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Step-economic and cost effective synthesis of coumarin based blue emitting fluorescent dyes

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ABSTRACT

Cost effective and green protocols for the synthesis of two new series of coumarin based blue light emitting fluorophores named as 'Beta Fluors' and 'Alpha Fluors' are described. The coumarin alkylamide based Beta Fluors are developed using a one-step multi-component process in the presence of phenyl boronic acid as an efficient green catalyst. The Alpha Fluors are structured with coumarin–triazole–carboxamide peptidomimetics and their synthesis involves the 'click with MCR' concept. The new fluorophores gave high Stoke's shift values for the emission wavelengths and their structural features are promising for further fine tuning to obtain preferred emission maxima.

In the context of developing reactions that use catalysts based on inexpensive and non-toxic materials, boronic acids are attracting increased attention of both material and medicinal chemists.[1,2] Boronic acids are reported to be useful for catalyzing reactions such as direct activation of alcohols and carboxylic acids,[3] esterifications and amidations,[4] imine hydrolysis,[5] epoxide opening,[6] Biginelli reaction,[7a] cycloadditions,[7b,7c] aldol condensation,[8] Friedel Crafts alkylations,[9] Nazarov cyclization's, etc.[10] In spite of all these available examples, convenient methodologies based on BAC in terms of operational simplicity and economy for the development of advanced functional molecules are still in demand.

Fluorogenic probes are one of such advanced functional molecules capable of interacting with biological targets in vivo or in vitro to accomplish the identification of targets or analytes.[11] The interaction with targets causes changes in the spectroscopic properties of the probes and these changes can be used for decoding the information about the targets.[11] Fluorogenic probes are usually made by linking a signaling entity (which undergoes spectroscopic changes during interaction with target) with a labeling moiety (which enables reaction with the target) with or without the aid of a spacer.[11,12] Several fluorogenic probes are now available based on the use of fluorochromes such as coumarin, rhodamine, cyanine, naphthalene, quinoline, squaraine, xanthene, etc.[11b]

Among the various fluorogenic probes, the coumarin based ones are more prominent and the commercial versions of such fluorophores include Alexa Fluor® dyes, AMCA, (Fig. 1) and DyLight Fluors.[13,13a,13b] All these dyes are derivatives of aminomethylcoumarin carboxylic acids and most of them are substituted with electron attracting and electron repelling groups at their 3 and or 7 positions.

For organic fluorophores, it is necessary that, the absorbing part of the molecule must be structurally rigid. The structural rigidity is usually maintained by making the fluorophoric core as a substituted fused heterocyclic system[11b] or placing an exocyclic functional group that can impart rigidity to the whole system, for example, the exocyclic amide functionality in AMCA-X SE (Fig. 1). The synthesis of fused heterocycle based fluorophores requires multistep protocols leading to the escalation of cost of production and high market price. Similarly, the current versions of exocyclic rigid functionality fluorophores suffer the drawbacks such as autofluorescence, insufficient brightness for the emission wavelengths, low cell permeability and are used only with highly abundant targets.

Compared to the fused ring systems, the exocyclic rigid functionality based fluorophores are relatively cheap and researchers

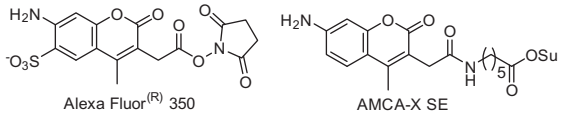

Figure 1. Representative examples of commercially available coumarin based blue emitting fluorescent dyes.

