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# A versatile standard for bathochromic fluorescence based on intramolecular FRET

Heinz Langhals,<sup>\*a</sup> Andreas Walter,<sup>a</sup> Erik Rosenbaum<sup>b</sup> and Lennart B.-Å. Johansson<sup>b</sup>

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A perylene and a terylene tetracarboxylic bisimide dyad was prepared in which an efficient energy transfer from the former to the latter is observed. The absorption spectrum of this compound covers a broad range. Bathochromic fluorescence with a high quantum yield was obtained independent of excitation wavelengths ( $\lambda < 655$  nm). The dyad can be recommended for the use of calibrating fluorescence spectrometers, as well as a fluorescence standard in the bathochromic region.

## Introduction

The determination of fluorescence quantum yields is conveniently related to the known quantum yield of a fluorescence standard.<sup>1</sup> Several fluorescent compounds<sup>2</sup> have been proposed for the calibration of fluorescence spectrometers in the hypsochromic visible region with quinine sulfate being highly prominent.<sup>3</sup> Perylene derivatives are stable, exhibit high quantum yields, are easy to work with, and have thus improved the range of fluorescence standards in the hypsochromic region.<sup>4</sup> However, there are inherent difficulties when measuring spectra in the bathochromic visible and the near infrared region, due to the lack of suitable reference standards. In this paper, we present a stable, easy-to-handle compound with a well-defined absorption and fluorescence spectrum in the bathochromic region.

## Materials and methods

### 2-(4-Amino-2,3,5,6-tetramethylphenyl)-9-(1-hexylheptyl)-anthra[2,1,9-def;6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (5)

2,3,5,6-Tetra-methylbenzene-1,4-diamine (197 mg, 1.20 mmol), 9-(1-hexylheptyl)-1*H*-isochromeno[6',5',4':10,5,6]anthra[2,1,9-def]-isoquinoline-1,3,8,10(9*H*)-tetraone (230 mg, 400  $\mu$ mol), zinc acetate (14.8 mg, 80  $\mu$ mol) and quinoline (4 mL) were stirred at 210 °C for 4 h under argon (dark red mixture), allowed to cool, added dropwise with intense stirring to 2 M aqueous HCl (300 mL), stirred for 2 h, allowed to stand for 16 h, collected by vacuum filtration, washed with 2 M aqueous HCl (300 mL), hot distilled water (300 mL) and a mixture of methanol/water 1 : 1, dried at 110 °C for 2 d *in vacuo*, dissolved in the minimal

