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Supporting Information

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Water-Soluble, Donor–Acceptor Biphenyl Derivatives in the 2-(*o*-Nitrophenyl)propyl Series: Highly Efficient Two-Photon Uncaging of the Neurotransmitter γ-Aminobutyric Acid at $\lambda = 800$ nm**

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SUPPORTING INFORMATION

General: All chemicals were purchased from Sigma-Aldrich, Alfa **A**esar 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 CARY 4000 spectrometer and fluorescence spectra on a Horiba Jobin Yvon Fluorolog.

Synthesis :

***tert*-butyl 2-(5-bromo-2-nitrophenyl)acetate (4)**

To a stirred solution of *t*-BuOK (12.8 g, 114 mmol) in anhydrous DMF (50 mL) was added through a cannula a solution of 4-Bromonitrobenzene (3.67 g, 18.2 mmol) and *tert*-butyl chloroacetate (4.26 g, 28.3 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. The crude was submitted to column chromatography on silica (Heptane / EtOAc: 9/1 to 8/2 in vol.) to afford a yellowish solid (5.52 g, 96 %).

RMN ^{1}H (400MHz, $CDCl_3$) : δ (ppm) = 1.41 (s, 9H), 3.89 (s, 2H), 7.48 (d, J=2.0 Hz, 1H), 7.57 (dd, J=8.5/2.0 Hz, 1H), 7.96 (d, J=8.5 Hz, 1H)

***tert*-butyl 2-(5-bromo-2-nitrophenyl)propanoate**

tert-butyl 2-(5-bromo-2-nitrophenyl)acetate (5.52g, 17.5 mmol) was dissolved in an anhydrous solution of sodium hydride (60 % in suspension in oil, 700 mg, 17.5 mmol) in 60 ml of THF. Methyl iodide (2.3 mL, 34 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 AcOEt. The extract was dried over

Na_2SO_4 and the combined extracts were evaporated. The crude was submitted to column chromatography on silica (Heptane / EtOAc: 9/1 to 8/2 in vol.) to afford a yellowish solid (4.78 g, 83 %).

RMN ^{1}H (400MHz, $CDCl_3$) : δ (ppm) = 1.40 (s, 9H), 1.57 (d, J=7.1 Hz, 3H), 4.21 (q, J=7.1 Hz, 1H), 7.54 (dd, J=8.5/2.0 Hz, 1H), 7.62 (d, J=2.0 Hz, 1H), 7.81 (d, J=8.5 Hz, 1H)

RMN ^{13}C (100MHz, $CDCl_3$) : δ (ppm) = 17.4, 27.7, 81.8, 126.3, 127.9, 131.0, 132.8, 137.7, 147.9, 171.6.

2-(5-bromo-2-nitrophenyl)propan-1-ol (3)

4.5 ml of DIBAL-H (4.5 mmol) were slowly added to a solution of *tert*-butyl 2-(5-bromo-2-nitrophenyl)propanoate (0.5 g, 1.51 mmol) in 19 ml of THF at 0°C. The mixture was stirred for 3 hours at the same temperature. 38 ml of an HCl solution (5 N) were added dropwise followed by 60 ml of ethyl acetate. The organic layer was washed with a solution of saturated NaCl and dried on anhydrous sodium sulfate. The organic layer was evaporated to obtain a dark red oil. The crude was submitted to column chromatography on silica (Heptane / EtOAc: 4:1 in vol.) to afford 0.33 g of bromo-2-nitrophenyl)propan-1-ol (**3**) (80 %) as a pale red oil.

RMN ^{1}H (400MHz, $CDCl_3$) : δ (ppm) = 1.32 (d, J=7.0 Hz, 3H), 3.55 (m, 1H), 3.78 (m, 2H), 7.49 (dd, J=8.5/2.1 Hz, 1H), 7.63 (d, J=2.1 Hz, 1H), 7.651 (d, J=8.5 Hz, 1H)

RMN ^{13}C (100MHz, $CDCl_3$) : δ (ppm) = 17.4, 36.4, 67.6, 125.7, 127.5, 130.4, 131.6, 140.5, 149.4

2-(4'-amino-4-nitro-[1,1'-biphenyl]-3-yl)propan-1-ol (5)

A mixture of (bromo-2-nitrophenyl)propan-1-ol (505 mg, 1.94 mmol), 4-amino-phenyl boronic acid hydrochloride (404 mg, 2.33 mmol), K_2CO_3 (725 mg, 5.24 mmol), Bu_4NBr (626 mg, 1.94 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 425 mg of the title compound in 81% yield.

RMN ^{1}H (400MHz, $CDCl_3$) : δ (ppm) = 1.40 (d, J=6.8 Hz, 2H), 2.19 (broad s, 2H), 3.69 (q, J=6.8 Hz, 1H), 3.81 (m, 2H), 6.75 (d, J=6.4 Hz , 2H), 7.44 (d, J=6.4 Hz, 2H), 7.50 (dd, J=8.4/2.0 Hz, 1H), 7.61 (d, J=2.0 Hz, 1H), 7.83 (d, J=8.4 Hz, 1H)

RMN ^{13}C (100MHz, $CDCl_3$) : δ (ppm) = 17.6, 36.5, 68.1, 111.8, 115.4, 124.7, 125.2, 125.7, 127.3, 129.0, 139.0, 145.9, 147.24.

