An investigation into the synthesis of azido-functionalised coumarins for application in 1,3-dipolar "click" cycloaddition reactions

Vikki Evans, Philip Duncanson, Majid Motevalli and Michael Watkinson*

The Joseph Priestley Building, School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London, E1 4NS, U.K.

Email: m.watkinson@qmul.ac.uk

Abstract

A range of reported methods have been assessed for the synthesis of two coumarin fluorophores containing azides, **1a** and **2**, for subsequent "click" modification. In the case of **1a** reported methods were successfully applied, but the resulting azide proved to be rather unstable and appears more suited to *in-situ* generation and conversion to the "click" triazole. In the case of **2**, reported methods for the synthesis of precursor **5** were ineffective in two cases and resulted in either no bromination α-to the carbonyl or the formation of multiple unwanted side-products, some of which were isolated, **6-13**. The use of $CuBr_2$ in excess or Br_2 in 50% HBr in acetic acid did result in the isolation of **5**, which could be efficiently converted to **2** using excess NaN_3 in THF.

Keywords

Coumarin, Fluorescent dye, Click chemistry, Azide.

1. Introduction

As a result of their operational simplicity and the low detection limits displayed for a wide range of analytes, small molecule fluorescent probes have revolutionised the field of bioimaging in recent years and are now firmly established experimental tools [for recent reviews see for example 1-6]. Despite the many advances that have been made, there continues to be considerable effort expended into the development of probes with enhanced properties for application in biological systems, such as increased brightness, lower energy excitation and emission profiles, biologically relevant K_d and specific cellular probe localisation. The use of the Huisgen-Sharpless-Meldal-Fokin Cu(I) catalysed 1,3-diploar cycloaddition between azides and alkynes (CuAAC) [7,8] has become a popular synthetic choice in many laboratories to generate probes due to its reliability and high, often almost quantitative, yields under mild reaction conditions and the area has been reviewed by us [9,10] and others [11]. Despite the many impressive and exciting probes reported to date based on click-triazole motifs, the methodology continues to be applied in the development of a wide range of new probes [e.g. 12-14]. Our interest in the field has focussed on the imaging of the biologically important d-block element zinc [15-18], the mis-regulation of which is associated with a wide range of disease states, including type 2 diabetes mellitus, prostate cancer, Alzheimer's disease, myocardial infarction, ischaemic stroke, epilepsy and infantile diarrhoea; this results in the ongoing interest in the field as chemistry researchers seek to develop better tools for biomedical scientists to apply in their research into understanding these societally important diseases [for recent reviews see for example 19-22]. We recently developed an efficient one-pot strategy using "click-click" chemistry to prepare a series of probes that were able to image zinc in specific cellular space [18]. Wishing to develop this chemistry further, we were keen to incorporate other fluorophores into our systems and were particularly attracted by the development and application of "click" coumarins. This is because coumarins have outstanding optical properties, are biocompatible and easy to modify synthetically [23,24]. Moreover, it is well-established that substitution at the 3- and 7-positions of the coumarin has a significant impact on their fluorescent properties, which has been

widely exploited including in the development of azide and alkyne derivatised analogues [e.g. 25-30]. We were particularly interested in the initial application of reported azide containing coumarins **1a** and **2** and herein report a short investigation into the development of effective methods for their synthesis.

2. Results and Discussion

The conjugated azide containing coumarin **1a** was successfully prepared by a slight modification of the reported procedure [26] via a diazo-coupling, see Electronic Supporting Information. The azide could be isolated in a good yield of 85% following purification by column chromatography, or in 65% yield through recrystallization, and although it was stable when stored at -20 ^{o}C it proved to be somewhat unstable. Although it was stable as a solid at room temperature for a week, when in $CDCl_3$ solution heated gently at 45 ^{o}C for five days complete decomposition occurred to give a complex mixture from which we could isolate two compounds by column chromatography. The first of these can be confidently assigned as aniline **1b**, by comparison with an authentic sample prepared in the stepwise synthesis of **1a** and we have observed such azide decomposition in related systems [18]. Very small quantities of a second compound were also isolated that we cautiously suggest to be **3** based on ^{1}H NMR spectroscopy and mass spectrometry, which we speculate forms from nitrene intermediates resulting from azide decomposition. This instability of **1a** therefore makes it an ideal candidate for *in situ* one-pot synthesis of triazole linkages, as reported by Moses *et al*. [31]. In contrast the synthesis of **2** proved to be much less straightforward. Coumarin **4** (Scheme 1) was readily prepared via Knœvenagal condensation of commercially available 4-(diethylamino) salicylaldehyde and ethyl 2-methylacetoacetate, as reported [32], in a good yield of 75%. A range of methods have been reported for the α-bromination of **4** and we were attracted by the report of Joshi *et al*.[33] who reported expedient bromide incorporation could be effected at room temperature using elemental bromine. In our hands this procedure proved ineffective, with unreacted starting material being the only material recovered. Laufer *et al*.[34], have reported

similar conditions for the effective α-bromination of a large number of compounds. However, the use of their procedure resulted in a very complex ^{1}H NMR spectrum of the crude reaction mixture that was indicative of multiple bromination products having been formed, although target coumarin **5** was not produced in significant amounts.

