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Abstract: Coumarin-based dye (Z)-N-(7-cyano-9,9,15,15-tetramethyl-9,10,11,13,14,15-hexahydro-6H-benzo[4",5"]imidazo[1",2":1',2']pyrido[3',4':5,6]pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-6-ylidene)pent-4-ynamide (3) was obtained in 81% yield by reaction between primary imine precursor 1 and pent-4-ynoic anhydride (2) under thermal conditions. The title compound was characterized by elemental analysis, melting point, 1H-NMR, 13C-NMR, FT-IR, mass, UV-vis and fluorescence spectroscopy.

Keywords: coumarin; fluorescent dye; alkyne; imine; anhydride

Introduction

Coumarin derivatives constitute one of the most important organic fluorescent materials [1–3]. During the last decades a number of substituted fluorescent coumarins have been reported to absorb and emit in most parts of the visible spectrum. In fact, their remarkable light emission properties and structural robustness have driven the use of these compounds in a wide range of applications, including, among others, the fabrication of fluorescent brightening agents, daylight fluorescent pigments for textiles, plastics, colouring paint and ink products, tuneable dye lasers, solar energy collectors, non-destructive flaw indicators, liquid crystal displays, organic electroluminescent materials and new diagnostic methods in medicine [1–7].
Nevertheless, some attractive commercial coumarin-related dyes lack a proper chemical unit to allow for the attachment of other molecular entities. In this sense, terminal alkynes have been proven to be one of the most versatile functional groups in synthetic organic and materials chemistry [8,9]. This is mainly due to (1) their facile introduction into organic molecules, and (2) their numerous characteristic reactions such as electrophilic additions of hydrogen, halogens, hydrogen halides or water, metathesis, hydroboration, oxidative cleavage, C–C coupling and cycloadditions. Of particular interest among different cycloaddition reactions, the Huisgen 1,3-dipolar cycloaddition has been greatly promoted in the last decade as the most representative example of the “click” reactions [10,11].

Herein, we report the synthesis and characterization of a new coumarin-related fluorescent dye bearing a terminal alkyne, which provides a remote functional group for further modification.

Results and Discussion

10,11,14,15-Tetrahydro-6-imino-9,9,15,15-tetramethyl-6H,9H,13H-benzimidazo[1″,2″:1′,2′]pyrido[4″,3″:2,3]pyrido[6,7,8-ij]quinolizine-7-carbonitrile (1) is a commercial coumarin-related fluorescent red dye that contains a primary ketimine group. This type of fluorescent dyes is suitable for application to polyester fibres [12]. In order to incorporate a terminal alkyne we successfully reacted 1 with pent-4-ynoic anhydride (2) at 90 °C during 16 h (Scheme 1). Although uncommon, similar reactivity using acetic anhydride has been also reported with other imine-containing compounds [13]. Thus, the desired fluorescent alkyne-derivative 3 could be obtained in 81% yield upon proper purification (see Experimental Section and Supplementary Files).


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It is worth to mention that attempts of direct alkylation of the imine group were fruitless due to fast decomposition of dye 1 in the presence of bases (e.g., LDA, tBuOK, NaOMe, NaHMDS, NaH) even at very low temperatures, presumably due to a ring-opening cascade reaction initiated by the nucleophilic unprotonated imine [14]. Hence, this process can be inhibited by acylation of the imine using anhydride 2 in the absence of base.

Alkyne-containing coumarin 3 was found to be well soluble in a wide range of polar organic solvents (e.g., acetone, EtOAc, THF, EtOH, CH₃CN, DMSO) or DMSO:H₂O mixtures (i.e., 30/70 v/v, c = 6.8 × 10⁻³ mol·L⁻¹) but insoluble in water or pentane. In terms of UV-vis absorption properties, the two characteristic absorption maxima of unmodified dye 1 in the green region were red-shifted ca. 10 and 16 nm respectively upon acylation of the imine, whereas the peaks intensity were kept nearly constant [14]. Moreover, modified dye 3 remained highly fluorescent with a quantum yield (ϕ) of 0.35
in EtOH as calculated from its excitation-emission fluorescence spectra [14]. In addition, a clear
dependence of absorption properties with solvent polarity was evidenced by a bathochromic (red) shift
of the absorption maximum with increasing the latter [14], ranging from 1,4-dioxane ($\lambda_{\text{max}} = 568 \text{ nm}$)
to DMSO ($\lambda_{\text{max}} = 580 \text{ nm}$) (Figure 1). This indicates that the excited state of the dye is more polar than
its ground state, and consequently it is better stabilized by polar solvation.

**Figure 1.** (A) UV-vis spectra of modified dye 3 in different organic solvents ($c = 3.8 \times 10^{-5} \text{ mol·L}^{-1}$)
showing a positive solvatochromism; (B) Appearance of solutions of dye 3 in different organic solvents ($c = 2.8 \times 10^{-4} \text{ mol·L}^{-1}$)
under (top) visible and (bottom) UV light irradiation ($\lambda_{\text{max}} = 365 \text{ nm}$).

