyl)ethyl-ATP taken as reference molecule.[17] A good uncaging quantum yields (Table 1) was determined in phosphate buffer (50 mM, pH 7.4) only for the for pHBP. Altogether, the high photolysis quantum yield and the significant molar absorption coefficient ($\varepsilon\Phi_u$ = 3100 $M^{-1}cm^{-1}$ at 315 nm) together with the efficient release of GABA (≥ 93%) make this photoremovable group an interesting cage for near UV photolysis. Therefore, we decided to fully characterize the two-photon sensitivity of pHBP-GABA. The TP uncaging action cross-section ($\delta_a\Phi_u$) of caged pHBP-GABA (**5**) was determined at 740 nm by HPLC analysis of the disappearance of the starting material during TP photolysis and compared to the disappearance of CouOAc as a reference ($\delta_a\Phi_u$ = 1.07 GM at 740 nm).[6] Unfortunately a modest two-photon uncaging action cross-section value of 0.24 GM at 740 nm was obtained, limiting further developments. (Table 1).

Donor–acceptor biphenyl derivatives in the *o*-nitrobenzyl series

One example has been selected in this series, that is based on a methoxy group as donor, and a nitro group as acceptor (Scheme 3).

The two-photon optimization of the donor–acceptor *o*-nitrobenzyl platform was investigated by the group of Jullien.[19] Using molecular engineering on this platform several strategies were investigated in order to induce a red-shifted absorbance that could allow an efficient photolytic reaction upon two-photon excitation. The donor–acceptor biphenyl platform was therefore investigated. The *p*-methoxynitrobiphenyl (**pMNB**) photoremovable group showed an interesting shift in absorbance compared to the usual *o*-nitrobenzyl (oNB) group (317 nm *versus* 262 nm) but with a very low quantum yield for the photolytic reaction. On the other hand, this series showed a poor hydrolytic stability on caged carboxylic acids like glutamate and GABA. Clearly, it



Scheme 3 Structure of the pMNB donor–acceptor biphenyl derivative in the *o*-nitrobenzyl series.

Table 1 Photochemical and photophysical properties of donor–acceptor biphenyl caged GABAs in the phenacyl series

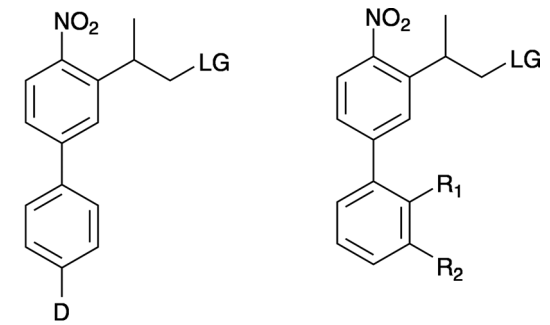
Compound	λmax (nm)	ε (M^{-1} cm^{-1})	% photo-release	Φ_u	$\delta_a\Phi_u$ (GM) 740 nm	Solubility (PBS pH 7.4)	Hydrolysis (*t*1/2 in PBS pH 7.4 at RT)
pHBP-GABA	313	14800	93	0.21	0.24	40 μM	10 h
pABP-GABA	369	18000	55	0.015	n.d.	13 mM	10 h

was very difficult to optimize both the absorbance and the uncaging properties in this series. Therefore, we decided to investigate the 2-(*o*-nitrophenyl)propyl series which, besides a good hydrolytic stability offered improved photolytic properties in the UV-visible region.[8]

Donor–acceptor biphenyl derivatives in the 2-(*o*-nitrophenyl) propyl series

In this series the caging platform is based on a biphenyl central core surrounded by a hydroxy, methoxy, tris-ethoxy(methoxy) or bis((bis-ethoxy)methyl)amino electron donating groups and nitro as electron attracting group (Scheme 4).

