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Synthesis of novel spirolactams by reaction of fluorescein methyl ester with amines

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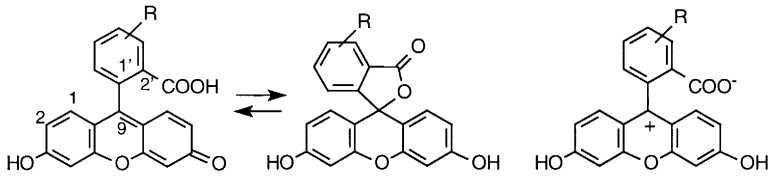
Abstract

A family of previously undescribed fluorescein spirolactams was synthesized by reaction of fluorescein methyl ester with a variety of primary amines. The structure of the non-fluorescent spirolactams was elucidated by NMR spectroscopy and X-ray crystallography.

Since their synthesis in the late 1800s, various fluorescent dyes have been utilized in many biotechnological applications.[1] Of these dyes, the fluoresceins are the most commonly used labels due to their solubility in aqueous buffers and high fluorescence quantum yield at physiological pH.[2] Traditionally, fluoresceins have been viewed to exist in two forms: a highly fluorescent, open, quinone form and a non-fluorescent, closed, spirolactone form (see below),[3] although more recently a third zwitterionic structure has been postulated to exist in aqueous solution.[4,5]



open form closed form zwitterionic form

A widespread biotechnological and medicinal chemistry interest in the preparation and properties of molecules related to fluorescein[2,6] lead us to investigate the reaction of a fluorescein 2′-ester with an amine, which, surprisingly, has not been previously described. The resulting 2′-amide might exist in its open or closed form, by analogy to fluorescein.

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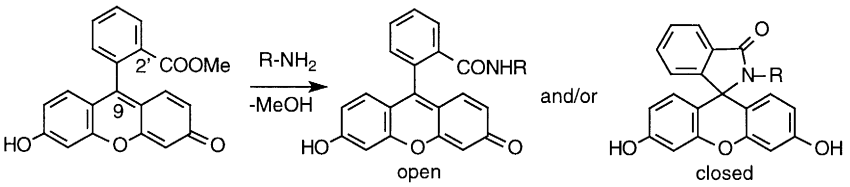
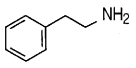
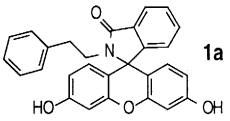
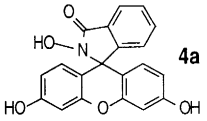
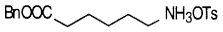
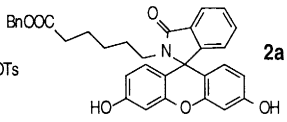
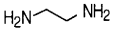
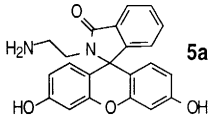
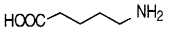
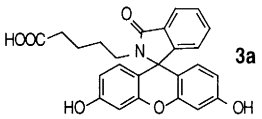
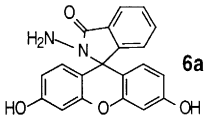


Reaction of fluorescein methyl ester[7] with a variety of primary amines (or primary amine salts with 1 equiv. diisopropylethylamine) in DMF at ambient temperature for 72 h or at 100°C for 14 h resulted in decolorization of the intensely red-orange solution. Unexpectedly, non-fluorescent white or off-white solids were isolated by preparative reversed-phase HPLC (acetonitrile/aqueous TFA mixtures, see Table 1).[8] In each case, electrospray mass spectrometry demonstrated that one equivalent of amine had added with concurrent loss of methanol. The new products were confirmed to be spirolactams by a characteristic peak near 64 ppm in the ^{13}C NMR spectrum, consistent for the 9-position carbon as a sp^3 carbon functionalized with a nitrogen atom.[4,9] ^{1}H and ^{13}C NMR spectra also displayed degenerate signals for the two symmetrical phenolic rings of the products. Additionally, X-ray crystallographic analysis of the spirolactam hydrazide **6a** clearly showed that the five-membered ring structure was present.

Table 1
Preparation of spirolactams from fluorescein methyl ester

Amine	Product	Yield[a]	Amine	Product	Yield[a]
	 **1a**	83		 **4a**	76[b]
	 **2a**	77[b]		 **5a**	84[c]
	 **3a**	64[b]		 **6a**	96

[a]72 hr, ambient temp. [b]with 1 eq DIEA. [c]12% formylated product in DMF; no side product in MeOH.

In summary, a previously undescribed family of xanthenes was synthesized by a new reaction of fluorescein methyl ester with a variety of primary amines. The non-fluorescent products were identified as spirolactams by a combination of mass spectrometry, NMR spectroscopy, and X-ray crystallography. The reactions proceed under mild conditions in good yield, and are tolerant of the different reactive functionalities (acid/ester, amine, hydroxy) required for further combinatorial elaboration adjacent to the core spirolactam pharmacophore. Moreover, these masked fluorescent substances[10] should find great utility in various biotechnological applications.

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8. Compound **2a**: Fluorescein methyl ester (25 mg, 72 μmol) was dissolved in anhydrous DMF (300 μL). Benzyl 6-aminocaproate tosylate (67 mg, 216 μmol) was added, followed by diisopropylethylamine (30 μL, 216 μmol), and the reaction stirred for 72 h at ambient temperature. Purification by C^{18} reversed phase HPLC (51% CH_3CN/49% 0.05% aq. TFA, v:v), followed by lyophilization provided 34 mg (77%) of a white solid. R_t 9.0 min (50% CH_3CN/50% 0.05% aq TFA) ^{1}H NMR δ 9.90 (s, 2H), 7.77 (m, 1H), 7.50 (m, 2H), 7.31 (s, 5H), 7.02 (m, 1H), 6.58 (d, 2H, J=2.2 Hz), 6.44 (dd, 2H, J=8.7, 2.3 Hz), 6.33 (d, 2H, J=8.6 Hz), 5.02 (s, 2H), 2.91 (br s, 2H), 2.15 (t, 2H, J=7.3 Hz), 1.24 (m, 2H), 0.96 (m, 4H). ^{13}C NMR δ 172.6, 166.7, 158.5, 152.9, 152.0, 136.3, 132.8, 130.6, 128.9, 128.5, 128.4, 127.9, 127.8, 123.6, 122.3, 112.4, 109.6, 102.2, 65.2, 63.7, 33.1, 27.4, 25.7, 23.9. ESMS 536.5 $(M+H)^+$. Anal. calcd for $C_{33}H_{29}NO_6$ 0.25 H_2O: C, 73.39, H, 5.51, N, 2.59. Found: C, 73.38; H, 5.48; N, 2.32.
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10. Preliminary studies indicate that compound **6a** can be fluorescently unmasked under Czarnik's conditions (Ref. 6d).