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A Mild Approach to the Synthesis of 4-Amino-8-(arylamino)pyrimido[5,4-*d*]-pyrimidine 3-Oxides

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The reaction of benzylhydroxylamine with 6-cyanopurines leads to the formation of 7-benzyloxy-8-imino-7,8-dihydropyrimido[5,4-*d*]pyrimidines. The hydrochloride of these compounds, isolated upon addition of aqueous hydrochloric acid, is a convenient precursor of the pyrimido[5,4-*d*]-pyrimidine *N*-oxides when a suspension of the salt is refluxed in ethanol or acetonitrile. Refluxing a solution of the same salt in ethanol, leads to the Dimroth-rearranged product.

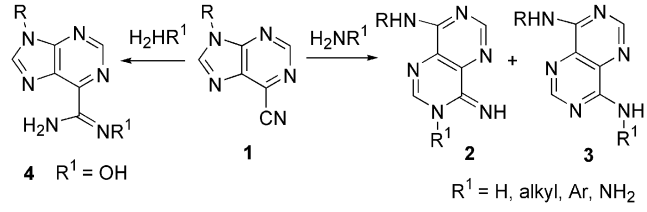
Introduction

Pyrimido[5,4-*d*]pyrimidine is a fused heterocyclic system of interest in the context of drug development because of its structural similarity to purines. Dipyridamole, a 2,4,6,8-tetrasubstituted pyrimido[5,4-*d*]pyrimidine is marketed nowadays as a coronary vasodilator.[1,2] Many other derivatives are biologically active as antiviral and antitumour agents,[3–13] as inhibitors of tyrosine kinase,[7,9,12] or as bronchodilators and antiallergic agents.[14] Several derivatives were synthesised from the 2,4,6,8-tetrachloro derivative by nucleophilic substitution of the chlorine atoms by the desired amine.[5,10,11,15] They were also synthesised from substituted pyrimidines by reaction with convenient electrophiles or nucleophiles.[12,16,17] The 6-cyanopurines were also used as precursors of the pyrimido[5,4-*d*]pyrimidines by reaction with an amine.[3,4,6,18–21] To the best of our knowledge, pyrimido[5,4-*d*]pyrimidine *N*-oxides were never reported in the literature. However, pyrimidine *N*-oxides are known. Most of them were obtained by *N*-oxidation of the appropriate pyrimidines, usually in low yields, using peroxides or peracids.[1,22–25] Pyrimidine *N*-oxides were also prepared from carboxamide oximes by reaction with an appropriate electrophile[26–28] and from 1,2,4-oxadiazoles by ring rearrangement/transformation.[29–33]

Herein we report a simple, mild and efficient method to synthesise the first example of pyrimido[5,4-*d*]pyrimidine *N*-oxides and the corresponding precursor.

Results and Discussion

In recent years, our research group developed a mild and efficient approach to 9-aryl- or 9-alkyl-6-cyanopurines **1**[34,35] and studied their reactivity with amines. Our studies[21,34] showed that nucleophilic attack of the amine to C8 of the purine ring was the first step of the cascade process leading to the formation of pyrimido-pyrimidine **2** through an ANRORC type mechanism (Scheme 1). Compounds **2** were isolated in very good yield, although in some cases the rearrangement product **3** could also be identified as a minor product. When hydroxylamine was used as nucleophile the reaction followed a different pathway leading to the 6-amidinopurine **4** as the only product. These results suggest that the nature of the amine nucleophile is decisive for the course of the reaction.



Scheme 1. Reaction of 6-cyanopurine with nucleophilic amines.

The present work reports the reaction of 6-cyanopurines **1** with benzylhydroxylamine ($R^1 = OCH_2Ph$), initially aiming at the formation of pyrimido-pyrimidines **2** and **3**, generated upon Dimroth rearrangement of **2**, or 6-amidinopurine **4** ($R^1 = OCH_2Ph$). It also describes the chemospecific reaction conditions to convert the pyrimido[5,4-*d*]pyrimidine **2** into the *N*-oxide derivative **5** or in the rearranged product **6**.

When purine **1c** was treated with 7 equiv. of benzylhydroxylamine, in the presence of a catalytic amount of DBU, using diethyl ether as solvent, the poor solubility of the reagents led to an exceptionally long reaction time. After 35 d at room temperature, the solid suspension was filtered and the ^1H NMR of the product mixture showed that

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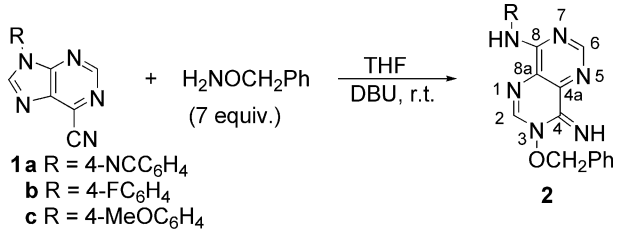
the purine **1c** was still the major compound, together with two new products, one of them identified as compound **2c** (Table 1, entry1). To overcome the solubility problem, THF was selected as solvent (Table 1). A careful control of the reaction conditions allowed the isolation of pyrimido-pyrimidine **2c** in 83% yield after 4 d at room temperature (Table 1, entry 5). The optimized reaction conditions were applied to the reaction of purines **1a**, **b** and **d** with benzylhydroxylamine and the derivatives **2a**, **b** and **d** were isolated in very good yield (Table 1, entries 6–8).