CANBP Cage (6)

The amino-nitro biphenyl derivative (195 mg, 0.7165 mmol), DIEA (375 µl, 2.16 mmol) and tertio-butyl bromoacetate (289 µl, 1.79 mmol) were dissolved in anhydrous DMF (10 mL). The mixture was stirred at 90 °C under argon for 5 h. The reaction was quenched with saturated $NaHCO_3$ and extracted with AcOEt. The extract was dried over Na_2SO_4 and the combined extracts were evaporated and purified by flash chromatography using gradient elution of heptane/EtOAc : 8/2 to 1/1 in vol. gave 190 mg of the title compound in 40 % yield.

RMN ^{1}H (400MHz, $CDCl_3$) : δ (ppm) = 1.35 (d, J=6.8 Hz, 2H), 1.45 (s, 18H), 3.63 (q, J=6.8 Hz, 1H), 3.82 (m, 2H), 4.03 (s, 4H), 6.65 (d, J=8.8 Hz , 2H), 7.40-743 (m, 1H), 7.57 (d, J=2.0 Hz, 1H), 7.86 (d, J=8.4 Hz, 1H)

RMN ^{13}C (100MHz, $CDCl_3$) : δ (ppm) = 17.6, 24.4, 30.9, 54.5, 68.1, 82.0, 112.6, 124. 7, 125.2, 125.7, 128.2, 139.0, 145.8, 139.1, 148.4, 148.6, 169.7

2-(2-nitro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propan-1-ol (8)

A mixture of 2-(5-bromo-2-nitrophenyl)propan-1-ol **(3)** (346 g**,** 1.33 mmol), $PdCl_2$(dppf) (97 mg, 0.1 mmol), KOAc (163 mg, 1.66 mmol), and bis(pinacolato)diboron (372 mg, 1.46 mmol) in DMSO (10 mL) was heated to 80 °C overnight. The reaction mixture was poor into 50 mL ice-water slush and extracted with ethyl acetate (3x100 mL) and dried over $MgSO_4$. After evaporation of solvent, crude product was purified by flash chromatography using gradient elution of heptane/EtOAc : 8/2 to 1/1 in vol. to give 200 mg of **8 (**49 %).

RMN ^{1}H (400MHz, $CDCl_3$) : δ (ppm) = 1.31-134 (m, 14H), 3.41 (q, J=6.8 Hz, 1H), 3.80 (m, 2H), 7.65 (d, J=8.4 Hz, 1H), 7.74 (dd, J=8.4/1.2 Hz, 1H), 7.85 (d, J=1.2 Hz, 1H)

RMN ^{13}C (100MHz, $CDCl_3$) : δ (ppm) = 17.6, 24.9, 36.5, 67.9, 84.5, 122.9, 133.6, 133.7, 134.4, 136.8, 162.6.

4-iodo-N,N-bis(methoxyethyl)aniline (9)

N,N-bis(methoxyethyl)aniline **7** (688 mg 2.31 mmol), obtained as described by Ros-Li *et al.*[1], was dissolved in dioxane (15 mL) and pyridine (15 mL) and the solution was cooled to 0 °C. Iodine (1.76 g, 6.15 mmol) was added in one portion. The solution progressively took a dark brown colour. After 1 h, the ice bath was removed and a supplementary portion of iodine (566 mg, 2.31 mmol) was added. The solution was further stirred for one hour at room temperature. A saturated solution of sodium thiosulfate was then added until the brown color disappeared. The mixture was extracted with dichloromethane (200 mL) and washed with water (200 mL). After evaporation, the product was filtered through a short plug of silica, eluted with ethyl acetate, to afford 827mg of the 4-iodo-N,N-bis(methoxyethyl)aniline (**9**) (84 %) as a brown oil.

RMN ^{1}H (400MHz, $CDCl_3$) : δ (ppm) = 3. 35 (s, 6H), 3,50-3,60 (m, 16H), 6.47 (d, J=9.2 Hz, 2H), 7.39 (d, J=9.2 Hz, 2H)

RMN ^{13}C (100MHz, $CDCl_3$) : δ (ppm) = 50.9, 59.1, 68.3, 70.6, 72.0, 92.1, 114.1, 137.7, 147.4

EANBP Cage (10)

A mixture of 2-(2-nitro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propan-1-ol **(8)** (97 mg, 0.32 mmol), 4-iodo-N,N-bis(methoxyethyl)aniline **(9)** (161 mg, 0.38 mmol), K_2CO_3 (65 mg, 0.48 mmol), Bu_4NBr (345 mg, 0.72 mmol), and $Pd(OAc)_2$ (catalytic) in EtOH (10 mL) and water (5mL) was heated under microwave conditions at 150 °C for 10 min. Water (100 mL) was added and the organic extracted into EtOAc (200 mL). Purification by flash chromatography using gradient elution of heptane/EtOAc : 2/8 in vol. gave 105 mg of the EANBP Cage (**10)** in 70 % yield.

