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Brief Article

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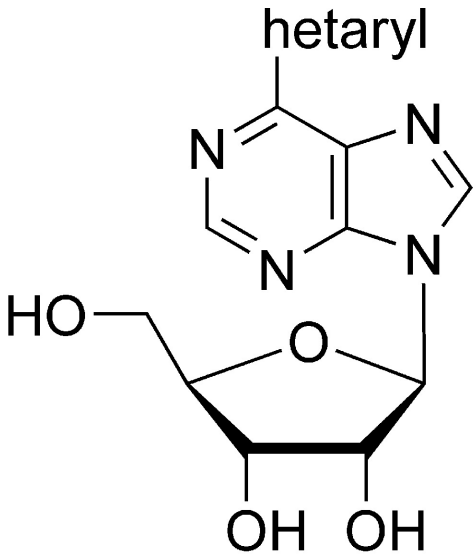

anti-HCV and cytostatic activity

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Brief Articles

Cytostatic 6-Arylpurine Nucleosides. 6.† SAR in Anti-HCV and Cytostatic Activity of Extended Series of 6-Hetarylpurine Ribonucleosides

Michal Hocek,‡,* Petr Nauš,‡ Radek Pohl,‡ Ivan Votruba,‡ Phillip A. Furman,⊥ Phillip M. Tharnish,⊥ and Michael J. Otto⊥

Centre for New Antivirals and Antineoplastics, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16610 Prague 6, Czech Republic, and Pharmasset Inc., 1860 Montreal Road, Tucker, Georgia 30084

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Significant anti-HCV activity of 6-hetarylpurine ribonucleosides has been discovered and is reported here for the first time and compared with cytostatic effect. An extended series of 6-hetarylpurine nucleosides has been prepared by heterocyclizations in position 6 of purine nucleosides or by cross-couplings of 6-chloropurine nucleosides with hetarylboronic acids, -stannanes, or -zinc halides. The most anti-HCV active were purine ribonucleosides bearing pyrrol-3-yl (**3k**) or 2-furyl (**3g**) groups exerting $EC_{90} = 0.14$ and 0.4 μM, respectively.

Introduction

Hepatitis C is a very common infection and a cause of chronic liver disease and liver transplantation. Current therapy is poorly tolerated and has limited efficacy. Therefore, there is a great need for more effective anti-HCV agents. Nucleoside analogues are a promising class of anti-HCV agents. In particular, 2′-β-*C*-methyl adenosine and their base-modified derivatives[1] are very promising examples inhibiting RNA-dependent RNA polymerase.[2] Very recently, related purine-modified nucleosides including 6-(het)aryl-9-(2-β-*C*-methyl-β-D-ribofuranosyl)purines were reported[3] to be moderate anti-HCV agents.

6-Arylpurine ribonucleosides were prepared[4,5] in our laboratory several years ago and found to possess significant cytostatic activity. Several 6-arylpurine nucleosides were later also reported to exert antimycobacterial activity.[6] This structural lead has been then extensively modified in order to study the SAR in cytostatic activity. It was found that also 6-hetaryl- and 6-benzylpurine ribonucleosides exerted cytostatic activity,[5] while any sugar modified derivatives (2′- or 5′-deoxyribonucleosides[7] or acyclic nucleoside or nucleotide analogues[8]) as well as 2- or 8-substituted 6-arylpurine ribonucleosides[9] were inactive. For compounds with the aryl groups in the position 6, the most active were 4-substituted phenyl derivatives, while 3- or 2-substituted phenyl as well as more bulky 1- or 2-naphthyl derivatives were much less active or entirely inactive. Only four examples of 6-hetarylpurine ribonucleosides were prepared in the previous study: 2-furyl and 2-thienyl derivatives possessed significant cytostatic activity, the 2-pyridyl derivative was somewhat less active, while (1-methylpyrrol-2-yl)purine nucleoside was inactive. Later on, these modified purine nucleosides were tested for anti-HCV activity in the replicon cell-based assay, and since several of them were active, the series of 6-hetarylpurine nucleosides was extended and the anti-HCV and cytostatic activity of both new and known compounds are compared in this report.

