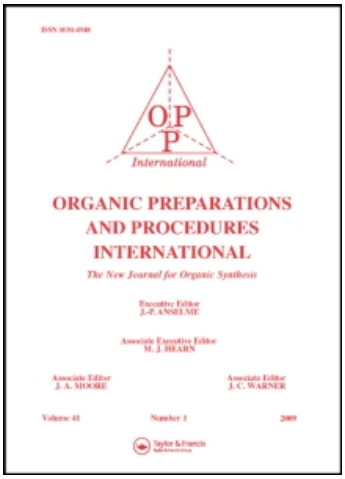

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SYNTHESIS OF COELENTERAZINE

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SYNTHESIS OF COELENTERAZINE

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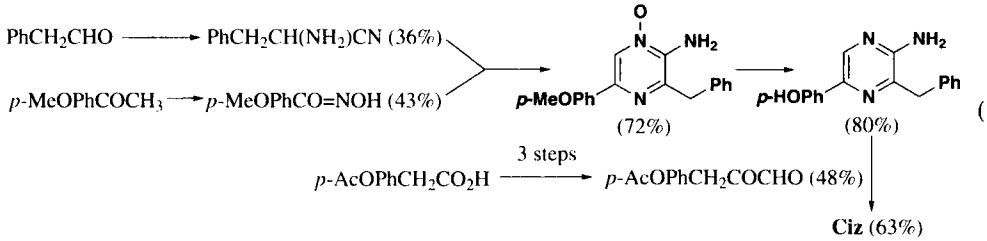
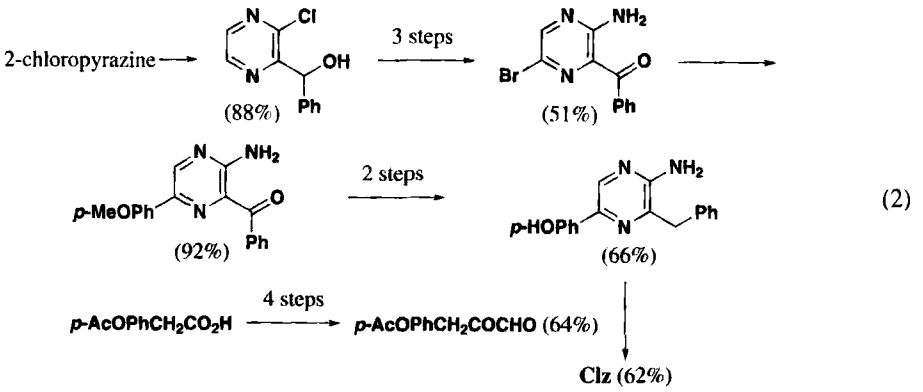
Coelenterazine [Clz, **9**, 8-benzyl-2-(4-hydroxybenzyl)-6-(4-hydroxyphenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one], a light producing compound originally found in jellyfish, *Aequorea victoria*,[1-4] is widely distributed in marine organisms, *e. g.*, coelenterates, fishes, squids and shrimps.[5-10] In these organisms, coelenterazine and molecular oxygen are incorporated into a photoprotein (*aequorin*, AQ) that emits blue light (λ_{max} 465 nm) in the presence of Ca^{2+}. The generated light aids the organism in a variety of activities including defense, feeding and breeding.[5-10] Coelenterazine-dependent luminescence (both chemiluminescence and bioluminescence) has found utility in many medical and biotechnological applications,[5-10] but a practical preparation of reasonable quantities of pure **9** has yet to be reported.

