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On pyridopyrazinol chemistry, synthesis of chemiluminescent substances

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Our work on new chemiluminescent substances related to the marine luciferin coelenterazine ($\lambda_{\text{max}} = 465$ nm) led us to attempt the synthesis of four nitrogen-rich pyridopyrazine-bearing analogues. Accordingly, we studied the preparation of the corresponding benzyl-bearing pyridopyrazinols. By varying the conditions of condensations of phenylpyruvic acid and 1,2-diaminopyrine or 3,4-diaminopyridine, all the possible pyridopyrazin-2-ol regioisomers were isolated and properly characterized including by means of crystallographic studies. The ensuing synthesis of the halogenated pyridopyrazines was fraught with difficulties ranging from extensive decomposition to an unexpected ring contraction. In one instance, the inherently reductive mixture of phosphorus oxychloride and phosphorus trichloride provided the 2-benzyl-3-chloropyrido[2,3-b]pyrazine. This precursor was then transformed into the target O-acetylated luciferin (6,8-dibenzylimidazo[1,2-a]pyrido[3,2-e]pyrazin-9-yl acetate). The “benzo” derivative of this analogue (e.g.: 2,12-dibenzylimidazo[1',2':1,6]pyrazino[2,3-c]isoquinolin-3-yl acetate) was also made and the chemiluminescence emission spectra of these compounds were determined in a phosphate buffer ($\lambda_{\text{max}} = 546$ and 462 nm).

Key words chemiluminescence, coelenterazine, imidazo[1,2-a]pyrazin-3(7H)-one, pyridopyrazine, luciferin, heterocycles, rearrangement.

We recently reported on the preparation and chemiluminescence properties of an array of heterocyclic analogues of the luciferin coelenterazine (1) such as compounds 2a-b depicted in figure 1.\textsuperscript{1} Because of their imidazolone component, such heterocyclic systems will, upon an oxidative decarboxylation process, lead to the production of a photon.\textsuperscript{1} Similarly, reports have described the synthesis and chemi- or bio- luminescence properties of altered luciferins such as the heteroatom-bearing analogues 4-6. In these cases, the structural alterations provided a degree of electron enrichment of the imidazo[1,2-a]pyrazine nucleus and, contrary to the blue-hued derivative 3 ($\lambda_{\text{max}} = 462$ nm), their chemiluminescence emission spectra was shifted toward redder wavelengths.\textsuperscript{2} On the other hand, with other type of analogues, we recently achieved a rather modest chemiluminescence shift with a $\lambda_{\text{max}}$ of 513 nm but only in the case of compound 2b which is featuring two
electron-attracting fluorine atoms.\textsuperscript{1} This result drove us to envisage the synthesis of the four possible aza analogues 7-10 in an attempt to study the effect of an additional nitrogen on their chemiluminescence in comparison with compounds 2a-b. In case of a tangible red shift of their chemiluminescence spectra, this would pave the way for a research program aiming at an extensive alteration of the catalytic sites of coelenterazine-using luciferases so that these compounds would become actual substrates and thus provide a red-shifted bioluminescent reporting system. Such approach has actually been successful in the case of a firefly-based luciferin/luciferase bioluminescence system.\textsuperscript{3} Accordingly, we focused on the preparation of the four halogenated pyridopyrazines 11-14 which, as we have demonstrated in other instances, are good intermediates to construct the imidazole component of imidazo[1,2-a]pyrazin-3(7H)-ones as well as many related heterocyclic systems.\textsuperscript{1, 4} Synthetic accesses to the chloropyridopyrazines 11 and 12 depicted were sought via a chlorination of the corresponding isomeric 2-benzylpyrido[3,4-b]pyrazin-3-ol (17) and 3-benzylpyrido[3,4-b]pyrazin-2-ol (20).

As depicted in scheme 1, to prepare these substances, we started from two reports describing regioselective condensations between 3,4-diaminopyrazine (15) and pyruvic acid or ethyl pyruvate in which the acidity of the reaction medium is actually governing the hydration state of the pyruvate and thus the orientation of the reaction.\textsuperscript{5} Accordingly, we tried these conditions using phenylpyruvic acid (16) instead of pyruvic acid.

Figure 1 Structures of the target compounds 1-10 and intermediates 11-14.
When heating compounds 15 and 16 in ethanol, the reaction led to the isolation of 34 % of the pyrido[3,4-b]pyrazin-2-ol 17 which structure was firmly established by means of X-ray based diffraction, as depicted here with the corresponding ORTEP representation. Moreover, sizable amount of the highly insoluble aminoacid 18 was also obtained although this compound could only be partially characterized (by 1H NMR and HRMS) as it turned out to decompose in the course of recrystallization attempts. A drastically different result was obtained when heating compounds 15 and 16 in ethanol along with 3 equivalent of hydrochloric acid 2N. Indeed, the thick precipitated turned out to be the hydrochloride salt of the imidazopyridine 19a (featuring a CH signal at 6.74 ppm) which was isolated in an 83% yield and fully characterized.

Scheme 1 i: PhCH$_2$COCO$_2$H (16), EtOH, reflux. ii: PhCH$_2$COCO$_2$H (16), EtOH, HCl 2N (3 eq.). iii: 1) EtOH, NaOH, reflux, 3 min, 2) NH$_4$Cl.

Interestingly, upon a basic treatment of this compound, the target isomeric 2-benzylpyrido[3,4-b]pyrazin-3-ol (20) was then isolated in a 66% yield after a simple extraction. A microanalysis was actually required to confirm this structure since, in solution, the pyridopyrazin-3-ol 20 (characterized by a CH$_2$ signal at 4.18 ppm and a broad singlet at 12.59 ppm) slowly isomerized into the free base form of imidazopyridine 19b (displaying a CH signal at 6.45 ppm as well as two broad singlets at 11.05 and 9.50 ppm) and appeared to reach a stable 1/1.6 ratio after 48 hours. To conclude this series of unexpected results, all our attempts to obtain the corresponding chloropyridopyrazines 11 or 12, from 17 or 20, using a variety of methods
unfortunately failed as extensive decompositions were the sole results. Similarly, methylation trials under basic conditions, or using methylorthocarboxylates, led to complex mixtures of compounds.