Results and Discussion

6-Hetarylpurine derivatives could be efficiently prepared by cross-coupling reactions of 6-halopurines with hetarylorganometallic reagents.[10] However, in some cases, the corresponding organometallic species might be less readily accessible or unstable, and therefore build-up of the heterocyclic moiety at the 6 position could be an alternative approach.[11] In this study we used both of these strategies. To thoroughly study the SAR of the biological activity of this class of compounds, we needed to prepare a series of derivatives bearing diverse types of five-membered heterocycles.

Because the cross-coupling approach is the most straightforward, we have used a variety of commercially available hetarylboronic acids or organozinc reagents in the Pd-catalyzed cross-couplings with acetyl-protected 6-chloropurine ribonucleoside **1** (Scheme 1) in analogy to the previously published syntheses.[4,5] The Suzuki−Miyaura reactions of 3-thienyl-, 3-furyl-, (3-methylthiophen-5-yl)-, and (4-methylthiophen-5-yl)boronic acids with nucleoside **1** proceeded very smoothly, affording the desired protected 6-hetarylpurine nucleosides **2a**−**d** in very good yields. An analogous reaction of (benzofuran-2-yl)boronic acid was much slower, and the product **2e** was inseparable from the starting compound **1**; thus, the obtained mixture was used in the deprotection step with a hope that the free nucleosides would be separable. Other hetarylboronic acids or esters (pyridine-3-boronic acid, pyridine-4-boronic acid, and pinacol pyrazole-4-boronate) have not reacted even when using a

† For Part V of this series, see ref 9b.

* To whom correspondence should be addressed. Phone: +420 220183324. Fax: +420 220183559. E-mail: hocek@uochb.cas.cz.
‡ Institute of Organic Chemistry and Biochemistry.
⊥ Pharmasset Inc.

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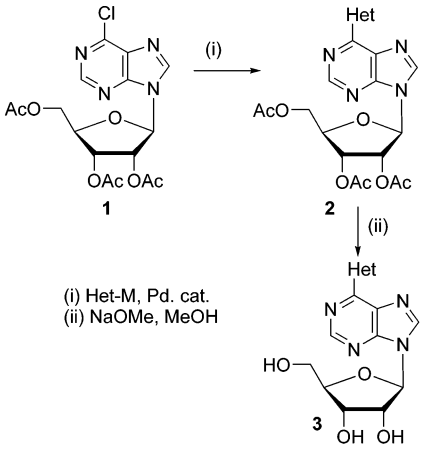
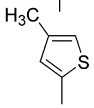
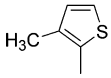
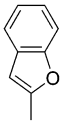
Scheme 1

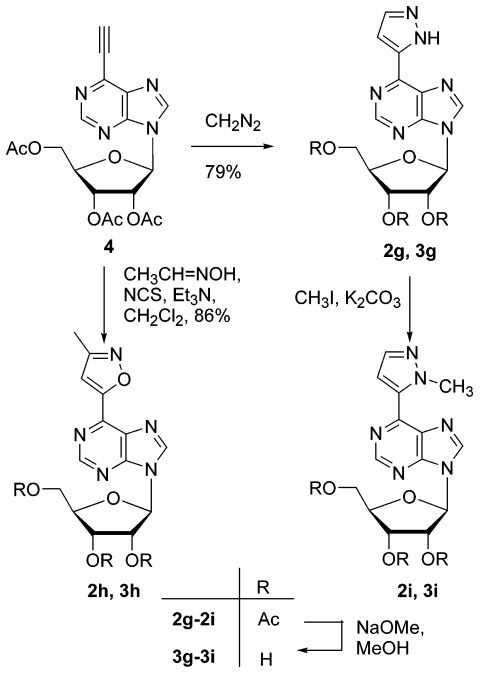


2-3	Het	M	Yield (i)	Yield (ii)
a		$B(OH)_2$	91%	84%
b		$B(OH)_2$	94%	82%
c		$B(OH)_2$	90%	83%
d		$B(OH)_2$	83%	78%
e		$B(OH)_2$	ca. 45%* (not isolated)	30%** (overall)
f		ZnBr	39%	75%