amount of CHCl<sub>3</sub>/methanol 80 : 1, and purified by column separation (short column of basic alumina CHCl<sub>3</sub>/methanol 80 : 1 and a further column separation with silica gel, CHCl<sub>3</sub>/methanol 40 : 1, dark red, non-fluorescent band). Yield 250 mg (87%), mp > 300 °C. *R<sub>f</sub>*-value (silica gel, CHCl<sub>3</sub>/methanol 40 : 1): IR (ATR):  $\tilde{\nu}$  = 3482 (w), 3395 (w), 2953 (m), 2924 (m), 2856 (m), 2363 (w), 1698 (s), 1653 (s), 1592 (s), 1577 (s), 1507 (w), 1458 (w), 1432 (m), 1405 (m), 1346 (m), 1328 (s), 1250 (m), 1198 (w), 1174 (m), 1138 (w), 1107 (w), 962 (w), 855 (w), 839 (w), 810 (m), 802 (w), 748 (m), 722 (w), 671 cm<sup>-1</sup> (w). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>, 27 °C, TMS):  $\delta$  = 8.78–8.66 (m, 8 H, CH<sub>arom.</sub> perylene), 5.22–5.16 (m, 1 H, CH), 3.71 (br, 2 H, NH<sub>2</sub>), 2.29–2.21 (m, 2 H), 2.16 (s, 6 H, 2  $\times$  CH<sub>3</sub>), 2.06 (s, 6 H, 2  $\times$  CH<sub>3</sub>), 1.91–1.83 (m, 2 H), 1.38–1.19 (m, 16 H), 0.83 ppm (t, <sup>3</sup>*J*<sub>(H–H)} = 6.7 Hz, 6 H, 2  $\times$  CH<sub>3</sub>). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>, 27.0 °C):  $\delta$  = 163.5, 143.4, 135.0, 134.5, 132.0, 131.2, 130.8, 130.1, 129.6, 126.8, 126.5, 124.3, 123.4, 123.2, 123.1, 118.9, 54.8, 32.4, 31.8, 29.2, 26.9, 22.6, 15.1, 14.0, 13.9 ppm. UV/VIS (CHCl<sub>3</sub>):  $\lambda_{\text{max}}$  (*E*<sub>rel</sub>) = 458.6 (0.22), 490.6 (0.60), 527.2 nm (1.00). Fluorescence (CHCl<sub>3</sub>):  $\lambda_{\text{max}}$  (*I*) = 535.8 (1.00), 575.7 nm (0.56). Fluorescence quantum yield (CHCl<sub>3</sub>,  $\lambda_{\text{exc}}$  = 491 nm, *E*<sub>491 nm, 1 cm</sub> = 0.0164, reference 2,9-bis-(1-hexylheptyl)anthra[2,1,9-def;6,5,10-d'e'f']diisoquinoline-1,3,8,10(2*H*,9*H*)-tetraone, RN 110590-84-6, with  $\Phi$  = 1.00): < 0.01. MS (DEP/EI) *m/z* (%): 719 (100) [M<sup>+</sup>], 538 (40) [M<sup>+</sup> + H – C<sub>13</sub>H<sub>26</sub>], 391 (29) [M<sup>+</sup> + H – C<sub>13</sub>H<sub>26</sub> – C<sub>10</sub>H<sub>14</sub>N]. HRMS (C<sub>47</sub>H<sub>49</sub>N<sub>3</sub>O<sub>4</sub>): calcd 719.3723, found 719.3729,  $\Delta$  = 0.0006. C<sub>47</sub>H<sub>49</sub>N<sub>3</sub>O<sub>4</sub> (719.4): calcd C 78.41, H 6.86, N 5.84%; found C 78.22, H 6.89, N 5.78%.</sub>

### 2,11-Bis(1-nonyldecyl)benzo[13,14]pentapheno[3,4,5-def;10,9,8-d'e'f'] diisoquinoline-1,3,10,12(2*H*,11*H*)-tetraone (3)

Oxygen was carefully excluded even in trace amounts and potassium-*tert*-butoxide (570 mg, 5.08 mmol), diazabicyclo-[4.3.0]non-5-ene (DBN, 840 mg, 6.77 mmol) and anhydrous

<sup>a</sup> Department of Chemistry, University of Munich, Butenandstr. 13, D-81377 Munich, Germany. E-mail: Langhals@lrz.uni-muenchen.de

<sup>b</sup> Department of Chemistry, Biophysical Chemistry, Umeå University, S-901 87 Umeå, Sweden. E-mail: Lennart.Johansson@chem.umu.se