Table 1. Synthesis of pyrimido[5,4-*d*]pyrimidines **2** from 6-cyanopurines **1**.



1a R = 4-NCC_6H_4
b R = 4-FC_6H_4
c R = 4-$MeOC_6H_4$
d R = Ph

2

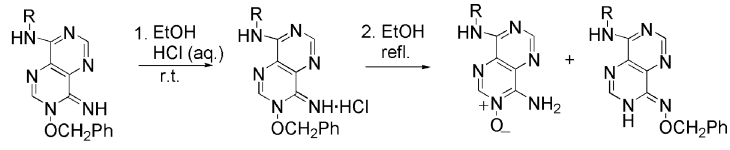
Entry	1	Reaction conditions	Product	% Yield
1	**c**	Diethyl ether, 35 d, r.t.	**2c**	[a]
2	**c**	THF(4 mL), DBU (75 μL), 9 d, r.t.	**2c**	40
3	**c**	THF(8 mL), DBU (25 μL), 6.5 d, r.t.	**2c**	60
4	**c**	THF(4 mL), DBU (20 μL), 9 d, r.t.	**2c**	68
5	**c**	THF(4 mL), DBU (25 μL), 4 d, r.t.	**2c**	83
6	**a**	THF(4 mL), DBU (25 μL), 22 h, r.t.	**2a**	89
7	**b**	THF(4 mL), DBU (25 μL), 22 h, r.t.	**2b**	84
8	**d**	THF(4 mL), DBU (25 μL), 26 h, r.t.	**2d**	75

[a] Product **2c** was detected by ^{1}H NMR of the reaction mixture.

When a concentrated aqueous hydrochloric acid solution was added to an ethanolic suspension of compound **2a**, an exothermic reaction occurred generating a yellow solid. This product was identified as the hydrochloride of **2a** (Table 2, entry 1). The ^{1}H NMR spectrum was very similar to that of the starting material, but the signals at δ = 10.95 ppm (1 H, 4-NH) and δ = 10.82 ppm (2 H, 4-NH + 8-NH) confirmed the formation of the salt **2a**·HCl.

In a different experiment, the reaction conditions leading to the hydrochloride of **2a** were reproduced, and the suspension was refluxed for 90 min (Table 2, entry 2). A light yellow solid was isolated and ^{1}H NMR showed it to be a mixture of two products. The minor component was identified as the rearranged product **6a** (10%). Besides the benzylic group at δ = 5.16 ppm, two signals at δ = 9.9 ppm and 11.6 ppm could be assigned to the acidic 8-NH and 3-NH protons, respectively. The major compound showed an aromatic pyrimido-pyrimidine core ($\delta_{CH} \geq 8.7$ ppm, 2-H and 6-H) and the benzylic group was no longer present. The arylamine substituent and an amino group (δ = 8.97 and

Table 2. Reaction of of pyrimido[5,4-*d*]pyrimidines **2** with aqueous hydrochloric acid.



2 a R = 4-NCC_6H_4
b R = 4-FC_6H_4
c R = 4-$MeOC_6H_4$
d R = Ph

2·HCl **5** **6**

Entry	Reagent	Reaction conditions	Product	% Yield
1	**2a**	1. EtOH (4 mL), **2** (0.38 mmol), HCl (21 equiv.); 5 min, r.t.	**2a**·HCl	96
2	**2a**	1. EtOH (4 mL), **2** (0.40 mmol), HCl (12 equiv.); 15 min, r.t. 2. EtOH, reflux, 90 min	**5a** + **6a**	90:10[a]
3	**2c**	1. EtOH (2 mL), **2** (0.40 mmol), HCl (13 equiv.), 20 min, r.t. 2. EtOH, reflux, 30 min	**5c** + **6c**	85:15[a]
4	**2b**	1 EtOH (4 mL), **2** (0.27 mmol), HCl (17 equiv.), 5 min, r.t. 2. EtOH, reflux, 30 min	**5b** + **2b**·HCl + **6b**	53:15:32[a]
5	**2d**	1. EtOH (4 mL), **2** (0.87 mmol), HCl (6.9 equiv.), 10 min, r.t. 2. EtOH, reflux, 60 min	**5d** + **6d**	74:26[a]
6	**2c**	1. EtOH (2 mL), **2** (0.32 mmol), HCl (15 equiv.), 5 min, r.t. 2. EtOH, reflux, 60 min	**5c** + **2c**·HCl + **6c**	19:13:68[a]
7	**2c**	1. EtOH (4 mL), **2** (0.35 mmol), HCl (17 equiv.), 15 min, r.t. 2. EtOH, reflux, 35 min	**5c** + **6c**	20:80[a]
8	**2c**	1. EtOH (4 mL), **2** (0.79 mmol), HCl (7.6 equiv.), 10 min, r.t.	**2c**·HCl	92
9	**2c**·HCl	2. EtOH (20 mL), reflux, 105 min	**5c**	73
10	**2b**	1. EtOH (4 mL), **2** (0.33 mmol), HCl (15 equiv.), 5 min, r.t. 2. EtOH, reflux, 60 min	**6b**	80
11	**2b**·HCl	2. EtOH (15 mL), reflux, 75 min	**5b** + **6b**	80:20[a]

[a] Ratio of products based on ^{1}H NMR of the mixture.