Synthetic pathways. The synthesis of 2-(4′-((di(tris-ethoxy (methyl))amino)-4-nitro-[1,1′-biphenyl]-3-yl)propyl (**EANBP**), 3-(2-Propyl-1-ol)-4′-methoxy-4-nitrobiphenyl (**PMNB**) and 3-(2-propyl-1-ol)-4′-hydroxy-4-nitrobiphenyl (**PHNB**) cages and their corresponding caged neurotransmitters were previously reported by Donato *et al.*[15] and Gug *et al.*[9] respectively. The



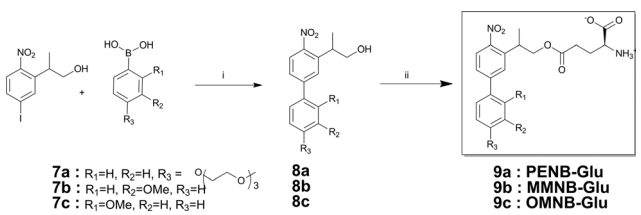
PHNB : D= OH
PMNB : D = OMe
PENB : D = O(2-(2-(2-methoxyethoxy)ethoxy)ethyl))
EANBP : D = N(2-(2-methoxyethoxy)ethyl)$_2$
mMNB : R_1 = H ; R_2 = OMe
oMNB : R_1 = OMe ; R_2 = H

Scheme 4 Structure of the donor–acceptor biphenyl derivatives in the 2-(*o*-nitrophenyl)propyl series: pHBP, PENB, EANBP, mMNB and oMNB.

syntheses of three donor–acceptor biphenyl 2-(*o*-nitrophenyl) propyl cages: 3-(2-propyl-1-ol)-4′-tris-ethoxy(methoxy)-4-nitrobiphenyl (**PENB**), 3-(2-propyl-1-ol)-*m*-methoxy-4-nitrobiphenyl (**mMNB**) and 3-(2-propyl-1-ol)-*o*-methoxy-4-nitrobiphenyl (**oMNB**) are summarized in Scheme 5. The 4-iodo-2-(2-propyl-1-ol)nitrobenzene[13] was coupled to the corresponding phenylboronic acids to give the biphenyl derivatives **8**. Protected glutamate (*N*-α-*t*-Boc-L-glutamic acid γ-*t*-butyl ester) was then grafted to this cage to give the caged glutamates **9** after deprotection in acidic media.

Photochemical and photophysical properties. The one-photon properties of caged glutamate **8a–c** have been investigated by UV-visible spectroscopy and HPLC. The absorption maxima and the molar extinction coefficients of **PENB-Glu**, **mMNB-Glu** and **oMNB-Glu** are summarized in Table 2. The photolytic release of glutamate was analyzed quantitatively by HPLC using derivatization with *o*-phthaldialdehyde and mercaptoethanol[16] after irradiation in neutral buffered medium. All caged glutamates **9a–c** afforded a 90% yield of glutamate release. The photochemical and photophysical properties of **EANBP-GABA**, **PMNB-Glu** and **PHNB-Glu**, are summarized in Table 2 as previously reported by Donato *et al.*[15] and Gug *et al.*,[9] respectively.

The 2-(*o*-nitrophenyl)propyl series was an ideal photoremovable protecting group to be connected to the donor–acceptor biphenyl plateform to generate powerful TPE-sensitive caging groups. First, we were able to improve the two-photon sensitivity by elongating the conjugated system on methoxynitrobiphenyl platforms, without substantially affecting the yield of photorelease and the quantum yield of the 2-(*o*-nitrophenyl)propyl photolytical reaction.[9] The *p*-methoxynitrobiphenyl platforms showed the best two-photon properties at 740 nm compared to the other methoxy regioisomers. However, the substitution of the methoxy group by a better electron donating hydroxy group which should also increase the δ_a, did not increase the two-



Scheme 5 Synthesis of donor–acceptor biphenyl caged glutamates in the 2-(*o*-nitrophenyl)propyl series. i) $Pd(PPh_3)_3$, $NaHCO_3$, toluene, 110 °C, 2–10 h, ρ = 54–80%. ii) a) *N*-Boc-Glu-OtBu, DCC, DMAP, CH_2Cl_2, 19 h, RT, b) TFA, CH_2Cl_2, RT, 5 h, ρ = 80–90%.

