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Boron-Containing Two-Photon-Absorbing Chromophores. 3.[1] One- and Two-Photon Photophysical Properties of *p*-Carborane-Containing Fluorescent Bioprobes

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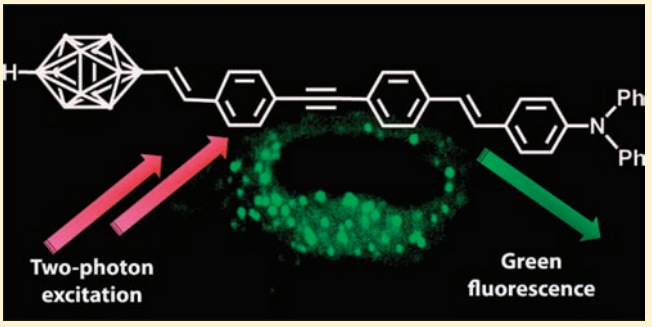
ABSTRACT: Boron-containing two-photon-absorbing fluorophores have been prepared as new bifunctional molecules, potentially useful in two-photon excited microscopy (TPEM) and boron neutron capture therapy. They are based on a one-dimensional conjugated system containing a *p*-carborane entity at one end of the molecule and various electron-donating groups containing oxygen or nitrogen atoms at the other end. We investigated their one- and two-photon photophysical properties. They showed efficient fluorescence in an organic solvent, as well as in water for two of them, allowing microscopy on cell cultures. High two-photon absorption cross sections were determined in the 700−900 nm range. TPEM images were obtained with these new *p*-carborane-containing fluorophores, with laser intensities in the submilliwatt range.



INTRODUCTION

Nowadays, the conception and synthesis of original nonlinear optical (NLO) chromophores is a very stimulating area in organic chemistry, at the interface of chemical engineering, physics, and, more recently, biology. Indeed, this research field is based on the multiple potential applications of NLO chromophores, particularly by using multiphoton absorption processes such as microfabrication,[2] 3D optical data storage,[3] optical limiting,[4] photodynamic therapy,[5] or bioimaging.[6] For many years, we have worked on the molecular engineering of efficient two-photon-absorbing (TPA) fluorophores for uses in in vitro and in vivo bioimaging.[7] Most often the explored fluorophores consist of a one-dimensional (1D) symmetric conjugated structure, in which a pair of electron-donor or -acceptor groups are placed at both extremities, so that an electronic interaction can occur through the whole 1D molecular conjugated system.[8] It should be noticed, however, that dissymmetric donor/acceptor systems, well-known in quadratic nonlinear optics, should also be efficient in cubic nonlinear optics, hence showing significant TPA properties. In most cases, the electronic interaction proceeds classically via a π-conjugated system arising from a succession of aromatic (or heteroaromatic) rings linked by single, double, or triple bonds. Recently, we have added a new property in such molecules that could permit one to exploit the two-photon fluorophores not only in two-photon microscopy but also for another potential use like therapy. We have chosen to introduce boron atoms into the structure of TPA fluorophores because boron-containing drugs are often used as sensitizers in boron neutron capture therapy (BNCT).[9] We have first reported such new kinds of TPA chromophores in which the interaction between the two donor moieties proceeds through the cyclodiborazane core, which is a pseudoconjugated boron-containing heterocycle.[10] Recently, we have reported the two-photon photophysical properties of new 1D fluorescent bioprobes centered on a pyrazabole central core.[11] However, the cyclodiborazane and pyrazabole heterocycles contain only two boron atoms, which could be insufficient for efficient BNCT. That is why we have been considering the introduction of 1,12-dicarba-*closo*-dodecaborane (1,12-$B_{10}C_2H_{12}$, known as *p*-carborane; Figure 1), which contains 10 boron atoms, to the TPA fluorophores. This unit is known to give derivatives with good thermal and chemical stability and offers wide possibilities of functionalization. π systems that bear *closo*-dodecaborate entities have already been reported by one of us as potential candidates for TPA processes.[12] The introduction of carborane into conjugated dyads has evidenced the electron-withdrawing characteristics of carboranes.[13] Recently, Tour et al. have prepared new highly fluorescent BODIPY-based nanocars containing four *p*-carborane units, but their TPA properties were not reported.[14] So, *p*-carborane **1** appears as an interesting building block to investigate novel conjugated fluorescent chromophores containing a great number of boron atoms and having significant NLO properties such as TPA.

These new *p*-carborane derivatives would lead to bifunctional molecules, potentially useful in two-photon excited microscopy

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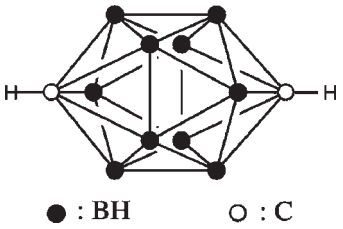


Figure 1. Structure of *p*-carborane **1** (1,12-dicarba-*closo*-dodecaborane, $B_{10}C_2H_{12}$).

