ORGANIC LETTERS

2002
Vol. 4, No. 5
719−722

Synthesis and Photophysical Properties of New Conjugated Fluorophores Designed for Two-Photon-Excited Fluorescence

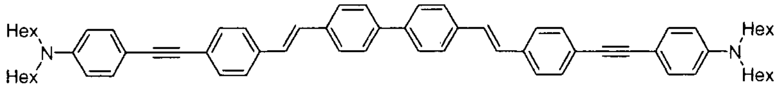
Olivier Mongin,[†] Laurent Porrès,[†] Laurent Moreaux,[‡] Jerome Mertz,[‡] and Mireille Blanchard-Desce*,[†]

Synthèse et ElectroSynthèse Organiques (CNRS, UMR 6510), Université de Rennes 1, Campus de Beaulieu, Bât. 10A, F-35042 Rennes Cedex, France, and Neurophysiologie et Nouvelles Microscopies (INSERM EPI 00-02), Ecole Supérieure de Physique et Chimie Industrielles, 10 rue Vauquelin, 75005 Paris, France

mireille.blanchard-desce@univ-rennes1.fr

Received November 29, 2001

ABSTRACT



9 $\sigma_2 = 1050$ GM; $\Phi = 0.80$

Novel elongated push-push fluorophores (e.g., 9) were synthesized by 2-fold Sonogashira or Wittig–Horner reactions. Modulation of the length and topology of the conjugated connectors allows tuning of their photophysical properties. In addition, their photoluminescence can be adjusted by playing on polarity. Derivatives combining enhanced two-photon absorption cross section (σ_2) in the visible red and high fluorescence quantum yield (Φ) have been obtained. Such fluorophores hold promise for nonlinear imaging of biological systems.

Research into molecular two-photon absorption (TPA) has received increasing attention owing to numerous applications in various areas such as optical data storage,[1] microfabrication,[2] optical power limiting,[3] photodynamic therapy,[4] and two-photon laser scanning fluorescence imaging.[5] Among these, two-photon-excited fluorescence (TPEF) has gained widespread popularity in the biology community owing to its many advantages. This includes a capacity for a highly confined excitation and intrisic 3-D high resolution when used in microscopic imaging, as well as the ability to image at increased penetration depth in tissue with reduced photodamage while avoiding background fluorescence by operating in the visible red-NIR region. Initially TPEF was developed using conventional fluorophores whose TPA characteristics were not optimized and thus led to the use of either high laser intensity and/or high fluorophore concentration. It was soon realized that molecules specifically engineered for TPEF may significantly outperform standard fluorophores optimized for one-photon excitation. Within this context, we have implemented a molecular engineering approach toward nanometric fluorophores combining a high fluorescence quantum yield (Φ) and enhanced TPA cross section (σ_2) in the visible red. We herein present the design, synthesis, and photophysical properties of a series of novel fluorophores.

Following the innovating route for molecular TPA optimization opened by Marder, Perry, and Brédas,[6,7] our approach was based on quasi one-dimensional quadrupolar systems[8] (i.e., symmetrical conjugated molecules bearing two electron-releasing (D) or electron-withdrawing (A) end groups). Such derivatives are liable to display very high TPA cross section in relation with a quadrupolar intramolecular

---

[†] Université de Rennes I.

[‡] Ecole Supérieure de Physique et Chimie Industrielles.

[1] (a) Parthenopoulos, D. A.; Rentzepis, P. M. *Science* **1989**, *245*, 843−845. (b) Strickler, J. H.; Webb, W. W. *Opt. Lett.* **1991**, *16*, 1780−1782.

[2] Cumpston, B. H.; Ananthavel, S. P.; Barlow, S.; Dyer, D. L.; Ehrlich, J. E.; Erskine, L. L.; Heikal, A. A.; Kuebler, S. M.; Lee, I.-Y. S.; McCord-Maughon, D.; Qin, J.; Röckel, H.; Rumi, M.; Wu, X. L.; Marder, S. R.; Perry, J. W. *Nature* **1999**, *398*, 51−54.

[3] (a) He, G. S.; Xu, G. C.; Prasad, P. N.; Reinhardt, B. A.; Bhatt, J. C.; Dillard, A. G. *Opt. Lett.* **1995**, *20*, 435−437. (b) 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.

