ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 6713–6715

www.rsc.org/chemcomm

COMMUNICATION

A novel and unusually long-lived chemiluminophore based on the 7-hydroxycoumarin scaffold†

Jan Kočí,[ab] Virgile Grandclaude,[ab] Marc Massonneau,[b] Jean-Alexandre Richard,[ab] Anthony Romieu*[ac] and Pierre-Yves Renard*[acd]

Received 4th April 2011, Accepted 21st April 2011
DOI: 10.1039/c1cc11919b

Synthesis and chemiluminescent properties of a new 1,2-dioxetane chemiluminophore bearing a 7-hydroxycoumarin moiety are presented. The 1,2-dioxetane decomposition ended up with strong and long-lived emission of light. This new structure opens way to the development of a new generation of bright chemiluminescent bio-probes.

Over the past decade, based on the strained and unstable intermediate polyoxygenated rings formed in the bioluminescence processes, many 1,2-dioxetane structures have been developed as convenient tools for chemiluminescence (CL) studies[1] and to design chemoluminogenic probes suitable for sensitive detection and quantitation of various bio-analytes in complex biological mixtures.[2] The origin of the CL for these 1,2-dioxetanes is a Chemically Initiated Electron Exchange Luminescence phenomenon (CIEEL): the deprotonation of a hydroxyaryl group bearing a 1,2-dioxetane unit (preferentially at the *meta* position of the aromatic relative to the phenolate) generates an unstable oxidoaryl-substituted 1,2-dioxetane which undergoes intramolecular charge-transfer-induced decomposition (CTID) to produce a singlet-excited carbonyl fragment while effectively emitting light.[3]

Among the required stabilising moieties, the dihydrofuran ring has been described in the late 90's as a convenient scaffold to obtain 1,2-dioxetanes exhibiting a remarkable thermal stability.[4] Thus, many examples of such oxy-substituted dioxetanes have been synthesised during these last few years.[5]

The main drawback of these structures is their light emission wavelengths, which are too close to the UV region for their successful use in some bioanalyses and bioimaging applications due to the significant absorption of the emitted light by some biomaterials.[6] The use of relay fluorophores can be an alternative to red-shift this short-wavelength emission. Thus, two different strategies are generally adopted to transfer the energy released by the chemiluminescent reaction to the fluorophore moiety. The first strategy relies on the connection of chemiluminophore and fluorescent dye by a linker which allows an efficient chemiluminescence resonance energy transfer (CRET)[7] or through bond energy transfer (TBET).[8,9] The second strategy is based on the concomitant encapsulation of chemiluminophore and fluorescent dye within the same confined environment of nanoparticles or micelles.[10]

To expand the scope of the first strategy to a wider range of fluorescent reporter molecules, we wish to describe in this communication the challenging synthesis and luminescent properties of a new chemiluminophore **1** based on a dihydrofuran ring directly bound at the 5-position to a pro-fluorescent 7-hydroxycoumarin unit (Fig. 1).

Indeed, the 7-hydroxycoumarin moiety is well-known for its pro-fluorescent properties: substitution of its phenol by any electron-withdrawing group completely abolishes its fluorescence.[11,12] By attaching a 1,2-dioxetane moiety at the 5-position of a 7-acetoxycoumarin unit (*i.e.*, in the *meta* position to the 1,2-dioxetane required for efficient CIEEL), it will be possible to study the unveiling luminescence associated to the phenol deprotection reaction which should end up with a simultaneous release of the fluorescent properties of the 7-hydroxycoumarin and of the electron exchange based luminescence.

