

Synthesis and Tautomeric Equilibrium of 6-Amino-5-benzyl-3-methylpyrazin-2-one. An Acceptor-Donor-Donor Nucleoside Base Analog

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6-Aminopyrazin-2-one, when incorporated as pyrimidine base analog into an oligonucleotide, might participate in a nonstandard base pair that retains a Watson-Crick geometry but is joined by a nonstandard hydrogen bonding pattern. Such base pairs can, at least in principle, be recognized independently in duplex nucleic acids. To explore the tautomeric properties that govern hydrogen bonding of this heterocycle, 6-amino-5-benzyl-3-methylpyrazin-2-one was synthesized. The equilibrium constant for the interconversion of the keto and hydroxyl tautomeric forms was estimated by comparing its ultraviolet spectrum with those of *N*- and *O*-methyl derivatives in water, methanol, ethanol, dioxane, and water-dioxane mixtures. A plot of the logarithm of the tautomeric equilibrium constant versus Dimroth's microscopic dielectric constant ($E_T(30)$) was linear. On the basis of an extrapolation of this relationship to the microscopic dielectric of water, 6-amino-5-benzyl-3-methylpyrazin-2-one is expected to favor at equilibrium the keto form over the hydroxyl form by a factor of ca. 2000 under conditions where DNA and RNA polymerases operate. This is substantially better than the tautomeric ratio observed with isoguanosine, where the minor form has been observed to create tautomeric ambiguity with some polymerase systems.

Introduction

The four bases found in natural oligonucleotides form two base pairs in the double helix according to complementarity rules defined both by size (purines pair with pyrimidines) and hydrogen bonding potential (hydrogen bond donor groups pair with hydrogen bond acceptor groups). This complementarity permits template-directed replication of oligonucleotides with the high fidelity essential for life.

At least four additional base pairs fit the Watson-Crick geometry (Figure 1). These pairs retain the size complementarity and the base-stacking potential found in the two natural base pairs. However, in each of the nonstandard base pairs, the large and small heterocycles are joined by a distinct pattern of hydrogen bonding. In principle, therefore, a nucleic acid containing the 12 bases shown in Figure 1 could be accurately copied by mechanisms used to copy natural DNA.¹⁻⁵

However, peculiarities in the chemical behavior of some nonstandard bases suggest certain limitations to an expanded genetic alphabet. In particular, a minor tautomeric form of isoguanosine,⁶⁻⁸ complementary to thymine and uracil, was found to cause infidelity in template-directed polymerization reactions.² In some cases, the predominant oligonucleotide products arose via mismatching of iso-G with T or U.

This paper concerns tautomerism in the 6-aminopyrazin-2-one ring system, a component of two of the nonstandard base pairs in Figure 1. Pyrazines were chosen over the analogous 6-amino-2-pyridones in view of the oxidizability of the aminopyridones in neutral and alkaline systems.⁹ The additional nitrogen of the pyrazine system decreases this reactivity. However, neither the parent pyrazine nor any derivative without an electron-withdrawing group was known. Further, aminopyrazinones might exist to an appreciable extent in the O²-H "enol" form complementary to xanthosine. Particularly worrisome were estimates based on tautomeric ratios in simple pyrazinone and aminopyridone systems^{10,11} that suggested that the O(2)-H tautomer might contribute 15% of the total at equilibrium.

We have therefore prepared 6-amino-5-benzyl-3-methylpyrazin-2-one (6) as a model for the riboside soluble in a range of organic media and studied its tautomeric behavior.

Results and Discussion

Synthesis. A synthesis for 5-substituted 6-aminopyrazin-2-ones compatible with protected ribose ring systems is shown in Figure 2. Pyrazine *N*-oxide 4 was prepared by condensing phenylalaninenitrile hydrochloride (2),¹² the Strecker synthesis product from phenylacetaldehyde (54%), with the 1-oxime of pyruvaldehyde, using a procedure generalized from the literature.¹³⁻¹⁷ The *N*-oxide was rearranged to yield 5. Similar rearrangements have been observed both in pyrazines activated by acceptor groups para to the position of attack by the acetate¹⁸⁻²¹