Coelenterazine was first isolated from marine organisms, but the process is laborious,[11] and low yielding (200 g of lyophilized squid livers gave only 15 mg of **9**). Two approaches have been disclosed for the chemical synthesis of Clz (**9**). The approach reported by Inoue *et al.*[11-13] (*Eq. 1*) involved the construction of an appropriately substituted 2-pyrazinamine derivative *via* classical pyrazine-ring formation[14-19] and subsequent elaboration to the imidazo[1,2-*a*]pyrazin-3(7*H*)-one ring system by treatment with a 1,2-dicarbonyl compound (4.6% overall yield for 9 steps). An improved synthesis of the two intermediates utilized in this approach, α-aminohydrocinnamonitrile and *p*-acetoxybenzylglyoxal, was reported subsequently.[20]



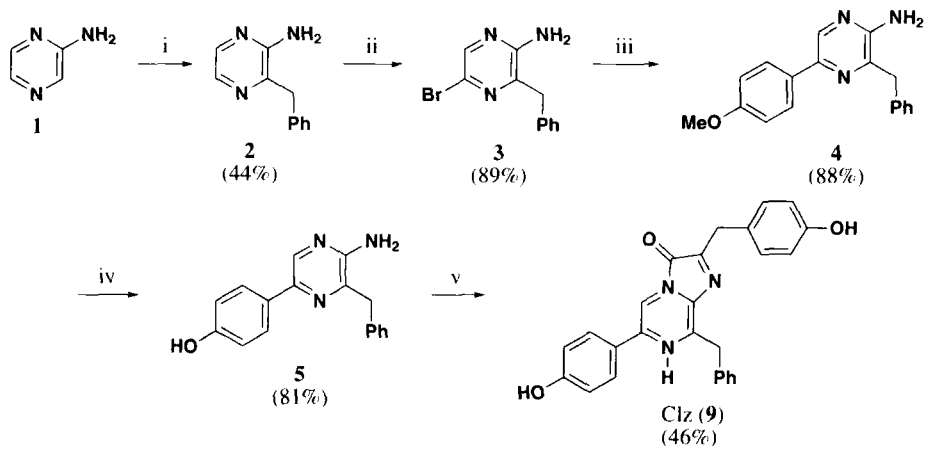
(1)

The second approach to the synthesis of Clz involved the introduction of appropriate substituents on pyrazine ring *via* organometallic chemistry to form a 2-pyrazinamine derivative[21,22] and its subsequent condensation with a 1,2-dicarbonyl compound,[23] as described in the first route.[11] In this approach, (*Eq. 2*) Jones *et al.*[23] began the synthesis with commercially available 2-chloropyrazine, which was metallated at the 3-position and condensed with benzaldehyde. The resulting alcohol was oxidized to the ketone and the 2-chloro group was displaced with ammonia. The 5-position was then brominated and subjected to Suzuki coupling with 4-methoxyphenylboronic acid to afford a 2-pyrazinamine derivative,[21,22] that was subsequently condensed with a 1,2-dicarbonyl compound to yield Clz (overall yield 11% for 12 steps).[23] These two approaches[11,20-23] involved a number of steps to produce Clz with low overall yield through processes that were not well documented. Though coelenterazine is now available commercially, it is still very expensive (1.0 mg/$1240.00, Molecular Probes, Eugene, OR, USA). This paper describes an improved synthesis of Clz (**9**) from a commercially available 2-pyrazinamine (**1**) and known 4-{[*tert*-butyl(dimethyl)silyl]oxy}phenylmethanol (**6**).[24]



(2)

