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Facile and Efficient Synthesis of 6-(Hydroxymethyl)purines

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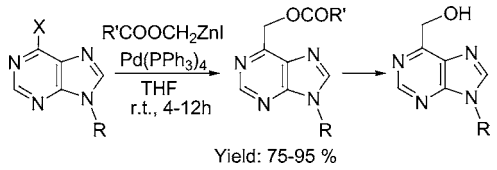
ABSTRACT



A facile and efficient methodology of the synthesis of 6-(hydroxymethyl)purine derivatives (bases and nucleosides) was developed based on Pd-catalyzed cross-coupling reactions of 6-halopurines with acyloxymethylzinc iodides followed by deprotection. Several title compounds are inhibitors of adenosine deaminase and exert cytostatic activity.

Purines bearing carbon substituents in position 6 possess a broad spectrum of biological activities. 6-(Arylalkynyl)-, 6-(arylalkenyl)-, and 6-(arylalkyl)purines show cytokinin[1] and antioxidant[2] activity, and 9-benzyl-6-arylpurines exhibit antimycobacterial, antibacterial, and cytotoxic effects.[3] 6-Aryl and 6-(arylakyl)purine ribonucleosides,[4] as well as some arylalkynyl-9-benzylpurines,[5] display significant cytostatic activity. However, little is known about the biological activity of purines bearing functionalized carbon substituents.

6-(Hydroxymethyl)purine can be considered a homologue of hypoxanthine and a potential transition-state analogue for adenosine deaminase.[6] 6-Hydroxymethyl-9-(β-D-ribofuranosyl)purine isolated from *Collybia maculata* was reported to possess antifungal, cytotoxic, and antiviral (vesicular stomatitis virus) properties.[7] Furthermore, Wolfenden et al. reported this compound as an adenosine deaminase inhibitor.[8] Synthetic approaches to 6-(hydroxymethyl)purines published so far are based on the radical photoaddition of methanol to unsubstituted purine in aerobic conditions[7a,9] or on the recently reported reaction of 6-magnesiated purine with paraformaldehyde.[10] Unfortunately, the photoaddition in aerobic condition proceeds with low chemoselectivity and a very complex reaction profile,[8b] whereas in an argon atmosphere, the main product is 1,6-dihydro-6-(hydroxymethyl)purine, which can be oxidatively converted on the 6-hydroxymethyl purine.[8b] The yield of nucleophilic addition of 6-magnesiated purine to formaldehyde is low because of low reactivity of the purinylmagnesium reagent.[10] On the other hand, 6-lithiated purine is much more reactive but is stable only for a few minutes at −130 °C.[11] Indirect routes

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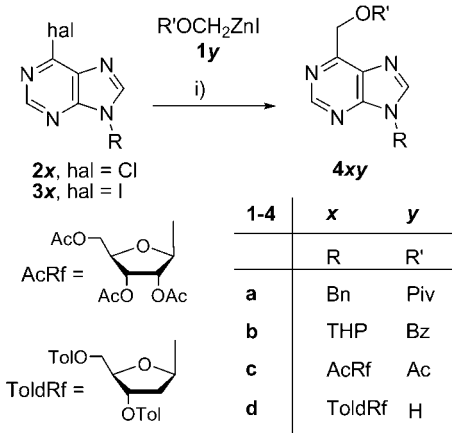
from 6-methylpurine: oxidation to aldehyde followed by reduction[12] or rearrangement of its *N*-oxide with Ac_2O to 6-(acetoxymethyl)purine[13] gave low yields.

Cross-coupling reactions of halopurines with various organometallics is an efficient approach for the preparation of purines bearing carbon substituents in the position 2, 6, or 8.[14] However, the use of cross-coupling reactions for the introduction of highly functionalized alkyl substituents is so far underdeveloped and still remains a synthetic challenge. The reason is the incompatibility of most of the functional groups with the highly reactive organozinc,[15] -magnesium,[16] -copper,[17] or -aluminum[18] groups necessary for an effective transmetalation of sp^3-hybridized substituents (tolerant and mild Suzuki−Miyaura or Stille couplings are usually not efficient for such substituents). Therefore, suitable protective or masking groups must be used.

