Studies on the Acetylation of 3,6-Diamino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile Derivatives

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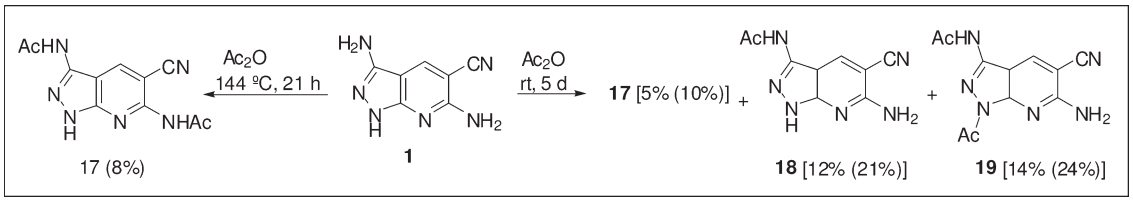

The acetylation reaction of the differently substituted 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile derivatives **1**–**6** is reported. The structure of the resulting acetamides has been investigated and confirmed by analytical, spectroscopic, and chemical transformations. From these studies, we conclude that, in general, under mild conditions, and using acetic anhydride, when free, the *N*(1)H moiety is a more reactive center respect to the C(3)NH_2 and C(6)NH_2 groups. This trend is reversed when no steric hindrance due to presence of a phenyl group at C4 drives the preferred acetylation to C(3)NH_2, as it is evident by comparing the observed results from precursor **1** with **3**. When N1 is blocked, the (C3)NH_2 group undergoes preferential acetylation over the (C6)NH_2 site, which only has been mono (or diacetylated) at reflux. Computational analyses based on DFT studies have been extensively used to explain the observed reactivities.

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INTRODUCTION

The pyrazolo[3,4-*b*]pyridine ring system [1] is present in a number of pharmaceutically important compounds targeted to inhibit VEGFR/PDGFR kinases [2] or GSK-3 [3]. In a current project aimed at the synthesis, design, and biological evaluation of new GSK-3 inhibitors, we have recently synthesized a series of known and new 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridines [4] (**A**) (Chart 1). To carry out basic SAR studies, we decided to explore the acylation reaction of 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridines (**1**–**6**) (Chart 2) to get presumably more potent inhibitors as previously demonstrated by other authors [3].

In this context, previous reports from other laboratories have described that the acylation (C_3H_7COCl, pyridine, reflux) of precursor **7** gave the C(3)NH_2 acetylated derivative **8** in 80% yield (Chart 3). Note, however, that the carbamoylation of the free amino groups on the fused pyrazole ring system in compound **9** showed a reversed regioselectivity, providing compound **10** (Chart 3) [5]. In addition, it has been also shown that under mild conditions, 5-substituted 3-aminopyrazoles are almost simultaneously acylated at N1 and at C(3)NH_2 to give diacylated derivatives [6]. Substituted 3-amino-1*H*-pyrazolo[3,4-*b*]quinoxalines have been selectively N-acylated at N1 [7]. Compound **2** has been reported to give exclusively the C(3)-*N*-formyl derivative **11** or the acetamide **13** from related amide **12** (Chart 3) [8]. Finally, a more complex situation was described for 4,5-diphenyl-1*H*-pyrazolo[3,4-*c*]pyridazine (**14**) [9] when using acetyl chloride. Depending on the base used, the acetylation goes to C(3)NH_2 to give compound **15** (for triethylamine as base) or to **16** (for pyridine as base) (Chart 3) [9]. Note also that the position of the acetyl residue at N1 was not unambiguously confirmed by X-ray analysis neither discussed in depth; however, 4,5-diphenyl-1*H*-pyrazolo[3,4-*c*]pyridazines have been regioselectively N-alkylated at N1 [9], a result that is in good agreement with similar reaction on 3-amino-1*H*-pyrazolo[3,4-*b*]quinoxalines [7].

In summary, all these data confirm that a simple reaction such as the acetylation in a complex, polyfunctionalized, heterocyclic framework can be more complicated than presumed at first glance [10]. This is in fact what we have observed in the acetylation of 3,6-diamino-pyrazolopyridines **1**–**6** (Chart 2), and in this work, we report our results.

RESULTS AND DISCUSSION

Synthesis, structural analysis, and reactivity. The synthesis of 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**1**) (Chart 2) proceeded uneventually as described by reacting 2-amino-6-chloropyridine-3,5-

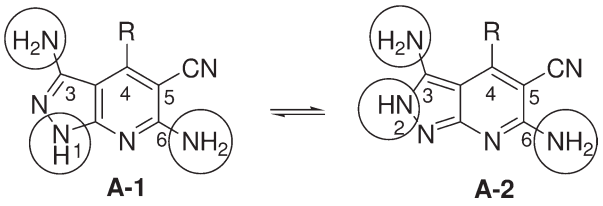


Chart 1. 3-6 Diamino-1*H*-pyrazolo[3,4-*b*]pyridines.

dicarbonitrile with hydrazine hydrate [8]. In the HMBC experiment of pyrazolopyridine **1**, we assigned the chemical shifts for the protons at C(3)NH_2 and C(6)NH_2, at δ 5.56 and 6.70, as cross-peaks were observed with the signals at δ 99.8 (C3a) and 83.0 (C5), respectively; in agreement with this, the signal at 99.8 ppm (C3a) showed a cross-peak with the signal at δ 11.60, corresponding to N(1)H. Similar chemical shifts and effects have been observed for protons in the other related compounds described here, and this trend [δ C(6)NH_2 >> δ C(3)NH_2] has served as diagnostic for proton assignments and structure determination (see later).

The reaction of compound **1** [8] with acetic anhydride, after 21 h, at 144°C, was complete, but extensive decomposition was observed by TLC analysis, and only *N*,*N*′-(5-cyano-3a,7a-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-3,6-diyl)diacetamide (**17**) could be isolated in 8% yield (Chart 4). Based on the analytical (MS/elemental analysis) and the NMR spectra, compound **17** was clearly a diacetamide derivative of precursor **1**, showing in its ^{1}H NMR spectrum two acetyl groups integrating for six protons (2xNHCOCH_3), as a singlet at δ 2.12; the singlet at δ 10.96 was assigned to C(3)N*H*COCH$_3$ as it showed cross-peaks in the HMBC spectrum with C3a (δ 104.7) and NH*C*OCH$_3$ (δ 169.2); consequently, the singlet at δ 10.79 corresponded to the proton at C(6)N*H*COCH$_3$; finally, the singlet for one proton at δ 13.62 was assigned to N(1)*H*. In this reaction, we detected traces of a second compound that was identical to the compound isolated when the reaction was carried at out at room temperature for 5 days and identified as *N*-(6-amino-5-cyano-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)acetamide (**18**), isolated in 12% yield (21% taking into account the recovered starting material); in this reaction, we also isolated diacetamide **19** [14% (24%)] and compound **17** [5% (10%)] (Chart 4). In the ^{1}H NMR spectrum of monoacetamide **18**, we could analyzed only one singlet for two protons at δ 6.95, indicating that the only free NH_2 group was at C6, leaving free the N(1)H (δ 12.66), the acetamido group being thus at C3, as in the ^{1}H NMR spectrum the signals for NHCOCH$_3$ appeared at δ (NH) 10.69 and δ (COCH$_3$) 2.12. These assignments were confirmed in the HMBC experiment, as the signal at δ 12.66 showed cross-peaks with C3a, C3, and C7a, while the singlet at δ 10.69 showed cross-peaks with C3a and NH*C*OCH$_3$. In the ^{1}H NMR spectrum of compound **19**, in addition to similar signals to those described for compound **18** (see Experimental), no N(1)H resonance was observed at low field, but a new singlet appeared for three protons at 2.77 ppm, characteristic for N(1)COCH$_3$ (see compound **16** in Chart 3) [9]. In summary, we conclude that the acetylation of 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**1**) is relatively complex, and a number of differently substituted acetamido derivatives were isolated and characterized. Under mild reaction conditions, preferent acetylation at C(3)NH_2 followed at N(1) positions was observed; the acetylation at C(6)NH_2 being also possible, at reflux, to give diacetamide **17** (Chart 4), as the only detected, in a poor chemical yield. Overall, these facts are in good agreement with the previous results [5–7] on the acylation of related substrates (see Chart 3) and strongly point to the absence of a substituent at C4, the key to favor the acetylation at C(3)NH_2 (see later).

