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Synthesis of non-purine analogs of 6-aryl-9-benzylpurines, and their antimycobacterial activities. Compounds modified in the imidazole ring

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ABSTRACT

Purine analogs modified in the five-membered ring have been synthesized and examined for antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ in vitro employing the microplate alamar blue assay (MABA). The 9-deaza analogs were only found to be weak inhibitors, but the 8-aza-, 7-deaza- and 8-aza-7-deazapurine analogs studied displayed excellent antimycobacterial activities, some even substantially better than the parent purine. In the 7-deazapurine series, MIC values between 0.08 and 0.35 μM, values comparable or better than the reference drugs used in the study (MIC rifampicin 0.09 μM, MIC isoniazid 0.28 μM and MIC PA-824 0.44 μM). The five most active compounds were also examined against a panel of drug-resistant *Mtb* strain, and they all retained their activity. The compounds examined were significantly less active against *M. tuberculosis* in a state of non-replicating persistence (NRP). MIC in the low-oxygen-recovery assay (LORA) $\geqslant$60 μM. The 7-deazapurines were somewhat more toxic towards mammalian cells, but still the selectivity indexes were excellent. The non-purine analogs exhibit a selective antimycobacterial activity. They were essentially inactive against *Staphylococcus aureus* and *Escherichia coli*.

1. Introduction

We have previously reported that certain 6,9-disubstituted purines are potent inhibitors of *Mycobacterium tuberculosis* (*Mtb*) in vitro.[1] These antimycobacterial purines display several properties which make them highly interesting as potential drugs against tuberculosis. These are high selectivity towards *Mtb* compared to other microorganisms, activity against several drug-resistant strains of *Mtb*, generally low toxicity towards mammalian cells, and ability to affect *Mtb* inside macrophages. Figure 1 summarizes the current SAR knowledge as well as the structures of some of the most active compounds in this series.

Tuberculosis (TB) still claims ca. 2 million deaths per year worldwide and resistance to existing drugs is a growing problem. Thus there is an urgent need for novel drugs for the treatment of TB. Agents that reduce the duration and complexity of the current therapy would have a major impact on the overall cure rate.[2] After exploring SAR of intact purines (Fig. 1), we now focus on non-purine analogs of compounds **1**.[3] Certain pyrimidine analogs (general structure **B**, Fig. 1) display activity comparable with the parent purine,[3b,d] but simple imidazole analogs (general structure **C**, Fig. 1) are only moderately active.[3c] Herein, we report the synthesis and antimycobacterial activities for purine analogs modified in the five-membered ring (general structure **D**, Fig. 1).

2. Chemistry

The 8-azapurine **5** was readily available from the pyrimidine **2** as shown in Scheme 1. The synthesis of 7-deaza-8-azapurine **6** (Fig. 2) has been published by us before.[3a]

In contrast with what has been reported for alkylation of the 7-deazapurine **7a**,[4,5] the benzylation shown in Scheme 2 went to completion at ambient temperature, and was reasonably regioselective. Compound **8a** was isolated in 81% and only minor amounts of more polar products (not isolated) were observed. Compound **8b** was prepared in high yield from the corresponding dichloro-7-deazapurine **7b**. Compounds **9a–9d** were synthesized by Stille couplings on the chlorides **8**. Reactions on the dichloride **8b** were conducted at milder conditions in order to achieve complete regioselectivity. Finally the methoxy compound **9e** was formed by exchange of the chloride in compound **9c**. This is analogous with the previously reported synthesis of the 2-methoxypurine **1e** (Fig. 1).[1f]

9-Alkyl-9-deazapurines have been synthesized by time-consuming construction of the bicyclic ring system via pyrroles[6] or pyrimidines,[7] and alkyl- or acyl substituents have been introduced

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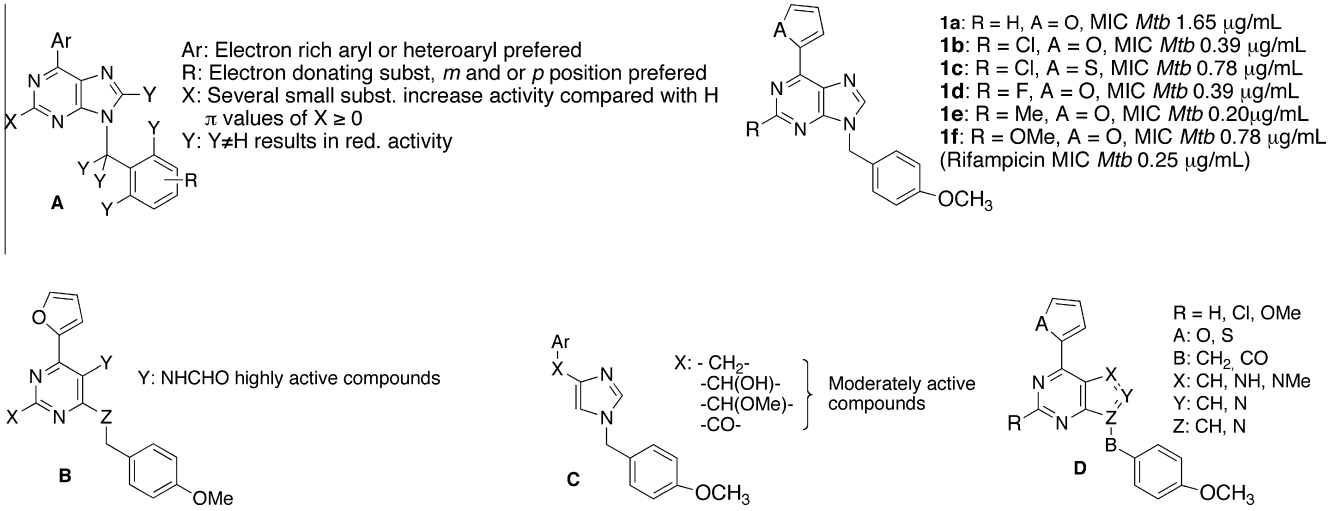
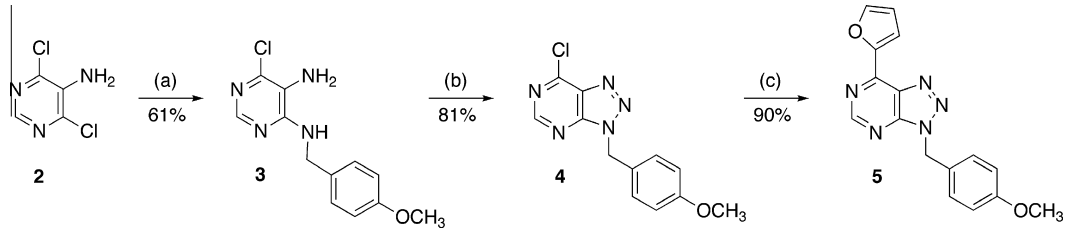


Figure 1. SAR summary for antimycobacterial purines **A**, structures of some of the most active purines (**1a**–**1f**), general structures of pyrimidine-**B** and imidazole analogs **C** and general structure of the target compounds **D** described in this study.



Scheme 1. Reagents and conditions: (a) *p*-MeO–C_6H_4–CH_2NH_2, Et_3N, *n*-BuOH, Δ; (b) $NaNO_2$, AcOH, CH_2Cl_2, H_2O; (c) (2-furyl)$SnBu_3$, $(Ph_3P)_2PdCl_2$; DMF, 90 °C.

