Diaroyl(methanato)boron Difluoride Compounds as Medium-Sensitive Two-Photon Fluorescent Probes

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Abstract: This paper evaluates the use of diaroyl(methanato)boron difluoride compounds for designing efficient fluorescent probes through two-photon absorption. Three different pathways allowing for the syntheses of symmetrical and dissymmetrical molecules are reported. The stable diaroyl(methanato)-boron difluoride derivatives can be easily obtained in good yields. They exhibit a large one-photon absorption that is easily tuned in the near-UV range. Their strong fluorescence emission covers the whole visible domain. In addition to these attractive linear properties, several diaroyl(methanato)-boron difluoride derivatives possess significant cross sections for two-photon absorption. The derived structure–property relationships are promising for designing new generations of molecules relying on the diaroyl(methanato)boron difluoride backbone.

Keywords: boron · fluorescent probes · structure–activity relationships · two-photon absorption

Introduction

Extrinsic fluorophores are increasingly used as reporter molecules for labeling, for probing environments, for evaluating intramolecular distances or molecular motions, and so forth.[1] Relatively small molecules exhibiting large fluorescence quantum yields are always sought after for such applications. In addition, other features have to be considered in answer to specific questions. A high sensitivity of the photophysical features to the surrounding medium is favored to evaluate the polarity or the viscosity of molecular environments. For fluorescence-depolarization experiments, fluorophores with well-defined electronic-transition dipoles in a molecular frame are preferred. Despite extensive studies, few series of molecules satisfy these criteria, as revealed by the catalog contents of the main purchasers of fluorescent molecular probes; therefore, searching for new series of fluorescent species is still an important issue. In fact, the lack of appropriate fluorescent labels or probes that cover the whole UV/Vis-wavelength range is regarded as critical for the development of promising experiments that rely on the observation of singly fluorescent molecules.[2] Finally, the growing interest in fluorescence imaging based on two-photon excitation[3,4] requires the identification of fluorophores exhibiting large cross sections for multiple-photon absorptions.[5,6]

In relation to the preceding paragraph, one of the most recently developed fluorescent chromophores is 4,4-difluoro-4-bora-3a-azonia-4a-aza-s-indacene (BODIPY) (Scheme 1).[7–11] BODIPY-based fluorophores exhibit spectral features that make them especially attractive when the excitation is performed in the 500–600 nm range, thus producing a fluorescence emission at 500–650 nm. Their molar absorption coefficient is very high ($\varepsilon > 8 \times 10^4\ \mathrm{M}^{-1}\mathrm{cm}^{-1}$) along with their fluorescence quantum yield (ϕ_F) that is close to one, even in water. For imaging, such properties are well suited for fluorescence microscopy relying on one-photon excitation, in which they compete with most fluorescein and rhodamine derivatives. In contrast, BODIPY-based

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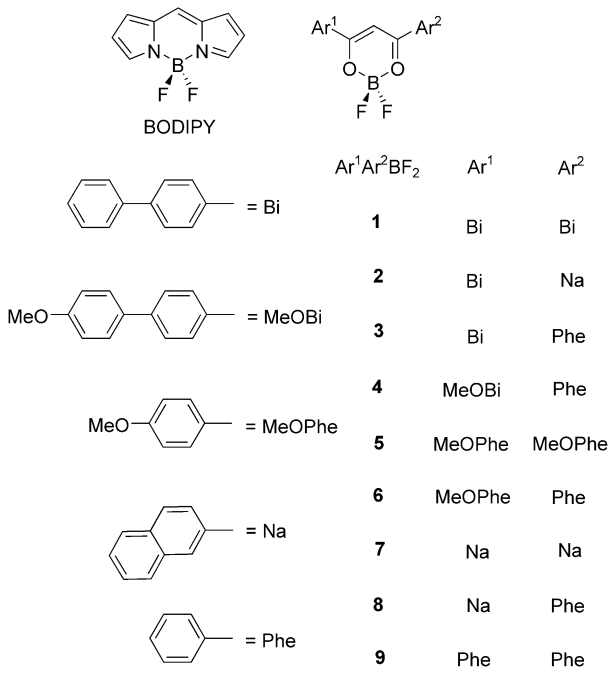

	$Ar^1Ar^2BF_2$	Ar^1	Ar^2
	1	Bi	Bi
	2	Bi	Na
	3	Bi	Phe
	4	MeOBi	Phe
	5	MeOPhe	MeOPhe
	6	MeOPhe	Phe
	7	Na	Na
	8	Na	Phe
	9	Phe	Phe

Scheme 1. Generic structures of the BODIPY and the diaroyl(methanato)boron difluoride $Ar^1Ar^2BF_2$ compounds prepared during this study.

fluorophores are less satisfactory when two-photon excitation is used. For instance, the two-photon fluorescence absorption (TPA) cross section (δ) of the disodium salt of 4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene-2,6-disulfonic acid is about two and ten times lower than the TPE cross sections of fluorescein and rhodamine B, respectively.[12]

Aiming at keeping the satisfactory aspects of the BODIPY chromophores while improving their features toward two-photon absorption, we became interested in a series of diaroyl(methanato)boron difluoride compounds ($Ar^1Ar^2BF_2$ **1**–**9**), with structures related to BODIPY (Scheme 1). β-Diketone–boron difluoride complexes have been known for more than 75 years;[13] nevertheless, the diaroyl series remains largely unexplored, excepting the investigations performed on the parent compound of this series, dibenzoyl(methanato)boron difluoride (**9**), whose emission[14,15] and reactivity[16] of the singlet excited state have been investigated. Some related compounds were reported to exhibit attractive absorption and emission features, such as large molar absorption coefficients, or large fluorescence quantum yields.[17–20] In particular, the range of absorption wavelengths (ca. 350–500 nm) is especially suitable for two-photon excitation with femtosecond Ti–sapphire pulsed lasers that can be tuned between 700 and 1100 nm.

The present paper is organized as follows: We first describe three different synthetic pathways to access the diaroyl(methanato)boron difluoride derivatives **1**–**9**. Some photophysical properties of these compounds are then reported and discussed (one- and two-photon absorption, steady-state fluorescence emission). In view of the possible use of $Ar^1Ar^2BF_2$ compounds as labels, we also examined both the dependence of the emission features upon the environment and the chemical stability of the present fluorescent molecules.

Abstract in French: *Cet article évalue l'intérêt de complexes difluoroboroniques de diaroyl-β-dicétones dans l'élaboration de sondes fluorescentes après excitation à deux photons. Trois différentes voies de synthèse de dérivés symétriques et dissymétriques sont décrites. Elles permettent d'obtenir de grandes quantités de complexes avec de bons rendements. Ces composés présentent une absorption intense à un photon, accordable dans le proche UV. Leur forte émission de fluorescence couvre la gamme du visible. En sus de ces intéressantes propriétés linéaires, certains des complexes difluoroboroniques de diaroyl-β-dicétones synthétisés possèdent une importante section efficace d'absorption à deux photons. Les relations structure-propriétés obtenues permettent d'envisager le développement de molécules performantes construites à partir du squelette étudié.*

Results and Discussion

Synthesis: To derive structure–property relationships, we synthesized eight compounds differing in 1) the aryl backbones: phenyl (Phe), biphenyl (Bi), and naphthalene (Na), 2) the aromatic substituents: H, or methoxy (MeO), and 3) their symmetry ($Ar^1 = Ar^2$ or $Ar^1 \neq Ar^2$). The diaroyl(methanato)boron difluoride compounds ($Ar^1Ar^2BF_2$ **1**–**9**) were easily obtained in good yields from the reaction of boron trifluoride/diethyl ether (BF_3/Et_2O) with the corresponding 1,3-diketones (Ar^1Ar^2) in benzene or dichloromethane (Scheme 2).[21] The Ar^1Ar^2 derivatives were synthesized by three different pathways (Scheme 2).

