DOI: 10.1021/ic900627g

Inorganic Chemistry
Article

Boron Containing Two-Photon Absorbing Chromophores. 2. Fine Tuning of the One- and Two-Photon Photophysical Properties of Pyrazabole Based Fluorescent Bioprobes

Ali Hayek,[†] Frédéric Bolze,[†] Cyril Bourgogne,[‡] Patrice L. Baldeck,[§] Pascal Didier,[†] Youri Arntz,[†] Yves Mély,[†] and Jean-François Nicoud*[,†]

[†]*Laboratoire de Biophotonique et Pharmacologie, Université de Strasbourg* (*UMR CNRS 7213*), *Faculté de Pharmacie, 74 route du Rhin, 67401 Illkirch, France,* [‡]*Institut de Physique et Chimie des Matériaux de Strasbourg, Université de Strasbourg* (*UMR CNRS 7504*), *23 rue du Loess, 67034 Strasbourg cedex 2, France, and* [§]*Laboratoire de Spectrométrie Physique, Université Joseph Fourier* (*UMR CNRS 5588*), *BP 87, 38402 Saint-Martin d'Hères, France*

Received March 31, 2009

New boron containing two-photon absorbing fluorophores have been prepared. Centered on a pyrazabole central core, various conjugated systems and end groups were investigated to modulate their physicochemical properties (alkoxy, diphenylamino, and boron dipyromethene groups). One and two-photon photophysical characterizations were performed, showing efficient fluorescence in organic solvents. High two-photon absorption cross sections were determined in the 500−800 nm range. Two-photon excited microscopy images were also obtained with these new boron containing fluorescent bioprobes with laser intensities in the milliwatt range.

Introduction

Nowadays the design and synthesis of original and useful multiphoton absorbing chromophores is a very stimulating field of research in synthetic chemistry, allowing strong interactions with physics and biology.[2] Indeed, this research area is based on the multiple applications of multiphoton absorbing chromophores, such as data storage,[3] microfabrication,[4] optical limiting,[5] photodynamic therapy,[6] or bioimaging.[7] Most explored and efficient one-dimensional (1D) fluorophores for two-photon absorption (TPA) have a symmetrical rigid rod structure and can be described as a couple of donor or acceptor groups in interaction through a conjugated central core (Figure 1).[8] In most cases the interaction proceeds classically via a π-conjugated system such as a succession of single and double bonds.

We have recently reported new TPA chromophores in which the interaction between the two donor moieties does not proceed via an entirely conjugated π-system but through

*To whom correspondence should be addressed. E-mail: jfnicoud@unistra.fr. Phone: +33 (0)3 90 24 42 76. Fax: +33 (0)3 90 24 43 13.

(1) Part 1, see: Hayek, A.; Nicoud, J.-F.; Bolze, F.; Bourgogne, C.; Baldeck, P. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 6466–6469.

(2) (a) Pawlicki, M.; Collins, H. A.; Dennings, R. G.; Anderson, H. L. *Angew. Chem., Int. Ed.* **2009**, *48*, 3244–3266. (b) Kim, H. M.; Cho, B. R. *Chem. Commun.* **2009**, 153–164. (c) He, G. S.; Tan, L.-S.; Zheng, Q.; Prasad, P. N. *Chem. Rev.* **2008**, *108*, 1245–1330. (d) Terenziani, F.; Katan, C.; Badaeva, E.; Tretiak, S.; Blanchard-Desce, M. *Adv. Mater.* **2008**, *20*, 4461–4678.

(3) (a) Corredor, C. C.; Huang, Z.-L.; Belfield, K. D.; Morales, A. R.; Bondar, M. V. *Chem. Mater.* **2007**, *19*, 5165–5173. (b) Belfield, K. D.; Schafer, K. J. *Chem. Mater.* **2002**, *14*, 3656–3662. (c) Strickler, J. H.; Webb, W. W. *Adv. Mater.* **1993**, *5*, 479–81.

(4) (a) Xing, J.-F.; Dong, X.-Z.; Chen, W.-Q.; Duan, X.-M.; Takeyasu, N.; Tanaka, T.; Kawata, S. *Appl. Phys. Lett.* **2007**, *90*, 131106(1−3). (b) Lemercier, G.; Martineau, C.; Mulatier, J.-C.; Wang, I.; Stéphan, O.; Baldeck, P.; Andraud, C. *New J. Chem.* **2006**, *30*, 1606–1613. (c) Nguyen, L. H.; Straub, M.; Gu, M. *Adv. Funct. Mater.* **2005**, *15*, 209–216. (d) Zhou, W.; Kuebler, S. M.; Braun, K. L.; Yu, T.; Cammack, J. K.; Ober, C. K.; Perry, J. W.; Marder, S. R. *Science* **2002**, *296*, 1106–1109. (e) Kawata, S.; Sun, H.-B.; Tanaka, T.; Takada, K. *Nature* **2001**, *412*, 697–698.

(5) (a) Bouit, P. A.; Wetzel, G.; Berginc, G.; Loiseaux, B.; Toupet, L.; Feneyrou, P.; Bretonnière, Y.; Kamada, K.; Maury, O.; Andraud, C. *Chem. Mater.* **2007**, *19*, 5325–5335. (b) Guyen, L. H.; Straub, M.; Gu, M. *Adv. Funct. Mater.* **2005**, *15*, 209–216. (c) Oenjarts, C. A.; Ober, C. K. *Chem. Mater.* **2004**, *16*, 5556–5558. (d) Ehrlich, J. E.; Wu, X. L.; Lee, I.-Y. S.; Hu, Z.-Y.; Röckel, H.; Marder, S. R.; Perry, J. W. *Opt. Lett.* **1997**, *22*, 1843–1845.

(6) (a) Karotki, A.; Khurana, M.; Lepock, J. R.; Wilson, B. C. *Photochem. Photobiol.* **2006**, *82*, 443–452. (b) Belfield, K. D.; Corredor, C. C.; Morales, A. R.; Dessources, M. A.; Hernandez, F. E. *J. Fluoresc.* **2006**, *16*, 105–110. (c) Oar, M. A.; Serin, J. M.; Dichtel, W. R.; Frechet, J. M. J.; Ohulchanskyy, T. Y.; Prasad, P. N. *Chem. Mater.* **2005**, *17*, 2267–2275. (d) Fournier, M.; Pepin, C.; Houde, D.; Ouellet, R.; van Lier, J. E. *Photochem. Photobiol. Sci.* **2004**, *3*, 120–126.

(7) (a) Helmchen, F.; Denk, W. *Nat. Methods* **2005**, *2*, 932–940. (b) Cruz, H. G.; Luescher, C. *Front. Biosci.* **2005**, *10*, 2263–2278. (c) Molitoris, B. A.; Sandoval, R. M. *Am. J. Physiol. Renal Physiol.* **2005**, *288*, F1084–F1089.

(8) (a) Rumi, M.; Ehrlich, J. E.; Heikal, A. A.; Perry, J. W.; Barlow, S.; Hu, Z.-Y.; McCord-Maughon, D.; Parker, T. C.; Röckel, H.; Thayumanavan, S.; Marder, S. R.; Beljonne, D.; Brédas, J.-L. *J. Am. Chem. Soc.* **2000**, *122*, 9500–9510. (b) Albota, M.; Beljonne, D.; Brédas, J.-L.; Ehrlich, J. E.; Fu, J.-Y.; Heikal, A. A.; Hess, S. E.; Kogej, T.; Levin, M. D.; Marder, S. R.; McCord-Maughon, D.; Perry, J. W.; Röckel, H.; Rumi, M.; Subramaniam, G.; Webb, W. W.; Wu, X.-L.; Xu, C. *Science* **1998**, *281*, 1653–1656.