Table 2 Photochemical and photophysical properties of donor–acceptor biphenyl caged neurotransmitters in the 2-(*o*-nitrophenyl)propyl series

Compound	λ_{max} (nm)	ε (M^{-1} cm^{-1})	% photo-release	Φ_u	$\delta\Phi_u$ (GM) (λ)	Solubility (in PBS pH 7.4)	Hydrolysis (24 h in PBS pH 7.4)
PHNB-Glu	330	n.d.	<10%	n.d.	n.d.	n.d.	n.d.
mMNB-Glu	296	7150	90	n.d.	1.8 (740 nm)	n.d.	n.d.
oMNB-Glu	302	6300	90	n.d.	2.2 (740 nm)	n.d.	n.d.
PMNB-Glu and PENB-Glu	317	9900	90	0.09	3.2 (740 nm)	> 5 mM for PENB-Glu	3%
EANBP-GABA	397	7500	95	0.15	11 (800 nm)	10 mM	≤ 1%

photon uncaging action cross-section, mainly due to undesirable photochemical reactions leading to an inefficient photolytic reaction. The *p*-dialkylaminonitrobiphenyl platforms, on the other hand, led to a 90 nm bathochromic shift compared to the *p*-methoxynitrobiphenyl platforms (Fig. 2), with no significant efficiency modification of the photolytic reaction (95% release with a 15% quantum yield for one photon photoconversion). Most importantly, the substitution of the methoxy group by a better electron-donating group (NR_2) significantly increased the TP sensitivity of this photoremovable group, leading to an unprecedented TP uncaging action cross-section at 800 nm (up to 11 GM). In addition, the presence of the dialkylamino group opens the possibility for improving significantly the aqueous solubility of these cages by grafting oligoethyleneglycol chains on the amino function. Finally, the photolytic kinetics for the more interesting photoremovable **EANBP** and **PENB** protecting groups were investigated using a fluorescent reporter: 1,3-dichloro-9,9-dimethyl-9*H*-acridin-2(7)-one (**DDAO**). This red emitting fluorophore showed a large and interesting spectral difference between the caged **DDAOs** and the free **DDAO**, allowing an easy estimation of the photolytic reaction kinetics by following the appearance of a red fluorescent signal after laser flash photolysis.[14–15] A time scale shorter than 5 μs and 15 μs for **EANB-DDAO** and **PENB-DDAO** respectively, were obtained for the release of the fluorescent **DDAO**, value in concordance with the reported kinetics for other *o*-(nitrophenyl) propyl photoremovable groups,[8–9] in agreement with temporal control of most neuronal processes.

In summary, the best photophysical properties in the 2-(*o*-nitrophenyl)propyl series, were observed for the *p*-alkoxy and *p*-bisalkylamino-4-nitro-[1,1′-biphenyl]-3-yl)propyl derivatives. Therefore oligoethylene glycol chains were used to alkylate those two scaffolds, leading the more soluble **PENB** and **EANBP** cages. The **EANBP** and **PENB** photoremovable protecting group are to our knowledge the best caging group for two-photon excitation at 800 and 740 nm respectively, offering attracting perspectives in chemical biology. They have already been used for the two-photon **GABA** release in intact brain tissue at 800 nm,[15] and for live-cell 740 nm two-photon uncaging of a far-red emitting acridinone fluorophore,[14] respectively.

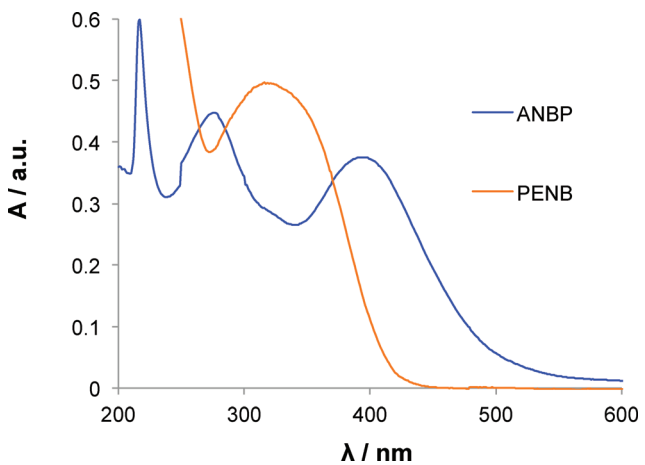


Fig. 2 UV-visible spectra of donor–acceptor biphenyl in the 2-(*o*-nitrophenyl)propyl series at 50 μM in PBS pH = 7.4, 50 mM.