Diketones BiBi, BiNa, MeOBiPhe, MeOPhePhe, NaNa, and NaPhe were prepared in three steps according to pathway A. In the first step, the ethyl aroyl acetates 1A-Ar^1 (1 = step 1, A = pathway A, Ar^1 = Bi or Na) were obtained from condensation of the dianion of monoethyl malonate with aroyl chloride.[22] The monoanions from the 1A-Ar^1 species were then condensed with the appropriate aroyl chloride to yield 2A-BiBi, 2A-BiNa, 2A-MeOBiPhe, 2A-MeOPhePhe, 2A-NaNa, and 2A-NaPhe.[23] Finally, the 2A-Ar^1Ar^2 compounds were decarboxylated in the presence of sodium chloride in DMSO to yield the desired diketones.[24]

BiPhe was synthesized in three steps (pathway B). The condensation of 4-acetylbiphenyl with benzaldehyde first provided the chalcone 1-biphenyl-4′-yl-3-phenylprop-2-enone 1B-BiPhe.[25] This compound was subsequently brominated[26] to yield 2B-BiPhe, which finally gave BiPhe after elimination under basic conditions.[27] Lastly, MeOPheMeOPhe was directly obtained in one step from acylation of ethenyl acetate by 4-methoxybenzoyl chloride (pathway C).[28]

Chemical stability against hydrolysis: During experiments performed in our laboratory, some BODIPY derivatives were prone to color fading in the presence of water. For example, during preparation of egg lecithin vesicles by detergent removal, the signal arising from a BODIPY-labeled

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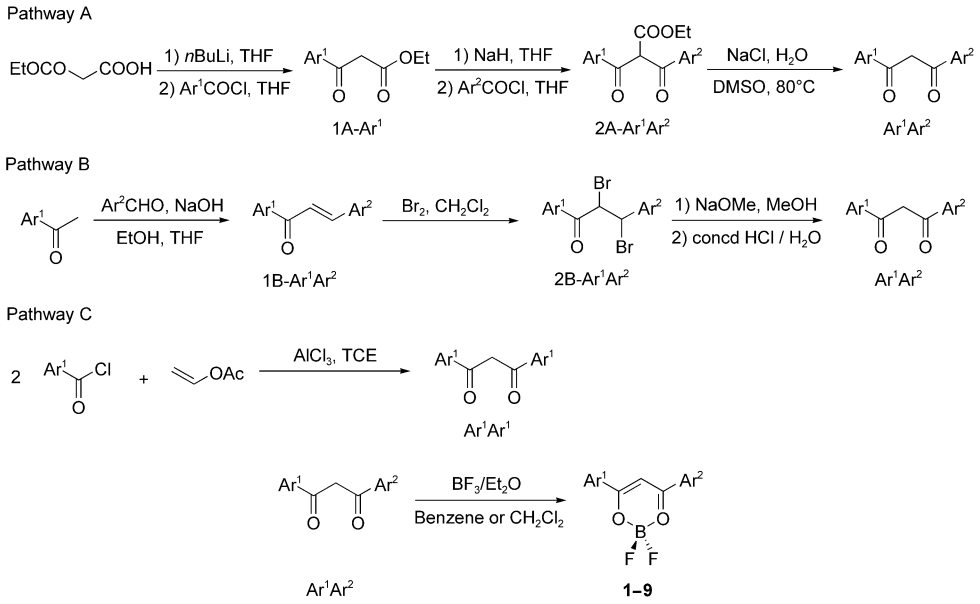

Scheme 2. Pathways for syntheses of the target compounds $Ar^1Ar^2BF_2$ **1–9**.

lipid essentially disappeared in less than 12 h, even when protected from light with aluminium foil. This behavior probably originates from slow hydrolysis of the boron difluoride bridge. In view of the structural similarity between the BODIPY dyes and the present $Ar^1Ar^2BF_2$ series, we investigated the stability of the series towards water hydrolysis. In view of the poor water solubility of the presently synthesized $Ar^1Ar^2BF_2$ compounds, we considered incorporating some of them into micelles that are known to remain quite permeable to water molecules, while solubilizing hydrophobic molecules. Compounds **4** and **7** were dissolved in an aqueous solution of α-D-octylglucopyranoside (OG 25 mM), which forms micelles above 23 mM at room temperature,[29,30] to yield dye solutions (80 μM). The resistance to hydrolysis was evaluated by following the fluorescence emission (vide infra) as a function of time at room temperature. In fact, no significant drop of the signal was noticed over a few days, so it was concluded that $Ar^1Ar^2BF_2$ compounds are rather stable. The better resistance to hydrolysis of the present series could be related to the greater affinity of boron to oxygen, compared with nitrogen.[31,32]

Table 1. Single-photon absorption maxima (λ_{max}), steady-state fluorescence emission maxima (λ_{em}), molar absorption coefficients for single-photon absorption ($\varepsilon(\lambda_{max})\pm5\%$ [$\mu M^{-1}cm^{-1}$]), fluorescence quantum yields ($\phi_F\pm5$ [%]), Stokes shift values ($\Delta\nu$), two-photon excitation spectra maxima (λ^2_{max}), two-photon excitation cross sections ($\delta_{max}\pm20\%$ in GM; $1\,GM=10^{-50}cm^4s(photon-molecule)^{-1}$) for the $Ar^1Ar^2BF_2$ derivatives in dichloromethane at 298 K.

Ar^1Ar^2	λ_{max} [nm]	$\varepsilon(\lambda_{max})$ [$\mu M^{-1}cm^{-1}$]	λ_{em} [nm]	ϕ_F [%]	$\Delta\nu$ [cm^{-1}]	λ^2_{max} [nm]	δ_{max} [GM]
1	411	75000	458	50	2500	795	30
2	416	45000	473	35	2900	745	60
3	394	61000	450	70	3160	775	30
4	410	59000	540	75	5900	835	200
5	410	89000	436	85	1050	785	20
6	396	62000	431	85	2050	787	25
7	420	47000	476	40	2800	775	85
8	398	40000	482	35	4380	805	30
9	364	46000	395	20	2150	–[a]	<5

[a] The signal remained in the 2—3 GM range between 700 nm and 750 nm.

Linear absorption and emission properties: Derivatives **1–9** exhibit a strong one-photon absorption ($\varepsilon>50000\,M^{-1}cm^{-1}$) in the 350–450 nm range (Figure 1 and Table 1). The corresponding band is rather narrow (50–75 nm at half-width). This band was interpreted as corresponding to a $\pi\rightarrow\pi^*$ transition in the parent compound **9**.[14,33] As anticipated, the maximum one-photon absorption (λ_{max}) is redshifted when the conjugation path length is increased. Linear dependence of the absorption on concentration without any spectrum alteration was observed in dichloromethane in the 1–20 μM range, suggesting that only monomeric species are present in this concentration range, as reported for **9**.[15] We investigated the solvatochromism of some $Ar^1Ar^2BF_2$ derivatives by recording the UV/Vis absorption spectra in several solvents (Table 2 and Figure 2a and b). The correlation between the transition energy associated with light absorption and the empirical parameter $E_T(30)$ (representing solvent polarity)[34] is displayed in Figure 2a for **3**, **8**, and **9**. It is reasonably linear except for a few deviating points originating from the solvent dependence of the largest vibronic band used to determine the absorption maximum. The observed slopes are negative (positive solvatochromism). In the absence of any evidence suggesting the formation of aggregates (except for **4** and **7** in cyclohexane), this result was interpreted as revealing an increase of molecular-dipole

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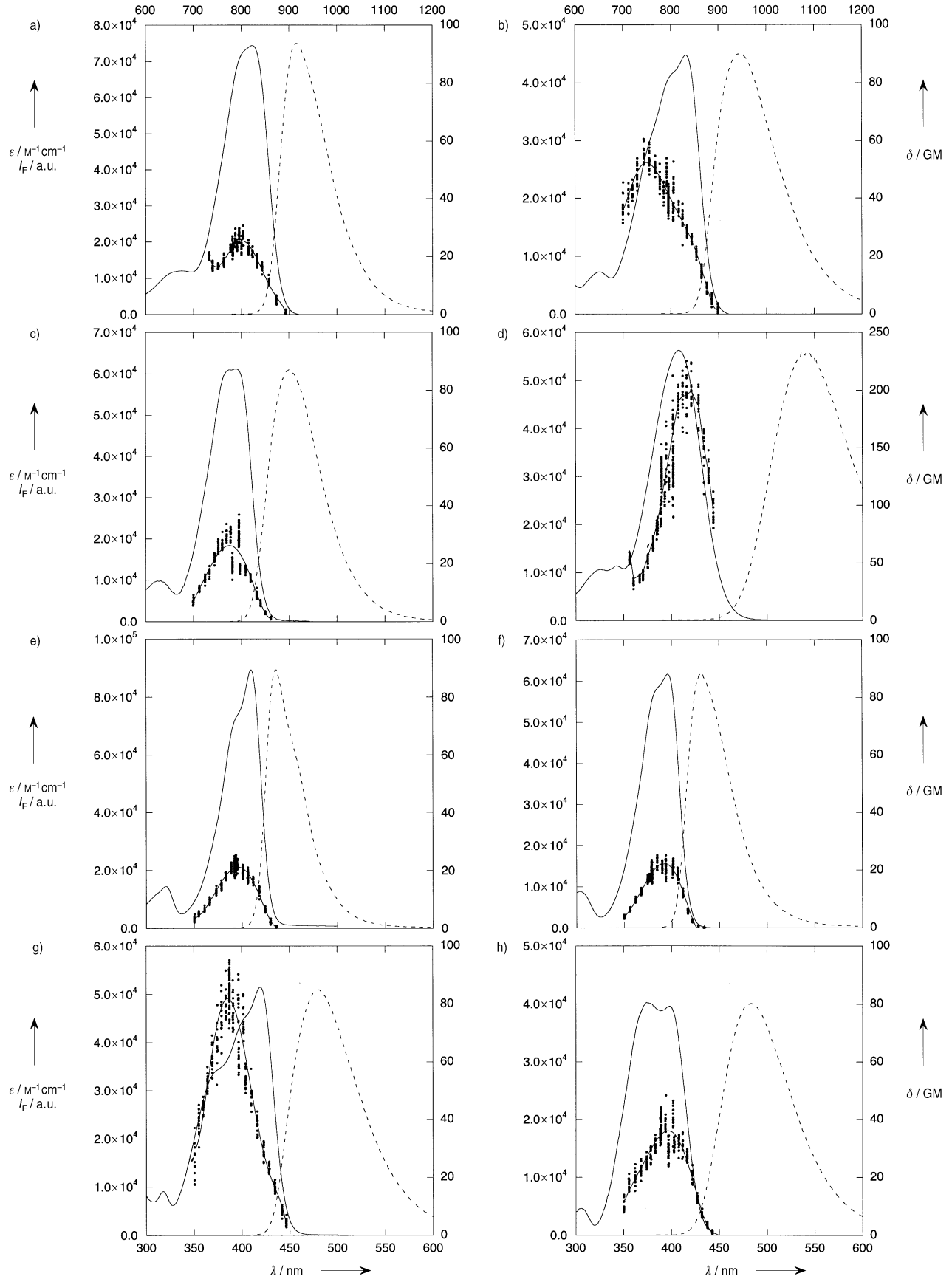

Figure 1. Single-photon absorption (molar absorption coefficient ε in $M^{-1}cm^{-1}$, solid line), steady-state fluorescence emission (fluorescence emission intensity I_F in arbitrary units, dotted line), and two-photon excitation spectra (clouds of points) of a)–h) **1**–**8**, respectively, in dichloromethane at 298 K. Spectra a)–h) all have the same scale unless stated otherwise.