(TPEM) and BNCT. The TPA imaging capabilities and BNCT sensitizing ambivalence could be of great interest for studying the mechanism of the processes involved and allowing an accurate localization of the sensitizer during the therapy.[15] Such molecules (as boron-containing porphyrins) are in growing emphasis.[16] TPEM takes advantage of the TPA properties in living tissues and animals, such as deep penetration or low photodamage.[17] Intravital TPEM was used to study the effects of tomographic synchrotron irradiation on mouse brain microvasculature, pointing out the interest of this microbeam radiation therapy.[18] We report here the synthesis and characterization of new 1D donor/acceptor TPA chromophores **6a**−**6c** containing a *p*-carborane moiety at one end and bearing various donor groups at the other end that could be used as such bifunctional molecules. Preliminary TPEM images will also be presented.

EXPERIMENTAL SECTION

Synthesis. All chemicals and reagents were purchased from Aldrich or Acros Organics and were used as received unless specified. The starting material *p*-carborane **1** was obtained from Katchem Inc. Tetrahydrofuran (THF) was distilled over sodium under an argon atmosphere prior to use. ^{1}H, ^{13}C, 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 NMR spectra and to $CDCl_3$ (δ 77 ppm) for ^{13}C NMR spectra. The chemical shifts for ^{11}B NMR spectra are reported in ppm at 128.37 MHz. Mass spectra were recorded with either a Bruker MicrOTOF-Q (ESI), an Agilent MSD (MM-ES-ACPI), or a Bruker Autoflex (MALDI-TOF) spectrometer. Thin-layer chromatography was run on Merck precoated aluminum plates (Silica 60 F_{254}). Column chromatography was run on Merck silica gel (60−120 mesh) or Merck neutral alumina (70−230 mesh). Compounds **2**,[19] **3**,[10] and **5a**[10] were prepared as previously described.

*(E)-C-4-iodostyryl-p-carborane (**4**).* Under an argon atmosphere, diethyl (4-iodobenzyl)phosphonate (**3**; 70 mg, 0.19 mmol) was dissolved in 30 mL of anhydrous THF, and then 10 mg of NaH (60% in oil, 0.25 mmol) was added. After the effervescence ceased, the suspension was refluxed for 1 h. C-monoformyl-*p*-carborane (**2**) in 20 mL of anhydrous THF was added dropwise (25 mg, 0.16 mmol), and then the mixture was refluxed for 2 h. After filtration, the solvent was evaporated under vacuum. The crude product was purified by column chromatography on silica gel with dichloromethane as the eluent, to give **4** as a white solid (40 mg, 70% yield).

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.59 (d, 2H, J = 8.4 Hz), 6.98 (d, 2H, J = 8.4 Hz), 6.27 (d, 1H, J = 15.5 Hz), 5.85 (d, 1H, J = 15.5 Hz), 3.10−1.21 (m, 11H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 137.7, 134.7, 131.0, 128.3, 127.5, 93.9, 58.9. ^{11}B NMR (128 MHz, $CDCl_3$, 25 °C): δ 11.8, 13.1, 14.3, 15.6. ^{11}B{H} NMR (128 MHz, $CDCl_3$, 25 °C): δ 12.4, 15.0. HRMS: m/z 372.13845 (calcd for $C_{10}H_{17}B_{10}I$: m/z 372.13781).

*Chromophore **6a**.* In a two-necked flask, under an argon atmosphere, 148 mg of **5a** (0.39 mmol), 120 mg of **4** (0.19 mmol), 7 mg of $PdCl_2$ (0.04 mmol), 1.19 mg of copper acetate monohydrate (0.006 mmol), and 41.76 mg of triphenylphosphine (0.16 mmol) were suspended in 50 mL of a THF/triethylamine mixture (15/45, v/v). The mixture was heated to 80 °C for 12 h, and the solvent was evaporated under vacuum. The product was purified by column chromatography on alumina, with a mixture of cyclohexane/dichloromethane (60/40, v/v) as the eluent, to give **6a** as a yellow solid (90 mg, 42% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.48−7.37 (m, 6H), 7.33 (d, 2H, J = 8.2 Hz), 7.27−7.23 (m, 5H), 7.16 (d, 2H, J = 8.6 Hz), 7.07 (d, 4H, J = 8.5 Hz), 7.03 (d, 4H, J = 8.5 Hz), 6.92 (d, 1H, J = 15.9 Hz), 6.32 (d, 1H, J = 15.9 Hz), 5.89 (d, 1H, J = 15.9 Hz), 3.40−1.21 (m, 11H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 147.65, 147.46, 137.75, 135.01, 132.43, 131.77, 131.72, 131.08, 129.30, 128.34, 126.59, 126.19, 124.62, 123.34, 123.16, 121.62, 90.95, 90.01. ^{11}B NMR (128 MHz, $CDCl_3$, 25 °C): δ 11.7, 13.0, 14.3, 15.6. ^{11}B{H} NMR (128 MHz, $CDCl_3$, 25 °C): δ 12.4, 15.0. HRMS: m/z616.3913 (calcd for $C_{38}H_{37}B_{10}N$: m/z 616.3913).

