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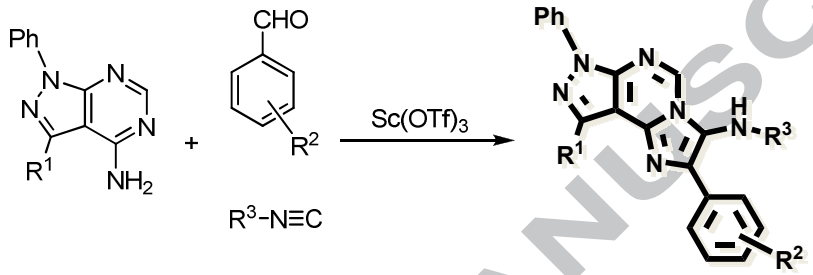

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Sc(OTf)3 promoted multicomponent synthesis of fluorescent imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine

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ABSTRACT

A three-component condensation reaction between aminopyrazolo[3,4-d]pyrimidine, an aldehyde and an isocyanide catalyzed by scandium triflate yields a series of heterocyclic derivatives of imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine in good yields, exhibiting interesting fluorescent properties.

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1. Introduction

Multicomponent reactions (MCRs) offer a convenient strategy to easily obtain complex molecules with interesting properties.[1] MCRs offer the elegance of one-pot reactions, atom and bond-forming economy, and the feasibility of introducing maximum diversity elements in one chemical event operation,[2] allowing the construction of architecturally complex molecules having a wide range of biological and pharmaceutical activities.[3]

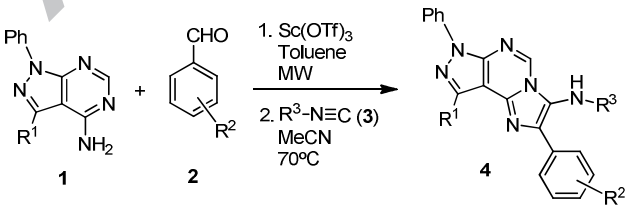
The reaction involving an isocyanide, an aldehyde and a 2-aminoazine was reported simultaneously by Groebke *et al*,[4] Bienayme *et al*,[5] and Blackburn *et al*.[6] The pre-formation of the imine was not necessary, but the reaction requires the use of either a Brönsted acid ($AcOH$,[4] $HClO_4$ [5]) or a Lewis acid ($Sc(OTf)_3$).[6] This one-step coupling constitutes a very efficient route for imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine derivatives. The presence of multi-functional groups increases the range of possible applications. On one hand, imidazopyrimidines are important structural moieties, found in pharmacological compounds such as anti-inflammatory agents,[7] anti-bacterials,[8] anticancer,[9] antimicrobial agents[10] and also used for their antituberculosis activity.[11] These compounds also provide a route to synthesize condensed nucleobases, such as adenine, guanine and cytosine,[12] and can also be used as Adenosine Receptor Antagonists due to their similarity to purine derivatives.[13] On the other hand, pyrazolopyrimidines have been shown to inhibit glycogen synthase kinase-3 (GSK-3),[14] and act as potent ligands for the peripheral benzodiazepine receptor.[15]

Development of an MCR-based approach to new fluorescent substituted imidazo[1,2-c]pyrazolo[3,4-d]pyridimidines capable of further transformations was the goal of our present work. In particular, we were interested in the synthesis of imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine derivatives catalyzed by $Sc(OTf)_3$, which are easily accessible thanks to the development of microwave assisted synthetic methodologies.

2. Results and discussion

2.1. Synthesis

The imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine derivatives were prepared using either a multi-component coupling reaction between an aldehyde **2**, an isocyanide **3** and an amino pyrazolo[3,4-d]pyrimidine[16] **1**, or using a two-steps synthesis via the formation of an imine. When optimizing the reaction conditions we have faced problems in both methods. In the two-step method, different catalysts and temperatures were tested, but the pre-formation of the imine needs higher temperature and the best results were obtained using $Sc(OTf)_3$ as catalyst and microwave. The one-step method was also tested using different catalysts, with the best results again obtained using $Sc(OTf)_3$.[17] Scheme 1 shows the synthetic strategy to obtain the target compounds by the two-steps method (Table 1).