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Table 2. Solvatochromism of some $Ar^1Ar^2BF_2$ derivatives. UV/Vis single-photon absorption and emission features under different conditions. Maxima of absorption λ_{max} [nm], molar absorption coefficients $\varepsilon(\lambda_{max}) \pm 5\%$ [$\mu M^{-1} cm^{-1}$], steady-state fluorescence emission λ_{em} [nm], and fluorescence quantum yields $\phi_F \pm 5$ [%]. Also see Table 1.

Ar^1Ar^2	Acetone, 42.2[a]	Acetonitrile, 45.6[a]	Cyclohexane, 30.9[a]	DMSO, 45.1[a]	Ethanol, 51.9[a]	OG micelles
3	385/51 000/451/50	385/60 000/468/65	375/52 000/408/15	406/60 000/469/1	378/45 000/454/40	–
4	–	–	394/–[b]/432/–[b]	420/56 000/610/15	–	–[c]/–[c]/566/40[d]
7	–	–	409/–[b]/420/–[b]	–	–	–[c]/–[c]/510/50[d]
8	397/41 000/501/50	370/38 000/523/50	395/39 000/407/35	373/38 000/502/30	406/41 000/530/1	
9	364/46 000/394/10	364/45 000/402/10	359/43 000/407/5	371/46 000/405/1	371/46 000/405/ < 1	

[a] $E_T(30)$ values from ref. [34]. [b] The Ar^1Ar^2 compounds were sometimes insoluble or only partially soluble in cyclohexane. In the latter case, only λ_{max} and λ_{em} are indicated. [c] The light scattering induced by the presence of the OG micelles prevented extraction of reliable data from the absorption spectrum. [d] The fluorescence quantum yields of **4** and **7** in OG micelles were crudely evaluated using Equation (1) under the assumption that the absorbances of the micellar solutions at the excitation wavelength were identical to the corresponding CH_2Cl_2 solutions at the same concentration.

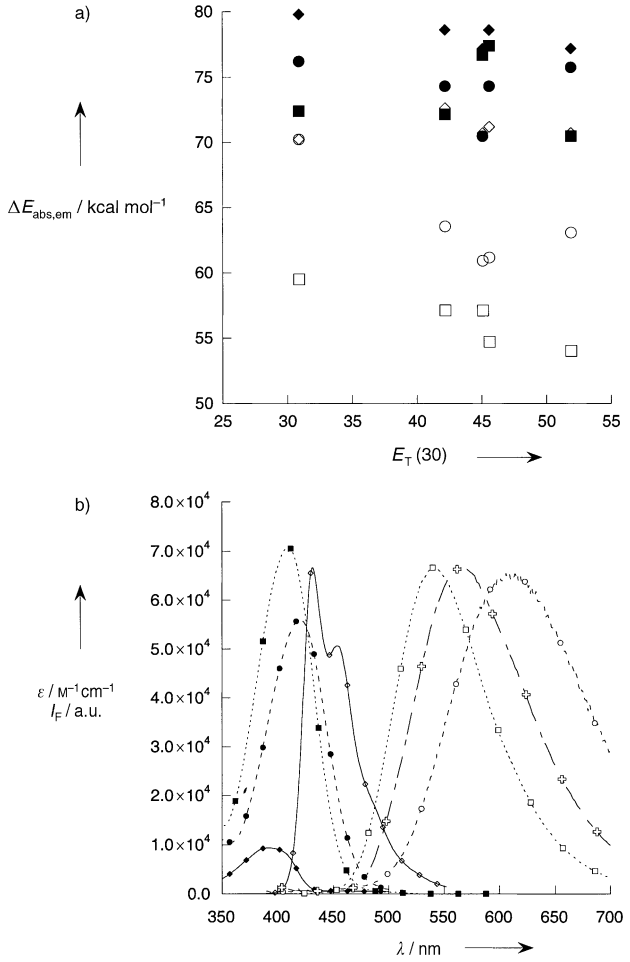

Figure 2. a) Dependence of the transition energies associated with the processes of absorption ($\Delta E_{abs} = hc/\lambda_{abs}$, filled markers) and emission ($\Delta E_{em} = hc/\lambda_{em}$, empty markers) on the empirical parameter $E_T(30)$ representing solvent polarity[34] for **3** (circles), **8** (squares), and **9** (diamonds); b) single-photon absorption (molar absorption coefficient ε in $M^{-1} cm^{-1}$, filled markers) and steady-state fluorescence emission (fluorescence emission intensity I_F in arbitrary units, empty markers) of **4** in cyclohexane (diamonds), dichloromethane (squares), DMSO (circles), and OG micelles (crosses) at 298 K.

moment upon light absorption. These observations are in accordance with the donor–acceptor structure of the $Ar^1Ar^2BF_2$ molecules. In fact, **9** possesses a ground dipole moment of 6.7 debye;[21] the BF_2 moiety has a withdrawal effect, whereas the aromatic rings act as donating groups.

Compounds **1–9** are strongly fluorescent in the 400–550 nm range in dichloromethane at 298 K (Figure 1 and Table 1). The fluorescence quantum yields are good-to-excellent for the most conjugated molecules ($0.35 < \phi_F < 0.85$).

As observed for one-photon absorption, fluorescence emission becomes redshifted when the solvent polarity is increased, consistent with an increase of dipole moment upon excitation (Table 2). In addition, a more pronounced solvatochromism is observed for the emission than for the absorption, as revealed by an increase of the Stokes shift with increasing polarity for the derivatives bearing the most donating groups (Table 2). The large Stokes shift values can be related to a major reorganization of the solvent shell during the excitation lifetime. Interestingly, **4** exhibits both the larger Stokes shift values (220 cm^{-1} in cyclohexane, 5900 cm^{-1} in dichloromethane, 7400 cm^{-1} in DMSO), as well as the most definite fluorescence solvatochromism (Figure 2b). This behavior can be attributed to a large nuclear reorganization after excitation prior to emission, in addition to a significant electronic redistribution occurring upon excitation. Within this framework, the more pronounced features of **4** can be tentatively interpreted as resulting from the larger donor character of the methoxybiphenyl moiety, leading to a larger excited-state dipole.[35]

The sensitivity of the fluorescence maxima to the solvent polarity makes such compounds attractive as polarity probes. From this point of view, the fluorescence characteristics of **4** suggest that α-D-octylglucopyranoside (OG) acts as a medium of intermediate polarity between dichloromethane and dimethylsulfoxide (Tables 1 and 2). In fact, although being structurally related to cyclohexane, the micellar core is strongly permeable to polar water molecules. In addition to the shift of the emission band, the solvent was found to exert a significant influence on the fluorescence quantum yield, hence supporting the view of $Ar^1Ar^2BF_2$ molecules as polarity reporters.

The fluorescence quantum yields were highest in dichloromethane and considerably decreased in polar media, such as DMSO (Tables 1 and 2). The fluorescence quantum yields in OG micelles are difficult to evaluate owing to light scattering precluding the evaluation of sample absorbances; nevertheless, the crude estimates derived suggest the corresponding quantum yields are intermediary between apolar and polar media. A possible explanation to account for such sensitivity to polarity relies on the presence of two excited

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states whose energy would be solvent dependent: a non-fluorescent excited state arising from a $n \rightarrow \pi^*$ transition, and a fluorescent excited state arising from $\pi \rightarrow \pi^*$. Such an explanation was already proposed in closely related series, to discuss the relationships between molecular conformation and photophysical features.[36]

As a partial conclusion, the linear properties of the present $Ar^1Ar^2BF_2$ series satisfactorily compare with the corresponding existing fluorescent series such as stilbene, coumarin, or pyrene derivatives, which exhibit comparable photophysical features.