As depicted in scheme 2, our attempts to prepare the chloropyridopyrazines 13-14 were somewhat more successful. Again, a regioselective synthesis of the corresponding 2-benzylpyrido[2,3-b]pyrazin-3-ol (22) and 3-benzylpyrido[2,3-b]pyrazin-2-ol (23) relied on the reported mechanistic investigations explaining the orientation of the condensations between 2,3-diaminopyrazine (21) and ethyl pyruvate. Of note is that if these reports described rather optimists yields and selectivity, a subsequent publication using these reagents reported far more sober results. In our case, from 2,3-diaminopyrazine (21) and phenylpyruvic acid (16), we obtained mixtures of the target isomers 22 and 23 in proportion dependent on the reaction conditions. Heating the diamine 21 phenylpyruvic acid (16) in ethanol at 85 °C in a closed reactor for 26 hours (conditions i) gave the pyridopyrazine isomers 22 and 23 in respectively 40 and 15% isolated yield. As depicted with its ORTEP, an X-ray based diffraction analysis again firmly established the structure of compound 22. Moreover, the more polar imidazopyridine derivative 24 was also detected in the crude reaction products. Under acidic conditions (conditions ii) the proportion of isomer was inversed and compound 22 and 23 were isolated in respectively 4 and 33% yield. Moreover, we could also isolate in this trial 7% of the rather insoluble imidazopyridine derivative 24. Interestingly, upon treatment of compound 24 with sodium hydroxide, and contrary to its isomers 19, this imidazopyridine mostly led to an extensive decomposition. Chlorination of the hydroxypyridopyrazine isomer 22 was initially problematic, although upon LC/MS monitoring we observed the occurrence of side compounds plausibly arising from oxidation processes. Accordingly, we undertook this reaction in a closed vessel in the presence of phosphorus trichloride as a reducing agent very much compatible with the phosphorus oxychloride used for this reaction. This combination proved to be rewarding and we could isolate compound 13 in a 55% yield. On the other hand, the use of these conditions, or many others, to obtain the corresponding chlorinated derivative 14 from compound 23 all failed. In every cases, the imidazopyridine 24 resulting from a remarkable rearrangement was the sole product detected in 1H NMR analysis of the reaction (more is suggested regarding this observation in the conclusion). Nevertheless, from the halogenated pyridopyrazine 13 we undertook the preparation of the O-acetylated derivative 27, the stabilized precursor of the target compound 9. This proceeded via a Buchwald-Hartwig N-arylation of phenylalanine ethyl ester (25) to give compound 26 in a rather modest 12% yield. Then, a saponification followed in situ by the addition of an excess of acetic anhydride gave the O-acetylated derivative 27 in a 33% yield after a chromatography.
As depicted in scheme 3 and to conclude this work, we also undertook the preparation of the benzo derivative 34 structurally related to compound 27. To achieve this, we envisaged a copper chloride-catalyzed condensation between the readily available 8-bromoisoquinolin-3-amine (28) and phenylalanine (29) to give the 1,4-dihydropyrazinoisoquinoline derivative 31. The first report describing similar transformations mentions the use of 1% of copper chloride, and in most of the examples, the yields described for the corresponding “dihydro” derivative are quite remarkable. However, in our specific case we had to resort to the use of 10% of copper chloride to achieve a degree of transformation along with rather low yields. Moreover, we always observed, by LC/MS and $^1$H NMR spectra, the occurrence of sizable amount of the
Aromatized derivative 32 which is in accordance with related results described in a subsequent publication.\textsuperscript{10} Along with these two compounds, we also noticed the occurrence of the aminoisoquinoline 30 plausibly resulting from high temperature DMSO-based reduction process.\textsuperscript{11} Eventually, we employed a different set of reported conditions\textsuperscript{12} involving copper oxide instead of copper chloride, a rather larger excess of amino acid and the much lower reaction temperature of 90 °C along with a rather extended 40 hours reaction time. In our case, this led to the expected mixture of the dihydro and the aromatized derivatives 31 and 32 along with some unreacted 4-bromoisooquinolin-3-amine (28). As fully detailed below, a procedure was then designed to isolate 31 and 32 from the reaction mixture and a treatment of the dihydro derivative 31 with N-bromosuccinimide in ethanol, followed by a filtration, gave additional amount of the aromatized product 32. With this two stages procedure an overall yield of 30\% of compound 32 was thus achieved from compound 28. Chlorination of compound 32, using the same combination of PCl\textsubscript{3} and POCl\textsubscript{3} mentioned above then gave the halogenated derivative 33 in 61\%. The next step turned out to be problematic as the Buchwald-Hartwig N-arylation of phenylalanine ethyl ester (25) with compound 33, using our previously reported conditions,\textsuperscript{4} invariably led to an extensive decomposition.

\begin{align*}
\text{28} & \xrightarrow{i} \text{30} + \text{31} \\
& \xrightarrow{\text{ii}} \text{32} \\
& \xrightarrow{\text{iii}} \text{33} \\
& \xrightarrow{\text{iv}} \text{34} \\
& \xrightarrow{\text{v}} \text{35}
\end{align*}

Scheme 3 i: PhCH\textsubscript{2}CHNH\textsubscript{2}CO\textsubscript{2}H (29), Cu\textsubscript{2}O, K\textsubscript{3}PO\textsubscript{4}, DMSO, 90°C. ii: NBS, EtOH, 20 °C. iii: PCl\textsubscript{3}, POCl\textsubscript{3}, 80 °C. iv: PhCH\textsubscript{2}CHNH\textsubscript{2}CO\textsubscript{2}Et (25, as a free base), NMP, 120 °C, 4 days. v: 1) NaOH, THF, 20 °C, 2) Ac\textsubscript{2}O, 20 °C.