toluene (5.6 mL) were stirred at 130 °C for 15 min, treated dropwise within 20 min with 2-(1-nonyldecyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**1**, 524 mg, 1.13 mmol) and 2-(1-nonyldecyl)-1*H*-benzo[5,10]anthra[2,1,9-*def*]isoquinoline-1,3(2*H*)-dione (**2**, 332 mg, 565 μmol) in degassed toluene (2 mL, dark, clear blue mixture), allowed to react for 15 min, treated dropwise with 2-(1-nonyldecyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (**1**, 524 mg, 1.13 mmol) in anhydrous, degassed toluene (0.1 mL), stirred at 130 °C for 2.75 h, allowed to cool, quenched by the addition of 2 M aqueous HCl (80 mL), extracted three times with chloroform (40 mL each), dried with MgSO<sub>4</sub>, evaporated *in vacuo*, dissolved in the minimal amount of chloroform and purified by column separation (fine silica gel, chloroform, third fraction, and a further column separation with toluene, bright blue band). Yield 296 mg (50%), mp 207 °C. *R<sub>f</sub>*-value (CHCl<sub>3</sub>): 0.7. IR (ATR):  $\tilde{\nu}$  = 2952 (m), 2919 (s), 2850 (m), 2361 (w), 1691 (s), 1649 (s), 1583 (s), 1572 (s), 1505 (w), 1456 (m), 1418 (w), 1350 (s), 1323 (s), 1302 (m), 1248 (m), 1205 (m), 1172 (w), 1144 (w), 1104 (w), 1022 (w), 954 (w), 852 (w), 840 (w), 806 (s), 790 (m), 748 (m), 723 (w), 693 (w), 680 (m), 667 cm<sup>-1</sup> (w). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>, 27 °C, TMS):  $\delta$  = 8.63–8.52 (m, 4 H, CH<sub>arom. terrylene</sub>), 8.48–8.34 (m, 8 H, CH<sub>arom. terrylene</sub>), 5.26–5.20 (m, 2 H, CH), 2.31–2.23 (m, 4 H, CH<sub>2</sub>), 1.93–1.85 (m, 4 H, CH<sub>2</sub>), 1.40–1.15 (m, 56 H, CH<sub>2</sub>), 0.82 ppm (t, <sup>3</sup>*J*<sub>(H–H)</sub> = 7.1 Hz, 12 H, CH<sub>3</sub>). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>, 27.0 °C):  $\delta$  = 164.8, 163.8, 135.3, 131.7, 131.0, 130.8, 129.7, 128.4, 125.8, 124.0, 122.5, 121.7, 121.2, 54.6, 32.4, 31.9, 29.6, 29.3, 27.1, 22.6, 14.1 ppm. UV/VIS (CHCl<sub>3</sub>):  $\lambda_{\text{max}}$  ( $\epsilon$ ) = 555.2 (22 200), 598.9 (69 100), 651.8 nm (136 700). Fluorescence (CHCl<sub>3</sub>):  $\lambda_{\text{max}}$  (*I*) = 666.2 (1.00), 731.7 nm (0.47). Fluorescence quantum yield (CHCl<sub>3</sub>,  $\lambda_{\text{exc}}$  = 599 nm,  $c$  = 6.5 × 10<sup>-7</sup> mol L<sup>-1</sup>): 0.94. Fluorescence lifetime (CHCl<sub>3</sub>,  $\lambda_{\text{exc}}$  = 633 nm) = 4.60 ns. MS (DEP/EI) *m/z* (%): 1047 (28) [M<sup>+</sup>], 781 (16) [M<sup>+</sup> – C<sub>19</sub>H<sub>38</sub>], 514 (100) [M<sup>+</sup> – 2 × C<sub>19</sub>H<sub>38</sub>]. HRMS (C<sub>72</sub>H<sub>90</sub>N<sub>2</sub>O<sub>4</sub>): calcd 1046.6901, found 1046.6891,  $\Delta$  = –0.0010. C<sub>72</sub>H<sub>90</sub>N<sub>2</sub>O<sub>4</sub> (1046.7): calcd C 82.56, H 8.66, N 2.67%; found C 82.71, H 8.49, N 2.69%.

#### 11-(1-Nonyldecyl)-1*H*-benzo[13,14]isochromeno[6',5',4':8,9,10]pentapheno[3,4,5-*def*]isoquinoline-1,3,10,12(11*H*)-tetraone (**4**)

2,11-Bis(1-nonyldecyl)benzo[13,14]pentapheno[3,4,5-*def*:10,9,8-*d'e'f'*]diisoquinoline-1,3,10,12(2*H*,11*H*)-tetraone (671 mg, 642 μmol) in *tert*-butylalcohol (25 mL) was stirred under argon at 110 °C for 1 h, treated with finely powdered 85% KOH (440 mg, 6.66 mmol), stirred with refluxing for 20 min, quenched by the addition of 2 M aqueous HCl/acetic acid (1:1, 200 mL), collected by vacuum filtration, washed with 2 M aqueous HCl (200 mL) and distilled water, dried in air at 110 °C for 2 d, purified by column separation (silica gel, chloroform for removing the starting material and chloroform/acetic acid 20:1 for collecting the product), evaporated *in vacuo*, dissolved in a small amount of chloroform, precipitated with methanol and collected by vacuum filtration. Yield 322 mg (64%) dark blue solid, mp > 300 °C. *R<sub>f</sub>*-value (CHCl<sub>3</sub>/methanol 60:1): 0.4. IR (ATR):  $\tilde{\nu}$  = 2920 (s), 2851 (m), 1762 (s), 1726 (m), 1694 (s), 1651 (s), 1582 (s), 1562 (s), 1504 (m), 1454 (m), 1396 (w), 1376 (m), 1351 (s), 1297 (s), 1262 (m), 1209 (m), 1162 (w), 1144 (w), 1127 (m), 1028 (w), 1008 (m), 844 (w), 807 (m),