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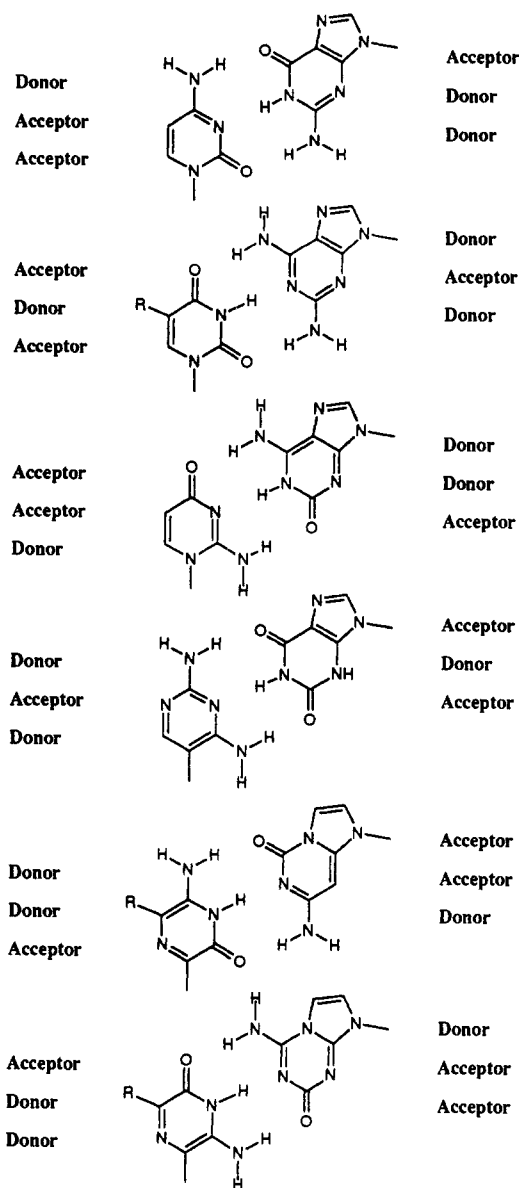


Figure 1. Nucleotide base pairs joined by various combinations of hydrogen bond donor and acceptor groups as possible independently replicatable constituents of an extended genetic alphabet. The first two base pairs represent the patterns used by Nature.

and in unactivated pyrazine derivatives.²²⁻²⁴ Yields in our unactivated system were greatly increased by adding acetate. Only two of the three acetyl groups of 5 could be removed in methanolic ammonia. Therefore, the more reactive hydrazine nucleophile was used to synthesize the target compound 6.

6-Amino-5-benzyl-3-methylpyrazin-2-one (6) was methylated with diazomethane to obtain the two methylated

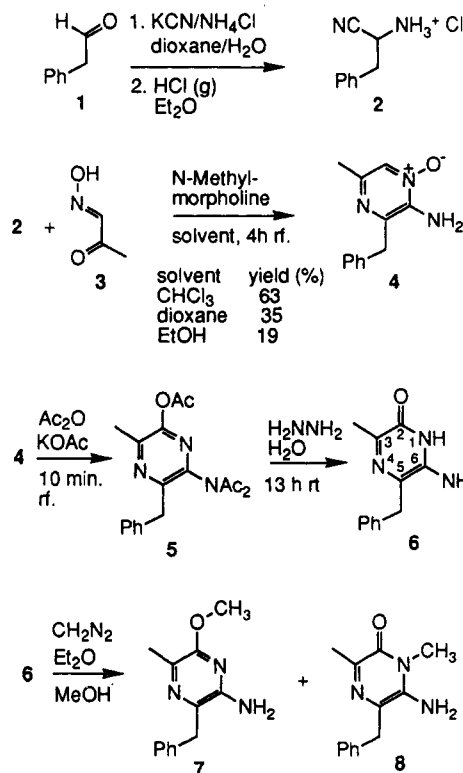


Figure 2. Synthesis of 6-amino-5-benzyl-3-methylpyrazin-2-one and methylated derivatives.

derivatives 7 and 8 which were separated by column chromatography (Figure 2). As with 3-aminopyrazin-2-one, diazomethane yields both the *N*-methyl and the *O*-methyl derivatives in equal amounts; the exocyclic amino function is not alkylated.²⁵ A long range ¹H-¹³C shift correlated NMR spectrum (HMBC²⁶) was obtained for 8 and used to assign the quaternary carbon atoms (see Experimental Section).

Estimation of the Tautomeric Equilibrium. Most methods for estimating tautomeric equilibrium constants rely on measurements made with model compounds where the proton is "fixed" by replacement with a methyl group. These models for the pyrazinone discussed here are shown in Figure 3. Spectroscopic properties (e.g., λ_{\max} , NMR chemical shifts) of a mixture of tautomers are then assumed to be linear functions of the fraction of the two tautomers in the mixture. The spectroscopic properties of the *N*-methyl and *O*-methyl derivatives 7 and 8 are taken as extrema for the range of intermediate tautomeric ratios.

