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An Efficient Synthesis of 2-Substituted 6-Methylpurine Bases and Nucleosides by Fe- or Pd-Catalyzed Cross-Coupling Reactions of 2,6-Dichloropurines

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Abstract: Fe-catalyzed cross-coupling reactions of 9-substituted or protected 2,6-dichloropurines with 1 equiv of methylmagnesium chloride gave regioselectively 2-chloro-6-methylpurines in good yields. The same reactions with 3 equiv of methylmagnesium chloride or Pd-catalyzed reactions with trimethylaluminum afforded 2,6-dimethylpurines. The 2-chloro-6-methylpurines underwent another coupling with phenylboronic acid to give 6-methyl-2-phenylpurines. All reactions were perfomed for Bn- and THP-protected purine bases as well as for acyl-protected ribosides and 2-deoxyribosides. After deprotection, free purine bases and nucleosides were obtained.

6-Methylpurine is highly cytotoxic.[1] Its liberation from the 2′-deoxyribonucleoside by purine nucleoside phosphorylases is used for detection of mycoplasma in cell cultures.[2] It is highly potent and toxic to nonproliferating and proliferating tumor cells. Recently, the use of cytotoxic 6-methylpurine base liberated by purine nucleoside phosphorylases from its nontoxic deoxyribonucleoside was proposed as a novel principle in the gene therapy of cancer.[3] 6-Methylpurines are also versatile starting materials for further modifications of the methyl group leading to 6-formyl-[4] or 6-halomethylpurines,[5] purine-6-carboxamides,[6] etc.

Traditionally, the 6-methylpurine bases were prepared[7,8] by low-yielding multistep heterocyclization approaches consisting in chlorination of 6-methyluracil followed by amination, nitration, reduction, and finally cyclization of the intermediate 4,5-diamino-6-methylpyrimidine with orthoformate. In 1974, the coupling of 6-chloropurines with ylides from alkyltriphenylphosphonium halides offered[9] the first straightforward method for the synthesis of 6-alkylpurines. In the last two decades, the development of cross-coupling reactions led to general and efficient methodology for the synthesis of 6-alkylpurines.[10] Thus, 6-methylpurine derivatives were prepared by Pd-catalyzed cross-couplings of 6-chloropurines with methylzinc bromide[11] or trimethylaluminum.[12] Other 6-alkylpurines were also prepared by Ni-[13] or Cu-catalyzed[14] couplings of 6-halo- or 6-methylsulfanylpurines with Grignard reagents. Very recently, Fe-catalyzed cross-couplings of Grignard reagents with aryl halides (including 6-chloropurines) have been described[15] as an efficient and general methodology.

This paper reports on the general synthesis of 2-substituted 6-methylpurines by regioselective cross-coupling methylations of 2,6-dichloropurines. In the past, only several compounds of this class have been prepared by cyclization[7,8] or by methylation of protected 2-methyl-6-chloropurine ribonucleoside with a Wittig reagent.[16] The only example of regioselective methylation of 9-benzyl-2,6-dichloropurine with methylzinc bromide leading to 9-benzyl-2-chloro-6-methylpurine was reported in a preliminary communication[17] without details. Our aim was to study and compare the regioselectivity of methylation of 2,6-dichloropurines with diverse methylorganometal reagents and to develop a practical and general methodology for the synthesis of 2-chloro-6-methylpurines as well as of some 2-C-substituted 6-methylpurines applicable both to purine bases and nucleosides.

Cross-coupling reactions of 2,6- and 6,8-dihalopurines are chemo- and regioselective.[17,18] Thus, the reactions of

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SCHEME 1

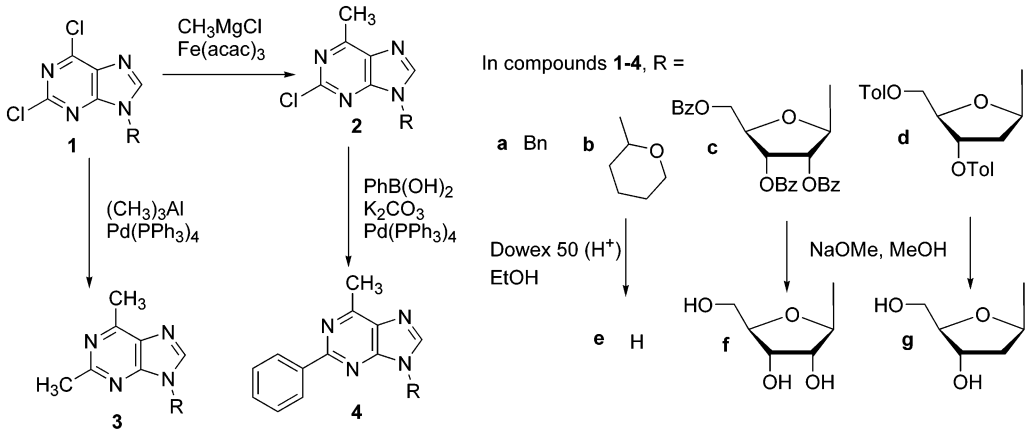

TABLE 1. Cross-Coupling Reactions of Chloropurines 1 and 2 with Organometallic Reagents