The acetylation of 3,6-diamino-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**2**) [8], under mild conditions, gave only monoacetamide **20** in good yield (Chart 4). In the ^{1}H MNMR of this compound, we could

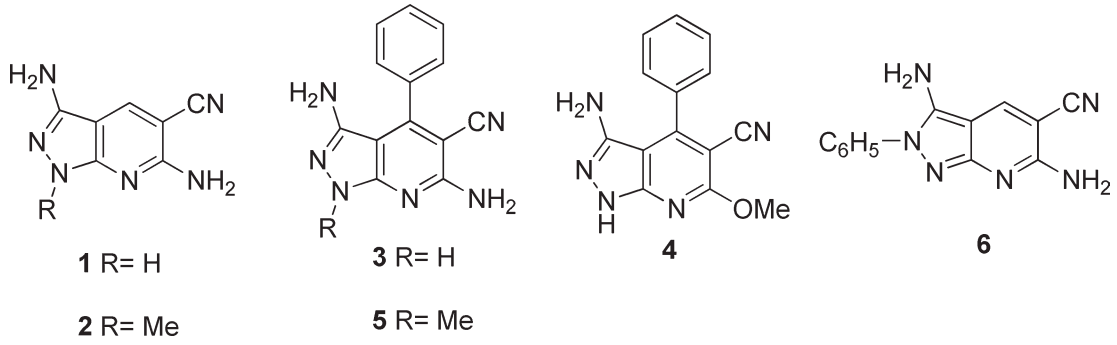


Chart 2. Structure of compounds **1–6**.

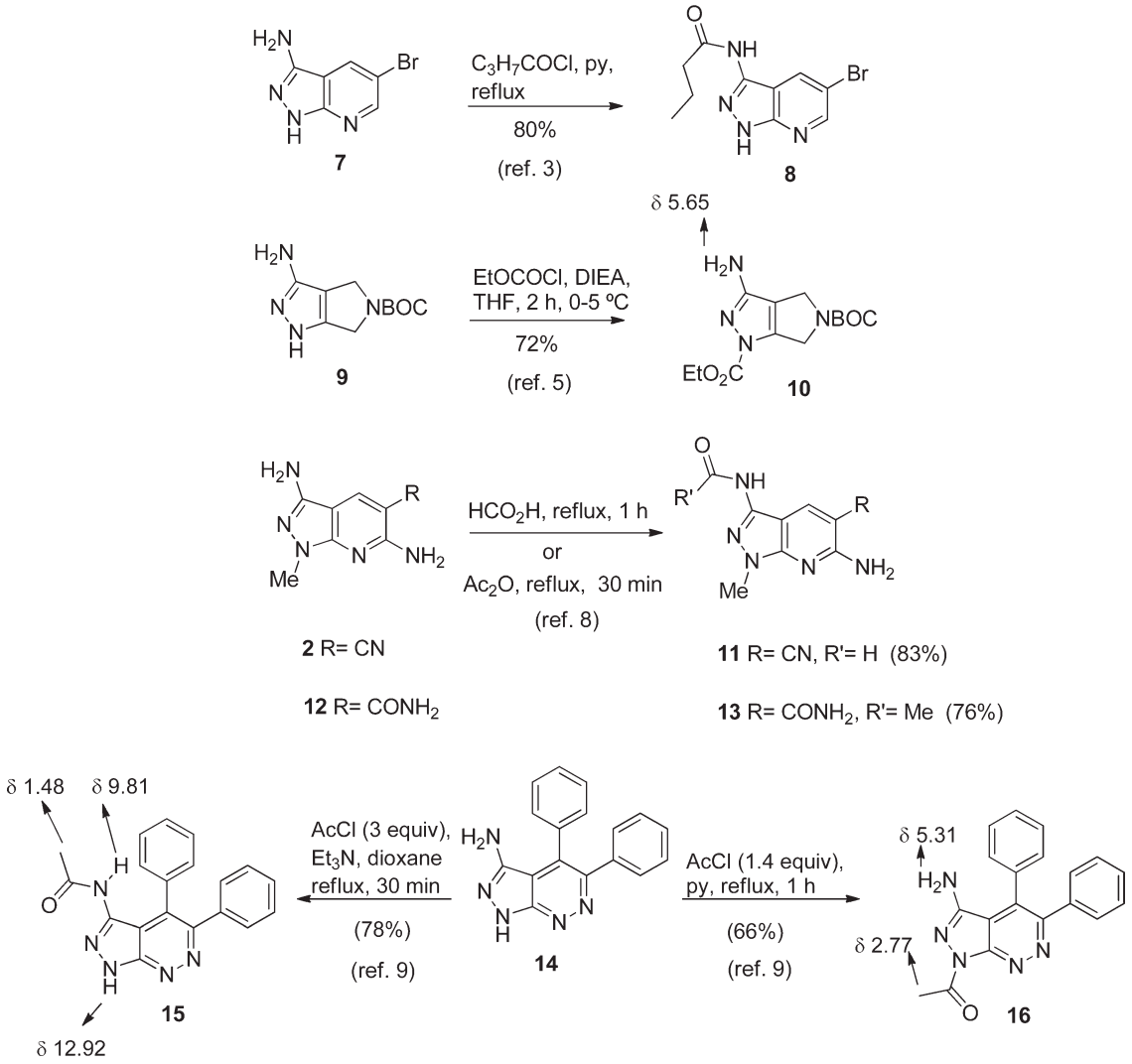


Chart 3. Transformation of compounds **2**, **7**, **8**, **12**, and **14**.

analyze two singlets for proton at δ 10.74 and for two protons at δ 7.08, corresponding to (C3)N*H*COCH$_3$ and (C6)NH$_2$ protons, respectively. In agreement with this assignment, in the HMBC-NMR experiment of this pyrazolopyridine, the NH proton at C(3)N*H*COCH$_3$ resonated at δ 10.74 and showed cross-peaks with C3a (100.3 ppm) and NH*C*OCH$_3$ (168.1 ppm), whereas for the proton at C(6)NH$_2$ (δ 7.08) cross-peaks appeared with the signals at δ 86.4 (C5), 158.3 (C6), and 150.7 (C7a). In addition, a small but evident selective nOe experiments between the NH proton at δ 10.74 [(C3)N*H*COCH$_3$] and H4 (δ 8.57) confirmed the position of the acetyl group on the nitrogen at C3. This result and reactivity is thus also in good agreement with the reported reactivity of precursor **2** in the formylation reaction (Chart 3) [8].

The small nOe observed between both protons in the N*H*COC*H*$_3$ group and H4 in compound **20** could be rationalized after computational analysis, which showed that, in fact, rotamer **20b** was 7.0 kcal/mol more stable than conformer **20a** (Fig. 1), possibly because of the lone pair–lone pair electronic repulsion between the carbonylic oxygen and the N2 present in conformer **20a**. On the other hand, the calculated chemical shifts for protons in NHCOC*H*$_3$ are in good agreement with the experimental values.

The reaction of 2-amino-6-chloro-4-phenylpyridine-3,5-dicarbonitrile [11] with hydrazine hydrate [4] gave 3,6-diamino-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**3**) (Chart 2) [12]. In the acetylation of compound **3** with Ac$_2$O, at 0°C, for 20 h (Method A, see Experimental), a solid was formed; it was filtered, washed with cold water/EtOH, and recrystallized from ethanol to give compound **21** in 25% yield (Chart 4). The analytical and spectroscopic data of this molecule