790 (w), 750 (w), 738 (w), 692 (w) 673 cm<sup>-1</sup>(w). <sup>1</sup>H NMR (400 MHz, C<sub>2</sub>D<sub>2</sub>Cl<sub>4</sub>, 120 °C):  $\delta$  = 8.74–8.59 (m, 12 H, CH<sub>arom. terrylene</sub>), 5.26–5.19 (m, 1 H, CH), 2.34–2.25 (m, 2 H), 2.01–1.90 (m, 2 H), 1.46–1.25 (m, 28 H), 0.90 ppm (t, <sup>3</sup>*J*<sub>(H–H)</sub> = 6.7 Hz, 6 H, CH<sub>3</sub>). UV/VIS (CHCl<sub>3</sub>):  $\lambda_{\text{max}}$  (*E*<sub>rel</sub>) = 599.0 (0.52), 651.6 nm (1.00). Fluorescence (CHCl<sub>3</sub>):  $\lambda_{\text{max}}$  (*I*) = 672.4 (1.00), 733.9 (0.58). Fluorescence quantum yield (CHCl<sub>3</sub>,  $\lambda_{\text{exc}}$  = 599 nm, *E*<sub>599 nm, 1 cm</sub> = 0.020(0), reference **3** with  $\Phi$  = 0.94): 0.94. MS (DEP/EI) *m/z* (%): 781 (47) [M<sup>+</sup>], 515 (100) [M<sup>+</sup> – C<sub>19</sub>H<sub>38</sub>]. HRMS (C<sub>53</sub>H<sub>51</sub>NO<sub>5</sub>): calcd 781.3767; found 781.3755,  $\Delta$  = –0.0012.

#### 2-{4-[2-(1-Hexylheptyl)-9-ylanthra[2,1,9-*def*:6,5,10-*d'e'f'*]-diisoquinoline-1,3,8,10(2*H*,9*H*)-tetraone]-2,3,5,6-tetramethylphenyl}-11-(1-nonyldecyl)benzo[13,14]pentapheno[3,4,5-*def*:10,9,8-*d'e'f'*]diisoquinoline-1,3,10,12(2*H*,11*H*)-tetraone (**6**)