Here we wish to report a novel efficient method for preparation 6-hydroxymethyl-9-substituted purines by the Negishi cross-coupling reactions of *O*-acyl-protected hydroxymethylzinc iodides **1** with 6-halo-9-substituted purines **2** or **3** (Scheme 1). As the first protected organozinc reagent

Scheme 1[a]



1-4	x	y
	R	R'
a	Bn	Piv
b	THP	Bz
c	AcRf	Ac
d	ToldRf	H

[a] (i) **1y**, $Pd(PPh_3)_4$, THF, rt, 4−12 h.

we used (pivaloyloxymethyl)zinc iodide **1a** prepared by a known procedure[19] from iodomethyl pivalate (easily available from commercial chloromethyl pivalate by Finkelstein reaction[20]). The reagent **1a** has been previously[19] prepared, transmetalated to zinc-cuprate, and used for conjugate additions or for uncatalyzed couplings with vinyl or allyl halides, as well as acyl chlorides. To the best of our knowledge, it has not yet been used for Pd-catalyzed cross-coupling reactions.

The Negishi reaction of this organozinc reagent with 6-halopurines **2** or **3** proceeded[21] smoothly at room temperature in about 6−8 h to give the 6-(pivaloyloxymethyl)-purines **4xa** in excellent yields (Table 1, entries 1−8). The

Table 1. Cross-Couplings of Acyloxymethylzinc Iodides with 6-Halopurines

entry	halopurine	1 (eq.)	*t* (h)	product (yield %)[a]
1	**2a**	1a (2.5)	8	**4aa**(83)
2	**2a**	1a (4)	8	**4aa** (95)
3	**3a**	1a (3)	6	**4aa** (88)
4	**2b**	1a (4)	8	**4ba** (90)
5	**3b**	1a (3)	6	**4ba** (94)
6	**2c**	1a (3)	8	**4ca** (95)
7	**2d**	1a (3)	8	**4da** (92)
8	**3d**	1a (3)	6	**4da** (95)
9	**2a**	1b (3)	8	**4ab** (81)
10	**3a**	1b (3)	6	**4ab** (96)
11	**2b**	1b (3)	8	**4bb** (72)
12	**3b**	1b (3)	6	**4bb** (95)
13	**2c**	1b (3)	8	**4cb** (94)
14	**2d**	1b (3)	8	**4db** (91)
15	**3d**	1b (3)	6	**4db** (84)/**4dd** (9)
16	**2a**	1c (3)	8	**4ac** (76)/**4ad** (15)
17	**3a**	1c (3)	6	**4ac** (60)/**4ad** (35)
18	**2b**	1c (3)	8	**4bc** (35)/**4bd** (33)
19	**3b**	1c (3)	6	**4bc** (76)/**4bd** (20)
20	**2c**	1c (3)	8	**4cc** (68)/**4cd** (25)
21	**2d**	1c (3)	8	**4dc** (42)/**4dd** (50)
22	**3d**	1c (3)	6	**4dc** (64)/**4dd** (30)

[a] Isolated yield.

effect of the leaving group was not crucial: 6-iodopurines **3x** usually gave just slightly better yields than 6-chloropurines **2x**. The reaction was applied on protected (Bn or THP) halopurine bases **2a**/**3a** and **2b**/**3b**, as well as on protected