Published on Web 09/03/2009

Figure 1. Typical structure of a 1D two-photon chromophore. Two donor parts (blue) are linked to a central core (green) by a conjugated system (white).

the cyclodiborazane core, which is a boron containing heterocyle that behaves as a pseudo-conjugated system.[1] This type of chromophore has led to high TPA cross sections. Recently, some studies reported that the *pyrazabole* moiety, another boron-containing non-conjugated system, could be interesting for obtaining new luminescent materials.[9] This system appeared particularly interesting because it could be used as a central core for donor−donor TPA systems, but in addition, this boron containing entity could be used as sensitizer for boron neutron capture therapy (BNCT), leading to a bifunctional molecule by association of two-photon excited microscopy (TPEM) and boron neutron capture therapy (BNCT). The boron content of such chromophores can be increased by adding to the pyrazabole core some donor moieties containing also boron atoms, such as the boron-dipyrromethene (BODIPY) group, widely used in bioimaging. The dual function given by TPEM and BNCT sensitizing would be of great interest in studies on the localization and the mechanism of action of sensitizers.[10] The use of TPA chromophores could take advantage of TPEM in living tissues and animals. Indeed, this technique allows a deep penetration in tissues and low laser induced photodamage.[7a,11] The combination of TPEM and tomographic synchrotron irradiation are actually used to point out the effect of this therapy on brain vascularization.[12] We report here the synthesis and one and two-photon photophysical characterizations of new symmetrical TPA chromophores built around a pyrazabole moiety, as well as preliminary TPEM bioimaging results.

Experimental Section

Synthesis. 3,4,5-Tridodecyloxystyrene,[13] 4-(*N*,*N*-diphenylamino)styrene[14] and diethyl-4-iodobenzyl-phosphonate[1] were prepared as described in literature. Tetrahydrofuran (THF), xylenes, and benzene were dried by distillation over a sodium benzophenone complex under an argon atmosphere. Diethylamine and triethylamine were dried by distillation over potassium hydroxide. Solvents were deaerated by argon bubbling in a syringe before introduction in the reaction flasks. TLC analyses were run on Merck precoated aluminum plates (Si 60 F254). Column chromatography was run on Merck silica gel (60−120 mesh) or Merck neutral alumina (70−230 mesh). ^{1}H, ^{13}C, ^{19}F, and ^{11}B NMR spectra were recorded with a Bruker US+ 400 instrument (BBFO+ probe) in $CDCl_3$. The chemical shifts for the ^{1}H and ^{13}C NMR spectra are reported in ppm at 400.13 and 100.63 MHz. Spectra are referenced internally to residual protic solvent ($CHCl_3$, δ 7.26) for ^{1}H spectra, and to $CDCl_3$ (δ 77 ppm) for ^{13}C spectra. The chemical shifts for the ^{19}F NMR spectra are reported in ppm at 376.50 MHz and chemical shifts for ^{11}B NMR spectra in ppm at 128.37 MHz. FT IR spectra were recorded on a Perkin-Elmer spectrum BX instrument. Mass spectra were recorded with either a Bruker MicrOTOF-Q (ESI) or an Agilent MSD (MM-ES-ACPI).

4,4,8,8-Tetrabutyl-2,6-diiodopyrazabole (2). To a mixture of 4-iodopyrazole **1** (4.0 g, 10.3 mmol) and 80 mL of xylenes, 21 mL of a 1 M THF solution of tributylborane was added and refluxed at 120 °C for 12 h. After removing the solvent under reduced pressure, the resulting product **2** was washed with methanol to yield a white solid (4.6 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.61 (s, 4H), 1.19 (qt, 8H, $J_1 = 7.3$ Hz, $J_2 = 7.3$ Hz), 0.84−0.72 (m, 20H), 0.66−0.61 (m, 8H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 138.5, 56.7, 27.3, 26.0, 14.0 ppm. ^{11}B-NMR (128 MHz, $CDCl_3$, 25 °C): δ 2.5 ppm. MS: m/z 579.0 (calcd for $C_{50}H_{84}IO_3$/M-Bu 579.0). IR (KBr pellet) ν cm^{-1}: 3130 (C−H); 1663, 1518, 1389, 1461, 1425, 1389, 836 (pz). Mp = 139 °C.

4,4,8,8-Tetrabutyl-2,6-bis(4-bromophenyl)pyrazabole (3). A 2.0 g portion (2.67 mmol) of diiodopyrazabole **2** was dissolved into dried benzene (50 mL), $Pd(PPh_3)_4$ (234 mg, 0.2 mmol) and saturated Na_2CO_3 solution (10 mL) was added. The mixture was deaerated and warmed to 80 °C. A solution of 4-bromophenylboronic acid (1.15 g, 5.7 mmol) in EtOH (10 mL) was then added. The resulting mixture was cooled and then stirred for 48 h at room temperature. The organic phase was then washed with water (3 × 50 mL), dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography (silica, MeOH−CH_2Cl_2 5−95 in vol.) to give the desired product **3** as a white powder (1.18 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.84 (s, 4H), 7.57 (d, 4H, $J = 6.9$ Hz), 7.45 (d, 4H, $J = 6.9$ Hz), 1.22 (qt, 8H, $J_1 = 7.1$ Hz, $J_2 = 7.1$ Hz), 0.90−0.70 (m, 28H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 132.1, 130.3, 128.5, 127.3, 122.1, 120.9, 27.6, 26.3, 26.1, 14.0 ppm. ^{11}B-NMR (128 MHz, $CDCl_3$, 25 °C): δ 1.5 ppm. HRMS: m/z 694.2038 (calcd for $C_{34}H_{48}B_2Br_2N_4$ m/z 694.2017). IR (KBr pellet) ν cm^{-1}: 3126 (C−H); 1569, 1166, 1069 (ar, pz) cm^{-1}. Mp = 222 °C.

4,4,8,8-Tetrabutyl-2,6-bis(trimethylsilylethynyl)-pyrazabole (4). A solution of diiodopyrazabole **2** (2.0 g, 3.14 mmol) in dry THF (30 mL) was prepared. A 110 mg portion of $Pd(PPh_3)_2Cl_2$ (0.16 mmol), CuI (30 mg, 0.16 mmol), and trimethylsilylacetylene (0.83 mL, 5.88 mmol) in diethylamine (20 mL) were then added. The mixture was stirred for 48 h at 60 °C. After evaporating the solvent under vacuum, the crude product was extracted with diethyl ether and washed with water and dried over Na_2SO_4. The crude product was purified by column chromatography (silica, cyclohexane-MeOH 98−2 in vol.) to give the desired product **4** as a white powder (1.63 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.70 (s, 4H), 1.18 (qt, 8H, $J_1 = 7.3$ Hz, $J_2 = 7.3$ Hz), 0.82−0.72 (m, 20H), 0.70−0/59 (m, 8H), 0.26 (s, 18H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 136.6, 104.1, 97.8, 94.8, 27.5, 26.2, 14.1, 0.0 ppm. ^{11}B-NMR (128 MHz, $CDCl_3$, 25 °C): δ 2.8 ppm. MS: m/z 518.2 (calcd for $C_{50}H_{84}IO_3$/M- Bu m/z 519.3). IR (KBr pellet) ν cm^{-1}: 3142 (C−H), 2175 (C≡C), 1464, 1438, 1356 (pz). Mp = 160 °C.