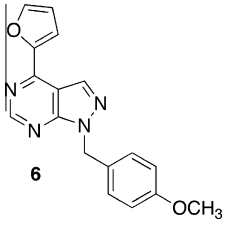
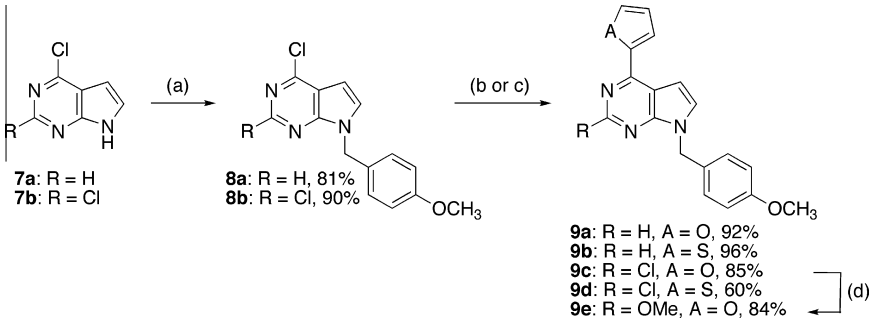
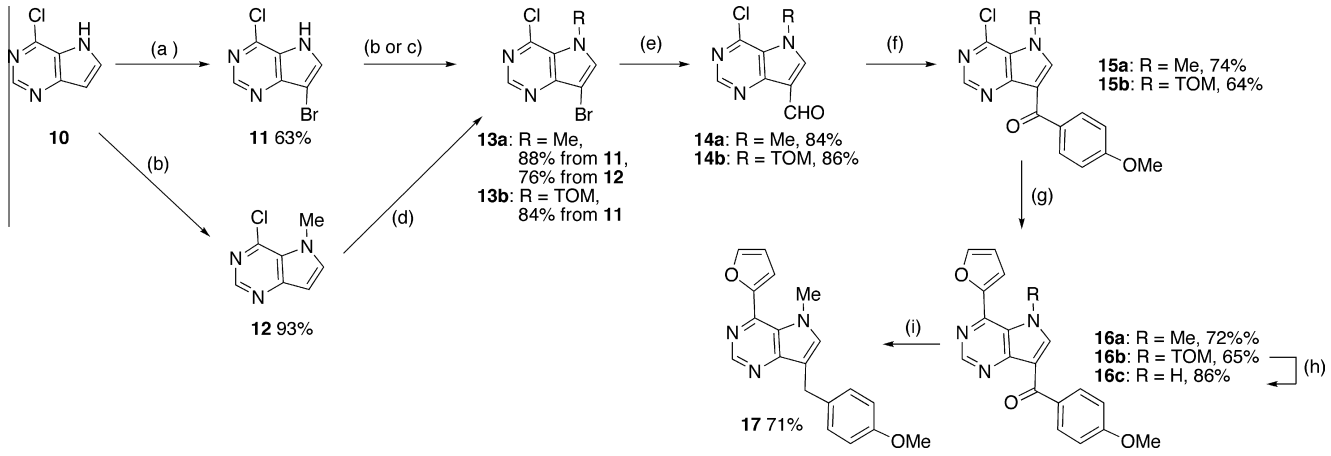


Figure 2. Structure of compound **6**.

at C-9 in 6-oxo-9-deazapurines be Friedel–Craft alkylation or acylation, but the yields are generally quite modest.[8] Instead, we evaluated several routes for the synthesis of our target 9-deazapurines **16** and **17** (Scheme 3), starting from 6-chloro-9-deazapurine (4-chloropyrrolo[3,2-*d*]pyrimidine) (**10**). The 4,7-dihalopyrrolo[3,2-*d*]pyrimidine **13a** were available be N-methylation and halogenation of compound **10**. Attempts to introduce the *p*-methoxybenzyl substituent at C-9 by Negishi couplings on the halide **13a** or the corresponding 7-iodo compound (not shown), met with little success. 9-Deazapurine can be lithiated at C-9 by metal-halogen exchange and the lithiated species may react with for instance imines[7b,9] or amides.[6c] However, attempts to introduce the desired C-9 substituent by lithiation of compound **13a** followed by reaction with *p*-methoxybenzyl halides failed. This was in part due to migration of lithium to C-8 before reaction with the alkyl halide. Also reactions between lithiated **13a** and anisaldehyde, the corresponding Weinreb amide, or benzoyl chloride turned out to be sluggish reactions. In our hands, the best route to the target molecule **17** is the one shown in Scheme 3. Lithiated **13a** was trapped with DMF to give the aldehyde **14a** in a high yield. Compound **14a** was subsequently reacted with *p*-methoxyphenylmagnesium



Scheme 2. Reagents and conditions: (a) *p*-MeO–C_6H_4–CH_2Cl, K_2CO_3, DMF; (b) R = H: (2-furyl)$SnBu_3$ or (2-thienyl)$SnBu_3$ $(Ph_3P)_2PdCl_2$, DMF, 90 °C; (c) R = Cl: (2-furyl)$SnBu_3$ or (2-thienyl)$SnBu_3$, $[(2\text{-furyl})_3P]_4Pd$ or $(Ph_3P)_2PdCl_2$, DMF, 50 °C; (d) MeONa, MeOH, Δ.



Scheme 3. Reagents and conditions: (a) NBS, THF; (b) NaH, MeI, DMF; (c) NaH, TOM-Cl, THF, 0 °C to rt; (d) NBS, CH_2Cl_2; (e) (1) *n*-BuLi, Et_2O, anisole, −78 °C, (2) DMF; (f) (1) *p*-MeO–C_6H_4–MgBr, LiCl, THF, 0 °C to rt, (2) PhCHO; (g) (1) (2-furyl)$SnBu_3$, $(Ph_3P)_2PdCl_2$, DMF, 90 °C; (h) KF, MeOH; (i) NH_2NH_2, NaOH, H_2O, $HOCH_2CH_2OH$, 120 °C.

bromide. The secondary alcohol thus formed turned out to be difficult to isolate in pure form, but the adduct was instead subjected to in situ Oppenaur oxidation by benzaldehyde in the presence of LiCl[10] to give the ketone **15a**. After introduction of the furyl group by a Stille coupling on the chloride **15a**, ketone **16a** was reduced to the target **17** under Wolff–Kishner conditions. Several other reduction methods were unsuccessful for this transformation.

Silyloxymethyl chlorides have been developed as reagents for N-[11] and O-protection,[12] and the protected compounds can be cleaved when treated with fluoride ions under milder conditions than normally required to cleave the analogous SEM-protected derivatives. The only commercially available of these reagents is (triisopropylsilyloxy)methyl chloride (TOM-Cl),[13] and we chose the TOM group as N-protection group in the synthesis of target compound **16c**. The N-protected ketone **16b** was synthesized as described for compound **16a** above, and the protection group was conveniently removed by KF in methanol. The protection group was not compatible with the harsh conditions required in a Wolff–Kishner reduction, nor was the reduction of the carbonyl group compound **16c** successful. However, the 9-deazapurines **16a**, **16c** and **17** all turned out to be far less active as antimycobacterial compounds compared to the parent purine and the other non-purine analogs reported herein (see below), and no further attempts were made to synthesize an analog of compound **17** with a free NH-group.

3. Biological evaluation

The target compounds **5**, **6**, **9a**–**9e**, **16a**, **16c** and **17** were screened for activity against *M. tuberculosis* $H_{37}Rv$ (ATCC #27294) in the microplate alamar blue assay (MABA)[14] and the MIC values are given in Table 1. The previously synthesized purines **1a** and **1b** were included for comparison. The substituents are defined in Fig. 1, and the detailed structures can be found in Fig. 2 and Schemes 1–3.

The deazapurines **16** and **17** were only weak inhibitors of antimycobacterial growth (MIC >90 μM). The low activities found discouraged us from synthesis of more compounds in this series as described above, and no further examination of antimycobacterial activities was done for these substances.

The 8-azapurine **5** and the 8-aza-7-deazapurine **6** were slightly less active than the parent purine **1a**, showing that the purine C-8 may be exchanged with a nitrogen without a significant loss of activity. However, the most intriguing results were found for the 7-deazapurines **9**. Compound **9a** was ca. 8 times more active than the parent purine **1a**. The thienyl derivative **9b** appeared to be a

Table 1
Antibacterial activity against *M. tuberculosis*, and cytotoxic activity against VERO cells for compounds **1a**, **1b**, **5**, **6**, **9a-d**, **16a**, **16c**, and **17**

Compound No.	R	A	B	X	Y	Z	MIC (MABA) *M. tuberculosis* H37Rv[a] (μM)	MIC (LORA) *M. tuberculosis* H37Rv pFPCA-luxAB (μM), inhib. at 128[b] (μM)	EC_{50} VERO cells[c] (μM)	SI (EC_{50}:MIC)
1a	H	O	CH_2	N	CH	N	2.1	>128 (88%)	>206[d,e]	>98
1b	Cl	O	CH_2	N	CH	N	0.36	>128 (82%)	>117[f]	>325
5	H	O	CH_2	N	N	N	2.4	>128 (61%)	>130	>54
6	H	O	CH_2	CH	N	N	3.6	>128 (60%)	>327	>91
9a	H	O	CH_2	CH	CH	N	0.16	>128 (82%)	>131	>756
9b	H	S	CH_2	CH	CH	N	0.35	60	40	114
9c	Cl	O	CH_2	CH	CH	N	0.080	>128 (88%)	85	1063
9d	Cl	S	CH_2	CH	CH	N	0.11	120	76	691
9e	OMe	O	CH_2	CH	CH	N	0.22	87	45	205
16a	H	O	CO	NMe	CH	N	192	n.d.	n.d.	n.d.
16c	H	O	CO	NH	CH	N	>300	n.d.	n.d.	n.d.
17	H	O	CH_2	NMe	CH	N	97	n.d.	n.d.	n.d.