8.28 ppm) were also identified. Structure **5a** was proposed for this compound, on the basis of analytic and spectroscopic data.

A detailed study on the reaction of compounds **2b**–**d** with aqueous hydrochloric acid/ethanol was performed (Table 2). The use of a large volume of solvent and/or hydrochloric acid solution improved the yield of the rearranged product **6** (entries 3, 6 and 7). A similar situation was observed when the hydrochloride of **2** was more soluble in the reaction mixture (entries 5, 10). When the hydrochloride of **2c** was isolated and then refluxed as a suspension in ethanol, where it was practically insoluble, compound **5c** was isolated exclusively (entry 9).

In order to understand the role of water and hydrochloric acid in the reaction pathway, an aqueous suspension of the hydrochloride of **2b** was stirred at room temperature for 24 h. The yellow colour of the salt gradually faded leading to a white solid identified as **2b** (85%). This result shows that, in aqueous solution, the pyrimido-pyrimidine **2** and its hydrochloride are present as an equilibrium mixture, and the concentration of HCl in the reaction mixture may determine the concentration of each species.

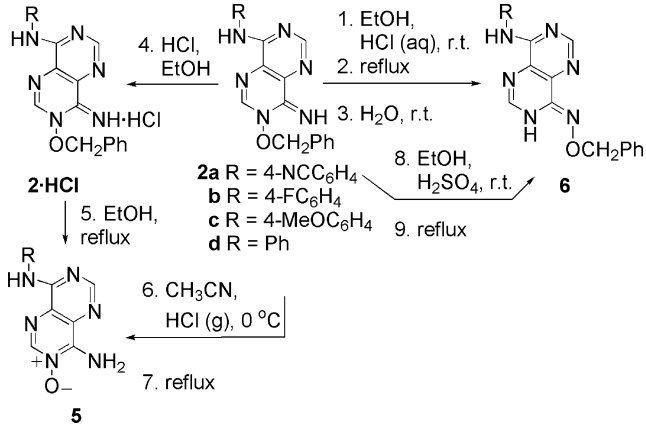
This information enabled us to optimize the reaction conditions in order to prepare selectively pyrimido-pyrimidine **6** or the *N*-oxide **5** (Table 3).

A general approach to the synthesis of pyrimido-pyrimidine **6** required the addition of a large excess of aqueous hydrochloric acid solution to a suspension of compound **2** in ethanol. The mixture was refluxed until the starting material was totally consumed (by TLC). The yellow solid that precipitates upon cooling corresponds to compound **6**, which could be contaminated with the corresponding hydrochloride. Neutralization was achieved simply by washing with water and the product was isolated in 82–85% yield (Table 3, entries 1–3). Product **6b** was also obtained from an ethanolic solution of the hydrogeno sulfate of **2b** under reflux conditions (entry 10). The non-aromatic structure of the compound **6** was confirmed by ^{1}H and ^{13}C NMR spectroscopy. The chemical shift registered for C^2-H ($\delta_H \approx 7.64$, $\delta_C \approx 144$ ppm) and the coupling observed between C^2-H and N^3-H ($J \approx 3$ Hz) supports the non-aromatic structure assigned to this pyrimidine ring.

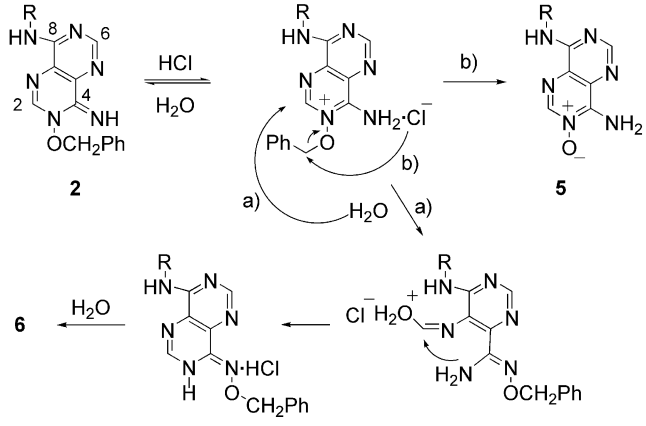
The formation of the *N*-oxide **5** (Table 3) occurred by refluxing the hydrochloride of **2** in a solvent where it was practically insoluble. The hydrochlorides of **2a** and **2b** were prepared by adding aqueous or gaseous hydrochloric acid to an ethanolic suspension of **2a** or **2b**. These salts were isolated, dried and refluxed in absolute ethanol to generate the corresponding *N*-oxide **5a** and **5b** (Table 3, entries 4–7). Compounds **5c** and **5d** were obtained from **2c** and **2d**, using acetonitrile as solvent. Gaseous hydrochloric acid was bubbled for 15 min, at 0 °C, followed by reflux for 15 min to 1 h (Table 3, entries 8, 9).

The mechanism for booth reactions involves the formation of the hydrochloride of pyrimido-pyrimidine **2** (Scheme 2). When this compound is partially soluble in a polar protic solvent, both the anion and cation are solvated and nucleophilic attack of water/ethanol to C2 of the activated pyrimidine ring may occur (pathway a). Ring opening followed by ring closure generates the final product **6**, either as the free base or as the hydrochloride. The acid-base equilibrium can be displaced in water to generate the free base **6**.