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(21) **Typical procedure for cross-coupling** of pivaloyloxymethylzinc iodide (**1a**) and 6-chloro-9-benzylpurine (**2a**). A solution of iodomethyl pivalate (538 mg, 2.22 mmol) in THF (3 mL) was added at 15 °C to a suspension of zinc dust (288 mg, 4.4 mmol) in THF (1 mL) that was preactivated with dibromoethane (20 μL) and trimethylsilyl chloride (18 μL). After 1 h, the solution of **1a** (4 mL, 0.5 mmol/mL) in THF was added at room temperature to the solution of **2a** (122 mg, 0.5 mmol) and $Pd(PPh_3)_4$ (29 mg, 5%) in THF (1 mL) and stirred at room temperature for 8 h. The reaction was quenched with 1 M NH_4Cl (30 mL) and extracted with ethyl acetate (3 × 25 mL). Collected organic phases were dried over $MgSO_4$, filtered, and evaporated. Crude oil was purified by chromatography on silica gel (hexanes/ethyl acetate 2/1−3/2), affording a yellowish oil (154 mg, 95%), which was crystallized from ethyl acetate−hexanes to give 123 mg (80%) of white crystals (mp 68−71 °C). Anal. Calcd for $C_{18}H_{20}N_4O_2$: C, 66.65; H, 6.21; N, 17.27. Found: C, 66.43; H, 6.12; N, 16.90. FAB MS *m*/*z* (%): 325 (M^+, 35); 241 (7); 224 (6); 91 (100); 57 (100). ^{1}H NMR ($CDCl_3$, 400 MHz): 1.29 (s, 9H, *t*-Bu); 5.44 (s, 2H, N-CH_2); 5.62 (s, 2H, O-CH_2); 7.33−7.36 (m, 5H, Ph); 8.03 (s, 1H, H-8); 8.97 (s, 1H, H-2). ^{13}C NMR ($CDCl_3$, 100 MHz): 27.2 (3 × CH_3); 38.9 (*C*Me_3); 47.3 (N-CH_2); 62.4 (O-CH_2); 127.9; 128.7; 129.2 (CH-phenyl); 131.7 (C-5); 134.9 (C-1-phenyl); 144.4 (C-8); 151.6 (C-4); 152.5 (C-2); 155.7 (C-6); 178.3 (C=O). IR (CCl_4): ν = 2934, 1739, 1594, 1500, 1480, 1332, 1146 cm^{-1}.

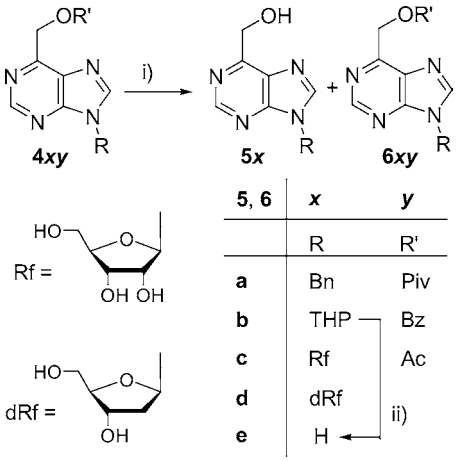
(acylated) ribo- or 2-deoxyribonucleosides **2c** and **2d**/**3d**, respectively.

Analogously, (benzoyloxymethyl)zinc iodide (**1b**) was prepared by insertion of zinc into the iodomethyl benzoate[22] at 5−10 °C. The yields of cross-couplings of 6-halopurines with **1b** were comparable or somewhat lower than with **1a** (entries 9−15), and in the case of synthesis of protected 2-deoxyribonucleoside **4db** (entry 15), partial hydrolysis to **4dd** was observed.

(Acetyloxymethyl)zinc iodide **1c** (prepared from iodomethyl acetate[20b] and activated zinc) was also used as reagent in the cross-coupling reactions with 6-halopurines. Unfortunately, the resulting 6-(acetyloxymethyl)purines appeared to be less stable and suffered from facile de-*O*-acetylation during the workup and purification steps. Thus, besides the desired 6-(acetyloxymethyl)purines **4xc**, significant amounts of deacetylated byproducts **4xd** were isolated by column chromatography. From the yields of products **4xc** and **4xd**, overall conversions of these cross-coupling reactions in the range of 90−96% are obtained (Table 1, entries 16−22).

Standard deprotection of 6-(acyloxymethyl)purine intermediates **4*xy*** was used to prepare the final free 6-(hydroxymethyl)purine bases and nucleosides **5a**−**5e** (Scheme 2, Table 2). The ester groups were cleaved by NaOMe in

Scheme 2 [a]



[a] (i) NaOMe, MeOH, rt; (ii) Dowex (H^+ form), EtOH.

methanol at room temperature.[23] In case of 6-(pivaloyloxymethyl)purines **4xa**, the cleavage of the bulky pivaloyl ester was quite slow, and even after 2 days significant amounts of partly deprotected compounds **6ca**, **6da**, and some yet unidentified byproducts were observed in addition to the desired fully deprotected purines **5*x*** (Table 2, entries 1−5). On the other hand, cleavage of benzoates **4xb** and acetates **4xc** proceeded smoothly to give the 6-(hydroxymethyl)-purines in good yields (Table 2, entries 6−13).