Two-photon absorption properties: In contrast to single-photon absorption, two-photon absorption is a nonlinear optical process causing a molecule to simultaneously absorb two photons to access an excited state. As a consequence, the two-photon absorption is related quadratically to light intensity. Provided that satisfactory chromophores will be available, the favorable features of two-photon absorption are anticipated to be useful for application in various fields, such as two-photon fluorescence microscopy,[3,37,38] two-photon-induced biological caging investigations,[39,40] and optical limiting.[41–46] Despite work already reported dealing with the same issue, there is still a need to accumulate experimental data to derive relevant structure–property relationships for optimizing two-photon absorption.[46–58]

Two-photon excitation spectra of **1**–**9** were recorded in dichloromethane at 298 K with a home-built set up relying on the design of Webb et al.[60] (see Figures 1S–3S in the Supporting Information and Experimental Section). Figure 1a–h displays the spectra (for **1**–**8**) together with the corresponding single-photon absorption and fluorescence emission spectra. The quadratic dependence of two-photon-excited fluorescence of the compounds listed in this paper was checked at several wavelengths (see Figure 3S in the Supporting Information for a typical example); it revealed satisfactory behavior over the 20–200 mW range. The position of the two-photon absorption maximum ($\lambda_{max}^{(2)}$) and the peak two-photon absorptivity maximum (δ_{max}) are summarized in Table 1. The observed values of $\lambda_{max}^{(2)}$ satisfactorily compare with twice the corresponding values of λ_{max}; this suggests that the same excited states are reached regardless of the excitation mode. Such an observation goes against the behavior of symmetrical molecules, such as fluorescein, but is in line with other reports making use of a comparable technique on unsymmetrical donor–acceptor compounds. For instance, coumarin 307 is reported to exhibit $\lambda_{max}^{(2)} = 800$ nm in methanol[12] and $\lambda_{max} = 395$ nm in ethanol.[61] In the present series, the δ_{max} range from 20 to 200 GM ($1\,GM = 10^{-50}\,cm^4\,s\,(photon-molecule)^{-1}$).

The effectiveness of electron-donating substituents on the aryl group to increase the two-photon fluorescence absorptivity (δ_{max}), is supported by the trend observed in both series of 1) dissymmetrically substituted molecules: **6** (25) versus **9** (<5) and **4** (200) versus **3** (30) and 2) symmetrically substituted molecules: **5** (20) versus **9** (<5). Thus, much lower TPA values are observed in the absence of donating substituents on the aryl moieties, suggesting that the dipolar contribution is a dominant effect, as observed in other push–pull systems.[47,54,55] We observe that the substituent effect is much more pronounced in the case of the elongated derivative **4**, leading to an increase of nearly one order of magnitude in the TPA response. This effect can be tentatively attributed to a synergetical effect between donating substituent and conjugated linker length, as observed for optical nonlinearities of push–pull derivatives.[62]

The nature and length of the aryl moieties also influences the TPA response. Increasing its donating strength or its size leads to an increase of the TPA response, as indicated by the comparison of 1) symmetrical molecules in the series **9** (<5), **1** (30), and **7** (85) and 2) dissymmetrical molecules in the series **9** (<5), **3** (30), and **8** (30), as well as in the series **6** (25) and **4** (200). It should be stressed that the length effect is much more pronounced in the case of a dissymmetrical compound with a donating substituent, leading again to an order of magnitude increase of the TPA cross section. We conclude from this observation that the distinctly larger TPA characteristics of **4**, among the nine related derivatives, could be related to the major electronic redistribution occurring upon excitation (vide supra), supporting the role of the dominant dipolar contribution. In contrast, the present investigation suggests that symmetrization of the structure does not bring any distinct advantage. In fact, δ_{max} values are identical for the couples (**1**,**3**) and (**5**,**6**).

Conclusion

We have prepared a series of novel fluorophores of definite interest for various applications in biological imaging (TPEF microscopy, microenvironment probes). Their absorption and emission properties have been investigated, demonstrating that these molecules can be used as sensitive fluorescent probes of micropolarity. These new fluorochromes combine large fluorescence quantum yields and enhanced chemical stability against hydrolysis in aqueous environment, as compared to the most popular BODIPY probes. Their TPA cross-section action has been determined using an original experimental protocol. Structure–property relationships have been derived providing evidence that their TPA cross sections can be significantly enhanced in elongated dissymmetrical derivatives bearing an electron-donating group. These combined features of the new series of fluorophores provide an interesting route towards optimized TPA fluorophores/probes for various applications.

Experimental Section

General procedures: Anhydrous solvents were freshly distilled before use. Column chromatography (CC): silica gel 60 (0.040–0.063 mm) Merck or silica gel SDS. Analytical: TLC: silica gel plates (Merck), detection by UV (254 nm); melting point: Büchi 510; ^{1}H NMR spectra: AM-250 Bruker, chemical shifts in ppm related to the protonated solvent as internal reference (^{1}H: $CHCl_3$ in $CDCl_3$, 7.27 ppm, CHD_2SOCD_3 in CD_3SOCD_3, 2.49 ppm; ^{13}C: $^{13}CDCl_3$ in $CDCl_3$, 76.9 ppm, $^{13}CD_3SOCD_3$ in CD_3SOCD_3, 39.6 ppm); coupling constants (J) in Hz; mass spectrometry (chemical ionization with NH_3) was performed at the Service de Spectro-

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métrie de masse de l'ENS; microanalyses were obtained from the Service de Microanalyses de l'Université Pierre et Marie Curie, Paris. Acyl chlorides were prepared from the corresponding acids by reaction with thionyl chloride.[65] Compound **9** was synthesized according to the presently reported procedure starting from the commercially available β-diketone PhePhe.

Syntheses of the diketones Ar^1Ar^2 along pathway A

Ethyl 3-(biphenyl-4′-yl)-3-oxo propionate (1A-Bi): Butyllithium in hexane (1M, 16 mL) was added dropwise to a cooled (−78°C) solution of monoethyl malonate (1.32 g, 10 mmol; 2 equiv) in THF (40 mL) with a crystal of 2,2′-bipyridine as indicator.[22] The solution was allowed to warm to −5°C and stirred for 15 min. After cooling (−70°C), biphenyl-4-carbonyl chloride (1.08 g, 5 mmol) in THF (5 mL) was slowly added. The cooling bath was removed and the mixture was stirred for 1 h. The mixture was poured into diethyl ether (35 mL) and hydrochloric acid (15 mL, 1M). The organic phase was separated and washed with saturated sodium hydrogencarbonate, then dried over magnesium sulfate. After filtration and evaporation, the crude product was purified by CC (cyclohexane/dichloromethane (C_6H_{12}/CH_2Cl_2), gradient 40:60 to 0:100) to yield 1A-Bi as a white powder (942 mg, 70%). M.p. 76°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): $\delta = 1.27$ (t, $^3J(H,H) = 7.2$ Hz, 3H), 4.02 (s, 2H), 4.23 (q, $^3J(H,H) = 7.2$ Hz, 2H), 7.32–7.52 (m, 3H), 7.54–7.74 (m, 4H), 7.97–8.07 ppm (m, 2H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS) (CE: enol-ester tautomer; CK: keto-ester tautomer): $\delta = 14.1$, 14.3, 46.0 (CK), 60.3, 61.5, 87.3 (CE), 126.5, 127.1, 127.2, 127.3, 127.4, 127.9, 128.4, 128.9, 129.0, 129.1, 132.3, 134.8, 139.6, 140.1, 144.0, 146.4, 167.5, 171.1, 173.2, 192.1 ppm; MS (CI, NH_3): *m*/*z* (%): 286 (24) [M^++18], 269 (100) [M^++1], 197 (40).

Ethyl naphthalene-2-carbonyl acetate (1A-Na):[66] Same procedure as for 1A-Bi, but with naphthalene-2-carbonyl chloride (952.5 mg, 5 mmol). Purified by CC (C_6H_{12}/CH_2Cl_2, 50:50 to 0:100). 1A-Na obtained as a white powder (930 mg, 76%). ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS) (HE: enol-ester tautomer; HK: keto-ester tautomer): $\delta = 1.27$ (t, $^3J(H,H) =$ 7.15 Hz, 3HK), 1.36 (t, $^3J(H,H) = 7.15$ Hz, 3HE), 4.13 (s, 2HK), 4.24 (q, $^3J(H,H) = 7.15$ Hz, 2HK), 4.30 (q, $^3J(H,H) = 7.15$ Hz, 2HE), 5.82 (s, 1HE), 7.48–7.70 (m, 2HK+2HE), 7.74–8.07 (m, 4HK+4HE), 8.37 (brs, 1HE), 8.47 (brs, 1HK), 12.69 ppm (s, 1HE); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS) (CE: enol-ester tautomer; CK: keto-ester tautomer): $\delta =$ 14.2, 14.4, 46.1 (CK), 60.4, 61.5, 87.9 (CE), 122.6, 123.9, 126.7 (2C), 126.8, 127.0, 127.6, 127.7, 127.9, 128.3, 128.7, 128.9, 129.1, 129.7, 130.6, 132.5, 132.7, 133.5, 134.7, 135.7, 167.7, 171.3, 173.3, 192.5 ppm.

