Purines bearing carbon substituents in the position 2, 6 and/or 8 are extensively studied as biologically active compounds and as tools in chemical biology. They are efficiently prepared by cross-coupling reactions of halopurines with diverse organometallics. Also 2,6- and 6,8-disubstituted purines could be prepared by cross-coupling reactions of dihalopurines. General regioselectivity of these reactions differs by the type of the dihalopurines. While the 2,6- and 6,8-dichloropurines react in the position of the better leaving group (I > Br > Cl), the (more reactive) position 6, chloro- bromo- and iodopurines react in the position of the better leaving group (I > Br > Cl). In this way, by the selection of a proper starting compound, regioselective reactions can be accomplished either in position 2, 6 or 8, leaving the other halogen (Cl) available for another coupling or nucleophilic substitution. This approach has been recently used for the synthesis of 2- or 8-substituted 6-phenylpurine nucleosides, and carba-analogues of Myoseverin.

As a continuation of the systematic study of regioselective cross-couplings of dihalopurines, we have focused on the synthesis of 8-substituted derivatives of important 6-phenyl- and 6-methylpurines. 6-Phenylpurine derivatives were found to exhibit cytostatic, antimycobacterial and antibacterial activity, while 6-methylpurine is a strongly cytotoxic compound considered for application in gene therapy in the form of its non-toxic 2-deoxyribonucleoside prodrug. The synthesis of 8-substituted derivatives of these purines should not only extend the knowledge of SAR (structure activity relationship) of this class of compounds but also complement the known regioselective cross-couplings of 6,8-dichloropurines with stannanes by hitherto unpublished reactions with boronic acids and Grignard reagents.

9-(Tetrahydropryan-2-yl)-protected 6,8-dichloropurine (1) was chosen as the starting compound for the regioselective cross-coupling reactions (Scheme 1). The first reaction under study was the Suzuki–Miyaura coupling with phenylboronic acid. The reaction of dichloropurine with 1.1 equivalents of PhB(OH)2 under [Pd(PPh3)4] catalysis in toluene gave a mixture of the expected 8-chloro-6-phenylpurine in an acceptable yield of 60% followed by 6,8-diphenylpurine (11%) and traces of the unreacted starting compound (6%). The analogous reaction of 1 with four equivalents of PhB(OH)2 afforded the protected 6,8-diphenylpurine in 67% yield.

Fe-catalyzed reactions of aryl halides with Grignard reagents were recently developed as an efficient cross-coupling methodology. It was also found to be superior for regioselective methylation of 2,6-dichloropurines and our next goal was to study analogous reaction of 6,8-dichloropurines. Thus the reaction of 1 with 1.1 equivalents of methylmagnesium chloride in the presence of Fe(acac)3 gave unexpectedly 6-chloro-8-methyl-9-(tetrahydropryan-2-yl)purine (3a) in a moderate yield of 37%, followed by 6,8-dimethylpurine (14%) and unreacted starting compound (34%). As the products were easily separable by column chromatography and the starting compound was recovered, despite the moderate yield, this reaction is still practical for the synthesis of 3a. Reaction of 1 with 3–5 equivalents of MeMgCl gave mixtures of products, while finally the use of 9 equivalents of MeMgCl gave complete conversion to 6,8-dimethylpurine in 90% yield.

The dichotomy in regioselectivity of coupling reactions of 1 with phenylboronic acid (leading to 6-substitution) and with methylmagnesium chloride (leading to unexpected 8-substitution) is very interesting. Our next goal was to...
study the scope and to explain the course of the unexpected Fe-catalyzed reaction. Attempted reaction of 1 with one equivalent of phenylmagnesium chloride afforded a complex mixture of products in which two products [6,8-dibenzyl-9-(tetrahydropryan-2-yl)purine and 6-benzyl-8-chloro-9-(tetrahydropryan-2-yl)purine] were identified (but not completely characterized).\(^\text{15}\) Apparently, the scope of a synthetically useful application of this regioselective cross-coupling is limited to methylmagnesium chloride.

One of the possible explanations for the direction of the Fe-catalyzed reaction to position 8 was a complexation of either the Grignard reagent or the proposed catalytic species [Fe(MgX)\(_2\)] with the THP-oxygen. A model experiment reacting 9-benzyl-6,8-dichloropurine 4 (6, lacking the oxygen atom in proximity to the position 8) with one equivalent of methylmagnesium chloride was performed (Scheme 2). Major product of this reaction was 9-benzyl-6-chloro-8-methylpurine (7, 29%), accompanied by a minor amount (<10%) of an inseparable complex mixture of other products and recovered starting compound (45%). This result excludes the effect of the oxygen and thus the mechanism remains unclear.