Table 3. Optimized reaction conditions to generate selectively the compounds **5** and **6**.



Entry	Reagent	Reaction conditions	Product	% Yield
1	**2b**	1. EtOH, HCl (aq.), r.t., 15 min 2. reflux, 3 h 40 min 3. H_2O, r.t., 20 min	**6b**	82
2	**2c**	1. EtOH, HCl (aq.), r.t., 15 min 2. reflux, 15 min 3. H_2O, r.t., 20 min	**6c**	85
3	**2d**	1. EtOH, HCl (aq.), r.t., 15 min 2. reflux, 25 min 3. H_2O, r.t., 20 min	**6d**	83
4	**2a**	4. EtOH, HCl (aq.), r.t., 15 min	**2a**·HCl	82
5	**2a**·HCl	5. EtOH, reflux, 50 min	**5a**	92
6	**2b**	4. EtOH, HCl (g), 0 °C, 15 min	**2b**·HCl	77
7	**2b**·HCl	5. EtOH, reflux, 3 h 30 min	**5b**	63
8	**2c**	6. CH_3CN, HCl (g), 0 °C, 15 min 7. reflux, 15 min	**5c**	75
9	**2d**	6. CH_3CN, HCl (g), 0 °C, 15 min 7. reflux, 1 h	**5d**	61
10	**2b**	8. EtOH, H_2SO_4, r.t., 5 min 9. reflux, 30 min	**6b**	100



Scheme 2. Mechanism proposed for the reaction.

When the hydrochloride of **2** is insoluble in the solvent, a tight ionic pair is formed (Figure 1). The repulsion between the chloride anion and the non-bonding electrons of the oxygen atom determines their opposite position in the molecule. This geometry favours nucleophilic attack to the benzylic carbon which occurs upon heating, with subsequent elimination of the benzyl group (Scheme 2, pathway b).

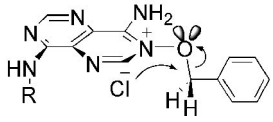


Figure 1. Ionic pair responsible for the formation of the *N*-oxide.

This mechanistic proposal is supported by the perception that the nucleophilicity of the counterion is important for the formation of the *N*-oxide. In a separate experiment, compound **2b** was combined with sulfuric acid in ethanol, and the dihydrogen sulfate of **2b** was isolated and dried. A suspension of this salt in ethanol was refluxed until the starting material was no longer present (17 d) and the solid was filtered and analysed by ^{1}H NMR spectroscopy. Compound **6b** was identified as a minor component in a complex reaction mixture.

Conclusions

The treatment of pyrimido[5,4-*d*]pyrimidines **2** with hydrochloric acid leads to the selective formation of pyrimido[5,4-*d*]pyrimidine **6** upon Dimroth rearrangement if the hydrochloride of **2** is partially soluble in the solvent (water or ethanol) and the reaction mixture is heated under reflux conditions. When the hydrochloride of **2** is insoluble in the solvent (acetonitrile or ethanol) heating causes cleavage of the benzyl group possibly as the alkyl chloride, leading to the regiospecific formation of the pyrimido[5,4-*d*]pyrimidine *N*-oxide **5**.

Experimental Section

General: 9-Substituted 6-cyanopurines **1** were prepared according to previously reported procedures.[34,35] Solvents and other chemicals commercially available were used as shipped. The melting points were determined with a Gallenkamp melting point apparatus and are uncorrected. The reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F_{254} (Merck) with detection by UV light. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unit Plus for solutions in [D$_6$]DMSO [residual [D$_6$]-DMSO (δ_H = 2.49 ppm) or [D$_6$]DMSO (δ_C = 39.5 ppm) as internal standard] at 298 K. IR spectra were recorded with a FT-IR Bomem MB 104 using nujol mulls and NaCl cells. Elemental analyses were performed with a LECO CHNS-932 instrument.

General Procedure for the Synthesis of Pyrimido[5,4-*d*]pyrimidines 2a–d from 1a–d: The *O*-benzylhydroxylamine hydrochloride was neutralised with an aqueous sodium hydroxide solution (1 M) and the solution was then extracted with diethyl ether (4 × 35 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated to give an oil. The oil was dissolved in THF (4 mL), the 6-cyanopurine **1** was added to this solution together with a catalytic amount of DBU (25 μL, 0.18 mmol). The suspension was stirred at room temperature until TLC showed the absence of the starting material. The solid suspension was filtered and washed with ethanol followed by diethyl ether.