RMN ^{1}H (400MHz, $CDCl_3$) : δ (ppm) = 1.35 (d, J=7.2 Hz, 2H), 3.36 (s, 6H), 3.50-3.67 (m, 17H), 3.80-3.82 (m, 2H), 6.89 (d, J=8.8 Hz , 2H), 7.46-7.49 (m, 3H), 7.58 (d, J=2.0 Hz, 1H), 7.83 (d, J=8.8 Hz, 1H)

RMN ^{13}C (100MHz, $CDCl_3$) : δ (ppm) = 17.7, 36.5, 51.0, 59.1, 68.1, 68.4, 70.7, 72.0, 112.0, 124, 4, 125.3, 126.1, 128.3, 136.0, 139.1, 145.8, 148.1, 148.3

General procedure for coupling and deprotection of N-Boc-GABA's

The caged alcohols **6** or **10** (0.15 mmol) were dissolved in dry CH_2Cl_2 (15 mL), then N-Boc-GABA (61 mg, 0.3 mmol), DCC (46.5 mg, 0.22 mmol) an DMAP (2 mg) was added at 0°C. The solution was further stirred for 19 hours 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 : 8/2 or 3/7 in vol. as eluant for the synthesis of **1** or **2** respectively. Finally the protected caged GABAs were disolved in anhydrous CH_2Cl_2 (8 mL) and TFA (6 mL). After 5 h and 1 min. (for **1** and **2** respectively) at room temperature under argon the solution was evaporated to yield 84 mg (94 %) and 97 mg (96 %) of the TFA salt's of **1** and **2** respectively.

CANBP-GABA (1):

RMN ^{1}H (400MHz, MeOD) : δ (ppm) = 1.25 (d, J=7.2 Hz, 3H), 1.67-1.72 (m, 2H), 2.23 (t, J=7.2 Hz, 2H), 2.72 (t, J=7.2 Hz , 2H), 3.63 (q, J=7.2 Hz, 1H), 4.11 (s, 4H), 4.18-4.23 (m, 2H), 6.59 (d, J=8.8 Hz, 2H), 7.44-7.48 (m, 3H), 7.57 (d, J=1.6 Hz , 1H), 7.68 (d, J=8.4 Hz, 2H)
RMN ^{13}C (100MHz, MeOD) : δ (ppm) = 18.0, 23.7, 31.5, 34.2, 40.0, 55.6, 69.72, 97.6 (q, J=292 Hz), 113.5, 125.9, 126.1, 126.4, 129.1, 129.2, 139.2, 146.8, 149.3, 149.8, 163.3 (q, J= 37 Hz), 170.8, 173.7
MS (ESI-ACI): *m/z* = 474.2 [M+H]

EANBP-GABA (2):
RMN ^{1}H (300MHz, $CDCl_3$) : δ (ppm) = 1.34 (d, J=7.8 Hz, 3H), 1.86-1.92 (m, 2H), 2.34 (t, J=7.2 Hz, 2H), 2.92 (t, J=7.2 Hz , 2H), 3.50 (s, 6H), 3.57-3.63 (m, 16H), 3.81 (q, J=7.8 Hz, 1H), 4.18-4.28 (m, 2H), 6.76 (d, J=9.0 Hz, 2H), 7.42-7.46 (m, 3H), 7.51 (d, J = 3.0 Hz , 1H), 7.78 (d, J = 9.0 Hz, 2H)
RMN ^{13}C (75MHz, $CDCl_3$) : δ (ppm) = 14.4, 30.1, 31.2, 31.4, 39.5, 51.3, 59.5, 68.7, 70.3, 71.0, 72.3, 108.2 (q, J=292 Hz), 112.5, 124.9, 125.5, 125.7, 126.2, 128.6, 138.4, 146.2, 148.1, 148.8, 168.3 (q, J= 37 Hz), 173.1
MS (ESI-ACI): *m/z* = 562.2 [M+H], 584.2 [M+Na]

Quantification of GABA release:

A chromophoric derivative was coupled to the GABA moiety in CANBP-GABA and EANBP-GABA to quantify GABA release by HPLC.

Synthesis:

1*H*-benzo[1,2,3]triazol-1-yl-4-methoxybenzoate (4-methoxybenzoic 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 organics extracted into EtOAc (50 mL). Purification by flash chromatography using gradient elution of heptane/EtOAc : 1/1 in vol. gave 335 mg of a white powder in 95 % yield.

RMN ^{1}H (400MHz, $CDCl_3$) : 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 (EANPB-GABA and CANPB-GABA) (0.004 mmol) in anhydrous DMF or CH_2Cl_2, respectively, were stirred over night at room temperature. Purification by semi-preparative C-18 HPLC with a 250x10 BetaBasic-18 column from Thermo using elution at a flow rate of 4 mL/min with a linear gradient of acetonitrile in 0.1% TFA in water from 0 to 100% (v/v) over 30 min gave solids after evaporation. Retention time : 21.5 min for the EANPB-GABA-MBA, 19.7 min for the CANPB-GABA-MBA and 13.7min for GABA-MBA.

GABA-MBA

RMN ^{1}H (400MHz, $CDCl_3$) : 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).

EANPB-GABA-MBA

HRMS (ESI- QTof) calculated for [$C_{37}H_{49}N_3O_{10}$] 696.349, found 696.349;

CANPB-GABA-MBA

MS (ESI-ACI) calculated for [$C_{31}H_{32}N_3O_{10}$]$^-$ 606.2, found 606.0;

Quantification of GABA release

The yield of released GABA-MBA from irradiated EANPB-GABA-MBA and CANPB-GABA-MBA is determined by HPLC analysis. A 1.5mL portion of a 50 µM solution of EANPB-GABA-MBA or CANPB-GABA-MBA in phosphate buffer (50 mM) was irradiated at 405 nm to ensure a complete conversion of the starting compound using a LUMOS 43 (Atlas Photonics Inc.). 200µL of each irradiated solutions were analyzed by HPLC and compared to the HPLC calibration curve (SI Figure 1) analysis of a solution of GABA-MBA in phosphate buffer.