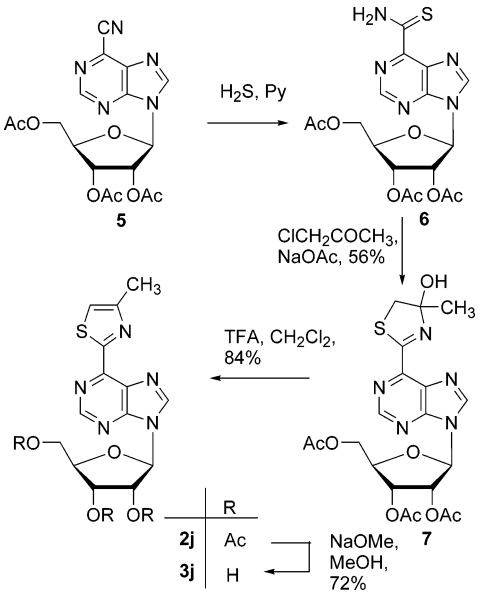
more reactive catalytic system ($Pd(OAc)_2$/2-(dicyclohexylphosphino)biphenyl).[12] For the synthesis of 6-(thiazol-2-yl)purine, a Pd-catalyzed cross-coupling of thiazol-2-ylzinc bromide was used. This reaction proceeded reasonably well, but the isolation of the product **2f** was difficult probably due to formation of stable complexes with zinc. Only after thorough extraction with EDTA and column chromatography was the product isolated in pure form with a moderate yield of 39%. All the acyl-protected 6-hetarylpurine nucleosides **2a**−**f** were easily deprotected by treatment with catalytic amount of sodium methoxide in methanol to afford free ribonucleosides **3a**−**f** in good yields. Also the benzofuran derivative **3e** was prepared by deacylation of the mixture of **2e** and **1**, and at this stage it was easily isolated from the byproducts by column chromatography.

For the synthesis of other 6-hetarylpurine nucleosides, heterocyclizations have been used. Alkynes are versatile building blocks for heterocycle formation by means of 1,3-dipolar cycloadditions. Thus, the 6-ethynylpurine **4**[13] reacted with diazomethane to give the pyrazole[14] **2g** and with in situ-generated nitrile oxide (formed from acetaldoxime, NCS, and triethylamine) to afford the 3-methylisoxazole[15] **2h** with yields of 79 and 86%, respectively (Scheme 2). The acylated nucleosides **2g** and **2h** were easily deprotected by sodium methoxide

Scheme 2



Scheme 3



in methanol to give the free nucleosides **3g** and **3h** in 78 and 92% yields, respectively. The acylated pyrrazole **2g** was also methylated by iodomethane in the presence of K_2CO_3 to give the 1-methylpyrazole **2i** that was without isolation deprotected to **3i**.

6-Cyanopurine nucleoside **5**[16] was treated with sulfane in pyridine to afford purine-6-thiocarboxamide **6**[17] that reacted with chloroacetone to give the 4-hydroxy-4-methyl-4,5-dihydrothiazole **7** (Scheme 3). Dehydration was achieved using TFA to afford the 4-methylthiazole **2j** that was deprotected to **3j**.