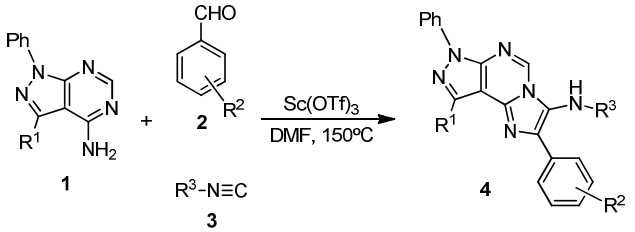


Scheme 1. Synthetic strategy used in the two-steps method

Table 1. Synthesis of imidazo[1,2-c]pyrazolo-[3,4-d]pyridimidine derivatives **4**

	R^1	R^2	R^3	Yield %	
				two-step method	one-pot method
4a	CH_3	4-Cl	*tert*-butyl	62	--
4b	CH_3	4-Cl	cyclohexyl	63	78
4c	CH_3	4-OMe	*tert*-butyl	60	73
4d	CH_3	4-OMe	cyclohexyl	55	61
4e	CH_3	2,5-di-Cl	cyclohexyl	56	--
4f	CH_3	3-NO_2	cyclohexyl	57	72
4g	CH_3	3-NO_2	*tert*-butyl	--	72
4h	H	4-Cl	cyclohexyl	--	73
4i	H	4-OMe	cyclohexyl	--	74
4j	H	4-NO_2	cyclohexyl	--	64

The two-steps strategy produces target compounds **4** in good yields (55% to 63%). However this method requires long reaction times and the purification of the imine is not straightforward. As part of our ongoing efforts on the development of new synthetic protocols for the synthesis of biologically active heterocyclic compounds through MCRs, we have used scandium triflate ($Sc(OTf)_3$) as catalyst for the synthesis of imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine derivatives **4**. A three component condensation of benzaldehydes, pyrazolopyrimidines, and isocyanides is reported for the synthesis of imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine derivatives **4** (Scheme 2), and catalyzed efficiently by $Sc(OTf)_3$, an environmental friendly catalyst.



Scheme 2. One-step three component condensation of benzaldehydes, pyrazolopyrimidines, and isocyanides catalyzed by $Sc(OTf)_3$.

As starting materials we used 4-aminopyrazolo-[3,4-d]pyrimidine derivatives **1**, which were subjected to condensation with substituted benzaldehydes **2**, in the presence of isocyanides **3** in presence of catalytic amount of $Sc(OTf)_3$ (5 mol%). The mixture was heated over night at 150°C in DMF, and after chromatographic purification **4** derivatives were obtained. Two isocyanides were tested successfully in this coupling, namely cyclohexyl isocyanide and *tert*-butyl isocyanide. Different benzaldehydes have been tested, with 4-chlorobenzaldehyde proving to be the most efficient in both methods. In Table 1 we show the different imidazo-[1,2-c]pyrazolo[3,4-d]pyridimidine **4** derivatives obtained by the one-pot condensation reaction. For example, in the synthesis of **4f** using 4-aminopyrazolo[3,4-d]pyrimidine, 4-nitrobenzaldehyde and cyclohexylisocyanide, the yields for the two-steps and one-pot methods were 57% and 72%, respectively. Likewise, the yields of imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine derivatives

4b, **4c** and **4d** synthesized by the one-pot procedure are always higher than those obtained in two-steps.

2.2. Fluorescence

The absorption and fluorescence properties of derivatives **4a**, **4b**, **4c**, **4d** and **4f** were studied in chloroform solution at 10 μM. We observed that these compounds feature similar molar absorptivities (in the range of ca. 4 $\times 10^4$ to 6 $\times 10^4$ $M^{-1}cm^{-1}$) but very different fluorescence intensities (Figure 1). The fluorescence intensity of **4f**, featuring a 3-NO_2 substituent in the R^2 position, is the lowest, although this derivative presents a slightly higher absorption efficiency. On the other hand, the highest fluorescence intensity is observed for **4d**, which contains a
4-OMe substituent at the R^2 position. Interestingly, maintaining the same substituent in the R^2 position but changing the cyclohexyl substituent in the R^3 position (**4d**) to a *tert*-butyl substituent (**4c**), the fluorescence intensity decreases markedly, probably because the bulky *tert*-butyl R^3 group of **4c** forces the phenyl ring containing R^2 to twist.