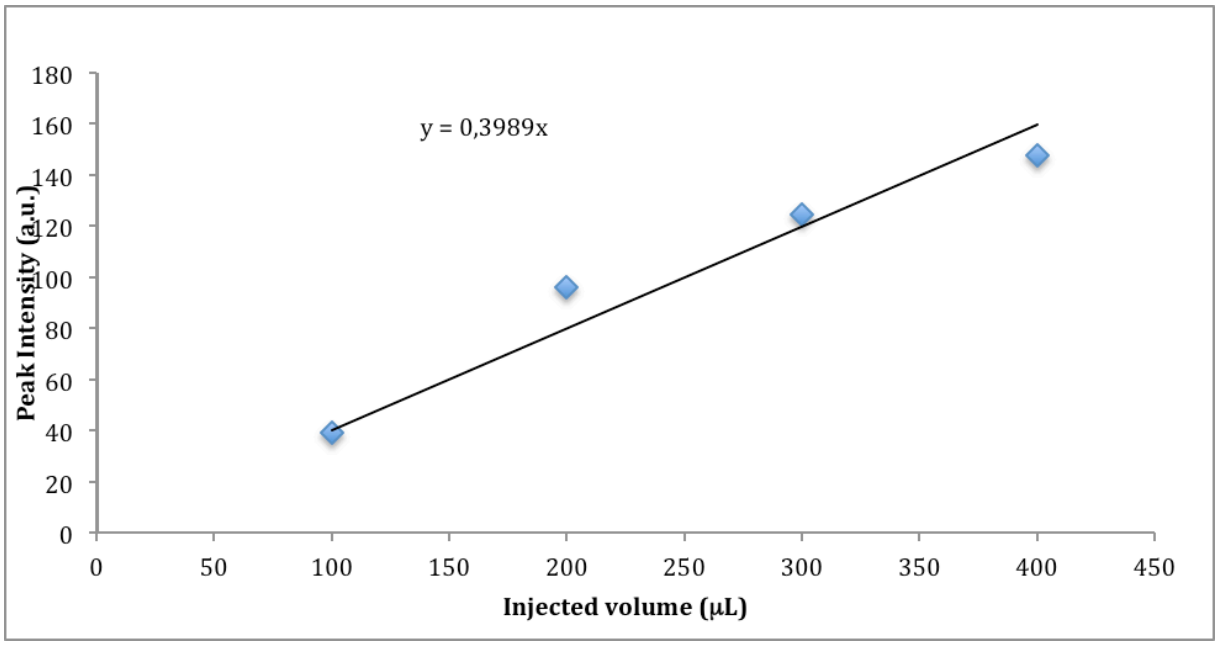
HPLC analysis was carried out on Acclaim Analytical SB-C18 (4.6 x 250 mm) column; elution was performed at a flow rate of 1 mL/min with a linear gradient of acetonitrile in 0.1% TFA in water from 0 to 100% (v/v) over 30 min.

Retention time: 25.9 min for EANPB-GABA-MBA, 23.1 min for CANPB-GABA-MBA and 15.8 min for GABA-MBA

GABA-MBA calibration curve

A solution of GABA-MBA at 100 µM in phosphate buffer (50 mM) / MeOH : 1/1 was prepared. 100µL to 400 µL of this solution was analyzed by HPLC.

SI Figure 1: GABA-MBA calibration curve.



Quantum yield determination :

The quantum yield for the photoconversion of compounds **9**, was determined by comparison with the photolysis of 1-(2-nitrophenyl)ethyl-ATP (NPE-ATP) (Φ = 0.63)[2] 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 **1** or **2** and 0.2 mM of NPE-ATP reference were used. Those mixtures were photolyzed by continuous irradiation at 315 nm using a 1000W 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 Zorbax (4.6 x 250 mm) column; elution was performed at a flow rate of 1 mL/min with a linear gradient of acetonitrile in 0.1 % TFA in water from 0 to 100 % (v/v) over 30 min. The retention times of **1**, **2** and NPE-ATP were 18.6, 20.5 and 11.8 min respectively. Quantum yields 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.

Two-Photon Uncaging Action Cross-Section at 800 nm.