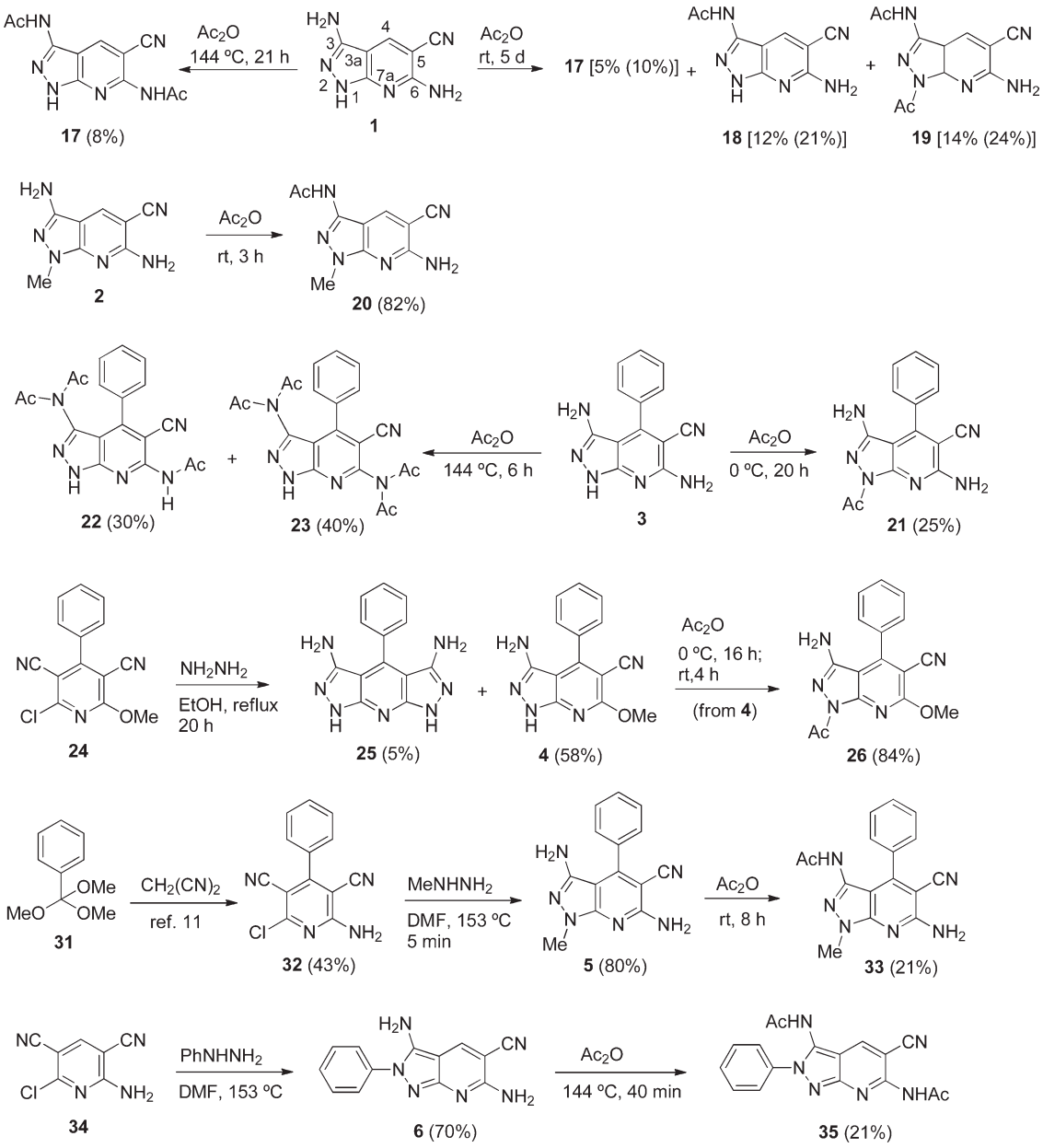


Chart 4. The acetylation reaction of compounds **1**–**6**.

clearly showed that **21** was a monoacetamide, as in the ^{1}H NMR, a methyl group was observed at an anomalous low field (δ 2.58) and two broad singlets (7.40 and 4.82 ppm) for two protons, each one corresponding to the NH_2 groups at carbons C6 and C3, respectively. These data along with the absence of a free NH, or a NH resonance for a N*H*COCH$_3$ moiety, clearly supported the structure of **21** as 1-acetyl-3,6-diamino-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile.This structure has also been confirmed by the data obtained in the ^{13}C NMR, HMQC, and HMBC experiments.

To improve the chemical yield, the acetylation reaction was carried out with Ac_2O at rt for 5 h or with AcCl, in pyridine, at 0°C for 5 days, but the yields (21 and 24%, respectively) of compound **21** were not better. Finally, the acetylation reaction was performed with Ac_2O at reflux for 6 h; after work-up and purification, peracetylated derivatives **22** (30%) and **23** (40%) were isolated (Chart 4). The structure of compound **22** was established as a triacetamide derivative of precursor **3** by its analytical and spectroscopic data. As in the ^{1}H NMR spectrum, we observed a broad singlet for one

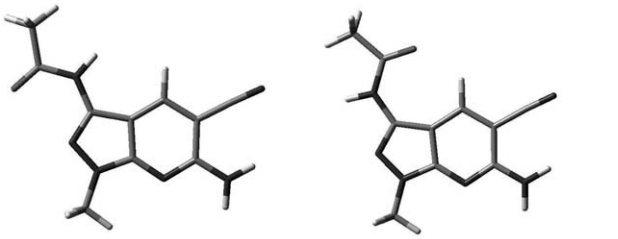


Figure 1. Conformers for compound **20**.

proton at 14.45 ppm, and three acetyl groups [δ 2.15 (singlet, one acetyl) and 1.91 (singlet, two acetyl groups)]; we concluded that N(1) was free, the only structural problem that remained to be established was to determine if the $NHCOCH_3$ group was at C3 or at C6. In the HMBC spectrum, the signal al δ 10.98 (N*H*$COCH_3$) showed cross-peaks with signals at 100.0, 152.3, and 169.5 (N*C*OCH_3). In the case that the group $NHCOCH_3$ was at C6, the signals at 100.0 and 152.3 should be assigned to C5 and C6, respectively, the signal appearing at 106.5 ppm corresponding thus to C3a, an assignment that seems reasonable, as we have routinely observed in the compounds investigated here that in the ^{13}C NMR spectrum δ C3a >> C5. To prove this hypothesis, a series of nOe experiments were carry out. When the N*H*$COCH_3$ was irradiated, only the singlet at 2.15 ppm showed a weak effect; the irradiation of this signal also showed only a weak effect at 10.98 ppm. However, the irradiation of the singlet at 1.91 ppm integrating for six protons (two $NHCOCH_3$ groups) produced a sharp effect on the aromatic protons; the reverse irradiation also produced the same effect. From these experiments, we conclude that the two acetamido groups should be in the same carbon (C3). For compound **23**, as in the ^1H MNR spectrum, we observed a broad singlet at δ 14.93 for one proton, clearly assigned to N(1)H, the location of the four acetamido groups present in the molecule at C3 and C6 was evident.

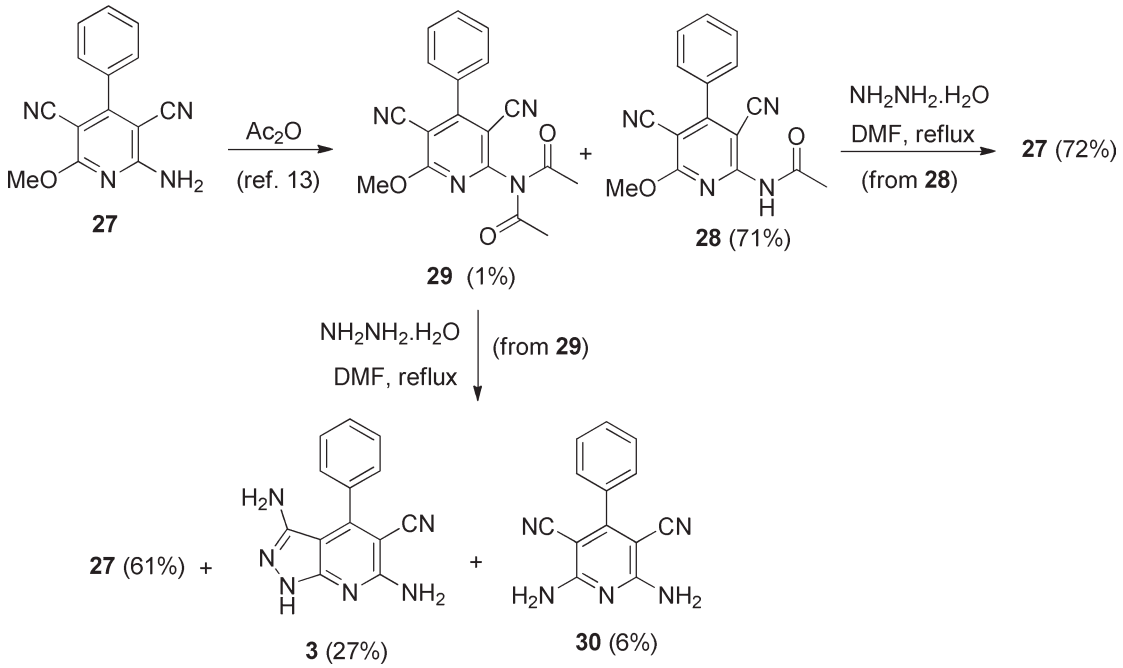
The regioselective acetylation at N1 in compound **3**, respect to both NH_2 groups at C3 and C6, under mild conditions, prompted us to investigate the same reaction on precursor **4**, where only two positions at N1 and C(3)NH_2 were available for the acetylation. This compound has been prepared from readily available 2-chloro-6-methoxy-4-phenylpyridine-3,5-dicarbonitrile (**24**) [13] after reaction with hydrazine hydrate; in this reaction, we isolated also traces of the bis-pyrazolopyridine **25** (Chart 4). When compound **4** was acetylated with Ac_2O (see Experimental) compound **26** was isolated in good yield (84%) (Chart 4). The analytical and spectroscopic data clearly showed that compound **26** is a monoacetamide bearing the acetyl group at N1, as a broad singlet appeared at 5.10 ppm integrating for two protons [C(6)NH_2], and the singlet for the acetyl groups integrating for three protons resonated at δ 2.69, a value that we have found in compound **21** (see earlier) and in compound **16** [9] (Chart 3).