4-{[7-(Benzyloxy)-8-imino-7,8-dihydropyrimido[5,4-*d*]pyrimidin-4-yl]amino}benzonitrile (2a): From **1a** (0.83 g, 3.4 mmol) and benzylhydroxylamine (2.95 g, 24 mmol), **2a** was isolated as an off-white solid (1.12 g, 3.0 mmol, 89%); m.p. 211.8–212.2 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.13 (s, 1 H, 8-NH), 8.66 (s, 1 H, 6-H), 8.57 (s, 1 H, 4-NH), 8.21 (s, 1 H, 2-H), 8.19 (d, $^3J_{H,H}$ = 8.7 Hz, 2 H, H$_o$), 7.79 (d, $^3J_{H,H}$ = 8.7 Hz, 2 H, H$_m$), 7.56 (m, 2 H, H$_o$), 7.42 (m, 3 H, H$_{m+p}$), 5.35 (s, 2 H, OCH_2) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 156.21, 154.48, 151.99, 146.15, 142.77, 140.84, 133.69, 132.53, 129.69, 128.96, 128.35, 124.66, 120.90, 118.85, 104.80, 76.88 ppm. IR (nujol mull): $\tilde{\nu}$ = 3314, 3291, 3273, 2223, 1644, 1601, 1585, 1556, 1521 cm^{-1}. $C_{20}H_{15}N_7O$ (369.41): calcd. C 65.02, H 4.10, N 26.55; found C 65.07, H 4.20, N 26.67.

7-(Benzyloxy)-*N*-(4-fluorophenyl)-8-imino-7,8-dihydropyrimido-[5,4-*d*]pyrimidin-4-amine (2b): From **1b** (0.73 g, 3.0 mmol) and benzylhydroxylamine (2.58 g, 21 mmol), **2b** was isolated as an off-white solid (0.92 g, 2.6 mmol, 84%); m.p. 208.7–210.4 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 9.80 (s, 1 H, 8-NH), 8.53 (s, 1 H, 6-H), 8.47 (s, 1 H, 4-NH), 8.18 (s, 1 H, 2-H), 7.86 (m, 2 H, H$_o$), 7.56 (m, 2 H, H$_o$), 7.43 (m, 3 H, H$_{m+p}$), 7.18 (t, $^3J_{H,H}$ = 9.0 Hz, 2 H, H$_m$), 5.35 (s, 2 H, OCH_2) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 159.47 (d, $^1J_{C,F}$ = 239.10 Hz), 156.70, 155.07, 152.34, 146.05, 140.43, 134.83 (d, $^4J_{C,F}$ = 2.55 Hz), 133.89, 130.03, 129.27, 128.62, 124.35, 123.74 (d, $^3J_{C,F}$ = 8.03 Hz), 115.03 (d, $^2J_{C,F}$ = 21.98 Hz), 76.97 ppm. IR (nujol mull): $\tilde{\nu}$ = 3355, 3251, 3065, 3035, 1635, 1613, 1588, 1558, 1533, 1508 cm^{-1}. $C_{19}H_{15}FN_6O$ (362.39): calcd. C 62.97, H 4.18, N 23.20; found C 62.60, H 4.28, N 23.06.

7-(Benzyloxy)-8-imino-*N*-(4-methoxyphenyl)-7,8-dihydropyrimido-[5,4-*d*]pyrimidin-4-amine (2c): From **1c** (0.58 g, 2.3 mmol) and benzylhydroxylamine (1.97 g, 16 mmol), **2c** was isolated as an off-white solid (0.73 g, 1.94 mmol, 83%); m.p. 200.3–201.8 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 9.61 (s, 1 H, 8-NH), 8.48 (s, 1 H, 6-H), 8.44 (s, 1 H, 4-NH), 8.15 (s, 1 H, 2-H), 7.71 (d, $^3J_{H,H}$ = 9.00 Hz, 2 H, H$_o$), 7.56 (m, 2 H, H$_o$), 7.42 (m, 3 H, H$_{m+p}$), 6.91 (d, $^3J_{H,H}$ = 9.00 Hz, 2 H, H$_m$), 5.33 (s, 2 H, OCH_2), 3.74 (s, 3 H, OMe) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 156.59, 155.73, 155.05, 152.19, 145.70, 140.08, 133.82, 131.29, 129.81, 129.06, 128.44, 124.09, 123.32, 113.56, 76.89, 55.15 ppm. IR (nujol mull): $\tilde{\nu}$ = 3449, 3358, 3237, 3053, 1628, 1600, 1579, 1553, 1522, 1509 cm^{-1}. $C_{20}H_{18}N_6O_2$ (374.42): calcd. C 64.15, H 4.86, N 22.45; found C 64.28, H 5.03, N 22.64.

7-(Benzyloxy)-8-imino-*N*-phenyl-7,8-dihydropyrimido[5,4-*d*]pyrimidin-4-amine (2d): From **1d** (0.40 g, 1.8 mmol) and benzylhydroxylamine (1.55 g, 12.6 mmol), **2d** was isolated as an off-white solid (0.46 g, 1.4 mmol, 75%); m.p. 196.0–197.1 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 9.67 (s, 1 H, 8-NH), 8.54 (s, 1 H, 6-H), 8.48 (s, 1 H, 4-NH), 8.18 (s, 1 H, 2-H), 7.86 (d, $^3J_{H,H}$ = 7.80 Hz, 2 H, H$_o$), 7.57 (m, 2 H, H$_o$), 7.42 (m, 3 H, H$_{m+p}$), 7.34 (t, $^3J_{H,H}$ = 7.80 Hz, 2 H, H$_m$), 7.09 (t, $^3J_{H,H}$ = 7.50 Hz, 1 H, H$_p$), 5.35 (s, 2 H, OCH_2) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 156.66, 155.06, 152.31, 146.02, 140.39, 138.44, 133.88, 129.97, 129.21, 128.58, 128.42, 124.36, 123.72, 121.63, 76.93 ppm. IR (nujol mull): $\tilde{\nu}$ = 3445, 3359, 3231, 1676, 1629, 1600, 1579, 1552, 1519 cm^{-1}. $C_{19}H_{16}N_6O$ (344.40): calcd. C 66.26, H 4.69, N 24.40; found C 66.20, H 4.77, N 24.42.