*(E)-1,2,3-Tris(dodecyloxy)-5-(4-iodostyryl)benzene (**8b**).* Under an argon atmosphere, 1 g of **3** (2.80 mmol) was dissolved in anhydrous THF (5 mL), and then 565 mg of NaH (60% in oil, 14 mmol) was added slowly. After 1 h of heating at 50 °C, 1.85 g of 3,4,5-tris(dodecyloxy)benzaldehyde (**7b**; 2.80 mmol) in 40 mL of anhydrous THF was added. The mixture was refluxed for 2 h and quenched with 1 mL of methanol. After cooling, the precipitate was filtered over Celite, and the solvent was removed under reduced pressure. The crude product was purified by recrystallization in methanol. The desired product **8b** was obtained as a white solid (2.04 g, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.66 (d, 2H, J = 8.3 Hz), 7.23 (d, 2H, J = 8.3 Hz), 7.03 (d, 1H, J = 16.2 Hz), 6.85 (d, 1H, J = 16.2 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 = 6.4 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, 29.33, 26.08, 22.66, 14.08. HRMS: m/z 858.5368 (calcd for $C_{50}H_{83}IO_3$: m/z 858.5386).

*(E)-1,2,3-Tris[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-5-(4-iodostyryl)-benzene (**8c**).* Under an argon atmosphere, 660 mg of **3** (1.86 mmol) was dissolved in anhydrous THF (20 mL), and then 565 mg of NaH (60% in oil, 14 mmol) was added slowly. After 1 h of heating at 50 °C, 1.22 g of 3,4,5-tris[2-(2-methoxyethoxy)ethoxy]benzaldehyde (**7c**; 1.86 mmol) in 20 mL of anhydrous THF was added. The mixture was refluxed for 2 h and quenched with 1 mL of methanol. After cooling, the precipitate was filtered over Celite, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel with dichloromethane/methanol ratios of 97/3 to 50/50 (v/v) as the eluent. The desired product **8c** was obtained as a yellow oil (1.10 g, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.66 (d, 2H, J = 8.3 Hz), 7.22 (d, 2H, J = 8.3 Hz), 7.15−6.83 (d, 1H, J = 16.2 Hz), 6.83 (d, 1H, J = 16.2 Hz), 6.74 (s, 2H), 4.22−4.08 (m, 6H), 3.86 (t, 4H, J = 5.5 Hz), 3.79 (t, 2H, J = 5.5 Hz), 3.75−3.62 (m, 18H), 3.55−3.52 (m, 6H), 3.36 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 152.75, 138.68, 137.65, 136.71, 132.39, 129.22, 128.61, 128.04, 126.84, 126.34, 106.41, 92.57, 72.33, 71.88, 71.87, 70.76, 70.64, 70.62, 70.50, 70.48, 70.46, 69.71, 68.87, 67.88, 58.95. HRMS: m/z 810.29248 (calcd for $C_{35}H_{57}INO_{12}$: m/z 810.29254, M + NH_4).

*(E)-Trimethyl[[4-[3,4,5-tris(dodecyloxy)styryl]phenyl]ethynyl]silane (**9b**).* In a three-necked flask, 0.08 mL of (trimethylsilyl)acetylene (51.9 mg, 0.53 mmol), 500 mg of **8b** (0.63 mmol), 24.51 mg of $Pd(PPh_3)_2Cl_2$ (0.035 mmol), and 10.2 mg of CuI (0.052 mmol) were suspended in 30 mL of anhydrous THF and 5 mL of isopropylamine. The mixture was deaerated with argon and then sonicated for 30 min at room temperature. The solvents were evaporated under vacuum, and the crude product was purified by column chromatography on alumina with

97/3 (v/v) cyclohexane/methanol as the eluent, to yield the desired product **9b** as a white solid (360.1 mg, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.45 (d, 2H, J = 8.8 Hz), 7.40 (d, 2H, J = 8.8 Hz), 7.06 (d, 1H, J = 16.2 Hz), 6.90 (d, 1H, J = 16.2 Hz), 6.70 (s, 2H), 4.04−3.95 (m, 6H), 1.85−1.73 (m, 6H), 1.54−1.44 (m, 6H), 1.27 (m, 48 H), 0.88 (t, 9H, J = 6.3 Hz), 0.27 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 153.28, 138.59, 137.88, 132.39, 132.09, 130.08, 126.74, 126.13, 120.73, 105.31, 80.65, 73.50, 69.16, 31.90, 31.88, 30.30, 29.71, 29.69, 29.66, 29.62, 29.57, 29.39, 29.35, 29.32, 26.08, 22.64, 14.07, −0.05. HRMS: m/z 829.147 (calcd for $C_{55}H_{92}SiO_3$: m/z 829.403).

*(E)-Trimethyl[[4-[3,4,5-tris[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-styryl]phenyl]ethynyl]silane (**9c**).* In a three-necked flask, 0.09 mL of (trimethylsilyl)acetylene (62 mg; 0.63 mmol), 500 mg of **8c** (0.63 mmol), 26 mg of $Pd(PPh_3)_2Cl_2$ (0.037 mmol), and 12 mg of CuI (0.063 mmol) were suspended in 30 mL of anhydrous THF and 5 mL of isopropylamine. The mixture was deaerated with argon and sonicated for 30 min at room temperature. The solvents were evaporated under vacuum, and the crude product was purified by column chromatography on alumina with 90/10 (v/v) cyclohexane/methanol as the eluent, to yield the desired product **9c** as a colorless oil (408 mg, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.46 (d, 2H, J = 8.5 Hz), 7.39 (d, 2H, J = 8.5 Hz), 7.02 (d, 1H, J = 16.2 Hz), 6.89 (d, 1H, J = 16.2 Hz), 6.75 (s, 2H), 4.22−4.15 (m, 6H), 3.87 (t, 4H, J = 5.3 Hz), 3.79 (t, 2H, J = 5.3 Hz), 3.76−3.61 (m, 18H), 3.56−3.53 (m, 6H), 3.37 (s, 3H), 3.37 (s, 6H), 0.25 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 152.74, 138.65, 137.32, 132.57, 132.25, 129.38, 128.63, 127.34, 126.36, 126.11, 121.94, 106.44, 105.12, 95.02, 72.35, 71.89, 70.77, 70.66, 70.63, 70.52, 70.49, 70.46, 69.71, 68.86, 58.97, 53.37, −0.05. HRMS: m/z 762.816 (calcd for $C_{40}H_{62}SiO_{12}$: m/z 762.999).

