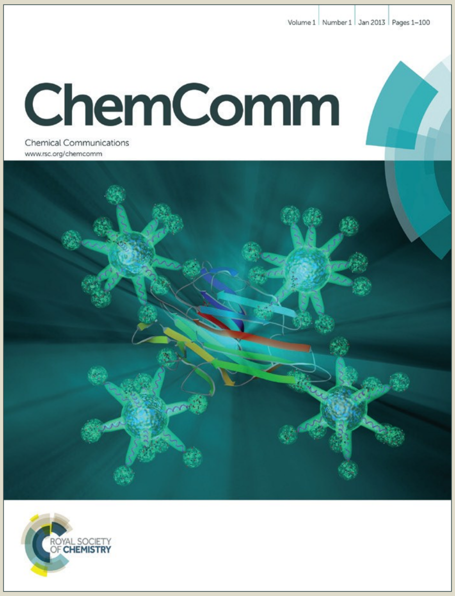
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

ChemComm

COMMUNICATION

Design and synthesis of a new chromophore, 2-(4-nitrophenyl)benzofuran, for two-photon uncaging using near-IR light†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Naomitsu Komori,[a] Satish Jakkampudi,[a,b] Ryusei Motoishi,[a] Manabu Abe,*[a,b,c] Kenji Kamada,*[d] Ko Furukawa,[e] Claudine Katan,*[f] Wakako Sawada,[g] Noriko Takahashi,[g] Haruo Kasai,[b,g] Bing Xue,[b,h] and Takayoshi Kobayashi[b,h]

A new chromophore, 2-(4-nitrophenyl)benzofuran (NPBF), was designed for two-photon (TP) uncaging using near-IR light. The TP absorption (TPA) cross-sections of the newly designed NPBF chromophore were determined to be 18 GM at 720 nm and 54 GM at 740 nm in DMSO. The TP uncaging reaction of a caged benzoate with the NPBF chromophore quantitatively produced benzoic acid with an efficiency (δ_u) of ~5.0 GM at 740 nm. The TP fragmentation of a EGTA unit was observed with δ_u = 16 GM. This behavior makes the new chromophore a promising TP photoremovable protecting group for physiological studies.

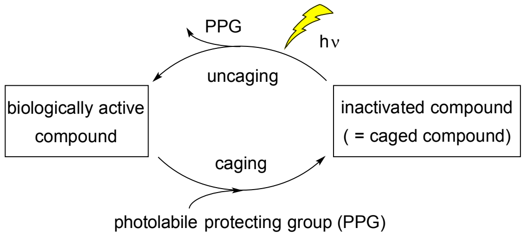


Fig 1. Caging and uncaging of biologically active compounds upon photolysis.

Caged compounds[1] have attracted considerable interest in physiological studies, e.g. in neuroscience, due to their many applications.[2-5] These compounds, in which biologically active molecules are inactivated by photoremovable protecting groups (PPGs)[6], can uncage (= release) the bioactive molecules upon photolysis (Fig. 1).[4] Spatial and temporal control of the uncaging process allows detailed investigation of the role of bioactive molecules *in vivo*.

Two-photon absorption (TPA) by near-IR irradiation of light (approximately 700-1300 nm) is increasingly used for photolysis in biological studies because most living tissues have low absorption and scattering in this wavelength region.[5,7] In addition, TPA enables spatial selective excitation of the small volume near the focal point of the laser beams. Thus two-photon (TP) excitation affords uncaging with higher spatial resolution, less photodamage to cells and tissues, and deeper penetration depth than one-photon (OP) excitation processes.[5,7] However, to work under the physiological conditions, a high uncaging efficiency is required. This efficiency is defined as the uncaging quantum yield (ϕ_u) multiplied by excitation probability.[8] The excitation probability can be expressed with the molar extinction coefficient (ε) for OP excitation or with TPA cross-section (σ_2) for TP excitation. From this convention, TP-efficiency (δ_u = ϕ_u⍰⍰⍰σ_2) has the same unit as the TPA cross section (GM). For biological applications, the minimum δ_u threshold value currently accepted is 3 GM.[9,10] This calls for development of biocompatible caged compounds with sizeable δ_u to ensure efficient release with tissue-permeable near-IR irradiation, providing valuable targets for applications in *in vivo* physiological studies.