[a] MIC rifampicin 0.09 μM, MIC isoniazid 0.28 μM, MIC PA-824 0.44 μM.
[b] MIC rifampicin 0.97 μM, MIC isoniazid >128 μM, MIC PA-824 3.11 μM.
[c] EC_{50} hyamine 0.01 μg/mL (ca. 0.03 μM).
[d] Taken from Ref. 1f.
[e] Low solubility preceded determination of the actual value.
[f] Taken from Ref. 1g.

slightly weaker inhibitor than the furyl derivative **9a**. The same tendency has been observed in the purine series before.[1] In our study of the purines, we have generally seen that antimycobacterial activity is substantially increased when chlorine is introduced in the 2-position,[1] and compounds **9c** and **9d** were the among the most active in this series. The values were in the same range as for the drug rifampicin and better than for isoniazid or the promising drug candidate PA-824.[15]

In case of tuberculosis, a sub-population of the bacteria isolate in a state of non-replicating persistence (NRP). NRP is considered to be an important factor contributing to the long treatment duration ($\geqslant$6 months required). Hence we wanted to investigate if the antimycobacterial purines and non-purine analogs described herein also could affect *Mtb* in NRP. Compounds **1a**, **1b**, **5**, **6** and **9a**–**9e** were thus tested in the low-oxygen-recovery assay (LORA)[16] (Table 1). Unfortunately, as also seen for many known antituberculosis drugs[16] including isoniazid, the compounds were not found to be very active in this assay. Interestingly, it seems likely that the thienyl substituent results in somewhat more active compounds, and that the chloride is not beneficial for activity in the LORA. The best inhibitor identified in this assay was the thienyl-7-deazapurine **9b** (MIC 60 μM).

The antimycobacterial purine analogs **5**, **6** and **9a**–**9e** were examined for toxicity against mammalian cells (VERO cells), and low EC_{50} values were found (Table 1). All deazapurines **9** were more toxic towards VERO cells than compounds **1**, **5** and **6**, but the selectivity indexes (SI) were still very good for these antimycobacterial compounds. For the best inhibitor of *Mtb* growth, compound **9c**, the SI was found to be >1000.

The five most active deazapurines (**9a**–**9e**) as well as the parent purine **1b** were examined against a panel of *Mtb* strains resistant to currently used anti-TB drugs; rifampicin (RMP), isoniazid (INH), streptomycin (SM), kanamycin (KM) and clofazimine (CLF) (Table 2). All compounds examined retained their activity against all the drug-resistant strains applied in this study.

In accordance with previous findings on the structurally related purines,[1] the non-purine analogs exhibit a selective antimycobacterial activity. Compounds **5**, **6**, **9a**, **9c**, **16a**, **16c** and **17** were tested against *Staphylococcus aureus* and *Escherichia coli* and were found to be essentially inactive (all MICs >32 μg/mL; >90 μM). Even though the mode of action for our purines and non-purine analogs is not known, the lack of cross resistance and lack of activity towards other bacteria points towards a novel mechanism of action and a target not found in all bacteria.

Table 2
Activity against drug-resistant strains of *M. tuberculosis*

Compound No.	MABA MIC (μM)				
	r-RMP[a]	r-INH[b]	r-SM[c]	r-KM[d]	r-CLF[e]
1b	0.15	0.15	0.56	0.16	0.47
9a	0.14	0.13	0.18	0.10	0.16
9b	0.13	0.14	0.26	0.12	0.18
9c	0.10	0.12	0.080	0.10	0.060
9d	0.14	0.090	0.060	0.080	<0.050
9e	0.090	0.090	0.090	0.10	0.070
RMP	>4	0.020	0.040	0.020	0.020
INH	0.25	>8	0.24	0.39	0.23
MOX[f]	0.24	0.30	0.25	0.34	0.25
SM	0.21	0.13	>16	0.98	0.33
PA-824	0.17	0.070	0.10	0.22	0.54

[a] Rifampicin resistant strain.
[b] Isoniazid resistant strain.
[c] Streptomycin resistant strain.
[d] Kanamycin resistant strain.
[e] Clofazimine resistant strain.
[f] Monofloxacin.

In summary, novel 8-aza-, 7-deaza- 9-deaza and 8-aza-7-deazapurines have been synthesized and their biological activities have been compared with those of known antimycobacterial purines with similar substitution patterns. It was found that the purine N-9 is important for activity, since the 9-deazapurines studied were only weak inhibitors of *Mtb* in the MABA. The purine C-8 may be exchanged with nitrogen without significant loss of activity and removal of the purine N-7 results in substantially improved growth inhibition. The best results were obtained for some of the 7-deazapurines with MIC in the MABA comparable with rifampicin. The compounds studied were generally of low toxicity towards mammalian (VERO) cells and essentially inactive against other bacteria (*S. aureus*, *E. coli*). Unfortunately, the compounds studied were not found to be very active against *Mtb* isolates in NRP.

4. Experimental

The ^{1}H NMR spectra were recorded at 500 MHz with a Bruker Avance DRX 500 instrument, at 300 MHz with a Bruker Avance DPX 300 instrument, or at 200 MHz with a Bruker Avance DPX 200 or a Varian Gemini instrument. The decoupled ^{13}C NMR spectra were recorded at 125, 75 or 50 MHz using instruments mentioned above. Mass spectra under electron impact conditions were recorded with a VG Prospec instrument at 70 eV ionizing voltage, and are presented as *m/z* (% rel. int.). Elemental analyses were performed by Ilse Beetz Mikroanalytisches Laboratorium, Kronach, Germany, or School of Chemistry, University of Birmingham, UK. Melting points were determined with a C. Reichert melting point apparatus or a Büchi Melting Point B-545 apparatus and are uncorrected. Triethylamine, DMF and CH_2Cl_2 were distilled from CaH_2 and stored over molecular sieves (3 Å). THF and anisole and diethyl ether were distilled from Na/benzophenone. Alternatively dry THF DMF, Et_2O and CH_2Cl_2 were obtained from a solvent purification system, MB SPS-800 from MBraun. Silica gel for flash chromatography was purchased from Merck, Darmstadt, Germany (Merck No. 09385). All other reagents were commercially available and used as received. Compounds synthesized by literature procedures: 5-amino-4-chloro-6-(4-methoxybenzylamino)pyrimidine (**3**),[3d] 4-(2-furyl)-1-(4-methoxybenzyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**6**).[3a] Activities against *S. aureus*,[17] *E. coli*[17] and VERO cells[1] were determined as reported before.

4.1. Antimycobacterial data

Minimum inhibitory concentrations (MIC) against replicating and non-replicating cultures of *M. tuberculosis* were determined using the microplate Alamar Blue assay (MABA)[14] and low-oxygen-recovery assay (LORA),[16] respectively. The former was determined against *M. tuberculosis* H_{37}Rv ATCC 27294 (American Type Culture Collection) as well as drug-resistant *Mtb* strains following 7 days incubation with test samples. The latter was determined against low oxygen adapted *M. tuberculosis* H_{37}Rv luxAB carrying a luciferase reporter gene following 10 days incubation under low oxygen followed by 28 h of normoxic recovery. Both assays were conducted in microplate format in 7H12 medium.[14b] The MIC was defined as the lowest concentration effecting a reduction of $\geqslant$90% in fluorescence (MABA) or luminescence (LORA) relative to untreated controls.