Ethyl benzoylnaphthoyl acetate (2A-NaPhe): A solution of ethyl benzoyl acetate (960 mg, 5 mmol) dissolved in THF (2 mL) was added dropwise to a suspension of sodium hydride (220 mg, 5.5 mmol) in THF (3 mL), under an atmosphere of nitrogen at 0°C.[23] The mixture was stirred until the hydrogen evolution ceased (10 min). Naphthalene carbonyl chloride (952 mg, 5 mmol) in THF (5 mL) was then added and the stirring continued at RT for 16 h. THF was evaporated and the mixture was hydrolyzed with sulfuric acid (2M, 10 mL). The residue was extracted three times with CH_2Cl_2 and the organic phase was washed with water. After drying (magnesium sulfate), the solvent was evaporated and the crude residue was washed several times with cyclohexane/diethyl ether (50:50, 3 mL) to yield 2A-NaPhe as a white powder (986 mg, 58%). M.p. 121°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): $\delta = 1.24$ (t, $^3J(H,H) = 7.1$ Hz, 3H), 4.31 (q, $^3J(H,H) = 7.1$ Hz, 2H), 6.36 (s, 1H), 7.4–7.52 (m, 2H), 7.52–7.68 (m, 3H), 7.82–8.04 (m, 6H), 8.44 ppm (brs, 1H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): $\delta = 14.0$, 62.5, 64.5, 123.9, 127.2, 127.9, 128.7, 129.0, 129.2, 129.8, 130.7, 132.5, 133.1, 134.1, 135.7, 136.0, 165.9, 190.7, 190.9 ppm; MS (DEI): *m*/*z* (%): 346 (3) [M^+], 300 (14), 155 (100), 127 (38), 105 (45), 77 (14); elemental analysis calcd (%) for $C_{22}H_{18}O_4$ (346): C 76.28, H 5.24; found: C 76.33, H 5.14.

Ethyl di(biphenyl-4-carbonyl) acetate (2A-BiBi): Same procedure as for 2A-NaPhe, but with ethyl biphenyl-4-carbonyl acetate (804 mg, 3 mmol), sodium hydride (132 mg, 3.3 mmol), biphenyl-4-carbonyl chloride (650 mg, 3 mmol). Compound 2A-BiBi was obtained as colorless crystals after recrystallization in ethanol (1.27 g, 94%). M.p. 163°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): $\delta = 1.28$ (t, $^3J(H,H) = 7.1$ Hz, 3H), 4.33 (q, $^3J(H,H) = 7.1$ Hz, 2H), 6.24 (s, 1H), 7.37–7.53 (m, 6H), 7.58–7.67 (m, 4H), 7.67–7.74 (m, 4H), 7.98–8.07 ppm (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): $\delta = 14.0$, 62.5, 64.6, 127.3, 127.6, 128.5, 129.0, 129.3, 134.3, 139.6, 146.8, 165.8, 190.5 ppm; MS (CI, NH_3): *m*/*z* (%): 466 (8) [M^++18], 449 (37) [M^++1], 377 (100), 269 (78), 198 (28); elemental analysis calcd (%) for $C_{30}H_{24}O_4$ (448): C 80.34, H 5.54; found: C 78.06, H 5.55.

Ethyl 3-(2-naphthyl)-3-oxo-2-(biphenyl-4-carbonyl) propionate (2A-BiNa): Same method as for 2A-NaPhe, but using ethyl naphthalene-2-carbonyl acetate (795 mg, 3.28 mmol), and biphenyl-4-carbonyl chloride (710 mg, 3.28 mmol). Compound 2A-BiNa was obtained as colorless crystals after recrystallization in ethanol (478 mg, 35%). M.p. 154°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): $\delta = 1.27$ (t, $^3J(H,H) = 7.1$ Hz, 3H), 4.33 (q, $^3J(H,H) = 7.1$ Hz, 2H), 6.38 (s, 1H), 7.37–7.74 (m, 9H), 7.84–8.04 (m, 6H), 8.47 ppm (brs, 1H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): $\delta = 14.0$, 62.5, 64.6, 123.9, 127.1, 127.3, 127.6, 127.8, 128.5, 129.0, 129.1, 129.3, 129.8, 130.7, 132.4, 133.0, 134.3, 135.9, 139.5, 146.7, 165.8, 190.3, 190.6 ppm; MS (CI, NH_3): *m*/*z* (%): 440 (33) [M^++18], 424 (34) [M^+ +2], 423 (100) [M^++1]; elemental analysis calcd (%) for $C_{28}H_{22}O_4$ (422): C 79.60, H 5.25; found: C 79.59, H 5.23.

Ethyl 3-(4′-methoxybiphenylyl)-3-oxo-2-benzoyl propionate (2A-MeOBiPhe): Same method as for 2A-NaPhe, but with ethyl benzoyl acetate (960 mg, 5 mmol), 4′-methoxybiphenylcarbonyl chloride (1.23 g, 5 mmol) to yield 2A-MeOBiPhe as white crystals after washing several times with C_6H_{12}/Et_2O (50:50) and after recrystallization in ethanol (1.105 g, 54%). M.p. 135°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): $\delta = 1.26$ (t, $^3J(H,H) = 7.1$ Hz, 3H), 3.87 (s, 3H), 4.25 (q, $^3J(H,H) = 7,1$ Hz, 2H), 6.22 (s, 1H), 6.98–7.03 (m, 2H), 7.43–7.70 (m, 7H), 7.90–8.02 ppm (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): $\delta = 14.0$, 55.4, 62.4, 64.4, 114.5, 126.9, 128.4, 128.6, 130.0, 129.3, 131.8, 133.6, 134.0, 135.6, 146.3, 160.1, 165.8, 190.1, 190.7 ppm; MS (CI, NH_3): *m*/*z* (%): 420 (25) [M^++18], 403 (71) [M^++1], 331 (100), 299 (41); elemental analysis calcd (%) for $C_{25}H_{22}O_5$ (402): C 74.61, H 5.51; found: C 74.08, H 5.54.

Ethyl benzoyl-4-methoxybenzoyl acetate (2A-MeOPhePhe): Same method as for 2A-NaPhe, but with ethyl benzoyl acetate (960 mg, 5 mmol) and 4-methoxybenzoyl chloride (853 mg, 5 mmol). Compound 2A-MeOPhePhe was obtained as white crystals after recrystallization in ethanol (1.25 g, 76%). M.p. 104°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): $\delta = 1.25$ (t, $^3J(H,H) = 7.1$ Hz, 3H), 3.87 (s, 3H), 4.29 (q, $^3J(H,H) =$ 7.1 Hz, 2H), 6.14 (s, 1H), 6.88–6.99 (m, 2H), 7.43–7.53 (m, 2H), 7.56–7.65 (m, 1H), 7.87–7.96 ppm (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): $\delta = 14.0$, 55.6, 62.4, 64.4, 114.2, 128.8, 128.9, 131.1, 133.9, 135.7, 164.2, 165.9, 189.0, 190.7 ppm; MS (CI, NH_3): *m*/*z* (%): 344 (42) [M^+ +18], 327 (100) [M^++1], 279 (51); elemental analysis calcd (%) for $C_{19}H_{18}O_5$ (326): C 69.92, H 5.56; found: C 69.78, H 5.57.

Ethyl di(naphthalene-2-carbonyl) acetate (2A-NaNa): Same method as for 2A-NaPhe, but with ethyl naphthalene-2-carbonyl acetate (930 mg, 3.84 mmol) and naphthalene-2-carbonyl chloride (731.5 mg, 3.84 mmol). Compound 2A-NaNa was obtained as a white powder after washing several times with C_6H_{12}/Et_2O 50:50 (1.02 g, 67%). M.p. 150°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): $\delta = 1.26$ (t, $^3J(H,H) = 7.12$ Hz, 3H), 4.33 (q, $^3J(H,H) = 7.12$ Hz, 2H), 6.50 (s, 1H), 7.49–7.69 (m, 4H), 7.81–8.07 (m, 8H), 8.48 ppm (brs, 2H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): $\delta =$ 14.0, 62.5, 64.7, 123.9, 127.1, 127.8, 129.0, 129.1, 129.8, 130.7, 132.5, 133.1, 135.9, 165.9, 190.6 ppm; MS (CI, NH_3): *m*/*z* (%): 414 (3) [M^++18], 397 (11) [M^++1], 260 (60), 243 (100); elemental analysis calcd (%) for $C_{26}H_{20}O_4$ (396): C 78.77, H 5.085; found: C 78.74; H 5.19.

1-(2-Naphthyl)-3-phenylpropane-1,3-dione (NaPhe): A solution of ethyl benzoylnaphthoyl acetate (692 mg, 2 mmol), water (72 mg, 4 mmol; 2 equiv) and sodium chloride (120 mg, 2.2 mmol; 1.1 equiv) in DMSO (2 mL) was heated at 140°C until the release of carbon dioxide ceased.[24] After cooling to room temperature, water was added and the mixture was extracted with ethyl acetate and cyclohexane (1:1). The organic phase was washed three times with water, then dried over magnesium sulfate. After filtration and evaporation of the solvent, NaPhe was obtained as a white solid after recrystallization in ethanol (510 mg, 93%). M.p. 98°C (ref. [67] 99°C); ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): $\delta =$ 4.77 (s, 2H), 7.45–7.65 (m, 5H), 7.87–8.11 (m, 6H), 8.55 ppm (brs, 1H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): $\delta = 93.5$, 123.2, 126.8, 127.2, 127.8, 128.1, 128.3, 128.5, 128.7, 129.4, 132.5, 132.75, 132.8, 135.3, 135.6, 185.5, 185.8 ppm.