In view of these results, and as we were interested in the synthesis of the monoacetamide at C6 in these pyrazolopyridines, we considered an alternative synthetic route based on the acetylation of 2-amino-6-methoxy-4-phenylpyridine-3,5-dicarbonitrile (**27**) [13] (Scheme 1). Carrying out the reaction as reported [13], we obtained a mixture of monoacetamide **28** (71%) and imide **29** (1%) (Scheme 1) that were easily separated by column chromatography and submitted to reaction with hydrazine hydrate, in DMF at reflux, aiming at the ''one-pot'' methoxy displacement and simultaneous pyrazolopyridine formation. For compound **28**, we obtained compound **27** in 72% yield (Scheme 1). For compound **29**, we isolated and characterized compounds **27**, **30**, and **3** (Scheme 1). The formation of these compounds is the result of a series of deacetylation reactions followed by pyrazolo formation. The structure of compound **30** has been unequivocally established by comparison of the reported data in literature [11] and with an authentic sample prepared in the reaction of DMF [14] with 6-amino-2-chloro-4-phenylpyridine-3,5-dicarbonitrile (**32**) [11,15] (see Experimental), obtained from the reaction of trimethylorthobenzoate (**31**) with malononitrile [11] (see Experimental) (Chart 4).

Next, we have investigated the acetylation of precursor **5**, prepared as usual from compound 6-amino-2-chloro-4-phenylpyridine-3,5-dicarbonitrile (**32**) [11] and *N*-methylhydrazine (Chart 4). As expected, in the ^1H NMR spectrum of compound **5**, a positive nOe effect between the broad singlet at δ 4.36 and the aromatic protons allowed us to assign this chemical shift for protons at C(3)NH_2, the signal at 6.90 ppm corresponding to C(6)NH_2. The acetylation of pyrazolopyridine **5** (Ac_2O, rt, 8 h) provided compound **33** in low yield (Chart 4). Not unexpectedly, in the ^1H NMR of compound **33**, the singlet for two protons at δ 7.12 [(C6)NH_2] clearly demonstrated that the acetylation has taken place in the C(3)NH_2 group. Selective nOe experiments also showed the small but evident effect between the protons at the acetamide group (CON*H*CH_3).

For compound **33** (Fig. 2), rotamer **b** is 1.8 kcal/mol more stable than **a**, possibly because of the electronic repulsion between the carbonylic oxygen and the N2 present in conformer **a**, as previously shown for compound **20**, although the effect here should be less strong, because the presence of the phenyl ring prevents a coplanar arrangement between the amide group and the azole plane, being rotated with a dihedral angle of 58°.

Scheme 1. Synthesis of compounds **28** and **29** and their reaction with hydrazine hydrate.

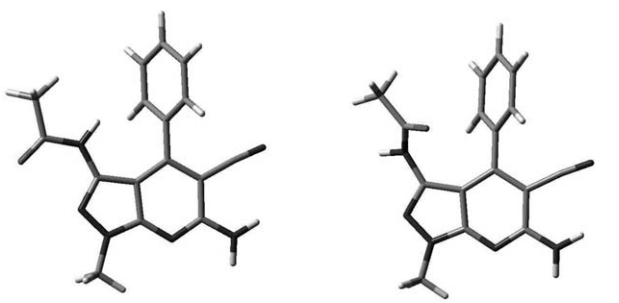


This fact increases the energy of conformer **b** and reduces the energy difference between the two possible conformers. On the other hand, the calculated and the experimental chemical shifts for the methyl groups in NHCOCH_3 are in good agreement; the low δ chemical shift observed for this methyl group possibly due to the shielding effect of the aromatic ring at C4.

Finally, we have prepared precursor **6** in good yield in the reaction of 2-amino-6-chloropyridine-3,5-dicarbonitrile (**34**) with *N*-phenylhydrazine (Chart 4). After selective nOe experiments in the ^{1}H NMR spectrum of this compound, the resonance at δ 6.97 was assigned to the amino group at C3, while the singlet integrating for two protons at δ 6.48 corresponded to (C6)NH_2. This analysis is the reverse that we have observed in the other precursors investigated in this work and must ascribed to the presence of a phenyl ring at N2. In fact, it is well known that the reaction of 2-halogeno-3-cyanopyridines with *N*-arylhydrazines respect to *N*-alkylhydrazines provides 2-aryl-2*H*-pyrazolo[3,4-*b*]pyridines instead of 1-alkyl-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridines [16]. The acetylation of compound **6** at reflux for 40 min gave the diacetylated derivative **35** in 21% yield (Chart 4), which showed spectroscopic and analytical data in good agreement with this structure (see Experimental).

Computational studies. In view of the results obtained for pyrazolopyridine **3** (Chart 4), we next addressed the reaction mechanisms to explain the observed regioselectivities during the acetylation reactions.

All calculations were carried out with Gaussian03 package [17]. All the minima and transition states involving were fully optimized with the B3LYP hybrid functional [18]. As the key aspect to account for reactivity and regioselectivity concerns atoms bearing lone-pair electrons, we have applied the extended 6-31+G(d,p) basis set to get reliable structures and energy values. Then, to optimize computational resources, we have selected AcCl as acetylated agent instead of Ac_2O. Treatment of precursor **1** with both electrophiles has shown similar results (see main text). Zero-point energies and thermal contributions to thermodynamic functions and activation parameters, as well as harmonic frequencies to assess the nature of the stationary points, were computed at the same level of theory on the



33 (conformer **a**)
Predicted: δ[(NHCOCH_3)]= 1.93

33 (conformer **b**)
Predicted: δ [(NH COCH_3)] = 1.43

Figure 2. Conformers for compound **33**.

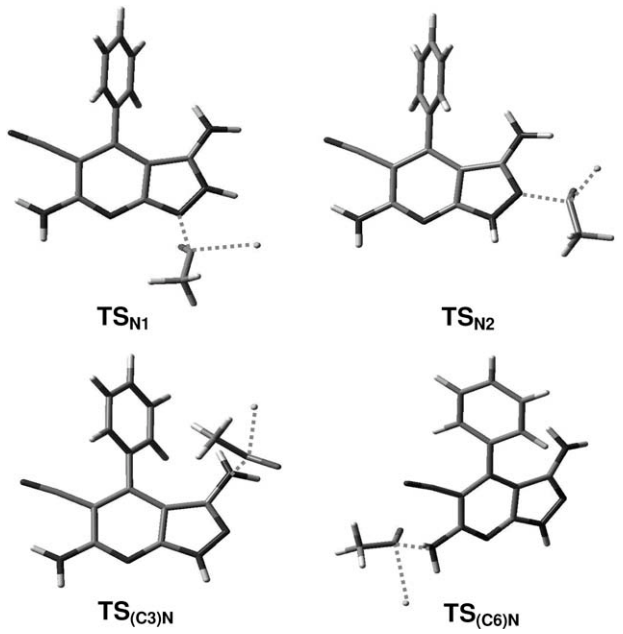


Figure 3. Transition structures for the N-acetylation of **3**.

optimized structures. To test the influence of solvation effects, we have calculated solvation free energies in solution ΔG_{solv} for the ground state and transition structures at the PCM/UAHF/B3LYP/6-31G(d) level [19] using the previously optimized gas-phase structures. Combination of these solvation free energies with gas-phase free energies obtained at B3LYP/6-31+G(d,p) level yields the relative free energy in solution $\Delta G^{\ddagger}_{sol}$ compiled in Table 2 in column 4 and Figure 3. Natural bond orbital (NBO) analyses [20] have been performed by the module NBO v.3.1 implemented in Gaussian 03 to evaluate the NPA charges at the optimization level.

We have initially carried out the study of the tautomeric equilibrium for compound **3**. We have focused on the prototropy tautomerism between the **3**-1*H* and **3**-2*H* forms involving the azole moiety (Scheme 2) as the structure with the hydrogen atom attached to the pyridine nitrogen at the position 7 is energetically unfavorable, as was expected from valence bond resonance considerations and verified by calculations on this [our calculations (B3LYP/6-31+G(d,p) reveal a structure 24.5 kcal mol^{-1} less stable than **3**-1*H*] and related structures [21].

Both structures **3**-1*H* and **3**-2*H* have in common that the amino groups are partially planarized (out-of-plane deviation of (C3)N/(C6)N: 29.4°/10.8° and 28.9°/14.2° in **3**-1*H* and **3**-2*H*, respectively) as the N electron pairs are part of the aromatic system, and that the aromatic ring attached to the C4 position is rotated with respect to the pyridine plane (with dihedral angles of 57.5 and 59.7°, respectively) to avoid steric repulsions with substituents at C3 and C5.

At the B3LYP/6-31+G(d,p) level, the tautomer **3**-2*H* is predicted to be less stable than **3**-1*H* by 9.5 and 4.0 kcal/mol in the gas phase and in DMSO, respectively. According to the Boltzmann distribution, at a temperature of 298 K, this difference in energy corresponds to a **3**-1*H*:**3**-2*H* ratio of >99:<1, with the population of the minor tautomer below the limit of detection for conventional NMR spectroscopy. Thus, according to the calculations, tautomer **3**-2*H* is unlikely to coexist with the other tautomer.