In addition to bromination, it was also apparent that a significant quantity of the starting material had undergone *N*-dealkylation of one of the ethyl groups of the tertiary amine, as a characteristic broad signal in the crude NMR spectrum of NH protons could be observed around 5 ppm. Through purification of the reaction mixture by extensive column chromatography we were able to isolate and characterise a number of these compounds, **6-9**, Figure 1, and to corroborate unequivocally that *N*-dealkylation had occurred in **7-9**. Additionally, we were further able to corroborate the structure of **7** unequivocally following the formation of crystals suitable for single crystal X-ray diffraction studies from an ethanolic solution, Figure 2a. Whilst this *N*-dealkylation process has been reported previously during aromatic bromination, it is rare and to our knowledge limited to a very small number of cases [35].

Subsequent repetition of the reaction again gave complex mixtures of multiple brominated products, which proved very difficult to separate, but after extensive column chromatography an additional four further compounds could be identified, which we were able to tentatively assign as **10-13**. Unfortunately as they were susceptible to decomposition and produced in very small quantities they proved impossible to fully characterise in all cases.

We then applied the procedure reported by Lin *et al*.[36] for the formation of **5** using $CuBr_2$ in excess as the bromine source, which we had initially viewed as a less attractive protocol. Gratifyingly, this procedure proved effective, although the target coumarin **5** was produced in a rather modest yield of 45% given the reaction stoichiometry (reported yield 47% [36]), with the only other material isolated from the crude reaction mixture being unreacted starting material and required labour

intensive chromatography to effect separation of the reaction mixture. This also allowed us to confirm the structure of **5** by single crystal X-ray diffraction, Figure 2b.

We finally turned to the procedure first reported by Takechi et al. [37], which has more recently been adapted by Pajik [38] to use elemental bromine, although no experimental conditions or spectroscopic data were presented. The procedure we developed based on this report proved to be the most effective, with **5** being isolated through a combination of recrystallisation and column chromatography in a total yield of 71% (see Electronic Supporting Information).

The conversion of **5** into **2** has also been previously reported by simple addition of excess NaN_3 to **5** in a DMF/AcOH mixture[37], which surprisingly failed in our hands and unreacted starting material was consistently re-isolated (Table 1, entry 1). We therefore screened a range of other conditions traditionally used for the preparation of azides from bromides in polar aprotic solvents, but in all cases only unreacted starting material was recovered (Table 1, entries 2-4). Switching to a polar protic solvent also proved unsuccessful (Table 1, entry 5) and it was only when excess NaN_3 was employed in dry THF that we met with success (Table 1, entry 6), with **2** being isolated in good yield, following a straightforward purification protocol.

3. Conclusions

Although a range of methods have already been reported for the synthesis of coumarins **1a** and **2** containing azide functionality that can be utilised in 1,3-dipolar 'click' cycloadditions, a number of synthetic issues have been identified with these. Firstly, whilst the reported procedures for the synthesis of **1a** work well, the resultant azide is susceptible to decomposition and may be more suited to *in situ* generation followed by immediate cycloaddition. In contrast, the apparently simple reported procedures to produce **5** using elemental bromine as the electrophile were ineffective and either gave none of the desired product or resulted in multiple unwanted materials from both

aromatic bromination and rare *N*-dealkylation, with **6-13** being identified. Use of excess $CuBr_2$ as the brominating agent gave the target bromide **5**, as reported and we have been able to develop an effective procedure using elemental bromine in HBr/acetic acid that is the highest yielding. Bromide **5** proved to be surprisingly unreactive to nucleophilic substitution with NaN_3 under a range of conditions typically used for such reactions, including a previously reported protocol [37], but was successfully converted to the target azide **2** using dry THF and an excess of NaN_3.

4. Experimental

4.1 General

All solvents were purchased from Sigma Aldrich, Alfa Aesar and Merck, and did not undergo any further purification. Anhydrous solvents were obtained from an MBraun MB SPS-800 solvent purification system. Flash chromatography was performed on silica gel (VWR, 40-63 µm), and monitored via TLC (silica gel 60 F254 plastic backed plates, Merck) and analysed by UV light or potassium permanganate staining. Melting points were measured on a Gallenkamp melting point apparatus. Both ^{1}H (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded on either Bruker AMX400 or AV400 spectrometers and are referenced to residual $CHCl_3$ (^{1}H NMR) or $CDCl_3$ (^{13}C NMR). Coupling constants (J) are reported in Hertz (Hz). IR spectra were obtained using a Perkin Elmer Spectrum 65 spectrometer fitted with an ATR attachment. UV-vis spectra were obtained using an HP 8453 spectophotometer. High resolution mass spectrometry was obtained from the EPSRC National Mass Spectrometry Service Swansea University using electrospray ionisation on a Thermofisher LTQ Orbitrap XL.

