New reactive fluorophores in the 1,2,3-triazine series

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Abstract—A one-pot synthesis of new fluorescent 2,5-dihydro-1,2,3-triazines with reactive functional groups and a large Stokes shift of 200 nm is described.

Fluorescent molecules with reactive functional groups are of particular interest because of their potential for use as real-time sensors in biomolecular systems.1–3 Among the triazine series the 1,2,3-triazine system is the least studied in comparison with the 1,2,4- and 1,3,5-triazine structures because the ring system is the least stable of the three and synthetic routes are limited.4 Herein, we describe the first fluorophores of the 1,2,3-triazine series from an experimentally simple one-pot reaction developed from our work on azolium ylide 1,3-dipole systems.5

Solutions of the 1,2,3-triazolium-1-aminide 1,3-dipoles 1 and alkylpropiolates in dry acetone when heated under reflux for 24 h produced the new fluorescent 1,2,3-triazine derivatives 2 and 3 along with lesser yields of the fused pyrrolo[2,3-d]-1,2,3-triazolines 4 and 5 (Table 1).6 When the reaction was carried out in undried acetone, small quantities of the products 6 and 7, which were also fluorescent, were encountered from hydrolytic degradation of the imine function in 2 and 3 to

Table 1. 2,5-Dihydro-1,2,3-triazines and pyrrolo-triazoline products

<table>
<thead>
<tr>
<th>Compd</th>
<th>Yield (%)</th>
<th>Mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>52</td>
<td>122–123</td>
</tr>
<tr>
<td>2b</td>
<td>35</td>
<td>191–194</td>
</tr>
<tr>
<td>2c</td>
<td>45</td>
<td>179–180</td>
</tr>
<tr>
<td>3a</td>
<td>38</td>
<td>146–147</td>
</tr>
<tr>
<td>3b</td>
<td>26</td>
<td>163–164</td>
</tr>
<tr>
<td>3c</td>
<td>33</td>
<td>158–159</td>
</tr>
<tr>
<td>4a</td>
<td>8</td>
<td>186–187</td>
</tr>
<tr>
<td>4b</td>
<td>23</td>
<td>157–158</td>
</tr>
<tr>
<td>4c</td>
<td>27</td>
<td>174–175</td>
</tr>
<tr>
<td>5a</td>
<td>13</td>
<td>164–166</td>
</tr>
<tr>
<td>5b</td>
<td>35</td>
<td>169–170</td>
</tr>
<tr>
<td>5c</td>
<td>25</td>
<td>163–164</td>
</tr>
<tr>
<td>5d</td>
<td>22</td>
<td>142–143</td>
</tr>
<tr>
<td>6</td>
<td>92</td>
<td>193–196</td>
</tr>
</tbody>
</table>

Table 2. Fluorescent properties of substituted 2,5-dihydro-1,2,3-triazines

<table>
<thead>
<tr>
<th>Compd</th>
<th>λabs max a</th>
<th>λabs max a</th>
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<tbody>
<tr>
<td></td>
<td>Band 1 (nm)</td>
<td>Band 2 (nm)</td>
<td>(est)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>3a</td>
<td>307</td>
<td>392</td>
<td>1.5</td>
<td>19,914</td>
<td>483.6</td>
<td>47.5</td>
<td>528.7</td>
<td>75.1</td>
</tr>
<tr>
<td>3b</td>
<td>310</td>
<td>391</td>
<td>3.1</td>
<td>25,971</td>
<td>482.6</td>
<td>48.6</td>
<td>528.1</td>
<td>74.1</td>
</tr>
<tr>
<td>3c</td>
<td>309</td>
<td>391</td>
<td>1.7</td>
<td>26,540</td>
<td>482.8</td>
<td>48.3</td>
<td>528.1</td>
<td>75.8</td>
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<tr>
<td>7</td>
<td>322</td>
<td>377</td>
<td>1.1</td>
<td>19,192</td>
<td>478.9</td>
<td>42.3</td>
<td>516.7</td>
<td>57.8</td>
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a formyl group, which was oxidised in situ and decarboxylated. The products 6 and 7 could be readily obtained in over 90% yield by separately heating solutions of 2 and 3 in

\[ \text{Stokes Shift} \]

\[ \lambda_{\text{ex}} \]

\[ \lambda_{\text{em}} \]

\[ \text{Wavelength (nm)} \]

\[ \text{Intensity} \]

\[ \text{Stokes Shift} \]

\[ 200 \text{ nm} \]

\[ \lambda_{\text{ex}} \]

\[ \lambda_{\text{em}} \]

\[ \text{Wavelength (nm)} \]

\[ \text{Intensity} \]

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\[ \lambda_{\text{ex}} \]

\[ \lambda_{\text{em}} \]

\[ \text{Wavelength (nm)} \]

\[ \text{Intensity} \]

\[ 200 \text{ nm} \]

\[ \lambda_{\text{ex}} \]

\[ \lambda_{\text{em}} \]
1:1 (v/v) aqueous ethanol. The structures of the products 2, 3, 6 and 7 are new fluorophores, which display a bright green fluorescence (Table 2), and contain reactive functional groups (ester and imine) with potential for binding as biomarkers. The UV absorption of these structures showed a dual absorption at ca. 310 nm with a shoulder at ca. 390 nm. The fluorescence emission (for excitation at 317 nm) displayed a dual band at ca. 480 and 528 nm. Hence, the complex system comprises at least two ground states and two excited states with the 310 nm absorption correlating with the 480 nm emission and the 520 nm emission and thereby providing a convenient one-pot synthesis of these triazines. We envisage that the ring-expansion involves an intermediate of type 8, which can arise from the 1,3-dipoles 1 and 2 undergoing a 1,4-N cleavage of the C(3a)–N(4) bond in 4.