1,3-Di(biphenyl-4-yl)propane-1,3-dione (BiBi): Same method as for NaPhe, but with ethyl di(biphenyl-4-carbonyl) acetate (248 mg,

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0.55 mmol). Compound BiBi was obtained as pale yellow plates (172 mg, 83%). M.p. 220°C (ref. [68] 218–220°C); ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS):δ = 6.95 (s, 1H), 7.37–7.54 (m, 6H), 7.57–7.82 (m, 8H), 8.04–8.13 (m, 4H), 14.58–14.76 ppm (m, 1H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): δ = 93.2, 127.3, 127.4, 127.8, 128.2, 129.0, 134.4, 140.0, 145.3, 185.3 ppm.

1-(Biphenyl-4′-yl)-3-(2-naphthyl)propane-1,3-dione (BiNa): Same method as for NaPhe, but with ethyl 3-(2-naphthyl)-3-oxo-2-(biphenyl-4-carbonyl) propionate (336 mg, 0.8 mmol). Compound BiNa was obtained as colorless crystals after recrystallization in ethanol (140 mg, 50%). ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 7.06 (s, 1H), 7.37–7.72 (m, 7H), 7.75 (d, ^3J(H,H) = 8.4 Hz, 2H), 7.86–8.10 (m, 4H), 8.13 (d, ^3J(H,H) = 8.4 Hz, 2H), 8.57 (brs, 1H), 14.60 ppm (brs, 1H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): δ = 64.7, 123.9, 127.1, 127.3, 127.6, 127.9, 128.5, 129.0, 129.1, 129.3, 129.8, 130.7, 132.5, 133.1, 134.4, 136.0, 139.6, 146.7, 165.8, 190.3, 190.6 ppm.

(4′-Methoxybiphenylyl)-3-phenylpropane-1,3-dione (MeOBiPhe): Same method as for NaPhe, but with ethyl benzoyl-4-methoxybenzoyl acetate (1.08 g, 2.7 mmol). Compound MeOBiPhe was obtained as pale yellow crystals after recrystallization in ethanol (515 mg, 57%). M.p. 176°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 3.87 (s, 3H), 6.90 (s, 1H), 6.98–7.06 (m, 2H), 7.44–7.66 (m, 5H), 7.65–7.72 (m, 2H), 7.97–8.09 ppm (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): δ = 55.4, 93.0, 114.4, 126.7, 127.2, 127.8, 128.3, 128.7, 132.3, 132.4, 133.6, 135.7, 144.8, 159.9, 185.4, 185.5 ppm; MS (CI, NH_3): *m/z* (%): 348 (2) [M^++18], 331 (100) [M^++1]; elemental analysis calcd (%) for $C_{22}H_{18}O_3$ (330): C 79.98, H 5.49; found: C 79.82, H 5.69.

1-(4-Methoxyphenyl)-3-phenylpropane-1,3-dione (MeOPhePhe): Same method as for NaPhe, but with ethyl benzoyl 4-methoxybenzoyl acetate (654 mg, 2 mmol). Compound MeOPhePhe was obtained as colorless crystals after recrystallization in ethanol (393 mg, 77%). M.p. 137°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 3.89 (s, 3H), 6.80 (s, 1H), 6.95–7.04 (m, 2H), 7.43–7.60 (m, 3H), 7.94–8.06 ppm (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): δ = 55.5, 92.4, 114.0, 127.0, 128.2, 128.6, 129.3, 132.1, 135.6, 163.3, 184.0, 186.2 ppm; MS (CI, NH_3): *m/z* (%): 272 (21) [M^++18], 256 (37) [M^++2], 255 (100) [M^++1]; elemental analysis calcd (%) for $C_{16}H_{14}O_3$ (254): C 75.57, H 5.55; found: C 75.56, H 5.65.

1,3-Di(2-naphthyl)propane-1,3-dione (NaNa): Same method as for NaPhe, but with ethyl di(naphthalene-2-carbonyl) acetate (792 mg, 2 mmol). Compound NaNa was obtained as colorless crystals after recrystallization in ethanol (501 mg, 77%). M.p. 171°C (ref. [69] 172°C); ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 7.16 (s, 1H), 7.51–7.68 (m, 4H), 7.84–8.20 (m, 8H), 8.60 (brs, 2H), 14.58 ppm (brs, 1H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): δ = 93.6, 123.3, 126.6, 127.6, 128.2, 128.4, 128.5, 129.4, 132.8, 132.9, 135.4, 185.6 ppm.

Syntheses of the diketones Ar^1Ar^2 along pathway B

1-Biphenyl-4′-yl-3-phenylprop-2-enone (1B-BiPhe):[25] Sodium hydroxide (5M, 4 mL) was slowly added to a heated (50°C) solution of benzaldehyde (1.06 g, 10 mmol) and 4-acetylbiphenyl (1.96 g, 10 mmol) in ethanol (50 mL) and THF (10 mL). The mixture was stirred for 20 h at room temperature. After evaporation of the solvents, the residue was extracted with CH_2Cl_2. The organic phase was washed several times with water and was dried (magnesium sulfate). After filtration and evaporation of the solvent, 1B-BiPhe was obtained as a white solid (2.67 g, 94%) that was used without further purification for the following step. ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 7.35–7.53 (m, 6H), 7.60 (d, ^3J(H,H) = 15.7 Hz, 1H), 7.58–7.78 (m, 6H), 7.86 (d, ^3J(H,H) = 15.7 Hz, 1H), 8.06–8.17 ppm (m, 2H).

1-(Biphenyl-4-yl)-2,3-dibromo-3-phenylpropanone (2B-BiPhe): An excess of bromine (0.75 mL, 20 mmol) in CH_2Cl_2 (7.5 mL) was slowly added at RT to a solution of 1B-BiPhe (2.67 g, 9.4 mmol) in CH_2Cl_2 (30 mL).[26] The mixture was stirred for 80 min. After evaporation of the solvent, 2B-BiPhe was obtained as a white solid (4.17 g) that was used without further purification for the following step. ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 5.67 (d, ^3J(H,H) = 11.3 Hz, 1H), 5.88 (d, ^3J(H,H) = 11.3 Hz, 1H), 7.34–7.64 (m, 8H), 7.60–7.70 (m, 2H), 7.72–7.82 (m, 2H), 8.12–8.23 ppm (m, 2H).

1-(Biphenyl-4-yl)-3-phenylpropane-1,3-dione (BiPhe): Sodium methylate (460 mg, 18.8 mmol; 2 equiv) in methanol (6 mL) was added to a suspension of 2B-BiPhe (4.17 g, 9.4 mmol) in methanol (30 mL).[27] After being under reflux for 2 h, concentrated hydrochloric acid (0.5 mL) was added to the mixture. Reflux was continued for 5 min and water (5 mL) was added. After evaporation of the solvent, the crude residue was purified by CC (C_6H_{12}/CH_2Cl_2 75:25 to 30:70) to yield BiPhe as a white solid (620 mg, 21%). M.p. 111°C (ref. [69] 112°C); ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 6.91 (s, 1H), 7.37–7.63 (m, 8H), 7.60–7.70 (m, 2H), 7.68–7.78 (m, 4H), 7.93–8.12 (m, 4H), 14.73 ppm (brs, 1H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): δ = 93.1, 127.0, 127.15, 127.2, 127.25, 127.3, 127.7, 128.1, 128.2, 128.7, 128.9, 128.95, 130.1, 132.4, 134.3, 135.6, 139.9, 145.2, 145.6, 185.3, 185.7 ppm.

Syntheses of the diketones Ar^1Ar^2 along pathway C

1,3-Di(4-methoxyphenyl)propane-1,3-dione (MeOPheMeOPhe):[28] A suspension of 4-methoxybenzoyl chloride (1.67 g, 9.8 mmol) and aluminium chloride (1.4 g, 10.25 mmol) in tetrachloroethane (5 mL) was stirred at 55°C for 2 h. Then, ethenyl acetate (430 mg, 5 mmol) was added and the mixture was further stirred at 55°C for 20 h. After cooling to room temperature, hydrochloric acid (2M, 2 mL) and water (20 mL) were successively added. After extraction with ethyl acetate, the organic phase was washed with sodium hydrogencarbonate and water. After drying (magnesium sulfate) and filtration, the organic solvent was evaporated to yield MeOPheMeOPhe as pale yellow crystals after recrystallization in ethanol (357 mg, 25%). M.p. 114°C (ref. [28] 116–117°C); ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 3.89 (s, 6H), 6.74 (s, 1H), 6.93–7.02 (m, 4H), 7.92–8.01 ppm (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): δ = 55.5, 91.5, 114.0, 128.2, 129.1, 131.4, 163.0, 184.6 ppm.

