

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

02 January 2020 (02.01.2020)



(10) International Publication Number

WO 2020/006534 A1

(51) International Patent Classification:

C07D 487/04 (2006.01) A61F 13/42 (2006.01)

(21) International Application Number:

PCT/US2019/040016

(22) International Filing Date:

11 July 2019 (11.07.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/692,485	29 June 2018 (29.06.2018)	US
62/845,189	08 May 2019 (08.05.2019)	US
16/457,788	28 June 2019 (28.06.2019)	US

(71) Applicant: INTERNATIONAL PAPER COMPANY

[US/US]; 6400 Poplar Avenue, Memphis, TN 38197 (US).

(72) Inventors: DUVVURU, Rajagopala, Reddy; C-4 & 8

Stone Valley Rd, No. 4 Banjara Hills, Hyderabad TS 500034 (IN). BHATT, Rama; 13907 SE 87th Street, Newcastle, WA 98059 (US). KUMAR, Anil; 13701 113th Ct E., Puyallup, WA 98374 (US).

(74) Agent: ZHENG, Juan et al.; Christensen O'Connor Johnson

Kindness PLLC, 1201 Third Avenue, Suite 3600, Seattle, WA 98101 (US).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- the filing date of the international application is within two months from the date of expiration of the priority period (Rule 26bis.3)

(54) Title: SYNTHESIS OF COELENTERAZINE

(57) Abstract: Disclosed herein are synthesis methods for coelenterazine. Also disclosed are articles including the coelenterazine and coelenterazine derivatives. Representative absorbent articles include disposable diapers and adult incontinence products.



WO 2020/006534 A1

SYNTHESIS OF COELENTERAZINE

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/845189, filed May 8, 2019, U.S. Provisional Application No. 62/692485, filed June 29, 2018, and U.S. Application No. 16/457788, filed June 28, 2019; each of which applications is expressly incorporated herein by reference in its entirety.

BACKGROUND

Personal care absorbent products, such as infant diapers, adult incontinent pads, and feminine care products, typically contain a fluid absorbent core. Many absorbent articles include the fluid absorbent core disposed between a top sheet and a back sheet. The top sheet can be formed from a fluid-permeable material adapted to promote fluid transfer into the absorbent core, such as upon a liquid insult, usually with minimal fluid retention by the top sheet. U.S. southern pine fluff pulp is commonly used in the absorbent core, generally in the form of a fibrous matrix, and sometimes in conjunction with a superabsorbent polymer (SAP) dispersed throughout the fibrous matrix. This fluff pulp is recognized worldwide as the preferred fiber for absorbent products, based on factors such as the fluff pulp's high fiber length, fiber coarseness, and its relative ease of processing from a wet-laid and dried pulp sheet to an air-laid web. The raw material for this type of cellulosic fluff pulp is Southern Pine (e.g., Loblolly Pine, *Pinus taeda* L.). The raw material is renewable, and the pulp is easily biodegradable. Compared to SAP, these fibers are inexpensive on a per mass basis but tend to be more expensive on per unit of liquid held basis. These fluff pulp fibers mostly absorb within the interstices between fibers. For this reason, a fibrous matrix readily releases acquired liquid on application of pressure. The tendency to release acquired liquid can result in significant skin wetness during use of an absorbent product that includes a core formed exclusively from cellulosic fibers. Such products also tend to leak the acquired liquid because liquid is not effectively retained in such a fibrous absorbent core.

SAPs are water-swellaable, generally water-insoluble absorbent materials having a high absorbent capacity for fluids. They are used in absorbent articles like baby diapers or adult incontinent products to absorb and hold body fluids. SAP, upon absorption of fluids,

swells and becomes a gel holding more than its weight of such fluids. The SAPs in common use are mostly derived from acrylic acid. Acrylic acid-based polymers also comprise a meaningful portion of the cost structure of diapers and incontinent pads. SAPs are designed to have high gel strength (as demonstrated by high absorbency under load or AUL). The high gel strength (upon swelling) of currently used SAP particles helps them to retain significant void space between particles, which is helpful for rapid fluid uptake. However, this high “void volume” simultaneously results in significant interstitial (between particles) liquid in the product in the saturated state. When there is interstitial liquid the “rewet” value or “wet feeling” of an absorbent product is compromised.

Some absorbent articles, such as diapers or adult incontinence pads, also include an acquisition and distribution layer (ADL) for the collection, and uniform and timely distribution of fluid from a fluid insult to the absorbent core. An ADL is usually placed between the top sheet and the absorbent core, and normally takes the form of composite fabric with most likely the top-one third of the fabric having low density (higher denier fiber) with relatively large voids and higher void volume for the effective acquisition of the presented fluid, even at relatively higher discharge rates. The middle one-third of the composite fabric of the ADL is usually made of higher density (low denier) fibers with smaller voids, while the lower one-third of the fabric is made of even higher density (lower and smaller denier) fibers and yet with finer voids. The higher density portions of the composite have more and finer capillaries and hence develop greater capillary pressure, thus moving greater volumes of fluid to the outer regions of the structure thus enabling the proper channelization and distribution of the fluid in an evenly fashion to allow the absorbent core to take up all of the liquid insult in a time bound manner to allow SAP within the absorbent core to hold and to gel the insult neither too slow nor too fast. The ADL provides for more rapid liquid acquisition (minimizing flooding in the target zone), and ensures more rapid transport and thorough distribution of the fluid into the absorbent core.

As noted above, the absorbent core is adapted to retain fluid, and as such may consist of one or more layers, such as layers to acquire, distribute, and/or store fluid. In many cases, a matrix of cellulose fibers, such as in the form of an air-laid pad and/or non-woven web, is used in (or as) the absorbent core of absorbent articles. In some cases, the different layers may consist of one or more different types of cellulose fibers, such as

cross-linked cellulose fibers. The absorbent core may also include one or more fluid retention agents, such as one or more SAPs, distributed throughout the fiber matrix, usually as particles. Advances in SAP technology have allowed the design of absorbent core configurations in which fluff pulp contributes less to the absorbent capability of the core and more to providing a matrix structure in which the SAP is stably held. Fluff pulp fibers also provide fluid distribution functionality, to direct fluid to the SAP. However, it has been found that these structural and fluid distribution functions may be provided, in some configurations, by synthetic fibers, leading to the development of absorbent cores containing both fluff pulp fibers and synthetic fibers, and even “fluff-less” absorbent cores containing no fluff pulp fibers. These configurations may offer the advantage of being less physically bulky, without sacrificing absorbency.

The back sheet is typically formed from a fluid-impermeable material to form a barrier to prevent retained fluid from escaping.

Whatever the structure, when the absorbent article is wet from one or more liquid insults, the chances for the fluid coming in contact with the skin increases profoundly, and if left unchanged for a long time can result in diaper rash for infants or dermatitis problem in adults, thereby posing a skin wellness hazard. However, in general, the only way to know whether the diaper or the incontinent pad is dry or wet is to physically inspect it. During day time this may not pose a significant problem because a caregiver can check the diapers or adult incontinent products as many times as desired. However, inspections during night time can be a discomfort to the baby as well as to the adult, disturbing their sleep. Moreover, frequent night time inspections, such as several times in a single night, can disrupt the wearer’s sleeping pattern, which poses health hazard to baby as well as the adult patient.

In addition, it is typical that an article of clothing, such as pants, pajamas, and/or undergarments, is worn over the diaper or absorbent article. Accordingly, even absorbent articles that incorporate different types of wetness and/or moisture indicators pose difficulties in timely discovery of an insult.

As a result, there is typically a time lapse between the insult and its discovery. If this time period is prolonged, then there exists the possibility of developing diaper rash, skin irritation, and/or skin flaking. These conditions can be very painful for those affected. This is particularly true for babies and those adults in care-giving facilities, and

particularly true for night time insults, which can lead to longer periods prior to changing the absorbent article.

Previous moisture indicators incorporated into absorbent articles use color change as a visual indication of wetness detection. Inks that appear, or disappear, based on contact with liquid are popular mechanisms for wetness detection. Fluorescence has also been used for wetness detection, such as by incorporating a compound that fluoresces in the presence of a liquid. The mechanisms for such indicators generally fall into three broad categories: (1) imprinting a moisture indicating pattern on one of the piles of the absorbent article; (2) discrete moisture- indicating strips or layers that are incorporated between the layers of the absorbent article; and (3) a discrete (i.e., not part of the absorbent article's construction) indicating strip that is fastened to the interior of the absorbent article immediately prior to use.

Whatever the mechanism, these visual indicators are deficient in low-light (e.g., night time) situations. Appearing or disappearing inks must be directly visually detected, such that the caregiver can see the absorbent product. In low-light situations, this may require both a light source (e.g., overhead light or flashlight), as well as the removal of covering garments (e.g., pajamas or undergarments). Fluorescent indicators suffer similar issues, in that they require an external light source to excite the fluorescent compound. Such excitation is typically provided by exposing the indicator to UV light (which presents health concerns to the wearer and caregiver) and must be in direct optical communication with the fluorescent compound, which then requires removal of covering garments, blankets, etc. Therefore, the use of visual indicators previously used to detect wetness in absorbent garments suffers many disadvantages in low-light situations, which greatly reduces the usefulness of their indication mechanisms.

There is a need for chemiluminescent materials that can generate light upon exposure to moisture, where the chemiluminescent materials are readily synthesized in large yields and good purity. The present disclosure seeks to fulfill these needs and provides further advantages.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not

intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

In one aspect, the present disclosure features a method of making coelenterazine, including:

- (a) reacting 3-benzylpyrazin-2-amine (25) with N-bromosuccinimide to provide 3-benzyl-5-bromopyrazin-2-amine (2);
- (b) reacting the 3-benzyl-5-bromopyrazin-2-amine (2) in two sequential steps to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine); and
- (c) coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one to provide coelenterazine, or a salt thereof.

In another aspect, the present disclosure features a method of making coelenterazine, including:

coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 3-(4-((tert-butyl)dimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) to provide coelenterazine (16), or a salt thereof;

wherein the 3-(4-((tert-butyl)dimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) is synthesized by

- i. reacting 4-hydroxybenzaldehyde (8) with tert-butyl)dimethylsilyl chloride to provide 4-((tert-butyl)dimethylsilyl)oxy)benzaldehyde (20a);
- ii. reacting the 4-((tert-butyl)dimethylsilyl)oxy)benzaldehyde with sodium borohydride to provide (4-((tert-butyl)dimethylsilyl)oxy)phenyl)methanol (20b);
- iii. reacting the (4-((tert-butyl)dimethylsilyl)oxy)phenyl)methanol with methanesulfonyl chloride to provide tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21);
- iv. reacting the tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane with magnesium to provide (4-((tert-butyl)dimethylsilyl)oxy)benzyl)magnesium chloride (22); and

v. reacting the (4-((tert-butyldimethylsilyl)oxy)benzyl)magnesium chloride with ethyl 2,2-diethoxyacetate to provide the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23).

In yet another aspect, the present disclosure features a method of making coelenterazine, including:

coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) to provide coelenterazine, or a salt thereof.

In yet a further aspect, the present disclosure features a method of making coelenterazine, including:

(a) reacting 3,5-dibromopyrazin-2-amine and (bromomethyl)benzene in the presence of zinc, iodine, and a first palladium catalyst to provide 3-benzyl-5-bromopyrazin-2-amine (2);

(b) reacting 3-benzyl-5-bromopyrazin-2-amine (2) in a first step to provide a hydrochloride salt of 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5), and reacting the hydrochloride salt of 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) in a second step to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7); and

(c) coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) to provide coelenterazine (16), or a salt thereof.

In yet another aspect, the present disclosure features a method of making coelenterazine, including:

coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) for a duration of 16 hours to 28 hours, to provide coelenterazine, or a salt thereof;

wherein the coupling reaction is monitored by reverse phase HPLC and is quenched when 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) stops depleting, or when coelenterazine starts to decompose.

In yet another aspect, the present disclosure features a method of making coelenterazine, including:

reacting 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) for a duration of 16 hours to 28 hours to provide crude coelenterazine, or a salt thereof;

tritulating crude coelenterazine with ethyl acetate; and

isolating a coelenterazine, or salt thereof.

In yet another aspect, the present disclosure features a method of making coelenterazine, including (a) reacting pyrazin-2-amine (24) with benzyl chloride to provide 3-benzylpyrazin-2-amine (25) (e.g., under Grignard conditions, such as by first providing a solution of magnesium, iodine, and ethyl bromide in a solvent before reacting pyrazin-2-amine (24) with benzyl chloride to provide 3-benzylpyrazin-2-amine (25); (b)

reacting 3-benzylpyrazin-2-amine (25) with N-bromosuccinimide to provide 3-benzyl-5-bromopyrazin-2-amine (2); (c) reacting the 3-benzyl-5-bromopyrazin-2-amine (2) in two sequential steps to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine); and (d) coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one or silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one to provide coelenterazine (16), or a salt thereof.

In another aspect, the present disclosure features a method of making an absorbent article, including incorporating the coelenterazine made according to the methods of the present disclosure into an absorbent article.

In yet another aspect, the present disclosure features an absorbent article, including the coelenterazine synthesized by the methods of the present disclosure.

DETAILED DESCRIPTION

Disclosed herein are synthesis methods for making coelenterazine, in high yield and with good purity. Also disclosed are articles including the coelenterazine and coelenterazine derivatives. Representative absorbent articles include disposable diapers and adult incontinence products.

Chemiluminescence results from a chemical reaction that produces light and therefore provides a lighted indication of moisture that can be seen in low light and/or in the absence of light, and through clothes. Furthermore, chemiluminescence requires no external excitation light, as is required for photoluminescent (e.g., fluorescence) indicators. Accordingly, by generating light upon contact with an aqueous system (e.g., urine), the disclosed embodiments greatly enhance the ability of absorbent articles to indicate the occurrence of an insult in darkened conditions (e.g., at night). Moreover, by generating light that can be seen through clothing, a caregiver may be able to ascertain

the occurrence of an insult without having to move or disturb the infant or adult wearer of such an absorbent article, such as during sleep.

The articles provided herein may provide the distinct advantages of insult indication at night and through clothes, which may reduce or even eliminate the need for caregivers to disturb the sleep (e.g., by pulling down clothes and/or shining a light) of one wearing an absorbent article in order to test for an insult. Further, because light (e.g., visible light) is produced by the chemiluminescent systems disclosed herein, there is no need to expose the absorbent article and/or the wearer to UV light in order to determine whether an insult has occurred, allowing health concerns associated with UV radiation to be avoided. Examples of articles including chemiluminescent materials are described, for example, in U.S. Application No. 14/516,255, the disclosure of which is herein incorporated in its entirety.

The articles of the present disclosure provide improved ease with which an insult can be detected, which allows the caregiver to check for an insult more frequently, due to the reduced interruption required. More frequent checks may allow an insult to be detected sooner and the absorbent article changed soon after the insult, thereby reducing the amount of time the insult contacts the wearer's skin, as well as reducing the possibility of fluid from multiple insults contacting the wearer's skin. The skin health and general comfort of the wearer are improved when the length of time that fluid is in contact with the skin is reduced. In some embodiments, the component amounts/ratios can be calibrated so that instead of seeing a peak and then a fade after each of a sequence of insults, once there is sufficient water present in the absorbent article, the luminescence can be maintained at a relatively steady intensity (e.g., the luminescence can vary less than about 30%, less than about 20%, less than about 10%, less than 30%, less than 20%, or less than 10%,) over a period of time (e.g., about 24 hours, about 12 hours, about 6 hours, about 3 hours, about 2 hours, about 1 hour, about 30 minutes, about 15 minutes, 24 hours, 12 hours, 6 hours, 3 hours, 2 hours, 1 hour, 30 minutes, or 15 minutes). As used herein, the word "about" as it relates to a quantity indicates a number within range of minor variation above or below the stated reference number. For example, "about" can refer to a number within a range of 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% above or below the indicated reference number. In some embodiments, "about" refers to a number within a range of 5% above or below the indicated reference number. In some

embodiments, “about” refers to a number within a range of 10% above or below the indicated reference number. In some embodiments, “about” refers to a number within a range of 1% above or below the indicated reference number.

Synthesis of Coelenterazine

In one aspect, the present disclosure features a method of making coelenterazine, including coupling coelenteramine, or a salt thereof, with a protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one; or coupling coelenteramine, or a salt thereof, with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14), to provide coelenterazine, or a salt thereof.

In another aspect, the present disclosure features a method of making coelenterazine, including coupling coelenteramine, or a salt thereof, with a protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one (e.g., a silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one), to provide coelenterazine, or a salt thereof.

The coelenteramine can be made via different routes, as outlined below. The methods can provide coelenterazine in good yield and at good purity.

Coelenteramine synthesis

In some embodiments, the coelenteramine is made by first (a1) reacting 3-benzylpyrazin-2-amine (25) with N-bromosuccinimide to provide 3-benzyl-5-bromopyrazin-2-amine (2), or a salt thereof; or by (a2) reacting 3,5-dibromopyrazin-2-amine and (bromomethyl)benzene in the presence of zinc, iodine, and a palladium catalyst to provide the 3-benzyl-5-bromopyrazin-2-amine (2), or a salt thereof. In a step (b), the 3-benzyl-5-bromopyrazin-2-amine (2) is then reacted in two sequential steps to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine).

The reaction of (a1) 3-benzylpyrazin-2-amine (25) with N-bromosuccinimide can be carried out in an organic solvent, such as CHCl₃ (chloroform) at room temperature (e.g., about 22 °C to 23 °C, 22 °C to 23 °C, or 22 °C) at atmospheric pressure (*i.e.*, about 1 atm, or 1 atm). Once the reaction is complete, the reaction mixture can be washed with water, and 3-benzyl-5-bromopyrazin-2-amine (2) can be isolated by removing the organic solvent under reduced pressure. In some embodiments, (a1) provides 3-benzyl-5-bromopyrazin-2-amine (2) in a yield of about 60% to about 85 % (e.g., 60% to 85 %) relative to 3-benzylpyrazin-2-amine (25) and in a purity of at least about 85 % (e.g., a purity of about 85% to about 95%, a purity of about 85% to about 100%, a purity of 85%

to 95%, or a purity of 85% to 100%). As used herein, it is understood that % yield refers to mole % yield. As used herein, the purity of a given compound (when accompanied by the % yield of the given compound) refers to the weight percent purity relative to the mass of the pure compound calculated based on mole % yield.

In some embodiments, in (a2), the palladium catalyst is bis(triphenylphosphine)palladium(II) dichloride. The palladium catalyst can be present in an amount of about 5 to about 10 mole percent (e.g., 5 to 10 mole percent) relative to 3,5-dibromopyrazin-2-amine. In some embodiments, (a2) includes about 1:2 to about 1:3 molar equivalent (e.g., 1:2 to 1:3 molar equivalent) of 3,5-dibromopyrazin-2-amine to (bromomethyl)benzene. (a2) can provide 3-benzyl-5-bromopyrazin-2-amine (2) in a yield of about 55% to about 75% (e.g., 55% to 75%) relative to the 3,5-dibromopyrazin-2-amine at a purity of about 80% to about 95% (80% to 95%). In some embodiments, (a2) includes reacting 3,5-dibromopyrazin-2-amine and (bromomethyl)benzene for a duration of about 18 hours to about 30 hours (e.g., 18 hours to 30 hours). The reaction of 3,5-dibromopyrazin-2-amine and the (bromomethyl)benzene can occur at a temperature of about 25 to about 40 °C (e.g., 25 to 40 °C) at a pressure of about 1 atm (e.g., 1 atm).

In some embodiments, step (b) includes a first step of reacting the 3-benzyl-5-bromopyrazin-2-amine (2) with 4-methoxyphenyl boronic acid (4) in the presence of a palladium catalyst to provide 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5), or salt thereof. The palladium catalyst can be a palladium (0) catalyst, for example, tetrakis(triphenylphosphine)palladium(0). The palladium catalyst can be present in an amount of about 5 to about 10 percent by weight (e.g., 5 to 10 percent by weight) relative to the 3-benzyl-5-bromopyrazin-2-amine (2). The first step in (b) can include about 1:1 to about 1:1.3 molar equivalent (e.g., 1:1 to 1:1.3 molar equivalent) of the 3-benzyl-5-bromopyrazin-2-amine (2) to 4-methoxyphenyl boronic acid (4). In some embodiments, the 3-benzyl-5-bromopyrazin-2-amine (2) and the 4-methoxyphenyl boronic acid (4) are reacted together for a duration of about 120 minutes to about 300 minutes (e.g., 120 minutes to 300 minutes). The 3-benzyl-5-bromopyrazin-2-amine (2) and the 4-methoxyphenyl boronic acid (4) can be reacted together at a temperature of about 60 °C to about 90 °C (e.g., 60 °C to 90 °C) and at atmospheric pressure (*i.e.*, a pressure of about 1 atm, or 1 atm). The first step in (b) can provide the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5), or salt thereof, in a yield of about 60% to about

85% (e.g., 60% to 85%) relative to the 3-benzyl-5-bromopyrazin-2-amine (2) of the product at a purity of about 80 to about 95% (*i.e.*, a yield of 60% to 85% of the 80%-95% pure product). As used here, yield at a given purity range refers to the yield of the product having the described purity range.