This preference of the 1*H* tautomer in pyrazolopyridines agrees with structural analysis of 4-aryl-5-cyano-pyrazolo[3,4-*b*]pyridines [22], theoretical studies of pyrazolo[3,4-*b*]pyridines bearing a variety of substituents [23], and crystallographic data of protein–ligand complexes [24], which indeed confirm that in this class of compounds, the (N1)H forms key H-bonds with the enzymes (cyclin-dependent kinases).

This picture contrasts with that for the related structures pyrano[2,3-c]pyrazoles (6-amino-5-cyano-3-methyl-4-aryl/heteroaryl-2*H*,4*H*-dihydropyrano[2,3-*c*]pyrazoles) as they exist predominantly in the 2*H* tautomeric form [25]. To confirm the reliability of our calculations in the prediction of tautomeric equilibria, we have performed further computations on the 6-amino-5-cyano-3-methyl-4-phenyl-dihydropyrano[2,3-*c*]pyrazole ring system [25–27]. Our results indicate that the 2*H* tautomer is 3.7 kcal mol^{-1} more stable than the 1*H* form in the gas phase. These data are in agreement with the crystallographic results [25–27], thus supporting our theoretical protocol in the estimation of the tautomeric equilibrium.

The reactivity of the 3,6-diamino-pyrazolo[3,4-b]pyridines against acetylation merits a careful analysis as four nucleophilic positions can undergo acetylation: besides both amino moieties [(C3)N and (C6)N], the piridinic- and pyrrole-type N of the pyrazole (N1, N2). The electron pair of the pyridinic-type N makes this position more nucleophilic than the pyrrole-type N, whose electron pair is part of the aromatic system.

In an attempt to rationalize the regioselectivity, DFT calculations were performed to determine both the atomic charges and the HOMO of **3**. In general, reactions with hard (high-lying LUMOs) electrophiles are charged

Scheme 2. Tautomer equilibrium in compound **3**.

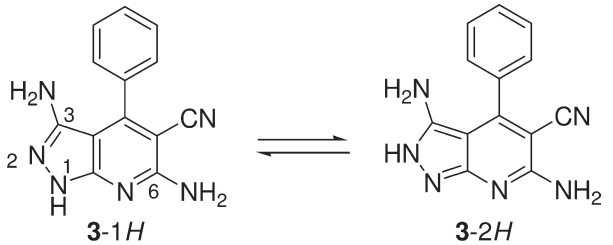


Table 1

Calculated NPA charges and HOMO coefficients for the N atoms of **3** prone to undergo attack by the acylating agent.

	NPA charges		HOMO coefficients	
	3-1*H*	**3**-2*H*	**3**-1*H*	**3**-2*H*
N1	−0.397	−0.327	0.47	0.73
N2	−0.340	−0.380	0.64	0.34
(C3)N	−0.861	−0.861	0.58	0.46
(C6)N	−0.829	−0.837	0.30	0.36

controlled, whereas reactions with soft (low-lying LUMOs) electrophiles are under frontier molecular orbital (FMO) control. The calculated atomic charges are not consistent with the experimentally determined regioselectivity. Thus, the greatest amount of negative charge is found at the (C3)N position (atomic charge = −0.861, Table 1), while reaction occurs selectively at the azole moiety. On the other hand, the observed selectivity fits well with the computed HOMO coefficients on both tautomers **3**-1*H* and **3**-2*H* (Table 1) as the largest value, and hence providing better overlapping orbital with the electrophile, is situated at the pyridinic-type nitrogen.

Although orbital properties can account only for the electronic factors, the steric effects (if exist) are included in the free energy of activation for the reaction. From a mechanistic point of view, when the reaction is carried out in the absence of a deprotonating agent, it should be expected that the N—H of the azole becomes acidic enough to be deprotonated only when the pyridinic-type N has been attacked by the acylated agent [28]. According to this assumption, we have considered the attack on every N of the neutral structure and have selected acetyl chloride (see Experimental) as electrophile to simulate the acetylation reaction.

The calculated transition structures (Fig. 3) provide free energy differences that suggest a kinetically favored attack on the azole ($\mathbf{TS_{N1}}$ and $\mathbf{TS_{N2}}$) rather than on the amino moieties ($\mathbf{TS_{(C3)N}}$ and $\mathbf{TS_{(C6)N}}$) in the gas phase and in solution (Table 2). In fact, as has been described earlier, these groups are rather planarized as the lone pair is partially delocalized in the aromatic system, thus being less nucleophilic groups than expected. This observation agrees with the experimental selectivity shown earlier (Chart 4). In the azole, the kinetically preferred site for the acetylation, in the gas phase and in solution, is N2 in the **3**-1*H* form (Table 2), which indeed is the proposed predominant tautomer. Also, according to these results, a small amount of the regioisomeric product could be formed by attacking at N1 in the **3**-2*H* form (estimation of the Boltzmann distribution N2:N1 93:7). In summary, these results suggest that **21** should be mostly the N2-substituted structure that results from N-acetylation of the major tautomer of **3** (**3**-1*H*). In view of these theoretical results and to support the position of the acetyl group at N1/N2, we tried to crystallize compound **21** as a free base or as its hydrochloride, but without success; consequently, the location of the acetyl groups at N1 is a tentative hypothesis that still needs to be experimentally confirmed.

Bases on these data, if the azole positions are blocked to undergo reaction, the (C3)N should be the preferred reactive site. This hypothesis agrees with the experimental observations as (C3)N is the acetylation site found in **20**. To further shed light on this result, we have performed calculations for the formation of **20** from the pyrazolopyridine **2**. The HOMO coefficients are parallel to those found for **3**-1*H*: (C3)N = 0.62, (C6)N = 0.27. Likewise, the calculated transition structure for the attacking on the amino group at C3 is 8.3 kcal mol^{-1} more stable than on the amino at C6 (Fig. 4). Accordingly, **20** is acetylated at (C3)N, which is supported by the experimental evidence.

In conclusion, in this work, we have described the acetylation of differently substituted 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile derivatives (**1**–**6**. The structure of the resulting acetamides has been investigated and confirmed by analytical, spectroscopic, and chemical transformations. From these studies, we conclude that, in general, under mild conditions, and using acetic anhydride, when free, the *N*(1)H moiety is the more reactive center respect to the C(3)NH$_2$ and C(6)NH$_2$ groups. This trend is reversed when no steric hindrance due to presence of a phenyl group at C4 drives the preferred acetylation to C(3)NH$_2$, as it is evident by comparing the observed results from precursor **1** with **3**. When N1 is blocked, the (C3)NH$_2$ group undergoes preferential acetylation over the (C6)NH$_2$ site, which only has been mono (or diacetylated) at reflux.

On the basis of these data, we have also undertaken a computational analysis to explain the observed selectivities during the acetylation reactions. The calculations of frontier orbital coefficients on the reactants pyrazolopyridines and activation barriers agree with the regiochemistry observed. The regioselectivity on the acetylation of the amino groups can be explained by the availability of

Table 2

Thermodynamic data (in kcal mol^{-1}) in gas phase and in solution for the potential transition structures for the N-acetylation of **3**.

Transition structures	$\Delta H^{\ddagger}_{gas}$	$\Delta G^{\ddagger}_{gas}$	$\Delta G^{\ddagger}_{sol}$
TS_{N1}	1.7	1.6	1.6
TS_{N2}	0.0	0.0	0.0
$TS_{(C3)N}$	6.2	6.5	3.2
$TS_{(C6)N}$	11.2	11.1	7.3

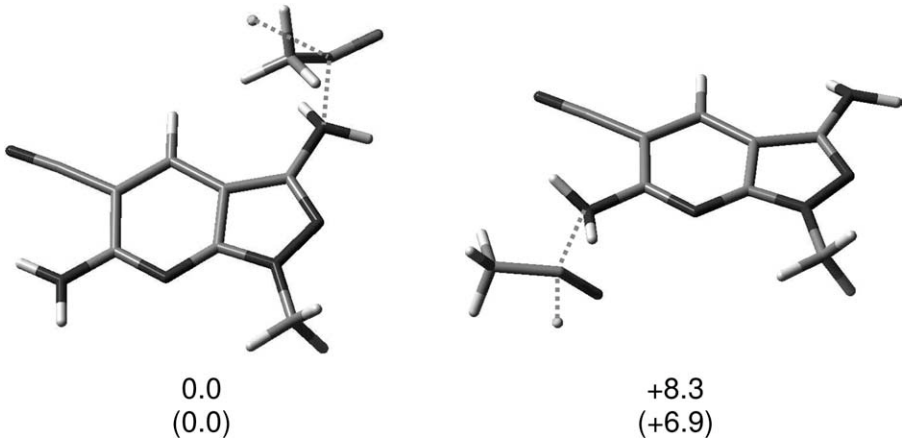


Figure 4. Transition structures for the acetylation of **2** at (C3)N (left) and (C6)N (right). Free-energy differences (in kcal mol^{-1}) are shown in the gas phase and in solution (in parenthesis).

the N lone pair. A NBO analysis on **2**, **3**-1*H* and the unsubstituted pyrazole[3,4-*b*]pyridine (Chart 5, values in blue) reveals that for the cyano derivatives the lone-pair orbital at (C6)N is less populated, and hence less prone to act as nucleophile, than (C3)N because of a higher delocation on the aromatic system. This induces a higher planarization of the amino group at C6 (Chart 5, values in parenthesis). Conversely, the absence of the electron-withdrawing nitrile substituent allows a more populated lone-pair orbital at (C6)N, in accordance with a decreased *N*-planarization. Therefore, the regioselectivity could be modulated by a careful choice of substituents [29].