4.1.1. 7-Chloro-[3-(4-methoxybenzylamino)]-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidine (4)

A mixture of 5-amino-4-chloro-6-(4-methoxybenzylamino)pyrimidine **3** (670 mg, 2.53 mmol), AcOH (50% in water, 12 mL) and $NaNO_2$ (192 mg, 2.78 mmol) in dichloromethane (12 mL) was stirred at ambient temperature under N_2-atm for 30 min, diluted with dichloromethane (20 mL), washed with water

(10 mL) and brine (10 mL), dried ($MgSO_4$) and evaporated in vacuo. The product was purified by flash chromatography on silica gel eluting with dichloromethane; yield 562 mg (81%), mp 110–112 °C, off-white powdery crystals. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.75 (s, 3H, OCH_3), 5.81 (s, 2H, CH_2), 6.85 (d, J = 8.4 Hz, 2H, Ar), 7.42 (d, J = 8.4 Hz, 2H, Ar), 8.90 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 51.0 (CH_2), 55.3 (OCH_3), 114.3 (CH in Ar), 125.8 (C-1 in Ar), 130.0 (CH in Ar), 134.1 (C-7a), 149.4 (C-3a/C-7), 154.0 (C-3a/C-7), 155.3 (C-5), 159.9 (C-4 in Ar); MS EI m/z (rel.%) 277/275 (12/36, M^+), 248 (39), 246 (92), 234 (6), 232 (18), 218 (7), 216 (22), 134 (13), 122 (9), 121 (199); HRMS calcd for $C_{12}H_{10}ClN_5O$ 275.0574, found 275.0582.

4.1.2. 7-(2-Furyl)-[3-(4-methoxybenzylamino)]-3*H*-[1,2,3] triazolo[4,5-*d*]pyrimidine (5)

A mixture of 7-chloro-[3-(4-methoxybenzylamino)]-3*H*-[1,2,3] triazolo[4,5-*d*]pyrimidine **4** (278 mg, 1.00 mmol), 2-furyl(tributyl)tin (0.48 mL, 1.5 mmol) and $(Ph_3P)_2PdCl_2$ (36 mg. 0.050 mmol) in DMF (4 mL) was stirred at 90 °C under N_2-atm for 4 h, and evaporated in vacuo. KF in MeOH (satd soln, 10 mL) was added to the residue and the mixture stirred for 18 h. The product was purified by flash chromatography on silica gel eluting with EtOAc/hexane (1:2) followed by EtOAc/hexane (1:1) and finally pure EtOAc; yield 275 mg (90%), mp 166–168 °C, colorless powdery crystals. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.75 (s, 3H, OCH_3), 5.81 (s, 2H, CH_2), 6.72 (dd, J = 3.6 and 1.8 Hz, 1H, furyl), 6.84 (d, J = 8.6 Hz, 2H, Ar), 7.43 (d, J = 8.6 Hz, 2H, Ar), 7.82 (br s, 1H, furyl), 8.11 (br d, J = 3.6 Hz, 1H, furyl), 9.07 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 50.1 (CH_2), 55.0 (OCH_3), 113.1 (CH in furyl), 114.0 (CH in Ar), 120.3 (CH in furyl), 126.3 (C-1 in Ar), 129.7 (CH in Ar), 130.3 (C-7a), 147.2 (CH in furyl) 147.7 (C-3a/C-7/C-2 in furyl), 148.4 (C-3a/C-7/C-2 in furyl), 149.1 (C-3a/C-7/C-2 in furyl), 155.9 (C-5), 159.5 (C-4 in Ar); MS EI m/z (rel.%) 307 (30, M^+), 279 (26), 278 (82), 264 (15), 250 (17), 159 (7), 146 (9), 121 (100); HRMS calcd for $C_{16}H_{13}N_5O_2$ 307.1069, found 307.1065. Anal. calcd for $C_{16}H_{13}N_5O_2$: C, 62.53; H, 4.26; N, 22.79. Found: C, 62.29; H, 4.38; N, 22.47

4.1.3. 4-Chloro-7-(4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (8a)

A mixture of 4-chloro-7-deazapurine **7a** (618 mg, 3.90 mmol) and K_2CO_3 (1.62 g, 11.7 mmol, oven-dried at 110 °C for 5 h) in DMF (20 mL) was stirred at ambient temperature under N_2-atm for 30 min, before 4-methoxybenzyl chloride (1.00 mL, 7.80 mmol) was added. The resulting reaction mixture was stirred at ambient temperature for 24 h and poured into water (200 mL). The aqueous phase was extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL), dried ($MgSO_4$) and evaporated in vacuo. The product was purified by flash chromatography on silica gel eluting with hexane followed by EtOAc/hexane (1:4); yield 865 mg (81%), colorless wax. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.76 (s, 3H, OCH_3), 5.37 (s, 2H, CH_2), 6.58 (d, J = 3.6 Hz, 1H, H-5), 6.84 (d, J = 8.7 Hz, 2H, Ar), 7.15–7.18 (m, 3H, Ar and H-6), 8.66 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 48.0 (CH_2), 55.3 (CH_3), 99.9 (C-5), 114.3 (CH in Ar), 117.5 (C-4a), 128.2 (C-1 in Ar), 129.0 (C-6), 129.2 (CH in Ar), 150.7 (C-2), 151.0 (C-4/C-7a), 152.0 (C-4/C-7a), 159.5 (C-4 in Ar); MS EI m/z (rel.%) 275/273 (14/38, M^+), 154 (14), 122 (16), 121 (100), 91 (6), 90 (2), 98 (3), 78 (13), 77 (9); HRMS calcd for $C_{14}H_{12}ClN_3O$ 273.0669, found 273.0668.

4.1.4. 2,4-Dichloro-7-(4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (8b)

The title compound was synthesized from 2,4-7-deazapurine **7b** (1.25 g, 6.65 mmol) following the procedure for synthesis of compound **8a**. The product was purified by flash chromatography on silica gel eluting with EtOAc/hexane (1:9); yield 1.85 g, (90%), mp 103–105 °C, colorless crystalline solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.77 (s, 3H, OCH_3), 5.31 (s, 2H, CH_2), 6.56 (d, J = 3.6 Hz, 1H, H-5), 6.85 (d, J = 8.6 Hz, 2H, Ar), 7.12 (d, J = 3.6 Hz, 1H, H-6), 7.17 (d, J = 8.6 Hz, 2H, Ar); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 48.1 (CH_2), 55.3 (CH_3), 100.3 (C-5), 114.4 (CH in Ar), 116.3 (C-4a), 127.6 (C-1 in Ar), 129.4 (CH in Ar and C-6), 152.0 (2 × C, C-2/C-4/C-7a), 152.6 (C-2/C-4/C-7a), 159.6 (C-4 in Ar); MS EI m/z (rel.%) 309/307 (6/10, M^+), 122 (9), 121 (100), 91 (3), 90 (1), 78 (7), 77 (5); HRMS calcd for $C_{14}H_{11}Cl_2N_3O$ 307.0279, found 307.0278.

4.1.5. 4-(2-Furyl)-7-(4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (9a)

The compound was synthesized by Stille coupling between compound **8a** (277 mg, 1.01 mmol) and 2-furyl(tributyl)tin (0.48 mL, 1.5 mmol) following the procedure for synthesis of compound **5**. The product was purified by flash chromatography on silica gel eluting with EtOAc/hexane (2:3); yield 280 mg (92%), mp 130–131 °C, colorless small needles. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.76 (s, 3H, OCH_3), 5.39 (s, 2H, CH_2), 6.61 (dd, J = 3.2 and 1.6 Hz, 1H, furyl), 6.83 (d, J = 8.6 Hz, 2H, Ar), 6.99 (d, J = 3.6 Hz, 1H, H-5), 7.14–7.19 (m, 3H, Ar and H-6), 7.38 (br d, J = 3.2 Hz, 1H, furyl), 7.68 (br s, 1H, furyl), 8.87 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 47.3 (CH_2), 55.2 (CH_3), 101.1 (C-5), 112.1 (CH in furyl), 112.6 (CH in furyl), 112.8 (C-4a), 114.1 (CH in Ar), 128.5 (C-6), 128.7 (C-1 in Ar), 128.9 (CH in Ar), 144.8 (CH in furyl), 147.1 (C in furyl), 151.3 (C-2), 151.8 (C-4/C-7a), 153.2 (C-4/C-7a), 159.0 (C-4 in Ar); MS EI m/z (rel.%) 305 (59, M^+), 290 (3), 198 (2), 185 (7), 184 (4), 157 (4), 156 (3), 153 (2), 129 (4), 122 (14), 121 (100); HRMS calcd for $C_{18}H_{15}N_3O_2$ 305.1164, found 305.1160; Anal. Calcd for $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.95; N, 13.76. Found: C, 70.53; H, 5.29; N, 13.48.