Alternatively, in some embodiments, (b) includes a first step of reacting the 3-benzyl-5-bromopyrazin-2-amine (2) with (4-methoxyphenyl)boronic acid in the presence of a palladium catalyst (e.g., a palladium (II) catalyst, such as bis(benzonitrile) palladium(II) dichloride) to provide 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) (coelenteramine), or a salt thereof. The palladium (II) catalyst can be present in an amount of about 5 to about 10 (e.g., 5 to 10) mole percent relative to the 3-benzyl-5-bromopyrazin-2-amine (2). The reaction mixture can further include 1,4-bis(diphenylphosphino)butane, for example, in an amount of about 5 to about 10 (e.g., 5 to 10) mole percent relative to the 3-benzyl-5-bromopyrazin-2-amine (2). In some embodiments, the reaction mixture further includes toluene, aqueous sodium carbonate, and ethanol. The 3-benzyl-5-bromopyrazin-2-amine (2) can be reacted with the (4-methoxyphenyl)boronic acid for a duration of about 200 minutes to about 350 minutes (e.g., 200 to 350 minutes), and/or at a temperature of about 80 to about 110 °C (e.g., 80 to 100 °C) and at a pressure of about 1 atm (e.g., 1 atm). When the palladium (II) catalyst is used, 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) can be provided in a yield of about 65% to about 85% (e.g., 65% to 85%) relative to the 3-benzyl-5-bromopyrazin-2-amine (2). The 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) can be isolated by diluting the reaction mixture with water and extracting with ethyl acetate. The ethyl acetate extract can be removed from the extract under reduced pressure to provide 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5).

The 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5), or salt thereof, regardless of whether obtained with a palladium (0) or palladium (II) catalyst, can be isolated by precipitation of the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) as a hydrochloride salt. Precipitation is advantageous as it can eliminate column chromatography, which can be laborious, costly, and time-consuming; and/or can also increase yield relative to column chromatography. In some embodiments, isolating the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) does not include chromatography (e.g., liquid chromatography), does not include recrystallization, or does not include chromatography

and recrystallization. In some embodiments, the reaction mixture from the reaction of 3-benzyl-5-bromopyrazin-2-amine (2) with (4-methoxyphenyl)boronic acid is diluted with aqueous sodium chloride solution (e.g., an about 20% (e.g., 20%) by weight sodium chloride solution) and extracted with ethyl acetate. The ethyl acetate extract can then be treated with HCl aqueous solution (e.g., an about 3N (e.g., 3N) HCl_(aq) solution) and the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine hydrochloride salt product (5) can be isolated by filtration with a purity of about 75% to about 95% (e.g., 75% to 95%).

In some embodiments, (b) further includes a second step of deprotecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5), or salt thereof, to provide the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine). The deprotection can include subjecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) to pyridinium chloride. Treatment with pyridinium chloride can proceed at an elevated temperature of about 180 to about 220 °C (e.g., 180 to 220 °C, or 200 °C) at atmospheric pressure. In some embodiments, the elevated temperature can cause the pyridinium chloride to separate from the reaction mixture by evaporation from the reaction mixture. Alternatively, in certain embodiments, deprotection can include subjecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) to sodium hydride and ethanethiol in N,N'-dimethylformamide (DMF). When treated with sodium hydride and ethanethiol in DMF, the reaction mixture can be at a temperature of about 90 °C to about 120 °C (e.g., about 100 °C to 110 °C, 90 °C to 120 °C, or 100 °C to 110 °C) and/or for a period of about 15 minutes to about 5 hours (e.g., about 30 minutes to about 2 hours, about 30 minutes to about 1 hour, 30 minutes to 2 hours, 30 minutes to 1 hour, or 30 minutes). Once the reaction is complete, the mixture can be cooled to about 30 °C to about 50 °C (e.g., about 35 °C to about 45 °C, about 40 °C, 35 °C to 45 °C, or 40 °C), extracted with water and an organic solvent (e.g., ethyl acetate). The organic layer can then be separated, refluxed, and then cooled to about 5 °C to 20 °C (e.g., about 10 °C to 15 °C, 5 °C to 20 °C, or 10 °C to 15 °C), the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine) can be isolated by filtration. In both deprotection methods, the coelenteramine can be optionally purified by washing with an aqueous sodium hydroxide/dioxane solution, stirring with activated charcoal/silica, filtration, followed by precipitation by acidification of the filtrate, and isolation of the precipitated product by filtration. The deprotection can provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine) in a yield of about

90% to about 100% (e.g., 90% to 100%, at least about 95%, at least 95%, at least about 98%, at least 98%, at least about 99%, or at least 99%) at a purity of about 85% to about 100% (e.g., 85 % to 100 %, about 90%, or 90%) relative to 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5).

In some embodiments, coelenteramine is synthesized by (a) reacting 3,5-dibromopyrazin-2-amine and (bromomethyl)benzene in the presence of zinc, iodine, and a first palladium catalyst to provide 3-benzyl-5-bromopyrazin-2-amine (2); (b) reacting 3-benzyl-5-bromopyrazin-2-amine (2) with 4-methoxyphenyl boronic acid (4) in the presence of a palladium catalyst in a first step to provide a hydrochloride salt of 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5), and deprotecting the hydrochloride salt of 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) in a second step to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine).

In some embodiments, coelenteramine is synthesized by reacting pyrazin-2-amine with benzyl chloride to provide 3-benzylpyrazin-2-amine; reacting 3-benzylpyrazin-2-amine (25) with N-bromosuccinimide to provide 3-benzyl-5-bromopyrazin-2-amine (2); and reacting the 3-benzyl-5-bromopyrazin-2-amine (2) in two sequential steps to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine). The two sequential steps for providing coelenteramine from 3-benzyl-5-bromopyrazin-2-amine (2) can include a first step of reacting the 3-benzyl-5-bromopyrazin-2-amine (2) with silyl-protected 4-bromophenol in the presence of magnesium and a palladium catalyst to provide silyl-protected 4-(5-amino-6-benzylpyrazin-2-yl)phenol. In some embodiments, the palladium catalyst is tetrakis(triphenylphosphine)palladium(0), which can be present in an amount of 1 percent or more (e.g., 2 percent or more, 3 percent or more, 4 percent or more, 5 percent or more, 6 percent or more, 7 percent or more, 8 percent or more, or 9 percent or more) and/or 10 percent or less (e.g., 9 percent or less, 8 percent or less, 7 percent or less, 6 percent or less, 5 percent or less, 4 percent or less, 3 percent or less, or 2 percent or less) by weight relative to the 3-benzyl-5-bromopyrazin-2-amine (2). For example, for every 100 grams of 3-benzyl-5-bromopyrazin-2-amine (2), 1 to 10 grams (e.g., 1 gram, 2 grams, 3 grams, or 5 grams) of palladium catalyst can be used. As an example, for every 100 grams of 3-benzyl-5-bromopyrazin-2-amine (2), 1 gram of palladium catalyst can be used. The two sequential steps can include a second step of deprotecting the silyl-protected 4-(5-amino-6-benzylpyrazin-2-yl)phenol to provide the 4-(5-amino-6-

benzylpyrazin-2-yl)phenol (7), for example by subjecting the silyl-protected 4-(5-amino-6-benzylpyrazin-2-yl)phenol to aqueous HCl. This synthesis procedure reduces or eliminates the use of: n-butyl lithium-in the first step of the synthesis, by replacing the n-butyl lithium reaction in toluene with benzyl chloride and THF (tetrahydrofuran). Thus, this synthesis presents the ability to scale up the reaction chemistry and can reduce the cost of the synthesis. In addition, the changes can improve the overall safety of the chemistry by replacing highly reactive materials with more stable materials. The omission of boronic acid compounds in the synthesis can greatly reduce the amount of expensive palladium catalyst in the reaction.

Protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one synthesis

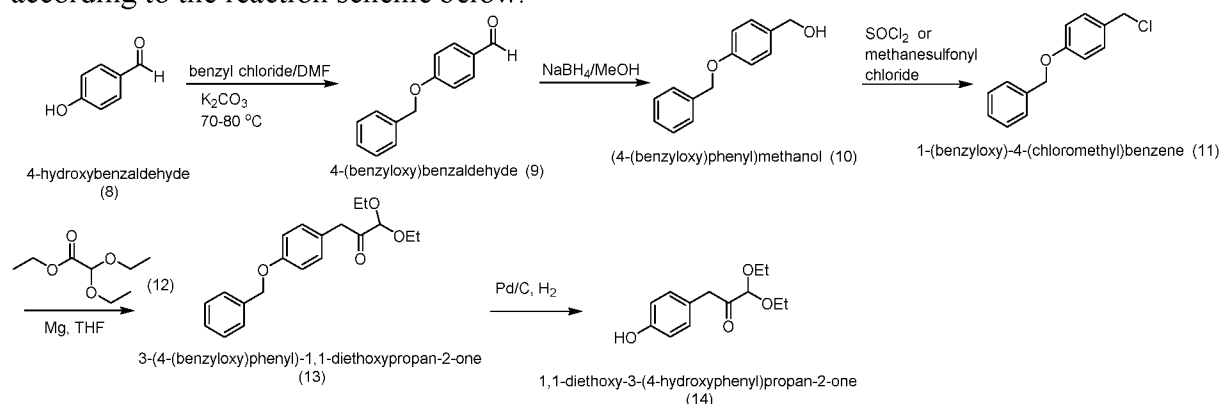
In some embodiments, the silyl protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one is 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23). The 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) can be synthesized by: i) reacting 4-hydroxybenzaldehyde (8) with tert-butyldimethylsilyl chloride to provide 4-((tert-butyldimethylsilyl)oxy)benzaldehyde (20a); ii) reacting the 4-((tert-butyldimethylsilyl)oxy)benzaldehyde with sodium borohydride to provide 4-((tert-butyldimethylsilyl)oxy)phenylmethanol (20b); iii) reacting the 4-((tert-butyldimethylsilyl)oxy)phenylmethanol with methanesulfonyl chloride in the presence of a base (e.g., triethylamine) to provide tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21); iv) reacting the tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane with magnesium, to provide 4-((tert-butyldimethylsilyl)oxy)benzylmagnesium chloride (22); and v) reacting the 4-((tert-butyldimethylsilyl)oxy)benzylmagnesium chloride with ethyl 2,2-diethoxyacetate to provide the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23).

In some embodiments, the tert-butyldimethylsilyl protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one can degrade in acidic conditions, and can be stabilized in a basic environment. Thus, a base can be added to the reaction mixture and/or during purification. For example, the tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21) can be purified by chromatography with an eluant that includes triethylamine. In some embodiments, the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) is further purified by chromatography with an eluant comprising triethylamine.

The 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) can be provided in a yield of about 20% to about 40% (e.g., 20% to 40%, about 20% to 25%, or 20% to 25%) relative to tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21), at a purity of about 85% to about 95% (e.g., 85% to 95%, about 90%, or 90%).

1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14)

1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) can be made, for example, according to the reaction scheme below.



Synthesis of 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-dimethoxypropan-2-one)

In some embodiments, 4-(tert-butyldimethylsiloxy) benzyl chloride can be as synthesized above, and can be reacted in a Grignard reaction with methyl 2,2-dimethoxyacetate to provide 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-dimethoxypropan-2-one). For example, the 4-(tert-butyldimethylsiloxy) benzyl chloride can be reacted with methyl 2,2-dimethoxyacetate in a Grignard reaction with Mg, and I₂ and dibromoethane as Grignard initiators to provide 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-dimethoxypropan-2-one).

Coelenterazine

In some embodiments, the coelenterazine is obtained by coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one or silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one to provide coelenterazine, or a salt thereof. Alternatively, in some embodiments, the coelenterazine is obtained by coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) to provide coelenterazine, or a salt thereof. The coupling step, whether with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one, silyl-protected 1,1-dimethoxy-

3-(4-hydroxyphenyl)propan-2-one, or with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14), can be conducted in the presence of dioxane, water, and HCl (e.g., at a ratio of about 90:5:5 dioxane to water to HCl; or at an HCl at 35%-38% concentration (*i.e.*, concentrated HCl) to water ratio about 10: about 1 (e.g., 10:1) to about 1:about 1 (e.g., 1:1), where the dioxane:(HCl+H₂O) ratio is about 9:1); and/or can be conducted at a temperature of about 60 to about 90 °C (e.g., 60 to 90 °C) and at a pressure of about 1 atm (e.g., 1 atm); and/or can proceed for a duration of about 16 hours to about 28 hours (e.g., 16 to 28 hours).

In some embodiments, whether the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) is coupled with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one, silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one, or with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14), the coupling reaction can be conducted in a solvent mixture of dioxane, methanol, and isopropyl alcohol. The methanol and the isopropyl alcohol can each independently be in the solvent mixture at a concentration of 3% or more (e.g., 5% or more, 7% or more, or 9% or more and/or 10% or less (e.g., 9% or less, 7% or less, or 5% or less) by volume. In certain embodiments, the methanol and the isopropyl alcohol are each independently in the solvent mixture at a concentration of about 5% (e.g., 5%) by volume. In some embodiments, the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) is reacted in the solvent mixture of dioxane, methanol, and isopropyl alcohol with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one, such as 3-(4-((tert-butyl)dimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23). Without wishing to be bound by theory, it is believed that methanol can favor the reaction kinetics and yield by increasing the solubility of the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) in the solvent mixture, thereby increasing its availability for reaction with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one, silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one, or with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14). In some embodiments, the coupling reaction can be conducted at a temperature of about 60 to about 90 °C (e.g., about 70 °C to 85 °C, about 80 °C, or 80 °C) and at a pressure of about 1 atm (e.g., 1 atm); and/or can proceed for a duration of about 24 hours to about 36 hours (e.g., 24 to 36 hours, or 36 hours).

In some embodiments, the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) is coupled with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (e.g., 3-(4-((tert-

butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23)), silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one, or with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) at a molar ratio of about 1:1.3 to about 1:2. In certain embodiments, the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) is coupled with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (e.g., 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23))) at a molar ratio of about 1:1.3 to about 1.2 (e.g., about 1:1.3, or 1:1.3).

In certain embodiments, whether the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) is coupled with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one, silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one, or with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14), the coupling reaction is monitored by reverse phase HPLC and stopped when 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) stops depleting, or when coelenterazine starts to decompose. Without wishing to be bound by theory, it is believed that the reverse phase HPLC monitoring can be important, as the coelenterazine can start to decompose or degrade after the coelenteramine stops depleting.

In some embodiments, stopping the reaction mixture includes cooling the reaction mixture (e.g., to room temperature, about 23 °C, or 23 °C), and optionally stirring the reaction mixture with activated carbon and silica. The coelenterazine or a salt thereof can then be isolated by filtering the reaction mixture (if activated carbon and silica are used), by removal of solvents (e.g., under reduced pressure), trituration with ethyl acetate, followed by filtration to obtain the solid coelenterazine. In some embodiments, the coelenterazine is isolated as a salt, such as a hydrochloride salt.

In some embodiments, when silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one or silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one is used as one of the starting materials in the coupling reaction, the coelenterazine or a salt thereof is obtained in a yield of about 50% to about 70 % (e.g., 50% to 70%) relative to the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7), at a purity of about 55% to about 70% (e.g. 55% to 70%).

In some embodiments, when 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) is used as one of the starting materials in the coupling reaction, coelenterazine or a salt thereof is obtained in a yield of about 60% to about 70 % (e.g., 60% to 70 %) relative to the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7), at a purity of about 60% to about 75%

(e.g., 60% to 75%). In some embodiments, the coelenterazine (or salt thereof) is obtained at a purity of 60% to 65% in the isolated composition. Without wishing to be bound by theory, it is believed that the isolated coelenterazine, or salt thereof, is stabilized (e.g., protected from degradation) by the presence of an amount of 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine). Thus, the isolated composition can include coelenterazine (or salt thereof) at an amount of 60% to 65% by weight and 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine). In some embodiments, the isolated composition consists essentially of coelenterazine (or salt thereof) at an amount of 60% to 65% by weight and 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine); such that impurities present in the composition do not substantially contribute (e.g., contribute more than 10%, more than 5%, or more than 1%) to the luminescence of the coelenterazine, or salt thereof.

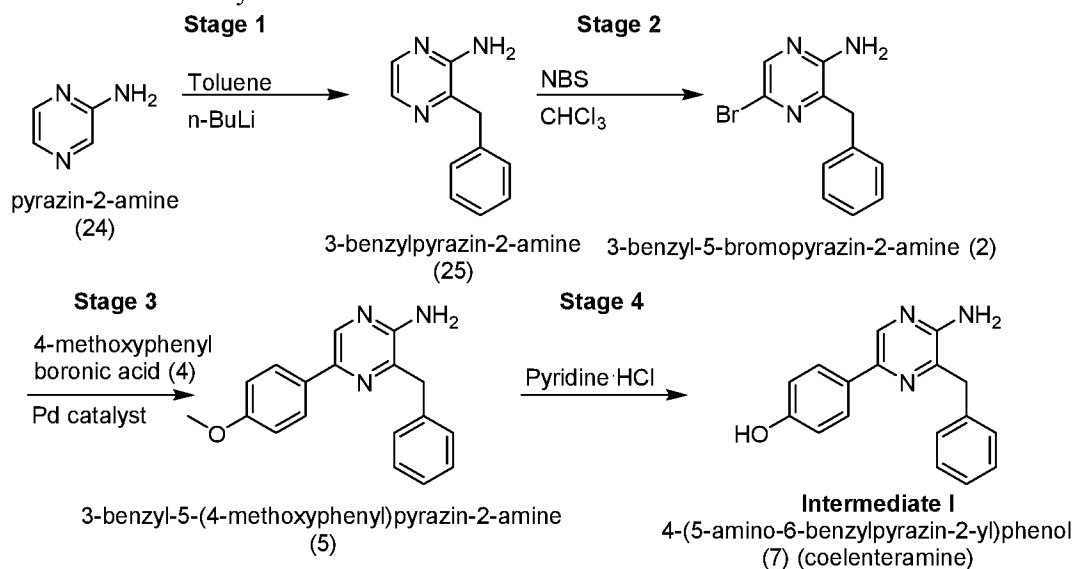
The relative amount of coelenterazine (or salt thereof) to 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine) in the isolated composition can be assessed by liquid chromatography-mass spectrometry (LC-MS). For example, a 1 mg/ml methanolic solution of the isolated coelenterazine composition can be diluted ten times into an injection solvent consisting of 70:30 reagent water : acetonitrile (v/v), each supplemented with 0.05% formic acid. The diluted solution including the isolated coelenterazine composition is separated by LC on a C-18 reverse phase column using a gradient elution. In some embodiments, the separation provides a response for coelenterazine (or salt thereof) at about 1.7 min and a response for coelenteramine (7) at about 2.5 min. Tandem MS is configured to monitor the (M+H)⁺ parent ion of each compound which is subsequently fragmented into its characteristic daughter ion. The daughter ion intensity creates the chromatographic signal for each compound which is then integrated to produce an area for the signal. The parent ion for coelenterazine is 424.1 Da with a daughter ion at 302.2 Da. The parent ion for coelenteramine is 278.1 Da and its daughter is 132.0 Da. In some embodiments, the ratio of the coelenterazine (or salt thereof) to coelenteramine in the isolated composition is about 20:1 or more (e.g., about 24:1 or more, about 30:1 or more, about 40:1 or more, about 50:1 or more, about 60:1 or more, about 70:1 or more, about 80:1 or more, or about 90:1 or more) and/or about 100:1 or less (e.g., about 90:1 or less, about 80:1 or less, about 70:1 or less, about 60:1 or less, about 50:1 or less, about 40:1 or less, about 30:1 or less, or about 24:1 or

less). In certain embodiments, the ratio of the coelenterazine (or salt thereof) to coelenteramine in the isolated composition is 24:1 or more and/or 80:1 or less. The isolated composition can be incorporated into an article, such as an absorbent article, as will be described below.