*4.2 General method for the bromination of **4** to yield multiple bromination products [34].*

3-Acetyl-7-diethylamino-chromen-2-one, **4**, [32] (0.288 g, 1.12 mmol) was dissolved in glacial acetic acid (20 mL) and bromine (0.268 g, 1.68 mmol) was added dropwise to the solution. The mixture was

left to stir at room temperature for 18 h. The solvent was removed *in vacuo* and the crude mixture was purified by flash chromatography (1:4 EtOAc: 40-60 Petroleum ether), to give products **6-9** as yellow solids. Repetition of the reaction and modification of purification protocols allowed the isolation of **10-13**.

4.2.1 3-Acetyl-8-bromo-7-(diethylamino)-2H-chromen-2-one **(6)**. Yellow powder, yield: 0.10 g (21 %) mp 178 °C. UV: λ_{max} (CH_2Cl_2)/nm (ε/ $mol^{-1}cm^{-1}dm^3$) 408 (30 402). IR ν_{max} / cm^{-1} 1745 (C=O), 1685 (C=O), 1225 (CN), 1204, 1040 (C-O). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.42 (s, 1H, C*H*$_{Ar}$), 7.45 (d, *J* = 8.6 Hz, 1H, C*H*$_{Ar}$), 6.96 (d, *J* = 8.6 Hz, 1H, C*H*$_{Ar}$), 3.37 (q, *J* = 7.1, 4H, C*H*$_2$CH$_3$), 2.70 (s, 3H, C*H*$_3$), 1.15 (t, *J* = 7.1 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 195.4, 159.3, 156.2, 154.5, 147.4, 129.1, 120.7, 118.5, 113.2, 104.5, 46.3, 30.7, 12.8. HRMS (ESI) *m/z* found 338.0391, $[M + H]^+$; $C_{15}H_{16}BrNO_3$ requires 338.0386.

4.2.2 3-Acetyl-8-bromo-7-(ethylamino)-2H-chromen-2-one **(7)**. Yellow powder, yield: 0.24 g (48 %) mp 206 °C. UV: λ_{max} (CH_2Cl_2)/nm (ε/ $mol^{-1}cm^{-1}dm^3$) 412 (38 970). IR ν_{max}/ cm^{-1} 3351 (NH), 1729 (CO), 1668 (CO), 1616 (NH), 1199 (CN), 1134 (CO). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.42 (s, 1H, C*H*$_{Ar}$), 7.43 (d, *J* = 8.6 Hz, 1H, C*H*$_{Ar}$), 6.62 (d, *J* = 8.6 Hz, 1H, C*H*$_{Ar}$), 5.26 (br s, 1H, N*H*), 3.37 (q, *J* = 7.2 Hz, 2H, C*H*$_2$CH$_3$), 2.69 (s, 3H, C*H*$_3$), 1.38 (t, *J* = 7.2 Hz, 3H, CH$_2$C*H*$_3$). ^{13}C NMR (101MHz, $CDCl_3$): δ_C 195.7, 159.9, 154.5, 150.7, 148.3, 131.2, 117.9, 110.0, 108.3, 94.7, 38.7, 30.8, 14.8. HRMS (ESI) *m/z* found 310.0077, $[M + H]^+$; $C_{13}H_{12}BrNO_3$ requires 310.0073.

4.2.3 3-Acetyl-6-bromo-7-(ethylamino)-2H-chromen-2-one **(8)**. Yellow powder, yield: 0.01 g (3 %) mp 201 °C. UV: λ_{max} (CH_2Cl_2)/nm (ε/ $mol^{-1}cm^{-1}dm^3$) 411 (22 830). IR ν_{max} / cm^{-1} 3405 (NH), 1726 (CO), 1672 (CO), 1613 (NH), 1205 (CN). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.37 (s, 1H, C*H*$_{Ar}$), 7.66 (s, 1H, C*H*$_{Ar}$),

6.47 (s, 1H, CH_{Ar}), 5.14 (br s, 1H, N*H*), 3.31 (q, *J* = 7.2 Hz, 2H, CH_2CH$_3$), 2.68 (s, 3H, CH$_3$), 1.39 (t, *J* = 7.2 Hz, 3H, CH$_2$CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 195.5, 160.2, 157.8, 150.0, 147.2, 133.4, 118.3, 109.8, 106.5, 96.6, 38.6, 30.7, 14.2. HRMS (ESI) *m/z* found 310.0078 $[M + H]^+$; $C_{13}H_{12}BrNO_3$ requires 310.0073.