Several PPGs with TPA character (TP-PPGs) have been reported so far.[11,12] For instance, in 2006, Ellis-Davis and co-workers reported a nitro-dibenzofuran (NDBF) skeleton with a planar biphenyl structure (Fig. 2). A caged calcium with the NDBF chromophore has high ε (18,400 $M^{-1}cm^{-1}$) and ϕ_u (0.7) in the OP excitation process,[12] but a low δ_u (~0.6 GM) in an aqueous buffer solution at 720 nm photolysis. Such a small δ_u is not surprising, given that NDBF has a dipolar character with an OPA maximum at 331 nm,[13] that predicts the lowest energy TPA maximum near 660 nm, far away from 720 nm.[14a] This prompted us to design a new chromophore having a red-shifted absorption band.

The extension of the π-conjugation system is an efficient way to bathochromically shift the absorption maximum and

---

[a] Department of Chemistry, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-hiroshima, Hiroshima 739-8526, Japan.
[b] JST-CREST, K's Gobancho, 7, Gobancho, Chiyoda-ku, Tokyo 102-0076, Japan.
[c] Research Centre for Smart Materials, Hiroshima University, 1-3-1 Kagamiyama, Higashi-hiroshima, Hiroshima 739-8526, Japan.
[d] IFMRI, National Institute of Advanced Industrial Science and Technology (AIST), 1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan.
[e] Centre for Instrumental Analysis, Niigata University, 8050 Ikarashi 2-no-cho, Nishi-ku, Niigata 950-2181, Japan.
[f] Institut des Sciences Chimiques de Rennes, UMR 6226 CNRS-Universite Rennes 1, 35042 Rennes, France.
[g] Laboratory of Structural Physiology, CDBIM, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan.
[h] Advanced Ultrafast Laser Research Center, The University of Electro-Communications, Chofugaoka 1-5-1,Chofu,Tokyo 182-8585, Japan.

† Electronic Supplementary Information (ESI) available: Experimental section, compound characterizations and computational details. See DOI: 10.1039/x0xx00000x

also improve the TP properties of chromophores.[15] At the same time, one may want to retain a simple synthetic scheme.

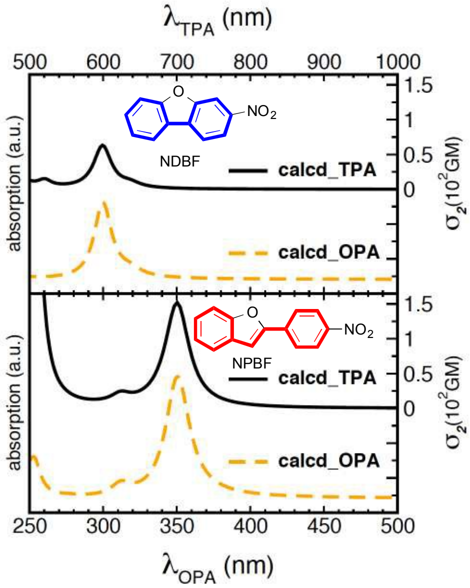


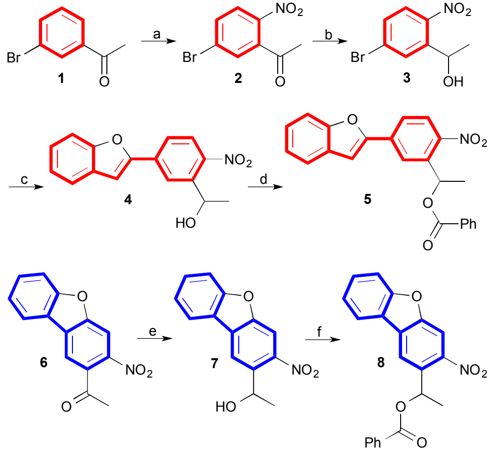
Fig. 2 Calculated OPA (dashed lines) and TPA (solid lines) spectra of NDBF (top panel) and NPBF chromophores (bottom panel).