*(E)-1,2,3-Tris(dodecyloxy)-5-(4-ethynylstyryl)benzene (**5b**).* Under an argon atmosphere, 400 mg of **9b** (0.55 mmol) was dissolved in 20 mL of THF, and then 0.28 mL of 1 M tetrabutylammonium fluoride (TBAF) was added. The mixture was stirred at room temperature for 1 h. The solvents were evaporated under vacuum, and the crude product was purified by column chromatography on silica gel with cyclohexane as the eluent. The pure product **5b** was obtained as a yellow oil (356 mg, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.49 (d, 2H, J = 8.77 Hz), 7.43 (d, 2H, J = 8.77 Hz), 7.02 (d, 1H, J = 16.2 Hz), 6.92 (d, 1H, J = 16.2 Hz), 6.71 (s, 2H), 4.04−3.95 (m, 6H), 3.13 (s, 1H), 1.87−1.70 (m, 6H), 1.54−1.44 (m, 6H), 1.27 (m, 48H), 0.88 (t, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 153.28, 138.59, 137.88, 132.39, 132.09, 130.08, 126.74, 126.13, 120.73, 105.31, 83.71, 73.50, 69.16, 31.90, 31.88, 30.30, 29.71, 29.69, 29.66, 29.62, 29.57, 29.39, 29.35, 29.32, 26.08, 22.64, 14.07. HRMS: m/z 756.64205 (calcd for $C_{52}H_{84}O_3$: m/z 756.64265).

*(E)-1,2,3-Tris[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]-5-(4-ethynyl-styryl)benzene (**5c**).* Under an argon atmosphere, 420 mg of **9c** (0.55 mmol) was dissolved in 20 mL of THF, and then 0.19 mL of 1 M TBAF was added. The mixture was stirred at room temperature for 1 h. The solvent was evaporated under vacuum, and the crude product was purified by column chromatography on silica gel with cyclohexane as the eluent. The pure product **5c** was obtained as a yellow oil (372 mg, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.49 (d, 2H, J = 8.8 Hz), 7.42 (d, 2H, J = 8.8 Hz), 7.02 (d, 1H, J = 16.2 Hz), 6.94 (d, 1H, J = 16.2 Hz), 6.78(s, 2H), 4.23−4.15 (m, 6H), 3.85 (t, 4H, J = 4.8 Hz), 3.79 (t, 2H, J = 4.8 Hz), 3.76−3.61 (m, 18H), 3.56−3.53 (m, 6H), 3.37 (s, 3H), 3.37 (s, 6H), 3.13 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 152.77, 138.74, 137.68, 132.49, 132.41, 129.61, 127.20, 126.36, 126.19, 120.89, 106.52, 83.68, 72.36, 71.89, 70.78, 70.66, 70.64, 70.52, 70.49, 70.47, 69.73, 68.89, 58.98. HRMS: m/z 690.3615 (calcd for $C_{37}H_{54}O_{12}$: m/z 690.36257).

*Chromophore **6b**.* In a two-necked flask, under an argon atmosphere, 220 mg of **5b** (0.29 mmol), 87 mg of **4** (0.14 mmol), 5 mg of $PdCl_2$ (0.028 mmol), 0.85 mg of copper acetate monohydrate (0.004 mmol), and 30 mg of triphenylphosphine (0.11 mmol) were suspended in 60 mL of THF/triethylamine (15/45, v/v). The mixture was heated to 80 °C for 12 h, and the solvent was evaporated under vacuum. The product was purified by column chromatography on alumina, with a mixture of cyclohexane/dichloromethane (50/50, v/v) as the eluent, to give **6b** as a pale-yellow solid (100 mg, 37% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.47 (d, 2H, J = 8.3 Hz), 7.44 (d, 2H, J = 8.3 Hz), 7.41 (d, 2H, J = 8.3 Hz), 7.2 (d, 2H, J = 7.3 Hz), 7.02 (d, 1H, J = 15.3 Hz), 6.93 (d, 1H, J = 15.3 Hz), 6.69 (s, 2H), 6.32 (d, 1H, J = 15.8 Hz), 5.89 (d, 1H, J = 15.8 Hz), 4.01 (t, 4H, J = 6.5 Hz), 3.96 (t, 2H, J = 6.5 Hz), 3.40−2.10 (m, broad, 11H), 1.78 (m, 6H), 1.47 (m, 6H), 1.25 (m, 48H), 0.87 (t, 9H, J = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 153.36, 138.68, 137.45, 135.07, 132.36, 132.18, 131.92, 131.80, 129.96, 127.77, 127.50, 126.61, 126.30, 123.30, 121.85, 105.42, 90.89, 90.11, 73.58, 69.25, 31.96, 30.30, 29.74, 29.62, 19.40, 29.33, 26.16, 22.72, 14.13. ^{11}B NMR (128 MHz, $CDCl_3$, 25 °C): δ 11.7, 13.0, 14.3, 15.6. ^{11}B{H} NMR (128 MHz, $CDCl_3$, 25 °C): δ 12.3, 15.0. HRMS: m/z 1001.8706 (calcd for $C_{62}H_{100}O_3B_{10}$: m/z 1001.8676).