2,6-Diethynyl-4,4,8,8-tetrabutylpyrazabole (5). To a THF (30 mL) solution of **4** (300 mg, 0.52 mmol) was added 0.82 mL of 1.0 M THF solution of tetrabutylammonium fluoride (TBAF) (0.82 mmol) drop by drop and stirred for 15 min at room temperature. The crude product was obtained by extraction with diethyl ether followed by evaporation of the solvent. It was purified by column chromatography (silica, cyclohexane) to

(9) (a) Matsumi, N.; Chujo, Y. *Polym. J.* **2008**, *40*, 77–89. (b) Cavero, E.; Lydon, D. P.; Uriel, S.; de la Fuente, M. R.; Serrano, J.-L.; Gimenez, R. *Angew. Chem., Int. Ed.* **2007**, *46*, 5175–5177. (c) Matsumoto, F.; Nagata, Y.; Chujo, Y. *Polym. Bull.* **2005**, *53*, 155–160.

(10) Smith, D. R.; Chandra, S.; Barth, R. F.; Yang, W.; Joel, D. D.; Coderre, J. A. *Cancer Res.* **2001**, *61*, 8179–8187.

(11) Xu, C.; Zipfel, W.; Shear, J. B.; Williams, R. M.; Webb, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10763–10768.

(12) Serduc, R.; Vérant, P.; Vial, J.-C.; Farion, R.; Rocas, L.; Rémy, C.; Fadlallah, T.; Brauer, E.; Bravin, A.; Laissue, J.; Blattmann, H.; van der Sanden, B. *Int. J. Rad. Oncol. Biol. Phys.* **2006**, *64*, 1519–1527.

(13) Lincker, F.; Bourgun, P.; Masson, P.; Didier, P.; Guidoni, L.; Bigot, J.-Y.; Nicoud, J.-F.; Donnio, B.; Guillon, D. *Org. Lett.* **2005**, *7*, 1505–1508.

(14) Tew, G. N.; Pralle, M. U.; Stupp, S. I. *Angew. Chem., Int. Ed.* **2000**, *39*, 517–521.

give the desired product **5** as a white powder (202 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.71 (s, 4H), 3,12 (s, 2H), 1.19 (qt, 8H, J_1 = 7.2 Hz, J_2 = 7.2 Hz), 0.81−0.75 (m, 20H), 0.68−0.63 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 136.64; 102.76; 79.47; 73.74; 27.25; 25.97; 13.95. ^{11}B-NMR (128 MHz, $CDCl_3$, 25 °C): δ 2.5 ppm. MS: *m*/*z* 375.2 (calcd for $C_{22}H_{33}B_2N_4$/M- Bu *m*/*z* 375.3).

(Z)-4-Iodo-3′,4′,5′-tridodecyloxystilbene (6). Diethyl 4-iodobenzylphosphonate[1] (1 g, 2.80 mmol) was dissolved in dry THF (10 mL), then NaH (60% in oil, 565 mg, 14 mmol) was added. After the effervescence ceased, the suspension was stirred for 1 h at 50 °C under an argon atmosphere. Then a solution of 3,4,5-tridodecyloxybenzaldehyde (1.85 g, 2.80 mmol) in anhydrous THF (40 mL) was added. The mixture was stirred under reflux for 2 h. After cooling, the solid is filtered off and the filtrate was quenched by EtOH. Solvents are evaporated under vacuum, and the solid residue was dissolved in CH_2Cl_2 and washed with water (10 mL). The organic phase was dried over $MgSO_4$ and the solvent evaporated. The resulting solid product was recrystallized in methanol to give **6** as a white solid (2.04 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.66 (d, 2H, J = 8.33 Hz), 7.23 (d, 2H, J = 8.55 Hz), 7.03−6.85 (AB, 2H, J = 16.23 Hz), 6.70 (s, 2H), 4.04−3.95 (m, 6H), 1.87−1.71 (m, 6H), 1.50−1.48 (m, 6H), 1.36−1.27 (m, 48H), 0.89 (t, 9H, J = 6.36 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 153.29; 138.55; 137.66; 136.90; 132.04; 129.69; 128.02; 126.42; 105.26; 92.41; 73.49; 69.15; 31.91; 31.89; 30.30; 29.72; 29.70; 29.68; 29.66; 29.62; 29.58; 29.39; 29.36; 29.33; 26.08; 22.66; 14.08. HRMS: *m*/*z* 859.5453 (calcd for $C_{50}H_{84}IO_3$ *m*/*z* 859.5460).

Chromophore 8. In a three necked flask under an argon atmosphere, **3** (430 mg, 0.62 mmol) $Pd(OAc)_2$ (50 mg, 0.22 mmol) and tri-*o*-tolylphosphine (TOP) (144 mg, 0.47 mmol) were deaerated. Triethylamine (30 mL) and xylenes (60 mL) were then added. The mixture was warmed up to 60 °C for 1.5 h. A solution of 3,4,5-tridodecyloxystyrene[13] (895 mg, 1.36 mmol) in xylenes (10 mL) was added slowly, and the temperature was raised to 120 °C. This mixture was stirred at 120 °C for a week. The solvents were removed under vacuum, and then the crude product was dissolved in methylene chloride and washed with water. After evaporation of the solvents, the product was purified by column chromatography (silica, cyclohexane) to give **8** in 20% yield as a yellow oil (228 mg). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.86 (s, 4H), 7.56 (s, 8H), 7.08−6.96 (AB, 4H, J = 16.01 Hz), 6.74 (s, 4H), 4.04 (t, 8H, J = 6.57 Hz), 3.98 (t, 4H, J = 6.58 Hz), 1.89−1.60 (m, 12H), 1.50−1.45 (m, 8H), 1.40−1.15 (m, 124H), 0.89 (t, 18H, J = 6.58 Hz), 0.79 (t, 12H, J = 7.24 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 153.30; 138.39; 136.30; 132.37; 130.31; 130.20; 128.79; 127.04; 126.91; 125.84; 122.74; 105.20; 73.50; 69.17; 43.42; 31.90; 31.89; 30.31; 30.13; 29.72; 29.72; 29.70; 29.67; 29.66; 29.63; 29.61; 29.58; 29.42; 29.39; 29.36; 29.33; 27.53; 26.87; 26.85; 26.09; 22.65; 14.07; 14.03. HRMS: *m*/*z* 1884.5805 (calcd for $C_{122}H_{206}B_2N_4O_6$ + K *m*/*z* 1884.5755). IR (KBr pellet) ν cm^{-1}: 3029 (C−H); 1663, 1568 1369 (pz). Mp = 198 °C.