4.2.4 3-Acetyl-6,8-dibromo-7-(ethylamino)-2H-chromen-2-one **(9)**. Yellow solid, yield: 0.009 g (2 %) mp 176-180 °C. UV: λ_{max} (CH_2Cl_2)/nm (ε/ $mol^{-1}cm^{-1}dm^3$) 399 (33 899). IR ν_{max} / cm^{-1} 3354 (NH), 1738 (C=O), 1671 (C=O), 1600 (NH), 1220 (CN). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.33 (s, 1H, CH_{Ar}), 7.70 (s, 1H, CH_{Ar}), 4.91 (br s, 1H, N*H*), 3.77 (q, *J* = 7.1 Hz, 2H, CH_2CH$_3$), 2.69 (s, 3H, CH_3), 1.32 (t, *J* = 7.2 Hz, 3H, CH$_2$CH_3). Insufficient material could be isolated to obtain a satisfactory ^{13}C NMR spectrum. HRMS (ESI) *m/z* found $[M + H]^+$ 387.9180; $C_{13}H_{12}Br_2NO_3$ requires 387.9178.

4.2.5 8-Bromo-3-(2-bromoacetyl)-7-(ethylamino)-2H-chromen-2-one (**10**).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.53 (s, 1H, CH_{Ar}), 7.47 (d, *J* = 9.2 Hz, 1H, CH_{Ar}), 6.65 (d, *J*= 9.1 Hz, 1H, CH_{Ar}), 5.38 (br s, 1H, NH), 4.75 (s, 2H, CH_2Br), 3.39 (q, *J* = 7.2 Hz, 2H, CH_2CH$_3$), 1.39 (t, *J* = 7.2 Hz, 3H, CH$_2$CH_3). Insufficient material could be isolated to obtain a satisfactory ^{13}C NMR spectrum.

4.2.6 6,8-Dibromo-3-(2-bromoacetyl)-7-(ethylamino)-2H-chromen-2-one **(11)**.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.44 (s, 1H, CH_{Ar}), 7.74 (s, 1H, CH_{Ar}), 5.06 (br s, 1H, N*H*), 4.73 (s, 2H, CH_2Br), 3.82 (q, *J* = 7.2 Hz, 2H, CH_2CH$_3$), 1.33 (t, *J*= 7.2 Hz, 3H, CH$_2$CH_3). Insufficient material could be isolated to obtain a satisfactory ^{13}C NMR spectrum. HRMS (ESI) *m/z* found $[M + H]^+$ 465.8289; $C_{13}H_{11}Br_3NO_3$ requires 465.8284.

4.2.7 8-Bromo-3-(2-bromoacetyl)-7-(diethylamino)-2H-chromen-2-one **(12)**.

A band collected by column chromatography using DCM/MeOH mixtures as the eluent gave a mixture of **12** and **13** in a 5:1 ratio. Slow crystallisation from an acetonitrile solution gave **12**. Yellow crystals, yield: 0.050 g (10%) mp 176-177 °C. IR ν_{max} / cm^{-1}: 2967, 1721 (C=O), 1678 (C=O), 1609, 1573, 1487, 1459, 1378, 1352, 1241, 1175, 1035, 988, 967, 809, 761. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.54 (s, 1H, C*H*$_{Ar}$), 7.49 (d, *J* = 8.7 Hz, 1H, C*H*$_{Ar}$), 6.99 (d, *J* = 8.7 Hz, 1H, C*H*$_{Ar}$), 4.76 (s, 2H, C*H*$_2$Br), 3.43 (q, *J* = 7.2 Hz, 4H, C*H*$_2$CH$_3$), 1.19 (t, *J* = 7.2 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 188.8, 159.2, 156.9, 154.9, 149.4, 129.5, 118.9, 117.9, 113.0, 103.7, 46.4, 36.3, 13.0. HRMS (ESI) *m/z* found 415.9305 $(M-H)^-$; $C_{15}H_{14}Br_2NO_3$ requires 415.9326.

4.2.8 6-Bromo-3-(2-bromoacetyl)-7-(diethylamino)-2H-chromen-2-one **(13)**.

Despite repeated efforts we were unable to purify **13** from the mixture with **12** by chromatography, however, it was possible to extract NMR data from spectra of the mixture.

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.50 (s, 1H, C*H*$_{Ar}$), 7.82 (s, 1H, C*H*$_{Ar}$), 6.88 (s, 1H, C*H*$_{Ar}$), 4.74 (s, 2H, C*H*$_2$Br), 3.38 (q, *J* = 7.2 Hz, 4H, C*H*$_2$CH$_3$), 1.19 (t, *J* = 7.2 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 188.7, 159.5, 156.2, 156.1, 148.5, 135.8, 118.4, 113.5, 113.2, 108.2, 46.3, 36.2, 12.6.