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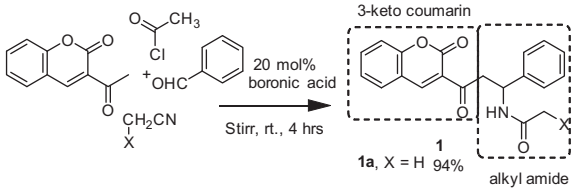
are now giving lots of attention to the development of their newer and cheaper versions. Among the various bioactive rigid functionalities, amide group or its surrogates are highly useful as privileged scaffolds for imparting stability, rigidity, and red shift in the emission maxima of small molecular probes. The recent activities of our research group are focused on the development of bioactive linear and cyclic peptidomimetics based on the fusion of amide groups or its surrogates on carbonyl compounds.[14] As part of our continuing interest in this area, here we report the synthesis of two new series of blue light emitting fluorogenic probes based on the fusion of amide and or its isosteres based peptidomimetics on coumarin core. The representative synthesis of fluorophore **1** is given in Scheme 1.

As shown in Scheme 1, compound **1** is prepared by condensing 3-acetyl coumarin with an aromatic aldehyde, acetyl chloride, and acetonitrile in the presence of 20 mol % of phenyl boronic acid at room temperature.[15] The aqueous work-up of the reaction mixture afforded near quantitative amount of the coumarin acetamide **1a** in high purity. Optimization reactions for the synthesis of **1a** were carried out for finding out the amount of catalyst and temperature requirements. The efficiency of phenyl boronic acid (**1A**) and 2,6-difluoro phenyl boronic acid (**1B**) for catalyzing this reaction at room temperature and at the boiling point of acetonitrile was studied. As given in Table 1, the room temperature reactions with 20 mol % of both the catalysts afforded maximum amount of products and the performance of both the catalysts was found to be almost equal. Since, **1A** is cheaper than **1B**, we used **1A** for further studies.

Following this protocol, we have synthesized 10 coumarin alkyl amides with various substitution patterns at the alkyl amide part (Table 2). The products with an electron withdrawing group at the acetamide phenyl ring gave better yield, compared to the products with electron donating groups at the same phenyl. A marginal decrease in yield was observed in the case of **1e** which was formed in 52% yield. In this case, we have isolated a side product from the reaction mixture (α-β-unsaturated ketone, 26%) formed via an aldol type reaction.

The fluorophoric properties of all the compounds were studied by measuring the absorption and emission spectra in dichloromethane from 0 to 10 pH. As a representative example, the normalized absorption and emission spectra of **1a** recorded in dichloromethane at neutral pH are given in Figure 2. Compound **1a** showed an absorption maxima centered at 345 nm and an emission maxima centered at 436 nm with high Stoke's shift values. These values were found to be stable to the changes in pH from 0 to 10. Fluorophores with high Stoke's shift values (the distance between the excitation maxima and emission maxima) are highly useful for bio-imaging applications, because, when using such compounds, there is no possibility of overlapping the excitation wavelengths with the emission wavelengths and therefore it is very easy to detect the fluorescence emission from biological targets.[16] All the compounds **1a–i** gave fluorescence emission at the blue emitting region with high Stoke's shift values and remain intact to changes in pH.

The substituent effects on the absorption/emission properties of **1a-j** did not follow any pattern. The molecules **1b, 1c**, **1f**, **1g**, and **1j**

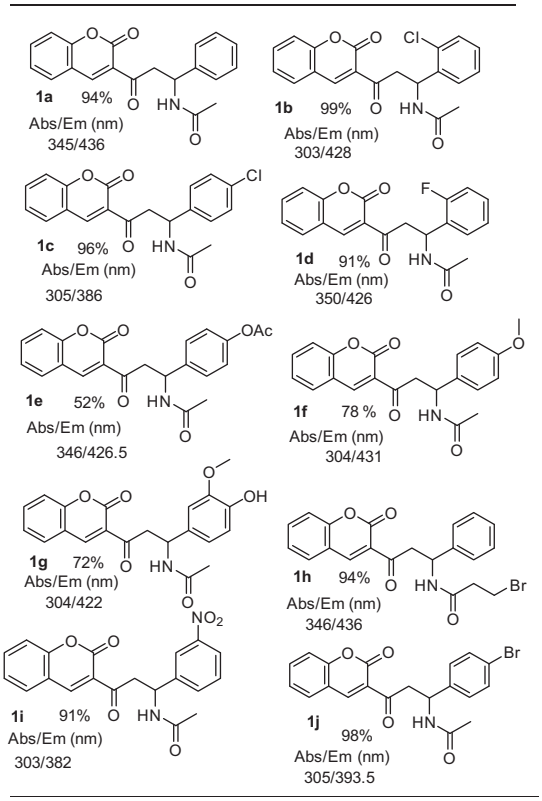


Scheme 1. Synthesis of fluorogenic coumarin alkyl amides based on a one pot four component reaction.