In our previous study,[16] a stilbene skeleton was chosen for its inherently high TPA cross-section of 12 GM at 514 nm, in spite of its small π-conjugated portion.[17,18] To avoid cis-trans isomerization in the excited state, the 1,2-dihydronaphthalene cyclic structure was chosen for the TP-uncaging reaction.[16]

We report herein the design and synthesis of a new TP-PPG with a 2-(4-nitrophenyl)benzofuran (NPBF) chromophore. The dipolar character of the donor-π-acceptor skeleton with a cyclic stilbene structure is promising to increase the TPA cross-section (Fig. 2).[14,15] For the comparison, the TP uncaging reaction of a caged benzoate **5** with NPBF is also investigated under irradiation in the 700-760 nm range.

First, the OPA and TPA spectra of the parent NPBF were computed at the TD-B3LYP/6-31G*//HF/6-31G* level of theory and compared with those of the NDBF core (Fig. 2). This level of theory has been shown to provide good predictions for structure-TPA relationships.[14a] A detailed discussion of the accuracy of calculated TPA cross sections can be found in the ESI. As expected, the extended π-conjugation system of the NPBF chromophore led to a sizable TPA cross-section of about 150 GM at longer wavelength of approximately 700 nm in gas phase.[19] On the other hand, the NDBF chromophore showed a relatively small TPA cross-section of about 50 GM at a shorter wavelength of about 600 nm. The NPBF calculations prompted us to synthesize the caged benzoate **5** and investigate its TP photochemical reactivity. To compare the TP uncaging reaction of the NPBF chromophore with the NDBF chromophore, the caged benzoate **8** was also prepared.

The syntheses of the caged benzoates **5** and **8** are summarized in Scheme 1. The key intermediate 1-(5-bromo-2-nitrophenyl)ethane-1-ol **3** was prepared using a modified method from our previous work, starting with 3-bromoacetophenone **1**.[16] Next, 1-(5-(benzofuran-2-yl)-2-nitrophenyl)ethane-1-ol **4**, a compound exhibiting a rigid stilbene structure, was synthesized in 95% yield by reacting **3** with commercially available 2-benzofuranboronic acid through a Suzuki-Miyaura cross-coupling.[20] 1-(3-Nitrodibenzo[b,d] furan-2-yl)ethane-1-one **6** was prepared according to a previously reported synthetic method,[12] and was then rapidly reduced with $NaBH_4$ to provide the corresponding alcohol **7**, exhibiting an NDBF core. Finally, the benzoic acid (BA) moiety was introduced to alcohols **4** and **7** to provide the caged benzoates NPBF-BA **5** and NDBF-BA **8** in 64% and 56% yields, respectively.[21]



Scheme 1 Synthesis of **5** and **8**. a) KNO_3, H_2SO_4, 0 °C to RT, 2h, 68%. b) $NaBH_4$, MeOH, 0 °C to RT, 1 h, 95%. c) $Pd(PPh_3)_4$, K_2CO_3, 2-benzofuranboronic acid, THF/water (1:1), 80 °C, 15 h, 95%. d) Benzoic acid, DCC, DMAP, CH_2Cl_2, RT, 16 h, 64%. e) $NaBH_4$, CH_2Cl_2/MeOH (1:1), 0 °C to RT, 1 h, 76%. f) Benzoic acid, DMAP, DCC, CH_2Cl_2, RT, 23 h, 56%. THF = tetrahydrofuran, DMAP = 4-dimethylaminopyridine, DCC = N,N'-dicyclohexylcarbodiimide.

The absorption spectra of **5** and **8** were measured in DMSO (Fig. S14, ESI). The absorbance maxima of **5** and **8** are found at 364 nm (ε = 12000 $M^{-1}cm^{-1}$) and 320 nm (ε = 9000 $M^{-1}cm^{-1}$), respectively. The red shift and increased absorbance of **5** were found and consistent with the theoretical predictions.