![Scheme 2](image)

Both protected monochloropurines 2a and 3a could be used in another cross-coupling or nucleophilic substitution reaction. Thus 8-chloro-6-phenyl-9-(tetrahydropryan-2-yl)purine (2a) was subjected to the Pd-catalyzed reaction with BnZnCl or to the Fe-catalyzed reaction with MeMgCl to give the corresponding 8-benzyl-2d or 8-methylpurines 2c in good yields of 89 or 77%, respectively. Attempted ammonolysis of 2a in methanolic ammonia gave a mixture of 8-methoxy-2f (58%) and 8-aminopurine 2e (34%) derivatives. The use of ethanolic ammonia did not lead to any 8-ethoxypurine side-product and gave the 8-amino derivative 2e in 69% yield. Analogously, the 6-chloro-8-methyl-9-(tetrahydropryan-2-yl)purine (3a) was subjected to the Pd-catalyzed reactions with PhB(OH)\(_2\) or BnZnCl to give the corresponding 6-phenyl-

### Table 1  Regioselective Cross-Coupling Reactions of 6,8-Dichloropurines

<table>
<thead>
<tr>
<th>Entry</th>
<th>Dichloropurine</th>
<th>Reagent</th>
<th>Ratio</th>
<th>Products, Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>PhB(OH)(_2)(^a)</td>
<td>1:1.1</td>
<td>2a (60) 2b (11) 1 (6)</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>PhB(OH)(_2)(^a)</td>
<td>1:4</td>
<td>– 2b (67) –</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>MeMgCl(^b)</td>
<td>1:1.1</td>
<td>3a (37) 3e (14) 1 (34)</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>MeMgCl(^b)</td>
<td>1:9</td>
<td>– 3e (90) –</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>MeMgCl(^b)</td>
<td>1:1.1</td>
<td>7 (29) – 6 (45)</td>
</tr>
</tbody>
</table>

\(^a\) Catalyzed by Pd(PPh\(_3\))\(_4\).

\(^b\) Catalyzed by Fe(acac)\(_3\).
3b (88%) or 6-benzylpurine 3d (96%) derivatives in good yields. Ammonolysis of 3a in ethanolic ammonia gave the protected 8-methyladenine 3e in 59% yield accompanied by some polar easily separable by-products (no 6-ethoxy derivative was detected).

All the THP protected derivatives 2a–f and 3a–e were depleted by reflux of their ethanolic solutions in the presence of a catalytic amount of wet Dowex 50WX8 (H+ form) to give the 8-substituted 6-phenylpurine bases 4a–f and 6-substituted 8-methylpurine bases 5a–e in good yields.

In order to verify the structures of the 6,8-disubstituted purines (in particular, the structure of unexpected 6-chloro-8-methylpurine 3a), several NMR experiments were performed in several model compounds. For the key intermediate 3a, 1H-13C HMBC showed clear cross-peaks of OCHN to C-4 and C-8 and of CH3 to C-8. Moreover, significant NOE interactions between CH3 and THP-methyl-8 and for substitution. In 6-phenyl-8-chloropurine derivative 5, many purine (in particular quaternary) carbon signals were extremely weak and some even did not appear in their 13C NMR spectra (albeit combinations of several experiments).

In conclusion, the Suzuki–Miyaura cross-coupling reaction of THP-protected 6,8-dichloropurine (1) with one equivalent of phenylboronic acid follows the same regioselectivity as reported for the reactions of 1 with stananes to give the 6-phenyl-8-chloropurine 2a as the major product accompanied by minor amounts of the dissubstituted derivative 2b. On the other hand, Fe-catalyzed coupling of 1 with one equivalent of methylenemagnesium chloride proceeded with lower conversion to give the 6-chloro-8-methylpurine 3a in a moderate yield of 37%. As substantial amounts of the starting compound could be recovered, even this lower yielding reaction could be used for the practical synthesis of 3a. Both monochloropurines 2a and 3a could be used for another cross-coupling or nucleophilic substitution to give, after deprotection, a series of 8-substituted-6-phenylpurines 4 or 6-substituted-8-methylpurines 5 in good yields. None of the purine bases 4 and 5 showed any considerable cytostatic activity (in vitro inhibition of the cell growth in the following cell cultures: 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)).