Three tautomeric forms are conceivable for 6-amino-5-benzyl-3-methylpyrazin-2-one (6) (Figure 3). Precedents in other heterocycles suggest that the imino form (structure **Z** in Figure 3) is the least likely form,^{10,27-31} and the available evidence ruled out this structure in our pyrazine as well. In the ¹H NMR spectra of 6-8, a broad signal (two

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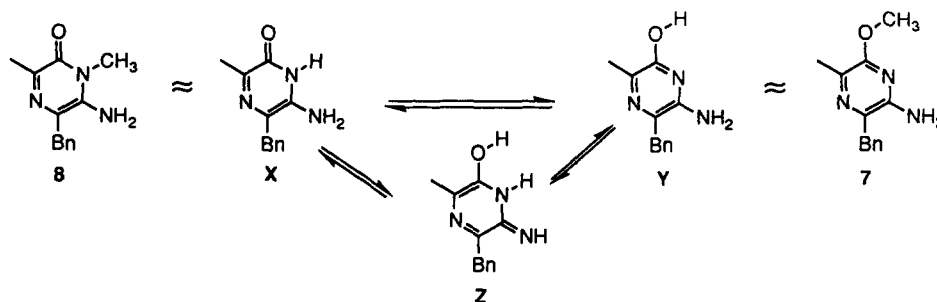


Figure 3. Fixed model compounds 7 and 8 and possible tautomeric forms of the 6-aminopyrazin-2-one ring system.

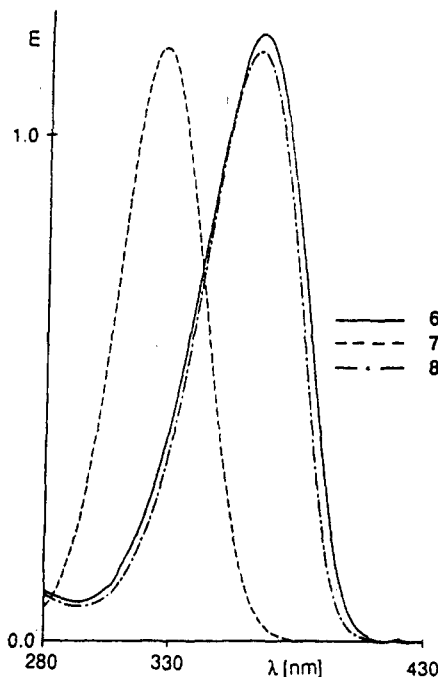


Figure 4. UV spectra of compounds 6–8 in aqueous buffer: pH 6.0, Tris 8 mM, spermidine 0.2 mM, $MgCl_2$ 4 mM; concentrations of 6–8 ca. 0.1 mM.

protons by integration) was assigned in each case to the exocyclic NH_2 protons. In 8, this assignment was confirmed via 1H - ^{13}C heterocorrelation NMR spectroscopy. Coupling was observed between these protons and C(5) and C(6) (see Experimental Section). These data essentially exclude the imino tautomer, which has no NH_2 group. Further, the ultraviolet spectra of 7 and 8 are quite different (Figure 4). If these two compounds existed as imino tautomers, the spectra should be nearly identical.

The IR spectra of 7 and 8 (in dilute chloroform solution) in the region of the NH stretching vibrations can also be interpreted in terms of an NH_2 group. Imines absorb weakly near 3300 cm^{-1} , while amines show absorption 5–10 times stronger³¹ at 3500 – 3400 cm^{-1} . Both 7 and 8 show two IR transitions with rather high probabilities in the region of 3500 – 3400 cm^{-1} , suggesting that they bear an NH_2 group. Confirmation of this assignment comes from analysis of these absorbances using the empirical rule developed by Bellamy and Williams³² for the coupling of N -H stretches in NH_2 groups: $\nu_{\text{symmetric}} = 0.876 \nu_{\text{asymmetric}} + 345.5$ (standard deviation ca. 5 cm^{-1}). Exocyclic NH_2 groups on pyridine and pyrimidine rings follow this rule.³¹ Both 7 and 8 in chloroform show good agreement with this

Table I. IR Absorption of Compounds 6–8 in the Range of 1700 – 1550 cm^{-1} . (abbreviations: s = strong, m = medium, w = weak, br = broad)

	IR absorption (cm^{-1})	tautomeric form	medium
6	1650 (s, br), 1620 (s, br), 1605 (s, br)	lactam–lactim	$CHCl_3$
6	1650 (s, br), 1625 (s, br), 1600 (s, br)	lactam–lactim	KBr
7	1605 (m), 1555 (m)	lactim	$CHCl_3$
7	1620 (m), 1600 (w), 1580 (m)	lactim	KBr
8	1685 (s, br), 1645 (s, br), 1615 (s, br)	lactam	$CHCl_3$
8	1700 (s, br), 1580 (s, br)	lactam	KBr

relationship; the deviations of 3 and 12 cm^{-1} are typical for heterocyclic amines.