The photolytic release of carboxylic acid[22] from compounds **5** and **8** was investigated by irradiation with a Xe-lamp at 360 ± 10 nm in DMSO-d_6 (Figs. 3 and 4). The reaction was monitored using ^1H NMR (400 MHz). The NMR signals of benzoic acid in the aromatic region are shown in Figs. 3e and 4e, with the overall time evolution of the reaction demonstrating a quantitative uncaging, larger than 90%. The uncaging quantum yields ϕ_u were determined in DMSO using the photochemical actinometer ferrioxalate coupled with high-performance liquid chromatography (HPLC), which led to 0.09 and 0.21 for **5** and **8**, respectively. Although the reported uncaging quantum yield of NDBF-EGTA[12] is 0.7, that of the measured NDBF-BA species is 0.21, despite having the same core. This indicates substituent effects on the benzyl position of the *o*-nitrobenzyl caging groups.[4] The OP photochemical efficiency (ε_{360} x ϕ_u) of the uncaging reaction of **5** (ε_{360} = 12000) at 360 nm was 1080, which was nearly the same as the value of 1071 obtained for **8** (ε_{360} = 5100).

The TPA cross-sections of the parent cores of NPBF and NDBF were measured at 720 nm by the Z-scan method, in which the sample was moved along the path of the laser beam and the light intensity was measured by a detector as a function of its position on the Z-axis.[23] Values of 18 ± 3 and 6 ± 1 GM were obtained for the NPBF and NDBF cores, respectively, at 720 nm in DMSO. Detailed information for the determination of the values can be found in the ESI.

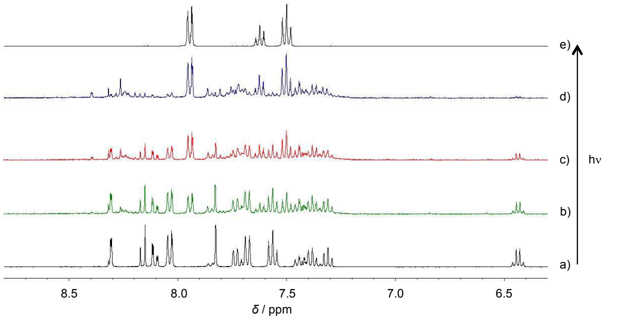


Fig. 3 ^{1}H NMR spectra of compound **5** in DMSO-d_6 (a) initially and after (b) 6, (c) 12, (d) and 24 h of irradiation at 360 nm; (e) the ^{1}H NMR spectrum of benzoic acid in DMSO-d_6.

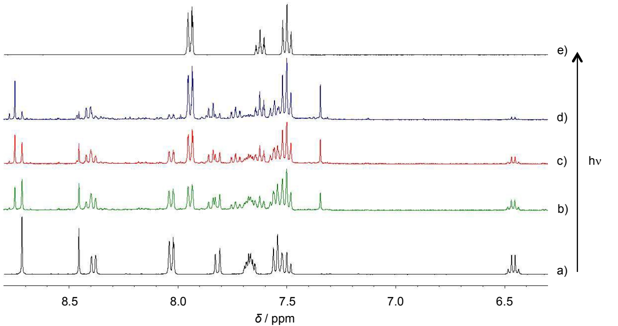


Fig. 4 ^{1}H NMR spectra of compound **8** in DMSO-d_6 (a) initially and after (b) 1, (c) 4, (d) and 6 h of irradiation at 360 nm; (e) the ^{1}H NMR spectrum of benzoic acid in DMSO-d_6.

TP photolysis of **5** and **8** was carried out in DMSO using 700, 710, 720, 730, 740, 750, and 760 nm light from a Ti:sapphire laser (pulse width 100 fs, 80 MHz) emitting at the average of 700 mW. The consumption of **5** and **8** and the production of benzoic acid upon photolysis were monitored by HPLC (Fig. 5).