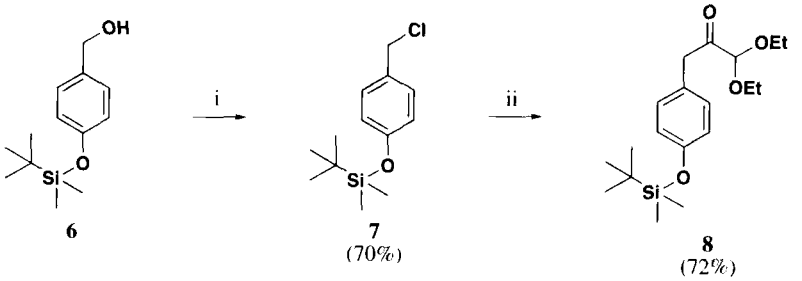
There are limited examples of the addition of alkyllithium reagents direct to the pyrazine nucleus.[25,26] When commercially available 2-pyrazinamine (**1**) was treated with benzyllithium[26] (prepared from toluene and *n*-butyllithium in THF) to consistently afford 3-benzyl-2-pyrazinamine (**2**) in 44% yield on a 50 mmol scale. Benzyllithium is unstable and should be prepared freshly and used immediately.[27] Next step in the synthesis of Clz (**9**) was to introduce the 4-hydroxyphenyl group at 5-position of the pyrazine ring. Although, the 4-hydroxyphenyl group can be incorporated using stannane reagents,[21] this transformation has practical limitations due to the additional steps involved in the preparation and lack of commercial source for these reagents. Alternatively, we decided to use a commercially available boronic acid reagent to introduce the 4-hydroxyphenyl group under Suzuki coupling conditions.[28-30] Thus, 2-amino-3-benzylpyrazine (**2**) was selectively brominated using tetra-*n*-butylammonium tribromide to afford 3-benzyl-5-bromo-2-pyrazinamine (**3**) in excellent yield (89%). Treatment of **3** with commercially available 4-(methoxy)phenylboronic acid in the presence of 1,4-*bis*(diphenylphosphino)butane and *bis*(benzonitrile)dichloropalladium (II) in a mixture of toluene-ethanol afforded 3-benzyl-5-(4-methoxyphenyl)-2-pyrazinamine (**4**) in 88% yield.



i) toluene, *n*-butyllithium, TMEDA, THF; ii) tetra-*n*-butylammonium tribromide, pyridine, $CHCl_3$; iii) *p*-$MeOC_6H_4B(OH)_2$, $(PhCN)_2$, $PdCl_2$, $(Ph)_2P(CH_2)_2P(Ph)_2$, 1.0 M aq. Na_2CO_3, toluene, ethanol; iv) pyridine hydrochloride; v) **8** (*Scheme 2*), aq. HCl, 1,4-dioxane

Scheme 1

The methoxy group in **4** was cleaved using pyridine hydrochloride[14] to afford the key intermediate, 4-(5-amino-6-benzyl-2-pyrazinyl)phenol (**5**) in 81% yield. The masked 1,2-dicarbonyl compound, 3-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-1,1-diethoxyacetone (**8**) needed for construction of imidazo[1,2-*a*]pyrazin-3(*7H*)-one ring system present in Clz was prepared from known 4-{[*tert*-butyl(dimethyl)silyl]oxy}phenylmethanol (**6**)[24] in two steps (Scheme 2). Thus, the hydroxy group in **6** was converted to the chloride by treatment with methanesulfonyl chloride in the presence of triethylamine to afford *tert*-butyl[4-(chloromethyl)phenoxy]dimethylsilane (**7**). Unlike the corresponding bromo compound {*tert*-butyl[4-(bromomethyl)phenoxy]dimethylsilane}, which was reported to be unstable and isolated only in 48% yield,[24] the chloro compound **7** was found to be stable and was obtained in 70% yield. The chloride **7** was treated with magnesium to form the corresponding Grignard reagent and then subjected to a reaction with the commercially available ethyl diethoxyacetate[31] at –78° for 1 h. Purification of the crude compound by silica gel column chromatography afforded 3-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-1,1-diethoxyacetone (**8**) in 72% yield.



i) MsCl, Et_3N, CH_2Cl_2; ii) a. Mg, THF; b. ethyl diethoxyacetate

Scheme 2

Finally, treatment of 4-(5-amino-6-benzyl-2-pyrazinyl)phenol (**5**) with the masked 1,2-dicarbonyl compound **8** in 1,4-dioxane in the presence aqueous hydrochloric acid, followed by two purifications (silica gel column chromatography and preparative reversed phase HPLC) and lyophilization afforded coelenterazine (**9**) in 46% yield as an orange powder.

In summary, an improved synthesis of coelenterazine (Clz, **9**) was developed from a 2-pyrazinamine (**1**) and 4-{[*tert*-butyl(dimethyl)silyl]oxy}phenylmethanol (**6**) in seven steps in 6.5% overall yield.

EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz) and the chemical shifts (δ) reported in ppm relative to TMS. Electrospray ionization mass spectrometry (ESI-MS) was carried out on a Perkin-Elmer (Norwalk, CT) Sciex API 100 Benchtop system, employing a Turbo IonSpray ion source and the HRMS were obtained on a Nermang 3010 MS-50, JEOL SX102-A. Thin layer chromatography was performed on a pre-coated Whatman MK6F silica gel 60 Å plates (layer thickness: 250 μm) and visualized with UV light and/or using 0.2% ninhydrin in ethanol. Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). Anhydrous solvents were freshly distilled [(THF from a purple solution of sodium and benzophenone) and (CH_2Cl_2 from CaH_2)] under nitrogen. All reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO). All the solvents employed were of HPLC grade purchased from EM Science (Gibbstown, NJ). Virtis Freezemobile-25SL purchased from The Virtis Company, Gardiner, NY was used for lyophilization.

3-Benzyl-2-pyrazinamine (2).- Toluene (53.0 mL, 0.50 mol) and *N,N,N,N*-tetramethylethylenediamine (TMEDA, 38.0 mL, 0.25 mol) were combined in a three-necked 1.0 L round bottom flask equipped with a reflux condenser and nitrogen inlet. The mixture was cooled with an ice bath. *n*-Butyllithium (2.5 M in hexanes, 100.0 mL, 0.25 mol) was added slowly by means of a syringe. After the addition was complete, the mixture was gently heated to 60° (oil bath temperature) for 30 min. During this period, a stream of butane gas evolved and the resulting dark brown mixture was allowed to cool to room temperature. In a separate flask, 2-pyrazinamine (**1**, 4.76 g, 50 mmol) in THF (400 mL) was cooled to 0° (ice bath) and the above-prepared brown colored benzyllithium solution was added by means of a cannula. After stirring for 30 min, the mixture was quenched with water at 0–5° (bath temperature). The mixture was extracted with EtOAc (3 x 400 mL), the combined organic layers were dried (Na_2SO_4), and evaporated on a rotary evaporator. The crude material was purified by silica gel column chromatography (3% MeOH in CH_2Cl_2) to afford 4.05 g (44%) of 3-benzyl-2-pyrazinamine (**2**) as a pale yellow solid, mp. 83–85°, *lit.*[26] 114–116°, R_f: 0.43 (5% MeOH in CH_2Cl_2); Analytical RP HPLC (Waters, Novapak, C18, 6.0 μ, 3.9 x 150 mm column): MeCN:water:0.1% aq trifluoroacetic acid/60:30:10, 1.0 mL/min at 215 nm, R_t: 1.81 min, 91%; ^1H NMR ($CDCl_3$): δ 7.95–7.92 (m, 1 H), 7.38–7.20 (m, 5 H), 4.39 (br s, 2 H), 4.10 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 153.2, 141.5, 140.5, 136.5, 133.7, 128.9, 128.4, 127.0, 40.9; ESI-MS (*m/z*): 186 $(M + H)^+$; HRMS (FAB, *m/z*): calcd for $C_{11}H_{12}N_3$: 186.1031 $(M + H)^+$; observed: 186.1030.

Anal. Calcd for $C_{11}H_{12}N_3$: C, 71.33; H, 5.99; N, 22.69; Found: C, 71.06; H, 5.87; N, 22.33