[4] Bhalwalkar, J. D.; Kumar, N. D.; Zhao, C. F.; Prasad, P. N. *J. Clin. Laser Med. Surg.* **1997**, *15*, 201−204.

[5] Denk, W.; Strickler, J. H.; Webb, W. W. *Science* **1990**, *248*, 73−76.

10.1021/ol017150e CCC: $22.00

charge transfer.[6−8] Giant σ_2 values can be obtained with DAAD or ADDA systems having strong D and A terminal and median groups but unfortunately often at the drastic expense of fluorescence quantum yield. On the basis of these observations, we have designed novel push-push fluorophores consisting of nanometric systems in which two electron-donating end groups are connected to a conjugated core via rigid and/or semirigid elongated conjugated rods (Figure 1).

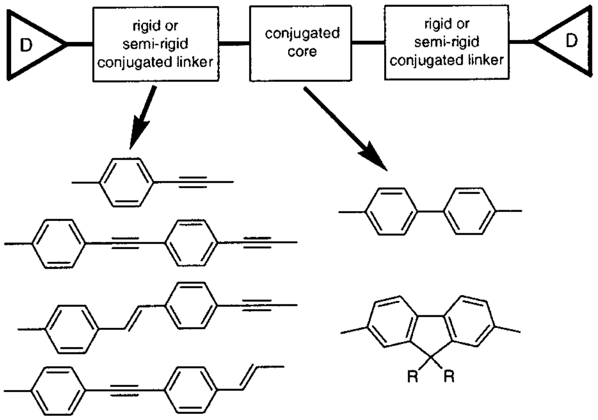
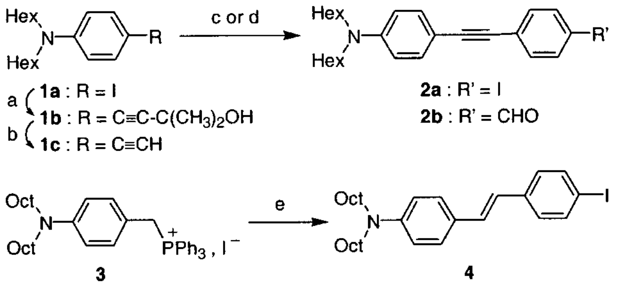


Figure 1. Molecular engineering of quadrupolar fluorophores designed for TPEF.

We selected biphenyl or fluorene central units, which allow the tuning of the electronic delocalization along the conjugated backbone by modulation of the twist angle between the two sections of the molecules. Conjugated rods built from phenylene−ethynylene and/or phenylene−vinylene oligomers were investigated in order to preserve fluorescence and modulate the electronic communication between the end and the center of the molecules. One of our goals was to ascertain the appropriate combination of core and rods moieties for optimized luminescence and/or TPA properties. Long alkyl chains could be added on the end groups and on the central block in order to obtain highly soluble derivatives.

Scheme 1[a]



[a] Reagents and conditions: (a) 2-methyl-3-butyn-2-ol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 40 °C, 12 h (82%); (b) NaOH, toluene/*i*-PrOH, reflux, 1 h (87%); (c) 1,4-diiodobenzene (3 equiv), $Pd(PPh_3)_2Cl_2$, CuI, toluene/Et_3N, 30 °C, 6 h (71% of **2a**); (d) 4-bromobenzaldehyde (1.2 equiv), $Pd(PPh_3)_2Cl_2$, CuI, toluene/Et_3N, 40 °C, 15 h (74% of **2b**); (e) 4-iodobenzaldehyde, *t*-BuOK, CH_2Cl_2, 20 °C, 5 h, then I_2 cat., *hν*, 2 h (85%).

End groups, linkers, and cores were assembled by means of Sonogashira, Wittig, and Wittig−Horner−Emmons reactions. The electron-donating moiety **1a** bearing an iodo group was converted to the ethynyl derivative **1c** in a two-step cross-coupling/deprotection sequence (Scheme 1). Extended rigid building blocks (tolanes **2a** and **2b**) were prepared by palladium(II)-catalyzed reaction of **1c** with 1,4-diiodobenzene and 4-bromobenzaldehyde, respectively. In parallel, the semirigid stilbene building block **4** was readily obtained by means of a Wittig condensation between phosphonium salt **3** and 4-iodobenzaldehyde, followed by catalytic isomerization. Double Sonogashira coupling of 4,4′-diethynylbiphenyl (**5**) with **1a**, **2a**, and **4** afforded fluorophores **6a**, **6b**, and **7**, respectively, whereas **9** was prepared by a double Wittig−Horner−Emmons condensation of bisphophonate **8** with aldehyde **2b** (Scheme 2).