As shown in Figure 5, the TP-uncaging rates were found to vary depending on the excitation wavelength. The fastest rate of **5** (k_{740} = 9.4 x 10^{-6} s^{-1}) was found at 740 nm while that of **8** was at 700 nm (k_{700} = 8.0 x 10^{-6} s^{-1}). The data shows the improved performance of the NPBF structure in **5** for the new chromophore. The other rate constants of TP uncaging reaction of **5** and **8** were measured in similar ways using the near-IR photolyses (Fig. 5).

The rate constant of **5** at 720 nm was 3.1 x 10^{-6} s^{-1} (Fig. 5a), about one third of the value that was observed at 740 nm. Since the absolute value of the TPA cross-section of the NPBF core was 18 GM at 720 nm, the corresponding value at 740 nm can be extrapolated to be about 54 GM. This would give a δ_u value of 5.0 GM (54 GM x 0.09), which is higher than the threshold value of δ_u > 3 GM.[9,10]

The TPA spectra of **5** and **8** over a wavelength range of 700-760 nm were extrapolated based on the TPA cross-section values at 720 nm of the two species (Fig. 6). The new NPBF species clearly shows highly improved TPA in the near-IR region when compared to the previously reported NDBF derivatives.

The new TP-PPG, NPBF chromophore, was successfully applied for the photochemical decomposition of ethylene glycol tetraacetic acid (EGTA) derivative NPBF-EGTA **9** (Figure 6b). The EGTA unit is important for caging Ca^{2+}.[12,24] The TP-induced fragmentation of the EGTA unit was observed with δ_u = 16 GM (ϕ_u = 0.3) at 740 nm in benzene (Figures S17,18).

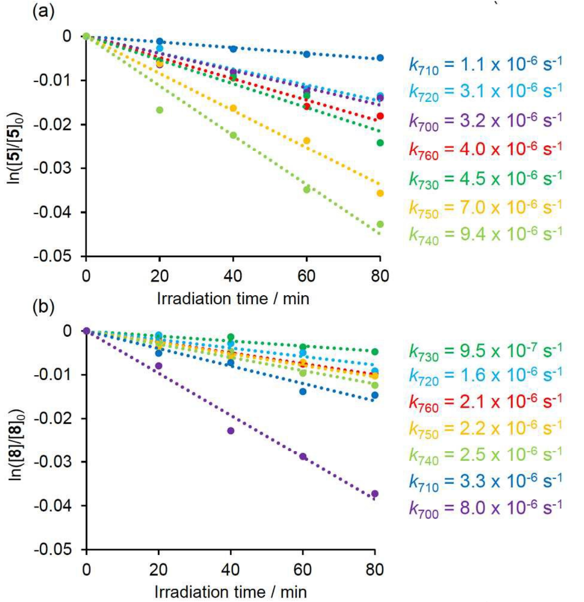


Fig. 5 Time profile of TP uncaging of (a) **5** and (b) **8**, ln([sub]/[sub]$_0$) versus irradiation time at wavelengths of 700, 710, 720, 730, 740, 750, and 760 nm and at a power of 700 mW.

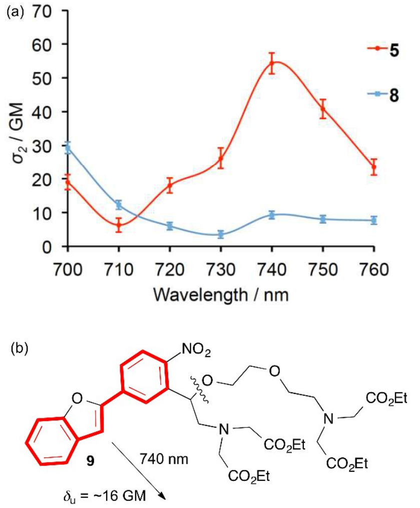


Fig. 6 (a) TPA spectra of **5** (red line) and **8** (blue line), as determined by the relative rate constants of the corresponding uncaging reactions; (b) TP reaction of **9** at 740 nm, δ_u = ~16 GM, ϕ_u = 0.3.