*Chromophore **6c**.* In a two-necked flask, under an argon atmosphere, 300 mg of **5c** (0.43 mmol), 130 mg of **4** (0.21 mmol), 18 mg of $Pd(PPh_3)_2Cl_2$ (0.026 mmol), and 8.25 mg of copper iodide (0.0043 mmol) were suspended in 50 mL of THF/triethylamine (15/45, v/v). The mixture was sonicated for 2 h and stirred for 2 days. The solvents were then evaporated under vacuum. The product was purified by column chromatography on alumina, with a mixture of cyclohexane/methanol (90/10, v/v) as the eluent, to give **6c** as a yellow oil (167 mg, 45% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.47 (d, 2H, J = 9.1 Hz), 7.43 (d, 2H, J = 9.1 Hz), 7.41 (d, 2H, J = 7.4 Hz), 7.19 (d, 2H, J = 8.4 Hz), 6.99 (d, 1H, J = 15.7 Hz), 6.92 (d, 1H, J = 15.7 Hz), 6.74 (s, 2H), 6.32 (d, 1H, J = 15.7 Hz), 5.88 (d, 1H, J = 15.7 Hz), 4.19 (t, 4H, J = 5.4 Hz), 4.15 (t, 2H, J = 4.9 Hz), 3.85 (t, 4H, J = 5.4 Hz), 3.77 (t, 2H, J = 4.5 Hz), 3.71 (m, 6H), 3.64 (m, 12H), 3.52 (m, 6H), 3.35 (s, 3H), 3.34 (s, 6H), 3.2−2.21 (m, broad, 11H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 152.7, 138.4, 137.27, 135.08, 132.83, 132.41, 131.93, 131.79, 129.36, 128.70, 128.35, 127.56, 126.55, 126.60, 126.37, 123.25, 122.03, 106.50, 106.38, 90.83, 90.12, 83.03, 72.35, 71.90, 71.85, 70.75, 70.63, 70.51, 70.44, 70.41, 69.68, 68.85, 59.04. ^{11}B NMR (128 MHz, $CDCl_3$, 25 °C): δ 11.8, 13.0, 14.3, 15.6. ^{11}B{H} NMR (128 MHz, $CDCl_3$, 25 °C): δ 12.4, 15.0. HRMS: m/z 958.5745 (calcd for $C_{47}H_{70}O_{12}B_{10}$: m/z 958.5761).

One-Photon Photophysics. 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 the reference. Values of the molar extinction coefficients, $\varepsilon_{\lambda max}$, of the 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 (φ = 0.54)[20] as the reference. For these measurements, the slit widths were adjusted so that the spectral bandwidths of the absorption and emission were identical at 1.0 nm, and the absorbances of the sample and the reference were chosen so that 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.[20] Fluorescence lifetime measurements were performed using the time-correlated single-photon-counting technique, according to the method described by Lami and Piémont.[21]

Two-Photon Excitation Characterizations. The TPA cross-sectional spectra were obtained by up-conversion fluorescence measurements using a Ti:sapphire femtosecond laser in the range 700−900 nm.