4.1.6. 7-(4-Methoxybenzyl)-4-(2-thienyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine (9b)

The compound was synthesized by Stille coupling between compound **8a** (740 mg, 2.70 mmol) and 2-thienyl(tributyl)tin (1.2 mL, 3.5 mmol) following the procedure for synthesis of compound **5**. The product was purified by flash chromatography on silica gel eluting with EtOAc/hexane (1:3); yield 850 mg (96%), mp 109–110 °C, colorless crystals. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.77 (s, 3H, OCH_3), 5.40 (s, 2H, CH_2), 6.84 (d, J = 8.7 Hz, 2H, Ar), 6.87 (d, J = 3.5 Hz, 1H, H-5), 7.19 (d, J = 8.7 Hz, 2H, Ar), 7.21 (m, 2H, H-6 and 1H in thienyl), 7.55 (d, J = 4.9 Hz, 1H, thienyl), 8.02 (br s, 1H, thienyl), 8.88 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 47.5 (CH_2), 55.3 (CH_3), 100.4 (C-5), 113.3 (C-4a), 114.2 (CH in Ar), 128.3–129.5 (3 × CH in thienyl, C-6 and C-1 in Ar), 129.1(CH in Ar), 142.8 (C-2 in thienyl), 151.0 (C-2), 151.2 (C-4/C-7a), 151.8 (C-4/C-7a), 159.4 (C-4 in Ar); MS EI m/z (rel.%) 321 (39, M^+), 306 (1), 214 (1), 200 (1), 122 (9), 121 (100), 78 (7), 77 (6); HRMS calcd for $C_{18}H_{15}N_3OS$ 321.0936, found 321.0940. Anal. Calcd $C_{18}H_{15}N_3OS$: C, 67.27; H, 4.70; N, 13.07. Found: C, 66.95; H, 4.98; N, 12.82.

4.1.7. 2-Chloro-4-(2-furyl)-7-(4-methoxybenzyl)-7*H*-pyrrolo [2,3-*d*]pyrimidine (9c)

A mixture of tris(dibenzylideneacetone)dipalladium chloroform adduct (16 mg, 0.015 mol) and tri(2-furyl)phosphine (26 mg, 0.11 mmol) in DMF (2 mL) was stirred at ambient temperature under N_2-atm for 15 min, before compound **8b** (155 mg, 0.500 mmol) in DMF (2 mL) and 2-furyl(tributyl)tin (0.20 mL, 0.60 mmol) were added. The resulting mixture was stirred for 8 h at 50 °C and evaporated in vacuo. A satd solution of potassium fluoride in methanol was added to the residue and the mixture was stirred overnight and evaporated in vacuo together with a small amount of silica gel. The residue was added on top of a chromatography column and the product was isolated by flash chromatography on silica

eluting with EtOAc/isohexane (1:12) followed by EtOAc/isohexane (1:6) and EtOAc/isohexane (1:3); yield 145 mg (85%), mp 128–130 °C, colorless crystalline solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.77 (s, 3H, OCH_3), 5.33 (s, 2H, CH_2), 6.60 (dd, J = 3.6 and 1.6 Hz, 1H, H-4 in furyl), 6.84 (d, J = 8.7 Hz, 2H, Ar), 6.96 (d, J = 3.6 Hz, 1H, H-5), 7.11 (d, J = 3.6 Hz, 1H, H-6), 7.18 (d, J = 8.8 Hz, 2H, Ar), 7.45 (dd, J = 3.6 and 0.5 Hz, 1H, H-3 in furyl), 7.68 (dd, J = 1.7 and 0.6 Hz, 1H, H-5 in furyl); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 47.5 (CH_2), 55.3 (CH_3), 101.9 (C-5), 111.7 (C-4a), 112.4 (C-4 in furyl), 114.3 (CH in Ar and C-3 in furyl), 128.3 (C-1 in Ar), 129.1 (C-6), 129.3 (CH in Ar), 145.6 (C-5 in furyl), 148.8 (C-2), 152.3 (C-2 in furyl), 153.5 (C-4/C-7a), 153.4 (C-4/C-7a), 159.4 (C-4 in Ar); MS EI m/z (rel.%) 341/339 (7/21, M^+), 218 (1), 122 (9), 121 (100), 91 (3), 89 (1), 78 (6), 77 (6); HRMS calcd for $C_{18}H_{14}ClN_3O_2$ 339.0775, found 339.0777. Anal. Calcd for $C_{18}H_{14}ClN_3O_2$: C, 63.63; H, 4.15; N, 12.37. Found: C, 63.76; H, 4.38; N, 12.11.

4.1.8. 2-Chloro-7-(4-methoxybenzyl)-4-(2-thienyl)-7*H*-pyrrolo [2,3-*d*]pyrimidine (9d)

The compound was synthesized by Stille coupling between compound **8b** (800 mg, 2.60 mmol) and 2-thienyl(tributyl)tin (0.10 mL, 3.1 mmol) and $PdCl_2(PPh_3)_2$ (5.5 mg, 0.078 mmol) as catalyst, otherwise following the procedure for synthesis of compound **9c**. The product was isolated by flash chromatography on silica eluting with EtOAc/isohexane (1:6) followed by EtOAc/isohexane (1:3); yellow crystals, yield 550 mg (60%), mp 124–126 °C, colorless crystalline solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.77 (s, 3H, OCH_3), 5.34 (s, 2H, CH_2), 6.82–6.86 (m, 3H, Ar and H-5), 7.13 (d, J = 3.7 Hz, 1H, H-6), 7.17–7.21 (m, 3H, Ar and 1H in thienyl), 7.57 (dd, J = 5.0 and 1.0 Hz, 1H, thienyl), 7.98 (dd, J = 3.6 and 1.0 Hz, 1H, thienyl); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 47.6 (CH_2), 55.3 (CH_3), 100.8 (C-5), 112.2 (C-4a), 114.3 (CH in Ar), 128.2 (CH in thienyl/C-1 in Ar), 128.3 (CH in thienyl/C-1 in Ar), 129.2 (C-6/CH in thienyl), 129.4 (CH in Ar), 129.7 (C-6/CH in thienyl), 130.4 (CH in thienyl), 141.4 (C-2 in thienyl), 152.9 (C-2/C-4/C-7a), 153.3 (C-2/C-4/C-7a), 153.4 (C-2/C-4/C-7a), 159.5 (C-4 in Ar); MS EI m/z (rel.%) 357/355 (7/19, M^+), 307 (2), 121 (100), 91 (5), 78 (6), 77 (6); HRMS calcd for $C_{18}H_{14}ClN_3OS$ 355.0546, found 355.0551. Anal. Calcd for $C_{18}H_{14}ClN_3OS$: C, 60.76; H, 3.97; N, 11.81. Found: C, 60.53; H, 4.31; N, 11.64.

4.1.9. 4-(2-Furyl)-2-methoxy-7-(4-methoxybenzyl)-7*H*-pyrrolo [2,3-*d*]pyrimidine (9e)

A solution of **9c** (150 mg, 0.440 mmol) in a freshly prepared solution of sodium methoxide in methanol (ca. 1.5 M, 15 mL) was heated at reflux under N_2-atm for 24 h. The reaction mixture was cooled to ambient temperature and quenched with satd aq NH_4Cl (30 mL). The resulting mixture was evaporated in vacuo, the suspension obtained was dissolved in EtOAc (30 mL) and washed with satd aq NH_4Cl (20 mL). The aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phase was dried ($MgSO_4$) and concentrated in vacuo. The product was purified by flash chromatography eluting with EtOAc (1:4); yield 125 mg (84%), mp 92–94 °C, off-white crystalline solid. ^{1}H NMR (Me_2CO-d_6, 300 MHz) δ 3.75 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 5.34 (s, 2H, CH_2), 6.69 (dd, J = 3.5 and 1.76 Hz, 1H, H-4 in furyl), 6.87 (d, J = 8.7 Hz, 2H, Ar), 6.92 (d, J = 3.6 Hz, H-5), 7.30–7.34 (m, 3H, H-6 and 2H in Ar), 7.39 (dd, J = 3.5 and 1.6 Hz, 1H, H-3 in furyl), 7.87 (dd, J = 1.7 and 0.74 Hz, H-5 in furyl); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 47.7 (CH_2), 54.6 (CH_3), 55.5 (CH_3), 101.7 (C-5), 109.5 (C-4a), 113.0 (C-4 in furyl), 113.5 (C-3 in furyl), 114.8 (CH in Ar), 128.7 (C-6), 130.1 (C-H in Ar), 130.6 (C-1 in Ar), 146.3 (C-5 in furyl), 149.0 (C-7a), 154.5 (C-2 in furyl), 155.2 (C-4), 160.3 (C-4 in Ar), 162.9 (C-2); MS EI m/z (rel.%) 335 (56, M^+), 299 (6), 122 (8), 121 (100); HRMS cacld for $C_{19}H_{15}N_3O_3$ 335.1270, found 335.1269; Anal. Calcd for $C_{19}H_{15}N_3O_3$: C, 68.05; H, 5.11. Found: C, 67.85; H, 5.00.