The synthesis of the fluorene core **10d** was achieved from 9,9-dinonylfluorene (**10a**) in a three-step sequence (diiodination, Pd(II)-catalyzed cross-coupling reaction with 2-methyl-3-butyn-2-ol and base-promoted deprotection). Push-push molecules **11** and **12** were finally obtained by means of double Sonogashira coupling with **2a** and **4**, respectively. These derivatives show spectacularly higher solubility than their biphenyl analogues **6b** and **7**. All new fluorophores (**6**, **7**, **9**, **11**, **12**) have been fully characterized by NMR, HRMS, and elemental analysis data.

As shown in Figure 2a, the novel fluorophores exhibit an intense absorption band in the UV blue region and show high transparency in the remaining range of the visible region. In addition, all molecules exhibit high fluorescence quantum yields, ranging between 0.50 and 0.87 (Table 1).[9] Changing the biphenyl core to a fluorene one results in a slight red-shift of the absorption band (Figure 2a), indicative of improved electronic conjugation, but in nearly no shift of the emission band (Figure 2b). In contrast, modulation of the linkers allows the spectral tuning of both the absorption and emission characteristics, as noted from Figure 2 and Table 1. Increasing the conjugated rods' length induces a bathochromic and hyperchromic shift of both the absorption and emission bands, in agreement with an extended electronic conjugation. A similar effect is obtained when replacing a triple bond with a double bond in the conjugated linkers.

Lengthening of the conjugated rods based on phenylene−ethynylene oligomers does not decrease the high fluorescence

---

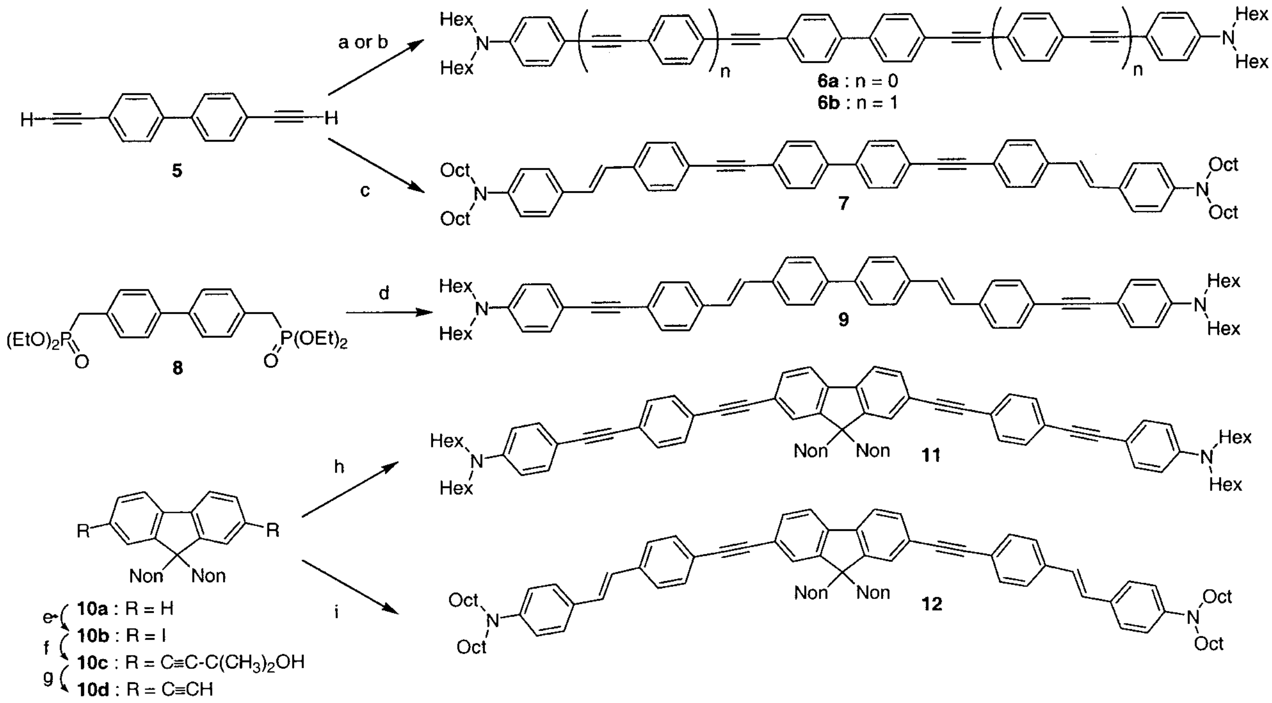
[6] Albota, M.; Beljonne, D.; Brédas, J.-L.; Ehrlich, J. E.; Fu, J.-Y.; Heikal, A. A.; Hess, S. E.; Kogej, T.; Levin, M. D.; Marder, S. R.; McCord-Maughon, D.; Perry, J. W.; Röckel, H.; Rumi, M.; Subramaniam, G.; Webb, W. W.; Wu, X.-L.; Xu, C. *Science* **1998**, *281*, 1653−1656.

