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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

Compd	Yield (%)	Mp (°C)
2a	52	122–123
2b	35	193–194
2c	45	179–180
3a	38	146–147
3b	26	163–164
3c	33	158–159
4a	8	186–187
4b	23	157–158
4c	27	174–175
5a	13	164–166
5b	35	169–170
5c	25	163–164
6	72	142–143
7	92	193–196

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

Compd	λ_{abs} max[a] Band 1 (nm)	λ_{abs} max[a] Band 2 (nm)	Area ratio[b] (est)	ε_{abs} max (M^{-1} cm^{-1})	λ_{em} max[c] Band 1 (nm)	FWHM[d]	λ_{em} max[c] Band 2 (nm)	FWHM[d]
3a	307	392	1.5	19,914	483.6	47.5	528.7	75.1
3b	310	391	3.1	25,971	482.6	48.6	528.1	74.1
3c	309	391	1.7	26,540	482.8	48.3	528.1	75.8
7	322	377	1.1	19,192	478.9	42.3	516.7	57.8

[a] Steady state excitation data recorded at concentration 10^{-6} M in MeOH.
[b] Estimated approximate area ratios band 1:band 2 from best-fit Gaussian functions at the offset baseline.
[c] Steady state emission data recorded with 317 nm excitation at concentration 10^{-6} M in MeOH.
[d] FWHM, full width half maximum.

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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

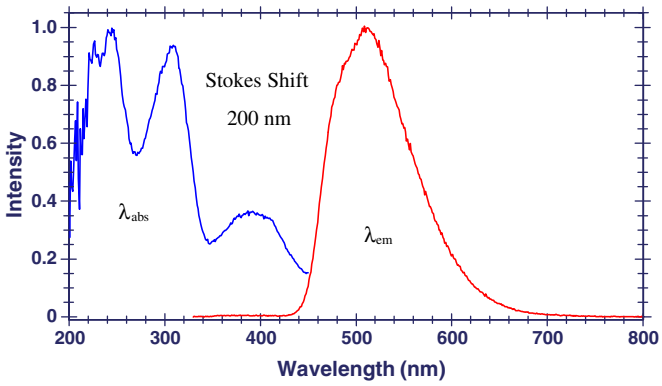
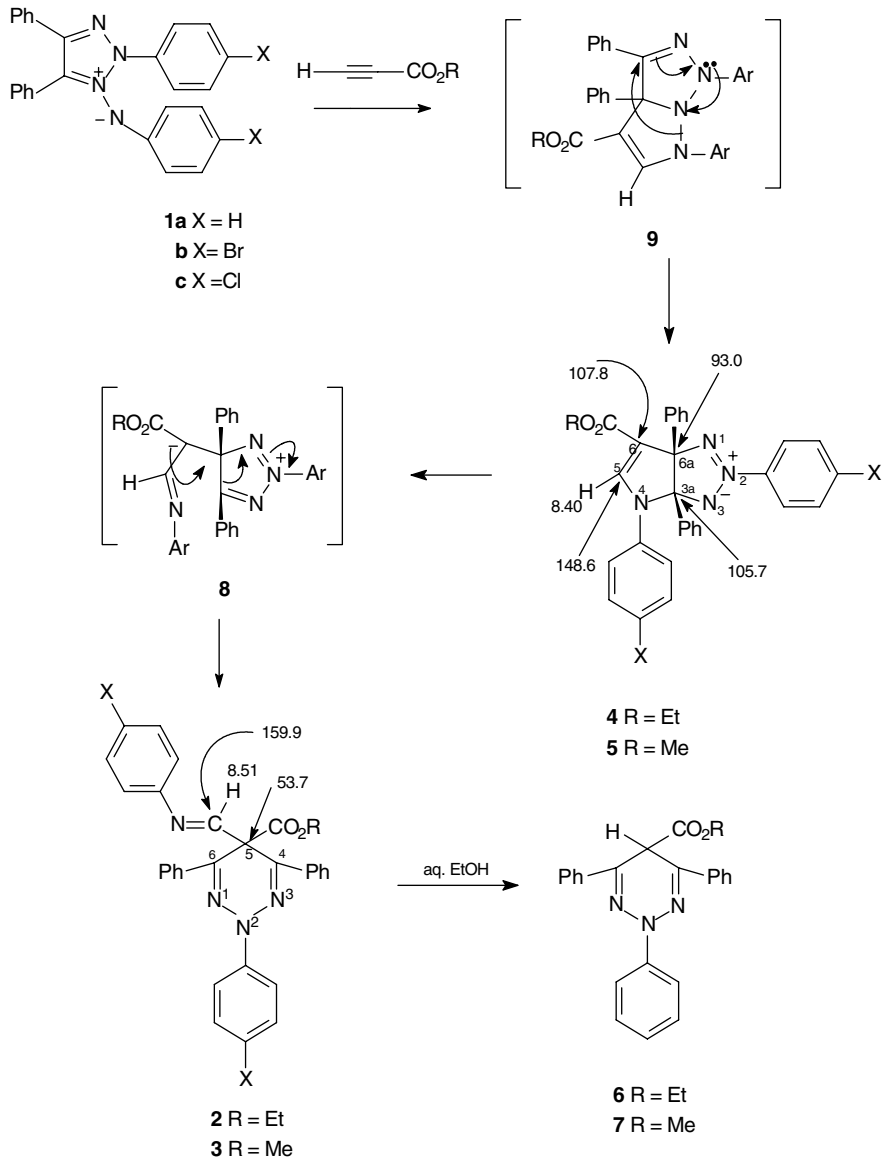