The two-photon uncaging action cross section was determined by comparison with a PENB-DDAO[3] taken as a reference. Each compound was dissolved in a 1/1 (in vol.) pH 7.1 phosphate buffer and acetonitrile mixture to reach the same optical density (O.D. = 0.49) at 400 nm. 100 μL of each was

separately irradiated at 800 nm using a mode-locked titanium:sapphire laser, Tsunami, Spectra Physics, 100 fs, 80 MHz. The measurements were performed at P = 400 mW, in the quadratic dependence regime. Each irradiated sample is analyzed by HPLC to determine the percentage of unreacted caged GABA and a calibration curve was established to determine the percentage of liberated DDAO (percentage of liberated DDAO=1-percentage of unreacted PENB DDAO). A linear regression is performed in the linear range (% of photolysis < 20 %), to limit as much as possible errors due to undesired light absorption during photolysis and to access to Grad(Substance) and Grad(Reference). Typically, the uncaging is quantified by HPLC after 15 to 60 minutes of irradiation. The two-photon uncaging action cross-section of the studied caged compound is given by the formula :

$$\delta_a\phi_u(\text{Substance}) = \delta_a\phi_u\ (\text{Reference}) \times \text{Grad[Substance]/Grad[Reference]}$$

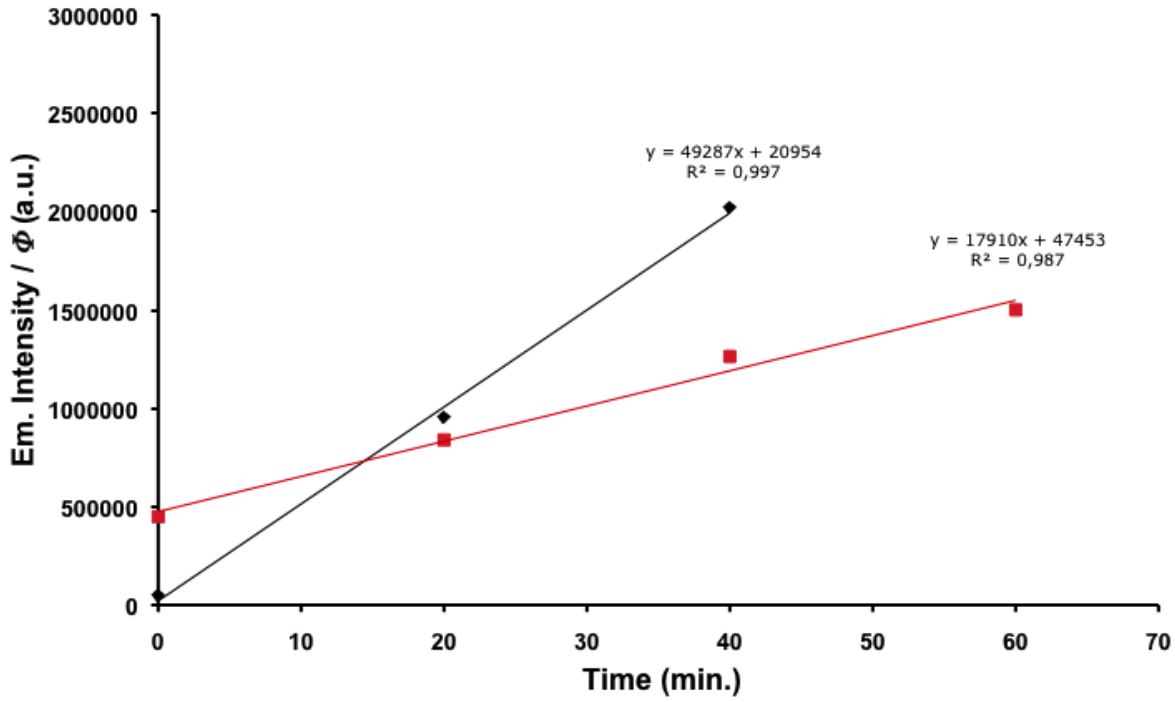
Where $\delta_a\phi_u$ (Reference) = 0.45 for PENB-DDAO

The two-photon uncaging action cross-section of the reference used in this study: PENB- 1,3-dichloro-9,9-dimethyl-9*H*-acridin-2(7)-one was cross-correlated with the one of a other caged fluorophore obtained by a different method : ethyl 3,5-dibromo-2,4-dihydroxycinnamate (measured by FCS, 1 GM at 800 nm).[4] Each compound was dissolved in a 1/1 (in vol.) pH 7.1 phosphate buffer and acetonitrile mixture to reach the same optical density (O.D. = 0.24) at 400 nm. 100 μL of each was separately irradiated at 800 nm using the same setup previously described. Each irradiated sample was analyzed by spectrofluorimetry to determine the percentage of released fluorescent moieties. A linear regression is performed in the linear range to access to Grad(Em. Intensity/Φ_f)$_{\text{Substance}}$ and Grad(Em. Intensity/Φ_f)$_{\text{Reference}}$. Where Φ_f is the fluorescence quantum yield and Em. Intensity is the integral of the corrected fluorescence spectrum. The two-photon uncaging action cross-section of the studied caged compound is given by the formula :

$$\delta_a\Phi_{u(\text{Substance})} = \delta_a\Phi_{u(\text{Reference})} \times \text{Grad(Em.Intensity/}\Phi_f)_{\text{Substance}} / \text{Grad(Em.Intensity/}\Phi_f)_{\text{Reference}}$$

$$\delta_a\Phi_{u(\text{PENB-DDAO})} = 1 \times 17910 / 49287 = 0.36\ \text{GM}$$

this value of 0.36 GM is in good agreement with the previously published one (0.45 GM) with 15 % uncertainties on the two-photon uncaging action cross-sections.