For a straightforward synthesis of pyrrol-3-yl derivative **3k**, we have adopted a method developed originally by van Leusen for the synthesis of 3,4-disubstituted pyrroles.[18] The reaction of tosylmethyl isocyanide (TosMIC) with the known TBS-protected 6-vinylpurine nucleoside **8**[19] in the presence of sodium *tert*-butoxide as a base in the mixture of DMSO/THF afforded the

Table 1. Cytostatic and Antiviral Activity of 6-R-Substituted Purine Ribonucleosides **3a**−**t**

compd	R	cytostatic activity IC_{50} (μM)[a,b] L1210	HL60	HeLa S3	CCRF-CEM	HCV EC_{90} (μM)[c]	rRNA CC_{50} (μM)[d]
3a	furan-3-yl	n.a.[e]	3.3 ± 0.17	n.a.	1.65 ± 0.11	1.4	6.9
3b	thiophen-3-yl	10.8 ± 0.78	3.8 ± 0.19	n.a.	1.64 ± 0.11	0.82	<0.1
3c	4-methylthiophen-2-yl	n.a.	5.6 ± 0.39	11.4 ± 0.89	2.9 ± 0.17	40.7	7.0
3d	3-methylthiophen-2-yl	n.a.	4.5 ± 0.36	>20	2.5 ± 0.19	>100	>100
3e	benzofuran-2-yl	n.a.	n.a.	n.a.	15 ± 1.8	>100	>100
3f	thiazol-2-yl	16.0 ± 0.64	6.3 ± 0.29	n.a.	2.73 ± 0.14	6.0	6.7
3g	pyrazol-5-yl	19.8 ± 1.81	1.17 ± 0.15	n.a.	0.75 ± 0.056	0.8	0.6
3h	3-methylisoxazol-5-yl	n.a.	n.a.	n.a.	n.a.	>100	>100
3i	1-methylpyrazol-5-yl	n.a.	n.a.	n.a.	n.a.	>100	>100
3j	4-methylthiazol-2-yl	n.a.	n.a.	n.a.	6.0 ± 0.49	>100	>100
3k	pyrrol-3-yl	17.5 ± 1.05	3.2 ± 0.18	n.a.	1.03 ± 0.06	0.14	<0.1
3l	phenyl	9.0 ± 0.36	6.3 ± 0.44	2.7 ± 0.13	0.7 ± 0.04	16.7	8.3
3m	4-methoxyphenyl	1.5 ± 0.06	7.6 ± 0.53	4.3 ± 0.17	0.25 ± 0.03	>100	<10
3n	4-fluorophenyl	4.5 ± 0.16	6.3 ± 0.44	2.7 ± 0.13	0.7 ± 0.04	49.5	9.2
3o	4-chlorophenyl	n.a.	n.a.	5.0 ± 0.35	0.9 ± 0.08	>100	>100
3p	4-hydroxyphenyl	5.0 ± 0.39	7.6 ± 0.49	32.5 ± 1.9	1.25 ± 0.053	2.6	5.2
3q	furan-2-yl	2.0 ± 0.16	2.7 ± 0.21	25 ± 1.26	0.6 ± 0.09	0.4	1.6
3r	thiophen-2-yl	0.37 ± 0.032	4.9 ± 0.35	25 ± 1.18	0.6 ± 0.11	0.7	1.1
3s	1-methylpyrrol-2-yl	n.a.	15.7 ± 0.94	n.a.	2.0 ± 0.019	>100	>100
3t	pyridin-4-yl	4.3 ± 0.31	5.6 ± 0.39	9.7 ± 0.43	0.9 ± 0.034	6.0	2.7
FUdR[f]		0.012 ± 0.003	0.012 ± 0.003	n.a.	0.017 ± 0.004	–	–
2′−C−CH_3-A[g]		–	–	–	–	1.14	15.0

[a] Concentration of a compound needed to reduce population growth by 50% in vitro. [b] Activities of compounds **3l**−**t** in L1210, HeLa, and CCRF-CEM cell lines are taken from refs 4, 5. [c] Effective concentration required for reducing the HCV by 90%. [d] Cytotoxic concentration required for reducing the rRNA level by 50%. [e] n.a. = not active (inhibition of the cell growthat $c = 10$ μM was lower than 20%). [f] 1-(β-D-2-Deoxy-*erythro*-pentofuranosyl)-5-fluorouracil.[23] [g] 2′-*C*-β-methyladenosine.[21]