4.3. Synthesis of 3-(2-Azidoacetyl)-7-(diethylamino)-2H-chromen-2-one *(**2**)*

Bromide **5** (2.00 g, 5.91 mmol) and NaN_3 (1.92 g, 29.57 mmol) were added to a flask and flushed with N_2. Dry THF (15 mL) was added to the flask and the reaction mixture was left to stir at 45 °C for 18 h. The reaction was quenched with H_2O (10 mL), washed with CH_2Cl_2 (3 x 15 mL) and the combined organic phase dried with $MgSO_4$. The solvent was removed *in vacuo* before redissolving in the minimal volume of CH_2Cl_2 and trituration with *n*-hexane (25 mL). The dark brown solid that precipitated was collected to give **2** as a dark brown powder (1.43 g, 80%). mp 172-174 °C. Lit 172-175 °C [37]. UV: λ_{max} (CH_2Cl_2)/nm (ε/ $mol^{-1}cm^{-1}dm^3$) 444 (45 976). IR ν_{max} / cm^{-1} 2099 (N_3), 1713 (C=O), 1615 (C=C), 1505 (C=C), 1351 (C-N), 1186 (C-O-C). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.55 (s, 1H, C*H*$_{Ar}$), 7.44 (d, *J* = 9.0, 1H, C*H*$_{Ar}$), 6.65 (dd, *J* = 9.0 Hz, 2.3, 1H, C*H*$_{Ar}$), 6.48 (d, *J* = 2.3 Hz, 1H, C*H*$_{Ar}$), 4.69 (s, 2H, C*H*$_2$N$_3$), 3.48 (q, *J* = 7.1 Hz, 4H, C*H*$_2$CH$_3$), 1.26 (t, *J* = 7.1 Hz, 6H, CH$_2$C*H*$_3$). ^{13}C NMR (101 MHz,

$CDCl_3$): δ_C 191.1, 175.9, 153.8, 149.1, 137.7, 132.5, 113.4, 110.5, 108.5, 96.8, 58.8, 45.4, 12.6. LRMS calcd *m/z* 300.1, found 301.6 [M + H].

4.4 Single crystal X-ray crystallography

The data were collected on a Bruker Kappa Apex Duo diffractometer with Apex II, area detector and a molybdenum sealed tube X-ray source (50 kV, 30 mA, λ=0.71073 Å). The crystal-to-detector distance was 30 mm and ϕ and Ω scans (2.0° increments, 10 s exposure time) were carried out to fill the Ewald sphere. Data collection and processing were carried out using the SAINT [39] and empirical absorption correction was applied using SADABS [39]. The structures were solved by the direct-method using the program SHELXS-2014/7 [40] and refined anisotropically (non-hydrogen atoms) by full-matrix least-squares on F^2 using SHELXL-2014/7 [40]. The H atom positions were calculated geometrically and refined with a riding model. The program SHELXTL [40] was used for drawing the molecules and preparing material for publication.

4.4.1 Data for **5**: Translucent intense yellow block, 0.080 x 0.160 x 0.350 mm; $C_{15}H_{16}BrNO_3$; M = 338.2; triclinic; a = 7.6131(7) Å, b = 8.7050(8) Å, c = 11.2410(11) Å, α = 77.542(2)°, β = 99.5630(10)°, γ = 68.871(2)°, V = 682.16(11) $Å^3$ are based upon the refinement of the XYZ-centroids of 9553 reflections above 20 σ(I) with 5.108° < 2θ < 56.76°;

P-1; Z= 2; T = 100(2) K; θ_{min} = 1.86 °; θ_{max} = 28.45 °;μ = 3.020 cm^{-1}; Total reflections measured = 12160; Independent reflections = 3369; Reflections used = 3264 (> 2σ(F^2)); The final anisotropic full-matrix least-squares refinement on F^2 with 183 variables converged at R1 = 1.81%, for the observed data and wR2 = 4.92% for all data. The goodness-of-fit was 1.083. The largest peak in the final difference electron density synthesis was 0.365 e^- $Å^{-3}$ and the largest hole was -0.271 e^- $Å^{-3}$ with an

RMS deviation of 0.061 e^- $Å^{-3}$. On the basis of the final model, the calculated density was 1.646 g cm^{-3} and F(000) 344 e^-. CCDC reference 1453090.

4.4.1 Data for **7**: Translucent light yellow shard, 0.260 x 0.120 x 0.090 mm; $C_{13}H_{12}BrNO_3$; M = 310.15; monoclinic; a = 9.9248(5) Å, b = 4.8313(2) Å, c = 25.4583(11) Å, β = 99.5630(10) °, V = 1203.75(9) $Å^3$ are based upon the refinement of the XYZ-centroids of 6157 reflections above 20 σ(I) with 4.711° < 2θ < 56.43°; P 1 21/n 1; Z= 4; T = 100(2) K; θ_{min} = 1.62 °; θ_{max} = 28.25 °;μ = 3.414 cm^{-1}; Total Reflections measured = 12588; Independent reflections = 2988; Reflections used = 2642 (> $2\sigma(F^2)$); The final anisotropic full-matrix least-squares refinement on F^2 with 165 variables converged at R1 = 2.69%, for the observed data and wR2 = 6.75% for all data. The goodness-of-fit was 1.046. The largest peak in the final difference electron density synthesis was 1.166 e^- $Å^{-3}$ and the largest hole was -0.478 e^- $Å^{-3}$ with an RMS deviation of 0.083 e^- $Å^{-3}$. On the basis of the final model, the calculated density was 1.711 g cm^{-3} and F(000) 624 e^-. CCDC reference 1453089.