Unless otherwise stated, solvents were evaporated at 40 °C/2 kPa and compounds were dried at 60 °C/2 kPa. Melting points were determined on a Kofer block and are uncorrected. NMR spectra were measured on Bruker AMX-3 400 (400 MHz for 1H and 100.6 MHz for 13C nuclei), and Bruker DRX 500 spectrometers (500 MHz for 1H and 125.8 MHz for 13C). TMS was used as an internal standard. Mass spectra were measured on ZAB-SEQ (VG Analytical). Microanalyses were performed on a Perkin-Elmer 240-II CHN Analyzer. Silica gel (ICN SiliTech, 32–63) was used for column chromatography. Toluen was degassed in vacuo and stored over molecular sieves under argon. THF was refluxed with Na and benzophenone under argon and freshly distilled prior to use. Methylmagnesium chloride, benzylimagemine magnesium chloride, phenylmagnesium bromide and benzylzinc chloride were commercial solutions in THF (Aldrich).

Cross-Coupling Reaction of Chloropurines with Methylmagnesium Chloride

Method A: MeMgCl (3 M solution in THF, 0.33 mL, 1 mmol, 1 mL, 3 mmol or 3 mL, 9 mmol) was added dropwise to a stirred solution of a chloropurine (1 mmol) and Fe(acac)3 (103 mg, 0.29 mmol) in THF (20 mL) and NMP (1 mL) under Ar and the resulting reaction mixture was stirred at r.t. for 8 h. Then the mixture was poured onto a mixture of ice (ca. 100 mL) and NH4Cl (1 g) and the products were extracted with CHCl3 (3 × 100 mL). Evaporation of the organic phase followed by a column chromatography on silica gel (100 g, EtOAc–hexanes, 1:3 → EtOAc) afforded the products.

Cross-Coupling Reaction of Chloropurines with Phenylboronic Acid

Method B: Toluene (10 mL) was added to an argon-purged flask containing a chloropurine (1 mmol), K2CO3 (300 mg, 2.2 mmol), phenylboronic acid (122 mg, 1 mmol, 366 mg, 4 mmol or 488 mg, 5 mmol) and Pd(PPh3)4 (59 mg, 0.05 mmol) and the mixture was stirred under argon at 100 °C for 8 h. After cooling to r.t., the solvent was evaporated in vacuo and the residue was chromatographed as in Method A.

Cross-Coupling Reaction of Chloropurines with Benzylzinc Bromide

Method C: BnZnCl (0.5 M solution in THF, 6 mL, 3 mmol or 2.2 mL, 1.1 mmol) was added dropwise to a stirred solution of a chloropurine (1 mmol) and Pd(PPh3)4 (60 mg, 0.05 mmol) in THF (20 mL) and the mixture was stirred at 80 °C for 8 h. Then the mixture was cooled to rt and poured onto a mixture of ice (ca. 100 mL) and NH4Cl (1 g) and the products were isolated in the same way as in Method A.

Ammonolysis of Chloropurines

Method D: A mixture of a chloropurine (1 mmol) in sat. methanolic or ethanolic ammonia (30 mL) was heated in a sealed tube at 80 °C for 15–24 h. The solvent was evaporated and the residue chromatographed as in Method A.

Cleavage of the THP-Protected Purines

Method E: A mixture of a THP-protected base 2 or 3 (0.5–1.5 mmol), Dowex 50WX8 (H+) (ca. 300 mg), EtOH (50 mL) and H2O (1 mL) was refluxed for 1 h, then filtered while hot and the resin was washed with hot EtOH (2 × 50 mL). The combined filtrates were evaporated and the residue codistilled with toluene. Crystallization of the residue afforded the free bases 4 or 5.

8-Chloro-6-phenyl-9-(tetrahydropyran-2-y)purine (2a)

Prepared from 1 (273 mg, 1 mmol) by Method B [1 equiv of PhBH(OH)2] in 60% yield (188 mg); colorless foam.

[1] H NMR (CDCl3, 500 MHz): δ = 1.63–1.93, 2.13–2.15 and 3.00–3.10 (5 H, CH3), 3.57–3.63 (dh, 1 H, J = 11.9, 2.0 Hz, H-5′a), 4.21–4.25 (m, 1 H, H-5′b), 5.82 (dd, 1 H, J = 11.3, 2.5 Hz, H-1′), 7.51–7.56 and 8.69–8.72 (5 H, H-2′, 3′, 4′, 5′ and 6′, 3H, arom), 9.00 (s, 1 H, H-2).

[2] 13C NMR (APT, CDCl3, 125.8 MHz): δ = 23.3, 24.7 and 28.9 (CH3), 69.4 (CH2O), 83.9 (NCHO), 128.7, 129.7 and 131.1 (CH arom), 129.9

Synthesis 2004, No. 6, 889–894 © Thieme Stuttgart · New York
HMBC cross-peaks: NCHO to C-4 and C-8, o-CH$_{arom}$ to C-6.