2-(4-Amino-2,3,5,6-tetramethylphenyl)-9-(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d'e'f'*]diisoquinoline-1,3,8,10-tetraone (**5**, 60 mg, 83 μmol), 11-(1-nonyldecyl)-1*H*-benzo[13,14]isochromeno[6',5',4':8,9,10]pentapheno[3,4,5-*def*]isoquinoline-1,3,10,12(11*H*)-tetraone (**4**, 43 mg, 55 μmol), zinc acetate (2 mg, 11 μmol) and quinoline (1 mL) were stirred under argon at 200 °C for 8 h (dark violet mixture), allowed to cool, while still warm the mixture was added dropwise with strong stirring to 2 M aqueous HCl (300 mL), stirred for 2 h, allowed to stand for 16 h, collected by vacuum filtration, washed with 2 M aqueous HCl (300 mL), hot distilled water and a methanol/water 1:1 (300 mL), dried *in vacuo* at 110 °C for 2 d, dissolved in the minimal amount of chloroform/methanol 80:1 and purified by column separation (short column of basic alumina, chloroform/methanol 80:1 and then silica gel, chloroform/methanol 60:1, blue violet, fluorescent band). Yield 6 mg (7%), mp > 300 °C. *R<sub>f</sub>*-value (silica gel, CHCl<sub>3</sub>/methanol 20:1): 0.8. IR (ATR):  $\tilde{\nu}$  = 2955 (m) 2924 (s), 2853 (m), 1697 (s), 1659 (s), 1586 (s), 1522 (w), 1507 (w), 1458 (w), 1405 (w), 1378 (m), 1354 (s), 1332 (m), 1304 (w), 1255 (m), 1204 (w), 1177 (w), 1104 (m), 1018 (m), 964 (w), 843 (m), 811 (m), 748 (m), 668 (w), 646 cm<sup>-1</sup> (w). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>, 27.0 °C, TMS):  $\delta$  = 8.82–8.56 (m, 20 H, CH<sub>arom. perylene</sub>), 5.24–5.18 (m, 2 H, CH), 2.31–2.23 (m, 4 H), 2.17 (s, 6 H, 2 × CH<sub>3</sub>), 2.16 (s, 6 H, 2 × CH<sub>3</sub>), 1.92–1.84 (m, 4 H), 1.40–1.17 (m, 44 H), 0.83 ppm (tt, <sup>3</sup>*J*<sub>(H–H)</sub> = 7.1 Hz, <sup>3</sup>*J*<sub>(H–H)</sub> = 7.1 Hz, 12 H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>, 27.0 °C):  $\delta$  = 163.1, 162.8, 135.3, 132.8, 132.7, 132.1, 123.4, 123.2, 121.7, 121.6, 118.9, 54.6, 32.4, 31.9, 31.8, 29.7, 29.6, 29.3, 29.2, 27.0, 26.9, 22.6, 22.6, 15.3, 14.1 ppm. UV/VIS (CHCl<sub>3</sub>):  $\lambda_{\text{max}}$  ( $\epsilon$ ) = 459.8 (14 900), 490.6 (42 000), 526.7 (71 700), 600.4 (63 700), 653.8 nm (124 400). Fluorescence (CHCl<sub>3</sub>):  $\lambda_{\text{max}}$  (*I*) = 670.4 (1.00), 733.5 nm (0.49). Fluorescence quantum yield (CHCl<sub>3</sub>,  $\lambda_{\text{exc}}$  = 490 nm,  $c$  = 6.99 × 10<sup>-7</sup> mol L<sup>-1</sup>, reference 2,9-bis-(1-hexylheptyl)anthra[2,1,9-*def*:6,5,10-*d'e'f'*]diisoquinoline-1,3,8,10(2*H*,9*H*)-tetraone, RN 110590-84-6, with  $\Phi$  = 1.00): 0.86 ± 0.03. MS (MALDI, matrix anthracene) *m/z*: 1483 [M<sup>+</sup>]. MS (FAB+) *m/z* (%): 1483 (20) [M<sup>+</sup>], 1302 (5) [M<sup>+</sup> + 1 – C<sub>13</sub>H<sub>26</sub>], 1217 (6), [M<sup>+</sup> + 1 – C<sub>19</sub>H<sub>38</sub>], 515 (45) [M<sup>+</sup> + 1 – C<sub>13</sub>H<sub>26</sub> – C<sub>19</sub>H<sub>38</sub> – C<sub>34</sub>H<sub>21</sub>N<sub>2</sub>O<sub>4</sub>]. HRMS (C<sub>100</sub>H<sub>98</sub>N<sub>4</sub>O<sub>8</sub>): calcd 1483.7418, found 1483.7419,  $\Delta$  = 0.0001.