General Procedure for the Synthesis of Pyrimido[5,4-*d*]pyrimidines 6b–d from 2b–d: A concentrated aqueous solution of hydrochloric

acid (16 equiv.) was added to a suspension of **2** in ethanol, kept under magnetic stirring at room temperature. The resulting yellow solution was stirred for 15 min and then was refluxed until TLC showed the absence of the starting material. The reaction mixture was then cooled in an ice bath leading to a yellow solid suspension which was filtered and washed with ethanol followed by diethyl ether. The isolated yellow solid was suspended in water (20 mL) and kept under magnetic stirring until the yellow colour faded, leading to a white solid. The solid was filtered, washed with water and diethyl ether.

8-[(4-Fluorophenyl)amino]pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one *O*-Benzyloxime (6b): From **2b** (0.11 g, 0.30 mmol), **6b** was isolated as a white solid (0.09 g, 0.25 mmol, 82%); m.p. 267.9–269.2 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 11.46 (s, 1 H, 3-H), 9.52 (s, 1 H, 8-NH), 8.34 (s, 1 H, 6-H), 7.88 (m, 2 H, H$_o$), 7.64 (d, $^3J_{H,H}$ = 3.00 Hz, 1 H, 2-H), 7.37 (m, 5 H, Ph), 7.16 (m, 2 H, H$_m$), 5.15 (s, 2 H, OCH$_2$) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 158.17 (d, $^1J_{C,F}$ = 238.50 Hz), 155.40, 154.61, 144.81, 141.04, 138.90, 138.03, 135.07 (d, $^4J_{C,F}$ = 2.65 Hz), 128.26, 127.91, 127.68, 125.26, 123.15 (d, $^3J_{C,F}$ = 7.73 Hz), 114.97 (d, $^2J_{C,F}$ = 22.35 Hz), 75.42 ppm. IR (nujol mull): $\tilde{\nu}$ = 3356, 3264, 3235, 3066, 1635, 1611, 1586, 1549, 1533, 1505 cm^{-1}. $C_{19}H_{15}FN_6O$ (362.39): calcd. C 62.97, H 4.18, N 23.20; found C 62.77, H 4.20, N 23.09.

8-[(4-Methoxyphenyl)amino]pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one *O*-Benzyloxime (6c): From **2c** (0.07 g, 0.19 mmol), **6c** was isolated as a white solid (0.07 g, 0.16 mmol, 85%); m.p. 239.8–241.2 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 11.43 (d, $^3J_{H,H}$ = 3.6 Hz, 1 H, 3-H), 9.30 (s, 1 H, 8-NH), 8.30 (s, 1 H, 6-H), 7.72 (m, 2 H, H$_o$), 7.63 (d, $^3J_{H,H}$ = 3.6 Hz, 1 H, 2-H), 7.37 (m, 5 H, Ph), 6.90 (m, 2 H, H$_m$), 5.14 (s, 2 H, OCH$_2$), 3.73 (s, 3 H, OMe) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 155.45, 155.44, 154.79, 144.65, 141.11, 138.56, 138.07, 131.66, 128.28, 127.89, 127.67, 125.13, 122.96, 113.61, 75.37, 55.19 ppm. IR (nujol mull): $\tilde{\nu}$ = 3361, 3220, 3077, 3031, 1636, 1603, 1581, 1550, 1531, 1507 cm^{-1}. $C_{20}H_{18}N_6O_2$ (374.42): calcd. C 64.15, H 4.86, N 22.45; found C 64.16, H 4.90, N 22.35.

8-Anilinopyrimido[5,4-*d*]pyrimidin-4(3*H*)-one *O*-Benzyloxime (6d): From **2d** (0.11 g, 0.32 mmol), **6d** was isolated as a white solid (0.09 g, 0.27 mmol, 83%); m.p. 260.0–261.4 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 11.46 (s, 1 H, 3-H), 9.38 (s, 1 H, 8-NH), 8.37 (s, 1 H, 6-H), 7.87 (d, $^3J_{H,H}$ = 7.80 Hz, 2 H, H$_o$), 7.65 (d, $^3J_{H,H}$ = 3.30 Hz, 1 H, 2-H), 7.38 (m, 7 H, Ph + H$_m$), 7.06 (t, $^3J_{H,H}$ = 7.50 Hz, 1 H, H$_p$), 5.15 (s, 2 H, OCH$_2$) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 155.39, 154.64, 144.87, 141.02, 138.68, 138.04, 128.47, 128.27, 127.90, 127.69, 125.32, 123.28, 121.11, 75.42 ppm. IR (nujol mull): $\tilde{\nu}$ = 3320, 3355, 3067, 3027, 2954, 2925, 2854, 1637, 1604, 1581, 1548, 1533 cm^{-1}. $C_{19}H_{16}N_6O{\cdot}0.1H_2O$ (346.20): calcd. C 65.91, H 4.67, N 24.28; found C 65.79, H 4.78, N 24.16.