EXPERIMENTAL

Melting points were determined on a microscope type apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at rt at 300, 400, or 500 MHz and at 75, 100, or 125 MHz. The assignment of chemical shifts is based on standard NMR experiments (^{1}H, ^{13}C-DEPT, ^{1}H,^{1}H-COSY, HSQC, HMBC). In the NMR spectra, values with (*) can be interchanged. Two-dimensional [^{1}H, ^{1}H] NMR experiments (NOESY) were carried out with the following parameters: a delay time of 1 s, a spectral width of 3000 Hz in both dimensions, 4096 complex points in t2 and 4 transients for each of 256 time increments, and linear prediction to 512. The data were zero-filled to 4096 × 4096 real points. NOESY experiments were acquired with a mix time of 300 ms. Mass spectra were recorded on a GC/MS spectrometer with an API-ES ionization source. Elemental analyses were performed at CQO (CSIC, Spain). TLC was performed on silica F254 and detection by UV light at 254 nm or by charring with ninhydrin, anisaldehyde, or phosphomolybdic-H_2SO_4 dyeing reagents. Where anhydrous solvents were needed, they were purified following the usual procedures. Column chromatography was performed on silica gel 60 (230 mesh).

Acetylation of 3,6-diamino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (1)

Method A. A solution of compound **1** [8] (100 mg, 0.57 mmol) in Ac_2O (4 mL, 26.52 mmol, 70 equiv) was heated at 144°C for 21 h. The Ac_2O in excess was removed, and the crude submitted to flash chromatography eluting with mixtures of CH_2Cl_2/MeOH from 1 to 4% to give *N*,*N*′-(5-cyano-3a,7a-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-3,6-diyl)diacetamide (**17**) (13 mg, 8%) [mp 294–296°C; IR (KBr) ν 3434, 3306, 32470, 2928, 1685, 1673, 1611, 1584, 1517, 1430, 1395, 1246, 1011 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 13.62 (s, 1H, N1H), 10.96 (s, 1H, C3NHAc), 10.79 (s, 1H, C6NHAc), 8.89 (s, 1H, H4), 2.12 (s, 6H, 2xCOCH$_3$); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 169.2 (2xN*C*OCH3), 168.4 (C6), 150.8 (C7a), 141.5 (C3),* 141.1 (C4),* 116.9 (CN), 104.7 (C3a), 97.2 (C5), 23.0

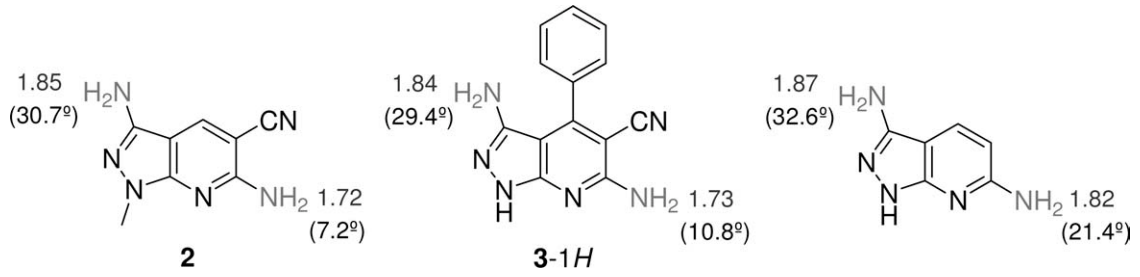


Chart 5. NBO analysis on 2,3-1*H* and the unsubstituted pyrazole[3,4-*b*]pyridine.

and 22.8 (2xNCO*C*H3); MS (ES): $[M + 1]^+$ 259.3, $[M + Na]^+$ 281.2, $[2M + Na]^+$ 539.5. Anal. Calcd. for $C_{11}H_{10}N_6O_2$: C, 51.16; H, 3.90; N, 32.54; found C, 51.29; H, 4.05; N, 32.81.

Method B. A solution of compound **1** (100 mg, 0.57 mmol) in Ac_2O (4 mL, 26.52 mmol, 70 equiv) was stirred at rt for 5 days. The Ac_2O in excess was evaporated. The crude was washed with water and ethanol to give a solid (84 mg) that was purified by chromatography eluting with $MeOH/CH_2Cl_2$ mixtures (from 1, 2 to 4%) affording compounds **17** [8 mg, 5% (10%)], **18** [15 mg, 12% (21%)], and unreacted precursor **1** (57 mg). The mother liquors were concentrated and recrystallized from ethanol to give compound **19** [20 mg, 14%, (24%)]. **18**: mp 253–256°C; IR (KBr) ν 3442, 2219, 1686, 1621, 1582, 1406, 1027 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ 12.66 (s, 1H, NH1), 10.69 [s, 1H, C(3)NHCOCH$_3$], 8.55 [s, 1H, 1CH (H4)], 6.95 (s, 2H, NH_2), 2.12 (s, 3H, NCOCH$_3$); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 168.2 (NH*C*OCH$_3$), 158.3 (C6), 152.6 (C7a), 141.7 (C4), 141.1 (C3), 117.7 (CN), 100.2 (C3a), 86.4 (C5), 22.8 (NHCO*C*H$_3$); MS (ES): $[M + 1]^+$ 217.1, $[M + Na]^+$ 239.3, $[2M + Na]^+$ 455.1. Anal. Calcd. for $C_9H_8N_6O$: C, 50.00; H, 3.73; N, 38.87; found C, 49.74; H, 3.54; N, 38.66. **19**: mp 244–246°C; IR (KBr) ν 3471, 3327, 2212, 1671, 1619, 1597, 1426, 1376, 1318, 1141 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 11.08 (s, 1H, NH), 8.63 (s, 1H, H4), 7.54 (s, 2H, NH_2), 2.67 [s, 3H, NCOCH$_3$)], 2.11 (s, 3H, NCOCH$_3$); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 169.1 [N(3)H*C*OCH$_3$], 166.9 [N(1)H*C*OCH$_3$], 159.4 (C6)*, 152.8 (C7a)*, 144.0 (C3), 141.9 (C4), 116.7 (CN), 103.3 (C3a), 88.3 (C5), 24.6 [N(1)HCO*C*H$_3$], 23.0 [N(3)HCO*C*H$_3$]; MS (ES): $[M + 1]^+$ 259.3, $[M + Na]^+$ 281.2, $[2M + Na]^+$ 539.5. Anal. Calcd. for $C_{11}H_{10}N_6O_2$: C, 51.16; H, 3.90; N, 32.54; found C, 50.98; H, 3.81; N, 32.38.

Acetylation of 3,6-diamino-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (2). A solution of compound **2** (100 mg, 0.53 mmol) in Ac_2O (2.5 mL, 26.52 mmol, 70 equiv) was stirred at rt for 3 h. The mixture was cooled at 0°C, the solid was filtrated, washed with cold ethanol, and recrystallized to give *N*-(6-amino-5-cyano-1-methyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)acetamide (**20**) (100 mg, 82%) as a white solid: mp 246–249°C; IR (KBr) ν 3429, 3332, 3222, 3128, 3086, 2217, 1658, 1617, 1586, 1443, 1276 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 10.74 (s, 1H, N*H*COCH$_3$), 8.57 (s, 1H, H4), 7.08 (s, 2H, C6NH$_2$), 3.70 (s, 3H, NCH$_3$), 2.07 (s, 3H, NHCOC*H*$_3$); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 168.1 (CO), 158.3 (C6), 150.7 (C7a), 142.1 (C3), 140.0 (C4), 117.6 (CN), 100.3 (C3a), 86.4 (C5), 32.8 (NCH$_3$), 22.9 (CH$_3$); MS (ES) $[M + 1]^+$ 231.0; $[M + Na]^+$ 253.0. Anal. Calcd. for $C_{10}H_{10}N_6O.1/2H_2O$: C, 50.20; H, 4.63; N, 35.13; found C, 50.49; H, 4.61; N, 34.65.