It is only by heating for four days at 120°C a solution of the phenylalanine ethyl ester (25) (as a free base) and 33 in N-methylpyrrolidone (NMP) without any palladium catalyst that a degree of N-arylation was achieved and compound 34 was isolated in a 10\% yield along with 25\% of the starting material 33.
Interestingly, no cyclized product (the target luciferin) could be observed in this step although we previously reported such reaction in another difficult instance under similar reaction conditions. From this ester, a last step gave the target O-acetylated luciferin 35 although in a rather modest 29% yield as a chromatography turned out to be necessary.

Unexpectedly and probably because of the inherent basicity of their neighboring nitrogen, the acetyl function of compounds 27 and 35 could not be hydrolyzed under the acidic conditions we previously used\textsuperscript{4} and even trials under harsher (acidic) conditions failed. On the other hand, these acetyl groups were readily hydrolyzed in a phosphate buffer (pH = 7.4) and, as expected, the resulting luciferin immediately reacted with oxygen to decompose and produce a photon. Accordingly, the determination of the chemiluminescence emission spectra of these two analogues was conducted using their O-acetylated derivatives in this buffer. The use of an Aqualog® (HORIBA Instruments, Inc.), a spectrometer endowed with a sensitive CCD detector (Water-Raman SNR > 20,000:1 - RMS method), was again\textsuperscript{1} crucial as well as the addition 2.5 mMol of cetyltrimethylammonium bromide to the buffer which led to a great enhancement of their signals. As depicted in the experimental part, the \( \lambda_{\text{max}} \) measured for the chemiluminescence of compounds 27 and 35 were respectively of 546 and 462 nm at 2.5 and 5 \( \mu \text{Mol} \) final concentration.

In conclusion, our attempt to prepare the four possible aza-analogues of luciferin 2a met quite a lot of unexpected problems. If the preparation and isolation of the benzylpyridopyrazinols 17, 20, 22 and 23 precursors could be achieved in rather modest yields, their transformation into the four corresponding halogenated derivatives turned out to be possible in only one instance. The rather unexpected rearrangement of compound 23 into the imidazopyridine 24 upon treatment with reagents such as POCl\textsubscript{3} or SOCl\textsubscript{2} is actually providing some insight in the reactivity of such heterocyclic systems and is actually reminiscent of various quinoxalinone ring contractions into benzimidazole previously reported.\textsuperscript{13} In our case, we suggest the mechanism depicted in scheme 4 to account for this experimental fact. The depicted second addition (ii) of phosphorus oxychloride on compound 23 nitrogen’s to give intermediate 37 could be the key accounting for the fragility of the pyrazine ring and its contraction into the imidazole component of compound 24 via penta-coordinated phosphor intermediates 38-40. A similar process can also be written with thionyl chloride leading then to a tetra-coordinated sulfur intermediate. Of note in this work is the use of the inherently reductive mixture of phosphorus oxychloride and phosphorus trichloride to achieve the preparation of compound 13 and 33. We are not aware of any reports describing the advantages of using such reaction conditions. Concerning the chemiluminescence study, even if the two O-acetylated proluciferins analogues made 27 and 35 could not be hydrolyzed under acidic conditions, we could determine their emission spectra by adding them to a neutral phosphate buffer which led in situ to the hydrolysis of their acetyl moiety. Interestingly, from compound 27 the luciferin 9 turned out be endowed with yellow colored chemiluminescence spectra (\( \lambda_{\text{max}} \) of 546 nm), even better than the green-emitting compound 2b (\( \lambda_{\text{max}} = 513 \) nm). On the other hand, in the same phosphate buffer, the benzo derivative 35 gave a blue signal at 462 nm, thus negating any gain from the insertion of the nitrogen in this heterocyclic system. In conclusion, the shift observed from the O-acetylated analogue 27 is encouraging although the chemistry to generate further derivatives will require to address the difficulties described in this report. In any case, the experimental values of these \( \lambda_{\text{max}} \) should again\textsuperscript{4} be useful for the design and validation of computer-based models\textsuperscript{14} of such light-producing decarboxylation reactions.
Scheme 4  Suggested mechanism for the rearrangement of 23 into 24. i: 1st addition of POCl₃, (- HCl). ii: 2nd addition of POCl₃, (- HCl). iii: cyclisation (+ HCl). iv and v: rearrangement of the carbon-nitrogen bond. vi: HCl elimination. vii: hydrolysis (upon treatment) of the two phosphoryl dichlorides.
Experimental

X-ray based structure determination. The structural elucidation of compounds 17 and 22 was achieved using the beamline PROXIMA-2 at Synchrotron SOLEIL and the data were deposited at the Cambridge Crystallographic Data Centre (CCDC), deposition number 2056217 and 2056386 respectively.

Chemistry. 1H NMR and 13C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz and 100 MHz, respectively. Shifts (δ) are given in ppm with respect to the TMS signal and cross-coupling constants (J) are given in Hertz. Column chromatography were performed either on Merck silica gel 60 (0.035 - 0.070 mm) or neutral alumina containing 1.5% of added water using a solvent pump and an automated collecting system driven by a UV detector set to 254 nm unless required otherwise. Sample deposition was carried out by absorption of the mixture to be purified on a small amount of the solid phase followed by its deposition of the top of the column. The low-resolution mass spectra were obtained on an Agilent 1100 series LC/MSD system using an atmospheric electrospray ionization system or an Agilent 1200 series LC/MSD system using an Agilent Jet-Stream atmospheric electrospray ionization system and the high-resolution mass spectra (HRMS) were obtained using a Waters Micromass Q-Tof with an electrospray ion source. When specified, the anhydrous solvents used were purchased. Experiments under inert atmosphere were carried out by purging the glassware with a stream of dry argon. Then, an argon balloon, fitted with a needle, was used to insure a positive pressure of inert gas during the reaction. Unless stated otherwise, a purity of at least 95% was obtained for all the compounds by means of chromatography or recrystallization and this level of purity was established by TLC, LC/MS and NMR spectroscopy.