Syntheses of the $Ar^1Ar^2BF_2$ compounds

Compound ***1***: A solution of BiBi (276 mg, 0.73 mmol) and BF_3/Et_2O (0.14 mL, 1.1 mmol) in benzene (2 mL) was heated to reflux for 1 h.[21] After evaporation of the solvent, **1** was obtained as yellow crystals after recrystallization in benzene (230 mg, 74%). M.p. >290°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 7.28 (s, 1H), 7.40–7.57 (m, 6H), 7.66–7.72 (m, 4H), 7.76–7.84 (m, 4H), 8.21–8.31 ppm (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): δ = 93.3, 127.4, 127.8, 128.9, 129.1, 129.6, 130.8, 139.2, 148.1, 182.4 ppm; ^{19}F NMR (235 MHz, $CDCl_3$, 25°C, $CFCl_3$): δ = −140.24 (75%), −140.17 ppm (25%); MS (CI, NH_3): *m/z* (%): 442 (100) [M^++18], 405 (18), 361 (5); elemental analysis calcd (%) for $C_{27}H_{19}BF_2O_2$ (424): C 76.44, H 4.51; found: C 76.45, H 4.51.

Compound ***2***: Same procedure as for **1**, but with BiNa (140 mg, 0.4 mmol) and BF_3/Et_2O (0.05 mL, 1.1 mmol). **2** was obtained as yellow crystals after recrystallization in benzene (113 mg, 71%). M.p. 258°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 7.39 (s, 1H), 7.43–7.57 (m, 3H), 7.57–7.64 (m, 4H), 7.82 (d, ^3J(H,H) = 8.5 Hz, 2H), 7.91–7.92 (m, 1H), 7.92–8.04 (m, 1H), 8.03–8.09 (m, 1H), 8.10–8.17 (m, 1H), 8.30 (d, ^3J(H,H) = 8.5 Hz, 2H), 8.81 ppm (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 25°C, TMS): δ = 93.6, 123.5, 127.3, 127.4, 127.7, 127.9, 128.9, 129.1, 129.1, 129.6, 129.8, 130.0, 130.65, 131.6, 132.5, 136.5, 139.5, 148.0, 182.4, 182.6 ppm; ^{19}F NMR (235 MHz, $CDCl_3$, 25°C, $CFCl_3$): δ = −140.185 (75%), −140.12 ppm (25%); MS (CI, NH_3): *m/z* (%): 416 (100) [M^++18], 379 (23), 351 (1), 335 (1); elemental analysis calcd (%) for $C_{25}H_{17}BF_2O_2$ (398): C 75.40, H 4.30; found: C 75.20, H 4.31.

Compound ***3***: Same method as for **1**, but with BiPhe (620 mg, 2.1 mmol) and BF_3/Et_2O (0.27 mL, 2.1 mmol) in CH_2Cl_2 (20 mL). **3** was obtained as orange crystals after recrystallization in benzene (556 mg, 76%). M.p. 228°C; ^{1}H NMR (250 MHz, $CDCl_3$, 25°C, TMS): δ = 7.25 (s, 1H), 7.40–7.63 (m, 5H), 7.63–7.77 (m, 3H), 7.77–7.83 (m, 2H), 8.13–8.21 (m, 2H), 8.21–8.28 ppm (m, 2H); ^{13}C NMR (63 MHz, $CDCl_3$, 25°C, TMS): δ = 93.3, 127.3, 127.7, 128.9, 129.1, 129.2, 129.6, 130.5, 132.0, 135.2, 139.1, 148.1, 182.7, 182.9 ppm; ^{19}F NMR (235 MHz, $CDCl_3$, 25°C, $CFCl_3$): δ = −140.20 (75%), −140.14 ppm (25%); MS (CI, NH_3): *m/z* (%): 366 (100) [M^++18], 329 (8), 300 (8), 285 (7); elemental analysis calcd (%) for $C_{21}H_{15}BF_2O_2$ (348): C 72.45, H 4.34; found: C 72.36, H 4.40.

Compound ***4***: Same method as for **1**, but with MeOBiPhe (330 mg, 1 mmol) and BF_3/Et_2O (0.14 mL, 1.1 mmol). **4** was obtained as a yellow powder after recrystallization in benzene (160 mg, 42%). M.p. 245°C; ^{1}H NMR (250 MHz, [D_6]DMSO, 25°C, TMS): δ = 3.81 (s, 3H), 7.01–7.12 (m, 2H), 7.59–7.72 (m, 2H), 7.77–7.88 (m, 3H), 7.90–8.01 (m,2H), 7.97 (s, 1H), 8.33–8.49 ppm (m, 4H); ^{13}C NMR (63 MHz, [D_6]DMSO, 25°C, TMS): δ = 55.7, 94.7, 115.0, 127.0, 129.0, 129.6, 129.8, 130.6, 130.7, 131.8, 136.1, 147.2, 160.6, 182.2, 182.4 ppm; ^{19}F NMR (235 MHz, $CDCl_3$ ($CFCl_3$), 25°C, $CFCl_3$): δ = −140.25 (67%), −140.19 ppm (33%); MS (CI,

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NH_3): *m*/*z* (%): 396 (100) [M^++18], 359 (9), 331 (8); elemental analysis calcd (%) for $C_{22}H_{17}BF_2O_3$ (378): C 68.87, H 4.53; found: C 69.73, H 4.70.

Compound ***5***: Same method as for **1**, but with MeOPheMeOPhe (284 mg, 1 mmol) and BF_3/Et_2O (0.14 mL, 1.1 mmol). **5** was obtained as yellow crystals after recrystallization in benzene (283 mg, 85%). M.p. 236 °C; ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.93 (s, 6H), 6.97–7.06 (m, 4H), 7.02 (s, 1H), 8.10–8.19 ppm (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C, TMS): δ = 55.8, 91.5, 114.6, 124.6, 131.3, 165.3, 180.9 ppm; ^{19}F NMR (235 MHz, $CDCl_3$, 25 °C, $CFCl_3$): δ = −141.53 (75%), −141.47 ppm (25%); MS (CI, NH_3): *m*/*z* (%): 350 (92) [M^++18], 313 (100), 269 (1); elemental analysis calcd (%) for $C_{17}H_{15}BF_2O_4$ (332): C 61.48, H 4.55; found: C 61.62, H 4.63.

Compound ***6***: Same method as for **1**, but with MeOPhePhe (254 mg, 1 mmol) and BF_3/Et_2O (0.14 mL, 1.1 mmol). **6** was obtained as yellow crystals after recrystallization in benzene (125 mg, 41%). M.p. 216 °C; ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.94 (s, 3H), 7.00–7.09 (m, 2H), 7.11 (s, 1H), 7.51–7,61 (m, 2H), 7.65–7.73 (m, 1H), 8.09–8.21 ppm (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C, TMS): δ = 55.8, 92.5, 114.7, 124.2, 128.6, 129.1, 131.7, 132.4, 134.7, 165.8, 181.6, 182.4 ppm; ^{19}F NMR (235 MHz, $CDCl_3$, 25 °C, $CFCl_3$): δ = −140.84 (75%), −140.78 ppm (25%); MS (CI, NH_3): *m*/*z* (%): 320 (58) [M^++18], 283 (18), 166 (100); elemental analysis calcd (%) for $C_{16}H_{13}BF_2O_3$ (302): C 63.62, H 4.34; found: C 63.71, H 4.31.

Compound **7**: Same method as for **1**, but with NaNa (324 mg, 1 mmol) and BF_3/Et_2O (0.14 mL, 1.1 mmol). **7** was obtained as a yellow powder after recrystallization in benzene (264 mg, 71%). M.p. >276 °C; ^{1}H NMR (250 MHz, [D_6]DMSO, 25 °C, TMS): δ = 7.73–7.97 (m, 4H), 8.18–8.26 (m, 2H), 8.27–8.43 (m, 4H), 8.37 (s, 1H), 8.49–8.58 (m, 2H), 9.30 ppm (brs, 2H); ^{13}C NMR (63 MHz, [D_6]DMSO, 25 °C, TMS): δ = 95.2, 123.9, 127.6, 127.9, 128.7, 129.1, 130.1, 130.2, 132.1, 132.2, 136.2, 182.1 ppm; ^{19}F NMR (235 MHz, $CDCl_3$ ($CFCl_3$), 25 °C, $CFCl_3$): δ = −143.32 (75%), −143.30 ppm (25%); MS (CI, NH_3): *m*/*z* (%): 390 (100) [M^++18], 353 (9), 309 (9); elemental analysis calcd (%) for $C_{23}H_{15}BF_2O_2$ (372): C 74.22, H 4.06; found: C 74.26, H 4.01.

Compound ***8***: Same method as for **1**, but with NaPhe (274 mg, 1 mmol) and BF_3/Et_2O (0.14 mL, 1.1 mmol). **8** was obtained as a yellow powder (261 mg, 81%). M.p. 229 °C; ^{1}H NMR (250 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.34 (s, 1H), 7.48–7.75 (m, 5H), 7.85–8.14 (m, 4H), 8.14–8.22 (m, 2H), 8.76 ppm (brs, 1H); ^{13}C NMR (63 MHz, $CDCl_3$, 25 °C, TMS): δ = 93.7, 123.4, 127.5, 127.9, 128.9, 129.1, 129.2, 129.8, 130.0, 131.6, 132.0, 132.5, 135.2, 136.5, 182.9 ppm; ^{19}F NMR (235 MHz, $CDCl_3$, 25 °C, $CFCl_3$): δ = −140.03 (75%), −139.98 ppm (25%); MS (CI, NH_3): *m*/*z* (%): 340 (100) [M^++18], 303 (12), 275 (45), 259 (63); elemental analysis calcd (%) for $C_{19}H_{13}BF_2O_2$ (322): C 70.85, H 4.07; found: C 70.72, H 4.10.