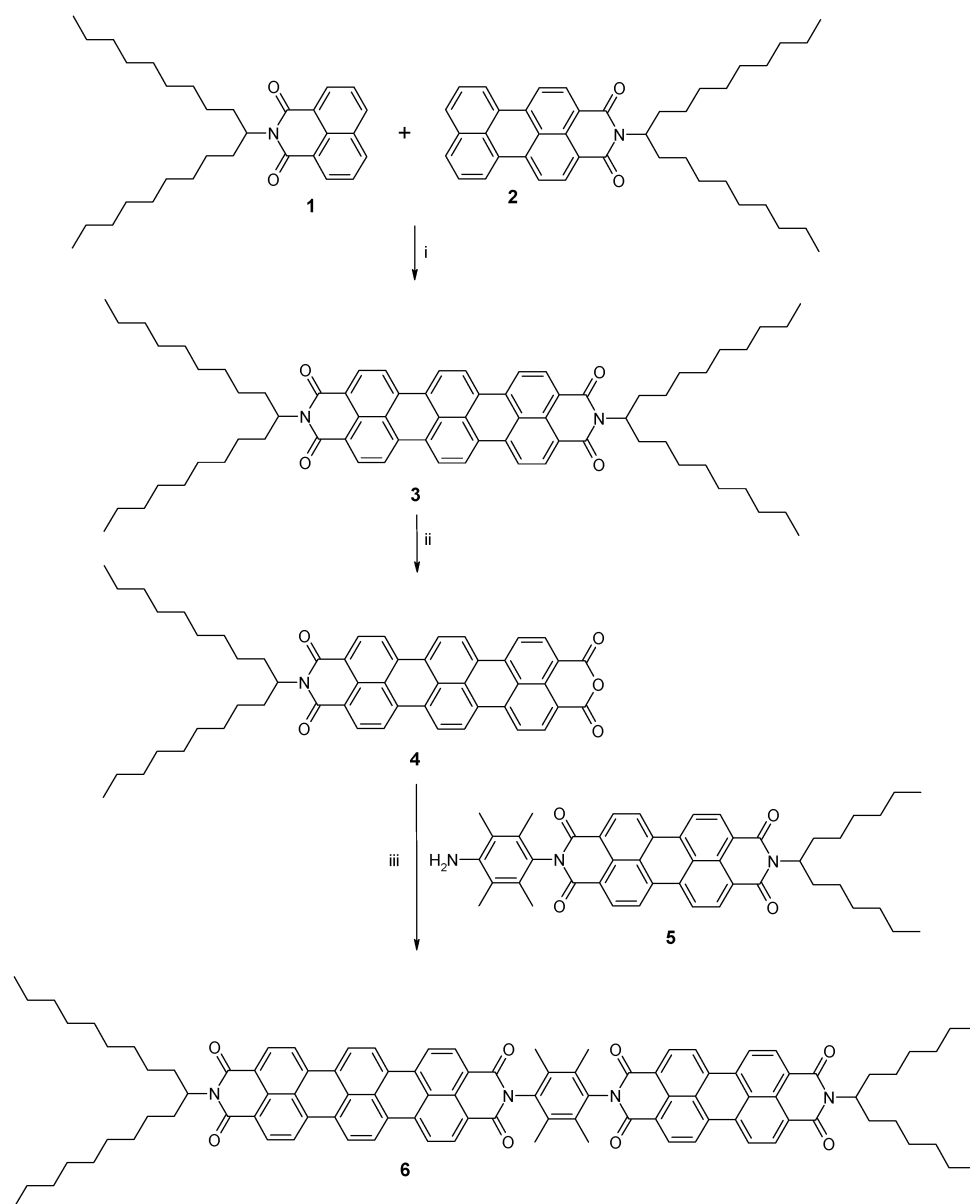
The absorption spectra were recorded on a Varian Cary, 5000 UV-VIS-NIR spectrometer. The fluorescence spectra

were transcribed by means of a Fluorolog<sup>®</sup>-3 spectrometer (Jobin Yvon) equipped with Glan-Thompson polarisers. The bandwidth of the excitation and emission light was 2 and 2 nm, respectively. All fluorescence spectra were corrected.

The time-resolved fluorescence decays were measured by means of the time-correlated single photon-counting technique<sup>5</sup> by using a Fluorolog<sup>®</sup>TCSPC (Horiba) spectrometer. The fluorescence decays were collected over 1024 channels, with a resolution of 50 ps per ch., with at least 20 000 photons in the peak maximum for the lifetime experiments, which were performed with the emission polariser set to a magic angle (54.7°) with respect to the excitation polariser. For the pulsed excitation, a PicoBrite laser (482 nm) in combination with an interference filter centred at 480 nm, and a NanoLED (635 nm) light source were used. The former excitation setup was

combined with longpass filters  $\lambda > 610$  nm and  $\lambda > 673$  nm. The fluorescence lifetime was calculated by means of a deconvolution method,<sup>6</sup> which is based on the Levenberg–Marquardt algorithm.<sup>7</sup>

The fluorescence quantum yields were calculated by means of comparison with a reference substance. Three different references were employed: C25 [2,10-bis(1-hexylheptyl)-6-[2-[3,8,9,10-tetrahydro-9-(1-octylonyl)-1,3,8,10-tetraoxanthra[2,1,9-*def*:6,5,10-*d'e'f'*]diisoquinolin-2(1*H*)-yl]ethyl]-1*H*-pyrrolo[3',4':4,5]pyreno[2,1,10-*def*:7,8,9-*d'e'f'*]diisoquinoline-1,3,5,7,9,11(2*H*,6*H*,10*H*)-hexone; RN 335458-21-4] dissolved in chloroform for excitation below 500 nm, rhodamine 101 dissolved in ethanol for excitation at 560 nm, and cresyl violet dissolved in ethanol for excitation at 600 nm. The fluorescence quantum yields for these reference substances were assumed to be 1.0, 1.0 and 0.88 respectively.