7-Hydroxycoumarins are usually synthesised by the Pechmann reaction, starting from the corresponding resorcinol and β-keto acid (or ester) and involving acidic conditions.[13]

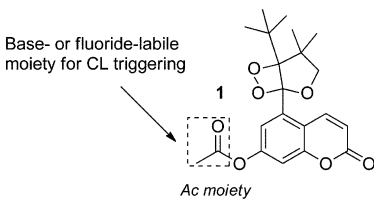
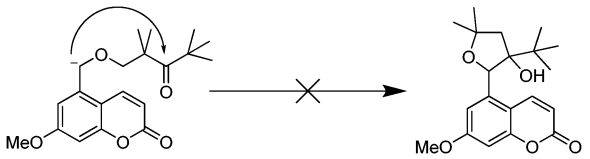


Fig. 1 Chemiluminophore–fluorophore hybrid studied in this work, whose CL reaction is based on the phenol deprotection.

[a] *Equipe de Chimie Bio-Organique, COBRA-CNRS UMR 6014 & FR 3038, rue Lucien Tesnière, 76131 Mont-Saint-Aignan, France. E-mail: anthony.romieu@univ-rouen.fr, pierre-yves.renard@univ-rouen.fr; Web: http://ircof.crihan.fr; Fax: +33 2-35-52-29-71; Tel: +33 2-35-52-24-27 or 24-76*
[b] *QUIDD, Pharmaparc II, bâtiment D, Voie de l'innovation, 27100 Val-de-Reuil, France. E-mail: mcmm@quidd.com; Web: http://www.quidd.com; Fax: +33 2-32-59-67-65; Tel: +33 2-32-63-46-91*
[c] *Université de Rouen, Place Emile Blondel, 76821 Mont-Saint-Aignan, France*
[d] *Institut Universitaire de France, 103 Boulevard Saint-Michel, 75005, Paris, France*

† Electronic supplementary information (ESI) available: Detailed synthetic procedures and characterisation/spectral data for compounds **1–11**. See DOI: 10.1039/c1cc11919b

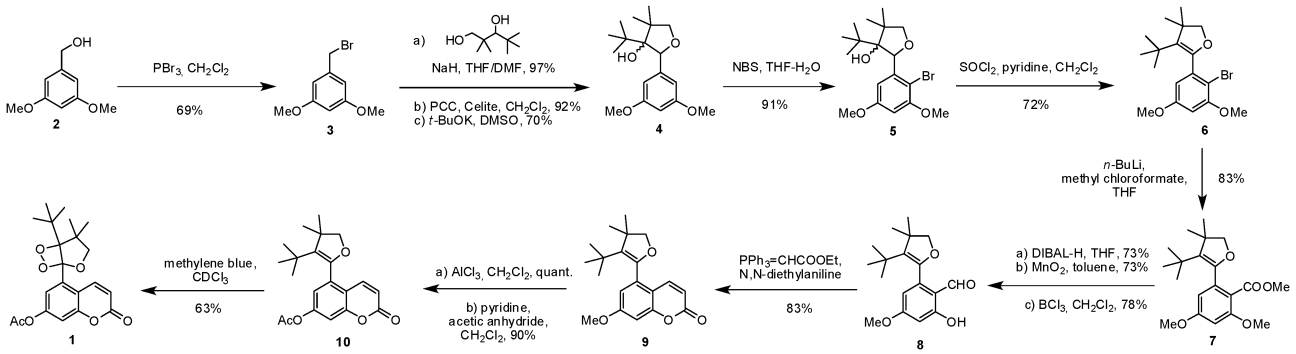


Scheme 1 Failure of the first synthetic route.

Thus, we first planned the preparation of **1** through the introduction of the functionalised alkyl chain required for the formation of the 1,2-dioxetane moiety, on the 5-position of a 7-methoxycoumarin derivative readily obtained from standard condensation (*vide supra*), and to build the tetrahydrofuran ring thereafter (Scheme 1).

Unfortunately and despite our efforts, the crucial synthetic step which consists of the intramolecular cyclisation of the benzylic anion to the ketone to form the tetrahydrofuran ring, which requires strongly basic conditions, did not generate the expected compound. Contrary to our expectations, the lactone ring did not suffer much from these conditions (especially ring opening), but we suspected that the electron delocalisation within the coumarin ring dramatically lessens the nucleophilicity of the formed benzyl-type carbanion, thus preventing the desired ring cyclisation. Using harsher reaction conditions eventually destroyed the coumarin unit. To overcome this failure, we turned our attention to an alternative and more challenging approach which involves: (1) the synthesis of the tetrahydrofuran ring, (2) the construction of the coumarin ring under unusual conditions and finally (3) the formation of the fragile 1,2-dioxetane moiety (Scheme 2).