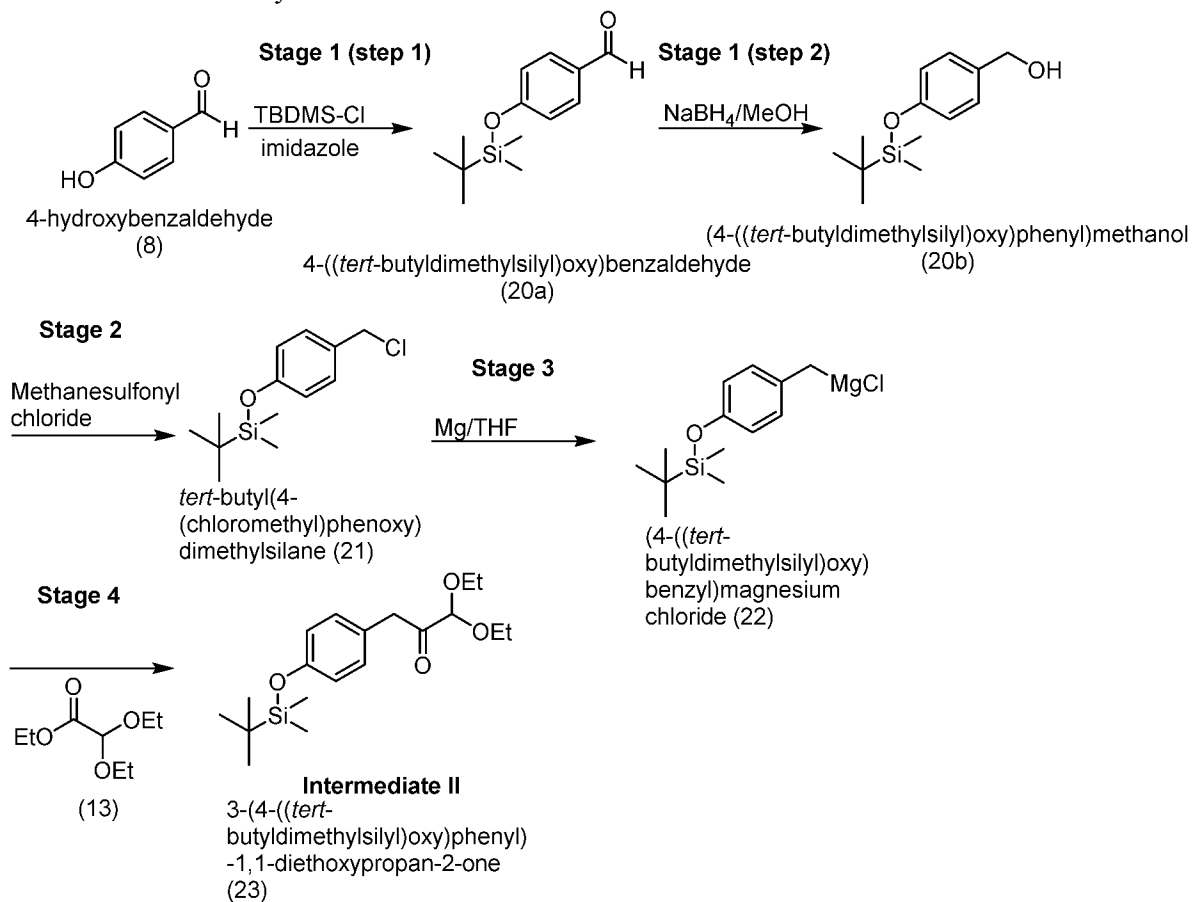
Representative syntheses

In some embodiments, the coelenterazine of the present disclosure can be made according to Schemes A, B, and C, below. Scheme A illustrates the synthesis of coelenteramine (Intermediate I), Scheme B illustrates the synthesis of silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (Intermediate II), and Scheme C illustrates the coupling of coelenteramine and silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one to generate coelenterazine.

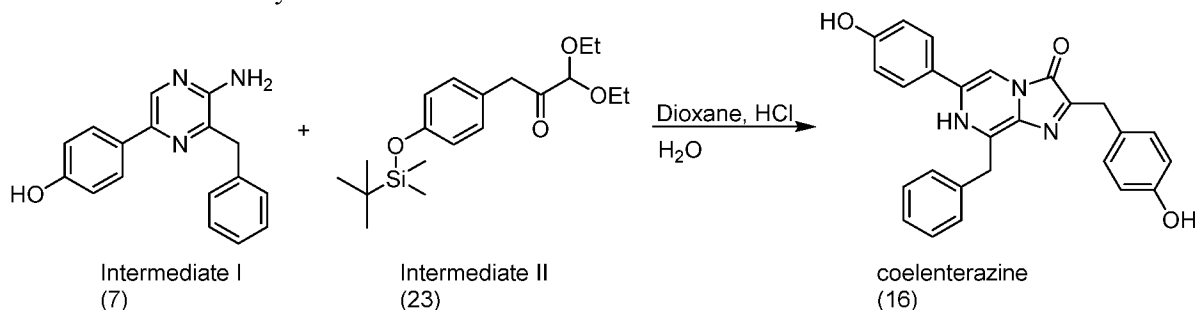
Scheme A. Synthesis of Intermediate I.



Scheme B. Synthesis of Intermediate II.

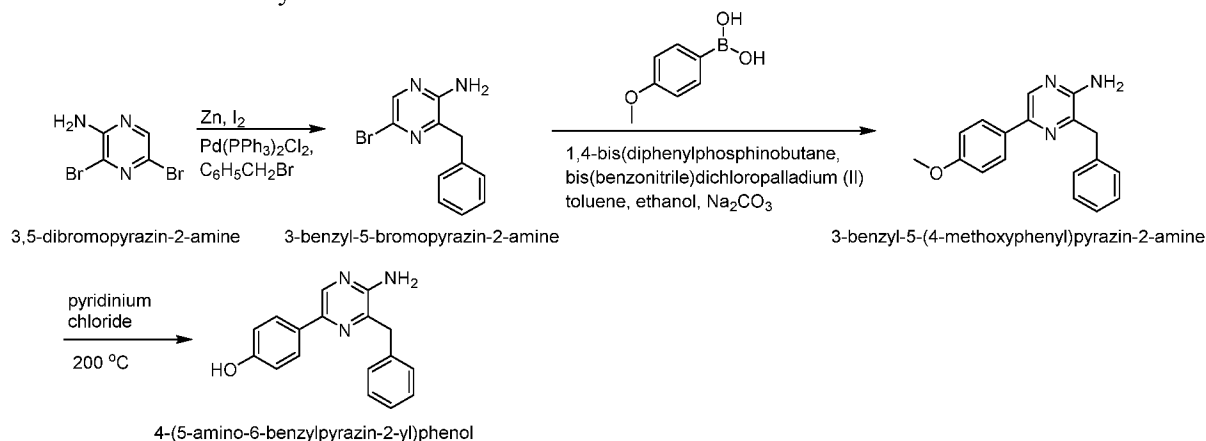


Scheme C. Synthesis of coelenterazine.

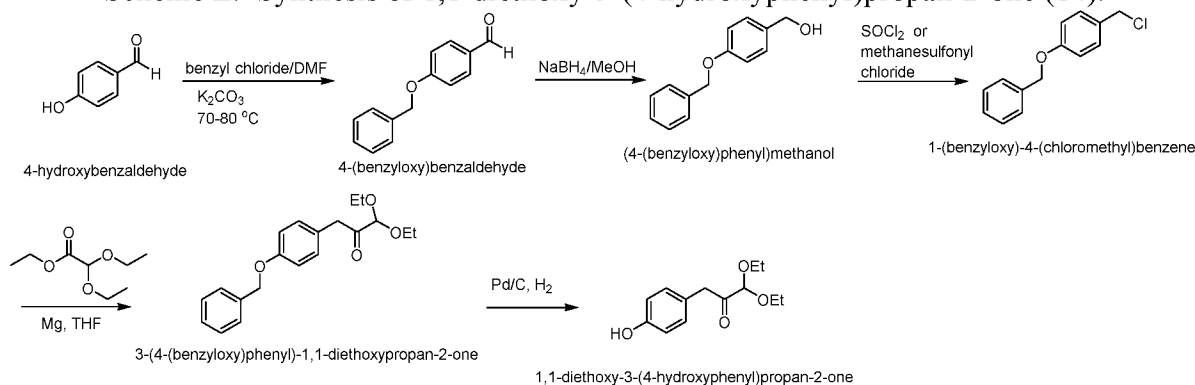


In some embodiments, the coelenterazine of the present disclosure can be made according to Schemes D, E, and F, below. Scheme D illustrates the synthesis of coelenteramine, Scheme E illustrates the synthesis of 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14), and Scheme F illustrates the coupling of coelenteramine and 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) to generate coelenterazine (16).

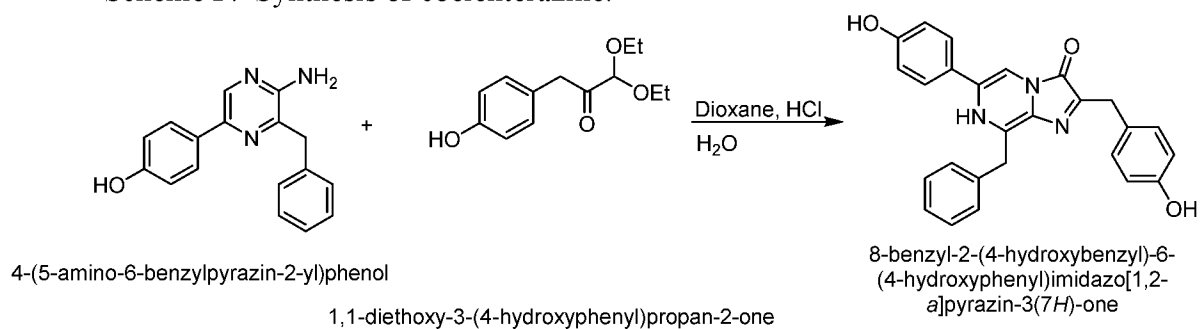
Scheme D. Synthesis of coelenteramine.



Scheme E. Synthesis of 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14).



Scheme F. Synthesis of coelenterazine.

Absorbent articles

The coelenterazine, or salt thereof, made according to the methods of the present disclosure can be incorporated into an absorbent article.

In some embodiments, the present disclosure features an absorbent article, including the coelenterazine, or salt thereof, synthesized by the methods of the present disclosure.

Materials and structural elements for absorbent articles that include one or more components of a chemiluminescent system are described, for example, in U.S. Provisional Application No. 62/692,502, titled “Chemiluminescent Wetness Indicator for Absorbent Products” and filed on June 29, 2018; US Provisional Application No. 62/753,024, titled "Chemiluminescent Wetness Indicator for Absorbent Products" and filed on October 30, 2018; and U.S. Application No. 16/457,732, titled "Chemiluminescent Wetness Indicator for Absorbent Products" and filed on June 28, 2019; the entire content of each of which is incorporated herein by reference.

Chemiluminescent System

The chemiluminescent system is configured to produce light upon contact with an aqueous system. The aqueous system initiates the chemiluminescence reaction in order to produce light. As used herein, the term “aqueous system” refers to water or water-containing compositions. In the context of this disclosure, such water-containing compositions are generally in the form of body fluid, such as urine, menses, fecal matter, and so forth. The occurrence of the release of bodily fluid (or the fluid itself) is referred to herein as an “insult,” or “liquid insult” or “fluid insult.” Accordingly, the chemiluminescent systems of the present disclosure produce light upon insult of an article in which the system is incorporated.

In being configured to produce light upon contact with an aqueous system, the chemiluminescent system includes at least one compound or material that luminesces when contacted with an aqueous system. In one embodiment, water initiates the chemiluminescence.

In one embodiment, the chemiluminescent system includes two or more materials that luminesce when contacted with an aqueous system. In this embodiment, there are two or more materials that together do not luminesce without the presence of the aqueous system.

Representative chemiluminescent systems that include two or more materials include bioluminescent systems, such as a system that includes a luciferin and a luciferase.

Bioluminescence is light that is produced by a chemical reaction that occurs within the body or in the secretions of certain type of organisms. Bioluminescence involves the combination of two types of substances in a light-producing reaction: a

luciferin and a luciferase. Luciferin is the compound that actually produces the light, for example, the luciferin can be coelenterazine. Luciferase is an enzyme that catalyzes the reaction. In some cases the luciferase is a protein known as a photoprotein, and the light making process requires a charged ion (e.g., a cation such as calcium) to activate the reaction. Photoprotein is a variant of luciferase in which factors required for light emission (including luciferin and oxygen) are bound together as one unit. Often, the bioluminescence process requires the presence of a substance such as oxygen or adenosine triphosphate (ATP) to initiate the oxidation reaction. The reaction rate for the luciferin is controlled by the luciferase or photoprotein. The luciferin-luciferase reaction can also create byproducts such as inactive oxyluciferin and water.

Luciferin and luciferase are generic names rather than specific materials. For example, the luciferin coelenterazine (natural form) is common in marine bioluminescence but variants can be chemically synthesized, and these various forms are collectively called luciferins. In another example, dinoflagellates (marine planktons) that obtain food through photosynthesis use a luciferin that resembles the chlorophyll structure.

The mechanism of light production through a chemical reaction differentiates bioluminescence from other optical phenomenon such as fluorescence or phosphorescence.

For example, fluorescent molecules do not emit their own light. They need an external photon source to excite the electrons to a higher energy state. On relaxation from the high energy state to their natural ground state, they release their acquired energy as a light source, but usually at a longer wavelength. Since the excitation and relaxation occurs simultaneously, fluorescent light is seen only when illuminated (excited).

The term phosphorescence technically refers to a special case of optically excited light emission where the relaxation from the excited state to ground state, unlike the fluorescence, is not immediate, and the photon emission persists for seconds to minutes after the original excitation.

The technical distinction between bioluminescence and fluorescence is sometimes blurred in a practical context but technically they are two distinct phenomena. In most cases, a bioluminescent can be an autofluorescent but the reverse is not true for a fluorescent; the latter still requires photon for excitation to emit light. In some cases a

bioluminescent cnidarians or crustaceans or fish can contain a fluorescent protein like Green Fluorescent Protein (GFP) and the light emitted from the bioluminescent would act as photons to excite the GFP. The GFP in turn under relaxed state would emit a light of different wave length (most probably of higher wave length) than the wavelength of the bioluminescent light that it has received as photon. In this example, the GFP may be excited by a blue light emitted by the bioluminescent (wavelength about 470 nm, or 470 nm) but in turn would emit a green light under its relaxed state (wavelength of about 510 nm to about 520 nm, or 510 nm to 520 nm).

Bioluminescent systems can be incorporated into fluff pulp compositions, fiber matrices, or absorbent articles in any manner that produces the desired chemiluminescence.

In one embodiment, the fluff pulp composition or absorbent product comprises a luciferin selected from the group consisting of coelenterazine, dinoflagellate luciferin, bacterial luciferin, fungal luciferin, firefly luciferin, and vargulin. With regard to coelenterazine, there are many variants, any of which can be used in the fluff pulp composition.

Certain embodiments of coelenterazine consistent with this disclosure comprise one or more of native coelenterazine, methyl coelenterazine, coelenterazine 400a (2-2'(4-dehydroxy)) coelenterazine, coelenterazine e, coelenterazine f, coelenterazine h, coelenterazine i, coelenterazine n, coelenterazine cp, coelenterazine ip, coelenterazine fcp, and coelenterazine hep. As a further example, the coelenterazine may be one or more of native coelenterazine, coelenterazine 400a, methyl coelenterazine, coelenterazine f, coelenterazine cp, coelenterazine fcp, and coelenterazine hep. As yet a further example, the coelenterazine may be one or more of coelenterazine 400a, methyl coelenterazine and coelenterazine fcp. As yet a further example, the coelenterazine may be one or more of coelenterazine 400a, methyl coelenterazine, and coelenterazine hep. In yet another example, the coelenterazine may be may be one or more of coelenterazine 400a and coelenterazine hep.

In one embodiment, the luciferin has a concentration of 0.0005% to 0.002%, by weight of the fluff pulp. In one embodiment, the luciferin has a concentration of 0.0005% to 0.0015%, by weight of the fluff pulp. In one embodiment, the luciferin has a concentration of 0.0005% to 0.001%, by weight of the fluff pulp.

In some embodiments, the luciferin can be incorporated in any component of an absorbent article. For example, the luciferin (e.g., coelenterazine, or a coelenterazine salt of the present disclosure) can be incorporated into an absorbent article in an amount of from about 0.01 to about 100 mg (e.g., from about 0.01 to about 75 mg, from about 0.01 to about 50 mg, from about 0.01 to about 25 mg, from about 0.01 to about 10 mg, or from about 0.01 to about 5 mg), or 0.01 to 100 mg (e.g., from 0.01 to 75 mg, from 0.01 to 50 mg, from 0.01 to 25 mg, from 0.01 to 10 mg, or from 0.01 to 5 mg).

In one embodiment, the fluff pulp composition or absorbent product comprises luciferase selected from the group consisting of *Gaussia* luciferase (Gluc), *Renilla* luciferase (RLuc), dinoflagellate luciferase, firefly luciferase, fungal luciferase, bacterial luciferase, and vargula luciferase. Certain embodiments of the luciferase consistent with this disclosure comprise one or more of *Gaussia* luciferase, *Renilla* luciferase, dinoflagellate luciferase, and firefly luciferase. As a further example, the luciferase may be one or more of *Gaussia* luciferase, *Renilla* luciferase, dinoflagellate luciferase, and firefly luciferase. In yet a further example, the luciferase may be one or more of *Gaussia* luciferase and *Renilla* luciferase.

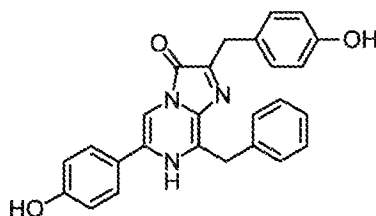
In one embodiment, the luciferase has a concentration of about 0.005% to about 0.04% (e.g., 0.005% to 0.04%) by weight of the fluff pulp. In one embodiment, the luciferase has a concentration of about 0.005% to about 0.02% (e.g., 0.005% to 0.02%) by weight of the fluff pulp. In one embodiment, the luciferase has a concentration of about 0.005% to about 0.01% (e.g., 0.005% to 0.01%) by weight of the fluff pulp.

In some embodiments, the luciferase can be incorporated in any component of an absorbent article. For example, the luciferase (e.g., GLuc) can be incorporated into an absorbent article in an amount of from about 0.2 mg to about 40 mg (e.g., from about 0.2 mg to about 30 mg, from about 0.2 mg to about 20 mg, from about 0.2 mg to about 15 mg, from about 0.2 mg to about 10 mg, from about 0.2 mg to about 5 mg, or from about 0.2 to about 2 mg); or from 0.2 mg to 40 mg (e.g., from 0.2 mg to 30 mg, from 0.2 mg to 20 mg, from 0.2 mg to 15 mg, from 0.2 mg to 10 mg, from 0.2 mg to 5 mg, or from 0.2 to 2 mg).

In one embodiment, the chemiluminescent system comprises coelenterazine as the luciferin and *Gaussia* or *Renilla* luciferase.

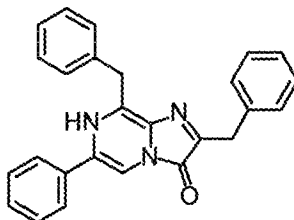
Representative luciferins include those of the coelenterazine family. Coelenterazine in its native form as well as its analogs have different luminescent characteristics due to variation in their structural moieties. Given structural variations within the coelenterazine family, some are good substrates for certain luciferases, whereas some are not. Below is a brief description of native coelenterazine and representative analogs.

Coelenterazine (native form) is a luminescent enzyme substrate for *Renilla (reniformis)* luciferase (Rluc). *Renilla* luciferase/coelenterazine has also been used as the bioluminescence donor in bioluminescence resonance transfer (BRET) studies.



Coelenterazine (native form)

Coelenterazine 400a is a derivative of coelenterazine and is a good substrate for *Renilla* luciferase, but does not oxidize well with *Gaussia* luciferase (Gluc). It is the preferred substrate for BRET (bioluminescence resonance energy transfer) because its emission maximum of about 400 nm (e.g., 400 nm) has minimal interference with the GFP emission.



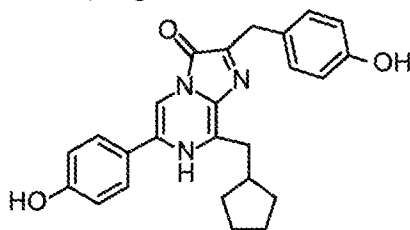
Coelenterazine 400a

Fluorescence resonance energy transfer (FRET), BRET, resonance energy transfer (RET), and electronic energy transfer (EET) are mechanisms describing energy transfer between two light-sensitive molecules (chromophores) and can define the interference of a luminescent chemical with another luminescent chemical's energy transfer. A donor chromophore, initially in its electronic excited state, may transfer energy to an acceptor chromophore through nonradiative dipole-dipole coupling. The efficiency of this energy transfer is inversely proportional to the sixth power of the distance between donor and

acceptor, making FRET extremely sensitive to small changes in distance. Measurements of FRET efficiency can be used to determine if two fluorophores are within a certain distance of each other.

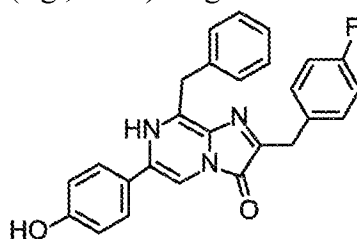
Such measurements are used as a research tool in fields including biology and chemistry.

Coelenterazine cp in a coelenterazine-aequorin complex generates luminescence intensity about 15 times (e.g., 15 times) higher than coelenterazine (native form).



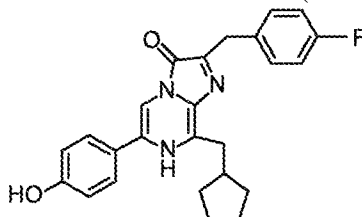
Coelenterazine cp

Coelenterazine f has about 20 times (e.g., 20 times) higher luminescence intensity (coelenterazine-apoaequorin complex) than the native form coelenterazine, while its emission maximum is about 8 nm (e.g., 8 nm) longer than that of the native form.



Coelenterazine f

Coelenterazine fcp is an analog wherein the α -benzene structure in the coelenterazine moiety of coelenterazine f structure is replaced with a cyclic pentane (similar to coelenterazine cp). Coelenterazine fcp has luminescence intensity about 135 times (e.g., 135 times) greater than that of coelenterazine (native form).