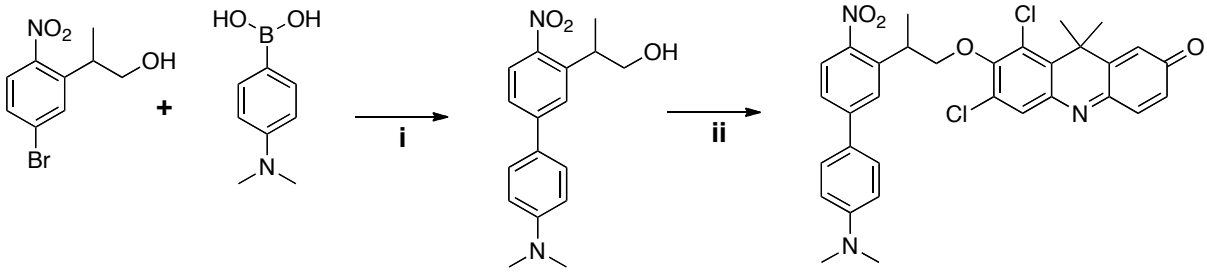


SI Figure 2: Spectrofluorimetric analysis of the fluorescent moieties release after 800nm two-photon excitation of PENB- 1,3-dichloro-9,9-dimethyl-9*H*-acridin-2(7)-one and ethyl 3,5-dibromo-2,4-dihydroxycinnamate

Kinetic Measurements.

Synthesis of ANBP-DDAO (12)

The synthesis of ANBP-DDAO is summarized in scheme S1



SI Scheme 1: Synthesis of ANBP-DDAO. i. K_2CO_3, Bu_4NBr, $Pd(OAc)_2$, $EtOH/H_2O$, ii. triphenylphosphine / diisopropyl azodicarboxylate, DDAO, THF.

2-(4'-(dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl)propan-1-ol (**11**):

A mixture of (5-bromo-2-nitrophenyl)propan-1-ol (**3**) (300 mg, 1.15 mmol), (4-(dimethylamino)phenyl)boronic acid (286 mg, 1.73 mmol), K_2CO_3 (397 mg, 2.87 mmol), Bu_4NBr (370 mg, 1.15 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 319 mg of the title compound in 92% yield.

RMN ^{1}H (400MHz, $CDCl_3$) : δ (ppm) = 1.40 (d, J=6.8 Hz, 3H), 3.71 (sex, J=6.8 Hz, 1H), 3.85 (m, 2H), 6.84 (d, J=8.0 Hz , 2H), 7.53 (m, 3H), 7.63 (d, J=2.0 Hz, 1H), 7.89 (d, J=8.0 Hz, 1H)

RMN ^{13}C (100MHz, $CDCl_3$) : δ (ppm) = 17.6, 36.5, 40.4, 68.1, 112.6, 124.4, 125.2, 125.4, 128.1, 139.0, 145.9, 148.1

ANBP-DDAO (**12**):

In a dry 25 mL round flask under argon, DDAO (50 mg ; 1.6×10^{-4} mol) and 2-(4'-(dimethylamino)-4-nitro-[1,1'-biphenyl]-3-yl)propan-1-ol (**11**) (49 mg ; 1.6×10^{-4} mol) are dissolved in 1.2 mL of anhydrous THF.

In a dry 10 mL round flask under argon, triphenylphosphine (87 mg ; 3.2×10^{-4} mol) and diisopropyl azodicarboxylate (52 μL ; 3.2×10^{-4} mol) are dissolved in anhydrous THF (1.2 mL) and stirred 5 minutes before being slowly added to the previous mixture. The 10 ml flask is washed with 1.2 mL of anhydrous THF, which is then added to the reaction mixture.

The mixture is stirred 2h at room temperature. A second addition of triphenylphosphine (87 mg ; 3.2×10^{-4} mol) and diisopropyl azodicarboxylate (52 μL ; 3.2×10^{-4} mol) dissolved in 1.2 mL of anhydrous THF is then performed. The mixture is then stirred under argon at room temperature for 4 additional hours.

After reaction, the solvent is removed under vacuum. The crude mixture is the purified by flash chromatography under a gradient of heptane/ethyl acetate (9/1 to 5/5 ; v/v). ANBP-DDAO is obtained as a dark-orange very viscous oil with a 35% yield (32mg; 5.42×10^{-5} mol).

RMN ^{1}H (300MHz, DMSO-d_6) : δ (ppm) = 9.91 (d ; J = 2 Hz ; 1H) ; 7.90 (d ; J = 6 Hz ; 1H) ; 7.77 (s ; 1H) ; 7.68 (dd ; J = 9 Hz and 2 Hz ; 1H) ; 7.64 (d ; J = 9 Hz ; 2H) ; 7.42 (d ; J = 10 Hz ; 1H) ; 6.87 (s ; J = 2 Hz ; 1H) ; 6.80 (d ; J = 9 Hz ; 2H) ; 6.68 (dd ; J = 10 Hz and 2 Hz ; 1H) ; 4.44 (t ; J = 8 Hz ; 1H) ; 4.28 (dd ; J = 9 Hz and 6 Hz ; 1H) ; 3.91 (m ; 1H) ; 2.96 (s ; 6H) ; 1.68 (d ; J = 4 Hz ; 6H) ; 1.48 (d ; J = 6 Hz ; 3H).