4.1.10. 4-Chloro-7-bromo-5*H*-pyrrolo[3,2-*d*]pyrimidine (11) [18]

N-Bromosuccinimide (0.30 g, 1.7 mmol) was added in small portions to a well stirred solution of **10** (0.20 g, 1.3 mmol) in THF (10 mL) under N_2-atm, the resulting reaction mixture was stirred at ambient temperature for 2 h. A small amount of silica gel was added and the mixture was evaporated. The residue was added on top of a silica gel flash chromatography column, and the product was isolated after elution with CH_2Cl_2 followed by 5% MeOH in CH_2Cl_2; yield 190 mg (63%), mp 230–232 °C, colorless crystalline solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.23 (s, 1H, H-6), 8.71 (s, 1H, H-2), 12.9 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 89.7 (C-7), 123.9 (C-4a), 113.9 (C-6), 142.5 (C-4), 147.5 (C-7a), 149.9 (C-2); MS EI m/z (rel.%) 233/231 (100/76, M^+), 198 (50), 196 (50), 117 (24); HRMS calcd for $C_6H_3BrClN_3$ 230.9199, found 230.9199. Anal. Calcd for $C_6H_3BrClN_3$: C, 31.00; H, 1.30; N, 18.08. Found: C, 31.21; H, 1.20; N, 17.93.

4.1.11. 4-Chloro-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidine (12)

NaH (ca. 60% in oil, 260 mg, ca. 6.5 mmol) was added in small portions to a stirring mixture of 4-chloro-5*H*-pyrrolo[3,2-*d*]pyrimidine **10** (500 mg, 3.25 mmol) in dry DMF (10 mL) at 0 °C under N_2-atm The resulting mixture was stirred for 1 h at 0 °C before iodomethane (0.46 mL, 3.5 mmol) was added drop wise. The reaction mixture was allowed to gradually reach ambient temperature over 2 h and again cooled to 0 °C. Acetic acid (1 mL) was added and the resulting suspension was stirred for 15 min, before the solvents were evaporated in vacuo. The residue was dissolved in EtOAc (50 mL) and washed with satd aq $NaHCO_3$ (25 mL) and water (25 mL). The aq. phases were extracted with EtOAc (25 mL) and the combined EtOAc extracts were dried ($MgSO_4$) and evaporated in vacuo. The product was used without further purification; yield 508 mg (93%), off-white solid. ^{1}H NMR (CD_3COD, 200 MHz) δ 4.17 (s, 3H, CH_3), 6.66 (d, J = 3.2 Hz, 1H, H-7), 7.78 (d, J = 3.2 Hz, 1H, H-6), 8.54 (s, 1H, H-2); MS EI m/z (rel.%) 169/167 (28/83, M^+), 132 (100). Spectral data are in good agreement with those reported before.[19]

4.1.12. 7-Bromo-4-chloro-5-methyl-5*H*-pyrrolo[3,2-*d*] pyrimidine (13a)

Method A: NaH (27 mg, ca. 0.68 mmol, ca. 60% in oil,) was added in small portions to a well stirred solution of **11** (80 mg, 0.34 mmol) in dry DMF (2 mL) at 0 °C under N_2-atm The reaction mixture was stirred for 1 h at 0 °C before iodomethane (0.021 mL, 0.34 mmol) was added dropwise. The reaction mixture was allowed to gradually reach ambient temperature over 1.5 h and subsequently cooled to 0 °C. Glacial acetic acid (0.1 mL) was added and resulting suspension was stirred for 10 min, before the solvents were evaporated in vacuo. The residue was dissolved in EtOAc (40 mL), washed with satd aq $NaHCO_3$ (40 mL) and brine (30 mL), dried ($MgSO_4$) and evaporated in vacuo. The product was purified by flash chromatography on silica gel eluting with CH_2Cl_2 followed by MeOH/CH_2Cl_2 (1:199); yield 74 mg (88%), mp 200–201 °C, colorless crystals. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.15 (s, 3H, CH_3), 7.45 (s, 1H, H-6), 8.74 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 175 MHz) δ 36.5 (CH_3), 87.8 (C-7), 123.5 (C-4a/C-7a), 138.3 (C-6), 142.1 (C-4a/C-7a), 148.1 (C-4), 149.6 (C-2); MS EI m/z (rel.%) 249/247/245 (25/100/77, M^+), 212 (49), 210 (51), 131 (22); HRMS calcd for $C_7H_5BrClN_3$ 244.9355, found 244.9359. Anal. Calcd for $C_7H_5BrClN_3$: C, 34.11, H, 2.04, N, 17.05. Found: C, 34.10; H, 1.99; N, 17.11.

Method B: NBS (436 mg, 2.45 mmol) was added in small portions to a stirring mixture of 4-chloro-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidine **12** (410 mg, 2.45 mmol) in dry dichloromethane (10 mL) at ambient temperature under N_2-atm The resulting mixture was stirred for 2 h, diluted with dichloromethane (10 mL) and washed with water (2 × 10 mL) and brine (10 mL), dried

$MgSO_4$) and evaporated. The product was purified by flash chromatography on silica gel eluting with MeOH/CH_2Cl_2 (1:99); yield 460 mg (76%).

4.1.13. 7-Bromo-4-chloro-5-[(triisopropylsilyloxy)methyl]-5*H*-pyrrolo[3,2-*d*]pyrimidine (13b)

NaH (51 mg, ca. 1.3 mmol, ca. 60% in oil) was added to a solution of **11** (250 mg, 1.07 mmol) in DMF (4 mL) at 0 °C. The reaction mixture was stirred at this temperature under N_2-atm for 1 h, before (triisopropylsilyloxy)methyl chloride (0.30 mL, 1.3 mmol) was added dropwise. The reaction mixture was allowed to reach ambient temperature over 2 h before it was cooled to 0 °C and few drops of water were added. The solvent was evaporated in vacuo, the residue was suspended in water (40 mL) and was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$) and evaporated in vacuo. The product was purified by flash chromatography on silica gel eluting with EtOAc/hexane (1:9) followed by EtOAc/hexane (1:4); yield 380 mg (84%), mp 164–166 °C, colorless crystals. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.02 (d, J = 6.3 Hz, 18H, 6 × CH_3), 1.11–1.18 (m, 3H, 3 × CH in *i*-Pr), 6.00 (s, 2H, CH_2), 7.68 (s, 1H, H-6), 8.79 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.9 (3 × CH in *i*-Pr), 17.8 (6 × CH_3), 72.3 (CH_2), 91.7 (C-7), 123.4 (C-4a), 134.6 (C-6), 142.9 (C-7a), 149.8 (C-4), 150.7 (C-2); MS ESI 422/420/418 [*M+H*]; HRMS calcd for $C_{16}H_{25}BrClN_3OSi$+H 418.0712, found 418.0722. Anal. Calcd for $C_{16}H_{25}BrClN_3OSi$: C, 45.88; H, 6.02; N, 10.03. Found: C, 45.95; H, 6.17; N, 9.86.