In addition **PENB-Glu** has been used for live-cell two-color photoactivation with 740 nm two-photon glutamate uncaging in combination with optogenetic methods (800 nm two-photon channel Rhodopsin activation) on brain slices.[22,23]

Conclusion

Molecular engineering of photoremovable protecting groups using the donor–acceptor biphenyl backbone was evaluated for the development of probes displaying highly efficient two-photon uncaging properties. In an early article[19] by the group of Jullien, the *o*-nitrobenzyl protecting group was investigated. Besides an interesting red-shift of the absorption maximum wavelength observed for the donor–acceptor biphenyl backbone, the quantum yield of uncaging dramatically dropped, leading to an inefficient two-photon uncaging process. Therefore the *o*-nitrobenzyl series seems not to be an adapted protecting group to enlarge the two-photon cross-section. The phenacyl protecting group was subsequently investigated. The *p*-hydroxybiphenyl backbone (**pHBP**) showed an interesting uncaging quantum yield but without an significant increase of the absorption maximum wavelength compared to the *p*-hydroxyphenacyl (**pHP**)[24] protecting group, also leading to an inefficient two-photon uncaging process at 740 nm. On the other hand, the *p*-dialkylaminobiphenyl backbone (**pABP**), a better electron donating group displaying a very interesting absorption maximum wavelength at 370 nm, showed a low uncaging quantum yield and only 55% yield of photocleavage, indicating that these functional groups induce some undesired competitive photochemical pathways. The 2-(*o*-nitrophenyl)propyl series was a more versatile platform to increase the two-photon sensitivity of photoremovable protecting groups, leading to the *p*-alkoxy and *p*-bisalkylamino-4-nitro-[1,1′-biphenyl]-3-yl)propyl derivatives: **PENB** and **EANBP** respectively. Those two photoremovable protecting groups are up to date the best caging groups for two-photon excitation at 800 and 740 nm respectively, offering attracting perspectives in chemical biology. In addition, they also could provide interesting applications using their two-photon orthogonality in order to trigger independently another effector with spatio-temporal control. The two-photon excitation around 800 nm of the **EANBP** cages could be selectively triggered without inducing a photocleavage of the **PENB** cage. But it will be unlikely that excitation around 740 nm will selectively cleave the **PENB** or any other cage. Therefore, a dual wavelength TP photoactivation can only be achieved through a spatial orthogonality of the biological process under investigation.

Acknowledgements

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References

1 J. E. T. Corrie and D. R. Trentham, Caged nucleotides and neurotransmitters, in *Bioorganic Photochemistry*, ed. H. Morrison, Wiley, New York, 1993, vol. 2, pp. 243–305; S. R. Adams and R. Y. Tsien, Controlling cell chemistry with caged compounds, *Annu. Rev. Physiol.*, 1993, 55,

755–784; G. Marriott, *Methods in Enzymology: Caged Compounds*, Academic Press, San Diego, 1998, vol. 291; A. P. Pelliccioli and J. Wirz, Photoremovable protecting groups: reaction mechanisms and applications, *Photochem. Photobiol. Sci.*, 2002, **1**, 441–458; M. Goeldner and R. Givens, *Dynamic Studies in Biology, Phototriggers, Phtoswitches and Caged Biomolecules*, Wiley-VCH, Weinheim, 2005; G. Mayer and A. Heckel, Biologically active molecules with a 'light switch', *Angew. Chem. Int. Ed.*, 2006, **45**, 4900–4921; H. M. Lee, D. R. Larson and D. S. Lawrence, Illuminating the chemistry of life: Design, synthesis, and applications of "caged" and related photoresponsive compounds, *ACS Chemical Biology*, 2009, **4**, 409–427.