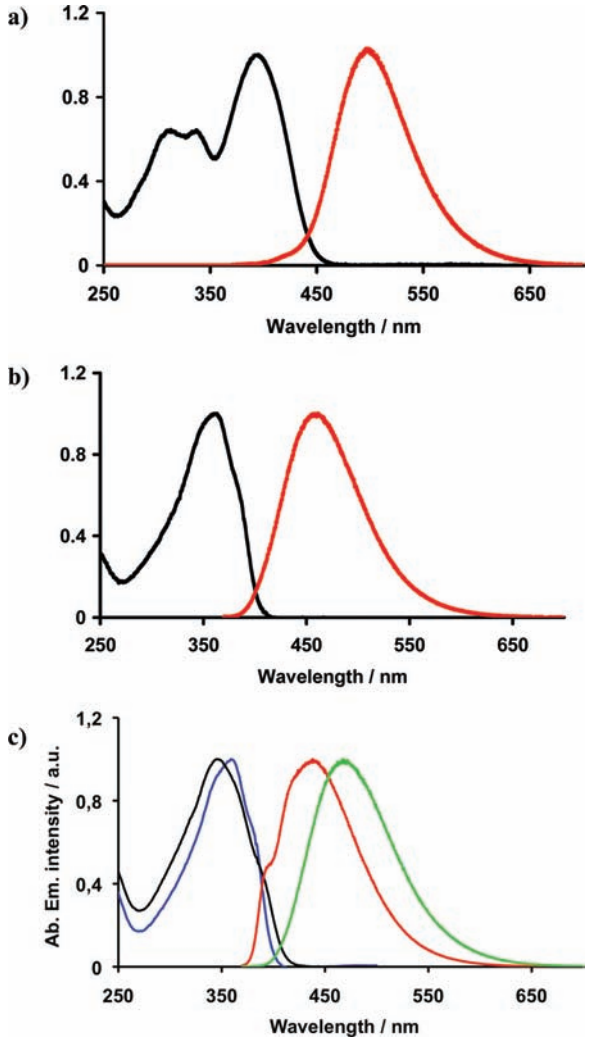


Figure 2. Absorption (black) and emission (red) spectra of **6a** (a), **6b** (b), and **6c** (c) in dichloromethane. Absorption (blue) and emission (green) spectra of **6c** in water (c).

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 at 570 nm, and with the published 700−900 nm Rhodamine B TPA spectrum.[22]

Bioimaging. One- and two-photon excited microscopies were performed on a Leica TCS SP2 AOBS MP microscope. For one-photon images, the excitation source was a continuous wave 405 nm laser diode, and for two-photon images, the excitation was performed by a Spectra Physics Tsunami laser coupled to the microscope by an optical fiber (repetition rate of 80 MHz and pulse duration of 1.5 ps). We used a 63× oil immersion objective HCX PL APO CS Lambda Blue (NA = 1.4).

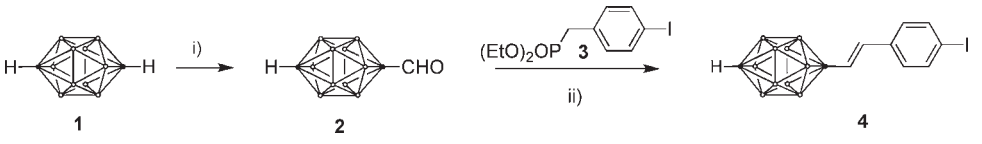
RESULTS AND DISCUSSION

Synthesis. We have prepared three 1D chromophores **6a**−**6c** containing at one end an acceptor *p*-carborane unit and at the other end various donor groups. All three are well-soluble in organic solvents, while only **6c** is sufficiently water-soluble to allow photophysical characterizations in water (Figure 2). The commercially available *p*-carborane **1** ($B_{10}C_2H_{12}$) was functionalized by monoformylation with 1 equiv of methyl formate to give the C-formylcarborane **2** in 65% yield. The reaction of 1 equiv of **3** with the monoformyl derivative **2** affords (*E*)-4-iodostyryl-*p*-carborane moiety **4** in 70% yield (Scheme 1). This compound can then be used as the starting material for the classical palladium cross-coupling reactions in order to build the targeted 1D conjugated chromophores **6a**−**6c**.

The iodo derivative **4** was coupled to three different conjugated building blocks bearing donor groups (Scheme 2): (*E*)-4-(4-ethynylstyryl)-*N*,*N*-diphenylaniline (**5a**) and **5b** to give chromophores **6a** and **6b**, which are soluble in organic solvents, and **5c** bearing three short oligo(ethylene glycol) (OEG) chains to give the nonionic water-soluble chromophore **6c**. The donor building block **5a** was prepared as described previously.[10]

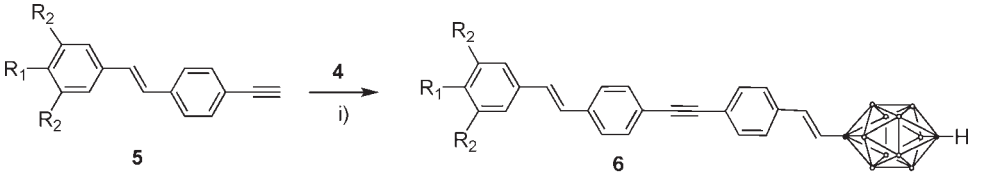
The synthetic pathway for the other oxygen-containing donor groups is shown in Scheme 3. All compounds were fully

Scheme 1. Synthesis of 4[a]



[a] (i) BuLi, methyl formate, and then 5 M HCl. (ii) THF, NaH, 1 h, reflux.

Scheme 2. Synthesis of chromophores 6a−6c[a]



5a, 6a : $R_1 = Ph_2N$, $R_2 = H$ **5b, 6b** : $R_1 = R_2 = C_{12}H_{25}O$ **5c, 6c** : $R_1 = R_2 = Me(OCH_2CH_2)_3O$

[a] (i) $PdCl_2$, $Cu(OAc)_2(H_2O)$, PPh_3, and THF/Et_3N for **6a** and **6b** and $Pd(PPh_3)_2Cl_2$, CuI, and THF/Et_3N for **6c**.