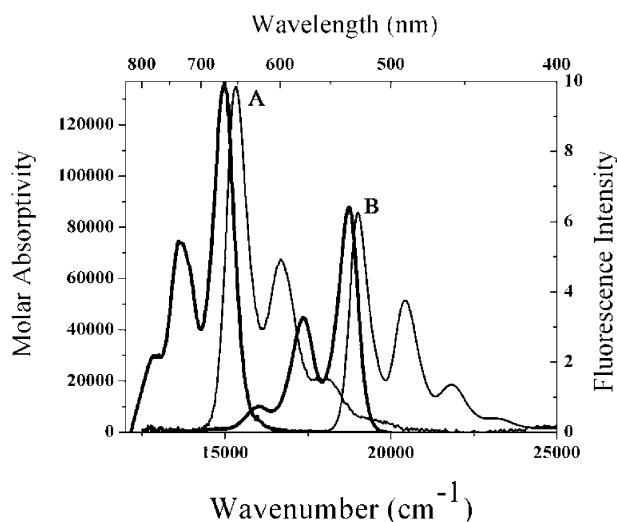
**Scheme 1** Synthesis of the terrylenetetracarboxylic bisimide **3** and the dyad **6**. (i)  $t\text{-C}_4\text{H}_9\text{OK}$ , DBU. (ii) 1. KOH,  $t\text{-C}_4\text{H}_9\text{OK}$ , 2. HCl. (iii) Zinc acetate, quinoline.

## Results and discussion

Terylenetetracarboxylic bisimides<sup>8</sup> are interesting basic chromophores for the development of fluorescence standards due to their high quantum yields, chemical, as well as photochemical stability. The obtained fluorescence quantum yields proved to be invariant to the presence of oxygen, which facilitates a simple handling. However, the inherently low solubility of these compounds is problematic for applications, which can however be solved by the attachment of long-chain *sec*-alkyl groups (swallow-tail substituents)<sup>9</sup> to the nitrogen atoms of the terylene derivative and to the target compound **3** (Scheme 1).

Compound **3** was prepared according to Sakamoto's method<sup>10</sup> from the *N*-1-nonyldecylnaphthalene-1,8-dicarboxylic imide and *N*-1-nonyldecylperylene-3,4-dicarboxylic imide<sup>11</sup> with the application of potassium-*tert*-butoxide and DBN, where the degassed solvent toluene gave an appreciable improvement compared to the literature.<sup>12</sup> Atmospheric oxygen must be carefully excluded even in trace amounts where it would otherwise interfere with the reaction as is indicated by a colour change of the reaction mixture to brownish and a strong lowering of the yield.

The UV/Vis spectra of substance **3** are bathochromically shifted as compared to the perylene bisimide as a consequence of the *peri*-anellation (*cf.* Fig. 1). The peak maximum molar absorptivity of substance **3** is increased by 55%, as compared to the perylene bisimide. The fluorescence spectrum is close to a mirror-image of the absorption spectrum and extends into the bathochromic limit of routine fluorescence spectrometers, so that the spectral sensitivity of such spectrometers can be calibrated by means of this compound. The fluorescence quantum yield in lipophilic solvents, such as chloroform, was found to be 94% and remains virtually unaffected by atmospheric oxygen. The fluorescence lifetime of this compound dissolved in degassed chloroform was found to be 3.1 ns, as