In conclusion, a new NPBF chromophore with TPA capability was designed and synthesized. The simple increase in the π-conjugation length and donating ability led to a bathochromic shift and a significant increase in TP sensitivity. The OP photolysis of **5** showed the quantitative release of benzoic acid with a high uncaging efficiency $\varepsilon\phi_u$ = 1080 at 360 ± 10 nm. Meanwhile, TP uncaging of **5** using near-IR light quantitatively produced benzoic acid with an efficiency of 5.0 GM at 740 nm, corresponding to a TPA cross-section of 54 GM. Furthermore, TP photolysis of **9** was found to induce the decomposition of

EGTA structure. This behaviour is superior to that of the NDBF derivatives, and makes it a promising TP-PPG, suggesting potential applications in physiology and neuroscience.

We thank N-BARD, Hiroshima University, for NMR and MS measurements. M.A. and K.K. gratefully acknowledge financial support from the Grant-in-Aid for Science Research on Innovative Areas "Stimuli-responsive Chemical Species" (No. 24109008 (MA); 25109544(KK)) from the MEXT, Japan. C.K. acknowledges the HPC resources of CINES and of IDRIS under the allocations 2014-[x2014080649] made by GENCI (Grand Equipement National de Calcul Intensif).

Notes and references

1 (a) J. Engels, E-J. Schlaeger, *J. Med. Chem.* 1977, **20**, 907–911. (b) H. Kaplan, B. Forbush and J. F. Hoffmann, *Biochemistry*, 1978, **17**, 1929-1935.

2 (a) W. J. McEntee and T. H. Crook, *Psychopharmacology*, 1993, **111**, 391-401. (b) V. Parpura, T. A. Basarsky, F. Liu, K. Jeftinija, S. Jeftinija and P. G. Haydon, *Nature*, 1994, **369**, 744-747.

3 (a) H. Mohler, *Cell Tissue Res.*, 2006, **326**, 505-516. (b) P. Somogyi and T. Klausberger, *J. Physiol.*, 2005, **562**, 9-26. (c) G. A. Ascoli, L. Alonso-Nanclares, S. A. Anderson, G. Barrionuevo, R. Benavides-Piccione, A. Burkhalter, G. Buzsaki, B. Cauli, J. DeFelipe, A. Fairen, D. Feldmeyer, G. Fishell, Y. Fregnac, T. F. Freund, D. Gardner, E. P. Gardner, J. H. Goldberg, M. Helmstaedter, S. Hestrin, F. Karube, Z. F. Kisvarday, B. Lambolez, D. A. Lewis, O. Marin, H. Markram, A. Munoz, A. Packer, C. C. H. Petersen, K. S. Rockland, J. Rossier, B. Rudy, P. Somogyi, J. F. Staiger, G. Tamas, A. M. Thomson, M. Toledo-Rodriguez, Y. Wang, D. C. West, R. Yuste, *Nat. Rev. Neurosci.*, 2008, **9**, 557-568.

4 (a) P. Klan, T. Solomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov and J. Wirz, *Chem. Rev.*, 2013, **113**, 119-191. (b) C. G. Bochet, A. Blanc, in *Handbook of Synthetic Photochemistry*, ed. A. Albini, M. Fagnoni, Wiley-VCH, Weinheim, 2010, 417-447.

5 (a) C. Brieke, F. Rohrbach, A. Gottschalk, G. Mayer and A. Heckel, *Angew. Chem. Int. Ed.*, 2012, **51**, 8446-8476. (b) G. Bort, T. Gallavardin, D. Ogden and P. I. Dalko, *Angew. Chem. Int. Ed.*, 2013, **52**, 4526-4537. (c) H. M. Kim, B. R. Cho, *Chem. Rev.*, 2015, **115**, 5014-5055.