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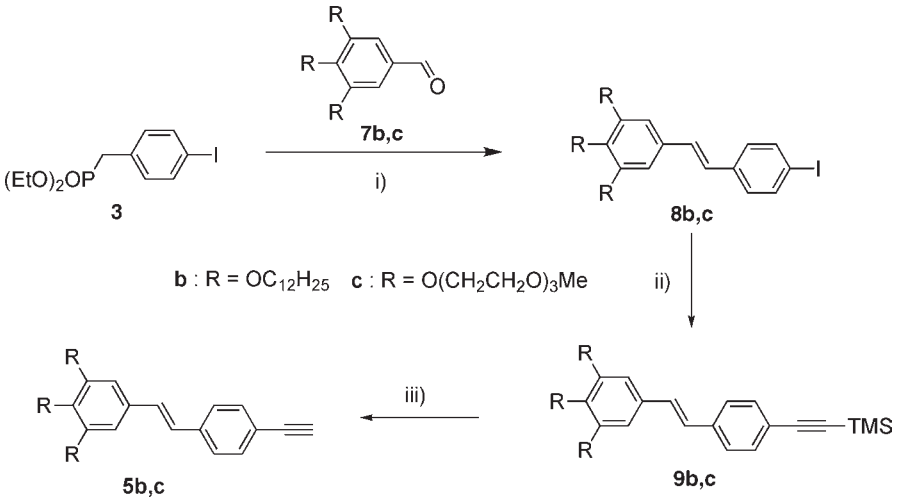
Scheme 3. Preparation of Oxygen-Containing Donor-Conjugated Systems 5b and 5c[a]



[a] (i) NaH, THF. (ii) (Trimethylsilyl)acetylene, $Pd(PPh_3)_2Cl_2$, CuI, THF/Et_3N, and ultrasound. (iii) TBAF and THF at room temperature.

Table 1. Photophysical Properties of Chromophores 6a–6c in Dichloromethane and Water

	absorption				emission			
	H_2O		CH_2Cl_2		H_2O		CH_2Cl_2	
chromophore	λ_{max} (nm)	$\varepsilon \times 10^{-4}$ (M^{-1} cm^{-1})	λ_{max} (nm)	$\varepsilon \times 10^{-4}$ (M^{-1} cm^{-1})	λ_{max} (nm)	Φ (%)	λ_{max} (nm)	Φ (%)
6a			395	9.9			496	58
6b			363	12.2			458	59
6c	346	7.2	360	10.7	467	24	438	69

characterized by ^1H, ^{13}C, and ^{11}B NMR spectroscopy, UV–visible spectroscopy, and high-resolution mass spectrometry (HRMS).

Photophysical Properties. The one-photon photophysical properties of chromophores **6a–6c** were studied in solution in dichloromethane, and **6c** was also studied in water. In an organic solvent, all three chromophores present an intense absorption maximum in the 360–395 nm range. The values of λ_{max} and the corresponding ε are presented in Table 1.

The UV–visible and emission spectra are depicted in Figure 2.

For chromophores **6b** and **6c**, those bearing alkoxy and OEG donor groups, the shapes of the UV–visible and emission spectra are similar in dichloromethane (229 cm^{-1} shift in λ_{max} values and 1.5×10^4 M^{-1} cm^{-1} difference for ε_{max}). For chromophore **6a**, bearing the diphenylamino donor group, we notice a 2461 cm^{-1} red shift of λ_{max} compared to the oxygenated chromophores **6b** and **6c**, as is expected for a better electron-donating group. Because chromophore **6c** is water-soluble, its photophysical properties were also investigated in water. There is a slight blue shift of 1124 cm^{-1} from dichloromethane to water. In addition, the molar extinction coefficient ε_{max} decreases from 10.7×10^4 M^{-1} cm^{-1} in dichloromethane to 7.2×10^4 M^{-1} cm^{-1} in water.

In dichloromethane, chromophores **6b** and **6c** present a blue fluorescence, while **6a** emits in the green region. Emission data are presented in Table 1. We can observe a blue shift in the emission from **6b** to **6c**, indicating a difference between the two chromophores in their excited states. In water, the emission of chromophore **6c** is red-shifted by 1418 cm^{-1} in association with a decrease of the fluorescence quantum yield compared to those measured in dichloromethane. Fluorescence lifetimes were also determined in dichloromethane. The three fluorophores present a fluorescence monoexponential decay, leading to lifetimes of 1.60, 1.25, and 1.17 ns for **6a–6c** respectively. In water, **6c** presents a multiexponential decay, leading to lifetimes of 0.12 ns (27%), 0.72 ns (41%), 2.32 ns (26%), and 6.35 ns (6%).

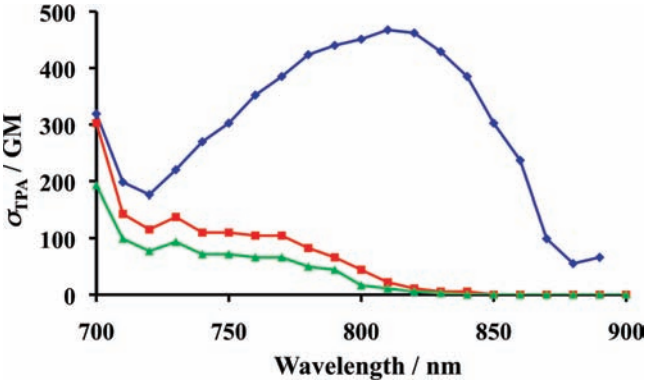


Figure 3. Two-photon excitation spectra of chromophores **6a** (blue), **6b** (red), and **6c** (green) in dichloromethane.