3,6-Diamino-4-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (3). A solution of 2-amino-6-chloro-4-phenylpyridine-3,5-dicarbonitrile [11] (100 mg, 0.39 mmol) and hydrazine hydrate (80 μL, 1,57 mmol, 4.0 equiv) in DMF (4 mL, 5mL/mmol) was warmed at 153°C for 1 h until complete reaction (TLC analysis). The mixture was cooled, the solid formed and filtered, washed with water/ethanol, dried, and purified by column chromatography eluting with CH_2Cl_2/MeOH (4%) to give compound **3** (70 mg, 74%): mp 282–283°C; IR (KBr) ν 3498, 3334, 3230, 2931, 1725, 1629, 1571, 1540, 1445, 1269, 1223, 1077 cm^{-1}; ^1H NMR (DMSO, 300 MHz): δ 11.88 [s, 1H, N(1)H], 7.58–7.50 (m, 5H, aromatic), 6.79 [s, 2H, C(6)NH$_2$], 4.26 [s, 2H, C(3)NH$_2$]; ^{13}C NMR (DMSO, 75 MHz): δ 159.9 (C6), 153.1 (C4), 152.1 (C3), 148.9 (C7a), 134.7 (C1′), 130.3 (C4′), 129.4 [2C (C2′,C6′)], 128.9 [2C (C3′,C5′)], 118.0 (CN), 98.5 (C3a), 85.1 (C5); MS (ES): $[M + 1]^+$ 251.3. Anal. Calcd. for $C_{13}H_{10}N_6$: C, 62.39; H, 4.03; N, 33.58; found C, 62.21; H, 4.08; N, 33.58.

Acetylation of 3,6-diamino-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (3)

Method A. A solution of compound **3** (100 mg, 0.4 mmol) in Ac_2O (2.5 mL, 28 mmol, 70 equiv) was stirred at 0°C for 20 h. Then, the solid was filtered, washed with water/ethanol, and recrystallized from ethanol to give compound **21** (29 mg, 25%). **21**: mp 221–223°C; IR (KBr) ν 3471, 3316, 3194, 2212, 1720, 1621, 1590, 1575, 1430, 1382, 1291 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ.7.61–7.50 (m, 5H, Ph), 7.40 (s, 2H, NH_2), 4.82 (s, 2H, NH_2), 2.58 (s, 3H, OCCH$_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 166.5 (N*C*OCH$_3$), 160.3 (C6)*, 152.7 (C7a)*, 151.5 (C4), 150.0 (C3), 132.9, 130.1, 129.0, 128.2 (aromatic, C_6H_5), 116.0 (CN), 101.3 (C3a), 87.6 (C5), 24.5 (NCO*C*H$_3$); MS (ES): $[M + 1]^+$ 293.2, $[M + Na]^+$ 315.2, $[2M + Na]^+$ 607.5. Anal. Calcd. for $C_{15}H_{12}N_6O.1/2H_2O$: C, 59.79; H, 4.35; N, 27.89; found C, 59.83; H, 4.50; N, 28.30.

Method B. A solution of compound **3** (75 mg, 0.3 mmol) in Ac_2O (2 mL) was refluxed for 6 h to give after column chromatography [hexane/ethyl acetate (6/4, 5/5, 4/6)] *N*-(6-acetamido-5-cyano-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)-*N*-acetylacetamide (**22**) (35 mg, 30%) and *N*,*N*′-(5-cyano-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-3,6-diyl)bis(*N*-acetylacetamide) (**23**) (50 mg, 40%). **22**: mp 183–185°C; IR (KBr) ν 3060, 3015, 2222, 1746, 1587, 1368, 1231, 1027 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ 14.46 [s, 1H, N(1)H], 10.98 [s, 1H, C(3)N*H*COCH$_3$)], 7.55–7.34 [m, 5H, aromatic), 2.15 [C(3)NHCOC*H*$_3$)], 1.91 [s, 6H, 2xC(6)NCOCH$_3$]; ^{13}C NMR (DMSO-d_6, 125 MHz): δ 171.6 [2x(C3)N*C*OCH$_3$], 169.5 [(C(6)N*C*OCH$_3$], 152.3 (C6), 151.2 (C7a)*, 150.9 (C3)*, 140.1 (C4), 132.4 (C1′), 129.9 (C4′), 128.6 [2C (C2′,C6′)], 128.1 [2C (C3′,C5′)], 115.4 (CN), 106.5 (C3a), 100.0 (C5), 25.5 [2xC(6)NCO*C*H$_3$], 23.1 [C(3)NCO*C*H$_3$]; MS (ES): $[M + 1]^+$ 377.2, $[M + Na]^+$ 399.2; $[2M + Na]^+$ 775.7. Anal. Calcd. for $C_{19}H_{16}N_6O_3$: C, 60.63; H, 4.28; N, 22.33; found C, 60.54; H, 4.39; N, 22.08. **23**: mp 137–139°C; IR (KBr) ν 3009, 2929, 2855, 2230, 1730, 1589, 1369, 1229, 1029 cm^{-1}; ^1H NMR (DMSO, 300 MHz) δ 14.93 [s, 1H, N(1)H], 7.60–7.43 (m, 5H, aromatic), 2.35 (s, 6H, 2xNCOCH$_3$), 1.95 (s, 6H, 2xNCOCH$_3$); MS (ES): $[M + 1]^+$ 419.2; $[M + Na]^+$ 441.2; $[2M + Na]^+$ 859.7. Anal. Calcd. for $C_{21}H_{18}N_6O$: C, 60.28; H, 4.34; N, 20.09; found C, 60.04; H, 4.18; N, 19.95.

Reaction of 2-chloro-6-methoxy-4-phenylpyridine-3,5-dicarbonitrile (24) hydrazine hydrate. A solution of compound **24** [12] (269 mg, 1 mmol) and hydrazine hydrate (0.1 mL, 2 mmol, 2 equiv) was refluxed in ethanol (20 mL) for 20 h until complete reaction (TLC analysis). The mixture was cooled at 0°C, water was added, and the solid was filtered, washed with water, and purified by chromatography eluting with CH_2Cl_2/MeOH (from 0.5 to 1%) to give compounds 4-phenyl-1,7-dihydrodipyrazolo[3,4-*b*:4′,3′-e]pyridine-3,5-diamine (**25**) (13 mg, 5%) and 3-amino-6-methoxy-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**4**) (95 mg, 58%): mp 248–250°C; IR (KBr) ν 3489, 3391, 3225, 3032, 2938, 2216, 1596, 1313, 1157 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 12.63 [s,

1H, N(1)H], 7.61–7.53 (m, 5H, Ph), 4.49 (s, 2H, NH_2), 4.01 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6,75 MHz): δ 164.0 (C6), 153.4 (C4), 150.9 (C3)*, 149.3 (C7a)*, 133.8, 130.7, 129.6, 129.1 (aromatic, C_6H_5), 116.5 (CN), 100.3 (C3a), 88.2 (C5), 55.3 (OCH_3); MS (ES): [M + 1]+ 266.0, [M + Na]+ 288.0, [2M + Na]+ 553.3. Anal. Calcd. for $C_{14}H11N_5O$: C, 63.39; H, 4.18; N, 26.40; found C, 63.15; H, 4.41; N, 26.37. **25**: mp 328–330°C; IR (KBr) ν 3428, 3249, 3037, 2948, 2593, 1597, 1099 cm^{-1}; ^1H NMR (DMSO, 300 MHz): δ 11.67 (s, 2H, 2 NH), 7.63–7.46 (m, 5H, aromatic), 4.24 (s, 4H, 2 NH_2); ^{13}C NMR (DMSO, 75 MHz): δ 153.4 (2C, C7a, C8a), 148.1 (2C, C3, C5), 139.3 (C4), 133.4 (C1′), 129.3 (C4′), 129.3 [C, (C2′,C6′)], 128.8 [C, (C3′,C5′)], 101.5 (2C C3a, C4a); MS (ES): $[M + 1]^+$ 266.2. Anal. Calcd. for $C_{13}H_{11}N_7$: C, 58.86; H, 4.18; N, 36.96; found: C, 58.79; H, 4.36; N, 36.72.

1-Acetyl-3-amino-6-methoxy-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (26). A solution of **4** (80 mg, 0.3 mmol) in Ac_2O (4 mL, 3.9 mmol, 13 equiv) was stirred at 0°C for 16 h and at rt for 4 h. Alter evaporation of the excess of Ac_2O, the crude was purified by column chromatography, eluting with CH_2Cl_2/MeOH 1% to afford compound **26** (63 mg, 84%): mp 236–238°C; IR (KBr) ν 3485, 3265, 3181, 2225, 1715, 1624, 1589, 1571, 1393, 1349, 1153 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 7.65–7.57 (m, 5H, Ph), 5.10 (s, 2H, NH_2), 4.10 (s, 3H, OCH_3), 2.69 (s, 3H, $NCOCH_3$); ^{13}C NMR (DMSO-d_6,75 MHz): δ 167.8, 164.9, 153.4, 151.0, 150.7, 132.7, 131.2, 129.8, 129.0 (aromatic, C_6H_5), 115.4 (CN), 104.8 (C3a), 92.5 (C5), 55.6 (OCH_3), 25.3 ($NCOCH_3$); MS (ES): $[M + 1]^+$ 308.3, $[M + Na]^+$ 370.2, $[2M + Na]^+$ 637.5. Anal. Calcd. for $C_{16}H_{13}N_5O.1/2H_2O$: C, 60.75; H, 4.46; N, 22.14; found: C, 60.59; H, 4.21; N, 22.07.