Chromophore 9. In a three necked flask under an argon atmosphere, **3** (250 mg, 0.62 mmol), $Pd(OAc)_2$ (32 mg, 0.14 mmol), and TOP (82 mg, 0.27 mmol) were deaerated. Triethylamine (40 mL) and xylenes (60 mL) were then added. The mixture was warmed up to 60 °C for 1.5 h. A solution of 4-(*N*,*N*)-diphenylaminostyrene[14] (215 mg, 0.79 mmol) in xylenes (20 mL) was added slowly and the temperature increased to 140 °C. This mixture was stirred at 140 °C for a week. The solvents were removed under vacuum, and the crude product dissolved in methylene chloride and washed with water. After evaporation of the solvents, the product is purified by column chromatography (silica, methylene chloride) to give **9** in 11% yield as a yellow solid (42 mg). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.86 (s, 4H), 7.56 (s, 8H), 7.42 (d, 4H, J = 8.77), 7.30−7.25 (m, 12H), 7.14−7.00 (m, 16H), 1.30−1.18 (m, 16H), 0.89−0.77 (m, 8H), 0.79 (t, 12H, J = 7.23 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 147.48; 147.40; 140.68; 139.94; 136.66; 136.52; 131.44; 131.34; 130.19; 130.17; 129.24; 128.74; 128.19; 128.05; 127.32; 127.28; 126.84; 126.67; 126.67; 126.46; 126.37; 125.84; 124.47; 123.52; 123.02; 122.76; 77.16; 29.79; 29.65; 27.53; 26.32; 26.11; 14.03. HRMS: *m*/*z* 1075.6725 (calcd for $C_{74}H_{81}B_2N_6$ *m*/*z* 1075.6725). IR (KBr pellet) ν cm^{-1}: 3129 (C−H); 1653, 1590 1369 (pz). Mp = 222 °C.

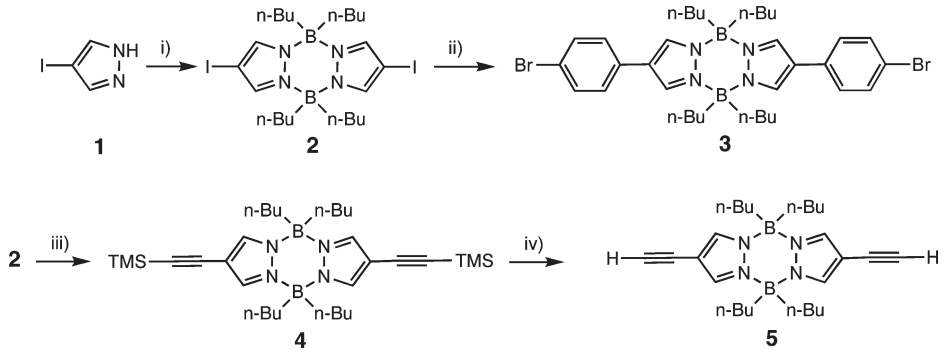
Chromophore 10. Under an argon atmosphere, 270 mg of 2,6-diethynyl-4,4,8,8-tetrabutylpyrazabole **5** (0.62 mmol) were dissolved in anhydrous THF (30 mL). Bis(triphenylphosphine)-palladium(II) chloride (43.8 mg, 0.06 mmol), CuI (11.8 mg, 0.06 mmol) and **6** (10 g, 28 mmol) in diethylamine (10 mL) were added. The mixture was heated at 60 °C for 24 h. The solvents were evaporated under vacuum, and the residue is dissolved in diethyl ether and washed with water. The crude product was purified by column chromatography (silica, cyclohexane) to give **10** as yellow oil (587 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.79 (s, 4H), 7.51 (s, 8H), 7.09−6.94 (AB, 4H, J = 16.00 Hz), 6.74 (s, 4H), 4.06−3.98 (m, 12H), 1.87−1.76 (m, 12H), 1.51−1.45 (m, 8H), 1.30−1.19 (m, 124H), 0.91 (t, 18H, J = 6.58 Hz), 0.82 (t, 12H, J = 7.23 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 153.31; 138.64; 137.59; 137.59; 137.55; 137.23; 136.04; 135.96; 132.18; 131.66; 130.00; 126.79; 126.31; 121.51; 105.37; 104.16; 104.07; 91.34; 80.03; 73.51; 69.18; 31.93; 31.92; 31.34; 29.74; 29.72; 29.70; 29.68; 29.66; 29.60; 29.44; 29.41; 29.38; 29.36; 27.39; 27.33; 26.11; 26.08; 26.02; 25.96; 22.68; 14.09; 14.03. HRMS: *m*/*z* 1894.6227 (calcd for $C_{126}H_{207}B_2N_4O_6$ *m*/*z* 1894.6229). Mp > 250 °C.

Chromophore 11. Under an argon atmosphere, 200 mg of 2,6-diethynyl-4,4,8,8-tetrabutylpyrazabole **5** (0.46 mmol) were dissolved in anhydrous THF (30 mL). Bis(triphenylphosphine)-palladium(II) chloride (34 mg, 0.046 mmol), CuI (10 mg, 0.046 mmol), and **7** (438 mg, 0.92 mmol) in diethylamine (10 mL) were added. The mixture was heated at 60 °C for 48 h. The solvents were evaporated under vacuum, and the residue was dissolved in diethyl ether and washed with water. The crude product was purified by column chromatography (silica, cyclohexane/MeOH 98/2 in vol.) to give **11** as a yellow solid (258 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.77 (s, 4H), 7.49 (s, 8H), 7.40 (d, 2H, J = 8.99 Hz), 7.30−7.24 (m, 8H), 7.14−6.95 (m, 20H), 1.26−1.15 (m, 8H), 0.86−0.71 (m, 16H), 0.80 (t, 12H, J = 7.24 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 147.40; 137.76; 137.23; 135.95; 131.64; 129.27; 127.44; 126.21; 126.03; 124.58; 123.28; 123.13104.02; 102.55; 91.35.79.93; 30.13; 29.66; 27.36; 27.30; 26.85; 26.08; 26.01. HRMS: *m*/*z* 1123.6701 (calcd for $C_{78}H_{80}B_2N_6$ *m*/*z* 1123.6708). M*p* > 250 °C.

Chromophore 13. Under an argon atmosphere, **12** (170 mg, 0.49 mmol), 4,4,8,8-tetrabutyl-2,6-diiodopyrazabole **2** (170 mg, 0.27 mmol), $Pd(PPh_3)_2Cl_2$ (10 mg, 0.015 mmol) were suspended in a mixture of THF (30 mL) and *i*-Pr_2NH (5 mL). Then CuI (4.7 mg, 0.024 mmol) was added. This mixture was sonicated 30 min and stirred for 2 days at room temperature. The solvents were removed under vacuum, and the crude product was purified by column chromatography (silica, cyclohexane-MeOH 95−5 in vol.) to give **13** as a reddish solid (131 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.80 (s, 4H), 7.67 (d, 4H, J = 8.11 Hz), 7.31 (d, 4H, J = 7.90 Hz), 6.00 (s, 4H), 2.57 (s, 12H), 1.43 (s, 12H), 1.22−1.15 (m, 8H), 0.89−0.69 (m, 16H), 0.80 (t, 12H, J = 7.23 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 155.79; 142.89; 140.58; 136.12; 135.16; 132.04; 128.41; 128.30; 123.62; 121.35; 103.61; 90.49; 80.68; 77.16; 31.39; 30.14; 29.65; 27.36; 26.04; 22.64; 14.56; 14.50; 14.01. ^{11}B-NMR (128 MHz, $CDCl_3$, 25 °C): δ 0.8 (t, J = 32 Hz) ppm. ^{19}F-NMR (376 MHz, $CDCl_3$, 25 °C): δ −146 (q, J = 32 Hz) ppm. HRMS: *m*/*z* 1077.6574 (calcd for $C_{64}H_{77}B_4F_4N_8$ *m*/*z* 1077.6579). Mp > 250 °C.