Table 1
Results of the optimization studies for the synthesis of **1a** using catalysts **1A** and **1B**

Entry	Catalyst	Loading (mol %)	T °C	Time (h)	Yield (%)
1	**1A**	5	rt	4	76
2	**1A**	10	rt	4	78
3	**1A**	15	rt	4	79
4	**1A**	20	rt	4	94
5	**1A**	20	70	4	76
6	**1B**	20	rt	4	95
7	**1B**	20	70	4	83

Table 2
List of Beta Fluors and their absorption/emission maxima values



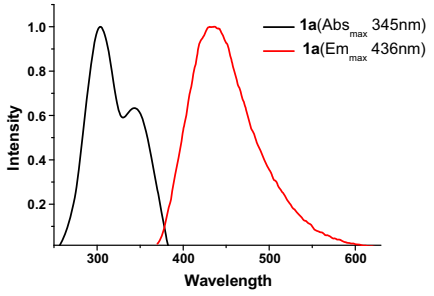
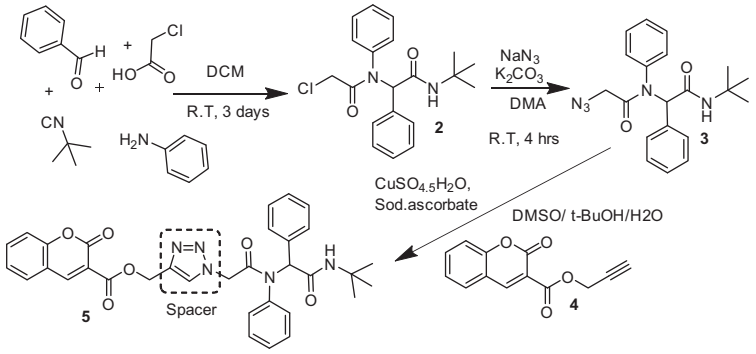


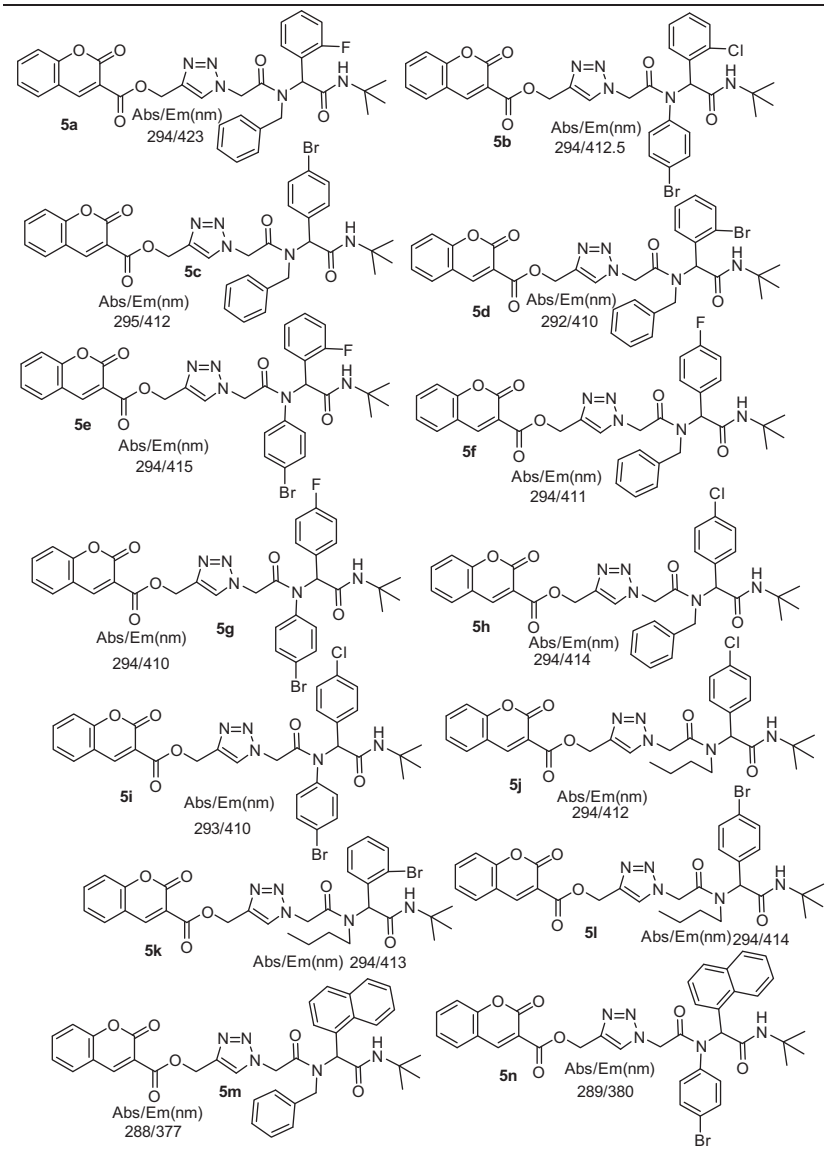
Figure 2. The normalized absorption and emission spectra of blue emitting 'Beta Fluor' **1a** in dichloromethane at neutral pH.