Figure 1. Excitation spectrum (emission set at 500 nm) and fluorescence emission spectrum (excitation set at 317 nm) for compound **3a**.



Scheme 1. ^{1}H and ^{13}C NMR shifts in $CDCl_3$ for **2a** and **4a**.

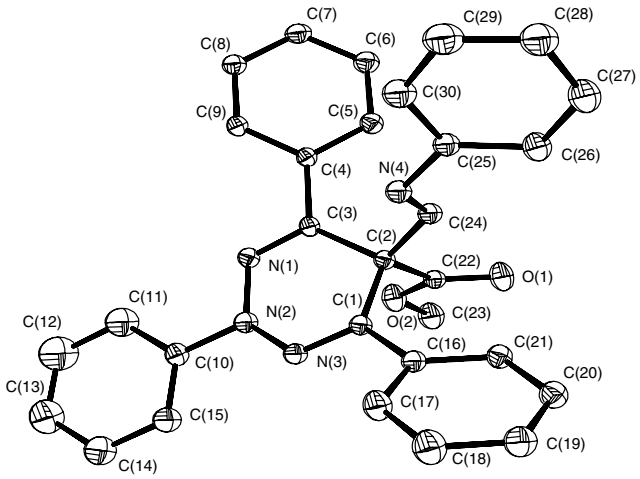


Figure 2. X-ray crystal structure of **3a**.

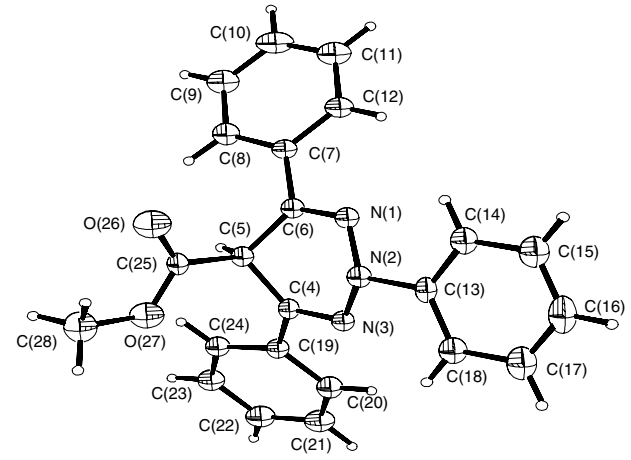


Figure 3. X-ray crystal structure of **7**.

1:1 (v/v) aqueous ethanol.[6] The structures **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 520 nm emission and the 390 nm absorption correlating with the 480 nm emission. The very significant Stokes shift of ca. 200 nm (Fig. 1) shows the potential of these molecules for biological fluorescent labelling experiments.

The structures of the products **2**–**7** were established from microanalyses, IR, proton and ^{13}C NMR spectra.[6] Some representative NMR shifts are shown in Scheme 1. X-ray crystal structure determinations[7,8] on compounds **3a** and **7a** supported the structures (Figs. 2 and 3).