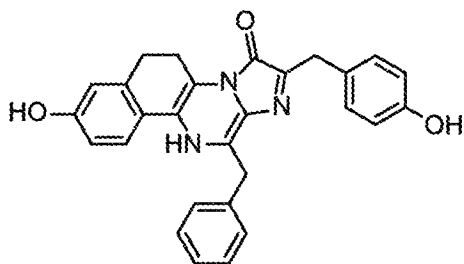


Coelenterazine fcp

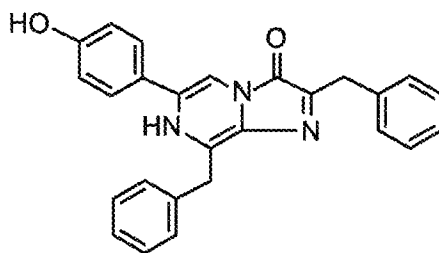
Coelenterazine fcp complexes with aequorin to form a coelenterazine fcp-apoaequorin complex, and, as a substrate for aequorin, has a relative luminescence

intensity of about 135 times (e.g., 135 times) that of native coelenterazine. However, coelenterazine fcp is a poor substrate for *Renilla* luciferase.

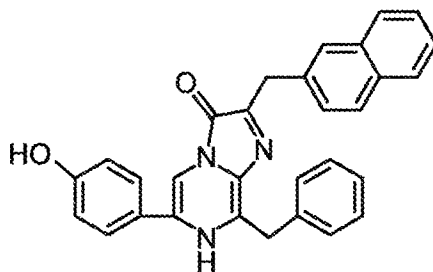
Other representative analogs of coelenterazine, as a substrate for *Renilla* Luciferase enzyme, are coelenterazine e, h and n. While these three analogs are good to excellent substrates for *Renilla* luciferase, they are poor substrates for apoaequorin.



Coelenterazine e



Coelenterazine h



Coelenterazine n

The luminescent properties of coelenterazine analogs vary. For example, certain analogs emit less light (as measured as lumens) but with higher luminescent intensity (lumens/steradian). Table A lists the luminescent properties of coelenterazine (native form) and its analogs with *Renilla* Luciferase. Luminescent intensity is reported as a % initial intensity. For example, an analog having an initial intensity of 900% is about 20 times (e.g., 20 times) intense as compared to the native coelenterazine with an initial intensity of about 45% (e.g., 45%).

Table A: Luminescent Properties of Selected Coelenterazine Analogs with *Renilla*

Luciferase

Analog	Aem(nm)	Total Light(%)	Initial Intensity(%)
native	475	100	45
e	418,475	137	900
f	473	28	45
h	475	41	135
n	475	47	900
cp	470	23	135

Light is produced by the chemiluminescent system. The light is visually detectable by a caregiver in the dark and through clothing, and as such the light has a wavelength, intensity, and duration sufficient to provide the necessary indication. These spectral characteristics of the chemiluminescent system can be tailored based on the chemiluminescent compound or compounds. For example, in bioluminescent systems, the luciferin and luciferase can be selected to produce the desired light characteristics. Depending on the bioluminescent system used, different spectral characteristics can occur. In the presence of superoxide anions and/or peroxy nitrile compounds, coelenterazine can also emit light independent of enzyme (luciferase) oxidation, a process known as autoluminescence.

The chemiluminescent system can be tailored to produce particular colors of light. As noted above in Table A, even within the coelenterazine family, the emission wavelength can range from about 400 nm (violet, e.g., 400 nm) to about 475 nm (blue with green tint, e.g., 475 nm).

With regard to duration, the duration of the light emitted may be controlled by the selection of the coelenterazine (luciferin), in native form versus its analogues, and the enzyme (Luciferase), for example *Gaussia* versus *Renilla*. The ratio and the concentration of luciferin and luciferase used may also modify the duration of light emission. To give an illustrative and non-limiting example, the luciferin analogue, coelenterazine e, has a total light of 130% and initial intensity of 900% over native coelenterazine. By judiciously selecting the concentration of coelenterazine e and *Renilla* luciferase, the duration of the light emitted can last as long as about 8 to about 10 hours (e.g., 8 to 10 hours).

In one embodiment, the light has a duration of about 0.5 to about 6 hours (e.g., 0.5 to 6 hours). In another embodiment, the light has a duration of about 1 to about 4 hours (e.g., 1 to 4 hours). In another embodiment, the light has a duration of about 2 to about 3 hours (e.g., 2 to 3 hours).

With regard to intensity, quantum efficiency of the chemiluminescence contributes to the intensity, depth, and hue of the color of the emission.

Quantum efficiency (QE) is the fraction of photon flux used to excite a luminescence chemical to elevate it to higher energy state. Quantum efficiency is one of the most important parameters used to evaluate the quality of a detector and is often called the “spectral response” to reflect its wavelength dependence. It is defined as the number of signal electrons created per incident photon. In some cases it can exceed 100% (i.e. when more than one electron is created per incident photon). If the spectral response exceeds 100%, then the intensity and depth of the color emitted is vivid, but depending on the status of the excited state of the primary electron, the duration of the emission will be determined (i.e., the higher the excited state, the more time it takes to return to the ground (normal) state).

Spectral responsivity is a similar measurement, but it has different units; the metric being the amperes or watts (i.e., how much current comes out of the device per incoming photon of a given energy and wavelength).

Both the quantum efficiency and the spectral responsivity are functions of the photons' wavelength. For example, in the case of the luciferin coelenterazine, between the native form and one of its analogs, coelenterazine e, the latter has not only high light intensity but emits 30% more light energy than the former, because the latter upon excitation by a given quanta ($h\nu$) of incident photon generates two electrons and the primary electron at wavelength 475 has the same emission intensity as native coelenterazine but with lumen intensity about 20 times (e.g., 20 times) greater than that of the native product. Accordingly, the light emitted by the excited coelenterazine analog would be twenty times brighter than the native form but with a total light energy of about 130% (e.g., 130%) will last longer than the native form.

The wavelength determines the color of the emitted light.

In one embodiment, the fluff pulp composition includes a luciferin and a luciferase. Such a fluff pulp has both elements of the chemiluminescent system required

to luminesce upon contact with an aqueous system. However, in another embodiment, the fluff pulp composition includes at least one component selected from a luciferin and a luciferase. In such an embodiment, the fluff pulp composition may include only one of a luciferin and a luciferase. Such a fluff pulp composition may be incorporated into an absorbent article such that the other one of the luciferin and the luciferase may be disposed in a top sheet or other layer of the absorbent article, such that the two components are combined only when carried by a liquid insult (e.g., water from an aqueous system passing through the top sheet into the fluff pulp composition). In one embodiment the fluff pulp composition comprises a luciferin but not a luciferase. In one embodiment the fluff pulp composition comprises a luciferase but not a luciferin.

Fluff Pulp

The fluff pulp of the fluff pulp composition can be formed from any pulp. In one embodiment, the fluff pulp is derived from a lignocellulosic fiber. In one embodiment, the fluff pulp is derived from a lignocellulosic fiber derived from wood. In one embodiment, the fluff pulp is derived from a lignocellulosic fiber derived from wood by chemical, mechanical, chemimechanical, or thermomechanical means. In one embodiment, the fluff pulp is derived from a cellulosic fiber derived from wood by chemical pulping. In one embodiment, the fluff pulp is derived from a cellulosic fiber derived from chemical pulping of wood either by alcohol pulping, organo-solve pulping, acid sulfite pulping, alkaline sulfite pulping, neutral sulfite pulping, alkaline peroxide pulping, Kraft pulping, Kraft-AQ pulping, polysulfide pulping, or polysulfide-AQ pulping.

In one embodiment, the fluff pulp is derived from a cellulosic fiber derived from chemical pulping of wood by further removing lignin from the said pulp either by alcohol pulping, organo-solve pulping, acid sulfite pulping, alkaline sulfite pulping, neutral sulfite pulping, alkaline peroxide pulping, Kraft pulping, Kraft-AQ pulping, polysulfide pulping, or polysulfide-AQ pulping for the preparation of absorbent articles (fluff pulp). In one embodiment, the fluff pulp is derived from a cellulosic fluff pulp derived from Kraft pulping. In one embodiment, the fluff pulp is derived from a cellulosic bleached fluff pulp derived from Kraft pulping. In one embodiment, the fluff pulp is derived from a cellulosic bleached fluff pulp derived from Kraft pulping of softwoods. In one embodiment, the fluff pulp is derived from a cellulosic bleached fluff pulp derived from

Kraft pulping of Southern softwoods. In one embodiment, the fluff pulp is derived from a cellulosic bleached fluff pulp derived from Kraft pulping of Southern pine. In one embodiment, the fluff pulp is derived from a Southern softwood. In one embodiment, the fluff pulp is derived from Southern pine.

The fluff pulp composition can be produced from pulp in any form, such as a wet-laid sheet which is dried to achieve a moisture content ranging from about 6% to about 11% (e.g., 6% to 11%).

In another aspect, methods of preparing the fluff pulp composition are provided. The fluff pulp composition is prepared by incorporating at least one component of the chemiluminescent system into the fluff pulp.

This can be accomplished using various methods that allow the fluff pulp to be treated with one or more components of the chemiluminescent system. One challenge in the chemical treatment of fluff pulp is to maintain the chemicals in a state in which the intended chemiluminescent reaction is not prematurely triggered, for example, before the treated fluff pulp is incorporated into an absorbent article that is then subjected to a liquid insult. For a wet end application, the chemicals typically cannot be comingled with water and be applied together. Accordingly, in an illustrative example, either the luciferase or luciferin may be microencapsulated and introduced during the wet-laying process, with the non-encapsulated component applied to the sheet in a non-aqueous environment by standard methods such as coating, dipping, spraying, or printing (or combination thereof), prior to the air-laid operation during absorbent article manufacture. In another illustrative example, a two sheet system, one containing luciferase and the other containing luciferin, may be made and processed further before the air-laid operation during absorbent article manufacture. In yet other examples, one of the chemicals maybe added during the wet-laying process and the other during the subsequent processing of the pulp; or the two components may be added to the pulp during or prior to the air-laid process, such as by rinsing and/or spraying the pulp in fluffed form with non-aqueous solutions of one or both the respective components.

Absorbent Articles

In one embodiment, the fluff pulp composition; the isolated composition including the coelenterazine, or salt thereof; and/or the coelenterazine, or salt thereof, can be incorporated into absorbent articles. Representative absorbent articles include child

diapers, adult diapers, adult incontinence products, feminine hygiene products, absorbent underpads, and wound care dressing articles. For example, the fluff pulp composition and/or the coelenterazine, or salt thereof, can be incorporated into one or more absorbent layers or portions of an absorbent article.

In another aspect, an absorbent article is provided. In one embodiment, the absorbent article includes a top sheet that is liquid permeable, a back sheet that is liquid impermeable, fluff pulp disposed between the top sheet and the back sheet and/or a fluffless or near fluffless non-woven fabric matrix disposed between the top sheet and the back sheet, and a chemiluminescent system configured to produce light upon contact with an aqueous system.

The chemiluminescent system (e.g., a luciferin such as coelenterazine, or a salt thereof of the present disclosure, and a luciferase) of the absorbent article is as described herein. However, the chemiluminescent system need not be disposed, in whole or in part, within the fluff pulp. As discussed above, structural and fluid distribution functions may be provided, in some configurations, by synthetic fibers, leading to the development of absorbent cores containing both fluff pulp fibers and synthetic fibers, and even “fluff-less” absorbent cores containing no fluff pulp fibers. In some embodiments, the chemiluminescent system, or parts of the chemiluminescent system, can be independently integrated in the liquid permeable top sheet, the liquid impermeable back sheet, the SAP, or another structure in the absorbent article.

In one embodiment, the chemiluminescent system comprises a luciferin and a luciferase. In one embodiment the luciferin and the luciferase are both disposed within the fluff pulp. In another embodiment, one of the luciferin and the luciferase is disposed within the fluff pulp and the other is disposed in a different layer (e.g., top sheet or ADL) of the absorbent product such that the two components are combined only when at least one of the two components is carried by a liquid insult (e.g., passing through the top sheet or ADL into the fluff pulp composition). In one embodiment the fluff pulp comprises a luciferin but not a luciferase. In one embodiment the fluff pulp comprises a luciferase but not a luciferin.

In yet another embodiment, at least one component of the chemiluminescent system is disposed on (for example, printed onto) the inner surface of the backsheet.

In one embodiment, one of the luciferin and the luciferase is disposed within the fluff pulp and the other is associated with the top sheet or another structure within an article, and configured to travel into the fluff pulp upon exposure to a liquid insult.

In one embodiment, the absorbent article further comprises a pH buffer, as disclosed herein. In one embodiment, the pH buffer is disposed within the fluff pulp. As discussed above, structural and fluid distribution functions may be provided, in some configurations, by synthetic fibers, leading to the development of absorbent cores containing both fluff pulp fibers and synthetic fibers, and even “fluff-less” absorbent cores containing no fluff pulp fibers. In some embodiments, the chemiluminescent system, or parts of the chemiluminescent system, can be independently integrated in the liquid permeable top sheet, the liquid impermeable back sheet, the SAP, or another structure in the absorbent article.

In one embodiment, the absorbent article further comprises a photoluminescent compound, as disclosed herein. In one embodiment, the photoluminescent compound is disposed within the fluff pulp.

In one embodiment, the absorbent article further comprises a photoluminescent compound and a pH buffer, as disclosed herein. In one embodiment, the photoluminescent compound and the pH buffer are disposed within the fluff pulp.

In one embodiment, the pH buffer, the photoluminescent compound, the luciferin, and the luciferase are disposed within the fluff pulp.

In one embodiment, at least one of the pH buffer, the photoluminescent compound, the luciferin, and the luciferase are not disposed within the fluff pulp. In some embodiments, at least one of the pH buffer, the photoluminescent compound, the luciferin, and the luciferase can be independently incorporated into synthetic fibers, “fluff-less” absorbent cores containing no fluff pulp fibers, in the liquid permeable top sheet, the liquid impermeable back sheet, and/or the SAP of the absorbent article. In some embodiments, the pH buffer, the photoluminescent compound, the luciferin, and/or the luciferase can migrate to an absorbent core from a different structure of the article. In some embodiments, the pH buffer, the photoluminescent compound, the luciferin, and/or the luciferase can migrate from an absorbent core to a different structure of the article.

In one embodiment, the absorbent article further includes a superabsorbent polymer (SAP), such as incorporated in the absorbent core. In such an embodiment, at

least one component of the chemiluminescent system may be disposed in the SAP, such that the chemiluminescence is generated upon the fluid from an insult traveling to the absorbent core.

In one embodiment the chemiluminescent system is contained entirely within an absorbent core of the absorbent article. As the absorbent core is almost always a multi-component system there exist more than one approach to incorporate the chemiluminescent system into the absorbent core. For instance the fluff pulp fibers could be the carrier of the chemiluminescent system. Alternatively, the chemiluminescent system could be contained within superabsorbent particles incorporated into the absorbent article. In some embodiments, the absorbent core can include cellulose fibers, cellulose fiber derivatives (rayon, lyocell, etc.), nonwoven cellulose fibers and/or cellulose fiber derivatives, non-cellulose fibers, or any combination thereof.

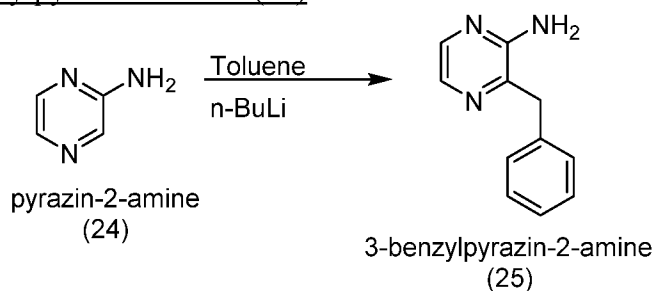
Furthermore, if only a portion of the SAP particles or fibers contained the chemiluminescent system chemistry, a desired pattern, such as an aesthetically pleasing pattern, can be achieved.

The chemiluminescent system can be added to the fluff pulp fibers at the time of absorbent article manufacture or during an upstream process entirely separated from final product assembly. As noted above, for example, the chemiluminescent system may be sprayed onto or otherwise incorporated into a fluff pulp sheet prior to hammermilling.

In another aspect, methods of manufacturing absorbent articles are also provided.

Absorbent articles are manufactured according to general techniques known to those of skill in the art that allow the incorporation of the chemiluminescent system to be incorporated into the absorbent article in the manner disclosed herein.

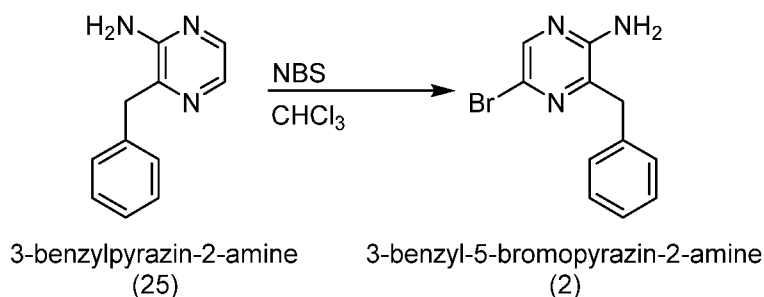
The following examples are intended to be illustrative, not limiting. Example 1 describes the synthesis of coelenterazine on a 1 Kg scale. Example 2 describes the synthesis of coelenterazine on a 25 gram scale. Example 3 describes the synthesis of coelenterazine on a 100 gram scale. Example 4 describes the synthesis of coelenterazine on a multi-kilogram scale.

EXAMPLESExample 1. Synthesis of Coelenterazine (1 Kilogram)Synthesis of 3-benzylpyrazin-2-amine (25)

A 100 L round bottom flask was charged with 10 L of toluene followed by 10 L tetramethylethylenediamine (TMEDA). The mixture was cooled at 0-5 °C with ice water. To this stirred mixture was added 20 L of n-butyl lithium (2.5 M in n-hexane) solution at 0°C to 8°C drop wise over 1 -1 ½ hrs under nitrogen. After the complete addition, the reaction mixture was allowed to come to room temperature (22 °C). After 20 minutes at room temperature, the mixture slowly heated up to 60°C. (During this time butane gas was released). The reaction mixture was maintained at 60°C ± 1°C for 30 minutes and then allowed to cool to room temperature.

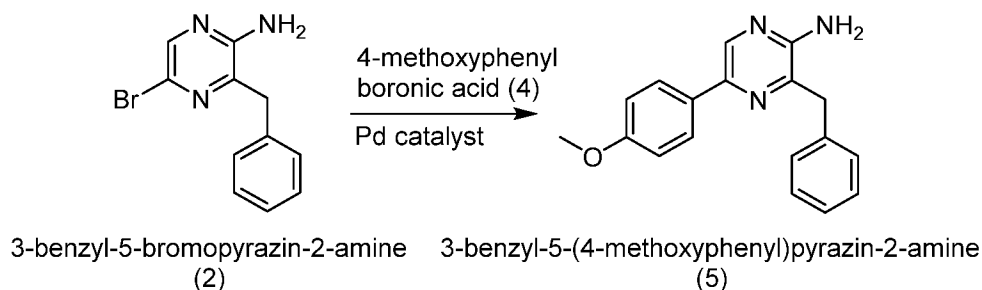
Meanwhile another 30 L flask was charged with tetrahydrofuran (THF) (15 L) and pyrazin-2-amine (24) (also named 2-amino pyrazine (24)) (1 Kg). The mixture was stirred at 25-35°C for 20 minutes. This solution was added over 1 – 1.5 hrs to the above benzyl lithium solution using addition flask. The mixture was stirred at room temperature for 1hr. Water (16 L) was added to the reaction mixture at 20°C dropwise while maintaining the temperature between 20-25°C. The mixture was stirred at room temperature for 30 min, and then the organic layer was separated, and distilled off excess solvents. The residue was treated with toluene (8 L) and water (3 L) and stirred for 10 minutes. The organic layer was separated, and solvent was distilled off at reduced pressure to yield the desired 3-benzylpyrazin-2-amine (25) in 60%-65% yield. Purity 94%-95%.

Synthesis of 3-benzyl-5-bromopyrazin-2-amine (2)



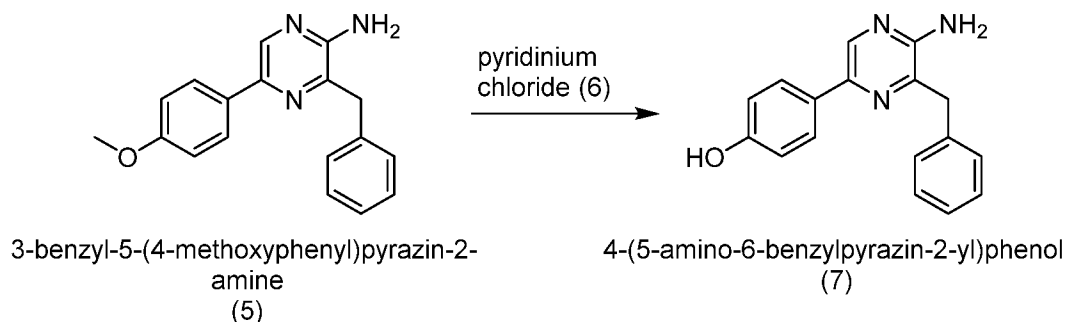
A 20 L round bottom flask was charged with chloroform (6 L) and 3-benzylpyrazin-2-amine (25) (also named 2-amino-3-benzyl pyrazine, (25)) (1 Kg), and stirred the mixture at room temperature (22 °C). N-bromosuccinimide (NBS) (800 grams) was added slowly over 1 to 1.5 hrs. After the complete addition, the mixture was stirred for 30 minutes. Water (2 L) was added and stirred for 10 minutes. The organic layer was separated and washed with water (2 x 1L). The chloroform layer was concentrated under reduced pressure and the oily residue was dried under vacuum. Yield 77%-85%. Purity 93%-95%.