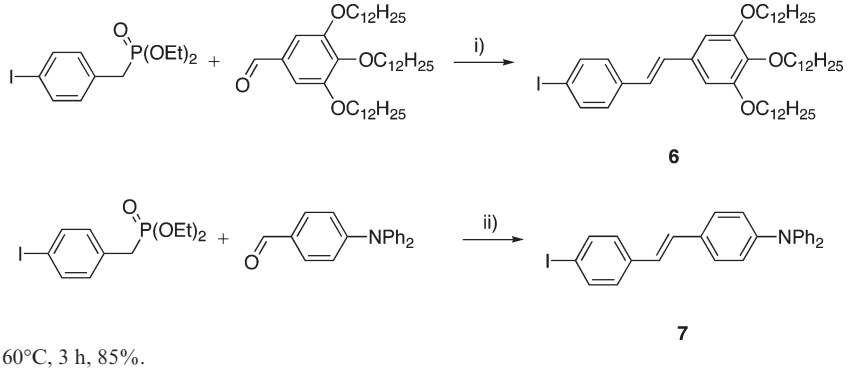
Theoretical Calculations. Molecular orbital (MO) calculations were computed using the semiempirical quantum chemistry

Scheme 1. Preparation of Pyrazabole Central Cores **3** and **5**[a]



[a] (i) BBu_3, xylenes, reflux, 12h, 70% (ii) 4-bromophenylboronic acid, $Pd(PPh_3)_4$, benzene, Na_2CO_3, EtOH, 80°C then RT 48 h, 60% (iii) trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, THF/Et_2NH, 60°C, 48 h, 90% (iv) TBAF, THF, 25°C, 15 min, 90%.

Scheme 2. Preparation of the 3,4,5-Trialkoxyphenyl and 4-*N*,*N*-diphenylaminophenyl Donor Conjugated Systems[a]



[a] (i) and (ii) NaH, THF, 60°C, 3 h, 85%.

package MOPAC6 with the AM1 Hamiltonian[15] incorporating the boron element parameters.[16] The optimized geometry of compound **11** was found to be in either the C_2 or the C_i symmetry point group depending of the starting conformation, and both resulting structures remained very close in energy. MO representation was performed with Cerius[2] (www.accelrys.com).

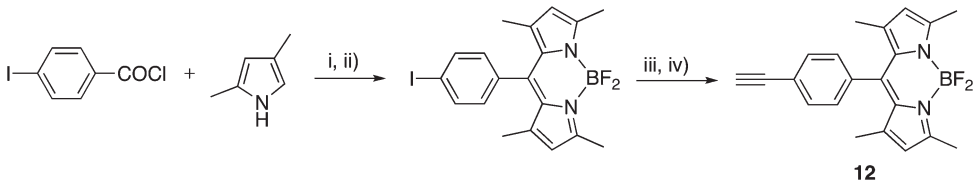
One and Two-Photon Photophysics. One-Photon Properties. UV−visible absorption spectra were recorded on a Cary 400 spectrophotometer in a dual beam mode using a matched pair of 1 × 0.5 cm quartz cells. Pure solvent was used as reference. Values of the molar extinction coefficients, $\varepsilon_{\lambda max}$, of compounds of interest were obtained as the average of at least five independent measurements with absorbance in the range 0.5−1. Fluorescence emission and excitation spectra were performed on a Fluorolog (Jobin-Yvon) spectrofluorimeter with optically dilute solutions (Abs. < 0.15) in 1 × 0.5 cm cells. Fluorescence quantum yields were measured by the relative method, using recrystallized quinine sulfate in 0.5 M aqueous H_2SO_4 ($\varphi = 0.54$) as reference.[17] In these measurements, the slit widths were adjusted so that the spectral bandwidth of the absorption and emission instruments were identical at 1.0 nm, and the absorbance of the samples and the reference were chosen so they were in the 0.1−0.15 range and nearly identical at the same excitation wavelength. Emission quantum yields were then calculated according to the method described by Crosby and Demas, taking into account the differences between the refractive indices of the sample and reference solutions.[17]

Two-Photon Properties. The TPA cross-section spectra were obtained by up-conversion fluorescence using a Nd:YAG pumped optical parametric oscillator that produces 2.6 ns [full width at half-maximum (fwhm)] pulses in the 450−650 nm spectral range and using a Ti:Sapphire femtosecond laser in the range 700−900 nm. This setup does not allow TPA measurements between 650 and 700 nm. The excitation beam is collimated over the cell length (10 mm). The fluorescence, collected at 90° of the excitation beam, was focused into an optical fiber connected to a spectrometer. The incident beam intensity was adjusted to ensure an intensity-squared dependence of the fluorescence over the whole spectral range. Calibration of the spectra was performed by comparison with *p*-bis-(*o*-methylstyryl)benzene, for which $\sigma_2 = 70$ GM (Göppert-Mayer) at 570 nm (1 GM = 10^{-50} cm^4.s.$photon^{-1}$) and with the published 700−900 nm Rhodamine B two-photon absorption spectrum.[18]

Two-Photon Imaging. Two-photon microscopy was performed on a home-built setup. Two-photon excitation (TPE) is provided by a Tsunami Ti:Sapphire laser pumped with a Millennia V solid-state laser (Spectra-Physics, Mountain View, CA). Pulses of ≈100 fs are produced with 80 MHz frequency at 760 nm. After a beam expander, the infrared light is focused into the sample by a water immersion Olympus objective (60×, NA = 1.2) mounted on an Olympus IX70 inverted microscope. The back aperture of the objective is slightly overfilled, creating a diffraction-limited focal spot. Samples were placed in eight wells Lab-Tek chambered cover glass (Nalge Nunc International, Rochester, NY) positioned in the *X* and *Y* directions by a motorized stage (Märzhäuser, Germany). The fluorescence from the samples was collected through the same objective and directed by a COWL750 dichroic mirror (Coherent, Orsay, France) toward a 50 μm diameter optical fiber coupled to an avalanche photodiode (SPCM 200 FC, EG&G, Canada). The residual infrared light was rejected by a BG39 Filter (Coherent). The imaging system on the same setup is based on the use of two