Scheme 4

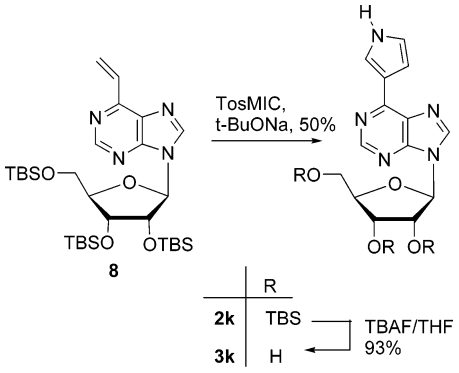
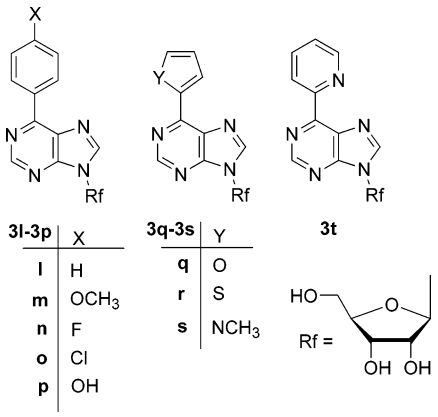


Chart 1



protected (pyrrol-3-yl)purine nucleoside **2k** in an acceptable yield of 50% (Scheme 4). Deprotection using TBAF gave the free nucleoside **3k** in excellent yield.

Biological Activity. Antiviral activity of the new series of 6-hetarylpurine ribonucleosides **3a**−**k**, as well as the previously reported 6-aryl- and 6-hetarylpurine nucleosides **3l**−**t**, was evaluated in a HCV subgenomic replicon assay,[20,21] and the results are presented in Table 1. Also the cytostatic effect of the new set of compounds **3a**−**k** was studied (inhibition of cell growth of the following cell cultures: (i) mouse leukemia L1210 cells (ATCC CCL 219); human promyelocytic leukemia HL60 cells (ATCC CCL 240); human cervix carcinoma HeLaS3 cells (ATCC CCL 2.2); human T lymphoblastoid CCRF-CEM cell line (ATCC CCL 119)). For comparison, Table 1 includes also previously reported effects of compounds **3l**−**t** in L1210, HeLa, and CCRF-CEM assays and data on this set of compounds in the HL60 cell line.

Many compounds of this series showed significant antiviral activity in the cell-based HCV replicon assay. In particular, purine ribonucleosides bearing a five-membered heterocyclic moiety in the 6 position, i.e. compounds **3a**, **3b**, **3g**, **3q**, and **3r**, were the most active ones with EC_{90} in the submicromolar range which is about 1 order of magnitude more effective than 2′-C-CH_3-A.[21] Unfortunately, any anti-HCV activity was accompanied by effects on cellular rRNA, suggesting a lack of specificity for these compounds. Surprisingly, the 6-(4-substituted phenyl)purine ribonucleosides **3l**−**p**, as well as 6-(pyridin-2-yl)purine derivative **3t**, that were the most potent cytostatics, exerted much lower or negligible anti-HCV activity. Introduction of a methyl group on a five-membered heterocycle in the 6 position (in compounds **3c**, **3d**, **3i**, **3j**, and **3s**) or benzo-annulation (compound **3e**) resulted in loss of antiviral activity but not necessarily loss of effects on rRNA. A significant decrease in the synthesis of intracellular HCV replicon RNA was seen in association with reduced cell growth. Some degree of cytotoxicity or cytostasis was observed for those compounds, demonstrating anti-HCV replicon activity. This would indicate that the anti-HCV activity is associated with the cytostatic affect of these compounds. This relationship was previously observed with N^3,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5]pyridin-5-one.[22]

The extension of this series of compounds allowed us to revisit the SAR of the cytostatic effect. Apparently, the 6-(4-substituted phenyl)purine nucleosides **3l**−**o** are the most active against CCRF-CEM and the only active examples in HeLa S3 cell lines. On the other hand, the 6-hetarylpurine nucleosides showed comparable or even better activity in L1210 and HL60 assays than the 6-phenylpurine nucleosides. Compounds bearing additional methyl substituents or larger aryl or hetaryl groups are much less active. However, even the most active compounds (e.g. **3m**, **3q**, and **3r**) are less active than FUdR[23] by ca. 1−2 orders of magnitude.