RMN ^{13}C (100MHz, DMSO-d_6) : δ (ppm) = 186.8 ; 152.5 ; 152.5 ; 150.7 ; 147.9 ; 147.5 ; 144.8 ; 140.7 ; 139.6 ; 137.9 ; 133.5 ; 132.1 ; 131.8 ; 129.1 ; 128.4 ; 127.9 ; 127.8 ; 127.0 ; 125.0 ; 125.0 ; 124.9 ; 124.1 ; 112.4 ; 77.1 ; 37.6 ; 34.6 ; 29.0 ; 28.0 ; 28.0 ; 17.8.

MS (ESI+): m/z = 590.2 $[M+H]^+$ (^{35}Cl-^{35}Cl); 592.2 $[M+H]^+$ (^{35}Cl-^{37}Cl).

λ_{max} = 402 nm ; ε = 33000 $M^{-1}.cm^{-1}$.

In order to prepare a stock solution in DMSO, ANBP-DDAO was purified by HPLC with a gradient of aqueous ammonium acetate 0.2M / acetonitrile from 0 to 100% of acetonitrile to insure perfect purity of the samples.

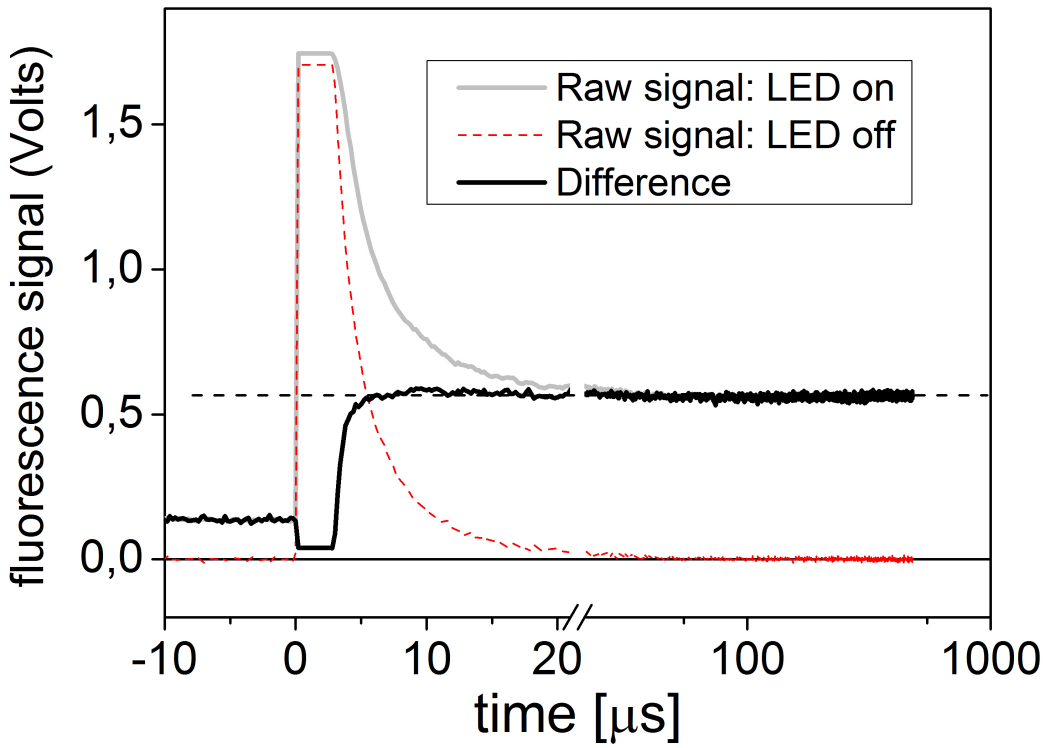
Purified ANPB-DDAO was then dissolved in DMSO (concentration : 1.96×10^{-3}M) and stored at room temperature in the dark.

The samples used in kinetics measurements were prepared by a 100 times dilution of the DMSO solution in a mixture of phosphate buffer (pH7.4) / acetonitrile (1:1 in vol.).

Kinetics measurement for the DDAO photorelease.

The kinetics of the photolysis reaction is tentatively followed in time by monitoring the fluorescence of the released DDAO. A 100-µL solution of the caged fluorophore ANBP-DDAO (**12**) in a mixture of phosphate buffer / acetonitrile (1:1 in vol.; OD = 0.35 at 355 nm) is homogeneously and continuously illuminated by a 596-nm Light Emitting Diode (LED). Hardly any fluorescence is emitted by the caged compound. At time zero, the photolysis is triggered by a single, nanosecond laser pulse at 355 nm (15 mJ/cm^2; third harmonic of a nanosecond YAG laser). The photolysis pulse is absorbed in the entire volume by the ANBP cage, triggering the homogeneous photorelease of the DDAO fluorophore. Its fluorescence is detected as a function of time with a PhotoMultiplier Tube (PMT) at λ>630 nm (color filter).

Figure S3 shows the detected fluorescence as a function of delay time. At time zero, the very large excitation intensity of the photolysis UV pulse generates intense parasitic nanosecond-long emission, which saturates the detector over microseconds. This initial intense signal can be recorded separately (red dashed line), and subtracted to the overall detected signal. Such data processing does not yield reliable kinetics for time delays shorter than 10 to 15 µs due to detector saturation. However, it clearly shows (solid black line) that the asymptotic fluorescence level is reached within the signal-to-noise level (< 5%) in less than 15 µs. Simply assuming that the photolysis obeys a monoexponential kinetics, we conclude that the corresponding time constant is less than 5 µs.