6 (a) J. A. Barltrop, P. Schofield, *Tetrahedron Lett.*, 1962, **3**, 697-699. (b) D. H. R. Barton, Y. L. Chow, A. Cox, G. W. Kirby, *Tetrahedron Lett.*, 1962, **3**, 1055-1057. (c) J. C. Sheehan, R. M. Wilson, *J. Am. Chem. Soc.*, 1964, **86**, 5277-5281. (d) J. A. Barltrop, P. J. Plant and P. Schofield, *Chem. Commun.*, 1966, 822-823. (e) A. Patchornik, B. Amit and R. B. Woodward, *J. Am. Chem. Soc.*, 1970, **92**, 6333-6335.

7 (a) M. Matsuzaki, G. C. R. Ellis-Davis, T. Nemoto, Y. Miyashita, M. Iino, H. Kasai, *Nature. Neurosci.* 2001, **4**, 1086-1092. (b) K. Svoboda and R. Yasuda, *Neuron*, 2006, **50**, 823-839. (c) G. C. R. Ellis-Davies, *Nat. Methods*, 2007, **4**, 619-628. (d) A. Specht, F. Bolze, Z. Omran, J. F. Nicoud and M. Goeldner, *HFSP J.*, 2009, **3**, 255-264. (e) M. Pawlicki, H. A. Collins, R. G. Denning and H. L. Anderson, *Angew. Chem. Int. Ed.*, 2009, **48**, 3244-3266. (f) D. Warther, S. Gug, A. Specht, F. Bolze, J. F. Nicoud, A. Mourot and M. Goeldner, *Bioorg. Med. Chem.*, 2010, **18**, 7753-7758. (g) G. C. R. Ellis-Davies, *ACS Chem. Neurosci.*, 2011, **2**, 185-197. (h) J. P. Olson, H. B. Kwon, K. T. Takasaki, C. Y. Q. Chiu, M. J. Higley, B. L. Sabatini and G. C. R. Ellis-Davies, *J. Am. Chem. Soc.*, 2013, **135**, 5954-5957. (i) J. M. Amatrudo, J. P. Olson, G. Lur, C. Q. Chiu, M. J. Higley and G. C. R. Ellis-Davies, *ACS Chem. Neurosci.*, 2014, **5**, 64-70.

8 TP uncaging efficiency (δ_u) is expressed by the multiplication of a TPA cross-section (σ_u in GM, 1 GM = 10^{-50} cm^{4}s per photon molecule) with the uncaging quantum yield ϕ_u, $\delta_u = \sigma_2 \times \phi_u$. OP uncaging efficiency, $\varepsilon \times \phi_u$, is expressed by the multiplication of an extinction coefficient ε in M^{-1}cm^{-1} with uncaging quantum yield ϕ_u.

9 Goeppert-Mayer (GM) unit: 1 GM = 10^{-50} cm^4 s photon^{-1} molecule^{-1}; named in honor of Maria Goeppert-Mayer, who set the theoretical basis of the TPA process, see: M. Goeppert-Mayer, *Ann. Phys.*, 1931, **401**, 273-294.

10 N. I. Kiskin, R. Chillingworth, J. A. McCray, D. Piston and D. Ogden, *Eur. Biophys. J.*, 2002, **30**, 588-604.

11 (a) L. Donato, A. Mourot, C. M. Davenport, C. Herbivo, D. Warther, J. Leonard, F. Bolze, J. F. Nicoud, R. H. Kramer, M. Goeldner and A. Specht, *Angew. Chem. Int. Ed.*, 2012, **51**, 1840-1843. (b) A. Specht, F. Bolze, L. Donato, C. Herbivo, S. Charon, D. Warther, S. Gug, J. F. Nicoud and M. Goeldner, *Photochem. Photobiol. Sci.*, 2012, **11**, 578-586. (c) C. Tran, T. Gallavardin, M. Petit, R. Slimi, H. Dhimane, M. Blanchard-Desce, F. C. Acher, D. Ogden and P. I. Dalko, *Org. Lett.*, 2015, **17**, 402-405. (d) H. J. Yin, B. C. Zhang, H. Z. Yu, L. Zhu, Y. Feng, M. Z. Zhu, Q. X. Guo and X. M. Meng, *J. Org. Chem.*, 2015, **80**, 4306-4312. (e) K. A. Korzycka, P. M. Bennett, E. J. Cueto-Diaz, G. Wicks, M. Drobizhev, M. Blanchard-Desce, A. Rebane and H. L. Anderson, *Chem. Sci.*, 2015, **6**, 2419-2426.

