# Highly efficient and flexible total synthesis of coelenterazine

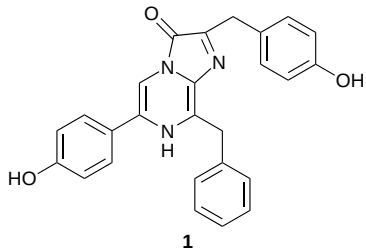
**Martine Keenan, Keith Jones* and Frank Hibbert**

*Department of Chemistry, King's College London, Strand, London, UK WC2R 2LS*

**A new total synthesis of the bioluminescent chromophore coelenterazine 1 is described.**

Coelenterazine **1** found in the jellyfish *Aequorea victoria* was the second imidazopyrazine-based chromophore to be isolated from luminescent marine organisms.[1] Its structure was determined in 1974[2] and it has been synthesised using an approach developed by Kishi[3] in which the fully-substituted pyrazine ring is synthesised by a condensation reaction between an α-amino nitrile and an α-nitroso ketone. In the jellyfish, coelenterazine is bound covalently to a protein which is activated to bioluminescence by reaction with calcium ions. The fact that the protein has been cloned and over-expressed means that a very sensitive system for the detection of calcium ions within cells is available.[4] In addition, the gene for this protein has recently been suggested to be an ideal reporter of gene expression.[5] Both these techniques require the addition of coelenterazine itself to the system in order to observe the desired luminescence. In addition coelenterazine on its own undergoes chemiluminescence with superoxide anion and this has been used as an assay of respiratory burst in neutrophils.[6] These wide-ranging analytical uses make coelenterazine a sought-after molecule for both medical and molecular biological use. Our interest in this area arose from a desire to couple the useful luminescent properties of coelenterazine, with a metal-binding site in the same molecule to explore the possibility of luminescent detection of specific metals. To achieve this goal, we needed a flexible, high-yielding synthesis of coelenterazine which could be adapted to incorporate suitable metal-binding sites. We chose to adopt a different strategy to the Kishi synthesis and start with the pyrazine ring intact to allow late introduction of the substituents.

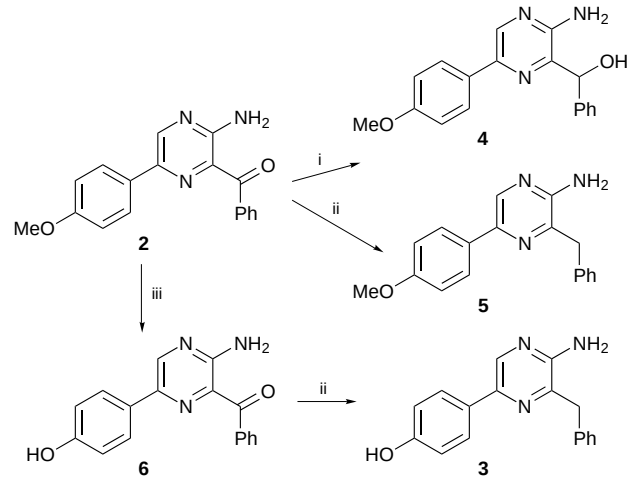
We recently reported the synthesis of the aminopyrazine **2** involving as a key step the formation of the aryl–heteroaryl bond by a high yielding Suzuki coupling reaction between a bromopyrazine and 4-methoxyphenylboronic acid.[7] We further demonstrated that this coupling could give a range of aryl-substituted pyrazines in excellent yield by varying the boronic acid. In order to convert this compound into the desired aminopyrazine **3** representing the southern hemisphere of coelenterazine, two transformations were required, namely reduction of the ketone to a methylene unit and deprotection of the methyl ether (Scheme 1). A two-step procedure was explored involving reduction of the ketone to the alcohol **4** in 60% yield using $NaBH_4$ in MeOH for 15 min.[8] However, reduction of the alcohol **4** with a variety of reagents ($Et_3SiH$ and $CF_3CO_2H$,[9] $ZnBr_2$ and $NaBH_3CN$[10] and radical-based deoxygenation[11]) failed to give any trace of **3**. Reduction of the ketone *via* the alcohol was abandoned and direct reduction to the alkane was investigated. After the failure of a number of methods, a Wolff–Kishner reduction of **2** was explored. Reaction of **2** with hydrazine hydrate and KOH in ethylene glycol at 240 °C for 3 h[12] gave an extremely insoluble red compound, the mass spectrum of which indicated reduction had occurred to give some **5**. However, the insolubility of this material led us to believe that **5** was a minor component and that extensive decomposition had occurred. Consequently, we decided to deprotect the methyl ether prior to reduction of the ketone moiety. Cleavage of methyl aryl ethers has been reported under a variety of conditions but the only successful deprotection of **2** was achieved using ethanethiolate in DMF at 100 °C for 10 h.[13] This gave phenol **6** as a brown solid (mp 176–177 °C) in 81% yield. Finally we were gratified to find that exposure of **6** to the Wolff–Kishner conditions as described above gave the aminopyrazine **3** as a powdery yellow solid (mp 220–222 °C) in 84% yield. This compound was readily purified by chromatography and was fully characterised.