[7] 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.

[8] (a) Ventelon, L.; Moreaux, L.; Mertz, J.; Blanchard-Desce, M. *Chem. Commun.* **1999**, 2055−2056. (b) Ventelon, L.; Charier, S.; Moreaux, L.; Mertz, J.; Blanchard-Desce, M. *Angew. Chem.* **2001**, *113*, 2156−2159; *Angew. Chem., Int. Ed.* **2001**, *40*, 2098−2101.

[9] Comparative experiments conducted in degassed, nondegassed, and aerated solutions of fluorophores in toluene revealed that the presence of oxygen has no effect on the fluorescence properties.

Scheme 2[a]



[a] Reagents and conditions: (a) **1a** (2.3 equiv), $Pd(PPh_3)_2Cl_2$, CuI, toluene/Et_3N, 20 °C, 2 h (84% of **6a**); (b) **2a**, conditions as in a (86% of **6b**); (c) **4**, conditions as in a, 3.5 h (81%); (d) **2b** (2.3 equiv), NaH, THF, 18-crown-6, 40 °C, 3 h (84%); (e) I_2, H_5IO_6, AcOH, H_2SO_4, H_2O, 75 °C, 2 h (69%); (f) 2-methyl-3-butyn-2-ol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 20 °C, 15 h (70%); (g) KOH, toluene, reflux, 0.5 h (71%); (h) **2a**, conditions as in a, 20 h (82%); (i) **4**, conditions as in a, 15 h (83%).

quantum yield, presumably owing to the stiffness of the oligomeric backbone. On the other hand, replacement of a triple bond by a double bond produces very different effects depending on its location in the conjugated rods: the double bond does not significantly affect the fluorescence quantum yield when positioned next to the central block but a decrease of 25−40% in the fluorescence quantum yield is obtained when the double bond is situated close to the end groups (Table 1). This provides evidence that the topology of the conjugated rods significantly influences the photoluminescence properties of the series of push-push fluorophores investigated in the present work.

Solvent polarity also allows tuning of the luminescence properties in a significant way. As illustrated in Figure 3 for compound **6a**, a pronounced positive solvatochromism (i.e., bathochromic shift with increasing polarity) is observed in emission, whereas only a slight red shift is observed in absorption. A parallel increase of the Stokes shift and of the