Acknowledgements

The authors are grateful to the EPSRC National Mass Spectrometry Service, University of Swansea for the provision of all MS data reported herein. MW would like to acknowledge the contribution of the COST Action TD1304 Zinc-Net.

References

[1] Domaille DW, Que EL, Chang CJ. Synthetic fluorescent sensors for studying the cell biology of metals. Nat Chem Biol 2008;4:168-75.

[2] Chan J, Dodani SC, Chang CJ. Reaction-based small-molecule fluorescent probes for chemoselective bioimaging. Nat Chem 2012;4, 973-84.

[3] Carter KP, Young AM, Palmer AE. Fluorescent Sensors for Measuring Metal Ions in Living Systems. Chem Rev 2014;114:4564–4601.

[4]Pak YL, Swamy KMK, Yoon, J. Recent Progress in Fluorescent Imaging Probes. Sensors 2015;15:24374-96.

[5]Qian XH, Xu ZC. Fluorescence imaging of metal ions implicated in diseases. Chem Soc Rev 2015;44:4487-93.

[6] Roopa, Kumar N, Bhalla V, Kumar M. Development and sensing applications of fluorescent motifs within the mitochondrial environment. Chem Commun 2015;51:15614-28.

[7] Kolb HC, Finn MG, Sharpless KB. Click chemistry: Diverse chemical function from a few good reactions. Angew Chem Int Ed Engl 2001;40:2004-21.

[8] Worrell BT, Malik JA, Fokin VV. Direct evidence of a dinuclear copper intermediate in Cu(I)-catalyzed azide-alkyne cycloadditions. Science 2013;340:457-60.

[9] Lau YH, Rutledge P, Watkinson M, Todd MH, Chemical Sensors that Incorporate Click-Derived Triazoles. Chem Soc Rev 2011;40:2848-66.

[10] Watkinson M. Click generated triazoles as chemo-sensors. Top Heterocycl Chem 2012;28,109-36.

[11] Bryant JJ, Bunz UH. Click to bind: metal sensors. Chem Asian J 2013;8:1354-67.

[12] Maity D, Govindaraju T. Conformationally constrained (coumarin-triazoyl-bipyridyl) click fluoroionophore as a selective Al^{3+} sensor. Inorg Chem 2010;49:7229-31.

[13] Lau YH, Price JR, Todd MH, Rutledge PJ. A click fluorophore sensor that can distinguish Cu^{2+} and Hg^{2+} via selective anion-induced demetallation. Chem Eur J 2011;17:2850-58.

[14] Xu ZC, Liu X, Pan J, Spring DR. Coumarin-derived transformable fluorescent sensor for Zn^{2+}. Chem Commun 2012;48:4764-66.

[15] Tamanini E, Katewa A, Sedger, L Todd MH, Watkinson M. A synthetically simple, click-generated cyclam-based zinc(II) sensor. Inorg Chem 2009;48:319-24.

[16] Tamanini E, Flavin K, Motevalli M, Piperno S, Gheber LA, Todd MH, Watkinson M. Cyclam-based "clickates": homogeneous and heterogeneous fluorescent sensors for Zn(II)", Inorg Chem 2010;49:3789-3800.

[17] Jobe K, Brennan CH, Motevalli M, Goldup SM, Watkinson M. "Modular 'click' sensors for zinc and their application in vivo. Chem Commun 2011;47:6036-38.

[18] Pancholi J, Hodson DJ, Jobe K, Rutter GA, Goldup SM, Watkinson M. Biologically targeted probes for $Zn^{2+:}$ A diversity oriented modular "click-S_NAr-click" approach. Chem Sci 2014;5:3528-35.

[19] Tomat E, Lippard SJ. Imaging Mobile Zinc in Biology. Curr Opin Chem Biol 2010;14:225–30.

[20] Xu ZC, Yoon J, Spring DR. Fluorescent chemosensors for Zn^{2+}. Chem Soc Rev 2010;39:1996-2006.

[21] Maret W. Analyzing free zinc(II) ion concentrations in cell biology with fluorescent chelating molecules. Metallomics 2015;7:202-11.

[22] Chen YC, Bai Y, Han Z, He WJ, Guo ZJ. Photoluminescence imaging of Zn^{2+} in living systems. Chem Soc Rev 2015; 44:4517-46.

[23] Chen Y, Clouthier CM, Tsao K, Strmiskova M, Lachance H, Keillor JW. Coumarin-based fluorogenic probes for no-wash protein labelling. Angew Chem Int Ed Engl 2014;53:13785-88.