4.1.14. 4-Chloro-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidine-7-carbaldehyde (14a)

To a suspension of compound **13a** (0.175 g, 0.700 mmol) in dry diethyl ether (8 mL) and anisole (3 mL) was added *n*-BuLi (0.63 mL, 0.91 mmol, 1.44 M soln) at −78 °C under N_2-atm. The reaction mixture was stirred at −78 °C for 40 min, before DMF (0.27 mL, 3.5 mmol) was added drop wise. The reaction mixture was stirred at −78 °C for an additional 1 h, was quenched by the addition of water (2 mL) and diluted with EtOAc (20 mL). The mixture was washed with water (20 mL) and the aqueous phase was extracted with EtOAc (2 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo and the product was isolated by flash chromatography eluting with CH_2Cl_2 followed by MeOH/CH_2Cl_2 (1:99); yield 115 mg (84%), mp 212–213 °C, off-white crystals. ^{1}H NMR (Me_2CO-d_6, 300 MHz) δ 4.31 (s, 3H, CH_3), 8.46 (s, 1H, H-6), 8.76 (s, 1H, H-2), 10.26 (s, 1H, CHO); ^{13}C NMR (Me_2CO-d_6, 75 MHz) δ 37.9 (CH_3), 116.9 (C-7), 142.7 (C-6), 144.2 (C-4), 151.5 (C-7a), 152.3 (C-2), 183.4 (CHO), C-4a was hidden; MS EI *m/z* (rel.%) 197/195 (4/13 M^+), 169 (32), 167 (100), 166 (39), 158 (8); HRMS cacld for $C_8H_6ClN_3O$ 195.0199, found 195.0194. Anal. Calcd for $C_8H_6ClN_3O$: C, 49.12; H, 3.09; N, 21.48. Found: C, 49.20; H, 2.95; N, 21.46. Compound **14a** was also synthesized from the iodide **13c** (130 mg, 0.44 mmol) otherwise following the same procedure; yield 59 mg (69%).

4.1.15. 4-Chloro-5-[(triisopropylsilyloxy)methyl]-5*H*-pyrrolo [3,2-d]pyrimidine-7-carbaldehyde (14b)

The product was synthesized from compound **13b** (240 mg, 0.570 mmol) as described for the synthesis of compound **14a** above and the product was isolated by flash chromatography eluting with EtOAc/hexane (1:9) followed by EtOAc/hexane (1:4); yield 180 mg, (86%), mp 140–141 °C, colorless crystals. ^{1}H NMR (Me_2CO-d_6, 300 MHz) δ 1.07 (d, J = 7.0 Hz, 18H, 6 × CH_3), 1.19–1.26 (m, 3H, 3 × CH in *i*-Pr), 6.27 (s, 2H, CH_2), 8.70 (s, 1H, H-6), 8.81 (s, 1H, H-2), 10.31 (s, 1H, CHO); ^{13}C NMR (Me_2CO-d_6, 75 MHz) δ 12.6 (3 × CH in *i*-Pr), 18.1 (6 × CH_3), 74.0 (CH_2), 117.7 (C-7), 125.1 (C-4a), 140.2 (C-6), 144.2 (C-4), 152.1 (C-7a), 152.8 (C-2), 183.8 (CHO); MS ESI 370/368 [*M+H*]; HRMS calcd for $C_{17}H_{26}ClN_3O_2Si$+H 368.1556, found 368.1568. Anal. Calcd for $C_{17}H_{26}ClN_3O_2Si$: C, 55.49; H, 7.12; N, 11.42. Found: C, 55.61; H, 7.16; N, 11.21.

4.1.16. (4-Chloro-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)(4-methoxyphenyl)methanone (15a)

LiCl (224 mg, 5.30 mmol) was dried under high vacuum for 1 h and then at 110 °C for 30 min. The flask fitted with air condenser was cooled under nitrogen and magnesium turnings (128 mg, 5.30 mmol) were introduced. A crystal of iodine and THF (7.5 mL) was added and the reaction mixture stirred vigorously. 4-Bromoanisol (0.67 mL, 5.3 mmol) was added slowly over a period of 10 min. The resulting reaction mixture was stirred at ambient temperature until the disappearance of magnesium (ca. 1 h). The resulting Grignard reagent was titrated against cyclohexanol using 1,10-phenanthroline as indicator. To a solution of compound **14a** (170 mg, 0.870 mmol) was added the 4-methoxyphenylmagnesium bromide-LiCl solution described above (2.2 mL, 1.0 mmol, 0.47 M in THF) at 0 °C under N_2-atm. The reaction mixture was stirred at 0 °C for 20 min, before benzaldehyde (0.13 mL, 1.3 mmol) was added. The resulting mixture was stirred at ambient temperature under N_2-atm for 40 h, a small amount of silica gel was added and the mixture evaporated. The residue was added on to of a flash chromatography column and the product eluted with a MeOH/CH_2Cl_2 mixture gradually increasing the amount of MeOH from 0.5% to 1%; yield 198 mg (74%), mp 199–200 °C, colorless crystals. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (s, 3H, OCH_3), 4.19 (s, 3H, NCH_3), 6.91 (d J = 8.8 Hz, 2H, Ar), 7.90 (d J = 8.8 Hz, 2H, Ar), 7.96 (s, 1H, H-6), 8.75 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 37.5 (NCH_3), 55.4 (OCH_3), 133.5 (CH in Ar), 116.4 (C-7), 124.9 (C-4a), 131.3 (C-1 in Ar), 132.2 (CH in Ar), 142.5 (C-6), 143.5 (C-4), 149.4 (C-7a), 151.4 (C-2), 163.4 (C-4 in Ar), 187.3 (CO); MS EI *m/z* (rel.%) 303/301 (29/86 M^+), 274 (37), 272 (100), 194 (36), 135 (21); HRMS cacld for $C_{15}H_{12}ClN_3O_2$ 301.0618, found 301.0617.

4.1.17. {4-Chloro-5-[(triisopropylsilyloxy)methyl]-5*H*-pyrrolo [3,2-d]pyrimidin-7-yl}(4-methoxyphenyl)methanone (15b)

The compound was synthesized from **14b** (260 mg, 0.700 mmol) following the procedure for the synthesis of **15a** above. The product was isolated by flash chromatography eluting with EtOAc/hexane (1:9) followed by EtOAc/hexane (1:4); yield 214 mg (64%), mp 135–138 °C, off-white crystals. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.05 (d, J = 6.5 Hz 18H, 6 × CH_3), 1.13–1.20 (m, 3H, 3 × CH in *i*-Pr), 3.89 (s, 3H, OCH_3), 6.08 (s, 2H, CH_2), 6.95 (d J = 8.9 Hz, 2H, Ar), 7.95 (d J = 8.9 Hz, 2H, Ar), 8.19 (s, 1H, H-6), 8.83 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 12.5 (3 × CH in *i*-Pr), 17.8 (6 × CH_3), 55.5 (OCH_3), 72.9 (CH_2), 113.6 (CH in Ar), 117.2 (C-7), 124.0 (C-4a), 131.1 (C-1 in Ar), 132.3 (CH in Ar), 140.3 (C-6), 143.3 (C-4), 150.3 (C-7a), 151.7 (C-2), 163.5 (C-4 in Ar), 187.4 (CO); MS EI *m/z* (rel.%) 473 (7, M^+), 432 (41), 430 (100), 402 (21), 400 (54), 137 (21), 135 (26); HRMS cacld for $C_{24}H_{32}ClN_3O_3Si$ 473.1901, found 473.1901. Anal. Calcd for $C_{24}H_{32}ClN_3O_3Si$: C, 60.80; H, 6.80; N, 8.86. Found: C, 60.69; H, 6.80; N, 8.60.