Chemiluminescence spectra determination. The time-dependent chemiluminescence signal was recorded using an Aqualog (HORIBA Instruments, Inc.) equipped with thermoelectrically cooled charge-coupled device (CCD) array detector. The system uses a xenon-arc light source to excite samples and two detectors for simultaneous Absorbance, Transmission and emission analysis was adapted to the chemiluminescence analysis by blocking the excitation source. The 2D emission spectra were recorded from 250 to 800 nm using 5 nm step, high gain and 30 s integration time for emission detection. A detector accumulation of 2 has been chosen to improve the Signal-to-noise Ratio and increase the photons counts. Chemiluminescence data were corrected for the instrumental emission spectral response, detector dark currents and blank sample emission. All samples were analyzed using 1 cm path length fluorescence quartz cuvettes and the signal was recorded immediately after addition of either 1 or 2 μL of a 5 mM stock solution in DMSO of the O-acetylated luciferin on 2 mL of a phosphate buffer DPBS GIBCO 1X which also contained 2.5 mM of cetyltrimethylammonium bromide. The chemiluminescence data were analyzed using Aqualog software based on OriginLab® and the signal was smoothed by 4 points fast Fourier transform (FFT).

Condensation of 3,4-diaminopyrazine (15) and phenylpyruvic acid (16) under neutral conditions. In a 60 mL tube fitted with a Teflon-covered screw cap, 3,4-diaminopyrazine (15) (1.82 g, 16.67 mmol) and phenylpyruvic acid (16) (2.73 g, 16.67 mmol) were dispersed in ethanol (40 mL, dried over 4 Å molecular sieves). The tube was closed tightly and heated at 85 °C for 24 hours. The resulting suspension was dispersed in 100 mL of boiling ethanol (100 mL) left to cool and filtered and this material was subjected again to this treatment to give, after drying, compound 18 (0.98 g) as a white powder which could not be further purified, probably because of the onset of an oxidative decarboxylation characterized by the appearance in LC/MS of a compound with a mass of m/z = 210. Both filtrates were collected, concentrated to dryness and purified by a chromatography over silica gel (dichloromethane – ethanol 97/3 to 94/6) to yield compound 17 (1.37 g, 34 %) as a white powder after dispersion of the corresponding fraction in boiling ethanol (100 mL) and filtration after cooling.
3-Benzylpyrido[3,4-b]pyrazin-2-ol (17)

Note: the structure of this compound (recrystallized in ethanol) was further established by X-ray crystallography. \(^1\)H NMR (DMSO-d6): 12.61 (s (br), 1H), 8.86 (s (br), 1H), 8.45 (d, 1H, \(J = 5.5\) Hz), 7.35-7.27 (m, 3H), 7.39 (m, 2H), 7.23-7.18 (m, 2H), 4.14 (s, 2H). \(^{13}\)C NMR (DMSO-d6): 162.8, 155.3, 150.2, 149.0, 137.9, 137.4, 129.7, 128.6, 126.9, 39.5. HRMS (m/z): [M+H]+ calcd for C\(_{14}\)H\(_{12}\)N\(_3\)O: 238.0980; found: 238.0975.

2-Benzyl-2,3-dihydro-1H-imidazo[4,5-c]pyridine-2-carboxylic acid (18)

\(^1\)H NMR (DMSO-d6, still containing some EtOH) 9.20 (s (br), 1H), 7.48 (d, 1H, \(J = 6.1\) Hz), 7.24 (m, 2H), 7.18-7.09 (m, 3H), 6.96 (s, 1H), 6.89 (s (br), 1H), 6.17 (d, 1H, \(J = 6.2\) Hz), 3.03 (m, 2H).

\(^{13}\)C NMR (DMSO-d6): not soluble enough. HRMS (m/z): [M+H]+ calcd for C\(_{14}\)H\(_{14}\)N\(_3\)O\(_2\): 256.1086; found: 256.1081.

Note: the growing occurrence of another compound (m/z = 210 and, amongst other, a singlet 1H signal at 4.23 ppm) was noticed when trying to further purify this batch using a third dispersion.

Condensation of 3,4-diaminopyrazine (15) and phenylpyruvic acid (16) under acidic conditions, preparation of 1H-imidazo[4,5-c]pyridin-2-yl)-2-phenylethen-1-ol hydrochloride salt (19): A mixture of 3,4-diaminopyrazine (15) (2.26 g, 20.70 mmol), phenylpyruvic acid (16) (3.39 g, 20.70 mmol), ethanol (40 mL) and 2N hydrochloric acid (31 mL, 62.12 mmol) was heated to reflux for 2 hours. This was left to cool, the precipitate was filtered, washed with ethanol and dried under vacuum at 60 °C to yield pure compound 19 (4.75 g, 83%) as a hydrochloride salt.

\(^1\)H NMR (DMSO-d6): 14.40 (s (br), 1H), 11.62 (s, 1H), 10.72 (s (br), 1H), 8.15 (dd, 1H, \(J = 1.0, 6.6\) Hz), 8.03 (d, 1H, \(J = 1.0\) Hz), 7.56 (m, 2H), 7.47 (m, 2H), 7.41 (d, 1H, \(J = 6.6\) Hz), 7.36 (m, 1H), 6.74 (s, 1H).

\(^{13}\)C NMR (DMSO-d6): 157.6, 141.6, 136.1, 133.8, 129.3 (two signals), 128.4, 127.0, 125.0, 124.6, 111.5, 109.1. HRMS (m/z): [M+H]+ calcd for C\(_{14}\)H\(_{11}\)N\(_3\)O + HCl: C, 61.43; H, 4.42; N, 15.35; O, 5.85. Found: C, 61.23; H, 4.42; N, 15.41; O, 6.14.

2-Benzylpyrido[3,4-b]pyrazin-3-ol (20)

Compound 19 (0.19 g, 0.69 mmol) and sodium hydroxide (0.2 g, 5 mmol) were brought to reflux in ethanol/water 1-1 (20 mL). This was left to cool, neutralized with solid ammonium chloride diluted in water and extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with brine (25 mL), dried over magnesium sulfate and concentrated to dryness to yield pure compound 20 (0.11 g, 66%) as a beige powder.