3-Benzyl-5-bromo-2-pyrazinamine (3)[21].- Pyridine (9.5 mL, 117.0 mmol, 3.0 equiv.) and tetra-*n*-butylammonium tribromide (18.9 g, 39.1 mmol, 1.0 equiv.) were added sequentially to a solution of 3-benzyl-2-pyrazineamine (**2**, 7.246 g, 39.1 mmol) in $CHCl_3$ (200 mL) at room temperature under nitrogen. After stirring the mixture for 45 min, it was transferred into a separatory funnel and washed with 20% aq NaCl solution (200 mL). The organic layer was dried (Na_2SO_4) and concentrated on a rotary evaporator. The crude material was purified by silica gel column chromatography (35% EtOAc in hexanes) to afford 9.21 g (89%) of 3-benzyl-5-bromo-2-pyrazinamine (**3**) as a gummy, pale yellow solid, R_f: 0.40 (40% EtOAc in hexanes); Analytical RP HPLC (Waters, Novapak, C18, 6.0 μ, 3.9 x 150 mm column): MeCN:water:0.1% aq trifluoroacetic acid/50:40:10, 1.0 mL/min at 215 nm, R_t: 3.16 min, 96%; ^{1}H NMR ($CDCl_3$): δ 8.03 (s, 1 H), 7.38–7.21 (m, 5 H), 4.39 (br s, 2 H), 4.08 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 152.1, 142.4, 141.7, 135.7, 129.1, 128.4, 127.3, 126.2, 40.7; ESI-MS (*m/z*): 264 and 266 $(M + H)^+$; HRMS (FAB, *m/z*): calcd for $C_{11}H_{11}N_3{}^{79}Br$: 264.0136 $(M + H)^+$; observed: 264.0130; calcd for $C_{11}H_{11}N_3{}^{81}Br$: 266.0116 $(M + H)^+$; observed: 266.0113.

3-Benzyl-5-(4-methoxyphenyl)-2-pyrazinamine (4).- 1,4-*bis*(Diphenylphosphino)butane (0.452 g, 1.06 mmol) was added to a suspension of *bis*(benzonitrile)dichloropalladium (II) (0.339 g, 0.885 mmol) in toluene (35 mL) at room temperature under nitrogen and stirred for 30 min. To this mixture, a solution of 3-benzyl-5-bromo-2-pyrazinamine (**3**, 4.68 g, 17.7 mmol) in tolune (30 mL), 4-(methoxy)phenylboronic acid (3.50 g, 23.0 mmol), EtOH (7.0 mL) and 1.0 M aq Na_2CO_3 (18 mL) were added sequentially at room temperature with stirring. The mixture was heated to reflux for 4 h and then allowed to cool to room temperature. The mixture was diluted with 20% aq NaCl solution (70 mL) and extracted with EtOAc (3 x 75 mL). The combined organic layers were dried (Na_2SO_4) and concentrated on a rotary evaporator. The crude material was dissolved in CH_2Cl_2 (30 mL) and purified by silica gel column chromatography (50% EtOAc in hexanes) to afford 4.539 g (88%) of 3-benzyl-5-(4-methoxyphenyl)-2-pyrazinamine (**4**) as a pale yellow solid, mp. 150–151°, *lit.*[16] 153–154°; R_f: 0.29 (50% EtOAc in hexanes); Analytical RP HPLC (Waters, Novapak, C18, 6.0 μ, 3.9 x 150 mm column): MeCN:water:0.1% aq trifluoroacetic acid/50:40:10, 1.0 mL/min at 215 nm, R_t: 2.51 min, 98%; ^{1}H NMR ($CDCl_3$): δ 8.33 (s, 1 H), 7.91–7.86 (m, 2 H), 7.36–7.25 (m, 5 H), 7.02–6.97 (m, 2 H), 4.35 (br s, 2 H), 4.18 (s, 2 H), 3.86 (s, 3 H); ^{13}C NMR ($CDCl_3$): δ 159.7, 151.2, 142.4, 140.4, 136.7, 136.6, 129.9, 128.9, 128.5, 126.9, 114.1, 55.3, 41.1; ESI-MS (*m/z*): 292 $(M + H)^+$; HRMS (FAB, *m/z*): calcd for $C_{18}H_{18}N_3O$: 292.1450 $(M + H)^+$; observed: 292.1441.

