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

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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.

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

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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 °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 °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**, Fig. 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, Fig. 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.

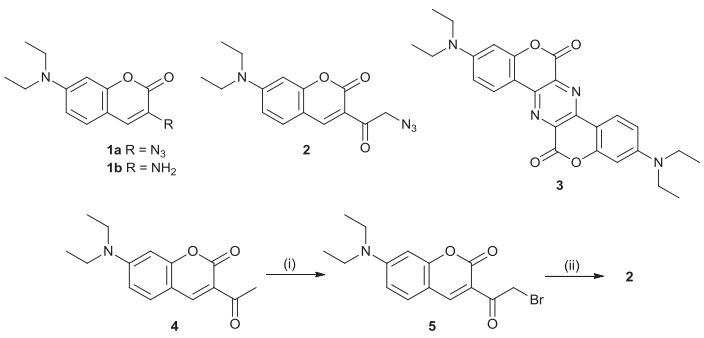
We then applied the procedure reported by Lin et al. [36] for the formation of **5** using $CuBr_2$ in excess as the bromine source, which we had initially viewed as a less attractive protocol. Gratifyingly, this procedure proved effective, although the target coumarin **5** was produced in a rather 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 its isolation 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, Fig. 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 recrystallization 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



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.