[24] Sun H, Fan H, Peng X. Quantitative DNA interstrand cross-link formation by coumarin and thymine: Structure determination, sequence effect and fluorescence detection. J Org Chem 2014;79:11359-69.

[25] Sivakumar K, Xie F, Cash BM, Long S, Barnhill HN, Wang Q. A fluorogenic 1,3-dipolar cycloaddition reaction of 3-azidocoumarins and acetylenes. Org Lett 2004;6:4603-06

[26] Key JA, Koh, S, Timerghazin QK, Brown A, Cairo CW. Photophysical characterization of triazole-substituted coumarin fluorophores. Dyes and Pigments 2009;82:196-203.

[27] Gordo J, Avó J, Parola AJ, Lima JC, Pereira A, Branco PS. Convenient synthesis of 3-vinyl and styrl coumarins. Org Lett 2011;13:5112-15.

[28] Xu ZC, Liu X, Pan J, Spring DR. Coumarin-derived transformable fluorescent sensor for Zn^{2+}. Chem Commun 2012;48:4764-66.

[29] Yin H, Zhang B, Yu H, Zhu L, Feng Y, Zhu M et al. Two-photon fluorescent probes for biological Mg^{2+} detection based on 7-substituted coumarin. J Org Chem 2015;80:4306-12

[30] Mallet AM, Davis AB, Davis DR, Panella J, Wallace KJ, Bonizzoni M. A cross metal reactive sensor array to probe divalent metal ions. Chem Commun 2015;51:16948-51.

[31] Barral K, Moorhouse AD, Moses JE. Efficient conversion of aromatic amines into azides: A one-pot synthesis of triazole linkages. Org Lett 2007;9:1809-11.

[32] Secci D, Carradori S, Bolasco A, Chimenti P, Yáñez M, Ortuso F, Alcaro S. Synthesis and selective human monoamine oxidase inhibition of 3-carbonyl, 3-acyl, and 3-carboxyhydrazido coumarin derivatives. Eur J Med Chem 2011;46:4846-52.

[33] Joshi SD, Manish K, and Badiger A. Synthesis and evaluation of antibacterial and antitubercular activities of some novel imidazo[2,1-b][1,3,4]thiadiazole derivatives. Med Chem Res 2013,22,869-78.

[34] Laufer SA, Hauser DRJ, Liedtke, AJ. Regiospecific and highly flexible synthesis of 1,4,5-trisubstituted 2-sulfanylimidazoles from structurally diverse ethanone precursors, Synthesis (Stuttg) 2008;2:253-66.

[35] Corrie JET, Munasinghe VRN. Synthesis and fluorescence properties of substituted 7-aminocoumarin-3-carboxylate derivatives. J Heterocyclic Chem 2000;37:1447-55 and references cited therein.

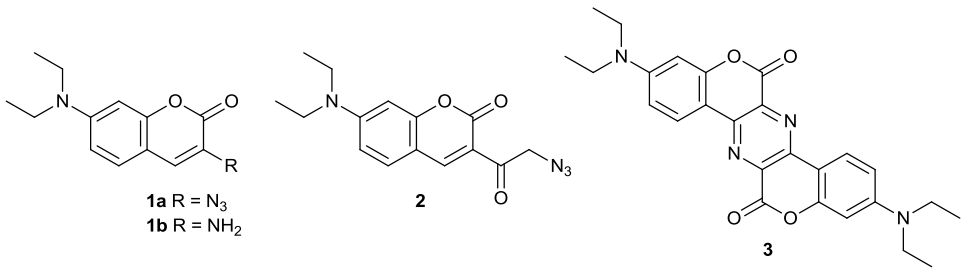
[36] Lin WY, Cao XW, Yuan L, Ding Y. Double functional group transformations for fluorescent probe construction: A fluorescence turn-on probe for thioureas. Chem Eur J 2010;16,6454–57.

[37] Takechi H, Oda Y, Nishizono N, Oda K, Machida M. Screening search for organic fluorophores: Syntheses and fluorescence properties of 3-azolyl-7-diethylaminocoumarin derivatives. Chem Pharm Bull 2000;48:1702–10.

[38] Pajik S. Synthesis and fluorescence properties of environment-sensitive 7-(diethylamino)coumarin derivatives. Tetrahedron Lett 2014;55:6044-47.

[39] Bruker: APEX2, SAINT and SADABS. Bruker AXS Inc., Madison.W1, 2009

[40] Sheldrick GM. A short history of SHELX. Acta Crystallogr Sect A: Found Crytstallogr 2008;64:112-22.