The coumarinic 1,2-dioxetane **1** has been synthesised from commercially available (3,5-dimethoxyphenyl)methanol **2**. This latter primary alcohol was brominated with PBr_3 to obtain the corresponding benzyl bromide derivative **3** in 69% yield. Substitution reaction with 2,2,4,4 tetramethylpentane-1,3-diol provided quantitatively the intermediate hydroxyl ether which was oxidised with pyridinium chlorochromate (PCC) to afford the corresponding ketone in 92% yield. Use of this rather drastic oxidant was required, since all attempts using milder conditions: Swern oxidation, IBX or TPAP/NMO system gave poor yields. Next, we found that the best conditions for the cyclisation involved the use of freshly prepared dimethyl sulfoxide anion (potassium dimsyl) as a strong base to form the corresponding tetrahydrofuran ring **4** in 70% yield.[14] In order to convert the resorcinol dimethyl ether **4** into the corresponding coumarin, we decided to generate the required lactone through a less commonly used Wittig reaction, which involved the introduction of a carbonyl moiety at the 1-position of the phenyl ring. Direct metallation of this position gave low yields. However, an efficient and selective bromination using *N*-bromosuccinimide in THF–water (95 : 5) gave the product **5** with a high yield (91%). This bromination reaction was best performed prior to the elimination process leading to the dihydrofuran derivative **6**. This latter reaction involved standard chlorination of the tertiary alcohol followed by spontaneous (and/or pyridine-mediated) elimination to form **6** with a good yield (72%). Thereafter, bromine–lithium exchange was performed using *n*-BuLi, and the aryllithium intermediate was trapped by methyl chloroformate to give methyl ester **7** in 83% yield. Further conversion into aldehyde was obtained by using a two-step reduction–oxidation sequence. Thus, methyl ester **7** was firstly treated with DIBAL-H and the resulting primary alcohol was oxidised with MnO_2 in suspension in anhydrous toluene under reflux to give the expected benzaldehyde in 53% overall yield. The formation of the lactone ring has required the selective deprotection of one of the two methoxy groups. We were pleased to observe that the presence of the *ortho*-aldehyde allowed the selective removal of the desired methyl group with BCl_3 to afford the key benzaldehyde derivative **8**. This product was directly submitted to a Wittig reaction, involving the ylide derived from (2-ethoxy-2-oxoethyl)triphenylphosphonium salt, in anhydrous *N*,*N*-diethylaniline as solvent. The lactone ring was readily formed to obtain 7-methoxycoumarin **9** in a good yield (83%). As expected, since the first methoxy group was very selectively removed, the second demethylation reaction of the 7-position of the coumarin core was by far trickier. We found that it could be achieved in quantitative yield by treatment of **9** with a large excess of $AlCl_3$ in dry CH_2Cl_2 under reflux. Subsequent protection of the released phenol with an acetyl group under standard conditions afforded the 7-acetoxycoumarin **10**. Finally, the 1,2-dioxetane moiety was generated through a [2 + 2] cycloaddition reaction between singlet oxygen and the enol ether of **10**. $^1O_2$ was generated under mild conditions by standard type II photosensitisation (*i.e.*, oxygen, visible light and methylene blue as photosensitiser). The 1,2-dioxetane **1** was found to be stable enough to be purified by chromatography on a silica gel column and finally recovered in 63% yield. All spectroscopic data, in particular NMR and mass spectrometry, are in agreement with the structure assigned (see ESI†). Interestingly, this chemiluminophore



Scheme 2 Synthesis of coumarinic chemiluminophore **1**.