entry	starting compd	reagent	ratio	condns[a]	catalyst	yield[b] (%) 1	2	3	4
1	**1a**	MeZnBr	1:1.2	A	$Pd(PPh_3)_4$		71	15	
2	**1a**	Me_3Al	1:1	B	$Pd(PPh_3)_4$	20		46	
3	**1a**	Me_3Al	1:4	B	$Pd(PPh_3)_4$			95	
4	**1a**	MeMgCl	1:1	C (+NMP)	$Fe(acac)_3$	21	72		
5	**1a**	MeMgCl	1:1	C	$Fe(acac)_3$	25	68		
6	**1a**	MeMgCl	1:3	C (+NMP)	$Fe(acac)_3$			96	
7	**1b**	MeMgCl	1:1	C (+NMP)	$Fe(acac)_3$	28	67		
8	**1b**	MeMgCl	1:1	C	$Fe(acac)_3$	26	57		
9	**1b**	Me_3Al	1:4	B	$Pd(PPh_3)_4$			86	
10	**1c**	MeMgCl	1:1	C (+NMP)	$Fe(acac)_3$	27	60		
11	**1c**	MeMgCl	1:1	C	$Fe(acac)_3$	41	41		
12	**1c**	Me_3Al	1:4	B	$Pd(PPh_3)_4$			74	
13	**1d**	MeMgCl	1:1	C (+NMP)	$Fe(acac)_3$	23	61		
14	**1d**	MeMgCl	1:1	C	$Fe(acac)_3$	35	50		
15	**1d**	Me_3Al	1:4	B	$Pd(PPh_3)_4$			87	
16	**2a**	$PhB(OH)_2$	1:2	D	$Pd(PPh_3)_4$				98
17	**2b**	$PhB(OH)_2$	1:2	D	$Pd(PPh_3)_4$				96
18	**2c**	$PhB(OH)_2$	1:2	D	$Pd(PPh_3)_4$				73
19	**2d**	$PhB(OH)_2$	1:2	D	$Pd(PPh_3)_4$				89

[a] Conditions: (A) THF, 50 °C; (B) THF, 75 °C; (C) THF, rt; (D) toluene, K_2CO_3, 90 °C. [b] Isolated yields.

2,6- or 6,8-dichloropurines with 1 equiv of an organometallic reagent lead to regioselective substitution in the position 6. On the other hand, 2- or 8-iodo-6-chloropurines react chemoselectively in the position of the iodine (2 or 8). These reactions were performed with arylboronic acids, aryl- and alkenylstannanes, and to certain extent, aryl- or alkylzinc halides.

The above-mentioned preliminary communication[17] reported a regioselective Pd-catalyzed methylation of 9-benzyl-2,6-dichloropurine (**1a**) with methylzinc bromide (formed in situ from methylmagnesium chloride and $ZnBr_2$) to form the 2-chloro-6-methylpurine in 69% yield. First of all, we have reproduced this reaction with 1.2 equiv of MeZnBr to confirm the formation of the desired 2-chloro-6-methylpurine **2a** in 71% yield (Scheme 1, Table 1, entry 1) accompanied by the 2,6-dimethylpurine **3a** (14%). The next atempt was the cross-coupling of **1a** with 1.2 equiv of trimethylaluminum. This reaction gave a complex mixture containing the 9-benzyl-2,6-dimethylpurine (**3a**) as a major product (46%) accompanied by some unreacted starting compound (20%) and hydrolytic products (entry 2). If this reaction was performed with 4 equiv of Me_3Al, the 2,6-dimethylpurine **3a** was isolated in an excellent yield of 95% (entry 3). Finally, the application of the Fürstner's Fe-catalyzed coupling[15] turned out to be the most practical for the regioselective methylation of 2,6-dichloropurines. Thus, the reaction of **1a** with 1 equiv of methylmagnesium chloride in the presence of a catalytic amount of $Fe(acac)_3$ in THF containing *N*-methylpyrrolidinone (NMP) at ambient temperature gave the 2-chloro-6-methylpurine **2a** in 72% yield accompanied by the unreacted starting compound **1a** (21%) (entry 4). The presence of NMP was beneficial but not crucial for this reaction—analogous reaction in absence of NMP gave a mixture of **2a** (68%) and **1a** (25%) (entry 5). When using 1.2 equiv of the Grignard, the yield of **2a** remained in the same level but the reaction mixture contained also some 2,6-dimethylpurine **3a** (ca. 10%)—as the unreacted **1a** can be easily separated and reused, it is more practical to use just one equivalent of the reagent. The Fe-catalyzed reaction of **1a** with 3 equiv of MeMgCl gave the dimethylpurine **3a** in an excellent yield of 96% (entry 6).