SI Figure 3: Kinetics of the impulsive photolysis of ANBP-DDAO (**12**) in acetonitrile/phosphate buffer (pH 7.4) mixture (1:1). The solid grey line shows the signal obtained while the LED is on: after a strong initial peak due to the intense photolysis pulse, the asymptotic fluorescence level (horizontal dashed line) is reached in less than 40 µs. The red dashed signal is recorded while the LED is off: only the initial peak is measured. The solid black line shows the difference of both, showing that the asymptotic fluorescence level is actually reached before 15 µs. This indicates that the photolysis occurs on a typical time scale shorter than 5 µs. Note, that between 0 and ~10 µs, the initial parasitic pulse is not reliably subtracted (detector saturation), and the retrieved kinetics is of no meaning.

Electrophysiology Methods :

Animal care and experimental protocols were approved by the University of California Berkeley Animal Care and Use Committee.

Slice preparation: Postnatal day 14-16 Sprague-Dawley rat pups were anaesthetized by isofluorane inhalation and decapitated. Brains were removed and sliced into 380 µm thick sections using a vibrating microtome (Leica, Buffalo Grove, IL). Slicing and recording were done in artificial cerebrospinal fluid (ACSF) containing in mM: 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 10 Glucose, 1.3 $MgCl_2$, 26 $NaHCO_3$ and 2.5 $CaCl_2$ equilibrated with 95% O2/5% CO_2. Slices were incubated at 32 ºC for 30 min and RT for 1 hr prior to recording.

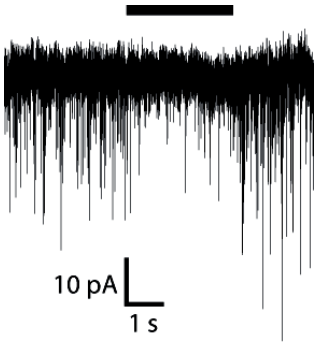
Primary culture: We prepared hippocampal neurons from neonatal Sprague-Dawley rats according to standard procedures[5], plated them at 100,000 cells cm^{-2} on poly(L-lysine)–coated coverslips and grew them in minimum essential medium containing 5% FBS, 20 mM glucose, B27 (Invitrogen), 2 mM glutamine and Mito+ Serum Extender (BD Biosciences). We recorded currents 2-3 weeks after plating.

Slice physiology: Slices were placed in a recoding chamber mounted on a custom-built microscope equipped with infrared optics for visualization of neurons and with a tunable Ti-sapphire laser (Chameleon, Coherent, Santa Clara, CA) for two photon fluorescence excitation and uncaging. Two photomultiplier tubes (Hamamatsu, Bridgewater, NJ) with bandpass filters (Chroma, Bellows Falls, VT) were used for fluorescence detection of green (475-545 nm) and red (545-680 nm) emission. Layer 2/3 cortical pyramidal cells were targeted for whole cell voltage clamp recording with glass microelectrodes (5-7 MΩ) filled with internal solution containing in mM: 108 Cs-gluconate, 20 HEPES, 5 TEACl, 2.8 NaCl, 0.4 EGTA, 4 ATP-Mg, 0.3 GTP-Na, 0.25 Alexa-fluor 488 hydrazide. Cells were held at 0 mV to isolate inhibitory currents. Cell morphology was visualized with 900 nm laser excitation of Alexa 488. 1 mM CANBP-GABA **1** in ACSF with 0.5 % DMSO was applied from a broken patch pipette placed lateral to the proximal apical dendrite. A red fluorescent dye (5 μM sulforhodamine 101) was included with CANBP-GABA **1** to visualize the application area. 800 nm laser (45 mW) was scanned over a dendritic area (total exposure time ~25 ms) to uncage GABA. Neuronal currents were amplified (Axopatch, Molecular Devices, Sunnyvale, CA), digitized (Digidata, Molecular Devices) and recorded (pClamp, Molecular Devices) to computer. TP uncaging and image acquisition were controlled by Scanimage software running in Matlab.

Cultured-neurons physiology: Voltage-clamp recordings were performed at room temperature and in the dark using standard whole cell patch-clamp techniques. Pipette resistance was 3-5 mΩ. Signals were amplified using a Patch Clamp PC-505B amplifier (Warner Instruments), filtered at 2 kHz, digitized at 10 kHz using a Digidata 1200 converter (Molecular Devices) and acquired using Clampex 8 (Molecular Devices). Perfusion was manually controlled. Holding potential was -60 mV. The extracellular solution contained (in mM): 138 NaCl, 1.5 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 5 HEPES, 10 Glucose; pH 7.4. Action potentials were blocked using 1 μM TTX. Excitatory events were blocked using AMPA (25 μM DNQX) and NMDA receptor antagonists (50 μM APV). The internal solution contained (in mM): 140 CsCl, 4 NaCl, 2 $MgCl_2$, 10 HEPES, 10 EGTA, 2 Mg-ATP; pH 7.4.

SI Figure 4: $GABA_A$ receptor antagonism by **1**. Spontaneous mIPSCs were recorded before, during and after bath perfusion of 100 μM **1**. Cells were held at -70 mV and pipette contained a high-chloride

concentration. GABAergic currents were isolated using NMDA and non-NMDA antagonists. GABAergic events were partially blocked by **1** but quickly recovered during washing (n = 3 cells).



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