**1**

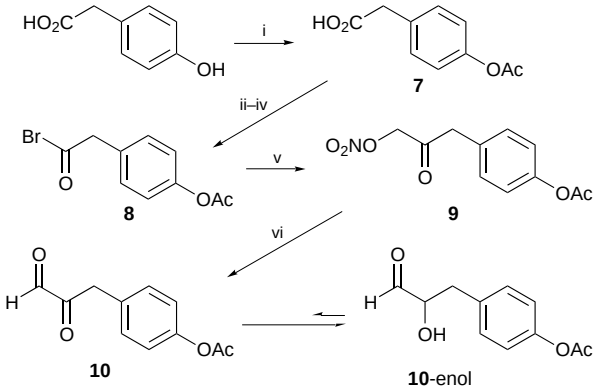
In order to complete the synthesis of coelenterazine, a suitable α-keto aldehyde representing the northern hemisphere was required. After exploring a variety of dithiane-based routes to this compound, we settled on the route shown in Scheme 2.[14] Acetylation of the phenol group in 4-hydroxyphenylacetic acid was carried out in 84% yield using NaOAc in $Ac_2O$. Formation of the acid chloride ($SOCl_2$) and reaction with $CH_2N_2$ gave the α-diazo ketone in high yield. This was not isolated but was reacted with dry HBr to give the bromomethyl ketone **8** in 89% overall yield from **7**. Reaction of the rather sensitive **8** with $AgNO_3$ in MeCN overnight gave the nitrate ester **9** as a colourless solid (mp 65–70 °C) in 98% yield. Finally, reaction of **9** with NaOAc in $Me_2SO$ at room temperature for 25 min gave the α-keto aldehyde **10** in 88% yield as a colourless solid (mp 134–136 °C). Interestingly, NMR studies on **10** indicated that it exists entirely in the enol form in a range of solvents.

The stage was now set for the condensation of **3** and **10** which should lead directly to coelenterazine.[14,15] Model condensations

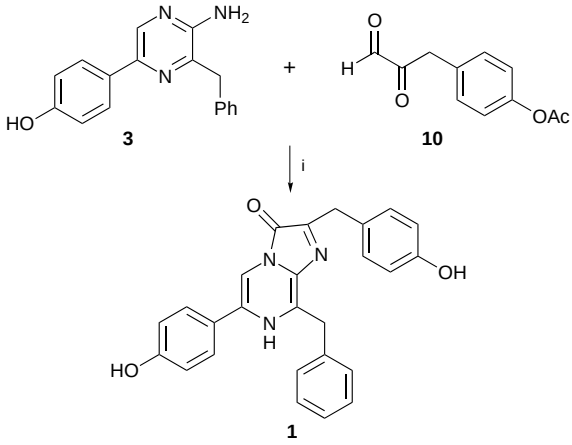


**Scheme 1** *Reagents and conditions*: i, $NaBH_4$, MeOH, 0 °C, 15 min; ii, hydrazine hydrate, KOH, ethanediol, 240 °C 3 h; iii, EtSH, NaH, DMF, 100 °C, 10 h