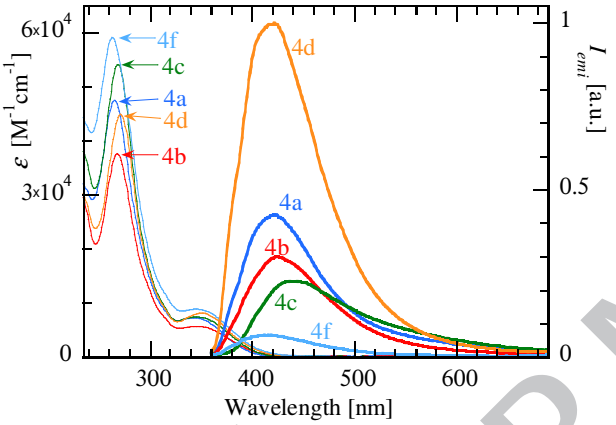


Figure 1. Absorption (thin lines) and emission (thick lines) spectra of imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine derivatives **4a** (dark blue), **4b** (red), **4c** (green), **4d** (orange) and **4f** (light blue) in chloroform solution (10 μM).

We also measured the fluorescence lifetimes of derivatives **4a**, **4b**, **4c**, **4d** and **4f** with laser excitation at λ_{exc} = 340 nm and collecting the emission at λ_{em} = 425 nm (Table 2). The decays were analyzed with a sum of two exponential functions, yielding good fitting results.[18] We observed very similar short and long lifetime components for all the samples, except **4f** which presented a faster decay, probably due to the R^2=NO_2 substituent. The decay of **4c**, featuring OMe and *tert*-butyl substituents, is somehow unexpected because the longer decay component has a weight of *ca.* 90%, contrarily to **4a**, **4b**, and **4d**, for which the weight of this component is *ca.* 10-20%. Therefore, **4c** has a much larger average decay lifetime. This causes the decrease in the radiative rate constant that results in lower fluorescence intensity of **4c** compared to that of **4d** in Figure 1.

Table 2. Fluorescence decay lifetimes obtained by fitting the decay curves of derivatives **4a**, **4b**, **4c**, **4d** and **4f** with a sum of two exponential functions (the weights w_1 and w_2 of each decay component are indicated). The average decay lifetime is calculated as $\langle\tau\rangle = w_1\times\tau_1 + w_2\times\tau_2$. R^1 is CH_3 in all cases.

	τ_1 [ns]	w_1	τ_2 [ns]	w_2	$\langle\tau\rangle$ [ns]	R^2	R^3
4a	1.5	80%	4.8	20%	2.1	4-Cl	*tert*-butyl
4b	1.5	89%	4.6	11%	1.8	4-Cl	cyclohexyl
4c	2.0	12%	5.7	88%	5.3	4-OMe	*tert*-butyl
4d	1.5	88%	5.2	12%	1.9	4-OMe	cyclohexyl
4f	1.1	47%	1.9	53%	1.5	3-NO_2	cyclohexyl

Conclusions

In summary, we have devised a simple, efficient and environmentally friendly protocol for the one-pot synthesis of imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine derivatives catalyzed by $Sc(OTf)_3$ under mild reaction conditions. Moreover, the present protocol possesses several unique merits such as simplicity, non-aqueous work-up and most importantly, high yield of the products. We obtained a new family of fluorescent imidazo[1,2-c]pyrazolo[3,4-d]pyridimidine derivatives that offer good prospects for fluorescence-based clinical and biomedical applications.

Experimental

*General procedure for the synthesis of imidazo-[1,2-c]pyrazolo[3,4-d]pyridimidine (**4**) using the **two-steps method***

Benzaldehyde derivative **2** (1 mmol) and scandium triflate (5 mol%) were added to a solution of 4-aminopyrazolo-[3,4-d]pyrimidine derivative **1**[16] (1 mmol) in toluene (1 mL) at room temperature. The mixture was heated with microwave radiation (200 W) for 30 min at 150°C. Then toluene was evaporated and isocyanide derivative **3** (1 mmol) and acetonitrile (1 mL) were added. The solution was heated at 70°C overnight, and afterwards the solvent was removed and the residue purified by flash chromatography on silica gel (petroleum ether/CH_2Cl_2 2:8).