If \(^1\)H and \(^{13}\)C NMR analysis were run using a freshly made sample of compound 20, the following spectra were obtained:

\(^1\)H NMR (DMSO-d6): 12.59 (s (br), 1H), 8.63 (d, 1H, \(J = 0.6\) Hz), 8.40 (d, 1H, \(J = 5.3\) Hz), 7.65 (dd, 1H, \(J = 0.6, 5.3\) Hz), 7.34-7.27 (m, 4H), 7.22 (m, 1H), 4.18 (s, 2H).

\(^{13}\)C NMR (DMSO-d6): 167.0, 154.6, 143.9, 139.1, 137.1, 135.7, 129.8, 128.8, 128.5, 127.0, 121.5, 39.7.

Following about 24 hours in the DMSO-d6 solution, the occurrence of a second product could be seen and subtraction of the signal described above gave the following spectra compatible with the free base form of compound 19:

\(^1\)H NMR (DMSO-d6): 11.05 (s (br), 1H), 9.50 (s (br), 1H), 7.99 (s, 1H), 7.92 (d, 1H, \(J = 5.5\) Hz), 7.52 (d, 2H, \(J = 7.9\) Hz), 7.41 (m, 2H), 7.26 (m, 2H), 7.06 (d, 1H, \(J = 5.5\) Hz), 6.44 (s, 1H). \(^{13}\)C NMR (DMSO-d6): 158.8, 144.5, 135.6, 135.2, 134.6, 129.2, 129.0, 128.7, 127.1, 122.8, 108.6, 105.2. HRMS (m/z): [M+H]+ calcd for C\(_{14}\)H\(_{12}\)N\(_3\)O: 238.0980; found: 238.0983. Note: when using formic acid as vector, a peak at m/z = 256.1074
was also present. Anal. calcd. for C_{14}H_{11}N_{3}O: C, 70.87; H, 4.67; N, 17.71; O, 6.74. Found: C, 70.39; H, 4.81; N, 17.63; O, 6.77.

Condensation of 2,3-diaminopyrazine (21) and phenylpyruvic acid (16) under neutral conditions. In a 60 mL tube fitted with a Teflon-covered screw cap, 1,2-diaminobenzene (21) (1.39 g, 12.73 mmol) and phenylpyruvic acid (16) (2.09 g, 12.73 mmol) were heated at 85 °C in ethanol (25 mL) for 26 hours. The resulting solution was concentrated to dryness and the residue purified by a chromatography over silica gel (dichloromethane - ethanol 98/2 to 96/4) to give, in that order of elution, compound 22 (1.21 g, 40%) and its isomers 23 (0.48 g, 15%) as described below.

Condensation of 2,3-diaminopyrazine (21) and phenylpyruvic acid (16) under acidic conditions. A mixture of 1,2-diaminobenzene (21) (1.32 g, 12.10 mmol), phenylpyruvic acid (16) (2.00 g, 12.10 mmol), and 2 N hydrochloric acid (18.1 mL, 36.29 mmol) were heated to reflux in ethanol (20 mL) for 26 hours. The resulting solution was cooled, diluted in water (100 mL), made basic with solid sodium hydrogenocarbonate. The resulting precipitate was filtered and the filtrate extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with water (30 mL), brine (30 mL), dried over magnesium sulfate and concentrated to dryness. This residue and the insoluble material described above, were both adsorbed over silica gel and purified by a chromatography over silica gel (dichloromethane - ethanol 98/2) to give an unclean fraction containing compound 22 (0.25 g) and then a clean fraction of compound 23 (0.95 g, 33%). Further purification of the first fraction by a second chromatography over silica gel (cyclohexane – ethyl acetate 2/1 to 1/0) gave pure compound 22 (0.11 g, 4%). In another trial, extensive dispersion of the initial precipitate in ethyl acetate (100 mL) and water (100 mL) followed by a filtration of the remaining insoluble material gave a solid which was purified by a chromatography over silica gel (dichloromethane - ethanol 97/3) to give a fraction (0.98 g) containing the imidazo[4,5-b]pyridine 24. This was further purified by a dispersion in boiling ethanol (40 mL) to give a pure sample of 24 (0.32 g, 7%) as described below.

2-benzylpyrido[2,3-b]pyrazin-3-ol (22)

Note: as described above, the structure of this compound (recrystallized in ethanol) was further established by X-ray crystallography and the 1H spectra is similar to the substance obtained using a different (but plausibly acid-containing) synthetic approach, endowed with very optimistic yields which we could not reproduce.\(^{15}\)

\(^{1}\)H NMR (DMSO-d6): 12.84 (s (br), 1H), 8.48 (dd, 1H, J = 4.7, 1.7 Hz), 8.14 (dd, 1H, J = 7.8, 1.7 Hz), 7.37-7.27 (m, 5H), 7.22 (m, 1H), 4.15 (s, 2H). \(^{13}\)C NMR (DMSO-d6): 164.1, 156.3, 150.1, 144.3, 137.5, 136.7, 129.7, 128.8, 127.3, 126.9, 102.1, 39.3. HRMS (m/z): [M+H]+ calcd for C_{14}H_{11}N_{3}ONa: 260.0800; found: 260.0805.

3-benzylpyrido[2,3-b]pyrazin-2-ol (23)

\(^{1}\)H NMR (DMSO-d6): 12.51 (s (br), 1H), 8.48 (dd, 1H, J = 4.5, 1.7 Hz), 7.69 (dd, 1H, J = 8.2, 1.7 Hz), 7.51 (dd, 1H, J = 8.1, 4.5 Hz), 7.37-7.20 (m, 5H), 4.18 (s, 2H). \(^{13}\)C NMR (DMSO-d6): 164.7, 154.6, 145.3, 143.0, 137.4, 129.8, 128.8, 128.3, 126.9, 125.2, 124.6, 39.6. HRMS (m/z): [M+H]+ calcd for C_{14}H_{12}N_{3}O: 238.0980; found: 238.0971.