With the imino tautomer excluded, the O(2)-H (phenolic) tautomer (structure Y in Figure 3) and the N(1)-H (keto) tautomer (structure X in Figure 3) are the remaining possibilities. Unfortunately, the classical approach to distinguish between these, where the properties of the O-protonated tautomer (structure Y) are assumed to be the same as those of the O-methylated analog 7, and the properties of the N-protonated tautomer (structure X) are the same as those of the N-methylated analog 8,³³ proved to be unreliable. For example, methylation substantially perturbed the chemical shifts of the signals in the ^{13}C NMR spectrum in a fashion unrelated to tautomerization (see Experimental Section).

Likewise, infrared spectra, summarized in Table I, proved to be difficult to interpret. Substituted pyrazines generally display one or more bands between 1550 and 1750 cm^{-1} . Weak bands in the IR between 1550 and 1640 cm^{-1} seem to be generally characteristic of 2-methoxy (and presumably hydroxy) forms. Strong bands between 1640 and 1750 cm^{-1} seem to be generally characteristic of 2-keto forms.³⁴ In contrast, absence of such strong bands down to 1497 cm^{-1} was used to conclude that 2-hydroxy-3,5,6-trifluoropyrazine mainly exists in the OH form in the solid state.³⁵ 6-Amino-5-benzyl-3-methylpyrazin-2-one (6) displays a rather strong band at 1650 cm^{-1} in chloroform solution, suggesting that it exists at least partially in the keto form in $CHCl_3$. However, no quantitative statement could be made.

Ultraviolet spectroscopy proved to be more useful. In an aqueous buffered solution similar to that used in template-directed enzymatic polymerization reactions, 6 has an absorbance around 360 nm . The ultraviolet spectrum was dependent on pH; in water, the heterocycle

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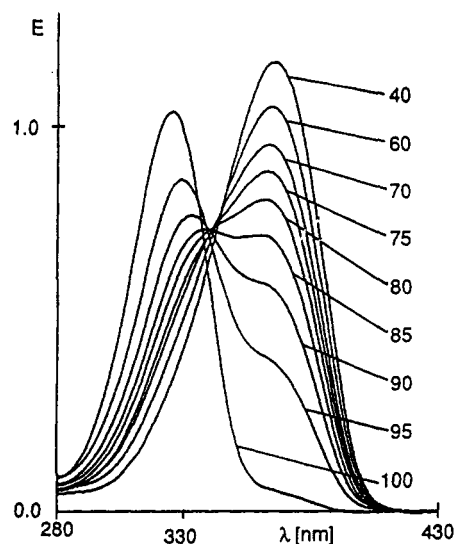


Figure 5. UV spectra of 6 in various aqueous buffer/dioxane mixtures (% dioxane indicated) at high dioxane concentrations.

deprotonates with a pK_a of ca. 8.2 ± 0.1 (by UV spectroscopy according to ref 36). To avoid complications involving deprotonation, the data were collected using aqueous buffer of pH 6. Under these conditions, the heterocycle is only negligibly deprotonated in all of the media examined, and deprotonated forms do not contribute significantly to the calculated tautomeric equilibrium constant.

Under these conditions, the spectrum of 6 (Figure 4) is essentially identical to that of the N-methylated keto derivative 8, and distinctly different from the spectrum of the O-methylated derivative 7. This implies that 6 exists largely in the desired carbonyl form.

Media with lower dielectric constants favor the enol form in 2-oxoazabenzene systems,³⁷ presumably because the resonance structure of the N(1)-H form that is formally aromatic involves charge separation favored by a medium with high dielectric. Evidence for a medium effect on the tautomeric equilibrium in 6 was readily obtained. In pure dioxane, the ultraviolet spectrum of 6 was similar to that of the O-methylated analog 7. In dioxane/aqueous buffer ratios ranging from 100:0 to 40:60, the ultraviolet spectra of 6 showed a mixture of the two tautomers (Figure 5); a tautomeric equilibrium constant near unity was observed at a dioxane/aqueous buffer ratio of 90:10. An isosbestic point is not expected in Figure 5, because the extinction coefficients and λ_{max} values of the two tautomers vary with medium polarity.

The tautomeric equilibrium ($K_{taut} = [keto]/[enol]$) was calculated in dioxane/water buffers (ratios from 95:5 to 60:40) according to the multiwavelength method of Dewar and Urch³⁸ at 300–330 nm, where the extinction constants of the enol and keto forms are most different. The UV spectrum of the O-methylated derivative 7 provided the standard for the pure enol form. The absorbance parameters were similar both in dioxane ($\lambda_{max} = 326$ nm, $\epsilon = 10910$) and in a mixture of dioxane/aqueous buffer 60:40 ($\lambda_{max} = 330$ nm, $\epsilon = 10690$). As can be seen from Figure 4, the UV spectrum of compound 6 resembles that of the

fixed keto derivative 8, in pure aqueous buffer solution. Additionally, the UV spectrum of 6 remains virtually unchanged in a mixture of aqueous buffer/dioxane 60:40. We therefore used the UV spectrum of 6 in aqueous buffer/dioxane 60:40 ($\lambda_{max} = 368$ nm, $\epsilon = 10990$) as a standard for the pure keto form.