UV/Vis spectroscopic measurements: All experiments were performed at 293 K in spectroscopic grade solvents. UV/Vis absorption spectra were recorded on a Kontron Uvikon-930 spectrophotometer. Molar absorption coefficients were extracted, while checking the validity of the Beer–Lambert law. Corrected fluorescence spectra were obtained from a Photon Technology International LPS 220 spectrofluorimeter. The concentrations of solutions for fluorescence measurements were adjusted so that the absorption was below 0.15 at the excitation wavelength. The overall fluorescence quantum yields ϕ_F were calculated from the relation shown in Equation (1):

$$\phi_F = \phi_{ref}\frac{A_{ref}(\lambda_{exc})}{A(\lambda_{exc})}\frac{D}{D_{ref}}\left(\frac{n}{n_{ref}}\right)^2 \quad (1)$$

in which the subscript *ref* stands for standard samples, $A(\lambda_{exc})$ is the absorbance at the excitation wavelength, D is the integrated emission spectrum, and n is the refractive index for the solvent. The uncertainty in the experimental value of ϕ_F was estimated to be ±10%. The standard fluorophore for the quantum yield measurements was quinine sulfate in 0.1 M H_2SO_4 with ϕ_S = 0.50.[70]

Samples for solvatochromism and stability studies were prepared in the following manner: Fixed volumes of a stock solution of dye in dichloromethane were transferred into pyrex hemolysis tubes. The volatile solvent was evaporated under a fume hood for one whole night or under vacuum for one hour. For solvatochromism investigations, the appropriate amount of desired spectroscopic grade solvent (Aldrich, except for the CH_2Cl_2 which was freshly home-distilled over calcium hydride) was added to each tube and the mixture was sonicated. For experiments involving micelles, β-octylglucopyranoside was added to stock solutions of dye before evaporation of dichloromethane. Transparent micellar solutions were obtained after addition of ultra-pure water and brief sonication (final concentration in detergent 25 mM).

Measurements of the cross sections for two-photon absorption

Method: In the absence of photobleaching, the photon flux $F(t)$ emitted under two-photon excitation is proportional to the fluorophore concentration c (assumed to be homogeneous and stationary) and to the square of the incoming power $I(\vec{r},t)$ [Eq. (2)]:

$$F(t) = \frac{1}{2}\alpha\phi_F\delta c\int_v I^2(\vec{r},t)\mathrm{d}V \quad (2)$$

in which ϕ_F is the fluorescence quantum yield (assumed to remain identical to the value determined by the study of the linear photophysical properties), δ is the two-photon absorption cross section to be measured (in $cm^4 s\,(photon-molecule)^{-1}$) and α is the set-up detection efficiency. During an acquisition, the amount of fluorescence detected is shown by using Equation (3):

$$\langle F(t)\rangle = \frac{1}{2}\alpha\phi_F\delta c\langle P^2(t)\rangle\int_v S(\vec{r})\mathrm{d}V \quad (3)$$

in which $S(\vec{r})$ is the temporal distribution of the incident light and $P(t)$ is the instantaneous photon flux for the sample (photon s^{-1}).

The signal detected is proportional to $\langle P^2(t)\rangle$, whereas most detectors are sensitive to $\langle P(t)\rangle^2$. An accurate measurement of δ thus requires the determination of the temporal structure of the excitation beam and its second-order temporal coherence: $g^{(2)} = \langle P^2(t)\rangle/\langle P(t)\rangle^2$. This is usually achieved by calibration at each wavelength by using a known standard, such as fluorescein or rhodamine, which causes heavy and repetitive manipulations. An alternative elegant method has been described by Xu et al.[60] By adding a Michelson interferometer to the laser path, the usual set up is transformed into an autocorrelator, using the fluorescent sample as the quadratic medium (Figure 1Sa and 1Sb in the Supporting Information). The major interest of this set-up configuration is evidently to get rid of the otherwise necessary determination of the pulse temporal shape, for this information is now intrinsically contained in the interference pattern. The only unknown quantity remaining is the α factor that may be determined by calibration at one single wavelength. In the interference conditions it was then shown that [Eq. (4)]:

$$\delta = \frac{\left(\int_{-T}^{T} F(t)\mathrm{d}t\right) - 2TF_\infty}{2\alpha cA\phi_F\left[\int_{-\infty}^{+\infty} P(t)\mathrm{d}t\right]^2} \quad (4)$$

in which T is the integration time (2T larger than the interference pattern width). $A = \int_v S^2(\vec{r})\mathrm{d}V \approx 2.55/\lambda$ when overfilling the back-aperture of the focusing lens (in the case of a plane wave truncated by an aperture, giving an Airy diffraction pattern). F_∞ is the mean fluorescence signal without any interference; it is then simply twice the fluorescence generated by one pulse from one of the Michelson paths. It was also shown that the ratio between the fluorescence maximum and F_∞ must be 8:1, which is also a probe for the quadratic dependence on the excitation power.

Set up and data treatment: The experiments were performed at room temperature in spectroscopic grade dichloromethane at 10^{-4} M. Freshly prepared solutions were sealed in square capillary tubes (1 mm × 1 mm; VitroCom, USA), kept at +5 °C, and away from light afterwards.

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The scheme of the home-made experimental set up was inspired from ref. [60] (Figure 1Sa and 1Sb in the Supporting Information). The excitation source was a pulsed Ti–sapphire laser (MIRA - Coherent, USA) pumped at 532 nm by a Nd-YVO_4 laser (VERDI - Coherent, USA). This femtosecond laser delivered pulses in the near-infrared region from 690 to 1000 nm.

The Michelson interferometer consisted of a beamsplitter (BS) and two corner-cubes (Figure 1Sb). One corner-cube (CC_1) was fixed on the diaphragm of a loudspeaker powered by a low-frequency generator. A continuous He-Ne laser was also included in the interferometer to calibrate the diaphragm displacement. The resulting interference pattern was recorded at the end of the Michelson interferometer on a photodiode (PD_1). Typical pulse widths at the sample were less than 200 fs.

After a beam-expander 6× (BE, Melles Griot), the IR laser beam overfilled the back aperture of the lens (Nikon, 20×, N.A. 0.35), which collimates it onto the sample. A lens focused most of the excitation beam on a second photodiode (PD_2; the fraction collected remains unchanged with wavelength). A long-pass dichroic filter (DC, Chroma, USA) separated the fluorescence in the visible range from the excitation light. After a set of short-pass filters (from Melles Griot or OmegaOptics), the remaining IR background (from elastic or inelastic scattering) was totally removed and the pure fluorescence emission was divided by a beam-splitter to be detected on two photomultiplier tubes (PMT_1, R1527P, and PMT_2, RC135 - Hamamatsu, Japan). PMT_2 is a photon-counting tube, but its minimal integration delay is limited to 10 ms, hence requiring the auxiliary use of PMT_1 with a faster rate cut energy-dependent signal. The signals of the two photodiodes and the fluorescence in energy and in photon counts from the two PMTs were thus recorded as functions of time on a PC, thanks to the acquisition card PCI-DAS1602/16 (ComputerBoards, USA), with a maximum acquisition rate of 200 kHz. The signals were then corrected by the sensitivities of the detectors given by the manufacturers. For PD_2, the excitation wavelength was first determined by the following method: both interference signals from the two photodiodes were treated by the Hilbert transform (HT) method. The phase of the HT was integrated as a function of time, and the slope was proportional to the interference spatial frequencies, that is, to the wavelength. The ratio of the slopes for the excitation IR to the He-Ne then gave the ratio between the wavelengths; the signal of the He-Ne on PD_2 was used as the reference wavelength ($\lambda_{\text{He-Ne}} = 632.8$ nm). With this determination of the excitation wavelength, we then referred to the constructor sensitivity charts to extract the required correction factor. For the two PMs, the correction was taken at the maximum emission, determined by fluorimetry (vide supra). We also checked that the correction was modified within the experimental uncertainty when integrating it on the whole emission spectrum.

The value of δ was computed according to Equation (4), directly from the input signals: PD_2 leads to $\langle P(t)\rangle$, PM_1 and PM_2 supply $F(t)$ and F_∞ (Figure 2S in the Supporting Information). To optimize its determination, the acquisition is triggered on the GBF signal, and the final value of δ was averaged over 15 to 20 successive acquisitions. The whole spectrum was obtained for steps in the wavelength between 5 and 10 nm, which corresponds to the width of the excitation wavelength distribution. It is then calibrated on one wavelength by comparison to fluorescein, whose TPA cross sections have been tabulated.[71] The accuracy of the measurements were first validated with rhodamine, whose spectrum was consistent with the one published.[12] The method was then applied to the newly synthesized chromophores.

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