Conclusions

An extended series of 6-hetarylpurine ribonucleosides was prepared either by cross-coupling reactions or heterocyclizations. Purine nucleosides bearing simple five-membered heterocyclic rings in the position 6 showed significant anti-HCV activity, significant inhibition of rRNA, and cytostatic effect against leukemia cell lines. On the other hand 6-phenylpurine nucleosides are strongly cytostatic for leukemia and cancer cell lines but of negligible effect against HCV. This structural lead will certainly attract further attention in the search for new antivirals and cytostatics.

Experimental Section

For general experimental and for characterization data of all new compounds, see Supporting Information. Cytostatic activity tests were performed as described in ref 4. The HCV replicon assay was performed as previously described by Stuyver et al.[20]

Cross-Coupling Reactions of 6-Chloropurine Nucleoside 1 with Arylboronic Acid. General Procedure. Toluene (10 mL) was added to an argon-purged flask containing the protected 6-chloropurine nucleoside **1**[24] (413 mg, 1 mmol), K_2CO_3 (200 mg, 1.5 mmol), arylboronic acid (1.5 mmol), and $Pd(PPh_3)_4$ (59 mg, 0.05 mmol), and the mixture was stirred under argon at 100 °C for 8 h. After being cooled to ambient temperature, the mixture was evaporated in vacuo and the residue was chromatographed on a silica gel column (50 g, ethyl acetate-light petroleum 1:2 to 9:1). Evaporation and drying of the product-containing fractions afforded the 6-arylpurines **2a**−**d** as foams or amorphous solids. Compound **2e** was not separable from the starting compound, and therefore the mixture was directly used in the deprotection step.

6-(Thiazol-2-yl)-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-purine (2f). THF (10 mL) was added to an argon purged flask containing the 6-chloropurine **1** (600 mg, 1.45 mmol) and $Pd(PPh_3)_4$ (59 mg, 0.05 mmol). The mixture was stirred at ambient temperature for 10 min, and then a solution of thiazol-2-ylzinc bromide (0.5 M solution in THF, 6 mL, 3 mmol) was added dropwise (within 10 min) at ambient temperature. The stirring at room temperature was continued for 15 min followed by stirring at 60 °C for 8 h. Then the reaction mixture was allowed to stand overnight at room temperature and poured into saturated aqueous NH_4Cl (10 mL) and saturated aqueous Na_2EDTA (10 mL). Then the mixture was extracted with ethyl acetate (3 × 20 mL), and the collected organic layers were washed with saturated aqueous Na_2EDTA (20 mL) and brine (20 mL), dried with $MgSO_4$, and evaporated in vacuo. Column chromatography of the residue on silica gel (50 g, ethyl acetate−light petroleum 1:2 to 9:1) afforded, after evaporation and drying, the product **2f** as amorphous solid, yield 260 mg (39%).

6-[1*H*(2*H*)-Pyrazol-3-yl]-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (2g). Ethereal diazomethane solution (3 mL) was added to **4**[13] (145 mg, 0.36 mmol) in chloroform (2 mL). Reaction mixture was left to stand for 3 h at room temperature. The volatiles were evaporated under reduced pressure, and chromatography on silica (AcOEt) afforded 126 mg (79%) of product **2g** as colorless amorphous glassy solid.