Ultraviolet spectra were also measured in mixtures of the aqueous buffer with methanol or ethanol (up to 40%). Line shapes, λ_{max} , and ϵ were virtually unchanged in these solvent mixtures. Thus, 6 exists largely in the keto form in these media as well. The keto form also predominates in both pure methanol and pure ethanol (see Experimental Section).

Such measurements are most sensitive for estimating tautomeric equilibrium constants near unity. However, as polymerases can amplify the significance of a minor tautomer³⁹ under physiological conditions, estimates of very small equilibrium constants in water are interesting. The medium dependence of K_{taut} provided a method for obtaining such an estimate.

Kosower's Z values, corresponding to the transition energies for the charge-transfer band of 1-alkylpyridinium iodide complexes, are an empirical measure of medium polarity on a microscopic scale.⁴⁰ The $E_T(30)$ values of Dimroth *et al.*⁴¹ are also measures of microscopic dielectric, derived from the $\pi \rightarrow \pi^*$ transition of pyridinium *N*-phenol betaines. A linear relationship between the $E_T(30)$ values and the Kosower Z value has been established,⁴² and the $E_T(30)$ values of 243 solvents⁴² and several binary solvent systems (see ref 42) have been tabulated.

In contrast to the macroscopic dielectric constant, both $E_T(30)$ and Z values correlate well with perturbations on chemical equilibria effected by media of different polarities. For example, using binary solvent mixtures, Gordon and Katritzky found an excellent linear free energy relationship between the tautomeric equilibrium constant for a series of pyridones and Z .⁴³ Using eight solvent media, a less linear but nevertheless satisfactory correlation was observed by Sepiol *et al.* between tautomer ratio and $E_T(30)$ with derivatives of isoguanine, and a K_{taut} for water was estimated by extrapolation.⁶

A plot of the logarithm of the tautomeric equilibrium constant for 6 versus the polarity parameter $E_T(30)$ of the set of dioxane-water mixtures⁴⁴ was remarkably linear in the region where the tautomeric ratio could be accurately measured (Figure 6). Assuming that this linear relationship can be extrapolated to the $E_T(30)$ of pure water, the tautomeric equilibrium constant for 6 in aqueous buffer is estimated to be $[keto]/[enol] \approx 2000$.

Extrapolation using mixed solvent systems to estimate tautomeric equilibrium constants in physiological media⁶ replaces to some extent the assumption that a methylated species is a good model for a protonated species. Instead, it assumes that the properties of the pure tautomers can be obtained in extremes of medium dielectric, that these are good end points, and that a linear extrapolation can be made as a function of a property of the medium (e.g.,

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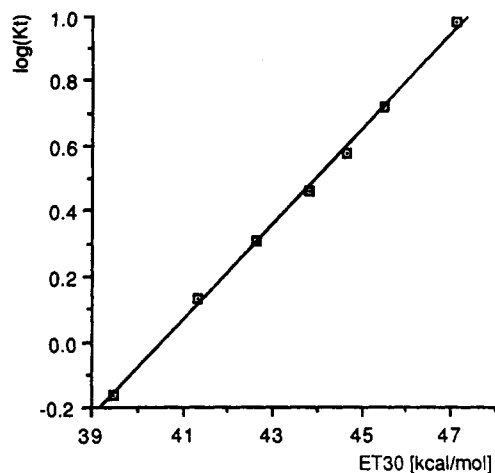


Figure 6. Plot of $\log(K_{\text{taut}})$ versus $E_T(30)$ for 6-amino-5-benzyl-3-methyl-pyrazin-2-one (6): slope = 0.15, intercept = -5.98, $R^2 = 0.998$.

$E_T(30)$ or Z values). While neither set of assumptions by itself is particularly secure, the two are quite independent. Taken together, a good case can be made both for the direction of a tautomeric equilibrium constant and for its magnitude.

A [keto]/[enol] ratio of ca. 2000 in aqueous media compares to estimates of 10^4 to 10^5 for guanine derivatives.⁸ This value is considerably higher than that obtained for isoguanosine derivatives ($K_T \approx 10$) by Shugar and his co-workers.⁶ Thus, we expect to encounter fewer problems with tautomeric equilibria in these systems than observed with the iso-C-iso-G base pair in enzyme-catalyzed polymerizations.

