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Sensing the chemical cleavage of fluorescent β-lactams via FRET/exciplex or excimer emission

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ABSTRACT

We report herein the design, synthesis of C6-triazolyl donor-acceptor fluorescent penicilines. Two of the fluorescent penicilines have been utilised for studying the photophysical outcome after ring cleavage by an external fluoregenic amine as nucleophile. Thus, the chemical cleavage of β-lactam rings is signaled by interesting photophysical phenomena-dual path to exciplex emission or via excimer emission.

Ever since the discovery of β-lactam antibiotic, it has got tremendous applications for treating microbial diseases [1]. However, resistance against almost all β-lactam antibiotics including the broadest spectrum carbapenems by metallo-β-lactamases (MBLs) is at an alarming stage that drives the continuous modification of known and the development of new effective β-lactam antibiotics [2,3]. Several research reports have depicted that modification of the N-acyl side chain and attaching a membrane-penetrating hybrid functionality may lead to a fruitful design of modified penicillines which could address the existing shortcomings of β-lactam anibiotics [1a,4]. Though there are few reports of such modified penicilines, however, these are still prone to hydrolysis by β-lactamases, thereby, demanding newer design concept and candidates to be produced to combat bacterial resistance [1a,5,6]. Furthermore, a number of fluorescent probes, though, have been reported for the detection of the activity of β-lactamases, however, most probes lack specificity or need helps of other inhibitors. [7,8] Given the challenges, the development of new fluorescent β-lactams would be valuable.

Considering both the challenges of β-lactam family and as a part of our continuous research efforts toward the design of fluorescent biomolecular building blocks [9a–c] and installation/modulation [9d] of fluorescence property via azide-alkyne 1,3-dipolar cycloaddition reaction, we took up a project to generate fluorescent β-lactams, particularly triazolyl donor/acceptor chromophore decorated fluorescent penicillanic acids. We envisaged that replacement of the acid sensitive N-6 acyl bond of peniciline with a triazole ring containing donor/acceptor aromatics might lead to stable and active β-lactams (Fig. 1) [1a,10]. It is also logical to think that the electron charge density at C-3 of β-lactam core might influence the electrophilicity of lactam carbonyl against the nucleophilic attack. Furthermore, we envisaged that the triazole unit would modulate the fluorescence property of the attached donor-acceptor aromatics as well as render stable electronic property onto the lactam ring [1a,6]. Thus, it would be worthwhile to investigate the stability of modified β-lactam ring toward chemical cleavage with a fluorogenic hydroxyl or amines as nucleophiles. The ring cleavage would then be signaled by a new fluorescence property possibly at a higher wavelength region by virtue of a photophysical interaction among the two fluorophores in the ring-opened form. Therefore, a fluorescence based method could be achieved for testing the stability of β-lactams in presence of an external fluorogenic nucleophile.

Our concept of generation of new fluorescence property in the ring opened form relies on the following fact (Fig. 1). The fluorescent β-lactams would show the emission signal of their respective triazolyl-chromophores. After nucleophilic ring opening the two fluorophores would be in close proximity which would result in dipolar photophysical/π-π-stacking interaction in the excited state. Therefore, for the case of hetero-chromophoric pair wherein the chromophore in the probe β-lactam and that in the incoming nucleophile are different, either dipolar photophysical

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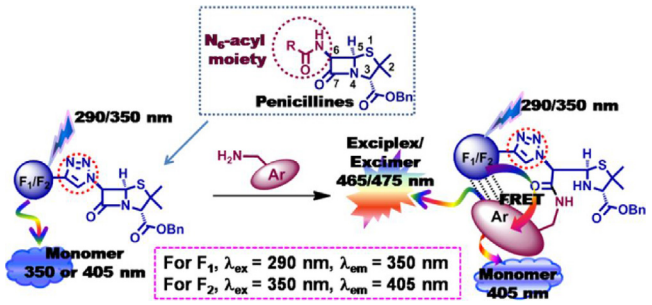


Fig. 1. The structure of N_6-acyl penicilline and the schematics of fluorescence discrimination of triazolyl β-lactam from the ring opened form.

interaction leading to FRET emission or π-π-stacking interaction leading to exciplex emission or both would be the result. On the other hand, if the second chromophore in the nucleophile becomes the same as in the probe β-lactam then a homo-chromophoric system would be generated which would be held by π-π-stacking interaction leading to an excimer emission (Fig. 1). Thus, the concept of change in fluorescence intensity and emission wavelength upon ring cleavage would provide insight into the stability of the β-lactams. It is worthwhile to mention that these model β-lactams can not be used directly for the fluorimetric detection ring opened status under the action of β-lactamases. A new design is obvious which is our future focus. However, this model chemical platform expectedly would shed light for the design of such other conceptual fluorescent β-lactam probes such as dual fluorescent labeled β-lactams which could be utilised to monitor the activity of β-lactam antibiotic fluorimetrically while β-lactamase would act upon ring opening.