6-(3-Methylisoxazol-5-yl)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (2h). Acetaldoxime (18 mg, 0.30 mmol) in 1 mL of CH_2Cl_2 was added to a strirred mixture of *N*-chlorosuccinimide (40 mg, 0.30 mmol) and pyridine (2 μL) in 1 mL of CH_2Cl_2, and the mixture was stirred for 10 min at room temperature. Then **4**[13] (100 mg, 0.25 mmol) in 2 mL of CH_2Cl_2 was added to the reaction mixture. Finally triethylamine (42 μL, 0.30 mmol) in 2 mL of CH_2Cl_2 was dropwise added during 30 min, and the reaction mixture was stirred overnight. The volatiles were evaporated in vacuo, the product was dissolved in 25 mL of ethyl acetate, and this solution was washed with water (2 × 25 mL). Organic phase was dried over $MgSO_4$, and column chromatography on silica (hexanes−ethyl acetate, 1:1) afforded 98 mg (86%) of product as white foam.

6-(4-Methylthiazol-2-yl)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (2j). To a solution of **7** (102 mg, 0.21 mmol) in dry dichloromethane (4 mL) was added trifluoroacetic acid (40 μL, 0.52 mmol). The solution was left standing at room temperature for 8 h and then was directly chromatographed on the column of silica (AcOEt), providing 83 mg (84%) of desired product as yellowish amorphous solid.

6-(1*H*-Pyrrol-3-yl)-9-[2,3,5-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]purine (2k). To a stirred suspension of *t*BuONa (160 mg, 1.67 mmol) in dry DMSO (5 mmol) was added a solution of TosMIC (211 mg, 1.08 mmol) and **8**[19] (518 mg, 0.83 mmol) in a mixture of dry DMSO/THF (5 mL/5 mL) at room temperature. The mixture was stirred 1 h and then was diluted with AcOEt (50 mL) and washed with saturated aqueous ammonium chloride solution (3 × 50 mL). The organic phase was dried over $MgSO_4$ and evaporated in vacuo. The residue was chromatographed on the column of silica (hexane−AcOEt, 2:1), affording 273 mg (50%) of product as amorphous foamy solid.

Deprotection of the Nucleosides 2a−h and 2j. General Procedure. A 1 M methanolic MeONa (200 μL, 0.2 mmol) solution was added to the solution of a protected nucleoside **2a**−**j** (0.5−0.8 mmol) in MeOH (20 mL), and the mixture was stirred at ambient temperature overnight. The solvent was then evaporated and the residue chromatographed on a siliga gel column (50 g, ethyl acetate/methanol 80:20). The products were recrystallized from EtOH/toluene/heptane to give the free nucleosides **3a**−**h** and **3j**.

6-[1-Methyl-1*H*-pyrazol-3-yl]-9-(β-D-ribofuranosyl)purine (3i). Iodomethane (17 μL, 0.26 mmol) was added to a mixture of compound **2g** (59 mg, 0.13 mmol) and K_2CO_3 (37 mg, 0.26 mmol) in acetonitrile (2 mL), and the solution was stirred at r.t. for 6 h. Then the solvent was evaporated, and the residue was dissolved in ethyl acetate and filtered through a small column of silica gel. The eluent was evaporated, dissolved in methanol (2 mL), and treated with 1 M methanolic NaOMe (20 μL, 0.02 mmol) for 6 h. Compound **3i** spontaneously crystallized from the mixture. Yield 25 mg (58%).

6-(1*H*-Pyrrol-3-yl)-9-(β-D-ribofuranosyl)purine (3k). To a stirred solution of **2k** (192 mg, 0.29 mmol) in dry THF (2 mL) was dropwise added a 1 M solution of TBAF·$3H_2O$ in THF (1.3 mL, 1.3 mmol) at room temperature. After 30 min, the reaction mixture was directly chromatographed on a column of silica (AcOEt−MeOH, 10:1), affording 86 mg (93%) of product that crystallized from MeOH.

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Supporting Information Available: General experimental and characterization data for all new compounds are available free of charge via the Internet at http://pubs.acs.org.

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