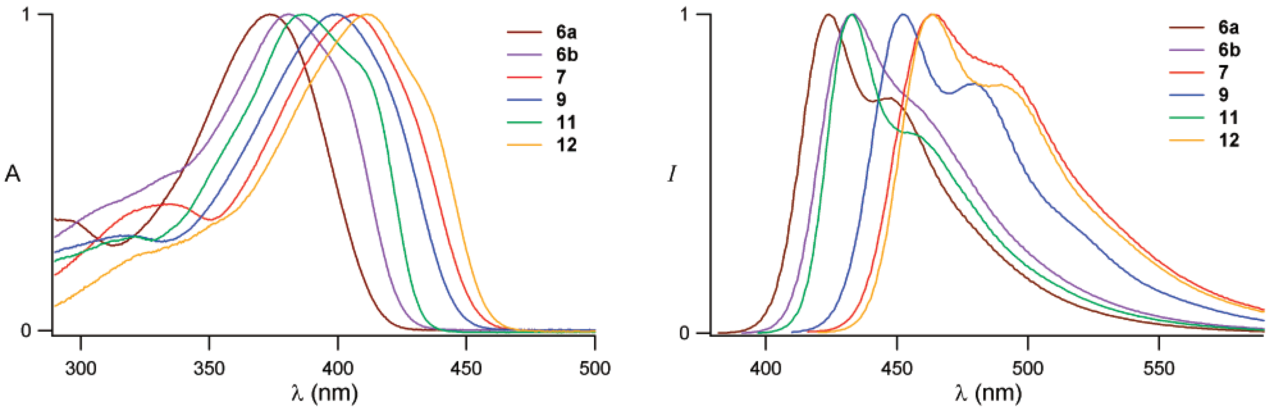


Figure 2. Normalized absorption (a) and fluorescence emission spectra performed at $\lambda_{ex} = \lambda_{max}$(abs) and $A_{\lambda_{ex}} = 0.1$ (b) of **6**, **7**, **9**, **11**, and **12** in toluene at room temperature.

Table 1. Photophysical Data for **6**, **7**, **9**, **11**, and **12**

compd	$\lambda_{\max}$ (nm)[a]	log ϵ	λ_{em} (nm)[b]	Φ_F[c]	τ (ns)[d]
6a	374	4.92	424, 448	0.87	1.0
6b	381	5.04	433	0.82	0.8
7	406	5.13	463	0.50	0.4
9	400	5.22	452, 480	0.80	0.5
11	387	5.11	433, 456 (sh)	0.82	0.7
12	411	5.10	464, 490 (sh)	0.61	0.5

[a] Absorption maximum in toluene. [b] Emission maxima in toluene. [c] Fluorescence quantum yield in toluene determined relative to fluorescein in 0.1 N NaOH as a standard ($\lambda_{\text{ex}} = 470$ nm). [d] Fluorescence lifetime.[10]

halfbandwith of the fluorescence spectra (from 2300 cm^{-1} in squalane to 4200 cm^{-1} in DMSO) is observed.[11] Such behavior is indicative of significant charge redistribution occurring upon excitation, prior to emission, and potentially large TPA cross sections.[6] On the basis of this observation, we have probed the TPEF properties of the lead compounds **7** and **9**. Their TPA cross sections were determined by comparing their TPEF to that of fluorescein (Figure 4).[12] The TPEF cross section values ($\sigma_2\Phi$) provided by this procedure were found to be of 500 and 800 GM at 740 nm (1 GM = 10^{-50} cm^4·s·photon^{-1}) for molecules **7** and **9**, respectively, and the corresponding σ_2 value derived from this result of 950 and 1050 GM, which corresponds to about 35 times that of fluorescein. These values rank among the highest TPA cross sections observed for other conjugated systems of either quadrupolar or octupolar or branched symmetries and comparable molecular weight.[13] Interestingly, replacement of a double bond by a triple bond in the conjugated system led to improved photostability as indicated by the absence of photodegradation either upon pulsed-laser or prolonged lamp irradiation for derivatives **6** and **11**.[14]

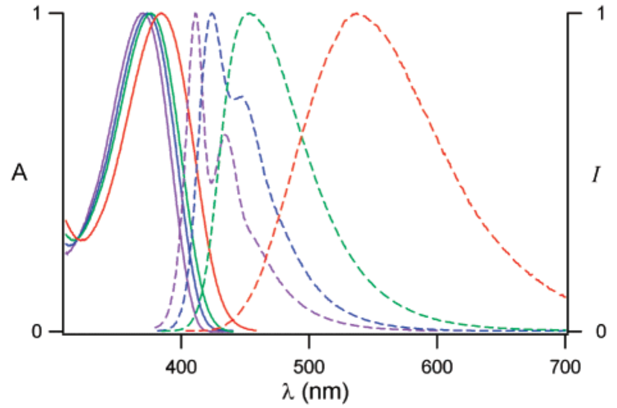


Figure 3. Solvatochromism of **6a**: normalized absorption (—) and emission spectra (- - -) at room temperature (squalane, purple; toluene, blue; CHCl$_3$, green; DMSO, red). Stokes shifts ($1/\lambda_{\text{abs}} - 1/\lambda_{\text{em}}$) in 10^3 cm^{-1}: 2.7 (squalane), 3.2 (toluene), 4.6 (CHCl$_3$), 7.4 (DMSO). Φ_F: 0.79 (squalane), 0.87 (toluene), 0.65 (CHCl$_3$), 0.67 (DMSO).