EI-MS: $m/z$ (%) = 470 (35), 286 (11), 270 (100). EIM-HRMS: calcd for C$_{16}$H$_{12}$N$_2$: 286.1100; found: 286.1095.

1H NMR (CDCl$_3$, 500 MHz): $\delta$ = 1.64–1.68, 1.93–1.99 and 2.37–2.42 (m, 6 H, CH$_3$), 3.73–3.64 (m, 1 H, H-5'a), 4.16–4.20 (m, 1 H, H-5'b), 4.54 (dd, 2 H, J = 5.7, 14.4 Hz, CH$_2$Ph), 5.68 (dd, 1 H, J = 11.1, 2.0 Hz, H-1'), 7.24–7.35, 7.50–7.57 and 8.79–8.82 (m, 10H$_{arom}$), 8.97 (s, 1 H, H-2).

1C NMR (APT, CDCl$_3$, 125.8 MHz): $\delta$ = 23.2, 24.8 and 29.9 (CH$_3$), 35.7 (CH$_2$Ph), 69.2 (CH$_2$O), 82.7 (NCHO), 127.0, 128.6, 128.7, 129.8, 130.6 (C$_{arom}$ and overlapped C$_{ipso}$), 135.9 (C-5), 151.5 (CH-2), 153.4 and 153.9 (C-8, C-4 and C-6). NOE: CH$_3$ to NCHO.

EI-MS: $m/z$ (%) = 458 (10), 211 (100), 85 (21). EI-HRMS: $m/z$ (%) = 458 (7), 211 (100), 85 (12). EI-HRMS: $m/z$ (%) = 458 (7), 211 (100), 85 (12). EI-HRMS: $m/z$ (%) = 458 (7), 211 (100), 85 (12). EI-HRMS: $m/z$ (%) = 458 (7), 211 (100), 85 (12).
1H, J = 2.2, 11.7 Hz, H-5'a), 4.17–4.22 (m, 1 H, H-5'b), 4.48 (s, 2 H, CH2Ph), 5.79 (dd, 1 H, J = 11.3, 2.3 Hz, H-1'), 7.16–7.70 (m, 5 H, arom), 8.80 (s, 1 H, H-2).

13C NMR (APT, CDCl3, 127.8 MHz): δ = 16.3 (CH3), 23.2, 24.9 and 30.2 (CH2), 39.0 (CH2Ph), 69.2 (CH2O), 82.8 (NCHO), 126.5, 128.4, 129.3 (CHarom), 131.6 (C-5), 138.0 (Cipso), 151.7 (CH-2), 152.1 (C-4), 153.6 (C-8), 156.8 (C-6).

HMBC cross-peaks: CH3 to C-8, CH2Ph to C-6 and C-5, NCHO to C-4 and C-8.

EI-MS: m/z (%) = 308 (51), 277 (56), 223 (100), 85 (20).

EI-HRMS: m/z calcd for C11H12N4O: 238.1637; found: 238.1632.

6-Amino-8-methyl-9-(tetrahydropyran-2-yl)purine (3e)
Prepared from 3a (253 mg, 1 mmol) by Method D (ethanolic ammonia) in 59% yield (137 mg); colorless oil.

1H NMR (CDCl3, 400 MHz): δ = 1.50–2.00 and 2.45–2.62 (m, 6 H, CH2), 2.59 (s, 3 H, CH3), 3.67 (br t, 1 H, CH2Ph), 4.04 (d, 1 H, J = 10.6 Hz, H-5'b), 4.07 (d, 1 H, J = 10.4 Hz, H-1'), 7.07 (s, 2 H, NH2), 8.09 (s, 1 H, H-2).

13C NMR (APT, CDCl3, 100.6 MHz): C-4 and C-8.

EI-MS: m/z (%) = 233 (7), 149 (100), 122 (10), 85 (10).


9-Benzyl-6-chloro-8-methylpurine (7)
Prepared from 6 (278 mg, 1 mmol) by Method A (1:1 equiv of MeMgCl) in 29% yield (75 mg); colorless foam.

1H NMR (CDCl3, 500 MHz): δ = 2.61 (s, 3 H, CH3), 5.45 (s, 2 H, CH2Ph), 7.17–7.35 (m, 5 H, arom), 8.72 (H-2).

13C NMR (APT, CDCl3, 100.6 MHz): δ = 14.8 (CH3), 46.5 (CH2), 127.1, 128.5 and 129.2 (CHarom), 134.7 (Cipso), 149.0 (C-6), 151.4 (CH-2), 153.3 (C-4) and 153.5 (C-8).