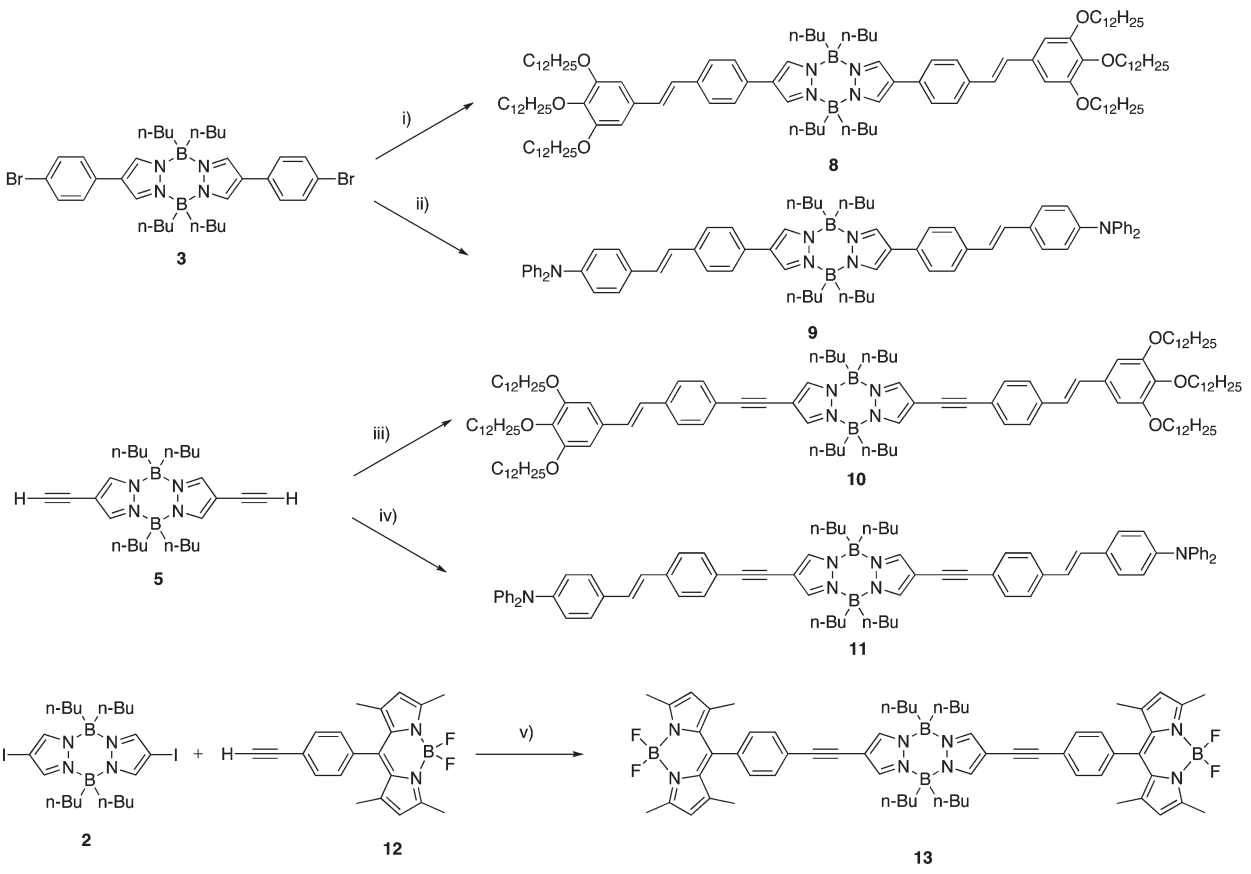
(15) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
(16) Dewar, M. J. S.; Jie, C.; Zoebisch, E. G. *Organometallics* **1988**, *7*, 513–521.
(17) Crosby, G. A.; Demas, J. N. *J. Phys. Chem.* **1971**, *75*, 991–1024.
(18) Xu, C.; Webb, W. W. *J. Opt. Soc. Am. B* **1996**, *13*, 481–491.

Scheme 3. Preparation of the Boron Containing Terminal Group[a]



[a] (i) Methylene chloride, 50°C, 2h (ii) NEt_3, toluene/methylene chloride, $BF_3 \cdot OEt_2$, 50°C, 1.5 h, 36% (iii) trimethylsilylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, THF/*i*-Pr_2NH, 30 min, ultrasound then 12 h at 25°C, 90% (iv) *n*-Bu_4NF, THF, 25°C, 15 min, 90%.

Scheme 4. Preparation of Fluorophores **8**−**13**[a]



[a] (i) 3,4,5-Tridodecyloxystyrene, $Pd(OAc)_2$, TOP, NEt_3/xylenes, 60−120°C, 1 week, 20% (ii) diphenylaminostyrene, $Pd(OAc)_2$, TOP, NEt_3/xylenes, 60−120°C, 1 week, 11% (iii) **6**, $Pd(PPh_3)_2Cl_2$, CuI, THF/$HNEt_2$, 24 h, 60°C, 50% (iv) **7**, $Pd(PPh_3)_2Cl_2$, CuI, THF/$HNEt_2$, 60°C, 48 h, 50% (v) $Pd(PPh_3)_2Cl_2$, CuI, THF/*i*-Pr_2NH, ultrasound, 30 min, RT then 2 days, RT, 50%.

galvo mirrors (model 6210, Cambridge Technology), in the so-called descanned detection mode. The two mirrors are used to deflect the beam along the *X*-axis and *Y*-axis, respectively. Each axis is driven by a closed loop power amplifier, and the position of the mirrors is controlled through two ADC electronic boards (PCI6711, National Instruments). The photons were counted using a counter/timer board (PCI6602, National Instruments), synchronized with the scan of the galvo-mirrors. Nominally, one picture is 512 × 512 pixels in size (corresponding to 70 × 70 μm), and the dwell time for each pixel is 4 μs (about 1 s per scan).

Results and Discussion

Synthesis. The pyrazabole central core **2** has been prepared by condensation of the iodoimidazole **1** with tributylborane in 70% yield as reported in the literature.[19] The π-system of this molecule was then extended either by a Suzuki coupling with 4-bromophenylboronic acid to give the dibromo central core **3** or by a Sonogashira coupling reaction with trimethylsilylacetylene followed by the deprotection[19] of the alkyne to give the diyne central core **5** as presented in Scheme 1.

The two donor parts used in this study were prepared by a Wadsworth−Emmons coupling between diethyl 4-iodobenzylphosphonate[1] and either 4-(*N*,*N*-diphenylamino)benzaldehyde or 3,4,5-tridodecyloxybenzaldehyde (Scheme 2).

The boron containing side group (BODIPY) was prepared by reaction between 4-iodobenzoyl chloride and 1,3-dimethylpyrrole, followed by reaction with $BF_3 \cdot OEt_2$.[20] This compound was then coupled with trimetyl-

(19) Matsumoto, F.; Chujo, Y. *Macromolecules* **2003**, *36*, 5516–5519.

(20) Burghart, A.; Thoresen, L. H.; Chen, J.; Burgess, K.; Bergström, F.; Johansson, L. B.-A. *Chem. Commun.* **2000**, 2203–2204.

Table 1. One and Two-Photon Photophysical Properties of New Fluorophores **8**−**11**, **13**, and Reference Chromophores **14**, **15** in Methylene Chloride

fluorophore	λ_{max} abs. (nm)	$\varepsilon \times 10^{-4}$ (M^{-1} cm^{-1})	λ_{max} em. (nm)	Φ (%)	σ_2 (GM)	τ (ns)
8	343	7.7	416	37	900 (525 nm)	0.57 (95%) 1.46 (5%)
9	379	9.2	456	60	209 (800 nm)	1.50
10	355	11.6	436	33	425 (525 nm)	0.58
11	387	10.4	479	57	314 (800 nm)	1.50
13	502	14.1	512	40	100 (740 nm)	2.33
14[22]	364		436			
15[23]	362	7.3	439	75	250 (510 nm)	

silylacetylene according to Sonogashira conditions, and subsequent deprotection of the alkyne gave **12** (scheme 3).[21]

Final fluorophores containing two stilbene units were obtained by double Heck coupling reactions between the bis-bromo central core **3** with 3,4,5-tridodecyloxystyrene[13] or 4-(*N*,*N*-diphenylamino)styrene[14] to give fluorophores **8** and **9** respectively. The fluorophores with elongated π-systems **10** and **11** were prepared by double Sonogashira coupling reactions between the central core **5** with **6** and **7**, respectively. The bis-BODIPY fluorophore **13** was obtained by double Sonogashira coupling reaction of **2** with **12** (Scheme 4).