However, the obvious point needs a statement: The actual dielectric in the base-stacked structure of DNA or RNA may well be different from the dielectric in water. Therefore the tautomeric ratio of a heterocycle at equilibrium in aqueous solution need not be the same as in a double helix or in the active site of a polymerase. Particularly noteworthy is the report that the keto/enol tautomeric equilibrium constants for the natural bases are not a function of medium polarity.^{45,46} This is unusual when compared with most other heterocycles,^{37,47} including our pyrazinone system. This difference may help explain why Nature has chosen A, G, C, and T (or U) as part of her coding system.

Experimental Section

General. NMR spectra were recorded at 9.394 T (400 MHz for ^1H , 100 MHz for ^{13}C) or 7.046 T (300 MHz for ^1H , 75 MHz for ^{13}C). TMS (δ 0.00 ppm) was used as internal reference for both ^1H and ^{13}C spectra. Hydroxyl and amino proton assignments were confirmed by D_2O exchange. Standard flash silica gel 60 of E. Merck was used for flash chromatography. Fluka solvents (puriss p.a., puriss, or purum) were used. CHCl_3 was extracted with water to remove EtOH, dried over CaCl_2 , and then distilled over P_2O_5 . Dioxane was distilled over Na/benzophenone.

Starting Materials. *anti*-Methylglyoxal 1-oxime was purchased from Aldrich. Methyl iodide (puriss p.a.), *N*-methylmorpholine (purum), and acetic acid anhydride (puriss p.a.) were

obtained from Fluka. Hydrazine hydrate was obtained from Siegfried AG.

(*R,S*)-2-Amino-3-phenylpropionitrile Hydrochloride (DL-Phenylalaninenitrile hydrochloride) (2). Phenylacetaldehyde (1) (0.50 mL, 4.3 mmol) and NH_4Cl (250 mg, 4.7 mmol) were suspended in a mixture of dioxane (11 mL) and water (2 mL), and the suspension was cooled to 0 °C. KCN (280 mg, 4.3 mmol) was dissolved in a mixture of dioxane (5 mL), and water (1 mL) was slowly added. The solution was warmed to rt and stirred for 2 days. The reaction mixture was then diluted with ice-water and extracted with ether at 0 °C. The ethereal phase was dried over Na_2SO_4 at 0 °C and the hydrochloride salt precipitated at 0 °C by introduction of HCl gas. The product was recovered by filtration and dried under high vacuum to yield the hydrochloride salt 2 as a white solid (421 mg, 54%). This was used directly for the next reaction. An analytical sample was obtained via recrystallization from acetonitrile: mp 164.5 °C dec; FAB-MS (glycerol) m/z 147 (M^+ of the cation), IR (KBr) 3430, 2900, 2030, 1500, 1475, 1210, 1180, 1030; ^1H NMR (DMSO) δ 3.17 (dd, $J = 10.5, 13.5$ Hz, 1H), 3.41 (dd, $J = 5.2, 13.4$ Hz, 1H), 4.88 (dd, $J = 5.2, 10.5$ Hz, 1H), 7.32–7.43 (m, 5H, arom H), 9.58 (s, br, 3H, NH_2); ^{13}C NMR (DMSO) δ 35.85 (t), 41.92 (d), 116.3 (s), 127.62 (d), 129.35 and 128.64 (d), 133.79 (s). Anal. Calcd for $\text{C}_9\text{H}_{11}\text{N}_2\text{Cl}$ (182.65): C, 59.18; H, 6.07; N, 15.34. Found: C, 59.37; H, 6.02; N, 15.09.

2-Amino-3-benzyl-5-methylpyrazine 1-Oxide (4). Phenylalaninenitrile hydrochloride (2) (100 mg, 0.55 mmol) was dissolved in CHCl_3 (1 mL) by addition of *N*-methylmorpholine (60.6 μL , 0.55 mmol). *anti*-Methylglyoxal 1-oxime (3) (47.9 mg, 0.55 mmol) was added and the reaction mixture heated under reflux for 4 h. The reaction mixture was diluted with CHCl_3 . The product was adsorbed on silica gel by addition of silica gel (450 mg) and removal of the solvent by rotary evaporation. Chromatography ($\text{CH}_2\text{Cl}_2/\text{EtOH}$ 15/1) yielded 4 (74 mg, 63%) as a slightly yellow solid. An analytical sample was obtained via recrystallization from ethyl acetate. The yields decreased if the reaction was run in dioxane (35%) or EtOH (19%): mp 144.5–145.5 °C; MS m/z (intensity) 216 ($M^+ + 1$; 30), 215 (M^+ ; 88), 199 (70), 198 (100), 171 (76), 91 (76); IR (CHCl_3) 3480, 3350, 2980, 1610, 1570, 1480, 1450, 1335, 1120, 990, 840; UV (EtOH) λ_{max} (ϵ) 255 (sh) nm (6350), 344 nm (8260); ^1H NMR (DMSO) δ 2.24 (s, 3H), 4.11 (s, 2H), 6.76 (s, 2H), 7.18–7.31 (m, 5H), 7.99 (s, 1H); ^{13}C NMR (DMSO) δ 19.92 (q), 38.20 (t), 126.20 (d), 127.76 (d), 128.18 and 128.64 (d), 137.71, 140.07, 141.62 and 143.53 (s). Anal. Calcd for $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}$ (215.25): C, 66.96; H, 6.09; N, 19.52. Found: C, 66.76; H, 6.13; N, 19.46.