Compound **4a** was obtained (62%) as a pale yellow solid, mp 249 °C. **^{1}H NMR ($CDCl_3$, 400 MHz)** δ 9.95 (s, 1H), 8.16 (d, *J*=8.4 Hz, 2H), 7.98 (d, *J*=8.4 Hz, 2H), 7.60-7.39 (m, 5H), 3.20 (s, 1H), 2.95 (s, 3H), 1.14 (s, 9H) ppm. **^{13}C NMR ($CDCl_3$, 100 MHz)** δ 145.5, 143.2, 138.9, 138.2, 137.6, 137.1, 133.6, 132.9, 129.5, 129.2, 128.7, 126.8, 123.1, 122.0, 103.9, 56.4, 30.4, 14.2 ppm. HRMS calculated for $C_{24}H_{23}ClN_6$: 430.1673; found: 430.1666. Elemental Analysis calculated (%): 66.89 C , 5.38 H , 19.50 N ; found (%): 66.34 C , 5.26 H , 19.72 N .

Compound **4b** was obtained (63%) as a pale yellow solid, mp 201 °C. **^{1}H NMR ($CDCl_3$, 400 MHz)** δ 8.83 (s, 1H), 8.16 (d, *J*=8.4 Hz, 2H), 8.08 (d, *J*=8.4 Hz, 2H), 7.60-7.40 (m, 5H), 3.16 (s, 1H), 3.03 (s, 1H), 2.94 (s, 3H), 1.90-1.20 (m, 10 H) ppm. **^{13}C NMR ($CDCl_3$, 100 MHz)** δ 143.2, 138.9, 137.7, 136.4, 134.9, 133.4, 132.2, 129.2, 128.9, 128.4, 126.7, 124.5, 122.0, 57.5, 34.2, 25.6, 24.8, 14.1 ppm. HRMS calculated for $C_{26}H_{25}ClN_6$: 456.1829; found 456.1834. Elemental Analysis calculated (%): 68.34 C , 5.51 H , 18.39 N ; found (%): 67.78 C , 5.41 H , 18.56 N .

Compound **4c** was obtained (60%) as a yellow solid, mp 227 °C. **^{1}H NMR ($CDCl_3$, 400 MHz)** δ 8.93 (s, 1H), 8.17 (d, *J*=8.8 Hz, 2H), 7.92 (d, *J*=8.8 Hz, 2H), 7.60-7.04 (m, 5H), 3.91 (s, 3H), 3.21 (s, 1H), 2.95 (s, 3H), 1.12 (s, 9H) ppm. **^{13}C NMR ($CDCl_3$, 100 MHz)** δ 159.3, 145.5, 143.2, 138.9, 138.6, 137.2, 129.5, 129.2, 126.6, 122.4, 121.9, 113.9, 103.9, 56.2, 55.3, 30.3, 14.2 ppm. HRMS calculated for $C_{25}H_{26}N_6$: 426.2168; found

426.2174. Elemental Analysis calculated (%): 70.40 C , 6.14 H , 19.70 N ; found (%): 69.7 C , 6.00 H , 19.68 N .

Compound **4d** was obtained (55%) as a pale yellow solid, mp 198 °C. **^{1}H NMR (CDCl$_3$, 400 MHz)** δ 8.86 (s 1H), 8.17 (d, *J*=8.0 Hz, 2H), 8.05 (d, *J*=8.0 Hz, 2H), 7.58-7.06 (m, 5H), 3.93 (s, 3H), 3.20 (s, 1H), 3.04 (s, 1H), 2.95 (s, 3H), 1.76-1.22 (m, 10H) ppm. **^{13}C NMR (CDCl$_3$, 100 MHz)** δ 159.2, 145.4, 143.2, 139.0, 136.5, 135.8, 129.2, 129.2, 128.4, 126.6, 126.3, 123.5, 121.9, 114.1, 103.9, 57.4, 55.4, 34.1, 25.7, 24.8, 14.2 ppm. HRMS calculated for $C_{27}H_{28}N_6O$: 452.2325; found: 452.2317. Elemental Analysis calculated (%): 71.66 C , 6.24 H , 18.57 N ; found (%): 71.10 C , 6.14 H , 18.86 N .