2 K. Svoboda and R. Yasuda, Principles of two-photon excitation primer microscopy and its applications to neuroscience, *Neuron*, 2006, **50**, 823–839; G. C. R. Ellis-Davies, Caged compounds: photorelease technology for control of cellular chemistry and physiology, *Nat. Methods*, 2007, **4**, 619–628; L. Sjulson and G. Miesenböck, Photocontrol of neural activity: Biophysical mechanisms and performance *in vivo*, *Chem. Rev.*, 2008, **108**, 1588–1602; A. Specht, F. Bolze, Z. Omran, J.-F. Nicoud and M. Goeldner, Photochemical tools to study dynamic biological processes, *HFSP J.*, 2009, **3**, 255–264; D. Warther, S. Gug, A. Specht, F. Bolze, J.-F. Nicoud, A. Mourot and M. Goeldner, Two-photon uncaging: New prospects in neuroscience and cellular biology, *Bioorg. Med. Chem.*, 2010, **18**, 7753–7758; G. C. R. Ellis-Davies, Two-Photon Microscopy for Chemical Neuroscience, *ACS Chem. Neurosci.*, 2011, **2**, 185–197.

3 C. Andraud, R. Anémian, A. Collet, J.-M. Nunzi, Y. Morel and P. Baldeck, Theoretical molecular engineering for nonlinear absorption by two-photon absorption in the visible, *J. Opt. A: Pure Appl. Opt.*, 2000, **2**, 284–288.

4 M. Göppert-Mayer and Über, Elementarakte mit zwei Quantensprüngen, *Ann. Phys.*, 1931, **401**, 273–294.

5 M. Pawlicki, H. A. Collins, R. G. Denning and H. L. Anderson, Two-photon absorption and the design of two-photon dyes, *Angew. Chem., Int. Ed.*, 2009, **48**, 3244–3266.

6 T. Furuta, S. S.-H. Wang, J. L. Dantzker, T. M. Dore, W. J. Bybee, E. M. Callaway, W. Denk and R. Y. Tsien, Brominated 7-hydroxycoumarin-4-ylmethyls: Photolabile protecting groups with biologically useful cross-sections for two-photon photolysis, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 1193–1200.

7 G. Papageorgiou and J. E. T. Corrie, Effects of aromatic substitution on the photocleavage of 1-acyl-7-nitroindolines, *Tetrahedron*, 2000, **56**, 8197–8205; M. Matsuzaki, G. C. R. Ellis-Davies, T. Nemoto, Y. Miyashita, M. Iino and H. Kasai, Dendritic spine morphology is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons, *Nat. Neurosci.*, 2001, **4**, 1086–1092.

8 A. Specht, J-S. Thomann, K. Alarcon, W. Wittayanan, D. Ogden, T. Furuta, Y. Kurakawa and M. Goeldner, New photoremovable protecting groups for carboxylic acids with high photolytic efficiencies at near-UV irradiation. Application to the photo-controlled release of glutamate, *ChemBioChem*, 2006, **7**, 1690–1695.

9 S. Gug, S. Charon, A. Specht, K. Alarcon, D. Ogden, B. Zietz, J. Léonard, S. Haacke, F. Bolze, J.-F. Nicoud and M. Goeldner, Photolabile glutamate protecting group with high one- and two-photon uncaging efficiencies, *ChemBioChem*, 2008, **9**, 1303–1307.

10 G. C. R. Ellis-Davies, M. Matsuzaki, M. Paukert, H. Kasai and D. E. Bergles, 4-Carboxymethoxy-5,7-dinitroindolinyl-Glu: An improved caged glutamate for expeditious ultraviolet and two-photon photolysis in brain slices, *J. Neurosci.*, 2007, **27**, 6601–6604.

11 S. Kantevari, M. Matsuzaki, Y. Kanemoto, H. Kasai and G. C. R. Ellis-Davies, Two-color, two-photon uncaging of glutamate and GABA, *Nat. Methods*, 2009, **7**, 123–125.

12 S. Gug, F. Bolze, A. Specht, C. Bourgogne, M. Goeldner and J.-F. Nicoud, Molecular engineering of photoremovable protecting groups for two photon uncaging, *Angew. Chem., Int. Ed.*, 2008, **47**, 9525–9529.

13 S. Buehler, I. Lagoja, H. Giegrich, K.-P. Stengele and W. Pfleiderer, New types of very efficient photolabile protecting groups based upon the [2-(2-nitrophenyl)propoxy]carbonyl (NPPOC) moiety, *Helv. Chim. Acta*, 2004, **87**, 620–659.