### Experimental

**General**

$^1$H-NMR spectra were recorded in CDCl$_3$ at 25 °C on a Bruker Avance 300 spectrometer. $^{13}$C-NMR spectra were recorded in CDCl$_3$ at 25 °C on a Bruker Avance III 600 spectrometer equipped with a
TCI cryo-sample head. Chemical shifts are reported relative to residual solvent peaks [14]. Mass
spectra were obtained with a Finnigan MAT 95 spectrometer. Infrared spectrum was recorded using a
Diamond ATR (attenuated total reflection) accessory (Golden Gate) on a Bio-Rad Excalibur FTS 3000
MX spectrophotometer. Melting point (m.p.) was measured in an Opti Melt MPA 100 instrument and
is uncorrected. Thin-layer chromatography was carried out on Merck aluminium sheets coated with
silica gel 60 F$_{254}$. Compounds were visualized by use of 254/365 nm UV light and phosphomolybdic
acid solution in ethanol (20 wt.%) with heating. Preparative TLC was carried out using precoated glass
plates of silica gel (SIL G-200 UV$_{254}$, 20 × 20 cm, 2 mm thick, Macherey-Nagel). All solvents used
during this research were of p.a. grade or purified by standard techniques [15]. UV-vis measurements
were performed on a Varian Cary 50 Instrument Excitation-emission fluorescence spectra were
obtained with an AMINCO Bowman Series 2 Luminescence spectrometer. Fluorescence quantum
yield was determined in EtOH using Rhodamine-6G ($\phi = 0.95$, $\lambda_{\text{ex}} = 530 \text{ nm}$) as standard sample.
Quarz glass cuvettes (Hellma 110-QS) of 0.5 and 1.0 cm pathlength were used for UV and
fluorescence measurements respectively. Anhydrous sodium sulphate was used for drying solutions. Although pent-4-ynoic anhydride (2) is commercially available, it was synthesized via DCC-coupling (DCC = 1,3-dicyclohexylcarbodiimide) of 4-pentynoic acid in CH2Cl2 at room temperature, affording the desired product as a colourless oil having spectroscopic data identical to those reported in the literature [16]. Fluorophore 10,11,14,15-tetrahydro-6-imino-9,9,15,15-tetramethyl-6H,9H,13H-benzimidazo[1",2":1',2']pyrido[4',3':2,3][1]benzopyrano[6,7,8-ij]quinolizine-7-carbonitrile (1) was purchased from FEW Chemicals GmbH and used without further purification.

Synthesis and characterization of (Z)-N-(7-cyano-9,9,15,15-tetramethyl-9,10,11,13,14,15-hexahydro-6H-benzo[4",5"]imidazo[1",2":1',2']pyrido[3',4':5,6]pyrano[2,3-f]pyrido[3,2,1-ij]quinolin-6-ylidene)pent-4-ynamide (3): 10,11,14,15-tetrahydro-6-imino-9,9,15,15-tetramethyl-6H,9H,13H-benzimidazo[1",2":1',2']pyrido[4',3':2,3][1]benzopyrano[6,7,8-ij]quinolizine-7-carbonitrile (1) (20 mg, 0.043 mmol) was mixed with pent-4-ynoic anhydride (2) (463 mg, 2.6 mmol) and stirred for 16 h at 90 °C. After this time, TLC-analysis indicated complete conversion of 1 into 3. The mixture was diluted with water (100 mL) and extracted with CH2Cl2 (100 mL). The organic layer was washed with water (3 × 50 mL) and brine (2 × 50 mL), dried over Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluting with EtOAc/hexanes mixtures ranging from 1:1 to 9:1 v/v) and subsequent preparative TLC (eluting with EtOAc/hexanes 4:1 v/v) affording product 3 (19 mg, 81% yield) as a dark purple solid [17]: TLC Rf (EtOAc/hexanes 3:1 v/v) 0.44; m.p. = 146 ± 1 °C; 1H-NMR (300 MHz, CDCl3) δ/ppm = 8.63 (s, 1H), 8.59 (d, J = 7.8 Hz, 1H), 7.81 (d, J = 7.7 Hz, 1H), 7.46 (td, J = 7.7, 1.2 Hz, 1H), 7.37 (td, J = 7.9, 1.3 Hz, 1H), 7.32 (s, 1H), 3.53 (t, J = 6.1 Hz, 2H), 3.42 (t, J = 5.9 Hz, 2H), 3.08–2.97 (m, 2H), 2.80–2.72 (m, 2H), 2.00 (t, J = 2.6 Hz, 1H), 1.88 (dd, J = 6.6, 4.7 Hz, 2H), 1.82 (t, J = 6.4 Hz, 2H), 1.67 (s, 6H), 1.35 (s, 6H); 13C-NMR (151 MHz, CDCl3) δ/ppm = 181.37, 163.07, 152.34, 149.90, 144.72, 144.24, 144.22, 136.93, 132.18, 131.36, 125.70, 125.35, 123.93, 118.84, 117.07, 115.63, 114.97, 112.11, 104.78, 83.84, 73.61, 70.61, 68.62, 48.19, 47.55, 38.86, 38.77, 34.55, 32.34, 32.32, 29.62, 28.19 (2C), 14.46; FT-IR (ATR) νmax (cm⁻¹) 3317, 2941, 2831, 2383, 2197, 1635, 1448, 1304, 1119, 952, 933, 660, 627, 540, 500, 469, 414; MS (ESI) m/z 542 [MH⁺]. HRMS calculated for C34H31N5O2 541.2475; found 541.2468. Elemental analysis calculated for C34H31N5O2: C, 75.38; H, 5.77; N, 12.94; found: C, 75.54; H, 5.93; N, 13.24; UV-vis (acetone, λmax, nm (ε, M⁻¹·cm⁻¹)) [sh = shoulder]: 576 (24200), 530 (sh).

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References and Notes

12. BASF German P 2 415 661 (1975).
14. See Supplementary Files for detailed information.
17. If necessary, the amount of pent-4-ynoic anhydride can be reduced (6-fold) using DMF as solvent over a period of 12 h.

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