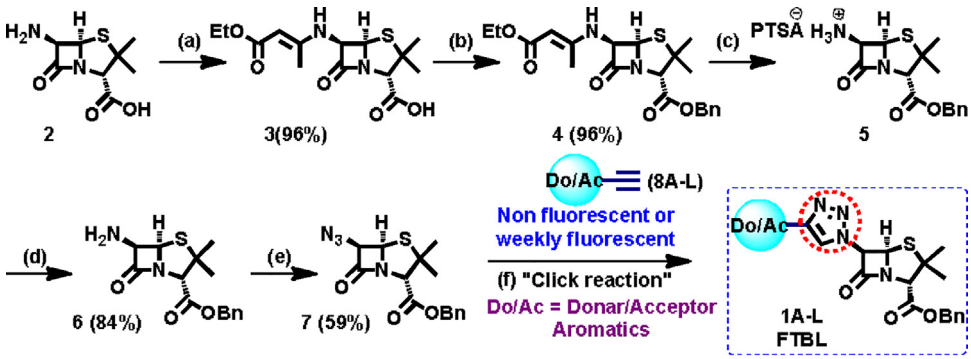
With this concept, we aimed to (a) generate triazolyl fluorescent β-lactams and (b) investigate the change in photophysical property upon chemical cleavage. The β-lactam ring was thus attempted to cleave with fluorogenic amine as nucleophile. We targeted two fluorescent β-lactams-one with triazolylmethoxy naphthyl (**TMNap**) and other with triazolyl pyrene (**TPy**) chromophore unit-for the model ring opening by a fluorogenic nucleophile, namely, aminomethyl pyrene (**10**, **AMePy**). Thus, upon ring cleavage we observed FRET as well as exciplex emission and established dual mechanism for exciplex emission in the β-lactam with triazolylmethoxy naphthalene (^{TMNap}**β-Lac^{Do}**, **1F**) and an excimer emission in triazolylpyrene (^{TPy}**β-Lac^{Do}**, **1H**) containing β-lactam.

The synthesis of the triazolyl β-lactams started with 6-aminopenicillanic acid (**2**), the amino functionality of which was converted to azide (**7**) following a modified literature protocol (Scheme 1). First of all the 6-aminopenicillanic acid (**2**) was converted to *N,-C-* diprotected penicillanic acid **4** in good yield. Next, the 6-amino penicillanic-3-benzyl carboxylate ester **6** was obtained via deprotection of 6-*N*-protecting group with *p*-toluene sulfonic acid and subsequent neutralization of PTSA salt **5** with $NaHCO_3$ in water. Ultimately, the free 6-amino group of **6** was converted to azide via diazo-transfer reaction with triflylazide in toluene to afford only azide **7** in very good yield (Scheme 1). All the intermediates and the azide were purified by silica gel column chromatography and were well characterized by NMR and mass spectrometry. The azido transfer reaction underwent with retention of configuration which was supported by the β-orientation of the azide group in **7** as was revealed from coupling constant value from ^{1}H NMR ($J_{5,6}$ = 4.0 Hz) [11]. After having the 6-azido penicillanic-3-benzyl carboxylate ester **7** in pure form various alkynes of donor/acceptor substituted aromatics and polyaromatic hydrocarbons were subjected to react with **7** under click reaction condition to obtain the desired designed 6-(donor/acceptor aromatic substituted)triazolyl- penicillanic-3-benzyl carboxylates **1A-L** in very good to excellent yields (Scheme 1 and SI, Fig. S1). All the compounds were characterized by NMR and mass spectrometry.

After getting all the β-lactams in pure form we had chosen two fluorescent β-lactams, namely, **1F** (^{TMNap}**β-Lac^{Do}**) and **1H** (^{TPy}**β-Lac^{Do}**) to examine the change in fluorescence photophysical property upon chemical cleavage of β-lactam ring by a nucleophilic amine. In case of β-lactam **1F** we used benzyl amine **9** as a test reaction which underwent smoothly at room temperature in absence of any base in dry dichloromethane (Scheme 2).

An attempt to cleave with benzyl alcohol was unsuccessful even at stringent condition. Next, the same reaction was carried out in dry THF solvent in presence of Et_3N at 80–85 °C using aminomethyl pyrene **10** as a pro-fluorescent nucleophile envisioning that in the ring-opened form **12** the triazolylmethoxy naphthalene (**TMNap**) and the aminomethyl pyrene (**AMePy**) moieties would show photophysical interaction property and expectedly would result in a FRET emission from aminomethyl pyrene (**AMePy**) upon excitation at **TMNap** as well as an exciplex emission either via FRET or via direct excitation at FRET acceptor, **AMePy** (Fig. 1). On the other hand, in the second example of β-lactam **1H**, we expected an excimer formation in the ring-opened form **13** between the triazolyl pyrene (**TPy**) and the aminomethyl pyrene (**AMePy**) moieties which would ultimately lead to the generation of an excimer emission upon excitation at 350 nm (Fig. 1). The structures of all the ring-opened β-lactams are depicted in Scheme 2.

To test our concept of fluorimetric detection of ring-opened status and to discriminate fluorescent β-lactams, we next, studied



Scheme 1. Synthesis of targeted fluorescent triazolyl β-lactams.