14 D. Warther, F. Bolze, J. Léonard, S. Gug, A. Specht, D. Puliti, X.-H. Sun, P. Kessler, Y. Lutz, J.-L. Vonesch, B. Winsor, J.-F. Nicoud and M. Goeldner, Live-cell one- and two-photon uncaging of a far red emitting acridinone fluorophore, *J. Am. Chem. Soc.*, 2010, **132**, 2585–2590.

15 L. Donato, A. Mourot, C. M. Davenport, C. Herbivo, D. Warther, J. Léonard, F. Bolze, J.-F. Nicoud, R. H. Kramer, M. Goeldner and A. Specht, New water-soluble, donor–acceptor biphenyl derivatives in the 2-(*o*-nitrophenyl)-propyl series for highly efficient two-photon uncaging of the neurotransmitter GABA at 800 nm, *Angew. Chem. Int. Ed. Engl.*, DOI: 10.1002/anie.201106559.

16 R. F. Chen, E. Scott and E. Trepman, Fluorescence properties of *o*-phthaldialdehyde derivatives of amino acids, *Biochim. Biophys. Acta*, 1979, **576**, 440–445.

17 J. W. Walker, G. Reid, J. A. McCray and D. R. Trentham, Photolabile 1-(2-nitrophenyl)ethyl phosphate esters of adenine nucleotide analogues. Synthesis and mechanism of photolysis, *J. Am. Chem. Soc.*, 1988, **110**, 7170–7177.

18 M. Rumi, J. E. Ehrlich, A. A. Heikal, J. W. Perry, S. Barlow, Z. Hu, D. McCord-Maughon, T. C. Parker, H. Röckel, S. Thayumanavan, S. R. Marder, D. Beljonne and J.-L. Brédas, Structure-property relationships for two-photon absorbing chromophores: Bis-donor diphenylpolyene and bis(styryl)benzene derivatives, *J. Am. Chem. Soc.*, 2000, **122**, 9500–9510.

19 I. Aujard, C. Benbrahim, M. Gouget, O. Ruel, J.-B. Baudin, P. Neveu and L. Jullien, *o*-Nitrobenzyl photolabile protecting groups with red-shifted absorption: Syntheses and uncaging cross-sections for one- and two-photon excitation, *Chem.–Eur. J.*, 2006, **12**, 6865–6879.

20 T. I. Wallow and B. M. Novak, Highly efficient and accelerated Suzuki aryl couplings mediated by phosphine-free palladium sources, *J. Org. Chem.*, 1994, **59**, 5034–5037; G. M. Allan, N. Vicker, H. R. Lawrence, H. J. Tutill, J. M. Day, M. Huchet, E. Ferrandis, M. J. Reed, A. Purohit and B. V. L. Potter, Novel inhibitors of 17β-hydroxysteroid dehydrogenase type 1: Templates for design, *Bioorg. Med. Chem.*, 2008, **16**, 4438–4456.

21 D. S. Durden, A. V. Juorio and B. A. Davis, Thin-layer chromatographic and high resolution mass spectrometric determination of beta-hydroxyphenylethylamines in tissues as dansyl-acetyl derivatives, *Anal. Chem.*, 1980, **52**, 1815–1820.

22 F. Bolze, J.-F. Nicoud, S. Gug, S. Charon, A. Specht, M. Goeldner, D. Warther, X.-H. Sun, P. Kessler, Y. Lutz, J.-L. Vonesch and A. Losonczy, Two-photon excitation in life sciences: neurotransmitter and fluorescence uncaging, *Proc. SPIE–Int. Soc. Opt. Eng.*, 2011, 790339.

23 M. Lovett-Barron, G. F. Turi, P. Kaifosh, P. H. Lee, F. Bolze, X.-H. Sun, J.-F. Nicoud, B. V. Zemelman, S. M. Sternson and A. Losonczy, Regulation of neuronal input transformations by tunable dendritic inhibition, *Nature Neurosciences*, DOI: 10.1038/nn.3024.

24 R. S. Givens and C.-H. Park, *p*-Hydroxyphenacyl ATP: A new phototrigger, *Tetrahedron Lett.*, 1996, **37** (35), 6259–6262; R. S. Givens, J. F. Weber, A. H. Jung and C. H. Park, New photoprotecting groups: Desyl and *p*-hydroxyphenacyl phosphate and carboxylate esters, *Methods Enzymol.*, 1998, **291**, 1–29.