showed absorption maxima at 303–305 nm region and **1a**, **1d**, **1e**, and **1h** showed the same at 345–350 region. In the emission part, the molecules **1a**, **1b**, **1d**, and **1e–h** showed emission maxima at 422–436 region and **1c**, **1i**, and **1j** showed the same at 382–393 region. Since the alkyl amide part of **1a–j** are β-amido ketones



Scheme 2. Synthesis of 'Alpha Fluors' **5** based on azide–alkyne [3+2] cycloaddition between propargyl esters of coumarin and α-(azido) acylamino acetamides.

Table 3
List of Alpha Fluors prepared and their absorption/emission values



and are considered as β-amino acid derivatives, we decided to name our molecules as 'Beta Fluors'. During the bio-profiling experiments, these new fluorophores are expected to cleave at the alkyl amide bond to release highly fluorescent β-amino coumarin moieties.[17]

Encouraged by the fluorescence properties obtained for the coumarin alkyl amides **1a–j**, we decided to study the fluorophoric properties of the peptidomimetic versions of the structure **1.** In one of our previous communications,[18] we reported the synthesis of such peptidomimetics using click chemistry[19] as a ligation tool

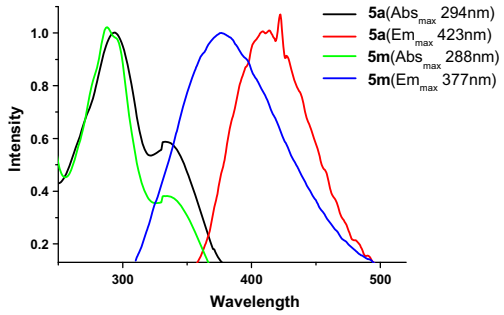


Figure 3. The normalized absorption and emission spectra of Alpha Fluor **5a** and **5m** in dichloromethane at neutral pH.

for connecting multicomponent reaction products. Following our reported methodology, we have analyzed 14 such coumarin peptidomimetics with a general structure **5** and their representative synthetic route is given in Scheme 2.