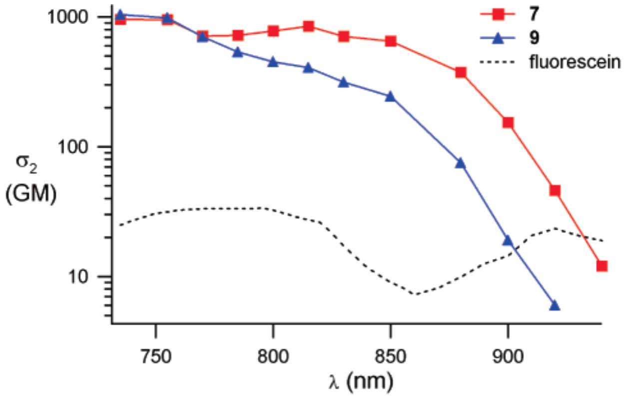


Figure 4. Two-photon excited fluorescence spectra of fluorophores **7** and **9** calibrated from that of fluorescein.

Finally, a series of novel nanometric fluorophores combining high linear transparency in the visible range and tunable luminescence properties have been designed via push-push functionalization of elongated conjugated systems derived from a biphenyl or fluorene core. Their photophysical properties can be tuned by playing on the nature, length, and topology of the conjugated rods connecting the terminal and central moieties. The dinonylfluorene core provides derivatives with high solubility while high photostability can be achieved by playing on the conjugated system. Preliminary TPEF experiments provide indication that such derivatives can also exhibit a very large TPA cross section. These derivatives hold promise for the development of new molecular probes specifically engineered for biological nonlinear microscopy[15] as well as for several applications including optical power limitation or elaboration of luminescent displays.

Acknowledgment. We acknowledge financial support from CNRS and MENRT. L.P. and L.M. received fellowships from MENRT and CNRS, respectively. We gratefully thank H. Le Bozec for access to fluorescence facilities.

Supporting Information Available: Photophysical methods (absorption, fluorescence, and TPEF). Description of all new fluorophores. Detailed protocols for the synthesis of reagents **3** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL017150E

---

(10) Calculated from the fluorescence quantum yield and intrinsic fluorescence lifetime (τ_0) values ($\phi = \tau/\tau_0$).

(11) A loss of defined vibronic structure parallels the broadening effect, in agreement with pronounced interactions of the emissive excited state with polar solvent environment. Fluorescence lifetimes of 1 ns have been derived for compound **6a** in low polarity solvents (in squalane and toluene) whereas a slightly smaller value (0.7 ns) is obtained in CHCl$_3$.

(12) Xu, C.; Webb, W. W. *J. Opt. Soc. Am. B* **1996**, *13*, 481−491.

(13) (a) Chung, S.-J.; Kim, K.-S.; Lin, T.-C.; He, G. S.; Swiatkiewicz, J.; Prasad, P. N. *J. Phys. Chem. B* **1999**, *103*, 10741−10745. (b) Kim, O.-K.; Lee, K.-S.; Woo, H. Y.; Kim, K.-S.; He, G. S.; Guang, S. H.; Swiatkiewicz, J.; Prasad, P. N. *Chem. Mater.* **2000**, *12*, 284−286. (c) Drobizhev, M.; Karotki, A.; Rebane, A.; Spangler, C. W. *Opt. Lett.* **2001**, *26*, 1081−1083.

(14) Whereas substitution of all triple bonds by double bonds is expected to lead to even higher TPA cross sections,[8b] the improved photostability and lower sensitivity to oxygen make the present flurorophores particularly attractive for practical imaging purposes.

(15) (a) Xu, C.; Zipfel, W.; Shear, J. B.; Williams, R. M.; Webb, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10763−10768. (b) Wang, X.; Krebs, L. J.; Al-Nuri, M.; Pudavar, H. E.; Ghosal, S.; Liebow, C.; Nagy, A. A.; Schally, A. V.; Prasad, P. N. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11081−11084.