HMBC cross-peaks: CH3Ph with C-8 and C-4, Me with C-8.

FAB-MS: m/z (%) = 250 (45), 91 (100).

FAB-HRMS: m/z calcd for C13H12ClN4: 259.0750; found: 259.0739 [M + H+].

8-Chloroo-6-phenylpurine (4a)
Prepared from 2a (157 mg, 0.5 mmol) by Method E in 87% yield (100 mg); colorless crystals; mp 288–290 °C (MeOH–toluene–heptane).

1H NMR (DMSO-d6, 400 MHz): δ = 2.62 (s, 3 H, CH3), 7.53–7.60 (m, 3 Harom), 8.70–8.80 (br m, 2 Harom), 8.85 (s, 1 H, H-2), ca. 13.2 (br s, 1 H, NH).

13C NMR (APT, DMSO-d6, 100.6 MHz): δ = 15.1 (CH3), 128.5, 129.0 and 130.4 (CHarom), 153.8 (C), 151.0 (CH-2). Other quaternary carbon signals did not appear due to tautomerism.

EI-MS: m/z (%) = 238 (100), 169 (53), 142 (15).


8-Benzyl-6-phenylpurine (4d)
Prepared from 2d (300 mg, 0.81 mmol) by Method E in 85% yield (197 mg); colorless crystals; mp 244–246 °C (MeOH–toluene–heptane); colorless crystals.

1H NMR (CDCl3, 400 MHz): δ = 4.30 (s, 2 H, CH2Ph), 7.23–7.60 (m, 8 H, arom), 8.74–8.82 (br m, 2 Harom), 8.87 (s, 1 H, H-2), ca. 13.4 (br s, 1 H, NH).

13C NMR (APT, CDCl3, 100.6 MHz): δ = 35.1 (CH2), 126.7, 128.5, 128.7, 129.1 and 130.6 (CHarom), 135.7 and 136.6 (Cipso), 151.3 (CH-2). Quaternary carbon signals did not appear due to tautomerism.

EI-MS: m/z (%) = 286 (100), 91 (25), 57 (31).

EI-HRMS: m/z calcd for C13H12N4: 286.1218; found: 286.1221.


8-Amino-6-phenylpurine (4e)
Prepared from 2e (148 mg) by Method E in 61% yield (64 mg); colorless crystals; mp >300 °C (MeOH–H2O); colorless crystals.

1H NMR (DMSO-d6, 400 MHz): δ = 6.96 (s, 2 H, NH2), 7.42–7.54 (m, 3 Harom), 8.53 (s, 1 H, H-2), 8.50–8.70 (br m, 2 Harom), 11.94 (br s, 1 H, NH).

13C NMR (APT, DMSO-d6, 100.6 MHz): δ = 128.2 and 129.1 (CHarom), 136.7 (C). Other quaternary carbon signals did not appear due to tautomerism.

EI-MS: m/z (%) = 211 (80), 184 (35), 57 (95), 43 (100).

EI-HRMS: m/z calcd for C13H12N4: 211.0858; found: 211.0860.

8-Methoxy-6-phenylpurine (4f)
Prepared from 2f (155 mg, 0.5 mmol) by Method E in 69% yield (78 mg); colorless crystals; mp 201–203 °C (MeOH–H2O).

1H NMR (DMSO-d6, 400 MHz): δ = 4.19 (s, 3 H, CH3), 7.50–7.57 (m, 3 Harom), 8.70 (br m, 2 Harom), 8.74 (s, 1 H, H-2).

13C NMR (APT, DMSO-d6, 100.6 MHz): δ = 57.2 (CH3), 128.5, 128.7 and 130.2 (CHarom), 135.8 (Cipso), 150.2 (CH-2). Other quaternary carbon signals did not appear due to tautomerism.

EI-MS: m/z (%) = 226 (100), 211 (60).

Synthesis 2004, No. 6, 889–894 © Thieme Stuttgart · New York
6-Chloro-8-methylpurine (5a)\textsuperscript{17}  
Prepared from 3a (270 mg, 1.1 mmol) by Method E in 78% yield (175 mg); colorless crystals; mp 204–208 °C (MeOH–toluene–heptane).

Found: C, 63.56; H, 4.54; N, 24.36.  
Anal. Calcd for C\textsubscript{13}H\textsubscript{13}N\textsubscript{4}: C, 69.99; H, 5.50; N, 24.51.

References

(15) Holec M.; Dvořáková H.; unpublished results.