As shown in Scheme 2, the α-(azido) acylamino acetamides **3** were synthesized by following the classical four-component Ugi reaction[20] between chloroacetic acid, alkyl/aryl amines, substituted aromatic aldehydes, and *t*-Butyl isocyanaide. The chloro derivatives **2** thus obtained were converted to the corresponding azides **3** by base catalyzed reaction with sodium azide.[21] The azides were then clicked with propargyl coumarin esters **4** under the conditions given in Scheme 2 to afford the coumarin–triazole–carboxamide conjugates **5**.[21] These compounds belong to the category of fluorophores with a spacer (1,2,3 triazole in the present case) in-between the signaling and labeling entities. Following the same protocol, we synthesized compounds **5a**–**n** and their structures are given in Table 3.

The absorption and emission spectra of **5a**–**n** were recorded in dichloromethane at 0–10 pH. For example, **5a** when subjected to excitation at 294 nm showed fluorescence emission in the blue region with a maxima centered at 423 nm and also with high Stoke's shift value (Fig. 3). Similar to Beta Fluors **1a**–**j,** the fluorescence properties of **5a**–**n** were also intact with changes in pH. Compounds **5m** and **5n** having a naphthyl group in the carboxamide part showed a blue shift of 46 and 49 nms, respectively, in the emission maxima with respect to the same obtained for **5a** and maintained the Stoke's shift values (Fig. 3). The presence of the spacer triazole in **5a**–**n** can impart better stability to the molecules and also it is possible to drive the enzyme action to the ester or amide sights to release highly fluorescent coumarin derivatives during imaging processes. Since compounds **5a**–**n** contain carboxamide moieties and are considered as derivatives of α-amino acids, we named our compounds **5a**–**n** as 'Alpha Fluors'.

The absorption and emission spectra of Beta Fluors **1a**–**j** and Alpha Fluor **5a**–**n** were compared with the same of the commercially available blue emitting coumarin based fluorescent probe Alexa Fluor 350 and DyLight∗350. The fluorescence spectra for the commercial molecules were obtained from web sources and preliminary comparisons revealed that, the fluorescence properties of **1a**–**j** are comparable with Alexa Fluor 350 and the same for **5a**–**n** are comparable with DyLight∗350. As pointed out by one of the referees during the evaluation process of this manuscript, detailed investigations under identical conditions are required to substantiate this claim and that will be addressed in the full paper version of this communication.

In conclusion, we have demonstrated a low-cost and green synthesis of two types of fluorogenic probes with blue emission wavelengths and comparable performance equivalency with commercial molecules of the same category. For the one step synthesis of the first category fluorophores named as 'Beta Fluors', we have established the usefulness of phenyl boronic acid as an effective green catalyst. For the three-step synthesis of the second category fluorophores named as 'Alpha Fluors', we effectively linked the concept of click chemistry with multi-component reaction strategies. The final molecules are featured with the presence of enough number of diversity points useful for substitution with electron withdrawing or donating groups for fine tuning the emission properties. We hope that the low cost and step-economic probes reported in this Letter will be useful for academic and industrial R&D researchers who are engaged in bio-imaging and cell tracking studies.

Supplementary data

Supplementary data (copies of ^{1}H NMR, ^{13}C NMR and FT-IR and MS of selected Alpha Fluors and Beta Fluors) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.06.071.

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15. *Typical experimental procedure for the synthesis of 'Beta Fluor' **1a**:* A 100 mL round-bottomed flask was charged with anhydrous acetonitrile (10 mL), benzaldehyde (0.212 g, 2 mmol), 3-acetyl coumarin (376 mg, 2 mmol), acetyl chloride (4 mL), and phenyl boronic acid (20 mol %) under constant stirring at room temperature. The reaction was monitored by TLC and was found to be complete after 4 h. The mixture was then poured into distilled water and kept for 1 h. The precipitated colorless solid was collected on a filter, washed with distilled water (3 × 25 mL), and dried under vacuum. The vacuum-dried solid was then washed with anhydrous diethyl ether (2 × 15 mL) and air dried to obtain **1a** in pure form (630 mg, 94%). FT-IR (KBr): 3318, 2953, 2851, 1747,