Compound **4e** was obtained (56%) as a brown solid, mp 194 °C. **^{1}H NMR (CDCl$_3$, 400 MHz)** δ 8.77 (s, 1H), 8.01 (d, *J*=8.4 Hz, 2H), 7.44-7.22 (m, 6H), 2.87 (s, 1H), 2.71 (s, 1H), 2.65 (s, 3H), 1.71-1.01 (m, 10H) ppm. **^{13}C NMR (CDCl$_3$, 100 MHz)** δ 156.8, 145.3, 143.2, 138.9, 136.6, 136.6, 130.4, 129.9, 129.2, 128.3, 126.8, 126.4, 122.1, 121.5, 101.5, 57.3, 33.8, 25.6, 24.6, 14.4 ppm. HRMS calculated for $C_{26}H_{24}Cl_2N_6$: 456.1829; found 456.1824.

Compound **4f** was obtained (57%) as a yellow solid, mp 204 °C. **^{1}H NMR (CDCl$_3$, 400 MHz)** δ 8.93 (s, 1H), 8.68 (s, 1H), 8.44 (d, *J*=8.0 Hz, 1H), 8.09 (d, *J*=8.0 Hz, 2H), 8.02 (m, 1H), 7.58-7.19 (m, 5H), 3.08 (s, 3H), 2.93 (s, 1H), 2.82 (s, 3H), 1.84-1.09 (m, 10H). **^{13}C NMR (CDCl$_3$, 100 MHz)** δ 148.5, 145.4, 143.3, 138.3, 137.9, 136.9, 135.5, 133.6, 132.9, 129.6, 129.2, 126.8, 125.3, 122.2, 121.9, 121.5, 103.9, 57.7, 34.3, 25.6, 24.8, 14.1 ppm. HRMS calculated for $C_{26}H_{25}N_7O_2$: 467.2070; found 467.2060. Elemental Analysis calculated (%): 66.79 C , 5.39 H , 20.97 N ; found (%): 66.34 C , 5.40 H , 20.91 N .

*General procedure for the synthesis of imidazo-[1,2-c]pyrazolo[3,4-d]pyridimidine (**4**) using the **one-pot method***

To a solution of 4-aminopyrazolo[3,4-d]pyrimidine **1**[16] (1 mmol) in DMF (1 mL), benzaldehyde derivative **2** (1 mmol), scandium triflate (5 mol %) and isocyanide derivative **3** (1 mmol) were added. The mixture was heated over night at 150°C, and after removing the solvent the residue was purified by flash chromatography on silica gel (petroleum ether/CH_2Cl_2 2:8).

Compound **4g** was obtained (72%) as a yellow solid, mp 234 °C. **^{1}H NMR (CDCl$_3$, 400 MHz)** δ 8.93 (s, 1H), 8.82 (s, 1H), 8.39 (d, *J*=7.6 Hz, 1H), 8.12 (dd, *J*=8.0 Hz, 1H), 8.04 (d, *J*= 7.6 Hz, 2H), 7.58-7.29 (m, 5H), 3.08 (s, 1H), 1.07 (s, 9H) ppm. **^{13}C NMR (CDCl$_3$, 100 MHz)** δ 148.3, 145.5, 143.3, 137.0, 136.1, 133.9, 129.5, 129.2, 126.9, 126.7, 123.8, 122.7, 122.4, 122.0, 103.8, 56.6, 30.6, 14.1 ppm. HRMS calculated for $C_{24}H_{23}N_7O_2$: 441.1913; found 441.1906. Elemental Analysis calculated (%): 65.29 C , 5.25 H , 22.21 N ; found (%): 64.86 C , 5.19 H , 22.38 N .

Compound **4h** was obtained (73%) as a pale yellow solid, mp 219 °C. **^{1}H NMR (CDCl$_3$, 400 MHz)** δ 8.74 (s, 1H), 8.43 (s, 1H), 8.06 (d, *J*=8.4 Hz, 2H), 7.80 (d, *J*=6 Hz, 2H), 7.45-7.35 (m, 5H), 3.05 (s, 1H), 1.74-1.07 (m, 10H) ppm. **^{13}C NMR (CDCl$_3$, 100 MHz)** δ 144.1, 141.3, 139.0, 137.3, 136.5, 135.8, 131.5, 130.9, 129.8, 129.2, 128.9, 128.3, 124.8, 122.2, 105.1, 57.5, 34.2, 27.3, 24.8 ppm. HRMS calculated for $C_{25}H_{23}ClN_6$: 442.1673; found 442.1673.