Synthesis of 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5)



A 50 L flask was charged with 1,4-dioxane (30 L) and 3-benzyl-5-bromopyrazin-2-amine (2) (1 Kg) at room temperature. Potassium carbonate (1.6 Kg) was added followed by water (5 L). The reaction mixture was stirred for 10 minutes. 4-methoxyphenyl boronic acid (4) (600 grams) was added followed by palladium catalyst (300 grams). The reaction mixture was slowly heated up to 82°C and stirred at 80-82°C for 5 hrs. The mixture was cooled to room temperature and transferred to 100 L round bottomed flask, and charged with ethyl acetate (15 L) and water (15 L) at room temperature and stirred for 20 minutes. The organic layer was separated and concentrated under reduced pressure to yield the desired product. Yield 85%-90%. Purity 92%-95%.

Synthesis of 4-(5-amino-6-benzyl-pyrazin-2-yl)phenol (7)



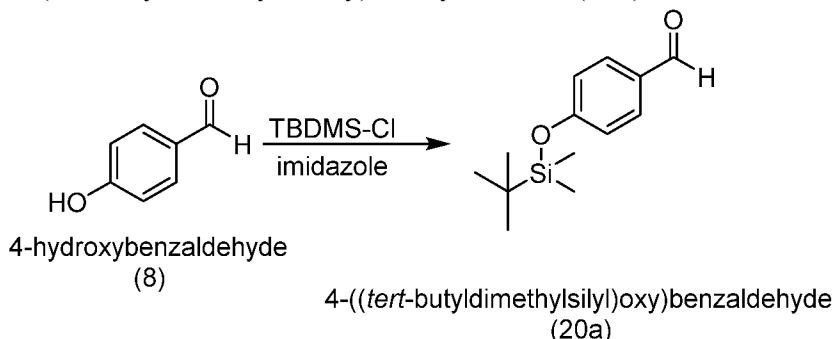
A 30L round bottom flask was charged with 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) (1Kg), and pyridine hydrochloride (6) (6.5 Kg). The reaction mixture was slowly heated to 200 °C in an oil bath. The reaction temperature was maintained at 200-210 °C for 30 min. The mixture was cooled to 40 °C and charged with water (13 L) and ethyl acetate (15 L) at 35-40 °C. The reaction mixture was stirred for 20 minutes. The ethyl acetate layer was separated and the aqueous layer was re-extracted with ethyl acetate (3L x 2). The combined ethyl acetate extracts were concentrated under reduced pressure. The residue was taken up with 2L of ethyl acetate and refluxed. The solution was slowly cooled to 10-15 °C and the solid formed was filtered to yield the desired product. Yield 90%-95%. Purity 90%.

Alternatively, instead of using pyridinium chloride, a 30L round bottom flask was charged with 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (1Kg), NaH (1Kg), DMF (20 L) and ethanethiol (2Kg). The reaction mixture was slowly heated to 100 °C in an oil bath. The reaction temperature was maintained at 100-110 °C for 30 min. The mixture was cooled to 40 °C and charged with water (13 L) and ethyl acetate (15 L) at 35-40 °C. The reaction mixture was stirred for 20 minutes. The ethyl acetate layer was separated, and the aqueous layer was re-extracted with ethyl acetate (3L x 2). The combined ethyl acetate extracts were concentrated under reduced pressure. The residue was taken up with 2L of ethyl acetate and refluxed. The solution was slowly cooled to 10-15 °C and the solid formed was filtered to yield the desired product. Yield 90%-95%. Purity 90%.

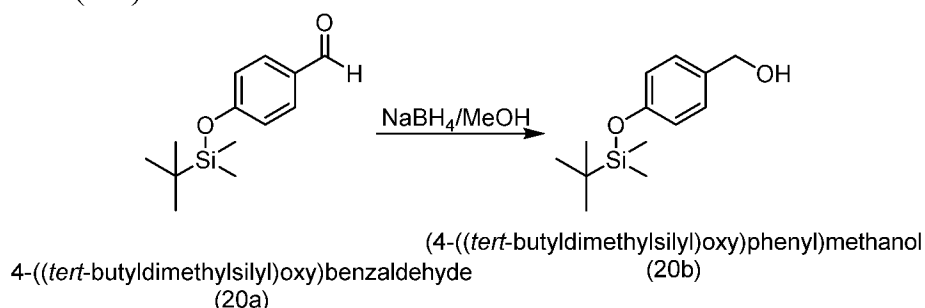
In both scenarios, the product can be optionally purified by charging 1 Kg of the product and 5 L 1,4-dioxane at room temperature into a 10L round bottom flask, adding sodium hydroxide solution (250g sodium hydroxide in 1L water) to the solution, charging the flask with 100g of charcoal and 100g silica gel at room temperature, stirring the mixture at room temperature for 20 mins, and filtering and washing the solids with 250 ml 1,4-dioxane.

The filtrate was then transferred to another 20.0 L round bottom flask and 200 ml HCl slowly added to the reaction mixture to adjust the pH to 7 to 7.5 at room temperature, during which a solid precipitate was observed. The reaction mixture was then stirred at room temperature for 30 mins, filtered, and washed with 200 ml 1,4-dioxane. The isolated solid product was dried under vacuum, and in the oven at 50-55°C for 4 hrs. Yield 90%-95%. Purity 90%.

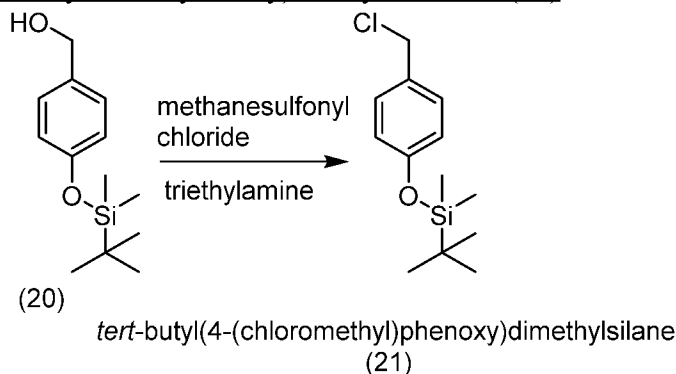
Synthesis of 4-(tert-butyldimethylsiloxy) benzyl alcohol (20b)



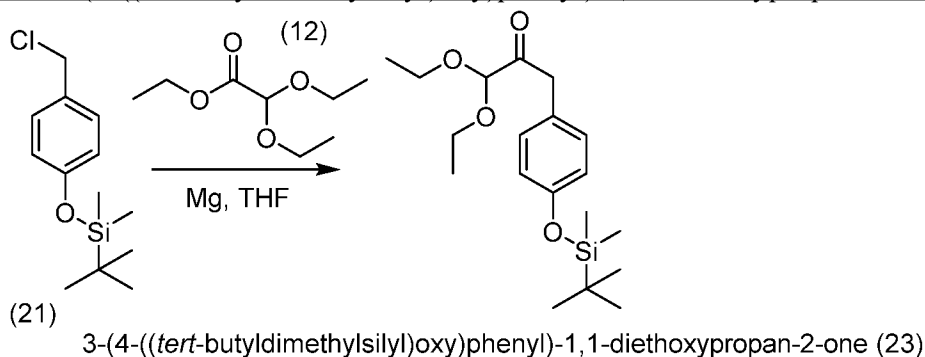
A 20 L round bottom flask was charged with dichloromethane (10 L), 4-hydroxy benzaldehyde (8) (1 Kg), N,N'-dimethylaminopyridine (50 grams) and imidazole (1.33 Kg). The reaction mixture was cooled to 20°C and stirred. To this stirred mixture was added portion wise tert-butyldimethylsilyl chloride (TBDMS-Cl, 500 grams x 3). After 1 hour, the reaction mixture was filtered, and concentrated under reduced pressure to get an oily product (20a).



The above product (20a) was taken in a 10 L round bottom flask and dissolved in methanol (6 L). The reaction mixture was cooled to 10-15 °C, and sodium borohydride (100 grams) was added with stirring. After 30 minutes the reaction pH was adjusted to 7.0 with acetic acid. After stirring for 20 minutes, methanol was distilled off to yield the desired 4-(tert-butyldimethylsiloxy) benzyl alcohol (20b). Yield 85%-90%, Purity 80%-85%.

Synthesis of 4-(tert-butyldimethylsiloxy) benzyl chloride (21)

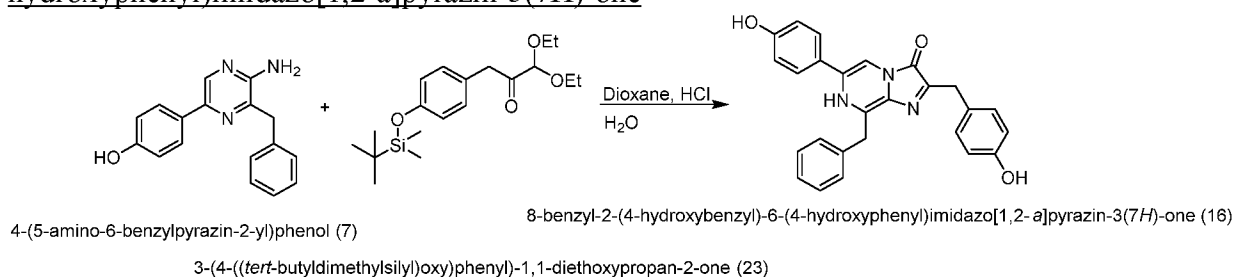
A 10 L round bottom flask was charged with 4-(tert-butyldimethylsiloxy)benzyl alcohol (20b) (1 Kg) and dichloromethane (6L) followed by triethylamine (1.4 L). The reaction mixture was stirred for 30 minutes at room temperature. Methanesulfonyl chloride (600 mL) was added slowly at 30-35 °C in about 1- 1.5 hours. After the completion of the reaction 30% aqueous sodium bicarbonate solution (400 ml) was added and stirred for 20 minutes. Dichloromethane layer was separated and washed with aqueous sodium chloride solution (2 x 500 ml). Dichloromethane was removed under reduced pressure. The residue was used for the next step without further purification.

Synthesis of 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23)

A 50 L round bottom flask was charged with magnesium turnings (1 Kg) and anhydrous tetrahydrofuran (3 L) followed by iodine (10 grams) and dibromoethane (50 ml). A solution of *tert*-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21) (1.6 Kg) in anhydrous tetrahydrofuran (12 L) was added drop wise at 40-45 °C over a period of 4 hours. The reaction mixture was cooled to 35 °C. Another 50 L round bottomed flask was charged with ethyl 2,2-diethoxyacetate (12) (2 Kg) and anhydrous tetrahydrofuran (10 L) and cooled to -35 °C. The above prepared Grignard reaction mixture was added to this solution at -35 °C over a period of 1-1.5 hours. After the completion of the reaction, the

reaction mixture was quenched with saturated aqueous ammonium chloride solution (1.2 Kg in water 7L). The organic layer was separated, washed with saturated sodium chloride solution and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography over silica gel with an eluant that includes triethylamine to yield 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) (0.48 Kg) Yield 20%-25%. Purity 90%.

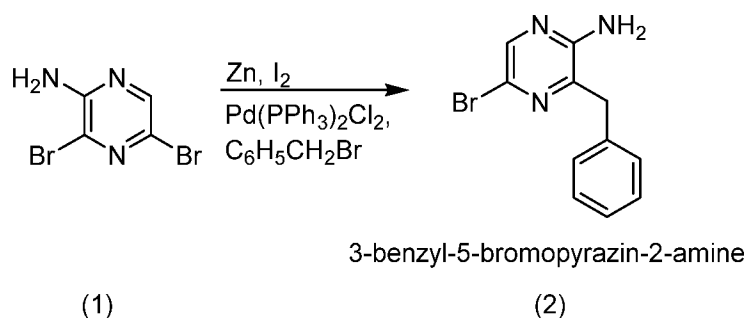
Synthesis of coelenterazine 8-benzyl-2-(4-hydroxybenzyl)-6-(4-hydroxyphenyl)imidazo[1,2-a]pyrazin-3(7H)-one



A 30 L round bottom flask was charged with 4-(5-amino-6-benzyl-pyrazin-2-yl)phenol (7) (0.9 Kg) and 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) (1.4 Kg) followed by 1, 4-dioxane (14 L). The reaction mixture was stirred at room temperature for 30 minutes. Concentrated hydrochloric acid (0.75 L) and water (0.75 L) was added and the reaction mixture was heated to 80-85 °C for 15 hours. The reaction mixture is cooled to 40 °C then activated carbon (100 g) and activated silica gel (100 g) are added and filtered. The solvent was removed under reduced pressure and the residue was precipitated by stirring with degassed ethyl acetate (2 L). Yield 60%-65%. Purity 60%-63%.

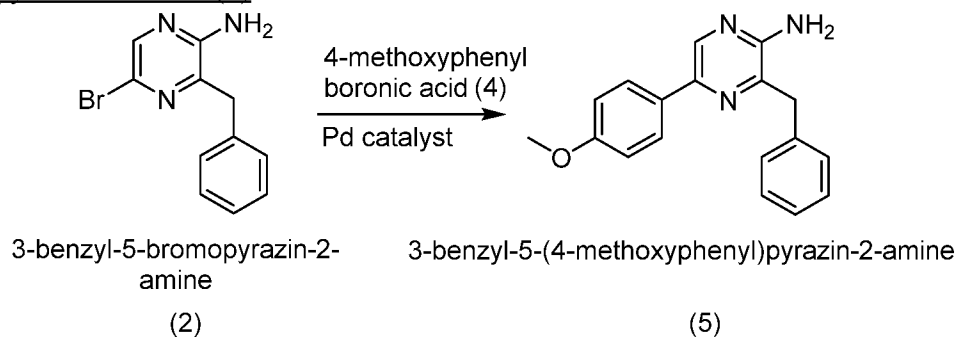
Example 2. Synthesis of Coelenterazine (16)

3-Benzyl-5-bromopyrazin-2-amine (2)



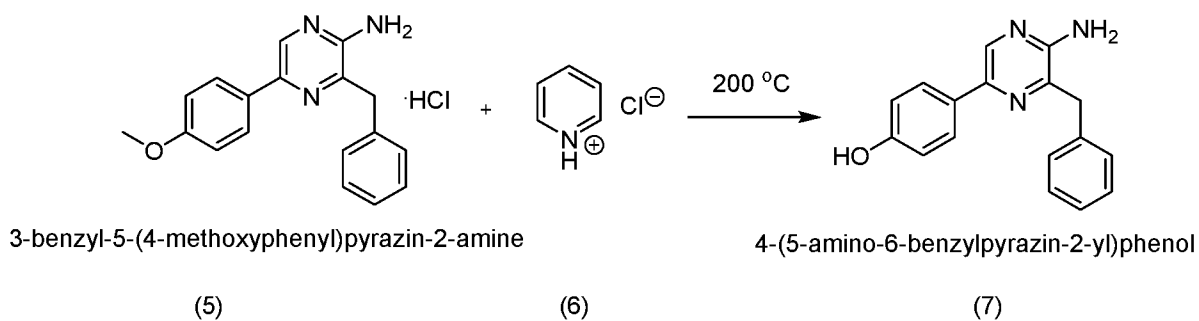
Zn dust and 30-mesh granular zinc (1:1, 16.05/16.05 g, 491.2 mmol, 3.5 equiv) and I₂ (6.23 g, 5% mol of Zn) were added to a dry 1 L 2-necked round-bottom flask under an argon atmosphere. *N,N*-dimethylacetamide (125 mL, freshly distilled over CaH₂) was added. The mixture was stirred at room temperature until the brown color of the I₂ disappeared. Benzyl bromide (61.04 g, 356.9 mmol, 2.5 equiv) was added dropwise, and the mixture was stirred at 80 °C for 4 h. The mixture was cooled to room temperature and a suspension of 3,5-dibromo-2-aminopyrazine (1) (36.0 g, 140.3 mmol, 1 equiv) and PdCl₂(PPh₃)₂ (5.04 g, 0.712 mmol, 5% of pyrazine) in *N,N*-dimethylacetamide (150 mL) was added. The mixture was stirred continuously under an argon atmosphere for 1 day. The thin layer chromatography (TLC) (30% ethyl acetate/hexane) indicated that the reaction was complete. The reaction mixture was filtered through a short bed of celite. The filtrate was slowly poured into ice cold water (1 L), and extracted with EtOAc (3 × 200 mL). The combined organic layer was washed with water (200 mL), brine (200 mL), and dried over anhydrous MgSO₄. The organic layer was filtered and concentrated on a rotary evaporator under reduced pressure. The purification of the brown residue on a silica gel column chromatography eluting with hexane/EtOAc, 2:1 gave 3-benzyl-5-bromopyrazin-2-amine (2) as brown viscous oil, 28.0 g 74% yield. ¹H NMR (CDCl₃): δ 8.05 (s, 1 H), 7.22-7.35 (m, 5 H), 4.41(s, 2 H), 4.11 (s, 2 H), 4.08 (s, 2H).

Palladium coupling reaction of p-methoxyphenyl boronic acid (4) with 3-benzyl-5-bromopyrazin-2-amine (2)



1,4-bis(diphenylphosphino)butane (2.71 g, 6.34 mmol) was added to a suspension of bis(benzonitrile)dichloropalladium (II) (2.03 g, 5.29 mmol) in toluene (210 mL) at room temperature under an argon atmosphere and stirred for 30 minutes. A solution of 3-benzyl-5-bromopyrazin-2-amine (2) (28.0 g, 106.0 mmol) in toluene (180 mL) was added to this mixture followed by 4-methoxyphenylboronic acid (20.94 g, 137.8 mmol), ethanol (42 mL), and 1.0 M aq. Na₂CO₃ (108 mL) were added sequentially at room temperature with stirring. The resulting 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 (400 mL) and extracted with ethyl acetate (EtOAc, 3 × 300 mL). The combined organic layer was washed with water (200 mL), brine (200 mL), and dried over anhydrous MgSO₄. The organic layer was filtered and the ethyl acetate solution was treated with 2 N HCl (200 mL) to precipitate the product as its hydrochloride salt. A yellow precipitate formed immediately. The precipitated solid was isolated by filtration and dried under vacuum. The dried solid was washed with ethyl acetate (2x100 mL) to remove non polar impurities and subsequently dried under vacuum to yield 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine hydrochloric acid salt (5), 24 g, 78% yield. ¹H NMR (CDCl₃): δ 8.05 (s, 1 H), 7.22-7.35 (m, 5 H), 4.41(s, 2 H), 4.11 (s, 2 H), 4.08 (s, 2H). The LCMS analysis indicated that the product was around 99% pure.

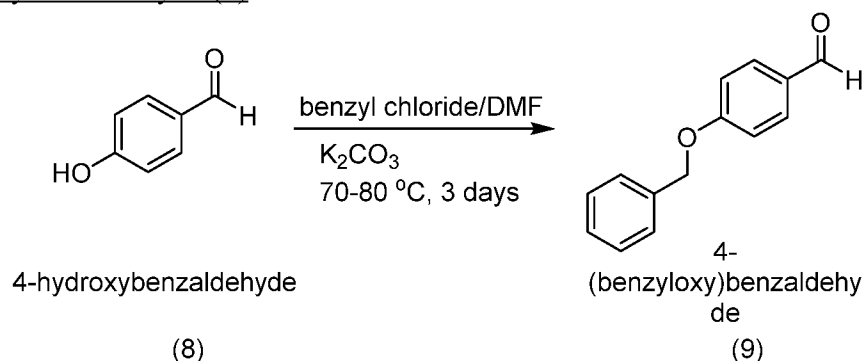
De-methoxylation of methyl ether hydrochloride salt to phenol using pyridinium hydrochloride



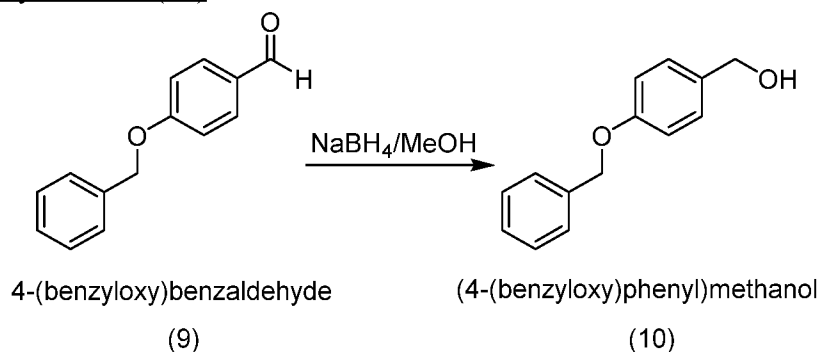
A mixture of 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine hydrochloric acid salt (5) (20.0 g, 61.0 mmol) and pyridinium hydrochloride (6) (70.5 g, 0.61 mol) was heated at 200 °C for 2 h under an argon atmosphere. The dark brown colored mixture was cooled to room temperature, and a saturated sodium bicarbonate solution (750 ml) was

added to the solid slowly followed by ethyl acetate (750 ml)). The organic layer was separated, and the aqueous layer was and extracted with EtOAc (3 ×200 mL). The combined ethyl acetate extracts were washed with water (2x300 mL), dried over anhydrous MgSO₄. The solution was filtered and concentrated on a rotary evaporator under reduced pressure. Purification of the brown residue on a silica gel column chromatography eluting with hexane–EtOAc, (3:7) gave 4-(5-amino-6-benzyl-pyrazin-2-yl)phenol (7), 14 g, 82% yield as light yellow solid., The solid was recrystallized from ethyl acetate /1% methanol to give a light brown powder. ¹H NMR (DMSO-D₆) δ 9.36 (s, 1 H), 8.17 (s, 1H), 7.60 (d, J = 8.4 Hz, 2H), 7.13-7.22 (m, 5H), 6.68 (d, J = 8.6 Hz, 2H), 6.09 (s, 2H), 3.94 (s, 2H). The LCMS analysis of the product indicated that it was around 99% pure.