4-(5-Amino-6-benzyl-2-pyrazinyl)phenol (5).- A mixture of 3-benzyl-5-(4-methoxyphenyl)-2-pyrazinamine (**4**, 2.46 g, 8.44 mmol) and pyridine hydrochloride (9.8 g, 85.0 mmol) was heated at 200° (bath temperature) with stirring for 30 min under nitrogen atmosphere. The reaction mixture was allowed to cool to room temperature and was diluted with saturated aqueous $NaHCO_3$ (100 mL) and EtOAc (100 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (4 x 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated on a rotary evaporator to about 20 mL volume, which was purified by silica gel column chromatography (60–70% EtOAc in hexanes) to afford 1.76 g (75%) of 4-(5-amino-6-benzyl-2-pyrazinyl)phenol (**5**) as a pale

yellow solid. Also, 0.188 g (7.6%) of starting material, 3-benzyl-5-(4-methoxyphenyl)-2-pyrazinamine (**4**) was isolated from silica gel column chromatography and was recycled. Based on the recovered starting material **4**, the yield of 4-(5-amino-6-benzyl-2-pyrazinyl)phenol (**5**) was 81%, mp. 220–222°, *lit.*[14] 217–219°; R_f: 0.28 (50% EtOAc in hexanes); Analytical RP HPLC (Waters, Novapak, C18, 6.0 μ, 3.9 x 150 mm column): MeCN:water:0.1% aq trifluoroacetic acid/30:60:10, 1.0 mL/min at 215 nm, R_t: 4.24 min, 99%; ^{1}H NMR (DMSO-d_6): δ 9.50 (s, 1 H), 8.29 (s, 1 H), 7.73 (d, 2 H, J = 8.4 Hz), 7.35–7.16 (m, 5 H), 6.79 (d, 2 H, J = 8.7 Hz), 6.21 (s, 2 H), 4.06 (s, 2 H); ^{13}C NMR (DMSO-d_6): δ 157.1, 152.0, 139.7, 139.5, 138.3, 135.9, 128.9, 128.2, 126.2, 126.1, 115.5, 38.7; ESI-MS (*m/z*): 278 (M + H)$^+$; HRMS (FAB, *m/z*): calcd for $C_{17}H_{15}N_3O$: 278.1211 (M)$^+$; observed: 278.1215.

Anal. Calcd for $C_{17}H_{15}N_3O$: C, 73.63, H, 5.45, N, 15.15; Found: C, 73.49, H, 5.38, N, 15.22

***tert*-Butyl[4-(chloromethyl)phenoxy]dimethylsilane (7).**- Imidazole (42.5 g, 0.62 mmol) and *tert*-butyldimethylsilyl chloride (45.0 g, 0.30 mol) were added to 4-hydroxybenzaldehyde (30.5 g, 0.25 mol) in anhydrous DMF (63 mL) at room temperature under nitrogen. After stirring the mixture for 48 h, it was diluted with water (400 mL) and extracted with hexanes (3 x 400 mL). The combined organic layers were washed with water (2 x 200 mL), dried ($MgSO_4$) and concentrated on a rotary evaporator. The resulting crude {4-[*tert*-butyl(dimethyl)silyl]oxy}benzaldehyde (64.5 g, pale yellow viscous oil) was dissolved in MeOH (625 mL) and cooled to 0° (ice bath) under nitrogen. $NaBH_4$ (11.82 g, 0.31 mol) was added in portions over 5 min period and the reaction mixture was stirred for 2 h. The mixture was quenched with brine (200 mL) and concentrated on a rotary evaporator to about 300 mL volume. The mixture was extracted with EtOAc (4 x 400 mL) and the combined organic layers were washed with brine (2 x 200 mL) and dried ($MgSO_4$). Solvent was removed on a rotary evaporator and the crude compound was purified by silica gel column chromatography (10–35% EtOAc in hexanes) to afford 43.6 g (73% for two steps) of (4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)methanol (**6**)[24] as a pale yellow viscous oil. R_f: 0.63 (40% EtOAc in hexanes); Analytical RP HPLC (Waters, Symmetry, C18, 7.0 μ, 8 x 100 mm column): MeCN:water/60:40, 2.0 mL/min at 225 nm, R_t: 9.99 min, 99.6%; ^{1}H NMR ($CDCl_3$): δ 7.28–7.23 (m, 2 H), 6.87–6.82 (m, 2 H), 4.63 (s, 2 H), 1.00 (s, 9 H), 0.21 (s, 6 H); ESI-MS (*m/z*): 239 (M + H)$^+$.