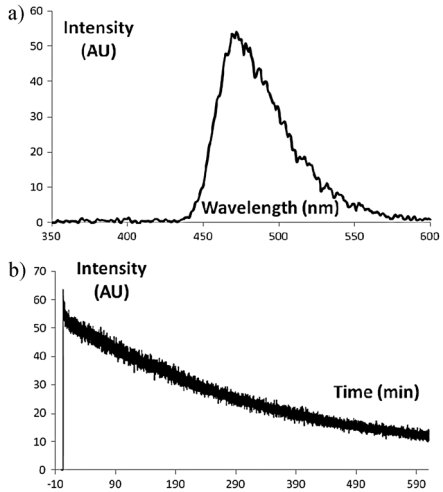
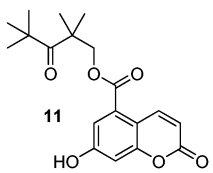


Fig. 2 CL spectra of **1** in DMSO (after adding 1.0 M TBAF in THF, 3% v/v) at 25 °C (concentration: 170 μM): (a) scan mode, (b) kinetic mode at λ = 470 nm.

was found to be full-stable for few days at room temperature as inferred from ^{1}H NMR analyses.

The photophysical properties of 7-acetoxycoumarin–dioxetane hybrid **1** were evaluated in DMSO. The UV-vis absorption spectrum displayed a broad band between 260 and 340 nm with two maxima located at 278 (ε 9700 dm^3 mol^{-1} cm^{-1}) and 321 nm (ε 8200 dm^3 mol^{-1} cm^{-1}) (see ESI†). The hypsochromic shift of this absorption spectrum relative to the free fluorophore (60 nm) is in agreement with the spectral behaviour currently observed for phenol-based pro-fluorescent probes.[12,15,16] Furthermore, as expected, this compound was found to be virtually non-fluorescent and its quantum yield was too low to be accurately measured. The chemiluminescent characteristics of **1** were also determined in DMSO after adding 1.0 M TBAF in THF (basic fluoride ions) which acts as a CL triggering through the removal of the acetyl group and subsequent generation of the phenolate species.[17] Fig. 2a shows the CL spectrum of **1**. This compound was confirmed to emit blue light with a single maximum at 470 nm. This luminescence peak apparently originated from the coumarin unit of **1**, as inferred from the similarity between Fig. 2a and the fluorescence emission spectrum of the coumarin **11** released by the TBAF-induced chemiluminescent reaction (see ESI†). Thus, the luminescence energy produced at the 1,2-dioxetane moiety was completely transferred to the energy acceptor coumarin.



Furthermore, light emission was very strong once the trigger was added, and its kinetic shape presents a long-lived decay, which is a significant advantage in biological assays (Fig. 2b). Indeed, significant light emission at 470 nm was still detected even 10 hours after the initial TBAF-mediated phenolate release.

We have successfully synthesised 7-hydroxycoumarin-attached 1,2-dioxetane **1** to develop a novel approach to red-shift emission of such chemiluminophores. Indeed, the CL studies have revealed that the luminescence energy produced at the 1,2-dioxetane moiety is efficiently transferred to the coumarin unit to emit light at the maximum wavelength of this fluorophore. Interestingly, functionalisation of its 4-position with a bio-orthogonal "handle" (*e.g.*, an acetic acid pendant arm) should make possible some applications in bio-labelling. Further efforts are also devoted to the implementation of this promising strategy to red-emitting fluorescent phenols such as conjugation-extended 7-hydroxycoumarin NIR dyes recently reported by us.[15]

This work was supported by La Région Haute-Normandie *via* the CRUNCh program (CPER 2007–2013) especially for a post-doctoral fellowship to Jan Kočí, and Institut Universitaire de France (IUF). We thank Annick Leboisselier (INSA de Rouen) for the determination of elemental analyses.