4-Benzyloxybenzaldehyde (9)



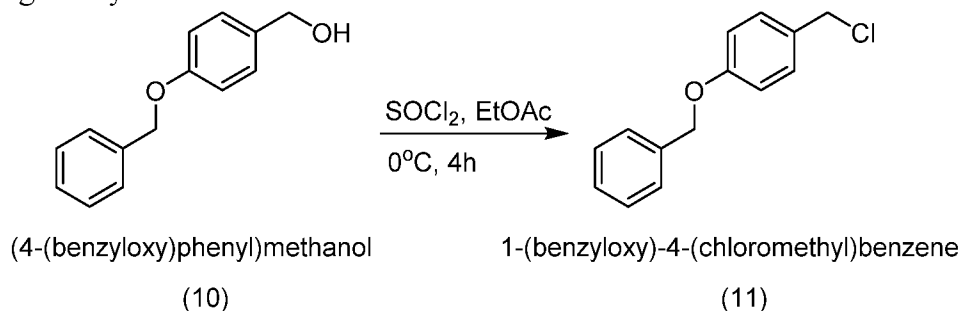
A mixture of 4-hydroxybenzaldehyde (8) (122.1 g, 1.0 mol), benzyl chloride (132.9 g, 1.05 mol), and anhydrous potassium carbonate (165.6 g, 1.2 mol) in N,N-dimethyl formamide (DMF, 1 L) was heated at 70-80 °C for 3 days with vigorous stirring. The progress of the reaction was monitored by TLC (20% ethyl acetate/hexane). The mixture was poured into ice cold water (4 L). The resulting solid was collected by filtration, washed with water (2x500 ml) and dried to yield the desired product as a white solid. Wt = 210 g, and yield 96.3%.

4-Benzyloxybenzyl alcohol (10)

Sodium borohydride (40.0 g, 1.08 mol) was slowly added portion wise to a solution of 4-(benzyloxy)benzaldehyde (9) (218.0 g, 1.02 mmol) in a mixture of ethyl acetate/methanol (1:1, 1 L) at 0-5 °C over a period of 1 h. After the complete addition of sodium borohydride, the reaction mixture was slowly warmed to room temperature and stirred for 2 h. The progress of the reaction was monitored by TLC (20% ethyl acetate/hexane). The mixture was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate (750 ml) and water (500 ml). The organic layer was separated. The aqueous layer was extracted with ethyl acetate (2x200 ml). The combined ethyl extracts were washed with water (500 ml) and dried over anhydrous MgSO_4 , filtered, and concentrated under reduced pressure. The residue was recrystallized with 20% ethyl acetate/hexane to yield desired product. Wt = 180.5 g, and yield 82.7%.

4-Benzyloxybenzyl chloride (11)

a) Using thionyl chloride.



Thionyl chloride (101.2 g, 0.86 mol) was added drop wise to a cooled solution of (4-(benzyloxy)phenyl)methanol (10) (167.0 g, 0.78 mol) in ethyl acetate (600 ml) at 0 °C over 1 h. After the addition the reaction mixture was warmed to room temperature and

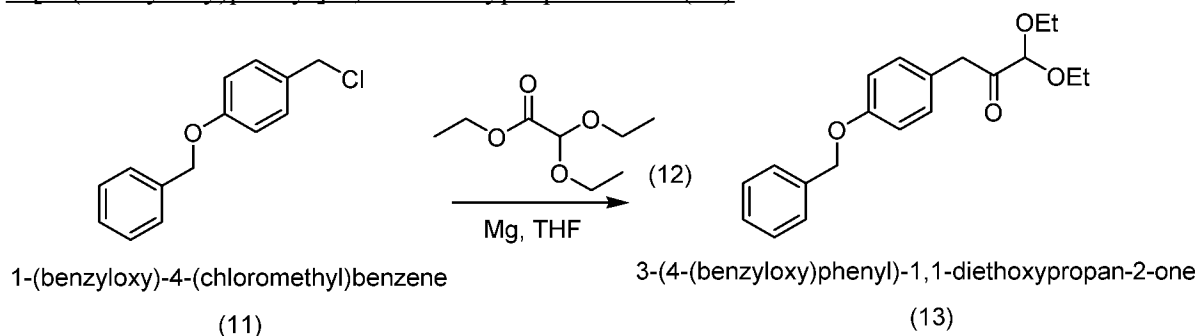
stirred. The progress of the reaction was monitored by TLC, after 4 h reaction was complete. The reaction mixture was concentrated under reduced pressure ($< 50\text{ }^{\circ}\text{C}$). The resulting residue was recrystallized twice with hexane to yield the desire product as white solid. Wt = 180 g, yield 77%. ^1H NMR (400 MHz, CDCl_3): $\delta = 4.57$ (s, 2 H), 5.08 (s, 2 H), 6.96(d, $J = 8.7$ Hz, 2 H), 7.32 (d, $J = 8.7$ Hz, 2 H), 7.43–7.37 (m, 5 H).

b) Using cyanuric chloride:

Cyanuric chloride (1.0 g) was added to anhydrous $\text{N,N}'$ -dimethylformamide (5ml) at room temperature under an atmosphere of argon and stirred for 30 minutes. A white suspension was formed. A solution of (4-(benzyloxy)phenyl)methanol (1.0 g) in dichloromethane (30ml) was added and stirred overnight. The precipitated solids were removed by filtration. The filtrate was diluted with hexane (50 ml) and washed with water, brine solution (20 ml each), dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure. The product was further purified by column chromatography over silica gel eluting with 5% ethyl acetate/hexane to give the product, Wt 650 mg Yield =59.5%

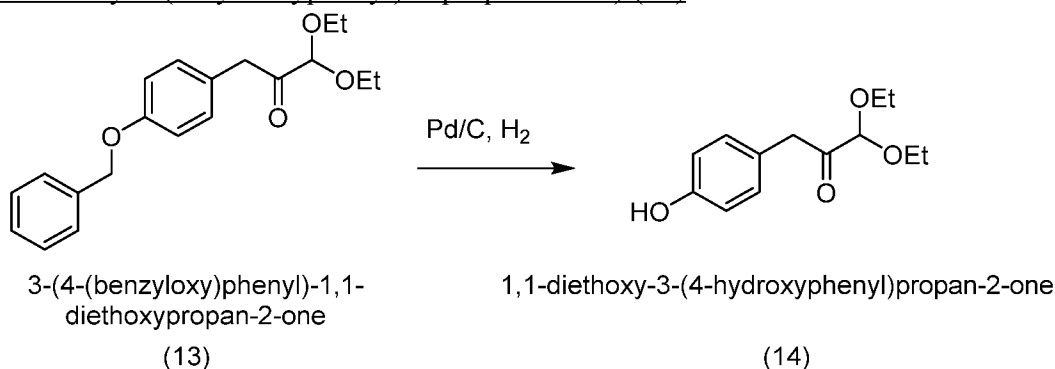
c) Using methanesulfonyl chloride:

Methanesulfonyl chloride (13.74 g; 0.12 mol) was added dropwise to a solution of (4-(benzyloxy)phenyl)methanol (21.8 g; 0.10 mol) and triethylamine (15.15 g; 0.15 mol) in dichloromethane (250 ml) at $0\text{ }^{\circ}\text{C}$. After the addition the reaction mixture was warmed to room temperature and stirred 12 h. The solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate (200ml) and water (100ml). The ethyl acetate layer was separated, washed with water, brine solution (75 ml each), dried over anhydrous magnesium sulfate. The crude product obtained after the removal of the solvent was recrystallized from hexane to obtain 18 gram of the product. Wt = 18 grams (Yield 70%).

3-[4-(Benzyloxy)phenyl]-1,1-diethoxypropan-2-one (13)

Mg turnings (10.57g, 0.435 mole, 1.5 equiv) were suspended in dry distilled THF (100 mL) in an argon-flushed, 500 mL two necked round-bottom flask. A solution of (11) (68.14 g, 0.29 mole) in THF (600 ml) was prepared and 25 ml of the solution was added at once and the flask was warmed to 40-50 °C with constant stirring until the Grignard reaction was initiated. After the initiation the remaining solution was added slowly at a rate so that the reaction mixture was warm to touch due to the heat of the reaction. After the addition mixture was stirred at room temperature for 30 min and then refluxed for 1 hour to complete the reaction. The pale yellow Grignard reagent was allowed to cool to room temperature and was then kept in an ice bath. A solution of ethyl diethoxy acetate (12) (51 g 0.29 mole,) in THF (200 mL) in a separate 1 liter round-bottom flask under an argon atmosphere and cooled to -78 °C. The Grignard reagent was transferred to dropping funnel and was added drop wise into the cooled flask over 45 min. The mixture was then stirred for 6 h at -78 °C and warmed to -20 °C and stirred for 2 h. The reaction was quenched with saturated ammonium chloride solution (200 mL). The reaction mixture was extracted with ethyl acetate (500 mL). The ethyl acetate layer was washed with H₂O (3 × 200 mL), followed by saturated brine solution (2 × 200 mL). The organic layer was dried (anhydrous magnesium sulfate) and the solvent was removed by rotary evaporation under reduced pressure. The product was heated to 150 °C under reduced pressure to remove the impurities and unreacted starting materials. NMR of the product showed it to be sufficiently pure to carry out the next reaction. Wt = 66.58 g Yield (70%)

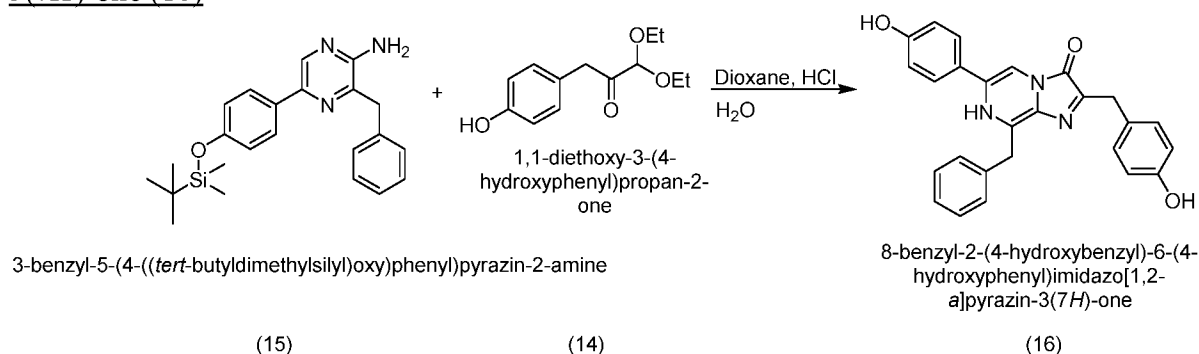
¹H NMR (400 MHz, CDCl₃): δ = 1.25 (t, *J* = 7.3 Hz, 6 H), 3.57 (m, 2 H), 3.69 (m, 2 H), 3.84 (s, 2 H), 4.64 (s, 1 H), 5.05 (s, 2 H), 6.94 (d, *J* = 8.4 Hz, 2 H), 7.14 (d, *J* = 8.4 Hz, 2 H), 7.43–7.35 (m, 5 H).

1,1-Diethoxy-3-(4-hydroxyphenyl)-2-propan-2-one (14)

Initial attempts to debenzylate under an atmosphere of hydrogen using palladium 10% on charcoal without the pressure reactor were not successful.

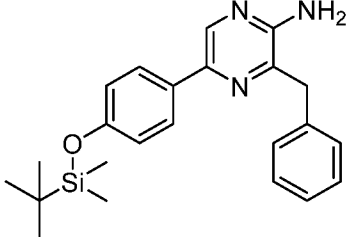
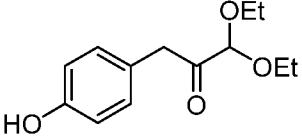
A solution of the compound (13) (66 g, 20.4 mole) in ethanol (400mL) was placed in a Parr hydrogenation flask and 10% Pd/C (7 g) was added. The mixture was hydrogenated for 24 hours under hydrogen atmosphere at 60 Psi. The black suspension was filtered and the solvent was removed using a rotary evaporator. The product was purified by passing through a small pad of silica gel to remove any suspended carbon particles eluting with 50% ethyl acetate/hexane. The desired product was isolated over a silica gel column eluting with 30% ethyl acetate/hexane to a colorless oil, 21.7 g.

^1H NMR (400 MHz, CDCl_3): δ = 1.25 (t, J = 7.0 Hz, 6 H), 3.55 (m, 2 H), 3.71 (m, 2 H), 3.82 (s, 2 H), 4.64 (s, 1 H), 5.11 (br s, 1 H), 6.77 (d, J = 8.6 Hz, 2 H), 7.07 (d, J = 8.6 Hz, 2 H).

Coelenterazine[8-Benzyl-6-(4-hydroxyphenyl)-2-[(4-hydroxyphenyl)methyl]imidazo[1,2-a]pyrazin-3(7H)-one (16)

The effect of dry hydrochloric acid, organic acids, vs. aqueous hydrochloric acid on the final condensation, rearrangement, and cyclization reaction of coelenterazine were evaluated. The following experiments were conducted either in ethanol or 1,4-dioxane. The results are summarized in the following Table 1.

Table 1. Coupling reaction conditions.

 (15)	 (14)	Reaction conditions	Result*
1 equivalent (eq)	1 eq	Ethanol (EtOH)/dry HCl, 80 °C, 8 h	--
1 eq	1 eq	EtOH/p-Toluenesulfonic acid (PTSA), 80 °C, 8 h	--
1 eq	1 eq	EtOH/Trifluoroacetic acid (TFA), 80 °C, 8 h	--
1 eq	1 eq	EtOH/ Conc. HCl, 80 °C, 8 h	~50% conversion
1 eq	1 eq	Dioxane/Conc. HCl, 80 °C, 8 h	Reaction is better than ethanol

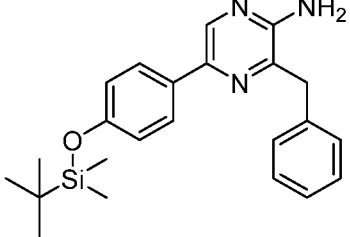
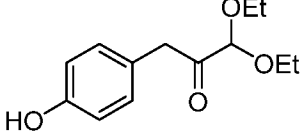
*The reaction mixture was analyzed by reverse phase HPLC. An authentic coelenterazine sample was used to identify the desired product.

The results from the above experiments (Table 1) indicated that the final condensation, rearrangement, and cyclization reaction of coelenterazine requires aqueous

hydrochloric acid and the desired solvent is 1,4-dioxane, which proved to be better than ethanol.

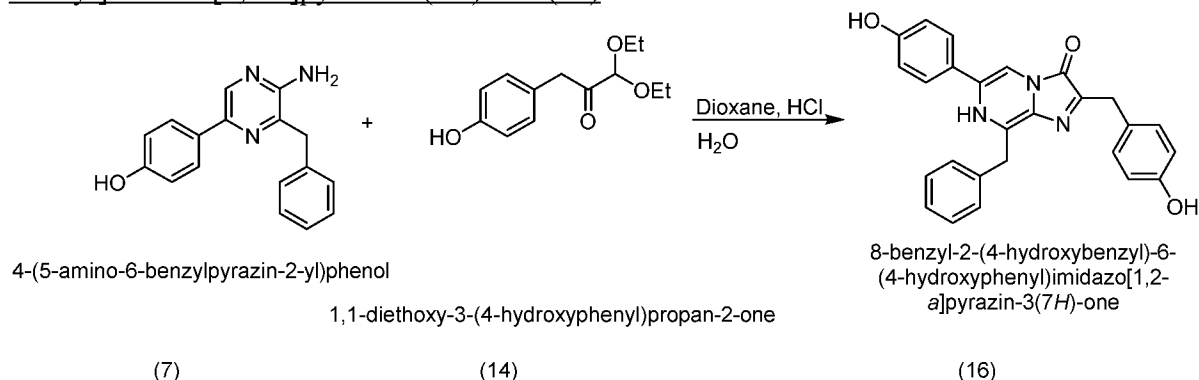
After finding right solvent and acid, experiments were conducted with varying amounts of starting material (14). Starting material (15) was kept constant at 1 equivalent and the amount of starting material (14) was increased. Unreacted excess of the reagent (starting material 14) could be removed by washing with organic solvent. Furthermore, under acidic reaction conditions only pyrazinamine starting material (15) derived product will form salt but not the acetal (14). To obtain insight into the correct amount of reagents required for optimal reaction conditions, the following experiments were conducted. The results are summarized in the following Table 2.

Table 2. Coupling reaction starting material ratios.

 (15)	 (14)	Reaction conditions	Result*
1 eq	1 eq	Dioxane (0.6 ml)/ 6N HCl (140 mg), water (0.1 ml), 80 °C, overnight	Good reaction, some side product
1 eq	1.3 eq	Dioxane (0.6 ml)/ 6N HCl (140 mg), water (0.1 ml), 80 °C, overnight	Better reaction, minimum side products
1 eq	1.7 eq	Dioxane (0.6 ml)/ 6N HCl (140 mg), water (0.1 ml), 80 °C, overnight	Best reaction, minimum side products

*The reaction mixture was analyzed by reverse phase HPLC. An authentic Coelenterazine sample was used to identify the desired product.

Coelenterazine, [8-benzyl-6-(4-hydroxyphenyl)-2-[(4-hydroxyphenyl)methyl]imidazo[1,2-*a*]pyrazin-3(7*H*)-one (16)

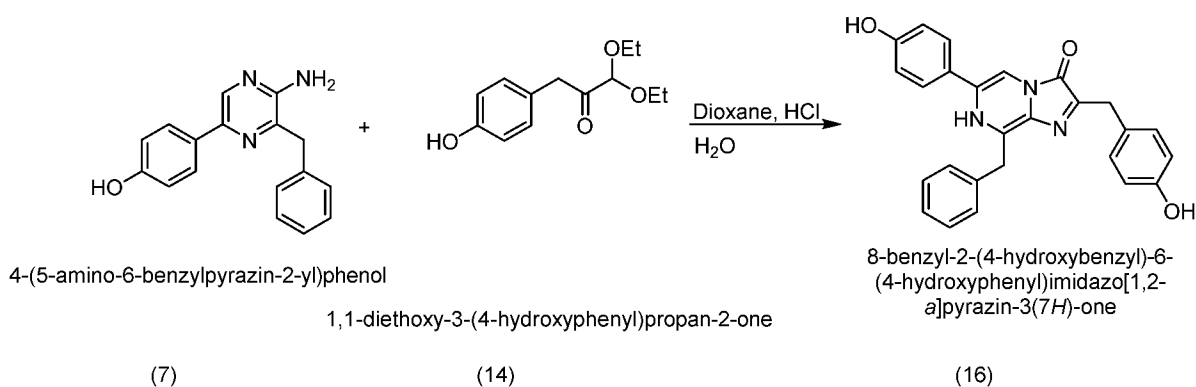


1,4-dioxane (2.3 mL), water (225 μ L), and conc. HCl (225 μ L) taken in a 25 ml round bottomed flask was degassed and filled with argon. 4-(5-amino-6-benzyl-pyrazin-2-yl)phenol ((7), 441 mg, 1.59 mmol) was added to this mixture. A solution of 1,1-diethoxy-3-(4-hydroxyphenyl)-2-propan-2-one ((14), 493 mg, 2.06 mmol, 1.3 eq) in 1,4-dioxane (2 mL) was added to this mixture. The resulting mixture was degassed and stirred under argon atmosphere at 78-82 $^{\circ}$ C for 14 hr. The dark brown solution was cooled to room temperature, and an aliquot was analyzed by reverse phase HPLC. The HPLC analysis indicated that the reaction mixture had little starting material. The reaction was degassed, and heating was continued for further 6 h (total 20 h). The reaction mixture was cooled to room temperature, and concentrated under reduced pressure. The dark brown residue was dried under high vacuum overnight. Ethyl acetate (40 mL) was added to the residue and triturated. The solid was allowed to settle decanted, and dried under vacuum to yield brown dry powder. Degassed ethyl acetate (40 mL) containing 1% methanol was added to the brown solid and triturated at 65 $^{\circ}$ C. The solid was allowed to settle, and decanted, and dried at pump to yield brown dry powder, 720 mg, quantitative yield. The proton NMR data is identical with that of reported values. On reverse phase HPLC it co-eluted with an authentic sample. The solid was again suspended in 40 ml of degassed ethyl acetate containing 1% methanol and stirred for 15 min., and decanted. The sample was dried at pump to yield brown solid.