Compound **4i** was obtained (74%) as a yellow solid, mp 251 °C. **^{1}H NMR (CDCl$_3$, 400 MHz)** δ 8.75 (s, 1H), 8.42 (s, 1H), 8.09 (d, *J*=8.0 Hz, 2H), 7.57-7.28 (m, 6H), 6.82 (d, *J*= 8.0 Hz, 1H) ppm.**^{13}C NMR (CDCl$_3$, 100 MHz)** δ 159.9, 144.6, 138.9, 136.5, 134.8, 132.7, 129.7, 129.2, 127.1, 125.0, 122.2, 119.4, 114.2, 112.1, 104.9, 57.5, 55.4, 33.0, 25.6, 24.8 ppm. HRMS calculated for $C_{26}H_{26}N_6O_2$: 438.2168; found 438.2160.

Compound **4j** was obtained (64%) as a brown solid, mp 182 °C. **^{1}H NMR (CDCl$_3$, 400 MHz)** δ 8.90 (s, 1H), 8.59 (s, 1H), 8.37 (m, 4H), 8.19 (d, *J*=8.4 Hz, 2H), 7.62 (t, *J*=7.6 Hz, 2H), 7.46 (d, *J*=8.4 Hz, 1H), 3.27 (s, 1H), 3.09 (s, 1H), 1.20-1.96 (m, 10H) ppm.**^{13}C NMR (CDCl$_3$, 100 MHz)** δ 153.8, 140.7, 146.83, 140.1, 136.4, 133.6, 132.8, 131.6, 129.3, 127.5, 127.4, 126.6, 124.1, 122.3, 104.9, 57.8, 34.3, 25.5, 24.8 ppm. HRMS calculated for $C_{25}H_{23}N_7O_2$: 453.1913; found 453.1934.

Absorption and Fluorescence Measurements

Absorption and steady-state fluorescence spectra, as well as time resolved fluorescence of the different imidazo[1,2-c]pyrazolo-[3,4-d]pyridimidine derivatives were measured in chloroform solutions. All the samples were prepared at the concentration of 10 μM, corresponding to an optical density of A ≈ 0.1 at 350 nm. UV-visible absorption spectra were obtained in a JASCO V-660 spectrometer, using quartz cells (l = 1 cm), unless indicated otherwise. The fluorescence spectra were recorded on a Horiba Jobin Yvon Fluorolog 3-22 spectrofluorimeter using quartz cells (l = 1 cm), excitation slits with 3 nm bandwidth, emission slits with 1 nm bandwidth, integration time of 1 s/point, maximum sensitivity, and right angle mode. The emission spectra were recorded between 360 and 690 nm using as excitation wavelength λ_{ex} = 350 nm. Fluorescence intensity decay curves with picosecond resolution were obtained by the single photon timing technique using two different laser excitation systems. One of the systems consists of a de-locked Coherent Inova 440-10 argon ion laser synchronously pumping a cavity dumped Coherent 701-2 dye laser using rhodamine, which delivers 5-6 ps pulses at a repetition rate of 460 kHz. The second experimental system consists of a Spectra-Physics Millenia Xs Nd:YVO_4 diode laser, pumping a pulse picked Spectra-Physics Tsunami titanium-sapphire laser, delivering 100 fs frequency-doubled in LBO crystal (360 nm–480 nm) pulses at a repetition rate of 4 MHz. In both cases, intensity decay measurements were made by alternate collection of excitation and decay curves, using an emission polarizer set at the magic angle. The excitation profile was recorded at the excitation wavelength with a scattering suspension. For the decays, a cut-off filter was used to remove all excitation light. The emission signal passed through a depolarizer, a Jobin Yvon HR-320 monochromator with a grating of 100 lines/mm and was detected with a Hamamatsu 2809U-01 microchannel plate photomultiplier (MCP-PT). The instrument response had as effective FWHM of 35 ps.

Fluorescence intensity decay curves were obtained by excitation light at 290 nm using the rhodamine dye laser and at 400 nm using the titanium sapphire laser, with emission collected at 525 nm. The decay curves were analyzed using home-made non-linear least-square reconvolution software based on the Marquard algorithm,[18] and the quality of the fit was evaluated by the reduced χ^2 the weight residuals and the autocorrelation of the residuals.

Acknowledgments

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