4-[(8-Amino-7-oxido-pyrimido[5,4-*d*]pyrimidin-4-yl)amino]benzonitrile (5a): A concentrated aqueous solution of hydrochloric acid (5.88 mmol, 21 equiv., 12 M) was added to a suspension of **2a** (0.10 g, 0.28 mmol) in ethanol (4 mL), under magnetic stirring at room temperature. The resulting yellow solution led to a yellow solid which was filtered, washed with ethanol and diethyl ether and identified by ^{1}H NMR as the hydrochloride of **2a** (0.09 g, 0.22 mmol, 82%). ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.95 (s, 1 H, 4-NH), 10.82 (s, 2 H, 4-NH, 8-NH), 8.93 (s, 1 H, 6-H), 8.89 (s, 1 H, 2-H), 8.21 (d, $^3J_{H,H}$ = 8.70 Hz, 2 H, H$_o$), 7.88 (d, $^3J_{H,H}$ = 8.70 Hz, 2 H, H$_m$), 7.70 (m, 2 H, H$_o$), 7.47 (m, 3 H, H$_{m+p}$), 5.46 (s, 2 H, OCH$_2$) ppm. A suspension of the hydrochloride of **2a** (0.08 g, 0.20 mmol) in ethanol (4 mL) was refluxed for 50 min. The resulting suspension was cooled, and the light yellow solid was filtered and washed with ethanol followed by diethyl ether. The isolated compound was identified as *N*-oxide **5a** (0.05 g, 0.18 mmol, 92%). M.p. > 300 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.58 (s, 1 H, 8-NH), 8.92 (s, 2 H, 2-H, 4-NH), 8.74 (s, 1 H, 6-H), 8.29 (d, $^3J_{H,H}$ = 8.70 Hz, 2 H, H$_o$), 8.22 (s, 1 H, 4-NH), 7.84 (d, $^3J_{H,H}$ = 8.70 Hz, 2 H, H$_m$) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 156.23, 154.80, 150.93, 145.48, 142.72, 132.60, 131.71, 124.08, 121.26, 118.83, 105.12 ppm. IR (nujol mull): $\tilde{\nu}$ = 3361, 2226, 1669, 1599, 1573, 1538, 1524 cm^{-1}. HRMS (ESI-TOF): calcd. for $C_{13}H_{10}N_7O$ [M + 1]$^+$ 280.0947; found 280.0943.

***N*-8-(4-Fluorophenyl)pyrimido[5,4-*d*]pyrimidine-4,8-diamine 3-Oxide (5b):** Dry hydrochloric acid was bubbled into a suspension of **2b** (0.15 g, 0.41 mmol) in ethanol (3 mL), kept under magnetic stirring at 0 °C, until a yellow solution was formed. From this solution, a yellow solid precipitated out and was filtered after 15 min. The product was washed with ethanol followed by diethyl ether and was identified as the hydrochloride of **2b** (0.17 g, 0.41 mmol, 77%). ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.74 (s, 2 H, 4-NH + 8-NH), 10.54 (s, 1 H, 4-NH), 8.86 (s, 1 H, 6-H), 8.76 (s, 1 H, 2-H), 7.86 (m, 2 H, H$_o$), 7.69 (m, 2 H, H$_o$), 7.47 (m, 3 H, H$_{m+p}$), 7.25 (t, $^3J_{H,H}$ = 8.70 Hz, 2 H, H$_m$), 5.46 (s, 2 H, OCH$_2$) ppm. A suspension of **2b**·HCl (0.17 g, 0.42 mmol) in ethanol (4 mL) was refluxed for 3.5 h and the mixture was cooled to room temperature. The yellow solid suspension was filtered, washed with ethanol and diethyl ether and identified as *N*-oxide **5b** (0.07 g, 0.26 mmol, 63%). m.p. > 300 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.25 (s, 1 H, 8-NH), 8.88 (s, 2 H, 2-H + 4-NH), 8.60 (s, 1 H, 6-H), 8.16 8 (s, 1 H, 4-NH), 7.97 (m, 2 H, H$_o$), 7.22 (m, 2 H, H$_m$) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 25 °C): δ = 158.55 (d, $^1J_{C,F}$ = 239.40 Hz), 156.47, 155.34, 151.04, 145.36, 134.74 (d, $^4J_{C,F}$ = 2.55 Hz), 131.53, 124.14, 123.91 (d, $^3J_{C,F}$ = 7.73 Hz), 115.07 (d, $^2J_{C,F}$ = 22.28 Hz) ppm. IR (nujol mull): $\tilde{\nu}$ = 3357, 3281, 3156, 1664, 1637, 1612, 1589, 1567, 1553, 1533, 1504 cm^{-1}. $C_{12}H_9FN_6O$ (272.26): calcd. C 52.93, H 3.34, N 30.87; found C 52.90, H 3.50, N 30.91.

General Procedure for the Synthesis of Pyrimido[5,4-*d*]pyrimidines 3-Oxide 5c–d from 2c–d: Dry hydrochloric acid was bubbled into a suspension of **2** in acetonitrile, kept under magnetic stirring at room temperature, until a yellow solution was formed. Shortly after, a yellow solid precipitated out from the yellow solution. The suspension was kept at room temperature for about 15 min and was then refluxed until TLC showed the absence of the starting material. The reaction mixture was cooled in an ice bath and the light yellow solid precipitated out of solution, was filtered and washed with ethanol followed by diethyl ether.