4.1.18. [4-(2-Furyl)-5-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl] (4-methoxyphenyl)methanone (16a)

The compound was synthesized by Stille coupling between compound **15a** (90 mg, 0.29 mmol) and 2-furyl(tributyl)tin (0.110 mL, 0.350 mmol) following the procedure for synthesis of compound **5**. The product was purified by flash chromatography on silica gel eluting with CH_2Cl_2, MeOH/CH_2Cl_2 (1:199), and finally MeOH/CH_2Cl_2 (1:99); yield 70 mg (72%), mp 252–254 °C, yellow crystals. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (s, 3H, OCH_3), 4.01 (s, 3H, NCH_3), 6.64 (dd, J = 3.5 and 1.8 Hz 1H, H-4 in furyl), 6.19 (d, J = 8.8 Hz, 2H, Ar), 7.28 (dd, J = 3.5 and 0.8 Hz, 1H, H-3 in furyl), 7.67 (br s, 1H, H-5 in furyl), 7.92 (d, J = 8.8 Hz, 2H, Ar), 7.95 (s,

1H, H-6), 9.00 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.9 (NCH_3), 55.4 (OCH_3), 112.6 (C-4 in furyl), 113.4 (CH in Ar), 114.3 (C-3 in furyl), 116.0 (C-7), 124.4 (C-4a), 131.4 (C-1 in Ar), 132.1 (CH in Ar), 140.6 (C-4), 143.3 (C-6), 144.7 (C-5 in furyl), 150.3 (C-7a), 150.5 (C-2 in furyl), 151.6 (C-2), 163.1 (C-4 in Ar), 187.7 (CO); MS EI *m/z* (rel.%) 333 (100 M^+), 332 (60), 305 (45), 304 (85), 276 (18); HRMS cacld for $C_{19}H_{15}N_3O_3$ 333.1113, found 333.1108. Anal. Calcd for $C_{19}H_{15}N_3O$: C, 68.46; H, 4.54; N, 12.61. Found: C, 66.70; H, 4.36; N, 12.21.

4.1.19. {4-(2-furyl)-5-[(triisopropylsilyloxy)methyl]-5*H*-pyrrolo [3,2-d]pyrimidin-7-yl}(4-methoxyphenyl)methanone (16b)

A mixture of compound **15b** (645 mg, 1.40 mmol), 2-furyl(tributyl)tin (0.52 mL, 1.6 mmol) and $(Ph_3P)_2PdCl_2$ (48 mg, 0.070 mmol) in DMF (4 mL) was stirred at 90 °C under N_2-atm for 18 h, and evaporated in vacuo. The residue was dissolved in MeCN (40 mL) and was washed with hexane (5 × 50 mL). Hexane (50 mL) was added to the MeCN layer and resulting mixture was stirred at ambient temperature for 1 h, the layers were separated and the MeCN layer was evaporated in vacuo. The product was isolated by flash chromatography eluting with EtOAc/hexane (1:3); yield 450 mg (65%), mp 108–110 °C, off-white crystalline solid. ^{1}H NMR (Me_2CO-d_6, 500 MHz) δ 0.93 (d, J = 7.2 Hz 18H, 6 × CH_3), 1.04–1.08 (m, 3H, 3 × CH in *i*-Pr), 3.91 (s, 3H, OCH_3), 6.28 (s, 2H, CH_2), 6.78 (dd, J = 3.5 and 1.8 Hz, 1H, H-4 in furyl), 7.04 (d, J = 8.9 Hz, 2H, Ar), 7.38 (dd, J = 3.5 and 0.9 Hz, 1H, H-3 in furyl), 7.95 (m, 3H, 2H in Ar and H-5 in furyl), 8.54 (s, 1H, H-6), 8.89 (s, 1H, H-2); ^{13}C NMR (Me_2CO-d_6, 125 MHz) δ 12.5 (3 × CH in *i*-Pr), 18.0 (6 × CH_3), 55.9 (OCH_3), 75.1 (CH_2), 113.2 (C-4 in furyl), 114.1 (CH in Ar), 114.4 (C-3 in furyl), 117.5 (C-7), 123.5 (C-4a), 132.6 (C-1 in Ar), 132.9 (CH in Ar), 141.8 (C-4), 142.6 (C-6), 146.2 (C-5 in furyl), 152.0 (C-7a), 152.2 (C-2 and C-2 in furyl), 164.2 (C-4 in Ar), 187.8 (CO); MS ESI 506 [*M+H*]; HRMS calcd for $C_{28}H_{35}N_3O_4Si$+H 506.2470, found 506.2458.

4.1.20. [4-(2-Furyl)-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl](4-methoxyphenyl)methanone (16c)

A solution of compound **16b** (225 mg, 0.440 mmol) in a saturated solution of KF in MeOH was stirred at ambient temperature for 12 h before few drops of methanolic ammonia were added and resulting mixture was stirred for 1 h at ambient temperature. The product was isolated by flash chromatography eluting with EtOAc/hexane (1:1) followed by pure EtOAc; yield 120 mg (86%), mp 230–233 °C (dec), pale yellow crystalline solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.85 (s, 3H, OCH_3), 6.84 (dd, J = 3.5 and 1.7 Hz 1H, H-4 in furyl), 7.06 (d, J = 8.8 Hz, 2H, Ar), 7.52 (d, J = 3.5, 1H, H-3 in furyl), 7.91 (d, J = 8.8 Hz, 2H, Ar), 8.09 (br s, 1H, H-5 in furyl), 8.33 (s, 1H, H-6), 8.86 (s, 1H, H-2); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 55.5 (OCH_3), 112.8 (CH in furyl), 113.1 (CH in furyl), 113.5 (CH in Ar), 115.7 (C-7), 121.1 (C-4a), 131.4 (C-1 in Ar), 131.9 (CH in Ar), 139.2 (C-4), 139.4 (C-6), 146.4 (C-5 in furyl), 148.6 (C-7a), 150.6 (C-2 in furyl), 151.4 (C-2), 162.7 (C-4 in Ar), 187.4 (CO); MS EI *m/z* (rel.%) 319 (100 M^+), 290 (70), 261 (17), 160 (9); HRMS calcd for $C_{18}H_{13}N_3O_3$ 319.0957, found 319.0957. Anal. Calcd for $C_{18}H_{13}N_3O_3$: C, 67.71; H, 4.10; N, 13.16. Found: C, 67.55; H, 4.00; N, 12.92.

4.1.21. 4-(2-Furyl)-7-(4-methoxybenzyl)-5-methyl-5*H*-pyrrolo [3,2-*d*]pyrimidine (17)

A suspension of **16a** (95 mg, 0.29 mmol) in ethylene glycol (3.0 mL) and hydrazine hydrate (0.28 mL, 5.7 mmol) was heated until a clear solution was obtained (ca. 10 min, ca. 70 °C) before crushed NaOH (228 mg, 5.70 mmol) was introduced. The resulting mixture was heated at 120 °C for 18 h before the reaction mixture was cooled to 0 °C and neutralized by dropwise addition of 10% aq HCl with stirring. After complete neutralization, water (20 mL) was added and resulting mixture was extracted with EtOAc (5 × 20 mL). The combined organic phases were washed with water (50 mL) followed by brine (50 mL), dried ($MgSO_4$) and evaporated in vacuo. The product was purified by flash chromatography on silica gel eluting with CH_2Cl_2, followed by MeOH/CH_2Cl_2 (1:199) and finally MeOH/CH_2Cl_2 (7:193); yield 65 mg, (71%), mp 114–115 °C, yellow crystalline solid. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.77 (s, 3H, OCH_3), 3.90 (s, 3H, NCH_3), 4.12 (s, 2H, CH_2), 6.65 (dd, J = 3.5 and 1.8 Hz 1H, H-4 in furyl), 6.83 (d, J = 8.7 Hz, 2H, Ar), 7.06 (s, 1H, H-6), 7.24 (d, J = 8.7 Hz, 2H, Ar),7.32 (br d, J = 3.5 Hz, 1H, H-3 in furyl), 7.66 (dd, J = 1.8 and 0.8 Hz, 1H, H-5 in furyl), 8.95 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 29.2 (CH_2), 38.2 (NCH_3), 55.7 (OCH_3), 112.9 (C-4 in furyl), 114.1 (C-3 in furyl), 114.4 (CH in Ar), 116.5 (C-7a), 124.6 (C-4a), 130.4 (CH in Ar), 133.4 (C-1 in Ar), 137.3 (C-6), 139.7 (C-4), 144.8 (C-5 in furyl), 149.6 (C-2), 151.4 (C-2 in furyl), 158.4 (C-4 in Ar), the signal from C-7 was hidden. MS EI *m/z* (rel.%) 319 (100, M^+), 318 (28), 304 (88), 288 (5), 160 (8); HRMS cacld for $C_{19}H_{15}N_3O_3$ 319.1321, found 319.1325. Anal. Calcd for $C_{19}H_{17}N_3O_2$: C, 71.46; H, 5.37; N, 13.16. Found: C, 71.18, H, 5.56; N, 12.69.

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