The products **4** and **5** arise from the 1,3-dipoles **1** by Huisgen cycloaddition[5] to give the unstable cycloadduct **9**, which undergoes a 1,4-N→C rearrangement[9] (Scheme 1). These are normally stable high melting solids[5] when there is a substitutent at C-5 but in the present first cases with a H-atom at C-5 they proved to be highly labile and ring expanded in situ into the fluorescent triazines **2** and **3** thereby providing a convenient one-pot synthesis of these triazines.[7] We envisage that the ring-expansion involves an intermediate of type **8**, which can arise from a cleavage of the C(3a)–N(4) bond in **4**.

Acknowledgements

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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 washed thoroughly with dichloromethane. Evaporation of the combined mother liquor and washings gave **1a** (80%), mp 178–179 °C (from toluene–petroleum spirit bp 60–80 °C). A suspension of 2,4,5-triphenyl-1,2,3-triazolium-1-phenyl aminide **1a** (0.3 g, 0.77 mmol) in dry acetone (10 cm^3) was treated with an excess of methyl propiolate (0.14 cm^3, 1.57 mmol). The reaction mixture was stirred under reflux for 24 h after which time the solvent was removed under reduced pressure. The residue (in 2 cm^3 of 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)/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); δ_H (400 MHz, $CDCl_3$) 3.54 (s, 3H, OCH_3), 6.77–6.79 (m, 2H, 2-*N*-phenylring, H_{ortho}), 7.11–7.42 (m, 12H, aromatic), 7.76–7.92 (m, 6H, aromatic), 8.47 (s, 1H, —N=CH); δ_C (400 MHz, $CDCl_3$) 53.6 (–OCH_3), 53.7 (C-5), 134.9 (C-6), 135.3 (C-4), 150.0, 120.3, 129.5, 126.3 (C-1′, C-2′, C-3′, C-4′, respectively, iminyl-*N*-phenylring), 145.4, 116.1, 128.9, 123.6 (C-1′, C-2′, C-3′, C-4′, respectively, 2-*N*-phenyl ring), 160.5 (—N=CH), 170.2 (C=O). Anal. Calcd for $C_{30}H_{24}N_4O_2$: C, 76.3; H, 5.1; N, 11.9. Found: C, 76.3; H, 4.8; N, 11.6.
A second product (**5a**) was isolated as a yellow solid (13%), mp 164–166 °C (from ethanol); δ_H NMR (400 MHz, $CDCl_3$) 3.6 (s, 3H, CO_2CH_3), 6.89–7.0 (m, 10H, aromatic),

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). δ_C NMR (400 MHz, $CDCl_3$): 51.2 (OCH_3), 92.9 (C-6a), 105.8 (C-3a), 107.4 (C-6), 135.9 (C-1′, 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), 149.1 (C-5), 165.5 (C=O). Anal. Calcd for $C_{30}H_{24}N_4O_2$: 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*-phenylformimidoyl)-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 cm^3) was stirred under reflux for 7 days. The product was extracted into dichloromethane (4 × 10 cm^3) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the product **7** was crystallized from (2:1 v/v) methylene chloride/hexane (92%) ethanol (40 cm^3) was crystallised from (2:1 v/v) methylene chloride/hexane (92%), mp 193–196 °C (from methylene chloride/hexane 2:1 v/v). δ_C NMR (400 MHz, $CDCl_3$) 38.4 (C-5), 53.2 ($-OCH_3$), 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 $C_{23}H_{19}N_3O_2$: C, 74.8; H, 5.2; N, 11.4. Found: C, 74.3; H, 5.1; N, 11.2.

7. Crystal structure determination for structure **3a**. Crystal data $C_{30}H_{24}N_4O_2$, $M = 472.53$, Monocyclic, $a = 10.2928(19)$, $b = 16.430(4)$, $c = 14.593(3)$ Å, $U = 2466.9(8)$ $Å^3$, $T = 298(2)$ K, space group $P21/n$, $Z = 4$, μ(Mo-Kα) = 0.082 mm^{-1}, 10,421 reflections collected, 2665 unique ($R_{int} = 0.0603$), which were used in all calculations. The final $wR(F_2)$ 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 $C_{23}H_{19}N_3O_2$, $M = 369.41$, orthorhombic, $a = 9.9253(13)$, $b = 10.973(7)$, $c = 17.458(4)$ Å, $U = 1901.4(14)$ $Å^3$, $T = 292(2)$ K, space group $P212121$, $Z = 4$, μ(Mo-Kα) = 0.084 mm^{-1}, 7353 reflections measured, 1886 unique ($R_{int} = 0.0796$), which were used in all calculations. The final $wR(F_2)$ 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].
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