Triethylamine (2.3 mL, 16.5 mmol) and methanesulfonyl chloride (0.95 mL, 12.37 mmol) were added sequentially to the above prepared 4-{[*tert*-butyl(dimethyl)silyl]oxy}phenylmethanol (**6**, 1.96 g, 8.25 mmol) in CH_2Cl_2 (45 mL) at room temperature. After stirring the mixture for 1.5 h, it was diluted with water (50 mL). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 50 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was removed on a rotary evaporator. The crude compound was purified by silica gel column chromatography (5% EtOAc in hexanes) to afford 1.49 g (70%) of *tert*-butyl[4-(chloromethyl)phenoxy]dimethylsilane (**7**) as a colorless viscous oil. R_f: 0.80 (10% EtOAc in hexanes); Analytical RP HPLC (Waters, Symmetry, C18, 7.0 μ, 8 x 100 mm column): MeCN:water/90:10, 2.0 mL/min at 225 nm, R_t: 4.86 min, 99.8%; ^{1}H NMR ($CDCl_3$): δ 7.27–7.23 (m, 2 H), 6.83–6.78 (m, 2 H), 4.56 (s, 2 H), 0.99 (s, 9 H), 0.20 (s, 6 H); ^{13}C NMR ($CDCl_3$): δ 155.7, 130.3, 130.0, 120.3, 46.3, 25.6, 18.2, –4.4; ESI-MS (*m/z*):

256 $(M)^+$, 221 $(M-Cl)^+$.

3-(4-{[*tert*-Butyl(dimethyl)silyl]oxy}phenyl)-1,1-diethoxyacetone (8)[24].- Magnesium (turnings, 0.754 g, 31.4 mmol.) was added to *tert*-butyl[4-(chloromethyl)phenoxy]dimethylsilane (**7**, 6.43 g, 25.12 mmol) in THF (50 mL) at room temperature under nitrogen. The reaction was initiated by 1,2-dibromoethane (0.010 mL) followed by sonication of the mixture for 1 min. The resulting warm reaction mixture was stirred for 10 min and heated at 50° (bath temperature) for 30 min. The heating bath was removed and mixture was cooled to room temperature in a water bath. In a separate flask, ethyl diethoxyacetate (6.63 g, 37.68 mmol) in THF (50 mL) was cooled to –78° under nitrogen. The Grignard reagent was added to this mixture via cannula over a 12 min period. The resulting pale yellow reaction mixture was stirred for 1 h and quenched with water (25 mL) at –78°. The mixture was allowed to warm to room temperature and diluted with EtOAc (400 mL) and water (50 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (300 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), and the solvent was removed on a rotary evaporator. The crude compound was purified twice by silica gel column chromatography (10–12% EtOAc in hexanes) to afford 6.40 g (72%) of 3-(4-{[*tert*-butyl (dimethyl)silyl]oxy}phenyl)-1,1-diethoxyacetone (**8**) in yield as a colorless viscous oil. R_f: 0.53 (5% EtOAc in hexanes); Analytical RP HPLC (Waters, Symmetry, C18, 7.0 µ, 8 x 100 mm column): MeCN:water/90:10, 2.0 mL/min at 225 nm, R_t: 5.06 min, 99.6%; ^1H NMR ($CDCl_3$): δ 7.10–7.05 (m, 2 H), 6.80–6.75 (m, 2 H), 4.63 (s, 1 H), 3.81 (s, 2 H), 3.73 – 3.63 (m, 2 H), 3.58 – 3.48 (m, 2 H), 1.24 (t, 6 H, J = 7.2 Hz), 0.97 (s, 9 H), 0.18 (s, 6 H); ^{13}C NMR ($CDCl_3$): δ 203.5, 154.6, 130.7, 126.3, 120.1, 102.1, 63.3, 43.1, 25.7, 18.1, 15.1, –4.4; ESI-MS (*m/z*): 256 $(M)^+$, 370 $(M + NH_4)^+$, 720 $(2 \times M + NH_4)^+$; HRMS (FAB, *m/z*): calcd for $C_{19}H_{32}O_4Si$: 375.1968 $(M + Na)^+$; observed: 375.1972.