***N*-8-(4-Methoxyphenyl)pyrimido[5,4-*d*]pyrimidine-4,8-diamine 3-Oxide (5c):** From **2c** (0.13 g, 0.36 mmol), **5c** was isolated as a light yellow solid (0.08 g, 0.27 mmol, 75%); m.p. > 300 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): δ = 10.06 (s, 1 H, 8-NH), 8.86 (s, 1 H, 2-H), 8.78 (s, 1 H, 4-NH), 8.55 (s, 1 H, 6-H), 8.10 (s, 1 H, 4-NH), 7.82 (d, $^3J_{H,H}$ = 9.30 Hz, 2 H, H$_o$), 6.95 (d, $^3J_{H,H}$ = 9.30 Hz, 2 H, H$_m$), 3.75 (s, 3 H, OMe) ppm. ^{13}C NMR (100.62 MHz, [D$_6$]-DMSO, 25 °C): δ = 156.38, 155.90, 155.49, 150.96, 145.18, 131.31, 131.26, 124.11, 123.61, 113.63, 55.21 ppm. IR (nujol mull): $\tilde{\nu}$ = 3367, 3125, 1664, 1615, 1603, 1555, 1507 cm^{-1}. HRMS (ESI-TOF): calcd. for $C_{13}H_{13}N_6O_2$ [M + 1]$^+$ 285.1100; found 285.1101.

***N*-8-Phenylpyrimido[5,4-*d*]pyrimidine-4,8-diamine 3-Oxide (5d):** From **2d** (0.28 g, 0.82 mmol), **5d** was isolated as a light yellow solid (0.22 g, 0.50 mmol, 61%); m.p. > 300 °C. ^{1}H NMR (300 MHz, [D$_6$]-DMSO, 25 °C): δ = 9.98 (s, 1 H, 8-NH), 9.00–7.50 (br. s, 2 H, 4-NH), 8.87 (s, 1 H, 2-H), 8.62 (s, 1 H, 6-H), 7.96 (d, $^3J_{H,H}$ = 7.80 Hz, 2 H, H$_o$), 7.38 (dt, $^3J_{H,H}$ = 7.80, $^3J_{H,H}$ = 7.50 Hz, 2 H, H$_m$), 7.13

(t, $^3J_{H,H}$ = 7.50 Hz, 1 H, H_p) ppm. ^{13}C NMR (75 MHz, [D_6]-DMSO, 25 °C): δ = 156.33, 155.21, 150.85, 145.12, 138.13, 131.32, 128.20, 123.97, 123.77, 121.66 ppm. IR (nujol mull): $\tilde{\nu}$ = 3353, 3285, 3159, 1667, 1638, 1608, 1582, 1567, 1556, 1533 cm^{-1}. $C_{12}H_{10}N_6O$ (254.27): calcd. C 56.68, H 3.97, N 33.06; found C 56.57, H 4.10, N 33.07.

Treatment of the Hydrochloride of Pyrimido[5,4-*d*]pyrimidine 2b with Water: A yellow suspension of **2b·HCl** (0.12 g, 0.31 mmol) in water (4 mL) was stirred at room temperature during 24 h. The white solid in suspension was filtered, washed with ethanol followed by diethyl ether and identified as **2b** (0.09 g, 0.26 mmol, 85%).

Reaction of 2b with Sulfuric Acid

7-(Benzyloxy)-*N*-(4-fluorophenyl)-8-imino-7,8-dihydropyrimido-[5,4-*d*]pyrimidin-4-amine Dihydrogen Sulfate (2b·H_2SO_4): Concentrated sulfuric acid (5.35 mmol) was added to an off-white suspension of **2b** (0.10 g, 0.28 mmol) in acetonitrile (3 mL) and ethanol (3 mL), kept under magnetic stirring at room temperature. A yellow solution was formed and after 5 min the reaction mixture was partially concentrated at 30 °C. The resulting solution was cooled to 0 °C leading to a yellow solid. The solid was filtered, washed with ethanol and diethyl ether and identified as **2b·H_2SO_4** (0.07 g, 0.14 mmol, 52%). ^1H NMR (300 MHz, [D_6]DMSO, 25 °C): δ = 10.83 (s, 1 H, 4-NH), 10.68 (s, 1 H, 4-NH), 10.54 (s, 1 H, 8-NH), 8.83 (s, 1 H, 6-H), 8.78 (s, 1 H, 2-H), 7.87 (m, 2 H, H_o), 7.68 (m, 2 H, H_o), 7.48 (m, 3 H, H_{m+p}), 7.26 (m, 2 H, H_m), 5.43 (s, 2 H, OCH_2) ppm.

8-[(4-Fluorophenyl)amino]pyrimido[5,4-*d*]pyrimidin-4(3*H*)-one *O*-Benzyloxime (6b): Concentrated sulfuric acid (5.35 mmol) was added to an off-white suspension of **2b** (0.05 g, 0.15 mmol) in ethanol (4 mL), kept with magnetic stirring at room temperature. A yellow solution was formed and after 5 min the reaction mixture was refluxed for 30 min. The yellow solution was concentrated leading to a yellow solid. The solid was filtered, washed with ethanol and diethyl ether, and identified as the **6b** (0.05 g, 0.15 mmol, 100%).

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