1a R = N_3
1b R = NH_2

2

3

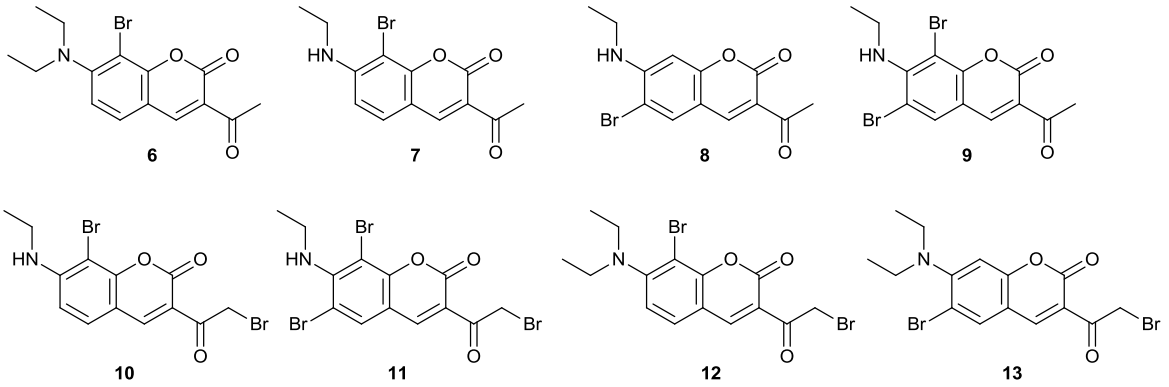
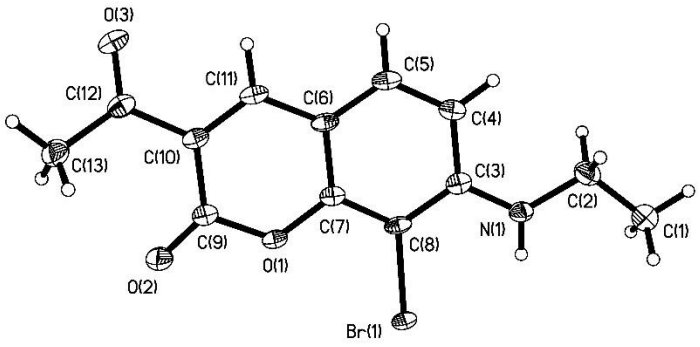


Figure 1. The structures of bromination side-products **6-13** isolated using the procedure of Laufer *et al*. [34].

a)



b)

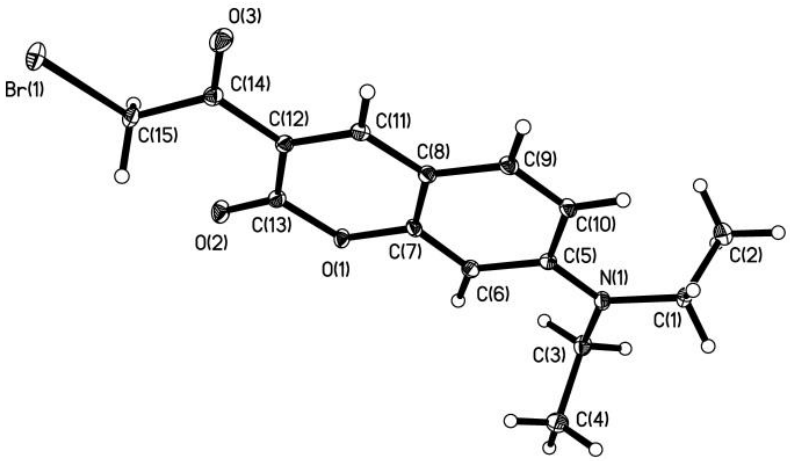
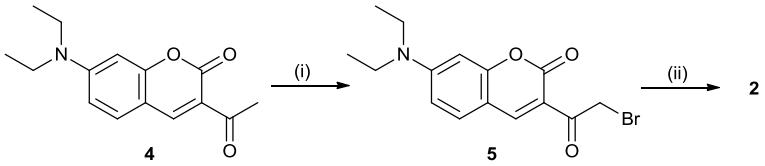


Figure 2. ORTEP plots of the single crystal structures of a) **7** and b) target coumarin **5** with 50% thermal elipsoids.



Scheme 1. Synthetic route to obtain the azidocoumarin **2**. *Regents and conditions*: i) $CuBr_2$, EtOH, 75 °C, 16 h [36] or Br_2, HBr/AcOH [37,38]; ii) NaN_3, THF, 45 °C, 18 h.

Table 1. Summary of the reaction conditions employed for the synthesis of **2**. All reactions were performed under an inert N_2 atmosphere and were either heated gently or were performed as previously reported [18, 37].

Entry	**Solvent**	**NaN_3 (Equiv.)**	**Temperature (°C)**	**Reaction time (h)**	**Yield of 2 (%)**
1[37]	DMF/AcOH	2	20	48	0
2	DMF	5	40	24	0
3	DMF	5	60	18	0
4[18]	NMP	1.5	20	24	0
5	EtOH	3	60	18	0
6	THF	5	45	16	80