8-Benzyl-2-(4-hydroxybenzyl)-6-(4-hydroxyphenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (Coelenterazine, Clz, 9).- Conc HCl (1.2 mL) was added to a solution of 4-(5-amino-6-benzyl-2-pyrazinyl)phenol (**5**, 0.50 g, 1.8 mmol) and 3-(4-{[*tert*-butyl(dimethyl)silyl]oxy} phenyl)-1,1-diethoxyacetone (**8**, 1.271 g, 3.61 mmol) in 1,4-dioxane (50 mL). The reaction mixture was heated to reflux for 8 h under nitrogen and concentrated on a rotary evaporator. The residue was purified with silica gel column chromatography (10% MeOH in CH_2Cl_2) to afford 0.336 g of Clz (**9**) as a dark brown solid. Also, 0.28 g of (4-hydroxy)benzylglyoxal (the hydrolyzed product of **8**) and 0.207 g (yield: 41%) of 4-(5-amino-6-benzyl-2-pyrazinyl)phenol (**5**) were recovered from the silica gel column chromatography. The Clz (**9**) (0.336 g) obtained from the silica gel column chromatography was further purified by preparative reversed phase HPLC (Waters, NovaPak RCM, C18, 6.0 µ, 40 x 100 mm) using MeCN:water/70:30, 45 mL/min at 225 nm. The fractions were collected and concentrated on a rotary evaporator (bath temperature: 30°) to about 200 mL volume and lyophilized to afford 0.205 g (27%) of **9** as an orange powder, mp. 176–181° (dec), *lit.*[11] 175–178° (dec.). The yield of Clz (**9**) based on the recovered starting pyrazine derivative **5** was 46%. R_f: 0.40 (10% MeOH in CH_2Cl_2). Analytical HPLC (Waters, NVC18 6.0 µ, 8 x 100 mm): MeCN:water/70:30, 45 mL/min at 225, R_t: 4.6 min, >99%; IR (KBr): 3191, 3063, 3029, 1611, 1564, 1513, 1453, 1242, 1172, 838, 698

cm^{-1}; ^{1}H NMR (CD_3OD): δ 7.58 (b, 1 H), 7.47 (d, 2 H, J = 8.2 Hz), 7.38 (d, 2 H, J = 6.9 Hz), 7.28 (d, 2 H, J = 7.7 Hz), 7.24 (m, 1 H), 7.15 (d, 2 H, J = 8.7 Hz), 6.87 (d, 2 H, J = 8.8 Hz), 6.69 (d, 2 H, J = 8.5 Hz), 4.39 (s, 2 H), 4.06 (s, 2 H); ^{1}H NMR (DMF-d_7): δ 8.01 (s, 2 H), 7.69 (b, 2 H), 7.53 (d, 2 H, J = 7.1 Hz), 7.29 (t, 2 H, J = 7.3 Hz), 7.22 (d, 2 H, J = 8.5 Hz), 6.94 (d, 2 H, J = 8.5 Hz), 6.76 (d, 2 H, J = 8.5 Hz), 4.40 (s, 2 H), 4.03 (s, 2 H); ESI-MS (*m/z*): 424 $(M + H)^+$; HRMS (FAB, *m/z*): calcd for $C_{26}H_{21}N_3O_3$, 423.1583; observed: 423.1598.

Anal. Calcd for $C_{26}H_{21}N_3O_3 \cdot H_2O$: C, 70.74, H, 5.25, N, 9.52; Found: C, 71.15, H, 5.26, N, 9.67

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