1-(1H-Imidazo[4,5-b]pyridin-2-yl)-2-phenylethen-1-ol (24)

\(^{1}\)H NMR (DMSO-d6): 11.06 (s (br), 1H), 9.20 (s (br), 1H), 7.80 (dd, 1H, J = 1.4, 4.9 Hz), 7.55 (m, 2H), 7.39 (m, 2H), 7.24 (m, 1H), 7.16 (dd, 1H, J = 1.4, 7.7 Hz), 6.80 (dd, 1H, J = 4.9, 7.7 Hz), 6.41 (m, 1H). \(^{13}\)C NMR (DMSO-d6): 158.6, 141.6, 135.4, 129.8, 129.3, 129.2, 128.5, 127.0, 121.5, 121.1, 116.3, 105.1. HRMS (m/z): [M+H]+ calcd for C_{14}H_{12}N_{3}O: 238.0980; found: 238.0969.
2-Benzyl-3-chloropyrido[2,3-b]pyrazine (13)

In a 60 mL tube fitted with a Teflon-covered screw cap, compound 23 (0.4 g, 1.68 mmol) was dispersed in a mixture of phosphorus oxychloride (4 mL) and phosphorus trichloride (2 mL). The tube was closed and heated at 80 °C with an oil bath for 40 min. The resulting solution was diluted in ethyl acetate (100 mL), poured onto an excess of ice and stirred for 15 minutes. The organic layer was made basic with 1N ammonia, washed with water (30 mL), brine (30 mL), dried over magnesium sulfate and concentrated to dryness. The residue was purified by a chromatography over silica gel (cyclohexane – ethyl acetate 3/1) to give compound 13 (0.35 g, 81%) as a white solid.

1H NMR (CDCl3): 9.13 (dd, 1H, J = 1.9, 4.2 Hz), 7.73 (dd, 1H, J = 4.2, 8.3 Hz), 7.73 (dd, 1H, J = 1.9, 8.3 Hz), 7.34 (m, 5H), 4.57 (s, 2H).

13C NMR (CDCl3): 155.9, 154.1, 150.6, 149.4, 137.9, 136.2, 136.1, 129.2, 128.6, 127.0, 125.4, 41.8.

HRMS (m/z): [M+H]+ calcd for C14H11N3Cl: 256.0641; found: 256.0634.

Ethyl (2-benzylpyrido[2,3-b]pyrazin-3-yl)phenylalaninate (26)

In a 20 mL sealable vial fitted with a septum, compound 13 (0.31 g, 1.21 mmol), phenylalanine ethyl ester hydrochloride salt (0.28 g, 1.21 mmol), cesium carbonate (1.3 g, 3.87 mmol), palladium diacetate (0.013 g, 0.06 mmol) and BINAP (0.053 g, 0.084 mmol) were weighted. The air was replaced by argon and, under an inert atmosphere, dry acetonitrile (4 mL) was injected. This was stirred and heated at 60 °C for 12 hours. The resulting suspension was diluted in ethyl acetate (75 mL), filtered, the filtrate was concentrated to dryness and the residue purified by a chromatography over silica gel (cyclohexane – ethyl acetate 2/1) to give compound 26 (0.06 g, 12%) as an oil.

1H NMR (CDCl3): 8.86 (dd, 1H, J = 2.0, 4.5 Hz), 8.25 (dd, 1H, J = 2.0, 8.1 Hz), 7.38 (dd, 1H, J = 8.1, 4.5 Hz), 7.29 (m, 3H), 7.18 (m, 5H), 6.89 (m, 2H), 5.64 (d, 1H, J = 7.4 Hz), 5.38 (m, 1H), 4.24 (s, 2H), 4.14 (m, 2H), 3.34 (dd, 1H, J = 5.4, 14.2 Hz), 3.17 (dd, 1H, J = 5.2, 14.2 Hz), 1.22 (t, 3H, J = 7.2 Hz).

13C NMR (CDCl3): 171.6, 152.0, 151.5, 151.0, 147.7, 137.2, 135.9, 135.0, 131.4, 129.3, 129.1, 128.6, 127.4, 126.8, 120.4, 61.5, 54.3, 41.3, 37.1, 14.2. HRMS (m/z): [M+H]+ calcd for C25H25N4O2: 413.1978; found: 413.1980.

6,8-Bibenzylimidazo[1,2-a]pyrido[3,2-e]pyrazin-9-yl acetate (27)

Compound 26 (0.06 g, 0.14 mmol) and powdered sodium hydroxide (0.023 g, 0.58 mmol) were weighted in a 100 mL flask. The air was replaced by argon and dry THF (0.2 mL) was injected. This was stirred for 19 hours and acetic anhydride (0.2 mL, 2.18 mmol) was then injected. The resulting suspension was stirred for 2 hours, diluted in water, stirred for 15 minutes and extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with water (30 mL), brine (30 mL), dried over magnesium sulfate and concentrated to dryness. The residue was purified by a chromatography over silica gel (cyclohexane – ethyl acetate 4/1) to give compound 27 (0.02 g, 33%) as a pale pink glass.

1H NMR (CDCl3): 8.52 (dd, 1H, J = 1.7, 4.7 Hz), 8.31 (dd, 1H, J = 1.7, 8.2 Hz), 7.60 (m, 2H), 7.47 (dd, 1H, J = 4.7, 8.2 Hz), 7.38–7.18 (m, 8H), 4.63 (s, 2H), 4.22 (s, 2H), 2.39 (s, 3H).

13C NMR (CDCl3): 168.7, 155.4, 146.9, 139.5, 138.4, 137.4, 137.3, 134.5, 134.4, 131.9, 131.1, 129.8, 129.0, 128.4, 128.3, 126.6, 126.4, 122.1, 39.6, 33.1, 20.4. HRMS (m/z): [M+H]+ calcd for C25H25N4O2: 409.1664; found: 409.1667.