Acknowledgements

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References and notes

6. A suspension of 1,2-bis(phenyl)hydrazone of benzil (1 g, 2.56 mmol) in dichloromethane (25 ml) was treated with lead dioxide (0.73 g, 3.07 mmol) and stirred for 18 h at ambient temperature. Insoluble salts were removed, and the residue (in 2 cm3 methylene chloride) was placed on a silica gel column (230–400 mesh ASTM). The column was eluted with a gradient mixture (1:0 to 0:1) (v/v) of petroleum spirit (bp 40–60 °C) and changed to 1:1 (v/v) methylene chloride using a 2.5% (v/v) changing gradient to give product 3a as a yellow solid (38%), mp 146–147 °C (from ethanol); δc (400 MHz, CDCl3) 3.54 (s, 3H, OCH3), 7.11–7.42 (m, 2H, 2-N-phenylring, Hortho), 7.76–7.92 (m, 6H, aromatic), 8.47 (s, 1H, N). Anal. Calcd for C30H24N4O2: C, 76.3; H, 5.1; N, 11.9. Found: C, 76.3; H, 4.8; N, 11.6.

7. A second product (5a) was isolated as a yellow solid (13%), mp 164–166 °C (from ethanol); δ1H NMR (400 MHz, CDCl3) 3.6 (s, 3H, CO2CH3), 6.89–7.0 (m, 10H, aromatic), 7.80 (d, 1.0) ppm, 2H, 2-N-phenylring, Hmeta. Anal. Calcd for C30H24N4O2: C, 76.3; H, 5.1; N, 11.9. Found: C, 76.3; H, 4.8; N, 11.6.
7.06–7.09 (m, 4H, aromatic), 7.25–7.26 (m, 4H, aromatic), 7.66–7.68 (m, 2H, 2-N-phenylring, H ortho), 8.40 (s, 1H, 5-CH, α-enamine). δC NMR (400 MHz, CDCl3): 51.2 (OCH3), 92.9 (C-6a), 105.8 (C-3a), 107.4 (C-6), 135.9 (C-10, 6a-Ph), 137.6 (C-1′, 3a-Ph), 140.6, 127.0, 123.2, 129.1 (C-1′, C-2′, C-3′, C-4′, respectively, 2-N-phenyl ring), 139.5, 118.2, 122.9, 128.5 (C-1′, C-2′, C-3′, C-4′, respectively, 4-N-phenyl ring), 132.0, 129.2, 127.7, 127.6 (remaining aromatics), 139.5, 118.2, 122.9, 128.5 (C-1′, C-2′, C-3′, C-4′, respectively, 4-N-phenyl ring), 141.9 (C-5), 165.5 (C=O). Anal. Calcd for C30H24N4O2: C, 76.3; H, 5.1; N, 11.9. Found: C, 76.1; H, 4.88; N, 11.8.

A solution of 5-methoxycarbonyl-5-(H-N-phenyl(formimidoyl)-2,4,6-triphenyl-2,5-dihydro-1,2,3-triazine 3a (0.15 g, 0.32 mmol) in a (1:1 v/v) mixture of aqueous ethanol (40 cm3) was stirred under reflux for 7 days. The product was extracted into dichloromethane (4·10 cm3) and dried over MgSO4. The solvent was evaporated under reduced pressure and the product 7 was crystallized from (2:1 v/v) methylene chloride/hexane (92%) ethanol (40 cm3) was crystallized from (2:1 v/v) methylene chloride/hexane (92%), mp 193–196 °C (from methylene chloride/hexane 2:1 v/v). δC NMR (400 MHz, CDCl3) 38.4 (C-5), 53.2 (–OCH3), 134.7 (C-6), 134.8 (C-4), 145.7, 116.2, 128.9, 123.5 (C-1′, C-2′, C-3′, C-4′, respectively, 2-N-phenylring), 126.6, 128.7, 129.8, 133.3 (remaining overlapping aromatics), 167.8 (C=O). Anal. Calcd for C23H19N3O2: C, 76.3; H, 5.1; N, 11.9. Found: C, 76.3; H, 5.1; N, 11.2.

7. Crystal structure determination for structure 3a. Crystal data C30H24N4O2, M = 472.53, Monocyclic, a = 10.292(19), b = 16.430(4), c = 14.593(3) Å, U = 2466.9(8) Å3, T = 298(2) K, space group P21/n, Z = 4, μ(Mo-Kα) = 0.082 mm–1, 10,421 reflections collected, 2665 unique (Rint = 0.0603), which were used in all calculations. The final wR(F2) was 0.1251 (all data). Crystallographic data (excluding structure factors) for the structures in this letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 286736. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

8. Crystal structure determination for structure 7. Crystal data C23H19N3O2, M = 369.41, orthorhombic, a = 9.9253(13), b = 10.973(7), c = 17.458(4) Å, U = 1901.4(14) Å3, T = 292(2) K, space group P212121, Z = 4, μ(Mo-Kα) = 0.084 mm–1, 7353 reflections measured, 1886 unique (Rint = 0.0796), which were used in all calculations. The final wR(F2) was 0.1406 (all data). Crystallographic data (excluding structure factors) for the structures in this letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 286737. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].