The two-photon photophysical properties of these fluorophores were investigated by way of their two-photon excited fluorescence. The two-photon excitation spectra are depicted in Figure 3. Chromophore **6a** presents a large and strong

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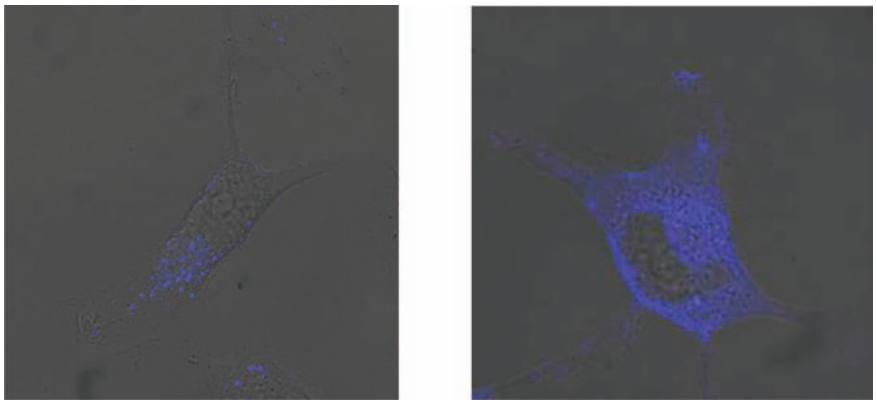

Figure 4. CLSM of HeLa cells dyed with fluorophores **6a** (left) and **6c** (right).

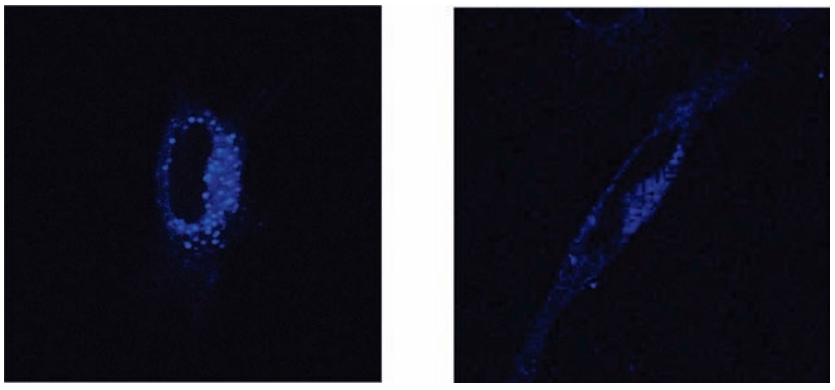

Figure 5. TPEM images of HeLa cell stained with fluorophores **6a** (left) and **6c** (right). Excitation wavelength at 800 nm (laser power for **6a**, 1−2 mW, and for **6c**, 15 mW).

two-photon absorption band in the wavelength region studied (700−900 nm) with a maximum σ_2 of 470 GM at 810 nm, while for chromophores **6b** and **6c**, only the absorption tails are observed. The TPA maxima are located under 700 nm, when the σ_2 values are 300 and 200 GM at 700 nm for **6b** and **6c**, respectively (1 GM = 10^{-50} cm^4 s photon^{-1}).

Bioimaging. Our aim was to check the behavior of **6a** and **6c** during preliminary fluorescence microscopy investigations. Fluorophore **6b** could not be used because of its insolubility in water. Fluorophore **6c** was readily soluble in an Hank's Balanced Salt Solution (HBSS) buffer (10^{-4} M). We have found that fluorophore **6a** was also soluble in HBSS in the presence of 1% dimethyl sulfoxide (for a final concentration of $\sim 10^{-5}$ M), allowing biological studies.

Dyes **6a** and **6c** were first studied by confocal laser scanning microscopy (CLSM) on a Leica LSP 2. They were internalized very rapidly (<5 min), but the localization behaviors of these two dyes were very different. Figure 4 shows their localization in HeLa cells (superposition of fluorescence and bright-field images). For **6a**, the distribution is heterogeneous, with visible vesicles, and for **6c**, the distribution is much more diffuse because of better water solubility.

TPEM was then tested, with an excitation wavelength of 800 nm. This wavelength lies in the most efficient domain of commercial laser microscopes (stability and power). With dye **6c**, the signal is low because of the mismatch in the excitation wavelength, while for **6a**, the signal is very intense down to the 1−2 mW excitation power range at the specimen (Figure 5). The distributions are similar to that obtained by one-photon excitation.

CONCLUSIONS

Three new 1D fluorescent boron-containing TPA chromophores of the donor/acceptor type have been designed, synthesized, and characterized. Their TPA cross sections are high in the near-IR range with σ_2 of 470 GM at 810 nm for the diphenylamino-substituted chromophore **6a** and 200−300 GM at 700 nm for the chromophores **6b** and **6c** containing oxygen donor groups. The inclusion of the *p*-carborane entity into TPA chromophores brings 10 boron atoms in the structure and does not reduce the TPA cross sections σ_2 of the whole conjugated systems. Two of the new chromophores (**6a** and **6c**) are sufficiently water-soluble for use in TPEM in living cells. The high σ_2 of **6a** and its high boron atom content make it a bifunctional molecule that could be of great interest in biology, to study by TPEM the mechanisms involved in BNCT. Further work is in progress to test it in two-photon bioimaging, and cellular results will be reported in a forthcoming paper.

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