As the Fe-catalyzed coupling of Grignard reagent was found to be quite practical for the synthesis of 2-chloro-6-methylpurines and both couplings with excess of trimethylaluminum or Grignard were suitable for the synthesis of 2,6-dimethylpurines, these methods were further explored in order to prepare the corresponding 2-substituted 6-methylpurine bases and nucleosides. The starting THP-protected 2,6-dichloropurine **1b**,[19] benzoyl-protected 2,6-dichloropurine riboside **1c**[18d] and toluoyl protected deoxyriboside **1d**[20] are known compounds. Analogously to **1a**, all protected 2,6-dichloropurines **1b**−**d** were subjected to the reactions with 1 equiv of MeMgCl under $Fe(acac)_3$ catalysis or with 4 equiv of Me_3Al under $Pd(PPh_3)_4$ catalysis (entries 7−15). In general, the reactions of **1b**−**d** with 1 equiv of MeMgCl in the presence of NMP gave the desired 2-chloro-6-methylpurines **2b**−**d** in about 60% yield, while in absence of NMP the yields of **2** were somewhat lower. The products **2b**−**d** were easily separated from the starting compound by column

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TABLE 2. Deprotection of Compounds 2b−4d

entry	starting compd	conditions[a]	product	yield[b] (%)
1	**2b**	E	**2e**	79
2	**3b**	E	**3e**	68
3	**4b**	E	**4e**	84
4	**2c**	F	**2f**	92
5	**3c**	F	**3f**	83
6	**4c**	F	**4f**	86
7	**2d**	F	**2g**	98
8	**3d**	F	**3g**	97
9	**4d**	F	**4g**	80

[a] Conditions: (E) Dowex 50X8 (H^+ form), EtOH, H_2O, reflux; (F) NaOMe, MeOH. [b] Isolated yields after crystallization.

chromatography and the starting compounds were reused. The Pd-catalyzed reactions of **1b−d** with trimethylaluminum gave the 2,6-dimethylpurines **3b−d** in good yields. The 2-chloro-6-methylpurines **2a−d** are good intermediates for further derivatizations in the position 2. It was demonstrated by the Suzuki−Miyaura cross-coupling reactions[21] with phenylboronic acid that furnished a series of protected 6-methyl-2-phenylpurine bases and nucleosides **4a−d** (entries 16−19) in very good yields.

The THP-protected purines **2b−b** were easily deprotected[22] making use of wet cation exchanger Dowex 50X8 (H^+ form) in boiling ethanol to give the free purine bases **2e−4e** by simple filtering off the ionex, evaporation of the filtrate and crystallization. The acyl-protected nucleosides **2c−4c** and **2d−4d** were cleaved by catalytic amount of sodium methoxide in dry methanol to give the free ribonucleosides **2f−4f** and deoxyribonucleosides **2g−4g**. The yields of the deprotection reactions are summarized in Table 2.

Though the regioselectivity of other cross-coupling reactions of 2,6-dichloropurines has been proved previously by means of long-range HETCOR[17] or $^1H-^{15}N$ HMBC[18e] spectra, an additional independent proof for these particular methylations was based on $^1H-^{13}C$ HMBC spectrum of compound **2a**. A charactetistic cross-peak of CH_3 to C-5 and C-6 signals has been found indicating the presence of the methyl group in the position 6. All other compounds were fully characterized by ^1H and ^{13}C NMR (assignment based on COSY and $^1H-^{13}C$ HMBC and HMQC experiments), MS, and microanalyses.