4-Bromoisoquinolin-3-amine (28)

The procedure described on page 67 of a patent8 was followed and compound 28 (7.09 g, 87%) was obtained as a yellow powder after a purification by chromatography over silica gel (cyclohexane – ethyl acetate 4/1).

1H NMR (CDCl3): 8.81 (s, 1H), 7.93 (m, 1H), 7.81 (m, 1H), 7.67 (m, 1H), 7.34 (m, 1H), 5.03 (s (br), 2H).

13C NMR (CDCl3): 151.7, 150.5, 137.0, 131.9, 128.1, 124.8, 123.8, 123.6, 97.6.
2-Benzylpyrazino[2,3-c]isoquinolin-3-ol (32)

In a 60 mL tube fitted with a Teflon-covered screw cap, compound 28 (1.61 g, 7.21 mmol), racemic phenylalanine (29, 3.6 g, 21.6 mmol), potassium phosphate (4.6 g, 7.21 mmol), copper I oxide (0.05 g, 0.36 mmol) were weighted. Dry DMSO (15 mL) was added, the tube closed and heated, using an oil bath, at 90 °C for 40 hours. The resulting orange suspension was dispersed in a mixture of water (100 mL), ethyl acetate (50 mL), ammonium chloride (1.1 g) and 30 % ammonia (10 mL) and stirred in open air for 30 minutes. The resulting precipitate was filtered, washed with water (2 x 50 mL), ethyl acetate (2 x 50 mL) and dried under vacuum at 50 °C to give pure compound 32 (0.38 g, 18 %) as a white powder. The filtrate was extracted with ethyl acetate (2 x 50 mL), the organic layer was washed with water (30 mL), brine (30 mL), dried over magnesium sulfate and concentrated to dryness. A chromatography over silica gel (cyclohexane – ethyl acetate 2/1) of the resulting residue gave an impure fraction containing the dihydro derivative 31 (0.47 g). This was dispersed in ethanol, N-bromosuccinimide was added (0.43 g) and the suspension stirred for 90 minutes. The resulting precipitate was filtered, washed with ethanol and dried under vacuum at 50 °C to give some more compound 32 (0.26 g, 12 %).

1H NMR (DMSO-d6): 13.1 (s, 1H), 9.29(s, 1H), 8.60 (m, 1H), 8.20 (m, 1H), 7.93 (m, 1H), 7.68 (m, 1H), 7.41 (m, 2H), 7.32 (m, 2H), 7.23 (m, 1H), 4.25 (s, 2H). 13C NMR (DMSO-d6): 160.2, 156.5, 154.5, 139.7, 137.8, 133.3, 132.7, 129.8, 128.8, 128.7, 126.9, 126.8, 126.4, 121.4, 119.9, 39.5. HRMS (m/z): [M+H]+ calcd for C18H14N3O: 288.1137; found: 288.1136.

2-Benzyl-3-chloropyrazino[2,3-c]isoquinoline (33)

In a 60 mL tube fitted with a Teflon-covered screw cap, compound 32 (1.4 g, 4.59 mmol) was dispersed in a mixture of phosphorus oxychloride (10 mL) phosphorus trichloride (5 mL). The tube was closed and heated at 120 °C with an oil bath for 23 hours. The resulting solution was diluted in ethyl acetate (100 mL), poured onto an excess of ice and stirred for 15 minutes. The organic layer was made basic with 1N ammonia, washed with water (2 x 30 mL), brine (30 mL), dried over magnesium sulfate and concentrated to dryness. The residue was purified by a chromatography over silica gel (cyclohexane – ethyl acetate 4/1) to give compound 33 (0.90 g, 61%) as a white solid.

1H NMR (CDCl3): 9.55 (s, 1H), 9.11 (m, 1H), 8.17 (m, 1H), 8.02 (m, 1H), 7.91 (m, 1H), 7.1 (m, 2H), 7.26 (m, 1H), 4.64 (s, 2H). 13C NMR (CDCl3): 158.0, 154.0, 148.4, 146.5, 136.7, 133.7, 132.2, 129.2, 129.9, 129.3, 128.6, 128.5, 128.2, 126.9, 123.8, 41.6. HRMS (m/z): [M+H]+ calcd for C18H13ClN3: 306.0798; found: 306.0793.

Ethyl (2-benzylpyrazino[2,3-c]isoquinolin-3-yl)phenylalaninate (34)

In a 60 mL tube fitted with a Teflon-covered screw cap, compound 33 (0.43 g, 1.41 mmol) and freshly extracted phenylalanine ethyl ester (25) (0.55 g, 2.81 mmol) were dissolved in dry N-methylpyrrolidone (4 mL). The tube was closed and heated, using an oil bath, at 120 °C for four days. The resulting black solution was diluted in ethyl acetate (100 mL), poured onto an excess of ice and stirred for 15 minutes. The organic layer was made basic with 1N ammonia, washed with water (2 x 30 mL), brine (30 mL), dried over magnesium sulfate and concentrated to dryness. The residue was purified by a chromatography over silica gel (cyclohexane – ethyl acetate 4/1) to give compound 34 (0.14 g, 10%) as an oil. In another chromatographic fraction pure starting material 33 (0.14 g, 25%) was recovered.

1H NMR (CDCl3): 9.40 (s, 1H), 9.00 (m, 1H), 8.05 (m, 1H), 7.88 (m, 1H), 7.69 (m, 1H), 7.28 (m, 5H), 7.19 (m, 3H), 6.94 (m, 2H), 5.46 (m, 2H), 4.33 (s, 2H), 4.17 (m, 2H), 3.38 (dd, 1H, J = 4.9, 14.0 Hz), 3.22 (dd, 1H, J = 5.3, 14.0 Hz), 1.24 (t, 3H, J = 7.3 Hz). 13C NMR (CDCl3): 172.0, 155.8, 151.2, 147.2, 144.6, 136.2,
135.8, 134.3, 131.3, 129.4, 129.0, 128.7, 128.6, 128.4, 127.9, 127.2, 127.0, 126.8, 126.7, 122.3, 61.4, 54.4, 41.1, 37.4, 14.1.