2-Acetoxy-5-benzyl-6-(diacetylamino)-3-methylpyrazine (5). Dry 4 (100 mg, 0.46 mmol) and KOAc (45.6 mg, 0.46 mmol) were dissolved in Ac_2O (1 mL) and refluxed for 10 min at 150 °C. The reaction mixture turned dark brown. After being cooled to rt, the anhydride was removed by distillation under high vacuum. To remove traces of anhydride, the mixture was suspended in EtOH and the EtOH removed by distillation at reduced pressure. After being dried under high vacuum, the product was dissolved in CH_2Cl_2 and the excess KOAc removed by filtration. By addition of silica gel (300 mg) and removal of the solvent, the product was adsorbed on silica gel. Flash chromatography (ether/hexane 4/5) yielded 5 (106 mg, 67%) as slightly yellow oil. An analytical sample was obtained via a second flash chromatography ($\text{AcOEt}/\text{hexane}$ 2/8): MS m/z (intensity) 341 (M^+ ; 11), 299 (43), 257 (76), 239 (51), 215 (68), 91 (56), 43 (100); IR (CHCl_3) 1770, 1725, 1415, 1390, 1320, 1185, 1145, 1020, 990; UV (EtOH) λ_{max} (ϵ) 276 nm (8050), 288 nm (7270); ^1H NMR (CDCl_3) δ 2.01 (s, 6H), 2.36 (s, 3H), 2.45 (s, 3H), 4.06 (s, 2H), 7.18–7.31 (m, 5H); ^{13}C NMR (CDCl_3) δ 18.97 (q), 20.81 (q), 26.29 (q), 40.14 (t), 127.09 (d), 128.80 and 129.06 (d), 136.48, 142.82, 147.51, 150.46 and 152.01 (s), 168.20 (s), 172.12 (s). Anal. Calcd for $\text{C}_{18}\text{H}_{19}\text{N}_3\text{O}_4$ (341.37): C, 63.33; H, 5.61; N, 12.31. Found: C, 63.06; H, 5.70; N, 12.27.

6-Amino-5-benzyl-3-methylpyrazin-2-one (6). Compound 5 (207 mg, 60.7 mmol) was dissolved in hydrazine hydrate (2 mL) and the solution stirred at rt overnight. The reaction mixture was neutralized with 2 M aqueous HCl and cooled to 0 °C. The precipitated product was recovered by filtration and dried under high vacuum to yield 6 (88 mg, 67%) as a light yellow solid. An analytical sample was obtained via recrystallization from

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acetonitrile: mp 208 °C; MS m/z (intensity) 216 ($M^+ + 1$; 18), 215 (M^+ ; 100), 186 (29), 145 (43), 129 (15), 110 (18), 91 (17), 77 (11); IR (KBr) 3450, 3020, 1650, 1625, 1600, 1550, 1490, 1450, 1390, 1370, 1245, 1140, 700; (CHCl_3) 3690, 3480, 3400, 3320, 2960, 2920, 2850, 1650, 1620, 1605, 1540, 1490, 1450, 1390, 1370, 1350, 1140, 1000; UV (EtOH) λ_{max} (ϵ) 240 nm (10140), 368 nm (9160); (MeOH) 240 nm (9950), 367 nm (9510); UV (aqueous buffer: pH 6.0, Tris 8 mM, spermidine 0.2 mM, MgCl_2 4 mM) 239 nm (10650), 366 nm (10580) (the compound was gently shaken at 40 °C overnight in the buffer solution for quantitative dissolution); $^1\text{H NMR}$ (DMSO) δ 2.10 (s, 3H), 3.83 (s, 2H), 5.58 (s, 2H), 7.11–7.24 (m, 5H), 10.74 (s, 1H); $^{13}\text{C NMR}$ (DMSO) δ 18.00 (q), 36.59 (t), 125.6 (d), 127.99 and 128.29 (d), 122.32 (s), 130.52 (s), 140.17 (s), 145.83 (s), 154.99 (s). Anal. Calcd for $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}$ (215.25): C, 66.96; H, 6.09; N, 19.52. Found: C, 66.74; H, 5.91; N, 19.55.