Reaction of *N*-acetyl-*N*-(3,5-dicyano-6-methoxy-4-phenylpyridin-2-yl)acetamide (29) with hydrazine hydrate. A solution of compound **29** (50 mg, 0.15 mmol) and hydrazine hydrate (20 μL, 0.22 mmol, 1.5 equiv) in DMF (5 mL) was refluxed (153°C) for 30 min until complete reaction. Then, the excess of DMF was removed, AcOEt was added, and washed with water. The organic phase was dried, filtered, and evaporated to give a solid that was submitted to chromatography eluting with (hexane/EtOAc, from 8/2 to 1/1) to give compounds **27** (23 mg, 61%), **30** [11] (2 mg, 6%), and **3** (10 mg, 27%).

2,6-Diamino-4-phenylpyridine-3,5-dicarbonitrile (30). In a 30-mL glass tube equipped with septa was placed a solution of 6-amino-2-chloro-4-phenylpyridine-3,5-dicarbonitrile (**32**) (0.382 g, 1.5 mmol) in 10 mL of DMF. The reaction mixture was stirred for 30 s before the irradiation to homogenize the solution and then exposed to MWI 250W at 180°C during 3 min. After completion showed by TLC (hexane/AcOEt, 3/2), the reaction mixture was diluted with water, and the precipitate was filtered and washed with water. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 25/1 to 10/1, v/v) to yield product **30** (155 mg, 44%), which showed spectroscopic data in good accord with those reported in literature [11].

6-Amino-2-chloro-4-phenylpyridine-3,5-dicarbonitrile (32). To a solution of trimethylorthobenzoate (**31**) (1.82 g, 0.01 mol) in pyridine (5 mL) was added malononitrile (1.32 g, 0.02 mol, 2 equiv). The mixture was heated at 110°C for 7 h. After cooling, concentrated aqueous hydrochloric acid (10 mL) was added, and the mixture was heated at 100°C for 2.5 h. After cooling to rt, the mixture was diluted with water and filtered to afford compound **32** (1.0 g, 40%), which showed spectroscopic data in agreement with those reported in literature [11].

3,6-Diamino-1-methyl-4-phenyl-1H-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (5). A mixture of 6-amino-2-chloro-4-phenylpyridine-3,5-dicarbonitrile (**32**) (254 mg, 1 mmol) and methylhydrazine (0.11 mL, 1.1 mmol, 1.1 equiv) in DMF (10 mL) was warmed at 153°C for 5 min. The mixture was cooled at rt; the solid was filtered and recrystallized from ethanol to give precursor **5** (211 mg, 80%): mp 279–281°C; IR (KBr) ν 3477, 3427, 3379, 3323, 3191, 2201, 1654, 1589, 1573, 1561, 1405, 1204 cm^{-1}; ^1H NMR (DMSO, 300 MHz): δ 7.58–7.48 (m, 5H, C_6H_5), 6.90 [s, 2H, C(6)NH_2], 4.36 [s, 2H, C(3)NH_2], 3.59 (s, 3H, NCH_3); ^{13}C NMR (DMSO, 75 MHz): δ 158.2 (C6), 151.7 (C4), 150.5 (C7a), 147.5 (C3), 133.8 (C1′), 129.7 (C4′), 128.8 [2C (C2′,C6′)], 128.2 [2C (C3′,C5′)], 117.3 (CN), 98.0 (C3a), 84.3 (C5), 32.4 (NCH_3); MS (ES): $[2M]^+$ 528.7, $[2M - 1]^+$ 527.7. Anal. Calcd. for $C_{14}H_{12}N_6$: C, 63.62; H, 4.58; N, 31.80; found C, 63.60; H, 4.65; N, 32.04.

***N*-(6-Amino-5-cyano-1-methyl-4-phenyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl)acetamide (33).** A solution of compound **5** (100 mg, 0.38 mmol) in Ac_2O (2.5 mL, 26.52 mmol, 70 equiv) was stirred at rt for 8 h. The crude was cooled at 0°C, and the precipitate was filtered, washed with EtOH, and submitted to chromatography (AcOEt) to give compound **33** (24 mg, 21%): mp 249–251°C; IR (KBr) ν 3489, 3414, 3330, 3263, 3052, 2218, 1664, 1625, 1590, 1571, 1518, 1444, 1399, 1379, 1259, 1196 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.49 (s, 1H, NH), 7.58–7.48 (m, 5H, C_6H_5), 7.12 (s, 2H, NH_2), 3.78 (s, 3H, NCH_3), 1.42 (s, 3H, $COCH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 169.1 ($NCOCH_3$), 159.8 (C6), 152.7 (C4), 151.7 (C7a), 139.0 (C3), 133.9 (C1′), 129.9 (C4′), 129.3 (2C, C2′,C6′), 128.6 (2C, C3′,C5′), 117.5 (CN), 102.9 (C3a), 88.3 (C5), 33.8 (NCH_3), 22.51 ($NCOCH_3$); MS (ES): $[M + 1]^+$ 307.1. Anal. Calcd. for $C_{14}H_{12}N_6$: C, 62.74; H, 4.61; N, 27.44; found C, 62.96; H, 4.68; N, 27.31.

3,6-Diamino-2-phenyl-2*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (6). A solution of precursor **34** [8] (100 mg, 0.56 mmol) and *N*-phenylhydrazine (82 μL, 0.84 mmol, 1.5 equiv) in DMF (2 mL, 5mL/mmol) was warmed at 153°C for 1 h until complete reaction (TLC analysis). The mixture was cooled at 0°C; the solid was recovered and submitted to chromatography eluting with CH_2Cl_2/MeOH (from 0.5 to 2%) to give product **6** (91 mg, 70%): mp 306–308°C; IR (KBr) ν 3467, 3353, 3299, 3154, 2211, 1620, 1596, 1455, 1343 cm^{-1}; ^1H NMR (DMSO, 300 MHz): δ 8.36 [s, 1H, H4), 7.57–7.37 (m, 5H, aromatic), 6.95 [s, 2H, (C3)NH_2], 6.47 [s, 2H, (C6)NH_2]; ^{13}C NMR (DMSO, 75 MHz): δ 157.9 (C6)*, 156.5 (C7a)*, 143.0 (C3), 140.0 (C4), 138.2 (C1′), 129.3 (C3′,C5′), 127.4 (C4′), 123.9 (C2′,C6′), 118.5 (CN), 96.9 (C3a), 84.6 (C5); MS (CI): *m/z* 251 [M^+, 100], 234 [M^+-NH_2, 8], 92 (14), 77(21). Anal. Calcd. for $C_{13}H_{10}N_6$: C, 62.39; H, 4.03; N, 33.58; found C, 62.10; H,4.32; N, 33.31.

***N*,*N*′-(5-Cyano-2-phenyl-2*H*-pyrazolo[3,4-*b*]pyridine-3,6-diyl)diacetamide (35).** A solution of compound **6** (100 mg, 0.4 mmol) in Ac_2O (2.5 mL, 28 mmol, 70 equiv) was heated at 144°C for 40 min. The mixture was cooled at rt, the solvent was removed under vacuo, and the crude submitted to chromatography (CH_2Cl_2/MeOH from 0.1 to 2%) to give product **35** (28 mg, 21%): mp 229–230°C; IR (KBr) ν 3467, 3353, 3299, 3154, 2211, 1619, 1596, 1454, 1343 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 10.82 (s, 1H, N*H*$COCH_3$), 10.68 (s, 1H, N*H*$COCH_3$), 8.84 (s, 1H, H4), 7.69–7.57 (m, 5 H, aromatic), 2.12 (s, 3H, NHCOC*H*$_3$), 2.08 (s, 3H, NHCOC*H*$_3$); ^{13}C NMR

(DMSO-d_6, 75 MHz): δ 169.8 (N*C*OCH$_3$), 169.4 (NC*O*CH$_3$), 154.6 (C6)*, 151.4 (C7a), 141.4 (C4), 137.8 (C1′), 131.8 (C3), 129.5 (2C, C3′,5′), 129.4 (C4′), 124.8 (2C, C2′,C6′), 116.7 (CN), 106.0 (C3a), 100.0 (C5), 22.9 (NCO*C*H$_3$), 22.7 (NCO*C*H$_3$); MS (ES): $[M + 1]^+$ 335.2, $[M + Na]^+$ 357.2, $[2M + Na]^+$ 691.5. Anal. Calcd for $C_{17}H_{14}N_6O$: C, 61.07; H, 4.22; N, 25.14; found C, 60.85; H, 4.36; N, 24.98.

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[29] The inhibition of a panel of protein kinases by the new compounds synthesized here is being evaluated by Dr. Francisco Wandosell (CBM, CSIC, Madrid, Spain), and will be reported elsewhere.