HRMS (m/z): [M+H]+ calcd for C_{29}H_{27}N_{4}O_{2}: 463.2129; found: 463.2124.

2,12-Dibenzylimidazo[1',2':1,6]pyrazino[2,3-c]isoquinolin-3-yl acetate (35)

Compound 34 (0.14 g, 0.30 mmol) and powdered sodium hydroxide (0.05 g, 1.2 mmol) were weighted in a 100 mL flask. The air was replaced by argon and dry THF (3 mL) was injected. This was stirred for 19 hours and acetic anhydride (0.43 mL, 4.51 mmol) was then injected. The resulting suspension was stirred for 2 hours, diluted in water (50 mL), stirred for 15 minutes and extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with water (30 mL), brine (30 mL), dried over magnesium sulfate and concentrated to dryness. The residue was purified by a chromatography over silica gel (cyclohexane – ethyl acetate 6/1) to give compound 35 (0.04 g, 29%) as a beige solid.

^1H NMR (CDCl₃): 9.07 (s, 1H) 9.4 (m, 1H), 8.0 (m, 1H), 7.87 (m, 1H), 7.67 (m, 3H), 7.41 (m, 2H), 7.35 (m, 4H), 7.26 (m, 2H), 4.75 (s, 2H), 4.28 (s, 2H), 2.42 (s, 3H). ^13C NMR (CDCl₃): 168.9, 153.3, 150.3, 138.5, 137.7, 134.9, 134.8, 134.1, 134.0, 131.5, 131.4, 129.9, 129.0, 128.4, 128.3, 127.5, 127.4, 126.5, 126.3, 125.4, 123.5, 39.5, 33.4, 20.5 (one signal missing). HRMS (m/z): [M+H]+ calcd for C_{29}H_{23}N_{4}O_{2}: 459.1821; found: 459.1822.

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Supplementary material

Crystallization, data collection, and structure determination

Chemiluminescence spectra

\(^1\)H and \(^{13}\)C spectra of the compounds described

Crystallization, data collection, and structure determination

Suitable crystals of compounds 17 and 22 were obtained by recrystallization in ethanol. Crystals were then flash-cooled in liquid nitrogen using Paratone-paraffin 50% (v/v) oil as the cryoprotectant. X-ray diffraction data were collected at a wavelength of 0.7293 Å on the beamline PROXIMA-2 at Synchrotron SOLEIL (St. Aubin, France). Diffraction data were integrated and reduced using the XDS program package.\(^1\) Structures were solved using SHELXT\(^2\) and refined with SHELXL.\(^3\)

Parameters for compound 17: Data Block Name: data_c17ca_a, Unit Cell Parameters: a 20.870(4) b 20.870(4) c 13.650(3) R-3, CCDC Deposition Number: 2056217 (see https://www.ccdc.cam.ac.uk/structures/), DOI: 10.5517/ccdc.csd.cc270nlj, CSD Entry: AKASAJ.

Parameters for compound 22: Data Block Name: data_c22ca, Unit Cell Parameters: a 32.03760(10) b 9.447(2) c 7.562(2) Pcca, CCDC Deposition Number: 2056386 (see https://www.ccdc.cam.ac.uk/structures/), DOI: 10.5517/ccdc.csd.cc270v15, CSD Entry: EKASIV.

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Chemiluminescence spectra
The time-dependent chemiluminescence signal was recorded using an Aqualog (HORIBA Instruments, Inc.) equipped with thermoelectrically cooled charge-coupled device (CCD) array detector. The system uses a xenon-arc light source to excite samples and two detectors for simultaneous Absorbance, Transmission and emission analysis was adapted to the chemiluminescence analysis by blocking the excitation source. The 2D emission spectra were recorded from 250 to 800 nm using 5 nm step, high gain and 30 s integration time for emission detection. A detector accumulation of 2 has been chosen to improve the Signal-to-noise Ratio and increase the photons counts.

Chemiluminescence data were corrected for the instrumental emission spectral response, detector dark currents and blank sample emission. All samples were analyzed using 1 cm path length fluorescence quartz cuvettes and the signal was recorded immediately after addition of, respectively, 1 or 2 µL of a 5 mM stock solution of the O-acteylated luciferins 27 or 35 on 2 mL of a phosphate buffer DPBS GIBCO 1X which also contained 2.5 mM of CTAB. The chemiluminescence data were analyzed using Aqualog software based on OriginLab® and as depicted below, the signal was smoothed by 4 points fast Fourier transform (FFT).

Spectra of compound 27

Spectra of compound 35
$^1$H and $^{13}$C spectra of the compounds described
3-Benzylpyrido[3,4-b]pyrazin-2-ol (17)

2-Benzyl-2,3-dihydro-1H-imidazo[4,5-c]pyridine-2-carboxylic acid (18)
1-(1H-imidazo[4,5-c]pyridin-2-yl)-2-phenylethen-1-ol hydrochloride salt (19)
2-benzylpyrido[3,4-b]pyrazin-3-ol (20)
5 minutes in solution

2-benzylpyrido[3,4-b]pyrazin-3-ol (20)
25 minutes in solution
2-benzylpyrido[3,4-b]pyrazin-3-ol (20)
1 hour in solution

2-benzylpyrido[3,4-b]pyrazin-3-ol (20)
1-(1H-imidazo[4,5-c]pyridin-2-yl)-2-phenylethen-1-ol
1 hour in solution
2-benzylpyrido[2,3-b]pyrazin-3-ol (22)
1-(1H-Imidazo[4,5-b]pyridin-2-yl)-2-phenylethen-1-ol (24)
2-Benzyl-3-chloropyrido[2,3-b]pyrazine (13)
6,8-Bibenzylimidazo[1,2-a]pyrido[3,2-e]pyrazin-9-yl acetate (27)
Note: add one to the compound number depicted
Note: add one to the compound number depicted
Note: add one to the compound number depicted

Ethyl (2-benzylpyrazolo[2,3-c]isoquinolin-3-yl)phenylalaninate (33)
Note: add one to the compound number depicted