Notes and references

1 For selected reviews, see: (*a*) M. Matsumoto, *J. Photochem. Photobiol., C*, 2004, **5**, 27; (*b*) W. J. Baader and E. L. Bastos, in *Science of Synthesis: Houben–Weyl Methods of Molecular Transformations*, ed. A. Berkessel, Thieme-Chemistry, Stuttgart, 2008, vol. 38, p. 323.
2 For selected recent examples, see: (*a*) L. Liu and R. P. Mason, *PLoS One*, 2010, **5**, e12024; (*b*) J.-A. Richard, L. Jean, A. Romieu, M. Massonneau, P. Noack-Fraissignes and P.-Y. Renard, *Org. Lett.*, 2007, **9**, 4853.
3 (*a*) F. McCapra, *J. Photochem. Photobiol., A*, 1990, **51**, 21; (*b*) A. P. Schaap and S. D. Gagnon, *J. Am. Chem. Soc.*, 1982, **104**, 3504; (*c*) A. P. Schaap, R. S. Handley and B. P. Giri, *Tetrahedron Lett.*, 1987, **28**, 935.
4 M. Matsumoto, N. Watanabe, N. C. Kasuga, F. Hamada and K. Tadokoro, *Tetrahedron Lett.*, 1997, **38**, 2863.
5 M. Matsumoto, N. Watanabe, N. Hoshiya and H. K. Ijuin, *Chem. Rec.*, 2008, **8**, 213.
6 G. A. Wagnières, W. M. Star and B. C. Wilson, *Photochem. Photobiol.*, 1998, **68**, 603.
7 X. Huang, L. Li, H. Qian, C. Dong and J. Ren, *Angew. Chem., Int. Ed.*, 2006, **45**, 5140.
8 J. Han, J. Jose, E. Mei and K. Burgess, *Angew. Chem., Int. Ed.*, 2007, **46**, 1684.
9 For the application of this concept to ROS-sensitive chemiluminescent probes, see: (*a*) R. Saito, A. Ohno and E. Ito, *Tetrahedron*, 2010, **66**, 583; (*b*) M. Sekiya, K. Umezawa, A. Sato, D. Citterio and K. Suzuki, *Chem. Commun.*, 2009, 3047.
10 (*a*) J. M. Baumes, J. J. Gassensmith, J. Giblin, J.-J. Lee, A. G. White, W. J. Culligan, W. M. Leevy, M. Kuno and B. D. Smith, *Nat. Chem.*, 2010, **2**, 1025; (*b*) M. Dasari, D. Lee, V. R. Erigala and N. Murthy, *J. Biomed. Mater. Res., Part A*, 2009, **89**, 561; (*c*) D. Lee, S. Khaja, J. C. Velasquez-Castano, M. Dasari, C. Sun, J. Petros, W. R. Taylor and N. Murthy, *Nat. Mater.*, 2007, **6**, 765.
11 X. Chen, M. Sun and H. Ma, *Curr. Org. Chem.*, 2006, **10**, 477.
12 K.-I. Setsukinai, Y. Urano, K. Kikuchi, T. Higuchi and T. Nagano, *J. Chem. Soc., Perkin Trans. 2*, 2000, 2453.
13 S. Sethna and R. Phadke, in *Org. React.*, ed. R. Adams, Wiley, New York, 1953, vol. VII, p. 1.
14 M. Matsumoto, N. Watanabe, A. Ishikawa and H. Murakami, *Chem. Commun.*, 1997, 2395.
15 J.-A. Richard, M. Massonneau, P.-Y. Renard and A. Romieu, *Org. Lett.*, 2008, **10**, 4175.
16 (*a*) J. A. Richard, Y. Meyer, V. Jolivel, M. Massonneau, R. Dumeunier, D. Vaudry, H. Vaudry, P. Y. Renard and A. Romieu, *Bioconjugate Chem.*, 2008, **19**, 1707; (*b*) Y. Meyer, J. A. Richard, M. Massonneau, P. Y. Renard and A. Romieu, *Org. Lett.*, 2008, **10**, 1517.
17 When aq. 1.0 M NaOH (3% v/v) was employed as CL trigger, two emission maxima at 430 and 470 nm were observed, respectively, assigned to cinnamate derivative (resulting from lactone ring-opening) and compound **11** (see ESI†).