The $\text{p}K_a$ of **6** (8.2 ± 0.1) was determined by UV spectroscopy according to described procedures.³⁶

Alkylation of 6-Amino-5-benzyl-3-methylpyrazin-2-one (6). A cooled ethereal solution (0 °C) of CH_2N_2 (2.5 mmol) was added slowly to a solution of **6** (215 mg, 1.0 mmol) at 0 °C in absolute MeOH (3 mL). The reaction mixture was stirred for 2 h at 0 °C and the excess of CH_2N_2 was removed by introduction of N_2 and simultaneous warming to rt. The solvent was removed by distillation and the two products separated by flash chromatography. **7** was eluted with a mixture of $\text{CH}_2\text{Cl}_2/\text{EtOH}$ 30/1 to yield 116 mg (50%) of a slightly impure light-yellow solid after removal of the solvent and drying under high vacuum. **8** was eluted with a mixture of $\text{CH}_2\text{Cl}_2/\text{EtOH}$ 10/1 to yield 115 mg (50%) of a yellow oil. An analytical sample of **7** was obtained by recrystallization from hexane. **8** was chromatographed a second time on silica gel.

6-Amino-5-benzyl-2-methoxy-3-methylpyrazine (7): mp 75–76 °C; MS m/z (intensity) 230 ($M^+ + 1$; 17), 229 (M^+ ; 100), 228 (10), 201 (13), 200 (18), 186 (10), 159 (15), 124 (20), 91 (37); IR (KBr) 3480, 3275, 3140, 3060, 3020, 2975, 2950, 2905, 1620, 1600, 1580, 1550, 1490, 1450, 1390, 1240, 1165, 1110, 1010, 760, 700; IR (CHCl_3) 3495, 3395, 2980, 2950, 1605, 1555, 1490, 1450, 1340, 1240, 1190, 1070, 1015, 990; UV (EtOH) λ_{max} (ϵ) 239 nm (10380), 329 nm (10590); UV (MeOH) 239 nm (10120), 329 nm (10660); UV (aqueous buffer: pH 6.0, Tris 8 mM, spermidine 0.2 mM, MgCl_2 4 mM): 235 nm (9630), 327 nm (9340) (the compound

was gently shaken at 40 °C overnight in the buffer solution for quantitative dissolution); $^1\text{H NMR}$ (DMSO) δ 2.18 (s, 3H), 3.79 (s, 3H), 3.92 (s, 2H), 5.81 (s, 2H), 7.10–7.26 (m, 5H); $^{13}\text{C NMR}$ (DMSO) δ 17.56 (q), 37.48 (t), 52.96 (q), 126.06 (d), 128.36 and 128.75 (d), 126.52 (s), 129.74 (s), 139.86 (s), 150.20 (s), 155.53 (s). Anal. Calcd for $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}$ (229.28): C, 68.10; H, 6.59; N, 18.33. Found: C, 68.05; H, 6.66; N, 18.07.

6-Amino-5-benzyl-1,3-dimethylpyrazin-2-one (8): MS m/z (intensity) 230 ($M^+ + 1$; 15), 229 (M^+ ; 100), 228 (21), 214 (29), 186 (15), 173 (16); IR (KBr) 3300 (br), 3080, 3060, 3030, 2920, 1700, 1580 (br), 1560, 1510, 1490, 1450, 1370, 1220, 1100, 1060, 880, 740, 700; (CHCl_3) 3485, 3395, 1685 (br), 1645, 1615, 1570, 1510, 1450, 1370, 1135, 1075, 1030, 1015, 910; UV (EtOH) λ_{max} (ϵ) 245 nm (8600), 367 nm (11360); UV (MeOH) 244 nm (8080), 367 nm (11260); UV (aqueous buffer: pH 6.0, Tris 8 mM, spermidine 0.2 mM, MgCl_2 4 mM) 241 nm, 364 nm (the compound was gently shaken at 40 °C overnight in the buffer solution for dissolution; a precise measurement of the extinction coefficient of **8** in aqueous solution was not possible due to the low solubility and the thermal instability of the compound); $^1\text{H NMR}$ (DMSO) δ 2.11 (s, 3H), 3.37 (s, 3H), 3.84 (s, 2H), 6.12 (s, 2H), 7.10–7.30 (m, 5H); $^{13}\text{C NMR}$ (DMSO) δ 19.46 (q), 28.42 (q), 36.56 (t), 125.56 (d), 127.97 and 128.12 (d), 114.4 (s), 134.3 (s), 140.48 (s), 142.04 (s), 154.35 (s). Relevant ^1H - ^{13}C heterocorrelations: NH_2 , C(5); NH_2 , C(6); CH_3N , C(2); CH_3N , C(6); CH_3C , C(2); CH_3C , C(3); CH_2 , C(5); CH_2 , C(6); CH_2 , C(1'); CH_2 , C(2'); HRMS calcd for $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}$ 229.1215, found 229.1246.

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