1681, 1648, 1610, 1560, 1493, 1448, 1369, 1174, 982,831,764 and 543 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 8.65 (s, 1H), 8.35–8.33 (d, J = 8 Hz, 1H), 7. 97–7.95 (d, J = 8 Hz, 1H), 7. 78–7.74 (t, 1H), 7.49–7.41 (m, 2H), 7.37–7.30 (m. 4H), 7.25–7.20 (t, 1H), 5.42–5.36(m, 1H), 3.59–3.53(dd, J = 8 Hz, J = 24 Hz, 1H) 3.45–3.38(dd, J = 12 Hz and J = 28 Hz, 1H), 1.79 (s, 3H)

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21. *Typical Experimental Procedure for the three stage synthesis of Alpha Fluor* ***5b****: Stage 1: Synthesis of carboxamide azide* ***3b****:* An equi-molar amount of 2-chloro benzaldehyde (700 mg, 0.005 mol) and 4-bromoaniline (851 mg, 0.005 mol) is dissolved in dichloromethane (8 mL) and stirred at room temperature for 20 min to form the Schiff-base. To this, one equivalent of *tert*-butyl isocyanide (0.83 g, 0.01 mol) and chloroacetic acid (0.95 g, 0.01 mol) was added and stirred at room temperature for 48 h. After 48 h, the solvent was removed under vacuum to obtain the crude product. It was washed with petroleum ether (5 × 15 mL) to afford the pure carboxamide chloride **2b** (876 mg, 92%). *Stage 2: Synthesis of carboxamide azide* ***2b****:* One equivalent of **2b** (236 mg, 0.05 mol) and sodium azide (1 g) is taken in dimethyl acetamide (6 mL). To this K_2CO_3 (1 g) was added and stirred at room temperature for 4 h. After 4 h, the reaction mixture was diluted with water to obtain **3b** as white precipitate. It was collected and washed repeatedly with water to obtain the pure azide **3b** (219 mg, 91%). Compound **3b:** FT-IR, KBr, γ_{max}: 3392.2, 3343.9, 2965.0, 2928.4, 2103.9, 1686.4, 1655.0, 1538.9, 1485.9, 1391.4, 1257.4, 1014.4, 743.4; ^1H NMR (400 MHz, DMSO-d_6): δ 8.09 (s, 1H), 7.37–7.35 (d, J = 7.6 Hz, 2H), 6.94–6.93 (d, J = 7.6 Hz, 2H), 7.20–7.172 (m, 2H), 7.10–7.07 (m, 2H), 6.61 (s, 1H), 3.8–3.53 (m, 2H), 1.26 (s, 9H). *Stage 3: Synthesis of Alpha Fluor* ***5b****:* One equivalent of propargyl coumarin ester **4** (114 mg, 0.05 mol) and 239 mg (0.05 mol) of the azide **3b** were dissolved in minimum amount of DMSO. To this, 2 ml of *t*-BuOH, 1 mL of water, $CuSO_4{\cdot}5H_2O$ (300 mg), and sodium ascorbate (300 mg) are added and stirred in room temperature for 20 h. After 20 h, the mixture was poured into cold water to obtain the crude precipitate of **5b**. It was then repeatedly washed with water and finally with a small amount of diethyl ether to obtain the white powder of **5b** (338 mg, 96%). FT-IR, KBr, γ_{max}: 3312, 2970, 2933, 2107, 1773, 1706, 1671, 1610, 1565, 1484., 1391, 1206, 764; ^1H NMR (400 MHz, DMSO-d_6): δ 8.74 (s, 1H), 8.13 (S, 1H), 8.01 (s, 1H), 7.92–7.94 (d, J = 8.0 Hz 2H),7.73–7.70 (m, 1H), 7.53–7.52 (d, J = 7.6 Hz, 1H), 7.42–7.36 (m, 3H), 7.11–7.08 (m, 3H), 6.92–6.90 (d, J = 7.2 Hz, 2H), 6.26 (s, 1H), 5.29 (s, 2H), 5.16–4.34 (m, 2H), 1.24 (s, 9H); MS: m/z Calcd for $C_{33}H_{29}N_5O_6BrCl$, 706.9; Found, 706.0.