12 A. Momotake, N. Lindegger, E. Niggli, R. J. Barsotti and G. C. R. Ellis-Davies, *Nat. Methods*, 2006, **3**, 35-40.

13 The absorption maximum of NDBF was observed at 331 nm (ε = 18945 M^{-1}cm^{-1}) in DMSO, see the UV-vis spectrum of NDBF in Supporting Information (Fig. S11a, ESI).

14 (a) F. Terenziani, C. Katan, E. Badaeva, S. Tretiak and M. Blanchard-Desce, *Adv. Mater.*, 2008, **20**, 4641-4678. (b) H. M. Kim and B. R. Cho, *Chem. Commun.*, 2009, 153-164. (c) S. Gug, F. Bolze, A. Specht, C. Bourgogne, M. Goeldner and J. F. Nicoud, *Angew. Chem. Int. Ed.*, 2008, **47**, 9525-9529.

15 G. S. He, L. S. Tan, Q. Zheng and P. N. Prasad, *Chem. Rev.*, 2008, **108**, 1245-1330.

16 S. Boinapally, B. Huang, M. Abe, C. Katan, J. Noguchi, S. Watanabe, H. Kasai, B. Xue and T. Kobayashi, *J. Org. Chem.*, 2014, **79**, 7822-7830.

17 M. Albota, D. Beljonne, J. L. Bredas, J. E. Ehrlich, J. Y. Fu, A. A. Heikal, S. E. Hess, T. Kogej, M. D. Levin, S. R. Marder, D. McCord-Maughon, J. W. Perry, H. Rockel, M. Rumi, C. Subramaniam, W. W. Webb, X. L. Wu and C. Xu, *Science*, 1998, **281**, 1653-1656.

18 R. J. M. Anderson, G. R. Holtom and W. M. McClain, *J. Chem. Phys.*, 1979, **70**, 4310-4315.

19 The absorption maximum of NPBF was observed at 371 nm (ε = 23260 M^{-1}cm^{-1}) nm in DMSO, see the UV-vis spectrum of NPBF in Supporting Information (Fig. S13b, ESI).

20 H. J. Lee, S. H. Kim, Y. R. Lee, X. Wang and W. S. Lyoo, *Bull. Korean Chem. Soc.*, 2010, **31**, 3027-3030.

21 A. K. Singh and P. K. Khade, *Tetrahedron*, 2005, **61**, 10007-10012.

22 Photo-release of carboxylic acid derivatives, see: (a) B. Amit, A. Patchornik, *Tetrahedron Lett.*, 1973, **14**, 2205-2208. (b) B. Amit, D. A. Ben-Efraim and A. Patchornik, *J. Am. Chem. Soc.*, 1976, **98**, 843-844. (c) J. Morrison, P. Wan, J. E. T. Corrie, G. Papageorgiou, *Photochem. Photobiol. Sci.*, 2002, **1**, 960-969. (d) A. D. Cohen, C. Helgen, C. G. Bochet, J. P. Tpscano, *Org. Lett.*, 2005, **7**, 2845-2848. (e) M. Matsuzaki, T. Hayama, H. Kasai, G. C. Ellis-Davies, *Nat. Chem. Biol.*, 2010, **6**, 255-257.

23 M. Sheik-Bahae, A. A. Said, T. H. Wei, D. J. Hagan and E. W. Van Stryland, *IEEE J. Quant. Electron.*, 1990, **26**, 760-769.

24 G. C. R. Ellis-Davies, *Chem. Rev.*, 2008, **108**, 1603-1613.