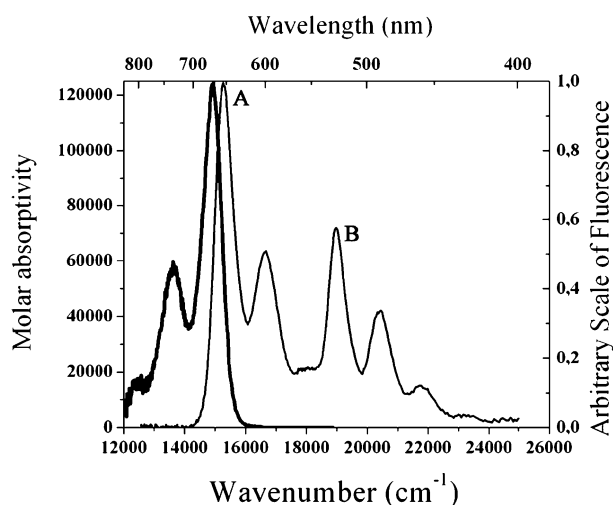


**Fig. 1** UV/Vis absorption (thin line, left) and fluorescence spectra (thick line, left) of compound **3**, as compared to the spectra of the perylene dye *N,N'*-bis(1-hexylheptyl)perylene-3,4:9,10-tetracarboxylic bisimide (thick and thin line, right). The molar absorptivity ( $\epsilon$ ) maxima of compound **3** and the latter compound are  $13\,670\text{ mol}^{-1}\text{ dm}^3\text{ cm}^{-1}$  at 652 nm (point A) and  $88\,000\text{ mol}^{-1}\text{ dm}^3\text{ cm}^{-1}$  at 526 nm (point B), respectively.

compared to 3.0 ns before the removal of oxygen. The chemical stability and the fastness to light of compound **3** are high enough for negligible degradation by extensive exposure to sunlight. Dye **3** exhibits many certifying properties for its application as a bathochromic fluorescence standard. However, the molar absorptivity of compound **3** is very low in the hypsochromic region, whereby the fluorescence excitation becomes problematic. As a consequence, the filling of this spectral gap would be beneficial. We aimed at creating a compound with a wide range of absorption by combining chromophores into dyads, according to ref. 4a and 13.

The synthesis started with compound **3** and applying the well established strongly hydrolysing mixture of KOH in *tert*-butyl alcohol<sup>14</sup> resulted in a stepwise degradation of the carboxylic imide groups. The primary product of the hydrolysis proved to be insoluble and thus became excluded from further reaction. This is advantageous since it is not necessary to consider a further progression of hydrolysis of the second carboxylic imide group. This also allows for high yields of the conversion. The carboxylic anhydride group in compound **4** was condensed with the  $\text{NH}_2$  group in compound **5**, when dissolved in quinoline at high temperature. This solvent proved to be a much better medium for this reaction, as compared to the alternatively applied imidazole,<sup>15</sup> which is useful in similar perylene chemistry. This may be a consequence of condensed aromatics being better solvents for terylene compounds.

The UV/Vis absorption spectrum of compound **6** exhibits an appreciable number of absorption maxima, due to the essential superposition of the absorbance of two linked chromophores: perylenetetracarboxylic bisimide and terylenetetracarboxylic bisimide (*cf.* Fig. 1 and 2). A high fluorescence quantum yield of  $0.86 \pm 0.03$  is observed, when the terylene chromophore dyad is excited at 480, 560 and 600 nm. Interference by aggregation can be excluded because a dilution of the solution from  $2.28 \times 10^{-7}$  to  $1.14 \times 10^{-7}\text{ mol L}^{-1}$  did not



**Fig. 2** UV/Vis absorption, thin line (right, right scale) and fluorescence spectrum, thick line (left, left scale) of compound **6** (internal name: Wan99) dissolved in chloroform. The two strongest molar absorptivity maxima are  $124\,400\text{ mol}^{-1}\text{ dm}^3\text{ cm}^{-1}$  at 654 nm (point A) and  $71\,540\text{ mol}^{-1}\text{ dm}^3\text{ cm}^{-1}$  at 527 nm (point B).

influence fluorescence quantum yields outside experimental error (+0.8% for irradiation at 600 nm and +2% at 490 nm). The two latter excitation wavelengths 560 and 600 nm refer to the absorption and fluorescence of compound **3**. On the other hand, the fluorescence of the perylene unit in chromophore **6** is completely quenched through intra molecular electronic energy transfer to the terylene unit from which all fluorescence is observed. The fluorescence lifetime of the dyad is 2.8 ns when excited throughout the absorption bands. As a consequence, there are many bands below 655 nm that are useful for excitation and therefore compound **6** becomes applicable for the calibration of fluorescence spectrometers, as well as a fluorescence reference standard.

## Conclusions

The readily soluble dyad composed of perylene and terylene tetracarboxylic bisimide (*i.e.* dyad number **6**) exhibits an absorption spectrum covering the visible region below 605 nm, which is essentially a superposition of the absorbance of each aromatic bisimide. This compound exhibits an efficient and fast intramolecular electronic energy transfer from the perylene to the terylene unit. A high fluorescence quantum yield is observed for all absorption bands. This dyad is recommended as a fluorescence standard in lipophilic media for the calibration of fluorescence spectrometers in the bathochromic region.

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