using the methoxy analogue of the acetoxy α-keto aldehyde **10** had proceeded in poor yield and subsequent cleavage of the methyl ether had proven impossible. The choice of the acetate group for protection of the phenol in the α-keto aldehyde unit was in order to allow deprotection to the phenol to occur under the acidic conditions of the coupling reaction. Reaction of **3** and **10** in EtOH containing conc. HCl and water for 4.5 h at 80 °C in the dark gave coelenterazine **1** as a methanol-soluble orange–brown solid (mp 177 °C, lit.,[14] 175–178 °C) in 62% yield after chromatography. This material forms brilliant yellow-coloured solutions which are both light- and air-sensitive but is relatively stable as a solid. The spectral properties of **1** agreed with those reported in the literature.[3,14] This new, highly convergent synthesis of coelenterazine involves 14 steps in total, although there are only 8 linear steps, and proceeds in 25% overall yield from commercially available 2-chloropyrazine. We are now in a position to prepare a wide range of analogues and explore their chemical and luminescent properties.




**Scheme 2** *Reagents and conditions*: i, NaOAc, $Ac_2O$, reflux, 2 h; ii, $SOCl_2$ (excess), room temp., 12 h; iii, $CH_2N_2$, $Et_2O$, 0 °C, 90 min; iv, dry HBr, 0 °C, 30 min; v, $AgNO_3$, MeCN, room temp., 12 h; vi, NaOAc·3 $H_2O$, $Me_2SO$, room temp., 25 min




**Scheme 3** *Reagents and conditions*: i, EtOH, conc. HCl, 80 °C, 4.5 h with light and air excluded

We thank the BBSRC for a studentship (M. K.) and Professor F. McCapra for useful discussions.

## References


1 O. Shimomura, F. H. Johnson and Y. Saiga, *J. Cell. Comp. Physiol*., 1962, **59**, 223; O. Shimomura and F. H. Johnson, *Tetrahedron Lett*., 1973, **14**, 2963.
2 O. Shimomura, F. H. Johnson and H. Morise, *Biochemistry*, 1974, **13**, 3278.
3 O. Shimomura, B. Musicki and Y. Kishi, *Biochem. J*., 1989, **261**, 913.
4 C. C. Ashley and E. B. Ridgway, *Nature (London)*, 1968, **219**, 1168; M. R. Knight, A. K. Campbell, S. M. Smith and A. J. Trewavas, *Nature (London)*, 1991, **352**, 524.
5 M. N. Badminton, G. B. Sala-Newby, J. M. Kendall and A. K. Campbell, *Biochem. Biophys. Res. Commun*., 1995, **217**, 950.
6 M. Lucas and F. Solano, *Anal. Biochem*., 1992, **206**, 273.
7 K. Jones, M. Keenan and F. Hibbert, *Synlett*, 1996, 509.
8 K. Teranishi and T. Goto, *Bull. Chem. Soc. Jpn*., 1990, **63**, 3132.
9 D. N. Kursanov, Z. N. Parnes and N. M. Loim, *Synthesis*, 1974, 633.
10 C. K. Lau, C. Dufresne, P. C. Bélanger, S. Piétré and J. Scheigetz, *J. Org. Chem*., 1986, **51**, 3038.
11 W. B. Motherwell and D. Crich, in *Free Radical Chain Reactions in Organic Synthesis*, Academic Press, London, 1992, pp. 37–52.
12 W. Schwaiger and J. P. Ward, *Recl. Trav. Chim. Pays-Bas*, 1972, **91**, 1175.
13 G. I. Feutrill and R. N. Mirrington, *Aust. J. Chem*., 1972, **25**, 1719.
14 S. Inoue, S. Sugiura, H. Kakoi and K. Hasizume, *Chem. Lett*., 1975, 141.
15 S. Inoue, S. Sugiura, H. Kakoi and T. Goto, *Tetrahedron Lett*., 1969, **10**, 1609.


*Received, 4th November 1996; Com. 6/07460J*