Photophysical Properties. The absorption and luminescence properties of fluorophores **8**−**11** and **13** are presented in Table 1. The modifications of both the central core skeleton and the electron donor groups allow a fine-tuning of the absorption and emission photophysical properties. Replacing the trialkoxyphenyl group by a diphenylamino group induces a red shift of about 30−40 nm in the absorption spectrum and 40 nm in the emission spectrum. Increasing the length of the conjugated system also leads to a red shift of 8−12 nm in the absorption spectrum and 20 nm for the emission. For the BODIPY derivative, we can notice the strong absorption band at 502 nm of the BODIPY moiety, and a moderate absorption band in the far UV. The absorption spectra of fluorophores **8**, **9**, **10**, **11**, and **13** are shown in Figure 2. All these pyrazabole containing compounds exhibit an intense fluorescence in solution. The fluorescence spectra are shown in Figures 2a,b, c, and the fluorescence quantum yields are presented in Table 1. The fluorescence quantum yield of **8** and **10** that bear trialkoxyphenyl donor groups are about 30−40% when it reaches 57−60% for **9** and **11** that bear diphenylamino donor groups. In these cases, the nature of the conjugated system around the pyrazabole core does not affect significantly the quantum yields of fluorescence.

Fluorescence lifetimes were determined for all fluorophores in methylene chloride. The shorter lifetimes were observed for fluorophores **8** and **10** (0.57 and 0.58 ns, respectively). For **8** a biexponential decay was found, with a dominant short lifetime of 0.57 ns (95%) and a longer one of 1.46 ns (5%). The diphenylamino derivatives **9** and **11** show mono-exponential decay with a 1.50 ns lifetime. The bis-BODIPY derivative **13** presents the longest lifetime of the series (2.33 ns). Interestingly, we

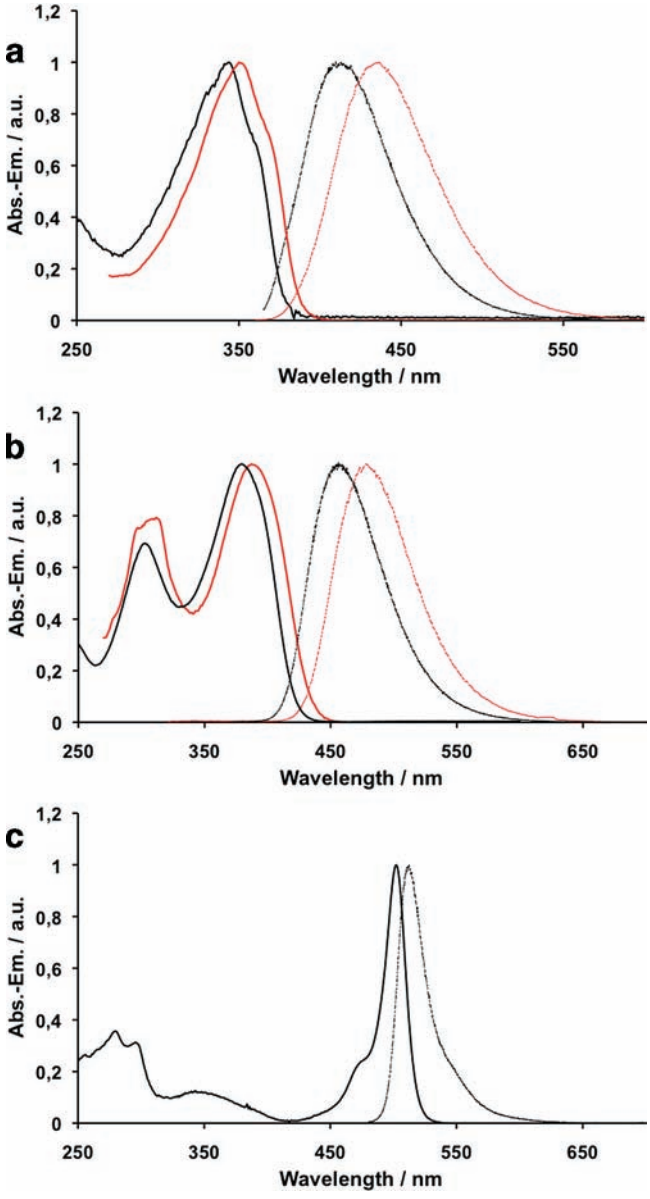


Figure 2. Absorption and emission spectra of fluorophores in methylene chloride (a) **8** (black) and **10** (red) (b) **9** (black) and **11** (red) (c) bis-BODIPY derivative **13**.

can notice a blue shift both in absorption and in emission for **8**, compare to 4,4′-bis-[2-(3,4,5-tridodecyloxyphenyl)-ethenyl]-biphenyl **14**, a parent molecule without the pyrazabole core.[22] This indicates a lower conjugation. To investigate deeper these results, molecular calculations were performed on **11** (Figure 3). The highest occupied molecular orbital (HOMO) and HOMO−1 are quasi-degenerate as well as the lowest unoccupied molecular

(21) Wan, C.-W.; Burghart, A.; Chen, J.; Bergstrom, F.; Johansson, L. B.; Wolford, M. F.; GyumKim, T.; Topp, M. R.; Hochstrasser, R. M.; Burgess, K. L. *Chem.—Eur. J.* **2003**, *9*, 4430–4441.

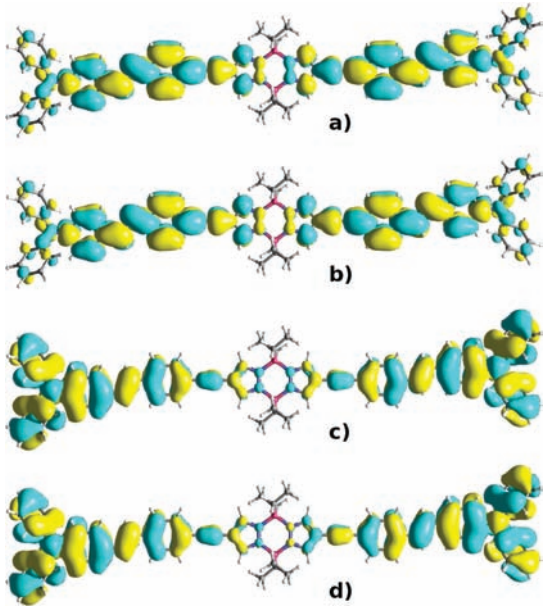


Figure 3. Molecular orbitals of fluorophore **11**. (a) LUMO+1 (E = −0.7398 eV). (b) LUMO (E = −0.7402 eV). (c) HOMO (E = −7.8633 eV). (d) HOMO−1 (E = −7.8647 eV).