Table 3. Mass spectra and LCMS analysis of 3 different samples.

Sample	Coelenterazine	Coelenterazine amine	Unknown
1	62%	11%	5.4%
2	65%	11%	6.2%
3	66%	10%	6.3%

Coelenterazine, [8-Benzyl-6-(4-hydroxyphenyl)-2-[(4-hydroxyphenyl)methyl]imidazo[1,2-*a*]pyrazin-3(7*H*)-one (16): 25 g scale synthesis



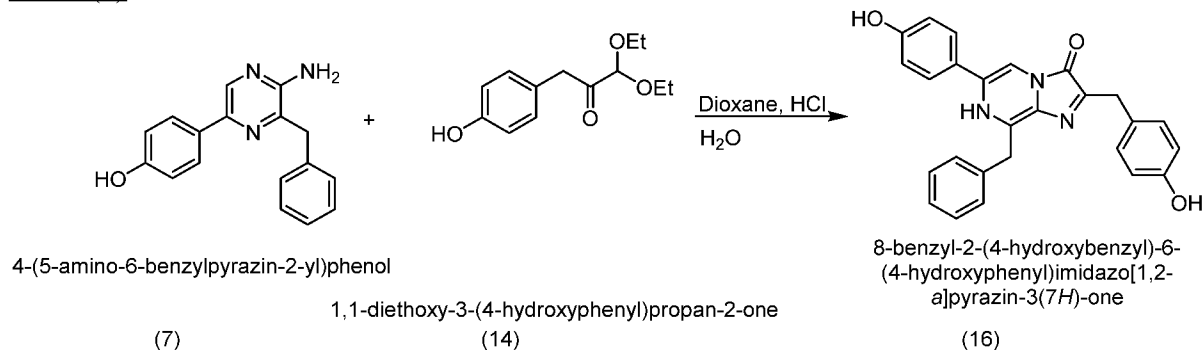
1,4-dioxane (80.0 mL), water (9.1 mL), and conc. HCl (9.1 mL) taken in 300 mL round bottomed flask was degassed and filled with argon. 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7, 15.94 g, 59.93 mmol) was added to this mixture and stirred. A degassed 1,4-dioxane (71 mL) solution of 1,1-diethoxy-3-(4-hydroxyphenyl)-2-propan-2-one (14, 16.4 g, 68.82 mmol, 1.2 eq) was added to this suspension. The resulting mixture was degassed and stirred under argon atmosphere at 78-84 °C for 34 h (the reaction progress was monitored by reverse phase HPLC). The reaction was cooled to room temperature, and concentrated under reduced pressure. The dark brown residue was dried under high vacuum overnight. Degassed ethyl acetate (250 mL) was added to this brown residue and triturated. The solid was allowed to settle, and decanted. This process was repeated second time, and the solid was dried at pump for 24 h to yield a brown dry powder, 25.8 g, in quantitative yield.

Example 3. Synthesis of Coelenterazine HCl salt, 100 g scale

Starting material synthesis:

The starting material 4-(5-amino-6-benzyl-pyrazin-2-yl)phenol (7) (53.0 g), and 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) (57.0 g) were synthesized by following the procedure described previously in Example 2.

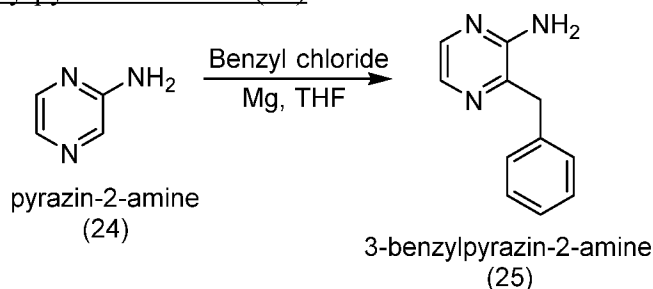
8-Benzyl-6-(4-hydroxyphenyl)-2-[(4-hydroxyphenyl)methyl]-7H-imidazo[1,2-a]pyrazin-3-one (3)



A 1 L round bottom flask equipped with a stirrer bar was charged with 4-(5-amino-6-benzyl-pyrazin-2-yl)phenol (7) (53.0 g, 191.25 mmol), followed by 1,4-dioxane (275 mL). The resulting mixture was degassed and filled with argon. To this stirred mixture was added degassed 1:1 water/conc. HCl (62.0 mL). The resulting mixture was again degassed and filled with argon, and stirred at room temperature for 15 min. To this stirred suspension was added a degassed 1,4-dioxane (244 mL) solution of 1,1-diethoxy-3-(4-hydroxyphenyl)-2-propan-2-one (14) (57.0 g, 239.21 mmol, 1.25 eq). The resulting mixture was degassed and stirred under argon atmosphere at 80-85 °C for 38 h (the reaction progress was monitored by reverse phase HPLC).

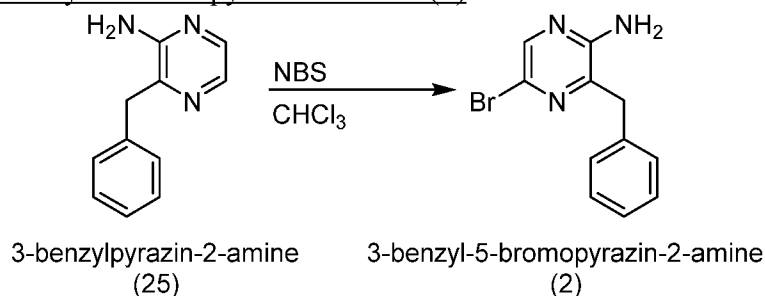
The reaction was cooled to room temperature, and concentrated under reduced pressure. The resulting dark brown residue was dried under high vacuum overnight. To this was added degassed ethyl acetate (250 mL) and triturated. The solid was allowed to settle, and decanted. This process was repeated one more time. Then added a degassed ethyl acetate (200 mL) containing 1% methanol to the brown solid and triturated. The solid was allowed to settle, and decanted. The residual dark brown solid was dried at high vacuum at 40 °C for 36 h, and stored under argon atmosphere, yield 101.8 g. On reverse phase HPLC it co-eluted with an authentic sample. The proton NMR spectral data were consistent with the reported values.

Example 4. Synthesis of Coelenterazine (Multiple Kilograms)

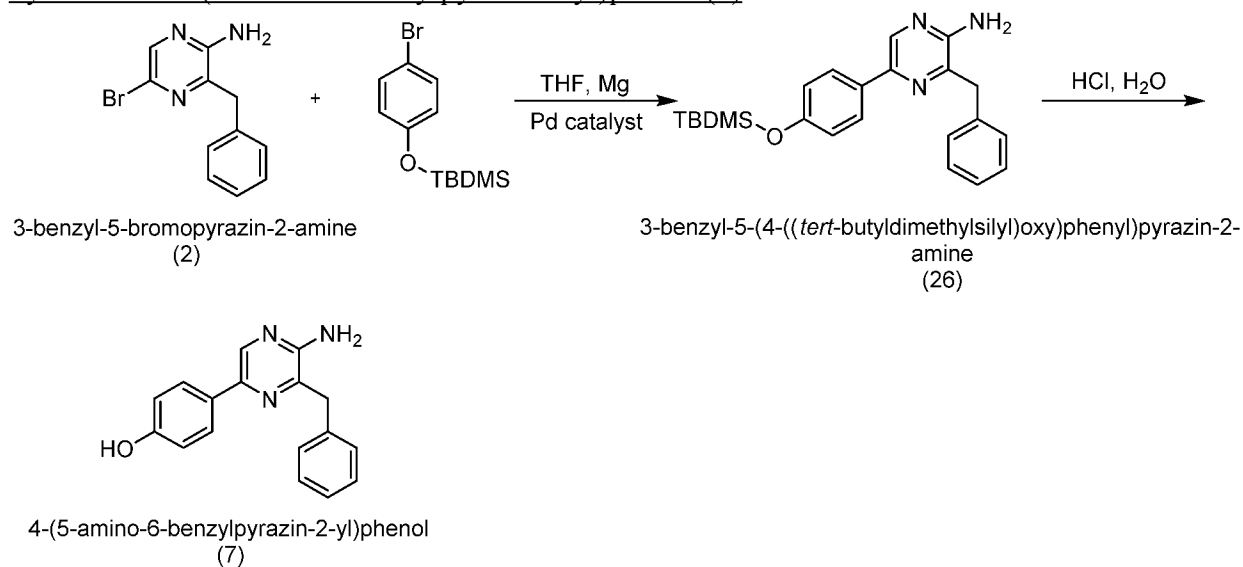
Synthesis of 3-benzylpyrazin-2-amine (25)

A 100 L round bottom flask was charged with 7.5 L of THF, 2.5 kg of magnesium metal, 10 g of iodine, and 200 ml ethyl bromide. The reaction mass was initiated. After initiation, benzyl chloride in THF solution (10 L benzyl chloride dissolved in 45 L of THF) was slowly added at 20-25 °C over a period of 4 to 4.5 hours and thereafter maintained for one hour at 30 – 35 °C. Then, 2-amino pyrazine solution (2.5 kg 2-amino pyrazine dissolved in 25L THF at 30-35 °C in 1 to 1.5 hours) was slowly added and the reaction mass was maintained for 5-6 hours at 30-35 °C. A TLC check was done, after compliance 10 L water was slowly added and the reaction mass was stirred for 20 minutes. The reaction mass was then allowed to settle for 30 minutes. The THF layer was separated and distilled out completely under vacuum below 70 °C. After completing the distillation, the reaction mass was then cooled to room temperature and charged with 20 L toluene and 5 L water and stirred for 10 minutes. The reaction mass was then allowed to settle for 20 minutes and the toluene layer was separated. The toluene layer was then charged with 3 L HCl and allowed to settle for 10 minutes. Then the toluene layer was separated and kept aside. The acidic HCl layer was then adjusted to a pH of about 8-9 with 3 kg soda ash and maintained for 30 minutes. The organic layer was then separated to yield the desired mono alkylated product in 40%-45% yield, purity 94%-95%.

The present modification reduces or eliminates the use of: n-butyl lithium-in the first step of the synthesis of Example 1, where n-butyl lithium reaction in toluene has been replaced by reaction with benzyl chloride and THF (tetrahydrofuran). Thus, the present Example improves the ability to scale up the reaction chemistry and reduces the cost of the synthesis. In addition, the changes improve the overall safety of the chemistry by replacing highly reactive materials with more stable materials.

Synthesis of 3-benzyl-5-bromopyrazin-2-amine (2)

The synthesis of 3-benzyl-5-bromopyrazin-2-amine is as described above, in Example 1. Specifically, a 20 L round bottom flask was charged with chloroform (6 L) and 3-benzylpyrazin-2-amine (25) (also named 2-amino-3-benzyl pyrazine, (25)) (1 Kg), and the mixture was stirred at room temperature (22 °C). N-bromosuccinimide (NBS) (800 grams) was added slowly over 1 to 1.5 hrs. After the complete addition, the mixture was stirred for 30 minutes. Water (2 L) was added and stirred for 10 minutes. The organic layer was separated and washed with water (2 x 1L). The chloroform layer was concentrated under reduced pressure and the oily residue was dried under vacuum. Yield 77%-85%. Purity 93%-95%.

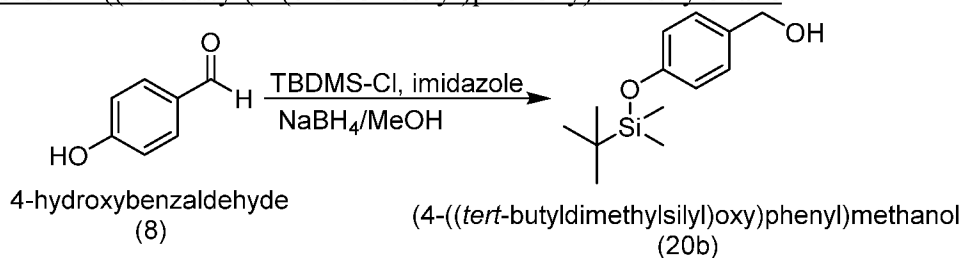
Synthesis of 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7)

2 kg of 3-benzyl-5-bromopyrazin-2-amine (2) was dissolved in 10 L THF and 20 g of palladium catalyst (significantly reduced from Example 1, 1/15 of the 300 g of palladium catalyst in Example 1) and stirred at room temperature for 30 minutes. This

solution was added to the Grignard reagent prepared using para bromo-phenol 3 kg, 4 kg of TBDMS chloride (t-butyldimethylsilyl chloride), and 2.5 kg of Mg metal. The reaction mixture was heated to 50 °C for 24 hours. After the completion of the reaction providing (26), Mg metal was filtered and diluted HCl 3L was added to reaction mixture and heated to 70 °C for 8 hours. To this reaction mixture containing (26) was added 2L of water and the product was extracted with ethyl acetate 2 L, this was repeated three times. The combined ethyl acetate layers were washed with water 1.5L, twice. The ethyl acetate is distilled to get the product (7) as a thick liquid with a weight of about 2.5 kg. Yield 70%-75% Purity 84%-88%.

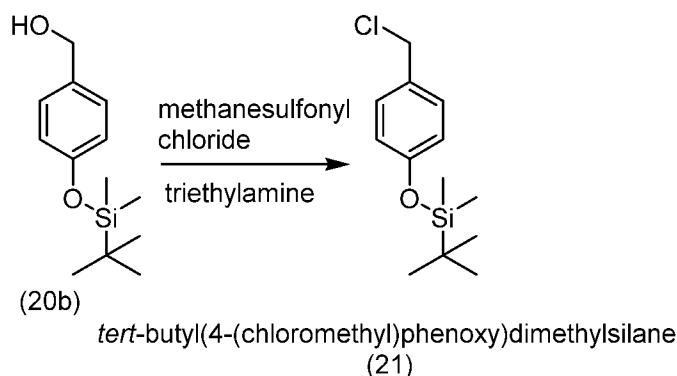
Thus, the present example omits the use of boronic acid compounds in the synthesis, and greatly reduces the amount of expensive palladium catalyst in the reaction.

Synthesis of 4-((tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane



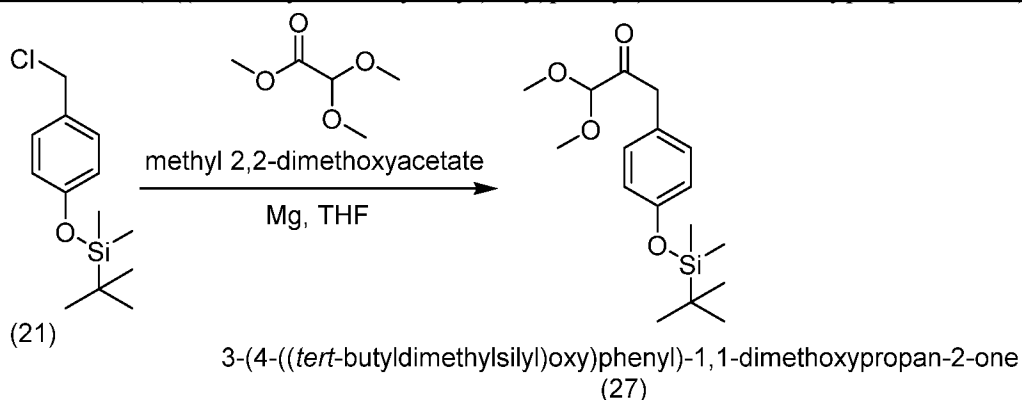
A 20 L round bottom flask was charged with dichloromethane (10 L), 4-hydroxy benzaldehyde (8) (1 Kg), N,N'-dimethylaminopyridine (50 grams) and imidazole (1.33 Kg). The reaction mixture was cooled to 20°C and stirred. To this stirred mixture was added portion-wise tert-butyldimethylsilyl chloride (TBDMS-Cl, 500 grams x 3). After 1 hour, the reaction mixture was filtered, and concentrated under reduced pressure to get an oily product (4-((tert-butyldimethylsilyl)oxy)benzaldehyde, (20a)).

The above product (20a) was taken in a 10 L round bottom flask and dissolved in methanol (6 L). The reaction mixture was cooled to 10-15 °C, and sodium borohydride (100 grams) was added with stirring. After 30 minutes the reaction pH was adjusted to 7.0 with acetic acid. After stirring for 20 minutes, methanol was distilled off to yield the desired product (20b). Yield 85%-90%, Purity 80%-85%.



A 10 L round bottom flask was charged with 4-(*tert*-butyldimethylsiloxy)benzyl alcohol (20b) (1 Kg) and dichloromethane (6L) followed by triethylamine (1.4 L). The reaction mixture was stirred for 30 minutes at room temperature. Methanesulfonyl chloride (600 mL) was added slowly at 30-35 °C in about 1-1.5 hours. After the completion of the reaction 30% aqueous sodium bicarbonate solution (400 ml) was added and stirred for 20 minutes. Dichloromethane layer was separated and washed with aqueous sodium chloride solution (2 x 500 ml). Dichloromethane was removed under reduced pressure. The residue containing *tert*-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21) was used for the next step without further purification.

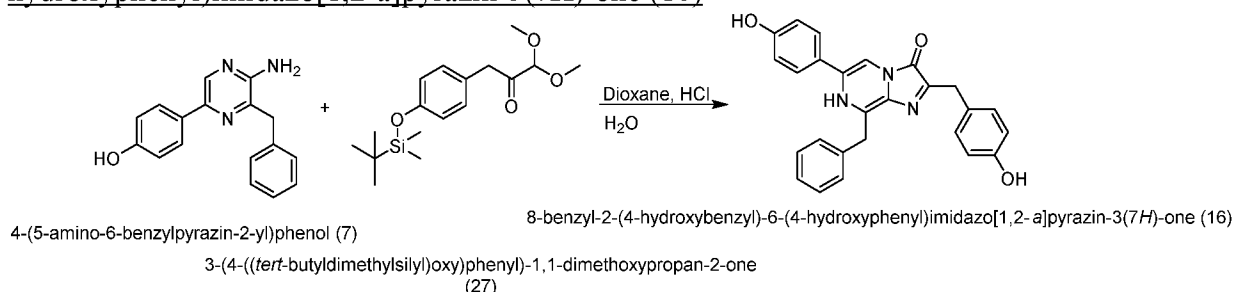
Synthesis of 3-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-1,1-dimethoxypropan-2-one (27)



A 50L round bottom flask was charged with magnesium turnings (1Kg) and anhydrous tetrahydrofuran (3L) followed by iodine (10 g) and dibromoethane (50 mL). A solution of *tert*-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21) (1.6 kg) in anhydrous tetrahydrofuran (12 L) was added drop wise at 40-45 °C over a period of 4 hours. The reaction mixture was cooled to 35 °C. Another 50 L round bottomed flask was charged with methyl 2,2-dimethoxyacetate (1.2 kg) and anhydrous tetrahydrofuran

(10 L) and cooled to 30-35 °C. The above-prepared Grignard reaction mixture was added to this solution at -10 °C over a period of 1-1.5 hours. After the completion of the reaction, the reaction mixture was quenched with saturated aqueous ammonium chloride solution (1.2 Kg in water 7L). The organic layer was separated, washed with saturated sodium chloride solution and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography over silica gel to yield 3-(4-((tert-butyl dimethylsilyl)oxy)phenyl)-1,1-dimethoxypropan-2-one (27) (0.48 Kg) Yield 40%-45%. Purity 90%.

Synthesis of coelenterazine, 8-benzyl-2-(4-hydroxybenzyl)-6-(4-hydroxyphenyl)imidazo[1,2-a]pyrazin-3(7H)-one (16)



A 30 L round bottom flask was charged with 4-(5-amino-6-benzyl-pyrazin-2-yl)phenol (7) (0.9 Kg) and 3-(4-((tert-butyl dimethylsilyl)oxy)phenyl)-1,1-dimethoxypropan-2-one (27) (1.4 Kg) followed by 1, 4-dioxane (14 L). The reaction mixture was stirred at room temperature for 30 minutes. Concentrated hydrochloric acid (0.75 L) and water (0.75 L) was added and the reaction mixture was heated to 80-85 °C for 15 hours. The reaction mixture was cooled to 40 °C then activated carbon (100 g) and activated silica gel (100 g) were added and filtered. The solvent was removed under reduced pressure and the residue (16) was precipitated by stirring with degassed ethyl acetate (2 L). Yield 60%-65%. Purity 60%-63%.

Example 5. LC-MS Characterization of Isolated Coelenterazine Compositions

The relative amount of coelenterazine to 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine) in the final isolated composition from the final coupling reaction to form coelenterazine in Examples 1-3 can be assessed by liquid chromatography-mass spectrometry (LC-MS).

A 1 mg/ml methanolic solution of isolated coelenterazine compositions resulting from coupling reactions of 4-(5-amino-6-benzyl-pyrazin-2-yl)phenol (7) and 3-(4-((tert-

butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23), as described, for example in Example 1 above, was diluted ten times into an injection solvent consisting of 70:30 reagent water : acetonitrile (v/v), each supplemented with 0.05% formic acid. The diluted solution including the isolated coelenterazine composition was separated by LC on a C-18 reverse phase column using a gradient elution. The separation provided a response for coelenterazine at about 1.7 min and a response for coelenteramine (7) at about 2.5 min. Tandem MS was configured to monitor the (M+H)⁺ parent ion of each compound which was subsequently fragmented into its characteristic daughter ion. The daughter ion intensity created the chromatographic signal for each compound which was then integrated to produce an area for the signal. The parent ion for coelenterazine is 424.1 Da with a daughter ion at 302.2 Da. The parent ion for coelenteramine is 278.1 Da and its daughter was 132.0 Da. In Table 4 below, the ratio of the coelenterazine to coelenteramine in the isolated composition was between about 24:1 and 80:1.