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Table 2. Cleavage of Ester Protecting Groups in Purines or Nucleosides **4*xy***

entry	**4*xy***	equiv of NaOMe	*t* (h)	product (yield, %)[a]
1	**4aa**	1	36	**5a** (22)
2	**4ba**	0.2	48	**5b** (36)
3	**4ba**	1.25	24	**5b** (42)
4	**4ca**	0.5	48	**5c** (38)/**6ca** (22)
5	**4da**	0.5	48	**5d** (35)/**6da** (12)
6	**4ab**	0.2	20	**5a** (84)
7	**4bb**	0.1	20	**5b** (85)
8	**4cb**	0.1	20	**5c** (77)
9	**4db**	0.2	20	**5d** (82)
10	**4ac**	0.1	12	**5a** (96)
11	**4bc**	0.1	12	**5b** (88)
12	**4cc**	0.1	12	**5c** (81)
13	**4dc**	0.1	12	**5d** (86)

[a] Isolated yield.

The THP protective group in position 9 of purine is easily cleavable under mild acidic conditions. Thus compounds **5b** and **4by** were deprotected using Dowex 50X8 (H^+ form)[24] in ethanol to afford 6-substituted-9*H*-purines **5e** and **6ey**, respectively (Scheme 2, Table 3).

Table 3. Cleavage of THP Protecting Groups

entry	THP derivative	product (yield, %)[a]
1	**5b**	**5e** (78)
2	**4ba**	**6ea** (89)
3	**4bb**	**6eb** (55)
4	**4bc**	**6ec** (56)

[a] Isolated yield.

The title 6-(hydroxymethyl)purines and nucleosides **5a** and **5c**−**5e**, as well as some of the partially deprotected 6-(acyloxymethyl)purines **6ca**, **6ea**, **6eb**, and **6ec**, were subjected to biological activity screening. In vitro cytostatic activity tests (inhibition of cell growth) were performed using 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). Adenosine deaminase (ADA) inhibition was studied[25] on calf intestinal adenosine aminohydrolase (EC 3.5.4.4.). The results are summarized in Table 4.

The most active was the 6-(hydroxymethyl)purine ribonucleoside (**5c**), which exerted very high antiproliferative activity against HL-60 and CCRF-CEM (IC_{50} = 0.01 and

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Table 4. Cytostatic and ADA Inhibitory Activity of Title Compounds

	IC_{50} (μmol/l)[a]		
compd	HL60	CCRF-CEM	ADA
5c	0.01 (±0.0011)	0.15 (±0.0011)	1.70 (±0.136)
5d	NA[b]	NA	10.0 (±0.90)
5e	12.0 (±0.96)	~30	NA

[a] Values are means of four experiments; standard deviation is given in parentheses. [b] NA = not active (inhibition of cell growth at 10 μM was lower than 30%).

0.15 μmol/L, respectively) but no considerable effect against L1210 and HeLa S3 cell lines. The corresponding purine base **5e** showed only weak cytostatic effects, and the other tested compounds were entirely inactive. Ribonucleoside **5c** was, in accord with the previous finding,[8] also found to exert considerable inhibitory effect on ADA (IC_{50} = 1.7 μmol/L), the corresponding 2-deoxyriboside **5d** was about 1 order of magnitude less potent, and all of the other compounds were inactive.

In conclusion, 6-(hydroxymethyl)purine bases and nucleosides can be prepared efficiently by this practical methodology (superior to the previously known approaches)[8−13] in two steps in overall yields of 75−87%. Benzoyloxymethylzinc iodide **1b** appears to be the most practical reagent because the benzoyl group is sufficiently stable during the metalation and cross-coupling but can be easily cleaved off at the end. 6-(Hydroxymethyl)purine nucleosides possess interesting cytostatic and ADA inhibitory effect, and thus other related derivatives and analogues, including acyclic nucleosides with side chains from known potent ADA inhibitors (EHNA, etc.) are a desirable target. Extension of this methodology to these purine derivatives, as well as to other related heterocycles, is under way.

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Supporting Information Available: Experimental and spectral data for compounds **4**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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