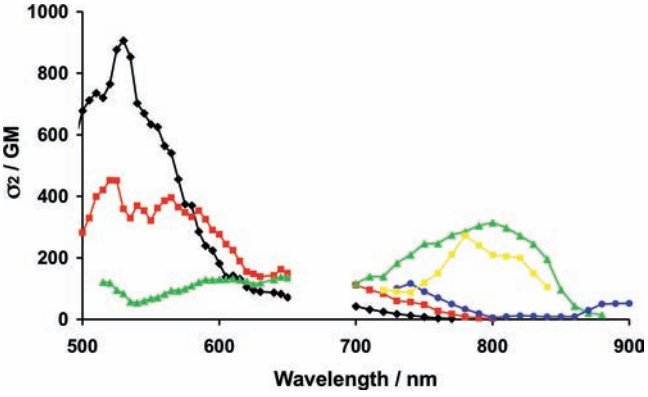


Figure 4. Two-photon action spectra of compounds **8** (black), **9** (yellow), **10** (red), **11** (green), and **13** (blue) in methylene chloride.

orbital (LUMO) and LUMO+1, and differ only from lobes symmetry considerations. This degeneracy was also observed with the chromophores bearing the cyclodiborazane central core.[1] However, contrary to what was pointed out with the cyclodiborazane derivatives, in the present case the calculations clearly indicate the absence of electron delocalization through the pyrazabole core in the ground state as well as in the excited state.

The two-photon properties of fluorophores were investigated experimentally by the two-photon fluorescence method (Figure 4). For **8** we can notice in the visible region a similar response to that of 4,4′-bis-{2-[3,4,5-tris-(1,4,7,10-tetraoxaundecyl)phenyl]ethenyl}-biphenyl **15**, another previously described two-photon fluorophore,[23] bearing the same π-system but without the

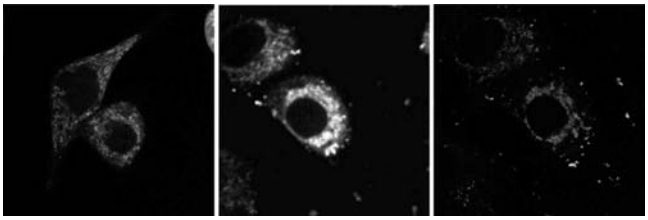


Figure 5. Two-photon images of HeLa cells stained with **10** (left, 740 nm, 15 mW), **11** (middle, 800 nm, 1 mW), and **13** (right, 900 nm, 15 mW).

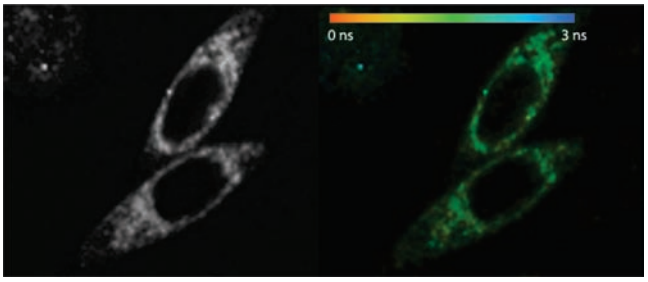


Figure 6. Two-photon intensity of fluorescence image (left) and fluorescence lifetime imaging (FLIM, right) images of HeLa cells stained with **11**.

central pyrazabole core. In this case there is no shift of the TPA λ_{max}, but the two-photon absorption cross-section is increased by a factor 2 for this higher energy band. The lower energy TPA band, present in **15**, is only present as a shoulder in **8**. This indicates that the pyrazabole core plays an important role for the two-photon absorption properties in such system, even without an actual electron delocalization. Similar conclusions have been proposed for the one-photon properties of pyrazabole polymers,[17] in which the pyrazabole moiety is crucial to the luminescence properties.

In the trialkoxyphenyl series, the introduction of two additional triple bonds (**10** vs **8**) lengthens the conjugated system inducing a red shift and a diminution of the higher energy TPA band, which is divided by a factor two. Interestingly the intensity of the lower energy TPA band centered at 675 nm is increased by a factor of two at 650 and 700 nm. For the diphenylamino derivatives, a strong TPA band (≈ 300 GM; 1 GM = 10^{-50} cm^4 s photon^{-1} molecule^{-1}) in the 800 nm range is observed. This wavelength is particularly interesting as most lasers use this wavelength range in commercial two-photon microscopes. The bis-BODIPY derivative **13** presents a maximum TPA efficiency of 100 GM at 740 nm in the near IR range. This value compares well with the ones of specific BODIPY derivatives that have been specifically engineered for two-photon excited fluorescence applications.[24]

Bioimaging. Two-photon microscopy with these lipophilic dyes was performed on HeLa cells. The fluorophores **10**, **11**, and **13** were dissolved in DMSO (10^{-2} M) and diluted to 10^{-4} M in water. This solution was added

(22) Lincker, F.; Masson, P.; Nicoud, J.-F.; Didier, P.; Guidoni, L.; Bigot, J.-Y. *J. Nonlinear Opt. Phys. Mater.* **2005**, *14*, 319–329.
(23) Hayek, A.; Bolze, F.; Nicoud, J.-F.; Baldeck, P. L.; Mély, Y. *Photochem. Photobiol. Sci.* **2006**, *5*, 102–106.
(24) (a) Zheng, Q.; Xu, G.; Prasad, P. N. *Chem.—Eur. J.* **2008**, *14*, 5812–5819. (b) Porres, L.; Mongin, O.; Blanchard-Desce, M. *Tetrahedron Lett.* **2006**, *47*, 1913–1917. (c) Meltola, N. J.; Wahlroos, R.; Soini, A. E. *J. Fluoresc.* **2004**, *14*, 635–647.

to cell cultures at a final concentration of about 10^{-6} M. For all fluorophores, we can notice a rapid penetration into the cells (< 5 min.), a staining of vesicles in the cytoplasm, and low residual fluorescence in the cytoplasm, in particular for **13**. Further investigations on the nature of theses vesicles are under investigation. For chromophore **11**, the laser power used to acquire images at 800 nm was 1 mW while it is 15 mW for chromophores **10** and **13**. This indicates that **11** is an efficient fluorophore for TPEF (Figure 5). In the case of the bis-BODIPY derivative **13**, we can notice a low residual fluorescence in the cytoplasm and an accumulation in the perinuclear region.

Lifetime imaging (FLIM) was also performed with fluorophore **11**. The fluorophore exhibits mono-exponential decay, around 1.6 ns, similar to the values obtained in organic solvent, indicating a hydrophobic environment in cells (Figure 6).

Conclusions

New 1D boron containing two-photon excited fluorophores centered on a pyrazabole core were prepared and characterized. Their one and two-photon photophysical properties were investigated. High two-photon absorption cross sections up to 325 GM in the 800 nm range permit efficient two-photon imaging, and TPEM images were performed with the more interesting fluorophores with laser power in the sub milliwatt range.

Acknowledgment. We thank Joëlle Fritz for HeLa cells culture, Joëlle Muller for IR spectra, Dr Etienne Piémont for lifetime determinations, Dr Cyril Anthaume for ^{11}B and ^{19}F NMR, and Dr Ludovic Richert for two-photon experiments. This work was supported by the CNRS and University Louis Pasteur of Strasbourg. A.H. is grateful to the French ministry of research for a fellowship.