Table 4. Integrated peak ratios of coelenterazine to coelenteramine as assessed by LC-MS.

Composition	Coelenterazine integrated peak area	Coelenteramine integrated peak area	Coelenterazine/coelenteramine peak ratio
Isolated composition 1	3.63E+07	1.52E+06	24
Isolated composition 2	3.15E+07	3.93E+05	80
Isolated composition 3	2.72E+07	5.46E+05	50
Isolated composition 4	2.87E+07	5.37E+05	53
Isolated composition 5	2.91E+07	4.45E+05	65

While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the disclosure.

CLAIMS

The embodiments of the disclosure in which an exclusive property or privilege is claimed are defined as follows:

1. A method of making coelenterazine, comprising:
 - (a) reacting 3-benzylpyrazin-2-amine (25) with N-bromosuccinimide to provide 3-benzyl-5-bromopyrazin-2-amine (2);
 - (b) reacting the 3-benzyl-5-bromopyrazin-2-amine (2) in two sequential steps to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine); and
 - (c) coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one to provide coelenterazine, or a salt thereof.
2. The method of Claim 1, wherein (a) provides 3-benzyl-5-bromopyrazin-2-amine (2) in a yield of 60% to 85% relative to 3-benzylpyrazin-2-amine (25) and in a purity of at least 85%.
3. The method of Claim 1, wherein (b) comprises a first step of reacting the 3-benzyl-5-bromopyrazin-2-amine (2) with 4-methoxyphenyl boronic acid (4) in the presence of a palladium catalyst to provide 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5).
4. The method of Claim 3, wherein the palladium catalyst is tetrakis(triphenylphosphine)palladium(0).
5. The method of Claim 3 or Claim 4, wherein the palladium catalyst is present in an amount of 5 to 10 percent by weight relative to the 3-benzyl-5-bromopyrazin-2-amine (2).
6. The method of any one of Claims 3 to 5, wherein the first step in (b) comprises 1:1 to 1:1.3 molar equivalent of the 3-benzyl-5-bromopyrazin-2-amine (2) to 4-methoxyphenyl boronic acid (4).

7. The method of any one of Claims 3 to 6, wherein the first step in (b) comprises reacting the 3-benzyl-5-bromopyrazin-2-amine (2) and the 4-methoxyphenyl boronic acid (4) for a duration of 120 minutes to 300 minutes.

8. The method of any one of Claims 3 to 7, wherein the first step in (b) comprises reacting the 3-benzyl-5-bromopyrazin-2-amine (2) and the 4-methoxyphenyl boronic acid (4) at a temperature of 60 to 90 °C and at a pressure of 1 atm.

9. The method of any one of Claims 3 to 8, wherein the first step in (b) provides the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) in a yield of 60% to 85% relative to the 3-benzyl-5-bromopyrazin-2-amine (2) at a purity of 80% to 95%.

10. The method of any one of Claims 3 to 9, wherein (b) further comprises a second step of deprotecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) to provide the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7).

11. The method of Claim 10, wherein deprotecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) comprises subjecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) to pyridinium chloride.

12. The method of Claim 10, wherein deprotecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) comprises subjecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) to sodium hydride and ethanethiol in N,N'-dimethylformamide.

13. The method of any one of Claims 1 to 12, wherein the silyl protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one is 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23).

14. The method of Claim 13, wherein the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) is synthesized by:

- i. reacting 4-hydroxybenzaldehyde (8) with tert-butyldimethylsilyl chloride to provide 4-((tert-butyldimethylsilyl)oxy)benzaldehyde;
- ii. reacting the 4-((tert-butyldimethylsilyl)oxy)benzaldehyde with sodium borohydride to provide (4-((tert-butyldimethylsilyl)oxy)phenyl)methanol;

- iii. reacting the 4-((tert-butyldimethylsilyl)oxy)phenyl)methanol with methanesulfonyl chloride to provide tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21);
- iv. reacting the tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21) with magnesium to provide 4-((tert-butyldimethylsilyl)oxy)benzyl)magnesium chloride; and
- v. reacting the 4-((tert-butyldimethylsilyl)oxy)benzyl)magnesium chloride with ethyl 2,2-diethoxyacetate to provide the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23).

15. The method of Claim 14, wherein tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21) is further purified by chromatography with an eluant comprising triethylamine.

16. The method of Claim 14, wherein 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) is further purified by chromatography with an eluant comprising triethylamine.

17. The method of any one of Claims 1 to 16, wherein (c) further comprises coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) in a solvent mixture comprising dioxane, water, and HCl; or in a solvent mixture comprising dioxane, methanol, and isopropyl alcohol.

18. The method of any one of Claims 1 to 17, wherein (c) further comprises coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) at a temperature of 60 to 90 °C and at a pressure of 1 atm.

19. The method of any one of Claims 1 to 18, wherein (c) further comprises coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) for a duration of 16 hours to 28 hours when the solvent mixture comprises dioxane, water, and HCl; or for a

duration of 24 hours to 36 hours when the solvent mixture comprises dioxane, methanol, and isopropyl alcohol.

20. The method of any one of Claims 1 to 19, wherein (c) further comprises stirring the reaction mixture with activated carbon and silica after coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) for a duration of 16 hours to 28 hours when the solvent mixture comprises dioxane, water, and HCl; or for a duration of 24 hours to 36 hours when the solvent mixture comprises dioxane, methanol, and isopropyl alcohol.

21. The method of Claim 20, wherein after stirring the reaction mixture with activated carbon and silica, coelenterazine or a salt thereof is isolated by filtration, removal of solvents from the filtrate, trituration with ethyl acetate, followed by filtration.

22. The method of any one of Claims 1 to 21, wherein (c) provides a coelenterazine or a salt thereof, in a yield of 50% to 70% relative to the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) at a purity of 55% to 70%.

23. A method of making an absorbent article, comprising incorporating the coelenterazine, or a salt thereof, made according to the method of any one of Claims 1 to 22 into an absorbent article.

24. An absorbent article, comprising the coelenterazine, or a salt thereof, synthesized by the method of any one of Claims 1 to 23.

25. A method of making coelenterazine, comprising:

coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) to provide coelenterazine, or a salt thereof;

wherein the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) is synthesized by

i. reacting 4-hydroxybenzaldehyde (8) with tert-butyldimethylsilyl chloride to provide 4-((tert-butyldimethylsilyl)oxy)benzaldehyde (20a);

ii. reacting the 4-((tert-butyldimethylsilyl)oxy)benzaldehyde (20a) with sodium borohydride to provide 4-((tert-butyldimethylsilyl)oxy)phenyl)methanol (20b);

iii. reacting the 4-((tert-butyldimethylsilyl)oxy)phenyl)methanol (20b) with methanesulfonyl chloride to provide tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21);

iv. reacting the tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane (21) with magnesium to provide 4-((tert-butyldimethylsilyl)oxy)benzyl)magnesium chloride (22); and

v. reacting the 4-((tert-butyldimethylsilyl)oxy)benzyl)magnesium chloride with ethyl 2,2-diethoxyacetate to provide the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23).

26. The method of Claim 25, wherein 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) is prepared by:

(a) treating 3-benzylpyrazin-2-amine (25) with N-bromosuccinimide to provide 3-benzyl-5-bromopyrazin-2-amine (2); and

(b) reacting the 3-benzyl-5-bromopyrazin-2-amine (2) in two sequential steps to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7).

27. The method of Claim 26, wherein (a) provides 3-benzyl-5-bromopyrazin-2-amine (2) in a yield of 60% to 85% relative to 3-benzylpyrazin-2-amine (25) and in a purity of at least 85%.

28. The method of Claim 26, wherein (b) comprises a first step of reacting the 3-benzyl-5-bromopyrazin-2-amine (2) with 4-methoxyphenyl boronic acid (4) in the presence of tetrakis(triphenylphosphine)palladium(0) catalyst to provide 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5), wherein the palladium catalyst is present in an amount of 5 to 10 percent by weight relative to the 3-benzyl-5-bromopyrazin-2-amine (2).

29. The method of Claim 28, wherein (b) further comprises a second step of deprotecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) to provide the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7).

30. The method of Claim 29, wherein deprotecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) comprises subjecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) to pyridinium chloride.

31. The method of Claim 29, wherein deprotecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) comprises subjecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) to sodium hydride and ethanethiol in N,N'-dimethylformamide.

32. The method of any one of Claims 25 to 31, wherein tert-butyl(4-(chloromethyl)phenoxy)dimethylsilane is purified by chromatography with an eluant comprising triethylamine.

33. The method of any one of Claims 25 to 32, wherein 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) is purified by chromatography with an eluant comprising triethylamine.

34. The method of any one of Claims 25 to 33, wherein (c) further comprises coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) in a solvent mixture comprising dioxane, water, and HCl; or in a solvent mixture comprising dioxane, methanol, and isopropyl alcohol.

35. The method of any one of Claims 25 to 34, wherein (c) further comprises coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) for a duration of 16 hours to 28 hours when the solvent mixture comprises dioxane, water, and HCl; or for a duration of 24 hours to 36 hours when the solvent mixture comprises dioxane, methanol, and isopropyl alcohol.

36. The method of Claim 35, wherein (c) further comprises stirring the reaction mixture with activated carbon and silica, after coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with the 3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1,1-diethoxypropan-2-one (23) for a duration of 16 hours to 28 hours when the solvent

mixture comprises dioxane, water, and HCl; or for a duration of 24 hours to 36 hours when the solvent mixture comprises dioxane, methanol, and isopropyl alcohol.

37. The method of Claim 36, wherein after stirring the reaction mixture with activated carbon and silica, coelenterazine, or salt thereof, is isolated by filtration, removal of solvents in the filtrate, trituration with ethyl acetate, followed by filtration.

38. A method of making coelenterazine, comprising:

coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) to provide coelenterazine, or a salt thereof.

39. The method of Claim 38, wherein the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) is prepared by (a) reacting 3,5-dibromopyrazin-2-amine and (bromomethyl)benzene in the presence of zinc, iodine, and a first palladium catalyst to provide 3-benzyl-5-bromopyrazin-2-amine (2), or a salt thereof; and (b) reacting 3-benzyl-5-bromopyrazin-2-amine (2) in two sequential steps to provide the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7).

40. The method of Claim 39, wherein the first palladium catalyst is bis(triphenylphosphine) palladium(II) dichloride.

41. The method of Claim 39 or Claim 40, wherein the first palladium catalyst is present in an amount of 5 to 10 mole percent relative to 3,5-dibromopyrazin-2-amine.

42. The method of any one of Claims 39 to 41, wherein (a) comprises 1:2 to 1:3 molar equivalent of 3,5-dibromopyrazin-2-amine to (bromomethyl)benzene.

43. The method of any one of Claims 39 to 42, wherein in (a), 3-benzyl-5-bromopyrazin-2-amine (2) is provided in a yield of 55% to 75% relative to the 3,5-dibromopyrazin-2-amine at a purity of 80% to 95%.

44. The method of any one of Claims 39 to 43, wherein (a) comprises reacting 3,5-dibromopyrazin-2-amine and (bromomethyl)benzene for a duration of 18 hours to 30 hours.

45. The method of any one of Claims 39 to 44, wherein (a) comprises reacting the 3,5-dibromopyrazin-2-amine and the (bromomethyl)benzene at a temperature of 25 to 40 °C at a pressure of 1 atm.

46. The method of any one of Claims 39 to 45, wherein (b) comprises a first step of reacting the 3-benzyl-5-bromopyrazin-2-amine (2) with (4-methoxyphenyl)boronic acid in the presence of a second palladium catalyst to provide 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) (coelenteramine), or a salt thereof.

47. The method of Claim 46, wherein (b) further comprises isolating the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) (coelenteramine) by precipitation of the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) as a hydrochloride salt.

48. The method of Claim 46, wherein isolating the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) does not include chromatography (e.g., liquid chromatography), does not include recrystallization, or does not include chromatography and recrystallization

49. The method of any one of Claims 46 to 48, wherein the second palladium catalyst is bis(benzonitrile) palladium(II) dichloride.

50. The method of any one of Claims 46 to 49, wherein the second palladium catalyst is present in an amount of 5 to 10 mole percent relative to the 3-benzyl-5-bromopyrazin-2-amine (2).

51. The method of any one of Claims 46 to 50, wherein the first step in (b) further comprises 1,4-bis(diphenylphosphino)butane.

52. The method of Claim 51, wherein the 1,4-bis(diphenylphosphino)butane is present in an amount of 5 to 10 mole percent relative to the 3-benzyl-5-bromopyrazin-2-amine (2).

53. The method of any one of Claims 46 to 52, wherein the first step in (b) further comprises toluene, aqueous sodium carbonate, and ethanol.

54. The method of any one of Claims 46 to 53, wherein the first step in (b) comprises reacting the 3-benzyl-5-bromopyrazin-2-amine (2) with the (4-methoxyphenyl)boronic acid for a duration of 200 minutes to 350 minutes.

55. The method of any one of Claims 46 to 54, wherein the first step in (b) comprises reacting the 3-benzyl-5-bromopyrazin-2-amine (2) with the (4-methoxyphenyl)boronic acid at a temperature of 80 to 110 °C and at a pressure of 1 atm.

56. The method of any one of Claims 46 to 55, wherein the first step in (b) provides a 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) yield of 65% to 85% relative to the 3-benzyl-5-bromopyrazin-2-amine (2).

57. The method of any one of Claims 46 to 56, wherein (b) further comprises a second step of deprotecting the 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7).

58. The method of any one of Claims 38 to 57, wherein coupling of the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with the 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) is conducted in a solvent mixture comprising dioxane, water, and HCl; or in a solvent mixture comprising dioxane, methanol, and isopropyl alcohol.

59. The method of any one of Claims 38 to 58, wherein coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with the 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) is conducted at a temperature of 60 to 90 °C and at a pressure of 1 atm.

60. The method of any one of Claims 38 to 59, wherein coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with the 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) is conducted for a duration of 16 hours to 28 hours when the solvent mixture comprises dioxane, water, and HCl; or for a duration of 24 hours to 36 hours when the solvent mixture comprises dioxane, methanol, and isopropyl alcohol.

61. The method of any one of Claims 38 to 60, wherein the coupling reaction of 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) is monitored by reverse phase HPLC and is quenched when 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) stops depleting or when coelenterazine starts to decompose.

62. The method of Claim 60 or Claim 61, further comprising triturating the reaction mixture after coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with the 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14).

63. The method of any one of Claims 38 to 62, comprising a coelenterazine, or a salt thereof, in a yield of 60% to 70% relative to the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7), at a purity of 60% to 75%.

64. The method of any one of Claims 38 to 63, wherein the coelenterazine is isolated as a salt.

65. The method of Claim 64, wherein the coelenterazine salt is a hydrochloride salt.

66. A method of making coelenterazine, comprising:

(a) reacting pyrazin-2-amine (24) with benzyl chloride to provide 3-benzylpyrazin-2-amine (25);

(b) reacting 3-benzylpyrazin-2-amine (25) with N-bromosuccinimide to provide 3-benzyl-5-bromopyrazin-2-amine (2);

(c) reacting the 3-benzyl-5-bromopyrazin-2-amine (2) in two sequential steps to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) (coelenteramine); and

(d) coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one or silyl-protected 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one to provide coelenterazine, or a salt thereof.

67. The method of Claim 66, wherein (a) further comprises first providing a solution of magnesium, iodine, and ethyl bromide in a solvent before reacting pyrazin-2-

amine (24) with benzyl chloride to provide 3-benzylpyrazin-2-amine (25).

68. The method of Claim 66 or 67, wherein (b) comprises a first step of reacting the 3-benzyl-5-bromopyrazin-2-amine (2) with silyl-protected 4-bromophenol in the presence of magnesium and a palladium catalyst to provide silyl-protected 4-(5-amino-6-benzylpyrazin-2-yl)phenol.

69. The method of Claim 68, wherein the palladium catalyst is tetrakis(triphenylphosphine)palladium(0).

70. The method of Claim 68 or Claim 69, wherein the palladium catalyst is present in an amount of from 1 to 10 percent by weight relative to the 3-benzyl-5-bromopyrazin-2-amine (2).

71. The method of any one of Claims 66 to 70, wherein (b) further comprises a second step of deprotecting the silyl-protected 4-(5-amino-6-benzylpyrazin-2-yl)phenol to provide the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7).

72. The method of Claim 71, wherein deprotecting the silyl-protected 4-(5-amino-6-benzylpyrazin-2-yl)phenol comprises subjecting the silyl-protected 4-(5-amino-6-benzylpyrazin-2-yl)phenol to aqueous HCl.

73. The method of any one of Claims 66 to 72, wherein (d) comprises coupling the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with silyl-protected 1,1-dimethoxy-3-(4-hydroxyphenyl)propan-2-one to provide coelenterazine, or a salt thereof.

74. A method of making an absorbent article, comprising incorporating the coelenterazine, or a salt thereof, made according to the method of anyone of Claims 38 to 73 into an absorbent article.

75. An absorbent article, comprising the coelenterazine, or a salt thereof, synthesized by the methods of any one of Claims 38 to 73.

76. A method of making coelenterazine, comprising:

(a) reacting 3,5-dibromopyrazin-2-amine and (bromomethyl)benzene in the presence of zinc, iodine, and a first palladium catalyst to provide 3-benzyl-5-bromopyrazin-2-amine (2);

(b) reacting 3-benzyl-5-bromopyrazin-2-amine (2) in a first step to provide a hydrochloride salt of 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5), and reacting the hydrochloride salt of 3-benzyl-5-(4-methoxyphenyl)pyrazin-2-amine (5) in a second step to provide 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7); and

(c) coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) to provide coelenterazine, or a salt thereof.

77. A method of making coelenterazine, comprising:

coupling 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) for a duration of 16 hours to 28 hours, to provide coelenterazine, or a salt thereof;

wherein the coupling reaction is monitored by reverse phase HPLC and is quenched when 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) stops depleting, or when coelenterazine starts to decompose.

78. A method of making coelenterazine, comprising:

reacting 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) with 1,1-diethoxy-3-(4-hydroxyphenyl)propan-2-one (14) for a duration of 16 hours to 28 hours to provide crude coelenterazine, or a salt thereof;

tritulating crude coelenterazine with ethyl acetate; and

isolating a coelenterazine, or salt thereof.

79. The method of any one of Claims 1-22, 25-73, and 76-78, wherein the coelenterazine, or salt thereof, is isolated in a composition; wherein the composition further comprises 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7).

80. The method of Claim 79, wherein when measured using liquid chromatography-mass spectrometry, the composition comprises a coelenterazine elution peak and a 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) elution peak, and the integrated

peak ratio of the coelenterazine elution peak to the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) elution peak is 20:1 or more and/or 100:1 or less.

81. An absorbent article, comprising the composition made according to the method of Claim 79 or Claim 80.

82. A composition, comprising coelenterazine or a salt thereof, and 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7).

83. The composition of Claim 82, wherein when measured using liquid chromatography-mass spectrometry, the composition comprises a coelenterazine elution peak and a 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) elution peak, and the integrated peak ratio of the coelenterazine elution peak to the 4-(5-amino-6-benzylpyrazin-2-yl)phenol (7) elution peak is 20:1 or more and/or 100:1 or less.

84. An absorbent article comprising the composition of Claim 82 or Claim 83.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/040016

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D487/04 A61F13/42
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MACIEJ ADAMCZYK ET AL: "SYNTHESIS OF COELENTERAZINE", ORGANIC PREPARATIONS AND PROCEDURES INTERNATIONAL: THE NEW JOURNAL FOR ORGANIC SYNTHESIS, vol. 33, no. 5, 1 October 2001 (2001-10-01), pages 477-485, XP055619683, US ISSN: 0030-4948, DOI: 10.1080/00304940109356615 Scheme 1, 2 ----- -/-	1-22, 25-73, 77-80, 82,83



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

9 September 2019

Date of mailing of the international search report

18/09/2019

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Lauro, Paola

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/040016

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VITO VECE ET AL: "Multicomponent synthesis of novel coelenterazine derivatives substituted at the C-3 position", TETRAHEDRON, vol. 71, no. 46, 1 November 2015 (2015-11-01), pages 8781-8785, XP055619709, AMSTERDAM, NL ISSN: 0040-4020, DOI: 10.1016/j.tet.2015.09.048 Scheme 1, 3 -----	38-65,76
X	WO 2016/060850 A1 (WEYERHAEUSER NR CO [US]) 21 April 2016 (2016-04-21) pages 6-8; figure 1A -----	23,24, 74,75, 81,84

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2019/040016

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2016060850	A1	21-04-2016	AR 103127 A1 19-04-2017
			BR 112017007755 A2 16-01-2018
			CL 2017000937 A1 12-01-2018
			CN 106999313 A 01-08-2017
			EP 3206647 A1 23-08-2017
			JP 2017536865 A 14-12-2017
			US 2016108375 A1 21-04-2016
			US 2019203187 A1 04-07-2019
			WO 2016060850 A1 21-04-2016