In conclusion, the application of the Fürstner Fe-catalyzed cross-coupling reaction of 2,6-dichloropurines with 1 equiv of methylmagnesium chloride is a practical method for the synthesis of 2-chloro-6-methylpurines, while the use of Pd-catalyzed reaction with trimethylaluminum is a good method for the synthesis of 2,6-dimethylpurines. The Fe-catalyzed cross-couplings are of comparable efficiency but much cheaper than the Pd-catalyzed ones. The 2-chloro-6-methylpurines could be used for further substitutions in the position 2 as demonstrated by the Suzuki−Miyaura reactions leading to 6-methyl-2-phenylpurines. The methods are easily applied for the synthesis of free purine bases, as well as for ribonucleosides and 2-deoxyribonucleosides. Preliminary cytostatic activity screening of compounds **2−4** on their in vitro inhibition of the cell growth in the following cell cultures did not show any considerable activity: mouse leukemia L1210 cells (ATCC CCL 219); human promyelocytic leukemia HL60 cells (ATCC CCL 240); human cervix carcinoma HeLa S3 cells (ATCC CCL 2.2); and human T lymphoblastoid CCRF−CEM cell line (ATCC CCL 119). However, this practical and versatile methodology could find much wider applications in the synthesis of other derivatives of the important class of 6-methylpurines.

Experimental Section

General Procedures. Cross-Coupling Reaction of 2,6-Dichloropurine 1a with Methylzinc Bromide (Method A). MeMgCl (3 M solution in THF, 1.2 mL, 3.6 mmol) was added dropwise to a stirred solution of $ZnBr_2$ (810 mg, 3.6 mmol) in THF (10 mL) at −78 °C under Ar, the stirring was continued for 30 min at −78 °C, and then the mixture was allowed to reach 0 °C. Then a solution of **1a** (837 mg, 3 mmol) and $Pd(PPh_3)_4$ (180 mg, 0.15 mmol) in THF (10 mL) was added, and the resulting reaction mixture was stirred at 50 °C for 8 h. The mixture was cooled to rt and poured onto a mixture of ice (∼100 mL) and NH_4Cl (1 g), and the products were exctracted with chloroform (3 × 100 mL). Evaporation of the organic phase followed by a column chromatography on silica gel (100 g, ethyl acetate/hexanes 1:1 → ethyl acetate → ethyl acetate/MeOH 9:1) afforded the products.

Cross-Coupling Reactions of 2,6-Dichloropurines 1 with Trimethylaluminum (Method B). Me_3Al (2 M solution in toluene, 0.5 mL, 1 mmol or 2 mL, 4 mmol) was added dropwise to a stirred solution of **1** (3 mmol) and $Pd(PPh_3)_4$ (180 mg, 0.15 mmol) in THF (20 mL) under Ar, and the resulting reaction mixture was stirred at 75 °C for 8 h. The workup and isolation was the same as in method A.

Cross-Coupling Reactions of 2,6-Dichloropurines 1 with Methylmagnesium Chloride (Method C). MeMgCl (3 M solution in THF, 0.33 mL, 1 mmol or 1 mL, 3 mmol) was added dropwise to a stirred solution of **1** (3 mmol) and $Fe(acac)_3$ (103 mg, 0.29 mmol) in THF (20 mL) either in the presence or in the absence of NMP (1 mL) under Ar, and the resulting reaction mixture was stirred at rt for 8 h. The workup and isolation was the same as in method A.

Cross-Coupling Reactions of 2-Chloro-6-methylpurines 2 with Phenylboronic Acid (Method D). Toluene (10 mL) was added to an argon-purged flask containing the chloropurine (1 mmol), K_2CO_3 (200 mg, 1.5 mmol), phenylboronic acid (220 mg, 2 mmol), and $Pd(PPh_3)_4$ (59 mg, 0.05 mmol), and the mixture was stirred under Ar at 100 °C for 8 h. After the mixture was cooled to ambient temperature, the solvent was evaporated in vacuo and the residue was chromatographed as in method A.

Cleavage of the THP-Protected Purines (Method E). A mixture of a THP-protected base **2b−4b** (0.6−0.8 mmol), Dowex 50X8 (H^+) (ca. 300 mg), ethanol (50 mL), and water (1 mL) was refluxed for 1 h and then filtered while hot, and the resin was washed with hot ethanol (2 × 50 mL). The combined filtrates were evaporated, and the residue was codistilled with toluene. Crystallization of the residue from methanol/toluene with an addition of heptane afforded the free bases **2e−4e**.

Deacylation of Nucleosides (Method F). A 1 M methanolic MeONa (100 μL, 0.1 mmol) was added to a solution of a protected nucleoside **2c−4c or 2d−4d** (0.5−1 mmol) in MeOH (20 mL), and the mixture was stirred at ambient temperature overnight. The solvent was evaporated, and the residue was chromatographed on a column (silica gel 50 g, ethyl acetate/MeOH 9:1−8:2). The crude products were recrystallized from EtOH/toluene/heptane to give free nucleosides.

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Yields of the particular products are summarized in Tables 1 and 2, and the characterization data are in the Supporting Information.

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Supporting Information Available: Complete characterization and spectroscopic data for compounds **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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